CHAPTER II

EXPERIMENTAL SECTION

2.1 General procedures

2.1.1 Measurements

All reactions were peformed in oven-dried glasswares. The weight of all substances chemical was determined on a Metler Toledo electrical balance. Evaporation of solvents was carried out on Büchi Rotavapor R-200 with a water aspirator model B-490 or a Refco Vacubrand pump. The magnetic stirrers were of Corning. The progress of the reaction was followed by thin layer chromatography (TLC) performed on Merck D.C. silica gel 60 F_{254} 0.2 mm. precoated aluminium plates cat. no. 1.05554 and visualized using UV light (254 nm). Column chromatography was performed on silica gel 230-400 mesh for flash column chromatography and 70-230 for column chromatography. Reverse phase HPLC experiments were performed on Water Delta 600™ system equipped with gradient pump and Water 996™ photodiode array detector; optionally alternate to Rheodyne 7725 manual sample loop (200 µL sample size for analytical scale). A Varian™ C_{18} HPLC column 3 µm particle size 4.6 × 50 mm was used for both analytical purposes. Peak monitoring and data processing were performed on the base Empower software. Fractions from HPLC were collected manually which was assisted by real-time HPLC chromatogram monitoring. The combined fractions were speed vaporized under reduced pressure using Heto Vacuum Centrifuge and MAXI dry-plus. Melting points were recorded on an electrothermal melting point apparatus model 9100. The optical rotations ($[\alpha]_D$) were measured at the ambient temperature on a Jasco P-1010 Polarimeter using sodium light (D line, 589.3 nm) and are reported in degrees; concentrations (c) are reported as g/100 mL. Elemental analysis results were analyzed on CHNS/O Analyzer (Perkin Elmer PE2400 Series II) at Scientific and

Technological Research Equipment Centre, Chulalongkorn University. ^{1}H and ^{13}C NMR spectra were recorded in deuterated solvents on Varian Mercury-400 plus operating at 400, 300 or 200 MHz (^{1}H) and 100, 75 or 50 MHz (^{13}C) respectively. Chemical shifts (δ) are reported in part per million (ppm) relative to tetramethylsilane (TMS) or using the residual protonated solvent signal as a reference ($CDCl_3$ δ 7.27, DMSO-d_6 δ 2.50). Coupling constant (*J*) are proton-proton coupling unless otherwise noted and reported in hertz (Hz). Multiplicities were abbreviated as followed: singlet (s), doublet (d), triplet (t), quartet (q). Splitting patterns that could not be interpreted or easily visualized are designated as multiplet (m) or broad (br). MALDI-TOF mass spectra of all PNA sequences were obtained on a Microflex MALDI-TOF mass spectrometry (Bruker Daltonics) using doubly recrystallized α-cyano-4-hydroxy cinnamic acid (CCA) as matrix. 0.1% trifluoroacetic acid in acetonitrile:water (1:2) was used as the diluents for preparation of MALDI-TOF samples.

2.1.2 Materials and Methods

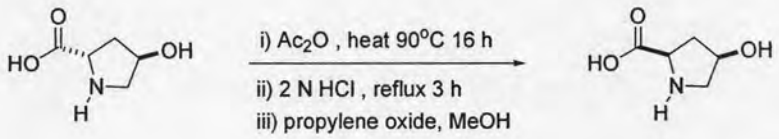
All chemicals were purchased from Fluka, Merck or Aldrich Chemical Co., Ltd., and were used as received without further purification. Commercial grade solvents were distilled before use for column chromatography. Solvents for reactions and crystallization were reagent grade and used without purification. Acetonitrile for HPLC experiment was HPLC grade, obtained for BDH and was filtered through a membrane filter (13 mm ϕ, 0.45 μm Nylon Lida) before use. Tetrahydrofuran and 1,4-dioxane for Mitsunobu reaction were dried with fresh thin-cut sodium metal and benzophenone under reflux. Anhydrous *N*,*N*-dimethylformamide ($H_2O \leq 0.01\%$) for solid phase peptide coupling reaction was obtained from Fluka and dried with activated 3Å molecular sieves. The solid support for peptide synthesis (TentaGel S RAM Fmoc resin) and trifluoroacetic acid were obtained from Fluka. The protected amino acids (Fmoc-L-Lys(Boc)-OPfp) was obtained form Calbiochem Novabiochem Co., Ltd. Fluorescence 5(6)-Carboxy-X-rhodamine *N*-succinimididyl ester for attach to PNA was purchased from Fluka. Acetic anhydride was synthesized from acetyl chloride and anhydrous sodium acetate according to the standard method. [77] Trifluoroacetic acid (98%) was obtained from Fluka. Nitrogen gas and hydrogen gas

was obtained from Thai Industrial Gas (TIG) with high purity up to 99.5 %. MilliQ water was obtained from ultrapure water system with Millipak® 40 filter unit 0.22 μm, Millipore (USA). All oligonucleotides were purchased from Bioservice Unit, National Science and Technology Development Agency (Thailand). N^4-Benzoylcytosine was prepared by Dr. Tirayut Vilaivan. (*N*-Fluoren-9-ylmethoxycarbonylamino)-*cis*-4-(N^2-isobutyrylguanin-9-yl)-D-proline pentafluorophenyl ester (**42**) and N^2-Isobutyryl-O^7-(*p*-nitroethyl)guanine were prepared by Miss Boonjira Boontha.

2.2 Synthesis of PNA monomers

2.2.1 Synthesis of Intermediate for Pyrrolidinyl monomers

***cis*-4-Hydroxy-D-proline (1)** [78]



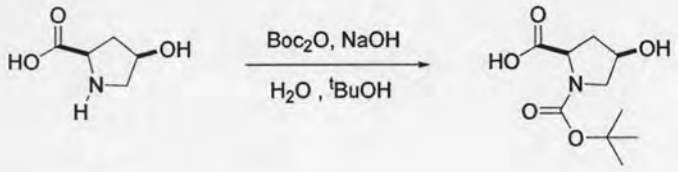
1

trans-4-Hydroxy-L-proline (26.65 g, 203.2 mmol) was heated in acetic anhydride (150 mL) at 90 °C under nitrogen atmosphere around 16-20 h to form cyclic lactone, then the mixture was allowed to cool at rt. The solvent was removed under reduced pressure to give dark thick oil, which was dissolved in 100 mL of 2 N hydrochloric acid and was then refluxed for another 3 h. The solvent was removed again to afford mixtures of *cis*-D and *trans*-L hydrochlorideas dark thick oil. This was redissolved in absolute methanol and then propylene oxide was slowly added. After stirring at rt for 8 h a pale brown solid was formed. Filtration and recrystallization from ethanol containing a small amount of water gave the desired product **1** as a white solid (17.12 g, 64% yield).

^{1}H NMR (400 MHz, D_2O) (Figure A-1) δ_H 2.08-2.12 [m, 1H, 1×C$\underline{H}_2$(3′)] 2.31-2.38 [m, 1H, 1×C$\underline{H}_2$(3′)] 3.19-3.32 [m, 2H, C$\underline{H}_2$(5′)] 4.04-4.07 [m, 1H, C$\underline{H}$(4′)] 4.42 [br,

1H, C<u>H</u>(2′)]; ^{13}C NMR (100 MHz, D_2O) (Figure A-2) δ_C 36.9 [<u>C</u>H$_2$(3′)] 52.7 [<u>C</u>H$_2$(5′)] 59.3 [<u>C</u>H(4′)] 68.9 [<u>C</u>H(2′)] 174.4 [<u>C</u>OOH]. $[\alpha]^{25}_D$ = 60.3 (c = 2.00 g/100 mL, H_2O), mp = 250-254 °C.

***N-tert*-Butoxycarbonyl-*cis*-4-hydroxy-D-proline (2)** [79]

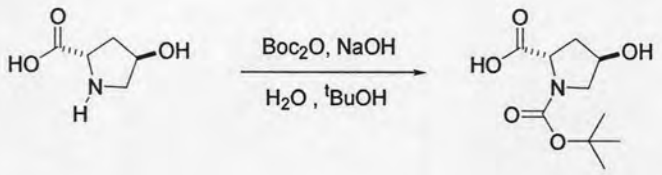


1 **2**

cis-4-Hydroxy-D-proline (1) (2.27 g, 17.3 mmol) was dissolved in aqueous 5% sodium hydroxide solution (20 mL). Di-*tert*-butyl bicarbonate (4.54 g, 20.8 mmol) was dissolved in *tert*-butanol (5 mL) and this solution was slowly added to the solution of *cis*-4-hydroxy-D-proline with stirring. Vigorous evolution of carbon dioxide and heat may be occurred during adding. If the solution was not homogeneous, sodium hydroxide solution or *tert*-butanol were added to keep the solution homogeneous. The reaction mixture was allowed to stir at rt for 8 h and then concentrated under reduce pressure. The residue was redissolved in water and acidified with 5% hydrochloric acid and the mixture was extracted with ethyl acetate (30 mL × 3). The combined organic layer was dried over sodium sulfate and evaporated by rotary evaporation. Scratching the oil or semisolid obtained with ice-cold hexanes affords the product **2** (3.83 g, 96% yield).

^{1}H NMR (400 MHz, DMSO-d_6) (Figure A-3) δ_H 1.31, 1.36 [2×s, 9H, C<u>H</u>$_3$ Boc rotamers] 1.78-1.81 [m, 1H, 1×C<u>H</u>$_2$(3′)] 2.21-2.33 [m, 1H, 1×C<u>H</u>$_2$(3′)] 3.07-3.09 [m, 1H, C<u>H</u>$_2$(5′)] 3.42-3.47 [m, 1H, C<u>H</u>$_2$(5′)] 4.03-4.09 [m, 1H, C<u>H</u> (2′)] 4.16-4.17 [m, 1H, C<u>H</u>(4′)]; ^{13}C NMR (100 MHz, DMSO-d_6) (Figure A-4) δ_C 28.3, 28.5 [<u>C</u>H$_3$ Boc rotamers] 38.0, 38.7 [<u>C</u>H$_2$(3′) rotamers] 54.0, 54.5 [<u>C</u>H$_2$(5′) rotamers] 57.5, 57.8 [<u>C</u>H(4′) rotamers] 68.0, 68.9 [<u>C</u>H(2′) rotamers] 79.0 [<u>C</u>CH$_3$ Boc] 153.5, 153.9 [<u>C</u>O Boc rotamers] 173.8, 174.2 [<u>C</u>OOH rotamers]. $[\alpha]^{25}_D$ = 50.2 (c = 1.05 g/100 mL, DMF), mp = 136-140 °C.

***N-tert*-Butoxycarbonyl-*trans*-4-hydroxy-L-proline (3)** [82]

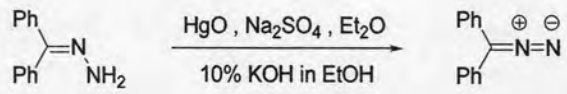


3

Synthesis of the title compound **3** was accomplished in the same way as described for compound **2** above. Starting from *trans*-4-hydroxy-L-proline (6.58 g, 50.0 mmol), aqueous 5% sodium hydroxide solution (70 mL) di-*tert*-butyl bicarbonate (13.19 g, 60.0 mmol) and *tert*-butanol (30 mL) afforded compound **3** (11.45 g, 99% yield).

^{1}H NMR (200 MHz, DMSO-d_6) (Figure A-5) δ_H 1.31, 1.35 [2×s, 9H, C$\underline{H}_3$ Boc rotamers] 1.82-1.92 [m, 1H, 1×C$\underline{H}_2$(3′)] 2.01-2.16 [m, 1H, 1×C$\underline{H}_2$(3′)] 3.21-3.42 [2×m, 2H, C$\underline{H}_2$(5′)] 4.07-4.12 [m, 1H, C$\underline{H}$(2′)] 4.20-4.23 [m, 1H, C$\underline{H}$(4′)]. ^{13}C NMR (50 MHz, DMSO-d_6) (Figure A-6) δ_C 28.2, 28.4 [$\underline{C}H_3$ Boc rotamers] 38.3, 39.1 [$\underline{C}H_2$(3′) rotamers] 54.7, 55.0 [$\underline{C}H_2$(5′) rotamers] 57.8, 58.0 [$\underline{C}$H(4′) rotamers] 68.2, 68.9 [$\underline{C}$H(2′) rotamers] 79.2 [$\underline{C}CH_3$ Boc] 153.6, 154.1 [$\underline{C}$O Boc rotamers] 174.3, 174.8 [$\underline{C}$OOH rotamers].

Diphenyldiazomethane (4) [83]

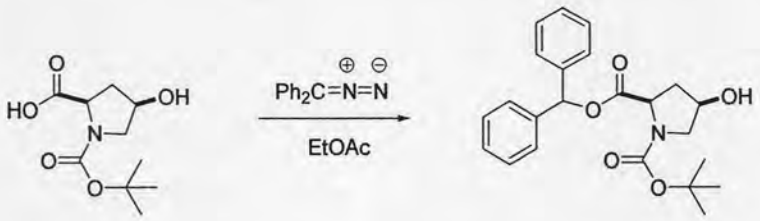


4

In a 1000 mL round bottle flask, wrapped with aluminium foil to protect from light, benzophenone hydrazone (13.74 g, 70.0 mmol), mercuric oxide (yellow) (15.16 g, 70.0 mmol) and anhydrous sodium sulfate (9.94 g, 70.0 mmol) were suspended in diethyl ether (150 mL) with stirring. Then 15 mL of 10% potassium hydroxide in ethanol was added until the solution turned to purple color. The reaction was stirred in

the dark for 6 h. The used mercuric oxide and sodium sulfate mixture were filtered off and washed with diethyl ether. Diethyl ether was removed by rotary evaporation without heating from the purple-colored filtrate to obtain product **4** as a purple liquid which was used for the next step without further purification.

***N-tert*-Butoxycarbonyl-*cis*-4-hydroxy-D-proline diphenylmethyl ester (5)** [80]



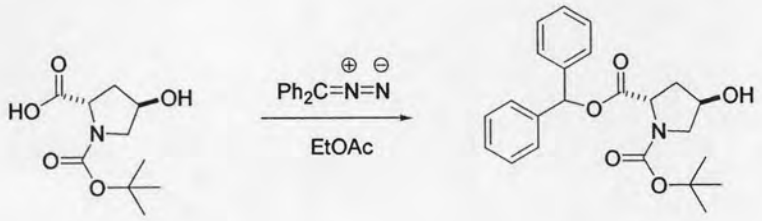
2 **5**

N-Boc-*cis*-4-hydroxy-D-proline (**2**) (12.69 g, 55.4 mmol) was stirred in ethyl acetate (70 mL) in a round bottom flask wrapped with aluminium foil and cooled in a cold-water bath to prevent the composition of the reagent. Freshly prepared diphenyldiazomethane (30 mL) diluting with ethyl acetate (30 mL) was gradually added to the solution and stirred at rt for 8 h. If the solution became colorless, more diphenyldiazomethane reagent was added to the reaction mixture until the purple color persists. The solvent was removed by rotary evaporator and the crude mixture was dissolved in a small amount of ethyl acetate. This solution was added dropwise to a flask containing vigorously stirred hexaness (1000 mL). A white solid precipitated which was collected by filtration and air-dried (20.47 g, 93% yield).

^{1}H NMR (300 MHz, $CDCl_3$) (Figure A-7) δ_H 1.17, 1.39 [2×s, 9H, C$\underline{H}_3$ Boc rotamers] 1.98 [t, J = 8.4 Hz, 1H, 1×C$\underline{H}_2$(3′)] 2.16-2.33 [m, 1H, 1×C$\underline{H}_2$(3′)] 3.47-3.55 [m, 2H, C$\underline{H}_2$(5′)] 4.22-4.26 [m, 1H, C$\underline{H}$(4′)] 4.34, 4.40 [dd, J = 23.6, 6.2 Hz, 1H, C$\underline{H}$(2′)] 6.81, 6.88 [2×s, 1H, C$\underline{H}$Ph$_2$ rotamers] 7.18-7.27 [m, 10H, phenyl C$\underline{H}$]; ^{13}C NMR (75 MHz, $CDCl_3$) (Figure A-8) δ_C 28.1, 28.4 [$\underline{C}H_3$ Boc rotamers] 37.7, 38.7 [$\underline{C}H_2$(3′) rotamers] 55.3, 55.7 [$\underline{C}H_2$(5′) rotamers] 58.0, 58.1 [$\underline{C}$H(4′) rotamers] 70.0, 70.9 [$\underline{C}$H(2′) rotamers] 77.9, 78.5 [$\underline{C}CH_3$ Boc rotamers] 80.3, 80.5 [$\underline{C}$H Dpm rotamers] 127.0-

128.6 [CH Ar Dpm] 139.4 [C Ar Dpm] 153.8, 154.4 [CO Boc rotamers] 173.4, 173.6 [CO rotamers]. $[\alpha]^{25}_{D}$ = +15.0 (c = 1.00 g/100 mL, $CHCl_3$), mp = 102-105 °C.

***N-tert*-Butoxycarbonyl-*trans*-4-hydroxy-L-proline diphenylmethyl ester (6)** [82]

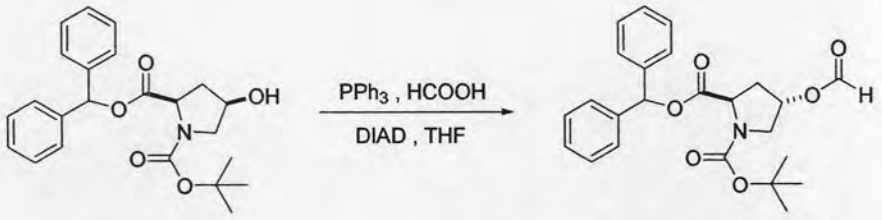


3 **6**

Synthesis of the title compound **6** was accomplished in the same way as described for compound **5** above. Starting from *N*-Boc-*cis*-4-hydroxy-D-proline (**3**) (7.68 g, 33.2 mmol) ethyl acetate (50 mL) and freshly prepared diphenyldiazomethane diluting with ethyl acetate (30 mL) afforded compound **6** (10.56 g, 80% yield).

^{1}H NMR (400 MHz, $CDCl_3$) (Figure A-9) δ_H 1.25, 1.48 [2×s, 9H, CH_3 Boc rotamers] 2.03-2.09 [m, 1H, 1×CH_2(3′)] 2.23-2.38 [m, 1H, 1×CH_2(3′)] 3.47-3.69 [m, 2H, CH_2(5′)] 4.42-4.51 [m, 1H, CH(4′)] 4.53-4.64 [dt, J = 22.8, 7.2 Hz, 1H, CH(2′)] 6.91, 6.96 [2×s, 1H, $CHPh_2$] 7.29-7.36 [m, 10H, phenyl CH]; ^{13}C NMR (50 MHz, $CDCl_3$) (Figure A-10) δ_C 28.0, 28.3 [CH_3 Boc rotamers] 38.2, 39.0 [CH_2(3′) rotamers] 54.6 [CH_2(5′)] 57.8, 58.1 [CH(4′) rotamers] 69.3, 70.0 [CH(2′) rotamers] 80.2 [CCH_3 Boc] 80.5 [CH Dpm] 126.7-128.5 [CH Ar Dpm] 139.7 [C Dpm] 154.1 [CO Boc] 171.8 [CO]. $[\alpha]^{25}_{D}$ = -49.0 (c = 1.00 g/100 mL, $CHCl_3$), mp = 93-95 °C.

***N-tert*-Butoxycarbonyl-*trans*-4-formyl-D-proline diphenylmethyl ester (7)** [80]

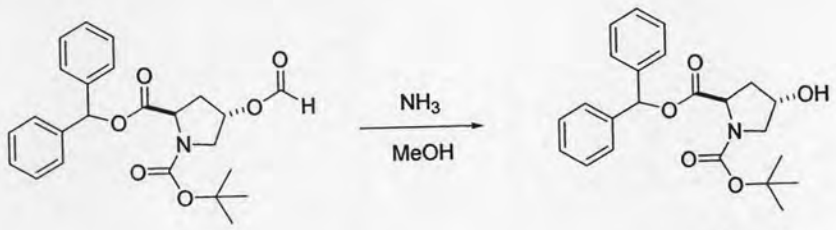


5 **7**

N-tert-butoxycarbonyl-*cis*-4-hydroxy-D-proline diphenylmethyl ester (**5**) (1.99 g, 5.0 mmol), triphenylphosphine (1.57 g, 6.0 mmol) and formic acid (0.2 mL, 6.0 mmol) were dissolved in dry THF (20 mL) in a dried 100 mL round bottom flask equipped with a magnetic bar and then cool down to 0 °C in an ice-salt bath. The solution was stirred under nitrogen balloon and DIAD (1.2 mL, 6.0 mmol) was added dropwise within 15 min and the reaction mixture was allowed to stir at 30 °C for 8 h. The solvent was evaporated and the residue was chromatographed on silica gel using hexanes:ethyl acetate (3:1) as eluent to give a clear viscous oil as *trans*-4-formate ester (7) (1.99 g, 93% yield).

^{1}H NMR (400 MHz, $CDCl_3$) (Figure A-11) δ_H 1.27, 1.48 [2×s, 9H, C$\underline{H}_3$ Boc rotamers] 2.13-2.23 [m, 1H, 1×C$\underline{H}_2$(3′)] 2.38-2.50 [m, 1H, 1×C$\underline{H}_2$(3′)] 3.61-3.80 [m, 2H, C$\underline{H}_2$(5′)] 4.51-4.63 [m, 1H, C$\underline{H}$(4′)] 5.34-5.41 [m, 1H, C$\underline{H}$(2′)] 6.93, 6.97 [2×s, 1H, C$\underline{H}$Ph$_2$ rotamers] 7.27-7.36 [m, 10H, phenyl C$\underline{H}$] 7.98 [s, 1H, C$\underline{H}$O]; ^{13}C NMR (100 MHz, $CDCl_3$) (Figure A-12) δ_C 28.0, 28.3 [$\underline{C}H_3$ Boc rotamers] 35.2, 36.4 [$\underline{C}H_2$(3′) rotamers] 51.9, 52.1 [$\underline{C}H_2$(5′) rotamers] 53.5 [$\underline{C}$H(4′) rotamers] 71.5, 72.3 [$\underline{C}$H(2′) rotamers] 77.4, 77.7 [$\underline{C}CH_3$ Boc rotamers] 80.5, 80.6 [$\underline{C}$H Dpm rotamers] 126.9-128.6 [$\underline{C}$H Ar Dpm rotamers] 139.6, 139.8 [$\underline{C}$ Dpm rotamers] 153.5, 154.0 [$\underline{C}$O Boc rotamers] 160.1 [$\underline{C}$HO] 171.0, 171.2 [$\underline{C}$O rotamers].

***N-tert*-Butoxycarbonyl-*trans*-4-hydroxy-D-proline diphenylmethyl ester (8)** [80]

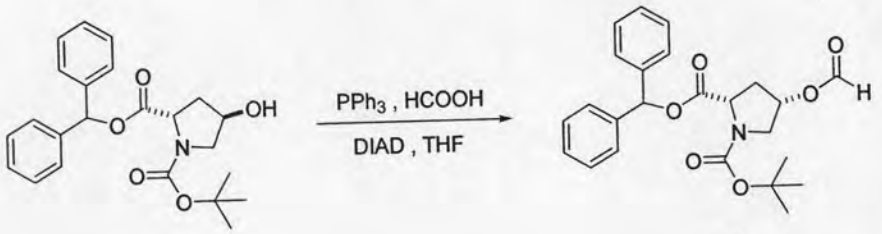


7 8

The *trans*-4-formate ester (7) was dissolved in a mixture of methanol (20 mL) and concentrated aqueous ammonia solution (2 mL). After 1 h, the ammonolysis was completed as indicated by TLC analysis. The solvent was removed under reduced pressure and the oily residue purified by column chromatography through silica gel using ethyl acetate:hexanes (1:2) as eluent to give a clear viscous oil. The product **8** was dried under vacuum (6.23 g, 78% yield 2 steps from compound **5**).

^{1}H NMR (400 MHz, $CDCl_3$) (Figure A-13) δ_H 1.25, 1.48 [2×s, 9H, C$\underline{H}_3$ Boc rotamers] 1.97-2.09 [m, 1H, 1×C$\underline{H}_2$(3′)] 2.24-2.38 [m, 1H, 1×C$\underline{H}_2$(3′)] 3.47-3.69 [m, 2H, C$\underline{H}_2$(5′)] 4.38-4.48 [m, 1H, C$\underline{H}$(4′)] 4.52-4.63 [dt, J = 26.8, 7.6 Hz, 1H, C$\underline{H}$(2′)] 6.91, 6.96 [2×s, 1H, C$\underline{H}$Ph$_2$ rotamers] 7.29-7.39 [m, 10H, phenyl C$\underline{H}$]; ^{13}C NMR (100 MHz, $CDCl_3$) (Figure A-14) δ_C 28.1, 28.4 [$\underline{C}$H$_3$ Boc rotamers] 38.2, 39.0 [$\underline{C}$H$_2$(3′) rotamers] 54.7 [$\underline{C}$H$_2$(5′)] 57.9, 58.2 [$\underline{C}$H(4′) rotamers] 69.2, 70.0 [$\underline{C}$H(2′) rotamers] 77.2, 77.6 [$\underline{C}$CH$_3$ Boc rotamers] 80.6 [$\underline{C}$H Dpm] 126.9-128.6 [$\underline{C}$H Ar Dpm] 139.7, 139.8 [$\underline{C}$ Dpm rotamers] 154.2 [$\underline{C}$O Boc] 171.9 [$\underline{C}$O]. $[\alpha]^{25}_D$ = +48.8 (c=1.00 g/100 mL $CHCl_3$); mp = 105-108 °C.

***N-tert*-Butoxycarbonyl-*cis*-4-formyl-L-proline diphenylmethyl ester (9)**

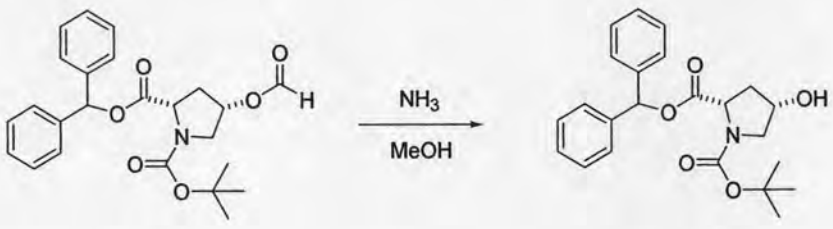


6 9

Synthesis of the title compound **9** was accomplished in the same way as described for compound 7 above. Starting from *N-tert*-Butoxycarbonyl-*trans*-4-hydroxy-L-proline diphenylmethyl ester (**6**) (1.21 g, 3.0 mmol), triphenylphosphine (1.00 g, 3.6 mmol) and formic acid (0.12 mL, 3.0 mmol) were dissolved in dry THF (10 mL) in a dried 100 mL round bottom flask equipped with a magnetic bar and then cool down to 0 °C in an ice-salt bath. The solution was stirred under nitrogen balloon and DIAD (0.70 mL, 3.6 mmol) was added dropwise within 15 min and the reaction mixture was allowed to stir at 30 °C for 8 h. afforded compound **9** (1.78 g, quantitative yield).

^{1}H NMR (400 MHz, $CDCl_3$) (Figure A-15) δ_H 1.32, 1.51 [2×s, 9H, C$\underline{H}_3$ Boc rotamers] 2.32-2.39 [m, 1H, 1×C$\underline{H}_2$(3′)] 2.45-2.50 [m, 1H, 1×C$\underline{H}_2$(3′)] 3.52-3.79 [m, 2H, C$\underline{H}_2$(5′)] 4.53-4.68 [2×m, 1H, C$\underline{H}$(4′)] 5.25-5.33 [m, 1H, C$\underline{H}$(2′)] 6.95, 6.99 [2×s, 1H, C$\underline{H}$Ph$_2$ rotamers] 7.24-7.37 [m, 10H, phenyl C$\underline{H}$]; ^{13}C NMR (100 MHz, $CDCl_3$) (Figure A-16) δ_C 28.1, 28.4 [$\underline{C}$H$_3$ Boc rotamers] 35.4, 36.3 [$\underline{C}$H$_2$(3′) rotamers] 52.0, 52.3 [$\underline{C}$H$_2$(5′) rotamers] 57.6, 57.9 [$\underline{C}$H(4′) rotamers] 71.4, 72.5 [$\underline{C}$H(2′) rotamers] 80.4, 80.5 [$\underline{C}$H Dpm rotamers] 127.1, 128.5 [$\underline{C}$H Ar Dpm rotamers] 139.7 [$\underline{C}$ Dpm] 153.6, 153.9 [$\underline{C}$O Boc rotamers] 159.9 [$\underline{C}$HO] 170.5, 170.7 [$\underline{C}$O rotamers].

N-tert-Butoxycarbonyl-_cis_-4-hydroxy-L-proline diphenylmethyl ester (10)

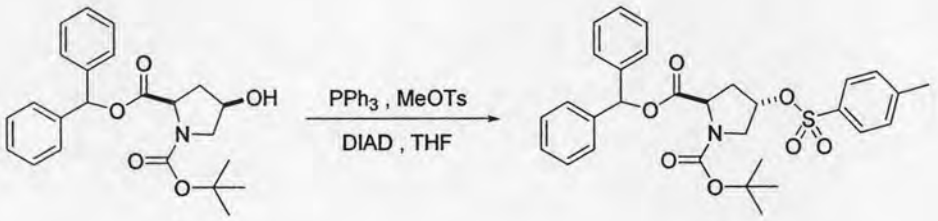


9 **10**

Synthesis of the title compound **10** was accomplished in the same way as described for compound **8** above. Starting from *N-tert*-Butoxycarbonyl-*cis*-4-formyl-L-proline diphenylmethyl ester (**9**) (1.21 g, 3.0 mmol), methanol (10 mL) and concentrated aqueous ammonia solution (1 mL) afforded title compound **10** (1.78 g, quantitative yield, 2 steps from compound **6**).

^{1}H NMR (400 MHz, $CDCl_3$) (Figure A-17) δ_H 1.27, 1.49 [2×s, 9H, C<u>H</u>$_3$ Boc] 2.09 [t, J = 7.6 Hz, 1H, 1×C<u>H</u>$_2$(3′)] 2.28-2.42 [m, 1H, 1×C<u>H</u>$_2$(3′)] 3.56-3.70 [m, 2H, C<u>H</u>$_2$(5′)] 4.31-4.38 [br, 1H, C<u>H</u>(4′)] 4.45, 4.57 [dd, J = 23.4, 5.0 Hz, 1H, C<u>H</u>(2′)] 6.91, 6.99 [2×s, 1H, C<u>H</u>Ph$_2$ rotamers] 7.29-7.37 [m, 10H, phenyl C<u>H</u>]; ^{13}C NMR (100 MHz, $CDCl_3$) (Figure A-18) δ_C 28.0, 28.3 [<u>C</u>H$_3$ Boc rotamers] 37.7, 38.6 [<u>C</u>H$_2$(3′) rotamers] 55.2, 55.6 [<u>C</u>H$_2$(5′) rotamers] 58.1 [<u>C</u>H(4′)] 69.9, 70.8 [<u>C</u>H(2′) rotamers] 77.9, 78.4 [<u>C</u>CH$_3$ Boc rotamers] 80.2, 80.4 [<u>C</u>H Dpm rotamers] 126.9-128.5 [<u>C</u>H Ar Dpm] 139.5 [<u>C</u> Dpm] 153.8, 154.3 [<u>C</u>O Boc rotamers] 173.3, 173.6 [<u>C</u>O rotamers]; Anal Calcd. for $C_{23}H_{27}NO_5$ requires C, 69.50; H, 6.85; N, 3.52 %; Found C, 69.51; H, 6.68; N, 3.51 %; $[\alpha]^{25}_D$ = -14.6 (c=3.285 g/100 mL $CHCl_3$); mp = 106-108 °C.

***N-tert*-Butoxycarbonyl-*trans*-4-tosyl-D-proline diphenylmethyl ester (11)** [80,85]



5 **11**

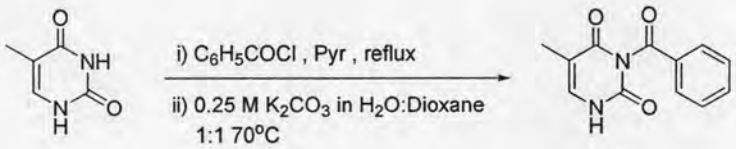
N-tert-butoxycarbonyl-*cis*-4-hydroxy-D-proline diphenylmethyl ester (**5**) (7.98 g, 20.0 mmol), triphenylphosphine (6.34 g, 24.0 mmol) and dry THF (30 mL) were dissolved in a dried 100 mL round bottom flask equipped with a magnetic bar and then cool down to 0 °C in an ice-salt bath. The solution was stirred under nitrogen balloon, methyl tosylate (3.1 mL, 20 mmol) and DIAD (4.7 mL, 24.0 mmol) were then added and the reaction mixture was allowed to stir at 30 °C for 8 h. The solvent was evaporated and residue was chromatographed on silica gel plate using hexanes:ethyl acetate (4:1) as eluent and the oily residue was purified by column chromatography through silica gel to give a white foamy solid as *trans*-4-tosylate ester (**11**) (9.06 g, 82% yield).

^{1}H NMR (400 MHz, $CDCl_3$) (Figure A-19) δ_H 1.20,1.41 [2×s, 9H, C<u>H</u>$_3$ Boc rotamers] 1.96-2.13 [m, 1H, 1×C<u>H</u>$_2$(3′)] 2.36-2.60 [2×m, 1H, 1×C<u>H</u>$_2$(3′)] 2.44 [s, 3H, C<u>H</u>$_3$ tosyl]

3.54-3.71 [m, 2H, C$\underline{H}_2$(5′)] 4.46-4.55 [m, 1H, C$\underline{H}$(4′)] 4.94-5.01 [m, 1H, C$\underline{H}$(2′)] 6.84, 6.89 [2×s, 1H, C$\underline{H}$ Dpm rotamers] 7.27-7.34 [m, 12H, C$\underline{H}$ Ar Dpm and tosyl] 7.74-7.76 [m, 2H, C$\underline{H}$ Ar tosyl]; ^{13}C NMR (100 MHz, $CDCl_3$) (Figure A-20) δ_C 21.6 [$\underline{C}H_3$ Ts] 27.9, 28.2 [$\underline{C}H_3$ Boc rotamers] 35.7, 37.1 [$\underline{C}H_2$(3′) rotamers] 51.8, 52.1 [$\underline{C}H_2$(5′) rotamers] 57.3, 57.5 [$\underline{C}$H(4′) rotamers] 77.5, 77.8 [$\underline{C}$H(2′) rotamers] 78.2, 78.8 [$\underline{C}CH_3$ Boc rotamers] 80.6, 80.8 [$\underline{C}$H Dpm rotamers] 126.8-130.0 [$\underline{C}$H Ar Dpm and Ts], 133.2, 133.4 [$\underline{C}$ Dpm rotamers] 139.4, 139.6 [$\underline{C}$ Dpm] 145.2, 145.3 [$\underline{C}$ tosyl rotamers] 153.2, 153.7 [$\underline{C}$O Boc rotamers] 170.8, 171.0 [$\underline{C}$O rotamers]; $[\alpha]^{25}_D$ = +27.4 (c = 0.99 g/100 mL $CHCl_3$); mp = 147-149 °C.

2.2.2 Synthesis of all four diastereomers of thymine pyrrolidinyl monomers

N^3-Benzoylthymine (12) [84]

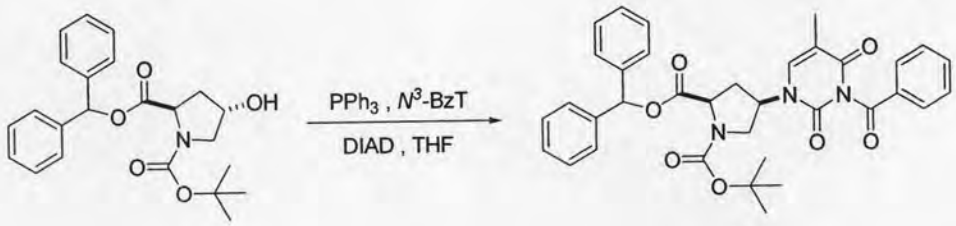


12

Benzoyl chloride (12.8 mL, 110.0 mmol) was added in one portion to a magnetically stirred suspension of powdered thymine (6.31, 50.0 mmol) in pyridine (30 mL) at reflux temperature. The reaction mixture remained heterogeneous throughout. After 3 h, the solvent was removed using a rotary evaporator. The residue was treated with 0.25 M potassium carbonate in H_2O and dioxane 1:1 (100 mL) and then heated at 70 °C in a water bath until the *N*-1 benzoyl group was completely cleaved, leaving only N^3-benzoyl thymine as the desired product. The progress of the reaction can be monitored by TLC in hexanes:ethyl aetate 1:2. Thus, the R_f of the N^1-benzoyl isomer was found to be higher than the R_f of the N^3-benzoyl isomer. After that, the solvent was evaporated and the pale yellow residue suspended in water and collected by filtration. The collected solid was washed with cold water and dried in vacuo at room temperature (10.22 g, 89% yield).

^{1}H NMR (400 MHz, DMSO-d_6) (Figure A-21) δ_H 4.00 [s, 3H, C<u>H</u>$_3$] 9.71 [s, 1H, C<u>H</u>] 9.78 [t, J = 7.9 Hz, 2H, C<u>H</u>(3,5) Ar] 9.96 [t, J = 7.4 Hz, 1H, C<u>H</u>(4) Ar] 10.12 [d, J = 7.3 Hz, 2H, C<u>H</u>(2,6) Ar]; ^{13}C NMR (100 MHz, DMSO-d_6) (Figure A-22) δ_C 12.1 [<u>C</u>H$_3$ Thymine] 108.4 [<u>C</u>5 Thymine], 129.8 [<u>C</u>4′ Ar] 130.6 [<u>C</u>1′ and <u>C</u>6′ Ar] 131.8 [<u>C</u>3′ and <u>C</u>5′ Ar] 135.7 [<u>C</u>1′ Ar] 139.1 [<u>C</u>6H Thymine] 150.5 [<u>C</u>2 Thymine] 164.0 [<u>C</u>4 Thymine] 170.6 [<u>C</u>O Bz Thymine]; mp = 152-155 °C.

N-tert-Butoxycarbonyl-_cis_-4-(N^3-benzoylthymin-1-yl)-D-proline diphenylmethyl ester (13) [80,86]



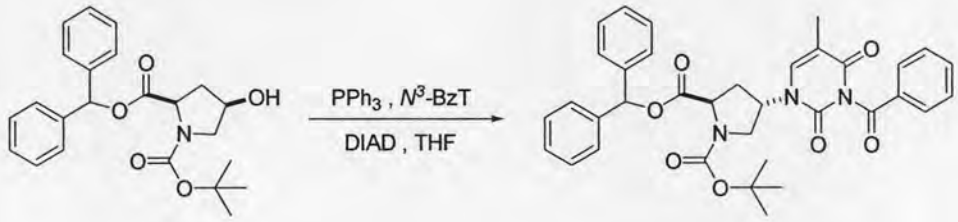
8 **13**

In a dried 100 mL round bottom flask equipped with a magnetic bar. The protected *trans*-4-hydroxy-D-proline (**8**) (6.23 g, 15.7 mmol), triphenylphosphine (4.96 g, 18.8 mmol) and N^3-benzoylthymine (**12**) (4.35 g, 18.8 mmol) were dissolved in dry THF (30 mL) with stirring and cooled down to 0 °C in an ice-salt bath. The reaction mixture was added DIAD (3.7 mL, 18.8 mmol) *via* syring slowly during within 15 min and allowed to stir at rt for 8 h. When the reaction was completed (checked by TLC using hexaness:ethyl acetate 2:1 with ammonium molybdate reagent), the solvent was evaporated and the residue recrystallized from methanol to afford the product **13** as white solid (4.76 g, 50% yield).

^{1}H NMR (400 MHz, $CDCl_3$) (Figure A-23) δ_H 1.33, 1.52 [2×s, 9H, C<u>H</u>$_3$ Boc rotamers] 1.76-1.84 [m, 3H, C<u>H</u>$_3$ Thymine] 1.99-2.14 [m, 1H, 1×C<u>H</u>$_2$(3′)] 2.78-2.93 [m, 1H, 1×C<u>H</u>$_2$(3′)] 3.48-3.70 [m, 1H, 1×C<u>H</u>$_2$(5′)] 3.99-4.04 [m, 1H, 1×C<u>H</u>$_2$(5′)] 4.48-4.67 [2×m, 1H, C<u>H</u>(4′)] 5.21-5.32 [m, 1H, C<u>H</u>(2′)] 6.96 [s, 1H, C<u>H</u>Ph$_2$] 7.14, 7.22 [2×s, 1H, C6<u>H</u> Thymine rotamers] 7.29-7.42 [m, 10H, Phenyl Dpm] 7.51 [t, J = 7.9 Hz, 2H, C<u>H</u>(3,5) Ar Bz] 7.67 [t, J = 7.4 Hz, 1H, C<u>H</u>(4) Ar Bz] 7.91 [d, J = 7.4 Hz,

2H, C<u>H</u>(2,6) Ar Bz]; ^{13}C NMR (100 MHz, $CDCl_3$) (Figure A-24) δ_C 12.5 [<u>C</u>H$_3$ Thymine] 28.0, 28.3 [<u>C</u>H$_3$ Boc rotamers] 33.9, 35.5 [<u>C</u>H$_2$(3′) rotamers] 49.4, 49.8 [<u>C</u>H$_2$(5′) rotamers] 52.1, 52.5 [<u>C</u>H(4′) rotamers] 57.4, 57.6 [<u>C</u>H(2′) rotamers] 78.0, 78.4 [<u>C</u>CH$_3$ Boc rotamers] 81.3 [<u>C</u>H Dpm] 111.6 [<u>C</u>5 Thymine] 126.8-128.6 [<u>C</u>H Ar Dpm] 129.1 [<u>C</u>4′ Ar] 130.4 [<u>C</u>1′ and <u>C</u>6′ Ar] 135.0 [<u>C</u>3′ and <u>C</u>5′ Ar] 135.8 [<u>C</u>1′ Ar] 139.2 [<u>C</u>6H Thymine] 149.8 [<u>C</u> Dpm] 153.3 [<u>C</u>2 Thymine] 162.3 [<u>C</u>4 Thymine] 168.7 [<u>C</u>O Boc] 171.2 [<u>C</u>O Proline]; $[\alpha]^{25}_D$ = +16.9 (c = 1.03 g/100 mL DMF); mp = 183-186 °C.

N-tert-Butoxycarbonyl-_trans_-4-(N^3-benzoylthymin-1-yl)-D-proline diphenyl methyl ester (14) [80]



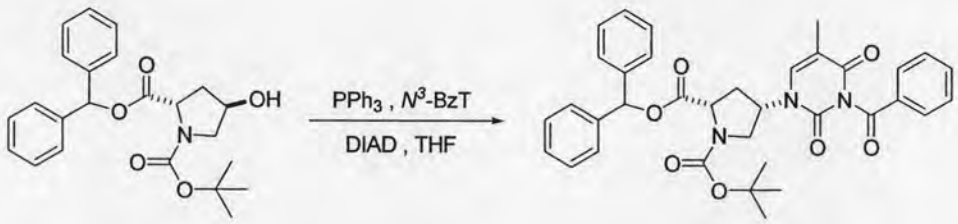
5 **14**

Synthesis of the title compound **14** was accomplished in the same way as described for compound **13** above. Starting from *N-tert*-Butoxycarbonyl-*cis*-4-hydroxy-D-proline diphenylmethyl ester (**5**) (1.19 g, 3.0 mmol), triphenylphosphine (0.97 g, 3.6 mmol) and N^3-Benzoylthymine (**12**) (0.86 mL, 3.6 mmol) and dry THF (20 mL) were dissolved in a dried 100 mL round bottom flask equipped with a magnetic bar and then cool down to 0 °C in an ice-salt bath. The solution was stirred under nitrogen balloon and DIAD (0.7 mL, 3.6 mmol) was added dropwise within 15 min and the reaction mixture was allowed to stir at 30 °C for 8 h. The solvent was removed under reduced pressure and the oily residue purified by column chromatography through silica gel using ethyl acetate:hexanes (1:2) as eluent to give a clear viscous oil afforded compound **14** (0.90 g, 49% yield).

^{1}H NMR (200 MHz, $CDCl_3$) (Figure A-25) δ_H 1.31, 1.46 [s, 9H, C<u>H</u>$_3$ Boc rotamers] 1.98 [s, 3H, C<u>H</u>$_3$ Thymine] 2.27-2.41 [m, 1H, 1×C<u>H</u>$_2$(3′)] 2.53-2.67 [m, 1H,

1×C<u>H</u>$_2$(3′)] 3.59-3.63 [2×m, 1H, 1×C<u>H</u>$_2$(5′)] 3.90-4.00 [m, 1H, 1×C<u>H</u>$_2$(5′)] 4.58-4.72 [2×m, 1H, C<u>H</u>(4′)] 5.12-5.20 [m, 1H, C<u>H</u>(2′)] 6.91, 6.96 [2×s, 1H, C<u>H</u>Ph$_2$ rotamers] 7.05 [s, 1H, C6<u>H</u> Thymine] 7.27-7.40 [m, 10H, Phenyl Dpm] 7.52 [t, J = 7.7 Hz, 2H, C<u>H</u>(3,5) Ar Bz] 7.68 [t, J = 7.3 Hz, 1H, C<u>H</u>(4) Ar Bz] 7.94 [d, J = 7.5 Hz, 2H, C<u>H</u>(2,6) Ar Bz]; ^{13}C NMR (50 MHz, $CDCl_3$) (Figure A-26) δ_C 12.7 [<u>C</u>H$_3$ Thymine] 28.0, 28.3 [<u>C</u>H$_3$ Boc rotamers] 33.4, 35.0 [<u>C</u>H$_2$(3′) rotamers] 49.1, 49.6 [<u>C</u>H(5′) rotamers] 53.8, 54.4 [<u>C</u>H(4′) rotamers] 57.8, 58.0 [<u>C</u>H(2′) rotamers] 77.9, 78.1 [<u>C</u>CH$_3$ Boc rotamers] 81.1, 81.2 [<u>C</u>H Dpm rotamers] 111.6 [<u>C</u>5 Thymine] 126.8-128.6 [<u>C</u>H Ar Dpm] 129.2 [<u>C</u>4′ Ar] 130.4 [<u>C</u>1′ and <u>C</u>6′ Ar] 131.3 [<u>C</u>3′ and <u>C</u>5′ Ar] 135.2, 136.2 [<u>C</u>1′ Ar] 149.6 [<u>C</u> Dpm] 153.4, 153.8 [<u>C</u>2 Thymine] 162.5 [<u>C</u>4 Thymine] 168.9 [<u>C</u>O Boc] 170.8 [<u>C</u>O Proline]. $[\alpha]^{25}_D$ = +32.0 (c=1.00 g/100 mL $CHCl_3$); mp=189-192 °C.

N-tert-Butoxycarbonyl-_cis_-4-(N^3-benzoylthymin-1-yl)-L-proline diphenylmethyl ester (15) [80]

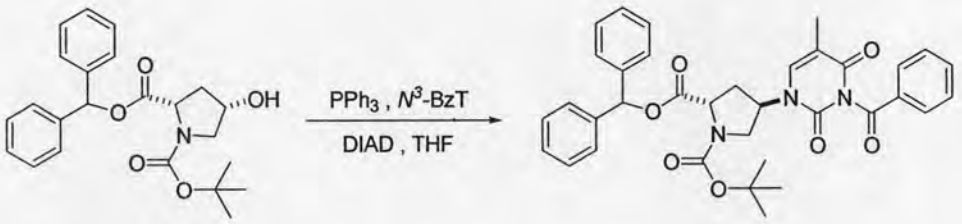


6 **15**

Synthesis of the title compound **15** was accomplished in the same way as described for compound **13** above. Starting from *N-tert*-Butoxycarbonyl-*trans*-4-hydroxy-L-proline diphenylmethyl ester (**6**) (3.99 g, 10 mmol), triphenylphosphine (3.15 g, 12 mmol) and N^3-Benzoylthymine (**12**) (2.30 g, 10 mmol) and dry THF (20 mL) were dissolved in a dried 100 mL round bottom flask equipped with a magnetic bar and then cool down to 0 °C in an ice-salt bath. The solution was stirred under nitrogen balloon and DIAD (2.3 mL, 12 mmol) was added dropwise over a period of 15 min. The reaction mixture was allowed to stir at rt for 8 h afforded. The solvent was removed under reduced pressure and the oily residue purified by column chromatography through silica gel using ethyl acetate:hexanes (1:2) as eluent to give a clear viscous oil **15** (3.61 g, 59% yield).

^{1}H NMR (400 MHz, $CDCl_3$) (Figure A-27) δ_H 1.22, 1.41 [2×s, 9H, C$\underline{H}_3$ Boc rotamers] 1.70-1.73 [m, 3H, C$\underline{H}_3$ Thymine] 1.90-2.03 [m, 1H, 1×C$\underline{H}_2$(3′)] 2.68-2.82 [m, 1H, 1×C$\underline{H}_2$(3′)] 3.38-3.59 [2×m, 1H, 1×C$\underline{H}_2$(5′)] 3.89-3.94 [m, 1H, 1×C$\underline{H}_2$(5′)] 4.40-4.54 [2×m, 1H, C$\underline{H}$(4′)] 5.09-5.21 [m, 1H, C$\underline{H}$(2′)] 6.84-6.85 [m, 1H, C$\underline{H}$Ph$_2$] 7.02, 7.11 [2×s, 1H, C6$\underline{H}$ Thymine] 7.18-7.27 [m, 10H, Phenyl Dpm] 7.40 [t, J = 7.5 Hz, 2H, C$\underline{H}$(3,5) Ar Bz] 7.56 [t, J = 7.3 Hz, 1H, C$\underline{H}$(4) Ar Bz] 7.81 [d, J = 7.3 Hz, 2H, C$\underline{H}$(2,6) Ar Bz]; ^{13}C NMR (100 MHz, $CDCl_3$) (Figure A-28) δ_C 11.4 [$\underline{C}$H$_3$ Thymine] 26.9, 27.2 [$\underline{C}$H$_3$ Boc rotamers] 32.8, 34.4 [$\underline{C}$H$_2$(3′) rotamers] 48.3, 48.7 [$\underline{C}$H$_2$(5′) rotamers] 51.1, 51.5 [$\underline{C}$H(4′) rotamers] 56.5 [$\underline{C}$H(2′)] 77.0, 77.3 [$\underline{C}$CH$_3$ Boc rotamers] 80.2 [$\underline{C}$H Dpm] 110.5 [$\underline{C}$5 Thymine] 125.7-127.5 [$\underline{C}$H Ar Dpm] 128.1 [$\underline{C}$4′ Ar] 129.3 [$\underline{C}$1′ and $\underline{C}$6′ Ar] 130.3 [$\underline{C}$3′ and $\underline{C}$5′ Ar] 134.0, 134.9 [$\underline{C}$1′ Ar] 138.1 [$\underline{C}$6H Thymine] 148.8 [$\underline{C}$ Dpm] 152.3 [$\underline{C}$2 Thymine] 161.3 [$\underline{C}$4 Thymine] 167.7 [$\underline{C}$O Boc] 170.2 [$\underline{C}$O Proline]; $[\alpha]^{25}_D$ = -23.8 (c = 1.00 g/100 mL $CHCl_3$); mp = 183-185 °C.

N-tert-Butoxycarbonyl-_trans_-4-(N^3-benzoylthymin-1-yl)-L-proline diphenyl methyl ester (16)



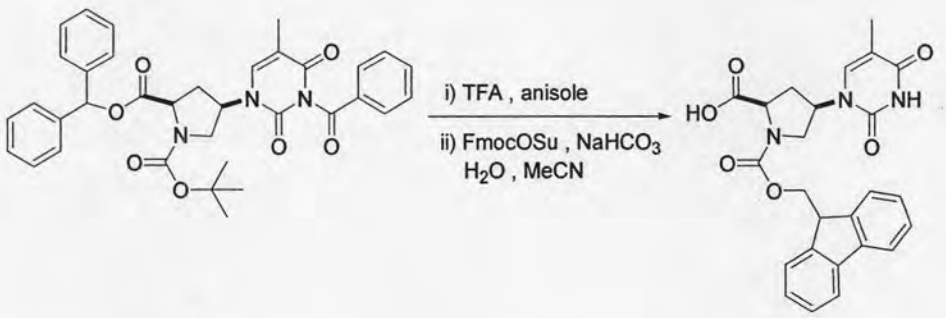
10 **16**

Synthesis of the title compound **16** was accomplished in the same way as described for compound **13** above. Starting from *N-tert*-Butoxycarbonyl-*cis*-4-hydroxy-L-proline diphenylmethyl ester (**10**) (1.21 g, 3.04 mmol), triphenylphosphine (1.23 g, 4.56 mmol) and N^3-Benzoylthymine (**12**) (1.05 mL, 4.56 mmol) and dry THF (20 mL) were dissolved in a dried 100 mL round bottom flask equipped with a magnetic bar and then cool down to 0 °C in an ice-salt bath. The solution was stirred under nitrogen balloon and DIAD (0.88 mL, 4.56 mmol) was added dropwise over a period of 15 min. The reaction mixture was allowed to stir at 30 °C for 8 h. The solvent was removed under reduced pressure and the oily residue purified by column

chromatography through silica gel using ethyl acetate:hexanes (1:2) as eluent to give a clear viscous oil afforded compound **16** (1.12 g, 60% yield).

^{1}H NMR (400 MHz, $CDCl_3$) (Figure A-29) δ_H 1.31, 1.50 [2×s, 9H, C<u>H</u>$_3$ Boc rotamers] 1.98 [s, 3H, C<u>H</u>$_3$ Thymine] 2.32-2.44 [m, 1H, 1×C<u>H</u>$_2$(3′)] 2.53-2.66 [m, 1H, 1×C<u>H</u>$_2$(3′)] 3.61-3.82 [2×m, 1H, 1×C<u>H</u>$_2$(5′)] 3.92-4.00 [m, 1H, 1×C<u>H</u>$_2$(5′)] 4.57-4.72 [2×m, 1H, C<u>H</u>(4′)] 5.11-5.19 [m, 1H, C<u>H</u>(2′)] 6.91, 6.96 [2×s, 1H, C<u>H</u>Ph$_2$] 7.05 [s, 1H, C6<u>H</u> Thymine] 7.29-7.37 [m, 10H, Phenyl Dpm] 7.52 [t, J = 7.7 Hz, 2H, C<u>H</u>(3,5) Ar Bz] 7.68 [t, J = 7.3 Hz, 1H, C<u>H</u>(4) Ar Bz] 7.94 [d, J = 7.5 Hz, 2H, C<u>H</u>(2,6) Ar Bz]; ^{13}C NMR (100 MHz, $CDCl_3$) (Figure A-30) δ_C 12.7 [<u>C</u>H$_3$ Thymine] 28.0, 28.3 [<u>C</u>H$_3$ Boc rotamers] 33.4, 35.0 [<u>C</u>H$_2$(3′) rotamers] 49.1, 49.6 [<u>C</u>H$_2$(5′) rotamers] 53.8, 54.4 [<u>C</u>H(4′) rotamers] 57.8, 58.0 [<u>C</u>H(2′) rotamers] 77.9, 78.1 [<u>C</u>CH$_3$ Boc rotamers] 81.1, 81.2 [<u>C</u>H Dpm rotamers] 111.6 [<u>C</u>5 Thymine] 126.8-128.6 [<u>C</u>H Ar Dpm] 129.2 [<u>C</u>4′ Ar] 130.4 [<u>C</u>1′ and <u>C</u>6′ Ar] 131.3 [<u>C</u>3′ and <u>C</u>5′ Ar] 135.2, 136.2 [<u>C</u>1′ Ar] 149.6 [<u>C</u> Dpm] 153.4, 153.8 [<u>C</u>2 Thymine] 162.5 [<u>C</u>4 Thymine] 168.9 [<u>C</u>O Boc] 170.8 [<u>C</u>O Proline]; Anal Calcd. for $C_{35}H_{35}N_3O_7$ requires C, 68.95; H, 5.79; N, 6.89 %; Found C, 68.97; H, 5.80; N, 6.89 %; $[\alpha]^{25}_D$ = -36.0 (c = 1.065 g/100 mL $CHCl_3$); mp = 103-105 °C.

(*N*-Fluoren-9-ylmethoxycarbonyl)-*cis*-4-(thymin-1-yl)-D-proline (17) [87]



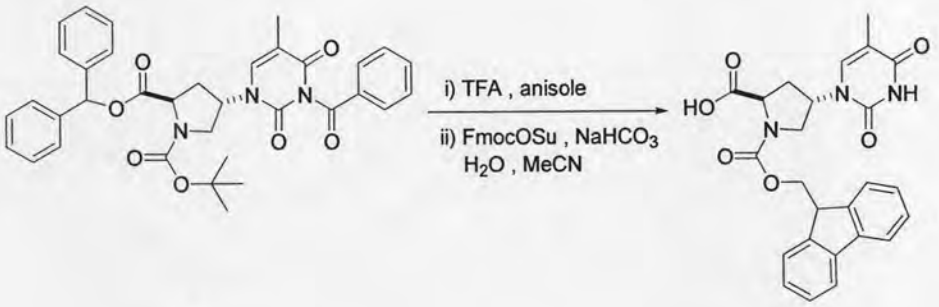
13 **17**

(*N-tert*-butyloxycarbonyl)-*cis*-4-(thymin-1-yl)-D-proline diphenylmethyl ester (**13**) (3.02 g, 5.00 mmol) was treated with trifluoroacetic acid containing 10 % anisole (5 mL) and left at rt for 8 h. After that, the solvent was removed by a gentle stream of

nitrogen. The residue was triturated and washed with diethyl ether. The white solid occurred and was collected by filtration, then was immediately dissolved in 1:1 H_2O:MeCN (5 mL/mmol) and treated with solid $NaHCO_3$ until pH 8 (pH paper). FmocOSu (1.84 g, 5.50 mmol) was then added in small portions with stirring. After stirring at 30 °C for 8 h, the solvent was removed by rotary evaporation. The residue was diluted with water (10 mL) and extracted with diethyl ether (3 × 20 mL). After purging the extracted aqueous layer to remove the dissolved ether with a gentle stream of N_2, the pH was adjusted to 2 with concentrated HCl. The precipitated Fmoc-amino acid was collected by filtration, washed with water, diethyl ether and dried under vacuum to afford the title compound **17** as white solid (1.81 g, 78% yield).

^{1}H NMR (400 MHz; DMSO-d_6) (Figure A-31) δ_H 1.75 [s, 3H, C$\underline{H}_3$ Thymine] 2.10-2.26 [m, 1H, 1×C$\underline{H}_2$(3′)] 2.56-2.74 [2×m, 1H, 1×C$\underline{H}_2$(3′)] 3.43-3.51 [m, 1H, 1×C$\underline{H}_2$(5′)] 3.82-3.89 [m, 1H, 1×C$\underline{H}_2$(5′)] 4.18-4.26 [m, 3H, C$\underline{H}$ Fmoc and C$\underline{H}_2$ Fmoc] 4.14-4.46 [2×m, 1H, C$\underline{H}$(2′)] 4.91-4.98 [m, 1H, C$\underline{H}$(4′)] 7.29-7.32 [m, 2H, C$\underline{H}$ Fmoc] 7.37-7.40 [m, 2H, C$\underline{H}$ Fmoc] 7.58 [s, 1H, $\underline{H}$6 Thymine] 7.64-7.67 [m, 2H, C$\underline{H}$ Fmoc] 7.82-7.87 [m, 2H, C$\underline{H}$ Fmoc] 11.30 [br, 1H, N$\underline{H}$ Thymine]; ^{13}C NMR (100 MHz; DMSO-d_6) (Figure A-32) δ_C 12.6 [$\underline{C}H_3$ Thymine] 33.5, 34.9 [$\underline{C}H_2$(3′) rotamers] 47.1 [$\underline{C}$H Fmoc] 48.8, 49.4 [$\underline{C}H_2$(5′) rotamers] 52.7, 53.3 [$\underline{C}$H(4′) rotamers] 57.4, 57.8 [$\underline{C}$H(2′) rotamers] 67.4, 67.7 [$\underline{C}H_2$ Fmoc rotamers] 109.5, 109.6 [$\underline{C}$5 Thymine rotamers] 120.6 [$\underline{C}$H Ar Fmoc] 125.6 [$\underline{C}$H Ar Fmoc] 127.7 [$\underline{C}$H Ar Fmoc] 128.2 [$\underline{C}$H Ar Fmoc] 138.3 [$\underline{C}$6H Thymine] 141.2 [$\underline{C}$ Ar Fmoc] 144.1 [$\underline{C}$ Ar Fmoc] 151.4 [$\underline{C}$2 Thymine] 154.4 [$\underline{C}$O Fmoc] 164.2 [$\underline{C}$4 Thymine] 173.1, 173.7 [$\underline{C}$O Proline rotamers]; HRMS (FAB$^+$) Calcd for $C_{25}H_{23}O_6N_3$ (M·H$^+$) 462.1666, Found 462.1656, $[\alpha]^{25}_D$ = -3.85 (c = 1.00 g/100 mL, DMF), mp = 201 °C (dec.).

(*N*-Fluoren-9-ylmethoxycarbonyl)-*trans*-4-(thymin-1-yl)-D-proline (18)

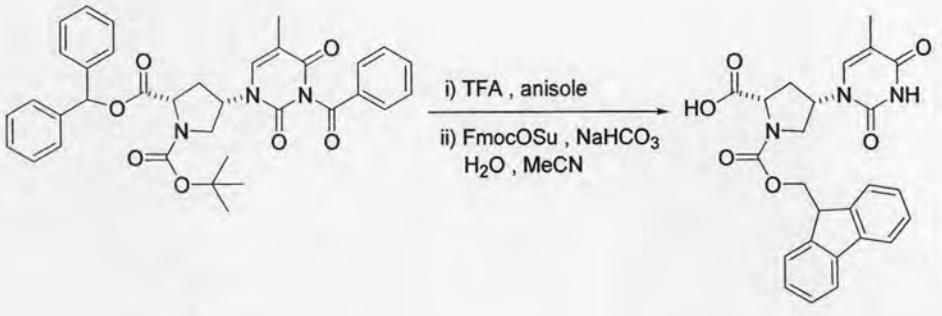


14 **18**

Synthesis of the title compound **18** was accomplished in the same way as described for compound **17** above starting from *N-tert*-Butoxycarbonyl-*trans*-4-(N^3-benzoylthymin-1-yl)-D-proline diphenylmethyl ester (**14**) (1.57 g, 2.57 mmol), 10% anisole in trifluoroacetic acid (5 mL). Purging with N_2 gas and triturating with diethyl ether gave the crude product which was used for the next reaction. The aqueous acetonitrile solution of the crude product was treated with solid $NaHCO_3$ until pH = 8 and FmocOSu (0.96 g, 2.83 mmol) was added to afford the title compound **18** as white solid (0.97 g, 82% yield).

^{1}H NMR (400 MHz, DMSO-d_6) (Figure A-33) δ_H 1.80 [s, 3H, C$\underline{H}_3$ Thymine] 2.21-2.33 [m, 1H, 1×C$\underline{H}_2$(3′)] 2.60-2.75 [m, 1H, 1×C$\underline{H}_2$(3′)] 3.45-3.53 [m, 1H, 1×C$\underline{H}_2$(5′)] 3.74-3.83 [m, 1H, 1×C$\underline{H}_2$(5′)] 4.18-4.41 [m, 3H, C$\underline{H}$ Fmoc and C$\underline{H}_2$ Fmoc] 4.41, 4.57 [2×m, 1H, C$\underline{H}$(2′)] 5.05-5.12 [m, 1H, C$\underline{H}$(4′)] 7.32-7.36 [m, 2H, C$\underline{H}$ Fmoc] 7.40-7.46 [m, 2H, C$\underline{H}$ Fmoc] 7.62 [m, 1H, $\underline{H}$6 Thymine] 7.65-7.67 [m, 2H, C$\underline{H}$ Fmoc] 7.89-7.92 [m, 2H, C$\underline{H}$ Fmoc] 11.36, 11.39 [2×s, 1H, N$\underline{H}$ Thymine rotamers]; ^{13}C NMR (100 MHz, DMSO-d_6) (Figure A-34) δ_C 12.6 [$\underline{C}H_3$ Thymine] 32.6, 33.7 [$\underline{C}H_2$(3′) rotamers] 46.9 [$\underline{C}$H Fmoc] 49.2, 49.4 [$\underline{C}H_2$(5′) rotamers] 52.2, 53.2 [$\underline{C}$H(4′) rotamers] 57.8, 58.0 [$\underline{C}$H(2′) rotamers] 67.3, 67.6 [$\underline{C}H_2$ Fmoc rotamers] 109.9 [$\underline{C}$5 Thymine] 120.5 [$\underline{C}$H Ar Fmoc] 125.6 [$\underline{C}$H Ar Fmoc] 127.6 [$\underline{C}$H Ar Fmoc] 128.1 [$\underline{C}$H Ar Fmoc] 138.0 [$\underline{C}$6H Thymine] 141.1 [$\underline{C}$H Ar Fmoc] 144.0, 144.1 [$\underline{C}$ Ar Fmoc rotamers] 151.3 [$\underline{C}$2 Thymine] 153.9, 154.2 [$\underline{C}$O Fmoc] 164.1 [$\underline{C}$4 Thymine] 173.5, 173.8 [$\underline{C}$O Proline rotamers]. $[\alpha]^{25}_D$ = -2.90 (c = 1.00 g/100 mL, DMF); mp = 134 °C (dec.).

(*N*-Fluoren-9-ylmethoxycarbonyl)-*cis*-4-(thymin-1-yl)-L-proline (19)

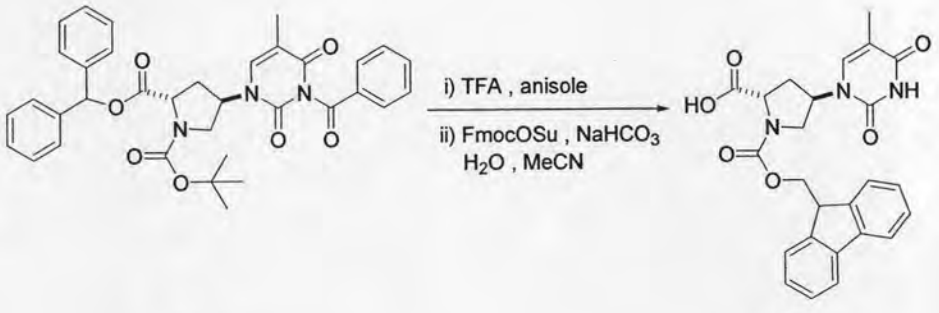


15 **19**

Synthesis of the title compound **19** was accomplished in the same way as described for compound **17** above starting from *N-tert*-Butoxycarbonyl-*cis*-4-(N^3-benzoylthymin-1-yl)-L-proline diphenylmethyl ester (**15**) (1.10 g, 1.80 mmol), 10% anisole in trifluoroacetic acid (5 mL). Purging with N_2 gas and triturating with diethyl ether gave the crude product which was used for the next reaction. The aqueous acetonitrile solution of the crude product was treated with solid $NaHCO_3$ until pH = 8 and FmocOSu (0.68 g, 1.97 mmol) was added to afford the title compound **19** as white solid (0.59 g, 72% yield).

^{1}H NMR (400 MHz, DMSO-d_6) (Figure A-35) δ_H 1.79 [s, 3H, C$\underline{H}_3$ Thymine] 2.15-2.31 [m, 1H, 1×C$\underline{H}_2$(3′)] 2.60-2.77 [2×m, 1H, 1×C$\underline{H}_2$(3′)] 3.47-3.55 [m, 1H, 1×C$\underline{H}_2$(5′)] 3.86-3.93 [m, 1H, 1×C$\underline{H}_2$(5′)] 4.18-4.50 [2×m, 1H, C$\underline{H}$(2′)] 4.24-4.34 [m, 3H, C$\underline{H}_2$ Fmoc and C$\underline{H}$ Fmoc] 4.94-5.00 [m, 1H, C$\underline{H}$(4′)] 7.35 [t, ^3J(H,H) = 7.2 Hz, 2H, C$\underline{H}$ Fmoc] 7.43 [t, ^3J(H,H) = 7.6 Hz, 2H, C$\underline{H}$ Fmoc] 7.61 [s, 1H, $\underline{H}$6 Thymine] 7.69 [d, ^3J(H,H) = 7.3 Hz, 2H, C$\underline{H}$ Fmoc]] 7.90 [d, ^3J(H,H) = 7.4 Hz, 2H, C$\underline{H}$ Fmoc] 11.36 [s, 1H, N$\underline{H}$ Thymine]; ^{13}C NMR (50 MHz, DMSO-d_6) (Figure A-36) δ_C 12.5 [$\underline{C}H_3$ Thymine] 33.1, 34.3 [$\underline{C}H_2$(3′) rotamers] 47.0, 47.1 [$\underline{C}$H Fmoc rotamers] 48.8, 49.4 [$\underline{C}H_2$(5′) rotamers] 52.7, 53.2 [$\underline{C}$H(4′) rotamers] 57.4, 57.8 [$\underline{C}$H(2′) rotamers] 67.4, 67.7 [$\underline{C}H_2$ Fmoc rotamers] 109.5, 109.6 [$\underline{C}$5 Thymine rotamers] 120.6 [$\underline{C}$H Ar Fmoc] 125.6 [$\underline{C}$H Ar Fmoc] 125.8 [$\underline{C}$H Ar Fmoc] 127.6 [$\underline{C}$H Ar Fmoc] 128.2 [$\underline{C}$H Ar Fmoc] 138.3 [$\underline{C}$6H Thymine] 141.1 [$\underline{C}$ Ar Fmoc] 144.1 [$\underline{C}$ Ar Fmoc] 151.4 [$\underline{C}$2 Thymine] 154.4 [$\underline{C}$O Fmoc] 164.2 [$\underline{C}$4 Thymine] 173.1, 173.6 [$\underline{C}$O Proline rotamers]. $[\alpha]^{25}_D$ = -9.80 (c = 1.00 g/100 mL, DMF).

(*N*-Fluoren-9-ylmethoxycarbonyl)-*trans*-4-(thymin-1-yl)-L-proline (20)

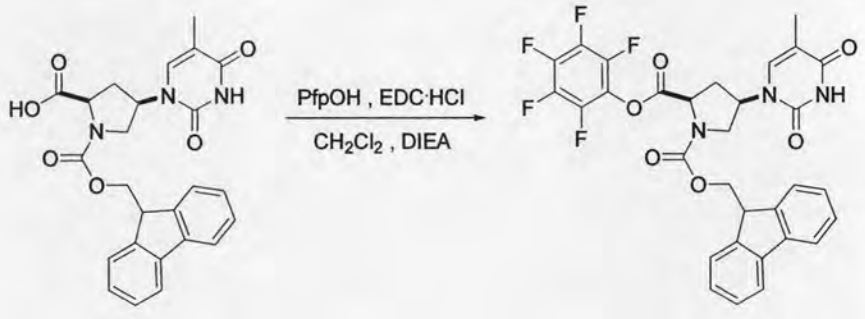


16 **20**

Synthesis of the title compound **20** was accomplished in the same way as described for compound **17** above starting from *N-tert*-Butoxycarbonyl-*trans*-4-(N^3-benzoylthymin-1-yl)-L-proline diphenylmethyl ester (**16**) (0.80 g, 1.32 mmol), 10% anisole in trifluoroacetic acid (5 mL). Purging with N_2 gas and triturating with diethyl ether gave the crude product which was used for the next reaction. The aqueous acetonitrile solution of the crude product was treated with solid $NaHCO_3$ until pH = 8 and FmocOSu (0.49 g, 1.45 mmol) was added to afford the title compound **20** as white solid (0.33 g, 53% yield).

^{1}H NMR (400 MHz, DMSO-d_6) (Figure A-37) δ_H 1.80 [s, 3H, C$\underline{H}_3$ Thymine] 2.20-2.33 [m, 1H, 1×C$\underline{H}_2$(3′)] 2.59-2.74 [m, 1H, 1×C$\underline{H}_2$(3′)] 3.46-3.50 [m, 1H, 1×C$\underline{H}_2$(5′)] 3.73-3.83 [m, 1H, 1×C$\underline{H}_2$(5′)] 4.24-4.31 [m, 3H, Fmoc C$\underline{H}$ and Fmoc C$\underline{H}_2$] 4.41-4.57 [2×m, 1H, C$\underline{H}$(2′) rotamers] 5.04-5.12 [m, 1H, C$\underline{H}$(4′)] 7.31-7.36 [m, 2H, Fmoc C$\underline{H}$] 7.41-7.46 [m, 2H, Fmoc C$\underline{H}$] 7.62 [s, 1H, Thymine $\underline{H}$6] 7.59-7.68 [m, 2H, Fmoc C$\underline{H}$] 7.90-7.92 [m, 2H, Fmoc C$\underline{H}$] 11.36, 11.39 [2×s, 1H, N$\underline{H}$ Thymine rotamers]; ^{13}C NMR (100 MHz, DMSO-d_6) (Figure A-38) δ_C 12.6 [$\underline{C}H_3$ Thymine] 32.6, 33.7 [$\underline{C}H_2$(3′) rotamers] 46.9, 47.0 [$\underline{C}$H Fmoc rotamers] 49.2, 49.4 [$\underline{C}H_2$(5′) rotamers] 52.2, 53.2 [$\underline{C}$H(4′) rotamers] 57.9, 58.1 [$\underline{C}$H(2′) rotamers] 67.3, 67.6 [$\underline{C}H_2$ Fmoc rotamers] 109.9 [$\underline{C}$5 Thymine] 120.5, 120.6 [$\underline{C}$H Ar Fmoc rotamers] 125.5-125.7 [$\underline{C}$H Ar Fmoc] 127.6 [$\underline{C}$H Ar Fmoc] 128.1 [$\underline{C}$H Ar Fmoc] 138.0, 138.1 [$\underline{C}$6H Thymine rotamers] 141.1 [$\underline{C}$ Ar Fmoc] 144.0 [$\underline{C}$ Ar Fmoc] 151.3 [$\underline{C}$2 Thymine] 153.9, 154.2 [$\underline{C}$O Fmoc rotamers] 164.1 [$\underline{C}$4 Thymine] 173.5, 173.8 [$\underline{C}$O Proline rotamers]; $[\alpha]^{25}_D$ = +1.80 (c = 1.00 g/100 mL, DMF); mp = 134 °C (dec.).

(*N*-Fluoren-9-ylmethoxycarbonyl)-*cis*-4-(thymin-1-yl)-D-proline pentafluoro phenyl ester (21)



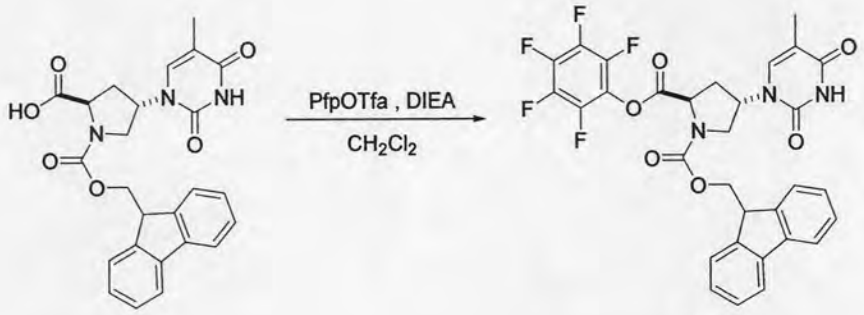
17 **21**

To a suspension of (*N*-fluoren-9-ylmethoxycarbonyl)-*cis*-4-(thymin-1-yl)-D-proline (**17**) (0.22 g, 0.48 mmol) and PfpOH (1.5 equiv) in dichloromethane (2 mL) was added EDC·HCl (1.5 equiv). The resulting mixture was stirred at 30 °C for 1 h. After the reaction was completed as indicated by TLC analysis, the solvent was removed and the residue purified by flash column chromatography eluting with hexanes:ethyl acetate (1:1) on silica gel to obtain the title compound **21** as white solid (0.26 g, 85% yield).

^{1}H NMR (400 MHz; $CDCl_3$) (Figure A-39) δ_H 1.92 [s, 3H, C$\underline{H}_3$ Thymine] 2.26-2.43 [m, 1H, 1×C$\underline{H}_2$(3′)] 2.90-3.01 [m, 1H; 1×C$\underline{H}_2$(3′)] 3.57-3.76 [2×m, 1H, 1×C$\underline{H}_2$(5′)] 3.89-4.06 [2×m, 1H, 1×C$\underline{H}_2$(5′)] 4.18-4.26 [m, 1H, Fmoc C$\underline{H}$] 4.46-4.55 [m, 2H, Fmoc C$\underline{H}_2$] 4.63-4.79 [2×m, 1H, C$\underline{H}$(2′)] 5.18-5.36 [2×m, 1H, C$\underline{H}$(4′)] 7.14 [br, 1H, Thymine $\underline{H}$6] 7.25-7.31 [m, 2H, C$\underline{H}$ Fmoc] 7.34-7.41 [m, 2H, C$\underline{H}$ Fmoc] 7.53-7.57 [m, 2H, C$\underline{H}$ Fmoc] 7.75 [d, ^3J(H,H) = 7.0 Hz, 2H, C$\underline{H}$ Fmoc] 10.16 [s, 1H, N$\underline{H}$ Thymine]; ^{13}C NMR (100 MHz; $CDCl_3$) (Figure A-40) δ_C 12.5 [$\underline{C}H_3$ Thymine] 34.2, 35.7 [$\underline{C}H_2$(3′) rotamers] 47.1 [$\underline{C}$H Fmoc] 48.9, 49.3 [$\underline{C}H_2$(5′) rotamers] 52.4, 52.8 [$\underline{C}$H(4′) rotamers] 56.9, 57.3 [$\underline{C}$H(2′) rotamers] 68.2, 68.5 [Fmoc $\underline{C}H_2$ rotamers] 111.9, 112.3 [$\underline{C}$5 Thymine rotamers] 120.1 [$\underline{C}$H Ar Fmoc] 124.8 [$\underline{C}$H Ar Fmoc] 127.1 [$\underline{C}$H Ar Fmoc] 127.9 [$\underline{C}$H Ar Fmoc] 136.6-142.2 [$\underline{C}$F Pfp] 135.7, 135.9 [$\underline{C}$6H Thymine rotamers] 141.3 [$\underline{C}$ Ar Fmoc] 143.5 [$\underline{C}$ Ar Fmoc] 151.3 [$\underline{C}$2 Thymine] 154.0, 154.5 [$\underline{C}$O Fmoc rotamers] 163.9 [$\underline{C}$4 Thymine] 168.1 [$\underline{C}$O Proline]; HRMS

(FAB$^+$) Calcd for $C_{31}H_{23}O_6N_3F_5$ ($M{\cdot}H^+$) 628.1508, Found 628.1505, $[\alpha]^{25}_D$ = -2.20 (c = 3.8 g/100 mL, $CHCl_3$), mp = 107-110 °C.

(*N*-Fluoren-9-ylmethoxycarbonyl)-*trans*-4-(thymin-1-yl)-D-proline pentafluoro phenyl ester (22)



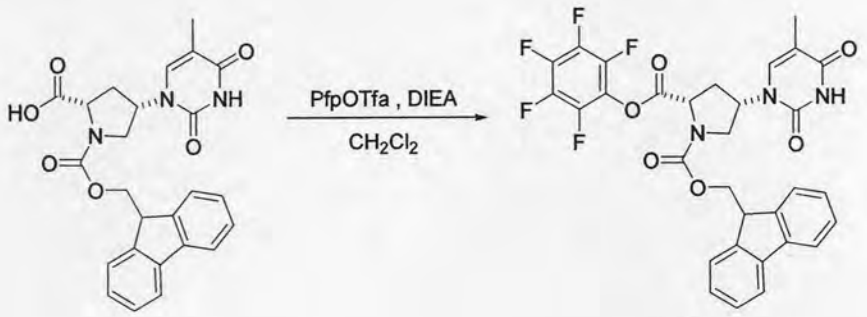
18 **22**

(*N*-fluoren-9-ylmethoxycarbonyl)-*trans*-4-(thymin-1-yl)-D-proline (**18**) (0.08 g, 0.18 mmol) was dissolved in dichloromethane (5 mL) and then added DIEA (36 μL, 0.21 mmol) and pentafluorophenyl trifluoromethyl acetate (PfpOTfa) (37 μL, 0.21 mmol) allowed to stir at rt around 1 h. After that, the solution mixture was diluted with dichloromethane and extracted with water. The crude was purified by column chromatography and scratched with ether/hexane obtained the product **22** as white solid (0.08 g, 75% yield).

^{1}H NMR (400 MHz, $CDCl_3$) (Figure A-41) δ_H 1.99 [s, 3H, C$\underline{H}_3$ Thymine] 2.57-2.70 [m, 1H, 1×C$\underline{H}_2$(3′)] 2.77-2.91 [m, 1H, 1×C$\underline{H}_2$(3′)] 3.67-3.87 [2×m, 1H, 1×C$\underline{H}_2$(5′)] 4.00-4.10 [m, 1H, 1×C$\underline{H}_2$(5′)] 4.24-4.31 [m, 1H, C$\underline{H}$ Fmoc] 4.48-4.60 [m, 2H, C$\underline{H}_2$ Fmoc] 4.80-4.98 [2×m, 1H, C$\underline{H}$(2′)] 5.19-5.33 [m, 1H, C$\underline{H}$(4′)] 6.98 [s, 1H, Thymine $\underline{H}$6] 7.29-7.35 [m, 2H, C$\underline{H}$ Fmoc] 7.41-7.47 [m, 2H, C$\underline{H}$ Fmoc] 7.57-7.60 [m, 2H, C$\underline{H}$ Fmoc] 7.79-7.80 [m, 2H, C$\underline{H}$ Fmoc] 8.69 [s, 1H, Thymine N$\underline{H}$]; ^{13}C NMR (100 MHz, $CDCl_3$) (Figure A-42) δ_C 12.6 [$\underline{C}H_3$ Thymine] 33.5, 34.9 [$\underline{C}H_2$(3′) rotamers] 46.9 [$\underline{C}$H Fmoc] 49.1, 49.2 [$\underline{C}H_2$(5′) rotamers] 54.0, 54.8 [$\underline{C}$H(4′) rotamers] 57.3, 57.6 [$\underline{C}$H(2′) rotamers] 68.1, 68.4 [Fmoc $\underline{C}H_2$ rotamers] 112.1, 112.2 [$\underline{C}$5 Thymine rotamers] 120.0 [$\underline{C}$H Ar Fmoc] 124.8 [$\underline{C}$H Ar Fmoc] 127.0, 127.1 [$\underline{C}$H Ar Fmoc rotamers] 127.7,

127.8 [CH Ar Fmoc rotamers] 136.4, 136.5 [C6H Thymine rotamers] 139.1-142.1 [CF Pfp] 143.0 [C Ar Fmoc] 143.4 [C Ar Fmoc] 143.5 [C Ar Fmoc] 143.8 [C Ar Fmoc] 150.7 [C2 Thymine] 153.7, 154.4 [CO Fmoc rotamers] 163.9 [C4 Thymine] 167.8 [CO Proline]; $[\alpha]^{25}_{D}$ = +26.6 (c = 1.015 g/100 mL, $CHCl_3$); mp = 102-103 °C.

(*N*-Fluoren-9-ylmethoxycarbonyl)-*cis*-4-(thymin-1-yl)-L-proline pentafluoro phenyl ester (23)



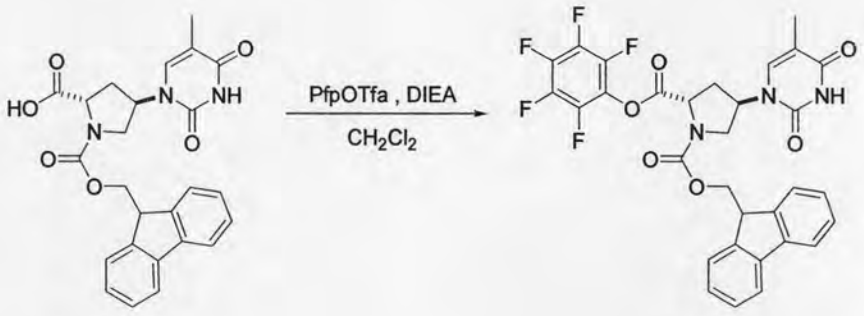
19 **23**

Synthesis of the title compound **23** was accomplished in the same way as described for compound **21** above starting from (*N*-fluoren-9-ylmethoxycarbonyl)-*cis*-4-(thymin-1-yl)-L-proline (**19**) (0.38 g, 0.82 mmol), PfpOTfa (1.2 equiv) and DIEA (1.2 equiv) in dichloromethane (5 mL) afforded compound **23** (0.23 g, 44% yield), as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) (Figure A-43) δ_H 1.92 [s, 3H, C$\underline{H}_3$ Thymine] 2.25-2.46 [m, 1H, 1×C$\underline{H}_2$(3′)] 2.88-3.04 [m, 1H, 1×C$\underline{H}_2$(3′)] 3.54-3.79 [2×m, 1H, 1×C$\underline{H}_2$(5′)] 3.87-4.08 [2×m, 1H, 1×C$\underline{H}_2$(5′)] 4.18-4.29 [m, 1H, C$\underline{H}$ Fmoc] 4.44-4.58 [m, 2H, C$\underline{H}_2$ Fmoc] 4.62-4.81 [2×m, 1H, C$\underline{H}$(2′)] 5.18-5.40 [2×m, 1H, C$\underline{H}$(4′)] 7.14 [s, 1H, $\underline{H}$6 Thymine] 7.25-7.31 [m, 2H, C$\underline{H}$ Fmoc] 7.37-7.41 [m, 2H, C$\underline{H}$ Fmoc] 7.53-7.57 [m, 2H, C$\underline{H}$ Fmoc] 7.75 [d, ^3J(H,H) = 7.6 Hz, 2H, C$\underline{H}$ Fmoc] 8.69 [s, 1H, N$\underline{H}$ Thymine]; ^{13}C NMR (100 MHz, $CDCl_3$) (Figure A-44) δ_C 12.5 [$\underline{C}H_3$ Thymine] 34.2, 35.7 [$\underline{C}H_2$(3′) rotamers] 47.0 [$\underline{C}$H Fmoc] 48.9, 49.2 [$\underline{C}H_2$(5′) rotamers] 52.4, 52.8 [$\underline{C}$H(4′) rotamers] 56.8, 57.2 [$\underline{C}$H(2′) rotamers] 68.1, 68.5 [Fmoc $\underline{C}H_2$ rotamers] 111.9, 112.3 [$\underline{C}$5 Thymine rotamers] 120.0 [$\underline{C}$H Ar Fmoc] 124.8 [$\underline{C}$H Ar Fmoc] 127.1 [$\underline{C}$H Ar Fmoc] 127.8 [$\underline{C}$H Ar Fmoc] 135.7, 135.9 [$\underline{C}$6H Thymine rotamers] 136.6-142.1 [$\underline{C}$F

Pfp] 143.0 [C Ar Fmoc] 143.3 [C Ar Fmoc] 143.5 [C Ar Fmoc] 143.8 [C Ar Fmoc] 151.1 [C2 Thymine] 153.9, 154.4 [CO Fmoc rotamers] 163.8 [C4 Thymine] 168.1 [CO Proline]; $[\alpha]^{25}_{D}$ = -0.69 (c = 1.01 g/100 mL, $CHCl_3$); mp = 106-107 °C.

(*N*-Fluoren-9-ylmethoxycarbonyl)-*trans*-4-(thymin-1-yl)-L-proline pentafluoro phenyl ester (24)



20 **24**

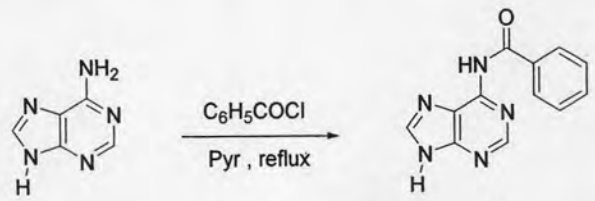
Synthesis of the title compound **24** was accomplished in the same way as described for compound **21** above. Starting from (*N*-fluoren-9-ylmethoxycarbonyl)-*trans*-4-(thymin-1-yl)-L-proline (**20**) (0.18 g, 0.40 mmol), PfpOTfa (1.2 equiv) and DIEA (1.2 equiv) in dichloromethane (5 mL) afforded compound **24** (0.13 g, 50% yield) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) (Figure A-45) δ_H 1.99 [s, 3H, C$\underline{H}_3$ Thymine] 2.60-2.69 [m, 1H, 1×C$\underline{H}_2$(3′)] 2.78-2.89 [m, 1H, 1×C$\underline{H}_2$(3′)] 3.66-3.87 [2×m, 1H, 1×C$\underline{H}_2$(5′)] 4.00-4.10 [m, 1H, 1×C$\underline{H}_2$(5′)] 4.28-4.32 [m, 1H, C$\underline{H}$ Fmoc] 4.49-4.61 [m, 2H, C$\underline{H}_2$ Fmoc] 4.82-4.98 [2×dd, ^3J(H,H) = 9.2, 2.8 Hz, 1H, C$\underline{H}$(2′)] 5.19-5.28 [m, 1H, C$\underline{H}$(4′)] 6.97 [s, 1H, $\underline{H}$6 Thymine] 7.32-7.36 [m, 2H, C$\underline{H}$ Fmoc] 7.40-7.46 [m, 2H, C$\underline{H}$ Fmoc] 7.57-7.61 [m, 2H, C$\underline{H}$ Fmoc] 7.80 [d, ^3J(H,H) = 7.2 Hz, 2H, C$\underline{H}$ Fmoc] 8.41, 8.44 [2×s, 1H, N$\underline{H}$ Thymine rotamers]; ^{13}C NMR (100 MHz, $CDCl_3$) (Figure A-46) δ_C 12.6 [$\underline{C}H_3$ Thymine] 33.6, 35.0 [$\underline{C}H_2$(3′) rotamers] 46.9 [$\underline{C}$H Fmoc] 49.0, 49.2 [$\underline{C}H_2$ (5′) rotamers] 53.9, 54.7 [$\underline{C}$H(4′) rotamers] 57.3, 57.5 [$\underline{C}$H(2′) rotamers] 68.1, 68.4 [Fmoc $\underline{C}H_2$ rotamers] 112.3 [$\underline{C}$5 Thymine] 120.0 [$\underline{C}$H Ar Fmoc] 124.8 [$\underline{C}$H Ar Fmoc] 127.1 [$\underline{C}$H Ar Fmoc] 127.8 [$\underline{C}$H Ar Fmoc] 136.2 [$\underline{C}$6H Thymine] 136.6-139.7 [$\underline{C}$F Pfp] 141.2 [$\underline{C}$ Ar Fmoc] 143.5 [$\underline{C}$ Ar Fmoc] 150.5 [$\underline{C}$2 Thymine] 153.6, 154.3 [$\underline{C}$O

Fmoc rotamers] 163.5 [C4 Thymine] 167.8 [CO Proline]; $[\alpha]^{25}_{D}$ = -28.7 (c = 1.035 g/100 mL, $CHCl_3$); mp = 101-103 °C.

2.2.3 Synthesis of *cis*-D pyrrolidinyl monomers with bases A, C and G

N^6-Benzoyladenine (25) [88]

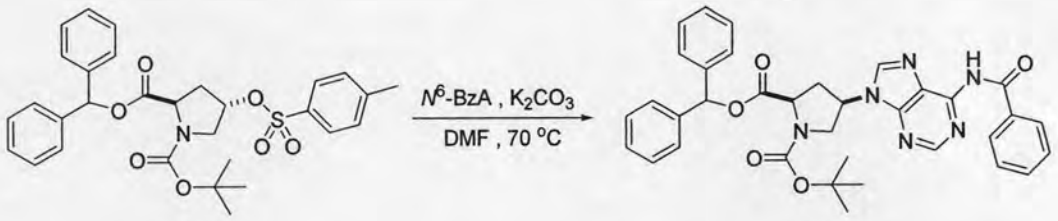


25

Benzoyl chloride (1.3 mL, 11.0 mmol) was added to a suspension of (1.35 g, (10.0 mmol) of adenine in 20 mL dry pyridine. The reaction mixture was refluxed for 2 h. The crude reaction mixture was treated with 10% sodium bicarbonate solution which caused the separation of an oily phase and then acidified with 5% HCl solution. The solution was poured into a beaker containing ice-water (500 mL), with vigorous stirring until crystallization occurred. The slightly yellow crystals were filtered off and washed with cold water. The residue was recrystallized from ethanol-water to yield the title compound **25** as colorless needles (1.54 g, 65% yield).

^{1}H NMR (400 MHz, DMSO-d_6) (Figure A-47) δ_H 7.55 [t, J = 7.8 Hz, 2H, CH(3,5) Ar Bz] 7.63 [t, J = 7.0 Hz, 1H, CH(4) Ar Bz] 8.09 [d, J = 7.6 Hz, 2H, CH(2,6) Ar Bz] 8.49 [s, 1H, CH8 Adenine] 8.71 [s, 1H, CH2 Adenine] 11.52 [s, 1H, NH9] 12.38 [s, 1H, NH6]; ^{13}C NMR (100 MHz, DMSO-d_6) (Figure A-48) δ_C 115.8 [C5 Adenine] 128.8 [CH(2,6) Ar Bz] 129.0 [CH(2,6) Ar Bz] 133.0 [CH(4) Ar Bz] 133.2 [CH(1) Ar Bz] 145.5 [C6 Adenine] 146.3 [C8 Adenine] 151.5 [C2 Adenine] 161.6 [C4 Adenine] 167.0 [CO Bz]; mp = 238-240 °C.

(*N*-*tert*-butyloxycarbonyl)-*cis*-4-(N^6-benzoyladenin-9-yl)-D-proline diphenyl methyl ester (26) [80]

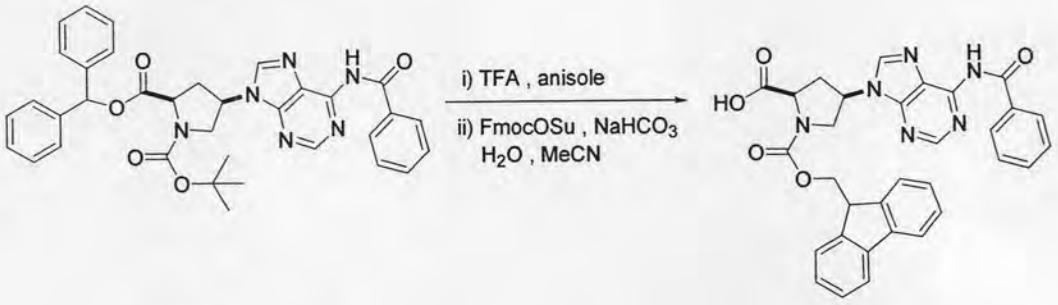


11 **26**

A mixture of the tosylate (**11**) (0.27 g, 0.50 mmol), N^6-benzoyladenine (0.30 g, 1.25 mmol), anhydrous K_2CO_3 (0.35 g, 2.50 mmol) in DMF (10 mL) was stirred under nitrogen at 80 °C for 24 h. Water (30 mL) was added to the reaction and the suspension was extracted with dichloromethane. The organic phase was washed with water, dried over $MgSO_4$ and evaporated to give the crude product, which was purified by column chromatography eluting with hexanes:ethyl acetate 1:3 on silica gel to obtain the title compound **26** as a white solid (0.12 g, 40% yield).

^{1}H NMR (300 MHz, $CDCl_3$) (Figure A-49) δ_H 1.18, 1.37 [2×s, 9H, C$\underline{H}_3$ Boc rotamers] 2.32-2.55 [m, 1H, 1×C$\underline{H}_2$(3′)] 2.71-2.93 [m, 1H, 1×C$\underline{H}_2$(3′)] 3.77-4.14 [m, 2H, C$\underline{H}_2$(5′)] 4.38-4.63 [m, 1H, C$\underline{H}$(2′)] 4.96-5.16 [m, 1H, C$\underline{H}$(4′)] 6.74 [s, 1H, C$\underline{H}$Ph$_2$] 7.07-7.18 [m, 10H, Phenyl Dpm] 7.29-7.43 [m, 3H, *m*- and *p*-CH Ar Bz] 7.89-7.96 [m, 3H, *o*-CH Ar Bz and C$\underline{H}$8 Adenine] 8.56 [s, 1H, C$\underline{H}$2 Adenine]; ^{13}C NMR (75 MHz, $CDCl_3$) (Figure A-50) δ_C 27.9, 28.3 [$\underline{C}H_3$ Boc rotamers] 34.5, 35.8 [$\underline{C}H_2$(3′) rotamers] 49.9, 50.5 [$\underline{C}H_2$(5′) rotamers] 52.3, 52.9 [$\underline{C}$H(4′) rotamers] 57.5 [$\underline{C}$H(2′)] 77.7, 78.0 [$\underline{C}$HPh$_2$ Dpm rotamers] 81.2 [$\underline{C}$CH$_3$ Boc] 122.8 [C5 Adenine] 126.7-128.6 [$\underline{C}$H Ar Dpm] 132.6 [$\underline{C}$H Ar] 133.6 [$\underline{C}$H Ar] 139.0, 139.2 [$\underline{C}$ Ar] 141.2 [$\underline{C}$4 Adenine] 149.6 [$\underline{C}$6 Adenine] 151.6 [$\underline{C}$2 Adenine] 152.2, 153.3 [$\underline{C}$O Boc rotamers] 165.0 [$\underline{C}$O Bz] 170.5 [$\underline{C}$O Proline]; $[\alpha]^{25}_D$ = +11.2 (c = 1.01 g/100 mL, $CHCl_3$), mp = 115-119 °C.

(*N*-Fluoren-9-ylmethoxycarbonyl)-*cis*-4-(N^6-benzoyladenin-9-yl)-D-proline (27)

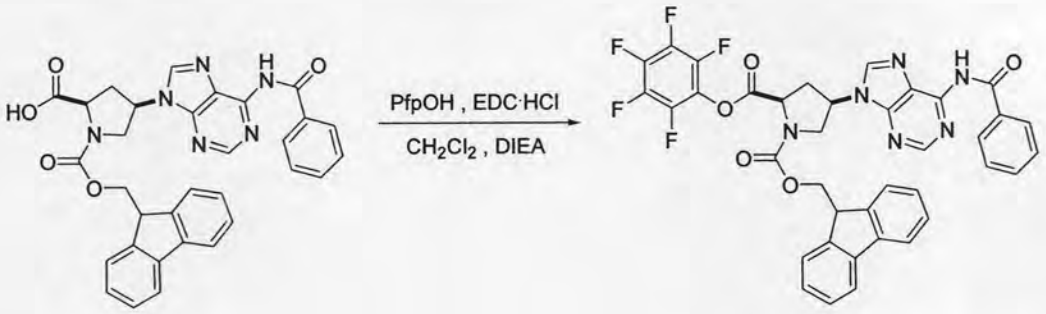


26 **27**

Synthesis of the title compound **27** was accomplished in the same way as described for compound **17** above starting from (*N-tert*-butyloxycarbonyl)-*cis*-4-(N^4-benzoyladenin-9-yl)-D-proline diphenylmethyl ester (**26**) (0.42 g, 0.69 mmol), and trifluoroacetic acid containing 10% anisole (3 mL) followed by FmocOSu (0.26 g, 0.76 mmol) and $NaHCO_3$ (3 equiv excess) in 1:1 H_2O:MeCN (5 mL/mmol). The product **27** was obtained as a white solid (0.36 g, 91% yiled).

^{1}H NMR (400 MHz, DMSO-d_6) (Figure A-51) δ_H 2.61-2.76 [m, 1H, 1×C$\underline{H}_2$(3′)] 2.87-3.04 [2×m, 1H, 1×C$\underline{H}_2$(3′)] 3.91-3.97 [m, 1H, 1×C$\underline{H}_2$(5′)] 4.15-4.22 [m, 2H, 1×C$\underline{H}_2$(5′)] 4.22-4.26 [m, 1H, C$\underline{H}$ Fmoc] 4.28-4.31 [m, 2H, C$\underline{H}_2$ Fmoc] 4.36-4.58 [2×m, 1H, C$\underline{H}$(2′)] 5.17-5.28 [m, 1H, C$\underline{H}$(4′)] 7.30-7.34 [m, 2H, C$\underline{H}$ Fmoc] 7.38-7.43 [m, 2H, C$\underline{H}$ Fmoc] 7.52-7.55 [m, 2H, C$\underline{H}$ Bz] 7.61-7.68 [m, 3H, C$\underline{H}$ Fmoc and Bz] 7.88 [d, ^3J(H,H) = 7.0 Hz, 2H, C$\underline{H}$ Fmoc] 8.03 [m, 2H, C$\underline{H}$ Bz] 8.54 [s, 1H, C$\underline{H}$ Adenine] 8.74, 8.76 [2×s, 1H, C$\underline{H}$ Adenine]; ^{13}C NMR (100 MHz, DMSO-d_6) (Figure A-52) δ_C 34.0, 35.2 [C$\underline{H}_2$(3′) rotamers] 47.1 [C$\underline{H}$ Fmoc] 50.0, 50.5 [C$\underline{H}_2$(5′) rotamers] 52.4, 53.0 [C$\underline{H}$(4′) rotamers] 57.7, 58.0 [C$\underline{H}$(2′)] 67.5, 67.8 [C$\underline{H}_2$ Fmoc] 120.6 [C$\underline{H}$ Ar Fmoc] 126.2 [C$\underline{5}$ Adenine] 125.8 [C$\underline{H}$ Ar Fmoc] 126.2 [C$\underline{H}$ Bz] 127.6 [C$\underline{H}$ Bz] 128.2 [C$\underline{H}$ Ar Fmoc] 128.9 [C$\underline{H}$ Ar Fmoc] 132.9 [C$\underline{}$ Bz] 133.6 [C$\underline{H}$ Bz] 141.1 [C$\underline{8}$H Adenine and C$\underline{}$ Ar Fmoc] 143.7 [C$\underline{}$ Ar Fmoc] 150.7 [C$\underline{4}$ Adenine] 151.8 [C$\underline{6}$ Adenine] 152.9 [C$\underline{2}$H Adenine] 154.3 [C$\underline{O}$ Fmoc] 166.0 [C$\underline{O}$ Bz] 173.0, 173.5 [C$\underline{O}$ Proline rotamers]; HRMS (FAB$^+$) Calcd for $C_{32}H_{26}O_5N_6$ (M·H$^+$) 575.2043, Found 575.2041, $[\alpha]^{25}_D$ = +7.40 (c = 0.995 g/100 mL, DMF), mp = 200 °C (dec.).

(*N*-Fluoren-9-ylmethoxycarbonyl)-*cis*-4-(N^6-benzoyladenin-9-yl)-D-proline pentafluorophenyl ester (28)

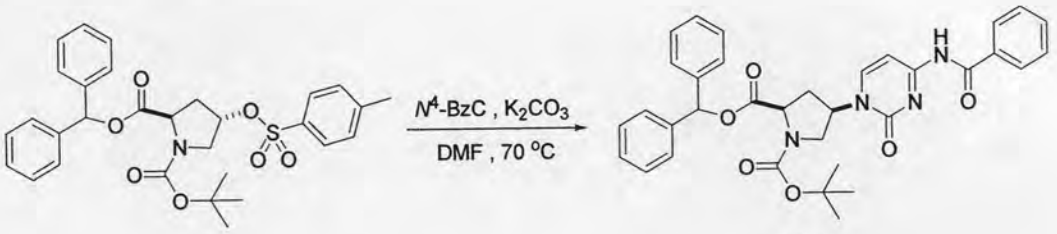


27 **28**

Synthesis of the title compound **28** was accomplished in the same way as described for compound **21** above starting from (*N*-fluoren-9-ylmethoxycarbonyl)-*cis*-4-(N^4-benzoyladenin-9-yl)-D-proline (**27**) (0.25 g, 0.43 mmol), pentafluorophenol (1.5 equiv) and EDC·HCl (1.5 equiv) in dichloromethane (2 mL). The product **28** was obtained as a white solid (0.26 g, 81% yield).

^{1}H NMR (400 MHz; $CDCl_3$) (Figure A-53) δ_H 2.80-2.96 [m, 1H, 1×C$\underline{H}_2$(3′)] 3.08-3.23 [m, 1H, 1×C$\underline{H}_2$(3′)] 4.02-4.15 [2×m, 1H, 1×C$\underline{H}_2$(5′)] 4.18-4.31 [m, 2H, C$\underline{H}$ Fmoc and 1×C$\underline{H}_2$(5′)] 4.46-4.55 [m, 2H, C$\underline{H}_2$ Fmoc] 4.58-4.87 [2×m, 1H, C$\underline{H}$(2′)] 5.25-5.35 [m, 1H, C$\underline{H}$(4′)] 7.26-7.30 [m, 2H, C$\underline{H}$ Fmoc] 7.35-7.37 [m, 2H, C$\underline{H}$ Fmoc] 7.46-7.47 [m, 2H, C$\underline{H}$ Bz] 7.55-7.56 [m, 3H, C$\underline{H}$ Fmoc and C$\underline{H}$ Bz] 7.73 [d, ^3J(H,H) = 7.0 Hz, 2H, C$\underline{H}$ Fmoc] 8.00-8.01 [m, 2H, C$\underline{H}$ Bz] 8.23, 8.31 [2×s, 1H, C$\underline{H}$ Adenine] 8.75 [s, 1H, C$\underline{H}$ Adenine]; ^{13}C NMR (100 MHz; $CDCl_3$) (Figure A-54) δ_C 34.6, 35.9 [$\underline{C}H_2$(3′) rotamers] 47.1 [$\underline{C}$H Fmoc] 49.8, 50.2 [$\underline{C}$H(5′) rotamers] 52.6, 53.3 [$\underline{C}$H(4′) rotamers] 56.9, 57.2 [$\underline{C}$H(2′) rotamers] 68.1, 68.6 [$\underline{C}H_2$ Fmoc rotamers] 120.0 [$\underline{C}$H Ar Fmoc] 122.3 [$\underline{C}$5 Adenine] 124.8 [$\underline{C}$H Ar Fmoc] 127.1 [$\underline{C}$H Bz] 127.8 [$\underline{C}$H Bz] 128.0 [$\underline{C}$H Ar Fmoc] 128.8 [$\underline{C}$H Ar Fmoc] 133.0, 133.1 [$\underline{C}$/$\underline{C}$H Bz] 136.5-142.0 [$\underline{C}$ Pfp] 141.3 [$\underline{C}$8H Adenine and $\underline{C}$ Ar Fmoc] 143.4 [$\underline{C}$H Ar Fmoc] 149.5 [$\underline{C}$4 Adenine] 151.7 [$\underline{C}$6 Adenine] 152.4 [$\underline{C}$2H Adenine] 153.9, 154.4 [$\underline{C}$O Fmoc] 165.1 [$\underline{C}$O Bz] 167.5, 167.7 [$\underline{C}$O Proline]; HRMS (FAB$^+$) Calcd for $C_{38}H_{26}O_5N_6F_5$ (M·H$^+$) 741.1885, Found 741.1898, $[\alpha]^{25}_D$ = -19.2 (c = 1.0 g/100 mL, $CHCl_3$), mp = 110 °C (dec.).

(*N-tert*-butyloxycarbonyl)-*cis*-4-(N^4-benzoylcytosin-9-yl)-D-proline diphenylmethyl ester (29) [80]

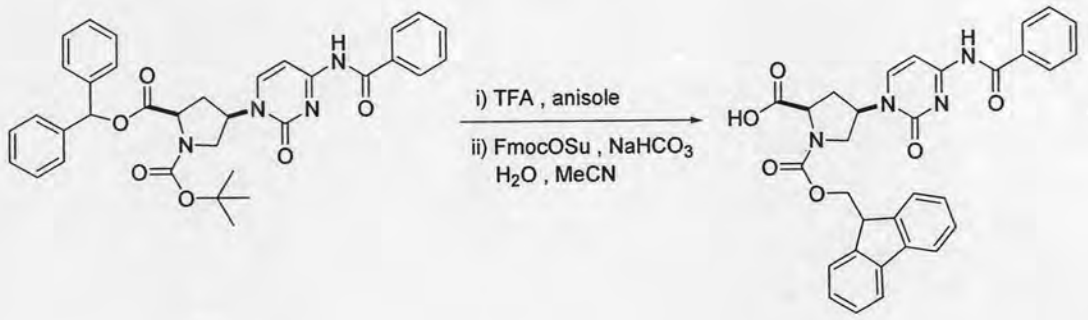


11 **29**

Synthesis of the title compound **29** was accomplished in the same way as described for compound **26** above starting from tosylate (**11**) (0.27 g, 0.50 mmol), N^4-benzoylcytosine (0.27 g, 1.25 mmol), anhydrous K_2CO_3 (0.35 g, 2.50 mmol) in DMF (10 mL). The reaction mixture was stirred under nitrogen at 80 °C for 6 h. The suspension was diluted with dichloromethane (20 mL), filtered through Celite. The organic phase was washed with water, dried over $MgSO_4$ and evaporated to give the crude product, which was purified by column chromatography eluting with hexanes: ethyl acetate to afford the desired N_1-isomer **29** as a white solid (0.08 g, 26% yield).

^{1}H NMR (400 MHz, $CDCl_3$) (Figure A-55) δ_H 1.29, 1.49 [2×s, 9H, C$\underline{H}_3$ Boc rotamers] 2.10-2.23 [m, 1H, 1×C$\underline{H}_2$(3′)] 2.80-2.96 [m, 1H, 1×C$\underline{H}_2$(3′)] 3.52-3.79 [2×m, 1H, 1×C$\underline{H}_2$(5′)] 3.94-4.04 [m, 1H, 1×C$\underline{H}_2$(5′)] 4.47-4.66 [m, 1H, C$\underline{H}$(2′)] 5.25-5.35 [m, 1H, C$\underline{H}$(4′)] 6.85 [s, 1H, C$\underline{H}$Ph$_2$] 7.19-7.35 [m, 10H, Phenyl Dpm] 7.51-7.55 [m, 3H, *m*- and *p*-C$\underline{H}$ Ar Bz] 7.61-7.73 [m, 2H, C$\underline{H}$5 and C$\underline{H}$6 Cytosine] 7.86-7.93 [m, 3H, *o*-C$\underline{H}$ Ar Bz] 8.60 [s br, 1H, N$\underline{H}$ Cytosine]; ^{13}C NMR (100 MHz, $CDCl_3$) (Figure A-56) δ_C 28.0, 28.3 [C$\underline{H}_3$ Boc rotamers] 34.4, 36.0 [$\underline{C}$H$_2$(3′) rotamers] 49.7, 50.4 [$\underline{C}$H$_2$(5′) rotamers] 54.3, 54.9 [$\underline{C}$H(4′) rotamers] 57.6 [$\underline{C}$H(2′)] 77.9, 78.3 [$\underline{C}$H Dpm] 81.2 [$\underline{C}$CH$_3$ Boc] 96.9 [$\underline{C}$5H Cytosine] 126.7-128.9 [$\underline{C}$H Ar] 133.1 [$\underline{C}$ Bz] 139.2, 139.5 [$\underline{C}$ Dpm] 145.1, 145.5 [$\underline{C}$6H Cytosine rotamers] 153.4, 154.8 [$\underline{C}$O Boc rotamers] 155.1 [$\underline{C}$2 Cytosine] 161.8 [$\underline{C}$4 Cytosine] 166.6 [$\underline{C}$O Bz] 170.9 [$\underline{C}$O Proline]; $[\alpha]^{25}_D$ = -8.50 (c = 1.0 g/100 mL, $CHCl_3$).

(*N*-Fluoren-9-ylmethoxycarbonyl)-*cis*-4-(N^4-benzoylcytosin-1-yl)-D-proline (30)

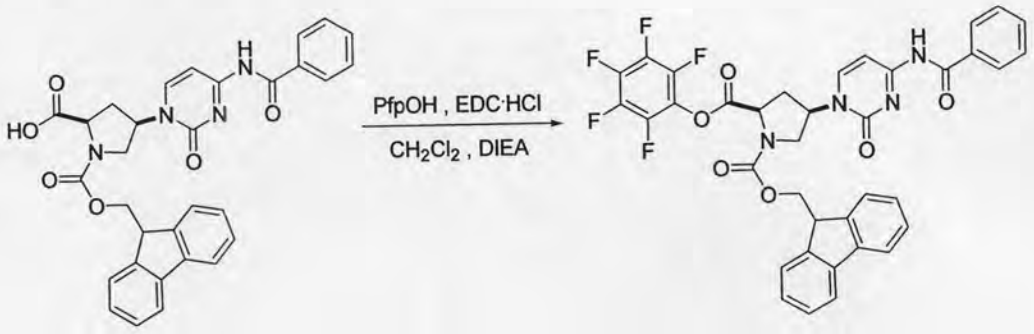


29 **30**

Synthesis of the title compound **30** was accomplished in the same way as described for compound **17** above starting from (*N-tert*-butyloxycarbonyl)-*cis*-4-(N^4-benzoylcytosin-9-yl)-D-proline diphenylmethyl ester (**29**) (0.21 g, 0.36 mmol), and trifluoroacetic acid containing 10% anisole (2 mL) followed by FmocOSu (0.11 g, 0.30 mmol) and $NaHCO_3$ (3 equiv excess) in 1:1 H_2O:MeCN (5 mL/mmol). The product **30** was obtained as a white solid (0.13 g, 83% yield).

^{1}H NMR (400 MHz, $CDCl_3$) (Figure A-57) δ_H 2.22-2.37 [m, 1H, 1×C$\underline{H}_2$(3′)] 2.66-2.84 [m, 1H, 1×C$\underline{H}_2$(3′)] 3.59-3.65 [m, 1H, 1×C$\underline{H}_2$(5′)] 3.93-3.99 [m, 1H, 1×C$\underline{H}_2$(5′)] 4.14-4.47 [2×m, 1H, C$\underline{H}$(2′)] 4.21-4.22 [m, 1H, C$\underline{H}$ Fmoc] 4.24-4.29 [m, 2H, C$\underline{H}_2$ Fmoc] 4.93-5.02 [m, 1H, C$\underline{H}$(4′)] 7.29-7.34 [m, 3H, C5$\underline{H}$ cytosine and C$\underline{H}$ Fmoc] 7.40 [t, ^3J(H,H) = 7.6 Hz, 2H, C$\underline{H}$ Fmoc] 7.49 [t, ^3J(H,H) = 7.6 Hz, 2H, C$\underline{H}$ Bz] 7.59-7.62 [m, 1H, C$\underline{H}$ Bz] 7.67 [d, ^3J(H,H) = 7.2 Hz, 2H, C$\underline{H}$ Fmoc] 7.86-7.89 [m, 2H, C$\underline{H}$ Bz] 7.99 [d, ^3J(H,H) = 7.6 Hz, 2H, C$\underline{H}$ Fmoc] 8.14 [t, ^3J(H,H) = 9.2 Hz, 1H, C6$\underline{H}$ Cytosine]; ^{13}C NMR (100 MHz, $CDCl_3$) (Figure A-58) δ_C 33.5, 33.6 [$\underline{C}H_2$(3′) rotamers] 47.1 [$\underline{C}$H Fmoc] 49.2, 49.8 [$\underline{C}H_2$(5′) rotamers] 55.5, 56.1 [$\underline{C}$H(4′) rotamers] 57.7, 58.0 [$\underline{C}$H(2′) rotamers] 67.4, 67.7 [$\underline{C}H_2$ Fmoc rotamers] 96.6 [$\underline{C}$5H Cytosine] 120.6 [$\underline{C}$H Ar Fmoc] 125.7 [$\underline{C}$H Ar Fmoc] 128.2 [$\underline{C}$H Bz] 128.8 [$\underline{C}$H Bz] 125.7 [$\underline{C}$H Ar Fmoc] 127.6 [$\underline{C}$H Ar Fmoc] 133.1 [$\underline{C}$ Bz] 133.6 [$\underline{C}$H Bz] 141.1 [$\underline{C}$ Ar Fmoc] 144.2 [$\underline{C}$ Ar Fmoc] 147.9 [$\underline{C}$6H Cytosine] 154.4 [$\underline{C}$O Fmoc] 155.4 [$\underline{C}$2 Cytosine] 163.1 [$\underline{C}$4 Cytosine] 167.9 [$\underline{C}$O Bz] 173.2, 173.8 [$\underline{C}$O Proline rotamers]; HRMS (FAB$^+$) Calcd for $C_{31}H_{26}O_6N_4$ (M·H$^+$) 551.1910, Found 551.1938, $[\alpha]^{25}_D$ = -7.04 (c = 1.02 g/100 mL, DMF), mp = 159 °C(dec).

(*N*-Fluoren-9-ylmethoxycarbonyl)-*cis*-4-(N^4-benzoylcytosin-1-yl)-D-proline pentafluorophenyl ester (31)

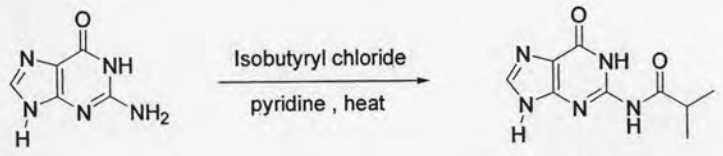


30 **31**

Synthesis of the title compound **31** was accomplished in the same way as described for compound **21** above. Starting from (*N*-fluoren-9-ylmethoxycarbonyl)-*cis*-4-(N^4-benzoylcytosin-9-yl)-D-proline (**30**) (0.25 g, 0.45 mmol), pentafluorophenol (1.5 equiv) and EDC·HCl (1.5 equiv) in dichloromethane (2 mL) afforded compound **31** (0.24 g, 76% yield) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) (Figure A-59) δ_H 2.42-2.57 [m, 1H, 1×C$\underline{H}_2$(3′)] 2.92-3.10 [m, 1H, 1×C$\underline{H}_2$(3′)] 3.64-3.70, 4.02-4.09 [2×m, 1H, 1×C$\underline{H}_2$(5′)] 3.90-3.99 [m, 1H, 1×C$\underline{H}_2$(5′)] 4.15-4.27 [m, 1H, C$\underline{H}$ Fmoc] 4.39-4.63 [m, 1H, C$\underline{H}_2$ Fmoc] 4.71-4.83 [m, 1H, C$\underline{H}$(2′)] 5.21-5.31 [m, 1H, C$\underline{H}$(4′)] 7.22-7.31 [m, 3H, C5$\underline{H}$ Cytosine and C$\underline{H}$ Fmoc] 7.36-7.38 [m, 2H, C$\underline{H}$ Fmoc] 7.43-7.47 [m, 2H, C$\underline{H}$ Bz] 7.54-7.57 [m, 3H, C$\underline{H}$ Ar Fmoc and C$\underline{H}$ Bz] 7.68-7.74 [m, 2H, C$\underline{H}$ Fmoc] 7.85-7.92 [m, 3H, C$\underline{H}$ Bz and C6$\underline{H}$ Cytosine]; ^{13}C NMR (100 MHz, $CDCl_3$) (Figure A-60) δ_C 34.8, 36.2 [$\underline{C}H_2$(3′) rotamers] 47.1 [$\underline{C}$H Fmoc] 49.3, 49.6 [$\underline{C}H_2$(5′) rotamers] 55.2, 55.8 [$\underline{C}$H(4′) rotamers] 56.9, 57.3 [$\underline{C}$H(2′) rotamers] 67.9, 68.5 [$\underline{C}H_2$ Fmoc rotamers] 97.1, 97.3 [$\underline{C}$5H Cytosine rotamers] 120.0 [$\underline{C}$H Ar Fmoc] 124.8 [$\underline{C}$H Ar Fmoc] 127.2 [$\underline{C}$H Bz] 127.8 [$\underline{C}$H Bz] 127.9 [$\underline{C}$H Ar Fmoc] 128.9 [$\underline{C}$H Ar Fmoc] 132.7 [$\underline{C}$ Bz] 133.3 [$\underline{C}$H Bz] 136.6-142.0 [$\underline{C}$ Pfp] 141.3 [$\underline{C}$ Ar Fmoc] 143.5 [$\underline{C}$ Ar Fmoc] 145.7, 145.7 [$\underline{C}$6H Cytosine rotamers] 154.2, 154.4 [$\underline{C}$O Fmoc rotamers] 155.2 [$\underline{C}$2 Cytosine] 162.4 [$\underline{C}$4 Cytosine] 166.8 [$\underline{C}$O Bz] 167.9, 168.0 [$\underline{C}$O Proline]; HRMS (FAB$^+$) Calcd for $C_{37}H_{26}O_6N_4F_5$ (M·H$^+$) 717.1773, Found 717.1758, $[\alpha]^{25}_D$ = -61.7 (c = 0.5 g/100 mL, $CHCl_3$), mp = 114-115 °C.

N^2-Isobutyrylguanine (32)

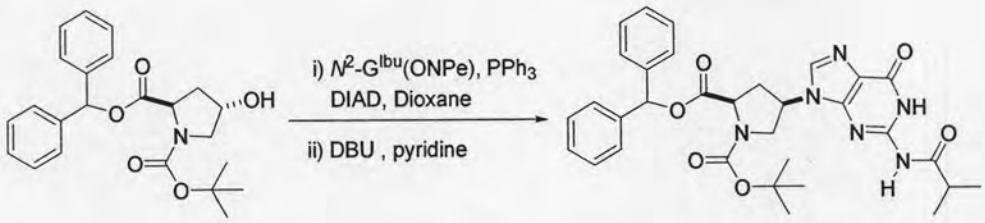


32

Isobutyryl chloride (16.6 mL, 100.0 mmol) was added to a suspension of 3.06 g (20.0 mmol) of guanine in 20 mL dry DMF. The reaction mixture was refluxed for 5 h. The crude mixture was treated with 10% sodium bicarbonate solution and filtered to get the white solid **32** (4.22 g, 94% yield).

^{1}H NMR (400 MHz, DMSO-d_6) (Figure A-61) δ_H 1.09 [d, J = 6.0 Hz, 6H, C<u>H</u>$_3$] 2.73 [h, J = 6.8 Hz, 1H, C<u>H</u> Isobutyryl] 6.64 [br, 1H, N<u>H</u>] 8.03 [s, 1H, C<u>H</u>(8)] 11.53 [s, 1H, N<u>H</u>] 12.06 [s, 1H, N<u>H</u>].

(*N-tert*-butyloxycarbonyl)-*cis*-4-(N^2-isobutylguanin-9-yl)-D-proline diphenyl methyl ester (33)



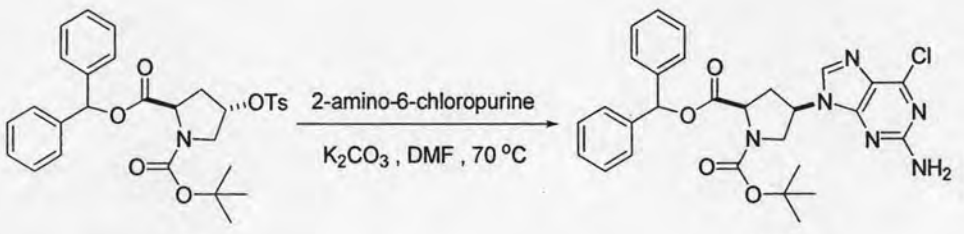
8 **33**

N-tert-butoxycarbonyl-*trans*-4-hydroxy-D-proline diphenylmethyl ester (**8**) (0.60 g, 1.50 mmol), N^2-isobutyryl-O-^{6}nitrophenylethylguanine which accomplished by Miss Boonjira Boonta (0.58 g, 1.50 mmol) and triphenylphosphine (0.46 g, 1.65 mmol) was stirred in anhydrous dioxane (10 mL) and was slowly added DIAD (0.32 mL, 1.65 mmol) under nitrogen at room temperature. Another aliquot of DIAD (0.11 mL, 0.55 mmol each) were added during a period of 24 h. The resulting clear yellow solution was evaporated and the residue chromatographed on silica gel using hexanes: ethyl acetate 1:5 as eluent to give the O^6-protected derivative as white foam

(contaminated with diethyl hydrazine dicarboxylate). This was dissolved in dry pyridine (5 mL) containing DBU (0.45 mL, 3.00 mmol) and the solution stirred at rt for 24 h under nitrogen. The reaction mixture was diluted with dichloromethane and washed with 5% HCl solution and water and then evaporated to dryness. The residue was purified by column chromatography on silica gel using hexanes:ethyl acetate 1:5 as eluent gradient to 2% methanol in ethyl acetate to give the product **33** as a white foam (0.16 g, 18% yield).

^{1}H NMR (400 MHz, $CDCl_3$) (Figure A-63) δ_H 1.25, 1.46 [2×s, 15H, C$\underline{H}_3$ Boc and ($C\underline{H}_3$)$_2$CH] 2.38-2.50 [m, 1H, 1×C$\underline{H}_2$(3′)] 2.65-2.92 [m, 2H, 1×C$\underline{H}_2$(3′) and (CH_3)$_2$ C$\underline{H}$] 3.87-3.93 [m, 1H, 1×C$\underline{H}_2$(5′)] 4.06-4.17 [m, 1H, 1×C$\underline{H}_2$(5′)] 4.49-4.64 [m, 1H, C$\underline{H}$(2′)] 4.88-4.97 [m, 1H, C$\underline{H}$(4′)] 6.82, 6.87 [2×s, 1H, C$\underline{H}$Ph$_2$] 7.23-7.29 [m, 10H, Phenyl Dpm] 7.69, 7.73 [2×s, 1H, C$\underline{H}$8 guanine] 9.32-9.35 [m, 1H, N$\underline{H}$1 guanine] 12.07 [m, 1H, N$\underline{H}_2$ guanine]; ^{13}C NMR (100 MHz, $CDCl_3$) (Figure A-64) δ_C 18.9, 19.0 [($\underline{C}H_3$)$_2$CH rotamers] 27.9, 28.3 [$\underline{C}H_3$ Boc rotamers] 30.9 [$\underline{C}H_2$(3′)] 35.5, 36.1 [(CH_3)$_2\underline{C}$H rotamers] 49.8, 50.3 [$\underline{C}H_2$(5′) rotamers] 52.2, 52.7 [$\underline{C}$H(4′) rotamers] 57.6 [$\underline{C}$H(2′)] 77.8, 78.1 [$\underline{C}$HPh$_2$ Dpm rotamers] 81.1 [$\underline{C}$CH$_3$ Boc] 121.0 [$\underline{C}$5 guanine] 126.7-128.5 [$\underline{C}$H Ar Dpm] 137.2 [$\underline{C}$8 guanine] 139.2 [$\underline{C}$ Ar Dpm] 147.6 [$\underline{C}$2 guanine] 148.4 [$\underline{C}$6 guanine] 153.3, 153.8 [$\underline{C}$O Boc rotamers] 155.5 [$\underline{C}$4 guanine] 170.9 [$\underline{C}$O Proline] 179.3 [$\underline{C}$O Isobutyryl]; $[\alpha]^{25}_D$ = +38.7 (c = 2.21 g/100 mL, $CHCl_3$); mp = 140-145 °C.

(*N-tert*-butyloxycarbonyl)-*cis*-4-(6-chloroguanin-9-yl)-D-proline diphenylmethyl ester (34)

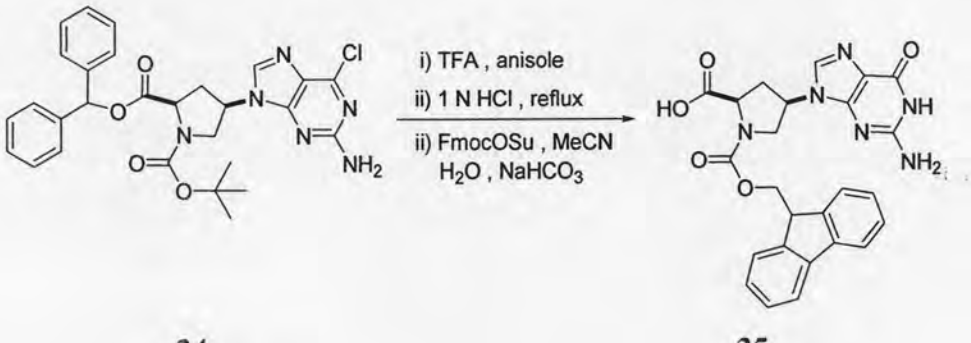


11 **34**

Synthesis of the title compound (**34**) was accomplished in the same way as described for compound **26** above starting from tosylate (**11**) (0.72 g, 1.30 mmol), 2-amino-6-chloropurine (0.61 g, 3.25 mmol), anhydrous K_2CO_3 (0.90 g, 6.49 mmol) in DMF (10 mL). The reaction mixture was stirred under nitrogen at 70 °C for 6 h. The suspension was diluted with dichloromethane (20 mL). The organic layer was washed with water, dried over $MgSO_4$ and evaporated to give the crude product, which was purified by column chromatography eluting with hexanes:ethyl acetate 1:2 to afford the product **34** (0.47 g, 65% yield).

^{1}H NMR (400 MHz, $CDCl_3$) (Figure A-65) δ_H 1.27, 1.47 [2×s, 9H, C$\underline{H}_3$ Boc rotamers] 2.37-2.53 [m, 1H, 1×C$\underline{H}_2$(3′)] 2.78-2.90 [m, 1H, 1×C$\underline{H}_2$(3′)] 3.86-4.01 [m, 1H, 1×C$\underline{H}_2$(5′)] 4.04-4.18 [m, 1H, 1×C$\underline{H}_2$(5′)] 4.50-4.64 [m, 1H, C$\underline{H}$(2′)] 4.88-4.97 [m, 1H, C$\underline{H}$(4′)] 6.87 [s, 1H, C$\underline{H}$Ph$_2$] 7.23-7.31 [m, 10H, Phenyl Dpm] 7.71, 7.76 [2×s, 1H, C$\underline{H}$8 guanine]; ^{13}C NMR (100 MHz, $CDCl_3$) (Figure A-66) δ_C 28.0, 28.3 [$\underline{C}H_3$ Boc rotamers] 34.2, 35.3 [$\underline{C}H_2$(3′) rotamers] 49.6, 50.1 [$\underline{C}H_2$(5′) rotamers] 52.3, 52.9 [$\underline{C}$H(4′) rotamers] 57.6 [$\underline{C}$H(2′)] 77.7 [$\underline{C}$HPh$_2$ Dpm] 81.3 [$\underline{C}$CH$_3$ Boc] 124.9 [$\underline{C}$5 guanine] 126.9-128.5 [$\underline{C}$H Ar Dpm] 139.1 [$\underline{C}$8 guanine] 139.3 [$\underline{C}$2 guanine] 140.7 [$\underline{C}$ Ar Dpm] 151.1 [$\underline{C}$6 guanine] 153.2, 153.3 [$\underline{C}$O Boc rotamers] 158.5 [$\underline{C}$4 guanine] 170.5 [$\underline{C}$O Proline]; $[\alpha]^{25}_D$ = +6.47 (c = 1.02 g/100 mL, $CHCl_3$).

(*N*-Fluoren-9-ylmethoxycarbonyl)-*cis*-4-(guanin-9-yl)-D-proline (35)



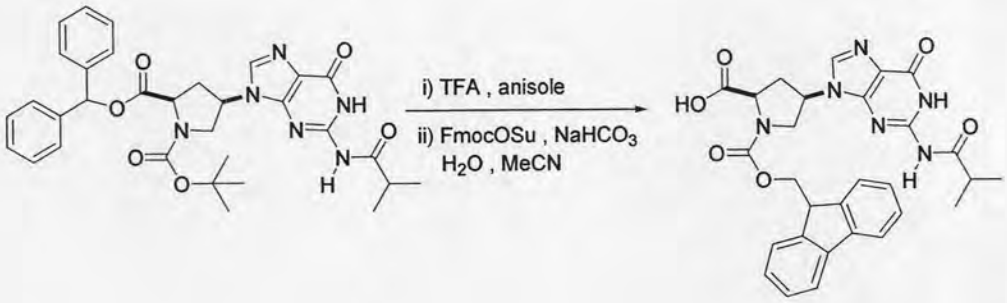
34 **35**

(*N-tert*-Butyloxycarbonyl)-*cis*-4-(6-chloroguanin-9-yl)-D-proline diphenyl methyl ester (**34**) (1.22 g, 2.23 mmol) was treated with trifluoroacetic acid containing 10 % anisole (5 mL) and left at rt for 8 h. After that, the solvent was removed by a

gentle stream of nitrogen. The residue was triturated and washed with diethyl ether. A white precipitate appeared and was collected by filtration. This was dissolved in 1 N HCl (15 mL) and refluxed for 3 h. Next, the reaction mixture was evaporated and then was immediately dissolved in 1:1 H_2O:MeCN (5 mL/mmol) and treated with solid $NaHCO_3$ until pH 8 (pH paper). FmocOSu (0.83 g, 2.45 mmol) was then added in small portions with stirring. After stirring at 30 °C for 8 h, the solvent was removed by rotary evaporation. The residue was diluted with water (10 mL) and extracted with diethyl ether (3 × 10 mL). After purging the extracted aqueous layer to remove the dissolved ether with a gentle stream of N_2, the pH was adjusted to 2 with concentrated HCl. The precipitated Fmoc-amino acid was collected by filtration, washed with water, diethyl ether and dried under vacuum to afford the title compound **35** as a white solid (0.26 g, 54% yield).

^{1}H NMR (400 MHz, DMSO-d_6) (Figure A-67) δ_H 2.77-2.84 [m, 1H, 1×C$\underline{H}_2$(3′)] 2.90-2.96 [m, 1H, 1×C$\underline{H}_2$(3′)] 3.68-3.81 [m, 1H, 1×C$\underline{H}_2$(5′)] 4.00-4.16 [m, 1H, 1×C$\underline{H}_2$(5′)] 4.25-4.27 [m, 1H, C$\underline{H}$ Fmoc] 4.30-4.35 [m, 2H, C$\underline{H}_2$ Fmoc] 4.19-4.53 [m, 1H, C$\underline{H}$(2′)] 4.84-4.91 [m, 1H, C$\underline{H}$(4′)] 7.33-7.37 [m, 2H, C$\underline{H}$ Ar Fmoc] 7.42-7.47 [m, 2H, C$\underline{H}$ Ar Fmoc] 7.69 [d, ^3J(H,H) = 7.6 Hz, 2H, C$\underline{H}$ Ar Fmoc] 7.91 [d, ^3J(H,H) = 8.0 Hz, 2H, C$\underline{H}$ Ar Fmoc] 10.70 [s, 1H; $\underline{H}$8 Guanine] 12.85 [br, 1H, N$\underline{H}$1 Guanine] 13.06 [br, 1H, N$\underline{H}$2 Guanine]; ^{13}C NMR (100 MHz, DMSO-d_6) (Figure A-68) δ_C 34.4, 35.3 [$\underline{C}H_2$(3′) rotamers] 47.0 [$\underline{C}$H Fmoc] 50.2, 50.8 [$\underline{C}H_2$(5′) rotamers] 51.7, 52.3 [$\underline{C}$H(4′) rotamers] 57.5, 57.9 [$\underline{C}$H(2′) rotamers] 67.4, 67.7 [$\underline{C}H_2$ Fmoc rotamers] 116.4 [$\underline{C}$5 Guanine] 120.6 [$\underline{C}$H Ar Fmoc] 125.7 [$\underline{C}$H Ar Fmoc] 127.6 [$\underline{C}$H Ar Fmoc] 128.2 [$\underline{C}$H Ar Fmoc] 136.0 [$\underline{C}$8 Guanine] 141.1, 144.1 [$\underline{C}$ Ar Fmoc] 172.8, 173.4 [$\underline{C}$O Proline rotamers] 151.5 [$\underline{C}$6 Guanine] 151.6 [$\underline{C}$2 Guanine] 154.1, 154.3 [$\underline{C}$O Fmoc rotamers] 156.9 [$\underline{C}$4 Guanine]; Anal Calcd. for $C_{25}H_{22}N_6O_5$ requires C, 61.72; H, 4.56; N, 17.28 %; Found C, 61.67; H, 4.58; N, 17.38 %.

(*N*-Fluoren-9-ylmethoxycarbonyl)-*cis*-4-(N^2-isobutyrylguanin-9-yl)-D-proline (36)

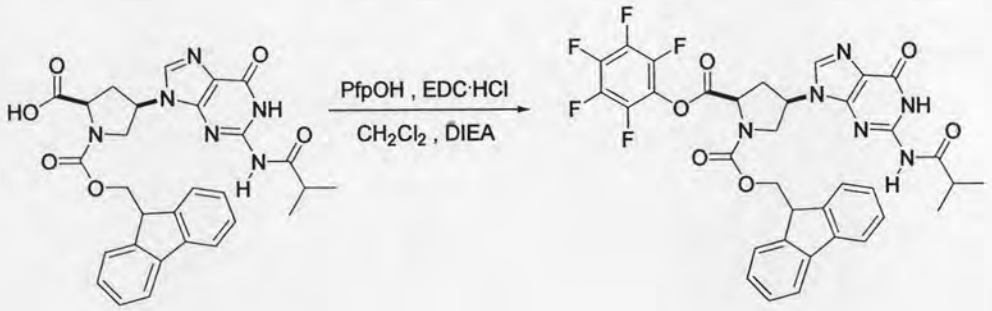


33 **36**

Synthesis of the title compound **36** was accomplished in the same way as described for compound **17** above. Starting from (*N*-*tert*-butyloxycarbonyl)-*cis*-4-(N^2-isobutylguanin-9-yl)-D-proline diphenylmethyl ester (**33**) (0.30 g, 0.49 mmol) and trifluoroacetic acid containing 10% anisole (2 mL) followed by FmocOSu (1.19 g, 0.54 mmol) and $NaHCO_3$ (3 equiv excess) in 1:1 H_2O:MeCN (5 mL/mmol) afforded compound **36** (0.13 g, 49% yield) as a white solid.

^{1}H NMR (400 MHz, DMSO-d_6) (Figure A-69) δ_H 1.10 [d, ^3J(H,H) = 6.4 Hz, 6H, (C$\underline{H}_3$)CH Ibu] 2.35-2.53 [m, 1H, 1×C$\underline{H}_2$(3′)] 2.74-2.80 [m, 1H, (CH$_3$)C$\underline{H}$ Ibu] 2.85-2.99 [m, 1H, 1×C$\underline{H}_2$(3′)] 3.71-3.87 [m, 1H, 1×C$\underline{H}_2$(5′)] 4.04-4.17 [m, 1H, 1×C$\underline{H}_2$(5′)] 4.17-4.21 [m, 1H, C$\underline{H}$ Fmoc] 4.28-4.37 [m, 2H, C$\underline{H}_2$ Fmoc] 4.34-4.47 [m, 1H, C$\underline{H}$(2′)] 4.88-4.97 [m, 1H, C$\underline{H}$(4′)] 7.29-7.33 [m, 2H, C$\underline{H}$ Ar Fmoc] 7.39 [t, ^3J(H,H) = 7.2 Hz, 2H, C$\underline{H}$ Ar Fmoc] 7.66 [d, ^3J(H,H) = 7.2 Hz, 2H; C$\underline{H}$ Ar Fmoc] 7.87 [d, ^3J(H,H) = 6.8 Hz, 2H, C$\underline{H}$ Ar Fmoc] 8.06 [s, 1H, $\underline{H}$8 Guanine]; ^{13}C NMR (100 MHz, DMSO-d_6) (Figure A-70) δ_C 19.3 [$\underline{C}$H$_3$ Ibu] 34.8, 35.6 [$\underline{C}$H$_2$(3′) rotamers] 35.1 [$\underline{C}$H Ibu] 47.0 [$\underline{C}$H Fmoc] 50.5, 51.1 [$\underline{C}$H$_2$(5′) rotamers] 52.1, 52.9 [$\underline{C}$H(4′) rotamers] 57.7, 58.2 [$\underline{C}$H(2′) rotamers] 67.4, 67.7 [$\underline{C}$H$_2$ Fmoc rotamers] 120.5 [$\underline{C}$5 Guanine] 120.2 [$\underline{C}$H Ar Fmoc] 125.8 [$\underline{C}$H Ar Fmoc] 127.6 [$\underline{C}$H Ar Fmoc] 128.1 [$\underline{C}$H Ar Fmoc] 138.3 [$\underline{C}$8 Guanine C8] 141.0, 144.1 [$\underline{C}$ Ar Fmoc] 148.2 [$\underline{C}$6 Guanine] 149.1 [$\underline{C}$2 Guanine] 154.4 [$\underline{C}$O Fmoc] 155.3 [$\underline{C}$4 Guanine] 173.0, 173.6 [$\underline{C}$O Proline rotamers] 180.6 [$\underline{C}$O Ibu]; HRMS (FAB$^+$) Calcd for $C_{29}H_{28}O_6N_6$ (M·H$^+$) 557.2149, Found 557.2149, $[\alpha]^{25}_D$ = -65.96 (c = 0.6 g/100 mL, DMF), mp = 184 °C (dec.).

(*N*-Fluoren-9-ylmethoxycarbonylamino)-*cis*-4-(N^2-isobutyrylguanin-9-yl)-D-proline pentafluorophenyl ester (37)



36 **37**

Synthesis of the title compound **37** was accomplished in the same way as described for compound **21** above. Starting from (*N*-fluoren-9-ylmethoxycarbonyl)-*cis*-4-(N^2-isobutyrylguanin-9-yl)-D-proline (**36**) (0.09 g, 0.17 mmol), PfpOH (1.5 equiv) and EDC·HCl (1.5 equiv) in dichloromethane (2 mL) afforded compound **37** (0.08 g, 66% yield), as a white solid.

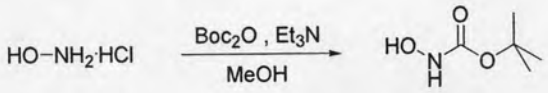
^{1}H NMR (400 MHz, $CDCl_3$) (Figure A-71) δ_H 1.20 [s, 6H, (C$\underline{H}_3$)CH Ibu] 2.63-2.84 [m, 1H, 1×C$\underline{H}_2$(3′)] 2.76-2.84 [m, 1H, (CH_3)C$\underline{H}$ Ibu] 3.11-3.20 [m, 1H, 1×C$\underline{H}_2$(3′)] 4.00, 4.04 [m, 1H, 1×C$\underline{H}_2$(5′)] 4.18-4.48 [m, 2H, 1×C$\underline{H}_2$(5′) and C$\underline{H}$ Fmoc] 4.36-4.50 [m, 2H, C$\underline{H}_2$ Fmoc] 4.77-4.86 [m, 1H, C$\underline{H}$(2′)] 5.10-5.24 [m, 1H, C$\underline{H}$(4′)] 7.22-7.27 [m, 2H, C$\underline{H}$ Ar Fmoc] 7.30-7.36 [m, 2H, C$\underline{H}$ Ar Fmoc] 7.52-7.56 [m, 2H, C$\underline{H}$ Ar Fmoc] 7.68-7.70 [m, 2H, C$\underline{H}$ Ar Fmoc] 8.00, 8.07 [2×s, 1H, $\underline{H}$8 Guanine rotamers] 10.01, 10.24 [2×s, 1H, N$\underline{H}$ Guanine rotamers] 12.27 [s, 1H, N$\underline{H}$ Guanine]; ^{13}C NMR (100 MHz, $CDCl_3$) (Figure A-72) δ_C 18.9 [($\underline{C}H_3$)CH Ibu] 34.5, 35.5 [$\underline{C}H_2$(3′) rotamers] 36.1 [(CH_3)$\underline{C}$H Ibu] 47.0 [$\underline{C}$H Fmoc] 49.9 [$\underline{C}H_2$(5′)] 52.8, 53.3 [$\underline{C}$H(4′) rotamers] 56.9, 57.4 [$\underline{C}$H(2′) rotamers] 68.2, 68.5 [$\underline{C}H_2$ Fmoc rotamers] 119.7 [$\underline{C}$5 Guanine] 120.0 [$\underline{C}$H Ar Fmoc] 124.9 [$\underline{C}$H Ar Fmoc] 127.1 [$\underline{C}$H Ar Fmoc] 127.8 [$\underline{C}$H Ar Fmoc] 136.4-142.1 [$\underline{C}$ Pfp] 137.5, 137.8 [$\underline{C}$8 Guanine rotamers] 141.2 [$\underline{C}$ Ar Fmoc] 143.5 [$\underline{C}$ Ar Fmoc] 148.2, 148.3 [$\underline{C}$6 Guanine] 148.5 [$\underline{C}$2 Guanine] 153.8, 154.4 [$\underline{C}$O Fmoc rotamers] 155.0, 155.1 [$\underline{C}$4 Guanine rotamers] 167.8, 168.0 [$\underline{C}$O Proline rotamers] 179.7, 179.9 [$\underline{C}$O Ibu rotamers]; HRMS (FAB^+) Calcd for

$C_{35}H_{28}O_6N_6F_5$ ($M{\cdot}H^+$) 723.1991, Found 723.2001, $[\alpha]^{25}_D$ = -10.81 (c = 0.5 g/100 mL, $CHCl_3$), mp = 140 °C (dec.).

2.3 Synthesis of *β*-amino acid spacers

2.3.1 Synthesis of *O*-spacer

N-tert-Butoxycarbonyl hydroxylamine (38)

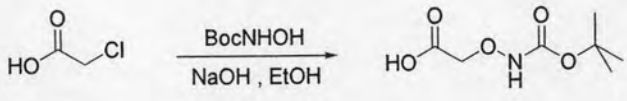


38

Hydroxylamine hydrochloride (2.08 g, 30 mmol) was dissolved in methanol (30 mL). The solution treated with triethylamine (4.6 mL, 33 mmol) and di-*tert*-butyl-dicarbonate (7.89 g, 36 mmol) and stirred for 8 h. The reaction mixture was concentrated under reduced pressure and residue was dissolved in dichloromethane followed by extracting with 5% hydrochloric acid solution. The organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give protected amino alcohol (**38**) as pale-yellow oil (3.46 g, 88% yield).

^1H NMR (200 MHz, $CDCl_3$) (Figure A-73) δ_H 1.29 [s, 9H, C$\underline{H_3}$ Boc]; ^{13}C NMR (50 MHz, $CDCl_3$) (Figure A-74) δ_C 28.2 [$\underline{C}H_3$ Boc] 82.1 [$\underline{C}CH_3$] 158.8 [$\underline{C}$O Boc].

N-tert-Butoxycarbonyl aminooxy-acetic acid (39) [91]

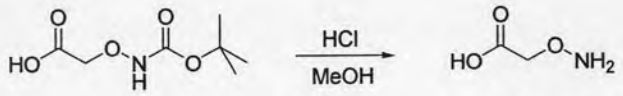


39

The *N*-protected hydroxylamine (**38**) (1.26 g, 9.46 mmol), chloroacetic acid (0.81 g, 8.6 mmol) and sodium hydroxide (1.89 g, 47.3 mmol) were dissolved in ethanol (30 mL) and refluxed for 5 h. The solvent was removed under reduced pressure to give a residue, which was dissolved in water (20 mL). The aqueous solution was neutralized with 5% hydrochloric acid solution and extracted with ethyl acetate (25 mL × 3). The organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give *N*-protected *β*-amino acid (**39**) as a white solid (1.12 g, 62% yield).

^{1}H NMR (300 MHz, DMSO-d_6) (Figure A-75) δ_H 1.48 [s, 9H, [(C<u>H</u>$_3$)$_3$C Boc] 4.48 [s, 2H, C<u>H</u>$_2$O] 7.25 [s, 1H, O<u>H</u>] 7.44 [s, 1H, N<u>H</u>]; ^{13}C NMR (75 MHz, DMSO-d_6) (Figure A-76) δ_C 28.3 [(<u>C</u>H$_3$)$_3$C Boc] 72.4 [<u>C</u>H$_2$O] 80.4 [<u>C</u>CH$_3$] 156.8 [<u>C</u>O Boc] 170.7 [<u>C</u>O$_2$H]; mp = 102 °C (dec.).

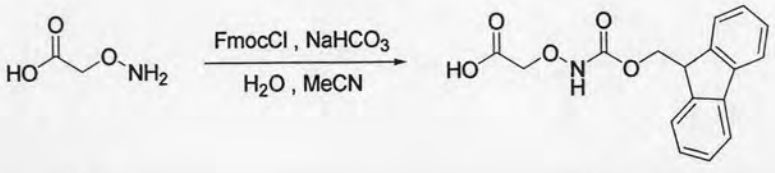
Amino-oxy-acetic acid (40)



39 **40**

To a stirred solution of *N*-protected *β*-amino acid (**39**) (1.18 g, 6.2 mmol) in methanol (5 mL) was added conc. hydrochloric acid (1.9 mL, excess). The reaction mixture was stirred at room temperature for over night. The methanol was removed via rotary evaporation to give the free *β*-amino acid (**40**) and was used for the next steps without further purification.

(*N*′-Fluoren-9-ylmethoxycarbonyl)-aminooxy acetic acid (41)

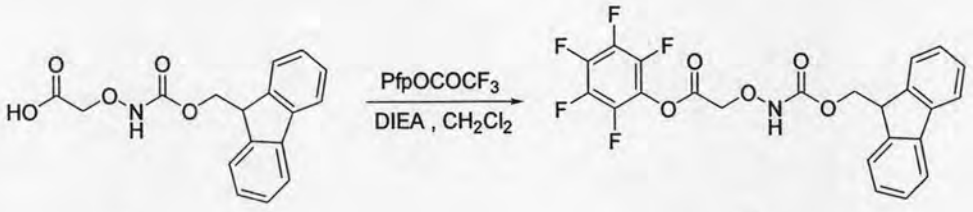


40 **41**

The free *β*-amino acid (**40**) (0.56 g, 6.2 mmol), (9-fluorenylmethyl)-chloro formate (FmocCl) (1.60 g, 6.2 mmol) and sodium hydrogen carbonate (1.04 g, 12.4 mmol) were dissolved in a mixture of water (10 mL) and acetonitrile (10 mL) and then stirred at 30 °C for 8 h. The mixture solution was extracted with diethyl ether (20 mL × 3) and the layers were separated. The aqueous layer was acidified with 5 % HCl solution, extracted with ethyl acetate, dried over $MgSO_4$ and concentrated to give *N*-Fmoc *β*-amino acid (**41**) as a white solid (1.10 g, 57% yield).

^{1}H NMR (300 MHz, $CDCl_3$) (Figure A-77) δ_H 4.12 [t, 1H, J = 6.3 Hz, C<u>H</u> Fmoc] 4.35 [s, 2H, CH_2O] 4.37 [d, 2H, J = 6.9 Hz, C<u>H</u>$_2$ Fmoc] 7.19 [t, 2H, J = 7.2 Hz, C<u>H</u> Ar Fmoc] 7.28 [t, 2H, J = 7.8 Hz, C<u>H</u> Ar Fmoc] 7.47 [d, 2H, J = 6.6 Hz, C<u>H</u> Ar Fmoc] 7.63 [d, 2H, J = 7.2 Hz, C<u>H</u> Ar Fmoc] 8.41 [s, 1H, O<u>H</u>]; ^{13}C NMR (75 MHz, $CDCl_3$) (Figure A-78) δ_C 68.2 [<u>C</u>H Fmoc] 73.2 [<u>C</u>H$_2$ Fmoc] 120.1 [<u>C</u>H Ar Fmoc] 124.9 [<u>C</u>H Ar Fmoc] 127.2 [<u>C</u>H Ar Fmoc] 127.9 [<u>C</u>H Ar Fmoc] 141.3 [<u>C</u> Ar Fmoc] 143.0 [<u>C</u> Ar Fmoc] 172.2 [<u>C</u>O$_2$H].

(*N*'-Fluoren-9-ylmethoxycarbonyl)-aminooxy acetic acid pentafluorophenyl ester (42)



41 **42**

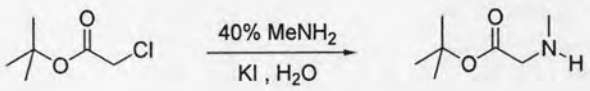
The *N*-Fmoc *β*-amino acid (**41**) (1.07g, 3.43 mmol) was dissolved in dichloromethane (10 mL) and added pentafluorophenyl trifluoroacetate (0.71 mL, 4.11 mmol), diisopropylethylamine (0.70 mL, 4.11 mmol). DMF was used as co-solvent for clear solution. The reaction mixture was stirred at 30 °C until the starting material disappeared (4 h, monitored by TLC). The solution was extracted with water (20 mL × 2) and the organic layer was concentrated under reduced pressure to give a yellow oil. Trituration with hexanes and filtration gave a solid which was further

purified by column chromatography (hexanes:ethyl acetate 4:1) to give the activated *β*-amino acid (**42**) as white solid (1.16 g, 71% yield).

^{1}H NMR (400 MHz, $CDCl_3$) (Figure A-79) δ_H 4.24 [t, 1H, J = 6.4 Hz, C<u>H</u> Fmoc] 4.54 [d, 2H, J = 6.7 Hz, C<u>H</u>$_2$ Fmoc] 4.75 [s, 2H, C<u>H</u>$_2$O] 7.37 [t, 2H, J = 6.2 Hz, C<u>H</u> Ar Fmoc] 7.41 [t, 2H, J = 6.2 Hz, C<u>H</u> Ar Fmoc] 7.57 [d, 2H, J = 7.2 Hz, C<u>H</u> Ar Fmoc] 7.76 [d, 2H, J = 7.0 Hz, C<u>H</u> Ar Fmoc] 7.95 [s, 1H, O<u>H</u>]; ^{13}C NMR (100 MHz, $CDCl_3$) (Figure A-80) δ_C 46.8 [CH_2O] 67.9 [<u>C</u>H Fmoc] 71.9 [<u>C</u>H$_2$ Fmoc] 120.0 [<u>C</u>H Ar Fmoc] 124.9 [<u>C</u>H Ar Fmoc] 127.1 [<u>C</u>H Ar Fmoc] 127.9 [<u>C</u>H Ar Fmoc] 136.6-142.3 [C Pfp] 141.3 [<u>C</u> Ar Fmoc] 143.2 [<u>C</u> Ar Fmoc] 157.2 [CO Fmoc] 165.4 [<u>C</u>O$_2$Pfp]; mp. 127.0-130.5 °C; HRMS (ES-TOF) calcd for $C_{23}H_{14}F_5NO_5$+Na^+, 502.0690; found 502.0147.

2.3.2 Synthesis of *N*-spacer

_N_-methyl glycine _tert_-butyl ester (43)

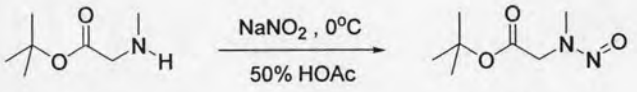


43

To a stirred solution of *tert*-butylchloroacetate (4.3 mL, 30.0 mmol) and methylamine (23 mL, 300.0 mmol) in water (20 mL) was added potassium iodide (4.98 g, 30.0 mmol) at 30°C. After stirring for 3 h, the solution was extracted with dichloromethane (30 mL × 3) and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure without heat to afford product **43** as yellow oil (3.94 g, 91% yield).

^{1}H NMR (200 MHz, $CDCl_3$) (Figure A-81) δ_H 1.38 [s, 9H, C<u>H</u>$_3$ Boc] 2.34 [s, 3H, C<u>H</u>$_3$N] 3.18 [s, 2H, C<u>H</u>$_2$].

(*N*-Nitroso)-*N*-methyl glycine *tert*-butyl ester (44)

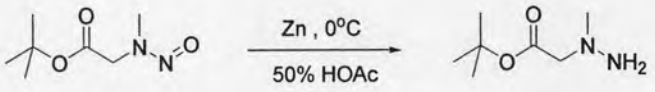


43 **44**

N-methyl glycine *tert*-butyl ester (**43**) (3.94 g, 27.0 mmol) was dissolved in 50% acetic acid solution (50 mL) in ice-bath. The mixture solution was added solution of sodium nitrite (18 g , 270.0 mmol) slowly. The solution was extracted with diethyl ether (30 mL × 3). Then, evaporation of diethyl ether without heating provided the nitroso compound **44** as yellow oil (4.14 g, 87% yield).

^{1}H NMR (200 MHz, $CDCl_3$) (Figure A-82) δ_H 1.44,1.46 [2×s, 9H, C<u>H</u>$_3$ Boc rotamers] 3.07, 3.85 [2×s, 3H, C<u>H</u>$_3$N rotamers] 4.10, 4.82 [2×s, 2H, C<u>H</u>$_2$N rotamers].

(*N*-Amino)-*N*-methyl glycine *tert*-butyl ester (45)

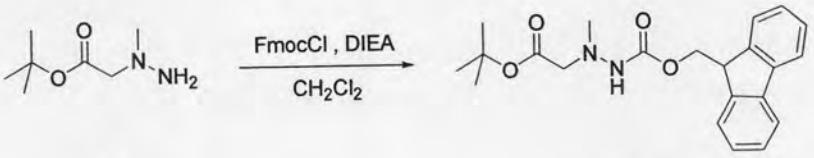


44 **45**

To a stirring solution of the nitroso compound **44** (1.74 g, 10.0 mmol) in 50% HOAc (20 mL) in ice-bath was added zinc powder (3.27 g, 50.0 mmol). The solution was stirred until all the nitroso compound was consumed (4 h). Concentrated aqueous ammonia was added until the solution was strongly basic. The reaction mixture was extracted with dichloromethane (20 mL × 3) and the combine organic layer was dried over anhydrous $MgSO_4$. The solution was concentrated under reduced pressure afforded a yellow oil which was a mixture of the hydrazine compound **45** (0.66 g, 41%) and other over-reduction product **43**. This product was used for the next step without further purification.

^{1}H NMR (400 MHz, $CDCl_3$) (Figure A-83) δ_H 1.39 [s, 9H, C<u>H</u>$_3$ Boc] 2.66 [s, 3H, C<u>H</u>$_3$N] 3.44 [s, 2H, C<u>H</u>$_2$N]; ^{13}C NMR (50 MHz, $CDCl_3$) (Figure A-84) δ_C 28.1 [<u>C</u>H$_3$ Boc] 44.5 [<u>C</u>H$_3$N] 59.1 [<u>C</u>H$_2$N] 81.9 [CH$_3$<u>C</u> Boc] 156.7 [<u>C</u>O]; mp = 86-88 °C.

(*N'*-Fluoren-9-ylmethoxycarbonyl)-(*N*-amino)-*N*-methyl glycine *tert*-butyl ester (46)

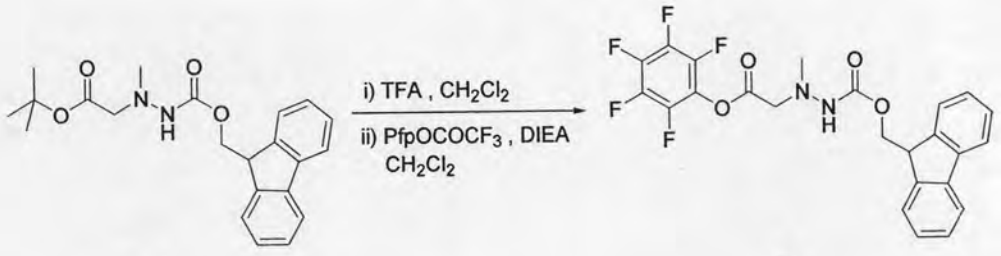


45 **46**

The hydrazine product **45** (0.80g, 5.0 mmol) was stirred in dichloromethane (10 mL) at 30 °C. The solution was added DIEA (0.9 mL, 5.0 mmol) to basic condition and added FmocCl (1.29 g, 5.0 mmol) respectively. The resulting mixture was stirred for 1 h and concentrated under reduced pressure. Purification of the residue by column chromatography (hexanes:ethyl acetate 3:1) provided *N*-protected *β*-amino derivative **46** as yellow oil (1.05 g, 55% yield).

^{1}H NMR (200 MHz, $CDCl_3$) (Figure A-85) δ_H 1.46 [s, 9H, C<u>H</u>$_3$C] 2.75 [s, 3H, C<u>H</u>$_3$N] 3.54 [s, 2H, C<u>H</u>$_2$N] 4.22 [t, 1H, J = 7.3 Hz, C<u>H</u> Fmoc] 4.38 [d, 2H, J = 7.3 Hz, C<u>H</u>$_2$ Fmoc] 7.27-7.36 [m, 4H, C<u>H</u> Ar Fmoc] 7.57 [d, 2H, J = 7.2 Hz, C<u>H</u> Ar Fmoc] 7.72 [d, 2H, J = 7.7 Hz, C<u>H</u> Ar Fmoc]; ^{13}C NMR (50 MHz, $CDCl_3$) (Figure A-86) δ_C 27.7 [<u>C</u>H$_3$C] 43.9 [<u>C</u>CH$_3$] 46.7 [<u>C</u>H$_3$N] 56.4 [<u>C</u>H$_2$N] 66.3 [<u>C</u>H$_2$ Fmoc] 81.6 [<u>C</u>H Fmoc] 119.5 [<u>C</u>H Ar Fmoc] 124.7 [<u>C</u>H Ar Fmoc] 126.6 [<u>C</u>H Ar Fmoc] 127.3 [<u>C</u>H Ar Fmoc] 140.8 [<u>C</u> Ar Fmoc] 143.4 [<u>C</u> Ar Fmoc] 155.3 [<u>C</u>O Fmoc] 169.54 [<u>C</u>O$_2$^{t}Bu].

(*N*'-Fluoren-9-ylmethoxycarbonyl)-(*N*-amino)-*N*-methyl glycine pentafluoro phenyl ester (47)



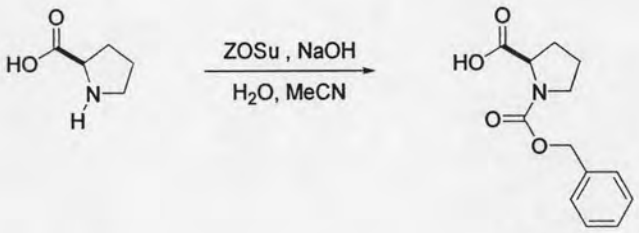
46 **47**

The *N*-protected *β*-amino derivative (**46**) (0.56 g, 1.47 mmol) was dissolved in dichloromethane (3 mL) and TFA (3 mL) was added. The solution was stirred until starting material disappeared (3 h) and the solvent was removed by flushing with N_2 gas and performed the next reaction by adding of dichloromethane (5 mL), DIEA (0.3 mL, 1.77 mmol) and $PfpOCOCF_3$ (0.3 mL, 1.77 mmol) respectively. The mixture was stirred for 3 h. After addition of dichloromethane, the reaction mixture was extracted with water (10 mL × 2). The organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure followed by chromatography (hexanes:ethyl acetate 3:1) to give activated *β*-amino acid spacer (**47**) (0.48 g, 68% yield).

^{1}H NMR (200 MHz, $CDCl_3$) (Figure A-87) δ_H 2.82 [s, 3H, C$\underline{H}_3$N] 4.03 [br, 2H, C$\underline{H}_2$N] 4.22 [t, 1H, J = 6.8 Hz, C$\underline{H}$ Fmoc] 4.45 [d, 2H, J = 6.8 Hz, C$\underline{H}_2$ Fmoc] 7.25-7.42 [m, 4H, C$\underline{H}$ Ar Fmoc] 7.57 [d, 2H, J = 6.91 Hz, C$\underline{H}$ Ar Fmoc] 7.75 [d, 2H, J = 7.35 Hz, C$\underline{H}$ Ar Fmoc]; ^{13}C NMR (50 MHz, $CDCl_3$) (Figure A-88) δ_C 47.1 [$\underline{C}H_3$N] 57.7 [$\underline{C}H_2$N] 67.1 [$\underline{C}H_2$ Fmoc] 120.0 [$\underline{C}$H Ar Fmoc] 125.0 [$\underline{C}$H Ar Fmoc] 127.0 [$\underline{C}$H Ar Fmoc] 127.8 [$\underline{C}$H Ar Fmoc] 135.4-142.0 [C Pfp] 140.4 [$\underline{C}$ Ar Fmoc] 143.6 [$\underline{C}$ Ar Fmoc] 155.5 [CO Fmoc] 166.3 [$\underline{C}O_2$Pfp]; anal calcd. for $C_{24}H_{17}F_5N_2O_4$ C;58.54 H;3.48 N;5.69; found C;58.51 H;3.46 N;5.69: HRMS (ES-TOF) calcd. for $C_{24}H_{17}F_5N_2O_4$+Na^+ 515.1006; found 515.0425; mp 122.4-125.2 °C.

2.3.3 Synthesis of D-APC spacer

_N_-Benzyloxycarbonyl-D-proline (48)

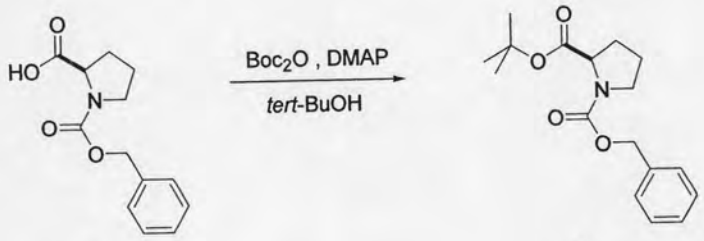


48

D-Proline (1.16 g, 10.0 mmol) was dissolved in H_2O (10 mL) and sodium hydroxide (0.40 g, 10.0 mmol) was added to the solution, after that acetonitrile (10 mL) and benzyloxycarbonyl succinimide (2.53 g, 10.0 mmol) were added to the reaction mixture. The reaction was stirred vigorously to get a clear solution and allowed to stir at rt for 24 h. When the reaction was completed, the solution was extracted with diethyl ether (30 mL × 3) and the aqueous layer was acidified with 2 N HCl. The aqueous phase was extracted with ethyl acetate (30 mL × 3) and the organic layer was evaporated under reduced pressure afford the *N*-protected D-proline (**48**) as a colorless oil (2.47 g, 99% yield).

^{1}H NMR (400 MHz, $CDCl_3$) (Figure A-89) δ_H 1.84-2.18 [m, 4H, C$\underline{H}_2$ Proline] 3.44-3.56 [m, 2H, C$\underline{H}_2$ Proline] 4.33-4.37 [m, 1H, C$\underline{H}$ Proline] 5.06-5.18 [m, 2H, C$\underline{H}_2$O Z] 7.28-7.34 [m, 5H, C$\underline{H}$ Ar Z]; ^{13}C NMR (100 MHz, $CDCl_3$) (Figure A-90) δ_C 23.4, 24.2 [$\underline{C}H_2$(4′) rotamers] 29.7, 30.8 [$\underline{C}H_2$(3′) rotamers] 46.6, 46.9 [$\underline{C}H_2$(5′) rotamers] 58.8, 59.4 [$\underline{C}$H(2′) rotamers] 67.1, 67.3 [$\underline{C}H_2$O] 127.5-128.4 [$\underline{C}$H Phenyl] 136.3 [$\underline{C}$ Phenyl] 154.7, 155.5 [$\underline{C}$O Z rotamers] 176.6, 177.1 [$\underline{C}$O Proline rotamers]; $[\alpha]^{25}_D$ = +57.7 (c = 1.10 g/100 mL $CHCl_3$).

_N_-Benzyloxycarbonyl-D-proline _tert_-butyl ester (49) [92]

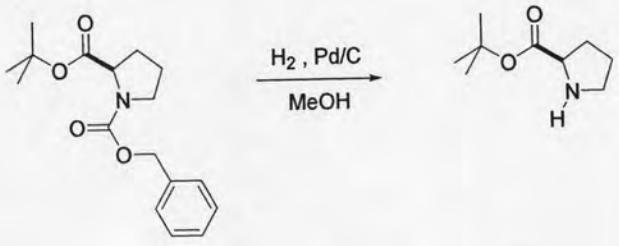


48 **49**

To a solution of *N*-benzyloxycarbonyl-D-proline (**48**) (2.30 g, 9.25 mmol) and Boc_2O (4.05 g, 18.50 mmol) in *tert*-butanol (20 mL) was added DMAP (0.35 g, 2.78 mmol) at rt. Evolution of carbon dioxide was immediately observed. After 1 h, the solvent was removed *in vacuo* and the residue was purified by column chromatography eluting with hexanes:ethyl acetate 4:1 to give a pale-yellow oil (**49**) (2.64 g, 93% yield).

^{1}H NMR (300 MHz, $CDCl_3$) (Figure A-91) δ_H 1.37, 1.47 [2×s, 9H, C$\underline{H}_3$ rotamers] 1.96-2.22 [m, 4H, C$\underline{H}_2$(4′) and C$\underline{H}_2$(3′)] 3.49-3.62 [m, 2H, C$\underline{H}_2$(5′)] 4.22-4.30 [m, 1H, C$\underline{H}$(2′)] 5.15 [t, 2H, J = 6.33 Hz, C$\underline{H}_2$O] 7.31-7.39 [m, 5H, C$\underline{H}$ Phenyl]; ^{13}C NMR (75 MHz, $CDCl_3$) (Figure A-92) δ_C 23.4, 24.2 [$\underline{C}H_2$(4′) rotamers] 27.9, 28.0 [$\underline{C}H_3$ rotamers] 29.9, 30.9 [$\underline{C}H_2$(3′) rotamers] 46.4, 46.9 [$\underline{C}H_2$(5′) rotamers] 59.6, 59.9 [$\underline{C}$H(2′) rotamers] 66.9 [$\underline{C}H_2$O] 81.2 [$\underline{C}CH_3$] 127.8-128.4 [$\underline{C}$H Phenyl] 136.7, 136.9 [$\underline{C}$ Phenyl rotamaers] 154.4, 154.8 [$\underline{C}$O Z rotamers] 171.9 [$\underline{C}$O Proline]; $[\alpha]^{25}_D$ = +51.1 (c = 2.1g/100 mL, EtOH).

D-Proline _tert_-butyl ester (50) [93]

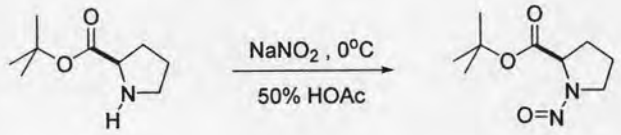


49 **50**

To a stirred solution of *N*-benzyloxycarbonyl-D-proline *tert*-butyl ester (**49**) (4.87 g, 16.0 mmol) in methanol (30 mL) was added palladium on charcoal (0.5 g, 10% by wight). The reaction round-bottom flask was sealed with a rubber septum and a hydrogen balloon was attached. The suspension was stirred at rt for 24 h. The mixture was filtered to remove the palladium and the filtrate was evaporated to get the product **50** as yellow oil (2.35 g, 86% yield).

^{1}H NMR (300 MHz, $CDCl_3$) (Figure A-93) δ_H 1.41 [s, 9H, C$\underline{H}_3$] 1.61-1.77 [m, 3H, C$\underline{H}_2$(4′) and 1×C$\underline{H}_2$(3′)] 1.98-2.10 [m, 1H, 1×C$\underline{H}_2$(3′)] 2.79-2.86 [m, 2H, C$\underline{H}_2$(5′)] 2.98-3.06 [m, 1H, N$\underline{H}$] 3.56-3.61 [m, 1H, C$\underline{H}$(2′)]; ^{13}C NMR (75 MHz, $CDCl_3$) (Figure A-94) δ_C 25.4 [$\underline{C}H_2$(4′)] 27.9 [$\underline{C}H_3$] 30.4 [$\underline{C}H_2$(3′)] 46.9 [$\underline{C}H_2$(5′)] 60.4 [$\underline{C}$H(2′)] 80.9 [$\underline{C}CH_3$] 174.6 [$\underline{C}$O Proline]; $[\alpha]^{25}_D$ = +39.0 (c = 1.0 g/100 mL $CHCl_3$).

_N_-Nitroso-D-proline *tert*-butyl ester (51) [94]



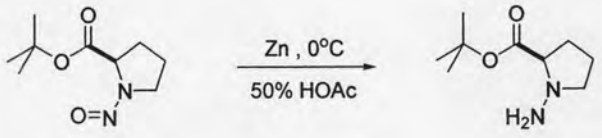
50 **51**

D-Proline *tert*-butyl ester (**50**) (1.81 g, 10.6 mmol) was dissolved in 50% acetic acid solution (20 mL) and cooled in an ice-bath. $NaNO_2$ (7.29 g, 105.6 mmol) was dissolved in water (10 mL) and this solution was slowly added to the solution of the amino acid. The brown-color gas ($NO+NO_2$) evolved and the reaction mixture was allowed to stir at 0 °C until the starting material was consumed. The crude reaction was neutralized with $NaHCO_3$ and the residue was extracted with dichloromethane (40 mL × 3). The organic layer was dried under reduced pressure afford the nitroso compound **51** as a yellow oil (2.62 g, quant. yield).

^{1}H NMR (300 MHz, $CDCl_3$) (Figure A-95) δ_H 1.44, 1.49 [2×s, 9H, C$\underline{H}_3$ rotamers] 2.03-2.13 [m, 2H, C$\underline{H}_2$(4′)] 2.20-2.44 [m, 2H, C$\underline{H}_2$(3′)] 3.65-3.79 [m, 1H, C$\underline{H}$(2′)] 4.33-4.46 [m, 2H, C$\underline{H}_2$(5′)]; ^{13}C NMR (75 MHz, $CDCl_3$) (Figure A-96) δ_C 20.5, 22.6 [$\underline{C}H_2$(4′) rotamers] 27.3, 27.4 [$\underline{C}H_3$ rotamers] 45.2, 49.4 [$\underline{C}H_2$(3′) rotamers] 54.1

[$\underline{C}H_2(5')$] 58.6, 62.1 [$\underline{C}H(2')$ rotamers] 81.0, 81.8 [$\underline{C}CH_3$ rotamers] 167.2, 169.3 [$\underline{C}O$ Proline rotamers]; $[\alpha]^{25}_D = +57.7$ (c = 1.1 g/100 mL $CHCl_3$).

_N_-Amino-D-proline _tert_-butyl ester (52) [94]

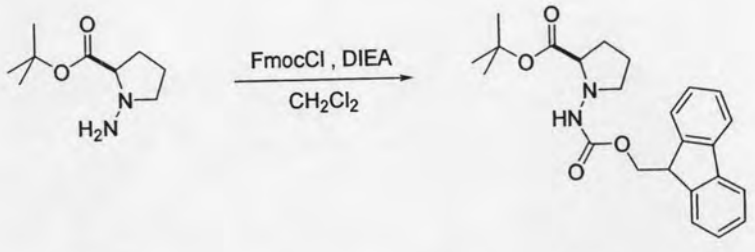


51 **52**

_N_-Nitroso-D-proline _tert_-butyl ester (**51**) (0.82 g, 4.1 mmol) was dissolved in 50 % acetic acid solution (10 mL) and cooled at 0 °C in an ice-bath. To the reaction mixture was slowly added zinc powder (2.13 g, 32.6 mmol). The progress of the reaction was monitored by TLC. More Zn powder was added if the reaction was not complete within 1-2 h. The crude reaction was filtered to remove Zn and to the filtrate was added conc ammonia until the solution was slightly basic (pH 8). The residue was extracted with dichloromethane (20 mL × 3) and the organic layer was dried under reduced pressure to give the product **52** as yellow oil (0.67 g, 89% yield).

^1H NMR (400 MHz, $CDCl_3$) (Figure A-97) δ_H 1.43, 1.46 [2×s, 9H, $C\underline{H}_3$ rotamers] 1.65-1.92 [m, 3H, $C\underline{H}_2(4')$ and 1×$C\underline{H}_2(3')$] 2.03-2.16 [m, 1H, 1×$C\underline{H}_2(3')$] 2.50-2.57, 2.82-2.88 [2×m, 1H, $C\underline{H}_2(5')$] 2.99-3.08 [m, 1H, $C\underline{H}_2(5')$] 3.23-3.27, 3.59-3.63 [2×m, 1H, $C\underline{H}_2(2')$]; ^{13}C NMR (100 MHz, $CDCl_3$) (Figure A-98) δ_C 20.9 [$\underline{C}H_2(4')$] 27.4, 28.0 [$\underline{C}H_3$ rotamers] 46.9 [$\underline{C}H_2(3')$] 58.1, 60.1 [$\underline{C}H_2(5')$ rotamers] 70.5 [$\underline{C}H(2')$] 80.9, 81.2 [$\underline{C}CH_3$ rotamers] 172.2, 174.1 [$\underline{C}O$ Proline rotamers].

(*N'*-Fluoren-9-ylmethoxycarbonyl)-*N*-Amino-D-proline *tert*-butyl ester (53)

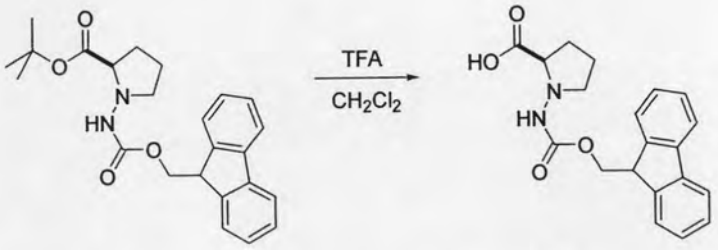


52 **53**

N-Amino-D-proline *tert*-butyl ester (**52**) (2.11 g, 11.4 mmol) was dissolved in dichloromethane (20 mL), and then FmocCl (3.53 g, 13.6 mmol) and DIEA (2.4 mL, 13.6 mmol) was added to the solution at rt. After 24 h, when the reaction was completed, the reaction mixture was extracted with water. The organic phase was evaporated and the residue purified by column chromatography using hexanes:ethyl acetate 3:1 as eluent to get compound **53** as a pale yellow oil (1.64 g, 35% yield).

^{1}H NMR (300 MHz, $CDCl_3$) (Figure A-99) δ_H 1.49 [s, 9H, C$\underline{H}_3$] 1.84-2.01 [m, 3H, C$\underline{H}_2$(4′) and 1×C$\underline{H}_2$(3′)] 2.18-2.35 [m, 1H, 1×C$\underline{H}_2$(3′)] 3.15-3.32 [m, 2H, C$\underline{H}_2$(5′)] 3.85-3.98 [m, 1H, C$\underline{H}_2$(2′)] 4.25 [t, J = 6.9 Hz, 1H, C$\underline{H}$ Fmoc] 4.43 [d, J = 6.4 Hz, 2H, C$\underline{H}_2$ Fmoc] 7.32 [t, J = 7.4 Hz, 2H, C$\underline{H}$ Ar Fmoc] 7.41 [t, J = 7.2 Hz, 2H, C$\underline{H}$ Ar Fmoc] 7.61 [d, J = 7.4 Hz, 2H, C$\underline{H}$ Ar Fmoc] 7.77 [d, J = 7.5 Hz, 2H, C$\underline{H}$ Ar Fmoc]; ^{13}C NMR (75 MHz, $CDCl_3$) (Figure A-100) δ_C 21.6 [$\underline{C}H_2$(4′)] 28.1 [$\underline{C}H_3$] 47.1 [$\underline{C}H_2$(3′)] 50.4 [CH Fmoc] 53.5 [$\underline{C}H_2$(5′)] 64.7 [$\underline{C}H_2$ Fmoc] 66.9 [$\underline{C}$H(2′)] 81.6 [$\underline{C}CH_3$] 119.9 [$\underline{C}$H Ar Fmoc] 125.1 [$\underline{C}$H Ar Fmoc] 127.0 [$\underline{C}$H Ar Fmoc] 127.7 [$\underline{C}$H Ar Fmoc] 141.3 [$\underline{C}$ Ar Fmoc] 143.8 [$\underline{C}$ Ar Fmoc] 155.6 [$\underline{C}$O Fmoc] 172.4 [$\underline{C}$O Proline].

(*N*′-Fluoren-9-ylmethoxycarbonyl)-*N*-Amino-D-proline (54)

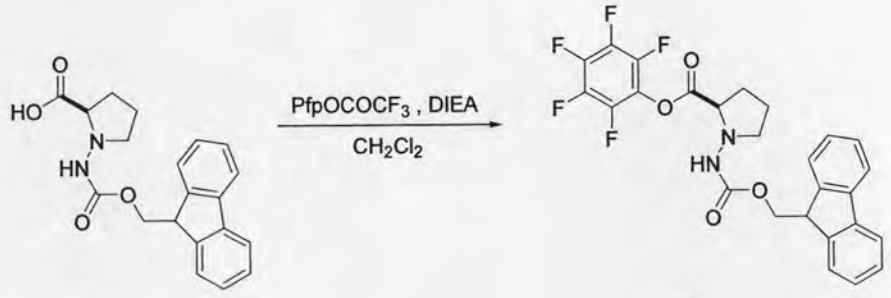


53 **54**

(*N*′-Fluoren-9-ylmethoxycarbonyl)-*N*-Amino-D-proline *tert*-butyl ester (**53**) (1.64 g, 4.02 mmol) and TFA (5 mL) were dissolved in dichloromethane (5 mL) and the solution was stirred at room temperature for 24 h. The reaction mixture was flushed with nitrogen to remove the volatile and the residue was used for next reaction without further purification.

^{1}H NMR (300 MHz, $CDCl_3$) (Figure A-101) δ_H 1.66-1.82 [m, 2H, C$\underline{H}_2$(4′)] 1.99-2.08, 2.15-2.27 [2×m, 2H, C$\underline{H}_2$(3′)] 2.78-2.87, 3.31-3.37 [2×m, 2H, C$\underline{H}_2$(5′)] 3.55-3.62 [m, 1H, C$\underline{H}$(2′)] 4.10-4.14 [m, 1H, C$\underline{H}$ Fmoc] 4.41-4.50 [m, 2H, C$\underline{H}_2$ Fmoc] 6.81 [br m, 1H, NH] 7.23 [t, J = 7.2 Hz, 2H, C$\underline{H}$ Ar Fmoc] 7.32 [t, J = 7.5 Hz, 2H, C$\underline{H}$ Ar Fmoc] 7.47 [d, J = 7.2 Hz, 2H, C$\underline{H}$ Ar Fmoc] 7.68 [d, J = 7.5 Hz, 2H, C$\underline{H}$ Ar Fmoc]; ^{13}C NMR (75 MHz, $CDCl_3$) (Figure A-102) δ_C 23.2 [$\underline{C}H_2$(4′)] 25.3 [$\underline{C}H_2$(3′)] 29.0 [CH Fmoc] 47.0 [$\underline{C}H_2$(5′)] 56.0 [$\underline{C}H_2$ Fmoc] 67.6 [$\underline{C}$H(2′)] 120.0 [$\underline{C}$H Ar Fmoc] 124.8 [$\underline{C}$H Ar Fmoc] 127.2 [$\underline{C}$H Ar Fmoc] 127.9 [$\underline{C}$H Ar Fmoc] 141.3 [$\underline{C}$ Ar Fmoc] 143.3 [$\underline{C}$ Ar Fmoc] 157.3 [$\underline{C}$O Fmoc] 172.4 [$\underline{C}$O Proline].

(*N*'-Fluoren-9-ylmethoxycarbonyl)-*N*-Amino-D-proline pentafluorophenyl ester (55)



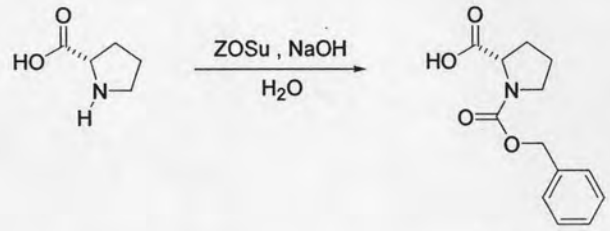
54 **55**

A solution of the Fmoc-protected acid (**54**) (1.42 g, 4.02 mmol), PfpOTfa (1.1 mL, 6.03 mmol) and DIEA (1.1 mL, 6.03 mmol) in dichloromethane (10 mL) was stirred at rt for 2-3 h. The reaction mixture was diluted with dichloromethane (20 mL × 3), washed with water and then evaporated to dryness. The residue was purifired by column chromatography on silica gel eluting with hexanes:ethyl acetate 3:1 to give the product **55** as a white solid (0.98 g, 47% yield).

^{1}H NMR (400 MHz, $CDCl_3$) (Figure A-103) δ_H 1.87 [m, 3H, C$\underline{H}_2$(4′) and 1×C$\underline{H}_2$(3′)] 2.38 [m, 1H, 1×C$\underline{H}_2$(3′)] 3.12, 3.29 [m, 2H, C$\underline{H}_2$(5′)] 4.14 [m, 1H, C$\underline{H}$ Fmoc] 4.36 [m, 3H, C$\underline{H}_2$ Fmoc and C$\underline{H}_2$(2′)] 6.51 [m, 1H, NH] 7.22 [t, J = 7.3 Hz, 2H, C$\underline{H}$ Ar Fmoc] 7.31 [t, J = 7.3 Hz, 2H, C$\underline{H}$ Ar Fmoc] 7.49 [d, J = 7.0 Hz, 2H, C$\underline{H}$ Ar Fmoc] 7.67 [d, J = 7.4 Hz, 2H, C$\underline{H}$ Ar Fmoc]; ^{13}C NMR (100 MHz, $CDCl_3$) (Figure A-104) δ_C 22.3 [$\underline{C}H_2$(4′)] 28.4 [$\underline{C}H_2$(3′)] 47.1 [CH Fmoc] 53.0 [$\underline{C}H_2$(5′)] 63.6 [$\underline{C}H_2$ Fmoc] 66.9 [$\underline{C}H_2$(2′)] 120.0 [$\underline{C}$H Ar Fmoc] 124.9 [$\underline{C}$H Ar Fmoc] 127.0 [$\underline{C}$H Ar Fmoc] 127.8 [$\underline{C}$H Ar Fmoc] 136.1-142.7 [$\underline{C}$F Pfp] 141.3 [$\underline{C}$ Ar Fmoc] 143.6 [$\underline{C}$ Ar Fmoc] 155.2 [$\underline{C}$O Fmoc] 169.6 [$\underline{C}$O Proline]. *m/z* (APCI+) 541 (M+Na$^+$, 12 %), 519 (M+H$^+$, 26), 341 (100), 307 (17), 297 (M-Fmoc$^+$, 5), 179 (34), 129 (26), 112 (12); ν_{max} (KBr)/cm^{-1} 3243s (N-H), 1795s (C=O), 1712s (C=O), 1527 and 1517s; Anal Calcd. for $C_{26}H_{19}N_2O_4F_5$ requires C, 60.20; H, 3.69; N, 5.40 %; Found C, 60.20; H, 3.72; N, 5.30 %; A small amount of Fmoc-D-Pro-OPfp was also isolated; $[\alpha]^{25}_D$ = +73.18 (c = 1.03 g/100 mL, $CHCl_3$); mp 138-140 °C (recrystallised from MeOH).

2.3.4 Synthesis of L-APC spacer

_N_-Benzyloxycarbonyl-L-proline (56)

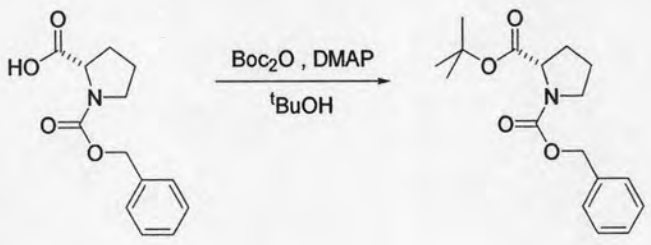


56

Synthesis of the title compound **56** was accomplished in the same way as described for compound **48** above. Starting from L-proline (1.15 g, 10.0 mmol), ZOSu (1.1 equiv) and NaOH (1.0 equiv) in dichloromethane (5 mL) afforded compound **56** (2.83 g, quantitative yield) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) (Figure A-105) δ_H 1.84-1.99 [m, 2H, C$\underline{H}_2$(4′)] 2.05-2.29 [m, 2H, C$\underline{H}_2$(3′)] 3.42-3.64 [m, 2H, C$\underline{H}_2$(5′)] 4.35-4.45 [m, 1H, C$\underline{H}$(2′)] 5.11-5.20 [m, 2H, C$\underline{H}_2$ Z] 7.24-7.36 [m, 5H, C$\underline{H}$ Z Ar] 11.64 [br, 1H, $CO_2\underline{H}$]; ^{13}C NMR (100 MHz, $CDCl_3$) (Figure A-106) δ_C 23.4, 24.2 [$\underline{C}H_2$(4′) rotamers] 29.6, 30.8 [$\underline{C}H_2$(3′) rotamers] 46.5, 46.9 [$\underline{C}H_2$(5′) rotamers] 58.6, 59.1 [$\underline{C}$H(2′) rotamers] 67.2, 67.3 [$\underline{C}H_2$O rotamers] 127.6-128.4 [$\underline{C}$H Phenyl] 136.3 [$\underline{C}$ Phenyl] 154.6, 155.4 [$\underline{C}$O Z rotamers] 176.9, 177.7 [$\underline{C}$O Proline rotamers]. $[\alpha]^{25}_D$ = -63.9 (c = 1.015 g/100 mL $CHCl_3$).

_N_-Benzyloxycarbonyl-L-proline *tert*-butyl ester (57) [92]

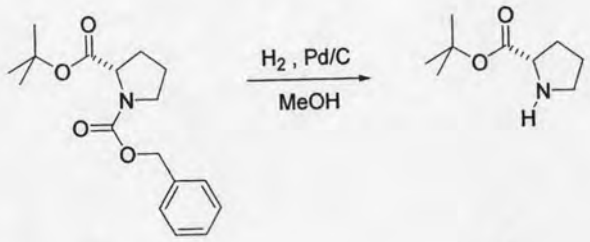


56 **57**

Synthesis of the title compound **57** was accomplished in the same way as described for compound **49** above. Starting from *N*-Benzyloxycarbonyl-L-proline **(66)** (1.95 g, 7.81 mmol), Boc_2O (2.0 equiv) and DMAP (0.3 equiv) in *tert*-butanol (10 mL) afforded compound **57** (2.06 g, 87% yield) as a pale-yellow oil.

^1H NMR (400 MHz, $CDCl_3$) (Figure A-107) δ_H 1.37, 1.48 [2×s, 9H, $C\underline{H}_3$ rotamers] 1.87-2.01, 2.06-2.16 [2×m, 4H, $C\underline{H}_2(4')$ and $C\underline{H}_2(3')$] 3.45-3.67 [m, 2H, $C\underline{H}_2(5')$] 4.23-4.30 [m, 1H, $C\underline{H}(2')$] 5.16 [t, 2H, J = 6.33 Hz, $C\underline{H}_2O$] 7.30-7.40 [m, 5H, C<u>H</u> Phenyl]; ^{13}C NMR (100 MHz, $CDCl_3$) (Figure A-108) δ_C 23.2, 24.0 [$\underline{C}H_2(4')$ rotamers] 27.6, 27.8 [$\underline{C}H_3$ rotamers] 29.7, 30.7 [$\underline{C}H_2(3')$ rotamers] 46.2, 46.8 [$\underline{C}H_2(5')$ rotamers] 59.3, 59.8 [$\underline{C}H(2')$ rotamers] 66.6 [$\underline{C}H_2O$] 80.9 [$\underline{C}CH_3$] 127.5-128.2 [<u>C</u>H Phenyl] 136.5, 136.7 [<u>C</u> Phenyl rotamaers] 154.2, 154.6 [<u>C</u>O Z rotamers] 171.6, 171.7 [<u>C</u>O Proline rotamers]. $[\alpha]^{25}_D$ = -54.3 (c = 2.20 g/100 mL EtOH).

L-Proline *tert*-butyl ester (58) [93]



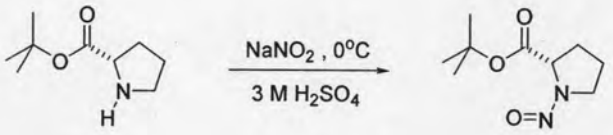
57 **58**

Synthesis of the title compound **58** was accomplished in the same way as described for compound **50** above. Starting from *N*-Benzyloxycarbonyl-L-proline *tert*-butyl ester (**57**) (0.37 g, 1.21 mmol), palladium on charcoal (0.1 equiv) in methanol (20 mL) under a hydrogen balloon afforded compound **58** (1.08 g, 79% yield) as a pale-yellow oil.

^1H NMR (400 MHz, $CDCl_3$) (Figure A-109) δ_H 1.43 [s, 9H, $C\underline{H}_3$] 1.65-1.79, 2.02-2.11 [2×m, 4H, $C\underline{H}_2(4')$ and $C\underline{H}_2(3')$] 2.82-2.87 [m, 1H, 1×$C\underline{H}_2(5')$] 3.02-3.07 [m, 1H, 1×$C\underline{H}_2(5')$] 3.58-3.62 [m, 1H, $C\underline{H}(2')$]; ^{13}C NMR (100 MHz, $CDCl_3$) (Figure A-110)

δ_C 25.4 [$\underline{C}H_2(4')$] 28.0 [$\underline{C}H_3$] 30.4 [$\underline{C}H_2(3')$] 47.0 [$\underline{C}H_2(5')$] 60.3 [$\underline{C}H(2')$] 80.8 [$\underline{C}CH_3$] 174.7 [$\underline{C}O$ Proline]. $[\alpha]^{25}_D$ = -37.3 (c = 1.10 g/100 mL $CHCl_3$).

_N_-Nitroso-L-proline _tert_-butyl ester (59) [94]

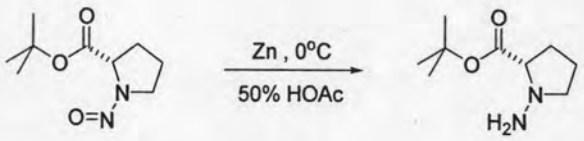


58 **59**

Synthesis of the title compound **59** was accomplished in the same way as described for compound **51** above. Starting from L-proline *tert*-butyl ester (**58**) (1.02 g, 5.93 mmol) in 2 N HCl (20 mL) and added $NaNO_2$ (10.0 equiv) solution (10 mL) at 0 °C afforded compound **59** (0.51 g, 43 % yield) as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) (Figure A-111) δ_H 1.43, 1.47 [2×s, 9H, C$\underline{H}_3$ rotamers] 1.99-2.08 [m, 2H, C$\underline{H}_2(4')$] 2.17-2.37 [m, 2H, C$\underline{H}_2(3')$] 3.60-3.70 [m, 1H, C$\underline{H}(2')$] 4.30-3.46 [m, 2H, C$\underline{H}_2(5')$]; ^{13}C NMR (100 MHz, $CDCl_3$) (Figure A-112) δ_C 21.0, 23.1 [$\underline{C}H_2(4')$ rotamers] 27.8, 27.9 [$\underline{C}H_3$ rotamers] 28.9 [$\underline{C}H_2(3')$] 45.6, 50.0 [$\underline{C}H_2(5')$ rotamers] 59.2, 62.8 [$\underline{C}H(2')$ rotamers] 82.2, 82.9 [$\underline{C}CH_3$ rotamers] 167.8, 169.5 [$\underline{C}O$ Proline rotamers].

_N_-Amino-L-proline _tert_-butyl ester (60) [94]



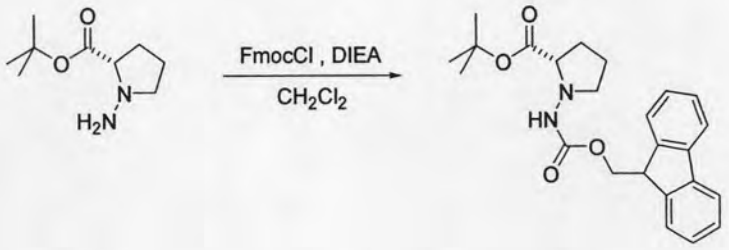
59 **60**

Synthesis of the title compound **60** was accomplished in the same way as described for compound **52** above. Starting from *N*-Nitroso-L-proline *tert*-butyl ester

(**59**) (0.47 g, 2.36 mmol) in 50% acetic acid (20 mL) and added zinc powder (12.0 equiv) at 0 °C afforded compound **60** (0.37 g, 84% yield) as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) (Figure A-113) δ_H 1.51 [s, 9H, C<u>H</u>$_3$] 1.78-1.95 [m, 3H, C<u>H</u>$_2$(4′) and 1×C<u>H</u>$_2$(3′)] 2.13-2.21 [m, 1H, C<u>H</u>$_2$(3′)] 2.55-2.61 [m, 1H, 1×C<u>H</u>$_2$(5′)] 3.04-3.08 [m, 1H, 1×C<u>H</u>$_2$(5′)] 3.28-3.32 [m, 1H, C<u>H</u>(2′)].

(*N*′-Fluoren-9-ylmethoxycarbonyl)-*N*-Amino-L-proline *tert*-butyl ester (61)

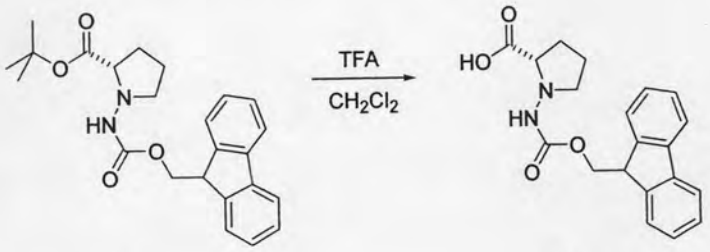


60 **61**

Synthesis of the title compound **61** was accomplished in the same way as described for compound **53** above. Starting from *N*-amino-L-proline *tert*-butyl ester (**60**) (0.36 g, 2.36 mmol), FmocCl (0.83 g, 2.47 mmol) and DIEA (0.32 mL, 2.47 mmol) in dichloromethane (5 mL) afforded compound **61** (0.37 g, 47% yield), as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) (Figure A-114) δ_H 1.56 [s, 9H, C<u>H</u>$_3$] 1.91-2.00 [m, 3H, C<u>H</u>$_2$(4′) and 1×C<u>H</u>$_2$(3′)] 2.20-2.38 [m, 1H, 1×C<u>H</u>$_2$(3′)] 3.19-3.41 [m, 2H, C<u>H</u>$_2$(5′)] 3.90-3.95 [m, 1H, C<u>H</u>(2′)] 4.26-4.30 [m, 1H, C<u>H</u> Fmoc] 4.43-4.50 [m, 2H, C<u>H</u>$_2$ Fmoc] 6.74 [br, 1H, N<u>H</u>] 7.33 [t, J = 7.6 Hz, 2H, C<u>H</u> Ar Fmoc] 7.42 [t, J = 7.6 Hz, 2H, C<u>H</u> Ar Fmoc] 7.58 [br, 2H, C<u>H</u> Ar Fmoc] 7.79 [d, J = 7.6 Hz, 2H, C<u>H</u> Ar Fmoc]; $[\alpha]^{25}_D$ = -39.4 (c = 1.00 g/100 mL $CHCl_3$).

(*N'*-Fluoren-9-ylmethoxycarbonyl)-*N*-Amino-L-proline (62)

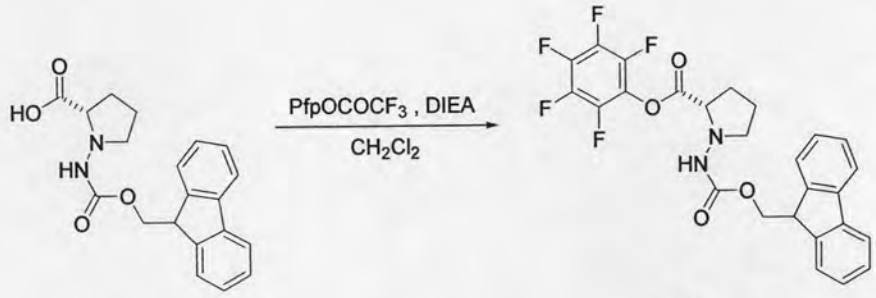


61 **62**

Synthesis of the title compound **62** was accomplished in the same way as described for compound **54** above. Starting from (*N'*-Fluoren-9-ylmethoxy carbonyl)-*N*-amino-L-proline *tert*-butyl ester (**61**) (0.13 g, 0.27 mmol), TFA (2 mL) in dichloromethane (2 mL) afforded compound **62** as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) (Figure A-115) δ_H 1.82-1.91 [2×m, 2H, C$\underline{H}_2$(4′)] 2.16-2.31 [2×m, 2H, C$\underline{H}_2$(3′)] 2.90 [br, 1H, $CO_2\underline{H}$] 3.43-3.65 [2×m, 2H, C$\underline{H}_2$(5′)] 4.22 [br, 1H, C$\underline{H}$ Fmoc] 4.50 [br, 2H, C$\underline{H}_2$ Fmoc] 6.75 [br, 1H, C$\underline{H}_2$(2′)] 7.34 [br, 2H, C$\underline{H}$ Ar Fmoc] 7.42 [br, 2H, C$\underline{H}$ Ar Fmoc] 7.58 [br, 2H, C$\underline{H}$ Ar Fmoc] 7.77-7.78 [m, 2H, C$\underline{H}$ Ar Fmoc]; ^{13}C NMR (100 MHz, $CDCl_3$) (Figure A-116) δ_C 23.4 [$\underline{C}H_2$(4′)] 29.2 [$\underline{C}H_2$(3′)] 47.0 [CH Fmoc] 56.1 [$\underline{C}H_2$(5′)] 67.6 [$\underline{C}H_2$ Fmoc] 67.8 [$\underline{C}$H(2′)] 120.0 [$\underline{C}$H Ar Fmoc] 124.9 [$\underline{C}$H Ar Fmoc] 125.0 [$\underline{C}$H Ar Fmoc] 127.2 [$\underline{C}$H Ar Fmoc] 127.9 [$\underline{C}$H Ar Fmoc] 141.3 [$\underline{C}$ Ar Fmoc] 143.3 [$\underline{C}$ Ar Fmoc] 157.5 [$\underline{C}$O Fmoc] 175.2 [$\underline{C}$O Proline]; Anal Calcd. for $C_{20}H_{20}N_2O_4$ requires C, 68.17; H, 5.72; N, 7.95 %; Found C, 68.21; H, 5.73; N, 7.92 %; $[\alpha]^{25}_D$ = -26.2 (c = 1.00 g/100 mL $CHCl_3$). mp = 123-124 °C.

(*N'*-Fluoren-9-ylmethoxycarbonyl)-*N*-Amino-L-proline pentafluorophenyl ester (63)



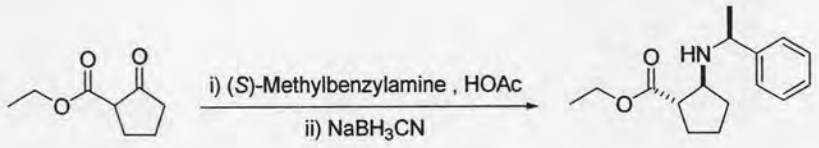
62 **63**

Synthesis of the title compound **63** was accomplished in the same way as described for compound **55** above. Starting from (*N'*-Fluoren-9-ylmethoxy carbonyl)-*N*-Amino-L-proline (**62**) (0.13 g, 0.27 mmol), PfpOTfa (48 μL, 0.27 mmol) and DIEA (47 μL, 0.27 mmol) in dichloromethane (2 mL) afforded compound **63** (0.06 g, 42% yield), as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) (Figure A-117) δ_H 2.00-2.55 [m, 4H, C$\underline{H}_2$(3′) and C$\underline{H}_2$(4′)] 3.50-3.80 [m, 2H, C$\underline{H}_2$(5′)] 4.20-4.55 [m, 2H, C$\underline{H}$ and 1×C$\underline{H}_2$ Fmoc aliphatic] 4.45-4.52, 4.70-4.75 [m, 2H, C$\underline{H}$(2′) and 1×C$\underline{H}_2$ Fmoc] 7.30-7.42 [2×m, 4H, C$\underline{H}$ Fmoc Ar] 7.57 [br, 2H, C$\underline{H}$ Fmoc Ar] 7.78 [br, 2H, C$\underline{H}$ Fmoc Ar]; ^{13}C NMR (100 MHz, $CDCl_3$) (Figure A-118) δ_C 22.2 [C$\underline{H}_2$(4′)] 28.3 [C$\underline{H}_2$(3′)] 47.1 [$\underline{C}$H Fmoc aliphatic] 53.0 [C$\underline{H}$(2′)CO_2] 63.6 [C$\underline{H}_2$(5′)] 66.9 [$\underline{C}H_2$ Fmoc] 120.2, 125.1, 127.3, 128.0 [$\underline{C}$H Fmoc Ar] 135.8, 137.0, 138.8, 141.0 [4×C-F] 141.6, 143.9 [$\underline{C}$ Fmoc Ar] 155.7 [$\underline{C}$O Fmoc] 170.0 [$\underline{C}$O Proline]; *m/z* (APCI+) 526 ($M+Na^+$, 2 %), 504 ($M+H^+$, 4), 326 (16), 282 (26), 179 (100), 116 (57); ν_{max} (KBr)/cm^{-1} 3244s (N-H), 1795s (C=O), 1713s (C=O), 1527 and 1520s; Anal Calcd. for $C_{26}H_{19}N_2O_4F_5$ requires C, 60.20; H, 3.69; N, 5.40 %; Found C, 60.10; H, 3.36; N, 5.26 %; A small amount of Fmoc-L-Pro-OPfp was also isolated; $[\alpha]^{25}_D$ = -73.8 (c = 1.00 g/100 mL $CHCl_3$); mp 140.5-141.5 °C.

2.3.5 Synthesis of (1*S*,2*S*)-ACPC spacer

Ethyl (1*S*,2*S*)-2-[(1′*S*)-phenylethyl]-aminocyclopentane carboxylate (64) [95]

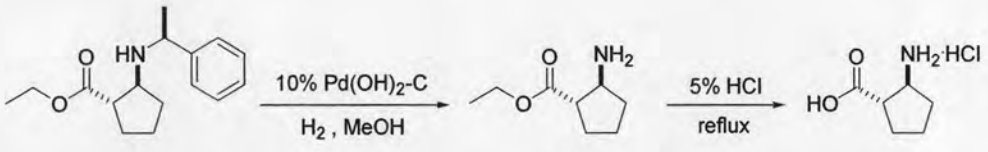


64

To a stirred solution of ethyl cyclopentanone-2-carboxylate (3.75 mL, 25.8 mmol) in absolute ethanol (30 mL) was added (*S*)-(-)-α-methylbenzylamine (3.82 mL, 30.0 mmol) and glacial acetic acid (3.0 mL, 32.0 mmol). The reaction mixture was stirred at 30 °C until the formation of enamine was completed (2 h, monitored by TLC, 3:1 hexanes/ethyl acetate, R_f = 0.60) The reaction mixture was heated to 72 °C and sodium cyanoborohydride (3.24 g, 52.0 mmol) was then added to the reaction mixture in five portions over a 5 h period. The disappearance of enamine was monitored by TLC. When the reaction was complete, water (40 mL) was added, and the ethanol was removed by rotary evaporation. The resulting mixture was extracted with diethyl ether. The ether was removed by rotary evaporation. The crude product was purified by flash column chromatography eluting with 10:1 hexanes:ethyl acetate on silica gel to obtain the title compound **64** as colorless liquid (1.40 g, 21% yield).

^{1}H NMR (400 MHz, $CDCl_3$) (Figure A-119) δ_H 1.30-1.35 [m, 3H, C$\underline{H}_3$ Ethyl] 1.44-1.62 [2×m, 2H, C$\underline{H}_2$ ring ACPC] 1.70-1.84, 1.92-2.06 [2×m, 4H, 2×C$\underline{H}_2$ ring ACPC] 2.74-2.85, 2.95-3.02 [2×m, 1H, C$\underline{H}$NH] 3.11-3.19 [m, 1H, C$\underline{H}$Ph] 3.85-3.92 [m, 1H, C$\underline{H}$CO$_2$Et] 4.17-4.31 [m, 2H, C$\underline{H}_2$ Ethyl] 7.25-7.35 [2×m, 5H, C$\underline{H}$ Ar]; ^{13}C NMR (100 MHz, $CDCl_3$) (Figure A-120) δ_C 14.2, 14.4 [C$\underline{H}_3$ Ethyl rotamers] 21.9, 22.4 [C$\underline{H}_3$ rotamers] 24.9, 25.0 [$\underline{C}H_2$ ring ACPC rotamers] 27.1, 27.8 [$\underline{C}H_2$ ring ACPC rotamers] 31.3, 32.4 [$\underline{C}H_2$ ring ACPC rotamers] 46.3, 47.9 [$\underline{C}$HCO$_2$Et rotamers] 55.8, 56.6 [$\underline{C}$HNH rotamers] 58.7 [$\underline{C}$HPh] 59.9, 60.2 [$\underline{C}H_2$O rotamers] 126.5-128.3 [$\underline{C}$H Phenyl] 145.8, 146.0 [$\underline{C}$ Phenyl] 174.3, 174.9 [$\underline{C}$O rotamers].

(1*S*,2*S*)-2-Aminocyclopentane carboxylic acid hydrochloride (66) [95]

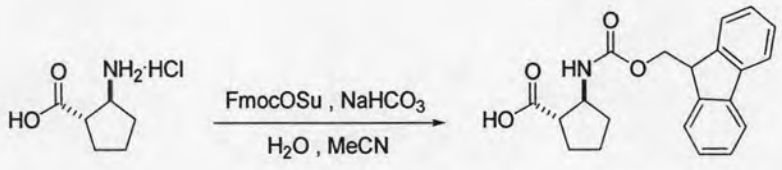


64 **65** **66**

Ethyl (1*S*,2*S*)-2-[(1′*S*)-phenylethyl]-aminocyclopentane carboxylate (**64**) (1.03 g, 3.95 mmol) was dissolved in methanol (5 mL) and palladium on charcoal (0.10 g) was added with stirring at 30 °C under H_2 balloon. TLC monitoring indicated disappearance of compound **64** and formation of ethyl (1*S*,2*S*)-2-aminocyclopentane carboxylate (**65**) were completed. The palladium on charcoal was filtered off with the aid of celite and washed with methanol. The filtrate was evaporated by rotary evaporation to obtain compound **65**. Next, a mixture of compound **65** and 5% HCl (20 mL) was refluxed for 2 h. Then the mixture was allowed to cool at 30 °C. The solvent was removed by rotary evaporation to obtain the title compound **66** as a white solid (0.39 g, 64% yield from compound **64**).

^{1}H NMR (400 MHz, D_2O) (Figure A-121) δ_H 1.50-1.74 [2×m, 4H, 2×C$\underline{H}_2$ ring ACPC] 1.97-2.05 [m, 2H, C$\underline{H}_2$ ring ACPC] 2.73-2.79 [m, 1H, C$\underline{H}$CO$_2$H] 3.69-3.74 [m, 1H, C$\underline{H}$NH]; ^{13}C NMR (100 MHz, D_2O) (Figure A-122) δ_C 22.5 [$\underline{C}$H$_2$ ring ACPC] 28.4 [$\underline{C}$H$_2$ ring ACPC] 30.2 [$\underline{C}$H$_2$ ring ACPC] 48.0 [$\underline{C}$HCO$_2$Et] 53.7 [$\underline{C}$HNH] 176.8 [$\underline{C}$O]; $[\alpha]^{25}_D$ = +61.3 (c = 1.00 g/100 mL H_2O).

(1*S*,2*S*)-2-(*N*-Fluoren-9-ylmethoxycarbonyl)-aminocyclopentanecarboxylic acid (67) [95]

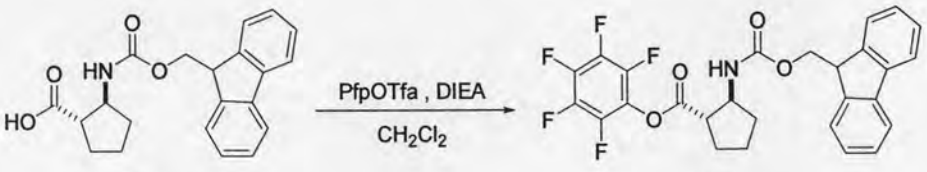


66 **67**

The (1*S*,2*S*)-2-aminocyclopentane carboxylic acid hydrochloride (**66**) (0.39 g, 2.54 mmol) was dissolved in acetone: water (1:1, 6 mL) and $NaHCO_3$ (3 equiv excess) was added until the solution was basic (pH = 8). FmocOSu (0.91 g, 2.54 mmol) was slowly added with stirring at 30 °C for 8 h. The acetone was removed by rotary evaporation under reduced pressure. The residue was diluted with water (20 mL) and extracted with diethyl ether (3 × 20 mL). The pH of the aqueous phase was adjusted to 2 with concentrated HCl. The precipitated white solid was extracted with dichloromethane (3 × 20 mL). Solvent was removed by rotary evaporation and dried in vacuum to afford the title compound **67** as a white solid (0.56 g, 63% yield).

^{1}H NMR (400 MHz, DMSO-d_6) (Figure A-123) δ_H 1.41-1.48, 1.55-1.69 [2×m, 4H, C$\underline{H}_2$×2 ring ACPC] 1.86-1.99 [m, 2H, C$\underline{H}_2$ ring ACPC] 2.48-2.61 [m, 1H, J = 7.7 Hz, C$\underline{H}$CO$_2$H] 4.01-4.04 [m, 1H, C$\underline{H}$NH] 4.19-4.28 [2×m, 3H, C$\underline{H}_2$ and C$\underline{H}$ Fmoc] 7.29-7.47 [3×m, 4H, C$\underline{H}$ Ar Fmoc] 7.68 [m, 2H, C$\underline{H}$ Ar Fmoc] 7.85 [d, 2H, J = 7.4 Hz, C$\underline{H}$ Ar Fmoc]; ^{13}C NMR (100 MHz, DMSO-d_6) (Figure A-124) δ_C 23.3 [$\underline{C}H_2$ ring ACPC] 28.9 [$\underline{C}H_2$ ring ACPC] 33.1 [$\underline{C}H_2$ ring ACPC] 47.2 [$\underline{C}$HCO$_2$H] 49.9 [$\underline{C}$H Fmoc] 55.9 [$\underline{C}$HNH] 65.7 [$\underline{C}H_2$ Fmoc] 120.6 [$\underline{C}$H Ar Fmoc] 125.5 [$\underline{C}$H Ar Fmoc] 125.6 [$\underline{C}$H Ar Fmoc] 127.5 [$\underline{C}$H Ar Fmoc] 128.0 [$\underline{C}$H Ar Fmoc] 141.2 [$\underline{C}$ Ar Fmoc] 144.3 [$\underline{C}$ Ar Fmoc] 144.4 [$\underline{C}$ Ar Fmoc] 155.9 [Fmoc $\underline{C}$O] 176.5 [$\underline{C}$O$_2$H]; Anal Calcd. for $C_{27}H_{10}NO_4F_5$ C, 62.67; H, 3.90; N, 2.71 %, Found C, 62.93; H, 3.86; N, 2.75 %, $[\alpha]^{25}_D$ = +36.4 (c = 1.00 g/100 mL, MeOH).

(1*S*,2*S*)-2-(*N*-fluoren-9-ylmethoxycarbonyl)-aminocyclopentanecarboxylic pentafluorophenyl ester (68)



67 **68**

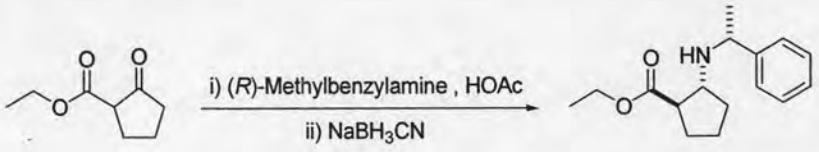
To a suspension of (1*S*,2*S*)-2-(*N*-fluoren-9-ylmethoxycarbonyl)-aminocyclo pentanecarboxylic acid (**67**) (0.36 g, 1.00 mmol) and DIEA (0.21 mL, 1.20 mmol) in

dichloromethane (5 mL) was added PfpOTfa (0.21 mL, 1.20 mmol). The resulting mixture was stirred at 30 °C for 1 h. The reaction was completed as indicated by TLC analysis and purified by flash column chromatography on silica gel eluting with hexanes:ethyl acetate (5:1) to obtain the title compound **68** as a white solid (0.50 g, 95% yield).

^{1}H NMR (400 MHz, $CDCl_3$) (Figure A-125) δ_H 1.64-1.74 [m, 1H, ring CH] 1.85-1.93 [m, 2H, ring CH] 2.08-2.13 [m, 1H, ring CH] 2.20-2.32 [m, 2H, ring CH] 3.06-3.17 [m, 1H, CHCO] 4.25 [t, ^3J(H,H) = 6.8 Hz, 1H, Fmoc CH] 4.33-4.41 [m, 1H, CHNH] 4.48 [d, ^3J(H,H) = 6.8 Hz, 2H, Fmoc CH_2] 4.94-4.98 [d ^3J(H,H) = 5.6 Hz, 1H, NH] 7.34 [t, ^3J(H,H) = 7.6 Hz, 2H, Fmoc Ar CH] 7.43 [t, ^3J(H,H) = 7.2 Hz, 2H, Fmoc Ar CH] 7.62 [d, ^3J(H,H) = 7.2 Hz, 2H, Fmoc Ar CH] 7.80 [d ^3J(H,H) = 7.2 Hz, 2H, Fmoc Ar CH]; ^{13}C NMR (100 MHz, $CDCl_3$) (Figure A-126) δ_C 23.2 [ring CH_2], 28.9 [ring CH_2], 32.8 [ring CH_2], 47.2 [CHCO], 49.8 [Fmoc CH], 56.4 [CHNH], 66.6 [Fmoc CH_2], 120.0 [Fmoc Ar CH], 125.0 [Fmoc Ar CH], 127.1 [Fmoc Ar CH], 127.7 [Fmoc Ar CH], 136.0-141.2 [Pfp C], 141.3 [Fmoc Ar C], 143.8 [Fmoc Ar C], 155.7 [Fmoc CO], 170.8 [Pro CO]; Anal Calcd. for $C_{27}H_{10}NO_4F_5$ C, 62.67; H, 3.90; N, 2.71 %, Found C, 62.93; H, 3.86; N, 2.75 %, $[\alpha]^{25}_D$ = +51.4 (c = 1.00 g/100 mL, $CHCl_3$); mp = 156-157 °C.

2.3.6 Synthesis of (1*R*,2*R*)-ACPC spacer

Ethyl (1*R*,2*R*)-2-[(1′*R*)-phenylethyl]-aminocyclopentane carboxylate (69) [95]



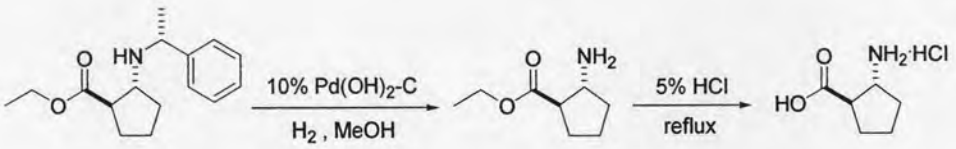
69

Synthesis of the title compound **69** was accomplished in the same way as described for compound **64** above. Starting from ethyl cyclopentanone-2-carboxylate (2.85 mL, 15.0 mmol), (*R*)-(+)-α-methylbenzylamine (2.21 mL, 17.4 mmol) in

ethanol (10 mL) afforded the intermediate enamine and then reduced with $NaBH_3CN$ (1.90 g, 30.0 mmol) to give compound **69** (1.14 g, 29% yield) as a colorless liquid.

^{1}H NMR (400 MHz, $CDCl_3$) (Figure A-127) δ_H 1.19 [t, J = 7.2 Hz, 3H, C$\underline{H}_3$ Ethyl] 1.31 [d, J = 6.4 Hz, 3H, C$\underline{H}_3$] 1.52-1.68 [m, 2H, C$\underline{H}_2$ ring ACPC] 1.72-1.97 [m, 4H, 2×C$\underline{H}_2$ ring ACPC] 2.55 [q, J = 8.8 Hz, 1H, C$\underline{H}$NH] 3.18 [q, J = 6.8 Hz, 1H, C$\underline{H}$Ph] 3.81 [q, J = 6.4 Hz, 1H, C$\underline{H}CO_2$Et] 4.03-4.12 [m, 2H, C$\underline{H}_2$ Ethyl] 7.17-7.28 [2×m, 5H, C$\underline{H}$ Ar]; ^{13}C NMR (100 MHz, $CDCl_3$) (Figure A-128) δ_C 14.2 [C$\underline{H}_3$ Ethyl] 23.6 [C$\underline{H}_3$] 24.6 [$\underline{C}H_2$ ring ACPC] 28.9 [$\underline{C}H_2$ ring ACPC] 34.3 [$\underline{C}H_2$ ring ACPC] 51.2 [$\underline{C}HCO_2$Et] 56.7 [C$\underline{H}$NH] 60.2 [C$\underline{H}$Ph] 61.5 [C$\underline{H}_2$O] 126.6-128.2 [$\underline{C}$H Phenyl] 145.7 [$\underline{C}$ Phenyl] 175.9 [$\underline{C}$O].

(1*R*,2*R*)-2-Aminocyclopentane carboxylic acid hydrochloride (71) [95]

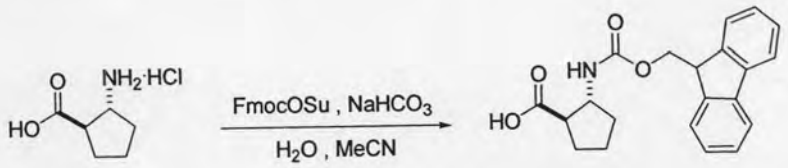


69 **70** **71**

Synthesis of the title compound **71** was accomplished in the same way as described for compound **66** above. Starting from Ethyl (1*R*,2*R*)-2-[(1′*R*)-phenylethyl]-aminocyclopentane carboxylate (**69**) (0.91 g, 3.49 mmol), palladium on charcoal (0.1 g, 0.35 mmol) in methanol (10 mL) under a hydrogen balloon afforded the intermediate free amine and then hydrolysis with 5% HCl solution (10 mL) to give compound **71** (0.44 g, 70% yield), as a white solid.

^{1}H NMR (400 MHz, D_2O) (Figure A-129) δ_H 1.57-1.66 [2×m, 4H, C$\underline{H}_2$×2 ring ACPC] 1.98-2.02 [m, 2H, C$\underline{H}_2$ ring ACPC] 2.73-2.77 [m, 1H, C$\underline{H}CO_2$H] 3.70-3.74 [m, 1H, C$\underline{H}$NH]; ^{13}C NMR (100 MHz, D_2O) (Figure A-130) δ_C 22.5 [$\underline{C}H_2$ ring ACPC] 28.4 [$\underline{C}H_2$ ring ACPC] 30.2 [$\underline{C}H_2$ ring ACPC] 48.0 [$\underline{C}HCO_2$Et] 53.7 [C$\underline{H}$NH] 176.8 [$\underline{C}$O].

(1*R*,2*R*)-2-(*N*-Fluoren-9-ylmethoxycarbonyl)-aminocyclopentanecarboxylic acid (72) [95]

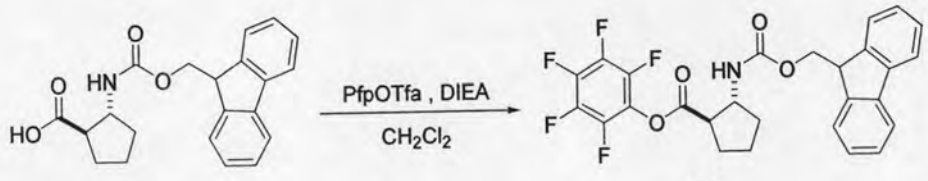


71 72

Synthesis of the title compound **72** was accomplished in the same way as described for compound **67** above. Starting from (1*R*,2*R*)-2-Aminocyclopentane carboxylic acid hydrochloride (**71**) (0.20 g, 1.16 mmol), FmocOSu (0.71 g, 2.10 mmol) and $NaHCO_3$ (3.0 equiv excess) in 1:1 H_2O:MeCN (5 mL/mmol) afforded compound **72** (0.37 g, 64% yield) as a white solid.

^1H NMR (400 MHz, DMSO-d_6) (Figure A-131) δ_H 1.40-1.88 [m, 4H, 2×C$\underline{H}_2$ ring ACPC] 1.92-2.02 [m, 2H, C$\underline{H}_2$ ring ACPC] 2.62-2.72 [m, 1H, C$\underline{H}$CO$_2$H] 4.07-4.14 [m, 1H, C$\underline{H}$NH] 4.20 [br, 1H, C$\underline{H}$ Fmoc] 4.28 [br, 2H, C$\underline{H}_2$ Fmoc] 7.31 [br, 2H, C$\underline{H}$ Ar Fmoc] 7.38 [br, 2H, C$\underline{H}$ Ar Fmoc] 7.68 [br, 2H, C$\underline{H}$ Ar Fmoc] 7.85 [br, 2H, C$\underline{H}$ Ar Fmoc]; ^{13}C NMR (100 MHz, DMSO-d_6) (Figure A-132) δ_C 23.3 [$\underline{C}H_2$ ring ACPC] 28.9 [$\underline{C}H_2$ ring ACPC] 33.0 [$\underline{C}H_2$ ring ACPC] 47.2 [$\underline{C}$HCO$_2$H] 49.8 [$\underline{C}$H Fmoc] 55.9 [$\underline{C}$HNH] 65.7 [$\underline{C}H_2$ Fmoc] 120.5 [$\underline{C}$H Ar Fmoc] 125.5 [$\underline{C}$H Ar Fmoc] 127.5 [$\underline{C}$H Ar Fmoc] 128.0 [$\underline{C}$H Ar Fmoc] 141.1 [$\underline{C}$ Ar Fmoc] 144.2 [$\underline{C}$ Ar Fmoc] 144.3 [$\underline{C}$ Ar Fmoc] 155.9 [Fmoc $\underline{C}$O] 176.5 [$\underline{C}O_2H$]; $[\alpha]^{25}_D$ = -47.5 (c = 1.025 g/100 mL, MeOH).

(1*R*,2*R*)-2-(*N*-fluoren-9-ylmethoxycarbonyl)-aminocyclopentanecarboxylic pentafluorophenyl ester (73)



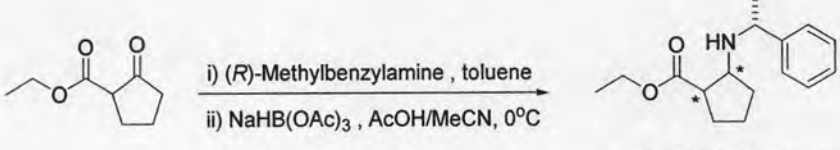
72 73

Synthesis of the title compound **73** was accomplished in the same way as described for compound **68** above. Starting from (1*R*,2*R*)-2-(*N*-Fluoren-9-ylmethoxy carbonyl)-aminocyclopentanecarboxylic acid (**72**) (0.36 g, 1.00 mmol), DIEA (1.2 equiv) and PfpOTfa (1.2 equiv) in dichloromethane (5 mL) afforded compound **73** (0.44 g, 84% yield) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) (Figure A-133) δ_H 1.68-1.75 [m, 2H, C$\underline{H}_2$ ring ACPC] 1.85-1.90 [m, 2H, C$\underline{H}_2$ ring ACPC] 2.06-2.13, 2.22-2.33 [2×m, 2H, C$\underline{H}_2$ ring ACPC] 3.10-3.17 [m, 1H, C$\underline{H}CO_2H$] 4.25 [t, ^3J(H,H) = 6.5 Hz, 1H, C$\underline{H}$ Fmoc] 4.36-4.40 [m, 1H, C$\underline{H}$NH] 4.48 [d, ^3J(H,H) = 6.2 Hz, 2H, C$\underline{H}_2$ Fmoc] 4.97-5.03 [m, 1H, N$\underline{H}$] 7.34 [t, ^3J(H,H) = 7.4 Hz, 2H, C$\underline{H}$ Ar Fmoc] 7.43 [t, ^3J(H,H) = 7.4 Hz, 2H, C$\underline{H}$ Ar Fmoc] 7.62 [d, ^3J(H,H) = 7.4 Hz, 2H, C$\underline{H}$ Ar Fmoc] 7.80 [d ^3J(H,H) = 7.4 Hz, 2H, Fmoc Ar C$\underline{H}$]; ^{13}C NMR (100 MHz, $CDCl_3$) (Figure A-134) δ_C 23.2 [CH_2 ring ACPC] 28.8 [CH_2 ring ACPC] 32.7 [CH_2 ring ACPC] 47.2 [$\underline{C}HCO_2H$] 49.7 [$\underline{C}$H Fmoc] 56.4 [CHNH] 66.6 [$\underline{C}H_2$ Fmoc] 119.9 [$\underline{C}$H Fmoc Ar] 124.9 [$\underline{C}$H Fmoc Ar] 127.0 [$\underline{C}$H Fmoc Ar] 127.7 [$\underline{C}$H Fmoc Ar] 138.5-139.4 [$\underline{C}$ Pfp] 141.3 [$\underline{C}$HFmoc Ar] 143.8 [$\underline{C}$ Fmoc Ar] 155.7 [$\underline{C}$O Fmoc] 170.8 [$\underline{C}$O Proline]; Anal Calcd. for $C_{26}H_{19}N_2O_4F_5$ requires 62.93; H, 3.86; N, 2.75%; Found C, 62.93; H, 3.85; N, 2.75 %; $[\alpha]^{25}_D$ = -46.5 (c = 0.54 g/100 mL, $CHCl_3$).

2.3.7 Synthesis of *cis*-(1*S*,2*R*)-2-aminocyclopentanecarboxylic acid spacer

Ethyl *cis*-(±)-2-[(1′*R*)-phenylethyl]-aminocyclopentane carboxylate (74) [96]



Both *cis*-isomer

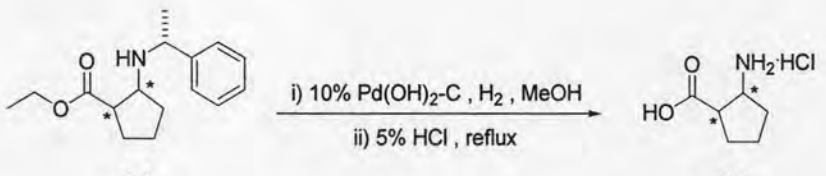
74

To a stirred solution of ethyl cyclopentanone-2-carboxylate (3.2 mL, 20.0 mmol) in toluene (20 mL) was added (*R*)-(+)-α-methylbenzylamine (2.8 mL, 22.0

mmol) and Na_2SO_4 (3.41 g, 24.0 mmol). The reaction mixture was stirred at 50 °C until the formation of enamine was completed (24 h, monitored by TLC, 3:1 hexanes/ethyl acetate, R_f = 0.50). Sodium triacetoxyborohydride (3.06 g, 80.8 mmol) was generated by dissolving sodium borohydride in glacial acetic acid (40 mL) at 10 °C for 30 min until hydrogen gas had evolved. The prepared sodium triacetoxy borohydride mixture was added acetonitrile (20 mL) and cooled down to 0 °C, and then the enamine was dissolved in acetonitrile (10 mL) and was transferred to the freshly prepared sodium triacetoxyborohydride flask. The reaction was stirred at 0 °C for 4 h and the disappearance of enamine was monitored by TLC. When the reaction was completed, the crude reaction was removed by rotary evaporation and was purified by flash column chromatography eluting with 20:1 hexanes:ethyl acetate on silica gel to obtain the title compound **74** as colorless liquid (3.11 g, 59% yield).

^{1}H NMR (400 MHz, $CDCl_3$) (Figure A-135) δ_H 1.31 [d, ^3J(H,H) = 6.4 Hz, 3H, C$\underline{H}_3$] 1.36 [t, ^3J(H,H) = 7.2 Hz, 3H, C$\underline{H}_3$ Ethyl] 1.42-1.51, 1.54-1.63 [2×m, 2H, C$\underline{H}_2$ ring ACPC] 1.69-1.86, 1.94-2.02 [2×m, 4H, 2×C$\underline{H}_2$ ring ACPC] 2.77-2.83, 2.93-2.98 [2×m, 1H, C$\underline{H}$NH] 3.08-3.14 [m, 1H, C$\underline{H}$Ph] 3.82-3.88 [m, 1H, C$\underline{H}$CO$_2$Et] 4.17-4.26 [m, 2H, C$\underline{H}_2$ Ethyl] 7.24-7.29, 7.33-7.36 [2×m, 5H, C$\underline{H}$ Ar]; ^{13}C NMR (100 MHz, $CDCl_3$) (Figure A-136) δ_C 14.2, 14.4 [C$\underline{H}_3$ Ethyl rotamers] 21.9, 22.4 [C$\underline{H}_3$ rotamers] 24.9, 25.0 [$\underline{C}H_2$ ring ACPC rotamers] 27.1, 27.8 [$\underline{C}H_2$ ring ACPC rotamers] 31.3, 32.4 [$\underline{C}H_2$ ring ACPC rotamers] 46.3, 47.9 [$\underline{C}$HCO$_2$Et rotamers] 55.8, 56.6 [$\underline{C}$HNH rotamers] 58.7 [$\underline{C}$HPh] 59.9, 60.2 [$\underline{C}H_2$O rotamers] 126.5-128.3 [$\underline{C}$H Phenyl] 145.8, 146.0 [$\underline{C}$ Phenyl rotamers] 174.3, 174.9 [$\underline{C}$O rotamers].

***cis*-(±)-2-Aminocyclopentane carboxylic acid hydrochloride (75)** [95]



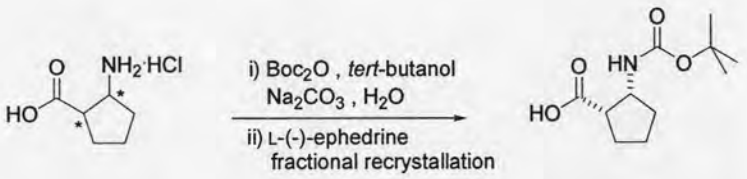
74 Both *cis*-isomer

75 Both *cis*-isomer

Synthesis of the title compound **75** was accomplished in the same way as described for compound **66** above. Starting from Ethyl (±)-2-[(1′*R*)-phenylethyl]-aminocyclopentane carboxylate (**74**) (3.11 g, 11.9 mmol), palladium on charcoal (0.31 g, 1.19 mmol) in methanol (10 mL) with hydrogen balloon afforded the intermediate free amine and then hydrolysis with 5% HCl solution (20 mL) to give compound **75** (1.86 g, 99% yield) as a white solid.

^{1}H NMR (400 MHz, D_2O) (Figure A-137) δ_H 1.09-1.30 [m, 2H, C$\underline{H}_2$ ring ACPC] 1.39-1.50 [m, 2H, C$\underline{H}_2$ ring ACPC] 1.54-1.59, 1.74-1.84 [m, 2H, C$\underline{H}_2$ ring ACPC] 2.74-2.84 [m, 1H, C$\underline{H}$CO$_2$H] 3.30-3.37 [m, 1H, C$\underline{H}$NH]; ^{13}C NMR (100 MHz, D_2O) (Figure A-138) δ_C 21.7, 21.8 [$\underline{C}H_2$ ring ACPC rotamers] 25.5 [$\underline{C}H_2$ ring ACPC] 26.8 [$\underline{C}H_2$ ring ACPC] 41.5, 41.6 [$\underline{C}$HCO$_2$H rotamers] 49.4, 49.5 [$\underline{C}$HNH rotamers] 176.2 [$\underline{C}O_2H$].

(1*S*,2*R*)-2-(*N*- *tert*-Butoxycarbonyl)-aminocyclopentanecarboxylic acid (76)



Both *cis*-isomer (1*S*,2*R*)-isomer

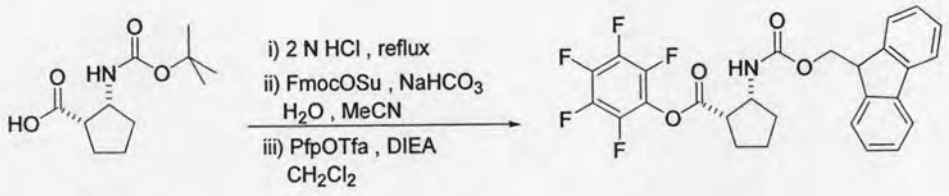
75 **76**

The compound **75** (1.05 g, 6.70 mmol) was dissolved in water and the solution was basified with Na_2CO_3 (3.00 g, 6.00 mmol). Boc_2O (1.61 g, 7.37 mmol) which dissolved in *tert*-butanol (5 mL) was slowly add to the solution mixture. The reaction was allowed to stir for 8 h and then extracted with ether. The aqueous residue was acidified with 5% hydrochloric acid and the mixture was extracted with ethyl acetate (20 mL × 3). The combined organic layer was dried over sodium sulfate and evaporated by rotary evaporation provided the pale-yellow solid (1.03 g, 71 % yield). The *N*-Boc protected (0.67 g, 2.94 mmol) was dissolved in acetonitrile and ether and then added L-(-)-ephedrine (0.94 g, 3.0 mmol). The resulting mixture was fractional

recrystallized and collected the solid by filtration obtained the optically pure (1*S*,2*R*)-isomer **76** as a white solid (0.43 g, 37% yiled, 2 steps)

^{1}H NMR (400 MHz, $CDCl_3$) (Figure A-139) δ_H 1.40, 1.44 [2×s, 3H, C<u>H</u>$_3$ Boc rotamers] 1.60-1.72, 1.83-2.05 [2×m, 6H, 3×C<u>H</u>$_2$ ring ACPC] 3.00-3.11 [m, 1H, C<u>H</u>CO_2H] 4.03-4.25 [m, 1H, C<u>H</u>NH]; ^{13}C NMR (100 MHz, $CDCl_3$) (Figure A-140) δ_C 19.7, 21.5 [<u>C</u>H_2 ring ACPC rotamers] 24.7 [<u>C</u>H_2 ring ACPC] 25.7 [<u>C</u>H_3 Boc] 27.1 [<u>C</u>H_2 ring ACPC] 46.5, 46.9 [<u>C</u>HCO_2H rotamers] 57.9 [<u>C</u>HNH] 76.8 [<u>C</u>CH_3] 152.8 [<u>C</u>O Boc] 176.7 [<u>C</u>O_2H].

(1*S*,2*R*)-2-(*N*-fluoren-9-ylmethoxycarbonyl)-aminocyclopentanecarboxylic pentafluorophenyl ester (77)



76 **77**

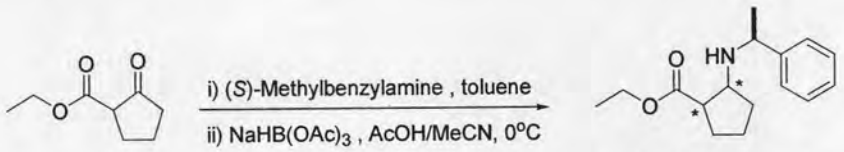
The *N*-Boc protected (**76**) (0.10 g, 0.60 mmol) was added 2 N HCl (5 mL) and was refluxed for 2 h. The reaction mixture was concentrated under reduced pressure and redissolved in water (5 mL). The aqueous residue was added FmoOSu (0.21 g, 0.61 mmol) in the presence of $NaHCO_3$ (0.15 g, 1.81 mmol) afforded the *N*-Fmoc protected amino acid which was further reacted with PfpOTfa (86 µL, 0.5 mmol), DIEA (87 µL, 0.5 mmol) in dichloromethane to give the compound **77** as a whit solid (0.14 g, 66 % yield, 3 steps)

^{1}H NMR (400 MHz, $CDCl_3$) (Figure A-141) δ_H 1.73-1.82, 1.92-1.97, 2.09-2.21 [3×m, 6H, C<u>H</u>$_2$(3′), C<u>H</u>$_2$(4′) and C<u>H</u>$_2$(5′)] 3.51 [dd, 1H, J = 7.1, 13.8 Hz, C<u>H</u>NH] 4.24 [m, 1H, C<u>H</u> Fmoc aliphatic] 4.44 [d, 2H, J = 7.0 Hz, C<u>H</u>$_2$ Fmoc] 4.51 [m, 1H, C<u>H</u>CO_2Pfp] 5.20 [d, 1H, J = 8.8 Hz, N<u>H</u>] 7.33 [t, 2H, J = 7.6 Hz, 4H, C<u>H</u> Fmoc Ar] 7.43 [t, 2H, J = 6.8 Hz, 4H, C<u>H</u> Fmoc Ar] 7.61-7.63 [m, 2H, C<u>H</u> Fmoc Ar] 7.80 [d, 2H, J = 8.0 Hz, C<u>H</u> Fmoc Ar]; ^{13}C NMR (100 MHz, $CDCl_3$) (Figure A-142) δ_C 22.0

[C<u>H</u>$_2$(4′)] 28.1 [CH_2(3′)] 31.7 [CH_2(5′)] 46.5 [C<u>H</u>CO_2Pfp] 47.1 [<u>C</u>H Fmoc aliphatic] 54.5 [C<u>H</u>NH] 67.0 [<u>C</u>H_2 Fmoc] 119.9, 125.0, 127.0 and 127.7 [<u>C</u>H Fmoc Ar] 141.3 and 143.8 [<u>C</u> Fmoc Ar] 136.6, 139.1, 139.7, 140.7 and 142.3 [4×C-F] 155.9 [<u>C</u>O Fmoc] 170.36 [<u>C</u>O_2Pfp]; Anal Calcd. for $C_{26}H_{19}N_2O_4F_5$ requires 62.93; H, 3.86; N, 2.75%; Found C, 62.79; H, 3.87; N, 2.51 %; $[\alpha]^{25}_D$ = +73.6 (c = 0.52 g/100 mL, $CHCl_3$); mp = 87-88 °C.

2.3.8 Synthesis of *cis*-(1*R*,2*S*)-2-aminocyclopentanecarboxylic acid spacer

Ethyl *cis*-(±)-2-[(1′*S*)-phenylethyl]-aminocyclopentane carboxylate (78)



Both *cis*-isomer

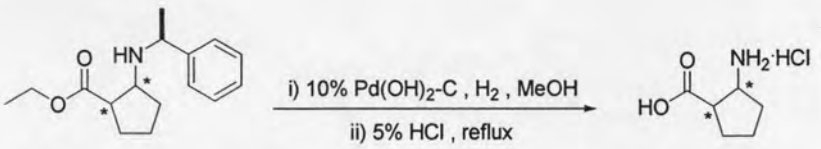
78

Synthesis of the title compound **78** was accomplished in the same way as described for compound **74** above but changed the chiral auxiliary from (*R*)-(+)- to (*S*)-(-)-α-methylbenzylamine. Starting from ethyl cyclopentanone-2-carboxylate (3.12 mL, 20.0 mmol), (*S*)-(-)-α-methylbenzylamine (2.80 mL, 22.0 mmol) in ethanol (10 mL) afforded the intermediate enamine and then reduced with $NaBH(OAc)_3$ (3.05 g, 80.5 mmol) to give compound **78** (3.22 g, 61% yield) as a colorless liquid.

^{1}H NMR (400 MHz, $CDCl_3$) (Figure A-143) δ_H 1.30-1.35 [m, 3H, C<u>H</u>$_3$ Ethyl and C<u>H</u>$_3$] 1.44-1.51, 1.54-1.64 [2×m, 2H, C<u>H</u>$_2$ ring ACPC] 1.70-1.82, 1.98-2.07 [2×m, 4H, 2×C<u>H</u>$_2$ ring ACPC] 2.79-2.82, 2.95-3.02 [2×m, 1H, C<u>H</u>NH] 3.11-3.19 [m, 1H, C<u>H</u>Ph] 3.85-3.92 [m, 1H, C<u>H</u>CO_2Et] 4.17-4.31 [m, 2H, C<u>H</u>$_2$ Ethyl] 7.22-7.27, 7.34-7.40 [2×m, 5H, C<u>H</u> Ar]; ^{13}C NMR (100 MHz, $CDCl_3$) (Figure A-144) δ_C 14.4 [C<u>H</u>$_3$ Ethyl] 21.9, 22.4 [C<u>H</u>$_3$ rotamers] 24.9, 25.0 [<u>C</u>H_2 ring ACPC rotamers] 27.1, 27.8 [<u>C</u>H_2 ring ACPC rotamers] 31.3, 32.4 [<u>C</u>H_2 ring ACPC rotamers] 46.3, 47.9

[C<u>H</u>CO$_2$Et rotamers] 55.8, 56.6 [C<u>H</u>NH rotamers] 58.7 [C<u>H</u>Ph] 59.9, 60.2 [C<u>H$_2$</u>O rotamers] 126.5-128.3 [<u>C</u>H Phenyl] 145.8, 146.0 [<u>C</u> Phenyl] 174.3, 174.9 [<u>C</u>O rotamers].

cis-(±)-2-Aminocyclopentane carboxylic acid hydrochloride (79)

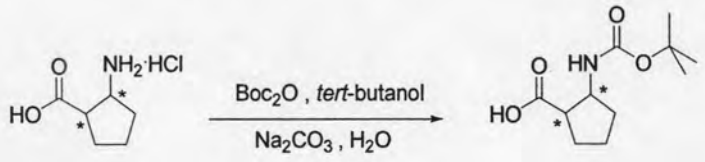


Both *cis*-isomer **78** → Both *cis*-isomer **79**

Synthesis of the title compound **79** was accomplished in the same way as described for compound **75** above. Starting from Ethyl (±)-2-[(1′*R*)-phenylethyl]-aminocyclopentane carboxylate (**78**) (2.17 g, 8.3 mmol), palladium on charcoal (0.21 g, 0.83 mmol) in methanol (10 mL) with hydrogen balloon afforded the intermediate free amine and then hydrolysis with 5% HCl solution (20 mL) to give compound **79** (1.10 g, 99% yield), as a white solid.

^{1}H NMR (400 MHz, D_2O) (Figure A-145) δ_H 1.55-1.86 [2×m, 4H, C<u>H$_2$</u>×2 ring ACPC] 1.97-2.04 [m, 2H, C<u>H$_2$</u> ring ACPC] 2.98-3.03 [m, 1H, C<u>H</u>CO$_2$H] 3.69-3.76 [m, 1H, C<u>H</u>NH]; ^{13}C NMR (100 MHz, D_2O) (Figure A-146) δ_C 22.5 [<u>C</u>H$_2$ ring ACPC] 28.4 [<u>C</u>H$_2$ ring ACPC] 30.2 [<u>C</u>H$_2$ ring ACPC] 48.0 [C<u>H</u>CO$_2$Et] 53.7 [C<u>H</u>NH] 176.8 [<u>C</u>O].

cis-(±)-2-(*N*- _tert_-Butoxycarbonyl)-aminocyclopentanecarboxylic acid (80)

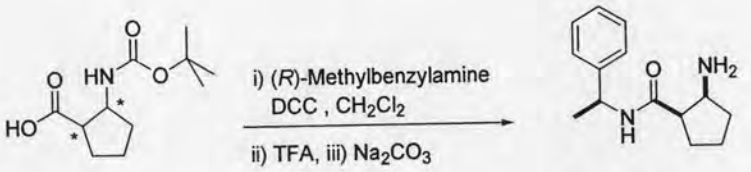


Both *cis*-isomer **79** → Both *cis*-isomer **80**

The compound **79** (1.86 g, 11.25 mmol) was dissolved in water (10 mL) and the solution was basified with Na_2CO_3 (4.20 g, 33.0 mmol). Boc_2O (2.951 g, 13.5 mmol) which dissolved in *tert*-butanol (5 mL) was slowly add to the solution mixture. The reaction was allowed to stir for 8 h and then extracted with ether. The aqueous residue was acidified with 5% hydrochloric acid and the mixture was extracted with ethyl acetate (20 mL × 3). The combined organic layer was dried over sodium sulfate and evaporated by rotary evaporation provided the pale-yellow solid **80** (2.17 g, 84% yield).

^{1}H NMR (400 MHz, $CDCl_3$) (Figure A-147) δ_H 1.40, 1.44 [2×s, 3H, C<u>H</u>$_3$ Boc rotamers] 1.60-1.72, 1.83-2.05 [2×m, 6H, 3×C<u>H</u>$_2$ ring ACPC] 3.00-3.11 [m, 1H, C<u>H</u>CO$_2$H] 4.03-4.25 [m, 1H, C<u>H</u>NH]; ^{13}C NMR (100 MHz, $CDCl_3$) (Figure A-148) δ_C 19.7, 21.5 [<u>C</u>H$_2$ ring ACPC rotamers] 24.7 [<u>C</u>H$_2$ ring ACPC] 25.7 [<u>C</u>H$_3$ Boc] 27.1 [<u>C</u>H$_2$ ring ACPC] 46.5, 46.9 [<u>C</u>HCO$_2$H rotamers] 57.9 [<u>C</u>HNH] 76.8 [<u>C</u>CH$_3$] 152.8 [<u>C</u>O Boc] 176.7 [<u>C</u>O$_2$H].

(1*R*,2*S*)-2-aminocyclopentanecarboxylic methylbenzyl ester (81)



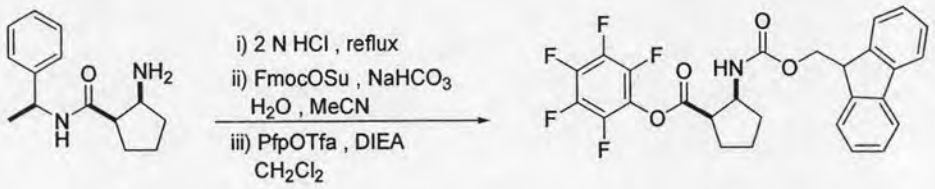
Both *cis*-isomer **80** → (1*R*,2*S*)-isomer **81**

To a stirred solution of compound **80** (1.49 g, 6.11 mmol) in dichloromethane (10 mL) was added (*R*)-(+)-α-methylbenzylamine (0.93 mL, 7.34 mmol) and EDC·HCl (1.41 mL, 7.34 mmol). The reaction mixture was stirred at 30 °C for 2 h and crude mixture was diluted with dichloromethane and extracted with water. The organic residue was concentrated under reduced pressure and treated with TFA (2 mL) in dichloromethane (2 mL). The reaction was allowed to stir for 2 h and then Na_2CO_3 solution was added to neutralize the mixture. The mixture was extracted with ethyl acetate and the organic layer was concentrated and purified by column

chromatography eluting with methanol:ethyl acetate 1:10 to afford the enantiopure product **81** as a white solid. (0.17 g, 48% yield, 2 steps)

^{1}H NMR (400 MHz, $CDCl_3$) (Figure A-149) δ_H 1.47-1.49 [m, 3H, C<u>H</u>$_3$] 1.50-1.55 [m, 2H, C<u>H</u>$_2$ ring ACPC] 1.85-1.95 [m, 4H, 2×C<u>H</u>$_2$ ring ACPC] 2.57-2.61 [m, 1H, C<u>H</u>Ph] 3.46-3.48 [m, 1H, C<u>H</u>NH] 5.09-5.13 [m, 1H, C<u>H</u>CO_2Et] 7.25-7.33 [m, 5H, C<u>H</u> Ar]; ^{13}C NMR (100 MHz, $CDCl_3$) (Figure A-150) δ_C 22.0 [<u>C</u>H_2 ring ACPC] 22.2 [<u>C</u>H_3] 27.1 [<u>C</u>H_2 ring ACPC] 34.6 [<u>C</u>H_2 ring ACPC] 48.6 [<u>C</u>HCO_2Et rotamers] 49.8 [<u>C</u>HNH] 54.7 [<u>C</u>HPh] 126.1-128.6 [<u>C</u>H Phenyl] 143.8 [<u>C</u> Phenyl] 173.0 [<u>C</u>O rotamers].

(1*R*,2*S*)-2-(*N*-fluoren-9-ylmethoxycarbonyl)-aminocyclopentanecarboxylic pentafluorophenyl ester (82)



81 **82**

Synthesis of the title compound **82** was accomplished in the same way as described for compound **77** above. Starting from compound **81**, 2 N HCl (5 mL), FmoOSu (0.21 g, 0.61 mmol), $NaHCO_3$ (0.15 g, 1.81 mmol), PfpOTfa (30 μL, 0.15 mmol), DIEA (22 μL, 0.15 mmol) to give the compound **82** as a whit solid (0.14 g, 74% yield, 3 steps)

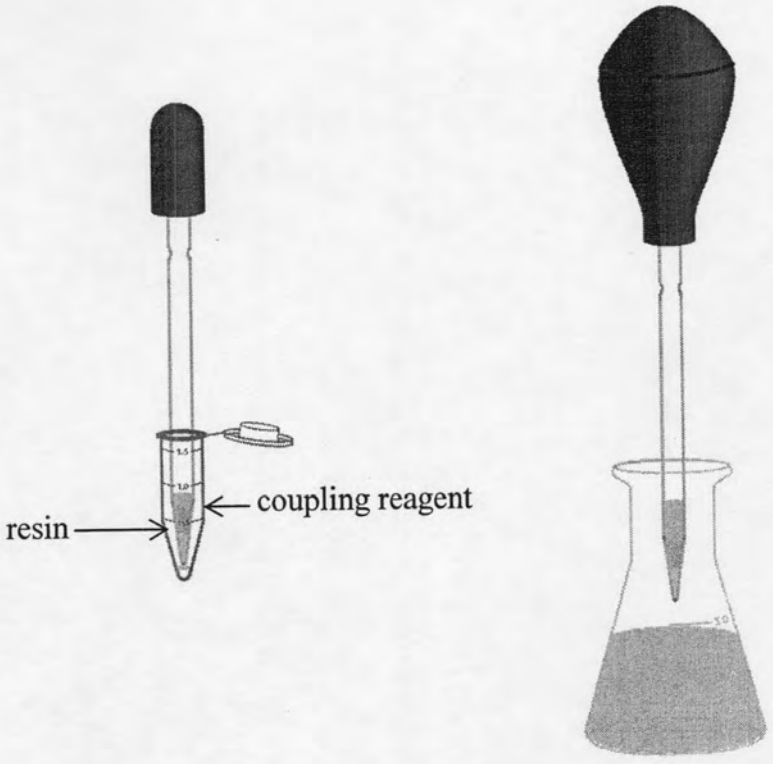
^{1}H NMR (400 MHz, $CDCl_3$) δ_H 1.72-2.20 [3×m, 6H, C<u>H</u>$_2$(3′), C<u>H</u>$_2$(4′) and C<u>H</u>$_2$(5′)] 3.50 [dd, J = 7.1, 13.8 Hz, 1H, C<u>H</u>NH] 4.24 [t, J = 6.7 Hz, 1H, C<u>H</u> Fmoc] 4.43 [d, 2H, J = 7.0 Hz, C<u>H</u>$_2$ Fmoc] 4.50 [m, 1H, C<u>H</u>CO_2Pfp] 5.18 [d, 1H, J = 8.8 Hz, N<u>H</u>] 7.33 [t, J = 6.8 Hz, 2H, C<u>H</u> Fmoc Ar] 7.43 [t, J = 6.8 Hz, 2H, C<u>H</u> Fmoc Ar] 7.60-7.63 [m, 2H, C<u>H</u> Fmoc Ar] 7.79 [d, J = 7.6 Hz, 2H, C<u>H</u> Fmoc Ar]; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 21.8 [CH_2(4′)] 27.9 [<u>C</u>H_2(3′)] 31.6 [<u>C</u>H_2(5′)] 46.5 [<u>C</u>HCO_2Pfp] 47.1 [<u>C</u>H Fmoc aliphatic] 54.5 [<u>C</u>HNH] 67.0 [<u>C</u>H_2 Fmoc] 120.1, 125.2, 127.2 and 127.9 [<u>C</u>H

Fmoc Ar] 136.8, 139.5, 140.5 and 142.3 [4×C-F] 141.5 and 144.1 [C Fmoc Ar] 156.2 [CO Fmoc] 170.6 [CO_2Pfp]; Anal Calcd. for $C_{26}H_{19}N_2O_4F_5$ requires 62.93; H, 3.86; N, 2.75%; Found C, 62.68; H, 3.92; N, 2.67 %; $[\alpha]^{25}_D$ = -66.5 (c = 0.50 g/100 mL, $CHCl_3$); mp = 110-112 °C (dec.).

2.4 Solid phase synthesis of PNA

2.4.1 Preparation of the reaction pipette and apparatus for solid phase synthesis

All peptide syntheses were carried out using a custom-made peptide synthesis column equipped with fritted glass as described below. A trimmed glass Pasteur pipette was plugged with a small amount of glass powder and sintered on a small flame. The length of the sintered glass should be around 3-5 mm. The resin was weighed accurately into the pipette and the pipette was equipped with a rubber teat. The resin in the pipette should be swollen in the required solvent at least 1 h before use. For each reactions, the reagent was directly sucked in, ejected out or hold on by manual control for the specified period of time. Occasional agitation may be performed using this device under manual control. All washings were performed by filling the solvent *via* the top of the pipette. The excess solvent was ejected out by squeezing the rubber teat as shown in **Figure 2.1**.



(a) (b)

Figure 2.1 A diagram showing the manual technique for solid phase peptide synthesis; (a) coupling, deprotection and cleaving process; (b) washing process.

2.4.2 Synthesis of diastereomer pyrrolidinyl with D-APC PNA

All synthesized PNA were named as the code name as shown in **Table 2.1** and abbreviation of the PNA in this research using an amino acid nomenclature; such as PNA that containing of *cis*-D isomer of pyrrolidine monomer of thymine with D-APC spacer five unit which have Fmoc group at *N*-terminal and $LysNH_2$ at the C-terminal can be called as *cis*-D/D-APC Fmoc-T_5-$LysNH_2$.

Table 2.1 All synthesized PNA sequences.

Code	Isomer	Spacer	N-Ter	Base Sequence	C-Ter
P1	CD	D-APC	Fmoc	TTTTT	$LysNH_2$
P2	TD	D-APC	Fmoc	TTTTT	$LysNH_2$
P3	TL	D-APC	Fmoc	TTTTT	$LysNH_2$
P4	CL	D-APC	Fmoc	TTTTT	$LysNH_2$
P5	CD	L-APC	Fmoc	TTTTT	$LysNH_2$
P6	CD	(1*S*,2*S*)-ACPC	H	TTTTT	$LysNH_2$
P7	CD	(1*R*,2*R*)-ACPC	H	TTTTT	$LysNH_2$
P8	CD	(1*S*,2*R*)-ACPC	H	TTTTT	$LysNH_2$
P9	CD	(1*R*,2*S*)-ACPC	H	TTTTT	$LysNH_2$
P10	CD	*N*-Spacer	Ac	TTTTT	$LysNH_2$
P11	CD	*O*-Spacer	Ac	TTTTT	$LysNH_2$
P12	CD	D-APC	Ac	AAAAA	$LysNH_2$
P13	CD	D-APC	Ac	TTTTTTT	$LysNH_2$
P14	CD	D-APC	Ac	TTTATTT	$LysNH_2$
P15	CD	D-APC	Ac	TTTCTTT	$LysNH_2$
P16	CD	D-APC	Ac	TTTGTTT	$LysNH_2$
P17	CD	D-APC	Ac	TTTTTTTTT	$LysNH_2$
P18	CD	D-APC	Ac	TTTTATTTT	$LysNH_2$
P19	CD	D-APC	Ac	TTTTCTTTT	$LysNH_2$
P20	CD	D-APC	Ac	TTTTGTTTT	$LysNH_2$
P21	CD	D-APC	Ac	TTTTATAT	$LysNH_2$
P22	CD	D-APC	Ac	TATATTTT	$LysNH_2$
P23	CD	D-APC	Ac	TTTTATA	Lys(FAM)-NH_2
P24	CD	D-APC	Ac-Ser	TTTTTTTTT	Lys(F)-$SerNH_2$
P25	CD	D-APC	Ac-Ser-Lys(F)	TTTTTTTTT	$SerNH_2$
P26	CD	(1*S*,2*S*)-ACPC	Ac	TTTTTTTTT	$LysNH_2$

2.4.2.1 Solid Phase Peptide Synthesis of *cis*-D/D-APC Fmoc-T_5-LysNH_2 (P1)

Synthesis of this PNA (P1) was carried out on 4.0 μmol scale. The synthesis was divided into steps as follows.

i Removing Fmoc protecting group form the resin

A reaction pipette prepared as described above was loaded with TentaGel S RAM Fmoc resin (15.6 mg, 4.0 μmol). The resin was treated with 20% piperidine in DMF (piperidine 50 μL and DMF 200 μL) in a 1.5 mL eppendorf tube at 30 °C for 15 min occasional agitation. After the specified period of time, the reagent was squeezed off and the reaction column was washed exhaustively with DMF.

For NovaSyn TGR resin which contains free NH_2 groups, this step can be skipped.

ii Anchoring with the first amino acid (Lys) residue

Fmoc-L-Lys(Boc)-OPfp (25.4 mg, 40 μmol) and HOAt (2.0 mg, 10 μmol) were dissolved in anhydrous DMF (30 μL) in a 1.5 mL eppendorf tube. Then DIEA (3.4 μL, 20 μmol) was added in this mixture. The prepared resin was soaked in this solution with occasional agitation at 30 °C for 1 h. After the specified period of time, the reagent was squeezed off and the reaction column was washed exhaustively with DMF.

iii Deprotection of the Fmoc protection group at *N*-terminal

After the coupling was completed, the resin was treated with 20% piperidine in DMF (piperidine 50 μL and DMF 200 μL) in a 1.5 mL eppendorf tube at 30 °C for 15 min occasional agitation. After the specified period of time, the reagent was squeezed off and the reaction column was washed exhaustively with DMF. The used deprotecting reagent can be used to determine the coupling efficiency by diluting with an appropriate volume of methanol (2 mL) and then the UV-absorbance of dibenzofulvene-piperidine adduct at 264 nm was measured. The first UV-absorbance

of the adduct, released from preloaded Fmoc-L-Lys(Boc)-resin, was assumed to be 100%. Such determination of coupling efficiency was advantageous in terms of determining how the solid phase reaction progress. The efficiency should be >95% for each step in order to give acceptable yield of the pentamer PNA (**P1**) from the synthesis. If the overall efficiency had dropped below 50%, the coupling must be stopped to save the valuable monomers.

iv Coulping with pyrrolidinyl monomer

The free amino group, generated from the deprotection step (iii) above, was further coupled with pyrrolidine thymine monomer. Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**) (7.5 mg, 16.0 μmol), HOAt (2.0 mg, 16.0 μmol) (for activated Pfp ester monomer) or HATU (2.0 mg, 16.0 μmol)/DIEA (5.6 μL, 32 μmol) (for Fmoc free acid monomer) were dissolved in anhydrous DMF (30 μL) in a 1.5 mL eppendorf tube. The reaction pipette was treated with this solution at 30 °C for 30 min with occasional agitation. After the specified period of time, the reagent was squeezed off and the reaction column was washed exhaustively with DMF.

v Coulping with D-APC spacer monomer

In the same way as described for (iv), D-APC-spacer monomer (**55**) (8.5 mg, 16 μmol) was coupled next to pyrrolidinyl T monomer. This constituted one unit of *cis*-D/D-APC PNA. Alternate couplings of the pyrrolidinyl monomer and D-APC spacer were performed in the subsequent steps until the complete sequence was obtained.

vi End capping

After anchoring or coupling step, the remaining free amino residue was capped with 10% lauroyl chloride/DIEA in anhydrous DMF (lauroyl chloride 5 μL, DIEA 5 μL and DMF 40 μL) in a 1.5 mL eppendorf tube to prevent formation of deletion sequences. The reaction pipette was occasionally agitated with this solution

at 30 °C for 10 min. After the specified period of time, the reagent was squeezed off and the reaction column was washed exhaustively with DMF.

After that, the next cycle (deprotection, coupling and capping) were carried out with the same method until the resin bound peptide had been extended up to desired pentamer.

vii Acetylation at *N*-terminal of pentamer PNA (P1)

The synthesis cycle was repeated until the growing peptide chain was extended up to pentamer. After final cleavage of Fmoc, the pentamer PNA (**P1**) was treated with 10 % Ac_2O/DIEA in anhydrous DMF (Ac_2O 5 μL, DIEA 5 μL and DMF 40 μL) in a 1.5 mL eppendorf tube. The reaction pipette was occasionally agitated with this solution at 30 °C for 15 min. After the specified period of time, the reagent was squeezed off and the reaction column was washed exhaustively with DMF.

For this PNA synthesis which had moderate efficiency yield, the final cleavage of Fmoc and acetylation at *N*-terminal can be skipped to leave the Fmoc group on the *N*-terminal for facilitating subsequent purification steps.

viii Method for cleavage the pentamer PNA (P1) from the resin

The resin bound PNA pentamer was released from the resin by treatment with trifluoroacetic acid (0.5 mL) at 30 °C for 2 h with occasional agitation. During the time, the resin becomes red. After the specified period of time, the trifluoroacetic acid was removed by a gently nitrogen stream (in fume hood).The cleavage was repeated again with another 0.5 mL of fresh TFA to ensure a complete cleavage of the peptide from the resin. The sticky residue was treated with diethyl ether (1 mL) to precipitate the crude PNA. The suspension was centrifuged and decanted. The crude peptide was centrifugally washed with diethyl ether 3 times. Finally the crude peptide was air dried at 30 °C and stored dried at -20 °C until used.

ix Purification and Characterization

The crude peptide was prepared for HPLC analysis by dissolving a mixture in 200 μL deionized water. The solution was filtered through a nylon membrane filter (0.45 μm). Analysis and purification was performed by reverse phase HPLC, monitoring by UV-absorbance at 260 nm and eluting with a gradient system of 0.01% TFA in acetonitrile/water. Conditions for HPLC gradient system;

Solvent A = 0.01% trifluoroacetic acid in acetonitrile

Solvent B = 0.01% trifluoroacetic acid in milliQ water

First A:B (10:90) for 5 min then linear gradient to A:B (90:10) over a period of 25 min then hold on for 5 min before revert back to A:B (10:90) in a period of 10 min.

Table 2.2 Gradient system for HPLC analysis of PNA.

Time (min)	Flow Rate (mL/min)	Solvent A (%)	Solvent B (%)
0	0.5	10	90
5	0.5	10	90
30	0.5	90	10
35	0.5	90	10
45	0.5	10	90
60	0.5	10	90

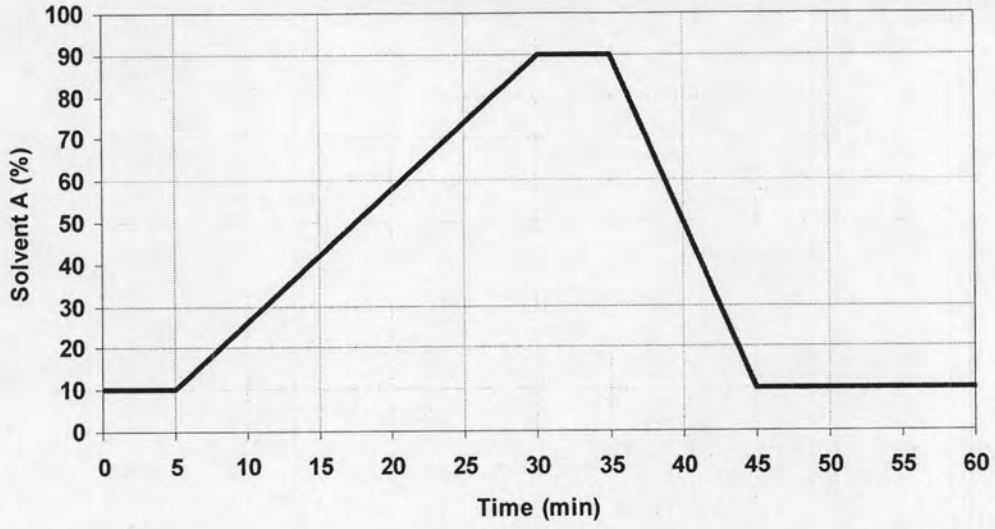


Figure 2.2 Diagram of gradient solvent in HPLC analysis of PNA condition-I.

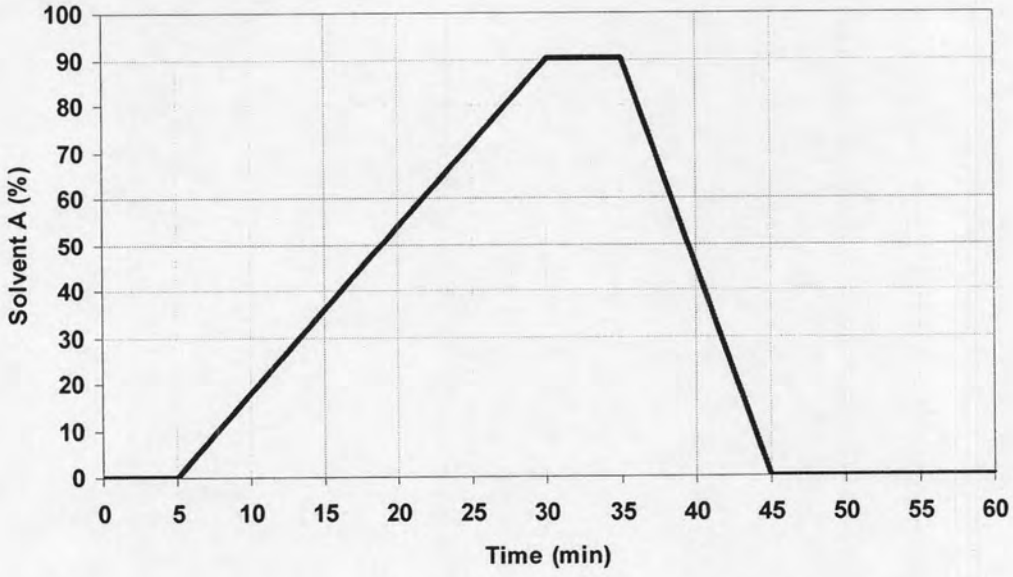


Figure 2.3 Diagram of gradient solvent in HPLC analysis of PNA condition-II (For PNA sequence which has any base A C or G).

After purification by HPLC, the peak of *cis*-D/D-APC Fmoc-T_5-LysNH_2 (**P1**) appeared at t_R = 22.283 min. The major product was collected and confirmed by MALDI-TOF mass spectrometry. MALDI-TOF $M{\cdot}H^+_{obs}$ = 2036.989; $M{\cdot}H^+_{calcd}$ = 2035.996.

2.4.2.2 Solid Phase Peptide Synthesis of *trans*-D/D-APC Fmoc-T_5-LysNH_2 (P2)

Synthesis of *trans*-D/D-APC Fmoc-T_5-LysNH_2 (**P2**) was accomplished in the same way as described for *cis*-D/D-APC Fmoc-T_5-LysNH_2 (**P1**) above. Starting from TentaGel S RAM Fmoc resin (13.7 mg, 4.0 μmol) and monomers as in following Table,

Cycle	Monomers	Amount
1	Fmoc-D-Pro-(*trans*-4-T)-OPfp (**22**)	8.1 mg (16 μmol)
2	Fmoc-D-APC-OPfp (**55**)	8.7 mg (16 μmol)
3	Fmoc-D-Pro-(*trans*-4-T)-OPfp (**22**)	8.4 mg (16 μmol)
4	Fmoc-D-APC-OPfp (**55**)	8.7 mg (16 μmol)
5	Fmoc-D-Pro-(*trans*-4-T)-OPfp (**22**)	7.4 mg (16 μmol)
6	Fmoc-D-APC-OPfp (**55**)	8.4 mg (16 μmol)
7	Fmoc-D-Pro-(*trans*-4-T)-OPfp (**22**)	7.6 mg (16 μmol)
8	Fmoc-D-APC-OPfp (**55**)	8.4 mg (16 μmol)
9	Fmoc-D-Pro-(*trans*-4-T)-OPfp (**22**)	7.6 mg (16 μmol)
10	Fmoc-D-APC-OPfp (**55**)	8.7 mg (16 μmol)

The monomers were used in each coupling cycle respectively. After cleavage from resin and purification by reverse phase HPLC, the chromatogram of (**P2**) appeared at t_R = 23.230 min. MALDI-TOF mass spectrum showed $M{\cdot}H^+_{obs}$ = 2036.197; $M{\cdot}H^+_{calcd}$ = 2035.996.

2.4.2.3 Solid Phase Peptide Synthesis of *trans*-L/D-APC Fmoc-T_5-LysNH_2 (P3)

Synthesis of *trans*-L/D-APC Fmoc-T_5-LysNH_2 (**P3**) was accomplished in the same way as described for *cis*-D/D-APC Fmoc-T_5-LysNH_2 (**P1**) above. Starting from TentaGel S RAM Fmoc resin (13.8 mg, 4.0 μmol) and monomers as in following Table,

Cycle	Monomers	Amount
1	Fmoc-L-Pro-(*trans*-4-T)-OPfp (**24**)	7.5 mg (16 μmol)
2	Fmoc-D-APC-OPfp (**55**)	8.5 mg (16 μmol)
3	Fmoc-L-Pro-(*trans*-4-T)-OPfp (**24**)	7.6 mg (16 μmol)
4	Fmoc-D-APC-OPfp (**55**)	8.4 mg (16 μmol)
5	Fmoc-L-Pro-(*trans*-4-T)-OPfp (**24**)	7.7 mg (16 μmol)
6	Fmoc-D-APC-OPfp (**55**)	8.6 mg (16 μmol)
7	Fmoc-L-Pro-(*trans*-4-T)-OPfp (**24**)	7.9 mg (16 μmol)
8	Fmoc-D-APC-OPfp (**55**)	8.4 mg (16 μmol)
9	Fmoc-L-Pro-(*trans*-4-T)-OPfp (**24**)	7.7 mg (16 μmol)
10	Fmoc-D-APC-OPfp (**55**)	8.3 mg (16 μmol)

The monomers were used in each coupling cycle respectively. After cleavage from resin and purification by reverse phase HPLC, the chromatogram of (**P3**) appeared at t_R = 22.264 min. MALDI-TOF mass spectrum showed $M{\cdot}H^+_{obs}$ = 2034.432; $M{\cdot}H^+_{calcd}$ = 2035.996.

2.4.2.4 Solid Phase Peptide Synthesis of *cis*-L/D-APC Fmoc-T_5-LysNH_2 (P4)

Synthesis of *cis*-L/D-APC Fmoc-T_5-LysNH_2 (**P4**) was accomplished in the same way as described for *cis*-D/D-APC Fmoc-T_5-LysNH_2 (**P1**) above. Starting from TentaGel S RAM Fmoc resin (14.1 mg, 4.0 μmol) and monomers as in following Table,

Cycle	Monomers	Amount
1	Fmoc-L-Pro-(*cis*-4-T)-OPfp (**23**)	7.7 mg (16 μmol)
2	Fmoc-D-APC-OPfp (**55**)	9.5 mg (16 μmol)
3	Fmoc-L-Pro-(*cis*-4-T)-OPfp (**23**)	8.4 mg (16 μmol)
4	Fmoc-D-APC-OPfp (**55**)	8.9 mg (16 μmol)
5	Fmoc-L-Pro-(*cis*-4-T)-OPfp (**23**)	8.6 mg (16 μmol)
6	Fmoc-D-APC-OPfp (**55**)	9.0 mg (16 μmol)
7	Fmoc-L-Pro-(*cis*-4-T)-OPfp (**23**)	8.4 mg (16 μmol)
8	Fmoc-D-APC-OPfp (**55**)	8.5 mg (16 μmol)
9	Fmoc-L-Pro-(*cis*-4-T)-OPfp (**23**)	8.5 mg (16 μmol)
10	Fmoc-D-APC-OPfp (**55**)	8.5 mg (16 μmol)

The monomers were used in each coupling cycle respectively. After cleavage from resin and purification by reverse phase HPLC, the chromatogram of (**P4**) appeared at t_R = 23.032 min. MALDI-TOF mass spectrum showed $M{\cdot}H^+_{obs}$ = 2036.003; $M{\cdot}H^+_{calcd}$ = 2035.996.

2.4.3 Synthesis of *cis*-D pyrrolidinyl with various spacers

2.4.3.1 Synthesis of *cis*-D/L-APC Fmoc-T_5-LysNH_2 (P5)

Synthesis of *cis*-D/L-APC Fmoc-T_5-LysNH_2 (**P5**) was accomplished in the same way as described for *cis*-D/D-APC Fmoc-T_5-LysNH_2 (**P1**) above. Starting from TentaGel S RAM Fmoc resin (14.0 mg, 4.0 μmol) and monomers as in following Table,

Cycle	Monomers	Amount
1	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	10.0 mg (16 μmol)
2	Fmoc-L-APC-OPfp (**63**)	8.2 mg (16 μmol)
3	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	10.2 mg (16 μmol)
4	Fmoc-L-APC-OPfp (**63**)	8.3 mg (16 μmol)
5	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	10.9 mg (16 μmol)
6	Fmoc-L-APC-OPfp (**63**)	7.4 mg (16 μmol)
7	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	10.2 mg (16 μmol)
8	Fmoc-L-APC-OPfp (**63**)	7.3 mg (16 μmol)
9	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	10.1 mg (16 μmol)
10	Fmoc-L-APC-OPfp (**63**)	6.0 mg (16 μmol)

The monomers were used in each coupling cycle respectively. After cleavage from resin and purification by reverse phase HPLC, the chromatogram of (**P5**) appeared at t_R = 21.715 min. MALDI-TOF mass spectrum showed $M{\cdot}H^+_{obs}$ = 2035.356; $M{\cdot}H^+_{calcd}$ = 2035.996.

2.4.3.2 Synthesis of *cis*-D/(1*S*,2*S*)-ACPC H-T_5-LysNH_2 (P6)

Synthesis of *cis*-D/(1*S*,2*S*)-ACPC H-T_5-LysNH_2 (**P6**) was accomplished in the same way as described for *cis*-D/D-APC Fmoc-T_5-LysNH_2 (**P1**) above. Starting from TentaGel S RAM Fmoc resin (19.9 mg, 4.0 μmol) and monomers as in following Table,

Cycle	Monomers	Amount
1	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	7.7 mg (16 μmol)
2	Fmoc-(1*S*,2*S*)-ACPC-OPfp (**68**)	6.1 mg (16 μmol)
3	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	7.9 mg (16 μmol)
4	Fmoc-(1*S*,2*S*)-ACPC-OPfp (**68**)	6.0 mg (16 μmol)
5	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	7.7 mg (16 μmol)
6	Fmoc-(1*S*,2*S*)-ACPC-OPfp (**68**)	6.3 mg (16 μmol)
7	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	7.7 mg (16 μmol)
8	Fmoc-(1*S*,2*S*)-ACPC-OPfp (**68**)	5.7 mg (16 μmol)
9	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	7.6 mg (16 μmol)
10	Fmoc-(1*S*,2*S*)-ACPC-OPfp (**68**)	6.0 mg (16 μmol)

The monomers were used in each coupling cycle respectively. After final cleavage of Fmoc, the pentamer PNA (**P6**) was not necessary to treat with 10 % Ac_2O/DIEA in anhydrous DMF (Ac_2O 5 μL, DIEA 5 μL and DMF 40 μL) in a 1.5 mL eppendorf tube. After cleavage from resin and purification by reverse phase HPLC, the chromatogram of (**P6**) appeared at t_R = 17.450 min. MALDI-TOF mass spectrum showed $M{\cdot}H^+_{obs}$ = 1809.032; $M{\cdot}H^+_{calcd}$ = 1808.996.

2.4.3.3 Synthesis of *cis*-D/(1*R*,2*R*)-ACPC H-T_5-LysNH_2 (P7)

Synthesis of *cis*-D/(1*R*,2*R*)-ACPC H-T_5-LysNH_2 (**P7**) was accomplished in the same way as described for *cis*-D/D-APC Fmoc-T_5-LysNH_2 (**P1**) above. Starting from TentaGel S RAM Fmoc resin (14.4 mg, 4.0 μmol) and monomers as in following Table,

Cycle	Monomers	Amount
1	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	7.5 mg (16 μmol)
2	Fmoc-(1*R*,2*R*)-ACPC-OPfp (**73**)	6.3 mg (16 μmol)
3	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	7.7 mg (16 μmol)
4	Fmoc-(1*R*,2*R*)-ACPC-OPfp (**73**)	7.3 mg (16 μmol)
5	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	8.1 mg (16 μmol)
6	Fmoc-(1*R*,2*R*)-ACPC-OPfp (**73**)	6.1 mg (16 μmol)
7	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	9.2 mg (16 μmol)
8	Fmoc-(1*R*,2*R*)-ACPC-OPfp (**73**)	5.7 mg (16 μmol)
9	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	7.9 mg (16 μmol)
10	Fmoc-(1*R*,2*R*)-ACPC-OPfp (**73**)	6.3 mg (16 μmol)

The monomers were used in each coupling cycle respectively. After final cleavage of Fmoc, the pentamer PNA (**P7**) was not necessary to treat with 10 % Ac_2O/DIEA in anhydrous DMF (Ac_2O 5 μL, DIEA 5 μL and DMF 40 μL) in a 1.5 mL eppendorf tube. After cleavage from resin and purification by reverse phase HPLC, the chromatogram of (**P7**) appeared at t_R = 27.202 min. MALDI-TOF mass spectrum showed $M{\cdot}H^+_{obs}$ = 1807.871; $M{\cdot}H^+_{calcd}$ = 1808.996.

2.4.3.4 Synthesis of *cis*-D/(1*S*,2*R*)-ACPC H-T_5-LysNH_2 (P8)

Synthesis of *cis*-D/(1*S*,2*R*)-ACPC H-T_5-LysNH_2 (**P8**) was accomplished in the same way as described for *cis*-D/D-APC Fmoc-T_5-LysNH_2 (**P1**) above. Starting from TentaGel S RAM Fmoc resin (14.0 mg, 4.0 μmol) and monomers as in following Table,

Cycle	Monomers	Amount
1	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	12.8 mg (16 μmol)
2	Fmoc-(1*S*,2*R*)-ACPC-OPfp (**77**)	9.5 mg (16 μmol)
3	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	11.2 mg (16 μmol)
4	Fmoc-(1*S*,2*R*)-ACPC-OPfp (**77**)	9.8 mg (16 μmol)
5	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	12.2 mg (16 μmol)
6	Fmoc-(1*S*,2*R*)-ACPC-OPfp (**77**)	9.0 mg (16 μmol)
7	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	11.0 mg (16 μmol)
8	Fmoc-(1*S*,2*R*)-ACPC-OPfp (**77**)	8.3 mg (16 μmol)
9	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	10.2 mg (16 μmol)
10	Fmoc-(1*S*,2*R*)-ACPC-OPfp (**77**)	8.8 mg (16 μmol)

The monomers were used in each coupling cycle respectively. After final cleavage of Fmoc, the pentamer PNA (**P8**) was not necessary to treat with 10 % Ac_2O/DIEA in anhydrous DMF (Ac_2O 5 μL, DIEA 5 μL and DMF 40 μL) in a 1.5 mL eppendorf tube. After cleavage from resin and purification by reverse phase HPLC, the chromatogram of (**P8**) appeared at t_R = 27.917 min. MALDI-TOF mass spectrum showed $M{\cdot}H^+_{obs}$ = 1807.900; $M{\cdot}H^+_{calcd}$ = 1808.996.

2.4.3.5 Synthesis of *cis*-D/(1*R*,2*S*)-ACPC H-T_5-LysNH_2 (P9)

Synthesis of *cis*-D/(1*R*,2*S*)-ACPC H-T_5-LysNH_2 (**P9**) was accomplished in the same way as described for *cis*-D/D-APC Fmoc-T_5-LysNH_2 (**P1**) above. Starting from TentaGel S RAM Fmoc resin (9.2 mg, 2.5 μmol) and monomers as in following Table,

Cycle	Monomers	Amount
1	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	6.6 mg (10 μmol)
2	Fmoc-(1*R*,2*S*)-ACPC-OPfp (**82**)	5.4 mg (10 μmol)
3	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	6.4 mg (10 μmol)
4	Fmoc-(1*R*,2*S*)-ACPC-OPfp (**82**)	6.0 mg (10 μmol)
5	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	8.7 mg (10 μmol)
6	Fmoc-(1*R*,2*S*)-ACPC-OPfp (**82**)	5.1 mg (10 μmol)
7	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	6.6 mg (10 μmol)
8	Fmoc-(1*R*,2*S*)-ACPC-OPfp (**82**)	5.2 mg (10 μmol)
9	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	8.8 mg (10 μmol)
10	Fmoc-(1*R*,2*S*)-ACPC-OPfp (**82**)	5.1 mg (10 μmol)

The monomers were used in each coupling cycle respectively. After final cleavage of Fmoc, the pentamer PNA (**P9**) was not necessary to treat with 10 % Ac_2O/DIEA in anhydrous DMF (Ac_2O 5 μL, DIEA 5 μL and DMF 40 μL) in a 1.5 mL eppendorf tube. After cleavage from resin and purification by reverse phase HPLC, the chromatogram of (**P9**) appeared at t_R = 18.401 min. MALDI-TOF mass spectrum showed $M{\cdot}H^+_{obs}$ = 1806.381; $M{\cdot}H^+_{calcd}$ = 1808.996.

2.4.3.6 Synthesis of *cis*-D/*N*-spacer Ac-T_5-LysNH_2 (P10)

Synthesis of *cis*-D/*N*-spacer Ac-T_5-LysNH_2 (**P10**) was accomplished in the same way as described for *cis*-D/D-APC Fmoc-T_5-LysNH_2 (**P1**) above. Starting from TentaGel S RAM Fmoc resin (4.2 mg, 1.0 μmol) and monomers as in following Table,

Cycle	Monomers	Amount
1	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	2.9 mg (4 μmol)
2	Fmoc-*N*-spacer-OPfp (**47**)	2.1 mg (4 μmol)
3	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	3.0 mg (4 μmol)
4	Fmoc-*N*-spacer-OPfp (**47**)	2.1 mg (4 μmol)
5	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	2.5 mg (4 μmol)
6	Fmoc-*N*-spacer-OPfp (**47**)	2.1 mg (4 μmol)
7	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	2.9 mg (4 μmol)
8	Fmoc-*N*-spacer-OPfp (**47**)	2.1 mg (4 μmol)
9	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	2.5 mg (4 μmol)
10	Fmoc-*N*-spacer-OPfp (**47**)	2.1 mg (4 μmol)

The monomers were used in each coupling cycle respectively. After final cleavage of Fmoc, the pentamer PNA (**P10**) was treated with 10 % Ac_2O/DIEA in anhydrous DMF (Ac_2O 5 μL, DIEA 5 μL and DMF 40 μL) in a 1.5 mL eppendorf tube. After cleavage from resin and purification by reverse phase HPLC, the chromatogram of (**P10**) appeared at t_R = 21.215 min. MALDI-TOF mass spectrum showed $M{\cdot}H^+_{obs}$ = 1723.760; $M{\cdot}H^+_{calcd}$ = 1725.536.

2.4.3.7 Synthesis of *cis*-D/*O*-spacer Ac-T_5-LysNH_2 (P11)

Synthesis of *cis*-D/*O*-spacer Ac-T_5-LysNH_2 (**P11**) was accomplished in the same way as described for *cis*-D/D-APC Fmoc-T_5-LysNH_2 (**P1**) above. Starting from TentaGel S RAM Fmoc resin (4.2 mg, 1.0 μmol) and monomers as in following Table,

Cycle	Monomers	Amount
1	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	2.9 mg (4 μmol)
2	Fmoc-*O*-spacer-OPfp (**42**)	2.1 mg (4 μmol)
3	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	3.0 mg (4 μmol)
4	Fmoc-*O*-spacer-OPfp (**42**)	2.1 mg (4 μmol)
5	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	2.5 mg (4 μmol)
6	Fmoc-*O*-spacer-OPfp (**42**)	2.1 mg (4 μmol)
7	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	2.9 mg (4 μmol)
8	Fmoc-*O*-spacer-OPfp (**42**)	2.1 mg (4 μmol)
9	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	2.5 mg (4 μmol)
10	Fmoc-*O*-spacer-OPfp (**42**)	2.1 mg (4 μmol)

The monomers were used in each coupling cycle respectively. However, for this particular spacer, the efficiency of the coupling yield was dropped lower 70 % for each step, therefore PNA containing *O*-spacer cannot be synthesized using the standard solid-phase synthesis method.

2.4.4 Synthesis of *cis*-D/D-APC with mixed base sequences

2.4.4.1 Synthesis of *cis*-D/D-APC Ac-A_5-LysNH_2 (P12)

Synthesis of *cis*-D/D-APC Fmoc-A_5-LysNH_2 (**P12**) was accomplished in the same way as described for *cis*-D/D-APC Fmoc-T_5-LysNH_2 (**P1**) above. Starting from TentaGel S RAM Fmoc resin (9.8 mg, 2.5 μmol) and monomers as in following Table,

Cycle	Monomers	Amount
1	Fmoc-D-Pro-(*cis*-4-A^{Bz})-OPfp (**28**)	8.5 mg (10 μmol)
2	Fmoc-D-APC-OPfp (**55**)	5.1 mg (10 μmol)
3	Fmoc-D-Pro-(*cis*-4-A^{Bz})-OPfp (**28**)	7.5 mg (10 μmol)
4	Fmoc-D-APC-OPfp (**55**)	5.3 mg (10 μmol)
5	Fmoc-D-Pro-(*cis*-4-A^{Bz})-OPfp (**28**)	7.7 mg (10 μmol)
6	Fmoc-D-APC-OPfp (**55**)	5.2 mg (10 μmol)
7	Fmoc-D-Pro-(*cis*-4-A^{Bz})-OPfp (**28**)	7.7 mg (10 μmol)
8	Fmoc-D-APC-OPfp (**55**)	5.6 mg (10 μmol)
9	Fmoc-D-Pro-(*cis*-4-A^{Bz})-OPfp (**28**)	8.0 mg (10 μmol)
10	Fmoc-D-APC-OPfp (**55**)	5.4 mg (10 μmol)

The monomers were used in each coupling cycle respectively. After final cleavage of Fmoc, the pentamer PNA (**P12**) was treated with 10 % Ac_2O/DIEA in anhydrous DMF (Ac_2O 5 μL, DIEA 5 μL and DMF 40 μL) in a 1.5 mL eppendorf tube. Before cleavage (**P12**) from resin, the nucleobase protecting groups (Bz for A and C, Ibu for G) must be removed by treatment of the resin with aqueous ammonia/dioxane 1:1 at 55 °C for 6 h. After cleavage from resin and purification by reverse phase HPLC by changing the ratio of the A:B at start from (10:90) to (0:100) for 5 min then linear gradient to A:B (90:10) over a period of 25 min then hold on for 5 min before revert back to A:B (0:100) condition-II, the chromatogram of (**P12**) appeared at t_R = 20.674 min. MALDI-TOF mass spectrum showed $M{\cdot}H^+_{obs}$ = 1899.507; $M{\cdot}H^+_{calcd}$ = 1899.998.

2.4.4.2 Synthesis of *cis*-D/D-APC Ac-T_7-LysNH_2 (P13), *cis*-D/D-APC Ac-T_3AT_3-LysNH_2 (P14), *cis*-D/D-APC Ac-T_3CT_3-LysNH_2 (P15) and *cis*-D/D-APC Ac-T_3GT_3-LysNH_2 (P16)

Synthesis of (**P13**), (**P14**), (**P15**) and (**P16**) were accomplished in the same way as described for *cis*-D/D-APC Fmoc-T_5-LysNH_2 (**P1**) above. Starting from TentaGel S RAM resin (14.1 mg, 4 μmol) and monomers as following table

Cycle	Monomers	Amount
1	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	10.1 mg (16 μmol)
2	Fmoc-D-APC-OPfp (**55**)	8.4 mg (16 μmol)
3	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	10.9 mg (16 μmol)
4	Fmoc-D-APC-OPfp (**55**)	8.3 mg (16 μmol)
5	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	9.6 mg (16 μmol)
6	Fmoc-D-APC-OPfp (**55**)	9.6 mg (16 μmol)

These monomers were used in each coupling cycle respectively until a T_3 sequence was obtained. The resin was split to four parts and then further coupled with **21** (2.5 mg, 1.0 μmol), **28** (3.4 mg, 1.0 μmol), **31** (3.4 mg, 1.0 μmol) and **37** (3.4 mg, 1.0 μmol) in each column separately. Then the coupling was continued with monomer **55** (2.4 mg, 1.0 μmol) before **21** (2.5 mg, 1.0 μmol), alternately until the peptide had been extended up to heptamer. Final Fmoc removed and acetylation was performed as usual. In case of mixed-base, before cleavage (**P14**), (**P15**) and (**P16**) from resin, the nucleobase protecting group was removed as described above. After cleavage from resin and purification by reverse phase HPLC, the chromatogram of these four PNA appeared as shown in a table below.

No.	PNA sequences	t_R	$M{\cdot}H^+_{calcd}$	$M{\cdot}H^+_{obs}$
(**P13**)	*cis*-D/D-APC Ac-T_7-LysNH_2	20.664	2521.618	2520.605
(**P14**)	*cis*-D/D-APC Ac-T_3AT_3-LysNH_2	26.215	2530.638	2532.827
(**P15**)	*cis*-D/D-APC Ac-T_3CT_3-LysNH_2	26.537	2506.608	2547.717
(**P16**)	*cis*-D/D-APC Ac-T_3GT_3-LysNH_2	22.314	2546.638	2547.717

2.4.4.3 Synthesis of *cis*-D/D-APC Ac-T_9-LysNH_2 (P17)

Synthesis of *cis*-D/D-APC Ac-T_9-LysNH_2 (**P17**) was accomplished in the same way as described for *cis*-D/D-APC Ac-T_7-LysNH_2 (**P13**) above starting from TentaGel S RAM Fmoc resin (4.2 mg, 1.0 μmol). The monomers used in this PNA sequence as shown in following Table.

Cycle	Monomers	Amount
1	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	3.2 mg (4 μmol)
2	Fmoc-D-APC-OPfp (**55**)	2.0 mg (4 μmol)
3	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	2.8 mg (4 μmol)
4	Fmoc-D-APC-OPfp (**55**)	2.4 mg (4 μmol)
5	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	3.3 mg (4 μmol)
6	Fmoc-D-APC-OPfp (**55**)	3.0 mg (4 μmol)
7	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	2.7 mg (4 μmol)
8	Fmoc-D-APC-OPfp (**55**)	2.7 mg (4 μmol)
9	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	2.9 mg (4 μmol)
10	Fmoc-D-APC-OPfp (**55**)	2.7 mg (4 μmol)
11	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	3.1 mg (4 μmol)
12	Fmoc-D-APC-OPfp (**55**)	2.0 mg (4 μmol)
13	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	3.3 mg (4 μmol)
14	Fmoc-D-APC-OPfp (**55**)	2.0 mg (4 μmol)
15	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	2.8 mg (4 μmol)
16	Fmoc-D-APC-OPfp (**55**)	2.4 mg (4 μmol)
17	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	2.7 mg (4 μmol)
18	Fmoc-D-APC-OPfp (**55**)	2.6 mg (4 μmol)

After cleavage from resin and purification by reverse phase HPLC, the chromatogram of (**P17**) appeared at t_R = 21.701 min. MALDI-TOF mass spectrum showed $M{\cdot}H^+_{obs}$ = 3188.306; $M{\cdot}H^+_{calcd}$ = 3188.308.

2.4.4.4 Synthesis of *cis*-D/D-APC Ac-T_4AT_4-LysNH_2 (P18)

The synthesis of *cis*-D/D-APC Ac-T_4AT_4-LysNH_2 (**P18**) were accomplished in the same way as described for *cis*-D/D-APC Ac-T_3AT_3-LysNH_2 (**P14**) above starting from TentaGel S RAM Fmoc resin (4.7 mg, 1.0 μmol) and monomers using in this synthesis as following Table.

Cycle	Monomers	Amount
1	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	3.2 mg (4 μmol)
2	Fmoc-D-APC-OPfp (**55**)	2.2 mg (4 μmol)
3	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	2.5 mg (4 μmol)
4	Fmoc-D-APC-OPfp (**55**)	2.0 mg (4 μmol)
5	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	2.9 mg (4 μmol)
6	Fmoc-D-APC-OPfp (**55**)	3.8 mg (4 μmol)
7	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	3.3 mg (4 μmol)
8	Fmoc-D-APC-OPfp (**55**)	2.1 mg (4 μmol)
9	Fmoc-D-Pro-(*cis*-4-A^{Bz})-OPfp (**28**)	4.1 mg (4 μmol)
10	Fmoc-D-APC-OPfp (**55**)	2.0 mg (4 μmol)
11	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	2.8 mg (4 μmol)
12	Fmoc-D-APC-OPfp (**55**)	2.1 mg (4 μmol)
13	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	2.5 mg (4 μmol)
14	Fmoc-D-APC-OPfp (**55**)	1.9 mg (4 μmol)
15	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	2.8 mg (4 μmol)
16	Fmoc-D-APC-OPfp (**55**)	2.4 mg (4 μmol)
17	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	2.8 mg (4 μmol)
18	Fmoc-D-APC-OPfp (**55**)	2.6 mg (4 μmol)

Final Fmoc removal and acetylation was performing as usual. Before cleavage from resin, the nucleobase protecting group was removed as described above. After purification by HPLC (condition-II), the peak of (**P18**) appeared at t_R = 21.898 min. MALDI-TOF mass spectrum showed $M{\cdot}H^+_{obs}$ = 3196.980; $M{\cdot}H^+_{calcd}$ = 3197.318.

2.4.4.5 Synthesis of *cis*-D/D-APC Ac-T_4CT_4-$LysNH_2$ (P19)

The synthesis of *cis*-D/D-APC Ac-T_4CT_4-$LysNH_2$ (**P19**) was accomplished in the same way as described for *cis*-D/D-APC Ac-T_3CT_3-$LysNH_2$ (**P15**) above starting from TentaGel S RAM Fmoc resin (4.8 mg, 1.0 μmol). The monomers used in this PNA sequence as shown in following Table.

Cycle	Monomers	Amount
1	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	3.1 mg (4 μmol)
2	Fmoc-D-APC-OPfp (**55**)	2.3 mg (4 μmol)
3	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	3.1 mg (4 μmol)
4	Fmoc-D-APC-OPfp (**55**)	2.0 mg (4 μmol)
5	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	2.7 mg (4 μmol)
6	Fmoc-D-APC-OPfp (**55**)	2.3 mg (4 μmol)
7	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	2.6 mg (4 μmol)
8	Fmoc-D-APC-OPfp (**55**)	2.1 mg (4 μmol)
9	Fmoc-D-Pro-(*cis*-4-C^{Bz})-OPfp (**31**)	3.0 mg (4 μmol)
10	Fmoc-D-APC-OPfp (**55**)	2.1 mg (4 μmol)
11	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	2.6 mg (4 μmol)
12	Fmoc-D-APC-OPfp (**55**)	2.4 mg (4 μmol)
13	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	3.1 mg (4 μmol)
14	Fmoc-D-APC-OPfp (**55**)	2.4 mg (4 μmol)
15	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	3.0 mg (4 μmol)
16	Fmoc-D-APC-OPfp (**55**)	2.6 mg (4 μmol)
17	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	3.0 mg (4 μmol)
18	Fmoc-D-APC-OPfp (**55**)	2.6 mg (4 μmol)

Final Fmoc removal and acetylation was performing as usual. Before cleavage from resin, the nucleobase protecting group was removed as described above. After purification by HPLC (condition-II), the peak of (**P19**) appeared at t_R = 22.112 min. MALDI-TOF mass spectrum showed $M{\cdot}H^+_{obs}$ = 3173.013; $M{\cdot}H^+_{calcd}$ = 3173.298.

2.4.4.6 Synthesis of *cis*-D/D-APC Ac-T_4GT_4-$LysNH_2$ (P20)

The synthesis of *cis*-D/D-APC Ac-T_4GT_4-$LysNH_2$ (**P20**) was accomplished in the same way as described for *cis*-D/D-APC Ac-T_4GT_4-$LysNH_2$ (**P16**) above starting from TentaGel S RAM Fmoc resin (4.6 mg, 1.0 μmol). The monomers used in this PNA sequence as shown in following Table.

Cycle	Monomers	Amount
1	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	3.1 mg (4 μmol)
2	Fmoc-D-APC-OPfp (**55**)	2.1 mg (4 μmol)
3	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	2.7 mg (4 μmol)
4	Fmoc-D-APC-OPfp (**55**)	2.0 mg (4 μmol)
5	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	3.0 mg (4 μmol)
6	Fmoc-D-APC-OPfp (**55**)	2.1 mg (4 μmol)
7	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	2.5 mg (4 μmol)
8	Fmoc-D-APC-OPfp (**55**)	2.1 mg (4 μmol)
9	Fmoc-D-Pro-(*cis*-4-G^{Ibu})-OPfp (**37**)	3.9 mg (4 μmol)
10	Fmoc-D-APC-OPfp (**55**)	2.2 mg (4 μmol)
11	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	2.9 mg (4 μmol)
12	Fmoc-D-APC-OPfp (**55**)	2.2 mg (4 μmol)
13	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	2.6 mg (4 μmol)
14	Fmoc-D-APC-OPfp (**55**)	2.7 mg (4 μmol)
15	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	3.5 mg (4 μmol)
16	Fmoc-D-APC-OPfp (**55**)	3.3 mg (4 μmol)
17	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	2.7 mg (4 μmol)
18	Fmoc-D-APC-OPfp (**55**)	2.2 mg (4 μmol)

Final Fmoc removal and acetylation was performing as usual. Before cleavage from resin, the nucleobase protecting group was removed as described above. After purification by HPLC (condition-II), the peak of (**P20**) appeared at t_R = 22.126 min. MALDI-TOF mass spectrum showed $M{\cdot}H^+_{obs}$ = 3213.138; $M{\cdot}H^+_{calcd}$ = 3213.318.

2.4.4.7 Synthesis of *cis*-D/(1*S*,2*S*)-ACPC H-T_{10}-LysNH_2 (P26)

Synthesis of *cis*-D/(1*S*,2*S*)-ACPC H-T_{10}-LysNH_2 (**P26**) was accomplished in the same way as described for *cis*-D/(1*S*,2*S*)-ACPC Fmoc-T_5-LysNH_2 (**P6**) above. Starting from TentaGel S RAM Fmoc resin (14.1 mg, 4.0 μmol) and monomers as in following Table,

Cycle	Monomers	Amount
1	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	11.0 mg (16 μmol)
2	Fmoc-(1*S*,2*S*)-ACPC-OPfp (**68**)	8.4 mg (16 μmol)
3	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	10.2 mg (16 μmol)
4	Fmoc-(1*S*,2*S*)-ACPC-OPfp (**68**)	8.5 mg (16 μmol)
5	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	11.6 mg (16 μmol)
6	Fmoc-(1*S*,2*S*)-ACPC-OPfp (**68**)	8.4 mg (16 μmol)
7	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	10.1 mg (16 μmol)
8	Fmoc-(1*S*,2*S*)-ACPC-OPfp (**68**)	8.5 mg (16 μmol)
9	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	10.2 mg (16 μmol)
10	Fmoc-(1*S*,2*S*)-ACPC-OPfp (**68**)	9.2 mg (16 μmol)
11	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	10.0 mg (16 μmol)
12	Fmoc-(1*S*,2*S*)-ACPC-OPfp (**68**)	8.8 mg (16 μmol)
13	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	10.5 mg (16 μmol)
14	Fmoc-(1*S*,2*S*)-ACPC-OPfp (**68**)	8.9 mg (16 μmol)
15	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	11.1 mg (16 μmol)
16	Fmoc-(1*S*,2*S*)-ACPC-OPfp (**68**)	10.0 mg (16 μmol)
17	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	10.4 mg (16 μmol)
18	Fmoc-(1*S*,2*S*)-ACPC-OPfp (**68**)	9.7 mg (16 μmol)
19	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	10.3 mg (16 μmol)
20	Fmoc-(1*S*,2*S*)-ACPC-OPfp (**68**)	9.3 mg (16 μmol)

The monomers were used in each coupling cycle respectively. After final cleavage of Fmoc, the decamer PNA (**P26**) was not necessary to treat with 10 % Ac_2O/DIEA in anhydrous DMF (Ac_2O 5 μL, DIEA 5 μL and DMF 40 μL) in a 1.5

mL eppendorf tube. After purification by HPLC (condition-II), the peak of (**P26**) appeared at t_R = 25.868 min. MALDI-TOF mass spectrum showed $M{\cdot}H^+_{obs}$ = 3467.838; $M{\cdot}H^+_{calcd}$ = 3469.757.

2.4.5 Synthesis of unsymmetrical PNA sequences

2.4.5.1 Synthesis of *cis*-D/D-APC Ac-T_4ATAT-LysNH_2 (P21)

Synthesis of *cis*-D/D-APC Ac-T_4ATAT-LysNH_2 (**P21**) was accomplished in the same way as described for *cis*-D/D-APC Ac-T_7-LysNH_2 (**P13**) above. Starting from TentaGel S RAM Fmoc resin (4.4 mg, 1.0 μmol) and monomers using in this PNA synthesis as shown in following Table,

Cycle	Monomers	Amount
1	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	3.7 mg (4 μmol)
2	Fmoc-D-APC-OPfp (**55**)	2.1 mg (4 μmol)
3	Fmoc-D-Pro-(*cis*-4-A^{Bz})-OPfp (**28**)	3.6 mg (4 μmol)
4	Fmoc-D-APC-OPfp (**55**)	2.1 mg (4 μmol)
5	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	3.1 mg (4 μmol)
6	Fmoc-D-APC-OPfp (**55**)	2.1 mg (4 μmol)
7	Fmoc-D-Pro-(*cis*-4-A^{Bz})-OPfp (**28**)	3.0 mg (4 μmol)
8	Fmoc-D-APC-OPfp (**55**)	2.0 mg (4 μmol)
9	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	2.7 mg (4 μmol)
10	Fmoc-D-APC-OPfp (**55**)	2.0 mg (4 μmol)
11	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	2.5 mg (4 μmol)
12	Fmoc-D-APC-OPfp (**55**)	2.4 mg (4 μmol)
13	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	3.2 mg (4 μmol)
14	Fmoc-D-APC-OPfp (**55**)	2.1 mg (4 μmol)
15	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	2.9 mg (4 μmol)
16	Fmoc-D-APC-OPfp (**55**)	2.4 mg (4 μmol)

Final Fmoc removal and acetylation was performing as usual. Before cleavage from resin, the nucleobase protecting group was removed as described above.After cleavage from resin and purification by reverse phase HPLC (condition-II), the chromatogram of (**P21**) appeared at t_R = 21.875 min. MALDI-TOF mass spectrum showed $M{\cdot}H^+_{obs}$ = 2874.024; $M{\cdot}H^+_{calcd}$ = 2872.988.

2.4.5.2 Synthesis of *cis*-D/D-APC Ac-TATAT$_4$-LysNH$_2$ (P22)

Synthesis of *cis*-D/D-APC Ac-TATAT$_4$-LysNH$_2$ (**P22**) was accomplished in the same way as described for *cis*-D/D-APC Ac-T$_7$-LysNH$_2$ (**P13**) above. Starting from TentaGel S RAM Fmoc resin (4.3 mg, 1 μmol) and monomer,

Cycle	Monomers	Amount
1	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	2.9 mg (4 μmol)
2	Fmoc-D-APC-OPfp (**55**)	2.3 mg (4 μmol)
3	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	2.9 mg (4 μmol)
4	Fmoc-D-APC-OPfp (**55**)	2.2 mg (4 μmol)
5	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	2.7 mg (4 μmol)
6	Fmoc-D-APC-OPfp (**55**)	2.1 mg (4 μmol)
7	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	2.7 mg (4 μmol)
8	Fmoc-D-APC-OPfp (**55**)	2.7 mg (4 μmol)
9	Fmoc-D-Pro-(*cis*-4-A^{Bz})-OPfp (**28**)	3.3 mg (4 μmol)
10	Fmoc-D-APC-OPfp (**55**)	2.3 mg (4 μmol)
11	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	2.7 mg (4 μmol)
12	Fmoc-D-APC-OPfp (**55**)	2.3 mg (4 μmol)
13	Fmoc-D-Pro-(*cis*-4-A^{Bz})-OPfp (**28**)	3.4 mg (4 μmol)
14	Fmoc-D-APC-OPfp (**55**)	2.7 mg (4 μmol)
15	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	2.8 mg (4 μmol)
16	Fmoc-D-APC-OPfp (**55**)	2.1 mg (4 μmol)

The monomers were used in each coupling cycle respectively. Final Fmoc removal and acetylation was performed as usual. Before cleavage from resin, the

nucleobase protecting group was removed as described above. After purification by HPLC (condition-II), the chromatogram of (**P22**) appeared at t_R = 22.236 min. MALDI-TOF mass spectrum showed $M{\cdot}H^+_{obs}$ = 2872.222; $M{\cdot}H^+_{calcd}$ = 2872.988.

2.4.5.3 Synthesis of Fluorescent *cis*-D/D-APC Ac-T_4ATA-Lys(FAM)-NH_2 (P23)

Synthesis of *cis*-D/D-APC Ac-T_4ATA-Lys(FAM)-NH_2 (**P23**) was carried out in the same way as described for *cis*-D/D-APC Ac-T_7-LysNH_2 (**P13**) above. Starting from TentaGel S RAM Fmoc resin (18.0 mg, 4.0 μmol) and monomer as following,

Cycle	Monomers	Amount
1	Fmoc-D-Pro-(*cis*-4-A^{Bz})-OPfp (**28**)	13.7 mg (16 μmol)
2	Fmoc-D-APC-OPfp (**55**)	8.8 mg (16 μmol)
3	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	11.5 mg (16 μmol)
4	Fmoc-D-APC-OPfp (**55**)	8.8 mg (16 μmol)
5	Fmoc-D-Pro-(*cis*-4-A^{Bz})-OPfp (**28**)	11.9 mg (16 μmol)
6	Fmoc-D-APC-OPfp (**55**)	8.3 mg (16 μmol)
7	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	11.0 mg (16 μmol)
8	Fmoc-D-APC-OPfp (**55**)	8.3 mg (16 μmol)
9	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	12.7 mg (16 μmol)
10	Fmoc-D-APC-OPfp (**55**)	8.6 mg (16 μmol)
11	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	13.9 mg (16 μmol)
12	Fmoc-D-APC-OPfp (**55**)	8.8 mg (16 μmol)
13	Fmoc-D-Pro-(*cis*-4-T)-OPfp (**21**)	12.5 mg (16 μmol)
14	Fmoc-D-APC-OPfp (**55**)	8.3 mg (16 μmol)

The monomers were used in each coupling cycle respectively. Before cleavage from resin, the nucleobase protecting group was removed as described above. After cleavage from resin, the crude PNA was dissolved in 0.5 M $NaHCO_3$ solution (50 μL) pH 9.0 and 5-carboxy fluorescien succinimidyl ester (1 mg) in DMSO (50 μL) was added to the crude PNA. The reaction was kept in dark at rt for 24 h, then filtered through sephadex (NAP-10 column) G-25 medium for desalting and buffer exchange

and purified by reverse phase HPLC (condition-II), MALDI-TOF mass spectrum showed $M{\cdot}H^{+}_{obs} = 2902.276$; $M{\cdot}H^{+}_{calcd} = 2913.718$.

2.5 Biophysical studies of PNA

2.5.1 UV-Titration

The UV titration experiment was performed on a MILTON ROY spectronic 3000 array UV spectrophotometer at 25 °C. To a solution containing the PNA *cis*-D/D-APC Fmoc-T_5-LysNH_2 (**P1**) (7.08 μM, $OD_{260} = 0.281$) and 10 mM sodium phosphate buffer pH 7.0 (2000 μL) was added a 5-20 μL aliquot of a concentrated stock solution of dA_{50} (23.51 μM, $OD_{260} = 11.424$) in 10 mM sodium phosphate buffer pH 7.0 at total volume 150 μL of titrant. After the absorbance is stabilized (10-15 min), the absorbance was read against a blank (10 mM sodium phosphate) and more dA_{50} aliquots were added until a total volume of 150 μL (corresponds to 1:3 PNA (**P1**):DNA) had been added. The ratio of the observed A_{260} and the calculated A_{260} were plotted as X-axis against the mole ratio of PNA (**P1**):DNA nucleotide as Y-axis and the stoichiometry was determined form the inflection point [97]

$$\text{Calculated } OD_{260} = \frac{OD_{260(P1)} \times V_{(P1)} + OD_{260(DNA)} \times V_{(DNA)}}{V_{(P1)} + V_{(DNA)}}$$

$$= \frac{0.281 \times 2000 + 11.424 \times V_{(DNA)}\,(\mu L)}{2000 + V_{(DNA)}\,(\mu L)}$$

$$\text{ratio of PNA:DNA} = \frac{\varepsilon_{(DNA)} \times OD_{260(P1)} \times V_{(P1)}}{\varepsilon_{(P1)} \times OD_{260(DNA)} \times V_{(DNA)}}$$

$$= \frac{486.0 \times 0.281 \times 2000}{39.6 \times 11.424 \times V_{(DNA)}\,(\mu L)}$$

Table 2.3 Data examples from the Calculation of UV-Titration experiment of *cis*-D/D-APC Fmoc-T_5-LysNH_2 (**P1**) with dA_{50}

Vol. of Titrant (μL)	Ratio of PNA: DNA	% mol of PNA	% mol of DNA	Calcd OD_{260}	Obs OD_{260}	Obs/ Calcd OD_{260}
0	-	100	0	0.281	0.281	1.000
5	12.055	92.340	7.660	0.308	0.293	0.949
10	6.027	85.770	14.230	0.336	0.308	0.917
15	4.018	80.073	19.927	0.363	0.325	0.895
20	3.014	75.085	24.915	0.391	0.342	0.874
25	2.411	70.682	29.318	0.418	0.362	0.866
30	2.009	66.768	33.232	0.445	0.377	0.848
35	1.722	63.264	36.736	0.472	0.402	0.851
40	1.507	60.109	39.891	0.499	0.426	0.853
45	1.339	57.254	42.746	0.526	0.452	0.859
50	1.205	54.658	45.342	0.552	0.479	0.868
55	1.096	52.287	47.713	0.579	0.501	0.865
60	1.005	50.114	49.886	0.605	0.527	0.872
70	0.861	46.267	53.733	0.657	0.582	0.886
80	0.753	42.968	57.032	0.709	0.632	0.891
90	0.670	40.109	59.891	0.760	0.683	0.899
100	0.603	37.607	62.393	0.811	0.733	0.904
120	0.502	33.434	66.566	0.911	0.838	0.92

2.5.2 T_m analysis

T_m experiments were performed on a CARY 100 Bio UV-Visible spectrophoto meter (Varian Ltd.) equipped with a thermal melt system. The sample for T_m measurement was prepared by mixing calculated amounts of stock oligonucleotide and PNA solutions together to give final concentration of nucleotides and sodium phosphate buffer (pH 7.0) and the final volumes were adjusted to 3.0 mL by addition

of deionized water. The samples were transferred to a 10 mm quartz cell with a Teflon stopper and equilibrated at the starting temperature for 10 min. The A_{260} was recorded in steps heating from 20-80 °C, cooling 80-20 °C and reheating 20-80 °C (block temperature) with a temperature ramp of 1 °C /min. The temperature recorded was the actual temperature measured by a built-in temperature probe. Only the result taken from the last heating cycle was used and was normalized by dividing the absorbance at each temperature by the initial absorbance (Table 2.4). T_m was obtain from derivative plot after smoothing using KaliedaGraph 3.6 (Synergy Software) and analysis of the data was performed on a PC compatible computer using Microsoft Excel XP (Microsoft Corp.). The independent experiments were accurate within ±0.5 °C.

Table 2.4 Data examples from T_m analysis of *cis*-D/D-APC Fmoc-T_5-LysNH_2 (**P1**) with dA_{50} by UV spectrophotometry.

entry	***cis*-D/D-APC Fmoc-T_5-LysNH_2 (P1) with d(A_{50}) at 20.00-80.00 °C**			
	Temperature (°C)	**Absorbance**	**Correct temp*(°C)**	**Nor Abs**
1	20.02	0.1400	18.97	1.0000
2	29.97	0.1413	28.70	1.0091
3	34.97	0.1419	33.59	1.0136
4	40.98	0.1430	39.47	1.0220
5	45.02	0.1441	43.42	1.0296
6	50.02	0.1459	48.31	1.0426
7	54.97	0.1487	53.15	1.0625
8	60.02	0.1512	58.09	1.0801
9	64.97	0.1614	62.93	1.1528
10	70.97	0.1765	68.80	1.2610
11	74.97	0.1877	72.71	1.3405
12	79.97	0.1965	77.60	1.4035

*The equation for determining the corrected temp was obtained by measuring the actual temp in the cuvette using a temperature probe and plotting against the set temperature (T_{block}) from 20-90 °C. The linear equation and relationship were obtained with Y = (0.978X-0.6068) and $r^2 > 0.99$.

Correct temperature and normalized absorbance are defined as follows.

$$\text{Correct. Temp.} = (0.978 \times T_{block}) - 0.6068$$

$$\text{Normalized Abs.} = Abs_{obs}/Abs_{init}$$

In entry 1; $T_{obs} = 20.02$ °C, $Abs_{init} = 0.1400$

$$Abs_{obs} = 0.1400$$

$$\text{Correct. Temp.} = (0.978 \times T_{obs}) - 0.6068$$

$$= (0.978 \times 20.02) - 0.6068$$

$$= 18.97 \text{ °C}$$

$$\text{Normalized Abs.} = Abs_{obs}/Abs_{init}$$

$$= 0.1400/0.1400$$

$$= 1.0000$$

In entry 2; $T_{obs} = 29.97$ °C, $Abs_{init} = 0.1400$

$$Abs_{obs} = 0.1413$$

$$\text{Correct. Temp.} = (0.978 \times T_{obs}) - 0.6068$$

$$\text{Correct. Temp.} = (0.978 \times 29.97) - 0.6068$$

$$= 28.70 \text{ °C}$$

$$\text{Normalized Abs.} = Abs_{obs}/Abs_{init}$$

$$= 0.1413/0.1400$$

$$= 1.0091$$

2.5.3 Circular dichroism spectroscopy

CD experiments were performed on a JASCO Model J-715 spectropolarimeter (Pharmaceutical Research Equipment Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University). The sample were prepared by mixing calculated amounts of stock oligonucleotide and PNA solutions together in a 10 mm quartz cell and the final volumes were adjusted to 2.5 mL by addition of deionized water containing an appropriate amount of sodium phosphate buffer pH 7.0 to give the appropriate concentration of each component as described in the text. The spectra were measured at 25 °C from 200 to 300 nm and averaged 4 times then subtracted from a spectrum of 10 mM sodium phosphate buffer pH 7.0 under the same condition.

2.5.4 Gel electrophoresis

The polyacrylamide gel electrophoresis experiments were performed on a electrophoresis machine (Department of Biology, Faculty of Sciences, Chulalongkorn University). Acrylamide and *N,N*-methylene bisacrylamide and *N,N,N',N'*-tetramethyl ethylenediamine (TEMED) and chemicals necessary for preparing buffers were of the highest purity available from BDH or Sigma Chemical Company. The stock 0.90 M Tris-Borate-EDTA (TBE) buffer pH 8.0 and loading buffer (15% Ficoll^{TM}, 0.002% bromophenol blue and 0.002% xylene cyanol FF in 90 mM TBE were prepared according to the literature [98]. The 15% polyacrylamide gel in 90 mM TBE was prepared according to the literature and run at 300 V and 44 mA. The samples were prepared by mixing calculated amount of concentrated stock of fluorescent labeled decaadenylic acid and PNA in eppendrof tube to give the total amount of 1 nmol and then mixed with appropriate volume of TBE buffer, loading buffer and deionized water. The experiments were constructed with hold glass plates 20 cm × 40 cm for pouring the 15% acrylamide monomer. The comb was inserted immediately after setting the gel in the gel mold and allowed the acrylamide to polymerize for 60 mins at room temperature. The comb was carefully removed and attached the gel to the electrophoresis tank filled with TBE. The DNA-PNA samples were loaded in the appropriate amount into the well at the top of the gel via microsyringe and then the system was connected to a power supply until the bromophenol blue marker dye moved to the desired distance through the gel. The power supply was then disconnected and the gel was removed from the glass plate using a thin spatula and the gel was visualized by a UV transilluminator and photographs taken.

2.5.5 Fluorescence experiment

Fluorescence experiment was performed on a Perkin-Elmer Luminescence Spectrometer LS50B using Perkin-Elmer Luminescence Spectrometry cells (Part No: B0631113) at School of chemistry, University of Southampton, United Kingdom. 5-Carboxy fluorescien succinimidyl ester (FAM-OSu) was reacted with target PNA using 0.5 M $NaHCO_3$ solution (50 μL) pH 9.0 in DMSO (50 μL) and kept in dark at rt

for 24 h, then filtered through sephadex (NAP-10 column) G-25 medium for desalting and buffer exchange and purified by reverse phase HPLC. The DNA probes attached with methyl red moiety were synthesized by an automated machine solid phase synthesis by Prof. Tom Brown. To a solution containing the *cis*-D/D-APC Ac-T_4ATA-Lys(FAM)-NH_2 (**P23**) (10.2 μM) 2000 μL was added DNA probe solution 10, 20 and 30 equiv and the intensity of fluorescence was measured at 490-650 nm after incubated for 10 min. The wavelength was plotted as X-axis against the intensity as Y-axis and the decrease of the signal of FAM at 520 nm was determined indicated that FAM moiety was quenchehed by methyl red.