Supporting Information

Pyrrolidine PNA: A novel conformationally restricted PNA analogue

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General Information. ^1H and ^{13}C NMR spectra were taken in $CDCl_3$ at 300 MHz and 75.0 MHz respectively unless specified otherwise. Chemical shifts are reported in parts per million using the solvent resonance internal standard (chloroform, 7.24 and 76.9 ppm). Pyridine, CH_2Cl_2, DMF and CH_3CN were dried over 4Å molecular sieves. THF was distilled from sodium. Reactions were carried out under nitrogen unless otherwise noted. Manual Boc-PNA Solid phase synthesis was carried out in a glass reactor. The references refers to those given in the Letter.

Preparation of compound **1**. Cs_2CO_3 (3.42 g, 10.5 mmol) was added to a stirred solution of N-Boc-cis-4-hydroxy-D-proline (2.31 g, 10.0 mmol) in dry DMF (36 ml). The reaction mixture was stirred 15 min after which MeI (0.75 ml, 12.0 mmol) was added dropwise. The reaction mixture was stirred overnight and then filtered through celite. The DMF was evaporated off and the residue was partitioned between sat $NaHCO_3$ (100 ml) and AcOEt (200 ml). The organic phase was washed with brine (2 x 100 ml), dried ($MgSO_4$) and evaporated in vacuo. Yield: 2.42 g (99%) off **1** as a white solid. $[\alpha]_D^{20} = 65.0$ (c 1, EtOH) (Litt:[9] $[\alpha]_D^{25} = 63.8$ (c 2.21, EtOH)).

Preparation of compound **2**.[11] Imidazol (1.44 g, 21.1 mmol), DIEA (2.5 ml, 14.4 mmol) and then tert-butyl-diphenylsilyl chloride (3.75 ml, 14.4 mmol) were added to a stirred solution of **1** (2.35 g, 9.60 mmol) in dry DMF (19 ml). The reaction mixture was stirred overnight and then filtered through celite. The DMF was evaporated off and the residue was partitioned between half sat $NaHCO_3$ (100 ml) and AcOEt (100 ml). The organic phase was washed with brine (50 ml), 10% citric acid (50 ml), brine (2 x 50 ml), and then dried ($MgSO_4$) and evaporated in vacuo. The crude material (6 g) was purified by chromatography (AcOEt:Hexan 1:9). Yield: 3.98 g (85%) of **2** as a white solid. NMR complicated by cis-trans isomeri around the Boc group: ^1H NMR ($CDCl_3$) δ 7.65-7.62 (m, 4H), 7.42-7.38 (m, 6H), 4.31-4.24 (m, 2 x H), 3.75 (s, 3H), 3.60-3.38 (m, 2H), 2.23-2.16 (m, 2H), 1.45 and 1.42 (2 x s, 9H), 1.07 and 1.04 (2 x s, 9H). ^{13}C NMR ($CDCl_3$) δ 174.9, 172.7, 172.3, 154.2, 153.5, 135.6, 135.5, 134.6, 133.4, 133.2, 133.0, 129.7, 129.4, 127.6, 127.5, 79.8, 71.5, 70.4, 57.6, 57.2, 54.2, 53.8, 52.0, 51.9, 39.1, 38.3, 28.3, 28.2, 26.6, 26.4, 18.8. FAB$^+$MS: 484.33 (MH^+). Calcd for $C_{27}H_{37}NO_5Si$: C, 67.05; H, 7.71; N, 2.90. Found: C, 66.90; H, 7.74; N, 2.94.

Preparation of compound **3**.[11] $LiBH_4$ (23.5 ml, 2.0 M in THF) was slowly added to a stirred solution of **2** (18.2 g, 37.6 mmol) in dry THF (100 ml) at 0 °C. The reaction mixture was allowed to warm to rt and then stirred 8 h. The reaction was quenched at 0 °C by the addition of H_2O (150 ml), followed by the slow addition of 1 M HCl (75 ml). The acidic solution was extracted with AcOEt (3 x 200 ml). The combined organic phases were washed with brine (100 ml), sat $NaHCO_3$ (100 ml), brine (100 ml) and dried (MgSO4) and evaporated. The crude material (17.6 g) was purified by chromatography (1-10% MeOH in CH_2Cl_2). Yield:

15.17 g (89%) off **3** as a white foam. NMR complicated by cis-trans isomeri around the Boc group: ^{1}H NMR ($CDCl_3$) δ 7.65-7.62 (m, 4H), 7.45-7.37 (m, 6H), 4.28 (m, 1H), 3.97 (m, 1H), 3.86 (m, 1H), 3.75 (m, 1H), 3.40-3.25 (m, 2H), 2.70 (br. s, 1H), 2.08 (m, 1H), 1.80-1.60 (m, 1H), 1.44 (s, 9H), 1.07 (s, 9H). FAB$^+$MS: 456.37 (MH^+).

Preparation of compound **4**. DIEA (8.7 ml, 50.1 mmol) and then methanesulfonyl chloride (3.1 ml, 40.0 mmol), was added to a stirred solution of **3** (15.2 g, 33.4 mmol) in dry CH_2Cl_2 (170 ml) at 0 °C. The reaction mixture stirred at 0 °C 40 min and then quenched by the addition of half sat $NaHCO_3$ (200 ml). The layers were separated and the aq layer was extracted with CH_2Cl_2 (2 x 150 ml). The combined organic phases were washed with brine (100 ml), 10% citric acid (2 x 100 ml), brine (100 ml), and then dried ($MgSO_4$) and evaporated. Yield: 17.2 g (97%) off **4** as a yellow foam. NMR complicated by cis-trans isomeri around the Boc group: ^{1}H NMR (CDCl3) δ 7.67-7.61 (m, 4H), 7.46-7.40 (m, 6H), 4.56 (m, 1H), 4.50-4.38 (m, 2H), 4.06 (m, 1H), 3.50-3.00 (m, 2H), 3.01 (s, 3H), 2.10-1.98 (m, 2H), 1.48 and 1.45 (2 x s, 9H), 1.07 (s, 9H). FAB$^+$MS: 534.20 (MH^+).

Preparation of compound **5**. NaN_3 (10.5 g, 162 mmol) was added to a stirred solution of **4** (17.2 g, 32.3 mmol) in dry DMF (160 ml) at rt. The reaction mixture was stirred at 90 °C 4 h after which the DMF was evaporated off. The residue was partitioned between half sat $NaHCO_3$ (100 ml) and AcOEt (200 ml). The aq phase was extracted with more AcOEt (200 ml). The combined organic phases were washed with brine (2 x 100 ml), dried (Na_2SO_4) and evaporated. The crude product (15.2 g) was purified by chromatography (AcOEt:Hexane 1:4). Yield: 10.97 g (71%) off **5** as a white solid. NMR complicated by cis-trans isomeri around the Boc group: ^{1}H NMR ($CDCl_3$) δ 7.59-7.54 (m, 4H), 7.38-7.30 (m, 6H), 4.24 (br. s, 1H), 3.80 (br. s, 1H), 3.57 (br. s, 1H), 3.40-3.10 (m, 2H), 2.00-1.90 (m, 2H), 1.38 (s, 9H), 0.99 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 153.9, 135.6, 133.0, 129.8, 127.7, 80.0, 71.2, 55.9, 54.8, 53.8, 52.6, 37.3, 36.5, 28.3, 26.7, 18.8. FAB$^+$MS: 481.32 (MH^+).

Preparation of compound **6**. TFA (4.6 ml, 58 mmol) was added to a stirred solution of **5** (2.14 g, 4.45 mmol) in dry CH_2Cl_2 (4.6 ml) at 0 °C . The ice bath was removed and the reaction mixture was stirred at rt 30 min. The reaction was quenched by the slow addition of sat $NaHCO_3$ (65 ml). The layers were separated and the aq phase was extracted with CH_2Cl_2 (2 x 100 ml). The combined organic phases were dried ($MgSO_4$) and evaporated. Yield: 1.70 g (100 %) of **6** as an oil. ^{1}H NMR ($CDCl_3$) δ 7.80-7.63 (m, 4H), 7.45-7.39 (m, 6H), 4.40 (m, 1H), 3.60 (br. s, 1H), 3.49-3.44 (m, 2H), 3.30 (m, 1H), 3.00-2.80 (m, 2H), 2.01 (m, 1H), 1.60 (m, 1H), 1.07 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 135.53, 135.50, 134.7, 135.5, 129.7, 127.6, 127.4, 73.5, 57.1, 55.1, 54.5, 38.6, 26.7, 18.9. FAB$^+$MS: 381.49 (MH^+).

Preparation of compound **7**. DIEA (4.69 ml, 27.0 mmol) and then methyl bromoacetate (2.35 ml, 24.8 mmol) was added to a stirred solution of **6** (8.78 g, 22.5 mmol) in dry THF (45 ml) at °C. The ice bath was removed and the reaction mixture was stirred at rt 4 h and then filtered through celite. The solvent was evaporated off and the crude product was purified by chromatography (AcOEt:Hexane 1:4). Yield: 9.31 g (91%) of **7** as a clear oil. ^{1}H NMR ($CDCl_3$) δ 7.75-7.67 (m, 4H), 7.47-7.36 (m, 6H), 4.43 (m, 1H), 3.67 (s, 3H), 3.57 (s, 2H), 3.54-3.36 (m, 2H), 3.14- 3.11 (m, 2H), 2.84-2.79 (m, 1H), 2.19-2.05 (m, 1H), 1.80-1.76 (m, 1H), 1.09 (s, 9H). FAB$^+$MS: 453.22 (MH^+).

Preparation of compound **8**. A degassed solution of **7** (1.50 g, 3.31 mmol), Boc_2O (1.45 g, 6.62 mmol) and 10% Pd/C (0.23 g) in AcOEt (33 ml) was hydrogenated at rt overnight using balloon technique. Occasionally the nitrogen that developed was lead out through a needle outlet. The catalyst was removed by filtering the solution through celite. The solvent was evaporated off and the crude product was purified by chromatography (1-10% MeOH in CH_2Cl_2). Yield: 1.32 g (76%) of **8** as a clear oil. ^{1}H NMR ($CDCl_3$) δ 7.69-7.63 (m, 4H), 7.45-7.34 (m, 6H), 5.43 (br. s, 1H), 4.30 (br. s, 1H), 3.66 (s, 3H), 3.60-3.40 (m, 1H), 3.40-3.00 (m,

5H), 2.68 (m, 1H), 2.15 (m, 1H), 1.82 (m, 1H), 1.43 (s, 9H), 1.08 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 171.2, 156.5, 135.3, 133.5, 129.6, 127.5, 78.9, 71.7, 61.6, 60.1, 52.8, 51.4, 41.6, 38.1, 28.3, 26.9, 18.9. FAB$^+$MS: 527.32 (MH^+). Calcd for $C_{29}H_{42}N_2O_5Si$: C, 65.56; H, 8.08; N, 5.27. Found: C, 65.64, 8.62, 5.41.

Preparation of compound **9**. A 1 M solution of TBAF in THF (16.3 ml, 16.3 mmol) was added to a stirred solution of **8** (7.18 g, 13.6 mmol) in THF (70 ml) at rt. The reaction mixture was stirred 4 h at rt and then quenched by the addition of ¼ sat NH_4Cl (200 ml) and CH_2Cl_2 (250 ml). The layers were separated and the aq phase was extracted with more CH_2Cl_2 (250 ml) and AcOEt (2 x 250 ml). The combined organic phases were dried (Na_2SO_4) and the solvent was evaporated off. The crude product (12.0 g) was purified by chromatography (1-10% MeOH in CH_2Cl_2). Yield: 3.47 g (88%) of **9** as a clear oil. ^{1}H NMR ($CDCl_3$) δ 5.51 (br. s, 1H), 4.23 (br. s, 1H), 3.64 (s, 3H), 3.60-3.20 (m, 4H), 3.10-3.00 (m, 2H), 2.90-2.85 (m, 1H), 2.71-2.66 (m, 1H), 2.27-2.17 (m, 1H), 1.67-1.61 (m, 1H), 1.37 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 171.4, 156.5, 78.9, 69.7, 62.4, 53.0, 51.5, 41.3, 37.8, 28.2. HR FAB$^+$MS: 289.1771 (MH^+) (Calcd for $C_{13}H_{25}N_2O_5$: 289.1763).

Preparation of compound **10**. p-toluenesulfonyl chloride (1.64 g, 8.62 mmol) was added to a stirred solution of **9** (1.24 g, 4.31 mmol) in dry pyridine (10.8 ml). The orange reaction mixture was stirred overnight at rt and then quenched by the addition of CH_2Cl_2 (100 ml) and sat $NaHCO_3$ (50 ml). The organic phase was extracted with more sat $NaHCO_3$ (50 ml), washed with brine (50 ml), and dried (Na_2SO_4). The solvent was evaporated off and the crude product was purified by chromatography (AcOEt:Hexane 1:1). Yield: 1.42 g (74%) of **10** as a clear oil. . ^{1}H NMR ($CDCl_3$) δ 7.75 (d, J=8.5 Hz, 2H), 7.30 (d, J=8.8 Hz, 2H), 5.14 (br. s, 1H), 4.96 (br. s, 1H), 3.65 (s, 3H), 3.42 (m, 2H), 3.26 (m, 2H), 3.06-2.93 (m, 3H), 2.41 (s, 3H), 2.33-2.24 (m, 1H), 1.84-1.79 (m, 1H), 1.41 (s, 9H). ^{13}C NMR (CDCl3) δ 170.4, 156.2, 144.6, 133.7, 129.7, 127.5, 79.7, 79.1, 60.3, 58.7, 51.9, 51.5, 41.0, 35.0, 28.1, 21.4. FAB$^+$MS: 443.21 (MH^+).

Preparation of compound **11**. 6-N-(Benzyloxycarbonyl)adenine (404 mg, 1.5 mmol), K_2CO_3 (186 mg, 1.35 mmol) and Cs_2CO_3 (49 mg, 0.15 mmol) was stirred in dry DMF (2 ml) 5 min at rt. A solution of **10** (662 mg, 1.50 mmol) in dry DMF (4 ml) was added dropwise and the suspension was stirred at rt 1h. The brown solution was further stirred at 80 °C 1.5 h and then 1.5 h at rt. The DMF was evaporated off and the crude product was purified by chromatograhy (6-15% MeOH in CH_2Cl_2 containing 0.5% DIEA). Yield: 278 mg of the Boc protected monomer adenine methylester: ^{13}C NMR and FAB$^+$MS showed that the benzyloxycarbonyl group had been lost: ^{13}C NMR ($CDCl_3$) δ 170.9, 156.0, 155.6, 152.3, 149.3, 138.6, 119.3, 78.9, 60.4, 57.9, 52.1 and 51.9, 51.3, 49.5, 41.3, 34.1, 28.0. FAB$^+$MS: 406.34 (MH^+). This intermediate (278 mg, 0.69 mmol) was dissolved in dry CH_2Cl_2 (5 ml). N-Benzyloxycarbonyl-N'-methylimidazolium triflate (757 mg, 2.1 mmol) was added and the solution was stirred at rt overnight. The reaction was diluted by adding more CH_2Cl_2 (50 ml) and then quenched by adding half sat $NaHCO_3$ (25 ml). The layers were separated and the aq phase was extracted with CH_2Cl_2 (50 ml) and AcOEt (50 ml). The combined organic phases were dried (Na_2SO_4) and the solvent was evaporated off. The crude product (781 mg) was purified by chromatography (AcOEt:MeOH 9:1). Yield: 195 mg (24%) of **11** as a white foam. ^{1}H NMR ($CDCl_3$) δ 10.0-9.8 (br. s, 1H), 8.69 (s, 1H), 8.01 (s, 1H), 7.40-7.26 (m, 5H) 5.24 (s, 2H), 5.14 (m, 1H), 4.97 (m, 1H), 3.67 (s, 3H), 3.62-3.35 (m, 5H), 3.06 (m, 2H), 2.28 (m, 2H), 1.41 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 170.9, 156.1, 152.2, 151.2, 149.5, 141.6, 135.2, 128.3, 128.24, 128.21, 122.1, 79.2, 67.4, 60.5, 57.8, 52.5, 52.1, 51.5, 41.1, 33.9, 28.1. HR FAB$^+$MS: 540.2586 (MH^+) (Calcd for $C_{26}H_{34}N_7O_6$: 540.2570). Calcd for $C_{26}H_{33}N_7O_6 0.25\ H_2O$: C, 57.39; H, 6.22; N, 18.02. Found: C, 57.71; H, 6.08; N, 17.37.

Preparation of compound **12**. A solution of $Ba(OH)_2 \cdot 8H_2O$ (166 mg) in H_2O (5 ml) was added dropwise to **11** (190 mg, 0.35 mmol) dissolved in THF (5 ml) at 0 °C. The ice bath was removed and the reaction mixture was stirred at rt 20 min. More H_2O (6 ml) was added and the THF was evaporated off. pH was adjusted to 2.3 by adding 4 N H_2SO_4 (0.35 ml) to the unclear solution. $BaSO_4$ was removed by centrifugation. The acidic solution was decanted and then lyopholized. The lyophilization was repeated from MeOH (1.2 ml) and H_2O (12 ml) to produce 86 mg (50%) of **12**·H_2SO_4 as a powder. ^{1}H NMR ($CDCl_3$) peaks shows considerable broadening probably due to the presence of H_2SO_4: δ 8.6 (2 x br. s, 2 x 1H), 7.4-7.0 (m, 5H), 5.6-5.4 (br. s, 1H), 5.3-5.0 (m, 3H), 4.6-3.4 (m, 7H), 2.6-2.4 (m, 2H), 1.27 (s, 9H). Pure on Tlc (Butanol:Acetic acid: H2O 4:1:1) R_f=0.41 (UV, ninhydrin reactive). 92% pure on RP-HPLC. HR FAB$^+$MS: 526.2405 (MH^+) (Calcd for $C_{25}H_{32}N_7O_6$: 526.2414).

Solid phase synthesis of H-TAC-TCA#-TAC-TCT-LysNH_2 (PNA 2104). This dodecamer was synthesized by the usual in situ neutralization method using HBTU and DIEA on a Boc-Lys-(2-Cl-Z)-MBHA-PS resin (25 mg, loading 0.12 mmol/g).[16] The novel monomer A# (6 mg, 11 μmol) was dissolved in DMF (140 μL). DIEA (8 μL, 45μmol) was added and this solution was added to HBTU (4 mg, 10 μmol). The solution was preactivated 2 min and then added to the resin (3 μmol). The coupling reaction was allowed to proceed for 2.5 h before the activated solution was drained out. A small amount of beads were subjected to the Kaiser test which produced a yellow color indicating complete reaction. Synthesis and cleavage (TFA:TFMSA:thioanisole:m-cresol 3:1:0.5:0.5) was continued the usual way.[16] After ether precipitation, the crude PNA was purified by RP-HPLC. Yield: 1.2 mg (12%). MALDI-MS: 3306 (Calcd for MH^+: 3303). Pure on RP-HPLC.

Solid phase synthesis of H-$(A\#)_{10}$-LysNH_2 (PNA 2110). This decamer was synthesized as described for PNA 2104. Yield: 4.4 mg (51%). MALDI-MS: 2873 (Calcd for MH^+: 2873). Pure on RP-HPLC.