Supplementary Material (ESI) for Chemical Communications

Clickable Peptide Nucleic Acids (cPNA) with tunable affinity

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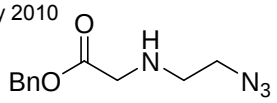
Supplementary information

General Techniques. All reactions were carried out under a nitrogen atmosphere with dry solvents under anhydrous conditions, unless otherwise noted. Anhydrous solvents were obtained by passing them through a commercially available alumina column (Innovative technology, MA). Reactions were monitored by TLC carried out on 0.25 mm E. Merck silicagel plates (60F-254) by using UV light as visualizing agent and ninhydrin or vanillin solution and heat as developing agents; or by LC-MS recorded using an Agilent 1100 HPLC. Unless otherwise stated, a Supelco C8 (5 cm x 4.6mm, 5 μm particles) column was used with a linear elution gradient from 95% H_2O (0.5% HCO_2H) to 100% MeCN in 8 min at a flow rate of 0.5 mL/min.E. Merck silica gel (60, particle size 0.040–0.063 mm) was used for flash-column chromatography. NMR spectra were recorded on a Bruker Avance-400 (400 MHz for ^1H, 100 MHz for ^{13}C) instrument and calibrated by using residual undeuterated solvent as an internal reference. The following abbreviations were used to explain the multiplicities: s=singlet, d= doublet, t= triplet, m=multiplet, dt= doublet of triplet and bs=broad singlet. MALDI spectra were measured using Bruker Daltonics AutoflexII TOF/TOF spectrometer. The samples were prepared using 2,5-dihydroxybenzoic acid (DHB) as a matrix. All the PNAs were synthesized by a Multipep RS Intavis AG bioanalytical instrument. Absorption spectra were recorded on a Uvicon XL spectrophotometer; melting curves were recorded at a 260-nm wavelength. The hybridization of PNA was monitored on Schott A+MDX 16 microarrays; hybridization data were mesured by Genepix personal 4100A (AXON/MDC) and analyzed with GenepixPro 6.0 software.

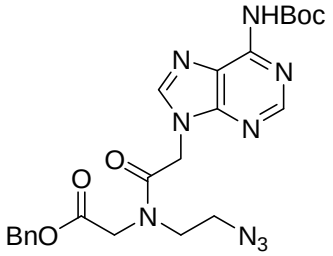
Abbreviations:

DBU = 1,8-Diazabicyclo[5.4.0]undec-7-ene
DMF = dimethylformamide,
HBTU = 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
Mtt = 4-Methyltrityl.
Cy3 = Carboxyl-indocarbocyanine
DCE = 1,2-dichloroethane
DIPEA = *N,N*-diisopropylethylamine
Lys(Fmoc)NHBoc = N-α-Fmoc-N-ε-t-Boc-L-lysine
NMP = *N*-methyl-2-pyrrolidone
TBTA = tris-(benzyltriazolylmethyl)amine
TFA = trifluoroacetic acid
THPTA = tris-(hydroxypropyltriazolylmethyl) amine
TNTU = 2-(5-norbornene-2,3-dicarboximido)-1,1,3,3-tetramethyluronium tetrafluoroborate



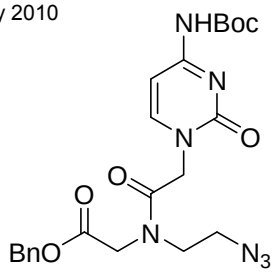
1

Azide backbone: To a solution of 2-aminoethylbromide hydrobromide (1.0 equiv, 10.0 g, 48.8 mmol,) in DMF (25 mL) was added NaN_3 (1.1 equiv, 3.49 g, 53.7 mmol) and the reaction mixture was heated to 60 ºC. After 3 hours, the reaction was cooled to 0 ºC and triethylamine (2.0 equiv, 13.7 mL, 97.6 mmol) was added followed by benzyl 2-bromoacetate (0.8 equiv, 6.21 mL, 39.0 mmol). The reaction mixture was stirred for 2 more hours at 0 ºC then diluted with Et_2O (200 mL) and washed with brine (200 mL). The organic layer was dried over Na_2SO_4 and concentrated *in vaccuo*. The compound was then purified by flash chromatography (30% EtOAc/petroleum ether to 100% EtOAc) to obtain **1** as a yellowish oil in 64% yield (5.87 g). R_f = 0.70 (EtOAc); ^{1}H NMR (400 MHz, DMSO-d6, 25 °C): δ = 7.40-7.36 (m, 5 H), 5.16 (s, 2 H), 3.46 (s, 2 H), 3.34 (t, J = 5.9 Hz, 2 H), 2.78 (t, J = 5.9 Hz, 2 H) ppm; ^{13}C NMR (100 MHz, DMSO-d6, 25 °C): δ = 172.5, 136.5, 128.8, 128.5, 128.4, 65.9, 50.9, 50.3, 48.1 ppm.



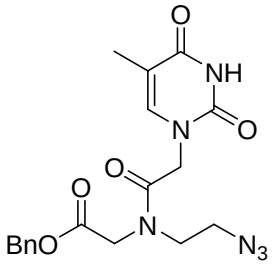
24A

Benzyl-protected azide 24A: To a solution of Boc-protected adenine acetic acid[1] (1.0 equiv, 0.63 g, 2.13 mmol) in DMF (5.0 mL) were added triethylamine (1.0 equiv, 0.30 mL, 2.13 mmol) and HBTU (1.0 equiv, 0.81 g, 2.13 mmol) at 23 °C. After stirring the reaction mixture for 20 min, a solution of azide backbone **1** (1.0 equiv, 0.50 g, 2.13 mmol) in DMF (2.0 mL) was added followed by triethylamine (1.0 equiv, 0.30 mL, 2.13 mmol) and the reaction mixture was stirred overnight at 23 °C. The reaction was quenched with water (8.0 mL), stirred for 20 min, and extracted with EtOAc (3 x 10 mL), then the combined organic layers were washed with brine (20 mL), dried over Na_2SO_4, and concentrated *in vaccuo*. The crude was purified by flash chromatography (50% petroleum ether/EtOAc to 5% MeOH/EtOAc) to afford **24A** as a pure white solid in 45% yield (0.48 g). R_f = 0.22 (EtOAc); ^{1}H NMR (400 MHz, DMSO-d6, 25 °C): δ = 8.56 (s, 2 H), 8.33 (s, 1.3 H), 8.28 (s, 0.7 H), 7.39-7.32 (m, 10 H), 5.44 (s, 2.6 H), 5.26 (s, 1.4 H), 5.23 (s, 1.4 H), 5.11 (s, 2.6 H), 4.61 (s, 1.4 H), 4.20 (s, 2.6 H), 3.73 (bs, 5.2 H), 3.52 (t, J = 5.8 Hz, 1.4 H), 3.44 (t, J = 5.8 Hz, 1.4 H), 1.47 (s, 18 H) ppm; ^{13}C NMR (100 MHz, DMSO-d6, 25 °C): δ = 169.3, 168.8, 167.4, 167.1, 152.2, 151.5, 151.1, 149.7, 144.9, 144.8, 135.7, 135.5, 128.5, 128.4, 128.2, 128.2, 128.0, 127.8, 127.6, 122.9, 80.0, 66.7, 65.9, 49.5, 49.1, 48.3, 48.1, 47.0, 46.5, 44.0, 27.8 ppm.



24C

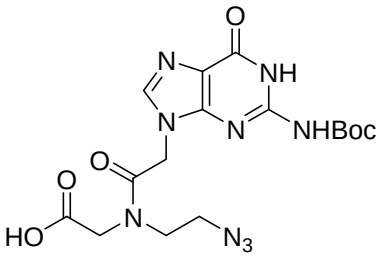
Benzyl-protected azide 24C: To a solution of Boc-protected cytosine acetic acid[1] (1.0 equiv, 1.15 g, 4.27 mmol) in DMF (10 mL) were added triethylamine (1.0 equiv, 0.59 mL, 4.27 mmol) and HBTU (1.0 equiv, 1.62 g, 4.27 mmol) at 23 °C. After stirring the reaction mixture for 20 min, a solution of azide backbone **1** (1.0 equiv, 1.00 g, 4.27 mmol) in DMF (3.0 mL) was added followed by triethylamine (1.0 equiv, 0.59 mL, 4.27 mmol) and the reaction mixture was stirred overnight at 23 °C. The reaction was quenched with water (10 mL), stirred for 20 min, and extracted with EtOAc (3 x 20 mL), then the combined organic layers were washed with brine (40 mL), dried over Na_2SO_4, and concentrated *in vaccuo*. The crude was purified by flash chromatography (50% petroleum ether/EtOAc to 5% MeOH/EtOAc) to afford **24C** as a pure white solid in 65% yield (1.33 g). R_f= 0.35 (EtOAc); ^{1}H NMR (400 MHz, DMSO-d6, 25 °C): δ = 7.88 (d, J = 7.4 Hz, 1.3 H), 7.81 (d, J = 7.4 Hz, 0.7 H), 7.43-7.29 (m, 10 H), 6.98-6.96 (m, 2 H), 5.20 (s, 1.4 H), 5.12 (s, 2.6 H), 4.86 (s, 2.6 H), 4.66 (s, 1.4 H), 4.50 (s, 1.4 H), 4.17 (s, 2.6 H), 3.67-3.60 (m, 5.2 H), 4.49 (t, J = 5.8 Hz, 1.4 H), 3.41(t, J = 5.8 Hz, 1.4 H), 1.45 (s, 18 H) ppm; ^{13}C NMR (100 MHz, DMSO-d6, 25 °C): δ = 169.3, 168.8, 167.9, 167.6, 163.3, 155.0, 152.1, 150.5, 150.4, 135.8, 135.5, 128.5, 128.4, 128.2, 128.1, 128.0, 127.8, 93.9, 80.9, 66.6, 65.9, 49.6, 49.2, 48.3, 48.1, 46.9, 46.3, 27.8 ppm.



24T

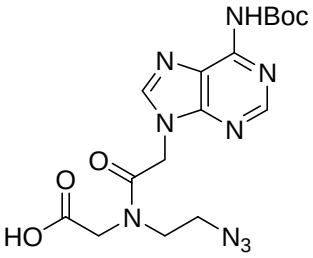
Benzyl-protected azide 24T: To a solution of thymine-1-acetic acid (1.0 equiv, 1.26 g, 6.83 mmol) in DMF (15 mL) were added triethylamine (1.0 equiv, 0.95 mL, 6.83 mmol) and HBTU (1.0 equiv, 2.59 g, 6.83 mmol) at 23 °C. After stirring the reaction mixture for 20 min, a solution of azide backbone **1** (1.0 equiv, 1.60 g, 6.83 mmol) in DMF (4.0 mL) was added followed by triethylamine (1.0 equiv, 0.95 mL, 6.83 mmol) and the reaction mixture was stirred overnight at 23 °C. The reaction was quenched with water (20 mL), stirred for 20 min, and extracted with EtOAc (3 x 30 mL), then the combined organic layers were washed with brine (60 mL), dried over Na_2SO_4, and concentrated *in vaccuo*. The crude was purified by flash chromatography (50% petroleum ether/EtOAc to 100% EtOAc) to afford **24T** as a pure white solid in 77% yield (2.10 g). R_f = 0.45(EtOAc); ^{1}H NMR (400 MHz, DMSO-d6, 25 °C): δ = 7.43-7.30 (m, 11.3 H), 7.22 (s, 0.7 H), 5.21 (s, 1.4 H), 5.12 (s, 2.6 H), 4.72 (s, 2.6 H), 4.52

(s, 1.4 H), 4.46 (s, 1.4 H), 4.16 (s, 2.6 H), 3.63-3.58 (m, 5.2 H), 3.49 (t, J = 6.1 Hz, 1.4 H), 3.41 (t, J = 6.1 Hz, 1.4 H), 1.74 (s, 6 H) ppm; ^{13}C NMR (100 MHz, DMSO-d6, 25 °C): δ = 169.3, 168.8, 167.9, 167.6, 164.4, 150.9, 142.1, 141.9, 135.7, 135.5, 128.5, 128.4, 128.2, 128.1, 128.0, 127.8, 108.2, 108.1, 66.6, 65.9, 49.0, 48.3, 48.0, 47.8, 46.8, 46.3, 11.8 ppm.



3G

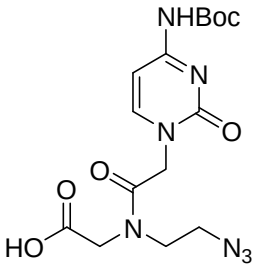
Benzyl-deprotected azide 3G: To a solution of Boc-protected guanine acetic acid[1] (1.0 equiv, 0.66 g, 2.13 mmol) in DMF (5.0 mL) were added triethylamine (1.0 equiv, 0.30 mL, 2.13 mmol) and HBTU (1.0 equiv, 0.81g, 2.13 mmol) at 23 °C. After stirring the reaction mixture for 20 min, a solution of azide backbone **1** (1.0 equiv, 0.50 g, 2.13 mmol) in DMF (2.0 mL) was added followed by triethylamine (1.0 equiv, 0.30 mL, 2.13 mmol) and the reaction mixture was stirred overnight at 23 °C. The reaction was quenched with water (10 mL) and the resulting precipitate was filtered and dried *in vaccuo* (0.49g), this residue was used in the next step without any further purification. To a solution of **24G** precipitate (1.0 equiv, 0.49 g, 0.93 mmol) in 1,4-dioxane (5.0 mL) was added a 2M NaOH solution (4.0 equiv, 1.86 mL, 3.73 mmol) and the resulting mixture was stirred at 23 °C for 2 hours. The solution was acidified until pH 3 with 20% aq citric acid and the resulting precipitate was filtered and dried under high vacuum to afford **3G** as a pure white solid in 50% yield over two steps (0.34 g). ^{1}H NMR (400 MHz, DMSO-d6, 25 °C): δ = 7.83 (s, 1.2 H), 7.78 (s, 0.8 H), 5.12 (s, 2.4 H), 4.92 (s, 1.6 H), 4.36 (s, 1.6 H), 4.02 (s, 2.4 H), 3.68-3.63 (m, 4.8 H), 3.47-3.42 (m, 3.2 H), 1.47(s, 18 H) ppm; ^{13}C NMR (100 MHz, DMSO-d6, 25 °C): δ = 170.7, 170.2, 167.3, 166.7, 155.1, 153.7, 149.5, 147.5, 140.4, 140.2, 118.9, 82.4, 49.3, 48.9, 48.2, 47.8, 46.7, 46.4, 43.9, 27.7 ppm.



3A

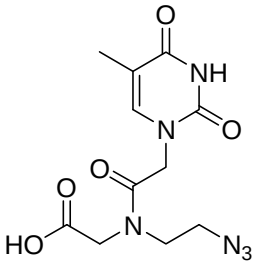
Benzyl-deprotected azide 3A: To a solution of benzyl-protected azide **24A** (1.0 equiv, 0.3 g, 0.58 mmol) in 1,4-dioxane (1.0 mL) was added a 2M NaOH solution (4.0 equiv, 1.17 mL, 1.6 mmol) and the resulting mixture was stirred at 23 °C for 2 hours. The solution was acidified until pH 3 with 20% aq citric acid. The reaction mixture was extracted with EtOAc (3 x 3 mL), and the combined organic layers were washed with brine (5.0 mL), dried over Na_2SO_4, and concentrated *in vaccuo*. The crude was purified by reverse phase chromatography (C18:

100% H_2O to 30% H_2O/CH_3CN) and lyophilized to afford **3A** as a pure white solid in 55% yield (1.37 g). ^{1}H NMR (400 MHz, DMSO-d6, 25 °C): δ = 10.05 (bs, 2 H), 8.54 (s, 1.2 H), 8.53 (s, 0.8 H), 8.32 (s, 1.2 H), 8.30 (s, 0.8 H), 5.39 (s, 2.4 H), 5.16 (s, 1.6 H), 4.41 (s, 1.6 H), 4.03 (s, 2.4 H), 3.72- 3.67 (m, 4.8 H), 3.48- 3.43 (m, 3.2 H), 1.47 (s, 18 H) ppm; ^{13}C NMR (100 MHz, DMSO-d6, 25 °C): δ = 170.6, 170.2, 167.3, 166.8, 152.2, 151.4, 151.1, 149.6, 145.0, 144.9, 122.9, 80.0, 49.1, 49.0, 48.2, 47.8, 46.8, 46.4, 43.9, 27.8 ppm.



3C

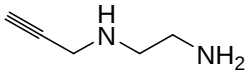
Benzyl-deprotected azide 3C: To a solution of benzyl-protected azide **24C** (1.0 equiv, 0.20 g, 0.41 mmol) in 1,4-dioxane (1.0 mL) was added a 2M NaOH solution (4.0 equiv, 0.82 mL, 1.65 mmol) and the resulting mixture was stirred at 23 °C for 1 hour. The solution was acidified until pH 3 with 20% aq citric acid and the resulting precipitate was filtered and dried under high vacuum to afford **3C** as a pure white solid in 61% yield (115 mg). ^{1}H NMR (400 MHz, DMSO-d6, 25 °C): δ = 10.27 (bs, 2 H), 7.98-7.83 (m, 2 H), 6.97-6.94 (m, 2 H), 4.83 (s, 2.4 H), 4.61 (s, 1.6 H), 4.29 (s, 1.6 H), 4.01 (s, 2.4 H), 3.65- 3.56 (m, 4.8 H), 3.47- 3.42 (m, 3.2 H), 1.45 (s, 18 H) ppm; ^{13}C NMR (100 MHz, DMSO-d6, 25 °C): δ = 170.6, 170.2, 167.8, 167.3, 163.1, 154.9, 152.1, 150.5, 150.4, 93.9, 93.8, 80.8, 49.4, 49.3, 49.0, 48.3, 47.8, 46.7, 46.3, 27.7 ppm.



3T

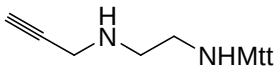
Benzyl-deprotected azide 3T: To a solution of benzyl-protected azide **24T** (1.0 equiv, 1.05 g, 2.62 mmol) in 1,4-dioxane (2.5 mL) at room temperature was added a 2M NaOH solution (4.0 equiv, 5.24 mL, 10.5 mmol) and the resulting mixture was stirred at 23 °C for 1 hour. The solution was acidified until pH 3 with 20% aq citric acid and the resulting precipitate was filtered and dried under high vacuum to afford **3T** as a pure white solid in 80% yield (654 mg). ^{1}H NMR (400 MHz, DMSO-d6, 25 °C): δ = 11.22 (bs, 2 H), 7.34 (s, 1 H), 7.26 (s, 1 H), 4.68 (s, 2 H), 4.46 (s, 1 H), 4.05 (s, 2H), 3.98 (s, 2 H), 3.61 (t, J = 5.7 Hz, 2 H), 3.53 (t, J =

5.7 Hz, 2 H), 3.45- 3.39 (m, 4 H), 1.75 (s, 6 H) ppm; ^{13}C NMR (100 MHz, DMSO-d6, 25 °C): δ = 170.3, 170.2, 167.8, 167.3, 164.3, 150.9, 142.2, 142.0, 108.0, 107.9, 50.2, 48.9, 48.2, 47.8, 47.7, 46.5, 46.3, 11.8 ppm.



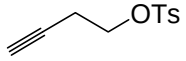
25

t1-Alkyne backbone 25: To a solution of propargyl chloride (1.0 equiv, 1.94 mL, 26.84 mmol) and DBU (1.0 equiv, 3.82 mL, 26.8 mmol) in toluene (15 mL) was added a solution of ethylene diamine (10 equiv, 18.0 mL, 268.4 mmol) in toluene (15 mL). The reaction was stirred for 4 hours at 23 °C. The mixture was concentrated *in vaccuo* and the product was isolated distillation (between 80 °C and 140 °C at 2 mbar) to afford **25** as a colorless oil in 52% yield (1.37 g). ^{1}H NMR (400 MHz, DMSO-d6, 25 °C): δ = 3.25 (d, J = 2.4 Hz, 2H), 3.01 (t, J = 2.4 Hz, 1 H), 2.60-2.57 (m, 2 H), 2.55-2.49 (m, 2 H), 1.54 (bs, 3 H) ppm; ^{13}C NMR (100 MHz, DMSO-d6, 25 °C): δ = 83.2, 73.2 , 51.3, 41.2, 37.3 ppm.



4

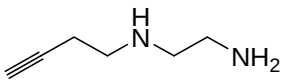
Mtt-protected t1-alkyne backbone 4: To a solution of t1-alkyne backbone **25** (1.0 equiv, 1.10 g, 11.21 mmol) and triethylamine (2.0 equiv, 3.11 mL, 22.4 mmol) in CH_2Cl_2 (6.0 mL) cooled in a water bath was added dropwise a solution of 4-methyltrityl chloride (1.3 equiv, 4.26 g, 14.57 mmol) in CH_2Cl_2 (6.0 mL) and the reaction mixture was stirred for 5 hours at 23 °C. The reaction was quenched with water (10 mL), stirred for 20 min, and extracted with CH_2Cl_2 (3 x 10 mL), then the combined organic layers were washed with brine (20 mL), dried over Na_2SO_4, and concentrated *in vaccuo*. The crude was purified by flash chromatography (EtOAc/petroleum ether 1:6 to 1:2) to afford **4** as a yellowish oil in 60% yield (2.37 g). R_f = 0.36 (petroleum ether/EtOAc 2:1); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.54 (d, J = 7.7 Hz, 4 H), 7.41 (d, J = 8.2 Hz, 2 H), 7.31 (t, J = 7.7 Hz, 4 H), 7.21 (t, J = 7.2 Hz, 2 H), 7.13 (d, J = 8.2 Hz, 2 H), 3.41 (d, J = 2.4 Hz, 2 H), 2.87 (t, J = 5.6 Hz, 2 H), 2.38-2.35 (m, 5 H), 2.24 (t, J = 2.4 Hz, 1 H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 146.3, 143.1, 135.7, 128.6, 128.5, 128.4, 127.7, 126.1, 82.3, 71.2, 70.5, 48.9, 42.9, 38.0, 20.9 ppm.



26

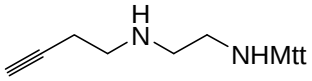
Tosyl-protected homopropargyl alcohol 26: To a solution of 3-butynol (1.0 equiv, 3.24 ml, 42.8 mmol), DMAP (0.01 equiv, 51.3 mg, 0.42 mmol) and triethylamine (1.3 equiv, 7.7 mL, 55.4 mmol) in CH_2Cl_2 (15 ml) at 0 °C was added a solution of *p*-toluenesulfonyl chloride (1.1

equiv, 8.97 g, 47.0 mmol) in CH_2Cl_2 (15 mL) and the reaction mixture was stirred for 4 hours at 23 °C. The reaction was quenched with water (30 mL), stirred for 20 min, and extracted with CH_2Cl_2 (3 x 40 mL), the combined organic layers were washed with brine (60 mL), dried over Na_2SO_4, and concentrated *in vaccuo* to afford **26** as a reddish-brown oil in 99% yield (9.51 g). R_f = 0.52 (petroleum ether/EtOAc 2:1); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.81 (d, J = 8.2 Hz, 2 H), 7.36 (d, J = 8.2 Hz, 2 H), 4.11 (t, J = 7.0 Hz, 2 H), 2.56 (dt, J = 7.0, 2.6 Hz, 2 H), 2.46 (s, 3 H), 1.97 (t, J = 2.6 Hz, 1 H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 145.0, 132.8, 129.9, 127.9, 78.3, 70.7, 67.4, 21.6, 19.4 ppm.



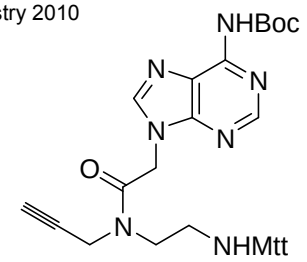
27

t2-Alkyne backbone: To a solution of tosylated alcohol **26** (1.0 equiv, 7.30 g, 32.5 mmol) and DBU (1.0 equiv, 4.63 mL, 32.5 mmol) in toluene (10 mL) was added a solution of ethylene diamine (10 equiv, 21.7 mL, 325 mmol) in toluene (10 mL) at 23 °C and stirred for 4 hours at the same temperature. The reaction mixture was then concentrated *in vaccuo* and purified by distillation (between 70° C and 120° C at 2 mbar) to afford **27** as a colorless oil in 53 % yield (1.90 g). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 2.81-2.76 (m, 4 H), 2.67 (t, J = 5.8 Hz, 2 H), 2.38 (dt, J = 6.6, 2.6 Hz, 2 H), 1.99 (t, J = 2.6 Hz, 1 H), 1.33 (bs, 3 H) ppm.



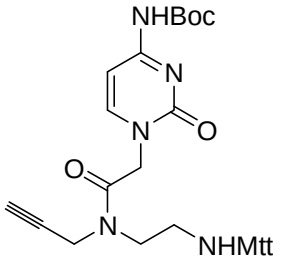
5

Mtt-protected t2-alkyne backbone 5: To a solution of t2 alkyne backbone **27** (1.0 equiv, 0.82 g, 7.31 mmol) and triethylamine (2.0 equiv, 2.04 mL, 14.6 mmol) in CH_2Cl_2 (9.0 mL) cooled in a water bath was added dropwise a solution of 4-methyltrityl chloride (1.3 equiv, 2.78 g, 9.50 mmol) in CH_2Cl_2 (12 mL) and the reaction mixture was stirred for 5 hours at 23 °C. The reaction was quenched with water (20 mL), stirred for 20 min, and extracted with CH_2Cl_2 (3 x 25 mL), then the combined organic layers were washed with brine (40 mL), dried over Na_2SO_4, and concentrated *in vaccuo*. The crude was purified by flash chromatography (EtOAc/petroleum ether 1:6 to 1:2) to afford **5** as a yellowish oil in 60% yield (1.65 g). R_f = 0.36 (EtOAc/petroleum ether 2:1); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.49 (d, J = 7.6 Hz, 4 H), 7.37 (d, J = 8.1 Hz, 2 H), 7.27 (t, J = 7.6 Hz, 4 H), 7.18 (t, J = 7.2 Hz, 2 H), 7.09 (d, J =8.1 Hz, 2 H), 2.76-2.70 (m, 4 H), 2.37 (dt, J = 6.6, 2.6 Hz, 2 H), 2.32-2.28 (m, 5 H), 1.99 (t, J = 2.6 Hz, 1 H), 1.75 (bs, 2 H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C) : δ = 146.2, 143.2, 135.6, 128.6, 128.5, 128.4, 127.7, 126.1, 82.5, 70.4, 69.4, 49.4, 47.5, 42.9, 20.9, 19.5 ppm.



6A

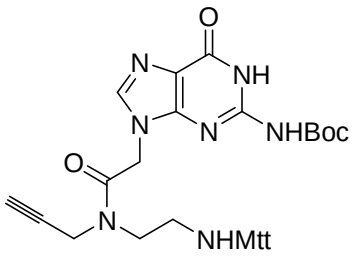
Mtt-protected t1-alkyne 6A: To a solution of Boc-protected adenine acetic acid[1] (1.0 equiv, 0.25 g, 0.85 mmol) in DMF (2.0 mL) were added triethylamine (1.0 equiv, 0.12 mL, 0.85 mmol) and HBTU (1.0 equiv, 0.32 g, 0.85 mmol) at 23 °C. After stirring the reaction mixture for 20 min, a solution of Mtt-protected t1-alkyne backbone **4** (1.0 equiv, 0.30 g, 0.85 mmol) in DMF (1.0 mL) was added followed by triethylamine (1.0 equiv, 0.12 mL, 0.85 mmol) and the reaction mixture was stirred overnight at 23 °C. The reaction was quenched with water (0.3 mL), stirred for 20 min, and extracted with EtOAc (3 x 10 mL), then the combined organic layers were washed with brine (20 mL), dried over Na_2SO_4, and concentrated *in vaccuo*. The crude was purified by flash chromatography (50% petroleum ether/EtOAc to 100% EtOAc) to afford **6A** as a pure white solid in 50% yield (478 mg). R_f = 0.49 (EtOAc); ^{1}H NMR (400 MHz, CD_3OD, 25 °C): δ = 8.55 (s, 0.7 H), 8.54 (s, 1.3 H), 8.30 (s, 0.7 H), 8.27 (s, 1.3 H), 7.56 (d, J = 7.5 Hz, 5.2 H), 7.47 (d, J = 7.5 Hz, 2.8 H), 7.42 (d, J = 8.3 Hz, 2.8 H), 7.34-7.28 (m, 9.2 H), 7.23 (t, J = 7.5 Hz, 4 H), 7.13 (t, J = 8.3 Hz, 4 H), 5.54 (s, 2.6 H), 5.45 (s, 1.4 H), 4.48 (d, J = 2.2 Hz, 1.3 H), 4.25 (d, J = 2.2 Hz, 2.6 H), 3.79 (t, J = 6.0 Hz, 2.6 H), 3.79 (t, J = 6.0 Hz, 1.3 H), 2.97 (t, J = 2.2 Hz, 0.7 H), 2.65 (t, J = 2.2 Hz, 1.3 H), 2.60 (t, J = 6.0 Hz, 2.6 H), 2.44 (t, J = 6.0 Hz, 1.4 H), 2.34 (s, 6 H), 1.66 (s, 18 H) ppm; ^{13}C NMR (100 MHz, CD_3OD, 25 °C): δ = 168.4, 168.3, 153.2, 152.4, 151.2, 151.1, 147.5, 146.1, 146.0, 144.3, 144.2, 137.1, 137.0, 129.9, 129.8, 129.7, 129.5, 129.4, 128.8, 128.7, 127.4, 127.2, 122.4, 82.7, 79.3, 79.0, 75.3, 73.7, 72.2, 71.8, 45.8, 45.6, 43.6, 43.1, 38.9, 36.3, 28.5, 20.9 ppm.



6C

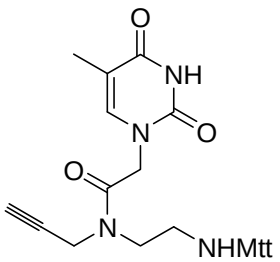
Mtt-protected t1-alkyne 6C: To a solution of Boc-protected cytosine acetic acid[1] (1.0 equiv, 0.23 g, 0.85 mmol) in DMF (2.0 mL) was added triethylamine (1.0 equiv, 0.12 mL, 0.85 mmol) and HBTU (1.0 equiv, 0.32 g, 0.85 mmol) at 23 °C. After stirring the reaction mixture for 20 min, a solution of Mtt-protected t1-alkyne backbone **4** (1.0 equiv, 0.30 g, 0.85 mmol) in DMF (1.0 mL) was added followed by triethylamine (1.0 equiv, 0.12 mL, 0.85 mmol) and the reaction mixture was stirred overnight at 23 °C. The reaction was quenched with water (3.0 mL), stirred for 20 min, and extracted with EtOAc (3 x 10 mL), then the combined organic layers were washed with brine (20 mL), dried over Na_2SO_4, and concentrated *in vaccuo*. The crude was purified by flash chromatography (50% petroleum ether/EtOAc to

100% EtOAc) to afford **6C** as a pure white solid in 80% yield (408 mg). R_f= 0.51 (EtOAc); ^{1}H NMR (400 MHz, CD_3OD, 25 °C): δ = 7.89 (d, J = 7.3 Hz, 0.7 H), 7.80 (d, J = 7.3 Hz, 1.3 H), 7.54-7.48 (m, 8 H), 7.41-7.31 (m, 14 H), 7.24 (t, J = 8.0 Hz, 4 H), 7.16 (t, J = 8.0 Hz, 4 H), 4.99 (s, 2.6 H), 4.97 (s, 1.4 H), 4.40 (d, J = 2.3 Hz, 1.4 H), 4.22 (d, J = 2.3 Hz, 2.6 H), 3.70 (t, J =6.1 Hz, 2.6 H), 3.64 (t, J =6.1 Hz, 1.4 H), 2.36 (s, 6 H), 1.6 (s, 18 H) ppm; ^{13}C NMR (100 MHz, CD_3OD, 25 °C): δ = 168.8, 168.6, 165.2, 158.5, 153.3, 151.3, 151.2, 147.4, 144.2, 137.1, 136.9, 129.9, 129.8, 129.7, 129.5, 129.4, 128.8, 128.7, 96.6, 83.2, 79.4, 79.0, 75.1, 73.6, 72.1, 71.8, 52.0, 51.6, 43.3, 43.0, 38.8, 36.1, 28.3, 21.0 ppm.



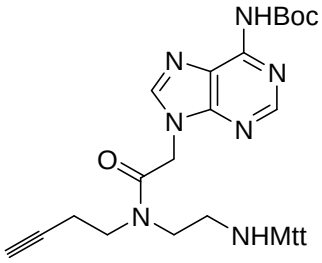
6G

Mtt-protected t1-alkyne 6G: To a solution of Boc-protected guanine acetic acid[1] (1.0 equiv, 0.78 g, 2.53 mmol) in DMF (6.0 mL) was added triethylamine (1.0 equiv, 0.35 mL, 2.53 mmol) and HBTU (1.0 equiv, 0.32 g, 2.53 mmol) at 23 °C. After stirring the reaction mixture for 20 min, a solution of Mtt-protected t1-Alkyne backbone **4** (1.0 equiv, 0.90 g, 2.53 mmol) in DMF (3.0 mL) was added followed by triethylamine (1.0 equiv, 0.35 mL, 2.53 mmol) and the reaction mixture was stirred overnight at 23 °C. The reaction was quenched with water (10 mL), stirred for 20 min, and extracted with EtOAc (3 x 20 mL), then the combined organic layers were washed with brine (40 mL), dried over Na_2SO_4, and concentrated *in vaccuo*. The crude was purified by flash chromatography (100% CH_2Cl_2 to 10% MeOH/CH_2Cl_2) to afford **6G** as a pure white solid in 87% yield (1.42 g). R_f = 0.49 (10% MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, CD_3OD, 25 °C): δ = 7.93 (s, 0.8 H), 7.87 (s, 1.2 H), 7.51-7.45 (m, 8 H), 7.38-7.29 (m, 12 H), 7.24 (t, J = 8.0 Hz, 4 H), 7.13 (t, J = 8.0 Hz, 4 H), 5.31 (s, 2.4 H), 5.29 (s, 1.6 H), 4.47 (d, J = 2.0 Hz, 1.6 H), 4.28 (d, J = 2.0 Hz, 2.4 H), 3.76 (t, J = 6.0 Hz, 2.4 H), 3.64 (t, J = 6.0 Hz, 1.6 H), 2.96 (t, J =2.0 Hz, 0.8 H), 2.68 (t, J =2.0 Hz, 1.2 H), 2.35 (s, 6 H), 1.62 (s, 10.8 H), 1.60 (s, 7.2 H) ppm; ^{13}C NMR (100 MHz, CD_3OD, 25 °C): δ = 168.6, 168.4, 157.7, 155.3, 151.6, 149.4, 147.5, 147.4, 144.3, 144.2, 142.1, 141.9, 137.2, 137.0, 129.9, 129.8, 129.7, 129.5, 129.4, 128.8, 128.7, 127.4, 127.3, 119.9, 84.6, 79.3, 79.1, 75.3, 73.7, 72.1, 71.7, 45.6, 45.5, 43.8, 43.0, 38.8, 36.5, 28.3, 21.0 ppm.



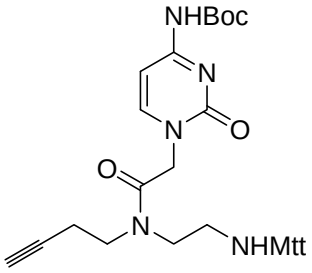
6T

Mtt-protected t1-alkyne 6T: To a solution of thymine-1-acetic acid (1.0 equiv, 0.12 g, 0.68 mmol) in DMF (2.0 mL) were added triethylamine (1.0 equiv, 94 µL, 0.68 mmol) and HBTU (1.0 equiv, 0.25 g, 0.68 mmol) at 23 °C. After stirring the reaction mixture for 20 min, a solution of Mtt-protected t1-alkyne backbone **4** (1.0 equiv, 0.24 g, 0.68 mmol) in DMF (1.0 mL) was added followed by triethylamine (1.0 equiv, 94 µL, 0.68 mmol) and the reaction mixture was stirred overnight at 23 °C. The reaction was quenched with water (3.0 mL), stirred for 20 min, and extracted with EtOAc (3 x 10 mL), then the combined organic layers were washed with brine (20 mL), dried over Na_2SO_4, and concentrated *in vaccuo*. The crude was purified by reverse phase chromatography (C18: 100% H_2O to 80% CH_3CN/H_2O) and dried under high vacuum to afford **6T** as a pure white solid in 75% yield (266 mg). ^{1}H NMR (400 MHz, DMSO-d6, 25 °C): δ = 7.40-7.35 (m, 8 H), 7.32-7.22 (m, 14 H), 7.17 (t, J = 6.8 Hz, 4 H), 7.08 (d, J = 8.0 Hz, 4 H), 4.69 (s, = 2.6 H), 4.66 (s, 1.4 H), 4.32 (d, J = 2.2 Hz, 1.4 H), 4.07 (d, J = 2.2 Hz, 2.6 H), 3.49 (t, J = 6.0 Hz, 2.6 H), 3.42 (t, J = 6.0 Hz, 1.4 H), 3.38 (t, J = 2.2 Hz, 0.7 H), 3.12 (t, J = 2.2 Hz, 1.3 H), 2.26-2.22 (m, 8.4 H), 2.17-2.12 (m, 1.6H), 1.74 (s, 6 H) ppm; ^{13}C NMR (100 MHz, DMSO-d6, 25 °C): δ =166.7, 166.6, 164.4, 151.0, 146.1, 142.9, 142.1, 135.1, 135.0, 128.3, 127.7, 126.0, 125.9, 108.0, 79.5, 79.2, 75.4, 74.2, 70.2, 70.0, 48.2, 48.0, 47.0, 46.4, 42.0, 41.8, 37.4, 34.5, 20.5, 12.0 ppm.



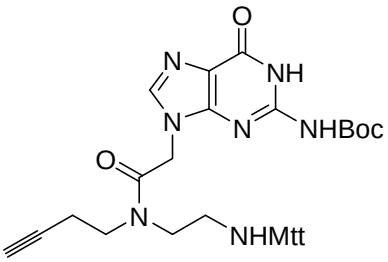
7A

Mtt-protected t2-alkyne 7A: To a solution of Boc-protected adenine acetic acid[1] (1.0 equiv, 0.25 g, 0.79 mmol) in DMF (2.0 mL) were added triethylamine (1.0 equiv, 100 µL, 0.79 mmol) and HBTU (1.0 equiv, 0.30 g, 0.79 mmol) at 23 °C. After stirring the reaction mixture for 20 min, a solution of Mtt-protected t2-alkyne backbone **5** (1.0 equiv, 0.29 g, 0.79 mmol) in DMF (1.0 mL) was added followed by triethylamine (1.0 equiv, 100 µL, 0.79 mmol) and the reaction mixture was stirred overnight at 23 °C. The reaction was quenched with water (3.0 mL), stirred for 20 min, and extracted with EtOAc (3 x 10 mL), then the combined organic layers were washed with brine (20 mL), dried over Na_2SO_4, and concentrated *in vaccuo*. The crude was purified by flash chromatography (50% petroleum ether/EtOAc to 100% EtOAc) to afford **7A** as a pure white solid in 50% yield (256 mg). R_f = 0.43 (EtOAc); ^{1}H NMR (400 MHz, CD_3OD, 25 °C): δ = 8.54 (s, 0.8 H), 8.53 (s, 1.2 H), 8.27 (s, 0.8 H), 8.26 (s, 1.2 H), 7.56 (d, J = 7.2 Hz, 4 H), 7.46-7.41 (m, 5.2 H), 7.34-7.28 (m, 8.8 H), 7.23 (t, J = 7.3 Hz, 4 H), 7.12 (t, J = 9.0 Hz, 4 H), 5.50 (s, 2.4 H), 5.47 (s, 1.6 H), 3.80 (t, J = 6.6 Hz, 1.6 H), 3.72 (t, J = 6.6 Hz, 2.4 H), 3.58-3.53 (m, 4 H), 2.71-2.68 (m, 1.6 H), 2.59-2.55 (m, 3.2 H), 2.47-2.40 (m, 4 H), 2.34-2.32 (m, 7.2 H), 1.66 (s, 10.8 H), 1.65 (s, 7.2 H) ppm; ^{13}C NMR (100 MHz, CD_3OD, 25 °C): δ = 168.8, 168.5, 153.2, 152.4, 151.2, 151.1, 147.5, 146.1, 146.0, 144.3, 144.2, 137.1, 137.0, 129.9, 129.8, 129.7, 129.5, 129.4, 128.8, 128.7, 127.4, 127.2, 122.4, 82.7, 82.1, 81.8, 72.8, 72.2, 71.8, 71.1, 49.9, 47.9, 47.6, 47.2, 45.7, 45.6, 44.1, 43.0, 28.5, 20.9, 19.1, 17.8 ppm.



7C

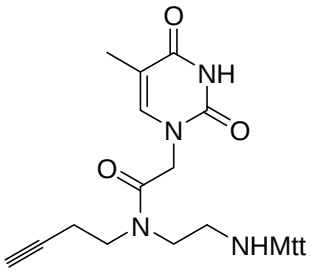
Mtt-protected t2-alkyne 7C: To a solution of Boc-protected cytosine acetic acid[1] (1.0 equiv, 0.21 g, 0.79 mmol) in DMF (2.0 mL) was added triethylamine (1.0 equiv, 100 µL, 0.79 mmol) and HBTU (1.0 equiv, 0.30 g, 0.79 mmol) at 23 °C. After stirring the reaction mixture for 20 min, a solution of Mtt-protected t2-alkyne backbone **5** (1.0 equiv, 0.29 g, 0.79 mmol) in DMF (1.0 mL) was added followed by triethylamine (1.0 equiv, 100 µL, 0.79 mmol) and the reaction mixture was stirred overnight at 23 °C. The reaction was quenched with water (3.0 mL), stirred for 20 min and extracted with EtOAc (3 x 10 mL), then the combined organic layers were washed with brine (20 mL), dried over Na_2SO_4, and concentrated *in vaccuo*. The crude was purified by flash chromatography (50% petroleum ether/EtOAc to 100% EtOAc) to afford **7C** as a pure white solid in 75% yield (378 mg). R_f = 0.54 (EtOAc); ^{1}H NMR (400 MHz, CD_3OD, 25 °C): δ = 7.85 (d, J = 7.4 Hz, 0.8 H), 7.80 (d, J = 7.4 Hz, 1.2 H), 7.54-7.47 (m, 8 H), 7.40-7.30 (m, 14 H), 7.24 (t, J = 8.3 Hz, 4 H), 7.16 (t, J = 8.3 Hz, 4 H), 4.96 (s, 2.4 H), 4.93 (s, 1.6 H), 3.72 (t, J = 6.8 Hz, 1.6 H), 3.61 (t, J = 6.8 Hz, 2.4 H), 3.55 (t, J = 6.8 Hz, 1.6 H), 3.50 (t, J = 6.8 Hz, 2.4 H), 2.65 (dt, J = 9.4, 2.5 Hz, 1.6 H), 2.55-2.50 (m, 3.2 H), 2.45-2.41 (m, 4 H), 2.36 (s, 6 H), 2.31 (t, J = 2.5 Hz, 2.4 H), 1.60 (s, 18 H) ppm; ^{13}C NMR (100 MHz, CD_3OD, 25 °C): δ = 169.0, 168.9, 165.4, 158.5, 153.4, 151.3, 151.2, 147.5, 144.3, 137.1, 136.9, 129.9, 129.8, 129.7, 129.5, 129.4, 128.8, 128.7, 127.3, 127.2, 96.6, 83.1, 82.1, 81.7, 72.4, 72.2, 71.9, 71.1, 52.0, 51.6, 49.8, 48.2, 47.8, 47.2, 43.8, 43.0, 28.3, 21.0, 19.1, 17.9 ppm.



7G

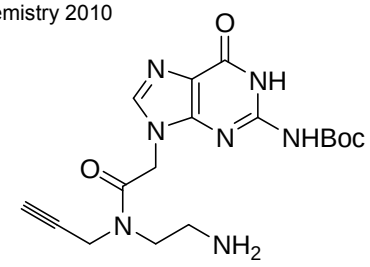
Mtt-protected t2-alkyne 7C: To a solution of Boc-protected guanine acetic acid[1] (1.0 equiv, 0.24 g, 0.79 mmol) in DMF (2.0 mL) was added triethylamine (1.0 equiv, 0.10 mL, 0.79 mmol) and HBTU (1.0 equiv, 0.30 g, 0.79 mmol) at 23 °C. After stirring the reaction mixture for 20 min, a solution of Mtt-protected t2-alkyne backbone **5** (1.0 equiv, 0.29 g, 0.79 mmol) in DMF (1.0 mL) was added followed by triethylamine (1.0 equiv, 0.10 mL, 0.79 mmol) and the reaction mixture was stirred overnight at 23 °C. The reaction was quenched with water (3.0 mL), stirred for 20 min and extracted with EtOAc (3 x 10 mL), then the combined

organic layers were washed with brine (20 mL), dried over Na_2SO_4, and concentrated *in vaccuo*. The crude was purified by flash chromatography (100% CH_2Cl_2 to 10% MeOH/CH_2Cl_2) to afford **7G** as a pure white solid in 80% yield (416 mg). R_f = 0.41 (10% MeOH/ CH_2Cl_2); ^{1}H NMR (400 MHz, CD_3OD, 25 °C): 7.87 (s, 1 H), 7.84 (s, 1 H), 7.51-7.43 (m, 8 H), 7.38-7.27 (m, 12 H), 7.22 (t, *J* = 8.3 Hz, 4 H), 7.11 (t, *J* = 8.3 Hz, 4 H), 5.28 (s, 2 H), 5.26 (s, 2 H), 3.76 (t, *J* = 6.0 Hz, 2 H), 3.67 (t, *J* = 6.0 Hz, 2 H), 3.56-3.54 (m, 4 H), 2.65-2.63 (m, 3.2 H), 2.53 (t, *J* = 5.8 Hz, 2 H), 2.46-2.39 (m, 4 H), 2.33 (s, 6.8 H). 1.60 (s, 9 H), 1.59 (s, 9 H) ppm; ^{13}C NMR (100 MHz, CD_3OD, 25 °C): δ = 168.8, 168.6, 157.6, 155.2, 151.5, 149.4, 149.2, 147.5, 147.4, 144.3, 144.2, 142.2, 142.0, 137.2, 137.0, 129.9, 129.8, 129.7, 129.5, 129.4, 128.8, 128.7, 127.4, 127.3, 119.9, 84.7, 82.1, 81.8, 73.0, 72.1, 71.7, 71.2, 49.9, 47.8, 47.5, 47.3, 45.6, 45.5, 44.2, 42.9, 28.3, 21.0, 19.2, 17.9 ppm.



7T

Mtt-protected t1-alkyne 7T: To a solution of thymine-1-acetic acid (1.0 equiv, 0.14 g, 0.79 mmol) in DMF (2.0 mL) were added triethylamine (1.0 equiv, 0.10 mL, 0.79 mmol) and HBTU (1.0 equiv, 0.25 g, 0.79 mmol) at 23 °C. After stirring the reaction mixture for 20 min, a solution of Mtt-protected t2-alkyne backbone **5** (1.0 equiv, 0.24 g, 0.79 mmol) in DMF (1.0 mL) was added followed by triethylamine (1.0 equiv, 0.10 mL, 0.79 mmol) and the reaction mixture was stirred overnight at 23 °C. The reaction was quenched with water (3.0 mL), stirred for 20 min, and extracted with EtOAc (3 x 10 mL), the combined organic layers were washed with brine (20 mL), dried over Na_2SO_4, and concentrated *in vaccuo*. The crude was purified by reverse phase chromatography (C18: 100% H_2O to 80% CH_3CN/H_2O) and dried under high vacuum to afford **7T** as a pure white solid in 85% yield (359 mg). ^{1}H NMR (400 MHz, DMSO-d6, 25 °C): δ = 7.39-7.33 (m, 8 H), 7.29-7.21 (m, 14 H), 7.19-7.13 (m, 4 H), 7.08 (d, *J* = 8 Hz, 4 H), 4.65 (s, 4H), 3.56 (t, *J* = 6.5 Hz, 1.6 H), 3.42 (t, *J* = 6.5 Hz, 1.6 H), 3.37-3.29 (m, 4.8 H), 2.95 (t, *J* = 2.5 Hz, 0.8 H), 2.81 (t, *J* = 2.5 Hz, 1.2 H), 2.30-2.11 (m, 14H), 1.73 (s, 6H) ppm; ^{13}C NMR (100 MHz, DMSO-d6, 25 °C): δ = 166.8, 166.5, 151.0, 146.1, 142.9, 142.2, 135.1, 135.0, 128.32, 127.6, 126.0, 125.9, 107.9, 81.8, 81.6, 80.4, 73.2, 72.2, 70.2, 70.0, 48.1, 47.9, 44.8, 42.5, 41.9, 20.4, 17.8, 16.6, 11.9 ppm.



18

Mtt-deprotected t1-Alkyne 18: To a solution of Mtt-protected t1 alkyne **6G** (1.0 equiv, 0.37 g, 0.57 mmol) in CH_2Cl_2 (7 mL) was added HFIP (30.0 equiv, 1.80 mL, 17.2 mmol). The reaction mixture was stirred for 4 hours at 23 °C. The reaction mixture was then diluted with EtOAc (5.0 ml) and concentrated *in vaccuo*. The crude was purified by reverse phase chromatography (C18: 100% H_2O to 40% H_2O/ CH_3CN) and lyophilized to afford **18** as a pure white solid in 60% yield (1.23 g); ^{1}H NMR (400 MHz, CD_3OD, 25 °C): δ = 7.97 (s, 1 H), 4.91 (s, 2 H), 3.47 (d, J = 2.5 Hz, 2 H), 3.42 (t, J = 6.3 Hz, 2 H), 2.87 (t, J = 6.3 Hz, 2 H), 2.67 (t, J = 2.5 Hz, 2 H), 1.63 (s, 9 H) ppm; ^{13}C NMR (100 MHz, CD_3OD, 25 °C): δ = 169.0, 157.7, 155.5, 151.6, 149.7, 141.9, 120.1, 84.6, 81.9, 73.3, 40.2, 46.7, 39.8, 38.1, 28.3 ppm.

PNA synthesis procedures:

All the resins were pre-swollen in CH_2Cl_2 for 20 minutes before being engaged in a reaction. Standard washing procedure: DMF (4 x), CH_2Cl_2 (4 x), DMF (2 x), CH_2Cl_2 (2 x). Capping solution: acetic anhydride (4.6 mL), 2,6-lutidine (7.5 mL) in DMF (100 mL)

Azide-PNA, resin 8: To a solution of Fmoc-Lys(Boc)-OH (1.0 equiv, adjusted to 0.2 mmol/g loading of resin) in NMP (15 µL/mg of resin) was added HOBt (5.0 equiv) followed by DIC (10 equiv). This mixture was shacked for 15 min prior to addition to a pre-swollen NovaPEG resin (0.44 mmol/g), and the resulting suspension was then shacked for 3 additional hours at 23 °C. The suspension was filtered, and the resin was washed following the standard washing procedure; then the unreacted amino groups were capped for 30 min (capping solution: 2 x 30µL/mg of resin) at 23 °C, and the resin was filtered, loaded on the synthesizer in 10 mg portions, and coupled with Fmoc-protected PNA monomers[1] using standard Fmoc chemistry as previously described.[2]

General procedure for copper-catalyzed click reaction on the resin: To a solution of alkynes **6** or alkynes **7** (7.5 equiv, 0.1 M sol. in NMP) were added sequentially sodium ascorbate (7.5 equiv, 1.0 M in H_2O), $CuSO_4$ (0.25 equiv, 0.13 M in H_2O) and TBTA ligand (0.50 equiv, 28.3 mM in NMP) and the resulting mixture was added to the corresponding resin after sonication. After 12 hours the resin was washed with CH_2Cl_2 (4 x), NMP (4 x), sodium diethyldithiocarbamate 0.02 M in NMP (4 x), NMP (4 x), MeOH (4 x), NMP (4 x) and CH_2Cl_2 (4 x). Cleavage of an analytical aliquot of resin and analysis by LC/MS indicated a complete conversion.

General procedure of Mtt deprotection: The Mtt was deprotected at 23 °C using HFIP/DCE solution (1:1) (20µL/mg), 4 times 2 min.

General Cy3 labelling procedure: To a solution of Cy3 fluorophore (5.0 equiv) in NMP (10µL/mg of resin) was added 2,6-lutidine (10 equiv) followed by TNTU (4.5 equiv) and the

mixture was shacked for 20 min. DMSO was added to the resin (2 µL/mg of resin) followed by the Cy3 solution and the reaction mixture was shacked overnight at 40 °C.

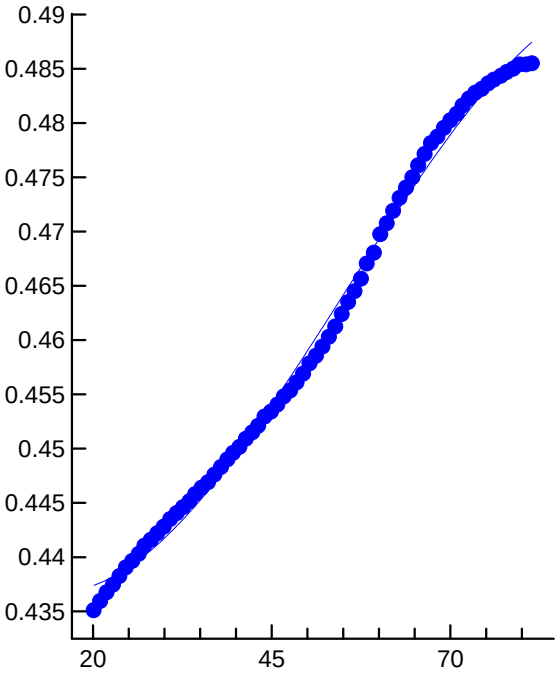
Synthesis of resin-bound alkyne 19: Mtt-deprotected t1-alkyne **18** (10 equiv, 34.3 mg, 88 µmol) was dissolved in 180 µL of NMP/MeOH/AcOH (80:19:1), added to the FMPB resin (10 mg, 0.88 mmol/g, 8.8 µmol) and shacked for 90 min at 23° C. A solution of $NaBH_3CN$ (10 equiv, 5.53 mg, 88 µmol) in 20 µL of NMP/MeOH (1:1) was then added and the reaction mixture was heated at 60 °C for 18 hours. The resin was allowed to cool down to room temperature, filtered, washed following the standard washing procedure, and dried.

General cleavage procedure: PNAs were cleaved from FMPB resin with TFA/H_2O (95:5) for 16 hours and from NovaPEG resin with neat TFA for 3 hours.
PNA purification: PNAs were purified when necessary by reverse phase chromatography (C18: 100% H_2O to 40% CH_3CN/ H_2O with 0.01 % TFA)

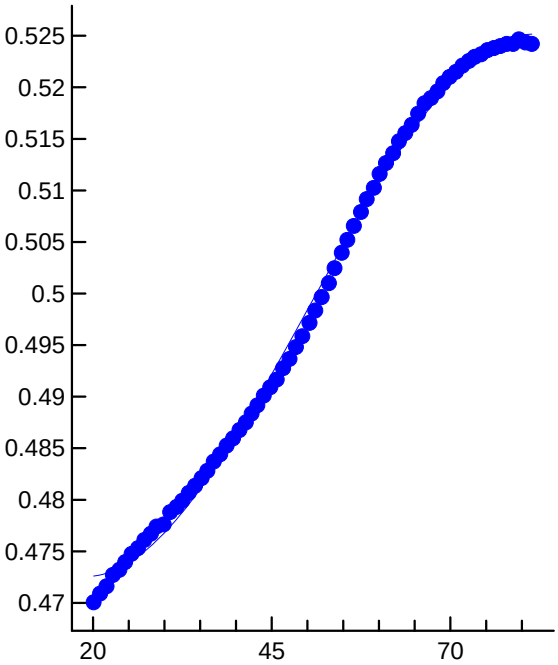
Melting temperature measurements: Stock solutions of PNAs **10**, **11**, **12** and of their complementary DNA were prepared in MilliQ water. Hybridized solutions containing 5 µM PNA–DNA duplexes were prepared in PBS buffer (10 mM phosphate buffer, 100 mM NaCl, pH = 7). All the hybridized samples were first incubated at 90 °C for 10 min, and then slowly cooled to room temperature. The samples were heated from 20 °C to 90 °C (0.5 °C/ min), then cooled down to 10° C and the UV signal variations at 260 nm were recorded. Melting temperatures were measured as the maximum of the first derivatives of the melting curves.

PNA hybridization on microarray slides: Stock solutions of PNAs **13-17** (10 mM) were prepared in DMSO/H_2O (1:1) and diluted in hybridization buffer [40 % formamide in PBS (137 mM NaCl, 2.7 mM KCl, 100 mM Na_2HPO_4, 2 mM KH_2PO_4, pH = 7.4)] to give final concentrations of 0.2, 1.0 and 5.0 nM. The slide was incubated with the different solutions (40 µL of each) and left overnight at 50 °C. After hybridization, the following washing steps were performed using cold solutions; washing solution **1**: 40 % Formamide, 0.01 % tween 20, in PBS 1X (3 x); washing solution **2**: 0.01 % tween 20, in PBS 1X (3 x); washing solution **3**: Quick wash with MilliQ water. The slide was dried and scanned.

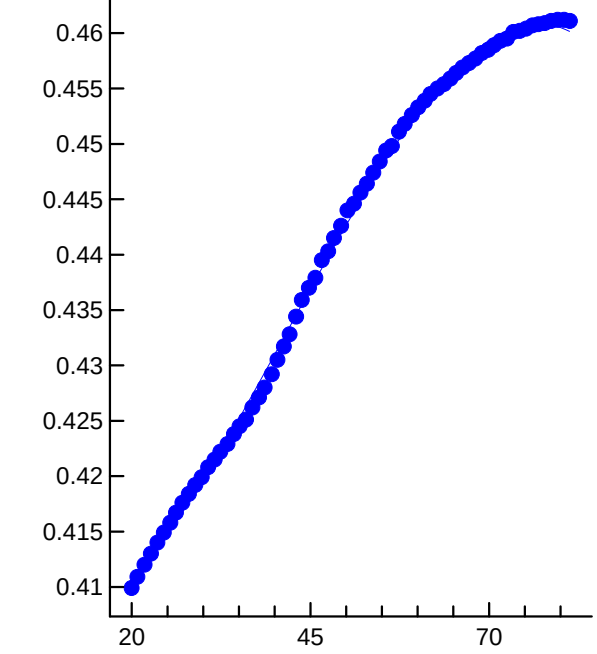
General procedure for templated click cycloaddition couplings: These couplings were performed in a total volume of 50 µL of PBS buffer (10 mM phosphate buffer, 100 mM NaCl, pH = 7), with final concentrations of: 5 µM for the DNA template, alkynicPNA and azidoPNA; 10 mM sodium ascorbate; 1 mM $CuSO_4$ and 7 mM THPTA ligand. Buffers were deoxygenated with a stream of nitrogen before use. Three hybridized solutions containing DNA template, alkynicPNA **20,** and azidoPNA **21** or **22** or **23** were mixed preheated to 90 °C for 10 min and recooled to room temperature. The solution containing sodium ascorbate, $CuSO_4$, and THPTA ligand was premixed and added to the above PNA/DNA mixture. The reactions were kept at 20 °C for 2 hours and then quenched with 50 µL of 10% TFA solution in H_2O/MeCN (1:1) before being spotted with matrix for analysis by MALDI.


0.49
0.485
0.48
0.475
0.47
0.465
0.46
0.455
0.45
0.445
0.44
0.435
20
45
70

PNA **10**

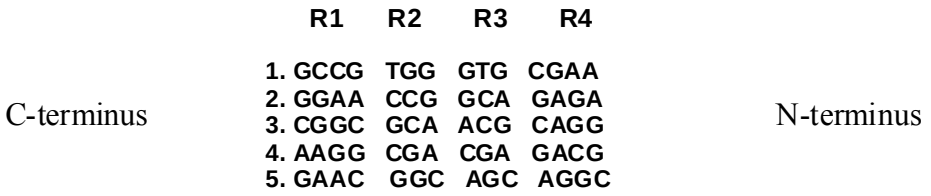

0.525
0.52
0.515
0.51
0.505
0.5
0.495
0.49
0.485
0.48
0.475
0.47
20
45
70

PNA **11**



PNA **12**

Figure S-1. Tm measurements of PNA 10 (top) 11 (middle) and 12 (bottom).



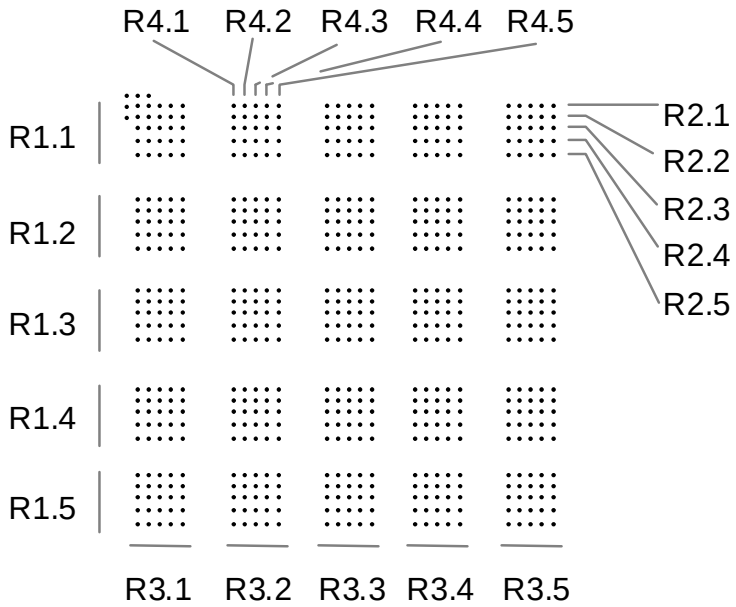


Figure S-2. Map of the microarray. The sequence of each PNA codon (R1-4) and its hybridization position on the microarray

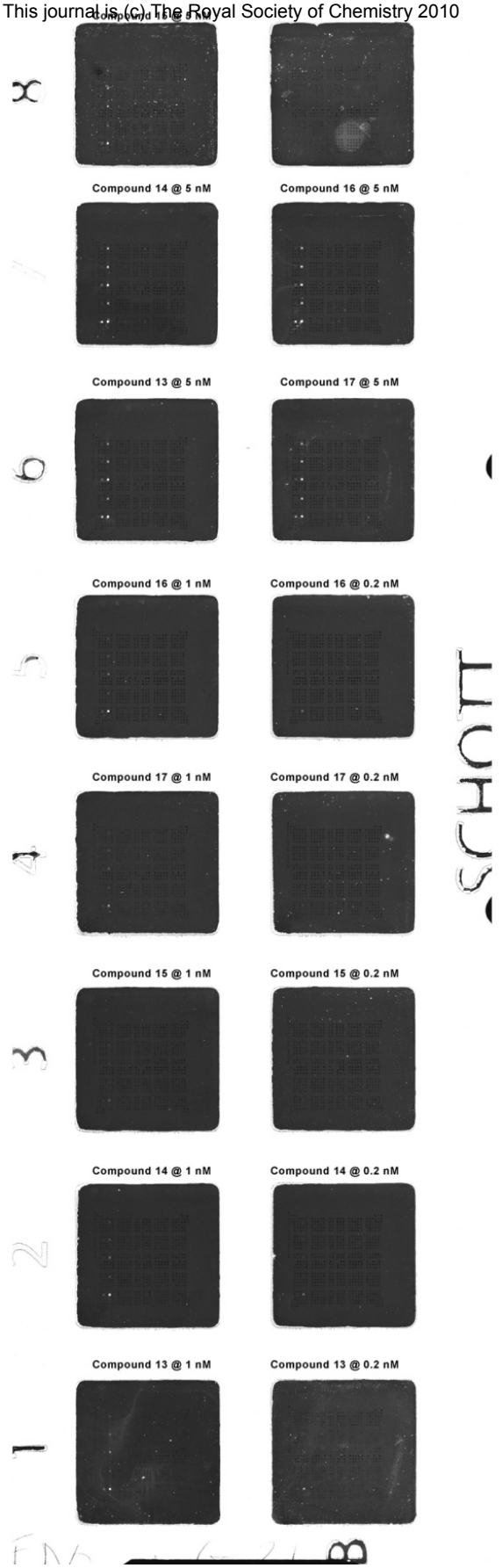
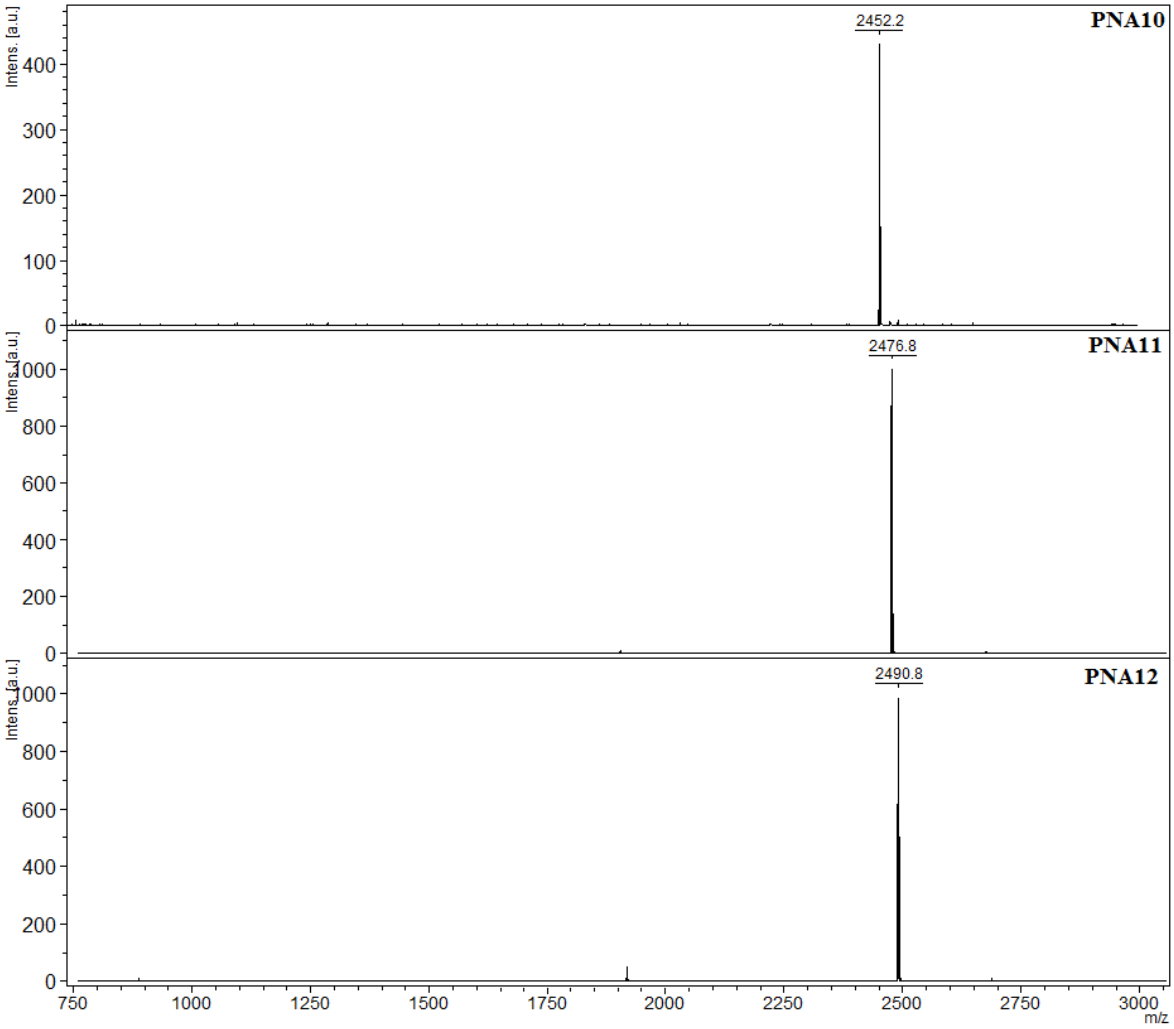
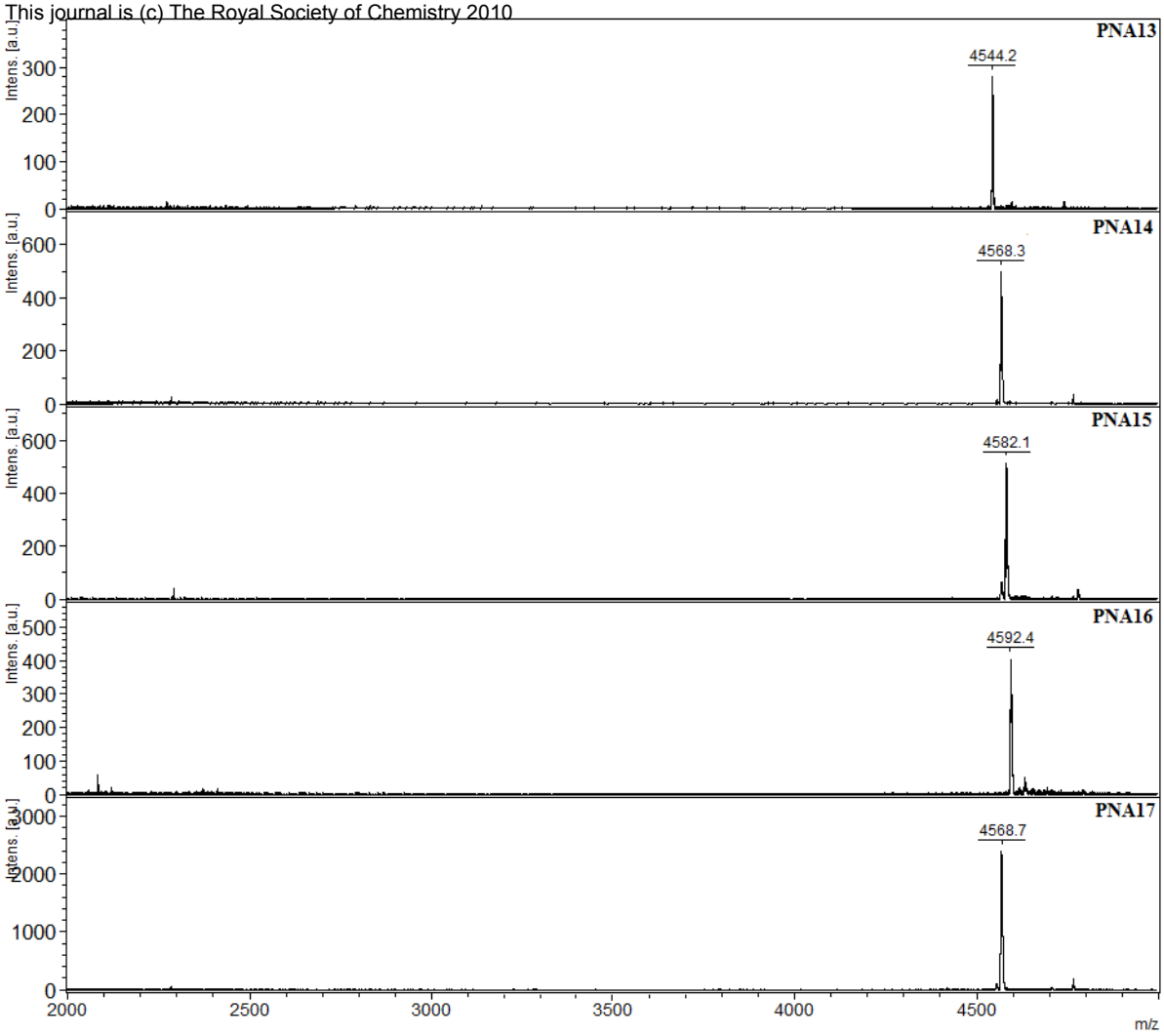


Figure S-3. Hybridization of compounds 13-17 at 0.2, 1 and 5 nM.

Figure S-4. MALDI analysis of PNA oligomers.




PNA13
4544.2
PNA14
4568.3
PNA15
4582.1
PNA16
4592.4
PNA17
4568.7
Intens. [a.u.]
m/z
2000
2500
3000
3500
4000
4500

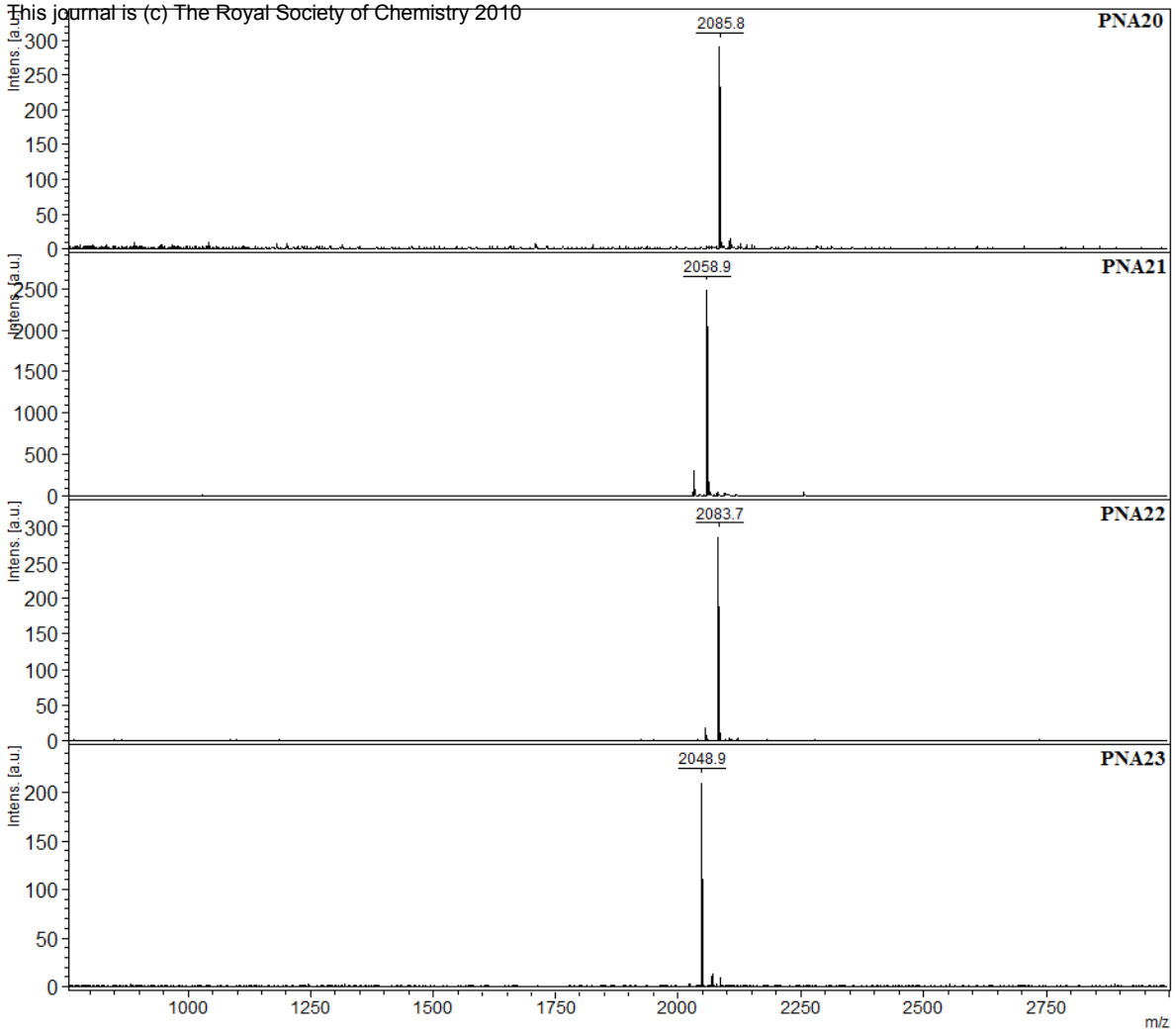
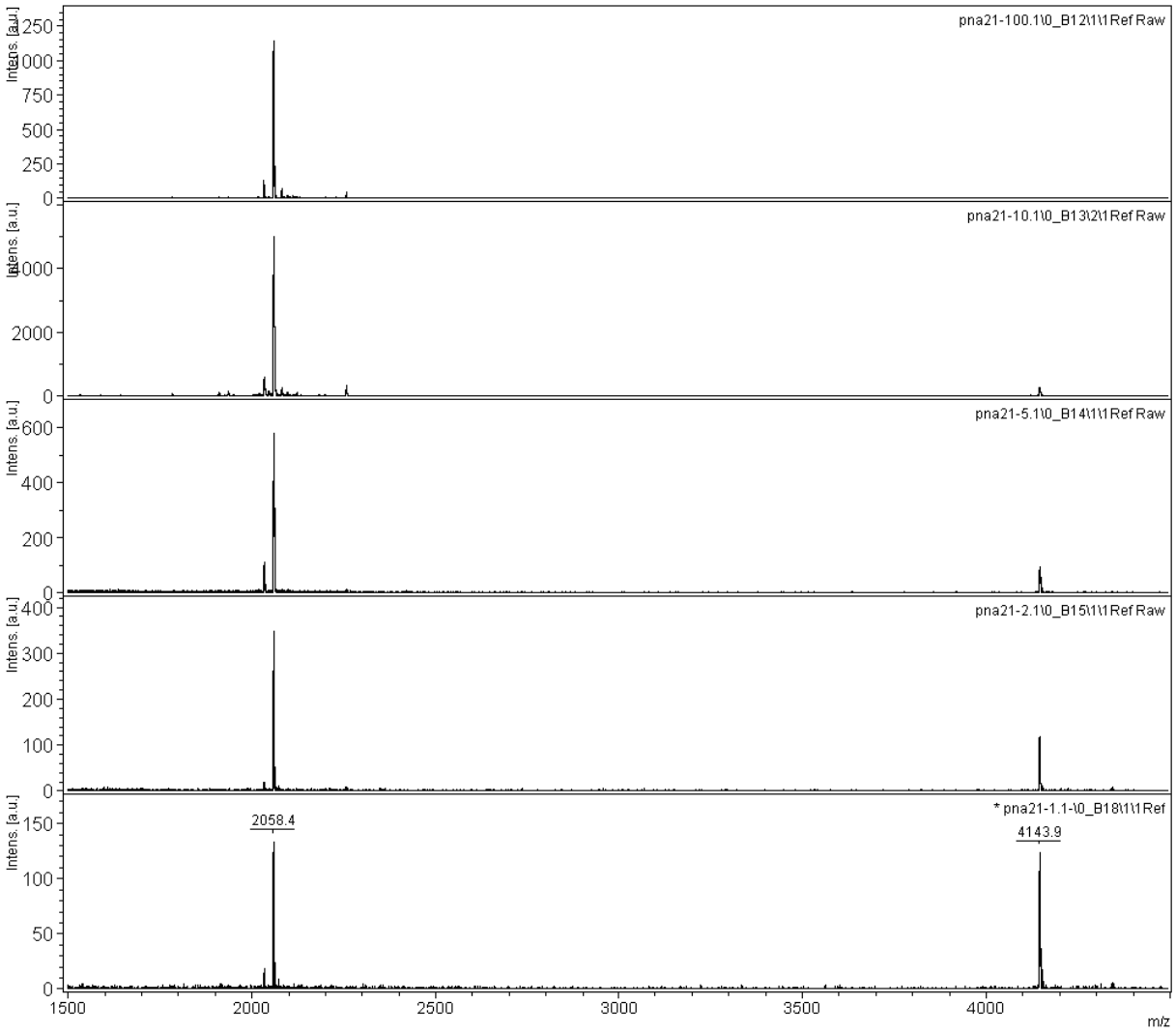
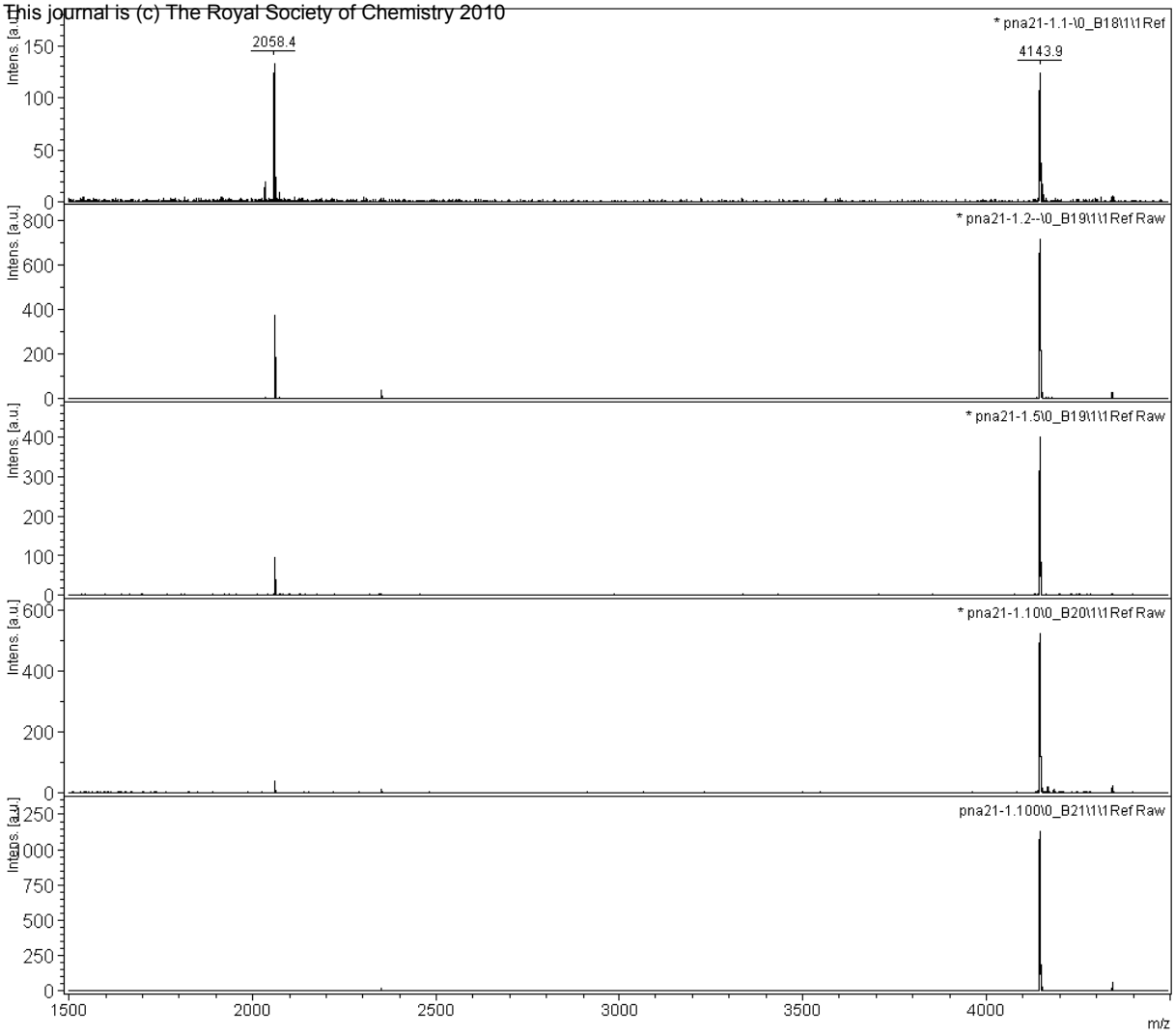


Figure S-5. Calibration of the MALDI signal for PNA **21** and the product of the reaction between **21** and **20** using mixture containing ratio ranging from 100:1 to 1:100 (ratio indicated in the upper right corner of each image)





1. Pothukanuri, S.; Pianowski, Z.; Winssinger, N., Expanding the scope and orthogonality of PNA synthesis. *Eur. J. Org. Chem*. 2008, (18), 3141-3148.

2. Pianowski, Z.; Winssinger, N., Fluorescence-based Detection of Single Nucleotide Permutation in DNA via Catalytically Templated Reaction. *Chem. Commun.* 2007, 3820-3822.