Supporting Information for :

"Polyamide Amino Acids": a new class of RNA Ligands

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Abbreviations:
NMP (N-methyl-2-pyrrolidinone), DIPEA (N,N-Diisopropylethylamine), DMF (Dimethylformamide), HBTU (2-(1H-benzotriazol-1-YL)-1,1,3,3-tetramethyluroniumhexafluorophosphate), NBSCl (Nitrobenzenesulfonyl chloride), NMM (N-methylmorpholine), Pyr (Pyridine), TEA (Triethylamine), TFA (Trifluoroacetic acid), TFMSA (Trifluoromethanesulfonic acid), Ac_2O (Acetic anhydride), Boc_2O (terbutyloxy anhydride), THF (Tetrahydrofurane), TIS (Triisopropylsilane), CH_2Cl_2 or DCM (Dichloromethane), EtOAc (Ethyl acetate), cyhex (Cyclohexane), DEA (Diethylamine), MeOH (Methanol), EtOH (Ethanol), DMSO (Dimethylsulfoxide), D_2O (Deuterium oxide), $CDCl_3$ (Deuterium Chloroform), Z (Benzyloxycarbonyl), Boc (Terbutyloxycarbonyl).

Materials and equipment

Synthesis of N-protected PAA monomers

Solvents and reagents were obtained from commercial sources and used without further purification. N-protected amino acids, were purchased from Novabiochem (VWR, Fontenay-sous-Bois, France). Dimethylformamide (peptide grade) used for coupling steps was purchased from IRIS Biotech GmbH.
Analytical thin-layer chromatography (TLC) was conduced on Merck (VWR) precoated silica gel 60F254 plates and compounds were visualized with ninhydrin test and/or under ultraviolet light (254 nm). Column chromatographies were carried out on silica gel (Merck, SDS 60A, 63-200µm, VWR). N-protected PAA monomers (**9**-**12**) were synthezised as described below and analysed by reverse-phase HPLC on a Thermo RP-18 column (3.2 x 250 mm, 5µm, 300 Å) using a Waters apparatus (St Quentin en Yvelines, France) including HPLC Alliance 2695, 996 photodiode array detector. Data were monitored using a Waters Millenium software. All HPLC analysis were run at room temperature. Solvent A and solvent B respectively, 0.1% TFA in water and 0.1% TFA in acetonitrile were used for HPLC studies. For monomers HPLC analysis, a gradient of A/B (80/20 to 0/100 for 30 min) was employed at a flow rate of 1 mL/min. ^1H, ^{13}C and HSQC NMR spectra of N-protected PAA monomers were recorded with Brucker AC 200 (^1H: 200 MHz, ^{13}C: 50 MHz) or AV 500 (^1H: 500 MHz, ^{13}C: 125 MHz) spectrometers using deuterated DMSO or $CDCl_3$ purchased from eurisotop. Chemical shift (δ) are reported in parts per million (ppm). ^1H NMR splitting patterns are designated as singlet (s), doublet (d), triplet (t), quartet (q). Splitting patterns that could not be interpreted or easily visualized were recorded as multiplet (m) or broad resonance (br). The NMR spectra of some compounds displayed a doubling of signals (or broad signals) cause by the presence of an equilibrium mixture of two major isomers generated by the substituted amide bonds. Their chemical shifts are described in italic when separated on spectra. ESI mass spectra were recorded with a Bruker Esquire 3000 plus, equipped with an atmospheric pressure ionization source. This method used in positive mode and negative mode gives respectively either $(M+H)^+$ and/or $(M+Na)^+$ signals and $(M-H)^-$ signals.

Synthesis of PAA oligomers

All solvents and reagents for solid-phase synthesis were of peptide grade and purchased from Iris Biotech GmbH. TFMSA, TIS and Ac_2O were obtained from Alpha aesar. PAA oligomers (**1**-**8**) were prepared by a solid-phase strategy as described below. They were synthesized in a 20-mL glass peptide vessel fitted with a polyethylene filter disk. Solvents and soluble reagents were removed by filtration under Argon. All syntheses and washes were done at 25°C.

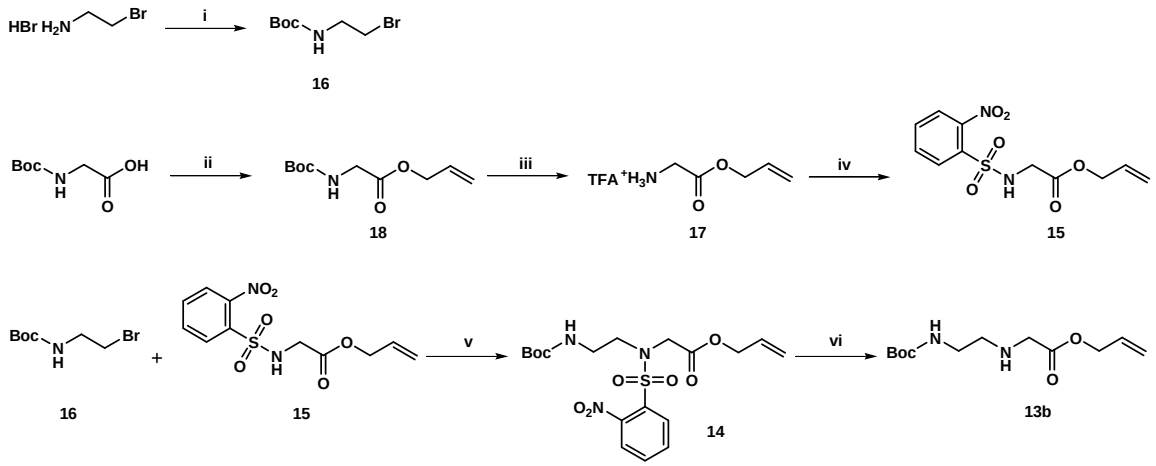
PAA oligomers were analysed by reverse-phase HPLC with the same equipment and solvents as for N-protected PAA monomers except that a gradient of A/B (100/0 for 7 min then 100/0 to 50/50 for 23 min) was employed at a flow rate of 1 mL/min. Preparative HPLC were performed on a Waters system (600E system controller, 2487 dual wavelength absorbance detector) with a Thermo RP-18 column (10 x 250 mm, 5 μm, 300 Å) at a flow rate of 3 mL/min, with the same solvent gradient as for analytical separations. ^1H, ^{13}C, COSY, HSQC, and HMBC NMR spectra were recorded with a Brucker AV 500(^1H: 500 MHz, ^{13}C: 125 MHz)

spectrometer using deuterated D_2O purchased from eurisotop. In the case of PAA oligomers, the presence of a mixture of several isomers cause signals multiplication, giving complex ^{1}H and ^{13}C NMR spectra. Thus, the reported data are those of the major conformers.

HRMS analysis was carried out on an LTQ Orbitrap hybrid mass spectrometer with an electrospray ionization probe (Thermo Scientific, San Jose, CA) by direct infusion from a pump syringue, to confirm correct molar mass and high purity of the compounds.

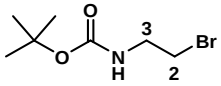
Synthesis

Synthesis of backbone 13b



Scheme S1: Synthesis of N-Boc allyl ester backbone. *Reagents and conditions:* (i) Boc_2O, MeOH, Et_3N, (86%); (ii) allyl bromide, Cs_2CO_3, DMF, 3h, rt, (94%); (iii) TFA/TIS 10%,1h, rt, (90%); (iv) NBSCl, NMM, CH_2Cl_2, (74%); (v) Cs_2CO_3, DMF, 20h, rt, (60%); (vi) PhSH, DIEA, DMF, overnight, rt, (66%)

tert-Butyl 2-bromoethylcarbamate (16)



To a stirred solution of 2-aminoethylbromide (2 g, 9.76 mmol) in MeOH (100 mL) was added TEA (14 mL, 100.5 mmol) followed by Boc_2O (4.1 g, 19.52 mmol). The reaction was stirred under reflux for 1 h then at room temperature for 14 h. The solvent was removed *in vacuo* and the obtained residue was diluted in DCM and washed successively with a 1M HCl solution (2 x 30 ml), a saturated $NaHCO_3$ solution (2 x 30 ml) and brine (2 x 30 ml). The organic layer was dried on $MgSO_4$ and concentrated under reduced pressure. The crude residue was purified by column chromatography (silica, CH_2Cl_2/MeOH, 100:0 to 98:2, v:v) to afford compound **16** as a yellow oil (1.87 g, 86%).

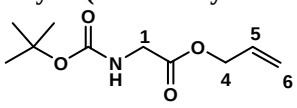
R_f (CH_2Cl_2) = 0.46

^{1}H RMN (500MHz, $CDCl_3$) δ= 5.01 (s, 1H, NH(Boc)), 3.51-3.43 (m, 4H, CH_2(2)+CH_2(3)), 1.42 (s, 9H, 3xCH_3(Boc))

^{13}C RMN (125MHz, $CDCl_3$) δ= 158.1 (1C, CO(Boc)), 79.8 (1C, C_{IV}(Boc)), 42.4 (1C, CH_2(3), 32.8 (1C, CH_2(2)), 28.3 (3C, 3xCH_3(Boc))

MS (ESI+) calcd for $C_7H_{14}NBrO_2$ $[M+H]^+$: 224.0, found : 224.1 $[M+H]^+$

Allyl 2-(tert-butoxycarbonylamino)acetate (18)



To a solution of BocGlyOH (3 g, 17.1 mmol) in DMF (40 mL) was added Cs_2CO_3 (6.1 g, 18.8 mmol) followed by allyl bromide (1.63 mL, 18.8 mmol). After stirring for 3 h at room temperature, the mixture was filtered on celite and the solvent was evaporated *in vacuo*. The residue was diluted in EtOAc and washed successively with

a 10% $NaHCO_3$ solution, water and brine. The organic layer was dried on $MgSO_4$ and evaporated under reduced pressure to afford compound **18** (4.6 g, 94%) as a colorless oil. **18** was subsequently used without further purification.

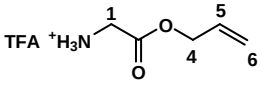
R_f (EtOAc/cyhex 1:1, v:v) = 0.78

^{1}H RMN (200MHz, $CDCl_3$) δ= 5.91 (tdd, *J* = 5.77, 10.34, 17.13 Hz, 1H, CH(5)), 5.36 (m, 2H, CH_2(6)), 5.04 (s, 1H, NH(Boc)), 4.63 (td, *J* = 1.27, 5.76 Hz, 2H, CH_2(4)), 3.94 (d, *J* = 5.62 Hz, 2H, CH_2(1)), 1.44 (s, 9H, 3xCH_3(Boc)) ;

^{13}C RMN (50MHz, $CDCl_3$) δ= 170.19 (1C, **C**O(OAll)), 158.3 (1C, **C**O(Boc)), 131.7 (1C, **C**H(5)), 118.9 (1C, **C**H_2(6)), 80.15 (1C, C_{IV}(Boc)), 65.9 (1C, CH_2(4)), 42.5 (1C, CH_2(1)), 28.4 (3C, 3xCH_3(Boc)) ;

MS (ESI+) calcd for $C_{10}H_{17}NO_4$ $[M+Na]^+$: 238.1, found : 238.0 $[M+Na]^+$

TFA. 2-(allyloxy)-2-oxoethanaminium (17)



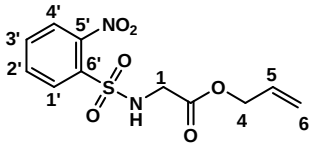
To a stirred solution of BocGlyOAll (4.6 g, 21.3 mmol) in CH_2Cl_2 (10 mL) cooled to 0°C, was added dropwise a 10% TFA/TIS solution (19 mL). The mixture was allowed to stir at room temperature for 1h, and the solvents were removed under reduced pressure. The residue was triturated in diethyl ether, filtered, and dried *in vacuo* to afford compound **17** (4.4 g, 90%) as a white solid.

R_f (EtOAc/MeOH, 1/1, v/v) = 0.39

^{1}H RMN (200MHz, D_2O) δ= 6.05-5.86 (m, 1H, CH(5)), 5.41-5.27 (m, 2H, CH_2(6)), 4.72 (d, *J* = 5.78Hz, 2H, CH_2(4)), 3.93 (s, 2H, CH_2(1)) ; **^{13}C RMN** (50MHz, D_2O) δ= 168.28 (1C, CO(OAll)), 131.3 (1C, CH(5)), 119.72 (1C, CH_2(6)), 67.62 (1C, CH_2(4)), 40.51 (1C, CH_2(1)) ;

MS (ESI+) calcd for $C_9H_9NO_2$ (without TFA anion) $[M+H]^+$: 116.1, found : 116.7 $[M+H]^+$

Allyl 2-(2-nitrophenylsulfonamido)acetate (15)



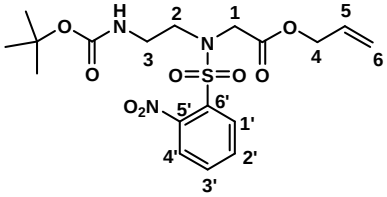
To a solution of TFA.HGlyOAll (4.4 g, 19.3 mmol) in CH_2Cl_2 (50 mL) was added NBSCl (4,3 g, 19.3 mmol). The reaction was cooled to 0°C and N-methylmorpholine (5.3 mL, 48.2 mmol) was added. The mixture was stirred for 2 h, then washed with a saturated $NaHCO_3$ solution, with water and with brine. The organic layer was dried on $MgSO_4$ and after filtration and concentration under reduced pressure, the residue was precipitated in diethyl ether and the resulting solid was collected, washed with diethyl ether and dried under vacuum to give compound **15** (4.3 g, 74%) as a colorless solid.

R_f (EtOAc/cyhex, 1/1, v/v) = 0.63

^{1}H RMN (200MHz, $CDCl_3$) δ= 8.12-8.07 (m, 1H, CH_{ar}(4')), 7.97-7.90 (m, 1H, CH_{ar}(1')), 7.76-7.72 (m, 2H, 2xCH_{ar}(2'+3'), 6.06 (t, *J* = 4.56Hz, 1H, NH(NBs)), 5.77 (tdd, J = 5.88, 10.24, 17.19Hz, 1H, CH(5)), 5.30-5.19 (m, 2H, CH_2(6)), 4.49 (td, J = 1.21, 5.89Hz, 2H, CH_2(4)), 4.05 (d, *J* = 5.80Hz, 2H, CH_2(1)) ; **^{13}C RMN** (50MHz, $CDCl_3$) δ= 168.38 (1C, **C**O(OAll), 158.3 (1C, **C**$_{IV}$(5')), 134.12 (1**C**, **C**$_{IV}$(6'), 133.8 (1C, **C**H(2')), 133.0 (1C, **C**H(3')), 131.1 (1C, **C**H(5)), 130.7 (1C, **C**H(1')), 125.8 (1C, **C**H(4')), 119.53 (1C, **C**H_2(6)), 66.49 (1C, CH_2(4)), 45.05 (1C, CH_2(1)) ;

MS (ESI+) calcd for $C_{11}H_{12}N_2O_6S$ $[M+Na]^+$: 323.0, found : 323.3 $[M+Na]^+$

Allyl 2-(N-(2-(tert-butoxycarbonylamino)ethyl)-2-nitrophenylsulfonamido)acetate (14)



To a stirred solution of NBSGlyOAll (1.34 g, 4.46 mmol) in DMF (10 mL) was added under inert atmosphere, first Cs_2CO_3 (2.92 g, 8.92 mmol) and then dropwise a solution of $BocNH(CH_2)_2Br$ (2.0 g, 8.92 mmol) in DMF (5 mL). The mixture was allowed to stir at room temperature for 20 h. Subsequent removal of solid via filtration on celite and concentration under reduced pressure afforded a crude product which was taken up in EtOAc and

washed twice with water and brine. The organic layer was dried on $MgSO_4$ and the solvent was evaporated off. The obtained oily residue was purified by column chromatography (silica, EtOAc/cyhex 3:7 to 5:5, v:v) to give compound **14** as a pale yellow oil (1.18 g, 60%).

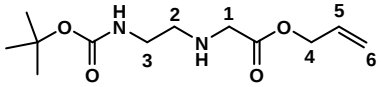
R_f (EtOAc/Cyhex 1/1, v/v) = 0.52

^{1}H RMN (200MHz, $CDCl_3$) δ= 8.07-8.03 (m, 1H, CH_{ar}(4')), 7.72-7.58 (m, 3H, 3xCH_{ar}(2'+3'+1')), 5.84 (tdd, *J* = 5.84, 10.32, 17.15Hz, 1H, CH(5)), 5.34-5.20 (m, 2H, CH_2(6)), 5.02 (m, 1H, NH(Boc)), 4.57 (td, *J* = 1.21, 5.84Hz, 2H, CH_2(4)), 4.23 (s, 2H, CH_2(1)), 3.49 (t, *J* = 5.71Hz, 2H, CH_2(2)), 3.33 (m, 2H, CH_2(3)), 1.41 (s, 9H, 3xCH_3(Boc)) ; **^{13}C RMN** (50MHz, $CDCl_3$) δ= 168.76 (1C, **C**OOAll), 155.98 (1C, **C**O(Boc)), 147.90 (1C, **C**$_{IV}$(5')), 133.04 (1C, **C**$_{IV}$(6')), 133.71 (1C, CH_{ar}(2')), 131.78 (1C, CH_{ar}(3')), 131.17 (1C, CH_{ar}(1')), 131.00 (1C, CH(5)), 124.18 (1C, CH_{ar}(4')), 119.14 (1C, **C**H_2(6)), 79.58 (1C, **C**$_{IV}$(Boc)), 66.15 (1C, **C**H_2(4)), 48.72, *48.54* (1C, **C**H_2(1)), 44.87 (1C, **C**H_2(2)), 38.40 (1C, **C**H_2(3)), 28.31(3C, 3xCH_3(Boc)) ;

MS (ESI+) calcd for $C_{18}H_{25}N_3O_8S$ [M+ Na]$^+$: 466.1, found: 465.9 [M+Na]$^+$

Synthesis of N-protected PAA monomers

Allyl 2-(2-(tert-butoxycarbonylamino)ethylamino)acetate (13b)



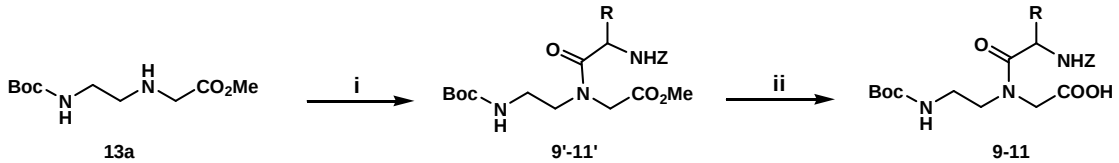
To a solution of Boc(NBS)OAll (1,07 g, 2,41 mmol) in DMF (8 mL) was added DIEA (1.68 mL, 9.64 mmol) and thiophenol (1.24 mL, 12.06 mmol). The reaction was stirred overnight at room temperature, and the solvent was removed under reduced pressure. The residue was taken up in EtOAc and extracted with a 1M $KHSO_4$ solution (2 x 30 mL). The aqueous layer was basified at 0°C with a saturated Na_2CO_3 solution until pH = 10 and extracted with DCM (4 x 40 mL). The combined organic layers were washed with brine, dried on $MgSO_4$ and concentrated under reduced pressure. The oily residue was purified by column chromatography (silica, EtOAc/cyhex 4:6 to 7:3, v:v*)* to afford compound **13b** as a yellow oil (0.37 g, 60%).

R_f (EtOAc)= 0.44

^{1}H RMN (200MHz, $CDCl_3$) δ= 5.91 (tdd, J = 5.80, 10.31, 17.15Hz, 1H, CH(5)), 5.37-5.21 (m, 2H, CH_2(6)), 5.03 (br s, 1H, NH(Boc)), 4.62 (td, J = 1.28, 5.80Hz, 2H, CH_2(4)), 3.42 (s, 2H, CH_2(1)), 3.24-3.16 (m, 2H, (CH_2(3)), 2.76-2.70 (m, 2H, CH_2(2)), 1.65 (s, 1H, NH), 1.42 (s, 9H, 3xCH_3(Boc)) ;

^{13}C RMN (50MHz, $CDCl_3$) δ= 172.3 (1C, **C**OOAll), 156.18 (1C, **C**O(Boc)), 131.9 (1C, **C**H(5)), 118.8 (1C, **C**H_2(6)), 79.3 (1C, **C**$_{IV}$(Boc)), 65.59 (1C, CH_2(4)), 50.54 (1C, CH_2(1)), 48.89 (1C, CH_2(2)), 40.31 (1C, CH_2(3)), 28.53 (3C, 3x**C**H_3(Boc)) ;

MS (ESI+) calcd for $C_{12}H_{22}N_2O_4$ [M+H]$^+$: 259.2, found : 259.1 [M+H]$^+$



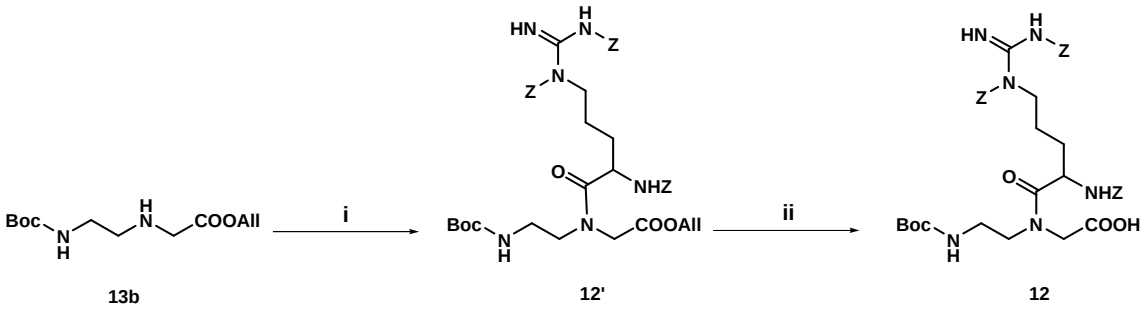
Z = benzyloxycarbonyl

(9) R = CH_3, PAA(Ala)

(10) R = CH_2-Ph, PAA(Phe)

(11) R = $(CH_2)_4$-NH-Z, PAA(Lys)

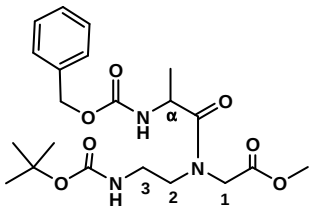
Scheme S2: Synthesis of N-protected PAA monomers. *Reagents and conditions:* (i) Z-NHCH(R)CO_2H/ HBTU/ DIPEA/ DMF, 2h, rt, (82%); (ii) LiOH/H_2O/THF, 0°C, 1h, (90%)



Scheme S3: Synthesis of N-protected PAA(Arg) monomer. *Reagents and conditions:* (i) Z-Arg(Z_2)-OH/ HBTU/ DIPEA/ DMF, 2h, rt, (80%); (ii) $Pd(PPh_3)_4$/DEA/DMF, 30min, rt, (80%)

General procedure for the synthesis of N-protected PAA methyl ester monomers (9'-12')
To a solution of the N-protected amino acid (5.16 mmol) in DMF (14 mL) was added DIPEA (17.2 mmol) and HBTU (5.16 mmol). The reaction was cooled to 0°C, stirred for 3 min and subsequently, a solution of backbone **13a**[1] or **13b** (4.3 mmol) in DMF (2 mL) was added. The mixture was stirred at room temperature for 2 h, and the solvent was removed under reduced pressure. The residue was taken up in EtOAc (100 mL), washed with a 1M $KHSO_4$ solution (3 x 30 mL), a saturated $NaHCO_3$ solution (3 x 30 mL), H_2O (2 x 30 mL) and brine (50 mL). The organic layer was dried on $MgSO_4$ and subsequent removal of solid via filtration and concentration under reduced pressure afforded an oily residue which was purified by flash column chromatography (silica, EtOAc/cyhex 1:1, v:v) to give the corresponding PAA monomer (**9'-12'**) as a pale yellow oil.

Boc(Z-Ala)OMe (9')



Synthesis of **9'** was prepared following the general procedure described above. **9'** (1.6 g, 85%).
R_f (EtOAc/cyhex, 6:4, v:v) = 0.45
^{1}H NMR (200MHz, DMSO-d_6) : δ (two major isomers)= *7.57*, 7.53 (d, J = 8.0Hz, 1H, NH(Z)) ; 7.40-7.27 (m, 5H, CH_{ar}(Z)) ; 6.85, *6.73* (t, J = 5.6Hz, 1H, NH(Boc)) ; 5.08-4.91 (m, 2H, CH_2(Z)) ; 4.65-4.45, *4.37-4.26* (m, 1H, CHα) ; *4.28*, 4.14 (d, J = 17.0Hz, 1H, CH_2(1)) ; *4.22*, 3.89 (d, J = 17.0Hz, 1H, CH_2(1)) ; *3.66*, 3.61 (s, 3H, OCH3) ; 3.51-2.95, (m, 4H, CH_2(2) et CH_2(3)) ; 1.36 (s, 9H, 3xCH_3(Boc)) ; 1.19, *1.13* (d, J = 6.8Hz, 3H, CH_3(Ala)) ; **^{13}C NMR** (50MHz, DMSO-d_6) : δ (two major isomers)= 173.23, *172.79* (1C, N-**C**O); *170.18*, 169.71 (1C, **C**OOMe); 155.61 ; 155.51 (1C, **C**ONH(Z+Boc)); 137.02, *136.93* (1C, **C**$_{IV}$(Z)); 128.33, 127.77, 127.70 (5C, 5x**C**H(Z)); 77.87, *77.69* (1C, **C**$_{IV}$(Boc)); *65.47*, 65.33 (1C, **C**H_2(Z)); *52.08*, 51.68 (1C, O**C**H_3); *49.31*, 47.74, 47.46, *46.65* (2C, **C**H_2(1)+**C**H_2(2)) ; *46.31*, 45.97 (1C, **C**Hα); *38.50* (1C, **C**H_2(3)); *28.15* (3C, 3x**C**H_3(Boc)); *17.71*, 17.59 (1C, **C**H_3(Ala)) ;
MS (ESI+) (m/z) : calcd for $C_{21}H_{31}N_3O_7$ $[M+Na]^+$: 460.2, found: 460.3 $[M+Na]^+$
$[\alpha]_D^{25}$ (C=1, MeOH) : -17.6
HPLC T_R = 13.9 min

[1] Uhlmann, E. ; Peyman, A. ; Breipohl, G. ; Will D.W. ; *Angew. Chem. Int. Ed.* **1998**, 37, (20), 2796-2823.

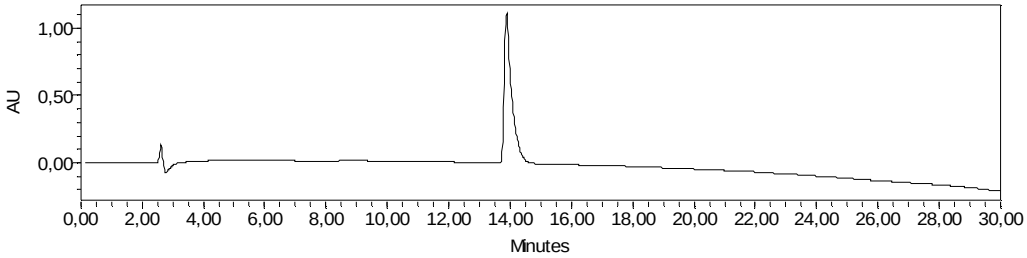


Figure 1: HPLC trace at 210nm of the purified **9'** monomer.

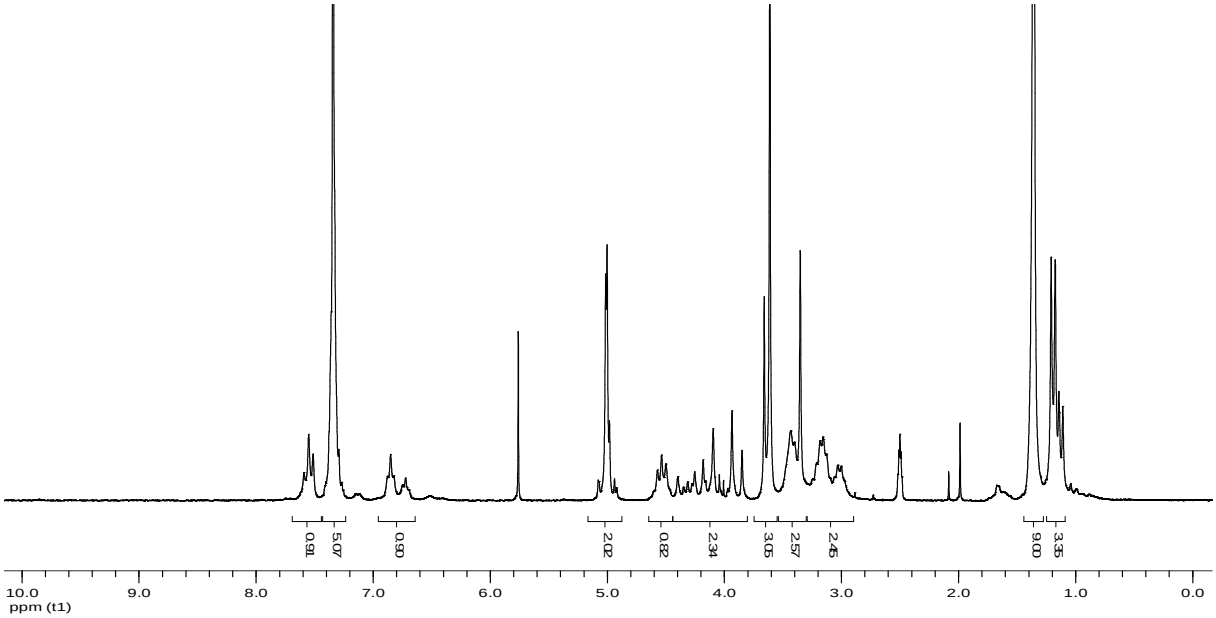
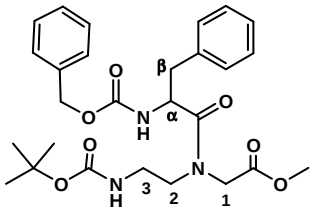


Figure 2: ^{1}H NMR of **9'** monomer.

Boc(Z-Phe)OMe (10')



10' was prepared following the general procedure described above. **10'** (1.95 g, 88%).
R_f (EtOAc/cyhex, 6:4, v:v) = 0.54
^{1}H NMR (500MHz, DMSO-d_6) : δ (two major isomers)= *7.75*, 7.67 (d, J = *8.8Hz*, J = 8.4Hz, NH(Z)) ; 7.36-7.19 (m, 10H, 5xCH_{ar}(Z)+5xCH_{ar}(Phe)) ; 6.91, *6.72* (t, J = 5.7Hz, NH(Boc)) ; 4.99-4.85 (m, 2H, CH_2 (Z)) ; 4.78-4.68, *4.43-4.35* (m, 1H, CHα) ; 4.45, 4.13 (d, J = 18.6Hz, J = 17.1Hz, 1H, CH_2(1)) ; 4.23, 3.97 (d, J = 18.6Hz, J = 17.1Hz, 1H, CH_2(1)) ; *3.67*, 3.64 (s, 3H, OCH_3) ; 3.56-2.98 (m, 4H, CH_2(2)+CH_2(3)) ; 2.95-2.73 (m, 2H, CH_2β) ; 1.37 (s, 9H, 3xCH_3(Boc)) ; **^{13}C NMR** (125MHz, DMSO-d_6) : δ (two major isomers)= 173.21, *172.86* (1C, N-**C**O) ; *171.05*, 170.51 (1C, **C**OOMe) ; 156.65 (1C, **C**O(Z)) ; 156.58, *156.46* (1C, **C**O(Boc)) ; *138.65*, 138.32 (1C, **C**$_{IV}$(Phe)) ; 137.85 (1C, **C**$_{IV}$(Z)) ; 130.32, *130.12*, 129.15, *128.96*, 128.89, 128.55, 128.32, *128.20*, 127.28, *127.17* (10C, 5x**C**H_{ar}(Z)+ 5x**C**H_{ar}(Phe)) ; 78.79, *78.60* (1C, **C**$_{IV}$(Boc)) ; *66.15*, 66.10 (1C, **C**H2(Z)) ; *53.11*, 52.92 (1C, **C**Hα) ; *53.03*, 52.60 (1C, O**C**H_3) ; *50.35*, 48.83 (1C, **C**H_2(1)) ; 48.56, *47.69* (1C, **C**H_2(2)) ; 39.30, *38.50* (1C, **C**H_2(3)) ; *38.02*, 37.86 (1C, **C**H_2β) ; *29.08*, 29.02 (3C, 3x**C**H_3(Boc)) ;
MS (ESI+) (m/z) : calcd for $C_{27}H_{35}N_3O_7$ $[M+Na]^+$: 536.2, found: 536.2 $[M+Na]^+$
$[\alpha]_D^{25}$ (C=1, MeOH) : -9.7
HPLC T_R = 17.8 min

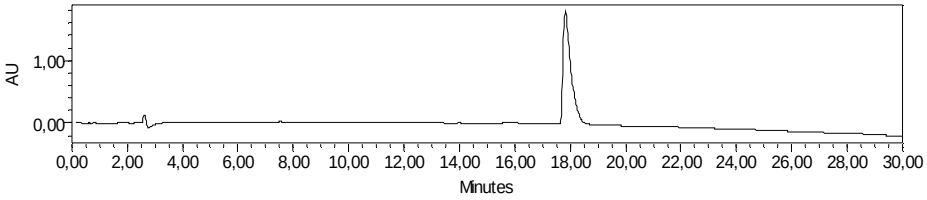


Figure 3: HPLC trace at 210nm of the purified **10'** monomer.

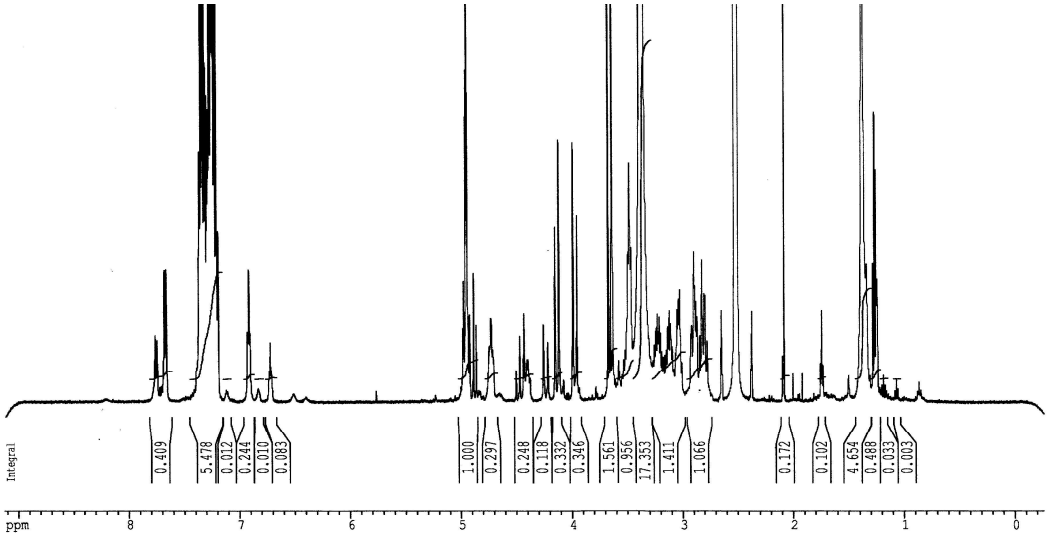
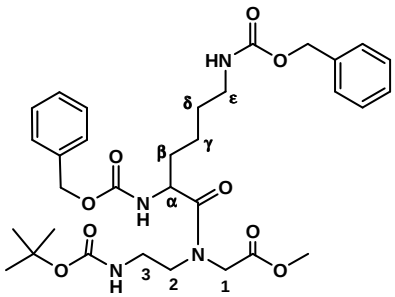


Figure 4: ^{1}H NMR of **10'** monomer.

Boc(Z-Lys(Z))OMe (11')



11' was prepared following the general procedure described above. **11'** (2,2 g, 80%).
R_f (EtOAc/cyhex, 6:4, v:v) = 0.52
^{1}H NMR (500MHz, DMSO-*d_6*) : δ (two major isomers)= *7.53*, 7.47 (d, J = *8.0Hz*, J = 7.0Hz, 1H, NH^{α}) ; 7.41-7.26 (m, 10H, 10xCH_{ar}(Z)) ; 7.23 (m, 1H, NH^{ε}) ; 6.84, *6.70* (m, 1H, NH(Boc)) ; *5.04*, 5.01 (s, 2H, CH_2(Z)) ; 4.99 (s, 2H, CH_2(Z)) ; 4.49-4.38 (m, 1H, CHα) ; 4.14 (d, J = 17.0Hz, 1H, CH_2(1)) ; 3.87 (d, J = 17.0Hz, 1H, CH_2(1)) ; *3.64*, 3.60 (s, 3H, OCH_3) ; 3.49-3,28 (m, 2H, CH_2(2)) ; 3.40-2.89 (m, 4H, CH_2(3)+CH_2ε) ; 1.61-1.45 (m, 2H, CH_2β) ; 1.45-1.24 (m, 4H, CH_2γ CH_2δ) ; 1.35 (s, 9H, 3xCH_3(Boc)) ; **^{13}C NMR** (50MHz, DMSO-*d_6*) : δ (two major isomers)= 172.75, *172.39* (1C, N-**C**O) ; *171.15*, 169.71 (1C, **C**OOMe) ; 156.09, 155.94 (1C, **C**O(Z)) ; 155.65 (1C, **C**O(Boc)) ; 137.27, 137.02, *136.97* (2C, $\mathbf{C}_{IV}$(Z)) ; 128.35, 127.75, *127.70* (10C, $\mathbf{C}H_{ar}$(Z)) ; 77.88, *77.73* (1C, $\mathbf{C}_{IV}$(Boc)) ; *65.50*, 65.40, 65.16 (1C, $\mathbf{C}H_2$(Z)) ; *52.08*, 51.69 (1C, O$\mathbf{C}H_3$) ; 50.56 (1C, **C**Hα) ; 47.81, 47.55 (2C, $\mathbf{C}H_2$(1)+$\mathbf{C}H_2$(2)) ; 39.30, 38.50 (1C, $\mathbf{C}H_2$(3)) ; 31.06 (1C, $\mathbf{C}H_2$β) ; 29.20 (1C, $\mathbf{C}H_2$γ) ; 28.15 (3C, 3x$\mathbf{C}H_3$(Boc)) ; 22.54 (1C, $\mathbf{C}H_2$δ) ; CH_2ε was not located because it was under DMSO signals.
MS (ESI+) (m/z) : calcd for $C_{32}H_{44}N_4O_9$ $[M+Na]^+$: 651.3, found: 651.2 $[M+Na]^+$
$[\alpha]_D^{25}$ (C=1, MeOH) : -10.3
HPLC T_R = 18.1 min

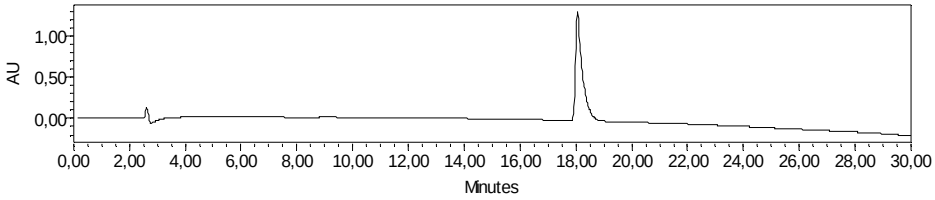


Figure 5: HPLC trace at 210nm of the purified **11'** monomer.

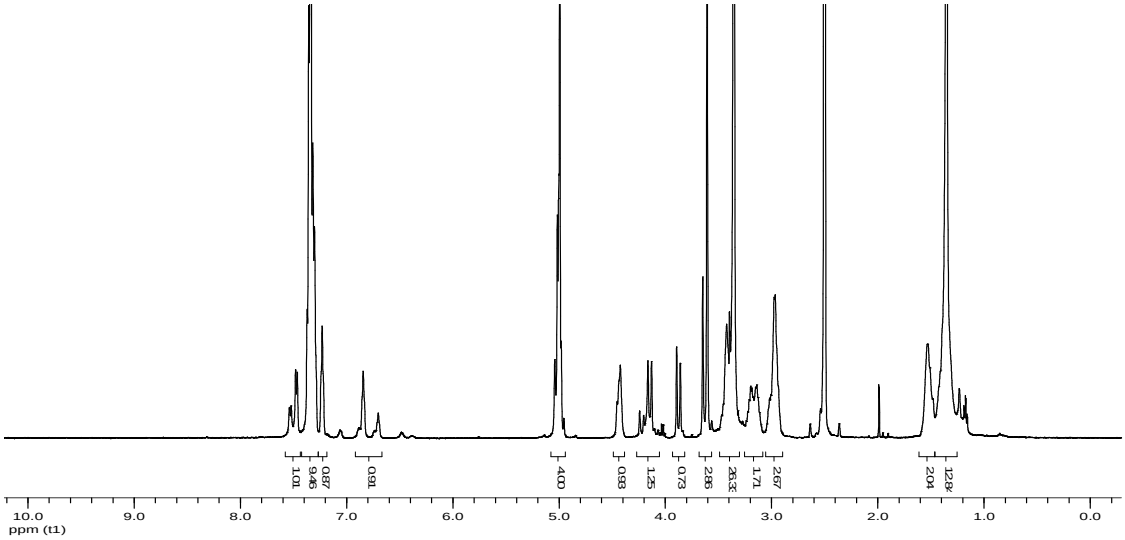
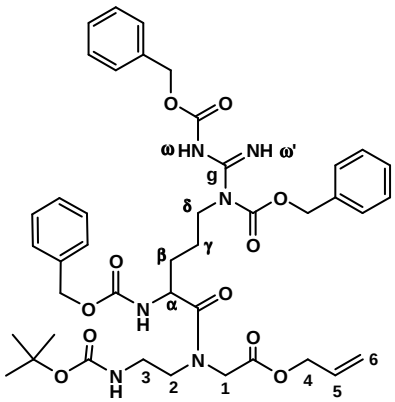


Figure 6: ^{1}H NMR of **11'** monomer.

Boc(Z-Arg(Z$_2$))OAll (12')



12' was prepared following the general procedure described above. **12'** (2.84 g, 81%).
R$_f$ (EtOAc/cyhex, 5:5, v:v) = 0.47
^{1}H NMR (500MHz, DMSO-*d$_6$*) : δ (two major isomers)= 9.18 (br s, 2H, NH=C-NHZ), *7.62*, 7.55 (d, J = *8.0Hz*, J = 7.5Hz, 1H, NH$^{\alpha}$(Z)) ; 7.44-7.22 (m, 15H, 15xCH$_{ar}$(Z)) ; 6.83, *6.71* (t, J = 5.5 Hz, J = *5.5 Hz*, 1H, NH(Boc)) ; 5.91-5.80 (m, 1H, CH(5)) ; 5.28 (dd, J = 18.0Hz and 1.5Hz, 1H, CH$_2$(6)) ; 5.22 (s, 2H, CH$_2$(Z)$^{\omega}$) ; 5.17 (dd, J = 10.5Hz and 1.5Hz, 1H, CH$_2$(6)) ; 5.05 (s, 2H, CH$_2$(Z)$^{\omega'}$) ; 4.99, *4.98* (s, 2H, CH$_2$(Z)$^{\alpha}$) ; 4.57-4.43 (m, 3H, CHα+CH$_2$(4)) ; 4.29-3.76 (m, 4H, CH$_2$(1)+CH$_2$δ) ; 3.47-2.92 (m, 4H, CH$_2$(2)+CH$_2$(3)) ; 1.75-1.45 (m, 4H, CH$_2$β CH$_2$γ) ; *1.35*, 1.33 (s, 9H, 3xCH$_3$(Boc)) ;
^{13}C NMR (125MHz, DMSO-*d$_6$*) : δ (two major isomers) 172.41, *171.98* (1C, **C**O(Arg)) ; *169.305*, 168.77 (1C, **C**OOAll); 162.87 (1C, **C**O(Z)$^{\delta}$; 159.68 (1C, **C**O(Z)$^{\omega}$; 155.85, *155.60* (1C, **C**O(Boc)) ; 154.96 (1C, **C**O(Z)$^{\alpha}$; 137.10, *136.93* (1C, **C**$_{IV}$g) ; 135.25 (3C, **C**$_{IV}$(Z)) ; 132.24, *132.09* (1C, **C**H(5)) ; 128.47, 128.27, *128.21*, 127.89, 127.79, *127.73*, 127.65, 127.62, *127.58* (15C, 15x**C**H(Z)) ; *118.01*, 117.72 (1C, **C**H$_2$(6)); 77.82, *77.65* (1C, **C**$_{IV}$(Boc)) ; 68.17 (1C, **C**H$_2$(Z)$^{\omega}$) ; 66.15 (1C, **C**H$_2$(Z)$^{\omega'}$) ; 65.41 (1C, **C**H$_2$(Z)$^{\alpha}$) ; 64.73 (1C, **C**H$_2$(4)) ; *50.41*,

50.27 (1C, **C**Hα) ; 49.46 (1C, **C**H_2(1)) ; 47.94, *47.50* (1C, **C**H_2(2)) ; 44.30 (1C, **C**H_2δ) ; 38.60, *37.63* (1C, **C**H_2(3)) ; 28.89, *28.76* (1C, **C**H_2β) ; *28.16*, 28.08 (3C, **C**H_3(Boc)) ; 24.85, *24.78* (1C, **C**H_2γ) ;
MS (ESI+) (m/z) : calcd for $C_{42}H_{52}N_6O_{11}$ $[M+Na]^+$: 839.4, found: 839.4 $[M+Na]^+$
$[\alpha]_D^{25}$ (C=1, MeOH) : -5.2
HPLC T_R = 21.6 min

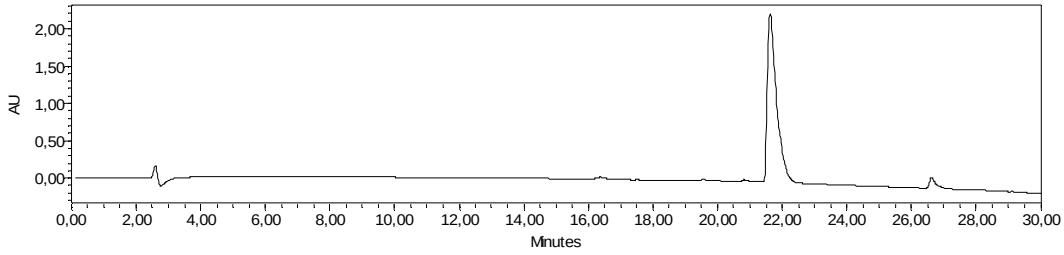


Figure 7: HPLC trace at 210nm of the purified **12'** monomer.

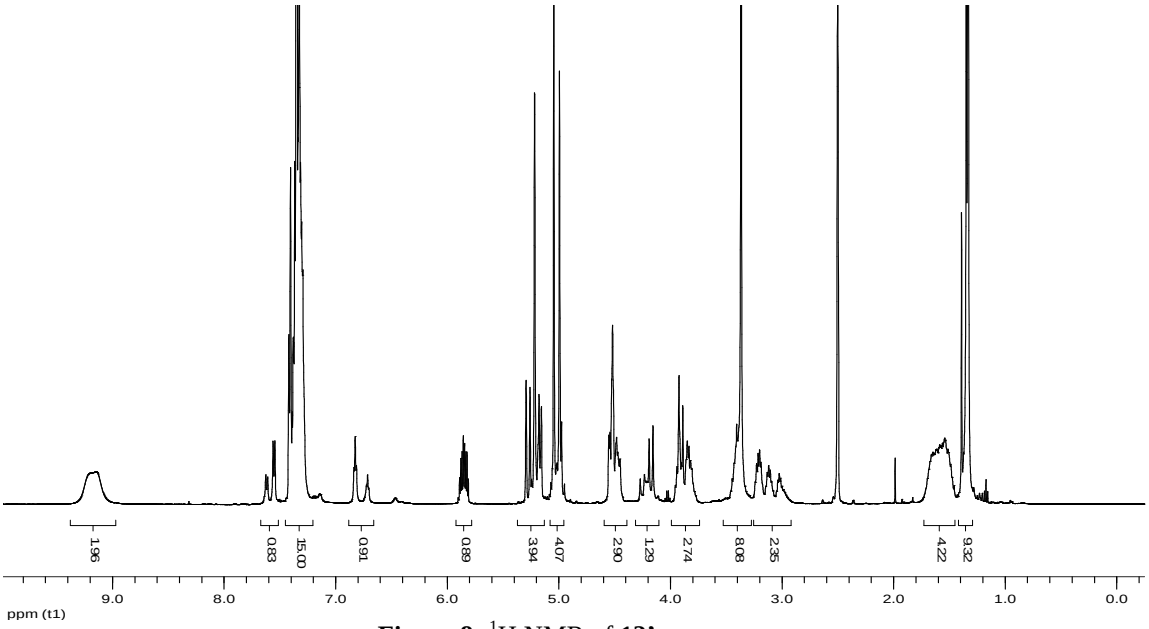
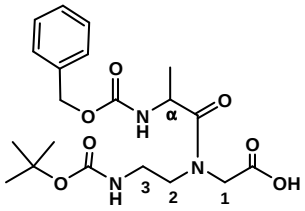


Figure 8: ^{1}H NMR of **12'** monomer.

General procedure for the synthesis of PAA acid monomers (9-11)
To a solution of PAA methyl ester monomer (**9'-11'**) (2.3 mmol) in THF (6 mL) cooled to 0°C was added slowly an aqueous 1M lithium hydroxide solution (4.6 mmol). After stirring for 1 h at 0°C, the mixture was neutralised (pH = 7) with a 1M $KHSO_4$ solution and the solvent was concentrated under reduced pressure. The remaining aqueous layer was acidified with a 1M $KHSO_4$ solution until pH = 2, then extracted with EtOAc (2 x 40 mL). The combined organic layers were washed with H_2O and brine, dried on $MgSO_4$ and the solvent was evaporated under reduced pressure to afford without further purification the corresponding PAA acid monomer (**9-11**) as a white solid.

Boc(Z-Ala)OH (9)



9 was prepared following the general procedure described above. **9** (0.88 g, 90%).
R_f (EtOAc/MeOH, 8:2, v:v) = 0.41
^{1}H NMR (500MHz, DMSO-d_6) : δ (two major isomers) 12.74 (br s, 1H) ; *7.47*, 7.46 (d, J = *7.9Hz*, J = 7.9Hz, NH(Z)) ; 7.39-7.28 (m, 5H, 5xCH_{ar}(Z)) ; 6.81, *6.69* (t, J = 5.3Hz, J = *5.3Hz*, 1H, NH(Boc)) ; 5.07-4.94 (m, 2H, CH_2(Z)) ; 4.58-4.48, *4.33-4.26* (m, 1H, CHα) ; *4.36*, 4.07 (d, J = 17.5Hz, 1H, CH_2(1)) ; *4.04*, 3.78 (d, J = 17.5Hz, 1H, CH_2(1)) ; 3.47-2.95 (m, 4H, CH_2(2)+CH_2(3)) ; 1.36 (s, 9H, 3xCH_3(Boc)) ; 1.20, *1.14* (d, *J* = 6.5Hz, 3H, CH_3(Ala)) ; **^{13}C NMR** (125MHz, DMSO-d_6) : δ (two major isomers) 173.32, *173.12* (1C, **C**O(Ala)) ; *171.52*, 170.98 (1C, **C**OOH) ; 155.94, *155.90* (1C, **C**O(Z)) ; 155.82 (1C, **C**O(Boc)) ; 137.34, *137.29* (1C, **C**$_{IV}$(Z)); 128.66, *128.09*, 128.03 (5C, 5x**C**H_{ar}(Z)) ; 78.17, *77.98* (1C, **C**$_{IV}$(Boc)) ; *65.46*, 65.35 (1C, **C**H_2(Z)); *49.61*, 47.67, 47.35, *46.70* (2C, **C**H_2(1)+**C**H_2(2)) ; *46.31*, 46.02 (1C, **C**Hα) ; 38.46, *37.60* (1C, **C**H_2(3)) ; *28.22*, 28.16 (3C, 3x**C**H_3(Boc)) ; *17.74*, 17.70 (1C, **C**H_3(Ala)) ;
MS (ESI-) (m/z): calcd for $C_{20}H_{29}N_3O_7$ [M-H]$^-$: 422.2, found: 421.9 [M-H]$^-$
$[\alpha]_D^{25}$ (C=1, MeOH) : -19.4
HPLC T_R = 11.8 min

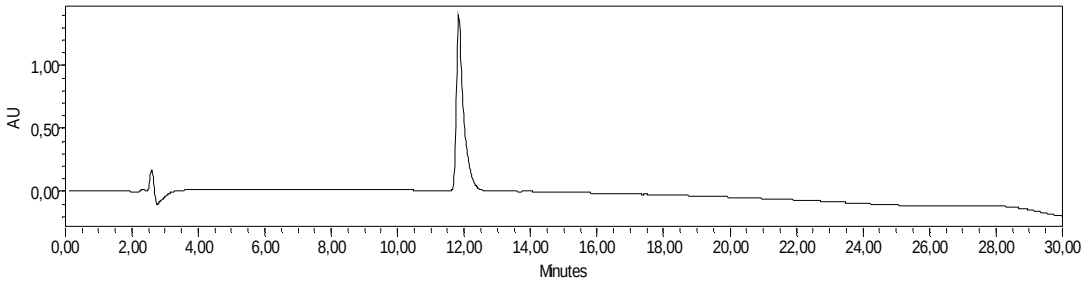


Figure 9: HPLC trace at 210nm of the purified **9** monomer.

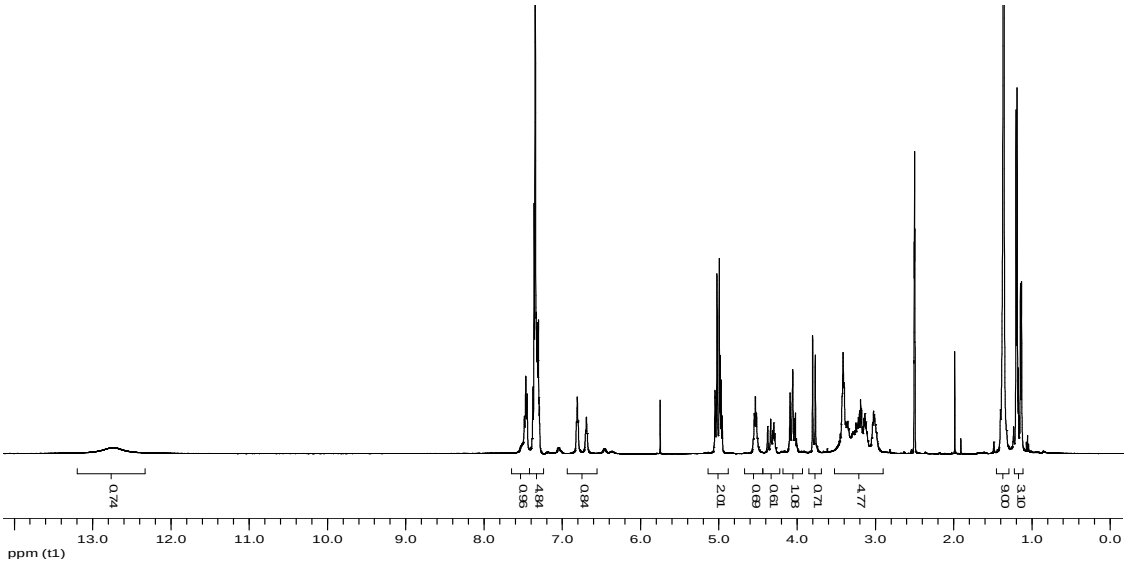
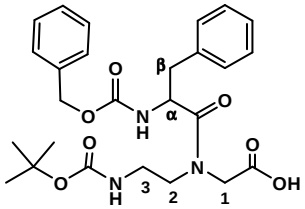


Figure 10: ^{1}H NMR of **9'** monomer.

Boc(Z-Phe)OH (10)



10 was prepared following the general procedure described above. **10** (0.98 g, 85%).

R_f (EtOAc/MeOH, 8:2, v:v) = 0.4
^{1}H NMR (500MHz, DMSO-d_6) : δ (two major isomers) 12.77 (br s, 1H, OH) ; *7.71*, 7.63 (d, J = 8.3Hz, 1H, NH(Z)) ; 7.39-7.14, (m, 10H, 10xCH_{ar}(Z)) ; 6.90, *6.72* (t, J = 6.0Hz, 1H, NH(Boc)) ; 5.02-4.83 (m, 2H, CH_2(Z)) ; 4.77-4.67, *4.41-4.33* (m, 1H, CHα) ; *4.43*, 4.06, (d, J = *18.0Hz*, J = 17.0Hz, 1H, CH_2(1)) ; *4.06*, 3.87 (d, J = *18.0Hz*, J = 17.0Hz, 1H, CH_2(1)) ; 3.52-2.98 (m, 4H, CH_2(2)+CH_2(3)) ; 2.94-2.85 (m, 1H, CH_2β) ; 2.85-2.73 (m, 1H, CH_2β) ; 1.37 (s, 9H, 3xCH_3(Boc)) ; **^{13}C NMR** (125MHz, DMSO-d_6) : δ (two major isomers) *172.19*, 172.12 (1C, **C**O(Phe)) ; *171.34*, 170.60 (1C, **C**OOH) ; *155.80*, 155.77 (1C, **C**O(Z)) ; 155.70, *155.58* (1C, **C**O(Boc)); *137.99*, 137.52 (1C, **C**$_{IV}$(Ph)) ; 136.98, *136.96* (1C, **C**$_{IV}$(Z)) ; 129.44, *129.24*, 128.28, 128.14, 128.06, 128.00, 127.66, 127.44, 127.35, 126.37, *126.27* (10C, 5x**C**H(Z)+5x**C**H(Ph)); *77.90*, *77.69* (1C, **C**$_{IV}$(Boc)) ; *65.28*, 65.22 (1C, **C**H_2(Z)) ; *52.37*, 52.11 (1C, **C**Hα) ; *49.83*, 47.87 (1C, **C**H_2(1)) ; 47.59, *46.87* (1C, **C**H_2(2)) ; 38.42, *37.67* (1C, **C**H_2(3)) ; 37.10, 37.07 (1C, **C**H_2β); *28.22*, 28.15 (3C, 3x**C**H_3(Boc)) ;
MS (ESI-) (m/z): calcd for $C_{26}H_{33}N_3O_7$ [M-H]$^-$: 498.2, found: 497.9 [M-H]$^-$
$[\alpha]_D^{25}$ (C=1, MeOH) : -7.7
HPLC T_R = 15.7 min

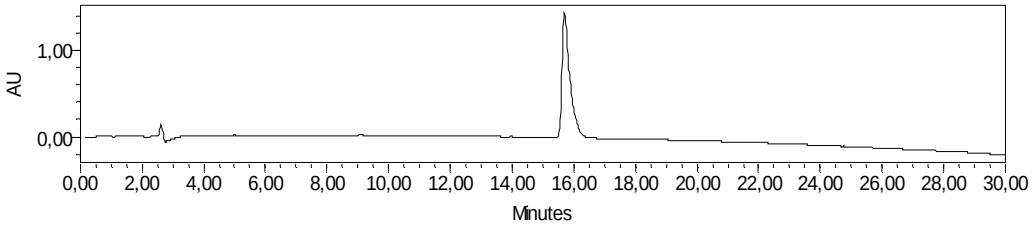


Figure 11: HPLC trace at 210nm of the purified **10** monomer.

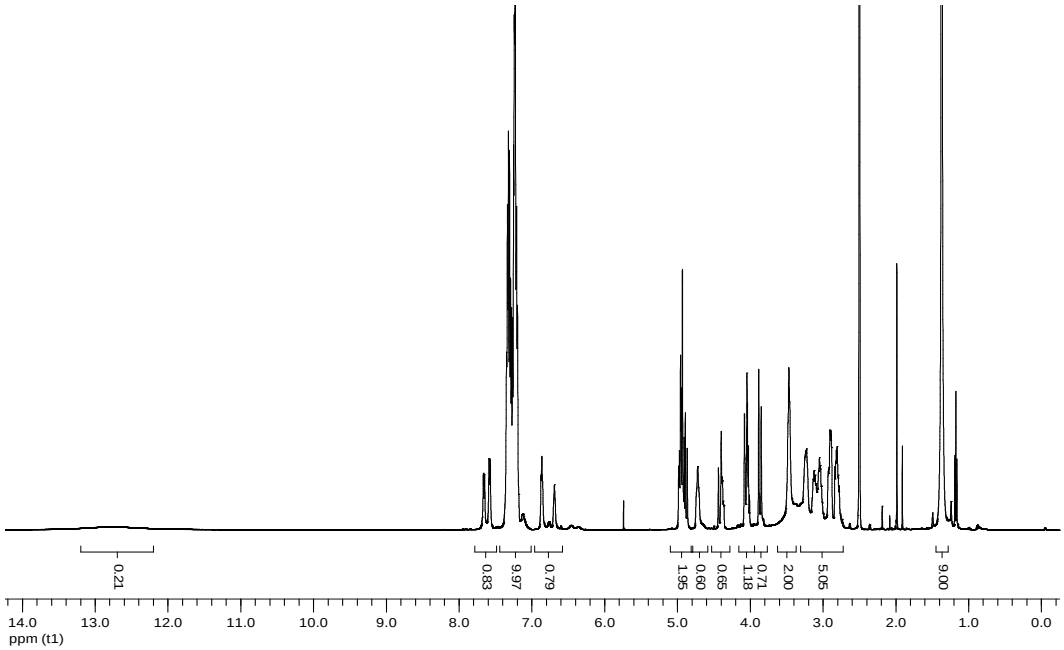
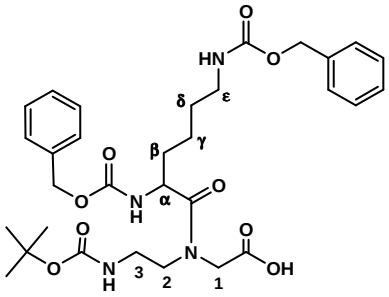


Figure 12: ^{1}H NMR of **10** monomer

Boc(Z-Lys(Z))OH (11)



11 was prepared following the general procedure described above. **11** (1.15 g, 81%).

R_f (EtOAc/MeOH, 8:2, v:v) = 0.48
^{1}H NMR (500MHz, DMSO-d_6) : δ (two major isomers) 12.77 (br s, 1H, OH) ; *7.44*, 7.43 (d, J = *9.6Hz*, J = 8.3Hz, 1H, NH^{α}(Z)) ; 7.39-7.28 (m, 10H, 10xCH_{ar}(Z)) ; 7.21 (t, J = 5.5Hz, 1H, NH^{ε}(Z)) ; 6.82, *6.71* (t, J = 5.5Hz, 1H, NH(Boc)) ; 5.70-4.95 (m, 4H, 2xCH_2(Z)) ; 4.46-4.39, *4.22-4.15* (m, 1H, CHα) ; *4.35*, 4.09 (d, J = *18.3Hz*, J = 17.4Hz, 1H, CH_2(1)) ; 4.03, 3.76 (d, J = *18.3Hz*, J = 17.3Hz, 1H, CH_2(1)) ; 3.47-3.34 (m, 2H, CH_2(2)) ; 3.26-2.91 (m, 4H, CH_2(3)+CH_2ε) ; 1.60-1.47 (m, 2H, CH_2β) ; 1.45-1.29 (m, 4H, CH_2γ CH_2δ) ; 1.36 (s, 9H, 3xCH_3(Boc)) ;
^{13}C NMR (125MHz, DMSO-d_6) : δ (two major isomers) 172.50, *172.38* (1C, **C**O(Lys)) ; *171.22*, 170.67 (1C, **C**OOH) ; *156.07*, 155.93 (2C, 2x**C**O(Z)) ; 155.63, *155.55* (1C, **C**O(Boc)) ; *137.27*, 137.25 (1C, $\mathbf{C}_{IV}(Z^{\alpha})$) ; 137.02, *136.98* (1C, $\mathbf{C}_{IV}(Z^{\varepsilon})$) ; 128.33, *127.76*, 127.73, 127.67, *127.65* (10C, 5x$\mathbf{C}H_{ar}(Z^{\alpha})$, 5x$\mathbf{C}H_{ar}(Z^{\varepsilon})$) ; 77.84, *77.64* (1C, $\mathbf{C}_{IV}$(Boc)) ; *65.47*, 65.37 (2C, **C**H_2(Z)) ; 65.13, *65.10* (1C, **C**H_2ε) ; *50.64*, 50.58 (1C, **C**Hα) ; 47.73, 47.42, *46.68* (2C, **C**H_2(1)+**C**H_2(2)) ; 38.50, *37.64* (1C, **C**H_2(3)) ; 31.09 (1C, **C**H_2β) ; 29.15 (1C, **C**H_2γ) ; *28.22*, 28.15 (3C, 3x**C**H_3(Boc)) ; 22.56 (1C, **C**H_2δ) ; **MS (ESI-)** (m/z): calcd for $C_{31}H_{42}N_4O_9$ $[M-H]^-$: 613.3, found: 613.0 $[M-H]^-$
$[\alpha]_D^{25}$ (C=1, MeOH) : -9.7
HPLC T_R = 16.4 min

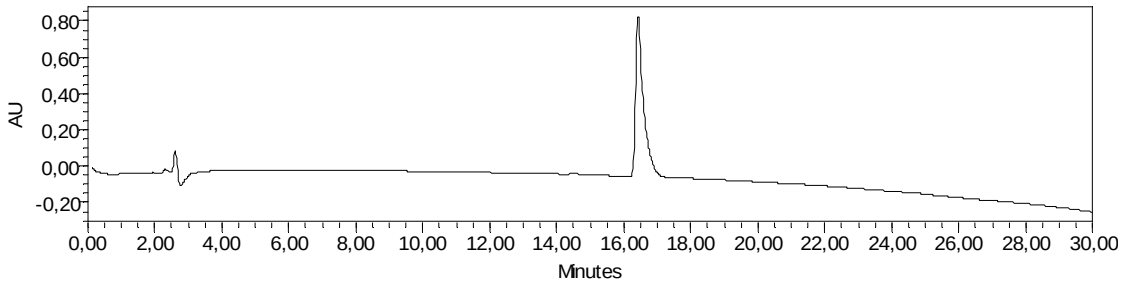


Figure 13: HPLC trace at 210nm of the purified **11** monomer.

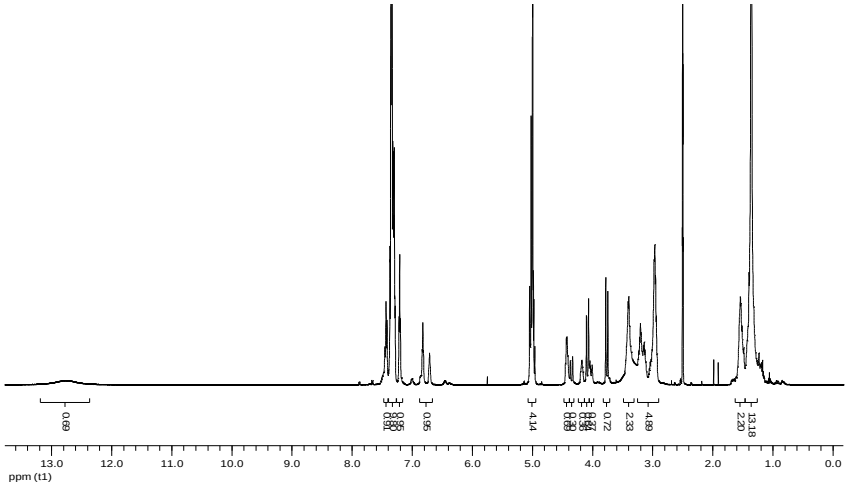
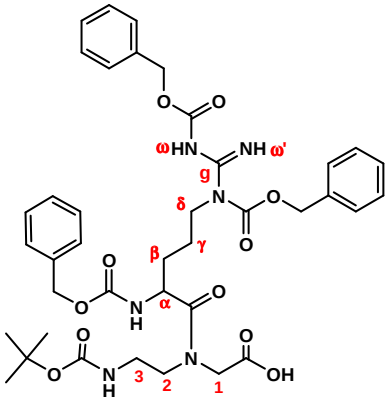


Figure 14: ^{1}H NMR of **11** monomer

Boc(Z-Arg(Z$_2$))OH (12)



To a solution of **12'** (1 g, 1.22 mmol) in degassed DMF (4 mL) was added DEA (2.5 mL, 24.4 mmol), and tetrakis(triphenylphosphine)palladium (140.3 mg ; 0.122 mmol) in the dark and under inert atmosphere. The reaction was stirred for 30 min at room temperature, and the solvent was removed under reduced pressure. The residue was purified by flash silica column chromatography (EtOAc to EtOAc/MeOH (9:1, v:v)) to afford **12** as yellow powder (760 mg, 80%).

R$_f$ (EtOAc/MeOH, 8:2, v:v) = 0.51

^{1}H NMR (500MHz, DMSO-*d$_6$*) : δ = (two major isomers) 9.20 (br s, 2H, (Z)NH-C=NH) ; 7.50-7.20 (m, 16H, 15CH$_{ar}$+NH$^{\alpha}$(Z)) ; *6.99*, 6.95 (br s, 1H, NH(Boc)) ; 5.21 (s, 2H, CH$_2$(Z)$^{\omega}$) ; 5.04 (s, 2H, CH$_2$(Z)$^{\omega'}$) ; *4.99*, 4.98 (s, 2H, CH$_2$(Z)$^{\alpha}$) ; *4.50-4.39*, 4.30-4.21 (m, 1H, CHα) ; 4.08-2.90 (m, 8H, CH$_2$(1)+CH$_2$δ+ CH$_2$(2)+CH$_2$(3)) ; 1.76-1.42 (m, 4H, CH$_2$β+CH$_2$γ) ; 1.33 (s, 9H, 3xCH$_3$(Boc)) ;

^{13}C NMR (125MHz, DMSO-*d$_6$*) *:* δ (two major isomers)= 172.14 (1C, **C**O(Arg)) ; 171.91, *171.68* (1C, **C**OOH) ; 162.91 (1C, **C**O(Z)) ; 159.79, 159.69 (1C, **C**O(Z)) ; 155.79, *155.58* (1C, **C**O(Boc)) ; 154.96, *154.86* (1C, **C**O(Z)) ; *137.10*, 136.97 (1C, **C**$_{IV}$g) ; 135.43, *135.30* (3C, **C**$_{IV}$(Z)) ; 128.51, 128.31, 128.23, 128.16, 127.94, 127.85, 127.73, 127.65, 127.60 (15C, 15x**C**H$_{ar}$(Z)) ; *77.74*, 77.43 (1C, **C**$_{IV}$(Boc)); *68.19*, 68.11 (1C, **C**H$_2$(Z)$^{\omega}$) ; 66.16 (1C, **C**H$_2$(Z)$^{\omega'}$) ; 65.43 (1C, **C**H$_2$(Z)$^{\alpha}$) ; 51.94, 48.75 (1C, **C**H$_2$(1)) ; 50.56, *50.39* (1C, **C**Hα) ; *47.47*, 47.05 (1C, **C**H$_2$(2)) ; 44.57, *44.40* (1C, **C**H$_2$δ) ; *38.33*, 37.59 (1C, **C**H$_2$(3)) ; *29.05*, 28.75 (1C, **C**H$_2$β; 28.22, *28.12* (3C, 3x**C**H$_3$(Boc)) ; *24.91*, 24.73 (1C, **C**H$_2$γ) ;

MS (ESI-) (m/z) : calcd for $C_{39}H_{48}N_6O_{11}$ [M-H]$^-$: 775.3, found: 775.1 [M-H]$^-$

[α]$_D^{25}$ (C=1, MeOH) : +1.0

HPLC T$_R$ = 16.4 min

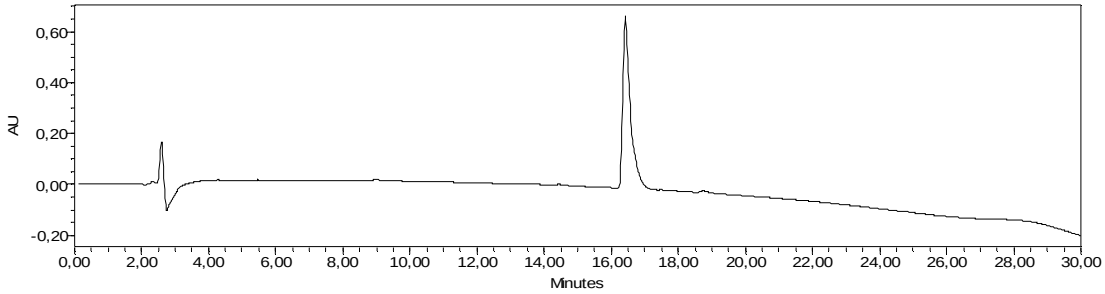


Figure 15: HPLC trace of the purified **12** monomer.

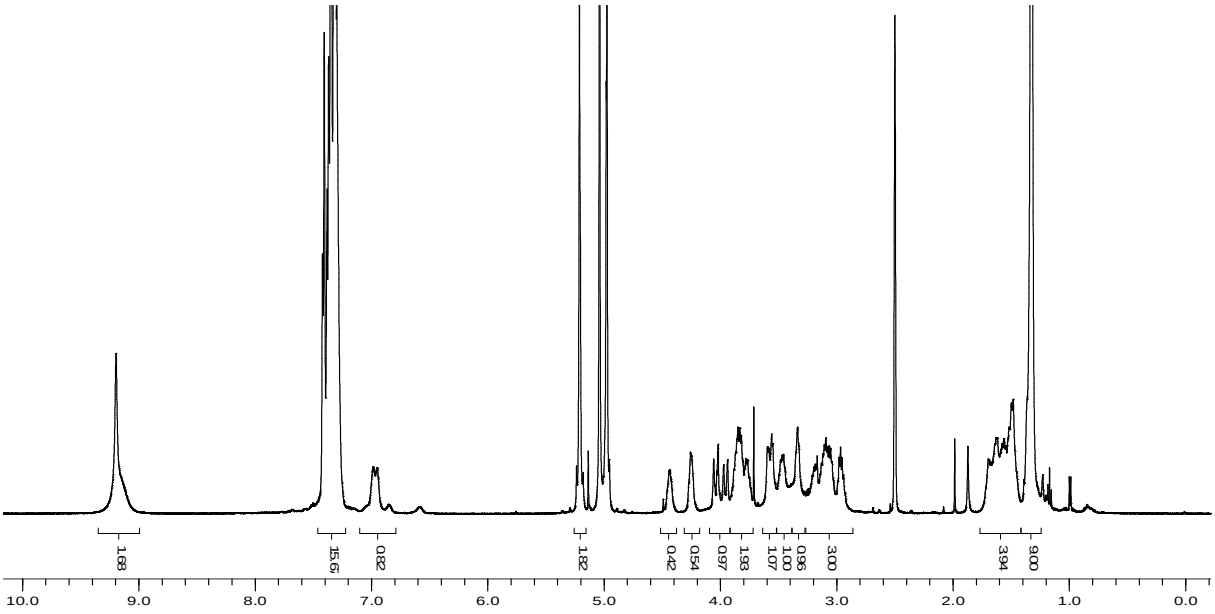


Figure 16: ^{1}H NMR of **12** monomer.

Synthesis of oligo-PAA

General method for solid-phase synthesis:
PAA oligomers were prepared following a standard solid-phase strategy. PAA oligomers were synthesized on an MBHA resin LL (100-200 mesh, 0.59-0.9 mmol/g, Novabiochem), which was downloaded to approximately half of its maximal capacity for the first building block. The remaining sites were capped using the capping mixture described below.
PAA oligomers were typically synthesized on 250 mg of resin using a standard Boc/Z protocol :
Boc cleavage*:* TFA/TIS (9:1, v:v), 2 x 15 min, DCM wash, NMP wash.
Coupling conditions*:* Couplings were performed for 1 h with a volume of 1.5 mL of a preactivated (3 min) mixture of PAA acid monomer (1.5 equiv), DIEA (7 equiv), and HBTU (1.4 equiv) in NMP. The couplings were monitored by a Kaiser test and repeated twice.
Capping: Ac_2O/Pyr/NMP 15:15:70 (v:v:v), 2 x 5 min, NMP wash, DCM wash.
Cleavage from the resin*:* Compounds were cleaved (and totally deprotected) using TFMSA/TFA/TIS (1:8:1, v:v:v) for 4 h. The resulting solution combined with 1 mL of TFA (used to wash the resin) was added to cold anhydrous diethyl ether (10 mL). Crude PAA oligomer was isolated by centrifugation (3,000 min^{-1}, 10 min), washed three times with diethyl ether (30 mL). After the last washing step, the solid was dried by lyophilization on a Flexy-Dry FTS system, to give a pale yellow powder

Note : Due to cleavage conditions from solid support and HPLC purifications, compounds **1**-**8** exhibit peaks in the ^{13}C spectra from TFA (163.3 ppm (q, J = 140.5Hz), 116.7 ppm (q, J = 1160Hz). These peaks are not listed in the spectra analysis below.

FRKA (1)

1 was prepared following the general solid-phase procedure described above. Crude **1** (95 mg) was obtained with a purity of 88.7% as determined by HPLC, and purified by semi-preparative HPLC.

HPLC T_R = 20.5 min

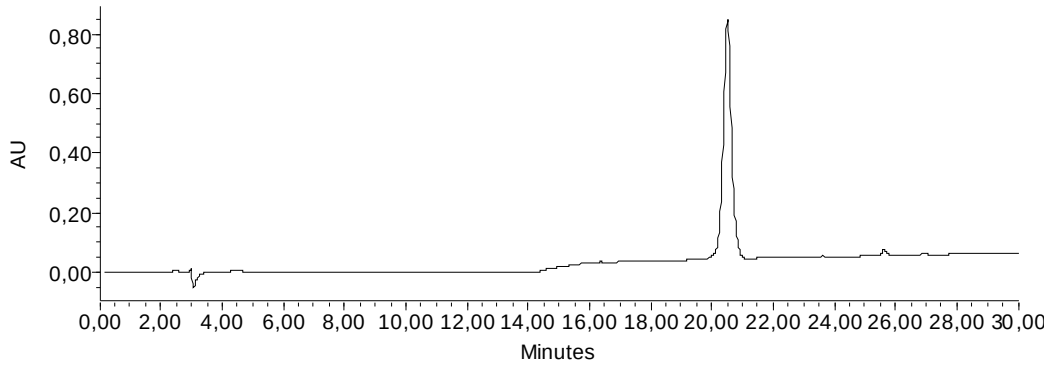


Figure 17: HPLC profile of **1** at 210nm

^{1}H NMR (500 MHz, D_2O): (two major isomers) δ= 7.50-7.30 (m, 5H, CH_{ar}) ; 4.76-4.00 (m, 12H, CHα Phe, CHα Ala, CHα Lys, CHα Arg, 4 x CH_2(1)) ; 3.80-3.10 (m, 22H, 4 x CH_2(2), 4 x CH_2(3), CH_2(5), CH_2δ Arg, CH_2β Phe) ; 3.09-2.98 (m, 2H, CH_2ε Lys) ; 2.56-2.48 (m, 2H, CH_2(4)) ; 2.02-1.65, (m, 11H, CH_3(Ac), CH_2β Lys, CH_2β Arg, CH_2γArg, CH_2δ Lys) ; 1.60-1.45, (m, 5H, CH_3 Ala, CH_2γLys).

^{13}C NMR (125 MHz, D_2O): (two major isomers) δ= 177.19, 174.97,*174.80*, 172.17, 171.77, 170.91, 170.85, 170.74, 170.64, 170.60, 170.55, 170.44, 169.89, 157.16, 133.60, 129.97, 129.84, 129.64, 128.65, *52.18*, 52.09, 51.20, 51.16, 50.92, 50.85, 50.71, 50.47, 50.40, 50.12, 49.89, 48.59, 48.54, 48.50, 48.34, 47.69, 47.64, 47.15, 47.11, 40.75, 39.33, 37.95, 37.83, 37.76, 37.72, 37.56, 37.10, 36.96, 36.90, 36.84, 36.42, 36.17, *34.87*, 34.80, *30.31*, 30.25, 27.96, 26.89, 23.90, *23.82*, *22.33*, 22.21, 21.42, *21.29*, *16.25*, 16.20.

HRMS *m/z* calcd for $C_{45}H_{80}N_{18}O_{10}$ $(M+H)^+$; 1033.63831 , found: 1033.63745

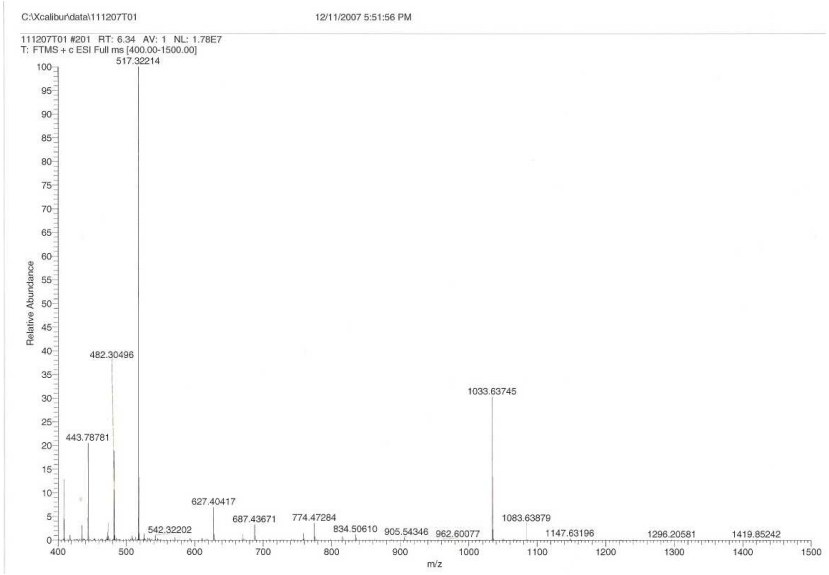


Figure 18: HRMS mass spectrum of **1**

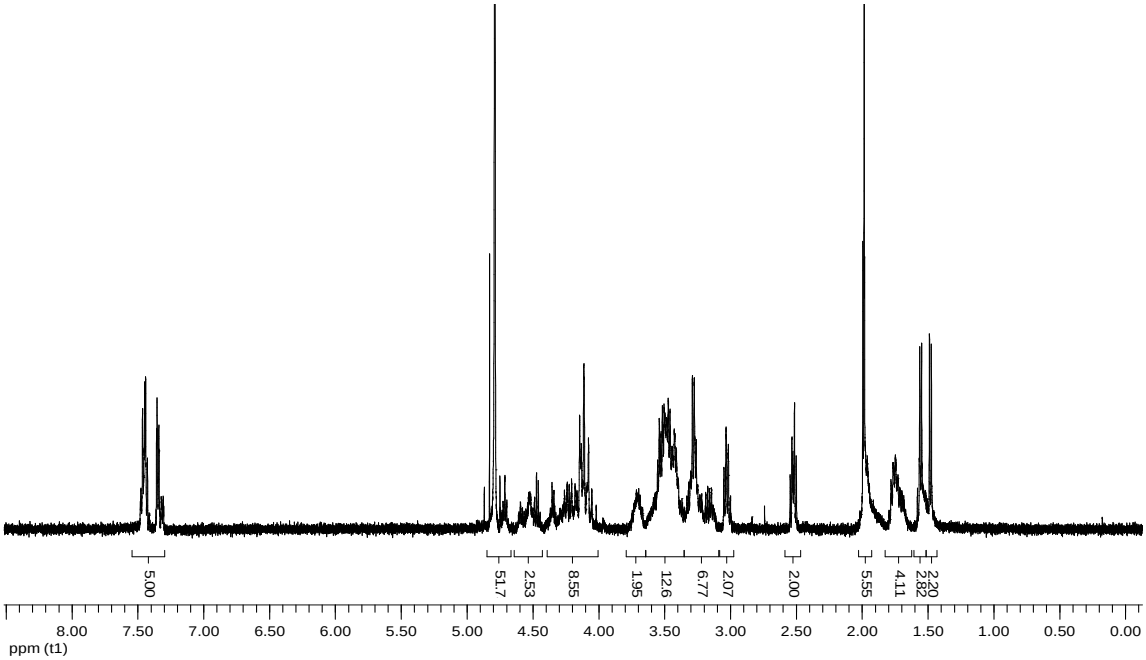


Figure 19: ^{1}H NMR 500MHz of **1**

RFAK (2)

2 was prepared following the general solid-phase procedure described above. Crude **2** (98.3 mg) was obtained with a purity of 90.6% as determined by HPLC, and purified by semi-preparative HPLC.

HPLC T_R = 19.7 min

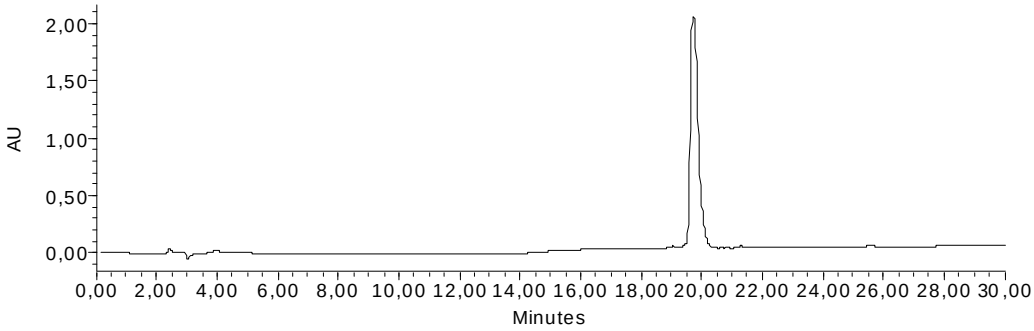


Figure 20: HPLC profile of **2** at 210nm

^{1}H NMR (500 MHz, D_2O): (two major isomers) δ= 7.50-7.30 (m, 5H, CH_{ar}) ; 4.72-4.00 (m, 12H, CH_α Phe, CH_α Ala, CH_α Lys, CH_α Arg, 4 x CH_2(1)) ; 3.80-3.14, (m, 22H, 4 x CH_2(2), 4 x CH_2(3), CH_2(5), CH_2δ Arg, CH_2β Phe) ; 3.10-3.00 (m, 2H, CH_2ε Lys) ; 2.60-2.49 (m, 2H, CH_2(4)) ; 2.05-1.82, (m, 7H, CH_3(Ac), CH_2β Lys, CH_2β Arg) ; 1.82-1.62 (m, 4H, CH_2γ Arg, CH_2δLys) ; 1.62-1.45 (m, 5H, CH_3 Ala, CH_2γ Lys).

^{13}C NMR (125 MHz, D_2O): (two major isomers) δ= 177.19, 175.04, 172.25, 172.16, 171.78, 170.93, 170.69, 170.49, 170.29, 169.76, 157.20, 133.66, 130.00, *129.70*, 129.61, 128.67, 52.34, 51.24, 50.65, 50.12, 49.87, 48.57, 48.53, 48.35, 47.61, 47.29, *40.81*, 40.76, 39.33, 37.93, 37.79, 37.58, 36.98, 36.91, 36.43, 36.16, 34.86, *34.78*, 30.18, *29.97*, 28.08, 26.82, 23.81, 22.25, *21.37*, 21.28, *16.34*, 16.26.

HRMS *m/z* calcd for $C_{45}H_{80}N_{18}O_{10}$ $(M+H)^+$; 1033.63831, found: 1033.64050

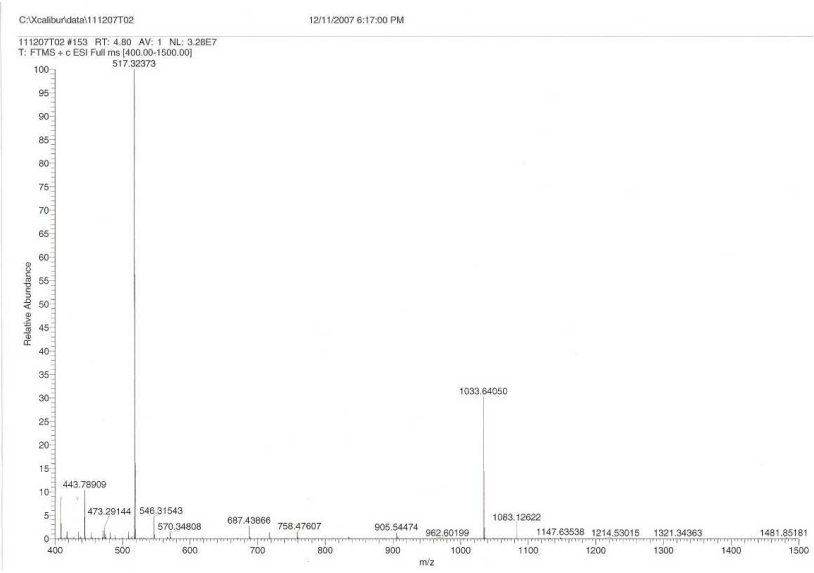


Figure 21: HRMS mass spectrum of **2**

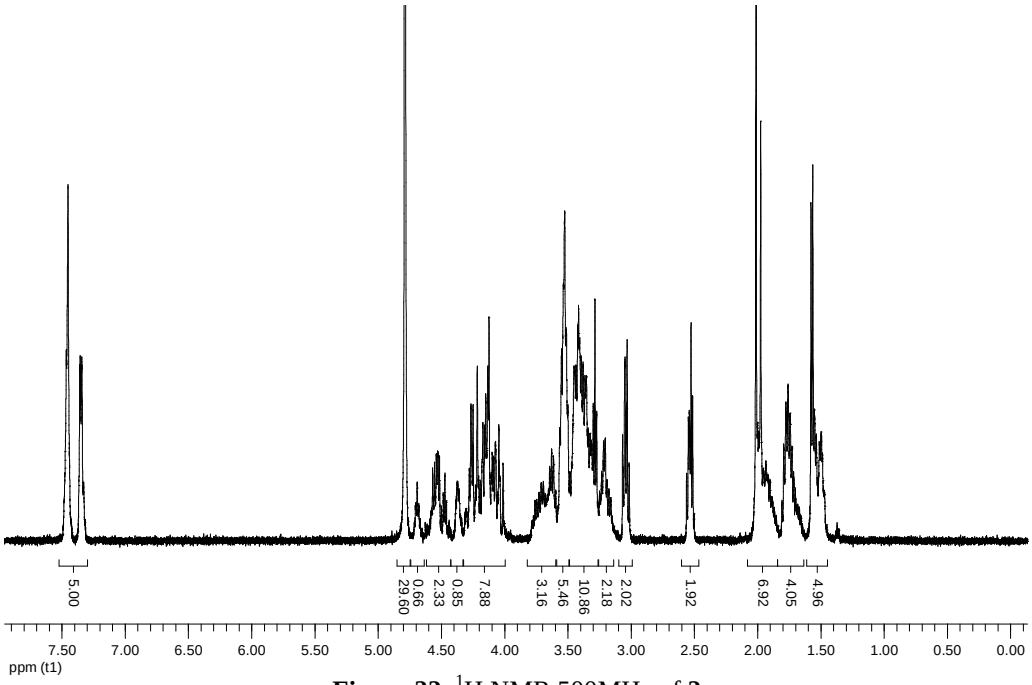


Figure 22: ^{1}H NMR 500MHz of **2**

KARF (3)

3 was prepared following the general solid-phase procedure described above. Crude **3** (101.5 mg) was obtained with a purity of 92% as determined by HPLC, and purified by semi-preparative HPLC.

HPLC T_R = 19.7 min

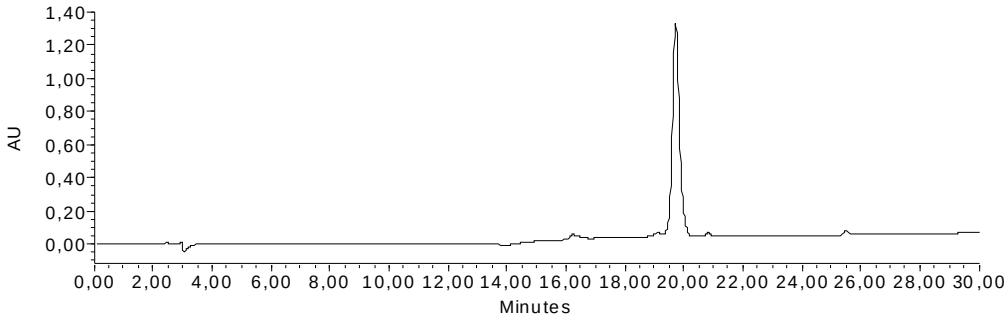


Figure 23: HPLC profile of **3** at 210nm

^{1}H NMR (500 MHz, D_2O): (two major isomers) δ= 7.60-7.27 (m, 5H, CH_{ar}) ; 4.74-3.90 (m, 12H, CH_α Phe, CH_α Ala, CH_α Lys, CH_α Arg, 4 x CH_2(1)) ; 3.85-3.20 (m, 22H, 4 x CH_2(2), 4 x CH_2(3), CH_2(5), CH_2δ Arg, CH_2β Phe) ; 3.16-3.05 (m, 2H, CH_2ε Lys) ; 2.65-2.55 (m, 2H, CH_2(4)) ; 2.10-1.89, (m, 7H, CH_3(Ac), CH_2β Lys, CH_2β Arg) ; 1.88-1.68 (m, 4H, CH_2γ Arg, CH_2δ Lys) ; 1.66-1.50, (m, 5H, CH_3 Ala, CH_2γ Lys).
^{13}C NMR (125 MHz, D_2O): (two major isomers) δ= 177.19, 175.10, *174.68* ; 170.98, 170.93, 170.61, 170.50, 169.72, 157.15, 133.78 , 129.93, 129.89, 129.63, 128.63, 52.44, *52.25*, 51.12, 50.92, 50.85, 50.70, 50.34, 50.22, 50.05, 49.78, 48.46, 48.36, 47.45, *40.84*, 40.80, 39.36, 38.02, 37.64, 37.55, 37.02, 36.87, 36.40, 36.20, 34.88, *34.77*, 30.54, *28.04*, 28.00, 26.80, 23.70, *23.58*, 22.28, 21.70, 16.34.
HRMS *m/z* calcd for $C_{45}H_{80}N_{18}O_{10}$ $(M+H)^+$; 1033.63831, found: 1033.63825

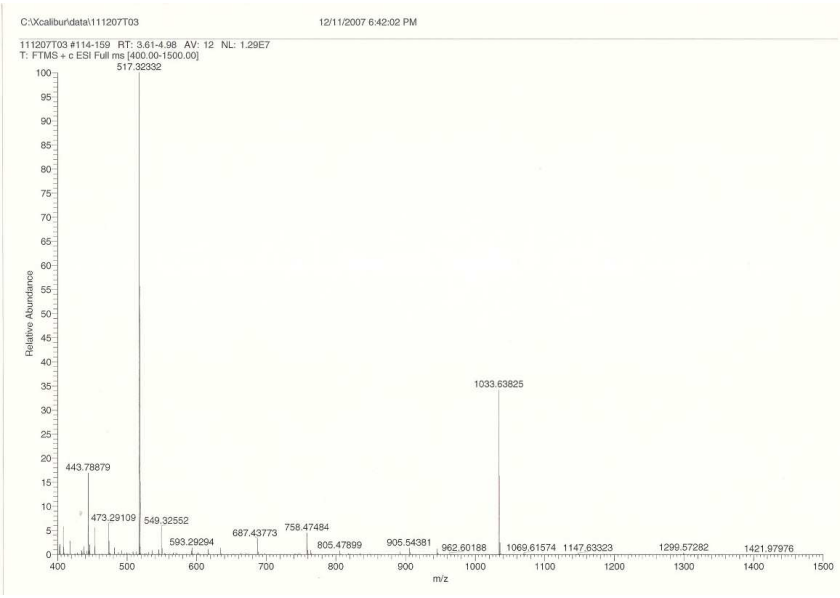


Figure 24: HRMS mass spectrum of **3**

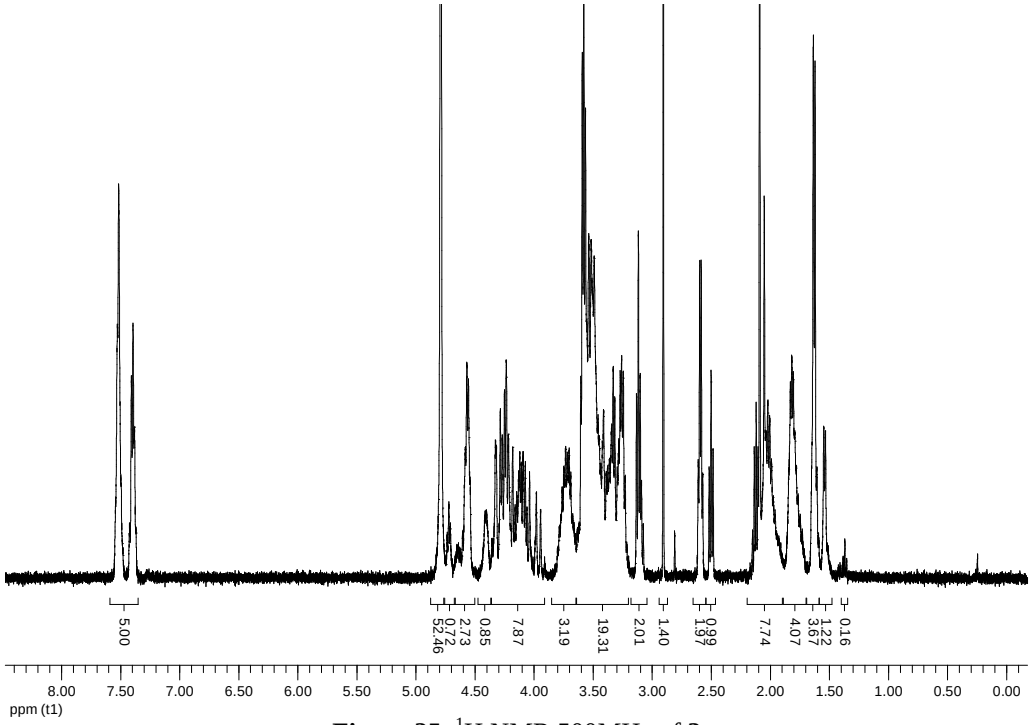


Figure 25: ^{1}H NMR 500MHz of **3**

AKFR (4)

4 was prepared following the general solid-phase procedure described above. Crude **4** (105 mg) was obtained with a purity of 89% as determined by HPLC, and purified by semi-preparative HPLC.

HPLC T_R = 19.6 min

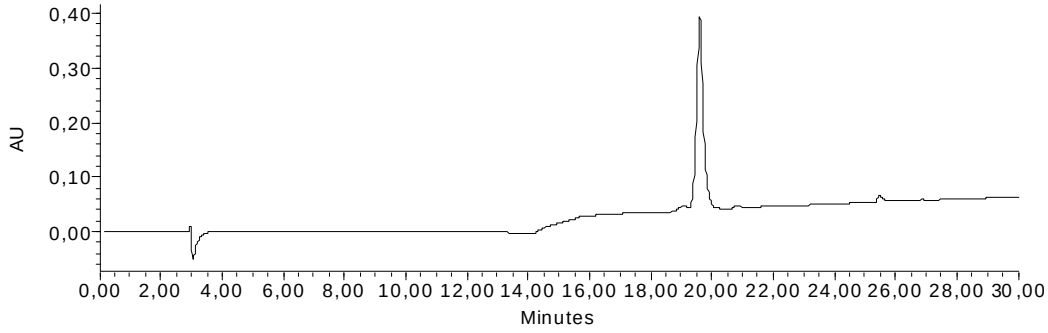


Figure 26: HPLC profile of **4** at 210nm

^{1}H NMR (500 MHz, D_2O): (two major isomers) δ= 7.50-7.30 (m, 5H, CH_{ar}) ; 4.79-3.60 (m, 12H, CH_{α}Phe, CH_{α} Ala, CH_{α} Lys, CH_{α} Arg, 4 x CH_2(1)) ; 3.80-3.10 (m, 22H, 4 x CH_2(2), 4 x CH_2(3), CH_2(5), CH_2δ Arg, CH_2β Phe) ; 3.10-2.96 (m, 2H, CH_2ε Lys) ; 2.57-2.46 (m, 2H, CH_2(4)) ; 2.04-1. 83 (m, 7H, CH_3(Ac), CH_2β Lys, CH_2β Arg) ; 1.82-1.62 (m, 4H, CH_2γ Arg, CH_2δ Lys) ; 1.60-1.44 (m, 5H, CH_3 Ala, CH_2γLys).

^{13}C NMR (125 MHz, D_2O): (two major isomers) δ= 177.17, *177.12*, 175.01, 171.72, 170.95, 170.90, 170.79, 170.72, 170.67, 170.62, 170.54, 170.44, 170.34, 170.24, 169.70, 157.19, *133.68*, 133.63, 130.00, 129.88, 129.66, 128.71, 52.41, 51.13, 51.06, 50.99, 50.52, 50.43, 50.20, 49.88, 49.74, 48.61, 48.46, 48.37, 47.34, 40.75, 39.33, 38.00, 37.91, 37.62, 37.54, 36.96, 36.90, 36.84, 36.75, *36.43*, 36.17, 34.86, *34.79*, 30.37, 27.76, *27.59*, 26.85, *26.79*, *23.63*, 23.58, 22.27, 21.59, 16.36, *16.21*.

HRMS *m/z* calcd for $C_{45}H_{80}N_{18}O_{10}$ $(M+H)^+$; 1033.63831, found: 1033.64075

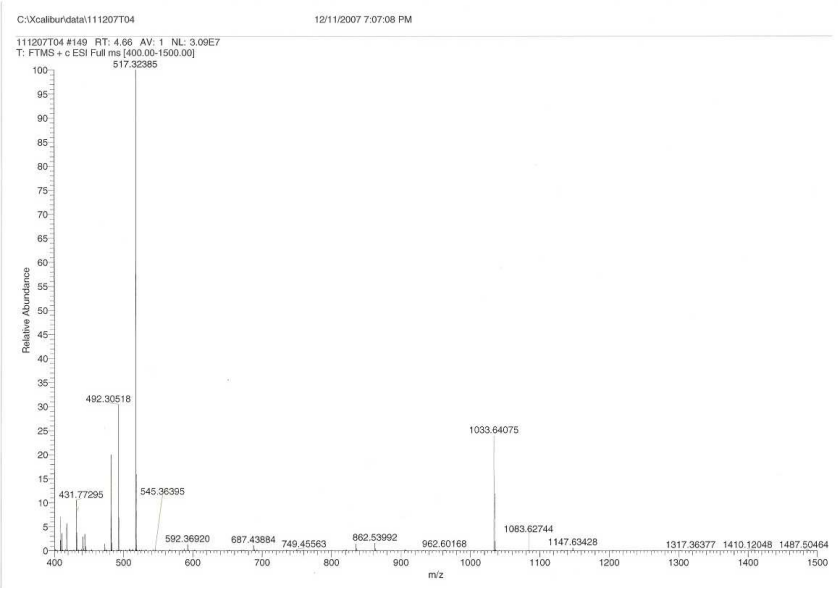


Figure 27: HRMS mass spectrum of **4**

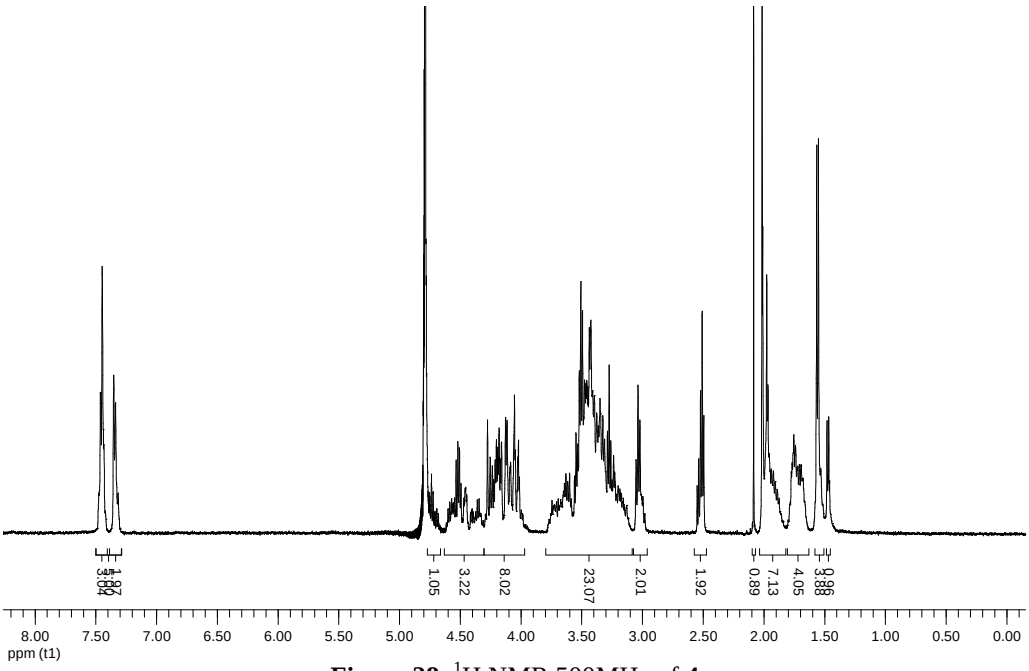


Figure 28: ^{1}H NMR 500MHz of **4**

KKKK (5)

5 was prepared following the general solid-phase procedure described above. Crude **5** (125 mg) was obtained with a purity of 86% as determined by HPLC, and purified by semi-preparative HPLC.

HPLC T_R = 17.5 min

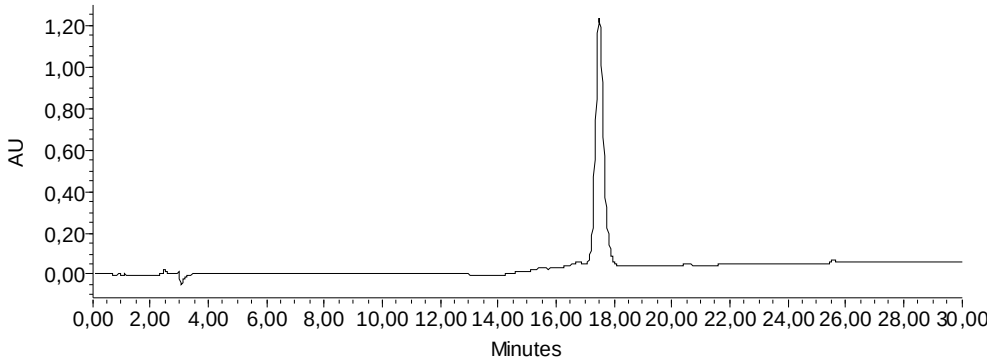


Figure 29: HPLC profile of **5** at 210nm

^{1}H NMR (500 MHz, D_2O): (two major isomers) δ 4.62-4.43, *4.39-4.31* (m, 4H, 4 x CHα Lys) ; 4.31-4.00 (m, 8H, 4 x CH_2(1)) ; 3.80-3.30 (m, 18H, 4 x CH_2(2), 4 x CH_2(3), CH_2(5)) ; 3.10-2.98 (m, 8H, CH_2ε Lys) ; 2.58-2.48 (m, 2H, CH_2(4)) ; 2.06-1.82 (m, 11H, CH_3(Ac), 4 x CH_2β Lys) 1.80-1.66, (m, 8H, 4 x CH_2δ Lys) ; 1.62-1.40, (m, 8H, 4 x CH_2γ Lys).
^{13}C NMR (125 MHz, D_2O): (two major isomers) δ 177.18, 175.08, 171.13, 170.89, 170.75, 170.67, 170.46, 170.33, 169.75, 51.20, 51.12, 51.08, 50.94, 50.78, 5.67, 50.45, 50.27, 49.92, 48.57, 48.48, 48.44, 48.23, 47.29, 47.12, 47.02, 46.92, 38.04, 37.99, 37.91, 37.86, 37.68, 37.62, 36.86, *36.42*, 36.16, 34.86, *34.77*, 30.48, 30.45, 30.38, 30.29, 30.14, 30.11, 30.06, 29.98, 26.85, 26.77, *22.32*, 22.26, 21.74, 21.61, 21.54, 21.50, 21.39, 21.34, 21.29, 21.21.
HRMS *m/z* calcd for $C_{45}H_{90}N_{18}O_{10}$ $(M+H)^+$; 1043.71656, found: 1043.71750

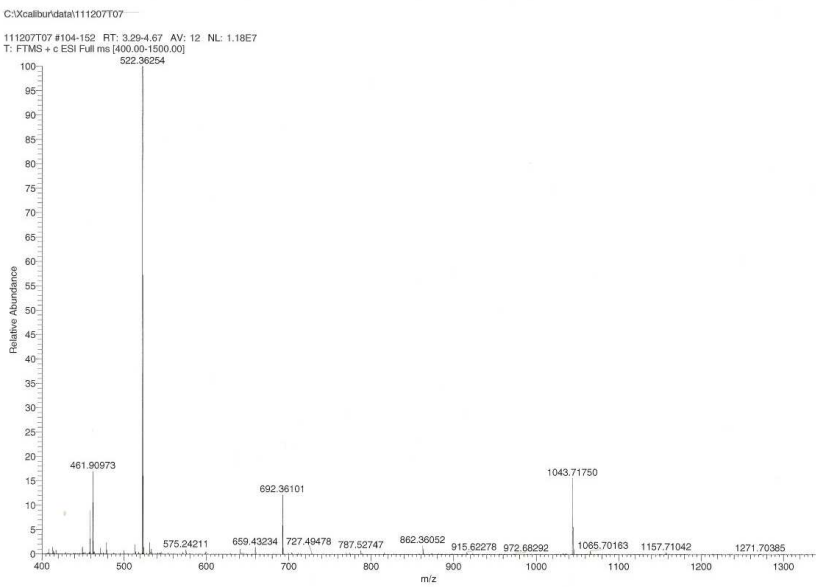


Figure 30: HRMS mass spectrum of **5**

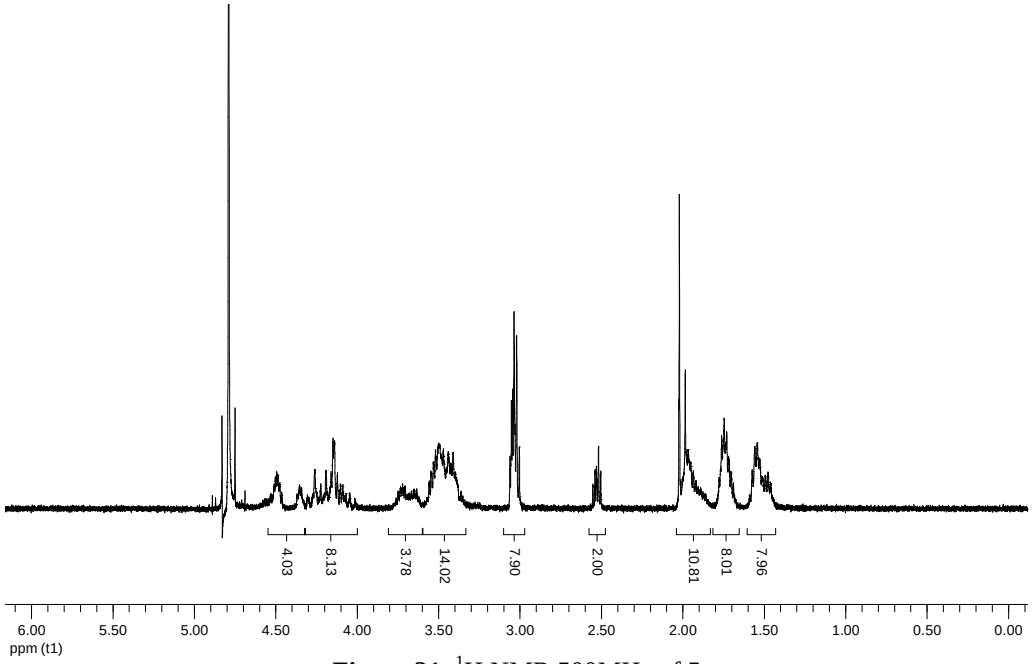


Figure 31: ^{1}H NMR 500MHz of **5**

RRRR (6)

6 was prepared following the general solid-phase procedure described above. Crude **6** (130 mg) was obtained with a purity of 60% as determined by HPLC, and purified by semi-preparative HPLC.
HPLC TR = 19.0 min

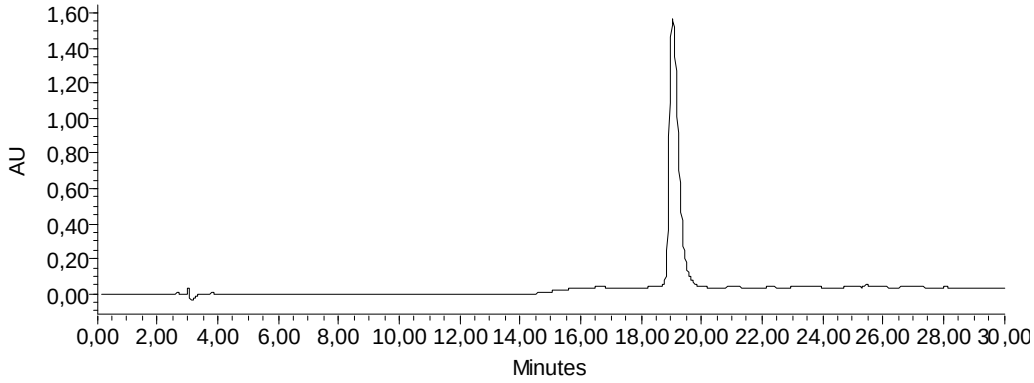


Figure 32: HPLC profile of **6** at 210nm

^{1}H NMR (500 MHz, D_2O): (two major isomers) δ 4.66-4.48, *4.43-4.36* (m, 4H, 4 x CHα Arg) ; 4.35-4.00 (m, 8H, 4 x CH_2(1)) ; 3.78-3.32 (m, 18H, 4 x CH_2(2), 4 x CH_2(3), CH_2(5)) ; 3.32-3.20 (m, 8H, CH_2δ Arg) ; 2.56-2.48 (m, 2H, CH_2(4)) ; 2.06-1.82 (m, 11H, CH_3(Ac), 4 x CH_2β Arg) ; 1.81-1.62 (m, 8H, 4 x CH_2γ Arg).
^{13}C NMR (125 MHz, D_2O): (two major isomers) δ 177.18, 175.07, 170.90, 170.84, 170.72, 170.57, 170.48, 170.43, 170.37, 169.67, 157.19, 51.11, 50.96, 50.90, 50.84, 50.71, 50.43, 50.28, 49.89, 49.73, 48.67, 48.54, 48.47, 48.42, 47.26, 46.99, 40.76, *40.71*, 38.02, 37.70, 36.84, 36.83, *36.42*, 36.16, 34.85, *34.78*, 28.01, 27.94, 27.89, 27.83, 27.72, 27.63, 27.58, 23.88, 23.70, 23.63, 23.59, 23.49, *22.28*, 22.24.
HRMS *m/z* calcd for $C_{45}H_{90}N_{26}O_{10}$ $(M+H)^+$; 1155.74115, found: 1155.73865

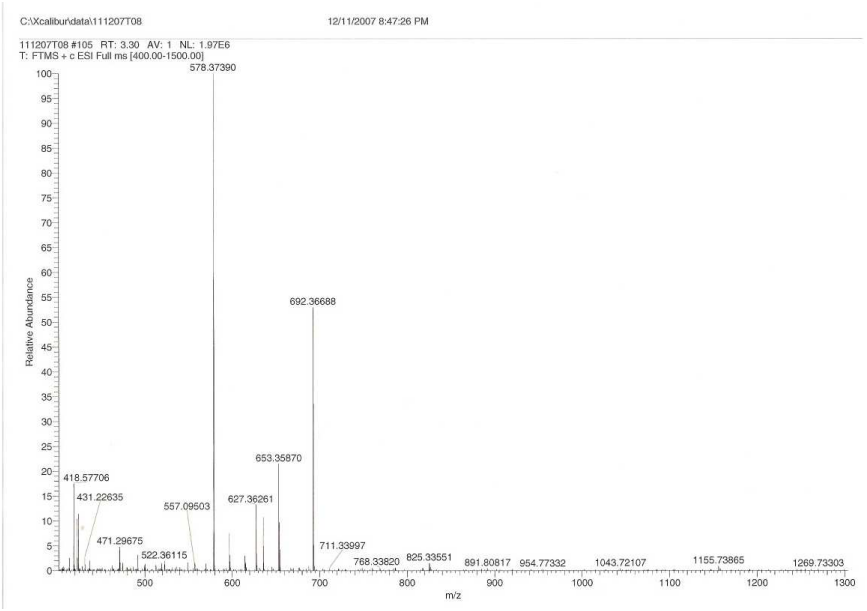


Figure 33: HRMS mass spectrum of **6**

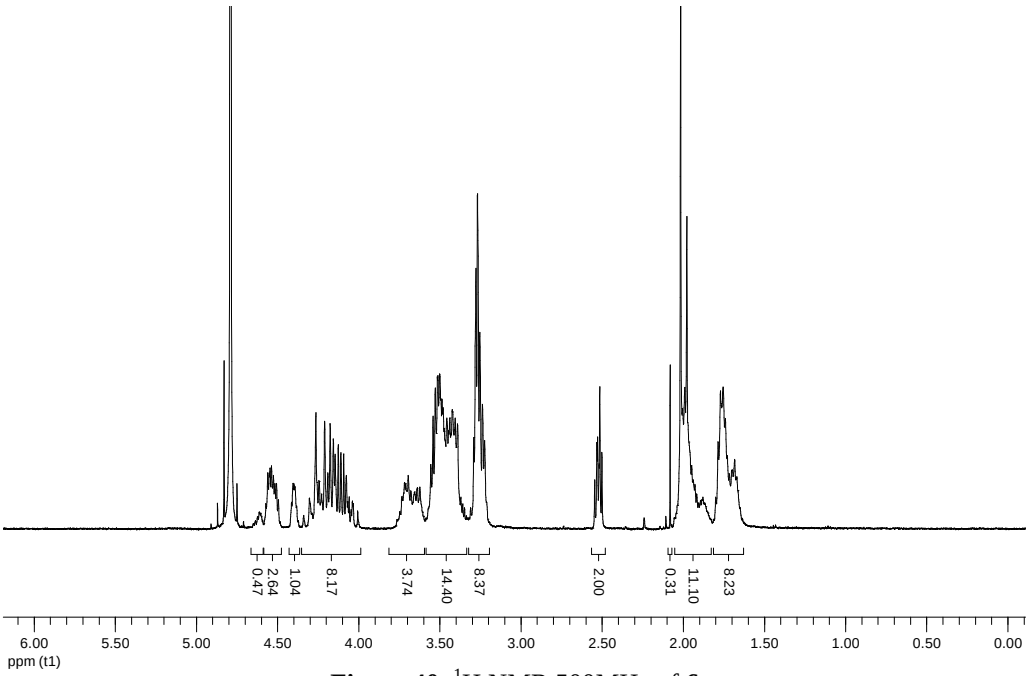


Figure 40: ^{1}H NMR 500MHz of **6**

AAAA (7)

7 was prepared following the general solid-phase procedure described above. Crude **7** (114 mg) was obtained with a purity of 89% as determined by HPLC, and purified by semi-preparative HPLC.

HPLC T_R = 17.1 min

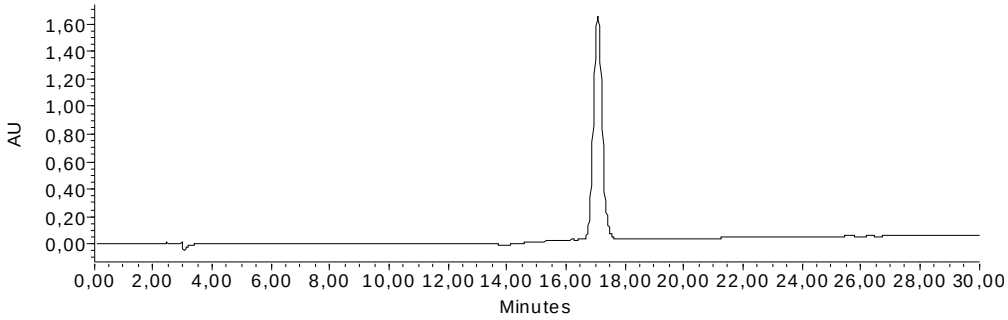


Figure 35: HPLC profile of **7** at 210nm

^{1}H NMR (500 MHz, D_2O): (two major isomers) δ= 4.64-4.42, *4.37-4.32* (m, 4H, 4 x CHα Ala) ; 4.32-4.00 (m, 8H, 4 x CH_2(1)) ; 3.78-3.28 (m, 18H, 4 x CH_2(2), 4 x CH_2(3), CH_2(5)) ; 2.56-2.48 (m, 2H, CH_2(4)) ; 2.02, *1.98* (s, 3H, CH_3(Ac)) ; 1.61-1.53, *1.50-1.44* (m, 12H, 4 x CH_3 Ala).

^{13}C NMR (125 MHz, D_2O): (two major isomers) δ= 177.21, 175.03, 172.13, 171.78, 171.70, 171.03, 170.90, 170.67, 170.45, 169.91, 50.80, 50.53, 50.37, 50.20, 50.16, 49.78, *49.69*, 48.47, 47.73, 47.63, 47.44 ,47.36, 47.11, 46.94, 46.87, 38.20, 37.92, 37.86, 37.82, 37.69, 37.60, 37.54, 37.43, 37.13, 36.96, 36.79, *36.42*, 36.16, 34.89, *34.82*, 22.27, *16.34*, 16.29, *16.25*, 16.19.

HRMS *m/z* calcd for $C_{33}H_{62}N_{14}O_{10}$ $(M+H)^+$; 815.48516, found: 815.48492

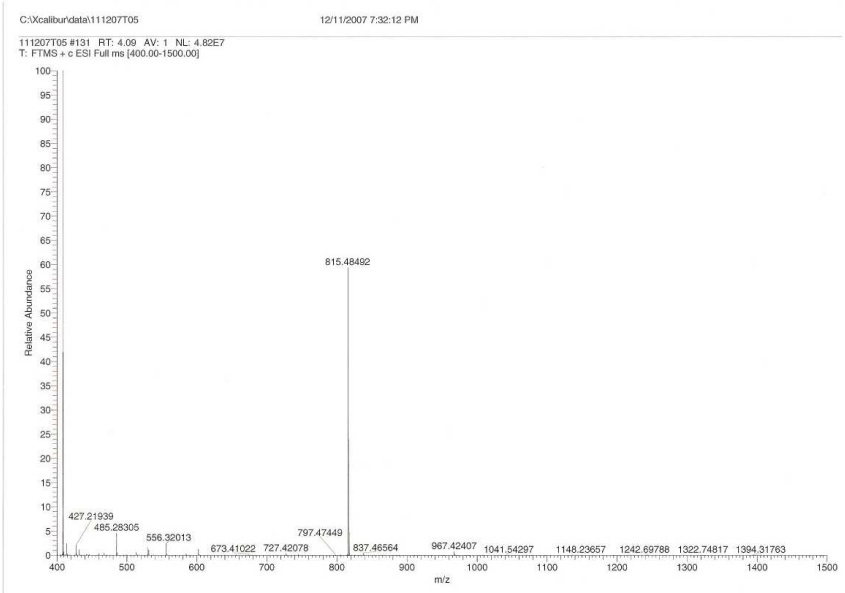


Figure 36: HRMS mass spectrum of **7**

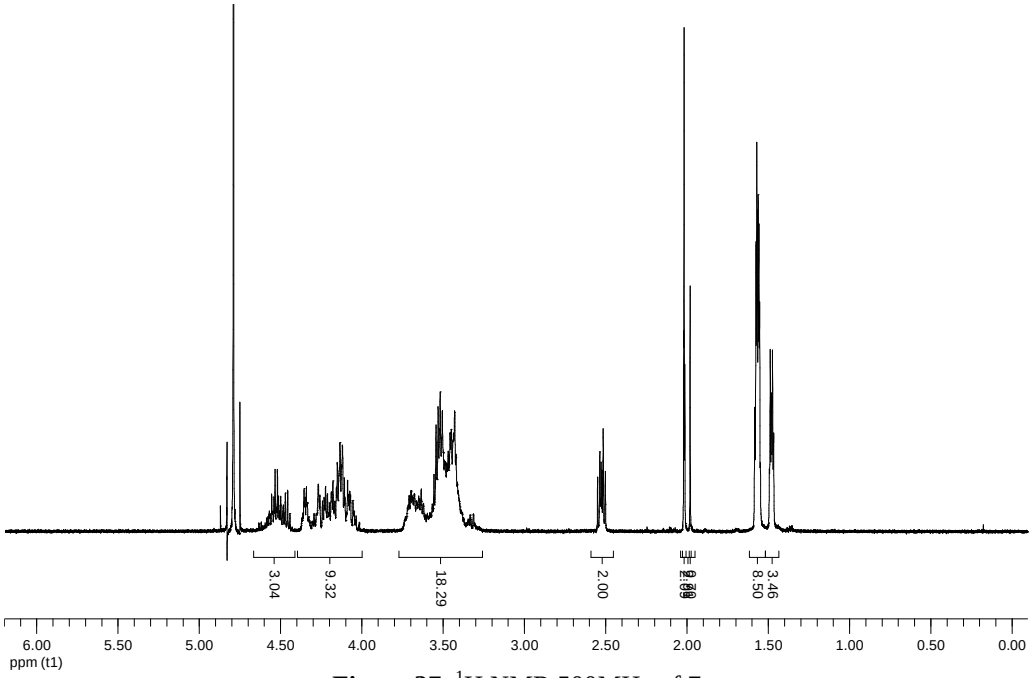


Figure 37: ^{1}H NMR 500MHz of **7**

FFFF (8)

8 was prepared following the general solid-phase procedure described above. Crude **8** (110 mg) was obtained with a purity of 82% as determined by HPLC, and purified by semi-preparative HPLC.

HPLC T_R = 25.2 min

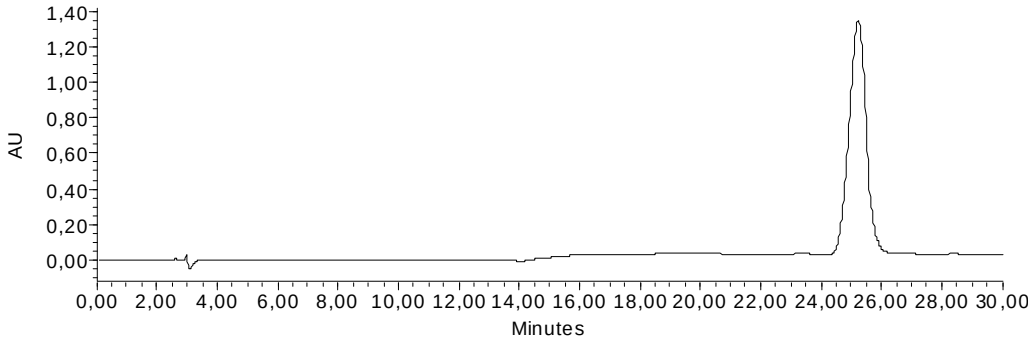


Figure 38: HPLC profile of **8** at 210nm

^{1}H NMR (500 MHz, D_2O): (two major isomers) δ= 7.50-7.20 (m, 20H, 4 x 5CH_{ar}) ; 4.76-4.60, *4.58-4.47* (m, 4H, 4 x CHα Phe) ; 4.24-3.79 (m, 8H, 4 x CH_2(1)) ; 3.67-3.00 (m, 26H, 4 x CH_2(2), 4 x CH_2(3), 4 x CH2β Phe, CH_2(5)) ; 2.55-2.46 (m, 2H, CH_2(4)) ; 1.97, *1.96* (s, 3H, CH_3(Ac)).

^{13}C NMR (125 MHz, D_2O): (two major isomers) δ= 177.14, *177.05*, 174.86, *174.67*, 170.85, 170.72, 170.65, 170.58, 170.49, 170.30, 170.26, 170.22, 170.18, 170.14, 169.93, 169.89, 169.67, 169.62, 133.65, 133.58, 129.95, 129.83, 129.67, 129.64, 128.66, 52.59, 52.52, 52.48, 52.24, 52.15, 52.10, 50.91, 50.61, 50.40, 50.25, 50.07, 49.89, 49.86, 49.74, 48.35, 48.24, 48.12, 47.86, 47.41, 47.28, 47.20, 37.84, 37.75, 37.64, 37.55, 37.50, 37.45, 37.08, 37.05, 36.90, 36.89, *36.38*, 36.17, 34.85, *34.74*, *22.30*, 22.18.

HRMS *m/z* calcd for $C_{57}H_{78}N_{14}O_{10}$ $(M+H)^+$; 1119.61036, found: 1119.60901

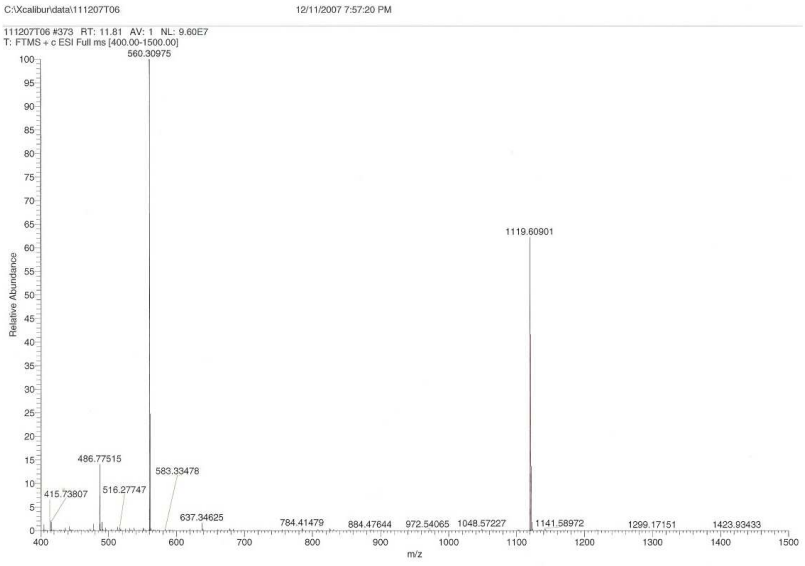


Figure 39: HRMS mass spectrum of **8**

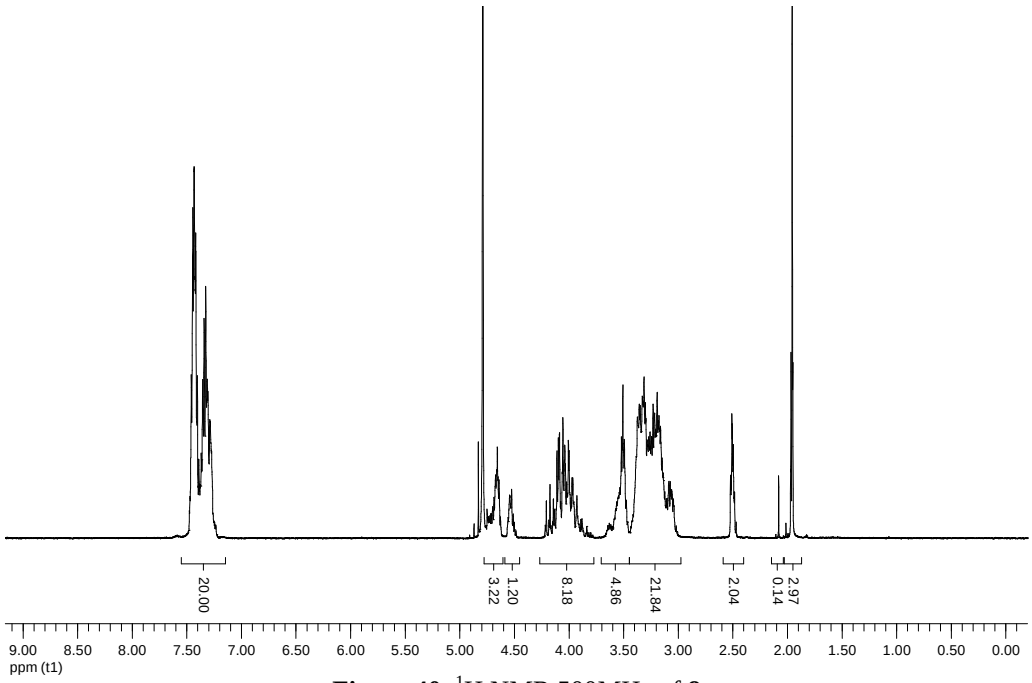


Figure 40: ^{1}H NMR 500MHz of **8**

FRK (1')

1' was prepared following the general solid-phase procedure described above. Crude **1'** (66 mg) was obtained with a purity of 75% as determined by HPLC, and purified by semi-preparative HPLC.

HPLC T_R = 21.2 min

HRMS : m/z calcd for $C_{38}H_{67}N_{15}O_8$ $[M+H]^+$ = 862,5375 ; found $[M+H]^+$ = 862,53748

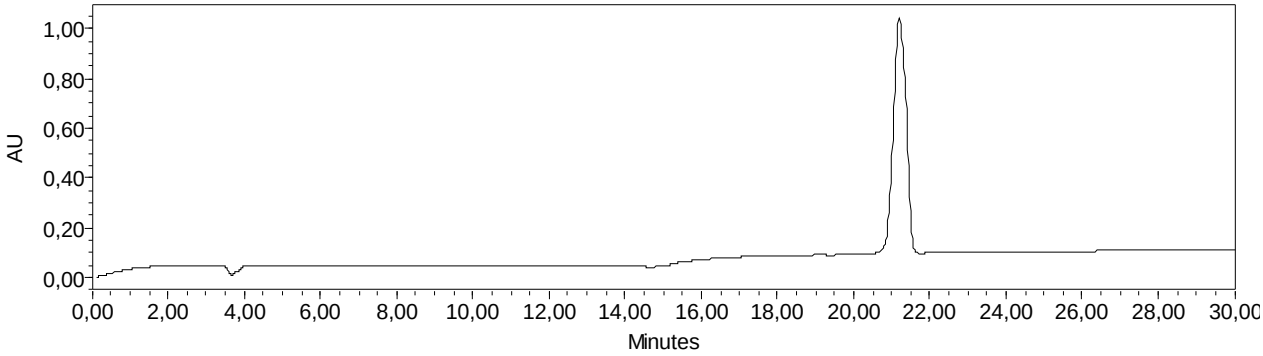


Figure 41: HPLC profile of **1'** at 210nm

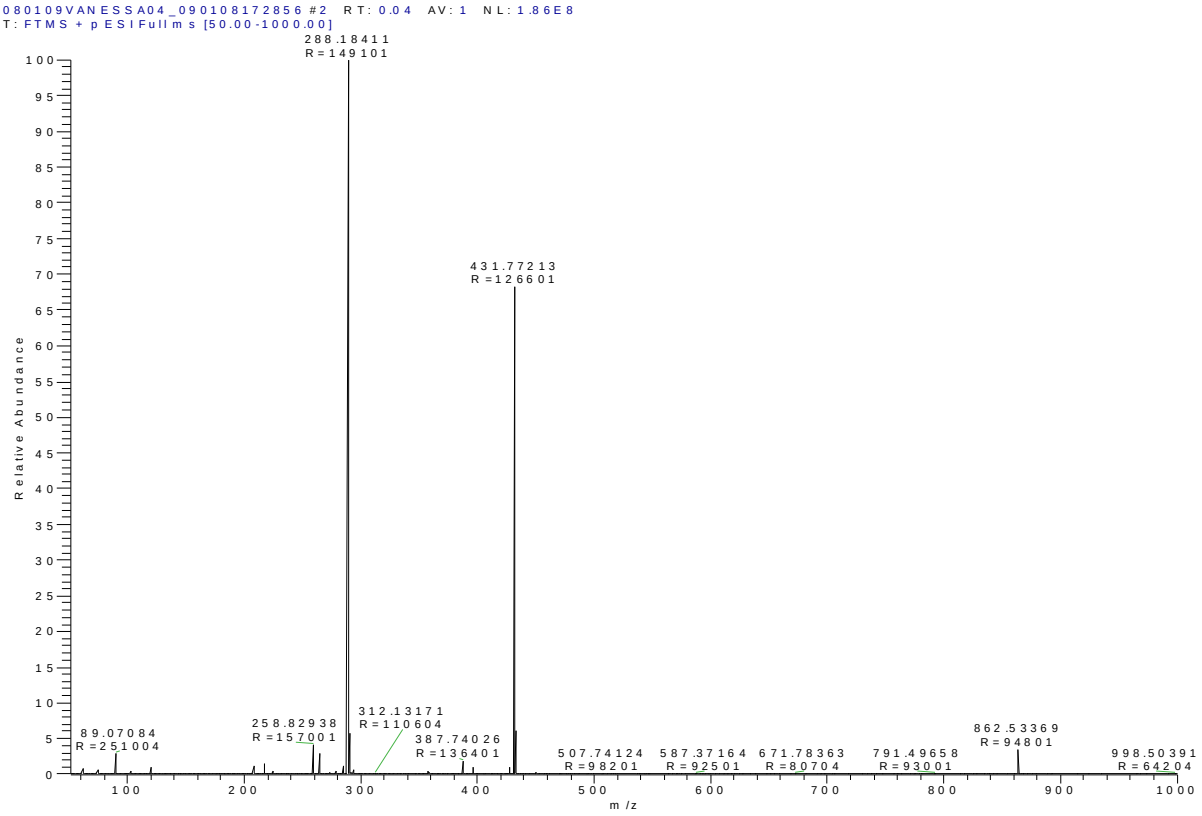


Figure 42: HRMS mass spectrum of **1'**

FAK (2')

2' was prepared following the general solid-phase procedure described above. Crude **2'** (80 mg) was obtained with a purity of 80% as determined by HPLC, and purified by semi-preparative HPLC.

HPLC T_R = 20.9 min

HRMS : m/z calcd for $C_{35}H_{60}N_{12}O_8$ $[M+H]^+$ = 777,4735 ; found $[M+H]^+$ = 777,47284.

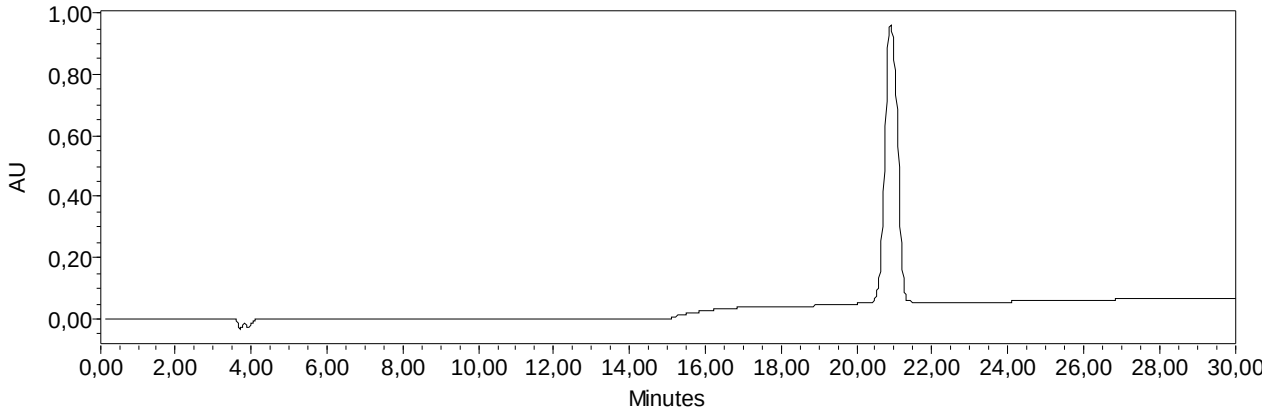


Figure 43: HPLC profile of **2'** at 210nm

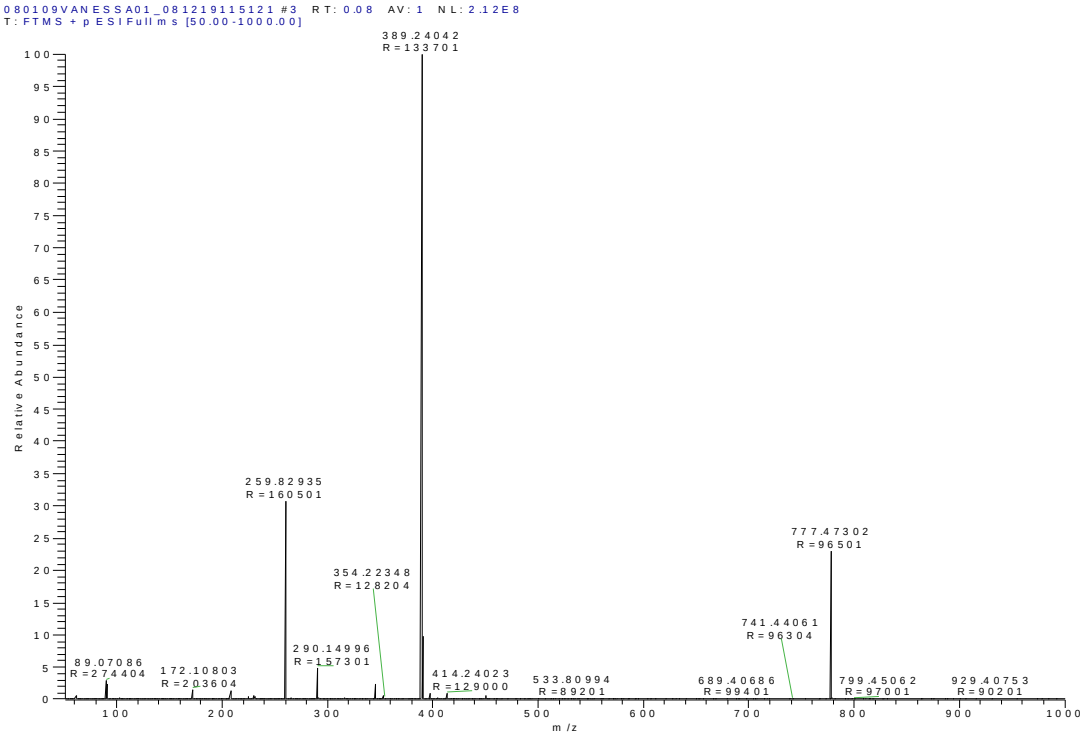


Figure 44: HRMS mass spectrum of **2'**

RRR (6')

8' was prepared following the general solid-phase procedure described above. Crude **8'** (39 mg) was obtained with a purity of 58% as determined by HPLC, and purified by semi-preparative HPLC.

HPLC T_R = 18.9 min

HRMS : m/z calcd for $C_{35}H_{70}N_{20}O_8$ $[M+H]^+$ = 899,5764 ; found $[M+H]^+$ = 899,57635

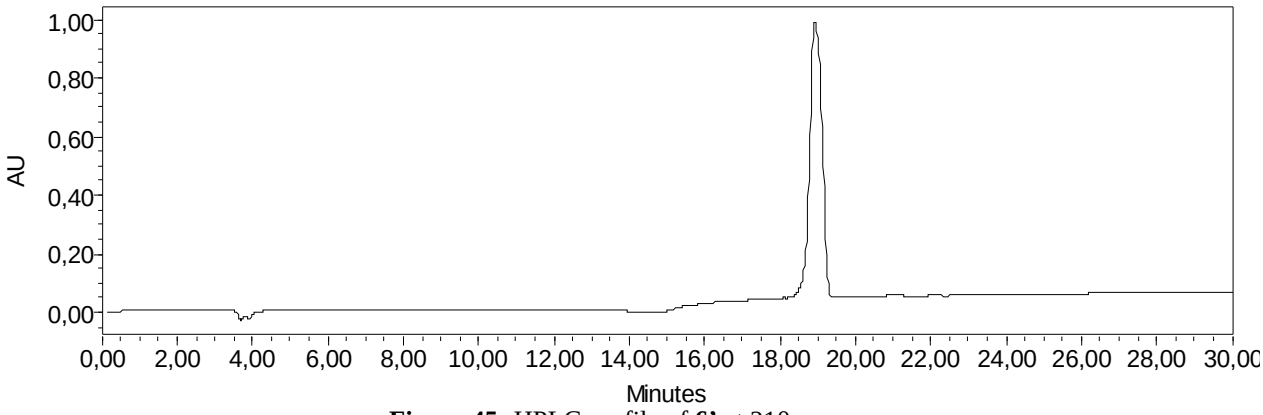


Figure 45: HPLC profile of **6'** at 210nm

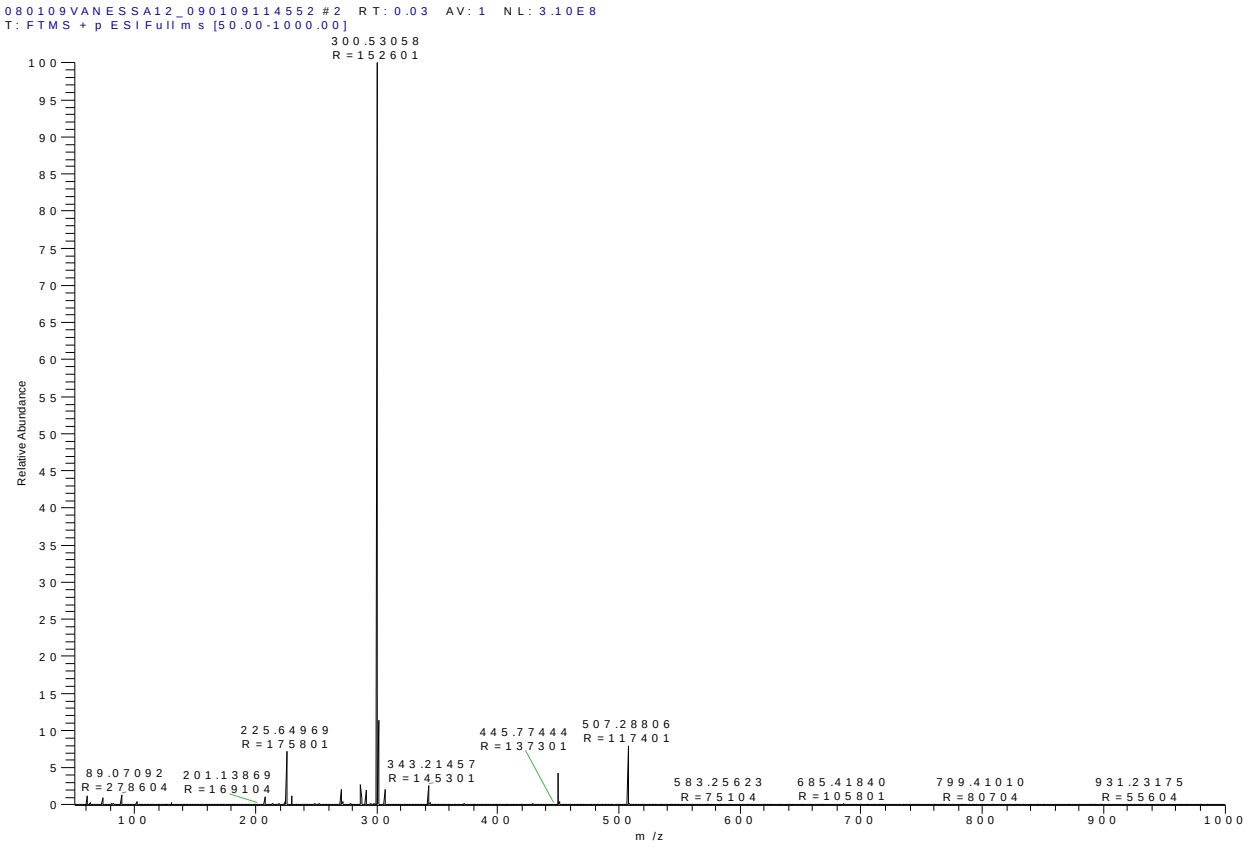


Figure 46: HRMS mass spectrum of **6'**

Binding studies

Unless otherwise stated, all reagents and solvents were of analytical grade and from Sigma (St Louis, U.S.A.). HEPES [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid] and all inorganic salts for buffers were purchased from Calbiochem (molecular biology grade). RNA (figure 47) and DNA oligonucleotides were purchased from IBA GmbH and used without further purification. A mixture of pre- and mature yeast tRNAs (containing over 30 different species) was purchased from Sigma (type X-SA). Stocks of tRNAmix can be quantified in its native form (without base hydrolysis) using an extinction coefficient of 9640 cm^{-1} M^{-1} per base[2].

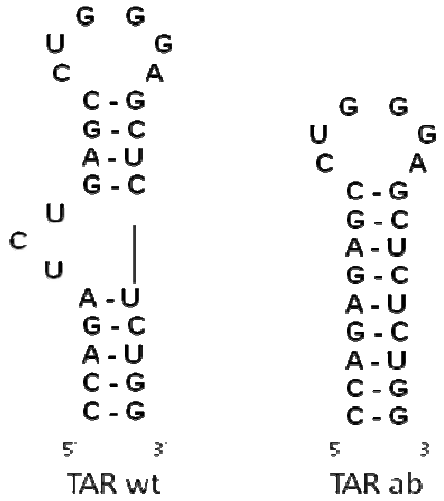


Figure 47: RNA structures

Buffers

All buffers were filtered through 0.22-µm Millipore filters (GP ExpressPLUS membrane). A small aliquot (50–100 ml) was first filtered and then discarded to avoid any contaminants that might be leached from the filter. The solutions to be used in the fluorescence experiments were prepared by diluting the concentrated stocks in Milli-Q water and filtered again as described above.

All standard fluorescence measurements were performed in buffer A (20 mM HEPES (pH 7.4 at 25 °C), 20 mM NaCl, 140 mM KCl and 3 mM $MgCl_2$).

For competitive experiments in the presence of a *ds*DNA, a 15-mer sequence (5'-CGTTTT TATTTTTGC-3') and its complement, annealed beforehand, were added to buffer A to obtain a 100-fold nucleotide excess regarding TAR RNA (900 nM duplex; 5 nM RNA).

For competitive experiments in the presence of a *t*RNA, a mixture of pre- and mature yeast *t*RNAs (containing over 30 different species from baker's yeast (*S. cerevisiae*, Sigma, type X-SA)) was added to buffer A to obtain a 100-fold nucleotide excess regarding TAR RNA. Stock solutions of *t*RNA were prepared in water and quantified using an extinction coefficient of 9640 cm^{-1} M^{-1} per base[2].

CD experiments were performed in buffer B (20 mM potassium phosphate buffer (pH 7.4 at 25 °C), 10 mM NaCl and 1 mM $MgCl_2$).

Circular dichroism spectra

CD measurements were performed with a Jasco J-810 spectropolarimeter equipped with a Jasco PTC 423S Peltier temperature controller. Samples were prepared in buffer B. Spectra were obtained at 3 µM RNA or PAA (for individual spectra) or a molar ratio of 1:1, 1:10 RNA:PAA for the complexes in a 1-mm path-length cell. RNA samples were heat-denatured and allowed to refold as described above prior measurement. Spectra were recorded at 20°C from 360 to 200 nm at 1 nm intervals with an integration time of 4 sec and a 50 nm/min speed. CD scans were repeated five times, then averaged and corrected by the subtraction of buffer background or the spectra of the appropriate ligand alone (figure 49).

[2] Luedtke, N. W., Liu, Q., and Tor, Y.; *Biochemistry* **2003** 42, 11391-11403.

Fluorescence binding assay.
Ligand solutions were prepared as serial dilutions in buffer A at a concentration two times higher than the desired final concentration to allow for the subsequent dilution during the addition of the RNA solution. The appropriate ligand solution (50µL) was then added to a well of a non-treated black 96-well plate (Nunc 237105), in triplicate. Refolding of the RNA was performed using a thermocycler (ThermoStat Plus Eppendorf) as follows: the RNA, diluted in 1mL of buffer A, was first denatured by heating to 90°C for 2 min; then cooled to 4 °C for 10 min followed by incubation at 20°C for 15 min. After refolding, the RNA was diluted to a working concentration of 10 nM through addition of the appropriate amount of buffer A. The tube was mixed and 50 μL of the RNA solution was added to each well containing ligand. This subsequent dilution lowered the final RNA concentration to 5 nM. The fluorescence was measured on a GeniosPro (Tecan) with an excitation filter of 485 ± 10 nm and an emission filter of 535 ± 15 nm. Each point was measured 10 times with a 500 µs integration time and averaged. Binding was allowed to proceed overnight at 5°C to achieve equilibrium (figure 50-54).
To study the temperature dependence, the plates were incubated after overnight equilibrium at different temperature ranging from 5°C to 35°C (figure 55).
The salt dependence was studied in 20 mM HEPES (pH 7.4 at 25 °C), 20 mM NaCl, 3 mM $MgCl_2$ with the KCl concentration varied between 70 and 250 nM (figure 56).

It has been shown that modest aggregations of labeled HIV-1 TAR fragments, occurring particularly at high concentrations, can potentially affect the fluorescence of the RNA. Thus, we first measured the fluorescence intensity of increasing concentrations (from 0.05 to 50 nM) of 5'-A488 labeled TAR at 519 nm, in the absence of tetra-PAA, in buffer A. A linear relationship was observed (figure 48), which was not affected upon addition of higher concentrations of unlabeled TAR fragment, demonstrating that aggregative effects of 5'-A488 labeled TAR fragment were minimal in these buffer conditions.

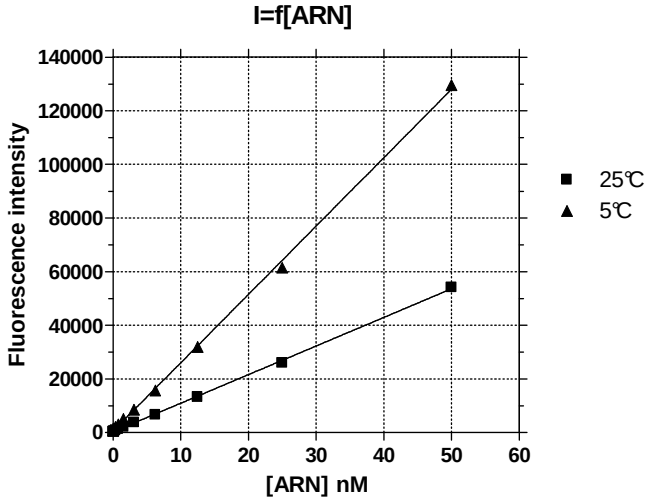


Figure 48: Fluorescence intensity of TAR RNA

Neomycin was taken as a control as its binding to TAR has already been studied using several methods. Its K_d value (17 ± 1µM) is in good agreement with previously reported values[3].

To check that the fluorescence quenching was not a result of nonspecific interactions between tetra-PAA and the fluorophore, titrations of a 5 nM solution of a A488 hexyl ester derivative with **1**-**8** ranging from 0.01 to 1500 µM were carried out. No significant changes of the fluorescence intensity was observed (less than 10 %, data not shown), thus confirming that the observed fluorescence decreases very likely reflect the binding of **1**-**8** to the labeled TAR fragment rather than nonspecific interactions with the fluorophore.
Moreover competitive binding experiments using the unlabeled TAR RNA fragment, showed that tetra-PAA **1**-**8** bind equally to both unlabeled and A488-labeled RNA fragments, confirming that the effect of the fluorescent probe on binding is negligible.

[3] Tassew, N.; Thompson, M.; *Biophysical Chemistry*, **2003**, 241–252

Data analysis

Binding data were analyzed using the non-linear least-squares numerical solver-based binding data global analysis program BIOEQS, in which the calculated binding surface is obtained using a numerical constrained optimization chemical equilibrium solver[4]. Unless otherwise stated, binding profiles were well modeled using a simple model assuming the one to one stoichiometry. $\Delta G°$ values were converted to K_d values as $K_d = e^{-(\Delta G°/RT)}$.

For thermodynamic analysis, $\Delta G°$ values were plotted versus T for the three-parameter fit[5]. Nonlinear regression in Prism 4 (GraphPad Software) was used to fit the following equation to the data:

$\Delta G°_T = \Delta H°_{Tr} + \Delta C_P (T - Tr) – T\Delta S°_{Tr} - T\Delta C_P \ln (T/Tr)$

where Tr is a constant reference temperature (in our study Tr = 293.15 K), and the three fit parameters are $\Delta H°_{Tr}$, the change in enthalpy upon binding at Tr ; $\Delta S°_{Tr}$, the change in entropy upon binding at Tr; and ΔC_P, the change in heat capacity. Starting values for the three parameters did not affect the final values. ΔC_P was assumed to be independent of temperature; inclusion of a $\Delta C_P/\Delta T$ term in the analysis did not improve the quality of the fits and gave larger standard errors for the returned parameters.

$\Delta H°_T$ and $\Delta S°_T$ were calculated by using:

$\Delta H°_T = \Delta H°_{Tr} + \Delta C_P (T - Tr)$

and

$\Delta S°_T = \Delta S°_{Tr} + \Delta C_P \ln (T/Tr)$.

Salt dependence of Kd was analyzed by the following equation[6](figure 56):

$\log(K_d) = \log(K_{nel}) - Z\psi \log [KCl]$

where K_{nel} is the dissociation constant at the standard state in 1 M KCl, *Z* is the number of ions displaced from the nucleic acid (essentially the number of intermolecular ion pairs) and ψ is the fractional probability of a counterion being thermodynamically associated with each phosphate of the RNA number of cations. K_{nel} and $Z\psi$ were treated as fitting parameters.

A higher initial fluorescence value is observed in the presence of *ds*DNA and *t*RNA which is consistent with the modification of the polarity of the solvent and a small fluorescence of the *t*RNA mixture.

[4] (a) Royer, C. A. *Anal. Biochem*., **1993**, 210, 91 ; (b) Royer, C. A. ; Beechem, J. M. *Methods Enzymol.* **1992**, 210, 481–505.

[5] (a) Boniface JJ, Reich Z, Lyons DS, Davis MM. *Proc Natl Acad Sci U S A.* **1999**, 96, 11446 ; (b) Yoo SH, Lewis MS. *Biochemistry* **1995**, 34, 632

[6] Record,M.T. Jr; Anderson,C.F. and Lohman,T.M. *Q. Rev. Biophys*., **1978**, 11, 103-178.

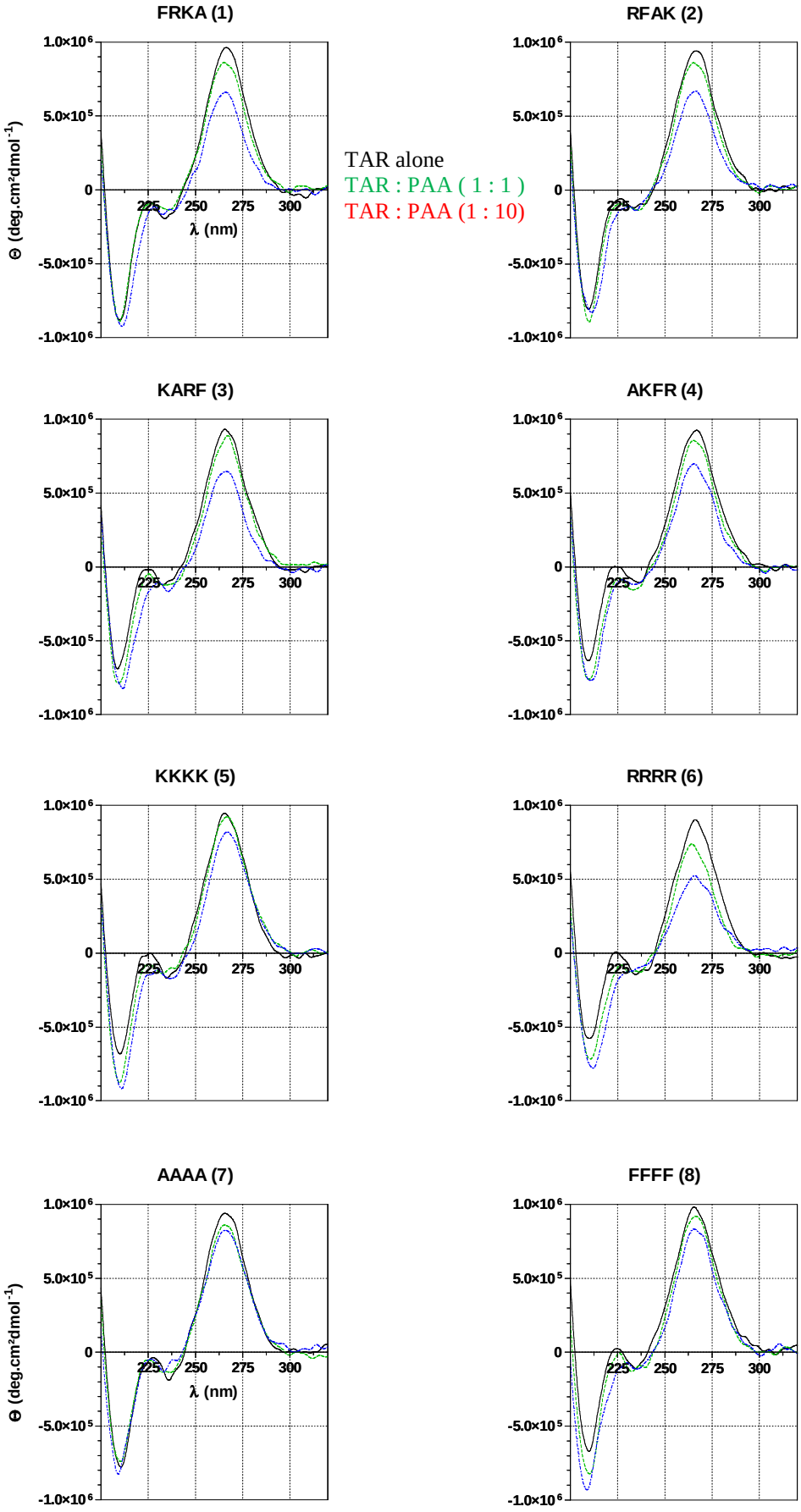
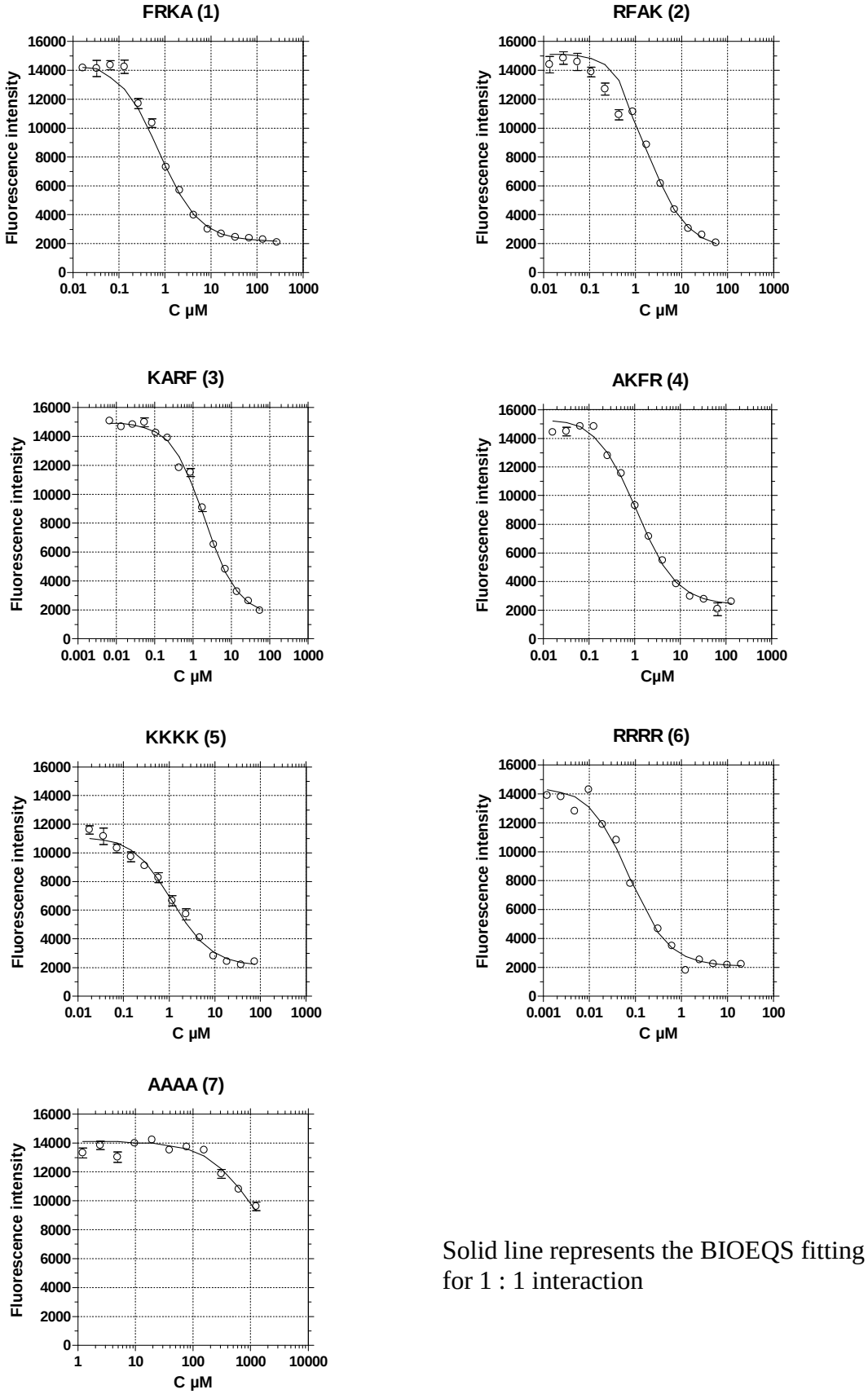


Figure 49. CD profiles of PAA **1-8**



Solid line represents the BIOEQS fitting for 1 : 1 interaction

Figure 50. Binding curves of PAA **1**-**7** without competitor

The profiles were obtained in 20 mM HEPES (pH 7.4), 20 mM NaCl, 140 mM KCl and 3 mM $MgCl_2$ at 25 °C in the presence of 5 nM of labeled TAR RNA.

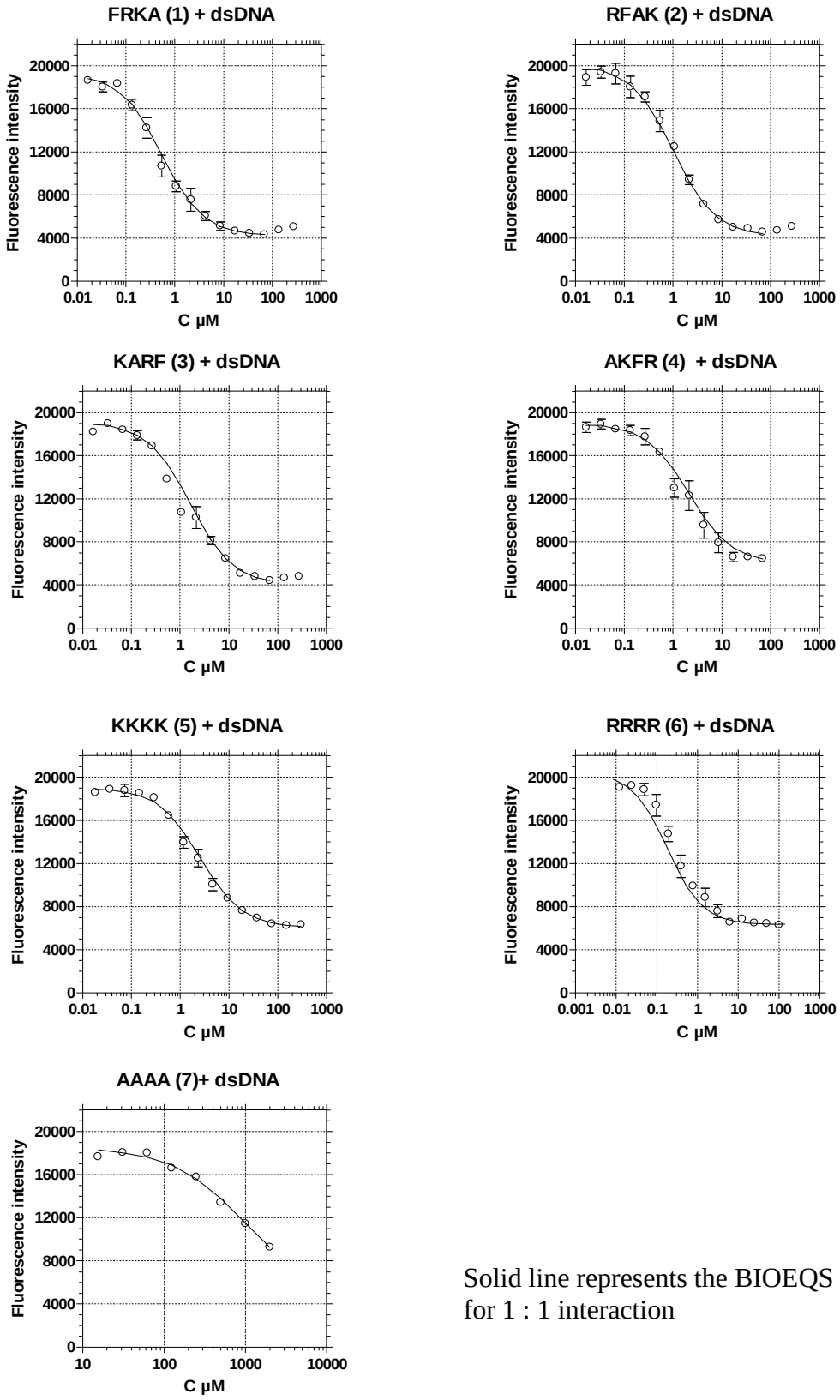


Figure 51. Binding curves of PAA 1-7 in the presence of *ds*DNA

The profiles were obtained in 20 mM HEPES (pH 7.4), 20 mM NaCl, 140 mM KCl and 3 mM $MgCl_2$ at 25 °C in the presence of 5 nM of labeled TAR RNA and 900 nM of duplex DNA.

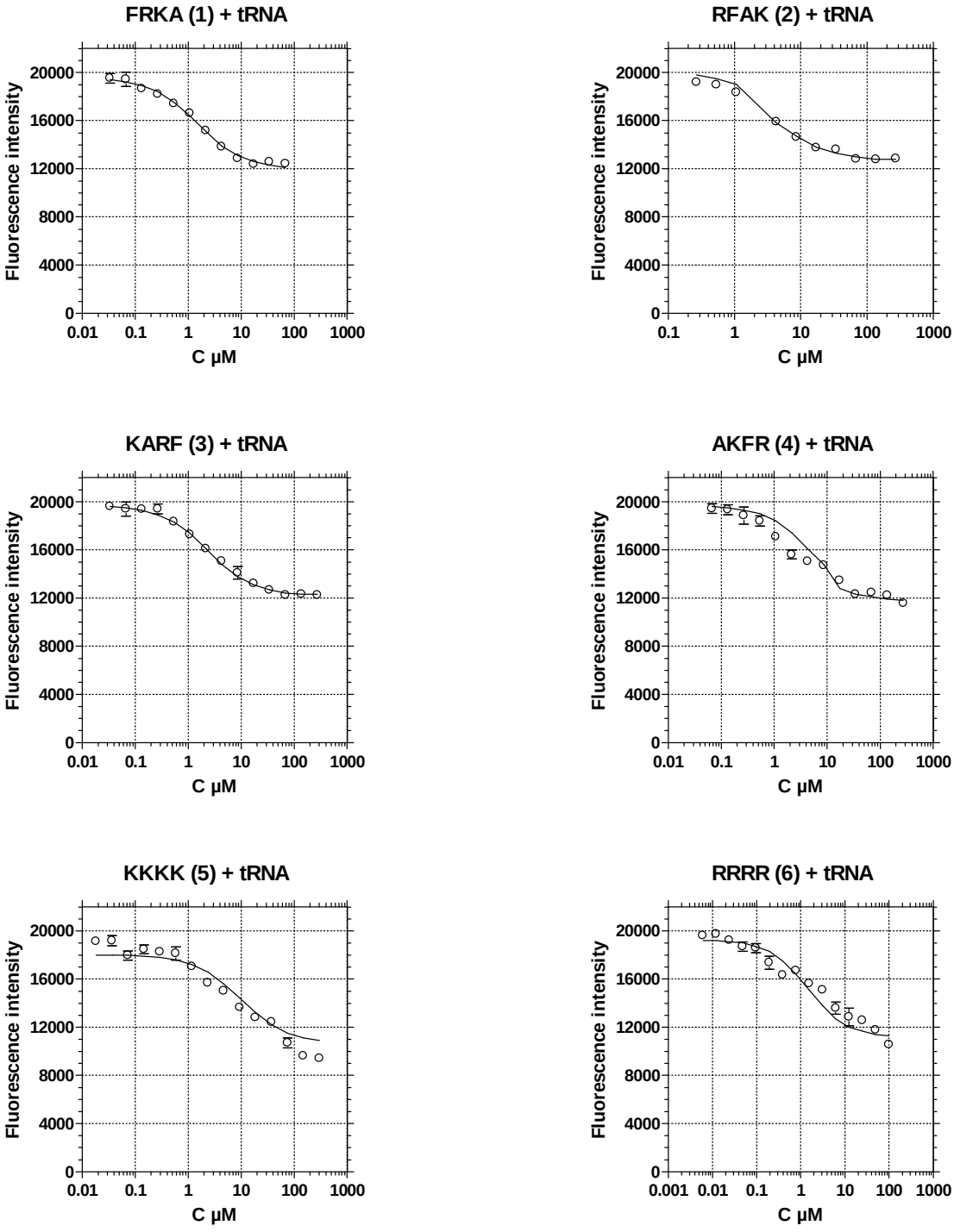


Figure 52. Binding curves of PAA 1-6 in the presence of *t*RNA

The profiles were obtained in 20 mM HEPES (pH 7.4), 20 mM NaCl, 140 mM KCl and 3 mM $MgCl_2$ at 25 °C in the presence of 5 nM of labeled TAR RNA and tRNA.

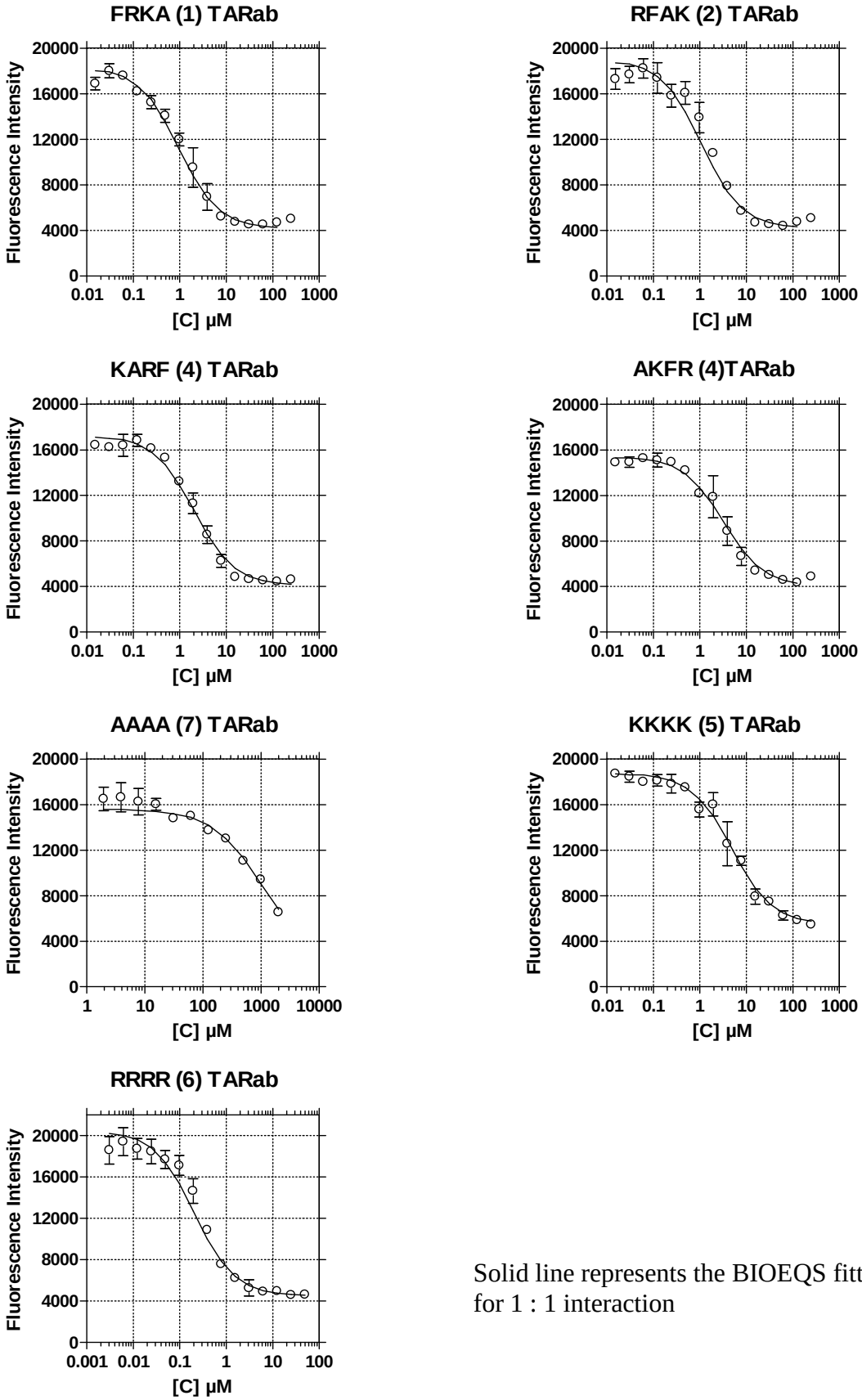


Figure 53. Binding curves of PAA 1-7 with TAR ab

The profiles were obtained in 20 mM HEPES (pH 7.4), 20 mM NaCl, 140 mM KCl and 3 mM $MgCl_2$ at 25 °C in the presence of 5 nM of labeled TAR RNAab.

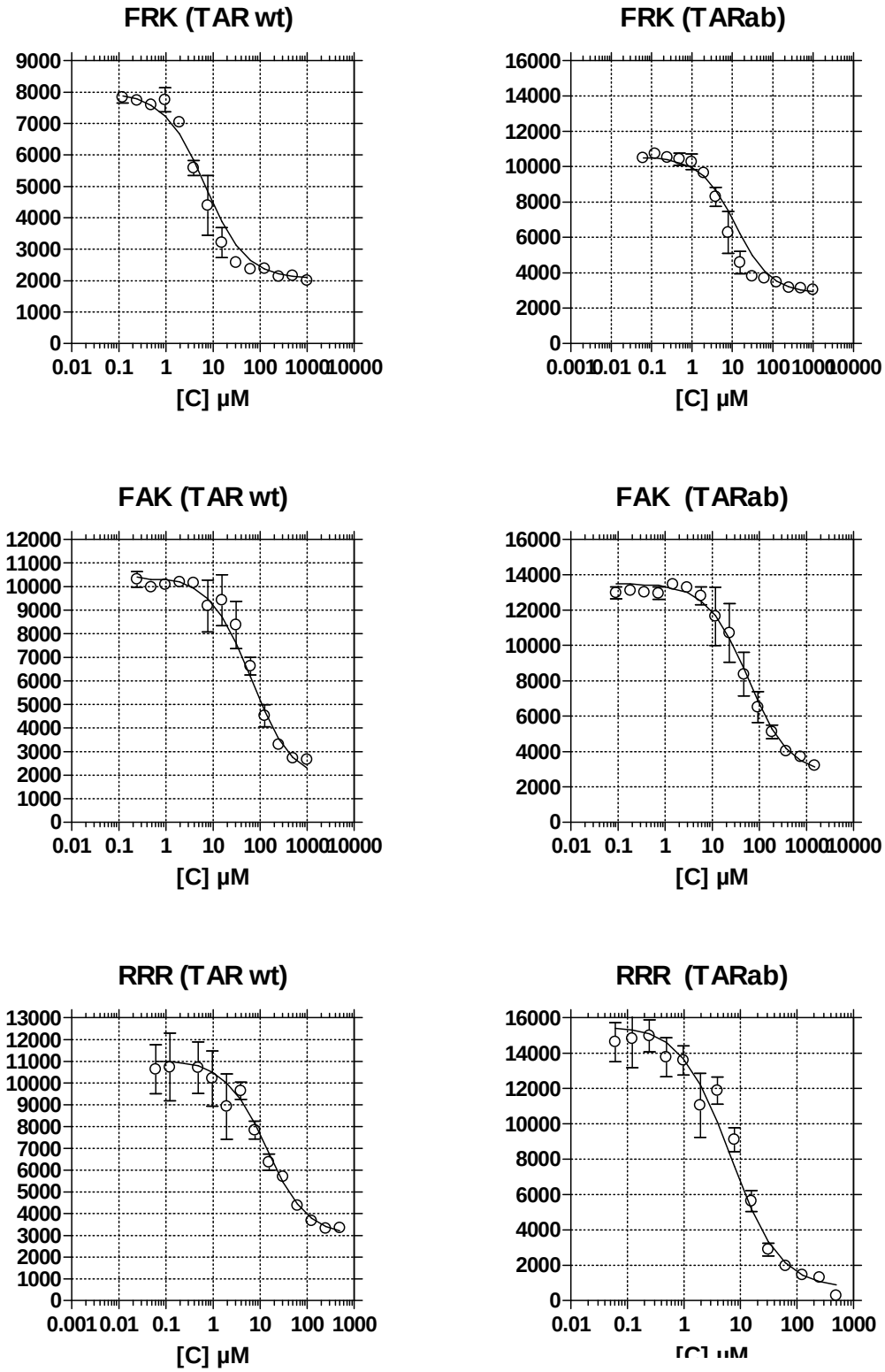
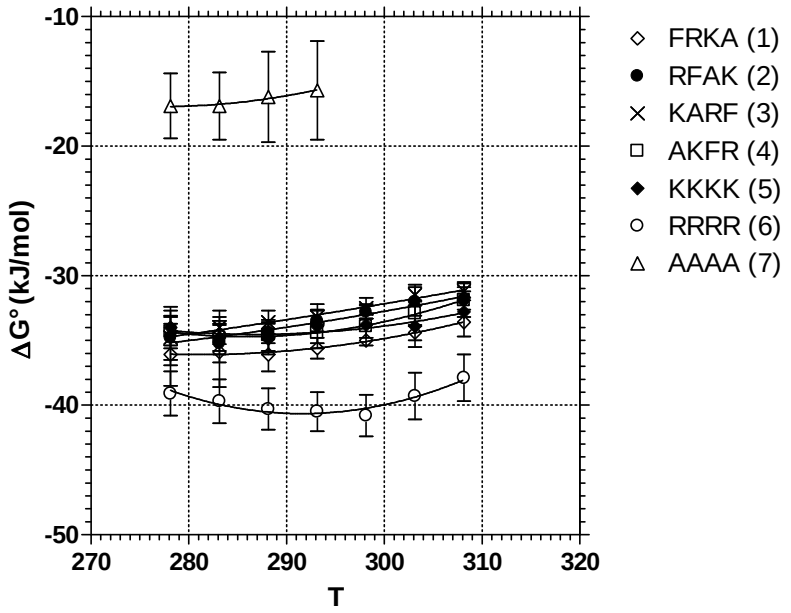


Figure 54. Binding curves of tri-PAA

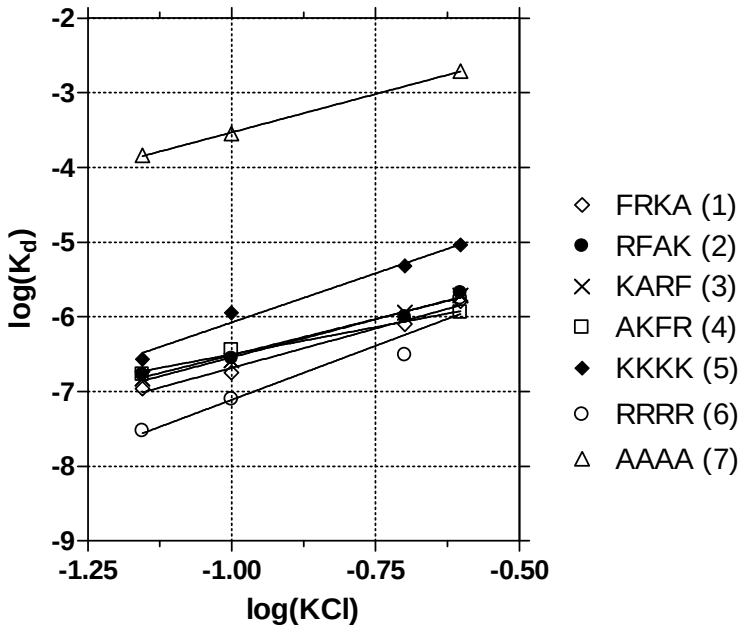
The profiles were obtained in 20 mM HEPES (pH 7.4), 20 mM NaCl, 140 mM KCl and 3 mM $MgCl_2$ at 25 °C in the presence of 5 nM of labeled RNA.



$$\Delta G_T = \Delta H_{Tr} + \Delta C_P (T - Tr) \quad - T\Delta S_{Tr} - T\Delta C_P \ln (T/Tr)$$

	FRKA (1)	RFAK (2)	KARF (3)	AKFR (4)	KKKK (5)	RRRR (6)	AAAA (7)
ΔH_{Tr}	-60.08	-68.65	-68.44	-59.24	-48.21	-49.35	-62.72
ΔC_P	-2.01	-0.60	-0.31	-3.38	-2.16	-5.65	-2.87
ΔS_{Tr}	-0.08	-0.12	-0.12	-0.08	-0.05	-0.03	-0.16
Std. Error							
ΔH_{Tr}	1.51	4.55	2.22	1.12	4.74	4.04	18.09
ΔC_P	0.35	1.05	0.51	0.26	1.09	0.93	2.29
ΔS_{Tr}	0.01	0.02	0.01	0.00	0.02	0.01	0.06
R^2	0.9866	0.9371	0.9846	0.9939	0.7529	0.911	0.961

Figure 55. Temperature effect for PAA 1-8



FRKA (1): y = 2.106 (± 0.1709) x - 4.578 (± 0.1709)	R^2 = 0.9870
RFAK (2): y = 1.930 (± 0.1794) x - 4.582 (± 0.1600)	R^2 = 0.9830
KARF (3): y = 2.031 (± 0.09434) x - 4.512 (± 0.08418)	R^2 = 0.9957
AKFR (4) : y = 1.455 (± 0.1829) x - 5.047 (± 0.1734)	R^2 = 0.9844
AAAA (7): y = 2.051 (± 0.04406) x - 1.480(± 0.04177)	R^2 = 0.9995
KKKK (5): y = 2.629 (± 0.2514) x - 3.446 (± 0.2244)	R^2 = 0.9820
RRRR (6): y = 2.891 (± 0.5488) x - 4.221 (± 0.4897)	R^2 = 0.9328

Figure 56. Salt effect for PAA 1-8