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Supporting Information

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Development and biological assessment of fully water soluble helical aromatic amide foldamers

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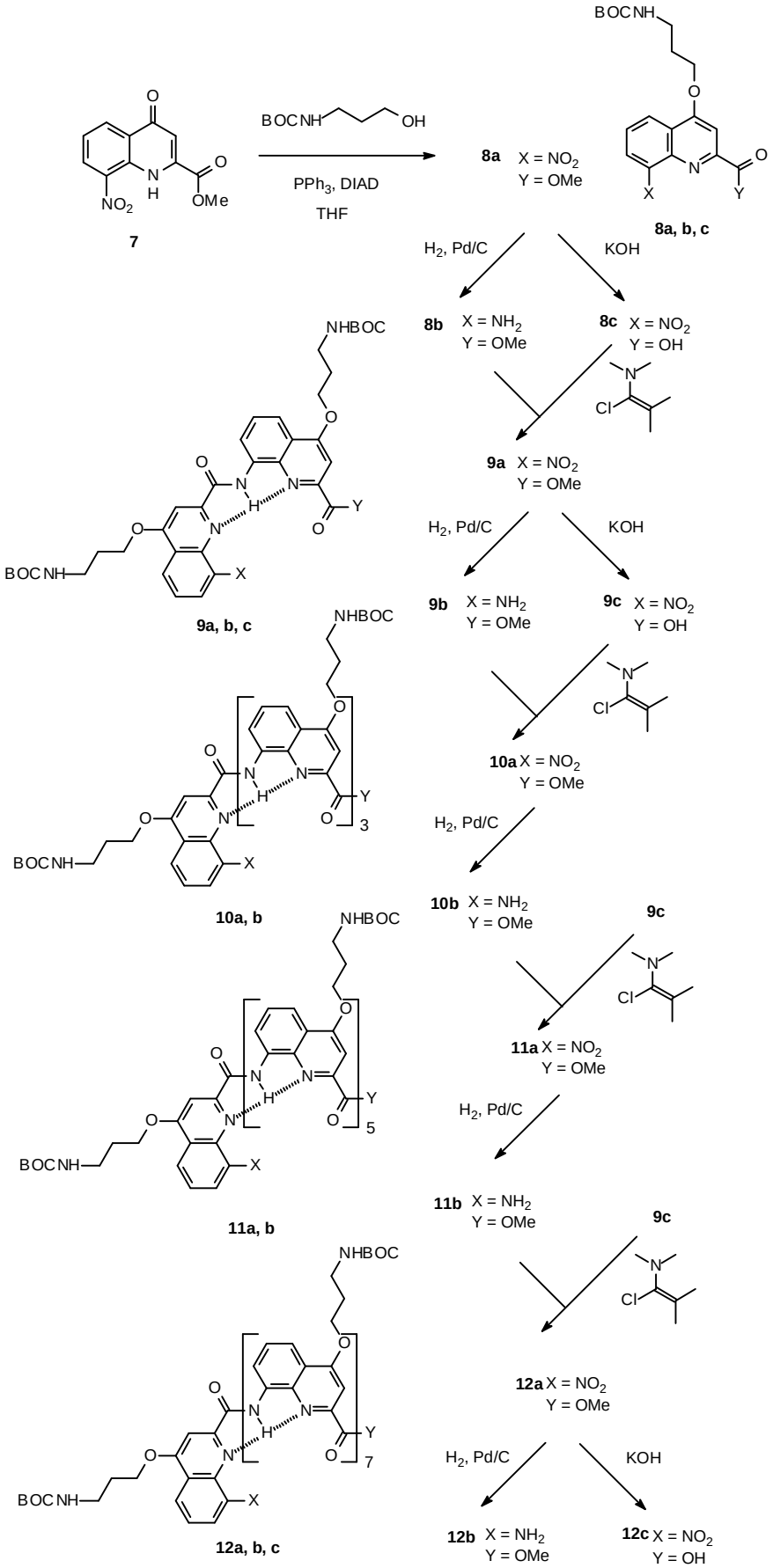
TABLE OF CONTENTS

Description of Syntheses

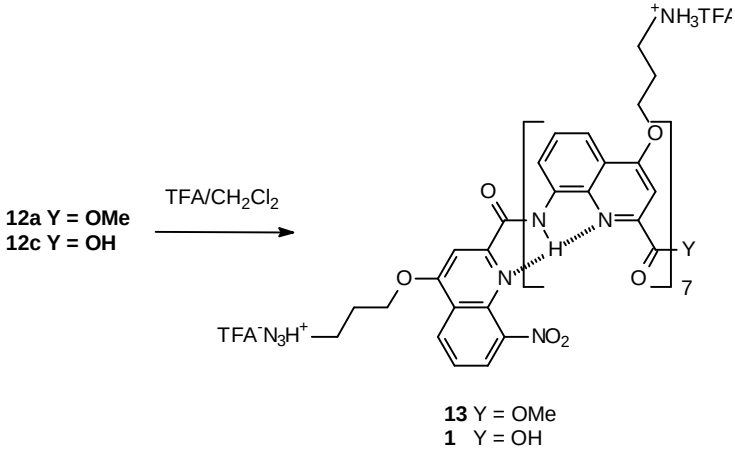
As shown in Scheme S1, the quinoline monomer **8a** was prepared by introduction of the BOC-protected aminopropoxy side chain to the quinolone **7**[1] under Mitsunobu conditions. The corresponding monomer amine **8b** was obtained by hydrogenation of **8a**, while the acid **8c** was prepared by saponification of the methyl ester. The Ghosez reagent was used to activate **8c** to the corresponding acid chloride, which was then coupled with **8b** to provide the dimer **9a**. No signs of BOC deprotection were observed under the activation or coupling conditions. The reaction was very clean and **9a** could be isolated by recrystallization, allowing the reaction to be easily performed on a multigram scale. Using the same procedures, **9a** could be converted to the corresponding amine **9b** and acid **9c**, which were coupled to form the tetramer **10a**. Rather than continuing in a convergent manner, due to difficulties in activation of the tetramer acid, the most efficient method to access the octamer was found to be two subsequent additions of the acid chloride of **9c** to the tetramer, providing first the hexamer **11a**, then the octamer **12a**.



Scheme S1

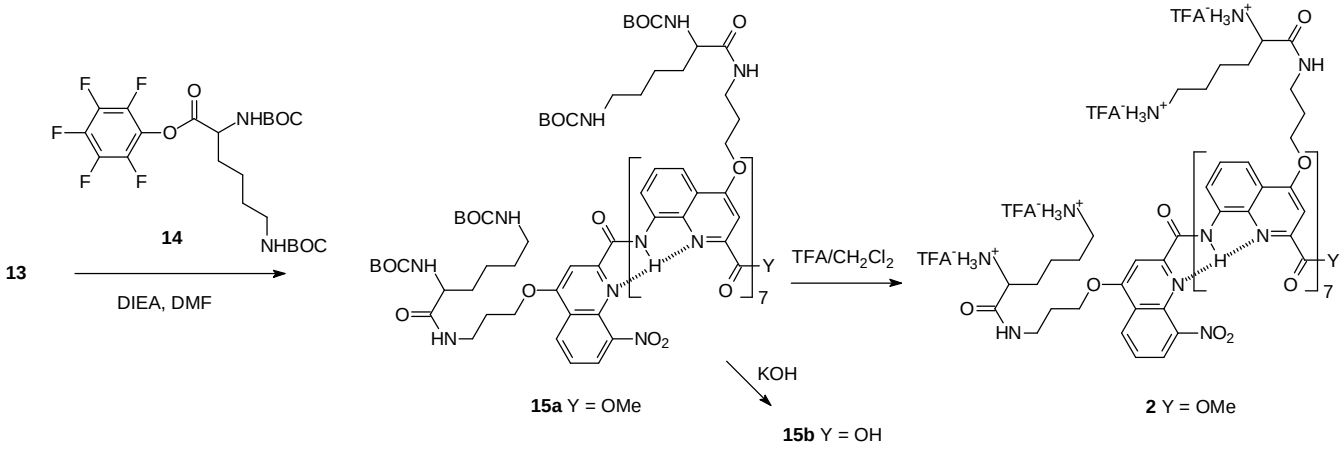
The BOC protecting groups were cleanly removed from the octamer **12a** using 1:1 TFA/CH_2Cl_2 to provide **13** as shown in Scheme S2. Although **13a** was highly soluble in pure water,

limited solubility was observed in phosphate buffers of neutral pH. It was hypothesized that an octamer having an ionizable acid terminus might exhibit improved solubility under biological conditions. Indeed, conversion of the methyl ester of **12a** to the acid **12c**, followed by deprotection provided octamer **1** which had good solubility (> 1 mg/mL) in biological buffers.



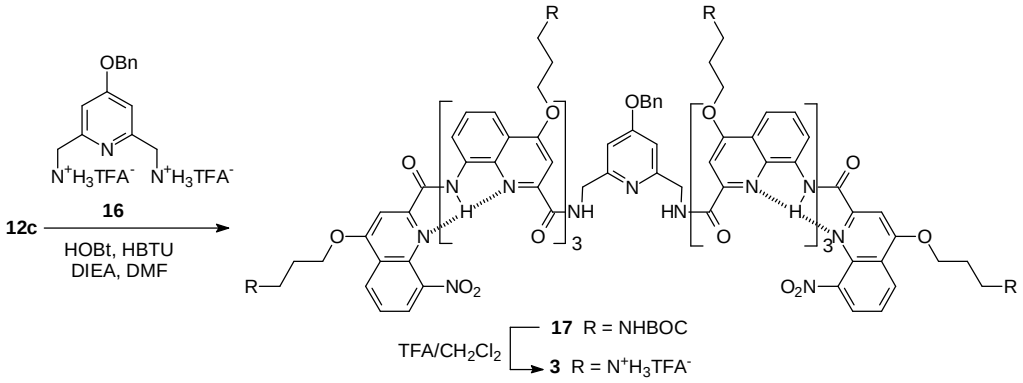
Scheme S2

To test the effect of charge density on the biological properties, the side chain amine groups of octamer **13** were functionalized with lysine groups using the BOC protected lysine pentafluorophenyl ester **14**[2] to give **15a** as shown in Scheme S3. Deprotection of **15a** provided **2**, having two cationic amine side chains per quinoline unit. **2** exhibited good solubility in pure water and in buffers.

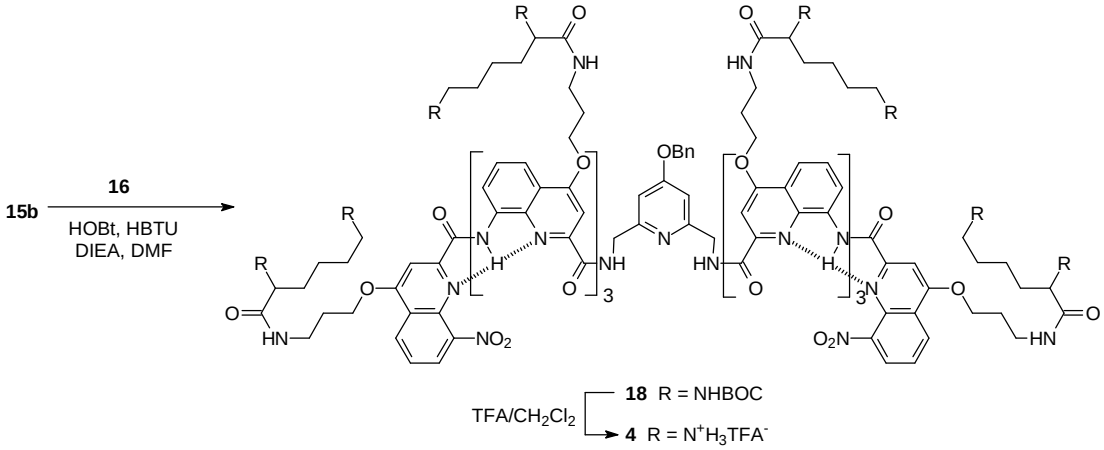


Scheme S3

While the synthesis of longer oligomers by sequential addition of dimer acid chlorides may eventually give long oligomers, a more convergent strategy is desirable for efficient growth. It has recently been demonstrated by our group that the introduction of 2,6-diaminomethylpyridine spacers within quinoline oligomers preserves the helical conformation.[3] In addition, the aliphatic amines of the spacer couple efficiently with the quinoline oligomer acids, thus allowing standard peptide coupling reagents to be used in place of acid chlorides. Indeed reaction of the pyridine spacer **16**[3] with octamer acid **12c** in the presence of HBTU and HOBt provided the heptadecamer **17**, which can be deprotected to provide **3** under standard conditions (Scheme S4). In an analogous manner, as shown in Scheme S5, the lysine functionalized octamer acid **15b** also reacted with pyridine **16** to provide the heptadecamer **18** which was deprotected to give **4**.

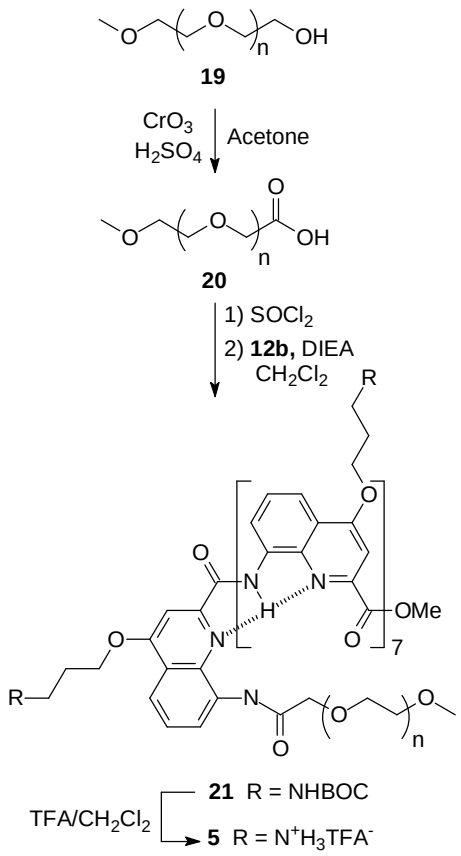


Scheme S4

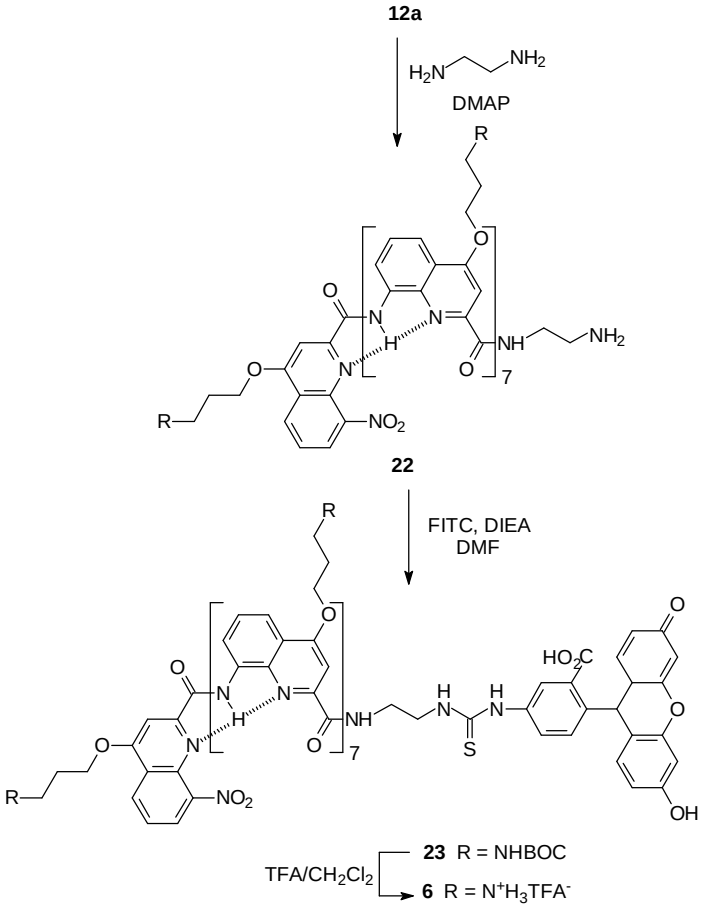


Scheme S5

To introduce PEO to the cationic quinoline-based oligoamides, a commercially available poly(ethylene glycol) monomethyl ether **19** with a MW of 1100 g/mol was first oxidized to the acid **20** under Jones conditions (Scheme S6). This acid was activated to the corresponding acid chloride using thionyl chloride, and then was coupled with the octamer amine **12b** to provide **21**. Standard deprotection conditions provided the oligocation **5.**



Scheme S6



Scheme S7

The reaction of **12a** with ethylene diamine in the presence of DMAP and gentle heating provided **22**, bearing an aliphatic amine functional handle which was reacted with fluorescein 5-isothiocyanate to provide **23**. **23** was deprotected to form **6** which was purified by HPLC in aqueous conditions (Scheme S7).

Protease degradation analysis.

The resistance of oligomer **1** to proteolytic enzymes was probed with proteases characterized by either low specificity (protease K, pepsin, subtilisin) or high specificity toward positively charged residues (trypsin). A 24h time-course experiment was monitored by on-line LC-ESI-MS with UV spectrophotometric detection (Figure S1), and off-line MALDI-MS. Control experiments were run simultaneously for each protease:

- incubation of oligomer **1** in buffer to check its potential own degradation
- incubation of protease in buffer to check its potential autolysis
- proteolysis of horse heart cytochrome C to check protease activity
- blank with buffer alone.

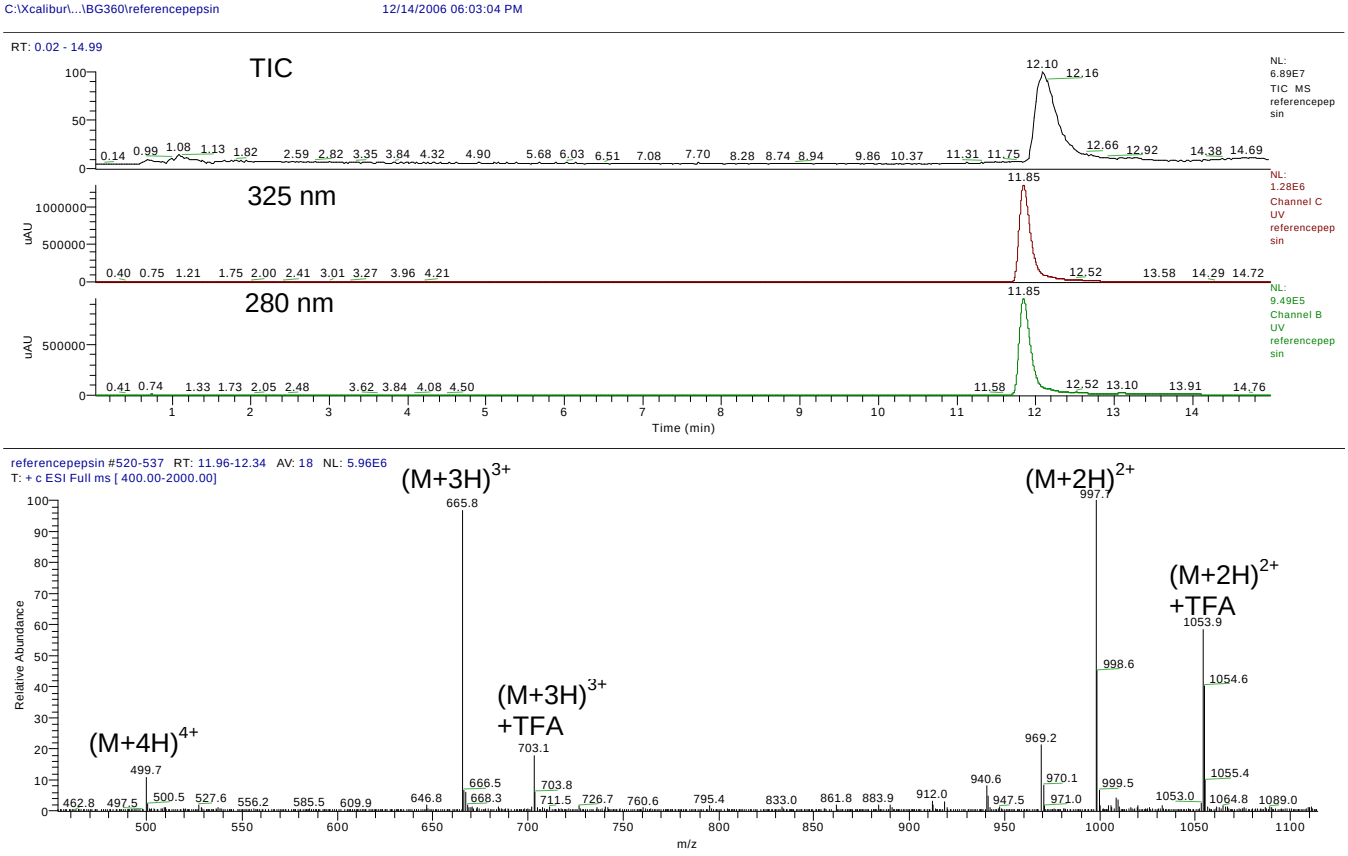


Figure S1. LC-MS analysis of oliomer **1**. The top graphs show HPLC chromatograms at two wavelengths (280 and 325 nm) and the subsequent (hence the small time gap) MS detection. The ESI mass spectrum showing several multicharged species is presented at the bottom.

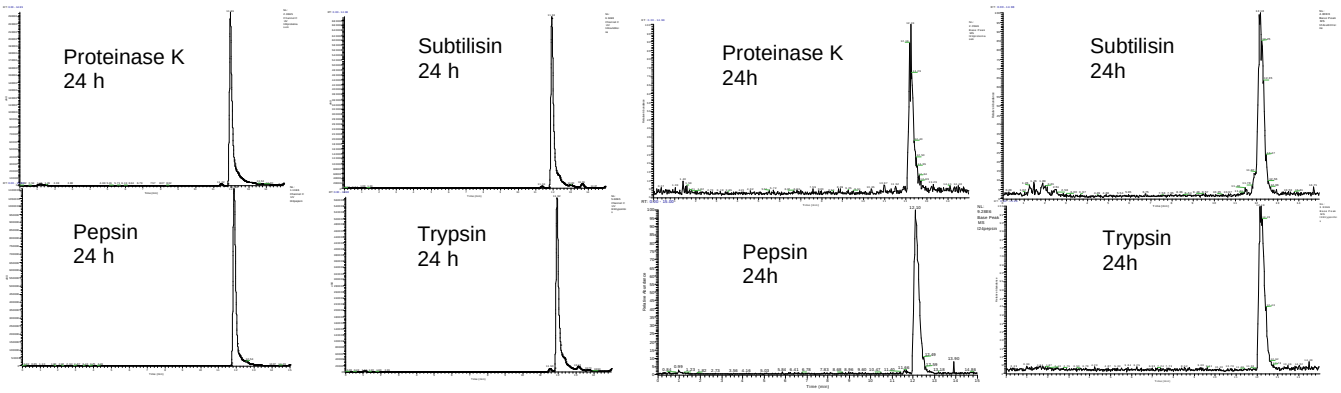


Figure S2. Chromatographic traces at 325 nm (left), mass spectrometric traces (right) of oligomer **1** after a 24h hour incubation with various proteases showing the absence of degradation of the oligomer.

Experimental section

General Procedures and Materials. Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. THF was distilled from Na/benzophenone while CH_2Cl_2 and diisopropylethylamine (DIEA) were distilled from CaH_2 prior to use. Chemical shifts are reported in ppm and are calibrated against residual solvent signals of $CDCl_3$ (*d* 7.26, 77.2), DMSO-d_3 (*d* 2.50, 39.4), or CD_3OD (*d* 3.31, 49.1). All coupling constants are reported in Hz. Silica gel chromatography was performed using Merck Kieselgel Si 60. Preparative size exclusion chromatography was performed using a Shimadzu LC-20AT pump and SPD-20A Uv-visible detector equipped with a manual recycling valve and Jaigel-1H and Jaigel-2H 20x600 mm columns (Japan Analytical Co.). Electrospray ionization (ESI) and high resolution electrospray ionization time of flight (HR-ESI) mass spectra were obtained in the positive ion mode and matrix assisted laser desorption ionization time of flight (MALDI) mass spectra were obtained in positive ion mode using α-cyanohydroxycinnamic acid as a matrix.

Monomer 8a. The quinolone 7[1] (8.0 g, 32 mmol, 1.0 equiv.)**,** triphenylphosphine (PPh_3) (9.3 g, 35 mmol, 1.1 equiv.) and *N*-(t-butoxycarbonyl)-3-aminopropanol[4] (6.2 g, 36 mmol, 1.1 equiv.) were combined under argon, then partially dissolved in dry THF (100 mL). The reaction mixture was cooled to 0 °C, then diisopropylazodicarboxylate (DIAD) (7.2 mL, 36 mmol, 1.1 equiv.) was added. The reaction mixture was stirred at 0 °C for 30 min, then was allowed to come to rt and was stirred overnight. The reaction mixture was evaporated, then the product was recrystallized from 130 mL of 3/1 MeOH/$CHCl_3$ to provide 9.1 g (77 % yield) of **8a** as a pale brown solid. ^{1}H NMR ($CDCl_3$, 400 MHz): *d* 1.41 (s, 9H), 2.15-2.21 (m, 2H), 3.38-3.44 (m, 2H), 4.01 (s, 3H), 4.37 (t, 2H, *J* = 6.0), 4.80 (br s, 1H), 7.61-7.65 (m, 2H), 8.08 (d, 1H, *J* = 7.3), 8.43 (d, 1H, *J* = 8.3). ^{13}C NMR ($CDCl_3$, 100 MHz): *d* 28.5, 29.5, 37.8, 53.5, 67.4, 79.7, 102.3, 123.3, 125.3, 126.2, 126.5, 140.1, 148.5, 151.4, 156.1, 162.6, 165.7. MS calcd $[M+H]^+$ ($C_{19}H_{24}N_3O_7$): 406.1614. Found: (HR-ESI) 406.1620.

General procedure for the nitro group reduction. The nitro precursor (eg. 5 mmol) was dissolved in 400 mL of EtOAc, and 200 mg of 10 wt % Pd/C was added. The reaction was stirred under a hydrogen atmosphere overnight (pressure provided by a balloon). Progress was monitored by TLC

and NMR, and additional Pd/C was added if necessary to complete the reaction. Upon completion the catalyst was removed by filtration over celite and the filtrate was evaporated to provide the amine. The product was characterized by ^{1}H NMR and used in the next step without further purification.

Monomer amine 8b from nitro 8a. Quantitative yield. Yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz): *d* 1.44 (s, 9H), 2.12-2.18 (m, 2H), 3.39-3.45 (m, 2H), 4.02 (s, 3H), 4.32 (t, 2H, *J* = 5.7), 4.80 (br s, 1H), 5.11 (br s, 2H), 6.94 (d, 1H, *J* = 5.9), 7.37 (t, 1H, *J* = 7.8), 7.47 (d, 1H, *J* = 7.6), 7.50 (s, 1H).

Dimer amine 9b from nitro 9a. Quantitative yield. Yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz): *d* 1.46 (s, 18H), 2.16-2.24 (m, 4H), 3.42-3.48 (m, 4H), 4.09 (s, 3H), 4.35-4.41 (m, 4H), 4.82 (br s, 2H), 5.56 (br s, 2H), 7.00 (d, 1H, *J* = 7.6), 7.38 (t, 1H, *J* = 7.8), 7.50 (d, 1H, *J* = 8.3), 7.56 (s, 1H), 7.66 (t, 1H, *J* = 8.1), 7.76 (s, 1H), 7.90 (d, 1H, *J* = 8.3), 9.04 (d, 1H, *J* = 7.6), 12.67 (s, 1H).

Tetramer amine 10b from nitro 10a. A Parr apparatus was used to provide a H_2 pressure of 4 bar. 93 % yield. ^{1}H NMR ($CDCl_3$, 400 MHz): *d* 1.39-1.49 (m, 32H), 2.10-2.32 (m, 8H), 3.38-3.68 (m, 11H), 3.79-3.86 (m, 2H), 4.04-4.18 (m, 4H), 4.38-4.48 (m, 3H), 4.50-4.64 (br s, 1H), 4.99 (br s, 2H), 5.39-5.46 (m, 1H), 5.74 (br s, 1H), 5.84 (d, 1H, *J* = 7.1), 6.58 (s, 1H), 6.72 (s, 1H), 6.93 (t, 1H, *J* = 7.7), 7.11-7.24 (m, 4H), 7.36 (d, 1H, *J* = 7.8), 7.57-7.70 (m, 4H), 7.85-7.89 (m, 3H), 8.42 (d, 1H, *J* = 7.3), 8.87 (d, 1H, *J* = 7.3), 11.66 (s, 1H), 11.79 (s, 1H), 12.26 (s, 1H).

Hexamer amine 11b from nitro 11a. A Parr apparatus was used to provide a H_2 pressure of 4 bar. 96 % yield. ^{1}H NMR (DMSO-d_6, 400 MHz): *d* 1.36-1.52 (m, 54H), 2.00-2.32 (m, 12H), 3.08 (s, 3H), 3.16-3.46 (m, 12H), 4.08-4.39 (m, 10H), 4.52-4.59 (m, 1H), 4.64-4.72 (m, 1H), 5.82 (d, 1H, *J* = 7.2), 6.34 (s, 1H), 6.46 (s, 1H), 6.71 (s, 1H), 6.75 (s, 1H), 6.85 (s, 1H), 6.92-7.02 (m, 3H), 7.08-7.16 (m, 5H), 7.28-7.46 (m, 5H), 7.56 (t, 1H, *J* = 8.1), 7.70 (d, 1H, *J* = 8.3), 7.74 (d, 1H, *J* = 7.6), 7.82-7.88 (m, 4H), 7.92-7.97 (m, 2H), 8.15 (d, 1H, *J* = 7.6), 8.58 (d, 1H, *J* = 7.3), 11.22 (s, 1H), 11.26 (s, 1H), 11.42 (s, 1H), 11.58 (s, 1H), 11.64 (s, 1H).

Octamer amine 12b from nitro 12a. A Parr apparatus was used to provide a H_2 pressure of 4 bar. 75 % yield. ^{1}H NMR (DMSO-d_6, 300 MHz): *d* 1.35-1.55 (m, 72H), 1.93-2.29 (m, 16H), 2.94 (s, 3H), 3.12-3.50 (m, 16H), 3.88-4.44 (m, 16H), 5.71 (d, 1H, *J* = 7.4), 6.00 (s, 1H), 6.19 (s, 1H), 6.28 (s,

1H), 6.36 (s, 1H), 6.57 (s, 1H), 6.58 (s, 1H), 6.74 (s, 1H), 6.83-7.86 (m, 30H), 8.03 (d, 1H, *J* = 6.8), 8.19 (d, 1H, *J* = 7.0), 10.86 (s, 2H), 10.94 (s, 1H), 10.98 (s, 1H), 11.18 (s, 1H), 11.20 (s, 1H), 11.29 (s, 1H).

General procedure for the methyl ester saponification. The methyl ester (eg. 6.4 mmol) was dissolved in 75 mL of THF and 25 mL of MeOH was added, followed by KOH (15.9 mmol). The reaction mixture was stirred at room temperature overnight, and then was acidified with acetic acid. The mixture was partitioned between CH_2Cl_2 and water, and then the CH_2Cl_2 layer was washed 3 times with water, dried with $MgSO_4$, filtered and evaporated to provide the product. The product was azeotroped with toluene to remove all traces of water and acetic acid, characterized by ^{1}H NMR, and used in the next step without further purification.

Monomer acid 8c from ester 8a. 96 % yield. Yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz): *d* 1.43 (s, 9H), 2.18-2.24 (m, 2H), 3.42-3.46 (m, 2H), 4.44 (t, 2H, *J* = 5.9), 4.70 (br s, 1H), 7.72 (d, 1H, *J* = 7.8), 7.75 (s, 1H), 8.24 (d, 1H, *J* = 7.6), 8.54 (d, 1H, *J* = 8.3), 11.06 (br s, 1H).

Dimer acid 9c from ester 9a. 89 % yield. Yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz): *d* 1.45 (s, 18H), 2.20-2.25 (m, 4H), 3.42-3.50 (m, 4H), 4.42 (t, 2H, *J* = 5.9), 4.48 (t, 2H, *J* = 5.9), 4.75 (br s, 2H), 7.70 (t, 2H, *J* = 8.8), 7.80 (s, 1H), 7.96 (s, 1H), 8.05 (d, 1H, *J* = 8.1), 8.27 (d, 1H, *J* = 7.3), 8.55 (d, 1H, *J* = 8.6), 9.16 (d, 1H, *J* = 7.6), 11.71 (s, 1H).

Octamer acid 12c from ester 12a. The reaction mixture was heated at 40 °C overnight, then an additional 2.5 equiv. of KOH were added and the reaction was stirred for an additional 2 h until TLC showed that it was complete. 91 % yield. Yellow solid. ^{1}H NMR (DMSO-d_6, 400 MHz): *d* 1.38-1.58 (m, 72H), 1.90-2.34 (m, 16H), 3.14-3.52 (m, 16H), 3.90-4.44 (m, 16H), 5.97 (s, 1H), 6.18 (s, 1H), 6.26 (s, 1H), 6.32 (s, 1H), 6.53 (s, 1H), 6.56 (s, 1H), 6.91-7.49 (m, 21H), 7.56-7.68 (m, 4H), 7.75-7.89 (m, 6H), 8.02 (d, 1H, *J* = 7.3), 8.12 (d, 1H, *J* = 7.3), 8.30 (d, 1H, *J* = 7.3), 10.79 (s, 1H), 10.81 (s, 1H), 10.88 (s, 1H), 10.99 (s, 1H), 11.19 (s, 1H), 11.22 (s, 1H), 11.87 (br s, 1H).

Lysine functionalized octamer acid 15b from ester 15a. The reaction mixture was heated at 40 °C overnight, then an additional 2.5 equiv. of KOH were added and the reaction was stirred for an additional 2 h until TLC showed that it was complete. Quantitative yield. Yellow solid. ^{1}H NMR

(DMSO-d_6, 400 MHz): *d* 1.10-1.79 (m, 192H), 1.95-2.35 (m, 16H), 2.80-3.01 (m, 16H), 3.22-3.64 (m, 16H), 3.68-3.48 (m, 24H), 5.99 (s, 1H), 6.19 (s, 1H), 6.28 (s, 1H), 6.33 (s, 1H), 6.53 (s, 1H), 6.55 (s, 1H), 6.66-7.19 (m, 19H), 7.24-8.34 (m, 30H), 10.78 (s, 1H), 10.83 (s, 2H), 10.87 (s, 1H), 10.98 (s, 1H), 11.17 (s, 1H), 11.23 (s, 1H).

General procedure for coupling an amine and an acid. The acid (eg. 1.2 mmol) was suspended in 40 mL of dry CH_2Cl_2 under an argon atmosphere and 1-chloro-N,N,2-trimethylpropenylamine (2.5 mmol) was added. The reaction mixture was stirred at rt for 2 h resulting in a homogeneous solution, then evaporated to provide the corresponding acid chloride. To a solution of the amine (1.1 mmol) in 20 mL of dry CH_2Cl_2 containing DIEA (3.7 mmol), the acid chloride in dry CH_2Cl_2 was added via cannula. The reaction mixture was stirred at rt overnight, then the solvent was evaporated.

Dimer 9a from amine 8b and acid 8c. The product was recrystallized from $CHCl_3$/MeOH. 88 % yield. Pale yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz): *d* 1.43 (s, 18H), 2.12-2.24 (m, 4H), 3.38-3.46 (m, 4H), 4.21 (s, 3H), 4.35 (t, 2H, *J* = 5.69), 4.43 (t, 2H, *J* = 5.87), 4.79-4.89 (m, 2H), 7.59-7.65 (m, 3H), 7.91 (s, 1H), 7.94 (d, 1H, *J* = 8.6), 8.16 (d, 1H, *J* = 7.3), 8.46 (d, 1H, *J* = 8.6), 9.06 (d, 1H, *J* = 7.8), 11.8 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): *d* 28.6, 29.5, 37.8, 38.0, 53.9, 67.0, 67.4, 79.6, 79.7, 100.3, 101.6, 116.8, 119.0, 122.2, 123.3, 125.69, 125.73, 126.8, 128.1, 135.0, 139.5, 139.8, 147.9, 148.5, 154.0, 156.1, 162.5, 163.0, 166.9. MS calcd $[M+H]^+$ ($C_{37}H_{45}N_6O_{11}$): 749.3146. Found: (HR-ESI) 749.3110.

Tetramer 10a from amine 9b and acid 9c. The product was purified by silica gel chromatography using a gradient from 80/20 to 60/40 toluene:EtOAc. 86 % yield. ^{1}H NMR ($CDCl_3$, 400 MHz): *d* 1.40 (s, 9H), 1.44 (s, 9H), 1.48 (s, 18H), 2.14-2.44 (m, 8H), 3.44-3.75 (m, 11H), 4.12-4.24 (m, 3H), 4.46-4.56 (m, 3H), 4.70-4.79 (m, 1H), 4.86-4.99 (m, 1H), 5.37 (br s, 1H), 5.62 (br s, 1H), 6.67 (br s, 1H), 6.80 (br s, 1H), 7.29 (d, 1H, *J* = 7.8), 7.39 (t, 1H, *J* = 7.9), 7.49 (s, 1H), 7.53 (d, 1H, *J* = 7.6), 7.64 (t, 1H, *J* = 7.9), 7.74 (t, 1H, *J* = 8.1), 7.82 (s, 1H), 7.86 (d, 1H, *J* = 8.3), 7.91 (d, 1H, *J* = 8.3), 7.96 (d, 1H, *J* = 8.6), 8.05 (d, 1H, *J* = 6.4). ^{13}C NMR ($CDCl_3$, 100 MHz): *d* 28.7, 29.1, 29.2, 29.4, 29.5, 37.4, 37.5, 38.0, 38.2, 52.8, 66.6, 66.8, 67.2, 79.4, 79.5, 79.7, 79.8, 97.6, 98.9, 100.3, 100.7, 116.0, 116.3, 116.7, 116.8, 116.9, 117.1, 118.0, 121.90, 121.91, 122.0, 123.7, 124.7, 125.9, 127.1,

127.7, 127.9, 128.6, 133.8, 134.1, 135.4, 138.4, 139.1, 139.2, 145.5, 148.8, 151.1, 153.9, 156.2, 156.4, 156.5, 160.8, 161.4, 162.2, 162.9, 163.1, 163.6, 164.5. MS calcd $[M+H]^+$ ($C_{73}H_{87}N_{12}O_{19}$): 1435.6210. Found: (HR-ESI) 1435.6227.

Hexamer 11a from amine 10b and acid 9c. The product was purified by silica gel chromatography using a gradient from 70/30 to 50/50 toluene/EtOAc. 91 % yield. ^{1}H NMR ($CDCl_3$, 400 MHz): *d* 1.35-1.50 (m, 54H), 2.06-2.38 (m, 12H), 3.14 (s, 3H), 3.32-3.74 (m, 12H), 3.96-4.54 (m, 11H), 4.66-4.74 (m, 1H), 5.01 (br s, 2H), 5.16 (br s, 1H), 5.36 (br s, 1H), 5.81 (br s, 1H), 5.87 (br s, 1H), 6.42 (s, 1H), 6.52 (s, 1H), 6.63 (s, 1H), 6.68 (s, 1H), 7.11 (s, 1H), 7.13-7.48 (m, 8H), 7.68 (t, 1H, *J* = 7.7), 7.72 (d, 1H, *J* = 8.3), 7.80 (d, 1H, *J* = 8.1), 7.84-7.92 (m, 2H), 7.96-8.04 (m, 2H), 8.12-8.18 (m, 1H), 8.25 (d, 1H, *J* = 7.3), 8.30 (d, 1H, *J* = 8.1), 8.50-8.58 (m, 1H), 11.28 (s, 1H), 11.33 (s, 1H), 11.54 (s, 2H), 11.78 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): *d* 28.6, 29.0, 29.4, 37.4, 38.0, 52.3, 66.3, 66.6, 66.8, 67.3, 79.1, 79.3, 79.4, 79.5, 97.4, 97.7, 98.00, 97.04, 99.7, 100.3, 115.5, 116.1, 116.3, 116.7, 116.9, 117.0, 121.4, 121.7, 121.9, 122.3, 123.4, 124.3, 126.0, 126.3, 126.6, 127.2, 127.5, 127.9, 132.6, 133.4, 133.5, 133.7, 134.1, 137.5, 138.0, 138.2, 138.3, 138.7, 138.8, 144.9, 145.0, 148.5, 148.8, 149.0, 150.6, 153.1, 156.2, 156.3, 156.5, 159.9, 160.3, 160.6, 161.0, 161.2, 161.8, 162.6, 162.8, 162.9, 163.0, 163.3, 164.0. MS calcd $[M+H]^+$ ($C_{109}H_{129}N_{18}O_{27}$): 2121.9275. Found: (HR-ESI) 2121.9001.

Octamer 12a from amine 11b and acid 9c. The product was purified by silica gel chromatography using a gradient from 50/50 to 40/60 toluene/EtOAc. 80 % yield. ^{1}H NMR (DMSO-d_6, 400 MHz): *d* 1.32-1.58 (m, 72H), 1.92-2.29 (m, 16H), 2.94 (s, 3H), 3.12-3.49 (m, 16H), 3.90-4.04 (m, 3H), 4.06-4.26 (m, 7H), 4.28-4.46 (m, 6H), 5.97 (s, 1H), 6.19 (s, 1H), 6.28 (s, 1H), 6.35 (s, 1H), 6.56 (s, 2H), 6.91-7.48 (m, 19H), 7.50 (t, 1H, *J* = 7.7), 7.55-7.68 (m, 5H), 7.77-7.89 (m, 6H), 8.03 (d, 1H, *J* = 7.3), 8.17 (d, 1H, *J* = 7.3), 8.30 (d, 1H, *J* = 7.3), 10.78 (s, 1H), 10.80 (s, 1H), 10.82 (s, 1H), 10.87 (s, 1H), 10.99 (s, 1H), 11.15 (s, 1H), 11.25 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): *d* 28.5, 28.6, 28.7, 29.0, 29.2, 29.3, 37.3, 37.4, 38.0, 38.2, 52.1, 66.1, 66.6, 66.7, 67.0, 67.1, 79.1, 79.3, 79.4, 97.2, 97.5, 97.7, 98.3, 98.9, 99.3, 99.6, 100.0, 115.2, 115.6, 116.0, 116.4, 116.6, 116.7, 121.1, 121.3, 121.4, 121.9, 122.1, 123.3, 124.2, 125.9, 126.1, 126.3, 126.5, 127.1, 127.4, 127.8, 128.0, 128.3, 128.5,

128.6, 129.1, 132.0, 132.1, 132.2, 132.5, 132.6, 133.1, 133.5, 134.0, 137.3, 137.4, 137.8, 137.9, 138.1, 138.57, 138.65, 144.7, 148.2, 148.4, 148.7, 149.1, 150.1, 153.0, 156.1, 156.17, 156.20, 156.3, 156.5, 159.2, 159.6, 160.1, 160.3, 160.75, 160.80, 161.6, 162.4, 162.6, 162.8, 164.0. MS calcd $[M+H]^+$ ($C_{145}H_{171}N_{24}O_{35}$): 2808.2339. Found: (HR-ESI) 2808.2434.

General procedure for the removal of the BOC protecting groups. The BOC protected oligomer (eg. 40 μmol) was dissolved in 4 mL of 1/1 CH_2Cl_2:TFA and was stirred at room temperature for 2 h. The solvent was evaporated to provide the TFA salt of the product as a glassy solid.

Deprotected octamer 13 from octamer 12a. Quantitative yield. ^{1}H NMR (CD_3OD, 400 MHz): *d* 2.20-2.60 (m, 16H), 3.04 (s, 3H), 3.16-3.58 (m, 16H), 4.04-4.12 (m, 3H), 4.18-4.56 (m, 13H), 6.13 (s, 1H), 6.32 (s, 1H), 6.42 (s, 1H), 6.43 (s, 1H), 6.56 (s, 1H), 6.57 (s, 1H), 7.06 (s, 1H), 7.11 (s, 1H), 7.15-7.19 (m, 2H), 7.24 (t, 1H, *J* = 8.0), 7.30-7.49 (m, 7H), 7.56 (t, 1H, *J* = 7.8), 7.69-7.85 (m, 6H), 7.95 (d, 2H, *J* = 8.1), 8.10 (d, 1H, *J* = 7.3), 8.25 (d, 1H, *J* = 7.6), 8.33 (d, 1H, *J* = 7.6), 11.07 (s, 1H), 11.12 (s, 1H), 11.15 (s, 1H), 11.20 (s, 1H), 11.27 (s, 1H), 11.32 (s, 1H), 11.51 (s, 1H). ^{13}C NMR (CD_3OD, 100 MHz): *d* 27.9, 28.0, 28.1, 28.2, 38.1, 38.2, 38.3, 38.4, 38.5, 38.6, 38.7, 38.8, 52.6, 66.9, 67.0, 67.2, 67.3, 67.4, 67.6, 67.8, 98.4, 98.5, 98.7, 99.2, 99.3, 100.0, 100.6, 101.3, 116.7, 116.9, 117.2, 117.3, 117.4, 117.5, 117.7, 117.8, 117.9, 118.1, 122.2, 122.3, 122.4, 122.7, 122.9, 123.0, 124.2, 126.1, 127.4, 127.9, 128.0, 128.1, 128.3, 128.7, 128.9, 133.2, 133.3, 133.8, 133.9, 134.2, 134.3, 134.4, 134.7, 138.5, 138.6, 138.9, 139.0, 139.4, 139.5, 145.5, 146.0, 149.1, 149.2, 149.4, 149.8, 150.8, 153.7, 160.5, 160.6, 160.7, 161.3, 161.4, 161.7, 161.8, 162.3, 162.4, 162.6, 163.5, 163.6, 163.7, 163.9, 164.0, 164.6. MS calcd $[M+H]^+$ ($C_{105}H_{107}N_{24}O_{19}$): 2007.81. Found: (MALDI) 2007.81.

Deprotected octamer 1 from octamer 12c. Quantitative yield. ^{1}H NMR (DMSO-d_6, 300 MHz): *d* 2.08-2.50 (m, 16H), 3.00-5.50 (m, 16H), 3.96-4.56 (m, 16H), 6.02 (s, 1H), 6.21 (s, 1H), 6.30 (s, 1H), 6.36 (s, 1H), 6.53 (s, 1H), 6.58 (s, 1H), 6.93 (s, 1H), 7.01 (s, 1H), 7.08 (d, 1H, *J* = 7.4), 7.21 (d, 1H, *J* = 7.9), 7.27 (d, 1H, *J* = 7.7), 7.35-7.92 (m, 18H), 8.05 (d, 1H, *J* = 7.6), 8.12-8.48 (m, 26H), 10.82 (s, 1H), 10.86 (s, 2H), 10.93 (s, 1H), 11.00 (s, 1H), 11.17 (s, 1H), 11.25 (s, 1H). ^{13}C NMR (CD_3OD, 100 MHz): *d* 27.7, 27.9, 28.0, 28.1, 38.2, 38.3, 38.5, 38.56, 38.61, 38.67, 66.8, 67.2, 67.4, 67.7, 98.2,

98.6, 99.0, 99.2, 100.1, 100.7, 100.8, 116.4, 116.6, 116.8, 116.9, 117.2, 117.4, 117.6, 117.9, 119.5, 121.9, 122.1, 122.56, 122.63, 122.8, 124.2, 126.2, 127.3, 127.9, 128.2, 128.5, 128.9, 132.9, 133.1, 133.2, 133.8, 133.9, 134.1, 134.2, 134.6, 138.4, 138.9, 139.0, 139.4, 145.4, 145.9, 146.2, 148.9, 149.1, 149.2, 149.3, 150.1, 150.8, 153.7, 160.2, 160.5, 160.7, 161.4, 162.0, 162.26, 162.33, 162.9, 163.57, 163.64, 163.7, 163.8, 163.9, 166.0. MS calcd $[M+H]^+$ ($C_{104}H_{105}N_{24}O_{19}$): 1993.80. Found: (MALDI) 1993.79.

Deprotected lysine functionalized octamer 2 from octamer 15a. Quantitative yield. ^{1}H NMR (CD_3OD, 400 MHz): *d* 1.40-2.10 (m, 48H), 2.24-2.52 (m, 16H), 2.84-3.06 (m, 16H), 3.09 (s, 3H), 4.43-3.96 (m, 20H), 4.00-4.12 (m, 7H), 4.18-4.60 (m, 13H), 6.17 (s, 1H), 6.39 (s, 1H), 6.48 (s, 1H), 6.51 (s, 1H), 6.63 (s, 0.5H), 6.66 (s, 1.5H), 7.10 (s, 1H), 7.15 (s, 1H), 7.17-7.22 (m, 2H), 7.34-7.50 (m, 8H), 7.56-7.61 (m, 2H), 7.72-7.78 (m, 2H), 7.82-7.96 (m, 5H), 7.99-8.03 (m, 2H), 8.12-8.15 (m, 1H), 8.26-8.29 (m, 1H), 8.41-8.45 (m, 1H), 11.12 (s, 1.5H), 11.15 (s, 0.5H), 11.19 (s, 1H), 11.20 (s, 1H), 11.33 (s, 1H), 11.40 (s, 0.5H), 11.42 (s, 0.5H), 11.56 (s, 0.5H), 11.58 (s, 0.5H). ^{13}C NMR (DMSO-d_6, 100 MHz): *d* 22.1, 22.2, 27.3, 27.36, 27.43, 29.1, 29.3, 29.3, 31.3, 31.5, 36.3, 36.4, 36.8, 36.9, 37.2, 39.2, 39.3, 39.4, 52.3, 53.0, 53.07, 53.14, 55.8, 66.8, 67.1, 67.4, 98.0, 98.4, 98.7, 99.0, 100.1, 100.4, 101.0, 116.1, 116.2, 116.7, 116.9, 117.1 (q, *J* = 294), 117.4, 121.6, 121.8, 122.1, 122.3, 122.5, 122.6, 123.7, 126.2, 126.9, 127.3, 127.4, 127.7, 132.8, 132.9, 133.70, 133.74, 133.9, 134.2, 137.46, 137.53, 137.6, 138.0, 138.1, 138.5, 138.8, 145.0, 145.5, 158.8, 149.0, 149.1, 150.5, 153.0, 159.6 (q, *J* = 35), 159.5, 160.5, 161.0, 161.2, 162.1, 162.56, 162.62, 162.7, 162.8, 162.98, 163.05, 163.3, 169.4, 169.46, 169.50, 169.6. MS calcd $[M+H]^+$ ($C_{153}H_{203}N_{40}O_{27}$): 3032.57. Found: (ESI) 3031.80.

Deprotected heptadecamer 3 from heptadecamer 17. Quantitative yield. ^{1}H NMR (CD_3OD, 400 MHz): *d* 2.28-2.50 (m, 32H), 3.24-3.48 (m, 32H), 3.78-4.52 (m, 32H), 4.68 (d, 1H, *J* = 11.5), 4.76 (d, 1H, *J* = 11.5), 5.37 (s, 2H), 5.67 (s, 2H), 5.92 (s, 2H), 5.95 (s, 2H), 6.09 (s, 2H), 6.14 (s, 2H), 6.18 (s, 2H), 6.73 (d, 2H, *J* = 7.1), 6.83-6.91 (m, 4H), 6.98-7.06 (m, 8H), 7.11 (d, 2H, *J* = 7.8), 7.14 (d, 2H, *J* = 7.6), 7.17-7.30 (m, 16H), 7.43-7.48 (m, 4H), 7.53-7.78 (m, 19H), 8.27 (d, 2H, *J* = 7.6), 10.11 (s, 2H), 10.47 (s, 2H), 10.56 (s, 2H), 10.65 (s, 2H), 10.74 (s, 2H), 10.92 (s, 2H), 10.98 (s, 2H).

^{13}C NMR (CD_3OD, 100 MHz): *d* 27.9, 28.0, 28.1, 28.3, 28.4, 38.3, 38.39, 38.44, 38.55, 38.65, 38.7, 38.9, 66.7, 67.3, 67.4, 67.6, 67.8, 70.9, 98.0, 98.4, 98.8, 99.0, 99.1, 99.2, 100.3, 100.7, 107.1, 116.5, 116.8, 116.9, 117.0, 117.1, 117.3, 117.4, 117.5, 117.7, 117.8, 117.9, 118.1, 119.9, 121.2, 122.0, 122.3, 122.8, 122.9, 123.0, 123.2, 124.4, 126.3, 127.3, 128.0, 128.1, 128.3, 128.4, 128.7, 128.8, 129.7, 130.0, 132.4, 132.8, 133.1, 133.2, 133.3, 134.3, 134.8, 137.6, 137.7, 138.2, 138.3, 138.4, 138.5, 138.7, 139.0, 139.6, 146.0, 147.7, 149.1, 149.2, 149.4, 151.1, 153.8, 153.9, 160.1, 160.4, 160.5, 160.7, 161.1, 161.6, 162.3, 162.6, 163.5, 163.7, 163.8, 163.9, 164.0, 164.3, 166.4. MS calcd $[M+H]^+$ ($C_{222}H_{222}N_{51}O_{37}$): 4193.7058. Found: (HR-ESI) 4193.7368.

Deprotected lysine functionalized heptadecamer 4 from 18. Quantitative yield. ^{1}H NMR (CD_3OD, 400 MHz): *d* 1.10-2.44 (m, 128 H), 2.96-3.16 (m, 32H), 3.20-4.48 (m, 80H), 5.30 (s, 2H), 5.40 (s, 2H), 5.63 (s, 2H), 5.79 (s, 2H), 5.93 (s, 2H), 5.96-6.14 (m, 4H), 6.60-7.92 (m, 53H), 8.35-8.46 (m, 2H), 10.16 (s, 2H), 10.42 (s, 4H), 10.52 (s, 2H), 10.68 (s, 2H), 10.92 (s, 2H), 11.01 (s, 2H). MS calcd $[M+H]^+$ ($C_{318}H_{414}N_{83}O_{53}$): 6243. Found: (ESI) 6246.

Deprotected PEO-functionalized octamer 5 from 21. Quantitative yield. ^{1}H NMR (CD_3OD, 400 MHz): *d* 2.10-2.65 (m, 16H), 2.76-2.82 (m, 2H), 3.02-3.80 (m, 5H), 3.12-3.70 (m, 110H), 4.08-4.68 (m, 16H), 6.12 (s, 1H), 6.34 (s, 1H), 6.48 (s, 1H), 6.52 (s, 1H), 6.63 (s, 1H), 6.65 (s, 1H), 7.09 (s, 1H), 7.19-7.51 (m, 10H), 7.60-7.68 (m, 3H), 7.73-7.83 (m, 6H), 7.91-7.93 (m, 2H), 7.80 (d, 2H, *J* = 7.6), 8.15 (d, 1H, *J* = 7.6), 8.32 (d, 1H, *J* = 7.3), 9.52 (s, 1H), 11.19 (s, 1H), 11.23 (s, 1H), 11.30 (s, 1H), 11.31 (s, 1H), 11.41 (s, 1H), 11.44 (s, 1H), 11.60 (s, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): *d* 27.4, 27.5, 27.7, 27.8, 27.9, 28.0, 36.9, 36.97, 37.01, 37.1, 37.2, 37.3, 52.4, 58.9, 66.5, 66.7, 66.8, 66.9, 68.8, 69.5, 69.7, 70.06, 70.11, 70.30, 70.34, 70.4, 70.5, 70.6, 72.1, 97.9, 98.5, 98.6, 98.8, 99.7, 99.8, 101.0, 113.0, 115.0, 115.6, 115.8, 115.9, 116.5, 116.7, 116.8, 117.4, 118.9, 121.3, 121.5, 121.7, 121.87, 121.94, 122.0, 122.1, 122.3, 127.4, 127.5, 127.6, 127.8, 128.6, 128.7, 132.7, 132.87, 132.91, 133.2, 133.39, 133.42, 133.7, 133.8, 136.9, 137.3, 137.4, 137.5, 137.57, 137.59, 138.0, 138.8, 145.3, 148.7, 148.8, 149.0, 150.1, 150.5, 159.0, 159.1, 159.4, 159.7, 160.1, 160.4, 160.9, 161.1, 161.4, 161.9, 162.5, 162.66, 162.74, 162.9, 163.1, 163.2, 163.3, 166.9. MS peak MW found: (MALDI) 2931.39.

Lysine functionalized octamer 15a. To a solution of the octamer **13** (0.15 g, 52 µmol, 1.0 equiv.) in 5 mL of DMF were added DIEA (80 mg, 0.62 mmol, 12 equiv.) and the lysine derivative **14**[2] (0.60 g, 1.2 mmol, 24 equiv.). The reaction mixture was stirred at room temperature overnight, then it was diluted with CH_2Cl_2 and washed three times with 1 M citric acid solution. The organic phase was dried with $MgSO_4$, filtered, and evaporated. The crude product was purified by silica gel chromatography using EtOAc to elute excess **14**, followed by 95/5 EtOAc/MeOH to provide 0.23 g of the product (99 % yield) as a 1:1 mixture of diastereomers. ^{1}H NMR (DMSO-d_6, 400 MHz): *d* 1.20-1.80 (m, 192H), 1.94-2.35 (m, 16H), 2.82-2.98 (m, 16H), 3.30-3.64 (m, 16H), 3.78-4.46 (m, 24H), 6.00 (s, 1H), 6.20 (s, 1H), 6.30 (s, 1H), 6.34 (s, 2H), 6.56 (s, 2H), 6.64-6.92 (m, 16H), 6.97 (s, 1H), 7.08-7.18 (m, 2H), 7.26-7.52 (m, 9H), 7.58-7.71 (m, 4H), 7.79 (t, 2H, *J* = 6.5), 7.85-8.22 (m, 14H), 8.29-8.32 (m, 1H), 10.75 (s, 1H), 10.84 (br s, 3H), 10.97 (s, 1H), 11.13 (s, 1H), 11.25 (s, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): *d* 23.7, 23.8, 29.0, 29.1, 29.5, 29.6, 29.9, 30.1, 30.2, 32.4, 32.6, 32.7, 32.8, 36.1, 36.3, 36.4, 36.6, 36.7, 41.6, 52.3, 55.4, 56.7, 67.5, 78.2, 78.8, 78.9, 80.0, 97.9, 98.5, 98.9, 99.1, 100.1, 100.5, 100.9, 115.8, 116.0, 116.3, 116.6, 117.0, 117.3, 121.4, 121.6, 121.8, 122.1, 122.3, 122.4, 122.6, 123.7, 126.2, 126.9, 127.2, 127.6, 128.4, 132.8, 132.9, 133.7, 133.9, 134.1, 137.4, 137.5, 138.0, 138.1, 138.5, 138.8, 145.1, 145.3, 145.4, 148.7, 148.9, 149.1, 150.6, 153.0, 155.6, 156.18, 156.23, 156.3, 156.5, 159.1, 159.3, 159.5, 160.4, 160.5, 161.0, 161.1, 162.1, 162.6, 162.7, 163.0, 163.3, 173.2, 173.3, 173.4. MS calcd $[M+H]^+$ ($C_{233}H_{329}N_{40}O_{59}Na_2$): 4677.3769. Found: (HR-ESI) 4677.3755.

General procedure for the synthesis of heptadecamers. The octamer acid (36 µmol), HBTU (50 µmol), and HOBt (33 µmol) were dissolved in 0.8 mL of dry DMF under an argon atmosphere and resulting solution was stirred at room temperature for 30 min. Diamine **16**[3] (14 µmol) was added and the reaction mixture was stirred at overnight at room temperature. Additional HBTU (50 µmol) was added, and the reaction mixture was stirred for an additional 24 h before diluting with toluene and washing three times with 1 M $NaHCO_3$ solution. The organic phase was dried with $MgSO_4$, filtered and evaporated. The product was purified by preparative size exclusion chromatography.

Heptadecamer 17 from octamer acid 12c. 60 % yield. ^{1}H NMR (DMSO-d_6, 400 MHz): *d* 1.28-1.63 (m, 144H), 1.88-2.12 (m, 32H), 3.10-3.38 (m, 32H), 3.72-4.28 (m, 32H), 4.69 (d, 1H, *J* = 10.8), 4.82 (d, 1H, *J* = 10.8), 5.33 (s, 2H), 5.66 (s, 2H), 5.69 (s, 2H), 5.73 (s, 2H), 5.87 (s, 2H), 5.92 (s, 2H), 6.06 (s, 2H), 6.54-7.62 (m, 73H), 7.69 (d, 2H, *J* = 6.4), 8.18 (d, 2H, *J* = 6.8), 9.80 (s, 2H), 10.03 (s, 2H), 10.24 (s, 2H), 10.29 (s, 2H), 10.32 (s, 2H), 10.35 (s, 2H), 10.54 (s, 2H), 10.65 (s, 2H). ^{13}C NMR (DMSO-d_6, 100 MHz): *d* 28.1, 28.16, 28.23, 28.3, 28.5, 28.7, 28.8, 28.9, 36.6, 36.7, 36.8, 37.0, 37.1, 37.4, 65.6, 66.3, 66.4, 66.8, 68.8, 69.7, 71.5, 77.4, 77.5, 77.57, 77.64, 79.1, 96.3, 96.6, 97.2, 97.5, 98.9, 99.5, 105.0, 114.2, 114.5, 114.9, 115.2, 115.8, 119.0, 119.9, 120.3, 120.8, 120.9, 121.1, 121.3, 122.7, 124.8, 125.5, 126.0, 126.3, 127.5, 128.1, 128.5, 130.5, 131.2, 131.5, 131.6, 132.5, 132.9, 135.7, 135.9, 136.1, 136.3, 136.7, 137.3, 144.1, 146.6, 147.4, 147.5, 147.56, 147.63, 149.5, 152.0, 152.1, 155.5, 155.6, 155.7, 157.5, 157.8, 158.1, 158.7, 159.4, 159.70, 159.75, 160.3, 160.9, 161.3, 161.4, 161.7, 162.0, 162.2. MS calcd $[M+H]^+$ ($C_{302}H_{349}N_{51}O_{69}Na$): 5816.52. Found: (ESI) 5816.92.

Heptadecamer 18 from octamer acid 15b. 68 % yield. Yellow solid. ^{1}H NMR (DMSO-d_6, 400 MHz): *d* 1.18-1.80 (m, 384H), 1.90-2.30 (m, 32H), 2.74-3.10 (m, 32H), 3.16-4.40 (m, 80H), 4.60-4.68 (m, 1H), 4.72-4.80 (m, 1H), 5.23 (s, 2H), 5.60 (s, 2H), 5.68 (s, 2H), 5.76 (s, 2H), 5.87 (s, 2H), 5.99 (s, 2H), 6.05 (s, 2H), 6.40-8.22 (m, 98H), 9.85 (s, 2H), 10.01 (s, 2H), 10.24 (s, 4H), 10.32 (s, 2H), 10.57 (s, 2H), 10.64 (s, 2H).

PEO acid 20. CrO_3 (5.5 g, 54 mmol, 6.0) was dissolved in 38 mL of H_2O and 4.7 mL of H_2SO_4 was added. After stirring for 10 min., this solution was added to solution of poly(ethylene oxide) monomethylether MW 1100 (**19**) (10 g, 9.1 mmol, 1.0 equiv.) in 36 mL of acetone and the reaction mixture was stirred at room temperature overnight. The reaction was quenched with isopropanol, and then the organic solvents were evaporated. NaCl was added to the aqueous phase, and then it was extracted with CH_2Cl_2 three times. The combined organic layers were dried with $MgSO_4$, filtered and evaporated. The product was purified by silica gel chromatography using 90/10 CH_2Cl_2/MeOH as an eluent to provide 5.0 g (50 % yield) of the product as a low melting white solid. ^{1}H NMR ($CDCl_3$, 400 MHz): *d* 3.15 (s, 3H), 3.31-3.47 (m, 60H), 3.85 (s, 2H). ^{13}C NMR ($CDCl_3$,

100 MHz): *d* 58.5, 60.9, 68.6, 69.7, 69.8, 69.85, 69.91, 70.0, 70.1, 71.2, 71.3, 71.4, 72.2, 172.9. MS peak MW found: (MALDI) 1125.65.

PEO functionalized octamer 21. PEO acid **20** (56 mg, 50 μmol, 2.0 equiv.) was dissolved in 100 μL of $SOCl_2$ and was heated at reflux for 15 min. The $SOCl_2$ was removed in vacuo to provide the corresponding acid chloride. The a solution of the amine **12b** (70 mg, 25 μmol, 1.0 equiv.) and DIEA (13 mg, 0.10 mmol, 4.0 equiv.) in 1 mL of dry CH_2Cl_2, a solution of the acid chloride in 1 mL of CH_2Cl_2 was added via cannula and the reaction mixture was stirred at rt overnight. The solvent was evaporated and the product was purified by silica gel chromatography using 80/20 EtOAc/toluene followed by 90/10 CH_2Cl_2/MeOH as an eluent. Size exclusion chromatography was performed to remove residual **20** to provide 50 mg (52 % yield) of the product. ^{1}H NMR (DMSO-d_6, 400 MHz): *d* 1.39-1.60 (m, 72H), 1.90-2.32 (m, 16H), 2.59-2.68 (m, 2H), 2.90-2.96 (m, 5H), 3.02-3.52 (m, 110H), 3.88-4.44 (m, 16H), 5.90 (s, 1H), 6.15 (s, 1H), 6.29 (s, 1H), 6.35 (s, 1H), 6.57 (s, 2H), 6.83 (s, 1H), 6.92 (s, 1H), 7.04-7.43 (m, 16H), 7.44-7.64 (m, 8H), 7.71-7.58 (m, 6H), 8.00 (d, 1H, *J* = 7.3), 8.18 (d, 1H, *J* = 7.3), 9.26 (s, 1H), 10.77 (s, 1H), 10.83 (s, 1H), 10.91 (s, 1H), 11.01 (s, 1H), 11.13 (s, 1H), 11.16 (s, 1H), 11.28 (s, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): *d* 29.0, 29.1, 29.2, 29.4, 29.8, 29.9, 30.1, 37.67, 37.73, 37.8, 37.9, 52.3, 58.9, 67.3, 67.5, 67.7, 68.8, 69.5, 69.8, 70.1, 70.3, 70.37, 70.45, 70.6, 72.1, 78.4, 78.5, 78.6, 97.9, 98.4, 98.6, 98.7, 98.9, 99.7, 99.8, 100.9, 114.5, 115.5, 115.9, 116.3, 116.5, 116.8, 117.3, 121.4, 121.5, 121.8, 122.0, 122.17, 122.22, 122.4, 127.0, 127.2, 127.3, 127.5, 128.3, 128.5, 132.8, 133.0, 133.2, 133.4, 133.5, 133.8, 133.9, 136.9, 137.3, 137.4, 137.5, 137.56, 137.59, 138.0, 138.8, 145.4, 148.7, 148.8, 149.0, 149.1, 150.2, 150.6, 156.56, 156.63, 156.67, 156.71, 158.96, 159.01, 159.3, 160.4, 161.0, 161.2, 161.3, 162.1, 162.6, 162.7, 162.8, 162.9, 163.0, 163.1, 163.3, 163.5, 166.8.

Amine functionalized octamer 22. To a solution of octamer **12a** (70 mg, 25 μmol, 1.0 equiv) in 0.20 mL of ethylene diamine was added DMAP (0.15 mg, 1.3 μmol, 0.05 equiv.) and the reaction mixture was heated at 50 °C overnight. The ethylene diamine was evaporated, then the product was purified by silica gel chromatography using 96/4 EtOAc:NEt_3. Size exclusion chromatography was used to remove residual DMAP, providing 45 mg (64 % yield) of the amine functionalized octamer

as a pale yellow solid. ^{1}H NMR (DMSO-d_6, 400 MHz): *d* 1.39-1.60 (m, 72H), 1.92-2.32 (m, 16H), 3.16-3.52 (m, 20H), 3.92-4.44 (m, 16H), 5.97 (s, 1H), 6.21 (s, 1H), 6.28 (s, 1H), 6.31 (s, 1H), 6.51 (s, 1H), 6.53 (s, 1H), 6.89 (s, 1H), 6.97-7.62 (m, 24H), 7.68 (d, 1H, *J* = 8.1), 7.76-7.87 (m, 5H), 8.03 (d, 1H, *J* = 7.3), 8.09 (d, 1H, *J* = 7.09), 8.30 (d, 1H, *J* = 7.8), 10.71 (s, 1H), 10.77 (s, 1H), 10.82 (s, 1H), 10.86 (s, 1H), 10.99 (s, 1H), 11.07 (s, 1H), 11.29 (s, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): *d* 29.17, 29.24, 29.6, 29.8, 29.9, 37.7, 37.8, 38.0, 38.1, 38.4, 67.3, 67.5, 67.9, 78.4, 78.5, 78.58, 78.63, 80.07, 97.8, 98.2, 98.4, 98.6, 98.8, 99.0, 100.0, 100.5, 115.5, 115.7, 115.8, 116.2, 116.4, 116.7, 116.8, 117.0, 117.1, 117.4, 121.4, 121.8, 122.0, 122.1, 122.3, 122.5, 123.7, 125.9, 126.6, 126.9, 127.3, 127.5, 127.7, 128.2, 128.3, 132.6, 132.8, 132.9, 133.4, 133.7, 134.1, 137.4, 137.5, 137.6, 138.0, 138.1, 138.2, 138.5, 145.2, 148.6, 148.7, 148.8, 149.0, 149.1, 150.6, 153.1, 156.6, 156.6, 156.68, 156.74, 159.0, 159.2, 159.4, 160.5, 160.6, 161.0, 162.2, 162.6, 162.8, 163.0, 163.1, 163.3, 163.4. MS calcd $[M+H]^+$ ($C_{146}H_{174}N_{26}O_{34}Na$): 2858.26. Found: (ESI) 2858.42.

Fluorescein functionalized octamer 6. To a solution of the amine functionalized octamer **22** (40 mg, μmol, 1.0 equiv.) in 0.5 mL of DMF was added fluorescein 5-isothiocyanate (6.1 mg, 16 μmol, 1.1 equiv.) and the reaction mixture was stirred at room temperature overnight. The DMF was evaporated in vacuo, then the resulting product was dissolved in 1/1 TFA/CH_2Cl_2 and stirred at rt for 2 h. The solvents were evaporated to provide the crude product. The product was purified by multiple semipreparative HPLC runs using a C18 column and a water/acetonitrile gradient with 0.1% TFA to provide approximately 20 mg of **6**. ^{1}H NMR (CD_3OD, 400 MHz): *d* 2.24-2.70 (m, 16H), 3.20-3.58 (m, 20H), 4.14-4.70 (m, 16H), 6.24 (s, 1H), 6.39-6.48 (m, 5H), 6.52 (s, 1H), 6.58 (s, 1H), 6.63 (s, 1H), 6.66 (s, 2H), 6.70 (s, 1H), 6.81 (d, 1H, *J* = 8.1), 7.11 (d, 1H, *J* = 8.1), 7.19 (s, 1H), 7.24-7.28 (m, 2H), 7.39-7.55 (m, 9H), 7.60-7.68 (m, 2H), 7.76-7.99 (m, 7H), 8.03-8.07 (m, 2H), 8.09-8.13 (m, 1H), 8.23 (d, 1H, *J* = 7.1), 8.31 (d, 1H, *J* = 6.8), 8.46 (d, 1H, *J* = 7.8), 11.22 (s, 1H), 11.25 (s, 2H), 11.31 (s, 1H), 11.37 (s, 1H), 11.42 (s, 1H), 11.79 (s, 1H). MS calcd $[M+H]^+$ ($C_{127}H_{122}N_{27}O_{23}S$): 2424.8928. Found: (HR-ESI) 2424.8892.

Protease degradation assays.

In a typical assay, a solution of protease (all purchased from Sigma) and oligomer **1** solution was prepared with ultra high quality water (Elga purification system, further referred as UHQ water). Stock solutions of trypsin, proteinase K and subtilisin were prepared at 1 μg/μl concentration in a 50 mM ammonium bicarbonate buffer at pH 8.2. A 1μg/μl pepsin stock solution was prepared in a 50 mM ammonium acetate solution adjusted at pH 2.5 with glacial acetic acid. An oligomer **1** stock solution was adjusted at 2.8 μg/μl in UHQ water. All degradations assays were performed in Eppendorf tubes held at 35°C (water bath) using 1 nanomole of oligomer **1** (volume adjusted to 100 μl) for each assay. For time-course analysis, 10 μl aliquots taken after 0.5, 3, and 24 h were analyzed by both MALDI-MS and LC-ESI-MS. Combined LC-MS was performed using a monolithic Styros C4 column (50 x 2 mm) directly coupled to the ESI source of a LCQ Advantage ion trap mass spectrometer (Thermo Fisher). Eluents A and B were 0.1% acetic acid in UHQ water and 0.1 % acetic acid in acetonitrile respectively, with a linear gradient from 0 to 90% B in 19 min at 200 μl/min flow rate). A UV5000 diode array spectrophotometer was used for detection at three wavelengths (214, 280 and 325 nm). MALDI-MS analysis was performed with a Reflex III mass spectrometer (Bruker) operated in the reflectron mode, using alpha-cyano-4-hydroxy-cinnamic acid as a matrix. Control experiments for the four enzymes, including proteolysis of horse heart cytochrome C (1 nmole, Sigma) were analyzed with the same protocol.

Cell Cytotoxicity Assay. HeLa cells were cultured in Dulbecco's Modified Eagle Medium (DMEM), containing 10 % FBS. Cells were seeded onto a 96-well plate at a density of 5000 cells per well and solutions of the cationic helices at varying concentrations in DMEM were added, providing a final volume of 100 μL per well. Following incubation in the presence of the helices for 24 h (37 °C, 5 % CO_2), 20 μL of Cell Titer96® AQ_{ueous} One Solution (Promega) was added to each well, and the cells were incubated for 1 hr. The absorbance at 490 nm was read on a plate reader. The tests were conducted in triplicate for each concentration. Optical densities measured for wells containing cells that received no helices were considered to represent 100 % growth.

DNA Transfection. The pGL3 plasmid DNA encoding for firefly luciferase (Promega, 1 μg/well) was mixed with polycations at varying charge ratios in 150 mM NaCl in polystyrene tubes and

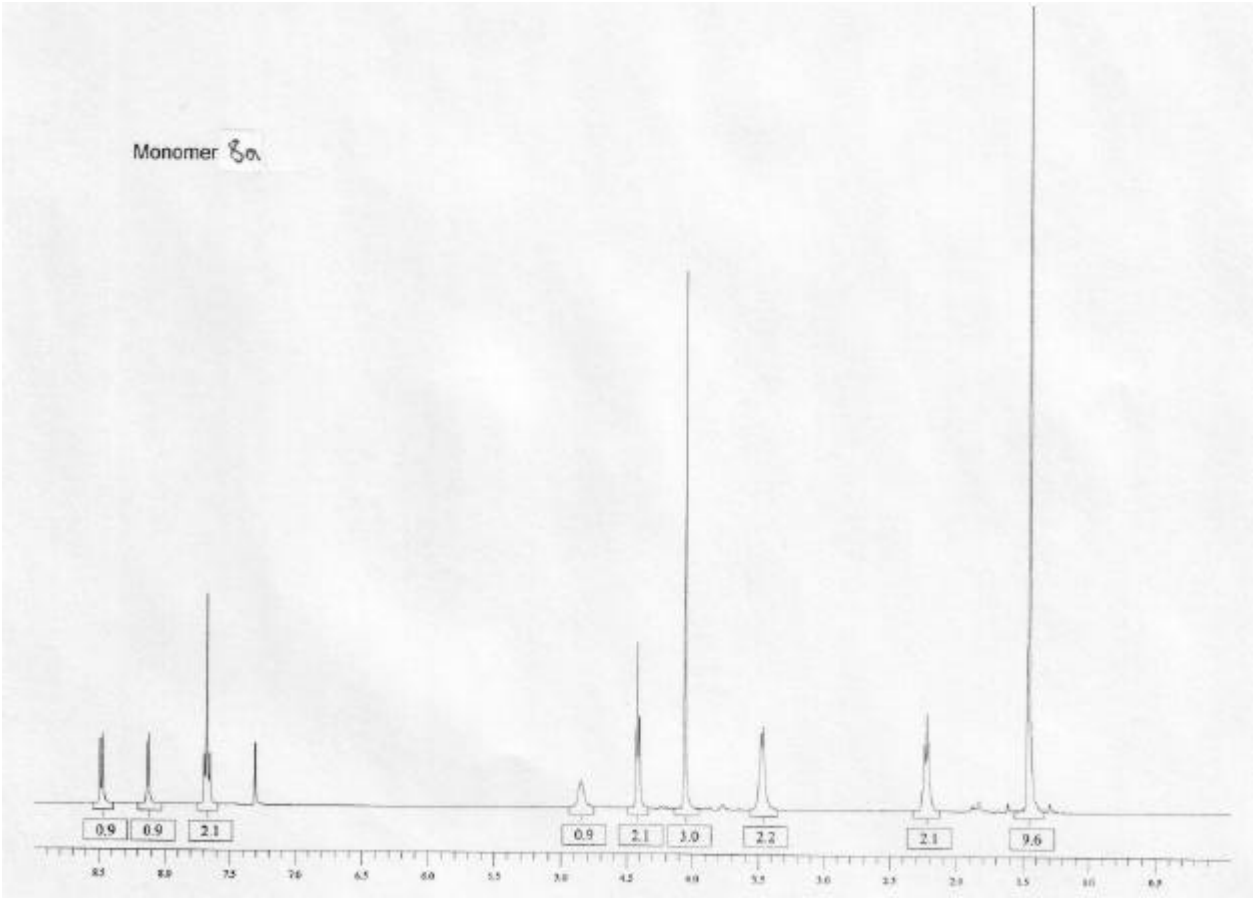
allowed to sit for 30 minutes. Lipofectamine (Invitrogen) was used as a positive control, as recommended by the manufacturer. HeLa cells were plated in a 24-well plate at a density of 7.5×10^4 cells/well, and grown at 37°C for 24 h prior to transfection in Dulbecco's Modified Eagle Medium (DMEM), supplemented with 10 % FCS, 2mM L-glutamine and 1% non-essential aminoacids (all from Invitrogen), in a 5%CO_2 atmosphere. The medium was carefully aspirated and the cells were rinsed with serum-free medium. Serum-free medium (250 µL/well) was added, followed by 50 µL of the DNA-polycation complex and the cells were incubated at 37°C for 2 hours before 300 µL/well of medium containing 20 % FBS was added. After 48 hours the medium was removed; the cells were washed with cold phosphate buffered saline (PBS) and lysed in 100 µL/well of lysis solution provided in the Luciferase Assay System (Promega). The samples were placed on ice for 30 min, homogenized by pipeting and firefly luciferase activity was measured in a luminometer (Berthold, Lumat LB9501) on 20 µL of cell lysate using the Luciferase assay kit. The results, expressed as arbitrary light units normalized for the number of cells (evaluated from the amount cell protein quantified the Bradford reaction) in each well are given below, indicating luciferase activity.

Transfection agent	Cationic charge ratio	Luminescence (n = negligible)
Lipofectamine	Optimized as recommended by manufacturer	514952
1	10	N
1	15	N
1	20	N
1	25	N
1	30	N
3	10	N
3	15	N
3	20	N
3	25	N
3	30	N
4	10	12195
4	15	16570
4	25	30938
4	30	31184

Cell viability test. Cells were grown in the 96 well microplates, 500 cells/well. Cell viability was assessed using the CEllTiter96 Aqueous one Solution Reagent (Promega) and a spectrophotometric microplate reader at 490 nm (Bio-Tek).

Confocal Microscopy. HeLa cells (2.0×10^4/well) were seeded onto a 16-well Lab-tek chamber slide (Nalge Nunc International) and grown for 24h before addition of the fluorescein-labeled cationic helix **6** at varying concentrations. Following incubation of the cells in the presence of the helices for the desired time period, the medium was carefully removed, the wells were rinsed three times with PBS and fixed for 5 min at room temperature in a 4 % formaldehyde solution in PBS. The wells were rinsed with PBS, and the slide was mounted with a coverslip using the S7461 antifading solution (Molecular Probes). Images were obtained using a Leica SP5 confocal microscope and a 100X oil immersion objective.

Cell uptake assay. HeLa cells (8.0×10^4/well) in DMEM were seeded onto a 24-well plate and incubated overnight (37 °C, 5 % CO_2). The following day, **18b** and FITC-Tat_{47-57} were added at the concentrations of 0.1 µM, 1.0 µM or 10 µM in a total volume of 200 µL of DMEM/well. The cells were incubated (37 °C, 5 % CO_2) for either 10 min or 1 hr, then the medium was carefully removed and the cells were washed 3 times with PBS. The cells were detached from the well by a trypsine/EDTA solution, suspended in 0.5 ml PBS and placed on ice. The cells were analyzed for FITC fluorescence by flow cytometry using a Beckman Coulter Epics XL cytometer. Basal fluorescence was given by Hela cells that were not incubated with fluorescent compounds. Each sample was run in triplicate2.0×10^4.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2.1
0.9
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3.0
2.2
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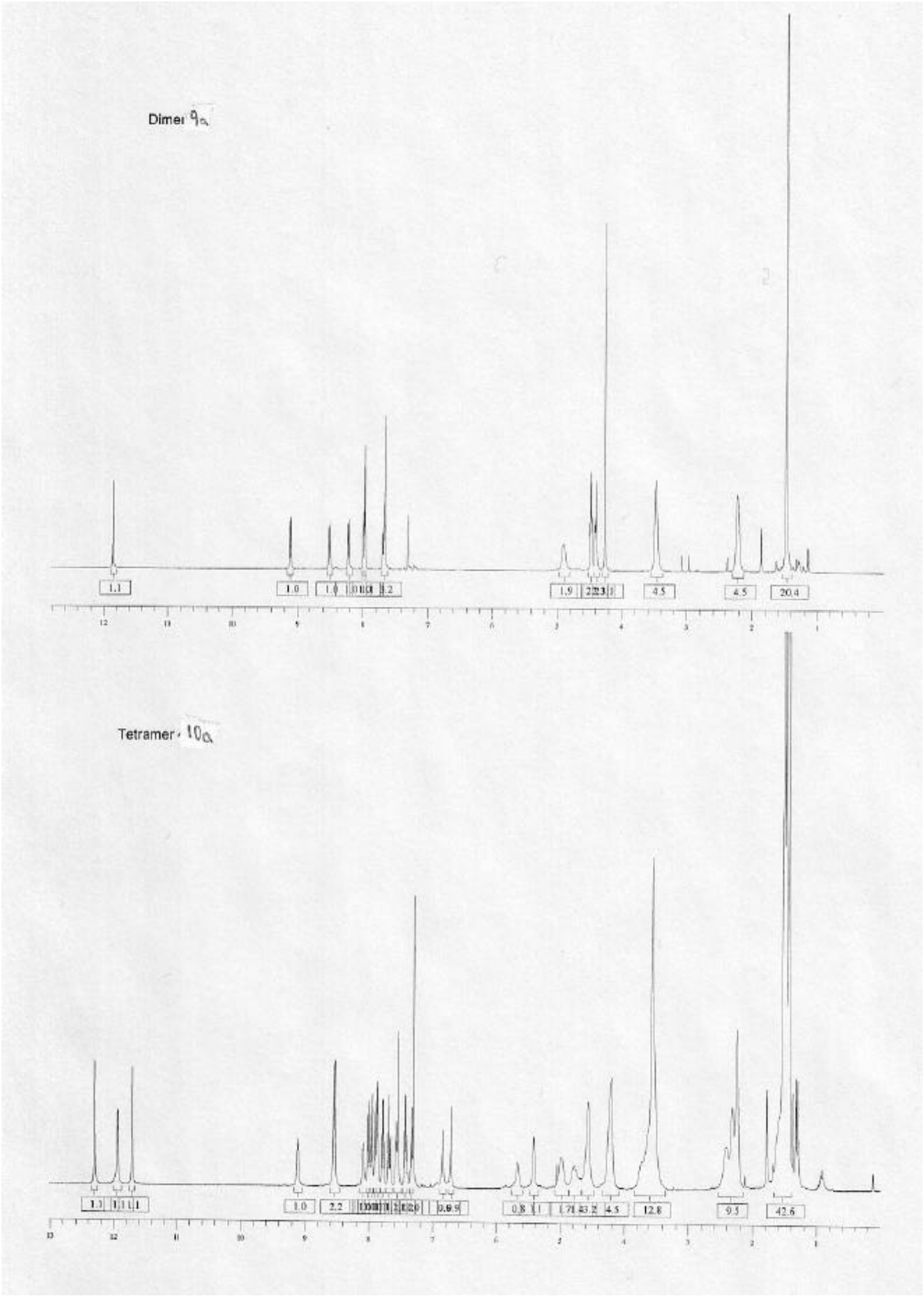
9.6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Tetramer 10a
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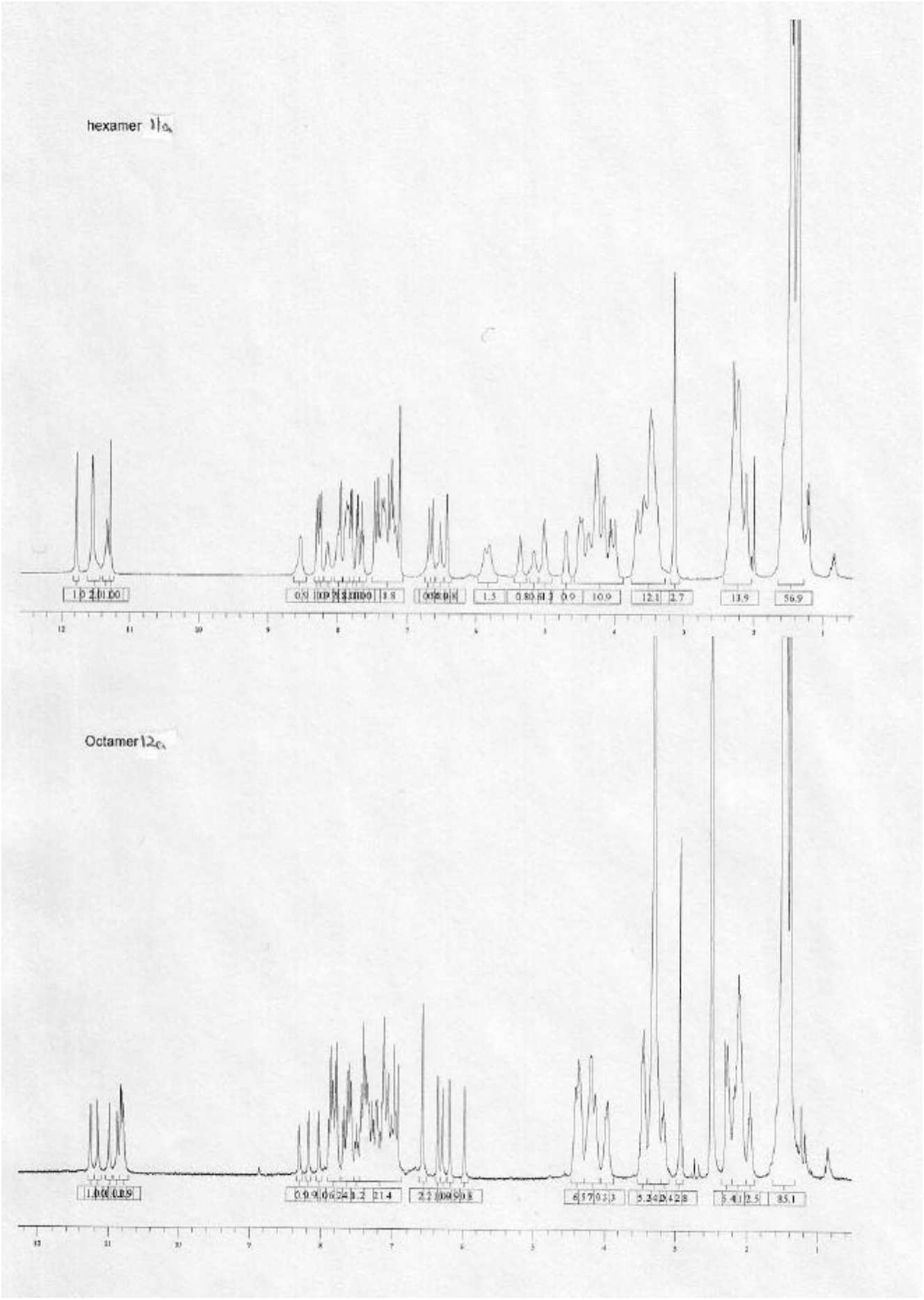
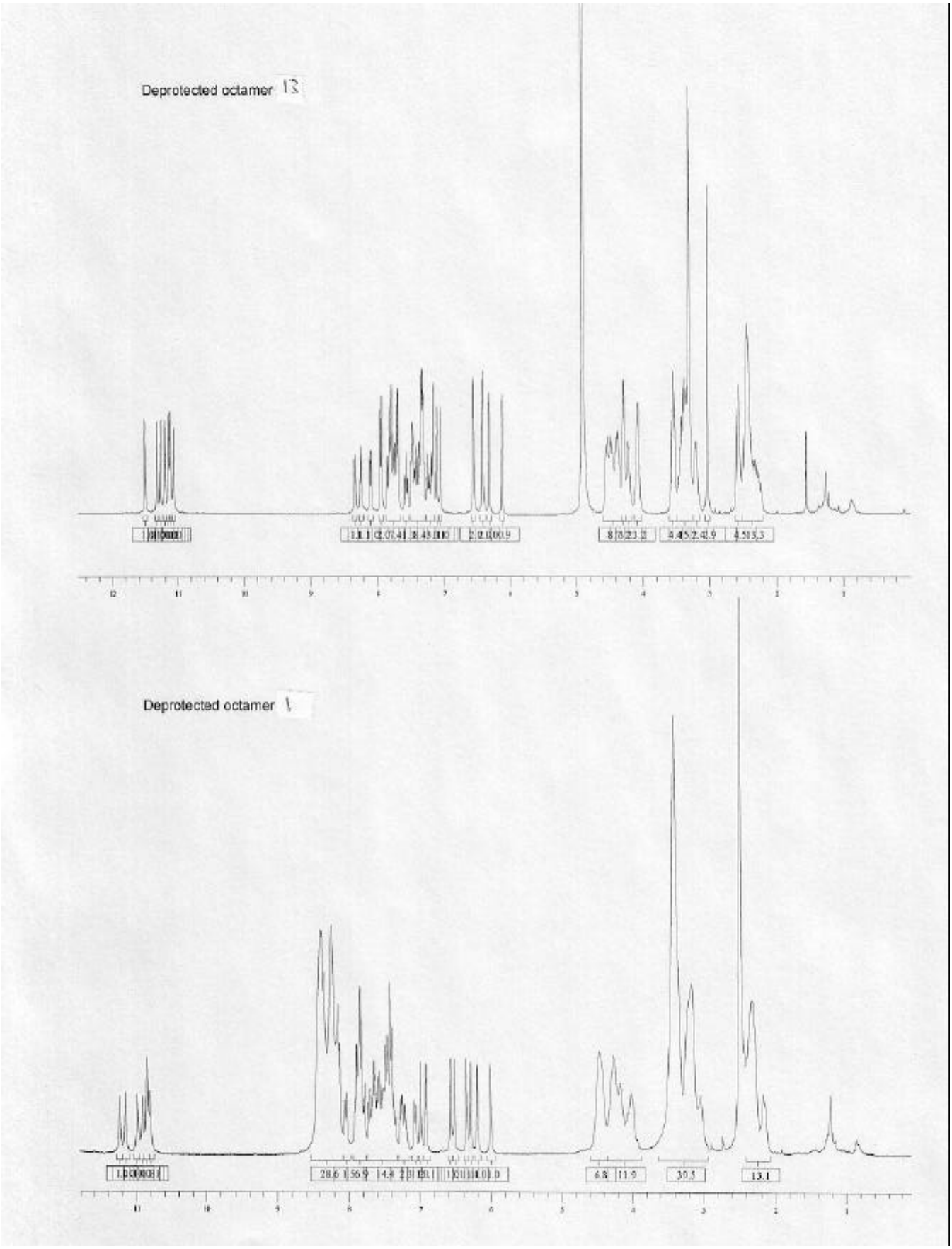
9.5
42.6


hexamer
Octamer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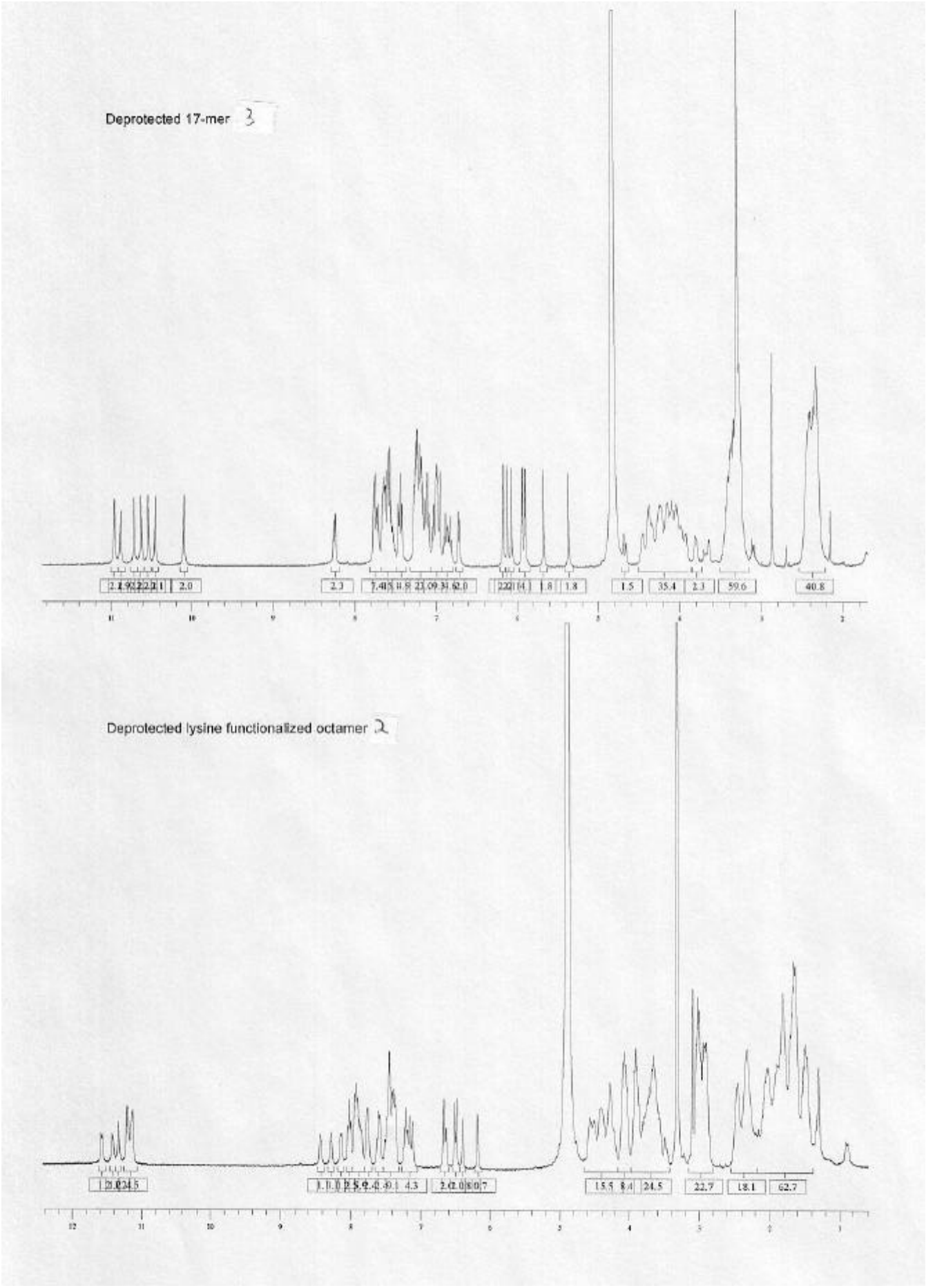
Deprotected octamer 1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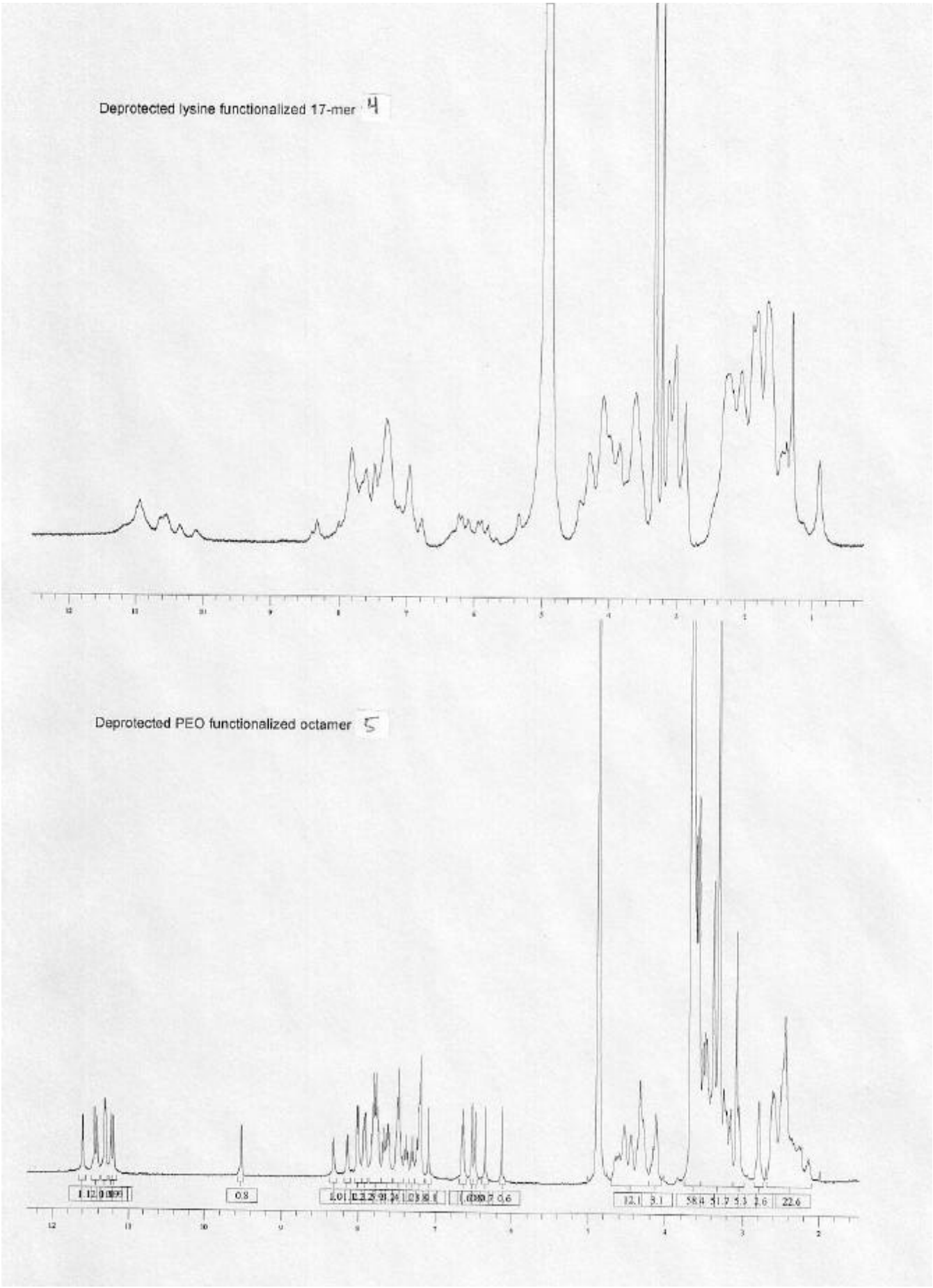
Deprotected lysine functionalized octamer 2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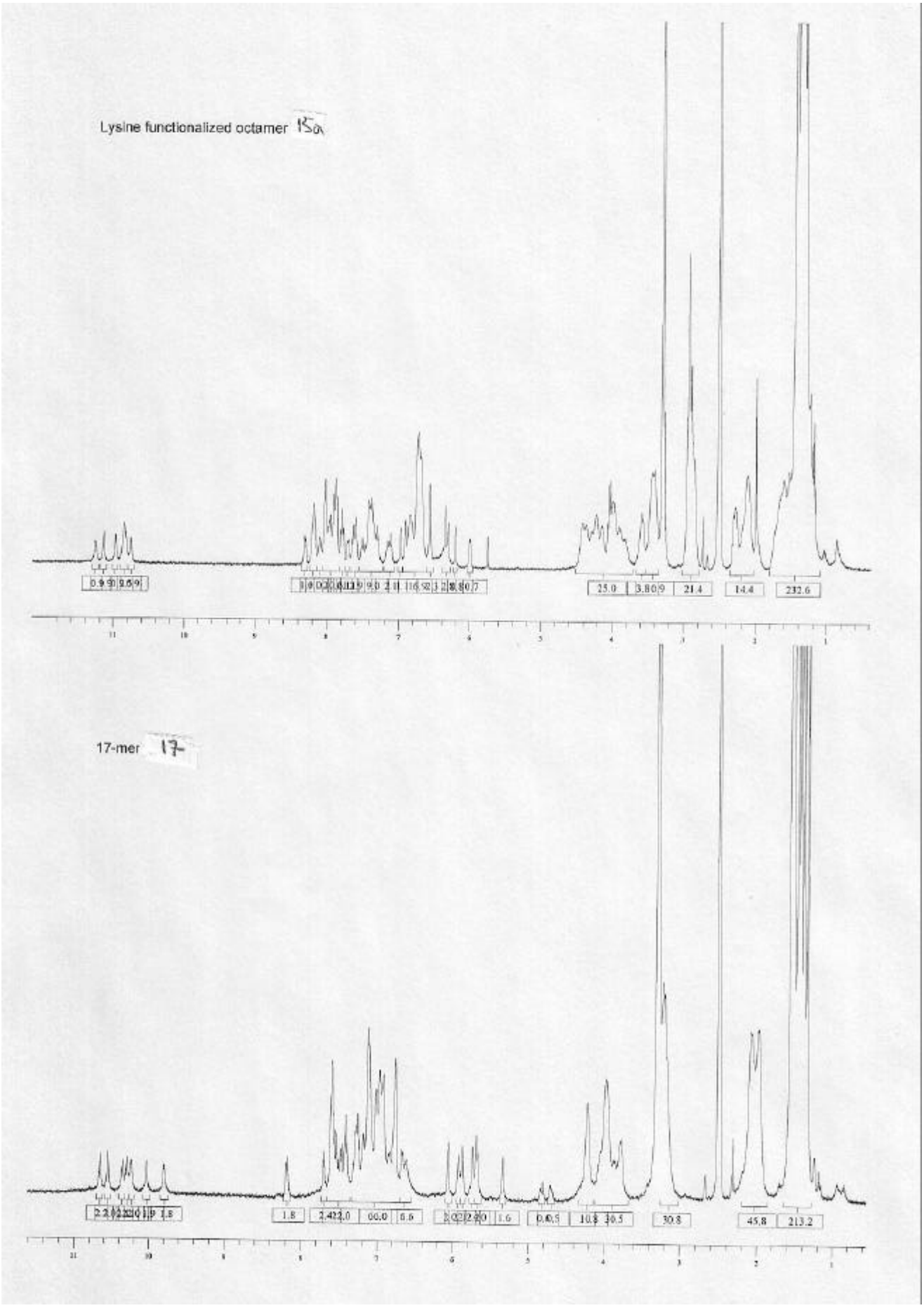
Deprotected PEO functionalized octamer 5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14.4
232.6
17-mer 17
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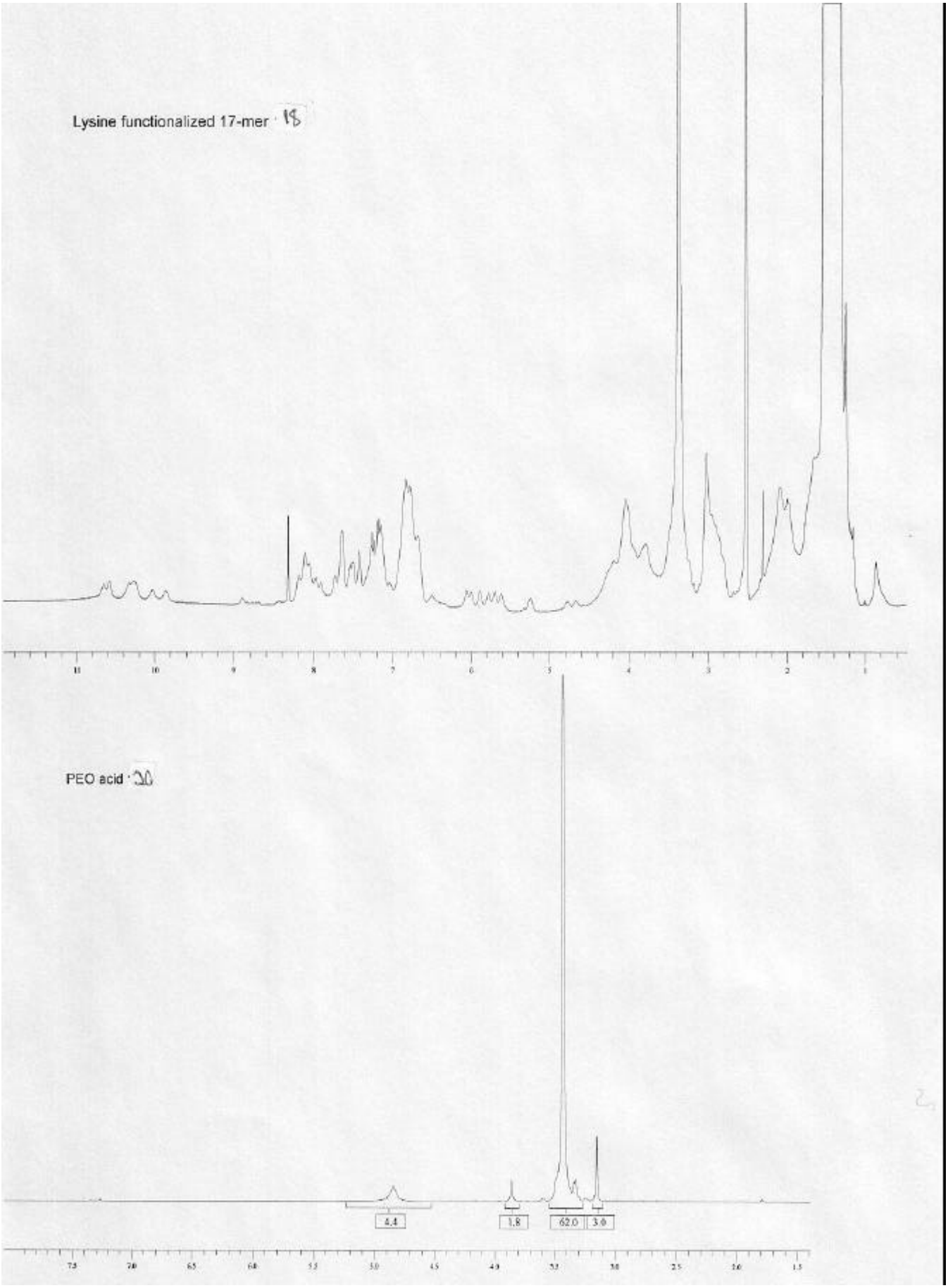
45.8
213.2


Lysine functionalized 17-mer · 18
PEO acid · 20
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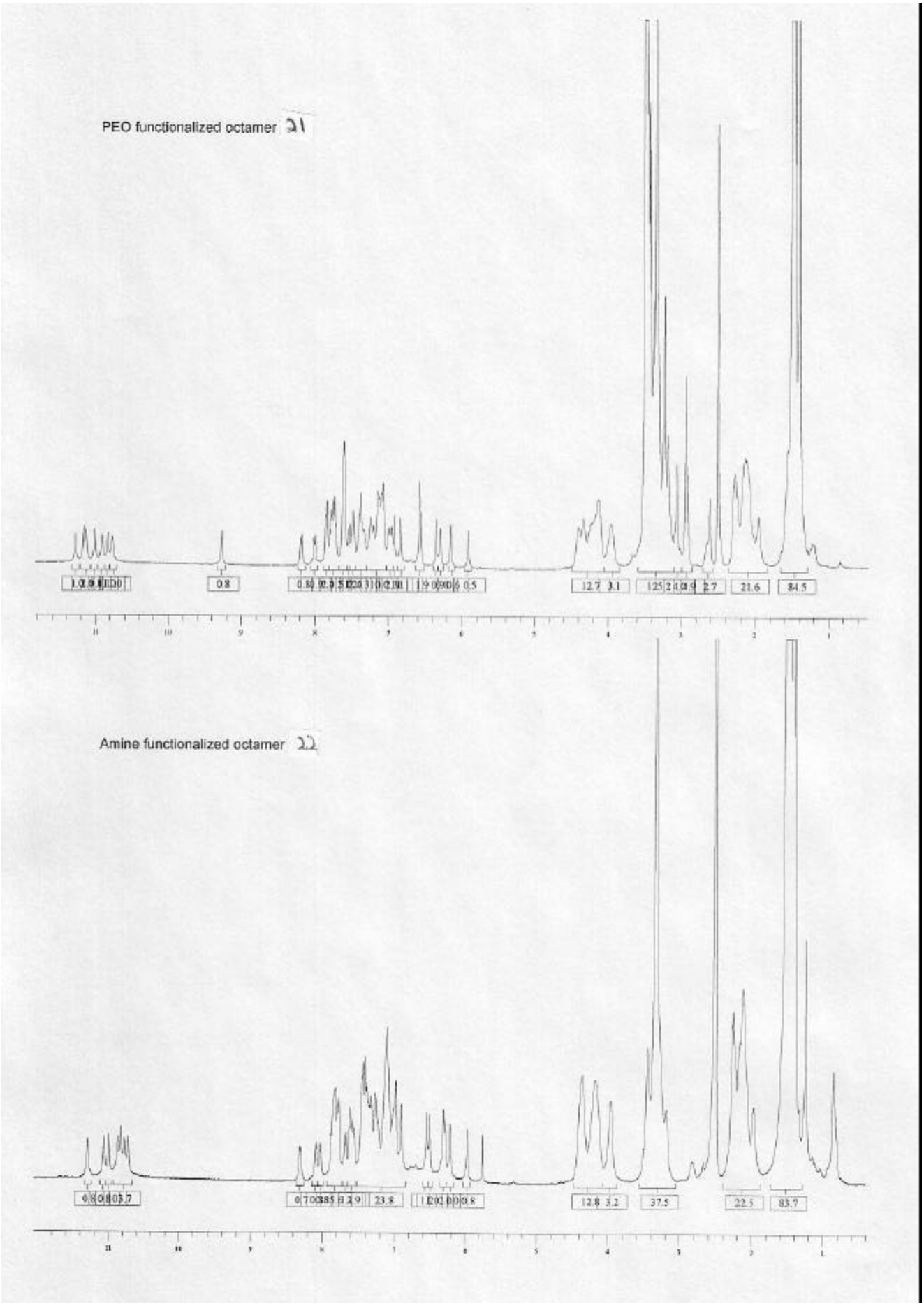
1.8
62.0
3.0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84.5
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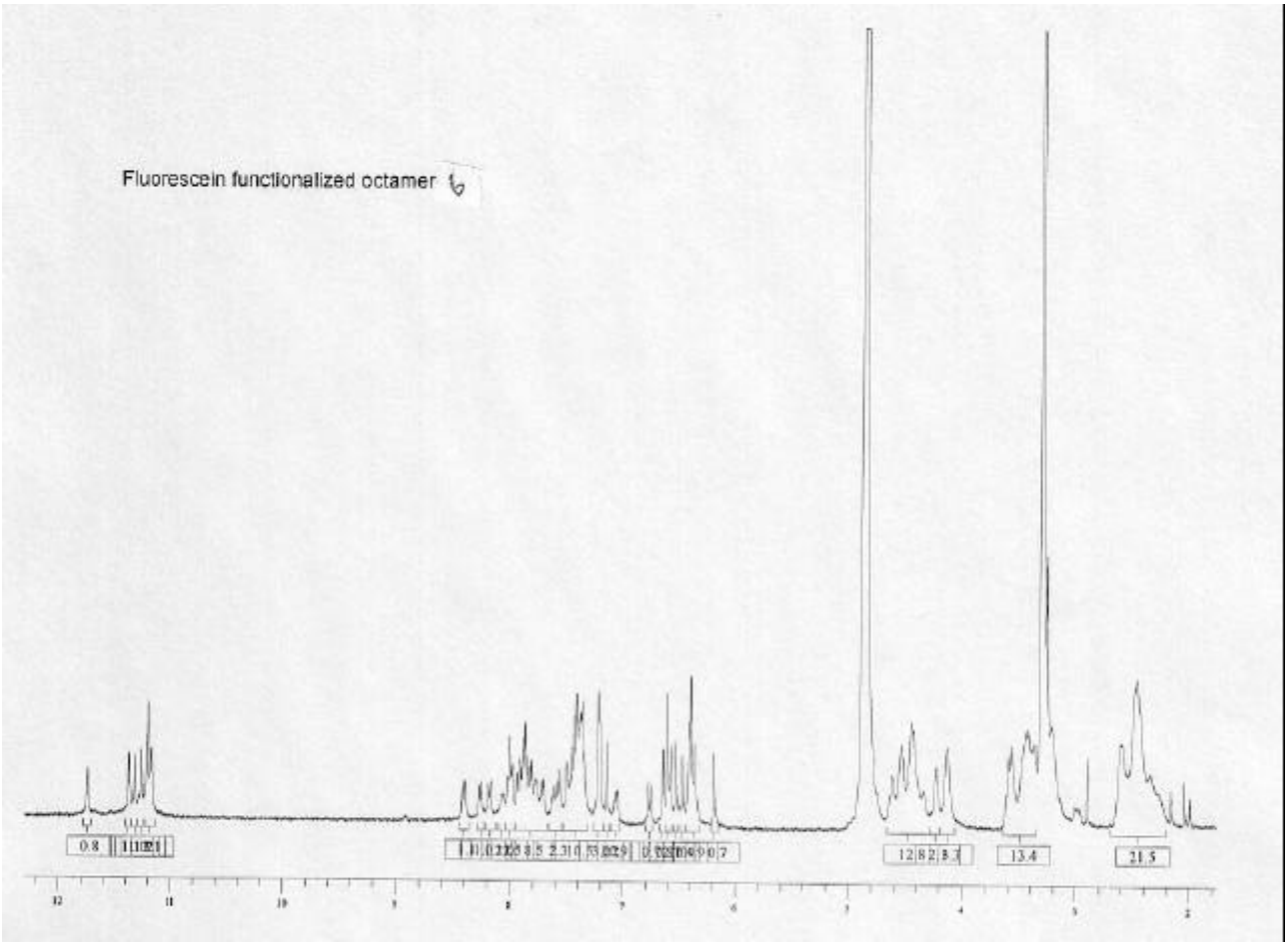
3.2
37.5
22.5
83.7



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