7.4 Gewald reaction

7.4.1 Synthesis of aminotiophenes- method A

Synthesis of Methyl 2-Amino-5-(2-methoxy-2-oxoethyl)thiophene-3-carboxylate (111a)

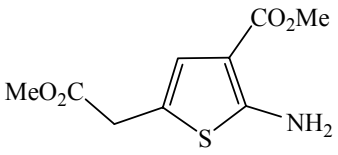
E 43 (IV 69)

Starting amounts:

0.188 g	(1.00 mmol) Siloxycyclopropanecarboxylate **53**
2.0 ml	DMF
0.163 g	$NEt_3 \cdot 3HF$
0.3 ml	NEt_3
0.099 g	(1.00 mmol) Methyl cyanoacetate
0.032 g	(1.00 mmol) Sulfur, pulverized

Procedure: Siloxycyclopropanecarboxylate **53** was dissolved in dimethylformamide and triethylamine and $NEt_3 \cdot 3HF$ were added simultaneously. After one hour stirring at room temperature, methyl cyanoacetate and sulfur were added to the reaction mixture, which was heated at 55 °C for 5 hours, then stirred over night at room temperature. The solvent was removed under reduced pressure, ethyl acetate and water were added and the layers separated. The aqueous layer was extracted with ethyl acetate, the combined organic layers were dried with Na_2SO_4 and the solvent was evaporated.

Purification: Column chromatography on silica gel with hexane/ethyl acetate 2:1
Yield: 128 mg (56 %) of **111a** as yellow solid



111a

Melting range: 74-78 ° C

^{1}H-NMR (250 MHz, $CDCl_3$): δ = 3.60 (s, 2 H, CH_2), 3.72, 3.78 (2 s, 3 H, 3 H, OMe), 5.94 (bs, 2 H, NH_2), 6.77 (s, 1 H, 4-H).
^{13}C-NMR (62.9 MHz, $CDCl_3$): δ = 35.1 (t, CH_2), 50.9, 52.2 (2 q, OMe), 105.9 (s, C-5), 116.1 (s, C-3), 124.8 (d, C-4), 162.8 (s, C-2), 165.5, 170.9 (2 s, C=O).

IR (KBr): ν = 3440–3335 cm^{-1} (N-H), 3070–2855 (C-H), 1740 (C=O), 1680 (C=O), 1590, 1505, 1440 (N-H, CS-NH).

MS (EI, 80 eV, 90 °C): *m/z* (%) = 229 (49, $[M]^+$), 170 (100, $[M^+ - CO_2Me]$), 138 (67).
HRMS (EI, 80 eV) *m/z* calculated for $[M]^+$: 229.04088, found: 229.04321.

$C_9H_{11}NO_4S$ (229.3)	calc.	C 47.15	H 4.84	N 6.11
	found	C 47.00	H 4.63	N 5.92

Synthesis of Benzyl 2-Amino-5-(2-methoxy-2-oxoethyl)thiophene-3-carboxylate (111b)

E 44 (IV 180)

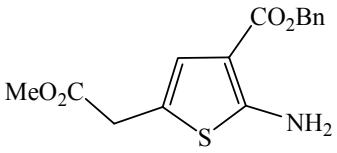
Starting amounts:

0.828 g	(4.40 mmol) Siloxycyclopropanecarboxylate **53**
10 ml	DMF
0.816 g	$NEt_3 \cdot 3HF$
1.5 ml	NEt_3
0.701 g	(4.00 mmol) Benzyl cyanoacetate
0.128 g	(4.00 mmol) Sulfur, pulverized

Procedure: Siloxycyclopropanecarboxylate **53** was dissolved in dimethylformamide and triethylamine and $NEt_3 \cdot 3HF$ were added simultaneously. After one hour stirring at room temperature, methyl cyanoacetate and sulfur were added to the reaction mixture which was heated at 55 °C for 6 hours, then stirred over night at room temperature. The solvent was

removed under reduced pressure, ethyl acetate and water were added and the layers separated. The aqueous layer was extracted with ethyl acetate, the combined organic layers were dried with Na_2SO_4 and the solvent was evaporated.

Purification: Column chromatography on silica gel with hexane/ethyl acetate 2:1
Yield: 776 mg (64 %) of **111b** as brownish oil



111b

^{1}H-NMR (250 MHz, $CDCl_3$): δ = 3.56 (s, 2 H, CH_2), 3.67 (s, 3 H, OMe), 5.23 (s, 2 H, C*H*$_2$Ph), 6.12 (bs, 2 H, NH_2), 6.80 (s, 1 H, 4-H), 7.21–7.46 (m, 5 H, Ph).
^{13}C-NMR (62.9 MHz, $CDCl_3$): δ = 34.7 (t, CH_2), 51.9 (q, OMe), 65.0 (t, *C*H$_2$Ph), 105.1 (s, C-5), 115.7 (s, C-3), 124.6 (d, C-4), 127.6, 127.7, 128.2 (3 d, Ph), 136.3 (s, Ph), 163.3, (s, C-2), 164.6, 170.8 (2 s, C=O).

IR (KBr): ν = 3445–3340 cm^{-1} (N-H), 3065–2850 (C-H), 1740 (C=O), 1675 (C=O), 1590, 1500, 1455 (N-H, -CS-NH-).

MS (EI, 80 eV, 140 °C): *m/z* (%) = 305 (23, $[M]^+$), 245 (19), 91 (100 $[Bn]^+$).
HRMS (EI, 80 eV) *m/z* calculated for [M^+, $C_{15}H_{15}NO_4S$]: 305.07217, found: 305.07366.

$C_{15}H_{15}NO_4S$ (305.3)	calc.	C 59.00	H 4.95	N 4.59
	found	C 58.42	H 4.79	N 4.32

Synthesis of *tert*-Butyl 2-Amino-5-(2-methoxycarbonyl-propyl)thiophene-3-carboxylate (111c)

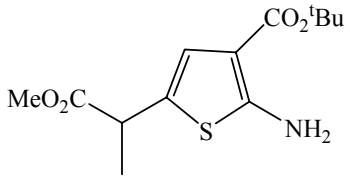
E 45 (IV 152)

Starting amounts:

0.100 g	(0.495 mmol) Siloxycyclopropanecarboxylate **55**
1.0 ml	DMF
0.082 g	$NEt_3 \cdot 3HF$
0.2 ml	NEt_3
0.070 g	(0.50 mmol) *tert*-Butyl cyanoacetate
0.016 g	(0.50 mmol) Sulfur, pulverized

Procedure: Siloxycyclopropanecarboxylate **55** was dissolved in dimethylformamide and triethylamine and $NEt_3 \cdot 3HF$ were added simultaneously. After one hour stirring at room temperature, methyl cyanoacetate and sulfur were added to the reaction mixture, which was heated at 55 °C for 5 hours, then stirred over night at room temperature. The solvent was removed under reduced pressure, ethyl acetate and water were added and the layers separated. The aqueous layer was extracted with ethyl acetate, the combined organic layers were dried with Na_2SO_4 and the solvent was evaporated.

Purification: Column chromatography on silica gel with hexane/ethyl acetate 2:1
Yield: 97 mg (69 %) of **111c** as brownish oil



111c

^{1}H-NMR (250 MHz, $CDCl_3$): δ = 1.48 (d, *J* = 8.1 Hz, 3 H, Me), 1.51 (s, 9 H, C*Me*$_3$), 3.68 (s, 3 H, OMe), 3.73 (q, *J* = 8.1 Hz, 1 H, CH), 5.79 (bs, 2 H, NH_2), 6.71 (s, 1 H, 4-H).

^{13}C-NMR (62.9 MHz, $CDCl_3$): δ = 18.7, 28.4 (2 q, Me, C*Me*$_3$), 40.6 (d, CH), 52.2 (q, OMe), 80.1 (s, *C*Me$_3$), 108.0 (s, C-5), 123.4 (d, C-4), 156.4 (s, C-3), 161.3 (s, C-2), 164.9, 174.0 (2 s, C=O).

IR (KBr): ν = 3450–3340 cm^{-1} (N-H), 2980–2935 (C-H), 1740 (C=O), 1670 (C=O), 1590, 1500, 1455 (N-H, CS-NH).

MS (EI, 80 eV, 40 °C): *m/z* (%) = 285 (6, $[M]^+$), 229 (26), 212 (12), 171 (11), 170 (100), 152 (28).
HRMS (EI, 80 eV) *m/z* calculated for $[M]^+$: 285.10349, found: 285.10466.

$C_{13}H_{19}NO_4S$ (285.4)	calc.	C 54.72	H 6.71	N 4.91
	found	C 55.34	H 5.86	N 4.36

7.4.2 Synthesis of aminothiophenes-method B

Synthesis of *tert*-Butyl 2-Amino-5-(2-methoxy-2-oxoethyl)thiophene-3-carboxylate (111d)

E 46 (IV 170)

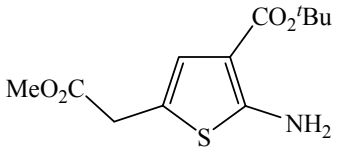
Starting amounts:

0.395 g	(2.10 mmol) Siloxycyclopropanecarboxylate **53**
0.282 g	(2.00 mmol) *tert*-Butyl cyanoacetate
0.064 g	(2.00 mmol) Sulfur, pulverized
4.0 ml	Methanol
5 drops	Diethylamine

Procedure: Siloxycyclopropanecarboxylate **53**, *tert*-butyl cyanoacetate and sulfur were suspended in methanol, then diethylamine was added. The reaction mixture was refluxed for 7 hours, and then stirred over night at room temperature. Ethyl acetate and water were added and the layers separated. The aqueous layer was extracted with ethyl acetate, the combined organic layers were dried with Na_2SO_4 and the solvent was evaporated.

Purification: Column chromatography on silica gel with hexane/ethyl acetate 2:1

Yield: 384 mg (71 %) of **111d** as brownish oil



111d

^{1}H-NMR (250 MHz, $CDCl_3$): δ = 1.53 (s, 9 H, C*Me*$_3$), 3.59 (s, 2 H, CH_2), 3.72 (s, 3 H, OMe), 5.81 (bs, 2 H, NH_2), 6.74 (s, 1 H, 4-H).

^{13}C-NMR (62.9 MHz, $CDCl_3$): δ = 28.4 (q, C*Me*$_3$), 35.0 (t, CH_2), 52.1 (q, OMe), 79.9 (s, *C*Me$_3$), 107.7 (s, C-5), 115.5 (s, C-3), 125.5 (d, C-4), 161.9 (s, C-2), 164.7, 170.9 (2 s, C=O).

IR (KBr): ν = 3445–3255 cm^{-1} (N-H), 3070–2845 (C-H), 1740 (C=O), 1670 (C=O), 1590, 1500, 1455 (N-H, CS-NH).

MS (EI, 80 eV, 70 °C): *m/z* (%) = 271 (14, $[M]^+$), 215 (64), 198 (20), 197 (26), 156 (100), 138 (36), 57 (31), 41 (11).

HRMS (EI, 80 eV) *m/z* calculated for $[M]^+$: 271.08783, found: 271.08633.

$C_{12}H_{17}NO_4S$ (271.3)	calc.	C 53.12	H 6.32	N 5.16
	found	C 53.37	H 6.28	N 4.85

Synthesis of *tert*-Butyl 2-Amino-5-(1-benzyl-2-methoxy-2-oxoethyl)thiophene-3-carboxylate (111e)

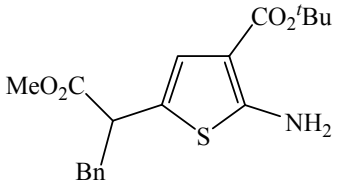
E 47 (IV 172)

Starting amounts:

0.160 g	(0.58 mmol) Siloxycyclopropanecarboxylate **57**
0.073 g	(0.52 mmol) *tert*-Butyl cyanoacetate
0.017 g	(0.52 mmol) Sulfur, pulverized
1.2 ml	Methanol
3 drops	Diethylamine

Procedure: Siloxycyclopropanecarboxylate **57**, *tert*-butyl cyanoacetate and sulfur were suspended in methanol, then diethylamine was added. The reaction mixture was refluxed for 8 hours, and then stirred over night at room temperature. Ethyl acetate and water were added and the layers were separated. The aqueous layer was extracted with ethyl acetate, the combined organic layers were dried with Na_2SO_4 and the solvent was evaporated.

Purification: Column chromatography on silica gel with 10% *i*-propanol/hexane
Yield: 69 mg (37 %) of **111e** as brownish oil



111e

^{1}H-NMR (250 MHz, $CDCl_3$): δ = 1.51 (s, 9 H, C*Me*$_3$), 3.00 (dd, *J* = 13.1, 6.6 Hz, 1 H, CH_2), 3.25 (dd, *J* = 13.1, 8.8 Hz, 1 H, CH_2), 3.61 (s, 3 H, OMe), 3.87 (dd, *J* = 8.8, 6.6 Hz, 1 H, CH), 5.81 (bs, 2 H, NH_2), 6.70 (s, 1 H, 4-H), 7.11–7.32 (m, 5 H, Ph).
^{13}C-NMR (62.9 MHz, $CDCl_3$): δ = 28.4 (q, C*Me*$_3$), 40.1 (t, CH_2), 48.7 (d, CH), 52.1 (q, OMe), 80.1 (s, *C*Me$_3$), 107.7 (s, C-5), 121.1 (s, C-3), 124.4 (d, C-4), 126.6, 128.4, 128.8 (3d, Ph), 138.2 (s, Ph), 161.5 (s, C-2), 164.8, 172.9 (2 s, C=O).

IR (KBr): ν = 3450–3340 cm^{-1} (N-H), 3090–2850 (C-H), 1740 (C=O), 1670 (C=O), 1590, 1500, 1455 (N-H, -CS-NH-).

MS (EI, 80 eV, 120 °C): *m/z* (%) = 361 (11, $[M]^+$), 270 (10 [M^+ - Bn]), 215 (12), 214 (100), 196 (12), 91 (16 $[Bn]^+$), 59 (16), 57 (44), 42 (12), 41 (16).
HRMS (EI, 80 eV) *m/z* calculated for $[M]^+$: 361.13477, found: 361.13522.

$C_{19}H_{23}NO_4S$ (361.5)	calc.	C 63.14	H 6.41	N 3.87
	found*	C 61.59	H 6.47	N 3.86

**It was not possible to obtain better results for elemental analysis*

Synthesis of *tert*-Butyl 2-Amino-5-(2-methoxy-2-oxoethyl)-4-methyl-thiophene-3-carboxylate (113)

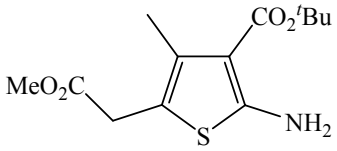
E 48 (IV 110)

Starting amounts:

0.260 g	(2.00 mmol) Levulinic acid methyl ester **112**
0.2 ml	Diethylamine
0.282 g	(2.00 mmol) *tert*-Butyl cyanoacetate
0.064 g	(2.00 mmol) Sulfur, pulverized
0.6 ml	Methanol, dry

Procedure: Levulinic acid methyl ester **112**, *tert*-butyl cyanoacetate and sulfur were suspended in methanol, then diethylamine was added. The reaction mixture was heated (t_{bath}=45 °C) for 78 hours. Ethyl acetate and water were added and the layers were separated. The aqueous layer was extracted with ethyl acetate, the combined organic layers were dried with Na_2SO_4 and the solvent was evaporated.

Purification: Column chromatography on silica gel with hexane/ethyl acetate 2:1, then HPLC (3 % *i*-propanol/hexane, 64 ml/min, 80 bar)
Yield: 54 mg (10 %) of **113** as brownish solid



113

Melting point: 110-112 ° C

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.52 (s, 9 H, C*Me*$_3$), 2.16 (s, 3 H, 4-Me), 3.52 (s, 2 H, CH_2), 3.67 (s, 3 H, OMe), 5.96 (bs, 2 H, NH_2).
^{13}C-NMR (125.8 MHz, $CDCl_3$): δ = 15.3, 28.5 (2 q, Me, C*Me*$_3$), 32.6 (t, CH_2), 52.1 (q, OMe), 79.9 (s, *C*Me$_3$), 108.2 (s, C-5), 109.6 (s, C-4), 133.5 (s, C-3), 161.7 (s, C-2), 165.4, 171.1 (2 s, 2 C=O).

IR (KBr): ν = 3445–3330 cm^{-1} (N-H), 2975–2930 (C-H), 1740 (C=O), 1665 (C=O), 1580, 1525, 1480 (N-H, CS-NH).

MS (EI, 80 eV, 90 °C): *m/z* (%) = 285 (24, [M^+]), 229 (69), 212 (17), 211 (35), 170 (100), 152 (45), 126 (11), 57 (34), 41 (17), 29 (15), 28 (17).
HRMS (EI, 80 eV) *m/z* calculated for [M^+, $C_{13}H_{19}NO_4S$]: 285.10349, found: 285.10522.

7.4.3 DCC and BOP mediated syntheses of tri- and tetrapeptide analogues

Synthesis of *tert*-Butyl 2-[(*tert*-Butoxycarbonyl)amino]-5-(2-methoxy-2-oxoethyl)thiophene-3-carboxylate (114)

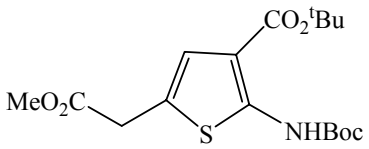
E 49 (IV 223)

Starting amounts:

0.293 g	(1.08 mmol) *tert*-Butyl 2-amino-5-(2-methoxy-2-oxoethyl)thiophene-3-carboxylate (**111d**)
1.20 g	(5.50 mmol) Boc_2O
1.01 g	(8.25 mmol) DMAP
0.3 ml	Et_3N
10 ml	CH_2Cl_2

Procedure: *tert*-Butyl 2-amino-5-(2-methoxy-2-oxoethyl)thiophene-3-carboxylate (**111d**) was dissolved in dichloromethane and then Boc_2O, DMAP and finally triethylamine were added. The reaction mixture was stirred at room temperature over 7 days. The solvent was evaporated, the residue was dissolved in ethyl acetate and this solution was successively washed with 2 M HCl and brine. The organic phase was dried with Na_2SO_4 and the solvent was evaporated under reduced pressure.

Purification: Column chromatography on silica gel with hexane/ethyl acetate 4:1
Yield: 268 mg (67 %) of **114** as brownish oil



114

^{1}H-NMR (250 MHz, $CDCl_3$): δ = 1.50, 1.53 (2 s, 9 H, 9H, C*Me*$_3$), 3.65 (s, 2 H, CH_2), 3.69 (s, 3 H, OMe), 6.90 (s, 1 H, 4-H), 10.01 (s, 1 H, NH).

^{13}C-NMR (62.9 MHz, $CDCl_3$): δ = 28.2, 28.3 (2 q, C*Me*$_3$), 34.9 (t, CH_2), 52.3 (q, OMe), 81.3, 82.0 (2 s, *C*Me_3), 112.2 (s, C-3), 123.1 (s, C-2), 124.0 (d, C-4), 150.0 (s, C=O), 152.5 (s, C-5), 164.7, 170.7 (2 s, C=O).

IR (KBr): ν = 3440–3335 cm^{-1} (N-H), 2980–2935 (C-H), 1800 (C=O), 1745 (C=O), 1710 (C=O), 1590, 1560, 1455 (N-H, CS-NH).

MS (EI, 80 eV, 120 °C): *m/z* (%) = 371 (9, $[M]^+$), 259 (30), 219 (48), 200 (11), 156 (32), 138 (11), 57 (100), 41 (24), 29 (19).
HRMS (EI, 80 eV) *m/z* calculated for [M^+, $C_{17}H_{25}NO_6S$]: 371.14026, found: 371.14244.

N-Boc deprotection of 114

E 50 (IV 248)

Starting amounts:

0.066 g	(0.18 mmol) *tert*-Butyl 2-[(*tert*-butoxycarbonyl)amino]-5-(2-methoxy-2-oxoethyl)thiophene-3-carboxylate **114**
0.041 g	(0.18 mmol) Me_3SiOTf
0.040 g	(0.36 mmol) 2,6-Lutidine
1.5 ml	CH_2Cl_2

Procedure: To a solution of *tert*-butyl 2-[(*tert*-butoxycarbonyl)amino]-5-(2-methoxy-2-oxoethyl)thiophene-3-carboxylate (**114**) and 2,6-lutidine in dry dichloromethane, Me_3SiOTf was dropwise added at room temperature. The reaction mixture was stirred for 15 min, quenched with saturated aqueous NH_4Cl solution and extracted with diethyl ether several times. The combined organic phases were washed with water and brine, dried with $MgSO_4$ and concentrated under reduced pressure. The crude silyl carbamate was dissolved in dry methanol, stirred for 20 min at room temperature and the solvent was evaporated.

Purification: Column chromatography on silica gel with hexane/ethyl acetate 4:1
Yield: 26 mg (53 %) of **111d** as brownish oil - for analytical data see E46 (IV 170)

Synthesis of {4-(*tert*-Butoxycarbonyl)-5-[(*tert*-butoxycarbonyl)amino]thien-2-yl}acetic acid (118)

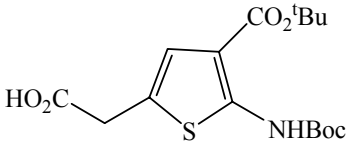
E 51 (IV 243)

Starting amounts:

0.214 g	(0.58 mmol) *tert*-Butyl 2-[(*tert*-butoxycarbonyl)amino]-5-(2-methoxy-2-oxoethyl)thiophene-3-carboxylate (**114**)
0.073 g	(1.74 mmol) $LiOH \cdot H_2O$
1 ml	H_2O
1 ml	MeOH
3 ml	THF

Procedure: Compound **114** was dissolved in a mixture of methanol and tetrahydrofurane, a solution of LiOH in water was added, and the resulting mixture was stirred for 24 hours at room temperature. 2 M HCl was added to adjust pH 7. Diethyl ether was added and the layers were separated. The aqueous layer was extracted with diethyl ether, the combined organic phases were dried with $MgSO_4$ and the solvent was evaporated.

Purification: Column chromatography on silica gel with hexane/ethyl acetate 4:1, then methanol/dichloromethane 4:1
Yield: 203 mg (98 %) of **118** as orange solid



118

Melting range: 169–172 °C

^{1}H-NMR (500 MHz, CD_3OD): δ = 1.57, 1.59 (2 s, 9 H, 9 H, C*Me*$_3$), 3.57 (s, 2 H, CH_2), 6.89 (s, 1 H, 4-H).

^{13}C-NMR (125.8 MHz, CD_3OD): δ = 28.7, 28.9 (2 q, C*Me*$_3$), 49.5 (t, CH_2), 82.6, 83.6 (2 s, *C*Me_3), 113.7 (s, C-3), 123.6 (d, C-4), 127.1 (s, C-2), 150.6 (s, C=O), 153.6 (s, C-5), 166.5, 180.6 (2 s, C=O).

IR (KBr): ν = 3390–3300 cm^{-1} (O-H, N-H), 2980–2850 (C-H), 1720 (C=O), 1670 (C=O), 1565, 1540, 1475 (N-H, CS-NH), 1250 (C-O).

MS (EI, 80 eV, 170 °C): *m/z* (%) = 357 (0.2, $[M]^+$), 57 (15), 56 (57), 55 (25), 44 (86), 43 (13), 41 (100), 40 (10), 39 (51).
HRMS (EI, 80 eV) *m/z* calculated for [M^+, $C_{16}H_{23}NO_6S$]: 357.12460, found: 357.12533.

Synthesis of Benzyl 2-{[*N*-(*tert*-Butoxycarbonyl)-L-alanyl]amino}-5-(2-methoxy-2-oxoethyl)thiophene-3-carboxylate (119b)

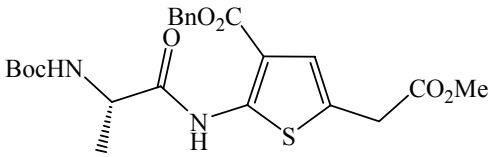
E 52 (IV 169)

Starting amounts:

0.192 g	(0.63 mmol) Benzyl 2-amino-5-(2-methoxy-2-oxoethyl)thiophene-3-carboxylate (**111b**)
0.179 g	(0.95 mmol) N-Boc L-Alanine
0.195 g	(0.95 mmol) DCC
3 ml	CH_2Cl_2

Procedure: To a stirred solution of the benzyl 2-amino-5-(2-methoxy-2-oxoethyl)thiophene-3-carboxylate (**111b**) and N-Boc L-alanine in dichloromethane, DCC was added at 0 °C. The resulting solution was stirred over 4 days at room temperature.

Purification: Flash chromatography on silica gel with 0.8 % methanol/dichloromethane, then 30 % ethyl acetate/dichloromethane, then 10 % *i*-propanol/hexane
Yield: 168 mg (56 %) of **119b** as yellow solid
60 mg (31 %) of **111b** as brownish oil was recovered



119b

Melting range: 75-78 ° C

Optical rotation: $[\alpha]_D^{20}$ = -61.9 (c = 1.05, MeOH).

^{1}H-NMR (250 MHz, $CDCl_3$): δ = 1.43 (s, 9 H, C*Me*$_3$), 1.44 (d, *J* = 8.9 Hz, 3 H, Me), 3.65 (s, 2 H, CH_2), 3.67 (s, 3 H, OMe), 4.37–4.41 (m, 1 H, CH), 5.24 (s, 2 H, C*H*$_2$Ph), 5.27 (s, 1 H, NH), 7.01 (s, 1 H, 4-H), 7.22–7.38 (m, 5 H, Ph), 11.40 (s, 1 H, NH).
^{13}C-NMR (125.8 MHz, $CDCl_3$): δ = 17.9 (q, Me), 28.1 (q, C*Me*$_3$), 34.7 (t, CH_2), 50.4 (d, CH), 52.2 (q, OMe), 66.1 (t, *C*H$_2$Ph), 80.3 (s, *C*Me$_3$), 112.4 (s, C-5), 123.0 (d, C-4), 125.3 (s, C-2), 128.0, 128.2, 128.5 (3 d, Ph), 135.7 (s, Ph), 155.1 (s, C-3), 148.1, 164.6, 170.2, 170.4 (3 s, C=O).

IR (KBr): ν = 3350–3290 cm^{-1} (N-H), 2980–2850 (C-H), 1720 (C=O), 1680 (C=O), 1560, 1530, 1455 (N-H, CS-NH).

MS (EI, 80 eV, 170 °C): *m/z* (%) = 476 (16, $[M]^+$), 420 (12), 307 (27), 306 (81), 305 (90), 247 (11), 246 (33), 245 (35), 91 (100 $[Bn]^+$), 57 (27).
HRMS (EI, 80 eV) *m/z* calculated for [M^+, $C_{23}H_{28}N_2O_7S$]: 476.16171, found: 476.16333.

Synthesis of *tert*-Butyl 2-{[*N*-(*tert*-Butoxycarbonyl)-L-alanyl]amino}-5-(2-methoxy-1-methyl-2-oxoethyl)thiophene-3-carboxylate (119c)

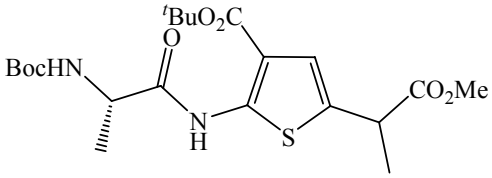
E 53 (IV 153)

Starting amounts:

0.285 g	**(**1.00 mmol) *tert*-Butyl 2-amino-5-(2-methoxycarbonyl-propyl)thiophene-3-carboxylate (**111c**)
0.218 g	(1.15 mmol) N-Boc L-alanine
0.237 g	(1.15 mmol) DCC
4 ml	CH_2Cl_2

Procedure: To a stirred solution of compound **111c** and N-Boc L-alanine in dichloromethane, DCC was added, at 0 °C. The resulting solution was stirred for 20 hours at room temperature.

Purification: Flash chromatography on silica gel with 30 % ethyl acetate/dichloromethane, then HPLC (4 % *i*-propanol/hexane, 64 ml/min, 95 bar)
Yield: 242 mg (53 %) of **119c** as yellow solid
22 mg (8 %) of **111c** as brownish oil was recovered



119c

Melting range: 45-47 °C

Optical rotation: $[\alpha]_D^{20}$ = -39.3 (c = 1.34, MeOH).

^{1}H-NMR (500 MHz, CD_3OD): δ = 1.46 (d, *J* = 7.3 Hz, 3 H, Me), 1.52 (s, 9 H, C*Me*$_3$), 1.54 (d, *J* = 7.4 Hz, 3 H, Me), 1.62 (s, 9 H, C*Me*$_3$), 3.74 (s, 3 H, OMe), 3.97 (q, *J* = 7.4 Hz, 1 H, CH), 4.30 (q, *J* = 7.3 Hz, 1 H, CH), 7.00 (s, 1 H, 4-H).
^{13}C-NMR (125.8 MHz, CD_3OD): δ = 17.4, 19.7 (2 q, Me), 29.0, 29.2 (2 q, C*Me*$_3$), 41.9, 49.4 (2 d, CH), 49.6 (q, OMe), 81.4, 83.1 (2 s, *C*Me$_3$), 115.8 (s, C-5), 122.9 (d, C-4), 134.3 (s, C-2), 158.1 (s, C-3), 148.1, 166.2, 173.0, 175.6 (4 s, C=O).

IR (KBr): ν = 3455–3260 cm^{-1} (N-H), 2980–2880 (C-H), 1745 (C=O), 1675 (C=O), 1590, 1505, 1455 (N-H, CS-NH).

MS (EI, 80 eV, 80-120 °C): *m/z* (%) = 425 (4, $[M^+ - OMe]$), 286 (17), 285 (49), 231 (10), 230 (45), 229 (91), 228 (42), 214 (11), 212 (37), 211 (12), 172 (14), 171 (23), 170 (100), 152 (55).
HRMS (EI, 80 eV) *m/z* calculated for $[M^+ - OMe]$: 425.17462, found: 425.17633.

$C_{21}H_{32}N_2O_7S$ (456.6)	calc.	C 55.25	H 7.06	N 6.14
	found	C 55.01	H 6.59	N 5.34

Synthesis of *tert*-Butyl 2-{[*N*-(*tert*-Butoxycarbonyl)-L-alanyl]amino}-5-(2-methoxy-2-oxoethyl)thiophene-3-carboxylate (119d)

Method A

E 54 (IV 157)

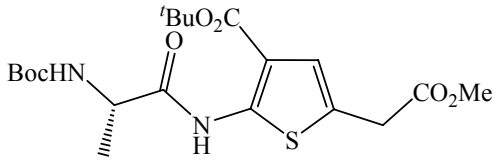
Starting amounts:

0.464 g	(1.71 mmol) *tert*-Butyl 2-amino-5-(2-methoxy-2-oxoethyl)thiophene-3-carboxylate (**111d**)
0.372 g	(1.97 mmol) N-Boc L-Alanine
0.406 g	(1.97 mmol) DCC
6.5 ml	CH_2Cl_2

Procedure: To a stirred solution of the compound **111d** and N-Boc L-alanine in dichloromethane, DCC was added at 0 °C. The resulting solution was stirred for 20 hours at room temperature.

Purification: Column chromatography on silica gel with 30 % ethyl acetate/dichloromethane, then HPLC (10 % *i*-propanol/hexane, 64 ml/min, 80 bar)
Yield: 454 mg (60 %) of **119d** as yellow solid

74 mg (16 %) of **111d** was recovered as brownish oil



119d

Melting range: 85-90 °C

Optical rotation: $[\alpha]_D^{20}$ = -16.7 (c = 0.42, MeOH).

^{1}H-NMR (250 MHz, $CDCl_3$): δ = 1.41 (s, 9 H, C*Me*$_3$), 1.43 (d, *J* = 7.2 Hz, 3 H, Me), 1.49 (s, 9 H, C*Me*$_3$), 3.65 (s, 2 H, CH_2), 3.66 (s, 3 H, OMe), 4.38 (q, *J* = 7.2 Hz, 1 H, CH), 5.18–5.20 (m, 1 H, NH), 6.92 (s, 1 H, 4-H), 11.47 (s, 1 H, NH).
^{13}C-NMR (125.8 MHz, $CDCl_3$): δ = 18.1 (q, Me), 28.1, 28.2 (2 q, C*Me*$_3$), 34.8 (t, CH_2), 50.4 (d, CH), 52.2 (q, OMe), 80.3, 81.4 (2 s, *C*Me$_3$), 114.4 (s, C-5), 123.6 (d, C-4), 124.7 (s, C-2), 155.1 (s, C-3), 147.0, 164.5, 170.1, 170.6 (4 s, C=O).

IR (KBr): ν = 3440–3290 cm^{-1} (N-H), 2980–2930 (C-H), 1670 (C=O), 1560, 1530, 1455 (CS-NH).

MS (EI, 80 eV, 130 °C): *m/z* (%) = 442 (12, $[M]^+$), 330 (17), 271 (16), 216 (12), 215 (100), 197 (10), 156 (33), 56 (10).
HRMS (EI, 80 eV) *m/z* calculated for $[M]^+$: 442.17737, found: 442.17546.

$C_{20}H_{30}N_2O_7S$ (442.5)	calc.	C 54.28	H 6.83	N 6.33
	found*	C 50.50	H 6.21	N 5.78

**It was not possible to obtain better results for elemental analysis*

Method B

E 55 (IV 199)

Starting amounts:

0.188 g	(1.00 mmol) Siloxycyclopropanecarboxylate **53**
0.128 g	(0.91 mmol) *tert*-Butyl cyanoacetate
0.029 g	(0.91 mmol) Sulfur, pulverized
2.0 ml	Methanol
5 drops	Diethylamine
0.172 g	(0.91 mmol) N-Boc L-Alanine
0.187 g	(0.91 mmol) DCC
4.5 ml	CH_2Cl_2

Procedure: The siloxycyclopropanecarboxylate **53**, *tert*-butyl cyanoacetate and sulfur were suspended in methanol, and then diethylamine was added. The reaction mixture was refluxed for 6 hours, then stirred overnight at room temperature. The solvent was evaporated. The structure of the crude product **111d** has been proved by NMR.
To a stirred solution of the Benzyl *tert*-butyl 2-amino-5-(2-methoxy-2-oxoethyl)thiophene-3-carboxylate (**111d**) as a crude product from previous step and N-Boc L-alanine in dichloromethane, DCC was added, at 0 °C. The resulting solution was stirred over one week at room temperature.

Purification: Column chromatography on silica gel with 30 % ethyl acetate/dichloromethane, then 5 % *i*-propanol/hexane
Yield: 225 mg (51 %) of **119d** as yellow oil

Synthesis of *tert*-Butyl 5-(1-Benzyl-2-methoxy-2-oxoethyl)-2-{[*N*-(*tert*-butoxycarbonyl)-L-alanyl]amino}thiophene-3-carboxylate (119e)

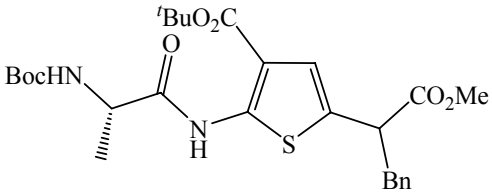
E 56 (IV 179)

Starting amounts:

0.066 g	(0.18 mmol) *tert*-Butyl 2-amino-5-(1-benzyl-2-methoxy-2-oxoethyl)thiophene-3-carboxylate (**111e**)
0.039 g	(0.21 mmol) N-Boc L-Alanine
0.043 g	(0.21 mmol) DCC
1 ml	CH_2Cl_2

Procedure: To a stirred solution of the compound **111e** and N-Boc L-alanine in dichloromethane DCC was added at 0 °C. The resulting solution was stirred over 4 days at room temperature.

Purification: Column chromatography on silica gel with 30% ethyl acetate/dichloromethane
Yield: 80 mg (84 %) of **119d** as yellow oil
7 mg (10 %) of **111e** was recovered as brownish oil



119e

Optical rotation: $[\alpha]_D^{20}$ = -6.1 (c = 0.17, MeOH).

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.44 (d, *J* = 8.2 Hz, 3 H, Me), 1.52, 1.54 (2 s, 9 H, 9 H, C*Me*$_3$), 3.38–3.51 (m, 2 H, CH_2), 3.62 (s, 3 H, OMe), 3.96–4.04 (m, 1 H, C*H*Bn), 4.37–4.47 (m, 1 H, C*H*Me), 5.29 (bs, 1 H, NH), 6.94 (s, 1 H, 4-H), 7.07–7.36 (m, 5 H, Ph), 11.53 (s, 1 H, NH).

^{13}C-NMR (125.8 MHz, $CDCl_3$): δ = 18.8 (q, Me), 28.4, 29.8 (2 q, C*Me*$_3$), 49.0 (d, *C*HBn), 49.4 (d, *C*HMe), 50.7 (q, OMe), 80.2, 80.4 (2 s, *C*Me$_3$), 116.9 (s, C-5), 122.6 (d, C-4), 126.7, 128.5, 128.9 (3 d, Ph), 129.2 (s, C-2), 139.9 (s, Ph), 154.7 (s, C-3), 149.7, 163.6, 172.2, 175.8 (4 s, C=O).

IR (KBr): ν = 3430–3325 cm^{-1} (N-H), 2980–2855 (C-H), 1740 (C=O), 1670 (C=O), 1590, 1560, 1530, 1455 (N-H, C=C, CS-NH).

MS (EI, 80 eV, 60 °C): *m/z* (%) = 532 (5, $[M]^+$), 460 (12), 442 (24), 441 (100), 288 (10).
HRMS (EI, 80 eV) *m/z* calculated for [M^+, $C_{27}H_{36}N_2O_7S$]: 532.22430, found: 532.22644.

Synthesis of 4-(*tert*-Butoxycarbonyl)-5-{[*N*-(*tert*-butoxycarbonyl)-L-alanyl]amino}thien-2-yl)acetic acid (120)

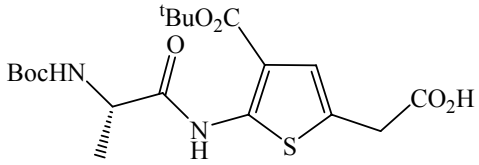
E 57 (IV 156)

Starting amounts:

0.047 g	(0.10 mmol) *tert*-Butyl 2-{[*N*-(*tert*-butoxycarbonyl)-L-alanyl]amino}-5-(2-methoxy-2-oxoethyl)thiophene-3-carboxylate (**119d**)
0.014	(0.32 mmol) $LiOH \cdot H_2O$
0.5 ml	H_2O
0.5 ml	MeOH
1.5 ml	THF

Procedure: The ester **119d** was dissolved in a mixture of methanol and tetrahydrofuran, a solution of LiOH in water was added, and the resulting mixture was stirred over night at room temperature. 2 M HCl was added to adjust pH 7. Diethyl ether was added and the layers were separated. The aqueous layer was extracted with diethyl ether, the combined organic phases were dried with $MgSO_4$ and the solvent was evaporated.

Purification: Column chromatography on silica gel with hexane/ethyl acetate 4:1, then methanol/dichloromethane 4:1
Yield: 40 mg (93 %) of **120** as yellow oil



120

Optical rotation: $[\alpha]_D^{20}$ = -5.0 (c = 0.10, MeOH).

^{1}H-NMR (500 MHz, CD_3OD): δ = 1.42 (d, *J* = 7.5 Hz, 3 H, Me), 1.49, 1.57 (2 s, 9 H, 9 H, C*Me*$_3$), 3.58 (s, 2 H, CH_2), 4.23 (q, *J* = 7.5 Hz, 1 H, CH), 6.92 (s, 1 H, 4-H).
^{13}C-NMR (125.8 MHz, CD_3OD): δ = 17.4, 28.6, 28.8 (3 q, Me, C*Me*$_3$), 38.9 (t, CH_2), 49.4 (d, CH), 82.3, 82.5 (2 s, *C*Me$_3$), 115.6 (s, C-5), 122.8 (d, C-4), 130.8 (s, C-2), 157.8 (s, C-3), 147.6, 166.1, 172.5 (3 s, C=O).

IR (KBr): ν = 3280 cm^{-1} (N-H, O-H), 2980–2930 (C-H), 1715 (C=O), 1675 (C=O), 1560, 1530, 1455 (N-H, CS-NH), 1250 (C-O).

MS (EI, 80 eV, 160 °C): *m/z* (%) = 428 (10, $[M]^+$), 316 (11), 257 (11), 201 (94), 183 (12), 156 (21), 57 (57), 56 (35), 55 (15), 44 (100), 41 (74), 39 (22), 29 (20), 28 (35), 27 (11).
HRMS (EI, 80 eV) *m/z* calculated for [M^+, $C_{19}H_{28}N_2O_7S$]: 428.16171, found: 428.16324.

Synthesis of *tert*-Butyl 2-({2-[(*tert*-butoxycarbonyl)amino]propanoyl}-amino)-5-[(methoxy-1-methoxyoxoethyl)amino](oxo)ethyl]-3-thiophenecarboxylate (121)

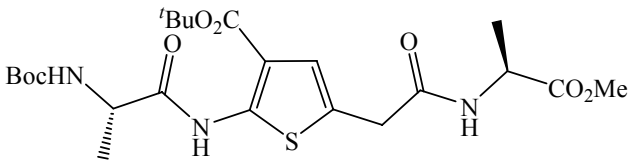
E 58 (IV 176)

Starting amounts:

0.069 g	(0.16 mmol) 4-(*tert*-Butoxycarbonyl)-5-{[*N*-(*tert*-butoxycarbonyl)-L-alanyl]amino}thien-2-yl)acetic acid (**120**)
0.026 g	(0.19 mmol) L-Ala-OMe·HCl
0.082 g	(0.19 mmol) BOP
0.08 ml	(0.48 mmol) DIEA
0.50 ml	CH_2Cl_2

Procedure: 4-(*tert*-Butoxycarbonyl)-5-{[*N*-(*tert*-butoxycarbonyl)-L-alanyl]amino}thien-2-yl)acetic acid (**120**), L-Ala-OMe·HCl and BOP were dissolved in dry dichloromethane, and then DIEA was added. The reaction mixture was stirred at room temperature for 30 min. Ethyl acetate and water were added and the layers were separated. The organic layer was successively washed with saturated $NaHCO_3$ solution, brine and water, dried with Na_2SO_4 and the solvent was removed under reduced pressure.

Purification: Column chromatography on silica gel with 30% ethyl acetate/ dichloromethane
Yield: 58 mg (71 %) of **121** as yellow oil



121

Optical rotation: $[\alpha]_D^{20}$ = -100.0 (c = 0.05, MeOH).

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.38 (d, *J* = 7.3 Hz, 3 H, Me), 1.48 (d, *J* = 7.4 Hz, 3 H, Me), 1.56, 1.57 (2 s, 9 H, 9 H, C*Me$_3$*), 3.64 (s, 2 H, CH_2), 3.73 (s, 3 H, OMe), 4.44 (q, *J* = 7.4 Hz, 1 H, CH), 4.58 (q, *J* = 7.3 Hz, 1 H, CH), 5.29, 6.13 (2 bs, 2 H, NH), 7.01 (s, 1 H, 4-H), 11.52 (s, 1 H, NH).

^{13}C-NMR (125.8 MHz, $CDCl_3$): δ = 18.4, 18.2 (2 q, Me), 28.4, 28.5 (2 q, C*Me$_3$*), 37.3 (t, CH_2), 40.9, 41.7 (2 d, CH), 52.5 (q, OMe), 80.9, 81.1 (2 s, *C*Me$_3$), 110.2 (s, C-5), 123.2 (d, C-4), 126.9 (s, C-2), 153.0 (s, C-3), 151.7, 164.7, 167.7, 171.3 (4 s, C=O).

IR (KBr): ν = 3300 cm^{-1} (N-H), 2980–2850 (C-H), 1745 (C=O), 1670 (C=O), 1560, 1530, 1455 (CS-NH).

MS (EI, 80 eV, 200 °C): *m/z* (%) = 513 (38, $[M]^+$), 401 (19), 384 (12), 342 (15), 287 (24), 286 (100), 271 (15), 204 (12), 183 (11), 157 (21), 156 (95), 138 (11), 59 (12), 57 (43), 56 (18), 44 (52), 43 (10), 41 (32), 29 (12), 28 (22).
HRMS (EI, 80 eV) *m/z* calculated for [M^+, $C_{23}H_{35}N_3O_8S$]: 513.21448, found: 513.21562.

7.4.4 TFFH mediated syntheses of tetra- and hexapeptide analogues

Synthesis of *tert*-Butyl 2-[(*tert*-Butoxycarbonyl)amino]-5-(2-{[3-(*tert*-butoxycarbonyl)-5-(2-methoxy-2-oxoethyl)thien-2-yl]amino}-2-oxoethyl)thiophene-3-carboxylate (122)

Method A

E 59 (IV 373)

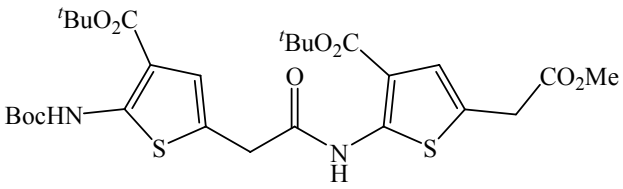
Starting amounts:

0.266 g	(0.74 mmol) {4-(*tert*-Butoxycarbonyl)-5-[(*tert*-butoxycarbonyl)amino]thien-2-yl}acetic acid (**118**)
0.202 g	(0.74 mmol) *tert*-Butyl 2-Amino-5-(2-methoxy-2-oxoethyl)thiophene-3-carboxylate (**111d**)
0.297 g	(1.13 mmol) TFFH
0.44 ml	DIEA
6 ml	CH_2Cl_2

Procedure: The acid **118**, amine **111d** and DIEA were dissolved in dichloromethane, cooled in an ice bath and TFFH was added. The temperature was allowed to rise to room temperature and then the reaction mixture was stirred over 6 days. The reaction mixture was successively washed with 1 M HCl, saturated $NaHCO_3$ solution and brine, dried with $MgSO_4$ and the solvent was removed under reduced pressure.

Purification: Column chromatography on silica gel with hexane/ethyl acetate 4:1

Yield: 325 mg (72 %) of **122** as colourless solid



122

Melting range: 78–85 °C

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.44, 1.45, 1.49 (3 s, 9 H, 9 H, 9 H, C*Me*$_3$), 3.62 (s, 2 H, CH_2), 3.64 (s, 3 H, OMe), 3.76 (s, 2 H, CH_2), 6.88, 7.00 (2 s, 1 H, 1 H, 4-H), 10.01, 11.14 (2 s, 1 H, 1 H, NH).
^{13}C-NMR (125.8 MHz, $CDCl_3$): δ = 28.2, 28.3, 28.4 (3 q, C*Me*$_3$), 35.0, 37.9 (2 t, CH_2), 52.4 (q, OMe), 81.4, 81.5, 81.7 (3 s, *C*Me$_3$), 112.7, 114.4 (2 s, C-5), 123.6, 124.9 (2 d, C-4), 125.1, 125.2 (2 s, C-2), 150.5, 151.4 (2 s, C-3), 147.3, 164.7, 166.6, 169.4, 170.6 (5 s, C=O).

IR (KBr): ν = 3290 cm^{-1} (N-H), 3000–2850 (C-H), 1745 (C=O), 1720 (C=O), 1670 (C=O), 1560, 1535, 1455 (CS-NH).

MS (EI, 80 eV, 200 °C): *m/z* (%) = 610 (4, $[M]^+$), 339 (21), 283 (20), 271 (21), 239 (23), 227 (28), 215 (58), 184 (20), 183 (100), 157 (11), 156 (54), 139 (11), 138 (26), 57 (26), 56 (12), 44 (12), 41 (26).
HRMS (EI, 80 eV) *m/z* calculated for $[M]^+$: 610.20190, found: 610.20355.

$C_{28}H_{38}N_2O_9S_2$ (610.7)	calc.	C 55.07	H 6.27	N 4.59
	found	C 54.58	H 5.94	N 4.36

´Methode B

Starting amounts:

0.055 g	(0.15 mmol) {4-(*tert*-Butoxycarbonyl)-5-[(*tert*-butoxycarbonyl)amino]thien-2-yl}acetic acid (**118**)
0.049 g	(0.15 mmol) *tert*-Butyl 2-amino-5-(2-methoxy-2-oxoethyl)thiophene-3-carboxylate (**111d**)
0.024 g	(0.18 mmol) HOBt
0.037 g	(0.18 mmol) DCC
2 ml	CH_2Cl_2

Procedure: To a stirred solution of the acid **118** and amine **111d** in dichloromethane, DCC and HOBt were added at room temperature. The resulting solution was stirred over 10 days at room temperature, and then the solvent was evaporated. The residue was suspended in ethyl acetate and filtered through Büchner funnel. The solvent was evaporated.

Purification: Column chromatography on silica gel with hexane/ethyl acetate 4:1, then HPLC (20 % *i*-propanol/hexane, 64 ml/min, 124 bar)
Yield: 14 mg (15 %) of **122** as colourless solid

Synthesis of *tert*-Butyl 2-Amino-5-(2-{[3-(*tert*-butoxycarbonyl)-5-(2-methoxy-2-oxoethyl)thien-2-yl]amino}-2-oxoethyl)thiophene-3-carboxylate (123)

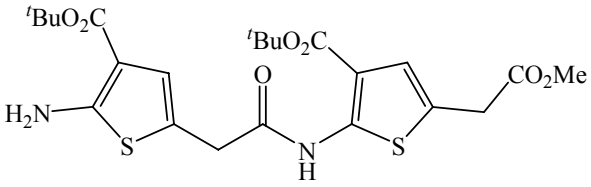
E 61 (IV 300)

Starting amounts:

0.127 g	(0.21 mmol) *tert*-Butyl 2-[(*tert*-butoxycarbonyl)amino]-5-(2-{[3-(*tert*-butoxycarbonyl)-5-(2-methoxy-2-oxoethyl)thien-2-yl]amino}-2-oxoethyl)thiophene-3-carboxylate (**122**)
0.047 g	(0.21 mmol) Me_3SiOTf
0.045 g	(0.42 mmol) 2,6-Lutidine
6 ml	CH_2Cl_2

Procedure: To a solution of N-Boc protected compound **122** and 2,6-lutidine in dry dichloromethane, Me_3SiOTf was added dropwise at room temperature. The reaction mixture was stirred for 30 minutes, quenched with saturated aqueous ammonium chloride solution and extracted with diethyl ether several times. The combined organic phases were washed with water and brine, dried with $MgSO_4$ and concentrated under reduced pressure. The crude silyl carbamate was dissolved in dry methanol, stirred for 20 minutes at room temperature and the solvent was evaporated.

Purification: Column chromatography on silica gel with hexane/ethyl acetate 1:2, then HPLC (10 % *i*-propanol/hexane, 64 ml/min, 80 bar)
Yield: 25 mg (23 %) of **123** as colourless solid
18 mg (14 %) of **122** was recovered as colourless solid



123

Melting range: 87–93 °C

^{1}H-NMR (500 MHz, CD_3OD): δ = 1.56, 1.57 (2 s, 9 H, 9 H, C*Me*$_3$), 3.74 (s, 3 H, OMe), 3.78, 3.81 (2 s, 2 H, 2 H, CH_2), 6.88, 6.97 (2 s, 1 H, 1 H, 4-H).
^{13}C-NMR (125.8 MHz, CD_3OD): δ = 28.9, 29.2 (2 q, C*Me*$_3$), 35.6, 38.2 (2 t, CH_2), 53.1 (q, OMe), 81.3, 83.2 (2 s, *C*Me$_3$), 116.0, 116.1 (2 s, C-5), 125.0, 127.3 (2 d, C-4), 128.4 (s, C-2), 148.4, 148.5 (2 s, C-3), 163.7 (s, C-2), 166.1, 166.8, 170.0, 173.0 (4 s, C=O).

IR (KBr): ν = 3445–3270 cm^{-1} (N-H), 3000–2930 (C-H), 1740 (C=O), 1670 (C=O), 1560, 1530, 1455 (CS-NH).

MS (EI, 80 eV, 170 °C): *m/z* (%) = 510 (5, $[M]^+$), 239 (23), 215 (20), 183 (73), 156 (55), 138 (28), 85 (13), 59 (15), 57 (28), 56 (43), 55 (20), 44 (36), 43 (17), 41 (100), 40 (10), 39 (42), 29 (18), 28 (26), 27 (19).

HRMS (EI, 80 eV) *m/z* calculated for [M^+, $C_{23}H_{30}N_2O_7S_2$]: 510.14944, found: 510.14799.

Synthesis of {4-(*tert*-Butoxycarbonyl)-5-[({4-(*tert*-butoxycarbonyl)-5-[(*tert*-butoxycarbonyl)amino]thien-2-yl}acetyl)amino]thien-2-yl}acetic acid (124)

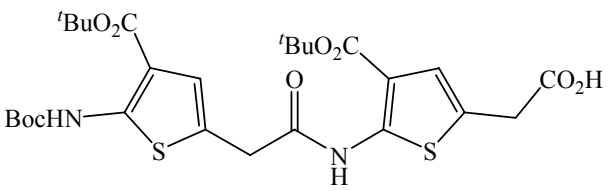
E 62 (IV 308)

Starting amounts:

0.228 g	(0.37 mmol) *tert*-Butyl 2-[(*tert*-butoxycarbonyl)amino]-5-(2-{[3-(*tert*-butoxycarbonyl)-5-(2-methoxy-2-oxoethyl)thien-2-yl]amino}-2-oxoethyl)thiophene-3-carboxylate (**122**)
0.047 g	(0.12 mmol) $LiOH \cdot H_2O$
1.5 ml	H_2O
1.5 ml	MeOH
4.5 ml	THF

Procedure: The ester **122** was dissolved in a mixture of methanol and tetrahydrofuran, a solution of LiOH in water was added, and the resulting mixture was stirred over night at room temperature. 2 M HCl was added to adjust pH 7. Diethyl ether was added and the layers were separated. The aqueous layer was extracted with diethyl ether, combined organic phases were dried with $MgSO_4$ and the solvent was evaporated.

Purification: Column chromatography on silica gel with hexane/ethyl acetate 4:1, then methanol/dichloromethane 4:1
Yield: 216 mg (98 %) of **124** as colourless solid



124

Melting point: 171–173 °C

^{1}H-NMR (500 MHz, CD_3OD): δ = 1.53, 1.55, 1.56 (3 s, 9 H, 9 H, 9 H, C*Me*$_3$), 3.67, 3.68 (2 s, 2 H, 2 H, CH_2), 6.63, 6.90 (2 s, 1 H, 1 H, 4-H).
^{13}C-NMR (125.8 MHz, CD_3OD): δ = 28.6, 28.7, 28.8 (3 q, C*Me*$_3$), 35.6, 35.7 (2 t, CH_2), 82.8, 82.9, 83.0 (3 s, *C*Me$_3$), 113.6, 113.7 (2 s, C-5), 124.9, 126.1 (2 d, C-4), 126.2, 127.7 (2 s, C-2), 150.1, 153.5 (2 s, C-3), 148.3, 166.2, 169.3, 174.3, 183.7 (5 s, C=O).

IR (KBr): ν = 3385–3290 cm^{-1} (N-H, O-H), 2980–2930 (C-H), 1725 (C=O), 1670 (C=O), 1560, 1535, 1480 (CS-NH), 1250 (C-O).

MS (EI, 80 eV, 180 °C): *m/z* (%) = 596 (0.2, [M]$^+$), 183 (11), 156 (12), 57 (22), 56 (69), 55 (27), 44 (66), 41 (100), 40 (11), 39 (45).
HRMS (EI, 80 eV) *m/z* calculated for [M$^+$, $C_{27}H_{36}N_2O_9S_2$]: 596.18622, found: 596.18729.

Synthesis of [5-({[5-Amino-4-(*tert*-butoxycarbonyl)thien-2-yl]acetyl}amino)-4-(*tert*-butoxycarbonyl)thien-2-yl]acetic acid (125)

E 63 (IV 394)

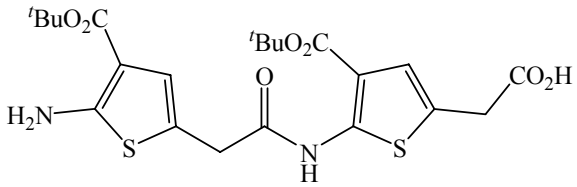
Starting amounts:

0.066 g	(0.11 mmol) {4-(*tert*-Butoxycarbonyl)-5-[({4-(*tert*-butoxycarbonyl)-5-[(*tert*-butoxycarbonyl)amino]thien-2-yl}acetyl)amino]thien-2-yl}acetic acid **124**
0.047 g	(0.22 mmol) Me_3SiOTf
0.036 g	(0.33 mmol) 2,6-Lutidine
5 ml	CH_2Cl_2

Procedure: To a solution of N-Boc protected compound **124** and 2,6-lutidine in dry dichloromethane, Me_3SiOTf was added dropwise at room temperature. The reaction mixture was stirred for 2 hours, quenched with saturated aqueous ammonium chloride solution and extracted with diethyl ether several times. The combined organic phases were washed with water and brine, dried with $MgSO_4$ and concentrated under reduced pressure. The crude silyl carbamate was dissolved in dry methanol, stirred for 2 hours at room temperature and the solvent was evaporated.

Purification: Column chromatography on silicagel with hexane/ethyl acetate 4:1, then methanol/dichloromethane 4:1, then HPLC (30 % *i*-propanol/hexane, 64 ml/min, 95 bar); after purification compound still contained some impurities which were visible in NMR and MS.

Yield: 51 mg (93 %) of **125** as pale yellow solid



125

Melting range: 180–185 °C

^{1}H-NMR (500 MHz, [D$_6$]DMSO): δ = 1.63, 1.65 (2 s, 9 H, 9 H, C*Me$_3$*), 3.85, 3.95 (2 s, 2 H, 2 H, CH_2), 7.16, 7.18 (2 s, 1 H, 1 H, 4-H), 11.07 (s, 1 H, NH).
^{13}C-NMR (125.8 MHz, [D$_6$]DMSO): δ = 28.9, 29.2 (2 q, C*Me$_3$*), 35.3, 37.0 (2 t, CH_2), 82.5, 82.7 (2 s, *C*Me$_3$), 116.7, 116.8 (2 s, C-5), 125.5, 127.8 (2 d, C-4), 136.2 (s, C-2), 146.1, 146.2 (2 s, C-3), 164.6 (s, C-2), 179.2, 183.2, 189.3 (3 s, 2 C=O).

IR (KBr): ν = 3430–3230 cm^{-1} (N-H, O-H), 3100–2930 (C-H), 1720 (C=O), 1670 (C=O), 1560, 1540, 1435 (CS-NH), 1250 (C-O).

MS (EI, 80 eV, 180 °C): *m/z* (%) = 496 (2, [M]$^+$), 183 (11), 339 (10), 228 (10), 227 (17), 184 (19), 183 (32), 170 (12), 157 (10), 156 (18), 138 (14), 91 (13), 59 (21), 58 (11), 57 (46), 56 (100), 55 (39), 53 (12), 51 (11), 50 (12).
HRMS (EI, 80 eV) *m/z* calculated for [M$^+$, $C_{22}H_{28}N_2O_7S_2$]: 496.13379, found: 496.13482.

Synthesis of *tert*-Butyl 2-[(*tert*-Butoxycarbonyl)amino]-5-(2-{[3-(*tert*-butoxycarbonyl)-5-(2-{[3-(*tert*-butoxycarbonyl)-5-(2-methoxy-2-oxoethyl)thien-2-yl]amino}-2-oxoethyl)thien-2-yl]amino}-2-oxoethyl)thiophene-3-carboxylate (126)

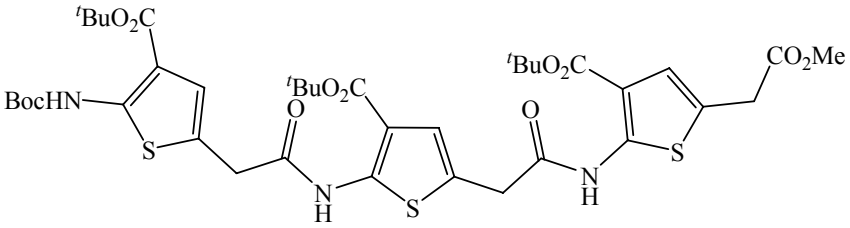
E 64 (IV 372)

Starting amounts:

0.079 g	(0.13 mmol) {4-(*tert*-Butoxycarbonyl)-5-[({4-(*tert*-butoxycarbonyl)-5-[(*tert*-butoxycarbonyl)amino]thien-2-yl}acetyl)amino]thien-2-yl}acetic acid (**124**)
0.035 g	(0.13 mmol) *tert*-Butyl 2-amino-5-(2-methoxy-2-oxoethyl)thiophene-3-carboxylate (**111d**)
0.044 g	(0.17 mmol) TFFH
0.07 ml	DIEA
2 ml	CH_2Cl_2

Procedure: The acid **124,** amine **111d** and DIEA were dissolved in dichloromethane, cooled in an ice bath and TFFH was added. The temperature was allowed to rise to room temperature and then the reaction mixture was stirred over 6 days. The reaction mixture was successively washed with 1 M HCl, saturated $NaHCO_3$ solution and brine, dried with $MgSO_4$ and the solvent was removed under reduced pressure.

Purification: Column chromatography on silica gel with hexane/ethyl acetate 4:1
Yield: 51 mg (46 %) of **126** as colourless solid



126

Melting point: 197-199 ° C

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.49, 1.50, 1.51, 1.54 (4 s, 9 H, 9 H, 9 H, 9 H, C*Me*$_3$), 3.66 (s, 2 H, CH_2), 3.69 (s, 3 H, OMe), 3.80, 3.83 (2 s, 2 H, 2 H, CH_2), 6.92, 7.05, 7.06 (3 s, 1 H, 1 H, 1 H, 4-H), 10.06, 11.17, 11.23 (3 s, 1 H, 1 H, 1 H, NH).
^{13}C-NMR (125.8 MHz, $CDCl_3$): δ = 28.2, 28.3, 28.4, 28.5 (4 q, C*Me*$_3$), 35.0, 35.4, 36.9 (3 t, CH_2), 52.3 (q, OMe), 81.4, 81.7 (2 s, *C*Me$_3$), 112.7, 114.8, 114.9 (3 s, C-5), 121.7, 121.9, 122.0 (3 d, C-4), 122.9, 123.6, 123.7 (3 s, C-2), 149.2, 150.1, 150.8 (3 s, C-3), 147.2, 164.7, 167.9, 168.7, 170.1, 172.1, 173.9 (7 s, C=O).

IR (KBr): ν = 3435–3200 cm^{-1} (N-H), 2975–2930 (C-H), 1715 (C=O), 1675 (C=O), 1565, 1530, 1450 (CS-NH).

MS (FAB (+)): *m/z* (%) = 850 (0.6, $[M + H]^+$), 818 (0.5, $[M – CH_3O]^+$), 199 (10), 183 (17), 182 (26), 158 (13), 157 (40), 156 (71), 138 (12), 57 (100).
MS (EI, 80 eV, 200 °C): *m/z* (%) = 749 (0.02, $[M^+ - CO_2CMe_3]$), 56 (60), 55 (25), 44 (44), 41 (100), 40 (12), 39 (49).
HRMS (EI, 80 eV) *m/z* calculated for $[M^+ - CO_2CMe_3, C_{34}H_{43}N_3O_{10}S_3]$: 749.21106, found: 749.21342.

Synthesis of {4-(*tert*-Butoxycarbonyl)-5-[({4-(*tert*-butoxycarbonyl)-5-[({4-(*tert*-butoxycarbonyl)-5-[(*tert*-butoxycarbonyl)amino]thien-2-yl}acetyl)amino]thien-2-yl}acetyl)amino]thien-2-yl}acetic acid (127)

E 65 (IV 378)

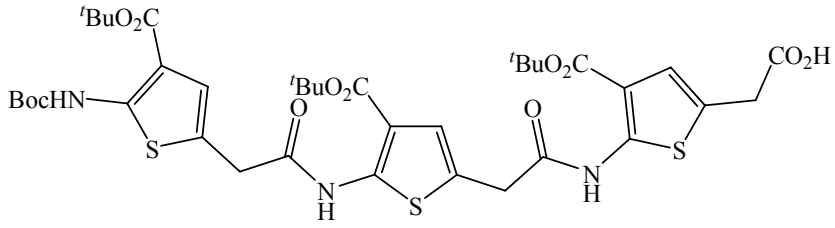
Starting amounts:

0.036 g	(0.042 mmol) *tert*-Butyl 2-[(*tert*-butoxycarbonyl)amino]-5-(2-{[3-(*tert*-butoxycarbonyl)-5-(2-{[3-(*tert*-butoxycarbonyl)-5-(2-methoxy-2-oxoethyl)thien-2-yl]amino}-2-oxoethyl)thien-2-yl]amino}-2-oxoethyl)thiophene-3-carboxylate (**126**)
0.005 g	(0.12 mmol) $LiOH \cdot H_2O$
0.5 ml	H_2O
0.5 ml	MeOH
1.5 ml	THF

Procedure: The ester **126** was dissolved in a mixture of methanol and tetrahydrofuran, a solution of LiOH in water was added, and the resulting mixture was stirred over night at room temperature. 2 M HCl was added to adjust pH 7. Diethyl ether was added and the layers were separated. The aqueous layer was extracted with diethyl ether, the combined organic phases were dried with $MgSO_4$ and the solvent was evaporated.

Purification: Column chromatography on silica gel with hexane/ethyl acetate 4:1, then methanol/dichloromethane 4:1; after purification compound still contained some impurities which were visible in NMR and MS.

Yield: 35 mg (98 %) of **127** as yellow solid



127

Melting point: >300 °C

^{1}H-NMR (500 MHz, CD_3OD): δ = 1.50, 1.51, 1.52, 1.54 (4 s, 9 H, 9 H, 9 H, 9 H, C*Me*$_3$), 3.40, 3.52, 3.60 (3 s, 2 H, 2 H, 2 H, CH_2), 6.60, 6.84, 6.85 (3 s, 1 H, 1 H, 1 H, 4-H).
^{13}C-NMR (125.8 MHz, CD_3OD): δ = 29.0, 29.1, 29.2, 29.3 (4 q, C*Me*$_3$), 39.6, 39.8, 39.9 (3 t, CH_2), 81.0, 82.7, 82.8, 83.5 (4 s, *C*Me$_3$), 113.8, 114.1, 115.1 (3 s, C-5), 123.7, 123.9 (2 d, C-4), 124.3 (s, C-2), 125.0 (d, C-4), 129.6, 129.9 (2 s, C-2), 150.57, 156.7, 156.8 (3 s, C-3), 145.9, 166.5, 166.6, 178.8, 178.9, 179.5, 179.7 (7 s, C=O).

IR (KBr): ν = 3435–3285 cm^{-1} (N-H, O-H), 2980–2850 (C-H), 1720 (C=O), 1675 (C=O), 1560, 1530, 1455 (CS-NH), 1250 (C-O).

MS (FAB (+)): *m/z* (%) = 834 (< 1, [M$^+$ - H]), 818 (< 1, [M$^+$ - OH]), 808 (< 1), 537 (< 1), 435 (< 1), 281 (1), 242 (2), 227 (< 1), 163 (10), 162 (10), 112 (11), 91 (9), 60 (21), 59 (10), 56 (11), 55 (11), 44 (100).
MS (EI, 80 eV, 190 °C): *m/z* (%) = 514 (<1), 413 (< 1), 340 (< 1), 284 (8), 257 (7), 241 (4), 201 (23), 184 (13), 183 (15), 157 (15), 156 (32), 138 (14), 60 (10), 59 (14), 57 (28), 56 (100), 53 (13), 51 (10).

Synthesis of [5-({[5-({[5-Amino-4-(*tert*-butoxycarbonyl)thien-2-yl]acetyl}amino)-4-(*tert*-butoxycarbonyl)thien-2-yl]acetyl}amino)-4-(*tert*-butoxycarbonyl)thien-2-yl]acetic acid (128)

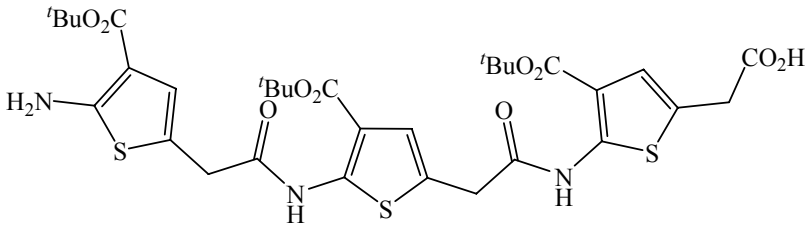
E 66 (IV 384)

Starting amounts:

0.039 g	(0.05 mmol) {4-(*tert*-Butoxycarbonyl)-5-[({4-(*tert*-butoxycarbonyl)-5-[({4-(*tert*-butoxycarbonyl)-5-[(*tert*-butoxycarbonyl)amino]thien-2-yl}acetyl)amino]thien-2-yl}acetyl)amino]thien-2-yl}acetic acid (**127**)
0.020 g	(0.09 mmol) Me_3SiOTf
0.015 g	(0.14 mmol) 2,6-Lutidine
2 ml	CH_2Cl_2

Procedure: To a solution of N-Boc protected compound **127** and 2,6-lutidine in dry dichloromethane, Me_3SiOTf was added dropwise at room temperature. The reaction mixture was stirred for 2 hours, quenched with saturated aqueous ammonium chloride solution and extracted with diethyl ether several times. The combined organic phases were washed with water and brine, dried with $MgSO_4$ and concentrated under reduced pressure. The crude silyl carbamate was dissolved in dry methanol, stirred for 2 hours at room temperature and the solvent was evaporated.

Purification: Column chromatography on silica gel with hexane/ethyl acetate 4:1, then methanol/dichloromethane 4:1; after purification compound still contained some impurities which were visible in NMR and MS.
Yield: 29 mg (86 %) of **128** as yellow solid



128

Melting range: 95-100 °C

^{1}H-NMR (500 MHz, CD_3OD): δ = 1.51, 1.52, 1.54 (3 s, 9 H, 9 H, 9 H, C*Me$_3$*), 3.56, 3.59, 3.63 (3 s, 2 H, 2 H, 2 H, CH_2), 6.84, 6.99, 7.06 (3 s, 1 H, 1 H, 1 H, 4-H).
^{13}C-NMR (125.8 MHz, CD_3OD): δ = 28.3, 28.4, 28.5 (3 q, C*Me$_3$*), 35.0, 35.2, 37.1 (3 t, CH_2), 80.2, 81.4, 81.7 (3 s, *C*Me$_3$), 108.0, 114.4, 115.8 (3 s, C-5), 123.6, 124.9, 125.7 (3 d, C-4), 147.3 (s, C-3), 162.0 (s, C-2), 166.7, 170.7, 171.1 (4 s, C=O).
missing signals for C-2 and C-3 were not possible unambiguously to determine; ^{13}C spectrum was of poor quality because of very poor solubility of the substance in CD_3OD.

IR (KBr): ν = 3440–3285 cm^{-1} (O-H, N-H), 2980–2855 (C-H), 1725 (C=O), 1675 (C=O), 1560, 1540, 1450 (CS-NH), 1250 (C-O).

MS (FAB (+)): *m/z* (%) = 523 (< 1), 469 (< 1), 408 (< 1), 320 (< 1), 209 (< 1), 217 (1), 176 (23), 154 (52), 136 (48), 107 (21), 91 (83), 57 (100), 48 (22).
MS (EI, 80 eV, 200 °C): *m/z* (%) = 357 (<1), 257 (< 1), 245 (< 1), 201 (< 1), 183 (< 1), 156 (< 1), 138 (< 1), 184 (14), 156 (43), 138 (28), 112 (9), 84 (13), 56 (100).
Fragmentation of the compound is in accordance with the structure.

Synthesis of *tert*-Butyl 2-[(3-{3-[(*tert*-Butoxycarbonyl)amino]imidazo[1,2-*a*]pyridin-2-yl}-3-methylbutanoyl)amino]-5-(2-methoxy-2-oxoethyl)thiophene-3-carboxylate (130)

E 67 (IV 307)

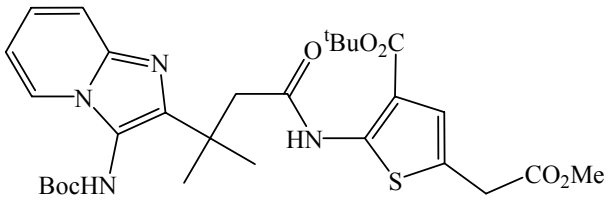
Starting amounts:

0.153 g	(0.46 mmol) 3-{3-[(*tert*-Butoxycarbonyl)amino]imidazo[1,2-*a*]pyridin-2-yl}-3-methylbutanoic acid (**96**)
0.124 g	(0.46 mmol) *tert*-Butyl 2-amino-5-(2-methoxy-2-oxoethyl)thiophene-3-carboxylate (**111d**)
0.158 g	(0.60 mmol) TFFH
0.23 ml	(1.38 mmol) DIEA
5 ml	CH_2Cl_2

Procedure: The acid **96**, amine **111d** and DIEA were dissolved in dichloromethane, cooled in an ice bath and TFFH was added. The temperature was allowed to rise to room temperature

and then the reaction mixture was stirred over 48 hours. The reaction mixture was successively washed with 1 M HCl, saturated $NaHCO_3$ solution and brine, dried with $MgSO_4$ and the solvent was removed under reduced pressure.

Purification: Column chromatography on silica gel with hexane/ethyl acetate 1:2, then methanol/dichloromethane 4:1, then HPLC (50 % *i*-propanol/hexane, 64 ml/min, 120 bar)
Yield: 38 mg (14 %) of **130** as brown oil



130

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.16, 1.18, 1.46, 1.47 (4 s, 3 H, 3 H, 9 H, 9 H, Me, C*Me$_3$*), 2.79, 3.59 (2 s, 2 H, 2 H, CH_2), 3.68 (s, 3 H, OMe), 6.84 (s, 1 H, 4-H), 6.91 (t, *J* = 6.7 Hz, 1 H, 6'-H), 7.22–7.36 (m, 1 H, 7'-H), 7.49 (d, *J* = 9.2 Hz, 1 H, 8'-H), 7.87 (d, *J* = 6.7 Hz, 1 H, 5'-H).

^{13}C-NMR (125.8 MHz, $CDCl_3$): δ = 28.0 (q, Me), 28.1, 28.3 (2 q, C*Me$_3$*), 29.6 (q, Me), 35.6, 39.7 (2 t, CH_2), 52.0 (q, OMe), 80.1, 81.9 (2 s, *C*Me$_3$), 113.2 (d, C-6'), 114.1 (s, C-3), 116.3 (d, C-8'), 122.2 (s, C-3'), 122.4 (d, C-5'), 126.4 (d, C-7'), 126.7 (d, C-4), 128.7, 129.6 (2 s, C-2, C-2'), 140.3 (s, C-8a'), 146.9 (s, C=O), 153.8 (s, C-5), 161.5, 162.5, 173.6 (3 s, C=O).

IR (KBr): ν = 3235 cm^{-1} (N-H), 2970–2855 (C-H), 1785 (C=O), 1740 (C=O), 1670 (C=O), 1560, 1525, 1455 (CS-NH).

MS (EI, 80 eV, 150 °C): *m/z* (%) = 586 (0.14, $[M]^+$), 513 (0.2, $[M^+ - C_3H_5O_2]$), 486 (0.4, $[M^+ - C_5H_8O_2]$), 260 (14), 216 (20), 215 (65), 200 (100), 78 (22).

HRMS (EI, 80 eV) *m/z* calculated for $[M^+, C_{29}H_{38}N_4O_7S]$: 586.24609, found: 586.24733.
m/z calculated for $[M^+ - C_3H_5O_2, C_{26}H_{33}N_4O_5S]$: 513.21716, found: 513.21682.