CHAPTER II

EXPERIMENTAL SECTION

2.1 General

The weight of all substances was determined on an Metler AT 200 electrical balance. Melting points were recorded on an electrothermal melting point apparatus model 9100. Rotary evaporator was of Eyela (Japan) equipped with a water aspirator model A-3S. UV cabinet for UV-visualization of TLC was made in-house by the author. The magnetic stirrers were of Ikamag RH and Corning. The high vacuum was delivered by an ATB-Loher Flender Vacubrand pump. Elemental analyses were performed by Ms. Amporn Ungpakornkaew at Chulalongkorn Research Equipment Centre on a Perkin-Elmer CHN analyzer model PE 2400 series II. Specific rotations were measured on Perkin-Elmer 241 polarimeter by Dr. Tirayut Vilaivan at Dyson Perrins Laboratory, University of Oxford. Thin layer chromatography was performed on Merck D.C. silica gel 60 F_{254} 0.2 mm. precoated aluminium plates cat no. 1.05554. Column chromatography was performed on silica gel 70-230 mesh or 230–400 mesh (for flash column chromatography). Reverse phase HPLC experiments were performed on Water 600^{TM} system equipped with gradient pump and Water 996^{TM} photodiode array detector; optionally alternate to Water^{TM} 717 plus autosampler for (20 µl sample size for analytical scale) or Rheodyne 7725 manual sample loop (variable sample size for preparative scale). An Altech C_{18} HPLC column 3 µm particle size 4.6 x 150 mm was used for both analytical and semi-preparative purpose. Peak monitoring and data processing were organized on Compaq prolinea 486 compatible computer operating a Millennium version 2.1 software. Fractions from HPLC were collected manually and was assisted by real-time HPLC chromatogram monitoring. The combined fractions were freeze dried under reduced pressure (0.1 mm Hg) using vacuum pump Vacubrand model ATB – Loher Flender.

IR spectra were recorded on Nicolet Magna 750 Fourier Transform Infrared spectrometer linked to Omnic software version 3.0. Routine UV experiment was measured

on Shimadzu model 160 spectrophotometer at the Institute for Biotechnology and Genetic Engineering. ^{1}H and ^{13}C spectra were recorded on Bruker ACF 200 operating at 200 MHz (^{1}H) and 50.28 MHz (^{13}C) by the author and JEOL JNM - A500 by Mrs. Wanna Sririnnut and Ms. Wanwimon Thabdee at Chulalongkorn Research Equipment Centre. Assignment of ^{1}H and ^{13}C chemical shift were assisted by COSY and DEPT experiment using JEOL JNM-A500. All chemical shifts are referred in ppm relative to tetramethylsilane (δ_H = 0 ppm, δ_C = 0 ppm) using the residual protonated solvent signal as reference. MALDI-TOF mass spectra of all cPNA were analyzed by Ms. Nanthiga Panchan on Bruker BIFLEX™ using doubly recrystallized α-cyano-4-hydroxy cinnamic acid (CCA) as matrix, and calibrated with human angiotensin II (M+H, 1047) and bovine insulin (M+H, 5734). 0.1% Trifluoroacetic acid in acetonitrile:water (70:30) was used as diluting agent for MALDI-TOF samples.

2.2 Materials

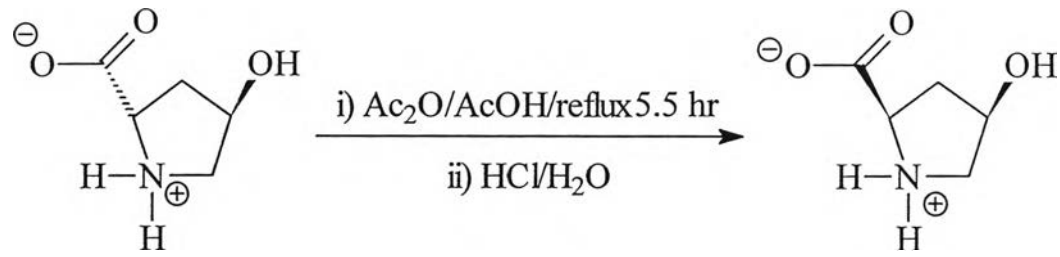
All chemicals were purchased from Fluka, Merck or Aldrich Chemicals Co., Ltd., and were purified as appropriate depending on reaction conditions and purposes. Acetic anhydride was synthesized from acetyl chloride and sodium acetate anhydrous according to the standard method.[60] Tetrahydrofuran for Mitsunobu reaction was dried with fresh thin-cut sodium metal and benzophenone under reflux. Commercial grade solvents were distilled before use for column chromatography. Pyridine, *N,N'*-dimethylformamide and acetonitrile were dried over calcium hydride overnight, distilled under reduced pressure and kept in dried close vessel over 4 °A molecular sieve. Nitrogen and hydrogen were obtained from TIG with high purity up to 99.5 %. Acetonitrile for HPLC experiment was HPLC grade, obtained from BDH and was filtered through a membrane filter (13 mm ϕ, 0.45 μm Nylon Lida) before use. Anhydrous *N,N'*-dimethylformamide for solid phase peptide coupling reaction was obtained from Fluka and was used without further purification. Reagent grade solvent was used for washing step. The solid support for peptide synthesis (Novasyn™ TGR resin 0.2 mmol substitution) and protected amino acids, Fmoc-L-Ser(O^{t}Bu)-OH, Fmoc-D-Ser(O^{t}Bu)-OH, Fmoc-L-Lys(Boc)-OPfp and Fmoc-L-Lys(Boc)-OH, were obtained from Calbiochem Novabiochem Co., Ltd. Trifluoroacetic

acid (98 %) was obtained from Fluka. Deionized water was obtained from Millipore system.

2.3 Synthesis of cPNA monomers

2.3.1 Synthesis of intermediate

***cis*-4-Hydroxy-D-proline (1b)**



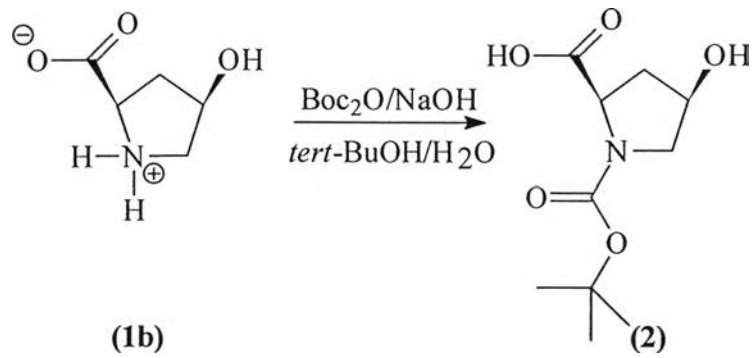
(1a) **(1b)**

A mixture of acetic anhydride (75 mL, 0.74 mol) and glacial acetic acid (218 mL) were heated to 50 °C and then *trans*-4-hydroxy-L-proline **(1a)** (18.00 g, 0.14 mol) was added. The suspension was refluxed for 5.5 hr. Then the mixture was allowed to cool at room temperature. The solvent was removed under reduced pressure to give a dark thick oil which was redissolved in 255 mL of 2 N hydrochloric acid and was then refluxed for another 3 hr. The solution was further decolourized by boiling with charcoal for 30 min. Filtration through celite while still hot to gave a clear solution which was evaporated to dryness by rotary evaporation. The yellow residue was redissolved in 50 mL of distilled water and the solution adjusted to pH 8 with triethylamine. To this basic solution was added absolute ethanol 450 mL and allowed to stand overnight at room temperature. White crystals so obtain were collected by suction filtration and recrystallized from methanol containing a little water to give the required product **(1b)** as colourless needles (6.55 g, 36 %).

mp.= 250-254 °C, δ_H (D_2O, 200 MHz); 1.94-2.02 [1H, m, 1xCH_2(3′)] and 2.24 [1H, ddd, J = 14.0, 10.5, 4.5 Hz, 1xCH_2(3′)], 3.09 [1H, ddd, J = 12.5, 2.0, 2.0 Hz, 1xCH_2(5′)] and 3.20 [1H, dd, J = 12.5, 4.0 Hz, 1xCH_2(5′)], 3.95 [1H, dd, J = 10.5, 4.0 Hz, CH(2′)], 4.31-4.57

[1H, m, CH(4′)]; δ_C (D_2O, 50.28 MHz); 39.6 [CH_2(3′)], 55.4 [CH_2(5′)], 62.1 [CH(2′)], 71.6 [CH(4′)], 176.8 [C=O(1)]; ν_{max} (KBr)/cm^{-1}; 3500 (OH), 3231 (NH stretch), 2935 (CH aliphatic), 1634 s (C=O), 1558 (NH bend), 1435, 1378 (OH bend), 1327 (C-O bend), 1176, 1088, 1041, 976, 868, 735; $[\alpha]_D^{23}$ +58.6 (c=2.0, H_2O), $[\alpha]_D^{25}$ +60.3 (c=2.0, H_2O).

N-tert-Butoxycarbonyl-_cis_-4-hydroxy-D-proline (2)



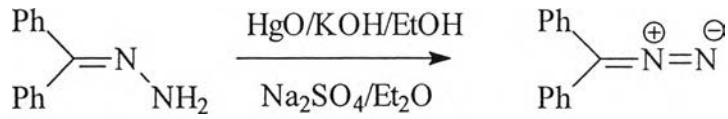
(1b) **(2)**

cis-4-Hydroxy-D-proline **(1b)** (6.55 g, 50.0 mmol) was added to a stirred mixture of 4 % sodium hydroxide (80 mL) and *tert*-butanol (20 mL). Di-*tert*-butyl dicarbonate (13.08 g, 60.0 mmol) was added to the solution dropwise to avoid too much increase of temperature. While the resulting emulsion became homogeneous, vigorous evolution of carbon dioxide was observed. The solution was allowed to stir overnight at room temperature. The solvent was removed under reduced pressure and the residue was redissolved in 10 mL of distilled water and acidified to pH 2 with 4 % hydrochloric acid. The acidified solution was extracted with 70 mL ethyl acetate. The ethyl acetate layers were combined, dried over magnesium sulfate and evaporated under reduced pressure to obtain clear thick oil. Scratching the oil with ice-cold hexane afford the product **(2)** as a white crystalline solid (11.17 g, 97 %).

mp.= 124-125 °C; δ_H (DMSO-d_6, 200 MHz); 1.32 and 1.37 [9H, 2x s, CH_3 Boc rotamers], 1.74-1.88 and 2.21-2.38 [2H, 2x m, CH_2(3′)], 3.06-3.11 [2H, m, CH_2(5′)], 3.45-3.50 [1H, m, CH(2′)], 4.03-4.17 [1H, m, CH(4′)]; δ_C (DMSO-d_6, 50.28 MHz); 33.1 and 33.3 [CH_3 Boc rotamers], 42.8 and 43.5 [CH_2(3′) rotamers], 58.8 and 59.3 [CH_2(5′) rotamers], 62.3

and 62.6 [CH(2′) rotamers], 72.9 and 73.7 [CH(4′) rotamers], 83.8 [Boc C], 158.3 and 158.7 [Boc CO rotamers], 178.6 and 179.0 [acid CO rotamers]; ν_{max} (KBr)/cm^{-1}; 3454 (OH stretch), 1731 (carboxylic CO), 1661 (amide/urethane CO), 1408, 1256 (^{t}Bu), 1094, 1008, 908, 846, 776, 631, 582.

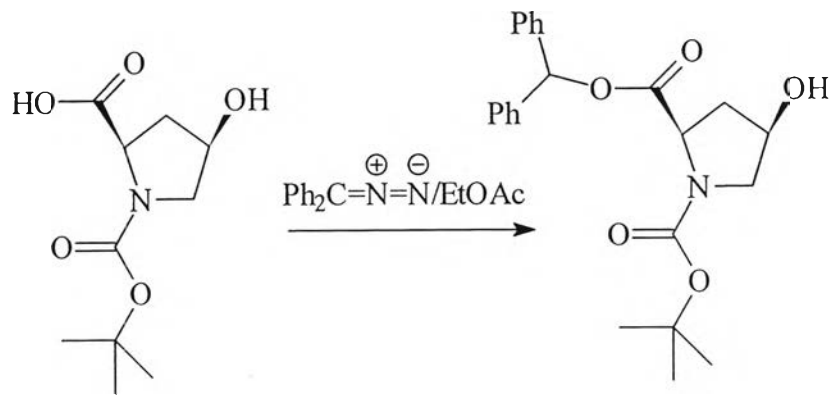
Diphenyldiazomethane (3)[61]



(3)

In a 100 mL round bottom flask, wrapped with aluminium foil to protect from light, benzophenone hydrazone (3.93 g, 20.0 mmol), mercuric oxide (4.33 g, 20.0 mmol) and anhydrous sodium sulfate (1.75 g, 20.0 mmol) were suspended in 50 mL diethyl ether with stirring. Then 0.5 mL of 10 % potassium hydroxide in ethanol was added. The mixture turned purple gradually and was allowed to stir in the dark for 6 hr. The work up procedure involved filtrating off the used mercuric oxide and sodium sulfate mixture which was then washed with ether. The purple filtrate was evaporated under reduced pressure without external heating. The required product was obtained as purple liquid (3.87 g, 99 %) which was used for the next step without characterization and purification.

***N-tert*-Butoxycarbonyl-*cis*-4-hydroxy-D-proline diphenylmethyl ester (4)**

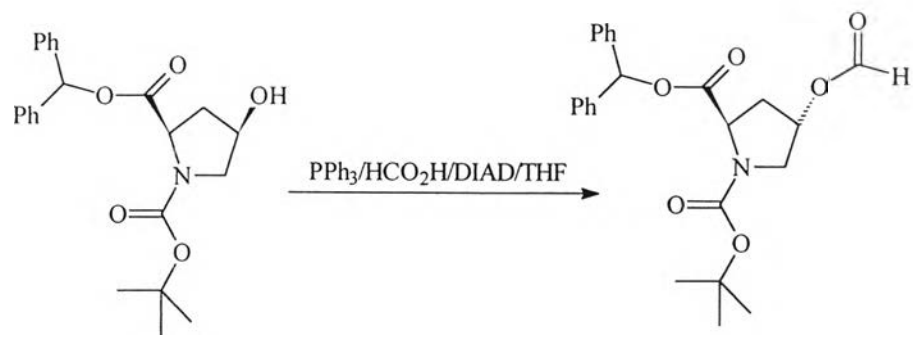


(2) **(4)**

To a stirred solution of freshly prepared diphenyldiazomethane **(3)** (2.94 g, 15.0 mmol) in ethyl acetate (12 mL) was added *N-tert*-butoxycarbonyl-*cis*-4-hydroxy-D-proline **(2)** (3.47 g, 15.0 mmol) portionwise. Small bubbles of nitrogen gas was slowly evolved from the solution and the purple color of the solution was gradually faded out. The reaction flask was then equipped with a drying tube and allowed to stir overnight in the dark. When the solution became colourless, a further portion of freshly prepared diphenyldiazomethane was added and the stirring was continued the reaction was completed as judged by TLC analysis. The solvent was evaporated under reduced pressure to obtain the crude product as a solid and then further purified by flash column chromatography on silica gel using hexane:ethyl acetate (7:3) as eluent. The pure fractions were collected and the solvent removed to give stricky oil which solidified after scratching with hexane. The white precipitate formed was collected by suction filtration and then washed twice with hexane to give a white fluffy foam **(4)** (5.18 g, 87 %). Drying under high vacuum was desirable in order to get a sufficiently dry material for the Mitsunobu reaction in the next step.

mp.= 102-105 °C; δ_H ($CDCl_3$, 200 MHz); 1.22 and 1.45 [9H, 2x s, C$\underline{H}_3$ Boc rotamers], 1.95-2.09 and 2.19-2.40 [2H, 2x m, C$\underline{H}_2$(3′)], 3.14 [1H, 2x d, J = 8.5 Hz, O$\underline{H}$ rotamers], 3.52-3.59 [2H, m, C$\underline{H}_2$(5′)], 4.20-4.40 [1H, m, C$\underline{H}$(2′)], 4.45-4.60 [1H, m, C$\underline{H}$(4′)], 6.86 and 6.94 [1H, 2x s, C$\underline{H}Ph_2$ rotamers], 7.31-7.62 [10H, m, phenyl C$\underline{H}$]; δ_C ($CDCL_3$, 50.28 MHz); 28.1 and 28.4 [Boc $\underline{C}H_3$ rotamers], 37.8 and 38.7 [$\underline{C}$H(3′) rotamers], 55.0 and 55.5 [$\underline{C}H_2$(5′) rotamers], 58.0 and 58.2 [$\underline{C}$H(2′) rotamers], 69.7 and 70.5 [$\underline{C}$H(4′) rotamers], 77.5 and 78.4 [$\underline{C}HPh_2$ rotamers], 80.2 and 80.4 [Boc $\underline{C}$], 127.1-128.5 [phenyl $\underline{C}$H rotamers], 139.7 [phenyl $\underline{C}$], 153.9 and 154.4 [Boc $\underline{C}$O rotamers], 172.8 and 173.2 [ester $\underline{C}$O rotamers]; ν_{max} (KBr)/cm^{-1}; 3435 br (OH and aromatic), 2981 (CH aliphatic), 1753 (ester CO), 1668 (amide CO), 1414, 1196, 1151, 702, $[\alpha]_D^{25}$ +41.2 (c=2.0, H_2O).

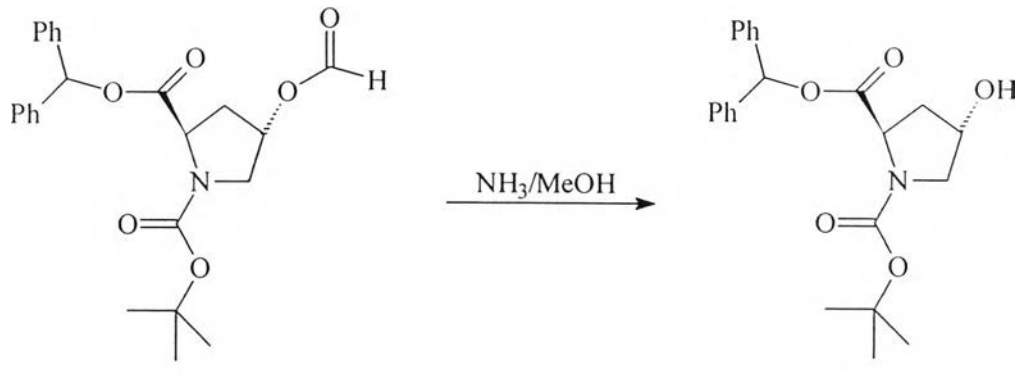
N-tert-Butoxycarbonyl-_trans_-4-hydroxy-D-proline diphenylmethyl ester (5b)



(4) **(5a)**

In a dried 100 mL round bottom flask, equipped with a magnetic bar, *N-tert*-butoxycarbonyl-*cis*-4-hydroxy-D-proline diphenylmethyl ester (3.57 g, 9.0 mmol) **(4)**, triphenylphosphine (3.06 g, 11.7 mmol) and formic acid (0.4 mL, 9.9 mmol) were dissolved in dry THF (50 mL) and cooled down to –10 °C in an ice salt bath. The solution was stirred under nitrogen balloon and DIAD (2.1 mL, 11.7 mmol) was added dropwise within 5 min and the reaction mixture was allowed to stir overnight. The solvent was evaporated and the residue was chromatographed on silica gel using hexane:ethyl acetate (7:3) as eluent to give the *trans*-4-formate ester **(5a)** as colourless oil (3.64 g, 95 %).

δ_H (200 MHz, $CDCl_3$); 1.30 and 1.47 [9H, 2x s, CH_3 Boc rotamers], 2.01-2.20 and 2.30-2.50 [2H, m, CH_2(3′)], 3.57-3.80 [2H, m, CH_2(5′)], 4.48-4.64 [1H, m, CH(2′)], 5.20-5.40 [1H, m, CH(4′)], 6.88 and 6.93 [1H, 2x s, $CHPh_2$ rotamers], 7.25-7.51 [10H, m, phenyl CH], 7.97 [1H, s, HCO formate]; δ_C ($CDCl_3$, 50.28 MHz); 28.0 and 28.4 [CH_3 Boc rotamers], 35.2 and 36.4 [CH_2(3′) rotamers], 52.1 [CH_2(5′)], 57.7 and 57.9 [CH(2′) rotamers], 71.6 and 72.3 [CH(4′) rotamers], 77.3 and 77.8 [$CHPh_2$ rotamers], 80.6 [Boc C], 127.1-128.6 [phenyl CH rotamers], 139.6 and 139.8 [phenyl C rotamers], 153.6 and 154.1 [Boc CO], 160.2 [CO formate], 171.2 [CO ester]; ν_{max} (KBr)/cm^{-1}; 3043 (aromatic CH), 2989 (aliphatic CH), 1751 (formate CO), 1697 (amide CO), 1533 (NH bend), 1439, 1385, 1250 (C-O stretch), 1190, 1120, 1070, 721, 696.

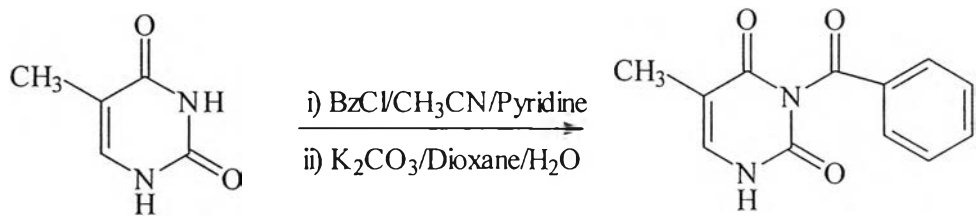


(5a) **(5b)**

The *trans*-4-formate ester **(5a)** was dissolved in a mixture of 7 mL of methanol and 28 % aqueous ammonia solution (1.8 mL). After 1 hr, 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 hexane:ethyl acetate (7:3) as eluent to give a clear viscous oil. Scratching with cold hexane gave a white fluffy solid which was collected by suction filtration. The product **(5b)** was dried under vacuum (3.30 g, 92 %).

mp.= 105-108 °C; δ_H ($CDCl_3$, 200 MHz); 1.19 and 1.43 [9H, 2x s, $C\underline{H}_3$ Boc rotamers], 1.80-2.05 and 2.19-2.45 [2H, 2x m, $C\underline{H}_2(3')$], 3.47-3.83 [2H, m, $C\underline{H}_2(5')$], 4.25-4.55 [2H, 2x m, $C\underline{H}(2')$ and $C\underline{H}(4')$], 6.87 and 6.92 [1H, 2x s, $C\underline{H}Ph_2$ rotamers], 7.05-7.52 [10H, m, phenyl C$\underline{H}$]; δ_C ($CDCl_3$, 50.28 MHz); 28.1 and 28.4 [$\underline{C}H_3$ Boc rotamers], 38.2 and 39.0 [$\underline{C}H_2(3')$ rotamers], 54.7 [$\underline{C}H_2(5')$], 57.9 and 58.2 [$\underline{C}H(2')$ rotamers], 69.1 and 69.9 [$\underline{C}H(4')$ rotamers], 77.3 and 78.1 [$\underline{C}HPh_2$ rotamers], 80.6 [Boc $\underline{C}$], 126.9-129.1 [phenyl $\underline{C}H$ rotamers], 135.2 and 139.7 [phenyl $\underline{C}$ rotamers], 154.3 [Boc $\underline{C}O$] and 172.0 [ester $\underline{C}O$]; ν_{max} (KBr)/cm^{-1}; 3492 (OH), 2978, 2908 and 2875 (aliphatic CH), 1726 (ester CO), 1693 (amide CO), 1408, 1213, 1157 (C-O stretch), 754, 708; $[\alpha]_D^{25}$ +53.0 (c=1.0, EtOH).

3-Benzoyl-5-methyl-(*1H*)-pyrimidine-2,4-dione (N^3-benzoylthymine) (6)

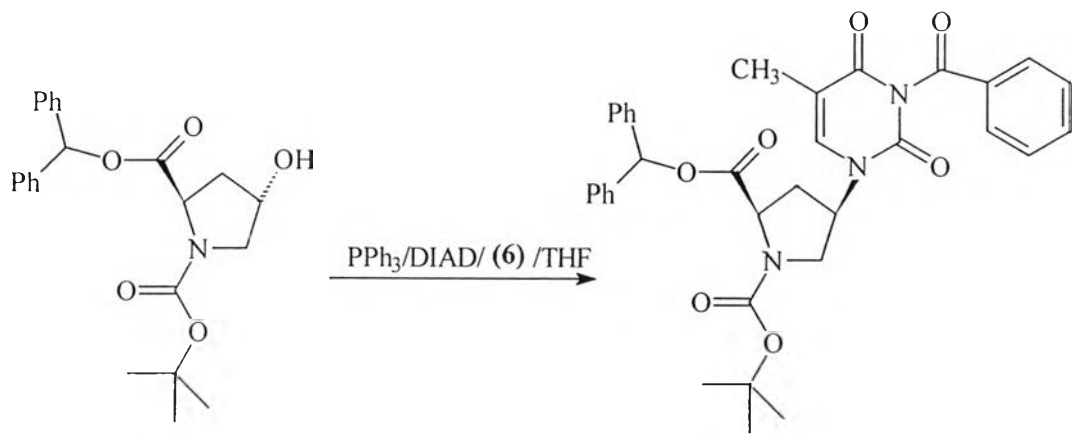


(6)

In a dried 100 mL round bottom flask, equipped with a magnetic bar, thymine (2.52 g, 20.0 mmol) was suspended in a mixture of anhydrous acetonitrile (36 mL) and pyridine (14 mL) followed by benzoyl chloride (6.3 mL, 44.0 mmol). The flask was stoppered and the reaction allowed to stir at room temperature. After the solution became clear, TLC analysis revealed the presence of both mono- and dibenzoylated products. The solvent was removed by rotary evaporation, the residue was subsequently redissolved in ethyl acetate and was extracted with 5 % hydrochloric acid, followed by washing with water (x3). The extracted organic layer was dried over magnesium sulfate and evaporated in *vacuo* to give a yellow viscous oil. The residue was suspended in 0.25 M potassium carbonate in dioxane:water (1:1) 80 mL and was warmed at 70 °C for 45 min, the solution was then evaporated to dryness and the residual solid collected by suction filtration. The white crystalline precipitate was recrystallized from absolute ethanol to give shining white crystals of the N^3-benzoylthymine **(6)** (3.42 g, 74 %).

mp.= 152-155 °C; δ_H (DMSO-d_6, 200 MHz); 1.81 [3H, s, C<u>H</u>$_3$], 7.51 [1H, d, J = 1.0 Hz, C<u>H</u>(6)], 7.58 [2H, dd, J = 8.0, 7.5 Hz, benzoyl *m*-C<u>H</u>], 7.77 [1H, t, J = 7.5 Hz, benzoyl *p*–C<u>H</u>], 7.92 [2H, d, J = 8.0 Hz, benzoyl *o*-C<u>H</u>]; δ_C (DMSO-d_6, 50.28 MHz); 16.9 [<u>C</u>H$_3$], 113.1 [<u>C</u>(5)], 134.6 and 135.4 [benzoyl *o*-<u>C</u>H and benzoyl *m*-<u>C</u>H], 136.6 [benzoyl <u>C</u>],140.5 [benzoyl *p*-<u>C</u>H], 143.9 [<u>C</u>(6)], 155.2 [<u>C</u>(2)], 168.8 [<u>C</u>(4)], 175.4 [benzoyl <u>C</u>O]; ν_{max} (KBr)/cm^{-1}; 3222 (NH), 3096 (CH aromatic), 2956 (CH$_3$), 1753, 1694 and 1646 (amide CO), 1489, 1421, 1252, 966, 841, 766, 690, 613.

N-tert-Butoxycarbonyl-_cis_-4-(N^3-benzoylthymin-1-yl)-D-proline diphenylmethyl ester (Intermediate) (7)



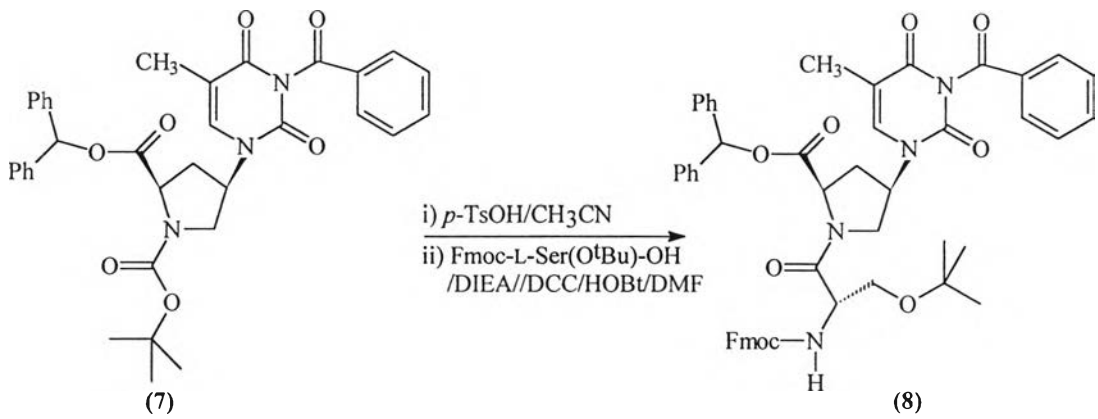
In dried 100 mL round bottom flask, equipped with a magnetic bar, *N-tert*-butoxycarbonyl-*trans*-4-hydroxy-D-proline diphenylmethyl ester **(5b)** (3.17 g, 8.0 mmol), triphenylphosphine (2.31 g, 8.8 mmol), and N^3-benzoylthymine **(6)** (1.84 g, 8.0 mmol) were dissolved in dry THF (50 mL) with stirring under nitrogen. The solution was cooled down in an iced-bath containing salt. DIAD (1.8 mL, 8.8 mmol) was added dropwise over a period of 5 min and was allowed to stir overnight. The solvent was removed under reduced pressure to complete dryness. The residue was recrystallized from methanol to afford white fluffy solid **(7)** (2.27 g, 47 %).

mp.= 183-186 °C; δ_H ($CDCl_3$, 500 MHz); 1.28 and 1.47 [9H, 2x brs, CH_3 Boc rotamers], 1.75 and 1.79 [3H, 2x s, thymine CH_3 rotamers], 1.95-2.07 [1H, m, 1xCH_2(3′)], 2.74-2.87 [1H, m, 1xCH_2(3′)], 3.48-3.63 [1H, 2x m, 1xCH_2(5′) rotamers], 3.97 [1H, dd, J = 12.0, 8.0 Hz, CH(2′)], 4.47-4.58 [1H, 2x m, 1xCH_2(5′) rotamers], 5.16-5.25 [1H, m, CH(4′)], 6.91 [1H, br s, $CHPh_2$], 7.08 and 7.17 [1H, 2x s, CH(6) rotamers], 7.27-7.36 [10H, m, Dpm aromatic CH], 7.45 [2H, t, J = 7.5 Hz, benzoyl *m*-CH], 7.61 [1H, t, J = 7.5 Hz, benzoyl *p*-CH], 7.85 [2H, dd, J = 7.5, 10.0 Hz, benzoyl *o*-CH]; δ_C ($CDCl_3$, 125.65MHz); 12.4 [thymine CH_3], 28.0 and 28.3 [Boc CH_3 rotamers], 33.9 and 35.1 [CH_2(3′) rotamers], 49.3 and 49.8 [CH_2(5′) rotamers], 52.1 and 52.5 [CH_2(2′) rotamers], 57.4 and 57.9 [CH(4′) rotamers], 78.0 and 78.3 [$CHPh_2$ rotamers], 81.3 [Boc C], 111.6 and 111.8 [C(5)], 126.8-130.4 [Dpm aromatic CH], 131.4 [benzoyl C], 135.0 [benzoyl *p*-CH], 135.6 and 135.8 [CH

(6)], 139.2 [phenyl C], 149.8 [C(2)], 153.4 and 153.7 [Boc CO rotamers], 162.3 [C(4)], 168.7 [benzoyl CO], 171.2 [ester CO]; ν_{max} (KBr)/cm^{-1}; 3534 (NH), 3068, 3032 (aromatic CH), 2988, 2933 (aliphatic CH), 1748 (ester CO), 1697 and 1656 (2x amide CO), 1601, 1491, 1477, 1451 (aromatic C=C), 1227 (C–N), 1157, 978, 897, 761, 699; $[\alpha]_D^{25}$ +16.9 (c=1.03, DMF).

2.3.2 Synthesis of serylproline cPNA monomers

***N*-(*N*-Fluoren-9-ylmethoxycarbonyl-*O*-*tert*-butyl-L-seryl)-*cis*-4-(*N*^{3}-benzoylthymin-1-yl)-D-proline diphenylmethyl ester (8)**



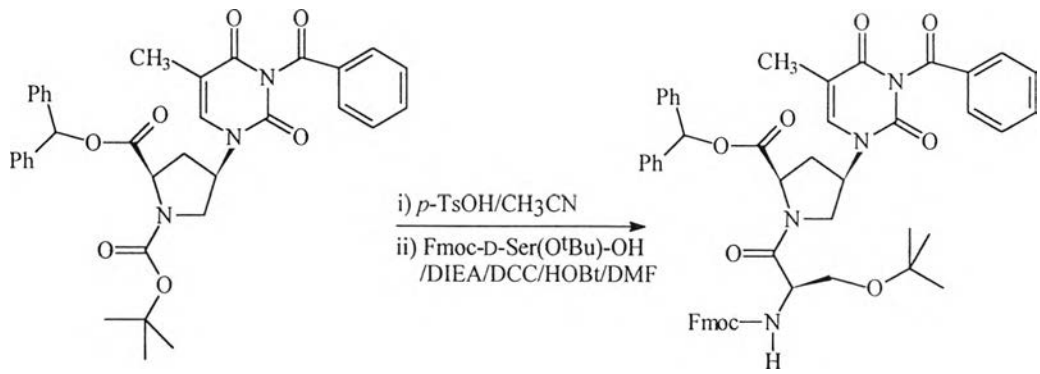
(7) (8)

Boc-D-Pro(*cis*-4-T^{Bz})-ODpm (7) (0.70 g, 1.16 mmol) was dissolved in acetonitrile (5 mL). *p*-Toluenesulfonic acid monohydrate (0.55 g, 2.90 mmol) was added and the solution stirred at room temperature for 1.5 hr, afterwhich TLC indicated complete deprotection of the Boc group. Diisopropylethylamine (DIEA) (530 μL, excess) and 2 mL of *N,N'*-dimethylformamide was then added with stirring under nitrogen. In a separate reaction vessel, a mixture of Fmoc-L-Ser(O^{t}Bu)-OH (0.50 g, 1.28 mmol), HOBt (0.20 g, 1.28 mmol) and DCC (0.26 g, 1.28 mmol) in *N,N'*-dimethylformamide (2 mL) was stirred at room temperature. After 2 hr, a white precipitate of dicyclohexylurea formed was removed by filtration and the filtrate was transfered to the first reaction vessel. The reaction mixture was stirred at room temperature for a further 3 hr then diluted with dichloromethane (50 mL) followed by washing with saturated aqueous sodium bicarbonate

and water. The organic phase was dried over magnesium sulfate and the solvent was removed under reduced pressure to give the crude product as an oil which was purified by column chromatography on silica gel using dichloromethane:acetone (20:1). The product **(8)** was obtained as a white foam (0.61g, 60 %).

mp. = 102–105 °C, (Found C, 71.8; H, 5.5; N, 6.3 %; $C_{52}H_{50}N_4O_9$ requires C, 71.4; H, 5.8; N, 6.4 %); δ_H ($CDCl_3$, 500 MHz) 1.14 [9H, s, ^tBu C$\underline{H}_3$], 1.81 [3H, s, thymine C$\underline{H}_3$], 2.00-2.09 [1H, m, 1xC$\underline{H}_2$(3′)], 2.78 [1H, ddd, *J* = 18.0, 14.5, 9.0 Hz, 1xC$\underline{H}_2$(3′)], 3.45 [1H, t, *J* = 9.0 Hz, 1xC$\underline{H}_2$(Ser)], 3.65 [1H, dd, *J* = 7.5, 5.5 Hz, 1xC$\underline{H}_2$ (Ser)], 3.77-3.84 [1H, m, 1xC$\underline{H}_2$ (5′)], 4.28-4.42 [3H, m, Fmoc aliphatic C$\underline{H}$, C$\underline{H}_2$], 4.55-4.59 [1H, m, 1xC$\underline{H}_2$(5′)], 4.66-4.72 [1H, m, Ser $C_\alpha\underline{H}$], 4.75 [1H, dd, *J* = 9.0, 6.5 Hz, 1xC$\underline{H}$(2′)], 5.23-5.31 [1H, m, C$\underline{H}$(4′)], 5.62 [1H, d, *J* = 8.5 Hz, peptide N$\underline{H}$], 6.88 [1H, s, C$\underline{H}Ph_2$], 7.12 [1H, s, C(6)$\underline{H}$], 7.26-7.36 [10H, m, Dpm aromatic C$\underline{H}$], 7.40 [2H, t, *J* = 7.5 Hz Fmoc aromatic C$\underline{H}$], 7.48 [2H, t, *J* = 7.5 Hz, benzoyl *m*-C$\underline{H}$], 7.58 [2H, t, *J* = 7.5 Hz, Fmoc aromatic C$\underline{H}$], 7.64 [1H, t, *J* = 7.5 Hz, benzoyl *p*-C$\underline{H}$], 7.73-7.79 and 7.86-7.91 [2x2H, 2x d, *J* = 7.5 Hz, Fmoc aromatic C$\underline{H}$]; δ_C ($CDCl_3$, 125.65 MHz) 12.4 [thymine $\underline{C}H_3$], 27.3 [^tBu $\underline{C}H_3$], 33.3 and 33.9 [$\underline{C}H_2$(3′) rotamers], 47.0 [Fmoc aliphatic $\underline{C}$H], 49.5 [$\underline{C}H_2$ (5′)], 52.4 and 52.5 [Ser C_αH and $\underline{C}$H(2′)], 57.5 [$\underline{C}$H(4′)], 63.3 [Ser $\underline{C}H_2$], 67.2 [Fmoc aliphatic $\underline{C}H_2$], 73.9 [^{t}Bu-$\underline{C}$], 78.5 [$\underline{C}HPh_2$], 112.0 [$\underline{C}$(5)], 120.0 [Fmoc aromatic $\underline{C}$H], 125.1-130.4 [Dpm aromatic $\underline{C}$H], 131.4 [$\underline{C}$(1) benzoyl], 135.0 and 135.3 [$\underline{C}$(6)H and benzoyl *p*-$\underline{C}$H], 139.0 and 139.4 [Dpm aromatic $\underline{C}$ rotamers], 141.2, 143.6 and 143.8 [Fmoc aromatic $\underline{C}$ rotamers], 149.7 [$\underline{C}$(2)], 155.7 [Fmoc $\underline{C}$O], 162.2 [$\underline{C}$(4)], 168.6 [benzoyl $\underline{C}$O], 170.1 [peptide $\underline{C}$O], 170.4 [ester $\underline{C}$O]; ν_{max} (KBr)/cm^{-1}; 3421 (amide NH), 3063 (aromatic CH), 2980, 2926 and 2873 (aliphatic CH), 1758 and 1702 (ester CO), 1646 (amide CO), 1463 (aromatic C=C), 1235 and 1173 (ester C-O), 768, 751, 706; $[\alpha]_D^{26}$ +12.3 (c=1.02, $CHCl_3$).

***N*-(*N*-Fluoren-9-ylmethoxycarbonyl-*O*-*tert*-butyl-D-seryl)-*cis*-4-(N^3-benzoylthymin-1-yl)-D-proline diphenylmethyl ester (9)**



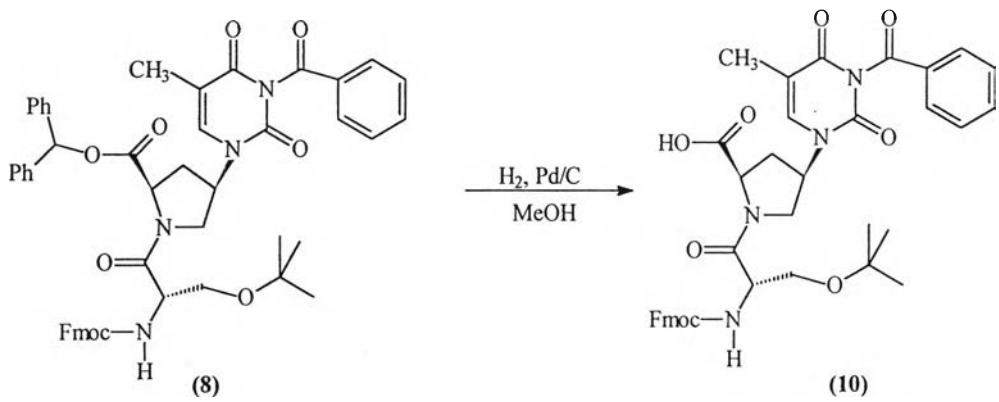
(7) (9)

Synthesis of titled compound **(9)** was achieved in the same manner as described for L-series above, Boc-D-Pro(*cis*-4-T^{Bz})–ODpm **(7)** (0.35 g, 0.58 mmol), *p*-toluene sulfonic acid monohydrate (0.28 g, 1.45 mmol), Diisopropylethylamine (268 μL, excess), Fmoc-D-Ser(O^{t}Bu)-OH (0.24, 0.64 mmol), DCC (0.13 g, 0.64 mmol), HOBt (0.10 g, 0.64 mmol) in *N,N'*-dimethylformamide (2 mL) afford **(9)** (10.33 g, 65 %), as a white foam.

mp. = 118–120 °C, (Found C, 71.3; H, 6.0; N, 6.4 %, $C_{52}H_{50}N_4O_9$ requires C, 71.4; H, 5.8; N, 6.4 %); δ_H ($CDCl_3$, 500 MHz); 1.14 and 1.17 [9H, 2x s, ^{t}Bu C$\underline{H}_3$ rotamers], 1.66 and 1.69 [3H, 2x s, thymine C$\underline{H}_3$ rotamers], 2.05-2.12 [2H, 2x m, 1xC$\underline{H}_2$(3′)], 3.43-3.70 [2H, m, C$\underline{H}_2$(Ser)], 3.98-4.04 [1H, m, 1xC$\underline{H}_2$(5′)], 4.17-4.31[2H, Fmoc aliphatic C$\underline{H}_2$], 4.33-4.39 [1H, d, J = 7.0 Hz, Fmoc aliphatic C$\underline{H}$], 4.58-4.66 [1H, m, 1xC$\underline{H}_2$(5′)], 4.76-4.82 [1H, dd, J = 9.5, 5.5 Hz, C$\underline{H}$(2′)], 5.10-5.25 [1H, m, Ser C$_\alpha\underline{H}$], 5.27-5.35 [1H, m, C$\underline{H}$(4′)], 5.60-5.63 [1H, d, J = 7.5 Hz, peptide N$\underline{H}$], 6.84-6.96 [1H, s, C$\underline{H}$Ph$_2$], 7.20 [1H, s, C(6)$\underline{H}$], 7.27-7.37 [10H, m, Dpm aromatic C$\underline{H}$], 7.37-7.41 [2H, m, Fmoc aromatic C$\underline{H}$], 7.46-7.51 [2H, m, Fmoc aromatic C$\underline{H}$], 7.57 [2H, d, J = 7.5 Hz, Fmoc aromatic C$\underline{H}$], 7.64 [1H, m, benzoyl *p*-C$\underline{H}$], 7.73-7.77 and 7.87-7.91 [2x2H, 2x d, J = 8.0, 8.0 Hz, Fmoc aromatic C$\underline{H}$]; δ_C ($CDCl_3$, 125.65 MHz); 12.4 and 12.5 [thymine C$\underline{H}_3$ rotamers], 27.2 and 27.3 [^{t}Bu C$\underline{H}_3$], 33.4 and 33.9 [C$\underline{H}_2$(3′)], 47.0 [Fmoc aliphatic C$\underline{H}$], 49.0 and 50.3 [C$\underline{H}_2$(5′) rotamers], 52.0 and 52.3 [Ser C$_\alpha\underline{H}$ rotamers], 53.0 and 53.3 [C$\underline{H}$(2′) rotamers], 57.6 and 57.8 [C$\underline{H}$(4′)

rotamers], 63.2 and 64.0 [Ser $\underline{C}H_2$ rotamers], 67.1 [Fmoc aliphatic $\underline{C}H_2$], 73.9 and 74.0 [^{t}Bu $\underline{C}$ rotamers], 78.4 and 79.5 [$\underline{C}HPh_2$ rotamers], 111.9 [$\underline{C}$(5)], 120.0 [Fmoc aromatic $\underline{C}$H], 129.1-130.4 [Dpm aromatic $\underline{C}$H], 131.4 [$\underline{C}$(1) benzoyl], 135.1, 135.2 and 139.8 [$\underline{C}$(6) H and benzoyl *p*-$\underline{C}$H], 139.1 and 139.5 [aromatic $\underline{C}$ rotamers], 143.6 and 143.8 [Fmoc aromatic $\underline{C}$], 149.7 [$\underline{C}$(2)], 155.8 [Fmoc $\underline{C}$O], 162.2 [$\underline{C}$(4)], 168.6 [benzoyl $\underline{C}$O], 170.3 [peptide $\underline{C}$O], 170.4 [ester $\underline{C}$O]; ν_{max} (KBr)/cm^{-1}; 3330 (amide NH), 3070 (aromatic CH), 2967 and 2925 (aliphatic CH), 1752 (ester CO) 1702 and 1656 (amide CO), 1504 and 1450 (aromatic C=C), 1280, 1256, 1185, 768, 743, 702; $[\alpha]_D^{26}$ +24.3 (c=1.05, $CHCl_3$).

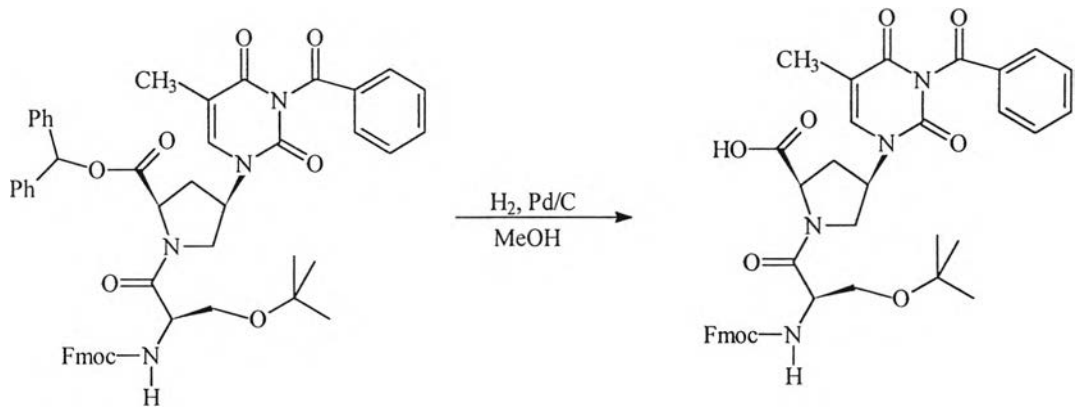
_N_-(_N_-Fluoren-9-ylmethoxycarbonyl-_O-tert_-butyl-L-seryl)-_cis_-4-(_N_^{3}-benzoylthymin-1-yl)-D-proline (10)



The protected L-serylproline dipeptide **(8)** (0.38 g, 0.43 mmol) and Pd/C (0.10 g, 30 %) were suspended in 10 mL of methanol with stirring under close system. The air was removed by connecting to a vacuum pump, and the atmosphere was replaced by nitrogen balloon followed by a hydrogen balloon respectively. The reaction was allowed to stir for 1 hr, afterwhich, TLC monitoring indicated complete deprotection. The reaction flask was carefully flushed with nitrogen and the used Pd/C catalyst was filtered off through celite and washed with methanol three times. The filtrate was evaporated by rotary evaporator to give a viscous residue which was further treated with hexane/ether to give white solids **(10)** (0.23 g, 74 %). The crude product was used for the next step without purification.

mp.= 148-151 °C; δ_H ($CDCl_3$, 500MHz); 1.11-1.34 [11H, m, ^tBu $C\underline{H}_3$, $C\underline{H}_2(3')$], 1.76-1.93 [3H, s, thymine $C\underline{H}_3$], 2.10-2.65 [1H, m, $1xC\underline{H}_2$(Ser)], 2.70-3.25 [3H, m, O$\underline{H}$ acid, $1xC\underline{H}_2$ (Ser), $1xC\underline{H}_2(5')$], 3.45-3.60 [1H, m, $1xC\underline{H}_2(5')$], 3.79-4.60 [5H, m, Fmoc aliphatic C$\underline{H}$, $C\underline{H}_2$, Ser$C_\alpha\underline{H}$, C$\underline{H}$(2′)], 4.78-5.19 [1H, m, C$\underline{H}$(4′)], 7.24-7.90 [14H, m, Fmoc aromatic C$\underline{H}$, benzoyl-C$\underline{H}$, thymine C(6)$\underline{H}$]; ν_{max} (KBr)/cm^{-1}; 3471 (OH acid), 3070 (aromatic CH), 2983 and 2925 (aliphatic CH), 1756 and 1706 (ester CO), 1656 (amide CO), 1541, 1450 (aromatic C=C), 1285 and 1247 (ester C-O), 1198, 772 and 739.

_N_-(_N_-Fluoren-9-ylmethoxycarbonyl-_O-tert_-butyl-D-seryl)-_cis_-4-(_N_^{3}-benzoylthymin-1-yl)-D-proline (11)

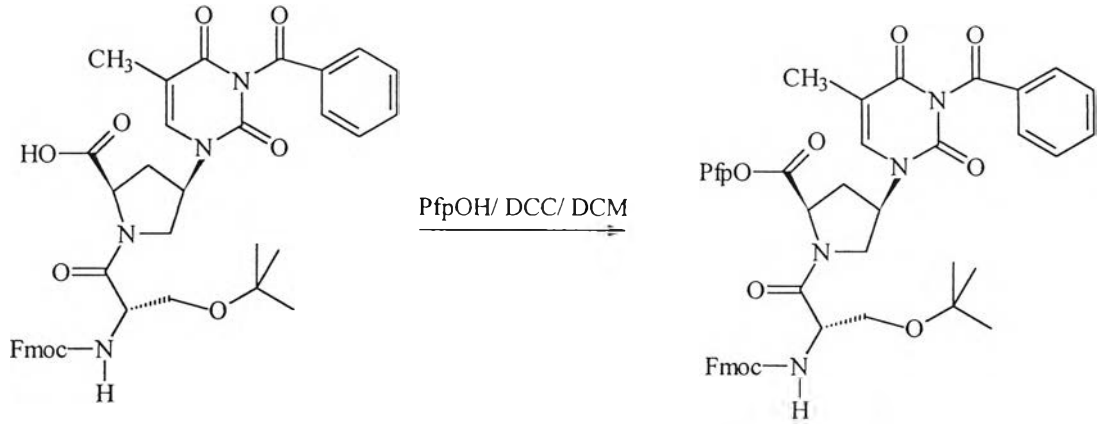


(9) **(11)**

Similar reaction sequence as for D-series was performed starting from the protected D- serylproline dipeptide **(9)** (0.79 g, 0.90 mmol), Pd/C (0.24 g, 30 %) and methanol (10 mL) to give the product **(11)** as a white solid (0.51 g, 80 %) .

mp.= 148-151 °C; δ_H ($CDCl_3$, 500 MHz); 1.17-1.25 [11H, m, ^tBu $C\underline{H}_3$, $C\underline{H}_2(3')$], 2.85 [3H, s, thymine $C\underline{H}_3$], 3.33-4.85 [7H, m, $C\underline{H}_2$(Ser), $C\underline{H}_2(5')$ Fmoc aliphatic C$\underline{H}$, $C\underline{H}_2$], 4.90-5.77 [3H, m, Ser$C_\alpha\underline{H}$, C$\underline{H}$(2′), C$\underline{H}$(4′)], 7.04-7.89 [14H, m, Fmoc aromatic C$\underline{H}$, benzoyl-C$\underline{H}$, thymine C(6)$\underline{H}$]; ν_{max} (KBr)/cm^{-1}; 3457 (OH), 3063 (aromatic CH), 2981 and 2934 (aliphatic CH), 1752 and 1705 (ester CO), 1658 (amide CO), 1455 (aromatic C=C), 1287 and 1244 (ester C-O), 768, 744.

N-(N-Fluoren-9-ylmethoxycarbonyl-O-*tert*-butyl-L-seryl)-*cis*-4-(N^3-benzoylthymin-1-yl)-D-proline pentafluorophenyl ester (12)



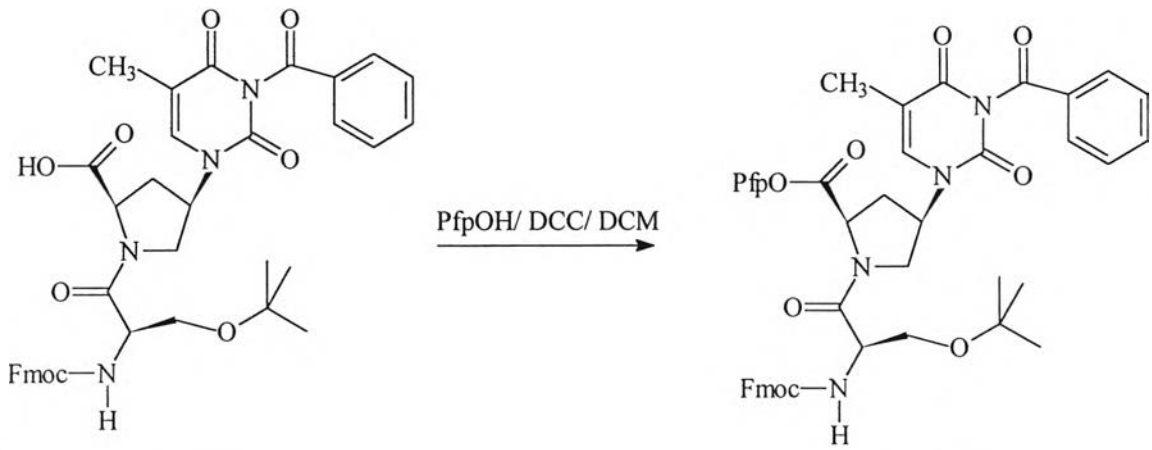
(10) **(12)**

In a dried 100 mL round bottom flask, equipped with a magnetic bar, a mixture of the Fmoc-L-serylproline free acid **(10)** (0.43 g, 0.61 mmol), and pentafluorophenol (0.13 g, 0.67 mmol) were dissolved in 5 mL dichloromethane and stirred at room temperature. DCC (0.10 g, 0.49 mmol) was added. After 30 min, the white precipitate of dicyclohexylurea formed was filtered off. The filtrate was evaporated to dryness by rotary evaporator and the residue was further purified by flash column chromatography (SiO_2, dichloromethane:ethyl acetate (20:1). The pure fraction was collected and evaporate to dryness, then scratched with hexane to give the active ester **(12)** as a white solid (0.16 g, 30 %).

mp.= 112-113 °C, (Found C, 61.7; H, 4.8; N, 6.5 %; requires C, 61.8; H, 4.5; N, 6.4 %); δ_H ($CDCl_3$, 500 MHz); 1.16 [9H, s, ^{t}Bu C$\underline{H}_3$], 1.97 [3H, s, thymine C$\underline{H}_3$], 2.33-2.42 [1H, m, 1xC$\underline{H}_2$(3′)], 2.90-2.99 [1H, m, 1xC$\underline{H}_2$(3′)], 3.51 [1H, t, J = 9.0 Hz, 1xC$\underline{H}_2$(Ser)], 3.64-3.67 [1H, m, 1xC$\underline{H}_2$(Ser)], 3.88-4.03 [1H, m, 1xC$\underline{H}_2$(5′)], 4.20 [1H, t, J = 7.0 Hz, Fmoc aliphatic C$\underline{H}$], 4.32-4.41 [2H, m, Fmoc aliphatic C$\underline{H}_2$], 4.46-4.54 [1H, m, 1xC$\underline{H}_2$(5′)], 4.68-4.76 [1H, m, Ser$C_\alpha\underline{H}$], 4.80-4.86 [1H, m, CH(2′)], 5.26-5.34 [1H, m, C$\underline{H}$(4′)], 5.60 [1H, d, J = 8.0 Hz, peptide N$\underline{H}$], 7.27-7.32 [1H, m, C(6)$\underline{H}$], 7.34-7.42 [2H, m, Fmoc aromatic C$\underline{H}$], 7.50 [2H, t, J = 7.5 Hz, benzoyl *m*-C$\underline{H}$], 7.56 [2H, t, J = 6.5 Hz, Fmoc aromatic C$\underline{H}$], 7.65 [1H, t, J = 7.5 Hz, benzoyl *p*-C$\underline{H}$], 7.73-7.78 and 7.89-7.93 [2x2H, 2x d, J = 7.5 Hz,

Fmoc aromatic C<u>H</u>]; δ_C ($CDCl_3$, 125.65 MHz); 12.3 [thymine <u>C</u>H_3], 27.3 [^{t}Bu <u>C</u>H_3], 33.7 [<u>C</u>H_2(3′)], 47.0 [Fmoc aliphatic <u>C</u>H], 49.1 [<u>C</u>H_2(5′)], 52.5 [Ser <u>C</u>$_\alpha$H], 53.2 [<u>C</u>H(2′)], 56.8 [<u>C</u>H(4′)], 63.0 [Ser <u>C</u>H_2], 67.4 [Fmoc aliphatic <u>C</u>H_2], 74.0 [^{t}Bu <u>C</u>], 112.1 [<u>C</u>(5)], 120.0 [Fmoc aromatic <u>C</u>H], 125.0-130.4 [Fmoc aromatic <u>C</u>H], 131.3 [<u>C</u>(1)-benzoyl], 135.1 and 135.4 [<u>C</u>(6)H and benzoyl *p*-<u>C</u>H], 141.2 and 141.3 [pentafluorophenyl <u>C</u> rotamers], 143.4 and 143.7 [Fmoc aromatic <u>C</u>], 149.8 [<u>C</u>(2)], 157.2 and 156.0 [Fmoc <u>C</u>O rotamers], 162.3 [<u>C</u>(4)], 167.1 [benzoyl <u>C</u>O], 168.5 [peptide <u>C</u>O], 171.1 [ester <u>C</u>O]; ν_{max} (KBr)/cm^{-1}; 3326 (amide NH), 3078 (aromatic CH), 2983, 2925 and 2855 (aliphatic CH), 1801 (pentafluorophenyl ester CO), 1752 and 1702 (ester CO), 1665 (amide CO), 955, 768 and 739.

_N_-(_N_-Fluoren-9-ylmethoxycarbonyl-_O-tert_-butyl-D-seryl)-_cis_-4-(_N_^{3}-benzoylthymin-1-yl)-D-proline pentafluorophenyl ester (13)

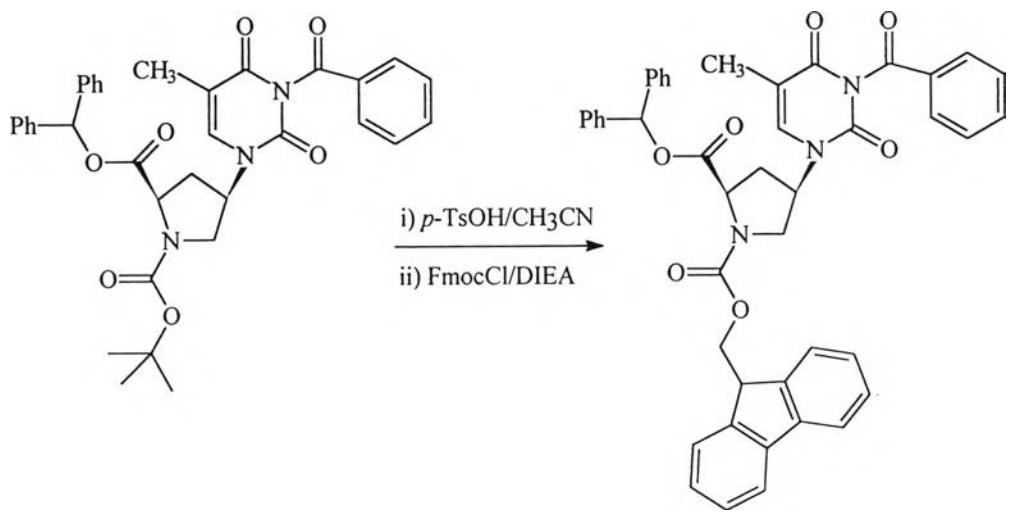


Synthesis of the D-series can be done in the same manner as above using Fmoc-D-serylproline free acid **(11)** (0.50 g, 0.71 mmol), pentafluorophenol (0.15 g, 0.78 mmol) and DCC (0.14 g, 0.78 mmol) to afford the active ester **(13)** as a white solid (0.11 g, 18 %).

mp = 109-110 °C, (Found C, 61.8; H, 4.6; N, 6.3 % requires C, 61.8; H, 4.5; N, 6.4 %); δ_H ($CDCl_3$, 500 MHz); 1.17 [9H, s, ^{t}Bu C<u>H</u>$_3$], 1.96 [3H, s, thymine C<u>H</u>$_3$], 2.34-2.42 [1H, m, 1xC<u>H</u>$_2$(3′)], 2.95 [1H, ddd, J = 16.9, 13.6, 8.5 Hz, 1xC<u>H</u>$_2$(3′)], 3.54 [1H, t, J = 8.5 Hz,

1xC$\underline{H}_2$(Ser)], 3.64 [1H, dd, J = 8.2, 5.2 Hz, 1xC$\underline{H}_2$(Ser)], 4.06-4.12 [1H, m, 1xC$\underline{H}_2$(5′)], 4.19 [1H, t, J = 7.0 Hz, Fmoc aliphatic C$\underline{H}$], 4.31-4.35 [1H, m, 1xC$\underline{H}_2$(5′)], 4.36-4.38 [2H, m, Fmoc aliphatic C$\underline{H}_2$], 4.62-4.69 [1H, m, SerC$_\alpha\underline{H}$], 4.88 [1H, t, J = 8.0 Hz, C$\underline{H}$(2′)], 5.28-5.35 [1H, m, C$\underline{H}$(4′)], 5.67 [1H, d, J = 8.0 Hz, peptide N$\underline{H}$], 7.22 [1H, s, C(6)$\underline{H}$], 7.28-7.33 [2H, 2x t, J = 7.5 Hz, Fmoc aromatic C$\underline{H}$], 7.39 [2H, t, J = 7.5 Hz, benzoyl *m*-C$\underline{H}$], 7.50 [2H, t, J = 7.5 Hz, Fmoc aromatic C$\underline{H}$], 7.57 [2H, d, J = 7.5 Hz, benzoyl *o*-C$\underline{H}$], 7.66 [1H, t, J = 7.5 Hz, benzoyl *p*-C$\underline{H}$], 7.73-7.78 and 7.89-7.94 [2x2H, 2x d, J = 7.5 Hz, Fmoc aromatic C$\underline{H}$]; δ_C ($CDCl_3$, 125.65 MHz); 12.6 [thymine C$\underline{H}_3$], 27.2 and 27.3 [^{t}Bu C$\underline{H}_3$ rotamers], 33.1 and 33.7 [C$\underline{H}_2$(3′) rotamers], 47.0 [Fmoc aliphatic C$\underline{H}$], 49.7 [C$\underline{H}_2$(5′)], 52.8 [Ser C$_\alpha\underline{H}$], 53.9 [C$\underline{H}$(2′)], 57.2 [C$\underline{H}$(4′)], 63.2 [Ser C$\underline{H}_2$ rotamers], 67.3 [Fmoc aliphatic C$\underline{H}_2$], 74.0 [^{t}Bu $\underline{C}$], 112.3 [$\underline{C}$(5)], 120.0 [Fmoc aromatic C$\underline{H}$], 125.0 and 130.4 [Fmoc aromatic C$\underline{H}$], 131.3 [$\underline{C}$(1) benzoyl], 135.2 and 135.6 [$\underline{C}$(6)H and benzoyl *p*-C$\underline{H}$], 141.3 [pentafluorophenyl $\underline{C}$], 143.6 and 143.7 [Fmoc aromatic $\underline{C}$], 149.7 [$\underline{C}$(2)], 155.8 [Fmoc $\underline{C}$O], 162.3 [$\underline{C}$(4)], 167.4 [benzoyl $\underline{C}$O], 168.5 [peptide $\underline{C}$O], 170.9 [ester $\underline{C}$O]; ν_{max} (KBr)/cm^{-1}; 3424 (amide NH), 3328 (aromatic CH), 2975 and 2931 (aliphaticCH), 1795 (pentafluorophenyl ester CO), 1756 and 1703 (ester CO), 1664 (amide CO), 1519 (aromatic C=C), 1451, 1369, 1240, 1001, 764 and 740.

_N_-(_N_-Fluoren-9-ylmethoxycarbonyl)-_cis_-4-(N^3-benzoylthymin-1-yl)-D-proline diphenylmethyl ester (14)

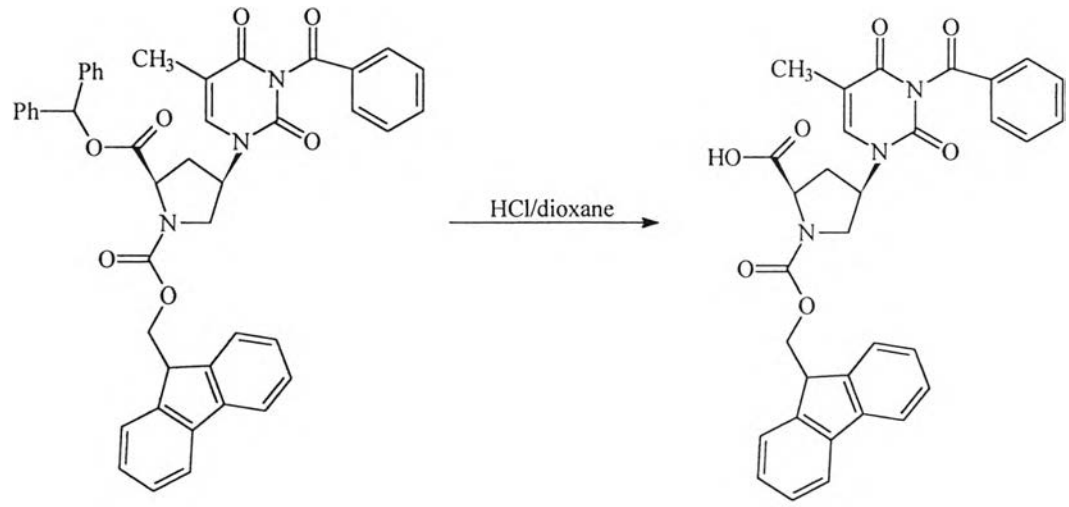


(7) (14)

Boc-D-Pro(*cis*-4-T^{Bz})-ODpm (**7**) (1.83 g, 3.0 mmol) and *p*-toluenesulfonic acid monohydrate (1.43 g, 7.5 mmol) were dissolved in anhydrous acetonitrile (2 mL). The deprotection was completed after stirring for 1.5 hr at room temperature as monitored by TLC analysis. Then acetonitrile (10 mL), diisopropylethylamine (1.8 mL, 10.5 mmol) and 9-fluorenylmethylchloroformate (0.85 g, 3.3 mmol) were added respectively. The solution was stirred for a further one hour then the solvent was removed by rotary evaporation, diluted with dichloromethane (50 mL) and extracted with 5% HCl (50 mL). The organic layer was washed with water (50 mL) three times and evaporated to dryness by rotary evaporation. The residue was chromatographed on silica gel eluting with hexane:ethyl acetate (7:3). The pure fractions was collected and evaporated to give viscous oil which after scratching with hexane gave a white solid **(14)** (1.53 g, 70 %).

mp.= 176-178 °C (Found C, 73.9; H, 5.2; N 5.8 %; $C_{45}H_{37}N_3O_7$ requires C, 73.9; H, 5.1; N 5.8 %); δ_H ($CDCl_3$, 500 MHz); 1.77 [3H, s, thymine C$\underline{H}_3$], 2.02-2.14 [1H, m, 1xC$\underline{H}_2$(3′)], 2.74-2.93 [1H, m, 1xC$\underline{H}_2$(3′)], 3.52-3.74 [1H, m, C$\underline{H}_2$(5′)], 3.85-4.05 [1H, m, Fmoc aliphatic C$\underline{H}$], 4.20-4.36 [1H, m, 1xFmoc aliphatic C$\underline{H}_2$], 4.40-4.66 [3H, m, 1xFmoc aliphatic C$\underline{H}_2$, 1xC$\underline{H}_2$(5′) and C$\underline{H}$(2′)], 5.16-5.30 [1H, m, C$\underline{H}$(4′)], 6.77-6.92 [1H, br d, C$\underline{H}$Ph$_2$ rotamers], 7.10 [1H, s, C(6)$\underline{H}$ thymine], 7.16-7.80 [18H, m, Dpm aromatic C$\underline{H}$, Fmoc aromatic C$\underline{H}$, benzoyl-C$\underline{H}$], 7.87 [2H, br s, Fmoc aromatic C$\underline{H}$]; δ_C ($CDCl_3$, 125.65 MHz); 12.3 [thymine $\underline{C}H_3$], 34.3 and 35.8 [$\underline{C}H_2$(3′) rotamers], 47.0 and 47.1 [Fmoc aliphatic $\underline{C}$H], 49.6 and 49.7 [$\underline{C}H_2$(5′) rotamers], 52.2 and 52.7 [$\underline{C}$H(2′) rotamers], 57.4 and 57.9 [$\underline{C}$H(4′) rotamers], 68.0 [Fmoc aliphatic $\underline{C}H_2$], 78.6 and 78.7 [$\underline{C}$HPh$_2$ rotamers], 111.6 and 112.0 [$\underline{C}$(5) rotamers], 120.0 and 120.1 [Fmoc aromatic $\underline{C}$H], 124.9-130.4 [Dpm aromatic and Fmoc aromatic $\underline{C}$H], 131.5 [$\underline{C}$(1) benzoyl], 135.1, 135.5 and 135.6 [$\underline{C}$(6)H, benzoyl *p*-$\underline{C}$H], 139.0 and 139.4 [Dpm aromatic $\underline{C}$ rotamers], 141.3 [Fmoc aromatic $\underline{C}$], 143.3 and 143.9 [Fmoc $\underline{C}$O rotamers], 150.0 [$\underline{C}$(2)], 162.3 [$\underline{C}$(4)], 168.7 [benzoyl $\underline{C}$O], 170.7 and 171.0 [ester $\underline{C}$O]; ν_{max} (KBr)/cm^{-1}; 3064 (aromatic CH), 2945 (aliphatic CH), 1749 and 1699 (ester CO), 1660 (amide CO), 1599 and 1450 (aromatic C=C), 1284 and 1176 (ester CO), 976, 762 and 741.

N-(N-Fluoren-9-ylmethoxycarbonyl)-*cis*-4-(N^3-benzoylthymin-1-yl)-D-proline (15)



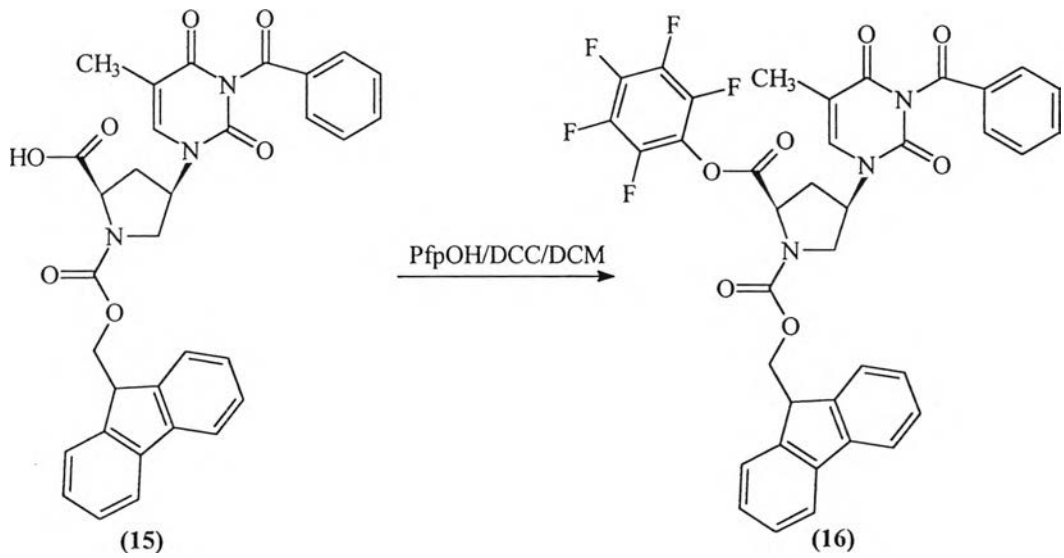
(14) **(15)**

In a dried 100 mL round bottom flask, equipped with a magnetic bar, Fmoc-D-Pro (*cis*-4-T^{Bz})ODpm **(14)** (0.46 g, 0.63 mmol) was dissolved in 5 mL of saturated anhydrous hydrogen chloride in dioxane (~4.4 M) at room temperature with stirring. Complete cleavage of diphenylmethyl ester was observed after stirring for 2 hr by TLC analysis. The reaction mixture was diluted with 20 mL of dichloromethane and evaporated to dryness by rotary evaporation. The procedure was repeated twice to remove any residual HCl. The residue was recrystallized from hexane/diethyl ether. The slightly yellow solid was obtained and was triturated with diethyl ether to give a white solid **(15)** (0.36 g, 94 %).

mp.= 119-120 °C, (Found C, 68.0; H, 4.9; N, 7.3 %; $C_{32}H_{27}N_3O_7$ requires C, 68.0; H, 4.8; N, 7.3 %); δH ($CDCl_3$, 500MHz); 1.89 [3H, s, thymine CH_3], 2.22-2.31 [1H, m, 1xCH_2(3′)], 2.65-2.75 [1H, m, 1xCH_2(3′)], 3.44-3.54 [1H, m, 1xCH_2(5′)], 3.56-3.78 [1H, m, OH], 3.82-3.98 [1H, m, Fmoc aliphatic CH], 4.11-4.54 [4H, m, Fmoc aliphatic CH_2, 1xCH_2(5′), CH(2′)], 5.00-5.19 [1H, m, CH(4′)], 6.75-7.00 [1H, m, C(6)H thymine], 7.20-7.42 [6H, m, Fmoc aromaticCH], 7.42-7.65 [3H, m, benzoyl *p*-CH and benzoyl *m*-CH], 7.68-7.77 [2H, m, benzoyl *o*-CH], 7.85-7.94 [2H, m, Fmoc aromatic CH]; δ_C ($CDCl_3$, 125.65); 12.5 and 14.0 [thymine CH_3 rotamers], 33.7 and 35.6 [CH_2(3′) rotamers], 47.0 [Fmoc aliphatic CH], 49.2 [CH_2(5′)], 53.0 [CH(2′)], 56.8 and 57.5 [CH(4′) rotamers], 67.9 and 68.1 [Fmoc

aliphatic $\underline{C}H_2$ rotamers], 111.0 and 111.7 [$\underline{C}$(5) rotamers], 120.0-130.4 [Fmoc aromatic $\underline{C}$H, benzoyl *m*-$\underline{C}$H and benzoyl *o*-$\underline{C}$H], 131.3 [benzoyl $\underline{C}$], 135.2 and 136.1 [$\underline{C}$(6)H and benzoyl *p*-$\underline{C}$H], 141.2 [Fmoc aromatic $\underline{C}$], 143.3, 143.5 and 143.7 [Fmoc $\underline{C}$O rotamers], 149.8 and 150.8 [$\underline{C}$H(2) rotamers], 162.5 and 162.7 [$\underline{C}$(4) rotamers], 168.8 [benzoyl $\underline{C}$O], 174.1 and 175.1 [acid $\underline{C}$O]; ν_{max} (KBr)/cm^{-1}; 3496 (acid OH), 3066 (aromatic CH), 1752 (ester CO),1690 (amide CO), 1462 (aromaticC=C), 764 and 739.

_N_-(_N_-Fluoren-9-ylmethoxycarbonyl)-*cis*-4-(N^3-benzoylthymin-1-yl)-D-proline pentafluorophenyl ester (16)



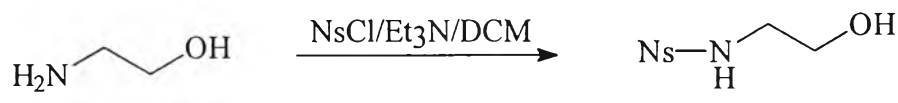
(15) (16)

In a dried 100 mL round bottom flask containing a magnetic bar, Fmoc-D-Pro(*cis*-4-T^{Bz})OH **(15)** (0.17 g, 0.30 mmol) and pentafluorophenol (0.06 g, 0.30 mmol), were dissolved in 5 mL dichloromethane. DCC (0.05 g, 0.24 mmol) was added and the solution was allowed to stir for 30 min at room temperature under nitrogen. After the reaction was completed as judged by TLC analysis, the white precipitate of dicyclohexyl urea was filtered off. The filtrate was evaporated by rotary evaporation and the residue was chromatographed using hexane:ethyl acetate (1:1) as eluent. The pure fractions was collected, evaporated and scratched with hexane to give the product **(16)** as a white solid (0.12 g, 55 %).

mp.= 68-70 °C, (Found C, 62.4; H, 3.8; N, 5.8 %; $C_{38}H_{26}N_3O_7F_5$ requires C, 62.4; H, 3.6; N, 5.7 %); δ_H ($CDCl_3$, 125.65 MHz); 1.95 [3H, s, thymine CH_3], 2.30-2.44 [1H, m, 1xCH_2 (3′)], 2.92-3.02 [1H, m, 1xCH_2(3′)], 3.58-4.07 [2H, m, CH_2(5′)], 4.18-4.28 [1H, m, Fmoc aliphatic CH], 4.50-4.57 [2H, m, Fmoc aliphatic CH_2], 4.60-4.79 [1H, m, CH(2′)], 5.14-5.29 [1H, m, CH(4′)], 7.18-7.26 [1H, m, Fmoc aromatic CH], 7.26-7.33 [1H, m, Fmoc aromatic CH], 7.39 [2H, t, J = 7.5 Hz, Fmoc aromatic CH], 7.49 [2H, t, J = 7.5 Hz, benzoyl *m*-CH], 7.55 [1H, d, J = 7.5 Hz, Fmoc aromatic CH], 7.61-7.68 [1H, m, benzoyl *p*-CH], 7.76 [2H, d, J = 7.5 Hz, Fmoc aromatic CH], 7.88-7.93 [3H, m, benzoyl *o*-CH, Fmoc aromatic CH]; δ_C ($CDCl_3$, 125.65 MHz); 12.6 [thymine CH_3], 33.7 and 34.5 [CH_2(3′) rotamers], 47.1 [Fmoc aliphatic CH], 49.0 and 49.4 [CH_2(5′) rotamers], 52.8 and 53.2 [CH (2′) rotamers], 56.8 and 57.2 [CH(4′) rotamers], 68.2 and 68.5 [Fmoc aliphatic CH_2 rotamers], 111.8 and 112.2 [C(5) rotamers], 120.1-130.4 [Fmoc aromatic CH], 131.3 [C(1) benzoyl], 135.2, 135.4 and 135.6 [C(6)H and benzoyl *p*-CH], 141.3 [Fmoc aromatic C], 143.0, 143.3, 143.5 and 143.8 [Fmoc CO rotamers], 149.9 [C(2)], 162.4 [C(4)], 168.1 [benzoyl CO], 168.6 [pentafluorophenyl ester CO]; ν_{max} (KBr)/cm^{-1}; 3475 (OH acid), 3074 (aromatic CH), 2934 (aliphaicCH),1756 and 1706 (ester CO),1652 (amide CO), 1524 and 1450 (aromatic C=C),1020, 995 and 979 (ester C-O), 768 and 739.

2.3.3 Synthesis of deoxyglycylproline cPNA monomer

_N_-(2-hydroxyethyl)-4-nitrobenzenesulfonamide (17)



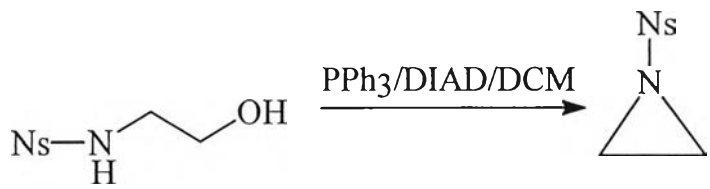
(17)

To a stirred solution of ethanolamine (1.47 g, 24.0 mmol) and triethylamine (2.8 mL, 20.0 mmol) in 50 mL of dried dichloromethane was carefully added 4-nitrobenzene sulfonyl chloride (4.44 g, 20.0 mmol) portionwise. The resulting clear solution was allowed to stir at room temperature for 30 min.The solution was diluted with 50 mL dichloromethane and washed with water. The aqueous layer was acidified with 5 %

hydrochloric acid and then re-extracted with 3x 50 mL of ethyl acetate. The combined organic extracts was washed with water and dried over magnesium sulfate. Evaporation of the solvent gave dark yellow crystalline solids **(17)** (4.03 g, 82 %) which was dried under reduced pressure for Mitsunobu reaction in next step.

mp.= 127-129 °C (Found C, 39.1; H, 4.3; N, 11.4 % $C_8H_{10}N_2O_5S$ requires C, 39.0; H, 4.1; N, 11.4 %); δ_H (DMSO-d_6, 500 MHz); 2.86 [2H, q, J = 12.0 and 6.0, NHC$\underline{H}_2$CH$_2$OH], 3.35-3.40 [2H, m, NHCH$_2$C$\underline{H}_2$OH], 4.71 [1H, t, J= 5.0 Hz, O$\underline{H}$], 8.00 [1H, t, J = 6.0 Hz, N$\underline{H}$], 8.02 and 8.41 [2x2H, q, (AA′XX′) J_{AX} = 9.0 Hz, 2xNosyl C$\underline{H}$]; δ_C (DMSO-d_6, 125.65 MHz); 45.2 [NH$\underline{C}$H$_2$CH$_2$ OH], 59.9 [NHCH$_2\underline{C}$H$_2$OH], 124.5 and 128.0 [Nosyl $\underline{C}$H], 146.4 and 149.5 [Nosyl $\underline{C}$]; ν_{max} (KBr)/cm^{-1}; 3458 (NH and OH), 3170, 3140 and 3050 (aromatic CH), 2902 (aliphatic CH), 1604 (aromatic C=C), 1529 (N=O stretch), 1477 (aromatic C=C), 1348 and 1165 (-SO_2- stretch), 1086 (C–O stretch), 854 (C–N stretch).

_N_-4-Nitrobenzene sulfonylaziridine (18)

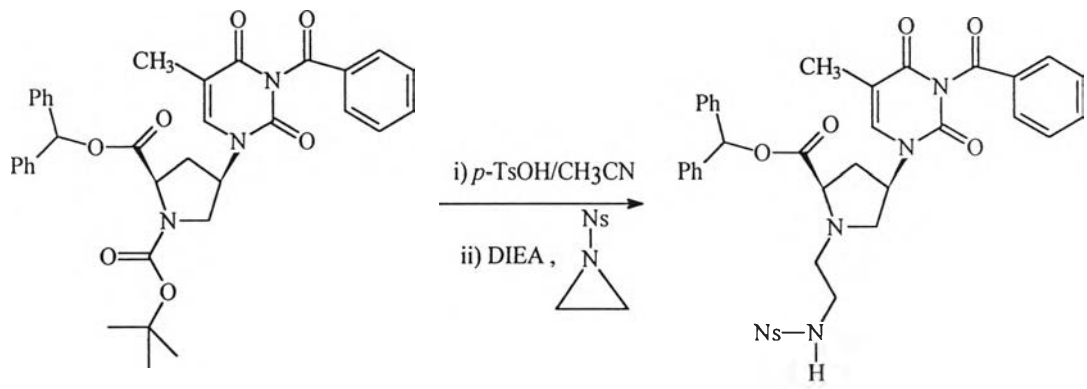


(17) **(18)**

In a dried 100 mL round bottom flask containing a magnetic bar, *N*-(2-hydroxylethylnitrobenzene sulfonamide **(17)** (1.23 g, 5.0 mmol) and triphenylphosphine (1.44 g, 5.5 mmol) were dissolved in dried dichloromethane. The solution was stirred in an ice-bath at 0 °C. DIAD (1.07 mL, 5.5 mmol) was added dropwise until over a period of 5 min. The solution was allowed to stir for 15 min when TLC analysis revealed that reaction was completed, the solvent was immediately evaporated to dryness under reduced pressure without heating. The crude product was purified by flash column chromatography on silica gel using dichloromethane:hexane (3:1) as eluent with dry-loading technique. The product **(18)** was obtained as bright yellow needles (0.77 g, 68 %) and was dried under reduced pressure.

mp. = 134-135 °C (Found C, 42.3; H, 3.5; N, 12.2; $C_8H_8N_2O_4S$ requires C, 42.1; H, 3.5; N, 12.2 %); δ_H ($CDCl_3$, 500 MHz); 2.45 [4H, s, 2xaziridine $C\underline{H}_2$], 8.12 and 8.39 [2x2H, q, J_{AX} = 9.0 Hz, Nosyl C<u>H</u> (AA'XX')]; δ_C ($CDCl_3$, 125.65 MHz); 28.1 [aziridine 2x$\underline{C}H_2$], 124.3 and 129.2 [Nosyl <u>C</u>H], 143.7 and 150.6 [Nosyl <u>C</u>]; ν_{max} (KBr)/cm^{-1}; 3120 and 3102 (aromatic CH), 2947 and 2862 (aliphatic CH), 1606 (aromatic C=C), 1529 (N=O str), 1329, 1167 (-SO_2- stretch), 1095, 916, 800, 742.

_N_-2-(4'-Nitrobenzenesulfonamidoethyl)-*cis*-4-(N^3-benzoylthymin-1-yl)-D-proline diphenylmethyl ester (19)



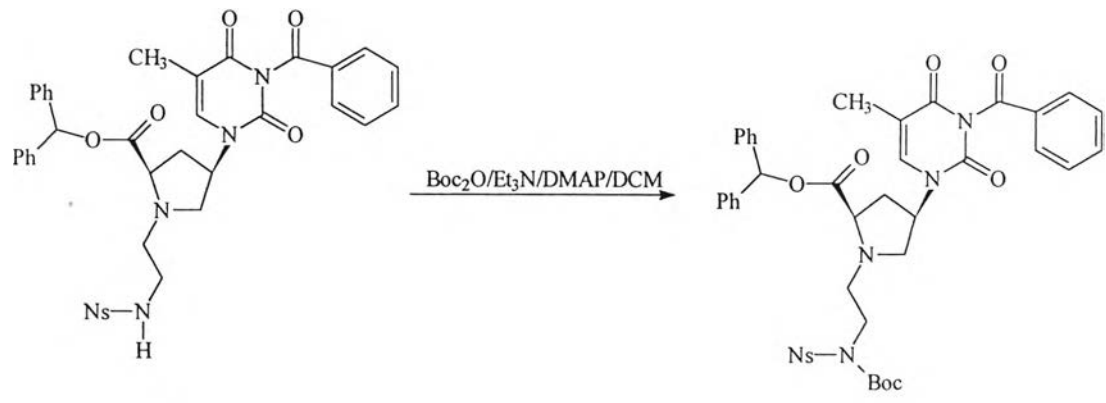
(7) **(19)**

In a dried 100 mL round bottom flask, Boc-D-Pro(*cis*-4-T^{Bz})-ODpm (**7**) (3.66 g, 6.0 mmol) and *p*-toluenesulfonic acid monohydrate (2.86 g, 15.0 mmol) were suspended in anhydrous acetonitrile (7 mL). The suspension was allowed to stir at room temperature which gradually became homogeneous. After 1 hr, a white solid reformed which indicated the complete deprotection of the Boc-group. The results corresponded to TLC analysis. The suspension was diluted with 50 mL of anhydrous acetonitrile and then diisopropylethylamine (3.6 mL, 21.0 mmol) was added to pH 8. *N*-Nosylaziridine **(18)** (1.66 g, 7.2 mmol) was added portionwise into the stirred solution. The solution was allowed to stir under nitrogen overnight at room temperature. The solvent was evaporated to dryness and the residue was redissolved in dichloromethane. The dichloromethane solution was extracted with 5 % hydrochloric acid and washed with water three times. The

organic layer was dried over magnesium sulfate and then evaporated to dryness. The residue was purified by flash column chromatography using dichloromethane:ethyl acetate (20:1) as eluent to afford slightly yellow crystalline solids. Drying under reduced pressure to yield **(19)** (3.54 g, 80 %).

mp.= 172-175 °C (Found C, 61.7; H, 4.8; N, 9.3 %; $C_{38}H_{35}N_5O_9S$ requires C, 61.9; H, 4.8; N, 9.5 %); δ_H ($CDCl_3$, 500 MHz); 1.91-1.94 [1H, m, 1xCH_2(3′)], 1.95 [3H, s, thymine CH_3], 2.60-2.67 and 2.68-2.75 [2H, 2x m, NsNHCH$_2$CH_2N], 2.76-2.80 [1H, m, 1x NsNHCH_2CH$_2$N], 2.81-2.86 [1H, m, 1xCH_2(5′)], 2.87-2.94 [1H, m, 1xCH_2(3′)], 3.18-3.23 [1H, m, 1xNsNHCH_2CH$_2$N], 3.24-3.27 [1H, m, 1xCH_2(5′)], 3.45 [1H, dd, J = 10.0, 7.0 Hz, CH(2′)], 5.18-5.26 [1H, m, CH(4′)], 5.91-5.94 [1H, m, NH], 6.87 [1H, s, CHPh$_2$], 7.24-7.38 [10H, m, Dpm aromatic CH], 7.46 [2H, t, J = 8.0 Hz, benzoyl *m*-CH], 7.63 [1H, t, J = 8.0 Hz, benzoyl *p*-CH], 7.75 [1H, s, thymine C(6)H], 7.88 [2H, d, J = 8.0 Hz, benzoyl *o*-CH], 7.98 and 8.25 [4H, q, 2xNosyl CH (AA′XX′), J_{AX} = 9.0 Hz]; δ_C ($CDCl_3$, 125.65 MHz); 12.6 [thymine CH_3], 36.7 [CH_2(3′)], 41.2 [NsNHCH$_2$CH$_2$N], 52.4 [CH(2′)], 52.9 [CH_2(5′)], 58.2 [CH(4′)], 64.5 [NsNHCH_2CH$_2$N], 78.5 [CHPh$_2$], 112.2 [C(5) thymine], 124.3-130.4 [Nosyl CH and Dpm aromatic CH], 131.6 [C(1)-benzoyl], 134.9 [benzoyl *p*–CH], 136.7 [CH(6) thymine], 138.9 and 139.0 [Dpm aromatic C], 145.9 [Nosyl C], 149.9 [C(2) thymine], 162.6 [C(4) thymine], 169.0 [benzoyl CO],173.0 [ester CO]; ν_{max} (KBr)/cm^{-1}; 3251 and 3068 (aromatic CH), 2943 and 2819 (aliphatic CH), 1755 (ester CO), 1693 and 1655 (amide CO), 1523 (N=O stretch), 1448 (aromatic C=C), 1281 (C-N), 1171 (-SO_2- stretch),1095, 737, 696; $[\alpha]_D^{23}$ +22.9 (c=1.04, $CHCl_3$).

***N*-2-(*N*-*tert*-Butoxycarbonylamino,*N*-4-Nitrobenzenesulfonamido)ethyl-*cis*-4-(N^3-benzoylthymin-1-yl)-D-proline diphenylmethyl ester (20)**



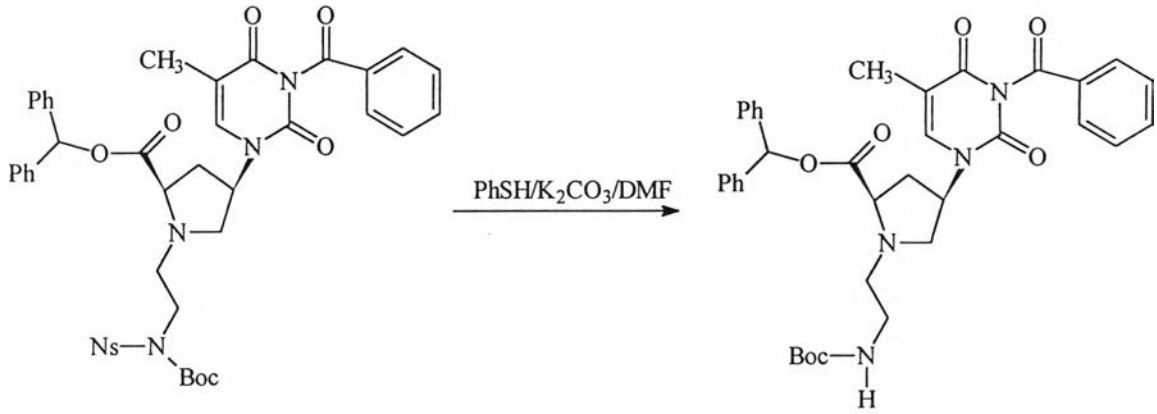
(19) (20)

Ns-(ψ-CH_2)Gly-D-Pro(*cis*-4-T^{Bz})-ODpm **(19)** (3.10 g, 4.2 mmol), 4-dimethylaminopyridine (0.05 g, 0.42 mmol) and triethylamine (0.7 mL, 5.04 mmol) were dissolved in dichloromethane (30 mL) with stirring. To the mixture was added di-*tert*-butyl dicarbonate (1.10 g, 5.04 mmol) dropwise. The resulting solution became orange and carbon dioxide bubbles emerged. The solution was allowed to stir under nitrogen at room temperature overnight. The solvent was removed under reduced pressure, the residue was dissolved in dichloromethane and extracted with 5% hydrochloric acid and washed with water. This organic layer was dried over magnesium sulfate and evaporated to give yellow oil. This oil was purified by flash column chromatography eluting with hexane:ethyl acetate (1:1) on silica gel to give clear viscous oil. Scratching with cold hexane gave the required product **(20)** as light yellow crystalline solids (3.38 g, 96 %).

mp.= 112-113 °C, (Found C, 61.2; H, 5.2; N, 8.1 %; $C_{43}H_{43}N_5O_{11}S$ requires C, 61.6; H, 5.2; N, 8.4 %); δ_H ($CDCl_3$, 500 MHz); 1.25 [9H, s, ^{t}Bu C$\underline{H}_3$], 1.63 [3H, s, thymine C$\underline{H}_3$], 1.95 [1H, ddd, *J* = 13.5, 8.5, 4.5 Hz, C$\underline{H}_2$(3′)], 2.51 [1H, dt, *J* = 13.0, 4.0 Hz, 1x(Ns/Boc) NCH$_2$C$\underline{H}_2$N], 2.74 [1H, dd, *J* = 1.0, 8.0 Hz, C$\underline{H}_2$(5′)], 2.87 [1H, ddd, J = 14.5, 9.0, 5.5, 1xC$\underline{H}_2$(3′)], 3.31 [1H, ddd, *J* = 13.0, 9.0, 5.0 Hz, 1x(Ns/Boc)NCH$_2$C$\underline{H}_2$N], 3.42 [1H, t, *J* = 8.5 Hz, C$\underline{H}$(2′)], 3.55 [1H, br d, *J* = 10.5 Hz, 1xC$\underline{H}_2$(5′)], 3.85 [1H, dt, *J* = 14.5, 4.0 Hz, (Ns/Boc)NC$\underline{H}_2$CH$_2$N], 4.07 [1H, ddd, *J* = 14.5, 9.5, 5.0 Hz, (Ns/Boc)NC$\underline{H}_2$CH$_2$N], 5.31 [1H, m, C$\underline{H}$(4′)], 6.93 [1H, s, C$\underline{H}$Ph$_2$], 7.23-7.39 [10H, m, Dpm aromatic C$\underline{H}$], 7.44 [2H, t,

J = 7.5 Hz, benzoyl *m*-C<u>H</u>], 7.59 [1H, t, J = 7.5 Hz, benzoyl *p*-C<u>H</u>], 7.78 [1H, s, thymine C (6)<u>H</u>], 7.87 [2H, d, J = 7.5 Hz, benzoyl *o*-C<u>H</u>], 8.22 and 8.29 [2x2H, q, J = 9.0 Hz, Nosyl C<u>H</u> (AA'XX')]; δ_C ($CDCl_3$, 125.65 MHz); 11.9 [thymine <u>C</u>H$_3$], 27.7 [Boc <u>C</u>H$_3$], 36.4 [<u>C</u>H$_2$ (3')], 44.8 [(Ns/Boc)NCH$_2$<u>C</u>H$_2$N], 52.1 [<u>C</u>H(2')], 53.9 [<u>C</u>H$_2$(5')], 58.6 [<u>C</u>H(4')], 66.0 [(Ns/Boc)N<u>C</u>H$_2$CH$_2$N], 77.9 [<u>C</u>HPh$_2$], 85.6 [Boc <u>C</u>], 111.6 [<u>C</u>(5) thymine], 124.0-130.8 [Nosyl <u>C</u>H and Dpm aromatic <u>C</u>H], 131.6 [<u>C</u>(1)-benzoyl], 134.8 [benzoyl *p*-<u>C</u>H], 137.5 [<u>C</u>H(6) thymine], 139.4 and 139.5 [Dpm aromatic <u>C</u>], 145.9 [Nosyl <u>C</u>], 150.1 [Boc <u>C</u>O], 162.7 [<u>C</u>(4) thymine], 169.1 [benzoyl <u>C</u>O], 171.2 [ester <u>C</u>O]; ν_{max} (KBr)/cm^{-1}; 3100 (aromatic CH), 2969 and 2827 (aliphatic CH), 1747(ester CO), 1699 and 1658 (amide CO), 1531 (N=O stretch), 1174 and 1149 (-SO$_2$- stretch), 742, 702, 634.

N-2-(N-*tert*-butoxycarbonylamino)ethyl–*cis*-4-(N^3-benzoylthymine-1-yl)-D-proline diphenylmethyl ester (21)



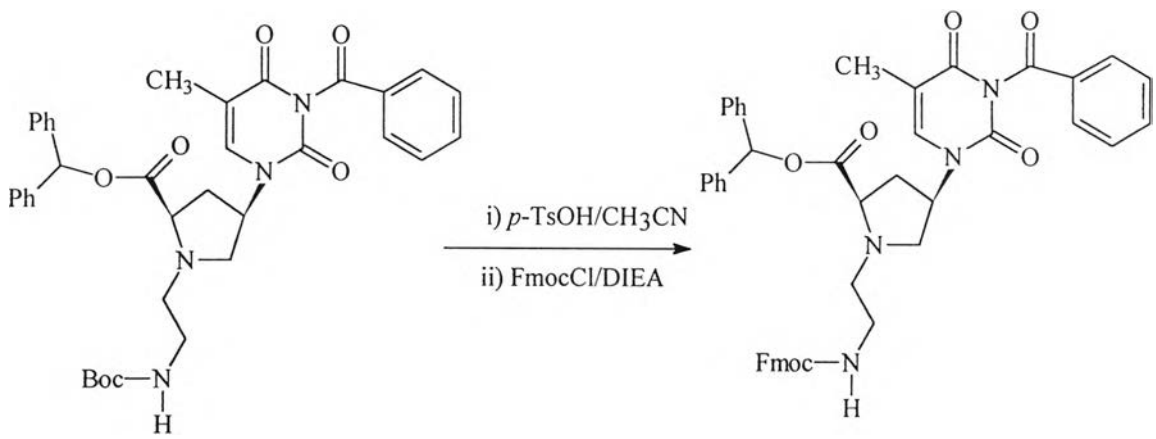
(20) **(21)**

In a dried 100 mL round bottom flask, Boc/Ns-(ψ-CH$_2$)Gly-D-Pro(*cis*-4-T^{Bz})-ODpm **(20)** (3.38 g, 4.03 mmol) was dissolved in anhydrous *N,N'*-dimethylformamide (2 mL) with stirring. This solution was treated with anhydrous potassium carbonate (0.61 g, 4.43 mmol) and thiophenol (0.45 mL, 4.43 mmol) for 2 hr at room temperature to afford a dark orange solution, afterwhich TLC analysis revealed that the nosyl group was completely removed. The mixture was diluted with dichloromethane (100 mL) and washed with water 20 mL. The aqueous layer was repeatedly extracted with dichloromethane. The dichloromethane extracts were combined and washed with large excess of water to

eliminate thiophenol residue. The organic phase was dried over magnesium sulfate and evaporate to dryness. Further purification was performed by column chromatography using hexane:ethyl acetate (1:1) on silica gel to give crude product as clear thick oil. Vigorous scratching with cold hexane gave the product **(21)** as a white amorphous solid (1.73 g, 63 %).

mp = 140-143 ° C, (Found C, 68.1; H, 6.2; N, 8.7 %, $C_{37}H_{40}N_4O_7$ requires C, 68.1; H, 6.3; N, 8.6 %); δ_H (500 MHz, $CDCl_3$); 1.43 [9H, s, ^tBu C$\underline{H}_3$], 1.87 [3H, s, thymine C$\underline{H}_3$], 1.92 [1H, ddd, *J* = 9.0, 6.5, 2.5 Hz, 1x C$\underline{H}_2$(3′)], 2.52-2.59 [1H, m, 1xBocNHCH$_2$C$\underline{H}_2$N], 2.71-2.80 [2H, m, 1xBocNHCH$_2$C$\underline{H}_2$N and 1xC$\underline{H}_2$(5′)], 2.85 [1H, ddd, *J* = 19.0, 15.0, 9.5 Hz, 1xCH$_2$(3′)], 3.03-3.12 [1H, m, 1xBocNHC$\underline{H}_2$CH$_2$N], 3.18-3.21 and 3.21-3.26 [2H, m, 1xBocNHC$\underline{H}_2$CH$_2$N and 1xCH$_2$(5′)], 3.40 [1H, dd, *J* = 7.0, 9.5 Hz, C$\underline{H}$(2′)], 5.09 [1H, br s, Boc N$\underline{H}$], 5.17-5.24 [1H, m, C$\underline{H}$(4′)], 6.96 [1H, s, C$\underline{H}$Ph$_2$], 7.24-7.38 [10H, m, Dpm aromatic C$\underline{H}$], 7.45 [2H, t, *J* = 8.0 Hz, benzoyl *m*-C$\underline{H}$], 7.61 [1H, t, *J* = 8.0 Hz, benzoyl *p*-C$\underline{H}$], 7.87 [2H, d, *J* = 8.0 Hz, benzoyl *o*-C$\underline{H}$], 7.96 [1H, s, thymine C(6)$\underline{H}$]; δ_C ($CDCl_3$, 125.65 MHz); 12.6 [thymine C$\underline{H}_3$], 28.4 [Boc C$\underline{H}_3$], 36.6 [C$\underline{H}_2$(3′)], 38.8 [BocNHCH$_2$C$\underline{H}_2$N], 52.3 [C$\underline{H}$(2′)], 52.7 [C$\underline{H}_2$(5′)], 57.9 [C$\underline{H}$(4′)], 64.9 [BocNHC$\underline{H}_2$CH$_2$N], 77.9 [C$\underline{H}$Ph$_2$], 79.3 [Boc C], 111.4 [C(5) thymine], 126.8-130.4 [Dpm aromatic C$\underline{H}$], 131.6 [C(1)-benzoyl], 134.9 [benzoyl *p*-C$\underline{H}$], 137.5 [C$\underline{H}$(6) thymine], 139.2 and 139.3 [Dpm aromatic C], 149.9 [C(2) thymine], 155.9 [Boc C$\underline{O}$], 162.6 [C(4) thymine], 169.0 [benzoyl C$\underline{O}$], 172.1 [ester C$\underline{O}$]; ν_{max} (KBr)/cm^{-1}; 3419 (NH stretch), 3066 (aromatic CH), 2969, 2920 and 2847 (aliphatic CH), 1743 (ester CO), 1694 and 1655 (amide CO), 1599, 1496 and 1446 (aromatic C=C), 1282, 1178, 773, 702; $[\alpha]_D^{22}$ +6.5 (c=1.06, $CHCl_3$).

N-2-(9-Fluorenylmethoxycarbonylamino)ethyl-*cis*-4-(N^3benzoylthymin-1-yl)-D-proline diphenylmethyl ester (22)



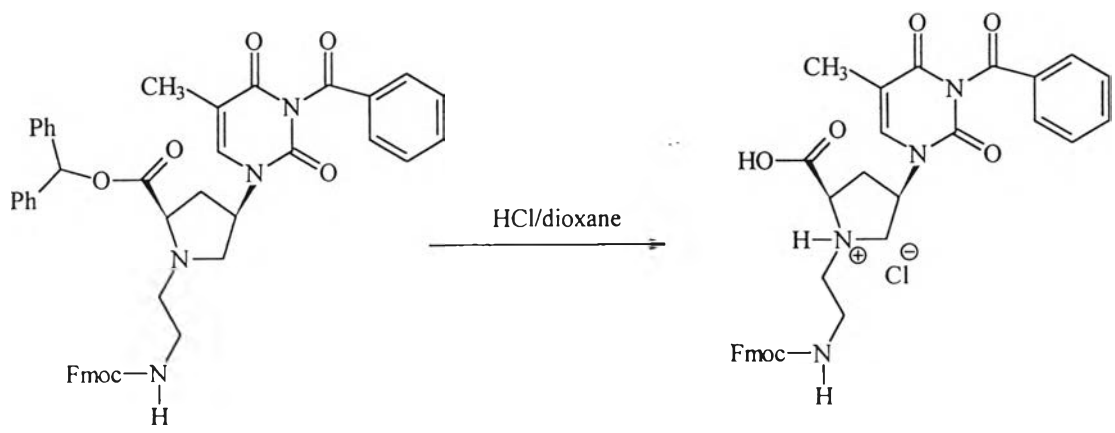
(21) **(22)**

Boc-(ψ-CH_2)Gly-D-Pro(*cis*-4-T^{Bz})-ODpm **(21)** (0.50 g, 0.78 mmol) was dissolved in acetonitrile (3 mL). *p*-Toluenesulfonic acid monohydrate (0.37 g, 1.96 mmol) was added and allowed to stir for 2.5 hr at room temperature, afterwhich Boc group was completely deprotected according to TLC analysis. Acetonitrile (30 mL) was then added to the solution, followed by diisopropylethylamine (0.6 mL, 2.74 mmol). To this basic solution was added 9-fluorenylmethylchloroformate (0.22 g, 0.86 mmol) and stirred for 6 hr at room temperature. The solvent was then removed by rotary evaporation and the residue dissolved in dichloromethane. The dichloromethane layer was washed with 5 % hydrochloric acid, saturated sodium bicarbonate and water respectively. The organic layer was dried over magnesium sulfate and the solvent was removed under reduced pressure. The residue was chromatographed with hexane:ethyl acetate (1:1) on silica gel to give a clear thick oil. Scratching with cold hexane gave the product **(22)** (0.28 g, 46 %) as a white crystalline solid which was dried under reduced pressure.

mp.= 94-98 ° C (Found C, 72.3; H, 5.7; N, 7.4 %; $C_{47}H_{42}N_4O_7$ requires C, 72.3; H, 5.5; N, 7.2 %); δ_H ($CDCl_3$, 500 MHz) 1.73 [3H, s, thymine CH_3], 1.85-1.93 [1H, m, 1xCH_2(3′)], 2.50-2.59 [1H, m, 1xFmocNHCH$_2$CH_2N], 2.65-2.76 [2H, m, 1xCH_2(5′) and 1xFmocNH CH_2CH_2N], 2.77-2.88 [1H, m, 1xCH_2(3′)], 3.02-3.11 [1H, m, 1xFmocNHCH_2CH$_2$N], 3.14-

3.24 [1H, m, 1xC$\underline{H}_2$(5′)], 3.25-3.33 [1H, t, 1xFmocNHC$\underline{H}_2$C$\underline{H}_2$N], 3.35-3.42 [1H, m, C$\underline{H}$ (2′)], 4.14 [2H, t, J = 7.0 Hz, Fmoc aliphatic C$\underline{H}_2$], 4.24 [1H, t, J = 8.5 Hz, Fmoc aliphatic C$\underline{H}$], 5.14-5.20 [1H, br m, C$\underline{H}$(4′)], 5.40 [1H, br s, Fmoc N$\underline{H}$], 6.92 [1H, s, C$\underline{H}$Ph$_2$], 7.18-7.30 [10H, m, Dpm aromatic C$\underline{H}$], 7.30-7.36 [4H, m, Fmoc aromatic C$\underline{H}$], 7.40 [2H, t, J = 7.5 Hz, benzoyl *m*-C$\underline{H}$], 7.52-7.58 [3H, m, benzoyl *p*-C$\underline{H}$, 2xFmoc aromatic C$\underline{H}$], 7.71 [2H, d, J = 7.5 Hz, benzoyl *o*-C$\underline{H}$], 7.81-7.87 [3H, m, thymine C(6)$\underline{H}$, 2xFmoc aromatic C$\underline{H}$]; δ_C ($CDCl_3$, 125.65 MHz); 12.5 [thymine $\underline{C}H_3$], 36.5 [$\underline{C}H_2$(3′)], 39.3 [Fmoc NHCH$_2\underline{C}$H$_2$N], 47.2 [Fmoc aliphatic $\underline{C}$H], 52.4 [$\underline{C}H_2$(5′)], 53.2 [$\underline{C}$H(2′)], 58.3 [$\underline{C}$H(4′)], 65.0 [Fmoc aliphatic $\underline{C}H_2$], 66.7 [FmocNH$\underline{C}H_2CH_2$N], 78.1 [$\underline{C}$HPh$_2$], 111.5 [$\underline{C}$(5)], 120.0 [Fmoc aromatic $\underline{C}$H], 125.1-130.4 [Dpm aromatic $\underline{C}$H], 131.6 [$\underline{C}$(1)-benzoyl], 134.9 [benzoyl *p*-$\underline{C}$H], 137.4 [$\underline{C}$H(6) thymine], 139.2 and 139.3 [Dpm aromatic $\underline{C}$], 141.3, 143.8 and 143.9 [Fmoc aromatic $\underline{C}$], 150.0 [$\underline{C}$(2)], 156.4 [Fmoc $\underline{C}$O], 162.6 [$\underline{C}$(4)], 169.1 [benzoyl $\underline{C}$O], 172.4 [ester $\underline{C}$O]; ν_{max} (KBr)/cm^{-1}; 3390 (NH stretch), 3064 (aromatic CH), 2951 and 2817 (aliphatic CH), 1747 (ester CO), 1697 and 1651 (amide CO),1516 and 1448 (aromatic C=C), 1282, 1257, 1176, 759, 742, 702; $[\alpha]_D^{23}$ +7.03 (c=0.92, $CHCl_3$).

_N_-2-(9-Fluorenylmethoxycarbonylamino)ethyl-*cis*-4-(N^3-benzoylthymin-1-yl)-D-proline hydrochloride (23)



(22) (23)

In a dried 50 mL round bottom flask equipped with a magnetic bar, Fmoc-(ψ-CH$_2$) Gly)-D-Pro(*cis*-4-T^{Bz})-ODpm **(22)** (0.30 g, 0.40 mmol) was dissolved in 5 mL of 4 M

anhydrous hydrogen chloride in dioxane for 2 hr at room temperature and then deprotection of diphenylmethyl ester was completely occurred as shown by TLC analysis. The volatiles were removed by rotary evaporation followed by co-evaporation with dichloromethane three times. The residue was precipitated with diethyl ether to give the desired product **(23)** (0.17 g, 68 %) as a white solid.

mp.= 150-151 °C (Found C, 60.9; H, 5.0; N, 8.2 % for first analysis and Found C, 59.8; H, 5.2; N, 8.2 % for second analysis (after vacuum dried) ;$C_{34}H_{33}N_4O_7Cl$ requires C, 63.3; H, 5.2; N, 8.7 % or $C_{34}H_{33}N_4O_7Cl.2H_2O$ requires C, 59.9; H, 5.5; N, 8.2 %); δ_H (DMSO-d_6, 500 MHz + 1 drop D_2O); 2.50 [3H, s, thymine C$\underline{H}_3$], 2.34-2.44 [1H, m, 1xC$\underline{H}_2$(3′)], 2.83 [1H, ddd, J =16.5, 14.0, 8.6 Hz, 1xC$\underline{H}_2$(3′)], 3.12-3.22, 3.26-3.36, 3.37-3.42 and 3.44-3.53 [4H, 4x m, FmocNHC$\underline{H}_2$C$\underline{H}_2$ N], 3.55-3.75 [1H, m, 1xC$\underline{H}_2$(5′) overlap with D_2O peak], 3.93-4.00 [1H, m, 1xC$\underline{H}_2$(5′)], 4.20 [1H, t, J = 6.5 Hz, Fmoc aliphatic C$\underline{H}$], 4.25-4.36 [2H, m, Fmoc aliphatic C$\underline{H}_2$], 4.46-4.54 [1H, m, C$\underline{H}$(2′)], 5.27-5.35 [1H, m, C$\underline{H}$(4′)], 7.24-7.42 [4H, m, Fmoc aromatic C$\underline{H}$], 7.57 [2H, t, J = 7.5 Hz, benzoyl *m*-C$\underline{H}$], 7.62-7.68 [2H, m, Fmoc aromatic C$\underline{H}$], 7.74 [1H, t, J = 7.5 Hz, benzoyl *p*-C$\underline{H}$], 7.86 [2H, d, J = 7.5 Hz, benzoyl *o*-C$\underline{H}$], 7.96 [2H, d, J = 7.5, Fmoc aromatic C$\underline{H}$], 8.03 [1$\underline{H}$, s, thymine C(6)$\underline{H}$]; δ_C (DMSO-d_6 + 1 drop D_2O, 125.65 MHz); 12.0 [thymine $\underline{C}H_3$], 32.8 [$\underline{C}H_2$(3′)], 36.6 [FmocNHCH$_2\underline{C}$H$_2$N], 46.6 [Fmoc aliphatic $\underline{C}$H], 51.5 [$\underline{C}$H(2′)], 54.2 [$\underline{C}H_2$(5′)], 55.9 [Fmoc aliphatic $\underline{C}H_2$], 65.5 [$\underline{C}$H(4′)], 65.7 [FmocNH$\underline{C}$H$_2$CH$_2$N], 109.6 [$\underline{C}$(5)], 120.1 [Fmoc aromatic $\underline{C}$H], 125.2-130.5 [Dpm aromatic $\underline{C}$H], 131.1 [$\underline{C}$(1)-benzoyl], 135.5 [benzoyl *p*-$\underline{C}$H], 139.2 [$\underline{C}$H(6) thymine], 140.7 and 143.8 [Dpm aromatic $\underline{C}$], 145.7 [Fmoc aromatic $\underline{C}$], 149.5 [$\underline{C}$(2)], 156.2 [Fmoc $\underline{C}$O], 162.4 [$\underline{C}$(4)], 168.2 [benzoyl $\underline{C}$O], 169.5 [Acid $\underline{C}$O]; ν_{max} (KBr)/cm^{-1}; 3411 (OH acid), 3061 (CH aromatic), 2918 and 2848 (CH aliphatic), 1749 (CO acid), 1694 and 1643 (amide CO), 1519 (NH bending), 1451 (C=C aromatic), 1247 (C-O stretch), 1018, 976, 762; $[\alpha]_D^{23}$ +13.5 (c=1.00, DMF).

2.4 Oligomerization of cPNA (Peptide synthesis)

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

All peptide syntheses were carried out using home-adapted pasteur pipette as described below. A new glass pasteur pipette was plugged with a small piece of glass wool. The resin was weighed accurately into the pipette and the pipette was equipped with the rubber sucker. The resin in the pipette should be swollen in the required solvent at least 1 hr 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 washing could be done by filling the solvent *via* the top of the pipette. In order to eliminate the excess reagent residue as much as possible, the reaction pipette was pierced through a rubber septum equipped with suction flask and aspirator. The excess solvent was then added *via* the top of pipette and flushed out rapidly. The pipette had to be nearly filled up with the solvent everytime before sucking dry according to figure 2.1

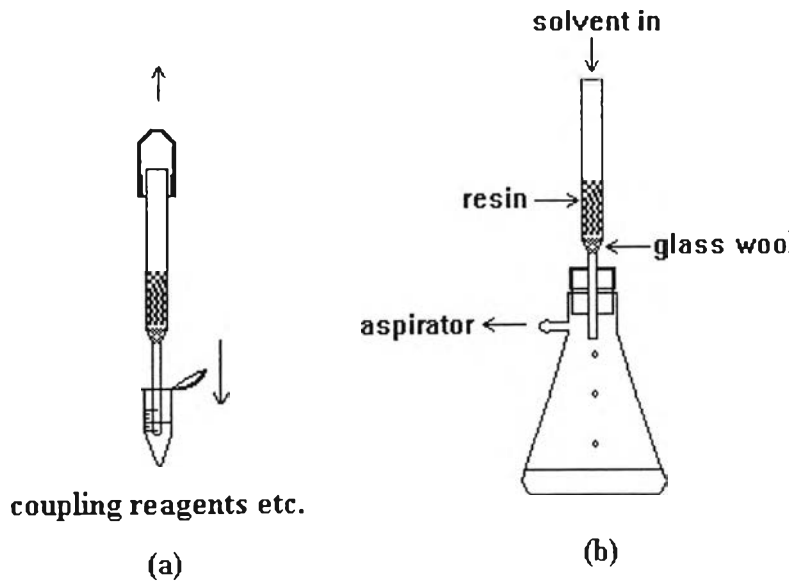


Figure 2.1 A diagram showing the manual technique for solid phase peptide synthesis; (a) coupling, deprotection and cleaving process; (b) washing process

2.4.2 Solid phase peptide synthesis of both L- and D-serylproline cPNA according to Fmoc/O^{t}Bu dipeptide fragment strategy.

2.4.2.1 The decamers of L-serylproline cPNA (25)

Synthesis of this cPNA was carried out on 4.0 μmol scale. The synthesis was divided into 4 steps as follows.

a) Anchoring with the first amino acid residue (Preloading)

The reaction pipette containing Novasyn TGR resin (20 mg, 4.0 μmol) was prepared as described above. Fmoc-L-Lysine was first attached to the free amino group on the resin employing Fmoc-L-Lys(Boc)-OPfp. Fmoc-L-Lys(Boc)OPfp (25 mg, 40 μmol) and HOBt (6 mg, 40 μmol) were dissolved in anhydrous DMF 100 μL in an eppendorf tube. The prepared resin was soaked in this solution with occasional agitation for 2 hr at room temperature. After the specified period of time, the resin was washed extensively with DMF. All procedures were repeated again to ensure that the coupling efficiency approached 100 %.

b) End capping

After anchoring or coupling step, the free amino residue was capped with 10 % Ac_2O/DIEA in anhydrous DMF (Ac_2O 18.6 μL, DIEA 27 μL and DMF 155 μL) in an eppendorf tube to prevent formation of deletion sequences. The reaction pipette was occasionally agitated with this solution for 30 min at room temperature. After the specified period of time, the reaction pipette was washed with DMF three times.

c) Deprotection of the Fmoc protecting group at N-terminal

After the coupling was completed, the resin was treated with 20 % piperidine in DMF (piperidine 70 μL and DMF 230 μL) in an eppendorf tube for 20 min at room

temperature with occasional agitation. After the specified period of time, the reaction pipette was washed extensively with DMF. The used deprotecting reagent can be used to determine the coupling efficiency by diluting with an appropriate volume of methanol and then the UV-absorbance of dibenzofulvene-piperidine adduct at 264 nm 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 at least 90 % for each step in order to give acceptable yield of the 10-mer cPNA from the synthesis. If the efficiency had dropped suddenly, the coupling must be stopped to save the valuable monomer.

d) Coupling with Fmoc-L-serylproline dipeptide monomer

The free amino group, generated from deprotection step, was further coupled with Fmoc-L-serylproline cPNA monomer. Fmoc-L-Ser(O^{t}Bu)-D-Pro(*cis*-4-T^{Bz})-OPfp **(12)** (14 mg, 16 μmol) and HOBt (2.2 mg, 16 μmol) were dissolved in 100 μL anhydrous DMF. The reaction pipette was treated with this solution for 2 hr at room temperature with occasional agitation. After the specified period of time, the reaction pipette was washed extensively with DMF.

After that, the next cycle (deprotecting, coupling and capping) were carried out with the same method until the resin bound peptide had been extended upto 10-mer. The synthesis was then stopped at the capping step without cleavage of the N-terminal Fmoc group.

e) Method for cleavage the 10-mer cPNA from the resin

The 10-mer resin bound peptide was released from the resin by treatment with trifluoroacetic acid 1 mL at room temperature for 6 hr with occasional agitation. After the specified period of time, the solution became orange and the trifluoroacetic acid was then evaporated to dryness with nitrogen stream (fume hood). The sticky residue was treated with diethyl ether (ten times the volume) to precipitate the peptide. The suspension was then centrifuged at 13,000 rpm for 5 min. The supernatant was decanted and the crude

peptide was centrifugally washed with diethyl ether twice. Finally the crude peptide was air dried at room temperature.

f) Purification and Identification

The crude peptide was prepared for HPLC analysis by dissolving a mixture in deionized water (135 μL) and 15 μL acetonitrile. The solution was filtered through a nylon membrane filter (0.45 μm), purified and further analyzed by reverse phase HPLC monitoring by UV-absorbance at 270 nm eluting with a gradient system of acetonitrile-0.1% trifluoroacetic acid in water. The major product, Fmoc-ON peptide (t_R = 21.5 min), was collected. After freeze drying, it was confirmed to be the desired Fmoc-ON peptide by MALDI-TOF mass spectrometry. To cleave the N-terminal Fmoc group, the Fmoc-ON peptide was treated with 20 μL piperidine for 20 min at room temperature. The solution was then diluted with 200 μL diethyl ether to eliminate the excess deprotecting agent and precipitate the crude Fmoc-OFF peptide. The suspension was centrifuged at 13,000 rpm for 10 min and then the supernatant was carefully decanted. The centrifugal-washed process was repeated twice. Finally the peptide was air-dried at room temperature and dissolved in a mixture of deionized water 135 μL and acetonitrile 15 μL acetonitrile. The sample was further purified by reverse phase HPLC eluting with the same gradient system. The major peak at t_R = 14.7 min was collected, freeze-dried and identified by MALDI-TOF mass spectrometry.

HPLC gradient system

solvent A = 0.1 % trifluoroacetic acid in acetonitrile

solvent B = 0.1 % trifluoroacetic acid in deionized water

First A:B (10:90) for 5 min then linear gradient to A:B (60:40) over a period of 25 min then hold on for 5 min before revert back to A:B (10:90)

2.4.2.2 The decamers of D-serylproline cPNA (26)

Procedure for D-series was carried out exactly the same way as described for L-series above. Smaller scale synthesis was performed due to the limited availability of the monomer.

a) Anchoring with the first amino acid residue

The procedure in 2.4.2.1(a) was followed using Novasyn TGR resin (9.6 mg, 1.8 μmol), Fmoc-L-Lys(Boc)-OPfp (11.4 mg, 18 μmol), HOBt (2.4 mg, 18 μmol) and anhydrous DMF 50 μL.

b) End capping

see section 2.4.2.1(b).

c) Deprotection of the Fmoc protecting group at N-terminal

see section 2.4.2.1(c).

d) Coupling with Fmoc-D-serylproline dipeptide monomer

The procedure was followed in 2.4.2.1(d) using a coupling solution containing Fmoc-D-Ser(O^{t}Bu)-D-Pro(*cis*-4-T^{Bz})-OPfp **(13)** (6.3 mg, 7.2 μmol), HOBt (1.0 mg, 7.2 μmol) and 50 μL anhydrous DMF for each coupling cycle.

e) Method for cleavage the 10-mer cPNA from the resin

The synthesized 10-mer of D-series was cleavaged with the same method as described in section 2.4.2.1.(e) with only 3 hr for TFA treatment.

f) Purification and identification

See section 2.4.2.1 (f), the peak of Fmoc-ON cPNA of the D-series appeared at t_R = 18.9 min and Fmoc/Bz-ON at 21.8 min. After deprotection of the N-terminal Fmoc and benzoyl group, the major peak at the t_R = 13.9 min was collected and was proved to be the desired product by MALDI-TOF mass spectrometry.

2.4.3 Solid phase peptide synthesis of deoxyglycylproline cPNA (24) according to Fmoc chemistry

Synthesis of this cPNA was carried out by Fmoc dipeptide fragment strategy similar to the serylproline route. No side-chain protection was required in this case. The steps for synthesis were described as follows.

a) Anchoring with the first amino acid residue

In this case, Fmoc-L-Lys(Boc)-OH was used instead of Fmoc-L-Lys(Boc)-OPfp in order to parallelized the coupling method to the deoxyglycylproline cPNA monomer **(23)**. The synthesis was performed on 5 μmol scale using Novasyn TGR resin (25 mg, 5 μmol), Fmoc-L-Lys(Boc)-OH (19 mg, 50 μmol) and HBTU (19 mg, 50 μmol) were dissolved in 100 μL of anhydrous DMF in an eppendorf tube followed by DIEA (17.2 μL, 100 μmol). The prepared resin was treated with this solution for 2 hr with occasional agitation and then washed with DMF. Double coupling was performed to ensure that the coupling efficiency approached 100 %.

b) End capping

See section 2.4.2.1 (b).

c) Deprotection of the Fmoc protecting group at N-terminal

See section 2.4.2.1 (c).

d) Coupling with Fmoc-deoxyglyclyproline cPNA monomer

In each coupling reaction, Fmoc-(ψ-CH_2)Gly-D-Pro(*cis*-4-T^{Bz})OH.HCl **(23)** (13 mg, 20 μmol), HBTU (8 mg, 20 μmol), were dissolved in 80 μL of anhydrous DMF in an eppendorf tube. DIEA (10.3 μL, 60 μmol), was added and the resin was treated with this solution for 2 hr with occasional agitation. After the specified period of time, the reaction pipette was extensively washed with DMF. For the best coupling efficiency, the double coupling should be performed with a new coupling solution.

e) Method for cleavage the 10-mer cPNA from the resin

In this case, the peptide-bound resin was treated with a mixture of 10 % thioanisole in trifluoroacetic acid for 3 hr and then evaporated with nitrogen stream. The residue was added with diethyl ether and followed by centrifugation-washing with diethyl ether and air dried as described in section 2.4.2.1 (e).

f) Purification and identification

The method was performed as described in section 2.4.2.1 (f). Two major peaks appeared at t_R = 18.3 and 20.7 min which corresponded to the Fmoc-ON and Fmoc/Bz-ON peptides respectively according to MALDI-TOF mass spectral data. After deprotection of the Fmoc and benzoyl groups by treatment with 20 μL piperidine for 20 min at room temperature, the Fmoc-OFF peptide product was purified by reverse phase HPLC (t_R = 18.8 min). The product was freeze dried and identified by MALDI-TOF mass spectrometry.

HPLC gradient system

solvent A = 0.1 % trifluoroacetic acid in acetonitrile
solvent B = 0.1 % trifluoroacetic acid in deionized water

1) System for Fmoc-ON and Fmoc/Bz-ON **(24)**

First A:B (10:90) for 5 min then linear gradient to A:B (90:10) over a period of 40 min then hold on for 5 min revert back to A:B (10:90)

2) System for Fmoc-OFF **(24)**

First A:B (10:90) for 5 min then linear gradient to A:B (60:40) over a period of 25 min then hold on for 5 min revert back to A:B (10:90)

2.5 Biophysical studies

Binding of all cPNAs were studied at Department of Biology, Faculty of Science, Chulalongkorn University by gel electrophoresis on SE-600 vertical slab gel electrophoresis unit (Hoefer Scientific Instruments). The gel dimensions were 10x9.5x 0.075 cm. The DC power sources was an electrophoresis power supply model EPS-301 (Amersham Pharmacia Biotech). Acrylamide and *N,N'*-methylene bisacrylamide were a concentrated aqueous solution obtained from Fluka. Ammonium persulfate was electrophoresis grade from Sigma Chemical Co., LTD. Tetramethylethylenediamine (TEMED) and chemical necessary for preparing buffers were of the highest purity available from BDH. The stock 0.90 M Tris-Borate-EDTA (TBE) buffer pH 8.0 and loading buffer (30 % glycerol, 0.025% bromophenol blue and 0.025% xylene cyanol FF in 0.09 M TBE were prepared according to the literature.[62] The 20 % polyacrylamide gel in 0.09 M TBE was prepared according to the literature.[62] The sample was prepared by mixing calculated amounts of the concentrated stock solutions of fluorescent labelled decaadenylic acid (1.21 μmol/mL) and cPNA in eppendorf tubes to give the total amounts

of $F(dA_{10})$ ~1 nmol and then mixed with 10% loading buffer. All samples were introduced into the well at the top of the gel by microsyringe. The system was connected to the power supply and run at 100 V until the bromophenol blue maker dye moved about half way through the gel (~2.5 hr). The power supply was stopped and disconnected from the system. The resulting gel was visualized by a UV-transilluminator through a yellow filter and the photograph was taken with a digital camera (Kodak model DC 240).