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), NBSCI (Nitrobenzenesulfonyl chloride), NMM (N-methylmorpholine), Pyr (Pyridine), TEA (Triethylamine), TFA (Trifluoroacetic acid), TFMSA (Trifluoromethanesulfonic acid), Ac₂O (Acetic anhydride), Boc₂O (terbutyloxy anhydride), THF (Tetrahydrofurane), TIS (Triisopropylsilane), CH₂Cl₂ or DCM (Dichloromethane), EtOAc (Ethyl acetate), cyclohexane, DEA (Diethylamine), MeOH (Methanol), EtOH (Ethanol), DMSO (Dimethylsulfoxide), D₂O (Deuterium oxide), CDCl₃ (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 conducted 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 synthesized 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, ¹³C and HSQC NMR spectra of N-protected PAA monomers were recorded with Bruker AC 200 (¹H: 200 MHz, ¹³C: 50 MHz) or AV 500 (¹H: 500 MHz, ¹³C: 125 MHz) spectrometers using deuterated DMSO or CDCl₃ 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 Ac₂O 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 absorbence 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, ¹³C, COSY, HSQC, and HMBC NMR spectra were recorded with a Bruker AV 500(¹H: 500 MHz, ¹³C: 125 MHz)

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spectrometer using deuterated D$_2$O 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 syringe, to confirm correct molar mass and high purity of the compounds.

Synthesis

Synthesis of backbone 13b

\[
\begin{align*}
&\text{HBr}_2\text{H}_2\text{N}_3\text{Br} \\
&\text{Boc} \quad \text{N} \quad \text{H} \\
&\quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \ quad
a 10% NaHCO₃ solution, water and brine. The organic layer was dried on MgSO₄ and evaporated under reduced pressure to afford compound 18 (4.6 g, 94%) as a colorless oil. 18 was subsequently used without further purification.

Rf (EtOAc/cyclohexane 1:1, v:v) = 0.78

1H RMN (200 MHz, CDCl₃) δ = 5.91 (tdd, J = 5.77, 10.34, 17.13 Hz, 1H, CH(5)), 5.36 (m, 2H, CH₂(6)), 5.04 (s, 1H, NH(Boc)), 4.63 (td, J = 1.27, 5.76 Hz, 2H, CH₂(4)), 3.94 (d, J = 5.62 Hz, 2H, CH₂(1)), 1.44 (s, 9H, 3xCH₃(Boc)) ; 13C RMN (50 MHz, CDCl₃) δ = 170.19 (1C, CO(OAll)), 158.3 (1C, CO(Boc)), 131.7 (1C, CH(5)), 118.9 (1C, CH₂(6)), 80.15 (1C, C(IV)(Boc)), 65.9 (1C, CH₂(4)), 42.5 (1C, CH₂(1)), 28.4 (3C, 3xCH₃(Boc)) ; MS (ESI+) calcd for C₁₀H₁₇NO₄ [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 CH₂Cl₂ (10 mL) was added dropwise a 10% TFA/TIS solution (19 mL). The mixture was allowed to stir at room temperature for 1 h, and the solvents were removed under reduced pressure. The residue was triturated in diethyl ether, filtered, and dried under vacuum to give compound 17 (4.4 g, 90%) as a white solid.

Rf (EtOAc/Methanol 1:1, v:v) = 0.39

1H RMN (200 MHz, D₂O) δ = 6.05-5.86 (m, 1H, CH(5)), 5.41-5.27 (m, 2H, CH₂(6)), 4.72 (d, J = 5.78 Hz, 2H, CH₂(4)), 3.93 (s, 2H, CH₂(1)) ; 13C RMN (50 MHz, D₂O) δ = 168.28 (1C, CO(OAll)), 131.3 (1C, CH(5)), 119.72 (1C, CH₂(6)), 67.62 (1C, CH₂(4)), 40.51 (1C, CH₂(1)) ;

MS (ESI+) calcd for C₉H₁₇NO₄ [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 CH₂Cl₂ (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 NaHCO₃ solution, with water and with brine. The organic layer was dried on MgSO₄ and after filtration and concentration under reduced pressure, the residue was triturated in diethyl ether, washed with diethyl ether and dried under vacuum to give compound 15 (4.3 g, 74%) as a colorless solid.

Rf (EtOAc/cyclohexane 1:1, v:v) = 0.63

1H RMN (200 MHz, CDCl₃) δ = 8.12-8.07 (m, 1H, CH₂(4′)), 7.97-7.90 (m, 1H, CH₂(1′)), 7.76-7.72 (m, 2H, 2xCH₂(2′,3′)), 6.06 (t, J = 4.56 Hz, 1H, NH(NB)), 5.77 (tdd, J = 5.88, 10.24, 17.19 Hz, 1H, CH(5)), 5.30-5.19 (m, 2H, CH₂(6)), 4.49 (td, J = 1.21, 5.89 Hz, 2H, CH₂(4)), 4.05 (d, J = 5.80 Hz, 2H, CH₂(1)) ; 13C RMN (50 MHz, CDCl₃) δ = 168.38 (1C, CO(OAll)), 158.3 (1C, C(IV)(5′)), 134.12 (1C, C(IV)(6′)), 133.8 (1C, CH(2′)), 133.0 (1C, CH(3′)), 131.1 (1C, CH(5)), 130.7 (1C, CH(1′)), 125.8 (1C, CH(4′)), 119.53 (1C, CH₂(6)), 66.49 (1C, CH₂(4)), 45.05 (1C, CH₂(1)) ;

MS (ESI+) calcd for C₁₁H₁₇NO₄S [M+Na]+ : 323.0, found : 323.3 [M+Na]+

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

To a stirred solution of NBGlyOAll (1.34 g, 4.46 mmol) in DMF (10 mL) was added under inert atmosphere, first CS₂CO₂ (2.92 g, 8.92 mmol) and then dropwise a solution of BocNH(CH₂)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 MgSO₄ 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%).

\[ R_f (\text{EtOAc/Cyhex 1/1, v/v}) = 0.52 \]

\[^1H\text{ RMN} (200\text{MHz, CDCl}_3) \delta = 8.07-8.03 \text{ (m, 1H, CH}(4')\text{)}, 7.72-7.58 \text{ (m, 3H, 3xCH}_2\text{(2'\text{-}3'\text{+1}')}\text{)}, 5.84 \text{ (tdd, } J = 5.84, 10.32, 17.15\text{Hz, 1H, CH}(5)\text{)}, 5.34-5.20 \text{ (m, 2H, CH}_2\text{(6)})\text{)}, 5.02 \text{ (m, 1H, NH(Boc))}, 4.57 \text{ (td, } J = 1.21, 5.84\text{Hz, 2H, CH}_2\text{(4)})\text{), 3.49 \text{ (t, } J = 5.71\text{Hz, 2H, CH}_2\text{(2)})\text{), 3.33 \text{ (m, 2H, CH}_2\text{(3)})\text{), 1.41 \text{ (s, 9H, 3xCH}_3\text{(Boc)}) ;}\]

\[^{13}C\text{ RMN} (50\text{MHz, CDCl}_3) \delta = 168.76 \text{ (1C, COAll), 155.98 \text{ (1C, CO(Boc))}, 147.90 \text{ (1C, C}_4\text{H}(5')\text{), 133.04 \text{ (1C, C}_4\text{H}(6')\text{), 133.71 \text{ (1C, CH}_2\text{(2')}\text{), 131.78 \text{ (1C, CH}_2\text{(3')}\text{), 131.17 \text{ (1C, CH}_2\text{(1')}\text{), 130.00 \text{ (1C, CH}(5)\text{)}, 124.18 \text{ (1C, CH}_2\text{(4')}\text{), 119.14 \text{ (1C, CH}_2\text{(6)})\text{)}, 79.58 \text{ (1C, C}_4\text{H}(Boc)), 66.15 \text{ (1C, CH}_2\text{(4)}\text{), 48.72, 48.54 \text{ (1C, CH}_2\text{(1)}\text{), 44.87 \text{ (1C, CH}_2\text{(2)})\text{), 38.40 (1C, CH}_2\text{(3)}\text{), 28.31 (3C, 3xCH}_3\text{(Boc)) ;}\]

**MS (ESI+)** calcd for C\textsubscript{18}H\textsubscript{25}N\textsubscript{3}O\textsubscript{8}S \[ [M+ Na]^+ : 466.1, \text{ found: 465.9 \[M+Na]^+ ]}\]

Synthesis of N-protected PAA monomers

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

\[ \text{To a solution of Boc(NBS)OAll (1.07 g, 2.41 mmol) in DMF (8 mL) was added DIEA (1.68 mL, 9.64 mmol) and thiophenol (1.24 mL, 12.06 mmol). The reaction was stirred overnight at room temperature, and the solvent was removed under reduced pressure. The residue was taken up in EtOAc and extracted with a 1M KHSO}_4 solution (2 x 30 mL). The aqueous layer was basified at 0°C with a saturated Na}_2CO}_3 solution until pH = 10 and extracted with DCM (4 x 40 mL). The combined organic layers were washed with brine, dried on MgSO}_4 and concentrated under reduced pressure. The oily residue was purified by column chromatography (silica, EtOAc/cyhex 4:6 to 7:3, v:v) to afford compound 13b as a yellow oil (0.37 g, 60%).} \]

\[ R_f (\text{EtOAc}) = 0.44 \]

\[^1H\text{ RMN} (200\text{MHz, CDCl}_3) \delta = 5.91 \text{ (tdd, } J = 5.80, 10.31, 17.15\text{Hz, 1H, CH}(5)\text{)}, 5.37-5.21 \text{ (m, 2H, CH}_2\text{(6)})\text{), 5.03 \text{ (br s, 1H, NH(Boc))}, 4.62 \text{ (td, } J = 1.28, 5.80\text{Hz, 2H, CH}_2\text{(4)})\text{), 3.42 \text{ (s, 2H, CH}_2\text{(1)})\text{), 3.24-3.16 \text{ (m, 2H, CH}_2\text{(3)})\text{), 2.76-2.70 \text{ (m, 2H, CH}_2\text{(2)})\text{), 1.65 \text{ (s, 1H, NH), 1.42 (s, 9H, 3xCH}_3\text{(Boc)}) ;}\]

\[^{13}C\text{ RMN} (50\text{MHz, CDCl}_3) \delta = 172.3 \text{ (1C, COOAll), 156.18 \text{ (1C, CO(Boc))}, 131.9 \text{ (1C, CH}(5)\text{), 118.8 \text{ (1C, CH}_2\text{(6)})\text{), 79.3 \text{ (1C, C}_4\text{H}(Boc)), 65.59 \text{ (1C, CH}_2\text{(4)}\text{), 50.54 \text{ (1C, CH}(2)\text{)}, 48.89 \text{ (1C, CH}_2\text{(2)})\text{), 40.31 \text{ (1C, CH}_2\text{(3)})\text{, 28.53 (3C, 3xCH}_3\text{(Boc)}) ;}\]

**MS (ESI+)** calcd for C\textsubscript{18}H\textsubscript{25}N\textsubscript{3}O\textsubscript{8}S \[ [M+H]^+ : 259.2, \text{ found : 259.1 \[M+H]^+ ]}\]

**Scheme S2**: Synthesis of N-protected PAA monomers. **Reagents and conditions**: (i) Z-NHCH(R)CO\textsubscript{2}H/ HBTU/ DIPEA/ DMF, 2h, rt, (82%); (ii) LiOH/H\textsubscript{2}O/THF, 0°C, 1h, (90%)
Scheme S3: Synthesis of N-protected PAA(Arg) monomer. *Reagents and conditions:* (i) Z-Arg(Z₂)-OH/ HBTU/ DIPEA/ DMF, 2h, rt, (80%); (ii) Pd(PPh₃)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 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 KHSO₄ solution (3 x 30 mL), a saturated NaHCO₃ solution (3 x 30 mL), H₂O (2 x 30 mL) and brine (50 mL). The organic layer was dried on MgSO₄ 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.

**Synthesis of Boc(Z-Ala)OMe (9')**

Boc(Z-Ala)OMe (9') was prepared following the general procedure described above. 9' (1.6 g, 85%).

**1H NMR (200MHz, DMSO-d₆)**: δ (two major isomers)= 7.57, 7.53 (d, J = 8.0Hz, 1H, NH(Z)) ; 7.40-7.27 (m, 5H, CH₆(Z)) ; 6.85, 6.73 (t, J = 5.6Hz, 1H, NH(Boc)) ; 5.08-4.91 (m, 2H, CH₂(Z)) ; 4.65-4.45, 4.37-4.26 (m, 1H, CHα) ; 4.28, 4.14 (d, J = 17.0Hz, 1H, CH₂(1)) ; 4.22, 3.89 (d, J = 17.0Hz, 1H, CH₂(1)) ; 3.66, 3.61 (s, 3H, OCH₃) ; 3.51-2.95, (m, 4H, CH₂(2) et CH₂(3)) ; 1.36 (s, 9H, 3xCH₃(Boc)) ; 1.19, 1.13 (d, J = 6.8Hz, 3H, CH₃(Ala)); 13C NMR (50MHz, DMSO-d₆): δ (two major isomers)= 173.23, 172.79 (1C, N-CO); 170.18, 169.71 (1C, COOMe); 155.61 ; 155.51 (1C, CONH(Z+Boc)); 137.02, 136.93 (1C, CIV(Z)); 128.33, 127.77, 127.70 (5C, 5xCH(Z)); 77.87, 77.69 (1C, CIV(Boc)); 65.47, 65.33 (1C, CH₂(Z)); 52.08, 51.68 (1C, OCH₃); 49.31, 47.74, 47.46, 46.65 (2C, CH₂(1)+CH₂(2)) ; 46.31, 45.97 (1C, CHₓ(Z)); 38.50 (1C, CH₂(3)); 28.15 (3C, 3xCH₂(Boc)); 21.71, 17.59 (1C, CH₃(Ala));

**MS (ESI+) (m/z)**: calcd for C₂₁H₂₃N₃O₇ [M+Na]⁺: 460.2, found: 460.3 [M+Na]⁺

**[α]D²⁵ (C=1, MeOH)**: -17.6

**HPLC Tₚ = 13.9 min**

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Figure 1: HPLC trace at 210nm of the purified 9\textsuperscript{'} monomer.

Figure 2: \textsuperscript{1}H NMR of 9\textsuperscript{'} monomer.

Boc(Z-Phe)OMe (10\textsuperscript{'}

10\textsuperscript{'} was prepared following the general procedure described above. 10\textsuperscript{'} (1.95 g, 88%).

R\textsubscript{R}(EtOAc/cyhex, v:v) = 0.54

\textsuperscript{1}H NMR (500MHz, DMSO-\textit{d}\textsubscript{6}) : \delta (two major isomers)= 7.75, 7.67 (d, J = 8.8Hz, J = 8.4Hz, NH(Z)) ; 7.36-7.19 (m, 10H, 5xCH\textsubscript{ar}(Z)+5xCH\textsubscript{ar}(Phe)) ; 6.91, 6.72 (t, J = 5.7Hz, NH(Boc)) ; 4.99-4.85 (m, 2H, CH\textsubscript{2}(Z)) ; 4.78-4.68, 4.43-4.35 (m, 1H, CH\alpha) ; 4.45, 4.13 (d, J = 18.6Hz, J = 17.1Hz, 1H, CH\textsubscript{2}(1)) ; 4.23, 3.97 (d, J = 18.6Hz, J = 17.1Hz, 1H, CH\textsubscript{2}(1)) ; 3.67, 3.64 (s, 3H, OCH\textsubscript{3}) ; 3.56-2.98 (m, 4H, CH\textsubscript{2}(2)+CH\textsubscript{2}(3)) ; 2.95-2.73 (m, 2H, CH\beta) ; 1.37 (s, 9H, 3xCH\textsubscript{3}(Boc));

\textsuperscript{13}C NMR (125MHz, DMSO-\textit{d}\textsubscript{6}) : \delta (two major isomers)= 173.21, 172.86 (1C, N-CO) ; 171.05, 170.51 (1C, COOMe) ; 156.65 (1C, CO(Z)) ; 156.58, 156.46 (1C, CO(Boc)) ; 138.65, 138.32 (1C, C\textsubscript{IV}(Phe)) ; 137.85 (1C, C\textsubscript{IV}(Z)) ; 130.32, 130.12, 129.15, 128.96, 128.89, 128.55, 128.32, 128.20, 127.28, 127.17 (10C, 5xCH\textsubscript{ar}(Z)+ 5xCH\textsubscript{ar}(Phe)) ; 78.79, 78.60 (1C, C\textsubscript{IV}(Boc)) ; 66.15, 66.10 (1C, CH2(Z)) ; 53.11, 52.92 (1C, CH\text{3}) ; 53.03, 52.60 (1C, OCH\textsubscript{3}) ; 50.35, 48.83 (1C, CH\textsubscript{2}(1)) ; 48.56, 47.69 (1C, CH\textsubscript{2}(2)) ; 39.30, 38.50 (1C, CH\textsubscript{2}(3)) ; 38.02, 37.86 (1C, CH\beta) ; 29.08, 29.02 (3C, 3xCH\textsubscript{3}(Boc));

MS (ESI+) (m/z) : calcd for C\textsubscript{27}H\textsubscript{35}N\textsubscript{3}O\textsubscript{7} [M+Na]\textsuperscript{+}: 536.2, found: 536.2 [M+Na]\textsuperscript{+}

[\alpha]\textsubscript{D}\textsuperscript{25} (C=1, MeOH) : -9.7

HPLC T\textsubscript{R} = 17.8 min
Figure 3: HPLC trace at 210nm of the purified 10' monomer.

Figure 4: 1H NMR of 10' monomer.

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

11' was prepared following the general procedure described above. 11' (2.2 g, 80%).

1H NMR (500MHz, DMSO-d6): δ (two major isomers)= 7.53, 7.47 (d, J = 8.0Hz, J = 7.0Hz, 1H, NHα) ; 7.41-7.26 (m, 10H, 10xCHar(Z)) ; 7.23 (m, 1H, NHε) ; 6.84, 6.70 (m, 1H, NH(Boc)) ; 5.04, 5.01 (s, 2H, CH2(Z)) ; 4.99 (s, 2H, CH2(Z)) ; 4.49-4.38 (m, 1H, CHα) ; 4.14 (d, J = 17.0Hz, 1H, CHβ(1)) ; 3.87 (d, J = 17.0Hz, 1H, CHβ(1)) ; 3.64, 3.60 (s, 3H, OCH3) ; 3.49-3.28 (m, 2H, CH2(2)) ; 3.40-2.89 (m, 4H, CH2(3)+CH2ε) ; 1.61-1.45 (m, 2H, CH2β) ; 1.45-1.24 (m, 4H, CH2γ CH2δ) ; 1.35 (s, 9H, 3xCH3(Boc)) ; 13C NMR (50MHz, DMSO-d6): δ (two major isomers) = 172.75, 172.39 (1C, N-CO) ; 171.15, 169.71 (1C, COOMe) ; 156.09, 155.94 (1C, CO(Z)) ; 155.65 (1C, CO(Boc)) ; 137.27, 137.02, 136.97 (2C, Cβ(Z)) ; 128.35, 127.75, 127.70 (10C, CHar(Z)) ; 77.88, 77.73 (1C, Cβ(Boc)) ; 65.50, 65.40, 65.16 (1C, CH2(Z)) ; 52.08, 51.69 (1C, OCH3) ; 50.56 (1C, CHα) ; 47.81, 47.55 (2C, CH2(1)+CH2(Z)) ; 39.30, 38.50 (1C, CH2(3)) ; 31.06 (1C, CH2β) ; 29.20 (1C, CH2γ) ; 28.15 (3C, 3xCH3(Boc)) ; 22.54 (1C, CH2δ) ; CH2ε was not located because it was under DMSO signals.


[α]D25 (C=1, MeOH) : -10.3

HPLC T_R = 18.1 min
**Figure 5:** HPLC trace at 210 nm 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%).

$^{13}$C NMR (125 MHz, DMSO-$d_6$): δ (two major isomers) = 172.41, 171.98 (1C, CO(Arg)); 169.305, 168.77 (1C, CO(All)); 162.87 (1C, CO($Z_6$)); 159.68 (1C, CO($Z_8$)); 155.85, 155.60 (1C, CO(All)); 154.96 (1C, CO($Z_2$)$_{\omega}$); 137.10, 136.93 (1C, C$_{IV}$(Boc)); 135.25 (3C, C$_{IV}$(Z)); 132.24, 132.09 (1C, CH(5)); 128.47, 128.27, 128.21, 127.89, 127.79, 127.73, 127.65, 127.62, 127.58 (15C, 15xCH(Z)); 118.01, 117.72 (1C, CH$_2$(6)); 77.82, 77.65 (1C, C$_{IV}$(Boc)); 68.17 (1C, CH$_2$(Z)$_{\omega}$); 66.15 (1C, CH$_2$(Z)$_{\omega}$); 65.41 (1C, CH$_2$(Z)$_{\omega}$); 64.73 (1C, CH$_2$(4)); 50.41,
50.27 (1C, CHα) ; 49.46 (1C, CH(1)) ; 47.94, 47.50 (1C, CH(2)) ; 44.30 (1C, CHδ) ; 38.60, 37.63 (1C, CH(3)) ; 28.89, 28.76 (1C, CHβ) ; 28.16, 28.08 (3C, CH3(Boc)) ; 24.85, 24.78 (1C, CHγ) ;

**MS (ESI+) (m/z) :** calc'd for C42H52N6O11 [M+Na]+: 839.4, found: 839.4 [M+Na]+

\[\alpha\]D25 (C=1, MeOH) : -5.2

**HPLC** TR = 21.6 min

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

**Figure 8:** 1H 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 KHSO₄ solution and the solvent was concentrated under reduced pressure. The remaining aqueous layer was acidified with a 1M KHSO₄ solution until pH = 2, then extracted with EtOAc (2 x 40 mL). The combined organic layers were washed with H₂O and brine, dried on MgSO₄ 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%).

**Rf** (EtOAc/MeOH, 8:2, v:v) = 0.41

1H NMR (500MHz, DMSO-d6) : 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, 5xCHar(Z)); 6.81, 6.69 (t, J = 5.3Hz, J = 5.3Hz, 1H, NH(Boc)); 5.07-4.94 (m, 2H, CH2(Z)); 4.58-4.48, 4.33-4.26 (m, 1H, CHα); 4.36, 4.07 (d, J = 17.5Hz, 1H, CH2(1)); 4.04, 3.78 (d, J = 17.5Hz, 1H, CH2(1)); 3.47-2.95 (m, 4H, CH2(2)+CH2(3)); 1.36 (s, 9H, 3xCH3(Boc)); 1.20, 1.14 (d, J = 6.5Hz, 3H, CH3(Ala)).

13C NMR (125MHz, DMSO-d6) : δ (two major isomers) 173.32, 173.12 (1C, CO(Ala)); 171.52, 170.98 (1C, COOH); 155.94, 155.90 (1C, CO(Z)); 155.82 (1C, CO(Boc)); 137.34, 137.29 (1C, Cq(Z)); 128.66, 128.09, 128.03 (5C, 5xCHar(Z)); 78.17, 77.98 (1C, Cq(Boc)); 65.46, 65.35 (1C, CH3(Z)); 49.61, 47.67, 47.35, 46.70 (2C, CH2(1)+CH2(2)); 46.31, 46.02 (1C, CHα); 38.46, 37.60 (1C, CH2(3)); 28.22, 28.16 (3C, 3xCH3(Boc)); 17.74, 17.70 (1C, CH3(Ala)).

MS (ESI-) (m/z): calcld for C20H29N3O7 [M-H]- : 422.2, found: 421.9 [M-H]

[α]D = -19.4

**HPLC** T_R = 11.8 min

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

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

Boc(Z-Phe)OH (10)

10 was prepared following the general procedure described above. 10 (0.98 g, 85%).
$R_t$ (EtOAc/MeOH, 8:2, v:v) = 0.4

$^1$H NMR (500MHz, DMSO-$d_6$) : $\delta$ (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$_2$(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, CH$\alpha$) ; 4.43, 4.06, (d, J = 18.0Hz, J = 17.0Hz, 1H, CH$_2$(1)) ; 3.87-2.73 (m, 1H, CH$_\beta$) ; 1.37 (s, 9H, 3xCH$_3$(Boc)) ; 

$^{13}$C NMR (125MHz, DMSO-$d_6$) : $\delta$ (two major isomers) 172.19, 172.12 (1C, CO(Phe)) ; 171.34, 170.60 (1C, COOH) ; 155.80, 155.77 (1C, CO(Z)) ; 155.70, 155.58 (1C, CO(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, 5xCH(Z)+5xCH(Ph)) ; 77.90, 77.69 (1C, C$_{IV}$(Boc)) ; 65.28, 65.22 (1C, CH$_2$(Z)) ; 52.37, 52.11 (1C, CH$_\alpha$) ; 49.83, 47.87 (1C, CH$_2$(1)) ; 47.59, 46.87 (1C, CH$_2$(2)) ; 38.42, 37.67 (1C, CH$_2$(3)) ; 37.10, 37.07 (1C, CH$_2$(1)) ; 28.22, 28.15 (3C, 3xCH$_3$(Boc)) ;

MS (ESI-) (m/z): calcd for C$_{26}$H$_{33}$N$_3$O$_7$ [M-H]$^-$: 498.2, found: 497.9 [M-H]$^-$

$[\alpha]$$^2$$^5_D$ (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%).

\[ R_f (\text{EtOAc/MeOH}, 8:2, v:v) = 0.48 \]

\[^1H\text{ NMR (500MHz, DMSO-}d_6): \delta \text{ (two major isomers) 12.77 (br s, 1H, OH) ; 7.44, 7.43 (d, J = 9.6Hz, J = 8.3Hz, 1H, NH}^{\alpha}(Z))) ; 7.39-7.28 (m, 10H, 10xCH}_2(Z)) ; 7.21 (t, J = 5.5Hz, 1H, NH}^\beta(Z)) ; 5.70-4.95 (m, 4H, 2xCH}_2(Z)) ; 4.46-4.39, 4.22-4.15 (m, 1H, CH\alpha(Z)) ; 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}_2(\varepsilon)) ; 1.60-1.47 (m, 2H, CH}_2(\beta)) ; 1.45-1.29 (m, 4H, CH}_2(\gamma)) ; 1.36 (s, 9H, 3xCH}_3(Boc)) ;

\[^{13}C\text{ NMR (125MHz, DMSO-}d_6): \delta \text{ (two major isomers) 172.50, 172.38 (1C, CO(Lys))) ; 171.22, 170.67 (1C, COOH) ; 156.07, 155.93 (2C, 2xCO(Z)) ; 155.63, 155.55 (1C, CO(Boc)) ; 137.27, 137.25 (1C, CIV(Z\alpha)) ; 137.02, 136.98 (1C, CIV(Z\varepsilon)) ; 128.33, 127.76, 127.73, 127.67, 127.65 (10C, 5xCH}_2(Z\alpha)+5xCH}_2(Z\varepsilon)) ; 77.84, 77.64 (1C, CIV(Boc)) ; 65.47, 65.37 (2C, CH}_2(Z)) ; 65.13, 65.10 (1C, CH\varepsilon(Z)) ; 50.64, 50.58 (1C, CH\alpha(Z)) ; 47.73, 47.42, 46.68 (2C, CH}_2(1)+CH}_2(2)) ; 38.50, 37.64 (1C, CH}_2(3)) ; 31.09 (1C, CH}_2(\beta)) ; 29.15 (1C, CH}_2(\gamma)) ; 28.22, 28.15 (3C, 3xCH}_3(Boc)) ; 22.56 (1C, CH}_2(\delta)) ; \text{MS (ESI-)} (m/z): \text{calcd for } C_{31}H_{42}N_4O_9 [M-H]: 613.3, \text{found: 613.0 [M-H]}\]

\[^{1}D\text{ (C=1, MeOH) : -9.7}\]

\[ \text{HPLC } T_R = 16.4 \text{ min} \]

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

**Rf** (EtOAc/MeOH, 8:2, v:v) = 0.51

**1H NMR** (500MHz, DMSO-<d6>): δ (two major isomers) 9.20 (br s, 2H, (Z)NH-C=NH) ; 7.50-7.20 (m, 16H, 15CH<ar> + NH<α>(Z)) ; 6.99, 6.95 (br s, 1H, NH(Boc)) ; 5.21 (s, 2H, CH<ω>(Z)) ; 5.04 (s, 2H, CH<ω>'(Z)) ; 4.99, 4.98 (s, 2H, CH<ω>'(Z)) ; 4.50-4.39, 4.30-4.21 (m, 1H, CH<α>) ; 4.08-2.90 (m, 8H, CH<2>(1)+CH<2>(2)+CH<2>(3)) ; 1.76-1.42 (m, 4H, CH<β> + CH<γ>) ; 1.33 (s, 9H, 3xCH<3>(Boc)) ;

**13C NMR** (125MHz, DMSO-<d6>): δ (two major isomers) = 172.14 (1C, CO(Arg)) ; 171.91, 171.68 (1C, COOH) ; 162.91 (1C, CO(Z)) ; 159.79, 159.69 (1C, CO(Z)) ; 155.79, 155.58 (1C, CO(Boc)) ; 154.96, 154.86 (1C, CO(Z)) ; 137.10, 136.97 (1C, C<γ>(Z)) ; 135.43, 135.30 (3C, C<γ>(Z)) ; 128.51, 128.31, 128.23, 128.20, 127.94, 127.85, 127.73, 127.65, 127.60 (15C, 15xCH<ar>(Z)) ; 77.74, 77.43 (1C, C<γ>(Boc)) ; 68.19, 68.11 (1C, CH<ω>′(Z)) ; 66.16 (1C, CH<ω>′(Z)) ; 65.43 (1C, CH<ω>'(Z)) ; 51.94, 48.75 (1C, CH<α>) ; 47.47, 47.05 (1C, CH<β>) ; 44.57, 44.40 (1C, CH<δ>) ; 38.33, 37.59 (1C, CH<γ>(Z)) ; 29.05, 28.75 (1C, CH<β>) ; 28.22, 28.12 (3C, 3xCH<3>(Boc)) ; 24.91, 24.73 (1C, CH<γ>);

**MS (ESI)** (m/z) : calcd for C<39>H<48>N<6>O<11>[M-H]: 775.3, found: 775.1 [M-H]<sup>-</sup>

[a] <sub>D</sub> <sup>25</sup> (C=1, MeOH) : +1.0

HPLC T<sub>R</sub> = 16.4 min
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:** Ac₂O/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 ¹³C 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) 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 210 nm

$^1$H NMR (500 MHz, D$_2$O): (two major isomers) $\delta$ = 7.50-7.30 (m, 5H, CH$_\alpha$); 4.76-4.00 (m, 12H, CH$_\alpha$ Phe, CH$_\alpha$ Ala, CH$_\alpha$ Lys, CH$_\alpha$ Arg, 4 x CH$_2$(1)); 3.80-3.10 (m, 22H, 4 x CH$_2$(2), 4 x CH$_2$(3), CH$_3$(5), CH$_2$ Arg, CH$_2$ Phe); 3.09-2.98 (m, 2H, CH$_2$ Lys); 2.56-2.48 (m, 2H, CH$_2$(4)); 2.02-1.65, (m, 11H, CH$_2$(Ac), CH$_2$ Lys, CH$_2$ Arg, CH$_2$Arg, CH$_2$ Lys); 1.60-1.45, (m, 5H, CH$_2$ Ala, CH$_2$Lys).

$^{13}$C NMR (125 MHz, D$_2$O): (two major isomers) $\delta$ = 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
**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<sub>R</sub> = 19.7 min

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**Figure 19:** $^1$H NMR 500MHz of 1

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

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**$^1$H NMR** (500 MHz, D$_2$O): (two major isomers) δ= 7.50-7.30 (m, 5H, CH$_\alpha$); 4.72-4.00 (m, 12H, CH$_\alpha$ Phe, CH$_\alpha$ Ala, CH$_\alpha$ Lys, CH$_\alpha$ 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$_2$δ Arg, CH$_2$β Phe); 3.10-3.00 (m, 2H, CH$_2$γ Lys); 2.60-2.49 (m, 2H, CH$_2$(4)); 2.05-1.82, (m, 7H, CH$_3$(Ac), CH$_3$β Lys, CH$_3$β Arg); 1.82-1.62 (m, 4H, CH$_2$γ Arg, CH$_2$δ Lys); 1.62-1.45 (m, 5H, CH$_3$ Ala, CH$_2$γ Lys).

**$^{13}$C NMR** (125 MHz, D$_2$O): (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, 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, 24.41, 23.81, 22.25, 21.37, 21.28, 16.34, 16.26.

**HRMS** m/z calcld for C$_{45}$H$_{80}$N$_{18}$O$_{10}$ (M+H)<sup>+</sup>: 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

**H NMR** (500 MHz, D$_2$O): (two major isomers) δ= 7.60-7.27 (m, 5H, CH$_\text{ar}$); 4.74-3.90 (m, 12H, CH$_\alpha$ Phe, CH$_\alpha$ Ala, CH$_\alpha$ Lys, CH$_\alpha$ 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$_3$δ Arg, CH$_3$β Phe); 3.16-3.05 (m, 2H, CH$_2$ε Lys); 2.65-2.55 (m, 2H, CH$_2$(4)); 2.10-1.89, (m, 7H, CH$_1$(Ac), CH$_3$β Lys, CH$_3$β Arg); 1.88-1.68 (m, 4H, CH$_2$γ Arg, CH$_3$δ Lys); 1.66-1.50, (m, 5H, CH$_3$ Ala, CH$_2$γ Lys).

**13C NMR** (125 MHz, D$_2$O): (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, 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
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

**1H NMR** (500 MHz, D$_2$O): (two major isomers) $\delta = 7.50$-7.30 (m, 5H, CH$_a$); 4.79-3.60 (m, 12H, CH$_a$Phe, CH$_a$Ala, CH$_a$Lys, CH$_a$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$_2$Arg, CH$_2$Phe); 3.10-2.96 (m, 2H, CH$_2$εLys); 2.57-2.46 (m, 2H, CH$_2$(4)); 2.04-1.83 (m, 7H, CH$_3$(Ac), CH$_2$βLys, CH$_2$βArg); 1.82-1.62 (m, 4H, CH$_2$γArg, CH$_2$δLys); 1.60-1.44 (m, 5H, CH$_3$Ala, CH$_2$γLys).

**13C NMR** (125 MHz, D$_2$O): (two major isomers) $\delta =$ 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

**H NMR** (500 MHz, D<sub>2</sub>O): (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<sub>2</sub>(1)) ; 3.80-3.30 (m, 18H, 4 x CH<sub>2</sub>(2), 4 x CH<sub>2</sub>(3), CH<sub>2</sub>(5)) ; 3.10-2.98 (m, 8H, CH<sub>2</sub> Lys) ; 2.58-2.48 (m, 2H, CH<sub>2</sub>(4)) ; 2.06-1.82 (m, 11H, CH<sub>3</sub>(Ac), 4 x CH<sub>2</sub>β Lys) 1.80-1.66, (m, 8H, 4 x CH<sub>2</sub>δ Lys) ; 1.62-1.40, (m, 8H, 4 x CH<sub>2</sub>γ Lys).

**13C NMR** (125 MHz, D<sub>2</sub>O): (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<sub>45</sub>H<sub>90</sub>N<sub>18</sub>O<sub>10</sub> (M+H)<sup>+</sup>; 1043.71656, found: 1043.71750

Figure 30: HRMS mass spectrum 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**

**1H NMR** (500 MHz, D$_2$O): (two major isomers) δ 4.66-4.48, 4.43-4.36 (m, 4H, 4 x CH$_2$ 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$_2$ Arg) ; 2.56-2.48 (m, 2H, CH$_2$ (4)) ; 2.06-1.82 (m, 11H, CH$_3$ (Ac), 4 x CH$_2$β Arg) ; 1.81-1.62 (m, 8H, 4 x CH$_2$γ Arg).

**13C NMR** (125 MHz, D$_2$O): (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

![HPLC profile of 7 at 210nm](image)

$^1$H NMR (500 MHz, D$_2$O): (two major isomers) $\delta = 4.64-4.42, 4.37-4.32$ (m, 4H, 4 x CH$_\alpha$ 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$_2$O): (two major isomers) $\delta = 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

![HRMS mass spectrum of 7](image)
**Figure 37**: $^1$H NMR 500MHz of 7

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

**FFF (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

$^1$H NMR (500 MHz, D$_2$O): (two major isomers) $\delta = 7.50-7.20$ (m, 20H, 4 x 5CH$_2$) ; 4.76-4.60, 4.58-4.47 (m, 4H, 4 x CH$_2$ 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 CH$_2$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$_2$O): (two major isomers) $\delta = 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 calc'd 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](image)

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 42: HRMS mass spectrum of 1’](image)
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 base².

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 MgCl₂).

For competitive experiments in the presence of a dsDNA, 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 tRNA, a mixture of pre- and mature yeast tRNAs (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 tRNA were prepared in water and quantified using an extinction coefficient of 9640 cm⁻¹ M⁻¹ per base².

CD experiments were performed in buffer B (20 mM potassium phosphate buffer (pH 7.4 at 25 °C), 10 mM NaCl and 1 mM MgCl₂).

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).

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 MgCl₂ 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](image)

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 values³.

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.

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. Unless otherwise stated, binding profiles were well modeled using a simple model assuming the one to one stoichiometry. \( \Delta G^o \) values were converted to \( K_d \) values as

\[
K_d = e^{(\Delta G^o / RT)}
\]

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

\[
\Delta G^o_T = \Delta H^o_T + \Delta C_P (T - T_r) - T \Delta S^o_T - T \Delta C_P \ln (T/T_r)
\]

where \( T_r \) is a constant reference temperature (in our study \( T_r = 293.15 \) K), and the three fit parameters are \( \Delta H^o_T \), the change in enthalpy upon binding at \( T_r \); \( \Delta S^o_T \), the change in entropy upon binding at \( T_r \); and \( \Delta C_P \), the change in heat capacity. Starting values for the three parameters did not affect the final values. \( \Delta C_P \) 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. \( \Delta H^o_T \) and \( \Delta S^o_T \) were calculated by using:

\[
\Delta H^o_T = \Delta H^o_{T_r} + \Delta C_P (T - T_r)
\]

and

\[
\Delta S^o_T = \Delta S^o_{T_r} + \Delta C_P \ln (T/T_r).
\]

Salt dependence of \( K_d \) was analyzed by the following equation (4)(figure 56):

\[
\log(K_d) = \log(K_{n_{el}}) - Z \psi \log [KCl]
\]

where \( K_{n_{el}} \) 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 \( \psi \) is the fractional probability of a counterion being thermodynamically associated with each phosphate of the RNA number of cations. \( K_{n_{el}} \) and \( Z \psi \) were treated as fitting parameters.

A higher initial fluorescence value is observed in the presence of dsDNA and tRNA which is consistent with the modification of the polarity of the solvent and a small fluorescence of the tRNA mixture.

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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
Figure 49. CD profiles of PAA 1-8

TAR alone
TAR : PAA (1 : 1)
TAR : PAA (1 : 10)
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 S51. Binding curves of PAA 1-7 in the presence of dsDNA.

The profiles were obtained in 20 mM HEPES (pH 7.4), 20 mM NaCl, 140 mM KCl and 3 mM MgCl₂ 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 tRNA

The profiles were obtained in 20 mM HEPES (pH 7.4), 20 mM NaCl, 140 mM KCl and 3 mM MgCl₂ at 25 °C in the presence of 5 nM of labeled TAR RNA and tRNA.

Solid line represents the BIOEQS fitting for 1 : 1 interaction
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.
\[ \Delta G_T = \Delta H_{Tr} + \Delta C_P (T - T_{Tr}) - T \Delta S_{Tr} - T \Delta C_P \ln \left( \frac{T}{T_{Tr}} \right) \]

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<th>RFAK (2)</th>
<th>KARF (3)</th>
<th>AKFR (4)</th>
<th>KKKK (5)</th>
<th>RRRR (6)</th>
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**Figure 55.** Temperature effect for PAA 1-8
Figure 56. Salt effect for PAA 1-8

FRKA (1): $y = 2.106 \pm 0.1709 \times - 4.578 \pm 0.1709$  \hspace{1cm} R² = 0.9870
RFAK (2): $y = 1.930 \pm 0.1794 \times - 4.582 \pm 0.1600$  \hspace{1cm} R² = 0.9830
KARF (3): $y = 2.031 \pm 0.09434 \times - 4.512 \pm 0.08418$  \hspace{1cm} R² = 0.9957
AKFR (4): $y = 1.455 \pm 0.1829 \times - 5.047 \pm 0.1734$  \hspace{1cm} R² = 0.9844
AAAA (7): $y = 2.051 \pm 0.04406 \times - 1.480 \pm 0.04177$  \hspace{1cm} R² = 0.9995
KKKK (5): $y = 2.629 \pm 0.2514 \times - 3.446 \pm 0.2244$  \hspace{1cm} R² = 0.9820
RRRR (6): $y = 2.891 \pm 0.5488 \times - 4.221 \pm 0.4897$  \hspace{1cm} R² = 0.9328