MOLECULAR MODELING:

To evaluate the "geometrical reasonableness" of α PNA **4**, molecular modeling was performed with the Insight® II (version 97.0)/Discover® (version 3.00) software packages (MSI) on a Silicon Graphics workstation using the following protocol: A model of the αPNA **4** was built with its 10 nucleobases fixed in the B-DNA helical geoemetry. This was accomplished by first building the α-helical peptide corresponding to **4** (minus the $-CH_2T$ appendages) using the program's biopolymer-building module. Next, B-helical dT10 was built then "stripped" of all atoms except C(1')-T. These two structures were then "combined" via the serine hydroxyl groups and the resulting "hybrid" αPNA (T10) was energy-minimized (consistent valence force field (CVFF), 50,000 iterations, convergence to 0.045 kcal•mol^{-1}•Å^{-1}) keeping the thymine atoms "fixed" as well as constraining the disulfide bond length (2.00-2.25 Å), the distance between the serine and linker methylenes (2.42-2.52 Å), and the distance between the n and n+4 α-carbons (4.50-5.80 Å). (This insured that the α-helix structure would not be disrupted prematurely during the building process.) A double-stranded B-helical $(dA)_{10}$•$(dT)_{10}$ was then built, overlayed with the αPNA(T10) structure at which point the $(dT)_{10}$ strand was removed. Two-stage minimization of the resulting αPNA(T10)•$(dA)_{10}$ duplex was performed first keeping the dA10 strand "fixed" and incorporating the PNA constraints then without any constraints at all to produce the structure shown in Figure 1 of the paper.

NUCLEOAMINOACID SYNTHESIS:

Reagents and Materials: All reactions were performed under an inert, moisture-free atmosphere (Ar). Solvents/reagents were purified beyond reagent grade as follows: THF (Fisher, ACS certified) was distilled from Na/benzophenone; DMSO (Fisher, ACS certified), 1,2-dichloroethane (Aldrich), acetonitrile (Fisher, HPLC grade) were distilled from CaH_2 (0-1 mm grain size, ACROS). All distillations were done in an inert (Ar), dry environment. HMDS (1,1,1,3,3,3,-hexamethyldisilazane, United Chemical Technologies), TMSCl (chlorotrimethylsilane, Aldrich), thymine (Aldrich), benzyl bromide (Aldrich), benzoyl peroxide (Aldrich), $Bu_4N^+I^-$ (Aldrich), cytosine (United States Biochemical Corp), DMAP (4-(dimethylamino)pyridine, Aldrich), di-*tert*-butyl dicarbonate (Aldrich), *N,O*-bis(trimethylsilyl)acetamide (Aldrich), formic acid (96%, Aldrich) were used as received. Analytical TLC was performed on JT-Baker Si250F plates (0.25 mm). TLC plates were visualized by UV illumination followed by charring with a 0.3% (w/v) ninhydrin solution in (97:3) EtOH-AcOH. Melting points were recorded on a Mel-Temp apparatus and are uncorrected. Spectrophotometric grade solvents (Aldrich) were used for measuring specific rotations. ^{1}H NMR assignments are based on selective homonuclear decoupling experiments and ^{13}C NMR assignments are based on HETCOR experiments. High resolution MS data are reported in units of m/z for M^+ or the highest mass species derived from M^+.

Fmoc-Ser-OBn.[1] To a 25 mL, Ar-flushed flask equipped with a magnetic stir bar and containing Fmoc-Ser-OH (0.481 g, 1.47 mmol), $KHCO_3$ (0.221 g, 2.20 mmol), and $Bu_4N^+I^-$ (0.0543 g, 0.147 mmol), was added 3 mL of dry DMSO via a gas-tight syringe. The resulting white suspension was stirred at RT for 10 min, when a clear solution was obtained. At this point, 0.524 mL (4.40 mmol) of benzyl bromide was added via gas-tight syringe. The colorless reaction mixture was stirred for 8 h at RT, at which point color of the reaction mixture was brownish-yellow and TLC analysis showed the formation of a single product and very little starting material. The reaction was quenched by addition of 25 mL of water and the DMSO/water layer was extracted with EtOAc (3 x 75 mL). The organic layers were combined and washed, first with saturated aq $NaHCO_3$ (3 x 50 mL), then saturated aq $Na_2S_2O_3$ (2 x 50 mL) and brine (1 x 50 mL), dried (Na_2SO_4) and concentrated by rotary evaporation. After removal of traces of EtOAc under high vacuum (8-9 h at 0.1 mm), a yellow oil was obtained. The yellow oil was cooled to -78 °C (dry ice/acetone) and 10 mL of hexanes added resulting in the precipitation of a yellow solid upon stirring with a glass rod. The yellow solid was transferred to a Büchner funnel (filter paper) and washed with hexanes under suction until a white solid was obtained (0.563 g, 92% yield). mp 96-96 °C; R_f = 0.20 in 3:7 EtOAc-hexanes; $[\alpha]_D^{26}$ +0.63° (*c* 4.45, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.77 (d, J = 7.6 Hz, 2H), 7.60 (d, J = 7.5 Hz, 2H), 7.26-7.43 (m, 9H), 5.75 (dd, J_1 = 7.0 Hz, J_2 = 1.1 Hz, NH), 5.23 (s, 2H, C<u>H</u>$_2$Ph), 4.20-4.50 (m, 3H, H-2 + 2 x H-10'), 4.22 (t, J = 6.7 Hz, 1H, H-9'), 4.07 (dd, J_1 = 16.5 Hz, J_2 = 1.8 Hz, C<u>H</u>$_2$OH), 2.15 (br s, 1H, OH). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 170.4, 156.2, 143.7, 143.6, 141.2, 135.0, 128.6, 128.4, 128.1, 127.0, 125.0, 119.9, 67.4 (<u>C</u>H$_2$Ph), 67.2 (C-10'), 63.1 (C-3), 56.1 (C-2), 47.0 (C-9'). HRMS (EI) m/z, calcd for $C_{25}H_{23}NO_5$ [M^+] 417.1576, obsd 417.1574.

<u>Recovery of unreacted Fmoc-Ser-OH:</u> The very first aq layer obtained during extraction and the sodium bicarbonate washings were combined and acidified to pH ~2-3 by slow addition of conc. HCl. The acidified aq layer was extracted with EtOAc (3 x 100 mL), washed with saturated aq $Na_2S_2O_3$ (2 x 50 mL) and brine (1 x 50 mL), dried (Na_2SO_4) and concentrated by rotary evaporation. After removal of traces of EtOAc under high vacuum (8-9 h at 0.1 mm), unreacted Fmoc-Ser-OH was obtained as a white solid (0.0124 g).

[1] Wang, S. S.; Gisin, B. F.; Winter, D. P.; Makofske, R.; Kulesha, I. D.; Tzougraki, C.; Meienhofer, J. *J. Org. Chem.* **1977**, *42*, 1286.

Fmoc-Ser(MTM)-OBn (11).[2] A 3-neck 250 mL round-bottom flask equipped with magnetic stir bar and solid addition assembly containing benzoyl peroxide (19.6 g, 0.0810 mol), was charged with Fmoc-Ser-OBn (9.66 g, 0.0232 mol). After flushing the system with Ar, 130 mL of dry MeCN was added with stirring at 0°C. At this point 11.9 mL (0.162 mol) of Me_2S was added, followed by addition of solid benzoyl peroxide over a 30 min period. After stirring for 1 h at 0° C, the reaction was judged to be complete by TLC analysis (double development with 3:7 EtOAc-hexanes). To the reaction mixture was added 100 mL of water and the excess acetonitrile was removed by rotary evaporation when a white precipitate formed. This mixture was extracted with Et_2O (3 x 250 mL), the organic layers were combined and washed with saturated aq. $NaHCO_3$ (3 x 75 mL) and brine (1 x 75 mL) respectively, dried (Na_2SO_4) and concentrated by rotary evaporation to give a white solid. The white solid was purified by flash chromatography on silica gel (6.5 x 17.5 cm bed, sample was loaded with $CHCl_3$; gradient elution, 1000 mL of 1:9 EtOAc-hexanes then 1000 mL of 1:4 EtOAc-hexanes followed by 500 mL of 3:7 EtOAc-hexanes) to afford 7.210 g of pure product and some mixed fractions which were combined and rechromatographed (4 x 20 cm bed; eluted with 600 mL of 3:7 EtOAc-hexanes) to afford an additional 2.575 g of pure product. Combined yield = 89%. mp 79-80 °C; R_f = 0.71, 3:2 hexanes-EtOAc; $[\alpha]_D^{25}$ -8.08° (*c* 1.36, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 (d, J = 7.5 Hz, 2H), 7.60 (d, J = 7.5 Hz, 2H), 7.24-7.42 (m, 9H), 5.70 (d, J = 8.7 Hz, NH), 5.34 (dd, J_1 = 19.5 Hz, J_2 = 12.3 Hz, C$\underline{H}_2$Ph), 4.33-4.64 (m, 5H, H-2 + 2 x H-10' + 2 x H-3), 4.22 (t, J = 7.0 Hz, 1H, H-9'), 4.04 (dd, J_1 = 9.6 Hz, J_2 = 3.0 Hz, H-3$_a$), 3.76 (dd, J_1 = 9.3 Hz, J_2 = 3 Hz, H-3$_b$), 2.00 (s, 3H, SCH_3). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 170.0, 155.9, 143.9, 143.7, 141.2, 141.2, 135.2, 128.6, 128.2, 127.7, 127.0, 125.1, 125.0, 119.9, 75.6 (C$\underline{H}_2$SMe), 67.8 (C-3), 67.4 (C$\underline{H}_2$Ph), 67.2 (C-10'), 54.2 (C-2), 47.1 (C-9), 13.7 (S$\underline{C}H_3$). HRMS (EI) m/z, calcd for $C_{27}H_{27}NO_5S$ [M^+] 477.1609, obsd 477.1619.

Fmoc-Ser(CH_2T)-OBn (12).[3] A flask containing 1.48 g of crushed 3Å MS was flame dried under vacuum, flushed with Ar, then cooled to RT. A solution of **11** (1.48 g, 3.11 mmol in 6 mL dry THF; 0.52 M) was transferred to the flask, followed by addition of a solution of thymine•2TMS[4] (4.21 g, 15.6 mmol, in 6.4 mL of dry THF; 1.45 M) and a solution of I_2 (1.18 g, 4.67 mmol, in 6 mL of dry THF; 7.78 M). The reaction mixture was stirred at RT under Ar for 48 h when TLC analysis showed only the product and some remaining starting material. The reaction mixture was poured into a 5% (w/v) aqueous solution of Na_2SO_3 (100 mL) and stirred vigorously whereupon a white precipitate formed. The mixture was suction-filtered and the cake washed with THF (200 mL). Excess THF was evaporated from the filtrate by rotary evaporation giving a further white precipitate. The aqueous layer was extracted with $CHCl_3$ (3 x 100 mL) and the combined organic layers washed with brine (2 x 100 mL) and dried over Na_2SO_4. The solid residue in the Büchner funnel was washed with THF until the washing was free of product (TLC). This THF washing was combined with the chloroform extract and concentrated by rotary evaporation to give a white solid. The white solid was purified by flash chromatography using silica gel (4 x 17.5 cm bed, column packed with 1:9 EtOAc-hexanes. The sample was loaded with $CHCl_3$ and 20 mL of additional $CHCl_3$ used to embed the sample onto the silica. This step is necessary to avoid precipitation of solid on the top of the column upon addition of eluting solvent). The column was eluted initially with 1:1 EtOAc-hexanes (200 mL) to recover 0.539 g of the starting material and then with 7:3 EtOAc-hexanes to give 1.036 g of pure product (60% yield; 95% based on recovered starting material). mp 69 °C (thawed) and 80 °C (formed glass); R_f = 0.46, 1:4 hexanes-EtOAc; $[\alpha]_D^{26}$ +2.20° (*c* 1.45, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.66 (s, 1H, NH_b), 7.77 (d, J = 7.2 Hz, 2H), 7.60 (d, J = 7.5 Hz, 2H), 7.27-7.43 (m, 9H), 6.97 (s, 1H, H-6'), 5.72 (d, J = 8.7 Hz, NH_a), 5.20 (AB quartet, J_{AB} = 12.3 Hz, 2 x H-1'''), 5.03 (s, C$\underline{H}_2$Ph), 4.60 (m, H-2), 4.33-4.48 (m, 2 x H-10''), 4.22 (t, J = 6.6 Hz, H-9''), 4.04 (dd, J_1 = 10.2 Hz, J_2 = 3.3 Hz, H-3$_a$), 3.88 (dd, J_1 = 9.9 Hz, J_2 = 3.3 Hz, H-3$_b$), 1.88 (s, 5'-Me). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 169.7, 163.7, 155.9, 151.0, 143.7, 141.3, 138.7, 128.7, 128.7, 128.3, 127.8, 127.1, 125.2, 125.1, 76.8 (C$\underline{H}_2$Ph), 69.6 (C-3), 67.6 (C-1'''), 67.3 (C-10''), 54.3 (C-2), 47.1 (C-9''), 12.3 (5'-C$\underline{H}_3$). HRMS (FAB, CsI/NaI/glycerol matrix) m/z, calcd for $C_{31}H_{29}N_3O_7$ [MCs^+] 688.1060, obsd 688.1046.

Fmoc-Ser(T)-OH (14). To a solution of **12** (1.52 g, 2.73 mmol) in 100 mL of 1:1 THF-MeOH was added 500 mg of Pd-C (10%) and then H_2 was bubbled through the reaction mixture at a moderate rate while stirring at RT for 45 min. A solution of 1:1 THF-MeOH was added to the reaction mixture periodically to replenish the evaporating solvent. At this point, TLC examination showed a clean conversion of starting material to product. The H_2 flow was stopped and Ar was bubbled through the reaction mixture for another 10 min. The reaction mixture was filtered through a pad of celite washing with THF until the wash was free of product (TLC). Upon evaporation of the solvent, a white foam was obtained which was dissolved in chloroform (2-3 mL) and concentrated by rotary evaporation (process repeated thrice to co-evaporate MeOH) to afford 1.280 g (quantitative) of a white solid. mp 80 °C (formed foam) and 110 °C (formed glass); R_f = 0.20, 90:8:2 $CHCl_3$-CH_3OH-AcOH; $[\alpha]_{578}^{23}$ +34.1° (*c* 1.11, $CHCl_3$), ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.22 (s, NH_b), 9.2 (br s, COOH), 7.73 (d, J = 7.1 Hz, 2H), 7.60 (t, J = 7.2 Hz, 2H), 7.37 (t, J = 7.8 Hz, 2H), 7.28 (t, J = 7.2 Hz, 2H), 7.05 (s, H-6'), 6.11 (d, 7.8 Hz, NH_a), 5.03 (AB quartet, J_{AB} = 9.9 Hz, 2 x H-1'''), 4.55 (m, H-2), 4.33-4.47 (m, 2

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[4] Garner, P.; Park, J. M. *J. Org. Chem*. **1990**, *55*, 3772.

x H-10"), 4.19 (t, 1H, J = 7.2 Hz, H-9"), 4.04 (br d, J = 7.8 Hz, H-3_a), 3.92 (br d, J = 8.1 Hz, H-3_b), 1.82 (s, 5'-Me).^{13}C NMR (75.4 MHz, $CDCl_3$) δ 165.0, 156.4, 151.8, 143.9, 143.8, 141.2, 140.0, 127.8, 127., 125.3, 125.2, 120.0, 111.5, 77.2, 69.5 (C-3), 67.2 (C-10"), 54.6 (C-2), 47.1 (C-9"), 12.1 (5'-Me); HRMS (FAB, CsI/NaI/Glycerol matrix) m/z, calcd for $C_{24}H_{23}N_3O_7$ [MCs^+] 598.0590, obsd 598.0620, calcd [MNa^+] 488.1433, obsd 488.1436.

***N*^{4}-BOC-Cytosine.**[5] A dry, 500 mL, one-necked, round-bottom flask, equipped with a magnetic stir bar and a rubber septum, was charged with 14.7 g (0.132 mol) of cytosine and 0.808 g (6.61 mmol) of DMAP. The apparatus was flushed with Ar and 240 mL of dry DMSO was added. The resulting white suspension was stirred at RT for 10 min, then 38 mL (0.165 mol) of di-*tert*-butyl dicarbonate was added whereupon the mixture became homogeneous. The light yellow solution was stirred for an additional 24 h at RT, during which time some white solid precipitated. Completion of the reaction was confirmed by analysis of ^{1}H NMR (1 mL of the reaction mixture was transferred to a 25 mL flask containing 10 mL water by syringe. The resulting white solid was filtered and washed with 10 mL each of water, MeOH, EtOAc, and hexane respectively. After drying under vacuum, the ^{1}H NMR of this white solid was taken). The reaction mixture was transferred to a 2 L flask and 1 L of water was added. The resulting suspension was stirred vigorously for 30 min and filtered. The yellow solid was washed first with 1 L of water and then with 500 mL each of MeOH, EtOAc and hexanes respectively to afford 14.97 g (54%) of pure product as a white solid. mp 270 °C (decomposed) ; R_f = 0.81, 4:1:1 *t*-BuOH-AcOH-H_2O; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.73 (d, J = 7.1 Hz, H-6), 6.87 (d, J = 7.1 Hz, H-5), 1.44 (s, ($C\underline{H}_3)_3$-). ^{13}C NMR (75.4 MHz, DMSO-d_6) δ 173.7 (C-6), 162.8 (C-2), 154.5, 146.3, 93.5 (C-5), 80.7 (($CH_3)_3\underline{C}$-), 27.8 (($\underline{C}H_3)_3$C-); HRMS (EI) m/z, calcd for $C_9H_{13}N_3O_3$ [M^+] 211.0957, obsd 211.0950.

***N*^{4}-BOC-Cytosine•2TMS.**[6] A dry 250 mL, one-necked, round-bottom flask, equipped with a magnetic stir bar and rubber septum was charged with 11.1 g (52.6 mmol) of *N* -BOC-cytosine. After flushing this system with Ar, 140 mL MeCN and 19.5 mL (78.9 mmol) of BSA (*N*,*O*-bis(trimethylsilyl)acetamide) was added. The resulting suspension was stirred at RT for 15 min, when a clear light-yellow solution was obtained. The reaction mixture was allowed to stir for another 4 h at RT under Ar atmosphere and then transferred to a flame-dried distillation assembly (flushed with Ar). The MeCN was first distilled under reduced pressure at RT and then the residual solvent/volatile-reagent (BSA) was removed by heating at 80-100 °C under reduced pressure (1-2 mm) to afford a white solid (17.2 g, 92 %) which was used directly in the next reaction without further purification.

Fmoc-Ser(C^{BOC})-OBn (13). A flask containing 0.63 g of crushed 3Å MS was flame dried under vacuum, flushed with Ar, then cooled to RT. A solution of **11** (0.628 g, 1.32 mmol in 3.0 mL dry THF, 0.44 M) was transferred to the flask, followed by the addition of a solution of *N*^{4}-BOC-cytosine•2TMS (0.701 g, 1.97 mmol in 1.4 mL of dry THF, 0.99 M) and a solution of I_2 (0.334 g, 1.32 mmol in 4.0 mL of dry THF). The reaction mixture was stirred at RT under Ar atmosphere for 48 h when TLC analysis showed only the presence of product and some starting material. The reaction mixture was then poured into a 5% (w/v) aqueous solution of Na_2SO_3 (40 mL) and stirred vigorously resulting the precipitation of a white solid. The mixture was suction-filtered and the cake washed with THF (200 mL). Excess THF was evaporated from the filtrate by rotary evaporation giving a further white precipitate. The aqueous layer was extracted with $CHCl_3$ (3 x 100 mL) and the combined organic layers washed with brine (2 x 100 mL) and dried over Na_2SO_4. The solid residue in the Büchner funnel was washed with THF until the washing was free of product (TLC). This THF washing was combined with the chloroform extract and concentrated by rotary evaporation to give a white solid. The white solid was purified by flash chromatography on silica gel (5.5 x 12 cm bed, sample loaded with $CHCl_3$), eluting first with 1:1 EtOAc-hexanes to afford 0.1122 g of starting material, and then with 7:3 EtOAc-hexanes to afford 0.402 g of pure **7** (48% yield, 58% based on recovered starting material). mp 177-178 °C R_f = 0.33, 7:3 EtOAc-hexanes; $[\alpha]_{578}^{25}$ +1.17° (*c* 1.96, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (d, J = 7.4 Hz, 2H), 7.60 (d, J = 7.1 Hz, 2H), 7.26-7.43 (m, 10H), 7.15 (d, J = 7.4 Hz, H-5'), 5.76 (d, J = 8.5 Hz, NH_a), 5.60-5.62 (m, 4H, $\underline{CH}_2$Ph, 2 x H-1"'), 4.58 (m, H-2), 4.32-4.48 (m, 2 x H-10"), 4.22 (t, J = 7.0 Hz, H-9"), 4.07 (dd, J_1 = 2.9 Hz, J_2 = 9.5 Hz, H-3a), 3.90 (dd, J_1 = 2.6 Hz, J_2 = 9.8 Hz, H-3b), 1.56 (s, 9H, ($C\underline{H}_3)_3$C-); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 169.7, 163.1, 155.9, 155.7, 150.9, 146.6, (C-6'), 143.8, 143.7, 141.3, 135.1, 128.6, 128.3, 127.7, 127.1, 125.1, 125.0, 119.9, 95.8 (C-5'), 83.0 (($CH_3)_3\underline{C}$-), 78.3, 69.9 (C-3'), 67.5, 67.2 (C-10") 54.3 (C-2) 47.1 (C-9") 28.0 (($\underline{C}H_3)_3$C-); HRMS (FAB, CsI/NaI/glycerol matrix) m/z, calcd for $C_{35}H_{36}N_4O_8$ [MH^+] 641.2611, obsd 641.2614.

Fmoc-Ser(C^{BOC})-OH (15).[7] A 250 mL round-bottomed flask, equipped with a magnetic stir bar was charged with 0.402 g (0.627 mmol) **13**. [Note: **13** must be a pure white solid, colored impurities appear to poison the Pd-catalyst.] and 25 mL of THF. The mixture was stirred at RT until a clear solution was obtained, when 10 mL of MeOH and 3.6 mL of formic acid were added. After bubbling Ar through this solution for 30 s, a mixture containing 0.803 g 10% Pd/C and 6 mL of 4.4% (v/v) formic acid in THF-MeOH (1:2, v/v) was added. The mixture was stirred while bubbling Ar for 5 min when reaction was complete as judged by TLC analysis. The reaction mixture was filtered

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[7] ElAmin, B.; Anantharamaiah, G. M.; Royer, G. P.; Means, G. *J. Org. Chem.*, **1979**, *44*, 3442.

through a pad of celite and the pad was washed with an additional 1000 mL of THF. To the filtrate 100 mL of water was added and excess THF and MeOH were removed by rotary evaporation giving a white solid. The product was suction-filtered and washed with water (2 x 100 mL). The residual white solid was dried over P_2O_5 under vacuum for 18 h (0.309 g, 90% yield). mp 130 °C (formed foam) and 156 °C (formed glass); R_f = 0.40, 4:1 $CHCl_3$-MeOH; $[\alpha]_{578}^{25}$ +29.6° (*c* 0.45, CH_2Cl_2); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.40 (br s, 1H), 8.03 (d, J = 6.9 Hz, H-6'), 7.86 (d, J = 7.4 Hz, 2H), 7.69 (d, J = 7.4 Hz, 2H), 7.39 (app t, J_1 = 7.4 Hz, J_2 = 7.2 Hz, 2H), 7.30 (app t, J_1 = 7.4 Hz, J_2 = 7.1 Hz, 2H), 7.08 (d, J = 6.9 Hz, H-5'), 6.97 (d, J = 7.4 Hz, NH_a), 5.16 (br s, 2 x H-1''') 4.20-4.30 (m, 1 x H-2), 4.03 (br s, H-9''), 3.80 (br s, 2 x H-10''), 3.58 (app t, J_1 = 6.0 Hz, J_2 = 4.1 Hz, 2 x H-3), 1.40 (s, ($C\underline{H}_3)_3$C-); ^{13}C NMR (75.4 MHz, DMSO-d_6) δ 163.6, 155.7, 155.2, 152.2, 148.7 (C-6'), 143.9, 140.7, 139.3, 127.6, 125.2, 124.9, 120.1, 94.6 (C-5'), 81.0 ($(CH_3)_3\underline{C}$-), 78.1, 69.8, 67.0 65.6, 55.5, 46.7 (C-9''), 27.8 ($(\underline{C}H_3)_3$C-). HRMS (FAB, CsI/NaI/glycerol matrix) m/z, calcd for $C_{28}H_{30}N_4O_8$ [MCs^+] 683.11180, obsd 683.11146; calcd for [M-H]Cs_2^+] 815.0094, obsd 815.0093.

SOLID PHASE PEPTIDE SYNTHESIS:

Reagents and Materials: DMSO (Fisher, ACS certified) was kept over 4Å MS for at least 12 h before use. NMP (1-methyl-2-pyrrolidinone, M-PYROL®, Aldrich) was distilled under reduced pressure and kept over 4Å MS for at least 12 h before use. DMF (*N*,*N*-dimethylformamide, Fisher, ACS certified) and DEA (*N*,*N*-diisopropylethylamine, Fluka, chemika) were heated to 60-70°C, while stirring, with ninhydrin (1,2,3-indantrione monohydrate, Pierce reagent, 500 mg/L of solvent) for one hour and then distilled under reduced pressure. Distilled DMF was kept over 4Å MS for 12 h before use. DIEA was redistilled from CaH_2 (0-1 mm grain size, ACROS) and kept over 4Å MS for 12 h before use. Piperidine (Aldrich) was distilled over KOH before use. DCM (Fisher, ACS certified), i-PrOH (Fisher, ACS certified), TFA (trifluoroacetic acid, Aldrich, protein sequencing grade), DBU (1,8-Diazabicyclo[5.4.0]undec-7-ene, Aldrich), bromophenol blue (Matheson Coleman & Bell) were used as received. All amino acids, their derivatives and the Rink Amide MBHA resin (4-(2',4'-dimethoxyphenyl-Fmoc-aminomethyl)-phenoxyacetamido-norleucyl-MBHA resin, 0.3-0.8 mmol/g loading capacity, 100-200 mesh) were obtained from Calbiochem-Novabiochem. HATU (O-(7-azabenzotriazol-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) was obtained from PerSeptive Biosystem.

HPLC Purification and Characterization. Crude peptides were purified by reversed phase HPLC using a semi-preparative C-18 column (Dynamax®-60A, C18-83-221C, 250 x 20 mm). A linear AB gradient (2.222% B/min) at a flow rate of 12 mL/min was used. Where solvent A was 0.036% aqueous TFA and solvent B was 0.045% TFA in acetonitrile. An UV detector (Spectroflow 757, Perkin Elmer) was used at 254 nm. Typically 5-6 mg of the crude sample was purified at a time (absorbance unit (FS) on the detector was kept at setting 2 during the separation for optimal performance). Analytical HPLCs were performed on either a Dynamax®-60A C-18 analytical column (C18-83-201C, 250 x 4.6 mm), a Thomson C8 analytical column (Advantage100-C-8, 250 x 4.6 mm, Thomson Liq. Chromatography), or a Thomson C4 analytical column (Advantage 300-C-4, 250 x 4.6 mm). For analytical HPLCs, the same gradient as that used for semi-preparative HPLC was employed but with a flow rate of 1 mL/min. MALDI-TOF mass spectra were recorded at the University of Georgia on a Kratos Kompact MALDI-TOF mass spectrometer. A 1:1 mixture of the sample and the sinapinic acid matrix were run in a linear mode and the spectrometer was calibrated with peptide standards.

The assembly of α-PNAs was carried out using an Advanced Chemtech Model 90 tabletop peptide synthesizer and the Rink Amide MBHA resin. The resin was swelled with DMSO for at least 16 h at RT before starting the synthesis. Coupling efficiency was monitored by a qualitative Kaiser test[8] except for the case of coupling to (ninhydrin inactive) Aib residue. In this case, coupling efficiency was monitored by bromophenol blue test.[9] Efficiency of the first amino acid loading was determined photometrically from the amount of Fmoc chromophore released upon treatment of resin with piperidine/DMF after the first coupling.[10] Amino acid coupling was done in a mixed solvent DMSO/NMP[11] to improve coupling rate (by reducing aggregation and folding of growing peptide chain) and solubility of DIPEA in DMSO. Fmoc deprotection was effected using DBU[12] followed by thorough washing of the resin to ensure complete removal of DBU. A standard method was used for N-acetylation (capping).[13] The following protocol was employed starting from 200 mg of Fmoc-protected resin (loading capacity 0.59 mmol/g).

A. Fmoc Deprotection

Reaction conditions:

2% DBU(v/v) in DMF, 8 min shake - flush

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[11] (a) Tam, J. P. *Int J. Peptide Protein Res.* **1987**, *29*, 421. (b) Bagley, J. C.; Otteson, K. M.; May, B. H.; McCurdy, S. N.; Pierce, L.; Ballard, F. J.; Wallace, J. C. *Ibid.* **1990**, *36*, 356. (c) Hyde, C; Johnson, T;, Sheppard, R. C. *J. Chem. Soc. Chem. Commun.* **1992**, 1573.

[12] Wade J. D.; Bedford, J.; Sheppard, R. C.; Tregear, G. W. *Pept. Res*, **1991**, *4*, 194.

[13] Eritja, R; Ziehler-Martin, J. P.; Walker, P. A.; Lee, T. D.; Legesse, K; Albericio, F.; Kaplan, B. E. *Tetrahedron*, **1987**, *43*, 2675.

Washing procedure:

1. 1 x 25 mL DMSO - flush
2. 2 x 15 mL DMSO, 1 min shake - flush
3. 1 x 25 mL ^{i}PrOH - flush
4. 1 x 15 mL ^{i}PrOH, 1 min shake - flush
5. 1 x 15 mL (0.5:1.0:8.5) DIPEA-^{i}PrOH-DMSO, 2 min shake - flush
6. 1 x 25 mL ^{i}PrOH - flush
7. 1 x 15 mL ^{i}PrOH, 1 min shake - flush
8. 2 x 15 mL (1:1) DMSO-^{i}PrOH, 2 min shake - flush
9. 2 x 25 mL DMSO - flush
10. 4 x 15 mL DMSO, 2 min shake - flush.

B. Peptide Coupling

Reaction conditions:

1. To a mixture of Fmoc amino acid (3 equiv) and HATU (3 equiv) was added a solution of DEA (9 equiv) in 4:1 NMP-DMSO (final concentration of amino acid was 0.2 M) and the resulting mixture was shaken for 5 min (pre-activation).

2. Pre-activated Fmoc amino acid was added to the resin-containing vessel and then shaken with N_2 bubbling for either 30 or 180 min. A 30 min coupling time was used when it was possible to monitor the coupling efficiency by either the Kaiser or Bromophenol blue test. In case of incomplete reaction, a second 30 min coupling was performed. In all other cases (overnight reactions), a single 180 min coupling was performed.

Washing procedure:

1. 1 x 25 mL DMSO flush
2. 4 x 15 mL DMSO, 2 min shake - flush

C. N-Terminus Acetylation

Reaction conditions:

After the final Fmoc cleavage, the resin was washed with NMP (3 x 15 mL, 1 min) to remove any DMSO. Acetylation (N-capping) was performed with Ac_2O (freshly distilled, 0.5 mL), DEA (0.85 mL), DMF (4.00 mL), for 15 min at RT.

Washing procedure:

1. 3 x 15 mL NMP, 1 min shake - flush
2. 5 x 15 mL DCM, 1 min shake - flush

D. Cleavage from Resin

The resin was transferred to a 25 mL round bottom flask with the aid of MeOH and DCM. After evaporation of the solvents under reduced pressure, the resin was treated with 95% TFA in water (v/v) [3 mL for 100 mg of resin] at room temperature for 1 h, with occasional shaking and sonicating. The orange red solution obtained was filtered through glass-wool and the resin was washed with 95% TFA in water [5 x 1 mL for 100 mg of resin]. TFA solution was evaporated on a rotary evaporator at RT to obtain an orange oil (1-2 mL). Cold ether (ice/water) was added to this orange oil with mixing until an off white precipitate formed. The precipitate was washed with ether (5 x 3 mL) to give an off white powder. The white powder was purified by reverse-phase HPLC as described above.

Ac-C(Acm)-G-S^{T}-D-A-E-S^{T}-A-A-K-S^{T}-A-A-E-S^{T}-A-Aib-A-S^{T}-K-G-NH_2 (1)

16% overall yield; t_R 16.2 min (C8); HRMS (MALDI-TOF) m/z, calcd for $[M + H]^+$ 2685.1236, obsd 2683.06.

Ac-Aib-G-S^{T}-D-A-E-S^{T}-A-A-K-S^{T}-A-A-E-S^{T}-A-Aib-A-S^{T}-K-C(Acm)-NH_2 (2)

15% overall yield; t_R 17.5 min (C18); HRMS (MALDI-TOF) m/z, calcd for $[M + H]^+$ 2714.1627, obsd 2713.15.

Ac-C(Acm)-G-S^{C}-D-A-E-S^{C}-A-A-K-S^{C}-A-A-E-S^{C}-A-Aib-A-S^{C}-K-G-NH_2 (3)

t_R 14.9 min (C4); HRMS (MALDI-TOF) m/z, calcd for $[M + H]^+$ 2610.1252, obsd 2613.65.

E. Disulfide Formation[14]

To a 100 µL of aqueous solution of peptide (1-3 µM), 100 µL of a iodine solution in methanol (iodine concentration should be only two equivalents to the peptide (Acm group) concentration, a higher iodine concentration will produce over oxidized products) was added at once and stirred vigorously for one hour at room temperature resulting in a turbid solution. HPLC analysis of an aqueous solution of the reaction mixture (diluted 10-fold) at this stage showed the reaction to be complete. The crude reaction mixture was purified directly by reverse-phase HPLC using a C-18 column. After lyophilization, the dimeric αPNA was isolated as a white powder.

[Ac-C-G-S^{T}-D-A-E-S^{T}-A-A-K-S^{T}-A-A-E-S^{T}-A-Aib-A-S^{T}-K-G-NH_2]$_2$ (4)

84% yield; t_R 17.5 min (C18); HRMS (MALDI-TOF) m/z, calcd for $[M + H]^+$ 5225.1494, obsd 5223.20.

[14] Kamber, B.; Hartmann, A.; Eisler, K.; Riniker, B.; Rink, H.; Sieber, P.; Rittel, W. *Helv. Chim. Acta*, **1980**, 63, 899.

Ac-Aib-G-S^T-D-A-E-S^T-A-A-K-S^T-A-A-E-S^T-A-Aib-A-S^T-K-C-NH_2]$_2$ (5)

49% yield; t_R 18.5 min (C18); HRMS (MALDI-TOF) m/z, calcd for $[M + H]^+$ 5281.2120, obsd 5282.26.

CIRCULAR DICHROISM (CD) SPECTROSCOPY:

CD spectra were recorded on a Jasco J-600 spectropolarimeter (Jasco) equipped with J-715 control driver version 1.00 and a Gateway 2000® computer running J-700 (system software version 1.50.00) for Windows® 95. To control the temperature of the cell a Neslab RTE-111M water bath/circulator was used. The instrument was routinely calibrated with an aqueous solution of recrystallized (+)-10-camphorsulfonic acid at 290 nm. HPLC grade water (Fisher) was used to make the solutions of αPNAs for CD measurements. CD spectra were the average of four scans obtained by collecting data at 0.10 nm intervals from 260 to 190 nm, with a response time of 2 s, bandwidth of 1 nm and sensitivity of 20 mdeg. Stoppered optical cells (Type: 21, Mat'l: H, Wilmad Glass Inc.) of path length 1 mm were used. Molar ellipticity is reported as the mean residue molar ellipticity ($[\theta]$, deg•cm^2•dmol^{-1}) and calculated from the equation:

$$[\theta] = [\theta]_{obs}(mrw)/10lc \qquad (1)$$

Where $[\theta]_{obs}$ is the ellipticity measured in degrees, mrw is the mean residue molecular weight (molecular weight of the peptide divided by the number of amino acid residues), c is the peptide concentration in grams per milliliter, and l is the optical path length of the cell in centimeters.

The α-helical nature of **4** was determined by CD spectroscopy in water. A thermal denaturation profile between 30 and 80 °C was obtained (see Figure 2 in paper). The double minimum at 219 and 206 nm and maximum at 193 nm are characteristic of an α-helix[15] while the isodichroic point at 202 nm was suggestive of a temperature-dependant α-helix to random coil transition. Helical content at 20 °C was estimated from a separate CD experiment by taking the ratio of $([\theta]_{219}-[\theta]_0)/[\theta]_{100}$, where $[\theta]_{219}$ is the mean residual helicity at 219 nm (= -14,850 deg•cm^2•dmol^{-1}), $[\theta]_0$ is the "background" mean residue ellipticity at 0% helicity (= -5,368 deg•cm^2•dmol^{-1}) as determined by a melting experiment (see Figure 2, insert). To account for the length dependence of CD for an α-helix, the following formula was used to calculate $[\theta]_{100}$:[16]

$$[\theta]_{100} = [\theta]_H^{\infty}\left(1 - k/n\right) \qquad (2)$$

Where $[\theta]_H^{\infty}$ is the molar ellipticity for an infinite helix (= -38,400 deg•cm^2•dmol^{-1}), k is a wavelength-dependent factor (= 2.69), and n is the number of helix amino acid residues (= 42). By this method of analysis, the helical content of **4** was estimated to be 26%.

UV-MELTING EXPERIMENTS:

Melting curves were obtained on a Perkin Elmer Lambda 20 UV-vis spectrophotometer equipped with a PTP-1 Peltier thermo-programmer. Perkin-Elmer's UV-WinLab software package was used to collect and process the data.

A solution of αPNA **4** and DNA $(dA)_{10}$ in water (2.2 μM, 1:1 ratio) was heated to 90 °C for 5 min then slowly cooled to room temperature. After equilibrating the solution at 4 °C for nearly 90 h (to maximize the hypochromic shift), a melting curve (A vs T) was obtained by heating the sample from 5 to 70 °C at a rate of 0.5 °C per minute. A melting temperature (T_m) of 51 °C was obtained from the first derivative of the melting curve. A similar experiment on a solution of αPNA **5** and DNA $(dA)_{10}$ in water (2.6 μM, 1:1 ratio) yielded a melting temperature of 39.1 ^{O}C.

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