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Design And Synthesis Of a Peptide that Binds Specific DNA Sequences Through Simultaneous Interaction In The Major and the Minor Groove

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General

Solvents used were purified using procedures described by Perrin,[1] and were dried under Ar by distillation over the corresponding drying agent immediately before their use. Reactions were performed under Ar unless specifically stated.

Peptide synthesis was made using standard Fmoc solid phase synthesis protocols. Using mixtures of HBTU/HOBt as coupling agents and DIEA as base in DMF. Cleavage/deprotection of peptides was performed by treatment of the peptide bound to the solid support for 2.5 h with the following mixture: 830 ìL TFA, 25 ìL EDT, 50 mg PhOH, 50 ìL tioanisole and 50 ìL H_2O (300 ìL of this mixture for each 10 mg of resin)

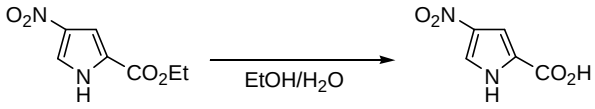
NMR spectrums were registered with *Brucker WM-250* and *Brucker AMX-300* spectrometers. HPLC analysis and purification were made using a *Hewlett-Packard Serial 1100* with a diode array detector. Column packing used was *Merck LiChropher WP 300 RP-18* (5 ìM) for analytical columns and *Nucleosil 120-10 C-18* for semipreparative columns. Mixtures of CH_3CN and water with 0.1% TFA were used as mobile phase with linear gradients. Detection was made at 220, 260 and 304 nm simultaneously.

Qualitative molecular modelling was made using the *Builder* module of *InsightII 98.0* from MSI Molecular Simulations Inc. using a Silicon Graphics Octane with two parallel R10000 processors. Partial energy minimizations of the linkers were made using the *Discover* module of *Insight 98.0* using AMBER forcefield.[2] Graphical representations were made using program WebLabViewer Lite 3.7 from Molecular Simulations Inc. X-Ray structures used were directly downloaded from the Protein Data Bank server (http://www.rcsb.org).

[1] Perrin, D.; Armarego, W. I. F. *Purification of Laboratory Chemicals*, **1998** Pergamon Press.

[2] Weiner, S. J.; Kollman, P. A.; Nguyen, D. T.; Case, D. A. *J. Comput. Chem*. **1986**, *7*, 230.

4-nitro-1 H-pyrrole-2-carboxylic acid (12)

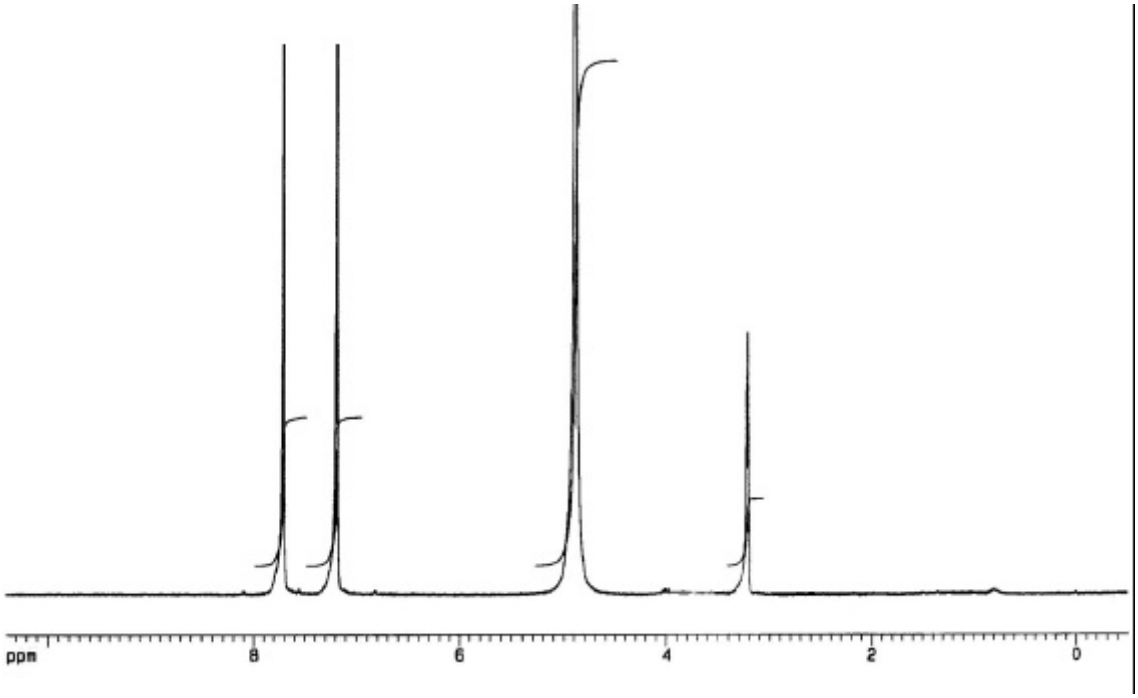


11 **12**

Pyrrole **11** (500 mg, 2.72 mmol) was treated with a 1M solution of NaOH in 70% aqueous EtOH (30 mL) at room temperature for 6 h. After, the resulting solution was carefully acidified with citric acid to pH=2 and was extracted with CH_2Cl_2. The combined organic layers were dried with $MgSO_4$. Removal of the solvent under reduced pressure gave product **12** as a white solid. (360 mg, 85%).[1]H-NMR was consistent with previously reported data.[3]

[1]H-NMR d: 3.2 (s 1H), 7.2 (d, *J* = 1.7 Hz, 1H), 7.7 (d, *J* = 1.7 Hz, 1H).
MS: m/z 156 (M^+, 63), 138 (13), 122 (12), 108 (9), 92 (6), 65, (100).
HRMS: calcd. for $C_5H_4N_2O_4$ 156.01711, found 156.01718.

[3] Lee, M.; Coulter, D. M.; Lown, J. W. *J. Org. Chem.* **1988**, *53*, 1855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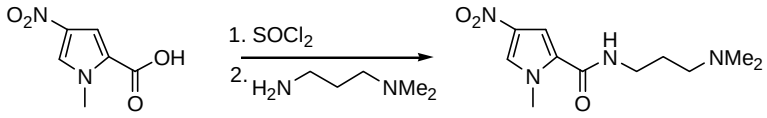
***N*-[3-(dimethylamino)propyl]-1-methyl-4-nitro-1 *H*-pyrrole-2-carboxamide (13)**

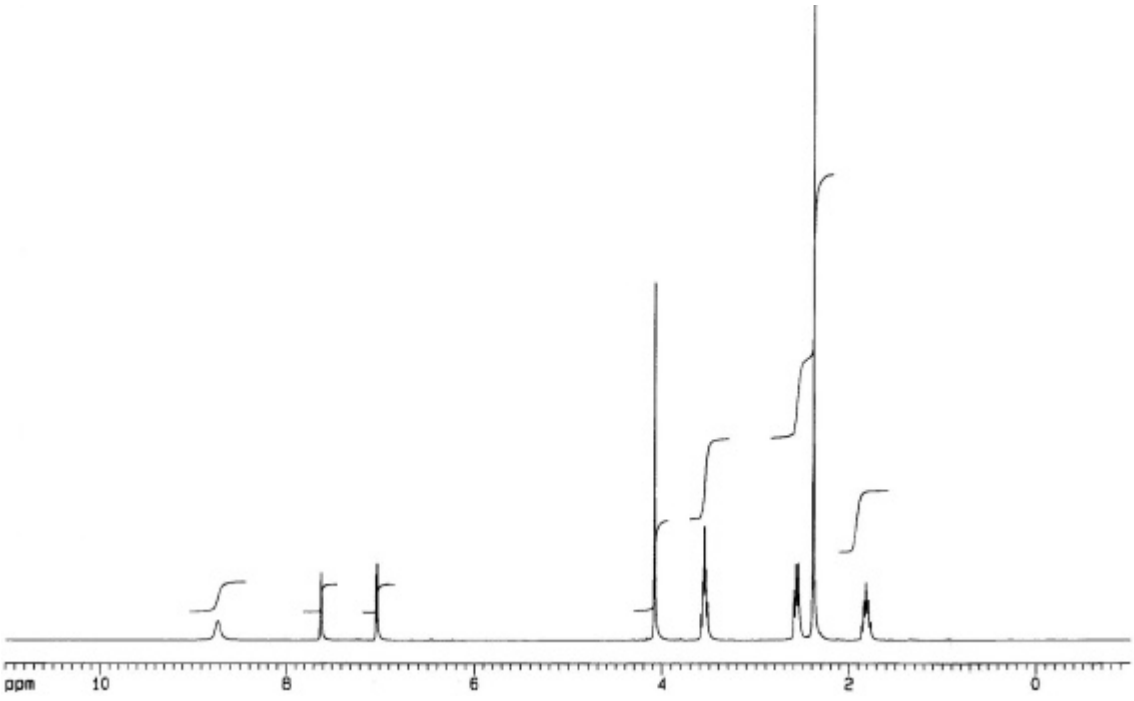


3 **13**

1-methyl-4-nitro-1 *H*-pyrrole-2-carboxylic acid **3** (1.5 g, 8.8 mmol) was dissolved in $SOCl_2$ (6 mL) and the resulting suspension was refluxed under argon for 6 h. Solvent was removed under reduced pressure and the orange residue was redissolved in CH_2Cl_2 which was again removed to ensure complete elimination of any residue of $SOCl_2$. The resulting solid residue was dissolved in DMF (7 mL) and cooled to 0 ºC. To this solution 3-dimethylaminopropylamine was added and the reaction mixture was allowed to stir at room temperature for 16 h. Water (20 mL) was added and the mixture was extracted with a mixture of EtOAc/hexane (3 x 20 mL). The combined organic layers were washed with aqueous $NaHCO_3$ (20 ml, 10%) and dried with $MgSO_4$. Solvent was removed under reduced pressure. The product was purified by flash column chromatography to give **13**. (1.47 g, 67%) Spectroscopic data match reported data.[4]

^{1}H-NMR d**:** 1.68-1.73 (m, 2H), 2.27 (s, 6H), 2.49 (t, *J* = 6.9, 2H), 3.46 (m, 2H), 4.01 (s, 3H), 6.9, 7.51 (2 s, 2H), 8.75 (s, 1H).
MS: m/z 254 (M^+, 10), 181 (14), 153 (11), 131 (17), 58 (100).
HRMS: calcd. for $C_{11}H_{18}N_4O_3$ 254.13789, found 254.13997.

[4] Bruice, T. C.; Mei, H.; He, G.; Lopez, V. *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 1700.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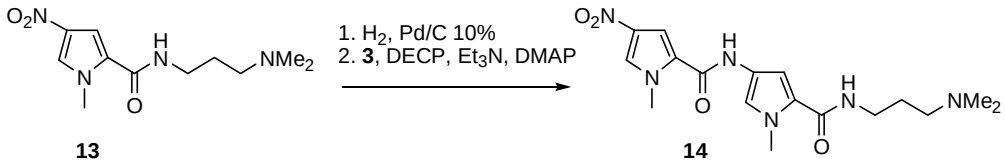
***N*-[(dimethylamino)propyl]-1-methyl-4-{[(1-methyl-4-nitro-1 *H*-pyrrole-2-yl)carbonyl]amino}-1*H*-pyrrole-2-carboxamide (14)**

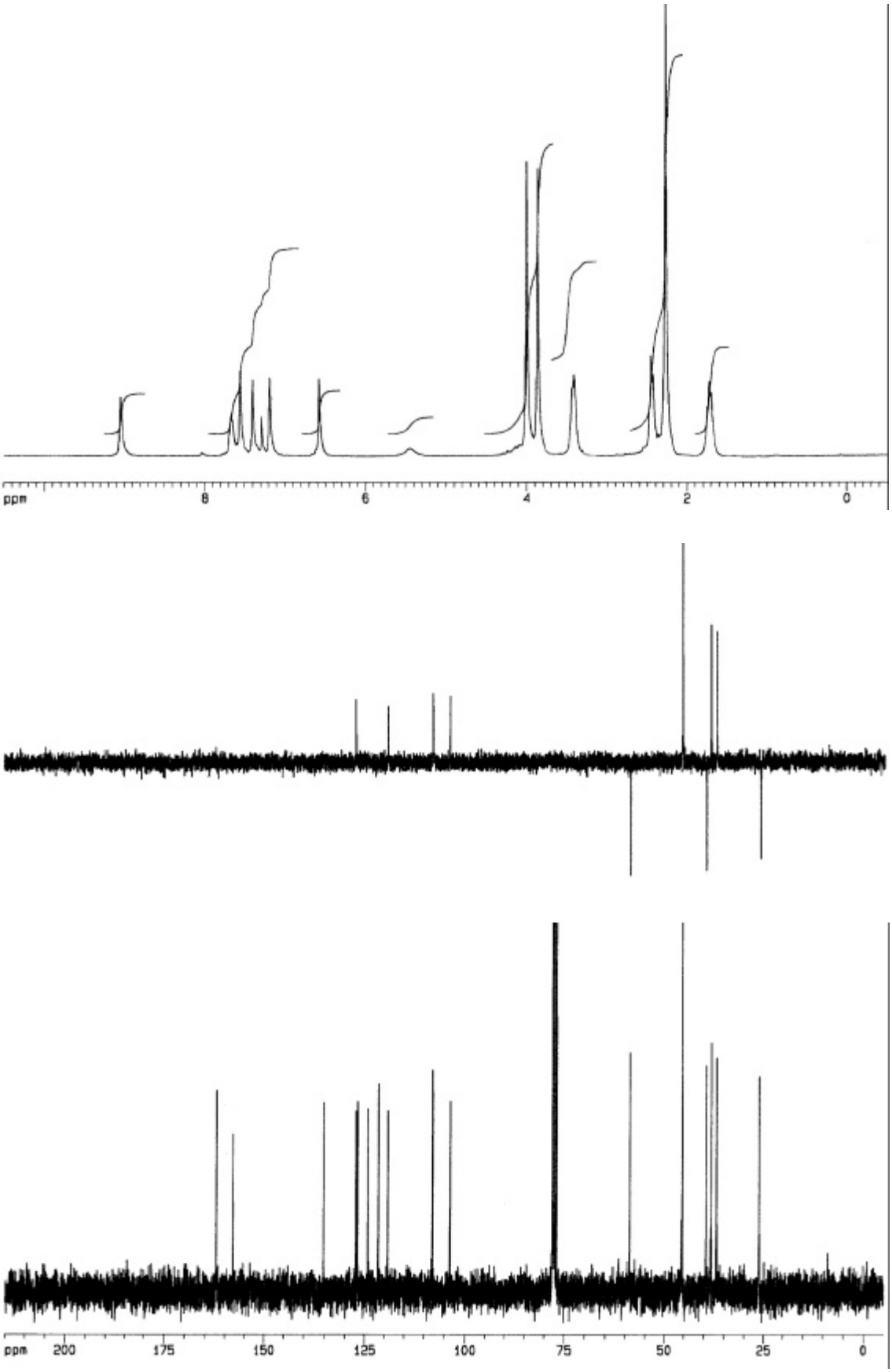


13 **14**

A solution of the pyrrole **13** (2.24 g, 8.84 mmol) in MeOH (100 mL) was hydrogenated over 10% palladium on charcoal (1.5 g) at room temperature for 1 h (balloon pressure). The catalyst was removed by filtration through celite, and the filtrate was concentrated under reduced pressure. The residue was dissolved in DMF (8 mL) and added over another solution previously prepared of 1-methyl-4-nitro-1 *H*-pyrrole-2-carboxylic acid **3** (1.66 g, 9.72 mmol), DECP (2 mL, 13.26 mmol), Et_3N (6.16 mL, 44.2 mmol) and DMAP (10 mg) in DMF (12 mL) cooled at 0 ºC. The resulting mixture was allowed to stir at room temperature for 12 h. MeOH (5 mL) was added and solvents were removed under reduced pressure, and the product was purified by flash column chromatography (1% Et_3N, 10% MeOH, CH_2Cl_2) to afford **14** (1.4 g, 80%) as a yellowish solid whose spectroscopic data match previously reported.[5]

^{1}H-NMR d**:** 1.6-1.7 (m, 2H), 2.27 (s, 6H), 2.42 (m, 2H), 3.46 (m, 2H), 3.82 (s, 3H), 4.01 (s, 4H), 6.53 (s, 1H), 7.17 (s, 1H), 7.39 (s, 1H), 7.54 (s, 1H), 9.06 (s, 1H).
^{13}C-NMR d**:** 26.1 (CH_2), 37.5 (CH), 38.6 (CH), 40.0 (CH_2), 45.1 (CH), 58.9 (CH_2) 103.6 (CH), 107.5 (CH), 119.1 (CH), 121.8 (C), 124.3 (C), 126.8 (CH), 127.2 (C), 135.0 (C), 157.7 (C), 161.2 (C).
MS: m/z 376 (M^+, 4), 307 (6), 155 (7), 154 (100).
HRMS: calcd. for $C_{17}H_{24}N_6O_4$ 376.1859, found 376.19469.

[5] Nishiwaki, E. T.; Shigeaki, L.; Hideaki, S.; Masayuki. *Heterocycles*, **1988**, *27*, 1945.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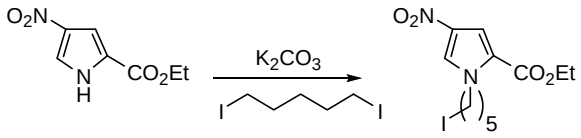
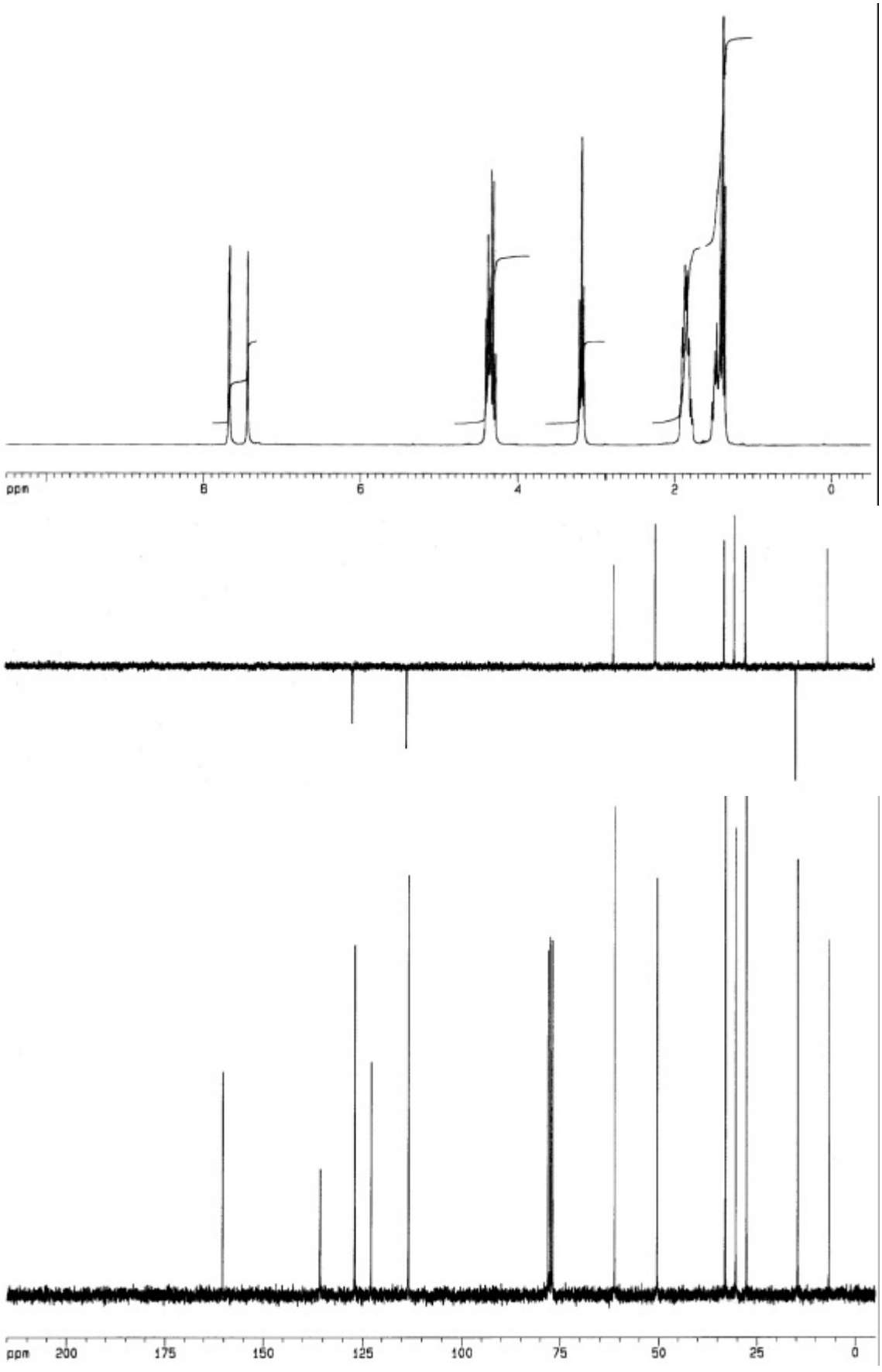
1-(5-iodopenthyl)-4-nitro-1 *H*-pyrrole-2-ethyl carboxylate (29)



11 **29**

To a solution of pyrrole **11** (1.7 g, 9.23 mmol) in dry acetone (60 mL) was added dry potassium carbonate (3.5 g) and 1,5-diiodopentane (11.96 g, 5.5 mL, 36.9 mmol). The reaction mixture was refluxed for 16 h. The suspension was filtered through celite and the filtrate was concentrated. The product was purified by flash column chromatography (EtOAc/hexane) to afford **29** as a pale-yellow oil that solidify on standing. (86%).

^{1}H-NMR d**:** 1.3-1.6 (m, 5H), 1.83 (m, 4H), 3.16 (t, *J* = 6.9 Hz, 2H), 4.22-4.37 (m, 4H), 7.42 (s, 1H), 7.68 (s, 1H).
^{13}C-NMR d**:** 6.7 (CH_2), 14.6 (CH), 27.6 (CH_2), 30.4 (CH_2), 33.0 (CH_2), 50.5 (CH_2), 61.3 (CH_2), 113.4 (CH), 122.8 (C), 127.1 (CH), 135.7 (C), 160.3 (C).
MS: m/z 381 (MH^+, 100), 364 (6), 307 (30), 289 (11), 253 (9).
HRMS: calcd. for $C_{12}H_{17}IN_2O_4$ 381.02331, found 381.02963.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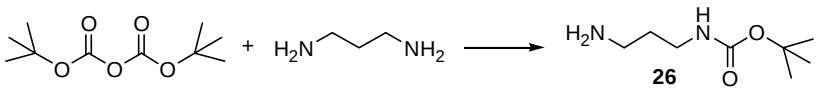
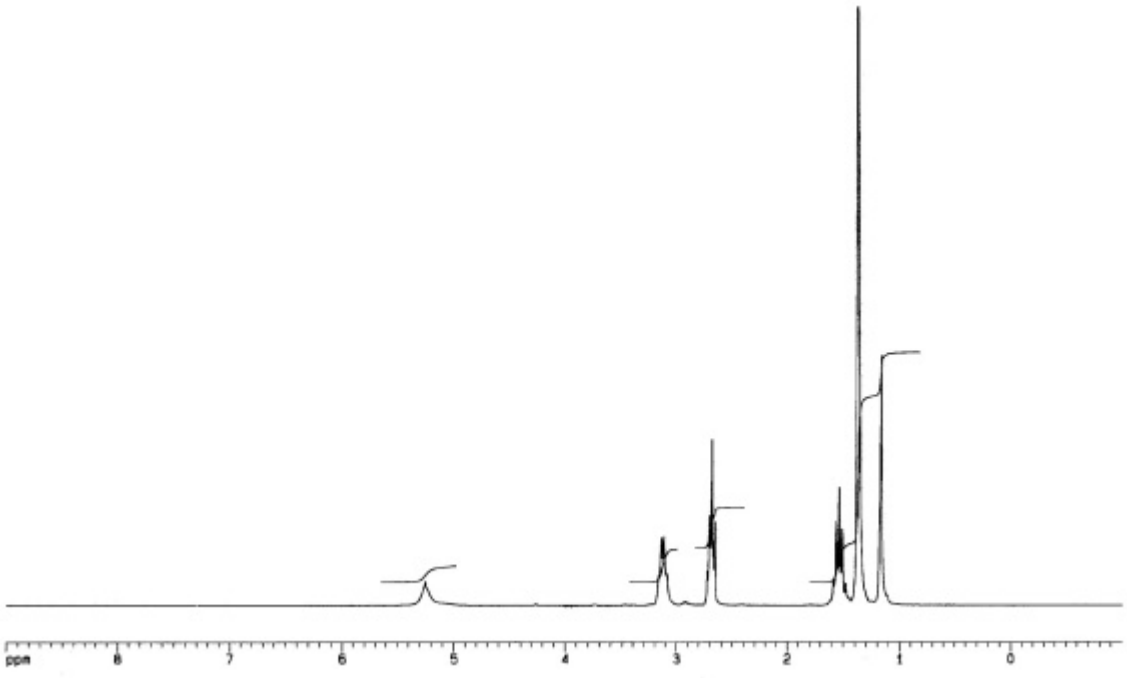
***tert*-buthyl 3-aminopropilcarbamate (26)**



A solution of 1,3-diaminopropane (20.4 g, 23.5 mL, 0.275 mmol) in dioxane (95 mL) was cooled to 0 ºC, and another solution of BOC_2O (7.46 g, 0.034 mmol) in dioxane (50 mL) was added dropwise. The reaction mixture was allowed to stir for 2 days. The solvent was removed under reduced pressure and the residue was redissolved in water (150 mL) and partitioned with CH_2Cl_2 (5 x 20 mL). Combined organic layers were dried with $MgSO_4$ and concentrated under reduced pressure to afford **26** as a clear oil. Spectroscopic data match previously reported data.[6]

^{1}H-NMR d ($CDCl_3$)**:** 1.14 y 1.28 (s, 9H), 1.50 (t, 2H), 2.62 (t, 2H), 3.11 (c, 2H), 5.22 (s broad, 1H).
MS: m/z 175 (MH^+, 13), 145 (52), 117 (40), 101 (68), 89 (16), 57 (100).
HRMS: calcd. for $C_8H_{18}N_2O_2$ 174.13683, found 174.13639.

[6] Lochner, M.; Geneste, H.; Hesse, M. *Helv. Chim. Acta*, **1998**, *81*, 2270.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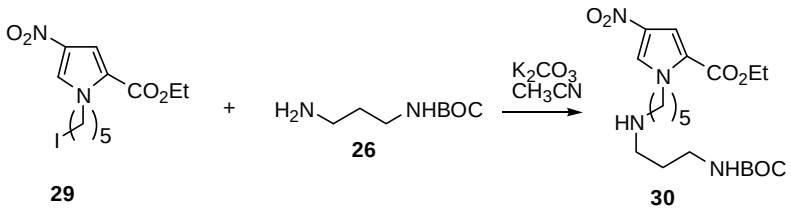
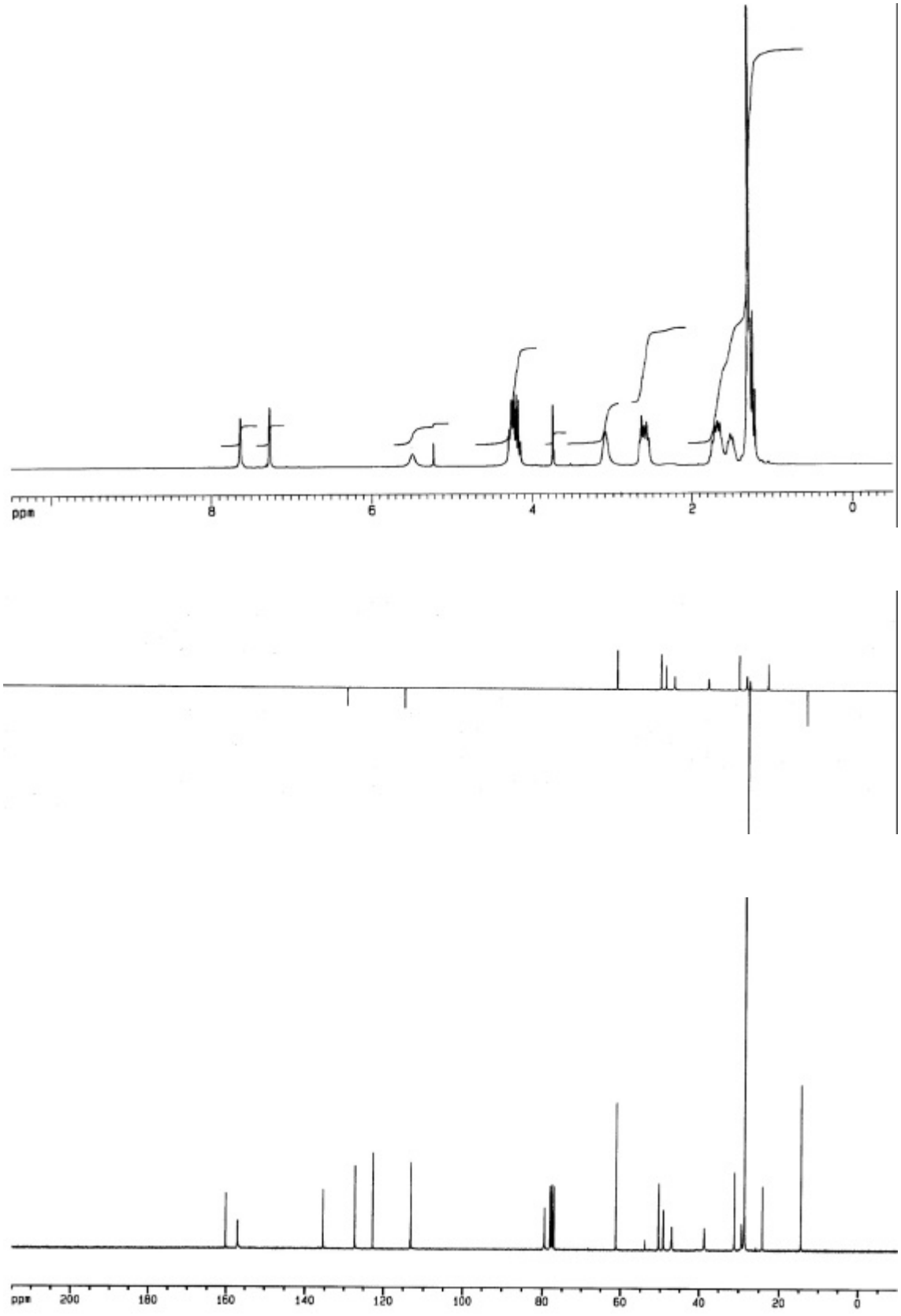
1-[5-({3-[(*tert*-butoxycarbonyl)amino]propyl}amino)penthyl]-4-nitro-1 *H*-pyrrole-2-ethyl carboxylate (30)



To a solution of iodide **29** (1 g, 2.63 mmol) in dry CH_3CN (50 mL) was added dry K_2CO_3 (5 g) and a solution of the amine **26** (1.22 g, 6.97 mmol) in CH_3CN (50 mL). The resulting reaction mixture was refluxed for 12 h and the suspension was filtered through celite. The filtrate was concentrated under reduced pressure. The product was purified by flash column chromatography (5% MeOH/CH_2Cl_2 → 1% Et_3N, 10% MeOH/CH_2Cl_2) to afford product **30** as a pale yellow oil (762 mg, 68%).

^{1}H-NMR d**:** 1.12-2.38 (m, 12H) 1.45 (m, 2H) 1.52-1.77 (m, 4H), 3.05-3.26 (m, 6H), 2.48-2.63 (m, 4H), 3.09 (m, 2H), 4.15 (m, 4H), 5.42 (s broad, 1H) 7.22 (s, 1H), 7.61 (d broad, 1H).
^{13}C-NMR d**:** 14.5 (CH), 24.2 (CH_2), 28.5 (CH_2), 28.6 (CH), 28.9 (CH_2), 31.1 (CH_2), 37.1 (CH_2), 47.1 (CH_2), 49.3 (CH_2), 50.5 (CH_2), 54.8 (CH_2), 61.2 (CH_2), 79.6 (C), 113.2 (CH), 122.7 (C), 127.3 (CH), 135.5 (C), 152.3 (C), 160.3 (C).
MS: m/z 427 (MH^+, 100), 413 (13), 371 (10), 307 (6).
HRMS: calcd. for $C_{20}H_{34}N_4O_6$ 426,24784, found 426.25674.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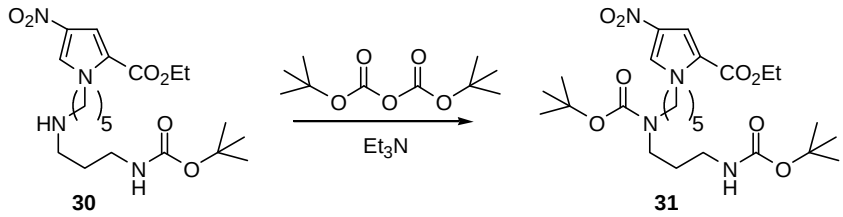
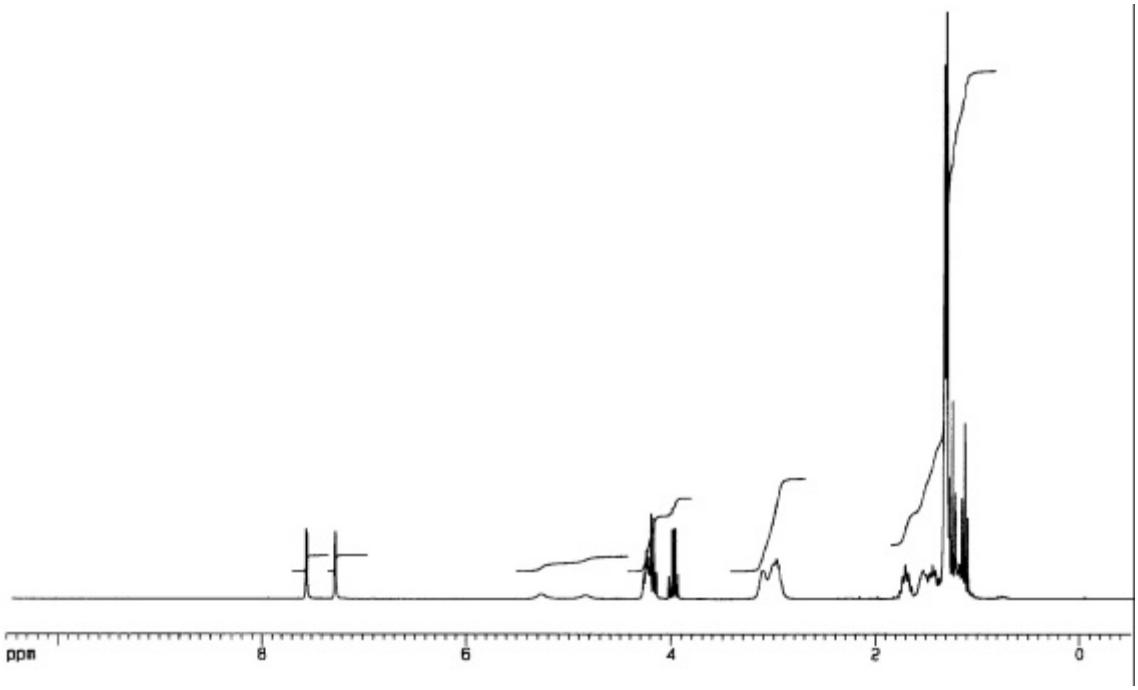
1-[5-((*tert*-butoxycarbonyl){3-[(*tert*-butoxycarbonyl)amino]propyl}amino)penthyl]-4-nitro-1 *H*-pyrrole-2-ethyl carboxylate (31)



To a solution of amine **30** (570 mg, 1.4 mmol) in CH_2Cl_2 (25 mL) was added Et_3N (0.97 mL, 7 mmol) and the solution was cooled to 0 ºC. A solution of BOC_2O (436 mg, 2.0 mmol) in CH_2Cl_2 (10 mL) was added and the resulting mixture was allowed to stir for 4 h at room temperature and was washed un with saturated aqueous solution of $NaHCO_3$. The combined organic layers were dried with $MgSO_4$ and solvent evaporated *in vacuo*. The crude residue was purified by flash column chromatography (20% EtOAc/hexane) affording product **31** as a oily solid (707 mg, 96%).

^{1}H-NMR d**:** 1.20-1.65 (m, 25H) 2.93-3.22 (m, 6H), 4.15-4.23 (m, 4H), 7.28 (s, 1H), 7.56 (s, 1H).
^{13}C-NMR d**:** 14.4 (CH), 21.2 (CH), 23.9 (CH_2), 28.7 (CH), 31.2 (CH_2), 37.7 (CH_2), 44.0 (CH_2), 44.1 (CH_2), 46.9 (CH_2), 50.6 (CH_2), 52.1 (CH), 60.6 (CH_2), 61.2 (CH_2), 77.6 (C), 79.8 (C), 113.3 (CH), 122.7 (C), 127.1 (CH), 135.6 (C), 156.3 (C), 156.7 (C), 160.2 (C).
MS: m/z 452 (0.1), 113 (12), 308 (3), 57 (100).
HRMS: calcd. for $C_{25}H_{42}N_4O_8$ 526.30027, found 526.30169.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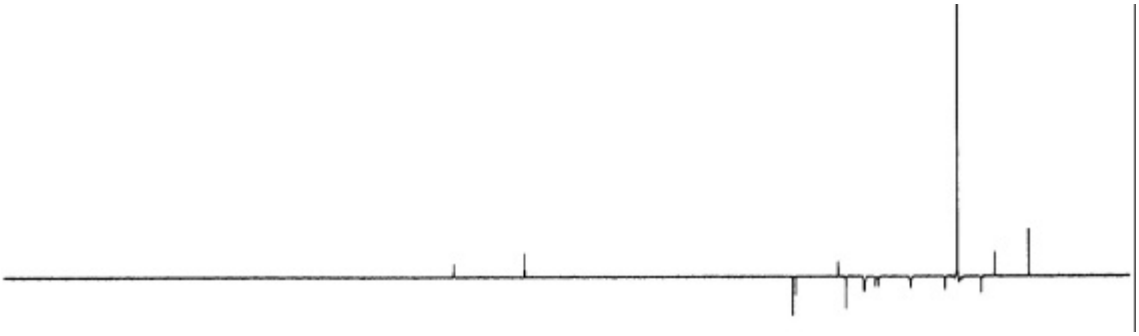

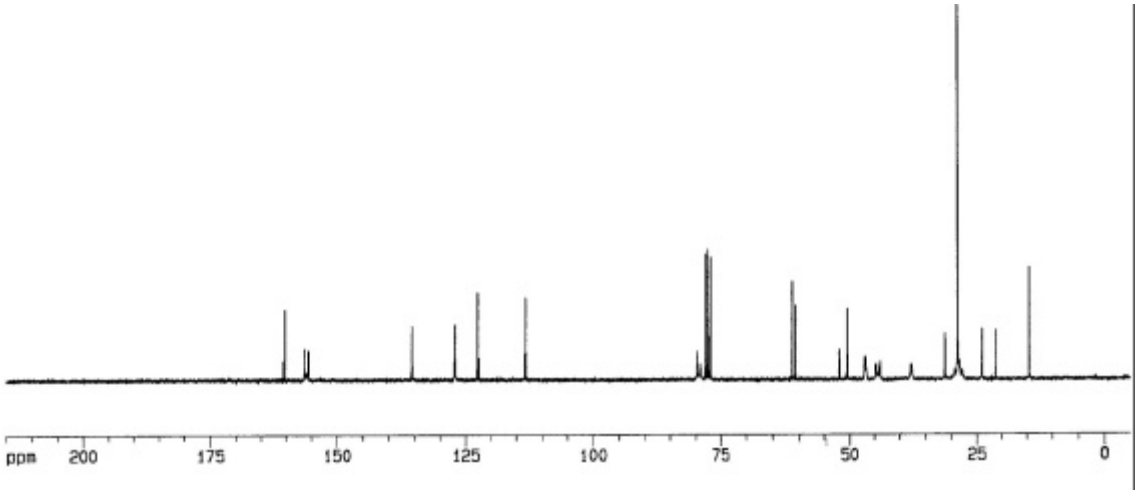
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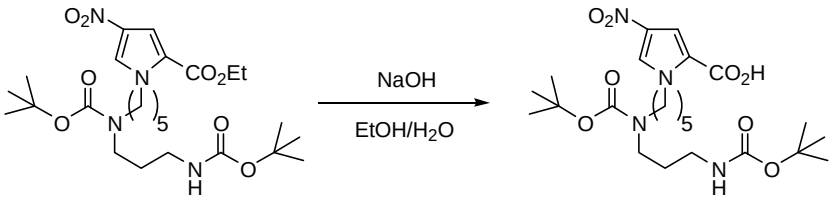
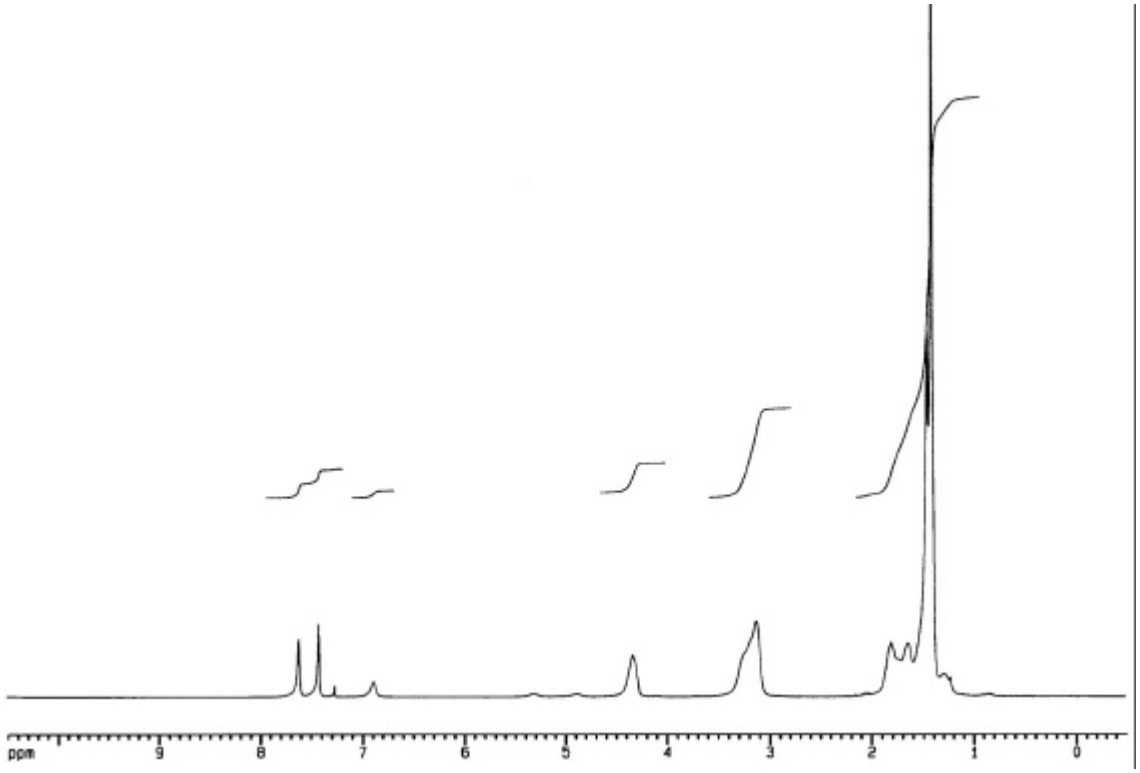
1-[5-((*tert*-butoxycarbonyl){3-[(*tert*-butoxycarbonyl)amino]propyl}amino)penthyl]-4-nitro-1*H*-pyrrole-2-carboxylic acid (32)



31 **32**

Pyrrole **31** (1.06g 2.03 mmol) was treated with a solution of NaOH (4 mL, 1 M en 70% EtOH/H_2O) at room temperature for 2 h. The solution was acidified with a saturated solution of citric acid to pH 5. This was then extracted with CH_2Cl_2. The combined organic extracts were dried with $MgSO_4$ and solvent was evaporated to give product **32** as a white powder (800 mg, 79%).

^{1}H-NMR d (CD_3OD)**:** 1.20 (m, 18H), 1.5-1.9 (m, 6H) 3.1-3.4 (m, 6H), 4.2-4.4 (m, 2H), 7.26 (s, 1H), 7.62 (s, 1H).
^{13}C-NMR d (CD_3OD)**:** 23.1 (CH_2), 24.2 (CH_2), 28.7 (CH), 30.2 (CH_2), 38.1 (CH_2), 44.1 (CH_2), 46.9 (CH_2), 47.5 (CH_2), 51.2 (CH_2), 79.5 (C), 81.8 (C), 114.1 (CH), 123.1, 126.9 (CH), 135.8 (C), 156.2 (C), 158.0 (C), 164.1 (C).
MS: m/z 499 (MH^+, 61), 343 (12), 154 (100).
HRMS: calcd. for $C_{23}H_{38}N_4O_8$ 498.26897, found 498.27919.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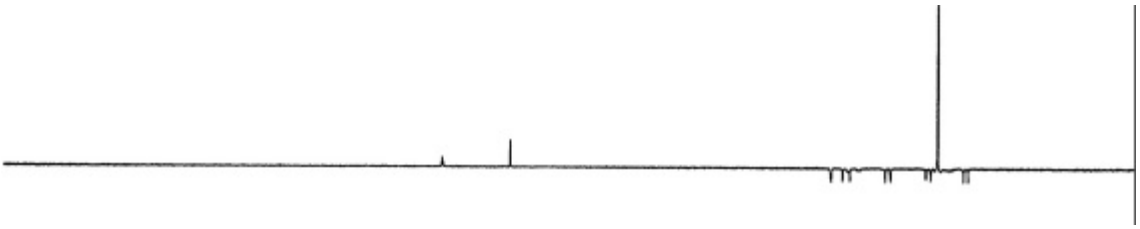

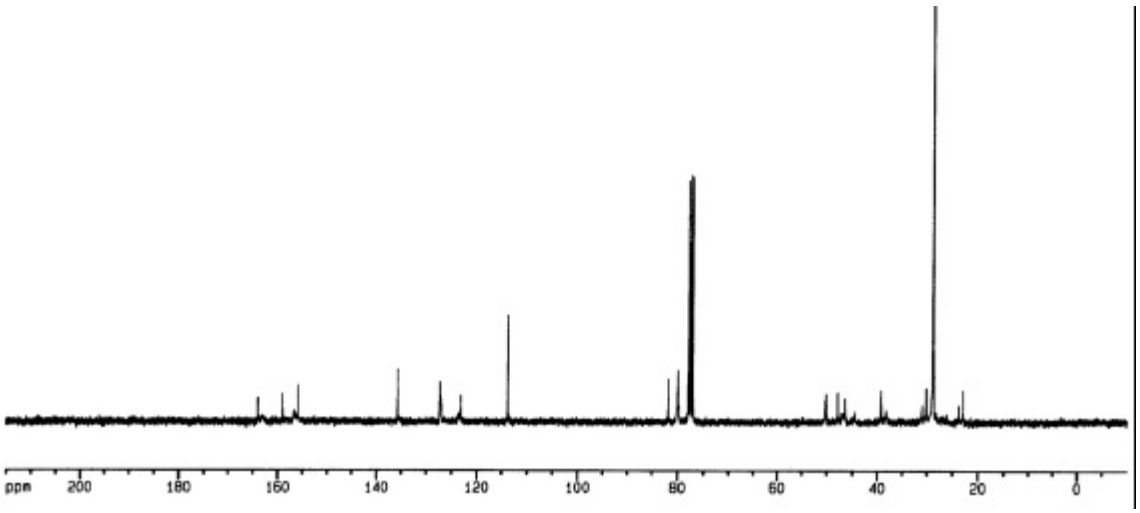
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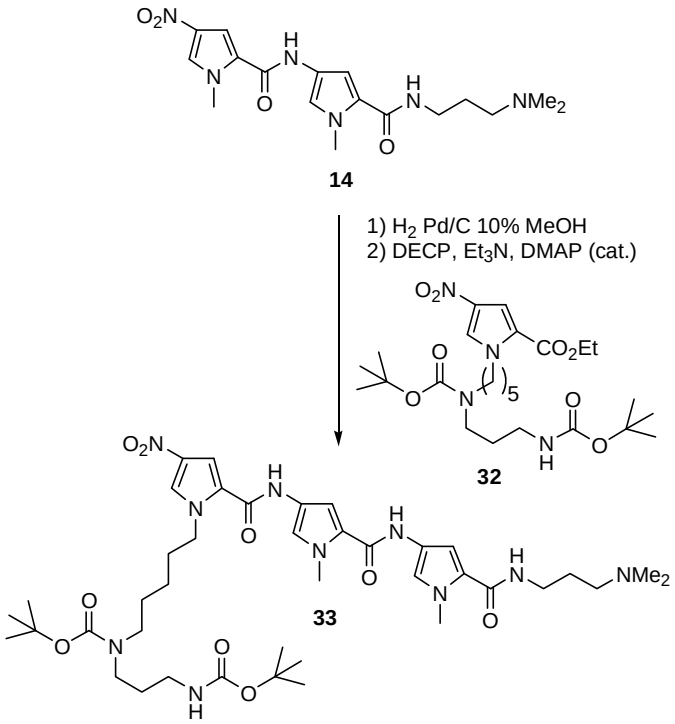
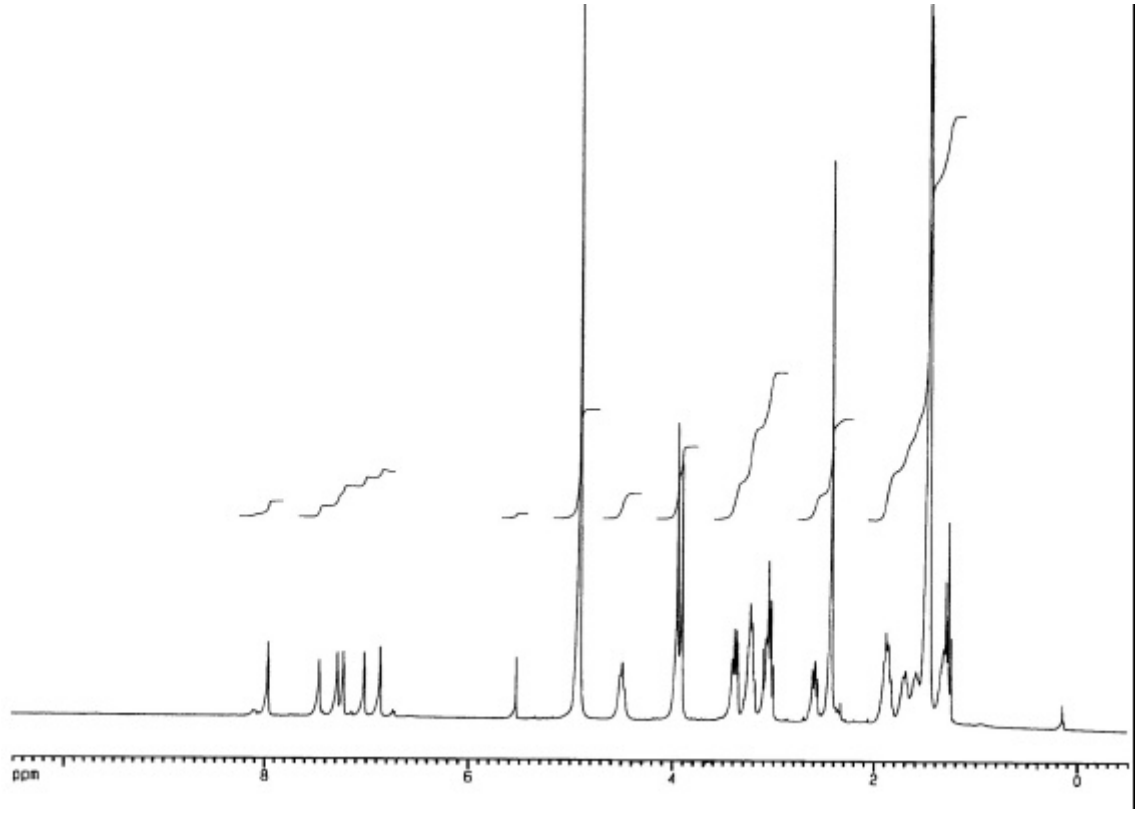
***N*2-(5-{[3-(dimetylamino)propyl]carbamoyl}-1-methyl-1 *H*-3 pyrrolyl-4-[(1-{5-[(*tert*-butoxycarbonyl) {3-[(*tert*-butoxycarbonyl)amino]propyl}amino]penthyl}-4-nitro-1 *H*-2-pyrrolyl) carboxamido] –1-methyl-1 *H*-2-pyrrolcarboxamido (33)**



A solution of dipyrrole **14** (430 mg, 1.131 mmol) in MeOH (50 mL) was hydrogenated over 10% palladium on charcoal (200 mg) at room temperature (balloon pressure). The catalyst was removed by filtration through celite and the filtrates were concentrated *in vacuo*. The residue was dissolved in THF/DMF (10 mL 8:2) and added over another solution previously prepared of acid **32** (470 mg, 0.943 mmol), DECP (184 mg, 172 µL, 1.131 mmol), Et_3N (476 mg, 0.65 mL, 4.71 mmol) and DMAP (10 mg) in THF/DMF (12 mL 8:2) cooled at 0 ºC. The resulting mixture was allowed to stir at room temperature for 10 h. MeOH (5 mL) was added and solvents were removed under reduced pressure, and the product was purified by flash column chromatography (1% Et_3N, 10% MeOH, CH_2Cl_2) to afford **33** (766 mg, 82% as a yellowish solid whose

^{1}H-NMR d (CD_3OD)**:** 1.2-1.4 (m, 4H), 1.5 (s, 18H), 1.6 (m, 2H), 1.7 (m, 2H), 1.8-2.0 (m, 4H), 2.38 (s, 6H), 2.56 (m, 2H), 3.05 (m, 4H), 3.21 (m, 4H), 3.30 (m, 2H), 3.79 (s, 3H), 3.81 (s, 3H), 4.42 (m, 2H), 6.81 (s, 1H), 7.0 (s, 1H), 7.19 (s, 1H), 7.26 (s, 1H), 7.41 (s, 1H), 7.91 (s, 1H).
^{13}C-NMR d (CD_3OD)**:** 25.0 (CH_2), 28.1 (CH_2), 29.0 (CH), 30.0 (CH_2), 32.6 (CH_2), 37.5 (CH), 38.0 (CH_2), 39.1 (CH_2), 45.2 (CH), 46.1 (CH_2), 50.0 (CH_2), 50.1 (CH_2), 51.1 (CH_2), 58.3 (CH_2), 80.0 (C), 81.1 (C), 106.4 (CH), 106.6 (CH), 109.7 (CH), 120.8 (CH), 121.1 (CH), 123.0 (C), 123.2 (C), 124.9 (C), 125.1 (C), 127.0 (CH), 128.1 (CH), 136.4 (C), 158.0 (C), 158.9 (C), 160.0 (C), 161.8 (C), 165.9 (C).
MS: m/z 827 (100), 627 (35), 307 (14), 179 (8).
HRMS: calcd. for $C_{40}H_{62}N_{10}O_9$ 826.46535, found 826.46597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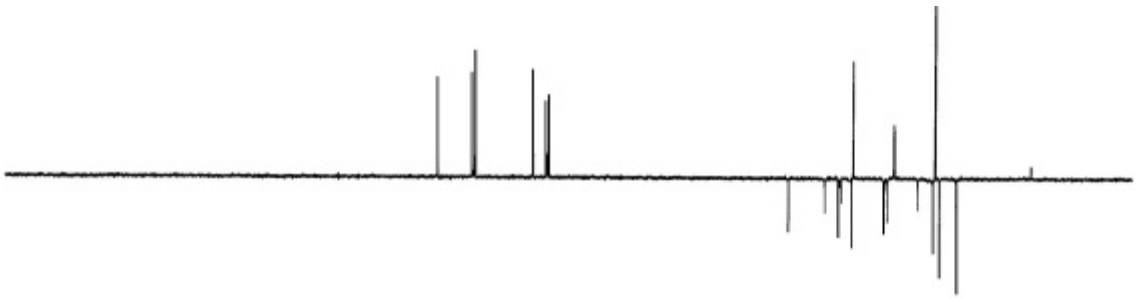

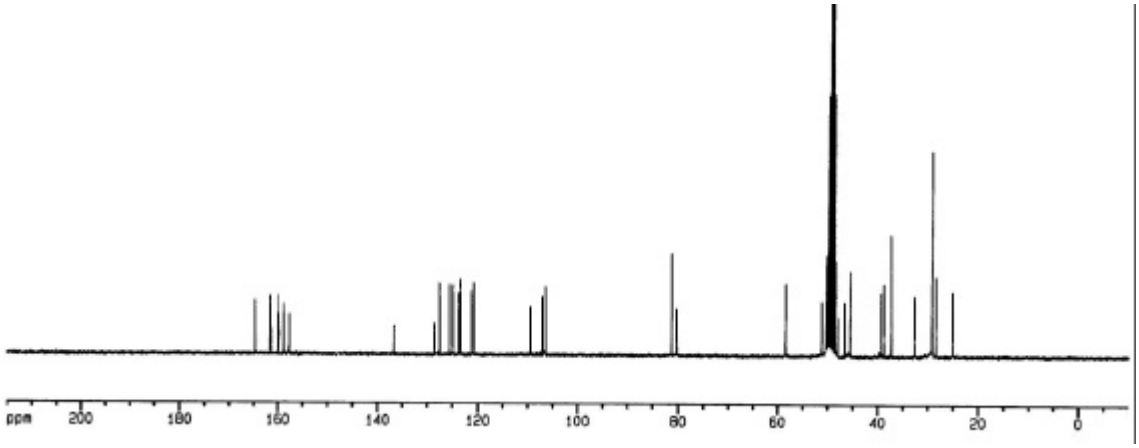
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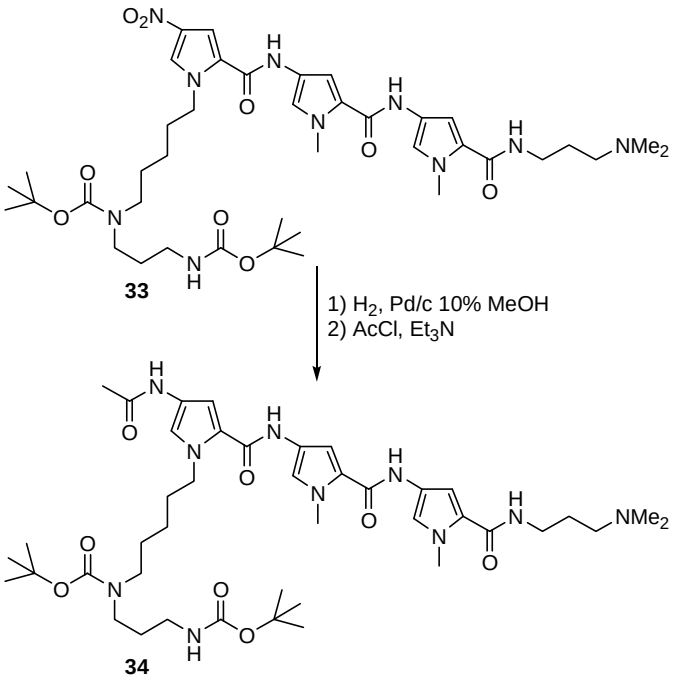
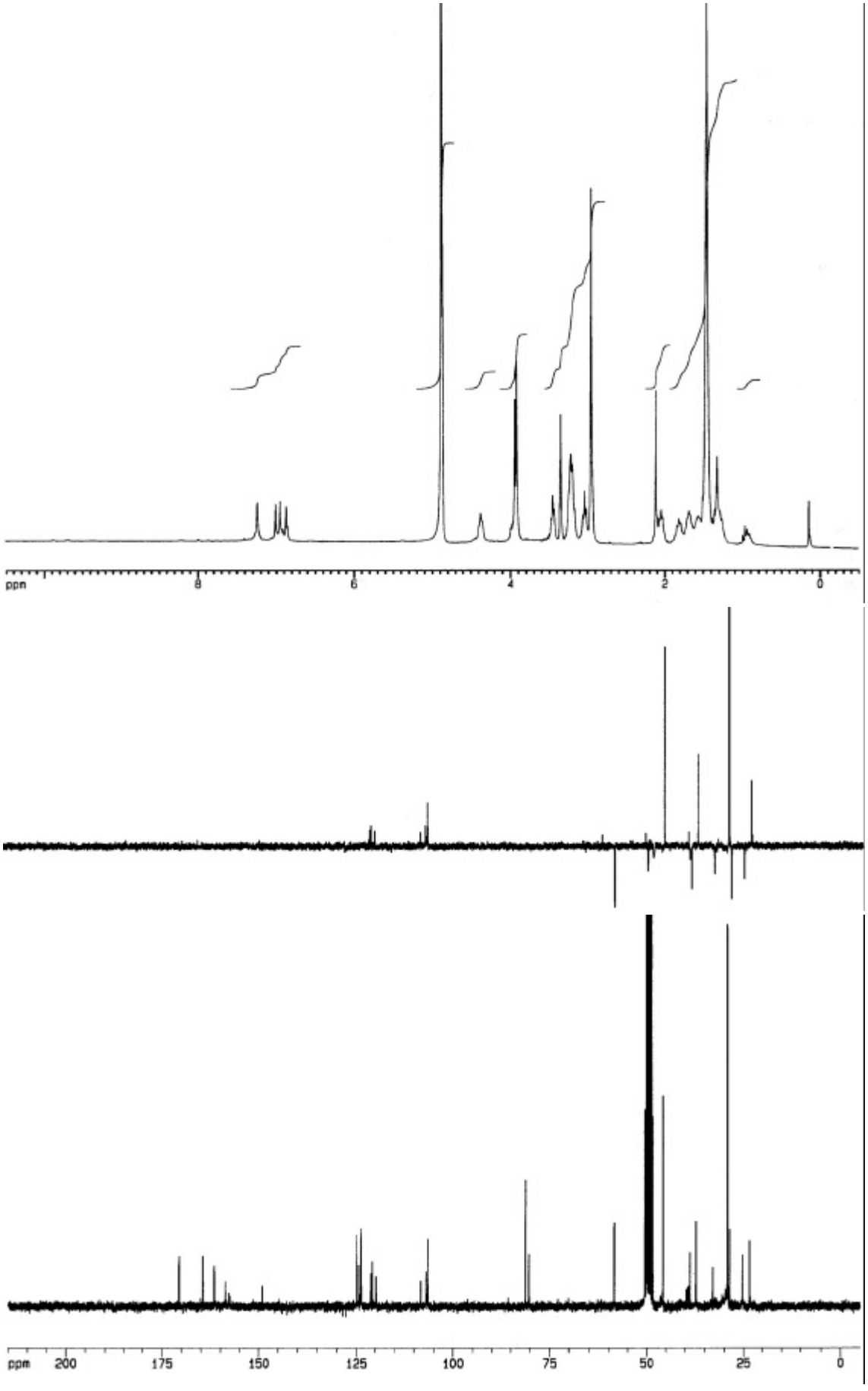
***N*2-(5-{[3-(dimethylamino)propyl]carbamoyl}1-methyl-1*H*-pyrrolyl)-4-{[1-{5-((*tert*-butoxycarbonyl) {3-[(*tert*-butoxycarbonyl)amino]propyl}amino)penthyl}-4- (Methyl carboxamido) -1*H*-2-pyrrolyl] carboxamido} -1-methyl-1 *H*-2-pyrrole carboxamido (34)**



A solution of tripyrrole **33** (100 mg, 0.121 mmol) in MeOH (40 mL) was hydrogenated for 45 min over 10% palladium on charcoal (50 mg) at room temperature (balloon pressure). The catalyst was removed by filtration through celite and the filtrate was concentrated. The residue was immediately dissolved in DMF (10 mL) containing Et_3N (0.17 mL, 1.209 mmol) and cooled to 0 ºC. AcCl (9.5 µL, 0.1331 mmol in 200 µL of DMF) was added and the solution was stirred at 0 ºC for 30 min and another at room temperature for another 10 h. Solvents were evaporated in vacuo and the resulting residue was purified by flash column chromatography (aluminium oxide, 5% MeOH, CH_2Cl_2) to give product **34** as a yellow solid (85 mg, 84%).

^{1}H-NMR d (CD_3OD)**:** 1.2-1.4 (m, 4H), 1.4-1.6 (m, 20H), 1.6-1.7 (m, 4H), 1.96 (s, 3H), 2.2 (s, 6H), 2.35 (m, 2H), 2.89 (m, 2H), 3.01 (m, 4H), 3.20 (m, 2H), 3.71 (s, 3H), 3.79 (s, 3H), 4.21 (m, 2H), 6.67 (m, 2H), 6.82 (s, 1H), 7.16 (m, 3H).
^{13}C-NMR d (CD_3OD)**:** 23.8 (CH), 25.1 (CH_2), 28.8 (CH_2), 29.2 (CH), 32.1 (CH_2), 37.5 (CH), 38.9 (CH_2), 39,2 (CH_2), 45.1 (CH), 45.2 (CH_2), 47.3 (CH_2), 50.0 (CH_2), 58.0 (CH_2), 80.3 (C), 81.2 (C), 106.5 (CH), 106.9 (CH), 108.2 (CH), 119.9 (CH), 120.8 (CH), 121.2 (CH), 123.8, (C) 124.4 (C), 124.9 (C), 158.7 (C), 161.6 (C), 161.7 (C), 164.5 (C), 170.6 (C).
MS: m/z 839.5 (100), 639 (19), 309 (12), 231 (48).
HRMS: calcd. for $C_{42}H_{66}N_{10}O_8$ 838.50651, found 838.46535.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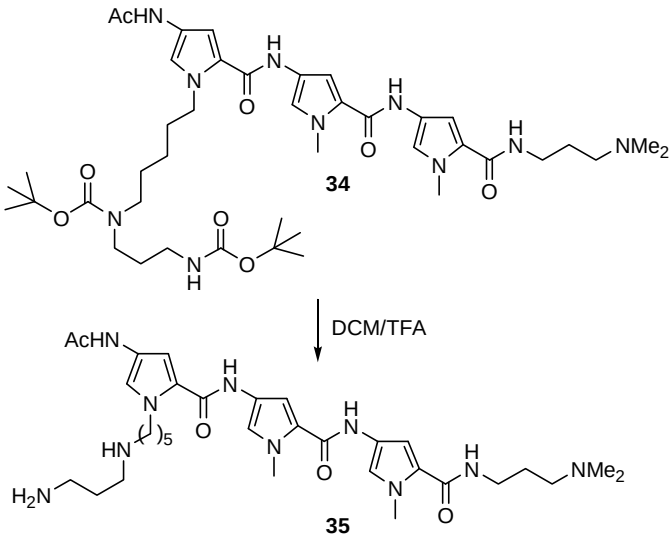
***N*2-(5-{[3-(dimetilamino)propil]carbamoil}1-metil-1 *H*-pirrolil)-4-{[1-{5-[(3-aminopropil) amino] pentil}-4-(metilcarboxamido)-1 *H*-2-pirrolil]carboxamido}-1-metil-1 *H*-2-pirrol carboxamido (35)**



A solution of tripyrrole **34** (84 mg, 0.1 mmol) in CH_2Cl_2 (3 mL) was cooled to 0 ºC and TFA (3 mL, 15 min) was added dropwise. The resulting orange solution was stirred at 0 ºC for 1 h and at room temperature for another 2 h. Solvents were removed in vacuo at room temperature and residual TFA was removed by codistillation with CH_2Cl_2. The residue was identified as the desired product **35**, showing by HPLC to be highly pure. HLPC conditions: analytical column C-18 5 → 95 % B, 30 min. t_R = 9.315.

^{1}H-NMR d (CD_3OD)**:** 1.14-1.35 (m, 4H), 1.5-1.9 (m, 4H), 2.0 (s, 3H), 2.79 (s, 6H), 2.8-3 (m, 2H), 3-3.1 (m, 2H), 3.3 (m, 4H), 3.70 (s, 3H), 3.72 (s, 3H), 4.2 (m, 2H), 6.6 (s, 1H), 6.65 (s, 1H), 6.7 (s, 1H), 7.09 (m, 3H).
^{13}C-NMR d (CD_3OD)**:** 22.1 (CH), 24.0 (CH_2), 24.9 (CH_2), 25.2 (CH_2), 29.8 (CH), 30.0 (CH_2), 35.0 (CH_2), 35.1 (CH), 35.2 (CH_2), 44.3 (CH), 46.3 (CH_2), 47.5 (CH_2), 48.1 (CH_2), 55.0 (CH_2), 107.3 (CH), 111.9 (C), 116.0 (C), 121.5 (CH), 122.7 (CH), 124.1 (C), 124.2 (C), 124.4 (C), 160.0 (C), 165.3 (C), 171.2 (C).
MS: m/z 639 (MH^+, 10), 422 (8), 391 (21), 309 (73), 278 (100).
HRMS: calcd. for $C_{32}H_{50}N_{10}O_4$ 638.40165, found 638.39561.

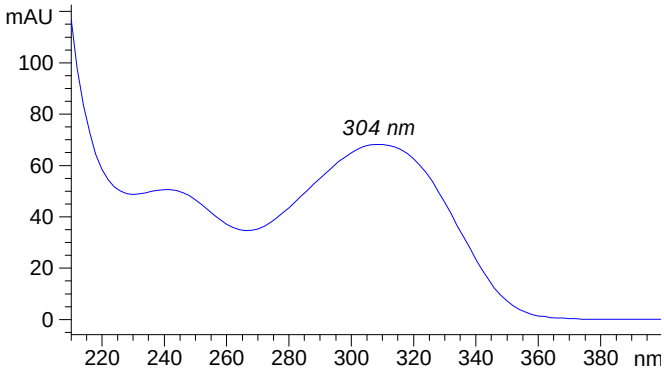
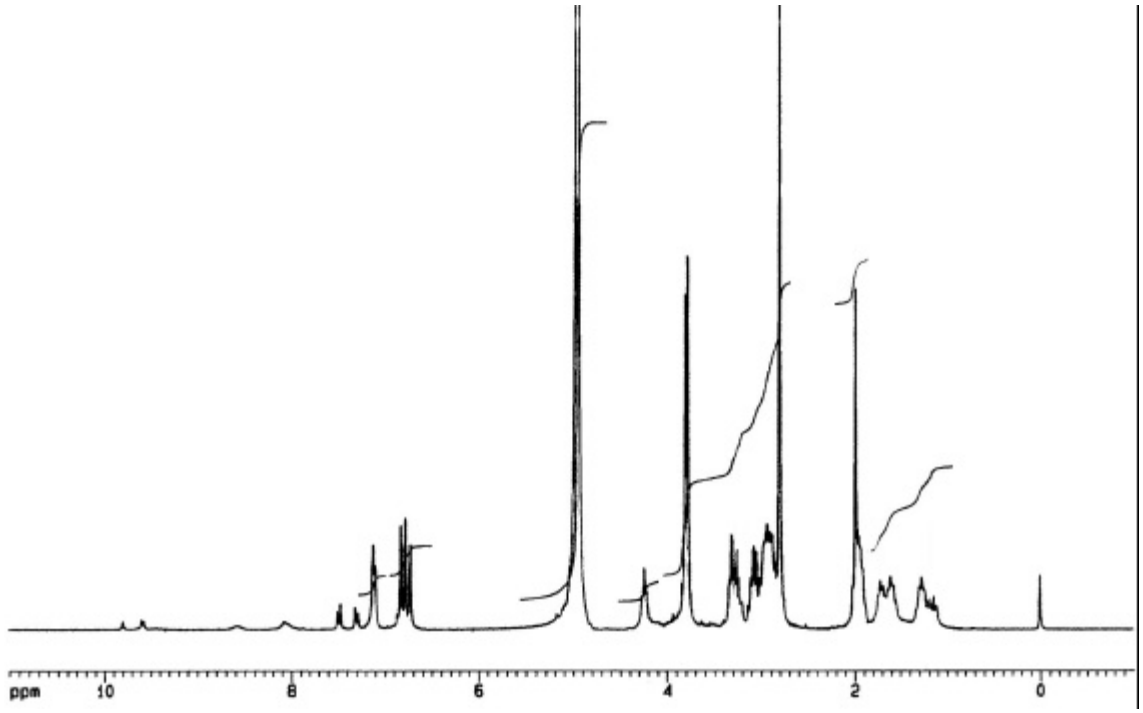


Figure 1. UV spectrum of **35**.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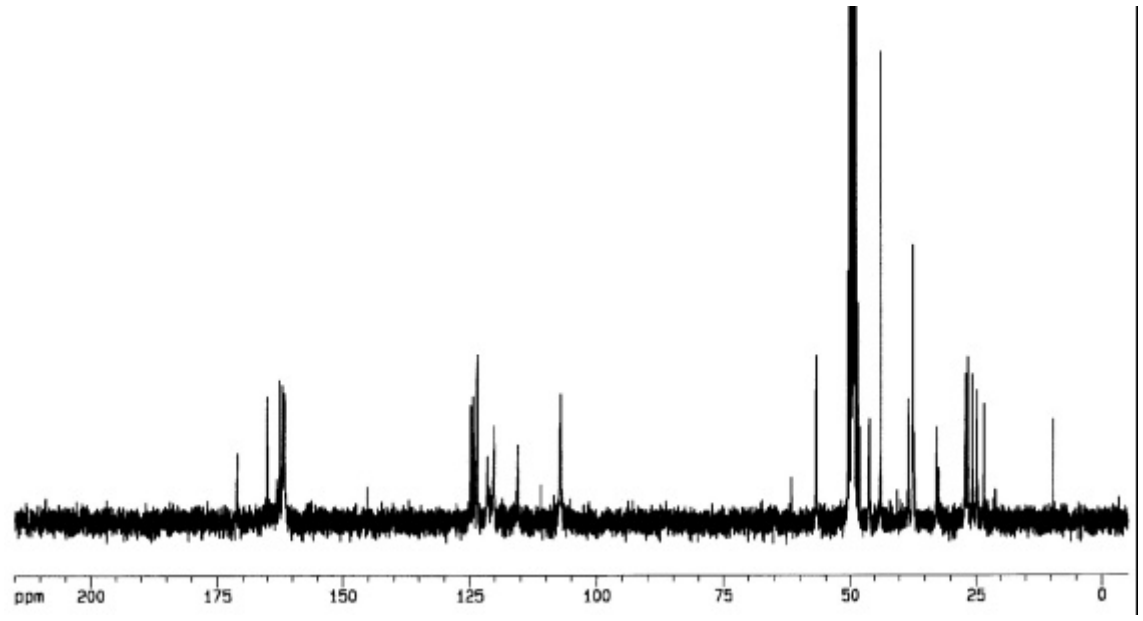
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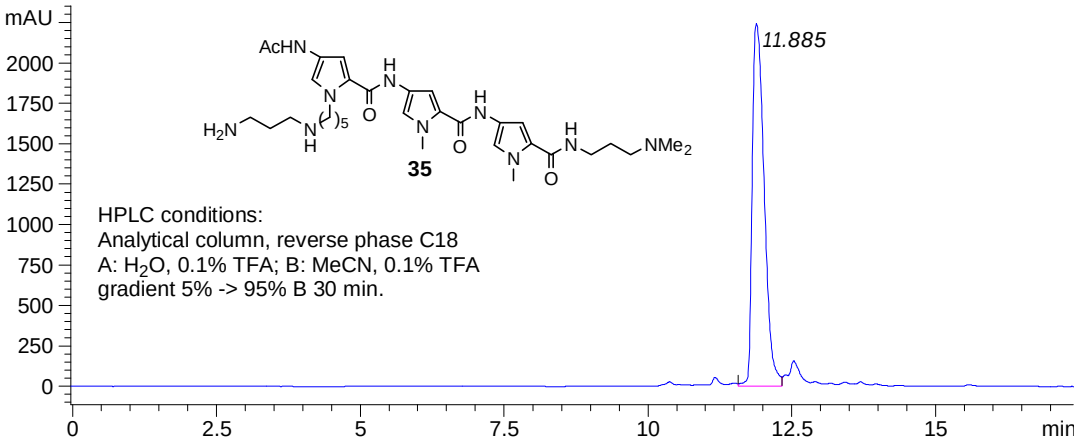
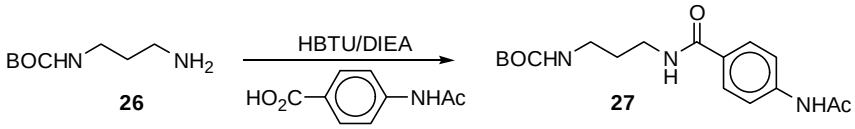


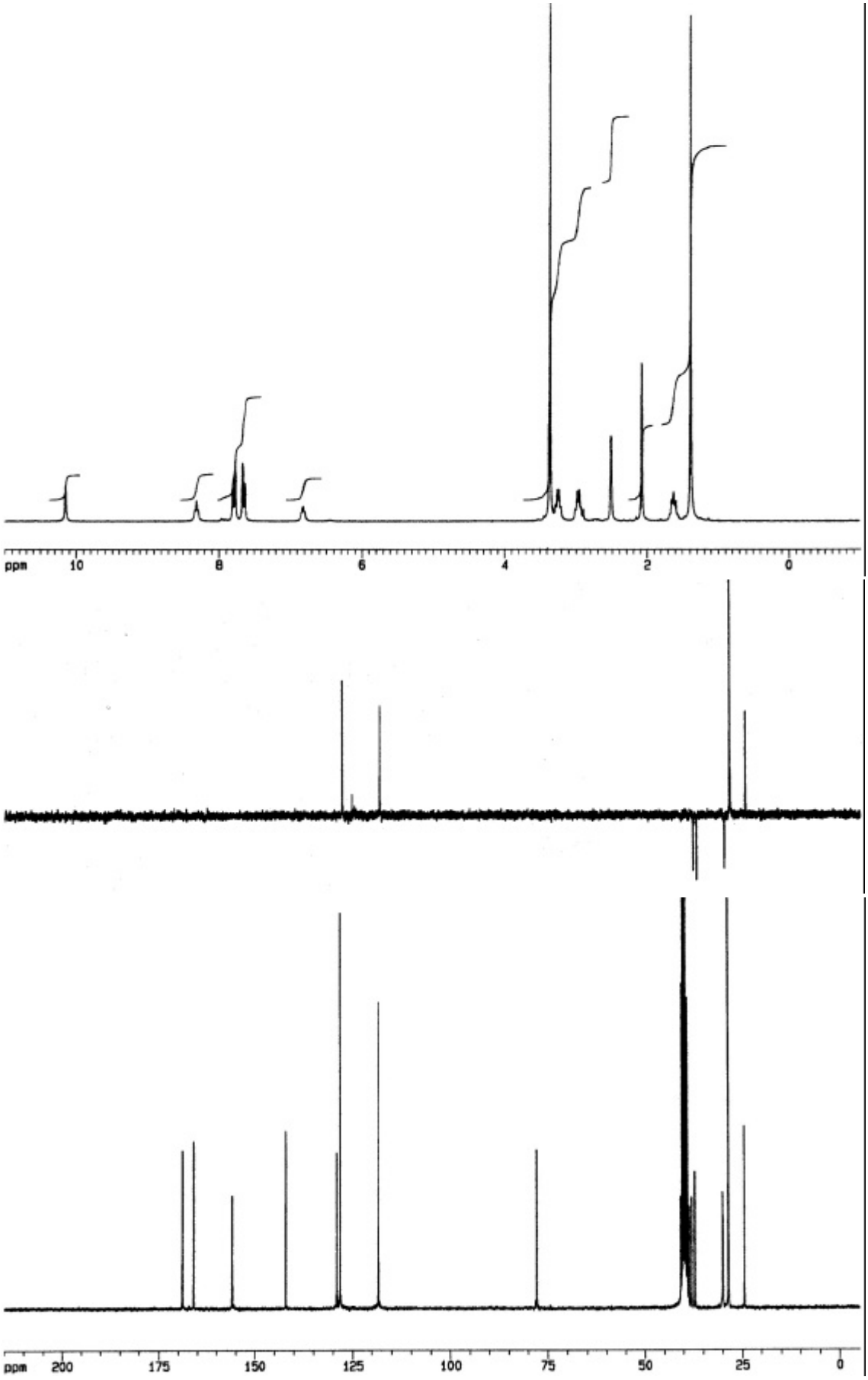
Figure 2. HPLC chromatogram of **35**.

***tert*-butyl 3-{[4-(acetylamino)benzoyl]amino}propylcarbamate (27)**



To a solution of amine **26** (250 mg, 1.435 mmol) and DIEA (1.252 mL, 7.17 mmol) in DMF (5 mL) was added a mixture of acetamidobenzoic acid (334 mg, 1.86 mmol) and HBTU (707 mg, 1.86 mmol) prepared 5 min before. The resulting solution was stirred for 4 h and H_2O (50 mL) was added and the mixture was extracted with CH_2Cl_2. The combined organic layers were dried with Na_2SO_4 and the solvents removed *in vacuo* to give product **27** as a light-yellow oil that solidified on standing. (336 mg, 70%).

^{1}H-RMN d (DMSO-D_6)**:** 1.38 (s, 9H), 1.60 (m, 2H), 2.84 (s, 3H), 2.96 (m, 2H), 3.24 (m, 2H), 6.78 (t, 1H), 7.67 (d, 2H), 7.78 (d, 2H), 8.28 (t, 1H), 10.13 (s, 1H).
^{13}C-RMN d (DMSO-D_6)**:** 24.43 (CH), 28.57 (CH), 29.99 (CH_2), 37.21 (CH_2), 38.05 (CH_2), 77.83 (C), 118.37 (CH), 128,27 (CH), 129.17 (C), 142.13 (C), 155.94 (C), 169.01 (C), 169.21 (C).
MS: 335 (M^+, 66), 201 (13), 101 (80), 58 (54), 57 (100).
HRMS: calcd. for $C_{17}H_{25}N_3O_4$ 335.18451, found 335.12455.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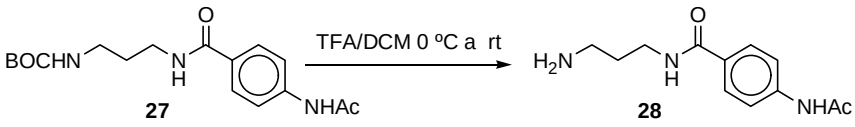
4-(acetylamino)-*N*-(3-aminopropyl)benzamide (28)



To a suspension of **27** (297 mg, 0.886 mmol) in CH_2Cl_2 (5 mL) cooled to 0 ºC, TFA (5 mL) was added dropwise, observing the formation of a clear solution that was stirred for 1 h and then heated to room temperature and stirred for another hour. Solvents were removed *in vacuo* and the residue redissolved in CH_2Cl_2 and removed again to ensure complete removal of TFA. HPLC of the white powder obtained showed only one product that was confirmed as product **28** by MS and NMR analysis.

^{1}H-RMN-d (DMSO-D6)**:** 1.84 (m, 2H), 2.12 (s, 3H), 2.85 (m, 2H), 3.37 (m, 2H), 7.56 (d, 2H), 7.69 (d, 2H), 7.88 (s, 1H).
MS: 235 (M^+, 62), 177 (34), 162 (23), 134 (63), 43 (100).
HRMS: calcd. for $C_{12}H_{17}N_3O_2$ 235.13208, found 235.13387.

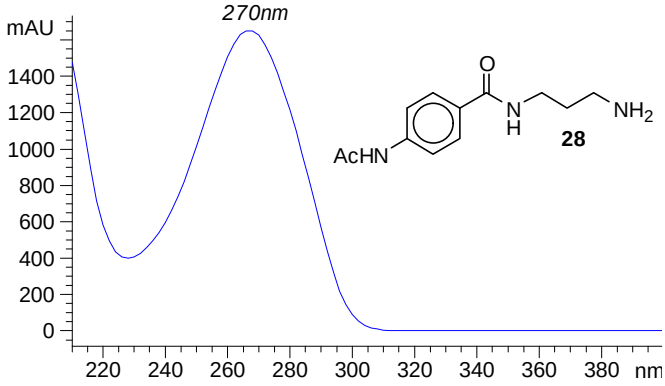
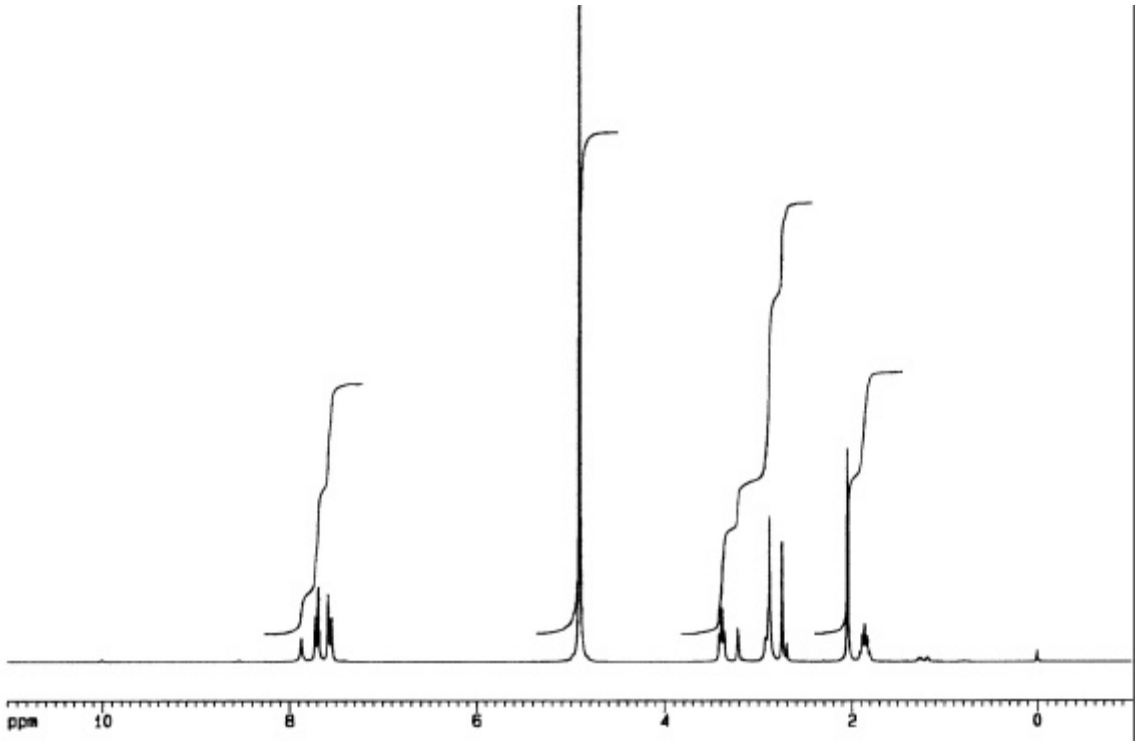


Figure 3. UV spectrum of amine **28**.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CD and UV spectra.

CD spectra were recorded using a *Jasco J-715* spectrometer coupled with a refrigerated water bath *Nestlab RTE-111*. All measurements were made using a 0.2 cm cell at 4 ºC. Concentration of the peptide hybrids was determined by UV spectroscopy. All UV spectra were recorded using a *Varian Cary 100 Bio* with 1 cm cells.

All CD measurements were made using 300 ìL of solutions of ds-DNA of known concentration (5 ìM) at pH = 7.5 (phosphate buffer 10 mM) and NaCl (100 mM). Over those solutions a known volume of the mother solution of the molecule studied was added. Final concentration of peptide and DNA were always near 5 ìM. The resulting mixtures were incubated for 5 min before registering.

CD spectra parameters:	
Cell Length:	0.2 cm
Concentration:	5×10^{-6} M
Solvent:	H_2O/buffer
Temperature:	4 º C
Data mode:	Mol. Ellip.
Range:	195-380 nm
Resolution:	0.2 nm
Band width:	2.0 nm
Sensitivity:	10 mdeg
Response:	0.25 sec
Speed:	100 nm/min
Accumulation:	10 scans

The spectra showed are the average of 10 scans and were slightly smoothed using the “smooth” macro implemented in the program *Kaleidagraph* (v 3.5 by Synergy Software). Spectra of the peptides in presence of DNA were calculated as the difference between the spectra of the peptide - DNA mixture and the spectrum of free DNA.

Determination of the affinity constant for tripyrrole derivatives.

CD spectroscopy was used to determine the affinity constant of the tripyrrole derivatives for selected DNA sequences. Considering the equilibrium L + M LM, where L is the ligand, M the macromolecule, and LM the complex formed by their association, the *affinity constant* K_{aff} is defined as:

$$K_{aff} = \frac{[ML]}{[M]\,[L]}$$

In the equilibrium, the exact concentration of free ligand [L] is given by the following expression:

$$[L] = 0.5 \left\{ ([M]_T + [L]_T + 1/K_{aff}) - \sqrt{K_{aff}\,([M]_T + [L]_T + 1/K_{aff})^2 - 4\,K_{aff}\,[M]_T\,[L]_T} \right\}$$

The recorded signal (CD in our case) can be related with that concentration resulting in the equation used to fit the data.[7]

$$[q] = 0.5\,[q]_\infty \left\{ ([M]_T + [L]_T + K_D) - \sqrt{([M]_T + [L]_T + K_D)^2 - 4\,[M]_T\,[L]_T} \right\}$$

Where $[q]_\infty$ represents the value of ellipticity at infinitum and $[q]$ the measured value of ellipticity at each point. $[M]_T$ is the total concentration of the macromolecule (DNA), $[L]_T$ the total concentration of ligand (tripyrrole), K_D is the dissociation constant. Data obtained for tripyrrole **4** are shown. Affinity constants are in both cases in the micromolar range (~0.8 ìM for tripyrrole **3** and ~1.0 ìM for tripyrrole **4**). Curve fitting was carried out using nonlinear least-squares analysis.

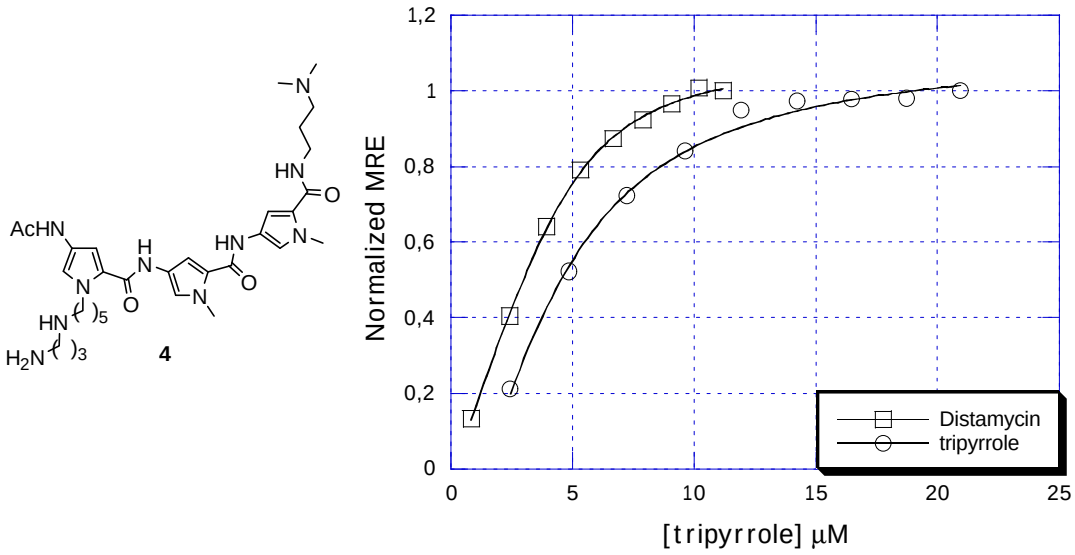
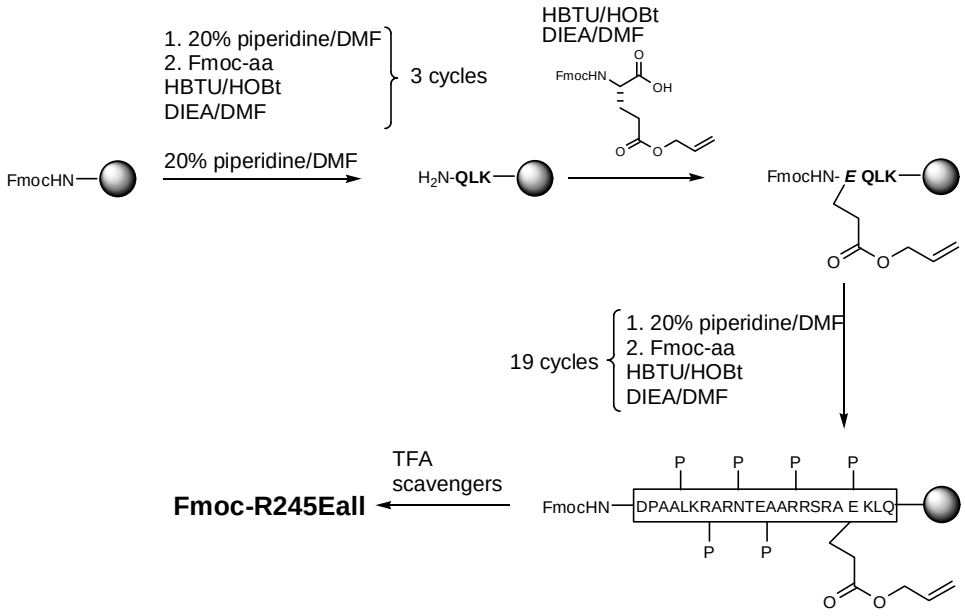


Figure 4. CD titration of tripyrrole **4** with ds-DNA 5'-CGCGAAAAAGCGC-3' compared with Distamycin. Values of MRE obtained at 330 nm are normalized and given arbitrary the value of 1 on saturation for easier comparison.

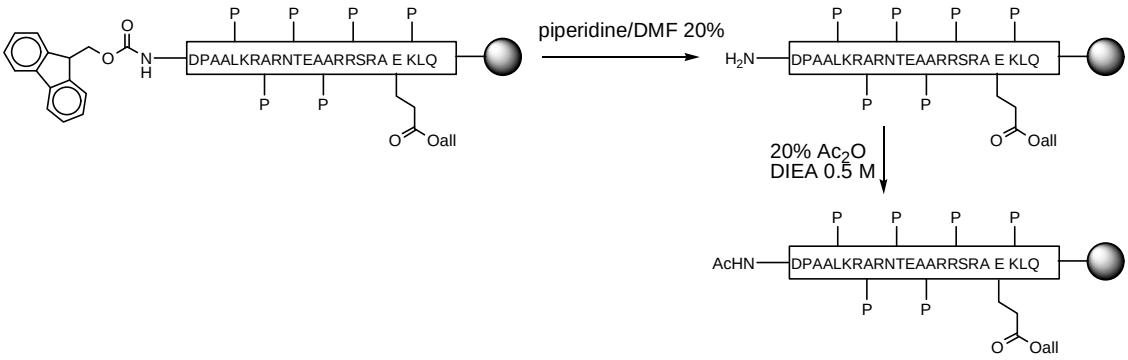
[7] Pilch, D. S.; Poklar, N., Baird, E. E.; Dervan, P. B., Breslauer, K. *Biochemistry*, **1999**, *38*, 2143.

Solid Phase Synthesis of peptide Fmoc-R245Eall



Synthesis of peptide **Fmoc-R245Eall** was performed using standard Fmoc SPPS conditions, starting with Rink MBHA amide resin (217 mg, 0.1 mmol, load 0.46 mmol/g). After completion of the synthesis deprotection/cleavage of 50 mg resin afforded after RP-HPLC 8 mg of a peptide identified by MS to be the desired product. (approx. 44%)

Preparation of peptide R245E: Acetillation of N terminal residue.



Over 165 mg of **Fmoc-R245E-all** peptide bound to the resin, a solution of piperidine (5 mL, 20% in DMF) was added and argon was passed through the mixture for 45 min. The resin was washed with DMF (3 x 5 mL x 5 min). Deprotection of Fmoc group can be confirmed by RP-HPLC analysis of the products by standard TFA/scavengers mixture treatment. Deprotection of Fmoc results in a shift on the t_R.

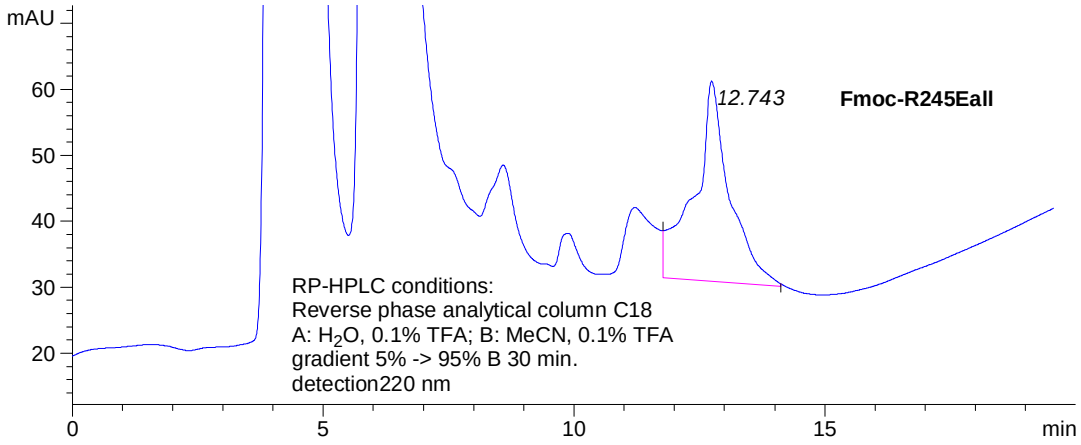


Figure 5. peptide **Fmoc-R245Eall**.

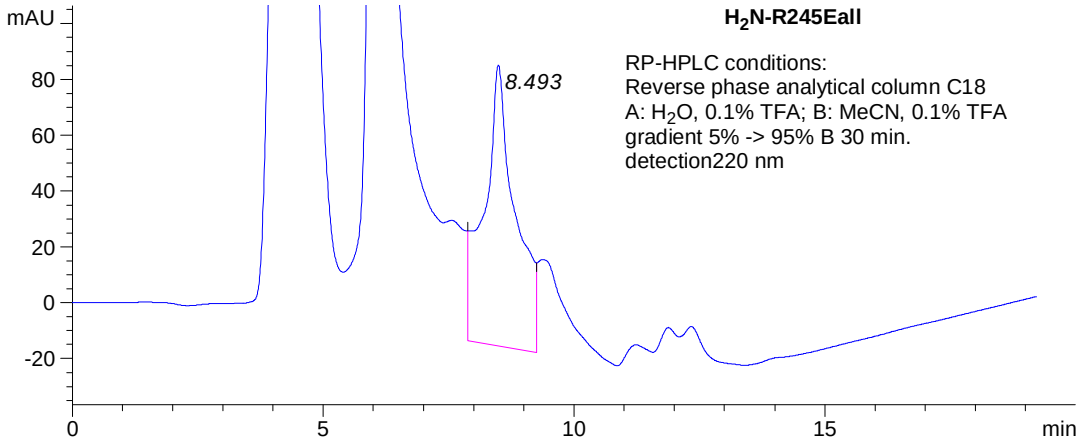


Figure 6. peptide **H_2N-R245Eall**.

Acetillation was carried out immediately after deprotection of the N terminal Fmoc protecting group. After washing the resin with DMF (3 x 5 mL x 5 min), DIEA (2.5 mL, 0.2 M in DMF) and Ac_2O (5 mL, 20% en DMF) were added. The resulting mixture was shacked for 1 h. After

filtration the resin was washed with DMF (3 x 5 mL x 5 min), *i*PrOH (5 mL x 5 min) and Et_2O (5 mL x 5 min.). HPLC analysis of the residue obtained after deprotection/cleavage clearly showed remove of the product at 8.5 min and a new product with retention time of 9.3 min, corresponding to the acetylated product as shown by MS (electrospray).

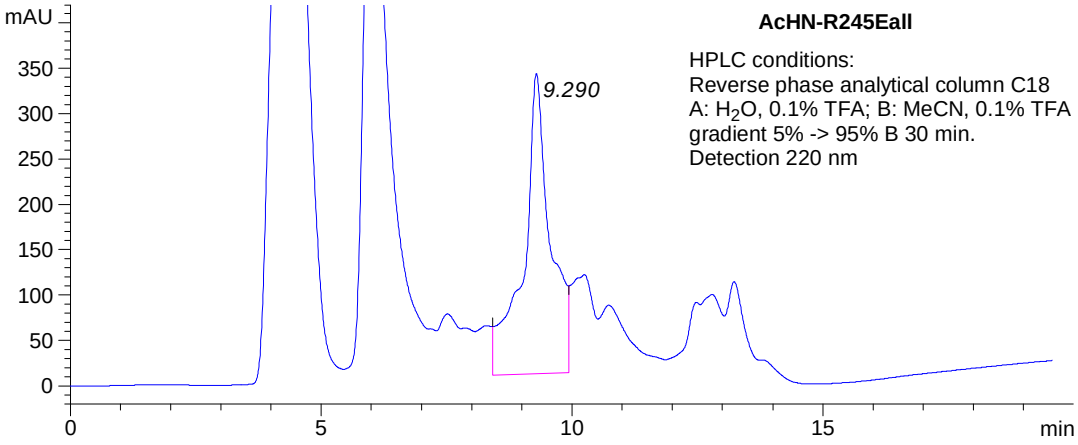


Figure 7. HPLC of **AcHN-R245EaII**

R245EaII MS (ES): [MH^+] m/z calcd. for $C_{112}H_{198}N_{43}O_{34}$ 2691.0, observed 2690.6.

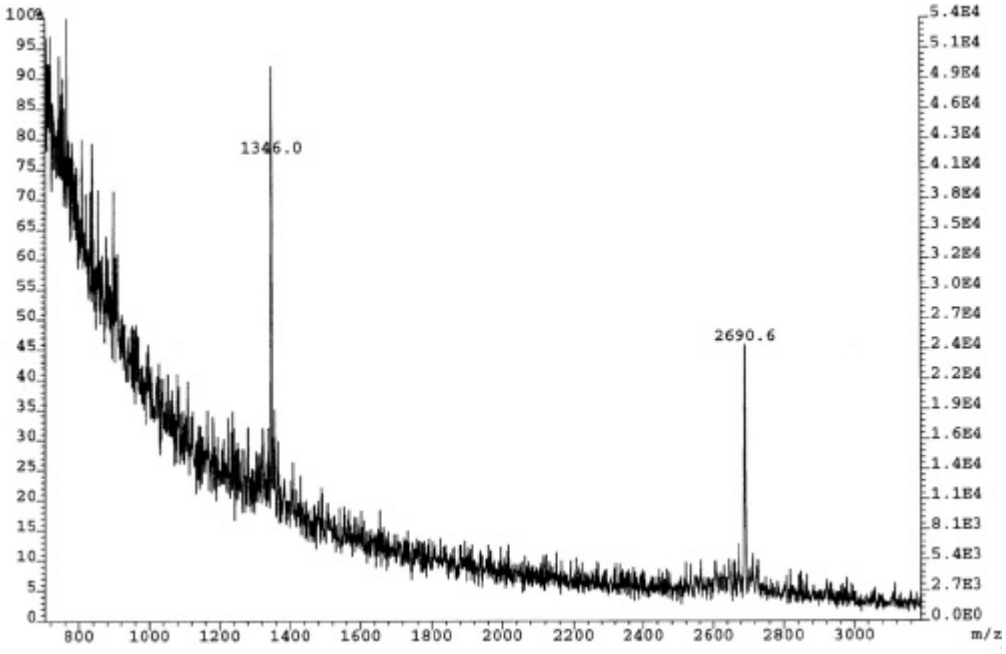
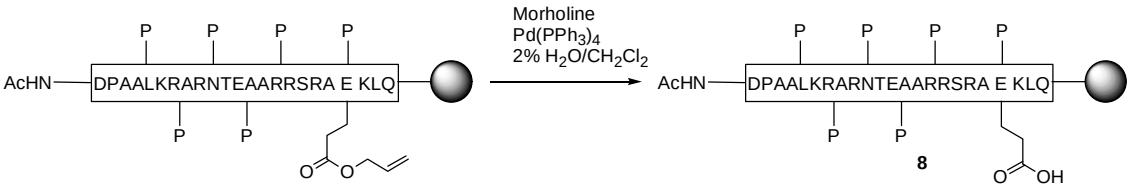


Figure 8. MS of peptide **AcHN-R245EaII**.

Preparation of peptide R245E: deprotection of allyl protecting group



Over 161 mg of resin (approx. 0.025 mmol of peptide) DMF (10 mL) was added and the resin was shacked for 4 h. The resin was filtrated and 2% H_2O/CH_2Cl_2 (6mL), morpholine (0.42 mL, 4.84 mmol) and $Pd(PPh_3)_4$ (29 mg, 0.025 mmol) were added. The mixture was shacked under argon for 12 h. The resin was filtered and washed with DMF (3 x 5 mL x 5 min), *i*PrOH (2 x 5 mL x 5 min) and CH_2Cl_2 (5 mL x 5 min).

Cleavage and deprotection of the product with the standard mixture, and its HLPC analysis shows a new product with retention time of 16.9 min.

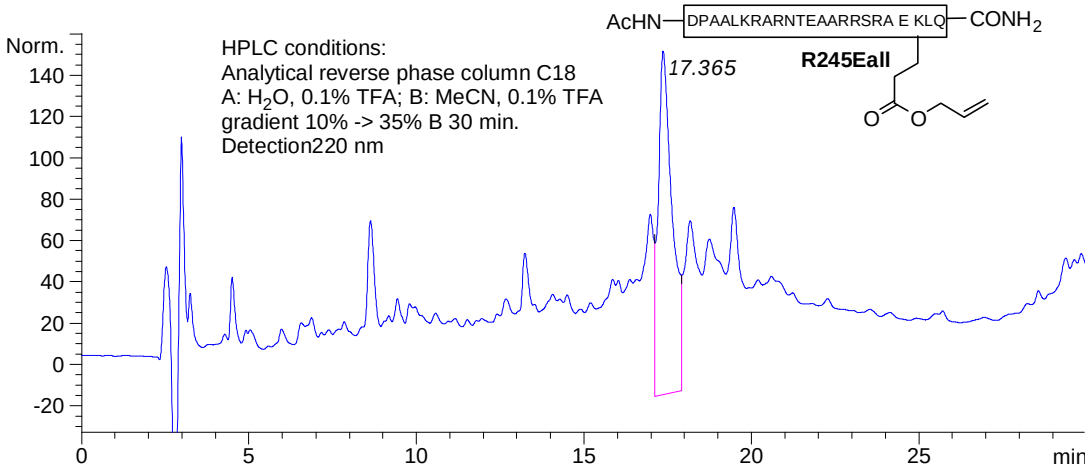


Figure 9. HPLC of peptide **R245Eal**.

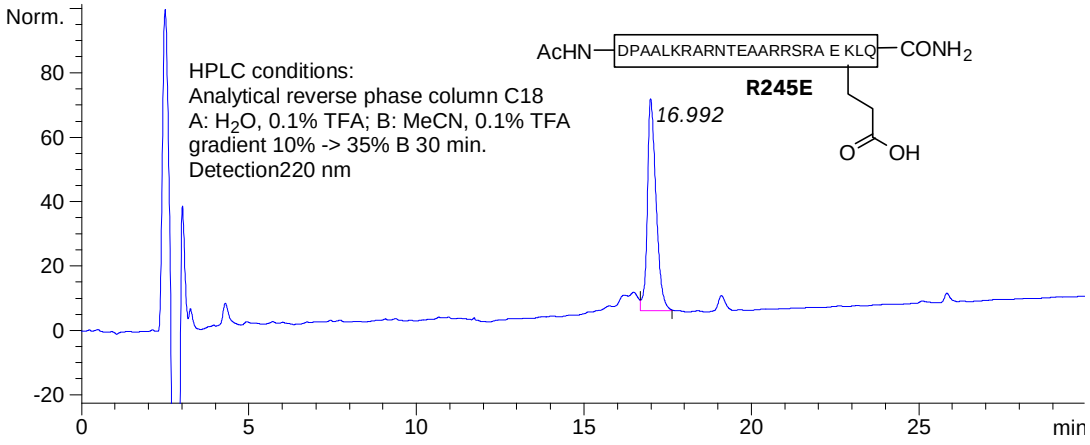
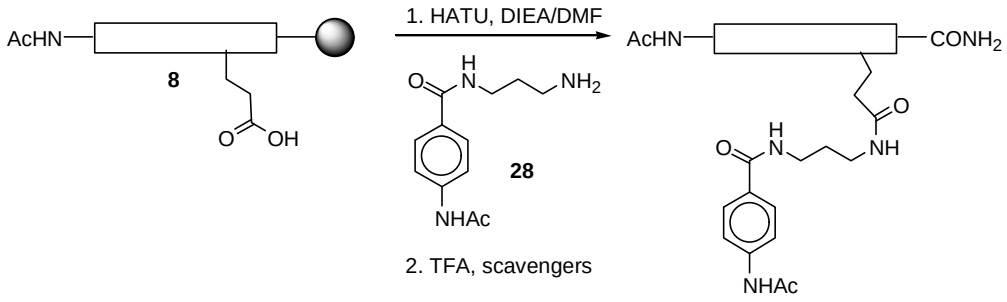


Figure 10. HPLC of peptide **R245E**.

R245E MS: $[MH^+]$ m/z calculated for $C_{109}H_{194}N_{43}O_{34}$ 2649.4, observed 2650.0.

Coupling of peptide R245E with 28



Resin with peptide **8** (15 mg) placed in an *Eppendorff* vial was treated with 1 mL of DMF during 1 h to ensure correct resin swelling. Resin was filtrated and DIEA was added (100 µL, 0.5M in DMF). HATU (2.1 mg in 100 µL of the same solution DIEA 0.5 M in DMF) was added and the mixture shaked for 5 minutes. Over the resulting activated acid, amine **28** was added (12 mg in 100 µL of the same solution DIEA 0.5 M in DMF) and the mixture shaked fot 3 h at room temperature. The resin was then filtrated and washed with DMF (3 x 0.6 mL x 5 min), *i*PrOH (1 x 0.6 mL x 5 min), finally with Et_2O and dried under Ar.

The cleavage/deprotection of the obtained resin was performed using standard conditions previously described, The residue was purified by RP-HPLC to give a white powder identified as the desired product **R245EABA** by MS (ES).

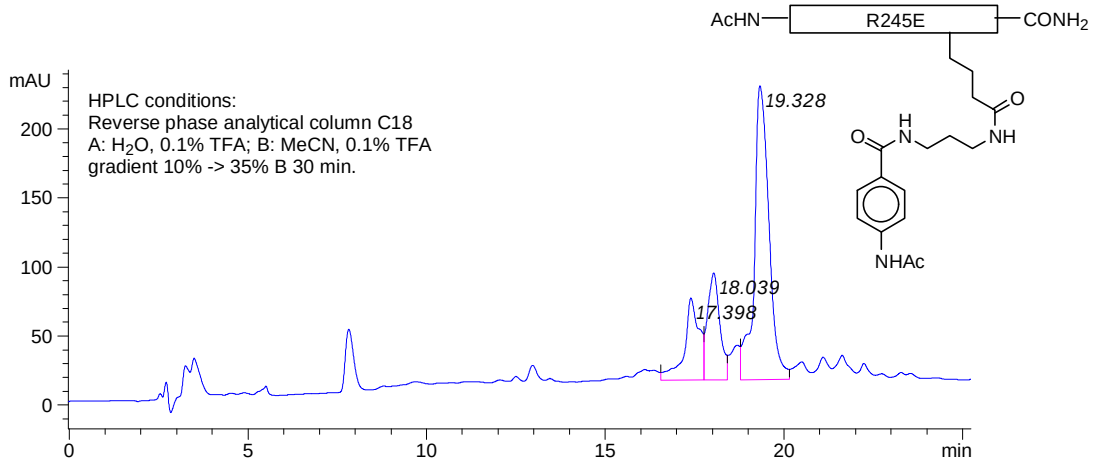


Figure 11. Coupling between **R245E** and amine **28**.

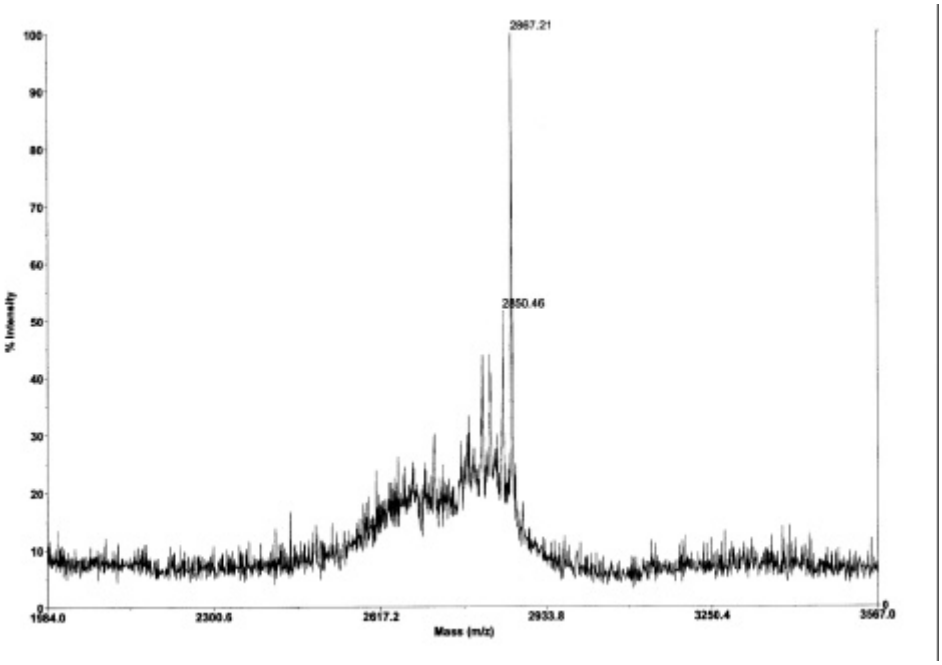
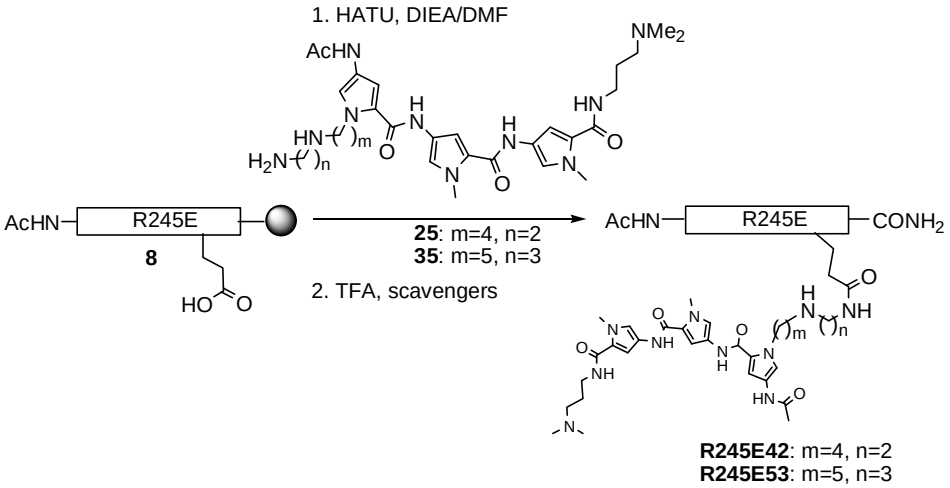


Figure 12. MS of peptide **R245EABA**.

MS(ES): [MH^+] m/z calcd. 2865.7, observed 2867.2.

Coupling of peptide R245E with tripyrrole 25 and 35.



Coupling procedure of tripyrrole **25** and **35** with peptide **R245E** was identical in both cases, as example, we report the experimental procedure followed with tripyrrole **35**.

Over 20 mg of **R245E** bound to the resin placed in an *Eppendorff,* DMF was added (1 mL) and the mixture was shacked for 1 h to ensure resin swelling. DMF was removed and HATU (3.5 mg in 100 ìL DIEA 0.5 M in DMF) was added. The resulting mixture was shacked for 5 min. and tripyrrole **35** (25 mg in 200 ìL DIEA 0.5 M in DMF) was added over the resin. The reaction mixture was shacked for 2 hours and then washed with DMF (3 x 0.6 mL x 5 min), *i*PrOH (1 x 0.6 mL x 5 min) and finally Et_2O. Cleavage/deprotection with standard conditions afforded a major product that was purified by RP-HPLC. MS analysis confirmed that product as the desired product **R245E53**. Yields were similar in both cases around 40-50%.

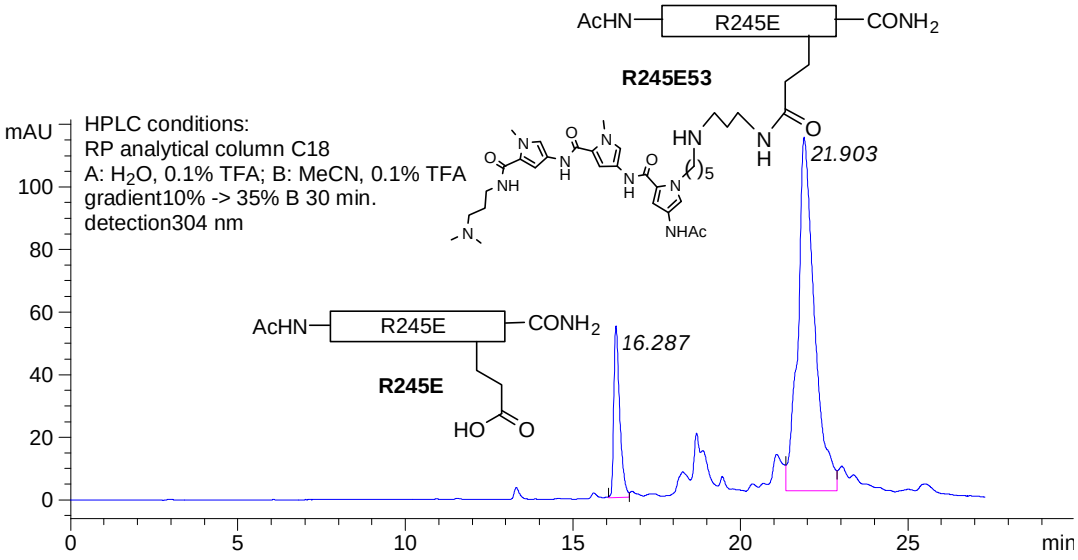


Figure 13. HPLC chromatogram of cleavage mixture.

R245E42 MS(ES): [MH^+] m/z calcd. for $C_{139}H_{238}N_{53}O_{37}$ 3241.8, observed 3243.4.
R245E53 MS(ES): [MH^+] m/z calcd. for $C_{141}H_{242}N_{53}O_{37}$ 3270.8, observed 3271.4.

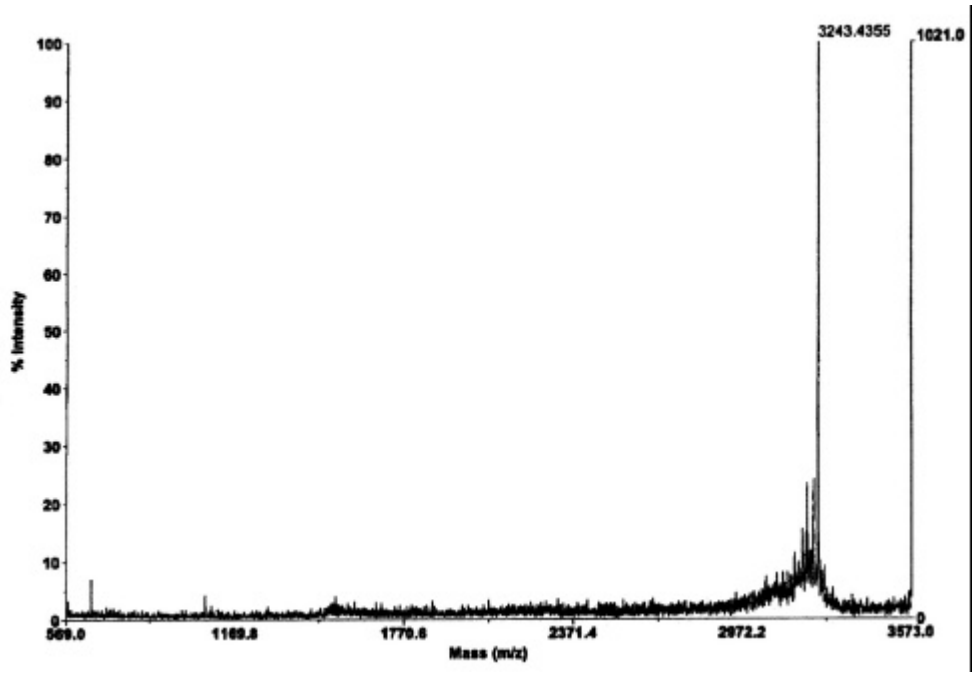


Figure 14. MS (ES) of peptide hybrid **R245E42**.

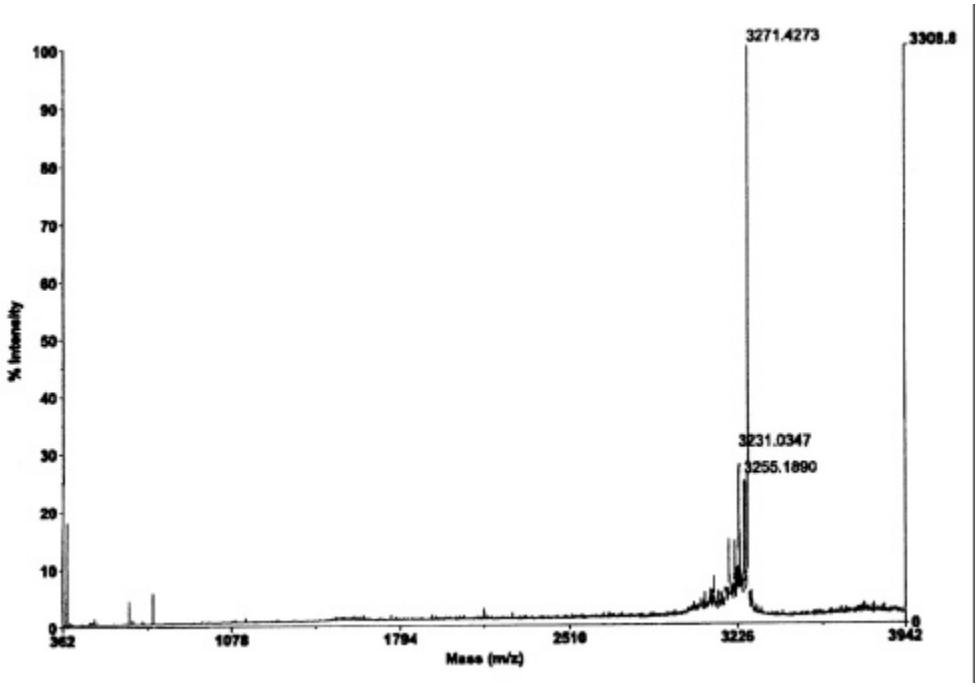


Figure 15. MS (ES) of peptide hybrid **R245E53**.