New Methods for Automated Glycan Assembly to Prepare Different Classes of Glycans

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by

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1st Reviewer: Professor Dr. Peter H. Seeberger

2nd Reviewer: Professor Dr. Kevin Pagel

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Declaration

This is to certify that the entire work in this thesis has been carried out by Mr. Heung Sik Hahm. The assistance and help received during the course of investigation have been fully acknowledged.

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(Date, Place) (Signature)

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List of Publications

Parts of this thesis have been published and communicated:

A. Scientific Publications

1. Y.-H. Tsai, S. Götze, N. Azzouz, **H. S. Hahm**, P. H. Seeberger, D. V. Silva A General Method for Synthesis of GPI Anchors Illustrated by the Total Synthesis of the Low-Molecular-Weight Antigen from *Toxoplasma gondii*. *Angew. Chem. Int. Edn Engl.* **2011**, *50*, 9961-9964. http://doi.org/10.1002/anie.201103483
2. S. Eller, M. Collot, J. Yin, **H. S. Hahm**, P. H. Seeberger, Automated Solid-Phase Synthesis of Chondroitin Sulfate Glycosaminoglycans. *Angew. Chem. Int. Edn Engl.* **2013**, *52*, 5858-5861. http://doi.org/10.1002/anie.201210132
3. O. Calin, S. Eller, **H. S. Hahm**, P. H. Seeberger, Total Synthesis of the Escherichia coli O111 O-Specific Polysaccharide Repeating Unit. *Chem. Eur.* **2013**, *19*, 3995-4002. http://doi.org/10.1002/chem.201204394
4. J. Kandasamy, F. Schuhmacher, **H. S. Hahm**, J. C. Klein, P. H. Seeberger, Modular Automated Solid Phase Synthesis of Dermatan Sulfate Oligosaccharides. *Chem. Commun.*, **2014**, *50*, 1875-1878. http://doi.org/10.1039/c3cc48860h
5. R J. Fair, **H. S. Hahm**, P. H. Seeberger, Combination of Automated Solid-Phase and Enzymatic Oligosaccharide Synthesis Provides Access to α-(2,3)-sialylated Glycans. *Chem. Commun.*, **2015**, *51*, 6183-6185. http://doi.org/10.1039/c5cc01368b
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7. M. P. Bartetzko, F. Schumacher, **H. S. Hahm**, P. H. Seeberger, F. Pfrengle, Automated Glycan Assembly of Oligosaccharides Related to Arabinogalactan Proteins. *Org. Lett.* **2015**, *17*, 4344-4347. http://doi.org/10.1021/acs.orglett.5b02185.
8. J. Hofmann*, **H. S. Hahm***, P. H. Seeberger, K. Pagel, Identification of Carbohydrate Anomers Using Ion Mobility-Mass Spectrometry. *Nature* **2015**, *526*, 241-244. http://doi.org/10.1038/nature15388. (*equal contribution)
9. M. W. Weishaupt, S. Matthies, M. Hurevich, C. L. Pereira, **H. S. Hahm**, P. H. Seeberger, Automated Glycan Assembly of *S. Pneumoniae* serotype 3 CPS Antigens. *Beilstein J. Org. Chem.* **2016**, *12*, 1440-1446. http://doi.org/10.3762/bjoc.12.139
10. **H. S. Hahm**, C.-F. Liang, C.-H. Lai, R. J. Fair, P. H. Seeberger, Automated Glycan Assembly of Complex Oligosaccharides. Related to Blood Group Determinants *J. Org. Chem.* **2016**, *81*, 5866-5877. https://doi.org/10.1021/acs.joc.6b00554

11. **H. S. Hahm**, M. Hurevich, P. H. Seeberger, Automated Assembly of Oligosaccharides Containing Multiple *cis*-Glycosidic Linkages. *Nat. Commun.* **2016**, *7*:12482. http://doi.org/10.1038/ncomms12482
12. **H. S. Hahm***, F. Bröcker*, F. Kawasaki, M. Mietzsch R. Heilbronn, M. Fukuda, P. H. Seeberger, Automated Glycan Assembly of Oligo-*N*-Acetyllacosamine and Kertan Sulfate Probes to Study Virus-Glycan Interactions. *Chem.* **2017**, *1*, 114-124. http://doi.org/10.1016/j.chempr.2016.12.004. (*equal contribution)
13. B. Schumann, **H. S. Hahm,** S. G. Parameswarappa, K. Reppe, A. Wahlbrink, S. Govindan, L.-A. Pirofski, M. Witzenrath, C. Anish, C. L. Pereira, P. H. Seeberger, A Semisynthetic *Streptococcus pneumoniae* Serotype 8 Glycoconjugate Vaccine. *Sci. Transl. Med.* **2017**, *9*, eaaf5347. http://doi.org/10.1126/scitranslmed.aaf5347
14. M. W. Weishaupt, **H. S. Hahm**, A. Geisner, P. H. Seeberger, Automated Glycan Assembly of Branched β-(1,3)-Glucans to Identify for Antibody Epitopes. *Chem. Commun.* **2017**, *36*, 3591-3594. http://doi.org/10.1039/c7cc00520b
15. **H. S. Hahm***, M. Hurevich*, M. Schlegel*, S. Eller, F. Schuhmacher, J. Hoffmann, K. Pagel, P. H. Seeberger, Automated Glycan Assemble Using the Glyconeer® Synthesizer. *Proc. Natl. Acad. Sci. USA.* **2017**, *114*, E3385-E3389. http://doi.org/10.1073/pnas.1700141114. (*equal contribution)
16. N. Kottari, F. Schuhmacher, F.; **H. S. Hahm,** P. H. Seeberger, Pushing the Limits of Automated Glycan Assembly: Synthesis of a 50mer Oligomannoside. *Chem. Commun.* **2017**, *53*, 9085-9088. http://doi.org/10.1039/c7cc04380e
17. E. Mucha, A. I. G. Flórez, M. Marianski, D. A. Thomas, W. Hoffmann, W. B. Struwe, **H. S. Hahm,** S. Gewinner, W. Schöllkopf, P. H. Seeberger, G. von Helden, G. K. Pagel, Glycan Fingerprinting Using Cold-Ion Infrared Spectroscopy. *Angew. Chem. Int. Ed.* **2017**, *56*, 11248-11251. http://doi.org/10.1002/anie.201702896
18. D. L. Riggs, J. Hofmann, **H. S. Hahm,** P. H. Seeberger, K. Pagel, R. R. Julian, Glycan Isomer Identification Using Ultraviolet Photodissociation Initiated Radical Chemistry. *Anal. Chem.* **2018**, *90*, 11581-11588. http://doi.org/10.1021/acs.analchem.8b02958
19. A. Geissner, A. Reinhardt, C. Rademacher, T. Johannsen, J. Monteiro, B. Lepenies, M. Thépaut, F. Fieschi, J. Mrázková, M. Wimmerova, F. Schumacher, S. Götze, D. Grünstein, X. Guo, **H. S. Hahm**, J. Kandasamy, D. Leonori, C. Martin, S. Parameswarappa, S. Pasari, M. Schlegel, H. Tanaka, G. Xiao, Y. Yang, C. Pereira1, C. Anish, P. H. Seeberger. A microbe-focused glycan array screening platform. *Proc. Natl. Acad. Sci. USA.* **2019**, *116*, 1958-1967. https://doi.org/10.1073/pnas.1800853116

B. Patents

1. P. H. Seeberger, C. L. Pereira, C. Anish, B. Schumann, S. G. Parameswarappa, **H. S. Hahm**, S. Govindan, Vaccine against *Streptococcus pneumoniae* serotype 8. WO2016046420A1
2. J. Hofmann, **H. S. Hahm**, P. H. Seeberger, K. Pagel, Method for Determining Carbohydrates Structure. WO2017036545A1

C. Oral Presentations

1. The discovery of new linker for the automated solid phase synthesis of glycosaminoglycans, **2013**, Most Surprising Discovery 2013 Award Presentation
2. The most immunogenic carbohydrate antigen discovery using automated synthesis, **2013,** MPIKG Ringberg Conference, Rottach-Egern, Germany.

D. Poster Presentations

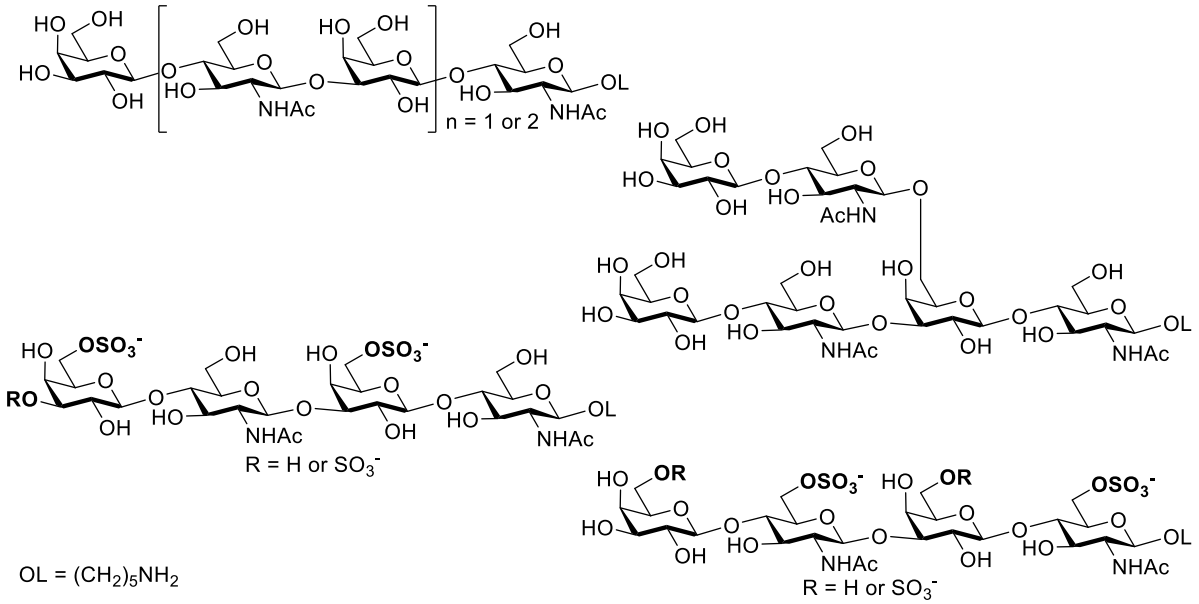
1. **H. S. Hahm**, P. H. Seeberger, The Logic of automated glycan Synthesis, 3rd international symposium of the collaborative research center 765 "Multivalency in Chemistry and Biochemistry", **2014**, Berlin, Germany.
2. **H. S. Hahm**, P. H. Seeberger, Installation of multiple 1,2-*cis* glycosidic bonds by automated synthesis, Poster Carb #72, 248th ACS National Meeting, **2014,** San Francisco, USA
3. **H. S. Hahm**, P. H. Seeberger, Installation of multiple 1,2-*cis* glycosidic bonds by automated synthesis, The 3rd Symposium of RIKEN-Max Planck Joint Research Center for System Chemical Biology, **2014**, Ringberg, Germany.
4. **H. S. Hahm**, P. H. Seeberger, Various LacNAc synthesis using automated solid-phase oligosaccharide synthesizer, The 2nd Symposium of Reiken-Max Planck Joint Research Center for System Chemical Biology, **2013**, Tokyo, Japan.
5. **H. S. Hahm,** F. Kawasaki, P. H. Seeberger, Automated solid-phase synthesis of *N*-Acetyllactosamine oligomers, The 26th International Carbohydrate, **2012**, Madrid, Spain.
6. S. Eller, M. Collot, J. Yin, **H. S. Hahm**, P.H. Seeberger, Automated solid-phase synthesis of chondroitin sulfates glycosaminoglycans, The 26th International Carbohydrate Symposium, **2012**, Madrid, Spain

Table of Contents

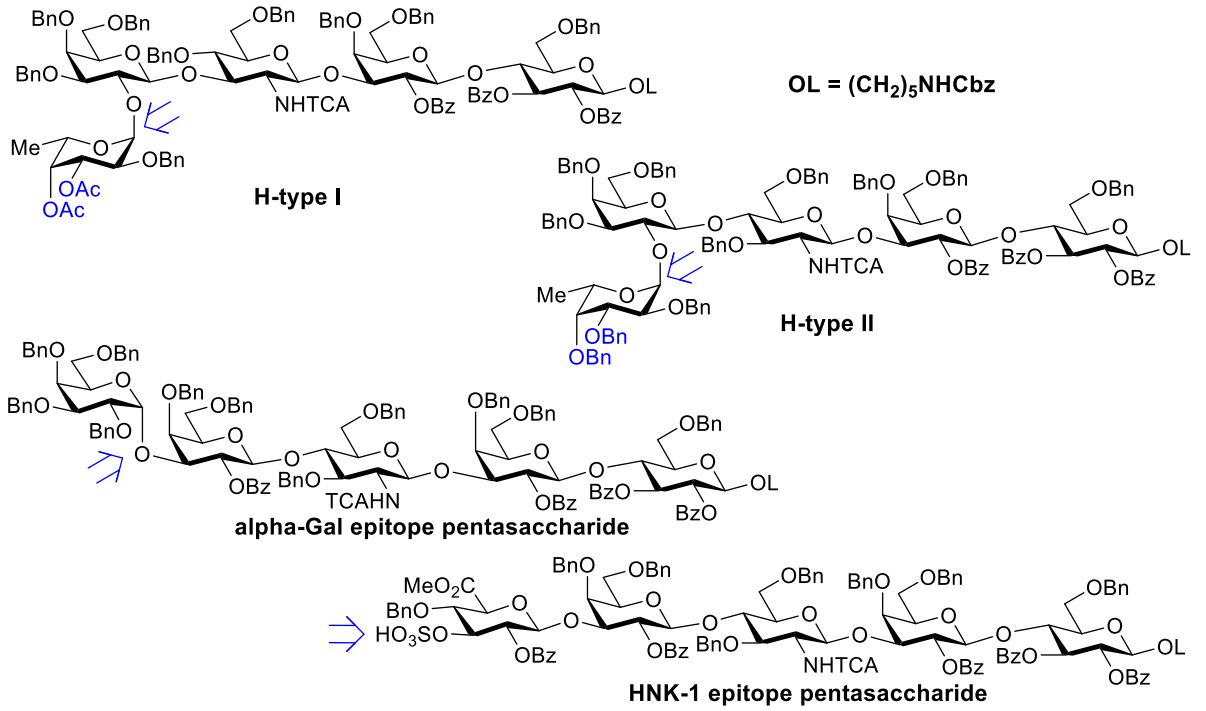
Summary

Carbohydrates are the most abundant biomolecules on earth and play pivotal roles in biological events. These carbohydrates exist as polymers, and furthermore as glycoconjugates, are connected to proteins, peptides, and lipids. Consequently, tremendous efforts to understand functions of carbohydrates have been made. Automated solid-phase oligosaccharide synthesis showed possibilities to make synthetic routes straightforward and streamlined for even non-specialists as a general and robust instrument like DNA and peptide synthesizers. Tremendous efforts were made over the past decades to advance this automated system and to produce diverse structures of oligosaccharides. In the extension of these investigations, the ultimate goal of this thesis is to establish the logic of automated glycan assembly (AGA) by which various synthetic oligosaccharides using generalized protocols have been procured and then, as obtained conjugation-ready carbohydrates, were shown to be molecular tools.

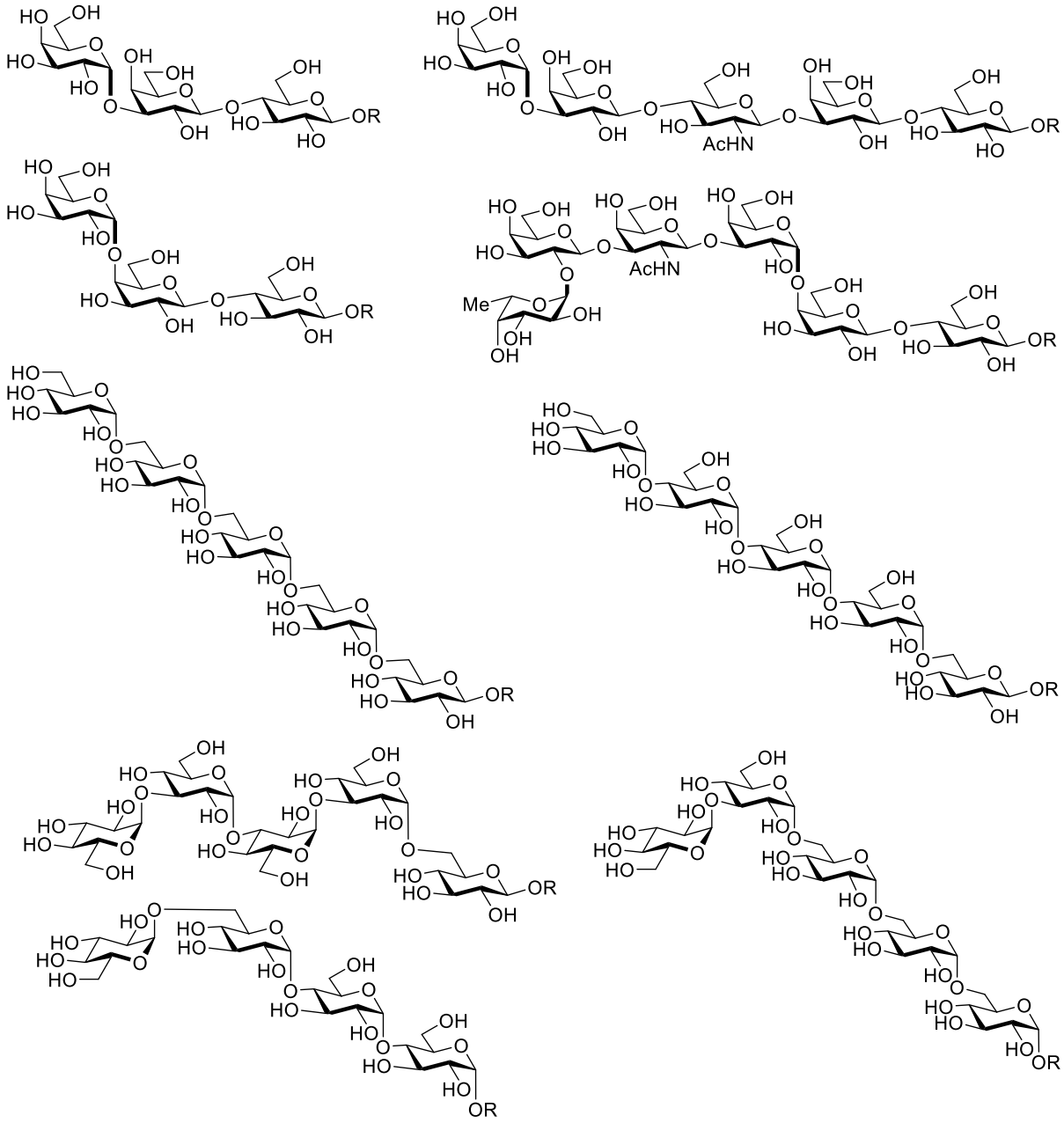
After a comprehensive introduction on the automated oligosaccharide synthesis in Chapter 1, Chapter 2 describes automated glycan assembly of oligo-*N*-acetyllactosamine (oligo-LacNAc) and keratan sulfates (KS) using building blocks bearing three temporary protecting groups. HPLC purification protocols allowed for access to a small library of linear and branch LacNAc structures, including sulfated keratans. Additionally, glycan microarray screening performed using seven LacNAc structures and fluorescence-labeled recombinant AAV particles revealed that one specific virus, AAV 10 identified disulfated tetrasaccharide LacNAc as a relevant epitope.



Automated synthesis of oligosaccharides containing challenging 1,2-*cis*-glycosidic linkages or disarmed building blocks is demonstrated in Chapter 3. Assembly of Lc_4 and nLc_4 core structures containing only 1,2-*trans*-glycosidic linkages was executed with building blocks employing a participating group at C2. Afterward, stereoselective installation of α-(1→2)-fucosidic linkages was achieved with the corresponding fucose building blocks depending on the sequence of the core structure, Lc_4 or nLc_4 in order to synthesize H-type I and II. For the synthesis of three α-Gal epitopes, the last glycosylation reaction formed α-(1→3)-galactosidic linkages. The stereoselectivity was found to be affected by the sequence of the acceptor bound to the solid support. Identification of the most reactive glucuronic acid building block, the phosphate employing Lev protecting group on 3-*O*, allowed for the synthesis of HNK-1 epitope pentasaccharide in a strictly linear approach. On the course of this investigation it was concluded that identification of approved building blocks was key towards accomplishing assembly of synthetically challenging oligosaccharides by using AGA.



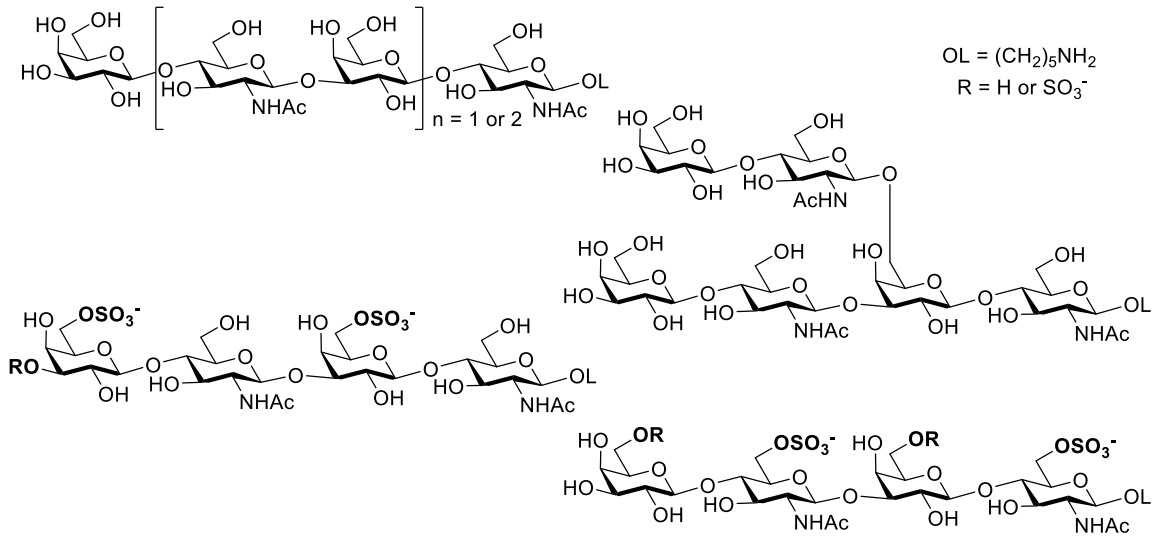
Chapter 4 of this dissertation presents the automated synthesis of oligosaccharides containing challenging 1,2-*cis* glycosidic linkages, one of the most challenging carbohydrates to date. In contrast to previous results, leaving groups affected the installation of α-galactosidic or β-mannosidic linkages using the automated fashion. Remote participation effects by acetyl group(s) at C3 and C4/C6 were studied in depth to identify a set of building blocks introducing 1,2-*cis* glycosidic linkages with good to excellent stereoselectivity. Taking advantage of "approved" building blocks enabled synthesis of Globo-H and α-glucans containing multiple 1,2-*cis* glycosidic linkages.



As a result of experiments performed in previous Chapters 2 to 4, Chapter 5 describes the reliable and rapid assembly of oligosaccharides using the commercially available automated glycan synthesizer, Glyconeer 2.1, monosaccharide building blocks, and a linker-functionalized polystyrene solid support. Protocols for purification using HPLC and quality-control using IM-MS for the oligosaccharide products have been standardized. Synthetic glycans prepared in this way are useful reagents as the basis for glycan arrays, diagnostics, and carbohydrate-based vaccines.

Zusammenfassung

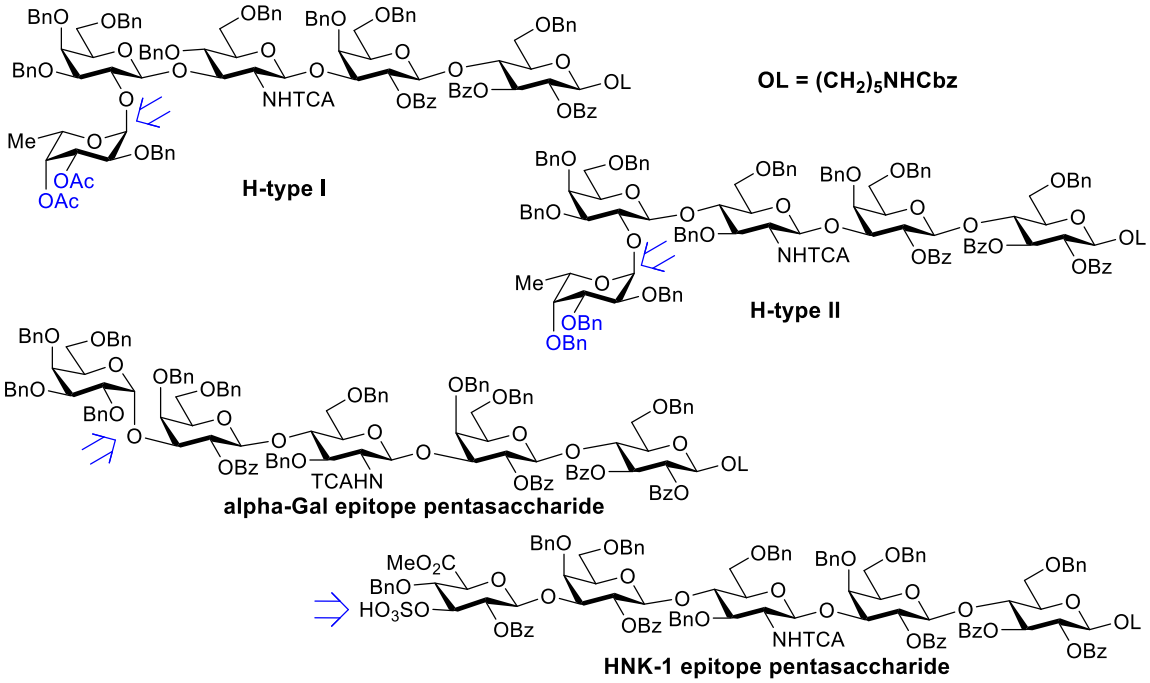
Kohlenhydrate sind die am häufigsten vorkommenden Biomoleküle auf der Erde und spielen wichtige Rollen in unzaehligen biologisches Prozessen. Kohlenhydrate sind als Polymere oder Glykokonjugate an Proteine, Peptide und Lipide gebunden und modulieren deren Funktionen. Folglich wurden signifikante Anstrengungen unternommen, um die verschiedenen Funktionen von Kohlenhydraten zu verstehen. In den vergangenen Jahren wurde umfangreich in die Forschung zur Entwicklung automatisierter Festphasen-Oligosaccharidsyntheseplattformen investiert. Diese Instrumente eröffnen Möglichkeiten zur Straffung und Automatisierung von Synthesewegen und machen komplexe Synthesen für Laien zugänglich, ähnlich wie DNA- und Peptidsynthesizers. Das Ziel der vorliegenden Arbeit liegt darin, eine Logik zur automatisierten Glycan-Assemblierung (AGA) zu entwickeln, wodurch die Synthese von Oligosacchariden unter Verwendung verallgemeinerter Protokolle moeglich wird. Konjugationsfertige Kohlenhydrate haben in den letzten Jahren als molecular Werkzeuge besonders in biomedizinischen Anwendungen an Bedeutung gewonnen.



Kapitel 1 dient einer umfassenden Einführung in die automatisierte Oligosaccharidsynthese. Kapitel 2 beschreibt eine automatisierte Glycansynthese von Oligo-N-acetyllactosamin (Oligo-LacNAc) und Keratinsulfaten unter Verwendung von Bausteinen, die drei temporäre Schutzgruppen tragen. Anschliessende Reinigungsprotokolle unter Verwendung von HPLC ermöglichten die Isolierung einer kleinen Library von linearen und verzweigten LacNAc-Strukturen einschließlich sulfatierter KS. Ein Glycan-Microarray screen unter Verwendung von sieben LacNAc-Strukturen und fluoreszenz-markierter

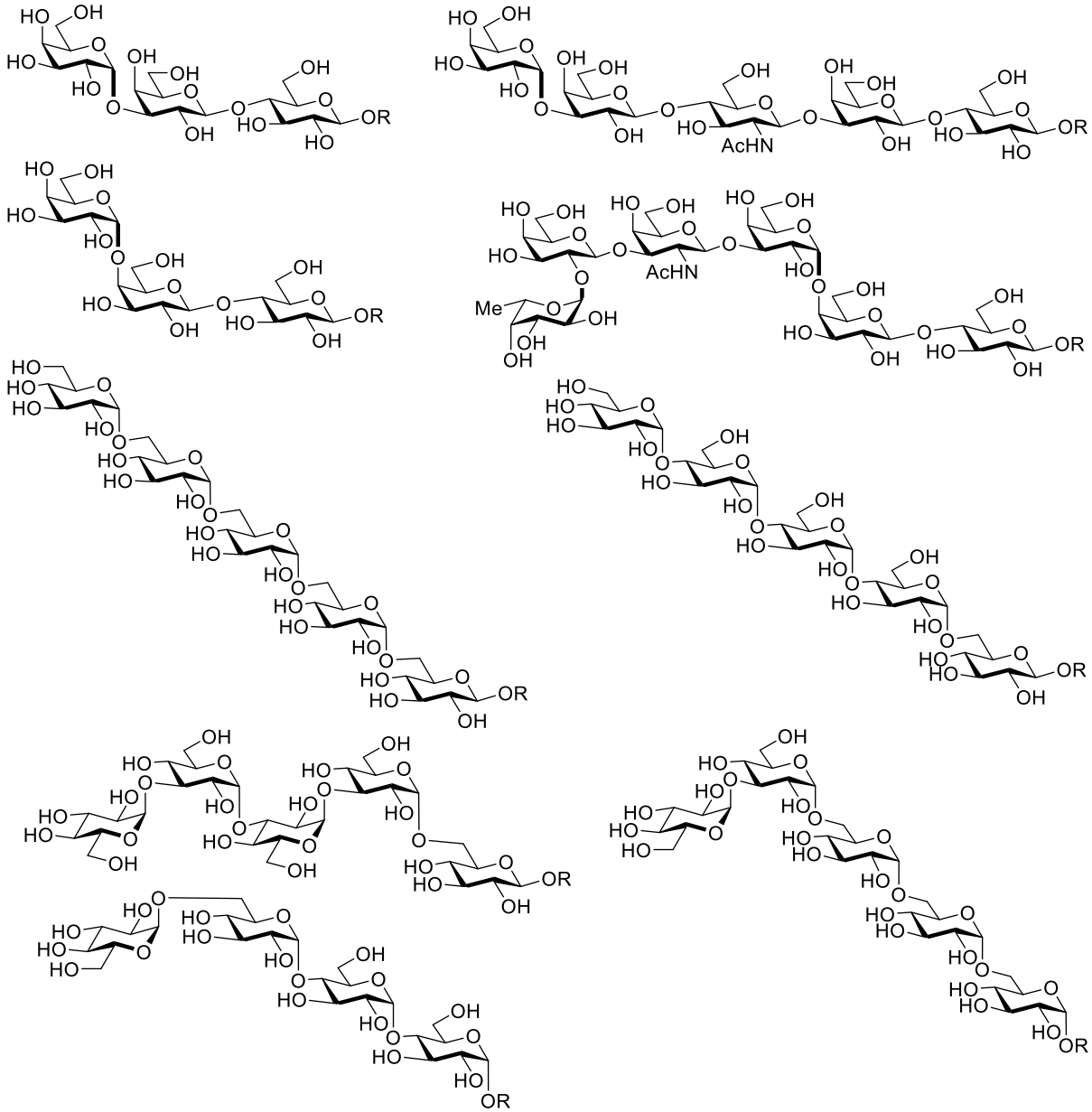
rekombinanten AAV-Partikel zeigte, dass ein spezifisches Virus, AAV 10, disulfatiertes Tetrasaccharid LacNAc als relevantes epitop erkannte.

Kapitel 3 beschreibt die automatisierte Synthese von Oligosacchariden mit schwierigen 1,2-cis-Glycosidbindung oder “disarmed” Bausteinen. Die Synthese von Lc 4 und nLc 4 Kernstrukturen, die nur 1,2-trans-glycosidische Bindungen enthielten, wurden mit Bausteinen durchgefuehrt die eine teilnehmende Gruppe bei C2 aufweisen. Die folgende stereoselektive Installation einer α-(1→2)-Fucosidbindung wurde mit den entsprechenden Fucose-Bausteinen erreicht, abhängig von der Sequenz der Kernstruktur, Lc 4 oder nLc 4, um H-Typ I und II zu synthetisieren. Für die Synthese von drei α-Gal-Epitopen, die letzte Glykosylierungsreaktion formte eine α-(1→3) -galactosidische Bindung. Die Stereoselektivität wurde durch die Sequenz des Akzeptors, das an die Festphase gebunden ist, beeinflusst. Identifizierung der reaktivsten Glucuronsäure-Baustein, das Phosphat das die Lev-Schutzgruppe an 3-O einsetzt, erlaubte die Synthese von HNK-1-Epitop-Pentasaccharid in einem streng linearen process. Diese Untersuchungen ergaben, dass die Identifizierung zugelassener Bausteine der Schlüssel zum Bau synthetisch herausfordernder Oligosacchariden mit AGA darstellt.



In Kapitel 4 dieser Dissertation wird die automatisierte Synthese von Oligosacchariden mit schwierigen 1,2-cis-glykosidischen Bindungen, einem der schwierigsten Kohlenhydrate, vorgestellt. Im Gegensatz zum vorherigen Ergebnis, die austretende Gruppen wirkten sich auf die Installation von α-Galaktosid oder β-mannosidische Bindungen unter Verwendung der automatisierten Fernbeteiligungseffekte von Acetyl

Gruppe (n) an C3 und C4 / C6 wurden eingehend untersucht, um eine Reihe von Bausteinen zu identifizieren, die einführten 1,2-cis-glycosidische Bindungen mit guter bis ausgezeichneter Stereoselektivität. Die Nutzung von "zugelassenen" Bausteinen ermöglichte die Synthese von Globo-H- und α-Glucanen mit mehreren 1,2-cis-glycosidische Bindungen.



Basierend auf der Arbeit die in den Kapiteln 2 bis 4 durchgeführten wurden, Kapitel 5 bschreibt eine zuverlässige und schnelle Synthese von Oligosacchariden unter Verwendung eines kommerziellen automatisierten Glykansynthesizer, Glyconeer 2.1, Monosaccharid-Bausteinen und einer linker-funktionalisierter Polystyrol-Festphase. Protokolle zur Reinigung mittels HPLC und Qualitaetskontrolle der Oligosaccharidprodukte mittels IM-MS wurden standardisiert. Auf diese Weise hergestellte Glykane sind nützliche Reagenzien für Glykanarrays, Diagnostika, und Kohlenhydrat-basierte Impfstoffe.

List of Abbreviations

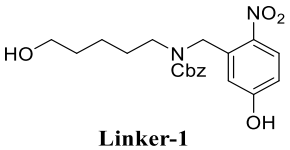
$[\alpha]_D^{20}$	Specific rotation
Ac	Acetyl
Ac_2O	Acetic anhydride
ACN/CH_3CN	Acetonitrile
AGA	Automated glycan assembly
All	Allyl
aq.	Aqueous
Ar	Aromatic
$BF_3.Et_2O$	Boron trifluoride diethyl etherate
BH_3	
Bn	Benzyl
BnBr	Benzyl bromide
Boc	*tert*-Butyloxycarbonyl
Boc_2O	*tert*-Butyloxycarbonyl anhydride
Bz	Benzoyl
BzCl	Benzoyl chloride
Bu_2SnO	Dibutyltinoxide
c	Concentration
cald	Calculated
CAN	Ceric (IV) ammonium nitrate
Cat.	Catalitic
Cbz	Carboxylbenzyl
$CHCl_3$	Chloroform
gCOSY	Gradient correlation spectroscopy
CPS	Capsular polysaccharide
CRM197	Cross-reactive material-197 Non-toxic mutant of diphtheria toxoid
CSA	Camphor sulfonic acid
CsF	Cesium fluoride
CsOAc	Cesium acetate
Cs_2CO_3	Cesium carbonate
δ	Chemical shift
d	Doublet
Da	Dalton
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCC	*N*,*N´*-Dicyclohexylcarbodiimide
DCM	Dichloromethane
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DIC	*N*,*N´*-Diisopropylcarbodiimide
DIPEA	Diisopropylethylamine
DMAP	4-(N,N-Dimethylamino)pyridine
DMF	*N*,*N*-Dimethylformamide
EDC	*N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide
equiv.	Equivalent
ESI	Electrospray ionization

Et	Ethyl
EtOH	Ethanol
Et_2O	Diethyl ether
Fmoc	9-Fluorenylmethyloxycarbonyl
Fuc	L-Fucose
GAG	Glycosaminoglycan
Gal	Galactose
GalNAc	*N*-Acetyl galactosamine
Glc	Glucose
GlcA	Glucuronic acid
GlcNAc	*N*-Acetyl glucosamine
$GlcNH_2$	*N*-Glucosamine
GPI	Glycosylphosphatidylinositol
h	Hour(s)
HRMS	High resolution mass spectroscopy
Hz	Hertz
HPLC	High-performance liquid chromatography
N_2H_4.AcOH	Hydrazine acetate
HCOOH	Formic acid
gHSQC	Gradient Heteronuclear Single Quantum Correlation
I_2	Iodine
IR	Infrared spectroscopy
J	Coupling constant
LG	Leaving group
Lev	Levulinoyl
LevOH	Levulinic acid
m	Multiplet
M	Molar
MALDI	Matrix assisted laser desorption/ionization
Man	Monnose
MeOH	Methanol
min	Minute(s)
mol	mole
Mg_2SO_4	Magnesium sulfate
$NaHCO_3$	Sodium bicarbonate
NAPBr	2-Naphthylmethyl
NBS	*N*-Bromosuccinimide
NHS	*N*-Hydroxysucinimide
NMR	Nuclear magentic resonance
NIS	*N*-Iodosuccinimide
NaOMe	Sodium methoxide
$NaBH_4$	Sodium borohydride
NP	Normal phase

PG	Protecting group
Ph	Phenyl
Piv	Pivaloyl
PivCl	Pivaloyl chloride
ppm	parts per million
PCV	Pneumococcal conjugate vaccine
Pn14PS	Capsular polysaccharide of *S. pneumoniae* serotype 14
PPV	Pneumococcal polysaccharide vaccine
Pd/C	Palladium on activated charcoal
$PhCH(OMe)_2$	Benzaldehyde dimethyl acetal
quant.	Quantitative
RP	Reverse phase
r.t.	Room temperature
RU	Repeating unit
s	Singlet
TBAF	Tetrabutylammonium fluoride
TBAI	Tetrabutylammonium iodide
TBS	*tert*-Butyldimethylsilyl
TBDMSCl	*tert*-Butyldimethylsilyl chloride
TCA	trichloroacetyl
Tf	trifluoromethanesulfonyl
TfOH	Trifluoromethanesulfonyl acid
TFA	Trifluoroacetic acid
TFAA	Trifluoroacetic anhydride
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMS	Trimethylsilyl
TMSOTf	
TOF	Time of flight
Tol	Toluene
Ts	Tosyl
TEA	Triethylamine
TEMPO	(2,2,6,6-Tetramethylpiperidin-1-yl)oxyl
UV	Ultraviolet
Zn	Zinc

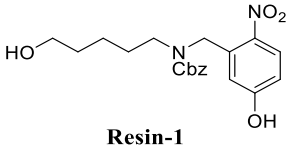
List of the Photolabile Linker and Building Blocks

Photolabile linker



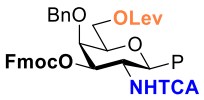
Linker-1

Linker-conjugated resin

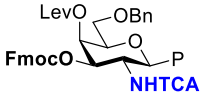


Resin-1

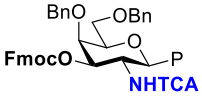
Building Blocks for 1,2-*trans* glycosidic linkage formation: GalNAc, and Gal



GalNAc-01

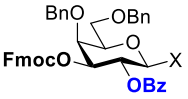


GalNAc-02

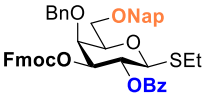


GalNAc-03

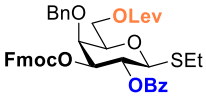
P = OPO(OBu)$_2$



Gal-01: X = SEt
Gal-01-p: X = OPO(OBu)$_2$

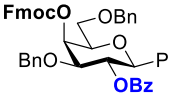


Gal-02



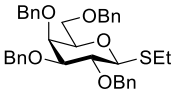
Gal-03

Gal-04

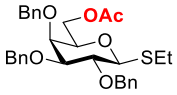


Gal-05

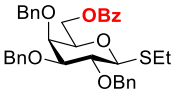
Building Blocks for 1,2-*cis* glycosidic linkage formation: Gal



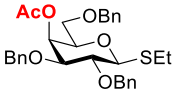
Gal-α-01



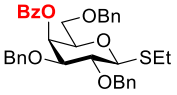
Gal-α-02



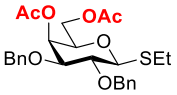
Gal-α-03



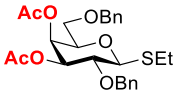
Gal-α-04



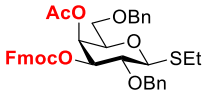
Gal-α-05



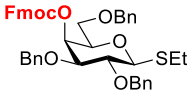
Gal-α-06



Gal-α-07

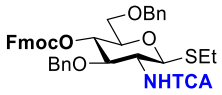


Gal-α-08

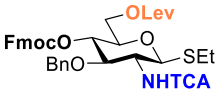


Gal-α-09

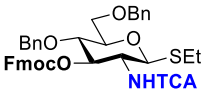
Building Blocks for 1,2-*trans* glycosidic linkage formation: GlcNAc, Glc, and GlcA



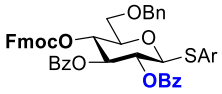
GlcNAc-01



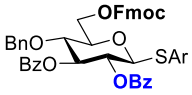
GlcNAc-02



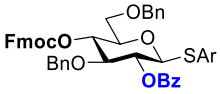
GlcNAc-03



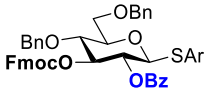
Glc-01



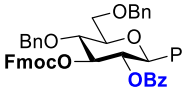
Glc-02



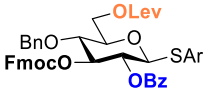
Glc-03



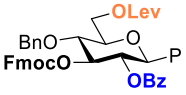
Glc-04



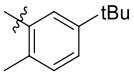
Glc-05



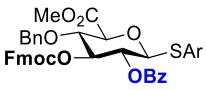
Glc-06



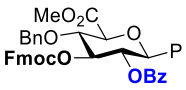
Glc-07

Ar = 

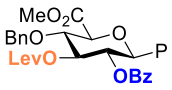
P = $OPO(OBu)_2$



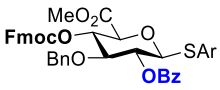
GlcA-01



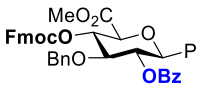
GlcA-02



GlcA-03

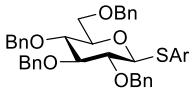


GlcA-04

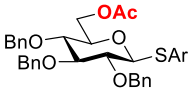


GlcA-05

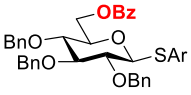
Building Blocks for 1,2-*cis* glycosidic linkage formation: Glc and Fuc



Glc-α-01

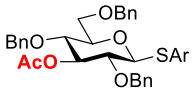


Glc-α-02

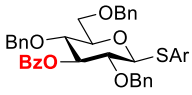


Glc-α-03

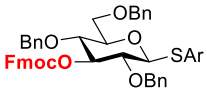
Glc-α-04



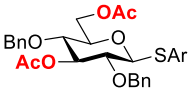
Glc-α-05



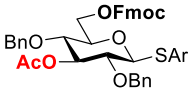
Glc-α-06



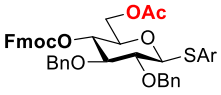
Glc-α-07



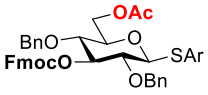
Glc-α-08



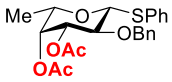
Glc-α-09



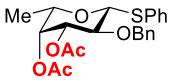
Glc-α-10



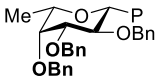
Glc-α-11



Fuc-01



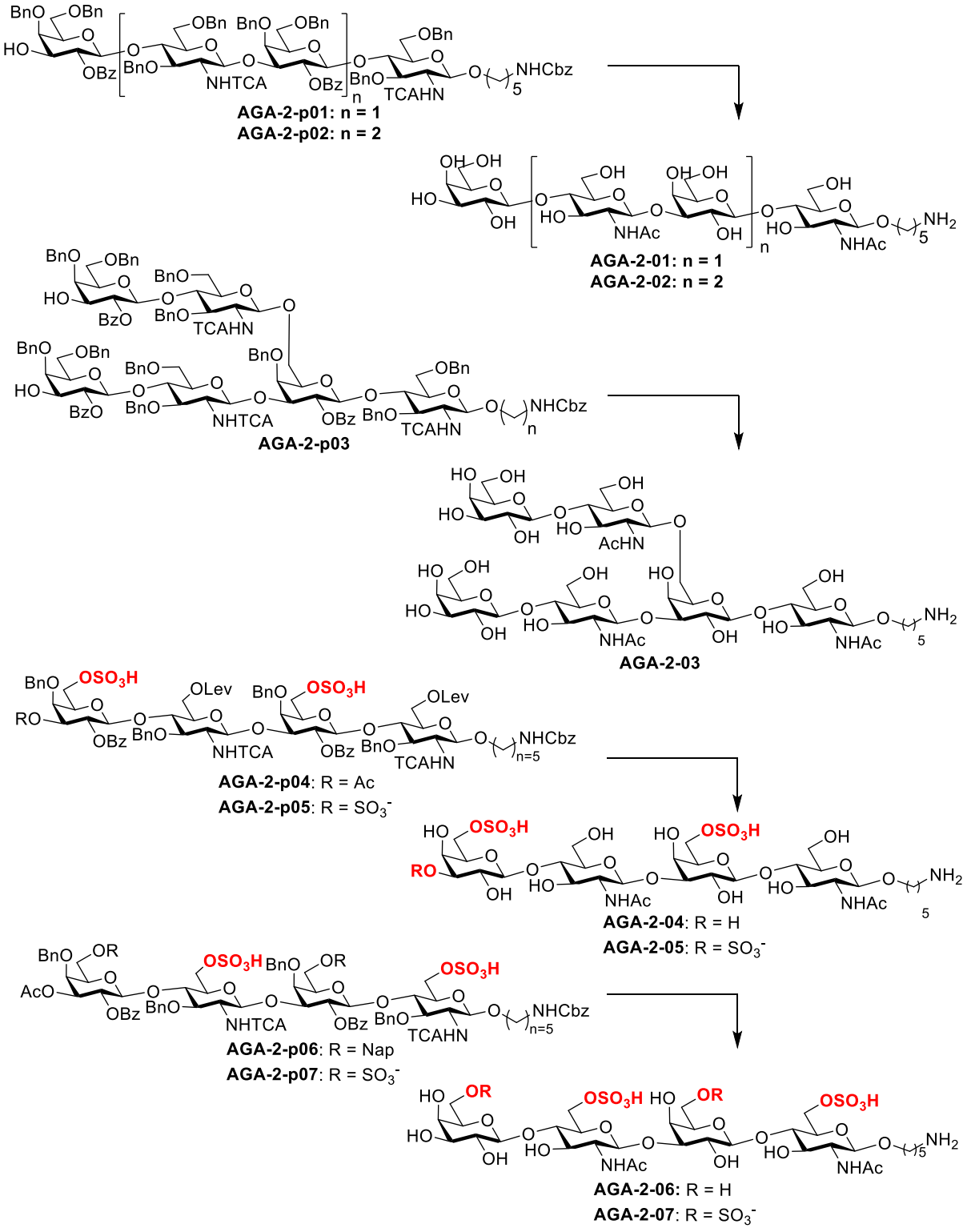
Fuc-02



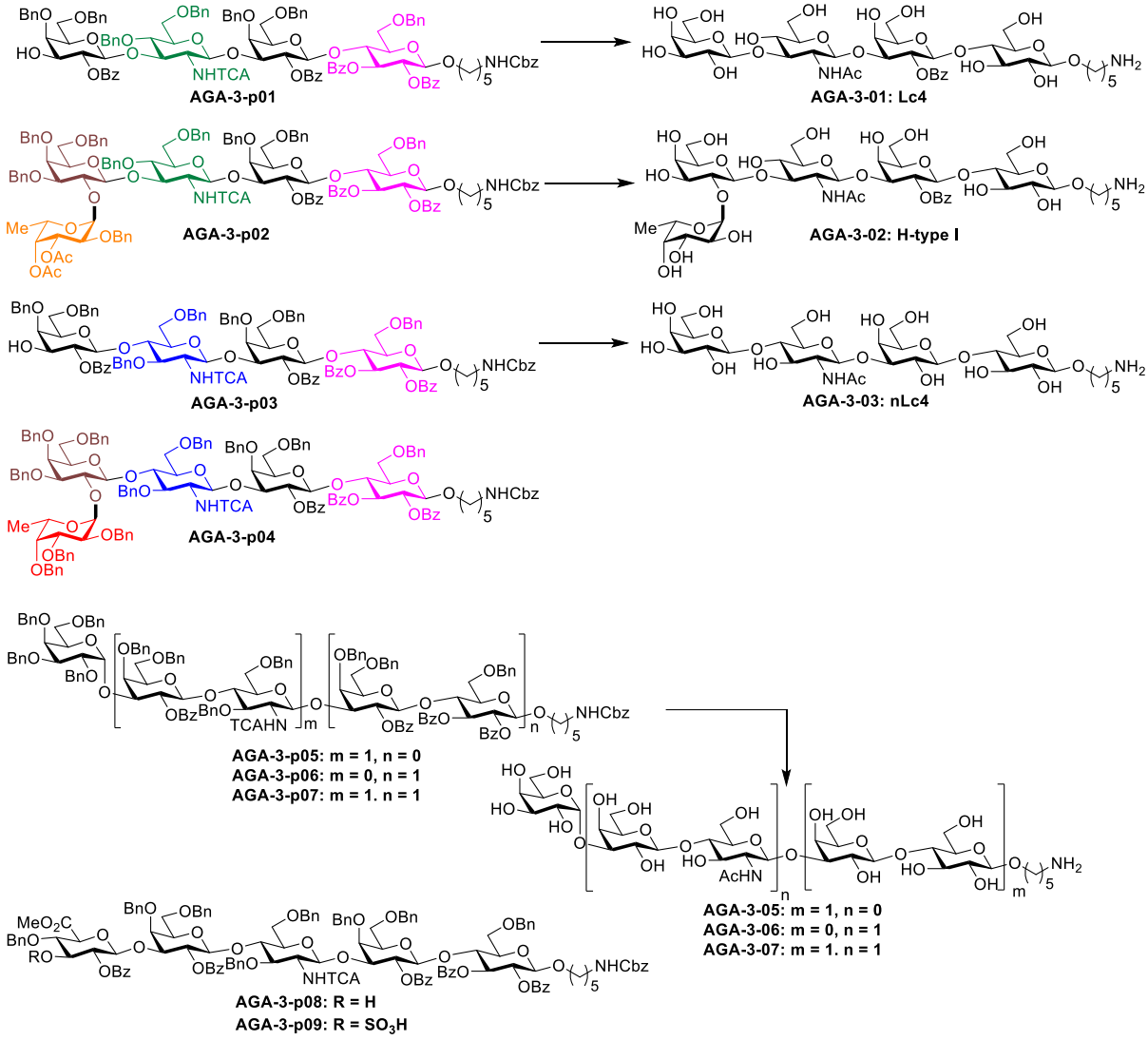
Fuc-03

List of Glycans Synthesized by AGA

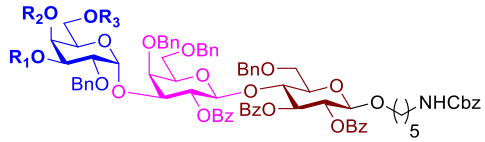
Chapter 2.



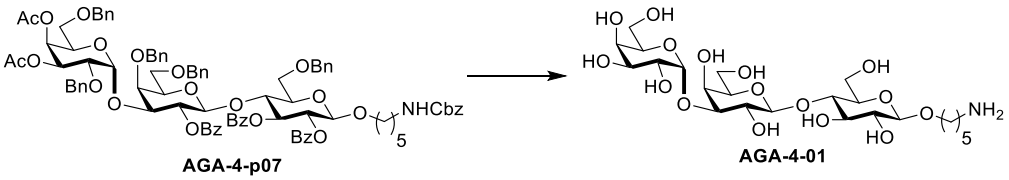
Chapter 3.


AGA-3-p01
AGA-3-01: Lc4
AGA-3-p02
AGA-3-02: H-type I
AGA-3-p03
AGA-3-03: nLc4
AGA-3-p04
AGA-3-p05: m = 1, n = 0
AGA-3-p06: m = 0, n = 1
AGA-3-p07: m = 1. n = 1
AGA-3-05: m = 1, n = 0
AGA-3-06: m = 0, n = 1
AGA-3-07: m = 1. n = 1
AGA-3-p08: R = H
AGA-3-p09: R = SO3H

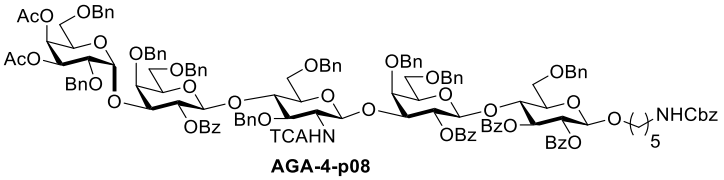
Chapter 4.



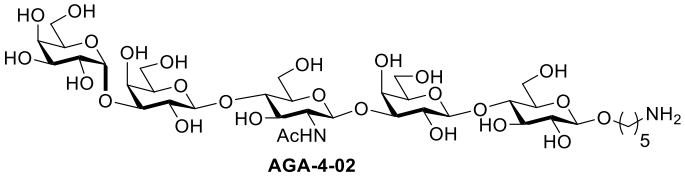
AGA-4-p01: R_1=Bn, R_2=Bn, R_3=Bn
AGA-4-p02: R_1=Bn, R_2=Bn, R_3=Ac
AGA-4-p03: R_1=Bn, R_2=Bn, R_3=Bz
AGA-4-p04: R_1=Bn, R_2=Ac, R_3=Bn
AGA-4-p05: R_1=Bn, R_2=Bz, R_3=Bn
AGA-4-p06: R_1=Ac, R_2=Ac, R_3=Bn
AGA-4-p07: R_1=Bn, R_2=Ac, R_3=Ac



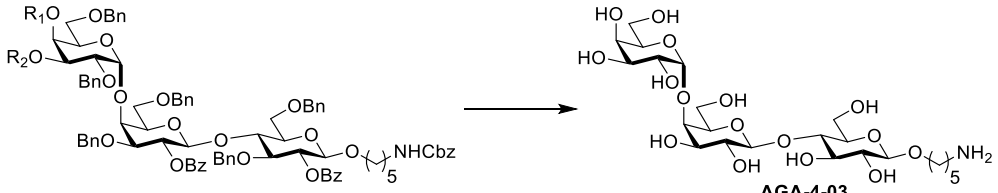
AGA-4-p07 → **AGA-4-01**



AGA-4-p08

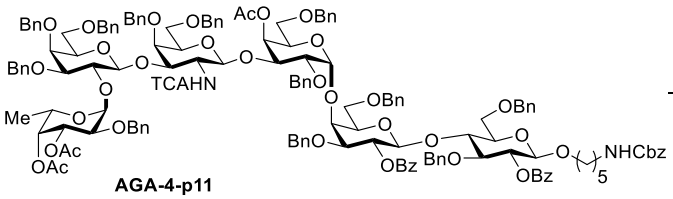


AGA-4-02

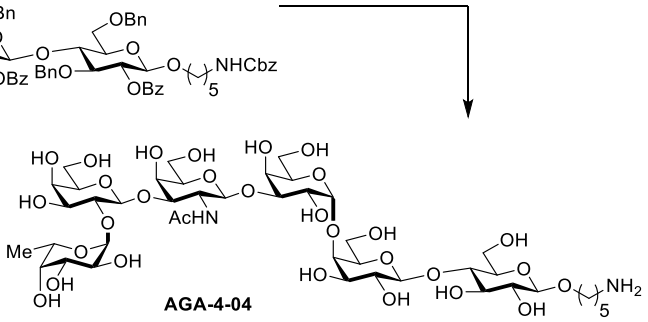


AGA-4-p09: R_1 = Bn, R_2 = Bn
AGA-4-p10: R_1 = Bn, R_2 = H

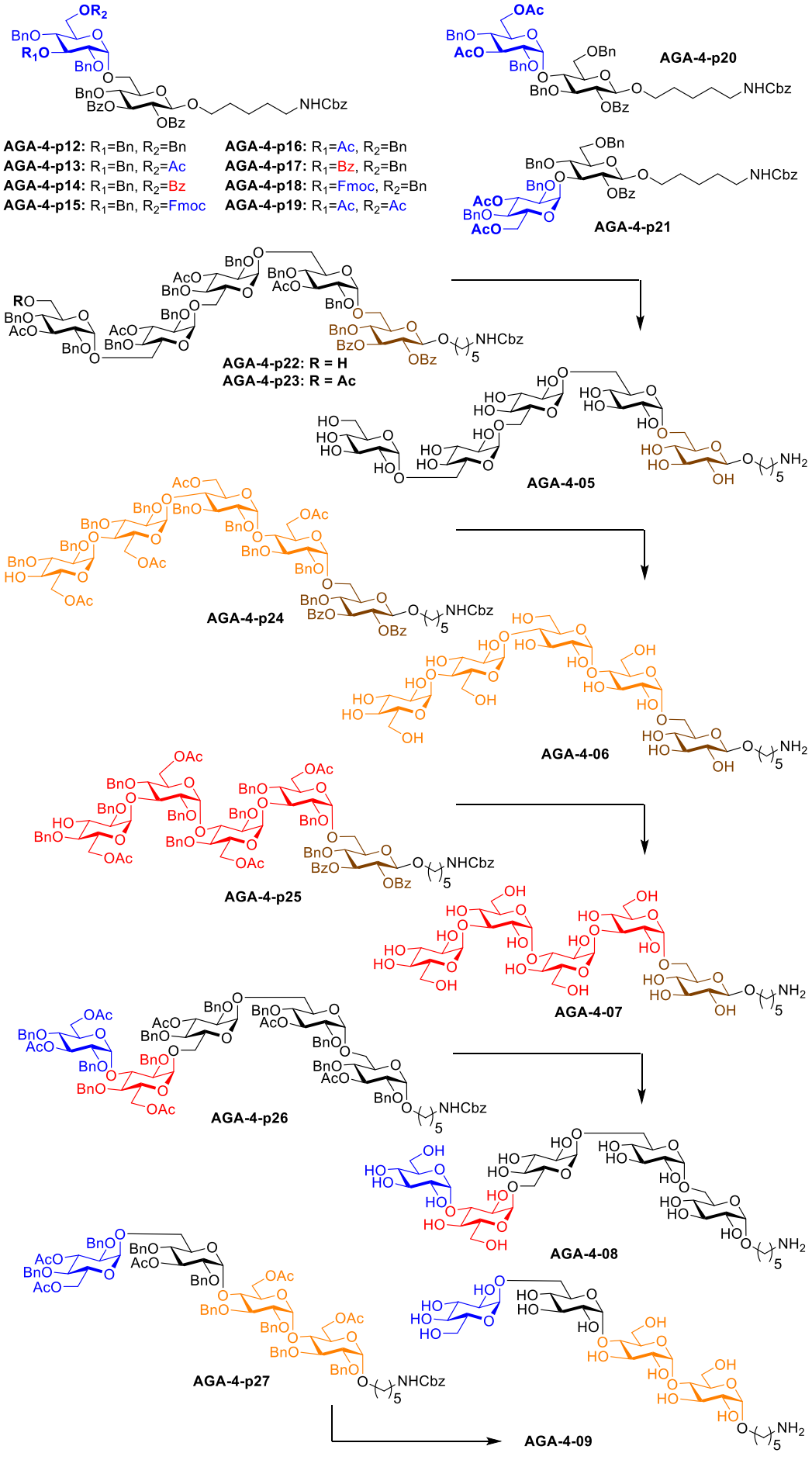
AGA-4-03



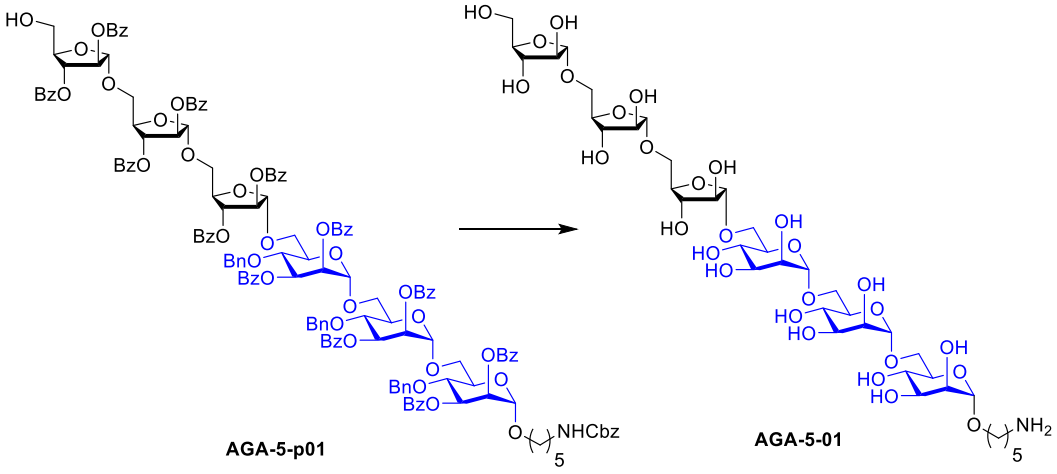
AGA-4-p11

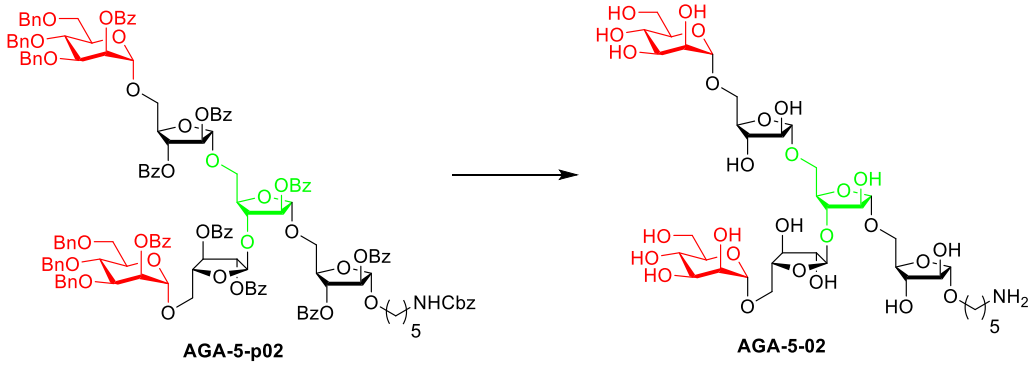


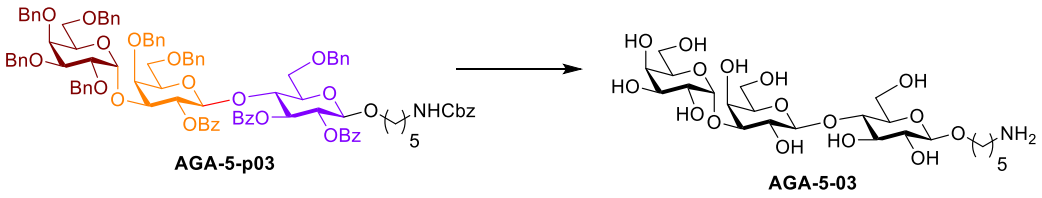
AGA-4-04


AGA-4-p12: R1=Bn, R2=Bn
AGA-4-p13: R1=Bn, R2=Ac
AGA-4-p14: R1=Bn, R2=Bz
AGA-4-p15: R1=Bn, R2=Fmoc
AGA-4-p16: R1=Ac, R2=Bn
AGA-4-p17: R1=Bz, R2=Bn
AGA-4-p18: R1=Fmoc, R2=Bn
AGA-4-p19: R1=Ac, R2=Ac
AGA-4-p20
AGA-4-p21
AGA-4-p22: R = H
AGA-4-p23: R = Ac
AGA-4-05
AGA-4-p24
AGA-4-06
AGA-4-p25
AGA-4-07
AGA-4-p26
AGA-4-08
AGA-4-p27
AGA-4-09

Chapter 5.







1. Introduction

Automation: The Past, The Present, and The Future

The Place where it has been shows you where to go.

온고이지신(溫故而知新), 가이위사의(可以僞師矣): **공자(孔子)**

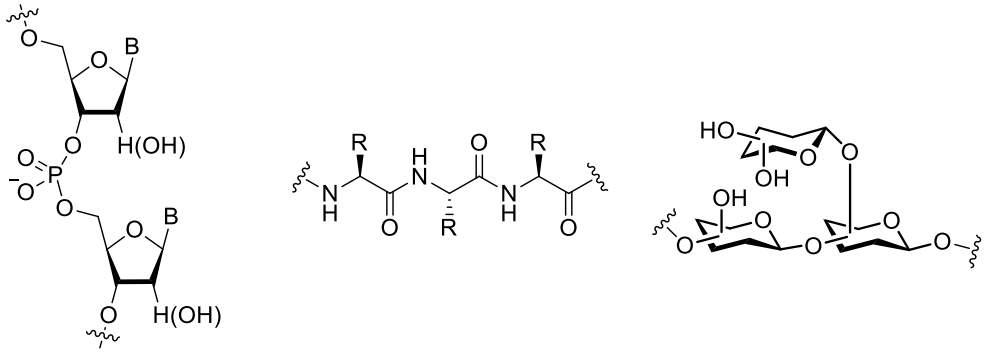
If advances are made by one who studies the old,
one deserves becoming the teacher.
Confucius

1. Introduction
Automation: The Past, The Present, and The Future

1.1 Impact of Automated Synthesis in Fields of -omics

Glycans are the most abundant biomacromolecules within living systems among nucleic acids, proteins, and lipids, and are constructed with individual monomers linked with one another in stereo- and region-connectivity.[1] They play diverse roles in biological processes including cell growth and proliferation, inflammation, immune response, and tumor cell metastasis.[1,2] Elucidation of functions of glycans associated with their structures is critical to understand biology, which is closely dependent on procurement of pure and structurally well-defined poly- and oligosaccharides and glycoconjugates. However, microheterogeneity of natural complex carbohydrate sources causes difficulties in obtaining acceptably pure carbohydrates in sufficient quantities.

However, nucleic acids (DNAs and RNAs) and proteins are greatly understood by many fundamental mechanisms existing in biological systems, along with instrumental advances, sequencing,[3] synthesis[4] and the polymerase chain reaction (PCR) amplification methods,[5] enabling to make progress in genomics and proteomics.



Potential #BBs	5+	20+	100s+
Branching	No	No	Yes
Linkage	Phosphate	Amide	Glycosidic
Stereochemistry	Set/Defined	Set/Defined	Cis or Trans
Complexity	Low	Middle	High

Figure 1.01 Structure of nucleic acids, peptides, and oligosaccharides, and their complexity.

The difficulties in synthesis of oligosaccharides become apparent in comparing glycans with oligonucleotides and peptides. **First**, glycans are both linear and branched

polymers whereas oligonucleotides and peptides are mostly linear. **Secondly**, building blocks of oligonucleotides and peptides are 5 nucleotides and 20 amino acids respectively. In stark contrast, hundreds of monosaccharides are known. **Third**, a new stereogenic center is newly formed on each glycosidic linkage that connects two monomers, while phosphate diesters in nucleic acids and amide linkages in proteins do not create a new stereocenter. Taken all together, combination of all possibilities, branched or linear bearing a new stereocenter with hundreds of building blocks shows highly complicated matters to be solved (**Figure 1.01**).[6]

1.2 Oligosaccharide Synthesis

Mentioned above, although chemical techniques enable one access to peptides and oligonucleotides, synthesis of oligosaccharides are more difficult because oligosaccharides often have branched structures varying with either α or β type of connectivity (**Figure 1.02 A**). In these oligosaccharide synthesis schemes, the fundamental reaction is the glycosylation reaction where the glycosidic linkage is formed between two monomers, the glycosyl acceptor (a nucleophile) and the glycosyl donor (an electrophile) in the presence of a promotor (or a Lewis acid) (**Figure 1.02 B**).[7,8] Since Michael[9] and Fisher[10] reported the glycosylation reactions, many glycosyl donors have been developed with various anomeric leaving groups that are activated by promotors. The most representative glycosyl donors are firstly glycosyl halides, thioglycoside, glycosyl imidates, and glycosyl phosphates and more.[11-15] Although various reaction conditions between leaving groups and promotors for the formation of glycosidic bonds have been developed, the formation of glycosidic linkages mainly remains in this mechanism (**Figure 1.02**). In general, it is considered that the glycosyl acceptor **1.02** (nucleophile) can react with the reaction intermediate oxocarbenium formed by activation of the glycosyl donor (glycosylating agent, electrophile) **1.01** in presence of a promotor (Lewis acid) which mainly drives to α-linear disaccharides **1.04** (**blue arrow**) or β-linear disaccharides **1.03** (**red arrow**) (**Figure 1.02, B**).

The stereoselective formation of glycosidic linkages is influenced by many factors. In general, the formation of 1,2-*trans* glycosidic linkages are more easily controlled in high selectivity because the neighboring group participation effect is well studied and applied (**Figure 1.02, red arrow**). Placement of a C2 participating protecting group such as acetate (Ac) and benzoate (Bz) in the glycosylation agent blocks one face of the dioxocarbenium ion intermediate, resulting in nucleophilic attack of the free hydroxyl group on the acceptor from the opposite face and the formation of the *trans*-glycosidic linkage.

Without neighboring group participation on the C2 position, the stereoselectivity of glycosidic bonds can be controlled by many reaction parameters. The α-anomer is the thermodynamically favored outcome due to the anomeric effect that stabilizes an axial configuration at the anomeric center by unpaired electrons of oxygen of the sugar, and the α-anomer is preferably obtained at the high reaction temperature, whereas the β-product is kinetically preferred to be formed at low temperatures. The reaction outcome can also result from the solvent, known as the solvent effect. Acetonitrile favors the formation of the α-product, however, the use of diethyl ether, THF, and 1,4-dioxane predominantly result in the β-product.[17-20] The remote participating groups, Ac or Bz, of the glycosyl donor also help increase the selective formation of 1,2-*cis* glycosidic bonds on glucose and galactose building blocks.[21] Other mechanically-proven protecting groups that influence the stereogenic outcome of the reaction through specific reaction mechanisms have been developed.[21-24] Therefore, the key to the carbohydrate chemistry is to prepare appropriately protected building blocks ensuring the desired regio- and stereochemical outcomes (**Figure 1.02, B**).

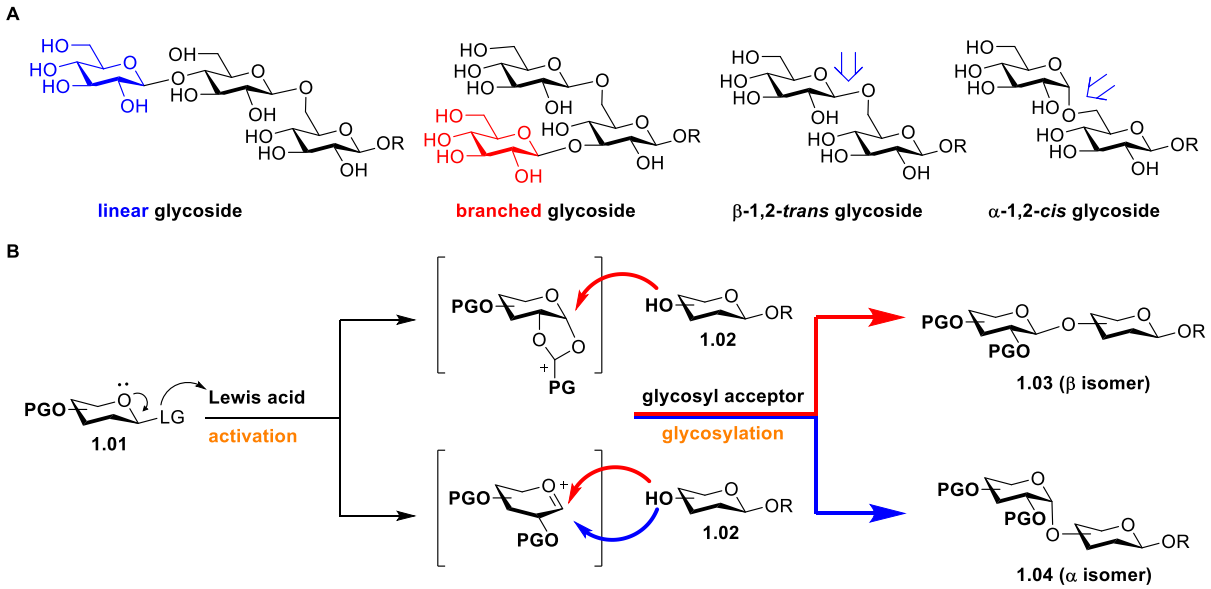


Figure 1.02 Mechanism of the glycosylation reaction with/without a participating group in the C2-position of the glycosyl donor (an electrophile).

Assembly of oligo- or polysaccharides using traditional carbohydrate chemistry composed of iterative processes: glycosylation, purification, deprotection to generate a free hydroxyl of the acceptor, and additional purification is still considered time-consuming and labor-intensive although the glycosylation mechanism has been developed. The two most

promising chemical synthetic approaches will be discussed here: one-pot oligosaccharide synthesis and automated solid-phase oligosaccharide synthesis.[25,26]

1.2.1 One-Pot Oligosaccharide Synthesis

In the one-pot strategy, multiple glycosylation reactions to form glycosidic bonds are performed by sequential addition of building blocks and activators in the one flask.[25] Therefore, the one-pot method can reduce multiple purifications and deprotection steps of intermediates in the iterative process of oligosaccharide synthesis. Three different one-pot methodologies have been developed to compensate limitations of each method: 1) chemoselective (programmed) one-pot method[27] where a building block (BB) having higher relative reactivity value (RRV) is selectively activated by a promoter rather than a BB bearing lower RRV to form a covalent bond; 2) preactivation (iterative) one-pot method[28] that activates the donor separately and transfers the acceptor into the same flask to create the glycosidic bond; 3) the orthogonal activation glycosylation[29] that uses building blocks having different leaving groups that are orthogonally activated over other leaving groups. In this section, the advantages of each of the one-pot strategies are briefly described and representative examples are presented to illustrate their contributions to oligosaccharides assembly with limitations (**Figure 1.03**).

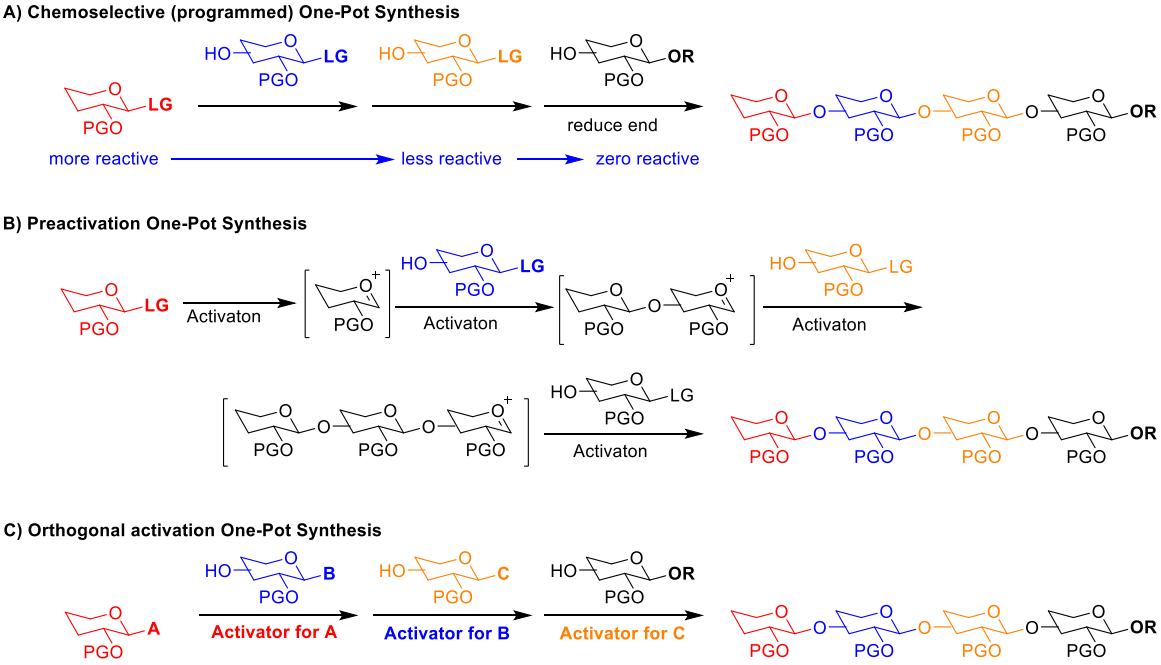


Figure 1.03 Schematic view of three one-pot strategies.[25,27-29]

Chemoselective (programmable) One-Pot Synthesis

The reactivity of each glycosylating agents bearing the same leaving group (for example: thioglycosides) can be controlled by introduction of protecting groups. The reactivity of the leaving group can be manipulated and the reactivity of building blocks can be differentiated by the nature of either the electron donating (generally acyl protecting groups such as Ac or Bz) or the electron withdrawing protecting groups (generally ether protecting groups such as benzyl ether), allowing for the oligosaccharide synthesis of the corresponding building blocks by chemoselective (programmable) one-pot synthesis (**Figure 1.03 A)**.

The concept of armed-disarmed glycosylating agents was introduced by Fraser-Reid who established the basics for chemoselective one-pot strategies where benzylated glycosides are electron-rich and more reactive, so-called armed, while their acetylated analogues, which are electron-poor and less reactive called as disarmed.[27a] Wong made advanced the concept of armed-disarmed into the relative reactivity of a set of thioglycoside building blocks by competitive glycosylation assays between the glycosylating donors of interest and a reference donor of known relative reactivity value (RRV).[27b] The research showed trends of building block's reactivity (fucose > galactose > glucose > mannose). Furthermore, general trends in reactivity have helped facilitate the design of an effective synthetic strategy.

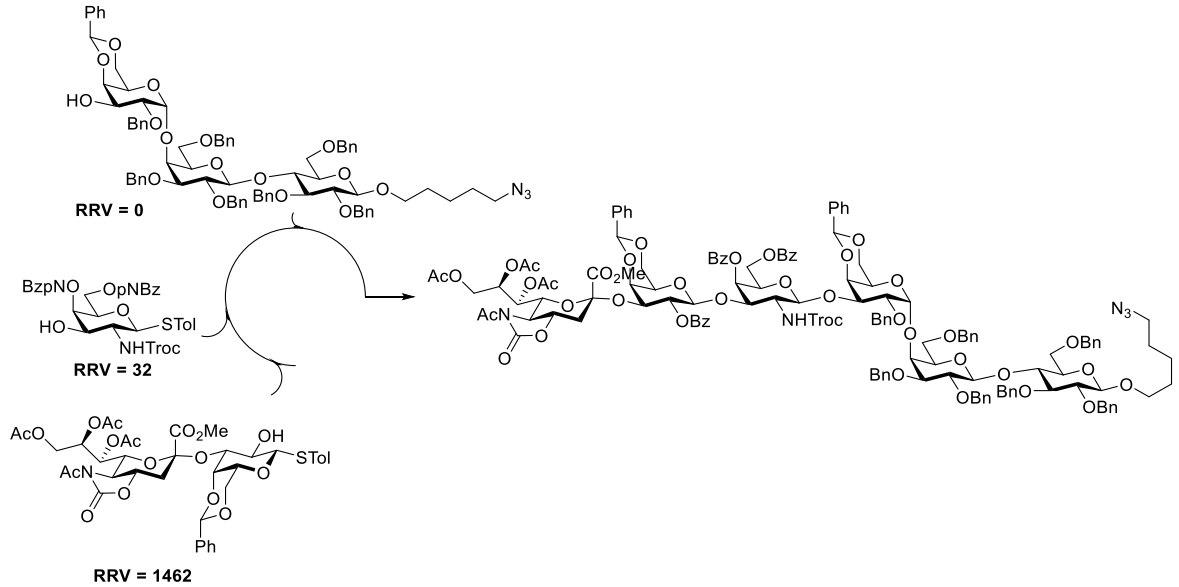


Figure 1.04 Representative examples SSEA-4 for programmed one-pot synthesis.[27c]

The programmable one-pot synthesis allowed for access to oligosaccharides such as the tumor-associated antigen stage-specific embryonic antigen-4 (SSEA-4) (**Figure 1.04**).[27c] The difference of reactivity between sialyl disaccharides (RRV = 1462), the galactoside (RRV = 32) and the reducing end disaccharide 109 (RRV = 0) made the one-pot assembly of

this oligosaccharide possible. Thus, sialyl disaccharides were coupled with the less reactive galactoside monomer by activation with N-iodosuccinimide/trifluoromethanesulfonic acid at two respective temperatures, which provided the targeted protected SSEA-4 in 43% overall yield.[27c]

Preactivation One-Pot Synthesis

The preactivation (iterative) synthesis is one of the most direct approaches towards the synthesis of oligosaccharides, compared to chemoselective one-pot strategies. The preactivation one pot strategy was developed to use building blocks having very close relative reactivity values (RRV) that cannot be used for the programmed one-pot method. Therefore, this approach is unrelated to the nature of the armed-disarmed protecting group in the programmable strategy. This strategy is dependent upon the preactivation of the glycosyl donor in the absence of the acceptor. When the second building block is added to the pre-activated glycosyl donor, a disaccharide is formed with an identical aglycon at the reducing end, and serves as a disaccharide donor. This process can be repeated in the same reaction flask to procure the target protected oligosaccharide (**Figure 1.03 B)**.

Homooligosaccharides such as β-(1→3)-glucan oligosaccharide that cannot be achieved using a single monomeric building block by the one-pot method were synthesized by the preactivation one-pot method recently (**Figure 1.05**).[28b]

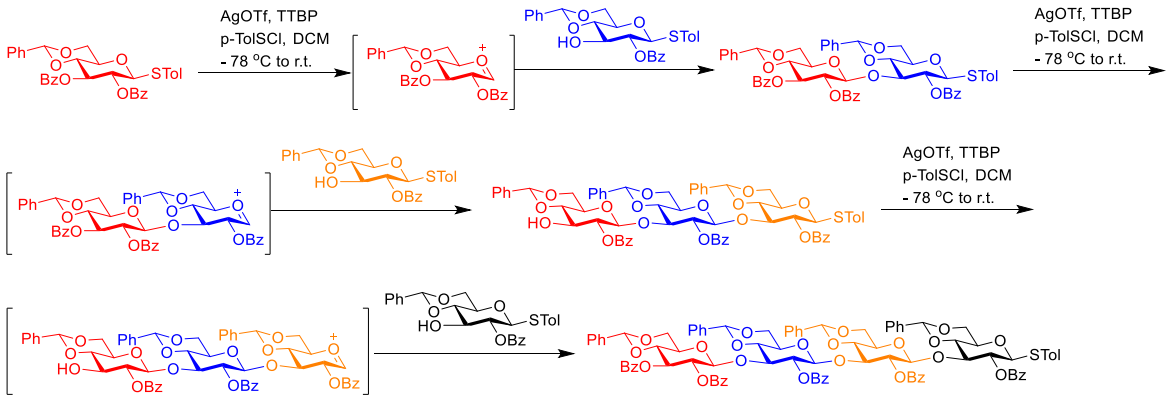


Figure 1.05 β-(1,3)-glucan synthesis using preactivation one-pot method.[28b]

Orthogonal One-Pot Synthesis

Lastly, the orthogonal one-pot method enables one to synthesize oligosaccharides in one flask where the corresponding building blocks bearing different leaving groups at the anomeric position can be selectively activated. In comparison with the programmable or preactivation strategy, where the leaving groups at the anomeric position would be the same,

the orthogonal one-pot synthesis of glycans is totally independent from the protection group pattern, but dependent on the activation conditions that activate exclusively one building block among other building blocks (**Figure 1.03 C)**. Three glycosylation reactions were performed to afford tetrasaccharide using the orthogonal activation method (**Figure 1.06**).[29b]

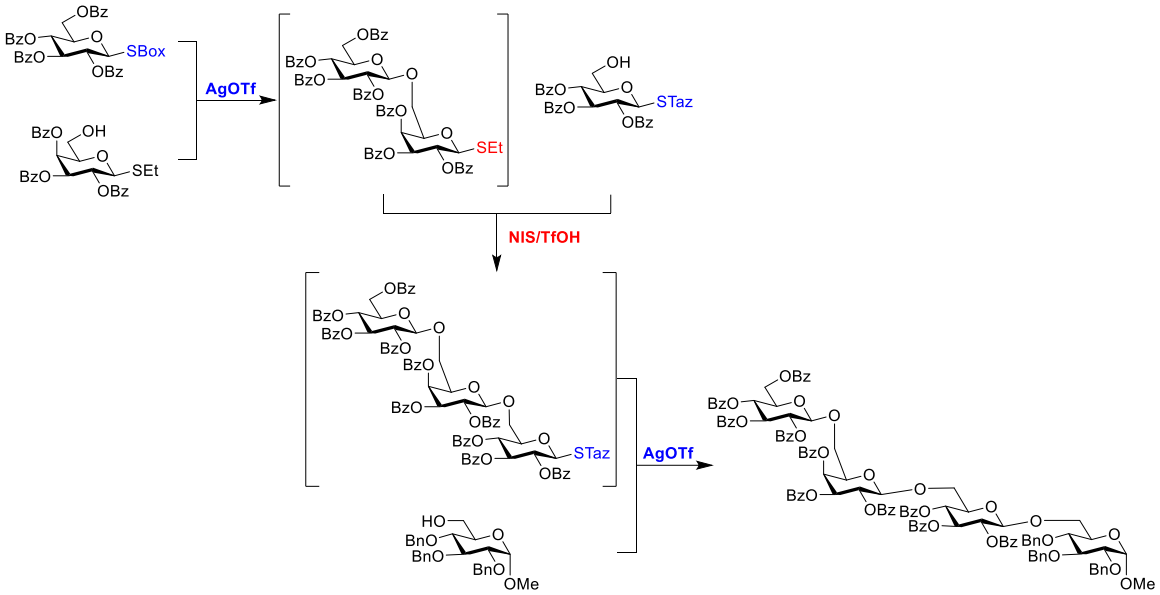


Figure 1.06 One-Pot Synthesis of Tetrasaccharide via Sequential Selective Activation of Building Blocks Equipped with Different Leaving Groups[29b]

Stereoselective formation of 1,2-*cis*-glycosidic linkages still remains challenging in the one-pot oligosaccharide synthesis to date although the one-pot method made great advances in assembly of oligosaccharides over the past two decades. The Wong group utilized two different approaches to assemble Globo-H. Interestingly, each approach performed three glycosylation reactions in one reaction vessel to assemble the target molecule (**Figure 1.07**).

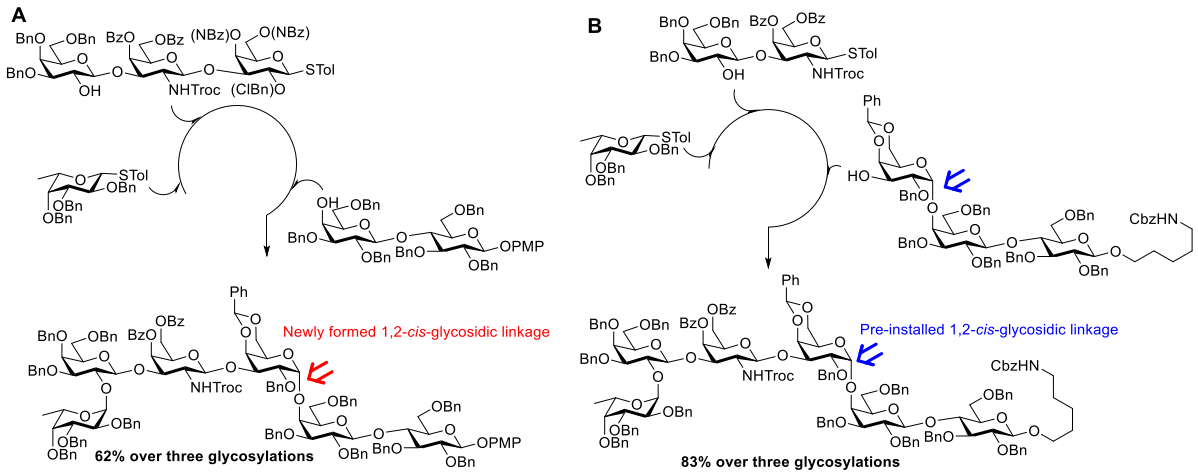


Figure 1.07 Representative examples to compare two different approaches. A) 1,2-*cis* glycosidic linkage was formed on the synthesis of Globo-H. B) Trisaccharide bearing 1,2-*cis* glycosidic linkage was prepared for programmed one-pot synthesis.

First the heptasaccharide Globo-H was assembled with a fucose monomer, one disaccharide unit, and one trisaccharide unit in a [1+3+2] glycosylation reaction in 62% overall yield (**Figure 1.07 A**).[30a] In this synthesis, neither the disaccharide unit nor the trisaccharide unit containing 1,2-*cis*-galactosidic linkages was used. However, an alternative approach using a [1+2+3] glycosylation reaction with the same fucose building block, one disaccharide unit, and one trisaccharide unit containing a 1,2-*cis*-galactosidic linkage gave Globo-H in 83% overall yield (**Figure 1.07 B**).[30b] Comparing these results, it is likely that the [1+3+2] glycosylation reaction yielded the undesired β-galactose isomer of Globo-H in about 20% (α:β = 3:1). Therefore, di- or trisaccharide building blocks bearing one or multiple 1,2-*cis* glycosidic linkages have been used to avoid low stereoselectivity of glycosidic linkage formation. This example clearly shows that stereoselective installation of 1,2-*cis* glycosidic linkages is still inaccessible by the one-pot strategy.

.

1.3 Automated Glycan Assembly

The first automated solid-phase oligosaccharide synthesizer[31] was created by the *Seeberger's group* in 2001 to satisfy one great goal: enable non-specialists to obtain pure, structurally defined oligosaccharides routinely. To do so, the newly created oligosaccharide synthesizer was designed to perform cycles of iterative processes: glycosylation and deprotection to expose a free hydroxyl for the next glycosylation. Automated synthesis using solid support required a single purification step to afford oligosaccharide structures that require further deprotection reactions and purification to obtain fully deprotected oligosaccharide. Despite making great strides in automated synthesis over the past decade, the development of automated synthesis is still in its infancy compared to the automated synthesis of nucleic acids and peptides. In this section, advances of the automated synthesis will be described with representative examples assembled using the specific linker.

1.3.1 The First Era of Automated Oligosaccharide Synthesis Using the Olefinic Linker

Multiple automated syntheses were achieved with the olefinic linker,[31,32] in the first era of automated synthesis. Representative oligosaccharide syntheses using the modified peptide synthesizer are described in order to use established knowledge to make today's advances.

The first automated synthesis using solid support was demonstrated to assemble protected α-mannosides up to decasaccharide, protected β-glucans up to dodecasaccharide **1.07**, and one fully deprotected trisaccharide containing solely 1,2-*trans* glycosidic linkages (**Figure 1.08**).[31] For the assembly of those oligosaccharides, an "acceptor bound strategy" was applied with an **Olefinic-Linker** and the phosphate building blocks, monomeric building block **1.05** and disaccharide building block **1.06**. The levulinic ester (Lev) was employed as a temporary protecting group. The disaccharide unit was pre-synthesized to make the branch point for β-glucan structures instead of using a sequential combination of monomeric building blocks. Automated oligosaccharide synthesis using solid-support established a new method to access synthetic oligosaccharides.

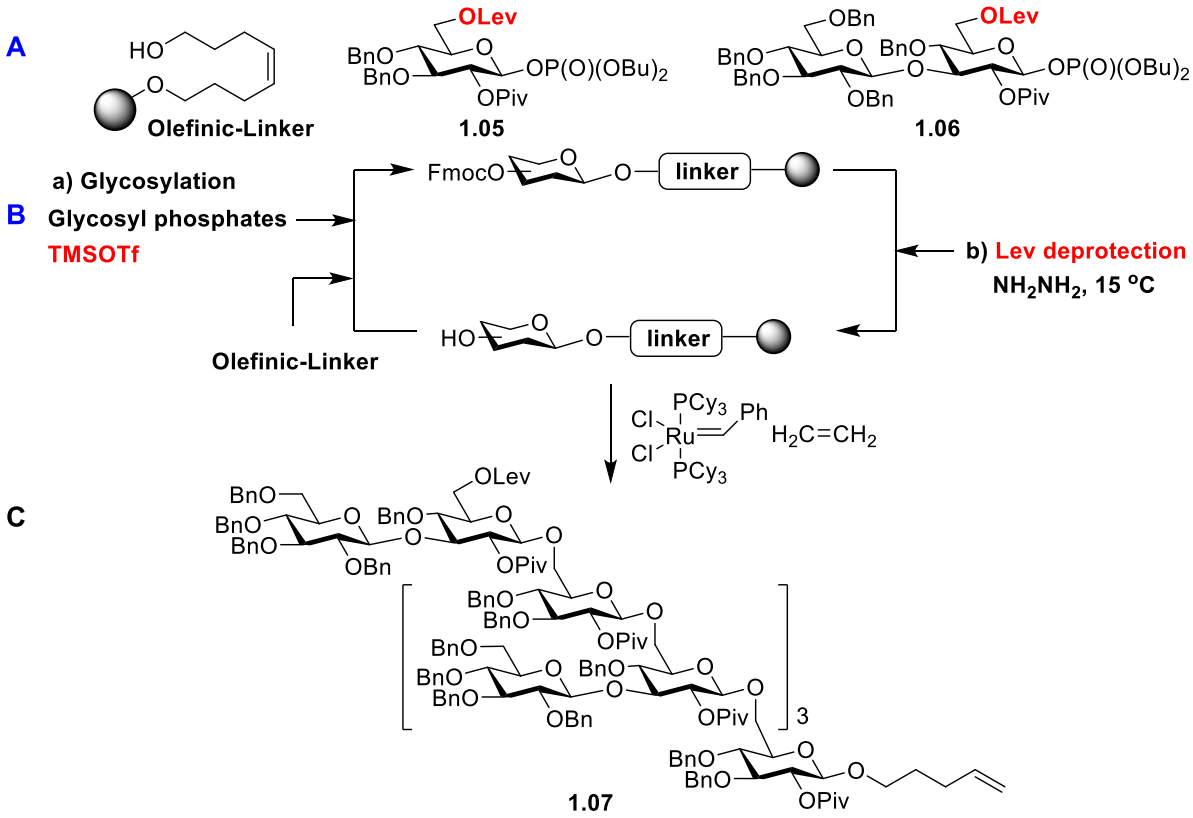


Figure 1.08 Automated oligosaccharide synthesis of phytoalexin elicitor (PE) β-glucan.[31]

The protected *Leishmania* cap branched tetrasaccharide **1.11** was assembled using only monomeric building blocks (**Figure 1.09**).[32a] For this purpose one mannose building block **1.08** was prepared to install a branched sugar with two orthogonal temporary protecting groups for the first time in automated synthesis without preparation of disaccharide building block.

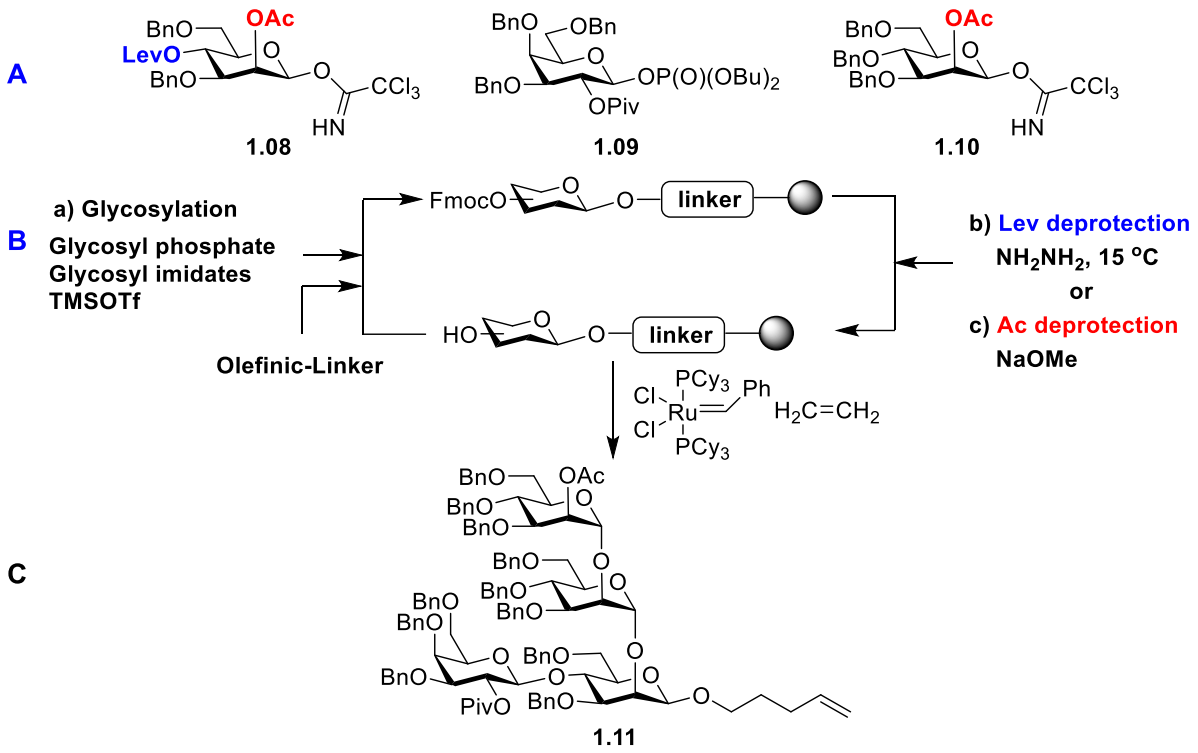


Figure 1.09 Automated oligosaccharide synthesis of the *Leishmania* cap tetrasaccharide.[32a]

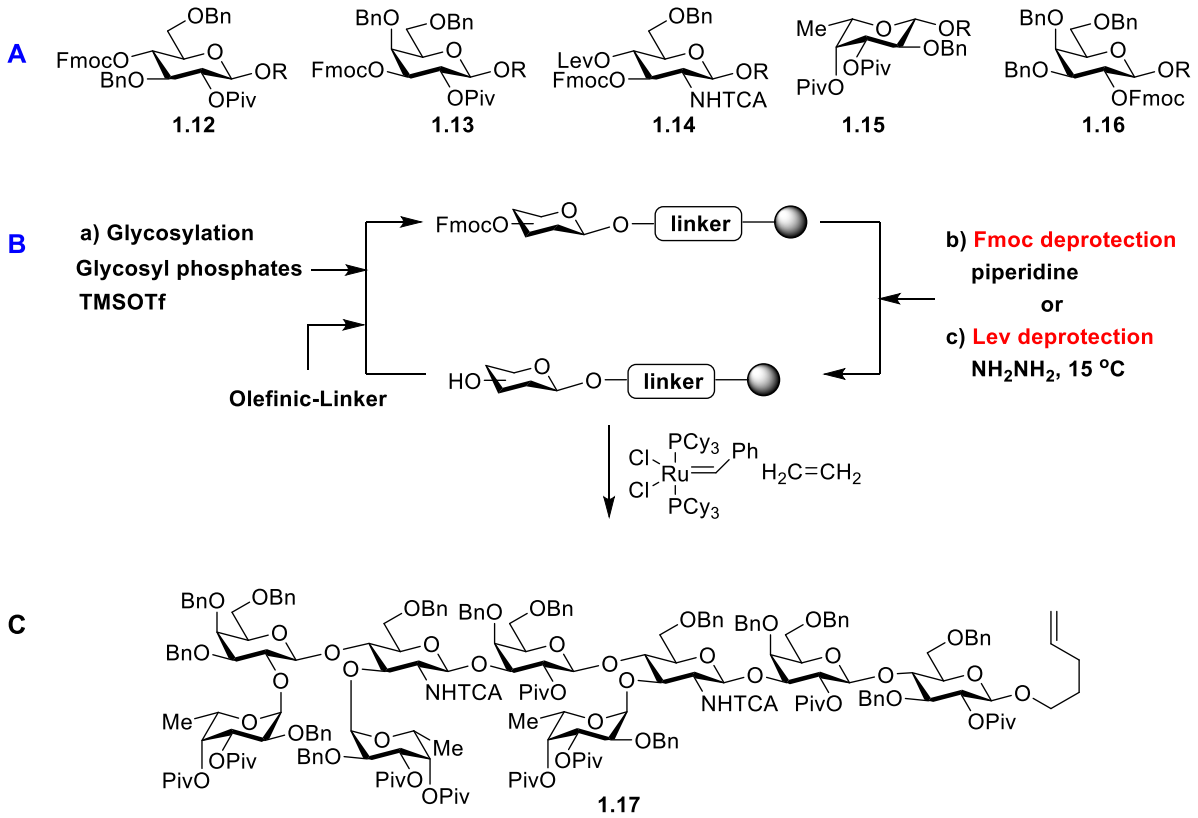


Figure 1.10 Automated synthesis of nonasaccharide Le^y-Le^x (R = P(O)(OBu)$_2$).[32b]

Tumor-associated antigen Le^y-Le^x and blood group determinants Le^y and Le^x contain α-(1→2) and/or α-(1→3) fucose linkages. Successful synthesis of Le^y-Le^x nonasaccharide 1.14 using automated synthesis made two great advancements (**Figure 1.10**).[32b] Stereoselective formation of 1,2-*cis* fucosidic linkages was achieved by automated synthesis on solid support. Additionally, the glycosylation efficiency for each glycosylation reaction was monitored by Fmoc quantification using UV/Vis spectroscopy.

Conventional carbohydrate synthesis and automated oligosaccharide synthesis were used synergistically to obtain a hexasaccharide for a malaria vaccine study (**Figure 1.11**).[32c] Following automated synthesis of a tetramannoside**,** conversion of the tetrasaccharide **1.22** into the glycosyl imidate **1.23** was achieved over two steps. A pseudohexasaccharide **1.25** was assembled with tetrasaccharide imidate **1.23** and disaccharide unit **1.24** in a [4+2] glycosylation reaction in solution. The hexasaccharide **1.25** was manipulated using multiple chemical steps to yield the fully deprotected hexasaccharide as a molecular probe for the malaria vaccine. It showed that automated synthesis of oligosaccharide could contribute to the preparation of fragment **1.22** as well as to the production of a molecular probe in the end.

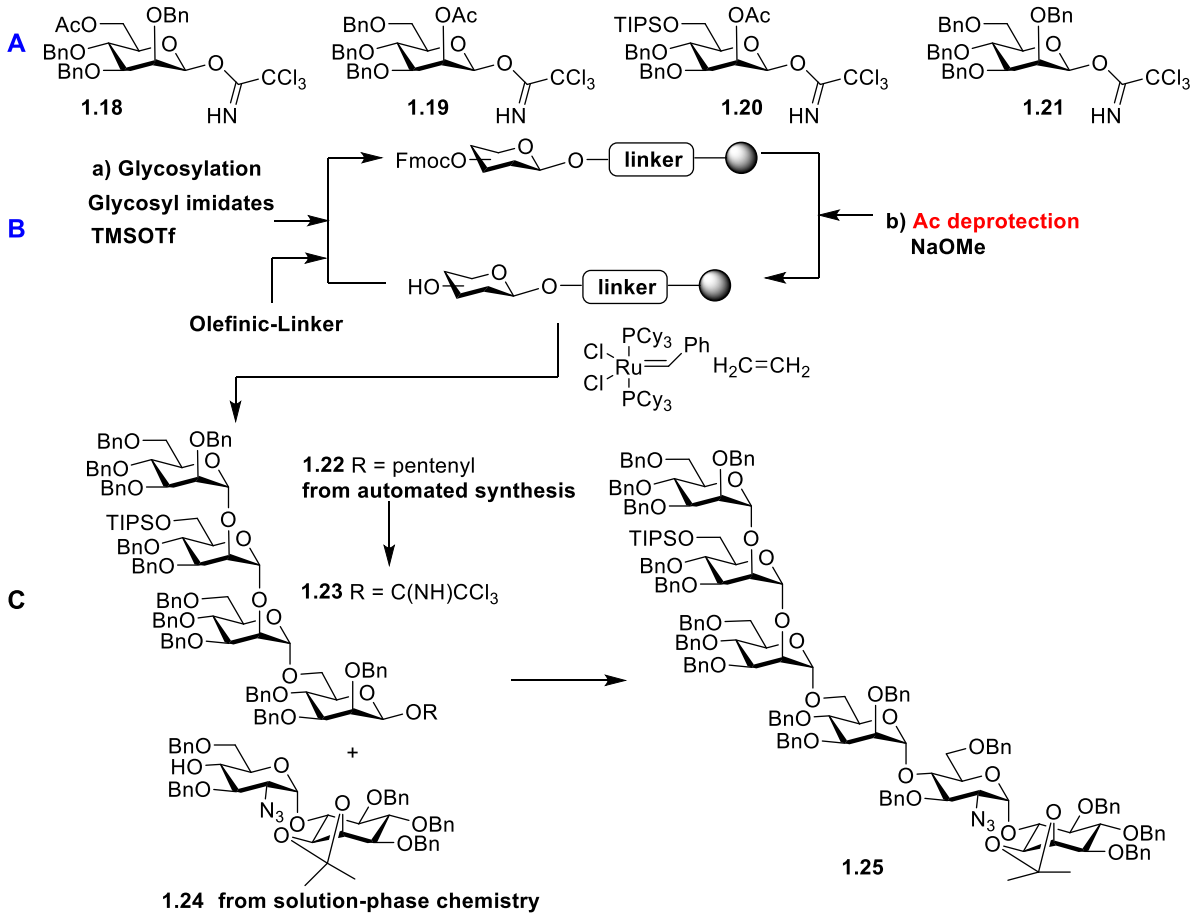


Figure 1.11 Synthesis of hexasaccharide for the biomedical research using combination of automated oligosaccharide synthesis and conventional solution-phase chemistry.[32]

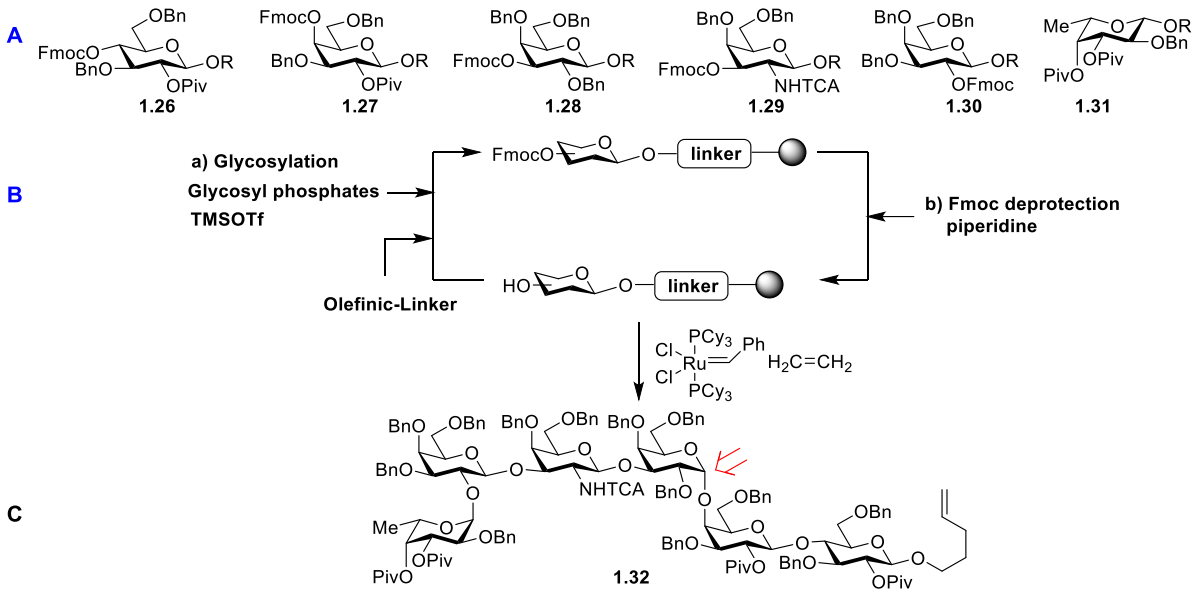


Figure 1.12 Automated synthesis of Globo-H.[32d]

Tumor-associated antigen Globo-H contains α-(1→2) fucose linkage and α-(1→4) galactose linkages. Automated synthesis of the protected tumor antigen Globo-H **1.32** was executed using the optimized galactose building block **1.28** that was obtained by systematic investigation of leaving group effects to install a stereoselective 1,2-*cis* galactosidic linkage (phosphate VS imidate) (**Figure 1.12**).[32d] This assembly showcased the importance of building block design. Similarly, the protected core pentasaccharide of a *N*-glycan containing β-1,2-*cis* mannosidic linkages was also assembled using mannose building block equipped with carboxybenxyl (CB) as an anomeric leaving group.[32e]

1.3.2 The Second Era of Automated Oligosaccharide Synthesis Using the Bi-functional Linker

As a result of dedication by the *Seeberger group* towards improving automated glycan synthesis since 2001, **a fully-automated synthesizer controlled by computer** with a user-friendly interface was developed and enabled installation of multiple reagents driven by syringe pumps as well as solvents by pressure.[33] This second generation synthesizer even allowed the resulting oligosaccharide to be collected to a fraction collector after performing a linker cleavage reaction using NaOMe. A representative example is shown (**Figure 1.13**).

The first automated synthesis using the bi-functional linker, **BiFunc-Linker**, was demonstrated to assemble a protected *N*-glycan core pentasaccharide **1.37** using thioglycoside **1.33**, carboxybenxyl (CB) glycoside **1.34**, and *N*-phenyl trifluoroacetimidate **1.35**.[33a] The resulting oligosaccharide was collected and purified by reverse-phase HPLC, and then

characterized by NMR and HPLC. Finally, following global deprotection of **1.36** using Pd/C and H_2 and purification by size-exclusion column or reverse-phase chromatography the conjugation ready form of oligosaccharide was made available **1.37**.

The second generation of automated synthesis pushed the limits to utilize thioglycosides as building blocks, and avoid linker modification by using the bi-functional linker that is tolerant to the activation conditions of the thioglycoside building blocks with NIS and TfOH.

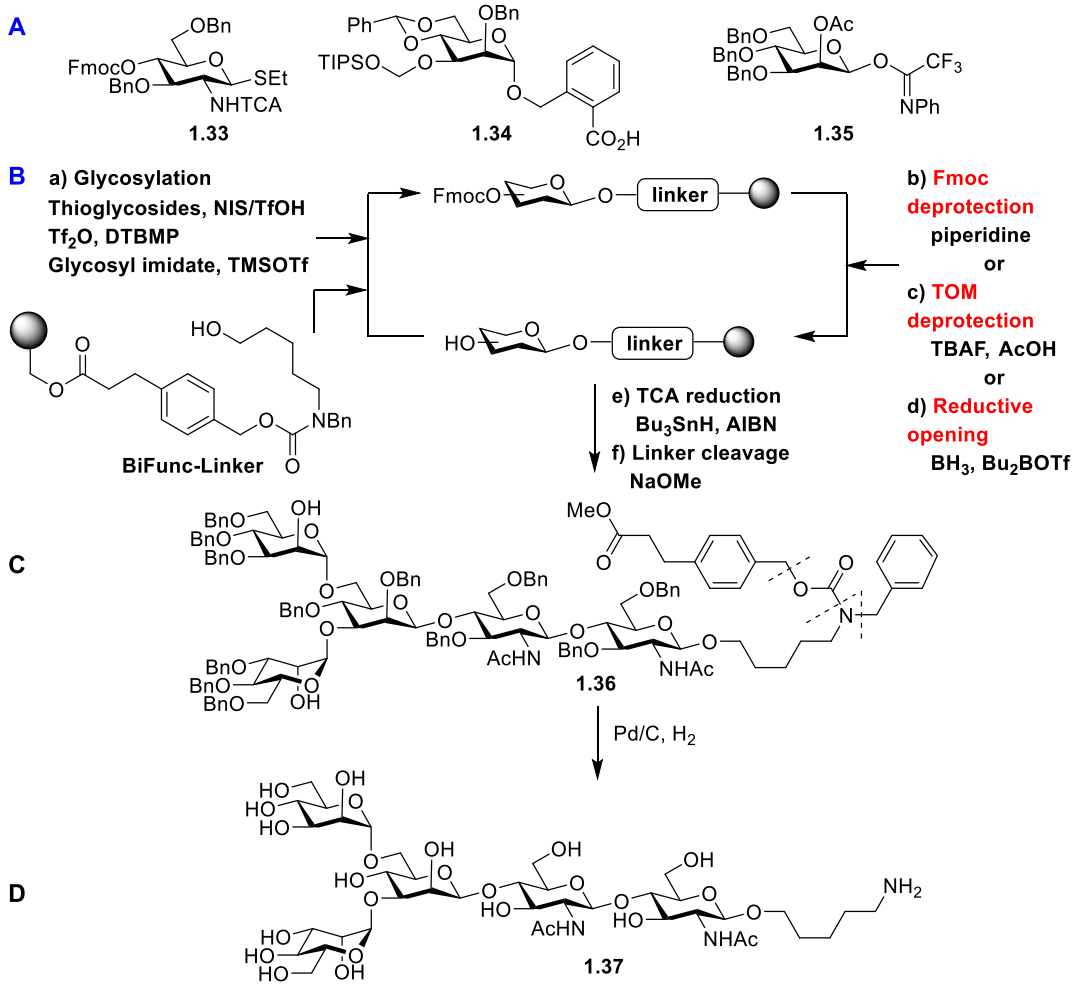


Figure 1.13 Automated synthesis of the *N*-glycan core pentasaccharide.[33b]

Development of the bi-functional linker simplified the process of automated synthesis and enhanced accessibility of the conjugation-ready oligosaccharides with many satisfactory aspects: (1) tolerance to the acidic conditions for the glycosylation reactions, and aqueous conditions for deprotection of Nap, (2) facilitation of an amino group as a functional handle provided by removal of protection groups. However, the linker cleavage condition using NaOMe sometimes provides the mixture of oligosaccharides assembled by AGA (**Figure 1.14**).

1.3.3 The Third Era of Automated Oligosaccharide Synthesis Using the Photolabile Linker

A new demand led to the development of a photolabile linker that is cleaved by UV irradiation. This linker gives two advantages resulting from each predecessor linker system; the olefinic linker and the bi-functional linker. First, on cleavage of the photolabile linker, the cleavage condition orthogonally drives the reaction on the photoactive functional group, *p*-nitrophenyl moiety rather than other protecting groups. Therefore, the fully protected oligosaccharides are released from the resin[34] as the olefinic linker does (**Figure 1.15**). Secondly, global deprotection provides the functional handle that is required for the various applications like microarray fabrication and conjugation on the carrier protein as the bi-functional linker does (**Figure 1.16**).

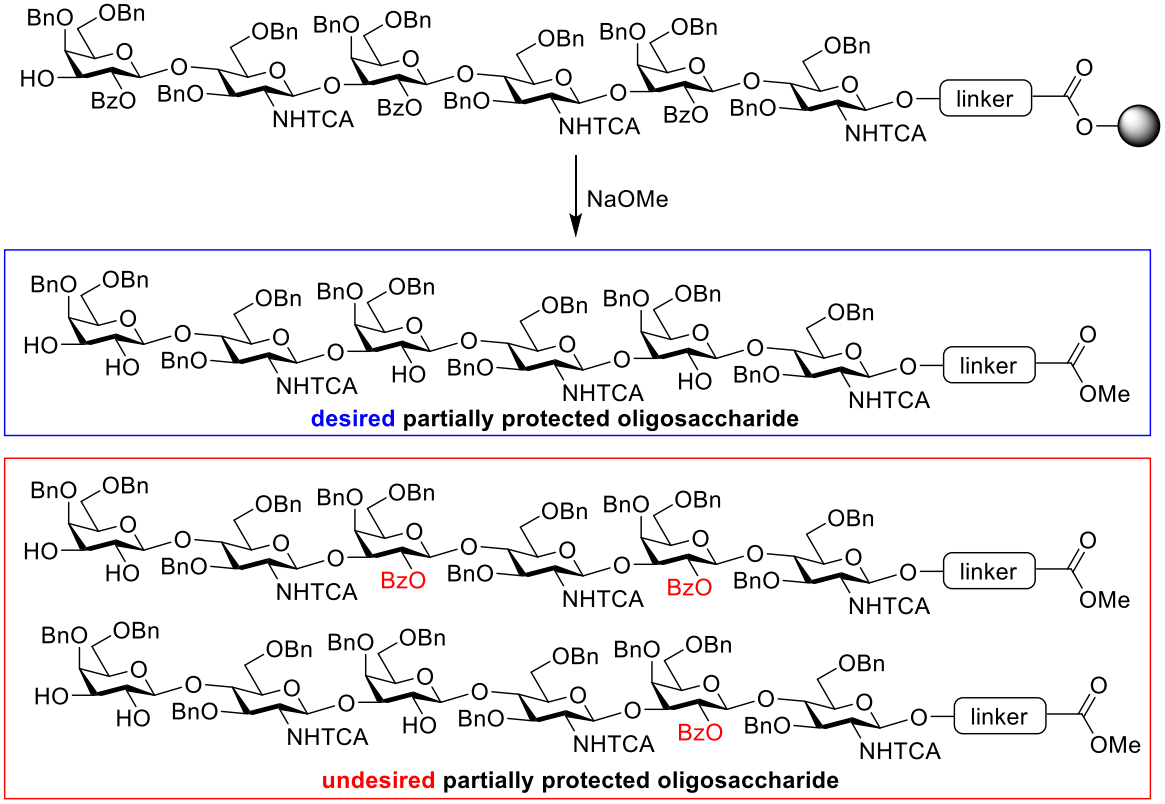


Figure 1.14 The obtained oligosaccharide by linker cleavage reactions.

The first automated synthesis using the photolabile linker was demonstrated to assemble protected chondroitin sulfates hexasaccharides **CS-C** and **CS-A** as representative examples of glycosaminoglycans (**Figure 1.15**).[34a] To synthesize these negative charged hexasaccharides, the orthogonally protected building blocks, **1.38**, **1.39**, and **1.40**, and a newly developed linker, so-called a photolabile linker, were introduced via an automated oligosaccharide synthesizer. The combination of each building block enabled to introduce

sulfation patterns. The photolabile linker is tolerate to reaction conditions and easily cleaved by UV irradiation in a continuous-flow photoreactor to provide the fully protected form of target oligosaccharides. The success of chondroitin sulfate using automated synthesis platform opened the new era of synthetic access to glycosaminoglycans (GAGs) oligosaccharides such as heparin, heparin sulfate, dermatan sulfate, or keratan sulfate.

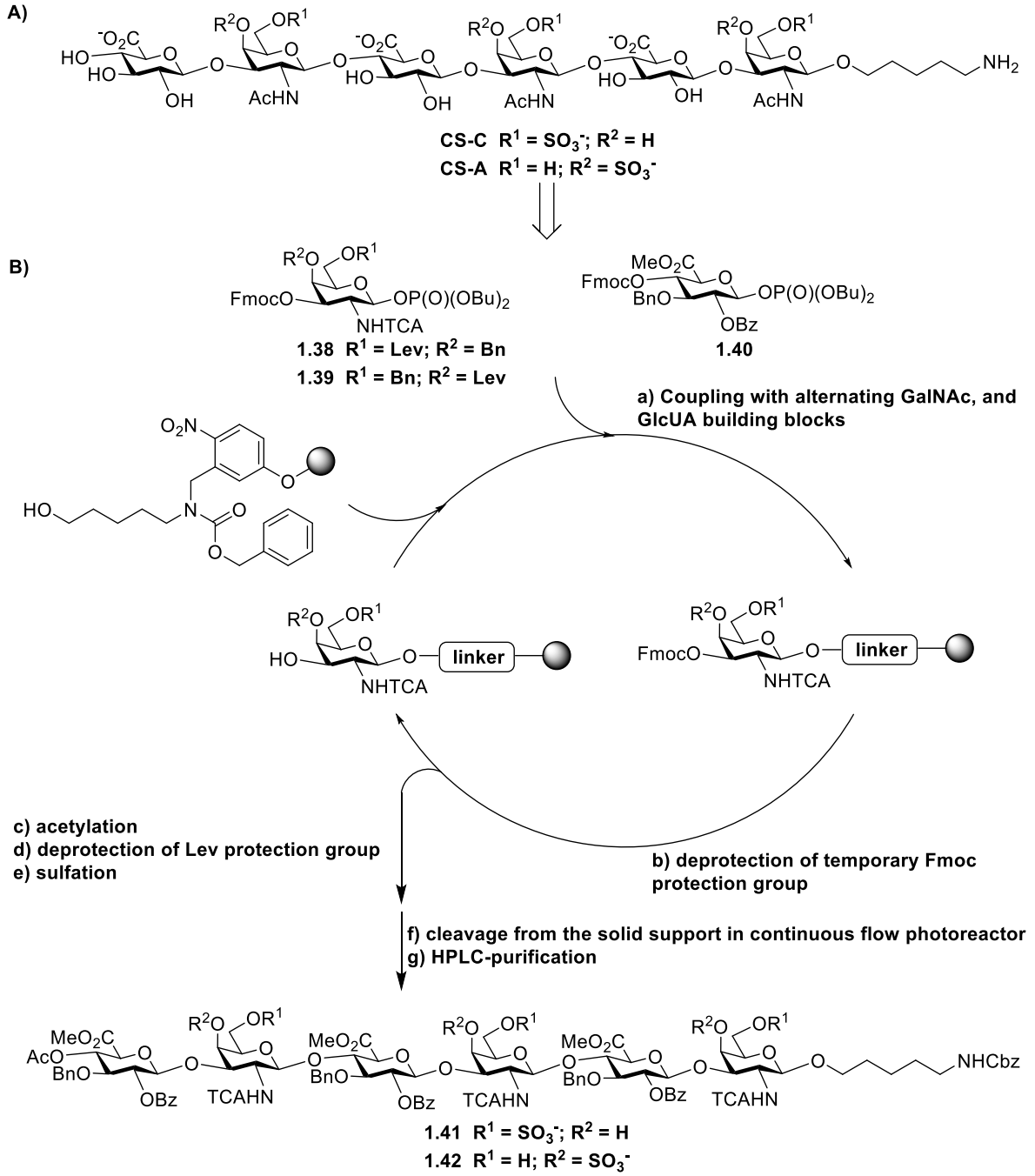


Figure 1.15 Automated Glycan Assembly of Chondroitin Sulfate C and A (CS-C and CS-A) Hexasaccharides. A) Retrosynthetic analysis of chondroitin oligosaccharide sequences with different sulfation patterns. B) Automated synthesis of chondroitin hexasaccharides.[34a]

α-(1,6)-30-mer of polymannoside, **1.45** was assembled by combination of the photolabile linker, **Linker-1**, and catch-and-release purification method as a proof-of-principle (**Figure 1.17**); proving that AGA enables access to homopolysaccharide chains. Introduction of a catch-and-release strategy is employed to purify the full-length oligosaccharide tagged and enriched by magnetic beads. Following separation from deletion sequences by magnet-assisted decanting, the desired product is released from the beads.[34b]

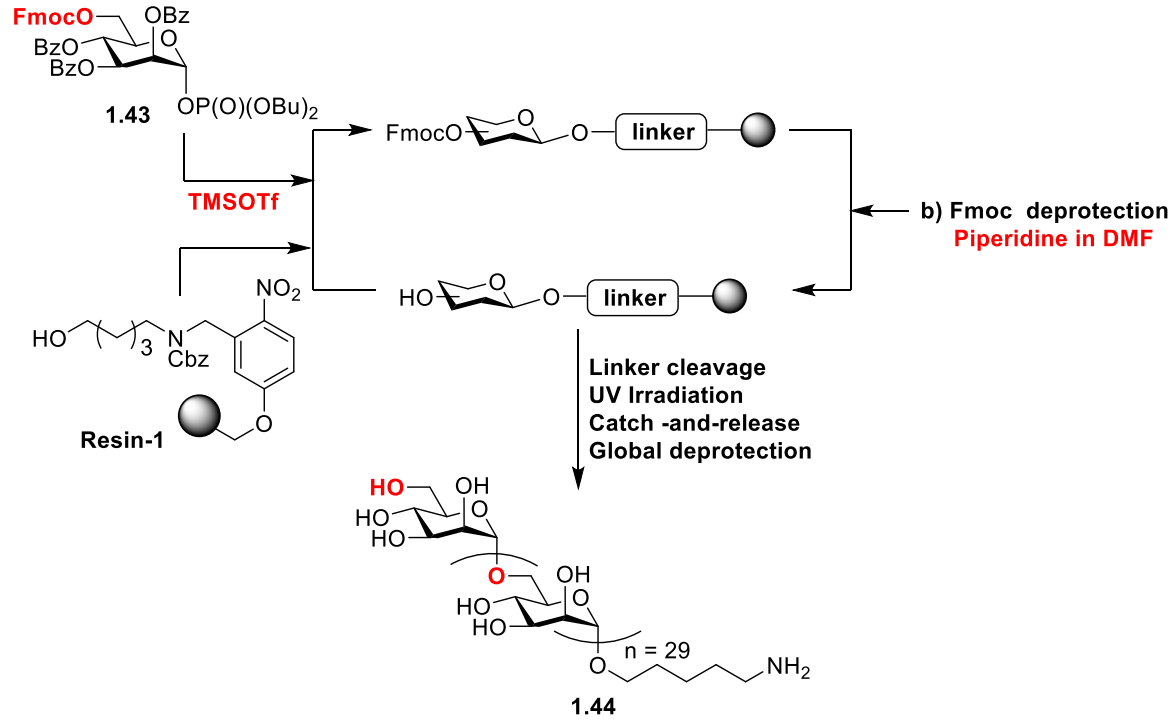


Figure 1.16 Automated oligosaccharide synthesis of 30-mer of polymannoside[34b]

1.3.4 Assignment of the Process

Over the advancement of automated synthesis of glycans, procurement of the final product, generation of conjugation-ready oligosaccharides, automated processing of the linker cleavage, deprotection of temporary protecting groups, purification, global deprotection, and final purification have not been firmly established yet. As a result, the mass production of conjugation-ready oligosaccharides remains elusive (**Figure 1.17**).

A general definition for each step has not been in demand because only a couple of research groups worldwide have been focusing on development of automated synthesis of oligosaccharides over the last decade. However, a common language could facilitate seamless communication between scientists from the interdisciplinary research area.

Prior to discussing the thesis project in detail, it should be noted that the automated synthesis process is often classified into three sub-steps: **Pre-automation steps**, **Automation**, and **Post-automation steps** (**Figure 1.17).** Selection of the best resin, linker development,

building block preparation, and the synthesizer are in the **pre-automation-step (blue box).** During **automation**, with these elements in hand, the instrument performs chemical reactions under optimized glycosylation and deprotection conditions for each building block. It is concluded that previous research on automated synthesis has been dedicated to developing pre-automation steps and automation. These two steps iteratively interplay to identify "approved" building blocks that ensure 1,2-*trans* stereoselectivity as well as regioselectivity. In contrast to advancement of pre-automation and automation steps, **post-automation step (red box)** mainly composed of purification followed by automation and deprotection chemistry and validation of purity of unprotected oligosaccharides, were not thoroughly established. In the following section, I will discuss what to develop during each of these steps to make automated oligosaccharide synthesis accessible to the field of glycoscience.

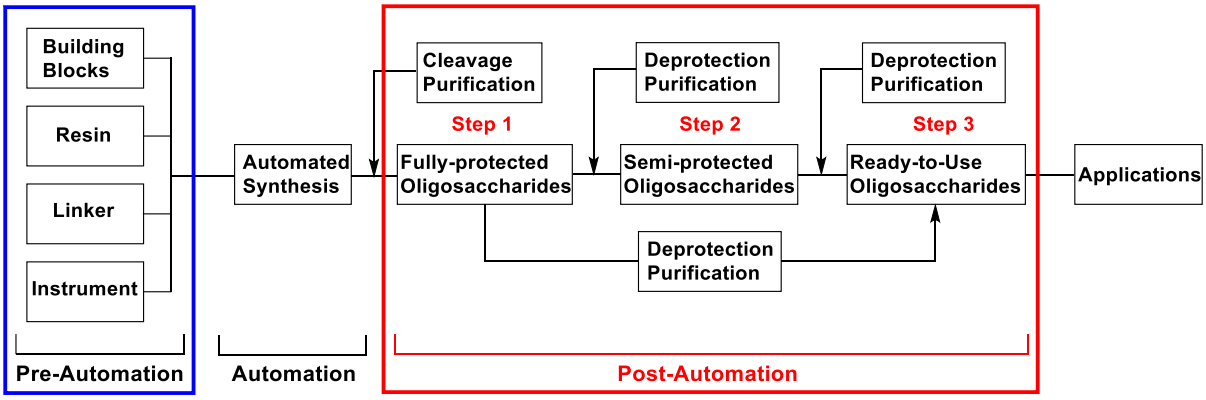


Figure 1.17 Schematic view of the first generation of automated synthesis process and assignment of the automated oligosaccharide synthesis process.

1.3.4.1 Preautomation: Various Target Oligosaccharides Require Expansion of Approved Building Blocks

The validation and scalable synthesis of the monomeric building blocks utilized for oligosaccharide synthesis using traditional solution-phase chemistry and automated glycan assembly using solid-phase chemistry is the most laborious, yet the most important requirement. These monomeric building blocks bear the Fmoc protected, tactically positioned hydroxyl group as a nucleophile for glycosylation, a proper leaving group, a thioglycoside group, which can be transformed to a phosphate, at the anomeric position, and appropriate protecting groups on the remaining hydroxyls. Once "approved" building blocks have been identified along with advancement in automated glycan assembly, various oligo- and polysaccharides require these "approved" building blocks that should be scalable and stable for a long time at reasonable storage conditions.

As shown in many examples, a building block for AGA bears permanent and temporary protecting groups. In the AGA approach, benzyl ethers (Bn), benzoylesters (Bz), acetylesters (Ac), azides, and *N*-trichloroacetyls (NHTCA) are utilized as permanent protecting groups that are removed on global deprotection during the post-automation step at the end of the streamlined AGA to obtain unprotected target glycans. 9-Fluorenylcarbyloxymethyl (Fmoc) and levulinoyl (Lev) serve as temporary protecting groups that are removed during automation to allow for the following glycosylation.

The abovementioned protecting groups are strategically introduced to achieve the designed connectivities. These protecting groups also control the stereochemistry of the glycosylation step to form glycosidic linkages and surprisingly influence the reactivity of a building block and affect the completion of each glycosylation reaction during the automation step. Therefore, in automated synthesis, protecting groups are classified as either permanent protecting groups and temporary protecting groups.

The leaving groups on an anomeric position have been developed. The glycosyl phosphates served for the first generation linker system. The glycosyl imidates and thioglycosides were used with the bi-functional linker over limitations of the olefinic linker. Unlike the glycosyl phosphates that require stoichiometric amounts of an activator, TMSOTf resulting in highly acidic reaction media, the trichlorocetimidates and thioglycosides are activated with catalytic amount of the acidic activator. All leaving groups show completion of the glycosylation step in AGA. Taken together, to obtain high empirical number of automated oligosaccharide synthesis, various building blocks will be prepared with proper protecting group strategy in this thesis.

1.3.4.2 Automation: Glycosylation Cycle Have Unknown Issues

The automation step is mostly composed of two chemical reactions: glycosylation and deprotection. The linker-conjugated resin is washed with various solvent in order to remove the glycosylating reagents, the donor, the activator, and a basic deprotection reagent. In the glycosylation step, the reaction with building blocks and the corresponding activator are repeated twice, which is called the double coupling. In the deprotection step, Fmoc is removed by piperidine solution (20% solution in DMF). The reaction mixture from each step is collected and then the reaction media containing dibenzofulvene, resulting from Fmoc cleavage, was measured by UV absorbance to monitor completion ratio of the glycosylation reaction, without further processing, followed by linker cleavage and HPLC analysis.

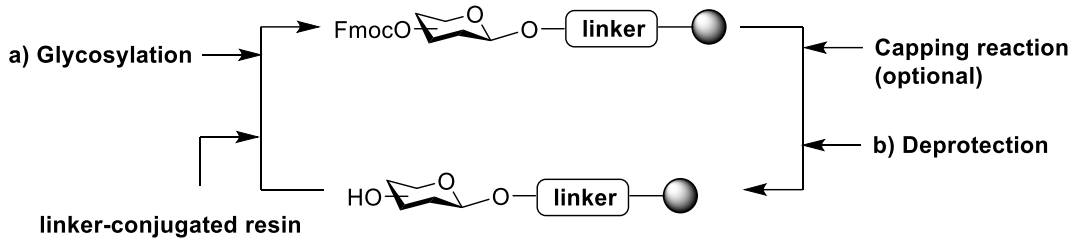


Figure 1.18 Automation step in the streamlined AGA

When automated synthesis was performed in the lab, it was observed that the glycosylation mixture of thioglycoside, NIS, and TfOH, was not properly activated, which can be easily monitored by the color of the collected solution from the activated glycosylation mixture. This issue should be solved to obtain target glycans in high yield with the least deletion sequence.

1.3.4.3 Postautomation: Purification and Quality Control of Target Oligosaccharides.

Postautomation steps on automated synthesis are generally known to be linker cleavage, purification and deprotection steps in the context of typical carbohydrate chemistry. Thus, completion of post-automation steps will provide unprotected, conjugation-ready oligosaccharides. Compared to the purification process of automated nucleic acids or peptides synthesis, the purification steps of automated carbohydrate synthesis are more complex because of various protecting groups on each monomer. After linker cleavage, the final form of nucleic acids and peptides are obtained by a single purification of the resulting mixtures using reverse-phase HPLC mostly without any chemical modification for deprotection of remaining protecting groups. However, linker cleavage following automated glycan assembly, provides either the fully protected **1.44/1.45** or the semi-protected oligosaccharide **1.46** that resulted from strategically protected building blocks (**Figure 1.19**). Thus, during post-automation, multiple purifications to obtain a protected or unprotected glycan and chemical reaction steps to remove all protection groups should be performed out of the synthesizer.

Based on the number of examples made over the past years, these post-automation steps require three purification conditions and two chemical reactions: i) the first purification to give the fully protected oligosaccharide; ii) the second purification to afford semi-protected oligosaccharide; iii) the last purification to procure the conjugation-ready glycan; iv) methanolysis using NaOMe to remove esters as temporary protection group; v) global deprotection using Pd/C and hydrogen gas (H_2). Therefore, standard conditions could be

established for purification as well as deprotection reactions for potential non-expert users. After procurement of the conjugation-ready oligosaccharide, purity should be confirmed prior to utilization or catalogue in an in-house library. To date NMR experiments serve to determine quality of synthetic carbohydrates, but this requires large quantities of sample and the use of high field NMR. NMR experiments can barely detect 5% of impurity for molecules. Therefore better, fast and reliable methods have to be developed and utilized routinely as general protocols for the glycoscience community.

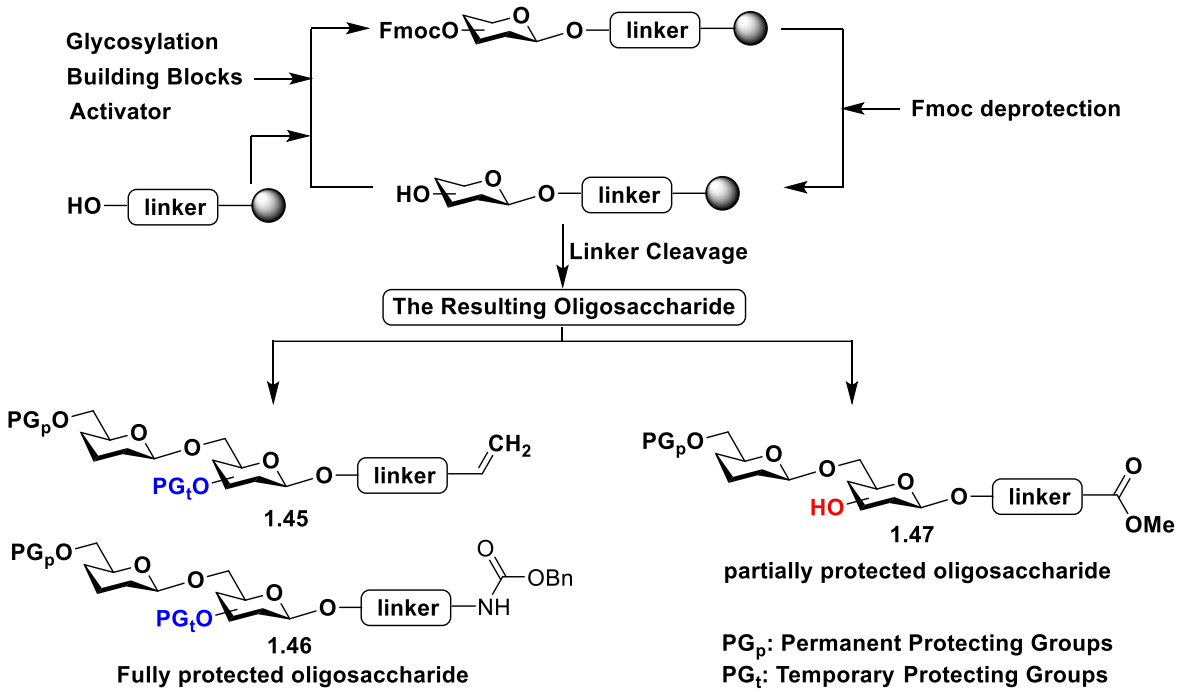


Figure 1.19 The obtained oligosaccharide by linker cleavage reactions.

In summary, the post-automation steps were less standardized other than pre-automated and automation steps in the field of the automated oligosaccharide synthesis. Standardized conditions for the post-automation steps are urgently required for access to conjugation-ready oligosaccharides routinely with guaranteed purity.

1.4 Aims of the Thesis

The ultimate goal of my thesis research is to help democratize the field of glycoscience by making a robust platform for automated glycan synthesis for scientists in order to obtain and to utilize structurally defined and pure glycans as biomedical/biochemical probes. To make this ultimate goal come true, the standardized process of automated synthesis should be established and facilitated with “approved” monomeric building blocks by both chemists and non-chemists.

Therefore, I focused on the synthesis of various oligosaccharides that require key elements: 1) the **photolabile linker** that tolerate many chemical manipulation conditions in addition to previously reported glycosylation and deprotection conditions; 2) the identification of the **"approved" building blocks** ensuring regio- and stereochemistry upon glycosylation reactions; 3) **establishment of the streamline process consisting of pre-automation, automation, and post-automation steps** as protocols that enable purification of fully protected, semi-deprotected or fully deprotected oligosaccharide to obtain final conjugation-ready oligosaccharide.

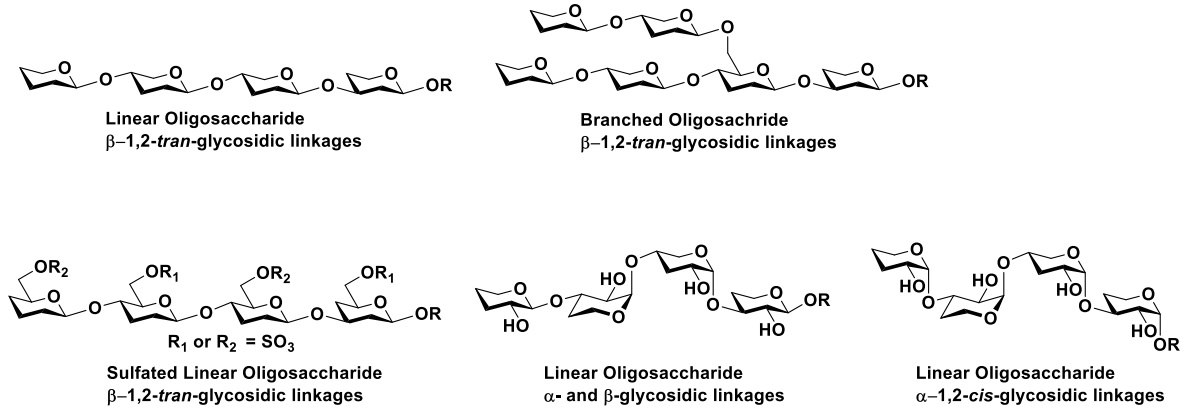


Figure 1.14 Schematic view of representative oligosaccharide structures.

First, diverse and challenging oligosaccharides were selected for automated glycan assembly. Four general structural archetypes were considered: i) linear structures having only β-1,2-*trans*-glycosidic linkages and branched structures having only β-1,2-*trans*-glycosidic linkages; ii) sulfated linear structures having β-1,2-*trans*-glycosidic linkages such as glycosaminoglycans; iii) linear structures containing a single 1,2-*cis*-glycosidic linkages; iv) linear structures containing multiple *cis*-glycosidic linkages (**Figure 1.14**). Following these automated syntheses, the second objective was to establish post-automation protocols that allow for the purification of any synthetic molecules by HPLC conditions. Taken together, these two objectives could provide the foundation to establish the standardized process for streamlined automated glycan assembly.

In Chapter 2, the automated synthesis of oligo-LacNAc structures including keratan sulfates as a member of glycosaminoglycans (GAGs) was demonstrated. A common tetrasaccharide backbone bearing three temporary protecting groups, Fmoc, Lev and Nap was transformed into differently sulfated tetrasaccharides. A microarray screening revealed the new binding to AAVth10 for the first time. This synthetic method will be applied to the synthesis of other GAGs oligosaccharides.

Chapter 3 showed the automated synthesis of oligosaccharides containing difficult linkages in the context of carbohydrate chemistry using “approved” building blocks. The H type-II pentasaccharide served as a synthetic challenge because of an α(1→2)-*cis* fucosidic linkage. Even though the synthesis of H-type II was achieved in a strictly linear manner in the solution-phase, the automated assembly of this pentasaccharide was not previously successful in the solid-phase (ref, JOC, 2005). Installation of a single 1,2-*cis* glycosidic linkage was achieved using “approved” building blocks that enable the synthesis of pentasaccharides such as H-type I, H-type II and α-Gal epitopes containing 1,2-*cis* linkages.

In Chapter 4, in the line of oligosaccharide synthesis bearing 1,2-*cis* glycosidic linkage, oligosaccharides containing multiple 1,2-*cis* glycosidic linkages were synthesized by AGA. In this process, many building blocks were systemically investigated and monomer building blocks bearing remote participating groups were identified to be “approved”. The mixture of stereoisomers was successfully purified by “normal-phase” HPLC to obtain the desired oligosaccharides. This approach showed the formation of 1,2-*cis* glycosidic linkages in AGA was no longer “sequence-dependent in Chapter 3”, but was led by “well-designed building blocks”.

Chapter 5 described the streamlined automated glycan synthesis using the commercial synthesizer, the Glyconeer 2.1®. The ultimate goal proposed above was achieved through a combination of the robust synthetic tool, the Glyconeer 2.1® and synthetic protocols comprising pre-automation, automation, and post-automation steps enabled the procurement of naturally occurring oligosaccharides and the tailored glycans. Furthermore, quality control using ion-mobility mass-spectrometry (IM-MS) showed purity and sequence of synthetic glycans.

2. Automated Glycan Assembly of Oligo-*N*-acetyllactosamine Glycans Rapidly Provides Probes to Characterize Virus-Glycan Interactions

This chapter has been modified in part from the following publication:

H. S. Hahm, F. Bröcker, F. Kawasaki, M. Mietzsch R. Heilbronn, M. Fukuda, P. H. Seeberger, Automated Glycan Assembly of Oligo-*N*-acetyllacosamine and Kertan Sulfate Probes to Study Virus-Glycan Interactions. *Chem.* **2017**, *1*, 114-124. Mr. Hahm and Dr. Broecker equally contributed.

https://doi.org/10.1016/j.chempr.2016.12.004

2.1 Introduction

2.1.1 Oligo-*N*-acetyllactosamine

Oligo-*N*-acetyllactosamine (LacNAc) and keratan sulfate (KS) glycans are major constituents of the mammalian glycocalyx that are crucial for cell-cell and host-pathogen interactions.[36-39] Both LacNAcs and KSs share a common [Galβ(1→4)GlcNAcβ(1→3)] disaccharide repeating unit and are part of cell-surface glycoproteins and glycolipids.[38-41] LacNAcs differ in the number of repeating units, mono- or oligosaccharide branching and terminal saccharides such as sialic acid or fucose.[40,41] These modifications are responsible for the differential recognition by glycan-binding proteins (GBPs) and dictate specific functions of glycans *in vivo*, including the regulation of the immune system[42,43] and inflammation.[44,45] Galectins, a family of GBPs involved in innate and adaptive immunity pathways[46-48] recognize various LacNAcs, including branched histo-blood group antigens (HBGAs), depending on the number of repeating units and terminal saccharides.[41,42] Different LacNAc glycans have been identified as cancer-specific antigens that include branched oligosaccharides such as Lewis antigens with or without terminal sialylation.[49] KS glycans are linear polymers of the disaccharide repeating unit that can be 6-*O*-sulfated at one or both Gal or GlcNAc residues with varying degrees and patterns of sulfation in different tissues.[36,37] In addition, the terminal Gal residue can be sulfated at the 3-position. KSs are specifically expressed on a subset of inflammation-associated dendritic cells[44] and aberrant sulfation patterns were shown to be implicated in pulmonary hypertension disorders.[45]

2.1.2 Virus-Glycan Interactions

Various mammalian viruses harness cell-surface glycans as entry receptors for infection.[50] Examples are noroviruses[51,52] and some rotavirus strains[53] that recognize HBGAs as well as influenza viruses interacting with sialylated LacNAc glycans[54,55]. Likewise, adeno-associated viruses (AAVs) that are promising gene therapy vectors utilize cell-surface glycans for attachment and entry to their hosts, and serotype-specific interactions with these glycans govern virus tropism.[56-59] Thirteen serotypes, AAV1-AAV13, are known[59], some of which recognize heparan sulfates or sialylated glycans.[56] The potential glycan receptors for other serotypes, including AAVrh10, remain to be identified.

2.2 Automated Glycan Assembly of Oligo-LacNAc Glycans

Because structural heterogeneity *in vivo*[36-41] impedes isolation in sufficient purity and quantity, structure-activity relationship studies of LacNAc/KS rely on the availability of synthetic oligosaccharides. Previously reported solution-phase syntheses yielded linear and branched LacNAcs up to hexasaccharides[60,61] as well as sialylated linear penta- and heptasaccharides.[62] Chemoenzymatic methods have been used to obtain a variety of sialylated LacNAc derivatives.[55,61,63-66] In addition, chemical syntheses of mono-, di- and tri-*O*-sulfated LacNAc/KS disaccharides[67,68], sialyl derivatives of the LacNAc disaccharide with 6-*O*-sulfation at either Gal or GlcNAc[69-72], including a complex ganglioside[73], have been reported. A LacNAc disaccharide with a 6-*O*-sulfated GlcNAc residue has been used as acceptor for enzymatic installment of terminal sialic acid.[74] Solution-phase synthesis approaches to obtain KS glycans from di- to hexasaccharides have been reported.[62,67,75-77] However, synthesis of these glycans is still generally a burden due to the time required for the synthesis and substantial loss of materials during multiple purification steps.

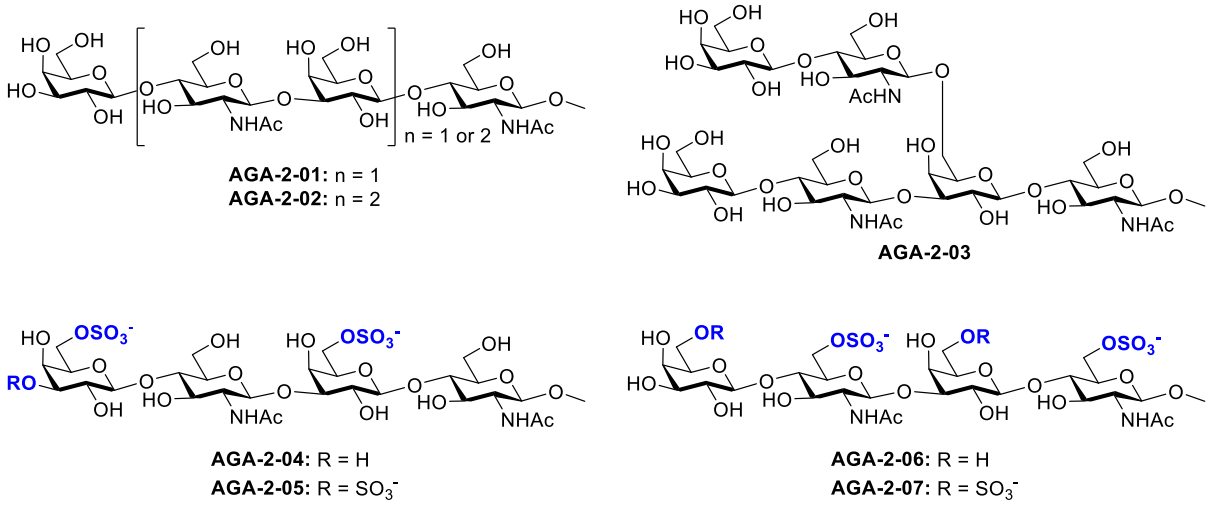


Figure 2.01. Chemical Structures of LacNAcs and KS Oligosaccharide Targets[EF]

To facilitate rapid access to structurally diverse LacNAc/KS glycans, we developed an automated glycan assembly (AGA)[78] strategy using three orthogonal protecting groups. This approach yielded a collection of linker-equipped linear, branched, and four differentially sulfated oligosaccharides **AGA-2-01 - AGA-2-07** (**Figure 2.01**) that bear a nucleophilic linker to enable efficient and orientation-specific attachment to surfaces including microarrays.[79,80] Such glycan microarrays provide a powerful tool to identify glycan ligands

of GBPs[54,55] or intact virus particles.[56] Microarrays presenting compounds **AGA-2-01** - **AGA-2-07** revealed glycan binding profiles of AAV particles. Disulfated KS tetrasaccharide **AGA-2-06** was specifically recognized by the AAVrh10 serotype, an interaction that was validated by surface plasmon resonance (SPR) measurements. Thus, we identified KS as a novel candidate glycan receptor for the AAVrh10 gene therapy vector.

2.2.1 Automated Glycan Assembly Strategy

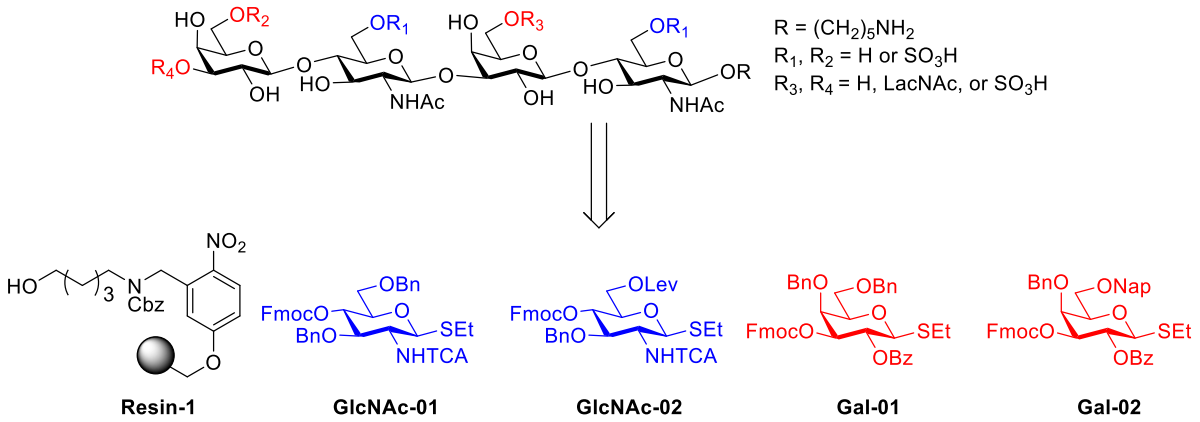


Figure 2.02. Retrosynthetic analysis of Linear, Branched, and Sulfated LacNAc and KS Oligosaccharides and the Complete Set of Building Blocks for AGA with Three Orthogonal Temporary Protecting Groups.

All regio- and stereochemical information during AGA resides in the monosaccharide building blocks. Aiming at a facile strategy to access diverse LacNAc/KS oligosaccharides, we designed a set of building blocks by introducing three orthogonal temporary protecting groups, which enabled structural diversification by the late-stage modification. Such orthogonal-protecting-group scenarios have been previously reported to diversify common oligosaccharide cores in solution-phase synthesis.[81,82] The three temporary protecting groups were employed as follows. The hydroxyl group that constitutes the next glycosylation site was protected with Fmoc. The glucosamine C-6 hydroxyl was masked with a Lev ester that was efficiently cleaved under mild conditions followed by sulfation reactions. The C-6 hydroxyl group of Gal was temporarily blocked by a 2-Naphthylmethyl (Nap) ether that was recently shown to be selectively removable in the presence of Fmoc,[83] in anticipation of branching or sulfation. Other hydroxyl groups were protected with either benzoyl esters (Bz) or benzyl ethers (Bn), depending on whether participation from C-2 to selectively install 1,2-*trans* glycosidic linkages was required or not. All these permanent protecting groups were removed either by methanolysis or hydrogenolysis using Pd/C and H_2 gas. To ensure C-2 participation, the amine of glucosamine was protected as *N*-trichloroacetyl amide (TCA) that

was converted to an acetyl (Ac) group during the hydrogenolysis step. Following these considerations, two differently protected building blocks of glucosamine (**GlcNAc-01** and **GlcNAc-02**) and galactose (**Gal-01** and **Gal-02**) were prepared on multi-gram scale (**Figure 2.02** and **Appendix**).

2.2.2 Automated Glycan Assembly of Linear LacNAc Glycans AGA-001 and AGA-002.

Linear LacNAc glycans **AGA-2-01** and **AGA-2-02** were prepared using polystyrene resin equipped with photolabile linker **Resin-1**[84] and building blocks **GlcNAc-01**[79] and **Gal-01.**[85] The monomers were added alternatingly by AGA using three reaction modules: acidic wash, glycosylation, and Fmoc deprotection (**Scheme 2.01** and **Table 2.01**).

Module	# of cycles	Reagents	Conditions
Acidic Wash	1	TMSOTf (2.5 equiv), DCM	-20 °C, for 1 min
Glycosylation-1	2	**GlcNAc-01** and **GlcNAc-02** (5 equiv), NIS (5.5 equiv), TfOH (0.55 equiv), DCM, dioxane (v/v, 9:1)	T_a = -30 °C, for 5 min T_i = -10 °C, for 25 min
Glycosylation-2	2	**Gal-01** and **Gal-02** (5 equiv), NIS (5.5 equiv), TfOH (0.55 equiv), DCM, dioxane (v/v, 9:1)	T_a = -40 °C, for 5 min T_i = -20 °C, for 25 min
Glycosylation-3	2	**Gal-02** (7.5 equiv), NIS (8.3 equiv), TfOH (0.83 equiv), DCM, dioxane (v/v, 9:1)	T_a = -40 °C, for 5 min T_i = -20 °C, for 25 min
Fmoc removal	3	20% of TEA in DMF	r.t. for 5 min
Nap removal	3	DDQ (8.0 equiv), DCE, MeOH, phosphate buffer (v/v/v, 64:16:1)	r.t. for 30 min
Lev removal	3	$NH_2NH_2 \cdot H_2O$ (11.2 equiv), AcOH, pyridine (v/v/, 3:2)	r.t. for 30 min
Capping	3	Ac_2O (1 mL)	r.t. for 30 min
Sulfation	3	SO_3·pyridine (40 equiv), DMF, pyridine (v/v/, 1:1)	50 °C for 30 min

Table 2.01. Automated Glycan Assembly Modules.

The acidic wash module added consecutive washing steps with *N*,*N*-dimethylformamide (DMF), tetrahydrofuran (THF), and dichloromethane (DCM) to remove any water from the reaction vessel, followed by a trimethylsilyl trifluoromethanesulfonate (TMSOTf) solution in DCM at -20 °C to neutralize basic residues present on the resin from previous synthesizer cycles. The glycosylation module used five equivalents of the respective

building block and a solution of *N*-iodosuccinimide (NIS) and trifluoromethanesulfonic acid (TfOH). First, the reaction was kept at the activation temperature (T_a) to allow for thorough mixing; then, it was increased to the incubation temperature (T_i) to complete glycosylation (Table 2.01).[86] The glycosylation module was performed twice in order to minimize the amount of unreacted glycosyl acceptor. Fmoc deprotection was achieved with 20% triethylamine (TEA) in DMF to liberate the hydroxyl group for subsequent chain elongation. These conditions replaced the conventional conditions used in AGA (20% piperidine in DMF) to suppress migration of benzoyl (Bz) groups in the terminal galactose residue. The cycles consisting of glycosylation and Fmoc deprotection were repeated four times to assemble tetrasaccharide **AGA-2-01** and six times for hexasaccharide **AGA-2-02**. Following AGA, the fully protected linear LacNAc oligosaccharides **AGA-2-p01** and **AGA-2-p02** were released from the resin by UV irradiation using a continuous flow reactor.[84] Formation of the desired molecules without any deletion sequences was confirmed by analytical normal-phase HPLC (**Figures 2.03** for **AGA-2-p01**).

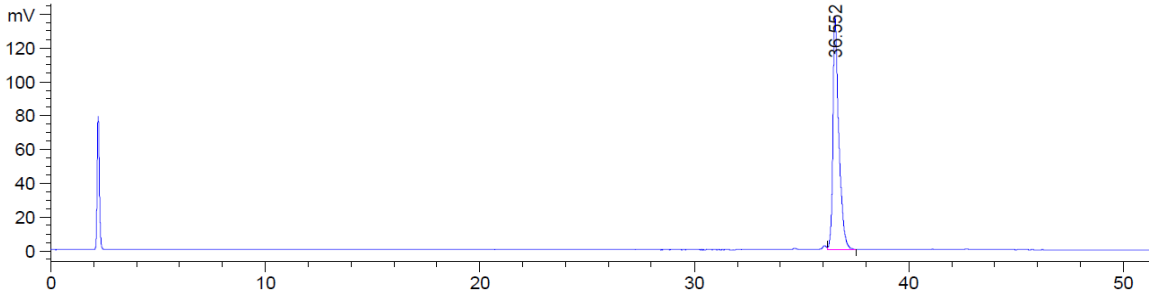


Figure 2.03. Analytical HPLC chromatogram of the crude protected tetrasaccharide AGA-2-p01

Without further purification, **AGA-2-p01** and **AGA-2-p02** were subjected to methanolysis with sodium methoxide to remove benzoyl esters as well as to hydrogenolysis with Pd/C and H_2 to both remove benzyl ethers and reduce trichloroacetamide to acetoamide. This afforded **AGA-2-01** (5.1 mg, 6.1 μmol, 25% over eleven steps) and **AGA-2-02** (4.8 mg, 4.0 μmol, 16% over fifteen steps) that were both purified by semi-preparative reverse-phase HPLC with Hypercarb® columns (**Figures 2.04** for **AGA-2-01**).

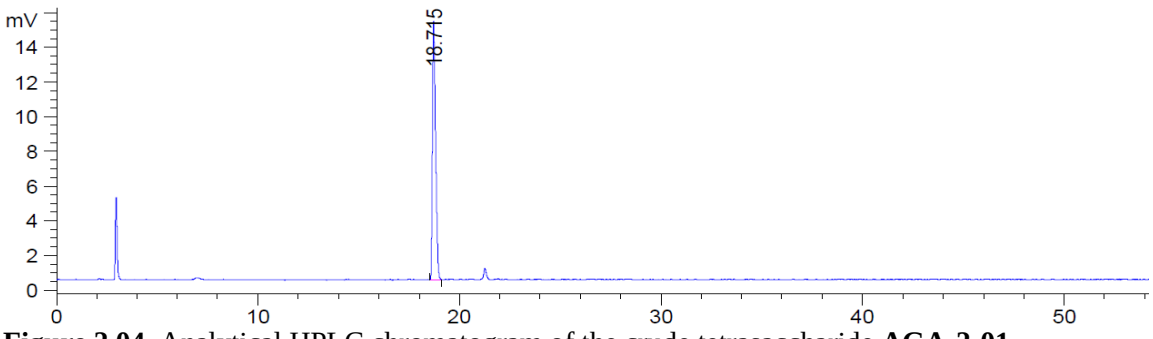
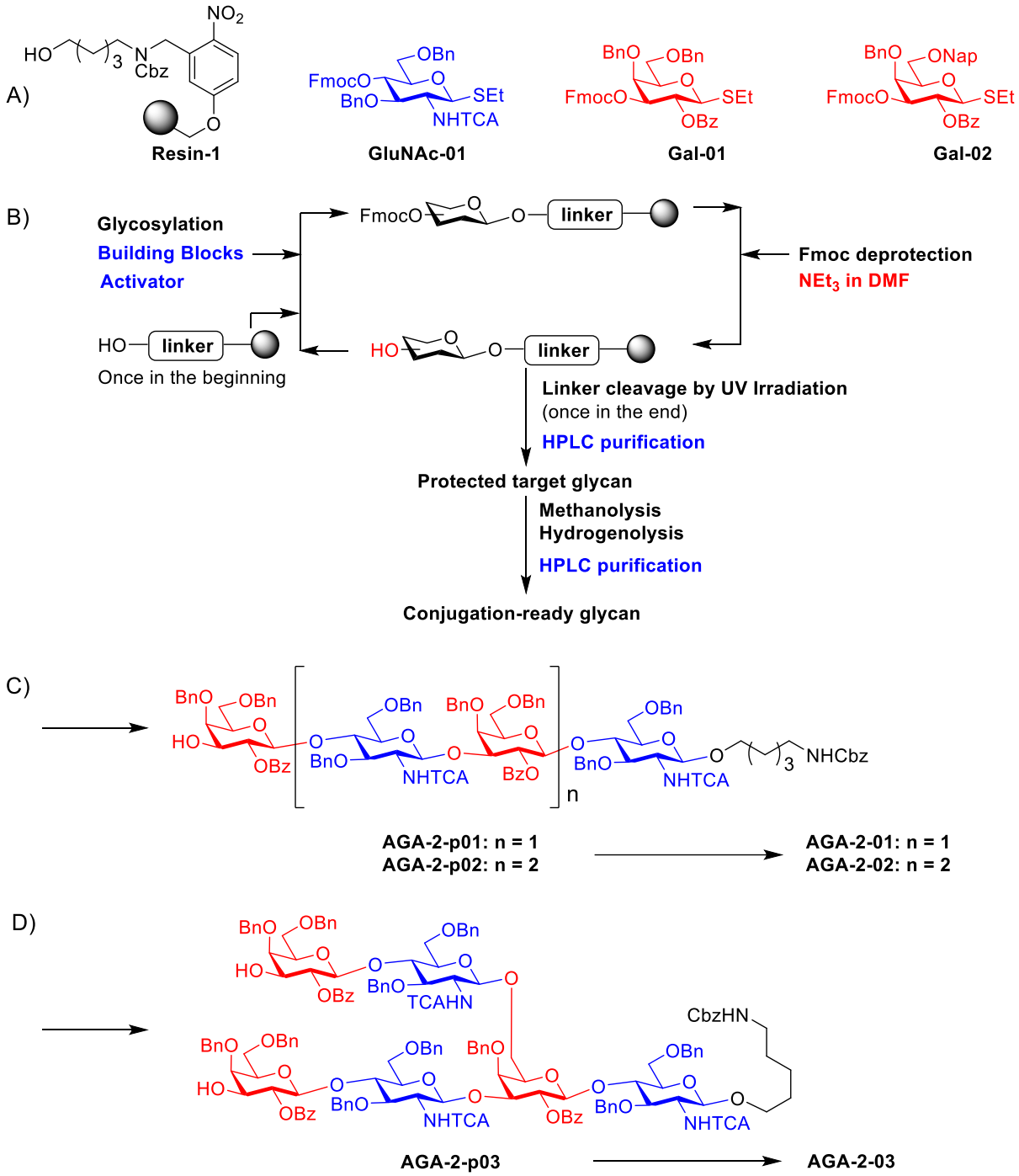


Figure 2.04. Analytical HPLC chromatogram of the crude tetrasaccharide **AGA-2-01**



Scheme 2.01. Automated glycan assembly of linear and branched LacNAc oligosaccharides[a]**.** (A) The photolabile linker conjugated resin **Resin-1** and building blocks **GluNAc-01**, **Gal-01** and **Gal-02**. (B) Schematic representation of automated glycan assembly (AGA). (C) Automated glycan assembly of two linear LacNAc oligosaccharides. (D) Automated glycan assembly of branched LacNAc oligosaccharides. [a]Reagents and conditions: Glycosylation reactions were repeated twice. (a) Acidic wash and glycosylation-1; (b) Acidic wash and glycosylation-2; (c) Acidic wash and glycosylation-3; (d) Fmoc deprotection; (e) Nap deprotection; (f) UV cleavage (305 nm); (g) NaOMe, MeOH, 40 °C; (h) Pd/C, H_2, MeOH/ethyl acetate/AcOH (30:4:1).

2.2.3 Automated Glycan Assembly of Branched Hexasaccharide AGA-2-03

Hexasaccharide **AGA-2-03** was synthesized using resin **Resin-1** and building blocks **GlcNAc-1**, **Gal-01** and **Gal-02** (Table 2.01 and Scheme 2.01). The Nap ether-protected galactose **Gal-02** allowed for branching at the C-6 position. In addition to the three modules used for linear LacNAc synthesis, the Nap protecting group was removed using a DDQ solution in DCE, methanol and phosphate buffer to introduce disaccharide branching during the Nap deprotection module (**Scheme 2.01**).

Three possible approaches were conceived with three building blocks. The first approach (A) relies on the complete assembly of the linear tetrasaccharide, followed by the removal of a specific Nap (or Lev) protective group and linear chain extension to place the disaccharide branch. This approach required six glycosylation cycles and **14 steps on resin in total**. Alternatively (B), simultaneous growth of both branches by placement of monosaccharides to a disaccharide was able to be pursued. For that purpose two hydroxyls were liberated by Fmoc cleavage followed and Nap (or Lev) removal to set the stage for extension. This strategy utilized **four glycosylation cycles to complete bis-glycosylation** at C-4 in glucosamine **GlcNAc-01**. Ultimately (C), a new approach was devised using a modified glycosylation conditions that performed **glycosylation reaction with 7.5 equivalents of a donor twice to save glycosylating agents and reduce assembly time**.

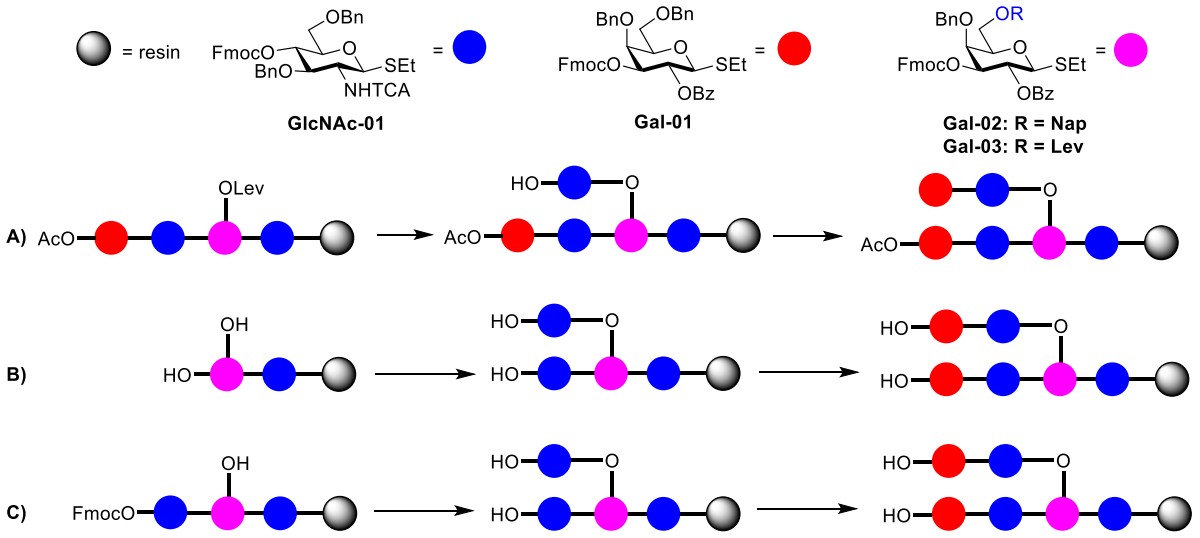


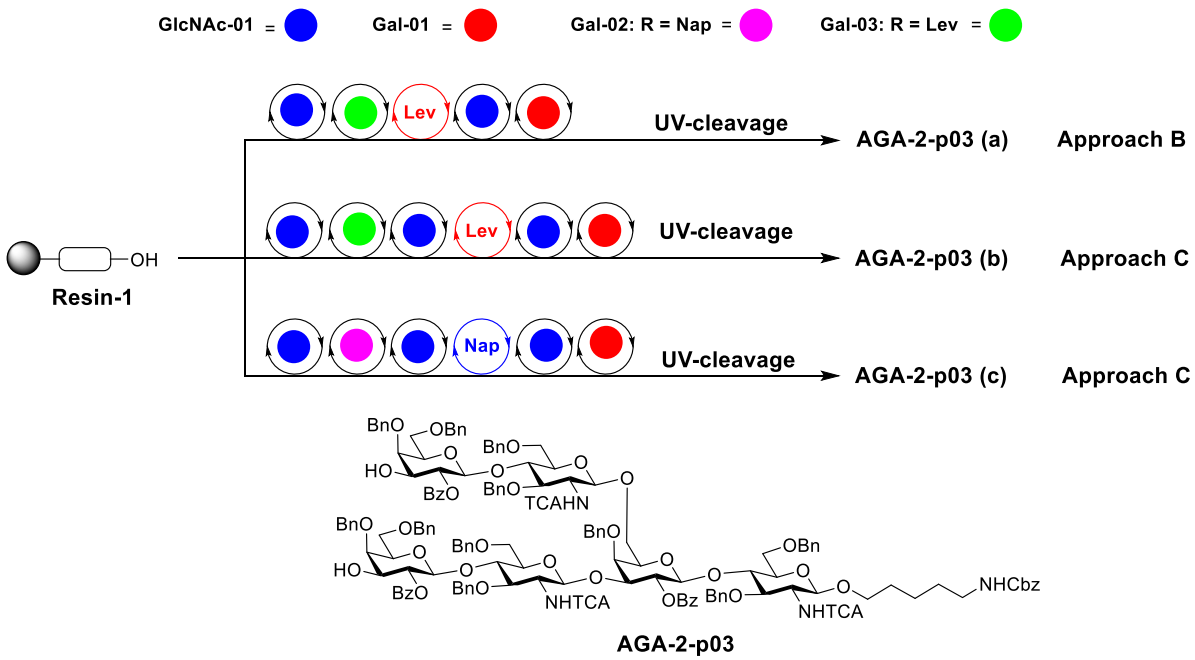
Figure 2.05. Three Approaches to Synthesize a Branched LacNAc Hexasaccharide**.** A) Step-wise approach; Following assembly of a tetrasaccharide backbone, branched disaccharide was elongated. B) Bis-glycosylation approach using 5 equiv. of building block four times. C) Bis-glycosylation approach using 7.5 equiv. of building block twice.

With regard to total reaction time and total consumption of building blocks, two approaches (**Figure 2.08** B and C) and two building blocks (**Gal-02** and **Gal-03**) were tested to assemble the branched hexasaccharide **AGA-2-03.** For this assembly, Lev and Nap deprotection modules were introduced to automated synthesis (Table 2.02). Lev deprotection removes a levulinoyl ester (Lev) using a solution of hydrazine in acetic acid and pyridine (v/v, 3/2) to allow for the other hydroxyl groups (module iv). Nap deprotection was performed to liberate the hydroxyl group to undergo glycosylation reaction using a homogeneous solution of DDQ in DCE, MeOH, and phosphate buffer (module v). Following AGA, the resulting oligosaccharide was released by UV irradiation.

Entry	Total Amount of Building Blocks		Glycosylation reactions	Total reaction time
	GluNAc-01 (equiv.)	**Gal-01(equiv.)**		
Approach A	30	20	12 times	360 min
Approach B	30	20	12 times	360 min
Approach C	30	**15**	**10** times	**300** min

Table 2.02. Comparison of three different approaches to assemble **AGA-2-03**.

To assemble the branched hexasaccharide following Approach B, the disaccharide LacNAc consisting of **GlcNAc-01** and **Gal-03** was initially assembled (**Figure 2.08**). For the bis-glycosylation, following Lev deprotection module, the glycosylation reactions, firstly using five equiv. of **GlcNAc-01** four times (4 x 5 eq. of **GlcNAc-01**) and five equiv. of **Gal-01** four times (4 x 5 eq. of **Gal-01**) were executed to afford the protected branched hexasaccharide **AGA-2-p03 (a)** (Scheme 2.02). To evaluate Approach C, a trisaccharide backbone consisting of **GluNAc-01** and **Gal-03** was substituted with glucosamine **GluNAc-01** at C-6 on galactose **Gal-03** to give a branched tetrasaccharide, and then a newly devised glycosylation module (2 x 7.5 eq. of **Gal-01**) afforded the desired compound **AGA-2-p03 (b)** (Scheme 2.02). Lastly, the branched hexasaccharide **AGA-2-p03 (c)** (Scheme 2.02) was assembled using the building blocks **Gal-02** instead of **Gal-03** by Approach C in order to investigate compatibility of Nap deprotection in this synthetic context (Scheme 2.02). Analysis of the crude hexasaccharide **AGA-2-p03** assembled by three different approaches using two respective building blocks (**Gal-02** and **Gal-03**) was indicated to be almost identical by analytical NP-HPLC (**Figure 2.06**). Removal of protecting groups provided the branched hexasaccharide **AGA-2-03** (5.3 mg, 4.4 μmol, 18% yield over 12 steps) through **AGA-2-p03 (c)**.



Scheme 2.02. Automated synthesis of linear and branched LanNAc oligosaccharides. Reactions and conditions: a) 2 x 5 equiv. building block **GlcNAc-01**, NIS, TfOH, DCM, -30 °C (5 min) → -10 °C (25 min); b) 2 x 5 equiv. building block **Gal-01, 02, and 03**, NIS, TfOH, DCM, -40 °C (5 min) → -20 °C (25 min); c) 2 x 7.5 equiv. building block **Gal-01**, NIS, TfOH, DCM, -40 °C (5 min) → -20°C (25 min); d) 3 x 20% triethylamine in DMF, 25 °C (5 min).

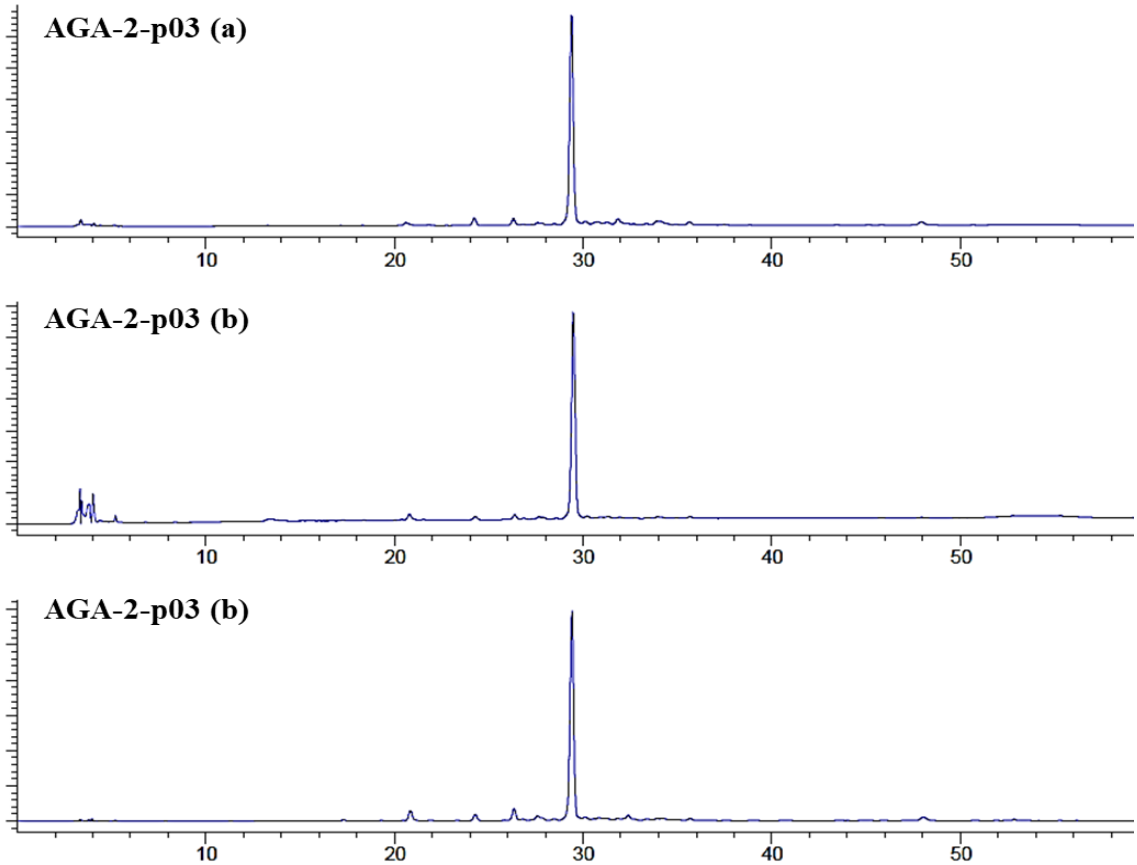


Figure 2.06. Analytical HPLC chromatogram of the crude protected hexasaccharides **AGA-2-03 (a), (b),** and **(c).**

2.2.4 Automated Glycan Assembly of Sulfated LacNAc Glycans AGA-2-04 – AGA-2-07.

Unlike prior synthesis of glycosaminoglycans by using each backbone to obtain each defined sulfated oligosaccharides,[80,88] KS oligosaccharides with defined sulfation patterns were obtained from the common tetrasaccharide backbone **AGA-common-Tet**, which harbors three orthogonal protecting groups (**Scheme 2.03**). Compound **AGA-common-Tet** was synthesized by AGA alternating building blocks **GluNAc-02** and **Gal-02**. After a cycle of an acidic wash glycosylation, Fmoc deprotection cycle, **AGA-common-Tet** was converted to four differentially sulfated tetrasaccharides (**AGA-2-p04 – AGA2-p07)** with a combination of four additional modules (**Scheme 2.03**). The capping module[84] was designed to block the free hydroxyl group when C-3 sulfation of Gal was not desired. Nap ethers or Lev esters were selectively removed with a homogeneous solution of DDQ[83] or hydrazine[84], respectively. The sulfation module[84] introduced sulfate groups at the defined positions. Compounds **AGA-2-p04 – AGA2-p07** were obtained after UV-mediated cleavage from the resin. Without further purification, **AGA-2-p04 (Figure 2.07)** and **AGA-2-p06** were subjected to methanolysis and hydrogenolysis to afford disulfated tetrasaccharides **AGA-2-04** (3.5 mg, 3.5 μmol, 14% over 14 steps) (**Figure 2.08**) and **AGA-2-06** (3.0 mg, 3.0 μmol, 12% over 14 steps), which were both purified by reverse phase HPLC using Hypercarb® columns. Protected trisulfated tetrasaccharide **AGA-2-p05** (21.9 mg, 10.1 μmol, 39% over eleven steps) and tetrasulfated tetrasaccharide **AGA-2-p07** (19.9 mg, 9.5 μmol, 38% over twelve steps) were both purified by preparative reverse phase HPLC. Deprotection of **AGA-2-p05** and **AGA-2-p07** afforded **AGA-2-05** (3.2 mg, 3.0 μmol, 30% over two steps) and **AGA-2-07** (2.8 mg, 2.4 μmol, 25% over two steps), respectively.

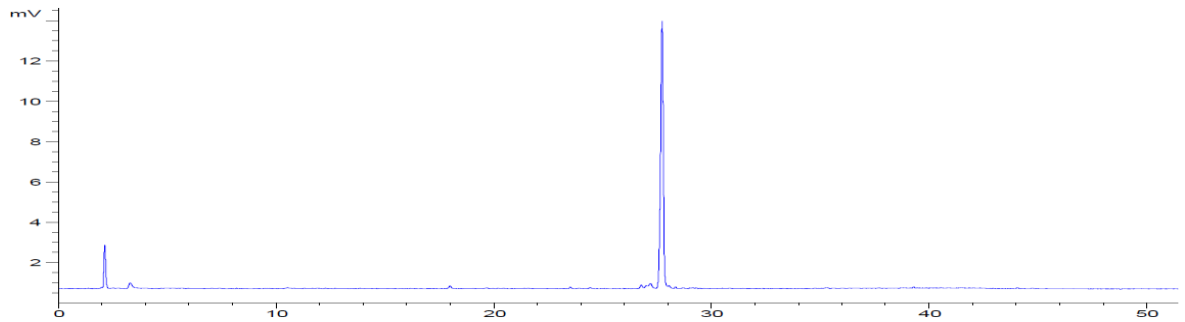
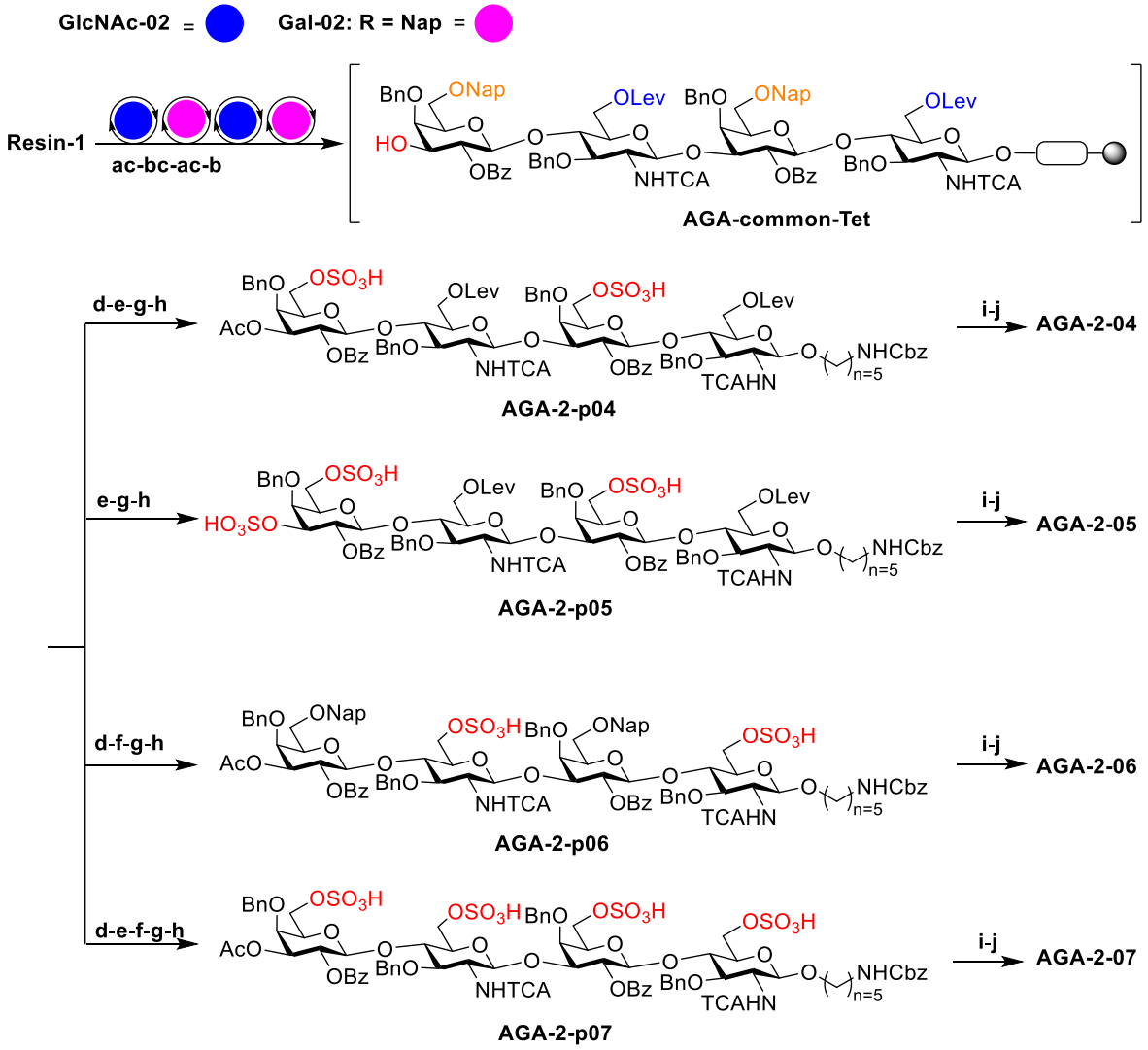


Figure 2.07. Analytical HPLC chromatogram of the crude tetrasaccharide **AGA-2-p04**



Scheme 2.03. Automated Glycan Assembly of Sulfated Oligo-*N*-Acetyllactosamines.[a] [a]Reagents and conditions: (a) 2 x 5 equiv of **GlcNAc-02**, NIS, TfOH, DCM/dioxane, -30 °C (5min) → -10 °C (25min); (b) 2 x 5 equiv of **Gal-02**, NIS, TfOH, DCM/dioxane, -40 °C (5min) → -20 °C (25min); (c) 3 x 20% triethylamine in DMF; (d) 3 x Ac_2O, pyridine; (e) 3 x 0.1 M DDQ in DCE/MeOH/Phosphate buffer (64:16:1) (30 min); (f) 3 x hydrazine hydrate ($NH_2NH_2 \cdot H_2O$), pyridine, AcOH, CH_2Cl_2, 25 °C (30 min); (g) 3 x SO_3·Pyridine, DMF/pyridine (1:1); (h) UV cleavage; (i) NaOMe, MeOH, 40 °C; (j) Pd/C, H_2, MeOH/TDW/AcOH (10:10:1).

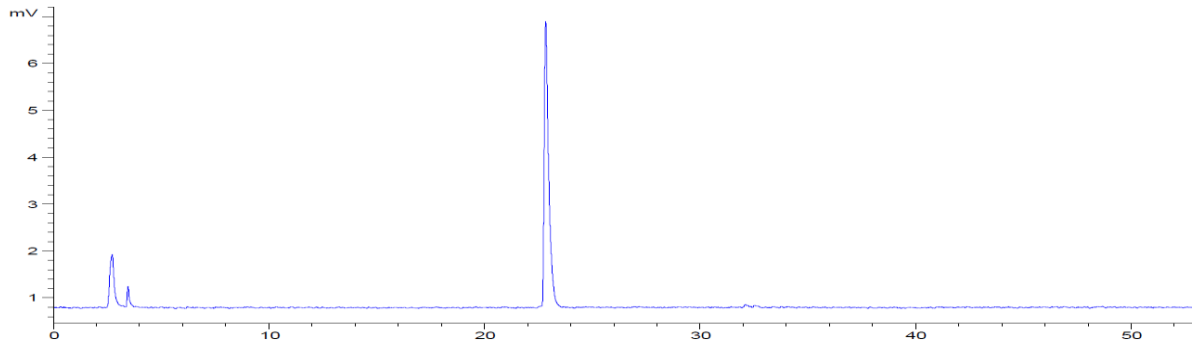


Figure 2.08. Analytical HPLC chromatogram of the crude tetrasaccharide **AGA-2-04**

The AGA approach described here allowed for the procurement of conjugation-ready sulfated KS tetrasaccharides within 3 days, including at most 16 h required for automated synthesis. AGA uses excess of glycosyl building block, which resulted in 2.5–3 hr for glycosylation and Fmoc deprotection in total. With the optimized protocol, protected oligosaccharides **AGA-2-p01, -p02, -p03, -p04** and **-p06** were obtained without any notable side products, as judged by analytical HPLC, indicating high conversion rates at each step during AGA. Minor impurities observed in crude protected oligosaccharides **AGA-2-p05** and **AGA-2-p07** were removed by reverse-phase HPLC (RP-HPLC) before global deprotection. Fully deprotected oligosaccharides were obtained with two HPLC purification steps at most. The syntheses of sulfated tetrasaccharides provided proof of concept that AGA is able to rapidly generate KS oligosaccharides with the most common sulfation patterns from two orthogonally protected monosaccharide building blocks. For future syntheses, it would be an option to scale up synthesis of the common intermediate **AGA-common-Tet**, followed by structural diversification on a smaller scale, which would contribute further to the rapid generation of sulfated oligosaccharide libraries. Additionally, different combinations of the four building blocks **GlcNAc-01, GlcNAc-02, Gal-01,** and **Gal-02** would allow for the synthesis of monosulfated tetrasaccharides (five possible sulfation patterns), disulfated tetrasaccharides (ten possible sulfation patterns), trisulfated tetrasaccharides (six possible sulfation patterns), and one pentasulfated tetrasaccharide via the described AGA strategy.

2.3 Interaction of LacNAc/KS Glycan with Adeno-associated Virus Particles

Custom glycan microarrays were fabricated by immobilizing RP-HPLC purified oligosaccharides **AGA-2-01 – AGA-2-07** via their primary amines on *N*-hydroxysuccinimide (NHS) ester-functionalized glass slides. The minor non-carbohydrate impurities from ion exchange and the resin observed in these preparations of **AGA-2-01 – AGA-2-07** were not expected to influence glycan binding signals. The microarrays were probed with fluorescence-labeled recombinant AAV particles representing serotypes AAV2, -7, -8, -rh10 and -12 to determine their binding specificities, as described.[76] AAVrh10 particles selectively and dose-dependently recognized KS oligosaccharide **AGA-2-06** (**Figure 2.09 A, B**). Binding to **AGA-2-06** was serotype-specific, as the other AAVs did not recognize this glycan (**Figure 2.09 C**). As observed before21, AAV2 bound to different heparin/heparan sulfate oligosaccharides. Successful spotting of LacNAc/KS glycans on the microarray slides was

qualitatively verified with the Bandeiraea (Griffonia) simplicifolia lectin-I (GS-I) that recognizes terminal Gal residues as present in 1-7.[89] The lectin bound to **AGA-2-01 – AGA-2-07** and appeared to favor the non-sulfated (**AGA-2-01 – AGA-2-03**) over the sulfated (**AGA-2-04 – AGA-2-07**) glycans. However, weaker binding signals observed for **AGA-2-04 – AGA-2-07** may also be due to less efficient immobilization of these glycans to the microarray surface.

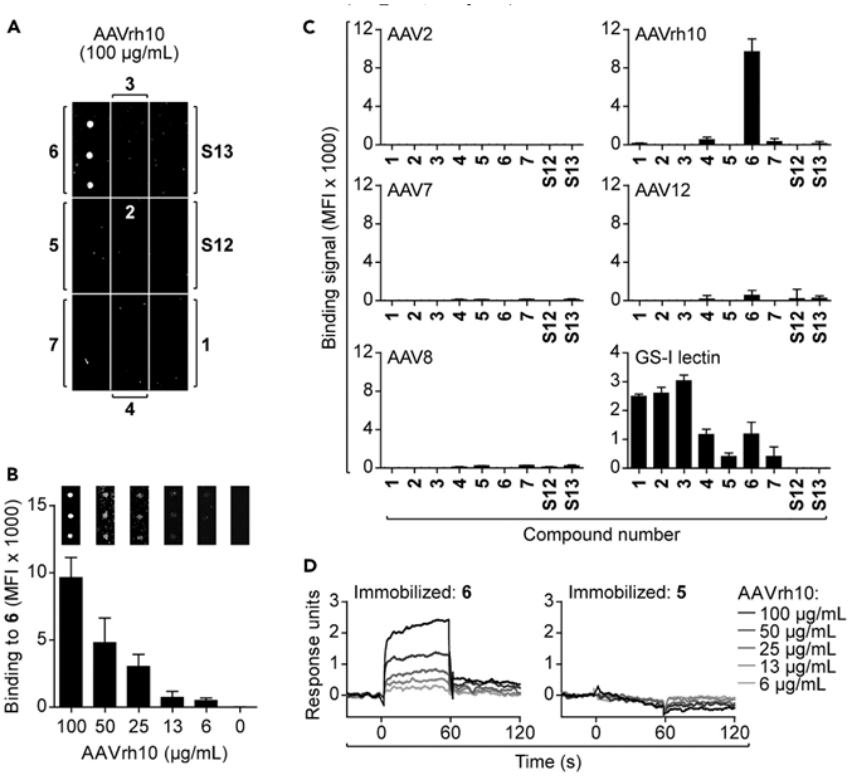


Figure 2.09. Binding patterns of AAV particles to AGA-2-01 – AGA-2-07 as determined by glycan microarray and SPR experiments. (A) Exemplary microarray scan of fluorescence-labeled AAVrh10 incubated at 100 µg/mL. The spotting pattern of **AGA-2-01 – AGA-2-07** and synthetic dermatan sulfate glycans[51]. Each compound was spotted in triplicate. Binding of AAVrh10 to **AGA-2-06** is seen as three white spots that represent fluorescence signals excited at 488 nm. (B) Binding signals of AAVrh10 to **6** as quantified by glycan microarray. Bars represent mean + SEM of mean fluorescence intensity (MFI) signals of six microarray spots. (C) Microarray-inferred binding signals of different AAV serotypes (100 µg/mL) and GS-I lectin (20 µg/mL) to the indicated glycans depicted as in panel (B). (D) SPR sensorgrams of non-labeled AAVrh10 that was passed at the indicated concentrations over sensor chip surfaces functionalized with either **AGA-2-06** (left) or **AGA-2-05** (right). The depicted sensorgrams are representative of two independent experiments with similar results. This experiment was performed by Dr. F. Bröcker.

The binding specificity of AAVrh10 observed by microarray was further studied by SPR using sensor chips functionalized with **AGA-2-05** or **AGA-2-06** (**Figure 2.09**). SPR has been used before to characterize interactions of virus particles with their receptor molecules.[90,91] Confirming the microarray studies, non-labeled AAVrh10 particles dose-

dependently recognized **AGA-2-06** but not **AGA-2-05** that differs from **AGA-2-06** only by its sulfation pattern.

2.4 Conclusion

We disclose the automated synthesis of LacNAc and KS glycans from monosaccharide building blocks bearing three orthogonal protecting groups. Automated glycan assembly provided access to diverse oligosaccharides with different sulfation patterns and varying length by sequential combination of building blocks **GlcNAc-01** and **-02 and Gal-01** and **-02,** and selective deprotection reactions followed by further chemical modification. Common tetrasaccharide **AGA-common-Tet** was transformed into four differently sulfated KS tetrasaccharides **AGA-2-03 – AGA-2-07** by orthogonal modifications. KS tetrasaccharide **AGA-2-06** was identified as specific interaction partner of AAVrh10 and represents a novel candidate glycan receptor of this virus. Of note, AAVrh10 has a distinct tropism for cells of the brain[92], a tissue that is known to express KS.[93] LacNAc/KS glycans, now available through AGA, will serve as molecular tools for investigating biological processes involving these structures. In a broader sense, this study highlights the power of AGA to provide access to versatile, conjugation-ready glycan probes suitable for biological interaction studies.

2.5 Experimental Part

2.5.1 Automation

Preparation of Reagent Solutions and Modules

Building Block Solution:

For the glycosylation using twice 5 equivalents, 0.25 mmol of building block was dissolved in 2.0 mL of CH_2Cl_2.

Acidic TMSOTf wash Solution

For the acidic TMSOTf wash 480 µL TMSOTf was dissolved in 20 mL CH_2Cl_2.

Activator Solution

For the thioglycoside monomer *N*-Iodosuccinimide (1.35 g) was dissolved in a 9:1 mixture of anhydrous CH_2Cl_2 and dioxane (40.0 mL) and then TfOH (60 µL) was added in ice bath.

Fmoc Deprotection Solution

Solution of 20% triethylamine in DMF (v/v) was prepared.

Nap deprotection Solution: 0.1 M of DDQ solution

For Nap deprotection, 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (454 mg, 2 mmol) was dissolved in 16 mL DCE, 4 mL MeOH and 0.25 mL phosphate buffer (pH 7.4).

Lev deprotection Solution: 0.56 M of hydrazine hydrate solution

For Lev deprotection a 0.56 M solution of hydrazine hydrate (0.68 mL) was dissolved in pyridine/acetic acid (25 mL, v/v, 3:2).

Acetylation capping Solution

Ac_2O was directly used.

Sulfation Solution: 0.5 M of sulfur trioxide pyridine complex in DMF/pyridine

For sulfation sulfur trioxide pyridine complex (1.6 g) was dissolved in DMF/pyridine (20 mL, v/v, 1:1).

Preparation of the resin and the synthesizer for automated synthesis: The functionalized resin was loaded into the reaction vessel of the synthesizer and swollen in 2 mL CH_2Cl_2. To start the synthesis sequence, the resin was washed using Module 1. The building blocks were co-evaporated with toluene three times, dissolved in DCM under an argon atmosphere and transferred into the vials that were placed on the corresponding port in the synthesizer. Reagents were dissolved in the corresponding solvents under an Ar atmosphere in bottles that were placed on the corresponding port in the synthesizer. Activation temperature ($\mathbf{T_a}$) and time ($\mathbf{t_1}$), incubation temperature ($\mathbf{T_i}$) and time ($\mathbf{t_2}$) were used.

Module I – Acidic TMSOTf Wash: The resin is washed with DMF, THF, DCM (three times each, with 2 mL for 10 s), and 0.350 mL of solution of TMSOTf in DCM for 1 minute at -20 °C. The resin is swollen in 2 mL DCM and the temperature of the reaction vessel is adjusted to $\mathbf{T_1}$.

Glycosylation conditions for building blocks.

Building block	Glycosylation conditions	
	Activation	Incubation
GlcNAc-01	5 min at – 30 °C	25 min at – 10 °C
GlcNAc-02		
Gal-01	5 min at – 40 °C	25 min at – 20 °C
Gal-02		
Gal-03		

Module II – Glycosylation using thioglycoside: For the glycosylation the DCM is drained and a solution of thioglycoside building block (BB) (5 eq. in 1.0 mL DCM) is delivered to the reaction vessel. After the set temperature is reached ($\mathbf{T_a}$), the reaction starts with the addition of 1 mL of activator solution. The glycosylation is performed at $\mathbf{T_a}$ for 5 min and at $\mathbf{T_i}$ for 25min. After the reaction the solution is drained and the resin is washed with DCM (six times with 2 mL for 15 s). This procedure is repeated twice.

Module III- Glycosylation (2 x 7.5 equivalents of the donor): For glycosylation the DCM is drained and a solution of thioglycoside **Gal-01** (**7.5 eq.** in 1.0 mL DCM) is delivered to the reaction vessel. After the set temperature is reached ($\mathbf{T_a}$), the reaction starts with the addition of **1.5 mL** of activator solution. The glycosylation is performed $\mathbf{T_a}$ for 5 min and at $\mathbf{T_i}$ for

25min. After the reaction the solution is drained and the resin is washed with DCM (six times with 2 mL for 15 s). This procedure is repeated twice.

Module IV - Fmoc Deprotection: The resin is washed with DMF (six times with 2 mL for 15 s), swollen in 2 mL DMF and the temperature of the reaction vessel is adjusted to 25°C. For Fmoc deprotection the DMF is drained and 3 mL of a solution of 20% Et_3N in DMF is delivered to the reaction vessel. After 5 min the reaction solution is collected in the fraction collector of the oligosaccharide synthesizer and 2 mL of a solution of 20% Et_3N in DMF is delivered to the resin. This procedure is repeated two times.

Module V – Lev deprotection: The resin is washed with DCM (six times with 2 mL for 25 s), swollen in 1.3 mL DCM and the temperature of the reaction vessel is adjusted to 25 °C. For Lev deprotection 0.8 mL of the hydrazine hydrate solution is delivered into the reaction vessel. After 30 min the reaction solution is drained and the resin is washed with 0.2 M acetic acid in DCM and DCM (six times each with 2 mL for 25 s). The entire procedure is performed three times.

Module VI – Nap deprotection: The resin is washed with DCM (six times with 2 mL for 15 s), swollen in 1.0 mL DCM and the temperature of the reaction vessel is adjusted to 25 °C. For Nap deprotection 2.0 mL of the DDQ solution is delivered. After 25 min the reaction solution is drained and the resin is washed with THF, DMF, and DCM (six times each with 2 mL for 15 s). The entire procedure is performed three times.

Module VII – Acetylation: The resin was washed pyridine (six times each with 2 mL for 15 s), swollen in 2 mL pyridine. The temperature of the reaction vessel was adjusted to 25 °C. The reaction was started by addition of 1 mL of acetic anhydride to the reaction vessel. After 30 min, the reaction solution was drained and the resin was washed with CH_2Cl_2 and pyridine (six times with 2 mL for 15 s). This acetylation procedure is performed three times.

Module VIII – Sulfation: The resin is washed with DMF and pyridine (three times each with 2 mL for 15 s), swollen in 2 mL pyridine and the temperature of the reaction vessel is adjusted to 50 °C. For sulfation, 2 mL of a 0.5 M solution of sulfur trioxide pyridine complex in DMF/pyridine, 1:1 is added. After 3 h, the reaction solution is drained and the resin is

washed with DMF and pyridine (three times each with 2 mL for 15 s). The entire procedure is performed for three times.

2.5.2 Post-Automation Steps

Cleavage and Purification

Resin Cleavage: To prepare the photoreactor, the FEP tubing is washed with 20 mL DCM using a flow rate of 5 mL/min. For the cleavage, the resin is slowly injected from the disposable syringe (20 mL) into the reactor and pushed through the tubing with 18 mL DCM (flow rate: 600 µL/min). The tubing is washed with 20 mL DCM (flow rate: 2 mL/min) to remove any remaining resin. The suspension leaving the reactor is directed into a filter where the resin is filtered off. The tubing is re-equilibrated with 20 mL DCM using a flow rate of 5 mL/min. The entire procedure is performed twice. The resulting solution is evaporated and the crude material was analyzed by NMR and HPLC

HPLC Conditions for linear and branched LacNAc structures:

For protected oligosaccharides AGA-2-p01, AGA-2-p02, and AGA-2-p03

Analytical NP-HPLC: The crude material was analyzed by HPLC (column: Luna 5µ Silica 100A, (260 X 4.60 mm); flow rate: 1 mL/min; eluents: 5% DCM in hexane / 5% DCM in EtOAc; gradient: 20% (5 min) 60% (in 40 min) 100% (in 5 min); detection: ELSD).

Deprotection Conditions: To a solution of the fully protected oligosaccharide **4** in MeOH (5 mL) was added 58 µL of 0.5 M NaOMe solution (0.25 eq. per acetyl or benzoyl group) in MeOH at 40 °C. The mixture is stirred until completed, then neutralized by 200 mg of Amberite (400 mg per 100 µL of NaOMe solution) after completion of the reaction. This crude mixture is dissolved in MeOH, EtOAc, and AcOH (v/v/v =5:0.5:0.2) added 5% Pd/C (W/V), purged first with argon and then with hydrogen, left to stir overnight at room temperature under balloon pressure. The reaction mixture was filtered through modified cellulose filter, washed with 20 mL of Water/MeOH, 9:1 and the combined solution was evaporated to provide the crude.

For protected oligosaccharides AGA-2-01, AGA-2-02, and AGA-2-03

Analytical RP-HPLC: The crude material was analyzed by HPLC (column: Hypercarb®, (150 X 4.60 mm); flow rate: 0.8 mL/min; eluents: 0.1% FA in Acetonitrile / 0.1% FA in TDW; gradient: 0% (10 min) 30% (in 30 min) 100% (in 5 min); detection: ELSD).

Preparative HPLC: The crude solution is purified by preparative HPLC (column: Hypercarb®, (150 X 10.00 mm); flow rate: 3.6 mL/min; eluents: 0.1% FA in Acetonitrile / 0.1% FA in TDW; gradient: 0% (10 min) 30% (in 30 min) 100% (in 5 min); detection: ELSD) to afford the unprotected oligosaccharide.

HPLC Conditions for sulfated LacNAc structures

For fully protected sulfated tetrasaccharide AGA-2-p04, AGA-2-p05, AGA-2-p06, and AGA-2-p07

Analytical RP-HPLC: The crude material was analyzed by HPLC (column: C18-Nucleodur (21x250 mm; 5 μm); flow rate: 1.0 mL/min; eluents: 3% isopropanol in Acetonitrile / 3% isopropanol in 0.01 $_M$ NH_4HCO_3 in TDW; gradient: 0% (10 min) 30% (in 30 min) 100% (in 5 min); detection: ELSD).

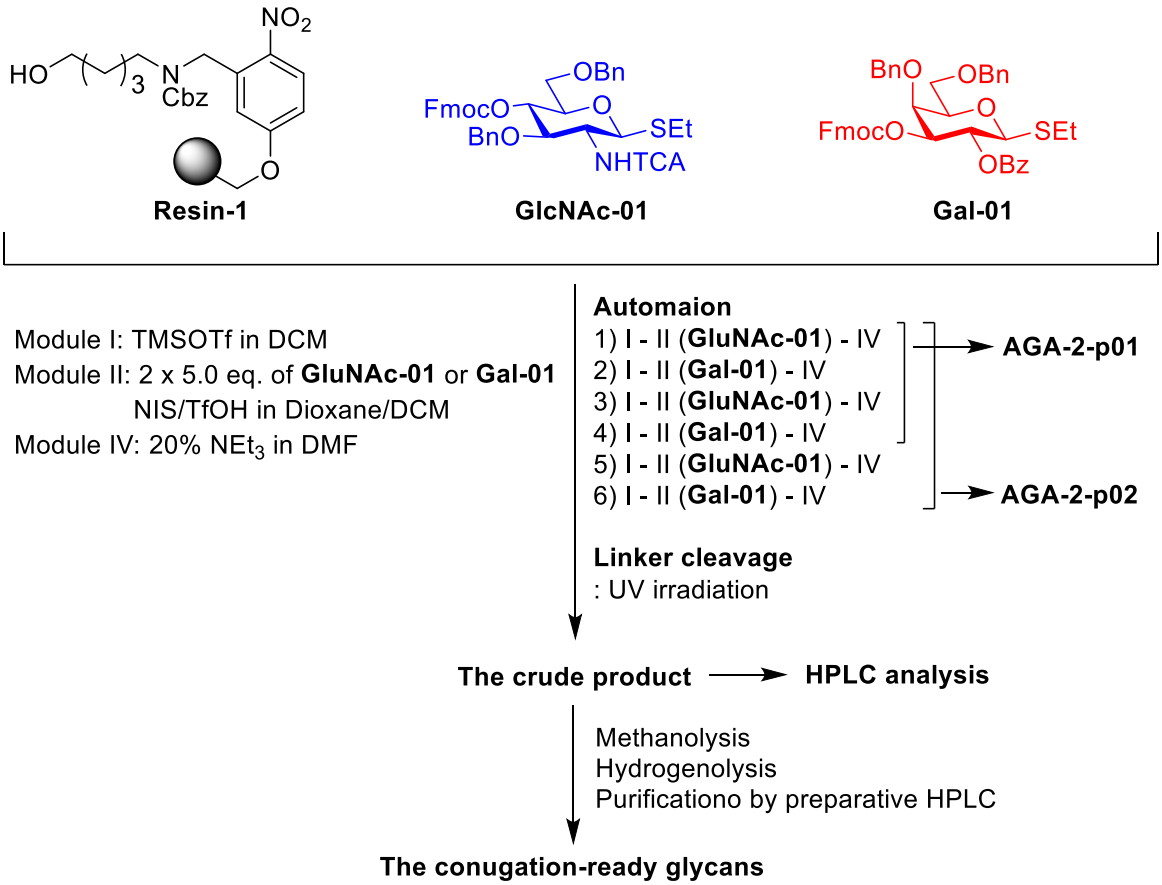
Preparative RP-HPLC: The crude material was analyzed by HPLC (column: C18-Nucleodur (21x250 mm; 5 μm); flow rate: 10.0 mL/min; eluents: 3% isopropanol in Acetonitrile / 3% isopropanol in 0.01 $_M$ NH_4HCO_3 in TDW; gradient: 0% (10 min) 30% (in 30 min) 100% (in 5 min); detection: ELSD).

For the conjugated-ready sulfated tetrasaccharide AGA-2-04, AGA-2-05, AGA-2-06, and AGA-2-07

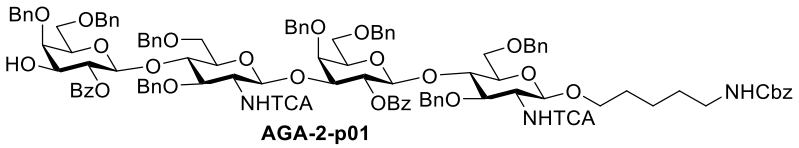
Analytical RP-HPLC: The crude material was analyzed by HPLC (column: Hypercarb®, (150 X 4.60 mm); flow rate: 0.8 mL/min; eluents: Acetonitrile / 0.01 M NH_4HCO_3 in TDW; gradient: 0% (10 min) 30% (in 30 min) 100% (in 5 min); detection: ELSD).

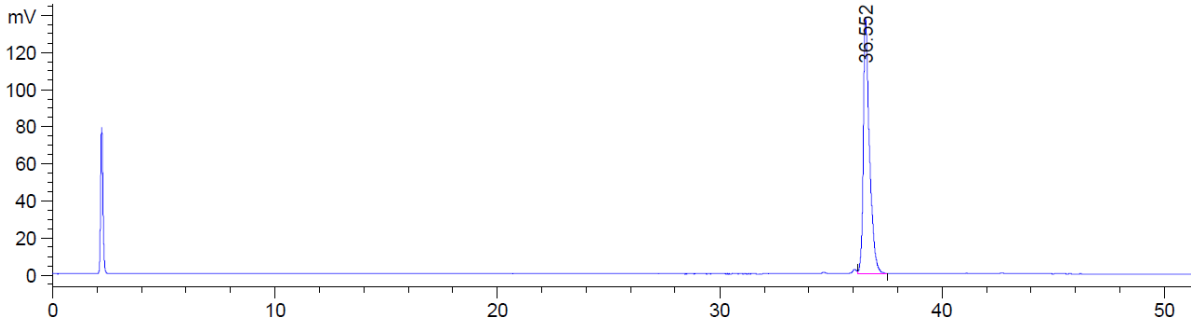
Preparative RP-HPLC: The crude solution is purified by preparative HPLC (column: Hypercarb®, (150 X 10.00 mm); flow rate: 3.6 mL/min; eluents: Acetonitrile / 0.01 M NH_4HCO_3 in TDW; gradient: 0% (10 min) 30% (in 30 min) 100% (in 5 min); detection: ELSD) to afford the unprotected sulfated tetrasaccharides. The collected solution was passed through Dowex-50WX8 resin (Na^+ form), then lyophilized.

2.5.3 Automated Synthesis of Linear LacNAc



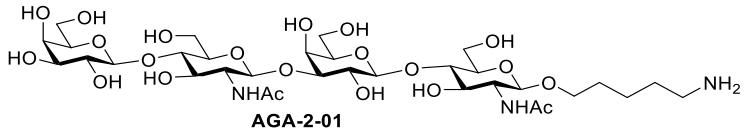
***N*-Benzyloxycarbonyl-5-amino-pentyl (2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-(3,6-di-*O*-benzyl-2-deoxy-2-trichloracetamido-β-D-glucopyranosyl)-(1→3)-(2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)- 3,6-di-*O*-benzyl-2-deoxy-2-trichloracetamido-β-D-glucopyranoside AGA-2-p01**



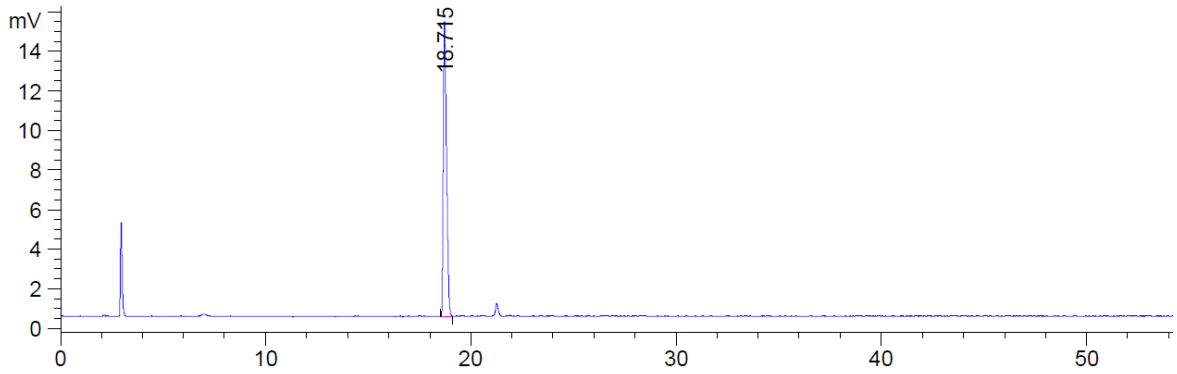


LC-MS Spectrum of AGA-2-p01.

5-Amino-pentyl β-D-galactopyranosyl-(1→4)-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-acetamido-2-deoxy-β-D-glucopyranoside AGA-2-01



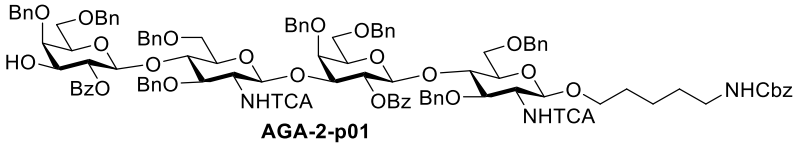
AGA-2-01



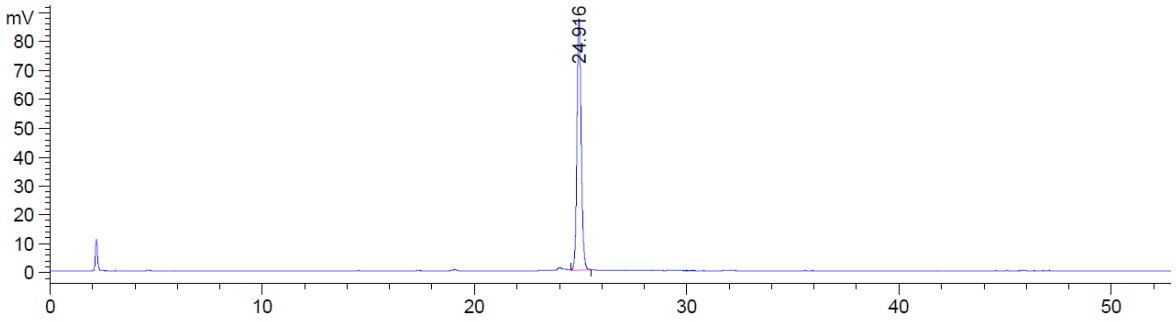
LC-MS Spectrum of AGA-2-01.

^{1}H NMR (600 MHz, D_2O) δ 4.72 (d, *J* = 8.4 Hz, 1H, **H-1**), 4.54 (d, *J* = 7.5 Hz, 1H, **H-1**), 4.50 (d, *J* = 7.9 Hz, 1H, **H-1**), 4.48 (d, *J* = 8.0 Hz, 1H, **H-1**), 4.17 (d, *J* = 2.8 Hz, 1H), 4.02 – 3.90 (m, 4H), 3.78 (dt, *J* = 31.4, 13.7, 11.1, 3.9 Hz, 17H), 3.64 – 3.54 (m, 5H), 3.02 – 2.98 (m, 2H), 2.05 (s, 6H), 1.72 – 1.66 (m, 2H), 1.61 (dt, *J* = 13.5, 6.6 Hz, 2H), 1.45 – 1.37 (m, 2H ^{13}C NMR (150 MHz, D_2O) δ 177.49 (NHAc), 177.01 (NHAc), 173.62 (Formic acid), 105.51 (**C-1**), 105.47 (**C-1**), 105.34 (**C-1**), 103.70 (**C-1**), 84.69, 81.13, 80.79, 77.96, 77.49, 77.36, 77.16, 75.12, 75.01, 74.79, 73.57, 72.70, 72.55, 71.15, 70.90, 63.63, 63.55, 62.68, 62.48, 57.80, 57.66, 41.94, 30.68, 28.98, 24.79, 24.76, 24.72.; MS ESI+-HRMS *m/z* $[M+H]^+$ calcd for $C_{38}H_{60}N_3O_{21}$ 834.3714, found 834.3722.

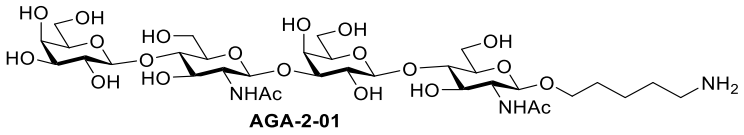
***N*-Benzyloxycarbonyl-5-amino-pentyl (2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-(3,6-di-*O*-benzyl-2-deoxy-2-trichloracetamido-β-D-glucopyranosyl)-(1→3)-(2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-(3,6-di-*O*-benzyl-2-deoxy-2-trichloracetamido-β-D-glucopyranosyl)-(1→3)-(2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-trichloracetamido-β-D-glucopyranoside AGA-2-p02**



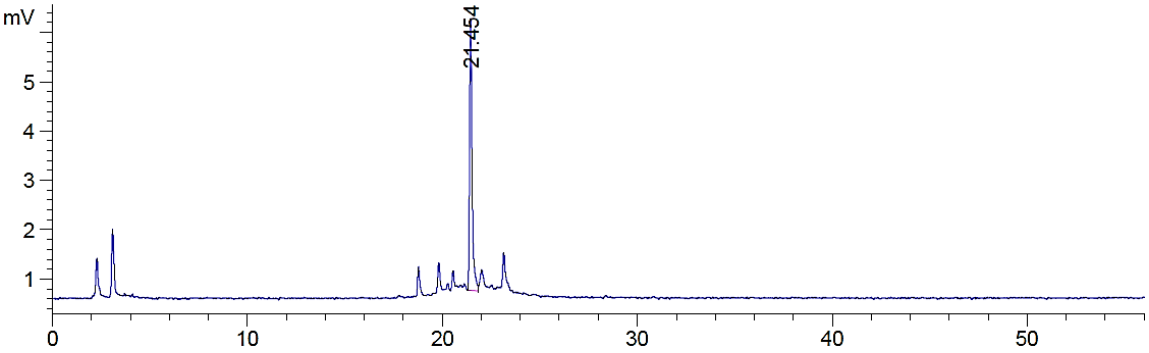
AGA-2-p01



LC-MS Spectrum of AGA-2-p02.



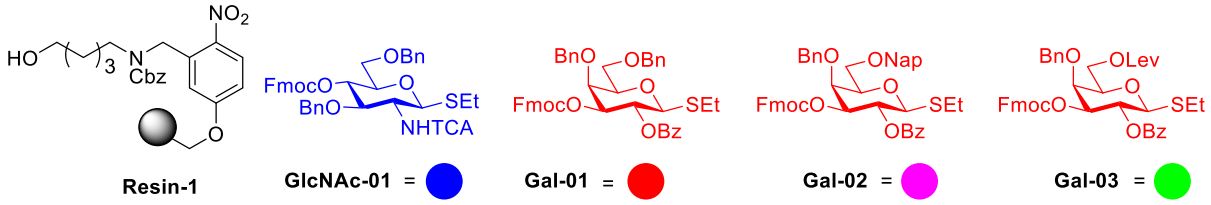
5-Amino-pentyl β-D-galactopyranosyl-(1→4)-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-acetamido-2-deoxy-β-D-glucopyranoside AGA-2-02



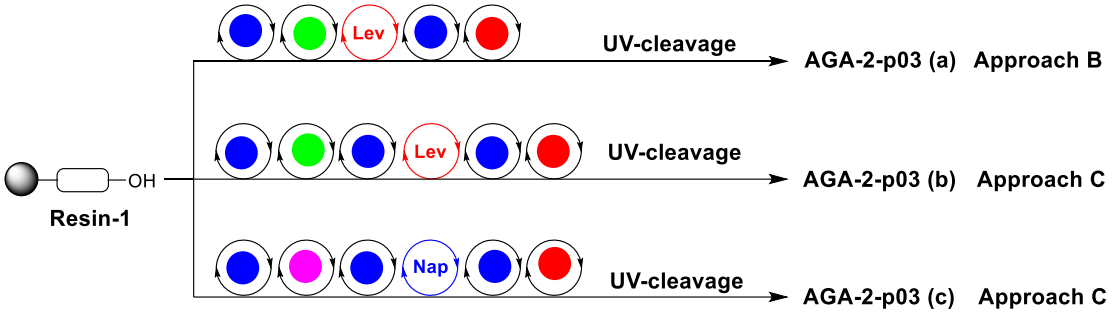
LC-MS Spectrum of AGA-2-02

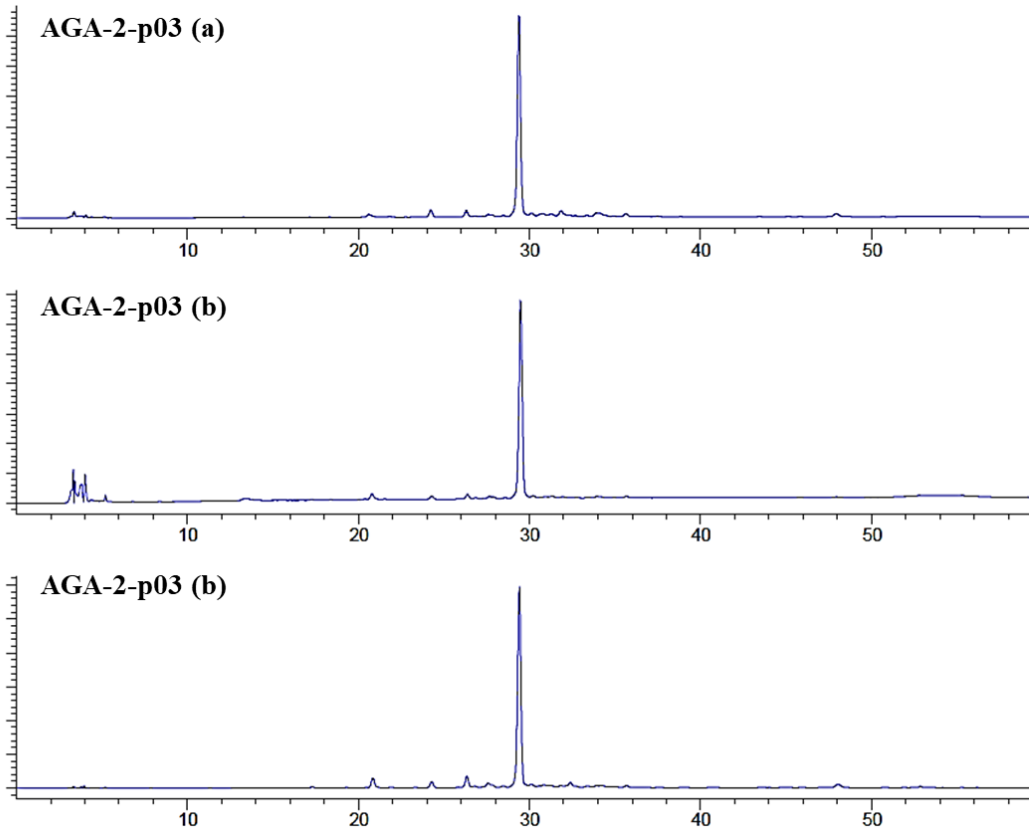
^{1}H NMR (600 MHz, D_2O) δ 4.57 (d, *J* = 8.1 Hz, 2H, 2 x **H-1**), 4.39 (d, *J* = 7.3 Hz, 1H, **H-1**), 4.34 (dd, *J* = 13.3, 6.8 Hz, 3H, 3 x **H-1**), 4.03 (s, 2H), 3.89 – 3.38 (m, 36H), 2.86 (t, *J* = 7.4 Hz, 2H), 1.91 (s, 9H), 1.58 – 1.51 (m, 2H), 1.50 – 1.42 (m, 2H), 1.31 – 1.22 (m, 2H). ^{13}C NMR (150 MHz, D_2O) δ 177.50 (2 x NHAc), 177.01 (NHAc), 173.63 (Formic acid), 105.50 (3 x **C-1**), 105.47 (**C-1**), 105.35 (**C-1**), 103.71 (**C-1**), 84.68, 81.12, 80.80, 77.96, 77.48, 77.36, 77.16, 75.12, 75.01, 74.79, 73.57, 72.71, 72.56, 71.15, 70.91, 63.63, 63.55, 62.67, 62.47, 57.80, 57.67, 41.94, 30.68, 28.99, 24.78.; MS ESI+-HRMS *m/z* [M+H]+ calcd for $C_{47}H_{84}N_4O_{31}$ 1199.5036, found 1199.4991.

Automated Synthesis of Branched LacNAc AGA-2-03



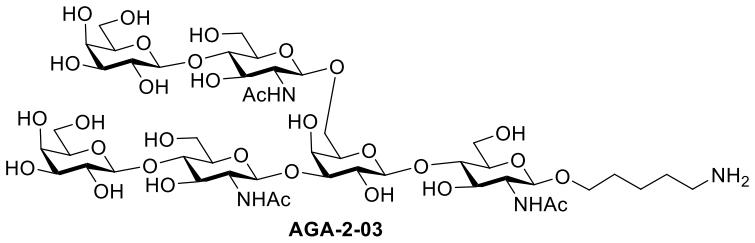
***N*-Benzyloxycarbonyl-5-amino-pentyl (2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-(3,6-di-*O*-benzyl-2-deoxy-2-trichloracetamido-β-D-glucopyranosyl)-(1→3)-[(2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-(3,6-di-*O*-benzyl-2-deoxy-2-trichloracetamido-β-D-glucopyranosyl)-(1→6)]-(2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)- 3,6-di-*O*-benzyl-2-deoxy-2-trichloracetamido-β-D-glucopyranoside AGA-2-p03**



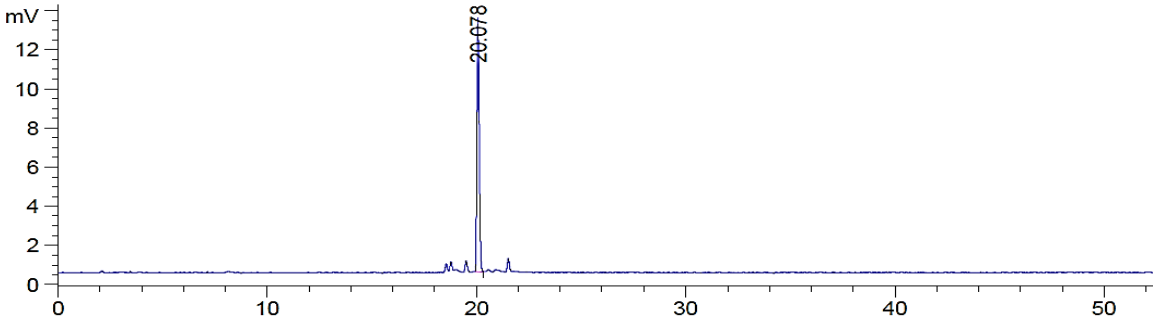


LC-MS Spectra of AGA-2-p03 (a), (b), and (c)

5-Amino-pentyl β-D-galactopyranosyl-(1→4)-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[β-D-galactopyranosyl-(1→4)-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)]-β-D-galactopyranosyl-(1→4)-acetamido-2-deoxy-β-D-glucopyranoside AGA-2-03



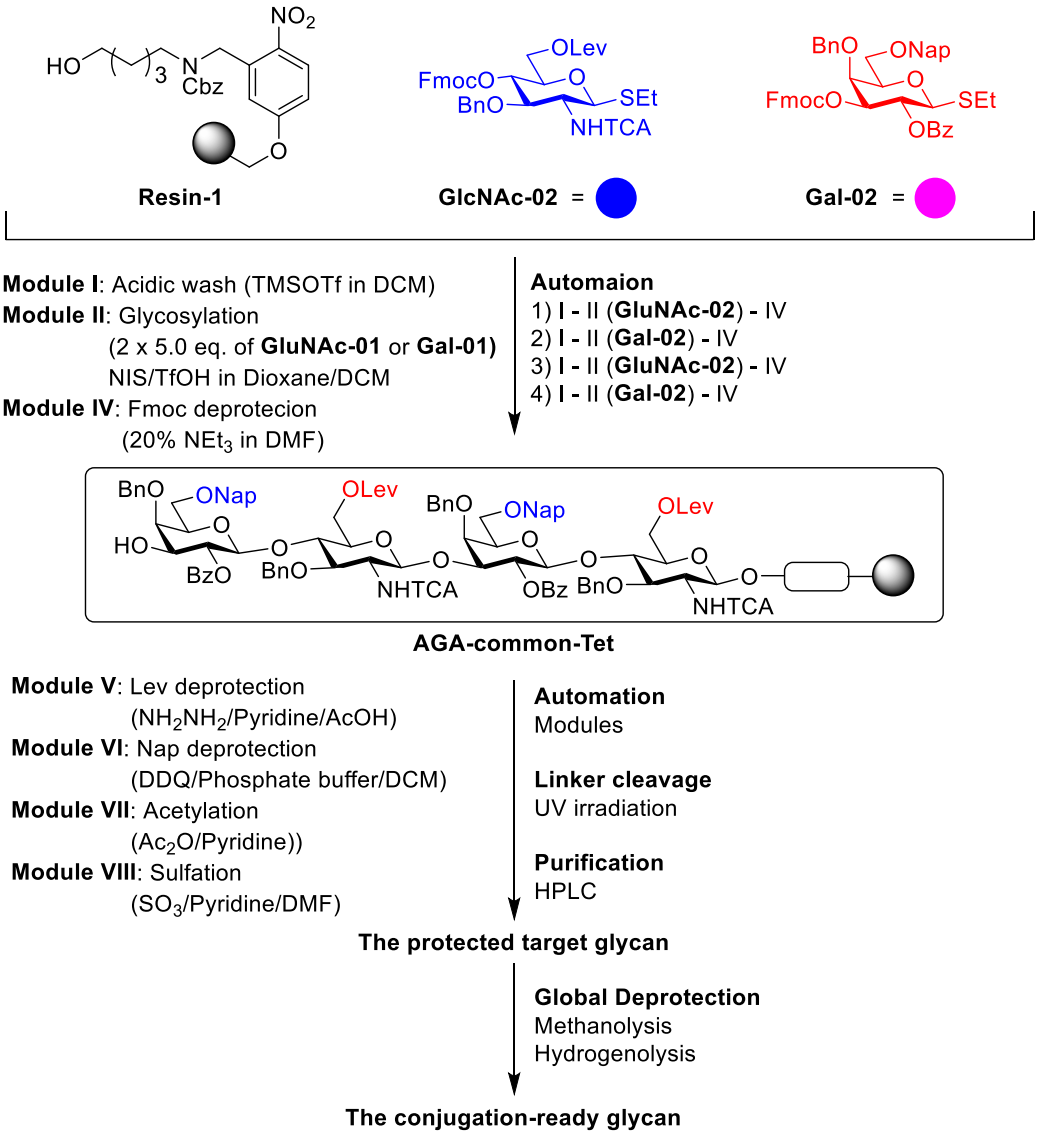
AGA-2-03



LC-MS Spectrum of AGA-2-03.

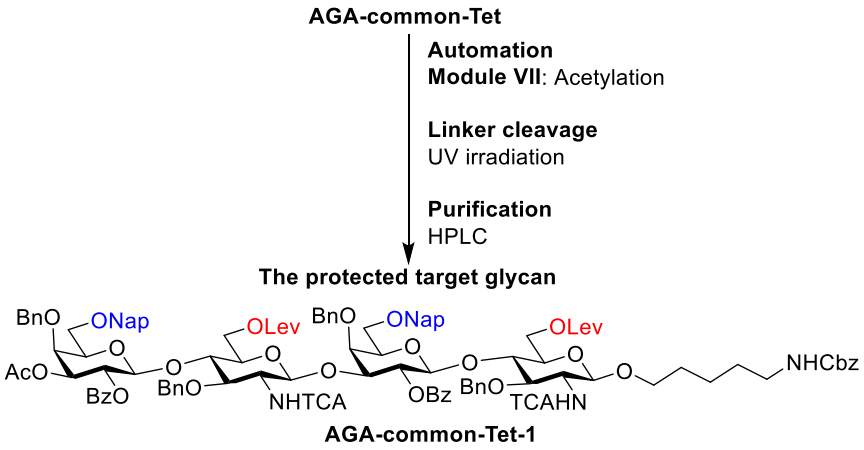
^{1}H NMR (600 MHz, D_2O) δ 4.72 (d, *J* = 8.4 Hz, 1H, **H-1**), 4.63 (d, *J* = 7.8 Hz, 1H, **H-1**), 4.54 (d, *J* = 8.2 Hz, 1H, **H-1**), 4.51 – 4.47 (m, 3H, 3 x **H-1**), 4.17 (d, *J* = 3.0 Hz, 1H), 4.02 – 3.90 (m, 7H), 3.89 – 3.54 (m, 28H), 3.01 (t, *J* = 7.6 Hz, 2H), 2.08 (s, 3H), 2.05 (d, *J* = 4.0 Hz, 6H), 1.69 (dt, *J* = 15.4, 7.7 Hz, 2H), 1.65 – 1.59 (m, 2H), 1.46 – 1.38 (m, 2H). ^{13}C NMR (150 MHz, D_2O) δ 177.47 (NHAc), 177.07 (NHAc), 176.90 (NHAc), 171.67 (formic acid), 105.53 (**C-1**), 105.47 (2 x **C-1**), 105.32 (**C-1**), 103.71 (**C-1**), 103.50 (**C-1**), 84.42, 81.60, 80.97, 80.77, 77.95, 77.28, 77.16, 76.32, 75.07, 74.95, 74.78, 73.57, 72.71, 72.41, 71.26, 71.15, 63.62, 62.59, 57.81, 57.64, 41.94, 30.68, 28.98, 25.03, 24.78, 24.72.; MS ESI+-HRMS *m/z* [M+Na]+ calcd for $C_{47}H_{83}N_4O_{31}Na$ 1221.4855, found 1221.4836.

2.5.4 Automated Synthesis of Sulfated LacNAc/KS Oligosaccharides

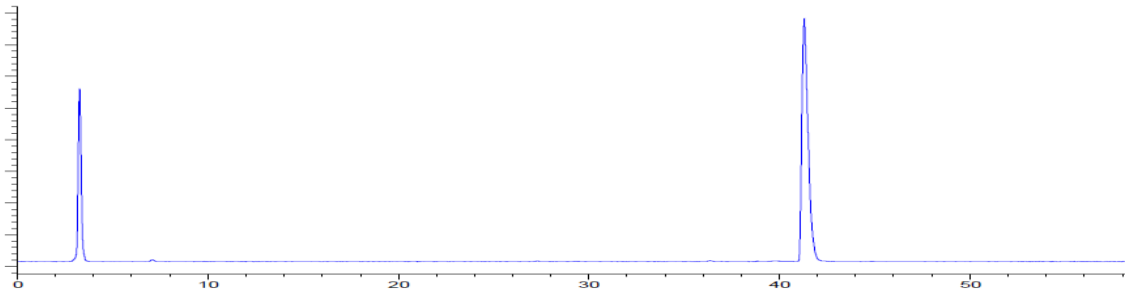


General Work Flow of Sulfated LacNAcs

***N*-Benzyloxycarbonyl-5-amino-pentyl 3-*O*-acetyl-2-*O*-benzoyl-4-*O*-benzyl-6-*O*-(2-methylnaphthyl)-β-D-galactopyranosyl-(1→4)-3-*O*-benzyl-6-*O*-levulinyl-2-deoxy-2-trichloracetamido-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-6-*O*-(2-methylnaphthyl)-β-D-galactopyranosyl-(1→4)-3-*O*-benzyl-6-*O*-levulinyl-2-deoxy-2-trichloracetamido-β-D-glucopyranoside AGA-common-Tet-1**

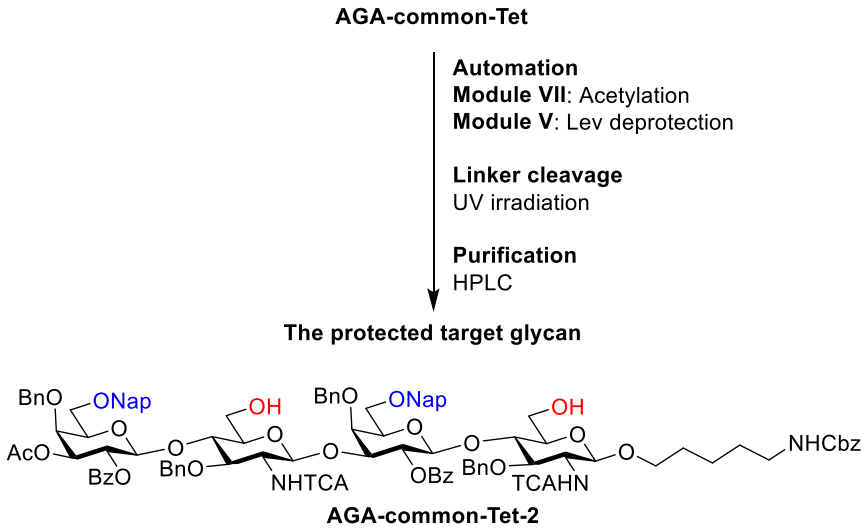


Automated glycan assembly of tetrasaccharides AGA-common-Tet-1



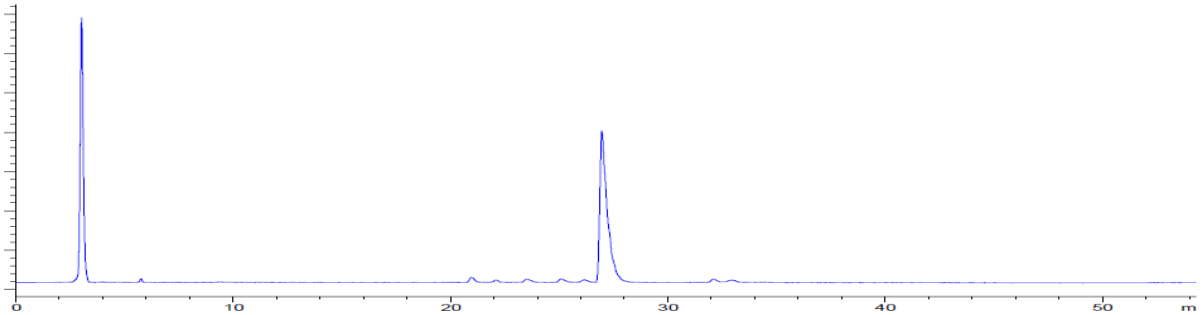
Analytical HPLC chromatogram of the crude tetrasaccharide AGA-common-Tet-1.

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.05 (dd, *J* = 23.0, 7.5 Hz, 4H), 7.78 (ddd, *J* = 12.0, 9.3, 3.5 Hz, 6H), 7.63 – 7.42 (m, 13H), 7.35 – 7.00 (m, 26H), 6.58 (d, *J* = 8.1 Hz, 1H), 5.62 (dd, *J* = 10.3, 7.9 Hz, 1H), 5.55 (dd, *J* = 9.9, 8.1 Hz, 1H), 5.29 (dd, *J* = 10.4, 3.0 Hz, 1H), 5.06 (s, 2H), 4.95 (dd, *J* = 11.0, 4.8 Hz, 2H), 4.85 (t, *J* = 8.8 Hz, 3H), 4.77 – 4.68 (m, 2H), 4.61 – 4.42 (m, 9H), 4.32 (dd, *J* = 12.0, 3.5 Hz, 2H), 4.25 (dd, *J* = 11.9, 3.8 Hz, 1H), 4.19 – 3.99 (m, 6H), 3.91 – 3.75 (m, 5H), 3.71 (t, *J* = 6.1 Hz, 1H), 3.69 – 3.55 (m, 3H), 3.52 – 3.45 (m, 1H), 3.44 – 3.32 (m, 4H), 3.29 (dd, *J* = 9.1, 5.3 Hz, 1H), 3.18 (dd, *J* = 15.7, 6.5 Hz, 1H), 3.11 (dd, *J* = 12.8, 6.3 Hz, 2H), 2.75 – 2.56 (m, 2H), 2.52 – 2.28 (m, 5H), 2.26 – 2.16 (m, 1H), 2.06 (s, 3H), 1.98 (s, 3H), 1.87 (s, 3H), 1.50 – 1.37 (m, 4H), 1.33 – 1.20 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 206.62, 206.56, 172.36, 170.40, 165.28, 165.14, 161.90, 161.81, 156.50, 138.96, 138.21, 136.76, 135.68, 135.39, 133.53, 133.29, 133.08, 130.17, 130.09, 129.52, 129.35, 128.80, 128.75, 128.61, 128.40, 128.25, 128.21, 128.09, 127.99, 127.80, 127.50, 127.33, 126.73, 126.64, 126.26, 126.06, 125.95, 100.91, 100.49, 99.60, 92.56, 92.09, 79.18, 77.85, 77.68, 75.86, 75.20, 75.11, 74.70, 74.49, 74.09, 73.68, 73.56, 73.45, 73.37, 73.24, 72.40, 70.99, 69.71, 68.73, 67.62, 66.65, 63.04, 62.32, 57.76, 56.76, 41.02, 37.82, 37.75, 29.83, 29.78, 29.64, 29.00, 27.82, 27.76, 23.28, 20.90.; MS ESI+-HRMS *m/z* $[M+Na]^+$ calcd for $C_{117}H_{121}N_3O_{30}Na$ 2280.6058, found 2280.6069.



Automated glycan assembly of tetrasaccharides AGA-common-Tet-2

***N*-Benzyloxycarbonyl-5-amino-pentyl 3-*O*-acetyl-2-*O*-benzoyl-4-*O*-benzyl-6-*O*-(2-methylnaphthyl)-β-D-galactopyranosyl-(1→4)-3-*O*-benzyl-2-deoxy-2-trichloracetamido-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-6-*O*-(2-methylnaphthyl)-β-D-galactopyranosyl-(1→4)-3-*O*-benzyl-2-deoxy-2-trichloracetamido-β-D-glucopyranoside AGA-common-Tet-2**

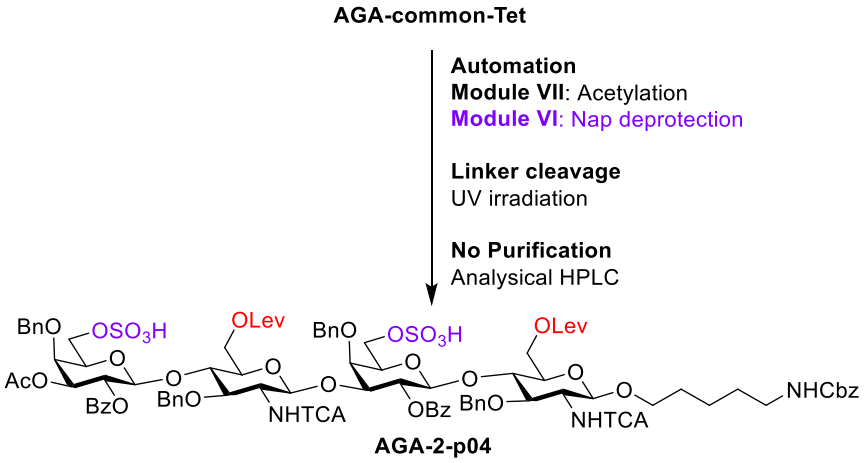


Analytical HPLC chromatogram of the crude tetrasaccharide AGA-common-Tet-2.

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.07 (d, *J* = 7.5 Hz, 2H), 8.02 (d, *J* = 7.5 Hz, 2H), 7.80 (dt, *J* = 18.4, 7.3 Hz, 6H), 7.63 – 7.55 (m, 4H), 7.52 – 7.43 (m, 9H), 7.26 (ddt, *J* = 37.6, 15.8, 6.0 Hz, 19H), 7.10 (dt, *J* = 21.9, 6.9 Hz, 4H), 7.03 (t, *J* = 7.3 Hz, 2H), 6.97 (d, *J* = 7.8 Hz, 1H), 6.55 (d, *J* = 8.1 Hz, 1H), 5.59 (dd, *J* = 18.2, 8.0 Hz, 2H), 5.18 (dd, *J* = 10.5, 3.0 Hz, 1H), 5.06 (s, 2H), 4.96 – 4.86 (m, 3H), 4.80 (d, *J* = 7.7 Hz, 1H), 4.76 (s, 1H), 4.73 (dd, *J* = 9.6, 5.4 Hz, 2H), 4.66 (d, *J* = 7.9 Hz, 1H), 4.57 (d, *J* = 7.6 Hz, 1H), 4.54 (d, *J* = 10.6 Hz, 1H), 4.49 (dt, *J* = 15.1, 8.0 Hz, 5H), 4.35 (dd, *J* = 11.9, 2.8 Hz, 2H), 4.03 (d, *J* = 2.6 Hz, 1H), 3.87 (ddd, *J* = 22.5, 14.7, 8.9 Hz, 5H), 3.76 – 3.72 (m, 1H), 3.66 (tt, *J* = 16.4, 8.3 Hz, 6H), 3.58 (s, 3H), 3.52 – 3.48 (m, 1H), 3.46 (t, *J* = 8.5 Hz, 2H), 3.35 (ddd, *J* = 18.8, 9.0, 5.3 Hz, 2H), 3.28 (d, *J* = 8.9 Hz, 1H), 3.18 (d, *J* = 8.1 Hz, 1H), 3.12 (d, *J* = 5.8 Hz, 3H), 1.89 (s, 3H), 1.50 – 1.41 (m, 4H),

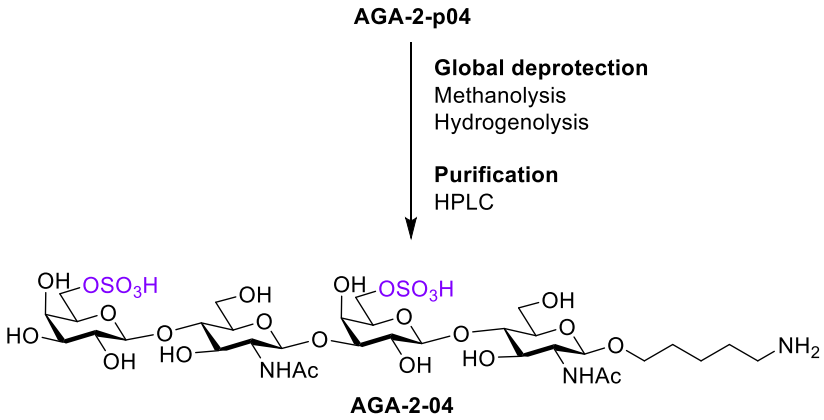
1.29 (dd, *J* = 15.5, 7.1 Hz, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.40 (Ac), 165.38 (Bz), 164.99 (Bz), 161.88 (2 x NHTCA), 156.56 (Cbz), 138.73, 138.30, 138.16, 138.11, 136.79, 135.52, 135.41, 133.71, 133.64, 133.39, 133.20, 129.95, 129.86, 129.68, 129.54, 128.88, 128.65, 128.45, 128.39, 128.37, 128.30, 128.22, 128.04, 128.00, 127.86, 127.62, 127.54, 127.48, 126.77, 126.72, 126.31, 126.10, 125.96, 125.92, 101.07, 101.00, 100.57, 99.84, 92.68, 92.24, 78.94, 78.01, 77.89, 77.37, 77.16, 76.95, 76.21, 76.12, 76.00, 75.87, 75.27, 74.95, 74.70, 74.52, 74.38, 73.93, 73.78, 73.74, 73.69, 72.70, 71.11, 69.83, 68.28, 67.72, 66.75, 61.02, 57.72, 57.55, 41.04, 29.63, 29.06, 23.26, 20.85.; MS ESI+-HRMS *m/z* [M+Na]+ calcd for $C_{107}H_{119}O_{26}Na$ 2084.5323, found 2084.5340.

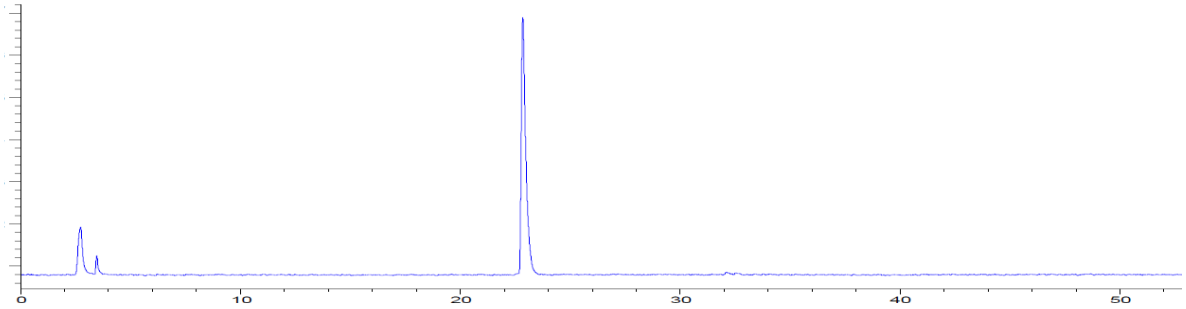
_N_-Benzyloxycarbonyl-5-amino-pentyl 3-_O_-acetyl-2-_O_-benzoyl-4-_O_-benzyl-6-_O_-sulfato-β-D-galactopyranosyl-(1→4)-3-_O_-benzyl-6-_O_-levulinyl-2-deoxy-2-trichloracetamido-β-D-glucopyranosyl-(1→3)-2-_O_-benzoyl-4-_O_-benzyl-6-_O_-6-_O_-sulfato-β-D-galactopyranosyl-(1→4)-3-_O_-benzyl-6-_O_-levulinyl-2-deoxy-2-trichloracetamido-β-D-glucopyranoside AGA-2-p04



Analytical HPLC chromatogram of the crude tetrasaccharide **AGA-2-p04**

5-Amino-pentyl 6-_O_-sulfato-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-6-_O_-sulfato-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside AGA-2-04 (3.5 mg, 3.5 μmol, 14% over fourteen steps)

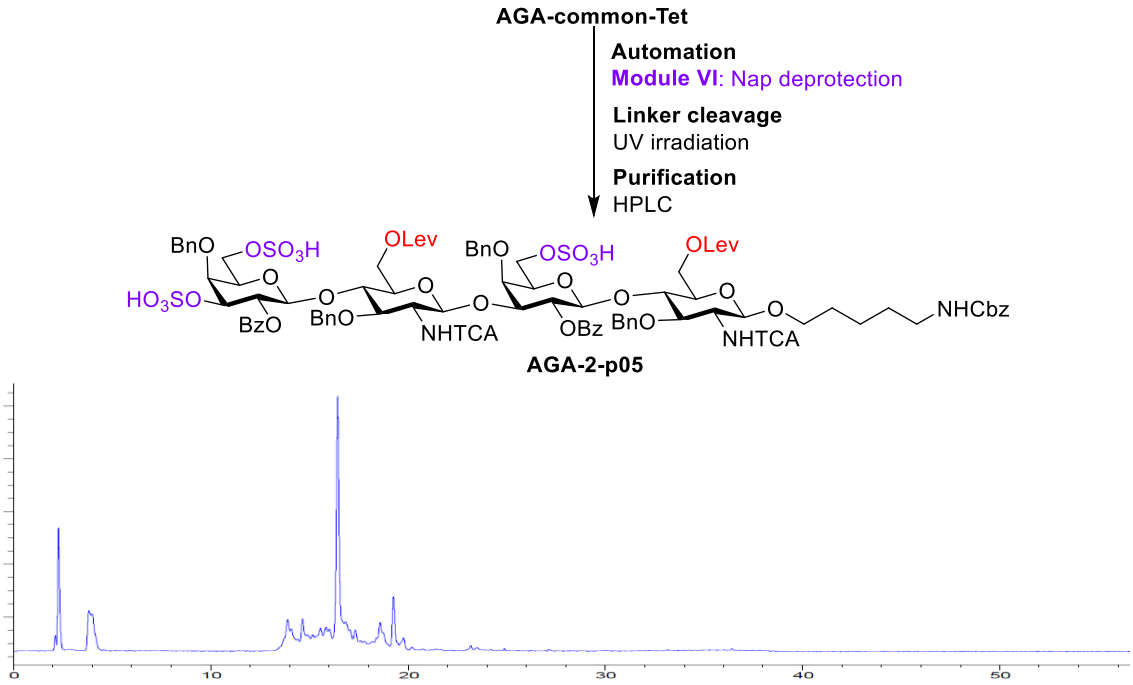




Analytical HPLC chromatogram of the crude tetrasaccharide **AGA-2-04**

^{1}H NMR (700 MHz, D_2O) δ 4.64 (d, *J* = 8.0 Hz, 1H, **H-1**), 4.44 (t, *J* = 8.5 Hz, 3H, 3 x **H-1**), 4.12 (dt, *J* = 21.2, 12.1 Hz, 4H), 3.94 – 3.86 (m, 4H), 3.85 – 3.81 (m, 1H), 3.80 – 3.59 (m, 12H), 3.57 – 3.50 (m, 4H), 3.50 – 3.46 (m, 1H), 2.92 (t, *J* = 7.5 Hz, 2H), 1.96 (s, 6H), 1.63 – 1.57 (m, 2H), 1.53 (dt, *J* = 12.9, 6.6 Hz, 2H), 1.36 – 1.30 (m, 2H). ^{13}C NMR (176 MHz, D_2O) δ 174.90, 174.43, 102.91 (**C-1**), 102.77 (**C-1**), 102.62 (**C-1**), 101.08 (**C-1**), 82.09, 79.18, 79.00, 74.74, 74.50, 72.80, 72.48, 72.31, 72.07, 70.81, 70.02, 69.73, 68.24, 67.97, 67.35, 67.17, 60.28, 60.00, 55.23, 55.17, 39.34, 28.06, 28.03, 26.36, 26.34, 26.32, 22.20, 22.17, 22.15, 22.09.; MS ESI+-HRMS *m/z* $[M-H]^-$ calcd for $C_{33}H_{58}N_3O_{27}S_2^-$ 992.2705, found 992.2708.

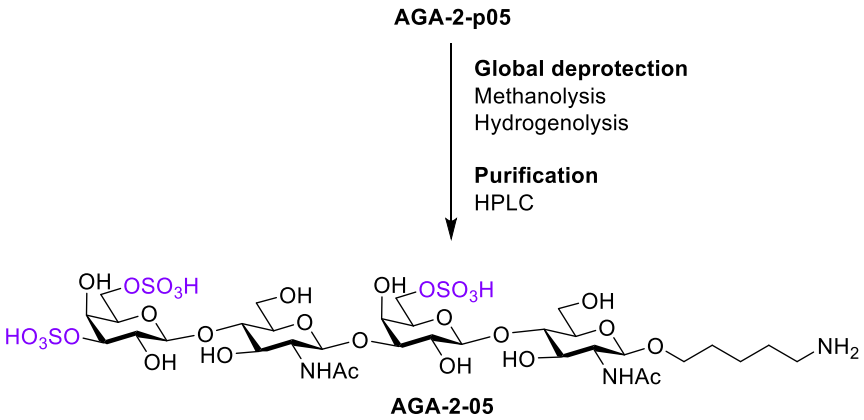
***N*-Benzyloxycarbonyl-5-amino-pentyl 2-*O*-benzoyl-4-*O*-benzyl-3,6-di-*O*-sulfato-β-D-galactopyranosyl-(1→4)-3-*O*-benzyl-6-*O*-levulinyl-2-deoxy-2-trichloracetamido-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-2,6-di-*O*-sulfato-β-D-galactopyranosyl-(1→4)- 3-*O*-benzyl-6-*O*-levulinyl-2-deoxy-2-trichloracetamido-β-D-glucopyranoside AGA-2-p05.** (21.1 mg, 9.7 μmol, 14% over eleven steps)

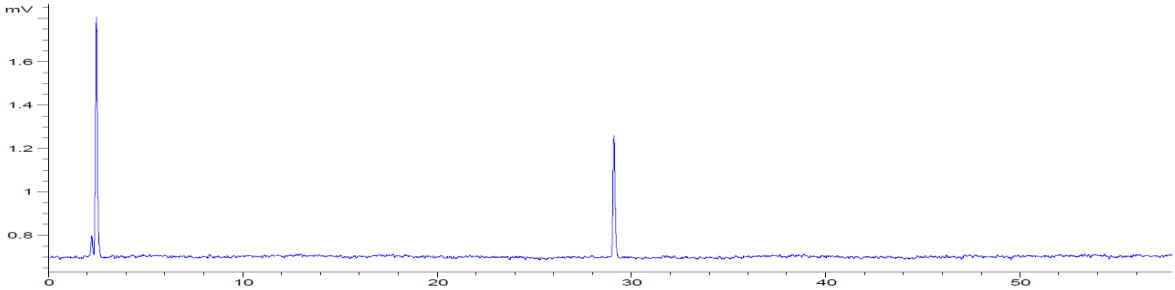


Analytical HPLC chromatogram of the crude tetrasaccharide AGA-2-p05

^{1}H NMR (600 MHz, CD_3OD) δ 8.13 (d, *J* = 7.4 Hz, 2H), 8.04 (d, *J* = 7.4 Hz, 2H), 7.74 – 7.69 (m, 1H), 7.62 (dt, *J* = 7.9, 3.0 Hz, 2H), 7.59 (t, *J* = 7.5 Hz, 1H), 7.50 (t, *J* = 7.7 Hz, 2H), 7.45 (dd, *J* = 16.3, 8.0 Hz, 6H), 7.32 (d, *J* = 4.3 Hz, 3H), 7.28 (dd, *J* = 15.1, 7.6 Hz, 5H), 7.22 (ddd, *J* = 7.6, 6.8, 4.4 Hz, 6H), 7.11 (dd, *J* = 14.5, 7.1 Hz, 5H), 5.51 (dd, *J* = 10.2, 7.9 Hz, 1H), 5.47 (dd, *J* = 10.2, 7.9 Hz, 1H), 5.13 (d, *J* = 10.8 Hz, 1H), 5.04 (d, *J* = 13.3 Hz, 3H), 4.93 (s, 2H), 4.76 – 4.69 (m, 3H), 4.64 (d, *J* = 11.0 Hz, 1H), 4.57 (s, 1H), 4.51 – 4.43 (m, 4H), 4.31 – 4.25 (m, 3H), 4.22 (dt, *J* = 9.9, 5.0 Hz, 3H), 4.17 – 4.11 (m, 2H), 4.04 (dt, *J* = 13.4, 6.5 Hz, 4H), 3.99 (dd, *J* = 8.9, 5.2 Hz, 2H), 3.92 – 3.83 (m, 3H), 3.79 – 3.72 (m, 3H), 3.69 (dd, *J* = 15.8, 6.2 Hz, 1H), 3.50 – 3.44 (m, 1H), 3.35 (dt, *J* = 11.6, 5.5 Hz, 1H), 3.27 (d, *J* = 8.4 Hz, 1H), 3.11 – 3.06 (m, 1H), 3.03 (t, *J* = 7.0 Hz, 2H), 2.84 – 2.66 (m, 4H), 2.56 – 2.37 (m, 4H), 2.15 (s, 3H), 2.07 (s, 3H), 1.48 – 1.39 (m, 6H).; ^{13}C NMR (150 MHz, CD_3OD) δ 209.73, 209.38, 174.28, 174.20, 169.34, 167.28, 166.79, 164.10, 164.02, 158.83, 140.44, 139.61, 139.50, 138.46, 134.48, 134.32, 133.58, 132.40, 131.35, 131.21, 131.07, 129.85, 129.77, 129.68, 129.60, 129.44, 129.20, 129.13, 129.09, 129.01, 128.90, 128.73, 128.40, 128.34, 128.28, 102.81, 101.94, 101.90, 101.57, 94.10, 93.57, 80.66, 80.23, 80.12, 79.23, 78.42, 78.19, 76.93, 76.52, 76.41, 75.94, 75.53, 75.39, 74.61, 74.43, 74.12, 73.98, 73.63, 72.67, 70.59, 69.12, 67.35, 67.26, 66.48, 63.85, 58.44, 58.06, 49.43, 49.28, 49.14, 49.00, 48.86, 48.72, 48.57, 47.93, 41.69, 40.18, 38.81, 38.62, 31.63, 30.47, 30.23, 30.14, 29.88, 28.97, 28.83, 25.55, 24.96, 24.24, 24.02.; MS ESI+-HRMS *m/z* $[M-3H]^{3-}$ calcd for $C_{93}H_{101}N_3O_{38}Cl_6S_3$ 725.1099, found 725.4409.

5-Amino-pentyl 3,6-di-*O*-sulfato-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-6-*O*-sulfato β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside AGA-2-05 (3.2 mg, 3.0 μmol, 30% over two steps)

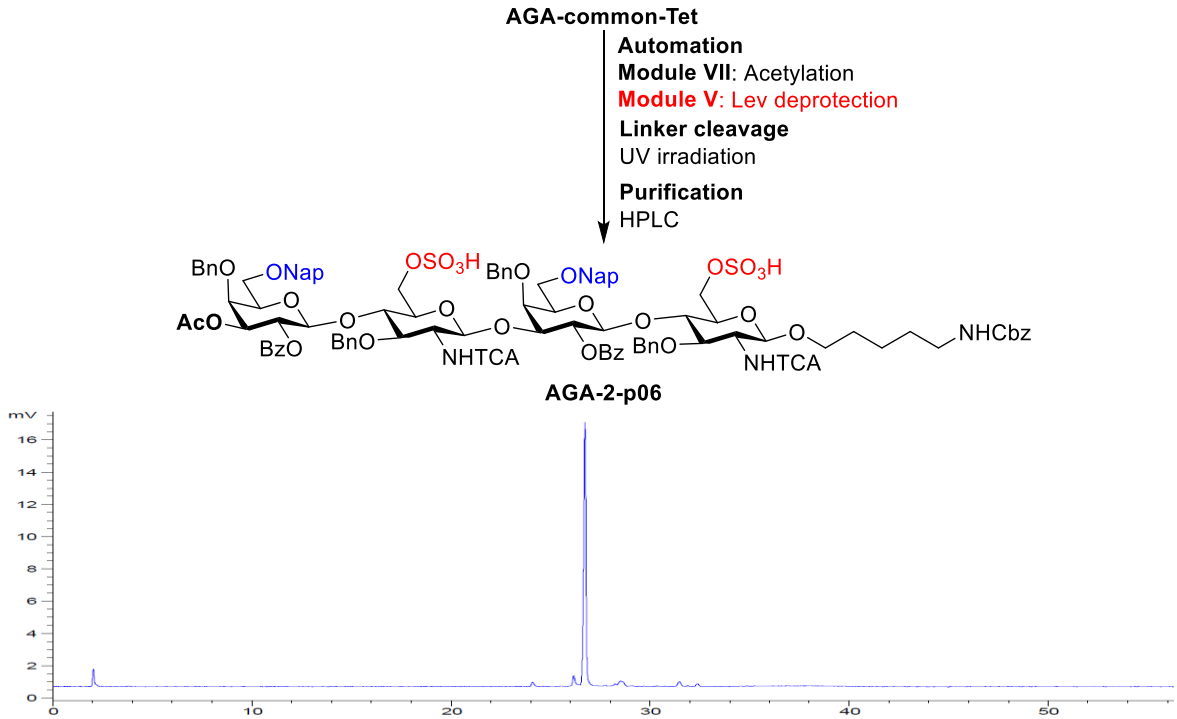




Analytical HPLC chromatogram of the crude tetrasaccharide AGA-2-05

^{1}H NMR (600 MHz, D_2O) δ 4.72 (d, *J* = 8.1 Hz, 2H), 4.65 (d, *J* = 7.9 Hz, 1H), 4.53 (t, *J* = 8.0 Hz, 2H), 4.37 (dt, *J* = 6.1, 3.3 Hz, 2H), 4.26 – 4.16 (m, 5H), 4.05 (dd, *J* = 7.1, 5.3 Hz, 1H), 3.99 (ddd, *J* = 12.1, 10.8, 2.1 Hz, 3H), 3.94 – 3.66 (m, 11H), 3.66 – 3.57 (m, 4H), 3.02 – 2.98 (m, 2H), 2.05 (s, 2H), 2.04 (s, 3H), 1.72 – 1.65 (m, 2H), 1.64 – 1.58 (m, 2H), 1.45 – 1.37 (m, 2H). ^{13}C NMR (150 MHz, D_2O) δ 177.48, 177.02, 105.43, 105.36, 105.19, 105.01, 103.67, 84.67, 82.27, 81.72, 81.56, 77.35, 77.11, 75.06, 74.63, 72.62, 72.31, 71.63, 71.54, 70.63, 70.56, 69.79, 69.22, 62.88, 57.79, 41.95, 30.63, 28.97, 24.79, 24.69.; MS ESI+-HRMS *m/z* $[M-2H]^{2-}$ calcd for $C_{33}H_{57}N_3O_{30}S_3^{2-}$ 535.6100, found 535.6101.

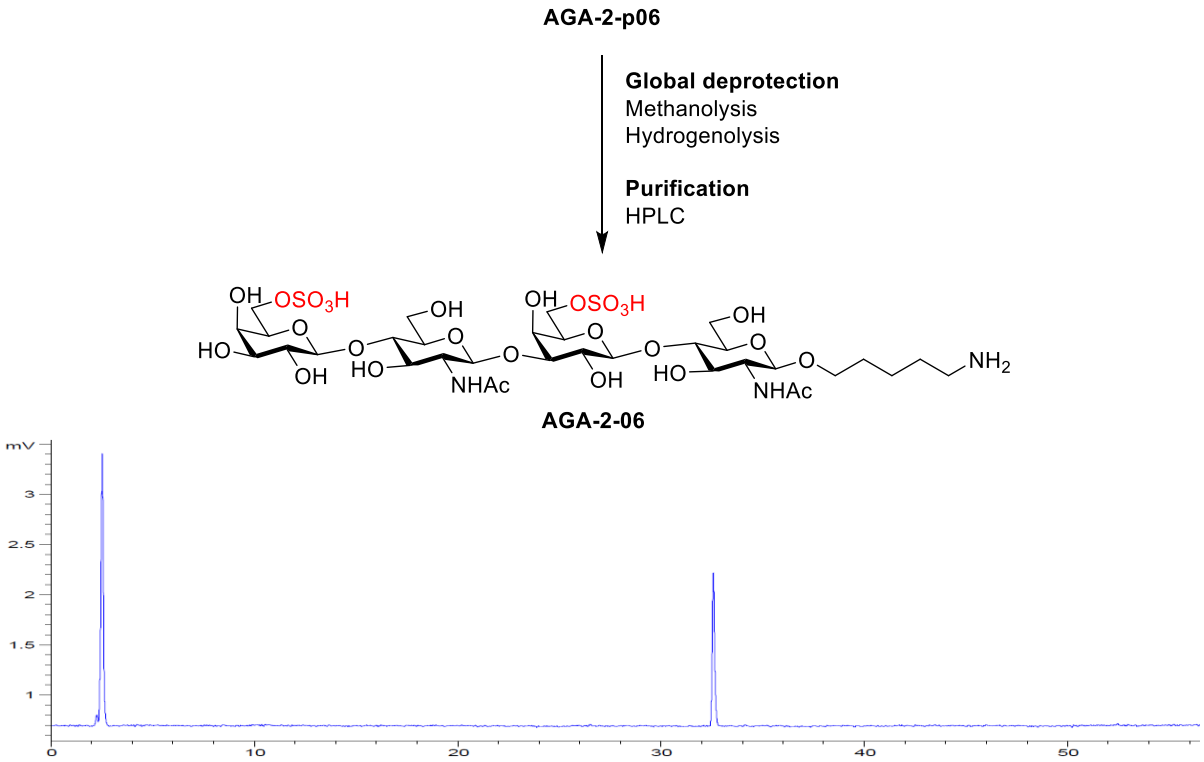
***N*-Benzyloxycarbonyl-5-amino-pentyl (2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-(3,6-di-*O*-benzyl-2-deoxy-2-trichloracetamido-β-D-glucopyranosyl)-(1→3)-(2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)- 3,6-di-*O*-benzyl-2-deoxy-2-trichloracetamido-β-D-glucopyranoside AGA-2-p06**



Analytical HPLC chromatogram of the crude tetrasaccharide **AGA-2-p06.**

^{1}H NMR (600 MHz, CD_3OD) δ 8.22 – 8.18 (m, 2H), 8.12 (d, *J* = 8.1 Hz, 2H), 7.84 – 7.74 (m, 6H), 7.72 – 7.61 (m, 3H), 7.60 – 7.53 (m, 4H), 7.50 – 7.30 (m, 14H), 7.26 – 7.18 (m, 11H), 7.12 (t, *J* = 7.3 Hz, 1H), 7.01 (t, *J* = 7.4 Hz, 1H), 6.98 – 6.87 (m, 5H), 5.59 (dd, *J* = 10.3, 7.9 Hz, 1H), 5.49 (dd, *J* = 10.1, 8.1 Hz, 1H), 5.29 (dd, *J* = 10.4, 3.2 Hz, 1H), 5.24 (d, *J* = 7.9 Hz, 1H), 5.05 (d, *J* = 11.4 Hz, 1H), 5.03 – 4.97 (m, 4H), 4.91 (d, *J* = 8.0 Hz, 1H), 4.69 (dd, *J* = 9.8, 5.2 Hz, 2H), 4.61 (d, *J* = 12.0 Hz, 1H), 4.49 (ddd, *J* = 23.6, 17.8, 11.5 Hz, 7H), 4.38 (d, *J* = 8.2 Hz, 1H), 4.30 (d, *J* = 12.0 Hz, 1H), 4.20 (ddd, *J* = 13.2, 10.4, 4.2 Hz, 6H), 4.15 – 4.09 (m, 3H), 4.03 (t, *J* = 6.7 Hz, 1H), 3.94 – 3.89 (m, 2H), 3.84 (d, *J* = 9.1 Hz, 1H), 3.81 (t, *J* = 5.9 Hz, 1H), 3.68 (dd, *J* = 20.0, 10.6 Hz, 3H), 3.64 – 3.59 (m, 1H), 3.53 – 3.48 (m, 2H), 3.44 – 3.38 (m, 3H), 1.86 (d, *J* = 0.5 Hz, 3H), 1.47 – 1.37 (m, 6H).^{13}C NMR (150 MHz, CD_3OD) δ 171.67, 167.08, 164.12, 158.85, 140.61, 139.94, 139.84, 137.42, 137.03, 134.73, 134.50, 134.33, 132.39, 131.59, 131.12, 130.63, 129.93, 129.85, 129.67, 129.62, 129.41, 129.26, 129.20, 129.13, 129.05, 129.02, 128.85, 128.71, 128.57, 128.11, 127.97, 127.78, 127.53, 127.49, 127.24, 127.00, 126.76, 126.54, 103.27, 101.93, 101.84, 101.15, 80.69, 78.98, 76.91, 76.76, 76.39, 76.24, 76.12, 75.87, 75.79, 75.59, 74.70, 74.55, 74.49, 73.69, 70.46, 70.37, 69.12, 68.85, 67.24, 66.14, 58.31, 58.01, 49.43, 49.28, 49.14, 49.00, 48.86, 48.72, 48.57, 47.90, 41.71, 40.19, 31.64, 30.49, 30.22, 30.14, 24.96, 24.27, 24.03, 20.69.; MS ESI+-HRMS *m/z* $[M-3H]^{3-}$ calcd for $C_{107}H_{107}N_3O_{32}Cl_6S_2$ 1111.2212, found 1111.2209.

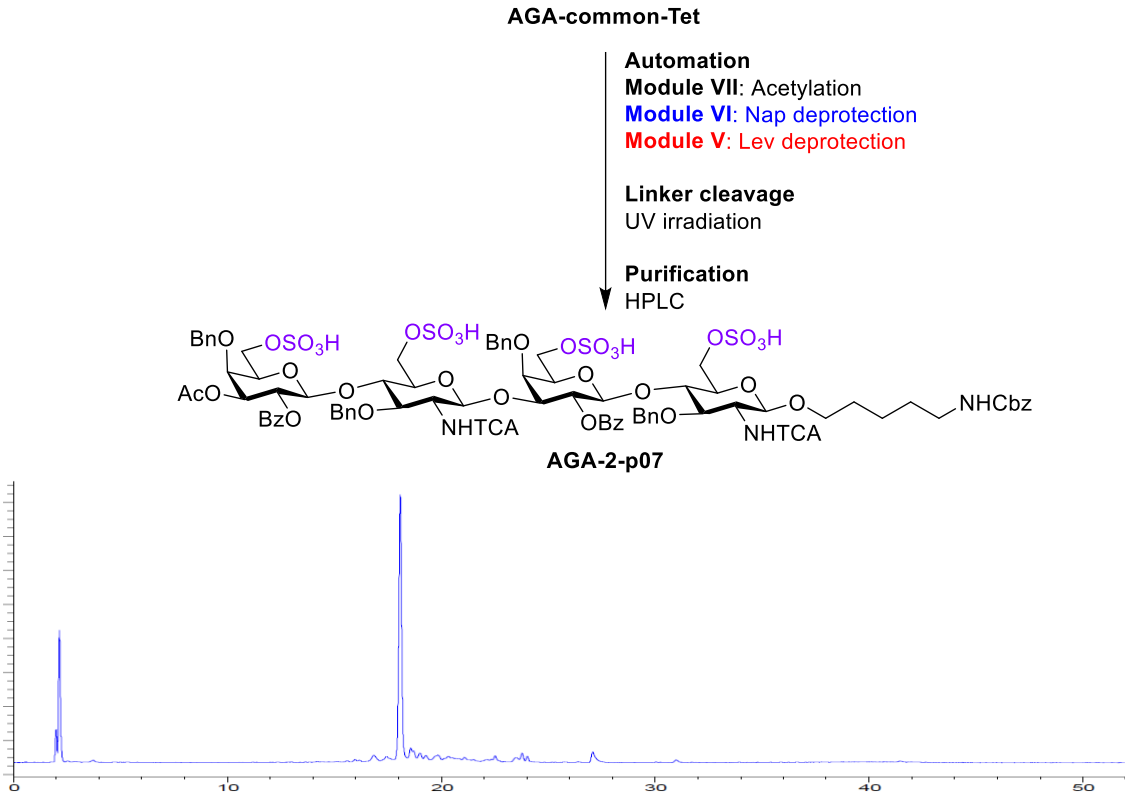
5-Amino-pentyl β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-6-*O*-sulfato-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy6-*O*-sulfato-β-D-glucopyranoside AGA-2-06 (3.0 mg, 3.0 µmol, 12% over fourteen steps)



Analytical HPLC chromatogram of the crude tetrasaccharide **AGA-2-06**

^{1}H NMR (600 MHz, D_2O) δ 4.72 (d, *J* = 8.7 Hz, 1H, **H-1**), 4.53 (dd, *J* = 18.2, 9.1 Hz, 3H, 3 x **H-1**), 4.45 – 4.28 (m, 4H), 4.19 (s, 1H), 3.93 (s, 1H), 3.90 – 3.62 (m, 18H), 3.56 (dt, *J* = 18.0, 8.8 Hz, 2H), 3.00 (t, *J* = 7.5 Hz, 2H), 2.04 (s, 3H), 2.03 (s, 3H), 1.71 – 1.65 (m, 2H), 1.63 – 1.56 (m, 2H), 1.49 – 1.35 (m, 2H).; ^{13}C NMR (150 MHz, D_2O) δ 177.53, 177.02, 105.46 (**C-1**), 105.26 (**C-1**), 105.11 (**C-1**), 103.76 (**C-1**), 85.02, 80.46, 79.91, 77.92, 77.63, 75.10, 75.05, 74.94, 74.72, 73.56, 72.83, 72.50, 71.20, 70.92, 68.98, 63.71, 63.63, 57.71, 57.60, 41.95, 30.67, 28.86, 24.78, 24.73, 24.61.; MS ESI+-HRMS *m/z* $[M-H]^-$ calcd for $C_{33}H_{58}N_3O_{27}S_2^-$ 992.2705, found 992.2707.

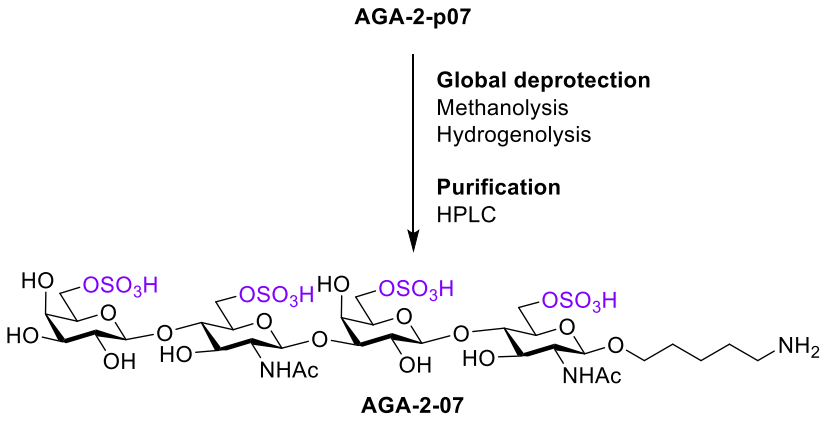
***N*-Benzyloxycarbonyl-5-amino-pentyl (2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-(3,6-di-*O*-benzyl-2-deoxy-2-trichloracetamido-β-D-glucopyranosyl)-(1→3)-(2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)- 3,6-di-*O*-benzyl-2-deoxy-2-trichloracetamido-β-D-glucopyranoside AGA-2-p07** (19.9 mg, 3.8 µmol, 29% over twelve steps)

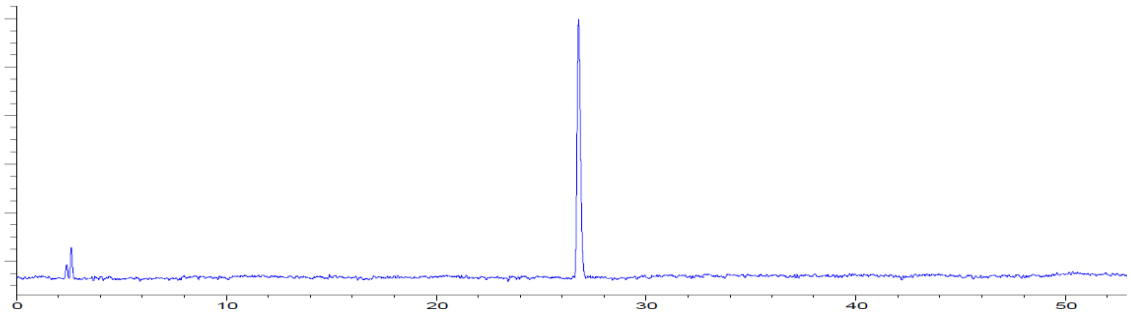


Analytical HPLC chromatogram of the crude tetrasaccharide **AGA-2-p07**

^{1}H NMR (600 MHz, CD_3OD) δ 8.18 (d, *J* = 8.0 Hz, 2H), 8.10 (d, *J* = 8.1 Hz, 2H), 7.73 – 7.60 (m, 2H), 7.60 – 7.52 (m, 3H), 7.49 – 7.42 (m, 6H), 7.33 – 7.21 (m, 13H), 7.18 (dd, *J* = 17.0, 9.9 Hz, 1H), 7.15 – 7.05 (m, 6H), 5.60 (dd, *J* = 10.0, 8.2 Hz, 1H), 5.50 (t, *J* = 8.8 Hz, 1H), 5.29 (dd, *J* = 10.3, 2.6 Hz, 1H), 5.24 (d, *J* = 7.9 Hz, 1H), 5.08 (d, *J* = 10.8 Hz, 1H), 5.02 (s, 2H), 4.95 (dt, *J* = 16.0, 8.9 Hz, 3H), 4.76 (dt, *J* = 28.5, 7.7 Hz, 2H), 4.68 (d, *J* = 8.2 Hz, 1H), 4.62 (d, *J* = 10.8 Hz, 1H), 4.47 (dd, *J* = 9.9, 6.3 Hz, 2H), 4.40 (dd, *J* = 21.6, 9.1 Hz, 2H), 4.18 (dt, *J* = 10.8, 9.1 Hz, 7H), 4.12 (d, *J* = 8.8 Hz, 1H), 4.11 – 4.00 (m, 4H), 3.94 – 3.85 (m, 4H), 3.68 (dd, *J* = 12.8, 6.0 Hz, 3H), 3.64 – 3.58 (m, 1H), 3.40 (d, *J* = 9.6 Hz, 1H), 3.34 – 3.31 (m, 1H), 3.06 – 2.99 (m, 2H), 1.85 (d, *J* = 2.9 Hz, 3H), 1.52 – 1.38 (m, 6H).^{13}C NMR (150 MHz, CD_3OD) δ 171.59, 169.33, 166.97, 164.14, 158.83, 140.45, 139.92, 139.43, 139.36, 138.44, 134.75, 134.42, 133.56, 132.40, 131.47, 131.08, 131.01, 130.60, 129.92, 129.85, 129.80, 129.71, 129.51, 129.39, 129.27, 129.14, 128.99, 128.88, 128.70, 128.59, 128.49, 128.40, 128.24, 102.80, 101.89, 101.50, 101.10, 94.08, 93.71, 80.62, 80.49, 79.96, 78.55, 76.79, 76.45, 76.42, 76.05, 75.52, 75.18, 74.72, 74.58, 73.81, 72.38, 70.54, 69.11, 67.67, 67.24, 66.16, 65.68, 58.31, 57.86, 41.67, 40.16, 31.61, 30.49, 30.20, 30.12, 24.94, 24.22, 24.01, 20.64, 14.39, 11.40, 9.41.; MS ESI+-HRMS *m/z* $[M-2H]^{2-}$ calcd for $C_{85}H_{91}N_3O_{38}Cl_6S_4$ 1050.6138, found 1050.6418.

5-Amino-pentyl 6-*O*-sulfato-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-6-*O*-sulfato-β-D-glucopyranosyl-(1→3)-6-*O*-sulfato-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-6-*O*-sulfato-β-D-glucopyranoside AGA-2-07 (2.8 mg, 2.4 μmol, 25% over two steps)





LC-MS chromatogram of AGA-2-07

^{1}H NMR (600 MHz, D_2O) δ 4.60 – 4.53 (m, 3H, 3 x **H-1**), 4.46 (d, *J* = 10.8 Hz, 1H), 4.42 (dd, *J* = 11.1, 2.1 Hz, 1H), 4.36 (dd, *J* = 11.1, 4.6 Hz, 1H), 4.31 (dd, *J* = 10.9, 5.5 Hz, 1H), 4.25 (d, *J* = 4.4 Hz, 1H), 4.22 (t, *J* = 8.7 Hz, 4H), 4.01 (d, *J* = 8.7 Hz, 3H), 4.92 – 3.88 (m, 4H), 3.81 – 3.71 (m, 8H), 3.70 – 3.65 (m, 1H), 3.64 – 3.60 (m, 1H), 3.59 – 3.54 (m, 1H), 3.02 (t, *J* = 7.6 Hz, 2H), 2.07 (s, 3H), 2.06 (s, 3H), 1.70 (dt, *J* = 14.5, 7.4 Hz, 2H), 1.66 – 1.60 (m, 2H), 1.44 (qd, *J* = 14.0, 7.3 Hz, 2H).; ^{13}C NMR (150 MHz, D_2O) δ 102.91 (**C-1**), 102.84 (**C-1**), 102.65 (**C-1**), 101.14 (**C-1**), 82.18, 79.18, 78.55, 74.47, 72.75, 72.67, 72.51, 72.37, 72.25, 72.15, 70.84, 70.19, 69.78, 68.21, 68.14, 67.86, 67.05, 66.89, 66.81, 66.49, 65.32, 55.09, 55.06, 39.37, 28.04, 26.23, 22.17, 21.99.; MS ESI+-HRMS *m/z* $[M-3H]^{3-}$ calcd for $C_{33}H_{56}N_3O_{33}S_4^{3-}$ 1150.1695, found 383.3905.

3. Automated Glycan Assembly of Complex Oligosaccharides Related to Blood Group Determinants

This chapter has been modified in part from the following publication:

H. S. Hahm, C.-F. Liang, C.-H. Lai, R. J. Fair, F. Schuhmacher, P. H. Seeberger, Automated Glycan Assembly of Complex Oligosaccharides Related to Blood Group Determinants *J. Org. Chem.* **2016**, *81*, 5866-5877.

https://doi.org/10.1021/acs.joc.6b00554

3.1 Introduction

Ten monosaccharide building blocks suffice to construct mammalian glycans of immense complexity.[94] Nature did not make all possible combinations of glycans but rather based on common biosynthetic pathways, some scaffolds are present in diverse glycans that serve very different biological functions. Two isomeric tetrasaccharides (Lc_4 and nLc_4) are the core structures of the lacto- and neo-lacto sub-families of glycosphingolipids (GSLs). GSLs are glycolipids that have been linked to many diseases.[95] Many GSL are tumor associated carbohydrate antigens (TACAs) that play critical roles in cancer development and survival.[96] Fucosylation of the Lc_4 and nLc_4 cores gives rise to the Lewis blood group antigens, as well as H-type I and II glycans (**Figure 3.01**).

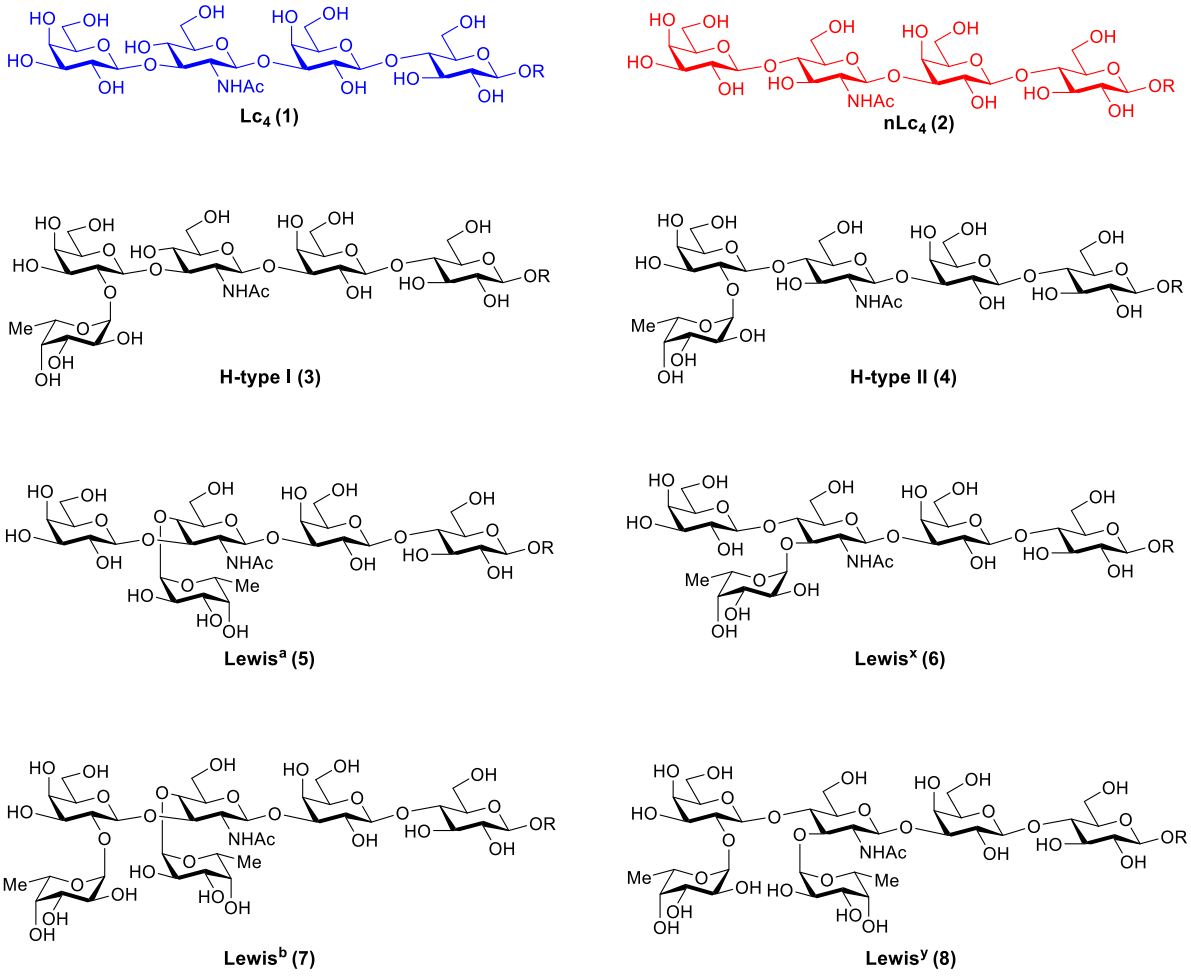


Figure 3.01 Oligosaccharides based on Lc4 and nLc4 core tetrasaccharides. (R = ceramide).

Some of these glycans serve as receptors for bacteria and viruses.[97,98] Sialylated Lewis blood group antigens are prevalent TACAs and cancer vaccine candidates[99] but are also associated with immunodeficiency disorders and atherosclerosis.[100] Sialyl-nLc4 (SPG)

for example is connected with certain cancers[96,100], the neurological disorder Guillain–Barré syndrome[101], and pathogen-cell recognition.[102] Lc4, nLc4, Lewis, and sialyl Lewis antigens can be sulfated directly or may contain additional sulfated saccharide units as is the case in HNK-1, a nLc4–based pentasaccharide containing a terminal 3-*O*-SO_3-glucuronic acid that is found in glycoproteins of the central nervous system.[101] Addition of a terminal α-galactose onto nLc4 gives rise to antigens such as the P^k blood group antigen that contains a terminal α(1,4)-galactose and has been associated with several forms of bacterial and viral pathogenesis.[103] Addition of an α(1,3)-galactose onto nLC4 is associated with hyperacute rejection of xenotransplanted organs.[104]

Pure, structurally defined glycans are critical to the study of these biologically significant molecules. Different methods are used to synthesize glycans: Enzymatic methods possess extraordinary regio- and stereoselectivity without the need for protecting group manipulations. However, accessing glycosyltransferases is laborious and mainly makes sense for large scale preparations of unmodified glycans.[105] Traditional solution-phase oligosaccharide synthesis is reliable and allows for the incorporation of unnatural glycans. However, it is a time consuming process that typically requires many purification steps and protecting group manipulations.[106] Sequential one-pot strategies that take advantage of varying building block reactivities obviate the need for intermediate purifications and greatly accelerate syntheses.[107a] Implementation of this method can be challenging as it requires multiple building blocks to install a particular linkage.[107b] Automated solid-phase strategies similarly accelerate syntheses and avoid intermediate purifications. High yields are ensured by mass action as multiple equivalents of building block are added during glycosylation reactions. Automated glycan assembly (AGA)[108] on solid support has progressed in recent years to provide access to increasingly complex structures.[109,110] Still, effective building block design, linker stability, and the development of improved glycosylation and deprotection conditions is ongoing.

A set of structurally diverse, biologically relevant glycans that contain the Lc4 and nLc4 cores served as challenge to further develop AGA methods. Previously, Lewis X, Lewis Y, and Le^y-Le^x antigens were successfully synthesized by AGA[109c] but H-type I and II pentasaccharides proved very challenging[111a] and automated syntheses were low yielding due to a lack of reliable methods to install α(1,2)-cis linkages.[111b] The synthesis of HNK-1 requires sulfation at the C-3 hydroxyl group of glucuronic acid and draws from our insights into the synthesis of chondroitin sulfate[110a] and dermatan sulfate[110b] fragments.

3.2 Automated Synthesis of Complex Oligosaccharides

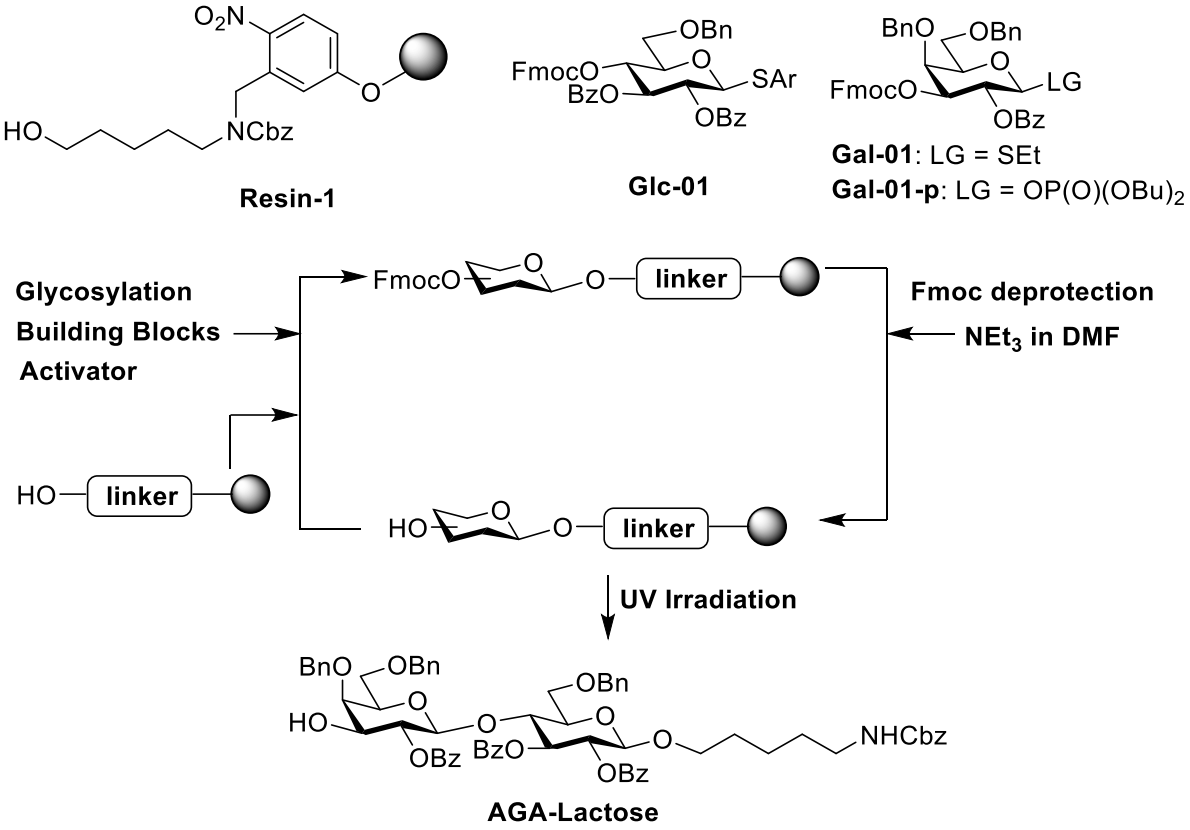
3.2.1 Assessment of Linker Stability

Merrifield resin equipped with a photolabile linker **Resin-1** has been the basis for AGA of many complex glycans.[110] However, the stability of **Resin-1** towards commonly utilized strong acid activators, trimethylsilyl triflate (TMSOTf) or triflic acid (TfOH) at room temperature had not been explored. Activation at ambient temperatures is required sometimes when deactivated building blocks are incorporated. Lactose disaccharide **AGA-Lactose** is present in all target molecules and was selected as a model for methodological studies (**Table 3.01**). Thioglucoside **Glc-01**[110b], thiogalactoside **Gal-01**[110b], and galactosyl phosphate **Gal-01-p**[110c] building blocks were prepared. Two different anomeric leaving groups were chosen that require either catalytic (thioglycoside) or stoichiometric (glycosyl phosphate) amounts of acid for activation.

All reactions were performed using an automated synthesizer[110a] that executes reaction modules. The acidic wash module adds a TMSOTf solution at -20 ^{o}C to neutralize basic residues and remove residual water from the resin. The glycosylation module introduces five equivalents of building block and an activator solution (TMSOTf for glycosyl phosphates, TfOH and N-iodosuccinimide (NIS) for thioglycosides) at an activation temperature ($\mathbf{T_a}$) to remain on the resin for the activation time ($\mathbf{t_1}$). The temperature is then increased to the incubation temperature ($\mathbf{T_i}$) for incubation time ($\mathbf{t_2}$) (Table 3.01). Each glycosylation cycle is repeated twice. During the Fmoc deprotection module the 9-fluorenemethylcarbonate (Fmoc) protecting group is removed using a solution of triethylamine (TEA). Following AGA, the protected disaccharide **AGA-Lactose** was cleaved from the resin by UV irradiation using a continuous flow photo-reactor.[110a] Product **AGA-Lactose** was quantitated by normal phase high-performance liquid chromatography (NP-HPLC) with an internal standard (**Figure 3.02**)

The result of the automated assembly of a simple disaccharide such as **AGA-Lactose** shows a clear dependence of the overall yield on the acid concentration and the reaction temperature. The linker stability to four sets of conditions was assessed (Table 3.01). The conditions did not change for glucose building block **Glc-01** or $\mathbf{T_a}$ for galactose building blocks **Gal-01** or **Gal-01-p**. The overall yield of **AGA-Lactose** did not change when thioglycoside **Gal-01** was used at $\mathbf{T_i}$ = -20 ^{o}C or 20 ^{o}C (42% vs. 40% overall yield). Apparently, linker **AGA-Lactose** is stable to catalytic quantities of TfOH at room temperature. Exposure of **Resin-1** to stoichiometric TMSOTf at room temperature may lead

to some cleavage as evidenced by slight drop in yield (36%) when glycosyl phosphate **Gal-01-p** was coupled at 20 °C. To test whether the exposure to strong acid at room temperature really results in linker cleavage, the disaccharide was prepared by coupling building block **Gal-01-p** at T_i = -20 °C, but during a subsequent cycle, five equivalents of TMSOTf were added at room temperature without building block. The drastically decreased yield (14%) confirmed the incompatibility of linker **Resin-1** with stoichiometric amounts of strong acid at room temperature.



Entry	Building block	T_a (°C)	T_i (°C)	Acidic wash[e]	Yield (%)[f]
1	**Gal-01**[b]	-40	-20	No	42
2	**Gal-01**[c]	-40	-20 → 20	No	40
3	**Gal-01-p**[d]	-40	20 → 20	No	36
4	**Gal-01**[b]	-40	-20	Yes	14

Table 3.01 Automated Glycan Assembly of Lactose to Assess Linker Stability[a]. [a]Reaction conditions: **Resin-1** (63.8 mg, 0.025 mmol, loading of resin: 0.392 mmol/g) was utilized. Building block **Glc-01, Gal-01, and Gal-01-p** (0.25 mmol, 10 equiv based on the resin) were dissolved in DCM (2 mL). Glycosylation: 2 x 5 equiv of **Glc-01**, NIS, TfOH, DCM/dioxane, -30 °C (5 min) → -10 °C (25 min). [b]2 x 5 equiv of **Gal-01**, NIS, TfOH, DCM/dioxane, -40 °C (5 min) → -20 °C (25 min). [c]2 x 5 equiv of **Gal-01** NIS, TfOH, DCM/dioxane, -40 °C (5 min) → -20 °C (25 min) → 20 °C (25 min). [d]2 x 5 equiv of **Gal-01-p**, TMSOTf, DCM, -40 °C (5 min) → -20 °C (25 min) → 20 °C (25 min). [e]2 x 5 equiv of TMSOTf, DCM, 20 °C (25 min). [f] Yields by preparative normal-phase HPLC.

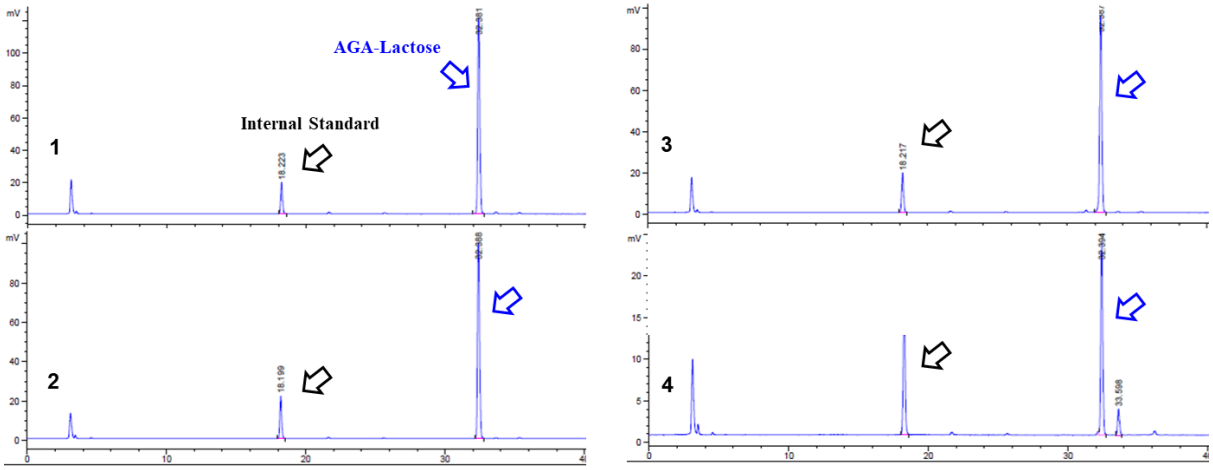


Figure 3.02. LC-MS of disaccharide **AGA-Lactose** (blue arrow) including building block **Gal-01** (black arrow) as an internal standard.

3.2.2 Synthesis of H type I and II

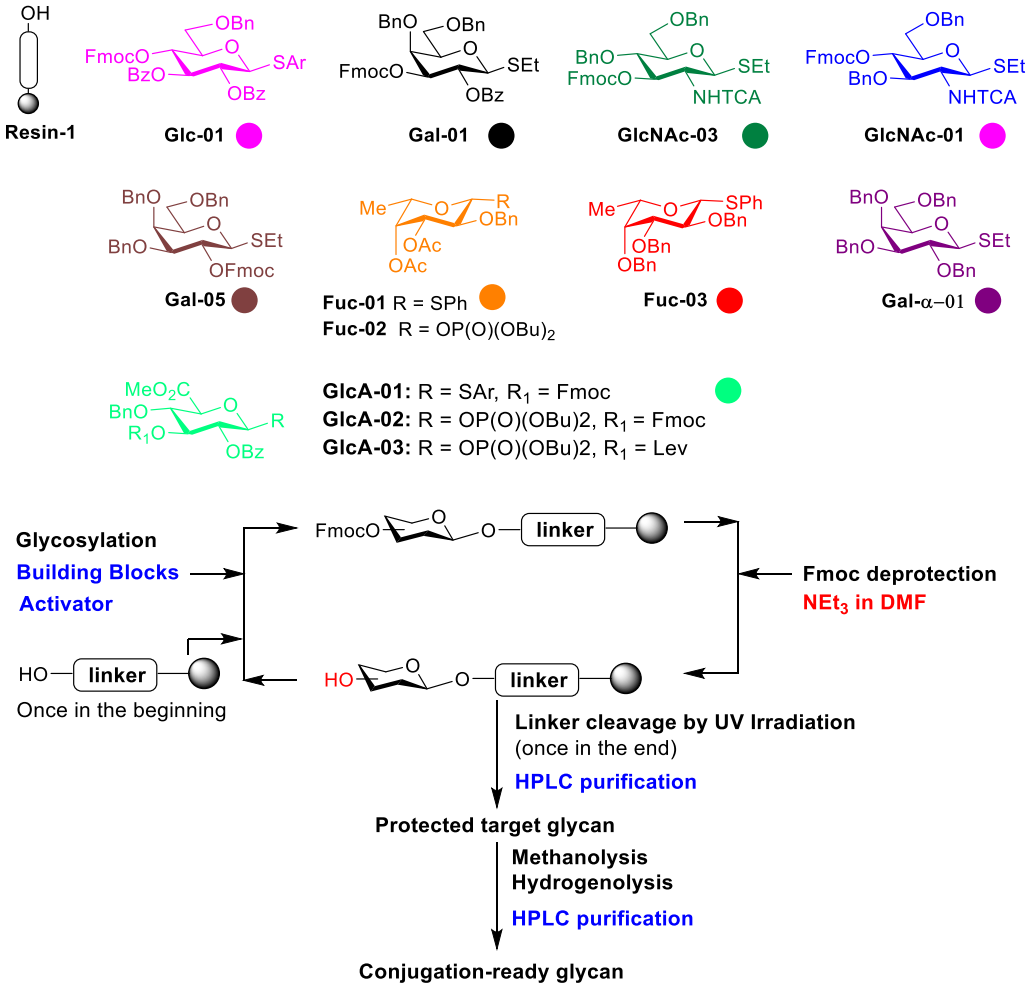
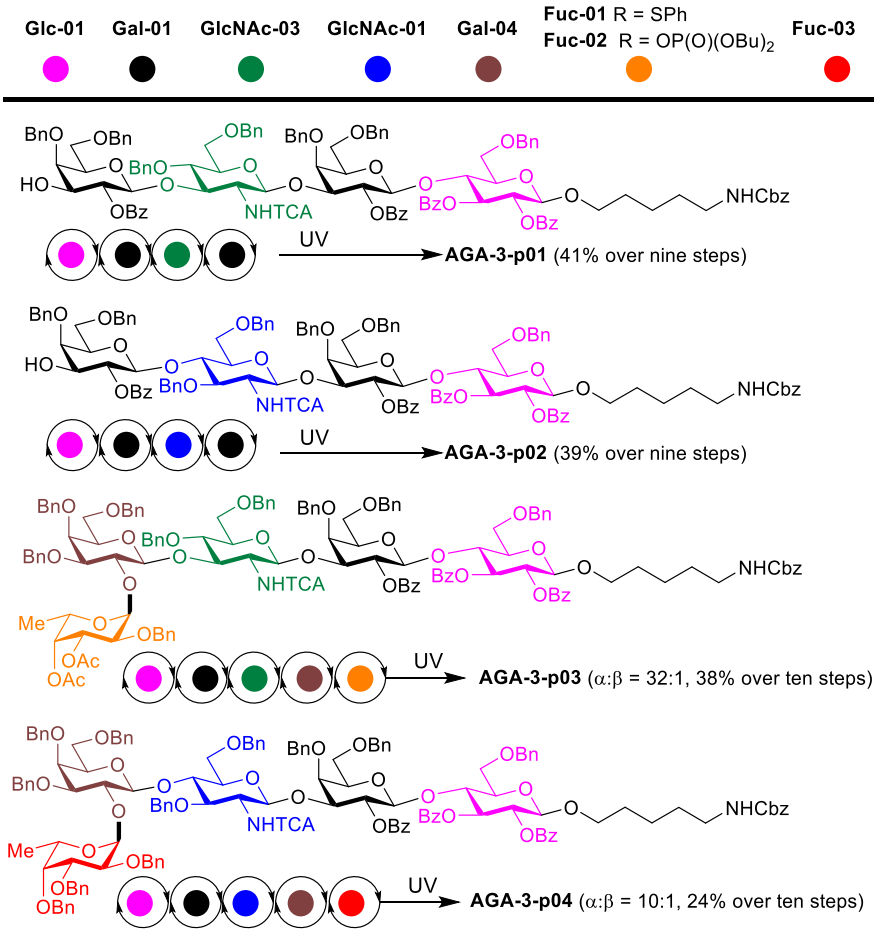


Figure 3.03 Retrosynthetic analysis of **Lc4, nLc4**, **H type I**, **H type II**, three **alpha-Gal epitopes**, and **HNK-1** Oligosaccharides and the Complete Set of Building Blocks for automated glycan assembly (AGA), and Schematic representation of AGA.

H-type I[112a,b], H-type II[112b,c], HNK-1[113], and α-Gal[114] epitope oligosaccharides were prepared using similar strategies but mono- and disaccharide building blocks that differed in anomeric leaving and protecting groups (**Figure 3.03**). The lack of a commonly agreed-upon set of "approved" building blocks, that can be prepared in large quantities, are stable for months, and can be activated at a specific temperature to reliably and selectively form the desired glycosidic linkage is a major impediment to carbohydrate chemistry. In the context of the oligosaccharide syntheses several reliable building blocks were identified.

Fully protected oligosaccharides were prepared by AGA. Following photo-cleavage from the resin and normal phase-HPLC purification the two core oligosaccharides Lc4 **AGA-3-p01** (41% over nine steps based on resin loading) and nLc4 **AGA-3-p02** (39% over nine steps) were obtained.



Scheme 3.01. Automated Glycan Assembly of the Protected Lc4 **AGA-3-p01**, H-type I **AGA-3-p02**, nLc4 **AGA-3-p03**, and H-type II **AGA-3-p04** Oligosaccharides[a]. [a]Reaction conditions: Building block**s** (0.025 mmol) were dissolved in DCM (2 mL); Glycosylation: 2 x 5 equiv of **Glc-01**, **GlcNAc-01**, or **GlcNAc-03**, NIS, TfOH, DCM/dioxane, -30 °C (5 min) → -10 °C (25 min). 2 x 5 equiv of **Gal-01**, **Gal-04**, **Fuc-01** or **Fuc-03**, NIS, TfOH, DCM/dioxane, -40 °C (5 min) → -20 °C (25 min). 2 x 5 equiv of **Fuc-02**, TMSOTf, DCM, -40 °C (5 min) → -20 °C (25 min); Deprotection: three cycle of 20% NEt_3 in DMF, 25 ºC for **Glc-01**, **Gal-01, GlcNAc-01,** or **GlcNAc-03,** or 30 ºC for **Gal-04**; UV-cleavage: hv (305 nm); Yields are based on resin loading and obtained by preparative normal-phase HPLC (detection: ELSD)

Fully protected H-type I pentasaccharide **AGA-3-p03** (38% over ten steps) was assembled by sequential incorporation of building blocks **Glc-01**, **Gal-01**, **GlcNAc-03**[22], **Gal-04**, and **Fuc-01**[23] (**Scheme 3.01**). NP-HPLC analysis of the initial attempt indicated a Fmoc-protected tetrasaccharide deletion sequence (black arrow, **Figure 3.04 A**) as well as the desired product **AGA-3-p03** (blue arrow, **Figure 3.04 A**) with the final fucose attached with good stereoselectivity (α:β = 11:1, B, **Figure 3.04**). Increasing the temperature of the Fmoc deprotection module from 25 to 30 °C eliminated the Fmoc-protected byproduct (**Figure 3.04 B**). Changing the glycosylation solvent for building block **Fuc-01** from a co-solvent (DCM/Et_2O, 1:3) to DCM also significantly improved the stereoselectivity of the fucosylation (α:β = 32:1, **Figure 3.04 C**).

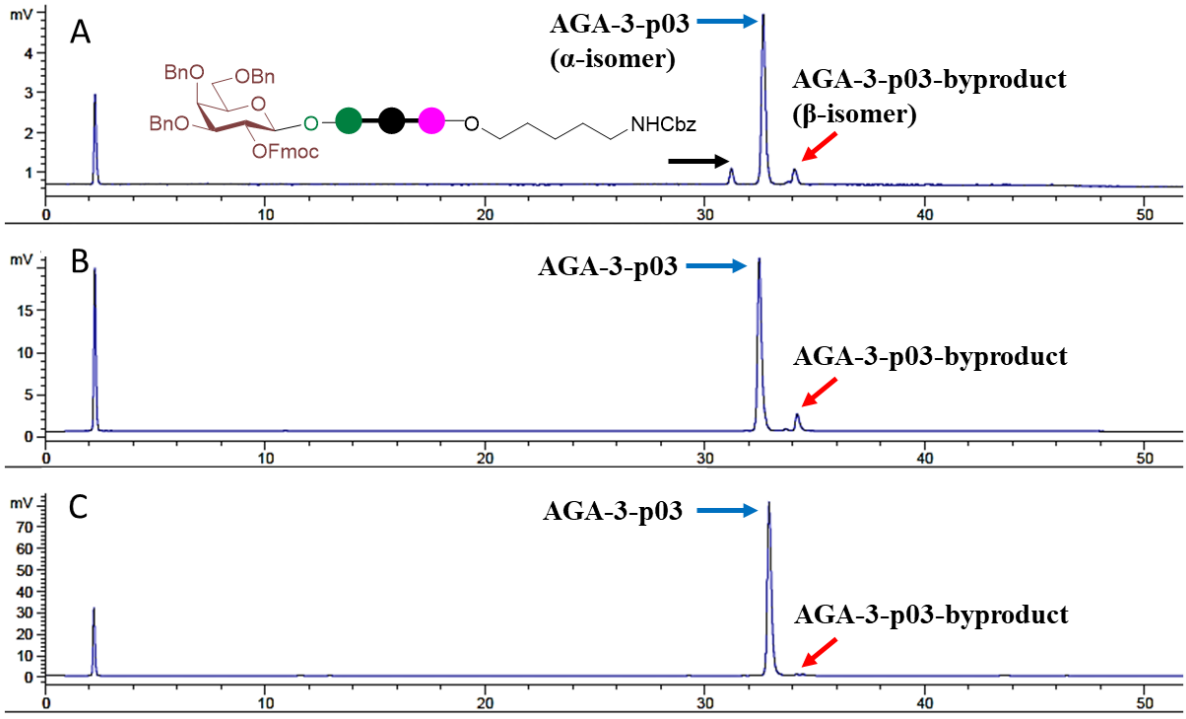


Figure 3.04. NP-HPLC of the protected pentasaccharide **AGA-3-p03**. HPLC was performed using Luna Silica. Conditions: eluents: hexane/ethyl acetate; gradient: 20% (5 min) → 45% (in 25 min) → 100% (in 5 min) at a rate 1 mL/min; detection: ELSD.

H-type II pentasaccharide **AGA-3-p04** was assembled using building blocks **Glc-01**, **Gal-01**, **GlcNAc-01**[109g], **Gal-04**, and **Fuc-03**[116] (**Scheme 3.01**). Prior automated synthetic efforts towards this target were unsuccessful.[111a] Our initial approach utilized fucose building block **Fuc-01** in line with the synthesis of pentasaccharide **AGA-3-p03**. However, this building block worked poorly due to the low reactivity as well as poor stereoselectivity (α:β = 1:1.8) (see 3.4 Experimental Section). Glycosyl phosphate **Fuc-02**, a variant of building block **Fuc-01**, resolved the reactivity issues, but resulted in even worse stereoselectivity (α:β = 1:2.8). Perbenzylated fucose building block **Fuc-03** was used to overcome these problems

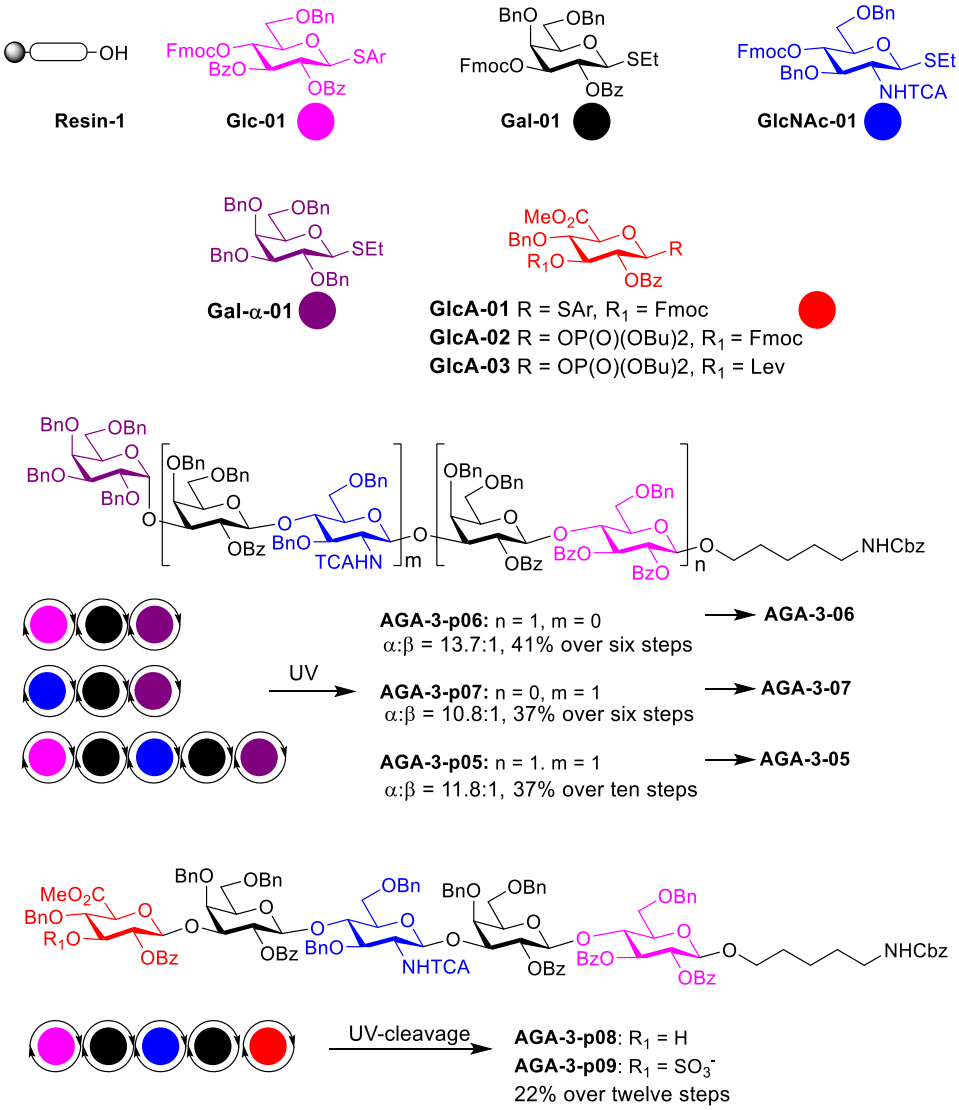
provided the H-type II pentasaccharide **AGA-3-p04** in good yield (24% over ten steps) with good stereoselectivity (α:β = 10:1). Perbenzylated fucose building block **Fuc-02** gave drastically improved stereoselectivity on AGA although 3,4-di-*O*-acetyl fucoside as well as perbenzyl fucoside on fucose are known to provide excellent α-stereoselectivity in solution-phase chemistry.[117]

The automated syntheses of H-type I and II pentasaccharides **AGA-3-p03** and **AGA-3-p04** illustrated that the formation of α(1,2)-fucosidic linkages is dependent upon oligosaccharide connectivity (Galβ1-3GlcNTCA or Galβ1-4GlcNTCA) even with the same sequence (Gal-GlcNTCA-Gal-Glc). The differences in stereoselectivity may be a result of mismatch between the donors and the acceptor on solid support**.**[119] By varying reaction conditions some challenges are overcome while in other cases building blocks have to be changed. By employing "approved" building blocks regarding to target oligosaccharides, protected H-type I (**AGA-3-p03**) and II (**AGA-3-p04**) pentasaccharides were synthesized in good yield with good to excellent stereoselectivity. The conjugation-ready unprotected glycans **AGA-3-01**[110b, 112a] (48**%** over two steps), **AGA-3-02**[110d] (39**%**), and **AGA-3-03**[112a] (46**%**) were obtained by using reverse-phase HPLC following deprotection of oligosaccharides **AGA-3-p01, -p02**, and **-p03** by methanolysis and hydrogenolysis.

3.2.3 Synthesis of α-Gal Epitopes

α-Gal pentasaccharide **AGA-3-05** and two α-Gal trisaccharide epitopes (**AGA-3-06** and **AGA-3-07**) were synthesized to investigate structural influence on 1,2-cis glycosylation (**Scheme 3.02**). Some acceptor sequence dependence on stereoselectivity was observed though the results were not as pronounced as during the synthesis of the H-type oligosaccharides. The three target epitopes were prepared by AGA employing building block **Gal-α-01**[109g] at lower temperature ($\mathbf{T_a}$ = -40 °C, $\mathbf{t_1}$ = 5 min, $\mathbf{T_i}$ = -20 °C, $\mathbf{t_2}$ = 25 min). The oligosaccharide targets were prepared in high yield with good stereoselectivity: pentasaccharide **AGA-3-p05** (37% over ten steps on resin, α:β = 11.8:1), trisaccharide **AGA-3-p06** (41% over six steps on resin, α:β = 13.7:1), and **AGA-3-p07** (38% over six steps on resin, α:β = 10.8:1). Coupling using **Gal-α-01** at higher temperature ($\mathbf{T_a}$ = -10 °C, $\mathbf{t_1}$ = 5 min, $\mathbf{T_i}$ = -10 °C, $\mathbf{t_2}$ = 25 min) led to incomplete glycosylation and the formation of deletion sequence nLc4 **AGA-3-p02** in addition to the desired product α-Gal epitope **AGA-3-p05**. Under these conditions, the decomposition of activated building block **Gal-α-01** was faster than glycosylation of the resin-bound acceptor. A change of solvents from DCM to a diethyl

ether/DCM (3/1) mixture during glycosylations with **Gal-α-01**[109d] had no effect on the stereochemical outcome of the coupling. The automated syntheses of the α-Gal epitopes demonstrated that the installation of a *cis*-galactosidic linkage is sequence dependent. Fully protected three α-Gal epitopes **AGA-3-p05, -p06,** and **–p07** were deprotected to furnish the conjugation-ready glycans **AGA-3-05** (51**%** over two steps), **AGA-3-06** (44**%**), and **AGA-3-07** (37**%**).



Scheme 3.02 Automated Glycan Assembly of the Protected Three α-Gal epitopes **AGA-3-p05**, **AGA-3-p06**, and **AGA-3-p07**, and Non-sulfated and Sulfated HNK-1 **AGA-3-p08** and **AGA-3-p09** oligosaccharide. [a]Reaction conditions: Glycosylation: 2 x 5 equiv of **Glc-01** or **GlcNAc-01**, NIS, TfOH, DCM/dioxane, -30 °C (5 min) → -10 °C (25 min). 2 x 5 equiv of **Gal-01** or **Gal-α-01**, NIS, TfOH, DCM/dioxane, -40 °C (5min) → -20 °C (25 min). 2 x 7.5 equiv of **GlcA-03**, TMSOTf, DCM, -10 °C (5 min) → 0 °C (50 min); Deprotection: three cycle of 20% NEt_3 in DMF, 25 °C; three cycle of hydrazine hydrate (0.56 M in pyridine/acetic acid (v/v, 3:2); Sulfation: three cycle of 0.5 M of sulfur trioxide pyridine complex in DMF/TEA (v/v, 1:1) at 50 °C; UV-cleavage: hv (305 nm); Yields are based on resin loading and obtained by preparative HPLC (detection: ELSD)

3.2.4 Synthesis of HNK-1

Addition of 3-*O*-SO_3-glucuronic acid on the non-reducing end of nLc4 tetrasaccharide backbone **AGA-3-p02** gives rise to HNK-1 **AGA-3-p09** (**Scheme 3.02**). An initial synthetic attempt employed the deactivated glucuronic acid thioglycoside building block **GlcA-01**[120] ($\mathbf{T_a}$ = -20 °C, $\mathbf{t_1}$ = 5 min, $\mathbf{T_i}$ = -10 °C, $\mathbf{t_2}$ = 50 min). Under these conditions, little of the non-sulfated pentasaccharide **AGA-3-p08** but mostly nLc4 **AGA-3-p02** (**AGA-3-p02**: **AGA-3-p08** = 90:10) was obtained. Use of glycosyl phosphate **GlcA-02** using the same coupling cycle based on time and temperature resulted in little improvement (**AGA-3-p02**:**AGA-3-p08** = 84:16). Phosphate building block **GlcA-03** bearing a C-3 levulinoyl ester instead of an Fmoc carbonate improved conversion to **AGA-3-p08** only incrementally (**AGA-3-p02**:**AGA-3-p08** = 64:36). By raising the coupling temperature for building block **GlcA-03** ($\mathbf{T_a}$ = -10 °C, $\mathbf{t_1}$ = 5 min, $\mathbf{T_i}$ = 0 °C, $\mathbf{t_2}$ = 50 min) the conversion of **AGA-3-p02** to **AGA-3-p08** (**AGA-3-p02**:**AGA-3-p08** = 39:61) improved. Finally, using these conditions with 15 equivalents of building block added over three cycles transformed tetrasaccharide backbone **AGA-3-p02** to **AGA-3-p08** in good yield (26% over eleven steps). With the glycosylation conditions of **GlcA-03** optimized, the automated glycan assembly of HNK-1 3-*O*-sulfated pentasaccharide **AGA-3-p09** was performed using a modified, sulfation module with the more reactive SO_3Py in a TEA/DMF rather than the standard pyridine/DMF solution. The crude product was purified by RP-HPLC to afford **AGA-3-p09** (22% over twelve steps).

The HNK-1 synthesis showed that building block design and optimal glycosylation conditions are crucial for achieving high glycosylation efficiencies. Modification of protecting groups (Fmoc to Lev), leaving groups (thioglycoside to glycosyl phosphate), and fine tuning of glycosylation conditions all played a central role in the automated synthesis of the HNK-1 pentasaccharide (**AGA-3-p09**).

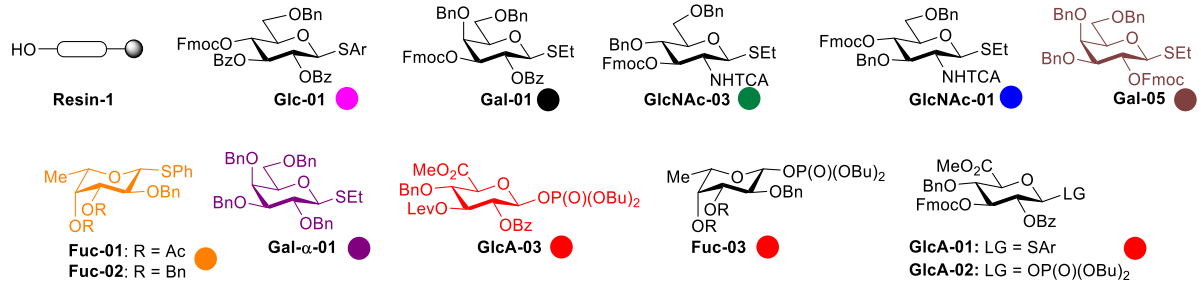
3.3 Conclusions

We describe the automated glycan assembly of fully protected H-type I and II, α-Gal epitopes, and the HNK-1 pentasaccharide using monosaccharide building blocks. During these syntheses, we discovered that the stereoselectivity of 1,2-*cis* glycosidic bond formation depends on the sequence of the resin-bound nucleophile. Glycosylation efficiencies were affected by leaving groups as well as protecting groups in the synthesis of the HNK-1. The identification of "approved" building blocks that are used in defined coupling cycles under conditions adjusted to the building block enables access to challenging oligosaccharides.

3.4 Experimental Section

3.4.1 Pre-Automation Steps

Preparation of the resin equipped with the photolabile linker and building block



A complete set of the resin and building blocks. For details, see appendix B.

3.4.2 Automation: Automated Glycan Assembly

Building Block Solution: For the glycosylation using twice 5 equivalents, 0.25 mmol of building block was dissolved in 2.0 mL of DCM.

Acidic TMSOTf wash Solution: For the acidic TMSOTf wash 480 µL TMSOTf was dissolved in 20 mL DCM.

Activator Solution: For the thioglycoside monomer *N*-Iodosuccinimide (1.35 g) was dissolved in a 9:1 mixture of anhydrous DCM and dioxane (40.0 mL) and then TfOH (60 µL) was added in ice bath. For the phosphate monomer 480 µL TMSOTf was dissolved in 20 mL DCM.

Fmoc Deprotection Solution: Solution of 20% trimethylamine (TEA) in DMF (v/v) was prepared.

Lev deprotection Solution: For Lev deprotection hydrazine hydrate (0.68 mL) was dissolved in pyridine/acetic acid (25 mL, v/v, 3:2) to give a 0.56 M solution.
Sulfation Solution (0.5 M of sulfur trioxide pyridine complex in DMF/TEA): For sulfation sulfur trioxide pyridine complex (1.6 g) was dissolved in DMF/TEA (20 mL, v/v, 1:1).

Preparation of the resin and the synthesizer for automated synthesis: The functionalized resin was loaded into the reaction vessel of the synthesizer and swollen in 2 mL DCM. To start the synthesis sequence, the resin was washed using Module 1. The building blocks were co-evaporated with toluene three times, dissolved in DCM under an argon atmosphere and transferred into the vials that were placed on the corresponding port in the synthesizer. Reagents were dissolved in the corresponding solvents under an Ar atmosphere in bottles that were placed on the corresponding port in the synthesizer. Activation temperature ($\mathbf{T_a}$) and time ($\mathbf{t_1}$), incubation temperature ($\mathbf{T_i}$) and time ($\mathbf{t_2}$) were used.

Building Block	Promotor	T_a (°C)	t_1 (min)	T_i (°C)	t_2 (min)
Glc-01, **GlcNAc-01**, and **GlcNAc-03**	NIS/TfOH	- 30	5	- 10	25
Gal-01, Gal-04, Fuc-01, Fuc-03, and **Gal-α-01**		- 40	5	- 20	25
GlcA-01		- 20	5	- 10	50
Fuc-02	TMSOTf	- 40	5	- 20	25
GlcA-02		- 10	5	0	50
GlcA-03		- 20	5	- 10	50

Sequence	Module	Details	Condition
I	1	2.5 eq. of TMSOTf solution	-20 °C, for 1 min
	2	5 eq. building block (**Glc-01**, **Gal-01**, **Gal-01-p**, **Gal-04**, **GlcNAc-01**, **GlcNAc-03**, and **GlcA-01**), 5 eq. of NIS Solution	
	3	Fmoc Removal	r.t for 5 min
II	1	2.5 eq. of TMSOTf solution	-20 °C, for 1 min
	4-1	5 eq. building block (**Gal-02** and **GlcA-02**) , 5 eq. of TMSOTf Solution	
	3	Fmoc Removal	r.t for 5 min
III	1	2.5 eq. of TMSOTf solution	-20 °C, for 1 min
	2	5 eq. building block (**Fuc-01**, **Fuc-03**, and **Gal-α-01**) 5 eq. of NIS Solution	
III	1	2.5 eq. of TMSOTf solution	-20 °C, for 1 min
	2	5 eq. building block (**Fuc-02**), 5 eq. of TMSOTf solution	
IV	1	2.5 eq. of TMSOTf solution	-20 °C, for 1 min
	4-1	5 eq. building block **GlcA-03**, 5 eq. of TMSOTf Solution	
	5	Lev Removal	r.t for 5 min
V	1	2.5 eq. of TMSOTf solution	-20 °C, for 1 min
	4-2	7.5 eq. building block **GlcA-03**, 7.5 eq. of TMSOTf Solution	
	5	Lev Removal	r.t for 5 min

Module 1 – Acidic TMSOTf Wash: The resin was washed with DMF, THF, DCM (three times each, with 2 mL for 25 s), and 0.350 mL of solution of TMSOTf in DCM once at -20 °C. The resin was swollen in 2 mL CH_2Cl_2 and the temperature of the reaction vessel is adjusted to $\mathbf{T_a}$.

Module 2 – Glycosylation using thioglycoside: For glycosylation the DCM was drained and a solution of thioglycoside building block (5 eq. in 1.0 mL DCM) was delivered to the reaction vessel. After the set temperature was reached ($\mathbf{T_a}$), the reaction starts with the addition of 1 mL of NIS (5 eq. in 1.0 mL DCM), and TfOH (0.1 eq. in 1.0 mL DCM) solution. The glycosylation was performed for $\mathbf{t_1}$ at $\mathbf{T_a}$ and for $\mathbf{t_2}$ at $\mathbf{T_i}$. After the reaction the solution was drained and the resin was washed with DCM (six times with 2 mL for 15 s). This procedure was repeated **twice.**

Module 3 - Fmoc Deprotection: The resin was washed with DMF (six times with 2 mL for 15 s), swollen in 2 mL DMF and the temperature of the reaction vessel was adjusted to 25°C. For Fmoc deprotection the DMF was drained and 2 mL of a solution of 20% Et_3N in DMF is delivered to the reaction vessel. After 5 min the reaction solution was collected in the fraction collector of the oligosaccharide synthesizer and 2 mL of a solution of 20% Et_3N in DMF was delivered to the resin. This procedure was repeated three times.

Module 4-1 - Glycosylation using phosphate: For glycosylation DCM was drained and a solution of phosphate building block (5 eq. in 1.0 mL DCM) was delivered to the reaction vessel. After the set temperature was reached ($\mathbf{T_a}$), the reaction starts with the addition of 1 mL of solution of TMSOTf. The glycosylation was performed for $\mathbf{t_1}$ at $\boldsymbol{T_a}$ and for $\mathbf{t_2}$ at $\boldsymbol{T_i}$. After the reaction the solution is drained and the resin was washed with DCM (six times with 2 mL for 15 s). This procedure was repeated **twice.**

Module 4-2 - Glycosylation using phosphate: For glycosylation DCM is drained and a solution of phosphate building block (BB) (5 eq. in 1.0 mL DCM) was delivered to the reaction vessel. After the set temperature was reached ($\mathbf{T_a}$), the reaction starts with the addition of 1 mL of solution of TMSOTf. The glycosylation is performed for $\mathbf{t_1}$ at $\boldsymbol{T_a}$ and for $\mathbf{t_2}$ at $\boldsymbol{T_i}$. After the reaction the solution was drained and the resin is washed with DCM (six times with 2 mL for 15 s). This procedure was repeated **three times.**

Module 5 – Lev deprotection: The resin was washed with DCM (six times with 2 mL for 25 s), swollen in 1.3 mL DCM and the temperature of the reaction vessel was adjusted to 25 °C. For Lev deprotection 0.8 mL of the hydrazine hydrate solution was delivered into the reaction vessel. After 30 min the reaction solution was drained and the resin was washed with 0.2 M acetic acid in DCM and DCM (six times each with 2 mL for 25 s). The entire procedure was performed **three times**.

Module 6 – Sulfation: The resin was washed with DMF and triethylamine (three times each with 2 mL for 15 s), swollen in 2 mL triethylamine and the temperature of the reaction vessel was adjusted to 50 °C. For sulfation, 2 mL of a 0.5 M solution of sulfur trioxide pyridine complex in DMF/triethylamine (v/v, 1:1) was added. After 1 h, the reaction solution was drained and the resin was washed with DMF and triethylamine (three times each with 2 mL for 15 s). The entire procedure was performed for three times.

3.4.3 Post-Automation Steps

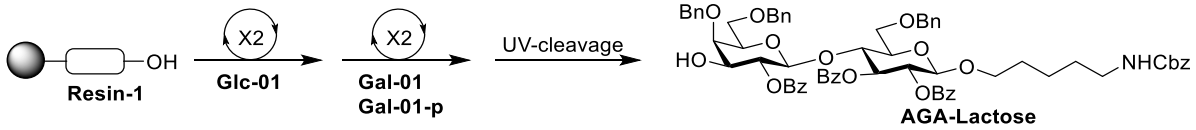
Cleavage and Purification

Resin Cleavage: For the cleavage, the resin is slowly injected from the disposable syringe (20 mL) into the reactor and pushed through the tubing with 18 mL DCM (flow rate: 600 µL/min). The tubing is washed with 20 mL DCM (flow rate: 2 mL/min) to remove any remaining resin. The suspension leaving the reactor is directed into a filter where the resin is filtered off. The entire procedure is performed twice. The resulting solution is evaporated and the crude material was analyzed by NMR and HPLC.

Analytical HPLC: The crude material was analyzed by HPLC (column: Luna 5µ Silica 100A, (260 X 4.60 mm); flow rate: 1 mL/min; eluents: hexane/ ethyl acetate; gradient: 20% (5 min) 60% (in 40 min) 100% (in 5 min); detection: 280 nm, and ELSD).

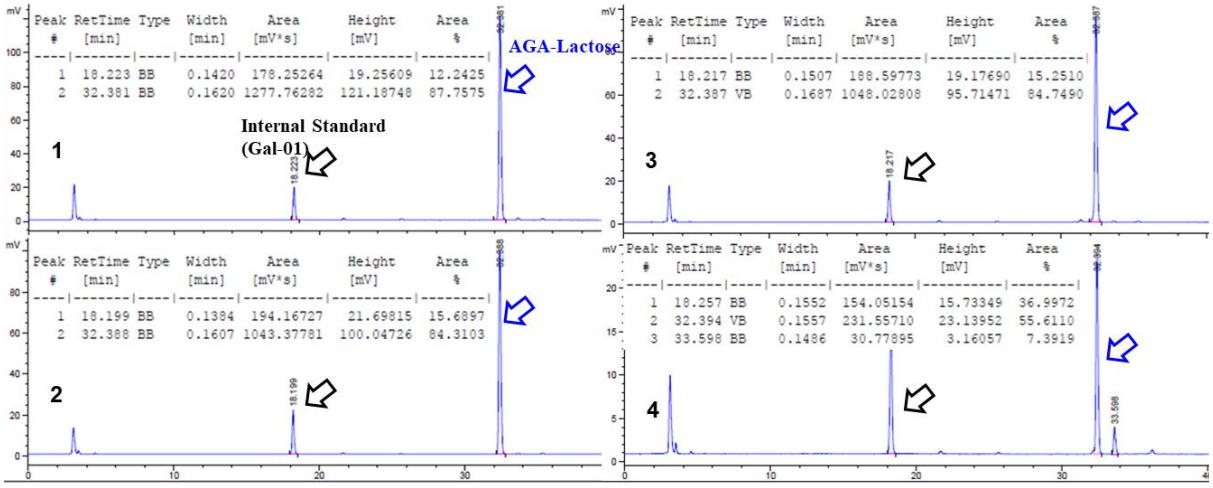
Preparative HPLC: The crude mixture was carefully dissolved in a minimum volume of DCM and 0.9 mL of 20% hexane in ethyl acetate. The crude solution was injected for purification using semi-preparative HPLC (column: Luna 5µ Sil (260 X 10 mm); flow rate: 5 mL/min; eluents: 5% DCM in hexane/5% DCM in ethyl acetate; gradient: 20% (5 min) 60% (in 40 min) 100% (in 5 min); detection: 280 nm, and ELSD) to afford the fully protected target oligosaccharide.

Stability Test: Automated synthesis of lactose disaccharide 5 under various conditions



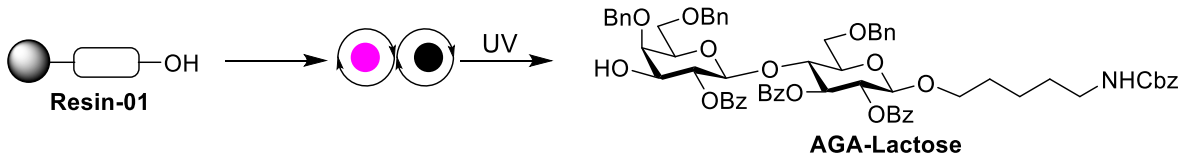
entry	BB 2		BB 3			BB 4			TMSOTf		Yield
	T_a	T_i	T_a	T_{i-1}	T_{i-2}	T_a	T_{i-1}	T_{i-2}	T_a	T_i	
1	-30	-10	-40	-20							42%
2	-30	-10	-40	-20	20						40%
3	-30	-10				-40	-20	20			36%
4	-30	-10	-40	-20					20	20	14%

Automated synthesis of disaccharide **AGA-Lactose**. Conditions: activation temperature ($\mathbf{T_a}$), incubation temperature ($\mathbf{T_i}$), the first incubation temperature ($\mathbf{T_{i-1}}$) for entry 2 and 3, the second incubation temperature ($\mathbf{T_{i-2}}$) for entry 2 and 3.



LC-MS of **AGA-Lactose** including building block **Gal-01** as an Internal standard.

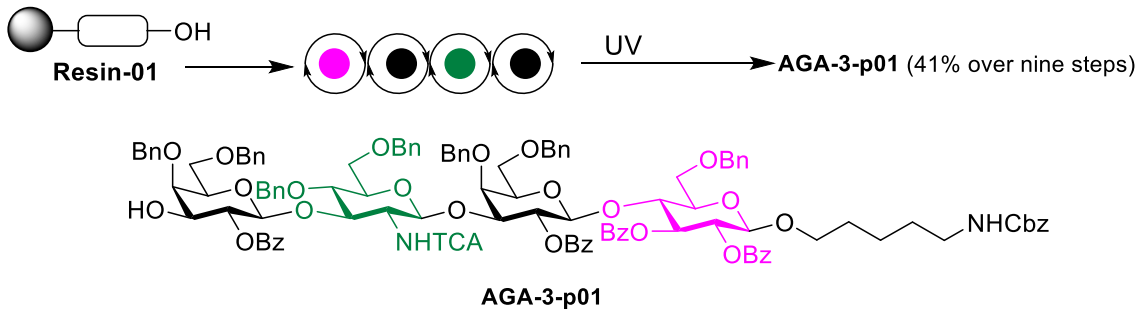
N-Benzyloxycarbonyl-5-amino-pentyl (2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranoside AGA-Lactose



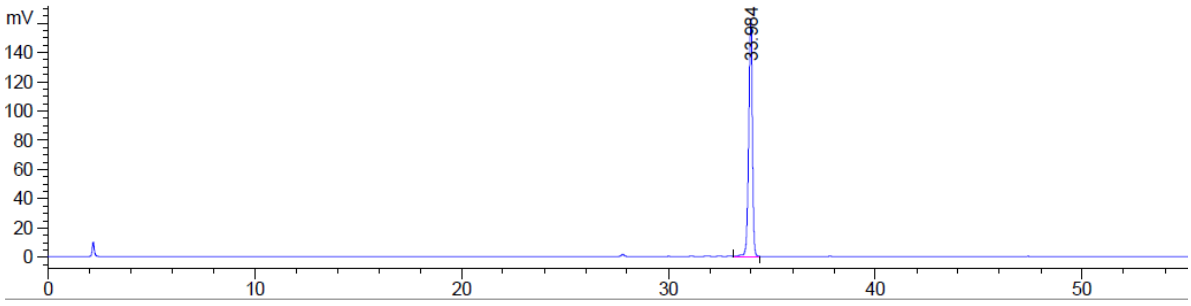
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 – 7.89 (m, 6H), 7.58 – 7.53 (m, 1H), 7.49 – 7.38 (m, 4H), 7.37 – 7.27 (m, 18H), 7.23 (dt, J = 8.3, 5.3 Hz, 6H), 5.59 (t, J = 9.4 Hz, 1H, H-3), 5.35 (dd, J = 9.8, 8.0 Hz, 1H, H-2), 5.11 (dd, J = 10.0, 7.9 Hz, 1H, H'-2), 5.06 (s, 2H, Cbz), 4.59 (d, J = 12.2 Hz, 1H, C*H*HPh), 4.57 – 4.49 (m, 5H, **H-1**, **H'-1**, CH_2Ph, NH), 4.36 (d, J = 12.2 Hz, 1H, C*H*HPh), 4.17 – 4.08 (m, 3H, H-4, 2 x C*H*HPh), 3.86 – 3.80 (m, 1H, OC*H*H, linker), 3.75 (d,

J = 3.4 Hz, 1H, H'-4), 3.71 (dd, J = 10.9, 3.9 Hz, 1H, H-6), 3.61 (dd, J = 10.9, 1.6 Hz, 1H, H-6), 3.56 – 3.49 (m, 2H, H-5, H'-3), 3.40 (dd, J = 15.5, 6.8 Hz, 1H, OC*H*H, linker), 3.31 (dd, J = 9.3, 5.2 Hz, 1H, H'-5), 2.96 (dd, J = 9.0, 4.9 Hz, 1H, H'-6), 2.91 (td, J = 12.7, 6.3 Hz, 2H, CH_2NHCbz), 2.85 (t, J = 9.2 Hz, 1H, H'-6), 2.24 (d, J = 10.4 Hz, 1H, OH), 1.48 (ddd, J = 20.5, 12.9, 6.5 Hz, 2H), 1.34 – 1.24 (m, 2H), 1.23 – 1.10 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.23 (Bz), 165.34 (Bz), 165.31 (Bz), 156.34 (Cbz), 138.22, 138.16, 137.73, 136.76, 133.32, 133.25, 132.77, 130.45, 129.98, 129.91, 129.84, 129.77, 129.57, 128.61, 128.58, 128.57, 128.51, 128.47, 128.22, 128.18, 127.99, 127.94, 127.87, 127.77, 127.67 (Ar), 101.15 (**C-1**), 100.63 (**C'-1**), 76.02 (C'-4), 75.61 (C-4), 75.14 (CH_2Ph), 74.72 (C'-3), 74.29 (C'-2), 73.63 (C-3), 73.54 (CH_2Ph), 73.21 (CH_2Ph), 72.93 (C-5), 72.74 (C'-5), 72.03 (C-2), 69.86 (OCH_2, linker), 67.82 (C-6), 66.89 (C'-6), 66.60 (Cbz), 40.88 (*C*H_2NHCbz), 29.47, 28.97, 23.15. MS ESI+-HRMS *m/z* $[M+Na]^+$ calcd for $C_{67}H_{69}O_{16}NNa$ 1166.4509, found 1166.1548.

Automated Synthesis of AGA-3-p01



AGA-3-p01

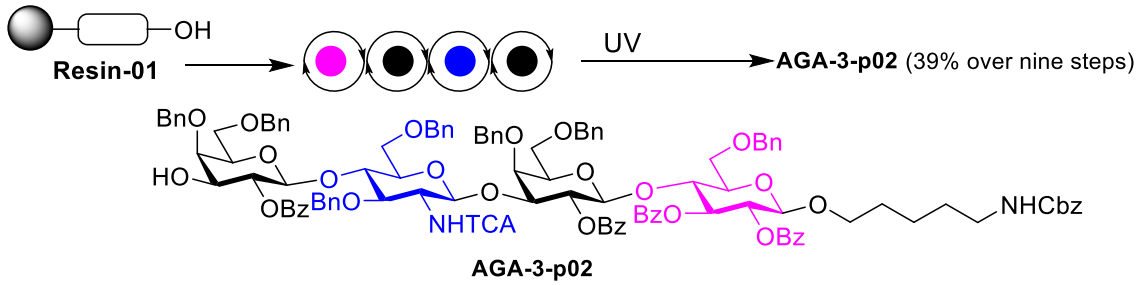


LC-MS of **AGA-3-p01**.

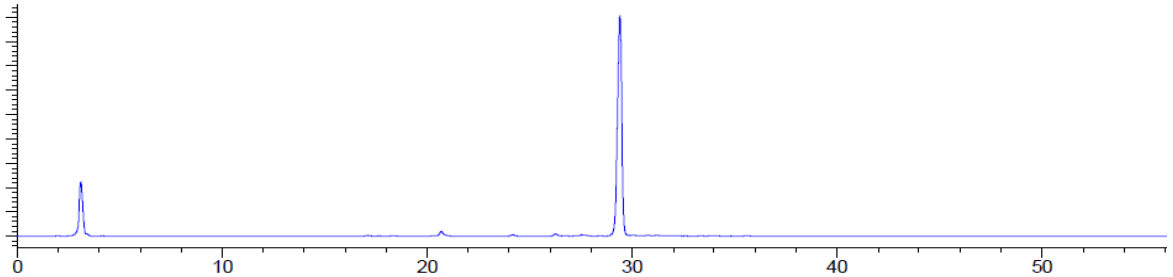
***N*-Benzyloxycarbonyl-5-amino-pentyl (2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-(4,6-di-*O*-benzyl-2-deoxy-2-trichloracetamido-β-D-glucopyranosyl)-(1→3)-(2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranoside AGA-3-p01**

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.04 (d, *J* = 7.2 Hz, 2H), 7.91 (d, *J* = 7.3 Hz, 2H), 7.85 (dd, *J* = 8.2, 7.3 Hz, 4H), 7.50 (td, *J* = 7.1, 1.1 Hz, 2H), 7.46 (t, *J* = 7.4 Hz, 1H), 7.40 – 7.18 (m, 37H), 7.18 – 7.08 (m, 12H), 6.60 (t, *J* = 6.8 Hz, 1H, NHTCA), 5.53 (t, *J* = 9.4 Hz, 1H, H-3), 5.31 (dt, *J* = 17.6, 8.9 Hz, 2H, 2 x H-2), 5.23 (dd, *J* = 10.0, 8.0 Hz, 1H, H-2), 5.06 (s, 2H, CH_2, Cbz), 4.90 (d, *J* = 10.4 Hz, 1H, C*H*HPh), 4.76 (d, *J* = 7.9 Hz, 1H, **H-1**), 4.74 – 4.59 (m, 4H, **H-1**, 3 x C*H*HPh), 4.57 – 4.53 (m, 2H, C*H*HPh, NHCbz), 4.48 (d, *J* = 7.9 Hz, 1H, **H-1**), 4.46 – 4.28 (m, 8H, **H-1**, 6 x C*H*HPh), 4.23 (d, *J* = 12.3 Hz, 1H), 4.05 (dt, *J* = 9.3, 8.5 Hz, 3H), 3.88 (dd, *J* = 8.0, 3.0 Hz, 2H), 3.82 – 3.74 (m, 2H), 3.70 (dd, *J* = 10.2, 1.7 Hz, 1H), 3.65 – 3.59 (m, 2H), 3.57 – 3.49 (m, 3H), 3.47 – 3.41 (m, 3H), 3.36 (d, *J* = 9.4 Hz, 3H), 3.31 (dd, *J* = 8.4, 5.1 Hz, 1H), 3.09 (d, *J* = 7.5 Hz, 1H, H-2), 2.90 (dt, *J* = 14.3, 7.3 Hz, 3H, H-6, CH_2NHCbz), 2.76 (t, *J* = 8.7 Hz, 1H, H-6), 2.41 (d, *J* = 7.9 Hz, 1H, OH), 1.52 – 1.36 (m, 2H, CH_2, pentane), 1.34 – 1.21 (m, 2H, CH_2, pentane), 1.20 – 1.05 (m, 2H, CH_2, pentane). ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.10 (Bz), 165.38 (Bz), 165.31 (Bz), 164.66 (Bz), 161.28 (TCA), 156.36 (Cbz), 139.31, 138.21, 138.10, 138.06, 137.75, 136.84, 133.58, 133.30, 133.16, 132.49, 130.59, 130.03, 130.00, 129.97, 129.90, 129.81, 129.70, 129.62, 128.65, 128.61, 128.59, 128.57, 128.55, 128.51, 128.50, 128.47, 128.43, 128.24, 128.20, 128.17, 128.06, 127.98, 127.96, 127.93, 127.88, 127.79, 127.77, 127.70, 127.17 (Ar), 101.05 (C-1), 100.83 (C-1), 99.72 (C-1), 98.95 (C-1), 92.37 (CCl_3), 77.81, 76.70, 76.41, 76.35, 75.68, 75.08, 74.86, 74.77, 74.68, 74.49, 73.59, 73.48, 73.42, 73.28, 73.16, 73.01, 72.67, 72.08, 69.73, 69.32, 67.83, 67.53, 67.32, 66.60, 59.37, 40.91, 29.83, 29.47, 28.97, 23.15.; MS ESI+-HRMS m/z $[M+Na]^+$ calcd for $C_{116}H_{117}N_2O_{27}$ 2097.6807, found 2097.6765.

Automated Synthesis of AGA-3-p02



AGA-3-p02



Lc-MS of **AGA-3-p02**

***N*-Benzyloxycarbonyl-5-amino-pentyl (2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-(3,6-di-*O*-benzyl-2-deoxy-2-trichloracetamido-β-D-glucopyranosyl)-(1→3)-(2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranoside AGA-3-p02**

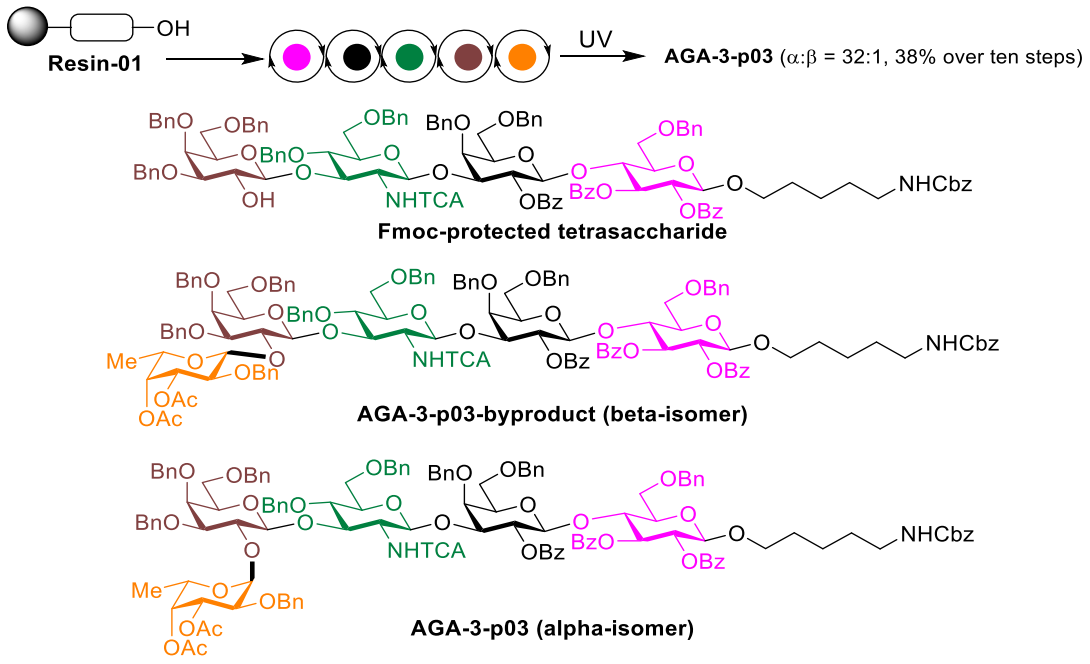
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, *J* = 7.8 Hz, 2H), 7.90 (t, *J* = 6.7 Hz, 4H), 7.84 (d, *J* = 7.9 Hz, 2H), 7.56 (t, *J* = 7.4 Hz, 1H), 7.51 (t, *J* = 7.5 Hz, 1H), 7.47 – 7.02 (m, 50H), 6.54 (d, *J* = 8.0 Hz, 1H, NHTCA), 5.55 (t, *J* = 9.3 Hz, 1H, H-3), 5.38 (dd, *J* = 9.9, 8.0 Hz, 1H, H-2), 5.29 (t, *J* = 8.7 Hz, 1H, H-2), 5.18 (dd, *J* = 9.8, 8.1 Hz, 1H, H-2), 5.06 (s, 2H, CH_2, Cbz), 4.87 (d, *J* = 10.7, 1H, C*H*HPh), 4.86 (d, *J* = 11.9 Hz, 1H, C*H*HPh), 4.75 (d, *J* = 7.6 Hz, 1H, **H-1**), 4.65 (s, 2H, CH_2Ph), 4.58 (d, *J* = 7.9 Hz, 1H, **H-1**), 4.53 – 4.41 (m, 6H, **2 x H**-1, 3 x C*H*HPh), 4.39 – 4.31 (m, 2H, 2 x C*H*HPh), 4.26 (dd, *J* = 11.9, 5.0 Hz, 3H, 3 x C*H*HPh), 4.07 – 3.97 (m, 3H, 2 x C*H*HPh), 3.95 (d, *J* = 8.0 Hz, 1H), 3.89 (s, 2H), 3.82 – 3.58 (m, 5H), 3.56 – 3.24 (m, 11H), 2.89 (dd, *J* = 8.6, 4.7 Hz, 3H, CH_2NHCbz, H-6), 2.82 (t, *J* = 8.7 Hz, 1H, H-6), 1.53 – 1.35 (m, 2H, CH_2, pentane), 1.35 – 1.23 (m, 2H, CH_2, pentane), 1.22 – 1.08 (m, 2H, CH_2, pentane). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.31 (Bz), 165.35 (Bz), 165.30 (Bz), 164.64 (Bz), 161.70 (TCA), 156.38 (Cbz), 139.27, 138.46, 138.36, 138.20, 138.17, 138.00, 137.79, 136.87, 133.45, 133.34, 133.15, 132.48, 130.61, 130.01, 129.95, 129.89, 129.83, 129.75, 128.70, 128.66, 128.61, 128.58, 128.45, 128.15, 128.12, 128.10, 128.04, 128.01, 127.90, 127.83, 127.80, 127.74, 127.67, 127.37, 127.19 (Ar), 101.07 (C-1), 101.00 (C-1), 100.43 (C-1), 100.21 (C-1), 92.18 (CCl_3), 79.23, 77.77, 77.36, 76.56, 76.50, 76.04, 75.61, 75.22, 75.02, 74.82, 74.57, 74.31, 73.60, 73.48, 73.40, 73.16, 73.02, 72.91, 72.53, 72.20, 69.72, 68.28, 67.80, 67.69, 67.39, 66.61, 57.96, 40.95, 29.50, 29.01, 23.17.; MS ESI+-HRMS m/z $[M+Na]^+$ calcd for $C_{116}H_{117}N_2O_{27}$ 2097.6807, found 2097.6811.

Automated Synthesis of AGA-3-p03

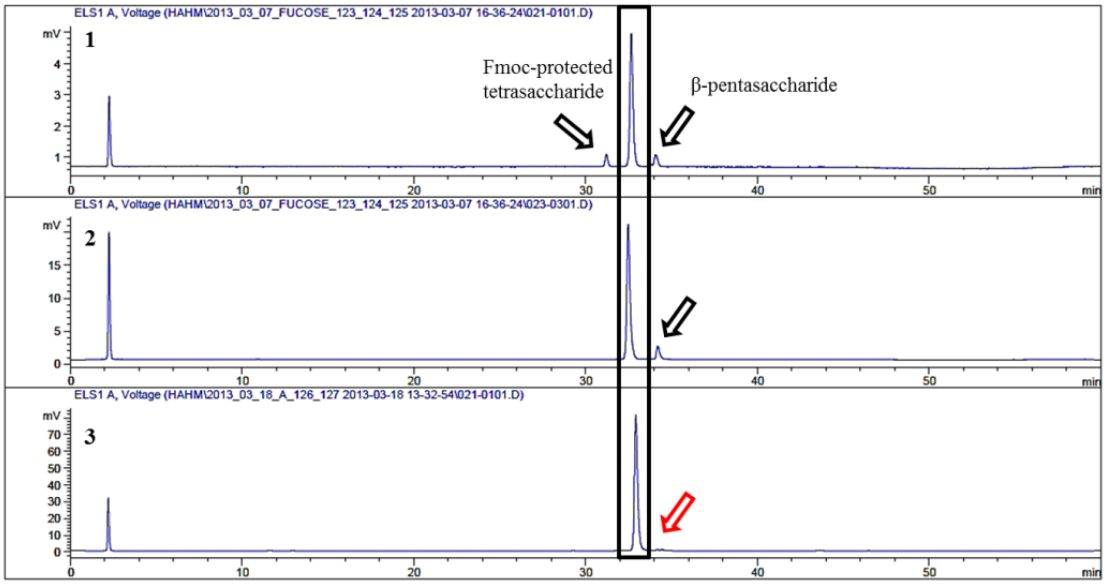
***N*-Benzyloxycarbonyl-5-amino-pentyl (3,4-di-*O*-acetyl-2-*O*-benzoyl-α-L-fucopyranosyl)-(1→2)-(4,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-(4,6-di-*O*-benzyl-2-deoxy-2-trichloracetamido-β-D-glucopyranosyl)-(1→3)-(2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranoside AGA-3-p03**

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.91 (d, *J* = 7.6 Hz, 2H), 7.87 (d, *J* = 4.4 Hz, 2H), 7.81 (d, *J* = 7.3 Hz, 2H), 7.66 (s, 1H), 7.50 (t, *J* = 6.7 Hz, 1H), 7.45 (t, *J* = 7.3 Hz, 1H), 7.42 – 7.08 (m,

52H), 7.02 (dd, *J* = 9.0, 6.0 Hz, 4H), 5.64 (d, *J* = 3.0 Hz, 1H, **H$_{Fuc}$-1**), 5.56 – 5.43 (m, 3H, H-3, H$_{Fuc}$-3, H'-2), 5.30 (dd, *J* = 11.5, 5.9 Hz, 1H, H-2), 5.20 (d, *J* = 1.3 Hz, 1H, H$_{Fuc}$-4), 5.15 (d, *J* = 12.0 Hz, 1H, C*H*HPh), 5.06 (s, 2H, CH$_2$, Cbz), 4.93 (d, *J* = 9.6 Hz, 1H, C*H*HPh), 4.88 (d, *J* = 11.3 Hz, 1H, C*H*HPh), 4.78 (dd, *J* = 12.4, 6.0 Hz, 2H, H$_{Fuc}$-5), 4.66 (d, *J* = 11.9 Hz, 1H, C*H*HPh), 4.55 – 4.44 (m, 6H, **H-1**, 4 x C*H*HPh), 4.41 – 4.28 (m, 8H, **2 x H-1**, 6 x C*H*HPh), 4.22 (dd, *J* = 29.7, 11.8 Hz, 2H, 2 x C*H*HPh), 4.15 – 4.10 (m, 1H), 4.04 (dt, *J* = 9.0, 8.1 Hz, 3H, 2 x C*H*HPh), 3.95 (d, *J* = 6.4 Hz, 3H, 2 x H-4), 3.81 – 3.71 (m, 4H), 3.54 (dd, *J* = 19.4, 10.9 Hz, 2H), 3.46 – 3.30 (m, 10H), 3.12 (s, 1H), 2.93 – 2.85 (m, 3H, H-6, CH$_2$NHCbz), 2.82 (t, *J* = 8.7 Hz, 1H, H-6), 2.10 (s, 3H, Ac), 1.57 (s, 3H, Ac), 1.51 – 1.38 (m, 2H, CH$_2$, pentane), 1.33 – 1.21 (m, 2H, CH$_2$, pentane), 1.19 – 1.10 (m, 2H, CH$_2$, pentane), 1.08 (d, *J* = 6.5 Hz, 3H, H$_{Fuc}$-6). ^{13}C NMR (150 MHz, CDCl$_3$) δ 171.37 (Ac), 170.51 (Ac), 165.36 (Bz), 165.34 (Bz), 164.57 (Bz), 162.19 (TCA), 156.37 (Cbz), 139.88, 139.04, 138.25, 138.15, 137.98, 133.12, 133.02, 132.39, 130.67, 130.18, 130.00, 129.85, 129.78, 128.93, 128.62, 128.56, 128.52, 128.48, 128.45, 128.28, 128.24, 128.20, 128.17, 128.05, 128.02, 127.92, 127.84, 127.68, 127.57, 127.48, 127.17, 126.86, 126.82 (Ar), 101.40 (C-1), 101.27 (C-1), 101.08 (C-1), 98.46 (C-1), 96.50 (C$_{Fuc}$-1), 92.28 (CCl$_3$), 84.11, 77.85, 77.65, 76.64, 75.45, 75.21, 75.00, 74.89, 74.74, 73.65, 73.55, 73.27, 73.24, 73.15, 72.95, 72.63, 72.44, 72.13, 72.04, 71.61, 69.73, 69.12, 67.72, 67.51, 66.61, 64.98, 60.78, 40.94, 29.49, 28.99, 23.18, 20.84, 20.39, 15.81 (C$_{Fuc}$-6).; MS ESI+-HRMS m/z [M+Na]$^+$ calcd for $C_{133}H_{139}N_2O_{32}Cl_3Na$ 2403.8269, found 2403.8303.



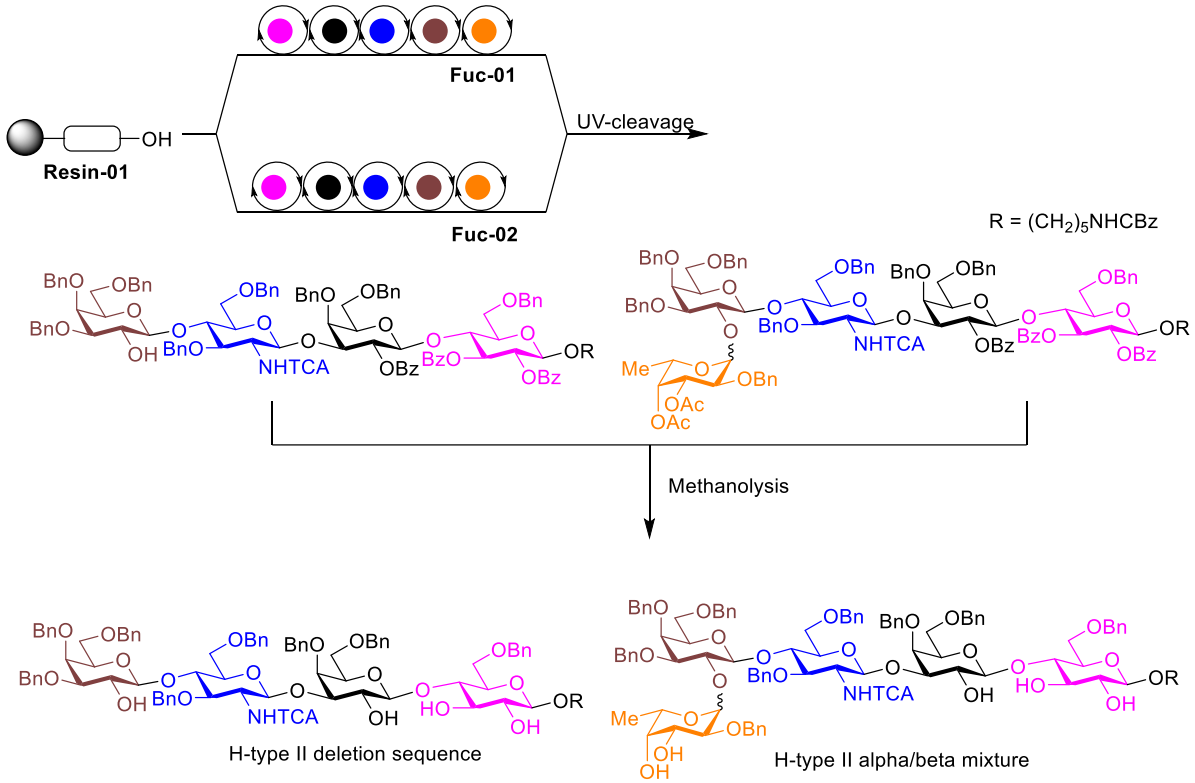
Automated synthesis of **AGA-3-p03**



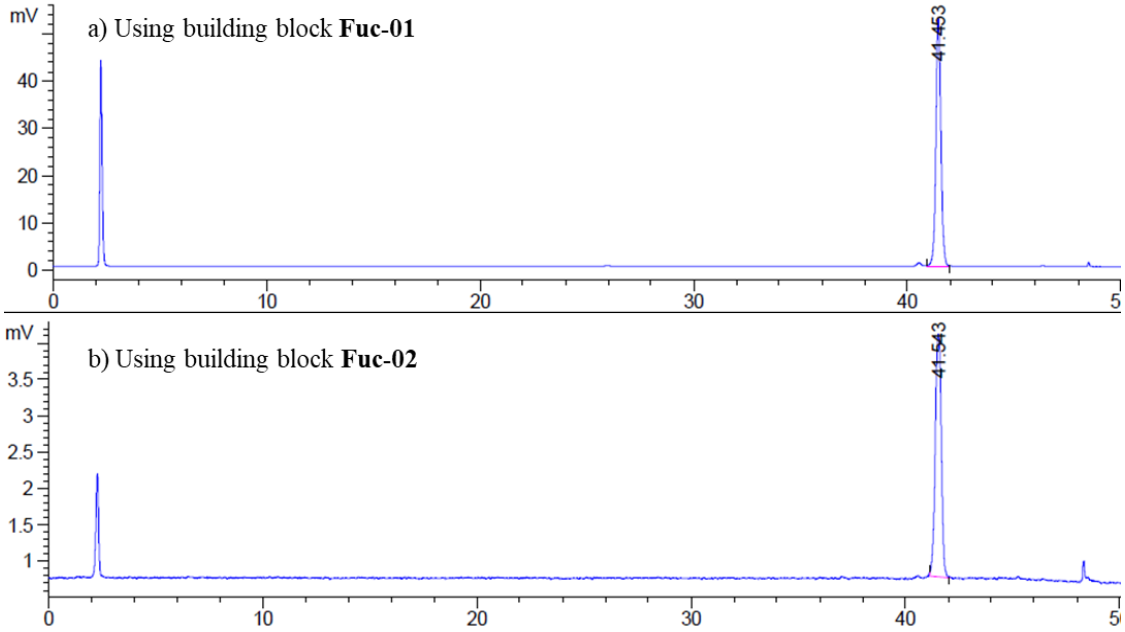
LC-MS of pentasaccharide **AGA-3-p03**. Condition: **Fuc-01** dissolved in DCM and Et_2O (v/v, 1/3) for entry 1 and 2. **Fuc-01** dissolved in DCM for entry 3.

Automated Synthesis of H-type II

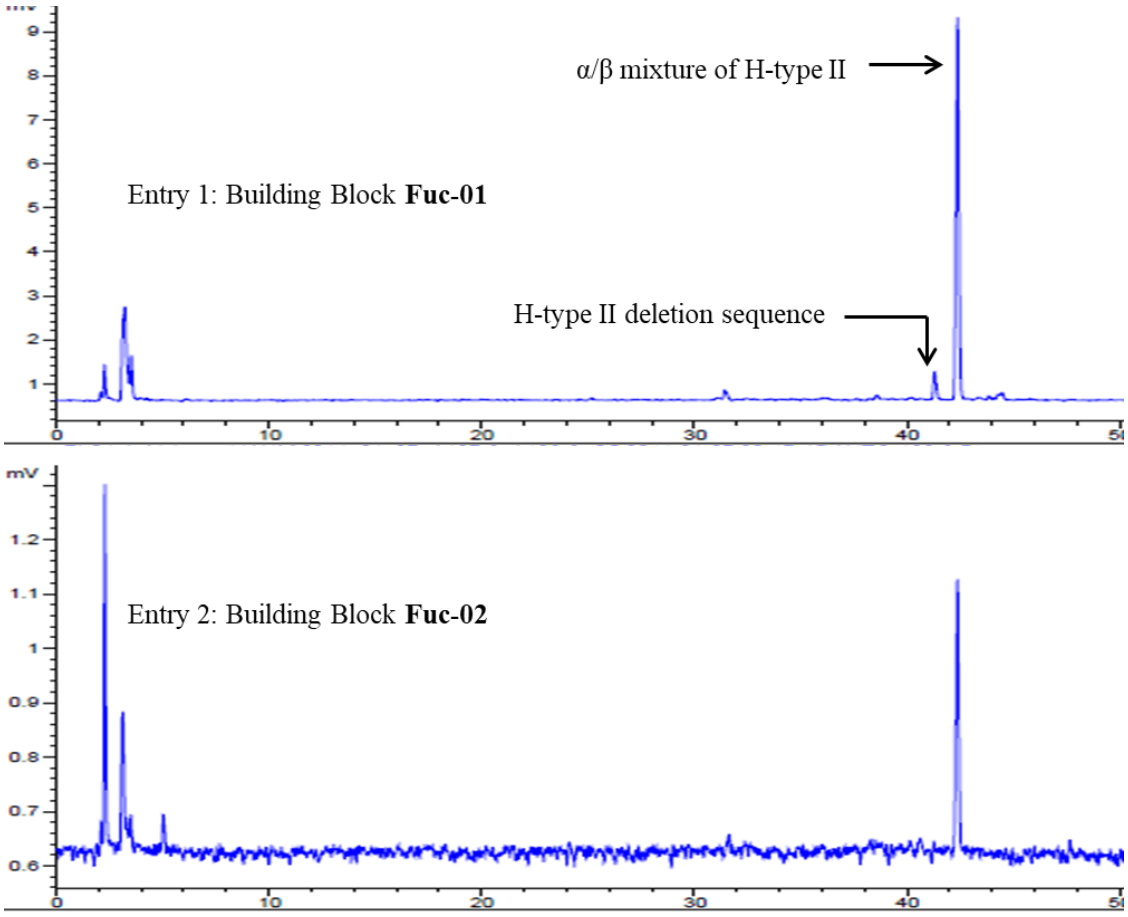
Automated Synthesis of H-type II with building block Fuc-01 and Fuc-02



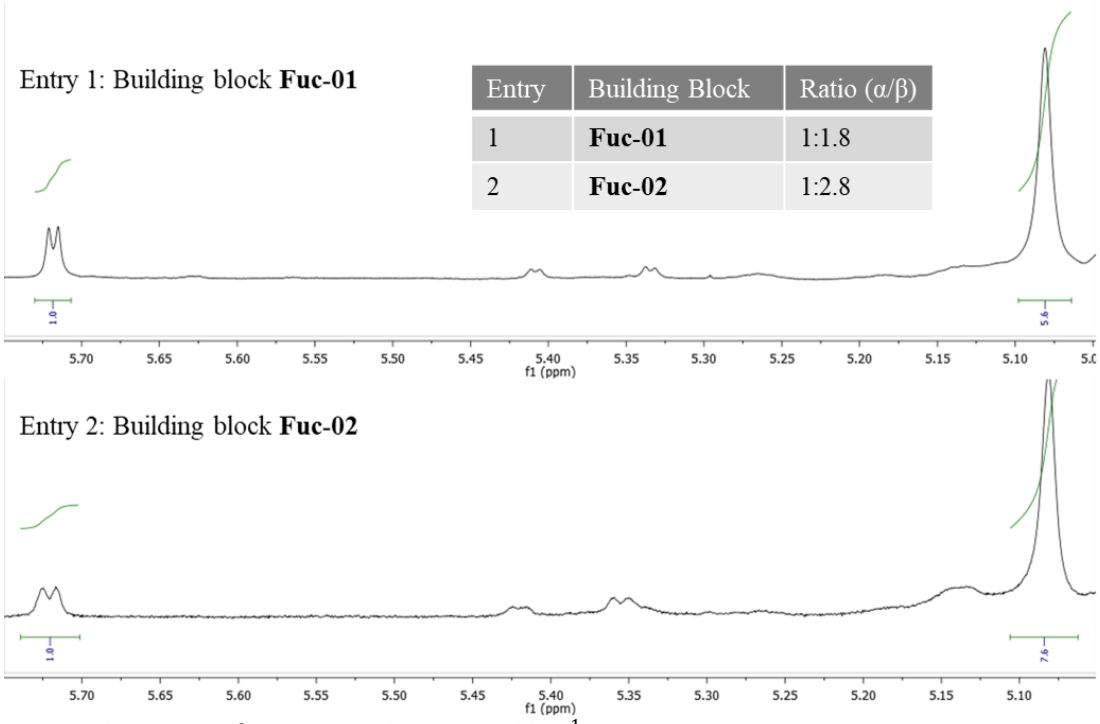
Automated synthesis of protected H-type II.



LC-MS of H-type II using fucose building block **Fuc-01** and **Fuc-02.** The mixture was not separable.



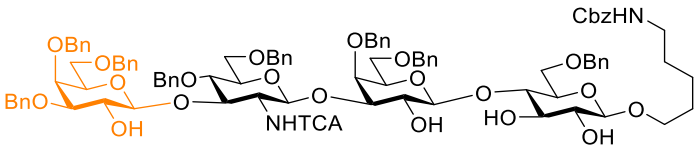
LC-MS of **H-type II deletion sequence and H-type II alpha/beta mixture**. Conditions: column: Luna C5 100A, (260 x 4.60 mm); flow rate: 1 mL/min; eluents: TDW/acetonitrile; gradient: 50% (5 min) 100% (in 40 min) 100% (in 5 min); detection: ELSD.



Entry	Building Block	Ratio (α/β)
1	**Fuc-01**	1:1.8
2	**Fuc-02**	1:2.8

Stereoselectivity of H-type II determined by ^{1}H NMR.

***N*-Benzyloxycarbonyl-5-amino-pentyl (3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-(4,6-di-*O*-benzyl-2-deoxy-2-trichloracetamido-β-D-glucopyranosyl)-(1→3)-(4,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-6-*O*-benzyl-β-D-glucopyranoside H-type II deletion sequence**

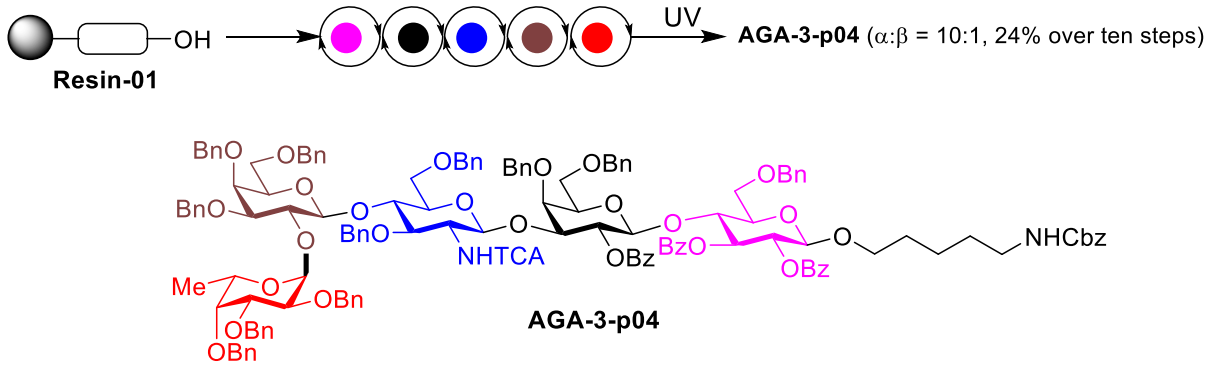


H-type II deletion sequence

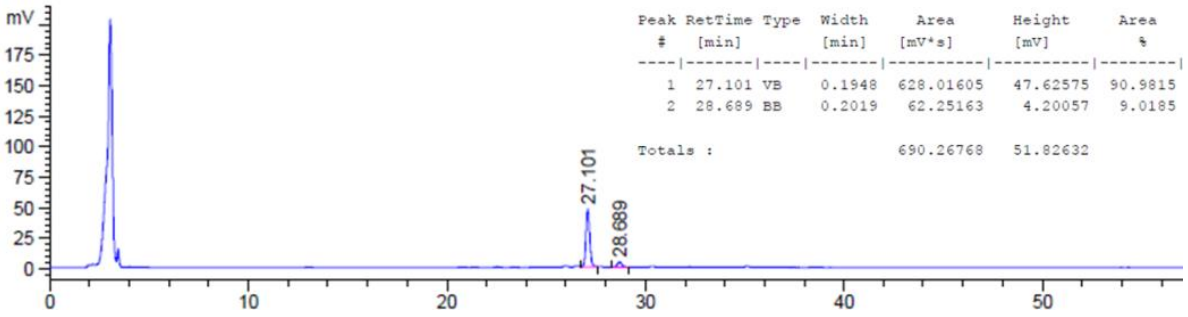
^{1}H NMR (600 MHz, $CDCl_3$) δ 7.39 – 7.13 (m, 45H, Ar), 7.01 (d, *J* = 7.9 Hz, 1H, NHTCA), 5.08 (s, 2H, CH_2, Cbz), 5.02 (d, *J* = 7.8 Hz, 1H), 4.93 (d, *J* = 10.9 Hz, 1H), 4.88 – 4.84 (m, 2H), 4.80 (s, 1H), 4.70 (d, *J* = 11.9 Hz, 1H), 4.64 – 4.57 (m, 4H), 4.54 (dd, *J* = 11.2, 6.4 Hz, 4H), 4.47 – 4.42 (m, 2H), 4.37 (d, *J* = 11.7 Hz, 1H), 4.31 (d, *J* = 11.7 Hz, 1H), 4.25 (t, *J* = 8.9 Hz, 2H), 4.21 (d, *J* = 7.7 Hz, 1H), 4.06 (t, *J* = 8.5 Hz, 1H), 3.97 – 3.71 (m, 10H), 3.61 – 3.48 (m, 9H), 3.39 – 3.32 (m, 3H), 3.32 – 3.26 (m, 2H), 3.18 (d, *J* = 4.1 Hz, 3H), 1.63 (d, *J* = 6.7 Hz, 2H), 1.54 – 1.46 (m, 2H), 1.40 (d, *J* = 7.3 Hz, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 162.03, 156.54, 138.81, 138.34, 138.17, 137.99, 137.91, 137.79, 137.59, 136.80, 128.79, 128.67, 128.65, 128.58, 128.55, 128.31, 128.25, 128.20, 128.12, 128.07, 128.02, 127.92, 127.88, 127.76, 127.63, 127.53 (Ar), 104.37 (C-1), 103.31 (C-1), 102.58 (C-1), 101.21 (C-1), 92.77, 83.80, 82.10, 81.64, 79.63, 75.19, 75.13, 75.02, 74.87, 74.75, 74.53, 74.27, 73.86,

73.75, 73.67, 73.60, 73.49, 73.41, 73.15, 72.78, 72.34, 71.98, 71.73, 70.44, 69.87, 68.83, 68.67, 68.23, 66.72, 57.86, 41.03, 29.85, 29.70, 29.23, 23.27.; MS ESI+-HRMS m/z $[M+Na]^+$ calcd for $C_{95}H_{107}N_2NaO_{23}$ 1771.6222, found 1771.6237.

Automated Synthesis of AGA-3-p04 using building block Fuc-03.



Automated synthesis of **AGA-3-p04**

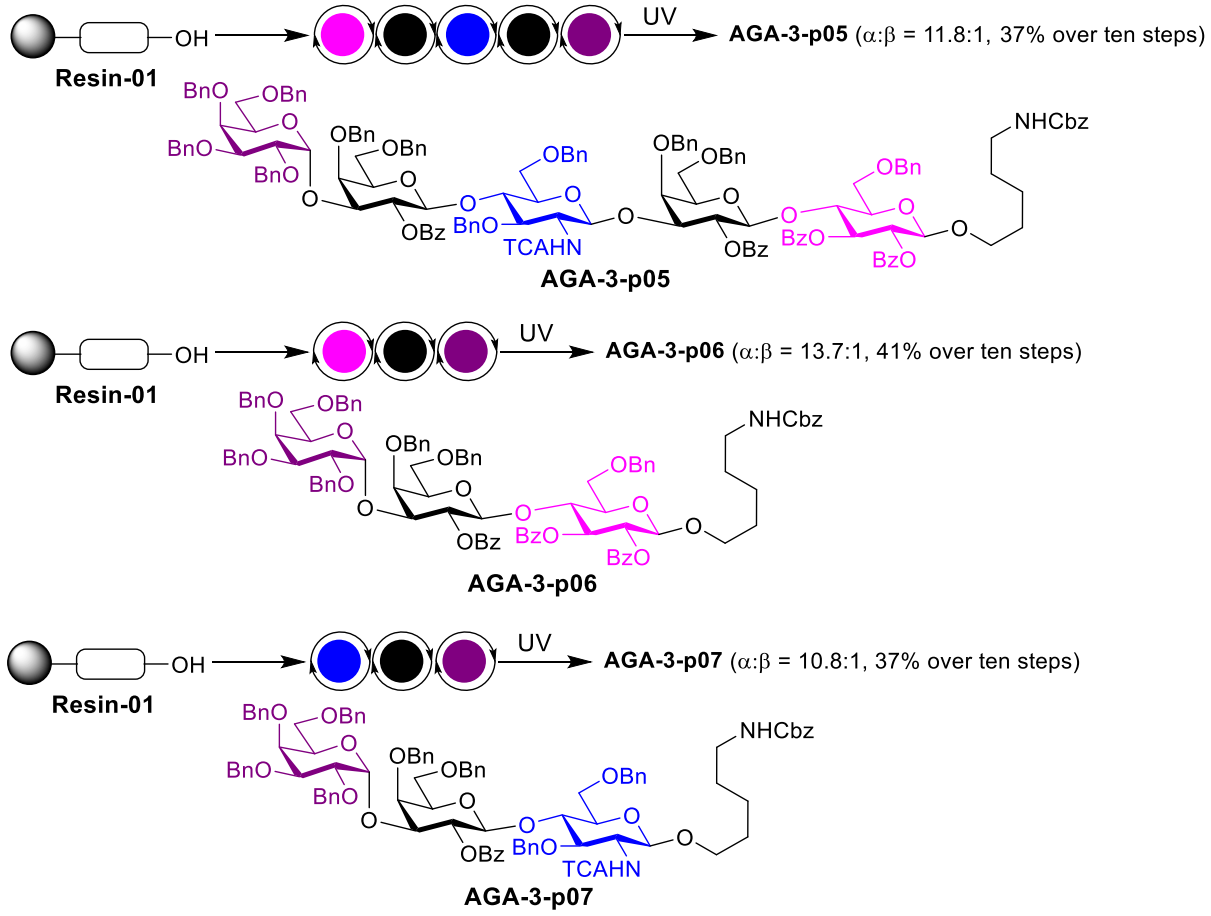


LC-MS of **AGA-3-p04**

***N*-Benzyloxycarbonyl-5-amino-pentyl (2,3,4-tri-*O*-benzoyl-α-L-fucopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-(4,6-di-*O*-benzyl-2-deoxy-2-trichloracetamido-β-D-glucopyranosyl)-(1→3)-(2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranoside AGA-3-p04**

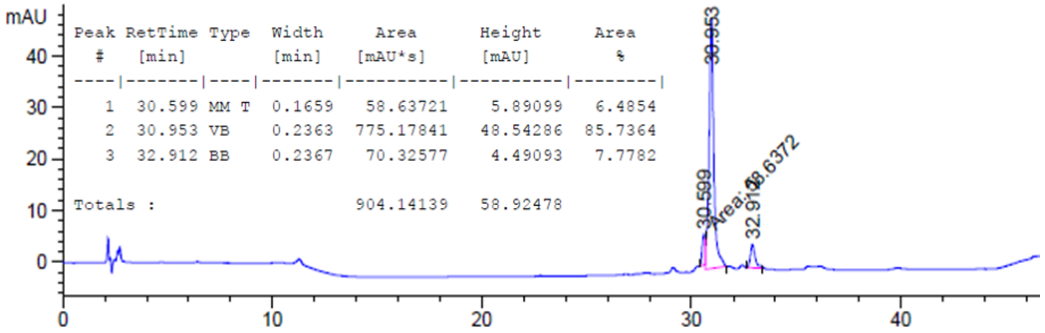
^{1}H NMR (600 MHz, $CDCl_3$) δ 7.94 (d, *J* = 7.2 Hz, 2H), 7.90 (d, *J* = 7.3 Hz, 2H), 7.85 – 7.80 (m, 2H), 7.52 – 7.48 (m, 2H), 7.45 (dd, *J* = 13.1, 5.7 Hz, 2H), 7.39 – 7.07 (m, 61H), 7.05 (d, *J* = 7.2 Hz, 2H), 7.00 (t, *J* = 7.6 Hz, 2H), 6.73 (d, *J* = 7.5 Hz, 1H), 5.68 (d, *J* = 3.7 Hz, 1H), 5.56 (t, *J* = 9.4 Hz, 1H), 5.43 (dd, *J* = 10.0, 8.0 Hz, 1H), 5.29 (dd, *J* = 9.6, 8.0 Hz, 1H), 5.05 (s, 2H), 4.94 (t, *J* = 10.1 Hz, 2H), 4.90 – 4.82 (m, 2H), 4.79 – 4.69 (m, 3H), 4.64 – 4.60 (m, 2H), 4.53 – 4.41 (m, 8H), 4.38 (d, *J* = 11.5 Hz, 2H), 4.34 (dd, *J* = 13.2, 4.8 Hz, 2H), 4.29 (d,

J = 2.0 Hz, 1H), 4.27 (d, *J* = 8.6 Hz, 3H), 4.19 (d, *J* = 11.9 Hz, 1H), 4.15 (dd, *J* = 9.4, 7.9 Hz, 1H), 4.06 – 4.00 (m, 4H), 3.96 (d, *J* = 2.3 Hz, 1H), 3.95 – 3.91 (m, 1H), 3.90 (d, *J* = 2.0 Hz, 1H), 3.86 – 3.76 (m, 4H), 3.72 – 3.64 (m, 3H), 3.55 (dd, *J* = 9.6, 2.7 Hz, 1H), 3.51 (dd, *J* = 11.0, 3.9 Hz, 1H), 3.43 (dd, *J* = 19.0, 10.4 Hz, 3H), 3.38 – 3.30 (m, 5H), 3.26 (dd, *J* = 8.7, 5.0 Hz, 1H), 2.93 – 2.87 (m, 3H), 2.83 (t, *J* = 8.7 Hz, 1H), 1.51 – 1.38 (m, 2H), 1.31 – 1.24 (m, 4H), 1.18 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 165.36 (Bz), 165.32(Bz), 164.75(Bz), 161.73 (NHTCA), 156.38 (Cbz), 139.21, 138.97, 138.91, 138.70, 138.60, 138.38, 138.10, 137.99, 133.33, 133.19, 132.55, 130.53, 130.11, 129.94, 129.84, 129.71, 128.63, 128.58, 128.51, 128.48, 128.45, 128.43, 128.35, 128.28, 128.15, 128.13, 128.09, 128.03, 127.95, 127.81, 127.78, 127.62, 127.55, 127.43, 127.35, 127.30, 127.22, 126.31, 101.35 (C-1), 101.07 (C-1), 99.85 (C-1), 97.52 (C-1), 92.10 (C_{Fuc}-1), 84.15, 79.42, 79.27, 78.23, 76.49, 76.02, 75.88, 75.51, 75.22, 74.98, 74.88, 74.82, 74.54, 73.63, 73.54, 73.41, 73.25, 73.05, 72.80, 72.52, 72.33, 72.19, 71.12, 69.76, 68.71, 68.14, 67.82, 67.34, 66.62, 66.54, 58.15, 40.94, 29.86, 29.51, 29.01, 23.19, 16.94.; MS ESI+-HRMS m/z $[M+Na]^+$ calcd for $C_{143}H_{147}N_2O_{30}Na$ 2499.8996, found 2499.9001.



Automated Synthesis of α-Gal Epitopes AGA-3-p05 to p07

Automated synthesis of AGA-3-p05

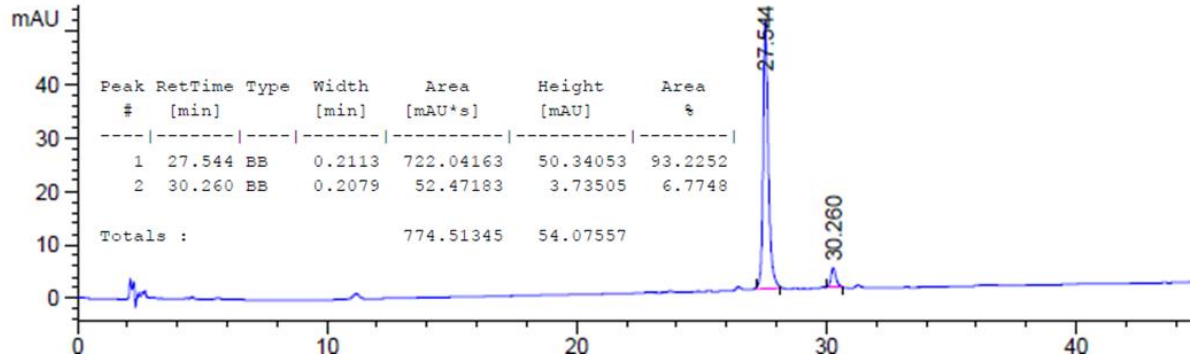


LC-MS of **AGA-3-p05**

***N*-Benzyloxycarbonyl-5-amino-pentyl (2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-*O*-benzoyl-4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-(4,6-di-*O*-benzyl-2-deoxy-2-trichloracetamido-β-D-glucopyranosyl)-(1→3)-(2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranoside AGA-3-p05**

^{1}H NMR ($CDCl_3$) δ 7.95 – 7.79 (m, 8H), 7.48 (ddd, *J* = 25.6, 16.1, 7.6 Hz, 4H), 7.39 – 7.06 (m, 66H), 7.05 – 6.98 (m, 2H), 5.55 (dt, *J* = 19.1, 9.4 Hz, 2H), 5.35 (dd, *J* = 9.8, 8.1 Hz, 1H), 5.29 (dd, *J* = 11.2, 5.0 Hz, 1H), 5.05 (s, 2H), 5.01 (s, 1H), 4.93 (d, *J* = 3.3 Hz, 1H), 4.88 – 4.78 (m, 4H), 4.69 (d, *J* = 7.6 Hz, 1H), 4.62 (d, *J* = 11.5 Hz, 1H), 4.56 – 4.52 (m, 3H), 4.50 – 4.46 (m, 3H), 4.43 – 4.38 (m, 3H), 4.32 (dd, *J* = 27.0, 11.8 Hz, 2H), 4.25 – 4.21 (m, 2H), 4.19 (d, *J* = 2.7 Hz, 1H), 4.14 (dd, *J* = 23.0, 10.1 Hz, 2H), 4.01 (ddd, *J* = 12.6, 8.6, 5.1 Hz, 4H), 3.95 – 3.87 (m, 3H), 3.83 (s, 1H), 3.78 (dd, *J* = 10.0, 2.4 Hz, 2H), 3.74 – 3.60 (m, 4H), 3.54 (dd, *J* = 10.6, 3.4 Hz, 1H), 3.50 – 3.29 (m, 9H), 3.28 – 3.17 (m, 4H), 2.97 (dd, *J* = 8.5, 5.2 Hz, 1H), 2.91 – 2.83 (m, 3H), 2.78 (t, *J* = 8.7 Hz, 1H), 1.51 – 1.35 (m, 2H), 1.34 – 1.22 (m, 2H), 1.20 – 1.05 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.36 (Bz), 165.31 (Bz), 165.09 (Bz), 164.62 (Bz), 161.68 (TCA), 156.40 (Cbz), 139.25, 139.20, 138.80, 138.65, 138.53, 138.32, 138.20, 138.17, 138.08, 133.34, 133.23, 133.17, 132.49, 130.59, 130.01, 129.95, 129.84, 129.72, 128.63, 128.58, 128.48, 128.46, 128.44, 128.27, 128.22, 128.07, 128.05, 128.00, 127.96, 127.92, 127.89, 127.85, 127.78, 127.74, 127.72, 127.65, 127.60, 127.55, 127.44, 127.31, 127.26, 127.15 (Ar), 101.08 (C-1), 100.99 (C-1), 100.81 (C-1), 100.24 (C-1), 99.66 (C-1), 92.16, 80.98, 79.21, 79.00, 77.70, 76.58, 76.17, 75.99, 75.18, 75.04, 74.84, 74.76, 74.41, 74.26, 73.76, 73.51, 73.45, 73.39, 73.28, 73.12, 73.00, 72.68, 72.47, 72.41, 72.17, 69.83, 69.74, 68.23, 68.11, 67.91, 67.75, 67.39, 66.64, 57.91, 40.94, 31.07, 29.85, 29.49, 28.99, 23.18.; MS ESI+-HRMS m/z [M+Na]+ calcd for $C_{150}H_{149}N_2O_{33}Cl_3Na$ 2633.9000, found 2633.9012.

Automated synthesis of AGA-3-p06

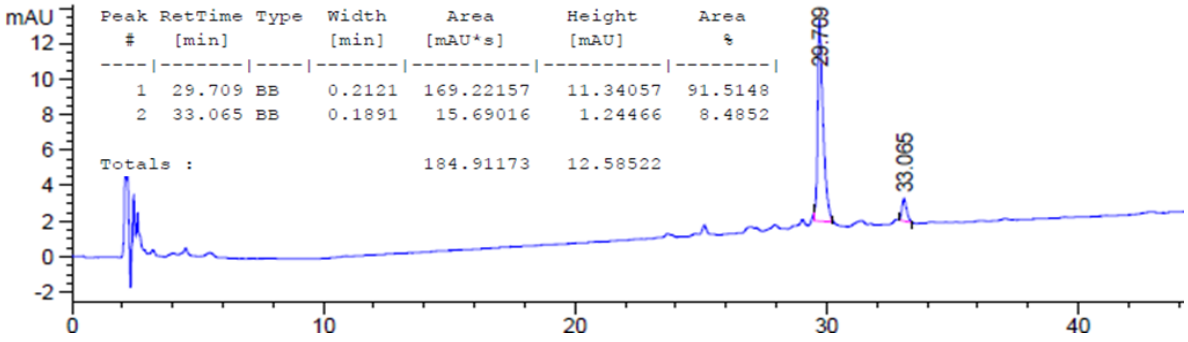


LC-MS of **AGA-3-p06**

***N*-Benzyloxycarbonyl-5-amino-pentyl (2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranoside AGA-3-p06**

^{1}H NMR ($CDCl_3$) δ 8.14 (d, *J* = 8.0 Hz, 2H), 7.97 (d, *J* = 7.7 Hz, 2H), 7.92 (d, *J* = 7.9 Hz, 2H), 7.61 (t, *J* = 7.0 Hz, 1H), 7.53 (dd, *J* = 13.1, 6.2 Hz, 2H), 7.47 (t, *J* = 7.4 Hz, 2H), 7.44 – 7.20 (m, 31H), 7.16 (t, *J* = 7.2 Hz, 2H), 7.12 (d, *J* = 7.2 Hz, 2H), 7.04 – 6.98 (m, 3H), 6.95 (t, *J* = 6.8 Hz, 1H), 5.48 (t, *J* = 8.8 Hz, 1H, H-2), 5.28 – 5.23 (m, 1H, H-2), 5.22 (d, *J* = 7.6 Hz, 1H, H''-1), 5.11 (t, *J* = 8.5 Hz, 1H, H-2), 5.06 (s, 2H, CH_2, Cbz), 4.92 (d, *J* = 11.5 Hz, 1H, C*H*HPh), 4.72 (d, *J* = 11.6 Hz, 1H, C*H*HPh), 4.66 (d, *J* = 12.2 Hz, 1H, C*H*HPh), 4.59 – 4.54 (m, 3H, H-1, 2 x C*H*HPh), 4.50 – 4.43 (m, 4H, H'-4, CH_2Ph, C*H*HPh), 4.40 (d, *J* = 12.0 Hz, 1H, C*H*HPh), 4.30 (d, *J* = 7.9 Hz, 1H, H-1), 4.26 (d, *J* = 12.0 Hz, 2H, 2 x C*H*HPh), 4.16 (d, *J* = 10.7 Hz, 1H, C*H*HPh), 3.93 (d, *J* = 2.4 Hz, 1H, H''-4), 3.90 (t, *J* = 9.0 Hz, 1H, H-4), 3.82 – 3.72 (m, 2H, H''-3, OC*H*H$(CH_2)_4$), 3.68 (dd, *J* = 12.4, 6.4 Hz, 1H, H-6), 3.65 – 3.57 (m, 3H, H-5, 2 x H-6), 3.57 – 3.50 (m, 2H, H-3, H-6), 3.49 – 3.42 (m, 3H, H-3, H-5, H-6), 3.34 – 3.27 (m, 2H, H-6, OC*H*H$(CH_2)_4$), 3.19 (d, *J* = 8.1 Hz, 1H, H-5), 3.00 (br, 1H, OH), 2.89 (d, *J* = 6.7 Hz, 2H, CH_2NHCbz), 1.51 – 1.35 (m, 2H, CH_2, pentane), 1.34 – 1.22 (m, 2H, CH_2, pentane), 1.20 – 1.06 (m, 2H, CH_2, pentane). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.92 (Bz), 165.09 (Bz), 164.57 (Bz), 156.35 (Cbz), 138.67, 138.47, 138.42, 138.35, 137.91, 137.66, 136.84, 133.16, 133.04, 132.87, 130.66, 130.25, 130.03, 129.81, 129.71, 128.59, 128.59, 128.52, 128.47, 128.44, 128.42, 128.36, 128.16, 128.12, 127.96, 127.92, 127.87, 127.77, 127.58, 127.46, 127.05 (Ar), 101.26 (C-1), 100.76 (C-1), 99.73 (C-1), 80.67, 80.13, 77.01, 76.66, 75.87, 75.25, 74.99, 74.97, 74.20, 73.79, 73.63, 73.60, 73.50, 73.44, 72.30, 71.79, 69.39, 69.11, 68.66, 68.53, 67.96, 66.55, 40.90, 29.81, 29.43, 28.94, 23.15, 22.81, 14.24.; MS ESI+-HRMS m/z [M+Na]+ calcd for $C_{94}H_{97}NO_{21}Na$ 1598.6451, found 1599.6567.

Automated synthesis of AGA-3-p07



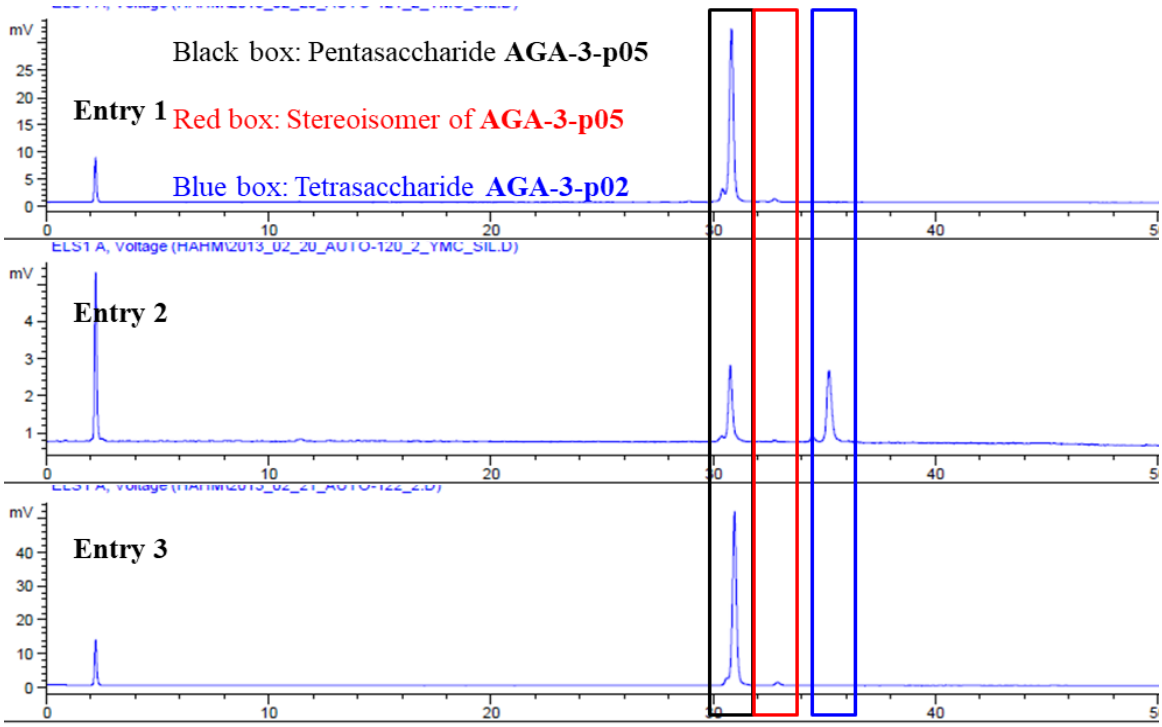
LC-MS of **AGA-3-p07**

***N*-Benzyloxycarbonyl-5-amino-pentyl (2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-trichloracetamido-β-D-glucopyranoside 19**

^{1}H NMR ($CDCl_3$) δ 7.93 (d, *J* = 7.5 Hz, 2H), 7.49 (t, *J* = 7.3 Hz, 1H), 7.36 – 7.14 (m, 45H), 7.10 (t, *J* = 7.3 Hz, 2H), 7.01 (s, 1H), 5.64 – 5.58 (m, 1H, H'-2), 5.08 – 5.03 (m, 3H, C*H*HPh, CH_2, Cbz), 4.95 (d, *J* = 3.2 Hz, 1H, **H''-1**), 4.90 (d, *J* = 10.6 Hz, 1H, C*H*HPh), 4.85 (d, *J* = 11.5 Hz, 1H, C*H*HPh), 4.79 (d, *J* = 11.2 Hz, 1H, C*H*HPh), 4.74 (s, 1H, NHCbz), 4.63 (d, *J* = 11.4 Hz, 2H, **H-1**, C*H*HPh), 4.56 (dd, *J* = 20.0, 9.0 Hz, 4H, **H'-1**, 3 x C*H*HPh), 4.49 (d, *J* = 11.9 Hz, 1H, C*H*HPh), 4.39 (dd, *J* = 11.4, 6.1 Hz, 2H, CH_2Ph), 4.34 (d, *J* = 12.1 Hz, 2H, 2 x C*H*HPh), 4.26 – 4.20 (m, 2H, 2 x C*H*HPh), 4.16 – 4.11 (m, 1H, C*H*HPh), 4.02 (dd, *J* = 10.2, 3.3 Hz, 1H, H''-2), 3.98 (d, *J* = 1.9 Hz, 2H), 3.93 – 3.90 (m, 2H), 3.79 (dd, *J* = 10.1, 2.5 Hz, 1H), 3.70 (d, *J* = 10.9 Hz, 3H), 3.62 – 3.49 (m, 3H), 3.48 – 3.43 (m, 2H), 3.35 (dd, *J* = 16.9, 13.1 Hz, 2H), 3.26 (t, *J* = 8.6 Hz, 1H), 3.21 (d, *J* = 8.0 Hz, 1H), 3.12 (d, *J* = 6.0 Hz, 2H), 2.99 (dd, *J* = 8.6, 5.3 Hz, 1H), 1.50 – 1.39 (m, 4H), 1.32 – 1.22 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.22 (Bz), 161.80 (Bz), 156.48 (Cbz), 139.19, 138.82, 138.66, 138.56, 138.34, 138.31, 138.28, 138.22, 136.80, 133.19, 129.98, 128.63, 128.56, 128.54, 128.49, 128.46, 128.43, 128.34, 128.27, 128.23, 128.18, 127.95, 127.91, 127.84, 127.79, 127.73, 127.59, 127.54, 127.50, 127.45, 127.34 (Ar), 100.59 (**C-1**), 99.63 (**C-1**), 99.60 (**C''-1**), 92.67, 80.85, 79.02, 77.99, 76.58, 75.79, 75.24, 75.03, 75.01, 74.85, 74.42, 74.23, 73.78, 73.58, 73.48, 73.29, 72.67, 72.41, 69.83, 69.57, 68.45, 68.22, 67.92, 66.69, 60.53, 57.33, 41.05, 29.85, 29.70, 29.07, 23.33, 14.35.; MS ESI+-HRMS m/z [M+Na]+ calcd for $C_{96}H_{101}N_2O_{19}Cl_3Na$ 1713.5956, found 1713.5928.

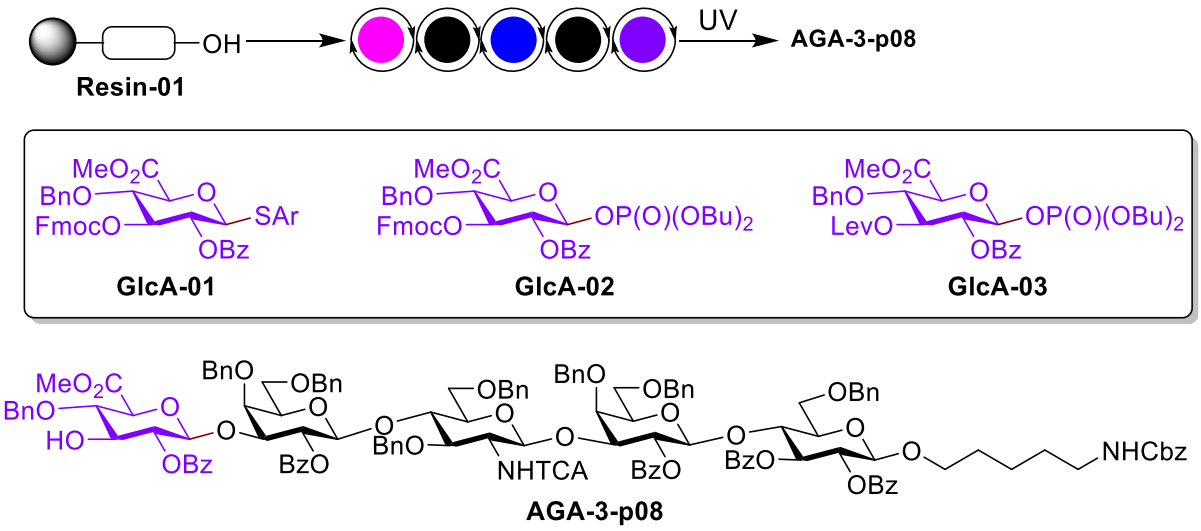
Entry	Sequencce	Ratio (α/β)
1	Galα1→3Galβ1→4Glcβ1→linker	13.7
2	Galα1→3Galβ1→4GlcNTCAβ1→linker	10.8
3	Galα1→3Galβ1→4GlcNTCAβ1→3Galβ1→4Glcβ1→linker	11.8

The acceptor dependency of the formation of 1,2-*cis*-galactosidic linkages.

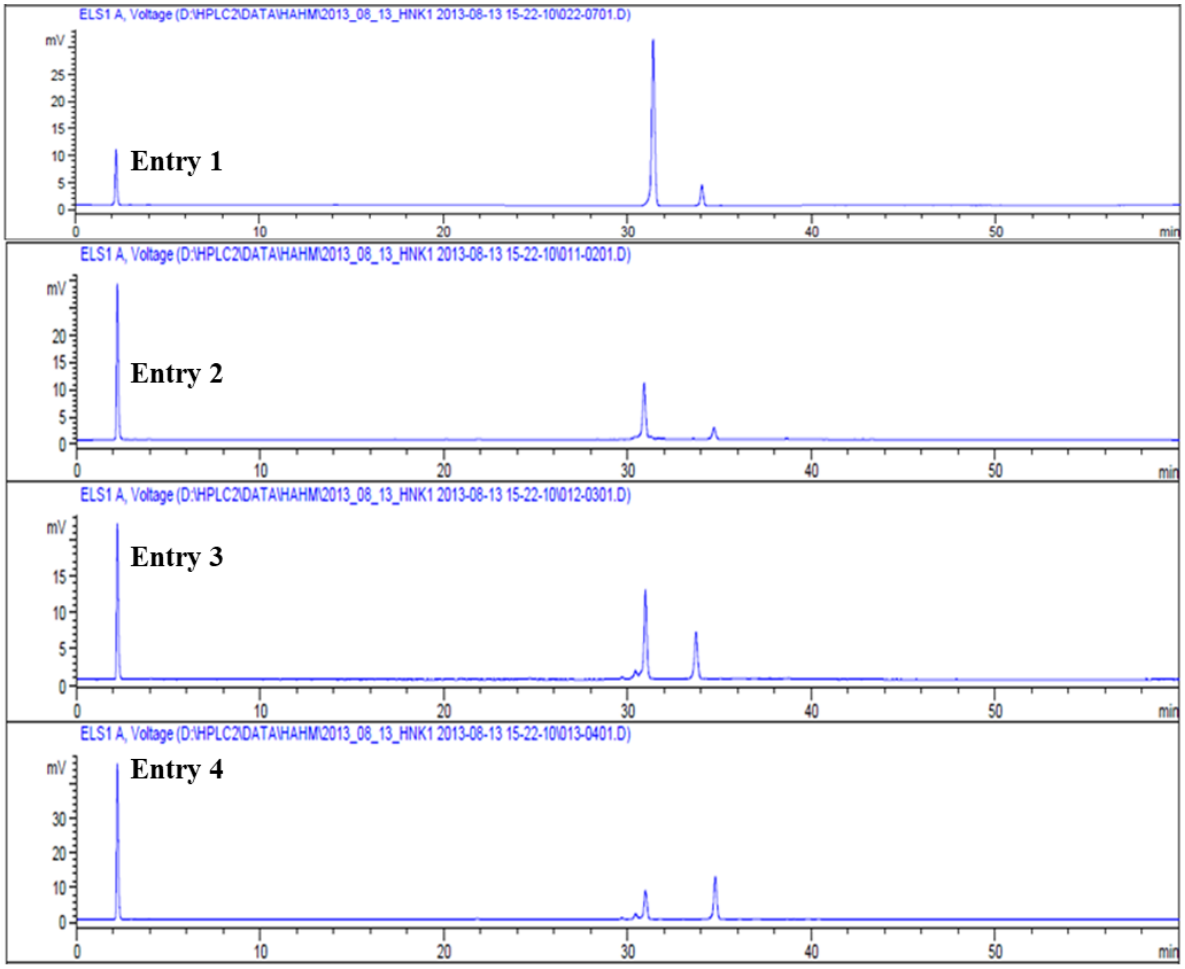


Temperature effect and a co-solvent effect on synthesis of **AGA-3-p05**

Automated Synthesis of HNK-1 Epitope Pentasaccharide AGA-3-p08 and –p09

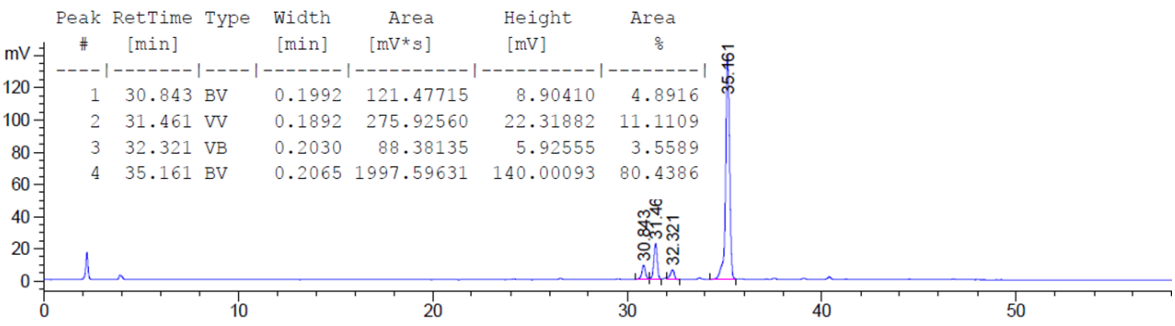


Automated Synthesis of HNK-1 Epitope Pentasaccharide **AGA-3-p08**



Entry	Leaving Group	BB	Temp.	Ratio
1	SAr	**GlcA-01**	-10	90:10
2	Phosphate	**GlcA-02**	-10	84:16
3	Phosphate	**GlcA-03**	-10	64:36
4	Phosphate	**GlcA-03**	0	39:61

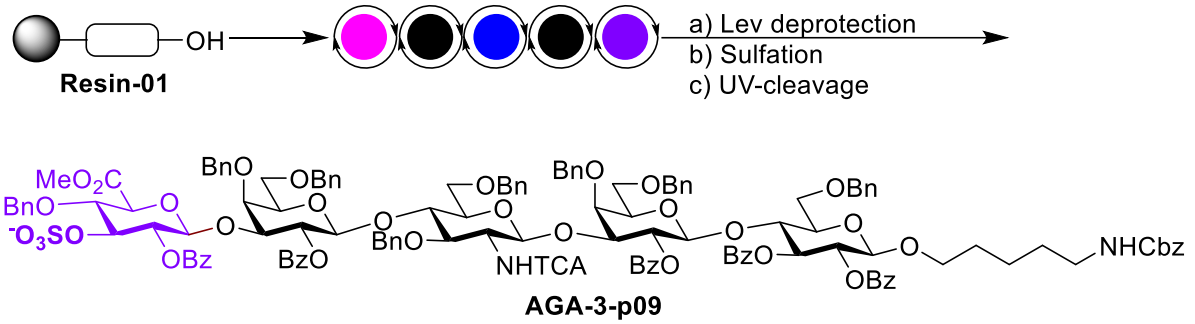
Optimization of automated synthesis of **AGA-3-p08** using **GlcA-01, -02,** and **-03**



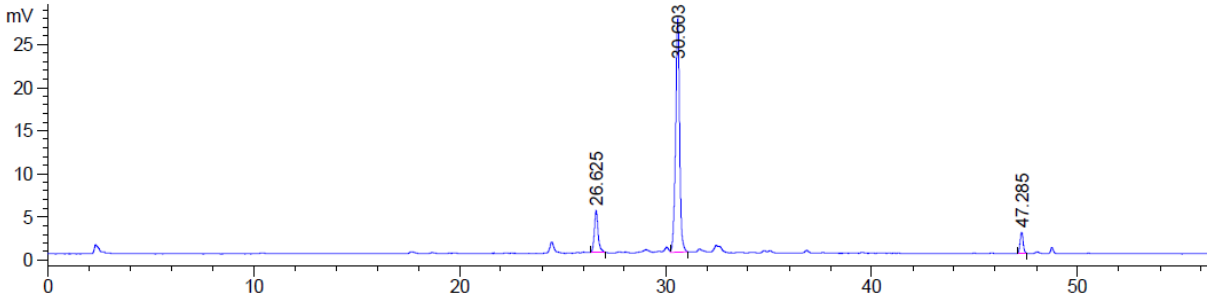
LC-MS of **AGA-3-p08**

***N*-Benzyloxycarbonyl-5-amino-pentyl (methyl 2-*O*-benzoyl-4-*O*-benzyl-β-D-glucopyranosyluronate)-(1→3)-(2-*O*-benzoyl-4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-(4,6-di-*O*-benzyl-2-deoxy-2-trichloracetamido-β-D-glucopyranosyl)-(1→3)-(2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranoside AGA-3-p08**

^{1}H NMR (600MHz, $CDCl_3$) δ 7.90 (dd, *J* = 14.7, 7.2 Hz, 4H), 7.83 (d, *J* = 7.5 Hz, 2H), 7.71 (d, *J* = 7.5 Hz, 4H), 7.58 – 7.42 (m, 5H), 7.42 – 7.08 (m, 52H), 7.07 – 7.01 (m, 3H), 6.53 (d, *J* = 7.3 Hz, 1H), 5.55 (t, *J* = 9.1 Hz, 1H), 5.48 – 5.43 (m, 1H), 5.38 – 5.33 (m, 1H), 5.30 (dd, *J* = 11.7, 5.2 Hz, 1H), 5.06 (t, *J* = 8.3 Hz, 3H), 4.98 (d, *J* = 11.4 Hz, 1H), 4.80 (ddd, *J* = 39.1, 23.4, 9.7 Hz, 4H), 4.69 (d, *J* = 11.2 Hz, 2H), 4.56 (s, 1H), 4.52 – 4.44 (m, 5H), 4.38 (dd, *J* = 16.3, 9.1 Hz, 2H), 4.30 (d, *J* = 11.9 Hz, 1H), 4.28 – 4.21 (m, 2H), 4.15 – 4.08 (m, 3H), 4.05 – 3.96 (m, 5H), 3.87 (dd, *J* = 20.1, 10.8 Hz, 4H), 3.80 – 3.72 (m, 5H), 3.68 – 3.59 (m, 2H), 3.48 (dd, *J* = 13.5, 6.1 Hz, 4H), 3.43 – 3.31 (m, 6H), 3.24 – 3.18 (m, 2H), 3.14 (s, 1H), 2.93 – 2.83 (m, 3H), 2.78 (t, *J* = 8.7 Hz, 1H), 1.52 – 1.38 (m, 2H), 1.33 – 1.22 (m, 2H), 1.19 – 1.10 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.29 (CO_2Me), 168.56 (Bz), 166.01 (Bz), 165.33 (Bz), 164.61 (Bz), 164.23 (Bz), 161.63 (TCA), 156.36 (Cbz), 139.21, 138.83, 138.29, 138.28, 138.16, 138.07, 138.02, 137.69, 136.80, 133.33, 133.15, 132.46, 130.54, 129.99, 129.92, 129.80, 129.66, 129.58, 129.55, 129.13, 128.66, 128.59, 128.55, 128.51, 128.48, 128.43, 128.40, 128.29, 128.26, 128.24, 128.21, 128.18, 128.13, 128.04, 127.98, 127.91, 127.87, 127.83, 127.75, 127.69, 127.62, 127.49, 127.21, 127.13, 124.06, 123.59, 116.02, 114.18, 101.46 (**C-1**), 101.04 (**C-1**), 100.99 (**C-1**), 100.65 (**C-1**), 100.26 (**C-1**), 92.08 (TCA), 79.44, 79.27, 78.97, 77.64, 77.37, 77.16, 76.95, 76.12, 75.95, 75.93, 75.25, 75.23, 75.17, 75.15, 74.96, 74.72, 74.58, 74.39, 74.36, 73.72, 73.55, 73.34, 73.08, 72.91, 72.73, 72.31, 72.12, 69.73, 68.10, 68.01, 67.71, 67.33, 66.58, 60.52, 57.81, 52.78, 40.89, 33.93, 32.03, 31.75, 31.55, 30.30, 29.77, 29.73, 29.62, 29.44, 29.27, 29.06, 28.96, 23.13, 22.80, 21.16, 18.01, 14.31, 14.24, 13.54.; MS ESI+-HRMS m/z [M+Na]+ calcd for $C_{137}H_{137}N_2O_{34}Cl_3Na$ 2481.8011, found 2481.7998.



Automated Synthesis of HNK-1 Epitope Pentasaccharide **AGA-3-p09**

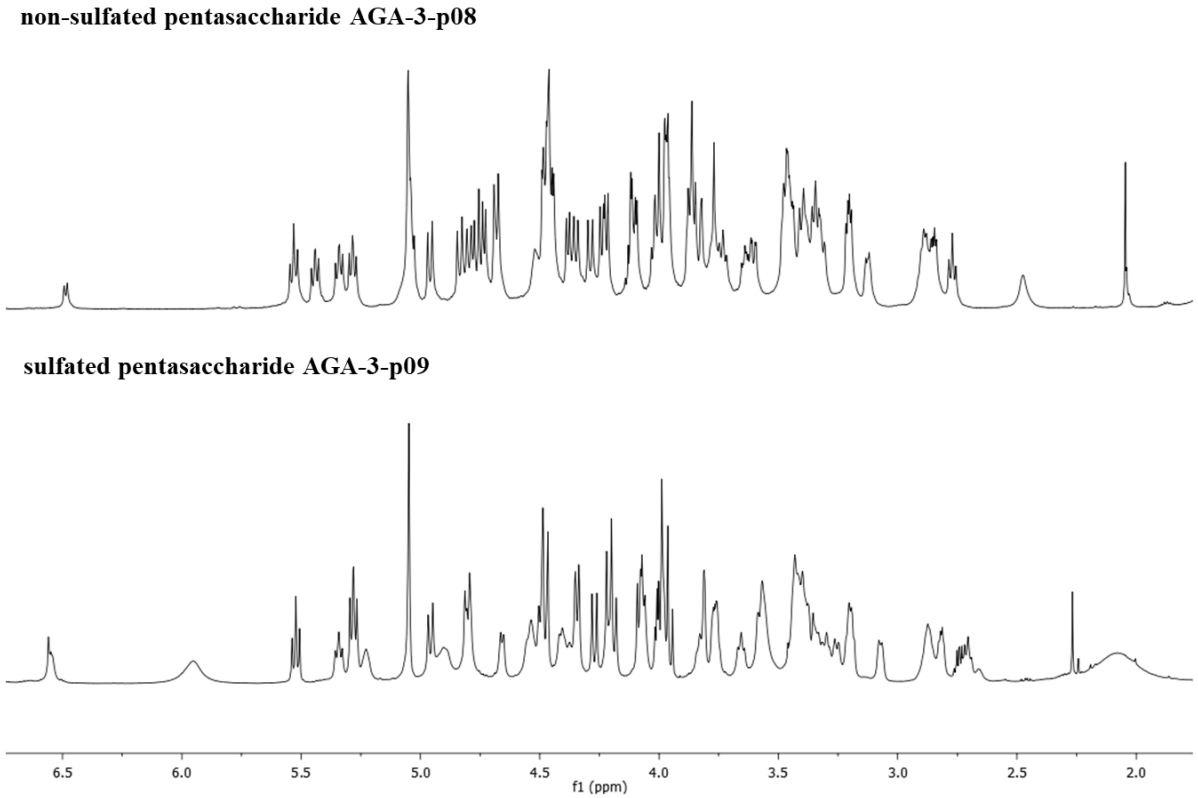


Purification of HNK-1epitope **AGA-3-p09.** Conditions: column: C18-Nucleodur (21×250 mm; 5 μm); flow rate: 10 mL·min^{-1}; eluents: 0.01 M NH_4HCO_3 in water/MeCN; gradient: 45% (5 min)→55% (in 40 min)→100% (in 5 min); detection: ELSD.

_N_-Benzyloxycarbonyl-5-amino-pentyl (methyl 2-_O_-benzoyl-4-_O_-benzyl-3-_O_-sulfato-β-D-glucopyranosyluronate)-(1→3)-(2-_O_-benzoyl-4,6-tri-_O_-benzyl-β-D-galactopyranosyl)-(1→4)-(4,6-di-_O_-benzyl-2-deoxy-2-trichloracetamido-β-D-glucopyranosyl)-(1→3)-(2-_O_-benzoyl-4,6-di-_O_-benzyl-β-D-galactopyranosyl)-(1→4)-2,3-di-_O_-benzoyl-6-_O_-benzyl-β-D-glucopyranoside AGA-3-p09

^{1}H NMR (600MHz, $CDCl_3$) δ 7.89 (d, *J* = 7.4 Hz, 2H), 7.82 (dd, *J* = 14.1, 7.4 Hz, 4H), 7.59 (t, *J* = 8.4 Hz, 3H), 7.49 (t, *J* = 7.3 Hz, 1H), 7.46 – 7.41 (m, 1H), 7.37 – 7.17 (m, 41H), 7.17 – 7.07 (m, 16H), 7.02 (dd, *J* = 15.2, 7.7 Hz, 2H), 6.56 (d, *J* = 6.2 Hz, 1H), 5.95 (s, 1H), 5.52 (t, *J* = 9.4 Hz, 2H), 5.40 – 5.32 (m, 1H), 5.28 (dd, *J* = 9.4, 8.2 Hz, 2H), 5.23 (s, 1H), 5.05 (s, 2H), 4.96 (d, *J* = 11.5 Hz, 1H), 4.90 (s, 2H), 4.83 – 4.77 (m, 2H), 4.66 (d, *J* = 7.2 Hz, 1H), 4.50 (dd, *J* = 26.5, 15.8 Hz, 5H), 4.43 – 4.32 (m, 4H), 4.27 (d, *J* = 11.9 Hz, 1H), 4.24 – 4.16 (m, 3H), 4.08 (dd, *J* = 11.4, 7.6 Hz, 2H), 3.99 (dt, *J* = 27.2, 8.3 Hz, 4H), 3.82 (d, *J* = 10.3 Hz, 2H), 3.79 – 3.73 (m, 2H), 3.69 – 3.63 (m, 1H), 3.58 (d, *J* = 10.4 Hz, 3H), 3.47 – 3.23 (m, 9H), 3.20 (d, *J* = 5.1 Hz, 2H), 3.07 (d, *J* = 7.7 Hz, 1H), 2.88 (s, 2H), 2.82 (d, *J* = 4.0 Hz, 1H), 2.77 – 2.68 (m, 2H), 1.49 – 1.36 (m, 2H), 1.29 (dd, *J* = 19.3, 8.5 Hz, 2H), 1.13 (dd, *J* = 13.8, 7.2 Hz,

2H). ^{13}C NMR (100 MHz, CD_3OD) δ 170.34 (CO_2Me), 167.32 (Bz), 167.08 (Bz), 166.89 (Bz), 166.45 (Bz), 166.41 (Bz), 163.98 (NHTCA), 158.74 (Cbz), 140.35, 140.19, 139.87, 139.45, 139.41, 139.39, 139.25, 139.23, 138.40, 134.57, 134.41, 134.05, 133.55, 131.50, 131.31, 131.13, 131.10, 131.04, 130.99, 130.81, 130.69, 130.58, 130.50, 129.95, 129.80, 129.73, 129.70, 129.61, 129.59, 129.48, 129.43, 129.41, 129.33, 129.29, 129.26, 129.19, 129.17, 129.10, 129.05, 128.94, 128.91, 128.89, 128.82, 128.76, 128.74, 128.72, 128.62, 128.59, 128.29, 128.09, 103.26 (**C-1**), 102.54 (**C-1**), 102.20 (**C-1**), 101.90 (**C-1**), 101.80 (**C-1**), 93.56 (CCl_3), 81.22, 80.96, 80.27, 80.13, 79.25, 77.75, 77.65, 76.12, 75.98, 75.91, 75.79, 75.76, 75.47, 75.40, 75.16, 75.06, 74.48, 74.42, 74.28, 74.13, 74.04, 73.88, 73.52, 73.46, 73.03, 70.80, 69.90, 69.18, 68.86, 68.47, 67.27, 64.91, 58.67, 53.17, 47.93, 44.66, 41.51, 30.16, 30.01, 29.95, 23.97.; MS ESI+-HRMS m/z [M+Na]+ calcd for $C_{137}H_{137}N_2O_{37}Cl_3SNa$ 2561.7579, found 2561.7601.



^{1}H NMR of pentasaccharide **AGA-3-p08** and sulfated pentasaccharide **AGA-3-p09**.

4. Automated Assembly of Oligosaccharides Containing Multiple cis-glycosidic linkages

This chapter has been modified in part from the following publication:

H. S. Hahm, M. Hurevich, P. H. Seeberger, Automated Assembly of Oligosaccharides Containing Multiple *cis*-Glycosidic Linkages. *Nat. Commun.* **2016**, *7*:12482.

https://doi.org/10.1038/ncomms12482

4.1 Introduction

Carbohydrates are the most abundant biomolecules on earth and serve many functions, including structure, nutrition, and information transfer.[121] The structural diversity and complexity of natural glycans, combined with the lack of amplification and expression methods, renders their isolation difficult and often impossible. The synthesis of complex glycans is time consuming and can be accessed only by highly trained chemists. Automated solid-phase assembly[122,123] has drastically accelerated the procurement of defined oligosaccharides to be used as molecular tools, on glycan arrays[124] and in applications such as oligosaccharide vaccines[125]. Using automated synthesis, oligosaccharides as large as 30-mers[126] that represent different subclasses of glycans, including glycolipids[127] and glycosaminoglycans such as chondroitin sulfate[128] are now accessible in hours rather than months. This strategy has been evolved to rely on the use of monomeric building blocks and a synthesizer run by a set of preprogrammed modules as a platform with minimal manual interventions that affects coupling efficiency and the stereoselectivity. To date a set of monosaccharide building blocks that reliably and efficiently introduce *trans*-glycosidic linkages on solid support has been identified and automated synthesis, therefore, ensures an access to ever more complex glycans with high degree of reproducibility. These monomeric building blocks are designed to take advantage of a C2 neighbouring group participation (NGP) in order to ensure exclusive *trans* glycosylation. Consequently, with few exceptions[129,130], most oligosaccharides assembled by automated synthesis to date contained either exclusively or predominantly *trans*-glycosidic linkages. The challenging formation of 1,2 *cis* glycosylic linkages in the automated synthesis of the tumor-associated hexasaccharide antigen Globo-H, containing one *cis*-galactosidic linkage[129], as well as a β-(1,4)-mannuronic acid alginate 12-mer containing exclusively 1,2-*cis*-mannosidic linkages[130] relied on C2 non-participating protecting groups and benefitted from the conformational influences and leaving group effects. To broaden the scope of automated glycan assembly (AGA) the stereoselective installation of *cis*-glycosidic linkages in glucose and galactose[131], the most prevalent 1,2-*cis* linkages in mammalian and bacterial glycomes[132] has to be addressed.

4.2 Developed Methods and Their Applications

Stereoselective formation of 1,2-*cis* glycosidic linkages still remains highly challenging[133-137], because non-participating C2 protecting groups generally lead to mixtures of stereoisomers that have to be separated at the end of the synthesis (**Figure 4.01 a**).

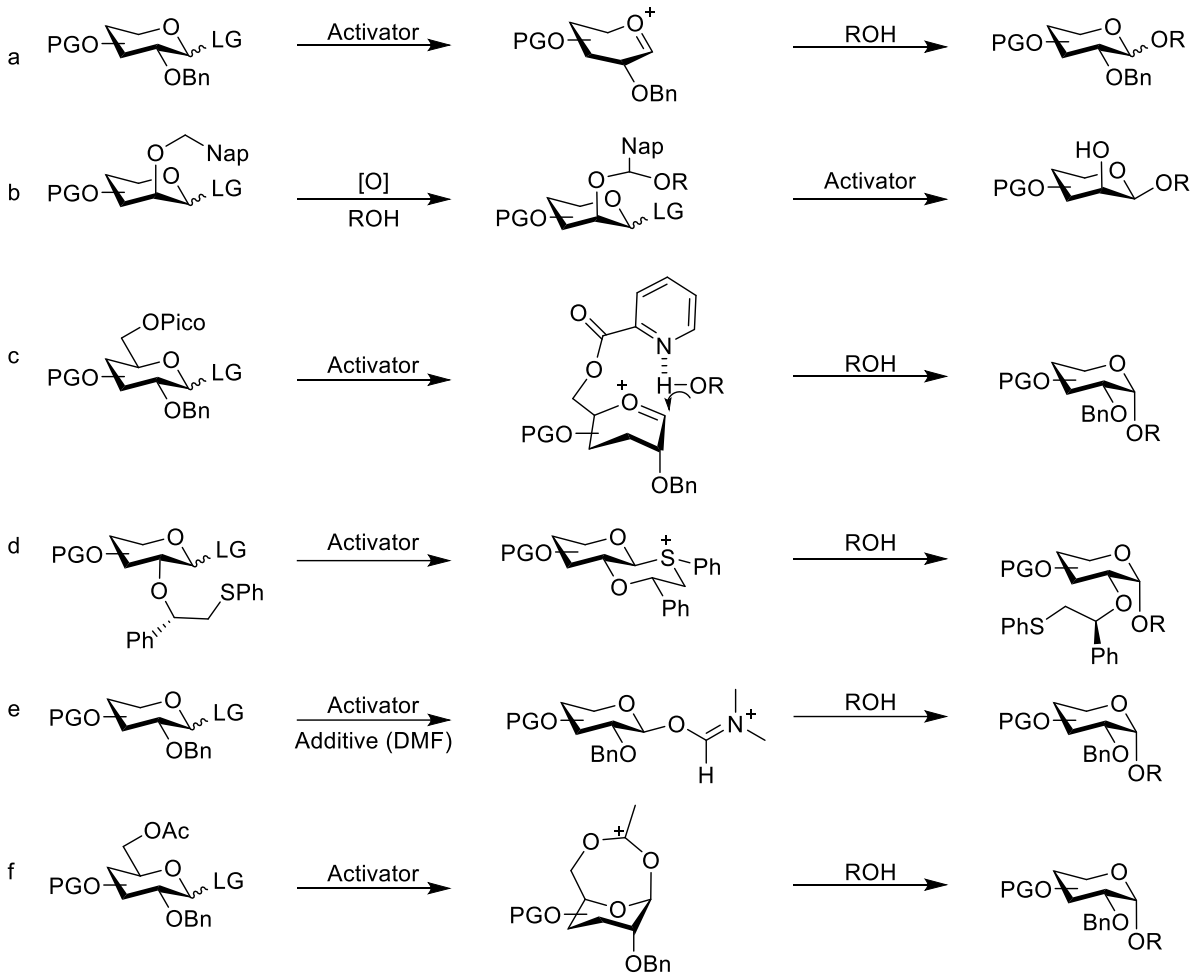


Figure 4.01. Methods for the formation of 1,2-*cis*-glycosides. a**,** A non-participating protecting group at C2 results in a mixture of anomers. **b,** Intramolecular aglycon delivery (IAD) with a C2 2-naphthylmethyl (NAP)-ether in mannose produces the β-anomer with high selectivity. **c,** Hydrogen-bond-mediated aglycan delivery (HAD) using picolinyl (Pic) and picoloyl (Pico) protecting groups at C4 position of glucose provide high selectivity. **d,** Chiral-auxiliaries ensure complete selectivity. **e,** Additive (or solvent)-modulation results in good to exclusive selectivity during 1,2-*cis*-glycoside formation. **f,** Remote participating groups result in varying selectivities depending on the acceptor. LG, leaving group; PG, protecting group. Pico, picoloyl; ROH, acceptor; Ph, phenyl.

Stereoselective formation of 1,2-cis glycosidic linkages still remains challenging[14-18], because non-participating C2 protecting groups generally lead to mixtures of stereoisomers that have to be separated at the end of the synthesis (**Figure 4.01 a**). Various strategies for the stereoselective synthesis of 1,2-cis glycosides have been described (**Figure 4.01**). Intramolecular aglycon delivery (IAD)[134], where the nucleophile is transferred from the adjacent C2 carbon to the anomeric position, is technically challenging when more than one cis-linkage is to be created. Hydrogen-bond-mediated aglycan delivery (HAD) method showed that installing picolinyl (Pic) and picoloyl (Pico) protecting groups on the C6 position is powerful for the synthesis of cis glucosidic linkage, but fails for cis galactosidic linkages[135], as none of remote hydroxyl groups (O3, O4, and O6) faces towards the bottom of the ring.

Chiral auxiliaries at C2 provide selectivity[136], but require two additional steps to be removed. Cleavage of the chiral auxiliary may result in a loss of benzyl ethers during solid-phase synthesis[137]. Additives can improve stereoselectivity by forming a less reactive intermediate in situ[138] but are hard to use during automated syntheses. Remote participation by protecting groups placed at the C3, C4, and/or C6 positions of glucose and galactose building blocks can control the stereoselectivity of glycosylations.[139-141] Building blocks containing common remote participating groups are attractive for automated synthesis as they fit the coupling – deprotection scheme and require no additional manipulations.

Here, we demonstrate that AGA of oligosaccharides containing 1,2-*cis*-glycosidic linkages is feasible when monosaccharide building blocks containing common remote participating groups are used. This work proves that identification and incorporation of reliable building blocks into AGA protocols enables accessibility to biologically relevant oligosaccharides containing *cis* linkages.

4.3 Results

To expand the scope of AGA to the stereoselective installation of cis-glycosidic linkages, we focused on cis-glucosides and cis-galactosides as the most prevalent 1,2-cis linkages in mammalian and bacterial glycomes[132,133]. Oligosaccharides (**AGA-4-01 - -04)** bearing α-galactosidic linkages and α-glucans examples (**AGA-4-05 - -09**) were selected as targets to develop automated methods for stereoselective *cis*-glycosidic bond formation (**Figure 4.02**).

4.3.1 Automated glycan assembly of various oligosaccharides containing α-galactoside.

Since the stereo- and regiochemical information for glycosidic bond formation mainly resides in the building blocks[139], the synthetic considerations focused initially on the identification of monomers containing remote participating groups. α-Gal trisaccharide epitope **AGA-4-01**[142] served as model system to optimize the formation of α-(1,3) galactosides. The influence of remote participation on the stereochemical outcome of AGA couplings was explored using seven thiogalactoside building blocks (**Gal-α-01-Gal-α-07**) containing Ac or Bz. Solid phase-bound disaccharide **AGA-Lactose** was assembled using building blocks **Glc-01** and **Gal-01** on a polystyrene resin equipped with a photocleavable linker **Resin-1.**[128]

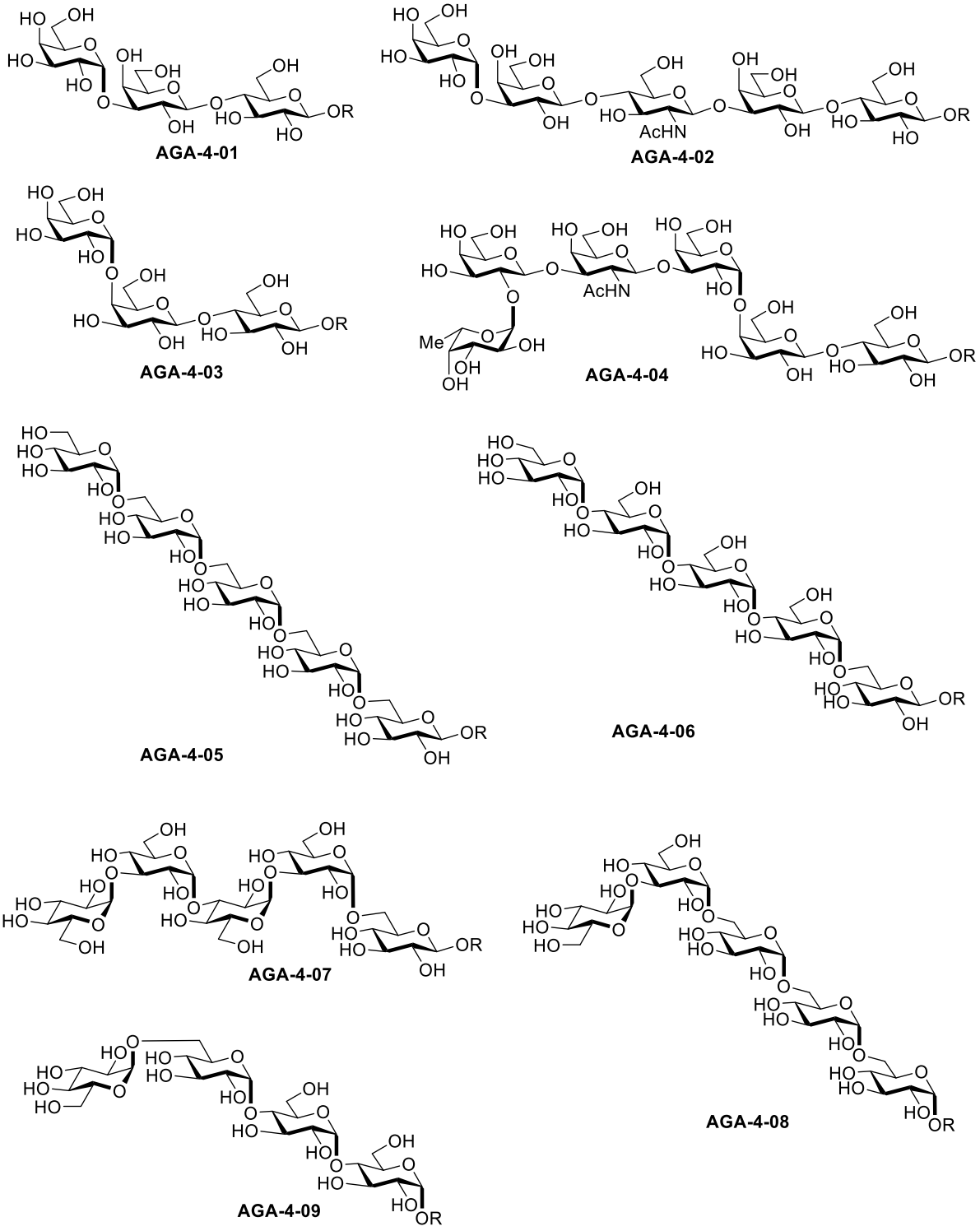
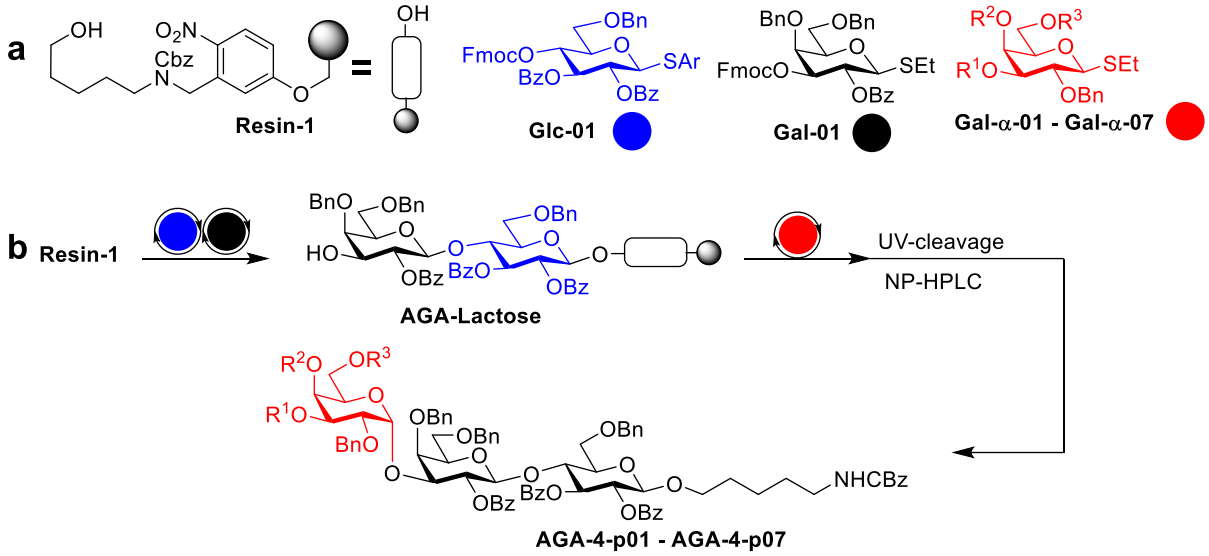


Figure 4.02 Oligosaccharides (**AGA-4-01**-**AGA-4-09**) containing different *cis*-glycosidic linkages were assembled by automated synthesis. R = $(CH_2)_5NH_2$.

Disaccharide **AGA-Lactose** served as the acceptor in glycosylations employing monomers **Gal**-α-**01 - Gal**-α-**07**. Incorporation of each building block was achieved by the addition of twice five equivalents monomer that was activated at activation temperature $\mathbf{T_a}$ (-40 ºC) for 5 min ($\mathbf{t_1}$) by the addition of a *N*-iodosuccinimide (NIS)/Trifluoromethanesulfonic acid (TfOH) activator solution, followed by incubation at the incubation temperature $\mathbf{T_i}$ (-20

°C)for 25 min (**t**$_2$). Following UV cleavage from the polystyrene resin, trisaccharides (**AGA-4-p01- AGA-4-p07**) were analyzed by normal-phase high-performance liquid chromatography (NP-HPLC) (**Table 4.01**) to determine conversion and stereoselectivity of the glycosylation reaction.



Entry	Building Block				Product	Ratio (α : β)	Yield[a]
		R^1	R^2	R^3			
1	**Gal-α-01**	Bn	Bn	Bn	**AGA-4-p01**	13.8:1	41%
2	**Gal-α-02**	Bn	Bn	Ac	**AGA-4-p02**	6.4:1	29%
3	**Gal-α-03**	Bn	Bn	Bz	**AGA-4-p03**	5.8:1	28%
4	**Gal-α-04**	Bn	Ac	Bn	**AGA-4-p04**	26.5:1	44%
5	**Gal-α-05**	Bn	Bz	Bn	**AGA-4-p05**	26.2:1	30%
6	**Gal-α-06**	Ac	Ac	Bn	**AGA-4-p06**	39.5:1	41%
7	**Gal-α-07**	Bn	Ac	Ac	**AGA-4-p07**	α only[b]	24%

Table 4.01 Identification of building blocks for installation of α-galactosidic linkages by automated synthesis. Conditions: Disaccharide **AGA-Lactose** was prepared using polystyrene resin equipped with photolabile linker (**Resin-1**) and building blocks **Glc-01** and **Gal-01**. Coupling with differentially protected building blocks **Gal-α-01 - Gal-α-07** was followed by UV cleavage to furnish trisaccharides **AGA-4-p01 - AGA-4-p07** that were analyzed by NP-HPLC. All couplings were performed using an automated synthesizer executing protocols delivering twice five equivalents of the building blocks. [a] Yield by preparative HPLC. [b] HPLC indicated *cis*-linked trisaccharide **AGA-4-p07** in addition to the disaccharide **AGA-Lactose** deletion sequence

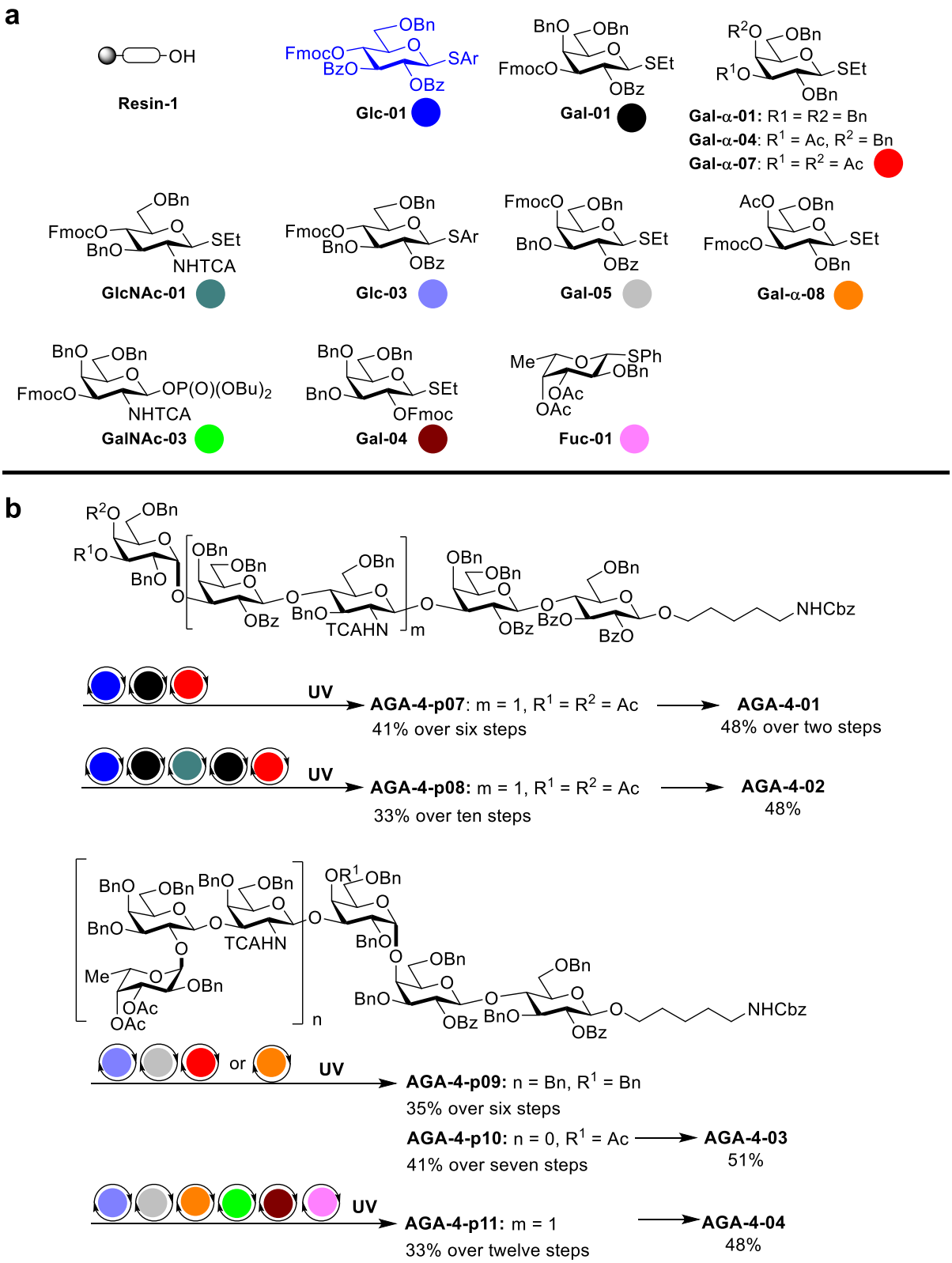
Galactose building block **Gal-α-01**[143], protected only with non-participating benzyl ether groups, relies exclusively on the anomeric effect to drive α-galactoside formation[143] and produced mainly the desired anomer **AGA-4-p01**[144] (α:β = 13.8:1) (entry 1).

Thiogalactosides **Gal-α-02**[145] and **Gal-α-03** bearing C6 acetyl or benzoyl esters resulted in lower selectivity (α:β = 6.4-5.8:1) (entries 2 and 3). These results are consistent with reports that C6 esters decrease the α-selectivity of galactose[140]. The presence of a C4-acetyl or benzoyl ester in thiogalactosides **Gal-α-04** and **Gal-α-05** drastically improved the α-selectivity (26.5-26.2:1). Expanding on the C4-ester effect, a second ester was placed on thiogalactose building blocks. Glycosylation of **AGA-Lactose** with C3, C4 bis-acetylated thiogalactose **Gal-α-06** proceeded with drastically improved selectivity (39.5:1) (**Table 4.01**, entry 6). Building block **Gal-α-07**, carrying C4 and C6 esters proceeded with complete stereoselectivity but significant amounts of the disaccharide deletion sequence remained after the coupling (entry 7). This study proved that the 1,2-*cis* galactosidic linkage can be installed by using building blocks that take advantage of remote participation groups but require no additives or chiral auxiliaries.

With optimized building blocks in hand, the α-Gal epitope pentasaccharide **AGA-4-02** was prepared by AGA (**Scheme 4.01**). All synthetic manipulations were executed on an automated oligosaccharide synthesizer working through preprogrammed steps that were combined into modules for each synthetic transformation. An initial acidic TMSOTf wash neutralizes basic residues that accumulate during DMF washes and removes any water present in the synthesizer. Glycosylation with twice five equivalents monomer was followed by Fmoc removal by treatment with a solution of triethylamine in DMF (v/v, 1/4). The glycosylation efficiency was estimated by quantitating the UV absorption of the released dibenzofulvene. Following automated assembly of the fully protected oligosaccharide targets, cleavage from the resin by UV irradiation was carried out using a continuous-flow photoreactor.[128] Purification by preparative normal phase-HPLC provided pentasaccharide **AGA-4-p01** (41% based on the resin loading over ten steps on resin) and **AGA-4-p08** respectively (33% over ten steps on resin). The conjugation-ready α-Gal epitope trisaccharide **AGA-4-01** (48**%** over two steps) and pentasaccharide **AGA-4-02** (48**%**) were purified by reverse-phase HPLC following deprotection of **AGA-4-p07** and **AGA-4-p08** by methanolysis and hydrogenolysis.

Building blocks carrying remote participation groups were employed in AGA of Globo-series oligosaccharides that contain a Gal-α-(1,4)-Gal-β-(1,4)-Glc common trisaccharide core (**AGA-4-03**)[146,147]. Globo-H (**AGA-4-04**), a hexasaccharide vaccine candidate currently in advanced clinical trials[148,149] contains the core trisaccharide **AGA-4-03**.

Thiogalactoside building block **Gal-α-08** was designed based on thiogalactoside **Gal-α-07** to allow for C3 elongation *en route* to Globo-H and to benefit from remote participation.



Scheme 4.01 Automated glycan assembly of α-galactoside containing oligosaccharides 1-4. (a) Resin functionalized with the photolabile linker (**Resin-1**) and monosaccharide building blocks. (b) Automated assembly and post-automation steps yield conjugation-ready oligosaccharides **AGA-4-01 – AGA-4-04**.

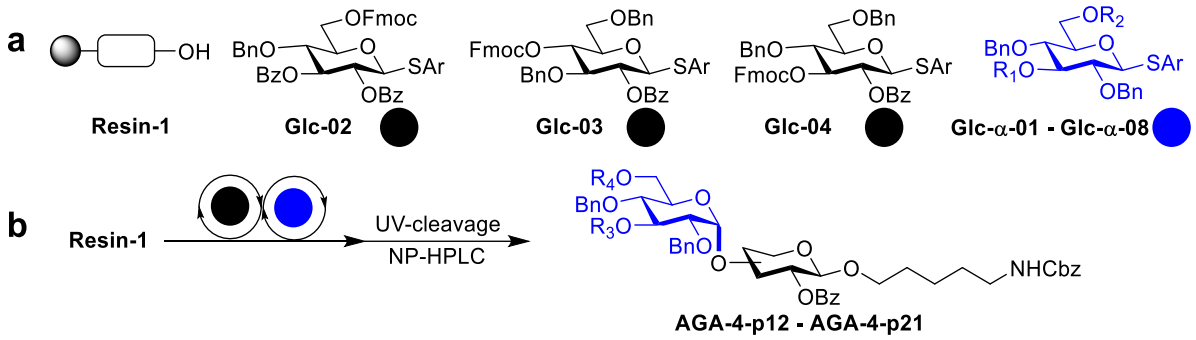
The influence of a remote C4 participation group present in monomer **Gal-05** was studied in the context of the AGA of trisaccharides **AGA-4-p09**[144] and **AGA-4-p10.** Trisaccharide **AGA-4-p10** using optimized building block **Gal-α-08** was obtained in 41% yield and with excellent selectivity (α:β = 19:1) while **AGA-4-p09** using perbenzylated thiogalactoside donor **Gal-α-01** was isolated in 35% yield and moderate selectivity (α:β = 4.3:1). This drastic improvement of stereoselective 1,2-*cis* galactosidic bond formation emphasizes the importance of the remote participating group on the C4 for the efficient synthesis of complex oligosaccharides containing *cis* galactoside(s) linkage using AGA. Similarly Globo-H hexasaccharide **AGA-4-04** was prepared by AGA using building blocks (**Scheme 4.01, Glc-03, Gal-04, GalNac-03, Gal-α-08**, **Gal-05**, and **Fuc-01**). Fully protected oligosaccharides **AGA-4-p03-Ac** and **AGA-4-p04** were deprotected to furnish **AGA-4-03 (**51% yield), and **AGA-4-04** (42%) respectively. The stereoselectivity of α-galactoside formation can dramatically benefit from the use of "approved" building blocks with remote participation.

4.3.2 Automated glycan assembly of various α-glucans containing multiple 1,2-*cis* glucosidic linkages.

The use of remote participation is not limited to the formation of cis-galactosidic linkages but can be generally employed as illustrated for 1,2-*cis* glucosidic linkages. Eight thioglucoside building blocks (**Glc-α-01**-**Glc-α-08**) exhibiting various protecting group patterns were synthesized to explore the influence of remote participation. AGA of disaccharides **AGA-4-p12-AGA-4-p21** was achieved using thioglycoside **Glc-02**, **Glc-03**, and **Glc-04** and building blocks **Glc-α-01**-**Glc-α-08** before photocleavage from the polystyrene resin yielded disaccharides **AGA-4-p12-AGA-4-p21** that were analyzed by HPLC (**Table 4.02**).

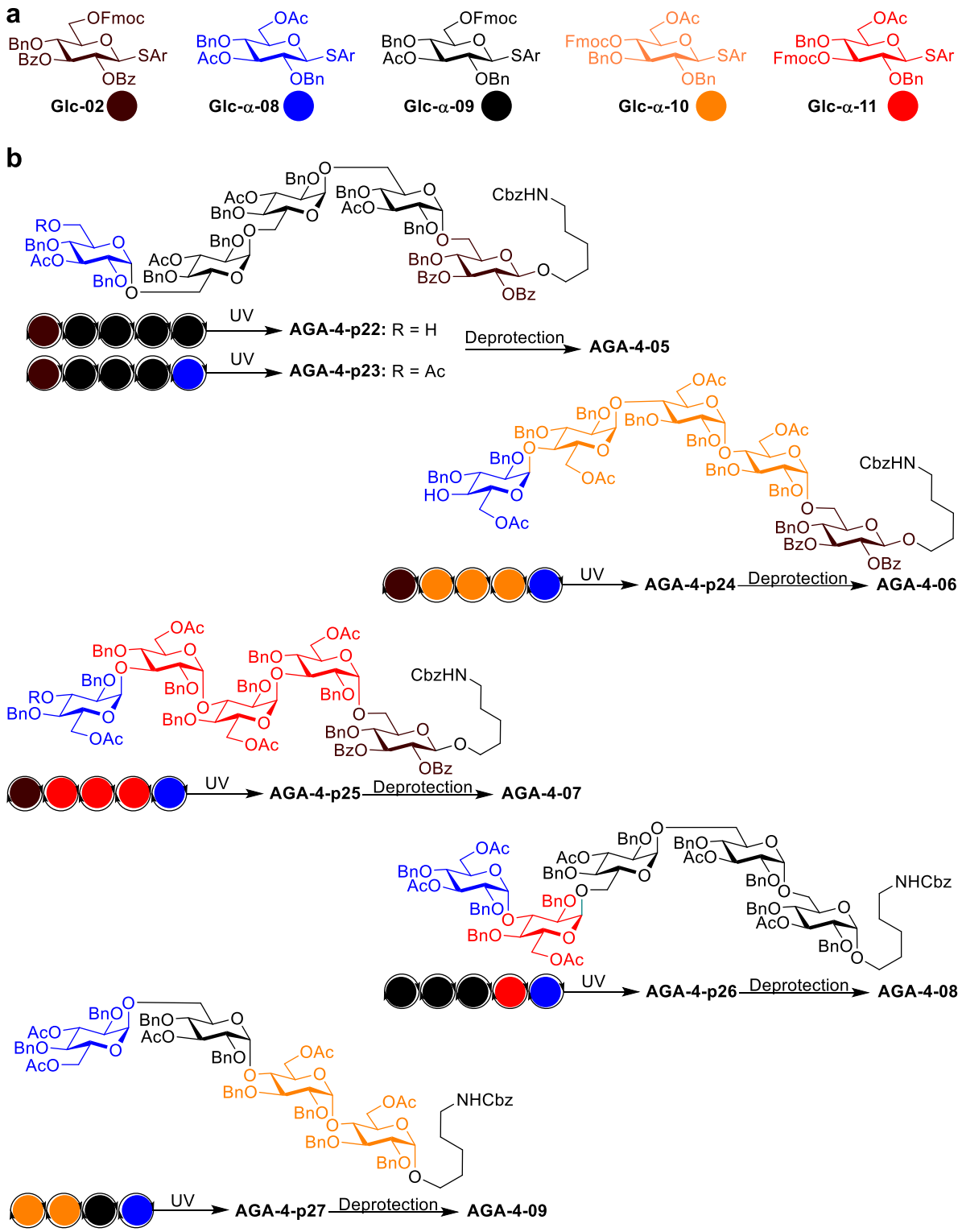
Glucose building block **Glc-α-01**, bearing only non-participating benzyl ether protective groups, relies exclusively on the influence of the anomeric effect to drive α -glucoside formation and yields the desired anomer **AGA-4-p12** (α:β = 2:1). Placement of a C6-acetyl ester in thioglucoside **Glc-α-02** drastically improved the (α:β) ratio (8:1). Sterically more demanding C6 protecting groups such as benzoate in monomer **Glc-α-02** or 9-fluorenemethylcarbonate (Fmoc) in building block **Glc-α-03** were less effective than the smaller acetate (**Glc-α-02**) but still improved the selectivity over building blocks without participation (**Glc-α-01**) (entries 3 and 4). Placement of participating protecting groups exclusively at the C3 hydroxyl group also resulted in improved selectivity (entries 5-7)

during the formation of disaccharides **AGA-4-p16-AGA4-p18**. This participation was less effective (α:β = 3.4-4.4:1) and the steric influence was less pronounced. These two positive factors were combined in building block **Glc-α-08** that carries two participating acetyl protecting groups in the C3 and C6 positions, and drastically improved the stereoselectivity to 11:1 (entry 9).[16,17] When diethyl ether was used as a co-solvent, the stereoselectivity was further improved to exceed 15: 1 (entry 10). α-(1→4) and α-(1→3)-glycosidic linkages formation was achieved effectively (α:β = 10.6:1, 9.5:1 respectively) using building blocks **Glc-03** and **Glc-04** and building block **Glc-α-08**. This study proved that the 1,2-*cis* glucosidic linkage can be installed by using building blocks that take advantage of remote participation groups as well.



Entry	1st BB	2nd BB	Product					Ratio (α : β)[a]	Yield[a]
			R1	R2		R3	R4		
1	Glc-02	**Glc-α-01**	Bn	Bn	**AGA-4-p12**	Bn	Bn	2.0 : 1	25%
2		**Glc-α-02**	Bn	Ac	**AGA-4-p13**	Bn	Ac	8.1 : 1	31%
3		**Glc-α-03**	Bn	Bz	**AGA-4-p14**	Bn	Bz	6.7 : 1	31%
4		**Glc-α-04**	Bn	Fmoc	**AGA-4-p15**	Bn	OH	3.5 : 1	30%
5		**Glc-α-05**	Ac	Bn	**AGA-4-p16**	Ac	Bn	4.4 : 1	27%
6		**Glc-α-06**	Bz	Bn	**AGA-4-p17**	Bz	Bn	3.4 : 1	27%
7		**Glc-α-07**	Fmoc	Bn	**AGA-4-p18**	OH	Bn	3.5 : 1	26%
8		**Glc-α-08**	Ac	Ac	**AGA-4-p19**	Ac	Ac	11.4 : 1	34%
9	**Glc-02**	**Glc-α-08**	Ac	Ac	**AGA-4-p19**	Ac	Ac	15.1 : 1[c]	34%
10	**Glc-03**	**Glc-α-08**	Ac	Ac	**AGA-4-p20**	Ac	Ac	10.6 : 1	34%
11	**Glc-04**	**Glc-α-08**	Ac	Ac	**AGA-4-p21**	Ac	Ac	9.5 : 1	35%

Table 4.02 Identification of building blocks for installation of α-glucosidic linkages by automated synthesis. Conditions: Using the resin equipped with photolabile linker (**Resin-1**) and glucose monomer **Glc-02** for the first coupling, differentially protected building blocks **Glc-α-01-Glc-α-08** were added to produce disaccharides **AGA-4-p12-AGA-4-p21** after cleavage from the solid support. [a] Building blocks were dissolved in DCM and ethyl ether (v/v, 1:3).



Scheme 4.02 Automated glycan assembly of fully protected α-glucans. a, Monosaccharide building blocks. b, AGA of conjugation-ready oligosaccharides **AGA-4-05-AGA-4-09**.

With reliable building blocks and protocols for the incorporation of α-glucosides by automated synthesis in place, the method was systematically challenged. The initial target, pentasaccharide **AGA-4-05,** containing one β-glycosidic and four consecutive α-(1→6)-

glycosidic linkages had been previously prepared using the chiral-axuiliary approach[132]. Using a polystyrene resin equipped with photocleavable linker (**Resin-1**), glucose building block **Glc-02** bearing a C2 benzoyl ester was incorporated. Subsequently, four consecutive α-1,2-*cis*-glucosidic linkages were formed using building block **Glc-α-09**.

All synthetic manipulations were executed on an automated oligosaccharide synthesizer working through preprogrammed steps that were combined into modules that are responsible for a synthetic transformation. An initial acidic TMSOTf wash neutralizes basic residues that accumulate during DMF washes and removes any water present in the synthesizer. Glycosylation with twice five equivalents monomer was followed by Fmoc removal by treatment with a solution of triethylamine in DMF (v/v, 1/4). The glycosylation efficiency was determined by quantitating the UV absorption of the released dibenzofulvene. Four couplings with monomer **Glc-α-09,** following the first coupling with monomer **Glc-02,** were performed to complete the automated assembly of α-glucan pentasaccharide **AGA-4-p22** in 15 h (**Scheme 4.02**). Following AGA, the fully protected oligosaccharide was liberated from the resin by UV irradiation using a continuous-flow photoreactor.[7] Pentasaccharide **AGA-4-p22** (13 mg, 23% over 11 steps on resin) was purified by preparative normal phase HPLC and characterized. When the most stereoselective building block **Glc-α-08** was used instead of **Glc-α-09** for the final coupling, protected pentasaccharide **AGA-4-p23** was produced of 21% overall yield over ten on-resin steps (**Scheme 4.02**). Removal of the protective groups from pentasaccharide **AGA-4-p22** yielded crude conjugation-ready pentasaccharide **AGA-4-05** that was purified by reverse-phase HPLC using a Hypercarb® column**.**

Pentasaccharide **AGA-4-06**, containing multiple α-(1→4) linkages, and **AGA-4-07** with multiple α-(1→3) glucosides were assembled using the corresponding building blocks (**Scheme 4.02**). The lower reactivity of the C4-hydroxyl group as glycosyl acceptor was compensated by prolonged glycosylation times ($\mathbf{t_2}$ = 50 min) during AGA of **AGA-4-06** using building blocks **Glc-02** and **Glc-α-10** (see Experimental Section). Cleavage from the resin and preparative normal phase HPLC yielded 9% of **AGA-4-06** over eleven steps. Using a similar AGA process, pentasaccharide **AGA-4-07** was obtained in 12% yield over eleven steps. The syntheses of **AGA-4-06** and **AGA-4-07** illustrated that glycosylations of secondary C3 or C4 hydroxyl groups are less efficient and less stereoselective than those involving primary C6 hydroxyl groups as nucleophiles. Such differences in glycosylation efficiency are well known for solution phase strategies. It is anticipated that the glycosylation modules (e.g. number of glycosylation, reaction time, or temperature) upon AGA will improve

glycosylation efficiency and seteroselectivity. Removal of the protective groups from pentasaccharide **AGA-4-p22, AGA-4-p24**, and **AGA-4-p25** yielded conjugation-ready pentasaccharides **AGA-4-05 (**47% yield), **AGA-4-06** (33%) and **AGA-4-07 (**31%) following reverse-phase HPLC**.** Two naturally occurring oligosaccharide fragments bearing multiple 1,2-*cis* glucosidic linkages were assembled using the building blocks and protocols established above. The immune-modulatory pentasaccharide **AGA-4-08**[150], containing α-(1→3) and α-(1→6) glucosidic linkages and tetrasaccharides **AGA-4-09**[151] known for activating toll-like receptor containing α-(1→4) and α-(1→6) glucosides were synthesized. Deprotection of oligosaccharides **AGA-4-p26** that was assembled in 17% yield over ten steps and **AGA-4-p27** yielding 20% over eight steps, furnished oligosacchardes **AGA-4-08 (**47% yield) and **AGA-4-09** (33%).

4.4 Conclusions

In conclusion, we demonstrate that building blocks bearing remote participating groups are effective in assembling oligosaccharides containing *cis*-glucosidic and *cis*-galactosidic linkages. Nine oligosaccharides (**AGA-4-01**-**AGA-4-09**) were assembled to illustrate the power of the AGA approach. Standardized synthesis as well as deprotection and purification protocols enabled us to procure conjugation-ready molecules. The reliable incorporation of *cis*-gluco- and *cis*-galactosides into oligosaccharides by automated synthesis complements earlier successes in installing *trans*-glycosides, to render AGA the method of choice for the procurement of complex glycans.

4.5 Experimental Section

4.5.1 Automation

Preparation of Reagent Solutions and Modules

Building Block Solution:

For the glycosylation using twice 5 equivalents, 0.25 mmol of building block was dissolved in 2.0 mL of DCM.

Acidic TMSOTf wash Solution

For the acidic TMSOTf wash 480 μL TMSOTf was dissolved in 20 mL DCM.

Activator Solution

For the thioglycoside monomer *N*-Iodosuccinimide (1.35 g) was dissolved in a 9:1 mixture of anhydrous DCM and dioxane (40.0 mL) and then TfOH (60 μL) was added in ice bath.
For the phosphate monomer 480 μL TMSOTf was dissolved in 20 mL DCM.

Fmoc Deprotection Solution

Solution of 20% triethylamine in DMF (v/v) was prepared.

Preparation of the resin and the synthesizer for automated synthesis: The functionalized resin was loaded into the reaction vessel of the synthesizer and swollen in 2 mL DCM. To start the synthesis sequence, the resin was washed using Module 1. The building blocks were co-evaporated with toluene three times, dissolved in DCM under an argon atmosphere and transferred into the vials that were placed on the corresponding port in the synthesizer. Reagents were dissolved in the corresponding solvents under an Ar atmosphere in bottles that were placed on the corresponding port in the synthesizer.

Module 1 – Acidic TMSOTf Wash: The resin was washed with DMF, THF, DCM (three times each, with 2 mL for 15 s). The resin was swollen in 2 mL DCM, and the temperature of the reaction vessel was adjusted to –20 °C. 0.5 mL of the TMSOTf solution in DCM was delivered to the reaction vessel at -20 °C. After one minute, the solution was drained. The

resin was swollen in 2 mL DCM and the temperature of the reaction vessel was adjusted to $\mathbf{T_a}$.

Module 2 – Glycosylation: During temperature adjustment, the DCM in the reaction vessel was drained and a solution of thioglycoside building block (5.0 eq. in 1.0 mL DCM) was delivered to the reaction vessel. After the set temperature was reached ($\mathbf{T_a}$), the reaction starts with the addition of 1 mL of NIS/TfOH (5.5 eq. in 1.0 mL DCM), and TfOH (0.2 eq. in 1.0 mL DCM) solution. The glycosylation mixture was activated for an activation time ($\mathbf{t_1}$ = 5 minutes) at **Ta**. The temperature was linearly ramped to the incubation temperature ($\mathbf{T_i}$) and finally incubated for an additional incubation time for $\mathbf{t_2}$ at $\mathbf{T_i}$. After the reaction the solution was drained and the resin is washed with DCM (six times with 2 mL for 15 s). This procedure was repeated **twice.**

Module 3 - Fmoc Deprotection: The resin was washed with DMF (six times with 2 mL for 15 s), swollen in 2 mL DMF and the temperature of the reaction vessel iwas adjusted to 25°C. For Fmoc deprotection the DMF is drained and 3.5 mL of a solution of 20% Et_3N in DMF was delivered to the reaction vessel. After 5 min the reaction solution was collected in the fraction collector of the oligosaccharide synthesizer. This procedure was repeated **twice**.

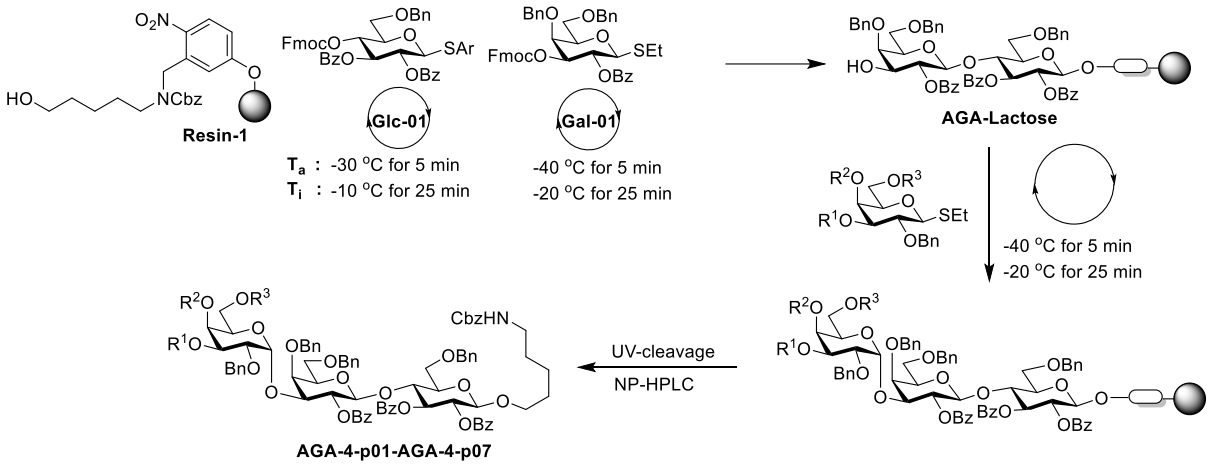
Resin Cleavage: To prepare the photoreactor, the FEP tubing was washed with 20 mL DCM using a flow rate of 5 mL/min. For the cleavage, the resin was slowly injected from the disposable syringe (20 mL) into the reactor and pushed through the tubing with 18 mL DCM (flow rate: 600 µL/min). The tubing was washed with 20 mL DCM (flow rate: 2 mL/min) to remove any remaining resin. The suspension leaving the reactor was directed into a filter where the resin was filtered off. The system was re-equilibrated by washing the tubes with 20 mL DCM using a flow rate of 5 mL/min. The entire procedure was performed twice. The resulting solution was evaporated and the crude material was analyzed by NMR and HPLC[1].

Analytical HPLC: The crude material was analyzed by HPLC (column: Luna 5µ Silica 100A, (260 X 4.60 mm); flow rate: 1 mL/min; eluents: 5% DCM in hexane / 5% DCM in ethyl acetate; gradient: 20% (5 min) 60% (in 40 min) 100% (in 5 min); detection: 280 nm). ***NOTE: The crude material were used to provide analytical HPLC data in supporting information to identify the desire oligosaccharide in the crude mixture following UV cleavage of the photolabile linker conjugated to the resin Resin-1.***

Preparative HPLC: The crude mixture was carefully dissolved in minimum volume of DCM and 0.9 mL of 20% hexane in ethyl acetate. The crude solution was injected for purification using preparative HPLC (column: Luna 5µ Sil (260 X 10 mm); flow rate: 5 mL/min; eluents: 5% DCM in hexane / 5% DCM in ethyl acetate; gradient: 20% (5 min) 60% (in 40 min) 100% (in 5 min); detection: 280 nm) to afford the fully protected target oligosaccharide.

Synthesis of trisaccharides AGA-4-p01-AGA-4-p07

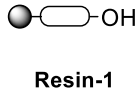
The building blocks **Glc-01**, **Gal-01** and the next corresponding building blocks **Gal-α-01-Gal-α-07** were placed on the corresponding building block vial location. Then the automated synthesis started performing reactions with module I, II, and III to afford disaccharides **AGA-4-p01-AGA-4-p07.**



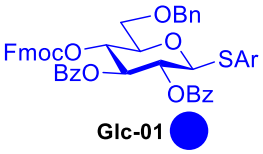
Sequence	Module	Details	Condition
I	1	2.5 eq. of TMSOTf solution	-20 °C, 1 min
	2	5 eq. building block **Glc-01**	T_a = -30 °C, t_1 = 5 min
		5 eq. of NIS Solution	T_i = -10 °C, t_2 = 25 min
	3	Fmoc Removal	r.t., 5 min
II	1	2.5 eq. of TMSOTf solution	-20 °C, 1 min
	2	5 eq. building block **Gal-01**	T_a = -40 °C, t_1 = 5 min
		5 eq. of NIS Solution	T_i = -20 °C, t_2 = 25 min
	3	Fmoc Removal	r.t., 5 min
III	1	2.5 eq. of TMSOTf solution	-20 °C, 1 min
	2	5 eq. building block **Gal-α-01-Gal-α-07**	T_a = -40 °C, t_1 = 5 min

5 eq. of NIS Solution	T_i = -20 °C, t_2 = 25 min

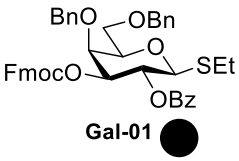
Modules of automated syntheses of trisaccharides AGA-4-p01-AGA-4-p07



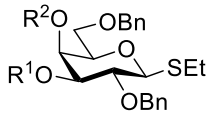
Resin-1



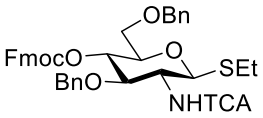
Glc-01



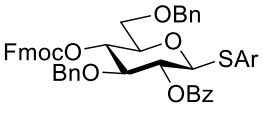
Gal-01



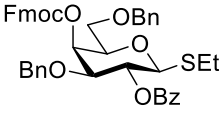
Gal-α-01: R1 = R2 = Bn
Gal-α-04: R^1 = Ac, R^2 = Bn
Gal-α-07: R^1 = R^2 = Ac



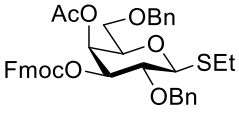
GlcNAc-01



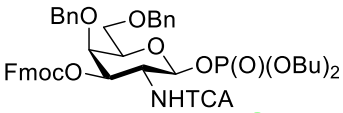
Glc-03



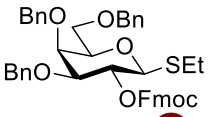
Gal-05



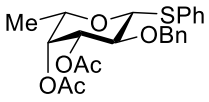
Gal-α-08



GalNAc-03



Gal-04



Fuc-01

Sequence	Module	Details	Conditions
I	1	2.5 eq. of TMSOTf solution	-20 °C, 1 min
	2	5 eq. BB **Glc-01, GlcNAc-01**, or **Glc-03** 5.5 eq. of NIS Solution	T_a = -30 °C, t_1 = 5 min T_i = -10 °C, t_2 = 25 min
	3	Fmoc Removal	r.t., 5 min
II	1	2.5 eq. of TMSOTf solution	-20 °C, 1 min
	2	5 eq. BB **Gal-01, Gal-05**, or **Gal-α-08** 5.5 eq. of NIS Solution	T_a = -40 °C, t_1 = 5 min T_i = -20 °C, t_2 = 25 min
	3	Fmoc Removal	r.t., 5 min
III	1	2.5 eq. of TMSOTf solution	-20 °C, 1 min
	2	5 eq. BB **Gal-α-01 - Gal-α-07** or **Fuc-01** 5.5 eq. of NIS Solution	T_a = -40 °C, t_1 = 5 min T_i = -20 °C, t_2 = 25 min
IV	1	2.5 eq. of TMSOTf solution	-20 °C, 1 min
	2	5 eq. BB **GalNAc-03** 5.5 eq. of TMSOTf solution	T_a = -30 °C, t_1 = 5 min T_i = -10 °C, t_2 = 25 min
	3	Fmoc Removal	r.t., 5 min
V	1	2.5 eq. of TMSOTf solution	-20 °C, 1 min
	2	5 eq. BB **Gal-05**	T_a = -40 °C, t_1 = 5 min

	5.5 eq. of NIS Solution	T_i = -20 °C, t_2 = 25 min
3	Fmoc Removal	**30 °C**, 5 min

Sequences of the glycosylation cycle with the corresponding monomers.

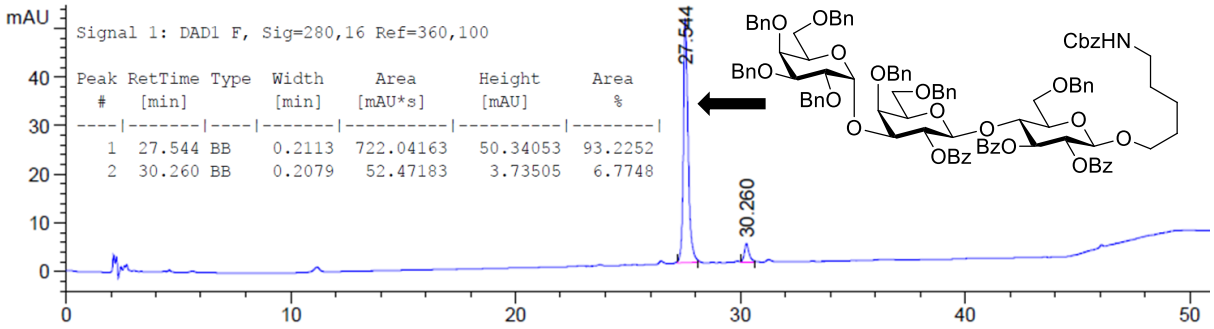
Entry	BB 2	Compound	Amounts	Yield
1	**Gal-α-01**	**AGA-4-p01**	16 mg	41%
2	**Gal-α-02**	**AGA-4-p02**	11 mg	29%
3	**Gal-α-03**	**AGA-4-p03**	11 mg	28%
4	**Gal-α-04**	**AGA-4-p04**	17 mg	44%
5	**Gal-α-05**	**AGA-4-p05**	12 mg	30%
6	**Gal-α-06**	**AGA-4-p06**	15 mg	41%
7	**Gal-α-07**	**AGA-4-p07**	9 mg	24%

Yields of trisaccharides AGA-4-p01-AGA-4-p07.

Compound		On-Resin Steps	Amounts	Yield
AGA-4-p08	Alpha-Gal pentasaccharide	10	20 mg	33%
AGA-4-p09	Gb-3	6	15 mg	35%
AGA-4-p10	Gb-3	7	17 mg	41%
AGA-4-p11	Globo-H	12	18 mg	33%

Yields of oligosaccharides **AGA-4-p08 - AGA-4-p11.**

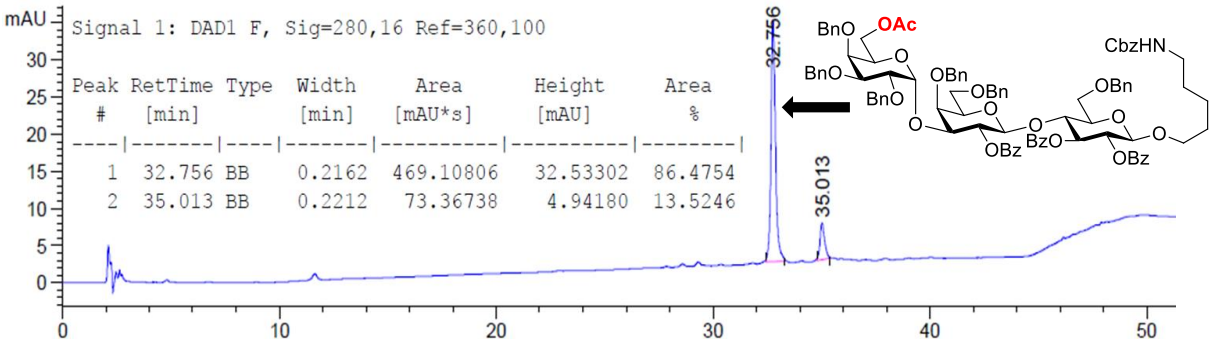
_N_-Benzyloxycarbonyl-5-amino-pentyl 2,3,4,6-tetra-_O_-benzyl-α-D-galactopyranosyl-(1→3)-2-_O_-bezoyl-4,6-di-_O_-benzyl-β-D-galactopyranosyl-(1→4)-2,3-di-_O_-benzoyl-6-_O_-benzyl-β-D-glucopyranoside AGA-4-p01



LC-MS chromatogram of **AGA-4-p01.**

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.97 (d, *J* = 7.7 Hz, 2H), 7.87 (d, *J* = 7.6 Hz, 2H), 7.61 (t, *J* = 7.4 Hz, 1H), 7.52 (t, *J* = 7.3 Hz, 1H), 7.46 (t, *J* = 7.6 Hz, 2H), 7.41 – 7.13 (m, 38H), 7.12 – 7.04 (m, 6H), 7.01 – 6.94 (m, 3H), 5.63 – 5.54 (m, 1H, H-2), 5.13 (t, *J* = 8.5 Hz, 1H, H'-2), 5.07 – 5.02 (m, 3H, H''-1, CH_2 of Cbz), 4.87 (dd, *J* = 11.6, 7.2 Hz, 2H, 2 x C*H*HPh), 4.80 (d, *J* = 12.2 Hz, 1H, C*H*HPh), 4.74 (d, *J* = 11.8 Hz, 1H, C*H*HPh), 4.71 – 4.62 (m, 3H, H-1, 2 x C*H*HPh), 4.60 (d, *J* = 12.1 Hz, 1H, C*H*HPh), 4.56 – 4.48 (m, 3H, H-,2 x C*H*HPh), 4.41 (d, *J* = 11.3 Hz, 1H, C*H*HPh), 4.35 – 4.04 (m, 13H), 3.78 – 3.73 (m, 1H, H-6), 3.69 (t, *J* = 8.9 Hz, 1H, H'-3), 3.65 – 3.59 (m, 1H, H-6), 3.56 (t, *J* = 9.1 Hz, 3H, 2 x H-6, $OCH_2(CH_2)_4NHCbz$), 3.44 (br, 1H, H-5), 3.40 (d, *J* = 10.2 Hz, 1H, H-3), 3.29 (d, *J* = 7.4 Hz, 2H, H-5, H-6), 3.19 (dd, *J* = 8.6, 4.6 Hz, 1H, H-6), 2.85 (d, *J* = 6.0 Hz, 2H, CH_2NHCbz), 1.50 – 1.32 (m, 2H, CH_2, pentane), 1.31 – 1.19 (m, 2H, CH_2, pentane), 1.18 – 1.02 (m, 2H, CH_2, pentane). ^{13}C NMR (150 MHz, $CDCl_3$) δ 165.16 (Bz), 165.10 (Bz), 156.37 (Cbz), 139.19, 138.81, 138.76, 138.65, 138.60, 138.35, 138.31, 138.03, 136.87, 133.25, 133.01, 130.22, 130.15, 129.95, 129.89, 128.75, 128.62, 128.56, 128.48, 128.37, 128.35, 128.32, 128.26, 128.22, 128.20, 128.19, 128.16, 127.95, 127.84, 127.80, 127.58, 127.41, 127.39, 127.28, 127.18, 101.31 (C'-1), 101.15 (C''-1), 100.71 (C-1), 79.87, 79.52, 78.90, 76.79, 76.56, 75.13, 75.08, 74.92, 74.29, 74.16, 73.92, 73.62, 73.52, 73.31, 73.22, 73.00, 72.68, 72.24, 71.20, 69.48, 69.23, 68.10, 67.83, 67.46, 66.59, 40.92, 29.45, 28.96, 23.18.; MS ESI$^+$-HRMS *m/z* $[M+Na]^+$ calcd for $C_{101}H_{105}NO_{20}Na$ 1674.7128, found 1674.7037.

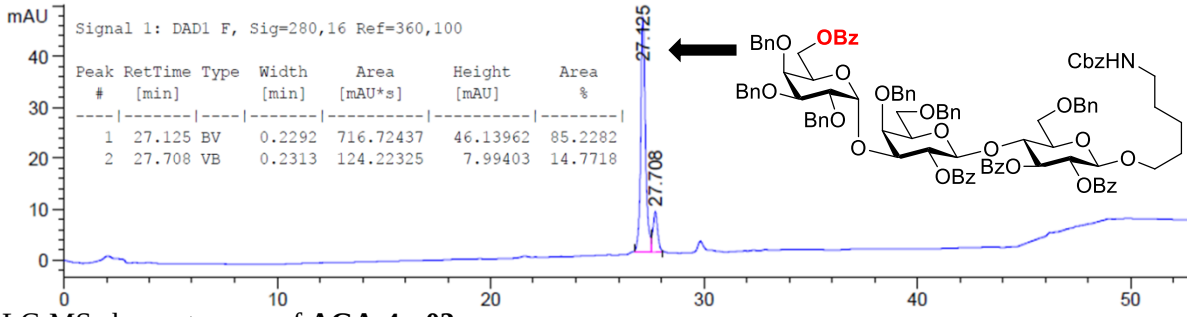
_N_-Benzyloxycarbonyl-5-amino-pentyl 6-_O_-acetyl-2,3,4-tri-_O_-benzyl-α-D-galactopyranosyl-(1→3)-2-_O_-bezoyl-4,6-di-_O_-benzyl-β-D-galactopyranosyl-(1→4)-2,3-di-_O_-benzoyl-6-_O_-benzyl-β-D-glucopyranoside AGA-4-p02



LC-MS chromatogram of **AGA-4-p02.**

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (s, 5H), 7.81 (d, *J* = 7.6 Hz, 2H), 7.56 – 7.03 (m, 43H), 5.52 (dt, *J* = 17.6, 9.1 Hz, 2H), 5.29 (s, 1H), 5.04 (s, 2H), 4.95 (d, *J* = 11.8 Hz, 1H), 4.89 (d, *J* = 2.6 Hz, 1H, **H''-1**), 4.78 (t, *J* = 11.4 Hz, 2H), 4.62 – 4.45 (m, 7H, , **H-1** and **H'-1**), 4.39 (d, *J* = 11.3 Hz, 1H), 4.28 (d, *J* = 12.3 Hz, 1H), 4.21 (d, *J* = 11.8 Hz, 1H), 4.01 (ddd, *J* = 13.1, 12.7, 7.3 Hz, 4H), 3.83 – 3.69 (m, 5H), 3.60 – 3.40 (m, 6H), 3.35 (s, 1H), 3.21 (d, *J* = 4.4 Hz, 1H), 2.83 (dd, *J* = 20.7, 11.9 Hz, 4H), 1.77 (s, 3H), 1.40 (dd, *J* = 15.9, 13.6 Hz, 2H), 1.26 (d, *J* = 12.7 Hz, 2H), 1.15 (d, *J* = 6.2 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.01 (Ac), 165.36 (Bz), 165.30 (Bz), 164.82 (Bz), 156.38 (Cbz), 139.38, 138.62, 138.38, 138.32, 138.18, 136.88, 133.27, 133.17, 132.59, 130.51, 129.93, 129.86, 129.83, 129.75, 128.62, 128.52, 128.49, 128.46, 128.39, 128.15, 128.08, 127.90, 127.83, 127.73, 127.63, 127.53, 127.48, 127.05 (Ar), 101.12 (**C-1** and **C'-1**), 98.69 (**C''-1**), 80.20, 79.06, 77.36, 76.43, 75.39, 74.89, 74.68, 74.39, 74.16, 73.74, 73.58, 73.51, 73.21, 73.16, 73.09, 72.22, 69.74, 68.95, 67.85, 67.27, 66.63, 62.16, 40.96, 29.53, 29.03, 23.19, 20.84.; MS ESI^{+}-HRMS *m/z* $[M+Na]^{+}$ calcd for $C_{96}H_{99}NO_{22}Na$ 1640.6551, found 1640.6558.

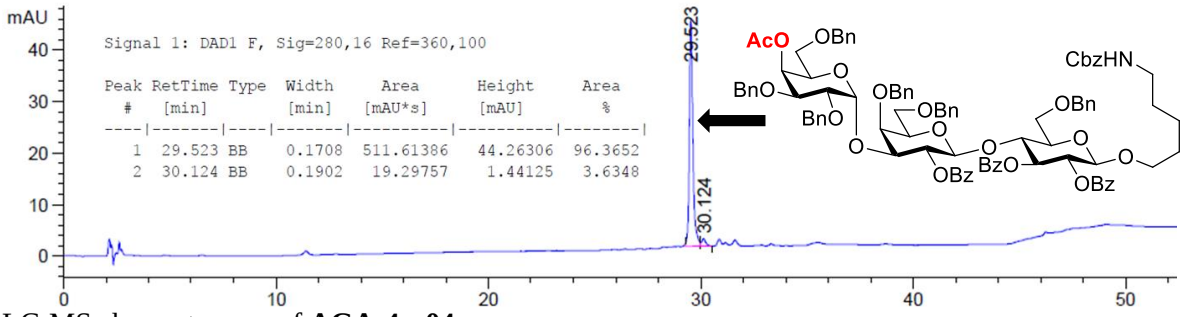
_N_-Benzyloxycarbonyl-5-amino-pentyl 6-_O_-benzoyl-2,3,4-tri-_O_-benzyl-α-D-galactopyranosyl-(1→3)-2-_O_-bezoyl-4,6-di-_O_-benzyl-β-D-galactopyranosyl-(1→4)-2,3-di-_O_-benzoyl-6-_O_-benzyl-β-D-glucopyranoside AGA-4-p03



LC-MS chromatogram of **AGA-4-p03**.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 – 7.87 (m, 4H), 7.84 – 7.80 (m, 4H), 7.62 – 7.57 (m, 1H), 7.44 (dd, *J* = 10.5, 5.0 Hz, 3H), 7.39 – 7.05 (m, 43H), 5.60 – 5.52 (m, 2H), 5.30 (dd, *J* = 9.7, 7.9 Hz, 1H), 5.06 (s, 2H), 5.01 (d, *J* = 11.8 Hz, 1H), 4.96 (d, *J* = 3.4 Hz, 1H, **H''-1**), 4.79 (t, *J* = 11.5 Hz, 2H), 4.63 (dd, *J* = 20.8, 11.7 Hz, 2H), 4.54 – 4.47 (m, 4H, **H-1**, **H'-1,** 2 x x C*H*HPh), 4.41 (dd, *J* = 17.5, 11.8 Hz, 2H), 4.23 (d, *J* = 12.1 Hz, 2H), 4.09 – 3.95 (m, 5H), 3.87 (dd, *J* = 12.8, 5.5 Hz, 1H), 3.84 – 3.74 (m, 4H), 3.66 (dd, *J* = 10.2, 2.5 Hz, 1H), 3.57 – 3.42 (m, 4H), 3.37 (dd, *J* = 15.8, 6.6 Hz, 1H), 3.19 (t, *J* = 6.8 Hz, 1H), 2.93 – 2.86 (m, 2H), 2.85 (d, *J* = 7.3 Hz, 2H), 1.54 – 1.37 (m, 2H), 1.28 (d, *J* = 8.6 Hz, 2H), 1.22 – 1.08 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.55 (Bz), 165.37 (Bz), 165.24 (Bz), 164.85 (Bz), 156.38 (Cbz), 139.40, 138.69, 138.36, 138.19, 138.16, 136.88, 133.30, 133.23, 133.16, 132.62, 130.48, 130.02, 129.92, 129.85, 129.75, 129.69, 128.62, 128.56, 128.50, 128.45, 128.39, 128.35, 128.20, 128.17, 128.07, 127.84, 127.74, 127.63, 127.60, 127.55, 127.03 (Ar), 101.35 (**C-1**), 101.06 (**C-1**), 98.18 (**C''-1**), 79.65, 79.09, 77.36, 76.32, 75.79, 74.83, 74.70, 74.51, 74.40, 73.79, 73.39, 73.27, 73.21, 73.07, 72.25, 72.01, 69.76, 68.98, 67.99, 67.21, 66.63, 62.38, 40.96, 29.52, 29.03, 23.19.; MS ESI^{+}-HRMS *m/z* $[M+Na]^{+}$ calcd for $C_{101}H_{101}NO_{22}Na$ 1702.6707, found 1702.6699.

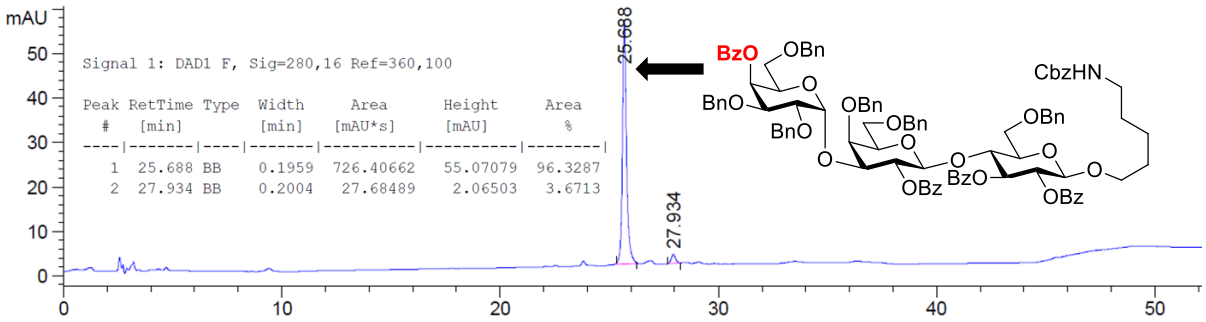
_N_-Benzyloxycarbonyl-5-amino-pentyl 4-_O_-acetyl-2,3,6-tri-_O_-benzyl-α-D-galactopyranosyl-(1→3)-2-_O_-bezoyl-4,6-di-_O_-benzyl-β-D-galactopyranosyl-(1→4)-2,3-di-_O_-benzoyl-6-_O_-benzyl-β-D-glucopyranoside AGA-4-p04



LC-MS chromatogram of **AGA-4-p04.**

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, *J* = 7.2 Hz, 2H), 7.93 – 7.89 (m, 2H), 7.83 (dd, *J* = 8.2, 1.1 Hz, 2H), 7.53 (t, *J* = 7.4 Hz, 1H), 7.46 (t, *J* = 7.4 Hz, 1H), 7.40 – 7.09 (m, 40H), 7.05 (dd, *J* = 7.2, 1.9 Hz, 2H), 5.59 (t, *J* = 9.4 Hz, 1H, H-3), 5.53 (dd, *J* = 10.0, 8.0 Hz, 1H, H'-2)), 5.31 (dd, *J* = 9.6, 8.0 Hz, 1H, H-2), 5.18 (s, 1H, H''-4), 5.06 (s, 2H, Cbz), 4.92 (d, *J* = 2.0 Hz, 1H, **H''-1**), 4.87 (d, *J* = 11.8 Hz, 1H), 4.76 (d, *J* = 11.6 Hz, 1H), 4.58 – 4.43 (m, 6H, **H-1**, **H'-1**), 4.34 – 4.15 (m, 5H), 4.10 – 3.95 (m, 4H), 3.86 – 3.76 (m, 2H), 3.74 – 3.63 (m, 3H), 3.62 – 3.47 (m, 3H), 3.38 (dd, *J* = 15.5, 6.6 Hz, 1H), 3.20 (t, *J* = 6.8 Hz, 1H), 3.08 – 2.97 (m, 2H), 2.90 (s, 2H), 2.85 (d, *J* = 6.6 Hz, 2H), 1.93 (s, 3H), 1.54 – 1.36 (m, 2H), 1.33 – 1.22 (m, 2H), 1.22 – 1.09 (m, 2H).^{13}C NMR (100 MHz, $CDCl_3$) δ 170.09 (Ac), 165.37 (Bz), 165.26 (Bz), 164.80 (Bz), 156.39, 139.23, 138.46, 138.42, 138.21, 138.13, 138.06, 136.87, 133.28, 133.17, 132.60, 130.47, 130.06, 129.95, 129.92, 129.85, 129.74, 128.62, 128.49, 128.39, 128.36, 128.16, 128.07, 128.03, 127.98, 127.82, 127.69, 127.61, 127.11, 101.25 (**C'-1**), 101.11 (**C-1**), 98.69 (**C''-1**), 80.29, 77.36, 76.23, 75.72, 75.61, 74.83, 74.66, 74.27, 73.77, 73.52, 73.36, 73.14, 73.10, 72.27, 71.69, 69.76, 68.26, 68.06, 67.84, 67.24, 66.63, 40.95, 29.51, 29.03, 23.19, 20.97.; MS ESI^{+}-HRMS *m/z* $[M+Na]^{+}$ calcd for $C_{96}H_{99}NO_{22}Na$ 1640.6551, found 1640.6561.

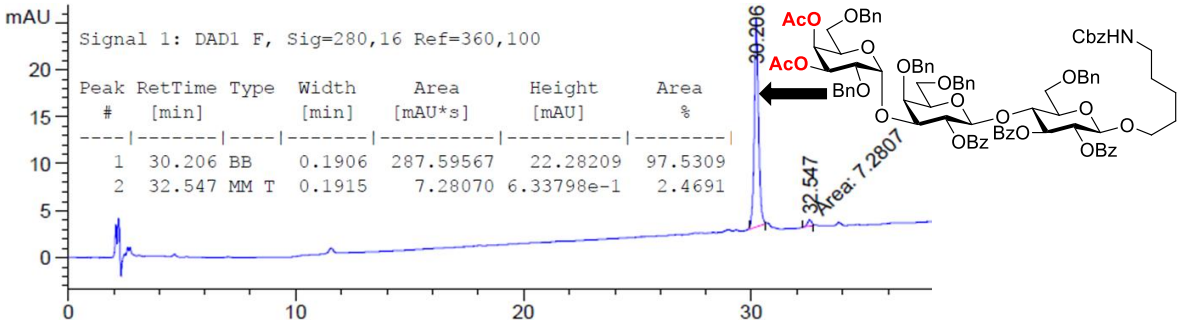
_N_-Benzyloxycarbonyl-5-amino-pentyl 4-_O_-benzoyl-2,3,6-tri-_O_-benzyl-α-D-galactopyranosyl-(1→3)-2-_O_-bezoyl-4,6-di-_O_-benzyl-β-D-galactopyranosyl-(1→4)-2,3-di-_O_-benzoyl-6-_O_-benzyl-β-D-glucopyranoside AGA-4-p05



LC-MS chromatogram of **AGA-4-p05.**

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (d, *J* = 7.6 Hz, 2H), 7.92 (d, *J* = 7.2 Hz, 4H), 7.83 (d, *J* = 7.4 Hz, 2H), 7.53 (t, *J* = 6.8 Hz, 2H), 7.46 (t, *J* = 7.3 Hz, 1H), 7.42 – 7.06 (m, 44H), 5.63 – 5.52 (m, 2H, H-3, H'-2), 5.36 (s, 1H, H''-4), 5.32 (d, *J* = 7.9 Hz, 1H, H-2), 5.06 (s, 2H, Cbz), 4.99 (s, 1H, **H''-1**), 4.94 (d, *J* = 11.7 Hz, 1H), 4.73 (d, *J* = 11.6 Hz, 1H), 4.61 – 4.48 (m, 5H, **H'-1**, **H'-1**, 2 x C*H*HPh), 4.43 (d, *J* = 12.2 Hz, 1H), 4.30 – 4.21 (m, 4H), 4.16 (d, *J* = 11.9 Hz, 1H), 4.11 – 3.98 (m, 4H), 3.85 (d, *J* = 9.1 Hz, 1H), 3.81 (d, *J* = 10.8 Hz, 3H), 3.75 (d, *J* = 10.2 Hz, 1H), 3.66 – 3.47 (m, 2H), 3.39 (dd, *J* = 16.0, 8.8 Hz, 1H), 3.20 (dd, *J* = 8.0, 5.3 Hz, 1H), 3.17 – 3.03 (m, 2H), 2.95 – 2.80 (m, 4H), 1.52 – 1.38 (m, 2H), 1.33 – 1.22 (m, 2H), 1.22 – 1.05 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.63 (Bz), 165.40 (Bz), 165.26 (Bz), 164.81 (Bz), 156.39 (Cbz), 139.25, 138.44, 138.36, 138.24, 138.16, 138.05, 136.89, 133.35, 133.18, 133.01, 132.64, 130.48, 130.28, 130.02, 129.94, 129.90, 129.77, 128.67, 128.63, 128.50, 128.47, 128.42, 128.36, 128.31, 128.27, 128.20, 128.11, 127.94, 127.77, 127.72, 127.68, 127.49, 127.15 (Ar), 101.37 (**C'-1**), 101.16 (**C-1**), 98.16 (**C''-1**), 79.55, 77.36, 76.50, 75.88, 75.16, 74.91, 74.67, 74.22, 73.88, 73.54, 73.35, 73.14, 72.99, 72.31, 72.15, 71.61, 69.79, 68.58, 68.47, 68.18, 67.26, 66.64, 40.97, 29.55, 29.06, 23.21.; MS ESI^{+}-HRMS *m/z* $[M+Na]^{+}$ calcd for $C_{101}H_{101}NO_{22}Na$ 1702.6707, found 1702.6705.

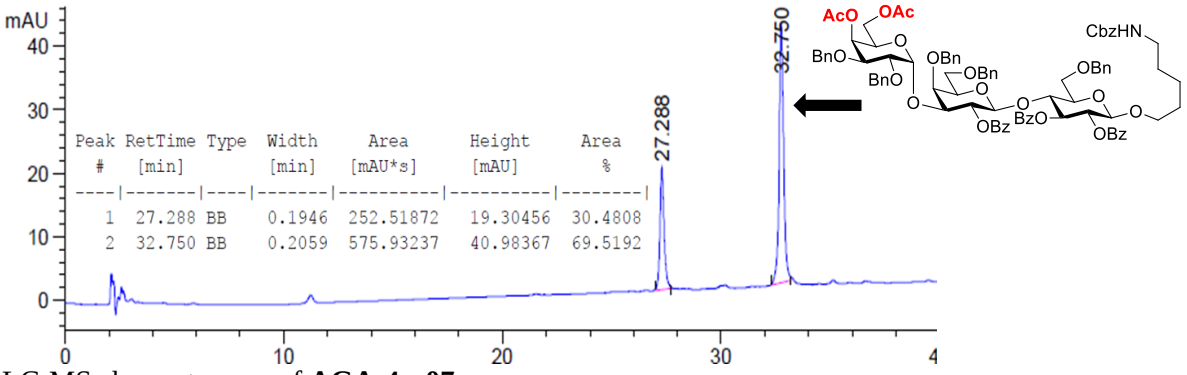
_N_-Benzyloxycarbonyl-5-amino-pentyl 3,4-di-_O_-acetyl-2,6-di-_O_-benzyl-α-D-galactopyranosyl-(1→3)-2-_O_-bezoyl-4,6-di-_O_-benzyl-β-D-galactopyranosyl-(1→4)-2,3-di-_O_-benzoyl-6-_O_-benzyl-β-D-glucopyranoside AGA-4-p06



LC-MS chromatogram of **AGA-4-p06.**

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (d, *J* = 7.4 Hz, 2H), 7.99 (d, *J* = 7.3 Hz, 2H), 7.94 (d, *J* = 7.2 Hz, 2H), 7.56 (dt, *J* = 20.3, 7.3 Hz, 2H), 7.50 – 7.20 (m, 37H), 5.68 (t, *J* = 9.3 Hz, 1H, H-3), 5.62 – 5.55 (m, 1H, H'-2), 5.42 – 5.36 (m, 1H, H-2), 5.28 (dd, *J* = 10.5, 3.2 Hz, 1H, H''-3), 5.22 (d, *J* = 2.0 Hz, 1H, H''-4), 5.14 (s, 2H, Cbz), 5.06 (d, *J* = 11.8 Hz, 1H), 4.99 (d, *J* = 3.3 Hz, 1H, **H''-1**), 4.71 (d, *J* = 11.9 Hz, 1H), 4.64 – 4.51 (m, 5H, **H-1**, **H'-1**, 3 x C*H*HPh), 4.42 (d, *J* = 11.8 Hz, 1H), 4.36 (d, *J* = 12.4 Hz, 2H), 4.21 (d, *J* = 12.0 Hz, 1H), 4.18 – 4.04 (m, 4H), 3.92 (d, *J* = 7.9 Hz, 1H), 3.91 – 3.82 (m, 2H), 3.71 – 3.53 (m, 4H), 3.46 (dd, *J* = 15.0, 6.7 Hz, 1H), 3.28 (t, *J* = 6.6 Hz, 1H), 3.06 (d, *J* = 6.4 Hz, 2H), 3.03 – 2.92 (m, 4H), 1.97 (s, 3H), 1.97 (s, 3H), 1.62 – 1.43 (m, 2H), 1.42 – 1.31 (m, 2H), 1.30 – 1.18 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.92 (Ac), 169.51 (Ac), 165.39 (Bz), 165.23 (Bz), 164.77 (Bz), 156.38 (Cbz), 139.18, 138.48, 138.19, 138.09, 138.04, 136.88, 133.14, 132.63, 130.49, 130.11, 129.99, 129.95, 129.87, 129.75, 128.63, 128.56, 128.48, 128.46, 128.19, 128.12, 127.96, 127.91, 127.89, 127.83, 127.76, 127.71, 127.67, 127.13 (Ar), 101.24 (**C'-1**), 101.12 (**C-1**), 99.00 (**C''-1**), 81.39, 77.36, 75.70, 74.85, 74.53, 73.96, 73.76, 73.37, 73.18, 73.13, 72.30, 69.79, 68.99, 68.07, 67.85, 67.30, 66.63, 40.96, 29.52, 29.04, 23.20, 20.88, 20.75.; MS ESI^{+}-HRMS *m/z* $[M+Na]^{+}$ calcd for $C_{91}H_{95}NO_{23}Na$ 1592.6187, found 1592.6199.

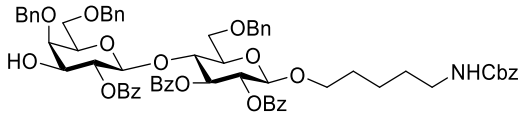
_N_-Benzyloxycarbonyl-5-amino-pentyl 4,6-di-_O_-acetyl-2,3-di-_O_-benzyl-α-D-galactopyranosyl-(1→3)-2-_O_-bezoyl-4,6-di-_O_-benzyl-β-D-galactopyranosyl-(1→4)-2,3-di-_O_-benzoyl-6-_O_-benzyl-β-D-glucopyranoside AGA-4-p07



LC-MS chromatogram of **AGA-4-p07.**

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, *J* = 7.4 Hz, 2H), 7.91 (d, *J* = 7.4 Hz, 2H), 7.84 (d, *J* = 7.5 Hz, 2H), 7.56 (t, *J* = 7.2 Hz, 1H), 7.48 – 7.11 (m, 35H), 7.05 (d, *J* = 5.5 Hz, 3H), 5.58 (t, *J* = 8.7 Hz, 1H, H-3), 5.55 – 5.46 (m, 1H, H'-2), 5.30 (d, *J* = 5.1 Hz, 1H, H''-4), 5.11 (d, *J* = 12.7 Hz, 1H, H-2), 5.06 (s, 2H, Cbz), 4.90 (d, *J* = 2.6 Hz, 1H, **H''-1**), 4.86 (d, *J* = 11.9 Hz, 1H), 4.77 (d, *J* = 11.5 Hz, 1H), 4.59 – 4.44 (m, 6H, **H-1**, **H'-1**, 4 x C*H*HPh), 4.29 (dd, *J* = 16.6, 11.8 Hz, 2H), 4.20 (d, *J* = 11.9 Hz, 1H), 4.06 (dd, *J* = 16.6, 11.8 Hz, 3H), 3.96 – 3.87 (m, 1H), 3.84 – 3.66 (m, 5H), 3.61 (s, 2H), 3.54 – 3.34 (m, 4H), 3.26 – 3.19 (m, 1H), 2.94 – 2.79 (m, 4H), 2.00 (s, 3H), 1.91 (s, 3H), 1.53 – 1.38 (m, 2H), 1.34 – 1.22 (m, 2H), 1.22 – 1.04 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.26 (Ac), 170.19 (Ac), 165.37 (Bz), 165.29 (Bz), 164.81 (Bz), 156.38 (Cbz), 139.25, 138.40, 138.36, 138.12, 138.00, 136.85, 133.44, 133.20, 132.62, 130.48, 129.94, 129.89, 129.82, 129.70, 128.70, 128.63, 128.54, 128.47, 128.44, 128.40, 128.23, 128.18, 128.16, 128.09, 128.07, 127.99, 127.89, 127.88, 127.76, 127.69, 127.49, 127.11 (Ar), 101.17 (**C'-1**), 101.05 (**C-1**), 98.79 (**C''-1**), 80.59, 75.90, 75.54, 75.45, 74.74, 74.40, 73.66, 73.51, 73.22, 73.05, 72.20, 71.85, 69.80, 67.86, 67.17, 67.15, 66.63, 61.13, 40.95, 29.53, 29.03, 23.20, 20.93, 20.83.; MS ESI^{+}-HRMS *m/z* $[M+Na]^{+}$ calcd for $C_{91}H_{95}NO_{23}Na$ 1592.6187, found 1592.6179.

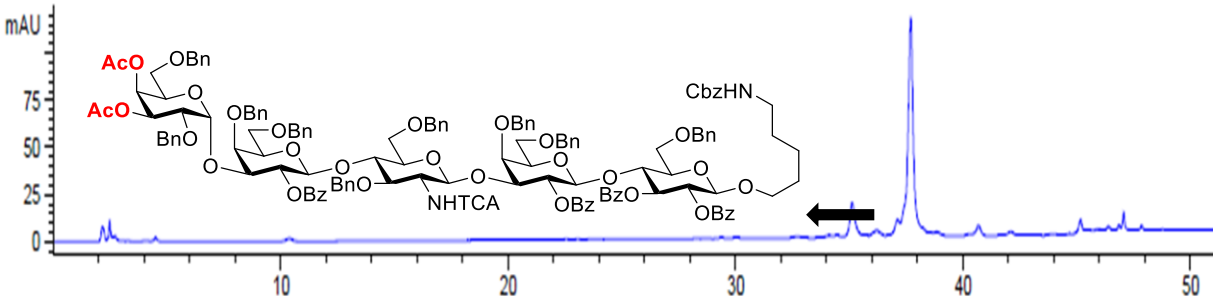
_N_-Benzyloxycarbonyl-5-amino-pentyl 2-_O_-benzoyl-4,6-di-_O_-benzyl-β-D-galactopyranosyl-(1→4)-2,3-di-_O_-benzoyl-6-_O_-benzyl-β-D-glucopyranoside 15g-byproduct Auto-Lactose



Deletion sequence of 15g: Auto-Lactose

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 – 7.89 (m, 6H), 7.58 – 7.53 (m, 1H), 7.49 – 7.38 (m, 4H), 7.37 – 7.27 (m, 18H), 7.23 (dt, J = 8.3, 5.3 Hz, 6H), 5.59 (t, J = 9.4 Hz, 1H, H-3), 5.35 (dd, J = 9.8, 8.0 Hz, 1H, H-2), 5.11 (dd, J = 10.0, 7.9 Hz, 1H, H'-2), 5.06 (s, 2H, Cbz), 4.59 (d, J = 12.2 Hz, 1H, C*H*HPh), 4.57 – 4.49 (m, 5H, **H-1**, **H'-1**, CH_2Ph, NH), 4.36 (d, J = 12.2 Hz, 1H, C*H*HPh), 4.17 – 4.08 (m, 3H, H-4, 2 x C*H*HPh), 3.86 – 3.80 (m, 1H, OC*H*H, linker), 3.75 (d, J = 3.4 Hz, 1H, H'-4), 3.71 (dd, J = 10.9, 3.9 Hz, 1H, H-6), 3.61 (dd, J = 10.9, 1.6 Hz, 1H, H-6), 3.56 – 3.49 (m, 2H, H-5, H'-3), 3.40 (dd, J = 15.5, 6.8 Hz, 1H, OC*H*H, linker), 3.31 (dd, J = 9.3, 5.2 Hz, 1H, H'-5), 2.96 (dd, J = 9.0, 4.9 Hz, 1H, H'-6), 2.91 (td, J = 12.7, 6.3 Hz, 2H, C*H*$_2$NHCbz), 2.85 (t, J = 9.2 Hz, 1H, H'-6), 2.24 (d, J = 10.4 Hz, 1H, OH), 1.48 (ddd, J = 20.5, 12.9, 6.5 Hz, 2H), 1.34 – 1.24 (m, 2H), 1.23 – 1.10 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.23 (Bz), 165.34 (Bz), 165.31 (Bz), 156.34 (Cbz), 138.22, 138.16, 137.73, 136.76, 133.32, 133.25, 132.77, 130.45, 129.98, 129.91, 129.84, 129.77, 129.57, 128.61, 128.58, 128.57, 128.51, 128.47, 128.22, 128.18, 127.99, 127.94, 127.87, 127.77, 127.67 (Ar), 101.15 (**C-1**), 100.63 (**C'-1**), 76.02 (C'-4), 75.61 (C-4), 75.14 (CH_2Ph), 74.72 (C'-3), 74.29 (C'-2), 73.63 (C-3), 73.54 (CH_2Ph), 73.21 (CH_2Ph), 72.93 (C-5), 72.74 (C'-5), 72.03 (C-2), 69.86 (OCH_2, linker), 67.82 (C-6), 66.89 (C'-6), 66.60 (Cbz), 40.88 (*C*H_2NHCbz), 29.47, 28.97, 23.15. MS ESI+-HRMS *m/z* $[M+Na]^+$ calcd for $C_{67}H_{69}O_{16}NNa$ 1166.4509, found 1166.1548.

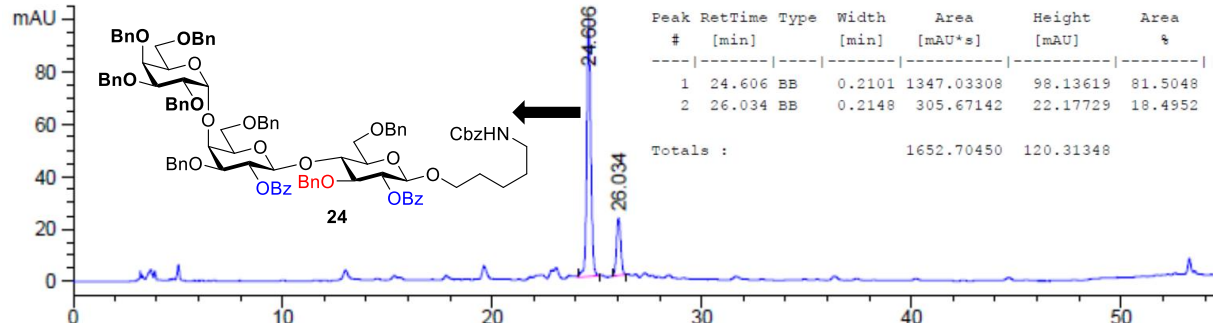
***N*-Benzyloxycarbonyl-5-amino-pentyl 3,4-di-*O*-acetyl-2,6-di-*O*-benzyl-α-D-galactopyranosyl-(1→3)-2-*O*-bezoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-3-*O*-benzoyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamino-β-D-glucopyranosyl-(1→3)-2-*O*-bezoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranoside AGA-4-p08**



LC-MS chromatogram of **AGA-4-p08**

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.97 (d, *J* = 8.0 Hz, 2H), 7.93 – 7.87 (m, 4H), 7.83 (d, *J* = 8.1 Hz, 2H), 7.52 (dt, *J* = 7.4, 3.7 Hz, 2H), 7.45 (t, *J* = 7.3 Hz, 1H), 7.41 – 7.10 (m, 57H), 7.05 (t, *J* = 7.4 Hz, 2H), 6.53 (d, *J* = 8.1 Hz, 1H, NHTCA), 5.56 (dt, *J* = 15.3, 8.9 Hz, 2H), 5.37 (dd, *J* = 9.9, 8.1 Hz, 1H), 5.32 – 5.25 (m, 2H), 5.24 – 5.21 (m, 1H), 5.07 (d, *J* = 7.9 Hz, 3H), 4.97 (d, *J* = 3.1 Hz, 1H, **H-1**), 4.90 – 4.83 (m, 2H), 4.68 (dd, *J* = 9.7, 5.3 Hz, 2H, **H-1**), 4.56 (dd, *J* = 9.7, 6.6 Hz, 3H, **H-1**), 4.49 (t, *J* = 8.2 Hz, 2H, **H-1**), 4.45 – 4.38 (m, 4H, **H-1**), 4.31 (d, *J* = 11.9 Hz, 1H), 4.27 – 4.20 (m, 4H), 4.15 (d, *J* = 11.8 Hz, 1H), 4.11 – 3.91 (m, 6H), 3.85 – 3.81 (m, 2H), 3.81 – 3.76 (m, 1H), 3.68 (dd, *J* = 17.0, 8.6 Hz, 2H), 3.63 (d, *J* = 10.2 Hz, 1H), 3.58 – 3.45 (m, 4H), 3.39 (dt, *J* = 16.9, 10.0 Hz, 4H), 3.27 – 3.21 (m, 3H), 3.04 – 2.96 (m, 2H), 2.89 (ddd, *J* = 13.5, 10.8, 5.5 Hz, 3H), 2.79 (t, *J* = 8.7 Hz, 1H), 1.92 (s, 3H), 1.91 (s, 3H), 1.52 – 1.36 (m, 2H), 1.34 – 1.23 (m, 2H), 1.16 (dd, *J* = 13.7, 6.2 Hz, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.93 (Ac), 169.61 (Ac), 165.33 (Bz), 165.29 (Bz), 164.98 (Bz), 164.60 (Bz), 161.70 (TCA), 156.35 (Cbz), 139.21, 138.94, 138.34, 138.30, 138.20, 138.12, 138.06, 137.99, 137.89, 136.83, 133.38, 133.31, 133.15, 132.46, 130.58, 129.96, 129.93, 129.81, 129.69, 128.68, 128.63, 128.60, 128.55, 128.48, 128.43, 128.42, 128.22, 128.19, 128.14, 128.05, 127.96, 127.94, 127.92, 127.90, 127.88, 127.82, 127.74, 127.70, 127.68, 127.63, 127.36, 127.24, 127.13, 101.04 (**C-1**), 100.97 (**C-1**), 100.83 (**C-1**), 100.32 (**C-1**), 99.24 (**C-1**), 92.12 (CCl_3), 81.49, 79.19, 77.89, 76.24, 75.94, 75.23, 75.16, 75.11, 74.73, 74.53, 74.32, 74.28, 74.15, 73.69, 73.53, 73.40, 73.38, 73.34, 73.08, 72.97, 72.39, 72.14, 69.85, 69.72, 68.90, 68.28, 68.03, 67.86, 67.70, 67.36, 67.13, 66.58, 57.69, 40.91, 29.47, 28.97, 23.15, 20.90, 20.75.; MS ESI^{+}-HRMS *m/z* $[M+Na]^{+}$ calcd for $C_{140}H_{141}N_2O_{35}Cl_3Na$ 2537.8273, found 2537.8301.

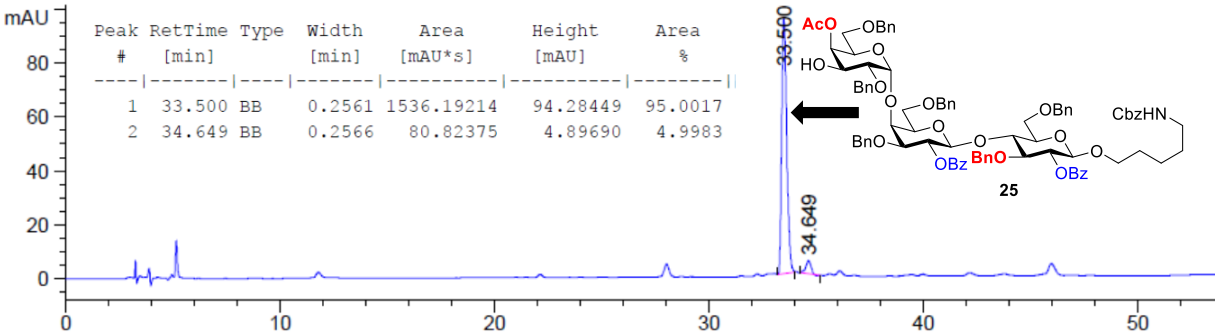
***N*-Benzyloxycarbonyl-5-amino-pentyl 2,3,4,6-*O*-tetra-benzyl-α-D-galactopyranosyl-(1→4)-2-*O*-bezoyl-3,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-benzyl-β-D-glucopyranoside AGA-4-p09**



LC-MS chromatogram of **AGA-4-p09**

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.97 (d, *J* = 7.7 Hz, 2H), 7.87 (d, *J* = 7.6 Hz, 2H), 7.61 (t, *J* = 7.4 Hz, 1H), 7.52 (t, *J* = 7.3 Hz, 1H), 7.46 (t, *J* = 7.6 Hz, 2H), 7.41 – 7.13 (m, 38H), 7.12 – 7.04 (m, 6H), 7.01 – 6.94 (m, 3H), 5.63 – 5.54 (m, 1H, H-2), 5.13 (t, *J* = 8.5 Hz, 1H, H'-2), 5.07 – 5.02 (m, 3H, H''-1, CH_2 of Cbz), 4.87 (dd, *J* = 11.6, 7.2 Hz, 2H, 2 x C*H*HPh), 4.80 (d, *J* = 12.2 Hz, 1H, C*H*HPh), 4.74 (d, *J* = 11.8 Hz, 1H, C*H*HPh), 4.71 – 4.62 (m, 3H, H-1, 2 x C*H*HPh), 4.60 (d, *J* = 12.1 Hz, 1H, C*H*HPh), 4.56 – 4.48 (m, 3H, H-,2 x C*H*HPh), 4.41 (d, *J* = 11.3 Hz, 1H, C*H*HPh), 4.35 – 4.04 (m, 13H), 3.78 – 3.73 (m, 1H, H-6), 3.69 (t, *J* = 8.9 Hz, 1H, H'-3), 3.65 – 3.59 (m, 1H, H-6), 3.56 (t, *J* = 9.1 Hz, 3H, 2 x H-6, $OCH_2(CH_2)_4NHCbz$), 3.44 (br, 1H, H-5), 3.40 (d, *J* = 10.2 Hz, 1H, H-3), 3.29 (d, *J* = 7.4 Hz, 2H, H-5, H-6), 3.19 (dd, *J* = 8.6, 4.6 Hz, 1H, H-6), 2.85 (d, *J* = 6.0 Hz, 2H, C*H*$_2$NHCbz), 1.50 – 1.32 (m, 2H, CH_2, pentane), 1.31 – 1.19 (m, 2H, CH_2, pentane), 1.18 – 1.02 (m, 2H, CH_2, pentane). ^{13}C NMR (150 MHz, $CDCl_3$) δ 165.16 (Bz), 165.10 (Bz), 156.37 (Cbz), 139.19, 138.81, 138.76, 138.65, 138.60, 138.35, 138.31, 138.03, 136.87, 133.25, 133.01, 130.22, 130.15, 129.95, 129.89, 128.75, 128.62, 128.56, 128.48, 128.37, 128.35, 128.32, 128.26, 128.22, 128.20, 128.19, 128.16, 127.95, 127.84, 127.80, 127.58, 127.41, 127.39, 127.28, 127.18, 101.31 (C'-1), 101.15 (C''-1), 100.71 (C-1), 79.87, 79.52, 78.90, 76.79, 76.56, 75.13, 75.08, 74.92, 74.29, 74.16, 73.92, 73.62, 73.52, 73.31, 73.22, 73.00, 72.68, 72.24, 71.20, 69.48, 69.23, 68.10, 67.83, 67.46, 66.59, 40.92, 29.45, 28.96, 23.18.; MS ESI$^+$-HRMS *m/z* $[M+Na]^+$ calcd for $C_{101}H_{105}NO_{20}Na$ 1674.7128, found 1674.7037.

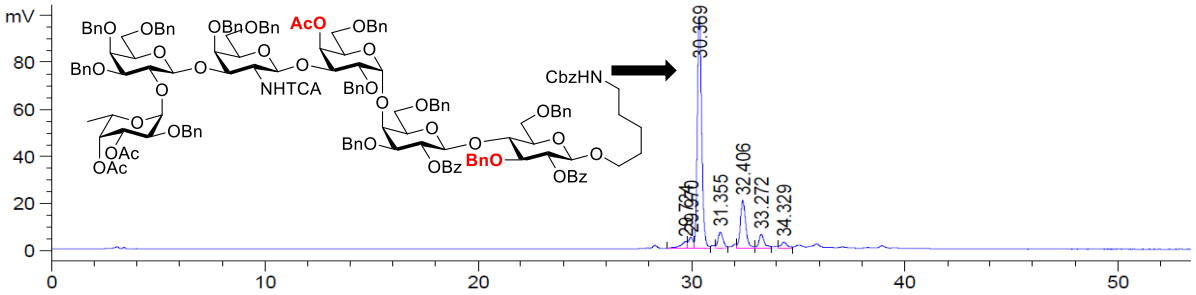
_N_-Benzyloxycarbonyl-5-amino-pentyl 4-_O_-acetyl-2,6-di-_O_-benzyl-α-D-galactopyranosyl-(1→4)-2-_O_-bezoyl-3,6-di-_O_-benzyl-β-D-galactopyranosyl-(1→4)-2-_O_-benzoyl-3,6-di-benzyl-β-D-glucopyranoside AGA-4-p10



LC-MS chromatogram of **AGA-4-p10.**

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.97 – 7.94 (m, 2H), 7.92 (d, *J* = 7.2 Hz, 2H), 7.62 (t, *J* = 7.4 Hz, 1H), 7.54 (t, *J* = 7.4 Hz, 1H), 7.47 (t, *J* = 7.8 Hz, 2H), 7.42 – 7.29 (m, 10H), 7.28 – 7.14 (m, 20H), 7.13 – 7.08 (m, 4H), 7.07 – 7.00 (m, 3H), 5.51 (q, *J* = 8.0 Hz, 2H, H-2, H''-4), 5.17 (dd, *J* = 9.1, 8.2 Hz, 1H, H'-2), 5.13 (d, *J* = 3.2 Hz, 1H, **H''-1**), 5.07 (s, 2H), 4.92 (d, *J* = 12.3 Hz, 1H), 4.75 – 4.50 (m, 9H, **H'-1**), 4.36 – 4.25 (m, 7H, **H-1**), 4.17 (d, *J* = 2.0 Hz, 1H), 4.12 – 4.03 (m, 3H), 3.75 (tdd, *J* = 14.6, 9.5, 5.0 Hz, 3H), 3.63 (dd, *J* = 10.9, 3.9 Hz, 1H), 3.59 – 3.48 (m, 2H), 3.43 (ddd, *J* = 12.9, 9.4, 4.0 Hz, 2H), 3.30 (t, *J* = 8.6 Hz, 3H), 3.21 (dd, *J* = 9.6, 4.8 Hz, 1H), 2.88 (d, *J* = 4.3 Hz, 2H), 1.98 (s, 3H), 1.51 – 1.34 (m, 2H), 1.27 (d, *J* = 14.7 Hz, 2H), 1.20 – 1.03 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.09 (Ac), 165.22 (Bz), 165.01 (Bz), 156.35 (Cbz), 138.90, 138.36, 138.21, 138.20, 138.19, 137.82, 136.86, 133.27, 133.06, 130.20, 130.03, 129.92, 129.85, 128.61, 128.59, 128.55, 128.46, 128.39, 128.37, 128.24, 128.20, 128.16, 128.04, 128.01, 127.92, 127.91, 127.88, 127.82, 127.76, 127.69, 127.57, 127.46, 127.22 (Ar), 101.29 (**C-1** or **C'-1**), 100.61 (**C'-1** or **C-1**), 100.11 (**C''-1**), 80.42, 78.72, 76.74, 74.90, 74.49, 74.07, 73.47, 73.45, 73.36, 73.29, 73.15, 72.13, 71.49, 71.36, 69.50, 68.63, 68.11, 67.86, 67.63, 67.33, 66.58, 40.91, 32.07, 29.84, 29.46, 28.96, 23.18, 22.83, 20.99, 14.26.; MS ESI^{+}-HRMS *m/z* $[M+Na]^{+}$ calcd for $C_{89}H_{95}NO_{21}Na$ 1536.6289, found 1536.6278.

***N*-Benzyloxycarbonyl-5-amino-pentyl 3,4-di-*O*-acetyl-2-*O*-benzoyl-α-L-fucopyranosyl-2-*O*-bezoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-trichloroacetamino-β-D-galactopyranosyl-(1→3)-4-*O*-acetyl-2,6-di-*O*-benzyl-α-D-galactopyranosyl-(1→4)-2-O-bezoyl-3,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-benzyl-β-D-glucopyranoside AGA-4-p11**



LC-MS chromatogram of **AGA-4-p11.**

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.93 (dd, *J* = 7.3, 5.4 Hz, 4H), 7.57 (t, *J* = 7.4 Hz, 1H), 7.49 (t, *J* = 8.1 Hz, 2H), 7.43 – 7.10 (m, 57H), 7.07 – 6.97 (m, 11H), 5.66 (d, *J* = 3.1 Hz, 1H, **H-1**), 5.57 (d, *J* = 2.5 Hz, 1H), 5.49 – 5.43 (m, 1H), 5.41 (dd, *J* = 10.6, 2.8 Hz, 1H), 5.19 (d, *J* = 1.5 Hz, 1H), 5.16 (t, *J* = 8.5 Hz, 1H), 5.10 (d, *J* = 8.0 Hz, 1H, **H-1**), 5.06 (s, 2H), 4.97 – 4.95 (m, 2H, **H-1**), 4.92 – 4.86 (m, 3H, **H-1**), 4.74 – 4.71 (m, 2H), 4.69 – 4.63 (m, 4H), 4.57 – 4.50 (m, 5H, **H-1**), 4.50 – 4.38 (m, 8H), 4.33 – 4.27 (m, 3H, **H-1**), 4.22 (dd, *J* = 17.3, 7.9 Hz, 4H), 4.18 (d, *J* = 2.7 Hz, 1H), 4.15 (d, *J* = 13.2 Hz, 1H), 4.09 (d, *J* = 11.7 Hz, 1H), 4.06 (d, *J* = 7.9 Hz, 1H), 4.05 – 4.01 (m, 2H), 3.98 – 3.93 (m, 2H), 3.92 (t, *J* = 9.0 Hz, 1H), 3.74 (dt, *J* = 11.6, 8.8 Hz, 4H), 3.69 – 3.64 (m, 2H), 3.63 – 3.59 (m, 1H), 3.59 – 3.47 (m, 6H), 3.42 – 3.25 (m, 8H), 2.87 (dd, *J* = 12.4, 6.1 Hz, 2H), 2.05 (s, 3H), 2.03 (s, 3H), 1.79 (s, 3H), 1.49 – 1.32 (m, 2H), 1.30 – 1.21 (m, 2H), 1.18 – 1.02 (m, 2H), 0.83 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.97 (Ac), 170.52 (Ac), 170.28 (Ac), 165.15 (Bz), 164.85 (Bz), 162.68 (NHTCA), 156.36 (Cbz), 139.18, 139.04, 138.93, 138.86, 138.77, 138.50, 138.37, 138.26, 138.03, 137.84, 136.88, 133.11, 133.06, 130.21, 129.98, 129.84, 128.63, 128.62, 128.54, 128.51, 128.46, 128.42, 128.35, 128.32, 128.26, 128.19, 128.15, 128.07, 128.01, 127.99, 127.90, 127.76, 127.70, 127.68, 127.64, 127.59, 127.53, 127.48, 127.36, 127.31, 127.07, 126.78, 102.41 (**C-1**), 101.46 (**C-1**), 101.10 (**C-1**), 100.67 (**C-1**), 98.66 (**C-1**), 96.71 (**C-1**), 92.58 (CCl_3), 83.99, 81.06, 78.56, 77.65, 75.98, 75.54, 75.29, 74.78, 74.71, 74.32, 74.25, 73.92, 73.85, 73.76, 73.45, 73.40, 73.24, 73.17, 73.11, 72.78, 72.53, 72.20, 72.02, 71.77, 71.45, 71.12, 70.33, 69.45, 69.18, 68.46, 68.35, 68.28, 67.90, 66.58, 64.88, 57.48, 40.93, 29.85, 29.47, 29.00, 23.20, 21.25, 20.88, 20.79, 15.61.; MS ESI^{+}-HRMS *m/z* $[M+Na]^{+}$ calcd for $C_{155}H_{165}N_2O_{37}Cl_3Na$ 2774.0049, found 2773.9809.

Deprotection Conditions: To a solution of the fully protected oligosaccharide in MeOH (5 mL) was added 58 μL of 0.5 M NaOMe solution (0.25 eq. per acetyl or benzoyl group) in MeOH at 40 ° C. The mixture is stirred until LC-MS analysis indicated complete deprotection, then neutralized by 200 mg of Amberite (400 mg per 100 μL of NaOMe solution) . The amberlite was filtered off and the crude filtrate evapoarted andre-dissolved in MeOH, ethyl acetate, and AcOH (v/v/v =5:0.5:0.2) added 5% Pd/C (W/V), purged first with argon and then with hydrogen, left to stir overnight at room temperature under balloon pressure. The reaction mixture was filtered through syringe filter, washed with 20 mL of Water/MeOH, 9:1 and the combined solution was evaporated to provide the crude product.

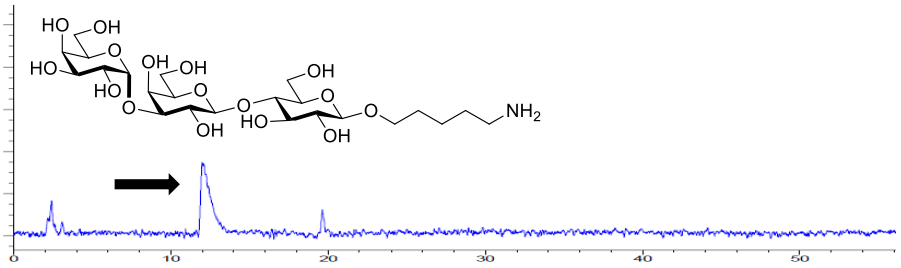
Analytical HPLC: The crude material was analyzed by HPLC (column: Hypercarb®, (150 X 4.60 mm); flow rate: 0.8 mL/min; eluents: 0.1% FA in Acetonitrile / 0.1% FA in TDW; gradient: 0% (10 min) 30% (in 30 min) 100% (in 5 min); detection: ELSD).
NOTE: The crude material was analysized to provide analytical HPLC data in supporting information to identify the desire oligosaccharide in the crude mixture following deprotection steps from pure protected oligosacchride.

Preparative HPLC: The crude solution is purified by preparative HPLC (column: Hypercarb®, (150 X 10.00 mm); flow rate: 3.6 mL/min; eluents: 0.1% FA in Acetonitrile / 0.1% FA in TDW; gradient: 0% (10 min) 30% (in 30 min) 100% (in 5 min); detection: ELSD) to afford the unprotected oligosaccharide.
Note: HPLC purifications using 0.1% formic acid (FA) sometime result in the fromation of formic acid salt with conjugation-ready oligosaccharide. The detection of formic acid by ^{1}H and ^{13}C NMR does not imply of impurity.

Compound	Amounts	Yield
AGA-4-01	2.5 mg	48%
AGA-4-02	4.1 mg	48%
AGA-4-03	3.3 mg	51%
AGA-4-04	2.9 mg	42%

Yields of oligosaccharides **AGA-4-01 - AGA-4-04.**

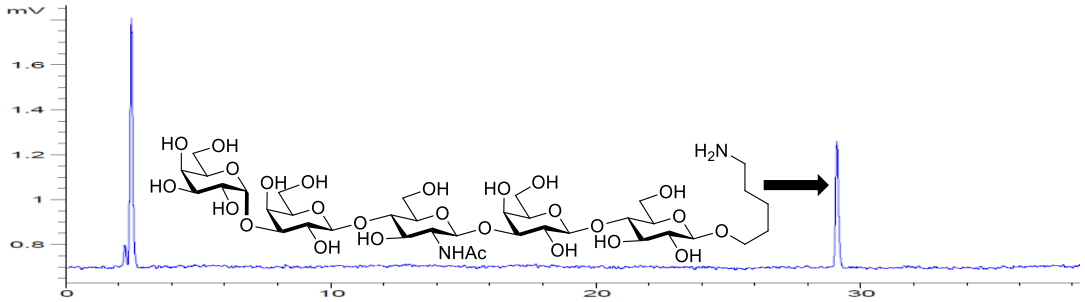
5-Amino-pentyl α-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside AGA-4-01



LC-MS chromatogram of **AGA-4-01.**

^{1}H NMR (600 MHz, D_2O) δ 8.48 (s, 1H, *H*CO_2H), 5.17 (d, *J* = 4.0 Hz, 1H, H, H''-1), 4.55 (d, *J* = 8.0 Hz, 1H, H-1), 4.52 (d, *J* = 8.1 Hz, 1H, H-1), 4.25 – 4.19 (m, 2H), 4.05 (d, *J* = 3.1 Hz, 1H), 4.02 (dd, *J* = 12.3, 1.8 Hz, 1H), 3.99 – 3.94 (m, 2H), 3.89 (dd, *J* = 10.4, 3.9 Hz, 1H), 3.86 – 3.65 (m, 12H), 3.64 – 3.61 (m, 1H), 3.36 – 3.32 (m, 1H), 2.98 (t, *J* = 7.3 Hz, 2H, O*CH*$_2$(CH_2)$_4$$NH_2$), 1.73 – 1.66 (m, 4H, $OCH_2CH_2CH_2CH_2CH_2NH_2$), 1.52 – 1.44 (m, 2H $OCH_2CH_2CH_2CH_2CH_2NH_2$). ^{13}C NMR (150 MHz, D_2O) δ 173.63 (CO_2H), 105.45 (C-1), 104.62(C-1), 98.03(C''-1), 81.28, 79.81, 77.66, 77.35, 77.13, 75.41, 73.44, 72.75, 72.18, 71.89, 71.73, 70.79, 67.41, 63.59, 63.53, 62.77, 42.09 (*C*H_2NH_2), 30.80, 29.63, 24.73.; MS ESI^+-HRMS *m/z* $[M+Na]^+$ calcd for $C_{23}H_{44}NO_{16}Na$ 590.2655, found 590.2644.

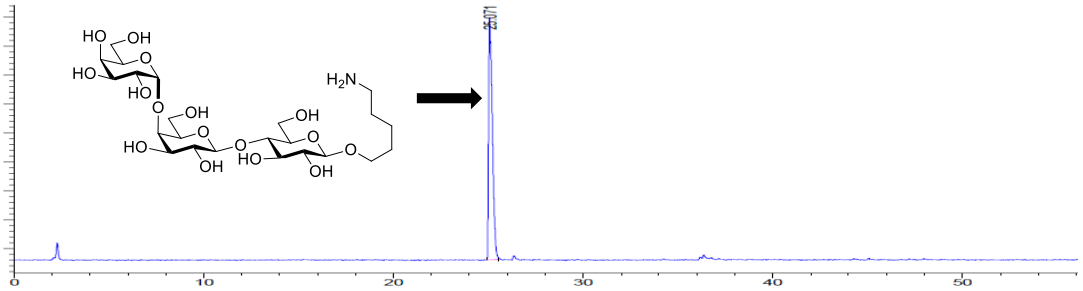
5-Amino-pentyl α-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→3)-2-deoxy-2-acetoamido-β-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside AGA-4-02



LC-MS chromatogram of **AGA-4-02.**

^{1}H NMR (500 MHz, D_2O) δ 5.13 (d, *J* = 3.8 Hz, 1H, **H’’’’-1**), 4.69 (d, *J* = 8.3 Hz, 1H, **H-1**), 4.54 (d, *J* = 7.8 Hz, 1H, **H-1**), 4.47 (d, *J* = 8.0 Hz, 1H, **H-1**), 4.42 (d, *J* = 7.9 Hz, 1H, **H-1**), 4.17 (dd, *J* = 8.3, 4.6 Hz, 2H), 4.14 (d, *J* = 2.9 Hz, 1H), 4.00 (d, *J* = 2.8 Hz, 1H), 3.98 – 3.89 (m, 4H), 3.86 – 3.53 (m, 23H), 3.29 (dd, *J* = 11.3, 5.7 Hz, 1H), 3.01 – 2.96 (m, 2H), 2.02 (s, 3H), 1.71 – 1.59 (m, 4H), 1.44 (dt, *J* = 15.2, 7.7 Hz, 2H). ^{13}C NMR (176 MHz, D_2O) δ 177.49 (NHAc), 105.54 (**C-1**), 105.38 (**C-1**), 105.34 (**C-1**), 104.59 (**C-1**), 98.04 (**C’’’’-1**), 84.67, 81.03, 79.81, 77.65, 77.49, 77.38, 77.14, 77.06, 75.41, 74.85, 73.45, 72.68, 72.55, 72.20, 71.89, 71.73, 70.91, 70.79, 67.41, 63.59, 63.55, 63.53, 62.69, 62.55, 57.78, 41.97, 30.75, 29.05, 25.87, 24.79, 24.68.; MS ESI+-HRMS *m/z* [M+Na]+ calcd for $C_{39}H_{70}N_2O_{26}Na$ 996.4242, found 996.4228.

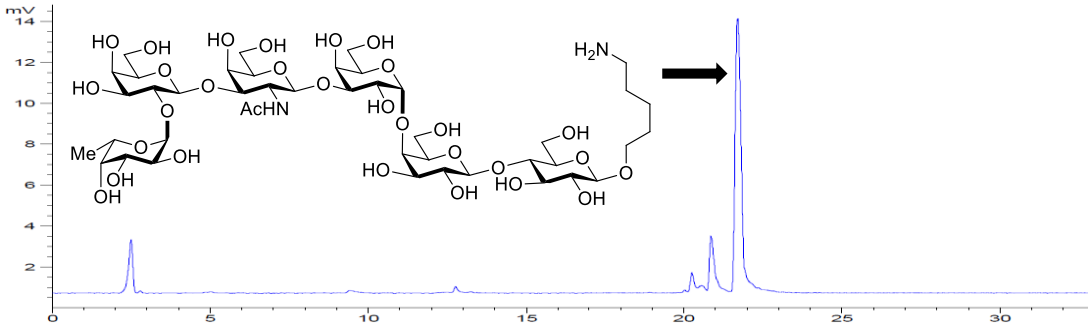
5-Amino-pentyl α-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside AGA-4-03



LC-MS chromatogram of **AGA-4-03**

^{1}H NMR (600 MHz, D_2O) δ 4.96 (d, *J* = 3.9 Hz, 1H, H''-1), 4.52 (d, *J* = 7.8 Hz, 1H, H-1), 4.50 (d, *J* = 8.0 Hz, 1H, H-1), 4.37 (t, *J* = 6.4 Hz, 1H), 4.07 – 3.56 (m, 18H), 3.34 – 3.29 (m, 1H), 3.04 – 3.00 (m, 2H, $OCH_2(CH_2)_4NH_2$), 1.75 – 1.64 (m, 4H, 2H $OCH_2CH_2CH_2CH_2CH_2NH_2$), 1.47 (dt, *J* = 15.3, 7.6 Hz, 2H, $-CH_2CH_2CH_2-$). ^{13}C NMR (150 MHz, D_2O) δ 108.44 (C-1), 107.14 (C-1), 105.48 (C''-1), 83.90, 82.54, 80.62, 79.99, 79.70, 78.09, 77.35, 76.10, 76.01, 75.25, 74.31, 74.12, 73.73, 65.69, 65.56, 65.24, 44.54 (CH_2NH_2), 33.33, 31.58, 28.43, 27.26.; MS ESI+-HRMS *m/z* [M+Na]+ calcd for $C_{23}H_{43}NO_{16}Na$ 612.2474, found 612.2484.

5-Amino-pentyl α-L-fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→3)-2-depxy-2-amidoaceto-β-D-galactopyranosyl-(1→3)-α-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside AGA-4-04



LC-MS chromatogram of **AGA-4-04.**

^{1}H NMR (500 MHz, D_2O) δ 8.46 (s, 1H, formic acid), 5.25 (d, *J* = 4.1 Hz, 1H, **H-1**), 4.82 (d, *J* = 3.9 Hz, 1H, **H-1**), 4.63 (d, *J* = 7.6 Hz, 1H, **H-1**), 4.56 (d, *J* = 7.1 Hz, 1H, **H-1**), 4.53 (d, *J* = 7.6 Hz, 1H, **H-1**), 4.50 (d, *J* = 8.1 Hz, 1H, **H-1**), 4.40 (t, *J* = 6.6 Hz, 1H), 4.25 (d, *J* = 7.1 Hz, 1H), 4.07 – 3.54 (m, 31H), 3.33 (d, *J* = 6.6 Hz, 1H), 3.02 (t, *J* = 7.7 Hz, 2H), 2.06 (s, 3H), 1.76 – 1.64 (m, 4H), 1.50 – 1.41 (m, 2H), 1.23 (d, *J* = 6.6 Hz, 3H). ^{13}C NMR (176 MHz, D_2O) δ 174.29 (NHAc), 170.81 (formic acid), 103.96 (**C-1**), 103.32 (**C-1**), 102.05 (**C-1**), 101.97 (**C-1**), 100.45 (**C-1**), 99.28 (**C-1**), 78.83, 78.26, 77.20, 76.38, 76.11, 75.50, 75.08, 74.81, 74.63, 74.55, 73.59, 72.95, 72.13, 71.86, 70.88, 70.18, 70.09, 69.53, 69.18, 69.12, 68.49, 68.03, 67.83, 66.79, 60.99, 60.96, 60.38, 60.07, 51.65, 39.35, 28.17, 26.40, 22.26, 22.08, 15.32.; MS ESI+-HRMS *m/z* [M+H]+ calcd for $C_{43}H_{78}N_2O_{30}$ 1101.4556, found 1101.4518.

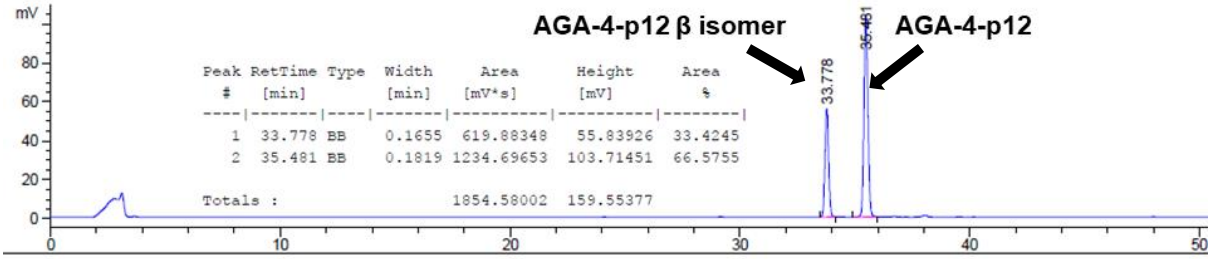
Synthesis of Disaccharides AGA-4-p12 – AGA-4-p21

The building blocks **Glc-02, Glc-03, or Glc-04** and the next corresponding building block (**Glc-α-01 to Glc-α-08**) were placed on the corresponding building block vial location. Then the automated synthesis started performing reactions with module 1, 2, and to afford disaccharides **AGA-4-p12 – AGA-4-p21.**

Entry	1st BB	2nd BB	Product					Ratio (α : β)[a]	Yield[a]
			R_1	R_2		R_3	R_4		
1	Glc-02	**Glc-α-01**	Bn	Bn	**AGA-4-p12**	Bn	Bn	2.0 : 1	25%
2		**Glc-α-02**	Bn	Ac	**AGA-4-p13**	Bn	Ac	8.1 : 1	31%
3		**Glc-α-03**	Bn	Bz	**AGA-4-p14**	Bn	Bz	6.7 : 1	31%
4		**Glc-α-04**	Bn	Fmoc	**AGA-4-p15**	Bn	OH	3.5 : 1	30%
5		**Glc-α-05**	Ac	Bn	**AGA-4-p16**	Ac	Bn	4.4 : 1	27%
6		**Glc-α-06**	Bz	Bn	**AGA-4-p17**	Bz	Bn	3.4 : 1	27%
7		**Glc-α-07**	Fmoc	Bn	**AGA-4-p18**	OH	Bn	3.5 : 1	26%
8		**Glc-α-08**	Ac	Ac	**AGA-4-p19**	Ac	Ac	11.4 : 1	34%
9	**Glc-02**	**Glc-α-08**	Ac	Ac	**AGA-4-p19**	Ac	Ac	15.1 : 1[c]	34%
10	**Glc-03**	**Glc-α-08**	Ac	Ac	**AGA-4-p20**	Ac	Ac	10.6 : 1	34%
11	**Glc-04**	**Glc-α-08**	Ac	Ac	**AGA-4-p21**	Ac	Ac	9.5 : 1	35%

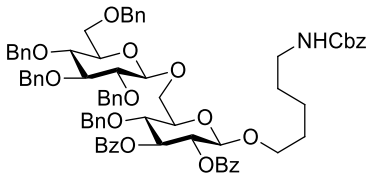
Yields of disaccharides **AGA-4-p12 – AGA-4-p21**

_N_-Benzyloxycarbonyl-5-amino-pentyl (2,3,4,6-tetra-_O_-benzyl-α-D-glucopyranosyl)-(1→6)-2,3-di-_O_-benzoyl-β-D-glucopyranoside AGA-4-p12



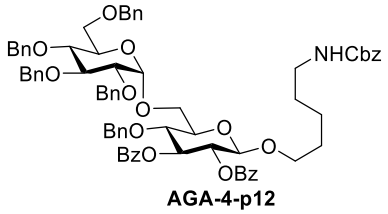
LC-MS chromatogram of **AGA-4-p12**

_N_-Benzyloxycarbonyl-5-amino-pentyl (2,3,4,6-tetra-_O_-benzyl-β-D-glucopyranosyl)-(1→6)-2,3-di-_O_-benzoyl-β-D-glucopyranoside AGA-4-p12 β isomer



^{1}H NMR (600 MHz, $CDCl_3$) δ 7.93 (d, *J* = 7.7 Hz, 2H), 7.89 (d, *J* = 7.8 Hz, 2H), 7.52 – 7.44 (m, 2H), 7.39 – 7.23 (m, 27H), 7.18 – 7.12 (m, 5H), 7.07 (dd, *J* = 6.4, 2.8 Hz, 2H), 5.72 – 5.68 (m, 1H, H-3), 5.35 – 5.30 (m, 1H, H-2), 5.06 (s, 2H, CH_2, Cbz), 5.01 (d, *J* = 11.1 Hz, 1H, C*H*HPh), 4.93 (d, *J* = 10.9 Hz, 1H, C*H*HPh), 4.84 – 4.77 (m, 3H, 3 x C*H*HPh), 4.64 (d, *J* = 12.2 Hz, 1H, C*H*HPh), 4.58 – 4.51 (m, 4H, H-1, NH, 2 x C*H*HPh), 4.50 – 4.43 (m, 3H, H'-1, CH_2Ph), 4.25 (d, *J* = 10.0 Hz, 1H, H-6_a), 3.83 – 3.69 (m, 6H, H-4, H-5, H-6_b, H'-6, OCHH$(CH_2)_4$NHCbz), 3.66 – 3.59 (m, 2H, H'-3. H'-4), 3.51 (t, *J* = 8.4 Hz, 1H, H'-2), 3.43 (d, *J* = 3.0 Hz, 1H, H'-5), 3.32 (dd, *J* = 15.2, 6.6 Hz, 1H, OCHH$(CH_2)_4$NHCbz), 2.86 (dd, *J* = 12.8, 6.4 Hz, 2H, CH_2NHCbz), 1.43 – 1.33 (m, 2H), 1.28 – 1.20 (m, 2H), 1.14 – 1.04 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 165.80 (Bz), 165.38 (Bz), 156.38 (Cbz), 138.69, 138.33, 138.26, 137.40, 136.90, 133.29, 133.25, 129.88, 129.85, 129.69, 129.58, 128.63, 128.56, 128.53, 128.50, 128.49, 128.45, 128.25, 128.17, 128.14, 128.12, 128.10, 128.01, 127.98, 127.94, 127.90, 127.76, 104.20 (C'-1), 101.19 (C-1), 84.94 (C'-3 or -4), 82.29 (C'-2), 77.98(C'-3 or -4), 76.84 (C-4 or -5), 75.84 (CH_2Ph), 75.35 (C-3), 75.26 (C-4 or -5), 75.17 (C'-5), 75.13 (CH_2Ph), 74.95 (CH_2Ph), 74.90 (CH_2Ph), 73.69 (CH_2Ph), 72.38 (C-2), 69.85 (OCH_2, linker), 69.03 (C'-6), 68.63 (C-6), 66.61 (CH_2Ph, Cbz), 40.96 (CH_2NHCbz), 29.47, 28.98, 23.17.; MS ESI+-HRMS m/z [M+Na]+ calcd for $C_{74}H_{77}NO_{15}$ 1242.5185, found 1242.5177.

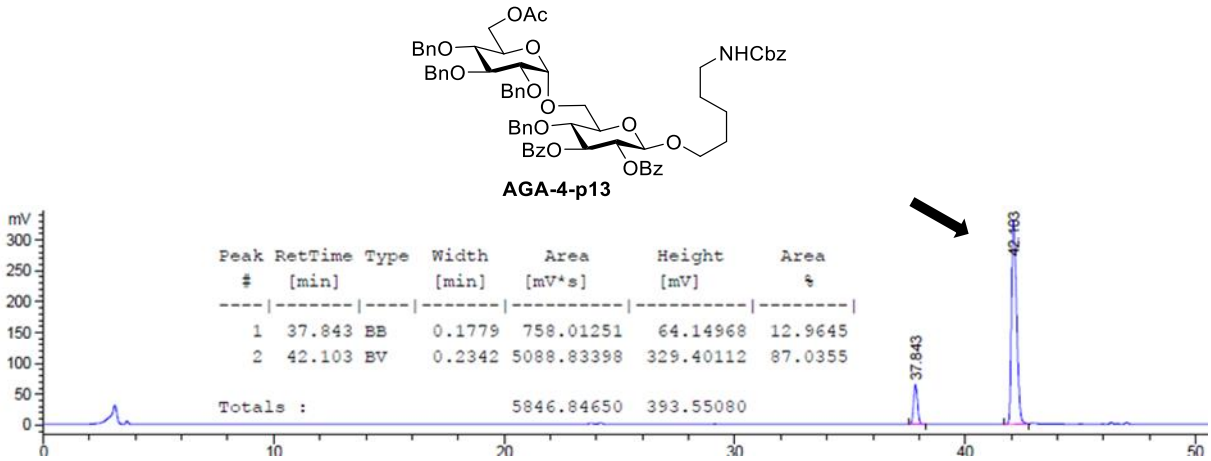
_N_-Benzyloxycarbonyl-5-amino-pentyl (2,3,4,6-tetra-_O_-benzyl-α-D-glucopyranosyl)-(1→6)-2,3-di-_O_-benzoyl-β-D-glucopyranoside AGA-4-p12



AGA-4-p12

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.93 (d, *J* = 7.9 Hz, 2H), 7.82 (d, *J* = 8.0 Hz, 2H), 7.48 (dd, *J* = 17.0, 7.3 Hz, 4H), 7.40 – 7.22 (m, 25H), 7.17 – 7.03 (m, 7H), 5.67 (t, *J* = 9.6 Hz, 1H, H-3), 5.33 – 5.28 (m, 1H, H-1), 5.16 (d, *J* = 3.3 Hz, 1H, H'-1), 5.06 (s, 2H, CH_2Ph, Cbz), 5.03 (d, *J* = 10.9 Hz, 1H, C*H*HPh), 4.83 (dd, *J* = 11.0, 5.7 Hz, 3H, 3 x C*H*HPh), 4.77 (d, *J* = 11.8 Hz, 1H, C*H*HPh), 4.64 – 4.53 (m, 5H, H-1, NH, 3 x C*H*HPh), 4.46 (dd, *J* = 11.4, 7.5 Hz, 2H, 2 x C*H*HPh), 4.02 (t, *J* = 9.3 Hz, 1H, H'-3), 3.96 – 3.83 (m, 5H, H-4, H'-5, H'-6, OCHH of linker), 3.73 – 3.63 (m, 5H, H-5, H-6, H'-2, H'-4), 3.43 (dd, *J* = 14.6, 7.0 Hz, 1H OCHH, linker), 2.88 (dd, *J* = 12.8, 6.4 Hz, 2H, CH_2NHCbz), 1.49 – 1.35 (m, 2H), 1.31 – 1.22 (m, 2H), 1.20 – 1.09 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 165.82 (Bz), 165.29 (Bz), 156.39 (Cbz), 138.97, 138.52, 138.36, 138.10, 137.47, 136.89, 133.21, 133.17, 129.92, 129.84, 129.72, 129.58, 128.71, 128.62, 128.52, 128.51, 128.47, 128.43, 128.38, 128.36, 128.25, 128.17, 128.09, 128.06, 127.94, 127.91, 127.83, 127.74, 127.70, 101.33 (C-1), 97.13 (C'-1), 82.00, 80.22, 77.77, 76.10, 75.74, 75.47, 75.17, 75.05, 74.74, 73.55, 72.94, 72.47, 70.36, 69.83, 68.66, 66.61, 64.98, 40.97, 29.52, 29.07, 23.27.; MS ESI+-HRMS m/z [M+Na]+ calcd for $C_{74}H_{77}NO_{15}$ 1242.5185, found 1242.5177.

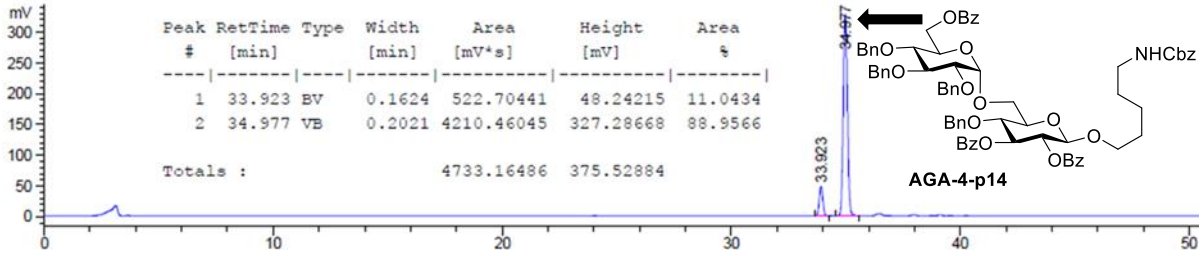
_N_-Benzyloxycarbonyl-5-amino-pentyl (6-_O_-acetyl-2,3,4-tri-_O_-benzyl-α-D-glucopyranosyl)-(1→6)-2,3-di-_O_-benzoyl-β-D-glucopyranoside AGA-4-p13



LC-MS chromatogram of **AGA-4-p13.**

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.93 (dd, *J* = 8.2, 1.0 Hz, 2H), 7.84 – 7.79 (m, 2H), 7.52 – 7.45 (m, 4H), 7.41 – 7.24 (m, 22H), 7.15 (dd, *J* = 7.3, 1.8 Hz, 2H), 7.12 – 7.06 (m, 3H), 5.68 (t, *J* = 9.6 Hz, 1H, H-3), 5.31 (dd, *J* = 9.9, 8.0 Hz, 1H, H-2), 5.16 (d, *J* = 3.1 Hz, 1H, H'-1), 5.07 (d, *J* = 10.0 Hz, 3H, C*H*HPh, CH_2Ph of Cbz), 4.89 (d, *J* = 10.9 Hz, 1H, C*H*HPh), 4.85 (dd, *J* = 11.2, 3.6 Hz, 2H, 2 x C*H*HPh), 4.78 (d, *J* = 11.7 Hz, 1H, C*H*HPh), 4.61 (d, *J* = 7.8 Hz, 2H, H-1, NHCbz), 4.59 (s, 2H, CH_2Ph), 4.56 (d, *J* = 10.9 Hz, 1H, C*H*HPh), 4.30 – 4.22 (m, 2H, H'-6), 4.05 (t, *J* = 9.2 Hz, 1H, H'-3), 3.99 (t, *J* = 9.5 Hz, 1H, H-4), 3.96 – 3.88 (m, 3H, H-6, H'-5), 3.88 – 3.83 (m, 1H, OCHH, linker), 3.72 – 3.67 (m, 1H, H-5), 3.62 (dd, *J* = 9.6, 3.5 Hz, 1H, H'-2), 3.53 (t, *J* = 9.4 Hz, 1H, H'-4), 3.45 (dd, *J* = 14.8, 7.0 Hz, 1H, OCHH, linker), 2.89 (m, 2H, CH_2NHCbz), 2.03 (s, 3H, Ac), 1.53 – 1.37 (m, 2H), 1.33 – 1.25 (m, 2H), 1.18 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.86 (Ac), 165.82 (Bz), 165.27 (Bz), 156.40 (Cbz), 138.76, 138.22, 138.09, 137.43, 136.88, 133.22, 133.19, 129.92, 129.83, 129.69, 129.55, 128.75, 128.62, 128.57, 128.47, 128.41, 128.40, 128.37, 128.26, 128.19, 128.17, 128.15, 128.14, 128.01, 127.97, 127.82, 101.43 (C-1), 97.08 (C'-1), 81.90 (C'-3), 80.17 (C'-2), 77.33 (C'-4), 75.96 (C-4), 75.81 (CH_2Ph), 75.53 (C-5), 75.20 (CH_2Ph), 75.03 (C-3), 74.78 (CH_2Ph), 72.90 (CH_2Ph), 72.43 (C-2), 69.94 (OCH_2, linker), 69.01 (C'-5), 66.61 (CH_2Ph, Cbz), 65.06 (C-6), 63.12 (C'-6), 40.97 (CH_2NHCbz), 29.53, 29.10, 23.29, 21.00 (Ac).; MS ESI+-HRMS m/z $[M+Na]^+$ calcd for $C_{69}H_{73}NO_{16}Na$ 1194.4822, found 1194.4798.

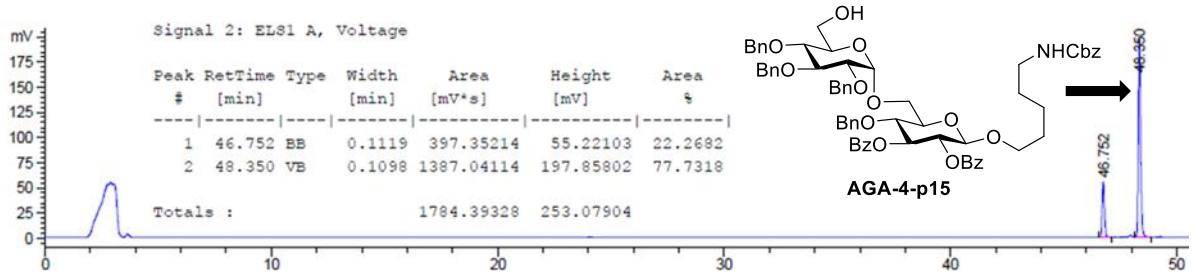
_N_-Benzyloxycarbonyl-5-amino-pentyl (6-_O_-benzoyl-2,3,4-tri-_O_-benzyl-α-D-glucopyranosyl)-(1→6)-2,3-di-_O_-benzoyl-β-D-glucopyranoside AGA-4-p14



LC-MS chromatogram of **AGA-4-p14.**

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.04 (d, *J* = 7.8 Hz, 2H), 7.93 (d, *J* = 7.9 Hz, 2H), 7.84 (d, *J* = 7.9 Hz, 2H), 7.58 (t, *J* = 7.1 Hz, 1H), 7.52 – 7.44 (m, 6H), 7.41 – 7.20 (m, 22H), 7.16 – 7.06 (m, 5H), 5.68 (t, *J* = 9.6 Hz, 1H, H-3), 5.33 – 5.28 (m, 1H, H-2), 5.13 (d, *J* = 3.1 Hz, 1H, H'-1), 5.09 – 5.04 (m, 3H, C*H*HPh, CH_2Ph of Cbz), 4.93 (d, *J* = 10.9 Hz, 1H, C*H*HPh), 4.88 – 4.83 (m, 2H, 2 x C*H*HPh), 4.79 (d, *J* = 11.7 Hz, 1H, C*H*HPh), 4.65- 51 (m, 6H, H-1, NH, 2 x C*H*HPh, CH_2Ph), 4.50 (dd, *J* = 11.9, 4.4 Hz, 1H, C*H*HPh), 4.13 - 05 (m, 2H, H'-3, H'-5), 3.93 – 3.89 (m, 3H, H-4, H-6), 3.89 – 3.84 (m, 1H, OCHH, linker), 3.71 (d, *J* = 9.6 Hz, 1H, H-5), 3.68 – 3.61 (m, 2H, H'-2, H'-4), 3.44 (d, *J* = 7.5 Hz, 1H, OCHH, linker), 2.87 (d, *J* = 5.7 Hz, 2H, CH_2NHCbz), 1.43 (ddt, *J* = 24.3, 17.9, 8.8 Hz, 2H), 1.26 (dt, *J* = 12.0, 6.7 Hz, 2H), 1.21 – 1.09 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.35 (Bz), 165.80 (Bz), 165.28 (Bz), 156.38 (Cbz), 138.69, 138.25, 138.04, 137.40, 136.89, 133.24, 133.22, 130.10, 129.91, 129.85, 129.84, 129.70, 129.57, 128.75, 128.61, 128.60, 128.57, 128.47, 128.42, 128.34, 128.26, 128.14, 127.99, 127.88, 101.36 (C-1), 96.97 (C'-1), 82.03 (C'-3), 80.38 (C'-2), 77.73 (C'-4), 76.16 (C-4), 75.97 (CH_2Ph), 75.40 (C-5), 75.34 (CH_2Ph), 75.06 (C-3), 74.81 (CH_2Ph), 72.99 (CH_2Ph), 72.45 (C-2), 69.93 (OCH_2, linker), 69.22 (C'-5), 66.59 (CH_2Ph), 65.20 (C-6), 63.59 (C'-6), 40.95 (CH_2Ph, CBz), 29.50, 29.08, 23.28.; MS ESI+-HRMS m/z $[M+Na]^+$ calcd for $C_{74}H_{75}NO_{16}Na$ 1256.4978, found 1256.4967.

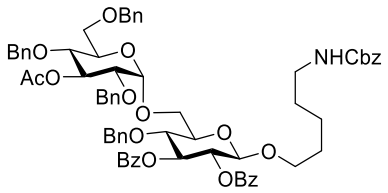
_N_-Benzyloxycarbonyl-5-amino-pentyl (2,3,4-tri-_O_-benzyl-α-D-glucopyranosyl)-(1→6)-2,3-di-_O_-benzoyl-β-D-glucopyranoside AGA-4-p15



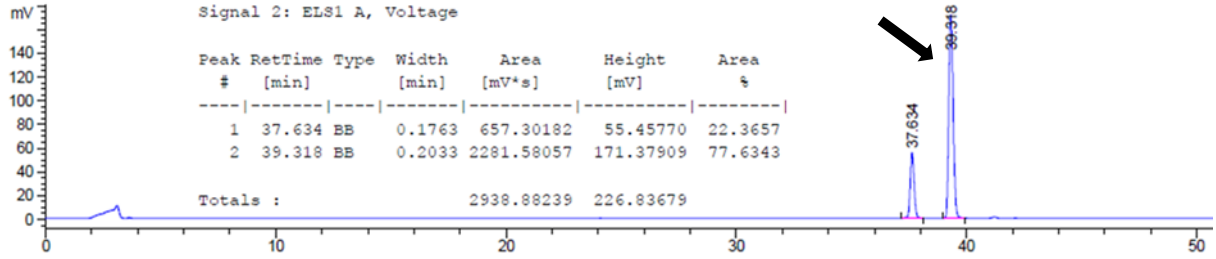
LC-MS chromatogram of **AGA-4-p15.**

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.93 (d, *J* = 7.9 Hz, 2H), 7.81 (d, *J* = 7.5 Hz, 2H), 7.52 – 7.45 (m, 5H), 7.42 – 7.25 (m, 21H), 7.15 (d, *J* = 6.9 Hz, 2H), 7.08 (d, *J* = 6.1 Hz, 3H), 5.68 (t, *J* = 9.5 Hz, 1H, H-3), 5.32 (t, *J* = 8.9 Hz, 1H, H-2), 5.16 (br, 1H, H'-1), 5.07 (s, 2H, Cbz), 5.05 (d, *J* = 11.0 Hz, 1H, C*H*HPh), 4.90 (t, *J* = 9.3 Hz, 1H, C*H*HPh), 4.88 – 4.82 (m, 2H, 2 x C*H*HPh), 4.79 (d, *J* = 11.7 Hz, 1H, C*H*HPh), 4.67 – 4.56 (m, 5H, H-1, NH, C*H*HPh, CH_2Ph), 4.06 (t, *J* = 9.1 Hz, 1H, H'-3), 4.01 (t, *J* = 9.4 Hz, 1H, H-4), 3.94 (d, *J* = 13.0 Hz, 2H, H-6), 3.85 (s, 1H, OCHH, linker), 3.78 (t, *J* = 9.4 Hz, 2H, H-5, H'-6), 3.74 – 3.65 (m, 2H, H'-5, H'-6), 3.62 – 3.55 (m, 2H, H'-2, H'-4), 3.49 (d, *J* = 7.7 Hz, 1H, OCHH, linker), 2.91 (d, *J* = 5.9 Hz, 2H, CH_2NHCbz), 1.47 (s, 2H), 1.37 – 1.12 (m, 4H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 165.81 (Bz), 165.27 (Bz), 156.40 (Cbz), 138.90, 138.39, 138.29, 137.47, 136.82, 133.21, 133.16, 129.92, 129.84, 129.69, 129.55, 128.75, 128.64, 128.57, 128.52, 128.46, 128.39, 128.28, 128.20, 128.13, 128.07, 127.93, 127.72, 101.47 (C-1), 97.11 (C'-1), 81.80 (C'-3), 80.30 (C'-2 or C'-4), 77.49 (C'-2 or C'-4), 75.93 (C-4), 75.72 (CH_2Ph), 75.62 (C'-5), 75.21 (CH_2Ph), 75.01 (C-3), 74.76 (CH_2Ph), 72.98 (CH_2Ph), 72.43 (C-2), 71.20 (C-5), 69.94 (OCH_2, linker), 66.67 (CH_2Ph), 64.86 (C-6), 61.95 (C'-6), 41.00 (CH_2Ph, Cbz), 29.50, 29.12, 23.28. MS ESI+-HRMS m/z $[M+Na]^+$ calcd for $C_{67}H_{71}NO_{15}Na$ 1152.4716, found 1152.4707.

_N_-Benzyloxycarbonyl-5-amino-pentyl (3-_O_-acetyl-2,4,6-tri-_O_-benzyl-α-D-glucopyranosyl)-(1→6)-2,3-di-_O_-benzoyl-β-D-glucopyranoside AGA-4-p16



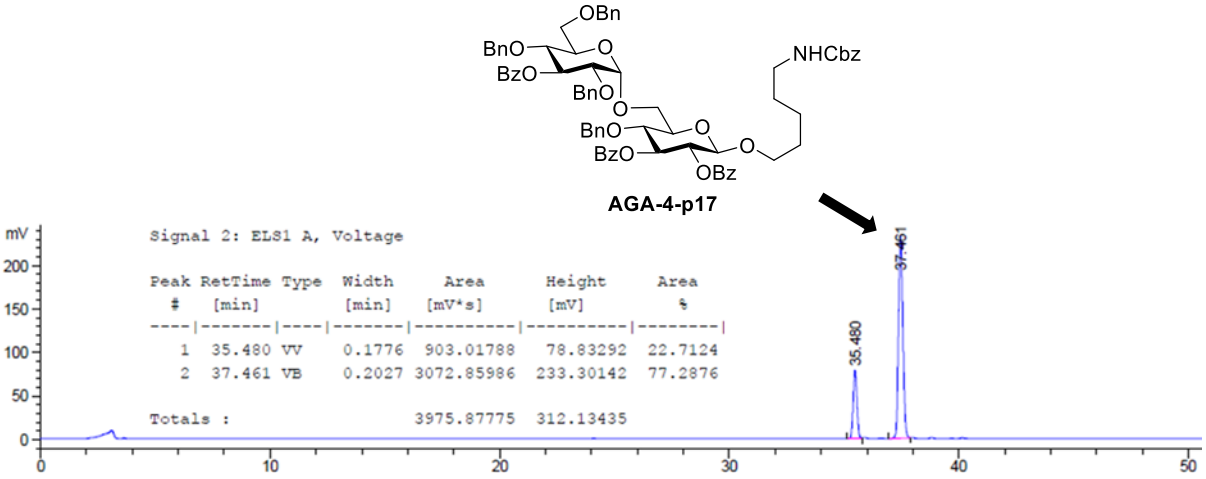
AGA-4-p16



LC-MS chromatogram of **AGA-4-p16**

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.93 – 7.90 (m, 2H), 7.81 (d, *J* = 7.2 Hz, 2H), 7.50 – 7.44 (m, 2H), 7.40 – 7.22 (m, 22H), 7.16 – 7.07 (m, 7H), 5.66 (t, *J* = 9.6 Hz, 1H, H-3), 5.59 (t, *J* = 9.6 Hz, 1H, H'-3), 5.24 (dd, *J* = 9.9, 8.0 Hz, 1H, H-2), 5.19 (d, *J* = 3.3 Hz, 1H, H'-1), 5.06 (s, 2H, CH_2Ph, Cbz), 4.78 (d, *J* = 12.4 Hz, 1H, C*H*HPh), 4.73 (br, 1H, NHCbz), 4.64 (m, 2H, , 2 x CHHPh), 4.61 – 4.55 (m, 3H, H-1, CH_2Ph), 4.48 (dd, *J* = 11.5, 6.0 Hz, 2H, 2 x C*H*HPh), 4.40 (d, *J* = 11.1 Hz, 1H, C*H*HPh), 3.95 – 3.87 (m, 3H, H-4, H'-5, H'-6), 3.86 – 3.80 (m, 2H, H'-6, OCH_2 of linker), 3.73 (dd, *J* = 10.7, 3.1 Hz, 1H, H-6), 3.71 – 3.66 (m, 2H, H-5, H'-4), 3.64 (dd, *J* = 10.6, 1.6 Hz, 1H, H-6), 3.53 (dd, *J* = 10.0, 3.4 Hz, 1H, H'-2), 3.45 (dd, *J* = 15.8, 6.6 Hz, 1H OCH_2, linker), 2.89 (dd, *J* = 12.8, 6.5 Hz, 2H, CH_2NHCbz), 1.98 (s, 3H, Ac), 1.49 – 1.35 (m, 2H), 1.33 – 1.21 (m, 2H), 1.20 – 1.10 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.24 (Ac), 165.83 (Bz), 165.27 (Bz), 156.43 (Cbz), 138.13, 138.11, 137.92, 137.49, 136.94, 133.18, 133.16, 129.93, 129.84, 129.71, 129.53, 128.64, 128.61, 128.58, 128.48, 128.45, 128.40, 128.39, 128.33, 128.24, 128.15, 127.97, 127.92, 127.88, 127.84, 127.84, 101.29 (C-1), 96.79 (C'-1), 77.34 (C'-2), 76.27 (C-5 or C'-4), 76.19 (C-4), 75.47 (C-5 or C'-4), 75.06 (C-3), 74.94 (CH_2Ph), 74.55 (CH_2Ph), 73.73 (C'-3), 73.68 (CH_2Ph), 72.45 (CH_2Ph), 72.36 (C-2), 70.02 (CH_2Ph), 69.96 (C'-5), 68.36 (OCH_2, linker), 66.58 (C-6), 64.96 (C'-6), 41.03 (CH_2Ph, Cbz), 29.44, 29.07, 23.26, 21.27 (Ac).; MS ESI+-HRMS m/z $[M+Na]^+$ calcd for $C_{69}H_{73}NO_{16}Na$ 1194.4822, found 1194.4825.

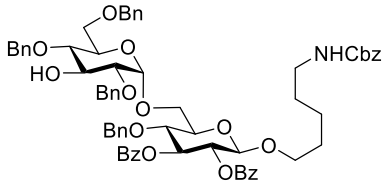
_N_-Benzyloxycarbonyl-5-amino-pentyl (3-_O_-benzoyl-2,4,6-tri-_O_-benzyl-α-D-glucopyranosyl)-(1→6)-2,3-di-_O_-benzoyl-β-D-glucopyranoside AGA-4-p17



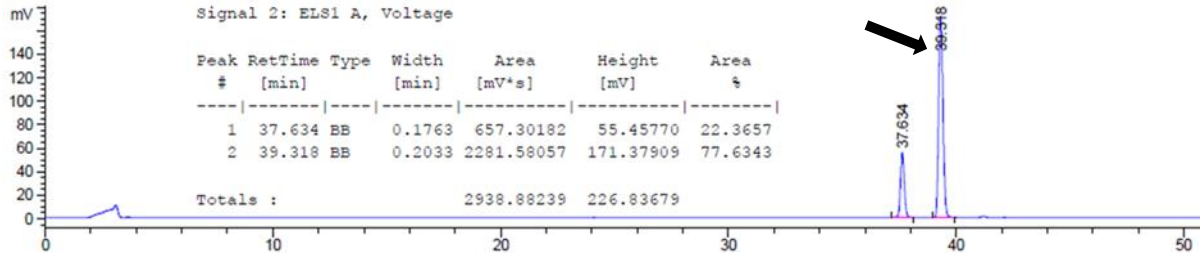
LC-MS chromatogram of **AGA-4-p17.**

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.05 (d, *J* = 7.8 Hz, 2H), 7.92 (d, *J* = 8.1 Hz, 2H), 7.84 (d, *J* = 8.1 Hz, 2H), 7.59 (t, *J* = 7.0 Hz, 1H), 7.47 (dt, *J* = 14.1, 4.1 Hz, 5H), 7.38 – 7.29 (m, 13H), 7.26 (dd, *J* = 6.6, 2.1 Hz, 1H), 7.18 (dd, *J* = 7.8, 6.8 Hz, 5H), 7.15 – 7.08 (m, 7H), 6.97 (d, *J* = 5.0 Hz, 2H), 5.87 (t, *J* = 9.5 Hz, 1H, H'-3), 5.69 (t, *J* = 9.6 Hz, 1H, H-3), 5.29 – 5.25 (m, 1H, H-2), 5.24 (d, *J* = 3.2 Hz, 1H, H'-1), 5.06 (s, 2H, Cbz), 4.76 (d, *J* = 12.5 Hz, 1H, C*H*HPh), 4.72 (br, 1H, NHCbz), 4.67 (d, *J* = 12.1 Hz, 1H, C*H*HPh), 4.64 – 4.59 (m, 4H, H-1, CH_2Ph, C*H*HPh), 4.52 – 4.45 (m, 2H, 2 x C*H*HPh), 4.37 (d, *J* = 10.8 Hz, 1H, C*H*HPh), 3.98 (dd, *J* = 12.2, 5.8 Hz, 2H, H-4, H'-5), 3.94 – 3.82 (m, 4H, H'-4, H'-6, OCHH of linker), 3.77 (dd, *J* = 10.7, 2.7 Hz, 1H, H-6), 3.72 (dd, *J* = 9.6, 4.2 Hz, 1H, H-5), 3.67 (dd, *J* = 9.6, 4.1 Hz, 2H, H-6, H'-2), 3.48 (dd, *J* = 15.4, 6.5 Hz, 1H, OC*H*H, linker), 2.89 (dt, *J* = 13.0, 6.5 Hz, 2H, CH_2NHCbz), 1.51 – 1.39 (m, 2H), 1.32 – 1.19 (m, 2H), 1.21 – 1.12 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 165.85 (Bz), 165.83 (Bz), 165.28 (Bz), 156.43 (Cbz), 137.88, 137.79, 137.50, 136.94, 133.18, 133.09, 130.44, 129.94, 129.83, 129.70, 129.53, 128.60, 128.59, 128.51, 128.45, 128.40, 128.39, 128.30, 128.20, 128.12, 128.09, 128.02, 127.99, 127.86, 127.81, 127.75, 101.33 (C-1), 96.86 (C'-1), 76.96 (C'-2), 76.18 (C-4 or C'-5), 76.14 (C'-4), 75.49 (C'-4), 75.09 (C-5), 74.96 (CH_2Ph), 74.67 (CH_2Ph), 74.53 (C'-3), 73.74 (CH_2Ph), 72.44 (C-2), 72.25 (CH_2Ph), 69.99 (C-4 or C'-5), 68.39, 66.55, 65.00, 41.01, 29.47, 29.08, 23.24.; MS ESI+-HRMS m/z $[M+Na]^+$ calcd for $C_{74}H_{75}NO_{16}Na$ 1256.4978, found 1256.4927.

_N_-Benzyloxycarbonyl-5-amino-pentyl (2,4,6-tri-_O_-benzyl-α-D-glucopyranosyl)-(1→6)-(2,3-di-_O_-benzoyl-β-D-glucopyranoside AGA-4-p18



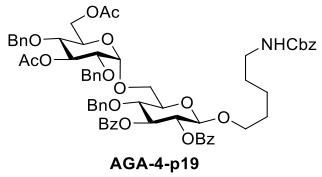
AGA-4-p18



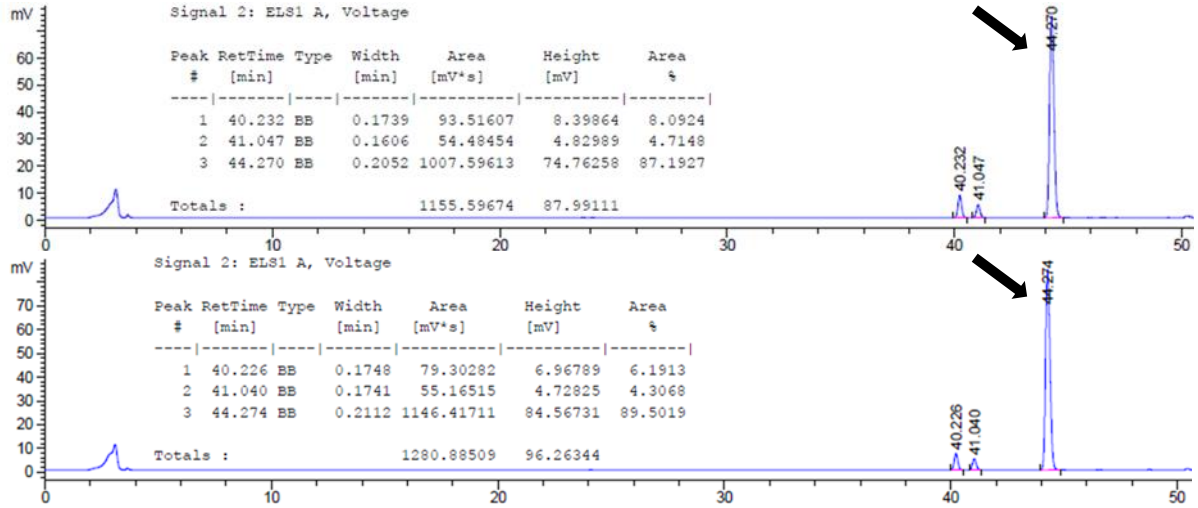
LC-MS chromatogram of **AGA-4-p18**

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (d, *J* = 8.0 Hz, 2H), 7.86 (d, *J* = 7.7 Hz, 2H), 7.48 (dd, *J* = 13.3, 6.1 Hz, 4H), 7.42 – 7.22 (m, 20H), 7.19 (d, *J* = 7.7 Hz, 2H), 7.10 (s, 5H), 5.69 (t, *J* = 9.6 Hz, 1H, H-3), 5.30 (dd, *J* = 9.7, 8.1 Hz, 1H, H-2), 5.14 (d, *J* = 2.6 Hz, 1H, H'-1), 5.06 (s, 2H, Cbz), 4.86 (d, *J* = 5.8 Hz, 1H, C*H*HPh), 4.83 (d, *J* = 6.4 Hz, 1H, C*H*HPh), 4.70 (d, *J* = 11.7 Hz, 1H, C*H*HPh), 4.62 (t, *J* = 9.2 Hz, 3H, H-1, 2 x C*H*HPh), 4.55 (s, 2H, CH_2Ph), 4.49 (t, *J* = 11.9 Hz, 2H, 2 x C*H*HPh), 4.17 – 4.09 (m, 1H, H'-3), 3.98 – 3.77 (m, 5H, H-4, H'-5, H'-6, OC*H*H of linker), 3.75 – 3.57 (m, 4H, H-5, H-6, H'-4), 3.52 – 3.40 (m, 2H, H'-2, OC*H*H of linker), 2.87 (dd, *J* = 12.8, 6.4 Hz, 2H, CH_2NHCbz), 2.61 (s, 1H), 1.50 – 1.35 (m, 2H), 1.33 – 1.19 (m, 2H), 1.19 – 1.05 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.65 (Bz), 165.15 (Bz), 156.24 (Cbz), 138.46, 137.89, 137.85, 137.26, 136.68, 133.08, 129.74, 129.69, 129.50, 129.35, 128.67, 128.48, 128.38, 128.32, 128.29, 128.26, 128.13, 128.02, 127.95, 127.83, 127.76, 127.71, 127.62, 101.11 (C-1), 96.50 (C'-1), 79.29 (C'-2), 77.22 (C'-4), 76.13 (C-4), 75.06 (C-5), 74.98 (C-3), 74.81 (CH_2Ph), 74.62 (CH_2Ph), 73.52 (C'-3), 73.40 (CH_2Ph), 72.42 (C-2), 72.26 (CH_2Ph), 69.83 (C'-5), 69.75 (OCH_2, linker), 68.43 (C-6), 66.48 (CH_2Ph, Cbz), 65.05 (C'-6), 40.80 (CH_2NHCbz), 29.32, 28.88, 23.06.; MS ESI+-HRMS m/z $[M+Na]^+$ calcd for $C_{67}H_{71}NO_{15}Na$ 1152.4716, found 1152.4700.

_N_-Benzyloxycarbonyl-5-amino-pentyl (3,6-di-_O_-acetyl-2,4-di-_O_-benzyl-α-D-glucopyranosyl)-(1→6)-2,3-di-_O_-benzoyl-β-D-glucopyranoside AGA-4-p19



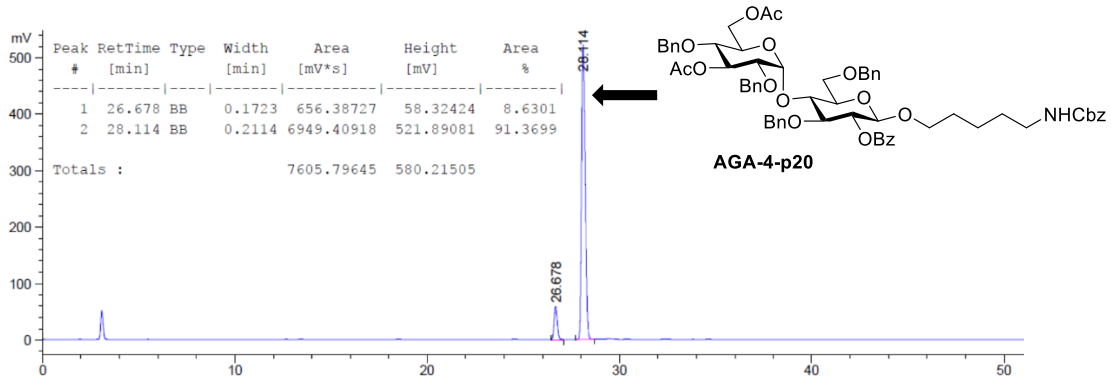
AGA-4-p19



LC-MS chromatogram of **AGA-4-p19** (entry 8 (top) and entry 9 (bottom))

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.91 (d, *J* = 7.4 Hz, 2H), 7.80 (d, *J* = 7.4 Hz, 2H), 7.47 (dd, *J* = 17.7, 7.5 Hz, 2H), 7.40 – 7.23 (m, 19H), 7.17 – 7.08 (m, 5H), 5.65 (dt, *J* = 19.2, 9.6 Hz, 2H, H-3, H'-3), 5.23 (dd, *J* = 9.8, 8.1 Hz, 1H, H-2), 5.20 (d, *J* = 2.9 Hz, 1H, H'-1), 5.06 (s, 2H, Cbz), 4.82 (d, *J* = 12.4 Hz, 1H, C*H*HPh), 4.71 (br, 1H, NH), 4.64 (d, *J* = 12.4 Hz, 1H, C*H*HPh), 4.62 – 4.54 (m, 4H, H-1, CH_2Ph, C*H*HPh), 4.50 (d, *J* = 11.0 Hz, 1H, C*H*HPh), 4.32 – 4.23 (m, 2H, H'-6), 4.00 – 3.94 (m, 2H, H'-3, H'-5), 3.89 (dd, *J* = 15.3, 7.6 Hz, 2H, H-6), 3.86 – 3.79 (m, 1H, OC*H*H, linker), 3.67 (dd, *J* = 9.6, 2.3 Hz, 1H, H-5), 3.57 – 3.50 (m, 2H, H'-2, H'-4), 3.49 – 3.42 (m, 1H, OC*H*H, linker), 2.90 (d, *J* = 6.0 Hz, 2H, CH_2NHCbz), 2.06 (s, 3H, Ac), 2.03 (s, 3H, Ac), 1.50 – 1.37 (m, 2H), 1.35 – 1.22 (m, 2H), 1.22 – 1.09 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.78 (Ac), 170.11 (Ac), 165.84 (Bz), 165.25 (Bz), 156.43 (Cbz), 138.02, 137.61, 137.47, 136.92, 133.18, 129.94, 129.84, 129.70, 129.50, 128.70, 128.64, 128.63, 128.47, 128.42, 128.41, 128.37, 128.28, 128.15, 127.94, 127.90, 127.81, 101.42 (C-1), 96.75 (C'-1), 77.22 (C'-2), 76.15 (C'-4), 76.01 (C'-3 or C'-5), 75.58 (C-5), 75.03 (C-3 or C'-3), 75.00 (CH_2Ph), 74.59 (CH_2Ph), 73.64 (C-3 or C'-3), 72.42 (CH_2Ph), 72.32 (C-2), 70.07 (OCH_2, linker), 68.70 (C'-3 or C'-5), 66.60 (Cbz), 65.01 (C-6), 62.94 (C'-6), 41.02 (CH_2NHCbz), 29.48, 29.11, 23.28, 21.30 (Ac), 21.03 (Ac).; MS ESI+-HRMS m/z $[M+Na]^+$ calcd for $C_{64}H_{69}NO_{17}Na$ 1146.4458, found 1146.4420.

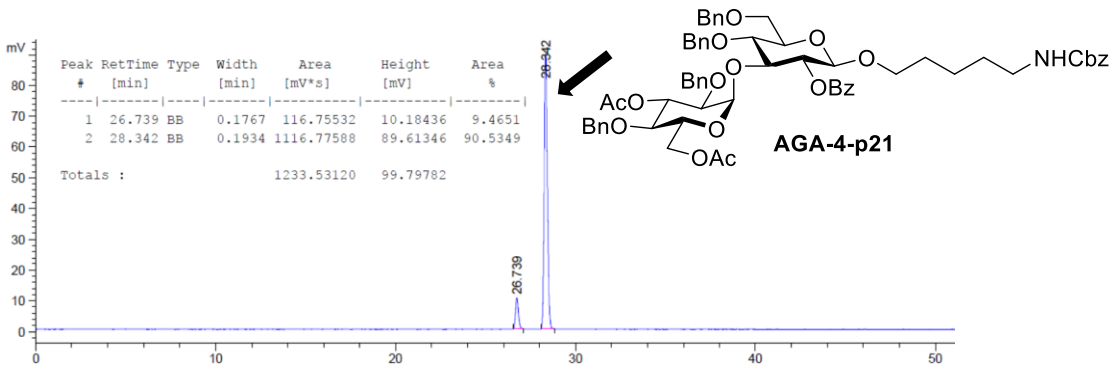
_N_-Benzyloxycarbonyl-5-amino-pentanyl (3,6-di-_O_-acetyl-2,4-di-_O_-benzyl-α-D-glucopyranosyl)-(1→4)-2-_O_-benzoyl-3,6-di-_O_-benzyl-β-D-glucipyranoside AGA-4-p20



LC-MS chromatogram of **AGA-4-p2** (entry 10)

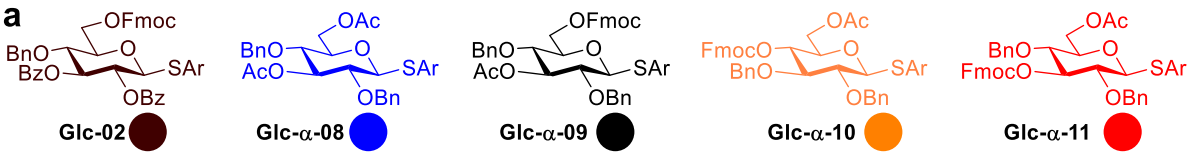
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, *J* = 7.7 Hz, 2H), 7.52 (t, *J* = 7.3 Hz, 1H), 7.42 – 7.21 (m, 20H), 7.10 (d, *J* = 8.1 Hz, 7H), 5.53 (t, *J* = 9.7 Hz, 1H), 5.50 (d, *J* = 3.3 Hz, 1H), 5.34 (t, *J* = 8.4 Hz, 1H), 5.07 (s, 2H), 4.75 (d, *J* = 11.3 Hz, 1H), 4.65 (d, *J* = 11.3 Hz, 1H), 4.61 – 4.53 (m, 4H), 4.53 – 4.41 (m, 3H), 4.25 (d, *J* = 12.4 Hz, 1H), 4.19 – 4.06 (m, 3H), 3.98 (t, *J* = 8.5 Hz, 2H), 3.89 (td, *J* = 12.1, 5.0 Hz, 2H), 3.78 (d, *J* = 10.7 Hz, 1H), 3.63 (d, *J* = 7.2 Hz, 1H), 3.44 (dd, *J* = 21.1, 11.7 Hz, 2H), 3.35 (dd, *J* = 10.1, 3.3 Hz, 1H), 2.91 (dd, *J* = 12.6, 6.3 Hz, 2H), 2.00 (s, 3H), 1.94 (s, 3H), 1.58 – 1.40 (m, 2H), 1.36 – 1.25 (m, 2H), 1.25 – 1.12 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.69, 169.99, 165.29, 156.37, 138.23, 138.21, 137.78, 137.54, 136.81, 133.27, 129.94, 129.82, 128.64, 128.62, 128.51, 128.44, 128.21, 128.17, 127.87, 127.84, 127.72, 127.68, 127.41, 127.31, 101.18, 96.75, 82.69, 77.36, 76.99, 76.11, 75.03, 74.56, 74.39, 73.50, 73.37, 73.31, 72.96, 72.48, 69.51, 69.29, 68.85, 66.61, 63.03, 40.93, 29.51, 29.05, 23.24, 21.23, 21.01.; MS ESI+-HRMS m/z $[M+Na]^+$ calcd for $C_{64}H_{71}NO_{17}Na$ 1132.4665, found 1132.4763.

_N_-Benzyloxycarbonyl-5-amino-pentanyl (3,6-di-_O_-acetyl-2,4-di-_O_-benzyl-α-D-glucopyranosyl)-(1→4)-2-_O_-benzoyl-4,6-di-_O_-benzyl-β-D-glucipyranoside AGA-4-p21



LC-MS chromatogram of **AGA-4-p21** (entry 11)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, *J* = 7.5 Hz, 2H), 7.50 (t, *J* = 7.0 Hz, 1H), 7.43 – 7.09 (m, 27H), 5.58 (t, *J* = 9.6 Hz, 1H), 5.23 (t, *J* = 8.0 Hz, 1H), 5.15 (d, *J* = 11.2 Hz, 1H), 5.07 (s, 3H), 4.65 – 4.50 (m, 4H), 4.40 (dt, *J* = 22.4, 11.1 Hz, 5H), 4.11 (d, *J* = 12.0 Hz, 1H), 4.06 – 3.91 (m, 3H), 3.91 – 3.83 (m, 1H), 3.81 – 3.66 (m, 3H), 3.65 – 3.54 (m, 1H), 3.46 (t, *J* = 9.6 Hz, 1H), 3.36 (d, *J* = 10.3 Hz, 2H), 2.87 (d, *J* = 5.8 Hz, 2H), 1.91 (s, 3H), 1.88 (s, 3H), 1.57 – 1.36 (m, 2H), 1.34 – 1.21 (m, 2H), 1.21 – 1.05 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.50, 169.78, 165.04, 156.37, 138.75, 138.16, 138.03, 137.62, 136.85, 133.11, 130.24, 129.68, 128.61, 128.50, 128.49, 128.44, 128.21, 128.15, 128.03, 127.96, 127.94, 127.90, 127.86, 127.74, 127.63, 127.30, 101.07, 98.65, 83.86, 77.81, 77.36, 77.28, 75.35, 75.13, 74.96, 74.03, 73.63, 73.52, 73.19, 72.88, 69.48, 69.18, 69.04, 66.57, 62.30, 40.89, 29.43, 29.02, 23.18, 21.19, 20.89.; MS ESI+-HRMS m/z $[M+Na]^+$ calcd for $C_{64}H_{71}NO_{17}Na$ 1132.4665, found 1132.4768.



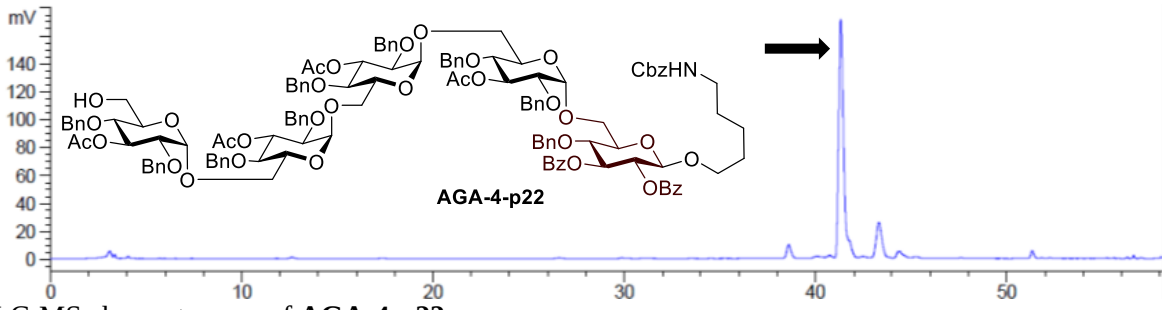
Sequence	Module	Details	Conditions
I	1	2.5 eq. of TMSOTf solution	-20 °C, 1 min
	2	5 eq. building block **Glc-02**, 5.5 eq. of NIS Solution	$\mathbf{T_a}$ = -30 °C, $\mathbf{t_1}$ = 5 min $\mathbf{T_i}$ = -10 °C, $\mathbf{t_2}$ = 25 min
	3	Fmoc Removal	r.t., 5 min
II	1	2.5 eq. of TMSOTf solution	-20 °C, 1 min
	2	5 eq. building block **Glc-α-09**, 5.5 eq. of NIS Solution	$\mathbf{T_a}$ = -30 °C, $\mathbf{t_1}$ = 5 min $\mathbf{T_i}$ = -10 °C, $\mathbf{t_2}$ = 25 min
	3	Fmoc Removal	r.t., 5 min
III	1	2.5 eq. of TMSOTf solution	-20 °C, 1 min
	2	5 eq. building block **Glc-α-10**, 5.5 eq. of NIS Solution	$\mathbf{T_a}$ = -30 °C, $\mathbf{t_1}$ = 5 min $\mathbf{T_i}$ = -10 °C, $\mathbf{t_2}$ = 50 min
	3	Fmoc Removal	r.t., 5 min
IV	1	2.5 eq. of TMSOTf solution	-20 °C, 1 min
	2	5 eq. building block **Glc-α-11**, 5.5 eq. of NIS Solution	$\mathbf{T_a}$ = -30 °C, $\mathbf{t_1}$ = 5 min $\mathbf{T_i}$ = -10 °C, $\mathbf{t_2}$ = 50 min
	3	Fmoc Removal	r.t., 5 min
V	1	2.5 eq. of TMSOTf solution	-20 °C, 1 min
	2	5 eq. building block **Glc-α-08**, 5.5 eq. of NIS Solution	$\mathbf{T_a}$ = -30 °C, $\mathbf{t_1}$ = 5 min $\mathbf{T_i}$ = -10 °C, $\mathbf{t_2}$ = 25 min

Sequences of the glycosylation cycle with the corresponding monomers.

Compound	On-Resin Steps	Amounts	Yield
AGA-4-p22	11	12.9 mg	23%
AGA-4-p24	11	5.3 mg	9%
AGA-4-p25	10	6.9 mg	12%
AGA-4-p26	10	9.6 mg	17%
AGA-4-p27	8	9.0 mg	20%

Yields of α-glucans **AGA-4-p22 - AGA-4-p27.**

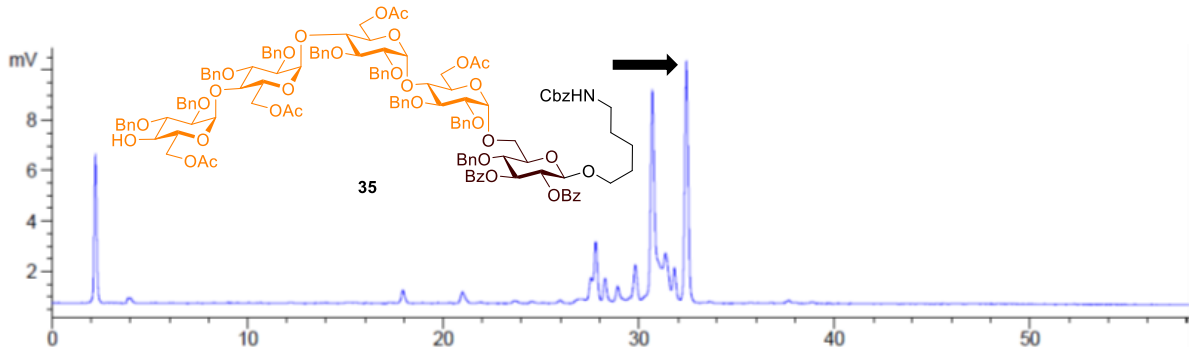
***N*-Benzyloxycarbonyl-5-amino-pentyl (3-*O*-acetyl-2,4-di-*O*-benzyl-α-D-glucopyranosyl)-(1→6)-(3-*O*-acetyl-2,4-di-*O*-benzyl-α-D-glucopyranosyl)-(1→6)-(3-*O*-acetyl-2,4-di-*O*-benzyl-α-D-glucopyranosyl)-(1→6)-(3-*O*-acetyl-2,4-di-*O*-benzyl-α-D-glucopyranosyl)-(1→6)-2,3-di-*O*-benzoyl-β-D-glucopyranoside AGA-4-p22**



LC-MS chromatogram of **AGA-4-p22**

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.92 (d, *J* = 7.9 Hz, 2H), 7.78 (d, *J* = 7.9 Hz, 2H), 7.38 – 7.17 (m, 50H), 7.15 – 7.07 (m, 4H), 5.68 – 5.61 (m, 2H, 2 x H-3), 5.57 – 5.49 (m, 3H, 3 x H-3), 5.25 – 5.20 (m, 1H, H-2), 5.14 (d, *J* = 3.3 Hz, 1H, H-1), 5.06 – 5.03 (m, 4H, 2 x H-1, CH_2 of Cbz), 4.99 (d, *J* = 3.2 Hz, 1H, H-1), 4.72 – 4.47 (m, 15H, H-1), 4.46 – 4.37 (m, 3H), 4.30 (d, *J* = 12.3 Hz, 2H), 3.93 (t, *J* = 9.5 Hz, 1H), 3.88 – 3.57 (m, 21H), 3.43 (dd, *J* = 9.9, 3.2 Hz, 2H), 3.22 – 3.11 (m, 3H), 2.85 (dd, *J* = 12.5, 6.2 Hz, 2H, CH_2NHCbz), 1.98 (s, 3H, Ac), 1.89 (s, 3H, Ac), 1.88 (s, 3H, Ac), 1.87 (s, 3H, Ac), 1.49 – 1.37 (m, 2H, CH_2, pentane), 1.31 – 1.19 (m, 2H, CH_2, pentane), 1.18 – 1.05 (m, 2H, CH_2, pentane). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.31 (Ac), 170.24 (Ac), 170.23 (Ac), 170.14 (Ac), 165.82 (Bz), 165.26 (Bz), 156.41 (Cbz), 138.44, 138.39, 138.36, 138.27, 138.18, 137.94, 137.59, 136.88, 133.19, 133.13, 129.92, 129.82, 129.70, 129.52, 128.64, 128.61, 128.59, 128.55, 128.54, 128.51, 128.50, 128.49, 128.48, 128.37, 128.35, 128.30, 128.24, 128.19, 128.16, 128.12, 128.10, 127.76, 127.74, 127.70, 127.68, 127.60, 127.56, 127.24, 127.02, 127.01, 101.36 (C-1), 97.18 (2 x C-1), 97.09 (C-1), 96.73 (C-1), 77.86, 77.85, 77.80, 77.63, 76.12, 75.93, 75.87, 75.72, 75.57, 75.02, 74.88, 74.70, 74.68, 74.63, 74.59, 73.55, 73.49, 73.46, 73.43, 72.51, 72.43, 72.03, 71.92, 71.81, 71.20, 70.98, 70.00, 66.58, 65.41, 65.20, 64.89, 61.59, 40.97, 29.42, 29.05, 23.22, 21.28 (Ac), 21.25 (3 x Ac).; MS ESI+-HRMS m/z [M+H]+ calcd for $C_{128}H_{140}NO_{34}$ 2234.9251, found 2234.9198.

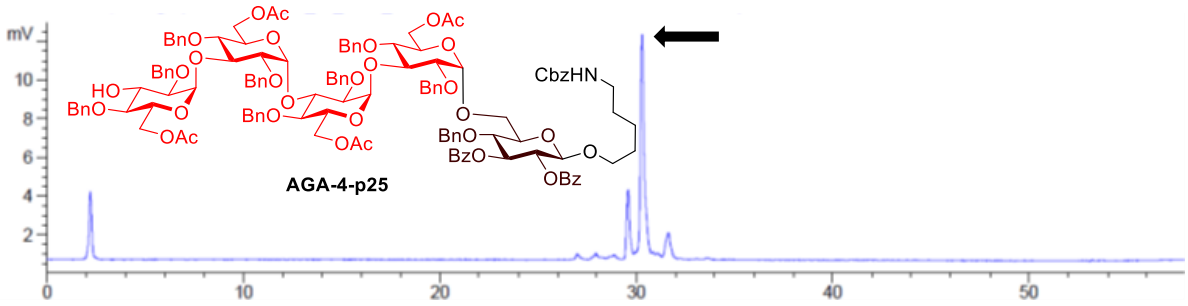
***N*-Benzyloxycarbonyl-5-amino-pentyl (6-*O*-acetyl-2,3-di-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-(6-*O*-acetyl-2,3-di-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-(6-*O*-acetyl-2,3-di-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-(6-*O*-acetyl-2,3-di-*O*-benzyl-α-D-glucopyranosyl)-(1→6)-2,3-di-*O*-benzoyl-β-D-glucopyranoside AGA-4-p24**



LC-MS chromatogram of **AGA-4-p24**

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.93 (d, *J* = 8.1 Hz, 2H), 7.81 (d, *J* = 8.1 Hz, 2H), 7.48 (dt, *J* = 13.2, 7.5 Hz, 2H), 7.42 – 7.01 (m, 54H), 5.69 (t, *J* = 9.6 Hz, 1H, H-3), 5.56 (d, *J* = 3.6 Hz, 1H, H-1), 5.42 (d, *J* = 3.4 Hz, 1H, H-1), 5.38 (d, *J* = 3.4 Hz, 1H, H-1), 5.35 – 5.30 (m, 1H, H-2), 5.28 (d, *J* = 3.2 Hz, 1H, H-1), 5.10 (d, *J* = 11.6 Hz, 1H), 5.06 (br, 2H, CH_2, Cbz), 4.88 – 4.51 (m, 16H, H-1), 4.44 (dt, *J* = 15.3, 10.6 Hz, 6H), 4.35 (t, *J* = 9.6 Hz, 2H), 4.26 (ddd, *J* = 20.9, 11.9, 4.3 Hz, 2H), 4.14 (t, *J* = 9.7 Hz, 2H), 4.08 (t, *J* = 9.5 Hz, 1H), 4.04 – 3.87 (m, 9H), 3.80 (dt, *J* = 15.3, 9.1 Hz, 4H), 3.70 – 3.64 (m, 2H), 3.50 – 3.39 (m, 4H), 2.91 – 2.84 (m, 2H, CH_2NHCbz), 2.70 (s, 1H, OH), 2.11 (s, 3H, Ac), 2.10 (s, 3H, Ac), 2.08 (s, 3H, Ac), 2.07 (s, 3H, Ac), 1.54 – 1.40 (m, 2H, CH_2, pentane), 1.33 – 1.22 (m, 2H, CH_2, pentane), 1.22 – 1.11 (m, 2H, CH_2, pentane). ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.62 (Ac), 170.88 (Ac), 170.73 (Ac), 170.71 (Ac), 165.84 (Bz), 165.23 (Bz), 156.37 (Cbz), 139.10, 138.90, 138.74, 138.07, 137.90, 137.50, 136.85, 133.17, 129.95, 129.82, 129.67, 129.54, 128.74, 128.63, 128.55, 128.47, 128.38, 128.31, 128.24, 128.11, 128.04, 127.98, 127.74, 127.71, 127.66, 127.27, 127.22, 127.17, 126.70, 126.55, 126.53, 101.55 (C-1), 98.26 (C-1), 97.38 (C-1), 97.27 (C-1), 96.77 (C-1), 81.24, 81.06, 80.78, 80.73, 80.00, 79.04, 78.95, 78.92, 75.66, 75.55, 75.34, 74.98, 74.82, 74.18, 74.17, 73.42, 73.32, 73.28, 72.67, 72.42, 70.89, 70.34, 70.01, 69.57, 68.58, 66.62, 64.72, 63.64, 63.55, 63.31, 40.94, 29.54, 29.11, 23.31, 21.13 (Ac), 21.12 (Ac), 21.06 (Ac), 21.00 (Ac).; MS ESI+-HRMS m/z [M+H]+ calcd for $C_{128}H_{140}NO_{34}$ 2234.9251, found 2234.9142.

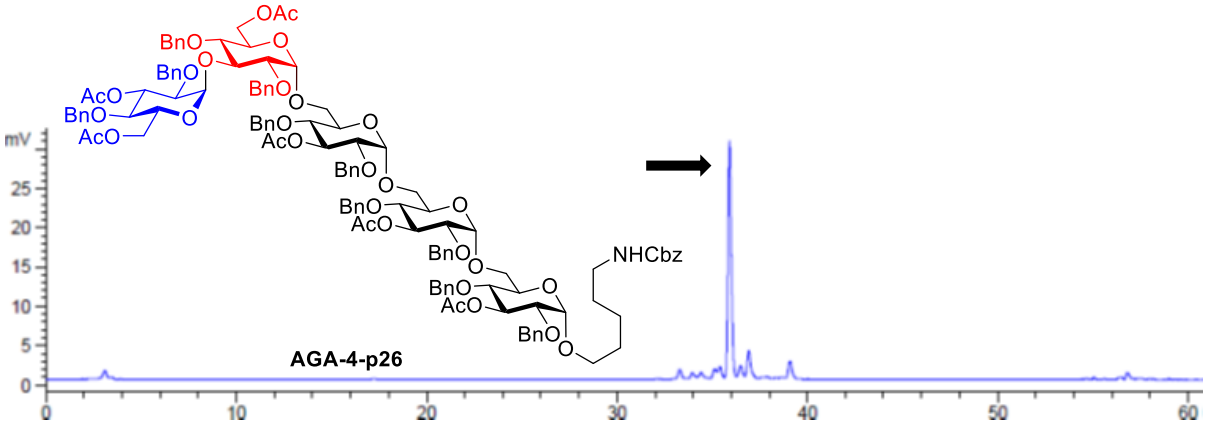
***N*-Benzyloxycarbonyl-5-amino-pentyl (3,6-di-*O*-acetyl-2,4-di-*O*-benzyl-α-D-glucopyranosyl)-(1→3)-(6-*O*-acetyl-2,4-di-*O*-benzyl-α-D-glucopyranosyl)-(1→3)-(6-*O*-acetyl-2,4-di-*O*-benzyl-α-D-glucopyranosyl)-(1→3)-(6-*O*-acetyl-2,4-di-*O*-benzyl-α-D-glucopyranosyl)-(1→3)-2,3-di-*O*-benzoyl-β-D-glucopyranoside AGA-4-p25**



LC-MS chromatogram of **AGA-4-p25.**

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.93 (d, *J* = 7.3 Hz, 2H), 7.88 (d, *J* = 7.5 Hz, 2H), 7.50 – 7.43 (m, 4H), 7.40 – 7.05 (m, 47H), 6.98 (s, 5H), 5.71 (t, *J* = 9.4 Hz, 1H, H-3), 5.67 (d, *J* = 0.4 Hz, 1H, H-1), 5.60 (d, *J* = 1.3 Hz, 1H, H-1), 5.52 (d, *J* = 1.3 Hz, 1H, H-1), 5.30 (t, *J* = 8.6 Hz, 1H, H-2), 5.06 (s, 3H, H-1, CH_2 of Cbz), 4.79 (dd, *J* = 18.8, 11.8 Hz, 2H), 4.73 – 4.22 (m, 25H, H-1), 4.17 (d, *J* = 11.8 Hz, 1H), 4.07 (dd, *J* = 30.6, 9.5 Hz, 3H), 3.86 (dt, *J* = 30.1, 18.3 Hz, 8H), 3.72 – 3.41 (m, 8H), 3.32 (dd, *J* = 18.1, 8.9 Hz, 2H), 2.86 (br, 2H, CH_2NHCbz), 2.05 (s, 3H, Ac), 2.05 (s, 3H, Ac), 2.03 (s, 3H, Ac), 1.97 (s, 3H, Ac), 1.47 – 1.34 (m, 2H, CH_2, pentane), 1.34 – 1.18 (m, 2H, CH_2, pentane), 1.17 – 1.05 (m, 2H, CH_2, pentane). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.80 (Ac), 170.76 (Ac), 170.70 (Ac), 170.61 (Ac), 165.83 (Bz), 165.23 (Bz), 156.36 (Cbz), 138.38, 138.06, 137.88, 137.79, 137.73, 137.65, 137.55, 137.32, 136.89, 133.22, 129.90, 129.81, 129.65, 129.46, 128.76, 128.60, 128.56, 128.45, 128.36, 128.28, 128.23, 128.14, 128.08, 127.97, 127.90, 127.74, 127.63, 127.53, 127.48, 127.00, 126.86, 126.74, 101.34 (C-1), 96.83 (C-1), 96.37 (C-1), 96.14 (C-1), 95.90 (C-1), 79.30, 79.24, 78.91, 78.63, 78.57, 78.20, 77.37, 77.16, 76.95, 76.56, 76.40, 76.09, 75.24, 75.18, 75.04, 74.95, 74.33, 73.76, 73.46, 73.42, 73.15, 72.96, 72.58, 72.45, 72.38, 69.93, 68.21, 68.16, 68.10, 66.56, 65.66, 63.25, 63.00, 40.90, 29.43, 29.03, 23.22, 21.12 (Ac), 21.09 (Ac), 21.05 (Ac).; MS ESI+-HRMS m/z [M+Na]+ calcd for $C_{129}H_{140}NO_{34}$ 2234.9251, found 2234.9178.

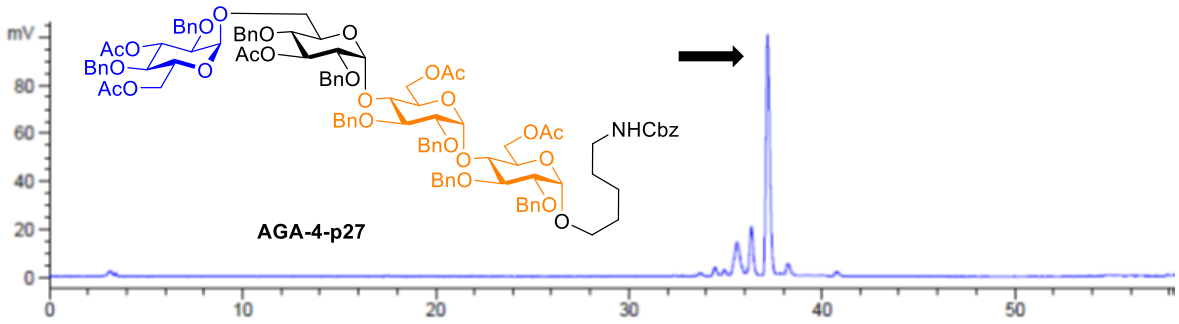
***N*-Benzyloxycarbonyl-5-amino-pentyl (3,6-di-*O*-acetyl-2,3-di-*O*-benzyl-α-D-glucopyranosyl)-(1→3)-(6-*O*-acetyl-2,4-di-*O*-benzyl-α-D-glucopyranosyl)-(1→6)-(3-*O*-acetyl-2,3-di-*O*-benzyl-α-D-glucopyranosyl)-(1→6)-(3-*O*-acetyl-2,3-di-*O*-benzyl-α-D-glucopyranosyl)-(1→6)-2,3-di-*O*-benzoyl-α-D-glucopyranoside AGA-4-p26**



LC-MS chromatogram of **AGA-4-p26**

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.37 – 7.13 (m, 53H), 7.09 – 7.05 (m, 2H), 5.65 (t, *J* = 9.7 Hz, 1H, H-3), 5.59 (t, *J* = 9.6 Hz, 1H, H-3), 5.55 (d, *J* = 3.4 Hz, 1H, H-1), 5.51 – 5.46 (m, 1H, H-3), 5.43 (t, *J* = 9.6 Hz, 1H, H-3), 5.19 (d, *J* = 3.4 Hz, 1H, H-1), 5.06 (s, 2H, CH_2, Cbz), 5.04 (d, *J* = 3.3 Hz, 1H, H-1), 4.93 (d, *J* = 11.7 Hz, 1H), 4.90 (d, *J* = 3.3 Hz, 1H, H-1), 4.67 – 4.56 (m, 8H, H-1, 7 x CHHPh), 4.50 – 4.32 (m, 12H), 4.28 – 4.22 (m, 3H), 4.07 (dd, *J* = 12.1, 3.9 Hz, 1H), 3.96 – 3.92 (m, 1H), 3.86 – 3.74 (m, 10H), 3.70 – 3.61 (m, 6H), 3.55 (dd, *J* = 9.6, 3.4 Hz, 1H, H'''-2), 3.52 – 3.43 (m, 2H), 3.30 (dd, *J* = 15.6, 6.4 Hz, 1H), 3.25 (dd, *J* = 10.0, 3.4 Hz, 1H), 3.17 – 3.07 (m, 4H), 2.02 (s, 3H, Ac), 1.99 (s, 3H, Ac), 1.94 (s, 3H, Ac), 1.91 (s, 3H, Ac), 1.86 (s, 3H, Ac), 1.82 (s, 3H, Ac), 1.63 – 1.55 (m, 2H, CH_2, pentane), 1.54 – 1.44 (m, 2H, CH_2, pentane), 1.41 – 1.31 (m, 2H, CH_2, pentane). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.75 (Ac), 170.53 (Ac), 170.36 (Ac), 170.21 (Ac), 170.11 (Ac), 170.03 (Ac), 156.58, 138.38, 138.31, 138.24, 138.17, 138.11, 137.93, 137.62, 137.54, 136.99, 128.60, 128.57, 128.52, 128.47, 128.45, 128.36, 128.22, 128.17, 128.08, 128.04, 128.00, 127.90, 127.87, 127.82, 127.75, 127.60, 127.09, 127.02, 126.86, 97.35 (C-1), 97.14 (C-1), 96.97 (C-1), 96.69 (C-1), 96.44 (C-1), 78.70, 78.05, 78.01, 77.97, 77.93, 77.87, 76.81, 76.31, 76.06, 75.68, 74.74, 74.58, 74.55, 74.18, 73.90, 73.81, 73.59, 73.53, 73.42, 72.63, 72.02, 71.95, 71.11, 70.91, 70.75, 68.65, 68.26, 66.54, 65.68, 65.23, 64.67, 62.74, 41.10, 29.66, 29.00, 23.61, 21.29 (Ac), 21.23 (Ac), 21.21 (Ac), 21.19 (Ac), 21.05 (Ac), 21.02 (Ac). ; MS ESI+-HRMS m/z $[M+NH_4]^+$ calcd for $C_{125}H_{145}N_2O_{34}$ 2217.9673, found 2217.9589.

***N*-Benzyloxycarbonyl-5-amino-pentyl (3,6-di-*O*-acetyl-2,3-di-*O*-benzyl-α-D-glucopyranosyl)-(1→6)-(3-*O*-acetyl-2,4-di-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-(6-*O*-acetyl-2,3-di-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-6-*O*-acetyl-2,3-di-*O*-benzyl-α-D-glucopyranoside AGA-4-p27**



LC-MS chromatogram of **AGA-4-p27**

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.38 – 7.17 (m, 32H), 7.17 – 7.07 (m, 9H), 7.04 (d, *J* = 7.5 Hz, 2H), 6.93 (d, *J* = 6.6 Hz, 2H), 5.63 (t, *J* = 9.4 Hz, 1H, H-3), 5.42 (d, *J* = 2.5 Hz, 1H, H-1), 5.41 (d, *J* = 2.0 Hz, 1H, H-1), 5.36 (t, *J* = 9.5 Hz, 1H, H-3), 5.22 (d, *J* = 1.3 Hz, 1H, H-1), 5.09 (br, 2H, CH_2, Cbz), 5.00 (d, *J* = 11.7 Hz, 1H), 4.90 (s, 1H, NHCbz), 4.81 (t, *J* = 10.7 Hz, 2H), 4.70 (d, *J* = 1.4 Hz, 1H, H-1), 4.67 – 4.49 (m, 9H), 4.44 – 4.38 (m, 4H), 4.33 – 4.27 (m, 2H), 4.22 (s, 2H), 4.18 (dd, *J* = 12.2, 3.5 Hz, 1H), 4.03 (dd, *J* = 19.0, 10.4 Hz, 2H), 3.98 – 3.84 (m, 6H), 3.82 – 3.68 (m, 5H), 3.66 (dd, *J* = 14.9, 6.9 Hz, 1H), 3.52 (t, *J* = 9.7 Hz, 2H), 3.48 – 3.41 (m, 2H), 3.38 (dd, *J* = 15.3, 6.9 Hz, 1H), 3.21 (d, *J* = 6.2 Hz, 2H, CH_2NHCbz), 2.96 (dd, *J* = 10.1, 2.3 Hz, 1H, H-2), 2.06 (s, 3H, Ac), 2.05 (s, 6H, 2 x Ac), 2.00 (s, 3H, Ac), 1.73 (s, 3H, Ac), 1.72 – 1.65 (m, 2H, CH_2, pentane), 1.61 – 1.53 (m, 2H, CH_2, pentane), 1.48 – 1.38 (m, 2H, CH_2, pentane). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.78 (Ac), 170.62 (Ac), 170.08 (Ac), 170.05 (Ac), 156.55 (Cbz), 139.33, 139.17, 138.23, 138.20, 138.13, 137.90, 137.79, 137.60, 136.84, 128.65, 128.63, 128.56, 128.54, 128.35, 128.31, 128.29, 128.23, 128.16, 128.15, 128.05, 127.85, 127.77, 127.70, 127.64, 127.32, 127.11, 127.08, 126.92, 126.65, 126.35 (Ar), 97.66 (C-1), 97.47 (C-1), 97.04 (C-1), 96.46 (C-1), 81.26, 80.88, 80.24, 79.23, 77.62, 77.37, 77.32, 77.16, 76.95, 76.19, 76.16, 75.40, 74.97, 74.64, 74.56, 74.42, 73.93, 73.50, 73.34, 73.31, 73.18, 73.13, 72.17, 71.24, 69.16, 68.74, 68.31, 68.27, 66.69, 65.06, 63.74, 63.64, 62.99, 41.06, 29.89, 29.05, 23.51, 21.28 (Ac), 21.11 (2 x Ac), 21.01 (2 x Ac).; MS ESI+-HRMS m/z $[M+NH_4]^+$ calcd for $C_{103}H_{118}NO_{28}$ 1816.7835, found 1816.7816.

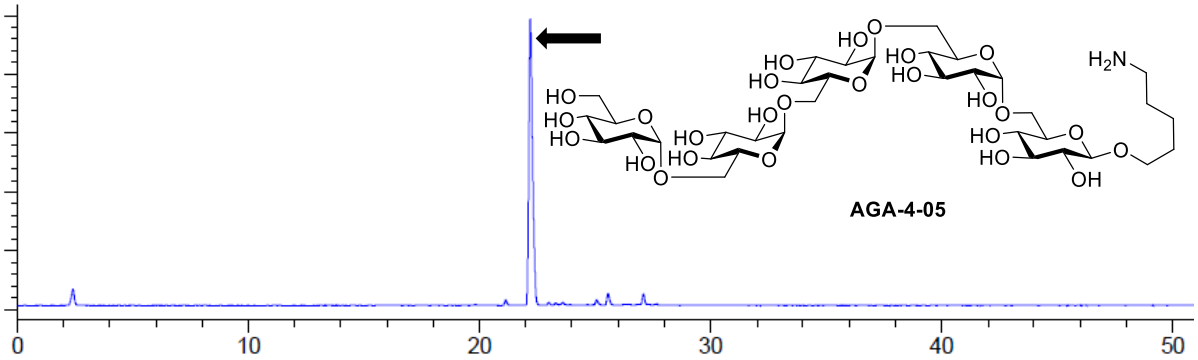
4.5.2 Post-Automation Steps: Removal of Protecting Groups, and Final Purification

Deprotection Conditions: To a solution of the fully protected oligosaccharide **4** in MeOH (5 mL) was added 58 μL of 0.5 M NaOMe solution (0.25 eq. per acetyl or benzoyl group) in MeOH at 40 ° C. The mixture is stirred utill completed, then neutralized by 200 mg of Amberite (400 mg per 100 μL of NaOMe solution) after completion of the reaction. This crude mixture is dissolved in MeOH, ethyl acetate, and AcOH (v/v/v =5:0.5:0.2) added 5% Pd/C (W/V), purged first with argon and then with hydrogen, left to stir overnight at room temperature under balloon pressure. The reaction mixture was filtered through modified cellulose filter, washed with 20 mL of Water/MeOH, 9:1 and the combined solution was evaporated to provide the crude mixture.

Analytical HPLC: The crude material was analyzed by HPLC (column: Hypercarb®, (150 X 4.60 mm); flow rate: 0.8 mL/min; eluents: 0.1% FA in Acetonitrile / 0.1% FA in TDW; gradient: 0% (10 min) 30% (in 30 min) 100% (in 5 min); detection: ELSD).
NOTE: The crude material were used to provide analytical HPLC data in supporting information to identify the conjugation-ready oligosaccharide in the crude mixture followed by deprotection steps from the pure protected oligosaccharide.

Preparative HPLC: The crude solution is purified by preparative HPLC (column: Hypercarb®, (150 X 10.00 mm); flow rate: 3.6 mL/min; eluents: 0.1% FA in Acetonitrile / 0.1% FA in TDW; gradient: 0% (10 min) 30% (in 30 min) 100% (in 5 min); detection: ELSD) to afford the unprotected oligosaccharide.
Note: HPLC purifications using 0.1% formic acid (FA) sometime result in the fromation of formic acid salt with conjugation-ready oligosaccharide. The detection of formic acid by ^{1}H and ^{13}C NMR does not imply of impurity.

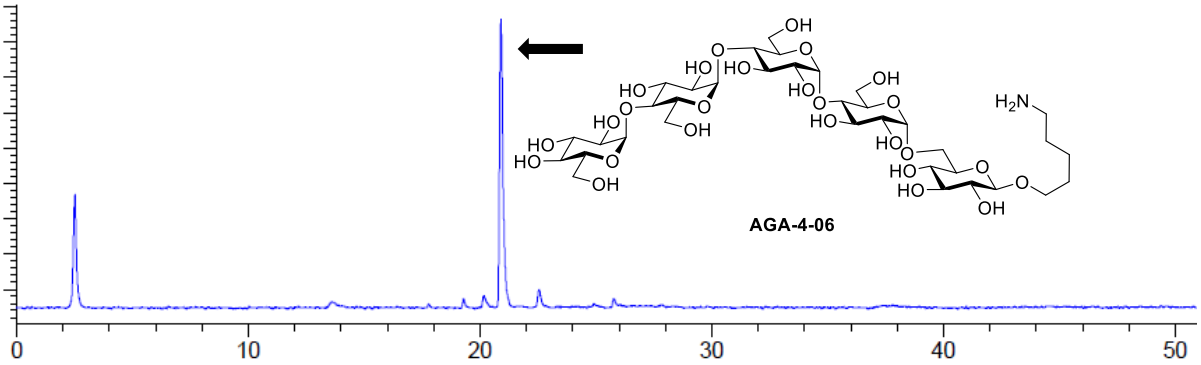
5-Amino-pentyl α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-β-D-glucopyranoside AGA-4-05



LC-MS chromatogram of **AGA-4-05**

^{1}H NMR (700 MHz, D_2O) δ8.31 (s, 1H, *H*CO_2H), 4.92 – 4.88 (m, 4H, 4 x H-1), 4.42 (d, *J* = 8.0 Hz, 1H, H-1), 3.93 – 3.89 (m, 4H), 3.86 – 3.82 (m, 4H), 3.78 (dd, *J* = 12.3, 2.2 Hz, 1H), 3.72 – 3.60 (m, 11H), 3.57 (ddd, *J* = 9.8, 4.4, 2.0 Hz, 1H), 3.53 – 3.40 (m, 9H), 3.37 – 3.34 (m, 1H), 3.20 (dd, *J* = 9.2, 8.1 Hz, 1H, H-2), 2.94 (t, *J* = 7.5 Hz, 2H, -CH_2NH_2), 1.66 – 1.57 (m, 4H, O$CH_2CH_2CH_2CH_2CH_2NH_2$), 1.42 – 1.36 (m, 2H, -$CH_2CH_2CH_2$-). ^{13}C NMR (175 MHz, D_2O) δ 168.62 (HCO_2H), 102.31 (C-1), 97.77 (C-1), 97.72 (C-1), 97.67 (2 x C-1), 76.06, 74.10, 73.37, 73.10, 73.06, 71.81, 71.45, 71.36, 70.23, 70.15, 70.12, 69.47, 69.30, 65.52, 65.42, 60.44, 39.34 (CH_2NH_2), 28.18, 26.41, 22.10.; MS ESI+-HRMS m/z $[M+Na]^+$ calcd for $C_{35}H_{64}NO_{26}$ 914.3711, found 914.3709.

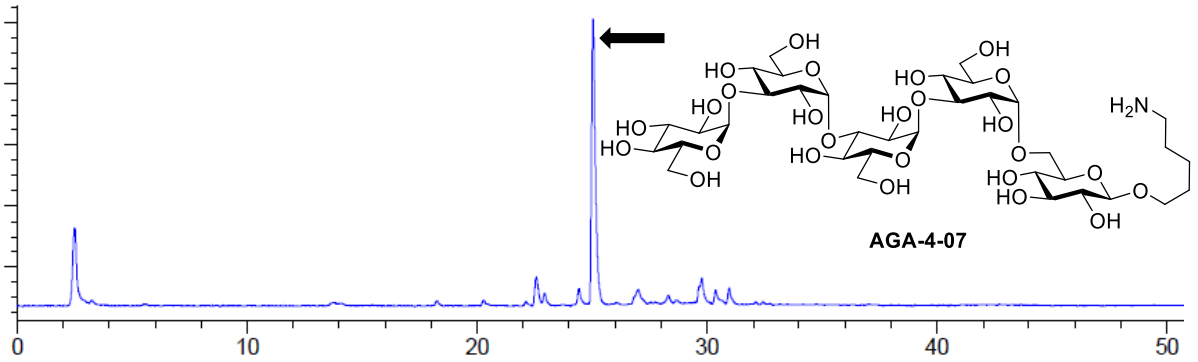
5-Amino-pentyl α-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl-(1→4)-β-D-glucopyranoside AGA-4-06



LC-MS chromatogram of **AGA-4-06**

^{1}H NMR (700 MHz, D_2O) δ 8.50 (s, 1H, *H*CO_2H), 5.40 (d, *J* = 4.0 Hz, 1H, H-1), 5.39 (d, *J* = 3.9 Hz, 1H, H-1), 5.37 (d, *J* = 3.9 Hz, 1H, H-1), 4.98 (d, *J* = 3.7 Hz, 1H, H-1), 4.52 (d, *J* = 8.0 Hz, 1H, H-1), 4.03 – 3.99 (m, 1H), 3.97 – 3.82 (m, 14H), 3.81 – 3.77 (m, 2H), 3.76 – 3.71 (m, 2H), 3.71 – 3.60 (m, 9H), 3.55 – 3.49 (m, 2H), 3.47 – 3.42 (m, 1H), 3.31 – 3.27 (m, 1H, H-2), 3.04 (t, *J* = 7.6 Hz, 2H, -CH_2NH_2), 1.76 – 1.68 (m, 4H, $OCH_2CH_2CH_2CH_2CH_2NH_2$), 1.56 – 1.46 (m, 2H, -$CH_2CH_2CH_2$-). ^{13}C NMR (175 MHz, D_2O) δ 170.16 (HCO_2H), 102.13 (C-1), 99.97 (C-1), 99.93 (C-1), 99.89 (C-1), 97.48 (C-1), 77.52, 77.09, 76.03, 74.20, 73.51, 73.41, 73.35, 73.15, 72.89, 72.73, 71.73, 71.52, 71.45, 71.26, 71.13, 70.24, 69.86, 69.44, 69.30, 65.45, 60.44, 60.33, 39.38 (CH_2NH_2), 28.16, 26.42, 22.15.; MS ESI+-HRMS m/z $[M+Na]^+$ calcd for $C_{35}H_{64}NO_{26}$ 914.3711, found 914.3700.

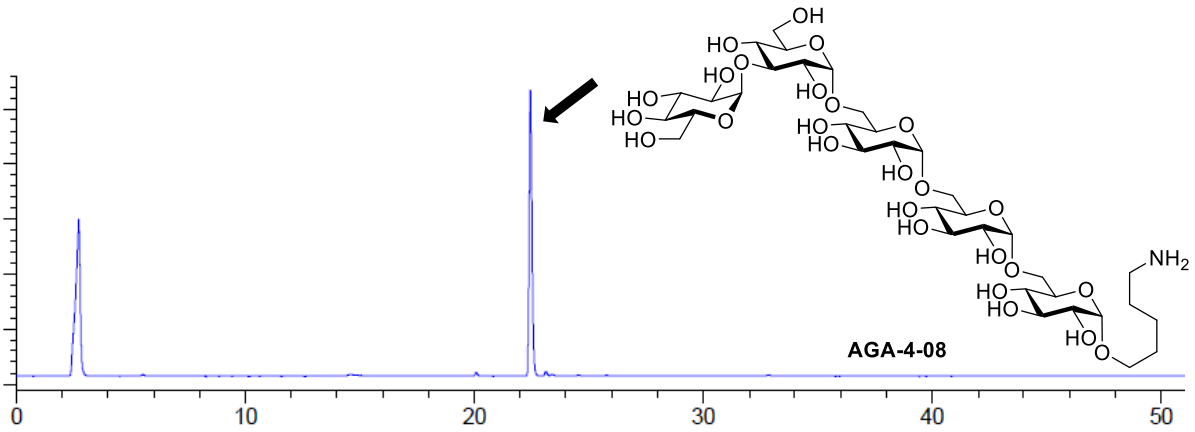
5-Amino-pentyl α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→3)-β-D-glucopyranoside AGA-4-07



LC-MS chromatogram of **AGA-4-07**

^{1}H NMR (700 MHz, D_2O) δ 8.48 (s, 1H, *H*CO_2H), 5.41 (d, *J* = 3.9 Hz, 1H, H-1), 5.40 (d, *J* = 4.0 Hz, 1H, H-1), 5.38 (d, *J* = 3.9 Hz, 1H, H-1), 4.98 (d, *J* = 3.6 Hz, 1H, H-1), 4.52 (d, *J* = 8.0 Hz, 1H, H-1), 4.08 – 4.03 (m, 3H), 4.01 (dd, *J* = 11.1, 4.3 Hz, 1H), 3.96 – 3.65 (m, 23H), 3.59 (dd, *J* = 9.9, 3.9 Hz, 1H), 3.56 (t, *J* = 9.5 Hz, 1H), 3.51 (t, *J* = 9.2 Hz, 1H), 3.46 (dd, *J* = 10.1, 9.2 Hz, 1H), 3.29 (dd, *J* = 9.3, 8.0 Hz, 1H, H-2), 3.05 – 3.01 (m, 2H, -CH_2NH_2), 1.76 – 1.66 (m, 4H, $OCH_2CH_2CH_2CH_2CH_2NH_2$), 1.52 – 1.43 (m, 2H, -$CH_2CH_2CH_2$-). ^{13}C NMR (175 MHz, D_2O) δ 171.04 (HCO_2H), 102.32 (C-1), 99.33 (C-1), 99.18 (2 x C-1), 97.94 (C-1), 79.94, 79.87, 79.48, 76.06, 74.14, 73.13, 72.85, 71.80, 71.68, 71.59, 71.42, 70.33, 70.10, 70.04, 69.85, 69.71, 69.47, 69.28, 65.30, 60.48, 60.27, 60.19, 60.07, 39.35 (CH_2NH_2), 28.17, 26.41, 22.10.; MS ESI+-HRMS m/z $[M+Na]^+$ calcd for $C_{35}H_{64}NO_{26}$ 914.3711, found 914.3719.

5-Amino-pentyl α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranoside AGA-4-08



LC-MS chromatogram of **AGA-4-08**

^{1}H NMR (700 MHz, D_2O) δ 8.37 (s, 1H, *H*CO_2H), 5.28 (d, *J* = 3.8 Hz, 1H, H-1), 4.91 (d, *J* = 3.6 Hz, 1H, H-1), 4.90 (d, *J* = 3.6 Hz, 1H, H-1), 4.89 (d, *J* = 3.5 Hz, 1H, H-1), 4.86 (d, *J* = 3.6 Hz, 1H, H-1), 3.97 – 3.88 (m, 4H), 3.87 – 3.83 (m, 2H), 3.80 – 3.57 (m, 17H), 3.53 – 3.42 (m, 8H), 3.38 (t, *J* = 9.6 Hz, 1H), 2.94 (t, *J* = 7.5 Hz, 2H, -CH_2NH_2), 1.67 – 1.59 (m, 4H, $OCH_2CH_2CH_2CH_2CH_2NH_2$), 1.47 – 1.34 (m, 2H, -$CH_2CH_2CH_2$-). ^{13}C NMR (175 MHz, D_2O) δ 169.99 (HCO_2H), 99.14 (C-1), 98.14 (C-1), 97.78 (2 x C-1), 97.72 (C-1), 79.71, 73.45, 73.37, 72.88, 71.67, 71.62, 71.34, 71.18, 70.14, 70.08, 69.93, 69.57, 69.49, 69.35, 67.90, 65.67, 65.58, 65.30, 60.30, 60.24, 39.37 (CH_2NH_2), 28.03, 26.51, 22.44.; MS ESI+-HRMS m/z $[M+Na]^+$ calcd for $C_{35}H_{64}NO_{26}$ 914.3711, found 914.3686.

5. Streamlined Automated Glycan Assembly Using Glyconeer 2.1®

This chapter has been modified in part from the following publication:

H. S. Hahm, M. K. Schlegel, M. Hurevich, S. Eller, F. Schuhmacher, J. Hoffmann, K. Pagel, and P. H. Seeberger, Automated Glycan Assembly Using the Glyconeer 2.1 Synthesizer. *Proc. Natl. Acad. Sci. USA* **2017**, *114 (17)*, E3385 – E3389. Mr Hahm, Dr. Schlegel, and Dr. Hurevish equally contributed.

https://doi.org/10.1073/pnas.1700141114

5.1 Introduction

Progress in genomics and proteomics was enabled by automated synthesis[153,154] and sequencing methods[155,156] for oligopeptides and oligonucleotides. In contrast, the structure-function relationships of carbohydrates, the most abundant biopolymers on earth, are less well understood than those of nucleic acids and proteins. Because the isolation of specific glycans is difficult and cannot rely on amplification methods, chemical and enzymatic syntheses, or combinations, have served to procure these molecules.[157] Efforts to accelerate traditional chemical syntheses, which is especially labor intensive for glycans, has focused mainly on automated glycan assembly (AGA) on solid support and programmable one-pot synthesis methods.[158,159]

The advantages of solid-phase synthesis are well appreciated since the invention of peptide assembly by Merrifield[160] and oligonucleotide synthesis on polymer supports.[161] Synthetic manipulations are executed by automated instrumentation and reactions can be driven to completion *via* the use of excess reagents that are simply removed by washing steps. The cleavage of the proper linker and purification protocols as a post-automation step is typically standardized. The keys to a successful automated synthesis are reliable building blocks and resins, selected based on a comprehensive protecting group strategy that encompasses a linker as resin-bound ”protecting group” anchoring the first monomer to the support, high yielding couplings, and reliable instrumentation.

Since introducing AGA in 2001[162] many aspects of the synthetic process have been systematically improved, ranging from strategic considerations to building blocks, linkers, purification methods, and quality control. Synthetic glycans of increasing length and complexity have been assembled by AGA.[158] Quality control of the stereo- and regiochemical composition of synthetic products has been greatly accelerated by employing ion mobility-mass spectrometry (IM-MS).[11] The streamlined AGA process described herein relies on the Glyconeer 2.1® automated oligosaccharide synthesizer utilizing monosaccharide building blocks[164] to rapidly assemble conjugation-ready oligosaccharides (**Figure 5.01,** the process is described in detail in **Experimental Section 5.4**).

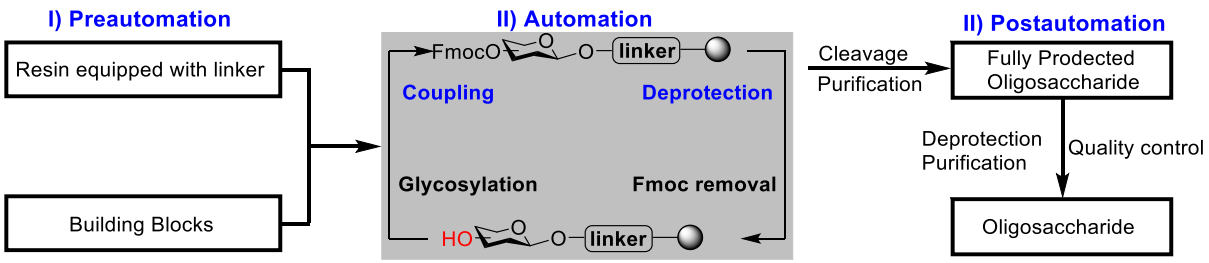


Figure 5.01. Schematic overview of Automated Glycan Assembly.

5.2 Results

5.2.1 Design and Preparation of Automated Oligosaccharide Synthesis Process.

The standardized AGA process is composed of the pre-automation, automation, and post-automation phases (**Figure 5.01**). The pre-automation phase includes preparation of the Glyconeer 2.1® by loading the reaction vessel with a functionalized polystyrene resin and the loading of vials with the appropriate building blocks to the building block unit based on the target oligosaccharide sequence. During the automation phase, the oligosaccharide is assembled as the Glyconeer 2.1® executes computer-controlled reaction cycles. The post-automation phase commences with cleavage of the resin-bound oligosaccharide to release semi-protected products that are analyzed and purified by normal-phase HPLC. Finally, removal of the remaining protecting groups, a final purification step, and confirmation of the structure via NMR and IM-MS produces the target molecules as conjugation-ready oligosaccharides.

The Glyconeer 2.1® synthesizer (see **Experimental Section 5.4**) was designed with flexible hardware and software to accommodate the complexities of glycan assembly, where it is essential that both the stereo- and regiochemistry of the newly formed glycosidic bond must be controlled during each addition to the growing oligosaccharide. The reaction temperatures can be widely varied, sensitive and/or corrosive reagents can be utilized, and coupling cycles can be adjusted as needed. The pressure driven system relies on argon pressure to push the solvents and reagents through valves and tubes that are inert to volatile and corrosive reagents for fast and accurate reagent delivery. Moisture is excluded from the reaction mixture by keeping the entire synthesizer under an argon atmosphere, thereby minimizing decomposition of activated building blocks via hydrolysis. Synthesis-ready building blocks are pre-weighed and either dissolved in the appropriate anhydrous solvent or kept as solids and placed in sealed vials on a 64 position carousel. “Approved” building blocks are those that can be synthesized in large quantities, are stable upon storage for extended periods of time, and upon activation result in very high coupling yields with excellent stereocontrol (Experimental section). Solvents and reagents are placed in various sizes of glass bottles and kept under argon pressure. The Glyconeer 2.1® also offers the flexibility to store bottles containing sensitive reagent mixtures at 4 °C in order to decrease decomposition. A polystyrene-based resin functionalized with an appropriate linker is loaded

into a triple-jacketed glass reaction vessel and can be mixed by a flow of argon from the bottom of the reactor. Building block-specific cycles are then loaded by the operator in the Glyconeer 2.1® software environment to be executed during the synthesis to provide the desired structure (for detailed set-up of the synthesizer see Experimental section).

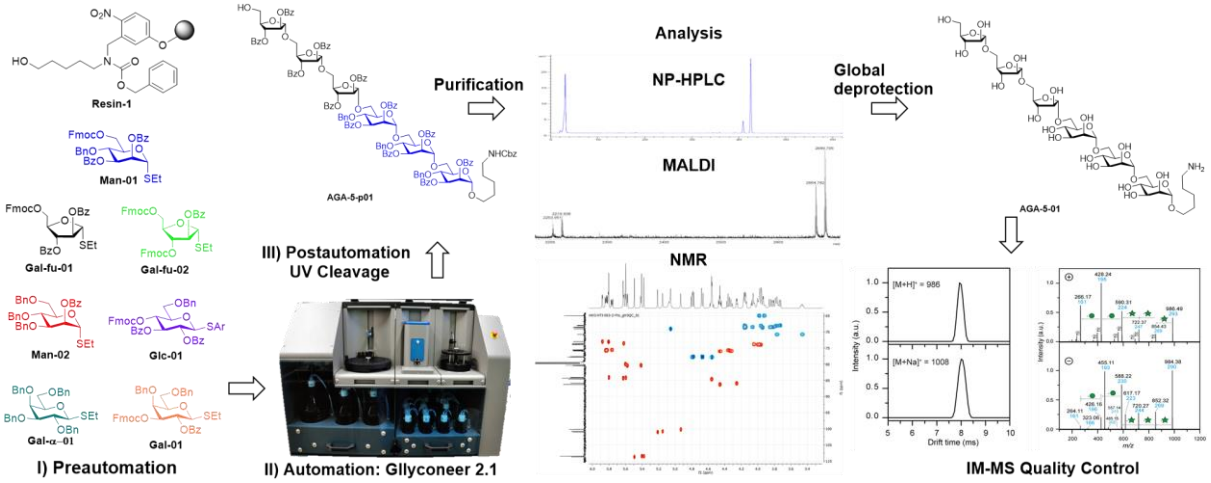


Figure 5.02. Detailed description of the full workflow demonstrated for the synthesis of oligosaccharide **AGA-5-01**. Preautomation phase: preparation of building blocks, resin with linker and the synthesizer. Automation phase: AGA using the Glyconeer 2.1 synthesizer. Postautomation phase: analysis and purification of fully protected oligosaccharide **AGA-5-p01**, procurement of the conjugation-ready oligosaccharide **AGA-5-01**, and quality control using IM-MS.

The completion of one synthetic cycle, that is, the extension of the growing oligosaccharide chain by one unit, relies on a successful glycosylation reaction followed by the removal of a specific temporary protecting group to reveal a nucleophile for upcoming glycosylation in the following cycle using pre-set modules. At the beginning of each glycosylation cycle, the reaction vessel (RV) is washed with anhydrous solvent and trimethylsilyl trifluoromethanesulfonate (TMSOTf) to quench any base that may be left over from previous steps and may interfere with acid-mediated activation. After washing the resin, a fresh portion of anhydrous solvent is added to the reaction vessel while the RV cools to the desired temperature (**Figure 5.03a** and **Figure 5.03c**). After purging the washing solvent from the RV, the building block solution is transferred to the cooled RV and constant argon bubbling is maintained. Adjustment of the RV to the activation temperature is crucial for glycosylation, hence, the activator solution is delivered only after the RV temperature reaches the desired temperature (**Figure 5.03a** and **Figure 5.03c**). Specific to each building block, the temperature is maintained throughout the glycosylation reaction, or may be gradually elevated to drive the glycosylation to completion. After completion of the glycosylation cycle, the reaction mixture is purged from the RV and transferred to the fraction collector where it

may be quenched using an appropriate base. This optional step allows the user to recover any hydrolyzed excess building block for possible regeneration of the pre-activated species. Analysis of the quenched building block solution provides information regarding the activation of the building block and thus very important feedback to unveil unreactive building blocks and/or inefficient coupling conditions.

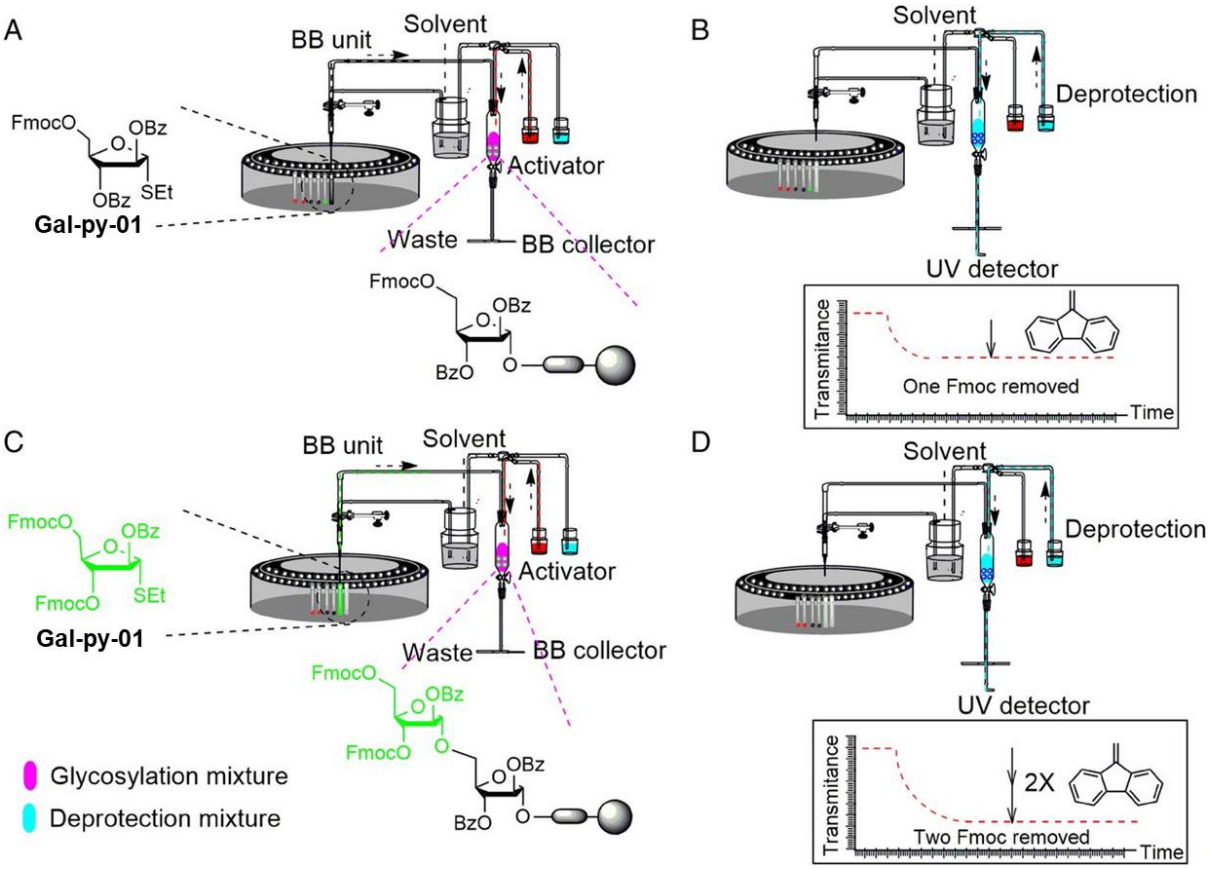


Figure 5.03. Delivery of reagents during glycosylation and removal of temporary protecting group during AGA of arabinomannan hexasaccharide **AGA-5-p01**. (A and C) Glycosylations: transparent tubes with colored bottoms represent building blocks (BB) stored in sealed vials before use, fully colored tubes represent dissolved building blocks, fully gray tubes represent empty vials after building-block delivery, red-colored bottles represents activator, and pink RV represents the activated glycosylation mixture. Below the reactor, the glycosylation product is shown. (B and D) Deprotection: light blue bottle represents the deprotection reagent; blue-colored RV represents the deprotection step. Dibenzofulvene formation as a result of Fmoc cleavage decreases UV transmittance as a quantitative measure of Fmoc release from the resin-bound glycan

Following glycosylation, the resin is washed free of excess reagents using different solvents. Thereafter, the removal of the desired temporary protecting group can be accomplished employing a solution of 20% triethylamine in dimethylformamid for the removal of Fmoc carbonate (**Figure 5.03b** and **Figure 5.03d**). The deprotection solution is subsequently drained from the reaction vessel through a UV-based flow cell to quantitate the

efficiency of the glycosylation step. In this manner, poor couplings can be identified so that the synthesis may be interrupted, thus, avoiding the loss of valuable building blocks.

5.2.2 Automated Synthesis of Selected Oligosaccharide Motifs

The streamlined AGA process is illustrated for the assembly of oligosaccharides representing motifs of the M. tuberculosis cell surface (**AGA-5-01** and **AGA-5-02**)[165] and cancer cells (**AGA-5-03**).[172] The three oligosaccharides constitute various degrees of complexity and each require several different building blocks. Linear hexamer **AGA-5-01** contains multiple 1,2-*trans* linkages, hexasaccharide **AGA-5-02** represents a branched structure with 1,2-*trans* linkages, while trisaccharide **AGA-5-03** contains both 1,2-*trans* and 1,2-*cis* linkages. The Glyconeer 2.1® instrument was loaded with a polystyrene resin functionalized with a photolabile linker (**Resin-1**)[164,173] and from a set of "approved" monosaccharide building blocks **Man-01, Gal-fu-01, Gal-fu-02, Man-02, Glc-01, Gal-01, and Gal-α-01** (**Figure 5.02**). Linear arabinomannan hexasaccharide **AGA-5-01** was assembled using thioglycoside building blocks **Man-01** and **Gal-fu-01**. Activation of these thioglycosides was initiated by the addition of a solution of N-iodosuccinimide (NIS) and trifluoromethanesulfonic acid (TfOH) in a dichloromethane/dioxane mixture (v/v, 9:1) to a suspension of resin and building block. The reaction was kept at an initial temperature of -40 °C for 5 minutes before the mixture was allowed to gradually warm up to -10 °C over 25 minutes. Each glycosylation reaction was performed twice using five equivalents of building block for each reaction. After removing the glycosylation mixture from the RV and washing the support, Fmoc removal was accomplished by treatment with triethylamine (20% in dimethylformamid). These glycosylation and deprotection cycles were repeated six times to assemble resin-bound hexasaccharide **AGA-5-p01** (**Figure 5.02**, for detailed modules and sequence program please see **Experimental Section**).

Following automated assembly, the products were cleaved from the resin by UV irradiation in a continuous-flow reactor[167,168] to afford the crude product mixture. Fully protected hexasaccharide **AGA-5-p01** (25.3 mg, 9.47 µmol, 38% overall yield based on resin loading, calc. 93% per coupling step: twelve on-resin steps) was obtained after preparative normal-phase HPLC (NP-HPLC) purification and was characterized by NMR and mass spectrometry (see **Experimental Section**).

Branched hexasaccharide **AGA-5-02** and linear trisaccharide **AGA-5-03** (**Figure 5.04**) were assembled using building blocks **Gal-fu-01, Gal-fu-02, Man-02** and **Glc-01, Gal-01, Gal-α-01**, respectively (for detailed modules and sequence program see **Experimental**

Section). Fully protected branched hexasaccharide **AGA-5-p02** (15.1 mg; 5.88 µmol; 24% overall yield based on resin loading, 83% per step: eighteen steps) and trisaccharide **AGA-5-p03** (10.4 mg; 6.24 µmol; 25% overall yield based on resin loading, 80% per step: six steps) were obtained in pure form following preparative NP-HPLC to remove both deletion sequences and undesired stereoisomers (for details see **Experimental Section**).

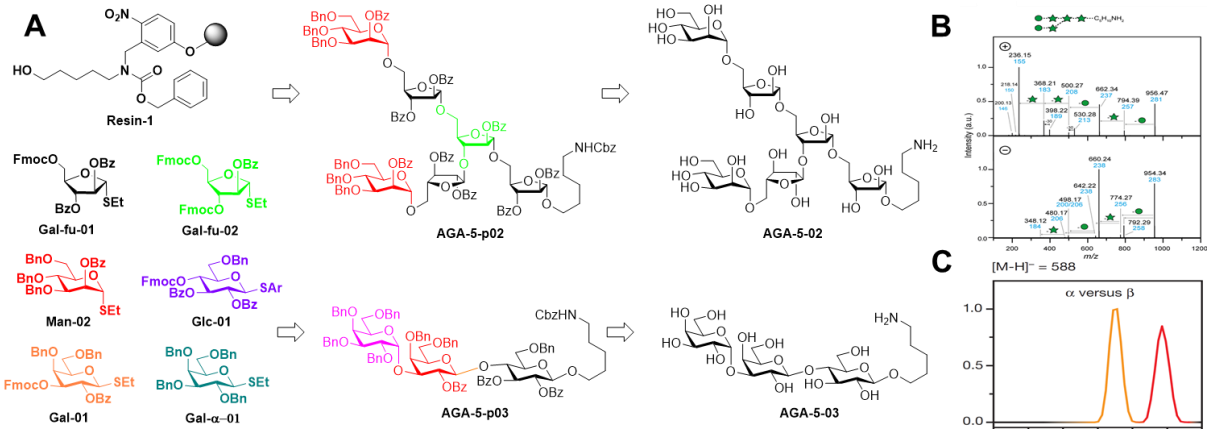


Figure 5.04. A. Protected oligosaccharides **AGA-5-p02** and **AGA-5-p03** are synthesized by selecting from a set of building blocks. AGA protocols and postautomation steps are performed to yield the conjugation-ready oligosaccharides **AGA-5-02** and **AGA-5-03**. MS/MS spectra of **AGA-5-02** in posivite and negative ion mode. **B.** Due to the branced structure of the molecule, two possible trisaccharide fragments can occour during collision-induced dissociation. **C**. IM–MS drift-time distributions of isomeric mixtures show baseline separation between stereoisomers. IM-MS experiment was performed by Dr. J. Hoffmann.

All remaining protecting groups were removed from oligosaccharides **AGA-5-p01**-**AGA-5-p03** via methanolysis using sodium methoxide and subsequent hydrogenolysis using palladium on carbon and hydrogen gas to remove benzoyl ester (Bz) and benzyl ether (Bn) protecting groups, respectively. Purification by semi-preparative HPLC using a Hypercarb® column furnished conjugation-ready linear hexasaccharide **AGA-5-01** (5.3 mg; 5.37 µmol; 56% over two steps), branched hexasaccharide **AGA-5-02** (3.1 mg; 3.24 µmol; 55% over two steps), and trisaccharide **AGA-5-03** (1.7 mg; 2.88 µmol; 46% over two steps). (NMR was used to confirm the identity and stereochemistry of the glycan products while IM-MS-based quality control of oligosaccharides **AGA-5-01**- **AGA-5-03** revealed purities above 99% (see Experimental Section).

5.2.3 Iterative Analysis and Feedback as Intergral Part of Automated Oligosaccharide Synthesis

Direct feedback during organic synthesis in solution is straightforward. Spectral, chromatographic, and other analytical methods have been developed to provide information that is used to estimate the reaction progress and selectivity of each step. While developing streamlined AGA, a systematic approach to optimize building blocks and reaction conditions for reproducible glycan synthesis evolved (**Figure 5.05**). This rational approach is based upon an ability to detect the steps that require optimization. This leads to appropriate modifications of building blocks, the automated cycle conditions, or both. The protocol and decision tree (**Figure 5.05**) aims to provide maximum information for each step of the synthesis to establish a reproducible and optimized AGA protocol. During automated solid-phase synthesis, UV quantification after the Fmoc deprotection step provides crucial feedback regarding an estimation of glycosylation yields based on the release of temporary protecting groups. A sharp increase in UV transmission indicates failed glycosylation or deprotection events. The instrument stops the assembly process following UV feedback that falls below a threshold set by the operator to conserve valuable building blocks, as is common during peptide syntheses. To obtain feedback concerning stereo- or regioselectivity of coupling reactions, a more detailed analysis following completion of the automated assembly process is performed. Crude, semiprotected glycan products, which have been cleaved from the resin, are analyzed by HPLC, MS, and NMR. The HPLC analysis assesses the amount of product compared with shorter oligosaccharide byproducts. The stereoselectivity of the reactions relies on an NMR analysis of the isolated compound(s) and together with the UV feedback and the HPLC analyses identifies any steps that may require further optimization. The final stage of the process removes remaining protecting groups from the oligosaccharides. MS analysis is then used to confirm the removal of these groups and NMR analysis is used to confirm the structure of the conjugation-ready oligosaccharide. For a more sensitive quality control, IM-MS is used to differentiate between oligosaccharide stereoisomers down to a level of 0.2% and to sequence the oligosaccharide (see **Experimental Section**).[170] The overall success of the synthesis provides feedback on the optimization process such that an AGA strategy based on approved building blocks, improved automated protocols, and standardized purification steps evolved.

Even though the AGA method has advanced to the point of commercialization, further improvements will accelerate and improve the process to procure glycans even quicker and cheaper. The insertion of a capping step that is common during oligonucleotide assembly will ensure that deletion sequences cannot accumulate and simplifies purification. Further streamlining and optimization of all washing steps, the cooling of the reaction vessel,

and all other manipulations will accelerate the syntheses and reduce the amount of building blocks and solvents used. The application of the instrument to procure ever more diverse glycans will be aided by the number of building blocks that are now becoming commercially available. Importantly, increasing adaptation of the technology will generate the demand for diverse structures and more building blocks. Increasing demand is expected to lower the cost per molecule due to the economy of scale, as was the case for oligonucleotide and peptide syntheses.

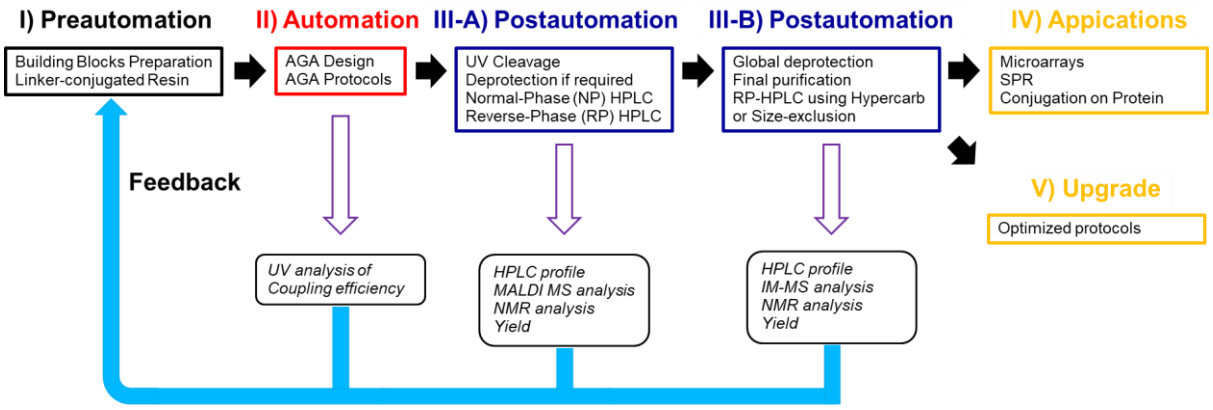


Figure 5.05. Flowchart of the standardized AGA process including purification and process feedback. Full forward arrows reflect the AGA process, synthesis (black solid) and analysis (purple), while solid skyblue backward arrows represent the various feedback levels. (I) Preautomated step is designed with standard building blocks, linker, and module system cycles (black box). (II) Automated synthesis with UV analysis provides the first-level feedback that reflects mostly on the success of glycosylation conversions (red box). (III-A) Following cleavage and partial deprotection, HPLC, MS, and NMR analysis of the crude products provides a second-level feedback reflecting on the conversion success and on the stereo- and regioselectivity (blue box). (III-B) After global deprotection HPLC, IM-MS, and NMR analysis provides third-level feedback that reflects on the overall process including isolated yields (blue box). (IV) After quality control of obtained pure glycans, various utilities will be able to be applied (orange box). (V) Using the combination of the three levels of feedback, an optimized protocol is produced in minimum effort to allow for the reproducible synthesis of a single-entity oligosaccharide (orange box).

5.3 Conclusions

The streamlined automated glycan assembly using a commercially available instrument (Glyconeer 2.1), resin with linkers, and a set of approved commercially available building blocks enables the synthesis of variety of oligosaccharides. The analysis and feedback process including quality control using NMR and IM-MS guarantee the purity of the ready-to-use oligosaccharides. The process described here enables access to large collections of tailor-made glycans for biological, biochemical, vaccinology, and materials research as well as many other applications.

5.4 Experimental Section

Synthesizer Set-up

Solvent preparation and assembly:

All solvents are purified in a Cycle-Tainer Solvent Delivery System or, alternatively, purchased in a safe/sure-seal bottles. Each solvent is transferred to the appropriate narrow-mouth glass bottle on the synthesizer's solvent drawer as fast as possible, fitted with two-way sealed cap and immediately pressurized with argon. The solvents are kept under a constant inert atmosphere.

Building Block preparation (125 umol/vial):

Option 1) building blocks are co-evaporated three times with toluene in a RB flask and kept under vacuum overnight. The dried BB powder is transferred into the designated synthesizer's vials and are further dried by placing the vials under vacuum overnight after which the vials are purged with argon stream and closed immediately with a designated Septum sealed cap. Option 2) building blocks are placed directly into the designated synthesizer's vials and co-evaporated three times with toluene before being placed under vacuum for overnight drying. The vials are purged with argon stream and closed immediately with a designated Septum sealed cap. In both cases the vials were placed in the BB carousel according to the specific oligosaccharide sequence.

Reagent preparation:

Reagent Bottle Preparation: In all cases, 250 mL amber glass narrow-mouth graduated laboratory bottles are used. The bottles are washed thoroughly and dried overnight in an oven before use. Shortly before filling the reagent solutions, the bottles are taken out of the oven and flushed with argon while cooling down to room temperature.

Acidic Washing Solution: A pre-dried reagent bottle is filled with anhydrous DCM (40 mL) under argon atmosphere and added trimethylsilyl trifluoromethanesulfonate (TMSOTf; 1.0 mL).

The prepared reagent bottle is placed in the predetermined acid-wash position in the reagent drawer, fitted with a two-way sealed cap, pressurized and stored under argon atmosphere.

NIS Activation Solution: Into a pre-dried reagent bottle recrystallized *N*-iodosuccinimide (NIS, 1.24 g) is added and filled with a mixture of anhydrous DCM and anhydrous dioxane (v/v 9/1, 40.0 mL) under an argon atmosphere. Triflic acid (TfOH) (50 μL) is added to the NIS solution at 0 °C. The bottle is placed in the cooling block on the reagent drawer, fitted with a two-way seal cap, pressurized and stored under argon atmosphere.

Triethylamine Solution: A pre-dried reagent bottle is filled with DMF (80 mL) and triethylamine (TEA, 20 mL) is added. This bottle is placed on the Fmoc deprotection bottle position, fitted with a two-way seal cap, pressurized and stored under argon atmosphere.

Automated synthesis working modules

The timing and quantity of solvents/reagents transferred to the reaction vessel in each step is controlled by the software. The delivery system is based on valve-pressured control in which the entire platform is constantly pressurized so that the specific solvent/reagent is transferred by timing the opening and closing of the appropriate valves.

Reaction Modules

Module 1: Acidic Washing: The resin is washed with DMF, THF, DCM (six times with 2 mL for 25 s). The resin is swollen in 2 mL DCM, and the temperature of the reaction vessel was adjusted to –20 °C. For acidic washing 0.5 mL of the solution of TMSOTf is delivered to the reaction vessel. After one minute, the solution is drained. Finally, 2 mL DCM is added to the reaction vessel.

Module 2: Glycosylation: Thioglycoside building block is dissolved in the proper solvent mixture (2 mL for two glycosylation cycles) in the designated building block vial on the carousel. The reaction vessel is set to reach the initial glycosylation temperature. During the adjustment of the temperature, the DCM in the reaction vessel is drained and half the solution of thioglycoside building block (5.0 eq. in 1.0 mL DCM) is delivered (slowly) from the building block vial to the reaction vessel. After the set temperature of -40 °C is reached, 1.0 mL NIS (5.5 eq. in 1.0 mL) and TfOH (0.2 eq. in 1.0 mL) solution in DCM and dioxane (v/v, 9:1) is delivered (slowly) to the

reaction vessel. The glycosylation mixture is incubated for 5 min at -40 °C, linearly ramped to -10 °C, and after reaching -10 °C the reaction mixture as incubated for additional 25 min. Once incubation time is finished, the reaction mixture is drained and the resin is washed with DCM (six times with 2 mL for 15 s). This procedure is repeated twice.

Module 3: Fmoc Deprotection: The resin is washed with DMF (six times with 2 mL for 25 s), swollen in 2 mL DMF and the temperature of the reaction vessel is adjusted to 25 °C. For Fmoc deprotection the DMF is drained and 2 mL of a solution of 20% triethylamine in DMF was delivered to the reaction vessel. After 5 min the reaction solution is collected in the fraction collector of the oligosaccharide synthesizer and 2 mL of a solution of 20% triethylamine in DMF is delivered to the resin. This procedure is repeated three times.

Post-automation protocols, purification and analysis

Cleavage from resin

Photoreactor set-up: A medium pressure mercury lamp (450 W) was placed in double jacked equipped with circulation water. A Pyrex filter rapped with or 1/8'' (inner diameter 0.06 inch) fluorinated-ethylene-propylene (FEP) tubing was placed around the double jacked apparatus. The entire setting was placed in a sealed aluminum box equipped with entry and exit points for the FEP tubing.

Oligosaccharide cleavage from solid support: The mercury lamp is turned on 30 min prior to the first cleavage event. The FEP tubing is washed with 20 mL DCM at a flow rate of 5 mL/min before cleavage. The solid support after AGA is pre-swelled in the dark in DCM for 30 min before being taken up with a 20 mL disposable syringe. The suspension of solid support in DCM is slowly injected from the disposable syringe (20 mL) into the FEP tubing using a syringe pump. The suspension is pushed through the FEP tubing into the photoreactor with additional 18 mL DCM (flow rate: 600 μL/min). The photocleavage takes place inside the reactor while solid support travels toward the exit point of the reactor. The suspension leaving the reactor is directed into a syringe equipped with polyethylene filter frit where the resin is filtered off and the solution containing the cleaved oligosaccharide is collected in a separate glass vial. The tubing is washed with 20 mL DCM (flow rate: 2 mL/min) until any remaining resin exits the reactor and the remaining oligosaccharide solution is collected. The tubing is re-equilibrated with 20 mL DCM using a flow rate of 5 mL/min and the entire cleavage procedure is repeated. The combined

solution that was collected in the photocleavage process is evaporated *in vacuo* and the crude material was analyzed by MALDI-TOF, NMR and HPLC.

Purification and characterization of fully protected oligosaccharides AGA-5-p01 - -p03

Analytical HPLC: The crude material was analyzed by normal-phase (NP) HPLC: Column: Luna 5μm Silica 100Å, (260 x 4.60 mm); flow rate: 1 mL/min; eluents: 5% DCM in hexane/5% DCM in ethyl acetate; gradient: 20% (5 min), 60% (in 40 min), 100% (in 5 min); detection: 280 nm, and ELSD.

Preparative HPLC: The crude mixture was dissolved in a minimum volume of DCM and 0.9 mL of 20% hexane in ethyl acetate. The crude solution was purified using preparative NP-HPLC. Column: Luna 5μm Silica (260 x 10 mm); flow rate: 5 mL/min; eluents: 5% DCM in hexane/5% DCM in ethyl acetate; gradient: 20% (5 min), 60% (in 40 min), 100% (in 5 min); detection: 280 nm, and ELSD) to afford the fully protected target oligosaccharide. The HPLC Chromatograms of oligosaccharides **AGA-5-p01**, **AGA-5-p02** and **AGA-5-p03** are presented in each synthesis result.

Final deprotection and purification protocols, compounds characterization, analysis and quality control.

Deprotection protocol

To the solution of the purified fully protected oligosaccharide (**AGA-5-p01**, **AGA-5-p02** or **AGA-5-p03**) in methanol (0.2 mL/μmol of oligosaccharide) was added 58 μL of 0.5 M NaOMe solution (0.25 eq. per acetyl of benzoyl group) in methanol at 40 °C. The mixture was stirred until complete conversion, neutralized by the addition of 200 mg of Amberite (400 mg per 100 μL of NaOMe solution). After filtration, the filtrate was dissolved in methanol, ethyl acetate, and acetic acid (v/v/v =5:0.5:0.2) followed by adding 5% palladium on carbon (Pd/C) (50% w/w = Pd/oligosaccharide), purged first with argon and then with hydrogen, and stirred overnight at room temperature under balloon pressure. The reaction mixture was filtered through modified cellulose filter, washed with 20 mL water/methanol (9:1) and the combined solution was evaporated *in vacuo* to provide the crude product.

Purification and characterization of oligosaccharides AGA-5-01, -02 and -03

Analytical HPLC: The crude material was analyzed by HPLC: column: Hypercarb®, (150 x 4.60 mm); flow rate: 0.8 mL/min; eluents: 0.1% formic acid (FA) in acetonitrile/0.1% FA in water; gradient: 0% (10 min), 30% (in 30 min), 100% (in 5 min); detection: ELSD.

Preparative HPLC: The crude solution is purified to afford the unprotected oligosaccharide by preparative HPLC: column: Hypercarb®, (150 X 10.00 mm); flow rate: 3.6 mL/min; eluents: gradient: 0.1% FA in acetonitrile/0.1% FA in water; gradient: 0% (10 min), 30% (in 30 min), 100% (in 5 min); detection: ELSD. HPLC Chromatograms of oligosaccharides **AGA-5-01**, **AGA-5-02** and **AGA-5-03** are presented in each synthesis result.

Quality control and sequence analysis of oligosaccharides AGA-5-01, -02 and -03

Ionmobility-mass spectrometry (IM-MS) was used to analyze the stereo-purity of the resulting oligosaccharides. In addition, MS/MS prior to the ion mobility separation was used to sequence the oligosaccharides. All analyses were performed on a Synapt G2-S HDMS (Waters Corporation, Manchester).[9,10] Samples were dissolved in water/methanol (1:1, v/v) and ionized using a nano-electrospray source (nESI) from platinum-palladium-coated borosilicate capillaries prepared in-house.

For each measurement, high and low intensity scans were performed. The high intensity scan (an average signal intensity of at least 10^4 counts per second) was used to identify components and impurities of low concentration. Low intensity scans (approximately 10^3 counts per second) are performed to avoid peak broadening caused by detector saturation, which results in narrower peaks for molecules of high concentration. As a result, a better separation, especially of poorly resolved species, can generally be obtained. Molecule **AGA-5-03** was studied previously[3]; IM-MS data of **AGA-5-01** and **AGA-5-02** are shown.

Typical settings for positive ion mode: Source temperature, 25 °C; needle voltage, 1.0 kV; sample cone voltage, 25 V; source offset, 25 V. Ion mobility parameters were: trap gas flow, 2 mL/min; helium cell gas flow, 180 mL/min; IM gas flow, 90 mL/min; drift time trimming, 5 bins; mobility delay after trap release, 0 µs; trap DC entrance, 3 V; trap DC bias, 35 V; trap DC exit, 0 V; IM wave velocity, 800 m/s; IM wave height, 40 V; for MS/MS: trap collision energy, 42 V.

Typical settings for negative ion mode: Source temperature, 25 °C; needle voltage, 0.8 kV; sample cone voltage, 25 V, source offset, 25 V. Ion mobility parameters were: trap gas flow, 2 mL/min; helium cell gas flow, 180 mL/min; IM gas flow, 90 mL/min; mobility delay after trap release, 1000 µs; trap DC entrance, 3 V; trap DC bias, 45 V; trap DC exit 0 V; IM wave velocity, 600 m/s; IM wave height, 40 V; for MS/MS: trap collision energy, 55-70 V.

Collision cross sections (CCSs) can be determined from the drift times of the ions by either using a calibration for travelling wave IM-MS instruments or using the Mason-Schamp equations when drift tube IM-MS instruments are used.[11-13] Here, a Synapt instrument that was modified with a linear drift cell, using a design reported previously[14], was used to measure absolute CCSs in the drift gas nitrogen. Drift times were measured at an IMS gas pressure of 2.2 Torr nitrogen at eight different drift voltages. Details of the experimental procedure can be found elsewhere.[11,15]

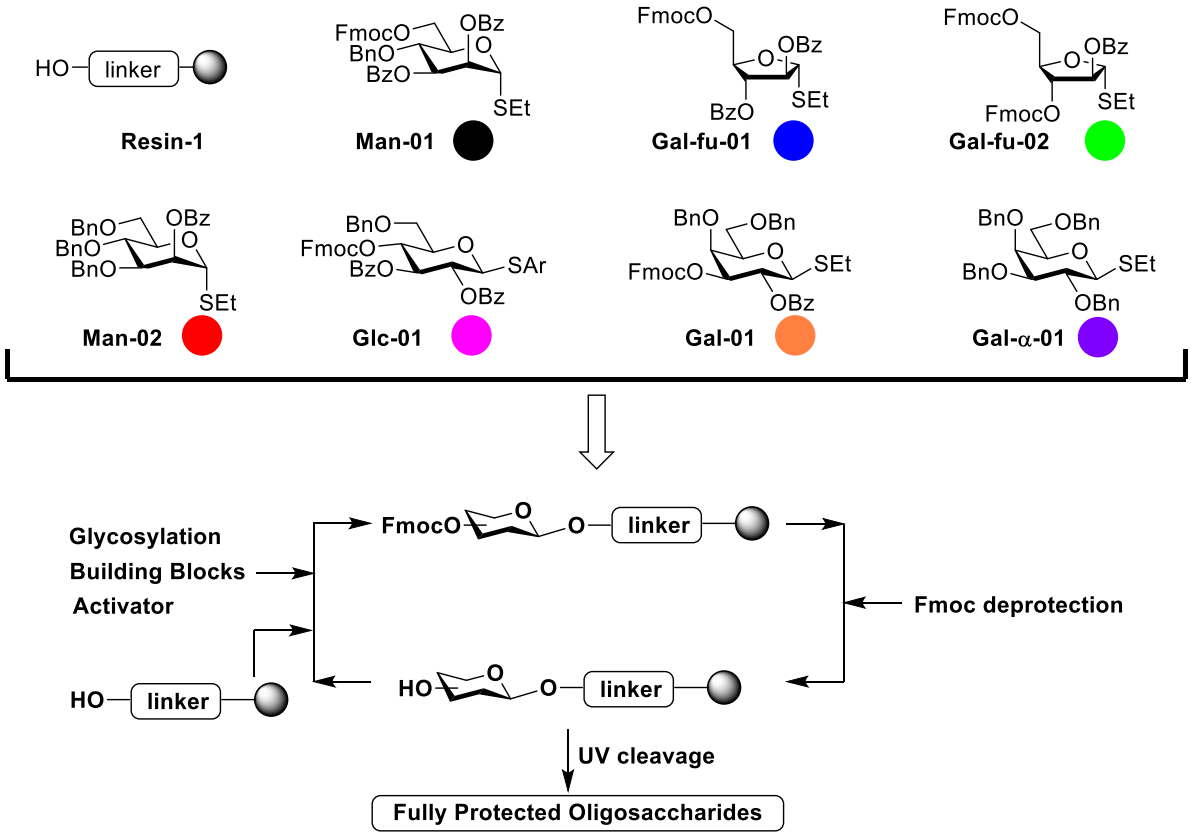
Overview of the main features of Glyconeer 2.1®

Glyconeer 2.1® automated glycan synthesizer with building block storage (a), temperature controlled reaction vessel within a cover to protect from ambient light and moisture (b), fraction collector (c), solvent storage (d) and reagent storage (e).

The Glyconeer 2.1® is a versatile automated platform for the synthesis of glycans on solid support. It features a collection of argon-pressurized bottles for the storage of up to eight solvents (**d**) and 16 reagent bottles of various sizes, two of which can be cooled to -4 °C (**e**). The solvents and reagents are delivered via argon pressure to a triple-jacketed reaction vessel (**b**). The reaction vessel is covered with a jacket that is connected to a computer controlled cryostat that allows to control the temperature in a range that varies from -40 °C to +80 °C. Building blocks can be stored as solids or solutions in the building block carousel for 64

individual vials (**a**). This carousel features a two-way needle to deliver both solvents (from any of the solvents currently attached to the synthesizer) to dissolve solid building blocks as well as deliver the building block solution to the reaction vessel. The contents of the reaction vessel may be mixed, and maintained under an inert argon atmosphere, via bubbling of argon gas through the bottom frit of the vessel. Coupling efficiency can be monitored via a programmable on-line UV detector for the detection of UV transmittance of specific protecting groups release from the resin-bound oligosaccharide. An additional fraction collector allows for the separation of specific reaction mixtures from the main waste stream (**c**). Finally, the Glyconeer 2.1® features user-friendly software allowing the user to program synthetic cycles, add additional "approved" building blocks and their reaction conditions, add and/or adjust reaction modules, and analyze previous runs and results.

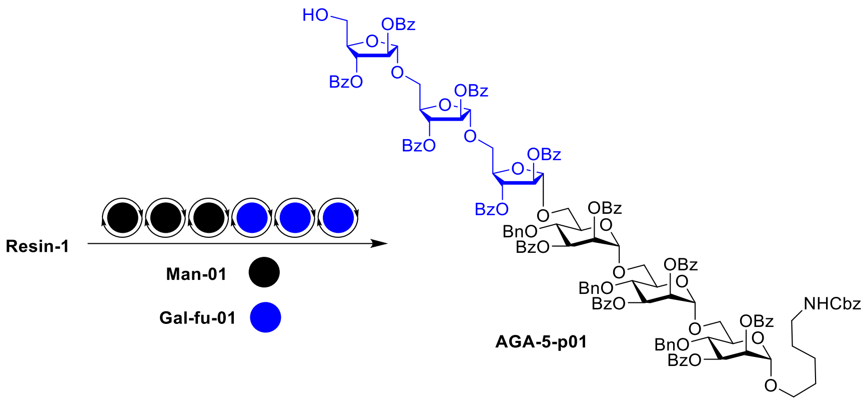
Detailed sequences used for the automated synthesis of oligosaccharides AGA-5-p01, AGA-5-p02 and AGA-5-p03



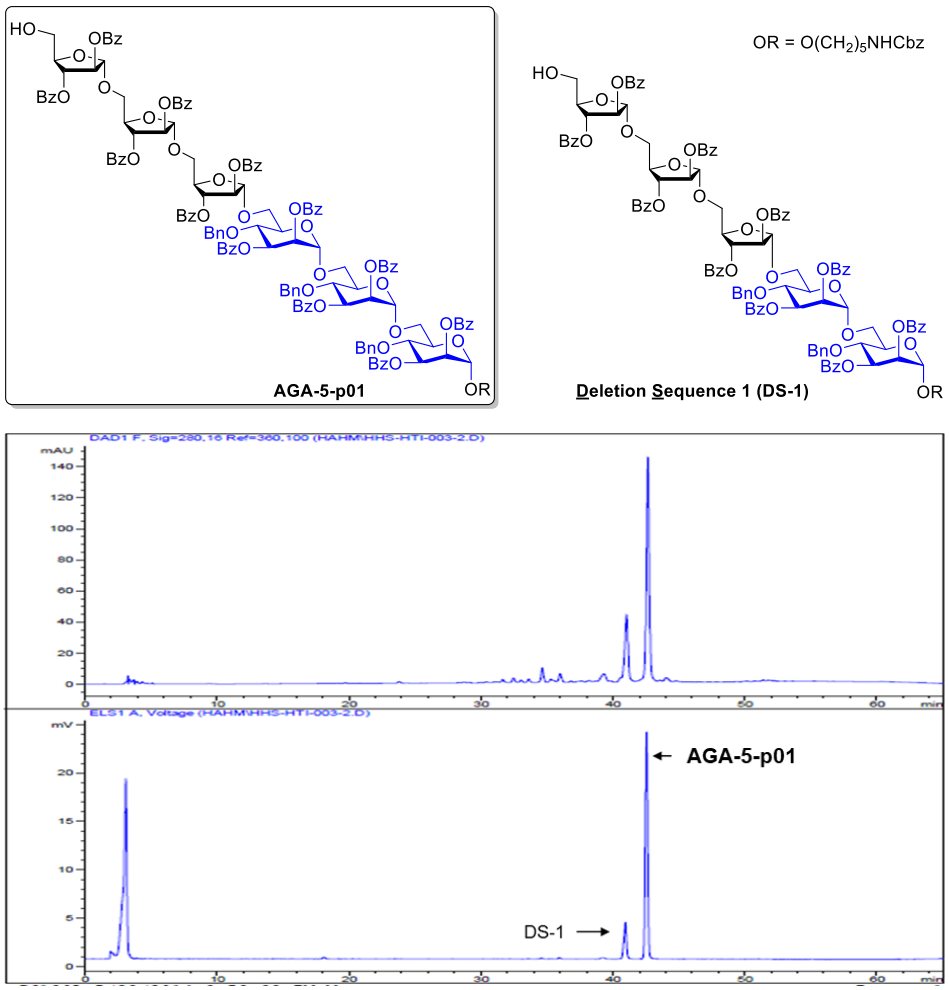
The overall process of the AGA using the Glyconeer 2.1® from the synthesis of oligosaccharides **AGA-5-p01**, **AGA-5-p02** and **AGA-5-p03** is presented. Functionalized resin **Resin-1** (64 mg; loading 0.392 mmol/g; 25.1 µmol) was loaded into the reaction vessel of the synthesizer and

swollen in 2 mL DCM. The sequences described in **Tables** were executed to assemble the target protected oligosaccharides **AGA-5-p01**, **AGA-5-p02** and **AGA-5-p03**, respectively.

***N*-Benzyloxycarbonyl-5-amino-pentyl (2,3-di-*O*-benzoyl-α-D-arabinofuranosyl)-(1→5)-(2,3-di-*O*-benzoyl-α-D-arabinofuranosyl)-(1→5)-(2,3-di-*O*-benzoyl-α-D-arabinofuranosyl)-(1→6)-(2,3-di-*O*-benzoyl-α-D-mannopyranosyl)-(1→6)-(2,3-di-*O*-benzoyl-α-D-mannopyranosyl)-(1→6)-2,3-di-*O*-benzoyl-α-D-mannopyranoside AGA-5-p01 (**25.3 mg; 9.47 μmol; 38%).



Glycosylation sequence	Module	Details	Condition	Repeating cycle
I	1	0.5 mL TMSOTf solution	- 20 °C for 1 min	*once*
	2	5 eq. BB **Man-01**, 5 eq. NIS solution	T_1 = - 40 °C T_2 = - 10 °C	*twice*
	3	20% TEA in DMF	r.t. for 5 min	*twice*
I	1	0.5 mL TMSOTf solution	- 20 °C for 1 min	*once*
	2	5 eq. BB **Man-01**, 5 eq. NIS solution	T_1 = - 40 °C T_2 = - 10 °C	*twice*
	3	20% TEA in DMF	r.t. for 5 min	*twice*
I	1	0.5 mL TMSOTf solution	- 20 °C for 1 min	*once*
	2	5 eq. BB **Man-01**, 5 eq. NIS solution	T_1 = - 40 °C T_2 = - 10 °C	*twice*
	3	20% TEA in DMF	r.t. for 5 min	*twice*
II	1	0.5 mL TMSOTf solution	- 20 °C for 1 min	*once*
	2	5 eq. BB **Gal-fu-01**, 5 eq. NIS solution	T_1 = - 40 °C T_2 = - 10 °C	*twice*
	3	20% TEA in DMF	r.t. for 5 min	*twice*
II	1	0.5 mL TMSOTf solution	- 20 °C for 1 min	*once*
	2	5 eq. BB **Gal-fu-01**, 5 eq. NIS solution	T_1 = - 40 °C T_2 = - 10 °C	*twice*
	3	20% TEA in DMF	r.t. for 5 min	*twice*
II	1	0.5 mL TMSOTf solution	- 20 °C for 1 min	*once*
	2	5 eq. BB **Gal-fu-01**, 5 eq. NIS solution	T_1 = - 40 °C T_2 = - 10 °C	*twice*
	3	20% TEA in DMF	r.t. for 5 min	*twice*

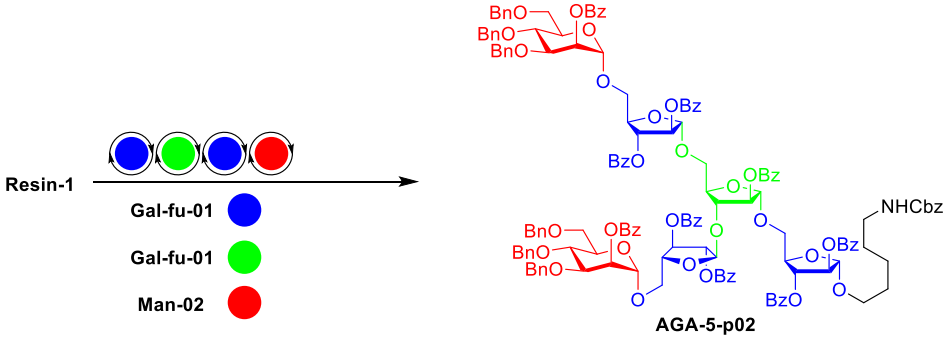


Purification of hexasaccharide **AGA-5-p01** by NP-HPLC.

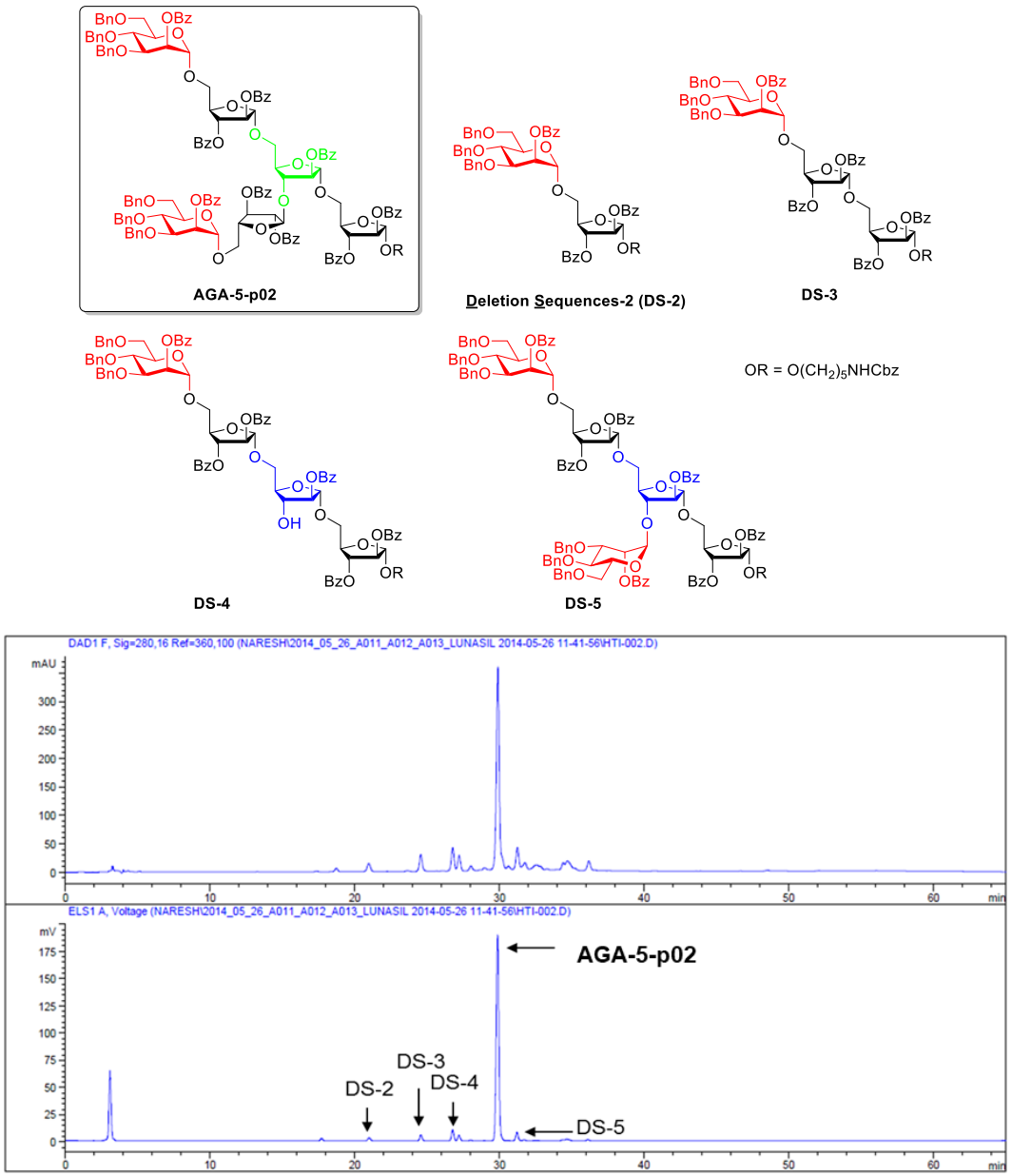
^{1}H NMR (600 MHz, $CDCl_3$) δ 8.15–8.09 (m, 2H), 8.08–8.03 (m, 8H), 8.01 (dd, *J* = 8.3, 1.2 Hz, 2H), 7.95–7.90 (m, 6H), 7.90–7.88 (m, 2H), 7.85 (dd, *J* = 8.3, 1.2 Hz, 2H), 7.81 (dd, *J* = 8.3, 1.2 Hz, 2H), 7.60–7.28 (m, 35H), 7.27–7.20 (m, 4H), 7.21–7.07 (m, 15H), 6.99–6.95 (m, 2H), 5.88 (dd, *J* = 3.1, 1.9 Hz, 1H), 5.86 – 5.80 (m, 4H), 5.77 (dd, *J* = 9.7, 3.4 Hz, 1H), 5.64 – 5.62 (m, 2H), 5.60 (d, *J* = 4.0 Hz, 2H), 5.58 (d, *J* = 4.3 Hz, 1H), 5.50 (s, 1H, **H$_{Araf}$-1**), 5.42 (dd, *J* = 5.8, 2.0 Hz, 2H, **H$_{Araf}$-1**), 5.38 (s, 1H, **H$_{Araf}$-1**), 5.22 (d, *J* = 1.6 Hz, 1H, **H$_{Man}$-1**), 5.16 (s, 1H, **H$_{Man}$-1**), 5.07 (d, *J* = 12.4 Hz, 2H), 4.94 (s, 1H, **H$_{Man}$-1**), 4.79 (dd, *J* = 11.2, 8.9 Hz, 2H), 4.67 (dd, *J* = 11.3, 1.3 Hz, 2H), 4.60 – 4.51 (m, 3H), 4.50 – 4.42 (m, 2H), 4.40 – 4.30 (m, 2H), 4.26 (dd, *J* = 6.7, 4.0 Hz, 1H), 4.19 – 4.12 (m, 3H), 4.07 (dd, *J* = 15.5, 6.4 Hz, 2H), 4.02 – 3.89 (m, 7H), 3.86 (d, *J* = 9.8 Hz, 1H), 3.82 (dd, *J* = 11.2, 2.4 Hz, 1H), 3.77 (t, *J* = 9.5 Hz, 2H), 3.73 (d, *J* = 10.1 Hz, 1H), 3.47 (dd, *J* = 15.5, 6.3 Hz, 1H), 3.19 (dd, *J* = 13.0, 6.6 Hz, 2H), 2.29 (s, 1H), 1.67 – 1.59 (m, 2H), 1.59 – 1.50 (m, 2H), 1.47 – 1.38 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.19 (Bz), 165.85 (Bz), 165.69 (Bz), 165.67 (Bz), 165.60 (Bz), 165.50 (Bz), 165.30 (Bz), 165.24 (Bz), 165.18 (Bz), 156.60 (Cbz), 137.81, 137.00, 133.66,

133.39, 133.31, 133.27, 133.20, 133.15, 130.00, 129.96, 129.87, 129.76, 129.72, 129.66, 129.62, 129.30, 129.25, 129.15, 129.11, 129.08, 128.84, 128.79, 128.71, 128.67, 128.55, 128.52, 128.48, 128.43, 128.37, 128.15, 128.01, 127.92, 127.89, 127.86, 127.80, 127.25 (Ar), 106.24 (**C_{Araf}-1**), 106.01 (2 x **C_{Araf}-1**), 98.54 (**C_{Man}-1**), 98.31 (**C_{Man}-1**), 97.74 (**C_{Man}-1**), 83.77, 83.41, 82.14, 81.83, 81.68, 81.61, 77.96, 77.84, 77.45, 75.30, 75.28, 75.16, 73.49, 73.43, 73.26, 73.15, 73.09, 73.05, 71.45, 71.33, 71.04, 70.55, 70.49, 68.29, 66.53, 66.18, 65.98, 65.57, 65.46, 62.46, 41.16, 29.85, 29.10, 23.70.; MS ESI+-HRMS *m/z* $[M+Na]^+$ calcd for $C_{151}H_{139}NO_{42}Na$ 2660.8664, found 2660.8608.

***N*-Benzyloxycarbonyl-5-amino-pentanyl (2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-α-D-manno-pyranosyl)-(1→5)-(2-*O*-benzoyl-3,5-di-*O*-benzyl-α-D-arabinofuranosyl)-(1→5)-(2-*O*-benzoyl-3-O-[(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→5)-(2-*O*-benzoyl-3,5-di-*O*-benzyl-α-D-arabinofuranosyl-(1→3)]-(α-D-arabinofuranosyl)-(1→5)-2,3-di-*O*-benzoyl-α-D-arabinofuranoside AGA-5-p02** (19.1 mg; 7.40 μmol; 31%)



Glycosylation sequence	Module	Details	Condition	Repeating cycle
II	1	0.5 mL TMSOTf solution	- 20 °C for 1 min	*once*
	2	5 eq. BB **Gal-fu-01**, 5 eq. NIS solution	T_1 = - 40 °C T_2 = - 10 °C	*twice*
	3	20% TEA in DMF	r.t. for 5 min	*twice*
III	1	0.5 mL TMSOTf solution	- 20 °C for 1 min	*once*
	2	5 eq. BB **Gal-fu-02**, 5 eq. NIS solution	T_1 = - 40 °C T_2 = - 10 °C	*twice*
	3	20% TEA in DMF	r.t. for 5 min	*twice*
II	1	0.5 mL TMSOTf solution	- 20 °C for 1 min	*once*
	2	5 eq. BB **Gal-fu-01**, 5 eq. NIS solution	T_1 = - 40 °C T_2 = - 10 °C	*four times*
	3	20% TEA in DMF	r.t. for 5 min	*twice*
IV	1	0.5 mL TMSOTf solution	- 20 °C for 1 min	*once*
	2	5 eq. BB **Man-02**, 5 eq. NIS solution	T_1 = - 40 °C T_2 = - 10 °C	*four times*
	3	20% TEA in DMF	r.t. for 5 min	*twice*

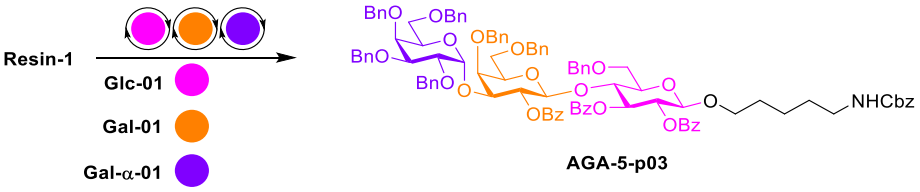


Purification of hexasaccharide **AGA-5-p02**

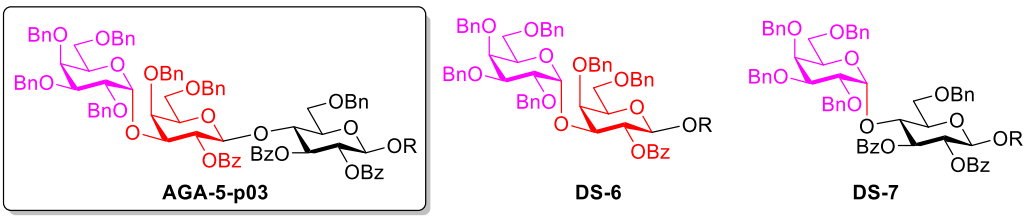
^{1}H NMR (600 MHz, $CDCl_3$) δ 8.04 (d, *J* = 8.0 Hz, 6H), 8.01 (dd, *J* = 7.2, 1.1 Hz, 4H), 7.99–7.96 (m, 4H), 7.95–7.89 (m, 4H), 7.51 (dt, *J* = 16.5, 7.1 Hz, 3H), 7.45–7.23 (m, 39H), 7.23–7.07 (m, 20H), 5.66 (d, *J* = 1.9 Hz, 1H), 5.63 (d, *J* = 1.8 Hz, 1H), 5.59 (d, *J* = 7.8 Hz, 2H, **H$_{Araf}$-1**), 5.52 (d, *J* = 0.9 Hz, 2H), 5.50 (s, 1H), 5.46 (d, *J* = 5.0 Hz, 1H), 5.43 (s, 2H), 5.39 (s, 1H, **H$_{Araf}$-1**), 5.37 (s, 1H, **H$_{Araf}$-1**), 5.18 (s, 1H, **H$_{Araf}$-1**), 5.07 (s, 2H), 5.05 (s, 1H, **H$_{Man}$-1**), 4.99 (s, 1H, **H$_{Man}$-1**), 4.85 (s, 1H), 4.75 (dd, *J* = 16.6, 11.0 Hz, 2H), 4.69 (dd, *J* = 19.5, 12.0 Hz, 2H), 4.54 – 4.36 (m, 11H), 4.15 (dd, *J* = 11.2, 4.7 Hz, 1H), 4.12 – 4.03 (m, 7H), 3.97 – 3.86 (m, 7H), 3.83 (td, *J* = 11.1, 3.3 Hz, 2H), 3.78 – 3.69 (m, 4H), 3.51 – 3.42 (m, 1H), 3.15 (dd, *J* = 12.7, 6.3 Hz, 2H), 1.66 – 1.55 (m, 2H), 1.55 – 1.46 (m, 2H), 1.45 – 1.36 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 165.73 (Bz), 165.68 (Bz), 165.62 (Bz), 165.59 (Bz), 165.57 (Bz),

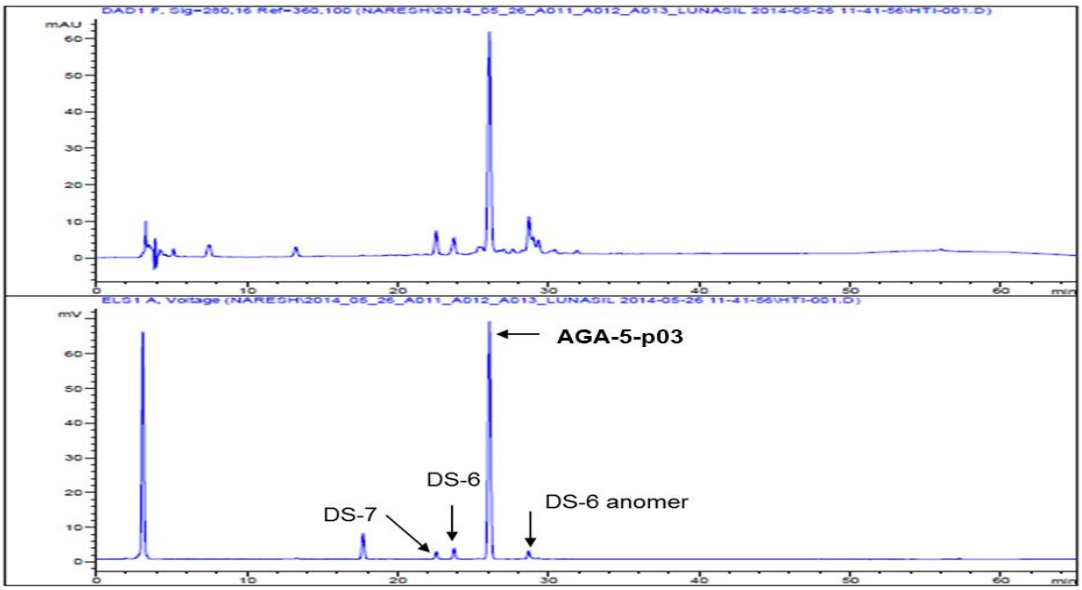
165.53 (Bz), 165.24 (Bz), 165.16 (Bz), 156.51 (Cbz), 138.68, 138.66, 138.18, 136.85, 133.62, 133.57, 133.42, 133.37, 133.16, 133.10, 130.08, 129.95, 129.46, 129.37, 129.29, 129.24, 128.74, 128.67, 128.62, 128.53, 128.49, 128.44, 128.43, 128.40, 128.33, 128.30, 128.24, 128.23, 128.16, 128.08, 128.05, 128.01, 127.57, 127.55, 127.53, 127.48 (Ar), 106.27 (**C_{Araf}-1**), 105.98 (**C_{Araf}-1**), 105.71 (**C_{Araf}-1**), 105.35 (**C_{Araf}-1**), 98.52 (**C_{Man}-1**), 98.47 (**C_{Man}-1**), 82.72, 82.40, 82.34, 82.21, 82.11, 81.89, 81.79, 80.96, 78.81, 78.75, 77.64, 77.37, 75.26, 75.19, 74.27, 74.20, 73.50, 73.45, 71.99, 71.57, 69.05, 68.99, 68.94, 67.42, 66.84, 66.66, 66.13, 65.97, 41.14, 29.80, 29.20, 23.52.; MS ESI+-HRMS *m/z* $[M+Na]^+$ calcd for $C_{150}H_{143}NO_{38}Na$ 2588.9180, found 2588.9158.

***N*-Benzyloxycarbonyl-5-amino-pentanyl (2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-*O*-benzoyl-3,6-di-*O*-benzyl-α-D-galactopyranosyl)-(1→4)-(2,3-di-*O*-benzoyl-6-*O*-benzyl-α-D-glucopyranoside AGA-5-p03** (10.4 mg; 6.00 μmol; 24%).



Glycosylation sequence	Module	Details	Condition	Repeating cycle
V	1	0.5 mL TMSOTf solution	- 20 °C for 1 min	*once*
	2	5 eq. BB **Glc-01**, 5 eq. NIS solution	T_1 = - 40 °C T_2 = - 10 °C	*twice*
	3	20% TEA in DMF	r.t. for 5 min	*twice*
VI	1	0.5 mL TMSOTf solution	- 20 °C for 1 min	*once*
	2	5 eq. BB **Gal-01**, 5 eq. NIS solution	T_1 = - 40 °C T_2 = - 10 °C	*twice*
	3	20% TEA in DMF	r.t. for 5 min	*twice*
VII	1	0.5 mL TMSOTf solution	- 20 °C for 1 min	*once*
	2	5 eq. BB **Gal-α-01**, 5 eq. NIS solution	T_1 = - 40 °C T_2 = - 10 °C	*twice*
	3	20% TEA in DMF	r.t. for 5 min	*twice*

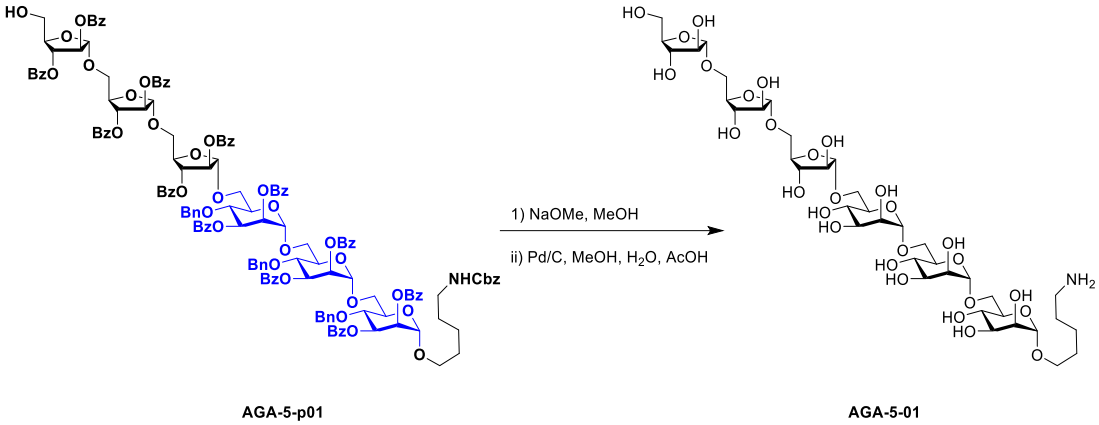


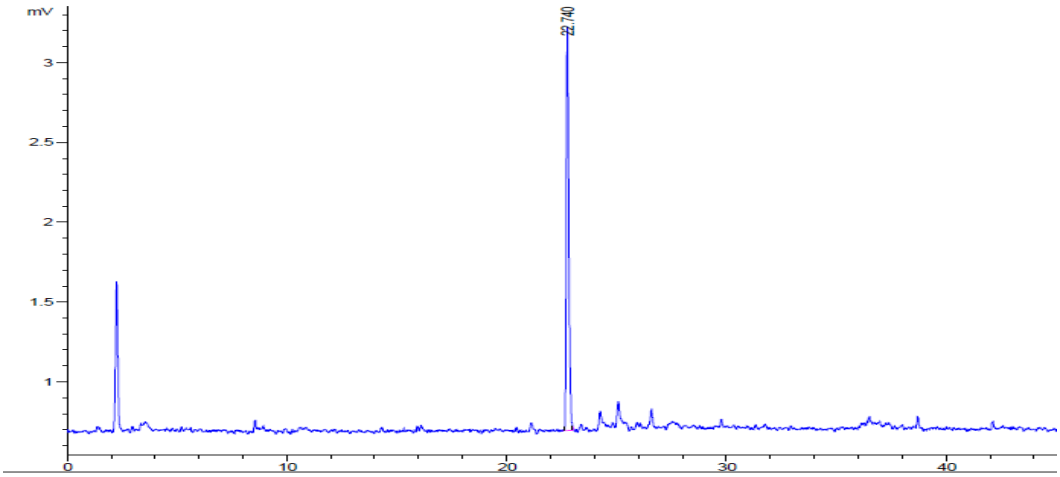


Purification of trisaccharide **AGA-5-p03**.

^{1}H NMR ($CDCl_3$) δ 7.97 – 7.88 (m, 4H), 7.83 (d, *J* = 7.3 Hz, 2H), 7.47 (dd, *J* = 15.3, 7.5 Hz, 2H), 7.40 – 7.06 (m, 47H), 5.55 (m, 2H, H'-2, H-3), 5.31 (br, 1H, H-2), 5.07 (s, 2H, CH_2, Cbz), 4.96 (d, *J* = 11.8 Hz, 1H, CHHPh), 4.90 (d, *J* = 3.3 Hz, 1H, H''-1), 4.78 (dd, *J* = 11.4, 6.0 Hz, 2H, 2 x CHHPh), 4.62–4.42 (m, 6H, H-1, H'-1), 4.37 (d, *J* = 11.3 Hz, 1H, CHHPh), 4.31–4.14 (m, 4H), 4.10-4.00 (m, 3H, H-4, CH_2Ph), 3.97 (dd, *J* = 10.1, 3.3 Hz, 1H, H''-2), 3.90–3.83 (m, 2H, H'-4, H''-5), 3.81 (br, 1H, -OCHH$(CH_2)_4$), 3.73 (dd, *J* = 10.1, 2.5 Hz, 1H, H''-3), 3.67–3.46 (m, 5H, H'-3, H''-4, H-5, H-6), 3.39 (s, 1H, -OCHH$(CH_2)_4$-NHCbz), 3.21 (dd, *J* = 9.9, 7.1 Hz, 2H, H'-5, H''-6), 2.99 (dd, *J* = 8.7, 5.5 Hz, 1H, H''-6), 2.91 (s, 2H, CH_2NHCbz), 2.86 (d, *J* = 6.6 Hz, 2H, H'-6), 1.54–1.37 (m, 2H, CH_2, pentane), 1.34 – 1.24 (m, 2H, CH_2, pentane), 1.18 (s, 2H, CH_2, pentane). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.36 (Bz), 165.29 (Bz), 164.84 (Bz), 156.39 (Cbz), 139.39, 138.85, 138.74, 138.55, 138.41, 138.26, 136.88, 133.15, 133.05, 132.58, 130.50, 130.19, 130.03, 129.92, 129.87, 129.76, 128.62, 128.48, 128.41, 128.23, 128.15, 128.12, 128.07, 127.85, 127.80, 127.75, 127.66, 127.61, 127.54, 127.49, 127.43, 127.05 (Ar), 101.27 (**C-1 or C'-1**), 101.14 (**C-1 or C'-1**), 99.11 (**C''-1**), 80.51, 79.04, 77.36, 76.53, 75.63, 74.95, 74.92, 74.85, 74.63, 74.25, 73.80, 73.60, 73.37, 73.28, 73.12, 72.72, 72.26, 69.82, 69.75, 68.11, 68.06, 67.30, 66.62, 40.95, 29.52, 29.03, 23.19.; MS ESI+-HRMS *m/z* $[M+Na]^+$ calcd for $C_{101}H_{103}NO_{21}Na$ 1688.6920, found 1688.6920.

5-Amino-pentanyl α-D-arabinofuranosyl-(1→5)-α-D-arabinofuranosyl-(1→5)-α-D-arabinofuranosyl-(1→6)-α-D-mannopyranosyl-(1→6)-α-D-mannopyranosyl-(1→6)-α-D-mannopyranoside AGA-5-01 (5.3 mg; 5.37 µmol; 56% over two steps)

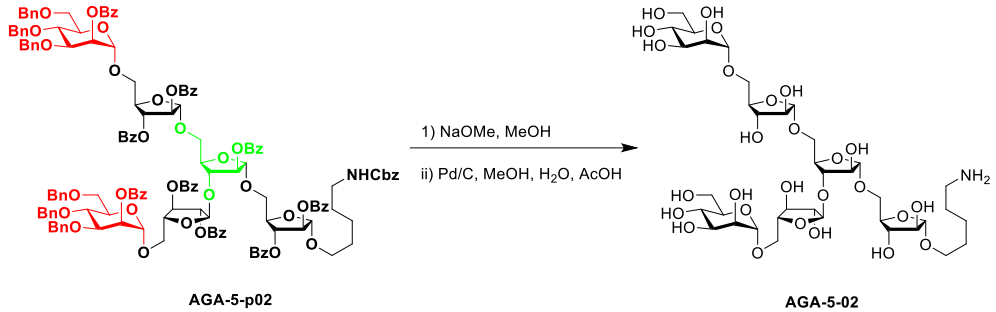


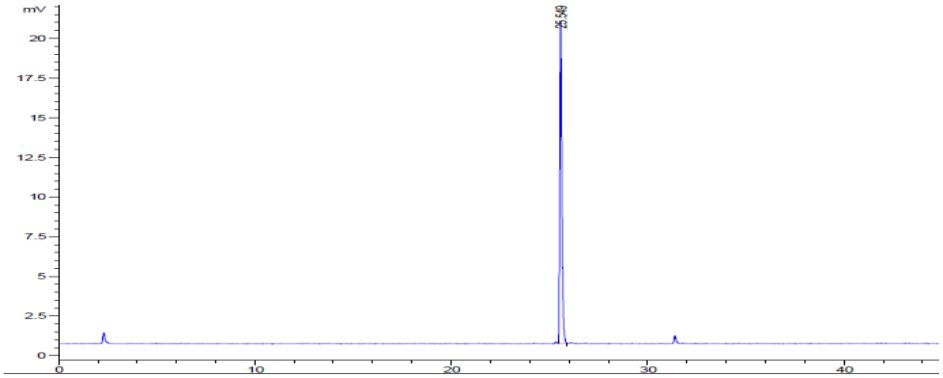


Purification of linear hexasaccharide **AGA-5-01** by Hypercarb HPLC. Column: Hypercarb®, (150 X 10.00 mm); flow rate: 3.6 mL/min; eluents: gradient: 0.1% FA in acetonitrile/0.1% FA in water; gradient: 0% (10 min), 30% (in 30 min), 100% (in 5 min); detection: ELSD.

^{1}H NMR (600 MHz, D_2O) δ 8.50 (s, 1H, HCO_2H), 5.14 (s, 2H, 2 x **H-1**), 5.13 (d, *J* = 1.5 Hz, 1H, **H-1**), 4.95 (d, *J* = 1.6 Hz, 1H, **H-1**), 4.93 (d, *J* = 1.5 Hz, 1H, **H-1**), 4.90 (d, *J* = 1.6 Hz, 1H, **H-1**), 4.26 (dq, *J* = 8.7, 2.9 Hz, 2H), 4.18 (tt, *J* = 15.2, 6.8 Hz, 3H), 4.14 (td, *J* = 5.9, 3.3 Hz, 1H), 4.06 (dt, *J* = 6.0, 2.9 Hz, 2H), 4.03 (dt, *J* = 3.4, 1.8 Hz, 2H), 4.02–3.97 (m, 5H), 3.95–3.74 (m, 19H), 3.61 (dt, *J* = 10.0, 6.2 Hz, 1H), 3.07–3.03 (m, 2H), 1.78–1.65 (m, 4H), 1.58–1.40 (m, 2H). ^{13}C NMR (600 MHz, D_2O) δ 173.62 (HCO_2H), 110.11 (**C-1**), 110.03 (**C-1**), 109.88 (**C-1**), 102.52 (**C-1**), 102.04 (**C-1**), 101.97 (**C-1**), 86.59, 84.96, 84.72, 83.54, 83.47, 83.44, 79.35, 79.30, 79.15, 73.61, 73.57, 73.52, 73.45, 73.35, 73.20, 72.70, 72.62, 72.57, 70.25, 69.49, 69.40, 69.28, 69.22, 69.21, 68.96, 68.32, 68.23, 63.81, 42.02, 30.66, 29.20, 25.16.; MS ESI+-HRMS *m/z* $[M+Na]^+$ calcd for $C_{38}H_{67}NO_{28}Na$ 1008.3742, found 1008.3778.

5-Amino-pentanyl α-D-mannopyranosyl-(1→5)-α-D-arabinofuranosyl-(1→5)-[α-D-mannopyranosyl-(1→5)-α-D-arabinofuranosyl-(1→3)]-(α-D-arabinofuranosyl)-(1→5)-α-D-arabinofuranoside AGA-5-02 (3.1 mg; 3.24 µmol; 55%)

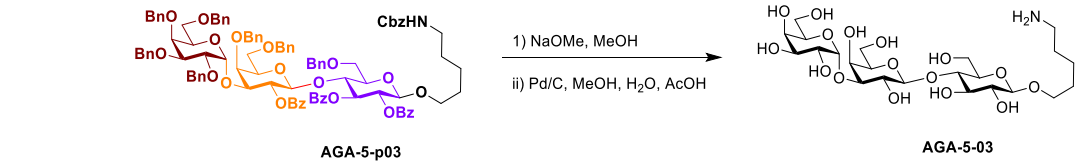


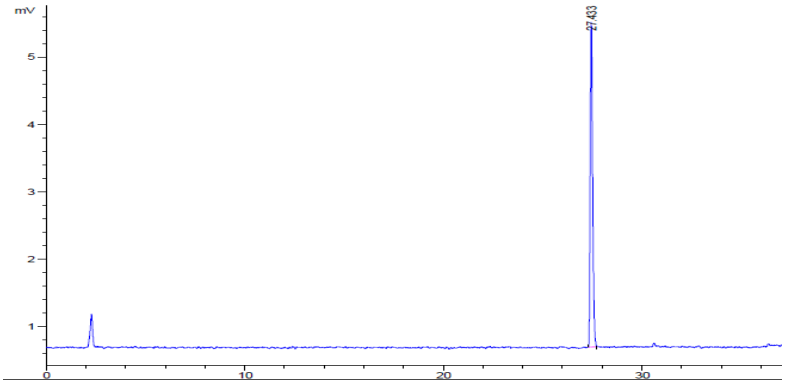


Purification of branched hexasaccharide **AGA-5-02**.

^{1}H NMR (600 MHz, D_2O) δ 8.32 (s, 1H, HCO_2H), 5.02 (d, *J* = 1.3 Hz, 1H**, H-1**), 4.98 (s, 1H**, H-1**), 4.96 (d, *J* = 1.3 Hz, 1H**, H-1**), 4.88 (d, *J* = 2.0 Hz, 1H**, H-1**), 4.79 (d, *J* = 1.7 Hz, 2H**, 2 x H-1**), 4.19–4.13 (m, 2H), 4.08 (td, *J* = 5.5, 3.0 Hz, 1H), 4.02 (ddd, *J* = 9.5, 4.5, 2.3 Hz, 4H), 3.95 (dd, *J* = 5.4, 2.1 Hz, 1H), 3.94–3.88 (m, 4H), 3.87–3.85 (m, 2H), 3.82 (dd, *J* = 11.4, 5.6 Hz, 1H), 3.73 (dddd, *J* = 15.5, 12.5, 9.0, 5.5 Hz, 8H), 3.66 (t, *J* = 4.2 Hz, 1H), 3.63 (ddd, *J* = 13.2, 6.8, 4.1 Hz, 5H), 3.57–3.50 (m, 4H), 3.45 (dt, *J* = 10.0, 6.4 Hz, 1H), 2.90–2.86 (m, 2H), 1.60–1.48 (m, 4H), 1.36–1.27 (m, 2H). ^{13}C NMR (600 MHz, D_2O) δ 173.62 (HCO_2H), 109.98 (**2 x C-1**), 109.83 (**C-1**), 109.72 (**C-1**), 102.42 (**C-1**), 102.39 (**C-1**), 85.07, 84.68, 84.55, 84.29, 84.26, 83.89, 83.61, 83.43, 81.70, 79.14, 79.09, 78.85, 75.53, 75.51, 73.15, 73.10, 72.50, 70.70, 69.30, 69.16, 68.88, 68.64, 68.47, 63.53, 41.97, 30.67, 29.04, 24.86.; MS ESI+-HRMS *m/z* $[M+H]^+$ calcd for $C_{38}H_{68}NO_{28}$ 956.3817, found 956.3834.

5-Amino-pentanyl α-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside AGA-5-03[7] (1.7 mg; 2.88 μmol; 46%)





Purification of trisaccharide **AGA-5-03.**

^{1}H NMR (600 MHz, D_2O) δ 8.48 (s, 1H, HCO_2H), 5.17 (d, *J* = 4.0 Hz, 1H, H, H''-1), 4.55 (d, *J* = 8.0 Hz, 1H, H-1), 4.52 (d, *J* = 8.1 Hz, 1H, H-1), 4.25 – 4.19 (m, 2H), 4.05 (d, *J* = 3.1 Hz, 1H), 4.02 (dd, *J* = 12.3, 1.8 Hz, 1H), 3.99–3.94 (m, 2H), 3.89 (dd, *J* = 10.4, 3.9 Hz, 1H), 3.86–3.65 (m, 12H), 3.64–3.61 (m, 1H), 3.36–3.32 (m, 1H), 2.98 (t, *J* = 7.3 Hz, 2H, $OCH_2(CH_2)_4NH_2$), 1.73–1.66 (m, 4H, $OCH_2CH_2CH_2CH_2CH_2NH_2$), 1.52–1.44 (m, 2H $OCH_2CH_2CH_2CH_2CH_2NH_2$). ^{13}C NMR (600 MHz, D_2O) δ 173.63 (CO_2H), 105.45 (C-1), 104.62(C-1), 98.03(C''-1), 81.28, 79.81, 77.66, 77.35, 77.13, 75.41, 73.44, 72.75, 72.18, 71.89, 71.73, 70.79, 67.41, 63.59, 63.53, 62.77, 42.09 (CH_2NH_2), 30.80, 29.63, 24.73.; MS ESI+-HRMS *m/z* $[M+Na]^+$ calcd for $C_{23}H_{44}NO_{16}Na$ 590.2655, found 590.2644.

Quality control and sequence analysis of oligosaccharides AGA-5-01-AGA-5-03 were performed by Dr. J. Hoffmann.

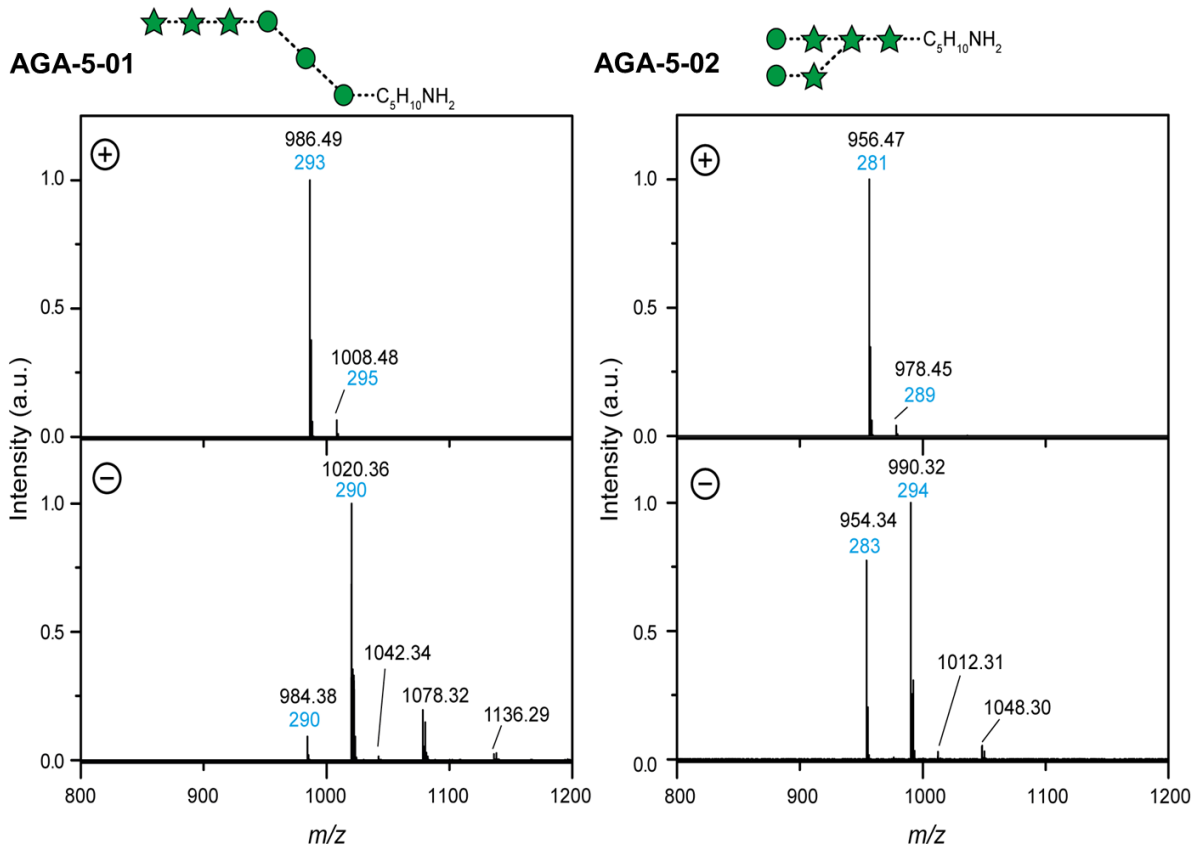
Ionmobility-mass spectrometry (IM-MS) was used to analyze the stereo-purity of the resulting oligosaccharides. In addition, MS/MS prior to the ion mobility separation was used to sequence the oligosaccharides. All analyses were performed on a Synapt G2-S HDMS (Waters Corporation, Manchester).[9,10] Samples were dissolved in water/methanol (1:1, v/v) and ionized using a nano-electrospray source (nESI) from platinum-palladium-coated borosilicate capillaries prepared in-house.

For each measurement, high and low intensity scans were performed. The high intensity scan (an average signal intensity of at least 10^4 counts per second) was used to identify components and impurities of low concentration. Low intensity scans (approximately 10^3 counts per second) are performed to avoid peak broadening caused by detector saturation, which results in narrower peaks for molecules of high concentration. As a result, a better separation, especially of poorly resolved species, can generally be obtained. Molecule **AGA-5-03** was studied previously[3]; IM-MS data of **AGA-5-01** and **AGA-5-02** are shown.

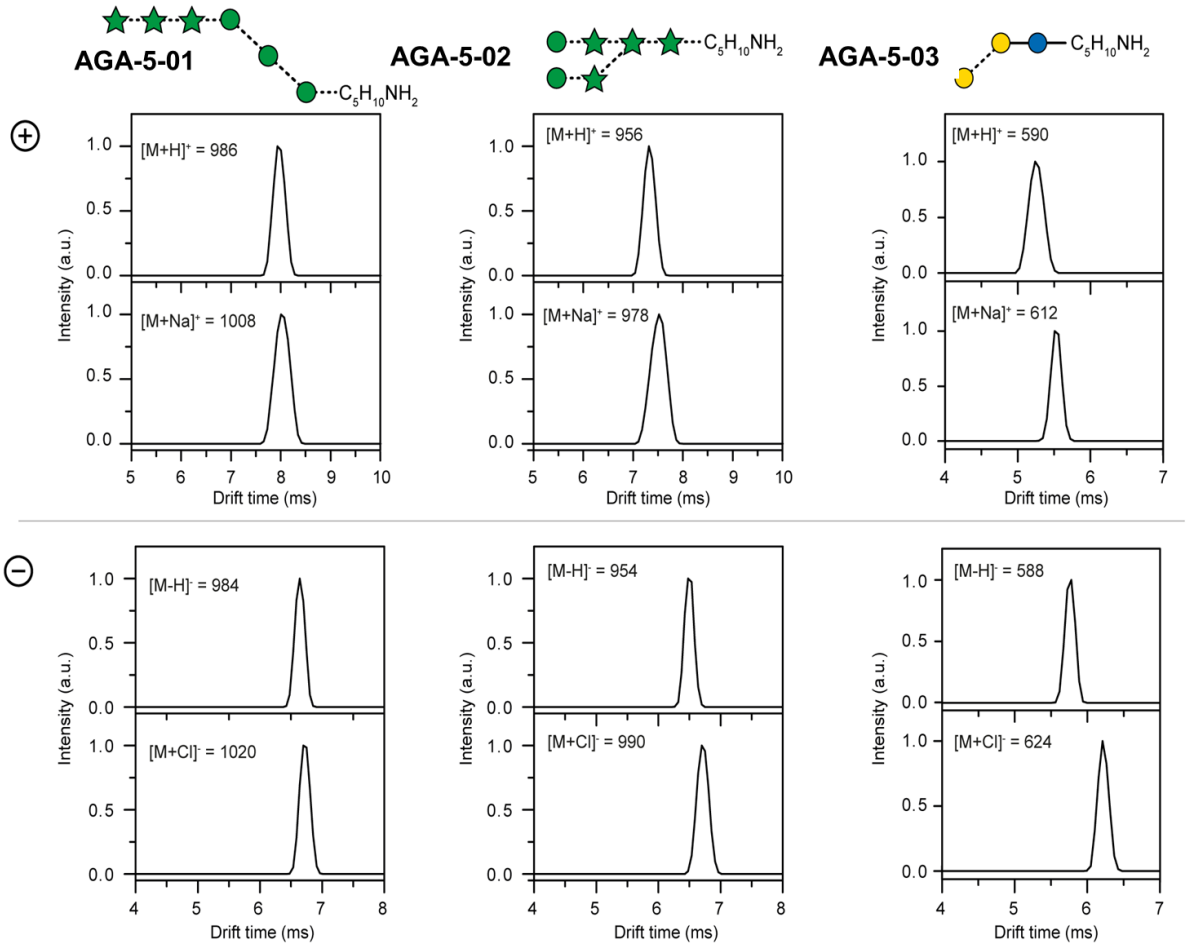
Typical settings for positive ion mode: Source temperature, 25 °C; needle voltage, 1.0 kV; sample cone voltage, 25 V; source offset, 25 V. Ion mobility parameters were: trap gas flow, 2 mL/min; helium cell gas flow, 180 mL/min; IM gas flow, 90 mL/min; drift time trimming, 5 bins; mobility delay after trap release, 0 µs; trap DC entrance, 3 V; trap DC bias, 35 V; trap DC exit, 0 V; IM wave velocity, 800 m/s; IM wave height, 40 V; for MS/MS: trap collision energy, 42 V.

Typical settings for negative ion mode: Source temperature, 25 °C; needle voltage, 0.8 kV; sample cone voltage, 25 V, source offset, 25 V. Ion mobility parameters were: trap gas flow, 2 mL/min; helium cell gas flow, 180 mL/min; IM gas flow, 90 mL/min; mobility delay after trap release, 1000 µs; trap DC entrance, 3 V; trap DC bias, 45 V; trap DC exit 0 V; IM wave velocity, 600 m/s; IM wave height, 40 V; for MS/MS: trap collision energy, 55-70 V.

Collision cross sections (CCSs) can be determined from the drift times of the ions by either using a calibration for travelling wave IM-MS instruments or using the Mason-Schamp equations when drift tube IM-MS instruments are used.[11-13] Here, a Synapt instrument that was modified with a linear drift cell, using a design reported previously[14], was used to measure absolute CCSs in the drift gas nitrogen. Drift times were measured at an IMS gas pressure of 2.2 Torr nitrogen at eight different drift voltages. Details of the experimental procedure can be found elsewhere.[11,15]

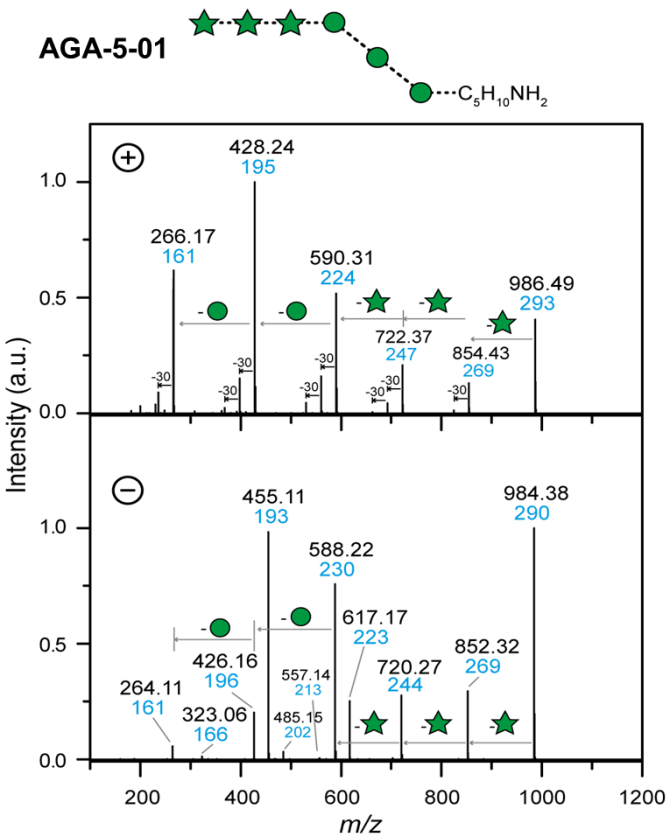


Mass spectra of **AGA-5-01** (Left) and **AGA-5-02** (right) in the positive (top) and negative (bottom) ion mode. *m/z* values are given in black and corresponding absolute nitrogen CCSs in blue. Chemical structures are depicted as cartoons according to the SNFG nomenclature: green circles, mannose; green star, arabinofuranose; horizontal lines, α1,4 bonds; upwards diagonals, α1→6 bonds; downwards diagonals, α1→3 bonds.

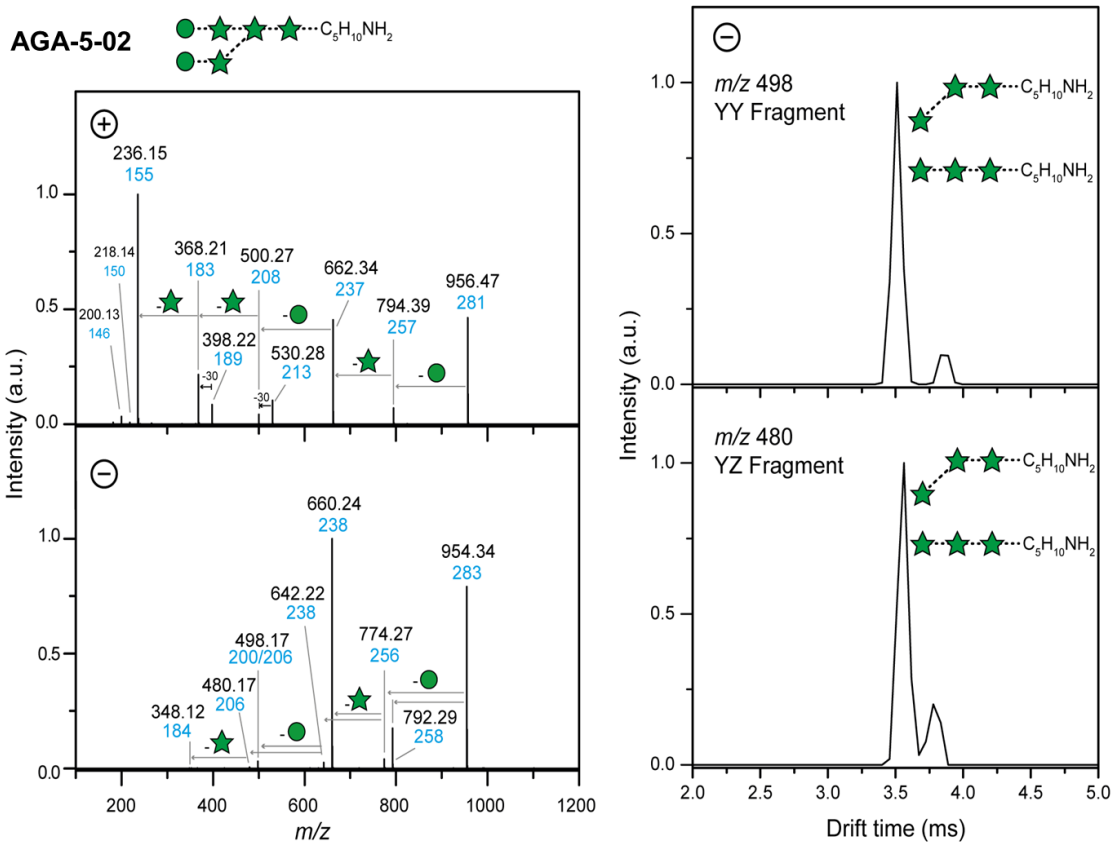


IM-MS arrival time distributions (ATDs) of the intact glycan ions **AGA-5-01**- **AGA-5-03**. Chemical structures are depicted as cartoons according to the SNFG nomenclature: green circles, mannose; green star, arabinofuranose; yellow circles, galactose; blue circles, glucose; horizontal lines, 1→4 bonds; upwards diagonals, 1→6 bonds; downwards diagonals, 1→3 bonds; dashed lines, α linkages; solid lines, β linkages.

An MS/MS study is performed to further confirm composition of the oligosaccharides **AGA-5-01** and **AGA-5-02**. The MS/MS study provides the monosaccharide fingerprint of the oligosaccharides. This analysis is very useful for the sequencing of **AGA-5-01** and **AGA-5-02** as it allows to clearly differentiate between the arabinose and mannose monosaccharides. For MS/MS experiments the species of interest were selected in the quadrupole and fragmented in the trap cell of the instrument by collision-induced dissociation using argon as a collision gas. The resulting fragments are subsequently separated in the ion mobility cell and their *m/z* and drift times are measured.



MS/MS spectra of **AGA-5-01** in posivite and negative ion mode. A sequential loss of monocaccharides is obverved. The corresponding ion mobility arrival time distributions of the fragments show no evidence for the presence of multiple structures (data not shown). *m/z* values are given in black and corresponding absolute nitrogen CCSs in blue.



MS/MS spectra of **AGA-5-02** in posivite and negative ion mode. Due to the branced structure of the molecule, two possible trisaccharide fragments can occour during collision-induced dissociation. As a result, two drift peaks are observed for the deprotonated ions *m/z* 498 and 480 (right panel). *m/z* values are given in black and corresponding absolute nitrogen CCSs in blue.

6. Final Conclusion and Outlook

6.1 Conclusion

This thesis was constructed on top of the successful solid road paved by the Seeberger group's consistent passion in order to democratize the field of glycoscience by providing robust synthetic technology platforms. The automated glycan assembly (AGA) using solid-phase chemistry was initiated with a small set of structures synthetized in 2001. The very initial success of AGA could not meet the high expectation of carbohydrate chemists and glycobiologists, and instead remained the opportunities to be advanced. The aim of this thesis was to develop the logic of automated glycan assembly rather than to synthesize a couple of highly challenging oligosaccharides, and then for non-chemists to make use of this method in order to procure customized glycans over the current limitations of automated oligosaccharide synthesis. Challenges with respects to the building blocks, reliable synthetic instrument, a various set of exemplary oligosaccharides synthesized by AGA including negative charged molecules, glycosaminoglycans (GAGs), oligosaccharides containing Lc4 and nLC4, oligosaccharides bearing one or more 1,2-*cis* glycosidic linkages on glucose and galactose, and a 50-mer polymannoside had to be showcased. Finally, the streamline synthetic protocols from building block preparation, automation program, linker cleavage, deprotection, purification, and quality control of glycan assembled by the commercial instrument, Glyconeer 2.1® was supposed to be demonstrated for non-organic chemists in order to provide conjugation-ready oligosaccharides for biomedical/biochemical studies as a ultimate goal of AGA (**Figure 6.01**)

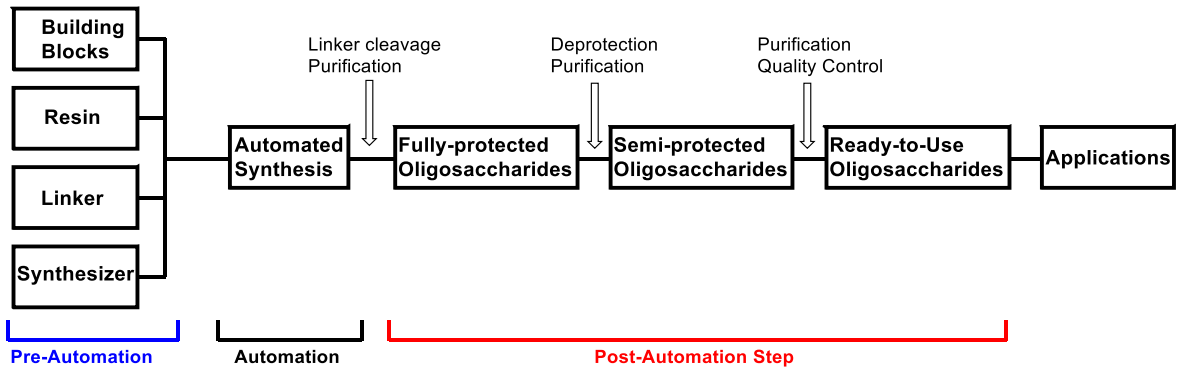


Figure 6.01. The automated synthesis process

The aim to synthesize oligo-*N*-acetyllactosamine glycans including four different sulfation patterns on one common tetrasaccharides motivated us to introduce (2-naphthyl)methyl (Nap) to the toolbox of orthogonal protecting groups for automated glycan assembly. The usage of Nap in the context of the anhydrous instrument condition of

automated glycan synthesizer required the mixture of organic solvent and water to deprotect Nap. This reaction condition caused the reaction vessel to be wet, which could hydrolyze the activated building blocks quickly on the glycosylation step. As a result, Nap protecting groups were not adapted in AGA since the first automated oligosaccharide synthesis debuted in 2001. Despite this potential risk, we decided to introduce Nap protecting group, because the Nap ether helps the building block become more reactive (armed) unlike Fmoc and Lev would disarm the glycosyl donor reactivity. To solve this expected problem of the reaction vessel becoming wet, we introduced the acidic wash using TMSOTf to remove basic residues and to keep the reaction vessel anhydrous at the same time. The glycosylation reaction following the challenging Nap deprotection and acidic wash was successfully achieved, and branched hexasaccharide was obtained without any deletion sequence. Furthermore, the orthogonal deprotection conditions over Lev and Fmoc helped four different keratan sulfates consisting of tetrasaccharide procured from one common tetrasaccharide (**Figure 6.02**).

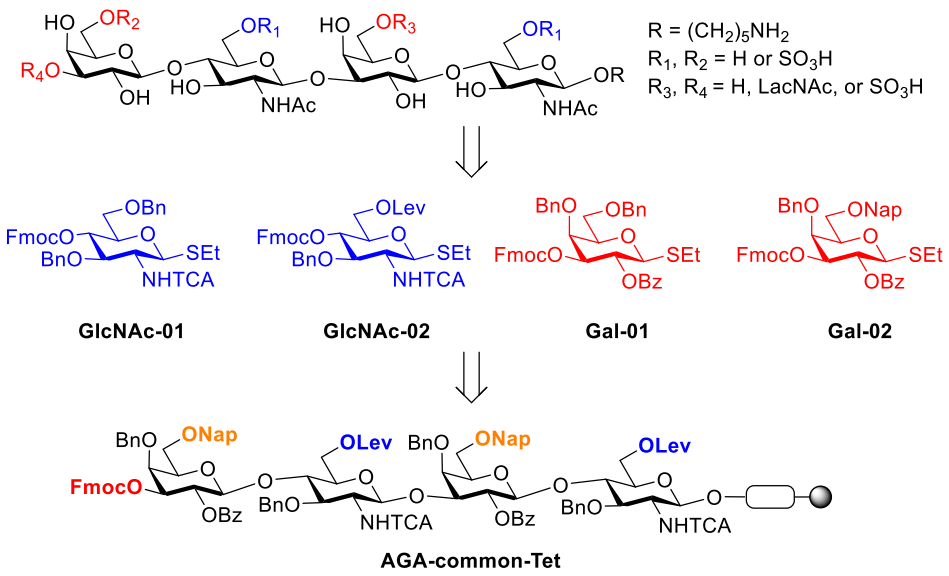


Figure 6.02. Retrosynthetic analysis of Linear, Branched, and Sulfated LacNAc and KS Oligosaccharides and the Complete Set of Building Blocks for AGA with Three Orthogonal Temporary Protecting Groups. Resin-conjugated **AGA-common-Tet** was orthogonally deprotected, and sulfated into KS.

In the third chapter, the automated synthesis of complex oligosaccharides H-type I and II, α-Gal epitopes, and the HNK-1 pentasaccharide using monosaccharide building blocks was successfully achieved. On the synthesis of H-type I and II, we discovered that the stereoselectivity of 1,2-*cis* fucosidic bond formation depends on the sequence of the resin-bound nucleophile. Fuc-01 and Fuc-03 were used to synthesize H-type I and II respectively. During α-Gal epitopes synthesis, the 1,2-*cis* galactosidic linkage formation again shows the

sequence-dependency. Leaving groups, Lev and Fmoc and protecting group affected efficiencies of glycosylation in the synthesis of the HNK-1. The results from various oligosaccharides emphasized that the identification of "approved" building blocks is key to automated glycan assembly.

In the third project, in order to overcome the sequence-dependency of 1,2-*cis* glycosylation with galactose building blocks, we showed that building blocks bearing remote participating groups effectively provide building block-dependent 1,2-*cis* glycosidic linkage formation in the context of AGA on synthesis of oligosaccharides containing 1,2-*cis*-glucosidic and 1,2-*cis*-galactosidic linkages. Standardized synthesis of alpha-glucan showcased the power of the AGA approach followed by deprotection and purification protocols that allowed for procurement of conjugation-ready molecules. The reliable incorporation of *cis*-gluco- and *cis*-galactosides into oligosaccharides by automated synthesis opened up many opportunities to study secondary glycan structures affected by combination of cis- and trans-glycosidic linkages on glycans to render AGA the method of choice for the procurement of complex glycans (**Figure 6.03**).

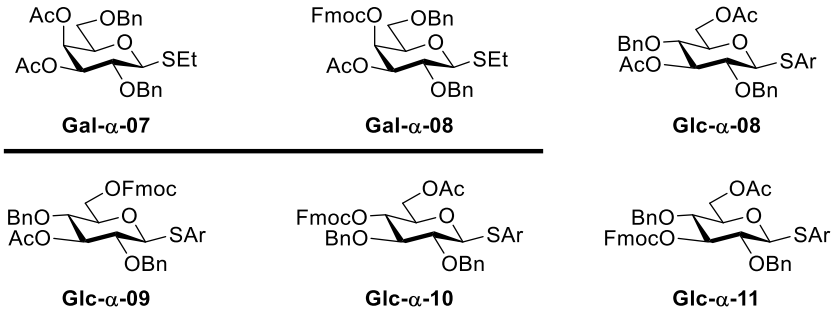


Figure 6.03 Identification of building blocks for installation of α-galactosidic linkages and α-glucosidic linkages toward Globo-H and α-Glucans

As the ultimate goal, the streamlined AGA using Glyconeer 2.1, photolabile linker conjugated resin (**Resin-1**), and a set of approved commercially available building blocks allows for the synthesis of variety of oligosaccharides. The purity of the ready-to-use oligosaccharides was analyzed by using IM-MS, and protocols of the streamlined AGA was updated by feedback process. The process described here helps glycoscientists access naturally or non-naturally occurring glycans for biochemical, biomedical, and materials science.

6.2 Outlook

New methods for automated glycan assembly to prepare different classes of glycans established in this thesis provides robust access to a diverse set of oligosaccharides and allows for various applications of tailored oligosaccharides, which contributes to the democratization of glycoscience demanding structurally well-defined oligosaccharides. Therefore, the presented automated glycan assembly synthetic platform has the potential to emerge a popular synthetic technology. In order to extend the logic of AGA, the following projects are anticipated:

1) Introduction of orthogonal protecting groups: A small set of the temporary protecting groups are used in automated glycan assembly. Fmoc, Lev, and AzBz disarm building blocks. Disarmed building blocks sometimes cause incomplete glycosylation using AGA. Chapter 2 showed that introduction of Nap protecting group keeps building blocks armed. More electron-donating temporary protecting groups are needed to be developed in order to be used in the context of AGA.

2) Methodology development of branched alpha-glucan synthesis: Chapter 4 showed that automated glycan assembly of linear alpha-glucans was successfully achieved with "approved" building blocks bearing remote participating groups. However, synthesis of branched alpha-glucans remains challenging. In order to overcome synthetic limitations, new remote participating groups will have to be introduced to the currently available toolbox of protecting groups.

3) Upgrading instrument: As described in Chapter 5, combination of Glyconeer 2.1® and well-established post-automation protocols paved the road of democratizing glycoscience. The next practical direction of the instrument will be to provide the extra reaction room where the building block can be preactivated, and then the activated building block will be delivered into the reaction vessel. This preactivation approach would provide the stereoselectivity of 1,2-*cis* glycosylation.

4) Longer-mers: In the Appendix, synthesis of 50-mer oligomannoside was successfully achieved by combination of AGA and armed mannose building blocks. This achievement still remains improvement on the capping process to increase the yield and on the branch-

structured polysaccharides. Their application as biosensors and/or to drug delivery will be able to be studied around the corner.

6.3 Final Conclusion

The new methods for automated glycan assembly were established to prepare different classes of glycans for biological and material utilities was the results from one visionary, Peter H, Seeberger, his former group members, and myself. This established logic will be evolved to become more mature, and finally open many new opportunities in biomedical and material science.

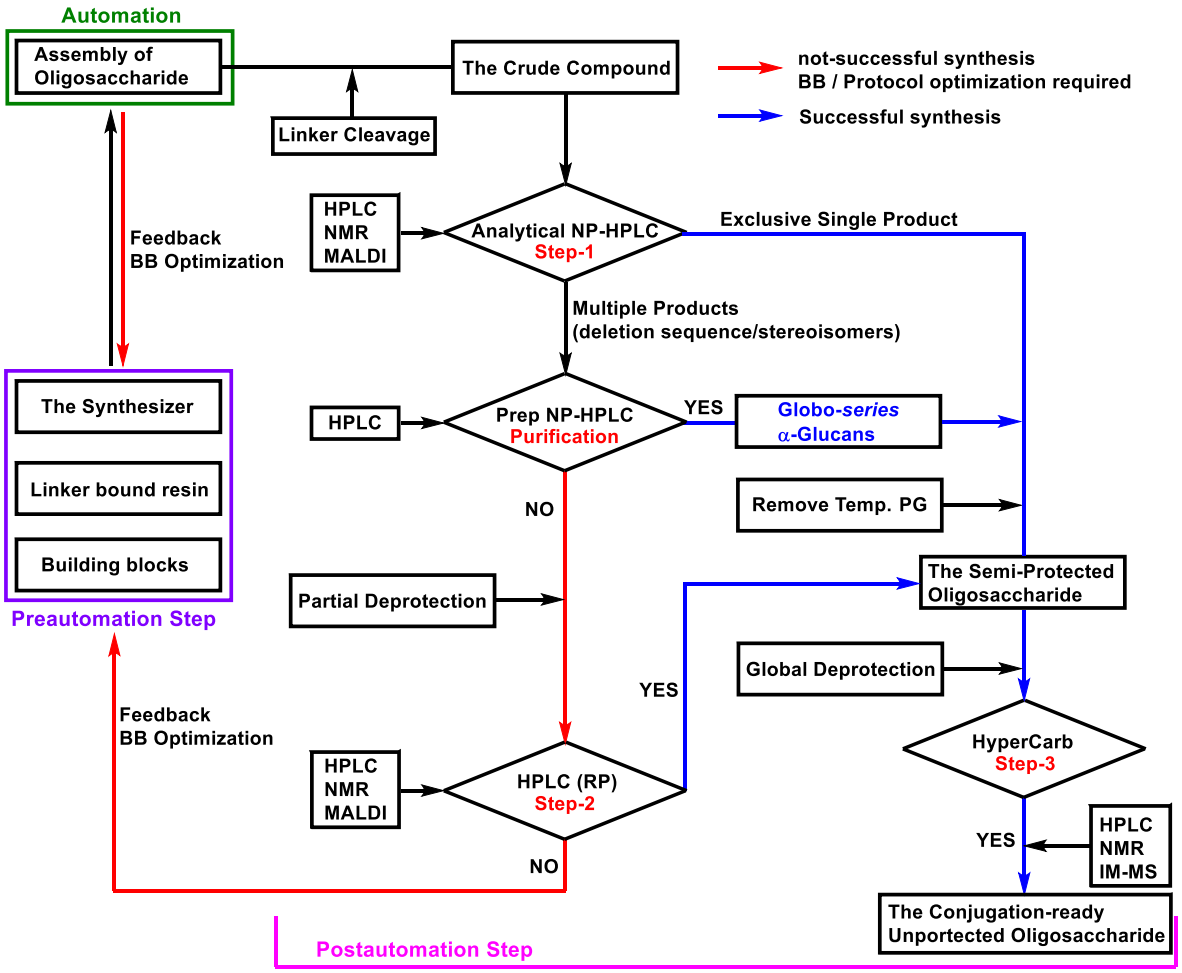


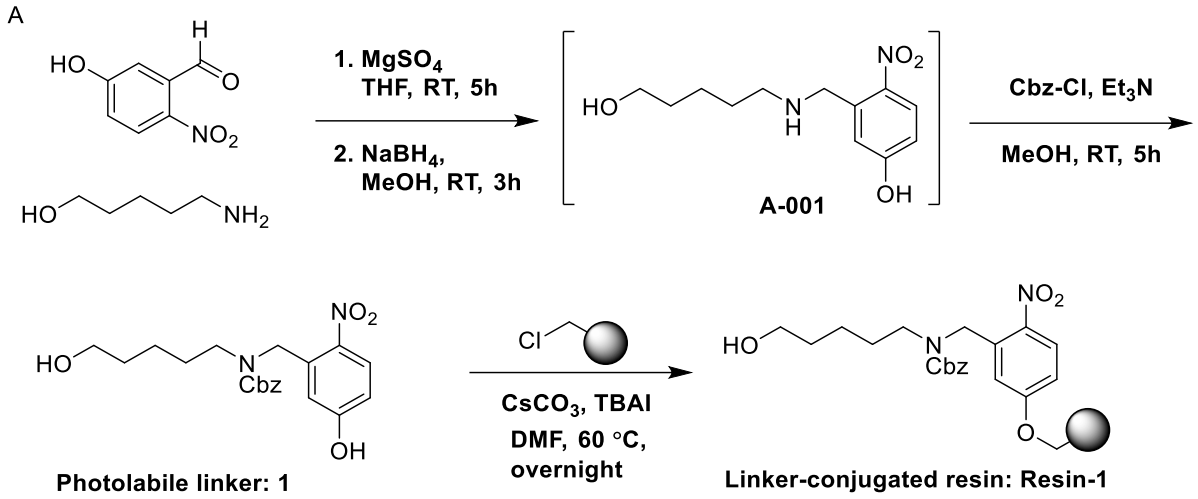
Figure 6.02. Detailed description of the full workflow established for Automated Glycan Assembly. Preautomation phase: preparation of building blocks, resin with linker and the synthesizer. Automation phase: AGA. Postautomation phase: analysis and purification of fully protected oligosaccharide, procurement of the conjugation-ready glycans, and quality control using IM-MS.

Appendix I: Preparation of Linker and Building Blocks

A.1 General Information

All chemicals used were reagent grade and used as supplied, exceptions are noted. All reactions were performed in oven-dried glassware under an argon atmosphere, unless noted otherwise. Prior to use, molecular sieves were activated by heating under high vacuum. *N*,*N*-dimethylformamide, (DMF) dichloromethane (DCM), toluene and tetrahydrofuran (THF) were purified in a Cycle-Tainer Solvent Delivery System. Analytical thin layer chromatography (TLC) was performed on Merck silica gel 60 F254 plates (0.25 mm). Compounds were visualized by UV-irradiation, or by dipping the plate either in a cerium sulfate ammonium molybdate (CAM) solution. Flash column chromatography was carried out using forced flow of the indicated solvent on Fluka silica gel 60 (230-400 mesh). All automated glycosylations were performed on a automated oligosaccharide synthesizer demonstrator unit using anhydrous solvents of the Cycle-Tainer Solvent Delivery System. LCMS chromatograms were recorded on an Agilent 1100 Series spectrometer. Preparative HPLC purifications were performed on an Agilent 1200 Series. Loading determination of functionalized resins was obtained using a Shimadzu UV-MINI-1240 UV spectrometer. ^{1}H, ^{13}C spectra were recorded on a Varian Mercury 400 (400 MHz), 600 (600 MHz) or a Bruker AVIII 700 (700 MHz) spectrometer in $CDCl_3$ or CD_3OD with chemical shifts (δ) referenced to internal standards ($CDCl_3$: 7.26 ppm ^{1}H, 77.16 ppm ^{13}C; CD_3OD: 4.87 or 3.31 ppm ^{1}H, 49.0 ppm ^{13}C; D_2O: 4.79 ppm ^{1}H) unless stated otherwise. Splitting patterns are indicated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; brs, broad singlet for ^{1}H-NMR data. NMR chemical shifts (δ) are reported in ppm and coupling constants (*J*) are reported in Hz. High resolution mass spectrometry (HRMS) analyses were performed by the MS-service in the Department of Organic Chemistry at Free University Berlin using an Agilent 6210 ESI-TOF (Agilent Technologies, Santa Clara, CA, USA). IR spectra were recorded on a Perkin-Elmer 1600 FTIR spectrometer. Optical rotations were measured with a UniPol L 1000 polarimeter (Schmidt & Haensch, Berlin, Germany), with concentrations expressed in g per 100 mL.

A.2 Polystyrene resin equipped with a photolabile linker 1



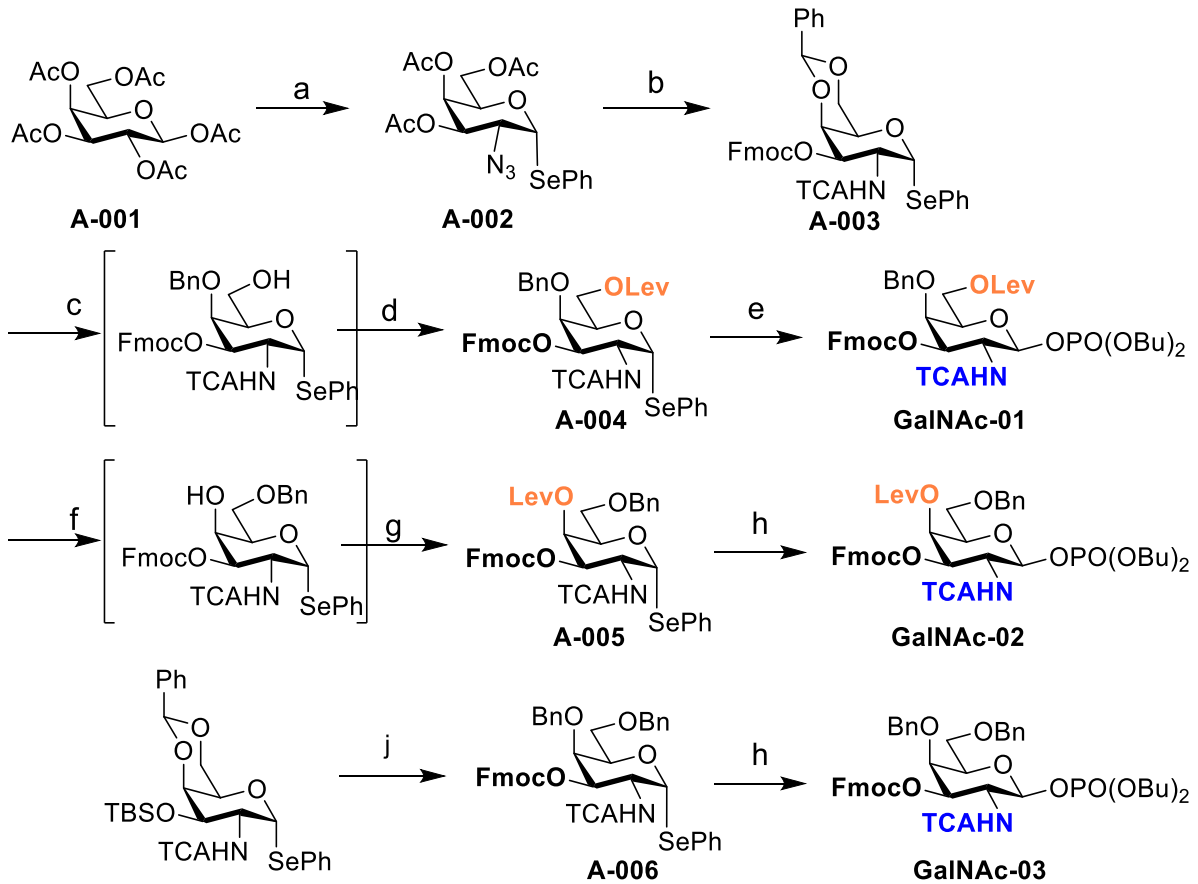
Scheme A.01. Preparation of a photolabile linker **1** and and linker bound resin **R**

5-Aminopentanol (10 g, 97.0 mmol, 1.0 eq.), 5-hydroxy-2-nitrobenzaldehyde (15.4 g, 92 mmol, 0.95 eq.) and $MgSO_4$ (19.0 g, 160 mmol, 2.1 eq.) were stirred in anhydrous THF (242 mL) at room temperature. After 5 h, the suspension was filtered and concentrated *in vacuo*. The crude was dissolved in MeOH (280 mL) and cooled to 0°C. $NaBH_4$ (3.67 g, 97 mmol, 1.0 eq.) was added portion-wise to the mixture and allowed to warm to room temperature. After 3 h, the mixture was quenched by addition of acetone (15 mL) and the solvents were evaporated to yield crude compound **A-001**. To a solution of the crude in MeOH (280 mL) was added triethylamine (41 mL, 291 mmol, 3.0 eq.) and benzyl chloroformate (Cbz-Cl, 34.6 mL, 243 mmol, 2.5 eq.) at room temperature. After 5 h, K_2CO_3 (40.0 g) was added to the reaction mixture and stirred for an hour. The reaction mixture was then filtered through celite and the solvents evaporated *in vacuo*. The crude was extracted with DCM. The organic layer was dried over $MgSO_4$, filtered, concentrated and subjected to flash chromatography (Silica/EtOAc:hexane) to obtain photo-cleavable linker **S1** in 73% yield (17 g, 71.1 mmol).

To a solution of the photolabile linker **1** (6.21 g, 16.0 mmol, 4.0 eq.) in DCM was added Merrifield resin (8.0 g, 4.0 mmol, loading 0.50 mmol/g). This suspension was carefully evaporated *in vacuo*, and redissolved in the minimum amount of DMF (4 mL DMF / 1 g resin). The suspension was then degassed by placing the flask under high vacuum for a couple of minutes, followed by refilling the evacuated flask with argon. After repeating this degassing procedure twice more, Cs_2CO_3 (5.21 g, 16 mmol, 4.0 eq.) and tetrabutylammonium iodide (TBAI) (1.48 g, 4.0 mmol, 1.0 eq.) were added to the flask and the entire suspension

rotated on a rotovap at 60°C and atmospheric pressure overnight. The next morning, water was added to the resin to dissolve all solids and the resin was subsequently washed with THF/water (1/1), THF, DMF, MeOH, DCM, MeOH, and finally DCM (six times each) to remove the yellow color. The resin was transferred again to a round bottom flask, swollen in a minimal amount of DMF (~4 mL DMF/g resin) and the flask degassed as above. Afterwards, CsOAc (1.54 g, 8.0 mmol) was added and the entire suspension rotated on a rotovap at 60 °C and atmospheric pressure overnight. The next morning, the resin was washed with THF/water (1/1), THF, DMF, MeOH, DCM, MeOH, and finally DCM (six times each) to remove the yellow color. The resin was then dried under high vacuum overnight and stored in the dark to obtain the photolabile linker bound resin **R (Scheme A.01)**. Loading (0.392 mmol/g) was determined.

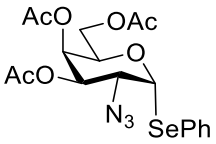
A.3 Building Block Preparation: GalNAc



Scheme A.02. Synthesis of galactosamine building blocks GalNac-01, -02, and -03. Reactions and conditions: a) (i) Ac2O, pyridine, 0 °C → r.t. (ii) HBr, AcOH, Ac_2O, 0 °C → r.t.; (iii) Zn, *N*-methyl-imidazole, EtOAc, reflux; (iv) Ph_2Se_2, TMS-N_3, $PhI(OAc)_2$, DCM, -30 °C → r.t. (a-d: 46% over 4 steps); b) (i) Me_3P, H_2O, THF, NEt_3, 0 °C; (ii) TCACl, pyridine, DCM, 0 °C → r.t.;(iii) NaOMe, MeOH, r.t.; (iv) $PhCH(OMe)_2$, *p*TsOH·H_2O, MeCN, r.t.; (v) FmocCl, pyridine, DCM, r.t. 86% over 5 steps; c) BH_3·THF, TMSOTf, DCM, THF, 0 °C → r.t.; d) Lev_2O, pyridine, DCM, r.t. 75% over 2 steps; e) HOP(O)OBu_2, NIS, DCM, 0 °C 63% for **GalNac-01**; f) (i)TFA, $(TFA)_2O$, Et_3SiH,DCM,

0 °C → r.t.; (ii) Lev_2O, pyridine, DCM; r.t. 62% over 2 steps); h) $HOP(O)OBu_2$, NIS, DCM, 0 °C 84% for **GalNac-02**. i) (i) BH_3·THF, TMSOTf, DCM, THF, 0 °C → r.t.; (ii) NaH, BnBr, THF/DMF, 0 °C → r.t.; (iii) BF_3OEt_2, MeCN, 0 °C; (iv) FmocCl, pyridine, DCM, 0 °C; h) $HOP(O)OBu_2$, NIS, DCM, 0 °C 54% over 5 steps for **GalNac-02**. Ph = phenyl; Fmoc = fluorenylmethyloxycarbonyl; TCA = trichloroacetyl; Bu = butyl Lev = levulinoyl; Bn = benzyl Ac = acetyl; EtOAc = ethyl acetate; TMS = trimethylsilyl; DCM = dichloromethane; THF = tetrahydrofuran; *p*Ts = *para*-toluenesolfonic acid; MeCN = acetonitrile; TMSOTf = trimethylsilyl trifluoromethanesulfonate; NIS = *N*-iodosuccinimide; TFA = trifluoroacetic acid.

Phenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-azido-1-seleno-α-D-galactopyranoside A-002

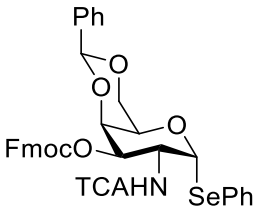


A-002

D-Galactose **A-001** (30 g, 167 mmol) was suspended in pyridine (180 mL, 2.23 mol) and cooled to 0 °C. Acetic anhydride (105 mL, 1.11 mol) was added, and the reaction was stirred and allowed to warm up to room temperature. After complete conversion of the starting material (TLC: *cyclo*-hexane/ethyl acetate, 1:3) the volatiles were removed *in vacuo* and the remainder was co-evaporated three times with toluene. The remainder was dissolved in DCM and extracted with 1 M aqueous HCl and saturated aqueous $NaHCO_3$. The organic phase was dried over $MgSO_4$ and the solvent was removed *in vacuo*. The crude product was used without further purification for the next step. The peracetylated galactose was dissolved in acetic anhydride (8 mL, 85 mmol) and DCM (170 mL), and cooled to 0 °C. An HBr solution (33% in acetic acid; 140 mL) was added and the reaction was stirred and allowed to warm up to room temperature. After complete conversion of the starting material (TLC: *cyclo*-hexane/ethyl acetate, 1:2), the solution was diluted with DCM (230 mL) and dumped into ice. After phase separation, the organic layer was washed with saturated aqueous $NaHCO_3$ and brine, and dried over $MgSO_4$. The solvents were removed *in vacuo* and the crude product was used without further purification for the next step. A suspension of zinc (55.9 g, 855 mmol) in ethyl acetate (460 mL) and *N*-methyl-imidazole (13.6 mL, 171 mmol) was stirred vigorously and heated to reflux. A solution of the galactosyl bromide in ethyl acetate (110 mL) was prepared. To activate the reaction, 3 mL of sugar solution were added quickly to the zinc suspension and the residual sugar solution was added dropwise 10 min later. The reaction was stirred under reflux, and after complete conversion of the starting material (TLC: *cyclo*-hexane/ethyl acetate, 1:1) the solution was cooled down to room temperature. The solution was decanted and the remaining solid was rinsed with ethyl acetate. The combined organic layers were washed with water, saturated aqueous $NaHCO_3$ and brine,

dried over $MgSO_4$, and the solvent was removed. The crude product was used without further purification for the next step. The galactal (10.8 g, 39.7 mmol) was dissolved in DCM (200 mL) and diphenyl diselenide (12.4 g, 39.7 mmol) was added. The solution was cooled to -30 °C under Ar atmosphere and iodobenzene diacetate (12.8 g, 39.7 mmol) and TMS-N_3 (10.5 mL, 79 mmol) were added. The solution was stirred overnight and allowed to warm up gradually. After complete conversion of the starting material (TLC: *cyclo*-hexane/ethyl acetate, 1:1), the solution was extracted with saturated aqueous $NaHCO_3$, dried over $MgSO_4$ and the solvent was removed *in vacuo*. The crude product was purified by crystallization from diethyl ether leading to the selenogalactoside **11** (7.2 g; 46% over four steps).

Phenyl 4,6-*O*-benzyliden-3-*O*-fluorenylmethoxycarbonyl-2-deoxy-2-trichloroacetamido-1-seleno-α-D-galactopyranoside A-003



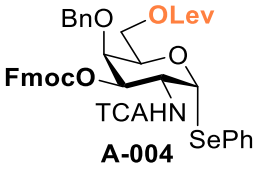
A-003

Selenogalactoside **A-002** (10 g, 21.3 mmol) was dissolved in THF (60 mL) and water (5 mL) and triethylamine (2.96 mL, 21.3 mmol) was added. The solution was cooled to 0 °C and a 1 M solution of trimethylphosphine in THF (25.5 mL, 25.5 mmol) was added. After stirring the reaction at 0 °C for 10 min (TLC: *cyclo*-hexane/ethyl acetate, 1:1) the volatiles were removed. The remainder was dissolved in DCM, washed with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$ and the solvent was removed *in vacuo*. The crude product was used without further purification for the next step. The amine was dissolved in DCM (100 mL), and pyridine (8 mL, 99 mmol) was added. The solution was cooled to 0 °C and trichloroacetyl chloride (3.6 mL, 31.9 mmol) was added dropwise. The solution was stirred overnight at room temperature. After complete conversion of the starting material (TLC: *cyclo*-hexane/ethyl acetate, 1:1), ice and 1 M aqueous HCl were added. After phase separation, the organic layer was washed with saturated aqueous $NaHCO_3$, dried over $MgSO_4$ and the solvent was removed *in vacuo*. The crude product was used without further purification for the next step. The trichloroacetamide was dissolved in MeOH (67 mL) and a solution of sodium methoxide (0.57 g, 10.63 mmol) in MeOH (33 mL) was added. The reaction was stirred at room temperature and after completion (TLC: DCM/MeOH, 95:1) the solution was neutralized with IR-120-H^+ amberlite resin. The resin was filtered off and the

solvent was removed *in vacuo* leading to the crude product that was used without further purification for the next step.

The triol was co-evaporated twice with toluene and dissolved in acetonitrile (100 mL). Benzaldehyde dimethyl acetal (6.4 mL, 42.5 mmol) and *para*-toluene sulfonic acid (0.4 g, 2.13 mmol) were added and the solution was stirred at room temperature overnight (TLC: DCM/MeOH, 95:1). After complete conversion of the starting material, the reaction was quenched by the addition of triethylamine (5.3 mL) and the volatiles were removed *in vacuo*. The remainder was dissolved in DCM and extracted with brine. The organic phase was dried over $MgSO_4$ and the solvent was removed *in vacuo*. The solid obtained was slurried in *n*-hexane and filtered off. The solid was then washed with *n*-hexane and dried *in vacuo*. The crude product was used without further purification for the next step. The benzylidene acetal was dissolved in DCM (117 mL) and 9-fluorenylmethyl chloroformate (8.3 g, 31.9 mmol) and pyridine (3.44 mL, 42.5 mmol) were added. The reaction was stirred at room temperature overnight and after complete conversion of the starting material (TLC: *cyclo*-hexane/ethyl acetate, 2:1) the solution was diluted with DCM and extracted with 1 M aqueous HCl and saturated aqueous $NaHCO_3$. The organic phase was dried over $MgSO_4$ and the solvent was removed *in vacuo*. The crude product was purified by silica gel flash column chromatography (DCM) affording the Fmoc-protected selenogalactoside **12** (14.2 g, 86%, over five steps). R_f = 0.60 (*cyclo*-hexane/ethyl acetate, 2:1); $[\alpha]_D^{20}$ = +43.8 (c = 0.19, $CHCl_3$); IR (thin film): υ = 3333, 3047, 1722, 1517, 1270, 1091 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.77 – 7.67 (m, 2H, Ar), 7.58 – 7.48 (m, 6H, Ar), 7.43 – 7.30 (m, 5H, Ar), 7.29 – 7.20 (m, 5H, Ar), 7.14 (d, *J* = 7.5 Hz, 1H, NH), 6.13 (d, *J* = 4.2 Hz, 1H, H-1), 5.61 (s, 1H, CHO_2Ph), 5.08 – 4.88 (m, 2H, H-2, H-3), 4.53 – 4.46 (dd, *J* = 2.9, 1.0 Hz, 1H, H-4), 4.46 – 4.06 (m, 6H, 2×CH_2O-Fmoc, H-6a, H-Fmoc, H-5, H-6b); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 161.81, 155.56 (C=O), 143.13, 143.05, 141.43, 141.34, 137.20, 133.90, 129.62, 129.36, 128.50, 128.42, 128.35, 128.16, 127.44, 127.36, 126.43, 125.36, 125.24, 120.10 (Ar), 120.06 (CCl_3), 101.16 (CHO_2Ph), 88.52 (C-1), 74.00 (C-3), 73.14 (C-4), 71.14 (CH_2O-Fmoc), 69.25 (C-6), 65.89 (C-5), 51.16 (C-2), 46.65 (CH-Fmoc); ESI-HRMS ($C_{36}H_{30}Cl_3NO_7Se$): calcd for [M+Na] 796.0151; found 796.0176.

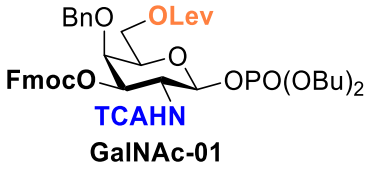
Phenyl 4-*O*-benzyl-3-*O*-fluorenylmethoxycarbonyl-6-*O*-levulinyl-2-deoxy-2-trichloroacetamido-1-seleno-α-D-galactopyranoside A-004



A-004

Fmoc-protected selenogalactoside **A-003** (14.2 g, 18.4 mmol) was co-evaporated with toluene and dissolved under an Ar atmosphere in DCM (170 mL). A 1 M solution of BH_3 in terahydrofuran (108 mL, 108 mmol) was added and the solution was cooled to 0 °C. After 10 min, trimethylsilyl triflate (1.66 mL, 9.2 mmol) was added and the reaction was stirred and allowed to warm to room temperature. After complete conversion of the starting material (TLC: *cyclo*-hexane/ethyl acetate, 2:1) the solution was diluted with DCM and extracted with saturated aqueous $NaHCO_3$. The organic phase was dried over $MgSO_4$ and the solvent was removed *in vacuo*. The crude product was used without further purification for the next step. The hydroxyl galactoside was dissolved in DCM (240 mL) then 4-oxopentanoic anhydride (11.8 g, 55.0 mmol) and pyridine (4.5 mL, 55.1 mmol) were added. The solution was stirred at room temperature overnight. After complete conversion of the starting material (TLC: *cyclo*-hexane/ethyl acetate, 2:1) the solution was diluted with DCM and extracted with 1 M aqueous HCl and saturated aqueous $NaHCO_3$. The organic phase was dried over $MgSO_4$ and the solvent was removed *in vacuo*. The crude product was purified by silica gel flash column chromatography (*cyclo*-hexane/ethyl acetate, 4:1→3:1→2:1) affording the Lev-protected selenogalactoside **13** (12.0 g, 75%, over two steps). R_f = 0.41 (*cyclo*-hexane/ethyl acetate, 2:1); $[\alpha]_D^{20}$ = +102.2 (c = 0.20, $CHCl_3$); IR (thin film): υ = 3358, 3005, 1739, 1716, 1514, 1260, 1086 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.81 – 7.23 (m, 18H, Ar), 7.20 (d, *J* = 7.4 Hz, 1H, NH), 6.03 (d, *J* = 4.1 Hz, 1H, H-1), 5.03 – 4.82 (m, 3H, H-3, CH_2O-Bn, H-2), 4.62 (d, *J* = 11.3 Hz, 1H, CH_2O-Bn), 4.50 – 4.39 (m, 3H, H-5, 2×CH_2O-Fmoc), 4.33 – 4.15 (m, 3H, H-Fmoc, H-6a, H-6b), 4.06 – 4.03 (m, 1H, H-4), 2.72 (t, *J* = 6.5 Hz, 2H, CH_2-Lev), 2.51 (t, *J* = 6.5 Hz, 2H, CH_2-Lev), 2.18 (s, 3H, CH_3-Lev); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 172.38, 161.88, 155.63 (C=O), 143.09, 142.97, 141.44, 137.25, 134.41, 129.52, 128.68, 128.41, 128.32, 128.23, 127.46, 127.39, 125.23 (Ar), 120.31 (CCl_3), 87.77 (C-1), 76.37 (C-3), 75.38 (CH_2O-Bn), 73.19 (C-4), 71.65 (C-5), 71.02 (CH_2O-Fmoc), 62.85 (C-6), 51.89 (C-2), 46.67 (CH-Fmoc), 37.97 (CH_2-Lev), 30.00 (CH_3-Lev), 27.83 (CH_2-Lev); ESI-HRMS ($C_{41}H_{38}Cl_3NO_9Se$): calcd for [M+Na] 896.0675; found 896.0687.

Di-*O*-butyl 4-*O*-benzyl-3-*O*-fluorenylmethoxycarbonyl-6-*O*-levulinyl-2-deoxy-2-trichloroacetamido- α/β-D-galactopyranosylphosphate GalNAc-01



GalNAc-01

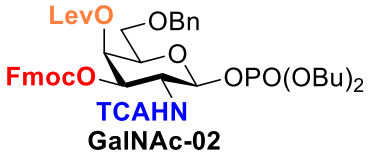
Selenoglycoside **A-004** (1.61 g, 1.85 mmol) was co-evaporated with toluene. The remainder and NIS (500 mg, 2.22 mmol) were dissolved in DCM (15.5 mL) under an Ar atmosphere and the solution was cooled 0 °C. Dibutyl hydrogen phosphate (1.1 mL, 5.56 mmol) was added and the reaction was stirred at 0 °C. After complete conversion of the starting material (TLC: *cyclo*-hexane/ethyl acetate, 1:1) the solution was diluted with DCM and extracted with 10% aqueous $Na_2S_2O_3$ and saturated aqueous $NaHCO_3$. The organic phase was dried over $MgSO_4$ and the solvent was removed *in vacuo*. The crude product was purified by silica gel flash column chromatography (*cyclo*-hexane/ethyl acetate, 4:1→3:1→2:1→1.5:1→1:1) affording the phosphate **GalNAc-01** (1.09 g, 63%). R_f = 0.38 (*cyclo*-hexane/ethyl acetate, 1:1); $[\alpha]_D^{20}$ = +132.2 (c = 0.16, $CHCl_3$); IR (thin film): υ = 3232, 2960, 2927, 2874, 1744, 1717, 1522, 1266, 1027 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$, α-anomer) δ 7.75 (d, *J* = 7.4 Hz, 2H, Ar), 7.58 (d, *J* = 7.5 Hz, 2H, Ar), 7.49 – 7.22 (m, 9H, Ar), 7.08 (d, *J* = 8.9 Hz, 1H, NH), 5.78 (dd, 1H, *J* = 5.7, 3.4 Hz, 1H, H-1), 5.18 (dd, *J* = 11.2, 2.9 Hz, 1H, H-3), 4.90 (d, *J* = 11.1 Hz, 1H, CH_2O-Bn), 4.86 – 4.79 (m, 1H, H-2), 4.61 (d, *J* = 11.1 Hz, 1H, CH_2O-Bn), 4.47 – 4.36 (m, 2H, 2×CH_2O-Fmoc), 4.33 – 4.16 (m, 6H, H-Fmoc, 4×CH_2O-Bu, H-5), 4.17 – 3.96 (m, 3H, H-6a, H-6b, H-4), 2.74 (t, *J* = 6.5 Hz, 2H, CH_2-Lev), 2.51 (t, *J* = 6.5 Hz, 2H, CH_2-Lev), 2.19 (s, 3H, CH_3-Lev), 1.76 – 1.60 (m, 4H, 4×CH_2-Bu), 1.47 – 1.32 (m, 4H, 4×CH_2-Bu), 1.00 – 0.87 (m, 6H, 6×CH_3-Bu); ^{13}C-NMR (100 MHz, $CDCl_3$, α-anomer): δ 206.46, 172.27, 162.23, 155.38 (C=O), 143.13, 142.99, 141.42, 137.23, 128.69, 128.32, 128.19, 127.43, 127.36, 125.24, 120.29 (Ar), 120.27 (CCl_3), 96.01 (C-1), 75.40 (CH_2O-Bn), 74.44 (C-3), 73.07 (C-4), 70.92 (CH_2O-Fmoc), 70.43 (C-5), 68.47 (C-6), 62.24 (CH_2O-Bu), 50.82 (C-2), 46.68 (CH-Fmoc), 37.94 (CH_2-Lev), 32.41 (CH_2-Bu), 29.95 (CH_3-Lev), 27.78 (CH_2-Lev), 18.76 (CH_2-Bu), 13.68 (CH_3-Bu); ESI-HRMS ($C_{43}H_{51}Cl_3NO_{13}P$): calcd for [M+Na] 948,2061; found 948.2034.

6-*O*-Benzyl-3-*O*-fluorenylmethoxycarbonyl-4-*O*-levulinyl-2-deoxy-2-trichloroacetamido-1-seleno-α-D-galactopyranoside A-005

A-005

Selenoglycoside **A-003** (500 mg, 0.646 mmol) was co-evaporated with toluene and dissolved in DCM (6.5 mL) under an Ar atmosphere. Activated ground molecular sieves 4 Å (1.5 g) and triethylsilane (0.62 mL, 3.88 mmol) were added and the solution was cooled to 0 °C. Trifluoroacetic anhydride (0.27 mL, 1.94 mmol) and trifluoroacetic acid (0.30 mL, 3.88 mmol) were added dropwise, and the reaction was stirred and allowed to warm to room temperature. After complete conversion of the starting material (DCM/MeOH, 95:5) the solution was diluted with DCM and the molecular sieves were filtered off. The solution was washed with saturated aqueous $NaHCO_3$, dried over $MgSO_4$ and the solvent was removed *in vacuo*. The crude product was used without further purification for the next step. The hydroxyl galactoside was co-evaporated with toluene and dissolved in DCM (8.9 mL). 4-Oxopentanoic anhydride (1.42 g, 6.61 mmol) and pyridine (0.45 mL, 5.56 mmol) were added. The solution was stirred at room temperature overnight. After complete conversion of the starting material (hexane/ethyl acetate, 2:1) the solution was diluted with DCM then extracted with 1 M aqueous HCl and saturated aqueous $NaHCO_3$. The organic phase was dried over $MgSO_4$ and the solvent was removed *in vacuo*. The crude product was purified by silica gel flash column chromatography (hexane/ethyl acetate, 5:1→3:1) affording the Lev-protected selenogalactoside **A-005** (348 mg, 62%, over two steps). R_f = 0.52 (hexane/ethyl acetate, 2:1); $[\alpha]_D^{20}$ = +110.6 (c = 1.58, CH_2Cl_2); IR (thin film): υ = 3347, 2923, 1748, 1719, 1688, 1529, 1381, 1261, 1155, 1083, 974, 821, 736, cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.6 Hz, 2H, Ar), 7.62 – 7.52 (m, 4H, Ar), 7.47 – 7.28 (m, 10H, Ar), 7.27 – 7.19 (m, 2H, Ar), 7.10 (d, *J* = 8.5 Hz, 1H, NH), 6.01 (d, *J* = 5.0 Hz, 1H, H-1), 5.72 (dd, *J* = 3.1, 1.1 Hz, 1H, H-4), 4.95 (dd, *J* = 11.6, 3.1 Hz, 1H, H-3), 4.76 (ddd, *J* = 11.6, 8.5, 5.0 Hz, 1H, H-2), 4.67 – 4.61 (m, 1H, H-5), 4.53 (s, 2H, 2×CH_2O-Bn), 4.46 (dd, *J* = 9.8, 6.6 Hz, 1H, CH_2O-Fmoc), 4.36 – 4.20 (m, 2H, CH_2O-Fmoc, H-Fmoc), 3.69 – 3.57 (m, 2H, H-6a, H-6b), 2.88 – 2.61 (m, 4H, CH_2-Lev), 2.16 (s, 3H, CH_3-Lev); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 206.15, 171.97, 162.00, 154.90 (C=O), 143.42, 142.98, 141.42, 141.33, 137.82, 134.70, 129.56, 128.58, 128.53, 128.13, 128.10, 128.06, 127.91, 127.37, 125.41, 125.29 (Ar), 120.20 (CCl_3), 87.90 (C-1), 73.69 (C-3), 73.57 (CH_2O-Bn), 71.56 (C-5), 71.05 (CH_2O-Fmoc), 67.85 (C-6), 67.31 (C-4), 51.60 (C-2), 46.60 (CH-Fmoc), 38.08 (CH_2-Lev), 29.92 (CH_3-Lev), 28.05 (CH_2-Lev); ESI-MS ($C_{41}H_{38}Cl_3NO_9Se$): calcd for [M+NH_4] 891.1; found 890.8. ESI-HRMS ($C_{41}H_{38}Cl_3NO_9Se$): calcd for [M+Na] 896.0675; found 896.0677.

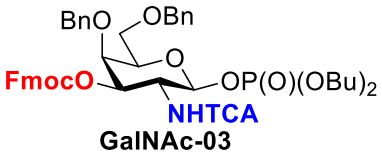
Di-*O*-butyl 6-*O*-benzyl-3-*O*-fluorenylmethoxycarbonyl-4-*O*-levulinyl-2-deoxy-2-trichloroacetamido-α/β-D-galactopyranosylphosphate GalNAc-02



GalNAc-02

Selenoglycoside **A-005** (2.0 g, 2.29 mmol) was co-evaporated with toluene. The remainder and NIS (618 mg, 2.75 mmol) were dissolved in DCM (19.1 mL) under an Ar atmosphere and the solution was cooled 0 °C. Dibutyl hydrogen phosphate (1.4 mL, 6.86 mmol) was added and the reaction was stirred at 0 °C. After complete conversion of the starting material (TLC: *cyclo*-hexane/ethyl acetate, 1:1) the solution was diluted with DCM and extracted with 10% aqueous $Na_2S_2O_3$ and saturated aqueous $NaHCO_3$. The organic phase was dried over $MgSO_4$ and the solvent was removed *in vacuo*. The crude product was purified by silica gel flash column chromatography (hexane/ethyl acetate, 4:1→1:1) affording phosphate **GalNAc-02** as a 1:1 α/β mixture (1.78 g, 84%). R_f = 0.57 (*cyclo*-hexane/ethyl acetate, 1:1); IR (thin film): υ = 3238, 2961, 1751, 1718, 1530, 1382, 1257, 1150, 1027, 958, 822, 732 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$, α/β = 1:1) δ 7.84 (d, *J* = 9.4 Hz, 1H, NH β), 7.75 (t, *J* = 6.6 Hz, 4H, Ar), 7.56 (t, *J* = 6.6 Hz, 4H, Ar), 7.46 – 7.24 (m, 18H, Ar,), 7.06 (d, *J* = 9.1 Hz, 1H, NH α), 5.83 (dd, *J* = 6.0, 3.3 Hz, 1H, H-1 α), 5.72 (dd, *J* = 2.3, 1.0 Hz, 1H, H-4 α), 5.59 – 5.45 (m, 2H, H-4b, H-1 β), 5.22 - 5.11 (m, 2H, H-3 α, H-3 β), 4.69 – 3.94 (m, 22H, H-2 α, 4×CH_2O-Bn, H-2 β, 4×CH_2O-Fmoc, 2×H-Fmoc, 8×CH_2O-Bu, H-5 α, H-5 β), 3.64 – 3.43 (m, 4H, 2×H-6a, 2×H-6b), 2.93 – 2.60 (m, 8H, CH_2-Lev), 2.16 (s, 6H CH_3-Lev), 1.79 – 1.55 (m, 8H, 8×CH_2-Bu), 1.41 – 1.29 (m, 8H, 8×CH_2-Bu), 0.95 – 0.83 (m, 12H, 12×CH_3-Bu); ^{13}C-NMR (100 MHz, $CDCl_3$, α/β = 1:1): δ 206.27, 206.16, 171.99, 171.94, 162.74, 162.41, 154.72, 154.32 (C=O), 143.62, 143.47, 143.10, 143.02, 141.42, 141.41, 141.33, 141.31, 137.71, 137.69, 128.58, 128.52, 128.12, 128.11, 128.07, 128.05, 128.01, 127.98, 127.96, 127.90, 127.36, 127.34, 125.47, 125.42, 125.29 (Ar), 120.20, 120.17 (CCl_3), 96.75 (C-1 β), 95.89 (C-1 α), 74.16 (C-3 β), 74.14 (CH_2O-Bn), 73.77 (C-5), 71.73 (C-3 α), 70.96 (CH_2O-Fmoc), 68.67 (CH_2O-Bu), 68.43 (C-6), 67.90 (C-4 α), 67.79 (C-4 β), 52.88 (C-2 β), 50.48 (C-2 α), 46.66 (CH-Fmoc), 38.03 (CH_2-Lev), 32.29 (CH_2-Bu), 29.84 (CH_3-Lev), 28.10 (CH_2-Lev), 18.70 (CH_2-Bu), 13.69 (CH_3-Bu); ESI-HRMS ($C_{43}H_{51}Cl_3NO_{13}P$): calcd for [M+Na] 948.2061; found 948.2058.

Di-*O*-butyl 4,6-di-*O*-benzyl-3-*O*-fluorenylmethoxycarbonyl-2-deoxy-2-trichloroacetamido-α/β-D-galactopyranosylphosphate GalNAc-03

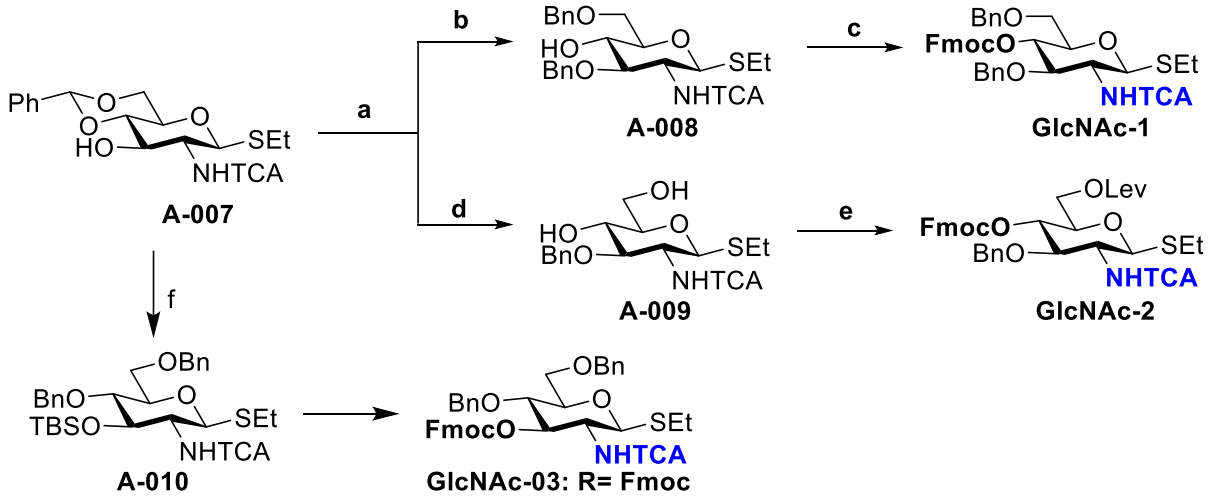


GalNAc-03

TBS-protected selenogalactoside (1.42 g, 1.84 mmol) was co-evaporated with toluene and dissolved under an Ar atmosphere in DCM (17 mL). A 1 M solution of BH_3 in terahydrofuran (11 mL, 10.8 mmol) was added and the solution was cooled to 0 °C. After 10 min, trimethylsilyl triflate (0.2 mL, 0.92 mmol) was added and the reaction was stirred and allowed to warm to room temperature. After complete conversion of the starting material (TLC: *cyclo*-hexane/ethyl acetate, 2:1) the solution was diluted with DCM and extracted with saturated aqueous $NaHCO_3$. The organic phase was dried over $MgSO_4$ and the solvent was removed *in vacuo*. The crude product was used without further purification for the next step. Sodium hydride (1.1 eq) was added portion-wise at 0 °C to a solution of the hydroxyl selenogalactoside and benzyl bromide (1.05 eq) in DMF. The reaction mixture was stirred for 3 h while the mixture was allowed to warm up to room temperature. The reaction was quenched by slowly adding aqueous NH_4Cl. After complete conversion of the starting material (TLC: *cyclo*-hexane/ethyl acetate, 2:1) the solution was diluted with DCM and extracted with 1 M aqueous HCl and saturated aqueous $NaHCO_3$. To a solution of the selenogalactoside in anhydrous acetonitrile was added boron trifluoride diethyl etherate ($BF_3{\cdot}OEt_2$) (1.2 eq.) at 0°C. After the reaction mixture was stirred for 20 min at 0°C the mixture was quenched with saturated aqueous $NaHCO_3$ (30 mL), diluted with DCM, and dried over $MgSO_4$. To a solution of the crude mixture in THF (11 mL, 0.4 M) was added tetra-*N*-butylammonium fluoride (2.0 eq.) at room temperature. After 4 h, the reaction mixture was concentrated *in vacuo*, re-dissolved in DCM, and extracted with 0.1 M of $CuSO_4$ aqueous solution. After the organic layer was dried over $MgSO_4$, and evaporated *in vacuo*. To a solution of this crude in anhydrous DCM (30 mL) was added 9-fluorenylmethylchloroformate (1.5 eq.) and pyridine (3.0 eq.) successively at 0°C, and stirred overnight at room temperature. After the mixture was quenched with 1 M aqueous HCl, and diluted with DCM. The organic layer was dried over $MgSO_4$ and the solvent evaporated *in vacuo*. Selenoglycoside was co-evaporated with toluene. The remainder and NIS (500 mg, 2.22 mmol) were dissolved in DCM (15.5 mL) under an Ar atmosphere and the solution was cooled 0 °C. Dibutyl hydrogen phosphate (1.1 mL, 5.56 mmol) was added and the reaction was stirred at 0 °C. After complete conversion of the starting material (TLC: *cyclo*-hexane/ethyl acetate, 1:1) the solution was diluted with DCM and extracted with 10%

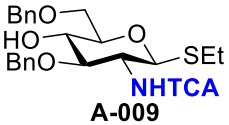
aqueous $Na_2S_2O_3$ and saturated aqueous $NaHCO_3$. The organic phase was dried over $MgSO_4$ and the solvent was removed *in vacuo*. The crude product was purified by silica gel flash column chromatography (*cyclo*-hexane/ethyl acetate, 4:1→1:1) affording the phosphate **GalNAc-03** (1.09 g, 54%).

A.4 Building Block Preparation: GlcNAc



Scheme A.03. Synthesis of glucosamine building blocks GlcNAc-001 and GlcNAc-002. a) BnBr, NaH, DMF, 0 °C; b) $HSiEt_3$, TFAA, TFA, DCM, 0 °C to r.t. 91% over two steps; c) FmocCl, pyridine, DCM, 0 °C to r.t. 92%; d) DCM, TFA, r.t. 86% over two steps; e) (i)2-chloro-1-methylpyridinium iodide, LevOH, DABCO, DCM, -15 °C; (ii) FmocCl, pyridine, DCM, 0 °C to r.t. 79% over two steps; f) (i) TBSCl, Imidazole, DCM, (ii) BH_3·THF, TMSOTf, DCM, THF, 0 °C → r.t.; (iii) BnBr, NaH, THF/DMF, 0 °C, 77% over two steps; g) (i) BF_3OEt_2, MeCN, -15 °C to 0 °C; (ii) FmocCl, pyridine, DCM, 0 °C to r.t. 92% over two steps; BnBr = benzyl bromide, DABCO = 1,4-diazabicyclo[2.2.2]octane, FmocCl = fluorenylmethyloxycarbonyl chloride, $HSiEt_3$ = triethylsilane, LevOH = levulinic acid, TFAA = trifluoroacetic anhydride, TFA = trifluoroacetic acid.

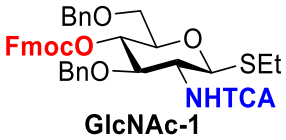
Ethyl 3,6-di-*O*-benzyl-2-deoxy-2-trichloroacetamino-1-thio-β-D-glucopyranoside A-009



To a solution of **A-007** (4.13 g, 9.04 mmol) in DMF (45 mL, 0.2 M) were added BnBr (1.77 mL, 14.92 mmol) and NaH (1.19 g 60% in mineral oil, 29.7 mmol) at 0 °C, and the reaction mixture was stirred at 0 °C for 2 h. The reaction was quenched by addition of MeOH (10 mL) at 0 °C, followed by the addition of H_2O (25 mL) at 0 °C to form a precipitate. The precipitate was filtered off and washed with MeOH–H_2O (2:1, v/v, 5 mL, five times), and cold MeOH (5 mL, three times) to give crude **A-008** as a colorless powder. The precipitate was dissolved in DCM, dried over $MgSO_4$, and evaporated *in vacuo*. The crude **A-008** was co-evaporated with toluene three times, and dissolved in DCM (41 mL, 0.18 M) under an Ar

atmosphere. To a solution of the crude mixture **S2** were added triethylsilane (12.0 mL, 75.0 mmol) and trifluoroacetic anhydride (0.64 mL, 4.52 mmol) at 0 °C. After 30 min at 0 °C trifluoroacetic acid (3.90 mL, 50.6 mmol) was added dropwise, and the mixture was allowed to warm to room temperature overnight. The mixture was quenched with saturated aqueous $NaHCO_3$, diluted with DCM, and the organic layer was separated. The aqueous layer was extracted with DCM. The organic layers were combined, dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane–ethyl acetate = 9:1 to 7:3) to afford **A-009** (4.37 g, 7.96 mmol, 88% over two steps). The analytical data was in agreement with literature data.

Ethyl 3,6-di-*O*-benzyl-4-*O*-fluorenylmethoxycarbonyl-2-deoxy-2-trichloroacetamino-1-thio-β-D-glucopyranoside GlcNAc-1



GlcNAc-1

Compound **A-009** (8.9 g, 16.2 mmol) and FmocCl (8.39 g, 32.4 mmol) were dissolved in 4 mL (49.6 mmol) of pyridine at 0 °C, and the reaction mixture was stirred at room temperature for 15 h. The reaction was quenched with saturated aqueous $NaHCO_3$, diluted with DCM, and the organic layer was separated. The aqueous layer was extracted with DCM. The organic layers were combined, washed with 1 M aqueous HCl, dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (hexane–ethyl acetate–DCM = 7:2:1 to 7:3:1, v/v/v) to give **GlcNAc-1** (3.50 g, 99%). The analytical data was in agreement with literature data.

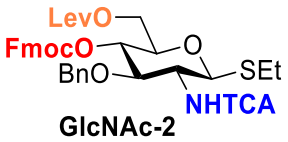
Ethyl 3-*O*-benzyl-2-deoxy-2-trichloroacetamino-1-thio-β-D-glucopyranoside A-010

A-005

To a solution of the compound **A-007**[S3] (2.1 g, 4.60 mmol) in DMF (10 mL) was added NaH (0.603 g, in oil 60 w%) at 0 °C. After the reaction mixture was stirred for 5 min at 0 °C, 0.9 mL (2.5 mmol) of benzyl bromide was added. The mixture was stirred 0 °C for 0.5 h and quenched with the addition of MeOH (10 mL) at 0 °C followed by addition of H_2O (25 mL) at 0 °C to form a precipitate. The precipitate was filtered off and washed with MeOH–H_2O

(2:1, v/v, 5 mL five times), cold MeOH (5 mL, three times) to give crude **A-007**. To a solution of **A-007** in DCM (100 mL) was added trifluoroacetic acid (5 mL) at 0 °C and the reaction mixture was stirred for 3 h at 0 °C. The reaction was quenched with saturated aqueous $NaHCO_3$, and extracted twice with DCM (150 mL). The organic layers were combined, washed with saturated aqueous $NaHCO_3$, and dried over Na_2SO_4. The crude mixture was concentrated *in vacuo* and re-dissolved in DCM–hexane (3:5, v/v, 80 mL) and stored at -20 °C overnight to form a precipitate. The precipitate was filtered, washed with DCM-hexane mixture (1:2, v/v), and hexane. The reprecipitation process was repeated twice to afford compound **A-010** (4.37 g, 3.96 mmol, 86% over two steps). R_f =0.1 (cyclohexane:ethyl acetate = 1:1) ^{1}H-NMR (400 MHz, **CD_3OD**) : δ 7.39 – 7.00 (m, 5H), 4.87 (d, *J* = 10.9 Hz, 1H, C*H*HPh), 4.71 (d, *J* = 10.6 Hz, 2H, H-1, C*H*HPh), 3.92 – 3.82 (m, 2H, H-2, H-6), 3.73 – 3.65 (m, 2H, H-3, H-6), 3.56 – 3.47 (m, 1H, H-4), 3.35 (ddd, *J* = 9.7, 5.9, 2.2 Hz, 1H, H-5), 2.82 – 2.64 (m, 2H), 1.25 (t, *J* = 7.4 Hz, 3H). ^{13}C-NMR (100 MHz, **CD_3OD**) : δ 163.85 ($NHCOCCl_3$), 139.84 (CCl_3), 129.15, 128.79, 128.49 (Ar), 84.71 (C-1), 84.57 (C-3), 82.36 (C-5), 76.10 (CH_2Ph), 72.07 (C-4), 62.80 (C-6), 57.35 (C-2), 24.89 (CH_2, SEt), 15.28 (CH_3, SEt); HR-ESI MS: *m/z* $[M+Na]^+$ calcd for $C_{17}H_{22}NO_9Cl_3SNa^+$ 480.0176, found 480.0184.

Ethyl 3-*O*-benzyl-4-*O*-fluorenylmethoxycarbonyl-6-*O*-levulinyl-2-deoxy-2-trichloro-acetamino-1-thio-β-D-glucopyranoside GlcNAc-2

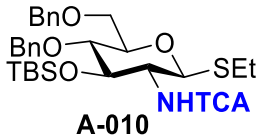


GlcNAc-2

To a solution of **A-010** (2.4 g, 5.23 mmol) in DCM (26 mL) were added levulinic acid (1.22 g, 10.5 mmol), 2-chloro-1-methylpyridinium iodide (2.94 g, 11.5 mmol), and 1,4-diazabicyclo[2.2.2]octane (DABCO) (2.35 g, 30.9 mmol) at –15 °C, and the mixture was stirred at –15 °C for 2 h. The mixture was quenched with saturated aqueous $NaHCO_3$, diluted with DCM, and the organic layer was separated. The aqueous layer was extracted with DCM. The organic layers were combined, washed with 1 M aqueous HCl, dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was dissolved in pyridine (0.85 mL). To the solution, FmocCl (2.91 g, 1.62 mmol) was added at 0 °C. The reaction mixture was stirred at room temperature for 15 h. The reaction was quenched with saturated aqueous $NaHCO_3$, diluted with DCM, and the organic layer was separated. The aqueous layer was extracted

with DCM. The organic layers were combined, washed with 1 M aqueous HCl, dried over $MgSO_4$ and concentrated. The crude product was purified by silica gel column chromatography (hexane–ethyl acetate–DCM = 7:2:1 to 7:3:1, v/v/v) to give **GlcNAc-2** (3.2 g, 4.11 mmol, 79%). R_f = 0.1 (cyclohexane:EtOAc = 9:1) ^{1}H-NMR (400 MHz, $CDCl_3$) : δ 7.75 (dd, *J* = 7.6, 3.6 Hz, 2H), 7.57 (ddd, *J* = 15.9, 7.5, 0.7 Hz, 2H), 7.38 (t, *J* = 7.5 Hz, 2H), 7.30 – 7.24 (m, 2H), 7.23 – 7.12 (m, 5H), 6.93 (d, *J* = 7.8 Hz, 1H, NH), 5.05 (d, *J* = 10.3 Hz, 1H, H-1), 4.91 (dd, *J* = 9.9, 9.0 Hz, 1H, H-4), 4.62 (s, 2H, CH_2Ph), 4.49 (dd, *J* = 10.5, 6z.8 Hz, 1H, C*H*H, Fmoc), 4.34 (dd, *J* = 10.5, 7.3 Hz, 1H, C*H*H, Fmoc), 4.31 – 4.16 (m, 4H, H-4, H-6, CH of Fmoc), 3.76 (ddd, *J* = 10.0, 5.2, 2.8 Hz, 1H, H-5), 3.63 (td, *J* = 10.1, 7.8 Hz, 1H, H-2), 2.80 – 2.65 (m, 4H, CH_2 of SEt, CH_2 of Lev), 2.60 (ddd, *J* = 10.3, 6.4, 3.1 Hz, 2H, CH_2 of Lev), 2.16 (s, 3H, CH_3, Lev), 1.29 (t, *J* = 7.4 Hz, 3H, CH_3, SEt). ^{13}C-NMR (100 MHz, $CDCl_3$) : δ 206.50 (CO, Lev), 172.46 (CO, Lev), 161.84 (Fmoc), 154.34 ($NHCOCl_3$), 143.41, 143.16, 141.46, 141.42, 137.29, 128.57, 128.09, 128.07, 127.97, 127.37, 125.25, 125.08, 120.23, 120.21 (Ar), 92.39 (CCl_3), 82.65 (C-1), 78.71 (CH, Fmoc), 75.94 (C-5), 75.32 (C-4), 74.97 (CH_2Ph), 70.48 (CH_2, Fmoc), 62.86 (C-6), 57.81 (C-2), 46.85 (C-3), 38.02 (CH_2, Lev), 29.97 (CH_3, Lev), 28.01 (CH_2, Lev), 24.97 (CH_2, SEt), 15.29 (CH_3, SEt).; HR-ESI MS: *m/z* $[M+Na]^+$ calcd for $C_{37}H_{38}NO_9Cl_3SNa^+$ 800.1225, found 800.1194.

Ethyl 4,6-di-*O*-benzyl-3-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-trichloroacetamino-1-thio-β-D-glucopyranoside A-010

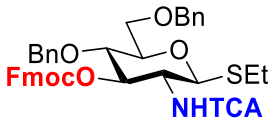


A-010

To a solution of **A-007** (6.0 g, 13.14 mmol) in DCM (66 ml) was added TBSCl (3.96 g, 26.3 mmol, 2 eq.), and Imidazol (2.146 g, 31.5 mmol, 2.4 eq.) at 0 ° C. After reaction was completed, the reaction mixture was quenched with the addition of MeOH (10 mL) at 0ºC, and extracted with DCM. The organic phase was dried over $MgSO_4$, and evaporated *in vacuo*. BF_3OEt_2 (52.5 ml of 1.0 M THF solution, 4 eq.) and TMSOTf (1.187 ml, 6.57 mmol, 0.5 eq.) were added to the solution of the crude at 0 º C, and stirred. After the reaction was completed, the reaction mixture was quenched with sat. $NaHCO_3$ (aq.), and extracted with DCM. The organic phase was dried over $MgSO_4$, and evaporated *in vacuo*. BnBr (4.69 ml, 39.4 mmol, 3 eq.), and NaH (1.577 g 60 % in mineral oil, 39.4 mmol, 3.0 eq.) were added to the solution of the crude (66 ml of DMF:THF 1:10(v:v)) at 0ºC. After the reaction was completed, MeOH was added to the mixture, and extracted with DCM. The organic phase was dried over

$MgSO_4$, and evaporated *in vacuo*. The crude was purified by silica gel column chromatography (hexane:ethylacetate:DCM = 9:0.5:1 to 9:1:1) to give **A-010** (6.7 g, 77%) for 5 steps. R_f: 0.41 (Hexane:EtOAc:DCM = 7:1:2). $[\alpha]_D$ = -2.71 (*c* = 3.52, $CHCl_3$). IR (thin film): υ = 3340, 2928, 2856, 1684, 1522, 1252, 1085, 836 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.36 – 7.11 (m, 10H), 6.98 (d, *J* = 8.8 Hz, 1Hn N*H*), 4.69 (d, *J* = 8.7 Hz, 1H, H-1), 4.62 (dd, *J* = 27.9, 11.6 Hz, 2H, CH_2), 4.55 – 4.45 (m, 2H, CH_2), 3.91 (t, *J* = 7.4 Hz, 1H, H-3), 3.82 (dd, *J* = 16.8, 8.3 Hz, 1H, H-2), 3.78 – 3.61 (m, 3H, H-5, H-6), 3.53 (t, *J* = 7.3 Hz, 1H, H-4), 2.80 – 2.58 (m, 2H), 1.22 (t, *J* = 7.4 Hz, 3H), 0.84 (s, 9H), 0.04 (s, 3H), -0.03 (s, 3H). ^{13}C NMR (100 MHz, cdcl$_3$) δ 161.51, 138.22, 137.93, 128.46, 127.90, 127.78, 127.77, 127.72, 127.55(Ar), 92.61(*C*Cl_3), 82.73(C-1), 78.65(C-5), 78.35(C-4), 74.05(C-3), 74.00(*C*H_2), 73.53(*C*H_2), 69.51(C-6), 57.45(C-2), 25.97(CH_3 of *t*-Bu), 24.84(*C*H_2), 18.07(Cq of *t*-Bu), 15.09(*C*H_3), -3.99(*C*H_3 of TBS), -4.18(*C*H_3 of TBS).; MS ESI+-HRMS *m/z* $[M+Na]^+$ calcd for $C_{30}H_{42}Cl_3NO_5SSiNa^+$ 684.1511, found 684.1921.

Ethyl 4,6-di-*O*-benzyl-3-*O*-fluorenylmethoxycarbonyl-2-deoxy-2-trichloroacetamino-1-thio-β-D-glucopyranoside GlcNAc-3

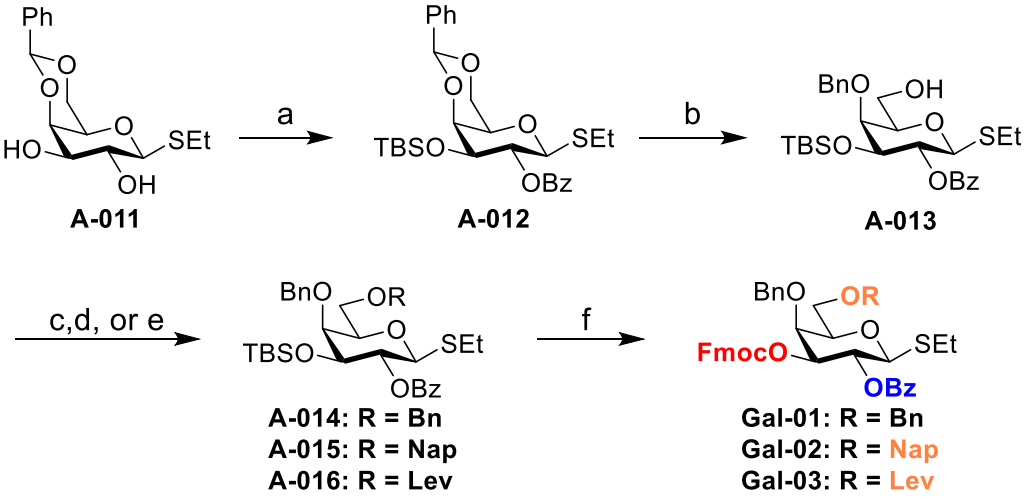


GlcNAc-3

To a solution of compound **A-010** (3.285 g, 4.95 mmol) in dry acetonitrile was added to BF_3OEt_2 (1.2eq). The reaction mixture was stirred in ice bath for 30 min, and quenched with sat. aq. $NaHCO_3$ (30 ml). The precipitated solid was filtered, and dried *in vacuo*. The crude product was used for next reaction. To a solution of the crude and FmocCl (2.56 g, 9.91 mmol, 2.0 eq.) was added anhydrous pyridine (1.202 ml, 4.62 mmol, 3.0 eq.) in ice bath. The reaction mixture was stirred at r. t. for 15h, and HCl(aq.) was added. The extracted organic layer was dried over Na_2SO_4, and concentrated *in vacuo*, was purified by silica gel column chromatography (hexane:ethylacetate:DCM = 7:2:1 to 7:3:1) to give **GlcNAc-03** (3.50 g, 92%). R_f: 0.29 (Hexane:EtOAc:DCM = 8:1:2). $[\alpha]_D$ = -27.57 (*c* = 2.67, $CHCl_3$). IR (thin film): υ = 3361, 3032, 2921, 2868, 1720, 1526, 1451, 1390, 1265 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.75 (dd, *J* = 7.6, 3.0 Hz, 2H), 7.55 (ddd, *J* = 10.3, 7.5, 0.6 Hz, 2H), 7.45 – 7.17 (m, 12H), 7.14 – 7.07 (m, 2H), 6.99 (d, *J* = 9.4 Hz, 1H, N*H*), 5.14 (dd, *J* = 10.4, 9.3 Hz, 1H, H-3), 4.70 – 4.50 (m, 4H, CH_2 x 2), 4.48 (d, *J* = 10.2 Hz, 1H, H-1), 4.30 (m, 2H, CH_2 of Fmoc), 4.22 – 4.09 (m, 2H, C*H* of Fmoc, H-2), 3.85 (t, *J* = 9.5 Hz, 1H, H-3), 3.67 (dd, *J* = 11.1, 3.9

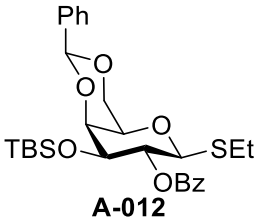
Hz, 1H, H-6a), 3.60 (dd, *J* = 11.1, 1.7 Hz, 1H, s3H). ^{13}C NMR (100 MHz, cdcl$_3$) δ 161.86, 155.53, 143.02, 142.83, 141.22, 141.17, 137.95, 137.42, 128.40, 128.39, 128.01, 127.99, 127.95, 127.80, 127.71, 127.33, 127.23, 125.16, 125.06, 120.08, 120.06(Ar), 92.29(*C*Cl$_3$), 83.79(C-1), 80.25(C-3), 79.13(C-5), 75.72(C-4), 75.13(*C*H$_2$), 73.53(*C*H$_2$), 70.83(*C*H$_2$ of Fmoc), 68.34(C-6), 54.91(C-2), 46.44(*C*H of Fmoc), 24.07, 14.87. MS ESI+-HRMS *m/z* [M+Na]$^+$ calcd for $C_{39}H_{38}NO_7Cl_3SNa^+$ 792.1327, found 792.1299.

A.5 Building Block Preparation: Gal



Scheme A.04. Synthesis of galactose building blocks Gal-01, -02, and -03. a) (i) TBDMSCl, Imidazole; (ii) Bz_2O, NEt_3, DMAP, DCM °C to r.t. 91% over two steps; b) BH_3 in THF, TMSOTf, DCM, 0 °C , 95%; c) BnBr, NaH, DMF, 0 °C, 98%; d) NapBr, THF, DMF, 0 °C, 86%; e) LevOH, DIC, DMAP, DCM, -15 °C 93%; f) (i) BF_3OEt_2, MeCN, 0 °C; (ii) FmocCl, pyridine, DCM, 0 °C, 95% for **Gal-01,** 95% for **Gal-02,** 94% for **Gal-03**.

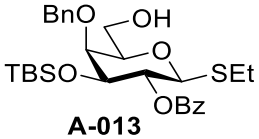
Ethyl 2-O-benzoyl-4,6-O-benzylidene-3-O-tert-butyldimethylsilyl-1-thio-β-D-galactopyranoside A-012



To a solution of compound **A-011** (13 g, 41.6 mmol, 1.0 eq.) in anhydrous DCM (104 mL, 0.4 M) was added TBDMSCl (7.53 g, 49.9 mmol, 1.2 eq.) and imidazole (3.97 g, 58.3 mmol, 1.4 eq.) successively at 0°C, stirred overnight at room temperature. After the mixture was quenched with saturated aqueous $NaHCO_3$, and diluted with DCM, the organic layer was dried over $MgSO_4$ and the solvent was evaporated *in vacuo*. To a solution of this crude in anhydrous DCM (100 mL, 0.4 M) was added benzoic anhydride (18.0 g, 80 mmol, 2.0 eq.), triethylamine (16.7 mL, 120 mmol, 3.0 eq.), and a catalytic amount of DMAP (0.974 g, 7.97 mmol, 0.2 eq.) at 0°C. The mixture was stirred overnight at room temperature. Afterwards, the mixture was quenched with saturated aqueous $NaHCO_3$ and diluted with DCM. The organic layer was dried over $MgSO_4$ and the solvent was evaporated *in vacuo*. The crude was purified by column chromatography on silica gel (hexane/ethyl acetate = 9:1 to 7:3) to afford **A-012** (19.2 g, 36.2 mmol, 91%) over two steps. R_f = 0.57 (hexane/ethyl acetate, 1:1); $[\alpha]_D^{20}$ = +35.44 (c = 1.00, chloroform); IR (thin film): υ = 3090, 2957, 2857, 1721, 1602, 1451, cm^-

[1]; ^{1}H NMR (CDCl3) δ ^{1}H NMR (400 MHz, cdcl$_3$) δ 8.07 – 8.01 (m, 2H), 7.60 – 7.52 (m, 3H), 7.46 – 7.33 (m, 5H), 5.60 (t, *J* = 9.6 Hz, 1H, H-2), 5.53 (s, 1H, C*H*Ph), 4.56 (d, *J* = 9.9 Hz, 1H, H-1), 4.39 (dd, *J* = 12.4, 1.5 Hz, 1H, H-6a), 4.14 (dd, *J* = 3.6, 0.7 Hz, 1H, H-4), 4.05 (dd, *J* = 12.4, 1.8 Hz, 1H. H-6b), 4.03 – 3.99 (m, 1H, H-3), 3.55 (d, *J* = 1.1 Hz, 1H, H-5), 2.99 – 2.65 (m, 2H, CH_2), 1.26 (t, *J* = 7.5 Hz, 3H, CH_3), 0.75 (s, 9H, *tert*-Bu), 0.04 (s, 3H, Me), -0.12 (s, 3H. Me). ^{13}C NMR (100 MHz, cdcl$_3$) δ 165.41(OBz), 138.01, 133.02, 130.45, 129.88, 128.99, 128.41, 128.29, 126.39(Ar), 101.24(*C*HPh), 82.90(C-1), 76.97(C-4), 73.65(C-3), 70.36(C-5), 70.20(C-2), 69.53(C-6), 25.57(*t*-Bu), 22.85(CH_2), 18.05(Cq), 15.00(CH_3), -4.44(Me), -4.58(Me); MS ESI+-HRMS *m/z* $[M+Na]^+$ calcd for $C_{28}H_{38}O_6SSiNa$ 553.2056, found 553.1976.

Ethyl 4-O-benzyl-3-O-tert-butyldimethylsilyl-2-O-benzoyl-1-thio-β-D-galactopyranoside A-013

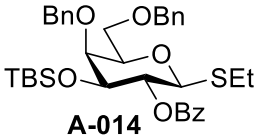


A-013

Compound **A-012** (20 g, 37.7 mmol, 1.0 eq.) was co-evaporated with toluene and dissolved under an Ar atmosphere in DCM (220 mL, 0.17 M). To a solution of compound **A-012** was added 1 M solution of BH_3 in THF (151 mL, 151 mmol, 4.0 eq.), and TMSOTf (3.40 mL, 18.84 mmol, 0.5 eq.) successively at 0°C. The mixture was stirred for 5 h at 0°C. After the mixture was quenched with saturated aqueous $NaHCO_3$ and diluted with DCM, the organic layer was dried over $MgSO_4$ and the solvent was evaporated *in vacuo*. The crude was purified by column chromatography on silica gel (hexane/ethyl acetate = 9:0.5:0.5 to 9:1:0.5) to afford **A-013** (19.5 g, 36.6 mmol, 95%). R_f = 0.28 (hexane/ethyl acetate, 1:1); $[\alpha]_D^{20}$ = +48.68 (c = 2.00, $CHCl_3$); IR (thin film): υ = 3030, 2930, 1719, 1602, 1452, 1352^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 – 8.08 (m, 2H), 7.69 – 7.61 (m, 1H), 7.55 – 7.49 (m, 2H), 7.48 – 7.34 (m, 6H), 5.72 (t, *J* = 9.5 Hz, 1H, H-2), 5.18 (d, *J* = 11.7 Hz, 1H, CH*H*Ph), 4.67 (d, *J* = 11.7 Hz, 1H, CH*H*Ph), 4.58 (d, *J* = 9.8 Hz, 1H, H-1), 4.09 – 4.00 (m, 1H, H-3), 3.92 (dd, *J* = 10.9, 6.4 Hz, 1H, H-6a), 3.85 (d, *J* = 2.2 Hz, 1H, H-4), 3.71 – 3.66 (m, 1H, H-5), 3.63 (dd, *J* = 10.9, 5.2 Hz, 1H, H-6b), 2.92 – 2.66 (m, 2H) 1.28 (t, *J* = 7.5 Hz, 3H, Me), 0.86 (d, *J* = 2.8 Hz, 9H, *t*-Bu), 0.20 (s, 3H, Me), -0.00 (s, 3H, Me). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.50 (Bz), 138.62, 133.10, 130.41, 129.95, 128.57, 128.44, 128.15, 127.94 (Ar), 83.91(C-1), 79.13(C-5), 77.00(C-4), 75.90(C-3), 74.97(*C*H_2Ph), 71.07(C-2), 62.40(C-6), 25.67(*t*-Bu), 23.76(CH_2),

17.94(Cq), 14.97(Me), -3.85(Me), -4.90(Me); MS ESI+-HRMS *m/z* $[M+Na]^+$ calcd for $C_{28}H_{40}O_6SSiNa$ 555.2213, found 555.2126.

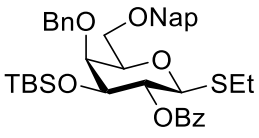
Ethyl 2-O-benzoyl-4,6-di-O-benzyl-3-O-tert-butyldimethylsilyl-1-thio-β-D-galactopyranoside A-014



A-014

To a solution of compound **A-013** (4.01 g, 7.51 mmol, 1.0 eq.) in anhydrous DMF and THF (58.7 mL, 1:9), were added BnBr (2.68 mL, 22.52 mmol, 3.0 eq.) and sodium hydride (0.721 mg, 18.02 mmol, 2.4 eq) portionwise at 0°C. After the reaction mixture was stirred for 2 h at 0°C the mixture was quenched with saturated aqueous NH_4C, diluted with DCM, over Mg_2SO_4. Following evaporation of the solvent, the crude product was purified by column chromatography on silica gel (hexane/ethyl acetate = 9:0.5:0.5 to 9:1:1) to afford **A-014** (4.58g, 7.36 mmol, 98%). R_f (hexane:ethylacetate:DCM = 8:2:1) = 0.75; $[\alpha]_D^{20}$= +19.04 (c = 1.0, $CHCl_3$). IR (thin film): υ = 3032, 1726, 1603, 1497, 1453 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.08 – 8.03 (m, 2H), 7.59 – 7.53 (m, 1H), 7.48 – 7.41 (m, 2H), 7.39 – 7.26 (m, 10H), 5.64 (t, *J* = 9.5 Hz, 1H, H-2), 5.10 (d, *J* = 11.5 Hz, 1H, CH*H*Ph), 4.54 (d, *J* = 11.5 Hz, 1H, CH*H*Ph), 4.50 (d, *J* = 9.7 Hz, 1H, H-1), 4.46 (dd, *J* = 16.0, 11.8 Hz, 1H, CH_2Ph), 3.97 (d, *J* = 9.3 Hz, 1H, H-3), 3.86 (d, *J* = 2.2 Hz, 1H, H-4), 3.74 (dd, *J* = 9.7, 3.6 Hz, 1H, H-5), 3.64 (d, *J* = 6.3 Hz, 2H, H-6), 2.81 – 2.60 (m, 1H, CH_2), 1.20 (t, *J* = 7.5 Hz, 2H, Me), 0.78 (s, 9H), 0.12 (s, 3H), -0.08 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.49(Bz), 139.02, 138.06, 133.02, 130.50, 129.94, 128.56, 128.40, 128.33, 128.04, 127.92, 127.79, 127.51(Ar), 83.78(C-1), 77.66(C-5), 77.44(C-4), 75.80(C-3), 75.30(*C*H_2Ph), 73.69(*C*H_2Ph), 71.19(C-2), 68.81(C-6), 25.67(*t*-Bu), 23.67(CH_2 thio), 17.94(Cq), 14.95(Me), -3.89(Me), -4.90(Me). MS ESI+-HRMS *m/z* $[M+Na]^+$ calcd for $C_{35}H_{46}O_6SSiNa$ 645.2682, found 645.2607.

Ethyl 2-*O*-benzoyl-4-*O*-benzyl-3-*O*-*tert*-butyldimethylsilyl-6-*O*-(2-naphthylmethyl)-1-thio-β-D-galactopyranoside A-015

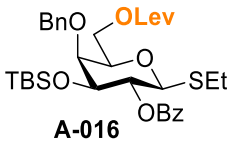


A-015

To a solution of **A-013** (3.92 g, 7.32 mmol) and NapBr (3.24 g, 14.64 mmol) in anhydrous DMF–THF (1:9, v/v, 53 mL) was added NaH (0.703 mg, 17.6 mmol) at 0 °C, and the

reaction mixture was stirred at 0 °C for 1 h. The reaction was quenched with saturated aqueous NH_4Cl, extracted with Et_2O, and dried with Mg_2SO_4. The organic layer was concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (hexane–ethylacetate–DCM = 9:0.5:1 to 9:1:1, v/v/v) to give **A-015** as a colorless solid (4.23 g, 86%). R_f (hexane:ethyl acetate:DCM = 8:2:1) = 0.79; $[\alpha]_D^{20}$= +10.64 (c = 1.0, $CHCl_3$). IR (thin film): υ = 3060, 2954, 2856, 1727, 1602, 1452, 1349, 1265, 1152, 1105, 1070, 1027, 910, 838, 708cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 – 8.00 (m, 2H), 7.84 – 7.75 (m, 3H), 7.71 (s, 1H), 7.59 – 7.49 (m, 1H), 7.49 – 7.36 (m, 5H), 7.31 – 7.17 (m, 5H), 5.61 (t, *J* = 9.4 Hz, 1H, H-2), 5.06 (d, *J* = 11.5 Hz, 1H, C*H*HPh), 4.59 (q, *J* = 12.0 Hz, 2H, CH_2NAP), 4.49 (d, *J* = 11.5 Hz, 1H, C*H*HPh), 4.48 (d, *J* = 9.7 Hz, 1H, H-1), 3.99 – 3.90 (m, 1H, H-3), 3.85 (d, *J* = 2.3 Hz, 1H, H-4), 3.74 (dd, *J* = 6.9, 5.9 Hz, 1H, H-5), 3.67 – 3.61 (m, 1H, H-6), 2.79 – 2.54 (m, 2H. CH_2 thio), 1.17 (t, *J* = 7.5 Hz, 3H, Me thio), 0.75 (s, 9H, *tert*-Bu Si), 0.09 (s, 3H, Me Si), -0.11 (s, 3H, Me Si). ^{13}C NMR (101 MHz, cdcl$_3$) δ 165.48(C=O), 138.95, 135.50, 133.37, 133.16, 133.01, 130.49, 129.93, 128.40, 128.36, 128.29, 128.02, 127.82, 127.76, 127.48, 126.91, 126.29, 126.09, 125.99(Ar), 83.79(C-1), 77.69(C-5), 77.43(C-4), 75.80(C-3), 75.27(CH_2Ph), 73.77(CH_2 of NAP), 71.21(C-2), 68.81(C-6), 25.67(*tert*-Bu Si), 23.67(CH_2 thio), 17.93(Cq Si), 14.94(Me thio), -3.89(Me Si), -4.91(Me Si). MS ESI+-HRMS *m/z* $[M+Na]^+$ calcd for $C_{39}H_{48}O_6SSiNa$ 695.2839, found 695.2759.

Ethyl 2-*O*-benzoyl-4-*O*-benzyl-3-*O*-*tert*-butyldimethylsilyl-6-*O*-levulinyl-1-thio-β-D-galactopyranoside A-016

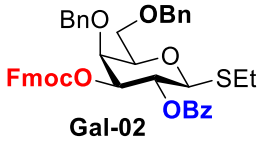


A-016

To a solution of compound **A-013** (4.28 g, 8.03 mmol) and DMAP (0.294 g, 2.41 mmol) in anhydrous DCM (40 mL) were added levulinic acid (2.80 g, 24.1 mmol) and DIC (3.75 mL, 24.10 mmol) at 0 °C, and the mixture was stirred for 1 h at 0 °C. The mixture was quenched with saturated aqueous $NaHCO_3$, diluted with DCM, and the organic layer was separated. The aqueous layer was extracted with DCM. The organic layers were combined, dried over $MgSO_4$, and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane–ethyl acetate-DCM = 9:1:1 to 7:3:1, v/v/v) to afford **A-016** (4.7 g, 7.45 mmol, 93%). R_f (hexane:ethyl acetate:DCM = 7:3:1) = 0.51; $[\alpha]_D^{20}$= +48.68 (c = 2.0, $CHCl_3$). IR (thin film): υ = 2886, 1719, 1452, 1352, 1259, 1069cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 – 7.99 (m, 2H), 7.62 – 7.50 (m, 1H), 7.48 – 7.41 (m, 2H), 7.41 – 7.31

(m, 4H), 7.31 – 7.25 (m, 1H), 5.63 (t, *J* = 9.5 Hz, 1H, H-2), 5.12 (d, *J* = 11.5 Hz, 1H, CH*H*Ph), 4.59 (d, *J* = 11.5 Hz, 1H, CH*H*Ph), 4.51 (d, *J* = 9.7 Hz, 1H, H-1), 4.27 (dd, *J* = 11.2, 6.6 Hz, 1H, H-6a), 4.19 (dd, *J* = 11.2, 5.8 Hz, 1H, H-6b), 3.98 (d, *J* = 9.2 Hz, 1H, H-3), 3.82 – 3.70 (m, 2H, H-4,5), 2.82 – 2.61 (m, 4H, CH_2 thio, CH_2 Lev), 2.57 – 2.46 (m, 2H, CH_2 of Lev), 2.18 (s, 3H, CH_3 of Lev), 1.21 (t, *J* = 7.5 Hz, 3H, CH_3 thio), 0.78 (s, 9H, *tert*-Bu of Si), 0.13 (s, 3H, Me of Si), -0.07 (s, 3H, Me of Si). ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.60(C=O, Lev), 172.61(C=O, Lev), 165.46(C=O, Bz), 138.66, 133.07, 130.41, 129.93, 128.42, 128.41, 127.94, 127.67(Ar), 83.85(C-1), 77.20(C-4), 76.23(C-15, 75.71(C-3), 75.16(CH_2Ph), 70.99(C-2), 63.62(C-6), 38.07(CH_2 Lev), 29.95(Me, Lev), 28.02(CH_2, Lev), 25.66(*tert*-Bu, Si), 23.86(CH_2, thio), 17.92(Cq, Si), 14.96(Me, thio), -3.89(Me, Si), -4.94(Me, Si). MS ESI+-HRMS *m/z* $[M+Na]^+$ calcd for $C_{33}H_{46}O8SSiNa$ 653.2580, found 653.2530.

Ethyl 2-O-benzoyl-4,6-di-O-benzyl-3-O-fluorenylmethoxycarbonyl-1-thio-β-D-galactopyranoside Gal-01

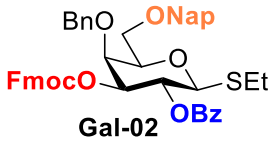


Gal-02

To a solution of compound **A-014** (3.77 g, 6.05 mmol, 1.0 eq.) in anhydrous acetonitrile was added boron trifluoride diethyl etherate ($BF_3{\cdot}OEt_2$) (0.920 ml, 7.26 mmol, 1.2 eq.) at 0°C. After the reaction mixture was stirred for 20 min at 0°C the mixture was quenched with saturated aqueous $NaHCO_3$ (30 mL), diluted with DCM, and dried over $MgSO_4$. The solvent was evaporated *in vacuo*. To a solution of this crude in anhydrous DCM (30 mL) was added 9-fluorenylmethylchloroformate (3.91 g, 15.12 mmol, 2.5 eq.) and pyridine (1.47 mL, 18.14 mmol, 3.0 eq.) successively at 0°C, and stirred overnight at room temperature. After the mixture was quenched with 1 M aqueous HCl, and diluted with DCM. The organic layer was dried over $MgSO_4$ and the solvent evaporated *in vacuo*. The crude was purified by column chromatography on silica gel (hexane:ethylacetate:DCM = 8:1:1 to 8:2:1) to afford **Gal-01** (4.21 g, 5.76 mmol, 95%). **NMR after first step A-014:** ^{1}H NMR (400 MHz, $cdcl_3$) δ 8.09 – 8.00 (m, 2H), 7.56 (t, *J* = 7.4 Hz, 1H), 7.44 (t, *J* = 7.7 Hz, 2H), 7.40 – 7.25 (m, 10H), 5.30 (t, *J* = 9.7 Hz, 1H, H-2), 4.73 (q, *J* = 11.8 Hz, 2H, Bn), 4.62 – 4.47 (m, 3H, H-1, Bn), 4.01 (d, *J* = 3.5 Hz, 1H, H-4), 3.84 – 3.68 (m, 3H, H-3,5,6), 2.87 – 2.57 (m, 3H, CH_2, thio), 2.37 (d, *J* = 9.7 Hz, 1H, *H*O-), 1.24 (t, *J* = 7.4 Hz, 3H, , Me, thio).^{13}C NMR (100 MHz, $CDCl_3$) δ 166.70(C=O, Bz), 138.22, 137.77, 133.34, 130.08, 129.92, 128.73, 128.64, 128.49, 128.10, 128.08, 128.05(Ar), 83.47(C-1), 77.56(C-5), 76.84(C-4), 75.58(Bn), 74.20(C-3), 73.74(Bn),

72.47(C-2), 68.30(C-6), 24.01(CH_2, thio), 15.10(Me, thio). **Product Gal-01:** R_f (hexane:ethylacetate:DCM = 8:2:1) = 0.49; $[\alpha]_D^{20}$= +34.08 (c = 2.69, $CHCl_3$). IR (thin film): υ = 2870, 1730, 1602, 1496, 1451 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.06 (dt, *J* = 8.5, 1.6 Hz, 2H), 7.74 – 7.66 (m, 2H), 7.62 – 7.27 (m, 9H), 7.13 (dtd, *J* = 8.6, 7.5, 1.1 Hz, 17H), 5.76 (t, *J* = 9.9 Hz, 1H, H-2), 5.09 (dd, *J* = 10.0, 3.0 Hz, 1H, H-3), 4.80 (d, *J* = 11.5 Hz, 1H, CH*H*Ph), 4.62 (d, *J* = 9.9 Hz, 1H, H-1), 4.58 – 4.42 (m, 3H, Bn, , CH*H*Ph), 4.31 (dd, *J* = 10.4, 7.1 Hz, 1H, H-6a), 4.23 (dd, *J* = 10.4, 7.8 Hz, 1H, H-6b), 4.18 – 4.14 (m, 1H, H-4), 4.07 (t, *J* = 7.4 Hz, 1H, H-5), 3.84 (t, *J* = 6.9 Hz, 1H, CH Fmoc), 3.68 (d, *J* = 6.7 Hz, 2H, CH_2 Fmoc), 2.76 (qq, *J* = 12.4, 7.5 Hz, 2H, CH_2), 1.25 (t, *J* = 7.5 Hz, 3H, Me). ^{13}C NMR (101 MHz, cdcl$_3$) δ 165.35(Bz), 154.64(Fmoc), 143.38, 142.95, 141.32, 141.21, 138.04, 137.84, 133.31, 130.05, 129.70, 128.57, 128.49, 128.42, 128.26, 128.00, 127.98, 127.92, 127.83, 127.22, 127.19, 125.28, 125.07, 120.07, 120.06(Ar), 83.84(C-1), 79.14(C-3), 77.36(CH, Fmoc), 75.20(Bn), 74.13(C-4), 73.67(Bn), 70.21(C-6), 68.70(C-2), 68.18(CH_2, Fmoc), 46.59(C-5), 23.99(CH_2), 14.93(CH_3). MS ESI+-HRMS *m/z* $[M+Na]^+$ calcd for $C_{44}H_{42}O_8SNa$ 753.2498, found 753.2442.

Ethyl 2-*O*-benzoyl-4-*O*-benzyl-3-*O*-fluorenylmethoxycarbonyl-6-*O*-(2-naphthylmethyl)-1-thio-β-D-galactopyranoside Gal-02

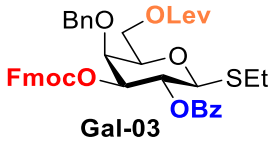


Gal-02

To a solution of **A-015** (4.23 g, 5.42 mmol) in anhydrous acetonitrile (68 mL, 0.08 M) was added $BF_3{\cdot}OEt_2$ (0.76 mL, 5.96 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 20 min. The reaction was quenched with saturated aqueous $NaHCO_3$ (30 mL), diluted with DCM and the organic layer was separated. The aqueous layer was extracted with DCM. The organic layers were combined, dried over $MgSO_4$, and concentrated *in vacuo*. The crude product was dissolved in anhydrous DCM (30 mL). To the solution was added 9-FmocCl (2.80 g, 10.8 mmol) and pyridine (1.10 mL, 13.6 mmol) successively at 0 °C, and the mixture was stirred overnight at room temperature. The mixture was quenched with saturated aqueous $NaHCO_3$, diluted with DCM, and the organic layer was separated. The aqueous layer was extracted with DCM. The organic layers were combined, washed with 1 M aqueous HCl, dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane–ethylacetate–DCM = 8:1:1 to 8:2:1, v/v/v) to afford **Gal-02** (3.97 g, 5.09 mmol, 94%). R_f: 0.31 (hexane: ethyl acetate:DCM = 9:1:1); $[\alpha]_D^{20}$=

+32.12 (c = 2.67, $CHCl_3$); IR (thin film): υ = 2870, 1745, 1450, 1272, 1154, 1095, 1027, 819, 740, 709cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.20 – 8.01 (m, 2H), 7.94 – 7.82 (m, 3H), 7.78 (s, 1H), 7.75 – 7.65 (m, 2H), 7.63 – 7.23 (m, 15H), 7.14 (dtd, *J* = 8.6, 7.5, 1.1 Hz, 2H), 5.78 (t, *J* = 9.9 Hz, 1H, H-2), 5.11 (dd, *J* = 10.0, 3.0 Hz, 1H, H-3), 4.82 (d, *J* = 11.4 Hz, 1H, CH*H*Ph), 4.67 (dd, *J* = 26.7, 11.0 Hz, 3H, H-1, CH_2Nap), 4.52 (d, *J* = 11.5 Hz, 1H, CH*H*Ph), 4.33 (dd, *J* = 10.4, 7.2 Hz, 1H, H-6a), 4.24 (dd, *J* = 10.4, 7.8 Hz, 1H, H-6b), 4.18 (dd, *J* = 2.9, 0.6 Hz, 1H, H-4), 4.08 (t, *J* = 7.4 Hz, 1H, H-5), 3.88 (dd, *J* = 7.4, 6.3 Hz, 1H, CH, Fmoc), 3.79 – 3.68 (m, 2H, CH_2 Fmoc), 2.84 – 2.69 (m, 2H, CH_2 thio), 1.26 (t, *J* = 7.5 Hz, 3H, Me thio). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.36(C=O, Bz), 154.65(C=O, Fmoc), 143.38, 142.96, 141.32, 141.22, 137.98, 135.29, 133.36, 133.32, 133.18, 130.06, 129.71, 128.50, 128.40, 128.23, 128.03, 127.92, 127.84, 127.82, 127.23, 127.20, 126.89, 126.32, 126.13, 125.95, 125.29, 125.07, 120.07(Ar), 83.86(C-1), 79.17(C-3), 77.42(*C*H, Fmoc), 75.20(CH_2, Bn), 74.13(C-4), 73.79(CH_2, Nap), 70.24(C-6), 68.72(C-2), 68.21(CH_2, Fmoc), 46.59(C-5), 24.01(CH_2 thio), 14.93(CH_3 thio). MS ESI+-HRMS *m/z* $[M+Na]^+$ calcd for $C_{48}H_{44}O_8SNa$ 803.2655, found 803.2601.

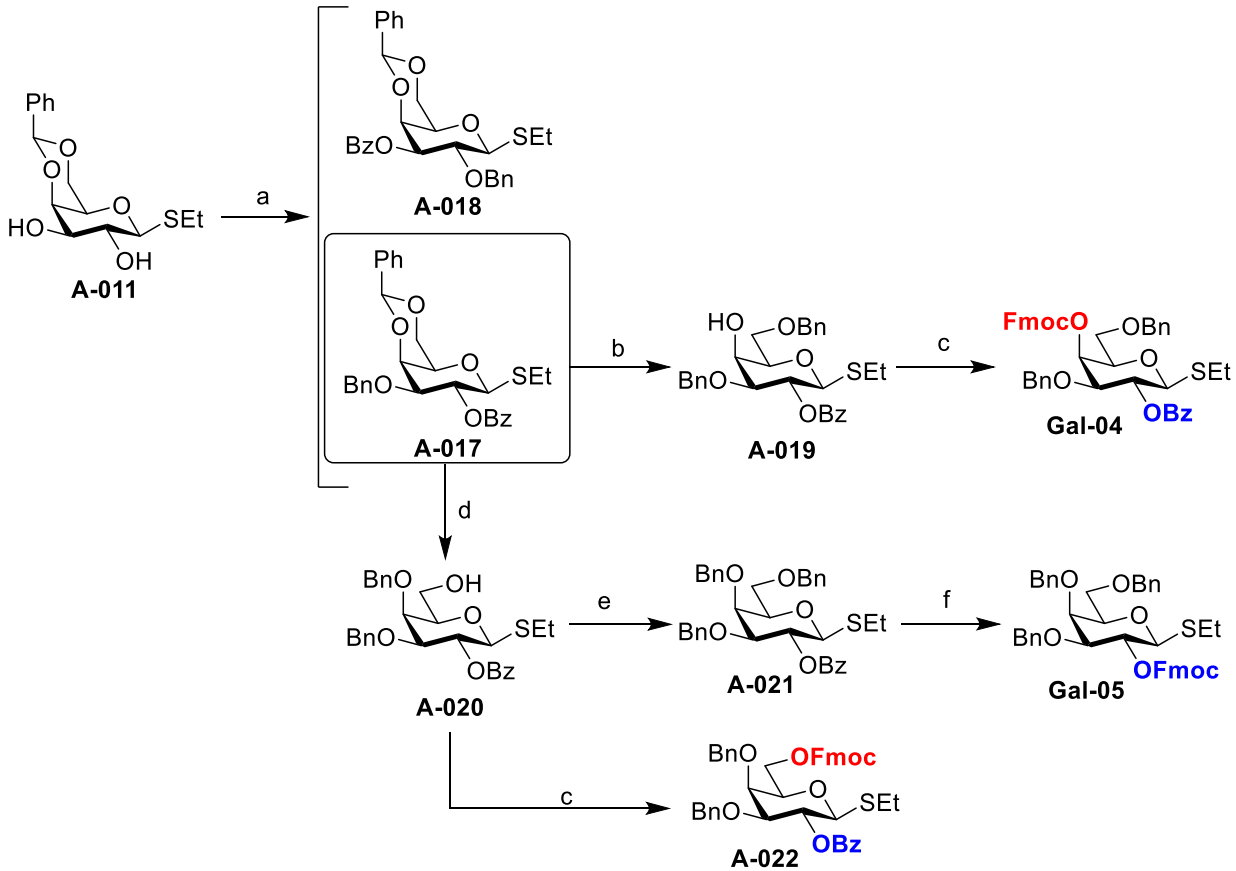
Ethyl 2-*O*-benzoyl-4-*O*-benzyl-3-*O*-fluorenylmethoxycarbonyl-6-*O*-levulinyl-1-thio-β-D-galactopyranoside Gal-03



Gal-03

To a solution of compound **A-016** (4.7 g, 7.45 mmol) in anhydrous acetonitrile (93 mL) was added $BF_3{\cdot}OEt_2$ (1.13 mL, 8.94 mmol) and the mixture was stirred at 0 °C for 20 min. The mixture was quenched with saturated aqueous $NaHCO_3$, diluted with DCM, and the organic layer was separated. The aqueous layer was extracted with DCM. The organic layers were combined, dried over $MgSO_4$, and concentrated *in vacuo*. The crude product was dissolved in anhydrous DCM (36 mL). To the solution was added 9-fluorenylmethylchloroformate (4.63 g, 17.91 mmol) and pyridine (1.74 mL, 21.49 mmol) successively at 0 °C, and the mixture was stirred overnight at room temperature. The mixture was quenched with saturated aqueous $NaHCO_3$, diluted with DCM, and the organic layer was separated. The aqueous layer was extracted with DCM. The organic layers were combined, washed with 1 M aqueous HCl, dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane–ethyl acetate–DCM = 8:1:1 to 8:2:1, v/v/v) to afford **Gal-03** (5.0 g, 6.77 mmol, 94%). R_f (hexane:ethyl acetate:DCM = 7:3:1) = 0.29; $[\alpha]_D^{20}$=

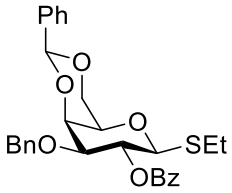
+33.58 (c = 2.0, $CHCl_3$). IR (thin film): υ = 3060, 1732, 1602, 1451, 1359, 1272, 1156 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.14 – 7.92 (m, 2H), 7.71 – 7.65 (m, 2H), 7.56 – 7.50 (m, 1H), 7.49 – 7.27 (m, 11H), 7.18 – 7.06 (m, 2H), 5.75 (t, *J* = 10.0 Hz, 1H, H-2), 5.08 (dd, *J* = 10.0, 2.9 Hz, 1H, H-3), 4.85 (d, *J* = 11.4 Hz, 1H, CH*H*Ph), 4.62 (d, *J* = 9.9 Hz, 1H, H-1), 4.55 (d, *J* = 11.4 Hz, 1H, CH*H*Ph), 4.37 – 4.20 (m, 3H, CH_2 Fmoc, H-6a), 4.16 (dd, *J* = 11.1, 6.4 Hz, 1H, H-6b), 4.07 (dd, *J* = 13.9, 5.2 Hz, 2H, C*H* Fmoc, H-4), 3.82 (dd, *J* = 6.8, 6.0 Hz, 1H, H-5), 2.84 – 2.66 (m, 4H, CH_2 Lev, CH_2 thio), 2.53 (t, *J* = 6.4 Hz, 2H, CH_2 Lev), 2.19 (s, 3H, Me Lev), 1.24 (t, *J* = 7.5 Hz, 3H, Me Lev). ^{13}C NMR ($CDCl_3$) ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.41(C=O, Lev), 172.30(C=O, Lev), 165.20(C=O, Bz), 154.49(C=O, Fmoc), 143.21, 142.75, 141.20, 141.08, 137.42, 133.25, 129.93, 129.47, 128.41, 128.39, 128.36, 127.94, 127.83, 127.11, 127.07, 125.15, 124.91, 119.98, 119.97(Ar), 83.82(C-1), 78.96(C-2), 75.90(C-5), 75.01(CH_2Ph), 73.52(C-4), 70.21(C-6), 68.36(C-2), 62.66(CH_2, Fmoc), 46.43(CH, Fmoc), 37.86(CH_2, Lev), 29.85(Me, Lev), 27.75(CH_2, Lev), 24.06(CH_2, thio), 14.82(Me, thio), MS ESI+-HRMS *m/z* $[M+Na]^+$ calcd for $C_{42}H_{42}O_{10}SNa$ 761.2396, found 761.2333.



Scheme A.05. Synthesis of galactose building blocks Gal-04 and Gal-05. a) (i) TBDMSCl, Imidazole, DCM; (ii) BnBr, NaH, DMF, 0 °C; (iii) TBAF, THF; (iv) Bz_2O, NEt_3, DMAP, DCM °C to

r.t. 79% for **A-017** over four steps, 17% for **A-018**; b)) $HSiEt_3$, TFAA, TFA, DCM, 0 °C to r.t. 97%; c) FmocCl, pyridine, DCM, 0 °C, 95% for **Gal-04**, 93% for **A-022**; d) BH_3 in THF, TMSOTf, DCM, 0 °C , 95%; e) BnBr, NaH, DMF, 0 °C, 98%; f) (i) NaOMe, MeOH; (ii) FmocCl, pyridine, DCM, 0 °C, 95% for **Gal-05.**

Ethyl 2-O-benzoyl-4,6-di-O-benzyl-4-O-fluorenylmethoxycarbonyl-1-thio-β-D-galactopyranoside A-017

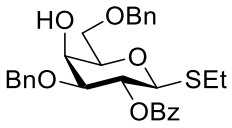


A-017

To a solution of **A-011** (14.7 g, 47.1 mmol, 1.0 eq.) in DCM (118 mL, 0.4 M) were added TBSCl (7.80 g, 51.8 mmol, 1.1 eq.) and imidazole (4.48 g, 65.9 mmol, 1.4 eq.). After the mixture was stirred for 12 h at room temperature, the mixture was quenched with saturated aqueous $NaHCO_3$, diluted with DCM, and dried over $MgSO_4$, the organic layer was evaporated *in vacuo*. To a solution of the crude in anhydrous DMF (234 mL, 0.2 M) were added BnBr (16.7 mL, 141 mmol, 3.0 eq.) and sodium hydride (4.50 g, 113 mmol, 2.4 eq) portionwise at 0°C. The reaction mixture was stirred at room temperature for 1 h and quenched with saturated aqueous NH_4Cl, extracted with Et_2O, and dried with Mg_2SO_4. To a solution of the crude mixture in THF (117 mL, 0.4 M) was added tetra-*N*-butylammonium fluoride (TBAF) (94 mL, 94 mmol, 2.0 eq.) at room temperature. After 4 h, the reaction mixture was concentrated *in vacuo*, re-dissolved in DCM, and extracted with 0.1 M of $CuSO_4$ aqueous solution. After the organic layer was dried over $MgSO_4$, and evaporated *in vacuo*, to a solution of this crude mixture in anhydrous DCM (157 mL, 0.4 M) were added benzoic anhydride (21.36 g, 94 mmol, 2.0 eq.), triethylamine (26.3 mL, 189 mmol, 4.0 eq.), and a catalytic amount of DMAP (1.15 g, 9.44 mmol, 0.2 eq.) at 0°C, and the mixture was stirred overnight at room temperature. After the mixture was quenched with saturated aqueous $NaHCO_3$, diluted with DCM, and dried over $MgSO_4$ the solvent was evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane:ethylacetate:DCM = 8/1/0.5 to 8/2/0.5) to give **A-018** (4.01g, 7.92 mmol, 16.8%) and **A-017** (19.0 g, 37.5 mmol, 79%) over four steps. **A-018** R_f: 0.19 (Hexane:EtOAc:DCM = 9:1:1); $[\alpha]_D^{20}$= +12.85 (c = 2.01, $CHCl_3$); IR (thin film): υ = 2871, 1719, 1453, 1295, 1261 cm-1; δ ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 – 8.03 (m, 2H), 7.60 – 7.55 (m, 1H), 7.52 – 7.41 (m, 4H), 7.39 – 7.32 (m, 3H), 7.25 – 7.15 (m, 5H), 5.51 (s, 1H), 5.23 (dd, *J* = 9.7, 3.6 Hz, 1H,

H-3), 4.91 (d, *J* = 10.5 Hz, 1H, C*H*HPh), 4.70 (d, *J* = 10.5 Hz, 1H, C*H*HPh), 4.60 (d, *J* = 9.6 Hz, 1H, H-1), 4.54 (dd, *J* = 3.6, 0.9 Hz, 1H, H-4), 4.37 (dd, *J* = 12.4, 1.6 Hz, 1H, H-6), 4.10 (d, *J* = 9.6 Hz, 1H, H-2), 4.07 – 4.00 (m, 1H, H-6), 3.60 (d, *J* = 1.1 Hz, 1H, H-5), 2.97 – 2.72 (m, 2H), 1.36 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.23 (Bz), 138.03, 137.93, 133.39, 129.97, 129.03, 128.58, 128.37, 128.24, 128.23, 127.85, 126.42 (Ar), 101.00 (CHPh), 84.86 (C-1), 76.01 (C-3), 75.74 (CH_2Ph), 75.57 (C-2), 74.34 (C-4), 69.76 (C-6), 69.38 (C-5), 24.42, 15.16. MS ESI+-HRMS *m/z* [M+Na]+ calcd for $C_{29}H_{30}O_6SNa$ 529.1661, found 529.1655. **A-017:** Rf = 0.18 (hexane/ethyl acetate/DCM, 9:1:1); $[\alpha]_D^{20}$ = +28.01 (c = 2.75, chloroform); IR (thin film): υ = 2871, 1719, 1261 cm-1; δ ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 – 8.02 (m, 2H), 7.62 – 7.53 (m, 3H), 7.50 – 7.43 (m, 2H), 7.43 – 7.34 (m, 3H), 7.26 – 7.16 (m, 5H), 5.74 (t, *J* = 9.7 Hz, 1H, H-2), 5.52 (s, 1H, CHPh), 4.66 (q, *J* = 12.8 Hz, 2H, CH_2Ph), 4.55 (d, *J* = 9.9 Hz, 1H, H-1), 4.36 (dd, *J* = 12.3, 1.5 Hz, 1H, H-6), 4.28 (dd, *J* = 3.4, 0.8 Hz, 1H, H-4), 4.02 (dd, *J* = 12.4, 1.7 Hz, 1H, H-6), 3.76 (dd, *J* = 9.6, 3.4 Hz, 1H, H-3), 3.47 (d, *J* = 1.1 Hz, 1H, H-5), 2.93 (dq, *J* = 12.3, 7.5 Hz, 1H, C*H*HCH_3), 2.78 (dq, *J* = 12.3, 7.5 Hz, 1H, C*H*HCH_3), 1.28 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.37 (Bz), 137.95, 137.89, 133.11, 130.27, 129.98, 129.15, 128.44, 128.42, 128.32, 127.83, 127.78, 126.59 (Ar), 101.47 (C*H*Ph), 83.04 (C-1), 78.29 (C-3), 73.57 (C-4), 71.13 (CH_2Ph), 70.28 (C-5), 69.50 (C-6), 68.91 (C-2), 22.88, 14.99.; MS ESI+-HRMS *m/z* [M+Na]+ calcd for $C_{29}H_{30}O_6SNa$ 529.1661, found 529.1656.

Ethyl 2-*O*-benzoyl-4,6-di-*O*-benzyl-1-thio-β-D-galactopyranoside A-019

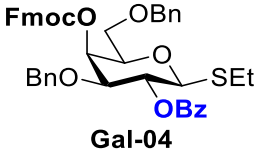


A-019

Compound **A-017** (8.25 g, 16.28 mmol, 1.0 eq.) was co-evaporated with toluene and dissolved under an Ar atmosphere in DCM (96 mL, 0.17 M). To a solution of **A-017** were added triethylsilane (15.6 mL, 98 mmol, 6.0 eq.) and trifluoroacetic anhydride (1.15 mL, 8.14 mmol, 0.5 eq.) at 0°C. After 30 min, trifluoroacetic acid (6.27 mL, 81 mmol, 5.0 eq.) was added dropwise, and the reaction mixture was stirred and allowed to warm to room temperature. After the mixture was quenched with saturated aqueous $NaHCO_3$, diluted with DCM, dried over $MgSO_4$ the solvent was evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane/ethyl acetate = 9:1 to 7:3) to afford **A-019** (8.01 g, 15.73 mmol, 97 %). R_f : 0.21 (Hexane/EtOAc/DCM : 8/2/0.5). $[\alpha]_D^{25}$ = 32.14

(C= 3.05, $CHCl_3$). IR (thin film): υ = 2871, 1723, 1453, 1268 cm-1 δ ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 – 8.00 (m, 2H), 7.62 – 7.56 (m, 1H), 7.49 – 7.43 (m, 2H), 7.39 – 7.28 (m, 5H), 7.23 – 7.14 (m, 5H), 5.53 (t, *J* = 9.7 Hz, 1H, H-2), 4.68 (d, *J* = 12.3 Hz, 1H, CH_2Ph) 4.60 (s, 2H, CH_2Ph), 4.54 (d, *J* = 12.3 Hz, 1H, CH_2Ph), 4.49 (d, *J* = 10.0 Hz, 1H, H-1), 4.19 (d, *J* = 2.8 Hz, 1H, H-4), 3.84 (dd, *J* = 9.8, 6.4 Hz, 1H, H-6), 3.77 (dd, *J* = 9.8, 5.6 Hz, 1H, H-6), 3.69 (d, *J* = 5.9 Hz, 1H, H-5), 3.66 (dd, *J* = 9.3, 3.2 Hz, 1H, H-3), 2.82 – 2.64 (m, 2H), 2.61 (br, 1H, OH), 1.22 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (100 MHz, cdcl3) δ 165.56 (Bz), 138.07, 137.27, 133.22, 130.09, 130.01, 128.58, 128.55, 128.48, 128.08, 127.97, 127.97, 127.93 (Ar), 83.59 (C-1), 79.46 (C-3), 77.43 (C-5), 73.88 (CH_2Ph), 71.46 (CH_2Ph), 69.67 (C-2), 69.12 (C-6), 66.49 (C-4), 23.76, 15.01. MS ESI-HRMS *m/z* $[M+Na]^+$ calcd for $C_{29}H_{32}O_6SNa$ 531.1817, found 531.1811.

Ethyl 2-O-benzoyl-4,6-di-O-benzyl-4-O-fluorenylmethoxycarbonyl-1-thio-β-D-galactopyranoside Gal-04

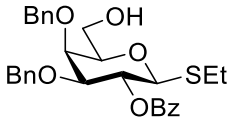


Gal-04

To a solution of compound **A-019** (7.7 g, 15.14 mmol, 1.0 eq) in anhydrous DCM (76 mL) were added 9-fluorenylmethylchloroformate (7.83 g, 30.3 mmol, 2.0 eq.) and pyridine (3.67 mL, 45.4 mmol, 3.0 eq.) successively at 0°C, and stirred overnight at room temperature. After the mixture was quenched with 1M aqueous HCl, and diluted with DCM he organic layer was dried over MgSO4 and the solvent was evaporated *in vacuo*. The crude was purified by column chromatography on silica gel (hexane:ethylacetate:DCM = 8:1:1 to 8:2:1) to afford **Gal-04** (10.2 g, 13.96 mmol, 92%). R_f = 0.15 (hexane/ethyl acetate/DCM, 9:1:0.5); $[\alpha]_D^{20}$ = +34.81 (c = 3.30, $CHCl_3$); IR (thin film): υ = 2871, 1748, 1727, 1451 cm-1; ^{1}H NMR (CDCl3) 8.01 (dd, *J* = 7.3, 1.1 Hz, 2H), 7.77 (dd, *J* = 7.2, 2.5 Hz, 2H), 7.70 (d, *J* = 7.4 Hz, 1H), 7.60 (ddd, *J* = 7.4, 6.6, 4.1 Hz, 2H), 7.50 – 7.28 (m, 10H), 7.26 – 7.20 (m, 1H), 7.16 – 6.99 (m, 5H), 5.60 (t, *J* = 9.8 Hz, 1H, H-2), 5.56 (d, *J* = 3.2 Hz, 1H, H-4), 4.70 (d, *J* = 12.6 Hz, 1H, C*H*HPh), 4.57 – 4.46 (m, 5H, H-1, C*H*HPh, CH_2Ph, C*H*H of Fmoc), 4.29 – 4.22 (m, 2H, C*H*H of Fmoc, CH of Fmoc), 3.85 (t, *J* = 6.5 Hz, 1H, H-5), 3.76 – 3.70 (m, 2H, H-3, H-6), 3.65 (dd, *J* = 9.3, 7.5 Hz, 1H, H-6)., 2.87 – 2.68 (m, 2H), 1.26 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (100 MHz, cdcl3) δ 165.33 (Bz), 155.17 (Fmoc), 143.80, 143.29, 141.45, 141.32, 137.67, 137.38, 133.21, 130.09, 130.07, 128.60, 128.46, 128.32, 128.11, 128.05, 127.96, 127.92,

127.77, 127.44, 127.40, 125.87, 125.42, 120.08, 120.04 (Ar), 84.02 (C-1), 77.61 (C-3), 76.11 (C-4), 73.95 (CH_2Ph), 71.15 (CH_2Ph), 70.72 (C-4), 70.38 (CH_2, Fmoc), 69.45 (C-2), 68.02 (C-6), 46.69 (CH, Fmoc), 24.08, 15.02.; MS ESI+-HRMS *m/z* [M+Na]+ calcd for $C_{44}H_{42}O_8SNa$ 753.2498, found 753.2474.

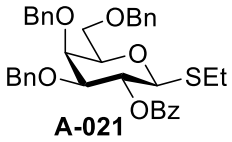
Ethyl 2-*O*-benzoyl-3,4-di-*O*-benzyl-1-thio-β-D-galactopyranoside A-020



A-020

Compound **A-017** (6.72 g, 13.26 mmol, 1.0 eq.) was co-evaporated with toluene and dissolved under an Ar atmosphere in DCM (78 mL, 0.17 M). To a solution of compound **A-017** was added 1M solution of BH_3 in THF (66ml, 66 mmol, 5.0 eq.), and TMSOTf (1.98 ml, 6.63 mmol, 0.5 eq.) successively at 0 °C. The mixture was stirred for 3 h at 0 °C. After the mixture was quenched with saturated aqueous $NaHCO_3$, diluted with DCM. The organic layer was dried over $MgSO_4$ and the solvent was evaporated *in vacuo*. The crude was purified by column chromatography on silica gel (hexane/ethyl acetate = 9:0.5:0.5 to 9:1:0.5) to afford **A-020** (6.5 g, 12.8 mmol, 96 %). R_f = 0.27 (hexane/ethyl acetate, 1:1); $[\alpha]_D^{20}$ = +10.38 (c = 2.10, chloroform); IR (thin film): υ = 2871, 1723, 1453, 1268 cm-1 δ ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (dd, *J* = 8.2, 1.1 Hz, 2H), 7.59 (ddd, *J* = 7.0, 2.5, 1.3 Hz, 1H), 7.46 (dd, *J* = 10.6, 4.7 Hz, 2H), 7.39 – 7.28 (m, 5H), 7.24 – 7.14 (m, 5H), 5.71 (t, *J* = 9.7 Hz, 1H, H-2), 5.01 (d, *J* = 11.9 Hz, 1H, C*H*HPh), 4.68 (d, *J* = 12.2 Hz, 1H, C*H*HPh), 4.66 (d, *J* = 11.9 Hz, 1H, C*H*HPh), 4.57 (d, *J* = 12.2 Hz, 1H, C*H*HPh), 4.51 (d, *J* = 9.9 Hz, 1H, H-1), 3.94 (d, *J* = 2.6 Hz, 1H, H-4), 3.84 (dd, *J* = 10.4, 6.0 Hz, 1H, H-6), 3.71 (dd, *J* = 9.6, 2.7 Hz, 1H, H-3), 3.54 (dd, *J* = 22.7, 5.6 Hz, 1H, H-6), 3.53 (br, 1H, H-5), 2.82 – 2.64 (m, 2H), 1.22 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (100 MHz, cdcl3) δ 165.53 (Bz), 138.33, 137.67, 133.18, 130.22, 130.02, 128.62, 128.58, 128.54, 128.49, 128.09, 127.96, 127.89 (Ar), 84.00 (C-1), 81.51 (C-3), 79.19 (C-5), 74.25 (CH_2Ph), 72.43 (C-4), 72.34 (CH_2Ph), 70.40 (C-2), 62.29 (C-6), 23.89, 15.00.; MS ESI+-HRMS m/z [M+Na]+ calcd for $C_{29}H_{32}O_6SNa$ 531.1817, found 531.1832

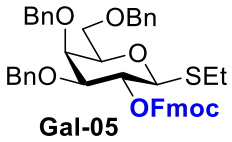
Ethyl 3,4,6-tri-*O*-benzyl-2-*O*-fluorenylmethoxycarbonyl-1-thio-β-D-galactopyranoside A-021



A-021

To a solution of compound **A-020** (1.5 g, 2.95 mmol, 1.0 eq.) in co-solvent of THF, and DMF (18 mL, v/v 9/1) was added benzyl bromide (1.05 ml, 8.83 mmol, 3.0 eq.), and sodium hydride (0.240 mg, 5.90 mmol, 2.0 eq.) dropwise at 0 °C. The mixture was stirred for 2 h at 0 °C. After the mixture was quenched with saturated aqueous NH_4Cl, diluted with DCM, the organic layer was dried over $MgSO_4$ and the solvent was evaporated *in vacuo*. The crude was purified by column chromatography on silica gel (hexane/ethyl acetate = 9:0.5:0.5 to 9:1:0.5) to afford **A-021** (1.59g, 2.66 mmol, 90 %). R_f : 0.29 (Hexane/EtOAc/DCM : 9/1/0.5). $[\alpha]_D^{25}$ = +28.50 (C= 3.13, $CHCl_3$). IR (thin film): υ = 2869, 1724, 1453, 1267, 1096, 1069 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.11 – 8.06 (m, 2H), 7.62 – 7.56 (m, 1H), 7.52 (d, *J* = 2.0 Hz, 1H), 7.46 (t, *J* = 7.8 Hz, 2H), 7.39 – 7.33 (m, 4H), 7.32 – 7.28 (m, 1H), 7.20 (dd, *J* = 7.9, 2.1 Hz, 1H), 7.07 (d, *J* = 8.0 Hz, 1H), 5.30 (t, *J* = 9.5 Hz, 1H, H-3), 4.89 (d, *J* = 11.5 Hz, 1H, CH*H*Ph), 4.78 (d, *J* = 10.2 Hz, 1H, H-1), 4.68 (d, *J* = 11.5 Hz, 1H, CH*H*Ph), 4.00 (t, *J* = 8.8 Hz, 1H, H-3), 3.89 (ddd, *J* = 11.9, 5.5, 2.3 Hz, 1H, H-6), 3.72 (ddd, *J* = 12.1, 7.4, 4.7 Hz, 1H, H-6), 3.65 (t, *J* = 9.2 Hz, 1H, H-4), 3.48 (ddd, *J* = 9.7, 4.6, 2.6 Hz, 1H, H-5), 2.17 (s, 3H, Me), 1.29 (s, 9H, *t*-Bu), 0.81 (s, 9H, *t*-Bu), 0.04 (s, 3H, Me), -0.13 (s, 3H, Me). ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 165.48 (Bz), 149.60, 138.12, 138.11, 136.46, 133.71, 133.08, 130.40, 129.97, 129.75, 129.17, 128.40, 128.35, 127.89, 127.64, 127.60, 127.53, 124.68 (Ar), 87.91 (C-1), 79.48 (C-5), 78.85 (C-4), 76.96 (C-3), 75.02 (*C*H_2Ph), 73.54 (*C*H_2Ph), 73.41 (C-2), 68.96 (C-6), 34.48 (Cq, *t*-Bu), 31.34 (Me, *t*-Bu), 25.79 (*t*-Bu of TBS), 20.25 (Me), 17.87 (Cq, TBS), -3.92 (Me, TBS), -4.18 (Me, TBS). MS ESI-HRMS m/z $[M+Na]^+$ calcd for $C_{36}H_{38}O_6SNa$ 621.2287, found 621.2297

Ethyl 3,4,6-tri-*O*-benzyl-2-*O*-fluorenylmethoxycarbonyl-1-thio-β-D-galactopyranoside Gal-05

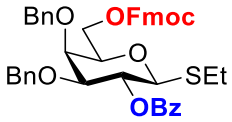


Gal-05

To a solution of compound **A-021** (1.3 g, 2.17 mmol, 1.0 eq.) in MeOH (7.2 ml, 0.3 M) was added 8.7 mL of 0.5 M sodium methoxide in MeOH (4.34 mmol, 2.0 eq.) at 40 °C. After completion, the mixture was neutralized with Amberlite IR-120 (H+) ion exchange resin. The mixture was filtered off, and concentrated, and co-evaporated with toluene. To a solution of

the crude in anhydrous DCM (15 mL, 0.3 M) were added 9-fluorenylmethylchloroformate (2.27 g, 8.78 mmol, 2.0 eq.) and pyridine (1.1 mL, 13.17 mmol, 3.0 eq.) successively at 0 °C, and stirred overnight at room temperature. After the mixture was quenched with 1M aqueous HCl, and diluted with DCM. The organic layer was dried over $MgSO_4$ and the solvent was evaporated *in vacuo*. The crude was purified by column chromatography on silica gel (hexane:ethylacetate:DCM = 8:1:1 to 8:2:1) to afford **Gal-05** (2.7 g, 3.77 mmol, 86%) over two steps. R_f = 0.17 (hexane/ethyl acetate/DCM, 9:1:0.5); $[\alpha]_D^{20}$ = -12.26 (c = 1.30, chloroform); IR (thin film): υ = 2869, 1752, 1452, 1251 cm-1; ^{1}H NMR (CDCl3) 8.03 (d, *J* = 7.5 Hz, 2H), 7.78 (d, *J* = 7.5 Hz, 2H), 7.59 (t, *J* = 7.7 Hz, 3H), 7.49 – 7.39 (m, 4H), 7.38 – 7.26 (m, 7H), 7.23 – 7.14 (m, 5H), 5.71 (t, *J* = 9.7 Hz, 1H, H-2), 5.04 (d, *J* = 11.7 Hz, 1H, C*H*HPh), 4.68 (d, *J* = 12.2 Hz, 1H, C*H*HPh), 4.67 (d, *J* = 11.7 Hz, 1H, C*H*HPh), 4.56 (d, *J* = 12.2 Hz, 1H, C*H*HPh), 4.52 (d, *J* = 9.9 Hz, 1H, 4.44 – 4.34 (m, 3H, H-6, CH_2 of Fmoc), 4.25 (t, *J* = 7.4 Hz, 1H, C*H* of Fmoc), 4.18 (dd, *J* = 11.1, 5.8 Hz, 1H, H-6), 3.97 (d, *J* = 1.9 Hz, 1H, H-4), 3.72 (dd, *J* = 13.2, 4.1 Hz, 2H, H-3, H-5), 2.82 – 2.64 (m, 2H), 1.22 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, cdcl3) 154.66 (Fmoc), 143.68, 143.47, 141.42, 141.40, 138.66, 137.95, 137.95, 128.56, 128.53, 128.32, 128.11, 128.04, 127.96, 127.94, 127.92, 127.85, 127.63, 127.57, 127.29, 127.26, 125.43, 125.31, 120.12 (Ar), 83.74 (C-1), 81.58 (C-3), 77.67 (C-5), 74.62 (CH_2Ph), 74.44 (C-2), 73.71 (CH_2Ph), 73.39 (C-4), 72.48 (CH_2Ph), 70.24 (CH_2, Fmoc), 68.65 (C-6), 46.93 (CH, Fmoc), 24.05, 15.05. MS ESI+-HRMS m/z [M+Na]+ calcd for $C_{44}H_{44}O_7SNa$ 739.2705, found 739.2699.

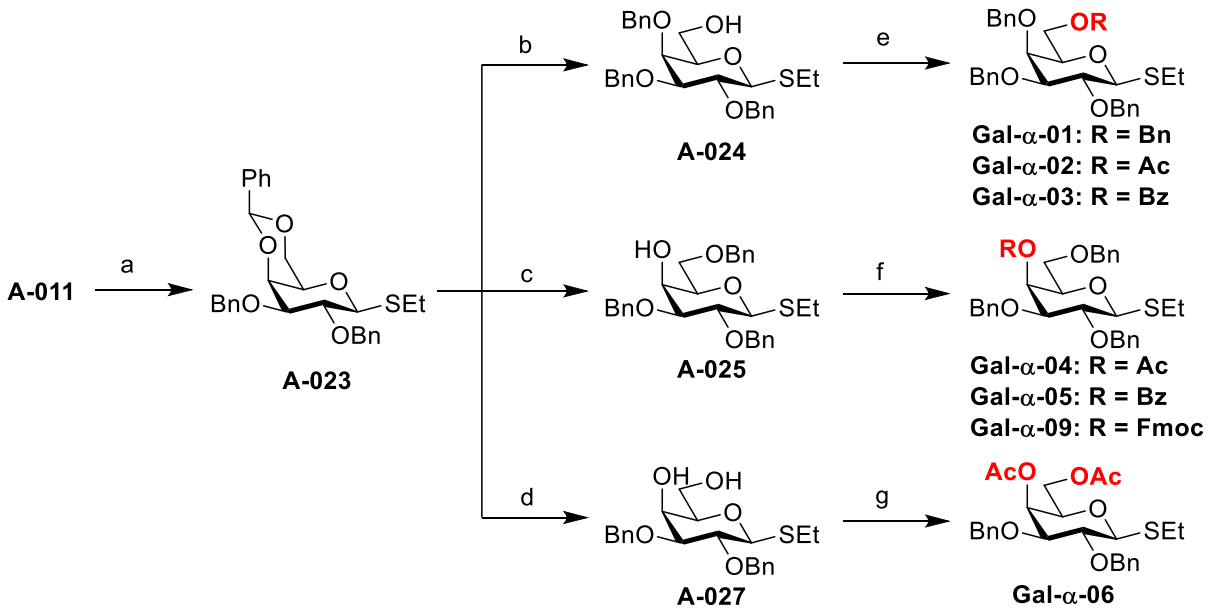
Ethyl 2-*O*-benzoyl-3,4-di-*O*-benzyl-6-*O*-fluorenylmethoxycarbonyl-1-thio-β-D-galactopyranoside A-022



A-022

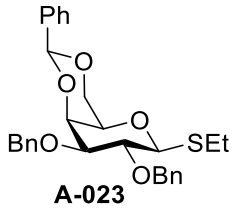
To a solution of compound **A-020** (4.0 g, 7.86 mmol, 1.0 eq) in anhydrous DCM (26 ml) was added 9-fluorenylmethylchloroformate (4.07 g, 15.73 mmol, 2.0 eq.) and pyridine (1.91 mL, 23.6 mmol, 3.0 eq.) successively at 0 °C, and stirred overnight at room temperature. After the mixture was quenched with 1M aqueous HCl, and diluted with DCM. The organic layer was dried over MgSO4 and the solvent was evaporated in vacuo. The crude was purified by column chromatography on silica gel (hexane:ethylacetate:DCM = 8:1:1 to 8:2:1) to afford **A-022** (5.35 g, 7.32 mmol, 93%). R_f = 0.27 (hexane/ethyl acetate/DCM, 9:1:0.5); $[\alpha]_D^{20}$ =

+22.41 (c = 2.50, chloroform); IR (thin film): υ = 2871, 1748, 1727, 1451 cm-1; ^{1}H NMR (CDCl3) 8.03 (d, *J* = 7.5 Hz, 2H), 7.78 (d, *J* = 7.5 Hz, 2H), 7.59 (t, *J* = 7.7 Hz, 3H), 7.49 – 7.39 (m, 4H), 7.38 – 7.26 (m, 7H), 7.23 – 7.14 (m, 5H), 5.71 (t, *J* = 9.7 Hz, 1H, H-2), 5.04 (d, *J* = 11.7 Hz, 1H, C*H*HPh), 4.68 (d, *J* = 12.2 Hz, 1H, C*H*HPh), 4.67 (d, *J* = 11.7 Hz, 1H, C*H*HPh), 4.56 (d, *J* = 12.2 Hz, 1H, C*H*HPh), 4.52 (d, *J* = 9.9 Hz, 1H, 4.44 – 4.34 (m, 3H, H-6, C*H*$_2$ of Fmoc), 4.25 (t, *J* = 7.4 Hz, 1H, C*H* of Fmoc), 4.18 (dd, *J* = 11.1, 5.8 Hz, 1H, H-6), 3.97 (d, *J* = 1.9 Hz, 1H, H-4), 3.72 (dd, *J* = 13.2, 4.1 Hz, 2H, H-3, H-5), 2.82 – 2.64 (m, 2H), 1.22 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, cdcl3) δ 165.49 (Bz), 154.93 (Fmoc), 143.40, 143.35, 141.43, 138.14, 137.59, 133.21, 130.12, 130.02, 128.52, 128.49, 128.43, 128.08, 127.94, 127.91, 127.82, 127.30, 125.24, 120.24 (Ar), 83.98 (C-1), 81.21 (C-3), 76.20 (C-5), 74.47 (CH_2Ph), 72.55 (C-4), 72.28 (CH_2Ph), 70.22 (CH_2 of Fmoc), 70.09 (C-2), 66.79 (C-6), 46.84 (CH of Fmoc), 24.05, 15.00; MS ESI+-HRMS m/z [M+Na]+ calcd for $C_{44}H_{42}O_8SNa$ 753.2498, found 753.2477.



Scheme A.06. Synthesis of galactose building blocks Gal-α-01 to -06 and Gal-α-09. a) BnBr, NaH, DMF, 0 °C, 98%; b) BH_3 in THF, TMSOTf, DCM, 0 °C , 98%; c) $HSiEt_3$, TFAA, TFA, DCM, 0 °C to r.t. 93%; d) DCM, TFA, r.t. 86%; TFA , 98%; e) i) BnBr, NaH, DMF, 0 °C, 91% for **Gal-α-01**; ii) Ac_2O, pyridine, DMAP, DCM, r.t , 91% for **Gal-α-02,** iii) Bz_2O, pyridine, DMAP, DCM, r.t , 88% for **Gal-α-03;** f) i) Ac_2O, pyridine, DMAP, DCM, r.t , 88% for **Gal-α-04,** ii) Bz_2O, pyridine, DMAP, DCM, r.t , 91% for **Gal-α-05,** iii) FmocCl, pyridine, DCM, 0 °C, 91% for **Gal-α-09;** g) Ac_2O, pyridine, DMAP, DCM, r.t , 84% for **Gal-α-06,**

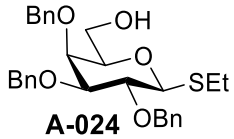
Ethyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside A-023



A-023

To a solution of compound **A-011** (4.01 g, 7.51 mmol, 1.0 eq.) in anhydrous DMF (58.7 mL), were added BnBr (2.68 mL, 22.52 mmol, 3.0 eq.) and sodium hydride (0.721 mg, 18.02 mmol, 2.4 eq) portionwise at 0°C. After the reaction mixture was stirred for 2 h at 0°C the mixture was quenched with saturated aqueous NH_4Cl, diluted with DCM, over Mg_2SO_4. Following evaporation of the solvent, the crude product was purified by column chromatography on silica gel (hexane/ethyl acetate = 9:0.5:0.5 to 9:1:1) to afford **A-023** (4.58g, 7.36 mmol, 98%). The analytical data were in agreement with those reported in the literature.

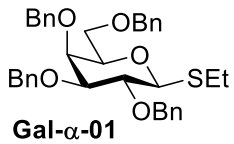
Ethyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-galactopyranoside A-024



A-024

Compound **A-023** (0.601 g, 1.22 mmol, 1.0 eq.) was co-evaporated with toluene and dissolved under an Ar atmosphere in DCM (7.2 mL, 0.17 M). To a solution of **A-023** was added 1M solution of BH_3 in THF (4.9 mL, 4.9 mmol, 5.0 eq.), and TMSOTf (0.11 ml, 0.61 mmol, 0.5 eq.) successively at 0 °C. The mixture was stirred for 3 hr at 0 °C. After the mixture was quenched with saturated aqueous $NaHCO_3$, diluted with DCM, and was dried over $MgSO_4$ The solvent was evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane/ethyl acetate = 9:0.5:0.5 to 9:1:0.5) to afford **A-024** (0.589 g, 1.19 mmol, 98 %). The analytical data was in agreement with the literature data.

Ethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside Gal-α-01

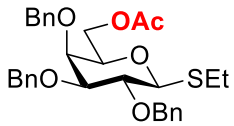


Gal-α-01

To a solution of compound **A-024** (1.02 g, 7.51 mmol, 1.0 eq.) in anhydrous DMF (16.1 mL), were added BnBr (0.25 mL, 8.9 mmol, 1.2 eq.) and sodium hydride (0.185 mg, 10.8 mmol, 1.5 eq) portionwise at 0°C. After the reaction mixture was stirred for 2 h at 0°C the mixture

was quenched with saturated aqueous NH_4Cl, diluted with DCM, over Mg_2SO_4. Following evaporation of the solvent, the crude product was purified by column chromatography on silica gel (hexane/ethyl acetate = 9:0.5:0.5 to 9:1:1) to afford **Gal-α-01** (0.98 g, 6.8 mmol, 91%). The analytical data were in agreement with those reported in the literature.

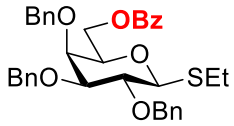
Ethyl 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-1-thio-β-D-galactopyranoside A-024



Gal-α-02

To a solution of compound **A-024** (0.20 g, 0.404 mmol, 1.0 eq.) in DCM (2.0 ml, 0.2 M) were added acetic anhydride (0.076 ml, 0.81 mmol, 2.0 eq.), triethylamine (0.113 ml, 0.809 mmol, 2.0 eq.), and DMAP (0.010 g, 0.081 mmol, 0.2 eq.) in the ice bath, and then the mixture was stirred overnight at room temperature. After the mixture was quenched with saturated aqueous $NaHCO_3$, diluted with DCM, and was dried over $MgSO_4$ the solvent was evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane:ethylacetate:DCM = 8/1/0.5 to 8/2/0.5) to give **Gal-α-02** (0.198 g, 0.369 mmol, 91%). The analytical data was in agreement with the literature data.

Ethyl 6-*O*-benzoyl-2,3,4-tri-*O*-benzyl-1-thio-β-D-galactopyranoside Gal-α-03

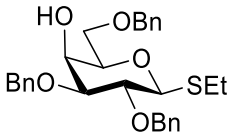


Gal-α-03

To a solution of compound **A-024** (0.21 g, 0.43 mmol) in anhydrous DCM (2.1mL, 0.2 M) were added were added benzoic anhydride (0.192 g, 0.849 mmol), triethylamine (0.12 mL, 0.849 mmol), and a catalytic amount of DMAP (10.4 mg, 0.085 mmol) at 0°C. After the mixture was stirred overnight at room temperature, the mixture was quenched with saturated aqueous $NaHCO_3$, diluted with DCM, dried over $MgSO_4$, and evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane/ethyl acetate = 9:1 to 7:3) to afford **Gal-α-03** (0.224 g, 0.374 mmol, 88%). R_f = 0.44 (hexane/ethyl acetate, 8:2); R_f = 0.21 (hexane/ethyl acetate/DCM, 9:1:0.5); $[\alpha]_D^{20}$ = -22.26 (c = 2.30, chloroform); IR (thin film): υ = 2869, 1724, 1453, 1267, 1096, 1069 cm-1; ^{1}H NMR ($CDCl_3$) δ 7.93 (dd, *J* = 8.2, 1.1 Hz, 2H), 7.57 (dd, *J* = 10.6, 4.3 Hz, 1H), 7.50 – 6.98 (m, 17H), 5.02 (d, *J* = 11.6 Hz, 1H, C*H*HPh), 4.90 (d, *J* = 10.2 Hz, 1H, C*H*HPh), 4.84 – 4.75 (m, 3H, 3 x C*H*HPh), 4.70 (d, *J* = 11.7 Hz, 1H, C*H*HPh), 4.52 – 4.45 (m, 2H, **H-1**, H-6), 4.32 (dd, *J* = 11.2, 6.0 Hz, 1H, H-6),

3.90 – 3.86 (m, 2H, H-2, H-4), 3.70 (t, *J* = 6.4 Hz, 1H, H-5), 3.61 (dd, *J* = 9.3, 2.7 Hz, 1H, H-3), 2.86 – 2.64 (m, 2H), 1.29 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.25 (Bz), 138.36, 138.34, 138.29, 133.26, 129.90, 129.75, 128.61, 128.59, 128.48, 128.45, 128.41, 127.93, 127.90, 127.86, 127.74 (Ar), 85.54 (C-1), 84.28 (C-3), 78.58 (C-2), 76.08 (CH_2Ph), 75.99 (C-5), 74.47 (CH_2Ph), 73.50 (C-4), 73.37 (CH_2Ph), 63.68 (C-6), 25.13, 15.27. MS ESI+-HRMS m/z [M+Na]+ calcd for $C_{36}H_{38}O_6SNa$ 621.2281, found 621.2292.

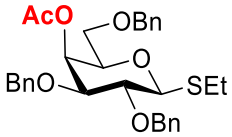
Ethyl 2,3,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside A-025



A-025

Compound **A-023** (0.356 g, 0.741 mmol, 1.0 eq.) was co-evaporated with toluene and dissolved under an Ar atmosphere in DCM (4.4 mL, 0.17 M). To a solution of **A-023** was added triethylsilane (0.710 mL, 4.45 mmol, 6.0 eq.) and trifluoroacetic anhydride (0.052 mL, 0.370 mmol, 0.5 eq.) at 0 °C. After 30 min., trifluoroacetic acid (0.285 mL, 3.7 mmol, 5.0 eq.) was added dropwise, and the reaction was stirred and allowed to warm to room temperature. After the mixture was quenched with saturated aqueous $NaHCO_3$, diluted with DCM, and was dried over $MgSO_4$ the solvent was evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane/ethyl acetate = 9:1 to 7:3) to afford **A-025** (0.342 g, 0.691 mmol, 93 %). The analytical data was in agreement with the literature data.

Ethyl 4-*O*-acetyl-2,3,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside Gal-α-04



Gal-α-04

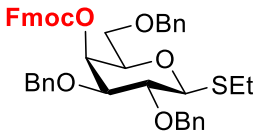
To a solution of compound **A-025** (0.20 g, 0.404 mmol, 1.0 eq.) in DCM (2.0 ml, 0.2 M) were added acetic anhydride (0.076 ml, 0.81 mmol, 2.0 eq.), triethylamine (0.113 ml, 0.809 mmol, 2.0 eq.), and DMAP (0.010 g, 0.081 mmol, 0.2 eq.) in the ice bath, and then the mixture was stirred overnight at room temperature. After the mixture was quenched with saturated aqueous $NaHCO_3$, diluted with DCM, and was dried over $MgSO_4$ the solvent was evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane:ethylacetate:DCM = 8/1/0.5 to 8/2/0.5) to give **Gal-α-04** (0.198 g, 0.369 mmol, 88%). The analytical data was in agreement with the literature data.

Ethyl 4-*O*-benzoyl-2,3,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside Gal-α-05

Gal-α-05

To a solution of compound **A-025** (0.21 g, 0.43 mmol) in anhydrous DCM (2.1mL, 0.2 M) were added were added benzoic anhydride (0.192 g, 0.849 mmol), triethylamine (0.12 mL, 0.849 mmol), and a catalytic amount of DMAP (10.4 mg, 0.085 mmol) at 0°C. After the mixture was stirred overnight at room temperature, the mixture was quenched with saturated aqueous $NaHCO_3$, diluted with DCM, dried over $MgSO_4$, and evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane/ethyl acetate = 9:1 to 7:3) to afford **Gal-α-05** (0.224 g, 0.374 mmol, 91%). R_f = 0.44 (hexane/ethyl acetate, 8:2); R_f = 0.21 (hexane/ethyl acetat, 9:1). T he analytical data was in agreement with the literature data.

Ethyl 2,3,6-tri-*O*-benzyl-4-*O*-fluorenylmethoxycarbonyl-1-thio-β-D-galactopyranoside Gal-α-09

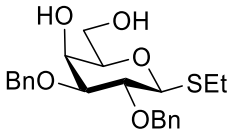


Gal-α-09

To a solution of compound **A-025** (0.610 g, 1.21 mmol) were added 9-fluorenylmethyl chloroformate (0.63 g, 2.43 mmol) and pyridine (0.29 mL, 3.64 mmol) successively at 0 °C. The reaction mixture was stirred overnight at room temperature. Then the mixture was diluted with DCM and it was quenched with 1M aqueous HCl. The combined organic layer was dried over $MgSO_4$ and the solvent was evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane/ethyl acetate/DCM = 9:0.5:0.5 to 8:2:0.5) to afford **Gal-α-09** (0.79 g, 1.11 mmol, 91 %). $[\alpha]_D^{25}$ = 5.59 (C= 3.60, $CHCl_3$). IR (thin film): υ = 3031, 2869, 1748, 1452, 1255, 1101 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.78 (d, *J* = 7.6 Hz, 2H), 7.65 (d, *J* = 7.5 Hz, 1H), 7.60 (d, *J* = 7.5 Hz, 1H), 7.44 – 7.37 (m, 4H), 7.34 – 7.27 (m, 11H), 7.25-23 (m, H), 7.21 – 7.17 (m, 3H), 5.49 (d, *J* = 2.9 Hz, 1H, H-4), 4.87 (d, *J* = 10.2 Hz, 1H, C*H*HPh), 4.82 (dd, *J* = 10.7, 5.7 Hz, 2H, 2 x C*H*HPh), 4.57 (d, *J* = 11.3 Hz, 1H, C*H*HPh), 4.50 (dt, *J* = 11.8, 9.1 Hz, 3H, H-1, CH_2Ph), 4.44 (dd, *J* = 10.4, 7.2 Hz, 1H, , C*H*H, Fmoc), 4.32 – 4.27 (m, 1H, C*H*H, Fmoc), 4.21 (t, *J* = 7.5 Hz, 1H, C*H*, Fmoc), 3.76 (t, *J* = 6.4 Hz, 1H, H-5), 3.68 (ddt, *J* = 12.4, 9.1, 6.2 Hz, 3H, H-2, H-3, H-6), 3.63 – 3.57

(m, 1H, H-6), 2.86 – 2.70 (m, 2H), 1.33 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) *δ* 155.23 (Fmoc), 143.80, 143.37, 141.37, 138.26, 137.86, 137.77, 128.57, 128.52, 128.47, 128.39, 128.07, 127.99, 127.95, 127.80, 127.28, 125.62, 125.33, 120.15, 120.12 (Ar), 85.53 (C-1), 81.17 (C-3), 77.89 (C-2), 76.14 (CH_2Ph), 75.80 (C-5), 73.92 (CH_2Ph), 72.13 (CH_2Ph), 71.42 (C-4), 70.21 (CH_2, Fmoc), 68.31 (C-6), 46.79 (CH, Fmoc), 25.11, 15.26. MS ESI-HRMS m/z $[M+Na]^+$ calcd for $C_{44}H_{44}O_7SNa$ 739.2700, found 739.2703.

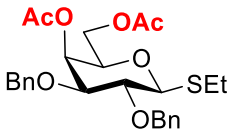
Ethyl 2,3-di-*O*-benzyl-1-thio-β-D-galactopyranoside A-027 (ref)



A-027

To a solution of compound **A-023** (0.502 g, 1.102 mmol, 1.0 eq.) in DCM (100 ml) and water (1.1 mL) was added TFA (1.6 mL) at 0 °C for 4 h. After the mixture was quenched with saturated aqueous $NaHCO_3$, diluted with DCM, and was dried over $MgSO_4$ the solvent was evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane/ethyl acetate = 9:1 to 7:3) to afford **A-027** (0.401 g, 0.991 mmol, 98 %). The analytical data was in agreement with the literature data.

Ethyl 4,6-di-*O*-acetyl-2,3-di-*O*-benzyl-1-thio-β-D-galactopyranoside Gal-α-06

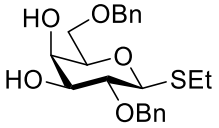


Gal-α-06

To a solution of compound **A-027** (0.411g, 1.02 mmol, 1.0 eq.) in DCM (5.1 ml, 0.2 M) were added acetic anhydride (0.48 ml, 5.08 mmol, 5.0 eq.), triethylamine (1.42 ml, 1.03 mmol, 10.0 eq.), and DMAP (0.025 g, 0.203 mmol, 0.2 eq.) in the ice bath, and the mixture was stirred overnight at room temperature. After quenched with saturated aqueous $NaHCO_3$, diluted with DCM, and was dried over $MgSO_4$ the solvent was evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane:ethylacetate:DCM = 8/1/0.5 to 7/3/0.5) to give **Gal-α-06** (0.416 g, 0.851 mmol, 84%). R_f = 0.22(hexane/ethyl acetate/DCM, 7:3); $[\alpha]_D^{20}$ = 28.56 (c = 3.10, chloroform); IR (thin film): υ = 2869, 1752, 1452, 1251 cm-1; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 – 7.25 (m, 10H), 5.55 (d, *J* = 2.3 Hz, 1H, H-4)), 4.79 (dd, *J* = 21.8, 10.4 Hz, 3H, C*H*HPh, CH_2Ph), 4.52

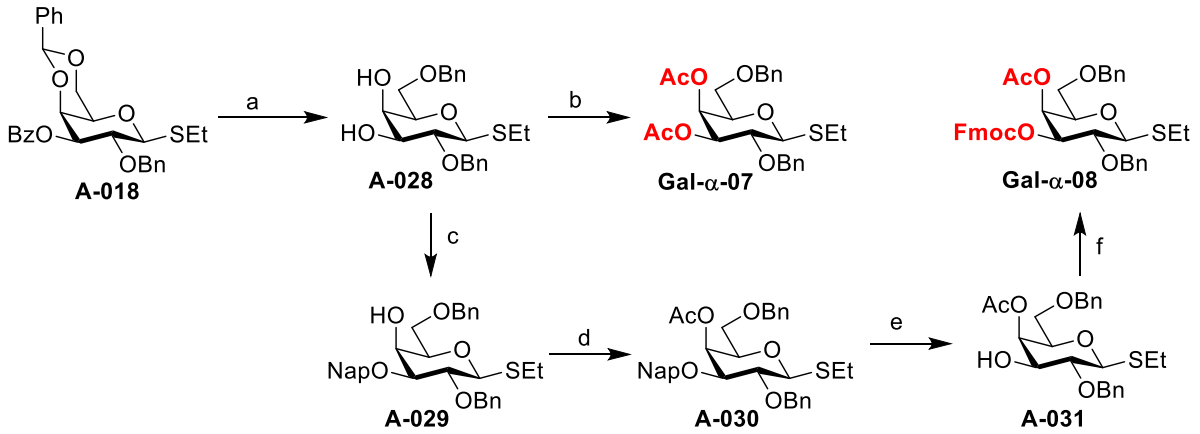
(d, *J* = 11.1 Hz, 1H, C*H*HPh), 4.48 (d, *J* = 8.9 Hz, 1H, H-1) 4.19 – 4.10 (m, 2H, H-6), 3.78 (t, *J* = 6.5 Hz, 1H, H-5), 3.66 – 3.55 (m, 2H, H-2, H-3), 2.76 (qd, *J* = 12.7, 7.1 Hz, 2H), 2.16 (s, 3H, Ac), 2.07 (s, 3H, Ac), 1.33 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.69 (Ac), 170.58 (Ac), 138.14, 137.69, 128.54, 128.48, 128.46, 128.26, 127.97 (Ar), 85.61 (C-1), 80.93 (C-3), 77.77 (C-2), 76.06 (CH_2Ph), 74.50 (C-5), 72.16 (CH_2Ph), 66.76 (C-4), 62.40 (C-6), 25.29, 21.08 (Ac), 20.91 (Ac), 15.24. MS ESI+-HRMS m/z [M+Na]+ calcd for $C_{26}H_{32}O_7SNa$ 511.1761, found 511.1765.

Ethyl 2,6-di-*O*-benzyl-3-*O*-(2-naphthylmathyl)-1-thio-β-D-galactopyranoside A-028



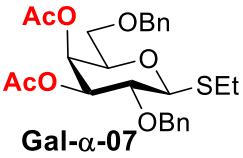
A-028

Compound **A-018** (2.1 g, 4.15 mmol) was co-evaporated with toluene and dissolved under an Ar atmosphere in DCM (24.4 mL, 0.17 M). To a solution of **A-018** were added triethylsilane (3.97 mL, 24.9 mmol) and trifluoroacetic anhydride (0.293 mL, 2.07 mmol) at 0°C. After 30 min, trifluoroacetic acid (1.60 mL, 20.7 mmol) was added dropwise, and the reaction mixture was stirred and allowed to warm to room temperature. After the mixture was quenched with saturated aqueous $NaHCO_3$, diluted with DCM, and dried over $MgSO_4$ the solvent was evaporated *in vacuo*. To a solution of the crude product in MeOH was added 0.5 M NaOMe (1.66 mL, 0.83 mmol) at room temperature, and the mixture was stirred for 2h. After the mixture was quenched with saturated aqueous $NaHCO_3$, diluted with DCM, and dried over $MgSO_4$ the solvent was evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane/ethyl acetate = 7:3 to 4:6) to afford **A-028** (1.98 g, 3.89 mmol, 94 %). The analytical data was in agreement with the literature data.



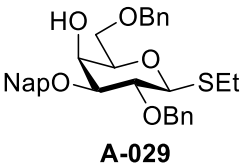
Scheme A.07. Synthesis of galactose building blocks Gal-α-07 and Gal-α-08. a) i) $HSiEt_3$, TFAA, TFA, DCM, 0 °C to r.t., ii) NaOMe, MeOH, r.t. 94% for two steps; b) Ac_2O, pyridine, DMAP, DCM, r.t , 95% for **Gal-α-07;** c) i) $SnO(n\text{-}Bu)_2$, in MeOH, reflux for 2 hrs; ii) 2-NapBr, NBu_4I, DMF, 60 °C, 85% for two steps; d)) Ac_2O, pyridine, DMAP, DCM, r.t , 94%; DDQ, DCM, 0 °C, 73%; f) FmocCl, pyridine, DCM, r.t , 92% for **Gal-α-08,**

Ethyl 3,4-di-*O*-acetyl-2,6-di-*O*-benzyl-1-thio-β-D-galactopyranoside Gal-α-07



To a solution of compound **A-028** (0.77 g, 1.90 mmol, 1.0 eq.) in DCM (9.5 ml, 0.2 M) was added acetic anhydride (0.72 ml, 7.61 mmol, 4.0 eq.), triethylamine (1.86 ml, 13.32 mmol, 7.0 eq.), and DMAP (0.023 g, 0.190 mmol, 0.1 eq.) in the ice bath, the mixture was stirred overnight at room temperature. After quenched with saturated aqueous $NaHCO_3$, the mixture was diluted with DCM. The organic layer was dried over $MgSO_4$ and the solvent was evaporated *in vacuo*. The crude was purified by column chromatography on silica gel (hexane:ethylacetate:DCM = 8/1/0.5 to 8/2/0.5) to give **Gal-α-07** (0.89 g, 1.82 mmol, 95%). The analytical data was in agreement with the literature data.

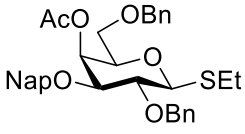
Ethyl 2,6-di-*O*-benzyl-3-*O*-(2-naphthylmathyl)-1-thio-β-D-galactopyranoside A-029



Dibutyltin oxide (2.2 g, 8.78 mmol, 1.2 eq.) was added to a solution of **A-028** (2.96 g, 7.32 mmol, 1.0 eq.) in mathanol (36.6 ml, 0.2 M) and the reaction mixture was heated to reflux for 4 h. Removal of solvent from the reaction mixture gave stannylene acetal that kept under vaccuem for 4 h. To a solution of the crude acetal in anhydrous DMF (37 mL, 0.2 M) were added to 2-naphtylmethyl bromide (2.43 g, 11.0 mmol, 1.5 eq.) and Tetrabutylammonium

iodide (NBu_4I) (3.24 g, 8.78 mmol, 1.2 eq.). The reaction mixture was stirred at 60 °C for 3 h. The reaction mixture was diluted with EtOAc (75 mL) and quenched with satureated aqueous NH_4Cl. The combined organic layer was wash with satureated aqueous NH_4Cl and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (hexane:ethylacetate:DCM = 8/1/0.5 to 8/2/0.5) to give **A-029** (3.4 g, 6.24 mmol, 85%). R_f = 0.31 (hexane/ethyl acetate/DCM, 7:3); $[\alpha]_D^{20}$ = 3.19 (c = 3.00, chloroform); IR (thin film): υ = 3479, 3030, 2869, 1453, 1366, 1093 cm-1; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.86 – 7.74 (m, 4H), 7.48 (tt, *J* = 3.9, 3.1 Hz, 4H), 7.44 – 7.40 (m, 2H), 7.37 – 7.27 (m, 7H), 4.91 (d, *J* = 10.2 Hz, 1H, C*H*HPh), 4.91 (d, *J* = 11.8 Hz, 1H, C*H*HPh), 4.86 (d, *J* = 11.8 Hz, 1H, C*H*HPh), 4.81 (d, *J* = 10.2 Hz, 1H, C*H*HPh), 4.58 (s, 2H, CHPh), 4.44 (d, *J* = 9.7 Hz, 1H, H-1), 4.13 (d, *J* = 2.6 Hz, 1H, H-4), 3.79 (dd, *J* = 9.9, 5.9 Hz, 1H, H-6), 3.75 – 3.70 (m, 2H, H-2, H-6), 3.60 (dd, *J* = 9.0, 3.2 Hz, 1H, H-3), 3.57 (t, *J* = 5.8 Hz, 1H, H-5), 2.84 – 2.70 (m, 2H), 2.57 (d, *J* = 2.4 Hz, 1H, OH), 1.32 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 138.33, 138.05, 135.33, 133.35, 133.20, 128.56, 128.49, 128.47, 128.05, 127.92, 127.90, 127.84, 126.83, 126.34, 126.19, 125.91 (Ar), 85.24 (C-1), 82.38 (C-3), 78.08 (C-2), 77.04 (C-5), 75.97 (CH_2Ph), 73.87 (CHPh), 72.28 (CH_2Ph), 69.49 (C-6), 67.15 (C-4), 24.90, 15.27. MS ESI+-HRMS m/z $[M+Na]^+$ calcd for $C_{33}H_{38}O_5SNa$ 567.2176, found 567.2201.

Ethyl 4-*O*-acetyl-2,6-di-*O*-benzyl-3-*O*-(2-naphthylmathyl)-1-thio-β-D-galactopyranoside A-030

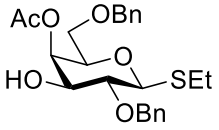


A-030

To a solution of **A-029** (3.4 g, 6.24 mmol, 1.0 eq.) in DCM (31 mL, 0.2 M) were added acetic anhydride (1.77 mL, 18.7 mmol, 3.0 eq.), triethylamine (5.22 mL, 37.5 mmol, 6.0 eq.), and DMAP (0.076 g, 0.624 mmol, 0.1 eq.) in the ice bath, and the reaction mixture was stirred overnight at room temperature. After the mixture was quenched with saturated aqueous $NaHCO_3$, diluted with DCM, and dried over $MgSO_4$ the solvent was evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane:ethylacetate:DCM = 8/1/0.5 to 8/2/0.5) to give **A-030** (3.44 g, 6.24 mmol, 94%). R_f = 0.19 (hexane/ethyl acetate/DCM, 7:3); $[\alpha]_D^{20}$ = 27.30 (c = 3.07, chloroform); IR (thin film): υ = 2867, 1741, 1454, 1372, 1231, 1097, 940 cm-1; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.84 – 7.69

(m, 4H), 7.49 – 7.43 (m, 3H), 7.42 – 7.29 (m, 10H), 5.70 (dd, *J* = 3.3, 0.8 Hz, 1H, H-4), 4.95 (d, *J* = 11.5 Hz, 1H, C*H*HPh), 4.83 (dd, *J* = 25.2, 10.2 Hz, 2H, CH_2Nap), 4.69 (d, *J* = 11.5 Hz, 1H, C*H*HPh), 4.58 (d, *J* = 11.8 Hz, 1H, C*H*HPh), 4.50 (d, *J* = 9.7 Hz, 1H, H-1), 4.48 (d, *J* = 11.8 Hz, 1H, C*H*HPh), 3.76 – 3.73 (m, 1H, H-5), 3.69 (dd, *J* = 9.2, 3.3 Hz, 1H, H-3), 3.64 – 3.59 (m, 2H, H-2, H-6), 3.52 (dd, *J* = 9.5, 6.9 Hz, 1H, H-6), 2.83 – 2.72 (m, 2H), 2.13 (s, 3H, Ac), 1.33 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.53 (Ac), 138.31, 137.75, 135.34, 133.38, 133.15, 128.58, 128.44, 128.41, 128.25, 128.17, 128.08, 127.99, 127.88, 127.76, 127.06, 126.29, 126.11, 126.00, 85.55, 81.04, 78.02, 76.00, 75.96, 73.84, 72.05, 68.33, 67.14, 25.22, 21.11 (Ac), 15.20. MS ESI+-HRMS m/z $[M+Na]^+$ calcd for $C_{35}H_{38}O_6SNa$ 609.2281, found 609.2245.

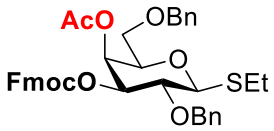
Ethyl 4-*O*-acetyl-2,6-di-*O*-benzyl-1-thio-β-D-galactopyranoside A-031



A-031

To a solution of **A-030** (1.4 g, 2.39 mmol, 1.0 eq.) in DCM and phosphate buffer (DCM:buffer, 5:1, 14 ml, 0.17 M) was added DDQ (1.4 g, 5.97 mmol, 2.5 eq.) at room temperature. After stirring for 1 h, the reaction mixture was quenched with saturated aqueous $NaHCO_3$, diluted with DCM. The combined organic layer was washed with saturated aqueous $NaHCO_3$, and brine, and dried over $MgSO_4$. After the solvent was evaporated *in vacuo* the crude product was purified by column chromatography on silica gel (hexane:ethylacetate:DCM = 8/1/0.5 to 8/2/0.5) to give **A-031** (0.78 g, 1.75 mmol, 73%). R_f = 0.11 (hexane/ethyl acetate/DCM, 9:1:0.5); $[\alpha]_D^{20}$ = -11.53 (c = 4.00, chloroform); IR (thin film): υ = 3472, 2869, 1741, 1454, 1374, 1233, 1099, 1047 cm-1; ^{1}H NMR ($CDCl_3$) δ 7.53 – 7.09 (m, 10H), 5.39 (d, *J* = 3.3 Hz, 1H, H-4), 4.94 (d, *J* = 10.7 Hz, 1H, C*H*HPh), 4.68 (d, *J* = 10.7 Hz, 1H, C*H*HPh), 4.55 (d, *J* = 11.8 Hz, 1H, C*H*HPh), 4.48 (d, *J* = 9.7 Hz, 1H, H-1), 4.44 (d, *J* = 11.8 Hz, 1H, C*H*HPh), 3.80 (dd, *J* = 9.1, 3.4 Hz, 1H, H-3), 3.75 (t, *J* = 6.3 Hz, 1H, H-5), 3.56 (dd, *J* = 9.6, 6.1 Hz, 1H, H-6), 3.53 – 3.46 (m, 2H, H-2, H-6), 2.87 – 2.69 (m, 2H), 2.07 (s, 3H, Ac), 1.34 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.30 (Ac), 137.99, 137.76, 128.64, 128.54, 128.41, 128.17, 128.01, 127.93, 85.31, 79.01, 76.07, 75.68, 73.88, 73.71, 70.26, 68.33, 25.41, 21.00 (Ac), 15.17. MS ESI+-HRMS m/z $[M+Na]^+$ calcd for $C_{24}H_{30}O_6SNa$ 469.1655, found 469.1754.

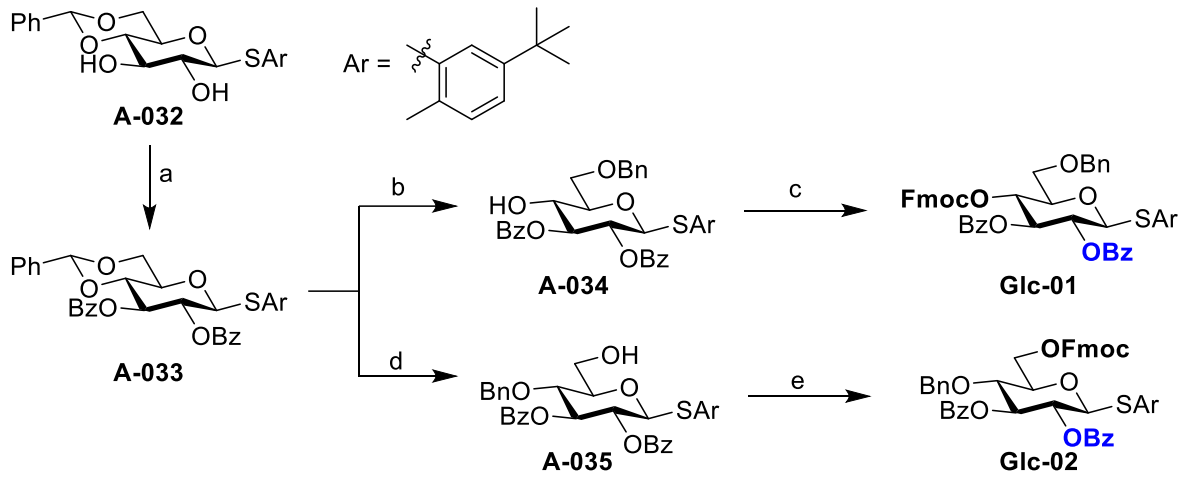
Ethyl 4-*O*-acetyl-2,6-di-*O*-benzyl-3-*O*-fluorenylmethoxycarbonyl-1-thio-β-D-galactopyranoside Gal-α-08



Gal-α-08

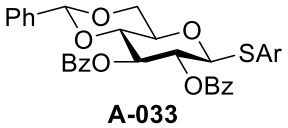
To a solution of **A-031** (0.78 g, 1.75 mmol, 1.0 eq) in anhydrous DCM (8.7 mL) were added 9-fluorenylmethylchloroformate (0.90 g, 3.49 mmol, 2.0 eq.) and pyridine (0.40 mL, 5.24 mmol, 3.0 eq.) successively at 0 °C, and stirred overnight at room temperature. After the mixture was quenched with 1M aqueous HCl, diluted with DCM, and was dried over $MgSO_4$ the solvent was evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane:ethylacetate:DCM = 8:1:1 to 8:2:1) to afford **Gal-α-08** (1.08 g, 1.62 mmol, 92%). R_f = 0.32 (hexane/ethyl acetate/DCM, 9:1:0.5); $[\alpha]_D^{20}$ = -1.01 (c = 3.45, chloroform); IR (thin film): υ = 1269, 1748, 1451, 1261, 1223, 1102 cm-1; ^{1}H NMR ($CDCl_3$) δ 7.77 (d, *J* = 7.5 Hz, 2H), 7.60 (dd, *J* = 17.2, 7.6 Hz, 2H), 7.44 – 7.38 (m, 3H), 7.37 – 7.23 (m, 11H), 5.63 (d, *J* = 2.5 Hz, 1H), 4.87 (d, *J* = 9.7 Hz, 2H), 4.71 (d, *J* = 10.6 Hz, 1H), 4.56 (d, *J* = 10.1 Hz, 2H), 4.54 – 4.48 (m, 1H), 4.43 (d, *J* = 12.0 Hz, 1H), 4.33 – 4.25 (m, 2H), 3.84 (t, *J* = 6.2 Hz, 1H), 3.71 (t, *J* = 9.7 Hz, 1H), 3.57 (dd, *J* = 9.3, 6.3 Hz, 1H), 3.49 (t, *J* = 8.1 Hz, 1H), 2.86 – 2.71 (m, 2H), 2.09 (d, *J* = 2.3 Hz, 3H), 1.34 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.33 (Ac), 154.27 (Fmoc), 143.88, 143.24, 141.46, 141.39, 137.79, 137.75, 128.59, 128.47, 128.26, 128.08, 128.02, 128.00, 127.98, 127.95, 127.31, 127.27, 125.44, 125.26, 120.14 (Ar), 85.53 (C-1), 78.70 (C-3), 76.34 (C-2), 75.85 (CH_2Ph), 75.78 (C-5), 73.72 (CH_2Ph), 70.42 (CH_2, Fmoc), 68.05 (C-6), 68.02 (C-4), 46.82 (CH, Fmoc), 25.47, 20.90 (Ac), 15.20. MS ESI+-HRMS m/z $[M+Na]^+$ calcd for $C_{39}H_{40}O_8SNa$ 691.2342, found 691.2245.

A.6 Building Block Preparation: Glc



Scheme A.07. Synthesis of glucose building blocks Glc-01 and Glc-02. a) Bz_2O, pyridine, DMAP, DCM, r.t , 93%; b) $HSiEt_3$, TFAA, TFA, DCM, 0 °C to r.t. 94%; c) FmocCl, pyridine, DCM, r.t., 96% for **Glc-01**; d) BH_3 in THF, TMSOTf, DCM, 0 °C , 94%; e) FmocCl, pyridine, DCM, r.t , 96% for **Glc-02.**

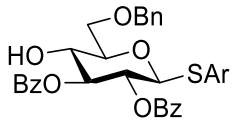
2-Methyl-5-tert-butylphenyl 2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside A-033



To a solution of compound **A-032** (10 g, 23 mmol, 1 eq.) in anhydrous DCM (116 mL, 0.2 M) were added benzoic anhydride (15.8 g, 70 mmol, 3.0 eq.), triethylamine (Et_3N) (13 mL, 93 mmol, 4.0 eq.), and a catalytic amount of DMAP (0.57 g, 4.65 mmol, 0.2 eq.) at 0°C, and then the mixture was stirred for 2 h at room temperature. After the mixture was quenched with saturated aqueous $NaHCO_3$, and diluted with DCM, the organic layer was dried over $MgSO_4$ and the solvent evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane/ethyl acetate = 9:1 to 7:3) to afford **A-033** (13.9 g, 21.7 mmol, 93%). R_f : 0.25 (Hexane/EtOAc/DCM : 1/9/0.5). $[\alpha]_D^{25}$ = 78.73 (C= 3.33, $CHCl_3$). IR (thin film): υ = 2962, 1727, 1270, 1095 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.97 (m, 4H), 7.58 (d, J = 2.1 Hz, 1H), 7.56 – 7.45 (m, 2H), 7.45 – 7.28 (m, 9H), 7.24 (dd, *J* = 8.0, 2.1 Hz, 2H), 7.11 (d, *J* = 8.0 Hz, 2H), 5.81 (t, *J* = 9.4 Hz, 1H, H-2), 5.64 – 5.46 (m, 2H, H-3, C*H*Ph), 4.99 (d, *J* = 10.1 Hz, 1H, H-1), 4.44 (dd, *J* = 10.5, 4.9 Hz, 1H, H-6), 3.96 (t, *J* = 9.5 Hz, 1H, H-4), 3.93 (t, *J* = 10.3 Hz, 1H, H-6), 3.74 (td, *J* = 9.8, 5.0 Hz, 1H, H-5), 2.22 (s, 3H, Me), 1.29 (s, 9H, *t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 165.54 (OBz), 165.20 (OBz), 149.64,

137.10, 136.66, 133.28, 133.08, 131.79, 130.10, 130.03, 129.83, 129.78, 129.35, 129.16, 129.02, 128.36, 128.27, 128.17, 126.11, 126.06, 125.50 (Ar), 101.46 (*C*Ph), 87.96 (C-1), 78.64 (C-4), 73.30 (C-2), 71.13 (C-3), 70.81 (C-5), 68.58 (C-6), 34.43 (Cq, *t*-Bu), 31.24 (Me, *t*-Bu), 20.24 (Me). MS ESI-HRMS *m/z* $[M+Na]^+$ calcd for $C_{38}H_{38}O_7S$ 661,2230, found 661.2255.

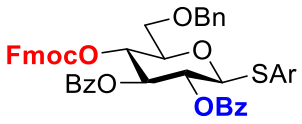
2-Methyl-5-tert-butylphenyl 2,3-di-*O*-benzoyl-6-*O*-benzyl-1-thio-β-D-glucopyranoside A-034



A-034

Compound **A-033** (4.7 g, 7.36 mmol, 1.0 eq.) was co-evaporated with toluene and dissolved under an Ar atmosphere in DCM (41 mL, 0.18 M). To a solution of compound **A-033** (4.7 g, 7.36 mmol, 1.0 eq.) were added triethylsilane (7.05 mL, 44.1 mmol, 6.0 eq.) and trifluoroacetic anhydride (0.520 mL, 3.68 mmol) at 0 °C for 30 min. Trifluoroacetic acid (2.83 mL, 36.8 mmol, 5.0 eq.) was added to the mixture slowly, and the mixture was stirred and allowed to warm to room temperature. After the mixture was quenched with saturated aqueous $NaHCO_3$, diluted with DCM, and dried over $MgSO_4$, the solvent was evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane/ethyl acetate = 9:1 to 7:3) to afford **A-034** (4.44 g, 6.94 mmol, 94%). R_f: 0.21 (Hexane/EtOAc/DCM : 8/2/0.5). $[\alpha]_D^{25}$ = 93.96 (C= 3.50, $CHCl_3$). IR (thin film): υ = 3458, 2961, 1729, 1274 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.07 – 7.82 (m, 4H), 7.59 (d, *J* = 2.0 Hz, 1H), 7.51 (ddd, *J* = 8.7, 2.5, 1.3 Hz, 2H), 7.40 – 7.27 (m, 8H), 7.22 (dd, *J* = 8.0, 2.1 Hz, 1H), 7.09 (d, *J* = 8.0 Hz, 1H), 5.63 – 5.21 (m, 2H, H-2, H-3), 4.88 (d, *J* = 9.7 Hz, 1H, H-1), 4.62 (q, *J* = 12.0 Hz, 2H, C*H*$_2$Ph), 4.00 (td, *J* = 9.3, 3.1 Hz, 1H, H-4), 3.86 (d, *J* = 4.6 Hz, 2H, H-6), 3.71 (dt, *J* = 9.3, 4.5 Hz, 1H, H-5), 3.23 (br, *J* = 3.4 Hz, 1H, OH), 2.23 (s, 3H, Me), 1.26 (s, 9H, *t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.29 (OBz), 165.41 (OBz), 149.79, 137.71, 137.22, 133.56, 133.38, 132.33, 130.20, 130.10, 130.06, 129.95, 129.48, 129.17, 128.62, 128.54, 128.50, 128.01, 127.99, 125.42 (Ar), 87.42 (C-1), 78.74 (C-5), 77.98 (C-3), 73.94 (*C*H_2Ph), 71.07 (C-4), 70.40 (C-2), 70.18 (C-6), 34.56 (Cq, *t*-Bu), 31.39 (Me, *t*-Bu), 20.45 (Me). MS ESI-HRMS *m/z* $[M+Na]^+$ calcd for $C_{38}H_{40}O_7S$ 663,2387, found 663.2392.

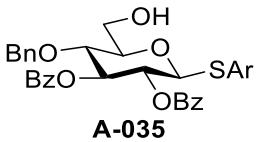
2-Methyl-5-tert-butylphenyl 2,3-di-*O*-benzoyl-6-*O*-benzyl-4-*O*-fluorenylmethoxycarbonyl-1-thio-β-D-glucopyranoside Glc-01



Glc-01

To a solution of compound **A-034** (4.45 g, 6.94 mmol) were added 9-fluorenylmethyl chloroformate (3.59 g, 13.88 mmol) and pyridine (1.68 mL, 20.82 mmol) successively at 0°C, and stirred overnight at room temperature. After the mixture was quenched with 1M aqueous HCl, and diluted with DCM the organic layer was dried over $MgSO_4$ and the solvent evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane/ethyl acetate/DCM = 9:0.5:0.5 to 9:1:0.5) to afford **Glc-01** (5.74 g, 6.65 mmol, 96%). R_f : 0.18 (Hexane/EtOAc/DCM : 9/1/0.5). $[\alpha]_D^{25}$ = 3.09 (C= 3.05, $CHCl_3$). IR (thin film): υ = 2960, 1755, 1732, 1278, 1249 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, *J* = 8.4 Hz, 2H), 7.88 (d, *J* = 8.4 Hz, 2H), 7.71 (dd, *J* = 7.6, 3.3 Hz, 2H), 7.61 (d, *J* = 1.7 Hz, 1H), 7.56 – 7.50 (m, 1H), 7.46 – 7.20 (m, 16H), 7.17 (t, *J* = 7.5 Hz, 1H), 7.10 (d, *J* = 8.0 Hz, 1H), 5.80 (t, *J* = 9.5 Hz, 1H, H-3), 5.54 (t, *J* = 9.8 Hz, 1H, H-3), 5.25 (t, *J* = 9.8 Hz, 1H, H-4), 4.94 (d, *J* = 10.1 Hz, 1H, H-1), 4.58 (q, *J* = 12.2 Hz, 2H, C*H*$_2$Ph), 4.22 (dd, *J* = 10.4, 7.3 Hz, 1H, C*H*HPh of Fmoc), 4.08 (dd, *J* = 10.4, 7.8 Hz, 1H, H-6, C*H*HPh of Fmoc), 4.00 – 3.85 (m, 2H, H-5, C*H* of Fmoc), 3.74 (d, *J* = 3.9 Hz, 2H, H-6), 2.25 (s, 3H, Me), 1.26 (d, *J* = 0.8 Hz, 9H, *t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 165.82 (OBz), 165.22 (OBz), 154.17, 149.86, 143.34, 143.04, 141.25, 141.20, 137.79, 137.34, 133.39, 132.13, 130.32, 130.09, 130.00, 129.98, 129.35, 128.93, 128.49, 128.46, 128.40, 127.90, 127.79, 127.23, 127.23, 125.54, 125.28, 125.11, 120.02 (Ar), 87.61 (C-1), 77.34 (C-5), 74.58 (C-3), 73.75 (*C*H$_2$Ph), 73.38 (C-4), 70.79 (C-2), 70.43 (*C*H$_2$ of Fmoc), 69.02 (C-6), 46.55 (*C*H of Fmoc), 34.54 (Cq, *t*-Bu), 31.37 (Me, *t*-Bu), 20.46 (Me). MS ESI-HRMS *m/z* $[M+Na]^+$ calcd for $C_{53}H_{50}O_9S$ 885.3068, found 885.3094.

2-Methyl-5-tert-butylphenyl 2,3-di-*O*-benzoyl-4-*O*-benzyl-1-thio-β-D-glucopyranoside A-035

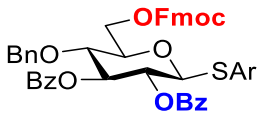


A-035

Compound **A-033**[1] (4.7 g, 7.36 mmol) was co-evaporated with toluene and dissolved under an Ar atmosphere in DCM (41 mL, 0.18 M). To a solution of compound **A-033** (10.0 g, 15.7

mmol) was added triethylsilane (7.05 mL, 44.1 mmol) and trifluoroacetic anhydride (0.520 mL, 3.68 mmol) at 0 °C. After 30 min, trifluoroacetic acid (2.83 mL, 36.8 mmol) was added slowly, and the reaction mixture was allowed to warm up to room temperature gradually. After 5 h, the mixture was quenched with saturated aqueous $NaHCO_3$, and then diluted with DCM. The organic layer was separated and aqueous phase was extracted twice with DCM. The organic layer was dried over $MgSO_4$ and the solvent was evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane/ethyl acetate /DCM = 9:1:0.5 to 7:3:0.5) to afford **A-035** (4.44 g, 6.94 mmol, 94%). R_f = 0.21 (hexane/ethyl acetate /DCM = 8:2:0.5). $[\alpha]_D^{25}$ = 93.96 (*c* = 3.50, $CHCl_3$). IR (thin film): υ = 3458, 2961, 1729, 1274 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.07 – 7.82 (m, 4H), 7.59 (d, *J* = 2.0 Hz, 1H), 7.51 (ddd, *J* = 8.7, 2.5, 1.3 Hz, 2H), 7.40 – 7.27 (m, 8H), 7.22 (dd, *J* = 8.0, 2.1 Hz, 1H), 7.09 (d, *J* = 8.0 Hz, 1H), 5.63 – 5.21 (m, 2H, H-2, H-3), 4.88 (d, *J* = 9.7 Hz, 1H, H-1), 4.62 (q, *J* = 12.0 Hz, 2H, CH_2Ph), 4.00 (td, *J* = 9.3, 3.1 Hz, 1H, H-4), 3.86 (d, *J* = 4.6 Hz, 2H, H-6), 3.71 (dt, *J* = 9.3, 4.5 Hz, 1H, H-5), 3.23 (br, *J* = 3.4 Hz, 1H, OH), 2.23 (s, 3H, Me), 1.26 (s, 9H, *t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.29 (OBz), 165.41 (OBz), 149.79, 137.71, 137.22, 133.56, 133.38, 132.33, 130.20, 130.10, 130.06, 129.95, 129.48, 129.17, 128.62, 128.54, 128.50, 128.01, 127.99, 125.42 (Ar), 87.42 (C-1), 78.74 (C-5), 77.98 (C-3), 73.94 (CH_2Ph), 71.07 (C-4), 70.40 (C-2), 70.18 (C-6), 34.56 (Cq, *t*-Bu), 31.39 (Me, t-Bu), 20.45 (Me). MS ESI-HRMS m/z $[M+Na]^+$ calcd for $C_{38}H_{40}O_7S$ 663,2387, found 663.2392.

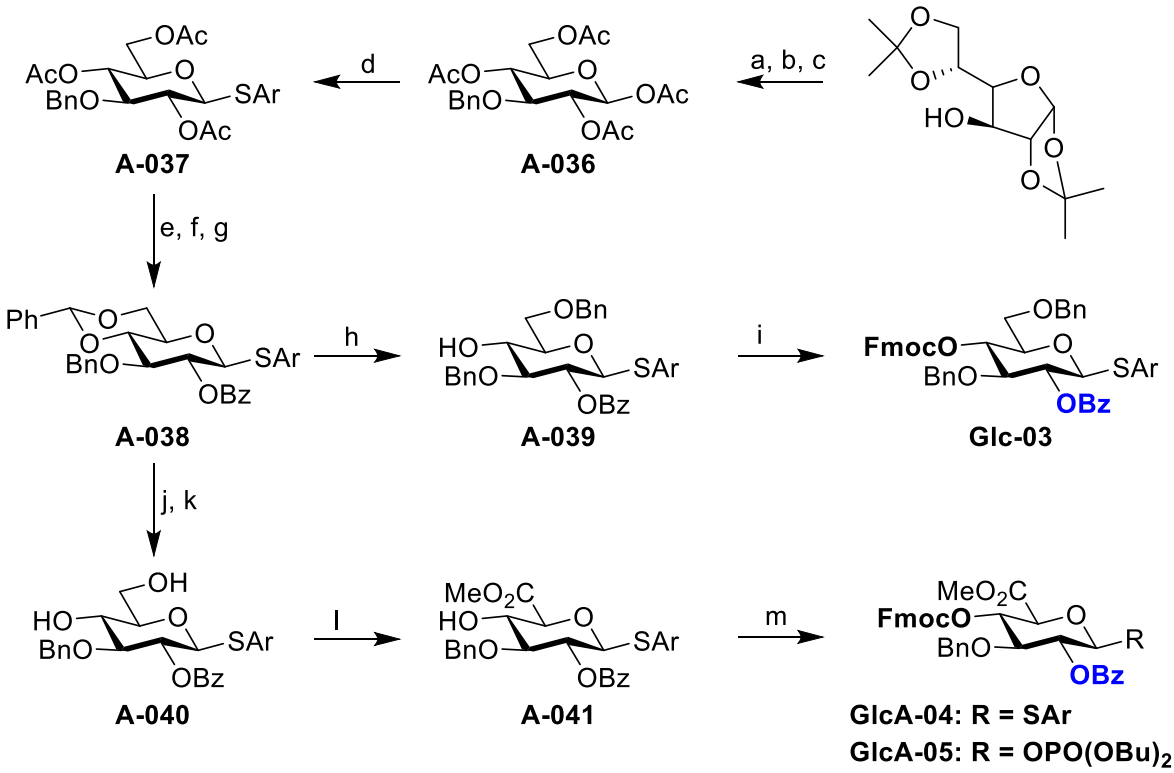
(2-Methyl-5-tert-butylphenyl) 2,3-di-*O*-benzoyl-4-*O*-benzyl-6-*O*-fluorenylmethoxycarbonyl-1-thio-β-D-glucopyranoside Glc-02



Glc-02

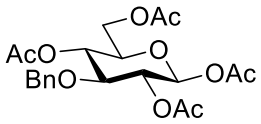
To a solution of compound **A-035** (4.45 g, 6.94 mmol) was added 9-fluorenylmethyl chloroformate (3.59 g, 13.88 mmol) and pyridine (1.68 mL, 20.82 mmol) at 0 °C, and stirred at room temperature for overnight. After the mixture was quenched with 1M aqueous HCl, it was diluted with DCM. The organic layer was separated and aqueous phase was extracted twice with DCM. The combined organic layer was dried over $MgSO_4$ and the solvent was evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane/ethyl acetate /DCM = 9:0.5:0.5 to 9:1:0.5) to afford **Glc-02** (5.74 g, 6.65 mmol, 96%). R_f = 0.18 (hexane/ethyl acetate /DCM = 9:1:0.5). $[\alpha]_D^{25}$ = 3.09 (*c* = 3.05, $CHCl_3$). IR (thin

film): ν = 2960, 1755, 1732, 1278, 1249 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, *J* = 8.4 Hz, 2H), 7.88 (d, *J* = 8.4 Hz, 2H), 7.71 (dd, *J* = 7.6, 3.3 Hz, 2H), 7.61 (d, *J* = 1.7 Hz, 1H), 7.56 – 7.50 (m, 1H), 7.46 – 7.20 (m, 16H), 7.17 (t, *J* = 7.5 Hz, 1H), 7.10 (d, *J* = 8.0 Hz, 1H), 5.80 (t, *J* = 9.5 Hz, 1H, H-3), 5.54 (t, *J* = 9.8 Hz, 1H, H-3), 5.25 (t, *J* = 9.8 Hz, 1H, H-4), 4.94 (d, *J* = 10.1 Hz, 1H, H-1), 4.58 (q, *J* = 12.2 Hz, 2H, CH_2Ph), 4.22 (dd, *J* = 10.4, 7.3 Hz, 1H, C*H*HPh, Fmoc), 4.08 (dd, *J* = 10.4, 7.8 Hz, 3H, H-6, C*H*HPh, Fmoc), 4.00 – 3.85 (m, 2H, H-5, CH, Fmoc), 3.74 (d, *J* = 3.9 Hz, 2H, H-6), 2.25 (s, 3H, Me), 1.26 (d, *J* = 0.8 Hz, 9H, *t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.82 (OBz), 165.22 (OBz), 154.17, 149.86, 143.34, 143.04, 141.25, 141.20, 137.79, 137.34, 133.39, 132.13, 130.32, 130.09, 130.00, 129.98, 129.35, 128.93, 128.49, 128.46, 128.40, 127.90, 127.79, 127.23, 127.23, 125.54, 125.28, 125.11, 120.02 (Ar), 87.61 (C-1), 77.34 (C-5), 74.58 (C-3), 73.75 (CH_2Ph), 73.38 (C-4), 70.79 (C-2), 70.43 (CH_2 of Fmoc), 69.02 (C-6), 46.55 (CH of Fmoc), 34.54 (Cq, *t*-Bu), 31.37 (Me, *t*-Bu), 20.46 (Me). MS ESI-HRMS m/z $[M+Na]^+$ calcd for $C_{53}H_{50}O_9S$ 885.3068, found 885.3094.



Scheme A.07. Synthesis of glucose building blocks Glc-03, GlcA-04, and GlcA-05. a) NaH, BnBr, DMF, 0 °C → r.t.; b) 60% AcOH in H_2O, 90 °C; c) Ac_2O, pyridine, r.t., 93% over 3 steps; d) (*t*Bu,Me)PhSH, $BF_3{\cdot}OEt_2$, 0 °C → r.t.; 90%; e) NaOMe, MeOH, r.t.; f) $PhCH(OMe)_2$, *p*TsOH·H_2O, DMF, 80 °C; g) Bz_2O, DMAP, NEt_3, DCM, 0 °C C → r.t.; 89% for 3 steps; h) $HSiEt_3$, TFAA, TFA, DCM, 0 °C to r.t. 94%; i) FmocCl, pyridine, DCM, r.t., 96% for **Glc-03**; j) 60% TFA, H_2O, DCM r.t. (e,f,g j: 81% over 4 steps); k) $PhI(OAc)_2$, TEMPO, H_2O, DCM r.t.; l) MeI, K_2CO_3, DMF r.t. 81% over 2 steps; m) FmocCl, pyridine, DCM, 0 °C (94%); l) $HOP(O)OBu_2$, NIS, TfOH, DCM, 0 °C (**4**: 87%); TEMPO = (2,2,6,6-tetramethylpiperidin-1-yl)oxyl;

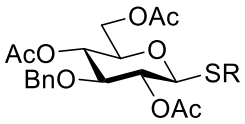
1,2,4,6-Tetra-*O*-acetyl-3-*O*-benzyl-β-D-glucopyranose A-036



A-036

Sodium hydride (1.1 eq) was added portion-wise at 0 °C to a solution of diacetone-glucose and benzyl bromide (1.05 eq) in DMF. The reaction mixture was stirred for 3 h while the mixture was allowed to warm up to room temperature. The reaction was quenched by slowly adding aqueous NH_4Cl and the mixture was diluted with ethyl acetate. After phase separation, the aqueous layer was extracted with ethyl acetate and the combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated. The crude product was dissolved in acetic acid/water, 3:2 and stirred overnight at 90 °C. The reaction mixture was co-evaporated three times with toluene and subsequently dissolved in pyridine. Acetic anhydride was added to the mixture and the reaction was stirred at room temperature for 6 h. The volatiles were removed *in vacuo* and the remainder was co-evaporated three times with toluene and dissolved in ethyl acetate. The organic phase was extracted with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated. The crude product was crystallized from ethanol to yield **A-036** (93% over three steps). The analytical data were in agreement with those reported in the literature

2-Methyl-5-*tert*-butylphenyl 2,4,6-tri-*O*-acetyl-3-*O*-benzyl-1-thio-β-D-glucopyranose A-037

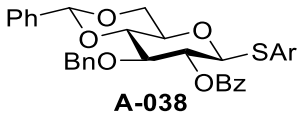


A-037

Acetylglucopyranose **A-036** (24.3 g, 55.4 mmol, 1.0 eq) was dissolved in anhydrous DCM (110 mL, 0.5 M) and cooled to 0 °C. 2-Methyl-5-*tert*-butyl-thiolphenol (13.2 mL, 66.5 mmol, 1.2 eq) and $BF_3 \cdot OEt_2$ (8.43 mL, 66.5 mmol, 1.2 eq) were added. The mixture was stirred for 3 h and was allowed to warm to room temperature. The reaction mixture was quenched by being poured into cold aqueous $NaHCO_3$. The layers were separated and the aqueous layer was extracted with DCM. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography on silica gel (*cyclo*-hexane/ethyl acetate, 4:1) to give a white foam containing two anomers

(90%). This product was dissolved in ethanol to crystallize the β-anomer (75%), **A-036**. α-Anomer: R_f = 0.38 (*cyclo*-hexane/ethyl acetate, 4:1). $[\alpha]_D^{20}$ = +143.0 (c = 1.10, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.46 (d, *J* = 2.0 Hz, 1H, Ar), 7.38-7.27 (m, 5H, Ar), 7.21 (dd, *J* = 8.0, 2.1 Hz, 1H, Ar), 7.13 (d, *J* = 8.0 Hz, 1H, Ar), 5.82 (d, *J* = 5.6Hz, 1H, H-1), 5.14 (dd, *J* = 10.2, 9.3 Hz, 1H, H-4), 5.07 (dd, *J* = 10.1 Hz, 1H, H-2), 4.79 (d, *J* = 11.8 Hz, 1H, CH_2O-Bn), 4.66 (d, *J* = 11.8 Hz, 1H, CH_2O-Bn), 4.45 (ddd, *J* = 10.1, 4.4, 2.3 Hz, 1H, H-5), 4.27 (dd, *J* = 12.4, 4.4 Hz, 1H, H-6a), 4.01 (dd, J_{gem} = 12.4, 2.3 Hz, 1H, H-6b), 3.98 – 3.91 (m, 1H, H-3), 2.38 (s, 3H, OAc), 2.15 (s, 3H, OAc), 2.07 (s, 3H, OAc), 2.02 (s, 3H, Me-thio), 1.34 (s, 9H, *t*Bu); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.5, 169.6, 169.2 (C=O), 149.7-124.9 (Ar), 85.4 (C-1), 77.7 (C-3), 74.9 (CH_2O-Bn), 73.3 (C-2), 69.3 (C-4), 68.8 (C-5), 61.9 (C-6), 34.3 (Cq *t*Bu), 31.1 (Me *t*Bu), 20.7, 20.6, 20.5 (OAc), 20.0 (Me-thio); MS ESI-HRMS *m/z* $[M+NH_4]^+$ calcd for $C_{30}H_{42}NO_8S$ 576.2631, found 576.2624. α-Anomer **17**: R_f = 0.25 (*cyclo*-hexane/ethyl acetate, 8/2); mp: 94-95°C. $[\alpha]_D^{25}$ = -3 (c = 4.5, $CHCl_3$); IR (thin film): υ = 2963, 1740, 1453, 1365, 1231, 1215, 1072, 1039, 897, 821, 698 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.55 (d, *J* = 2.1 Hz, 1H, Ar), 7.35-7.26 (m, 3H, Ar), 7.25-7.20 (m, 3H, Ar), 7.13 (d, *J* = 8.0 Hz, 1H, Ar), 5.17 (m, 2H, H-2, H-4), 4.69-4.65 (m, 3H, 2×CH_2O-Bn, H-1), 4.27 (dd, J_{gem} = 12.3 Hz, J_{H6-H5} = 5.3 Hz, 1H, H-6a), 4.14 (dd, J_{H6-H5} = 2.3 Hz, 1H, H-6b), 3.79 (t, J_{H3-H4} = J_{H3-H2} = 9.2 Hz, 1H, H-3), 3.67 (m, 1H, H-5), 2.40 (s, 3H, Me-thio), 2.08 (s, 3H, OAc), 2.08 (s, 3H, OAc), 1.99 (s, 3H, OAc), 1.36 (s, 9H, *t*Bu); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.3, 169.0, 168.8 (C=O), 149.3-124.9 (Ar), 86.9 (C-1), 81.2 (C-3), 75.7 (C-5), 73.8 (CH_2O-Bn), 71.1 (C-2), 69.1 (C-4), 62.3 (C-6), 34.1 (Cq *t*Bu), 31.0 (Me *t*Bu), 20.6, 20.4, 20.4 (OAc), 19.9 (Me-thio); ESI-HRMS m/z $[M+NH_4]^+$ calcd for $C_{30}H_{42}NO_8S$ 576.2631, found 576.2629.

2-Methyl-5-tert-butylphenyl 2-*O*-benzoyl-3-*O*-benzyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside A-038

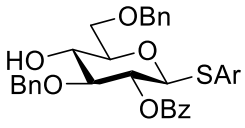


A-038

To a solution of compound **A-037** (9.59 g, 17.17 mmol, 1.0 eq.) in MeOH (86 ml, 0.2 M) was added sodium methoxide (NaOMe) (0.93 g, 17.17 mmol, 1.0 eq.) at 0 °C, and then the mixture was stirred overnight at 40 °C. After completion, the mixture was neutralized with Amberlite IR-120 (H+) ion exchange resin, filtered off, concentrated, and coevaporated with toluene. To a solution of the crude mixture in DMF (86 ml, 0.2 M) were added benzaldehyde dimethyl acetal (5.16 mL, 34.4 mmol, 2.0 eq.) and a catalytic amount of p-toluenesulfonic

acid (pTsOH) (0.65 g, 3.44 mmol, 0.2 eq.) and the mixture was heated at 80°C for 2 h. After the mixture was quenched with saturated aqueous $NaHCO_3$ and extracted with ethyl acetate three times, after which the combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated. To a solution of this crude mixture in anhydrous DCM (86 ml, 0.2 M) were added benzoic anhydride (Bz_2O) (7.77 g, 34.3 mmol, 2.0 eq.), triethylamine (7.2 mL, 51.5 mmol, 3.0 eq.), and a catalytic amount of DMAP (0.42 g, 3.43 mmol, 0.2 eq.) at 0°C, and the mixture was stirred overnight at room temperature. After the mixture was quenched by saturated aqueous $NaHCO_3$, and diluted with DCM, the organic layer was dried over $MgSO_4$ and the solvent was evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane/ethyl acetate/DCM = 9:0.5:0.5 to 9:1:0.5) to afford **A-038** (9.5 g, 15.21 mmol, 89% over three steps). R_f : 0.23 (hexane/EtOAc/DCM : 8/2/0.5). $[\alpha]_D^{25}$ = + 69.86 (c = 3.18, $CHCl_3$); IR (thin film): υ = 2962, 2870, 1729, 1452, 1265 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 8.06 – 8.00 (m, 2H), 7.65 – 7.57 (m, 1H), 7.56 – 7.37 (m, 8H), 7.21 (dd, *J* = 8.0, 2.1 Hz, 1H), 7.17 – 7.05 (m, 6H), 5.64 (s, 1H, CHPh), 5.38 (ddd, *J* = 10.2, 5.9, 2.8 Hz, 1H, H-2), 4.83 (d, *J* = 12.0 Hz, 1H, C*H*HPh), 4.82 (d, *J* = 10.2 Hz, 1H, H-2), 4.70 (d, *J* = 12.0 Hz, 1H, C*H*HPh), 4.40 (dd, *J* = 10.5, 5.0 Hz, 1H, H-6), 3.96 – 3.83 (m, 3H, H-3, H-4, H-6), 3.56 (dt, *J* = 14.4, 4.9 Hz, 1H, H-5), 2.19 (s, 3H), 1.28 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ : 165.07 (Bz), 149.50, 137.68, 137.11, 136.87, 133.17, 132.21, 129.91, 129.88, 129.76, 129.73, 129.03, 128.34, 128.27, 128.13, 128.06, 127.55, 125.96, 125.21 (Ar), 101.27 (CHPh), 87.86 (C-1), 81.48 (C-3 or C-4), 79.27 (C-3 or C-4), 74.20 (Bn), 72.08 (C-2), 70.50 (C-5), 68.65 (C-6), 34.39 (Cq, *t*-Bu), 31.23 (Me, *t*-Bu), 20.19 (Me). MS ESI-HRMS *m/z* $[M+NH_4]^+$ calcd for $C_{38}H_{40}O_6SNa$ 647.2443, found 647.2462.

2-Methyl-5-*turt*-butylphenyl-2-*O*-benzoyl -3, 6-di-*O*-benzyl-1-thio-β-D-glucopyranose A-039

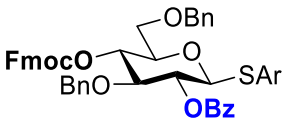


A-039

Compound **A-038** (4.7 g, 7.36 mmol, 1.0 eq.) was co-evaporated with toluene and dissolved under an Ar atmosphere in DCM (41 mL, 0.18 M). To a solution of compound **S6** (10.0 g, 15.7 mmol, 1.0 eq.) were added triethylsilane (7.05 mL, 44.1 mmol, 6.0 eq.) and trifluoroacetic anhydride (0.520 mL, 3.68 mmol) at 0°C. After 30 min, trifluoroacetic acid (2.83 mL, 36.8 mmol, 5.0 eq.) was added to the mixture that was stirred and allowed to warm

to room temperature for 8 h. After the mixture was quenched with saturated aqueous $NaHCO_3$, diluted with DCM, and dried over $MgSO_4$ the solvent was evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane/ethyl acetate = 9:1 to 7:3) to afford **A-039** (4.44 g, 6.94 mmol, 94%). R_f : 0.25 (Hexane/EtOAc/DCM : 8/2/0.5). $[\alpha]_D^{25}$ = 38.25 (c = 1.00, $CHCl_3$). IR (thin film): υ = 3458, 2961, 1729, 1274 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.09 – 8.05 (m, 2H), 7.63 – 7.56 (m, 2H), 7.50 – 7.43 (m, 2H), 7.39 – 7.27 (m, 5H), 7.22 – 7.16 (m, 6H), 7.07 (d, *J* = 8.0 Hz, 1H), 5.35 (dd, *J* = 10.1, 9.1 Hz, 1H, H-2), 4.76 (d, *J* = 10.1 Hz, 1H, H-1), 4.71 (dd, *J* = 20.0, 9.6 Hz, 2H, CH_2Ph), 4.60 (q, *J* = 12.0 Hz, 2H, CH_2Ph), 3.85 (t, *J* = 9.2 Hz, 1H, H-4), 3.80 (d, *J* = 4.7 Hz, 2H, H-6), 3.71 (t, *J* = 9.0 Hz, 1H, H-3), 3.57 (dt, *J* = 9.5, 4.7 Hz, 1H, H-5), 2.78 (br, 1H, OH), 2.20 (s, 3H), 1.25 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.38 (Bz), 149.69, 138.00, 137.76, 136.89, 133.34, 132.95, 129.98, 129.92, 129.70, 128.60, 128.55, 128.53, 128.17, 127.99, 127.97, 127.94, 125.08 (Ar), 87.60 (C-1), 83.70 (C-3), 78.23 (C-5), 74.73 (Bn), 73.89 (Bn), 72.29 (C-2), 72.22 (C-4), 70.49 (C-6), 34.53 (Cq, *t*-Bu), 31.38 (Me, *t*-Bu), 20.38 (Me). MS ESI-HRMS *m/z* $[M+Na]^+$ calcd for $C_{38}H_{42}O_6SNa$ 649,2600, found 649.2585.

2-Methyl-5-turt-butylphenyl-2-O-benzoyl -3, 6-di-O-benzyl-1-thio-β-D-glucopyranose Glc-03

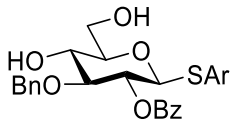


Glc-03

To a solution of compound **A-039** (4.45 g, 6.94 mmol, 1.0 eq.) were added 9-fluorenylmethyl chloroformate (3.59 g, 13.88 mmol, 2.0 eq.) and pyridine (1.68 mL, 20.82 mmol, 3.0 eq.) successively at 0°C, and then the mixture was stirred overnight at room temperature. After the mixture was quenched with 1M aqueous HCl, diluted with DCM, and dried over $MgSO_4$ the solvent was evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane/ethyl acetate/DCM = 9:0.5:0.5 to 9:1:0.5) to afford **Glc-03** (5.74 g, 6.65 mmol, 96%). R_f : 0.17 (Hexane/EtOAc/DCM : 9/1/0.5). $[\alpha]_D^{25}$ = 35.70 (c = 2.95, $CHCl_3$). IR (thin film): υ = 2960, 1754, 1732, 1451, 1248 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.03 (dd, *J* = 7.5, 0.9 Hz, 2H), 7.75 (dd, *J* = 7.5, 3.8 Hz, 2H), 7.63 – 7.51 (m, 4H), 7.45 (t, *J* = 7.7 Hz, 2H), 7.39 (td, *J* = 7.5, 2.3 Hz, 2H), 7.33 – 7.17 (m, 8H), 7.10 – 7.02 (m, 6H), 5.39 (t, *J* = 9.6 Hz, 1H, H-2), 5.03 (t, *J* = 9.5 Hz, 1H, H-4), 4.76 (d, *J* = 10.1 Hz, 1H, H-1), 4.57 (dd, *J* = 20.3, 12.6 Hz, 2H, CH_2Ph), 4.53 (s, 2H, CH_2Ph), 4.32 (d, *J* = 7.1 Hz, 2H,

CH_2, Fmoc), 4.12 (t, *J* = 7.1 Hz, 1H, CH, Fmoc), 3.92 (t, *J* = 9.2 Hz, 1H, H-3), 3.80 – 3.71 (m, 1H, H-5), 3.71 – 3.62 (m, 2H, H-6), 2.20 (s, 3H), 1.23 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 165.15 (Bz), 154.33 (Fmoc), 149.82, 143.43, 143.24, 141.44, 141.40, 137.89, 137.44, 137.03, 133.41, 132.74, 130.02, 129.98, 129.83, 128.56, 128.46, 128.29, 128.04, 127.88, 127.76, 127.31, 125.23, 125.14, 120.21 (Ar), 87.69 (C-1), 81.19 (C-3), 77.40 (C-5), 75.55 (C-4), 74.40 (Bn), 73.73 (Bn), 72.18 (C-2), 70.19 (CH_2, Fmoc), 69.76 (C-6), 46.84 (CH, Fmoc), 34.55 (Cq, *t*-Bu), 31.37 (Me, *t*-Bu), 20.43 (Me). MS ESI-HRMS *m/z* $[M+Na]^+$ calcd for $C_{53}H_{52}O_8SNa$ 871.3281, found 871.3311.

2-Methyl-5-*tert*-butylphenyl 2-*O*-benzoyl-3-*O*-benzyl-1-thio-β-D-glucopyranose A-040

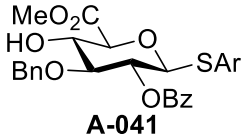


A-040

Thioglucopyranose **A-038** was dissolved in MeOH, NaOMe (1.0 eq) was added and the reaction mixture was stirred overnight. The mixture was neutralized with IR-120-H^+ amberlite resin, filtered off, concentrated and co-evaporated with toluene. The crude triol was dissolved in DMF. Benzaldehyde dimethyl acetal (2.0 eq) and a catalytic amount of *para*-toluene sulfonic acid were added and the mixture was stirred at 80 °C for 2 h. After the mixture was cooled to room temperature, saturated aqueous $NaHCO_3$ was added. After phase separation, the organic phase was extracted three times with ethyl acetate. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated. The remainder was dissolved in DCM, cooled to 0 °C and Bz_2O (2.0 eq), DMAP (0.5 eq) and triethylamine (4.0 eq) were added. After complete conversion, the reaction was quenched with saturated aqueous $NaHCO_3$ and the combined organic layers were washed with brine, dried over MgSO4, filtered and concentrated. The crude product was co-evaporated with toluene, dissolved in DCM and 60% aqueous TFA was added. The reaction was stirred for 2 h. Saturated aqueous $NaHCO_3$ was added and after phase separation the aqueous layers were extracted three times with DCM. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated. The residue was purified by flash column chromatography on silica gel to provide compound **A-040** (81% over four steps). R_f = 0.47 (hexane/ethyl acetate, 1:1); $[\alpha]_D^{20}$ = +49.5 (c = 1.9, CH_2Cl_2); IR (thin film): υ = 3263, 2946, 1723, 1452, 1384, 1359, 1269, 1069, 1042, 986, 827, 730, cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.15 – 8.03 (m, 2H, Ar), 7.66 – 7.55 (m, 1H, Ar), 7.52 (d, *J* = 2.1 Hz, 1H, Ar), 7.50 – 7.44

(m, 2H, Ar), 7.25 – 7.16 (m, 6H, Ar), 7.08 (d, *J* = 8.0 Hz, 1H, Ar), 5.35 (dd, *J* = 10.1, 8.8 Hz, 1H, H-2), 4.80 (d, *J* = 10.1 Hz, 1H, H-1), 4.75 (d, *J* = 11.5 Hz, 1H, CH_2O-Bn), 4.59 (d, *J* = 11.4 Hz, 1H, CH_2O-Bn), 3.95 (dd, *J* = 11.9, 3.5 Hz, 1H, H-6a), 3.83 (dd, *J* = 12.1, 5.3 Hz, 1H, H-6b), 3.78 (t, *J* = 9.2 Hz, 1H, H-4), 3.72 (t, *J* = 8.8 Hz, 1H, H-3). 3.47 (ddd, *J* = 9.0, 5.2, 3.5 Hz, 1H, H-5), 2.39 (d, *J* = 2.3 Hz, 1H, HO-4), 2.20 (s, 3H, Me-thio), 1.56 (br, 1H, HO-6), 1.27 (s, 9H, *t*Bu); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 165.38 (C=O), 149.83, 137.81, 136.85, 133.52, 130.13, 130.00, 129.83, 129.53, 128.75, 128.66, 128.24, 128.19, 125.35 (Ar), 87.43 (C-1), 84.12 (C-3), 79.45 (C-5), 74.91 (CH_2O-Bn), 72.62 (C-2), 70.61 (C-4), 62.95 (C-6), 34.59 (Cq *t*Bu), 31.43 (Me *t*Bu), 20.34 (Me-thio); ESI-HRMS *m/z* $[M+Na]^+$ calcd for $C_{31}H_{36}O_6S$ 559.2130, found 559.2122.

Methyl (2-Methyl-5-*tert*-butyl-phenyl)-2-*O*-benzoyl-3-*O*-benzyl-1-thio-β-D-glucopyranosyluronate A-041

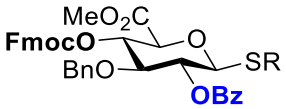


A-041

To a mixture of diol **A-040** (32.0 g, 62.1 mmol) and $PhI(OAc)_2$ (44.0 g, 137 mmol, 2.2 eq) in DCM (292 mL), water (68.5 mL) and TEMPO (2.91 g, 18.6 mmol, 0.3 eq) were added. After 30 min a saturated $Na_2S_2O_3$ solution was added and the mixture was extracted with DCM. The organic layer was dried over $MgSO_4$, filtered and the solvent was removed *in vacuo*. The residue was co-evaporated with toluene and dissolved in DMF (10 mL). Potassium carbonate (28.4 g, 206 mmol, 3 eq), MeI (8.58 mL, 137 mmol, 2 eq) were added and the reaction was stirred for 4 h. NH_4Cl was added and the reaction mixture was diluted and extracted with DCM, washed with water and brine. The organic layer was dried over $MgSO_4$, filtered and the solvent was evaporated. The residue was purified by column chromatography on silica gel (*cyclo*-hexane/ethyl acetate, 7:3) affording 29.2 g of compound **A-041** (81%) as a white foam that was crystallized from MeOH. R_f = 0.71 (hexane/ethyl acetate, 1:1); $[\alpha]_D^{20}$ = +31.9 (c = 2.4, CH_2Cl_2); IR (thin film): υ = 3408, 2956, 2866, 1753, 1725, 1602, 1452, 1360, 1254, 1068, 987, 824, 771, 708 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.18 – 7.91 (m, 2H, Ar), 7.71 – 7.53 (m, 2H, Ar), 7.51 – 7.38 (m, 2H, Ar), 7.24 – 7.11 (m, 6H, Ar), 7.07 (d, *J* = 8.1 Hz, 1H, Ar), 5.32 (dd, *J* = 10.2, 9.1 Hz, 1H, H-2), 4.76 (d, *J* = 10.2 Hz, 1H, H-1), 4.80 – 4.71 (m, 2H, CH_2O-Bn), 4.11 (ddd, *J* = 9.8, 8.9, 2.6 Hz, 1H, H-4), 3.91 (d, *J* = 9.8 Hz, 1H, H-5), 3.84 (s, 1H, OMe), 3.74 (t, *J* = 9.0 Hz, 1H, H-3), 3.11 (d, *J* = 2.7 Hz, 1H, HO-4), 2.18 (s, 3H Me-thio), 1.28 (s, 9H *t*Bu); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 169.52, 165.27 (C=O), 149.80, 137.89,

137.12, 133.40, 130.31, 130.01, 129.87, 128.56, 128.45, 128.23, 127.88, 125.44 (Ar), 88.34 (C-1), 82.28 (C-3), 77.64 (C-5), 74.86 (CH_2O-Bn), 72.16 (C-4), 71.59 (C-2), 52.93 (OMe), 34.58 (Cq *t*Bu), 31.34 (Me *t*Bu), 20.34 (Me-thio); ESI-HRMS *m/z* $[M+Na]^+$ calcd for $C_{32}H_{36}O_7S$ 587.2079, found 587.1089.

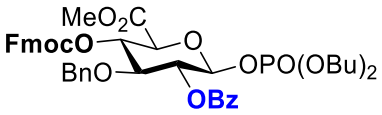
Methyl (2-methyl-5-*tert*-butyl-phenyl)-2-*O*-benzoyl-3-*O*-benzyl-4-*O*-fluorenylmethoxycarbonyl-1-thio-β-D-glucopyranosyluronate GlcA-04



GlcA-04

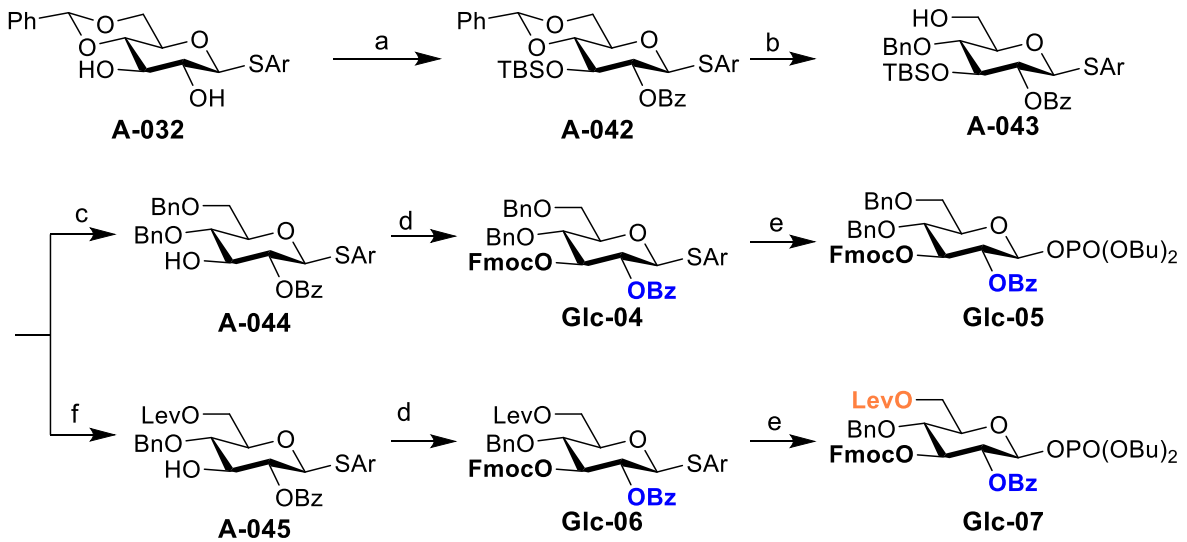
FmocCl (2.3 g, 9.0 mmol, 1.5 eq) and pyridine (0.97 mL, 12.0 mmol, 2.0 eq) were added to a solution of glucopyranosyluronate **A-041** (3.27 g, 6.0 mmol) in DCM (18 mL) at 0 ºC and stirred for 2 h. After the reaction was completed, a saturated $NaHCO_3$ solution was added. After phase separation the aqueous layer was extracted with DCM. The organic layer was washed with 1 M aqueous HCl, dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography on silica gel (*cyclo*-hexane/DCM/ ethyl acetate = 7:2:0.2) to give 4.32 g of compound **GlcA-04** (94%) as a white foam. R_f = 0.92 (*cyclo*-hexane/ethyl acetate, 1:1); $[\alpha]_D^{20}$ = +33.9 (c = 2.5, CH_2Cl_2); IR (thin film): υ = 2954, 1762, 1746, 1708, 1452, 1260, 1083, 1027, 962, 736, 719 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.03 (dt, *J* = 8.4, 1.5 Hz, 2H, Ar), 7.75 (dd, *J* = 7.5, 4.7 Hz, 2H, Ar), 7.66 – 7.55 (m, 4H, Ar), 7.54 – 7.43 (m, 2H, Ar), 7.43 – 7.36 (m, 2H, Ar), 7.29 (tdd, *J* = 7.5, 5.3, 1.1 Hz, 3H, Ar), 7.23 (dd, *J* = 8.0, 2.1 Hz, 1H, Ar), 7.11 – 7.02 (m, 5H, Ar), 5.38 (dd, *J* = 10.1, 9.0 Hz, 1H, H-2), 5.21 (dd, *J* = 9.8, 9.2 Hz, 1H, H-4), 4.77 (d, *J* = 10.1 Hz, 1H, H-1), 4.64 (d, *J* = 11.6 Hz, 1H, CH_2O-Bn), 4.56 (d, *J* = 11.6 Hz, 1H, CH_2O-Bn), 4.50 – 4.43 (m, 1H, CH_2O-Fmoc), 4.35 (dd, *J* = 10.5, 7.5 Hz, 1H, CH_2O-Fmoc), 4.24 (t, *J* = 7.2 Hz, 1H, CH-Fmoc), 4.10 (d, *J* = 9.9 Hz, 1H, H-5), 3.96 (t, *J* = 9.1 Hz, 1H, H-3), 3.74 (s, 3H, OMe), 2.22 (s, 3H, Me-thio), 1.28 (s, 9H *t*Bu); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 167.20, 165.00, 154.06 (C=O), 149.82, 143.49, 143.15, 141.46, 141.41, 137.62, 137.21, 133.50, 131.74, 130.98, 130.10, 130.02, 129.70, 128.59, 128.34, 128.14, 128.09, 128.06, 127.88, 127.35, 125.76, 125.30, 125.19, 120.22 (Ar), 87.89 (C-1), 80.37 (C-3), 76.27 (C-5), 75.10 (C-4), 74.51 (CH_2O-Bn), 71.65 (C-2), 70.54 (CH_2O-Fmoc), 52.94 (OMe), 46.79 (CH-Fmoc), 34.57 (Cq *t*Bu), 31.34 (Me *t*Bu), 20.43 (Me-thio); ESI-HRMS *m/z* $[M+Na]^+$ calcd for $C_{47}H_{46}O_9S$ 809,2760, found 809.2750.

Methyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-fluorenylmethoxycarbonyl-1-di-*O*-butyl-phosphatidyl-β-D-glucopyranosyluronate GlcA-05



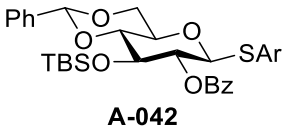
GlcA-05

Thioglycoside **GlcA-04** (5.0 g, 6.35 mmol) was co-evaporated twice with toluene. The remainder and NIS (1.72 g, 7.62 mmol) were dissolved in DCM (53 mL) under an Ar atmosphere and the solution was cooled 0 °C. Dibutyl hydrogen phosphate (3.8 mL, 19.1 mmol) and triflic acid (56 L, 0.635 mmol) were added, and the reaction was stirred at 0 °C. After complete conversion of the starting material (TLC: *cyclo*-hexane/ethyl acetate, 1:1) the solution was diluted with DCM and extracted with 10% aqueous $Na_2S_2O_3$ and saturated aqueous $NaHCO_3$. The organic phase was dried over $MgSO_4$ and the solvent was removed *in vacuo*. The crude product was purified by silica gel flash column chromatography (hexane/ethyl acetate, 3:1→2:1→1.5:1→1:1) affording the phosphate **GlcA-05** (4.51 g, 87%). R_f = 0.67 (*cyclo*-hexane/ethyl acetate, 1:1); $[\alpha]_D^{20}$ = +28.5 (c = 1.9, CH_2Cl_2); IR (thin film): υ = 2960, 1756, 1733, 1451, 1250, 1026, 904, 738, 710 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.00 (dd, *J* = 8.3, 1.3 Hz, 2H, Ar), 7.76 (dd, *J* = 7.0, 6.1 Hz, 2H, Ar), 7.71 – 7.53 (m, 3H, Ar), 7.50 – 7.37 (m, 4H, Ar), 7.34 – 7.24 (m, 2H, Ar), 7.18 – 6.98 (m, 5H, Ar), 5.51 – 5.38 (m, 2H, H-1, H-2), 5.16 (dd, *J* = 9.8, 9.2 Hz, 1H, H-4), 4.66 (d, *J* = 11.7 Hz, 1H, CH_2O-Bn), 4.55 (d, *J* = 11.7 Hz, 1H, CH_2O-Bn), 4.47 (dd, *J* = 10.5, 7.1 Hz, 1H, CH_2O-Fmoc), 4.39 (dd, *J* = 10.5, 7.4 Hz, 1H, CH_2O-Fmoc), 4.28 – 4.19 (m, 2H, CH-Fmoc, H-5), 4.15 – 4.01 (m, 2H, 2×CH_2O-Bu), 4.00 – 3.89 (m, 1H, H-3), 3.79 – 3.59 (m, 5H, 2×CH_2O-Bu, OMe), 1.68 – 1.59 (m, 2H, 2×CH_2-Bu), 1.48 – 1.15 (m, 4H, 4×CH_2-Bu), 1.07 – 0.96 (m, 2H, 2×CH_2-Bu), 0.91 (t, *J* = 7.4 Hz, 3H, 3×CH_3-Bu), 0.67 (t, *J* = 7.4 Hz, 3H, 3×CH_3-Bu); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 166.84, 164.85, 154.12 (C=O), 143.35, 143.08, 141.45, 141.41, 136.99, 133.66, 130.04, 129.21, 128.61, 128.39, 128.18, 128.12, 128.10, 127.98, 127.36, 125.23, 125.18, 120.25, 120.23 (Ar), 96.34 (C-1), 78.36 (C-3), 75.04 (CH_2O-Bn), 74.56 (C-4), 72.83 (C-5), 72.45 (C-2), 70.63 (CH_2O-Fmoc), 68.41 (CH_2O-Bu), 68.35 (CH_2O-Bu), 52.95 (OMe), 46.75 (CH-Fmoc), 32.14 (CH_2-Bu), 32.06 (CH_2-Bu), 18.66 (CH_2-Bu), 18.33 (CH_2-Bu), 13.66 (CH_3-Bu), 13.46 (CH_3-Bu); ESI-HRMS ($C_{44}H_{49}O_{13}P$): calcd for [M+Na] 839.2808; found 839.2828.



Scheme A.08. Synthesis of glucose building blocks Glc-04, -05, -06, and GlcA-07. a) i) TBSCl, Imidazole, DCM, r.t., ii)) Bz_2O, pyridine, DMAP, DCM, r.t , 89% for 98% for 2 steps; b) BH_3 in THF, TMSOTf, DCM, 0 °C , 95%; c) i) BnBr, NaH, THF/DMF (v/v = 9:1), 0 °C; ii) $BF_3{\cdot}OEt_2$, MeCN, 0 °C 94% for 2 steps; d) FmocCl, pyridine, DCM, 0 °C, 96% for **Glc-04,** 96% for **Glc-06**; e) $HOP(O)OBu_2$, NIS, TfOH, DCM, 0 °C, 94% for **Glc-05**, 983% for **Glc-07;** f) i) LevOH, DIC, DMAP, DCM, 0 °C; ii) $BF_3{\cdot}OEt_2$, MeCN, 0 °C 94% for 2 steps.

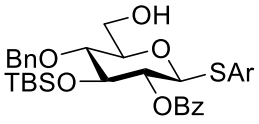
(2-Methyl-5-*tert*-butylphenyl) 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside A-042



To a solution of compound **A-032** (10 g, 23.2 mmol) in anhydrous DCM (46 ml, 0.5 M) were added TBSCl (4.20 g, 27.9 mmol) and imidazole (1.21 g, 32.5 mmol) at 0 °C, and the mixture was stirred for 12 h at room temperature. After the reaction mixture was quenched with MeOH and saturated aqueous $NaHCO_3$, diluted with DCM, and dried over $MgSO_4$, the combined organic layer was evaporated *in vacuo*. To a solution of this crude in anhydrous DCM (116 ml, 0.2 M) were added benzoic anhydride (Bz_2O) (10.51 g, 46.4 mmol), triethylamine (Et_3N) (9.70 ml, 69.7 mmol), and a catalytic amount of DMAP (0.57 g, 4.64 mmol) at 0 °C and the mixture was stirred at room temperature till completion. After the reaction mixture was quenched by saturated aqueous $NaHCO_3$, diluted with DCM and dried over $MgSO_4$, the solvent was evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane/EtOAc/DCM = 9:0.5:0.5 to 9.0:1.0:0.5) to afford **A-042** (12.1 g, 18.65 mmol, 89% over two steps). $[\alpha]_D^{25}$ = 13.68 (C= 1.00, $CHCl_3$). IR (thin film): υ = 3006, 2959, 1733, 1266, 1097 cm^{-1}; ^1H NMR (400 MHz, Chloroform-*d*) δ 8.10 – 8.05 (m, 2H), 7.61 – 7.54 (m, 2H), 7.52 – 7.43 (m, 4H), 7.41 – 7.33 (m, 3H), 7.20 (dd, *J* = 7.9,

2.1 Hz, 1H), 7.07 (d, *J* = 8.0 Hz, 1H), 5.57 (s, 1H, C*H*Ph), 5.38 – 5.29 (m, 1H, H-2), 4.83 (d, *J* = 10.2 Hz, 1H, H-1), 4.37 (dd, *J* = 10.5, 5.0 Hz, 1H, H-6), 4.06 (t, *J* = 8.8 Hz, 1H, H-3), 3.87 (t, *J* = 10.3 Hz, 1H, H-6), 3.69 (t, *J* = 9.2 Hz, 1H, H-4), 3.55 (td, *J* = 9.7, 5.0 Hz, 1H, H-5), 2.17 (s, 3H, Me), 1.28 (s, 9H, *t*-Bu), 0.69 (s, 9H, *t*-Bu), -0.05 (s, 3H, Me), -0.14 (s, 3H, Me). ^{13}C NMR (150 MHz, $CDCl_3$) δ 165.38 (Bz), 149.70, 137.16, 136.81, 133.26, 132.94, 130.18, 130.03, 129.99, 129.59, 129.21, 128.49, 128.29, 126.40, 125.22(Ar), 102.04 (*C*HPh), 88.28 (C-1), 81.51 (C-4), 74.50 (C-3), 73.75 (C-2), 70.77 (C-5), 68.83 (C-6), 34.59 (Cq, *t*-Bu), 31.42 (Me, *t*-Bu), 25.68 (*t*-Bu of TBS), 20.33(Me), 18.08(Cq, TBS), -4.06(Me, TBS), -4.78(Me, TBS). MS ESI-HRMS m/z $[M+Na]^+$ calcd for $C_{37}H_{48}O_6SSiNa$ 671.2833, found 671.2831.

(2-Methyl-5-*tert*-butylphenyl) 2-*O*-benzoyl-4-*O*-benzyl-3-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside A-043

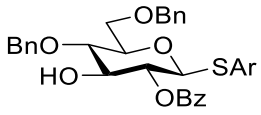


A-043

Compound **A-042** (11.3 g, 17.41 mmol) was co-evaporated with toluene and dissolved under an Ar atmosphere in DCM (78 mL, 0.2 M). To a solution of **A-042** was added 1M solution of BH_3 in THF (87ml, 87 mmol), and TMSOTf (1.94 ml, 8.71 mmol) successively at 0 °C. The mixture was stirred for 5 h at 0 °C. After the mixture was quenched with saturated aqueous $NaHCO_3$, diluted with DCM, and dried over $MgSO_4$. The combined organic layer was evaporated *in vacuo*. The crude was purified by column chromatography on silica gel (hexane/EtOAc/DCM = 9:0.5:0.5 to 9:1:0.5) to afford **A-043** (10.7g, 16.5 mmol, 95 %). R_f : 0.27 (Hexane/EtOAc/DCM : 8/2/0.5). $[\alpha]_D^{25}$ = 42.23 (C= 2.00, $CHCl_3$). IR (thin film): υ = 3476, 2958, 1732, 1263, 1090 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.11 – 8.06 (m, 2H), 7.62 – 7.56 (m, 1H), 7.52 (d, *J* = 2.0 Hz, 1H), 7.46 (t, *J* = 7.8 Hz, 2H), 7.39 – 7.33 (m, 4H), 7.32 – 7.28 (m, 1H), 7.20 (dd, *J* = 7.9, 2.1 Hz, 1H), 7.07 (d, *J* = 8.0 Hz, 1H), 5.30 (t, *J* = 9.5 Hz, 1H, H-3), 4.89 (d, *J* = 11.5 Hz, 1H, CH*H*Ph), 4.78 (d, *J* = 10.2 Hz, 1H, H-1), 4.68 (d, *J* = 11.5 Hz, 1H, CH*H*Ph), 4.00 (t, *J* = 8.8 Hz, 1H, H-3), 3.89 (ddd, *J* = 11.9, 5.5, 2.3 Hz, 1H, H-6), 3.72 (ddd, *J* = 12.1, 7.4, 4.7 Hz, 1H, H-6), 3.65 (t, *J* = 9.2 Hz, 1H, H-4), 3.48 (ddd, *J* = 9.7, 4.6, 2.6 Hz, 1H, H-5), 2.17 (s, 3H, Me), 1.29 (s, 9H, *t*-Bu), 0.81 (s, 9H, *t*-Bu), 0.04 (s, 3H, Me), -0.13 (s, 3H, Me). ^{13}C NMR (150 MHz, $CDCl_3$) δ 165.58 (Bz), 149.76, 137.99, 136.53, 133.24, 133.21, 130.28, 130.07, 130.03, 129.02, 128.54, 128.46, 127.90, 127.75, 125.07 (Ar), 87.66

(C-1), 79.69 (C-5), 78.53 (C-4), 76.81 (C-3), 75.25 (*C*H_2Ph), 73.43 (C-2), 62.24 (C-6), 34.56 (Cq, *t*-Bu), 31.42 (Me, *t*-Bu), 25.80 (*t*-Bu of TBS), 20.25 (Me), 17.93 (Cq, TBS), -3.88 (Me, TBS), -4.14 (Me, TBS). MS ESI-HRMS m/z $[M+Na]^+$ calcd for $C_{37}H_{50}O_6SSiNa$ 673.2990, found 673.2988.

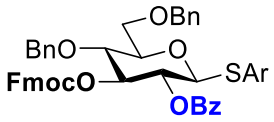
(2-Methyl-5-*tert*-butylphenyl) 2-*O*-benzoyl-4,6-di-*O*-benzyl-1-thio-β-D-glucopyranoside A-044



A-044

To a solution of compound **A-043** (3 g, 4.61 mmol) in co-solvent of THF and DMF (v/v, 9:1) were added benzyl bromide (1.64 ml, 13.83 mmol) and sodium hydride (0.265 mg, 11.06 mmol) at 0 °C, and the mixture was stirred for 2 h at 0 °C. After the reaction was quenched with saturated aqueous NH_4Cl, diluted with DCM, extracted with DCM, the combned organic layer was dried over $MgSO_4$ and evaporated *in vacuo*. To a solution of this crude product in anhydrous acetonitrile (57 ml, 0.08 M) was added boron trifluoride etherate ($BF_3{\cdot}OEt_2$) (0.70 mL, 5.54 mmol) at 0 °C, and the mixture was stirred for 20 min. at 0 °C. After the mixture was quenched with saturated aqueous $NaHCO_3$, and diluted with DCM, the combined organic layer was dried over $MgSO_4$ and evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane/EtOAc/DCM = 9:1:1 to 7:3:1) to afford **A-044** (2.89g, 4.32 mmol, 94 % over two steps). R_f : 0.11 (Hexane/EtOAc/DCM : 8/2/0.5). $[\alpha]_D^{25}$ = -2.85 (C= 2.67, $CHCl_3$). IR (thin film): υ = 3456, 2961, 1727, 1263 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.12 (d, *J* = 8.2 Hz, 2H), 7.65 (d, *J* = 1.6 Hz, 1H), 7.63 – 7.57 (m, 1H), 7.47 (t, *J* = 7.7 Hz, 2H), 7.43 – 7.25 (m, 10H), 7.23 (dd, *J* = 8.0, 1.9 Hz, 1H), 7.11 (d, *J* = 8.0 Hz, 1H), 5.17 (t, *J* = 9.5 Hz, 1H, H-2), 4.86 (d, *J* = 11.2 Hz, 1H, CH*H*Ph), 4.81 (d, *J* = 10.1 Hz, 1H, H-1), 4.64 (dd, *J* = 27.6, 12.0 Hz, 3H, , CH*H*Ph, 2 x C*H*$_2$Ph), 3.97 (dd, *J* = 12.4, 5.2 Hz, 1H, H-3), 3.86 – 3.76 (m, 2H, H-6), 3.71 (t, *J* = 9.2 Hz, 1H, H-4), 3.63 – 3.56 (m, 1H, H-5), 2.82 (br, 1H, OH), 2.28 (s, 3H, Me), 1.29 (s, 9H, *t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 166.45 (Bz), 149.68, 138.14, 138.10, 136.95, 133.47, 132.57, 130.09, 129.93, 129.75, 129.66, 128.59, 128.50, 128.47, 128.17, 128.02, 127.75, 125.07 (Ar), 86.68 (C-1), 79.16 (C-5), 78.07 (C-4), 77.57 (C-3), 74.97 (*C*H_2Ph), 73.70 (C-2), 73.62 (*C*H_2Ph), 68.95 (C-6), 34.50 (Cq, *t*-Bu), 31.33 (Me, *t*-Bu), 20.40 (Me). MS ESI-HRMS m/z $[M+Na]^+$ calcd for $C_{38}H_{42}O_6SNa$ 649.2594, found 649.2560.

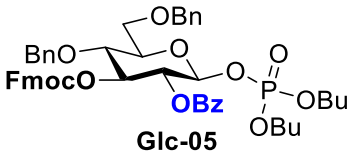
(2-Methyl-5-*tert*-butylphenyl) 2-*O*-benzoyl-4,6-di-*O*-benzyl-3-*O*-fluorenylmethoxycarbonyl 1-thio-β-D-glucopyranoside Glc-04



Glc-04

To a solution of compound **A-044** (2.74 g, 4.37 mmol) were added 9-fluorenylmethyl chloroformate (2.26 g, 8.74 mmol) and pyridine (2.14 mL, 26.4 mmol) successively at 0 °C, and the reactiom mixture was stirred overnight at room temperature. After the mixture was quenched with 1M aqueous HCl, diluted with DCM, and extracted with DCM, the combined organic layer was dried over $MgSO_4$ and evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane/ethyl acetate/DCM = 9:0.5:0.5 to 8.5:1.5:0.5) to afford **S5** (3.55 g, 4.18 mmol, 96 %). R_f : 0.18 (Hexane/EtOAc/DCM : 9/1/0.5). $[\alpha]_D^{25}$ = 55.96 (C= 2.79, $CHCl_3$). IR (thin film): υ = 2960, 1752, 1732, 1451, 1269 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.02 (dd, *J* = 8.3, 1.3 Hz, 2H), 7.70 (dd, *J* = 7.6, 3.5 Hz, 2H), 7.58 (d, *J* = 2.1 Hz, 1H), 7.52 – 7.45 (m, 2H), 7.42 (d, *J* = 7.4 Hz, 1H), 7.38 – 7.28 (m, 9H), 7.23 – 7.12 (m, 8H), 7.08 (d, *J* = 8.0 Hz, 1H), 5.39 (t, *J* = 9.7 Hz, 1H, H-2), 5.29 (t, *J* = 9.3 Hz, 1H, H-3), 4.82 (d, *J* = 10.0 Hz, 1H, H-1), 4.67 (d, *J* = 11.2 Hz, 1H, CH*H*Ph), 4.65 (d, *J* = 12.1 Hz, 1H, CH*H*Ph), 4.57 (d, *J* = 11.2 Hz, 1H, CH*H*Ph), 4.56 (d, *J* = 12.2 Hz, 1H, CH*H*Ph), 4.25 (dd, *J* = 10.4, 7.0 Hz, 1H, CH*H*Ph of Fmoc), 4.08 (dd, *J* = 10.4, 8.0 Hz, 1H, CH*H*Ph of Fmoc), 4.01 – 3.90 (m, 2H, H-4, , C*H*Ph of Fmoc), 3.77 (d, *J* = 3.0 Hz, 2H, H-6), 3.63 (dt, *J* = 9.8, 3.0 Hz, 1H, H-5), 2.23 (s, 3H, Me), 1.25 (s, 9H, *t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 165.22 (Bz), 154.63 (Fmoc), 149.62, 143.38, 142.95, 141.14, 141.04, 137.92, 137.50, 137.02, 133.25, 132.33, 129.95, 129.87, 129.32, 128.40, 128.33, 127.91, 127.82, 127.75, 127.71, 127.11, 125.24, 125.16, 124.95, 119.86 (Ar), 87.10 (C-1), 80.88 (C-3), 79.20 (C-5), 75.70 (C-4), 74.92 (*C*H_2Ph), 73.58 (*C*H_2Ph), 71.02 (C-2), 70.26 (*C*H_2 of Fmoc), 68.50 (C-6), 46.46 (*C*H of Fmoc), 34.41 (Cq, *t*-Bu), 31.24 (Me, *t*-Bu), 20.30 (Me). MS ESI-HRMS m/z $[M+Na]^+$ calcd for $C_{53}H_{52}O_8SNa$ 871.3275, found 871.3276.

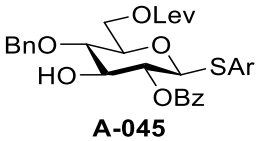
Dibutyl 2-*O*-benzoyl-4,6-di-*O*-benzyl-3-*O*-fluorenylmethoxycarbonyl-1-thio-β-D-glucopyranoside Glc-05



Glc-05

Thioglycoside **Glc-04** (6.40 g, 7.55 mmol) was co-evaporated twice with toluene. The remainder and NIS (1.87 g, 8.30 mmol) were dissolved in DCM (37.7 mL, 0.2 M) under an Ar atmosphere and the solution was cooled to 0 °C. Dibutyl hydrogen phosphate (2.99 mL, 15.1 mmol) and triflic acid (67 µL, 0.755 mmol) were added into reaction flask, the reaction was stirred at 0 °C for 1 h. After complete conversion of the starting material, it was diluted with DCM and extracted with 10% aqueous $Na_2S_2O_3$ and then saturated aqueous $NaHCO_3$. The organic phase was dried over $MgSO_4$ and the solvent was removed *in vacuo*. The crude product was purified by silica gel flash column chromatography (hexane/ethyl acetate, 3:1 to 1:1) to afford the title compound **Glc-05** (6.21 g, 94%). R_f : 0.35 (Hexane/EtOAc/DCM : 1/1/0.5). $[\alpha]_D^{25}$ = 150.12 (C= 2.10, $CHCl_3$). IR (thin film): υ = 2962, 1751, 1733, 1449 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.00 (dd, *J* = 8.3, 1.2 Hz, 2H), 7.73 (dd, *J* = 7.6, 5.0 Hz, 2H), 7.60 – 7.49 (m, 3H), 7.46 – 7.33 (m, 4H), 7.32 – 7.17 (m, 8H), 7.12 – 6.99 (m, 4H), 5.42 – 5.35 (m, 2H), 5.11 – 5.05 (m, 1H), 4.58 (d, *J* = 11.6 Hz, 1H), 4.54 – 4.46 (m, 3H), 4.33 (qd, *J* = 10.5, 7.1 Hz, 2H), 4.11 (t, *J* = 7.1 Hz, 1H), 4.06 – 3.96 (m, 2H), 3.92 – 3.80 (m, 2H), 3.76 – 3.60 (m, 4H), 1.61 – 1.49 (m, 2H), 1.37 – 1.20 (m, 4H), 1.05 – 0.95 (m, 2H), 0.85 (t, *J* = 7.4 Hz, 3H), 0.66 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 164.96, 154.24, 143.37, 143.19, 141.43, 141.40, 137.81, 137.24, 133.54, 130.02, 129.40, 128.57, 128.44, 128.42, 128.31, 128.07, 128.05, 128.04, 127.83, 127.78, 127.72, 127.31, 125.19, 125.11, 120.20, 96.66, 96.61, 79.25, 79.23, 77.48, 77.16, 76.84, 75.12, 74.36, 73.78, 73.72, 73.01, 72.93, 70.21, 69.18, 68.17, 68.11, 68.04, 67.98, 46.82, 32.14, 32.07, 31.90, 31.83, 18.67, 18.34, 13.67, 13.48. MS ESI-HRMS m/z $[M+Na]^+$ calcd for $C_{50}H_{55}O_{12}PNa$ 901.3323, found 901.3330.

(2-Methyl-5-*tert*-butylphenyl) 2-*O*-benzoyl-4-*O*-benzyl-6-*O*-levulinyl-1-thio-β-D-glucopyranoside A-045

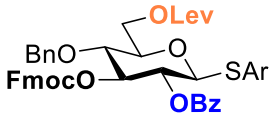


A-045

To a solution of compound **A-043** (1.7 g, 2.61 mmol) in anhydrous DCM (13 ml, 0.2 M) were added levulinic acid (0.910 g, 7.83 mmol), and DIC (1.22 ml, 7.83 mmol), and a

catalytic amount of DMAP (0.096 g, 0.78 mmol) at 0 °C, and the mixture was stirred for 1 h at 0 °C. After the mixture was quenched saturated aqueous $NaHCO_3$, diluted with DCM, extracted with DCM, the combined organic layer was dried over $MgSO_4$ and evaporated *in vacuo*. To a solution of this crude product in anhydrous acetonitrile (57 ml, 0.08 M) was added boron trifluoride etherate ($BF_3{\cdot}OEt_2$) (0.41 mL, 2.72 mmol) at 0 °C, and the mixture was stirred for 20 min. at 0 °C. After the mixture was quenched with saturated aqueous $NaHCO_3$, and diluted with DCM, the combined organic layer was dried over $MgSO_4$ and evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane/EtOAc/DCM = 9:1:1 to 7:3:1) to afford **A-045** (2.89g, 4.32 mmol, 94 % over two steps). R_f : 0.21 (Hexane/EtOAc/DCM : 8/2/0.5). $[\alpha]_D^{25}$ = 56.91 (C= 2.85, $CHCl_3$). IR (thin film): υ = 3482, 2961, 1721, 1263 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.10 (dt, *J* = 8.4, 1.5 Hz, 2H), 7.64 – 7.57 (m, 1H), 7.51 (d, *J* = 2.1 Hz, 1H), 7.49 – 7.42 (m, 2H), 7.38 – 7.26 (m, 5H), 7.21 (dd, *J* = 7.9, 2.1 Hz, 1H), 7.09 (d, *J* = 8.0 Hz, 1H), 5.08 (dd, *J* = 10.0, 9.1 Hz, 1H, H-2), 4.88 (d, *J* = 11.1 Hz, 1H, CH*H*Ph), 4.76 (d, *J* = 10.1 Hz, 1H, H-1), 4.72 (d, *J* = 11.1 Hz, 1H, CH*H*Ph), 4.45 – 4.37 (m, 1H, H-6), 4.36 – 4.25 (m, 1H, H-6), 3.97 (m, 1H, H-3), 3.66 – 3.54 (m, 2H, H-4, H-5), 2.78 – 2.69 (m, 2H, Lev), 2.60 (dd, *J* = 10.0, 3.6 Hz, 2H, Lev), 2.24 (s, 3H, Lev), 2.20 (s, 3H, Me), 1.27 (s, 9H, *t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 206.50 (CO, Lev), 172.64 (Lev), 166.63 (Bz), 149.74, 137.89, 137.48, 133.67, 132.04, 130.18, 130.15, 130.11, 129.55, 128.74, 128.62, 128.44, 128.23, 125.49 (Ar), 86.82 (C-1), 77.87 (C-3), 77.75 (C-4), 77.01 (C-5), 75.16 (*C*H_2Ph), 73.68 (C-2), 63.51 (C-6), 38.04 (Lev), 34.56(Cq, *t*-Bu), 31.41 (Me, *t*-Bu), 30.04 (Me, Lev), 28.04 (Lev), 20.46 (Me). MS ESI-HRMS m/z $[M+Na]^+$ calcd for $C_{36}H_{42}O_8SNa$ 657.2493, found 657.2489.

(2-Methyl-5-*tert*-butylphenyl) 2-*O*-benzoyl-4-*O*-benzyl-3-*O*-fluorenylmethoxycarbonyl-6-*O*-levulinyl-1-thio-β-D-glucopyranoside Glc-06

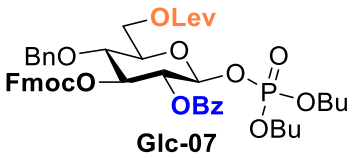


Glc-06

To a solution of compound **A-045** (1.61 g, 2.55 mmol) in DCM (12.5 ml, 0.2 M) were 9-fluorenylmethyl chloroformate (1.32 g, 5.11 mmol) and pyridine (0.62 mL, 7.66 mmol) successively at 0 °C, and the mixture was stirred overnight at room temperature. After the mixture was quenched with saturated aqueous $NaHCO_3$, diluted with DCM, and extracted with DCM, the combined organic layer was washed 1M aqueous HCl, dried with dried over

$MgSO_4$ and evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane/ EtOAc /DCM = 9:0.5:0.5 to 8.5:1.5:0.5) to afford **Glc-06** (2.411 g, 2.46 mmol, 96 %). R_f : 0.35 (hexane/EtOAc/DCM : 9/1/0.5). $[\alpha]_D^{25}$ = 59.08 (C= 2.80, $CHCl_3$). IR (thin film): υ = 2961, 1733, 1268 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.04 – 7.97 (m, 2H), 7.69 (dd, J = 7.6, 4.8 Hz, 2H), 7.52 – 7.46 (m, 3H), 7.42 (dd, J = 7.5, 0.8 Hz, 1H), 7.38 – 7.30 (m, 4H), 7.27 – 7.15 (m, 9H), 7.09 (d, J = 8.0 Hz, 1H), 5.36 (t, J = 9.6 Hz, 1H, H-3), 5.30 (t, J = 9.1 Hz, 1H, H-2), 4.81 (d, J = 9.7 Hz, 1H, H-1), 4.65 (dd, J = 42.3, 11.2 Hz, 2H, C*H*$_2$Ph), 4.39 (dd, J = 12.1, 2.1 Hz, 1H, H-6), 4.31 (dd, J = 12.1, 4.5 Hz, 1H, H-6), 4.26 (dd, J = 10.4, 7.0 Hz, 1H, CH*H* of Fmoc), 4.11 (dd, J = 10.4, 7.9 Hz, 1H, CH*H* of Fmoc), 3.96 (t, J = 7.4 Hz, 1H, C*H* of Fmoc), 3.88 – 3.79 (m, 1H, H-4), 3.68 (ddd, J = 9.8, 4.4, 2.2 Hz, 1H, H-5), 2.80 – 2.70 (m, 2H, Lev), 2.60 (dd, J = 9.9, 3.5 Hz, 2H, Lev), 2.23 (s, 3H, Me), 2.21 (s, 3H, Lev), 1.27 (s, 9H, *t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 206.40 (CO, Lev), 172.53 (Lev), 165.35 (Bz), 154.68 (Fmoc), 149.79, 143.47, 143.01, 141.30, 141.19, 137.55, 137.23, 133.48, 132.01, 130.33, 130.12, 130.09, 129.33, 128.59, 128.50, 128.20, 128.16, 127.91, 127.26, 125.63, 125.34, 125.06, 120.03 (Ar), 87.42 (C-1), 80.95 (C-2), 77.12 (C-5), 75.44 (C-5), 75.08 (*C*H_2Ph), 71.00 (C-3), 70.44 (*C*H_2 of Fmoc), 63.13 (C-6), 46.59 (*C*H of Fmoc), 38.02 (*C*H_2 of Lev), 34.55 (Cq, *t*-Bu), 31.41 (*t*-Bu), 30.03 (Me of Lev), 28.00 (*C*H_2 of Lev), 20.43 (Me). MS ESI-HRMS m/z $[M+Na]^+$ calcd for $C_{51}H_{52}O_{10}SNa$ 879.3173, found 879.3179.

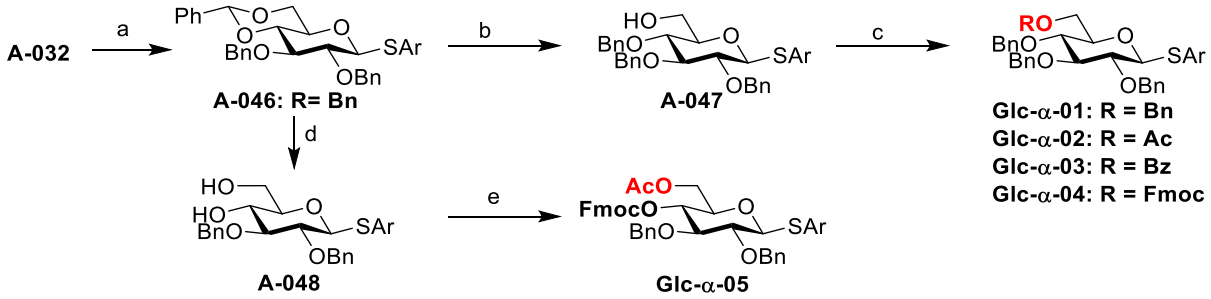
Dibutyl 2-*O*-benzoyl-4-*O*-benzyl-3-*O*-fluorenylmethoxycarbonyl-6-*O*-levulinyl-1-thio-β-D-glucopyranoside Glc-07



Glc-07

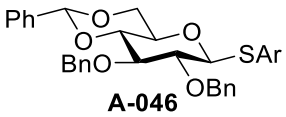
Thioglycoside **Glc-06** (3.42 g, 4.00 mmol) was co-evaporated twice with toluene. The remainder and NIS (0.99 g, 4.40 mmol) were dissolved in DCM (20.0 mL, 0.2 M) under an Ar atmosphere and the solution was cooled to 0 °C. Dibutyl hydrogen phosphate (1.58 mL, 7.99 mmol) and triflic acid (35 µL, 0.400 mmol) were added into reaction flask, the reaction was stirred at 0 °C for 1 h. After complete conversion of the starting material, it was diluted with DCM and extracted with 10% aqueous $Na_2S_2O_3$ and then saturated aqueous $NaHCO_3$. The organic phase was dried over $MgSO_4$ and the solvent was removed *in vacuo*. The crude product was purified by silica gel flash column chromatography (hexane/ethyl acetate, 3:1 to 1:1) to afford the title compound **Glc-07** (2.95 g, 83%). R_f : 0.28 (Hexane/EtOAc/DCM :

1/1/0.5). $[\alpha]_D^{25}$ = 33.02 (C= 2.40, $CHCl_3$). IR (thin film): υ = 2963, 1736, 1262 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.12 – 8.03 (m, 2H), 7.72 – 7.65 (m, 2H), 7.61 – 7.24 (m, 12H), 7.11 (dtd, *J* = 15.2, 7.5, 1.1 Hz, 2H), 5.80 (dd, *J* = 10.5, 8.0 Hz, 1H), 5.42 (dd, *J* = 8.0, 7.4 Hz, 1H), 5.03 (dd, *J* = 10.5, 3.0 Hz, 1H), 4.80 (d, *J* = 11.2 Hz, 1H), 4.52 (d, *J* = 11.2 Hz, 1H), 4.36 (dd, *J* = 10.4, 7.1 Hz, 1H), 4.32 – 4.19 (m, 3H), 4.11 (t, *J* = 7.0 Hz, 1H), 4.08 – 3.98 (m, 3H), 3.91 (td, *J* = 6.3, 1.2 Hz, 1H), 3.79 – 3.63 (m, 2H), 2.77 – 2.72 (m, 3H), 2.52 (dd, *J* = 6.9, 6.1 Hz, 2H), 2.19 (s, 3H), 1.65 – 1.56 (m, 2H), 1.42 – 1.32 (m, 2H), 1.32 – 1.24 (m, 2H), 1.07 – 0.97 (m, 2H), 0.90 (t, *J* = 7.4 Hz, 3H), 0.68 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 206.35, 172.19, 165.00, 154.35 (, 143.13, 142.70, 141.21, 141.10, 137.16, 133.45, 129.93, 129.11, 128.52, 128.48, 128.10, 127.85, 127.11, 127.07, 125.09, 124.90, 120.00 (Ar), 96.73, 96.68, 77.39, 77.37, 77.33, 77.22, 77.02, 76.70, 75.29, 73.02, 72.88, 70.25, 69.77, 69.68, 68.03, 67.97, 67.92, 67.85, 62.09, 46.45, 37.82, 32.02, 31.95, 31.77, 31.70, 29.82, 29.57, 27.69, 18.56, 18.21, 13.56, 13.36. MS ESI-HRMS m/z $[M+Na]^+$ calcd for $C_{48}H_{55}O_{14}PNa$ 909.3222, found 909.3219.



Scheme A.09. Synthesis of glucose building blocks Glc-α-01, -02, -03, -04, and Glc-α-05. a) BnBr, NaH, DMF, 0 °C, 96%; b) BH_3 in THF, TMSOTf, DCM, 0 °C, 91%; %; c) i) BnBr, NaH, DMF, 0 °C, 96% for **Gal-α-01**; ii) Ac_2O, pyridine, DMAP, DCM, r.t., 94% **Gal-α-02,** iii) Bz_2O, pyridine, DMAP, DCM, r.t , 98% for **Gal-α-03,** iv) FmocCl, pyridine, DCM, 0 °C, 95% for **Gal-α-04**; d) DCM, TFA, r.t. 82%; e) i) 2-Chloro-1-methylpyridium iodide, DABCO, DCM, -15 °C; ii) FmocCl, pyridine, DCM, 0 °C, 84% for **Gal-α-05.**

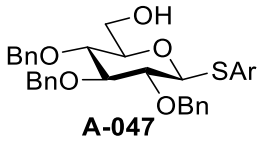
2-Methyl-5-tert-butylphenyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside A-046



To a solution of compound **A-032** (1.3 g, 3.02 mmol) in anhydrous DMF (15 mL, 0.2 M) was added BnBr (1.08 g, 9.06 mmol), NaH (0.604 g, 15.1 mmol) at 0 °C, and mixture was stirred for 2 h at room temperature. Then the mixture was quenched with saturated aqueous NH_4Cl,

it was diluted with DCM. The organic layer was separated and aqueous phase was extracted twice with DCM. The combined organic layer was dried over $MgSO_4$ and the solvent was evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane/ethyl acetate = 9:1 to 7:3) to afford **A-046** (1.77 g, 2.90 mmol, 96%). R_f : 0.35 (hexane/ethyl acetate/DCM = 8:2:0.5). $[\alpha]_D^{25}$ = -2.75 (*c* = 3.33, $CHCl_3$). IR (thin film): υ = 2962, 1454, 1089 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.65 – 7.11 (m, 13H), 5.62 (s, 1H, C*H*HPh), 5.01 – 4.80 (m, 4H, 2 X CH_2Ph), 4.77 (d, *J* = 9.9 Hz, 1H, H-1), 4.36 (dd, *J* = 10.5, 4.8 Hz, 1H, H-6), 3.97 – 3.81 (m, 2H, H-3, H-6), 3.78 (t, *J* = 9.2 Hz, 1H, H-4), 3.60 (t, *J* = 9.0 Hz, 1H, H-2), 3.49 (td, *J* = 9.3, 4.9 Hz, 1H, H-5), 2.41 (s, 3H, Me), 1.30 (s, 9H, t-Bu). ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.67, 138.49, 138.13, 137.36, 136.27, 132.89, 130.07, 129.12, 129.08, 128.51, 128.50, 128.40, 128.36, 128.22, 127.98, 127.88, 126.11, 124.88 (Ar), 101.28 (CHPh), 88.63 (C-1), 83.17 (C-3), 81.63 (C-4), 81.01 (C-2), 76.19 (CH_2Ph), 75.43 (CH_2Ph), 70.21 (C-5), 68.89 (C-6), 34.62 (Cq, t-Bu), 31.44 (Me, t-Bu), 20.46 (Me). MS ESI-HRMS m/z $[M+Na]^+$ calcd for $C_{38}H_{42}O_5S$ 633.2645, found 633.2644.

(2-Methyl-5-tert-butylphenyl) 2,3,4-tri-*O*-benzyl-1-thio-β-D-glucopyranoside A-047

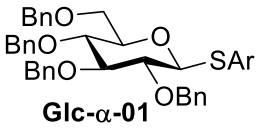


A-047

Compound **A-046** (1.81 g, 2.95 mmol) was co-evaporated with toluene and dissolved under an Ar atmosphere in DCM (78 mL, 0.2 M). To a solution of compound **S8** were added 1 M solution of BH_3 in THF (14.7 mL, 14.7 mmol), and TMSOTf (0.266 mL, 1.47 mmol) at 0 °C. After the mixture was stirred for 5 h at 0 °C, the mixture was quenched with saturated aqueous $NaHCO_3$, and then diluted with DCM. The organic layer was separated and aqueous phase was extracted twice with DCM. The combined organic layer was dried over $MgSO_4$ and the solvent was evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane/Ethyl acetate/DCM = 9:1:0.5 to 7:3:0.5) to afford **A-047** (1.64 g, 2.68 mmol, 91%). R_f : 0.41 (hexane/Ethyl acetate/DCM = 7:3:0.5). $[\alpha]_D^{25}$ = 16.17 (*c* = 3.33, $CHCl_3$). IR (thin film): υ = 3471, 2961, 1071 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.54 (s, 2H), 7.43 – 7.23 (m, 15H), 7.20 (d, *J* = 8.0 Hz, 1H), 7.12 (d, *J* = 7.9 Hz, 1H), 4.89 (ddd, *J* = 38.1, 21.9, 10.2 Hz, 5H, 2 x CH_2Ph, C*H*HPh), 4.70 (d, *J* = 9.9 Hz, 1H, H-1), 4.66 (d, *J* = 10.8 Hz, 1H, C*H*HPh), 3.86 (d, *J* = 11.7 Hz, 1H, H-6), 3.77 – 3.66 (m, 2H, H-3, H-6), 3.62 (t, *J* = 9.4 Hz, 1H, H-4), 3.54 (t, *J* = 9.3 Hz, 1H, H-2), 3.43 – 3.33 (m, 1H, H-5), 2.39 (s, 3H, Me), 1.28 (s, 9H, *t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.62, 138.33, 137.88,

137.82, 135.83, 132.95, 129.94, 128.50, 128.44, 128.38, 128.23, 128.20, 128.04, 127.95, 127.86, 127.77, 127.71, 124.59 (Ar), 87.64 (C-1), 86.60 (C-3), 81.39 (C-2), 79.13 (C-5), 77.54 (C-4), 75.80 (CH_2Ph), 75.67 (CH_2Ph), 75.10 (CH_2Ph), 62.18 (C-6), 34.45 (Cq, *t*-Bu), 31.29 (Me, *t*-Bu), 20.24 (Me). MS ESI-HRMS m/z $[M+Na]^+$ calcd for $C_{38}H_{44}O_5S$ 635.2802, found 635.2795.

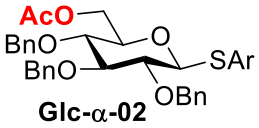
(2-Methyl-5-tert-butylphenyl) 2,3,4,6-tetra-*O*-benzyl-3-*O*-fluorenylmethoxycarbonyl-1-thio-β-D-glucopyranoside Glc-α-01



Glc-α-01

Compound **A-047** (0.2 g, 0.33 mmol), benzyl bromide (0.097 mL, 0.82 mmol), and sodium hydride (0.039 g, 0.979 mmol) were dissolved in anhydrous DMF (1.6 mL, 0.2 M) and stirred for 2 h at 0 °C. The mixture was quenched with saturated aqueous NH_4Cl, and then diluted with DCM. The organic layer was separated and aqueous phase was extracted twice with DCM. The combined organic layer was dried over $MgSO_4$ and the solvent was evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane/Ethyl acetate = 9:0.5 to 9.0:1.0) to afford **Glc-α-01** (0.22 g, 0.31 mmol, 96%). R_f = 0.48 (hexane/Ethyl acetate/DCM = 8:2:0.5). $[\alpha]_D^{25}$ = +9.43 (*c* = 3.50, $CHCl_3$). IR (thin film): υ = 2961, 1905, 1454, 1361, 1067 cm^{-1}; H NMR (600 MHz, $CDCl_3$) 7.66 (d, *J* = 1.9 Hz, 1H), 7.45 – 7.23 (m, 18H), 7.19 – 7.05 (m, 4H), 4.97 (d, *J* = 10.3 Hz, 1H, C*H*HPh), 4.93 (d, *J* = 11.0 Hz, 1H, C*H*HPh), 4.86 (d, *J* = 11.0 Hz, 1H, C*H*HPh), 4.83 (d, *J* = 10.7 Hz, 1H, C*H*HPh), 4.79 (d, *J* = 10.3 Hz, 1H, C*H*HPh), 4.67 (d, *J* = 9.9 Hz, 1H, H-1), 4.59 (dd, *J* = 11.3, 9.8 Hz, 2H, CH_2Ph), 4.53 (d, *J* = 12.3 Hz, 1H, C*H*HPh), 3.74 – 3.69 (m, 4H, H-3, H-4, H-6), 3.58 (dd, *J* = 9.7, 8.6 Hz 1H, H-2), 3.49 (ddd, *J* = 9.3, 3.9, 2.2 Hz, 1H, H-5), 2.40 (s, 3H), 1.26 (s, 9H, *t*-Bu). ^{13}C NMR (150 MHz, $CDCl_3$) δ 149.71, 138.64, 138.26, 138.19, 138.18, 135.92, 133.75, 129.86, 128.60, 128.57, 128.55, 128.51, 128.48, 128.37, 128.11, 128.04, 127.95, 127.91, 127.83, 127.80, 127.71, 124.39 (Ar), 88.18 (C-1), 86.99 (C-3), 81.39 (C-2), 79.17 (C-5), 77.96 (C-4), 75.92 (Bn), 75.71 (Bn), 75.20 (Bn), 73.61 (Bn), 69.01 (C-6), 34.61 (Cq, *t*-Bu), 31.43 (Me, *t*-Bu), 20.46 (Me). MS ESI-HRMS m/z $[M+Na]^+$ calcd for $C_{45}H_{50}O_5SNa$ 725.3277, found 725.3240

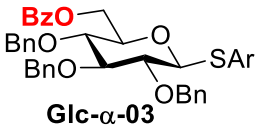
(2-Methyl-5-tert-butylphenyl) 3-*O*-Acetyl-2,4,6-tri-*O*-benzyl-1-thio-β-D-glucopyranoside Glc-α-02



Glc-α-02

To a solution of compound **A-047** (0.2 g, 0.33 mmol) in anhydrous DCM (1.6 mL, 0.2 M) were added acetice anhydride (0.062 mL, 0.65 mmol), triethylamine (Et_3N) (0.18 mL, 1.31 mmol), and a catalytic amount of DMAP (8 mg, 0.065 mmol) at 0 °C and the mixture was stirred for 2 h at room temperature. After the mixture was quenched with saturated aqueous $NaHCO_3$, it was diluted with DCM. The organic layer was separated and aqueous phase was extracted twice with DCM. The combined organic layer was dried over $MgSO_4$ and the solvent was evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane/Ethyl acetate/DCM = 9:1:0.5 to 7:3:0.5) to afford **Glc-α-02** (0.2 g, 0.31 mmol, 94%). R_f = 0.27 (hexane/Ethyl acetate/DCM = 8:2:0.5). $[\alpha]_D^{25}$ = 23.49 (*c* = 3.07, $CHCl_3$). IR (thin film): υ = 2961, 1743, 1236, 1067 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.59 (d, *J* = 1.8 Hz, 1H), 7.40 (d, *J* = 7.0 Hz, 2H), 7.36 – 7.24 (m, 10H), 7.20 (dd, *J* = 8.0, 1.9 Hz, 1H), 7.13 (d, *J* = 8.0 Hz, 1H), 5.00 (d, *J* = 10.2 Hz, 1H, C*H*HPh), 4.96 (d, *J* = 10.9 Hz, 1H, C*H*HPh), 4.88 (d, *J* = 10.8, 1H, C*H*HPh), 4.87 (d, *J* = 10.9, 1H, C*H*HPh), 4.80 (d, *J* = 10.2 Hz, 1H, C*H*HPh), 4.66 (d, *J* = 9.9 Hz, 1H, H-1), 4.59 (d, *J* = 10.8 Hz, 1H, C*H*HPh), 4.34 (dd, *J* = 11.9, 1.5 Hz, 1H, H-6), 4.24 (dd, *J* = 12.0, 5.0 Hz, 1H, H-6), 3.74 (t, *J* = 8.7 Hz, 1H, H-3), 3.65 – 3.49 (m, 3H, H-2, H-4, H-5), 2.39 (s, 3H, Me), 2.03 (s, 3H, Ac), 1.30 (s, 9H, *t*-Bu). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.90 (Ac), 149.69, 138.40, 138.04, 137.72, 136.25, 133.33, 130.00, 128.69, 128.67, 128.63, 128.54, 128.34, 128.24, 128.18, 128.02, 127.91, 124.71 (Ar), 88.38 (C-1), 86.91 (C-3), 81.32 (C-2), 76.96 (C-5), 75.96 (CH_2Ph), 75.77 (CH_2Ph), 75.27 (CH_2Ph), 63.66 (C-6), 34.61 (Cq, t-Bu), 31.48 (Me, t-Bu), 21.06 (Ac), 20.44 (Me). MS ESI-HRMS m/z $[M+Na]^+$ calcd for $C_{40}H_{46}O_6S$ 677.2907, found 677.2930

(2-Methyl-5-tert-butylphenyl) 3-*O*-Benzoyl-2,4,6-tri-*O*-benzyl-1-thio-β-D-glucopyranoside Glc-α-03

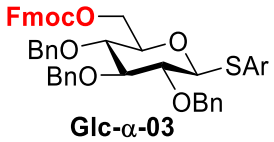


Glc-α-03

To a solution of compound **A-047** (0.2 g, 0.33 mmol) in anhydrous DCM (1.6 mL, 0.2 M) were added benzoic anhydride (0.15 g, 0.65 mmol), triethylamine (Et_3N, 0.18 mL, 1.31 mmol), and a catalytic amount of DMAP (8 mg, 0.065 mmol) at 0 °C and the mixture was stirred for 2 h at room temperature. After the mixture was quenched with saturated aqueous $NaHCO_3$, it was diluted with DCM. The organic layer was separated and aqueous phase was

extracted twice with DCM. The combined organic layer was dried over $MgSO_4$ and the solvent was evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane/Ethyl acetate/DCM = 9:1:0.5 to 7:3:0.5) to afford **Glc-α-03** (0.23 g, 0.32 mmol, 98%). R_f = 0.31 (hexane/Ethyl acetate/DCM = 8:2:0.5). $[\alpha]_D^{25}$ = 18.84 (*c* = 2.98, $CHCl_3$). IR (thin film): υ = 2961, 1722, 1272, 1068 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.99 (dd, *J* = 8.2, 1.1 Hz, 2H), 7.62 – 7.53 (m, 2H), 7.43 (m, 4H), 7.38 – 7.21 (m, 13H), 7.19 (dd, *J* = 7.9, 2.0 Hz, 1H), 7.09 (d, *J* = 7.9 Hz, 1H), 5.04 (d, *J* = 10.2 Hz, 1H, C*H*HPh), 4.97 (d, *J* = 10.8 Hz, 1H, C*H*HPh), 4.89 (d, *J* = 10.7 Hz, 1H, C*H*HPh), 4.88 (d, *J* = 10.8 Hz, 1H, C*H*HPh), 4.84 (d, *J* = 10.2 Hz, 1H, C*H*HPh), 4.69 (d, *J* = 9.9 Hz, 1H, H-1), 4.62 (d, *J* = 10.7 Hz, 1H, C*H*HPh), 4.58 (dd, *J* = 12.0, 1.9 Hz, 1H, H-6), 4.49 (dd, *J* = 12.0, 4.5 Hz, 1H, H-6), 3.78 (t, *J* = 8.8 Hz, 1H, H-3), 3.74 (t, *J* = 9.2 Hz, 1H, H-4), 3.65 – 3.63 (m, 1H, H-5), 3.61 (dd, *J* = 9.6, 8.8 Hz, 1H, H-2), 2.39 (s, 3H, Me), 1.24 (s, 9H, *t*-Bu). ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.36 (Bz), 149.67, 138.36, 138.09, 137.69, 136.99, 133.18, 132.88, 130.03, 129.93, 129.66, 128.67, 128.65, 128.57, 128.48, 128.34, 128.23, 128.14, 128.03, 127.98, 125.03, 88.71 (C-1), 86.97 (C-3), 81.65 (C-2), 77.72 (C-4), 77.13 (C-5), 76.11 (CH_2Ph), 75.82 (CH_2Ph), 75.37 (CH_2Ph), 63.83 (C-6), 34.54 (Cq, *t*-Bu), 31.42 (Me, *t*-Bu), 20.59 (Me). MS ESI-HRMS m/z $[M+Na]^+$ calcd for $C_{45}H_{48}O_6S$ 739.3064, found 739.3093.

(2-Methyl-5-tert-butylphenyl) 2,4,6-tri-*O*-Benzyl-3-*O*-fluorenylmethoxycarbonyl-1-thio-β-D-glucopyranoside Glc-α-04

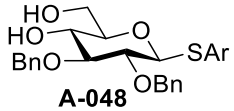


Glc-α-03

To a solution of compound **A-047** (0.2 g, 0.33 mmol) in anhydrous DCM (1.6 mL, 0.2 M) were added 9-fluorenylmethyl chloroformate (0.13 g, 0.49 mmol) and pyridine (0.053 mL, 0.65 mmol) at 0 °C, and the mixture was stirred for 3hr at room temperature. Then the mixture was quenched with 1M aqueous HCl, and then diluted with DCM. The organic layer was separated and aqueous phase was extracted twice with DCM. The combined organic layer was dried over $MgSO_4$ and the solvent was evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane/Ethyl acetate/DCM = 9:0.5:0.5 to 9:1:0.5) to afford **Glc-α-04** (0.26 g, 0.31 mmol, 95%). R_f = 0.35 (hexane/Ethyl acetate/DCM = 8:2:0.5). $[\alpha]_D^{25}$ = 19.11 (*c* = 3.05, $CHCl_3$). IR (thin film): υ = 2960, 1748, 1452, 1254, 1066 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.77 (dd, *J* = 7.5, 2.8 Hz, 2H), 7.66 – 7.57 (m, 3H), 7.43 – 7.37 (m, 4H), 7.35 – 7.24 (m, 15H), 7.19 (dd, *J* = 7.9, 1.9 Hz, 1H), 7.11

(d, *J* = 8.0 Hz, 1H), 5.00 (d, *J* = 10.3 Hz, 1H, C*H*HPh), 4.96 (d, *J* = 10.9 Hz, 1H, C*H*HPh), 4.89 (d, *J* = 10.9 Hz, 1H, C*H*HPh), 4.87 (d, *J* = 10.9 Hz, 1H, C*H*HPh), 4.80 (d, *J* = 10.2 Hz, 1H, C*H*HPh), 4.68 (d, *J* = 9.9 Hz, 1H, H-1), 4.61 (d, *J* = 10.9 Hz, 1H, C*H*HPh), 4.45 (dd, *J* = 11.6, 1.6 Hz, 1H, H-6), 4.38 (ddd, *J* = 24.2, 10.5, 7.5 Hz, 2H, CH_2Ph of Fmoc), 4.32 (dd, *J* = 11.6, 5.1 Hz, 1H, H-6), 4.24 (t, *J* = 7.4 Hz, 1H, CH of Fmoc), 3.75 (t, *J* = 8.7 Hz, 1H, H-3), 3.64 (t, *J* = 9.2 Hz, 1H, H-4), 3.61 – 3.54 (m, 2H, H-2, H-5), 2.39 (s, 3H, Me), 1.28 (s, 9H, *t*-Bu). ^{13}C NMR (150 MHz, $CDCl_3$) δ 155.21 (Fmoc), 149.82, 143.56, 143.48, 141.42, 138.43, 138.07, 137.75, 136.29, 133.29, 130.00, 128.92, 128.68, 128.64, 128.56, 128.35, 128.22, 128.15, 128.02, 127.92, 127.34, 127.32, 125.39, 125.34, 124.80, 120.18 (Ar), 88.46 (C-1), 86.89 (C-3), 81.29 (C-5), 77.55 (C-4), 76.88 (C-2), 75.98 (CH_2Ph), 75.78 (CH_2Ph), 75.35 (CH_2Ph), 70.12 (CH_2 of Fmoc), 67.01 (C-6), 46.86 (CH of Fmoc), 34.63 (Cq, *t*-Bu), 31.48 (Me, *t*-Bu), 20.48 (Me). MS ESI-HRMS m/z $[M+Na]^+$ calcd for $C_{45}H_{48}O_6S$ 857.3482, found 857.3505.

(2-Methyl-5-tert-butylphenyl) 2,3-di-*O*-benzyl-1-thio-β-D-glucopyranoside A-048

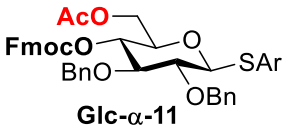


A-048

A solution of compound **A-046** (4.0 g, 6.55 mmol) in DCM/Water/TFA (62 mL: 4 mL: 16 mL, 0.08 M) was stirred for 2 h at 0 °C. After the reaction was completed, saturated aqueous $NaHCO_3$ was added and the aqueous layers were extracted three times with DCM. The organic layer was separated and aqueous phase was extracted twice with DCM. The combined organic layer was dried over $MgSO_4$ and the solvent was evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane/Ethyl acetate/DCM = 8:2:0.5 to 5:5:0.5) to afford **A-048** (2.8 g, 5.36 mmol, 82%). R_f = 0.35 (hexane/Ethyl acetate/DCM = 8:2:0.5). $[\alpha]_D^{25}$ = 2.02 (*c* = 2.34, $CHCl_3$). IR (thin film): υ = 3449, 2964, 1455, 1071 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 (d, *J* = 1.8 Hz, 1H), 7.45 – 7.26 (m, 9H), 7.22 (dd, *J* = 8.0, 1.9 Hz, 1H), 7.14 (d, *J* = 8.0 Hz, 1H), 5.03 (d, *J* = 10.9 Hz, 1H, C*H*HPh), 4.86 (d, *J* = 11.2 Hz, 1H, C*H*HPh), 4.76 – 4.70 (m, 2H, 2 x C*H*HPh), 4.69 (d, *J* = 6.2 Hz, 1H, H-1), 3.89 (dd, *J* = 12.0, 2.6 Hz, 1H, H-6), 3.81 (t, *J* = 8.8 Hz, 1H, H-4), 3.73 (dd, *J* = 12.1, 4.7 Hz, 1H, H-6), 3.53 (t, *J* = 9.3 Hz, 1H, H-3), 3.46 – 3.34 (m, 2H, H-2, H-5), 2.40 (s, 3H, Me), 1.29 (s, 9H, *t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.82, 138.15, 138.03, 135.85, 133.01, 130.14, 128.75, 128.71, 128.39, 128.26, 128.25, 128.21, 128.17, 124.75 (Ar), 87.25 (C-1), 81.06 (C-2), 79.02 (C-5), 78.74 (C-4), 75.47 (C-3), 74.88 (CH_2Ph), 62.40

(CH_2Ph), 34.62 (Cq, *t*-Bu), 31.45 (Me, *t*-Bu), 20.37 (Me). MS ESI-HRMS m/z $[M+Na]^+$ calcd for $C_{31}H_{38}O_5S$ 545.2332, found 545.2276.

(2-Methyl-5-tert-butylphenyl) 6-*O*-Acetyl-2,3-di-*O*-benzyl-4-*O*-fluorenylmethoxycarbonyl-1-thio-β-D-glucopyranoside Glc-α-11

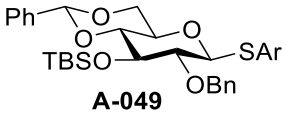


Glc-α-11

To a solution of compound **A-048** (1.2 g, 2.33 mmol), 2-Chloro-1-methylpyridium iodide (1.49 mL, 5.84 mmol), DABCO (1.05 g, 9.34 mmol) in anhydrous DCM (23 mL, 0.1 M) was added acetic acid (0.15 mL, 2.57 mmol) slowly at -15 °C and the mixture was stirred for 2 h at -15 °C. After the mixture was quenched using saturated aqueous $NaHCO_3$, it was diluted with DCM. The organic layer was separated and aqueous phase was extracted twice with DCM. The combined organic layer was dried over $MgSO_4$ and the solvent was evaporated *in vacuo*. To a solution of the crude product in anhydrous DCM (11 mL) was added 9-fluorenylmethyl chloroformate (1.10 g, 4.25 mmol) and pyridine (0.52 mL, 6.37 mmol) at 0 °C, and stirred at room temperature for overnight. After the mixture was quenched with 1M aqueous HCl, it was diluted with DCM. The organic layer was dried over $MgSO_4$ and the solvent was evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane/Ethyl acetate/DCM = 9:0.5:0.5 to 8:2:0.5) to afford **Glc-α-11** (1.54 g, 1.96 mmol, 84% over two steps). R_f = 0.35 (hexane/Ethyl acetate/DCM = 8:2:0.5). $[\alpha]_D^{25}$ = 7.41 (*c* = 2.65, $CHCl_3$). IR (thin film): υ = 2962, 1750, 1258, 1037 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.67 (d, *J* = 7.6 Hz, 1H), 7.53 (d, *J* = 1.9 Hz, 1H), 7.50 (d, *J* = 7.5 Hz, 1H), 7.46 (d, *J* = 7.5 Hz, 1H), 7.33 – 7.13 (m, 8H), 7.06 (d, *J* = 8.0 Hz, 1H), 4.89 (d, *J* = 9.7 Hz, 1H), 4.91 – 4.86 (m, 1H, H-4), 4.74 (d, *J* = 11.2 Hz, 1H, C*H*HPh), 4.70 (d, *J* = 10.3 Hz, 1H, C*H*HPh), 4.59 (d, *J* = 10.4 Hz, 1H, C*H*HPh), 4.57 (d, *J* = 9.7 Hz, 1H, H-1, C*H*HPh), 4.37 (dd, *J* = 10.5, 7.1 Hz, 1H, C*H*H, Fmoc), 4.26 – 4.21 (m, 2H, H-6, , C*H*H, Fmoc), 4.09 (m, 2H, H-6, CH of Fmoc), 3.66 (t, *J* = 9.1 Hz, 1H, H-3), 3.58 (ddd, *J* = 10.0, 5.0, 2.4 Hz, 1H, H-5), 3.56 – 3.50 (m, 1H, H-2), 2.33 (s, 3H, Me), 1.96 (s, 3H, Me of Ac), 1.23 (s, 9H, *t*-Bu). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.82 (Ac), 154.43 (Fmoc), 149.75, 143.40, 143.26, 141.41, 141.39, 137.94, 137.88, 136.72, 132.82, 130.11, 129.42, 128.56, 128.44, 128.39, 128.08, 128.04, 128.02, 127.85, 127.83, 127.32, 125.23, 125.10, 125.09, 120.20, 120.18 (Ar), 88.61 (C-1), 83.99 (C-3), 80.95 (C-2), 75.83 (CH_2Ph), 75.76 (CH_2Ph), 75.52 (C-5), 74.41 (C-4), 70.41 (CH_2 of Fmoc), 62.82 (C-6), 46.82 (CH of Fmoc), 34.62 (Cq, *t*-Bu), 31.49 (Me, *t*-Bu), 20.95 (Me of

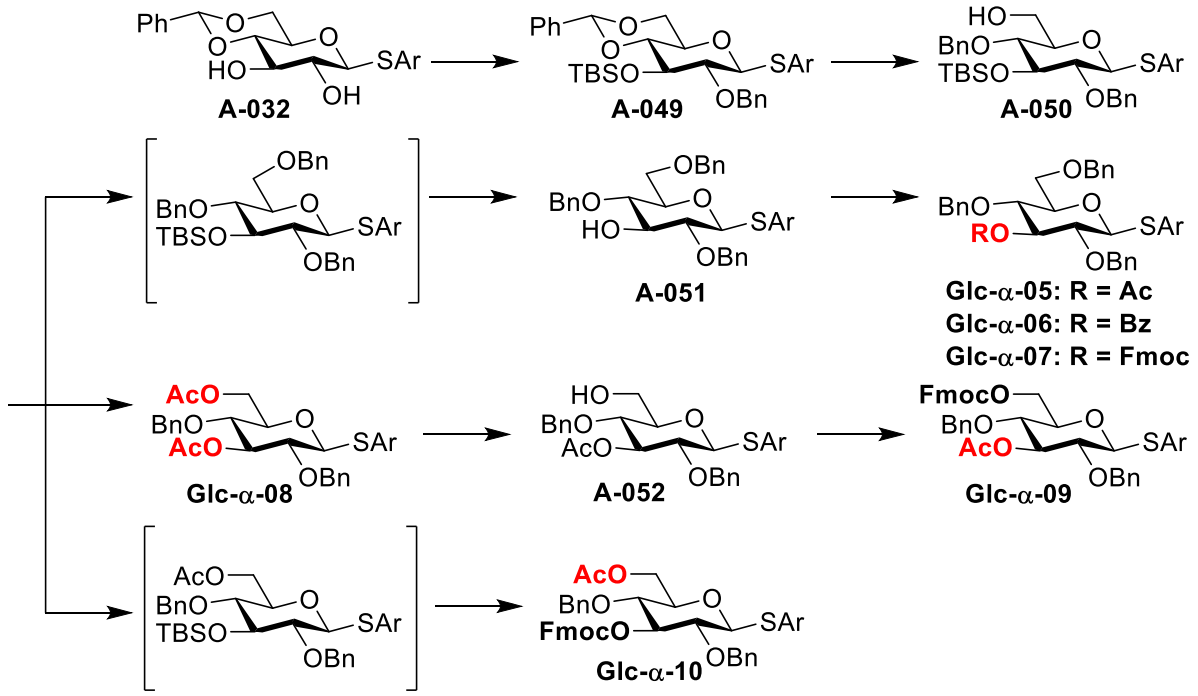
Ac), 20.52 (Me). MS ESI-HRMS m/z $[M+Na]^+$ calcd for $C_{48}H_{50}O_8S$ 809.3119, found 809.3137.

(2-Methyl-5-tert-butylphenyl) 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-tert-butyldimethylsilyl-1-thio-β-D-glucopyranoside A-049



A-049

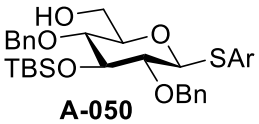
To a solution of compound **A-032** (9 g, 20.9 mmol) in anhydrous DCM (21 mL, 1.0 M) was added TBSCl (3.78 g, 25.mmol), imidazole (1.99 g, 29.3 mmol) dropwise at 0 °C, the mixture was gradually wormed up to room temperature, and stirred at room temperature for overnight. After the mixture was quenched with MeOH, it was diluted with DCM. The organic layer was separated and aqueous phase was extracted twice with DCM. The combined organic layer was dried over $MgSO_4$ and the solvent was evaporated *in vacuo*. To a solution of this crude product in anhydrous DMF (91 mL, 0.2 M) were added benzyl bromide (7.46 mL, 62.7 mmol, 3.0 eq.) and sodium hydride (2.01 g, 50.2 mmol, 2.4 eq.) at 0 °C for 2 h. The mixture was quenched by saturated aqueous NH_4Cl and then diluted with DCM. The organic layer was dried over $MgSO_4$ and the solvent was evaporated *in vacuo*. The crude product passed through silica pad, then utilized for the next reaction**.**



Scheme A.09. Synthesis of glucose building blocks Glc-α-05 to Glc-α-10. a) i) TBSCl, Imidazole, DCM, r.t.; ii) BnBr, NaH, DMF, 0 °C; b) BH_3 in THF, TMSOTf, DCM, 0 °C, 77%; c) i) BnBr, NaH, THF/DMF (v/v = 9:1), 0 °C; ii) $BF_3{\cdot}OEt_2$, MeCN, 0 °C, 90% for 2 steps; d) i) Ac_2O, pyridine, DMAP, DCM, r.t , 89% for **Gal-α-05,** ii) Bz_2O, pyridine, DMAP, DCM, r.t , 90% for **Gal-α-06,** iii) FmocCl,

pyridine, DCM, 0 °C, 88% for **Gal-α-07**; e) Ac_2O, pyridine, DMAP, DCM, r.t , 91% for **Gal-α-08**; f) NH_3, MeOH, . 86%; TFA , 95%; f) FmocCl, pyridine, DCM, 0 °C, 94% for **Gal-α-09**; g) i) Ac_2O, pyridine, DMAP, DCM, r.t , ; ii) $BF_3{\cdot}OEt_2$, MeCN, 0 °C, iii) FmocCl, pyridine, DCM, 0 °C, 84%, **Gal-α-10** for 3 steps.

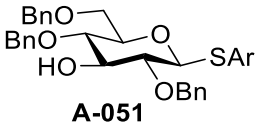
(2-Methyl-5-tert-butylphenyl) 2,4-di-*O*-benzyl-3-*O*-tert-butyldimethylsilyl-1-thio-β-D-glucopyranoside A-050



A-050

The crude **A-049** was co-evaporated with toluene and dissolved under an Ar atmosphere in DCM (53 mL, 0.2 M). To a solution of the crude **S11** were added 1 M solution of BH_3 in THF (52.6 mL, 52.6 mmol), and TMSOTf (0.95 mL, 5.26 mmol) at 0 °C and the mixture was stirred for 5 h at 0 °C. After completion, the mixture was quenched with saturated aqueous $NaHCO_3$, and then diluted with DCM. The organic layer was separated and aqueous phase was extracted twice with DCM. The combined organic layer was dried over $MgSO_4$ and the solvent was evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane/ethyl acetate/DCM = 9:1:0.5 to 7:3:0.5) to afford **A-050** (6.45 g, 10.1 mmol, 77%) over three steps. R_f : 0.27 (Hexane/ethyl acetate/DCM = 8:2:0.5). $[\alpha]_D^{25}$ = 42.23 (C= 2.00, $CHCl_3$). IR (thin film): υ = 3476, 2958, 1732, 1263, 1090 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.52 – 7.26 (m, 11H), 7.23 – 7.08 (m, 2H), 4.98 (d, *J* = 9.8 Hz, 1H, C*H*HPh), 4.88 (d, *J* = 11.5 Hz, 1H, C*H*HPh), 4.79 (d, *J* = 10.6 Hz, 1H, C*H*HPh), 4.74 (d, *J* = 9.8 Hz, 1H, H-1), 4.63 (d, *J* = 9.4 Hz, 1H, C*H*HPh), 3.86 – 3.75 (m, 2H, H-4, H-6), 3.64 (m, 1H, H-6), 3.49 (t, *J* = 9.2 Hz, 1H, H-3), 3.44 – 3.34 (m, 2H, H-2, H-5), 2.32 (s, 3H, Me), 1.28 (s, 9H, *t*-Bu), 0.96 (s, 9H, TBS), 0.05 (s, 3H, TBS), 0.00 (s, 3H, TBS). ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.28, 138.12, 135.54, 129.93, 128.42, 127.98, 127.75, 127.62, 127.39 (Ar), 87.72 (C-1), 81.93 (C-2), 79.22 (C-5), 78.71 (C-4), 78.66 (C-3), 75.86 (CH_2Ph), 75.36 (CH_2Ph), 61.60 (C-6), 34.53 (Cq, t-Bu), 31.52 (t-Bu), 26.29 (TBS), 20.11 (Me), 18.13 (Cq, TBS), -3.81 (TBS), -3.89 (TBS). MS ESI-HRMS m/z $[M+Na]^+$ calcd for $C_{37}H_{52}O_5S$ 659.3197, found 659.3185.

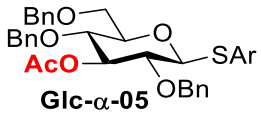
2-Methyl-5-tert-butylphenyl 2,4,6-tri-*O*-benzyl-1-thio-β-D-glucopyranoside A-051



A-051

To a solution of compound **A-050** (1.9 g, 2.98 mmol) in co-solvent of THF, and DMF (9:1) was added benzyl bromide (1.06 mL, 8.95 mmol), and sodium hydride (0.24 mg, 5.97 mmol) dropwise at 0 °C. The mixture was stirred for 2 h at 0 °C. After completion the mixture was quenched with saturated aqueous NH_4Cl, and then was diluted with DCM. The organic layer was dried over $MgSO_4$ and the solvent was evaporated *in vacuo*. To a solution of this crude in anhydrous acetonitrile (37 mL, 0.08 M) was added boron trifluoride etherate ($BF_3{\cdot}OEt_2$) (0.38 mL, 2.98 mmol) at 0 °C and the mixture was stirred at 0 °C for 20 min. After completion the mixture was quenched with saturated aqueous $NaHCO_3$ and then it was diluted with DCM. The organic layer was separated and aqueous phase was extracted twice with DCM. The combined organic layer was dried over $MgSO_4$ and the solvent was evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane/ethyl acetate/DCM = 9:1:1 to 7:3:1) to afford **A-051** (1.65 g, 2.69 mmol, 90% over two steps). R_f = 0.36 (Hexane/ethyl acetate/DCM = 8:2:0.5). $[\alpha]_D^{25}$ = +0.43 (C= 3.50, $CHCl_3$). IR (thin film): υ = 3447, 3033, 2963, 2869, 1455, 1069 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.68 (d, *J* = 1.9 Hz, 1H), 7.42 – 7.07 (m, 17H), 5.01 (d, *J* = 11.0 Hz, 1H, C*H*HPh), 4.81 (d, *J* = 11.1 Hz, 1H, C*H*HPh), 4.71 (d, *J* = 10.9 Hz, 1H, C*H*HPh), 4.65 (d, *J* = 9.9 Hz, 1H, H-1), 4.62 (d, *J* = 10.1 Hz, 1H, C*H*HPh), 4.60 (d, *J* = 8.8 Hz, 1H, CHHPh), 4.54 (d, *J* = 12.3 Hz, 1H, C*H*HPh), 3.82 – 3.69 (m, 3H, H-3, H-6), 3.60 (t, *J* = 9.3 Hz, 1H, H-4), 3.52 – 3.43 (m, 2H, H-2, H-5), 2.41 (s, 3H), 1.27 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.75, 138.32, 138.16, 135.75, 133.61, 129.90, 128.71, 128.60, 128.52, 128.47, 128.40, 128.19, 128.13, 128.08, 127.98, 127.75, 124.38 (Ar), 87.58 (C-1), 80.75 (C-2), 78.86 (C-5), 78.84 (C-3), 77.40 (C-4), 75.27 (Bn), 74.78 (Bn), 73.60 (Bn), 68.95 (C-6), 34.61 (Cq, t-Bu), 31.42 (Me, t-Bu), 20.43 (Me). MS ESI-HRMS m/z $[M+Na]^+$ calcd for $C_{38}H_{44}O_5SNa$ 635.2802, found 635.2795.

2-Methyl-5-tert-butylphenyl 3-*O*-Acetyl-2,4,6-tri-*O*-benzyl-1-thio-β-D-glucopyranoside Glc-α-05

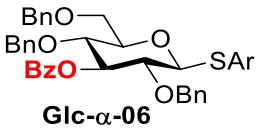


Glc-α-05

To a solution of compound **A-051** (0.2 g, 0.33 mmol) in anhydrous DCM (1.6 mL, 0.2 M) were added acetice anhydride (0.062 mL, 0.65 mmol), triethylamine (Et_3N, 0.18 mL, 1.31 mmol), and a catalytic amount of DMAP (8 mg, 0.065 mmol) at 0 °C, the mixture was stirred for 2 h at room temperature. After completion the mixture was quenched with saturated aqueous $NaHCO_3$ and then diluted with DCM. The organic layer was separated and aqueous

phase was extracted twice with DCM. The combined organic layer was dried over $MgSO_4$ and the solvent was evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane/ethyl acetate = 9:1 to 7:3) to afford **Glc-α-05** (0.19 g, 0.29 mmol, 89%). R_f = 0.34 (Hexane/ethyl acetate/DCM = 8:2:0.5). $[\alpha]_D^{25}$ = 3.20 (*c* = 3.00, $CHCl_3$). IR (thin film): υ = 2960, 1748, 1361, 1225, 1087 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 7.65 (d, *J* = 1.7 Hz, 1H), 7.41 – 7.04 (m, 17H), 5.28 (t, *J* = 9.3 Hz, 1H, H-3), 4.88 (d, *J* = 11.1 Hz, 1H, C*H*HPh), 4.68 (d, *J* = 9.8 Hz, 1H, H-1), 4.63 (d, *J* = 12.2 Hz, 1H, C*H*HPh), 4.57 (d, *J* = 11.1 Hz, 1H, C*H*HPh), 4.53 (d, *J* = 12.2 Hz, 1H, C*H*HPh), 4.51 (s, 2H, CH_2Ph), 3.76 – 3.68 (m, 3H, H-4, H-6), 3.59 – 3.46 (m, 2H, H-2, H-5), 2.40 (s, 3H, Me), 1.83 (s, 3H, Ac), 1.26 (s, 9H, *t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.86 (Ac), 149.52, 137.85, 137.70, 137.67, 136.04, 132.93, 129.79, 128.91, 128.35, 128.31, 128.16, 128.12, 127.93, 127.88, 127.75, 127.72, 127.63, 124.49 (Ar), 87.86 (C-1), 78.71 (C-2), 78.70 (C-5), 76.67 (C-3), 75.79 (C-4), 74.66 (Bn), 74.27 (Bn), 73.47 (Bn), 68.39 (C-6), 34.39 (Cq, *t*-Bu), 31.22 (Me, *t*-Bu), 20.93 (Me, Ac), 20.29 (Me). MS ESI-HRMS m/z $[M+Na]^+$ calcd for $C_{40}H_{46}O_6SNa$ 677.2913, found 677.2882.

2-Methyl-5-tert-butylphenyl 3-*O*-Benzoyl-2,4,6-tri-*O*-benzyl-1-thio-β-D-glucopyranoside Glc-α-06

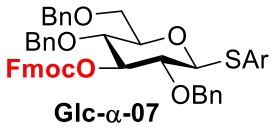


Glc-α-06

To a solution of compound **A-051** (0.2 g, 0.33 mmol) in anhydrous DCM (1.6 mL, 0.2 M) were added benzoic anhydride (0.15 g, 0.65 mmol), triethylamine (Et_3N, 0.18 mL, 1.31 mmol, 4.0 eq.), and a catalytic amount of DMAP (8 mg, 0.065 mmol) at 0 °C and the mixture was stirred for 2 h at room temperature. After completion the mixture was quenched with saturated aqueous $NaHCO_3$ and then diluted with DCM. The organic layer was separated and aqueous phase was extracted twice with DCM. The combined organic layer was dried over $MgSO_4$ and the solvent was evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane/ethyl acetate = 9:1 to 7:3) to afford **Glc-α-06** (0.21 g, 0.29 mmol, 90%). R_f = 0.40 (hexane/ethyl acetate/DCM = 7:3:0.5). $[\alpha]_D^{25}$ = 18.06 (*c* = 3.20, $CHCl_3$). IR (thin film): υ = 2960, 2867, 1728, 1453, 1266, 1088, 1069 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 7.97 (dd, *J* = 8.4, 1.2 Hz, 2H), 7.68 (s, 1H), 7.61 – 7.54 (m, 1H), 7.35 (dddd, *J* = 12.7, 11.5, 10.2, 5.6 Hz, 7H), 7.23 – 6.95 (m, 12H), 5.57 (t, *J* = 9.2 Hz, 1H, H-3), 4.82 (d, *J* = 10.7 Hz, 1H, C*H*HPh), 4.76 (d, *J* = 9.8 Hz, 1H, H-1), 4.65 (d, *J* = 12.2 Hz, 1H, C*H*HPh), 4.56 (d, *J* = 10.6 Hz, 1H, C*H*HPh), 4.55 (d, *J* = 12.4 Hz, 1H, C*H*HPh), 4.51 – 4.42 (m, 2H,

CH_2Ph), 3.88 (t, *J* = 9.5 Hz, 1H, H-4), 3.76 (s, 2H, H-6), 3.70 (t, *J* = 9.5 Hz, 1H, H-2), 3.58 (d, *J* = 9.8 Hz, 1H, H-5), 2.41 (s, 3H, Me), 1.27 (s, 9H, *t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.55 (Bz), 149.69, 138.03, 137.49, 137.41, 136.17, 133.22, 133.16, 130.07, 129.96, 129.84, 129.01, 128.54, 128.50, 128.48, 128.33, 128.26, 128.23, 128.13, 127.83, 127.81, 127.79, 124.63 (Ar), 88.12 (C-1), 78.87 (C-5), 78.69 (C-2), 78.18 (C-3), 75.83 (C-4), 74.90 (Bn), 74.57 (Bn), 73.68 (Bn), 68.61 (C-6), 34.58 (Cq, *t*-Bu), 31.40 (Me, *t*-Bu), 20.47 (Me). MS ESI-HRMS m/z $[M+Na]^+$ calcd for $C_{45}H_{48}O_6SNa$ 739.3069, found 739.3085.

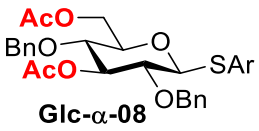
(2-Methyl-5-tert-butylphenyl) 2,4,6-tri-*O*-benzyl-3-*O*-fluorenylmethoxycarbonyl-1-thio-β-D-glucopyranoside Glc-α-07



Glc-α-07

To a solution of compound **A-051** (0.2 g, 0.33 mmol) in anhydrous DCM (1.6 mL, 0.2 M) were added 9-fluorenylmethyl chloroformate (0.13 g, 0.49 mmol) and pyridine (0.053 mL, 0.65 mmol) successively at 0 °C and the mixture was stirred for 3hr at room temperature. After completion the mixture was quenched with 1M aqueous HCl and then diluted with DCM. The organic layer was separated and aqueous phase was extracted twice with DCM. The combined organic layer was dried over $MgSO_4$ and the solvent was evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane/ethyl acetate/DCM = 9:0.5:0.5 to 9:1:0.5) to afford **Glc-α-07** (0.24g, 0.29 mmol, 88%). R_f = 0.51 (hexane/ethyl acetate/DCM : 7:3:0.5). $[\alpha]_D^{25}$ = 9.40 (*c* = 3.21, $CHCl_3$). IR (thin film): υ = 2954, 1752, 1451, 1252, 1072 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, *J* = 7.6 Hz, 2H), 7.64 (d, *J* = 1.9 Hz, 1H), 7.56 (d, *J* = 7.5 Hz, 2H), 7.41 – 7.16 (m, 23H), 7.14 – 7.09 (m, 3H), 5.12 (t, *J* = 9.3 Hz, 1H, H-3), 4.93 (d, *J* = 10.8 Hz, 1H, CHHPh), 4.68 (d, *J* = 9.9 Hz, 1H, H-1), 4.65 – 4.50 (m, 5H, 3 X C*H*HPh, CH_2Ph), 4.36 – 4.24 (m, 2H, CH_2, Fmoc), 4.13 (t, *J* = 7.2 Hz, 1H, CH, Fmoc), 3.79 (t, *J* = 9.6 Hz, 1H, H-4), 3.71 (d, *J* = 2.8 Hz, 2H, H-6), 3.62 (t, *J* = 9.5 Hz, 1H, H-2), 3.51 (dt, *J* = 9.8, 2.7 Hz, 1H, H-5), 2.40 (s, 3H, Me), 1.25 (s, 9H, *t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.77 (Fmoc), 149.74, 143.47, 143.42, 141.36, 138.05, 137.72, 136.33, 133.05, 129.98, 129.19, 128.51, 128.44, 128.37, 128.18, 128.07, 127.97, 127.90, 127.87, 127.81, 127.28, 125.21, 124.78, 120.15 (Ar), 88.01 (C-1), 82.71 (C-3), 79.04 (C-3), 78.84 (C-5), 75.92 (C-4), 75.21 (Bn), 74.79 (Bn), 73.66 (Bn), 70.19 (CH_2, Fmoc), 68.62 (C-6), 46.82 (CH, Fmoc), 34.59 (Cq, *t*-Bu), 31.41 (Me, *t*-Bu), 20.50 (Me). MS ESI-HRMS m/z $[M+Na]^+$ calcd for $C_{53}H_{54}O_7SNa$ 857.3488, found 733.3177 .

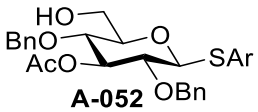
(2-Methyl-5-tert-butylphenyl) 3,6-di-*O*-Acetyl-2,4-di-*O*-benzyl-1-thio-β-D-glucopyranoside Glc-α-08



Glc-α-08

To a solution of compound **A-050** (3.6 g, 5.65 mmol) in anhydrous acetonitrile (71 mL, 0.08 M) was added boron trifluoride etherate ($BF_3 \cdot OEt_2$) (0.79 mL, 6.22 mmol) at 0 °C and the mixture was stirred for 20 min at 0 °C. After completion the mixture was quenched with saturated aqueous $NaHCO_3$ and then diluted with DCM. The organic layer was dried over $MgSO_4$ and the solvent was evaporated *in vacuo*. To a solution of the resulting crude protuct in anhydrous DCM (28 mL, 0.2 M) was added acetic anhydride (2.13 mL, 22.58 mmol), and triethylamine (4.72 mL, 33.9 mmol), and a catalytic amount of DMAP (0.069 g, 0.564 mmol) at 0 °C and the mixture was stirred for 1 h at 0 °C. After completion the mixture was quenched saturated aqueous $NaHCO_3$ and then diluted with DCM. The organic layer was separated and aqueous phase was extracted twice with DCM. The combined organic layer was dried over $MgSO_4$ and the solvent was evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane/ethyl acetate/DCM = 9:1:0.5 to 7:3:0.5) to afford **Glc-α-08** (3.12 g, 5.14 mmol, 91% over two steps). R_f = 0.27 (hexane/ethyl acetate/DCM = 8:2:0.5). $[\alpha]_D^{25}$ = +9.19 (*c* = 2.84, $CHCl_3$). IR (thin film): υ = 2958, 1742, 1455, 1363, 1219 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.58 (s, 1H), 7.39 – 7.01 (m, 12H), 5.32 (t, *J* = 8.4 Hz, 1H, H-3), 4.91 (d, *J* = 11.0 Hz, 1H, C*H*HPh), 4.68 (d, *J* = 9.8 Hz, 1H, H-1), 4.62 – 4.48 (m, 3H, C*H*HPh, CH_2Ph), 4.33 (d, *J* = 12.1 Hz, 1H, H-6), 4.22 (dd, *J* = 12.0, 3.5 Hz, 1H, H-6), 3.64 – 3.49 (m, 3H, H-2, H-4, H-5), 2.38 (s, 3H, Me), 2.04 (s, 3H, Ac), 1.88 (s, 3H, Ac), 1.29 (s, 9H, *t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.82 (Ac), 169.96 (Ac), 149.75, 137.77, 137.29, 136.53, 132.74, 130.11, 129.15, 128.71, 128.57, 128.32, 128.27, 128.19, 128.02, 125.01 (Ar), 88.29 (C-1), 78.91 (C-2), 77.30 (C-3), 76.79 (C-4), 76.07 (C-5), 75.03 (Bn), 74.64 (Bn), 63.45 (C-6), 34.62 (Cq, *t*-Bu), 31.47 (Me, *t*-Bu) 21.15 (Ac), 21.08 (Ac), 20.47 Me). MS ESI-HRMS m/z $[M+Na]^+$ calcd for $C_{35}H_{42}O_7SNa$ 629.2549, found 629.2522.

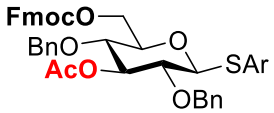
(2-Methyl-5-tert-butylphenyl) 6-di-*O*-Acetyl-2-*O*-benzoyl-4-*O*-benzyl-1-thio-β-D-glucopyranoside A-052



A-052

Through the solution of compound **Glc-α-08** (4.15 g, 6.84 mmol) in MeOH (34 mL, 0.2 M) was bubbled ammonia gas (NH_3) at 0 °C while the starting material was consumed. Excess NH_3 was expelled by bubbling Ar through the solution and then the mixture was evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane/ethyl acetate/DCM = 8:2:0.5 to 7:3:0.5) to afford **A-052** (3.35 g, 5.93 mmol, 87%). R_f = 0.32 (hexane/ethyl acetate/DCM = 7:3:0.5). $[\alpha]_D^{25}$ = +15.78 (C= 2.85, $CHCl_3$). IR (thin film): υ = 3489, 2960, 1748, 1455, 1362, 1231, 1072, 1030 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.55 (d, *J* = 2.0 Hz, 1H), 7.36 – 7.25 (m, 11H), 7.22 (dd, *J* = 7.9, 1.6 Hz, 1H), 7.14 (d, *J* = 8.0 Hz, 1H), 5.32 (t, *J* = 9.3 Hz, 1H, H-3), 4.91 (d, *J* = 11.0 Hz, 1H, C*H*HPh), 4.73 (d, *J* = 9.8 Hz, 1H, H-1), 4.63 – 4.58 (m, 3H, C*H*HPh, CH_2Ph), 3.88 (d, *J* = 12.1 Hz, 1H, H-6), 3.73 (d, *J* = 12.2 Hz, 1H, H-6), 3.65 (t, *J* = 9.6 Hz, 1H, H-4), 3.51 (t, *J* = 9.5 Hz, 1H, H-2), 3.44 (d, *J* = 9.7 Hz, 1H, H-5), 2.39 (s, 3H, Me), 1.87 (s, 3H, Ac), 1.29 (s, 9H, *t*-Bu). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.01 (Ac), 149.84, 137.81, 137.72, 136.30, 132.64, 130.20, 128.89, 128.66, 128.55, 128.29, 128.17, 128.15, 128.00, 125.04 (Ar), 87.84 (C-1), 79.30 (C-2), 79.16 (C-5), 77.26 (C-3), 75.79 (C-4), 75.07 (Bn), 74.67 (Bn), 62.00 (C-6), 34.62 (Cq, *t*-Bu), 31.45 (Me, *t*-Bu), 21.14 (Ac), 20.42 (Me). MS ESI-HRMS m/z $[M+Na]^+$ calcd for $C_{33}H_{40}O_6SNa$ 587.2443, found 587.2421.

(2-Methyl-5-tert-butylphenyl) 6-*O*-Acetyl-2,4-di-*O*-benzyl-3-*O*-fluorenylmethoxycarbonyl-1-thio-β-D-glucopyranoside Glc-α-09

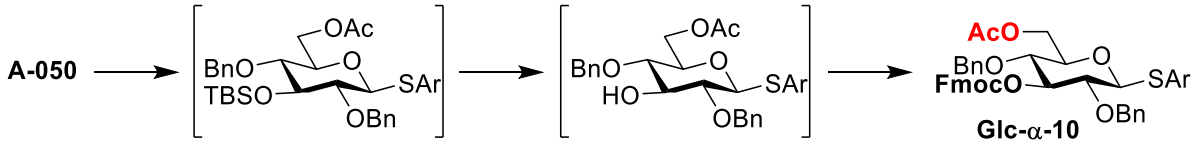


Glc-α-09

To a solution of compound **A-052** (2.2 g, 3.90 mmol) were added 9-fluorenylmethyl chloroformate (2.02 g, 7.79 mmol) and pyridine (0.95 mL, 11.69 mmol) at 0 °C and the mixture was stirred for 2 h at room temperature. After completion the mixture was quenched with 1M aqueous HCl and then diluted with DCM. The organic layer was separated and aqueous phase was extracted twice with DCM. The combined organic layer was dried over $MgSO_4$ and the solvent was evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane/ethyl acetate/DCM = 9:0.5:0.5 to 8.5:1.5:0.5) to afford **Glc-α-09** (2.88 g, 3.66 mmol, 94%). R_f : 0.27 (hexane/ethyl acetate/DCM : 8:2:0.5). $[\alpha]_D^{25}$ = +12.18 (C= 2.85, $CHCl_3$). IR (thin film): υ = 2960, 1747, 1452, 1255, 1226, 1090 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.75 (t, *J* = 7.2 Hz, 2H), 7.63 – 7.58 (m, 3H), 7.39 (dd, *J* = 15.9,

7.9 Hz, 2H), 7.34 – 7.21 (m, 12H), 7.19 (dd, *J* = 7.9, 2.0 Hz, 1H), 7.10 (d, *J* = 8.0 Hz, 1H), 5.31 (t, *J* = 9.0 Hz, 1H, H-3), 4.90 (d, *J* = 11.1 Hz, 1H, C*H*HPh), 4.68 (d, *J* = 9.8 Hz, 1H, H-1), 4.58 (d, *J* = 11.1 Hz, 1H, C*H*HPh), 4.57 – 4.51 (m, 2H, CH_2Ph), 4.45 – 4.36 (m, 3H, H-6, CH_2 of Fmoc), 4.30 (dd, *J* = 11.6, 4.3 Hz, 1H, H-6), 4.24 (t, *J* = 7.3 Hz, 1H, CH, Fmoc), 3.66 – 3.58 (m, 2H, H-4, H-5), 3.54 (t, *J* = 9.5 Hz, 1H, H-2), 2.37 (s, 3H, Me), 1.85 (s, 3H, Ac), 1.27 (s, 9H, *t*-Bu). ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.92 (Ac), 155.09 (Fmoc), 149.86, 143.54, 143.39, 141.43, 141.42, 137.82, 137.36, 136.58, 132.70, 130.09, 129.43, 128.69, 128.54, 128.30, 128.21, 128.03, 127.98, 127.33, 127.30, 125.32, 125.27, 125.06, 120.21, 120.20 (Ar), 88.36 (C-1), 78.92 (C-2), 77.22 (C-3), 76.71 (C-5), 75.98 (C-4), 75.01 (Bn), 74.67 (Bn), 70.12 (CH_2, Fmoc), 66.68 (C-6), 46.87 (CH, Fmoc), 34.60 (Cq, t-Bu), 31.45 (Me, *t*-Bu), 21.11 (Ac), 20.48 (Me). MS ESI-HRMS m/z $[M+Na]^+$ calcd for $C_{48}H_{50}O_8SNa$ 809.3124, found 809.3120.

2-Methyl-5-tert-butylphenyl 6-*O*-Acetyl-2,4-di-*O*-benzyl-3-*O*-fluorenylmethoxycarbonyl-1-thio-β-D-glucopyranoside Glc-α-010



To a solution of compound **A-050** (6 g, 9.42 mmol) in anhydrous DCM (47 mL, 0.2 M) were added acetic anhydride (1.78 mL, 18.84 mmol), and triethylamine (3.94 mL, 28.3 mmol), and a catalytic amount of DMAP (0.115 g, 0.942 mmol, 0.1 eq.) at 0 °C and the mixture was stirred for 1 h at 0 °C. After completion the mixture was quenched with saturated aqueous $NaHCO_3$ and then diluted with DCM. The organic layer was dried over $MgSO_4$ and the solvent was evaporated *in vacuo*. To a solution of the resulting crude product in anhydrous acetonitrile (114 mL, 0.08 M) was added boron trifluoride etherate ($BF_3{\cdot}OEt_2$) (1.27 mL, 10.04 mmol) at 0 °C. and the mixture was stirred for 20 min at 0 °C. After completion the mixture was quenched with saturated aqueous $NaHCO_3$ and then diluted with DCM. The organic layer was dried over $MgSO_4$ and the solvent was evaporated *in vacuo*. To a solution of the crude product in anhydrous DCM (47 mL) were added 9-fluorenylmethyl chloroformate (6.09 g, 23.55 mmol) and pyridine (2.24 mL, 28.3 mmol) at 0 °C and the mixture was stirred for 3 h at room temperature. After completion the mixture was quenched with 1M aqueous HCl and then diluted with DCM. The organic layer was separated and aqueous phase was extracted twice with DCM. The combined organic layer was dried over $MgSO_4$ and the solvent was evaporated *in vacuo*. The crude product was purified by column

chromatography on silica gel (hexane/ethyl acetate/DCM = 9:1:0.5 to 8:2:0.5) to afford **Glc-α-010** (6.2 g, 7.88 mmol, 84% over three steps). R_f : 0.26 (hexane/ethyl acetate/DCM = 9:1:0.5). $[\alpha]_D^{25}$ = +27.39 (C= 2.70, $CHCl_3$). IR (thin film): υ = 3034, 2960, 1748, 1489, 1453, 1254, 1092, 1070 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.78 – 7.73 (m, 2H), 7.60 – 7.55 (m, 3H), 7.40 – 7.35 (m, 2H), 7.30 – 7.19 (m, 13H), 7.13 (d, *J* = 8.0 Hz, 1H), 5.14 (t, *J* = 9.1 Hz, 1H, H-3), 4.95 (d, *J* = 10.8 Hz, 1H, C*H*HPh), 4.67 (d, *J* = 9.8 Hz, 1H, H-1), 4.63 (dd, *J* = 11.0, 2.4 Hz, 2H, 2 x C*H*HPh), 4.50 (d, *J* = 11.1 Hz, 1H, C*H*HPh), 4.40 – 4.31 (m, 3H, H-6, CH_2 of Fmoc), 4.21 (dd, *J* = 12.1, 4.9 Hz, 1H, H-6), 4.14 (t, *J* = 7.1 Hz, 1H, CH, Fmoc), 3.65 (t, *J* = 9.5 Hz, 1H, H-4), 3.62 – 3.54 (m, 2H, H-2, H-5), 2.39 (s, 3H, Me), 2.02 (s, 3H, Ac), 1.29 (s, 9H, *t*-Bu). ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.27 (Ac), 157.19 (Fmoc), 152.28, 145.96, 145.84, 143.91, 140.14, 139.71, 139.19, 135.16, 132.61, 131.85, 131.10, 130.92, 130.71, 130.68, 130.53, 130.44, 129.81, 127.66, 127.61, 122.70 (Ar), 90.75 (C-1), 85.20 (C-3), 81.54 (C-2), 79.23 (C-5), 78.17 (C-4), 77.83 (Bn), 77.26 (Bn), 72.72 (CH_2, Fmoc), 65.88 (C-6), 49.37 (CH, Fmoc), 37.11 (Cq, *t*-Bu), 33.97(Me, *t*-Bu), 23.53 (Ac), 22.98 (Me). MS ESI-HRMS m/z $[M+Na]^+$ calcd for $C_{48}H_{50}O_8SNa$ 809.3124, found 809.3089.

Appendix II: Key Co-author Purblications

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PUBLICATIONS **Reference for Publication List: 1 to 3 at Sogang Univ., 4 to 7 at TSRI; 8 to 26 during Ph.D.** §Co-authors and contributed equally to this work.

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