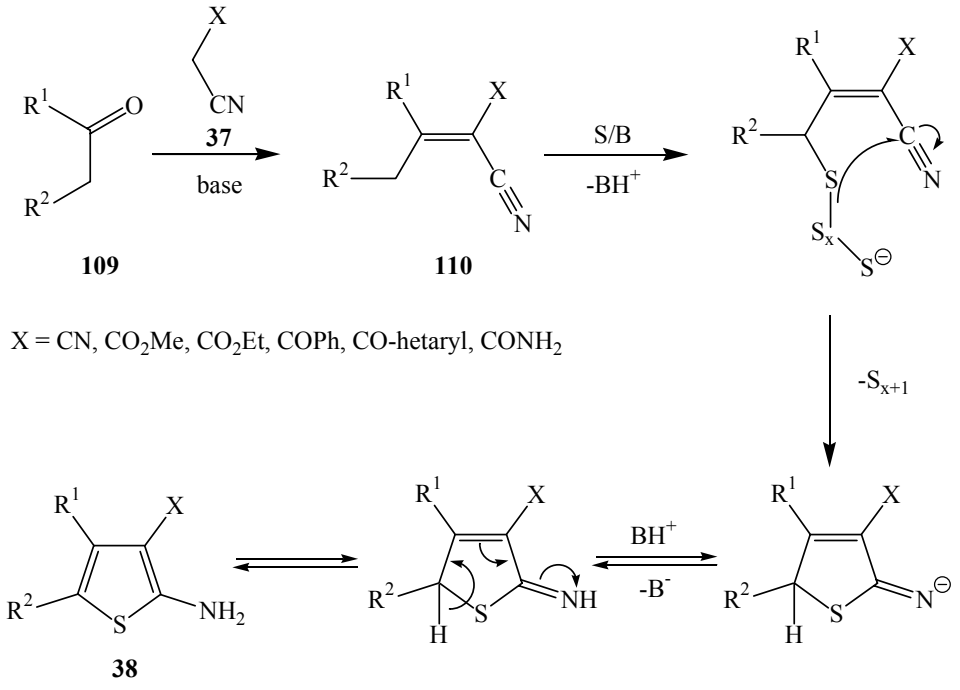
6 Gewald thiophene synthesis with siloxycylopropanes

The synthesis of polysubstituted thiophenes from the multicomponent condensation of ketones or aldehydes, activated nitriles and elemental sulfur was originally published in 1961 by Gewald and co-workers.[126] In addition to the industrial applicability of thiophenes as dyes and conducting polymers,[127] highly substituted thiophenes have shown extensive potential in the pharmaceutical industry. The core structure of these compounds is found in inhibitors of the phosphatase PTP1B,[128] serotonin receptor subtype 5-HT_{1A},[129] human leukocyte elastase[130] and adenosine receptor A_3,[131] as well as in many natural products.[132] It also proved to be an isosteric replacement for phenyl groups in medicinal chemistry – the couple benzene-thiophene represents one of the most prominent examples of bioisosterism.[133, 134] In addition, the basic framework of these compounds is prevalent in screening libraries available from commercial sources and is therefore a likely candidate for synthesis of analogs.[129] Furthermore, the application of polysubstituted 2-aminothiophenes as a starting point for further parallel synthesis has been recently described.[135]

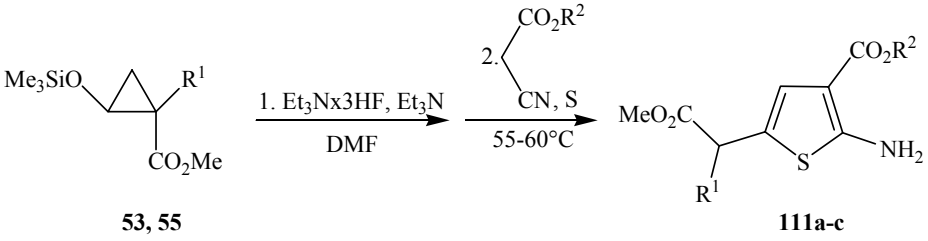
The mechanism of the Gewald reaction is outlined in Scheme 63.[32] It is likely that the first step of reaction is the Knoevenagel-Cope condensation of a carbonyl compound **109** with activated nitrile **37** yielding an α,β-unsaturated nitrile **110**. This intermediate is then thiolated at the methylene group by the electrophilic elemental sulfur, followed by ring closure to afford 2-aminothiophene **38**.



Scheme 63. Mechanism of Gewald's thiophene synthesis

6.1 Gewald thiophene synthesis – Method A

Siloxycyclopropanes **53** and **55** were reacted with Et_3Nx3HF and Et_3N in DMF in order to obtain γ-oxocarboxylates which have been used in the next reaction step without purification. After one hour, nitrile and sulfur have been added and the reaction mixture was heated at 55-60°C for 6 hours (Scheme 64).[126] This one-pot procedure gave 2,3,5-trisubstituted thiophenes **111a**, **b** and **c** as brownish oils after workup and purification by column chromatography. Yields obtained with this method were moderate and ranged between 56 and 69%.



53, 55 **111a-c**

Scheme 64. Gewald thiophene synthesis – Method A

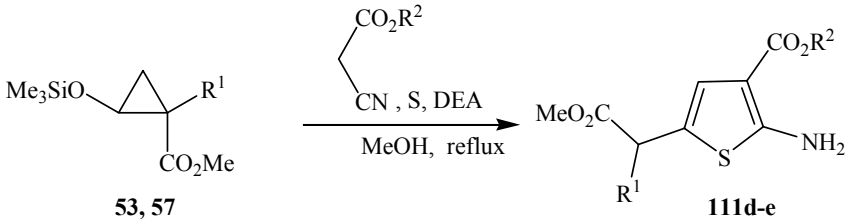
Table 8. Yields of 2-aminothiophenes **111** obtained with Method A (experiments: E43-E45)

Entry	Start. mat.	R^1	R^2	Product	Yield (%)
1	**53**	H	Me	**111a**	56
2	**53**	H	Bn	**111b**	64
3	**55**	Me	^{t}Bu	**111c**	69

6.2 Gewald thiophene synthesis – Method B

Method B included the use of methanol as a solvent. Under protic conditions 2-siloxycyclopropanes **53** and **57** were readily cleaved to provide the γ-oxocarboxylates which undergo reaction with nitrile and sulfur in the presence of diethylamine to afford 2-aminothiophenes **111d-e** (Scheme 65) as brownish oils after workup and purification by column chromatography. Reaction mixtures were in all cases refluxed for 6 hours.[136] The results are summarized in Table 9. While compound **111d** was obtained in a good yield of

71% (entry 1), benzyl-substituted compound **111e** was obtained in significantly lower yield of 37% (entry 2).



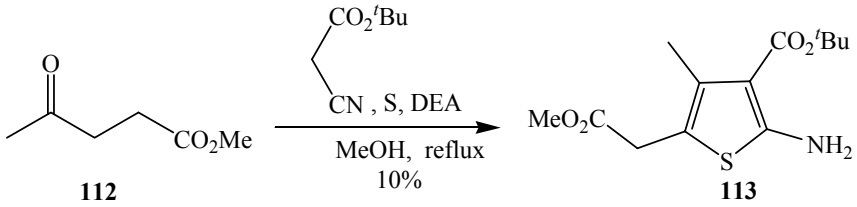
Scheme 65. Gewald thiophene synthesis – Method B

Table 9. Yields of 2-aminothiophenes obtained with Method B (experiments: E46, E47)

Entry	Start. mat.	R^1	R^2	Product	Yield (%)
1	**53**	H	^{t}Bu	**111d**	71
2	**57**	Bn	^{t}Bu	**111e**	37

6.3 Synthesis of 2,3,4,5-tetrasubstituted thiophene 113

The attempt to introduce a 4-alkyl substituent into the thiophene ring was performed using levulinic acid methyl ester **112** as a starting material. It was heated with *tert*-butyl cyanoacetate and sulfur in methanol in the presence of diethylamine for 78 hours, but even after increased reaction time this experiment afforded only 10% of 2,3,4,5-tetrasubstituted thiophene **113** as thick dark brownish oil (Scheme 66).



Scheme 66. Synthesis of 2,3,4,5-tetrasubstituted thiophene **113** (experiment: E48)

6.4 NMR and IR data of compounds 111 and 113

Characteristics in the ^{1}H NMR spectra of substituted 2-aminothiophenes are the signals of the amino group at δ = 5.8 to 6.1 ppm as broad singlet and of the 4-H proton at δ = 6.7 to 6.8 ppm as singlet. Also significant is the signal of the methyl ester in the range of δ = 3.6 to 3.8 ppm which appears as singlet.

Table 10. Characteristic ^{1}H chemical shifts of compounds **111a-e** and **113**; δ (ppm) ($CDCl_3$)

Thiophene	OMe	NH_2	4-H
111a	3.72, 3.78	5.94	6.77
111b	3.67	6.12	6.80
111c	3.68	5.79	6.71
111d	3.72	5.81	6.74
111e	3.61	5.81	6.70
113	3.67	5.98	/

In the ^{13}C NMR spectra characteristic are the chemical shifts of carbons at positions 2, 3, 4 and 5 in the thiophene ring (Table 11). Assignment of ^{13}C NMR signals was achieved with 2D NMR experiments, including HETCORR (C-4) and HMBC experiments (C-5), and through comparison with similar compounds previously reported (C-2).[133, 136-138]

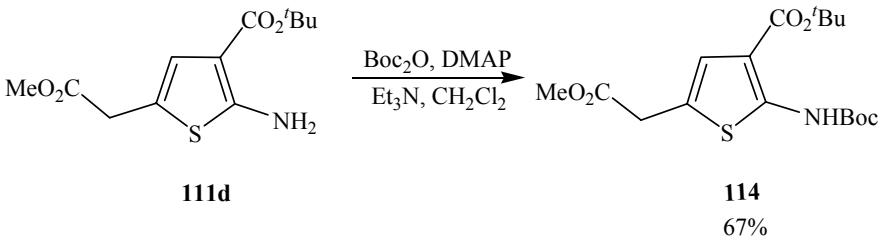
Table 11. Characteristic ^{13}C chemical shifts of compounds **111a-e** and **113**; δ (ppm) ($CDCl_3$)

Thiophene	C-2	C-3	C-4	C-5
111a	162.8	155.0	124.8	116.1
111b	163.3	155.0	126.4	115.7
111c	161.3	156.4	123.4	108.0
111d	161.9	153.5	125.5	115.5
111e	161.5	121.1	124.4	107.7
113	161.7	133.5	109.6	108.2

IR spectra (KBr) of the compounds **111a-e** and **113** show characteristic bands at 1740 and 1665-1680 cm^{-1} [ν(C=O)] and three characteristic bands 1580-1590, 1505-1525 and 1440-1480 cm^{-1} [ν(-CS-NH)].

6.5 Protection and deprotection of amino group

N-Protection of the amino-group of 2-aminothiophene **111d** was required in order to enable coupling reactions of this compound. N-Boc protection has been chosen again, mainly because of its resistance towards alkaline hydrolysis. Reaction of **111d** with 5 equivalents of Boc_2O and 7.5 equivalent of DMAP in the presence of triethylamine in dichloromethane afforded N-Boc protected compound **114** after stirring over 5 days at room temperature (Scheme 67). The crude product was purified by column chromatography and 67% of **114** were obtained as thick brownish oil.



111d **114** 67%

Scheme 67. Boc-protection of amino-group (experiment: E49)

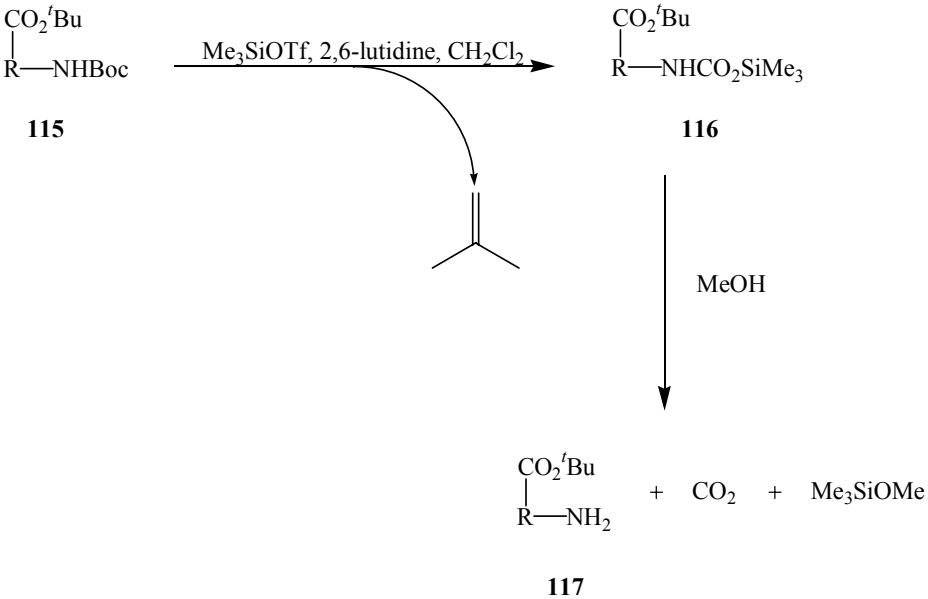
The structure of the compound **114** has been proven by the spectroscopic means. The most significant difference in ^1H NMR spectrum of **114** comparing to **111d** is the dissapearance of broad singlet at $\delta \approx 6$ ppm for NH_2 and displaying singlet at $\delta \approx 10$ ppm for NH.
The described reaction conditions provided the best yield of N-Boc protected compound **114**. In order to optimize this reaction, several reaction conditions have been tried (Table 12).

Table 12. Optimization of N-Boc-protecting reaction

Boc_2O (Eq)	DMAP (Eq)	Et_3N (Eq)	Solvent	T	Time (h)	Yield (%)
2.0	-	18.3	MeOH	Reflux	5	-
2.0	4.0	-	MeCN	Reflux	5	-
1.2	-	-	CH_2Cl_2	r.t.	12	-
1.2	-	2.4	CH_2Cl_2	r.t.	12	-
1.2	2.4	-	CH_2Cl_2	r.t.	48	40
2.4	3.6	-	CH_2Cl_2	r.t.	168	44
2.4	3.6	catalytic	CH_2Cl_2	r.t.	48	46
5.0	7.5	catalytic	CH_2Cl_2	r.t.	144	67

In her master thesis work Houda Al-Hajmi[139] converted **111d** to the N-Boc protected compound **114** in 70 % yield by DMAP-catalyzed reaction with $(Boc)_2O$ followed by addition of 1.4 eq of *tert*-butylalcohol. Attempts to obtain N-Boc protected compound **114** applying NaH, Boc_2O and DMAP in THF and using BOC-ON reagent resulted in negligible yields (lower than 10%).

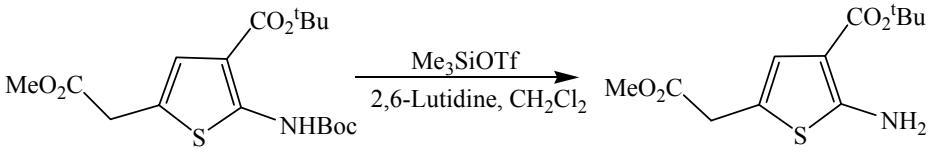
A *tert*-butyloxycarbonyl blocking of the amino-group had to be removed chemoselectively in this molecule, since the subsequent removal of this protecting group using TFA (trifluoroacetic acid) would also have caused cleavage of the *tert*-butyl ester in position 3. Several groups reported efficient chemoselective method for the deprotection of the N-*t*-Boc group (compound **115**) by use of 1.5 equivalents of Me_3SiOTf (trifluoromethanesulfonate) in the presence of 2.0 equivalents of 2,6-lutidine in dichloromethane at room temperature. After workup, the resultant trimethylsiloxycarbonyl group was removed by dissolving in methanol to give primary amine **117** (Scheme 68).[140]



115 **116** **117**

Scheme 68. Chemoselective removal of N-Boc group in the presence of a *t*-butyl ester

This method has been tested for N-Boc-deprotection of compound **114**. After reacting this compound with Me_3SiOTf and dissolving a crude trimethylsilyl carbamate in methanol, primary amine **111d** was obtained. Purification by column chromatography afforded **111d** in 53% yield as yellowish oil (Scheme 69).

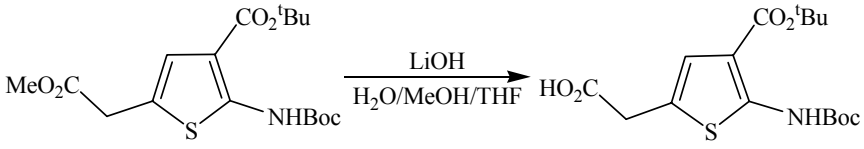


114 **111d**
53%

Scheme 69. Chemoselective removal of N- Boc group of compound **114** (experiment: E50)

6.6 Ester hydrolysis

Saponification of the ester **114** with 3 equivalents of LiOH in H_2O/MeOH/THF mixture as previously described,[89] generated the corresponding carboxylic acid **118** in 98% yield (Scheme 70).

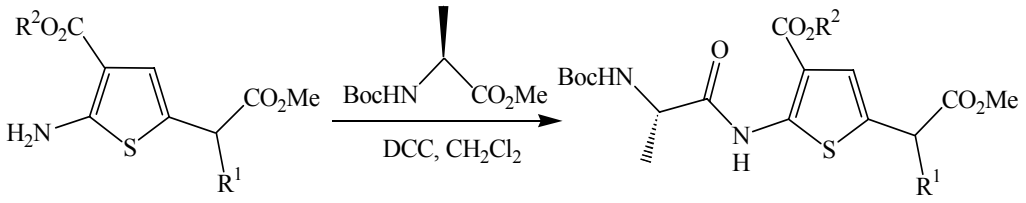


114 **118**
98%

Scheme 70. Base-mediated hydrolysis of compound **114 (e**xperiment: E51)

6.7 DCC-mediated couplings - Syntheses of tripeptide analogues

Tripeptide analogues **119b-e** were prepared employing the corresponding 2-aminothiophenes **111b-e**. In a typical procedure DCC was added to a solution of 2-aminothiophene and N-Boc L-alanine in dichloromethane at 0°C (Scheme 71). The reaction mixture was allowed to warm to room temperature and the product was purified by column chromatography.[141] All four synthesized tripeptide analogues are yellowish oils. Representative examples of this reaction scheme are shown in table 13.



111b-e **119b-e**

Scheme 71. DCC-mediated tripeptide analogue synthesis

Table 13. Results of the DCC-mediated tripeptide analogue synthesis (experiments: E52-E54, E56)

Entry	Start. mat.	R^1	R^2	Product	R. time (h)	Yield (%)	Rec. s.m. (%)
1	**111b**	H	Bn	**119b**	96	56	31
2	**111c**	Me	^{t}Bu	**119c**	20	53	8
3	**111d**	H	^{t}Bu	**119d**	20	60	16
4	**111e**	Bn	^{t}Bu	**119e**	96	84	10

The compounds **111c** and **111e** have been used as racemic mixtures which should result in products **119c** and **119e** as mixtures of diastereoisomers. In the NMR spectra of these compounds only one diastereoisomer could be observed, most probably because the overlap of all signals.

Yields obtained in these coupling reactions have been moderate to good (53-84%), but in all examples starting material was also recovered (8-31%). Elongation of reaction time and use of 1.5 equivalents of N-Boc alanine seems not to be beneficial for the outcome of these reactions. The best yield (84%) was obtained in reaction with benzyl-substituted compound **111e** (entry 4). On the other hand, synthesis of amine **111e** proceeded with the lowest yield (37%) compared with the syntheses of other amines involved in this coupling reaction. Amine **111d** afforded 60% of coupling product and itself was synthesized in a good yield (71%), so it has been chosen to be a model compound for further coupling reactions.

6.7.1 NMR data of tripeptide analogues 119

Characteristics in the ^{1}H NMR spectra of tripeptide analogues **119** are the signals of NH protons at δ ≈ 5.3 ppm and δ ≈ 11.5 ppm, of the 4-H proton at δ ≈ 7.0 and of the methoxy group at δ ≈ 3.7 ppm, all of them displayed as singlets (Table 14).

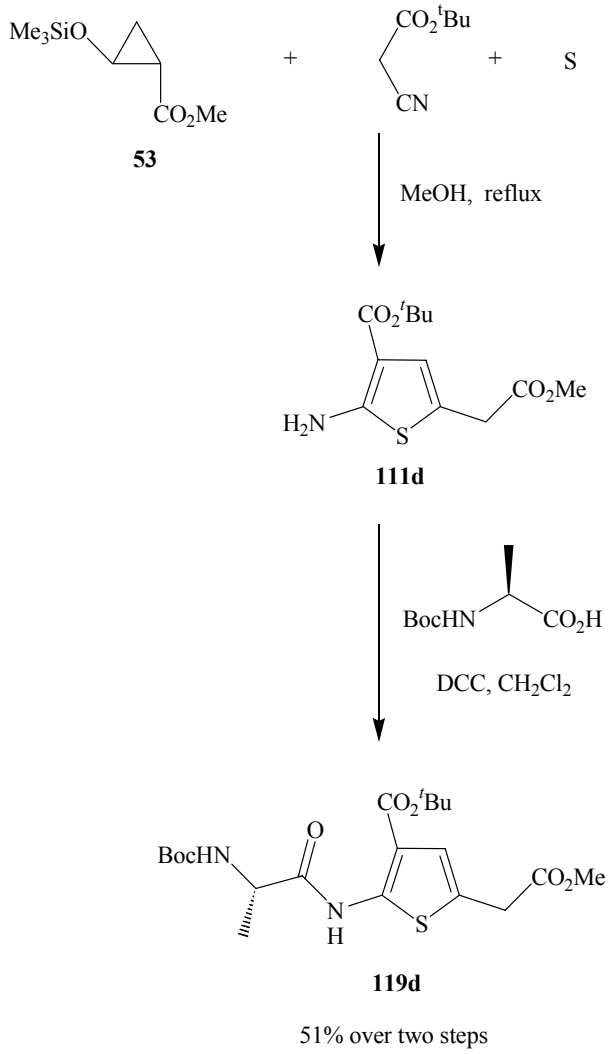
Table 14. Characteristic ^{1}H chemical shifts of tripeptide analogues **119b-e**; δ (ppm)

Compound	OMe	NH	4-H	NH
119b	3.67[a]	5.27[a]	7.01[a]	11.40[a]
119c	3.74[b]		7.00[b]	
119d	3.66[a]	5.19[a]	6.92[a]	11.47[a]
119e	3.62[a]	5.29[a]	6.94[a]	11.53[a]

[a] in $CDCl_3$; [b] in CD_3OD

6.7.2 One-pot protocol for synthesis of tripeptide analogue 119d

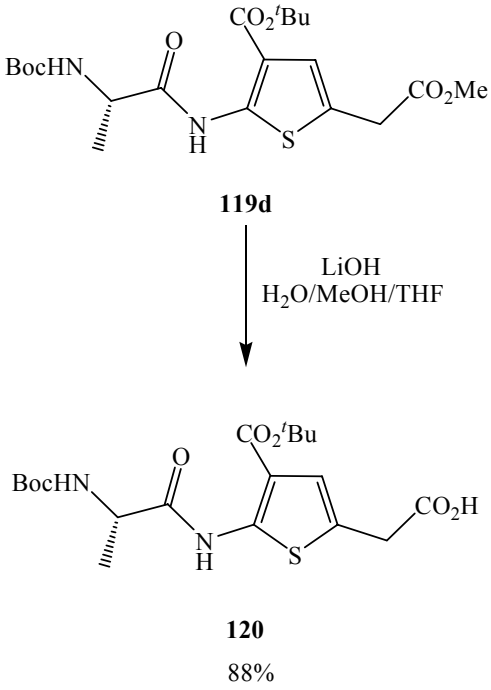
Synthesis of tripeptide analogue **119d** was carried out in a one-pot procedure by the sequence shown in Scheme 72. Siloxycyclopropane **53** was refluxed with *tert*-butyl cyanoacetate and sulfur in methanol in the presence of DEA (Gewald thiophene synthesis, Method B). After the completion of the reaction, the solvent was evaporated and the residue dissolved in dichloromethane. N-Boc L-alanine and DCC were added at 0°C; the reaction mixture was allowed to warm to room temperature and after one week crude coupling product has been obtained (Scheme 72). Purification by column chromatography provided compound **119d** in 51% yield (starting from the siloxycyclopropane **5**). The yield obtained in the described one-pot protocol (51%) was slightly higher than the yield obtained in two separated experiments (overall 43%) and one purification was sufficient.



Scheme 72. One-pot protocol for synthesis of tripeptide analogue **119d** (experiment: E55)

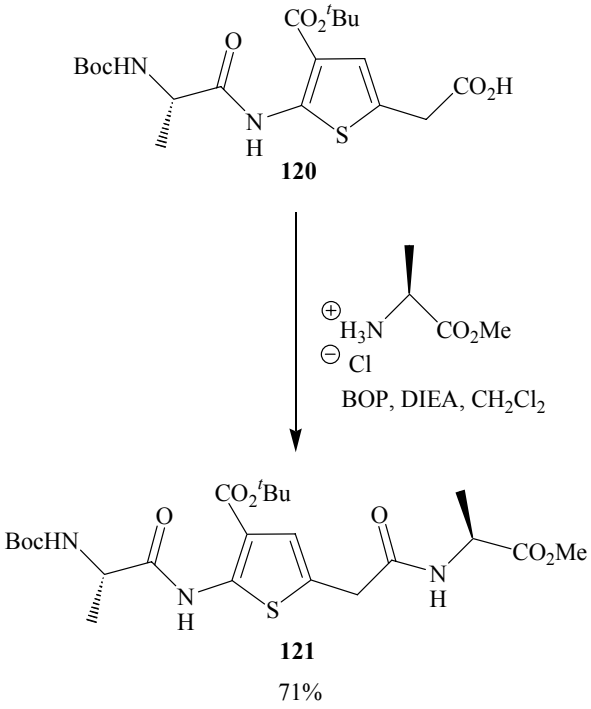
6.8 DCC- and BOP-mediated couplings – Syntheses of tri- and tetrapeptide analogues

To enable coupling of tripeptide analogue **119d** with L-alanine methyl ester it was treated with LiOH in H_2O/MeOH/THF solution to generate the carboxylic acid. Base mediated hydrolysis afforded carboxylic acid **120** in 88% yield (Scheme 73).[89]



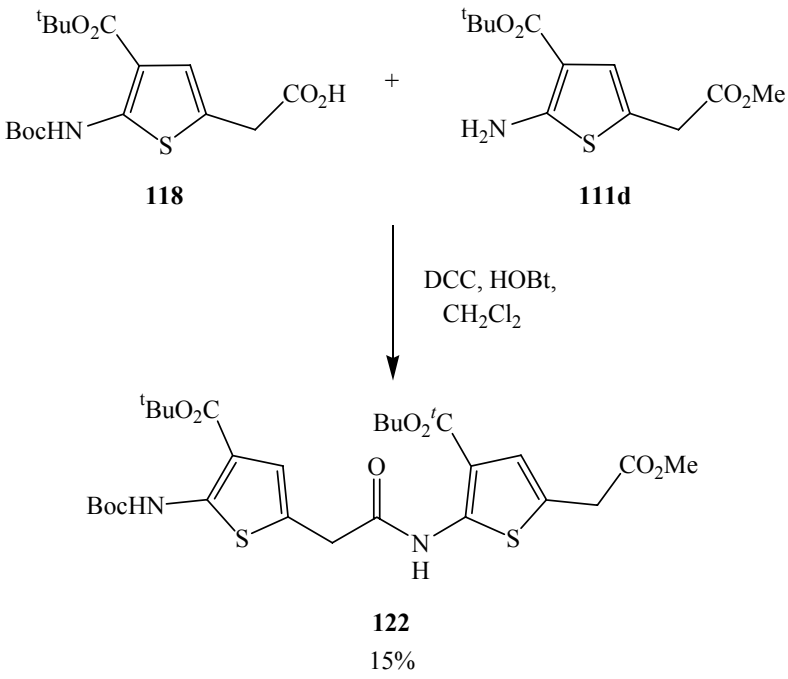
Scheme 73. Base-mediated hydrolysis of tripeptide analogue **119d** (experiment: E57)

In the next step carboxylic acid **120** was coupled with L-alanine methyl ester hydrochloride in the presence of BOP. To the solution of acid, amine and BOP in dichloromethane was added DIEA. The reaction was completed in 30 minutes at room temperature and after basic workup and purification by column chromatography tetrapeptide analogue **121** was obtained in 71% yield as yellowish oil (Scheme 74).[125]



Scheme 74. BOP-mediated synthesis of tetrapeptide analogue **121** (experiment: E58)

Finally, coupling of two thiophene compounds was attempted. N-Boc protected carboxylic acid **118** and δ-amino ester **111d** were mixed with DCC and HOBt in dichloromethane at 0°C. The reaction mixture was allowed to warm to room temperature and after 10 days dipeptide **122** was isolated in 15% yield after purification by column chromatography followed by HPLC (Scheme 75).[142] Attempts to obtain tetrapeptide analogue **122** in BOP- and TBTU-mediated reactions from the same starting materials failed.



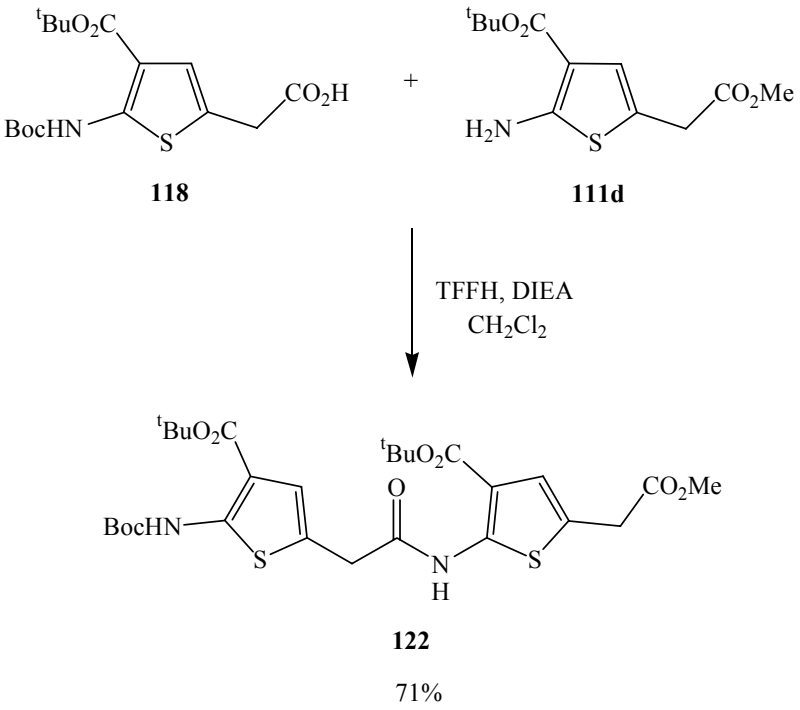
Scheme 75. DCC/HOBt-mediated synthesis of tetrapeptide analogue **122** (experiment: E60)

6.9 Couplings with TFFH

A number of coupling reactions involving aminothiophene **111d** mediated by TFFH have been performed.

6.9.1 TFFH-mediated synthesis and deprotection reactions of tetrapeptide analogue 122

Since the DCC/HOBt-mediated coupling of carboxylic acid **118** and amine **111d** furnished only 15% of tetrapeptide analogue **122** and BOP- and TBTU-mediated couplings were unsuccessful, coupling reactions *via* carboxylic acid fluorides were attempted. Starting compounds were mixed in dichloromethane with TFFH at 0°C in the presence of DIEA. Reaction mixture was allowed to warm to room temperature and after 6 days tetrapeptide analogue **122** was isolated. Purification by column chromatography afforded 71% of compound **122** as pale yellow solid, which could be further purified by HPLC to give a colorless solid[10] (Scheme 76).

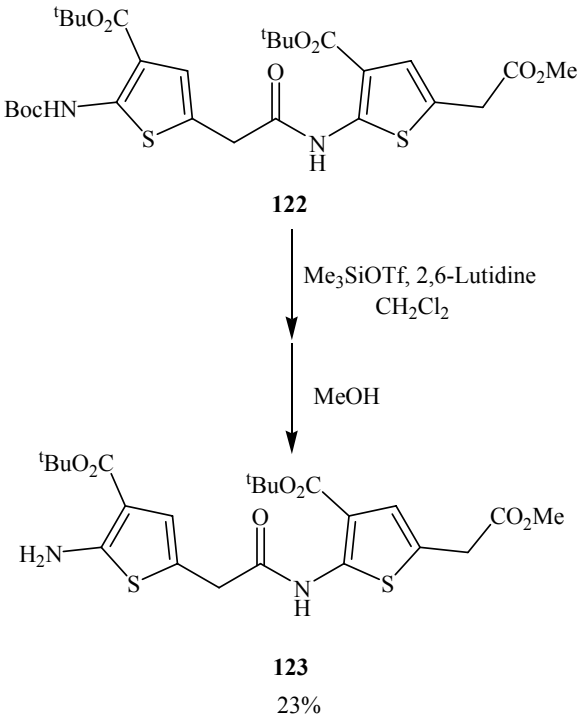


Scheme 76. TFFH-mediated synthesis of tetrapeptide analogue **122** (experiment: E59)

Tetrapeptide analogue **122** was characterized by NMR, MS and IR spectroscopy, as well as by elemental analysis. Characteristics in the ^{1}H NMR spectra of compound **122** are the signals of NH protons at δ = 11.14 and 10.1 ppm, of the 4-H protons at δ = 7.00 and 6.88 ppm, of CH_2 protons at δ = 3.62 and 3.76 ppm and of methyl ester at δ = 3.64 ppm, all of them displayed as singlets (in $CDCl_3$).

IR spectra (KBr) of the compound **122** show characteristic bands at 1745, 1720 and 1670 cm^{-1} [ν(C=O)] and three characteristic bands 1560, 1535 and 1455 cm^{-1} [ν(-CS-NH)].

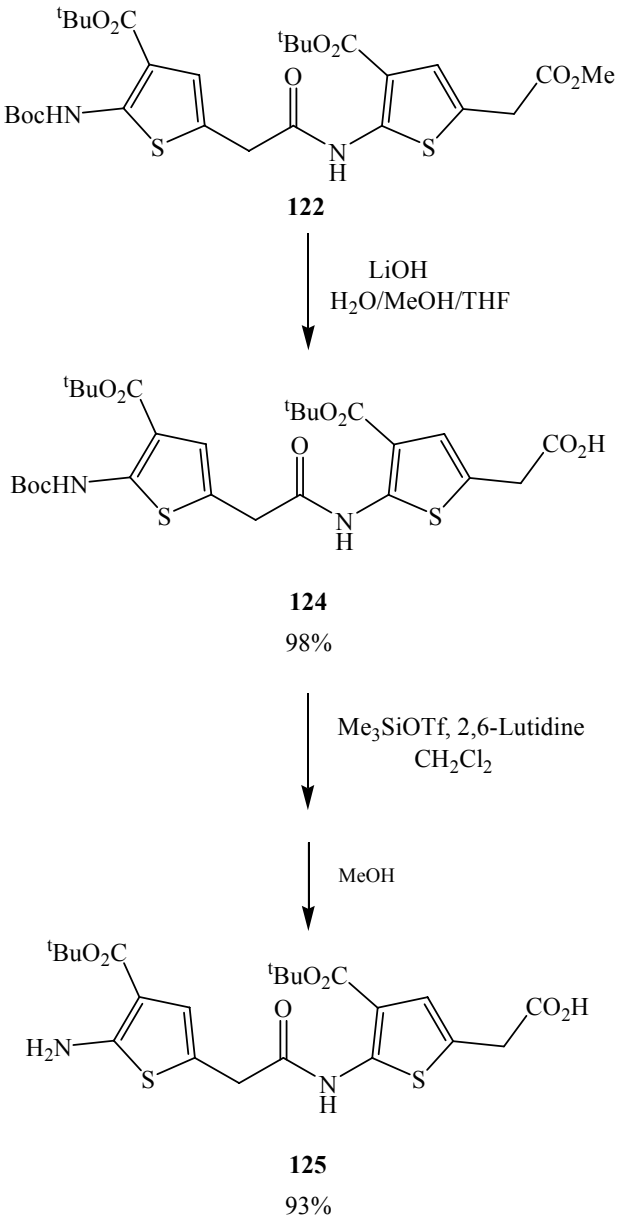
According to the above described chemoselective deprotection of N-Boc group *via* trimethylsilyl-carbamates, compound **122** was transformed to primary amine **123**. Reaction of **122** with trimethylsilyltrifluoromethanesulfonate in the presence of 2,6-lutidine was carried out at room temperature over 30 minutes and was quenched with saturated aqueous ammonium chloride solution. After dissolving a crude trimethylsilyl carbamate in methanol and purification by column chromatography and HPLC, primary amine **123** was isolated in 23% yield (Scheme 77).[140]



Scheme 77. Chemoselective removal of N- Boc group in compound **122** (experiment: E61)

Reaction conditions for this reaction have not been optimized. Subsequent deprotection of hexapeptide analogue **127** carried out according to the same procedure afforded a much higher yield (86%) of primary amine with increased reaction time (Scheme 81).

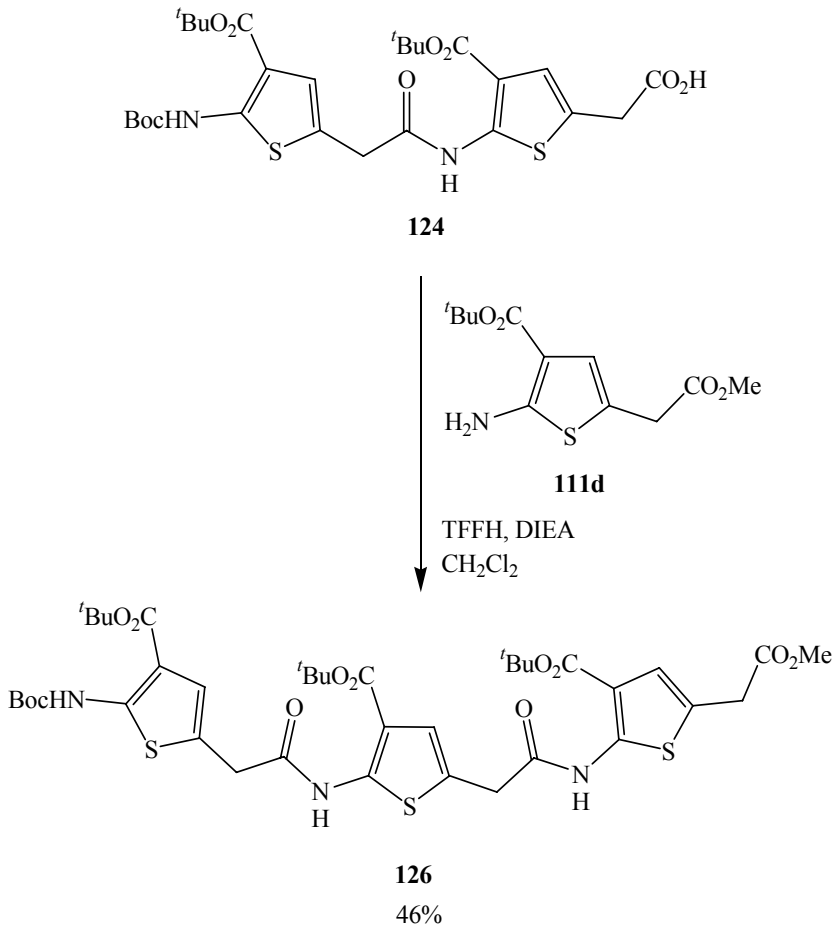
Conversion of methyl ester **122** into carboxylic acid **124** was easily accomplished using 3 equivalents of LiOH in H_2O/MeOH/THF mixture.[89] After 12 hours of reaction time, carboxylic acid **124** was obtained as orange solid in almost quantitative yield (Scheme 78). Further treatment of **124** with trimethylsilyltrifluoromethanesulfonate in the presence of 2,6-lutidine over 2 hours followed by dissolving the crude trimethylsilyl carbamate in methanol and purification by column chromatography and HPLC afforded fully deprotected tetrapeptide analogue **125** in 93 % yield.



Scheme 78. (experiment: E62, E63)

6.9.2 TFFH-mediated synthesis and deprotection reactions of hexapeptide analogue 126

Hexapeptide analogue **126** was synthesized in a similar manner as tetrapeptide analogue **122** – by reacting carboxylic acid **124** with amine **111d** in the presence of TFFH and DIEA in dichloromethane. After 6 days stirring at room temperature (TFFH was added at 0°C) and purification by column chromatography, compound **126** was obtained in 46 % yield as pale yellow solid (Scheme 79).

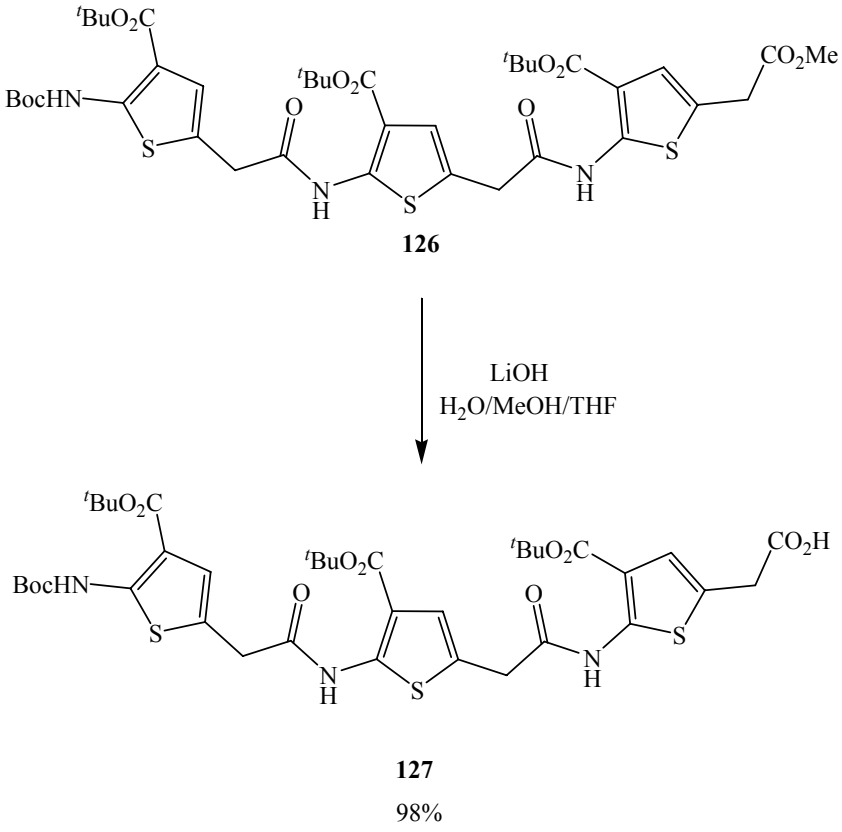


Scheme 79. TFFH-mediated synthesis of hexapeptide analogue **126** (experiment: E64)

Characteristics in the ^{1}H NMR spectra of hexapeptide analogue **126** are the signals of NH protons at δ = 11.23, 11.17 and 10.06 ppm, of the 4-H protons at δ = 7.06, 7.05 and 6.92 ppm and of CH_2 protons at δ = 3.83, 3.80 and 3.66 ppm, all of them displayed as singlets (in $CDCl_3$).

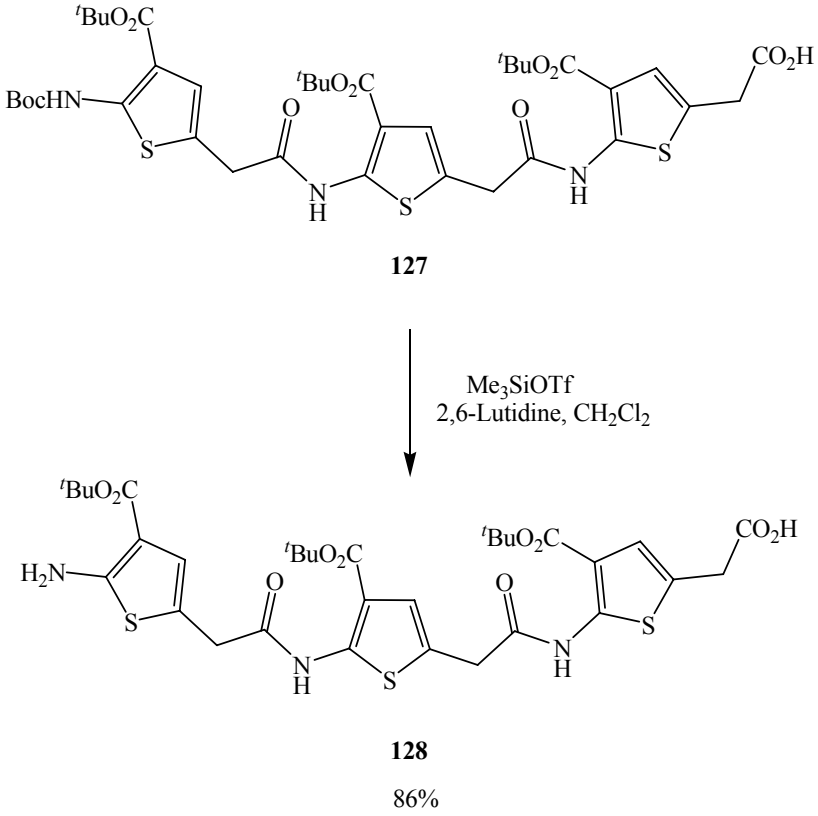
IR spectra (KBr) of the compound **126** show characteristic bands at 1715 and 1675 cm^{-1} [ν(C=O)] and three characteristic bands 1565, 1530 and 1450 cm^{-1} [ν(-CS-NH)].

Base-mediated hydrolysis of the compound **126** with LiOH in H_2O/MeOH/THF mixture as described above[89] afforded carboxylic acid **127** in almost quantitative yield as orange solid (Scheme 80).



Scheme 80. Base-mediated hydrolysis of the compound **126** (experiment: E65)

Finally, fully deprotected hexapeptide analogue **128** was obtained by reaction of **127** with trimethylsilyltrifluoromethanesulfonate in the presence of 2,6-lutidine and consequent dissolving of crude trimethylsilyl carbamate in methanol. After purification by column chromatography compound **128** was isolated in 86% yield (Scheme 81).

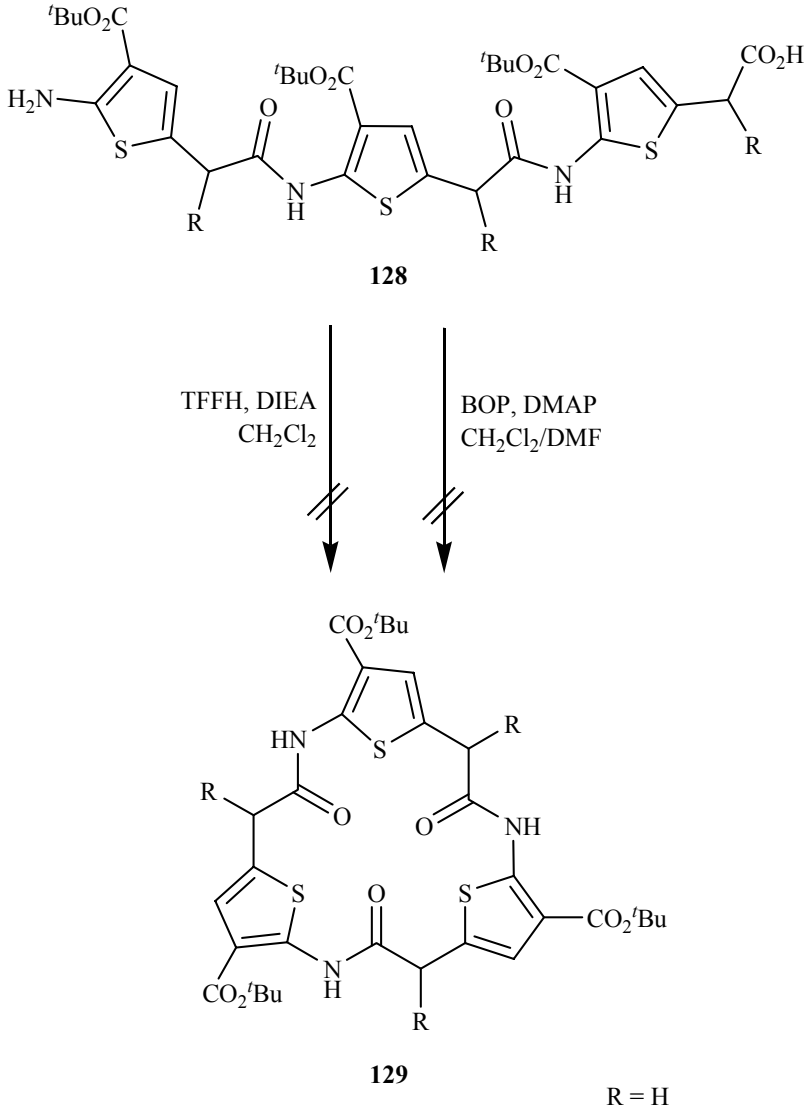


Scheme 81. Chemoselective deprotection of amino-group in hexapeptide analogue **127** (experiment: E66)

6.9.3 Attempts of cyclization of hexapeptide analogue 128 to macrolactam analogue 129

In order to obtain macrolactam analogue **129** cyclization of **128** has been attempted. The coupling procedure with TFFH and DIEA in diluted CH_2Cl_2 solution gave no cyclic trimer. Another attempt has been made by slow addition of **128** to a solution of BOP and DMAP in CH_2Cl_2/DMF (2/1) with a syringe pump,[60] again no cyclic compound was obtained.

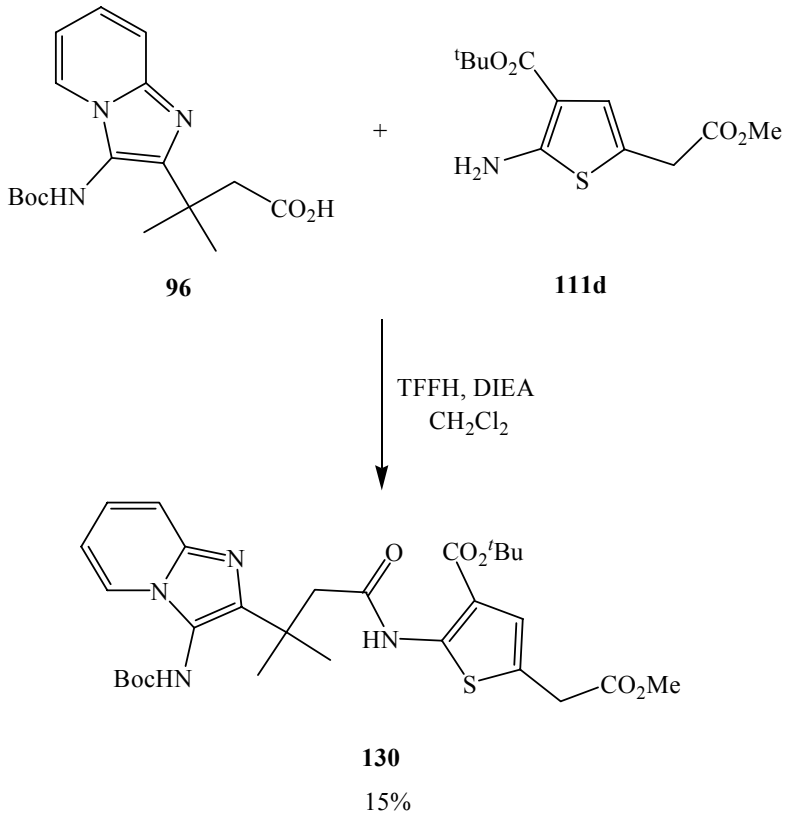
These attempts were unsuccessful most probably because of the nature of the starting compound – previous results showed that similar compounds where R = H produced no cyclic trimers independently from the coupling method applied and that it is necessary to use precursors where $R \neq H$ for a successful cyclotrimerization.[65]



Scheme 82. Attempts of cyclization of **128** to macrolactam analogue **129**

6.9.4 TFFH-mediated synthesis of tetrapeptide analogue 130

Synthesis of a compound combined from two newly synthesized unnatural δ-amino acids has been attempted. It has been chosen to react carboxylic acid **96** and amine **111d**. The first experiment was carried out in the presence of DCC and HOBt in dichloromethane[142] which afforded tetrapeptide analogue **130** in 9% yield after column chromatography followed by HPLC. Slightly improved result was obtained by employing the TFFH coupling method, when compound **130** was obtained in 15% yield (Scheme 82).[10]



Scheme 82. TFFH-mediated synthesis of tetrapeptide analogue **130** (experiment: E67)

Such low yields in this coupling reaction may be explained by poorly nucleophilic character of amino-group in **111d** combined with sterical hindrance present in both molecules.

6.10 Conclusion

2-Aminothiophenes have been synthesized from 2-siloxycyclopropanes **53**, **54** and **57** as starting materials and applied to Gewald's general reaction depicted in Scheme 15 according to the methods A and B. A number of compounds **111** which represent unnatural δ-amino acid methyl esters have been synthesized. Yields were moderate and ranged between 37 and 71 %. The 2,3,4,5-tetrasubstituted derivative **113** was synthesized starting from commercially available levulinic acid methyl ester **112**. These compounds, dipeptide analogues, could be

coupled with L-alanine to tripeptide analogues **119b-e**. Compound **111d** has been further utilized for synthesis of different peptidomimetics **120-130**.

This methodology provides a very efficient means to synthesize thiophene-containing unnatural amino acids, which represent dipeptide analogues and could be exploited in synthesis of various peptidomimetics and unnatural and hybrid oligomers. Such oligomers would be possible to apply in synthesis of macrolactam ring structures.