Supporting Information

Novel Fmoc-Polyamino Acids for Solid-Phase Synthesis of Defined Polyamidoamines

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Experimental Section

General:

All reactions were performed under a dry atmosphere of nitrogen. All chemicals and solvents purchased from Sigma-Aldrich or IRIS Biotech were used without further purification, unless otherwise stated. Rink amide MBHA resin was purchased from Novabiochem, Fmoc-(L)-Lys(fmoc)-OH and Fmoc-(L)-Trp(boc)-OH were purchased from IRIS Biotech GmbH, Germany. ^{1}H NMR and ^{13}C NMR spectra were recorded using a Jeol JNMR-GX 400 (400 MHz) or JNMR-GX 500 (500 MHz) in $CDCl_3$ or D_2O. The spectra were analyzed using MestreNova (Ver. 5.2.5-4119 by Mestrelab Research). Reactions were monitored by thin layer chromatography using silica gel 60 sheets F254 purchased from Merck, Darmstadt, Germany. The ESI-HRMS were measured on a ThermoScientific LTQ-FT Mass Spectrometer or on a Bruker Maxis ESI. MALDI-MS analysis was performed on a Bruker Autoflex MALDI-TOF.

Experimental Procedures:

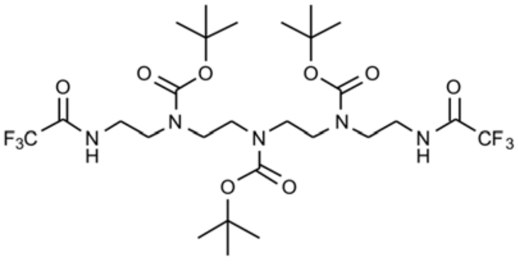
General Procedure for the Synthesis of (3) and (4)

Tetraethylenepentamine (Tp) **1** (10.18 g, 53.8 mmol, 1 eq, technical grade) or triethylenetetramine (Tt) **2** (7.86 g, 53.8 mmol, 1 eq) were weighed in a 1 L round bottom flask and dissolved in 500 mL DCM. The mixture was cooled down to 0°C. Trifluoroacetic ethyl ester (13.45 mL, 16.05 g, 112.6 mmol, 2.1 eq.) was diluted in 220 mL DCM and transferred into a dropping funnel. It was added dropwise to the cooled mixture in the round bottom flask over 2.5 h. After complete addition of the trifluoroacetic ethyl ester the reaction was stirred for an additional hour at room temperature.

Di-*tert*-butyl dicarbonate (47 g, 215.3 mmol, 4 eq.) was dissolved in 80 mL DCM and added dropwise over one hour. Afterwards 30 mL of triethylamine (21.8 g, 215.2 mmol, 4 eq) were added and the mixture was stirred over night.

The organic phase was reduced to approximately 150 mL and was washed three times with saturated sodium bicarbonate, 5 % sodium citrate solution and finally three times with water. The organic phase was dried over sodium sulfate and the solvent was evaporated to a yellowish viscous, waxy solid. The oily residue was recrystallized: Therefore it was dissolved in the minimal amount of boiling DCM (60 mL). Then *n*-hexane (140 mL) was added slowly to the boiling DCM till clouding was observed at the drop-in site. The crystallization solution was stored over night in a refrigerator at 4°C. The microcrystalline residue was filtered, washed with cooled *n*-hexane and dried.

Spectral and Analytical Data for Tert-butyl N-[2-[tert-butoxycarbonyl-[2-[tert-butoxycarbonyl-[2-[(2,2,2-trifluoroacetyl)amino]ethyl]amino]ethyl]amino]ethyl]-N-[2-[(2,2,2-trifluoroacetyl)amino]ethyl]carbamate (3)

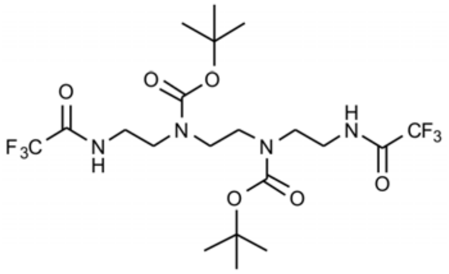


Yield: 30.4 g, 83%

^{1}H NMR (500 MHz, CDCl$_3$, 24 °C): δ = 8.34-8.20 (d, 1H, J = 56Hz, N$\underline{H}$), 8.00-7.92 (d, 1H, J = 32Hz, N$\underline{H}$), 3.31-3.47 (m, 16H, C$\underline{H}_2$), 1.44 (s, 27H, OC(C$\underline{H}_3$)$_3$) ppm

HRMS (ESI): Calculated for $C_{27}H_{45}F_6N_5O_8$, 681.6681, found: 682.3268, [M+H].

Spectral and Analytical Data for Tert-butyl N-[2-[tert-butoxycarbonyl-[2-[(2,2,2-trifluoroacetyl)amino]ethyl]amino]ethyl]-N-[2-[(2,2,2-trifluoroacetyl) amino] ethyl]carbamate (4)



Yield: 18.8 g, 65%

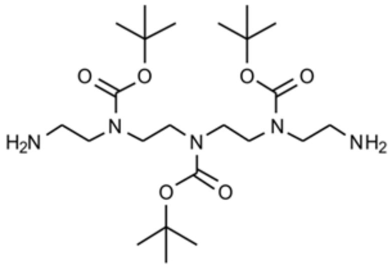
^{1}H NMR (500 MHz, CDCl$_3$, 24 °C): δ = 9.17 (sb, 1 H, N$\underline{H}$), 7.86 (sb, 1 H, N$\underline{H}$), 3.29-3.54 (m, 12H, C$\underline{H}_2$), 1.45-1.39 (m, 18H, OC(C$\underline{H}_3$)$_3$) ppm

HRMS (ESI): Calculated for $C_{20}H_{32}F_6N_4O_6$, 538.4838, found: 539.2312, [M+ H].

General Procedure for the Synthesis of (5) and (6)

To a suspension of compound **3** or **4** (22.1 mmol) in ethanol (150 mL) a 3 M aqueous sodium hydroxide solution (150 mL) was slowly added (30 min) under stirring. The reaction mixture was stirred for 20 h, the ethanol was evaporated and the aqueous phase was extracted with DCM (3x150 mL). The organic phase was dried over sodium sulfate and the solvent was evaporated, yielding a semi solid viscous liquid which was dried under high vacuum and solidified on storage at 4 °C.

Spectral and Analytical Data of tert-butyl N-(2-aminoethyl)-N-[2-[2-(2-aminoethyl(tert-butoxycarbonyl)amino)ethyl-tert-butoxycarbonyl-amino]ethyl]carbamate (5)

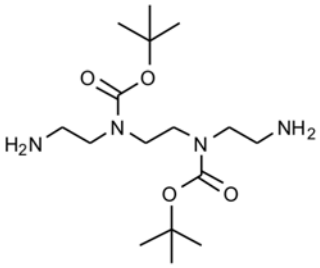


Yield: 10.7 g, 99%

^{1}H NMR (400 MHz, $CDCl_3$, 50 °C): δ = 3.21-3.41 (m, 12 H, $C\underline{H}_2$), 2.79-2.96 (t, J= 5.1Hz, 4H, $C\underline{H}_2$), 2.36-2.58 (bs, 4H, $N\underline{H}_2$), 1.40-1.50 (m, 27H, $OC(C\underline{H}_3)_3$) ppm

HRMS (ESI): Calculated for $C_{23}H_{47}N_5O_6$, 489.6516, found: 490.3610, [M+ H].

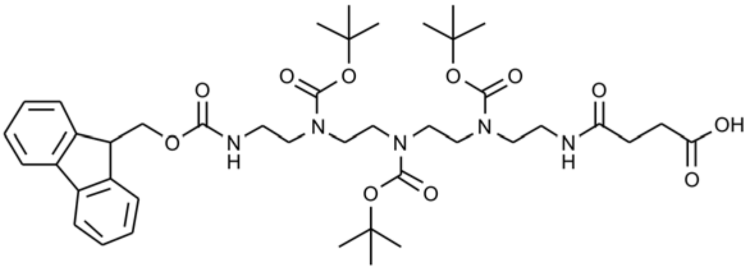
Spectral and Analytical Data of tert-butyl N-(2-aminoethyl)-N-[2-(2-aminoethyl(tert-butoxycarbonyl)amino)ethyl]carbamate (6)



Yield: 4.6 g, 60%

^{1}H NMR (400 MHz, $CDCl_3$, 50 °C): δ = 3.19-3.28 (m, 8 H, $C\underline{H}_2$), 2.78 (t, J= 6.52 Hz, 4H, $C\underline{H}_2$), 1.39 (s, 18H, $OC(C\underline{H}_3)_3$) ppm

Synthesis of 4-[2-[tert-butoxycarbonyl-[2-[tert-butoxycarbonyl-[2-[tert-butoxycarbonyl-[2-(9H-fluoren-9-ylmethoxycarbonylamino)ethyl]amino]ethyl] amino]ethyl]amino]ethylamino]-4-oxo-butanoic acid (7), Fmoc-Stp(boc)$_3$-OH



9.0 g of **3** (18.36 mmol, 1 eq) were dissolved in 100 mL of THF and cooled to -75 °C. 2.2 g (22 mmol, 1.1 eq) of succinic anhydride were dissolved in 200 mL THF and added dropwise over the course of 2 h. The reaction was stirred for an additional hour at -75 °C and then for 1 h at RT. DIPEA (7.11 g, 55.22 mmol, 3 eq) were added and the reaction mixture cooled to 0 °C. Fmoc-OSu (9.25 g, 27.42 mmol, 1.5 eq) was dissolved in a mixture of MeCN : THF (80 mL : 30 mL). This solution was added dropwise to the reaction mixture at 0 °C and stirred over night. The solution was concentrated to approximately 200 mL, mixed with 200 mL DCM and was washed 5 x with 0.1 M sodium citrate buffer (pH 5.2). The organic phase was dried over sodium bicarbonate, concentrated and purified by DCVC using a n-Heptane/EtOAc gradient to elute Fmoc-byproducts, followed by a EtOAc/MeOH gradient to isolate the product.

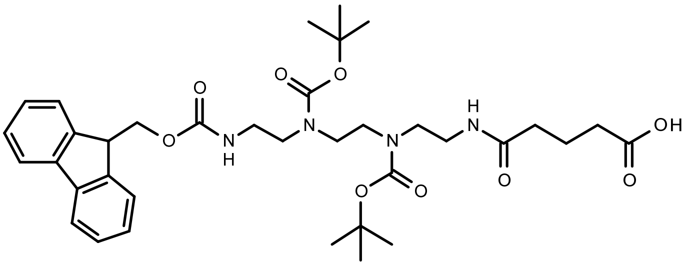
Yield: 6.84 g, 46 %

^{1}H NMR (400 MHz, $CDCl_3$, 50 °C): δ 7.67 (d, 2H, J=8Hz, Ar$\underline{H}$-Fmoc), 7.50 (d, 2H, J=8Hz, Ar$\underline{H}$-Fmoc), 7.29 (t, 2H, J=8Hz, Ar$\underline{H}$-Fmoc), 7.22 (t, 2H, J=8Hz, Ar$\underline{H}$-Fmoc), 4.30 (m, 2H, C$\underline{H}_2$-Fmoc), 4.11 (m, 1H, C$\underline{H}$-Fmoc), 3.20-3.25 (m, 12H, 8xC$\underline{H}_2$-Tepa), 2.57 (t, 2H, C$\underline{H}_2$-Suc), 2.39 (bm, 2H, C$\underline{H}_2$-suc), 1.44 (s, 27H, C$\underline{H}_3$-*tert*-but).

$^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$, 50 °C): δ 171.0, 155.3, 144.0, 141.4, 127.7, 127.0, 125.0, 119.9, 80.5, 60.3, 52.6, 47.4, 45.9, 30.7, 28.5, 21.0, 14.1

HRMS (ESI): Calculated for $C_{42}H_{61}N_5O_{11}$, 811.4367, found: 810.4318, [M-H].

Synthesis of 5-[2-[tboc-[2-[tboc-[2-(9H-fluoren-9-ylmethoxycarbonylamino) ethyl]amino]ethyl]amino]ethylamino]-5-oxo-pentanoic acid (8); Fmoc-Gtt(boc)$_2$-OH



Compound **4** (4.33 g, 12.5 mmol, 1 eq) was dissolved in ACN:THF (100:25 mL) at room temperature and cooled to -70 °C, followed by dropwise addition of a glutaric anhydride solution (1.57 g, 13.8 mmol, 1.1 eq) in ACN (25 mL) over 20 minutes. The reaction mixture was kept at -75 °C for additional 10 minutes, was then brought to room temperature and stirred for further 60 minutes to obtain a semi-solid mass. DIPEA (4.84 g, 37.5 mmol, 3 eq) was added and the mixture cooled to 0 °C while stirring. Fmoc-OSu (6.32 g, 18.75 mmol, 1.5 eq) was dissolved in acetonitrile (125 mL) and added dropwise (over the course of 1 h) at 0 °C. After stirring for additional 20 minutes at 0 °C the mixture was brought to room temperature and stirred over night. The solvent was evaporated and the resulting viscous product dissolved in 350 mL DCM. The organic phase was washed with 0.1 M sodium citrate buffer (pH 5.2) for 5 times, dried over sodium sulfate and evaporated to yield crude **6**. The crude product was purified by DCVC using a n-Heptane/EtOAc gradient to elute Fmoc-by-products, followed by a EtOAc/MeOH gradient to isolate the product.

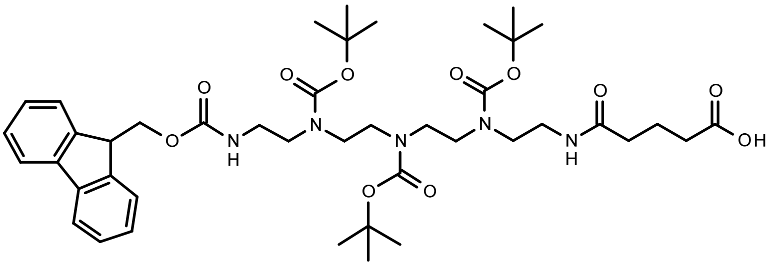
Yield: 3.58 g, 42%

^{1}H NMR (400 MHz, CDCl$_3$): δ 7.75 (d, 2H, J=8Hz, ArH-Fmoc), 7.56 (d, 2H, J=8Hz, ArH-Fmoc), 7.38 (t, 2H, J=8Hz, ArH-Fmoc), 7.28 (t, 2H, J=8Hz, ArH-Fmoc), 4.40 (m, 2H, CH$_2$-Fmoc), 4.20 (m, 1H, CH-Fmoc), 3.32-3.41 (m, 12H, 6xCH$_2$-Teta), 2.37-2.40 (m, 2H, CH$_2$-Glu), 2.26-2.29 (m, 2H, CH$_2$-Glu), 1.91-1.98 (m, 2H, CH$_2$-Glu), 1.45 (s, 27H, CH$_3$-*tert*-but).

^{13}C{^{1}H} NMR (100 MHz, CDCl$_3$): δ 173.1, 171.1, 144.1, 141.4, 127.7, 127.0, 125.0, 120.0, 80.6, 63.0, 60.3, 47.4, 28.5, 20.9, 20.5, 14.2.

HRMS (ESI): Calculated for $C_{36}H_{50}N_4O_9$, 682.3577, found: 681.3494, [M- H].

Synthesis of 5-[2-[tboc-[2-[tboc-[2-[tboc-[2-(9H-fluoren-9-ylmethoxycarbonylamino)ethyl]amino]ethyl]amino]ethyl]amino]ethylamino]-5-oxo-pentanoic acid (9); Fmoc-Gtp(boc)$_3$-OH



9.8 g of **3** (20.0 mmol, 1 eq) were dissolved in 250 mL of THF and cooled to -70 °C. 2.52 g (22 mmol, 1.1 eq) of glutaric anhydride were dissolved in 40 mL THF and added dropwise over the course of 15 min. The reaction suspension was stirred for additional 30 min at -75 °C and then for 1 h at RT. DIPEA (7.8 g, 60.0 mmol, 3.0 eq) was added to the RBF and the reaction mixture cooled to 0 °C. Fmoc-OSu (10.1 g, 30.0 mmol, 1.5 eq) was dissolved in 250 mL ACN. This solution was added dropwise to the reaction mixture at 0 °C and stirred over night. The solution was concentrated to approximately 200 mL, mixed with 200 mL of DCM and was washed 5 x with 0.1 M sodium citrate buffer (pH 5.2). The organic phase was dried over sodium bicarbonate, concentrated and purified by DCVC using an n-Heptane/EtOAc gradient to elute Fmoc by-products, followed by a EtOAc/MeOH gradient to isolate the product.

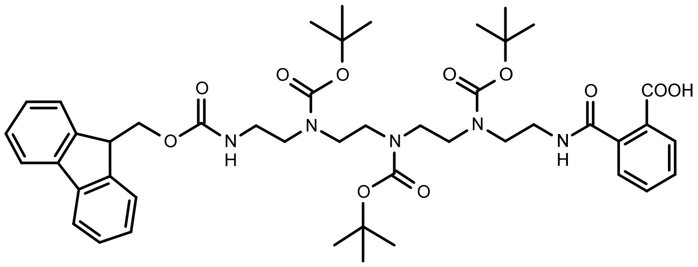
Yield: 7.42 g, 45%

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.73 (d, 2H, J=8Hz, Ar$\underline{H}$-Fmoc), 7.57 (d, 2H, J=8Hz, Ar$\underline{H}$-Fmoc), 7.34 (t, 2H, J=8Hz, Ar$\underline{H}$-Fmoc), 7.26 (t, 2H, J=8Hz, Ar$\underline{H}$-Fmoc), 4.37 (m, 2H, C$\underline{H}_2$-Fmoc), 4.17 (m, 1H, C$\underline{H}$-Fmoc), 3.30-3.43 (m, 16H, 8xC$\underline{H}_2$-Tepa), 2.35-2.38 (m, 2H, C$\underline{H}_2$-Glu), 2.20-2.24 (m, 2H, C$\underline{H}_2$-Glu), 1.91-1.95 (m, 2H, C$\underline{H}_2$-Glu), 1.43 (s, 27H, C$\underline{H}_3$-*tert*-but).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): δ 173.0, 170.8, 144.0, 141.4, 127.7, 127.0, 125.0, 119.9, 80.5, 60.3, 47.4, 45.5, 35.1, 33.0, 28.5, 20.9, 20.8, 14.2

HRMS (ESI): Calculated for $C_{43}H_{63}N_5O_{11}$, 825.4524, found: 824.4431, [M- H].

Synthesis of 2-[2-[tert-butoxycarbonyl-[2-[tert-butoxycarbonyl-[2-[tert-butoxy carbonyl-[2-(9H-fluoren-9-ylmethoxycarbonylamino)ethyl] amino]ethyl]amino] ethyl]amino]ethylcarbamoyl]benzoic acid (10); Fmoc-Ptp(boc)$_3$-OH



Compound **3** (2.45 g, 5 mmol, 1 eq) was dissolved in THF (25 mL) at room temperature and cooled to -70 ^{o}C, followed by dropwise addition of a phthalic anhydride solution (0.814g, 5.5 mmol, 1.1 eq) in ACN:THF (2:10 mL) over 30 minutes. The reaction mixture was stirred at that temperature for one hour, then brought to room temperature and stirred for an additional hour. DIPEA (1.95 g, 15 mmol, 3 eq) was added to the reaction mixture which was cooled to 0 ^{o}C. Fmoc-OSu (2.52 g, 7.5 mmol, 1.5 eq) in ACN: THF (25:10 mL) was added dropwise over one hour. The reaction mixture was stirred for additional 20 min at 0 °C, then brought to room temperature and stirred over night. The solvent was evaporated under reduced pressure and the resulting residue dissolved in 70 mL DCM. The organic phase was washed 5 x with 0.1 M sodium citrate buffer (pH 5.2). The organic phase was dried over sodium sulfate, concentrated and purified by DCVC using a n-Heptane/EtOAc gradient to elute Fmoc by-products, followed by an EtOAc/MeOH gradient to isolate the product.

Yield: 1.00 g, 24%

^{1}H NMR (400 MHz, CDCl$_3$): δ 7.92 (bs, 1H, N$\underline{H}$), 7.67 (d, 2H, J=8Hz, Ar$\underline{H}$-Fmoc), 7.51 (d, 2H, J=8Hz, Ar$\underline{H}$-Fmoc), 7.37 (s, 4H, Ar$\underline{H}$-Ph), 7.29 (t, 2H, J=8Hz, Ar$\underline{H}$-Fmoc), 7.20 (t, 2H, J=8Hz, ArH-Fmoc), 4.29 (m, 2H, $C\underline{H}_2$-Fmoc), 4.11 (m, 1H, $C\underline{H}$-Fmoc), 3.25-3.50 (m, 16H, $8xC\underline{H}_2$-Tepa), 1.27-1.36 (m, 27H, $C\underline{H}_3$-*tert*-but).

$^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ 171.0, 155.5, 144.0, 141.4, 127.7, 127.0, 125.1, 120.0, 80.8, 66.9, 60.3, 47.4, 45.8, 40.1, 28.5, 20.9, 14.2

HRMS (ESI): Calculated for $C_{46}H_{61}N_5O_{11}$, 859.4367, found: 858.4266, [M- H].

General Procedure for the Synthesis of Polyamidoamines (Sequences 11-15)

The polyamidoamines were synthesize on Rink Amide MBHA resin (100-200 mesh, loading 0.56 mmole/g) using a syringe reactor and a batch size of 25 µmole. The resin was pre-swollen for 40 minutes with DMF (peptide grade) before the first deprotection step. Fmoc was cleaved by two sequential piperidine treatments (20% piperidine in DMF, 10 min and 20 min), followed by extensive washing with DMF. Couplings of polyamine building blocks (**7**, **8, 9, 10**) or amino acids were done by dissolving of Fmoc-building block/amino acid (4 eq), PyBOP® (4 eq), HOBt (4 eq), DIPEA (8 eq) in the minimal amount of DMF, followed by transfer to the reactor. After 30 minutes the completeness of the coupling reaction was checked by Kaiser test. After the final Fmoc deprotection step the resin was washed with DMF followed by DCM washes and dried over KOH in a vacuum desiccator.

Like commonly used in standard Fmoc peptide synthesis protocols, 4 equivalents of building blocks were applied to reduce the required coupling reaction times. Pilot experiments demonstrated that 1.5-2.0 equivalents of Fmoc-Stp(boc)$_3$-OH (**7)** were sufficient for coupling, but required extended coupling times of 4-5 hrs.

Procedure for the Synthesis of Sequence (16)

EGFR targeting peptide GE11 was assembled in a fully automatic fashion using Fmoc/tBu chemistry on an Applied Biosystems 431A Peptide Synthesizer by employing the Applied Biosystems Small Scale FastMoc® protocols (100 µmol scale with 10-fold excess of amino acid building blocks and HOBT/HBTU chemistry). After successful synthesis the resin was transferred to a syringe reactor and was manually modified with two Stp units using PyBOP®/HOBt/DIPEA (4/4/8 eq) activation. After completing the sequence the resin was washed with DCM and dried in a vacuum desiccator.

Synthesis of Polyamidoamine H_2N-Stp-Stp-Stp-Lys-$CONH_2$

To prove whether the polyamidoamine assembly is compatible with automated synthesis H_2N-Stp-Stp-Stp-Lys-$CONH_2$ was successfully synthesized using the Applied Biosystems 431A Peptide Synthesizer on a 250 µmol scale (FastMoc protocols, 4 eq (1 mmol) of Stp building block and PyBOP/HOBt activation).

General Procedure for the Cleavage of Polyamidoamines from Resin (Sequences 11-16)

The dry resin was treated with a TFA: Water: TIS (95:2.5:2.5) mixture for 2-3 h (10 mL/g resin). The resin was filtered and washed three times with TFA and DCM. The combined filtrates were concentrated under reduced pressure and precipitated in ice cold MTBE. The precipitate was isolated by centrifugation and dissolved in water containing 2.5% acetic acid and lyophilized to obtain the crude product.

Products (**11-13**) were obtained with a very high crude purity of > 95% (for an exemplary ^1H NMR spectrum of **11** see **Figure S1.**

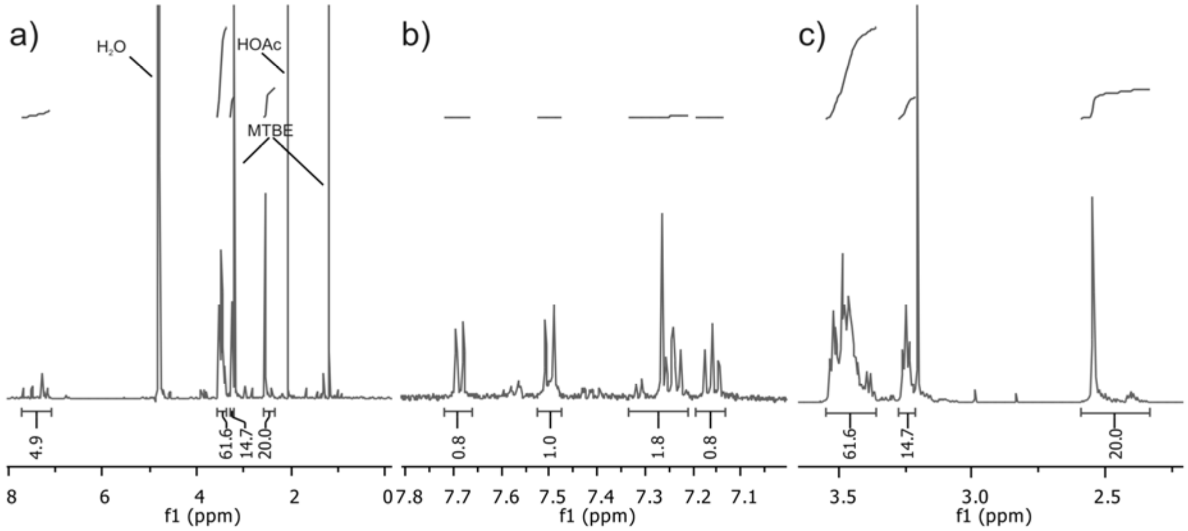
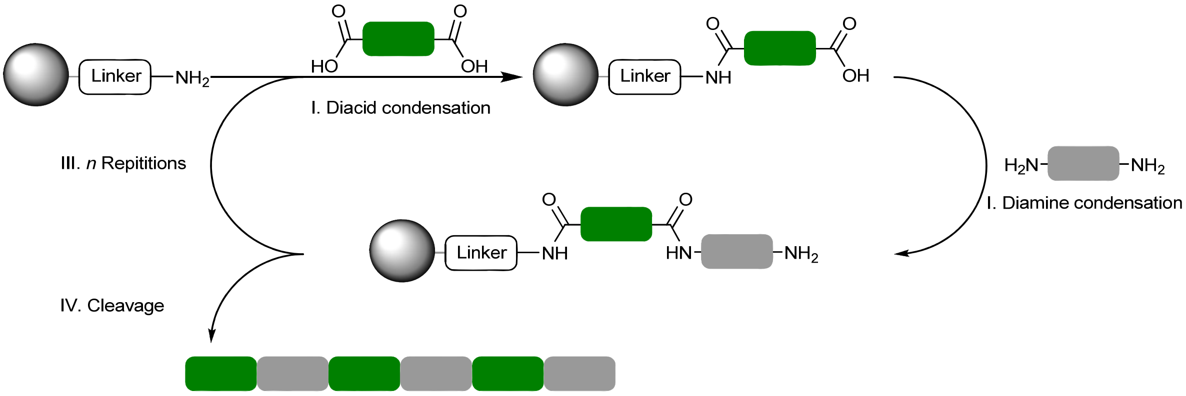


Figure S1. ^{1}H NMR spectrum of crude sequence **11** in D_2O after deprotection and cleavage from resin. a) Full spectrum. b) Aromatic protons of tryptophane c) Protons of Stp (tp CH2-N, succ CH2-CO).

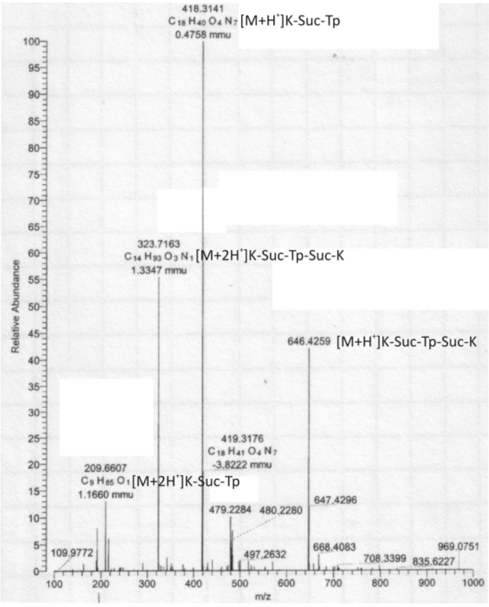
Solid-phase Synthesis using Alternating Condensation Approach:

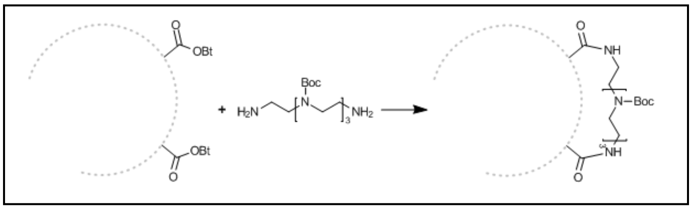


Scheme S1: PAA Synthesis using an alternating condensation approach analogously as described by Hartmann et al.[1]

Originally we attempted to apply a two step condensation synthesis strategy characterized by an alternating assembly of the diacid succinic acid and diamines, analogously as described by Hartmann et al.[1] The sequence H_2N-Tp-Succ-Tp-Succ-K-OH was chosen as simple model PAA. The building block **5**, H_2N-Tp(boc)$_3$-NH_2 was applied as diamine. In-synthesis reaction monitoring showed inconsistent results for the colorimetric assays (Malachit green assay, Kaiser test) in every step, accompanied by a drastically lowered resin mass gain (compared to calculated values). MS-analysis of the cleavage solution revealed the formation of the crosslinked product HO-K-Succ-Tp-Succ-K-OH (**Figure S2**).

The incubation of the activated carboxylic acid function with the diamine building block results in crosslinking of a large degree of the adjacent reaction sites, preventing further chain elongation. Using a Wang-Lys resin with a moderate loading (0.52 mmol/g) a product mixture of crosslinked and desired product could be identified by ESI-MS after the second coupling step, however accompanied by very low yields.





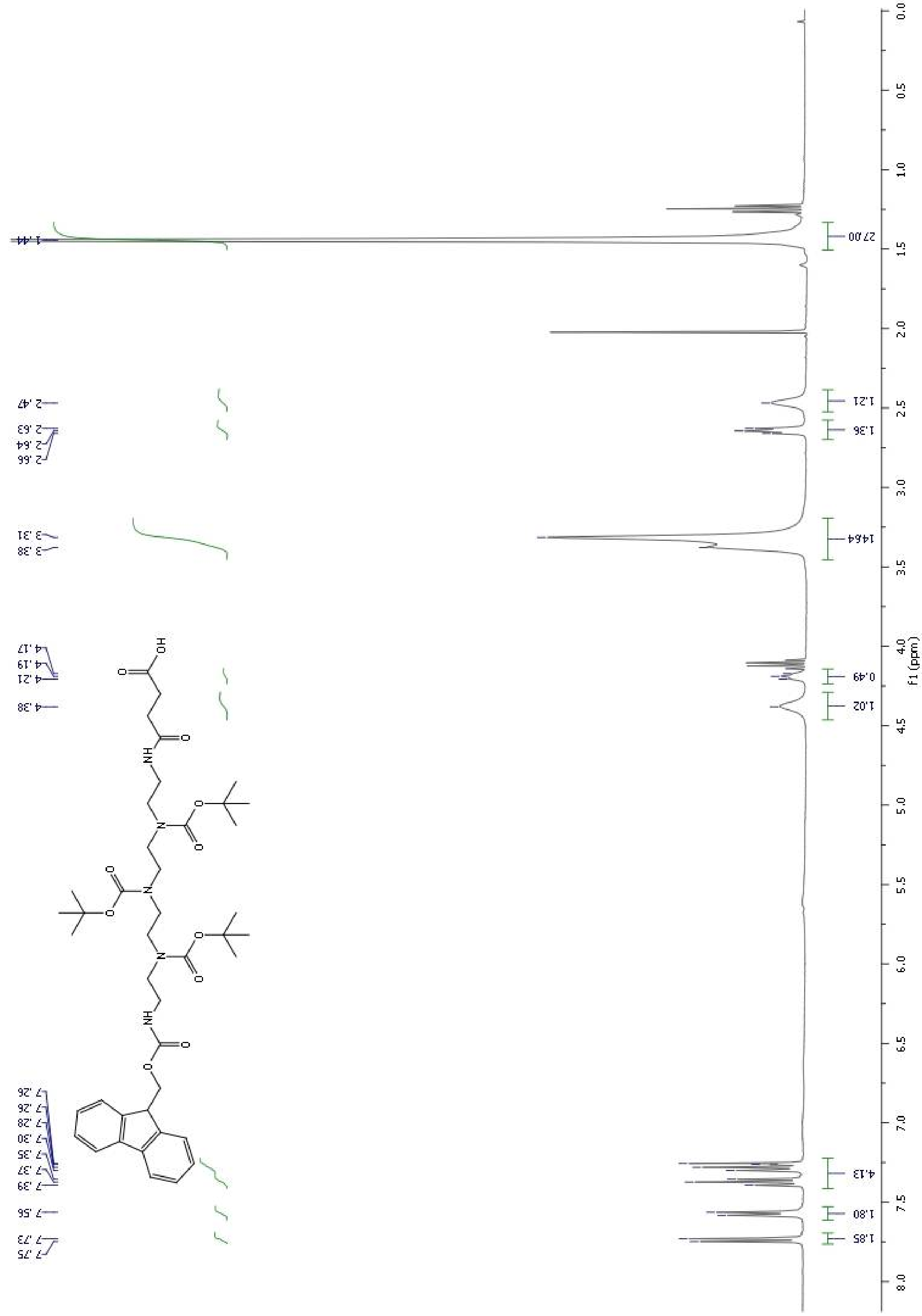
Reaction conditions	Crosslinking
PS-Wang-Lys-NH_2 10 eq	+
PS-Wang-Ala-NH_2 low load 20 eq	+
PS-Wang-Ala-NH_2 low load 10 eq DCM	+
PS-Wang-Cys(Trt)-NH_2 10 eq	+
PS-Trityl-Ethylenediamine 10 eq	+
PS-Wang-Ala-NH_2 LL 10 eq	+
Tentagel-Trt-Cys(Trt)-NH_2 LL	+

Figure S2: Left: Exemplary ESI-MS-spectrum of an alternating condensation reaction using a PS-Wang-K resin. **Upper Right:** Proposed mechanism for on resin crosslinking. **Lower right:** Formation of crosslinking product in dependency to various reaction conditions.

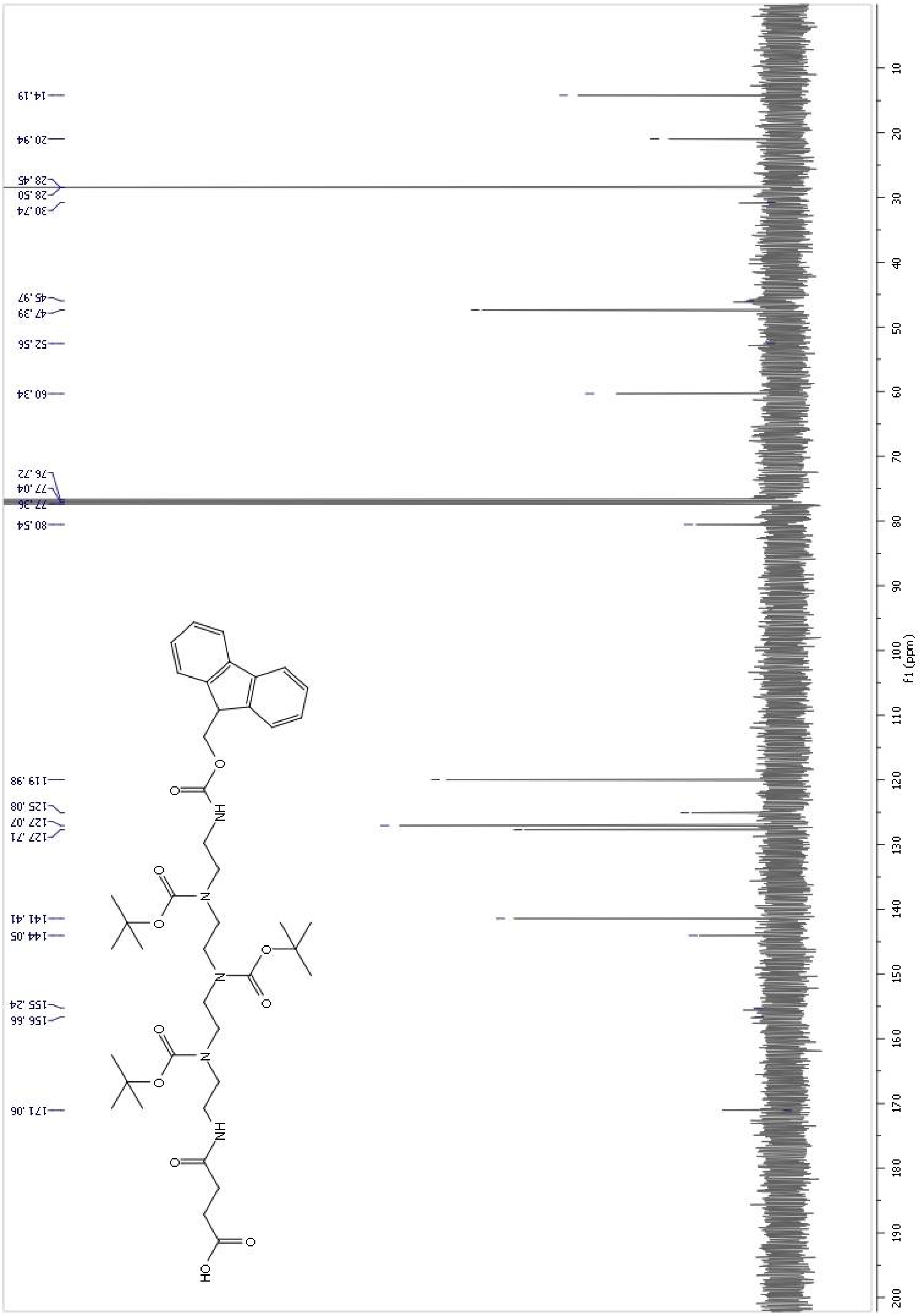
To evaluate the influence of experimental conditions on the formation of crosslinked side-products, different reaction conditions were tested. In each case the mass increase of the resin was less than predicted and colorimetric assays used for reaction monitoring showed unexpected behavior. Crosslinked product was always present. These results are consistent with literature for on-resin carboxy activation followed by coupling of unprotected diamines. Jørgensen et al.[2] describe crosslinking up to 60% (per step) of adjacent, activated carboxy sites on solid-phase using a related approach.

Thus, the synthetic strategy of alternating diacid / diamine couplings yielded no reliable results, at least in our hands using larger polyamine units.

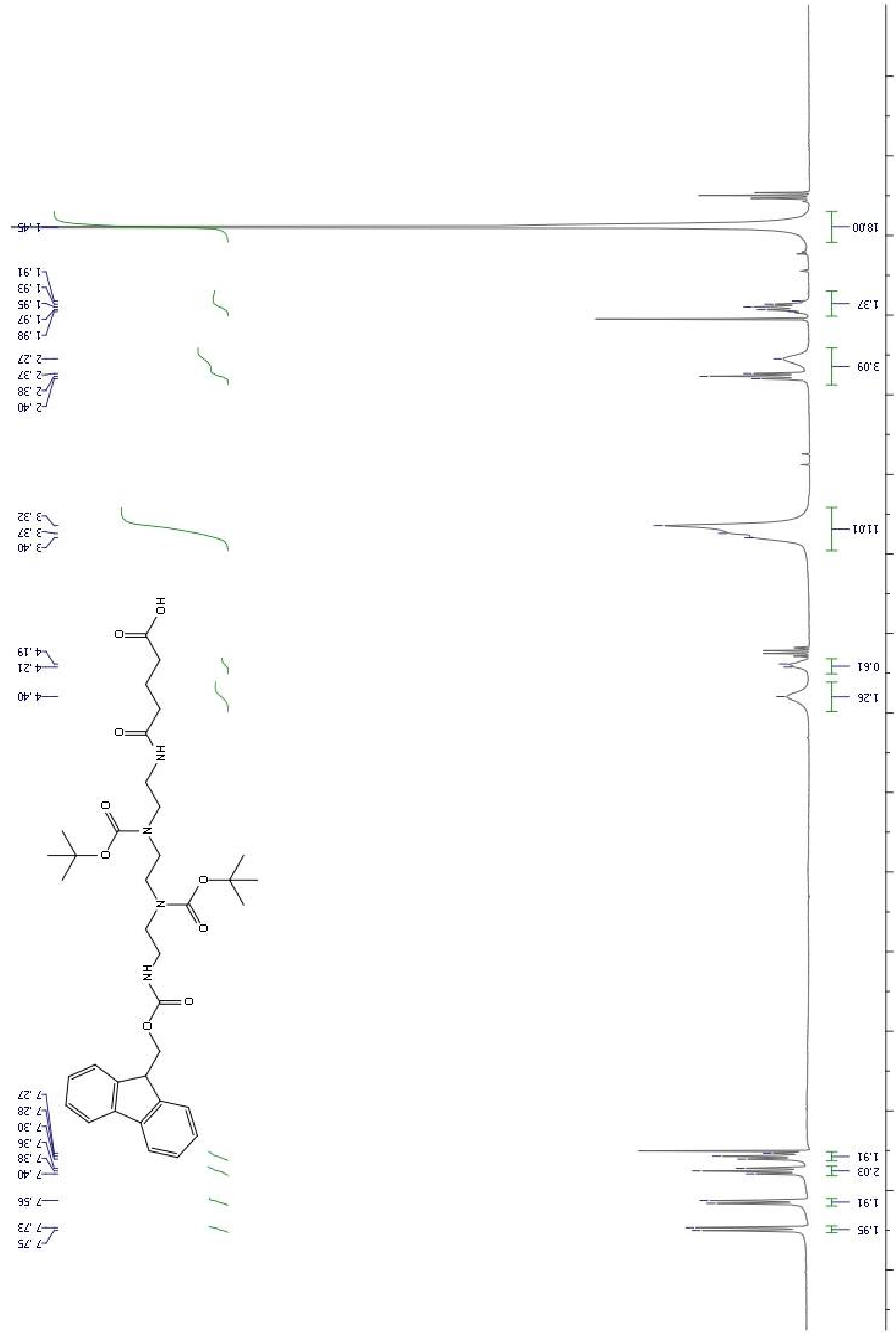
Proton NMR of Compound No. 07:



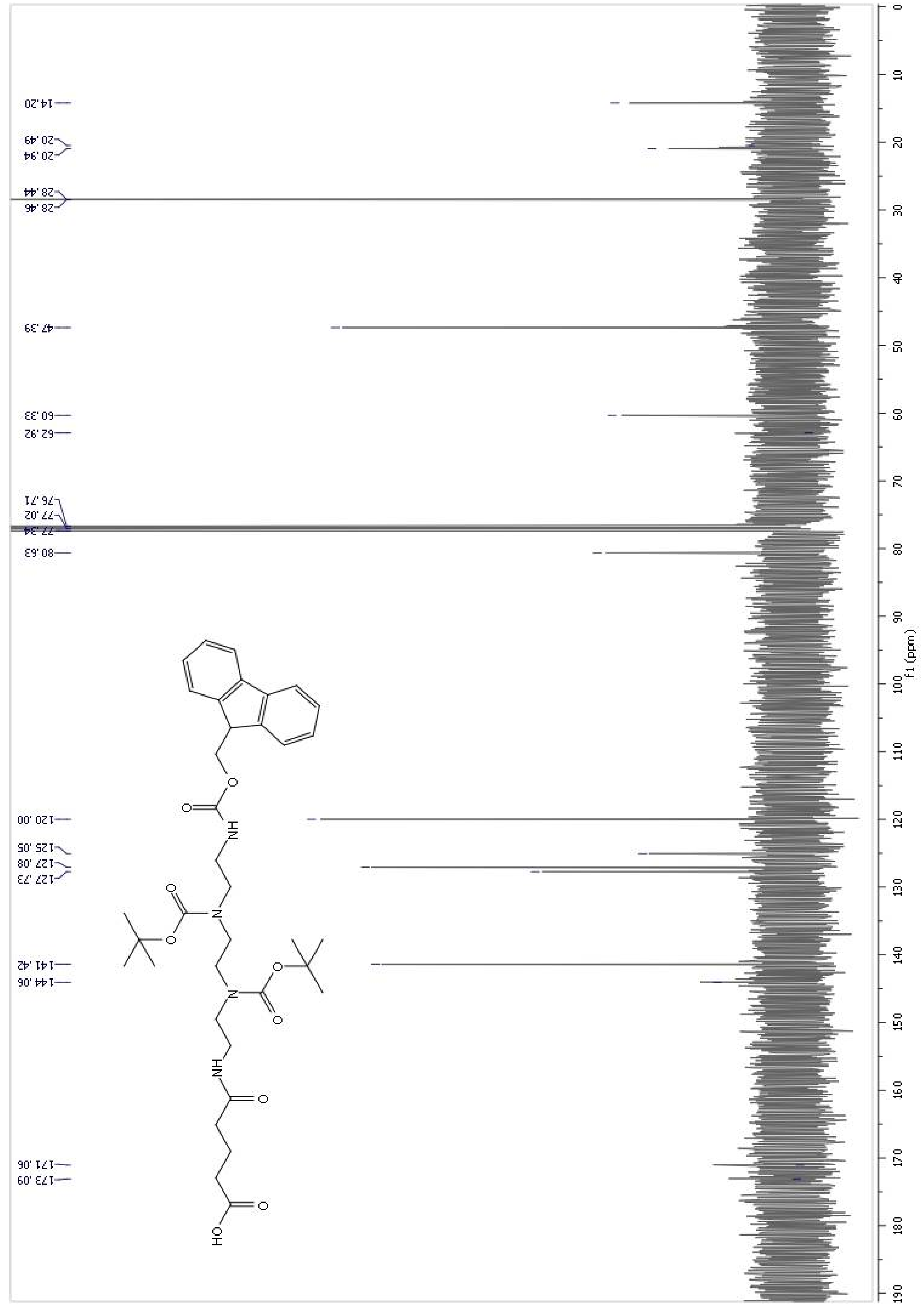
$^{13}C\{^1H\}$ NMR of Compound No. 7:



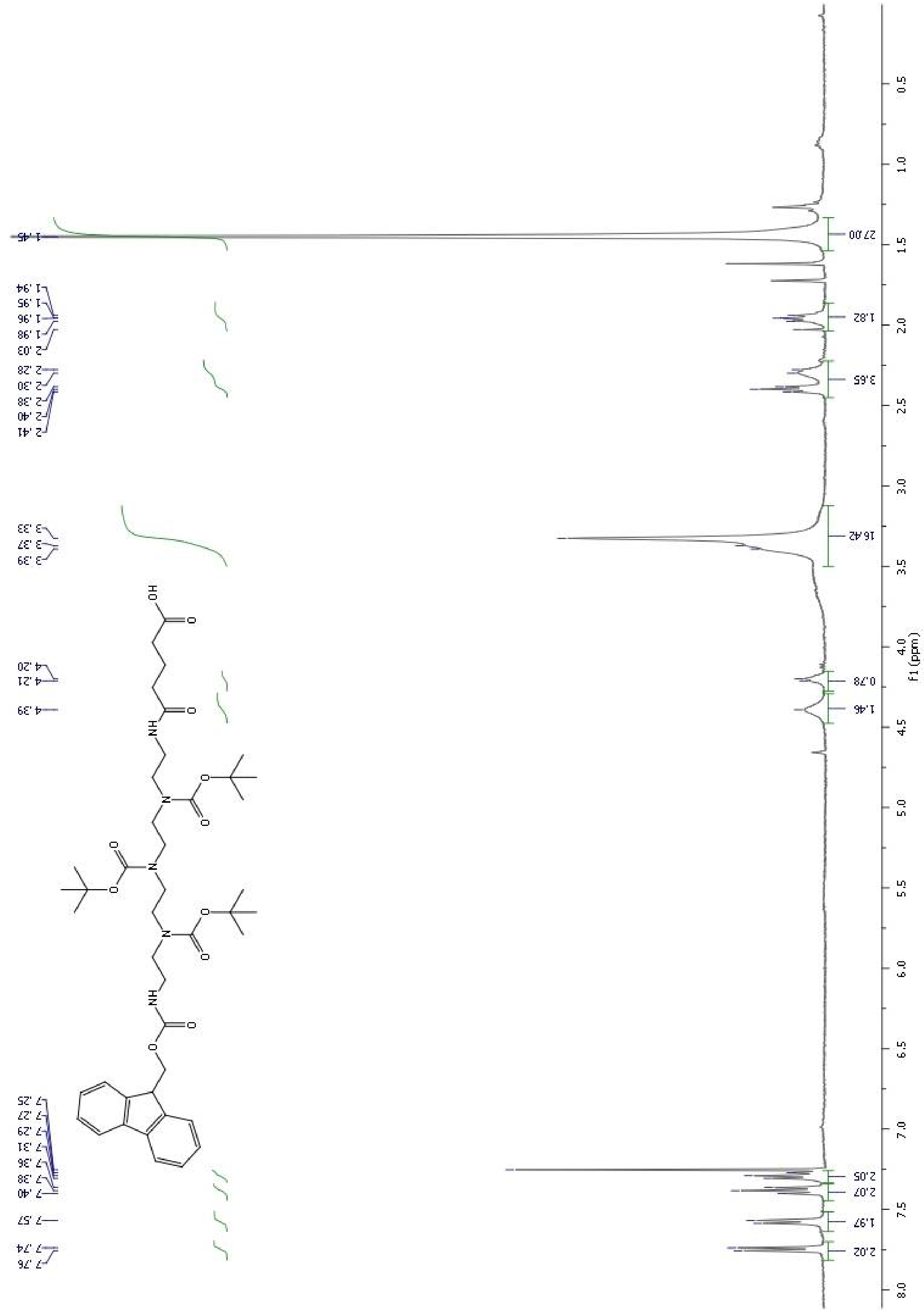
Proton NMR of Compound No. 8:



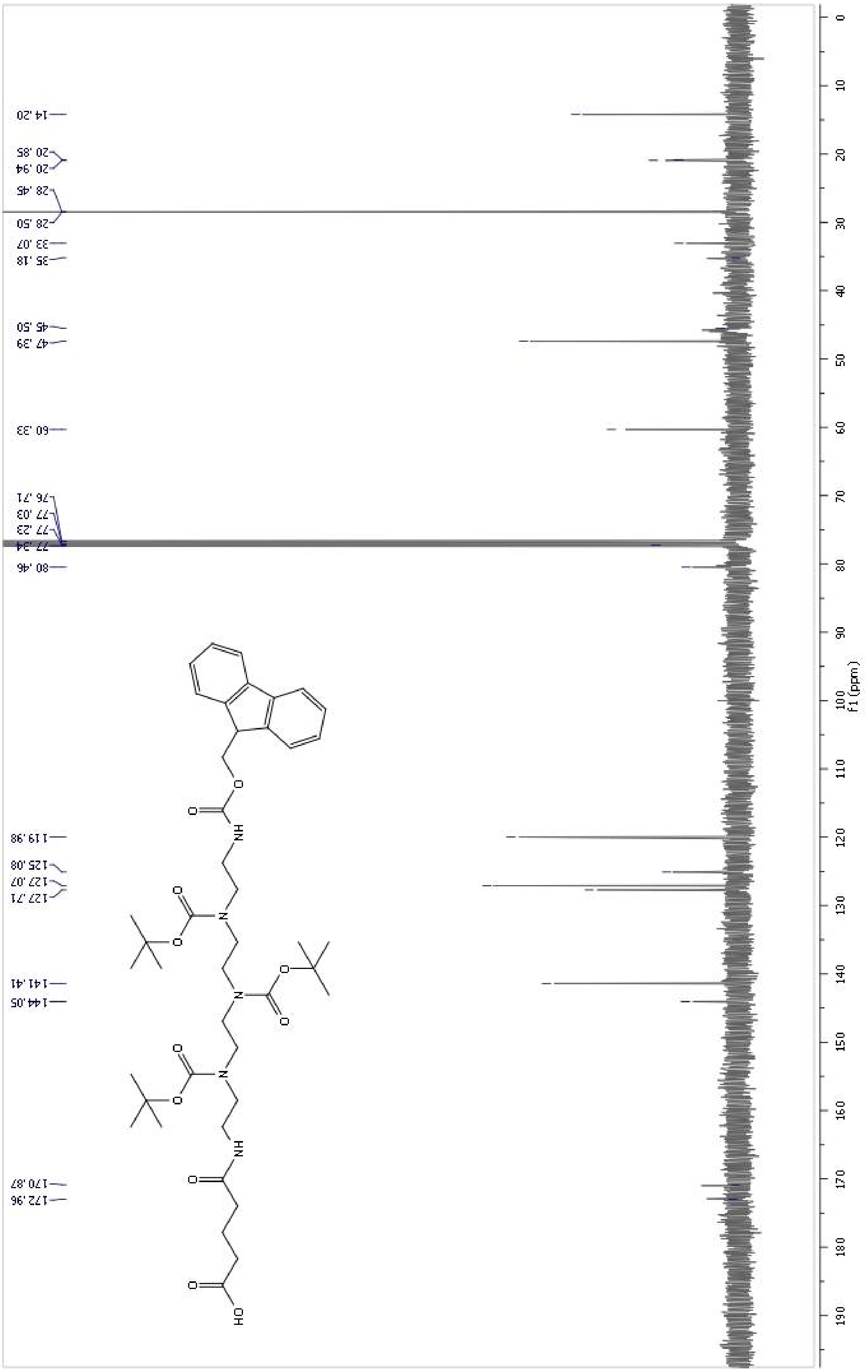
$^{13}C\{^1H\}$ NMR of Compound No. 8:



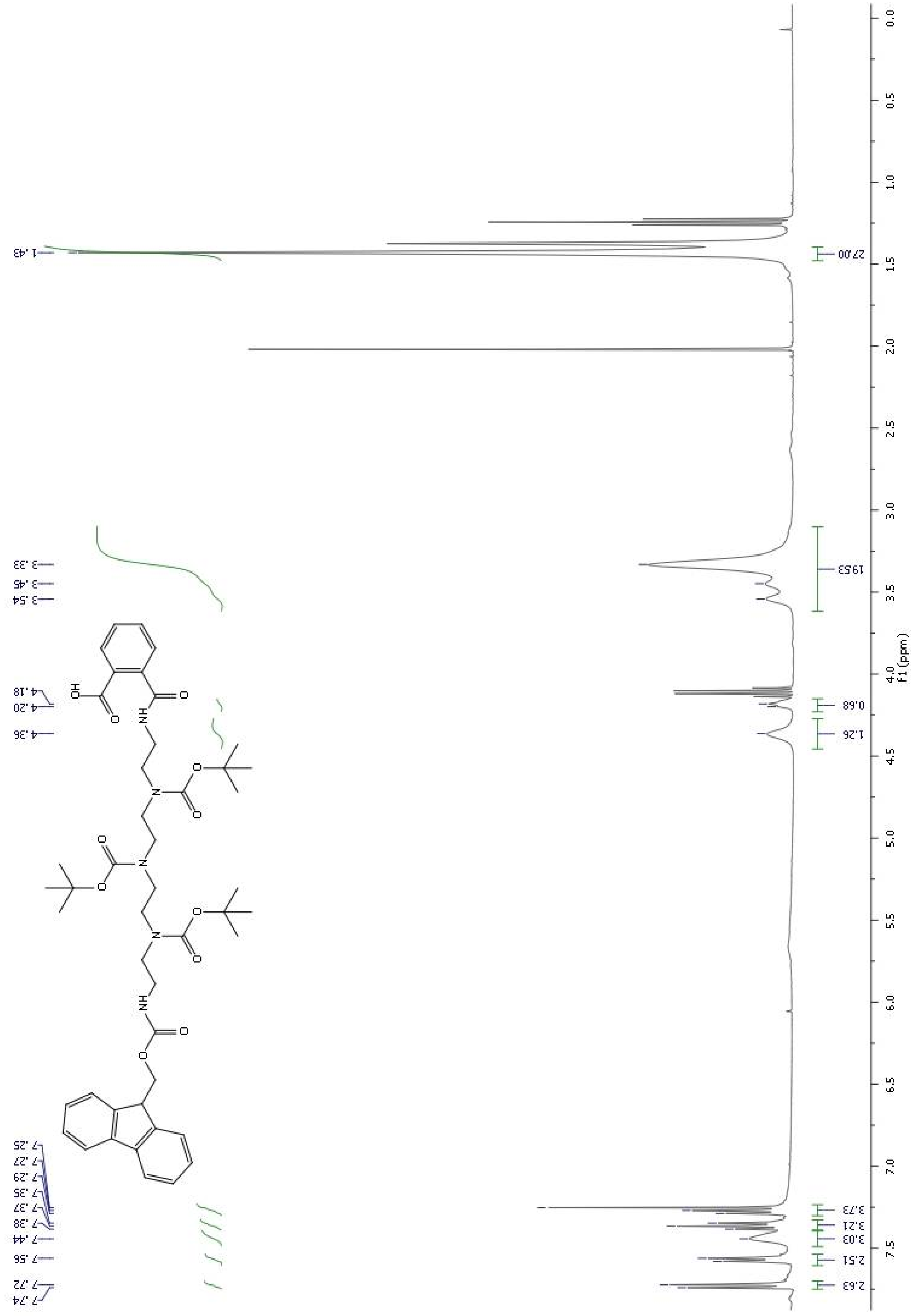
Proton NMR of Compound No. 9:



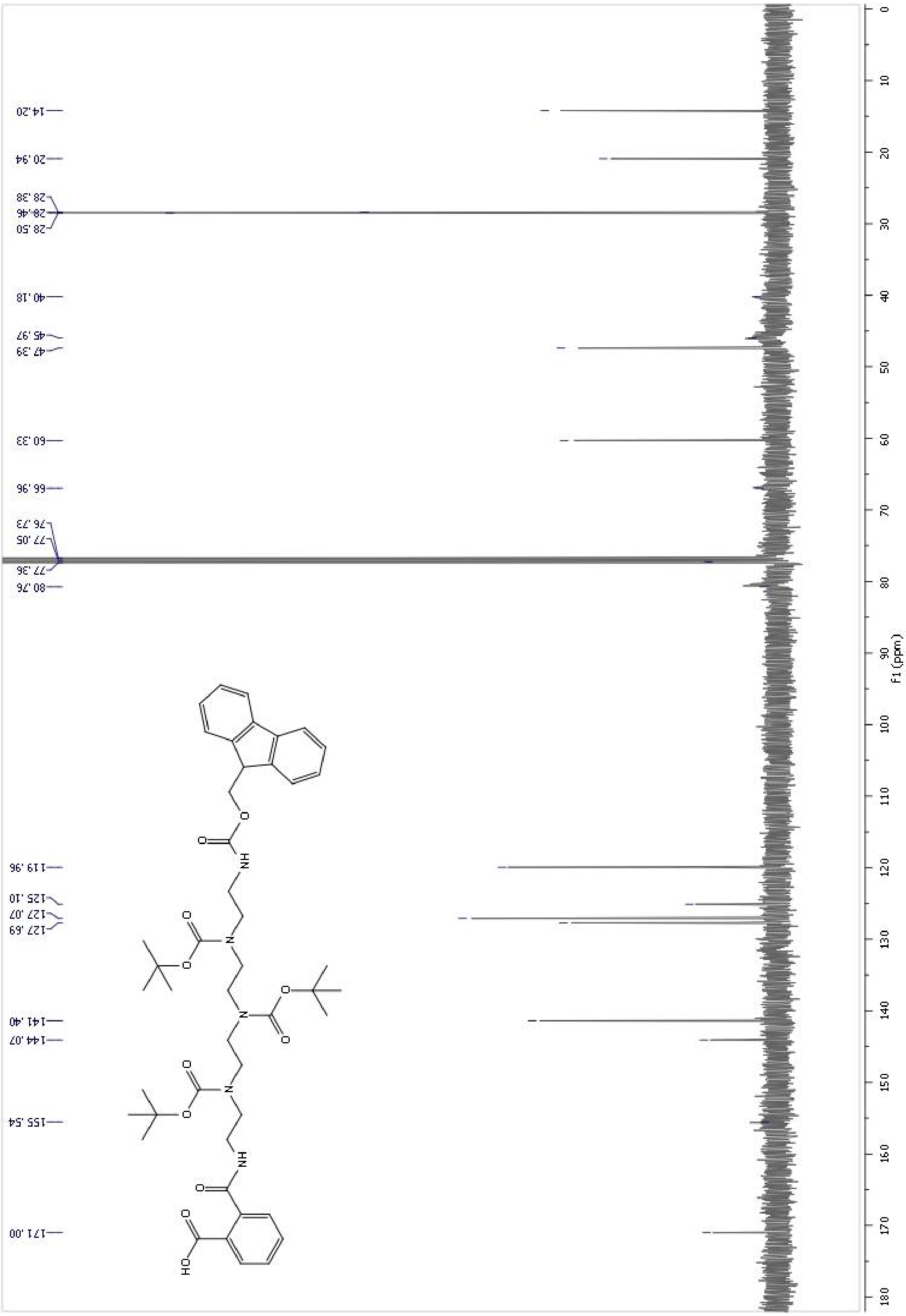
$^{13}C\{^1H\}$ NMR of Compound No. 9:



Proton NMR of Compound No. 10:

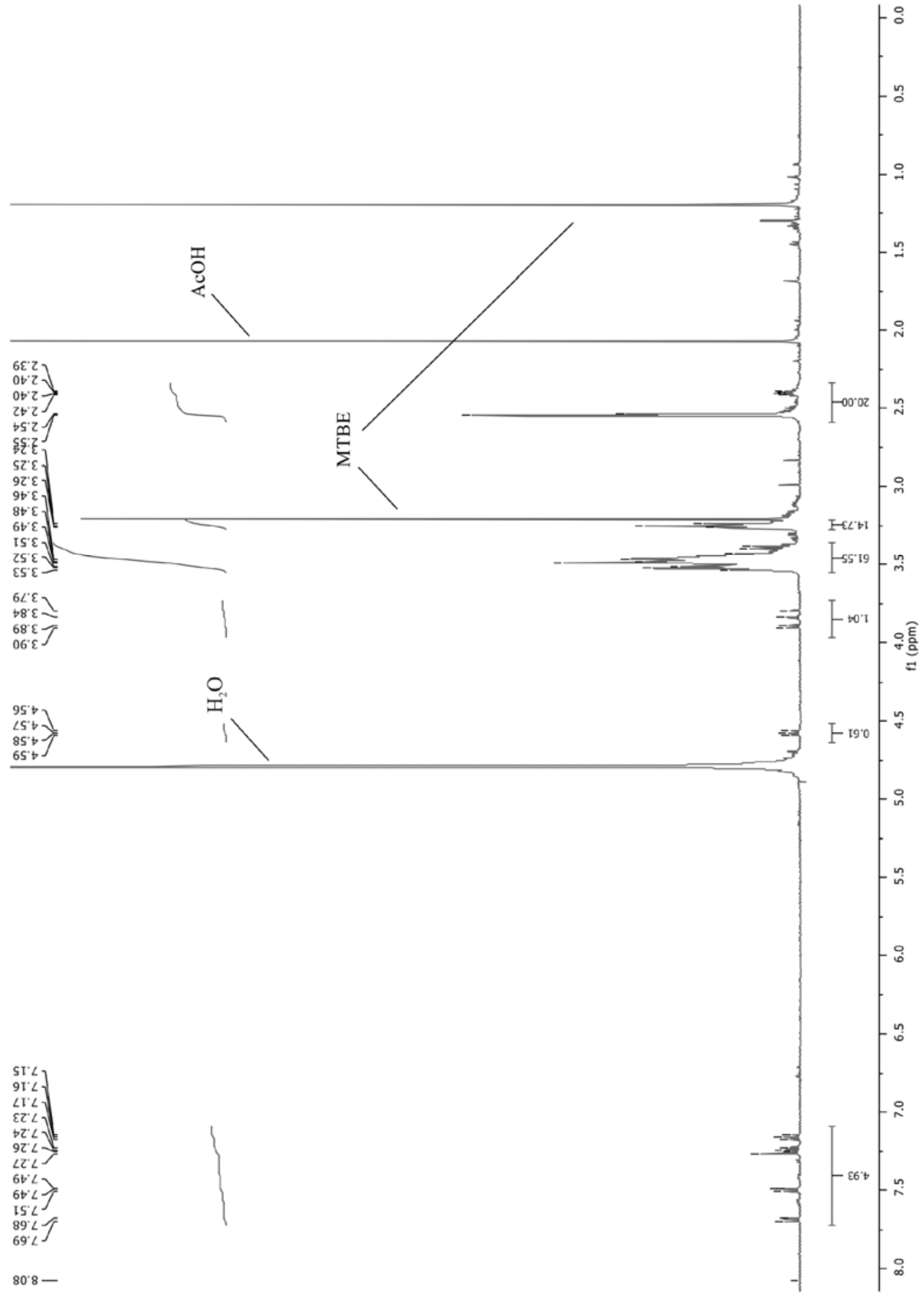


$^{13}C\{^1H\}$ NMR of Compound No. 10:



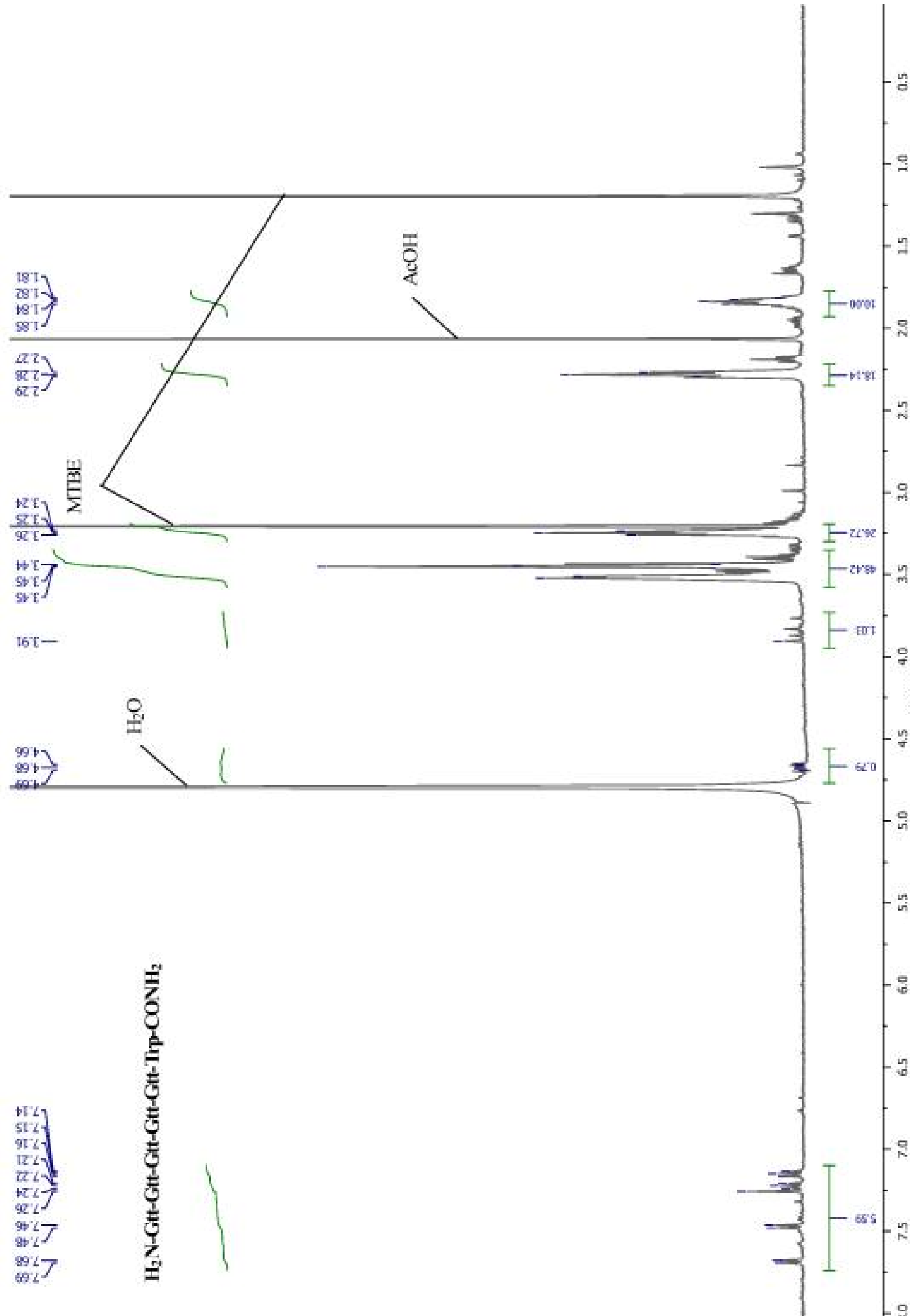
Proton NMR of Sequence No. 11 (Magnifications see Figure S1):

^{1}H NMR (400 MHz, D_2O at RT): δ 7.69 (d, 1H, J=8Hz, ε^3Ar$\underline{H}$-Trp), 7.50 (d, 1H, J=8Hz, ξ^2Ar$\underline{H}$-Trp), 7.15-7.28 (m, 3H, $\delta^1\xi^3\eta^2$Ar$\underline{H}$-Trp), 4.57-4.56 (m, 1H, $\alpha\underline{H}$ Trp), 3.20-3.53 (m, 80H, -C$\underline{H}_2$ of Tp, plus m, 2 H, βH Trp), 2.40-2.55 (m, 20H, succinyl -CO-C$\underline{H}_2$)



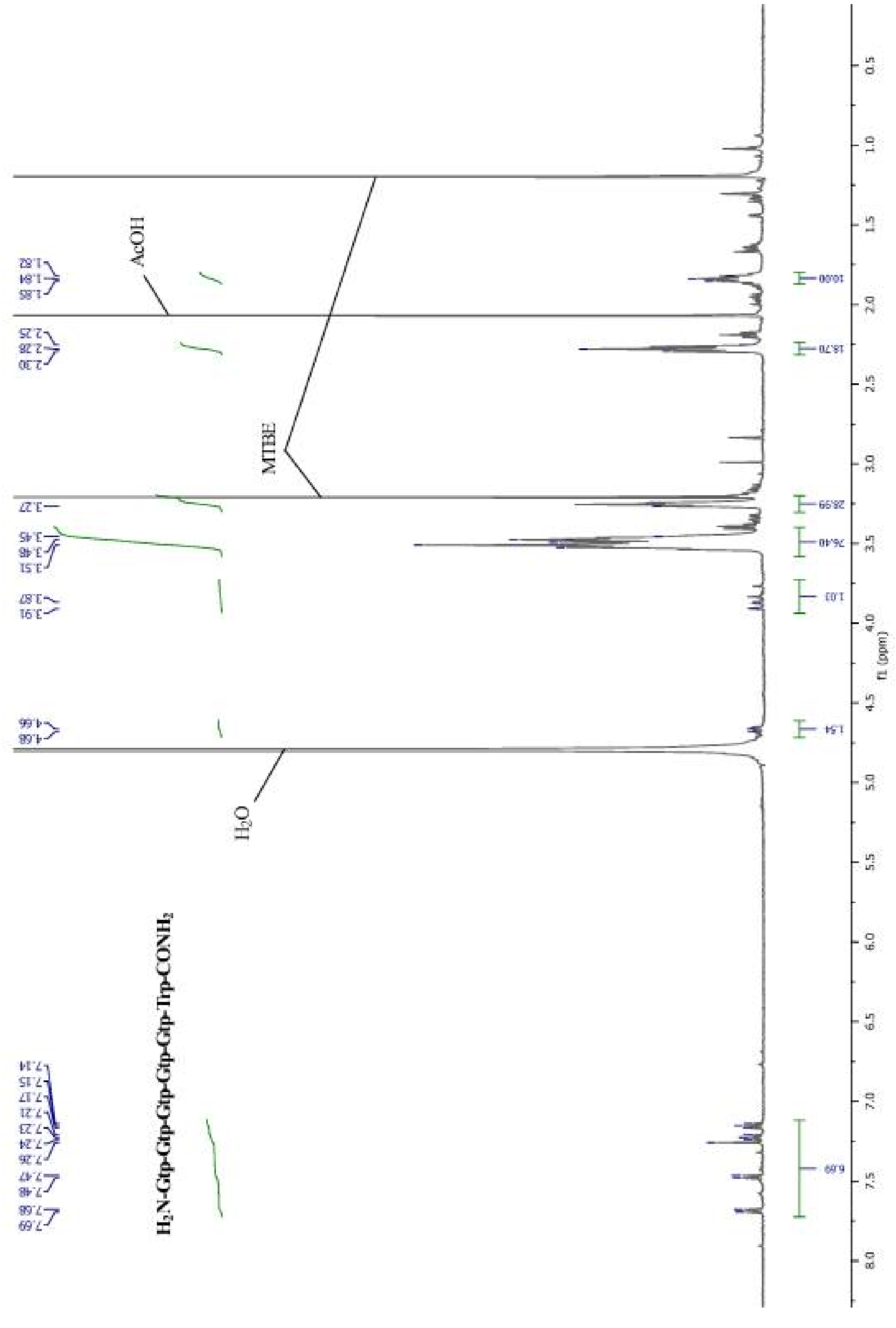
Proton NMR of Sequence No. 12:

^{1}H NMR (400 MHz, D_2O at RT): δ 7.68 (d, 1H, J=8Hz, ε^3Ar$\underline{\mathrm{H}}$-Trp), 7.46 (d, 1H, J=8Hz, ξ^2Ar$\underline{\mathrm{H}}$-Trp), 7.14-7.26 (m, 3H, $\delta^1\xi^3\eta^2$Ar$\underline{\mathrm{H}}$-Trp), 4.66-4.69 (m, 1H, $\alpha\underline{\mathrm{H}}$ Trp), 3.14-3.52 (m, 60H, -C$\underline{\mathrm{H}}_2$ of Tt plus m, 2 H, $\beta\underline{\mathrm{H}}$ Trp), 2.27-2.30 (m, 20H, glutaryl -CO-C$\underline{\mathrm{H}}_2$), 1.82-1.85 (m, 10H, C$\underline{\mathrm{H}}_2$-CH$_2$-CO).



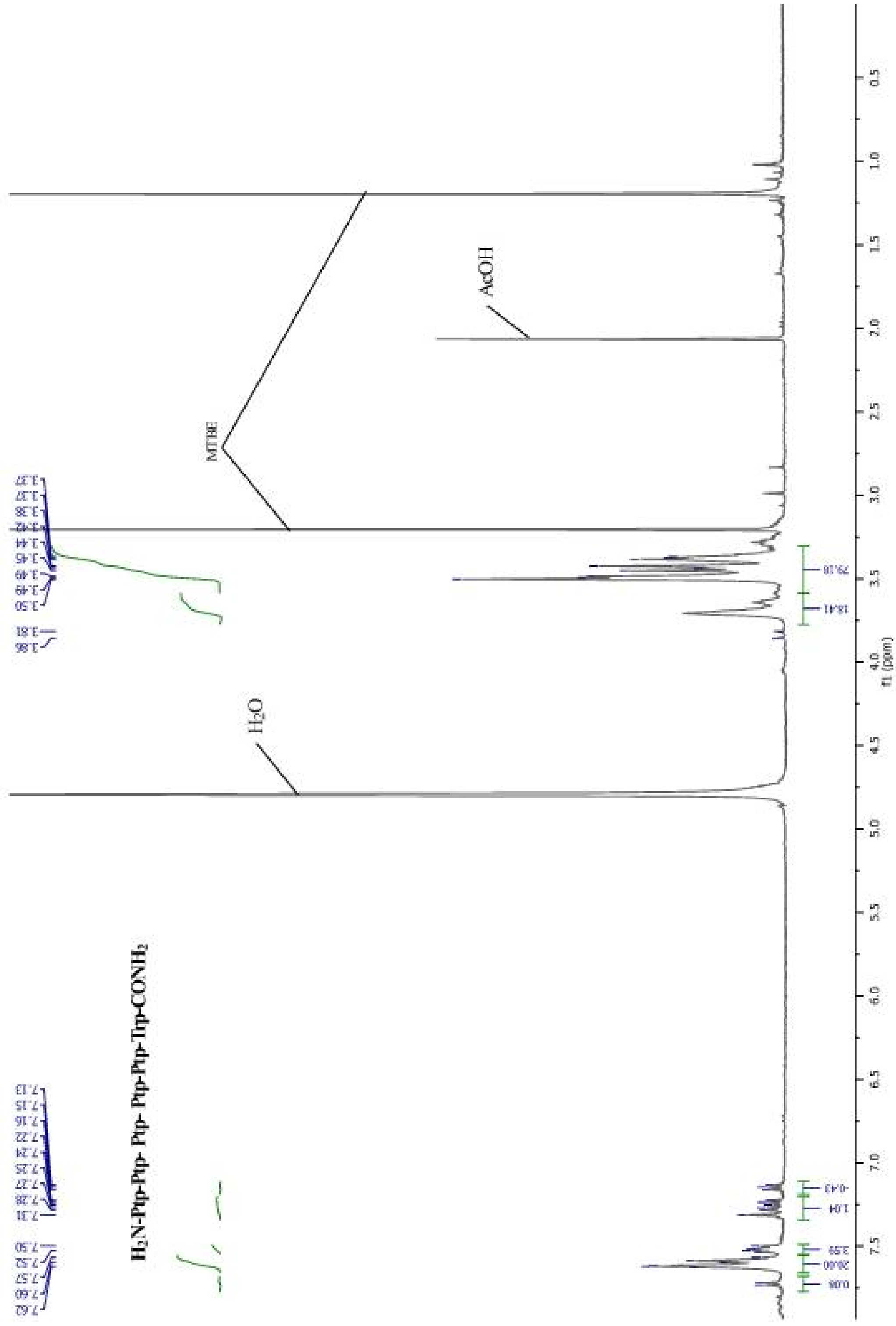
Proton NMR of Sequence No. 13:

^{1}H NMR (400 MHz, D_2O at RT): δ 7.70 (d, 1H, J=8Hz, ε^3Ar$\underline{H}$-Trp), 7.47 (d, 1H, J=8Hz, ξ^2Ar$\underline{H}$-Trp), 7.14-7.26 (m, 3H, $\delta^1\xi^3\eta^2$Ar$\underline{H}$-Trp), 4.66-4.68 (m, 1H, $\alpha\underline{H}$ Trp), 3.17-3.52 (m, 80H, -C$\underline{H}_2$ of Tp, plus m, 2 H, $\beta\underline{H}$ Trp), 2.25-2.30 (m, 20H, glutaryl CO-C$\underline{H}_2$-), 1.81-1.87 (m, 10H, C$\underline{H}_2$-CH$_2$-CO).



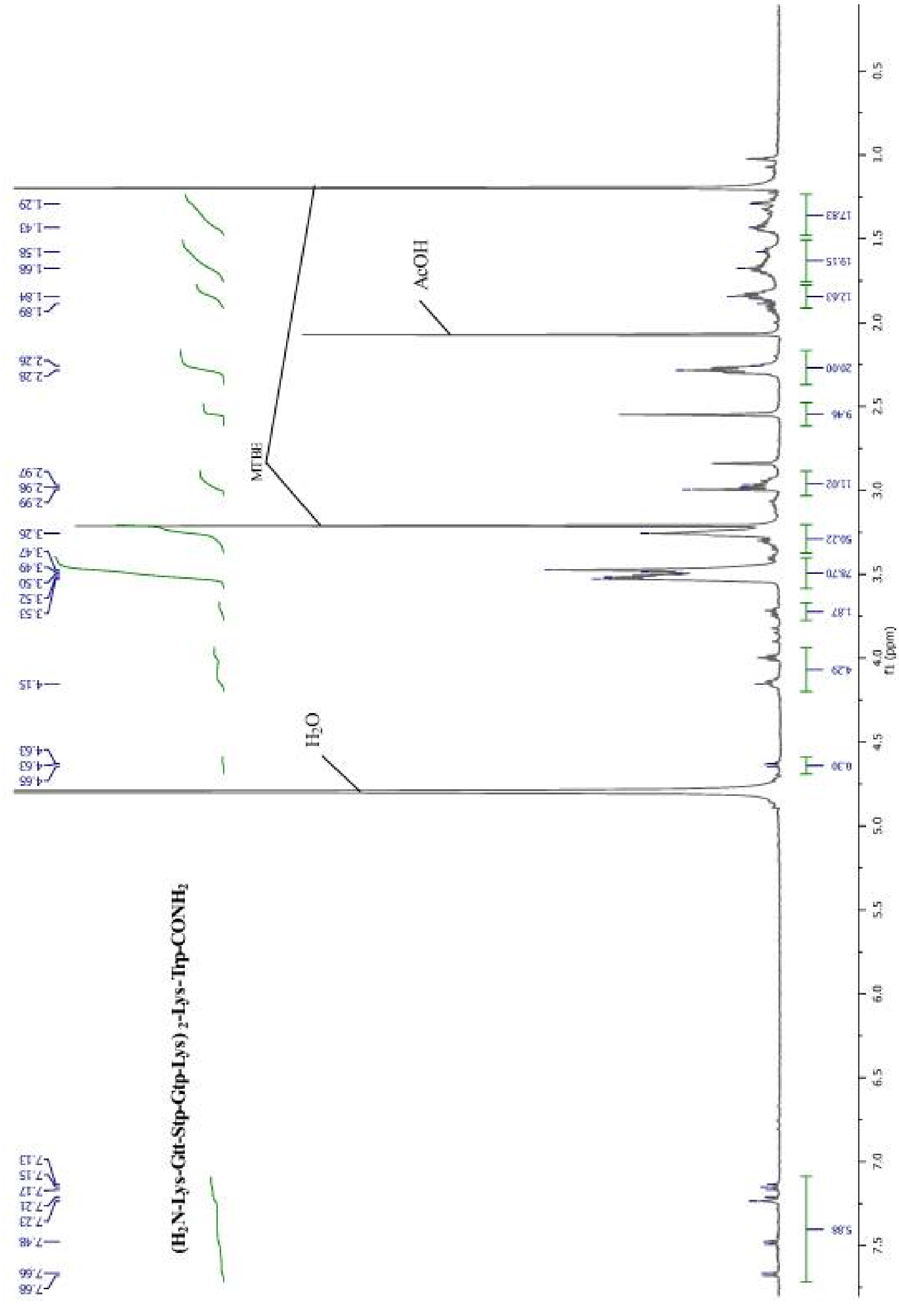
Proton NMR of Sequence No. 14:

^{1}H NMR (400 MHz, D_2O at RT): δ 7.73 (d, 1H, J=8Hz, ε^{3}ArH-Trp), 7.59-7.63 (m, 20H, ArH-Ptp), 7.52 (d, 1H, J=8Hz, ξ^{2}ArH-Trp), 7.13-7.27 (m, 3H, δ^{1}ξ^{3}η^{2}ArH-Trp), 3.27-3.70 (m, 80H, -CH_2 of Tp, plus m, 2 H, βH Trp).

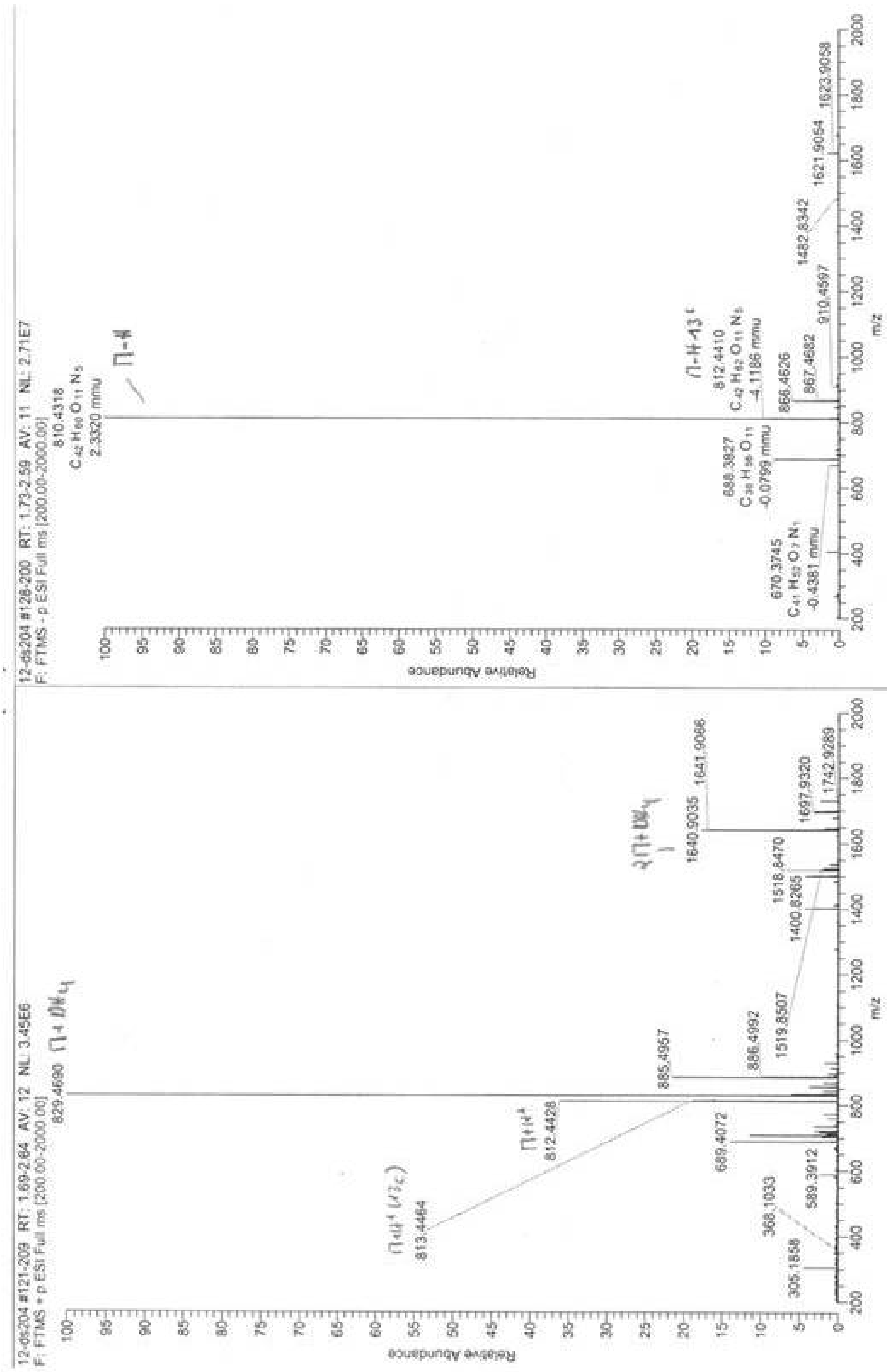


Proton NMR of Sequence No. 15:

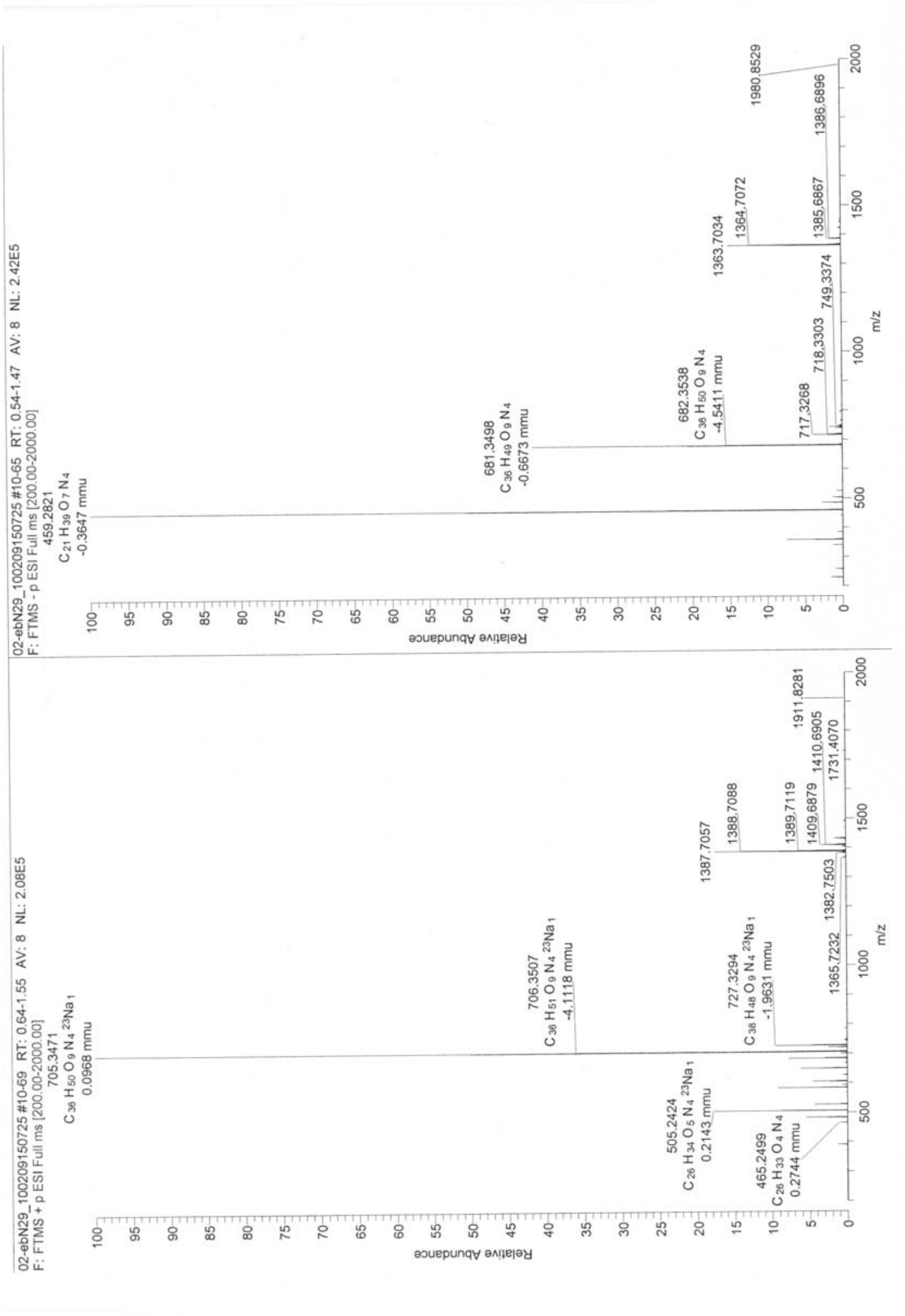
^{1}H NMR (400 MHz, D_2O): δ 7.68 (d, 1H, J=8Hz, ε^3Ar$\underline{H}$-Trp), 7.42 (d, 1H, J=8Hz, ξ^2Ar$\underline{H}$-Trp), 7.13-7.23 (m, 3H, $\delta^1\xi^3\eta^2$Ar$\underline{H}$-Trp), 4.63-4.65 (m, 1 H, α$\underline{H}$ Trp), 3.7-4.25 (m, 5H, α$\underline{H}$ Lys), 3.2-3.53 (m, 88H, -CH_2 of 4 Tp and 2 Tt, plus m, 2 H, βH Trp), 2.97-2.99 (m, 10 H, βH lys), 2.26-2.30 (m, 24, 6 x -CO-CH_2-), 1.80-1.84 (m, 8H, 4 x C$\underline{H}_2$-CH_2-CO), 1.29-1.65 (m, 30 H, γδεH lys)



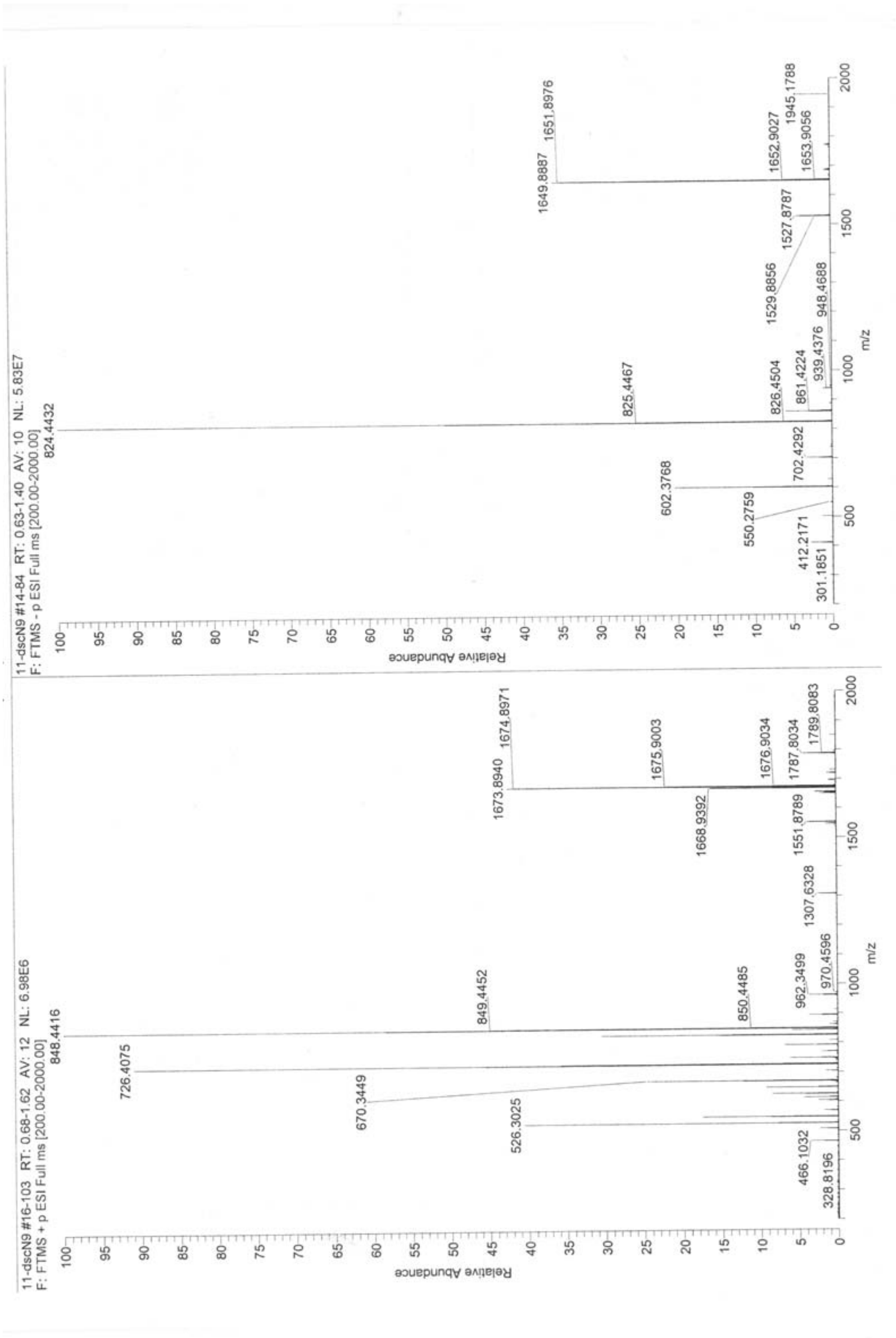
HRMS of 4-[2-[tert-butoxycarbonyl-[2-[tert-butoxycarbonyl-[2-[tert-butoxycarbonyl-[2-(9H-fluoren-9-ylmethoxycarbonylamino) ethyl]amino]ethyl]amino]ethyl]amino]ethylamino]-4-oxo-butanoic acid (7)



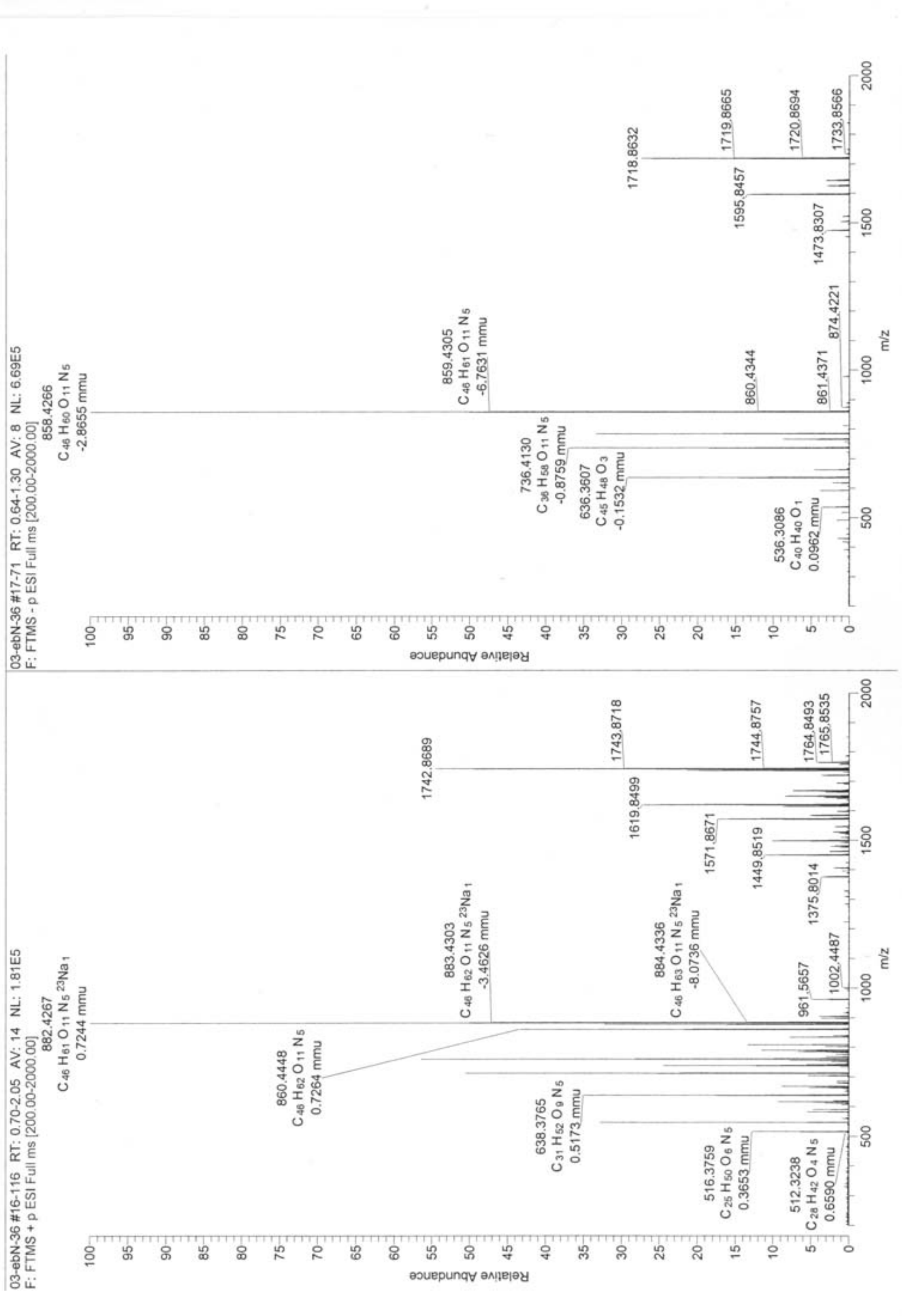
HRMS of 5-[2-[tboc-[2-[tboc-[2-(9H-fluoren-9-ylmethoxycarbonylamino) ethyl]amino]ethyl]amino]ethylamino]-5-oxo-pentanoic acid (8)



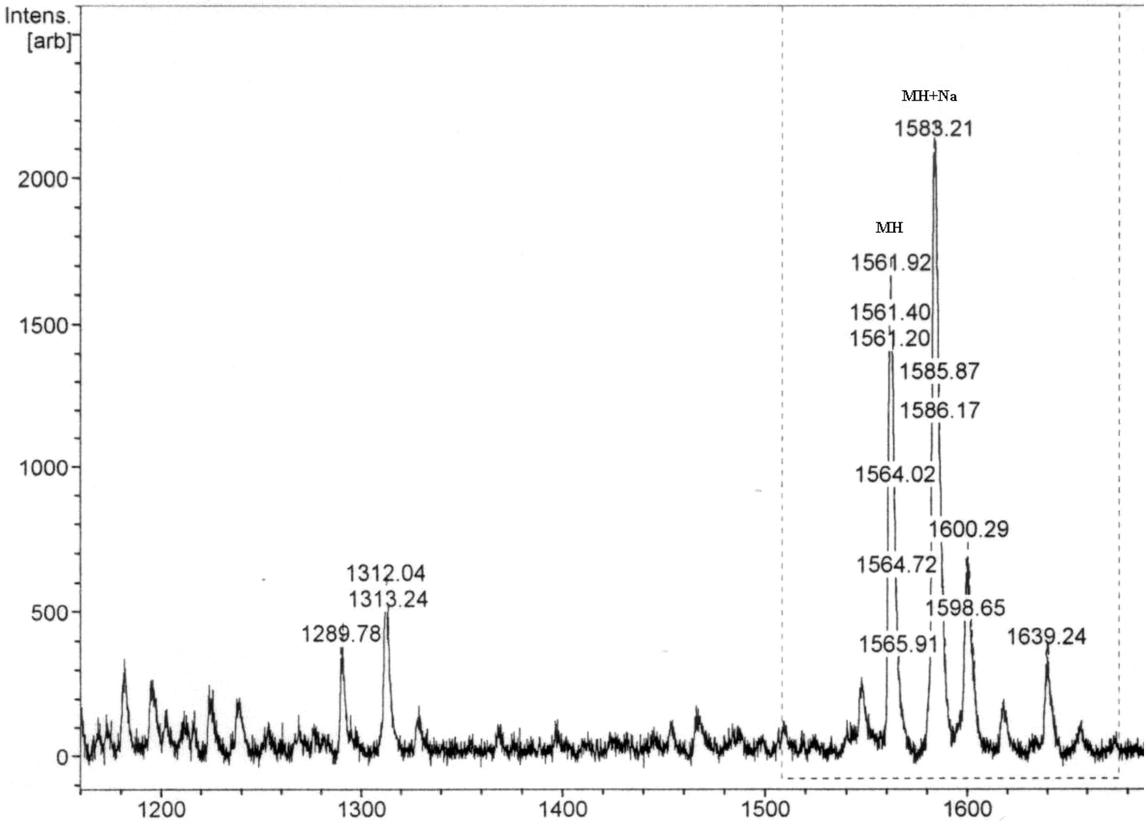
HRMS of 5-[2-[tboc-[2-[tboc-[2-[tboc-[2-(9H-fluoren-9-ylmethoxycarbonylamino)ethyl]amino]ethyl]amino]ethyl]amino]ethylamino]-5-oxo-pentanoic acid (9)



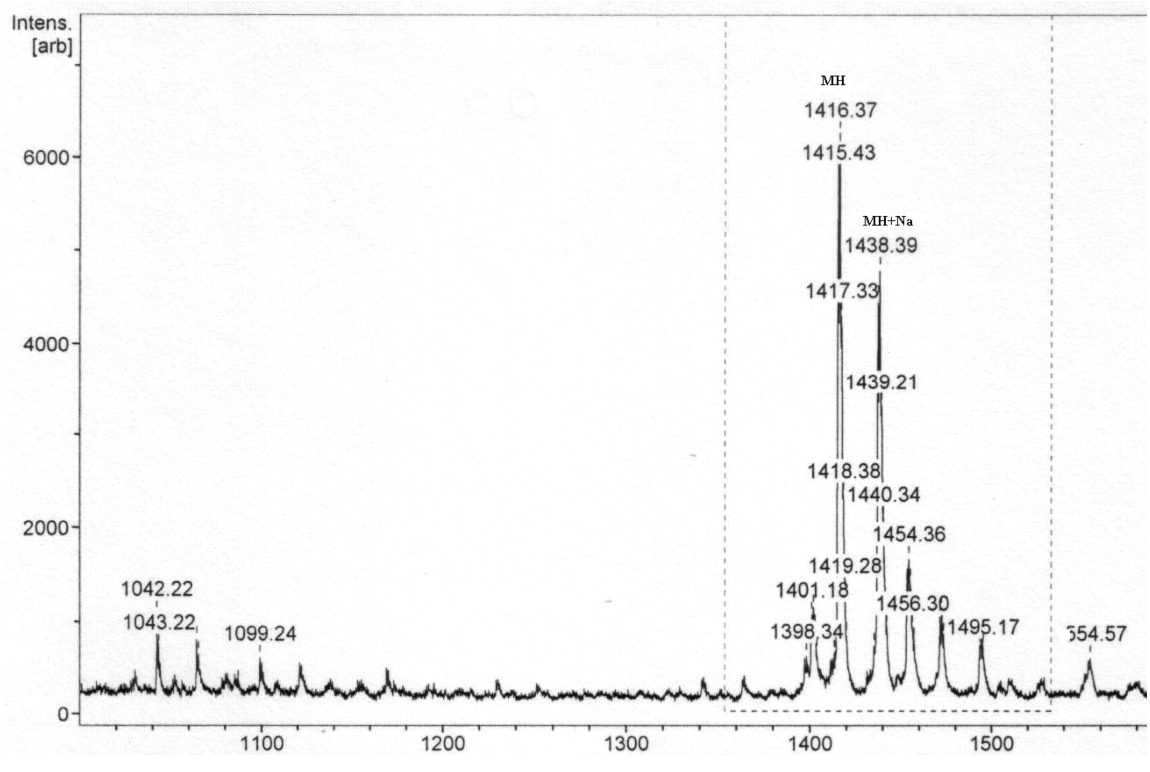
HRMS of 2-[2-[tert-butoxycarbonyl-[2-[tert-butoxycarbonyl-[2-[tert-butoxy carbonyl-[2-(9H-fluoren-9-ylmethoxycarbonylamino)ethyl] amino] ethyl]amino] ethyl]amino]ethylcarbamoyl]benzoic acid (10)



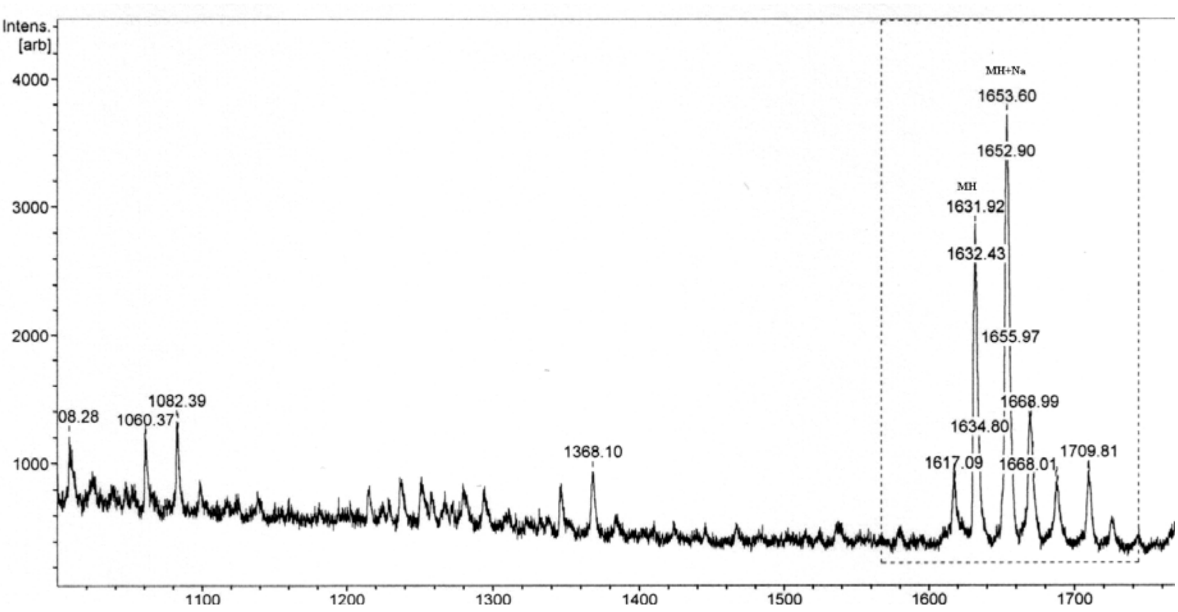
MALDI-MS of Sequence No. 11



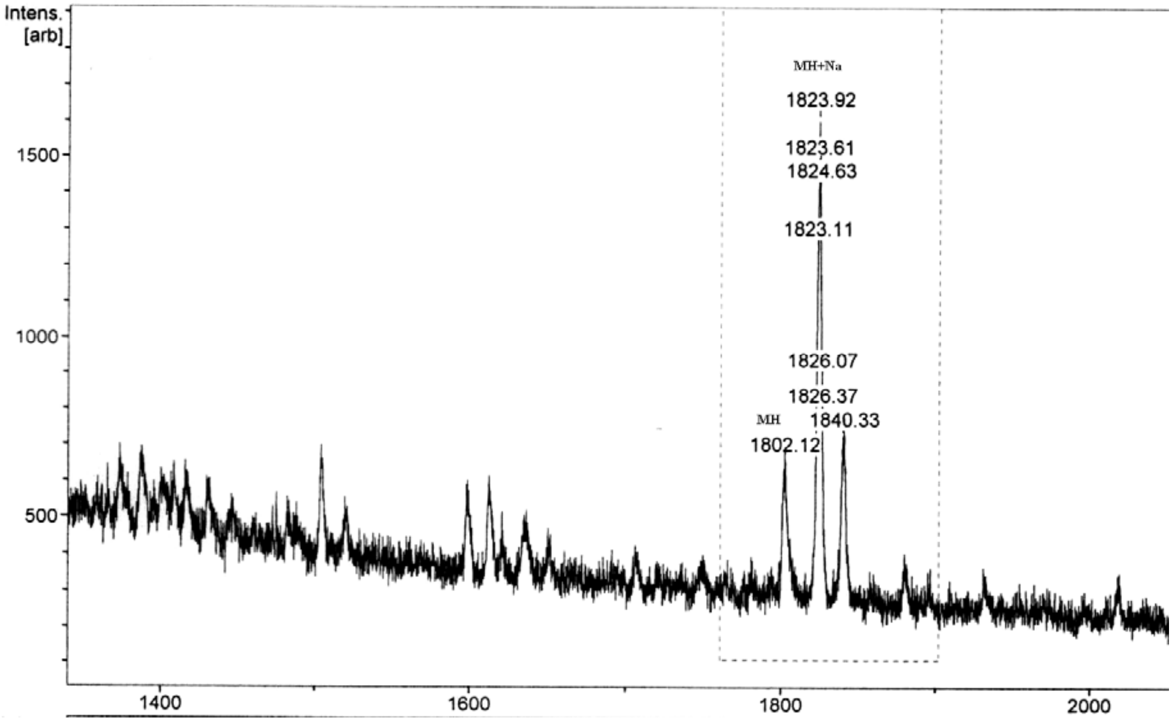
MALDI-MS of Sequence No. 12



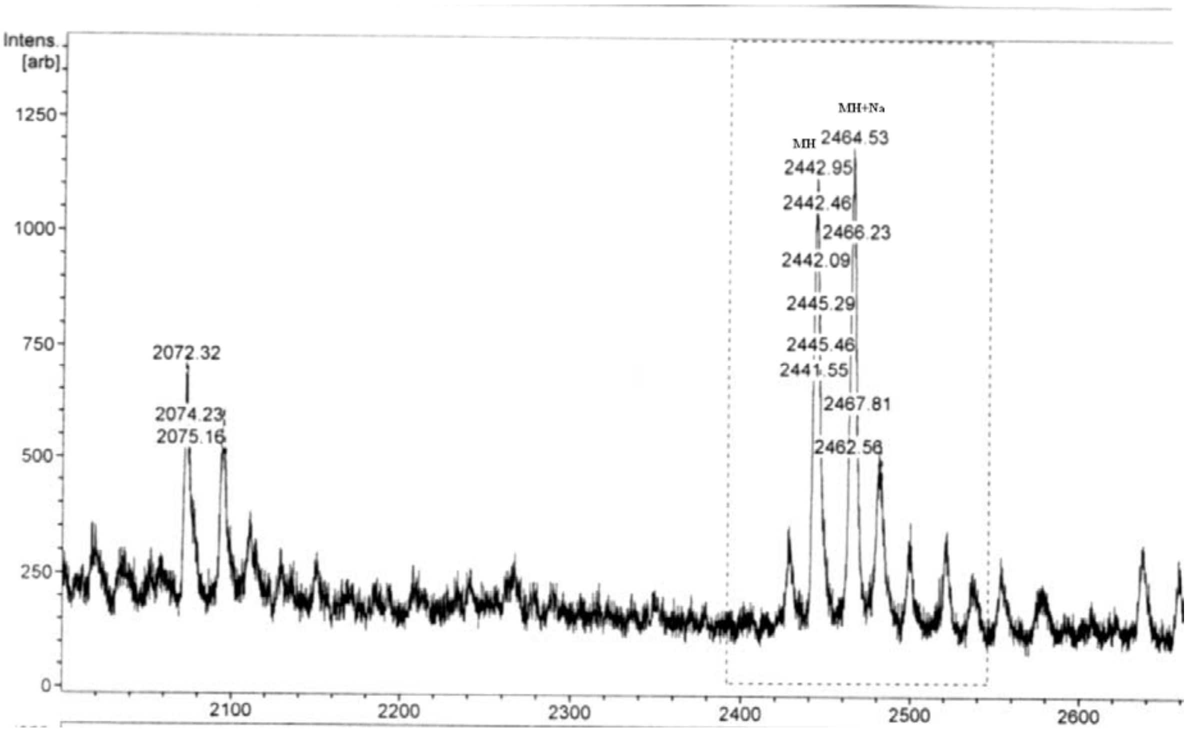
MALDI-MS of Sequence No. 13



MALDI-MS of Sequence No. 14

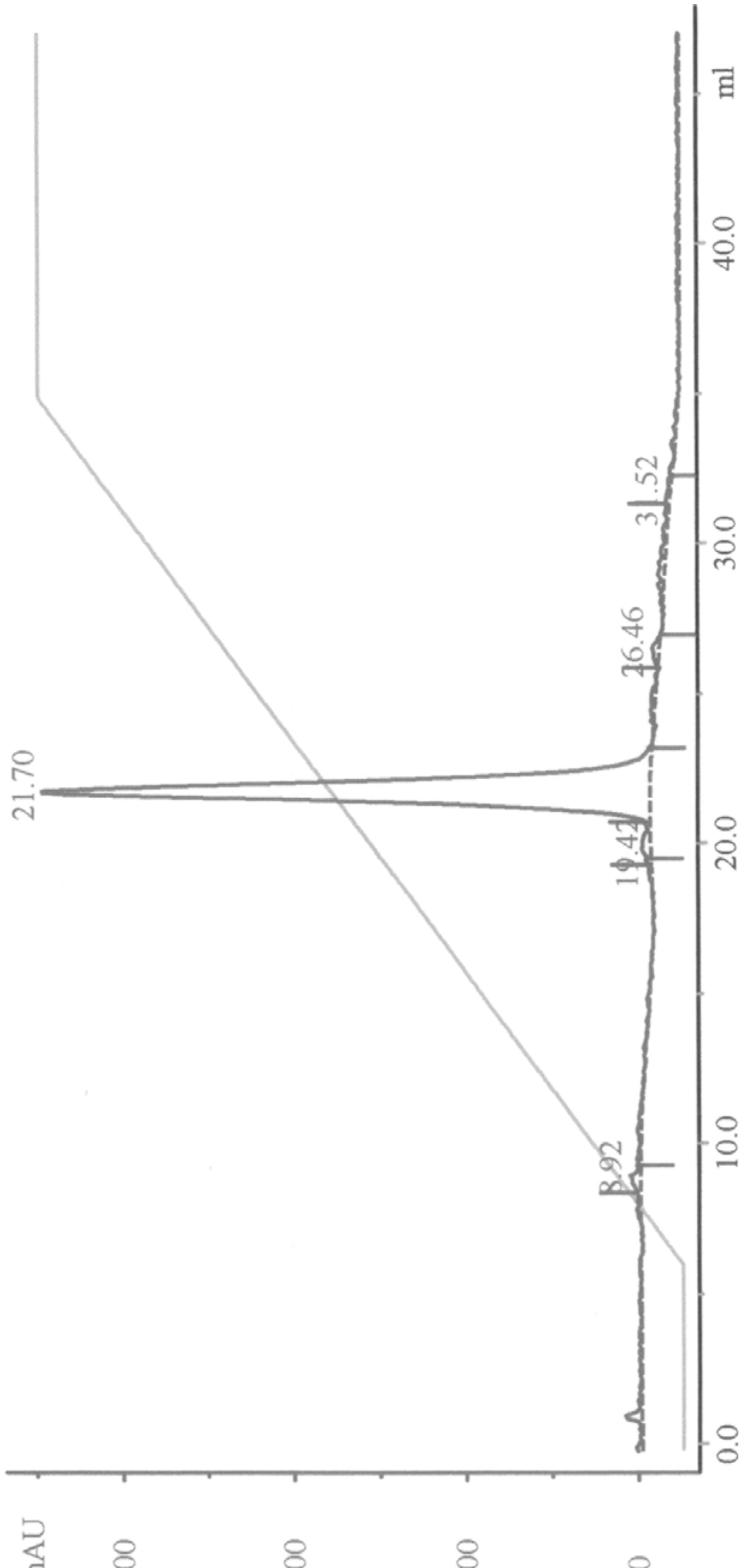


MALDI-MS of Sequence No. 15



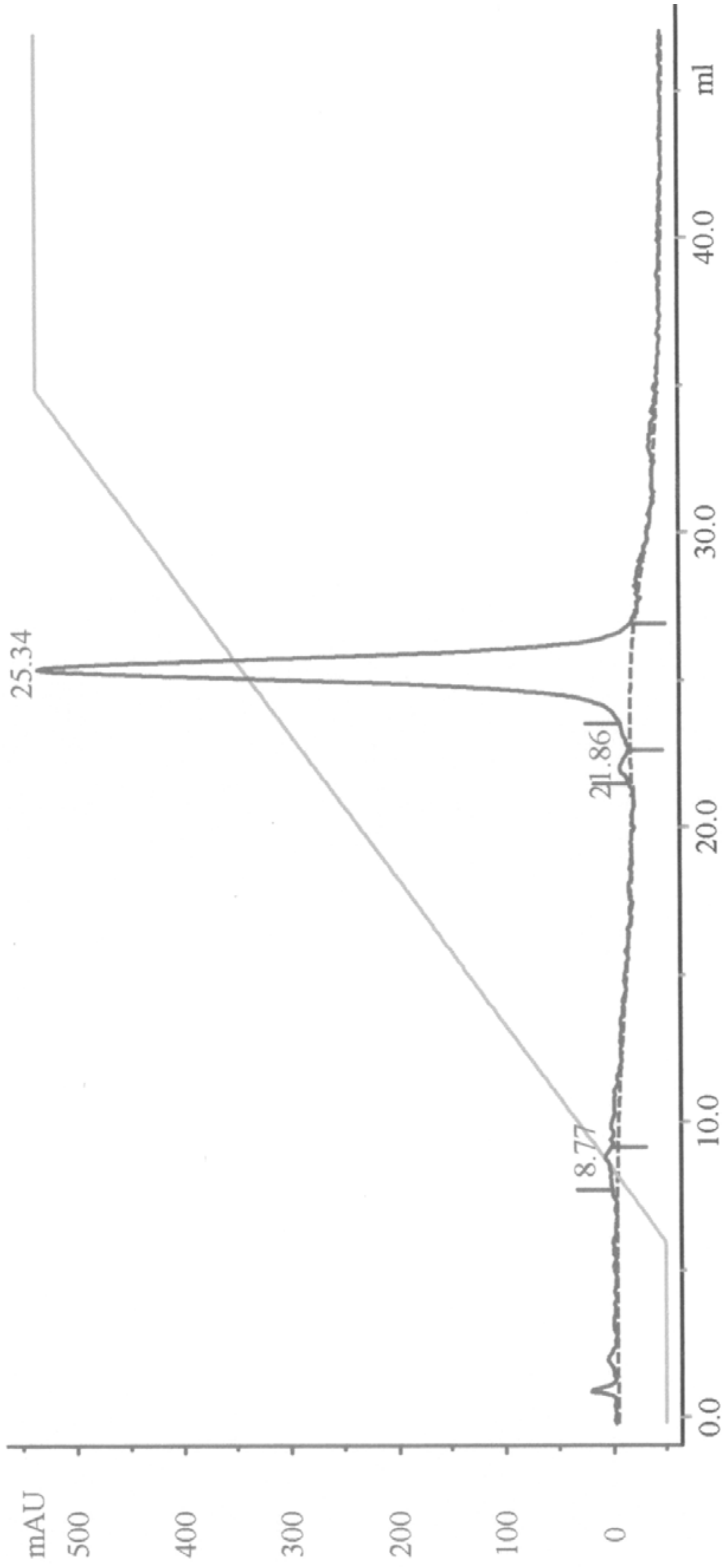
HPLC purity of Sequence No. 12

H_2N-Gtt-Gtt-Gtt-Gtt-Gtt-Trp-$CONH_2$



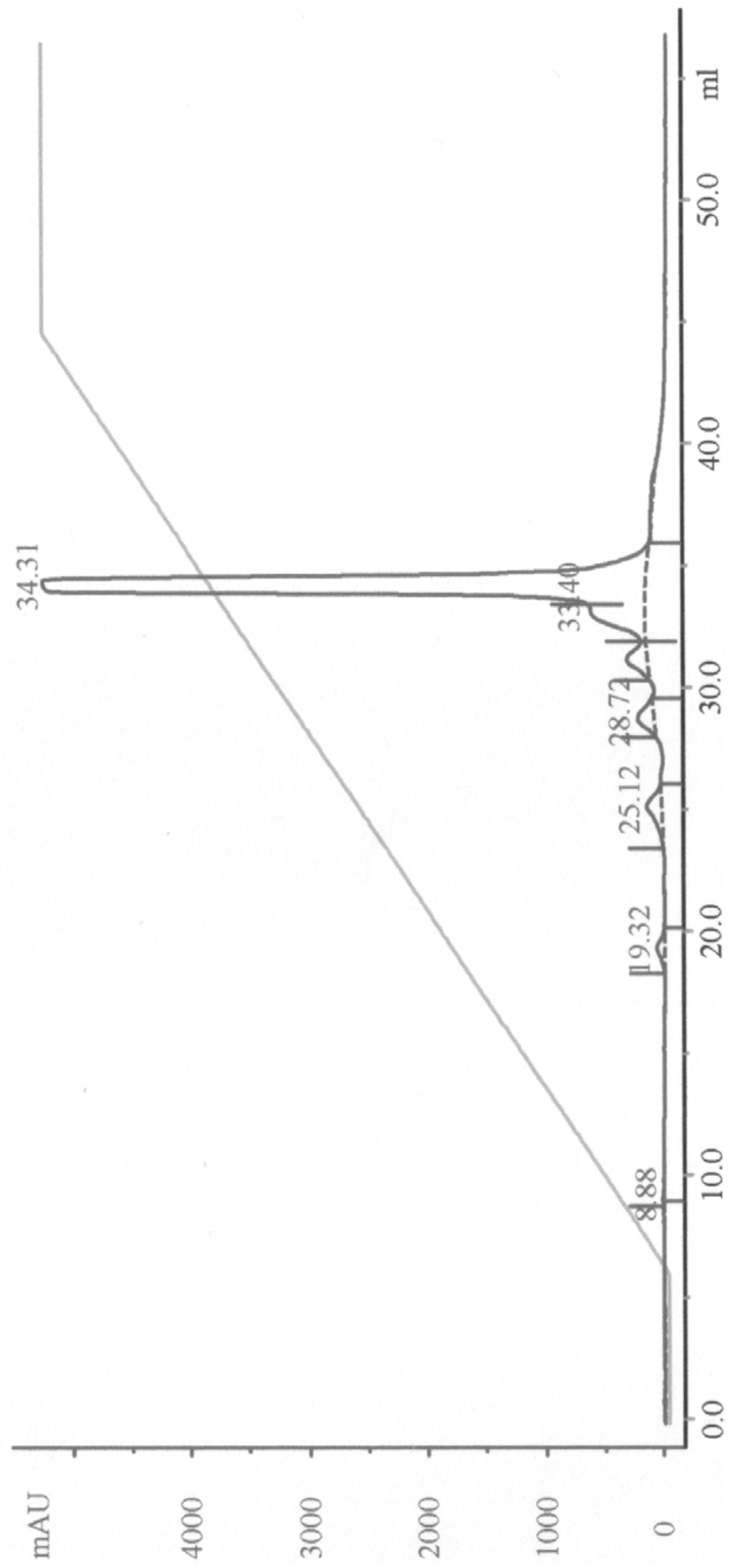
HPLC purity of Sequence No. 13

H_2N-Gtp-Gtp-Gtp-Gtp-Gtp-Trp-$CONH_2$



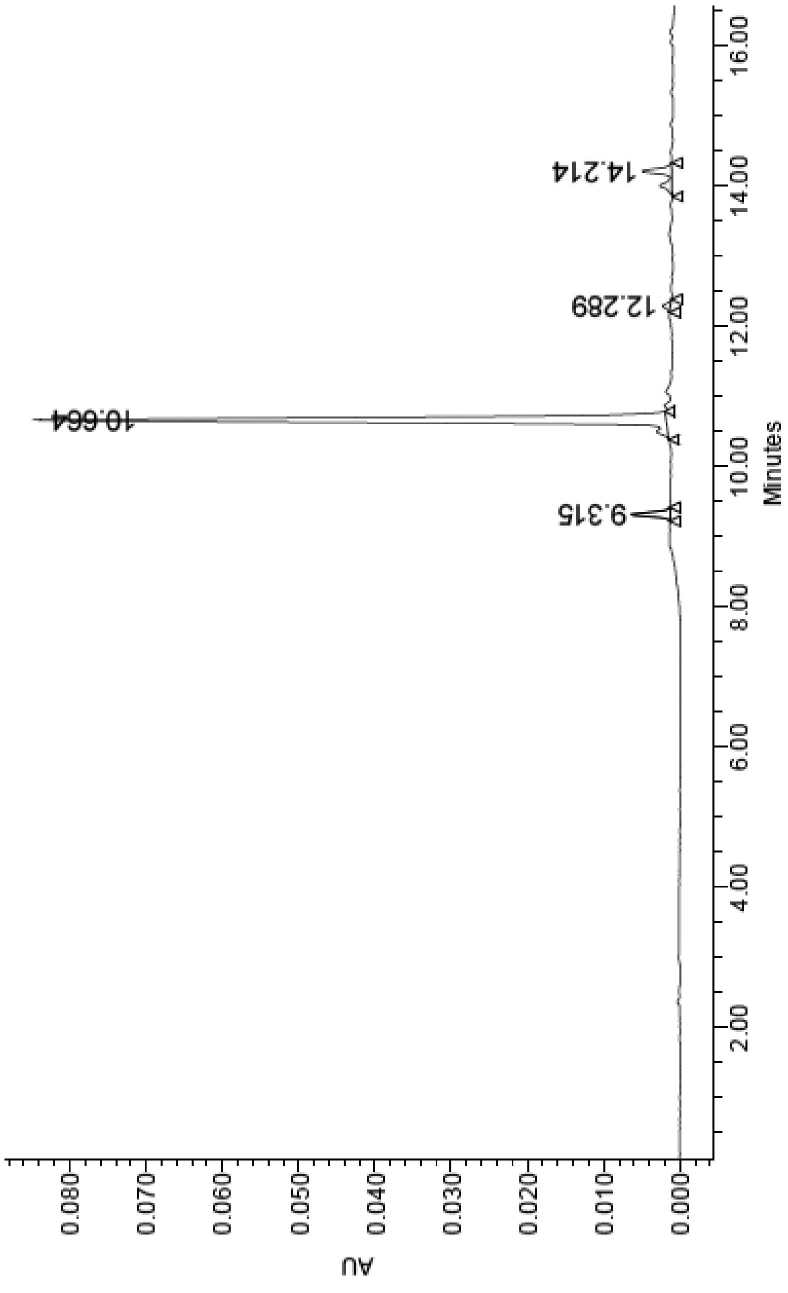
HPLC purity of Sequence No. 14

H_2N-Ptp-Ptp-Ptp-Ptp-Ptp-Trp-$CONH_2$



HPLC purity of GE11 peptide precursor 16

H_2N-Tyr-His-Trp-Tyr-Gly-Tyr-Thr-Pro-Gln-Asn-Val-Ile-COOH



References:

1) Hartmann, L.; Krause, E.; Antonietti, M.; Borner, H. G. *Biomacromolecules* **2006**, 7, 1239.

2) Jørgensen, M.R., Jaroszewski, J.W., Witt, M. and Franzyk, H. *Synthesis* **2005**, 2687-2694.