Exploration of the 1,3-dipolar cycloaddition reaction between azide and phosphorane partners and use in peptide ligation

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Abbreviations

Ac	Acetyl
Ac_2O	Acetic anhydride
AA-OH	Amino acid
BTFFH	Fluoro-*N,N,N',N'*-bis (Tetramethylen)formamidiniumhexafluorophosphat
*Cu*AAC	Copper catalyzed azide-alkyne cycloaddition
DCC	Dicyclohexylcarbodiimide
DIC	N,N'-Diisopropylcarbodiimide
DMAP	4-Dimethylaminopyridine
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
Equiv.	Equivalent
Fmoc	Fluorenylmethoxycarbonyl
FT-ATR-IR	Fourier-Transform-Attenuated-Total-Reflection-IR
HATU	N-[(Dimethylamino)-1H-1,2,3-triazolo[4,5b]pyridin-1-ylmethylene] N-methylmethanaminium, Hexafluorophosphate N-Oxide
HOBt	1-Hydroxybenzotriazole
HPLC	High-Performance-Liquid-Chromatography
MSNT	1-(Mesitylen-2-sulfonyl)-3-nitro-1H-1,2,4-triazole
TAS-F	Tris-(dimethylamino)sulfonium difluorotriethylsilicate
tert-Bu	*Tertiary* butyl
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TMSE	Trimethylsilylethylester
TGS	Target guided synthesis

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Summary

Our aim was to integrate triazole ligation into standard Fmoc-based peptide synthesis and develop a biocompatible triazole ligation. For this purpose phophorane chemistry which has already been integrated into solid phase peptide synthesis by our group for *C*-terminal variation of peptides, was used for the synthesis of peptidyl-phosphoranes. The dissertation deals with use of peptidyl-phosphoranes as a dipole in 1,3-dipolar cycloaddition reaction with azides.

Polymer-supported triphenylphosphane was alkylated with *tert*-butyl bromoacetate and resulting phophoranylidene acetate was acylated with activated amino acid through racemization-free *C*-acylation and the peptide chain was elongated through Fmoc-based solid phase peptide synthesis (SPPS). After *N*-acetylation of the amine terminus of the peptide chain or coupling azido acid at the *N*-terminus, the *tert*-butyl ester was saponified with TFA. Ester cleavage proceeded with instantaneous decarboxylation resulting in peptidyl phosphoranes or azido-peptidyl phosphoranes depending on *N*-terminus of peptide chain.

Peptidyl phosphoranes were first reacted with electron deficient azides such as triflic azide, tosyl azide, 4-nitro benzoyl azide and 4-carboxyl phenyl azide in unpolar solvents at room temperature with complete regioselectivity in favor of 1,5-disubstituted triazole. The reaction, however, needed polar solvents and gentle heating in case of relatively electron rich azides such as benzyl azide, methyl 2-azido acetate and 2-azido-acetamide but still with good yield and complete regioselectivity. Finally, this reaction was used for short peptides ligation by heating peptidyl phosphoranes with azido peptides in polar solvents like THF and DMF. The potential of ligation products, with 1,5-triazole incorporated, to form stable conformations in solution was investigated using 2D-ROESY spectroscopy and molecular dynamic simulations. All the structures consistently indicated a turn-like bent in short peptides.

Azido-peptidyl phosphoranes were subjected to cyclative-cleavage resulting in triazolyl-cyclopeptides. When azido-dipeptidyl phosphoranes were heated in DMF, they delivered exclusively dimeric products. In contrast to this, the azido-tripeptidyl phosphoranes yielded a mixture of monomeric and dimeric triazolyl-cyclopeptides through competition between intra-versus inter-site reactions. To minimize inter-site reactions by using a more rigid resin with higher percentage of cross linker, macroporous polystyrene resin (20% divinyl benzene) was used instead of microporous polystyrene (2% polystyrene) and a major shift towards monomeric product (intra-site reaction) in case of azido-tripeptidyl phosphorane was observed.

Azido-dipeptidyl phopsphoranes, however, still resulted in the formation of dimeric products (inter-site reaction) despite of using the macroporous resin. This observation indicates that azido dipeptides are too short to be cyclized through cyclative cleavage and inter-site cyclization is strongly favored in this case. As expected, azido-tetra-, penta- and octa-peptidyl phosphoranes resulted in monomeric triazolyl-cyclopeptides.

The solid phase chemistry described above was translated into solution phase to broaden its applications in chemical biology through a flexible scheme allowing variation of substituents on phosphorous centre. Wang resin was esterified with bromoacetyl bromide which was then used for alkylation of trivalent phosphane. Peptide chain was then constructed on support bound phosphoranylidene acetate. Finally, soluble peptidyl-phosphorane was released into solution by treatment with TFA and then used in solution phase triazole ligation.

Thus, the triazole ligation method established has potential to introduce β-turn in short peptide chains. Moreover, the reaction is devoid of any metal catalysis and has potential to be developed as biocompatible ligation. In this context soluble peptidyl phosphoranes were synthesized and used in azide-phosphorane triazole ligation in aqueous medium at room temperature.

The peptide cyclization method through triazole ligation completely avoids formation of soluble, non cyclized and oligomeric by-products and is superior to solution phase cyclizations. The method provides an easy access to cyclopeptides with locked *cis*-peptide mimetic.

Zusammenfassung

Ziel dieses arbeit war der Integration der Triazolligation in die Fmoc-basierte Peptidsynthese und die Entwicklung einer biokompatiblen Triazolliagtion. Zu diesem Zweck wurden polymergebundene Phosphorane, die bereits in der Festphasenpeptidsynthese für *C*-terminale Variationen von Peptiden in unserer Gruppe angewandt worden ist, für die Synthese von Peptidylphosphoranen verwendet. In dieser Dissertation ist die Anwendung der Peptidylphosphorane als Dipol bei 1,3-dipolaren Cycloadditionen mit Aziden beschrieben.

Polymer-unterstütztes Phosphoranylidenacetat wurde durch Alkylierung mit *tert*-Butyl-essigester und anschließender Deprotonierung mit Triethylamin erhalten. Das festphasen-gebundene Wittigylid wurde für eine razemisierungsfreie *C*-*C* Acylierung und eine weitere Festphasenpeptidsynthese (SPPS) erfolgreich verwendet. Nach Acetylierung oder Einführung einer Azido-gruppe am N-Terminus wurde die *tert*-Butyl-Schutzgruppe mit TFA abgespalten. Nach der Esterabspaltung und anschließender Decarboxylierung wurden.Peptidylphosphorane oder Azidophosphorane erhalten

Das Peptidylphosphoran wurde mit elektronenarmen Aziden wie Trifluoroacetylazid, Tosylazid, 4-Nitrobenzylazid und Carboxylphenylazid in unpolaren Lösungsmitteln bei Raumtemperatur regioselektiv zu 1,5-disubstituierten Triazolen umgesetzt. Bei elektronenreichen Aziden wie Benzylazid, Methyl-2-azidoacetat und 2-Azidoacetamid konnten 1,5-disubstituierte Triazole synthetisiert werden, allerdings unter Anwendung von polaren Lösungsmitteln bei 60℃.

Letztendlich wurde diese Reaktion für kurze Peptidligationen durch Erhitzen von Peptidylphosphoranen mit Azidopeptiden in polaren Lösungsmitteln wie THF oder DMF verwendet. Das Potential der Ligationsprodukte mit eingebauten 1,5-Triazolen stabile Konformationen in Lösung zu bilden wurde unter Anwendung der 2D-ROESY-Spektroscopie und die Molekulare Dynamische Simulation untersucht.

Die Azido-Peptidyl-Phosphorane wurden durch die cyclische Spaltung zu Tirazolyl-zyklopeptiden umgesetzt. Wenn Azido-dipeptidyl Phosphorane in DMF erhitzt werden, reagieren sie zu Dimeren. Im Gegensatz dazu, durch die Konkurenz zwischen intra-gegen inter-zentrenreaktionen, die Azido-tripeptidyl phophorane führten zu einer Mischung aus monomeren und dimeren Triazolyl-zyklopeptiden.

Ein rigideres macroporöses Harz mit höherer prozentualer Quervernetzung (20% DVB) wurde hier anstatt der bis jetzt benutzten mikroporöser Harz verwendet, um die interzentrenreaktionen zu vermeiden und eine höher Verschiebung der Reaktion

Richtung des monomeren Produktes im Falle des Azido-tripeptidylphosphorans. Azido-dipeptidylphosphorane wurden unter Anwendung des makroporösen Harz in der dimeren Form erhalten. Unsere Beobachtungen zeigen, dass Azido-dipeptide zu kurz für eine zyklische Abspaltung sind. In diesem Fall ist außerdem die interzentrenreaktion streng bevorzugt. Azido tetra-, penta- und octa-peptidyl-phosphorane wurden wie erwartet als monomere Triazolyl-zyklopeptide erhalten.

Die oben beschriebene Festphasensynthese wurde auch in Lösung durchgeführt, um die breite Anwendung der Methode in der chemischen Biologie und die Variation der Substituenten in relevanten Positionen zu zeigen. Das Wang Harz wurde mit Bromacetylbromid verestert, welches dann für die Alkylierung des dreiwertigen Phosphans.verwendet wurde Das peptidische Teil wurde am festphasengebundenen Phosphoranylidenacetat synthetisiert. Letztendlich wurden lösliche Peptidylphosphorane in Lösung gebracht unter Behandlung mit TFA und wurden in Lösung für die Triazolligation verwendet. Demzufolge hat die establierte Triazolligationmethode hat das Potential, die β-Turns in kurze Peptideketten einzubringen. Ein weiterer Vorteil dieser Methode ist, dass ohne Metallkatalyse gearbeitet werden kann. In diesem Zusammenhang wurden lösliche Peptidylphosphorane synthetisiert und in das Azido-phosphoran Triazolligation in wässrigem Medium bei Raumtemperatur eine Anwendung gefunden haben.

Die Zyklisierungsmethode durch die Triazolligation vermeidet Komplett die Bildung von löslichen nicht zyklisierten und oligomeren Nebenprodukten und ist sehr gut geeignet für Zyklisierungen in Lösung. Die Methode bietet einen einfachen Zugang zu zyklischen Peptiden mit formschlüssigen *cis*-Peptidmimetika.

1 Project introduction

1.1 Peptides

Peptides and proteins are involved in many biochemical functions in living organisms and have been shown to affect almost all the essential processes. They are, thus, of prime importance in understanding the physiological functions and pathological conditions and in the development of new therapeutic agents. A variety of peptide-based drugs are currently developed as therapeutics in the treatment of cancer, pain management, viral infections and other diseases.[1]

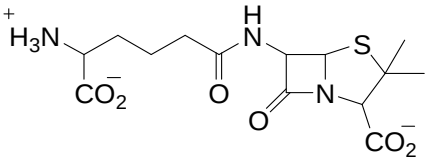
Due to wide range use of peptides in drug development, “peptide synthesis” enjoys prominent status in synthetic chemistry. The field was revolutionized after the introduction of solid phase peptide synthesis (SPPS) by Bruce Merrifield who was awarded with the Nobel Prize for this achievement. SPPS involves attachment of an amino acid to the polymer support through a linker and growth of peptide chain through repetitive coupling of activated amino acids by condensation agents such as DIC/HOBt. The process has been optimized to such an extent that it permits the development of automation and an extensive range of robotic instrumentation is now available. Synthetic peptides are widely used for: a) structure verification of naturally occurring peptides as determined by degradation techniques. b) structure-activity relationship of biologically active peptides and proteins to establish their molecular mechanisms. c) development of new peptide-based immunogens, hormones, vaccines etc.

Having this stated, use of peptides as drugs is, however, limited due to unfavorable properties associated with them, for example, poor oral bioavailability, rapid metabolism and low selectivity. These unfavorable characteristics can be overcome or minimized, at least, by the development of peptidomimetics and/or cyclopeptides. [2]

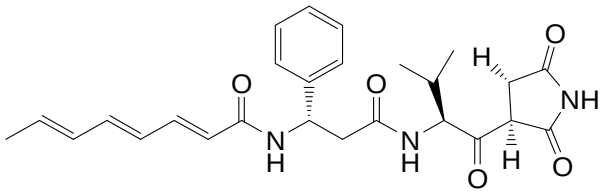
1.2 Peptidomimetics

According to Gante, “a peptidomimetic is a substance having secondary structure as well as other structural features analogous to that of original peptide, which allows it to displace the original peptide from its receptor or enzyme”.[3] Broadly, three distinct classes of peptidomimetics have been described over the years.[4] The first one includes structures that mimic the local topography about an amide bond (amide

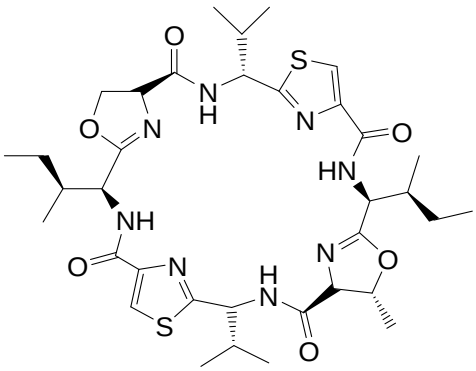
bond isosters, pyrrolinones etc.). These mimetics often match the peptide backbone atom-for-atom but retain the functionalities that are important in contacts with binding sites. Early examples from this class focused on transition state isosters.[5] The second type of peptidomimetics is the functional mimetic, which is a small non-peptide molecule that binds to a peptide receptor.[6] The third type includes those molecules which possess novel templates containing the necessary groups positioned on a novel non-peptide scaffold to serve as a topographical mimetics.[7] More specifically, small-molecule peptidomimetics are very important in drug discovery. There are many approaches for the development of small-molecule peptidomimetics including the use of peptoids and privileged substructures. In addition, introduction of small molecule fragment into a peptide chain, such as aromatic rings or heterocycles, has been and still is a famous target.[8] The motivation for the introduction of a heterocycle in the peptide chain comes from Nature (Fig. **1.1**). Many microbes use catalytic processes to transform conformationally flexible peptide chains into rigidified scaffolds which possess antibiotic or toxin activity. Important examples include the biosynthesis of the β-lactam antibiotics of the pencillin and the cephalosporins and the maturation of vancomycin, a non-ribosomal peptide, where structural modifications to the nascent peptide lead to the physiological function.[9]



Isopencillin N



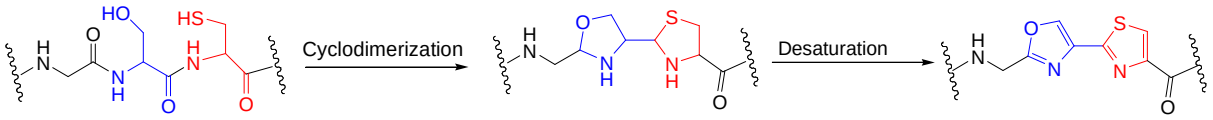
Andrimid



Patallamide A

Figure 1.1, Examples of peptides modification into heterocycles in Nature

An example of modification of the peptide segments into heterocycles is the posttranslational modification of McbA, a 69-residue pro-toxin, which affords the peptide toxin microcin B17 (MccB17).[10] Three different enzymes modify the nascent peptide and create four thiazole and four oxazole moieties from six glycines, four serines and four cysteines through cyclodehydration and desaturation processes (Scheme **1.1**). This two-step process of cyclodehydration and desaturation occurs also in patellamide and streptolysin S (SLS) posttranslational modifications.



Scheme 1.1: Conversion of Gly-Ser-Cys tripeptide into oxazole-thiazole pair through oxazoline-thiazoline intermediate

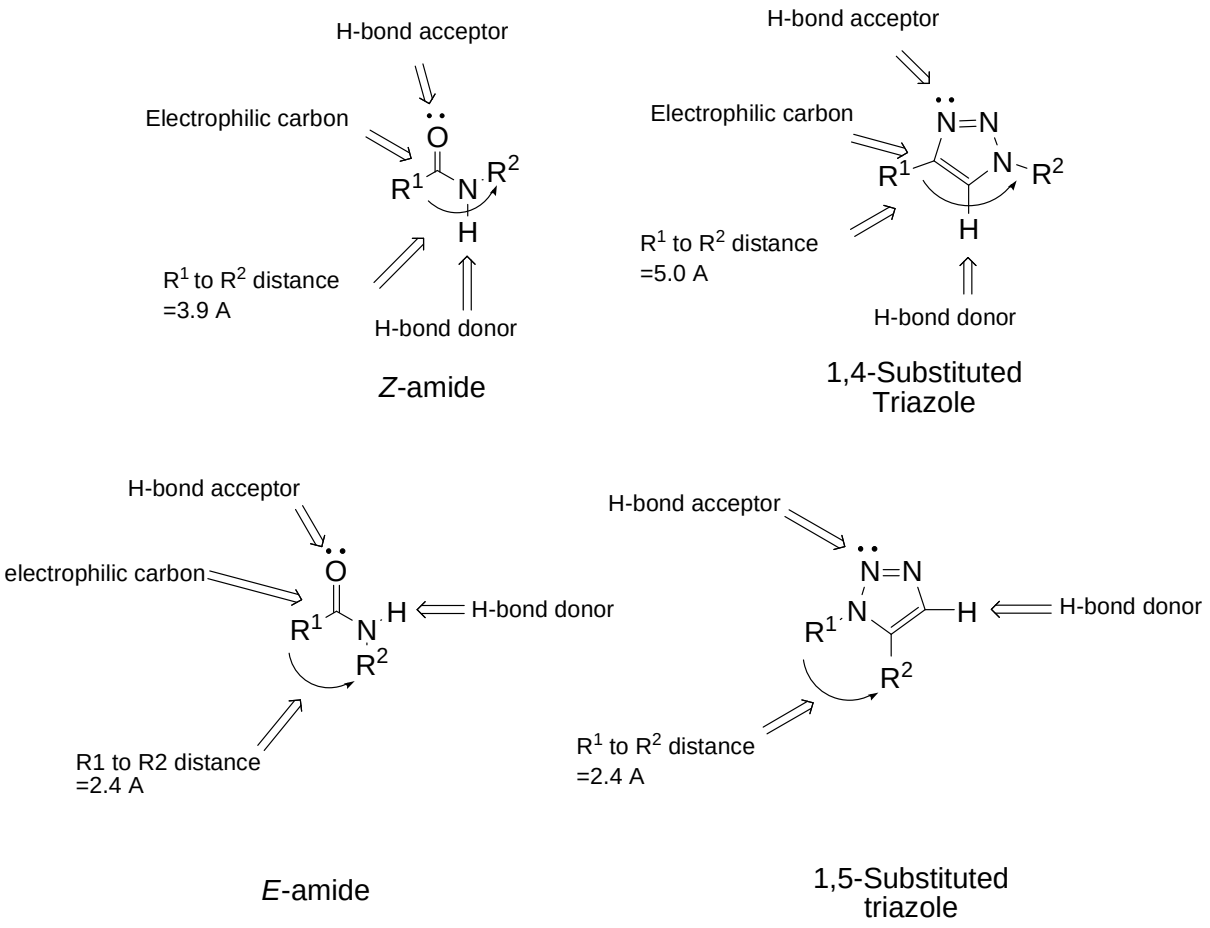
Inspired by Nature, heterocyclic moieties have been inserted into peptide chains in the laboratory, either terminally or flanked by peptide chain. Heterocyles, being drug like structures, enhance interactions with drug targets, in addition to granting conformational rigidity to the peptide. Various heterocylic moieties including furan, thiophene, pyrrole, pyrimidine, thiazole, oxazole, triazine, tetrazole, triazole etc. have been incorporated into peptide chains, synthetically. The triazole ring, however, stands prominent among the heterocycles incorporated into peptides.[11]

1.2.1 1,2,3-Triazole-based peptidomimetics

Triazoles possess a number of desirable features in the context of drug discovery. For example triazoles are stable to acidic and basic hydrolysis as well as reductive and oxidative conditions, indicating their stability. At the same time this heterocycle possesses a high dipole moment (about 5 D) and is supposed to participate actively in hydrogen bonding as well as dipole-dipole and π-stacking interactions. Moreover, it is resistant to metabolic degradation.

The structure of 1,2,3-triazole suggests that it can act as a surrogate for the peptide bond and mimic *cis*- or *trans*- amide bonds depending upon substitution pattern of the ring. The 1,4-disubstituted triazole ring shows similarity with *trans*-amide bond; the lone pair of the nitrogen mimics carbonyl oxygen of the amide, the polarized C(5)-H bond can act as hydrogen bond donor like the amide N-H bond and the C-(4) atom is polarized and resembles the carbonyl carbon, electronically. Since the dipole

moment of the triazole ring is larger than that of amide bond, its hydrogen donor and acceptor properties should be more ehhanced, thus, optimizing its peptide mimicry. The difference, however, between the triazole ring and a *trans*-peptide bond is the distance of substituents.[12]



Scheme 1.2: Hypothesis; how triazoles can act as bioisosters of amide bonds

On the other hand, 1,5-disubstituted triazole rings can mimic *cis*-peptide bonds. The link between substituents as well as hydrogen bond donor and acceptor sites resemble in the two entities, however, they seem to be different in terms of atom polarization.

The notion that 1,2,3-triazole can act as surrogate for peptide bond has also been proven experimentally. One exmple is reported by Raines' group where they replaced Asn113-Pro114 dipeptide segment with 1,5-triazole-Ala and 1,4-triazole-Ala surrogates in bovine pancreatic ribonuclease (RNase A, 124 residues), shown in

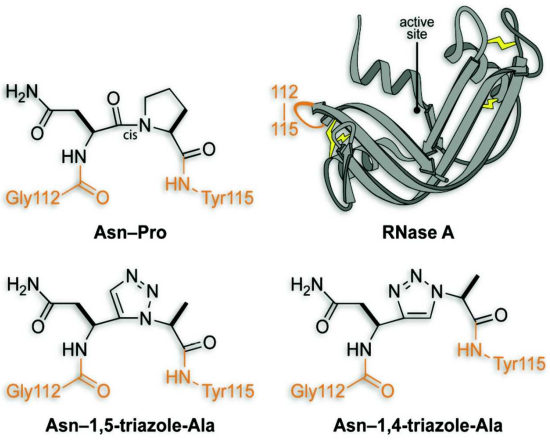


Figure 1.2: Analogy of cis-peptide with 1,5-triazole.

Comparison of the activity of wild type enzyme with semi-synthetic enzymes (triazoles incorporated) indicated that incorporation of the 1,5-triazole in place of *cis*-peptide bond maintains the enzymatic activity. On the other hand replacement with 1,4-triazole led to considerable decrease in the activity of the enzyme.[13]

Being peptide bond isosters, triazoles have been incorporated in peptides by a number of research groups. Different types of peptidic triazoles, synthesized for various purposes are shown in Fig. **1.3**.[14]

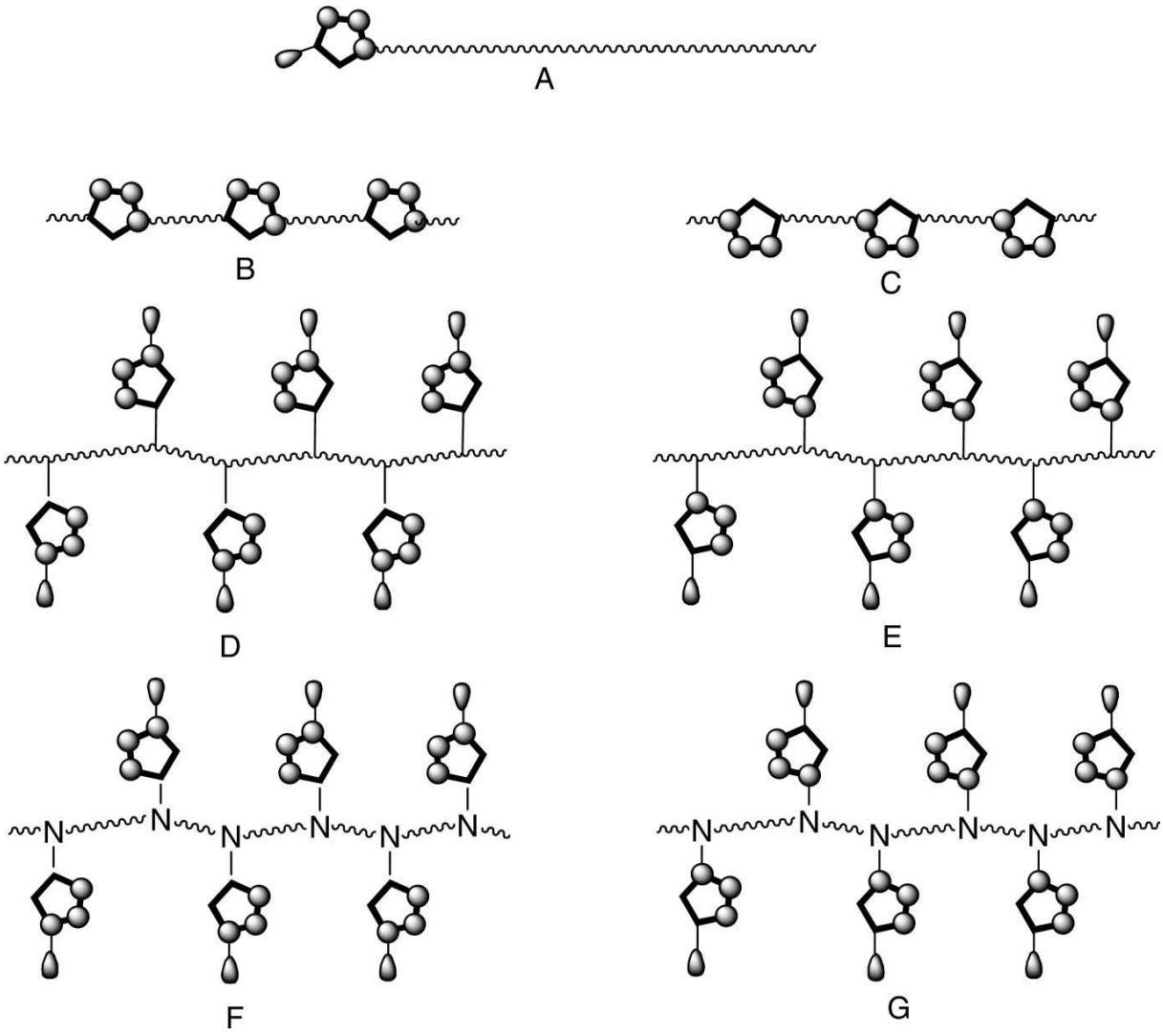


Figure 1.3: Different types of peptidic triazoles synthesized either in solution or solid phase.

Meldal's group, for example, prepared a huge library of triazole-based peptidomimetics *via* the split and mix approach and then screened them on resin against a protease from the pathogen, *Leishmania Mexicana*.[15]

Proline-derived triazoles (Fig. **1.4**) have been synthesized to study the impact of triazole on *cis/trans* proline-amide ratios.[16]

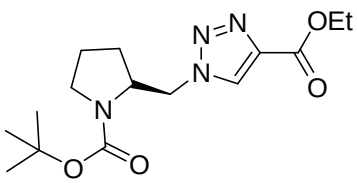


Figure 1.4: Proline-triazole dipeptide.

1.2.2 Triazole-based structural modification of peptides

Local structural motifs in protein loops are crucial for their interaction with other protein receptors and often β-turn mimetics are incorporated into peptide mimetics to stabilize the bioactive conformations. One of the motivations to incorporate triazoles in peptides is to mimic the secondary structure. An illustration of the idea is shown in Fig. **1.5**, where azido and alkyne functionalized peptides were combined to give β-turn mimics. The secondary structures of these peptide turns were examined in chloroform and showed cross peaks.[17]

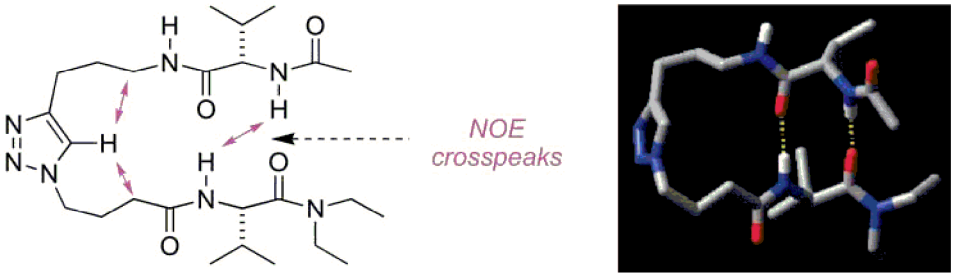


Figure 1.5: Triazole based β-turn mimetic.

Ghadiri's group incorporated triazoles into peptides (Fig. **1.6**) that form four helix bundles and solved the crystal structure of the bundled peptides.[18]

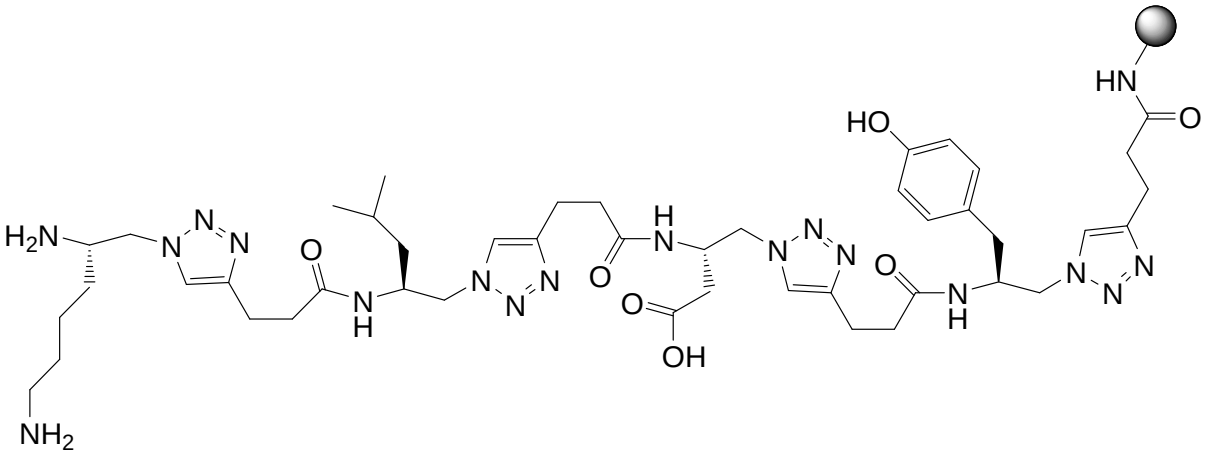


Figure 1.6: Triazole-based peptides shown to assume structures compatible with α-helices.

1.2.3 Triazolyl-cyclopeptides

Cyclopeptides can broadly be classified into homodetic and heterodetic cyclopeptides. Homodetic peptides are connected by amide bonds only while heterodetic peptides involve at least one non-amide link.[19] These non-amide links result either from the final cyclization process or can be incorporated in linear precursor during synthesis. Examples of heterodetic linkages include esters (depsi peptides),[20] thioethers[21] oxazoles[22, 23]and thiazoles.[24] Nature makes use of these linkages in cyclic peptides. Additionally, synthetic cyclopeptides incorporate alkene function[25] (resulting from cross metathesis[26, 27] or enyne cycloisomerization)[28], imidazoles[29], triazoles[30-32] and tetrazoles etc.[33] Triazoles, being amide bond mimetic, are frequently incorporated in the cyclic peptides. Maarseveen's group prepared three triazole analogues of the naturally occurring cyclo-[Pro-Tyr-Pro-Val], a potent tyrosinase inhibitor the synthesis of which had been problematic. Biological activity of the triazole analogues, shown in Fig. **1.7**, was not compromised but enhanced as compared to the naturally occurring cyclopeptide.[31]

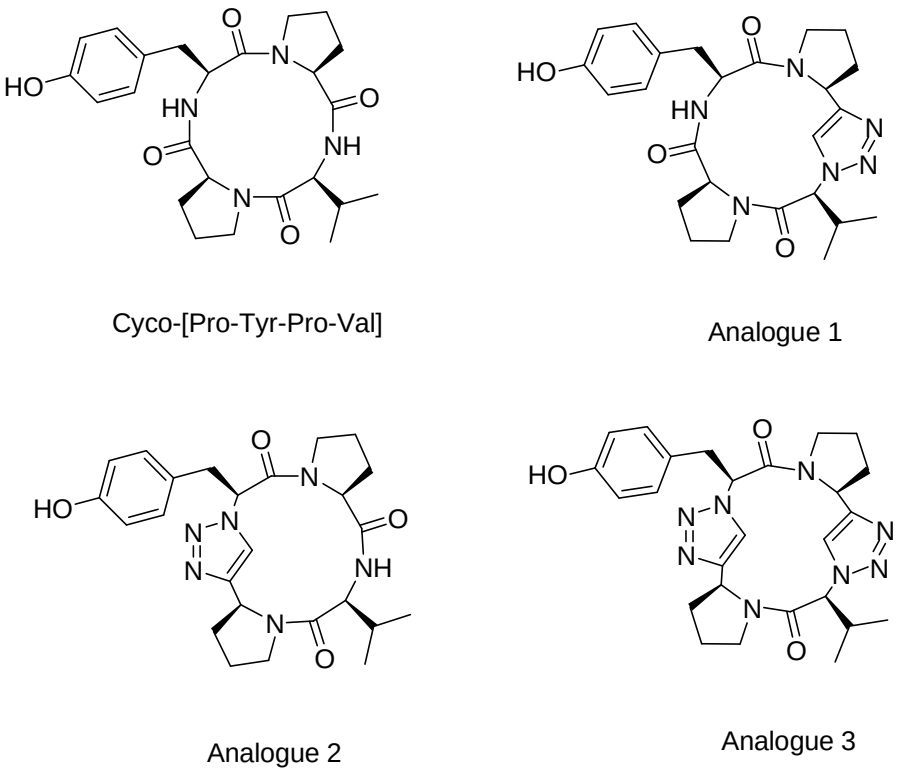


Figure 1.7: Triazole analogues of the naturally occuring cyclic peptide.

Pan's group synthesized the RGD containing triazolyl-cyclopeptides and studied their binding potential with integrin domain.[34]

Ghadiri's group highlighted the utility of triazolyl-cyclopeptidesby synthesizing the triazole analogues of apicidin. Apicidin is naturally occurring cyclotetrapeptide, an inhibitor of the histone deacetylases (HDACs). Apicidin was originally reported to adopt *t-t-t-t* conformation based on NMR measurements in CH_2Cl_2 or pyridine. Later on, it was found to adopt multiple conformations in DMSO with *t-t-t-t* as the major and *c-t-t-t* as the second most populated conformation, in which the tertiary amide bond of the pipecolic acid (Pip) residue adopts *cis* configuration (Fig. **1.8**). Ghadiri et al. addressed the question; which amide configuration is found in the dominant bioactive conformation.

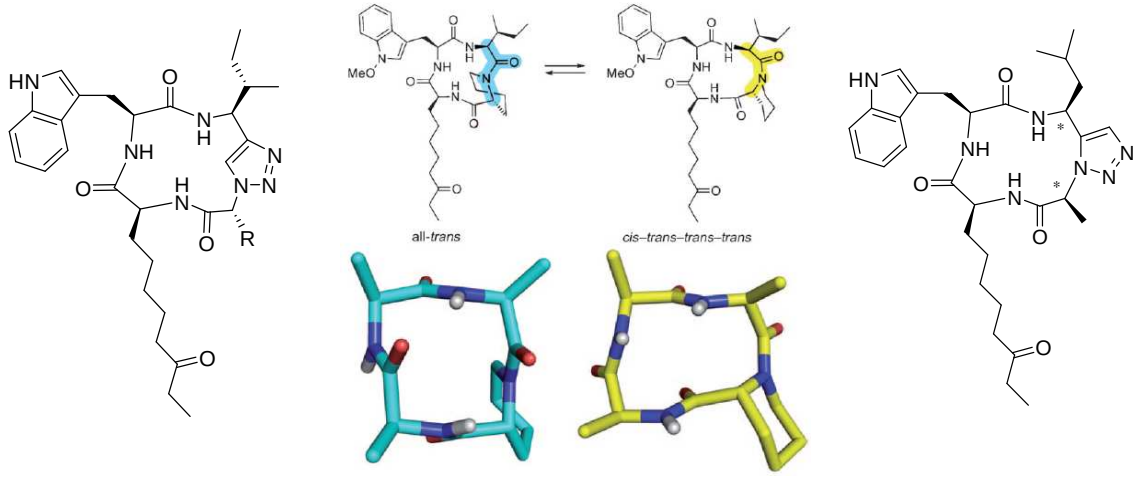


Figure 1.8: Cis-trans isomerization in Apicidin and its conformationally locked triazole analogues.

They replaced the peptide bond of Pip-residue with 1,4- and 1,5-substituted triazoles, *trans*- and *cis*-peptide bond surrogates, respectively. Numerous derivatives were synthesized with residue changes at the positions flanking the triazole. Triazole replacement not only maintained the activity but enhanced it in case of 1,5-triazole replacement, proving thereby the bioactive conformation was *c-t-t-t*.[35]

The incorporation of a triazole ring into the cyclopeptide renders it a distinct, rigid and conformationally homogeneous turn-like structure. Thus, structural homogeneity enables triazolyl cyclopeptides to serve as pharmacophore for rational drug design. This fact was observed by Ghadiri's group when they prepared a library of triazole containing pseudo-cyclo-tetrapeptides, investigated their homogeneity through NMR spectroscopy (Fig. **1.9**) and explored their potential as binders for the somatostatin receptor.[36]

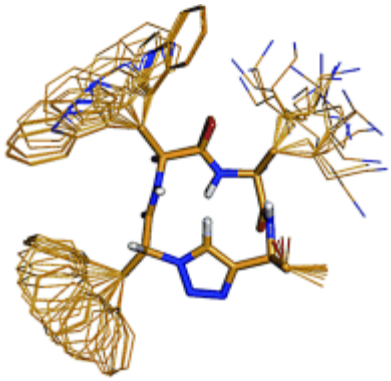


Figure 1.9: Representation of triazolyl-cyclopeptides as a homogeneous structure.

Having recognised the importance of 1,2,3-triazole incorporation into peptides, linear as well as cyclic, the synthetic strategies used for this incorporation come into question. Huisgen's 1,3-dipolar cycloaddition between azides and alkynes, original and its catalyzed versions, delivers 1,2,3-triazole.

1.3 Synthetic strategy leading to 1,2,3-triazoles: azide-alkyne 1,3-dipolar cycloaddition

Huisgen reported a reaction between an azide and an alkene/alkyne leading to 1,2,3-triazole formation, for the first time, and named it as 1,3-dipolar cycloaddition.[37] This report was followed by a series of reports using other dipoles in addition to azides and mechanism of 1,3-dipolar cycloaddition.

The first mechanistic study on 1,3-dipolar cycloadditon was also published by Huisgen.[38] A few years later, Woodward and Hoffman defined the concept of pericyclic reactions and orbital symmetry and developed the interacting π-electron model. Fukui discovered that the chemical reactivity can be explained in terms of interacting frontier molecular orbitals: the highest occupied molecular orbital (HOMO) of one reactant (dipole or dipolarophile) and the lowest unoccupied molecular orbital (LUMO) of the other reactant. According to this theory those orbitals will interact most which overlap best and are closest in energy, as shown in Fig. **1.10**. The reactivity of 1,3-dipoles and dipolarophiles varies and the variation has been explained with the Frontier Molecular Orbitals (FMO) model. Regiochemistry of the reaction depends on which frontier molecular orbital interaction is dominant. If the energy difference between HOMO and LUMO controlled reactions is small, a mixture of regioisomers is formed. The transition state of the cycloaddition reaction has been thoroughly studied and reviewed.

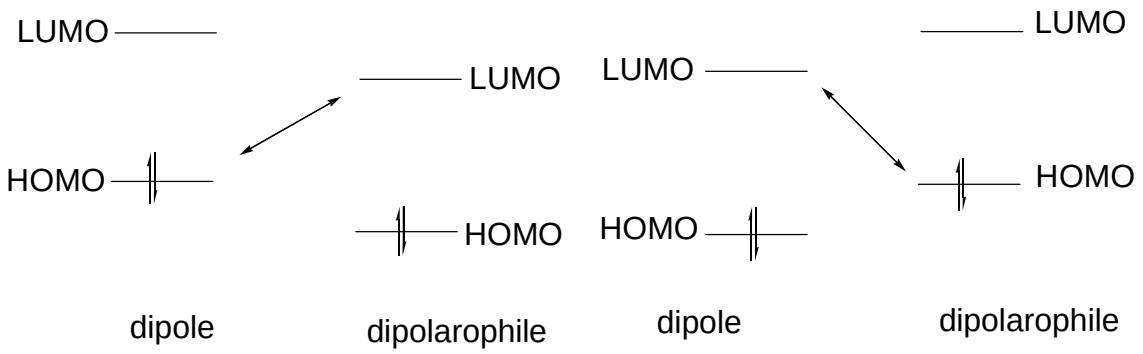


Figure 1.10: Frontier Molecular Orbitals (FMO) control of 1,3-dipolar cycloaddition.

Classically, cycloaddition mechanisms have been grouped into three categories: 1) the concerted mechanism, involving simultaneous formation of both new bonds and experimentally characterized by *cis*- stereospecificity and lack of solvent polarity effects on rate; 2) a stepwise diradical mechanism, involving a diradical intermediate and experimentally characterized by low or nonexistent stereospecificity; and 3) a stepwise zwitterionic mechanism, characterized by variable stereospecificity and a large effect of solvent polarity on reaction rate.

The proposal, by Firestone in the 1970s, of a diradical stepwise mechanism for 1,3-dipolar cycloadditions triggered a hot controversy between this author and Huisgen, who strongly and convincingly supported the concerted mechanism. The latter mechanism eventually emerged as the only mechanism that satisfactorily explains all the experimental observations (such as isotope effects, activation parameters, solvent effects, selectivity, reactivity sequences etc.), in particular the strict stereospecifity that as a rule characterizes these reactions. Now, there is general consensus that 1,3-dipolar cycloadditions follow a concerted path, but not necessarily a synchronous transition state. The most documented and convincing exceptions have been discovered by Huisgen. However, these exceptions are confined to a realm of stepwise via dipolar (zwitterion) intermediate mechanisms and not to that of stepwise via diradical intermediate processes. While several examples of stepwise diradical mechanism are known in the field of Diels-Alder reactions, no convincing experimental example for 1,3-dipolar cycloadditon has been reported so far.

Stepwise mechamism can also be explained by orbital symmetry rules because they allow for a concerted mechanism but don’t forbid a stepwise stepwise course. In the case where the dipole and/or dipolarophile are asymmetrical, the bond representing the lowest HOMO-LUMO gap is more completely formed in transition state. Thus,

increasing the electron donating ability at one end of the dipole (normal demand) or dipolarophile (inverse demand) and increasing electron withdrawing ability at the other end will encourage one bond to be formed faster than the other, this creats partial charges in the transition state and greater the difference in electron demand greater will be the partial charges. In the most extreme cases of difference in electron demand, asynchrony of bond formation becomes so large that a distinct intermediate with formal charge separation and with one of the two bonds formed exists.

Recently, more accurate density functional theory (DFT) has been applied in the mechanistic studies on 1,3-dipolar cycloadditions, and the reactivity and regiochemistry of the cycloadditiond has been predicted on the basis of electron densities derived from quantum mechanical calculations. Using density functional theory calculations, Houk has proposed a general distortion/interaction theory for the 1,3-dipolar cycloadditon reactivity.[39-41] According to this theory differences in the energy ($\Delta E_d^{\ddagger}$) required to distort the 1,3-dipole and dipolarophile into the transition state (TS) controls activation energy for different 1,3-dipoles. Relationship between the activation energy ($\Delta E^{\ddagger}$), distortion energy ($\Delta E_d^{\ddagger}$), and energy of interaction ($\Delta E_i^{\ddagger}$) of the distorted reaction componets for the azide-alkyne cycloaddition is shown in Fig. **1.11**. The activation energy is the sum of destabilizing distortions and stabilizing interactions (($\Delta E^{\ddagger} = \Delta E_d^{\ddagger} + \Delta E_i^{\ddagger}$).

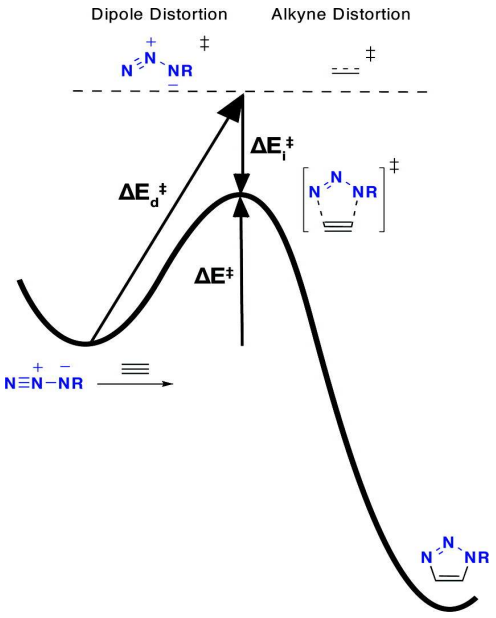


Figure 1.11: Relationship of different energies in azide-alkyne cycloaddition.

This theory elegantly explains the higher reaction rate for cycloalkynes as compared to linear alkynes in strain promoted cycloadditions.[42]

Azide-alkyne cycloaddition reaction has been exploited extensively in organic synthesis since its discovery but its applications increased exponentially when Meldal and Sharpless laboratories discovered that *Cu*-I catalysis of the azide-alkyne cycloaddition reaction results in the exclusive formation of one regioisomer, 1,4-disubstituted triazole.[43, 44] Prior to the use of copper, the reaction required elevated temperature for prolonged reaction time due to high activation barrier (25-26 kcal/mol for methyl azide and propyne) despite being highly exothermic in nature (*ca* 50-60 kcal/mol.). Typically, conditions involve heating in toluene or carbon tetrachloride for 12-48 hours. Furthermore, since the difference in HOMO-LUMO energy levels for alkynes and azides are of same order of magnitude, both dipole-HOMO- controlled and dipole-LUMO- controlled pathways operate and result into a mixture of two regioisomers, 1,4-disubstituted and 1,5-disubstituted triazoles. Some control of regio-specificity was obtainable using electron-withdrawing groups on acetylene in favour of 1,4- regio-isomer and electron-withdrawing groups at azides favoring the formation of 1,5-triazole.

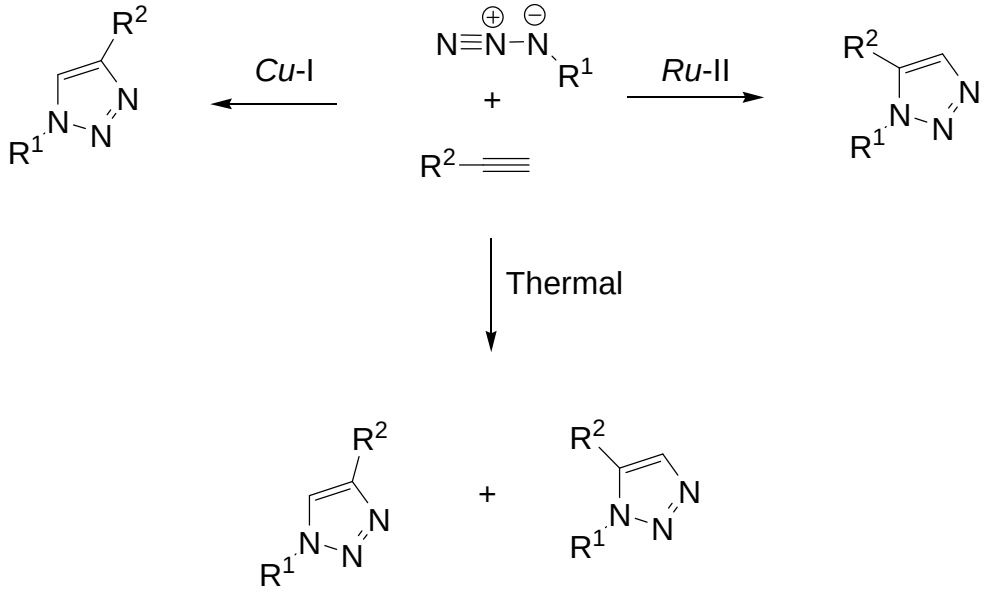
The first publication making use of copper catalysis for triazoles synthesis from alkynes and azides was by Meldal's group.[43] This was an organic-solvent-based procedure that used copper (I) iodide and *N,N*-diisopropylethylamine (DIPEA) in various solvents with terminal alkynes immobilized on a swollen solid support. Copper catalysis was observed not only to speed up the reaction but also resulting into the exclusive formation of 1,4-disubstituted triazole.

1.3.1 Azide-alkyne cycloaddition as "click reaction"

By definition, a click reaction must be of wide applications, giving consistently high yields with a variety of starting materials, should be easy to perform, insensitive to oxygen or water and require simple work up and product isolation.[45] Sharpless' laboratory working on the development of click reaction focused on azide-alkyne cycloaddition, deprived of attention by synthetic chemists due to "azidophobia". They identified this reaction as most useful among the family of 1,3-dipolar cycloadditons because azide is most convenient to introduce and to carry hidden through many synthetic steps. What makes azides unique for click chemistry purposes is their stability towards oxygen, water and the majority of synthetic conditions.

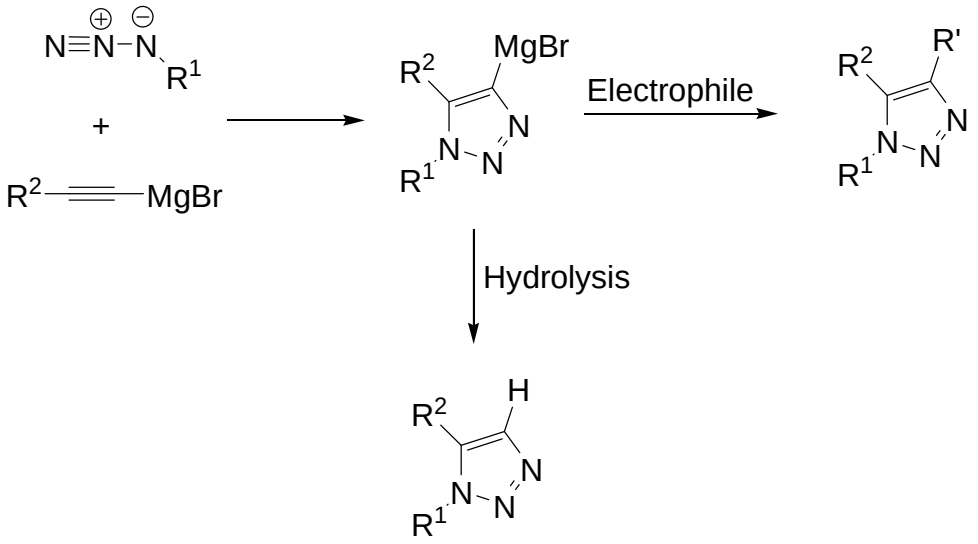
Meldal's report was closely followed by Sharpless et al.'s who presented a more refined and "click" harnessed version of the reaction. The reaction is very straightforward; add azides and terminal alkynes to the mixture of *tert*-butanol and

water (1:1 or 2:1) followed by sodium ascorbate (5-10 mol. %) and copper (II) sulphate pentahydrate solution (1-5 mol. %), seal the flask and stir vigorously at room temperature. The copper (I) is generated *in situ* by reduction of copper (II) with excess of sodium ascorbate.[14, 46] The exact mechanism is quite complex but the reaction is essentially non-concerted and is mediated by copper acetylide. A recent analysis suggests that both azide and alkyne are activated by copper within multinuclear copper-acetylide specie.[47, 48] Enormous rate acceleration (10^7 to 10^8 compared to un-catalyzed conversions), insensitivity to aqueous and oxidative conditions and exclusive regioselectivity have made this reaction widely applicable across disciplines such as bioorganic chemistry, organic synthesis and material and polymer science.[49, 50] In contrast to *Cu*-I, *Ru*-II catalysis of the azide-alkyne cycloaddition was observed to give 1,5-disubstituted triazoles exclusively depending upon ruthenium complex used. Unlike, copper catalyzed azide-alkyne cycloaddition (CuAAC), Ruthenium catalyzed azide-alkyne cycloaddition (RuAAC) is applicable to both terminal as well as internal alkynes.[51, 52] Scheme **1.3** summarizes the Huisgen's azide-alkyne cycloaddition and its catalyzed versions.



Scheme 1.3:Huisgen's azide-alkyne cycloaddition and its catalyzed versions.

In addition to *Ru*-II catalysis, addition of bromo-magnesium acetylide to azide was also reported to give 1,5-disubstituted triazoles exclusively, shown in scheme **1.4**.[53]

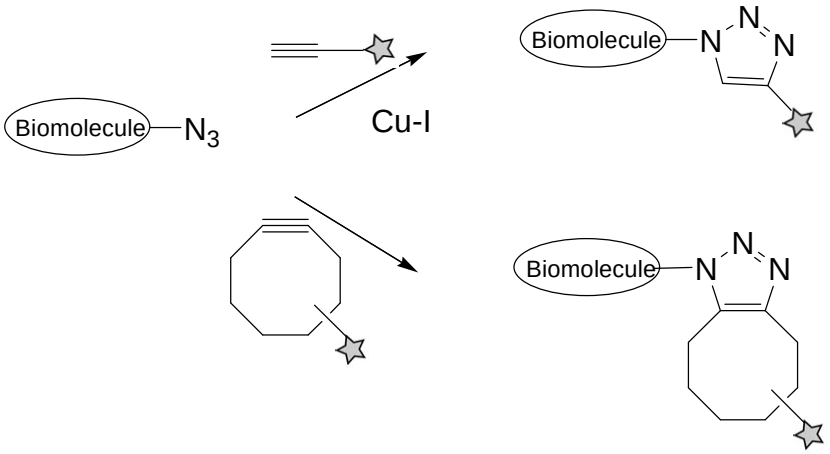


Scheme 1.4: 1,5-Disubstituted and trisubstituted triazoles from bromo-magnesium acetylide.

1.3.2 Biocompatible azide-alkyne cycloaddition

The exceptional utility of CuAAC (copper catalyzed azide-alkyne cycloaddition) stems from the simplicity of azide and alkyne reactants and their stability towards a broad range of synthetic conditions. This makes the reaction suitable for bioconjugation and was first harnessed by Finn and co-workers for this purpose where they attached dyes to the cowpea mosaic virus.[54] To be used for protein modifications, although *Cu*-I has potential to mediate oxidative side reactions with sensitive protein side chains but it can be managed by using short reaction times, stabilizing *Cu*-ligands, and anaerobic reaction conditions. Side effects of using copper as catalyst can be minimized to some extent but copper does pose problems for transformations involving living systems.[55] Bacterial and mammalian cells as well as zebrafish embryos have been experimented with CuAAC conditions. *E. coli* expressing protein-associated azides have been labelled with 100 µM CuBr for 16 h and survived for initial labelling but were no longer able to divide.[56, 57] Considerable cell death occured in the case of mammalian cells using optimized CuAAC conditions, though they survived initially for short period (1h) with low copper concentration (below 500 µM).[58] Zebrafish embryos were also found sensitive to copper. When embryos were treated with 1 mM $CuSO_4$, 1.5 mM sodium ascorbate and 0.1 mM tris(benzyltriazolylmethyl)amine ligand, all the embryos died within 15 minutes.[59] Thus, CuAAC in traditional formulation face limitations for labelling biomolecules in living systems.

To improve the biocompatibility of azide-alkyne cycloaddition, an alternative is to activate the alkynes by means other than metal catalysis. One option is to use alkynes incorporated into a ring as such strained alkynes have been reported to react faster than acyclic alkynes.



Scheme 1.5: . Copper catalyzed v/s strain-promoted cycloaddition.

This effect has already been observed in Huisgen's era, when Wittig and Krebs reported that cyclooctyne, the smallest stable cyclooctyne, reacted "like an explosion" when added to phenylazide.[60] It is calculated that alkyne in such a ring system induces 18 kcal/mol of ring strain, which activates the alkyne to react with azide at faster speed.[61] Bertozzi group made use of the old chemistry and synthesized a biotin conjugate of cyclooctyne to demonstrate labelling of cell surfaces without any observable cytotoxic effects.[62]

Limitations of the initial strain-promoted reactions include a relatively slow reaction rate as compared to CuAAC and loss of regioselectivity. This initial study, however, laid the foundation for the investigation of series of cyclooctynes (shown in Fig. **1.12**) that enabled the detection of azides in living systems through strain-promoted azide-alkyne cycloaddition.[63-68]

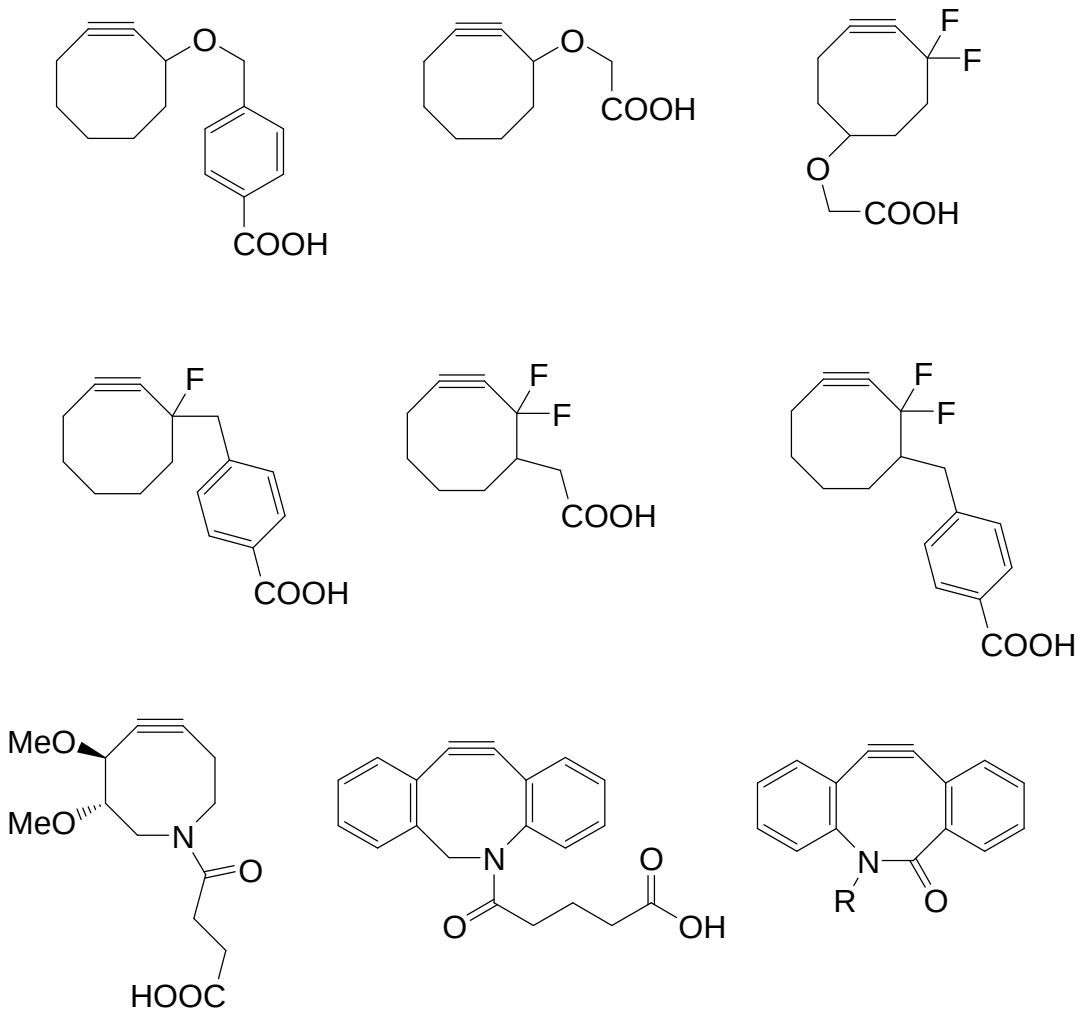


Figure 1.12: Some of the important cyclootynes used for Cu-free click chemistry.

Through labelling experiments reported by different groups in recent years, this chemistry is optimized to the extent that Bertozzi group recently demonstrated *Cu*-free click chemistry using a variety of cyclooctyne probes to label azido sugars in the physiological relevant context of mouse, sketched in Fig. **1.13**.[69]

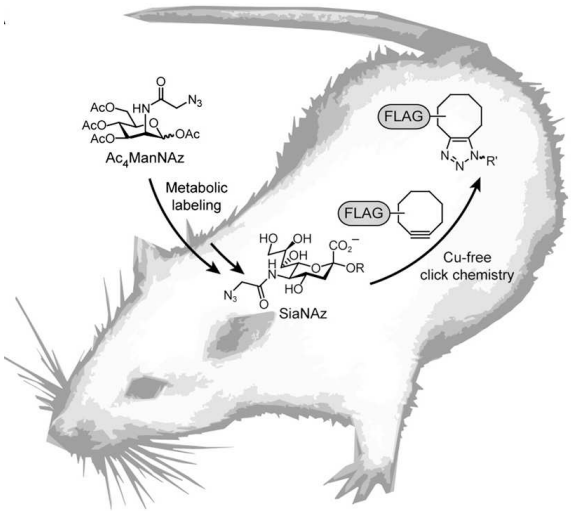


Figure 1.13: Cu-free click chemitry in mice.

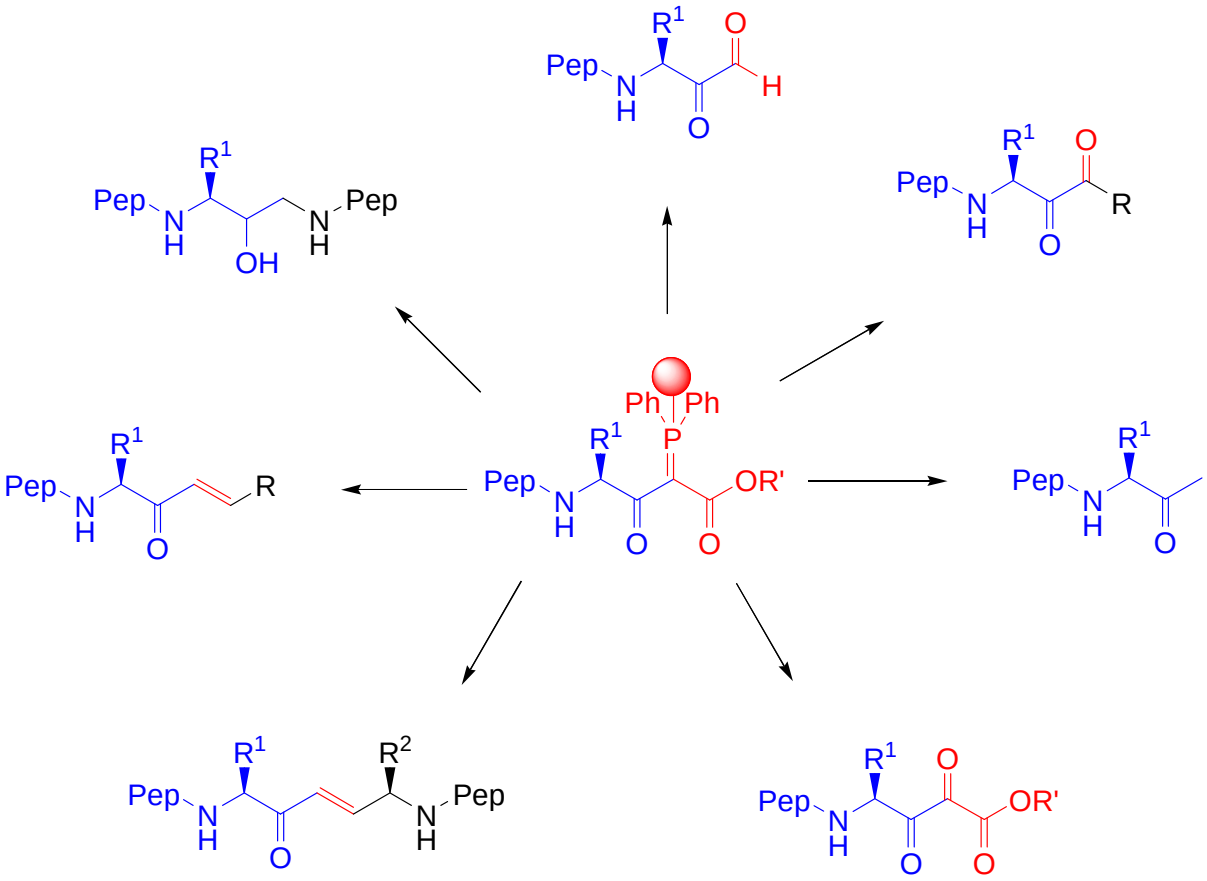
1.4 Project vision: to complement azide-alkyne cycloaddition reaction

The discussion reflects the importance of 1,2,3-triazoles, especially in peptide chemistry and chemical biology in general. Still there are certain limitations of the existing methods *e.g* azide-alkyne cycloaddition reactions need to be metal-catalyzed for regioselectivity which limits their applications in living systems. Strain-promoted cycloaddition avoids metal catalysis but regioselectvity is lost in this case. Moreover, cyclooctynes demand considerable synthetic efforts.

Taking these considerations into account, it is desirable to look for an alternative synthetic method for the target 1,2,3-triazoles. The method, ideally, should avoid use of heavy metal catalysis but retain regioselectivity for the either 1,4- or 1,5-disubstituted 1,2,3-triazole. As 1,2,3-triazole acts as surrogate for the peptide bond, it has broad range of utility in peptide chemistry. It would be an additional advantage of the method if it is integrated into usual Fmoc solid phase peptide synthesis.

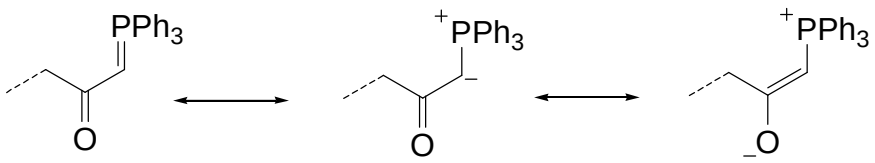
Phosphoranes are mostly known for their utility in Wittig reaction for alkene synthesis. This application dwarfed the exploration of their potential as intermediate to a variety of useful products. Our group has introduced their use for *C*-terminal variation of peptides and a variety of peptidomimetics thereof, summarized in scheme **1.6**.[70-76]

These Wittig ylides (phosphoranyl-ketones) can exist in two mesomeric forms as shown in scheme **1.7**.



Scheme 1.6: Phosphorane-based C-terminal variation in peptides.

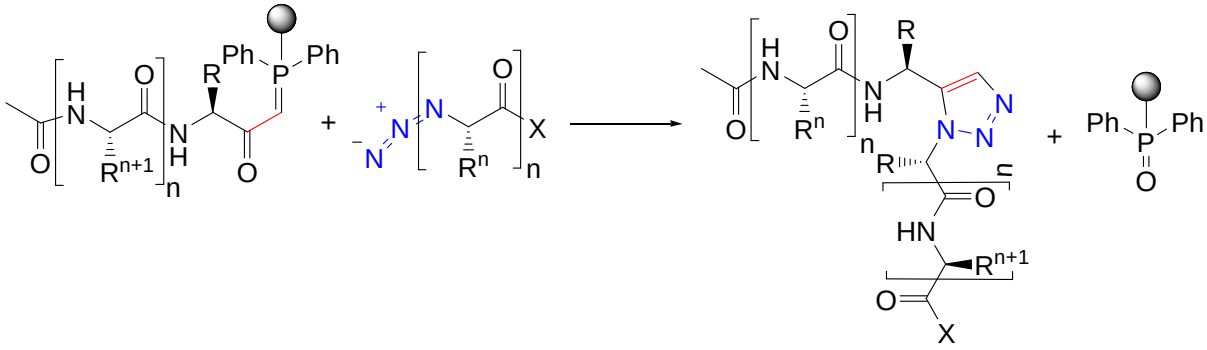
We envisioned the use of these peptidyl-phosphoranes as dipolarophiles in 1,3-dipolar cycloaddition with azide dipoles. We looked into the literature and found only three preliminary examples by Smets group about 40 years back where they had reported reaction between simple α-keto-phosphorous ylides and electron deficient azides.[77-79] This reaction was not investigated further for its potential in organic synthesis and was suppressed by the application of phosphorous ylides in alkenes synthesis.



Scheme 1.7: Mesomeric representation of phosphoranyl-ketones.

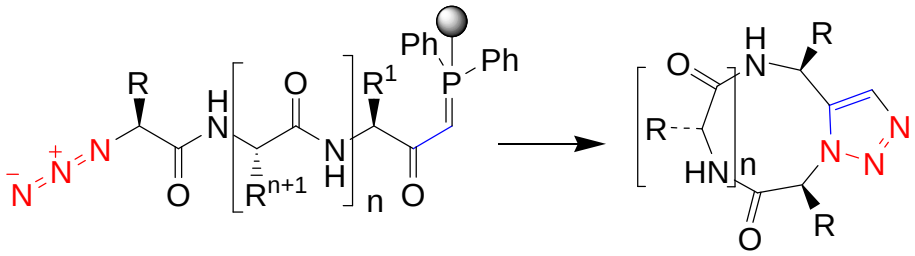
As, we have already integrated phosphorous ylides into Fmoc-based solid phase peptide synthesis. Reactions of peptidyl-ketones with azides will open new synthetic

strategies for access to 1,2,3-triazoles and strengthen their broad applications in chemical biology especially in peptide chemistry. Phosphorane-azide cycloaddition is supposed to deliver 1,5-triazoles as the exclusive regioisomer with simple removable phosphane oxide as the side product, shown in scheme **1.8**. The removal of by-product phosphane oxide would further be facilitated by using peptidyl-phosphorane bound to polymer support. If the azide derivatives used are part of peptide chain then 1,5-triazole would be incorporated internally into the peptide chain.



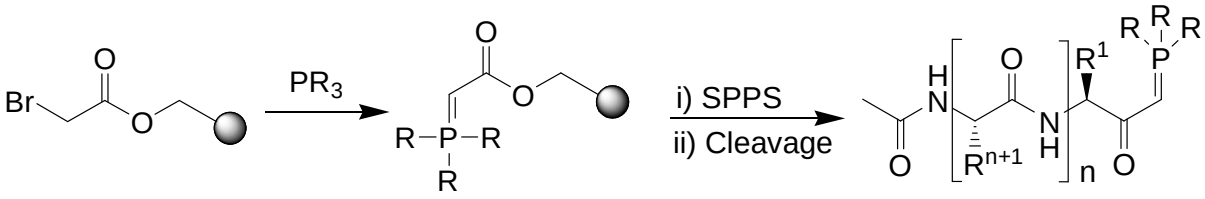
Scheme 1.8: Reaction between peptidyl-phosphoranes and azido-peptides.

Furthermore, if the terminal amino acid of the polymer-bound peptidyl-phosphorane would be converted into an azido acid, heating of such peptidyl-phosphorane is envisioned to deliver triazolyl-cyclopeptides via cyclative cleavage as shown in scheme **1.9**. This reaction would open an easy and elegant route to biologically privileged 1,5-triazolyl-cyclopeptides.



Scheme 1.9: Cyclative cleavage of peptidyl-phosphoranes giving 1,5-triazolyl-cyclopeptides.

As the reaction is metal free and regioselective, it may find applications in labelling experiments. So, it is desirable to translate this solid phase synthesis concept into solution phase chemsitry leading to soluble peptidyl-phosphoranes. Furthermore, this translation would enable reactivity-tuning of peptidyl-phosphoranes by the variation of substitution on phosphorous atom. This vision is summarized in scheme **1.10**.

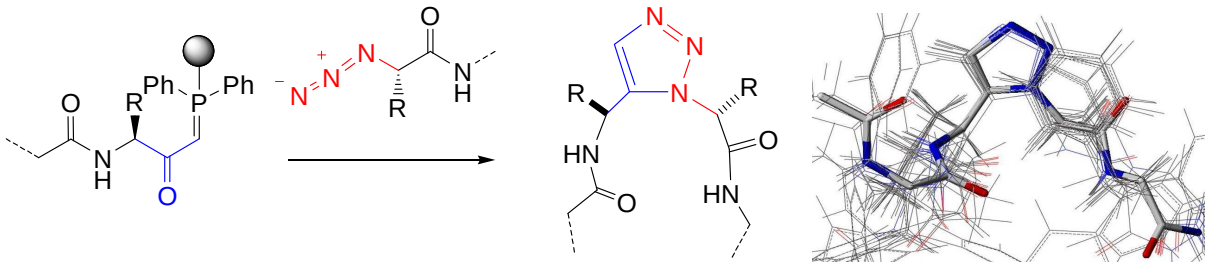


Scheme 1.10: Synthetic strategy for soluble peptidyl-phosphoranes.

These soluble peptidyl-phosphoranes may find applications in the labelling of proteins or other biomolecules with azide incorporated, *in vitro* and *in-vivo*, in the future.

2 Metal-free, regioselective, triazole ligations that delivering locked cis-peptide mimetics

Polymer-bound triphenylphosphane was alkylated with *tert*-butyl or trimethylsilylethyl bromoacetate. The immobilized 2-phosphoranylidene acetate was then acylated with an activated Fmoc- amino acid without racemisation. *C-C* Acylation was then followed by standard peptide synthesis via DIC/HOBt activation. The amino terminus of the peptide chain was capped with acetyl group. The ester group was saponified with trifluoroacetic acid or TSAF depending upon the ester protecting group, *tert*-butyl acetate or trimethylsilyl acetate, respectively. Saponification led to spontaneous decarboxylation giving peptidyl-phosphoranes. 1,5-Disubstituted peptidyl triazoles were obtained regioselectively from the 1,3-dipolar cycloaddition of peptidyl-phosphoranes and azides. When azido peptides were reacted with peptidyl-phosphoranes, peptide turns were formed which contained a conformationally locked *cis* peptide bond mimetic. The racetion conditions varied from room temperature to gentle heating depending on the relative electron density at azide function. Being regioselective and free of heavy metals, this reaction may find broad application in chemical biology and medicinal chemistry.



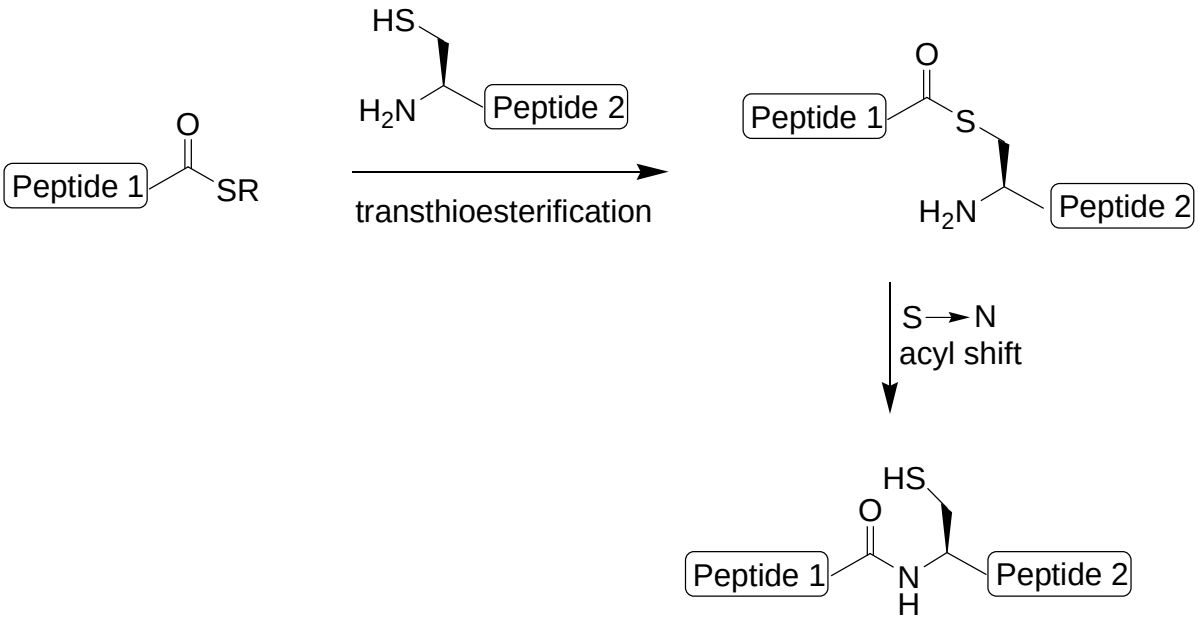
2.1 Chemical ligations

Selectively combining large molecules is a hot topic in chemical biology. Particularly when biopolymers and bioconjugates are involved, there is high need for biocompatible reactions. Chemical ligation reactions have become popular during recent years as they enable the formation of chemoselective linkages under mild, often aqueous reaction conditions and with high yields.[80-83] Chemical ligations are especially useful if they can couple complex functionalized molecules without protecting groups under physiological conditions or in the presence of living cells. Reversible ligations have been used successfully in fragment-based screening and drug discovery.[74] A ligation strategy furnishing conformationally locked, bioactive molecules would be an exciting extension of current methodology. Such a strategy could be especially valuable for the structure-based design and synthesis of inhibitors of protein-protein interactions and peptidyl isomerases.[84]

A toolbox comprising of several powerful chemical ligation techniques already exists and is continuously being extended. Most important constituents of the toolbox for research at the interface between chemistry and biology are native chemical ligation, Staudinger liagtion and triazole ligation.[85]

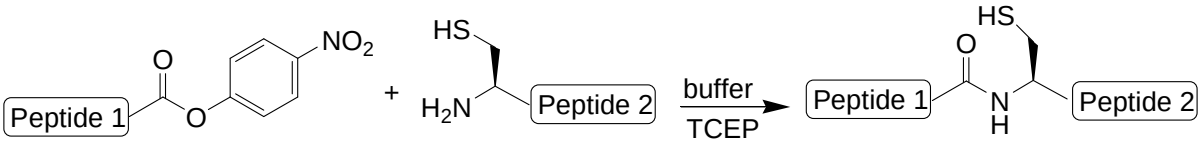
2.1.1 Native chemical ligation

Native chemical ligation is a widely employed technique for the synthesis of large polypeptide chains and small proteins. It forms the basis of modern chemical protein synthesis in fact. The reaction was introduced by Kent et al.[86], although Wieland had observed the condensation of peptide thioesters long time ago.[87] In native chemical ligation (NCL) a peptide containing a *C*-terminal thioester reacts with another peptide having *N*-terminal cysteine residue, in the presence of an added thiol catalyst as outlined in scheme 2.1.



Scheme 2.1: Native chemical ligation.

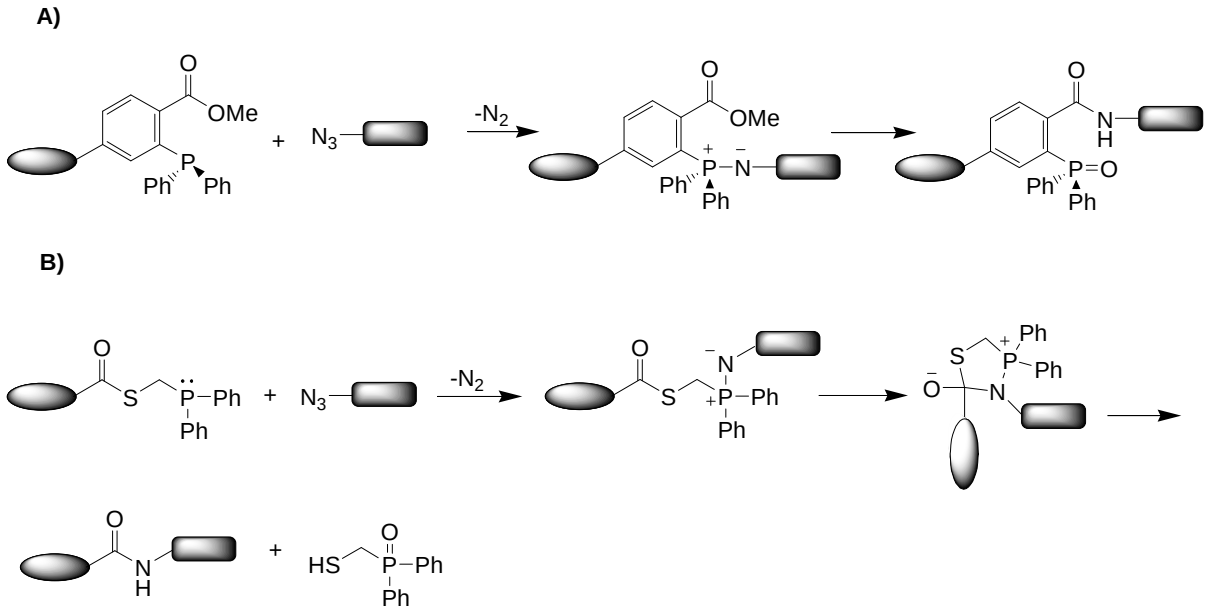
In the first reversible step, transthioesterification occurs to give a thioester-linked intermediate which rearranges under reaction conditions to give native peptide bond at the ligation site. NCL largely relies on the location of Xaa-Cys ligation sites, spaced at intervals no greater than about 40 residues in the target amino acids sequence.[88, 89] Cysteine residues are rare in many proteins or are found only at unsuitable positions. Kent's group introduced another approach that allowed Xaa-Ala ligation sites, with a cysteine residue used in place of alanine. Subsequent desulfurization of the ligation product gives native target sequence.[90] More recently other β-thiol[91] and γ-thiol[92] containing amino acids have been used for NCL followed by desulfurization. Although not as effective as the cystein residue but other thiol-containing ligation auxiliaries can be used which can be removed after synthesis. Modified versions of original NCL also exist, one example shown in scheme **2.2**, but are not widely employed as methods of choice.[93]



Scheme 2.2: Oxo-ester mediated native chemical ligation.

Staudinger ligation

Reaction between a phosphane and and azide to form iminophosphorane (aza-ylide), originally described by Staudinger and Meyer almost a century ago,[94] was harnessed as a highly chemoselective bioconjugation tool by Bertozzi and Saxon.[95] Both reacting functionalities are bioorthogonal to virtually all existing functionalities in biological systems and readily combine at room temperature tolerating an aqueous environment. However, when a traditional Staudinger reaction is performed in water, the iminophosphorane quickly hydrolyzes to form a primary amine. By introducing an electrophilic reactive group within the phosphane, an intramolecular cyclization reaction can out compete the hydrolysis to yield an amide-bond product as shown in scheme **2.3 (A)**. The Staudinger ligation has found many applications ranging from the fluorescent labelling of proteins and DNA, the immoblization of proteins to surfaces, labelling of cell-surface glycoproteins and activity-based proteasome profiling.[63, 96-104]



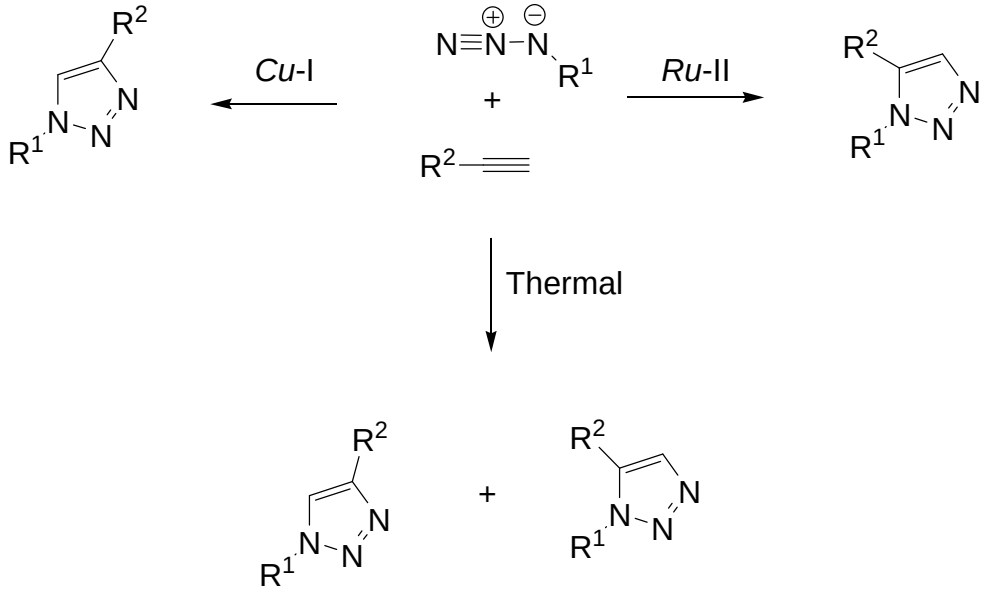
Scheme 2.3: A) Traditional Staudinger ligation. B) Traceless Staudinger ligation.

The Staudinger ligation has been refined to facilitate amide bond formation without the phosphane oxide remaining as part of the product, shown in scheme **2.3 (B)**. This traceless Staudinger ligation, developed by the Bertozzi and Raines laboratories independently, has been used in the preparation of glycoproteins and cyclic peptides as well as in the surface immobilization of azide-labelled proteins.[105-109] Despite

forming a non-native bond, original Staudinger ligation is more commonly applied for bioconjugation.

2.1.2 Triazole ligation

In addition to ligation reactions giving native bond in peptides' perspective e.g native chemical ligation and traceless Staudinger ligation, other ligation reactions resulting in unnatural (non-peptide) backbone structure at the ligation site have permitted the facile preparation of a wide range of backbone-modified peptides and proteins. Among the list of such ligation reactions, triazole ligation is of prime importance as triazole ring can act as surrogate for the *trans*- or *cis*-peptide bond depending upon the substitution pattern.[12, 110] Furthermore, 1,2,3-Triazoles are attractive ligation products as they are thermodynamically and physiologically stable. Accordingly, they are found in several orally administered drugs.[14] 1,3-Dipolar cycloadditions delivering 1,2,3-triazoles from alkynes and azides were first reported by Huisgen[37] and became especially popular after they were found to proceed regioselectively with copper (I) as catalyst and a base yielding 1,4-disubstituted 1,2,3-triazoles.[43, 44] 1,5-Disubstituted 1,2,3-triazoles can be formed regioselectively from alkynes and azides using a *Ru*(II) catalyst.[52]



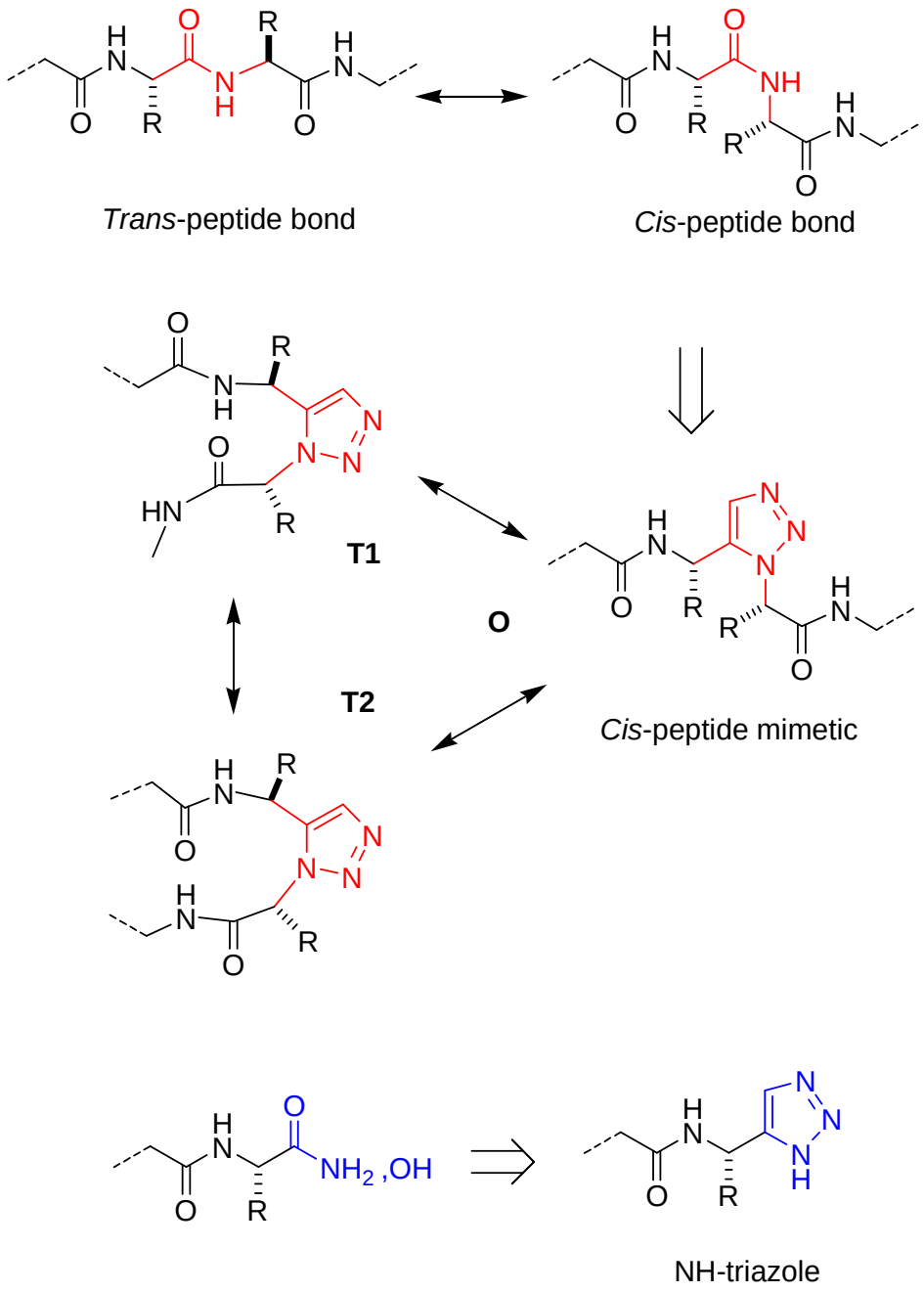
Scheme 2.4: Huisgen's azide-alkyne cycloaddition and its catalyzed versions.

Unfortunately, the published methods for regioselective triazole-ligations, both for the 1,4- and 1,5- isomers rely on the use of heavy metal salts, excluding applications in

the presence of living cells. Bertozzi's group managed to avoid metal catalysis by making use of strain promoted 1,3-dipolar cycloaddition reactions between azides and cyclooctynes at the cost of regioselectivity. Moreover, cyclooctyne precursors require much more synthetic effort than acyclic alkynes. Therefore, the development of a metal-free, regioselective, and biocompatible triazole ligation method was considered as highly attractive target.[64, 65]

2.2 Aim of the present work

Our aim was to establish the direct incorporation of 1,5-disubstituted triazoles into peptides starting from standard amino acid building blocks. A reaction yielding these products would open a straightforward access to locked *cis*-peptide mimetics or "clack-peptides" (Scheme **2.5**).[111] The obtained 5-peptidyl-(1*H*-1,2,3-triazol-1-yl)-peptides should allow to study and exploit the effects of *cis*-peptide geometry, either for open-chain conformations (O) or for peptide turns (T1 and T2) induced by the *cis*-peptide bond (Scheme **2.5**).[13, 16, 112-114]

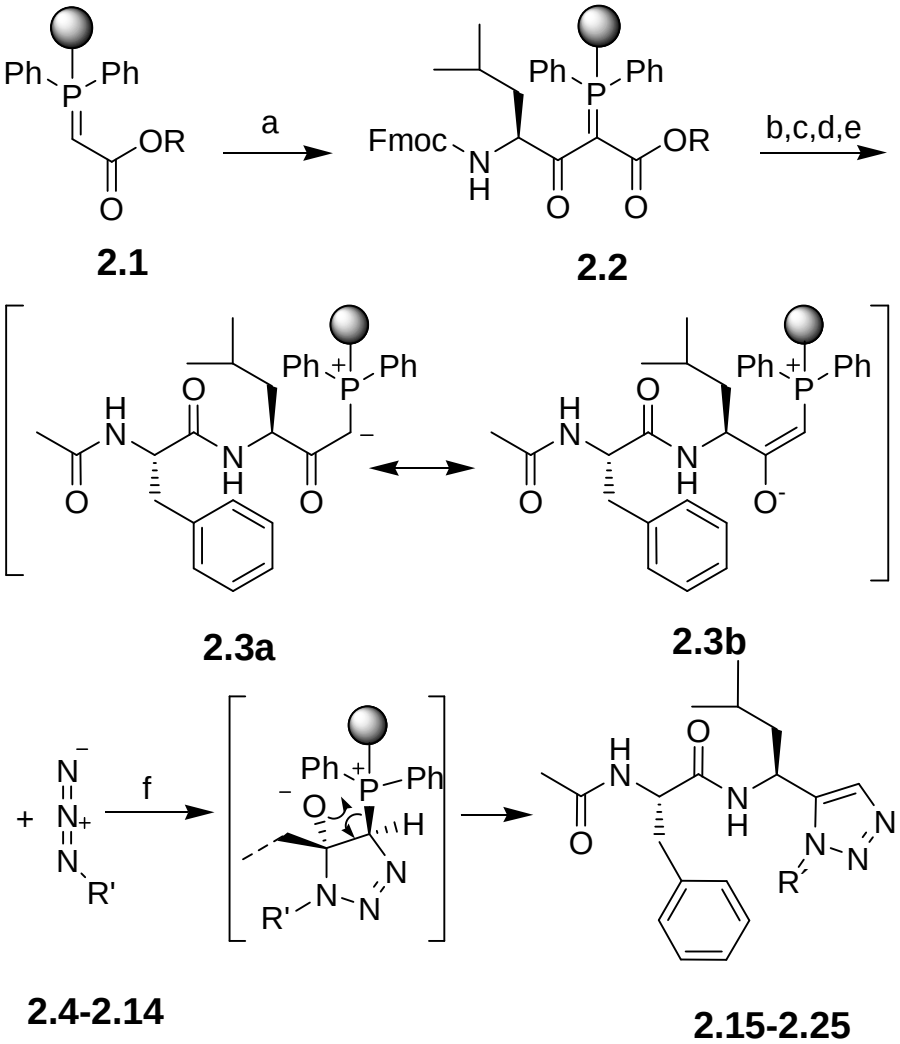


Scheme 2.5: Replacement of a peptide amide bond with 1,5-disubstituted 1,2,3-triazole furnishes a 5-peptidyl-1H-1,2,3-triazolyl peptide, a “locked cis-peptide mimetic” or clack-peptide. cis-peptide mimetics can be expected to populate either open-chain conformations (O) or turn structures (T1 or T2). 5-Peptidyl-1H-1,2,3-triazoles (NH-triazoles) are expected to constitute an enzymatically stable C-terminus with H-bonding potential similar to the wild-type. Moreover, the 1-unsubstituted 5-peptidyl-1H-1,2,3-triazoles which have not been synthetically prepared unitil today, might serve as proteolytically stable isosteres of the C-termini of the peptides and proteins (bottom).

❖

2.3 Results and discussion

We envisioned that 5-peptidyl-(1*H*-1,2,3-triazol-1-yl)-peptides should be accessible by solid phase synthesis starting from the polymer-supported phosphoranylidene acetate **2.1** (Scheme 2). Racemization-free C-acylations of polymeric phosphoranylidene acetates with Fmoc-protected amino acids have been demonstrated recently to yield Fmoc-amino acyl phosphoranylidene acetates **2.2**.[71, 115] Following Fmoc-cleavage of **2.2**, the free amino group could be elongated by standard peptide chemistry. Deprotection of the trimethylsilylethyl (TMSE) or *tert*-butyl ester, respectively, was found to furnish the decarboxylated peptidyl phosphorane **2.3** as single product.

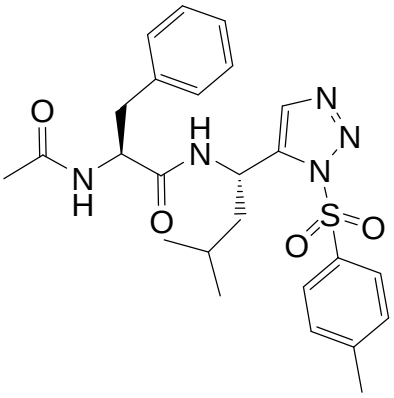
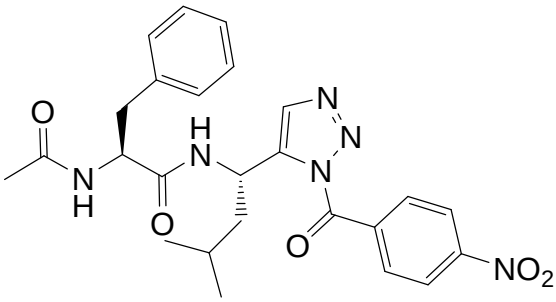
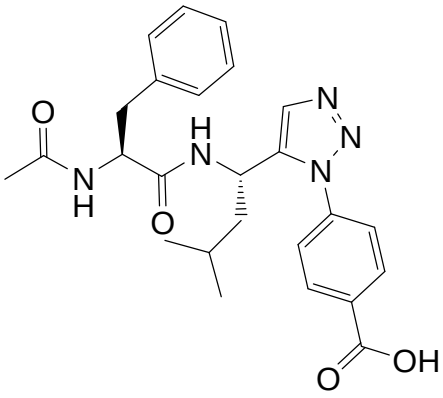
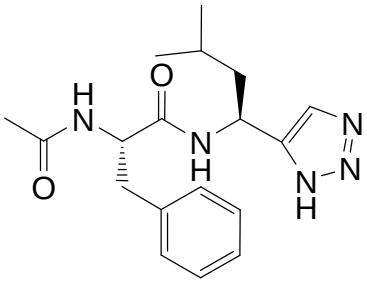


Scheme 2.6. Reaction conditions: a) Fmoc-Leu-OH and MSNT, lutidine in CH_2Cl_2 or: BTFFH, DIPEA, DMF (R = TMSE or *tert*-butyl, respectively), 14 h; b) 20% piperidine/DMF; c) Fmoc-Phe-OH, DIC, HOBT, DMF, 2h; d) 20% Ac_2O/DMF; e) TSA-

F/DMF or TFA/CH_2Cl_2 (R = TMSE or *tert*-butyl, respectively); f) Azides **2.4-2.14**, CH_2Cl_2, or THF.

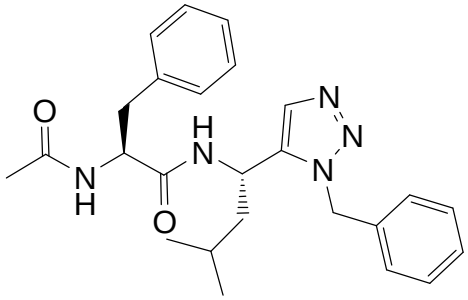
In this chapter we report reactions of peptidyl phosphorane **2.3** with azides **2.4-2.14**. At room temperature polymer **2.3** reacted smoothly with 4-toluene-sulfonyl azide **2.4** furnishing the 5-peptidyl-(1*H*-1,2,3-triazol-1-yl)-tosylate **2.15** in high yield. The product was obtained in excellent purity solely by washing the resin followed by evaporation of the solvent. Triphenylphosphane-oxide as the second product remained attached to the polymer. Neither traces of the 1,4-substituted triazole or of epimerized by-products were detected. Product formation can be rationalized by 1,3-dipolar cycloaddition reaction considering the phosphorous ylide **2.3a** as phosphonium enolate in accordance with the resonance structure **2.3b**. The cyclic intermediate can be formed either via a concerted or step-wise mechanism. To our best knowledge this reaction of peptidyl phosphoranes has not been reported to date and is also the first account of a dipolar cycloaddition of phosphoranes on polymeric support. Earlier works have reported 1,3-dipolar cycloadditions with electron-rich olefins and the transition states of such reactions have been calculated explaining the observed 1,5-selectivity by interaction of the olefin HOMO with the azide LUMO.[77-79, 116, 117] This reaction mode suggests a negative effect of azide electron density on the reaction rate. Thus we investigated the scope of this reaction by using azides with varying electron-density (Scheme **2.6**, Table **2.1**). Azides substituted with electron-withdrawing substitutents such as sulfonyl and acyl reacted rapidly in unpolar solvent as dichloromethane and yielded the 1,5-disubstitued triazolyl-sulfonamides, and –carboxamides, respectively. 4-Nitrobenzoyl-azide **2.6** led to the 1-acyl-1,2,3-triazole **2.16**. The aromatic azide, 4-azido benzoic acid **2.7** required prolonged reaction time to yield the 1-aryl-5-peptidyl triazole **2.17** with high purity and yield. Employing trifluoromethanesulfonyl azide **2.5** (triflylazide, Tf-N_3) as dipole furnished directly 5-peptidyl-1H-1,2,3-triazole **2.18** during aqueous work-up. Aliphatic azides such as benzylazide **2.8**, methyl 2-azido-acetate **2.9**, and 2-azido-acetamide **2.10** did not react in dichloromethane even after several days. Polar solvents such as THF or DMF, however, enabled efficient and clean reactions at slightly elevated temperatures furnishing triazoles **2.19-2.21**. The observed reactivity trends of azides as well as the effect of solvent polarity are in accordance with a polar transition state and with a LUMO-controlled 1,3-dipolar cycloaddition-reaction.

Table 2.1: Selected triazole ligation products

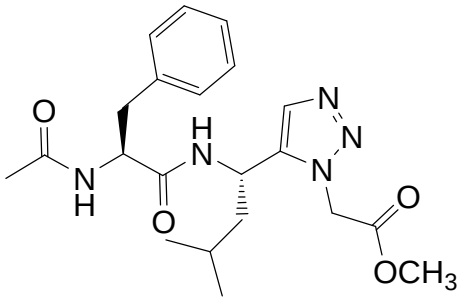
Entry	Products	Yield (%)	Purity[c] (%)
1	 **2.15**	86[a]	96
2	 **2.16**	95[a]	74[d]
3	 **2.17**	80[a]	92
4		88[a]	92[d]

❖

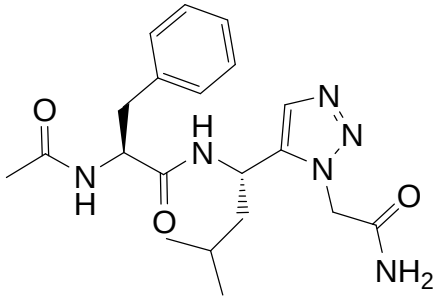
2.18

5  62[b] 88

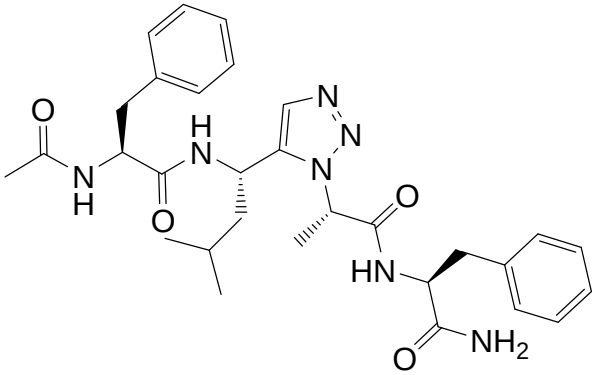
2.19

6  70[b] 91

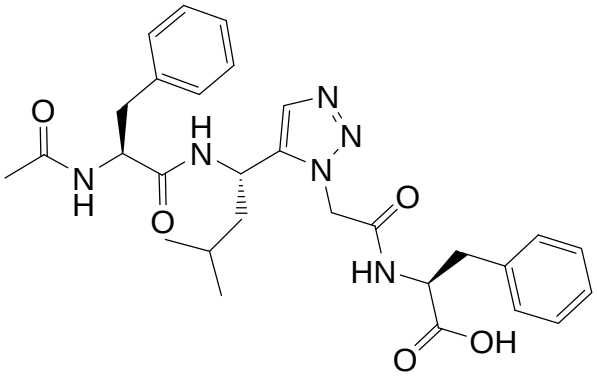
2.20

7  72[b] 95

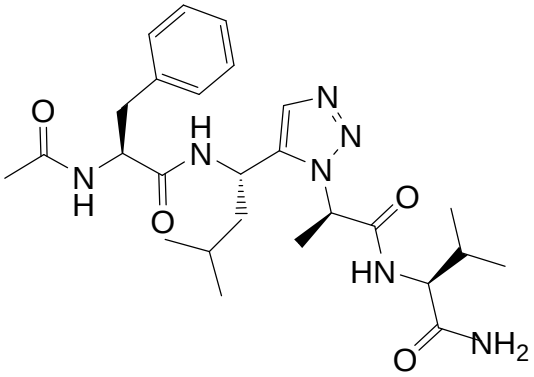
2.21

8  68[b] 82

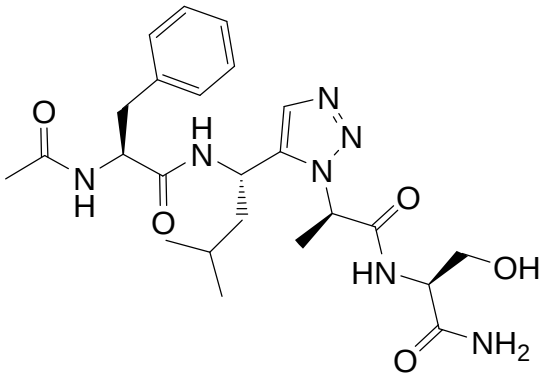
2.22

9		72[b]	96

2.23

10		74[b]	97

2.24

11		73[b]	98

2.25

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a. Reaction in CH_2Cl_2 at RT.

b. Reaction in THF at 60 ^oC.

c. Purities determined at 220 nm. Unless noted purities reported after HPL-chromatography. Compounds **2.15** and **2.17–2.21** were > 80% pure in crude products after trituration with acetonitrile.

d. Crude purity; compound **2.16** degraded partially to **2.18** during purification or storage.

Moreover, the polymer-supported peptidyl phosphorane **2.3** harbours the potential for a direct access to 5-peptidyl-(1,2,3-triazol-1-yl)-peptides. To verify this hypothesis 2-azido acids were synthesized from unprotected amino acids via diazo transfer reaction employing trifluoromethane-sulfonyl azide **2.5**.[118] The 2-azido-peptide amides **2.11-2.13** were prepared on Rink resin using the respective 2-azido acids in the final coupling step while 2-azido-peptide acid **2.14** was prepared on 2-chlorotrityl resin. Azido-peptides were reacted in excess with the peptidylphosphorane under the conditions defined for electron-rich azides and the desired peptidyl-triazolyl-peptides were isolated by reversed phase HPLC in good yields.

All novel products were purified by column chromatography – if necessary – and were characterized by HR-MS and by full assignment of their ^{1}H-NMR spectra. The potential of peptidyl-triazolyl peptides to form stable conformations in solution was investigated with respect to product **2.22** in DMSO using NMR spectroscopy. A complete assignment of all ^{1}H and ^{13}C resonances was obtained from a set of two-dimensional spectra. Based on this assignment, distances were extracted from a 2D-ROESY experiment. The molecule was well structured and more than 30 distances could be obtained that were used as distance restraints in a molecular dynamics simulation. Arrays of structures were calculated by a simulated annealing protocol. An overlay of ten calculated structures indicated consistently a turn-like bent of the triazole-flanking amino acid residues (Figure 2.1) which was characterized by the inward orientation of the -1 amide carbonyl and the +1 amide NH (numbering in N to C-terminal direction). Thus, the preferred conformation of peptidyl-triazol-1-yl-peptide **2.22** in DMSO solution resembled the T2 structure in Scheme 1 most closely. Precise rotational angels with their corresponding standard deviations are summarized in the supplemental. More detailed studies concerning the effects of elongated peptide tails, of amino acid stereochemistry and cyclization of 1,5-triazolyl peptides are under way and will deliver a more comprehensive picture of the potential of this compound class in conformation control.

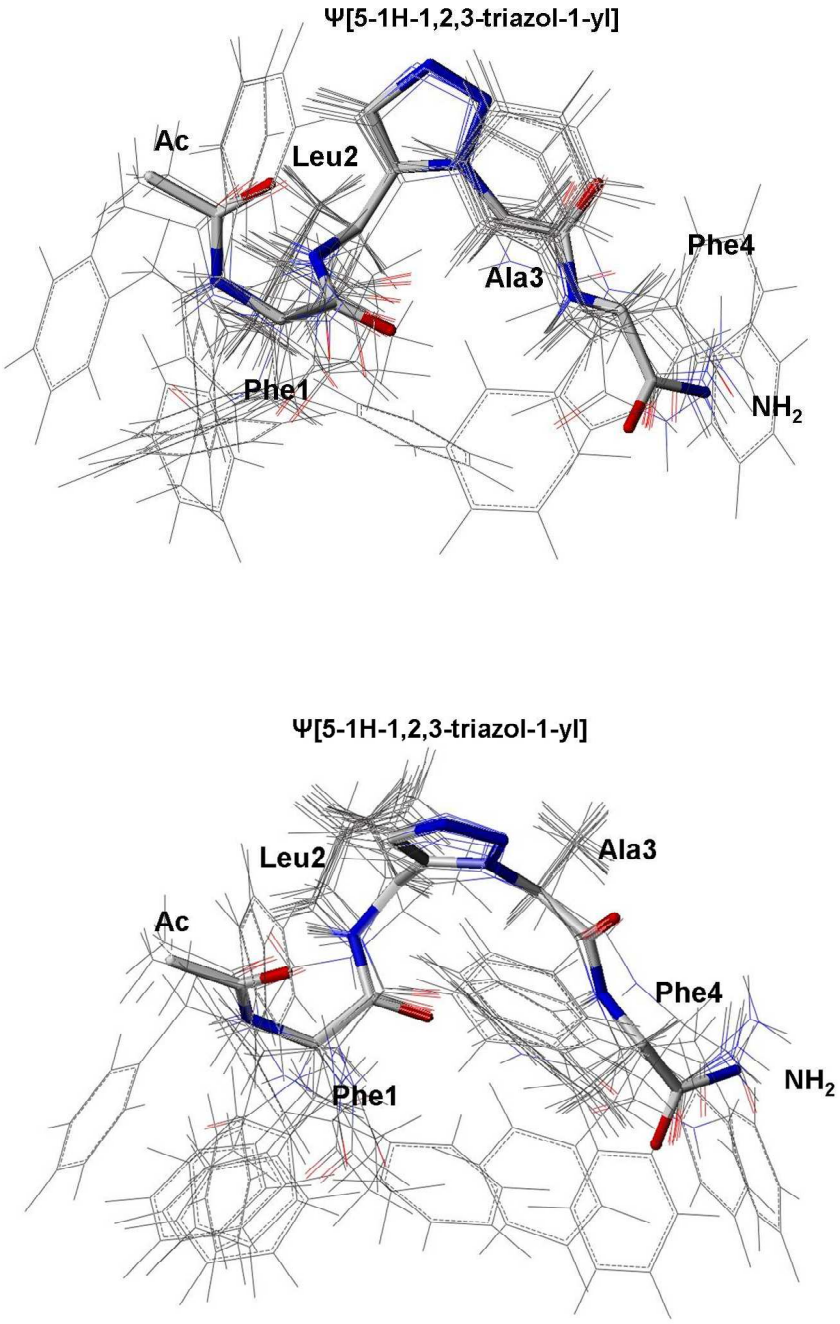


Figure 2.1: The solution structure of 5-peptidyl-triazol-1-yl peptide 2.22 was determined in DMSO by ROESY-NMR. Measured average distances were used as distance constraints in a molecular dynamics simulation. Shown is an overlay of 10 structures obtained by simulated annealing. All structures display a bent-like turn structure. Amino acids flaking the triazole motif are relatively rigid with the carbonyl of the -1 amino acid and the NH of the +1 amino acid pointing inwards in all ten structures.

2.4 Summary and outlook

In summary, we have presented the first metal-free and regioselective triazole ligation. The reaction has a broad scope and, most relevantly allows full integration into peptide synthesis avoiding the use of tediously prepared amino acid alkynes. The novel triazole ligation can be employed to yield products with carefully controlled conformations.

2.5 Experimental section

Reagents and general methods. Fmoc Rink amide resin (0.7 mmol/g), triphenylphosphine polystyrene resin (1.6 mmol/g), Trityl resin (1.6 mmol/g), Fmoc-amino acids and all other reagents were purchased from commercial suppliers, unless synthesis stated. Anhydrous solvents were purchased and stored over molecular sieves. Fmoc solid-phase peptide synthesis was conducted in plastic syringes equipped with PE filters. Loading was determined by photometric methods after Fmoc cleavage. Solid phase reactions were monitored through FT-ATR-IR spectra of the resins with a Bruker Vector 22/Harrick SplitPea ATR unit. Cleaved products were analyzed by an analytical HPLC column (5 µm, 250 x 2 mm, Nucleosil 100 RP-C18) with detection at 220 nm and 254 nm. Eluents A (0.1% TFA in water) and B (0.1% TFA in acetonitrile) were used in a linear gradient (20% B 100% B in 45 min.). Compounds were purified on a semipreparative HPLC column (10 µm, 250 x 20 mm, Grom-SIL 300 ODS-5 ST RP-C18) employing individual gradients derived from analytical runs (eluents A and B). LC-MS were recorded on an Agilent 1100 series chromatography workstation (Agilent Technologies) equipped with a single quadrupole mass spectrometer and electrospray ionization (ES). HRMS measurements were conducted with an Agilent 6220 ESI-TOF mass spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE 300 MHz, or 600 MHz instruments and chemical shifts (δ) were measured in parts per million (ppm) relative to trimethylsilane (TMS).

2D NMR spectrocopy

All measurements were performed using 5 mm sample tubes. NMR spectra were recorded on a Bruker DRX600 NMR spectrometer using a 5 mm inverse triple resonance probe equipped with a three-axis self-shielded gradient coil. XWINNMR 3.5 (Bruker, Karlsruhe, Germany) was used as the acquisition software.

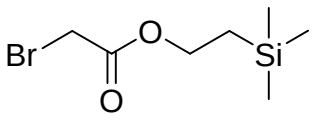
Homonuclear spectra included DQF-COSY, TOCSY and ROESY and were all recorded using States-TPPI to achieve quadrature detection in the indirect dimension. Spectra were recorded using 2k x 512 complex points and 10000 Hz and 8333 Hz spectral width in the direct and indirect dimension, respectively. 8 scans were used for the DQF-COSY and the TOCSY, 32 scans for the ROESY. MLEV-17 was used as the mixing sequence for the TOCSY experiments, the mixing time was 80 msec, in case of the ROESY the mixing time was 80 msec. Heteronuclear spectra were recorded for 13C and 15N and included HMQC and HMBC. They were

recorded using a BIRD pulse and States-TPPI to achieve quadrature detection in the indirect dimension in case of the HMQC and gradients for coherence selection and echo-antiecho as a means of quadrature detection in case of the HMBC. In case of ^{13}C 512 x 256 complex points, 32 scans and 10000 Hz and 20833 Hz spectral width were used for the HMQC and 2k x 512 complex points, 32 scans and 10000 Hz and 31250 Hz spectral width were used for the HMBC. In case of ^{15}N 512 x 64 complex points, 128 scans and 10000 Hz and 3012 Hz spectral width were used for the HMQC and 2k x 128 complex points, 80 scans and 10000 Hz and 12500 Hz spectral width were used for the HMBC.

All spectra were processed using TOPSPIN, a squared sine bell was using a weighting function, sifted by 90° in case of TOCSY, ROESY and HMQC and by 60° in case of the DQF-COSY. In case of the HMBC a sinebell was used in the acquisition dimension followed by a magnitude calculation.

Assignment of resonances and extraction of distances was performed using SPARKY.

2-(Trimethylsilyl) ethyl 2-bromoacetate



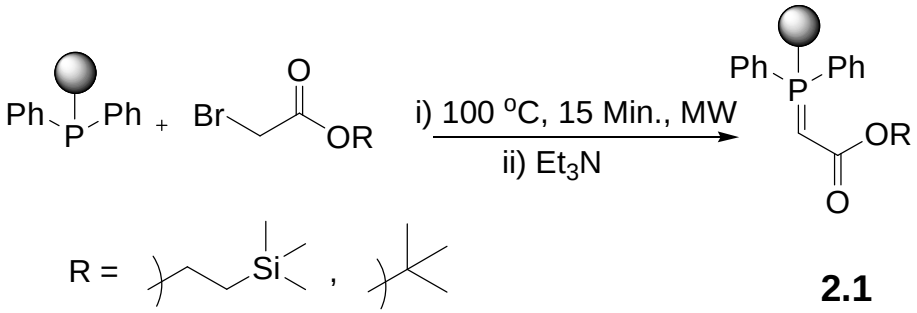
To a solution of 2-bromoacetic acid (5.7 g, 41 mmol) in dry CH_2Cl_2 (30 ml) at 0 ℃ was added, 2-(trimethylsilyl)ethanol (2.37 g, 20 mmol) and 4-(dimethylamino)pyridine (200 mg). To the stirred solution, DCC (9.5 g, 42 mmol) was added carefully in small portions over 5 min. The resulting mixture was stirred at 0 ℃ for 1 h. The formed mixture was filtered over celite and the remaining filter cake was washed with hexane/ether (100 mL, 4:1 v:v). Filtrate was washed with $KHCO_3$-solution. The organic layers were dried over sodium sulphate, and solvent removed under rotary evaporator. The crude product thus obtained was distilled under high vacuum and characterized by NMR.

^{1}H-NMR (300MHz, $CDCl_3$): δ = 0.08 (s, 9H, $(CH_3)_3Si$), 1.1 (m, 2H, $COOHCH_2CH_2Si$), 3.9 (s, 2H, $BrCH_2CO$), 4.2 (m, 2H, $COOCH_2CH_2Si$),

^{13}C-NMR: (75.5 MHz, $CDCl_3$): δ = 0.001, 18.7, 27.6, 66.3, 168.8. Yield (4.0 g, 83.4%)

Synthesis of polymer-supported peptidyl phosphoranes

Polymer-supported 2-phosphoranylidene acetate 2.1

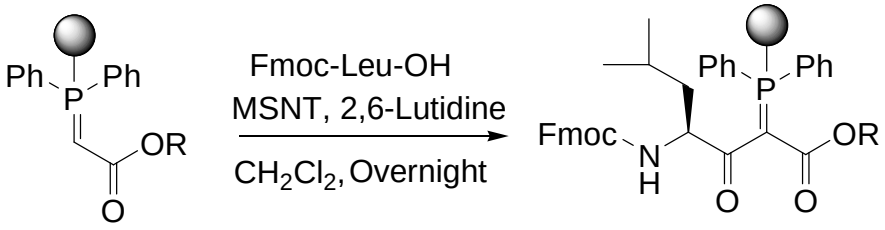


2.1

Triphenylphosphine polystyrene resin (0.5 g, 1.6 mmol/g, 0.8 mmol, 1% divinyl benzene, 100-200 mesh) was weighed into a microwave vial and suspended in dry toluene (4 mL). After addition of 2-bromoester (4 mmol, 5 equiv.), the vial was sealed and heated at 100 ℃ for 15 min in a microwave synt hesizer. The vial was cooled to room temperature before opening, the resin was filtered and washed with dry toluene and CH_2Cl_2. The obtained polymer phosphonium salt was resuspended in dry CH_2Cl_2 (5 mL) and TEA (557 µL, 4 mmol, 5 equiv.) was added. After shaking for 2 h at RT, the yellow-coloured resin **2.1** was filtered, washed and dried in vacuum.

Acylation of 1 by Fmoc amino acid

a) R = Trimethylsilylethyl

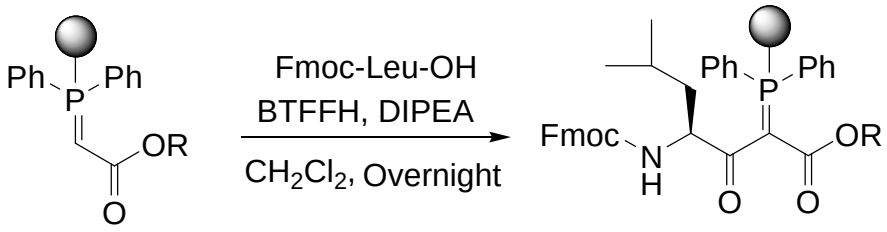


2.1

Resin **2.1** (200 mg, 1.43 mmol/g, 0.286 mmol) was pre-swollen in dry CH_2Cl_2. The Fmoc-Leucine (1.43 mmol, 5 equiv.) was suspended in dry CH_2Cl_2 (4 mL) and dissolved under addition of 2,6-lutidine (1.40 mmol, 4.9 equiv.). The clear solution obtained after addition of 1-(2-mesitylenesulfonyl)-3-nitro-1H-1,2,4-triazole (MSNT) (1.43 mmol, 5 equiv.) was directly mixed with the resin suspension and shaken overnight at room temperature. The resin was washed three times with each solvent (DMF, THF, CH_2Cl_2) after the coupling step and dried in vacuum. Acylation yields

were determined by photometric determination after Fmoc cleavage of small samples.

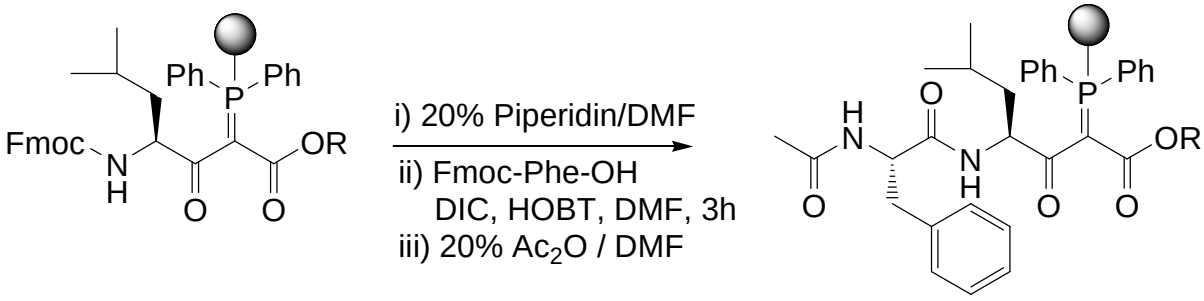
b) R = *t* Bu.



2.1

Resin **2.1** (300 mg, 1.32 mmol/g, 0.396 mmol) was pre-swollen in dry CH_2Cl_2. The Fmoc-Leucine (1.98 mmol, 5 equiv.) was dissolved in dry DMF (6 mL) after addition of diisopropylethyamine (DIPEA) (3.96 mmol, 10 equiv.) and BTFFH (1.98 mmol, 5 equiv.) The clear solution obtained was directly mixed with the resin suspension and shaken overnight at room temperature. The resin was washed thoroughly with CH_2Cl_2 after the coupling step and dried in vacuum.

Polymer-supported *N*-acetyl-L-phenylalanyl-2-(L-leucyl)-2-phosphoranylidene acetate 2.2



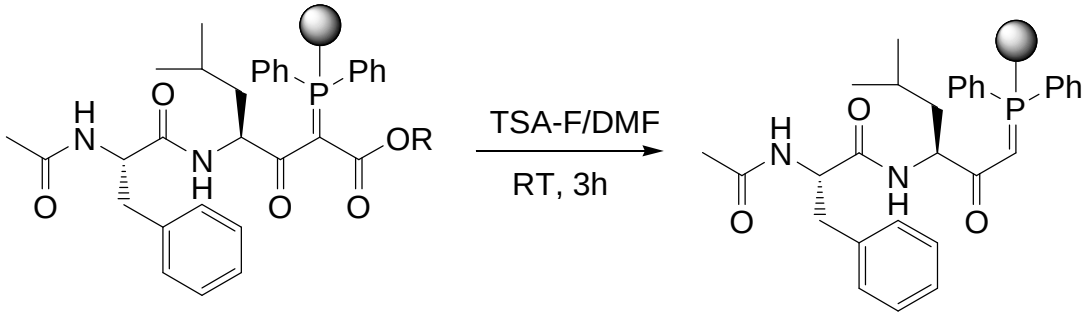
2.2

Fmoc protection was cleaved with 20 % piperidine/DMF (v/v, 2x6 min). The deprotected resin (300 mg, 0.396 mmol) was washed and suspended in dry DMF. Fmoc-phenylalanine (1.98 mmol, 5 equiv.) and $HOBt \cdot H_2O$ (1.98 mmol, 5 equiv.) were dissolved in dry DMF (6 mL), pre-activated under addition of DIC (1.98 mmol, 5 eq) and added to the resin following standard Fmoc solid-phase peptide synthesis. The mixture was shaken for 3 h at RT, filtered, and the resin was washed. After Fmoc deprotection, the resin was treated with 20 % acetic anhydride in DMF(2x30 minutes)

to cap the free amino group with acetyl. Quantitative couplings were verified by the Kaiser test.

Polymer-supported *N*-acetyl-L-phenylalanyl-2-(L-leucinyl)-2-phosphoranes 2.3

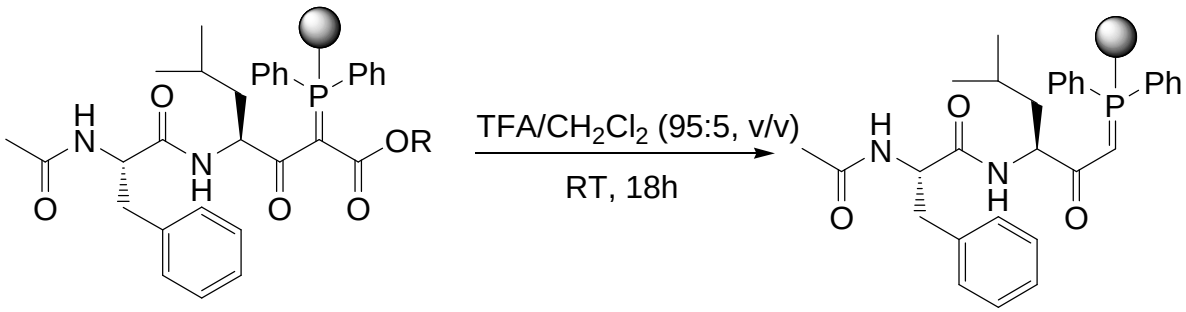
a) R = Trimethylsilylethyl



2.2 **2.3**

To a suspension of peptidyl-phosphoranylidene acetate resin **2.2** (200 mg, 1.27 mmol/g, 0.254 mmol) in dry DMF (5 mL) was added tris(dimethylamino)sulphur (trimethylsilyl)difluoride (TAS-F) (0.762 mmol, 3 equiv.) and mixture stirred for 3 h at RT. The resin was filtered, thoroughly washed (DMF, THF, CH_2Cl_2) and dried in vacuum.

b) R = *tert*-Butyl.



2.3

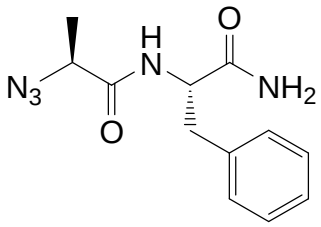
The resin **2.2** was treated with 8 mL of TFA : CH_2Cl_2 (95:5) for 18 h to remove the *tert*-butyl group. After washing the resin with CH_2Cl_2 (x 5), neutralization was carried out by treating the resin with DIPEA:CH_2Cl_2 (1:9, v:v) (3x5 min) followed by more CH_2Cl_2 washing (x 5).

Azides

4-Toluenesulfonyl azide **2.4**,[119] trifluoromethanesulfonyl azide **2.5**, 4-nitro-benzoyl azide **2.6**,[120] benzyl azide **2.8**, methyl 2-azido acetate **2.9**,[121] and 2-azido acetamide **2.10** [122] were synthesized according to standard reported procedures while 4-azido benzoic acid **2.7** was purchased from TCI, Europe.

General procedure for the synthesis of azido peptides (2.11-2.14)

Azido peptides **2.11-2.13** were prepared on Rink amide resin and **2.14** on 2-chlorotrityl resin through standard Fmoc chemistry using HOBT (1-hydroxy benzotriazole) and DIC (diisopropylcarbodiimide) activation. In all the cases final coupling was conducted with azido acids instead of amino acids. Azido acids were synthesized from amino acids employing trifluoromethyl-sulfonyl azide according to the reported procedure.[118]

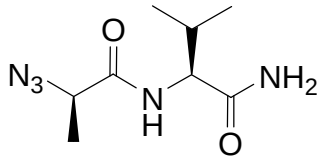


2.11

^{1}H-NMR: (300 MHz, $CDCl_3$): δ = 1.44 (d, *J* = 6.1 Hz, 3H, *CH_3*, Ala,), 3.04-3.16 (m, 2H, *CH_2*, Phe), 4.00-4.08 (m, 1H, *CH* , Ala), 4.6–4.73 (m, 1H, *CH* , Phe), 5.86 (d, 2H, *NH_2*), 7.02-7.23, 7.28-7.32 (2m, 5 H, arom.)

^{13}C-NMR: (75.5 MHz, $CDCl_3$): δ = 16.9, 38.2, 53.9, 58.8, 127.1, 128.7, 129.2, 136.10, 169.9, 172.6.

HRMS (ESI): Calculated for $C_{12}H_{15}N_5O_2$ $[M+H]^+$: 262.1304 Da. Found: 262.13038 m/z.

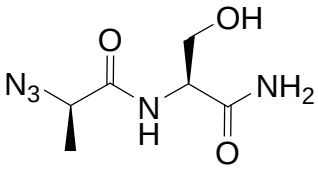


2.12

^{1}H-NMR: (300 MHz, Methanol-d_4): δ = 0.94, 0.96 (2d, *J* = 7.3 Hz, 6H, *2CH*$_3$, Val), 1.45 (d, *J* = 6.7 Hz, 3H, *CH*$_3$, Ala), 2.02-2.13 (m, 1H, *C*$^{\beta}$*H*, Val), 4.01–4.07 (q, *J* = 6.7 Hz, 1H, *CH*, Ala), 7.22 (m, 1H, *C*$^{\alpha}$*H*, Val)

^{13}C-NMR: (75.5 MHz, Methanol-d_4): δ = 17.5, 18.3, 19.7, 32.1, 59.3, 59.6, 173.1, 175.7.

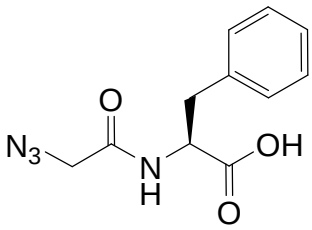
HRMS (ESI): Calculated for $C_8H_{15}N_5O_2$ $[M+H]^+$: 214.1304 Da. Found: 214.13035 m/z.



2.13

^{1}H-NMR: (300 MHz, Methanol-d_4): δ = 1.46 (d, *J* = 6.7 Hz, 3H, *CH*$_3$, Ala), 3.74-3.84 (m, 2H, *C*$^{\beta}$*H*$_2$, Ser), 3.99–4.12 (m, 1H, *CH*, Ala), 4.40–4.43 (m, 1H, *CH*, Ser).

^{13}C-NMR: (75.5 MHz, Methanol-d_4): δ = 17.2, 56.4, 59.4, 62.9, 173.1, 174.5.



2.14

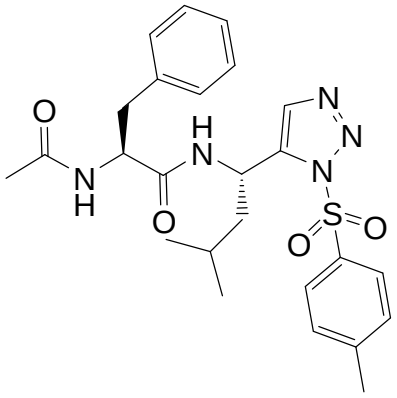
^{1}H-NMR: (300 MHz, $CDCl_3$): δ = 2.84-3.16 (m, 2H, *CH*$_2$, Phe), 3.77 (d, *J* = 3.6 Hz, 2H, *CH*$_2$, Gly), 4.58–4.65 (m, 1H, *CH*, Phe), 7.14-7.26 (m, 5H, arom.)

^{13}C-NMR: (75.5 MHz, $CDCl_3$): δ = 38.9, 52.7, 55.6, 127.8, 129.4, 130.2, 138.2, 169.8, 175.7.

HRMS (ESI): Calculated for $C_{11}H_{12}N_4O_3$ $[M+H]^+$: 249.09877 Da. Found: 249.09855 m/z.

General procedure for the synthesis of peptidyl-5-1H-1,2,3-triazoles (2.15, 2.16, 2.17):

Phosphorane **2.3** (200 mg, 0.254 mmol) was pre-swollen in CH_2Cl_2 (3 mL) and azides **2.4**, **1.6**, and **2.7** (1.2 equiv., 0.30 mmol) were added respectively. The mixture in CH_2Cl_2 was stirred at room temperature for 5 hours in a sealed glass vial. The polymer support was filtered off and washed with CH_2Cl_2. Solvents were removed under vacuum and crude products were triturated with acetonitrile (0.4 mL) to get 74–91% pure peptidyl triazoles. Compounds **2.15** and **2.17** were purified through HPLC. Compound **2.16** was not stable during HPLC purification and storage, the 4-nitro benzoyl group being cleaved off.



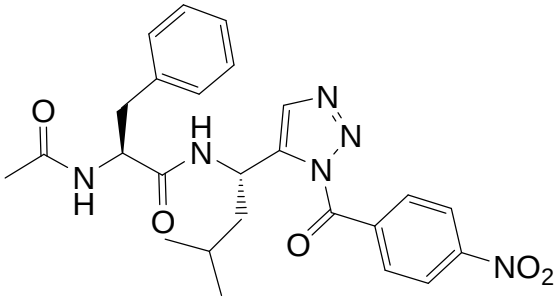
2.15

2.15 as white solid, Yield: (130 mg, 86%).

^{1}H-NMR: (300 MHz, DMSO-d_6): δ = 0.85, 0.87 (2d, *J* = 6.1 Hz, 6H, *CH*$_3$, Leu), 1.50–1.65 (m, 3H, *CH*$_2$, *CH*, Leu), 1.76 (s, 3H, *CH*$_3$, acetyl), 2.28 (s, 3H, *CH*$_3$, tosyl), 2.68-2.93 (m, 2H, *CH*$_2$, Phe), 4.46–4.53 (m, 1H, *CH*, Phe), 5.01–5.08 (m, 1H, *CH*, Leu), 7.10-7.23, 7.48–7.50 (2m, 9H, arom.), 7.43 (s, 1H, triazole), 8.08, .8.31 (2d, *J* = 8.5 2H, 2*NH*).

^{13}C-NMR: (75.5 MHz, DMSO-d_6): δ = 20.7, 21.7, 22.7, 30.6, 37.7, 43.0, 43.6, 54.0, 125.5, 127.3, 128.1, 130.6, 134.5, 137.8, 147.7, 169.0, 170.6.

HRMS (ESI): Calculated for $C_{25}H_{31}N_5O_4S$ $[M+H]^+$: 498.2175 Da. Found: 498.21721 m/z.



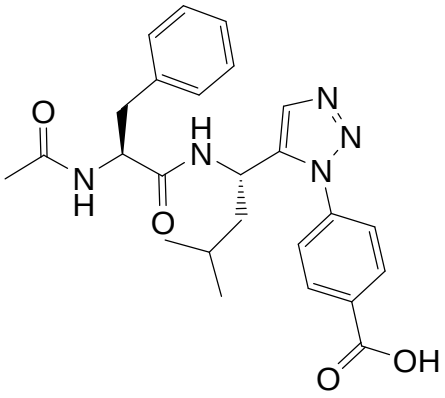
2.16

2.16 as pale yellow solid, Yield: (118 mg, 95%).

^{1}H-NMR: (300 MHz, Methanol-d_4): δ = 0.90–1.00 (m, 6H, *CH_3*, Leu), 1.58–1.88 (m, 3H, *CH_2, CH*, Leu), 1.92 (s, 3H, *CH_3*, acetyl), 2.82-3.04 (m, 2H, *CH_2*, Phe), 4.58 (t, *J* = 7.3 Hz, 1H, *CH*, Phe), 5.12–5.20 (m, 1H, *CH*, Leu), 7.15-7.25 (m, 5H, *arom*., Phe), 8.21–8.25, 8.30-8.36 (2m, 5H, triazole, Nitro Phenyl).

^{13}C-NMR: (75.5 MHz, Methanol-d_4): δ = 22.1, 22.3, 23.2, 25.7, 39.0, 44.7, 124.5, 124.8, 127.7, 129.4, 130.3, 131.6, 131.9, 138.1, 172.8, 173.0.

HRMS (ESI): Calculated for $C_{25}H_{28}N_6O_5$ $[M+H]^+$: 493.21994 Da. Found: 493.21993 m/z.



2.17

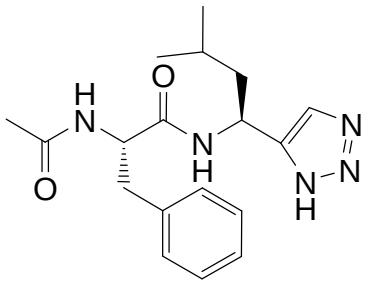
2.17 as white solid, Yield: (94 mg, 80%).

^{1}H-NMR: (300 MHz, Methanol-d_4): δ = 0.68–0.74 (m, 6H, *CH_3*, Leu), 1.32–1.69 (m, 3H, *CH_2, CH*, Leu), 1.90 (s, 3H, *CH_3*, acetyl), 2.75-2.91 (m, 2H, *CH_2*, Phe), 4.52 (dd, *J_1* = 7.3, *J_2* = 8.5 Hz,1H, *CH*, Phe), 5.13–5.18 (m, 1H, *CH* , Leu), 7.13-7.23 (m, 5H, *arom.*, Phe), 7.34 (s, 1H, *CH*, triazole), 7.65–7.68, 8.23-8.26 (2d, *J* = 8.5, 4H, *arom.*, carboxy-phenyl).

^{13}C-NMR: (75.5 MHz, $CDCl_3$): δ = 22.9, 24.6, 30.3, 34.2, 38.4, 54.4, 118.9, 125.4, 127.2, 128.6, 119.0, 131.5, 131.9, 133.4, 135.1, 139.3, 139.7, 145.2.

HRMS (ESI): Calculated for $C_{25}H_{29}N_5O_4$ $[M+H]^+$: 464.22978 Da. Found: 464.22947 m/z.

Peptidyl-5-1H-1,2,3-triazole 2.19:



2.18

To the phosphorane resin **2.3** (200 mg, 0.254 mmol), excess of freshly prepared trifluoromethanesulfonyl azide **2.5** (3 equiv.) in CH_2Cl_2 (5 mL) was added and the mixture was stirred for 5 hours at room temperature. The polymer support was filtered off and washed with CH_2Cl_2. Solvents were removed under vacuum and crude product dissolved in acetonitrile. Water (1 ml) was added and the mixture was lyophilized to deliver 92% pure **2.18** as a pale yellow solid (99 mg, 88%).

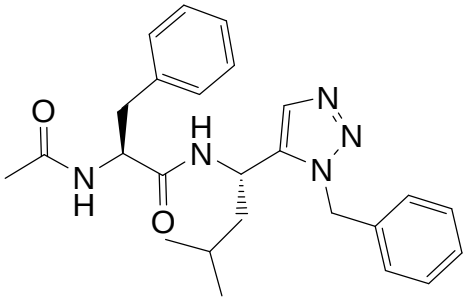
^{1}H-NMR: (300 MHz, CD_3CN): δ = 0.83, 0.88 (2d, *J* = 6.1 6.7, 6H, 2*CH*$_3$, Leu), 1.46–1.63 (m, 3H, *CH*, *CH*$_2$, Leu), 1.86 (s, 3H, *CH*$_3$, acetyl), 2.86-3.11 (m, 2H, *CH*$_2$, Phe), 4.23–4.32 (m, 1H, *CH* , Phe), 4.55–4.62 (m, 1H, *CH*, Leu), 6.93-7.49 (m, 6 H, arom.).

^{13}C-NMR: (75.5 MHz, $CDCl_3$): δ = 22.0, 23.2, 25.1, 30.7, 38.5, 46.7, 54.7, 117.7, 122.0, 125.9, 129.1, 129.8, 136.4, 171.2, 177.5.

HRMS (ESI): Calculated: for $C_{18}H_{25}N_5O_2$ $[M+H]^+$: 344.20865 Da. Found: 344.20845 m/z.

General procedure for the synthesis of peptidyl-5-1H-1,2,3-triazoles (2.20-2.22):

Phosphorane **3** (200 mg, 0.254 mmol) was pre-swollen in THF (3 mL) and azides **2.8-2.10** (1.2 equiv., 0.305 mmol) were added respectively and the mixture was heated overnight at 60 ^{o}C in a sealed glass vial. The polymer support was filtered off and washed with THF and CH_2Cl_2. Solvents were removed under vacuum and crude products were triturated with acetonitrile to yield compounds with 76–87% purity. Purification was conducted employing HPLC.



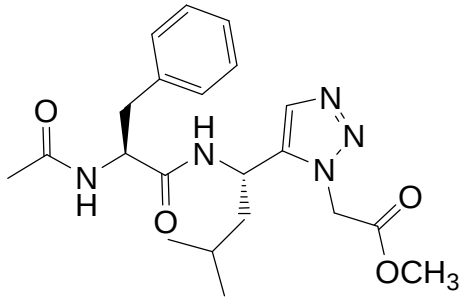
2.19

2.19 as white solid, yield: (70 mg, 68%).

^{1}H-NMR: (300 MHz, Methanol-d_4): δ = 0.62, 0.63 (2d, *J* = 6.1, 6H, 2*CH*$_3$, Leu), 1.18–1.71 (m, 3H, *CH*$_2$, *CH*, Leu), 1.92 (s, 3H, *CH*$_3$, acetyl), 2.80-2.93 (m, 2H, *CH*$_2$, Phe), 4.47 (dd, *J*$_1$ = 7.3, *J*$_2$ = 8.5 Hz,1H, *CH*, Phe), 5.02–5.08 (m, 1H, *CH*, Leu), 5.46 (d, *J* = 15.8 Hz, 1H, *CH*$_2$, benzyl), 5.63 (d, *J* = 15.8 Hz, 1H, *CH*$_2$, benzyl), 7.05-7.36 (m, 10 H, arom.), 7.48 (s, 1H, *CH*, triazole)

^{13}C-NMR: (75.5 MHz, Methanol-d_4): δ = 22.3, 22.5, 38.7, 41.8, 43.8, 52.9, 56.0, 127.8, 128.3, 129.3, 130.0, 130.1, 136.8, 137.7, 172.8, 172.9.

HRMS (ESI): Calculated for $C_{25}H_{31}N_5O_2$ $[M+H]^+$: 434.2556 Da. Found: 434.25598 m/z.



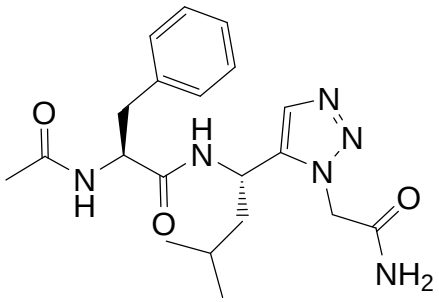
2.20

2.20 as white solid, yield: (73 mg, 70%).

^{1}H-NMR: (300 MHz, Methanol-d_4): δ = 0.73 (d, *J* = 6.1 Hz, 3H, *CH*$_3$, Leu), 0.77 (d, *J* = 7.3 Hz, 3H, *CH*$_3$, Leu),1.38–1.74 (m, 3H, *CH*$_2$, *CH*, Leu), 1.84 (s, 3H, *CH*$_3$, acetyl), 2.88 (d, *J* = *7.9* Hz, 2H, C*H*$_2$, Phe), 3.67 (s, 3H, *CH*$_3$, ester), 4.45-4.54 (m, 2H, 2C*H*, Phe, Leu), 5.22 (dd, 2H, *CH*$_2$, *J*$_1$ = *J*$_2$ = 17.7 Hz, acetate), 7.02-7.13 (m, 5 H, arom.), 7.46 (s, 1H, *CH*, triazole)

^{13}C-NMR: (75.5 MHz, Methanol-d_4): δ = 21.8, 22.3, 23.2, 25.6, 30.9, 38.7, 41.8, 43.4, 53.41, 56.0, 126.1, 127.8, 129.4, 130.0, 132.5, 137.8, 141.5, 168.8, 173.0.

HRMS (ESI): Calculated for $C_{21}H_{29}N_5O_4$ $[M+H]^+$: 416.22978 Da. Found: 416.22926 m/z.



2.21

2.21 as white solid, yield: (73 mg, 74%).

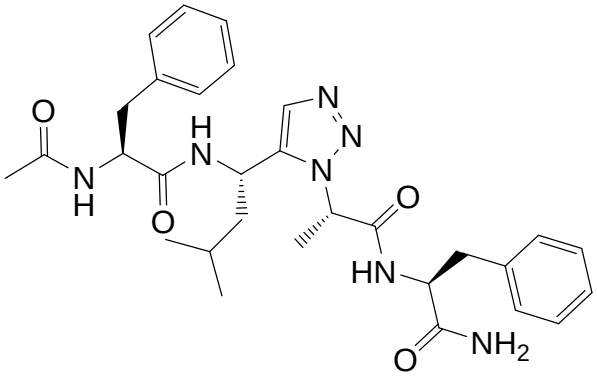
^{1}H-NMR: (300 MHz, Methanol-d_4): δ = 0.88 (d, *J* = 6.0 Hz, 3H, *CH*$_3$, Leu), 0.92 (d, *J* = 9.0 Hz, 3H, *CH*$_3$, Leu), 1.53–1.87 (m, 3H, *CH*$_2$, *CH*, Leu), 1.91 (s, 3H, *CH*$_3$, acetyl), 2.81-2.97 (m, 2H, *CH*$_2$, Phe), 4. 47 (dd, 1H, *CH*, *J*$_1$ = 6.0, *J*$_2$ = 9.0, Hz, Phe), 5.06 (dd, 1H, *CH* , *J*$_1$ = 4.8, *J*$_2$ = 9. 7, Hz, Leu), 5.20 (s, 2H, *CH*$_2$, acetamide), 7.03-7.12 (m, 5H, arom.), 7.47 (s, 1H, *CH*, triazole)

^{13}C-NMR: (75.5 MHz, Methanol-d_4): δ = 21.7, 22.3, 23.4, 25.6, 38.7, 42.0, 43.4, 51.1, 56.1, 127.8, 129.3, 130.0, 132.2, 137.8, 142.0, 170.0, 173.0, 173.0.

HRMS (ESI): Calculated for $C_{20}H_{28}N_6O_3$ $[M+H]^+$: 401.23011 Da. Found: 401.23007 m/z.

General procedure for the synthesis of peptidyl-5-1*H*-1,2,3-triazolyl-1-peptides (2.22-2.25):

Phosphorane resin **2.3** (100 mg, 0.127 mmol) was pre-swollen in THF (1 mL) in a glass vial and azido-peptides **2.11-2.14** (1.5 equiv., 0.19 mmol), dissolved in THF (1 mL), were added to the vial respectively. The mixture was heated overnight at 60 ^{o}C in sealed glass vial. After cooling to room temperature, the polymer support was filtered off and washed with THF and CH_2Cl_2. Solvents were removed under vacuum and products **2.22-2.25** were isolated by preparative reversed phase HPLC to remove remains of the azido-peptide reagents. All the compounds **2.22-2.25** were obtained as white lyophilisates.



2.22

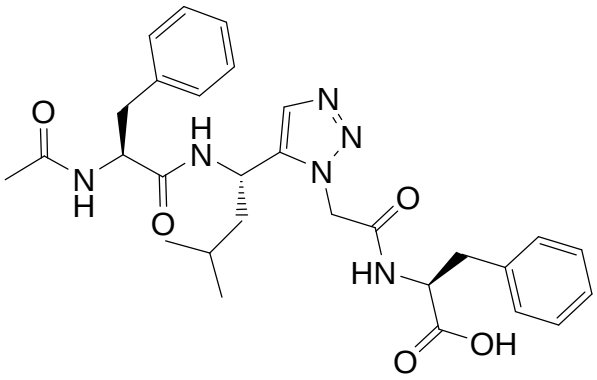
Yield: (48 mg, 68%).

^{1}H-NMR: (300 MHz, DMSO-d_6): δ = 0.82, 0.85 (2d, *J* = 6.1 Hz, 6H, 2*CH*$_3$, Leu), 1.35-1.52 (m, 2H, Leu), 1.60 (d, 3H, *J* = 7.3 Hz, *CH*$_3$, Ala), 1.65–1.67 (m, 1H, *C*$^{\beta}$*H*, Leu), 1.73 (s, 3H, *CH*$_3$, acetyl), 2.75–3.02 (m, 4H, 2*CH*$_2$, Phe), 1.53–1.87 (m, 3H, *CH*$_2$, *CH*, Leu), 2.50-3.31 (m, 4H, 2*CH*$_2$, Phe), 4.39–4.49 (m, 2H, 2*CH*, Phe), 5.04–5.12 (m, 1H, *C*$^{\alpha}$*H*, Leu), 5.43 (q, *J* =7.3, 1H, *CH*, Ala), 7.02 (d, *J* = 7.4, 2H, *NH*$_2$), 7.11–7.24 (m, 10H, *arom*., Phe), 7.55 (s, 1H, *CH*, triazole), 8.01, 8.04, 8.53 (3d, *J* = 7.9, 8.3, 7.9, 3H, 3*NH*).

^{13}C-NMR: (75.5 MHz, DMSO-d_6): δ = 17.7, 21.6, 22.3, 22.6, 24.2, 37.2, 41.4, 43.2, 53.7, 54.3, 56.7, 126.1, 127.9, 129.0, 131.6, 137.5, 139.7, 167.5, 169.1, 171.1, 172.2.

^{13}C-NMR: (75.5 MHz, DMSO-d_6): δ = 17.7, 21.6, 22.3, 22.6, 24.2, 37.2, 41.4, 43.2, 53.7, 54.3, 56.7, 126.1, 127.9, 129.0, 131.6, 137.5, 139.7, 167.5, 169.1, 171.1, 172.2.

HRMS (ESI): Calculated for $C_{30}H_{39}N_7O_4$ $[M+H]^+$: 562.31418 Da. Found: 562.31419 m/z.



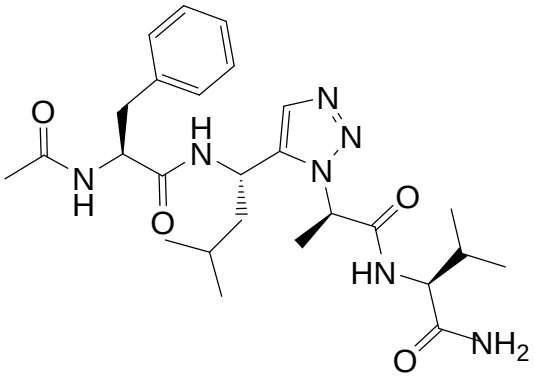
2.23

Yield: (50 mg, 72%).

^{1}H-NMR: (300 MHz, DMSO-d_6): δ = 0.80 , 0.85 (2d, *J* = 6.1, 7.3 Hz, 6H, *CH*$_3$, Leu), 1.44-1.80 (m, 6H, *CH*$_3$, acetyl, *CH*$_2$, *CH*, Leu), 1.53–1.87 (m, 3H, *CH*$_2$, *CH*, Leu), 2.61 - 3.10 (m, 4H, *2CH*$_2$, Phe), 4.38–4.47 (m, 2H, 2*CH*, Phe), 4.87–4.94 (m,1H, *CH*, Leu), 5.11 (s, 2H, *CH*$_2$, Gly), 7.06-7.30 (m, 10H, *arom.*, Phe), 7.52 (s, 1H, *CH*, triazole), 8.03, 8.41, 8.71 (3d, *J* = 8.5, 3H, 3*NH*.).

^{13}C-NMR: (75.5 MHz, DMSO-d_6): δ = 21.2, 22.3, 22.9, 23.9, 30.6, 36.8, 37.9, 41.9, 53.7, 54.1, 126.2, 126.5, 128.0, 128.2, 129.1, 131.1, 137.0, 137.4, 140.5, 165.4, 169.1, 170.9, 172.3.

HRMS (ESI): Calculated for $C_{29}H_{36}N_6O_5$ $[M+H]^+$: 549.28254 Da. Found: 549.28242 m/z.



2.24

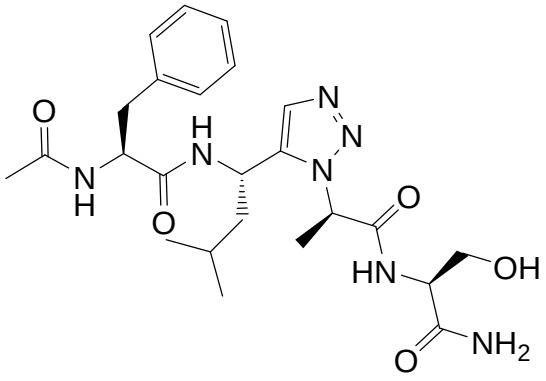
Yield: (48 mg, 74%).

^{1}H-NMR: (300 MHz, DMSO-d_6): δ = 0.78–0.85 (m, 12H, 4*CH*$_3$, Leu, Val), 1.25–1.67 (m, 3H, *CH*$_2$, *CH*, Leu), 1.75 (s, 3H, *CH*$_3$, acetyl), 1.82 (d, 3H, *J* = 7.3 Hz, *CH*$_3$, Ala), 1.94–2.01 (m, $C^{\beta}H$, 1H, Val), 4.19 (dd, $J_1 = J_2$ = 6.1 Hz, 1H, $C^{\alpha}H$, Val), 4.46 (m, 2H, 2$C^{\alpha}H$, Val, Phe), 4.76 (m, 1H, $C^{\alpha}H$, Leu),7.07–7.22 (m, 5H, arom.), 7.47 (s, 1H, *CH*, triazole), 7.53 (s, 2H, *NH*$_2$), 8.06, 8.22, 8.50 (3d, *J* = 8.5 Hz, 3H, 3*NH*).

❖

^{13}C-NMR: (75.5 MHz, DMSO-d_6): δ = 17.6, 17.8, 19.2, 21.2, 22.2, 22.3, 22.8, 23.9, 30.9, 37.3, 42.4, 53.7, 57.1, 126.2, 127.9, 128.9, 130.9, 139.6, 139.9, 168.9, 171.16, 172.2.

HRMS (ESI): Calculated for $C_{26}H_{39}N_7O_4$ $[M+H]^+$: 514.31418 Da. Found: 514.31397 m/z.



2.25

Yield: (46 mg, 73%).

^{1}H-NMR: (300 MHz, DMSO-d_6): δ = 0.76, 0.78 (2d, *J* = 7.3 Hz, 6H, 2CH_3, Leu), 1.21–1.60 (3m, 3H, CH_2, CH, Leu), 1.65 (d, 3H, *J* = 7.3 Hz, CH_3, Ala),1.75 (s, 3H, CH_3, acetyl), 2.72–2.91 (m, 2H, $C^{\beta}H_2$, Phe), 3.60–3.67 (m, 2H, $C^{\beta}H_2$, Ser), 4.20–4.26 (m, 1H, $C^{\alpha}H$, Phe), 4.43–4.50 (m, 1H, $C^{\alpha}H$, Ser), 4.95–5.03 (m, 1H, $C^{\alpha}H$, Leu), 5.42–5.49 (m, 1H, $C^{\alpha}H$, Ala), 7.19–7.25 (m, 7H, arom., NH_2), 7.34 (s, 1H, -OH), 7.64 (s, 1H, CH, triazole), 7.90, 8.02, 8.50 (3d, *J* = 7.3 Hz, 3H, 3NH_2).

^{13}C-NMR: (75.5 MHz, DMSO-d_6): δ = 18.2, 21.4, 22.3, 22.7, 23.9, 26.2, 30.6, 61.5, 62.4, 123.4, 126.3, 128.1, 129.0, 137.4, 168.1, 170.9, 171.6.

HRMS (ESI): Calculated for $C_{24}H_{35}N_7O_5$ $[M+H]^+$: 502.27779 Da. Found: 502.27755 m/z.

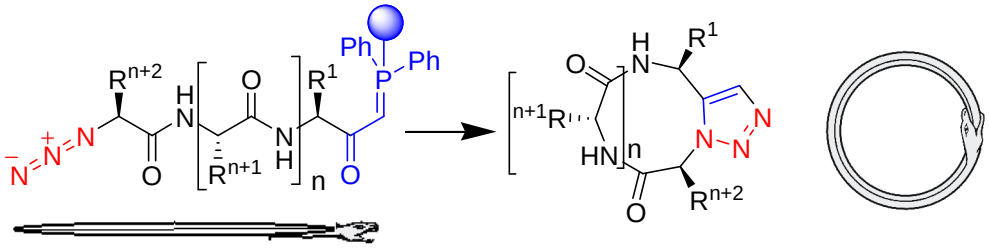
Backbone and side chain tortional angles calculated for **2.22**

Ac-Phe-Leu-Triazole-Ala-Phe-NH_2
1 2 3 4

	Φ1	Ψ1	Φ2	Ψ2	Φ3	Ψ3	Φ4	Ψ4
MEAN	258.6	280.7	267.5	116.7	226.0	136.8	192.3	69.0
STD. DEV.	40.0	84.4	46.9	6.0	4.9	2.4	55.3	26.5
HIGH	298.2	354.6	310.9	131.9	233.9	141.2	234.6	129.1
LOW	188.8	109.9	142.2	110.4	219.6	133.5	40.0	32.4

3 Cyclative cleavage via dipolar cycloaddition: polymeric azido-peptidyl-phosphoranes that delivered locked cis-triazolyl-cyclopeptides as privileged protein binders

An ourobouros, a snake (or dragon) which bites its own tail, is an ancient alchemist symbol for the circularity of many natural processes. Our snakes are azido-peptidyl phosphoranes which reacted via dipolar cycloadditions on solid support resulting in a cyclative cleavage. The reaction yielded cyclopeptides with an incorporated triazol ring very efficiently. 1,5-Disubstitution of the heterocycle locked one amide bond of the cyclopeptide in the *cis*-geometry. The solid support is advantageous favoring cyclization strongly over oligomerization reactions and thus released cyclized products only.



n= 1,2,3,6

3.1 Cyclopeptides

Cyclic peptides are important natural products displaying a broad range of biological effects including immunosuppression, antibacterial and anti-cancer activity.[123-126] Many cyclopeptides act as potent inhibitors of protein-protein interactions or enzymatic activities by constituting partially rigidified conformations thus providing improved protein target recognition together with favorable pharmacokinetic properties and metabolic stability.[127-129] As a result several cyclopeptides have been used successfully as clinically admitted drugs for decades and continuously cyclopeptides and pseudo-cyclopeptides supply the pipelines of pharmaceutical industry with novel drug candidates.[130, 131] Due to their importance in drug discovery, synthesis of cyclopeptides and pseudo-cyclopeptides has been an attractive target.[132-138] Generally, lactamization step for the cyclization of peptides is a limiting factor which is accompanied with intermolecular reactions leading to oligomers and polymers formation. These intermolecular reactions are encouraged by the breakage of circular conformations (in case of short linear peptides) resulting from the activation of peptide termini, as shown in Fig. **3.1**.[139-141]

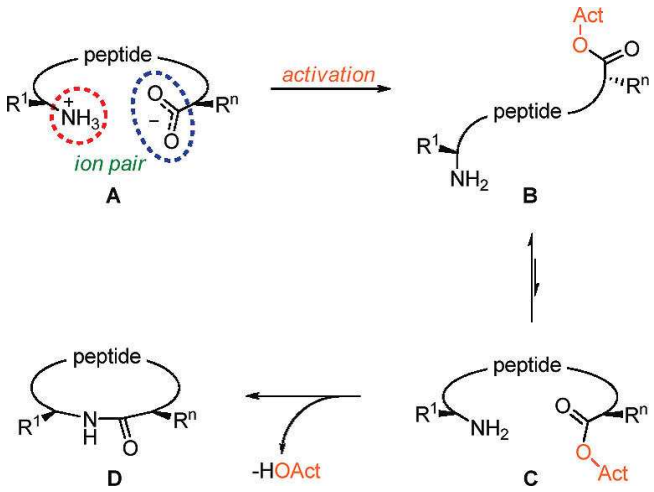


Figure 3.1: Common peptide cyclization strategy.

3.2 Ligation strategies used for peptide cyclization

Various synthetic strategies have been introduced to effect cylization of peptides and pseudo-peptides. Among these, ligation reactions are very prominent.

Native chemical ligation has been extensively applied for peptide cyclization. Muir's group, *e.g*, harnessed Native chemical ligation for intra-molecular cyclization of

unprotected polypeptide chain and synthesized circular proteins.[142, 143] Barany's group used on-resin peptide cyclization through NCL, shown in Fig **3.2**.[144]

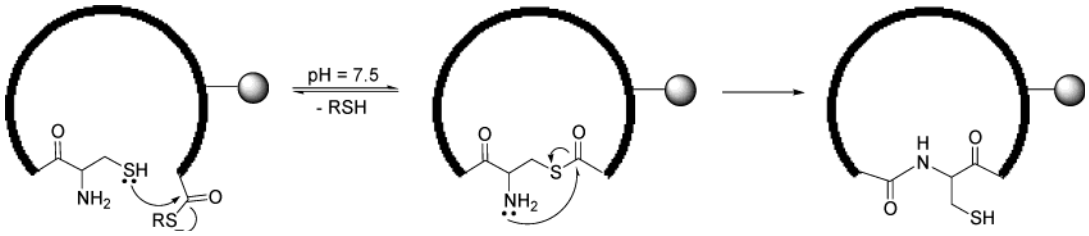
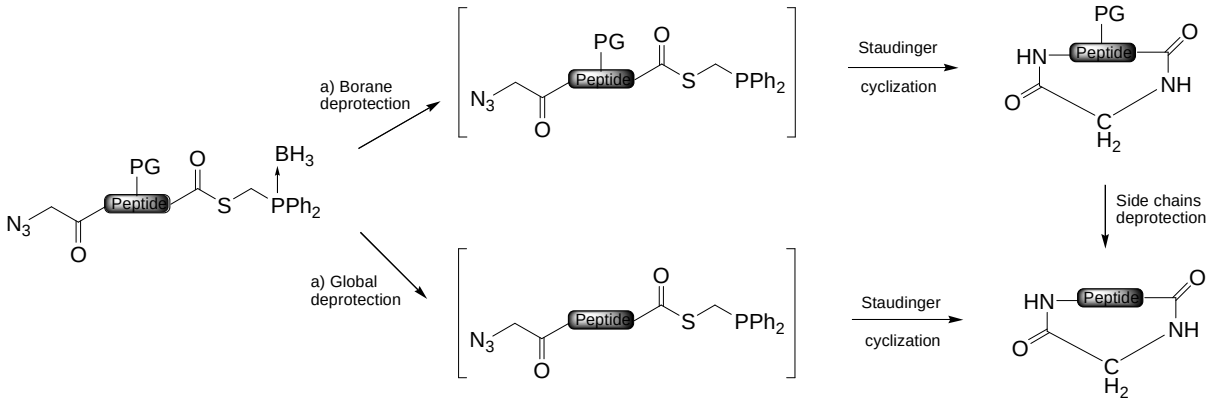


Figure 3.2: On-resin peptide cyclization through NCL.

Likewise, Marsaveen's group used traceless Staudinger ligation for lactam synthesis[145] which has recently been applied for peptide cyclization by Hackenberger's group, shown in Scheme. **3.1**.[109]



Scheme 3.1: Traceless Staudinger ligation for peptide cyclization.

Recently, triazole ligation has also been used for peptide cyclization, although with success only in few cases.

3.3 Aim of the present work

Triazolyl-cyclopeptides have been introduced as pseudo-cyclopeptides with decreased flexibility as one peptide bond is replaced with the heterocycle locking it either in *trans*-peptide or in *cis*-peptide geometry.[35, 36] For several biological targets, *cis*-locked cyclopeptides containing 1,5-disubstituted triazoles possessed significantly increased binding affinity and biological activity as compared to the 1,4-disubstituted derivatives or native cyclopeptides.[35] These observations constitute 1,5-disubstituted triazolyl-cyclopeptides as privileged protein binders.

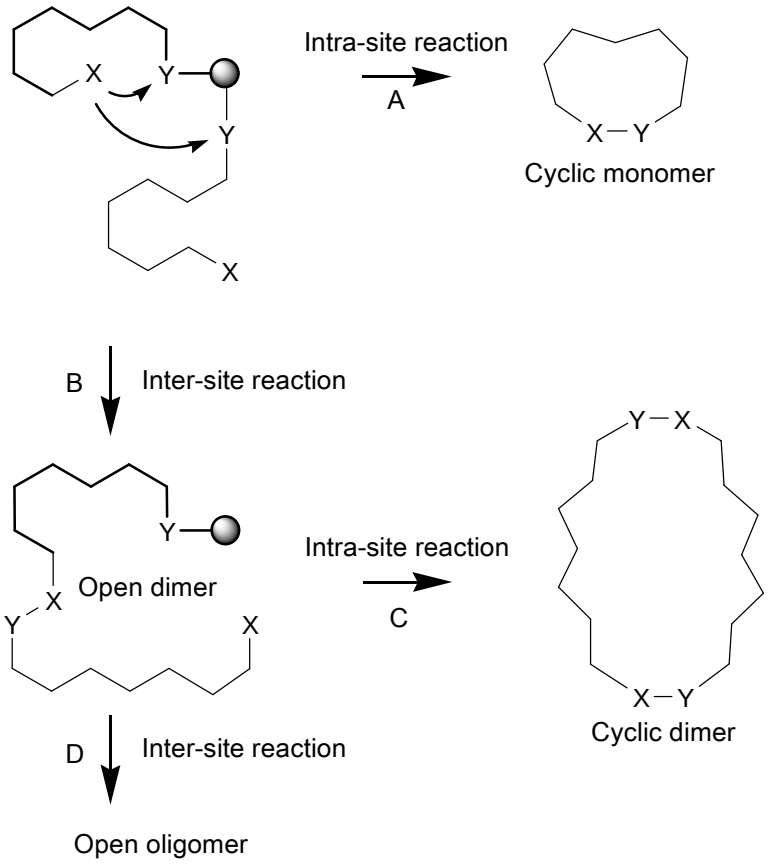
Despite of their broad biological significance the synthesis of triazolyl-cyclopeptides as well as wild-type cyclopeptides still can be a major challenge demanding for further improvement. Cyclization reactions in solution often deliver low yields and furnish mixtures of monomeric, oligomeric, cyclized, and open-chain products, if the inter-molecular reaction competes with an intra-molecular reaction path.[146, 147] Attachment to a solid support can reduce oligomer-formation considerably due to site-separation by the polymer backbone, also denominated as pseudo-dilution.[144, 148, 149] Nevertheless, highly swellable polymers such as low crosslinked polystyrene allow inter-site reactions depending critically on the length and flexibility of the attached molecules.[150-153] If, however, cyclization and cleavage proceed in the same chemical reaction (denominated as cyclative cleavage), open-chain oligomeric by-products remain attached to the solid support and are easily removed by washing the resin whereas cyclic monomers and eventually cyclic oligomers are released in solution (Scheme 1).[154-158] Moreover, the flexibility of the polymer support as well as the resin loading are parameters which can be exploited to favor intra-site versus inter-site reactions. In the light of these considerations, preparation of triazolyl-cyclopeptides employing cyclative cleavage appeared to be an attractive enterprise.

3.4 Results and Discussion

Though 1,3-dipolar cycloadditions of azides and alkynes under Cu(I) or Ru(II) catalysis succeeded to incorporate 1,4- and 1,5-disubstituted triazoles in peptides,[43, 44, 52] the efficacy of the Cu (I)-catalyzed, 1,4-selective reactions dropped significantly for cyclizations,[34] even more pronounced when conducted on solid support.[32, 159, 160] Therefore, in most of these cases the 1,4-disubstitued triazole has been introduced into cyclic peptides through the reaction of peptides with azide and alkyne termini in solution.[30-32, 159-162] Alternatively, the 1,4-disubstitued triazole was prepared first in the linear peptide sequence followed by a final lactamization step.[163, 164]

Unfortunately, the synthesis of the biologically privileged 1,5-disubstituted triazolyl-cyclopeptides has been proven especially difficult. No cyclizations of azido-alkynyl peptides to 1,5-disubstituted triazoles either in solution or on solid support have been reported yet. As the ruthenium-catalyzed cycloaddition has to be performed at a high concentration (0.5-1.0 M) it was not suitable for higher dilutions required to reduce oligomer formation.[165] On the contrary, the only synthesis of 1,5-disubstituted triazolyl cyclopeptides was realized via a final lactamization reaction.[35]

Recently, we have developed the synthesis of 1,5-peptidyl-triazolyl-peptides via metal-free, regioselective 1,3-dipolar cycloaddition reactions.[166] The method enabled preparation of triazolyl-peptides starting exclusively from commercially available amino acid building blocks and yielded the products in high purity employing the dipolar cycloaddition as cleavage reaction. As demonstrated by ROESY NMR and a simulated annealing protocol, the obtained 1,5-disubstituted triazole acting as *cis*-peptide bond mimetic induces turn structures already in short peptide stretches. Moreover, using azido-peptidyl-phosphorane resins for a cyclative cleavage should enable the selective and exclusive formation of cyclized products as all open-chain monomers and oligomers are expected to remain attached to the polymer support (Scheme **3.2**).

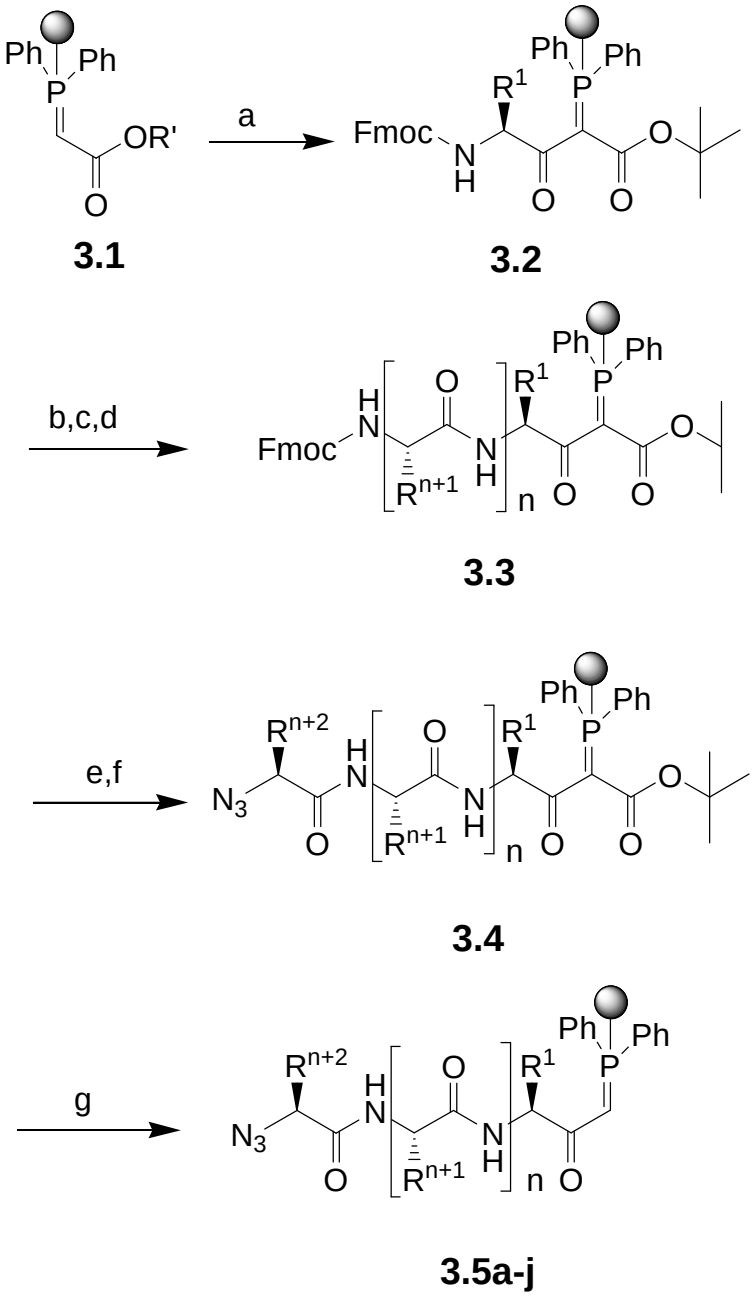


Scheme 3.2: In cyclizations on solid supports intra-site reactions (A) compete with inter-site reactions (B). In the special case of cyclative cleavage, pathway A yielding cyclic monomer competes with B, providing open chain dimer still attached to the polymer. The latter can react following pathway (C) leading to the cyclic dimer (intra-site) or pathway (D) leading to the attached oligomer in an inter-site reaction.

In order to test this hypothesis N-terminal azido-peptidyl-phosphoranes were prepared on the solid phase (Scheme **3.3**).[71, 115] Starting from *tert*-butyl phosphoranylidene acetate **3.1**, amino acyl phosphorane **3.2** was obtained by a non-racemizing *C*-acylation employing an Fmoc-protected amino acid and BTFFH for activation in 76-82 % yields depending on the use of the amino acids glycine, leucine, phenylalanine and *tert*-butyl serine in this step. **3.2** was elongated with further amino acids by employing standard couplings of Fmoc-amino acids with diisopropyl-carbodiimide/1-hydroxybenzotriazole activation furnishing resin **3.3**. For the elongation steps various amino acids with and without side chain protection were used including Pro, Leu, Val, Trp, Ser, Thr, Met, and Tyr. Following Fmoc-deprotection of **3.3**, the obtained free amine was acylated with one of the 2-azido acids **3.6** or **3.7** obtained by nucleophilic substitution of bromoacetic acid with sodium azide or via diazotransfer from freshly prepared triflyl azide, respectively.[118] The reaction furnished the azido-peptidyl phosphoranylidene acetate **3.4** which was treated with trifluoroacetic acid to remove all side chain protecting groups. Cleavage of the C-terminal acetate ester led to instantaneous decarboxylation of the phosphoranylidene acetate yielding azido-peptidyl-phosphoranes **3.5a-j** (Scheme **3.3**).

Cyclizations of **3.5a-j** were investigated with peptide chains of different lengths as well as varying amino acid sequences (Scheme **3.4**, Table **3.1**). Temperature between 60-80 ℃ together with polar solvents was s ufficient for cyclative cleavage of azido-peptidyl-phosphoranes. DMF was selected as preferred solvent as it assured good solubility of those products which were only partially soluble in other polar solvents swelling the polystyrene support. When the longer azido penta-, and octa-peptidyl-phosphoranes **3.5i,j** (n=3,6) were heated in DMF exclusively the expected monomeric triazolyl-cyclopeptides **3.15** and **3.16** respectively, were formed. These results are in full agreement with a considerable degree of site separation exerted by the solid support.

When, however, the azido-dipeptidyl-phosphoranes **3.5a,b** (n=0) were treated under identical conditions, exclusively the dimeric bis-triazolyl-cyclotetrapeptides **3.8** and **3.9** were formed resulting from inter-site reactions (see Scheme **3.4**).



Scheme 3.3: Preparation of azido-peptidylphoshoranes 5a-j on polystyrene support. Reaction conditions: a) Fmoc-AA-OH and BTFFH, DIPEA, DMF, 14 h; b) 20% piperidine/DMF; c) Fmoc-AA-OH, DIC, HOBt, DMF, 2h; d) b & c are repeated n-times; e) 20% piperidine/DMF ; f) Azido acid (3.6 or 3.7), DIC, HOBt, DMF, 2h; d) b &c are repeated n-times; e) 20% piperidine/DMF; f) azido acid (3.6 or 3.7), DIC, HOBt, DMF, 2h; g) TFA/CH_2Cl_2 (95% v:v), 5h followed by Et_3N treatment.

Azido-tripeptidyl- phosphoranes **3.5c** (n=1) delivered a 3:2 mixture of the monomeric triazolyl-cyclotripeptide with the respective dimeric product. Azido-tetrapeptidyl-phosphoranes **3.5e,f,g,h** (n=2) were cyclized under identical conditions and provided

the respective triazolyl-cyclotetrapeptides as the major products. While azido-tetrapeptidyl-phosphoranes **3.5g,h** furnished the cyclic monomer **3.13** and **3.14** as the exclusive product, reactions of **3.5e** and **3.5f** yielded the monomeric triazolyl-cyclopeptides **3.11** and **3.12** together with the corresponding dimeric products formed in minor amounts according to LC-MS analysis.

Table 3.1: Formation of monomeric and dimeric products by cyclative cleavage of phosphoranes 3.5a-j. Peptide sequences are assigned with the one-letter code using capital letters for L-amino acids and small letters for D-amino acids.

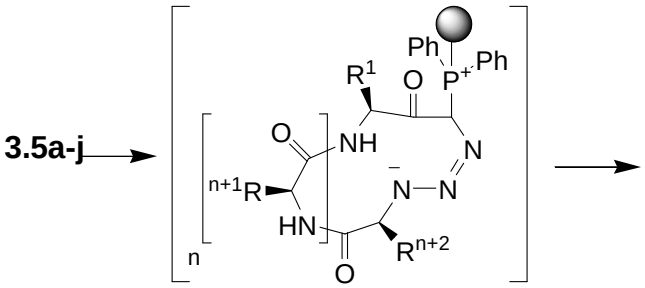
Phospho-rane	Peptide sequence	n (chain length)	Product	Ring size (# atoms)	Monomer (%)[a]	Dimer (%)[a]
3.5a,b	AG,Fa	0 (dipeptide)	**3.8,3.9**	12	0	100[b]
3.5c	LPG	1 (tri-)	**[d]**	9	60	40
3.5c	LPG	1 (tri-	**[d]**	9	80[c]	20[c]
3.5d	LSG	1 (tri-)	**3.10**	9	12	88
3.5d	LSG	1 (tri-)	**3.10**	9	75[c]	25[c]
3.5e	LaFG	2 (tetra-)	**3.11**	12	80	20
3.5f	GvaG	2 (tetra-)	**3.12**	12	85	15
3.5g	SMYG	2 (tetra-)	**3.13**	12	100	0
3.5h	lWMa	2 (tetra-)	**3.14**	12	100	0
3.5i	SMYTG	3 (penta-)	**3.15**	15	100	0
3.5j	LSASMYTG	6 (octa-)	**3.16**	24	100	0

[a] Ratios of monomeric to dimeric products were determined on the basis of the UV absorption signal at 220 nm in the LC-MS chromatogram. If not noted specifically, cleavages were conducted from low crosslinked, microporous polystyrene (2 % divinylbenzene, 1.6 mmol/g).

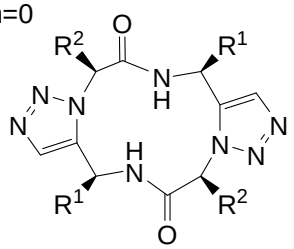
[b] Product ratio for cleavage from low crosslinked, microporous triphenyl phosphane polystyrene (2 % divinylbenzene, 1.6 mmol/g) and from highly crosslinked, macroporous polystyrene (>20 % divinylbenzene, 1.62 mmol/g).[c] Product ratio for cleavage from highly crosslinked, macroporous triphenyl phosphane polystyrene (> 20 % divinylbenzene, 1.62 mmol/g).

[d] Not isolated, products precipitate during purification.

The formation of dimeric products indicates a significant degree of site interaction within the polymer support. As site separation depends strongly on the flexibility of the polymer carrier, it should be enhanced by using more rigid supports such as macroreticular (macroporous) polystyrene. Use of macroreticular polystyrene is supposed to affect the cyclization reaction favoring monomeric over dimeric cyclic products. To test this assumption, azido-peptidyl-phosphoranes **3.5a,c,d** were prepared using macroporous resin with >20 % divinylbenzene (DVB) crosslinking, considerably more than the standard low crosslinked, microporous polystyrene resins with only 2 % DVB crosslinker. Both resins possessed an initial loading of *ca.* 1.6 mmol/g. Cyclization of the azido-tripeptidyl phosphorane **3.5c** on macroporous resin yielded 80 % of the monomeric product, indicating a significant shift towards the intra-site reaction pathway. Unfortunately, the cyclization products derived from **3.5c** precipitated during purification and thus could not be isolated. Therefore, the tripeptide precursor **3.5d** was synthesized both on micro- and macroporous resins replacing the proline residue by serine in order to improve the solubility of products. Here, cyclization on microporous resin delivered a ratio of 12:88 in favor of the dimeric (inter-site) cyclization product. Again the synthesis on macroporous support yielded the monomeric (intra-site) reaction product **3.10** in excess (75:25); **3.10** was isolated and characterized spectroscopically. On the other hand, the azido-dipeptidyl-phosphorane **3.5a** on macroporous resin upon heating in DMF still delivered exclusively the dimeric product **3.8**, albeit in significantly reduced yield (46 % instead of 78 %). Obviously, the inter-site cyclization pathway is favored strongly in these cases so that formation of the monomeric product was not detected even on the more rigid polymer. These results are surprising at first glance if one considers a concerted mechanism of the dipolar cycloaddition reaction. Experimental findings as well as calculations indicated, however, a step-wise reaction mechanism in the case of electron-rich dipolarophiles.[167-170] Considering phosphorous ylides as electron-rich dipolarophiles we can postulate a step-wise mechanism for their cycloadditions with azides as well. This step-wise mechanism, however, requires the attack of the ylide carbon at the terminal azide nitrogen (as depicted in Scheme **3**) and thus must disfavor intra-site reactions of shorter azido-peptidyl phosphoranes strongly compared to the concerted mechanism resulting in the formation of the dimeric products **3.8** and **3.9**.

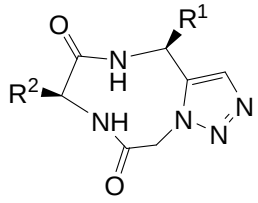


n=0



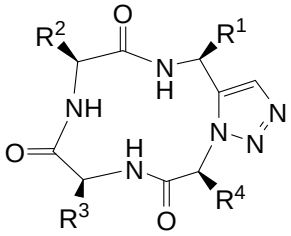
3.8: R^1, R^2: Ala, Gly;
Y: 78 %, P: 96 %
3.9: R^1, R^2: Phe, D-Ala;
Y: 54 %, P: 92 %

n=1



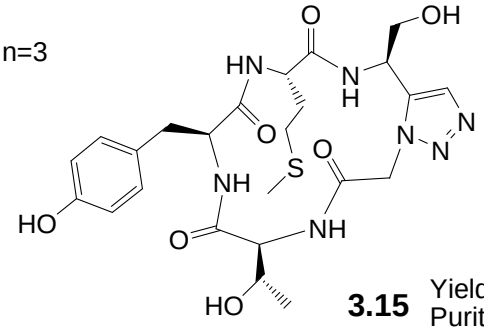
3.10 Y: 43%
P: 90%

n=2



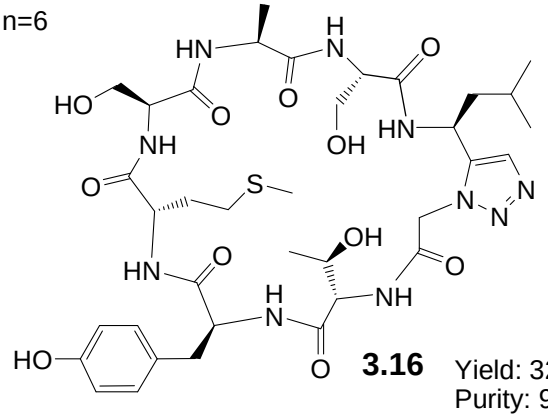
3.11: R^1- R^4: Leu, D-Ala, Phe, Gly; Y: 67%, P: 89%
3.12: R^1- R^4: Gly, D-Val, D-Ala, Gly; Y: 72%, P: 95%
3.13: R^1- R^4: Ser, Met, Tyr, Gly; Y: 48 %, P: 93 %
3.14: R^1- R^4: D-Leu, Trp, Met,D-Ala; Y: 64 %, P: 90 %

n=3



3.15 Yield: 37%
Purity: 91%

n=6



3.16 Yield: 32%
Purity: 94%

Scheme 3.4: Products formed by cyclative cleavage of azido-phosphoranes 3.5a-j. Yields and purities for 3.8, 3.9 and 3.11, 3.12, 3.14 are given for the crude products, data for compounds 3.10, 3.13, 3.15, and 3.16 are reported after HPLC purification.

❖

3.5 Summary and outlook

In summary, the work has investigated the power of the cyclative cleavage of azido-peptidyl-phosphoranes, a reaction reported here for the first time. Depending on the length of the starting material, on the building block sequence, and on the polymer rigidity the method delivered monomeric or dimeric products. Pure dimers were obtained from azido-dipeptidyl-phosphoranes (n=0). Tripeptidyl-phosphoranes (n=1) yielded mixtures of products, whereas longer starting materials provided monomeric products, either entirely pure (n>3) or with small dimeric impurities (n=3). Intra-site reaction products were significantly favored by using a more rigid, highly crosslinked polymer support. All products were isolated in good to excellent yields which exceeded strongly the reported results for comparable cyclizations in solution phase.[35] The solubility of the products was critical for the yields obtained and for the feasibility of chromatographic purification. All isolated products **3.8-3.16** were fully assigned in one- and two dimensional proton and ^{13}C-NMR spectra and characterized by HR-MS.

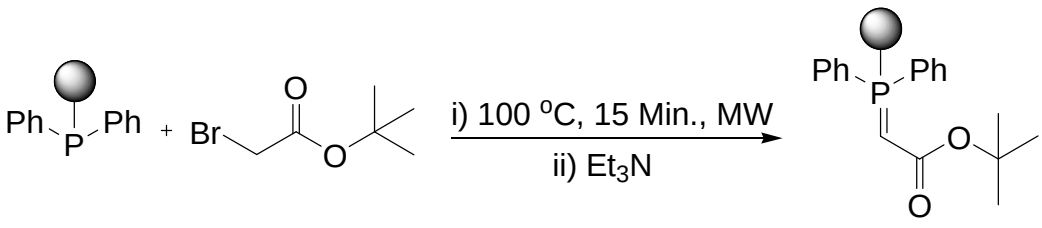
Moreover, the method completely avoided formation of soluble, non-cyclized, oligomeric by-products and is therefore superior to solution protocols with respect to synthetic efficiency, yields and purity. This access to *cis*-locked triazolyl-cyclopeptides should facilitate the systematic investigation of structural and biological properties of these compounds with already reported biological significance considerably.

3.6 Experimental section:

Reagents and general methods. Triphenylphosphane microporous polystyrene resin (2% DVB crosslinked, 1.6 mmol/g, Fluka), triphenylphosphane macroporous polystyrene resin (>20 % DVB crosslinked, 1.62 mmol/g, Varian), Fmoc-amino acids and all other reagents were purchased from commercial suppliers, unless synthesis stated. Anhydrous solvents were purchased and stored over molecular sieves. Fmoc solid-phase peptide synthesis was conducted in plastic syringes equipped with PE filters. Loading was determined by photometric methods after Fmoc cleavage. Solid phase reactions were monitored through FT-ATR-IR spectra of the resins with a Bruker Vector 22/Harrick SplitPea ATR unit. Cleaved products were analyzed by an analytical HPLC column (5 µm, 250 x 2 mm, Nucleosil 100 RP-C18) with detection at 220 nm and 254 nm. Eluents A (0.1% TFA in water) and B (0.1% TFA in acetonitrile) or (0.1% TFA in methanol) were used in a linear gradient (20% B 100% B in 45 min.). Compounds were purified on a semipreparative HPLC column (10 µm, 250 x 20 mm, Grom-SIL 300 ODS-5 ST RP-C18) employing individual gradients derived from analytical runs (eluents A and B). LC-MS were recorded on an Agilent 1100 series chromatography workstation (Agilent Technologies) equipped with a single quadrupole mass spectrometer and electrospray ionization (ES). HRMS measurements were conducted with an Agilent 6220 ESI-TOF mass spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE 300 MHz instrument and chemical shifts (δ) were measured in parts per million (ppm) relative to trimethylsilane (TMS). Spectra were assigned employing two-dimensional spectra.

Synthesis of polymer-supported peptidyl phosphoranes

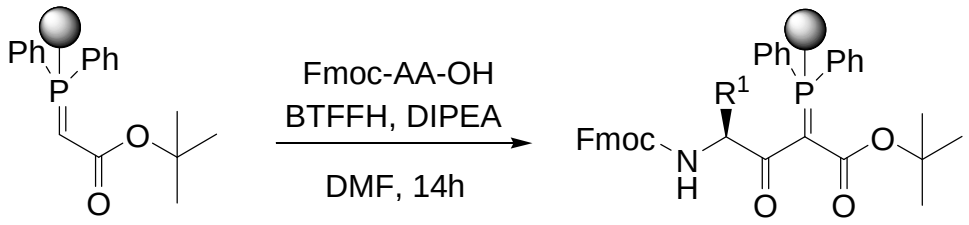
Polymer-supported 2-phosphoranylidene acetate 3.1



3.1

Triphenylphosphane polystyrene resin (0.5 g, 1.6 mmol/g, 0.8 mmol, 1% divinyl benzene, 100-200 mesh) was weighed into a microwave vial and suspended in dry toluene (4 mL). After addition of 2-bromoester (4 mmol, 5 equiv.), the vial was sealed and heated at 100 ℃ for 15 min in a microwave synt hesizer. The vial was cooled to room temperature before opening, the resin was filtered and washed with dry toluene and CH_2Cl_2. The obtained polymer phosphonium salt was resuspended in dry CH_2Cl_2 (5 mL) and TEA (557 µL, 4 mmol, 5 equiv.) was added. After shaking for 2 h at RT, the yellow-coloured resin **1** was filtered, washed with CH_2Cl_2 and dried in vacuum.

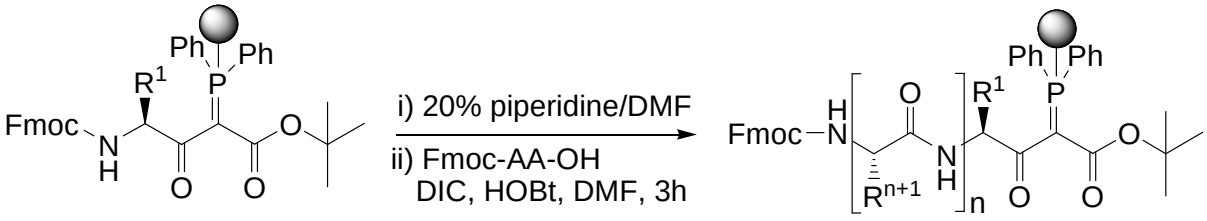
Acylation of 3.1 with Fmoc amino acid



3.1 **3.2**

Resin **3.1** (300 mg, 1.32 mmol/g, 0.396 mmol) was pre-swollen in dry DMF. The Fmoc amino acid (1.98 mmol, 5 equiv.) was dissolved in dry DMF (6 mL) after addition of diisopropylethyamine (DIPEA) (3.96 mmol, 10 equiv.) and BTFFH (1.98 mmol, 5 equiv.) The clear solution obtained was directly mixed with the resin suspension and shaken for 14h at room temperature. The resin was washed thoroughly with DMF and THF after the coupling step and dried in vacuum.

Polymer-supported peptidyl-2-phosphoranylidene acetate 3.3



3.2 **3.3**

Fmoc protection was cleaved with 20 % piperidine/DMF (v/v, 2x6 min). The deprotected resin (300 mg, 0.315 mmol) was washed and suspended in dry DMF.

Fmoc amino acid (1.98 mmol, 5 equiv.) and HOBt·H_2O (1.98 mmol, 5 equiv.) were dissolved in dry DMF (6 mL), pre-activated under addition of DIC (1.98 mmol, 5 equiv.) and added to the resin. The mixture was shaken for 3 h at RT, filtered, and the resin was washed with DMF and THF.

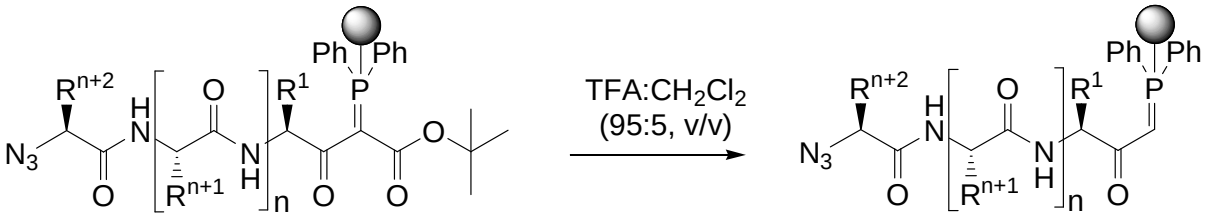
All the sequence of steps, stated, was repeated n-times. Quantitative couplings were verified by the Kaiser test.

N-terminal azido-peptidyl-2-phosphoranylidene acetate 3.4

3.3 **3.4**

After Fmoc deprotection of **3.3**, 2-azido acid (2-azido glycine **3.6** or 2-azido alanine **3.7**) was used instead of amino acid in the final coupling step. The remaining procedure was conducted as stated before.

Polymer-supported azido-peptidyl phosphoranes 3.5



3.4 **3.5**

The resin **3.4** was treated with 8 mL of TFA:CH_2Cl_2 (95:5) for 14 h to remove the *tert*-butyl group as well as side chain protections. After washing the resin with CH_2Cl_2 (x

5), neutralization was carried out by treating the resin with DIPEA:CH_2Cl_2 (1:9, v:v) (3x5 min) followed by CH_2Cl_2 washing (x 5).

Macroporous polymer-supported azido-peptidyl phosphoranes 3.5

N-terminal azido-peptidyl phosphorane bound to macroporous polystyrene resin was prepared by using the exactly similar protocol as described in previous steps with difference of only using polystyrene to which triphenylphosphane is attached. The yield of acylated phosphoranyldene acetate **2** drops to 0.76 mmol/g while peptide coupling steps are quantitative for macroporous polystyrene.

Preparation of triflic azide

Sodium azide (1.76 g, 27.1 mmol) was dissolved in water (5 ml), in a round bottom flask and placed in ice bath. Triflic anhydride (0.9 ml) was added drop-wise to the solution. The reaction mixture was stirred for 2 h at room temperature. Finally, the triflic azide was extracted with CH_2Cl_2 (4 mL x 2) and washed with saturated Na_2CO_3 solution (8 mL). CH_2Cl_2 solution containing triflic azide was used as such for azido-alanine synthesis.[171]

Preparation of azido acids[118]

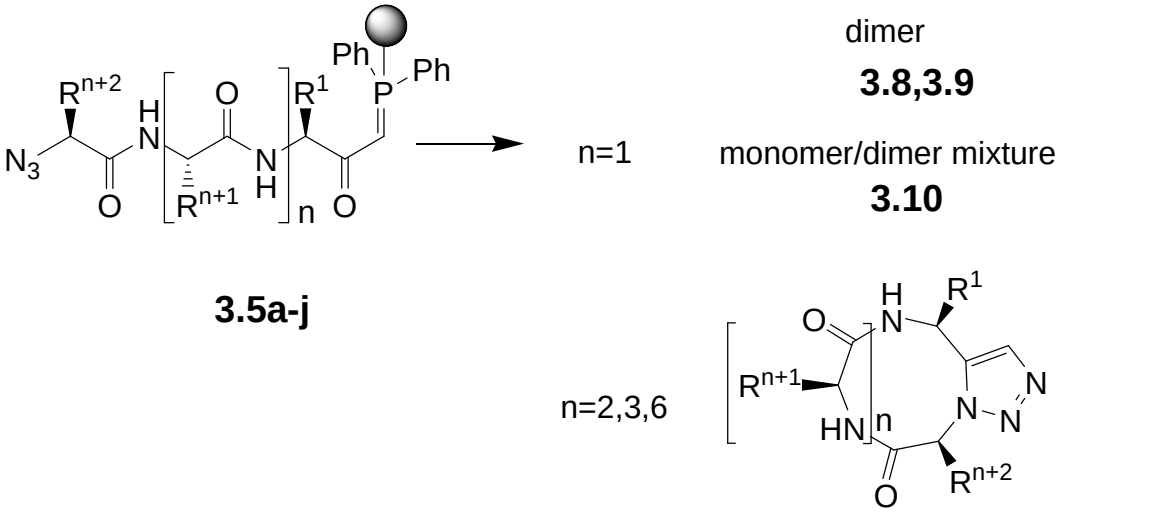
2-Azido glycine 3.6

To a solution of sodium azide (5.14 g, 79.2 mmol, 2.1 equiv.), partially dissolved in DMSO, bromoacetic acid (5.24 g, 37.7 mmol) was added and the mixture was stirred for 14 h at room temperature. The reaction mixture was diluted with water (250 mL), the pH was adjusted to 2.5 with conc. HCl, and the product was extracted with ethyl acetate (2 x 400 mL). The extract was washed with brine (x 4). After drying with $MgSO_4$, ethyl acetate was evaporated under reduced pressure to deliver pale yellow oil **3.6** (2.52 g, 65 %) which was used without further purification.

2-Azido alanine 3.7

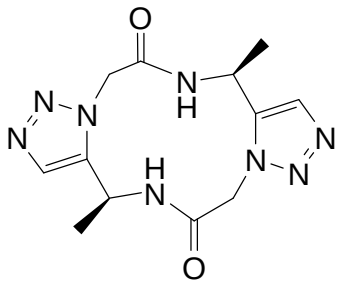
D-alanine (248.3 mg, 2.79 mmol), K_2CO_3 (477.5 mg, 4.19 mmol) and $CuSO_4$ pentahydrate (6.98 mg, 27.9 µmol) were dissolved in water (9 mL) and methanol (18 mL). Triflic azide in CH_2Cl_2 was added to the mixture and stirred for 14h at room temperature. Organic solvents were removed under reduced pressure and the obtained slurry was diluted with water (50 mL). The solution was acidified with conc. HCl to pH 6.5 and further diluted with phosphate buffer (50 mL, 0.25 M, pH 6.2). Sulfonamide by-product was extracted with ethyl acetate (20 mL x 2). The aqueous phase was acidified to pH 2.5 and product was extracted with ethyl acetate (20 mL x 3). The extract was dried with $MgSO_4$ and evaporated to give pale yellow oil (167 mg, 52 %).

Cyclization of azido-peptidyl phosphoranes 3.5



Azido-peptidyl phosphorane **3.5** (300 mg, 0.315 mmol) was swollen in dry DMF (4 mL) and heated in a sealed glass vial at 80 ^{o}C for 14 hours. After cooling to room temperature, the support was filtered off and washed with extra DMF (2 mL x 5) with shaking. All the washing fractions were combined and DMF was evaporated under reduced pressure to get the solid products. Compounds **3.8, 3.9** and **3.11, 3.12, 3.14** were pure enough in crude form to be characterized through NMR spectroscopy while **3.10**, **3.13, 3.15,** and **3.16** were purified through reverse phase preparative HPLC prior to NMR analysis.

When **3.5** was di-peptidyl phosphorane (n=0 in scheme), dimeric products **3.8** and **3.9** were obtained exclusively on cyclization.



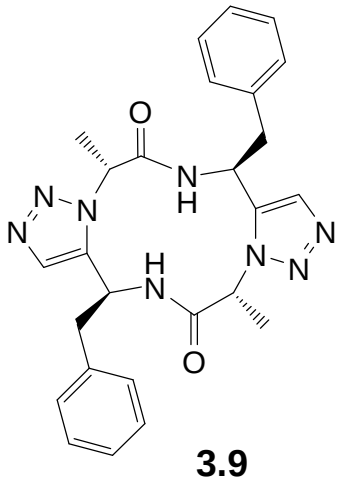
3.8

3.8: Yield: 75 mg (78%)

^{1}H-NMR: (300 MHz, DMSO-d_6): δ = 1.45 (d, *J* = 6.72 Hz, 6H, 2*CH*$_3$, 2Ala), 5.03 (dd, 4H, *J*$_1$,*J*$_2$ = 17.09 Hz, 2*CH*$_2$, 2Gly), 5.08–5.15 (m, 2H, 2*CH*, 2Ala), 7.63 (s, 2H, 2*CH*, triazole), 8.00 (bs, 2H, 2*NH*).

^{13}C-NMR: (75.5 MHz, DMSO-d_6): δ = 18.7, 40.4, 50.8, 132.6, 139.1, 164.7.

HRMS (ESI): Calculated for $C_{12}H_{16}N_8O_2$ $[M+H]^+$: 305.1475 Da. Found: 305.1478 m/z.



3.9

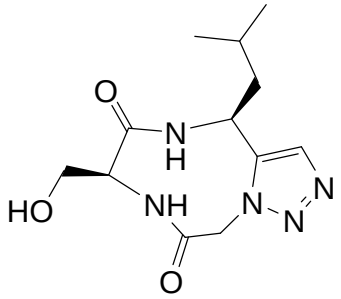
3.9: Yield: 82 mg (54%)

^{1}H-NMR: (300 MHz, DMF-d_7): δ = 1.46 (d, *J* =7.33 Hz, 6H, 2*CH*$_3$, 2 D-Ala), 3.20 (d, *J* =7.93 Hz, 4H, 2*CH*$_2$, 2Phe), 5.32 (dd, *J* =7.32, 7.93 Hz, 2H, 2*CH*, 2Phe), 5.54 (q, *J* =6.71 Hz, 2H, 2*CH*, 2 D-Ala), 7.14–7.49 (m, 10H, arom., 2Phe), 7.68 (s, 2H, 2*CH*, triazole), 8.54 (d, *J* =7.32 Hz, 2H, 2*NH*).

^{13}C-NMR: (75.5 MHz, DMSO-d_6): δ = 17.6, 40.7, 46.8, 59.1, 127.2, 128.8, 130.1, 134.7, 136.8, 137.9, 168.1.

HRMS (ESI): Calculated for $C_{26}H_{28}N_8O_2$ $[M+H]^+$: 485.2414 Da. Found: 485.2415 m/z.

When **3.5** was azido-tripeptidyl phosphorane (n=1 in scheme), compound **3.10** was found to be mainly dimeric product (88%). The use microporous resin, however, gave a mixture of monomer and the respective dimer in a ratio of 3:1 in favor of monomer.



3.10

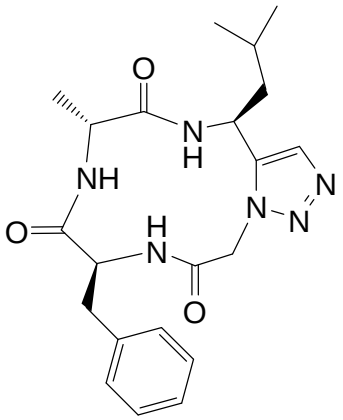
3.10: Yield: 48 mg (43%)

^{1}H-NMR: (300 MHz, DMSO-d_6): δ = 0.89 (dd, *J* = 5.5 Hz, 6H, *CH*$_3$, Leu), 1.39-1.67 (m, 3H, *CH*$_2$, *C*$^{\beta}$*H*, Leu), 3.54–3.61 (m, 2H, *CH*$_2$, Ser), 4.11-4.16 (m, 1H, *CH*, Ser), 5.07 (dd, *J* = 17.1 Hz, *CH*$_2$, Gly), 5.17-5.25 (m, 1H, *C*$^{\alpha}$*H*, Leu), 7.64 (s, 1H, *CH*, triazole), 7.89, 8.26 (2d, *J* = 7.3, 9.7 Hz, 2H, 2*NH*).

^{13}C-NMR: (75.5 MHz, DMSO-d_6): δ = 21.4, 23.5, 27.6, 30.8, 139.5, 162.3, 164.7, 168.2.

HRMS (ESI): Calculated for $C1_{12}$ $H_{19}N_5O_3$ $[M+H]^+$: 282.1566 Da. Found: 282.1568 m/z.

When **3.5** was tetra-, penta- and octa-azido-peptidyl phosphorane (n=2,3,6 in scheme), compounds **3.13-3.16** were found to be exclusively monomer when analyzed by LCMS while **3.11** and **3.12** were found to be monomer as major product with minor dimer (20% and 15% respectively) .



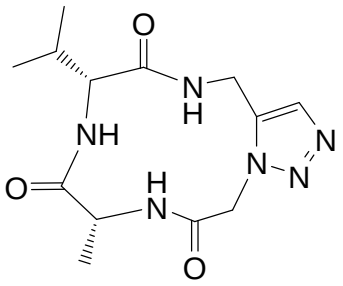
3.11

3.11: Yield: 87 mg (67%)

^{1}H-NMR: (300 MHz, DMF-d_7): δ = 0.94, 0.96 (2d, *J* = 6.7, 7.3 Hz, 6H, *CH*$_3$, Leu), 1.26 (d, 3H, *J* = 6.7 Hz, *CH*$_3$, D-Ala), 1.66-1.94 (m, 3H, *CH*$_2$, *C*$^{\gamma}$*H*, Leu), 2.96–3.17 (m, 2H, *CH*$_2$, Phe), 4.10-4.14 (m, 1H, *CH*, Phe), 4.68-4.74 (m, 1H, *C*$^{\alpha}$*H*, D-Ala), 5.12 (dd, *J* = 17.1 Hz, *CH*$_2$, Gly), 5.36-5.44 (m, 1H, *C*$^{\alpha}$*H*, Leu), 7.10-7.30 (m, 5H, arom., Phe) 7.72 (s, 1H, *CH*, triazole), 8.33, 8.53 (2d, *J* = 6.1, 9.1 Hz, 2H, 2*NH*), 8.36 (m, 1H, *NH*).

^{13}C-NMR: (75.5 MHz, DMF-d_7): δ = 17.4, 21.9, 23.1, 25.3, 39.0, 40.6, 42.3, 50.3, 54.8, 126.9, 128.5, 130.2, 131.9, 137.9, 140.5, 166.5, 170.7, 173.2.

HRMS (ESI): Calculated for $C_{21}H_{28}N_6O_3$ $[M+H]^+$: 413.2301 Da. Found: 413.2304 m/z.



3.12

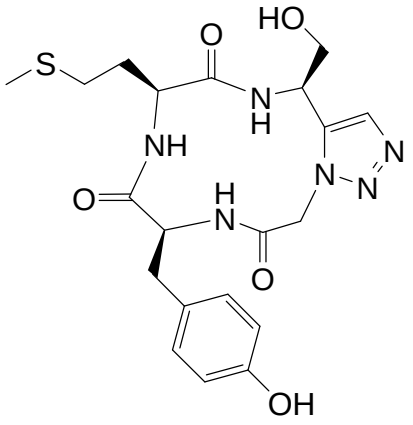
3.12: Yield: 71 mg (72%)

^{1}H-NMR: (300 MHz, DMF-d_7): δ = 0.82, 0.85 (2d, *J* = 6.7 Hz, 6H, *CH*$_3$, D-Val), 1.40 (d, 3H, *J* = 7.3 Hz, *CH*$_3$, D-Ala), 2.02–2.11 (m, 1H, *C*$^{\beta}$*H*, D-Val), 4.12 (dd, 1H, *J* = 6.7,

7.3 Hz, $C^{\alpha}H$, D-Val), 4.45–4.56 (m, 2H, CH_2, Gly), 5.41 (s, 1H, CH_2, Gly), 7.64 (s, 1H, *CH*, triazole), 8.08, 8.65 (2d, *J* = 6.7, 2H, 2*NH*), 8.36 (dd, *J* = 4.8, 5.4, 1H, *NH*).

^{13}C-NMR: (75.5 MHz, DMF-d_7): δ = 18.4, 18.7, 19.4, 31.6, 49.8, 50.8, 59.9, 134.0, 135.6, 166.5, 171.5, 173.3.

HRMS (ESI): Calculated for $C_{13}H_{20}N_6O_3$ $[M+H]^+$: 309.1675 Da. Found: 309.1677 m/z.



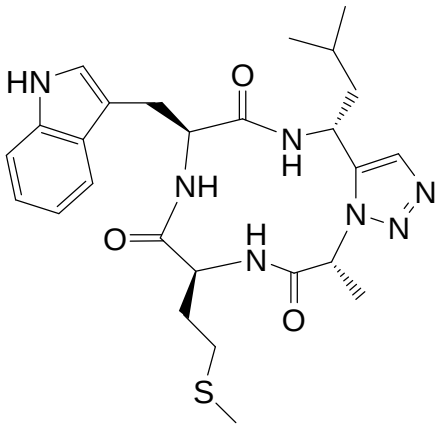
3.13

3.13: Yield: 70 mg (48%)

^{1}H-NMR: (300 MHz, Methanol-d_4): δ = 1.90-1.98, 2.09-2.22 (2m, 2H, CH_2, Met), 2.06 (s, 3H, CH_3, Met), 2.45-2.57 (m, 2H, CH_2, Met), 2.79-2.87, 3.04-3.11 (2m, 2H, CH_2, Tyr), 3.84-3.87 (m, 2H, CH_2, Gly), 3.88-3.91 (m, 1H, *CH*, Tyr), 4.43-4.63 (m, 3H, CH_2, Ser, *CH*, Met), 5.92 (bs, 1H, *CH*, Ser), 6.63-6.70, 7.01-7.08 (2m, 4H, arom., Tyr), 6.73 (s, 1H, triazole).

^{13}C-NMR: (75.5 MHz, Methanol-d_4): δ = 15.2, 24.1, 31.1, 31.6, 38.0, 52.6, 54.4, 56.2, 111.7, 116.3, 127.3, 128.5, 131.3, 139.9, 157.4, 170.0, 171.9, 173.9.

HRMS (ESI): Calculated for $C_{20}H_{26}N_6O_5S$ $[M+H]^+$: 463.1764 Da. Found: 463.1765 m/z.



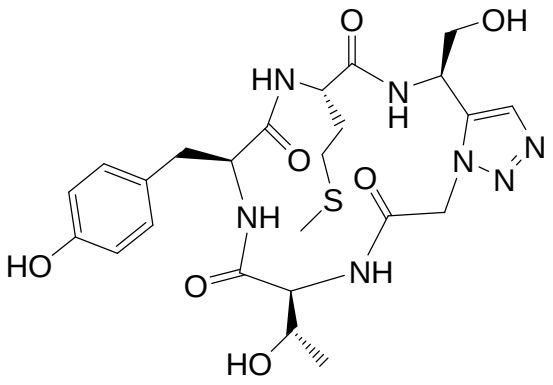
3.14

3.14: Yield: 105 mg (64%)

^{1}H-NMR: (300 MHz, DMF-d_7): δ = 0.99, 1.06 (2d, J_1=J_2 = 6.1 Hz, 6H, *CH*$_3$, Leu), 1.65-1.92 (m, 5H, *CH*$_2$, *C*$^{\gamma}$*H*, Leu, *C*$^{\beta}$*H*$_2$, Met), 1.95 (s, 3H, CH_3, Met), 1.97 (d, 3H, *J* = 6.7 Hz, *CH*$_3$, Ala), 2.30-2.41 (m, 2H, *C*$^{\gamma}$*H*$_2$, Met), 3.18 (dd, J_1=31.7 Hz, J_2=11.6 Hz, 2H, *CH*$_2$, Trp), 4.23-4.30 (m, 1H, *CH*, Trp), 4.54-4.62 (m, 1H, *CH*, Ala), 5.07-5.14 (m, 1H, *CH*, Met), 5.35-5.44 (m, 1H, *C*$^{\alpha}$*H*, Leu), 6.95-7.10, 7.37-7.41, 7.69-7.72 (3m, 5H, arom., Trp) 7.74 (s, 1H, *CH*, triazole), 7.85, 8.39, 8.63 (3d, *J* = 7.9, 9.1, 9.7 Hz, 3H, 3*NH*), 10.89 (s, 1H, *NH*, Trp).

^{13}C-NMR: (75.5 MHz, DMF-d_7): δ = 14.9, 17.4, 22.2, 23.6, 25.2, 32.1, 41.2, 43.0, 48.9, 56.6, 57.5, 110.6, 111.9, 119.0, 121.6, 125.2, 128.1, 132.2, 137.4, 139.5, 170.2, 171.8, 172.7.

HRMS (ESI): Calculated for $C_{26}H_{35}N_7O_3S$ $[M+H]^+$: 526.2600 Da. Found: 526.2603 m/z.



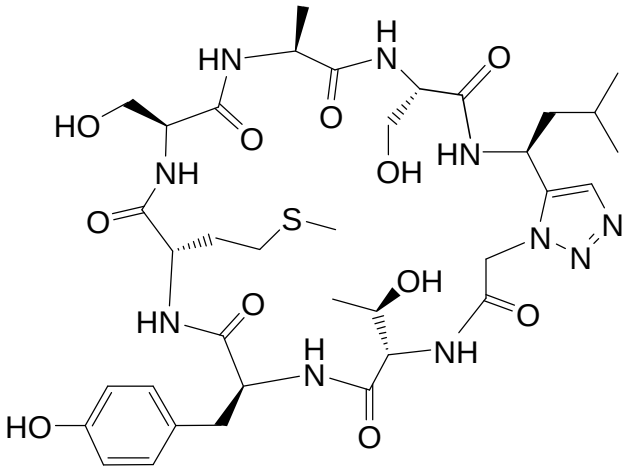
3.15

3.15: Yield: 65.8 mg (37%)

^{1}H-NMR: (300 MHz, Methanol-d_4): δ = 1.12 (d, *J* = 6.1 Hz, 3H, *CH3*, Thr), 1.87-2.01, 2.08-2.17 (2m, 2H, *CH_2*, Met), 2.06 (s, 3H, *CH_3*, Met), 2.40-2.57 (m, 2H, *CH_2*, Met), 2.85-2.91, 3.04-3.11 (2m, 2H, *CH_2*, Tyr), 3.96 (s, 2H, *CH_2*, Gly), 3.98-4.14 (m, 2H, *CH_2*, Ser), 4.28-4.35, 4.51-4.63 (2m, 3H, *3CH*, Met, Thr, Tyr), 5.91 (s, 1H, *CH*, Ser), 6.62-6.68, 7.00-7.08 (2m, 4H, arom., Tyr), 6.70 (s, 1H, triazole).

^{13}C-NMR: (75.5 MHz, Methanol-d_4): δ = 15.2, 19.9, 24.2, 31.1, 31.7, 36.9, 37.7, 52.8, 54.5, 56.3, 59.9, 68.4, 111.9, 116.3, 119.2, 128.6, 131.4, 139.9, 157.3, 164.8, 170.4, 171.9, 173.8.

HRMS (ESI): Calculated for $C_{24}H_{33}N_7O_7S$ $[M+H]^+$: 564.224 Da. Found: 564.2246 m/z.



3.16

3.16: Yield: 84.3 mg (32%)

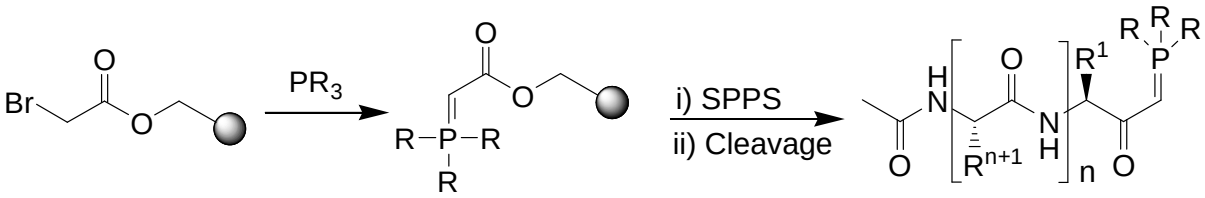
^{1}H-NMR: (300 MHz, DMSO-d_6): 0.83 (d, *J* = 5.5 Hz, 6H, *2CH$_3$*, Leu), 1.17 (d, *J* = 5.5 Hz, 3H, *CH$_3$*, Thr), 1.27 (d, *J* = 7.3 Hz, 3H, *CH$_3$*, Ala), 1.55-1.78 (m, 3H, *CH$_2$*, CH, Leu), 1.91-1.98, 2.04-2.33 (2m, 4H, *2CH$_2$*, Met), 2.0 (s, 3H, *CH$_3$*, Met), 2.86-2.95 (m, 2H, *CH$_2$*, Tyr), 3.51-3.68 (m, 4H, *2CH$_2$*, 2Ser), 4.01-4.14, 4.26-4.35, 4.42-4.51, 4.53-4.63, 4.88-4.98 (5m, 7H, *6C$^{\alpha}$H*, *C$^{\beta}$H*, 2Ser, Ala, Met, Tyr, Thr), 4.90 (dd, *J* = 17.7 Hz, 2H, *CH$_2$*, Gly), 5.5 (s, 1H, *C$^{\alpha}$H*, Leu), 5.71 (bs, -OH, Thr), 6.66, 7.05 (2d, *J* = 7.9, 8.5 Hz, 4H, arom., Tyr), 7.70 (s, 1H, triazole), 7.22, 7.73, 7.73, 8.35, 8.66, 8.66, 8.81 (7d, 7H, NH), 9.23 (bs, 1H, -OH, Tyr).

^{13}C-NMR: (75.5 MHz, DMSO-d_6): δ = 14.8, 16.5, 18.6, 21.6, 22.6, 23.9, 29.9, 31.6, 41.1, 49.7, 50.3, 51.5, 53.8, 55.4, 57.1, 60.1, 115.1, 127.2, 129.8, 138.8, 156.0, 165.9, 170.1, 170.3, 170.8, 173.2.

HRMS (ESI): Calculated for $C_{36}H_{54}N_{10}O_{11}S$ $[M+H]^+$: 835.3772 Da. Found: 835.3774 m/z.

4 An approach towards soluble peptidyl-phosphorane's synthesis and fine tuning of their reactivity for biocompatible ligations

Wang resin was esterified with bromoacetyl bromide and support-bound bromoesters were further used for alkylation of trivalent phosphanes. Supported phosphoranylidene acetates were then *C*-acylated with an activated amino acid without racemization. Peptide was built over the amino acyl phosphorane and amine terminus of the peptide was capped with acetyl group. TFA treatment of the resulting polymer support released the peptidyl-phosphoranes into solution, cleavage being result of ester saponification accompanied with decarboxylation. This synthetic strategy, thus, saved an extra protection and deprotection step involved in the synthesis of solid supported peptidyl-phosphorane, reported in last chapters. Further this scheme carries an electronic knob for reactivity control of the soluble peptidyl-phosphoranes which have potential to be used in biocompatible ligations.

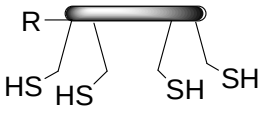
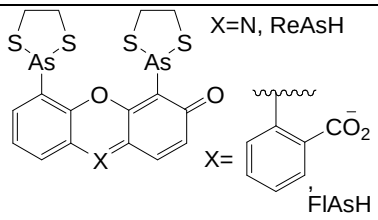
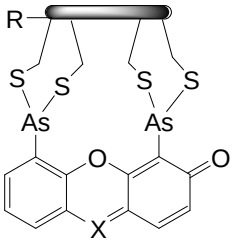
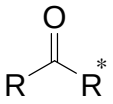
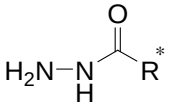
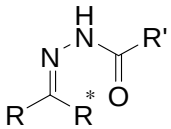
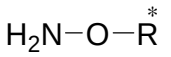
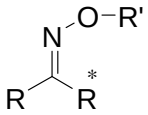
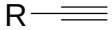
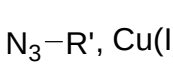
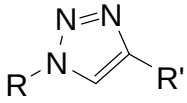
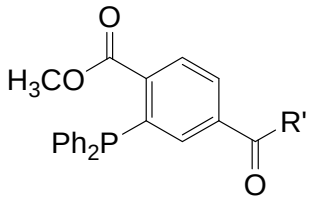
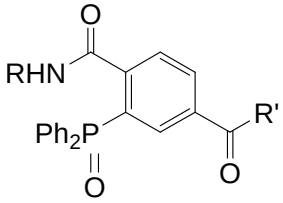
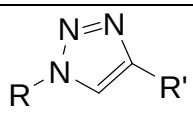
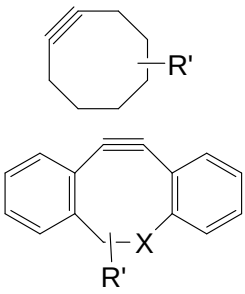
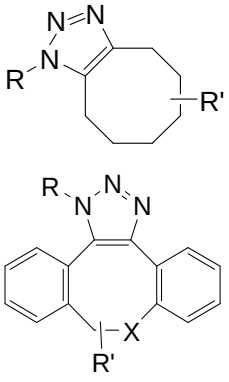


4.1 Bioorthogonal and biocompatible reactions

The term “click chemistry”, frequently encountered in literature, was coined by Sharpless for a set of reactions that meet the necessary criteria of being selective, high yielding and having good kinetics.[45] A subclass of click reactions which go unhindered and are inert to the surrounding biological milieu are termed as “bioorthogonal reactions”, thus, facing a higher degree of selection due to added complications of biocompatibility.[59, 63] Among the organic reactions, quite few reactions meet the criterion of biocompatibility due to strict checks *e.g* at the first place remove all the reactions sensitive to water. Secondly, with abundant supply of amines and thiols, reagents prone to nucleophilic attack must be avoided. Third, because of the reducing environment in the cytosol, redox sensitive reactions must be removed. A biocompatible reaction should not require heat (above 37 ^{o}C), pressure or high concentrations.[172]

So far only a handful of chemical motifs are known to possess the requisite qualities of biocompatibility and bioorthogonality. This elite group comprises peptide sequences that can be ligated with small molecule imaging probes,[173-181] cell surface electrophiles that can be tagged with hydrazide and aminooxy derivatives,[179, 182-186] nitrones that can be derivatized with activated alkynes,[68] azides that can be selectively modified with phosphines and activated alkynes,[57, 58, 63-67, 99, 187] and terminal alkynes that can be ligated with azides.[188] A brief account of bioorthogonal reactions is summarized in table **4.1**

Table 4.1: Common chemical reporters and bioorthogonal reactions used in living systems

No.	Chemical reporter	Reactive partner	Ligation product	Target (R)
1	 Tetracycteine motif	 X=N, ReAsH X= , FlAsH		Protein
2	 Ketone/ aldehyde			Protein Glycan
				
3	 Terminal alkyne	Click chemistry 		Protein
4	 Azide	Staudinger Ligation 		Protein, Glycan, Lipid
		Click chemistry 		
		Strain induced cycloaddition 		

4.2 Applications of biocompatible reactions

Living systems, consisting of biopolymers, metabolites, and ions form a complex and interacting network which is responsible of cellular processes. Role of these biomolecules is difficult to understand in their isolated and purified form, therefore, biocompatible reactions are being developed and applied to understand role and functioning of biomolecules in their native state and hence biological processes. Major application of biocompatible reactions is imaging biomolecules in their native state, target guided synthesis is another emerging area for the application of such mild reactions.[189]

4.2.1 Labelling and imaging of biomolecules

This approach involves incorporation of a unique chemical functionality, a bioorthogonal chemical reporter, into the target biomolecule using the cell's own synthetic machinery and modification of this reporter in living cells through highly selective chemical reactions with exogenously delivered probes.[59]

The bioorthogonal chemical reporter strategy involves the incorporation of unique functionalities into the target of interest, followed by chemical labelling with small molecule probe. Ideally, the reporter should be incorporated without any structural perturbation. To date, bioorthogonal chemical reporter have been non-native combinations of endogenous functionality or small, abiotic functional groups that can slip through existing biosynthetic pathways for their incorporation. Once installed in the target biomolecules, the chemical reporter must be reacted with a probe bearing a complementary functional group. Bioorthogonal reactions from the literature, used for labelling biomolecules, are briefly listed in Table **4.1**.

4.2.2 Target guided synthesis (TGS)

More than 60 % of the weight of a typical mammalian cell consists of proteins. Thus, proteins (especially enzymes) are prime targets to understand biological functions. Many drug molecules are enzyme inhibitors, so their discovery and improvement is an active area of research in biochemistry and pharmacology.

Target guided synthesis (TGS) is based on the concept of using target molecule (usually an enzyme) itself for forming its own inhibitors from a collection of building blocks reagents. Only building blocks which interact with separate binding sites on the protein's surface will react with each other, if their reactive group are in close

proximity. This will result in highly potent protein-effectors as the resulting polydentate ligand simultaneously addresses multiple binding pockets.[190-193] Rideout was the pioneer of TGS concept. Since then several approaches have been explored which can be classified broadly into three categories, described in the following.

4.2.2.1 Dynamic combinatorial chemistry

The Dynamic combinatorial chemistry approach, introduced by Lehn, is based on the principle of shifting a thermodynamic equilibrium by interactions with the target protein. Building blocks that react reversibly with each other form a thermodynamically controlled mixture of products. In the presence of target molecule, the equilibrium is shifted towards the compounds showing higher target affinity. In order to identify all the reaction products, the equilibrium has to be frozen (e.g by lowering pH). The approach is hampered by the analysis of a complex mixture of products and an additional step to freeze the equilibrium that might be limiting the number of building blocks due to incompatible functional groups. It is futher limited to reactions forming products in equilibrium.[74, 75, 194-196]

4.2.2.2 Stepwise target guided synthesis

The stepwise TGS make indirect use of the target molecule for ligand synthesis and consists of three steps; first a library of building blocks is screened to identify candidates that bind to the target. Then, building blocks with highest affinity are linked together using conventional combinatorial synthesis approaches. Resulting “divalent” effectors’ library is then screened to find ligands with high binding constant using traditional assays. This approach suffers from the drawback of screening of possibly lot of compounds.[197, 198]

4.2.2.3 Kinetically controlled target guided synthesis

This approach is based on the irreversible reaction between pairs of building blocks, influenced by target molecules that act like a catalyst, leading to a kinetic product.[199] Mostly, the target accelerates the formation of the product that best fits its binding pockets, as it lowers its energy of transition. The role of target is to bind with reagents and hold them in close proximity until they are connected through an “arranged” chemical reaction. Kinetically controlled TGS have been applied for different targets including glycinamide ribonucleotide transformylase (GAR Tfase), carbonic anhydrase, acetylcholinesterase etc.[200-210]

The scope of TGS is limited by the requirement of highly reactive groups (strong electrophiles or nucleophiles, metathesis catalysis etc.). As high reactivity goes hand in hand with low selectivity, those reagents can react in many pathways including ones that even destroy the protein. As proteins carry different functional groups like, amines, thiols, carboxylic acids etc., bioorthogonal reactions are best suited for TGS, being inert to biomolecules.[211, 212]

4.3 Design of bioorthogonal reagents

One of the most popular bioorthogonal functionality is the azide. Azides do not react appreciably with water and are resistant to oxidation. Additionally, azides are mild electrophiles; they do not react with hard nucleophiles that are abundant in biological systems.[213] Azides are succeptible to reduction by free thiols including ubiquitous glutathione.[214-216] However, reaction between mono thiols and alkyl azides requires vigorous heating (heating at 100 ^{0}C for several hours) or auxiliary catalysis.[217, 218] Despite these promising qualities, azides have only recently been used in chemistry of living systems and reason for the hesitation was the assumption of their instability and toxicity. However, it has been observed that azides are decomposed only at very high temperature but quite stable at physiological even moderately high temperature. Likewise, organic azides have been found to be non toxic unlike sodium azide.[213]
Additionally the azide has unique reaction modes with other bioorthogonal functionalities, most notably phosphanes and alkynes. Indeed the reaction of azides with triarylphosphines equipped with an ester, a process termed as Staudinger Ligation, is one of the early examples of bioorthogonal reactions performed in living systems.[99] Modification of azides incorporated in biomolecules does not hamper living systems but still Staudinger ligands face danger of oxidation to phosphane-oxide before ligation reaction, thereby, limiting the amount of reagent for the specified reaction. Moreover, Staudinger ligation is a relatively slow reaction (k = 2.5x10^{-3} M^{-1} s^{-1}) making it less favourite for being used in living systems where concentrations of the reagents are limited.[219] Improvements in the reaction kinetics and water solubility of the reagents are also troublesome. While improving the biocompatibility of the Staudinger ligation, chemical biologists harnessed another bioorthogonal reaction for the modification of azides in biomolecules. In Staudinger ligation, azide act as an electrophile and subject to react with soft nucleophiles (triphenylphosphane) but they are also dipoles and can react with dipolarophiles (such as alkynes). This azide-alkyne cycloaddition, discovered by Huisgen,[37] has found enormous applications as

a bioorthogonal reaction after Sharpless and Meldal discovery that *Cu*-I can catalyze the reaction to occur at physiological temperature and in aqueous medium.[43, 44] Despite advantages associated with click reaction in terms of kinetics and regioselectivity, it is not ideal for use in cellular systems (let alone the living organisms) due to copper toxicity. Scope of click materials is also limited due to the residual traces of copper.[54-57] To improve the biocompatibility of the azide-alkyne cycloaddition, Bertozzi and co-workers made use of strained alkynes instead of metal catalysis for rate acceleration but at the cost of regioselectivity.[62] This was an attractive extension of the azide-alkyne cycloaddition reaction in terms of biocompatibility but still the reaction is very slow as compared to Cu-catalyzed reaction and reaction rate is comparable to Staudinger ligation. Although, reaction kinetics is much improved after use of second generation cyclooctyne derivatives[63-68] however, it is not without disadvantages. It has *e.g* limitations for the tasks where regioselectivity really does matter.

4.4 Aim of the present work

Among bioorthogonal reagents, azides are the most popular ones. It is desirable to enrich the list of complementary functional groups to react with azides. Among available complementary reagents, triaryl-phosphanes react very slowly and alkynes either need metal catalysis or lose regioselectivity (in case of strained alkynes). We have reported 1,3-dipolar cycloaddition reaction between azides and solid supported peptidyl-phosphoranes to give 1,5-disubstituted triazoles regioselectively[166] and the reaction is devoid of any metal catalysis but retains regioselectivity, therefore has potential to be developed into the biocompatible reaction. Once developed, the reaction has potential to probe azides incorporated in biomolecules, especially in cases where regioselectivity does matter.

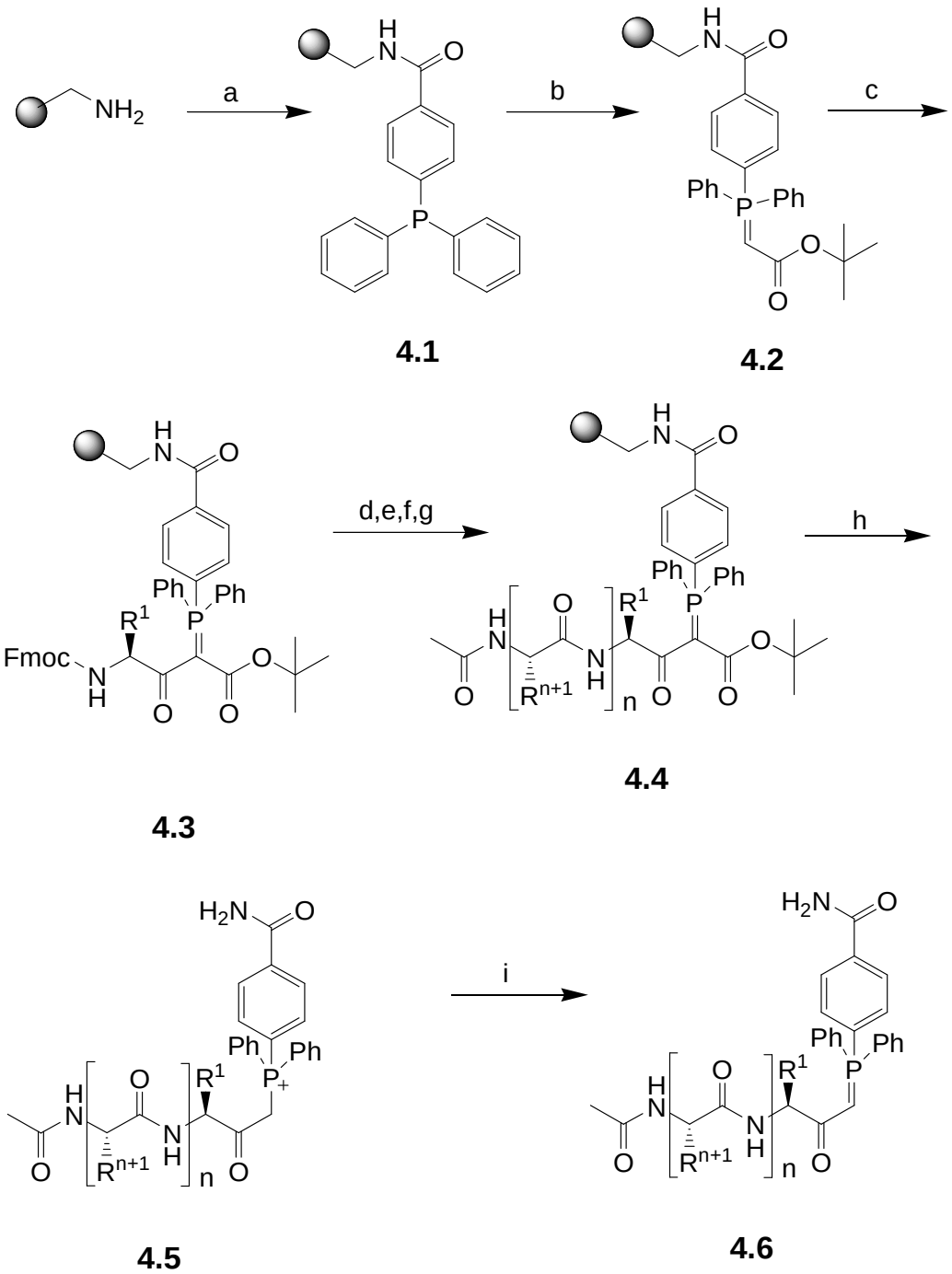
Moreover, it might be of special interest for in-situ inhibitor synthesis (target guided synthesis) in case of the enzyme acetylcholinesterase (AChE). Azide-alkyne reaction needs catalysis at room temperature for regioselectivity or heating in absence of catalyst resulting in the formation of mixture of 1,4- and 1,5-disubstituted triazoles. However, when the reaction is performed in AChE's active site at room temperature and in the absence of any metal catalyst, there is selective formation of 1,5-triazole.[201, 220] This indicates high affinity of the enzyme for 1,5-triazole (syn-triazole). It is, therefore, worth to investigate regioselective reaction resulting in 1,5-disubstituted triazole formation inside AChE's active site. Additionally, soluble

peptidyl-phosphoranes developed for use in the azide-phosphorane reaction can also be used in Wittig reaction for target guided synthesis.

4.5 Results and Discussion

To fine-tune the biocompatibility of our reported reaction (azide-phosphorane reaction) we, first of all, needed a strategy for the synthesis of soluble peptidyl-phosphoranes. Secondly, the reaction should be performable in aqueous media and thirdly it should proceed at room temperature.

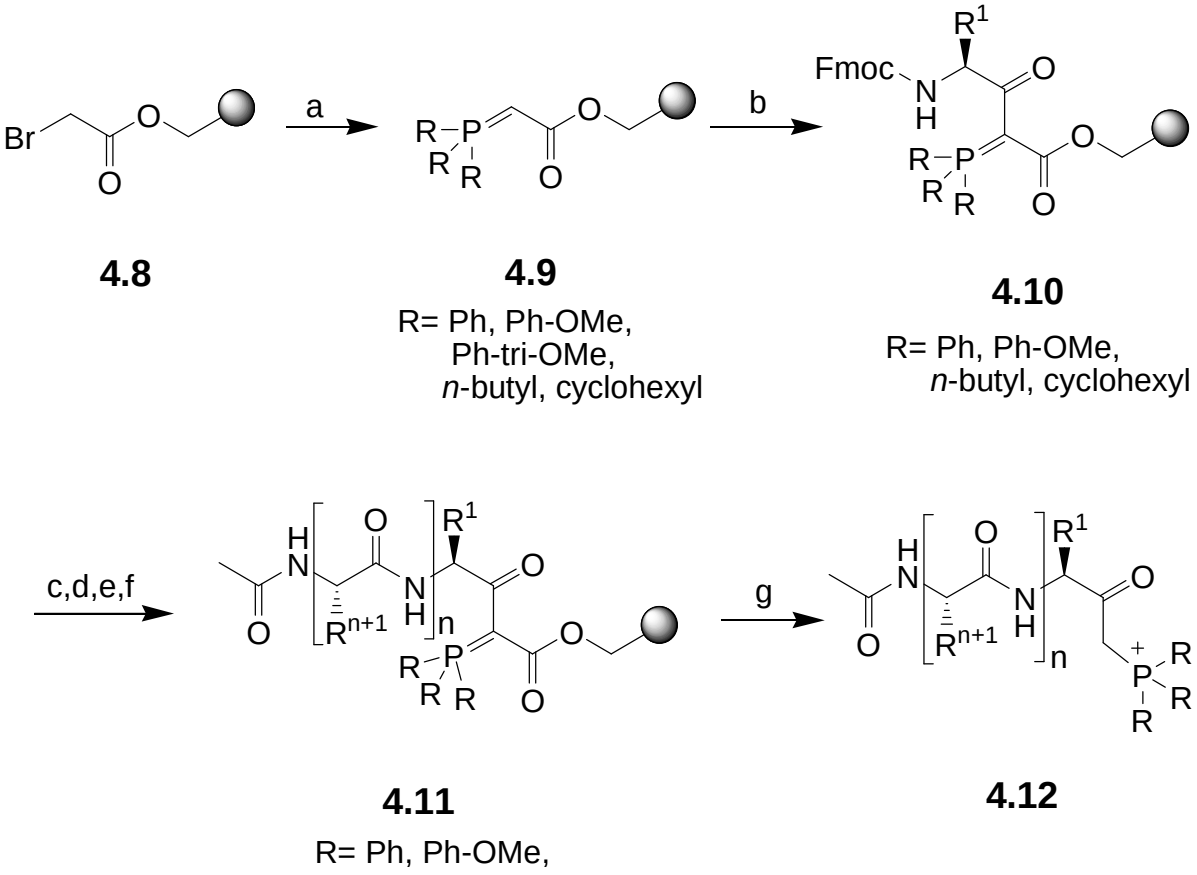
To achieve the goals, we immobilized diphenly-4-carboxylic-phenlyphosphane on Rink amide resin. The solid support bound triphenylphosphane **4.1**, immobilized through amide linkage, was alkylated with *tert*-butyl bromoacetate and deprotonated with Et_3N to give diphenyl-4-carboxamide-phenyl-phosphoranylidene acetate **4.2**. Amino acyl phosphorane **4.3** was obtained by a non-racemizing *C*-acylation of **4.2** employing an Fmoc-protected amino acid and BTFFH for activation which was elongated with further amino acids by employing standard couplings of Fmoc-amino acids with diisopropyl-carbodiimide/1-hydroxybenzotriazole activation and finally capping the *N*-terminus with acetyl group furnishing resin **4.4**. TFA treatment of the peptidyl-phosphoranylidene acetate **4.4** cleaved the phophoranes off from resin as well as resulted in saponification of the ester linkage which proceeded with instantaneous decarboxylation. Methanolic solution of the soluble peptidyl Wittig salt **4.5** was precipitated from NaOH solution delivering relatively pure peptidyl-Wittig ylide **4.6**. The peptidyl-phosphoranes **4.6** were initially reacted with 4-carboxylate phenyl azide selected on the basis of our previous investigations. We were pleased to see the outcome of the reaction with above 95% conversion in 16 h at room temperature in aqueous media. The reaction, however, got sluggish when the same peptidyl-phosphoranes were ligated with peptides having phenyl azide clipped at the amine terminus (**4.7**). There was not more than 50% conversion over long reaction time of 3 days. Slow kinetics of this reaction may be useful in case of kinetically controlled TGS where slow reactions are desireable, however, it must be improved for the applications in labelling of biomolecules.



Scheme 4.1: Reaction conditions: a) diphenyl-4-carboxy-phenylphosphane, DIC, HOBT, DMF, 2h; b) i) tert-butyl bromoacetate, toluene, MW, 10 min. ii) Et_3N, CH_2Cl_2, 2h; c) Fmoc-AA-OH, BTFFH, DIPEA, DMF, 14 h; b) 20% pip./DMF; d) Fmoc-Phe-OH, DIC, HOBt, DMF, 2h; e) (b) & (c) are repeated n-times; f) 20% Ac_2O/DMF; g) TFA/CH_2Cl_2; h) aq. NaOH solution.

Slow kinetics of the Staudinger ligation has limited its use for labelling of biomolecules, the same fate looked for our reaction. Many attempts to improve

kinetics of Staudinger ligation failed but we had enough space to play in for improving the kinetics of the azide-phosphorane cycloaddition reaction. Postulating that the reaction involved lowest unoccupied molecular orbital (LUMO) of the azide and highest occupied molecular orbital (HOMO) of the phosphorane, we had two options to increase the reactivity. Either one could make the azides more electron-deficient with electron withdrawing groups or one could increase electron density at phosphorane center with electron donating substituents. The first choice was, however, not reasonable based on our previous findings that triazoles with highly electron withdrawing substituents, like tosyl or triflyl groups, are prone to degradation in aqueous medium. We, therefore, pursued the second option of increasing electron density at phosphoranes. That required an alternate more flexible synthetic scheme allowing the variation of substituents at the phosphorous centre. In the new strategy, scheme **4.2**, Wang resin was esterified with bromoacetyl bromide and triphenyl phosphine was alkylated with support bound bromoacetate **4.8** and treated with Et_3N to give Wittig ylide **4.9**. Wittig ylide was *C*-acylated with activated amino acid followed by a sequence of peptide chain construction, *N*-acetylation and TFA cleavage of peptidyl-phosphorane **4.12**. Peptidyl-phosphorane **4.12** (R= Ph), lacking amide group as compared to **4.6**, was supposed to be more reactive but practically there was not much difference. Exploiting the fact that alkyl groups have higher electron donating ability, we used tri-*n*-butyl phosphane in place of triphenyl phosphane in scheme **4.2**. However, we failed to acylate solid support bound tributyl phosphoranylidene acetate **4.9** (R= *n*-butyl) with amino acid inspite of using a list of activating agents including BTFFH, TFFH, PyBOP, HATU, and MSNT. Separately synthesized and isolated amino acid fluorides, as an alternate, succeeded to *C*-acylate the ylide **4.9** (R= *n*-butyl). Quantitative acylation was observed on the basis of peaks integration in NMR spectrum when **4.10** (R= *n*-butyl) was treated with TFA. While **4.10** was stable towards strong acid, it was found prone to degradation on treatment with 20% piperidine while building the peptide chain on first amino acid. Use of tricyclohexyl phosphine in scheme **4.2**, met with the same fate and it was not possible to get **4.11** (R= *n*-butyl, cyclohexyl).



4.8

4.9

R= Ph, Ph-OMe,
Ph-tri-OMe,
n-butyl, cyclohexyl

4.10

R= Ph, Ph-OMe,
n-butyl, cyclohexyl

4.11

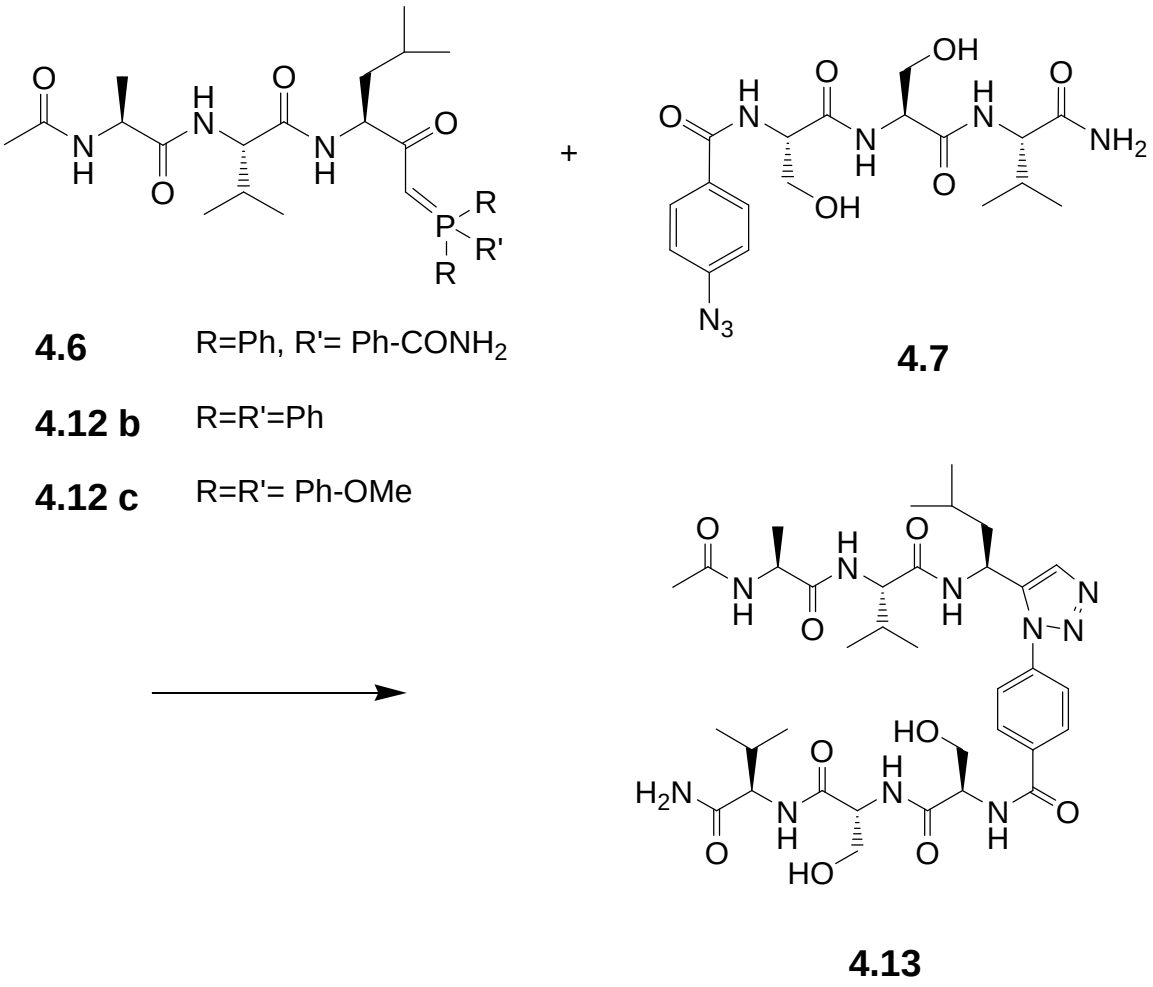
R= Ph, Ph-OMe,

4.12

Scheme 4.2: Reaction conditions: a) (i) Triaryl or trialkyl phosphane, toluene, 14 h, R.T (ii) Et_3N, CH_2Cl_2, 2h; b) Fmoc-AA-OH, BTFFH, DIPEA, DMF, 14 h or Fmoc-AA-F, CH_2Cl_2, 14h (for R= Ph, Ph-OMe and R= n-butyl, cyclohexyl respectively); c) 20% pip./DMF; d) Fmoc-Phe-OH, DIC, HOBt, DMF, 2h; e) (b) & (c) are repeated n-times; f) 20% Ac_2O/DMF; g) TFA/CH_2Cl_2.

Based on this observation, we decided to use triarylphosphanes and vary the substitutions on phenyl rings. *p*-Methoxy substitution was considered an optimal choice due to its electron-donating character and triphenylphosphine in scheme **4.2** was replaced with tris-(4-methoxy phenyl) phosphine that resulted in the peptidyl-phosphorane **4.12c** (R= Ph-OMe) in good yield. Peptidyl-tris(4-methoxy phenyl)phosphorane **4.12b** (R= Ph-OMe) was ligated with peptidyl-phenyl azide in various organic-aqueous media (Scheme **4.3**). There was a considerable improvement in reactivity as compared to un-substituted peptidyl-phosphoranes **4.12b** (R= Ph) with above 85 % conversion over 18 h. Ligation product **4.13** was purified by preparative HPLC and analysed through NMR spectroscopy. Motivated by these results, we were interested to see the outcome while using tris-(2,4,6-trimethoxy phenyl) phosphine in place of triphenylphosphine in scheme **4.2** but we

were unable to *C*-acylate the Wittig ylide **4.9** (R= Ph-tri-OMe) by any mean.



Scheme 4.3: Reaction conditions: DMF/H_2O (60:40), R.T., 18 h.

4.6 Summary and outlook

In short, we have presented the first synthesis of soluble peptidyl-phosphoranes. The synthetic scheme involved immobilization of trivalent phosphine on polymer support through an ester linkage followed by peptide construction on phosphoranylidene acetate. The scheme thus avoided an extra step of protection and deprotection for the synthesis of peptidyl-phosphorane.[71, 166, 221] Soluble peptidyl-phophoranes were then released by the treatment of TFA which performs three jobs; cleavage from resin, saponification of *tert*-butyl ester accompanied with decarboxylation and peptide side chain deprotection.

The scheme is very flexible having enough space to play in for the reactivity control over peptidyl-phosphoranes by altering the trivalent phophine. The phosphoranes

can be used to derivatize the azide function under physiological conditions, although need further activation for efficient labelling of biomolecules in living systems. Azide function, a unique chemical reporter in living systems, has found another reagent for its derivatization.

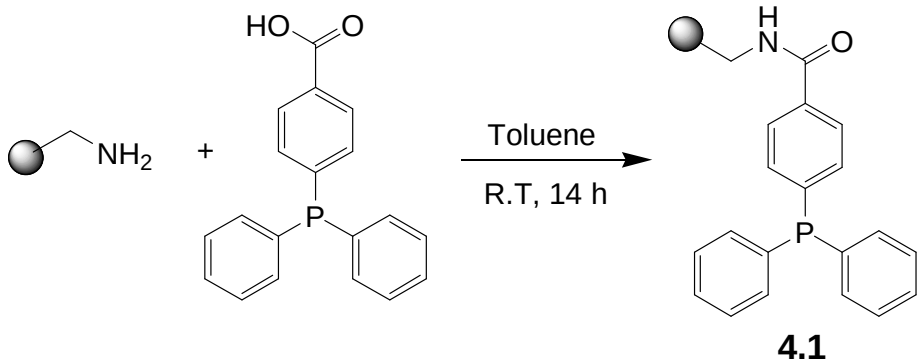
Additionally, these soluble peptidyl-phosphoranes have potential to be used in target guided synthesis either through azide-phophorane reaction or Wittig reaction.

4.7 Experimental section

Reagents and general methods. Fmoc Rink amide resin (0.7 mmol/g, 1 % divinyl benzene,100-200 mesh), Wang resin (1.1 mmol/g), trityl resin (1.6 mmol/g), Fmoc-amino acids and all other reagents were purchased from commercial suppliers, unless synthesis stated. Anhydrous solvents were purchased and stored over molecular sieves. Fmoc solid-phase peptide synthesis was conducted in plastic syringes equipped with PE filters. Loading was determined by photometric methods after Fmoc cleavage. Solid phase reactions were monitored through FT-ATR-IR spectra of the resins with a Bruker Vector 22/Harrick SplitPea ATR unit. Cleaved products were analyzed by an analytical HPLC column (5 µm, 250 x 2 mm, Nucleosil 100 RP-C18) with detection at 220 nm and 254 nm. Eluents A (0.1% TFA in water) and B (0.1% TFA in acetonitrile) were used in a linear gradient (20% B 100% B in 45 min.). Compounds were purified on a semipreparative HPLC column (10 µm, 250 x 20 mm, Grom-SIL 300 ODS-5 ST RP-C18) employing individual gradients derived from analytical runs (eluents A and B). LC-MS were recorded on an Agilent 1100 series chromatography workstation (Agilent Technologies) equipped with a single quadrupole mass spectrometer and electrospray ionization (ES). HRMS measurements were conducted with an Agilent 6220 ESI-TOF mass spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE 300 MHz, or 600 MHz instruments and chemical shifts (δ) were measured in parts per million (ppm) relative to trimethylsilane (TMS).

Synthesis of soluble peptidyl phosphoranes by using Rink amide resin

Immobilization of triphenylphosphine on Rinkamide resin 4.1

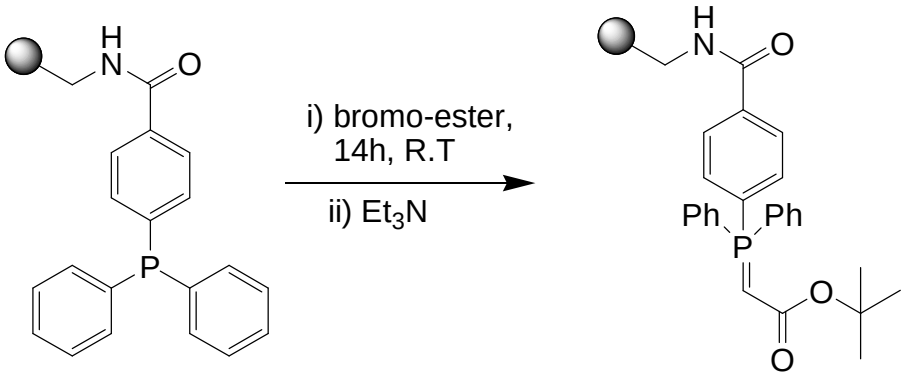


4.1

4-carboxy-phenyl-diphenylphosphine (1.05 mmol, 321.2 mg) was dissolved in DMF and activated with HOBt (1.05 mmol, 162.5 mg) and DIC (1.05 mmol, 162.3 uL) was

added to pre-weighed Rink amide resin (0.5 g, 0.7 mmol/g, 0.35 mmol) into a microwave vial. The vial was sealed and shaked at room temperature for 3 hours. The resin was then washed with dry DMF (4mL x2) and CH_2Cl_2 (4mL x2) and dried in vacuum.

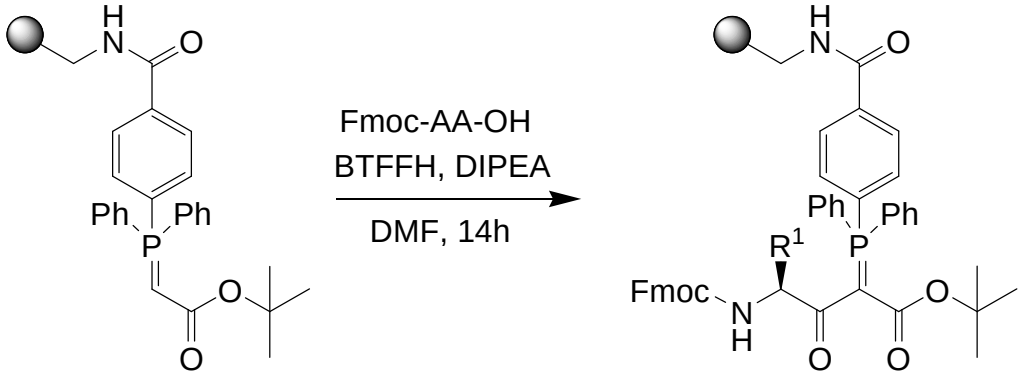
Alkylation of immobilized triphenylphosphine



4.1 **4.2**

Resin **4.1** (0.5 g, 0.7 mmol/g, 0.35 mmol) was weighed into a microwave vial and suspended in dry toluene (4 mL). After addition of *ter*-butyl 2-bromoacetate (1.75 mmol, 5 equiv., 258.3 uL), the vial was sealed and shaked at room temperature for 14 h and subsequently washed with dry toluene and CH_2Cl_2. The obtained polymer phosphonium salt was resuspended in dry CH_2Cl_2 (5 mL) and Et_3N (557 µL, 4 mmol, 5 equiv.) was added. After shaking for 2 h at RT, the yellow-coloured resin **4.2** was filtered, washed with CH_2Cl_2 and dried in vacuum.

Acylation of 4.2 by Fmoc amino acid

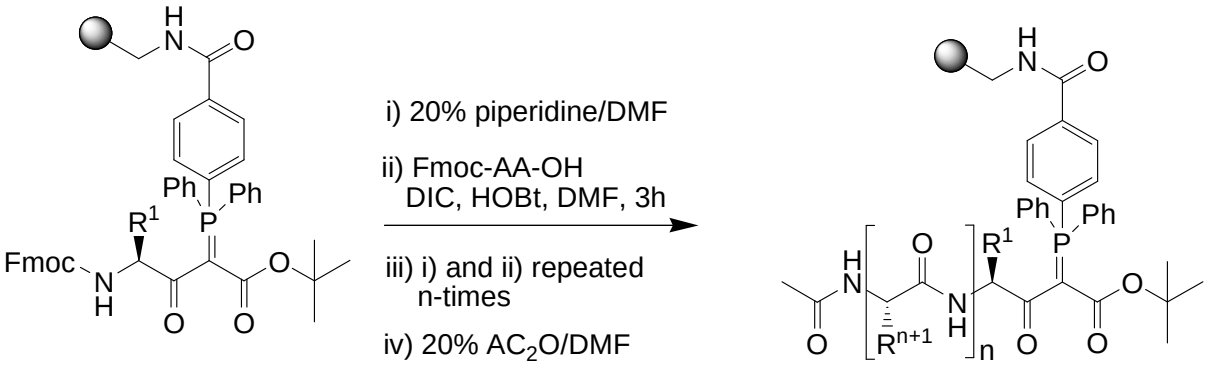


4.2 **4.3**

❖

Resin **4.2** (300 mg) was pre-swollen in dry DMF. The Fmoc amino acid (1.05 mmol, 5 equiv.) was dissolved in dry DMF (5 mL) after addition of diisopropylethyamine (DIPEA) (2.10 mmol, 10 equiv., 365 uL) and BTFFH (1.05 mmol, 5 equiv., 331.8 mg) The clear solution obtained was directly mixed with the resin suspension and shaken for 14h at room temperature. The resin was washed thoroughly with DMF and THF after the coupling step and dried in vacuum.

Polymer-supported peptidyl-2-phosphoranylidene acetate 4.4



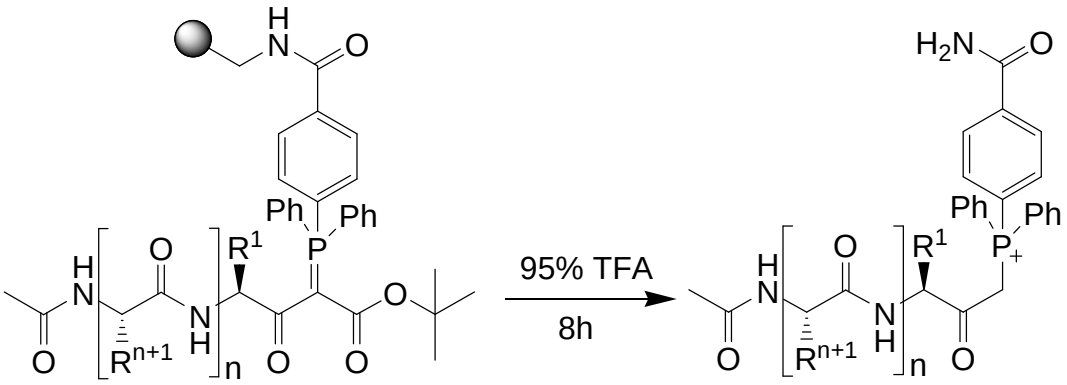
4.3 **4.4**

Fmoc protection of the resin **4.3** (0.51 mmol/g, 0.15 mmol) was cleaved with 20 % piperidine/DMF (v/v, 2x6 min) and deprotected was washed and suspended in dry DMF. Fmoc amino acid (0.75 mmol, 5 equiv.) and HOBt·H_2O (0.75 mmol, 5 equiv., 114.7 mg) were dissolved in dry DMF (5 mL), pre-activated under addition of DIC (0.75 mmol, 5 equiv., 116 uL) and added to the resin. The mixture was shaken for 3 h at RT, filtered, and the resin was washed with DMF and THF.

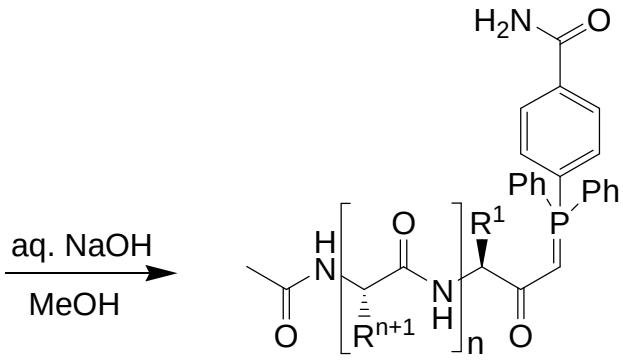
All the sequence of steps, stated, was repeated n-times. Quantitative couplings were verified by the Kaiser test.

After final Fmoc deprotection, the resin was treated with 20 % acetic anhydride in DMF (2x30 minutes) to cap the free amino group with acetyl.

Soluble N-acetyl peptidyl-phosphoranes 4.6

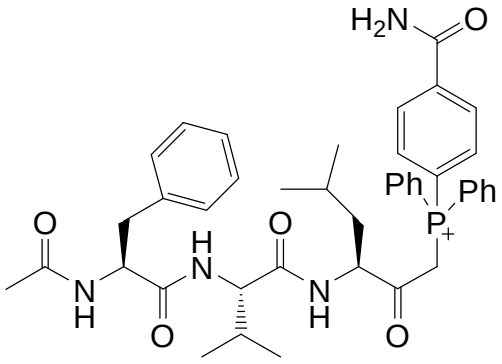


4.4 **4.5**



4.6

The resin **4.4** was treated with TFA:CH_2Cl_2 (95:5) (6 mL) for 8 h for cleavage of phpsphoranes and to remove the *tert*-butyl group as well as side chain protections. The resin was filtered off and filterate was evaporated to give Wittig salt **4.5**. Concentrated aqueous solution of NaOH was added dropwise to Wittig salt solution in methanol (1mL) and ylide **4.6** precipitated out which was filtered and dried in air.



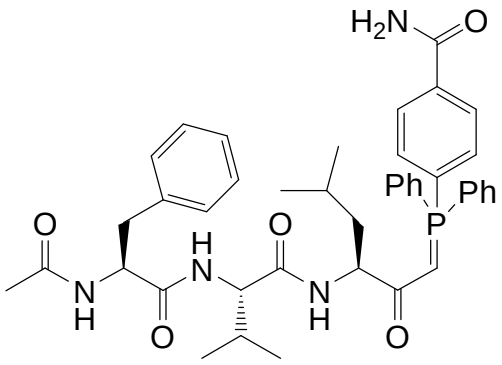
4.5

4.5: Yield: 84 mg (78%)

^{1}H-NMR: (300 MHz, acetonitrile-d_3): δ = 0.79, 0.85 (2d, J_1=J_2 = 6.1 Hz, 6H, *CH*$_3$, Val), 0.91 (d, *J* = 6.1 Hz, 6H, *CH*$_3$, Leu), 1.39-1.63 (m, 3H, *CH*$_2$, *C*$^{\gamma}$*H*, Leu), 1.85 (s, 3H, *CH*$_3$, acetyl), 2.05-2.16 (m, 1H, *C*$^{\beta}$*H*, Val), 2.76–3.01 (m, 2H, *CH*$_2$, Phe), 3.99 (t, *J* = 6.1 Hz, 1H, *C*$^{\alpha}$*H*, Leu), 4.27 (bs, 2H, *CH*$_2$, phosphoranyl), 4.81-4.91 (m, 1H, *C*$^{\alpha}$*H*, Phe), 5.18-5.28 (m, 1H, *C*$^{\alpha}$*H*, Val), 7.13-7.27 (m, 5H, arom., Phe) 6.55 (s, 1H, *NH2*), 7.19, 7.24, 7.50 (3d, *J* = 6.1 Hz, 3H, 3*NH*), 7.60-7.84, 8.00-8.04 (2m, 14H, *arom.*, triphenylphosphine).

^{13}C-NMR: (75.5 MHz, acetonitrile-d_3): δ = 18.5, 19.3, 21.1, 22.6, 23.0, 25.1, 30.2, 37.3, 37.8, 57.2, 59.1, 60.9, 115.1, 118.9, 119.4, 119.5, 127.4, 129.1, 129.8, 130.7, 130.8, 137.9, 140.2, 160.2, 160.7, 167.8, 172.6, 173.3, 173.7.

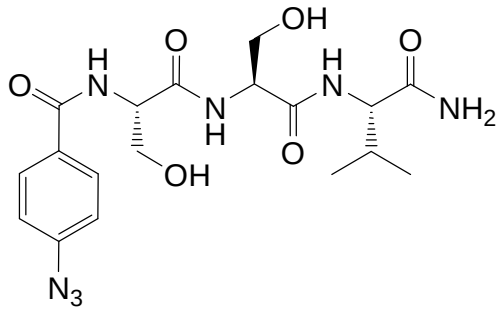
HRMS (ESI): Calculated for $C_{42}H_{50}N_4O_5P$ $[M]^+$: 721.3513 Da. Found: 721.3516 m/z.



4.6

4.6: Yield: 77 mg (92%)

^{1}H-NMR: (300 MHz, methanol-d_4): δ = 0.88, 0.97 (m, 12H, 4*CH*$_3$, Leu, Val), 1.53-1.75 (m, 3H, *CH*$_2$, *C*$^{\gamma}$*H*, Leu), 1.85 (s, 3H, *CH*$_3$, acetyl), 2.06-2.10 (m, 1H, *C*$^{\beta}$*H*, Val), 2.14 (s, 1H, *CH*, phosphoranyl) 2.78–3.13 (m, 2H, *CH*$_2$, Phe), 4.27-4.29 (m, 1H, *C*$^{\alpha}$*H*, Leu), 4.44-4.47 (m, 1H, *C*$^{\alpha}$*H*, Phe), 4.68-4.72 (m, 1H, *C*$^{\alpha}$*H*, Val), 7.21-7.99 (m, 19H, *arom.*, Phe, triphenylphosphine).



4.7

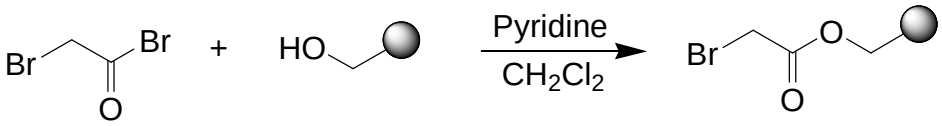
^{1}H-NMR: (300 MHz, DMSO-d_6): δ = 0.82 (dd, 6H, *J* = 7.3 Hz, 2CH_3, Val), 1.95-2.06 (m, 1H, $C^{\beta}H$, Val), 3.55-3.73 (m, 4H, 2$C^{\beta}H_2$, 2Ser), 4.07-4.12, 4.30-4.36 (2m, 2H, 2$C^{\alpha}H$, Ser, Ala), 4.56 (dd, *J* = 6.1 Hz, 1H, $C^{\alpha}H$, Ser), 7.17 (d, 2H, *J* = 42.1 Hz, NH_2), 7.21, 7.94 (2d, 4H, J_1=J_2= 8.5 Hz *arom.*), 7.60, 8.11, 8.40 (3d, 3H, J_1=8.5, J_2=7.3, 3*NH*).

^{13}C-NMR: (75.5 MHz, DMSO- d_6): δ = 17.6, 19.3, 30.0, 55.3, 55.9, 57.9, 61.4, 118.8, 129.4, 130.5, 142.5, 165.5,169.9, 170.5, 172.9.

HRMS (ESI): Calculated for $C_{18}H_{25}N_7O_6$ $[M+H]^+$: 436.1945 Da. Found: 436.1946 m/z.

Synthesis of soluble peptidyl phosphoranes using Wang resin

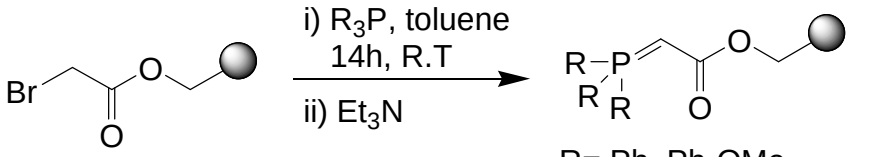
Esterification of Wang resin



4.7

Wang resin (300 mg, 1.1 mmol/g, 0.33 mmol) was swollen in dry CH_2Cl_2 and shaked at room temperature for 15 minutes after addition of dry pyridine (1.65 mmol, 132.6 uL) and then bromoacetyl bromide (1.65 mmol, 143.5 uL) was added to the same mixture at 0-4 ^{o}C and sealed vial was shaken for 5 hours at room temperature. The resin **4.7** was filtered and washed thoroughly with DMF and CH_2Cl_2.

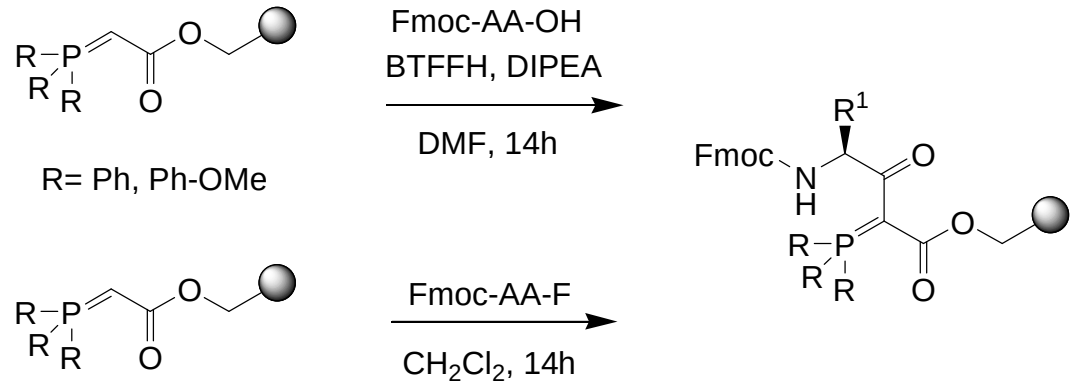
Phosphoranylation of immobilized bromo-esters 4.7



4.7 **4.8**

Resin **4.7** (300 mg, 0.33 mmol) was suspended in dry toluene (5ml) and one of the triaryl-phosphines or trialkyl-phosphines (1.65 mmol, 5 equiv.) was added under stream of nitrogen and sealed vial was shaked for 14 hours at room temperature. The resin was subsequently washed with dry toluene and CH_2Cl_2. The obtained phosphonium salt was resuspended in dry CH_2Cl_2 (5 mL) and Et_3N (557 µL) was added. After shaking for 2 h at RT, the deep purple-coloured resin **4.8** was filtered, washed with CH_2Cl_2 and dried in vacuum.

Acylation of 4.8 by Fmoc amino acid



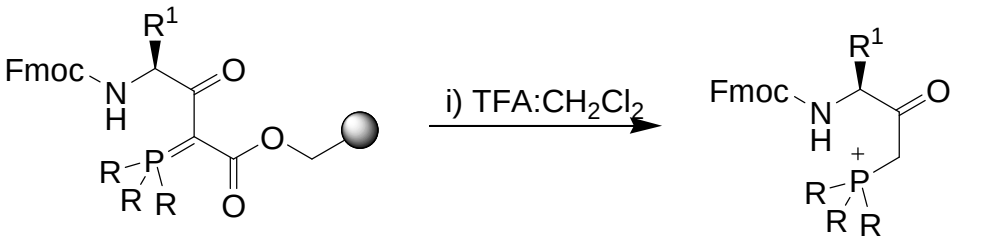
4.9

a) R = Ph or Ph-OMe

Resin **4.8** (300 mg, 0.33 mmol) was pre-swollen in dry DMF. The Fmoc amino acid (1.65 mmol, 5 equiv.) was dissolved in dry DMF (5 mL) after addition of diisopropylethyamine (DIPEA) (3.3 mmol, 10 equiv., 573 uL) and BTFFH (1.65 mmol, 5 equiv., 521.4 mg) The clear solution obtained was directly mixed with the resin suspension and shaken for 14h at room temperature. The resin was washed thoroughly with DMF and THF after the coupling step and dried in vacuum.

b) R = n-butyl, cyclohexyl

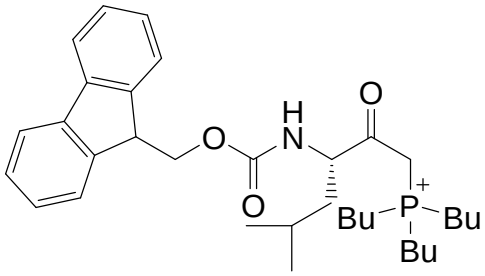
Resin **4.8** (300 mg, 0.33 mmol) was pre-swollen in dry CH_2Cl_2 and Fmoc-leucinyl fluoride (0.99 mmol, 3 equiv. 351 mg) was added to the mixture and sealed vial was shaken for 14 hour at room temperature. The resin was washed thoroughly with CH_2Cl_2, DMF and THF after the coupling step and dried in vacuum.



R= n-butyl, cyclohexyl

4.9 **4.10**

To see the efficacy of C-C acylation, The resin **4.9** (R= *n*-butyl, cyclohexyl) was treated with 6 mL of TFA:CH_2Cl_2 (95:5) for 8 h for cleavage of phpsphoranes from resin and decarboxylation as well as side chain deprotections. The resin was filtered off and filterate was evaporated to give Wittig salt **4.10**. The crude product thus obtained was characterized by NMR and showed quantitative acylation based on peaks integration.

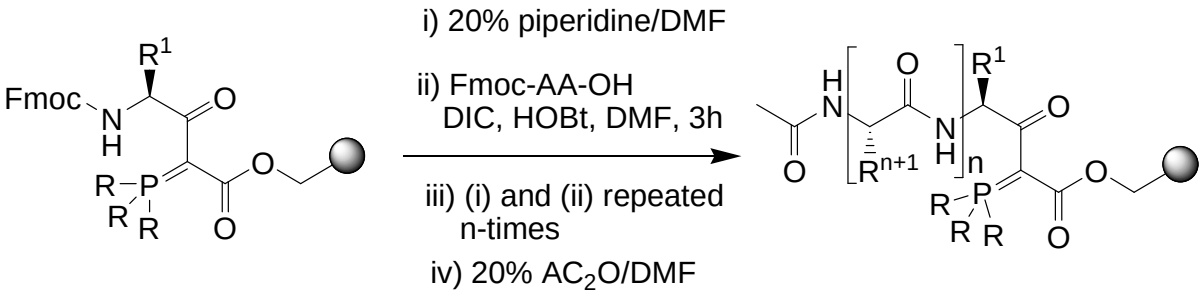


4.10

4.10: Yield: 178 mg (98%)

^{1}H-NMR (300MHz, Methanol-d_4): δ = 0.78-0.87 (m, 15H, 5CH_3, tributyl, Leu), 1.30-1.49 (m, 14H, 7CH_2, tributyl, Leu), 1.54-1.62 (m, 1H, $C^{\gamma}H$, Leu), 2.05-2.22 (m, 6H, 3$C^{\alpha}H_2$, tributyl), 3.43-3.47 (m, 1H, $C^{\alpha}H$, Leu), 4.02-4.29 (m, 5H, 2CH_2, CH, Fmoc, phosphoranyl), 7.13-7.26, 7.51-7.55, 7.64-7.66 (3m, 9H, *arom.*, 1N*H*)

Peptide elongation and acetyl capping (R= Ph or Ph-OMe)



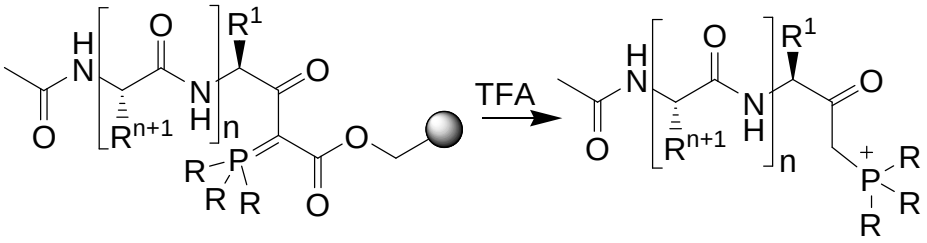
4.9 **4.11**

Fmoc protection of the resin **4.9** (0.82 mmol/g, 300 mg, 0.25 mmol) was cleaved with 20 % piperidine/DMF (v/v, 2x6 min) and deprotected was washed and suspended in dry DMF. Fmoc amino acid (1.25 mmol, 5 equiv.) and HOBt·H_2O (1.25 mmol, 5 equiv., 191 mg) were dissolved in dry DMF (5 mL), pre-activated under addition of DIC (1.25 mmol, 5 equiv., 193 uL) and added to the resin. The mixture was shaken for 3 h at RT, filtered, and the resin was washed with DMF and THF.

All the sequence of steps, stated, was repeated n-times. Quantitative couplings were verified by the Kaiser test.

After final Fmoc deprotection, the resin was treated with 20 % acetic anhydride in DMF (2x30 minutes) to cap the free amino group with acetyl.

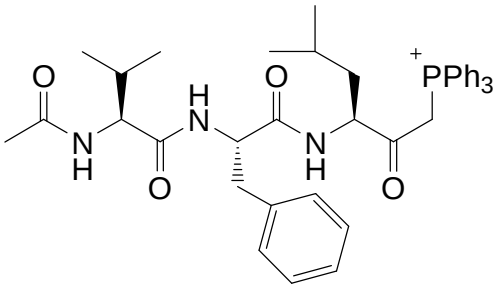
Cleavage of N-acetyl peptidyl-phosphoranes 4.11 (R= Ph or Ph-OMe)



4.11 **4.12a-d**

The resin **4.11** was treated with 6 mL of TFA:CH_2Cl_2 (95:5) for 8 h for for cleavage of phpsphoranes from resin and decarboxylation as well as side chain deprotections. The resin was filtered off and filterate was evaporated to give Wittig salt **4.12 a-d**

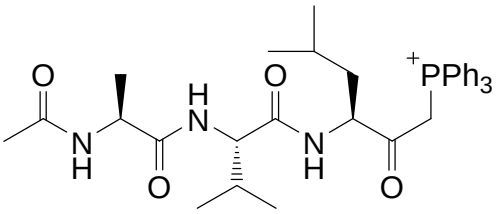
which was analyzed through NMR spectroscopy. Concentrated aqueous solution of NaOH was added dropwise to Wittig salt solution in methanol (1mL) and ylide was precipitated down.



4.12a

4.12a: Yield: 139 mg (82%)

^{1}H-NMR: (300 MHz, methanol-d_4): δ = 0.75 (d, *J* = 6.1 Hz, 6H, *CH*$_3$, Val), 0.80-0.86 (m, 6H, *CH*$_3$, Leu), 1.28-1.54 (m, 3H, *CH*$_2$, *C*$^{\gamma}$*H*, Leu), 1.80-1.91 (m, 1H, *C*$^{\beta}$*H*, Val), 1.96 (s, 3H, *CH*$_3$, acetyl), 2.95–3.19 (m, 2H, *CH*$_2$, Phe), 3.84 (d, *J* = 6.1 Hz, 1H, *CH*$_2$, phosphoranyl), 4.25-4.30 (m, 1H, *C*$^{\alpha}$*H*, Phe), 4.58-4.63 (m, 1H, *C*$^{\alpha}$*H*, Val), 7.16-7.24 (m, 5H, *arom.*, Phe), 7.64-7.88 (m, 15H, *arom.*, triphenylphosphane).



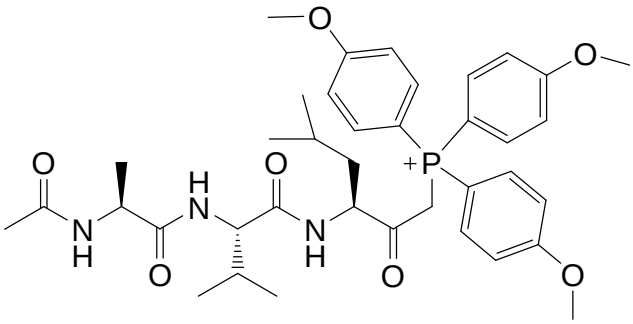
4.12b

4.12b: Yield: 120 mg (80%)

^{1}H-NMR: (300 MHz, DMSO-d_6): δ = 0.75-0.88 (m, 12H, *CH*$_3$, Val, Leu), 1.12 (d, *J* = 6.7 Hz, 3H, *CH*$_3$, Ala), 1.42-1.64 (m, 3H, *C*$^{\gamma}$*H, C*$^{\beta}$*H*$_2$, Leu), 1.81 (s, 3H, *CH*$_3$, acetyl), 1.93-2.03 (m, 1H, *C*$^{\beta}$*H*, Val), 3.74 (d, *J* = 25.6 Hz, 2H, *CH*$_2$, Phosphoranyl), 4.14-4.36 (m, 3H, 3*C*$^{\alpha}$*H*, Leu, Val, Ala), 7.54-7.64 (m, 15H, *arom.*, triphenylphosphane) 7.72, 7.84, 8.03 (3d, *J* = 7.3, 8.5, 9.1 Hz, 3H, 3*NH*).

^{13}C-NMR: (75.5 MHz, DMSO- d_6): δ = 17.9, 19.3, 21.7, 22.4, 23.2, 24.5, 30.6, 48.1, 57.5, 126.1, 127.3, 128.6, 128.8, 129.0, 131.5, 132.5, 132.6, 133.4, 168.9, 169.9, 172.2.

HRMS (ESI): Calculated for $C_{35}H_{45}N_3O_4P$ $[M]^+$: 602.3142 Da. Found: 602.3139 m/z.



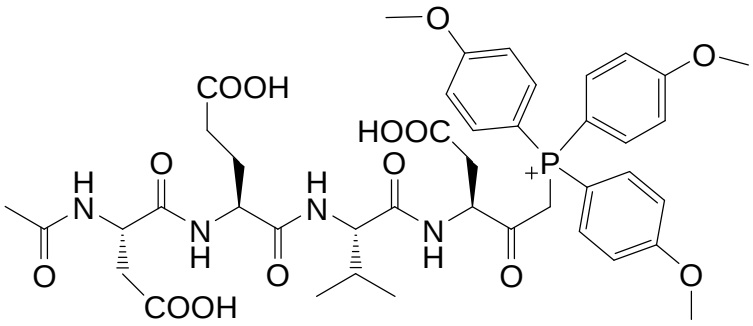
4.12 c

4.12c: Yield: 128 mg (73%)

^{1}H-NMR: (300 MHz, acetonitrile-d_3): δ = 0.79-0.92, 1.23-1.35 (2m, 12H, *CH_3*, Val, Leu), 1.39-1.61, 1.90-1.92 (2m, 3H, *$C^{\gamma}H$, $C^{\beta}H_2$*, Leu), 1.97 (s, 3H, *CH_3*, acetyl), 2.05-2.18 (m, 1H, *$C^{\beta}H$*, Val), 3.87 (s, 9H, 3*CH_3*, methoxy), 4.02-4.14, 4.23-4.32, 4.36-4.46 (3m, 3H, 3*$C^{\alpha}H$*, Leu, Val, Ala), 4.63 (d, *J* = 12.2 Hz, 2H, *CH_2*, Phosphoranyl),7.12-7.18, 7.51-7.63 (2m, 12H, *arom.*) 7.06, 7.30 (d, t, *J* = 8.5, 7.9 Hz, 2H, 2*NH*).

^{13}C-NMR: (75.5 MHz, acetonitrile-d_3): δ = 16.9, 17.7, 19.1, 21.0, 22.1, 23.0, 25.1, 31.1, 32.1, 37.9, 40.2, 49.2, 50.1, 51.5, 56.4, 58.2, 60.4, 109.5, 110.8, 114.5, 116.3, 136.3, 159.6, 160.1, 165.4, 173.0, 173.8, 200.9, 202.9.

HRMS (ESI): Calculated for $C_{35}H_{45}N_3O_4P$ $[M]^+$: 602.3142 Da. Found: 602.3139 m/z.



4.12 d

4.12d: Yield: 134 mg (62%)

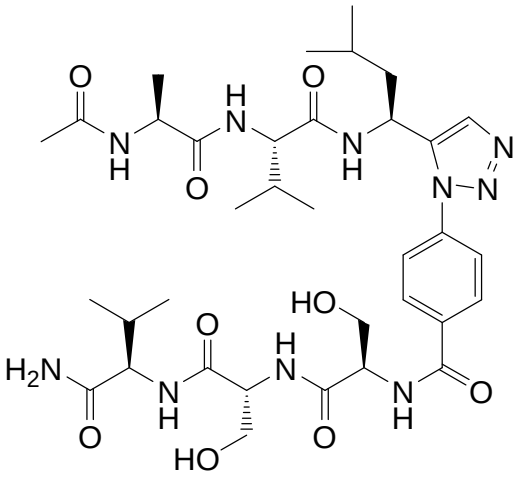
^{1}H-NMR: (300 MHz, DMSO-d_6): δ = 0.88 (dd,6H, *J* = 6.1 Hz, *CH_3*, Val), 1.54-1.75 (m, 2H, *$C^{\beta}H_2$*, Glu), 1.82 (s, 3H, *CH_3*, acetyl), 1.91-2.02 (m, 1H, *$C^{\beta}H_2$*, Val), 2.08-2.29 (m, 2H, *$C^{\gamma}H_2$*, Glu), 2.51-2.87 (m, 4H, 2*$C^{\beta}H_2$*, 2Asp), 3.86 (s, 9H, 3*CH_3*, -OMe), 3.93-3.97 (m, 1H, *$C^{\alpha}H$*, Asp), 4.16-4.23 (m, 1H, *$C^{\alpha}H$*, Val), 4.39-4.54 (m, 2H, 2*$C^{\alpha}H$*, Asp, Glu),

5.13-5.27 (m, 2H, CH_2, phosphoranyl), 7.21-7.25, 7.59-7.66 (2m, 12H, *arom.*), 7.80, 7.90, 8.28, 9.1 (4d, 4H, J_1=7.9, J_2= 7.3, J_3=6.7, 4*NH*).

^{13}C-NMR: (75.5 MHz, DMSO- d_6): δ = 18.6, 18.9, 22.4, 26.9, 29.7, 33.9, 35.7, 49.9, 51.3, 51.7, 55.8, 58.7, 109.2, 110.5, 115.5, 115.7, 135.4, 135.6, 158.0, 158.5, 163.8, 163.9, 169.9, 171.8, 172.7, 173.9.

Procedure for ligation reaction:

Peptidyl-phosphorane **4.12b** (0.1 mmol, 50 mg) was converted into ylide by treatment with NaOH solution.The resulting Wittig ylide and peptidyl-phenyl azide **4.7** (0.12 mmol, 52.2 mg) were dissolved in DMF (1 mL) and water (0.6 mL) was added to the solution. The reaction mixture was stirred at room temperature and monitored by LCMS. There was maximum conversion over 18h (85%), solvent was then evaporated and final product **4.13** was purified by preparative HPLC and analysed through NMR spectroscopy.



4.13

4.13: Yield: 44 mg (58%)

^{1}H-NMR: (300 MHz, DMSO-d_6): δ = 0.65-0.74, 0.80-0.85 (2m, 18H, CH_3, 2Val, Leu), 1.12 (d, J = 6.7 Hz, 3H, CH_3, Ala), 1.36-1.46 (m, 2H, $C^{\beta}H_2$, Leu), 1.63-1.75 (m, 1H, $C^{\gamma}H$, Leu), 1.81 (s, 3H, CH_3, acetyl), 1.85-1.91, 1.98-2.04 (2m, 2H, 2$C^{\beta}H$, 2Val), 3.57-3.77 (m, 4H, 2$C^{\beta}H_2$, 2Ser), 4.02-4.13 (m, 2H, 2$C^{\alpha}H$, Ala, Ser), 4.27-4.38 (m, 3H, 3$C^{\alpha}H$, 2Val, Ser), 4.99-5.06 (m, 1H, $C^{\alpha}H$, Leu), 7.17 (d, 2H, J = 45.7 Hz, NH_2), 7.71, 8.12 (2d, 4H, J_1=J_2= 8.5 Hz *arom.*), 7.80 (s, 1H, triazole), 7.60, 7.71, 8.03, 8.18, 8.52, 8.64 (6d, 6H, J_1=8.5, J_2= 7.9, J_3=7.3, 6*NH*).

^{13}C-NMR: (75.5 MHz, DMSO- d_6): δ = 17.6, 17.8, 19.2, 21.3, 22.4, 24.2, 30.0, 30.3, 41.4, 43.5, 48.1, 55.3, 56.1, 57.5, 57.7, 61.4, 125.2, 128.9, 135.0, 137.9, 140.8, 165.4, 169.1, 169.8, 170.3, 170.6, 172.4, 172.8.

HRMS (ESI): Calculated for $C_{35}H_{55}N_{10}O_9$ $[M+H]^+$: 759.4153 Da. Found: 759.4159 m/z.

5 Project outlook

We have successfully integrated triazole ligation into Fmoc-based solid phase peptide synthesis (SPPS) and introduced a triazole ligation method devoid of heavy metal catalysis. Azide-phosphorane reaction has opened up an elegant route to introduce *cis*-peptide mimetic and β–trun structure in peptide chains. The novel triazole ligation can be employed to yield products with carefully controlled conformations. Moreover, synthesis of soluble peptidyl-phosphoranes has facilitated the potential use of azide-phophorane in chemical biology. Although needs further refinement, the reaction can be used as biocompatible triazole ligation. The same reaction used for peptide cyclization, is an easy way to incorporate locked *cis*-peptide mimetics into cyclopeptides. This access to *cis*-locked triazolyl-cyclopeptides should facilitate the systematic investigation of structural and biological properties of these compounds with already reported biological significance considerably.

We have explored the potential of peptidyl-phosphoranes as a dipolarophile to react with azides in 1,3-dipolar cycloaddition. Phorphoranes reacted with electron deficient azides at room temperature in unpolar solvents but reaction needed heating in polar solvents for relatively electron rich azides. Thus, reactivity trend of azide-phosphorane cycloaddition and effect of solvent polarity reflects polar transition state involved. Moreover, the reaction is dipole-LUMO/dipolarophile-HOMO controlled 1,3-dipolar cycloaddition. It is, therefore, an example of reverse electron demand case where increase in electron density at dipolarophile and decrease in electron density at dipole activates the cycloaddition while azide-alkyne cycloaddition is a case of normal electron demand.

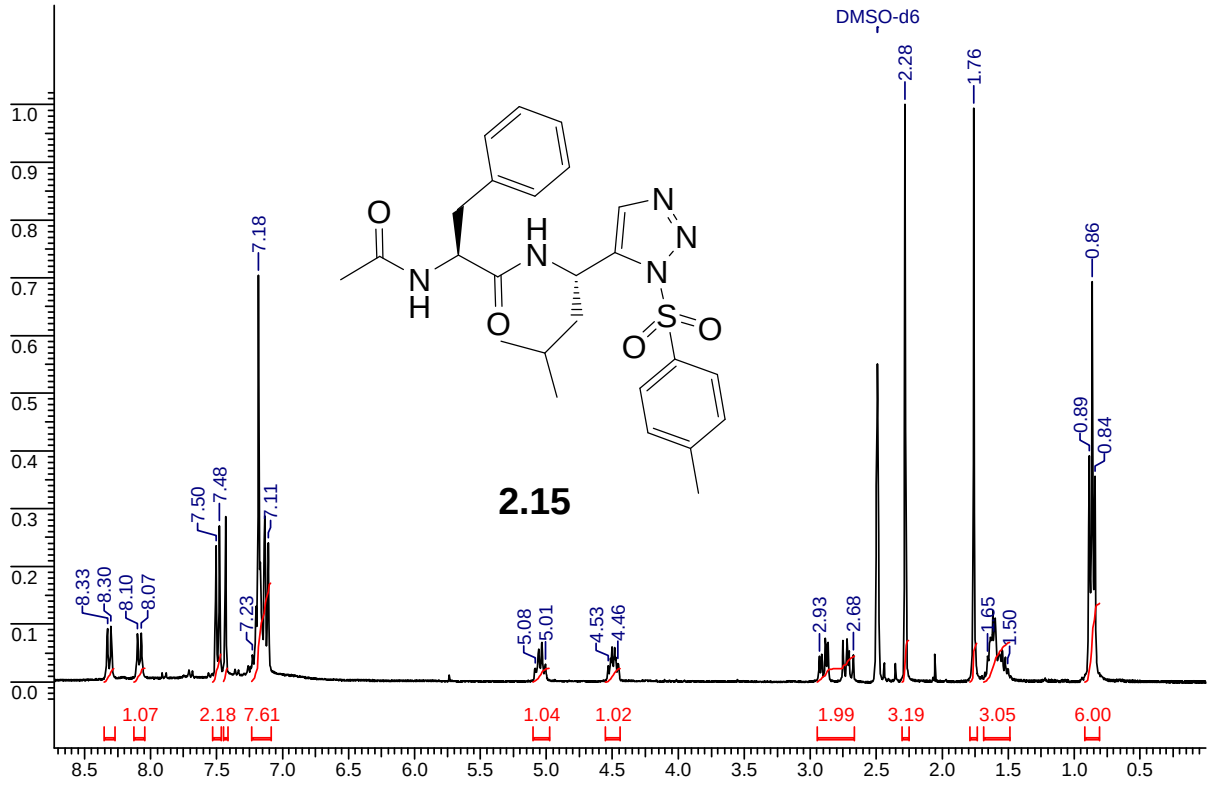
There is a rich prospectus of exploring cycloaddition reaction of phosphoranes with other dipoles in addition to azides. From the reactivity trend, it is percepted that phosphorane can react smoothly with relatively electron poor dipoles from the propargyl-allenyl type, to which azides do belong. Obvious examples would include nitrile oxides and nitrile imines.

As an example of the scope of phosphorane reaction with other dipoles, peptidyl-phosphoranes were found to react with nitrile oxide, generated *in-situ* from 2,4-dichloro-benzhydroxamoyl chloride, at room temperature and at a rate comparable with electron defficient azides (*e.g.* tosyl azide). Phosphoranes, thus, potentially can act as dipolarophile with other dipoles in addition to azides. Moreover, participation of

peptidyl-phosphoranes in 1,3-dipolarcyclo additions opens up an easy way to introduce five-membred heterocycles into peptide chains.

6 NMR spectra of the novel compounds

This chapter contains ^{1}H and ^{13}C spectra of the novel compounds reported in the dissertation.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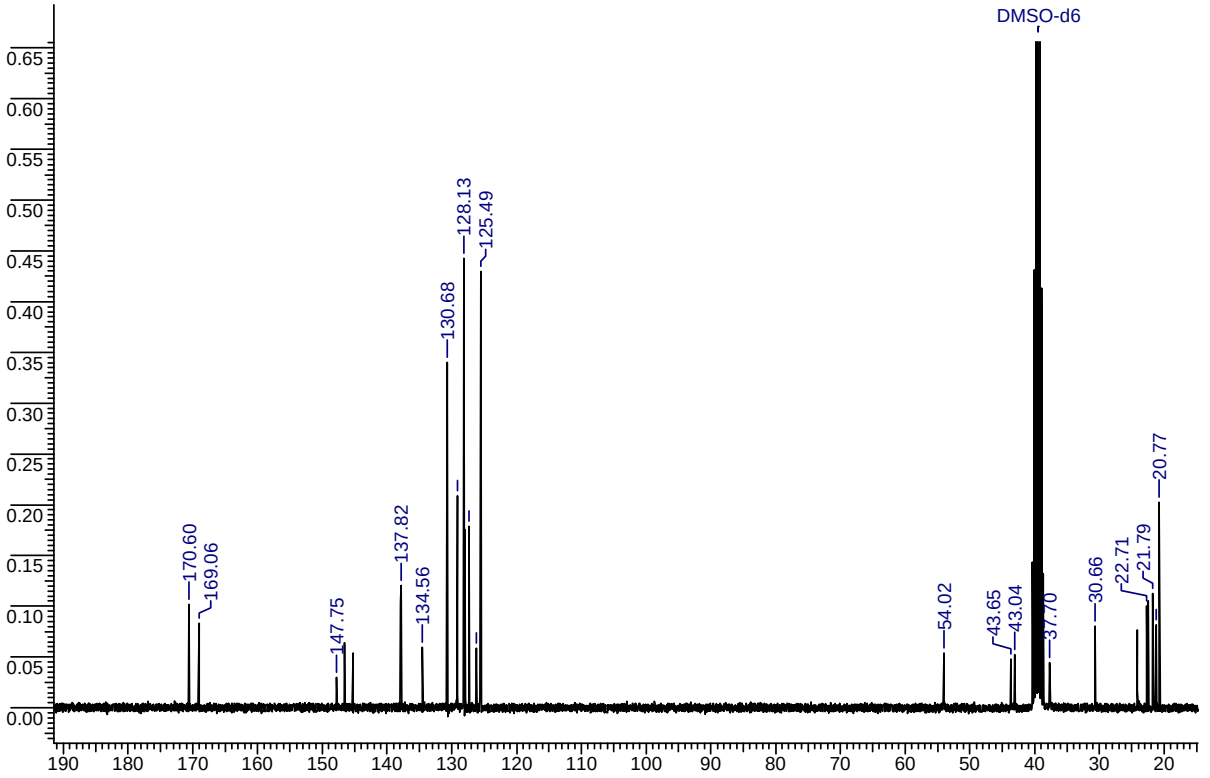
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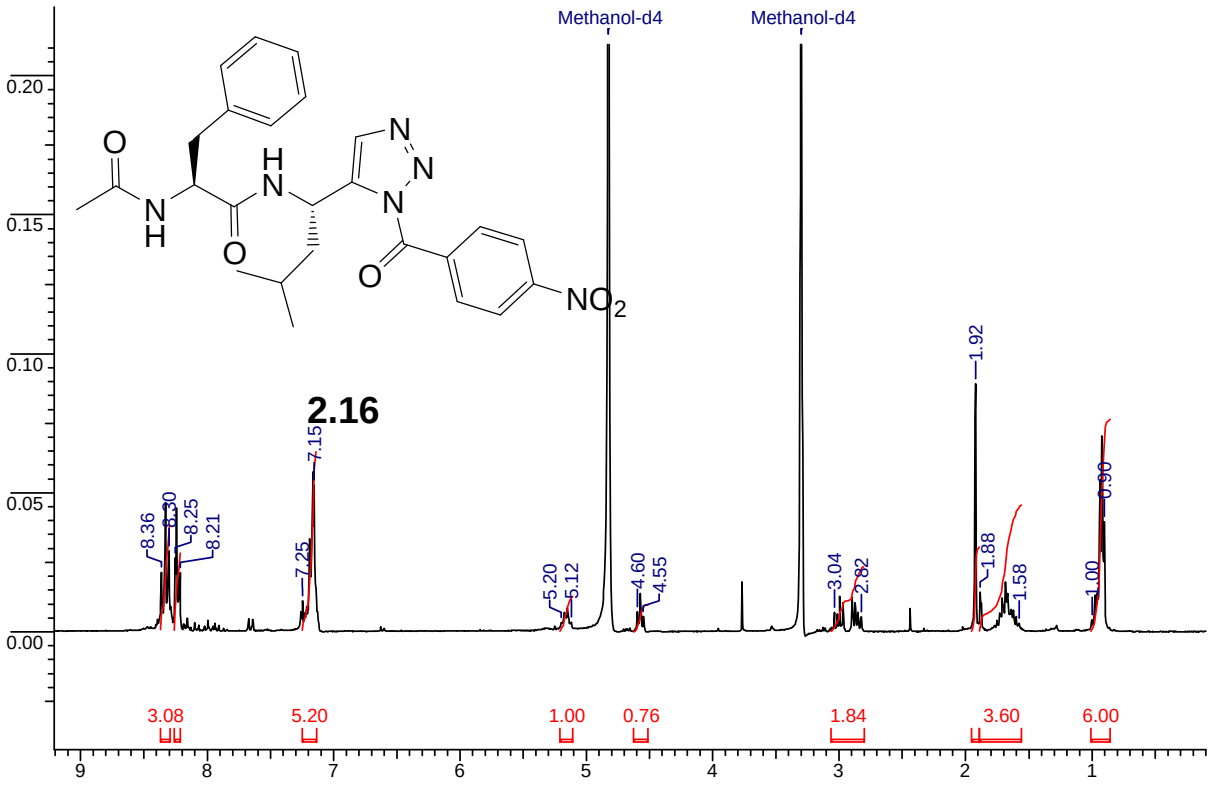
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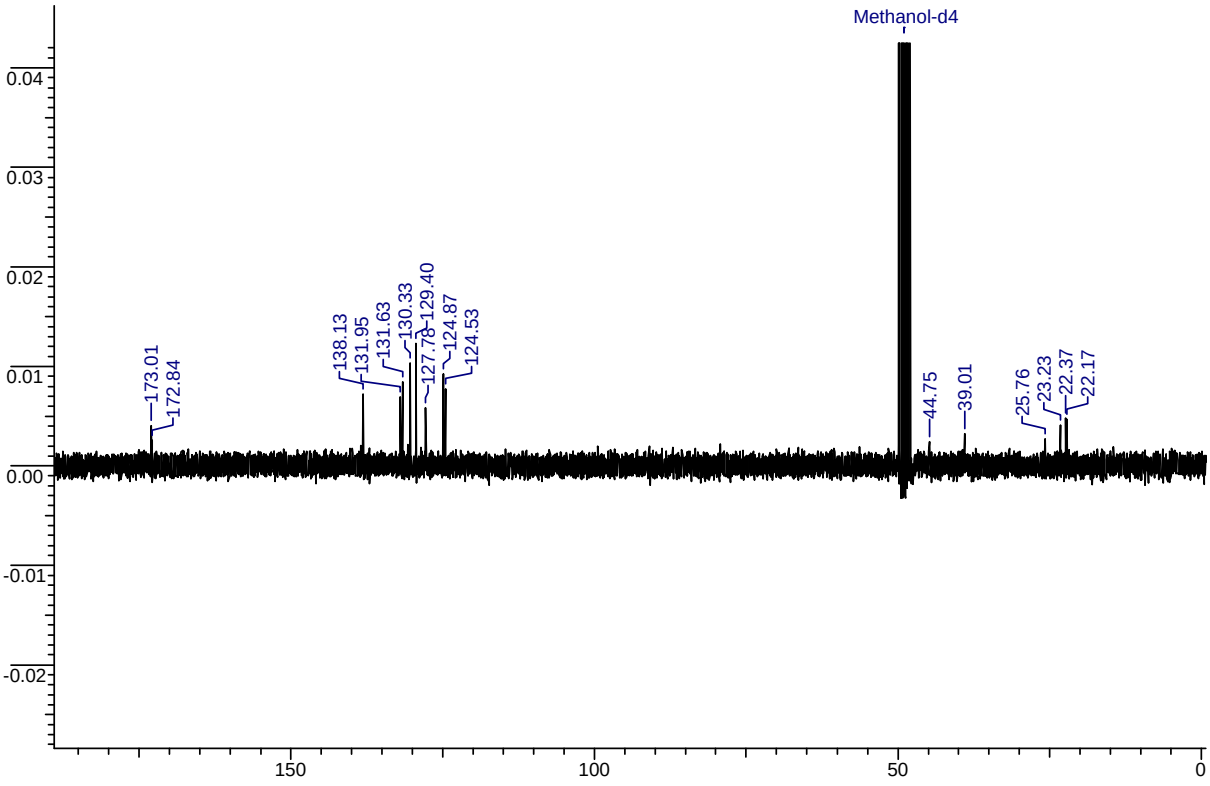
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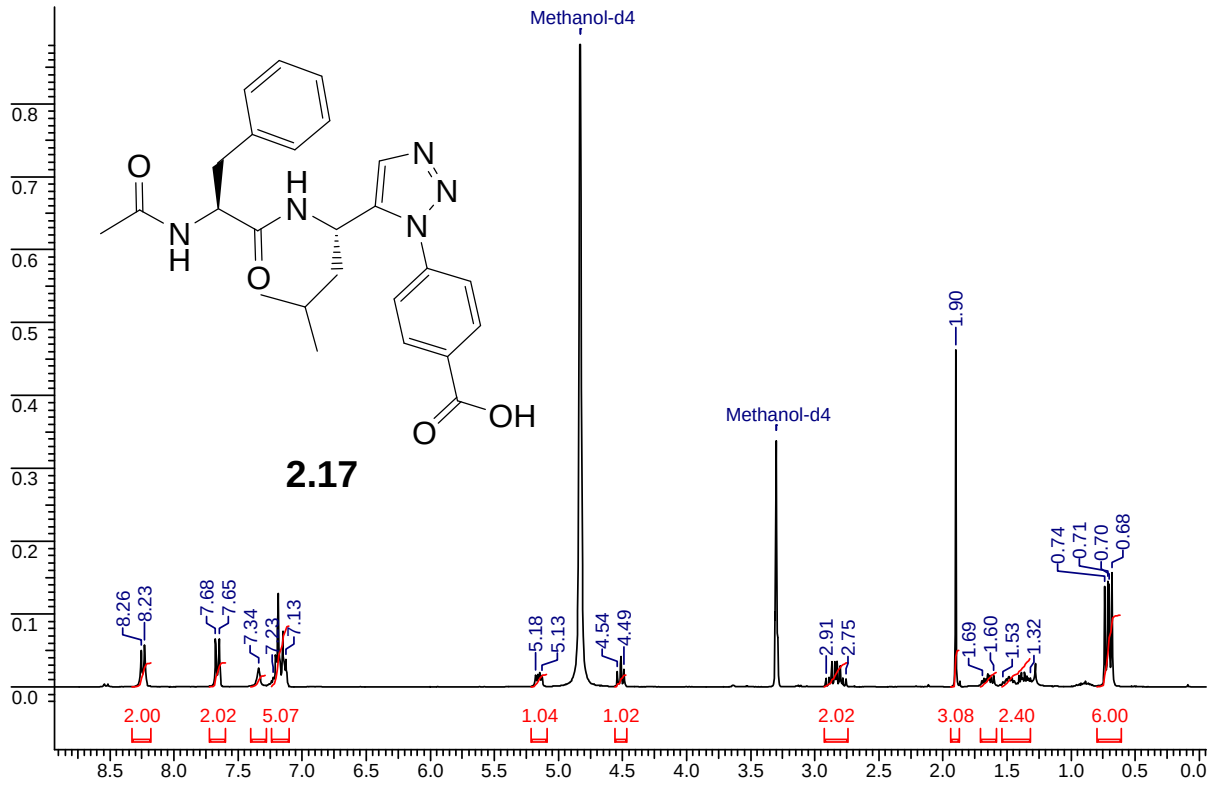
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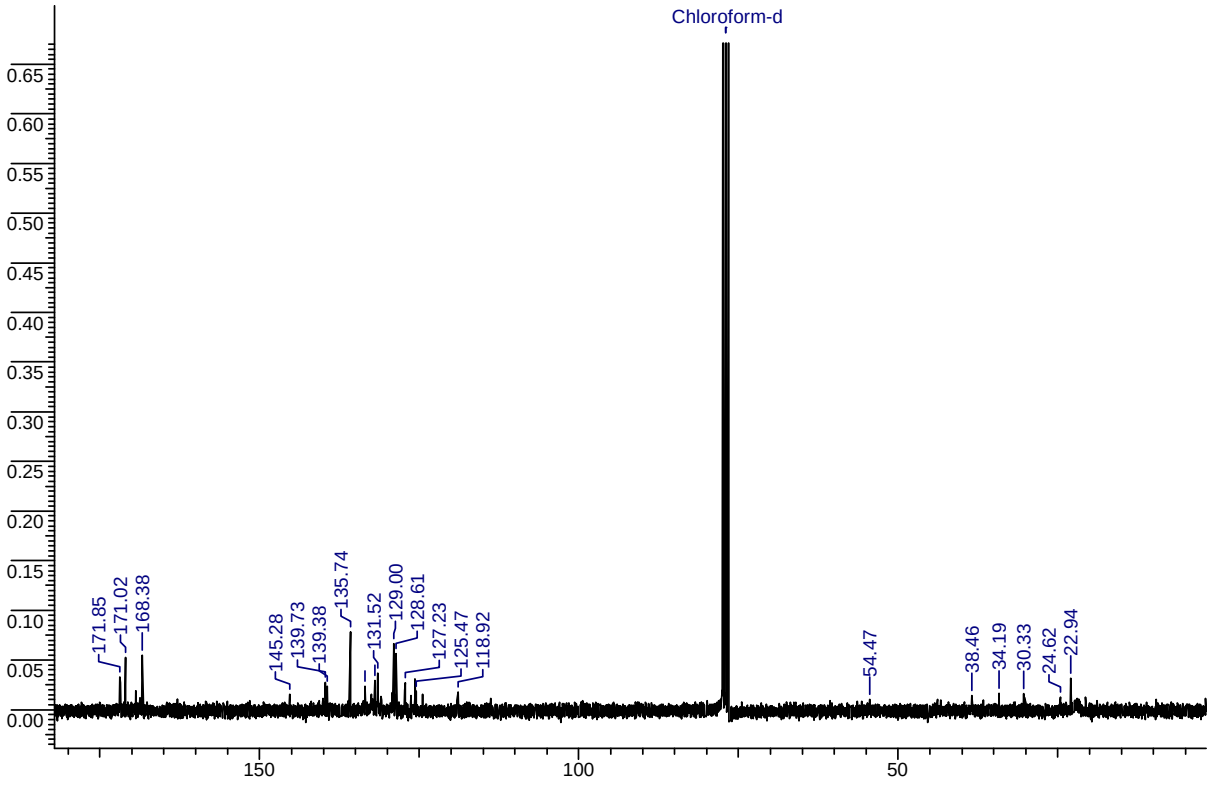
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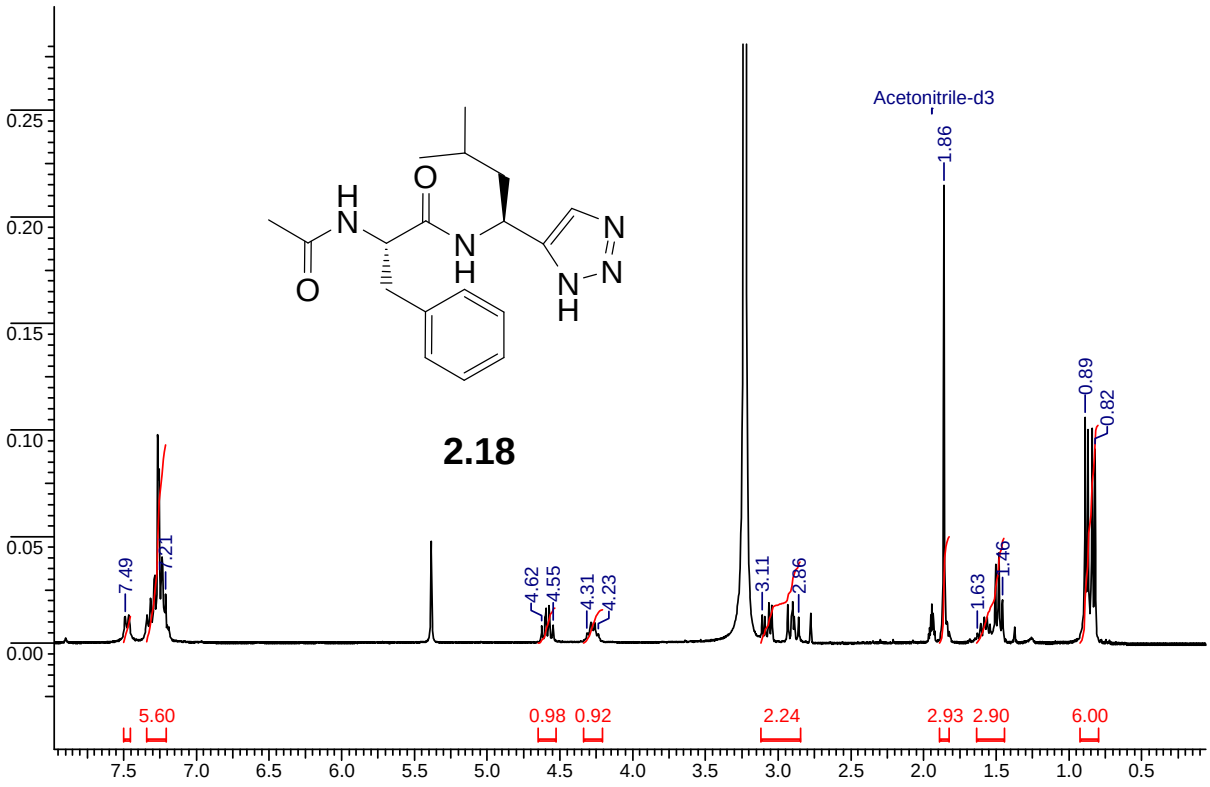
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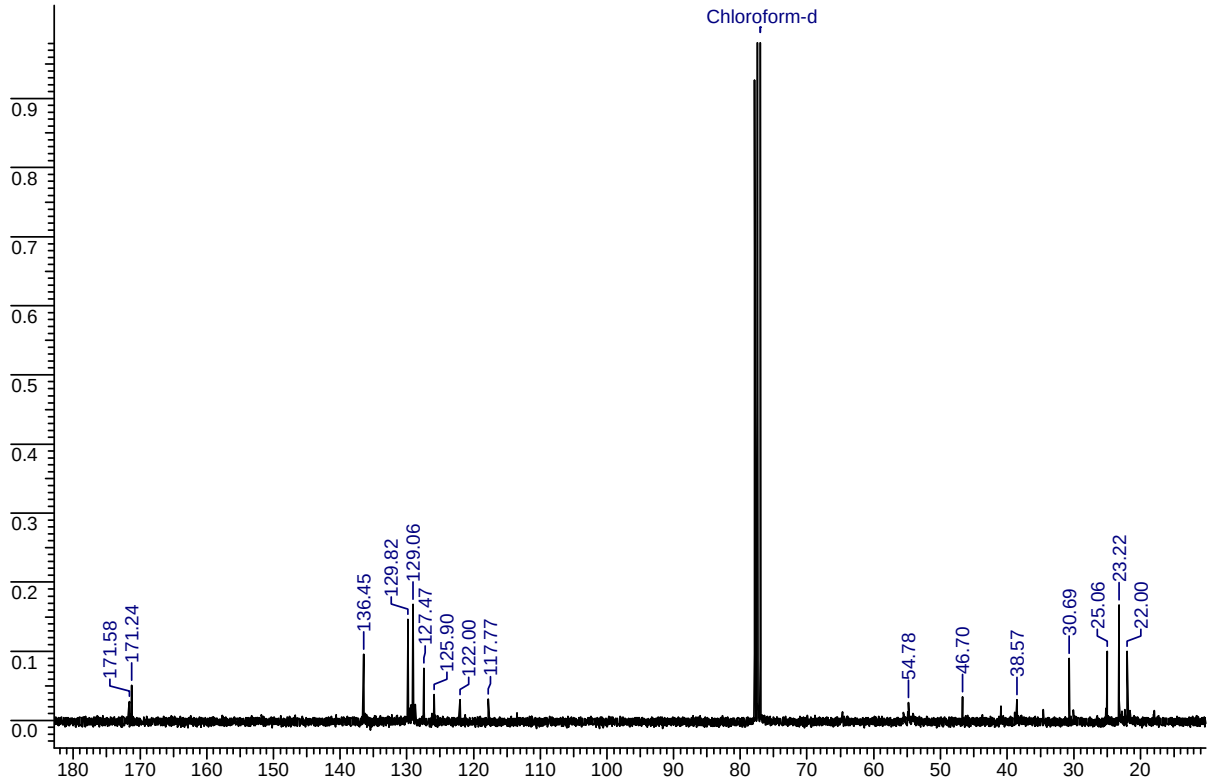
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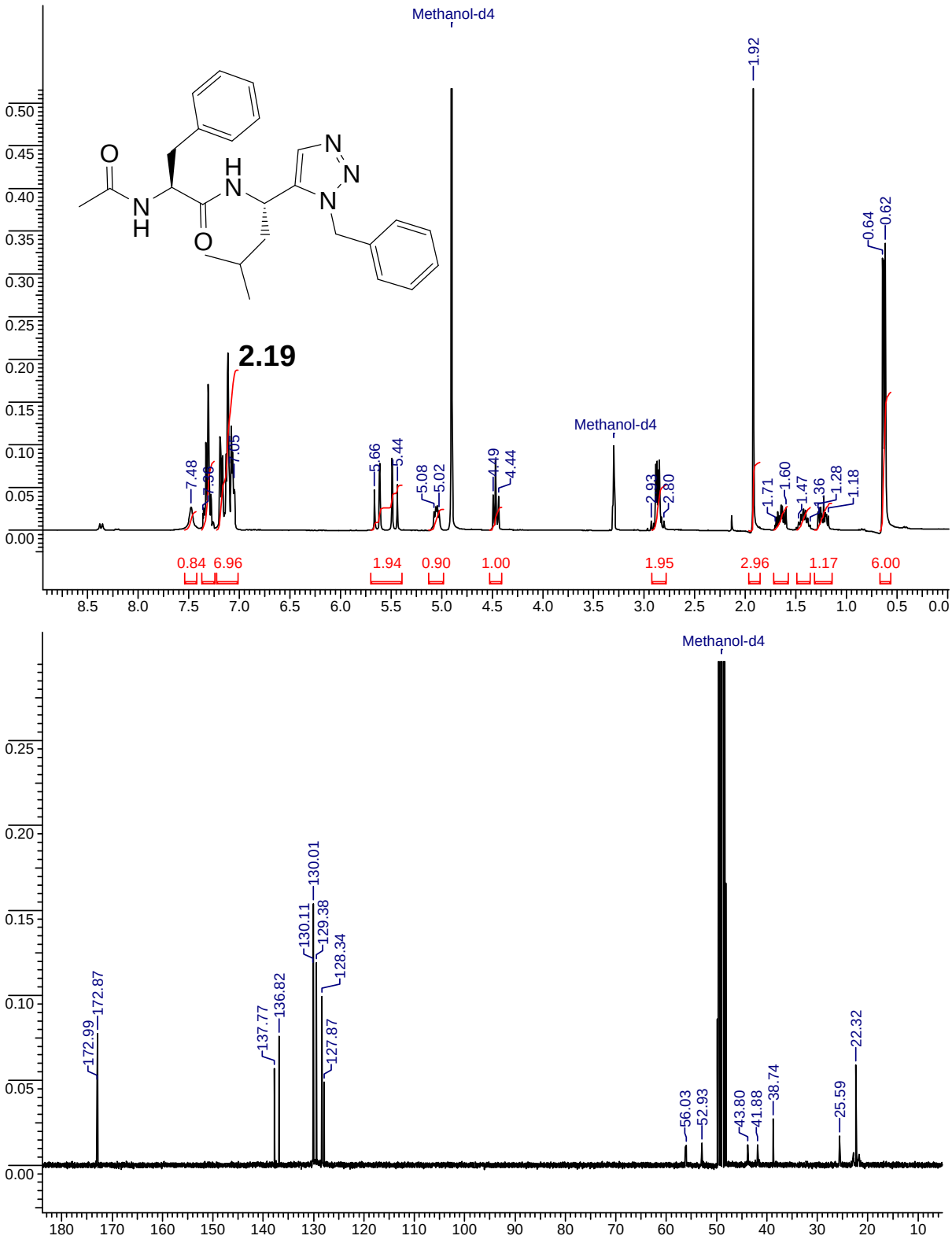
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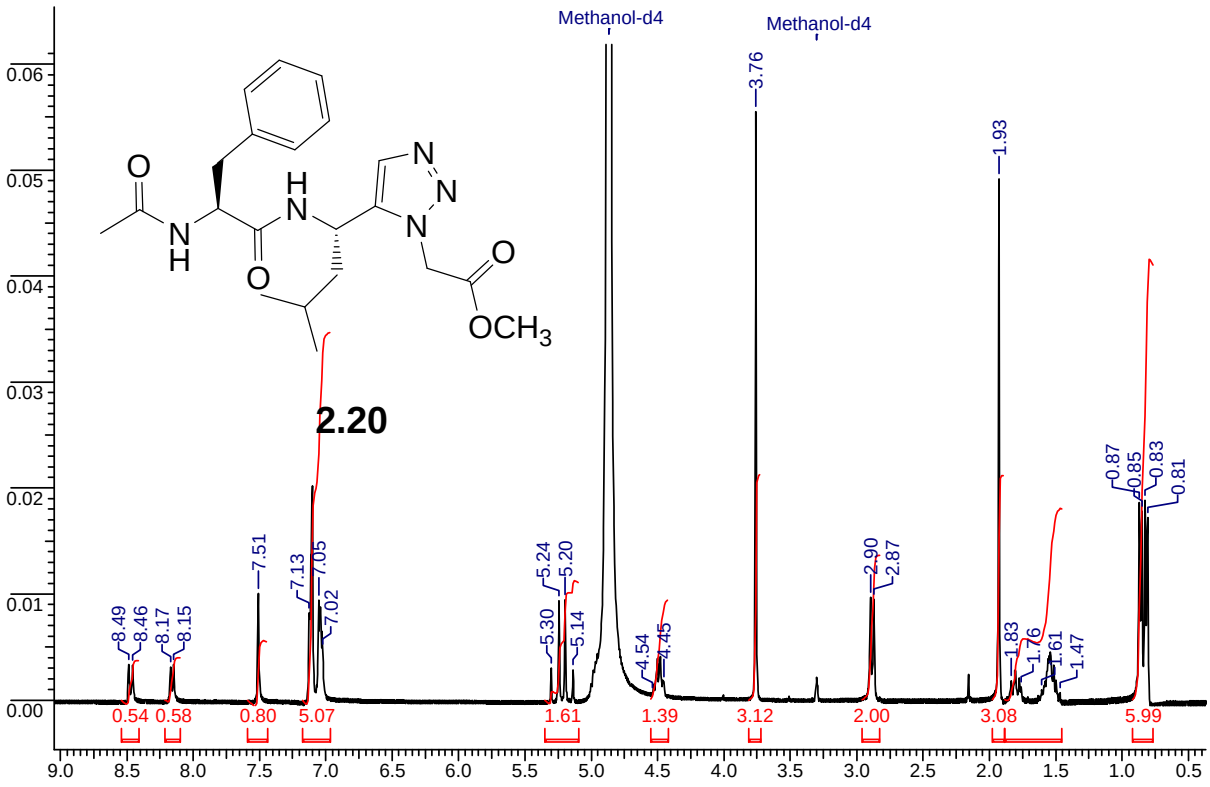
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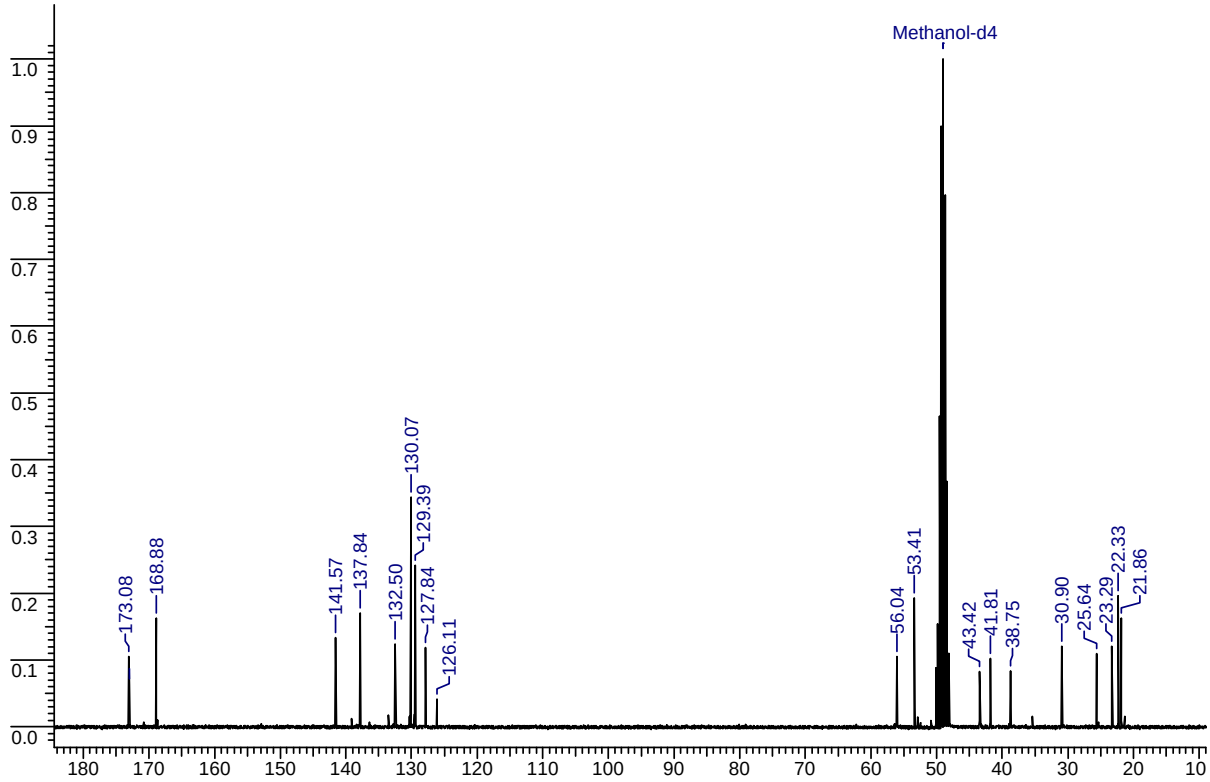
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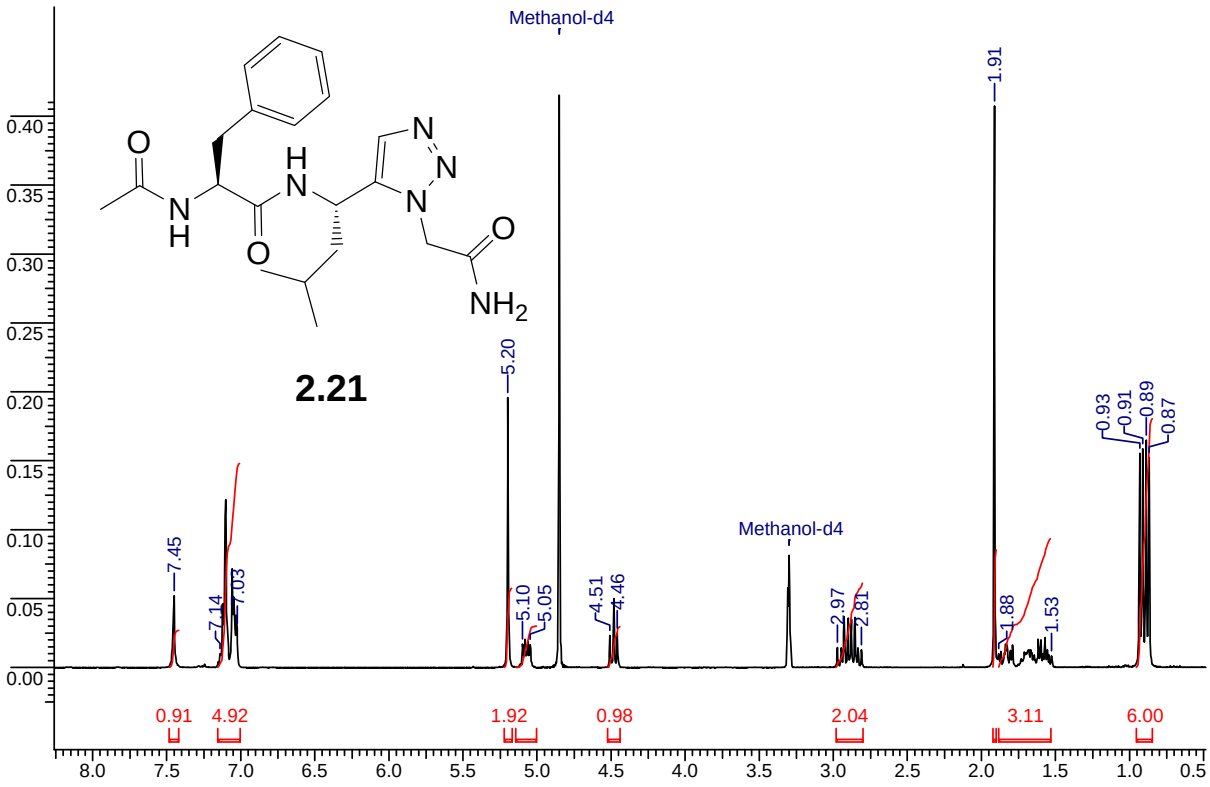
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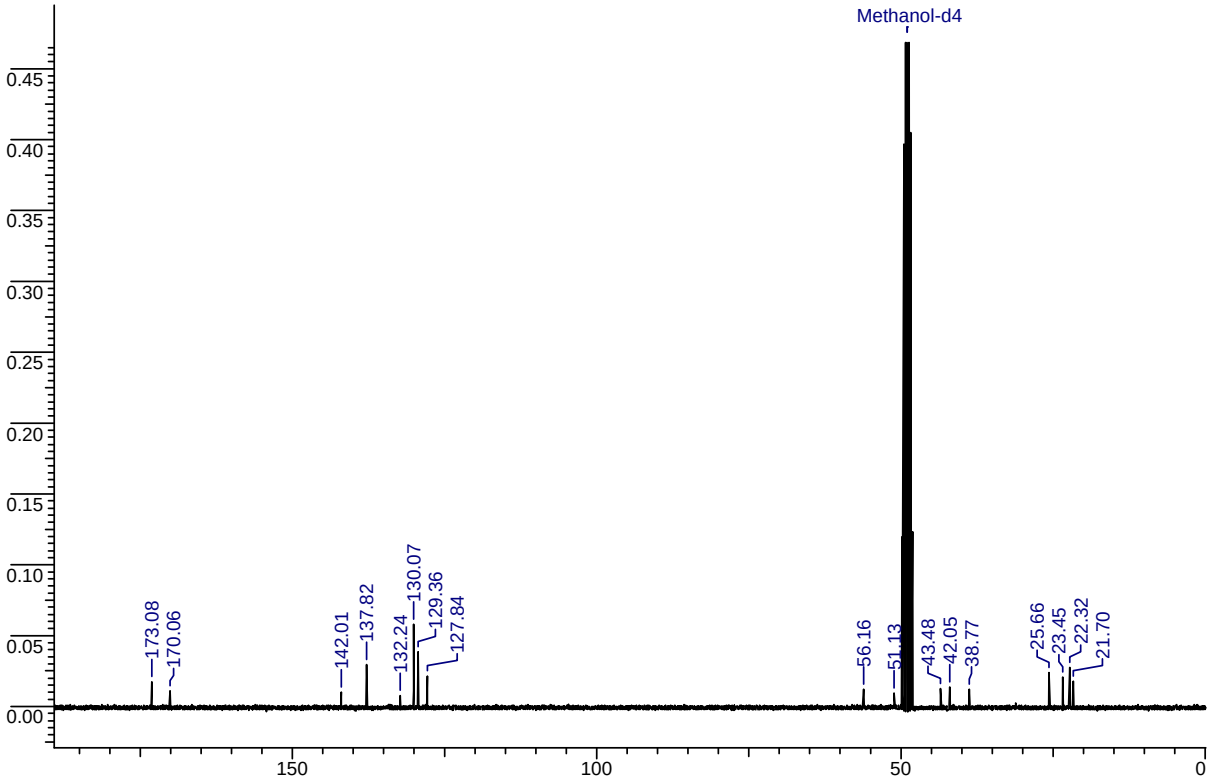
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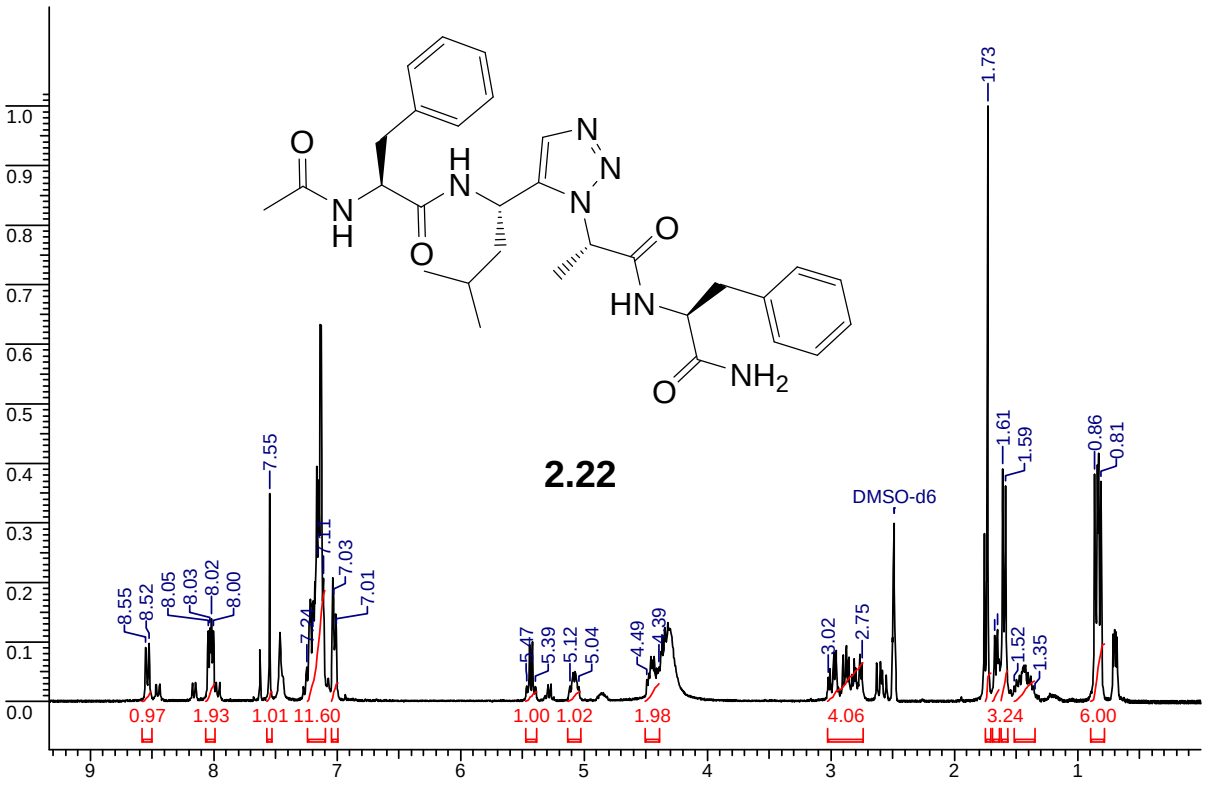
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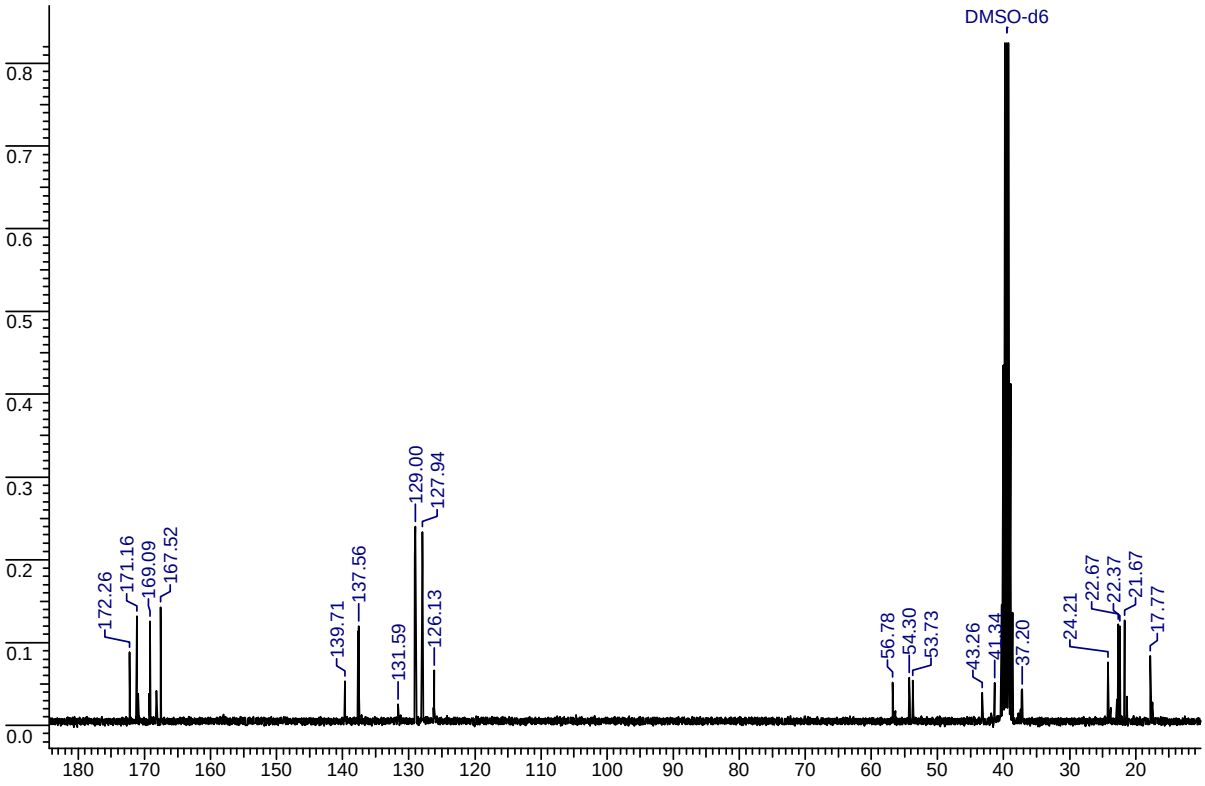
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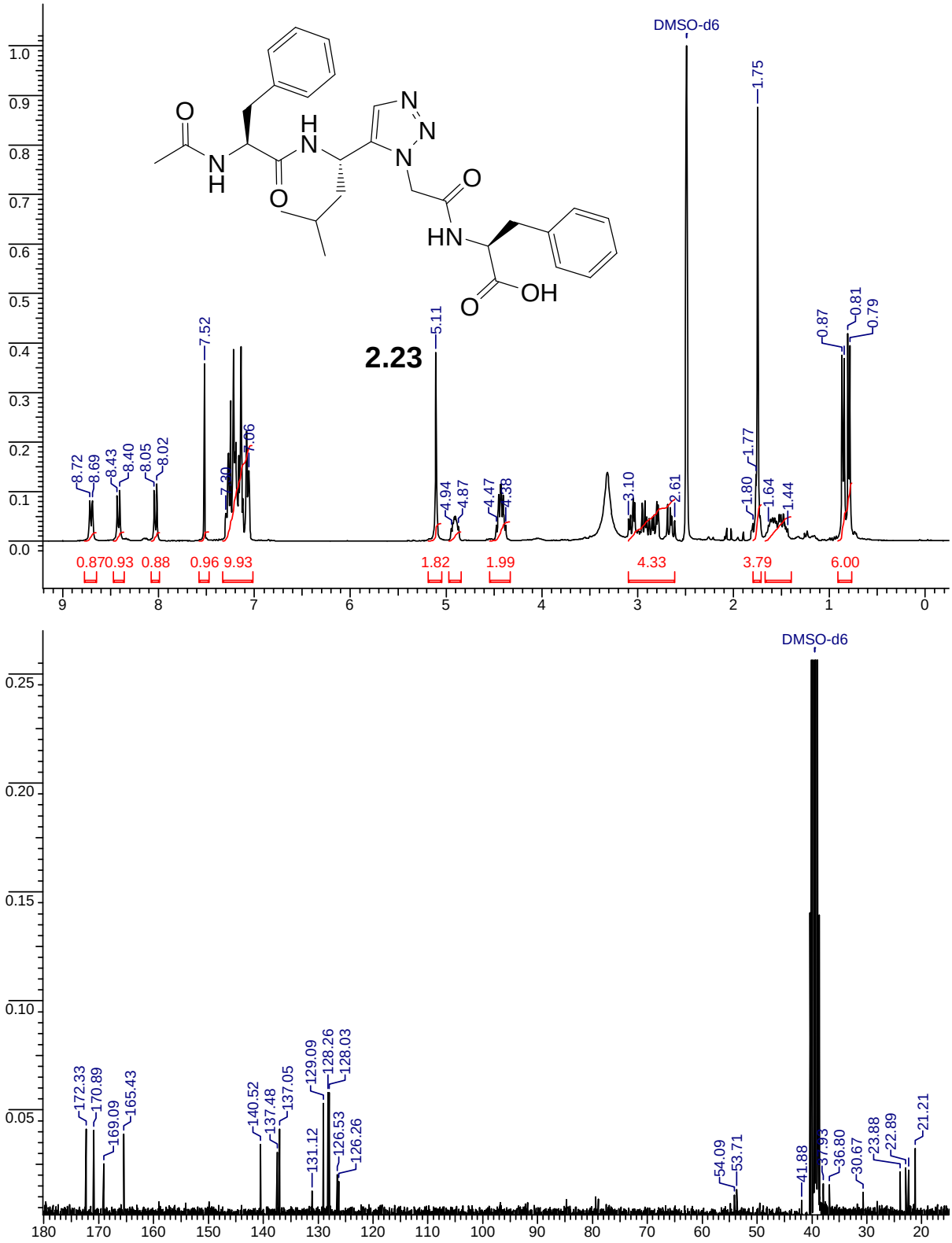
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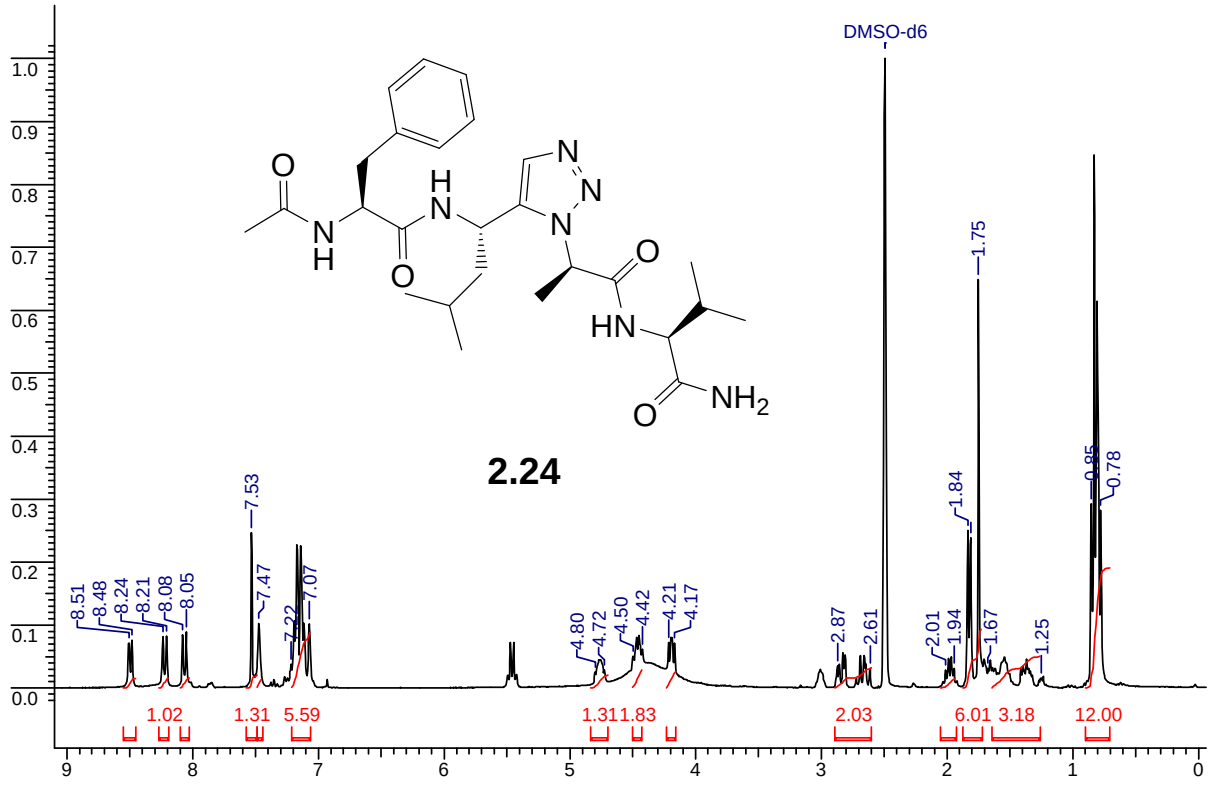
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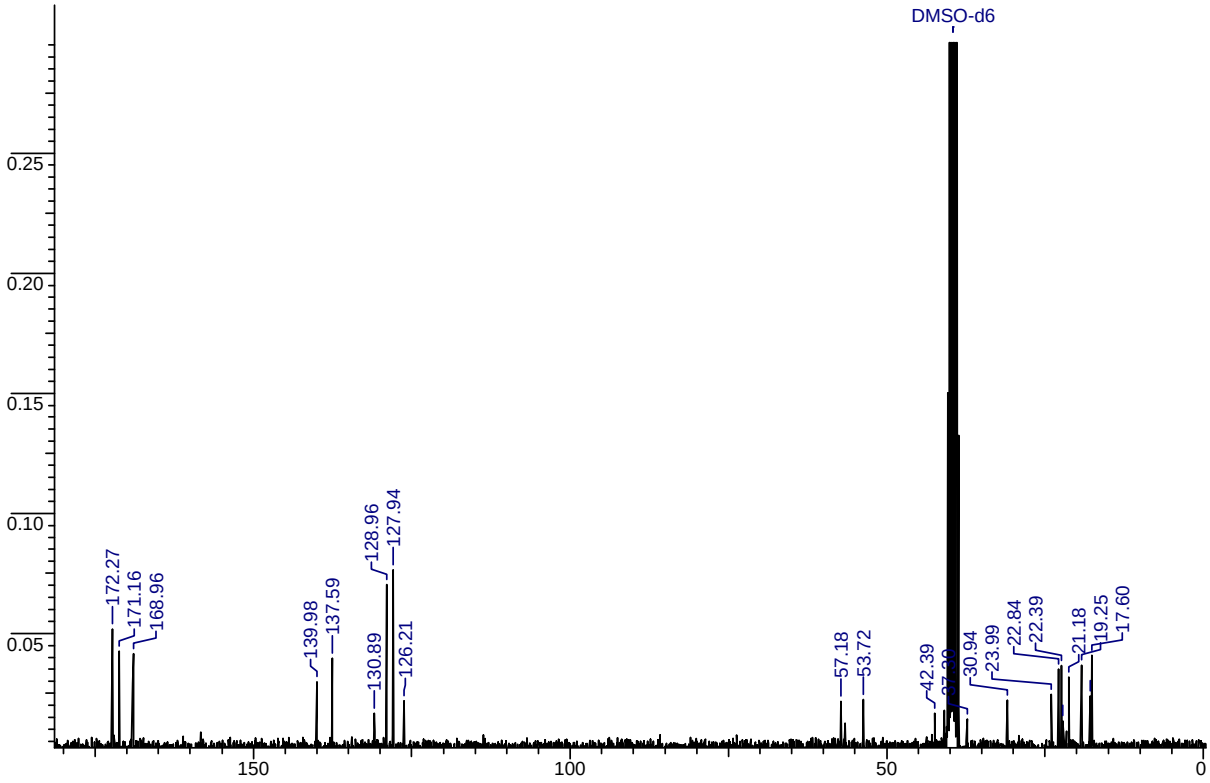
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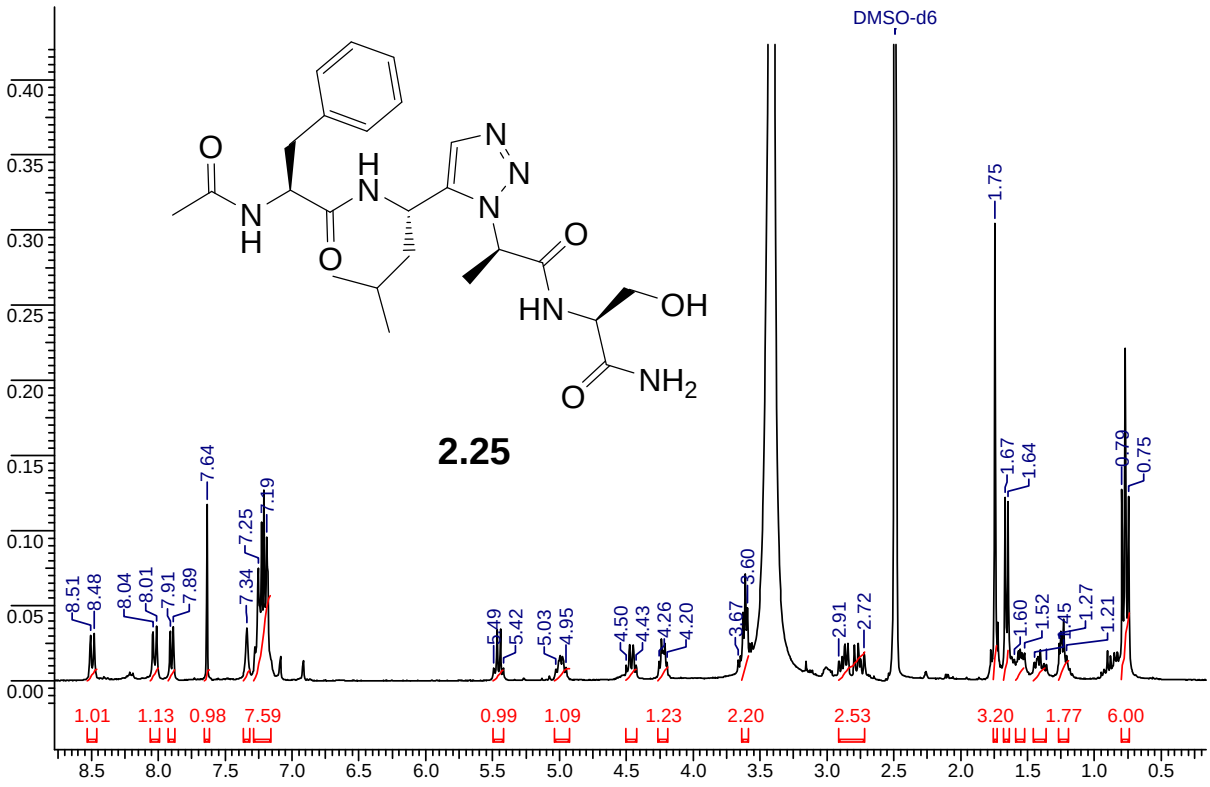
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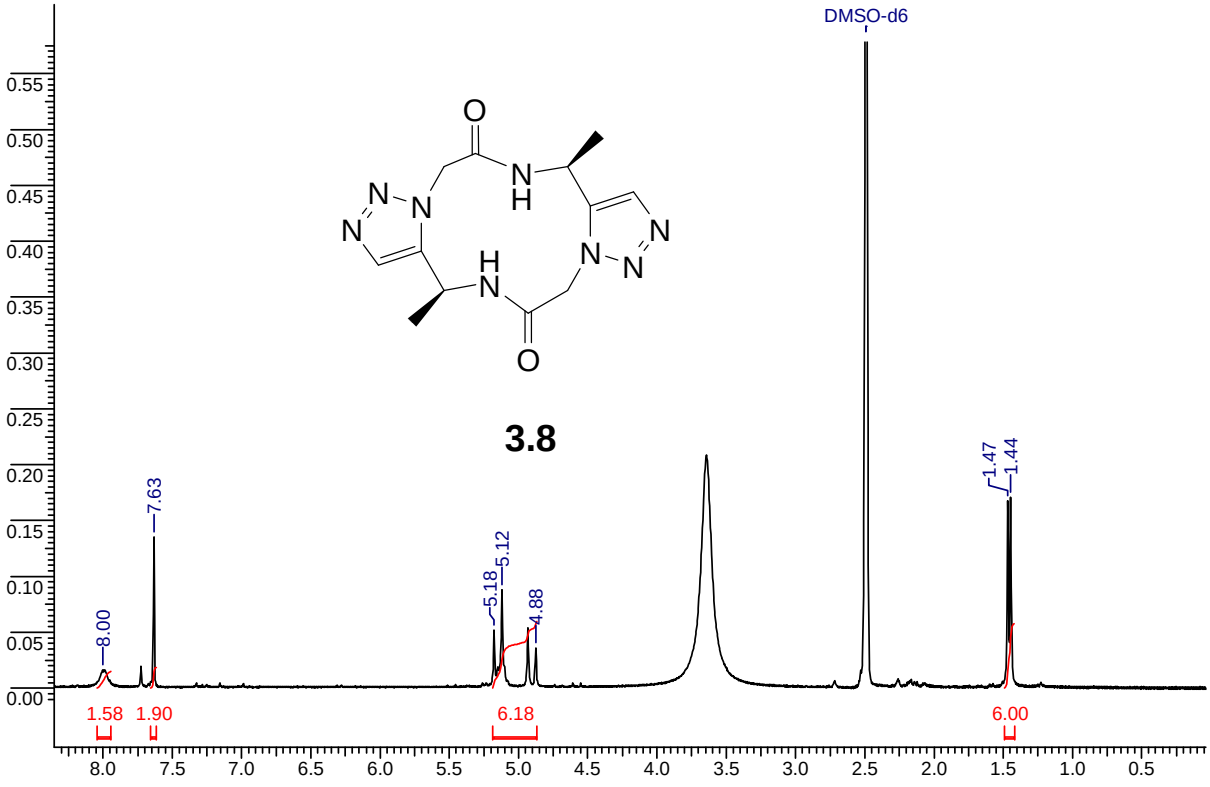
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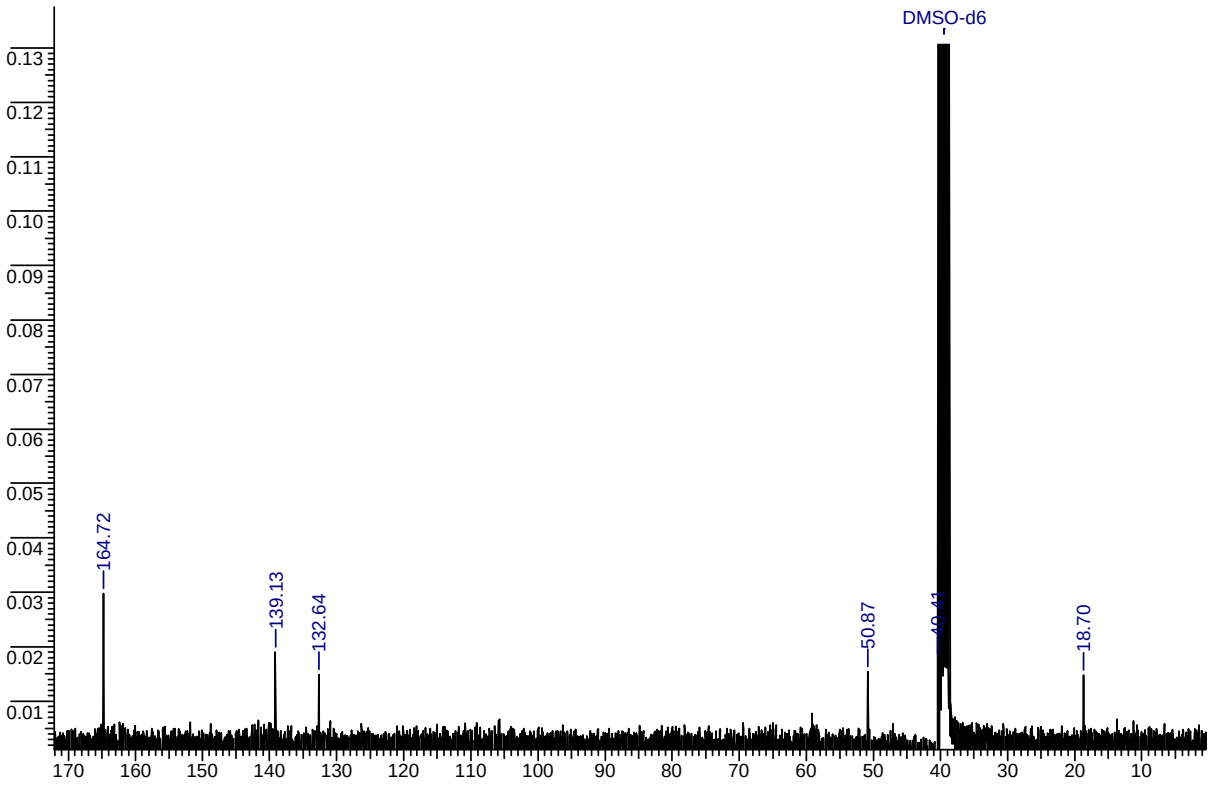
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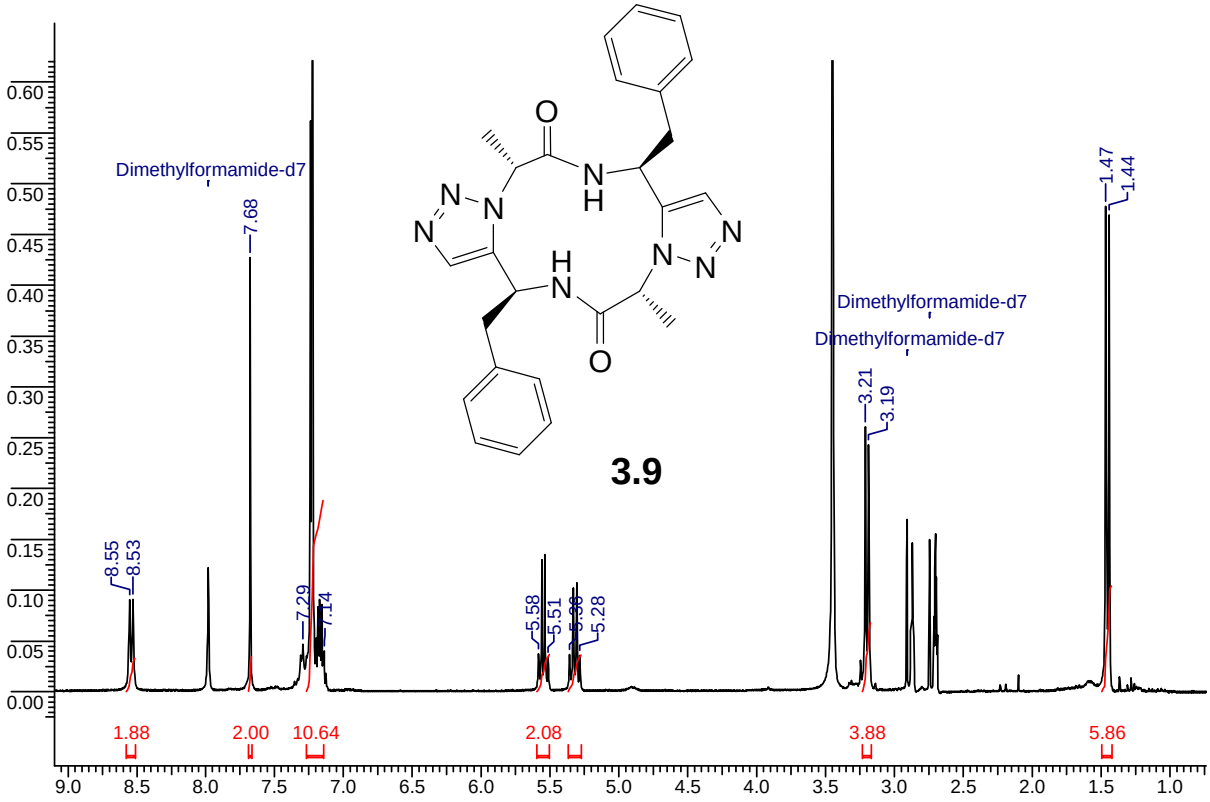
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Dimethylformamide-d7
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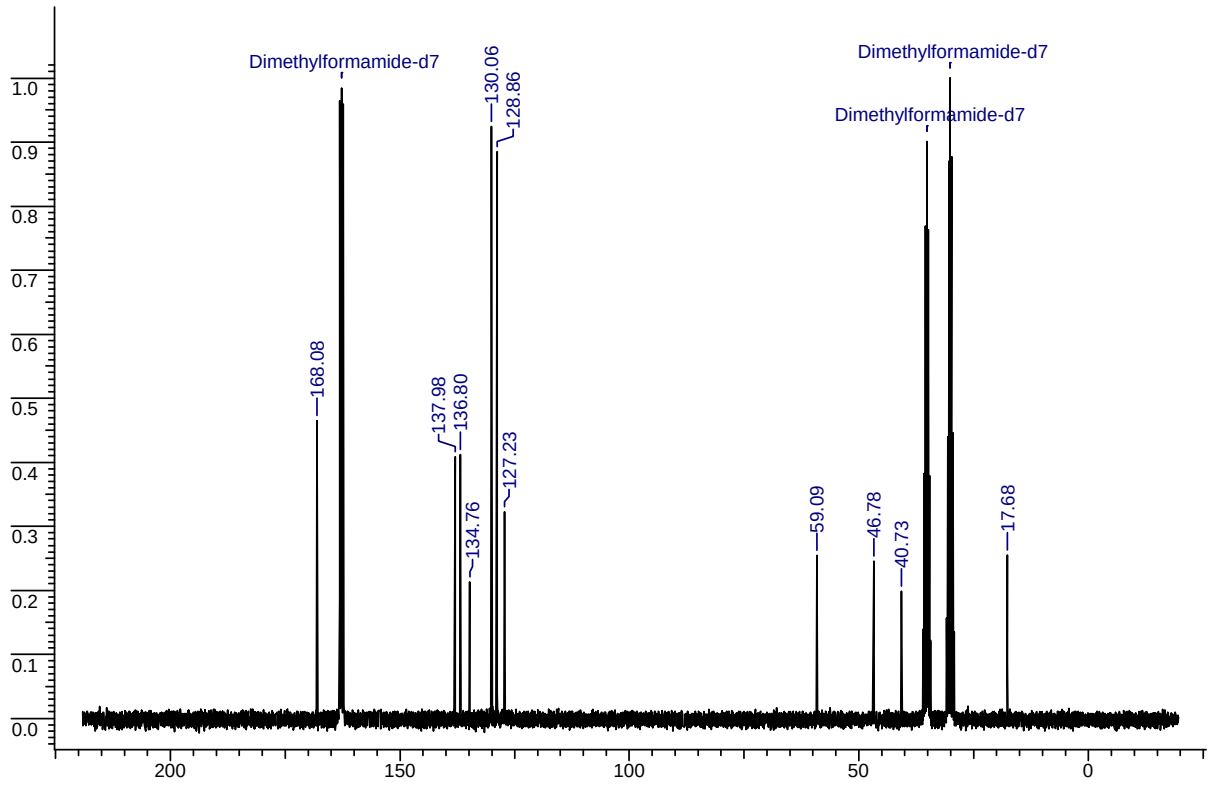
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Dimethylformamide-d7
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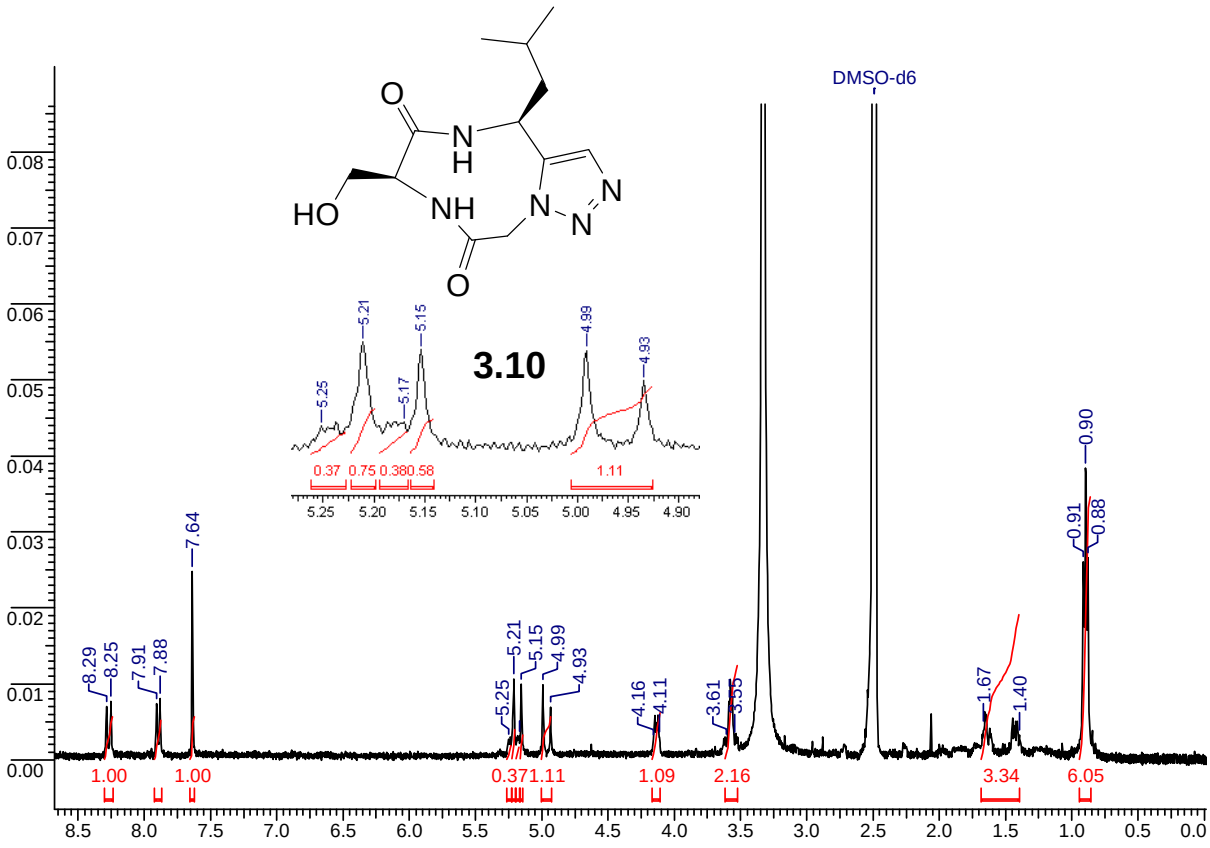
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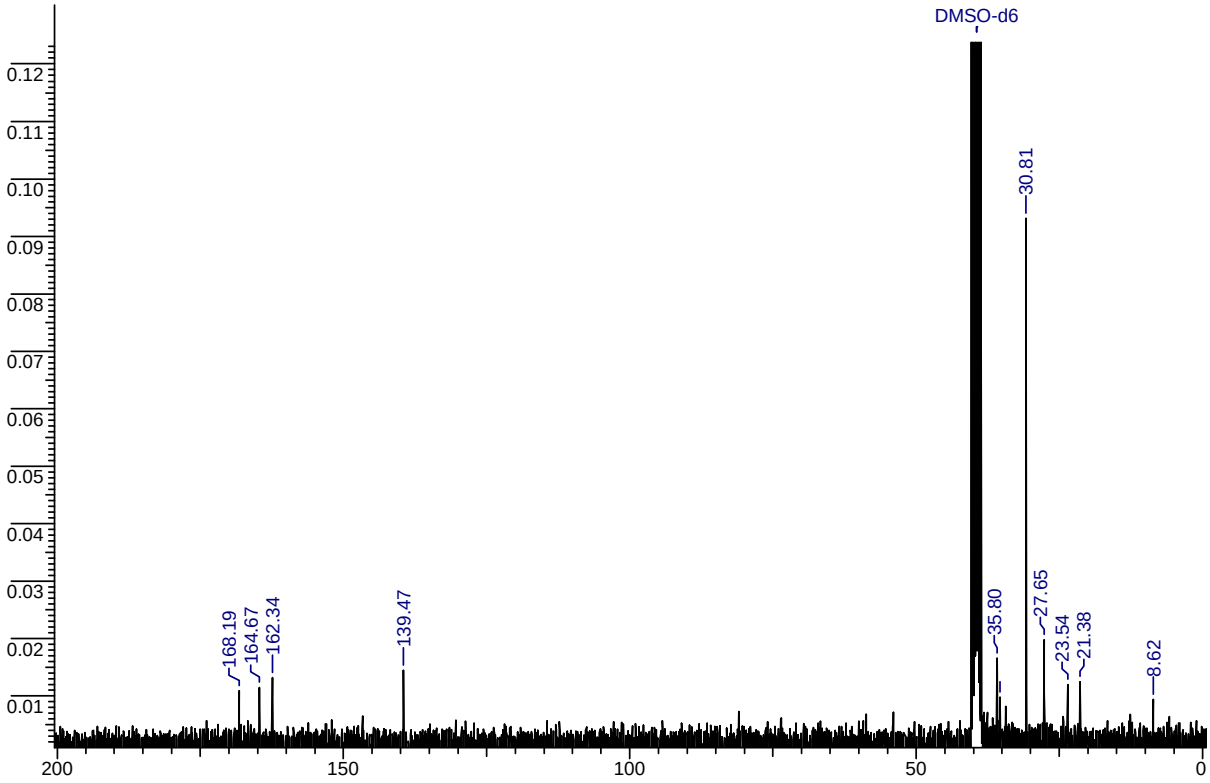
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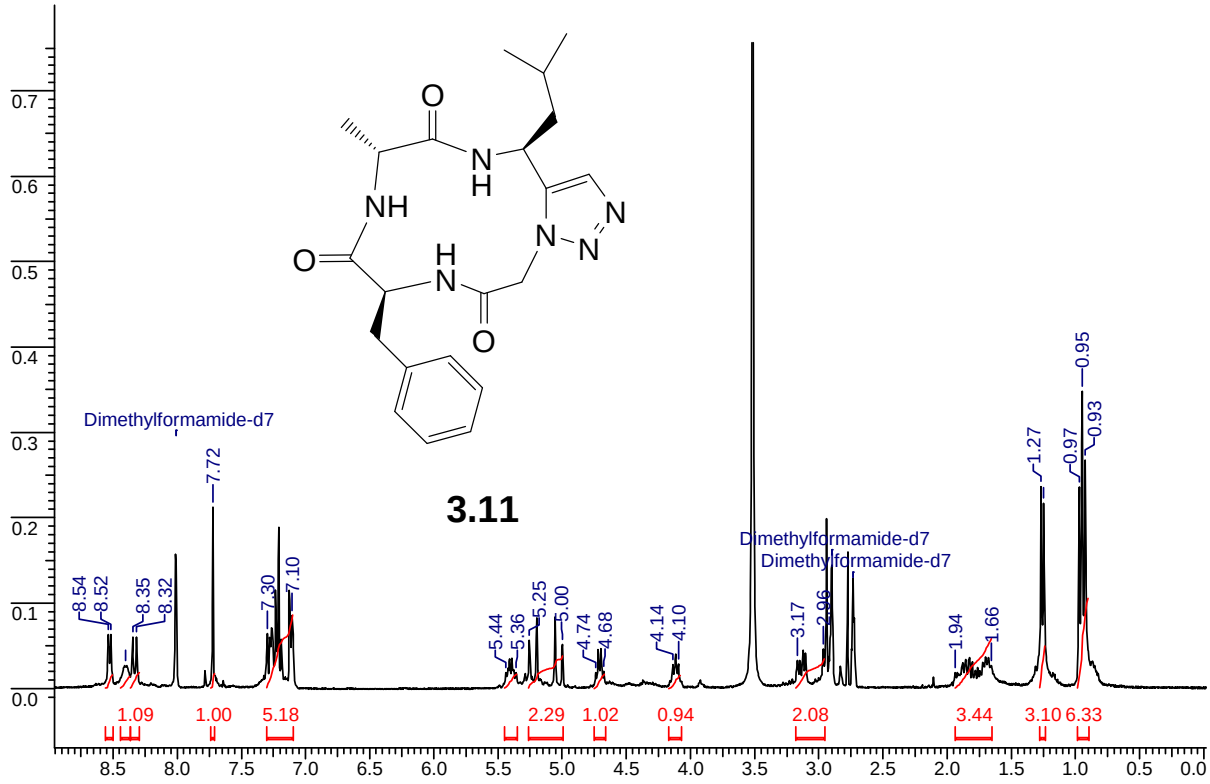
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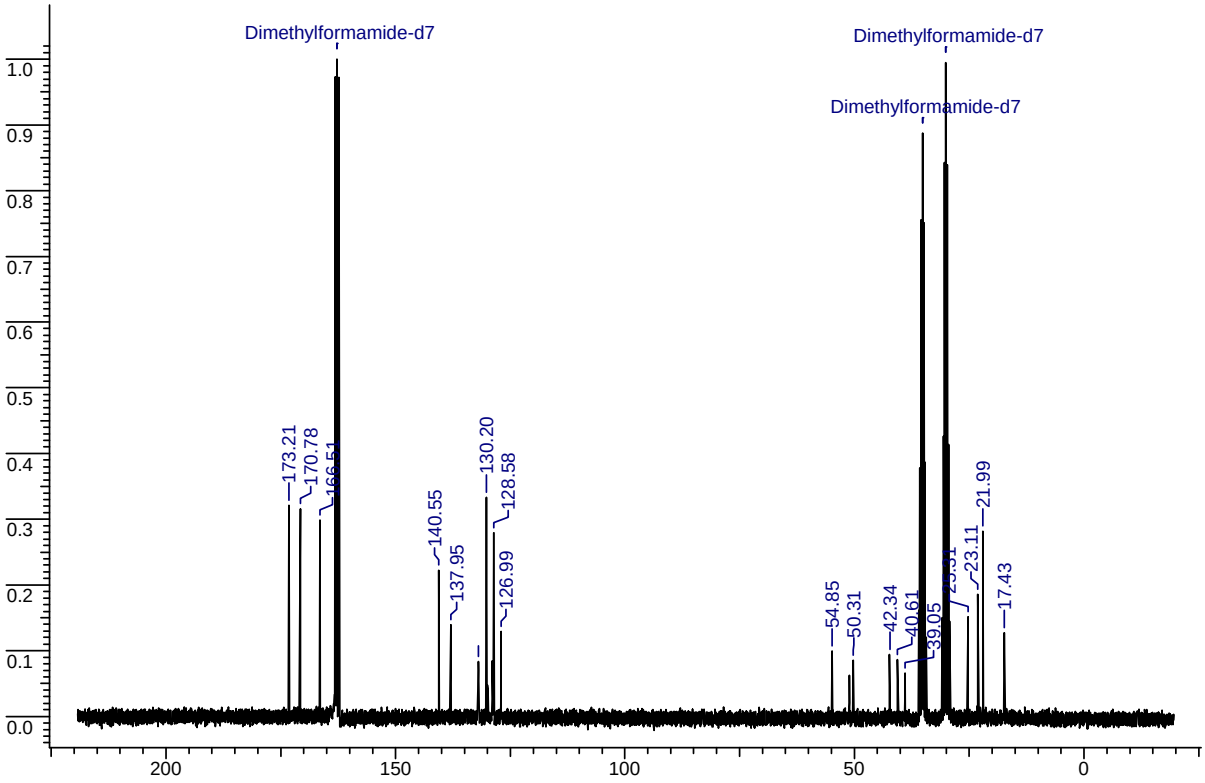
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Dimethylformamide-d7
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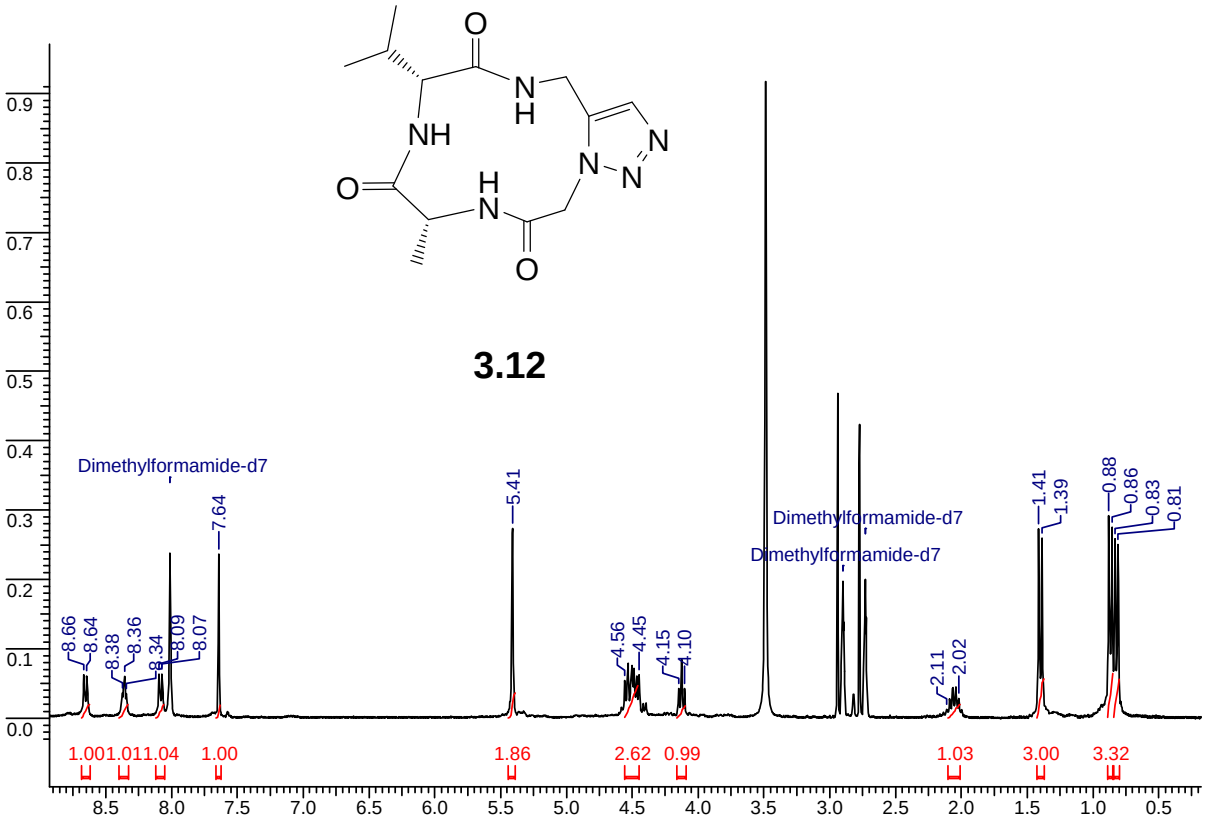
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Dimethylformamide-d7
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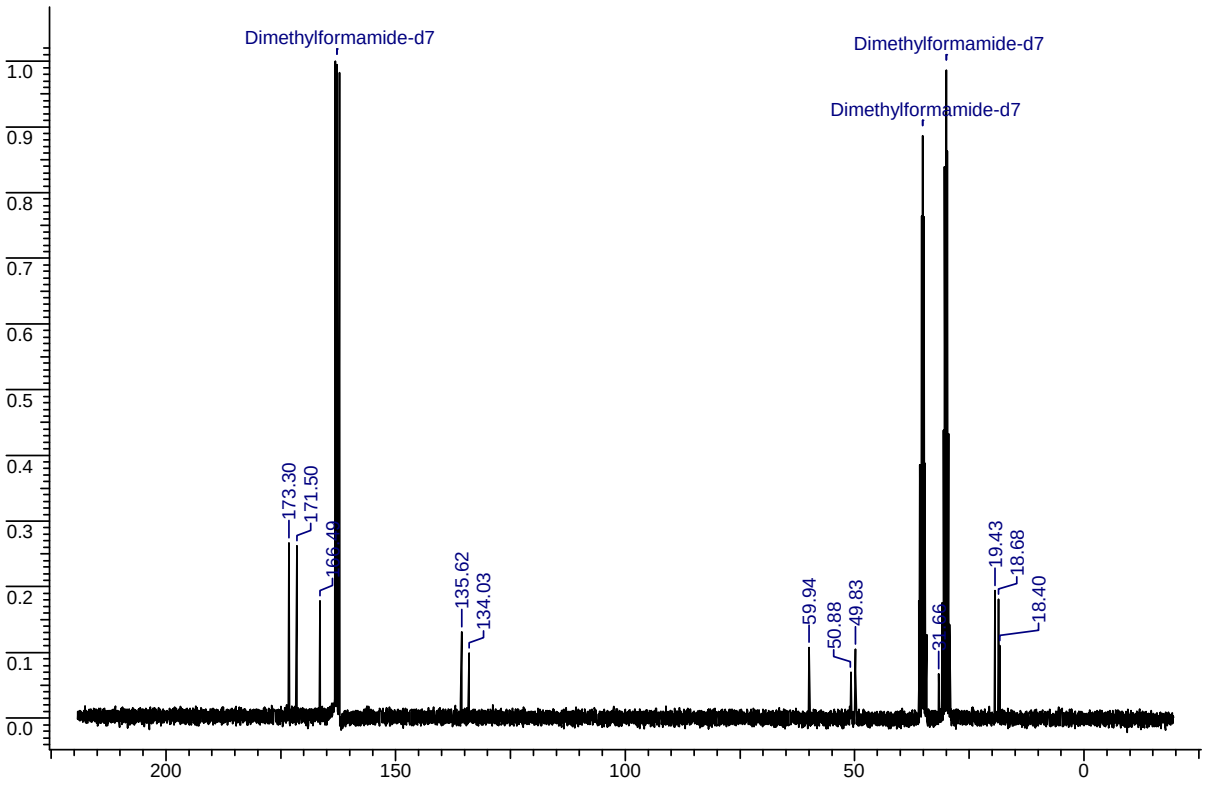
3.12


Dimethylformamide-d7
Dimethylformamide-d7
Dimethylformamide-d7
173.30
171.50
166.49
135.62
134.03
59.94
50.88
49.83
31.66
19.43
18.68
18.40
1.0
0.9
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0.4
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0.1
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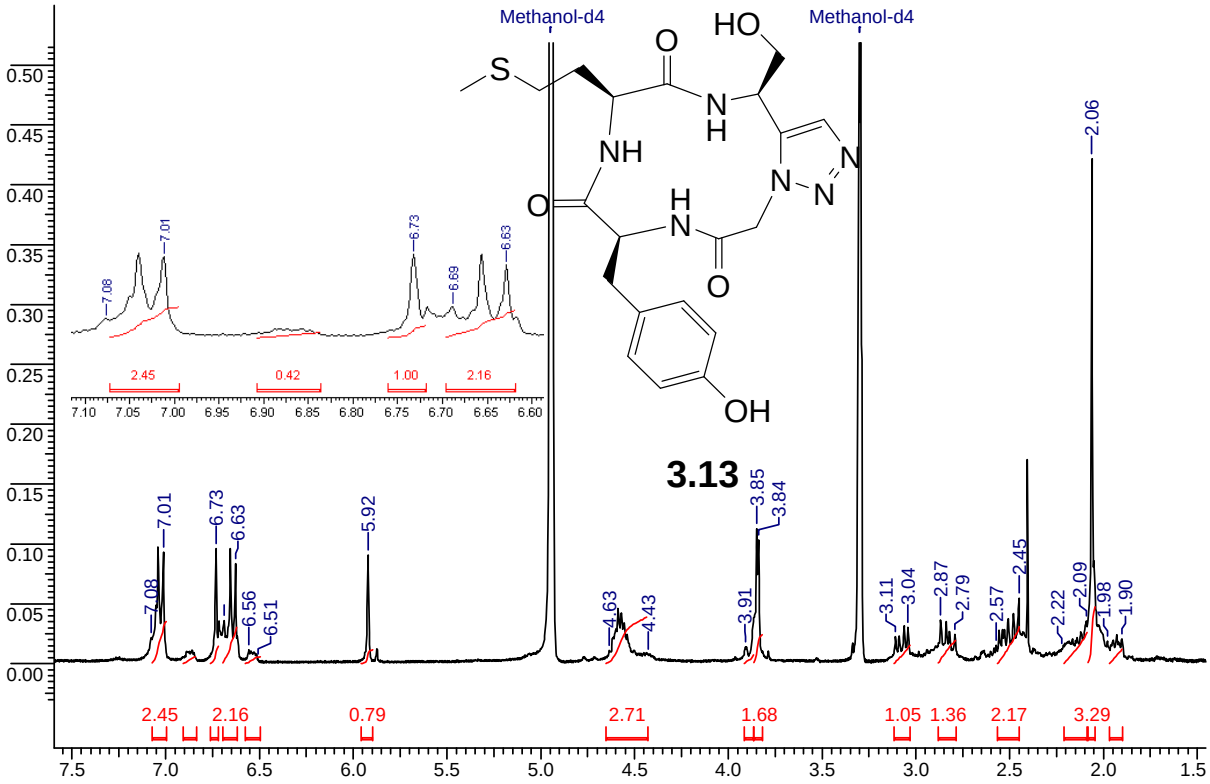
Methanol-d4
Methanol-d4
HO
O
S
N
H
NH
N
N
O
H
N
O
OH
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7.08
7.01
6.73
6.63
6.56
6.51
5.92
4.63
4.43
3.91
3.85
3.84
3.11
3.04
2.87
2.79
2.57
2.45
2.22
2.09
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1.68
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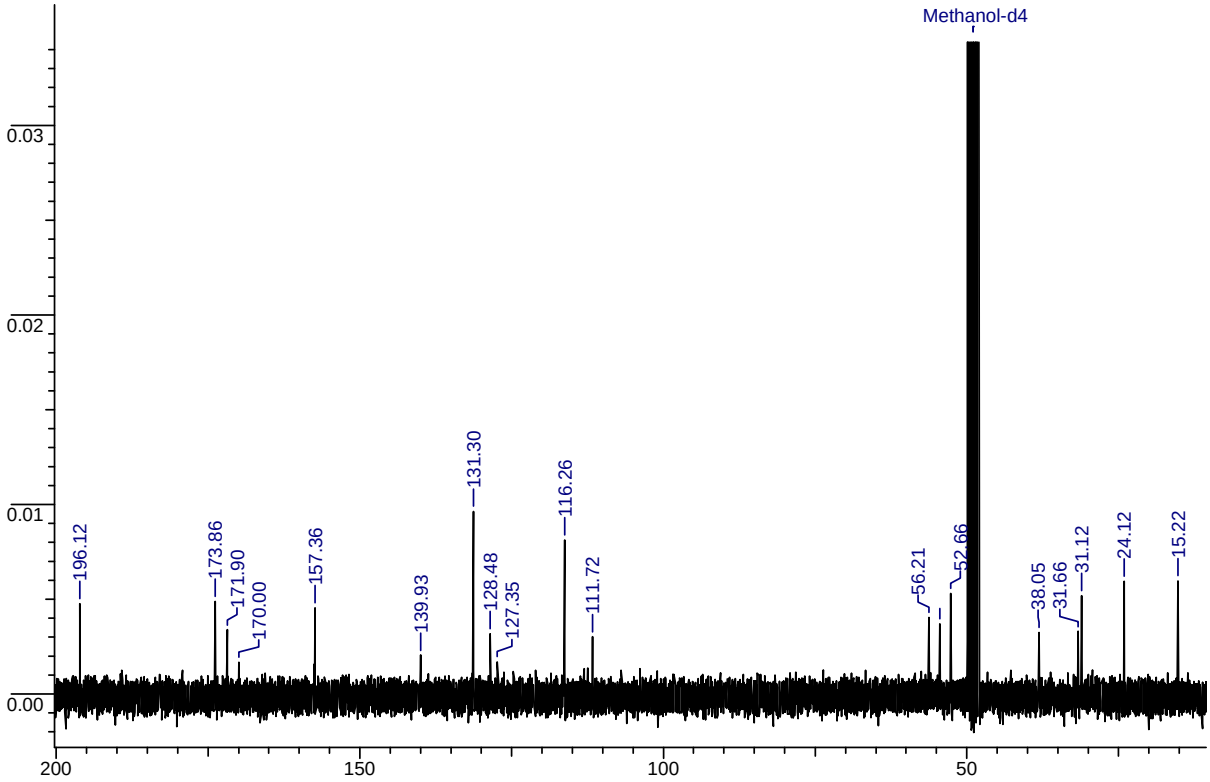
3.29


Methanol-d4
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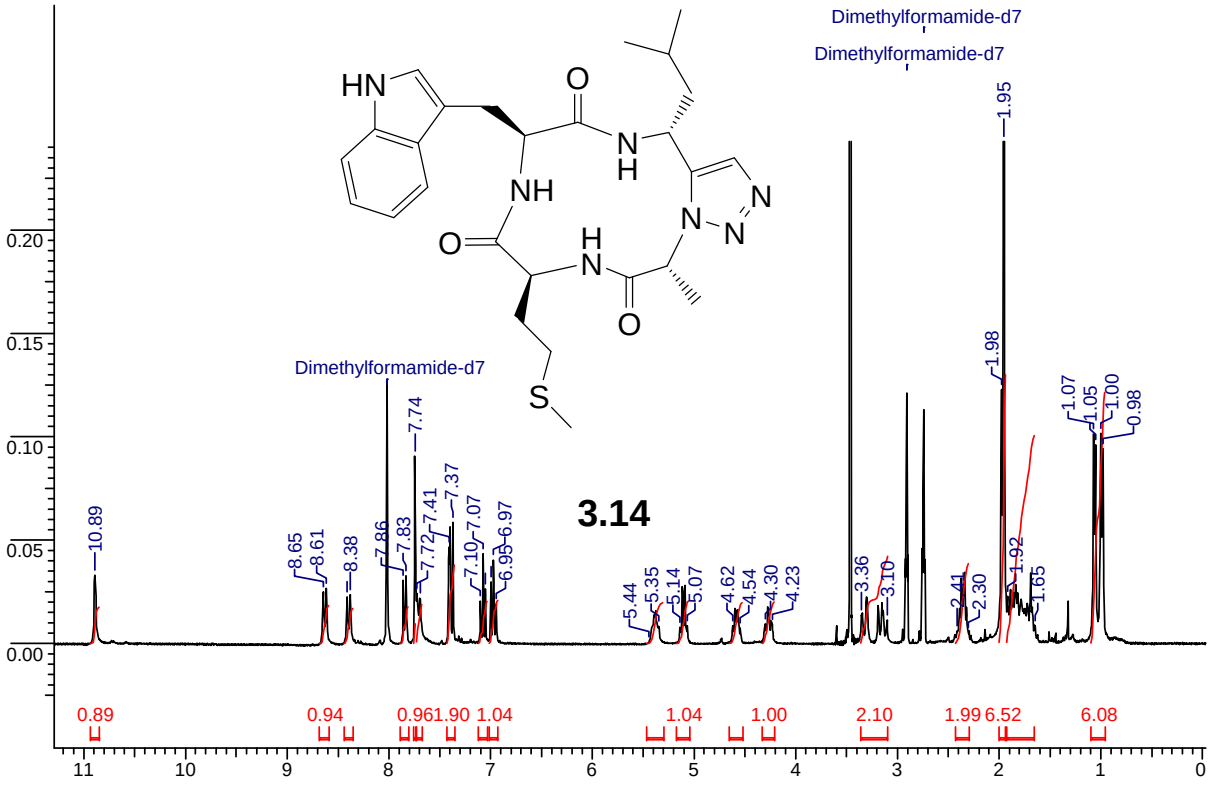
52.66
38.05
31.66
31.12
24.12
15.22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Dimethylformamide-d7
Dimethylformamide-d7
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10.89
8.65
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8.38
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7.83
7.74
7.72
7.41
7.37
7.10
7.07
6.99
6.97
5.44
5.35
5.14
5.07
4.62
4.54
4.30
4.23
3.36
3.10
2.41
2.30
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1.00
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1.04
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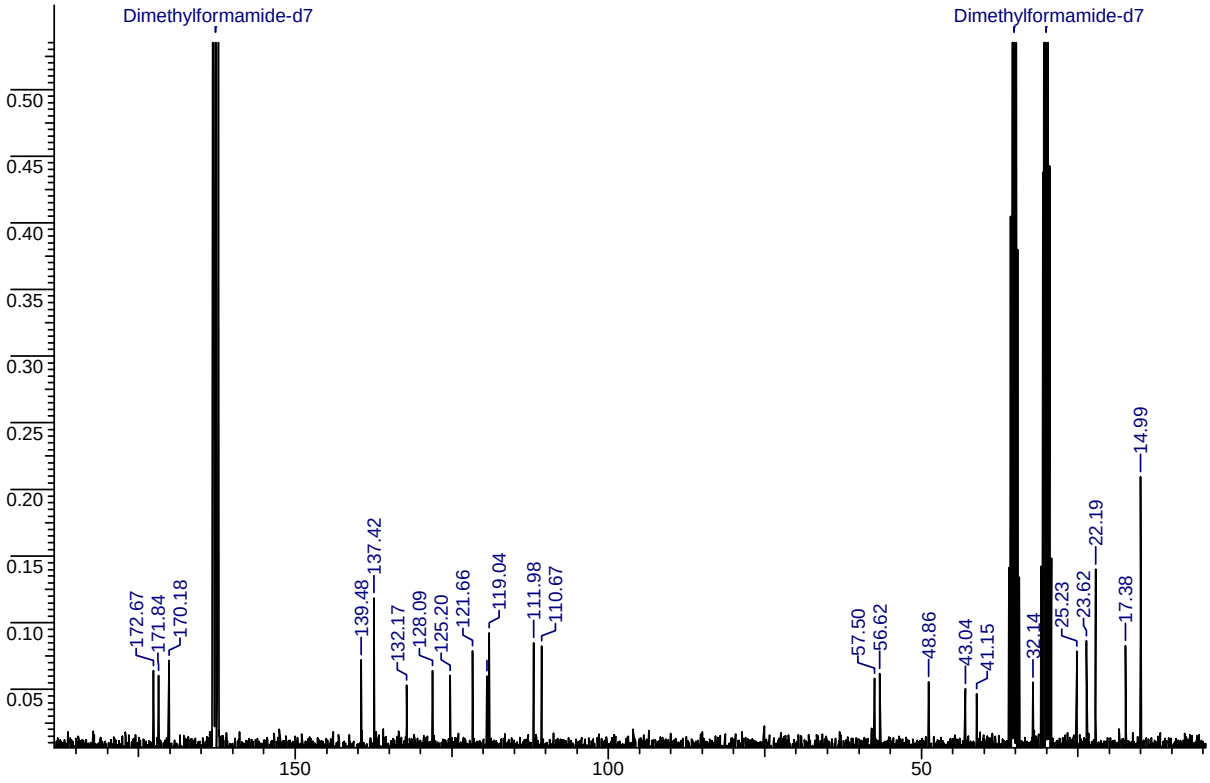
Dimethylformamide-d7
Dimethylformamide-d7
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139.48
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121.66
119.04
111.98
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25.23
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22.19
17.38
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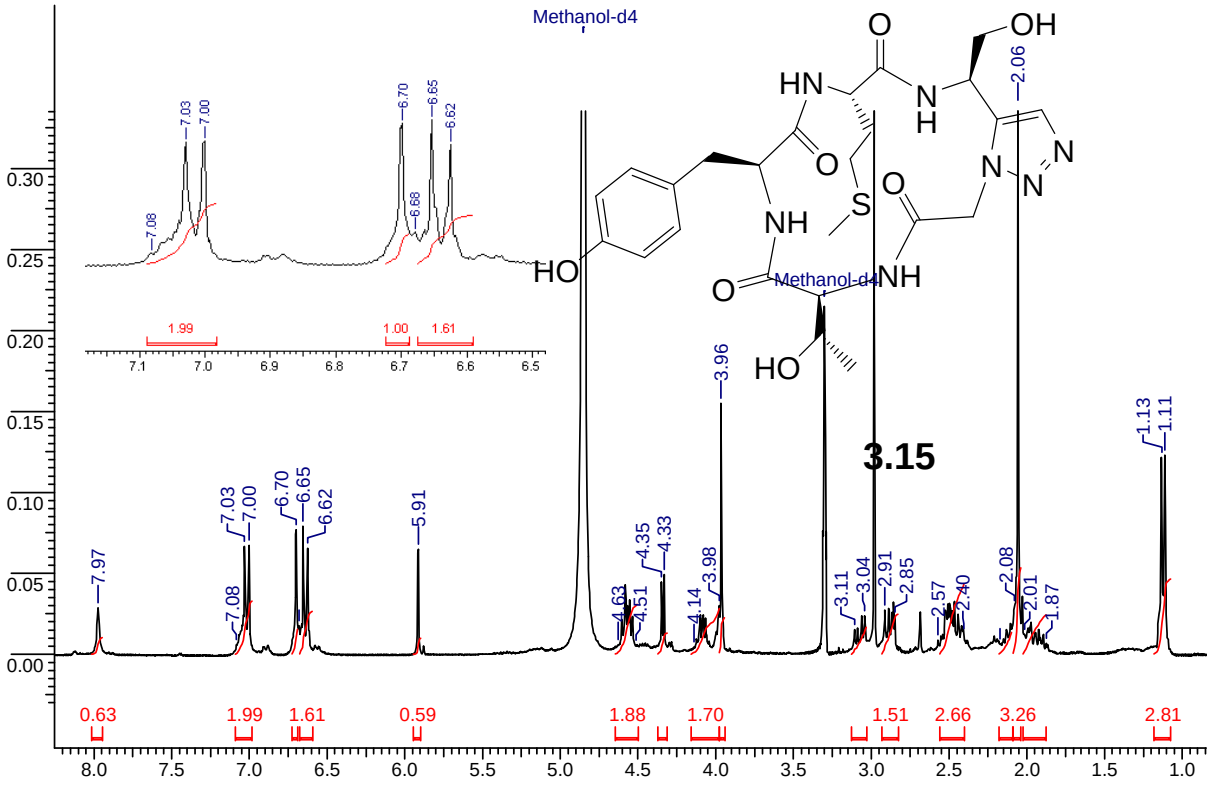
Methanol-d4
Methanol-d4
3.15
7.97
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7.00
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6.62
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4.63
4.51
4.35
4.33
4.14
3.98
3.96
3.11
3.04
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2.85
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2.01
1.87
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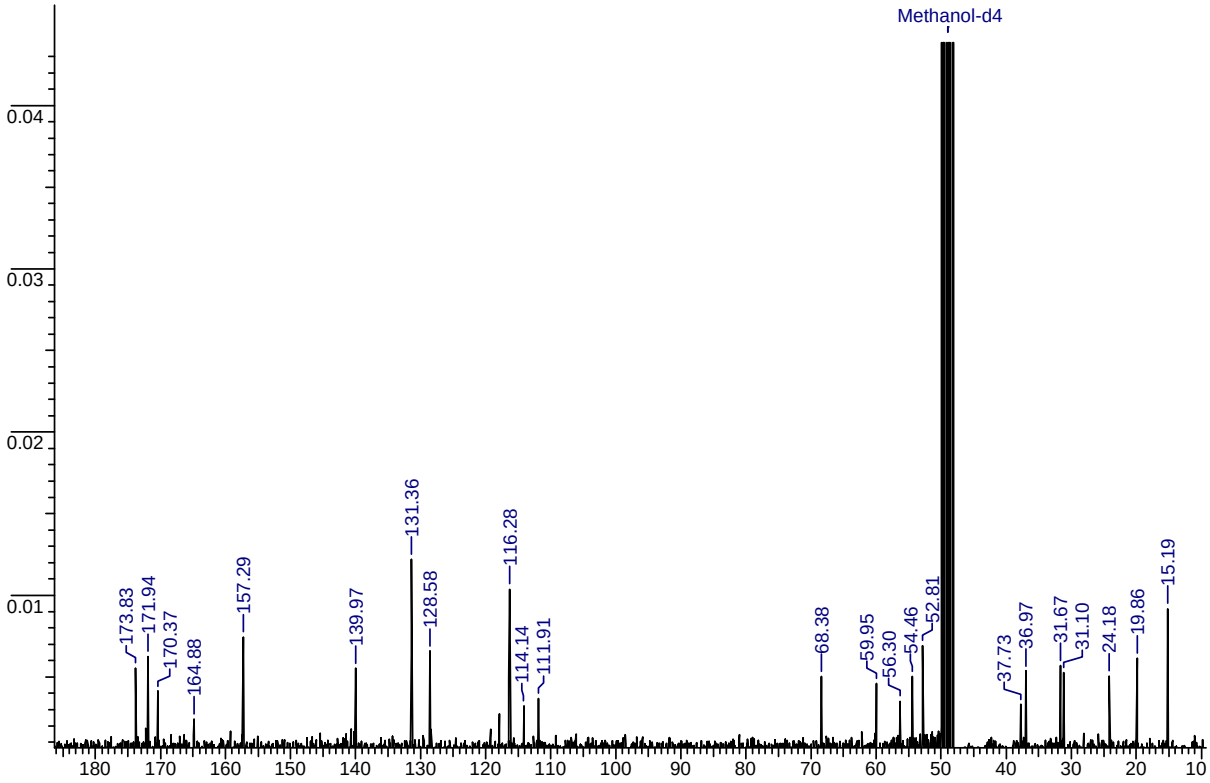
1.88
1.70
1.51
2.66
3.26
2.81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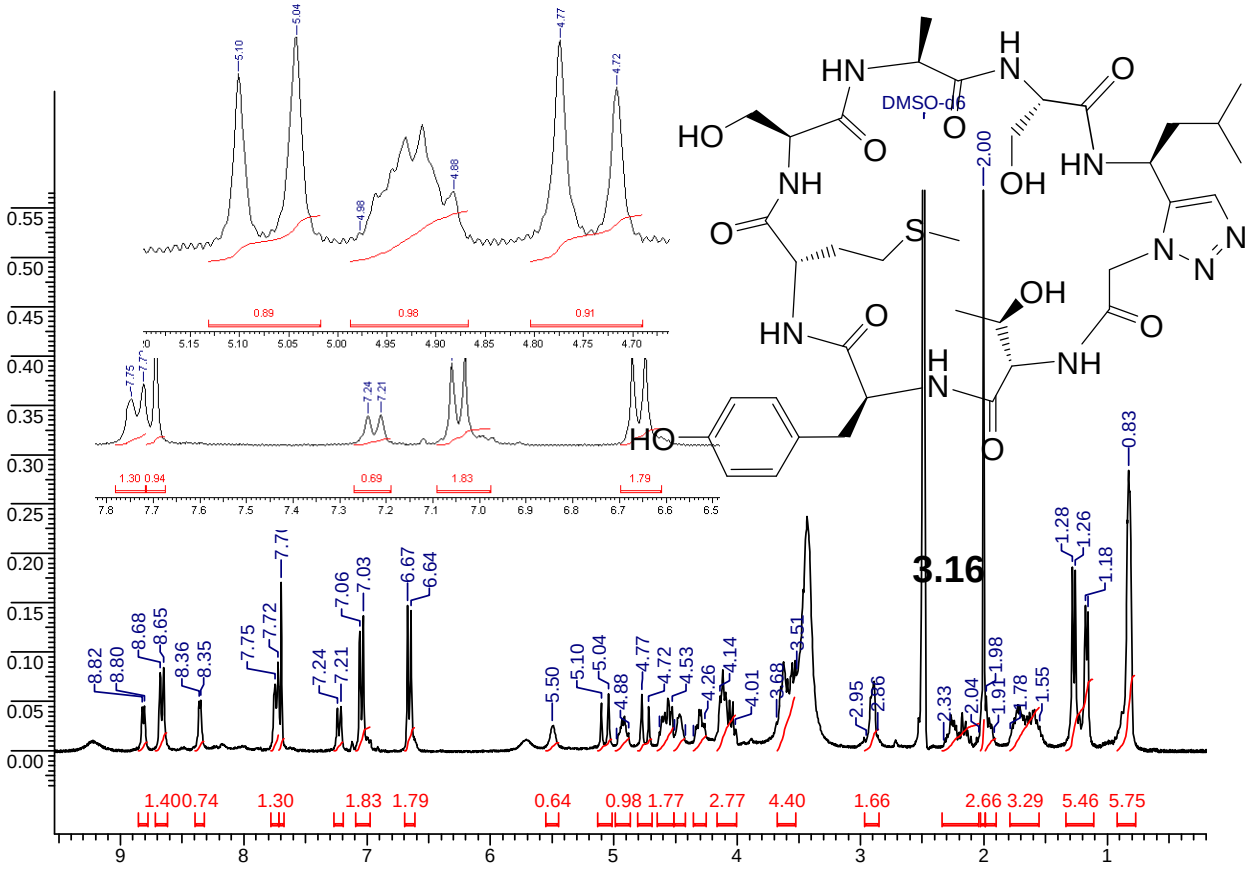
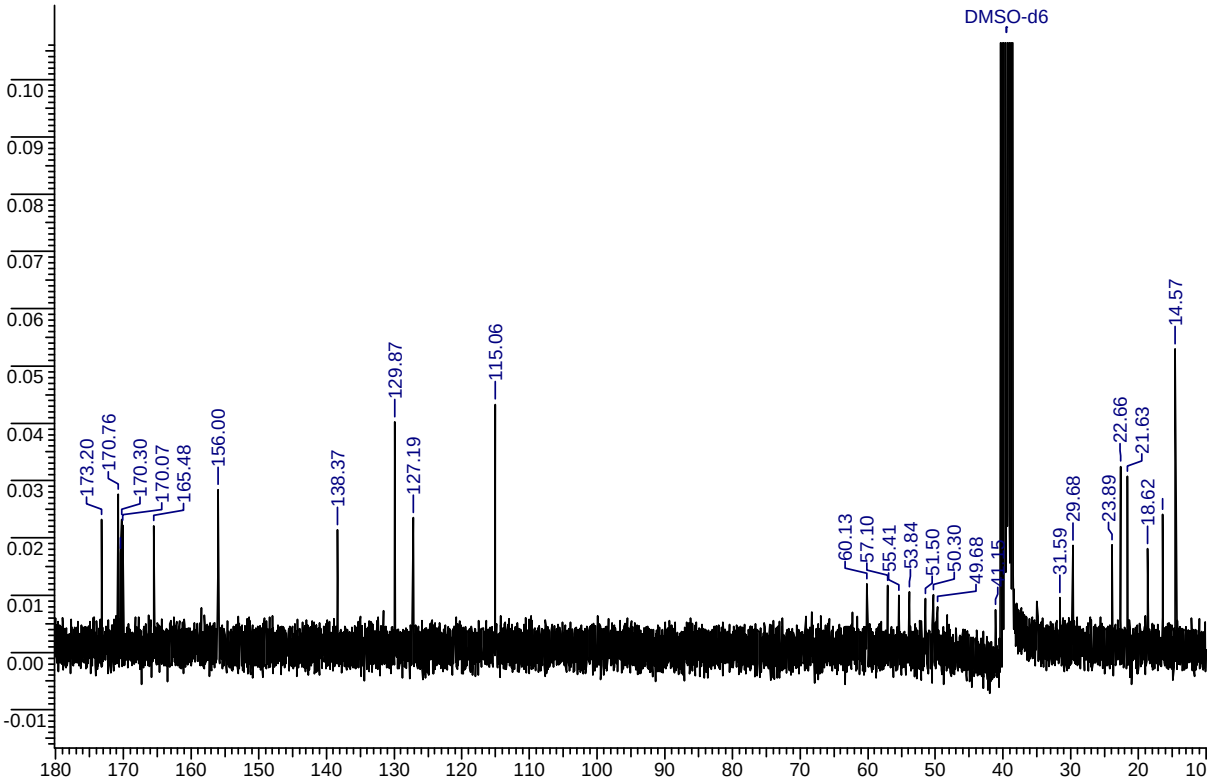
24.18
19.86
15.19


DMSO-d6
3.16
HO
HN
NH
OH
N
O
S


DMSO-d6
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170.76
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170.07
165.48
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138.37
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127.19
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57.10
55.41
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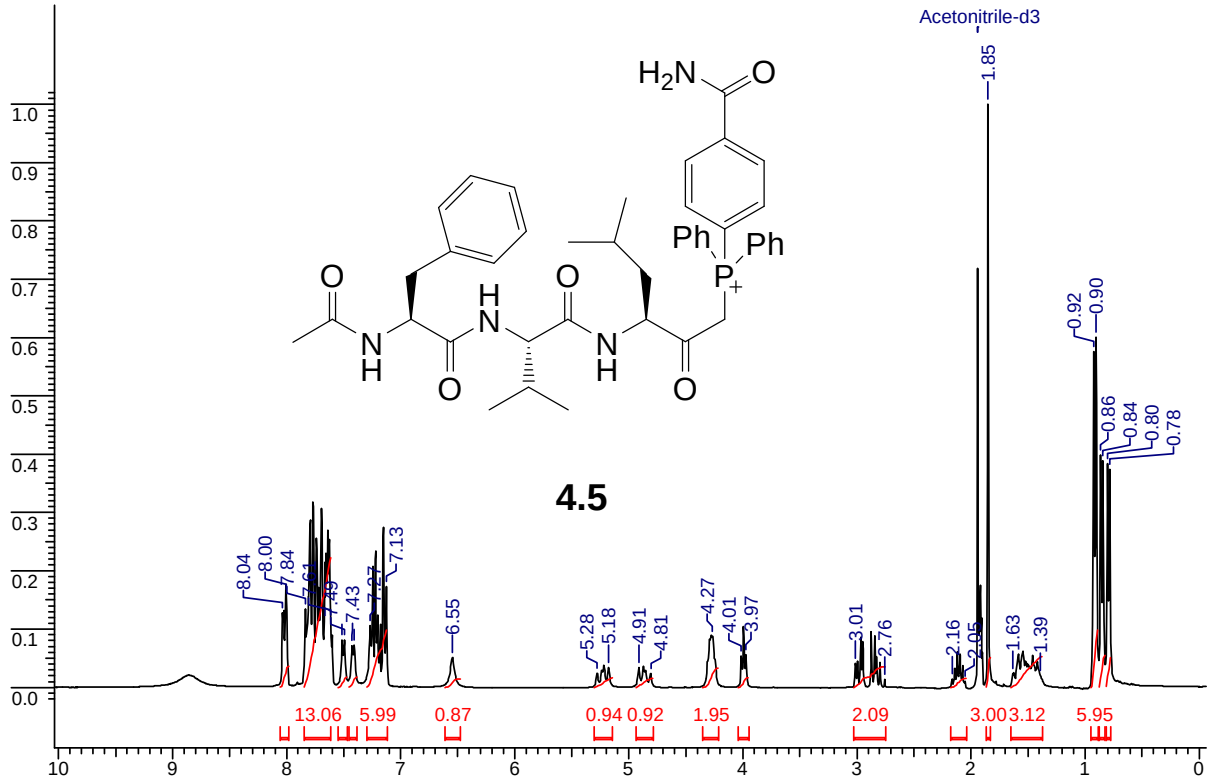
21.63
18.62
14.57


Acetonitrile-d3
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0.86
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0.78
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8.00
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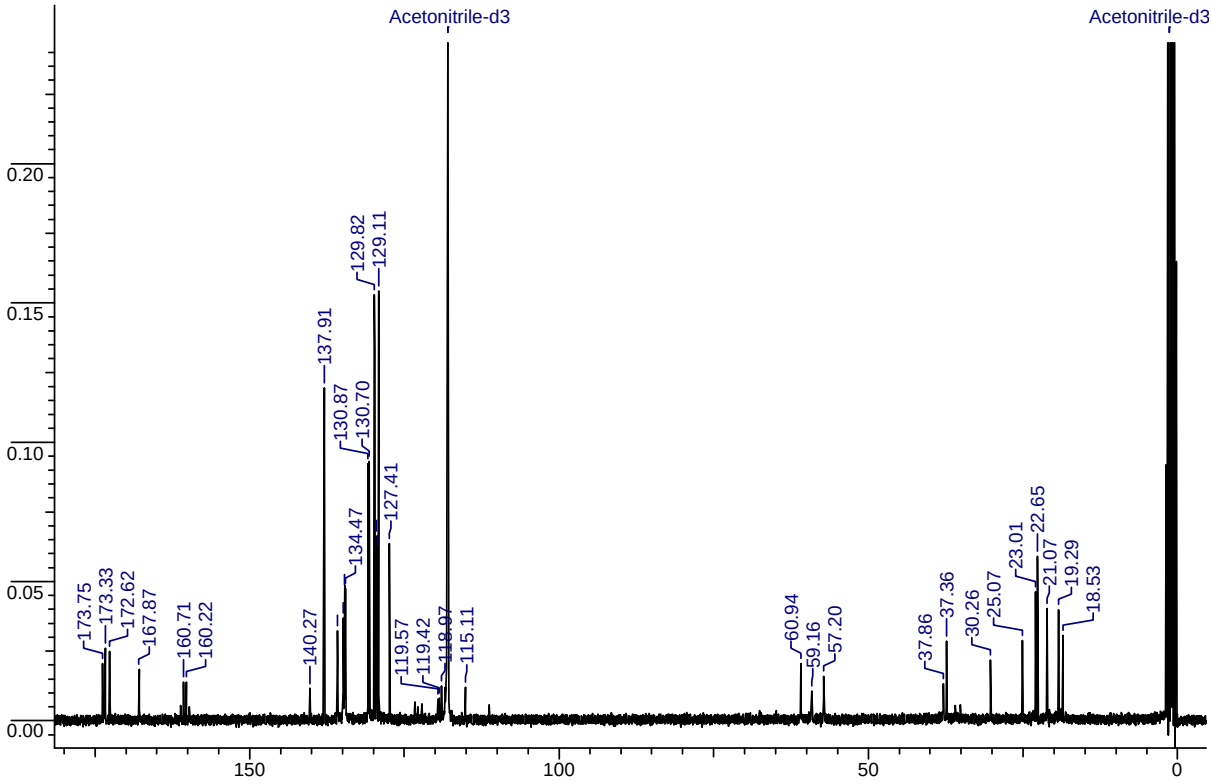
7.61
7.49
7.43
7.27
7.13
6.55
5.28
5.18
4.91
4.81
4.27
4.01
3.97
3.01
2.76
2.16
2.05
1.63
1.39
13.06
5.99
0.87
0.94
0.92
1.95
2.09
3.00
3.12
5.95

H2N
O
Ph
Ph
P+
O
H
N
O
N
H
O
N
H
O

4.5


Acetonitrile-d3
Acetonitrile-d3
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172.62
167.87
160.71
160.22
140.27
137.91
130.87
130.70
129.82
129.11
134.47
127.41
119.57
119.42
118.97
115.11
60.94
59.16
57.20
37.86
37.36
30.26
25.07
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0.05
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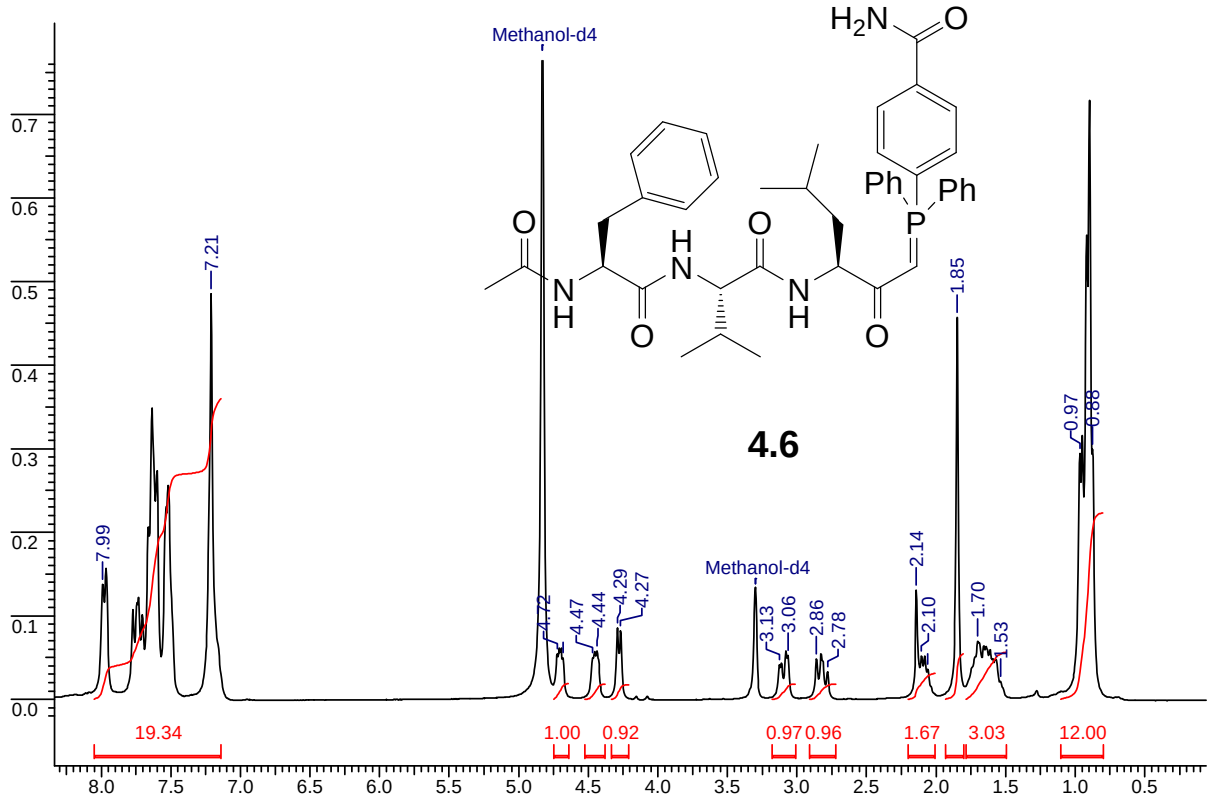
Methanol-d4
Methanol-d4
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4.47
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4.29
4.27
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3.06
2.86
2.78
2.14
2.10
1.85
1.70
1.53
0.97
0.88
19.34
1.00
0.92
0.97
0.96
1.67
3.03
12.00
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
8.0
7.5
7.0
6.5
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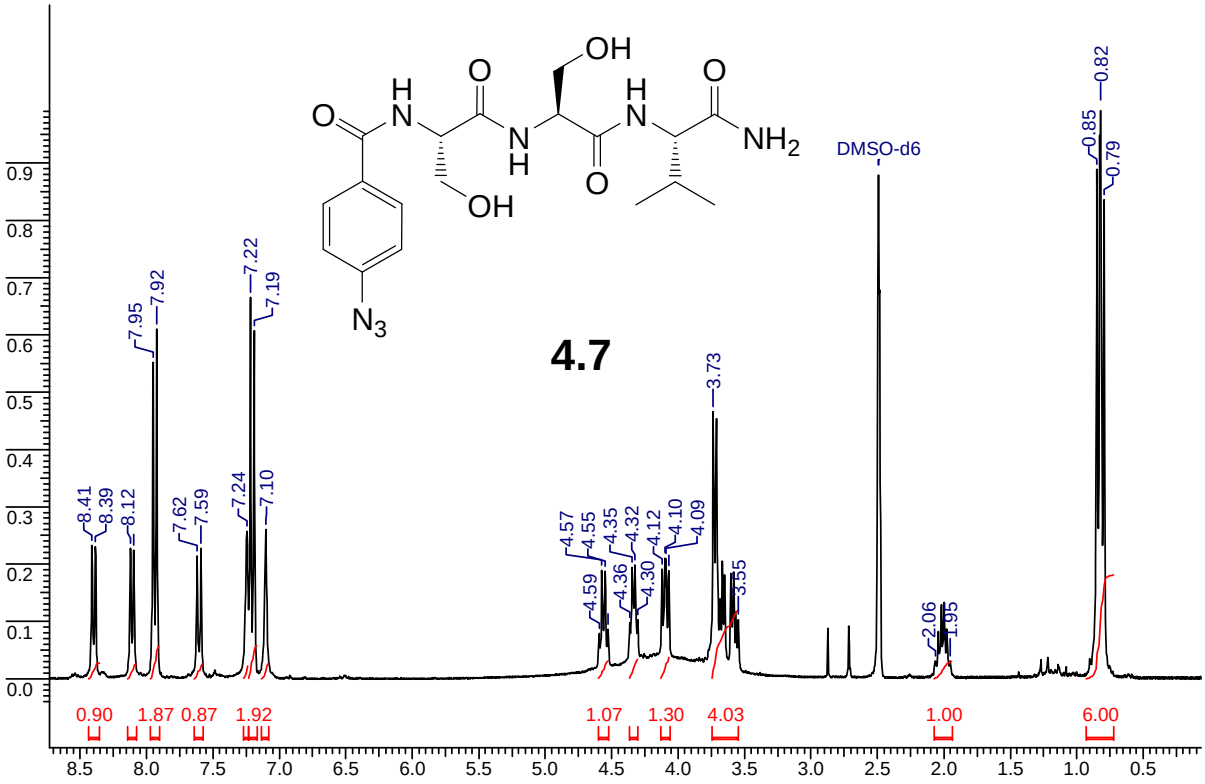
1.0
0.5


4.7
DMSO-d6
8.41
8.39
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7.62
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7.24
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7.19
7.10
4.57
4.55
4.59
4.36
4.35
4.32
4.30
4.12
4.10
4.09
3.73
3.55
2.06
1.95
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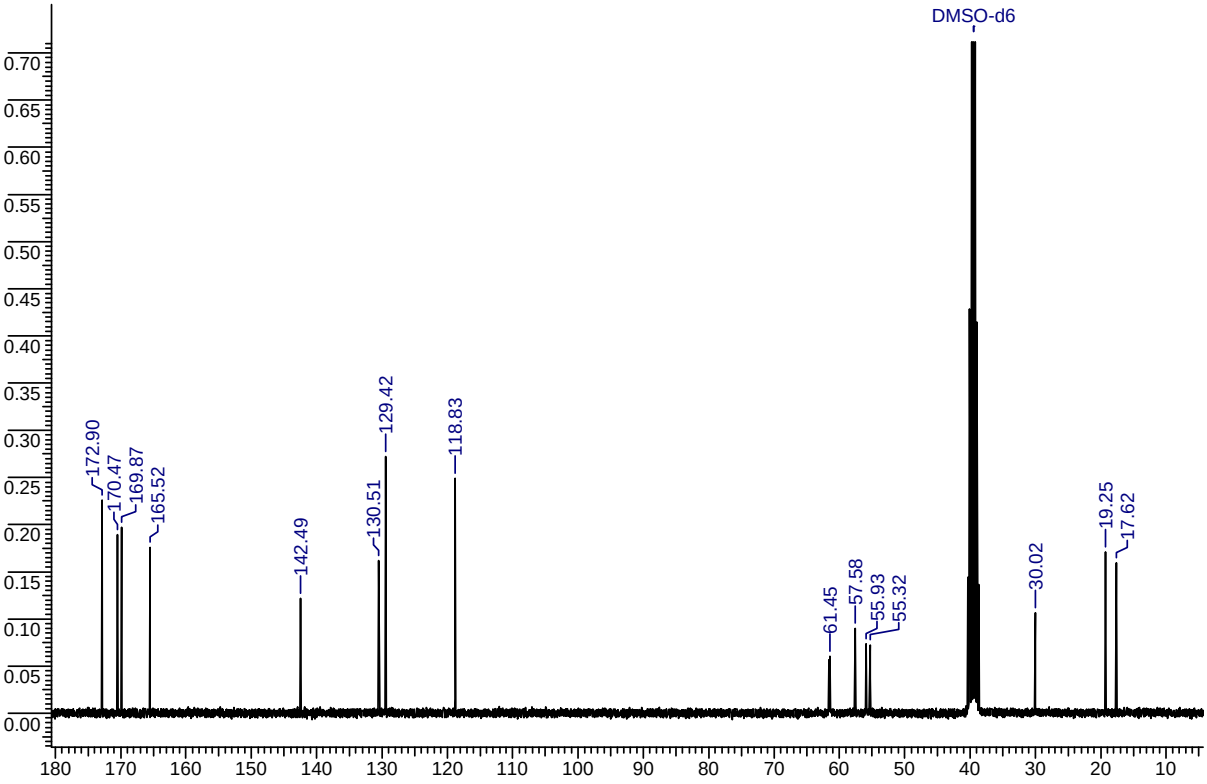
0.79
0.90
1.87
0.87
1.92
1.07
1.30
4.03
1.00
6.00


DMSO-d6
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55.93
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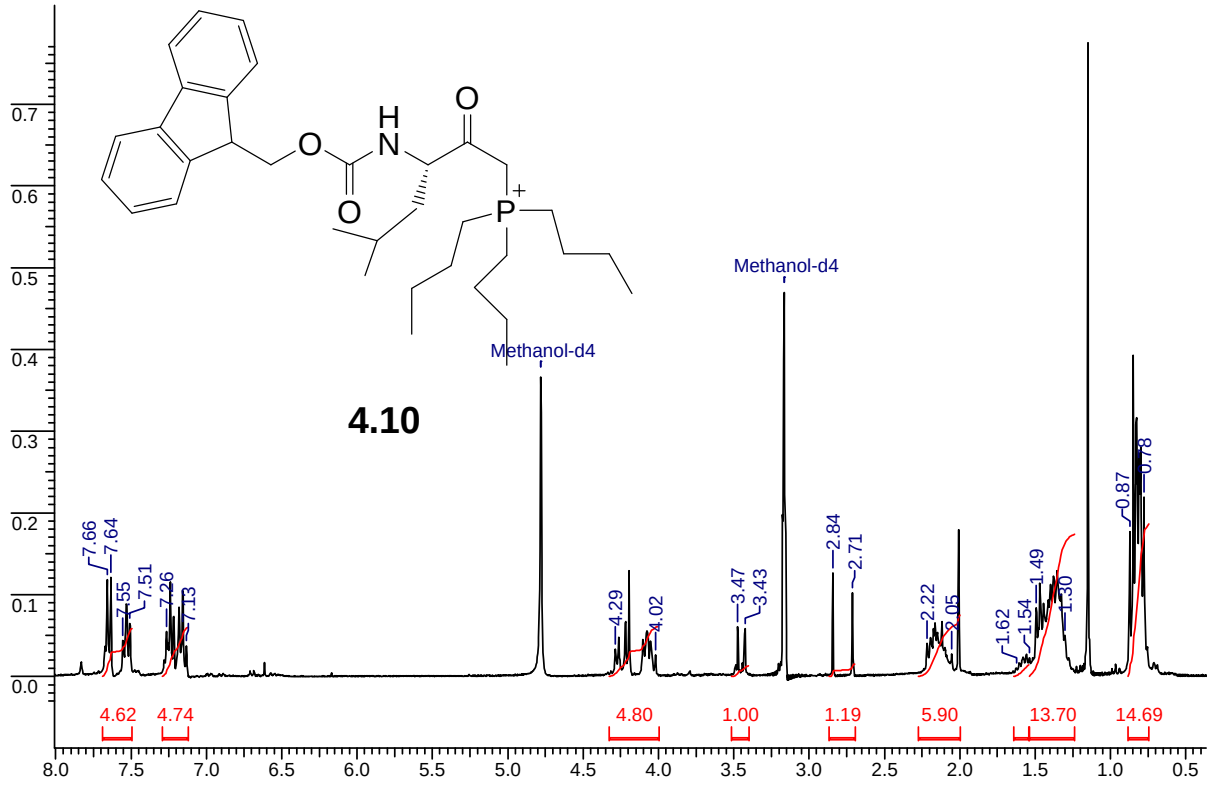
30.02
19.25
17.62


4.10
Methanol-d4
Methanol-d4
7.66
7.64
7.55
7.51
7.26
7.13
4.29
4.02
3.47
3.43
2.84
2.71
2.22
2.05
1.62
1.54
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0.78
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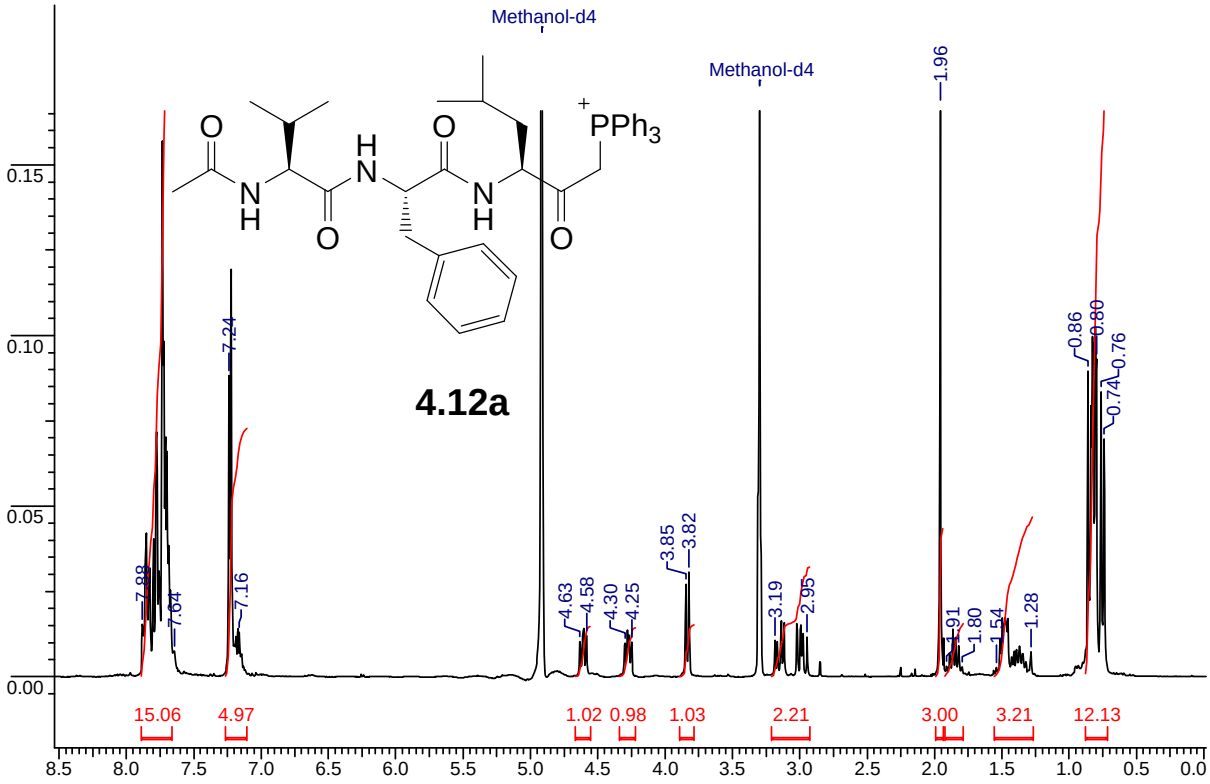
13.70
14.69


4.12a
Methanol-d4
Methanol-d4
PPh3
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7.64
7.24
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4.25
3.85
3.82
3.19
2.95
1.96
1.91
1.80
1.54
1.28
0.86
0.80
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0.74
15.06
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1.03
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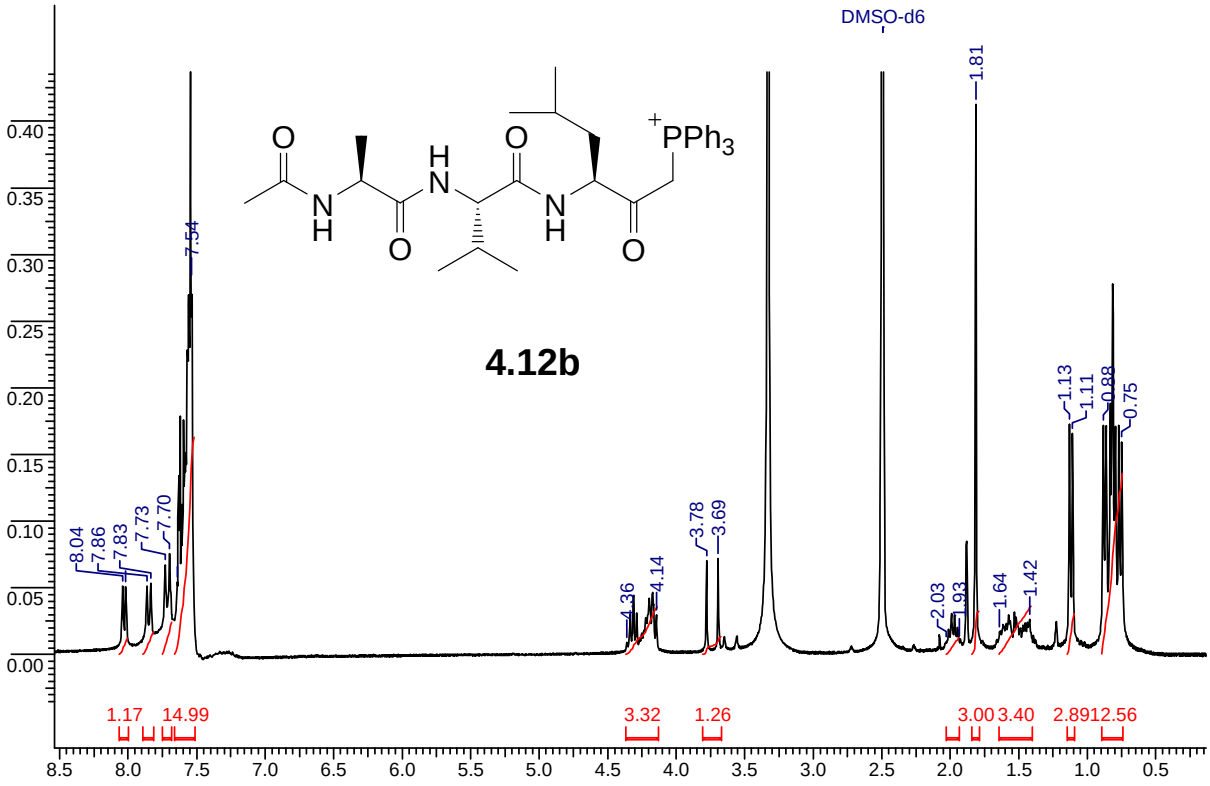
3.21
12.13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4.14
3.78
3.69
2.03
1.93
1.64
1.42
1.13
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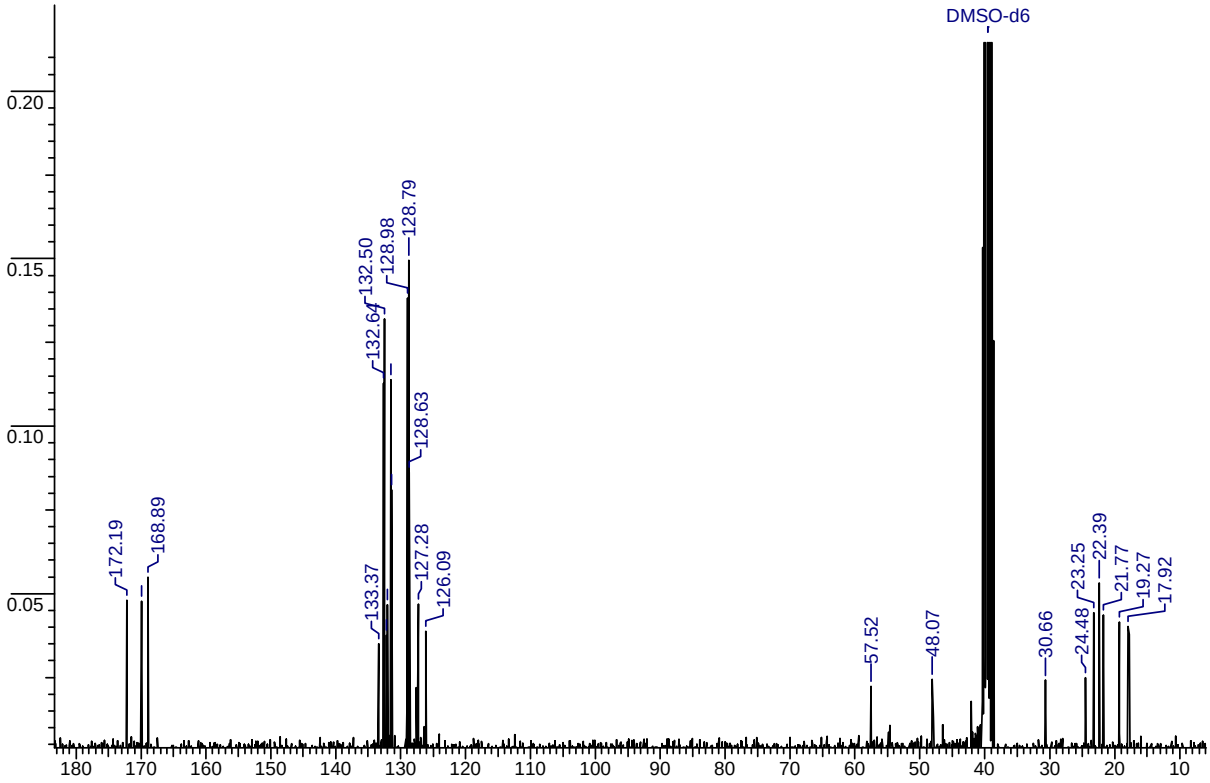
2.89
12.56
4.12b


DMSO-d6
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132.50
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128.79
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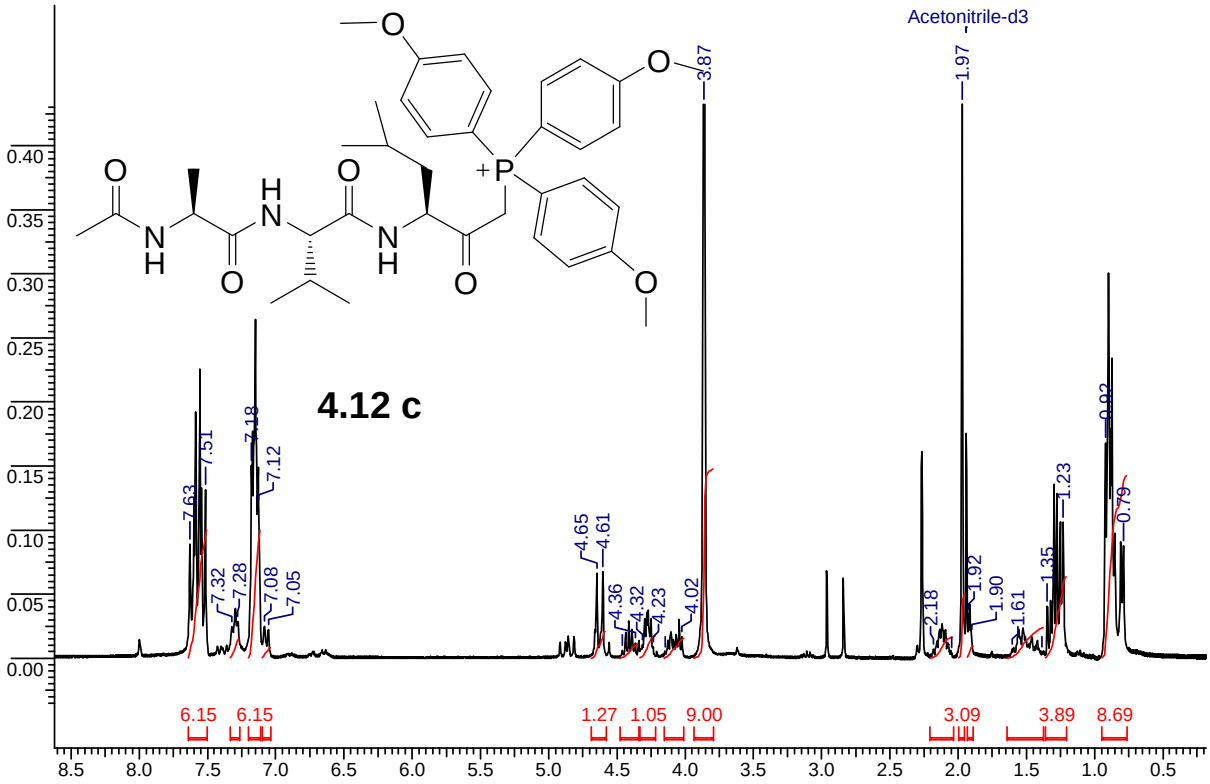
19.27
17.92


Acetonitrile-d3
4.12 c
7.63
7.51
7.18
7.12
7.32
7.28
7.08
7.05
4.65
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4.36
4.32
4.23
4.02
3.87
1.97
2.18
1.92
1.90
1.61
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1.05
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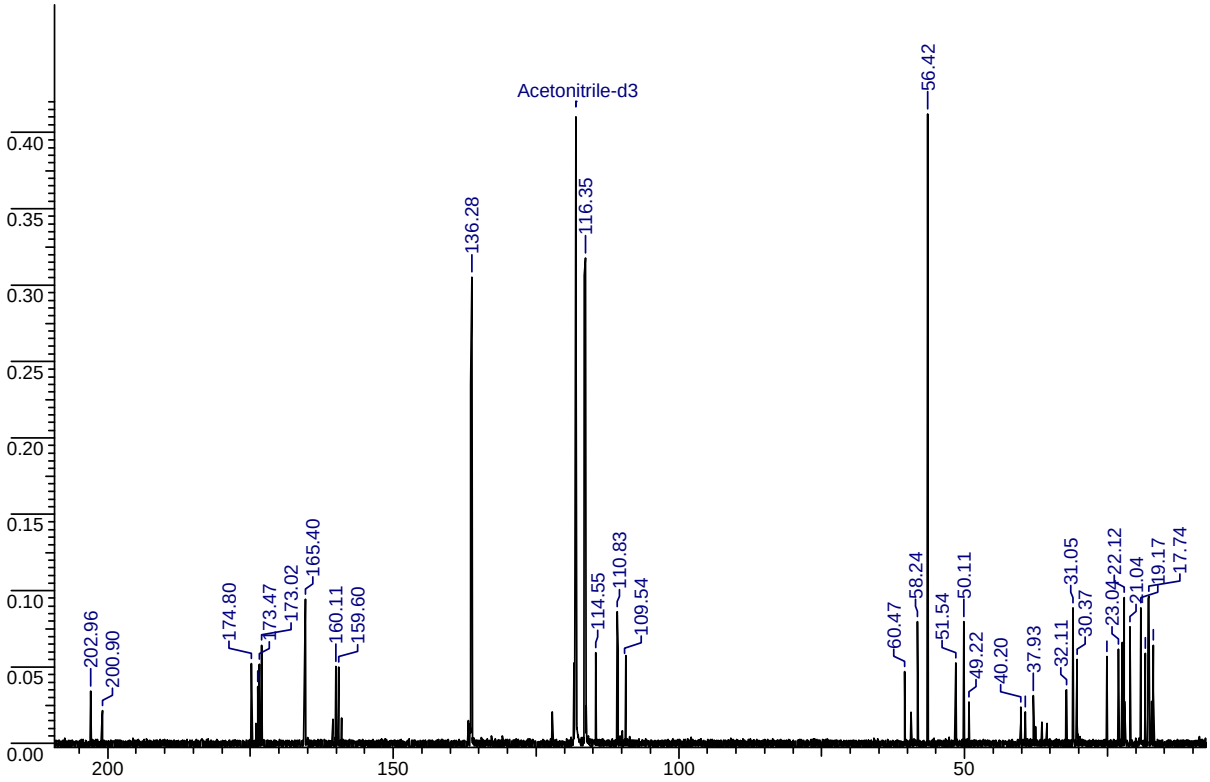
Acetonitrile-d3
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200.90
174.80
173.47
173.02
165.40
160.11
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114.55
110.83
109.54
60.47
58.24
51.54
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32.11
31.05
30.37
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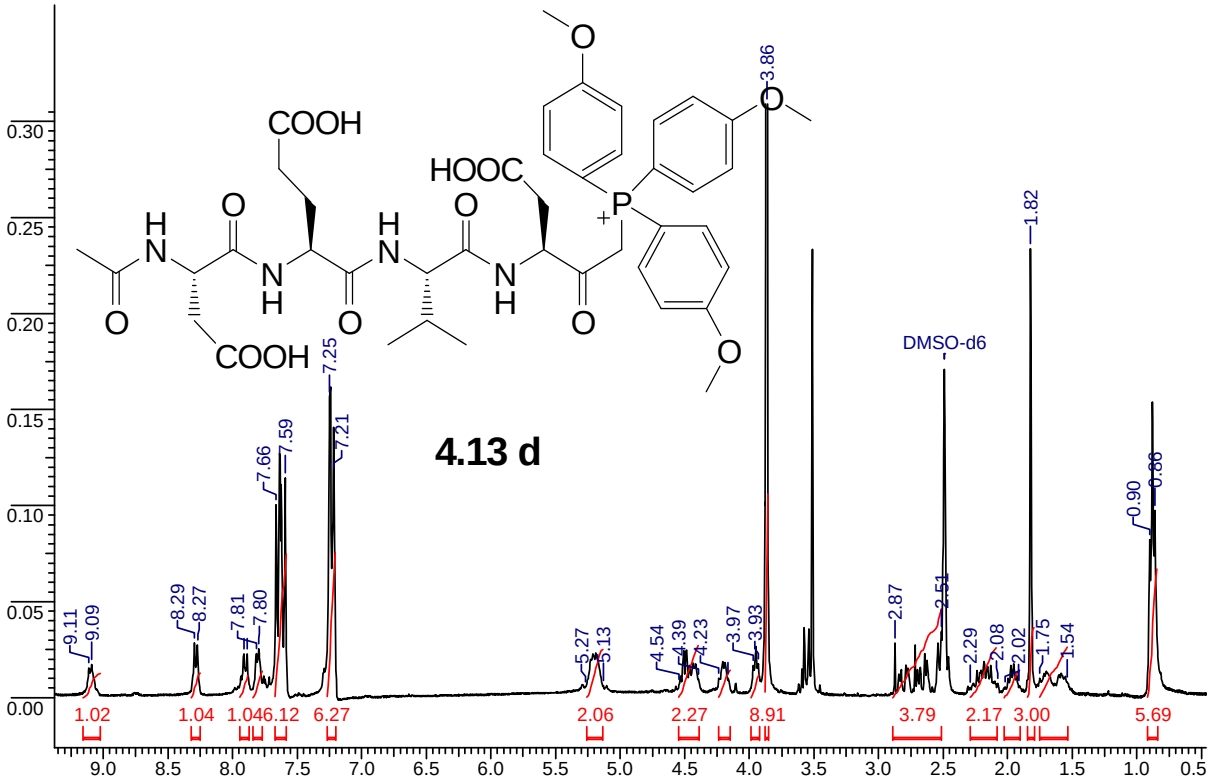
17.74


COOH
HOOC
COOH
P+
4.13 d
DMSO-d6
9.11
9.09
8.29
8.27
7.81
7.80
7.66
7.59
7.25
7.21
5.27
5.13
4.54
4.39
4.23
3.97
3.93
3.86
2.87
2.51
2.29
2.08
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1.82
1.75
1.54
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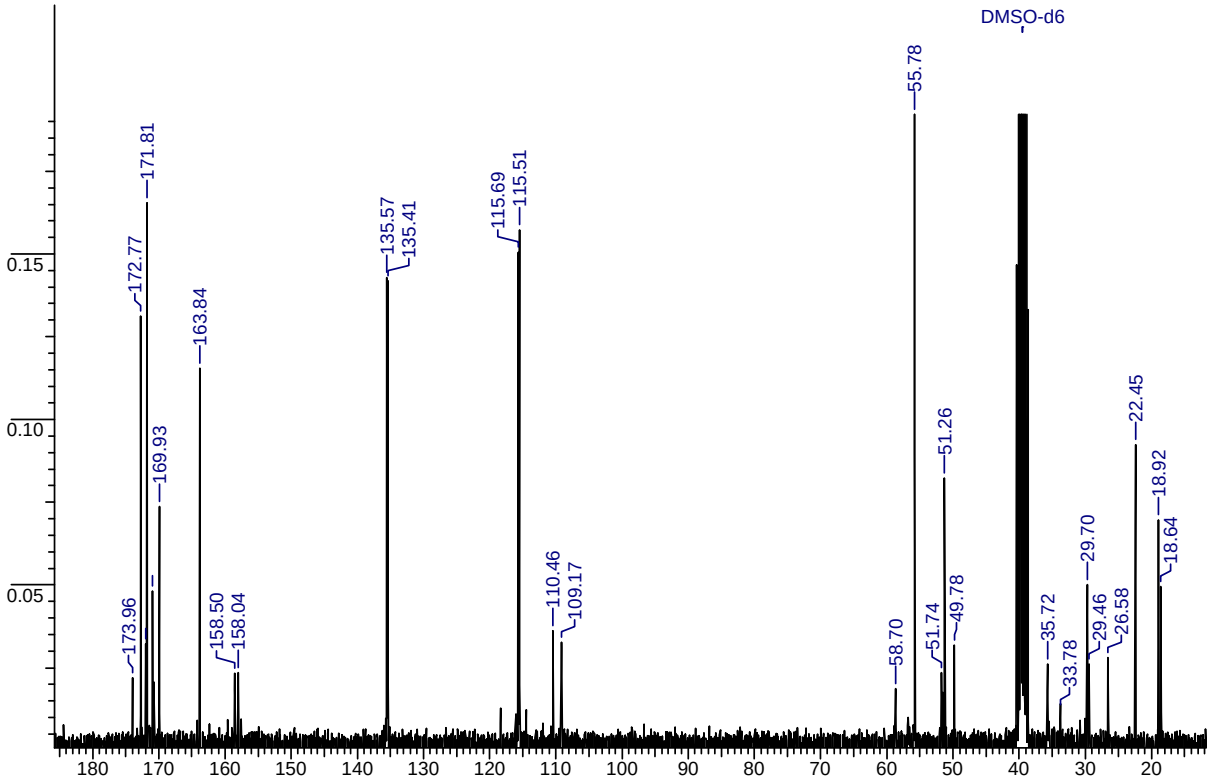
DMSO-d6
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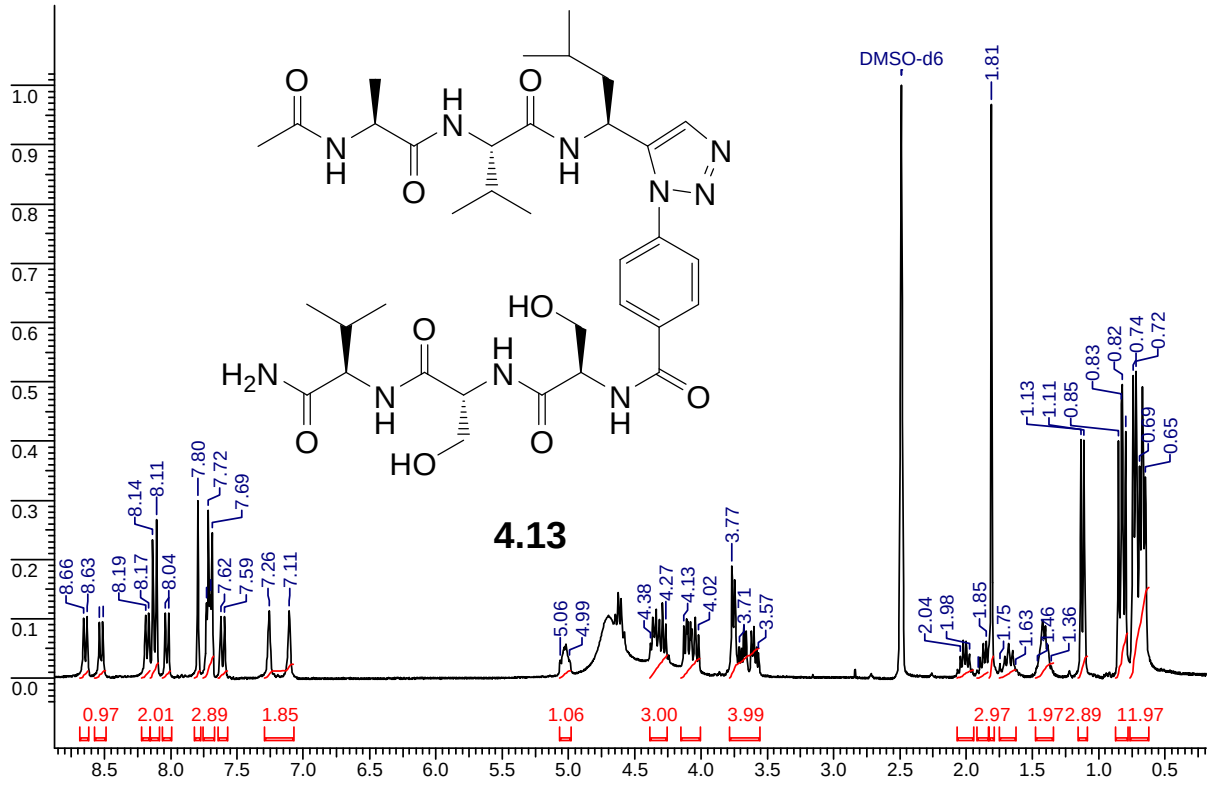
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DMSO-d6
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7.69
7.62
7.59
7.26
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5.06
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4.02
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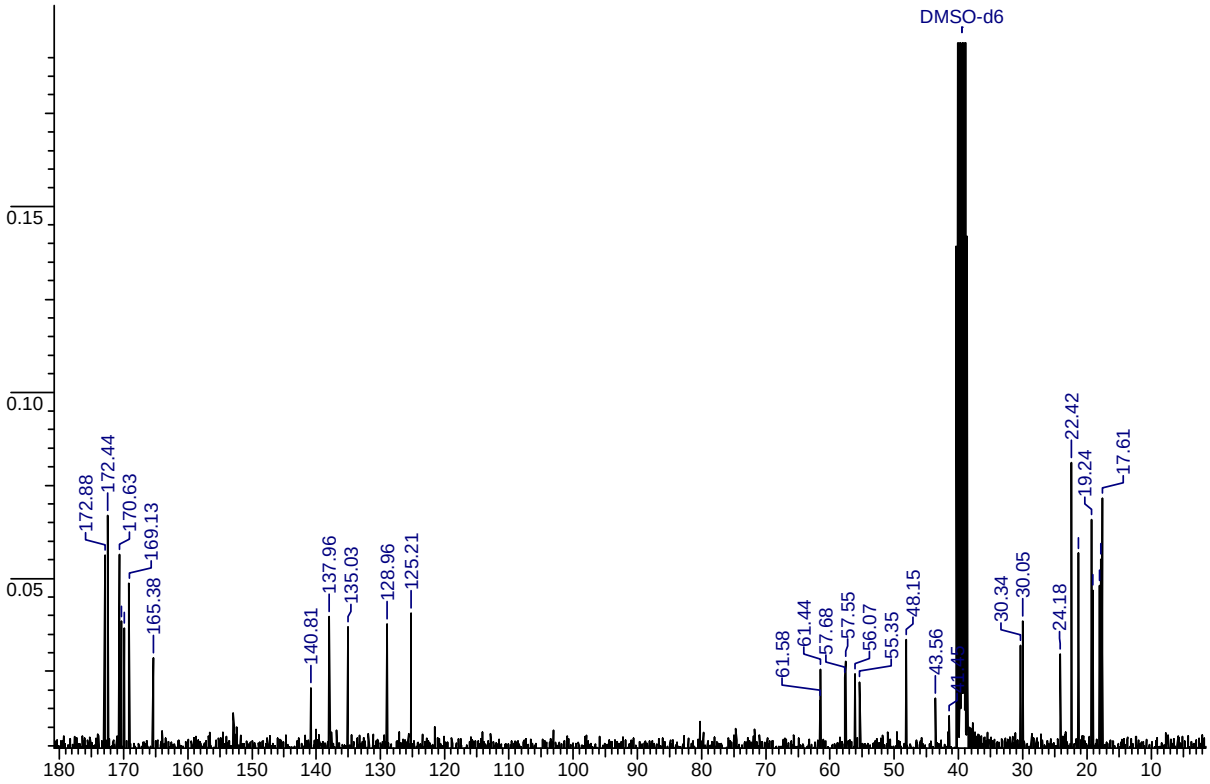
DMSO-d6
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