Azidopeptide Nucleic Acid. An Alternative Strategy for Solid Phase Peptide Nucleic Acid (PNA) Synthesis.

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Experimental Section

General Techniques. All reactions were carried out under a nitrogen atmosphere with dry, freshly distilled solvents under anhydrous conditions, unless otherwise noted. Tetrahydrofuran (THF), toluene and diethyl ether (Et_2O) were distilled from sodium-benzophenone, and methylene chloride (CH_2Cl_2) from calcium hydride. Anhydrous solvents were also obtained by passing them through commercially available alumina columns (Solv-Tek, Inc., VA). Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials, unless otherwise stated. Reagents were purchased at highest available commercial quality and used without further purification unless otherwise stated. Substituted polystyrene resins (100-200 mesh, 1% DVB) were purchased from Advanced Chemtech. Reactions were monitored by thin layer chromatography (TLC) carried out on 0.25 mm E. Merck silica gel plates (60F-254) using UV light as visualizing agent and 10% ethanolic phosphomolybdic acid or vanillin solution and heat, as developing agents. E. Merck silica gel (60, particle size 0.040-0.063 mm) was used for flash column chromatography. NMR spectra were recorded on Brucker Advance-400 instruments and calibrated using residual undeuterated solvent as an internal reference. DMSO was calibrated to 2.5 ppm for ^{1}H NMR and 39.97 ppm for ^{13}C NMR. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, qt = quintet, m = multiplet, b = broad. IR spectra were

recorded on a Perkin-Elmer 1600 series FT-IR spectrometer. LC-MS were recorder using an Agilent 1100 HPLC with a Bruker micro-TOF instrument (ESI). Unless otherwise stated, a Supelco C8 (5 cm x 4.6mm, 5 μm particules) column was used with a linear elution gradient from 100% H_2O (0.5% HCO_2H) to 100% MeCN in 10 min at a flow rate of 0.5 mL/min

Benzyl 2-azidoethylglycinate (2). To a solution of 2-aminoethylbromide hydrobromide (10.0 g, 48.8 mmol, 1.0 eq) in DMF (25 mL) was added NaN_3 (3.49 g, 53.7 mmol, 1.1 eq) and the reaction mixture was heated to 60 ºC. After 3 h, the reaction was cooled to 0 ºC and triethylamine (13.7 mL, 97.6 mmol, 2.0 eq) was added followed by benzyl 2-bromoacetate (6.21 mL, 39.0 mmol, 0.8 eq). The reaction was stirred for an additional 2 h at 0 ºC then diluted with Et_2O (200 mL) and washed with brine (200 mL). The organic phase was dried over $MgSO_4$ and concentrated *in vaccuo*. The compound was then purified by flash chromatography (gradient, 30% EtOAc in hexanes to 100% EtOAc) to obtain 5.87 g of **2** as a yellowish oil (64% yield). R_f = 0.70 (silica gel, EtOAc); FT-IR (neat) ν_{max}, 3334.5, 2940.4, 2100.6, 1739.8 cm^{-1}; ^1H NMR (400 MHz, D_6-DMSO, 25 °C) δ 7.40-7.36 (m, 5H, Ar), 5.16 (s, 2H, O-*CH*$_2$-C), 3.46 (s, 2H, NH-*CH*$_2$-CO), 3.34 (t, *J* = 5.9, 2H, N_3-*CH*$_2$-CH_2), 2.78 (t, *J* = 5.9, 2H, N_3-CH_2-*CH*$_2$); ^{13}C NMR (100 MHz, DMSO, 25 °C) δ 172.5, 136.5, 128.8, 128.5, 128.4, 65.9, 50.9, 50.3, 48.1; HRMS (ESI) calculated for $C_{11}H_{14}N_4O_2$: (MH^+) = 235.1111, found: 235.1301.

Thymine-1-acetic acid (3-T) was purchased from Aldrich (448958-25G)

4-*N*-(Benzhydryloxycarbonyl)cytosine-1-acetic acid (3-C).[1] To cytosine (20.6 g, 180 mmol, 1.0 eq) in DMF (180 mL) was added potassium *t*-butoxide (23.2 g, 207 mmol, 1.15 eq) and the reaction was heated to 100 °C for 2 h. The reaction was then cooled to 10 °C and benzyl bromoacetate (32.1 mL, 202 mmol, 1.12 eq) was added drop-wise over 30 min. The reaction was allowed to warm to room temperature while stirring for 12 h, then quenched with acetic acid (11.8 mL, 207 mmol, 1.2 eq), concentrated and

[1] This compound was synthesized from cytosine according to a modified procedure from Coull *et al.* (patent WO 96/40685).

thoroughly dried *in vaccuo*. The residues were re-suspended in H_2O (200 mL) and stirred for 4 h then filtered, washed with H_2O (4 x 300 mL), dried under high vacuum to recover 41.2g of benzyl cytosine-1-acetate as a pure compound (91.2% yield). To this material (41.2 g, 164 mmol, 1.0 eq) dissolved in DMF (320 mL) was added carbonyldiimidazole (42.5 g, 262.5 mmol, 1.6 eq). Progression of the reaction was monitored by TLC analysis of a reaction aliquot quenched with MeOH. After 1.5 h of stirring, TLC analysis indicated that the isocyanate formation was complete and benzhydrol (39.3 g, 213 mmol, 1.3 eq) was added. The reaction was heated at 60 °C and two more batches of benzhydrol (2 x 3.65 g, 19.8 mmol, 0.12 eq) were added at 1 h intervals. After 6 h, the heating was stopped and the reaction was allowed to proceed for 12 h and then quenched by the addition of MeOH (9.3 mL, 230 mmol, 1.4 eq). The solvents were removed *in vaccuo* and the product was recrystallized from ethanol. The mother liquor was concentrated and recrystallized from 180 mL of 3/1 MeOH/H_2O. The two batches of impure powder were combined and further crystallized in methanol (230 mL) to recover 58.7 g (125 mmol) of pure benzyl (4-*N*-(benzhydryloxycarbonyl)cytosine)-1-acetate (76% yield). This material (58.7 g, 125 mmol, 1.0 eq) was dissolved in acetonitrile:MeOH:H_2O:EtOH (2:2:1:1, 755 mL) with heat and then cooled to 0 °C and treated with $LiOH{\cdot}H_2O$ (51 g, 1.22 mol, 9.7 eq) dissolved in water (393 mL). The progression of the reaction was carefully monitored by TLC and quenched immediately upon consumption of the starting material with citric acid (117 g, 607 mmol, 4.9 eq) in water (580 mL) to recover 44.3 g (117 mmol) of (4-*N*-(benzhydryloxycarbonyl)cytosine)-1-acetic acid **3-C** (93% yield). R_f = 0.15 (30% MeOH in EtOAc); FT-IR (neat) ν_{max} 3422.0, 3151.8, 3063.4, 1764.2, 1708.6, 1673.0 cm^{-1}; ^1H NMR (400 MHz, D6-DMSO, 25 °C) δ 8.03-8.01 (d, *J* = 7.5, 1H, *C6*), 7.46 (d, *J* = 7.5, 4H, Ph), 7.38 (t, *J* = 7.5, 4H, Ph), 7.3 (d, *J* = 7.5, 2H, Ph), 6.96 (d, *J* = 7.5, 1H, *C5*), 6.82 (s, 1H, *CH*-$(C_6H_5)_2$), 4.50 (s, 2H, N-*CH*$_2$-CO); ^{13}C NMR (100 MHz, D_6-DMSO, 25 °C) δ 169.9, 163.5, 155.5, 152.8, 151.1, 140.8, 129.0, 128.3, 126.9, 94.2, 71.9, 51.3; HRMS (ESI) calculated for $C_{20}H_{17}N_3O_5$: (MN_a^+): 402.1060, found: 402.1010.

6-*N*-(Benzhydryloxycarbonyl)adenine-9-acetic acid (3-A).[1] Adenine (20.0 g, 148 mmol, 1.0 eq) in DMF (350 mL) was treated with sodium hydride (60% in mineral oil,

6.88 g, 172 mmol, 1.2 eq) in two portions at 1 h intervals. After 3 h, all hydrogen gas evolution had stopped and the solution was cooled in an ice bath and benzyl-bromoacetate (26.6 g, 163 mmol, 1.1 eq) was added drop-wise over 30 min The reaction was allowed to warm to room temperature while stirring for 12 h after which solvents were removed *in vaccuo* and water (160 mL) was added. After brief stirring, the water was decanted and the remaining sticky oil was dissolved in boiling ethanol and stirred overnight to obtain 29.3 g (99 mmol) of benzyl adenine-9-acetate as a solid (72% yield). This crude material was dissolved in DMF (200 mL), treated with carbonyldiimidazole (24.0 g, 148 mmol, 1.5 eq), heated slowly until 105 °C and stirred at that temperature for 2 h. The temperature was then decreased to 95 °C before adding benzhydrol (27.3 g, 148 mmol, 1.5 eq) and the reaction was stirred for 12 h without further heating. The reaction was quenched with the addition of water (500 mL) and stirred vigorously for 2 h after which the solution was decanted and the residues dried under high vacuum. The crude oil was then redissolved in ethanol/acetonitrile (1/1, 330 ml) with heating and once a homogenious solution was obtained, water (146 mL) was added and the solution was cooled to 10 °C. $LiOH{\cdot}H_2O$ (74.9 g, 1.79 mol, 17.7 eq) in water (365 mL) was then added. The progression of the reaction was carefully monitored by TLC and quenched immediately upon consumption of the starting material with a solution of citric acid (192 g, 0.99 mol, 10 eq) in water (400 mL), stirred 10 min after which more water (100 mL) was added, and the mixture was cooled at 4 °C and allowed to crystallized overnight to afford 30.5 g of 6-*N*-(benzhydryloxycarbonyl)adenine-9-acetic acid (**3-C**) as a pure white solid (76.4% yield for 2 steps). R_f = 0.13 (30% MeOH in EtOAc); FT-IR (neat) ν_{max} 3297.9, 3058.3, 1736.0, 1614.7 cm^{-1}; ^1H NMR (400 MHz, D_6-DMSO, 25 °C) δ 10.9 (s, 1H, C-*NH*-CO), 8.63 (s, 1H, C_2), 8.45 (s, 1H, C_8), 7.54 (d, *J* = 7.5, 4H, Ph), 7.39 (t, *J* = 7.5, 4H, Ph), 7.3 (t, *J* = 7.5, 2H, Ph), 6.83 (s, 1H, O-*CH*-$(C_6H_5)_2$), 5.09 (s, 2H, N -CH_2-CO_2H); ^{13}C NMR (100 MHz, D6-DMSO, 25 °C) δ 169.5, 152.6, 152.1, 151.6, 149.8, 145.3, 141.3, 128.9, 128.1, 126.9, 123.1, 77.7, 44.7; HRMS (ESI) calculated for $C_{21}H_{17}N_5O_4$: (MH^+): 404.1275, found: 404.1231.

2-*N*-(Benzhydryloxycarbonyl)guanine-9-acetic acid (3-G).[1] To 2-amino-6-chloropurine (27.9 g, 165 mmol, 1.0 eq) in DMF (280 mL) at 85 °C was added potassium

carbonate (3.34 g, 247 mmol, 1.5 eq). After brief stirring, the reaction mixture was cooled in an ice bath and benzyl-2-bromo-acetate (28.0 mL, 177 mmol, 1.07 eq) was added drop-wise, in three batches over 3 h while maintaining the temperature at 0 °C. The reaction was then allowed to warm to room temperature and stirred for 12 h after which, the reaction mixture was filtered to remove the salts and the product precipitated by pouring the reaction mixture in vigorously stirring acidic water (150 mL of 1 N HCl). After 2 h of stirring, a pink powder was filter and washed with water (1.5 L), then crystallized overnight from boiling acetonitrile while stirring (280 mL). The product was collected by filtration and washed with methanol and diethyl ether to yield 35.3 g (111 mmol) of benzyl 2-amino-6-chloropurine-9-acetate were obtained as white powder (67.7% yield). This product was dissolved in THF (435 mL) cooled to 0 °C and triphosgene (11.9 g, 40.1 mmol, 0.36 eq) was added. The reaction was stirred 0 °C for 1 h before adding drop-wise diisopropylethylamine (42.7 mL, 245 mmol, 2.2 eq). After another 30 min at 0 °C, benzhydrol (24.6 g, 133.8 mmol, 1.2 eq) was added and the reaction was stirred for 12 h while warming to room temperature. The reaction was quenched with ethanol (75 mL) and concentrated until dryness *in vaccuo*. The oil was dissolved in CH_2Cl_2 and stirred with 10% aq citric acid (200 mL), after which the organic phase was washed with 5% sodium bicarbonate (100 mL), dried with $MgSO_4$ and concentrated. The residues were recrystallized in boiling methanol (300 mL) to recovered 37.5 g (71.1 mmol) of benzyl 2-*N*-(benzhydryloxycarbonyl)-6-chloropurine-9-acetate (63.8% yield) as a pure solid. THF (425 mL) loaded with sodium hydride (60% in mineral oil, 13.8 g, 345 mmol, 5.0 eq) was cooled to –78 °C before adding 3-hydroxypropionitrile (23.6 mL, 345 mmol, 5.0 eq). After 2.5 h of stirring at 0 °C, 2-*N*-(benzhydryloxycarbonyl)-6-chloropurine-9-*N*-acetate (36.4 g, 68.96 mmol, 1.0 eq) was added and the reaction mixture was further stirred for 12 h at room temperature. The THF was removed *in vaccuo* and the reaction mixture thus obtained was poured in H_2O (250 mL), which was acidified until pH 3 with 20% aq citric acid to obtain a white precipitate. The precipitate was collected by filtration, washed with water and recrystallized 3 times in methanol to recover 25.1 g of 2-*N*-(benzhydryloxycarbonyl)guanine-acetic acid **3-G** (86.5% yield). R_f = 0.18 (30% MeOH in EtOAc); FT-IR (neat) ν_{max} 3422.0, 3229.8, 2925.5, 1686.1, 1611.1 cm^{-1}; ^1H NMR

(400 MHz, D6-DMSO, 25 °C) δ 7.94 (s, 1H, *C*$_8$), 7.48 (d, 4H, Ph, *J* = 7.5), 7.39 (t, 4H, Ph, *J* = 7.5), 7.31 (t, 2H, Ph, *J* = 7.5), 6.88 (s, 1H, O-*CH*-(C_6H_5)$_2$), 4.87 (s, 2H, N-*CH*$_2$-CO_2H); ^{13}C NMR (100 MHz, D6-DMSO, 25 °C) δ 169.9, 169.5, 155.5, 154.1, 149.7, 147.6, 140.7, 140.5, 138,4, 129.3, 128.4, 126.9, 78.5, 44.9; HRMS (ESI) calculated for $C_{21}H_{17}N_5O_5$: (MH^+): 420.1224, found: 420.1281.

Benzyl *N*-(2-azidoethyl)-*N*-(thymine-1-acetyl)glycinate 4-T. To a solution of thymine-1-acetic acid **3-T** (5.97 g, 32.4 mmol, 1.0 eq) in DMF (35.0 mL) was added benzyl 2-azidoethylglycinate **2** (7.59 g, 32.4 mmol, 1.0 eq), 4-DMAP (0.39 g, 3.24 mmol, 0.1 eq), and EDCI (7.46 g, 38.9 mmol, 1.2 eq). After 2 h, the reaction was diluted with CH_2Cl_2 (50 mL) and washed with HCl 1M (50 mL) and saturated $NaHCO_3$ (100 mL). Each aqueous phase was back extracted with CH_2Cl_2 (2 x 100 mL). The combined organic phases were dried over $MgSO_4$ and concentrated to recover 12.2 g of spectroscopically pure **4-T** as a white powder (94 % yield). R_f = 0.75 (10% MeOH in EtOAc); FT-IR (neat) ν_{max} 3162.5, 3032.8, 2128.8, 1746.5, 1458.0 cm^{-1}; ^{1}H NMR (400 MHz, D6-DMSO, 25 °C) δ 7.37 (m, 5H, Ar), 7.24 (s, 1H, *C*$_6$), 5. 23 (s, 0.7H, O-*CH*$_2$-Ar, rotamer 1), 5.15 (s, 1.3H, O-*CH*$_2$-Ar, rotamer 2), 4.74 (s, 1.3H, N-*CH*$_2$-CO, rotamer 1), 4.54 (s, 0.7H, N-*CH*$_2$-CO, rotamer 2), 4.48 (s, 0.7H, N-*CH*$_2$-CO), 4.18 (s, 1.3H, N-*CH*$_2$-CO, rotamer2), 3.64 (m, *J* = 4.8, 1.3H, N_3-*CH*$_2$-CH_2, rotamer 1), 3.50 (t, *J* = 5.4, 0.3H, N_3-*CH*$_2$-CH_2, rotamer 2), 3.61 (t, *J* = 4.8, 1.3H, N_3-CH_2-*CH*$_2$-N, rotamer 1), 3.44 (t, *J* = 5.4, 0.3H, CH_2-*CH*$_2$-N, rotamer 2) 8: 1.77 (s, 3H, C-*CH*$_3$); ^{13}C NMR (100 MHz, D6-DMSO, 25 °C) δ 169.8, 169.3, 168.4, 168.1, 164.9, 151.4, 142.6, 136.2, 136.0, 128.9, 128.9, 128.7, 128.6, 128.5, 128.3, 108.7, 108.5, 67.1, 66.4, 49.5, 48.7, 48.5, 48.3, 47.2, 46.8, 12.3; HRMS (ESI) calculated for $C_{18}H_{20}N_6O_5$: (MH^+): 401.1490, found: 401.1500.

Benzyl *N*-(2-azidoethyl)-*N*-[4-*N*-(benzhydryloxycarbonyl)cytosine-1-acetyl]glycinate 4-C. To a solution of 4-*N*-(benzhydryloxycarbonyl)cytosine-1-acetic acid **3-C** (5.31 g, 14.0 mmol, 1.2 eq) and NMM (3.26 mL, 25.6 mmol, 2.2 eq) in DMF/acetonitrile (1/1, 33.3 mL) at 0 ºC was added pivaloyl chloride (1.72 mL, 14.0 mmol, 1.2 eq) drop-wise. The mixture was stirred for 20 min at 0 ºC then benzyl 2-azidoethylglycinate **2** (2.73 g, 11.6 mmol, 1.0 eq) was added and the reaction stirred 90 min at room temperature. The

reaction was then diluted with CH_2Cl_2 and washed with 20% aq citric acid (50 mL) and saturated $NaHCO_3$ (100 mL). The organic phase was dried over $MgSO_4$, concentrated and purified by flash chromatography (100% EtOAc) to recover 4.01 g of **4-C** as a white powder (50% yield). R_f = 0.80 (10% MeOH in EtOAc); FT-IR (neat) ν_{max} 3032.1, 2944.7, 2103.6, 1744.5, 1668.6, 1628; ^{1}H NMR (400 MHz, D6-DMSO, 25 °C) δ 11.01 (s, 1H, CO-*NH*-C), 7.92 (d, *J* = 7.5, 0.7H, *C*$_6$, rotamer 1), 7.85 (d, *J* = 7.5, 0.3H, *C*$_6$, rotamer 2), 7.39 (m, 15H, Ar), 6.96 (m, 1H, *C*$_5$), 6.81 (s, 1H, O-*CH*-$(C_6H_5)_2$), 5.23 (s, 0.7H, O-CH_2-C_6H_5, rotamer 2), 5.14 (s, 1.3H, O-CH_2-C_6H_5, rotamer 1), 4.89 (s, 1.3H, N-*CH*$_2$-CO, rotamer 1), 4.69 (s, 0.7H, N-*CH*$_2$-CO, rotamer 2), 4.52 (s, 0.7H, N-*CH*$_2$-CO, rotamer 2), 4.19 (s, 1.3H, N-*CH*$_2$-CO, rotamer 1), 3.67 (m, 2.6H, N_3-*CH*$_2$-CH_2, rotamer 1), 3.51 (t, *J* = 5.4, 0.7H, N_3-*CH*$_2$-CH_2, rotamer 2), 3.43 (t, *J* = 5.36, 0.7H, CH_2-*CH*$_2$-N, rotamer 2); ^{13}C NMR (100 MHz, D6-DMSO, 25 °C) δ 169.8, 169.3, 168.3, 168.0, 163.5, 155.4, 152.8, 151.5, 140.8, 136.2, 136.0, 129.0, 128.9, 128.6, 128.5, 128.3, 126.9, 94.2, 77.9, 67.0, 66.4, 50.1, 49.5, 48.8, 48.6, 47.4, 46.8; HRMS (ESI) calculated for $C_{31}H_{29}N_7O_6$ (MH^+): 596.2174, found: 596.2157.

Benzyl *N*-(2-azidoethyl)-*N*-[6-*N*-(benzhydryloxycarbonyl)adenine-9-acetyl]glycinate 4-A. A solution of 6-*N*-(benzhydryloxycarbonyl)adenine-9-acetic acid **3-A** (0.20 g, 0.50 mmol, 1.0 eq) in acetonitrile at 0 ºC (1.3 mL) was treated sequentially with NMM (111 μL, 0.99 mmol, 2.0 eq) and pivaloyl chloride as a drop-wise addition (67.2 μL, 0.55 mmol, 1.2 eq). The mixture was stirred for 20 min at 0 ºC then benzyl 2-azidoethylglycinate **2** (0.12 g, 0.51 mmol, 1.02 eq) was added and the reaction was continued for 90 min at room temperature. The reaction was then diluted with CH_2Cl_2 and washed with 20% aq citric acid followed by saturated $NaHCO_3$. The organic phase was dried over $MgSO_4$, concentrated and purified by flash chromatography (10% MeOH/EtOAc) to obtain 0.522 g of **4A** as white powder (80% yield). R_f = 0.65 (10% MeOH in EtOAc); FT-IR (neat) ν_{max} 3397.3, 2927.1, 2105.3, 1751, 1675, 1612 cm^{-1}; ^{1}H NMR (400 MHz, D6-DMSO, 25 °C) δ 8.61 (1s, 1H, *C*$_2$), 8.38 (s, 0.7H, *C*$_8$, rotamer 1), 8.33 (s, 0.3H, *C*$_8$, rotamer 2), 7.56-7.30 (m, 15H, Ar), 6.83 (s, 1H, O-*CH*-$(C_6H_5)_2$), 5.50 (s, 1.3H, Ph-*CH*$_2$-CON, rotamer 1), 5.28 (s, 0.7H, Ph-*CH*$_2$-CON, rotamer 2), 6: 5.25 (s, 0.6H, N-*CH*$_2$-CON, rotamer 2), 5.13 (s, 1.4H, N-*CH*$_2$-CON, rotamer 1), 4.63 (s, 0.7H,

NCH_2CO_2, rotamer 2), 4.21 (s, 1.3H, NCH_2CO_2, rotamer 1), 3.74 (m, 2.6H, N_3-*CH_2*-*CH_2*-N, rotamer 1), 3.53 (t, *J* = 4.8, 0.6H, N_3-*CH_2*-CH_2-N, rotamer 2), 3.45 (t, *J* = 4.8, 0.6H, N3-CH2-*CH2*-N, rotamer 2); ^{13}C NMR (100 MHz, D6-DMSO, 25 °C) δ 169.8, 169.3, 167.8, 167.5, 152.8, 152.0, 151.6, 149.7, 145.7, 141.4, 136.2, 128.9, 128.6, 128.5, 128.3, 128.1, 126.9, 123.1, 77.7, 67.6, 66.4, 49.6, 48.7, 48.6, 47.4, 46.9, 44.5; HRMS (ESI) calculated for $C_{32}H_{29}N_9O_5$ (MH^+): 620.2286, found: 620.2113.

Benzyl *N*-(2-azidoethyl)-*N*-[2-*N*-(benzhydryloxycarbonyl)-guanine-9-acetyl]glycinate 4-G. To a solution of 2-*N*-(benzhydryloxycarbonyl)-guanine-9-acetatic acid **3-G** (0.40 g, 0.96 mmol, 1.1 eq) in DMF (2 mL), was added TOTU (0.32 g, 0.96 mmol, 1.1 eq) followed by Et_3N (0.2 mL, 1.50 mmol, 1.7 eq). The mixture was stirred for 10 min and benzyl 2-azidoethylglycinate **2** (0.2 g, 0.87 mmol, 1.0 eq) was added. After 2 h, the reaction was diluted with CH_2Cl_2 (50 mL) and washed with brine (100 mL), 20% aq citric acid (50 mL), $NaHCO_3$ (100 mL), dried over $MgSO_4$ and concentrated. The crude product was purified by flash chromatography (10% MeOH in EtOAc) to recover 0.27 g of **4-G** as a white powder (49% yield). R_f = 0.30 (10% MeOH in EtOAc); FT-IR (KBr pellet) ν_{max} 3245.4, 2925.1, 2104.5, 1685.7, 1609.1 cm^{-1}; ^{1}H NMR (400 MHz, D6-DMSO, 25 °C) δ 7.86 (s, 1H, *C_8*), 7.48-7.30 (m, 15H, Ar), 6.88 (s, 1H, O-*CH*-Ph), 5.26 (s, 0.8H, N-*CH_2*-CO, rotamer 1), 5.21 (s, 1.2H, N-*CH_2*-CO, rotamer 2), 5.14 (s, 1.4H, O-*CH_2*-Ar_{Bn}, rotamer 1), 5.03 (s, 0.6H, O-*CH_2*-Bn rotamer 2), 3.72 (s, 2H, N-*CH_2*-CO), 3.71 (m, 2H, N_3-*CH_2*-CH_2, rotamer 1), 3.71 (m, 2H, CH_2-*CH_2*-N, rotamer 1), 3.52 (t, *J* = 5.4, 0.5H, N_3-*CH_2*-CH_2, rotamer 2), 3.44 (t, *J* = 5.4, 0.5H, CH_2-*CH_2*-N, rotamer 2); ^{13}C NMR (100 MHz, D6-DMSO, 25 °C) δ 170.8, 169.9, 169.3, 167.4, 167.5, 155.5, 154.1, 147.4, 141.0, 140.5, 136.2, 136.0, 129.0, 128.9, 128.8, 128.6, 128.4, 128.3, 78.5, 67.2, 66.4, 53.0, 49.0, 48.7, 47.3, 46.9, 44.5, 44.6; HRMS (ESI) calculated for $C_{32}H_{29}N_9O_6$ (MH^+): 636.2235, found: 636.2055.

***N*-(2-Azidoethyl)-*N*-(thymine-1-acetyl)glycine 5-T.** To a solution of benzyl ester **4-T** (14.8 g, 37.1 mmol, 1.0 eq) in 1,4-dioxane (35 mL) at room temperature was added NaOH as a 2M solution (74.2 mL, 148.5 mmol, 4.0 eq). After 15 min, TLC analysis indicated that the reaction was complete and 20 mL of dioxane were evaporated. The

solution was acidified until pH 3 with 20% aq citric acid at which stage the desired product precipitated. Filtration followed by drying *in vaccuo* afforded 8.88 g as a pure white solid (77% yield). R_f = 0.08 (30% MeOH in EtOAc); FT-IR (neat) ν_{max} 3448.1,3171.7, 3041.3, 2105.9, 1696.4, 1655.2 cm^{-1}; ^1H NMR (400 MHz, D6-DMSO, 25 °C) δ 7.30 (bs, 0.5H, *C*$_6$, rotamer 1), 7.26 (bs, 0.5H, *C*$_6$, rotamer 2), 4.71 (s, 1.1H, N-*CH*$_2$-CO_2, rotamer 1), 4.49 (s, 0.9H, N-*CH*$_2$-CO_2, rotamer 2), 3.88 (s, 1.1H, N-*CH*$_2$-CON, rotamer 2), 3.86 (s, 0.9H, N-*CH*$_2$-CON, rotamer 1), 3.55 (t, *J* = 4.8, 1H, N_3-*CH*$_2$-CH_2, rotamer 1), 3.47 (t, *J* = 4.8, 1H, N_3-CH2-*CH*$_2$, rotamer 1), 3.41 (t, *J* = 5.4, 1H, N_3-*CH*$_2$-CH_2, rotamer 2), 3.36 (t, *J* = 5.4, 1H, N_3-CH_2-*CH*$_2$-N, rotamer 2), 1.75 (s, 3H, C- *CH*$_3$); ^{13}C NMR (100 MHz, D6-DMSO, 25 °C) δ 171.3, 170.8, 168.4, 167.8, 164.9, 151.4, 142.7, 142.6, 108.6, 50.2, 49.4, 48.7, 48.3, 47.0, 46.8, 12.3; HRMS (ESI) calculated for $C_{11}H_{14}N_6O_5$ (MH^+): 311.1020, found: 311.1075.

***N*-(2-Azidoethyl)-*N*-[4-*N*-(benzhydryloxycarbonyl)cytosine-1-acetyl]glycine 5-C.** A solution of benzyl ester **4-C** (21.0 g, 35.2 mmol, 1.0 eq) in 1,4-dioxane (35 mL) at room temperature was treated with NaOH 2 M (70.4 mL, 140.8 mmol, 4.0 eq). After 15 min, TLC analysis indicated that the reaction had proceeded to completion and the solution was acidified until pH 3 with 20% aq citric acid which resulted in the precipitation of the desired acid. Filtration followed by drying *in vaccuo* afforded 16.8 g of acid **5-C** as pure white powder (95% yield). R_f = 0.19 (30% MeOH in EtOAc); FT-IR (neat) ν_{max} 3227.5, 3029.7, 2106.2, 1740, 1633.8, 1572, 1507 cm^{-1}; ^1H NMR (400 MHz, D6-DMSO, 25 °C) δ 7.94 (d, 0.6H, *J* = 7.5, *C*$_6$, rotamer 1), 7.90 (d, 0.4H, *J* = 7.5, *C*$_6$, rotamer 2), 7.47 (d, *J* = 7.5, 4H, Ph), 7.38 (t, *J* = 7.5, 4H, Ph), 7.32 (t, 2H, *J* = 7.5, Ph), 6.96 (d, 0.6H, *J* = 7.5, *C*$_5$, rotamer 1), 6.95 (d, 0.4H, *J* = 7.5, *C*$_5$, rotamer 2), 6.81 (s, 1H, O-*CH*-$(C_6H_5)_2$), 4.87 (s, 1.2H, N-*CH*$_2$-CO, rotamer 1), 4.66 (s, 0.8H, N-*CH*$_2$-CO, rotamer 2), 4.03 (s, 1.2H, N-*CH*$_2$-CO, rotamer 2), 4.32 (s, 0.8H, N-*CH*$_2$-CO, rotamer 1), 3.66 (t, *J* = 4.8, 1.2H, N_3-*CH*$_2$-CH_2 rotamer 1), 3.61 (t, *J* = 4.8, 1.2H, CH_2-*CH*$_2$-N, rotamer 1), 3.50 (t, *J* = 5.4, 0.8H, N_3-*CH*$_2$-CH_2, rotamer 2), 3.45 (t, *J* = 5.4, 0.8H, CH_2-*CH*$_2$-N, rotamer 2); ^{13}C NMR (100 MHz, D6-DMSO, 25 °C) δ 171.2, 170.8, 168.2, 167.7, 163.5, 155.4, 152.8, 151.4, 143.0, 140.8, 129.0, 126.9, 128.5, 94.3, 77.9, 50.1, 50.1, 49.5, 49.7, 47.2, 46.8; HRMS (ESI) calculated for $C_{24}H_{23}N_7O_6$ (MH^+): 506.1704, found: 506.1606.

***N*-(2-Azidoethyl)-*N*-[6-*N*-(benzhydryloxycarbonyl)adenine-9-acetyl]glycine 5-A.** To a solution of benzyl ester **4-A** (0.25 g, 0.39 mmol, 1.0 eq) in dioxane (1 mL) at room temperature was added NaOH 2M (0.79 mL, 1.58 mmol, 4.0 eq). After 15 min, all starting material had been consumed as jugged by TLC and the desired compound was precipitated by acidification to pH 3 with 20% aq citric acid. Filtration of the white precipitate and drying *in vaccuo* afforded 0.16 g of acid **5-A** as a pure white solid (76 % yield). R_f = 0.14 (30% MeOH in EtOAc); FT-IR (neat) ν_{max} 3031.8, 2925.9, 2103.4, 1763.6, 1671.1, 1616 cm^{-1}; ^1H NMR (400 MHz, D6-DMSO, 25 °C) δ 8.61 (s, 0.6H, *C*$_2$, rotamer 1), 8.60 (s, 0.4H, *C*$_2$, rotamer 2), 8.39 (s, 0.6H, *C*$_8$, rotamer 1), 8.36 (s, 0.4H, *C*$_8$, rotamer 2), 7.54 (d, *J* = 7.5, 4H, Ph); 7.39 (t, *J* = 7.5, 4H, Ph), 7.30 (t, *J* = 7.5, 2H, Ph), 6.83 (s, 1H, O-*CH*-(C_6H_5)$_2$), 5.43 (s, 1.2H, N-*CH*$_2$-CO, rotamer 1), 5.20 (s, 0.8H, N-*CH*$_2$-CO, rotamer 2), 6: 4.39 (s, 0.8H, N-*CH*$_2$-CO, rotamer 1), 4.05 (s, 1.2H, N-*CH*$_2$-CO, rotamer 2), 3.72 (m, 2.4H, N_3-*CH*$_2$-*CH*$_2$-N, rotamer 1), 3.50 (t, *J* = 5.4, 0.8H, N_3-*CH*$_2$-CH_2, rotamer 2), 3.46 (t, *J* = 5.4, 0.8H, CH_2-*CH*$_2$-N, rotamer 2); ^{13}C NMR (100 MHz, D6-DMSO, 25 °C) δ 171.3, 170.8, 167.8, 167.2, 152.8, 152.0, 151.6, 149.7, 145.7, 141.3, 128.9, 128.1, 126.9, 123.1, 77.7, 49.5, 48.7, 48.3, 47.2, 46.9, 44.5; HRMS (ESI) calculated for $C_{25}H_{23}N_9O_5$: (MH^+): 530.1817, found: 530.1787.

***N*-(2-Azidoethyl)-*N*-[2-*N*-(benzhydryloxycarbonyl)-guanine-9-acetyl]glycine 5-G.** To a solution of benzyl ester **4-G** (0.18 g, 283 μmol, 1.0 eq) in 1,4-dioxane (725 μL) was added NaOH 2 M (566 μL, 1.13 mmol, 4.0 eq). After 20 min, the solution was acidified until pH 3 with 20% aq citric acid, were the compound precipited. Filtration of the white precipitate and drying *in vacuo* afforded 123 mg of desire acid **5-G** as a white solid (80% yield). R_f = 0.11(30% MeOH in EtOAc); FT-IR (neat) ν_{max} 3407, 3237.4, 2946.1, 2103.4, 1678.3, 1611.9, 1572 cm^{-1}; ^1H NMR (400 MHz, D6-DMSO, 25 °C) δ 7.87 (s, 0.6H, *C*$_8$, rotamer 1), 8.83 (s, 0.4H, *C*$_8$, rotamer 2), 7.48 (d, *J* = 7.5, 4H, Ph), 7.40 (t, *J* = 7.5, 4H, Ph), 7.32 (t, *J* = 7.5, 2H, Ph), 6.88 (s, 1H, O-*CH*-(C_6H_5)$_2$), 5.18 (s, 1.2H, N-*CH*$_2$-CON, rotamer 1), 4.98 (s, 0.8H, N-*CH*$_2$-CON, rotamer 2), 4.40 (s, 0.7H, N-*CH*$_2$-CO_2, rotamer 2), 4.05 (s, 1.3H, N-*CH*$_2$-CO_2, rotamer 1), 3.71 (t, *J* = 4.8, 1.2H, N_3-*CH*$_2$-CH_2, rotamer 1), 3.66 (t, *J* = 4.8, 1.2H, N_3-CH_2-*CH*$_2$, rotamer 1), 3.48 (t, *J* = 5.4, 0.8H, N_3-

CH$_2$-CH$_2$, rotamer 2), 3.45 (t, *J* = 5.4, 0.8H, CH$_2$-*CH*$_2$-N, rotamer 2); ^{13}C NMR (100 MHz, D6-DMSO, 25 °C) δ 171.2, 170.7, 169.5, 167.7, 167.2, 155.5, 154.2, 146.9, 149.7, 147.4, 147.6, 141.1, 140.9, 140.5, 140.4, 129.0, 128.4, 126.9, 78.5, 49.7, 49.4, 48.7, 48.3, 47.1, 46.9, 44.8, 44.4; HRMS (ESI) calculated for $C_{25}H_{23}N_9O_6$: (MH^+): 546.1766, found: 546.1713.

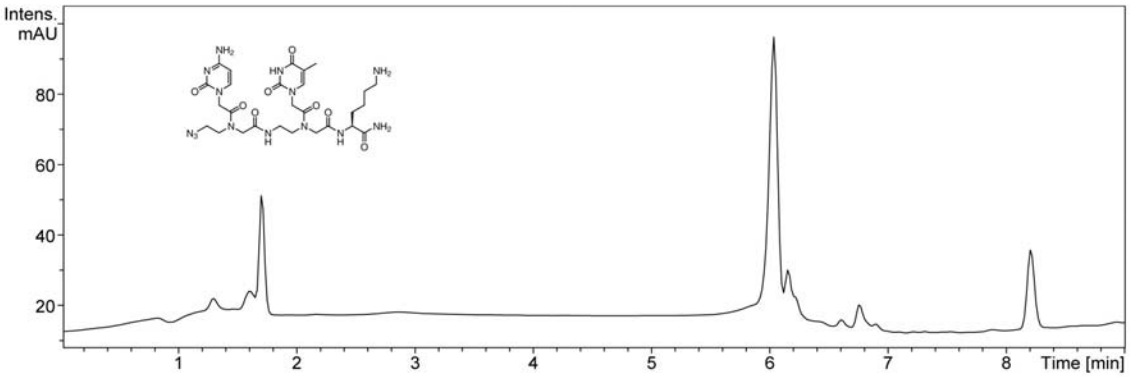
General procedure for oligomerization of azidoPNA.

Azide reduction. The resin was treated with a 1 M PMe_3 : H_2O (9:1; 1 mL/ g of resin) for 5 min then washed with THF (3 x 1 mL / g of resin) and treated immediately with the pre-activated solution of acid **5**.

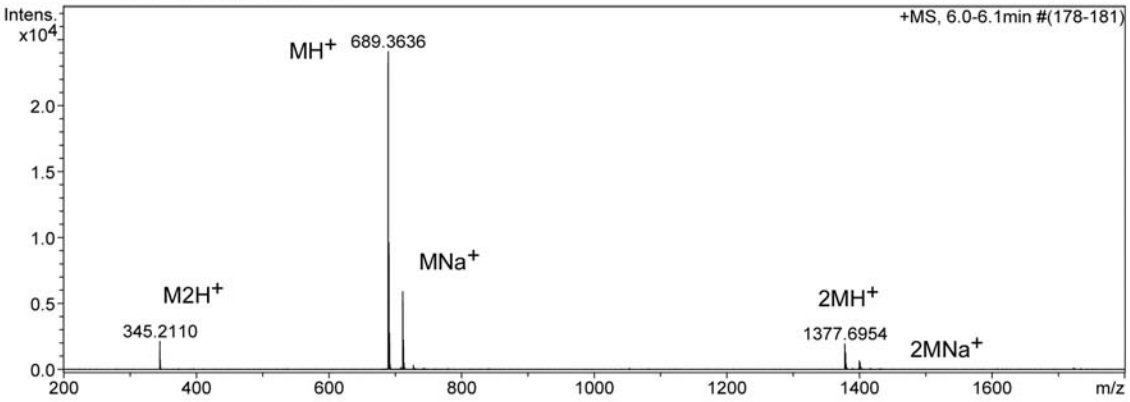
Activation-coupling. A solution of acid **5** (3.0 eq) in DMF (0.1 M) was treated with DIC (2.5 eq) and HOBt (3.0 eq) and agitated for 1 h prior to reaction. The activated acid solution was added to the freshly reduced or otherwise deprotected resin and the reaction was allowed to proceed for 1 h.

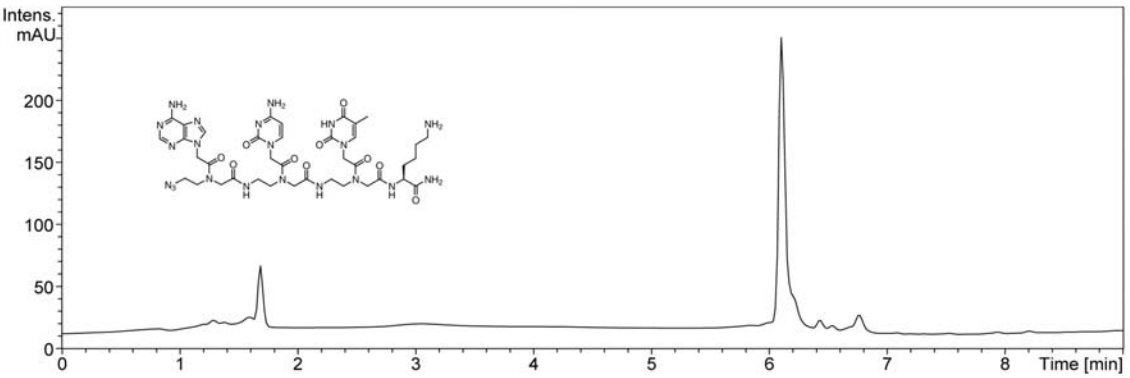
Cleavage. A sample of resin was treated with TFA: *m*cresol (9:1) for 1 h then precipitated in Et_2O and collected by centrifugation.

Synthesis of 6-mer PNA 14. Rink Amide resin loaded with Boc-protected lysine **13** was prepared using standard Fmoc chemistry. The PNA oligomerization was performed on 35 μmol according to the general procedure described above with double coupling at each step and capping with Ac_2O/pyridine (4.0 eq, 0.1 M in DMF for 5 min). An analytical sample was removed and cleaved at each step and analyzed by LC-MS. HRMS (ESI) calculated for: K-T (MH^+): 438.2130, found 438.2650; K-TC (MH^+): 689.3148, found 689.3639; K-TCA (MH^+): 964.4278, found 964.4671; K-TCAG (MH^+): 1255.5359, found 1255.5809; K-TCAGC (MH^+): 1506.6377, found 1506.6977; K-TCAGCT ($M2H^+$): 887.3696, found (M-C13-H^{++}) 887.4299.



UV Chromatogram, 254 nm





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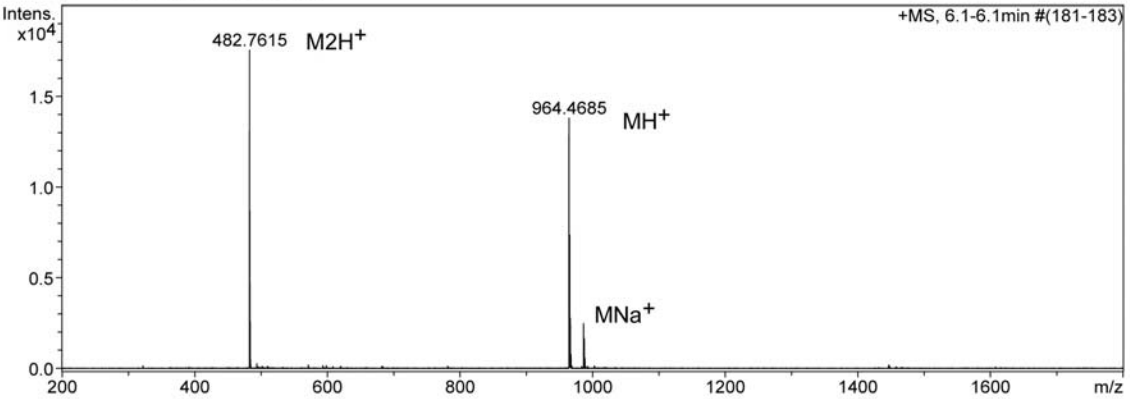

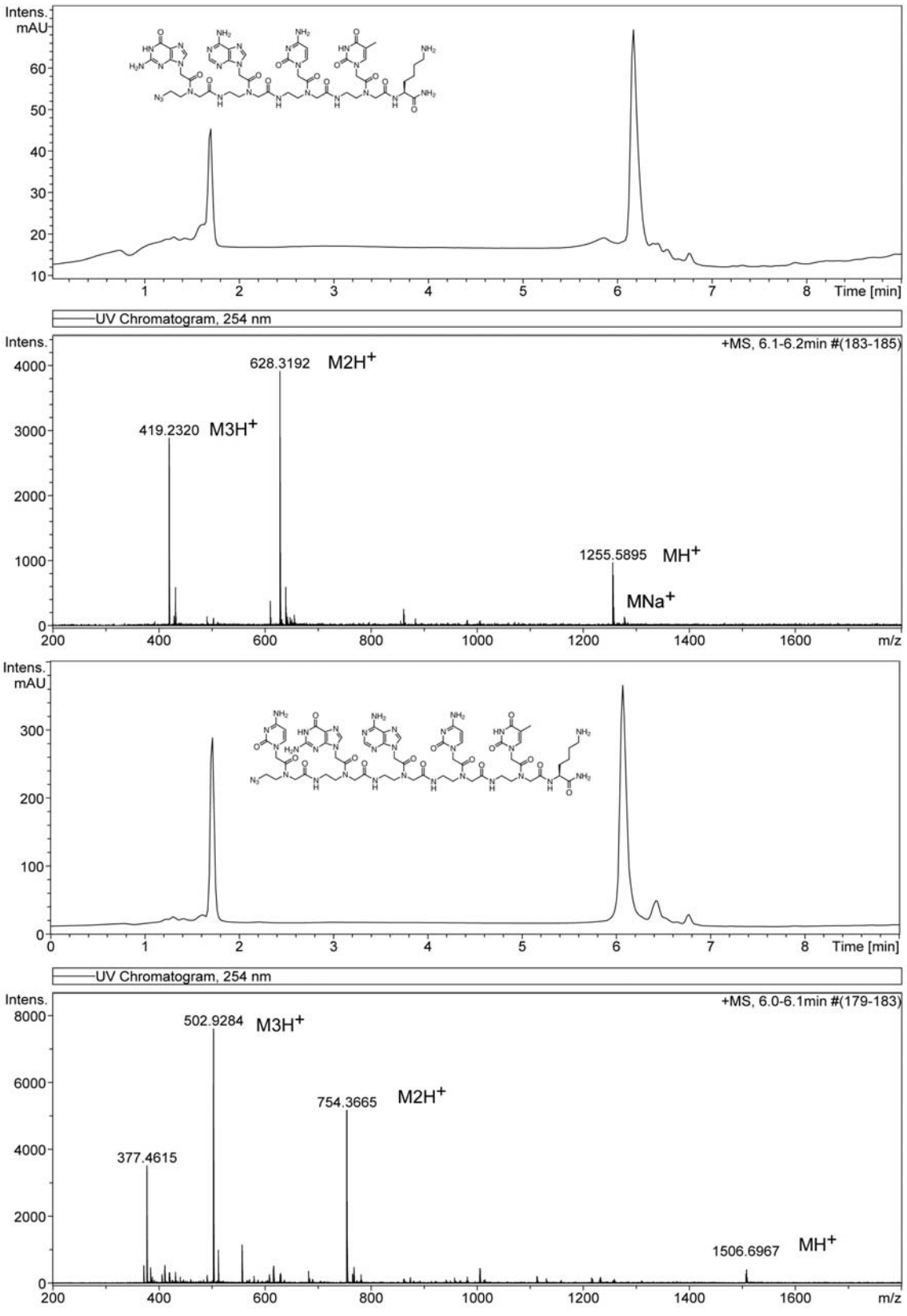
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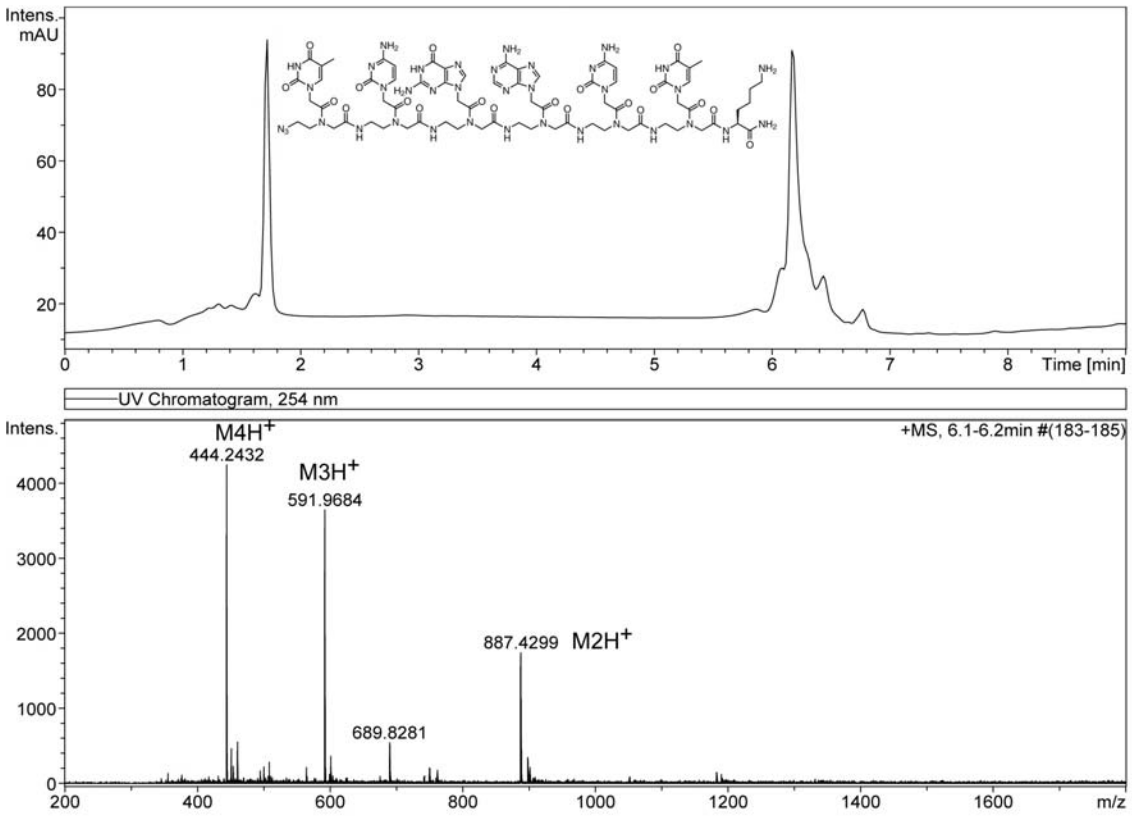
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