Supplementary data

Cyclic PNA-based compound directed against HIV-1 TAR RNA : Modelling, liquid-phase synthesis and TAR binding

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General.

Unless otherwise stated, all reagents were obtained from commercial suppliers and used without further purification. All solvents were freshly distilled. The following abbreviations are employed : allyloxycarbonyl (Alloc); benzyl (Bn); benzyloxy (BnO); benzyloxycarbonyl (Z); tertio-butyloxycarbonyl (Boc); di-ter-butyldicarbonate (Boc_2O); benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium-hexafluorophosphate (Bop); Bromo-tris-(dimethylamino)-phosphonium-hexafluorophosphate (Brop); N,N'-dicyclohexylcarbodiimide (DCC); diethylamine (DEA); diisopropylethylamine (DIEA); dimethylformamide (DMF); O-(7-Azabenzotriazol-1-yl)-N,N,N',N'-tetramethyl-uronium hexafluorophosphate (HATU); 1-hydroxy-7-azabenzo-triazole (HOAt); N-hydroxy succinimide (HOSu); Methoxytrityl chloride (Mmt-Cl); N-methylmorpholine (NMM); tetrakis(triphenylphosphine)-palladium(0) ($Pd[P(Ph)_3]_4$); triethyl amine (TEA); trifluoroacetic acid (TFA); triisopropylsilane (TIS); TLC were performed on 0.25-mm-thick silica gel plates (Merck, silica gel 60F254). Columns of chromatography were performed using Merck silica gel 60 (230-400 mesh ASTM) and on Sephadex (Sigma, LH20, 25-100 μm). Analytical HPLC chromatograms were obtained

using a WATERS 600 equipped with a 996 Photodiode Array Detector (PDA, UV detector from 195 to 290nm) and a column (250*4mm) packed with Lichrospher 100-RP-18 (5µm). A gradient with water (0.1% TFA) as solvent A and acetonitrile (0.1% TFA) as solvent B was used with a flow=1mL/min. In these slight acidic conditions, some of the N-Mmt amine compounds were deprotected during their HPLC analysis. In these cases, the retention time corresponds to the Mmt-deprotected compound. For semi-preparative HPLC purifications, a WATERS 600 equipped with a 2487 Dual λ Absorbance detector was used with a column (250*10mm) packed with Lichrospher 100-RP-18 (5µm) with a flow=2mL/min. ^{1}H (at 200 MHz) and ^{13}C (at 50.3 MHz) NMR spectra were recorded on a Bruker AC 200 Fourier Transform spectrometer and the chemical shifts δ are given in ppm. The NMR spectra of some compounds displayed a doubling of signals caused by the presence of an equilibrium mixture of the E and Z isomers generated by the substituted amide bond. The minor form was indicated in *italic*. In the case of polymers "Nmers", the putative 2^N isomers could not be differentiated, the corresponding broad signals were then designated by the shift displacements of the beginning and the end of the signal. Mass spectrometry analyses were carried out on a INCOS 500^E FINNIGAN MAT (EI, CI), on a TSQ 7000 FINNIGAN MAT (ESI) and performed by J.M. Guigonis of GUMPAC-Nice (France). MALDI-TOF spectra (Voyager DE perspective biosystems, N2 Laser 337nm) were kindly performed by J.M. Guigonis and Dr J.J. Vasseur of Laboratoire de Chimie Organique Biomoléculaire de Synthèse (UMR CNRS-UM II) of Montpellier (France).

The synthesis of backbones **8**, **13** and **37**, of base acetic acid units **14**, **20**, **23** and **11** and of triAlloc fragments **6** and **32** have been previously described.[12, 13b]

Chemistry.

Synthesis of cyclic hexaPNA 1:

Boc-NH(CH_2)$_5$CO-[H]-OMe (10): A solution of N-ε-Boc-ε-amicaproic acid **9** (1.0 g, 4.33 mmol), HOSu (747 mg, 6.60 mmol) and DCC (982 mg, 4.76 mmol) in DMF (10 mL) was stirred 12 hours at rt. After cooling to −15°C, compound **8** (976 mg, 4.76 mmol) and NMM (954 µL, 8.66 mmol) were added. The mixture was stirred at −15°C for 3h then allowed to warm to rt (ca 2 h). The precipitated dicyclohexylurea (DCU) was

filtered off on celite, washed with EtOAc and the filtrate were concentrated under reduced pressure. The residue was taken up in an aqueous (1M) $KHSO_4$ solution. The acidic solution was washed with EtOAc. The pH was adjusted to 8-9 with an aqueous 10% $NaHCO_3$ solution and the aqueous layer was extracted with EtOAc. The organic layers were washed with water, brine and dried over $MgSO_4$. The solvent was removed *in vacuo* and the residue was purified by silica gel column chromatography (EtOAc/MeOH 8:2 v:v) to yield compound **10** as an amorphous solid (1.30 g, 87%). TLC (EtOAc/MeOH 8:2 v:v): Rf =0.25. HPLC (A/B 80:20 to 30:70 over 30 min): Rt = 10.7 min. MS (ESI+) m/z 346.3 $(M+H)^+$; m/z 368.3 $(M+Na)^+$. ^{1}H NMR ($CDCl_3$) δ 7.20 (1H, t); 5.45 (1H, t); 3.70 (3H, s); 3.40 (2H, s); 3.30 (2H, q); 3.05 (2H, q); 2.75 (2H, t); 2.25 (1H, s); 2.20 (2H, t); 1.80-1.30 (6H, m); 1.40 (9H, s). ^{13}C NMR (DMSO d_6) δ 173.70; 173.15; 156.50; 77.85; 52.04; 50.65; 48.59; 40.47; 39.07; 36.53; 29.77; 26.42; 25.36; 28.54.

Boc-NH$(CH_2)_5$CO-[U]-OMe (12): To a cooled solution of the previous compound **10** (1.0 g, 2.90 mmol), uracil acetic acid **11** (543 mg, 3.19 mmol) and TEA (808.4 µL, 5.80 mmol) in CH_2Cl_2 (5 mL) was added Brop (1.24 g, 3.19 mmol). The mixture was stirred for two hours at rt. CH_2Cl_2 was evaporated and the crude residue was taken up in EtOAc. The organic layer was washed successively with a (1M) $KHSO_4$ solution, a 10% $NaHCO_3$ solution, brine and dried over $MgSO_4$. The solvent was evaporated *in vacuo*. The residue was purified by silica gel column chromatography (EtOAc/MeOH 9:1 v:v) to afford the methyl ester **12** as an amorphous solid (1.15g, 80%). TLC (EtOAc/MeOH 8:2 v:v): Rf=0.41. HPLC (A/B 80:20 to 0:100 over 30min): Rt=11.4min. MS (ESI+) m/z 498.2 $(M+H)^+$. ^{1}H NMR ($CDCl_3$) (two isomers) δ 7.40 (1H, d); 6.95-6.50 (1H, t); 5.65 (1H, d); 4.80 (1H, t); 4.55, *4.40* (2H, s); 4.20, *4.05* (2H, s); 3.75, *3.70* (3H, s); 3.60-3.20 (4H, m); 3.10 (2H, q); 2.20 (2H, t); 1.70-1.20 (15H, m). ^{13}C NMR (DMSO d_6) δ 173.90, 171.25, 170.43, 163.87, 157.51, 151.53, 146.11, 101.82, 79.03, 53.72, 52.51, 49.20, 48.81, 40.46, 37.29, 36.17, 29.69, 26.45, 25.22, 28.52.

Boc-NH$(CH_2)_5$CO-[U]-OH (5): The methyl ester **12** (1.65 g, 3.31 mmol) was dissolved in dioxane (50 mL) and 6.62 mL of aqueous (1N) LiOH was added at 0°C. The mixture was stirred for 1 hour, then slightly acidified with (1M) aqueous HCl until pH = 6. The solvent was evaporated *in vacuo* and the residue was purified on Sephadex (LH-20, MeOH) to yield **5** as an amorphous solid (1.60g, 100%). TLC (EtOAc/MeOH 1:1 v:v): Rf = 0.49. HPLC (A/B 80:20 to 0:100 over 30 min): Rt = 9.12 min, λ_{max} = 211 nm. MS (ESI-) m/z 482.3 $(M-H)^-$. ^{1}H NMR (DMSO d_6) (two isomers) δ 11.30 (1H, bs); 8.65 (1H, t); 7.50 (1H, d); 6.85 (1H, t);

5.60 (1H, d); 4.65, *4.55* (2H, s); 3.75, *3.70* (2H, s); 3.60-3.10 (4H, m); 3.00 (2H, q); 2.15 (2H, t); 1.70-1.20 (15H, m). ^{13}C NMR (DMSO d_6) (two isomers) δ 172.22, *171.67*, 171.82, *171.23*, 167.12, *165.74*, 163.59, 155.24, 150.75, 146.33, 100.71, 76.96, 55.81, 52.18, 47.63, 39.36, 36.02, 35.09, 28.91, 25.64, 24.52, 27.91.

Mmt-[Ad^Z]-OH (15): Compound **13** (625 mg, 1.55 mmol), Z-Ad-OH **14** (505 mg, 1.55 mmol) and DIEA (747.3 µL, 4.65 mmol) were mixed in DMF (4 mL) at 0°C. Then Brop (661.7 mg, 1.705 mmol) was added and the mixture was stirred at rt for 2 hours. DMF was evaporated and the crude product was suspended in $CHCl_3$ and washed alternatively with a aqueous 10% $NaHCO_3$ solution and water. The organic layer was dried over $MgSO_4$. The residue was purified by silica gel column chromatography (gradient: EtOAc/hexane 7:3 to 100% EtOAc) to give the PNA methyl ester (820 mg, 74%) as pure product. TLC (EtOAc 100%): Rf = 0.71. MS (ESI+) m/z 714.6 $(M+H)^+$. HPLC (A/B 80:20 to 0:100 over 30min): Rt = 9.8 min (MMt cleaved product), λ_{max} = 209.7 nm, 268.6 nm. ^{1}H NMR ($CDCl_3$) (two isomers) δ 9.45 (1H, bs); 8.75, *8.65* (1H, 2s); 8.1, *8.05* (1H, 2s); 7.6-6.7 (19H, m); 5.5 (2H, s); 4.25, *3.95* (2H, 2s); 3.9 (2H, s); 3.8-3.5 (8H, m); 2.2 (2H, td). This PNA methyl ester (150 mg, 0.21 mmol) was suspended in dioxane (4mL) and a (1N) LiOH (0.84 mL, 0.84 mmol) was added dropwise. The mixture was stirred for 2 hours at rt and was then cooled down to 0°C. The excess of LiOH was neutralized with a aqueous (0.2N) $KHSO_4$ solution. This mixture was washed with $CHCl_3$ (20ml three times). The organic layer was dried over $MgSO_4$ and evaporated under reduced pressure to afford the corresponding acid **15** (138.4 mg, 94%) as crude product. TLC (EtOAc/MeOH 1:1 v:v): Rf = 0.48. MS (ESI-) m/z 698.1 $(M-H)^-$. HPLC (A/B 80:20 to 0:100 over 30min): Rt = 9.3 min (Mmt cleaved product), λ_{max} = 209.6 nm, 268.6 nm. ^{1}H NMR (DMF d_7) (two isomers) δ 8.65-8.40 (2H, 2s); 7.60-6.85 (19H, m); 5.70, *5.20* (2H, 2s); 4.10, *3.95* (2H, 2s); 3.85-3.40 (5H, m); 2.30 (2H, td).

Mmt-[Alloc]-OH (16): To a cold solution (0°C) of N-Mmt backbone methyl ester **13** (900 mg, 2.22 mmol) and DIEA (895 µL, 5.57 mmol) in CH_2Cl_2 (10 mL) were added dropwise Alloc-Cl (307 µL, 2.89 mmol) in CH_2Cl_2. The mixture was stirred at rt till completion (reaction was monitored by TLC EtOAc/Hex 1:1 v:v). The solvent was concentrated in vacuo and the crude residue was taken up in EtOAc. The organic layer was washed with water and then dried over $MgSO_4$. The residue was purified by silica gel column chromatography (gradient Hex 100% to EtOAc/Hex 1:1 v:v) to afford the corresponding ester Mmt[Alloc]OMe as a white resin (996 mg, 92%). TLC (EtOAc/Hex 1:1 v:v): Rf = 0.79. MS (ESI+) m/z 511.2 $(M+Na)^+$. ^{1}H NMR ($CDCl_3$) δ

7.50-6.80 (14H, m); 5.80 (1H, m); 5.30-5.10 (2H, m); 4.60 (2H, d); 4.00 (2H, s); 3.80 (3H, s); 3.70 (3H, s); 3.40 (2H, t); 2.30 (2H, td); 1.80 (1H, bs). ^{13}C NMR ($CDCl_3$) (two isomers) δ 171.05, *171.52*, 156.05, 132.68, 117.75, *117.32*, 79.21, 60.35, 52.23, 49.87, *49.66*, 48.98, *48.62*, 39.20, 28.44. This compound (930mg, 1.9mmol) was dissolved in a 0.8M $CaCl_2$ solution of isopropanol/water (20 mL 7:3 v:v). An aqueous solution of (1N) liOH (4.75 mL, 4.75 mmol) was then added at rt. After 6 hours of stirring, the mixture was diluted with ice-cold water and then cautiously acidified until pH 5 using an aqueous solution of 5% citric acid. The aqueous layer was extracted with EtOAc and the organic solution was washed with water then dried over Na_2SO_4. The solvent was evaporated in vacuo. The residue was purified by silica gel column chromatography (gradient EtOAc 100% to MeOH 100%) to afford the corresponding acid **16** as a white resin (830 mg, 92%). TLC (EtOAc): Rf=0.17. MS (ESI-) m/z 473.3 $(M-H)^-$. ^1H NMR ($CDCl_3$) δ 7.60-7.05 (12H, m); 7.0-6.65 (2H, m); 6.15-5.70 (1H, m); 5.50-5.05 (2H, m); 4.65-4.40 (2H, d); 4.05-3.20 (7H, m); 2.90-2.60 (3H, bs).

TFA.H-[Alloc]-NH$(CH_2)_5CO_2$tBu (18): A mixture of Mmt-(Alloc)-OH **16** (820 mg, 1.73 mmol) and $H_2N(CH_2)_5CO_2$tBu **17** (355 mg, 1.9 mmol) in DMF (5 mL) was cooled down to 0°C. DIEA (1.4 mL, 8.64 mmol) and Bop (918 mg, 2.07 mmol) were then added. The mixture was stirred at 0°C for 5min and was allowed to warm to rt. After 2 hours, DMF was evaporated off and EtOAc (20 mL) was added. The organic layer was washed with a aqueous 10% $NaHCO_3$ solution, with water and dried over $MgSO_4$. The solvent was evaporated *in vacuo* and the residue was purified by silica gel column chromatography (gradient: EtOAc/hexane 3:7 to 7:3, v:v) to afford the ester Mmt-[Alloc]-NH$(CH_2)_5CO_2$tBu (820 mg, 74%) as an amorphous solid. TLC (EtOAc/Hex 1:1, v:v): Rf = 0.42. ^1H NMR ($CDCl_3$) δ 7.65-6.85 (14H, m); 6.40 (1H, bs); 5.90 (1H, m); 5.35-5.20 (2H, m); 4.60 (2H, d); 3.90 (2H, s); 3.85 (3H, s); 3.55-3.45 (2H, m); 3.15 (2H, t); 2.50 (3H, m); 2.20 (2H, t); 1.55-1.20 (15H, m). ^{13}C NMR ($CDCl_3$) δ 173.11; 169.39; 158.21; 156.90; 146.00; 138.87; 132.57; 129.89; 128.60; 128.05; 126.61; 118.13; 113.3; 80.19; 49.4; 42.5; 39.29; 35.54; 29.44; 28.29; 26.46; 24.80. MS (ESI+) m/z 644.7 $(M+H)^+$. This ester Mmt-[Alloc]-NH$(CH_2)_5CO_2$tBu (750 mg, 1.16 mmol) and TIS (41μL, 0.2 mmol) were stirred at rt in a solution of 1% TFA in CH_2Cl_2 for two hours. The N-deprotection was monitored by TLC and after completion the solvent was evaporated to dryness *in vacuo*. The residue was purified by flash chromatography (gradient: EtOAc 100% to MeOH 100%) to afford the corresponding TFA salt **18** (472mg, 83%). TLC (EtOAc/Hex 1:1, v:v): Rf = 0.11. HPLC (A/B 80:20 to 0:100 over 30min): Rt = 14.2 min. MS (ESI+) m/z 372.4 $(M+H)^+$. ^1H NMR ($CDCl_3$) δ 8.40-8.25 (3H, bs); 7.40-7.20

(1H, t); 5.75 (1H, m); 5.45-5.25 (2H, m); 4.65 (2H, d); 4.20-3.00 (8H, m); 2.10 (2H, t); 1.65-1.10 (15H, m). ^{13}C NMR ($CDCl_3$) δ 173.59; 171.26; 170.71; 162.59; 156.5; 131.94; 125.30; 128.60; 128.05; 126.61; 118.13; 113.3; 80.19; 49.4; 42.5; 39.29; 35.54; 29.44; 28.29; 26.46; 24.80.

Mmt-[A^Z, Alloc]-NH$(CH_2)_5CO_2$tBu (19): Compounds **15** (350 mg, 0.5 mmol), **18** (260 mg, 0.536 mmol) and DIEA (402 μL, 2.5 mmol) were mixed at 0°C in DMF (5 mL). Brop (233 mg, 0.6 mmol) was added and the mixture was stirred for 10 min at 0°C then two hours at rt. The reaction was monitored using TLC. After completion, the DMF was evaporated off and EtOAc (40ml) was added. The organic layer was washed with water, dried over $MgSO_4$ and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (gradient EtOAc/MeOH 8:2 to 1:1 v:v) to afford the tert-butyl ester **19** (378 mg, 72%) as an amorphous solid. TLC (EtOAc/MeOH 8:2 v:v): Rf = 0.81. HPLC (A/B 80:20 to 0:100 over 30min): Rt = 16.4 min. MS (ESI+) m/z 1075.98 $(M+Na)^+$. ^{1}H NMR ($CDCl_3$) δ 8.65 (1H, s); 8.20 (1H, s); 7.65-7.05 (19H, m); 6.50 (1H, bs); 5.95-5.50 (3H, m); 5.40-5.05 (4H, m); 4.50 (2H, m); 4.25-3.00 (15H, m); 2.60-2.45 (3H, m); 2.05 (2H, m); 1.60-1.20 (15H, m). ^{13}C NMR ($CDCl_3$) δ 173.19; 171.32-167.03; 158.04; 156.52; 152.53; 151.71; 148.91; 145.80; 144.50; 137.64; 135.32; 132.42; 129.76; 128.56; 128.44; 128.01; 127.89; 126.47; 121.12; 117.54; 113.33; 80.19; 70.78; 67.83; 65.82; 55.21; 52.50; 51.02-48.05; 44.14; 42.12; 39.41; 35.23; 28.85; 28.11; 26.13; 24.40.

Mmt-[A^Z,G^{OBn}]-NH$(CH_2)_5CO_2$tBu (21): Compound **19** (375 mg, 0.356 mmol) and DEA (553 μL, 5.34 mmol) were dissolved in CH_2Cl_2 (4 mL) at rt. $Pd[P(Phe)_3]_4$ (41 mg, 36 μmol) was added. The mixture was stirred for 30 min at rt. The solvent was concentrated under reduced pressure and the crude residue was purified by silica gel column chromatography (gradient EtOAc/MeOH 8:2 to 1:1 v:v) to afford the corresponding secondary amine Mmt-[A^Z, H]-NH$(CH_2)_5CO_2$tBu (270.4 mg, 80%) as a slightly yellow resin. TLC (EtOAc/MeOH 8:2 v:v): Rf = 0.13. MS (ESI+) m/z 991.6 $(M+Na)^+$. ^{1}H NMR ($CDCl_3$) (two isomers) δ 8.65 (1H, s); 8.05 (1H, s); 7.65-6.75 (19H, m); 5.40, *5.25* (2H, 2s); 5.25, *5.05* (2H, 2s); 4.15, *3.95* (2H, 2s); 3.70-3.05 (13H, m); 2.50 (4H, m); 1.90 (2H, t); 1.65-1.05 (15H, m). ^{13}C NMR ($CDCl_3$) δ 173.29; 171.71; 168.55; 167.59; 167.26; 158.21; 152.71; 151.94; 149.62; 146.01; 144.11; 137.78; 135.69; 129.91; 128.71; 128.59; 128.10; 126.65; 121.59; 113.45; 80.27; 76.93; 55.35; 52.12; 50.83; 50.42; 49.06; 44.61; 44.39; 39.30; 38.98; 35.49; 29.29; 28.26; 26.43; 24.77.

Mmt-[A^Z,H]-NH$(CH_2)_5CO_2$tBu (205 mg, 0.212 mmol), G^{OBn}-OH **20** (76 mg, 0.254 mmol) and DIEA (170 µL, 1.6 mmol) were mixed at 0°C in DMF (1.2 mL). HOAt (52 mg, 0.381 mmol) and HATU (97 mg, 0.254 mmol) were added at 0°C and the mixture was then allowed to warm to rt (ca. 1 h). After completion the DMF was evaporated off and $CHCl_3$ (20 mL) was added. The organic layer was washed with a aqueous 10% $NaHCO_3$ solution, water and dried over $MgSO_4$. Solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (EtOAc/MeOH 8:2 v:v) to afford **21** (216.5 mg, 82%) as an amorphous solid. TLC (EtOAc/MeOH 7:3 v:v): Rf = 0.81. HPLC (A/B 80:20 to 0:100 over 30min): Rt = 18.3 min (Mmt cleaved product). MS (ESI+) m/z 1272.63 $(M+Na)^+$. ^{1}H NMR ($CDCl_3$) (two isomers) δ 8.65, *8.60* (1H, 2s); 8.55 (1H, bs); 8.05, *8.05* (1H, 2s); 7.70-6.70 (26H, m); 5.50-5.20 (6H, m); 4.85, *4.60* (2H, 2s); 4.10-3.05 (16H, m); 2.65-2.40 (3H, m); 2.05 (2H, m); 1.50-1.00 (15H, m). ^{13}C NMR ($CDCl_3$) δ 173.06-172.92; 170.16; 169.14-168.41; 168.25-168.11; 167.71-167.45; 160.87-160.65; 159.51-159.40; 158.07; 154.26-154.22; 152.56; 151.72-151.27; 149.48-149.25; 145.80; 145.03-144.88; 142.03; 137.60; 136.43-136.21; 135.80-135.55; 129.77; 128.46-128.04; 126.50; 121.39-120.82; 114.22; 113.35; 80.12; 70.82; 68.04-67.59; 67.39; 55.21; 50.95-42.33; 39.61-39.37; 35.28-35.10; 28.88-28.80; 28.08; 26.31-26.15; 24.55-24.37.

TFA.H-[A^Z, G^{OBn}]-NH$(CH_2)_5CO_2$tBu (7): Mmt-[A^Z, G^{OBn}]-NH$(CH_2)_5CO_2$tBu **21** (210 mg, 0.168 mmol) and TIS (10 µL, 50 µmol) were stirred at rt in 5 mL of a solution of CH_2Cl_2 containing 1% TFA for 20 min. The N-deprotection was monitored by TLC and after completion the solvent was evaporated to dryness *in vacuo*. The residue was precipitated by the addition of EtOAc/Et_2O (10 mL 2:8 v:v) to afford the corresponding trifluoacetate salt **7** (52 mg, 87%). TLC (EtOAc/MeOH 1:1 v:v): Rf = 0.15. HPLC (A/B 80:20 to 0:100 over 30min): Rt = 18.3min, λ_{max} = 212.1nm, 252.6 nm, 269.8 nm. MS (ESI+) m/z 1001.01 $(M+Na)^+$.

HCl.H-[Alloc]$_3$-OMe (6): Acetyl chloride (5.2 mL, 73 mmol) was added dropwise to 10 mLml of MeOH at 0°C, then Boc-[Alloc]$_3$-OMe[12, 13b] **32** (500 mg, 0.73 mmol) was introduced. The mixture was stirred one hour at rt. The solvent was removed under reduced pressure and the residue was precipitated in Et_2O yielding the corresponding hydrochloride salt **6** (431 mg, 95%) as a white powder. TLC (EtOAc/MeOH 1:1 v:v): Rf = 0.41. HPLC (A/B 80:20 to 0:100 over 30 min): Rt = 11.9min, λ_{max} = 204.8nm. MS (ESI+) m/z 585.3 $(M+H, ^{35}Cl)^+$. ^{1}H NMR ($CDCl_3$) δ 9.05- 8.05 (3H, m); 7.70-7.20 (2H, m); 5.90-5.75 (3H, m); 5.25 (6H, m); 4.65 (6H, m);

4.05-3.95 (6H, m); 3.70-3.25 (15H, m). ^{13}C NMR ($CDCl_3$) δ 171.83-171.07; 156.35-156.04; 132.62-132.33; 117.81-117.43; 66.60; 52.42; 52.34-49.32; 39.46-38.63.

Boc-NH$(CH_2)_5$CO-[U,$(Alloc)^3$]-OH (22): Boc-NH$(CH_2)_5$-CO-[U]-OH **5** (1.0 g, 2.07 mmol), HCl.H-$[Alloc]^3$-OMe **6** (1.28 g, 2.07 mmol) and TEA (865 μL, 6.21 mmol) were dissolved in DMF (5 mL) at 0°C. Bop (916 mg, 2.0 7mmol) was added and the mixture was stirred at rt for two hours. The solvent was removed *in vacuo* and the residue was taken up in EtOAc (20 mL). The organic layer was then washed successively with a (1M) $KHSO_4$ solution, an aqueous 10% $NaHCO_3$ solution, brine and finally dried ($MgSO_4$). The solvent was removed under reduced pressure and the crude residue was purified by silica gel column chromatography (from 100% EtOAc to EtOAc/MeOH 1:1 v:v) to afford Boc-NH$(CH_2)_5$CO-[U, $(Alloc)^3$]-OMe as a white powder (1.41 g, 65%). TLC (EtOAc/MeOH 6:4 v:v): Rf = 0.53. HPLC (A/B 80:20 to 0:100 over 30 min): Rt = 14.8 min, λ_{max} = 206.2nm, 261.2 nm. MS (ESI+) m/z 1073.2 $(M+Na)^+$. ^{1}H NMR ($CDCl_3$) δ 8.40- 7.90 (2H, m); 7.60-7.15 (3H, m); 6.80 (1H, t); 6.00-5.70 (3H, m); 5.65 (1H, d); 5.35-5.05 (6H, m); 4.75-4.40 (8H, m); 4.20-3.75 (8H, m); 3.70 (3H, s); 3.00 (2H, q); 2.10 (2H, t); 1.80-0.30 (15H, m). ^{13}C NMR ($CDCl_3$) δ 173.82, 171.23, 170.34, 168.59, 167.71, 164.33, 156.42, 156.25, 151.48, 145.98, 132.82, 132.74, 132.50, 117.86, 117.43, 101.91, 79.02, 66.50, 53.65, 53.28, 51.64, 52.48, 49.14, 48.79, 40.46, 38.29, 37.48, 36.38, 36.00, 33.85, 29.69, 26.45, 25.02, 28.52. This compound (243 mg, 0.23 mmol) was dissolved in THF (2 mL) and 0.46 mL of aqueous (1N) LiOH was added at 0°C. The mixture was stirred for 1 hour at rt, then slightly acidified with aqueous (1M) HCl. The solvent was evaporated *in vacuo* and the residue was taken up in aqueous 10% $NaHCO_3$ solution (20 mL). The basic layer was washed with EtOAc and then adjusted to pH 3-4 with a (1M) $KHSO_4$ aqueous solution. The acidic phase was then extracted by EtOAc. The organic layers were washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography (from 100% EtOAc to EtOAc/MeOH 1:1 v:v) to afford the acid **22** (174 mg, 73%) as a white powder. TLC (MeOH 100%): Rf = 0.55. HPLC (A/B 80:20 to 0:100 over 30 min): Rt = 13.5 min, λ_{max} = 209.2 nm, 261.4 nm. MS (ESI-) m/z 1073.2 $(M-H)^-$. ^{1}H NMR ($CDCl_3$) δ 9.05-8.05 (2H, bs); 7.45-7.15 (3H, m); 6.80 (1H, t); 6.05-5.80 (3H, m); 5.70 (1H, d); 5.30-5.15 (6H, m); 4.95 (1H, bs); 4.65-4.40 (8H, m); 4.00-3.75 (8H, m); 3.60-3.20 (16H, m); 3.00 (2H, q); 2.10 (2H, t); 1.60-1.15 (15H, m). ^{13}C NMR ($CDCl_3$) δ 174.22, 174.08, 171.19-167.47, 156.42, 156.41-156.12, 151.39, 145.64, 132.71-132.33, 117.74-117.30, 102.01, 79.13, 66.50, 53.02-47.49, 40.42, 38.29- 36.21, 36.01, 31.32, 29.67, 28.46, 26.33, 25.10.

Boc-NH(CH$_2$)$_5$CO[U,(Alloc)^3,A^Z,G^{OBn}]-NH(CH$_2$)$_5$CO$_2$tBu (4): Mixed tetramer **22** (171 mg, 165 μmol), TFA salt of dimer **7** (180 mg, 165 μmol) and DIEA (133 μL, 0.825 mmol) were mixed at 0°C in DMF (1 mL). Bop (88 mg, 197 μmol) was added and the mixture was stirred for 10min at 0°C and then allowed to warm to rt. The reaction was monitored using HPLC (A/B 80:20 to 0:100 over 30 min). After completion, the solvent was evaporated *in vacuo* and the crude residue was triturated with an aqueous 10% $NaHCO_3$ solution then with water. EtOAc (10 mL) was added and the resulting solid was filtered off to give compound **4** (294 mg, 89%) which was used without any further purification for the next step. HPLC (A/B 80:20 to 0:100 over 30 min): Rt = 20.8 min, λ_{max} = 206nm, 254 nm. MS (ESI+) m/z 2020.13 $(M+Na)^+$. MALDI-TOF MS calcd average mass for $C_{92}H_{126}O_{25}N_{26}$: 1996.15, found: (positive mode): m/z 2602.25 $(M+H)^+$, (negative mode): m/z 1996.08 $(M-H)^-$. ^{1}H NMR (CD_3OD) δ 8.60 (1H, m); 7.95 (1H, m); 7.60-7.35 (12H, m); 6.15-5.80 (3H, m); 5.70 (1H, d); 5.75-4.85 (16H, m); 4.45 (6H, m); 4.00-2.75 (40H, m); 2.30 (2H, m); 1.60-1.15 (30H, m). ^{13}C NMR (CD_3OD) δ 175.01, 165.09, 160.32-159.77, 156.89-156.36, 154.03, 151.92-151.34, 149.18, 148.80, 146.41, 144.72, 142.02, 136.29-135.82, 132.60, 128.04-127.49, 121.02, 116.50-116.08, 113.01, 100.02, 79.78-78.17, 67.59-65.77, 51.41-42.32, 39.49- 38.78, 37.85-36.03, 35.42-34.74, 29.05, 24.22, 27.31-26.82.

Boc-NH(CH$_2$)$_5$CO-[U,(C^Z)^3,A^Z,G^{OBn}]-NH(CH$_2$)$_5$CO$_2$tBu (24): The previous mixed hexamer **4** (250 mg, 125 μmol) and DEA (583 μL, 5.63 mmol) were dissolved in $CHCl_3$/DMF (3 mL 98:2 v:v) at rt. Pd[P(Phe)$_3$]$_4$ (15 mg, 12 μmol) was added. The mixture was stirred for 30 min at rt. The reaction was monitored using HPLC (A/B 80:20 to 0:100 over 30 min). The solvent was concentrated under reduced pressure and the crude residue was taken off by EtOAc. The resulting precipitated was filtered off to afford the expected triamine Boc-NH(CH$_2$)$_5$CO-[U,(H)^3,A^Z, G^{OBn}]-NH(CH$_2$)$_5$CO$_2$tBu (218 mg, 96%) as an amorphous solid which was used without any further purification for the next step. HPLC (A/B 80:20 to 0:100 over 30 min): Rt = 18.7 min, λ_{max} = 208.6 nm, 268.6 nm. MALDI-TOF MS calcd average mass for $C_{80}H_{114}O_{19}N_{26}$: 1743.93, found: (positive mode): m/z 1745.98 $(M+H)^+$. ^{1}H NMR (CD_3OD) δ 8.60 (1H, m); 8.15 (1H, m); 7.70-7.25 (12H, m); 5.65-4.50 (11H, m); 4.45-2.65 (36H, m); 2.60 (4H, m); 2.30 (4H, m); 1.70-1.20 (30H, m). This compound (200 mg, 114 μmol), C^Z-OH **23** (132 mg, 436 μmol), HOAt (63 mg, 463.6 μmol) and DIEA (370 μL, 2.29 mmol) were mixed at 0°C in DMF (1 mL). HATU (152 mg, 401 μmol) was then added at 0°C and the mixture was allowed to warm to rt (ca. 2 h). The reaction was monitored using HPLC (A/B 80:20 to 0:100 over 30 min). After completion, The solvent was concentrated under reduced pressure and the crude residue was taken off by

EtOAc (10 mL). The resulting solid was filtered off and washed with an aqueous (1N) Na_2CO_3 solution, water and dried over P_2O_5 to give compound **24** (269 mg, 90%) as an amorphous solid. HPLC (A/B 80:20 to 0:100 over 30 min): Rt = 24.2 min, λ_{max} = 206 nm, 254 nm. MALDI-TOF MS calcd average mass for $C_{122}H_{147}O_{31}N_{35}$: 2599.69, found: (positive mode): m/z 2602.25 $(M+H)^+$, (negative mode): 2597.91 $(M-H)^-$.

TFA.$H_2N(CH_2)_5CO$-[U,$(C^Z)^3$,A^Z,G]-$NH(CH_2)_5CO_2H$ (3) : Previous compound **24** (250 mg, 96 µmol) was dissolved in $CHCl_3$/TFA mixture (4 mL 1:1 v:v). The mixture was stirred at rt and monitored using HPLC (A/B 80:20 to 0:100 over 30 min). After the reaction was completed, solvents were removed *in vacuo* and the residue was triturated with $CHCl_3$/MeOH/EtOAc (3:1:6 v:v:v). The resulting solid was filtered off giving **3** as a white amorph powder (222.5 mg, 94%). HPLC (A/B 80:20 to 0:100 over 30 min): Rt = 16.0 min, λ_{max} = 206 nm, 254 nm. MALDI-TOF MS calcd average mass for $C_{106}H_{125}O_{29}N_{35}$: 2353.35, found: (positive mode): m/z 2354.47 $(M+H)^+$.

[U,$(C^Z)^3$,A^Z,G]$_c$ (25): TFA salt **3** (210 mg, 85 µmol), HOAt (21 mg, 153 µmol) and DIEA (137 µL, 851 µmol) were mixed at 0°C in DMF (8 mL). HATU (42 mg, 110 µmol) was then added at 0°C and the mixture was allowed to warm to rt (ca. 1 h). The reaction was monitored using HPLC. After completion, the DMF was removed under pressure and EtOAc (10 mL) was added. The resulting solid was filtered off and was washed with an aqueous 10% $NaHCO_3$ solution, water and dried over P_2O_5 to give 181 mg (90%) of compound **25**, which was used in the next step without further purification. HPLC (A/B 80:20 to 0:100 over 30 min): Rt = 17.2min, λ_{max} = 206 nm, 254 nm. MALDI-TOF MS calcd average mass for $C_{106}H_{123}O_{28}N_{35}$: 2335.33, found: (positive mode): m/z 2336.46 $(M+H)^+$.

[U,C,C,C,A,G]$_c$ (1): The cyclic protected hexaPNA **25** (44.7 mg, 19.1 µmol) was dissolved in HBr/AcOH (2 mL) and the mixture was stirred at rt for 5 days. Periodically (once a day), fresh HBr/AcOH mixture (1 mL) was added. The reaction was monitored using HPLC until completion. Solvents were evaporated off under reduced pressure.

*Purification of compound **1** by semi-preparative HPLC and purity assessment*: the crude residue was purified by HPLC, using a column RP-18 (5 µm) Lichrospher (250X10 mm). A linear gradient of B in A (A/B

97/3 to 40/60 over 60 min) and a flow rate of 2 mL/min were used for elution. The absorbency was detected both at 205 and 254 nm. The purified solution was first concentrated *in vacuo*, then the remaining solvent was removed by lyophilization. Compound **1** was obtained as a colorless resin (17 mg, 57%). The purity of the purified material was assessed by analytical reverse phase HPLC. A single peak at 15.2 min was recorded for the system A/B 97/3 to 40/60 over 30 min (λ_{max} = 206nm, 254 nm). MALDI-TOF MS calcd average mass for $C_{74}H_{99}O_{20}N_{35}$: 1798.80, found: (positive mode): m/z 1799.04 $(M+H)^+$.

Synthesis of the linear hexaPNA 2:

TFA. H-[G^{OBn}]-OMe (30): To a cooled solution of N-Mmt backbone **13** (418 mg, 1.03 mmol), G^{OBn}-OH **20** (310 mg, 1.03 mmol) and DIEA (831 µL, 5.17 mmol) in DMF (3 mL) was added Bop (504 mg, 1.13 mmol). The mixture was stirred for two hours at rt. Then DMF was removed under pressure and the crude residue was taken up in CH_2Cl_2. The organic layer was washed successively with a 10% $NaHCO_3$ solution, brine and dried over $MgSO_4$. The solvent was evaporated *in vacuo*. The residue was purified by silica gel column chromatography (from EtOAc/hex 1:1 to 7/3 v:v) to afford the corresponding N-Mmt methyl ester PNA as a white solid (520 mg, 75%). TLC (EtOAc/MeOH 7:3 v:v): Rf = 0.25. ^{1}H NMR ($CDCl_3$) (two isomers) δ 7.75 *7.70* (1H, 2s); 7.5-6.70 (19H, m); 5.55 (2H, s); 4.85 (2H, s); 4.25-3.95 (4H, m); 3.75-3.30 (8H, m); 2.50 (3H, m); 2.35 (1H, bs). ^{13}C NMR ($CDCl_3$) (two isomers) δ 169.40, 167.31 *167.18*, 129.83-126.24, 114.90, 113.30 *113.11*, 70.72, 68.14, 55.21, 52.17, 50.36 *49.70*, 49.42 *48.28*, 44.13 *43.61*, 43.04 *42.11*. The Mmt protecting group of the last compound was cleaved following the same procedure as for compound **18**. Starting from 100 mg (0.146 mmol) of the previous N-Mmt methyl ester PNA, TFA salt **30** was obtained as a white solid (67.4 mg, 87%). HPLC (A/B 80:20 to 0:100 over 30 min): Rt = 9.7 min. MS (ESI+) m/z 414.3 $(M+H)^+$. ^{1}H NMR (CD_3OD) (two isomers) δ 7.70 (1H, s); 7.50-7.25 (5H, m); 5.45 *5.45* (2H, 2s); 5.00 *4.80* (2H, 2s); 4.25 *4.05*(2H, m); 3.80-3.40 (8H, m); 3.10 (2H, m).

Mmt-[A^Z,G^{OBn}]-OMe (31): Compound **15** (152 mg, 0.216 mmol), TFA salt **30** (109 mg, 0.206 mmol) and DIEA (166 µL, 1.03 mmol) were mixed at -15°C in DMF (1.5 mL). Brop (88.1 mg, 0.227 mmol) was added and the mixture was stirred for 10 min at -15°C° until completion. DMF was then evaporated off and the crude residue was suspended in $CHCl_3$ (40 mL). The organic layer was washed with a 10% $NaHCO_3$ solution, with

water then dried over $MgSO_4$ and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (from EtOAc/MeOH 8:2 to 1:1 v:v) to afford the diPNA **31** (207 mg, 91%) as a colorless resin. TLC (EtOAc/MeOH 1:1 v:v): Rf = 0.83. HPLC (A/B 80:20 to 0:100 over 30 min): Rt = 15.2min (Mmt cleaved product). MS (ESI+) m/z 1095.4 $(M+H)^+$ m/z 1117.5 $(M+Na)^+$. ^1H NMR (CD_3OD) δ 8.50 (1H, m); 8.20-7.95 (1H, m); 7.75-6.70 (25H, m); 5.50-4.70 (8H, m); 4.55-4.05 (4H, m); 3.95-3.00 (12H, m); 2.40 (2H, m). ^{13}C NMR (CD_3OD) (two isomers) δ 172.31-169.33; 161.89 *159.38*; 153.42-153.05; 150.94 *150.77*; 147.72; 146.69-146.37; 142.62-142.41; 139.28; 137.88-137.37; 131.20-127.32; 122.93; 114.91; 114.18-113.77; 71.86; 69.07-68.42; 55.67; 53.22-37.90.

TFA.H-[A^Z,G^{OBn}]-OMe (28): This compound was prepared following the same procedure as for compound **7**. Starting from **31** (100 mg, 0.091 mmol), the trifluoacetate salt **28** was obtained as a white solid (81 mg, 95%). HPLC (A/B 80:20 to 0:100 over 30 min): Rt = 15.2 min, MS (ESI+) m/z 823.2 $(M+H)^+$, m/z 845.2 $(M+Na)^+$. ^1H NMR (CD_3OD) δ 8.55-7.75 (3H, m); 7.60-7.10 (10H, m); 5.50-4.70 (8H, m); 4.60-4.10 (4H, m); 4.05-3.00 (11H, m); ^{13}C NMR (CD_3OD) δ 172.91-167.71; 163.38-161.49; 155.15-150.49; 146.10-145.93; 142.22-141.90; 137.88-137.37 129.80-128.02; 122.10-122.63; 114.09; 69.27-65.21; 53.16-44.79; 39.20-38.51.

Boc-[Alloc]$_3$-OH (33): Compound **33** was prepared following the same procedure as for compound **16**. Starting from the ester **32**[13b] (200 mg, 0.292 mmol), the corresponding acid **33** was obtained as a colorless resin (190 mg, 97%). TLC (EtOAc/MeOH 8:2 v:v): Rf = 0.41. MS (ESI-) m/z 669.1 $(M-H)^-$, ^1H NMR ($CDCl_3$) δ 9.50(1H, bs); 8.40-7.00 (3H, m); 6.00-5.50 (4H, m); 5.35 (6H, m); 4.55 (6H, m); 4.10-3.80 (6H, m); 3.60-3.20 (12H, m); 1.45 (9H, s).

Boc-[$(Alloc)^3$,A^Z,G^{OBn}]-OMe (34): Compound **33** (145 mg, 0.216 mmol), TFA salt **28** (114 mg, 0.216 mmol) and DIEA (166 µL, 1.03 mmol) were mixed at -15°C in DMF (1.5 ml). Bop (88.1 mg, 0.227 mmol) was added and the mixture was stirred for 10 min at -15°C until completion. DMF was then evaporated off and the crude residue was suspended in $CHCl_3$ (40 mL). The organic layer was washed successively with a (1M) $KHSO_4$ solution, a 10% $NaHCO_3$ solution, with water then dried over $MgSO_4$ and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (from $CHCl_3$ to $CHCl_3$/MeOH 1:1 v:v)

to afford the compound **34** (271 mg, 85%) as a colorless resin. HPLC (A/B 80:20 to 0:100 over 30 min): Rt = 20min, MS (ESI+) m/z 1497.4 $(M+Na)^+$.

Boc-[$(C^Z)^3$,A^Z,G^{OBn}]-OMe (35): Compound **34** (26.1 mg, 17.6 μmol) and DEA (82.3 μL, 0.79 mmol) were dissolved in CH_2Cl_2 (250 μL) at rt. $Pd[P(Phe)_3]_4$ (2 mg, 1.7 μmol) was added. The mixture was stirred for 30 min at rt. The solvent was concentrated under reduced pressure and the crude residue was triturated with AcOEt/Et_2O. After filtration, the triamine Boc-[$(H)^3$,A^Z,G^{OBn}]-OMe was isolated as a white amorph solid (21.4 mg, 98%), which was used without further purification. HPLC (A/B 80:20 to 0:100 over 30 min): Rt = 14.8 min, MS (ESI+) m/z 1245.3 $(M+Na)^+$. Triamine Boc-[$(H)^3$,A^Z,G^{OBn}]-OMe (22 mg, 18.1 μmol), C^Z-OH **23** (20.8 mg, 68.7 μmol), HOAt (9.8 mg, 72.3 mol) and DIEA (58.1 μL, 361 μmol) were mixed at 0°C in DMF (220 μL). HATU (24 mg, 63.4 μmol) was then added at 0°C and the mixture was allowed to warm to rt (ca. 1 h). After completion the DMF was evaporated off and the crude mixture was triturated with AcOEt/Et_2O. The solid was filtered off, then washed with an aqueous 10% $NaHCO_3$ solution, water and finally CH_3CN. Compound **35** was isolated as a yellow amorphous solid (25 mg, 66%). HPLC (A/B 80:20 to 0:100 over 30 min): Rt = 23.6min. MS (ESI-) m/z 2075.8 $(M-H)^-$.

H-[$(C^Z)^3$,A^Z,G]-OMe (36): Compound **35** (21 mg, 10.1 μmol) was dissolved in $CHCl_3$/TFA 1:1 (800 μL) at rt. The mixture was stirred two hours then the solvent was evaporated *in vacuo*. The crude residue was triturated with AcOEt then the precipitate was filtered off. The TFA salt **36** was isolated as a pale yellow solid (21 mg, 97%). HPLC (A/B 80:20 to 0:100 over 30 min): Rt = 17.5 min. MS (ESI+) m/z 2075.8 $(M+H)^+$.

Boc-[U]-OH (26): Compound **37**[13b] (153 mg, 0.66 mmol), U-OH **11** (115 mg, 0.66 mmol) and 2,6-lutidine (270 μL, 2.35 mmol) were mixed in DMF (mL) at 0°C. Then Brop (336 mg, 0.86 mmol) was added and the mixture was stirred at rt for 2 hours. DMF was evaporated and the crude product was suspended in AcOEt. The solution was washed alternatively with a (1M) $KHSO_4$ solution, a 10% $NaHCO_3$ solution and water. The organic layer was dried over $MgSO_4$. The residue was purified by silica gel column chromatography (from EtOAc to EtOAc/MeOH 8:2) to give the PNA methyl ester (208 mg, 82%) as a colorless resin. TLC (EtOAc/MeOH 8:2): Rf = 0.64. HPLC (A/B 80:20 to 0:100 over 30 min): Rt = 9.7 min. MS (ESI+) m/z 407.2 $(M+Na)^+$. ^1H NMR ($CDCl_3$) (two isomers) 7.25 (1H, d); 5.65 (1H, d); 5.60, *5.30* (4H, 2bs); 4.65 *4.50* (2H, 2s);

4.05 *3.00* (2H, 2s); 3.75-3.70 (3H, 2s); 3.60-3.20 (4H, m); 1.40 (9H, s). ^{13}C NMR ($CDCl_3$) (two isomers) δ 173.33 *173.19*; 170.30 *170.14*; 167.71; 156.55 *156.34*; 151.47; 145.28; 102.10; 80.05; 52.62-48.63; 28.30. This uracil PNA methyl ester (115 mg, 0.3 mmol) was suspended in dioxane (2 mL) and (1N) LiOH (331 µL, 0.662 mmol) was added dropwise. The mixture was stirred for 2 hours at rt and was then cooled down to 0°C. The excess of LiOH was neutralized with an aqueous (0.5N) HCl solution, then the mixture was lyophilized. The crude residue was purified using a LH-20 sephadex with MeOH as eluant. The Boc uracil PNA **26** was isolated as a white amorph solid (110 mg, 100%). TLC (EtOAc/MeOH 1:1 v:v): Rf = 0.64. HPLC (A/B 80:20 to 0:100 over 30 min): Rt = 7.4 min. MS (ESI-) m/z 369.2 $(M-H)^-$. ^{1}H NMR (CD_3OD) δ 7.60 (1H, dd); 5.80 (1H, d); 4.90, *4.75* (2H, 2s); 4.10 4.05 (2H, 2s); 3.60-3.15 (4H, m); 1.60 (9H, s).

Boc-[U,$(C^Z)^3$,A^Z,G]-OMe (38): Uracil PNA monomer **26** (12.3 mg, 34.2 mol), TFA salt **36** (66.3 mg, 31.2 µmol) and DIEA (97.8µL, 0.606 mmol) were mixed at in DMF (0.6 mL). Bop (14.7 mg, 34.2 µmol) was added and the mixture was stirred at rt until completion (ca one hour). DMF was then evaporated off and the crude residue was triturated with CH_3CN. The solid was filtered off, then washed with a 10% $NaHCO_3$ solution and water. Compound **38** was isolated as a pale yellow solid (52.5 mg, 75%). HPLC (A/B 80:20 to 0:100 over 30 min): Rt = 18.9 min. MS (ESI+) m/z 2242.8 $(M+H)^+$.

H-[U,$(C^Z)^3$,A^Z,G]-OH (2): The protected hexaPNA **38** (20 mg, 8.9 µmol) was dissolved in HBr/AcOH (500 µL) solution containing a few drops of water. The mixture was stirred at rt for 3 days. The reaction was monitored using HPLC (isochratic, A/B 90/10) until completion. Solvents were evaporated off under reduced pressure.

*Purification of compound **2** by semi-preparative HPLC and purity assessment*: the crude residue was purified by HPLC, using a column RP-18 (5 µm) Lichrospher (250X10 mm). An isochratic mixture of B and A (A/B 91/9) and a flow rate of 2 mL/min were used for elution. The absorbency was detected both at 205 and 254 nm. The purified solution was first concentrated *in vacuo*, then the remaining solvent was removed by lyophilization. Compound **2** was obtained as a colorless resin (8 mg, 40%). The purity of the purified material was assessed by analytical reverse phase HPLC. A single peak at 12.5 min was recorded for the system A/B 97:3 to 40:60 over 30 min or at 9.1 min for the system A/B 90/10; λ_{max} = 206 nm, 254 nm. MS (ESI+) m/z

1591.5 $(M+H)^+$, m/z 1613.5 $(M+Na)^+$. MALDI-TOF MS calcd average mass for $C_{62}H_{79}N_{33}O_{19}$: 1590.50; found (positive mode): 1590.67 $(M+H)^+$.

TAR Binding evaluation.

The following two biochemical protocols have been previously described.[25]

Melting Temperature Studies. Melting temperature measurements were performed with a 27-mer oligoribonucleotide (Eurogentec) as a RNA substrate, in BPE buffer pH 7.1 (6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM EDTA) using 0.5 μM RNA molecule and 0.5-2 μM ligand, in 1 mL quartz cuvettes at 260 nm with a heating rate of 1 °C/min.

RNase Footprinting. The 59-mer TAR RNA was produced by *in vitro* transcription using T3 RNA polymerase. Binding experiments were performed by a gel shift assay and sequence recognition was studied by RNase A footprinting.