CHAPTER II

EXPERIMENTAL SECTION

2.1 General Procedure

2.1.1 Measurement

The weight of all substances was determined on a Metler Toleclo electrical balance. Melting points were recorded on an electrothermal melting point apparatus model 9100. Rotary evaporator was of Büchi Rotavapor R-200 with a water aspirator model B-490. UV cabinet for UV-visualization of TLC was made in-house by Mr. Chanchai Khongdeesameor. The magnetic stirrers were of Corning. The high vacuum was delivered by a Refco Vacubrad pump. 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 gal 70-230 mesh (for general chromatography) or 230 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 Rheodyne 7725 manual sample loop (100 µl sample size for analytical scale). A Hypersil^{TM} C_{18} HPLC column 5 µm particle size 4.6 x 250 mm was used for both analytical purposes. Peak monitoring and data processing were available for integrated operating with the base Empower software. Fractions from HPLC were collected manually and were assisted by real-time HPLC chromatogram monitoring. The combined fractions were speed vaporization under reduced pressure using Heto Vacuum Centrifuge and MAXI dry-plus. T_m experiment was measured on a CARY 100 Bio UV-Visible spectrophotometer (Varian). ^1H and ^{13}C spectra were recorded on Varian Mercury-400 plus operating at 400 MHz by Dr. Tirayut Vilaivan and Miss Woraluk Mansawat. MALDI-TOF mass spectra of all *aep*PNA were analyzed by the author on Omniflex 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.

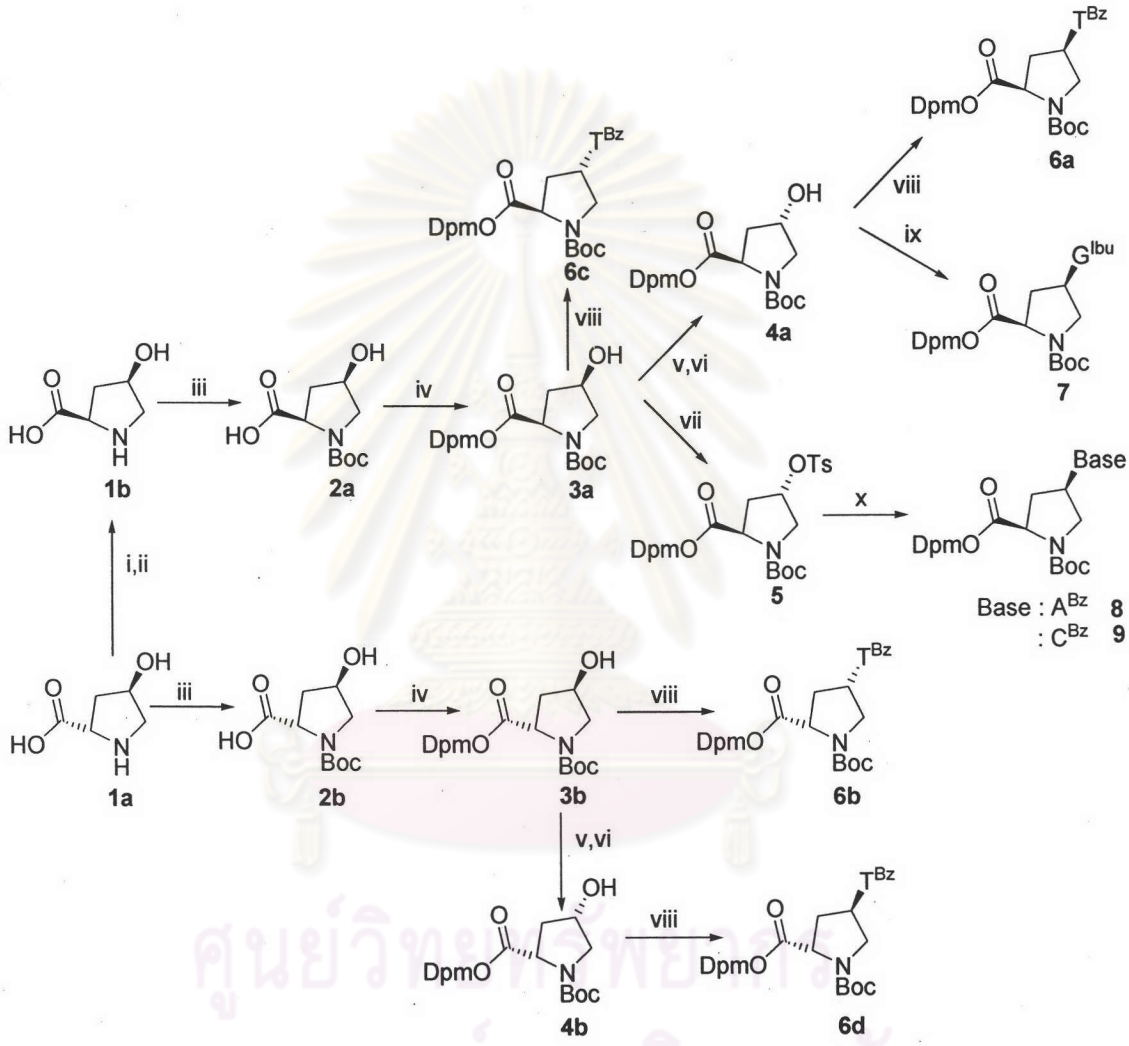
2.1.2 Materials

All chemical were purchased from Fluka, Merck or Aldrich Chemical Co., Ltd., and were purified as appropriate depending on reaction conditions and purposes. Acetic anhydride was synthesized from acetyl chloride and anhydrous sodium acetate according to the standard method [48]. 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. Solvents for reactions and crystallization were reagent grade and used without purification. Nitrogen was obtained from TLG with high purity up to 99.5 %. Acetonitrile for HPLC experiment was HPLC grade, obtained for BDH and was filered 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. The solid support for peptide synthesis (TentaGel S RAM Fmoc resin) and Trifluoroacitic acid were obtained from Fluka. The protected amino acids (Fmoc-L-Lys(Boc)-OPfp) was obtained form Calbiochem Novabiochem Co., Ltd.

2.2 Experimental procedure

2.2.1 Synthesis of intermediate

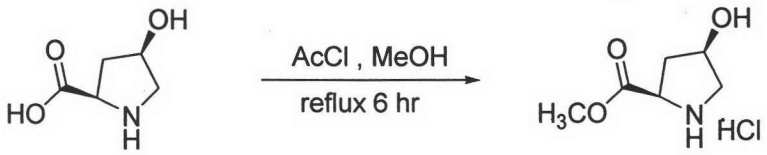
(a) Synthesis of proline derivatives modified with nucleobases



Scheme 1. i. Ac_2O, heat 90 °C 16 h; ii. 2 M HCl, reflux 5 h; iii. Boc_2O, ^{t}BuOH, NaOH (aq), overnight; iv. Ph_2CN_2, EtOAc, overnight; v. HCO_2H, Ph_3P, DIAD, THF, overnight; vi. NH_3, MeOH, 2 h; vii. TsCl, Ph_3P, DIAD, THF, overnight; viii. N^3-T^{Bz}, Ph_3P, DIAD, THF, overnight; ix. N^2-IbuG(ONpe), Ph_3P, DIAD, dioxane, overnight; x. N^6-A^{Bz} or N^4-C^{Bz}, K_2CO_3, DMF, heat 90 °C 5 h

The intermediate proline derivative of thymine (**6**), quinine (**7**), adenine (**8**) and cytosine (**9**) were synthesized as described previously [37-39].

***cis*-hydroxy-D-proline methyl ester (10) [49]**



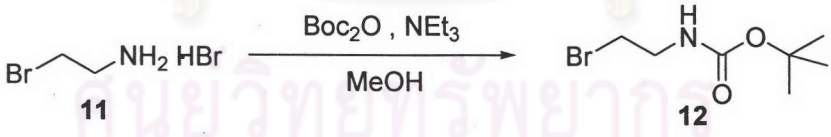
1b **10**

To a suspension of *cis*-hydroxy-D-proline (**1b**) (1.3113 g, 10 mmol) in methanol (10 mL) was added acetyl chloride (7.14 mL, 100 mmol) drop wise at 0 °C. The mixture was refluxed for 6 h. Then the mixture was allowed to cool at room temperature. The solvent was removed under reduced pressure to give the ester compound (**10**) as white solid (1.9436 g, 100 %).

δ_H (400 MHz, D_2O) 2.28-2.33 and 2.35-2.43 [2H, 2 x m, C$\underline{H}_2$(3'), 3.29-3.37 [2H, m, C$\underline{H}_2$(5')], 3.72 [3H, s, C$\underline{H}_3$O], 4.50-4.52 [1H, m, C$\underline{H}$(4') and 4.54-4.57 [1H, dd, J = 2.8, 9.6 Hz, C$\underline{H}$(2')].

(b) Synthesis of spacer

***N-tert*-butoxycarbonyl-bromoethylamine (12) [50]**

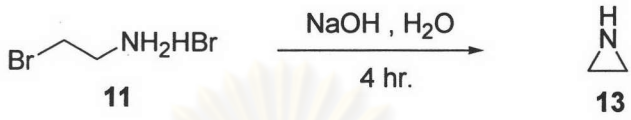


11 **12**

To bromoethylamine (**11**) (2.2539 g, 11 mmol) and Boc_2O (2.1825 g, 10 mmol) in 10 mL of MeOH was added NEt_3 (11 mL, 11 mmol). The mixture was stirred at ambient temperature overnight. The solvent was removed under reduced pressure and the residue was redissolved in 30 mL of 5 % HCl. The acidified solution was extracted with dichloromethane (3 x 30 mL) and the combined organic phase was dried over magnesium sulfate and evaporated under reduced pressure to obtain Boc-bromoethylamine (**12**) as colorless oil (1.7224 g, 77 %).

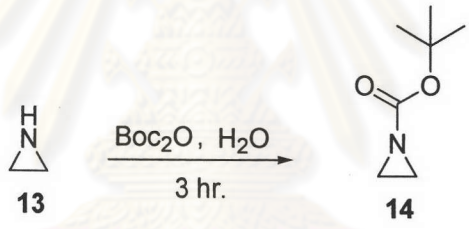
δ_H (400 MHz, $CDCl_3$)1.48 [9H, s, Boc $C\underline{H}_3$], 3.38-3.51 [4H, 2 x m, $BrC\underline{H}_2C\underline{H}_2NH$] and 5.00 [1H, s, N$\underline{H}$], δ_C (400 MHz, $CDCl_3$) 28.3 [Boc $\underline{C}H_3$], 35.6 [$\underline{C}H_2Br$], 42.3 [$\underline{C}H_2NH$], 79.7 [Boc $\underline{C}$] and 156.7 [Boc $\underline{C}O$].

aziridine (13)



To a solution of bromoethylamine (**11**) (2.0490 g, 10 mmol) in water (10 mL) was added NaOH (1.6 g, 40 mmol). The mixture was stirred at ambient temperature for 4 h.

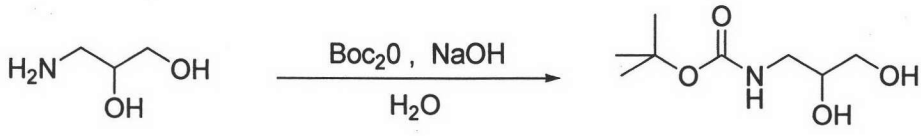
***N-tert*-butoxycarbonyl-aziridine (14)**



To a stirred solution of freshly prepared aziridine (**13**) in water was added Boc_2O (1.7460 g, 8 mmol). The mixture was stirred at ambient temperature. After 3 h. the reaction mixture was diluted with 20 mL of water and extracted with dichloromethane (3 x 30 mL). The combined organic extract was dried over magnesium sulfate and evaporated under reduced pressure to obtain Boc-aziridine (**14**) as colorless oil (0.9424 g, 82 %).

δ_H (400 MHz, $CDCl_3$) 1.49 [9H, s, Boc $C\underline{H}_3$] and 2.17 [4H, s, 2 x aziridine], δ_C (400 MHz, $CDCl_3$) 25.8 [Boc $\underline{C}H_3$], 27.9 [$\underline{C}H_2$ x aziridine], 81.2 [Boc $\underline{C}$] and 162.9 [Boc $\underline{C}O$].

***N-tert*-butoxycarbonylamino-1,2-propanediol (16) [51]**

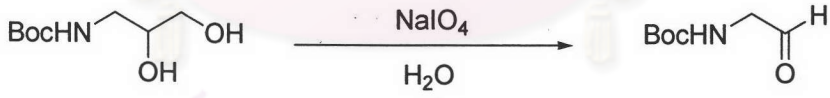


15 **16**

To an ice-cooled solution of racemic-3-amino-1,2-propanediol (1.8222 g, 20 mmol) in water (20 mL) was added Boc_2O (4.3650 g, 20 mmol). The mixture was allowed to warm to room temperature and the pH was maintained at 10.5 by the addition of aqueous NaOH (2 N). The solution was concentrated to a paste which was triturated with DCM (4 x 40 mL). The suspension was filtered and the organic phase qas dried ($MgSO_4$) then evaporated to give the diol (**16**) (3.3682 g, 88 %) as an oil.

δ_H (400 MHz, $CDCl_3$) 1.47 [9H, s, Boc $C\underline{H}_3$], 3.28 [2H, m, $C\underline{H}_2NH$], 3.60 [2H, m, $C\underline{H}_2OH$], 3.77 [1H, m, $C\underline{H}OH$] and 5.23 [1H, t, J = 5.4 Hz, $N\underline{H}$], δ_C (400 MHz, $CDCl_3$) 28.4 [Boc $\underline{C}H_3$], 42.8 [$\underline{C}H_2NH$], 63.6 [$\underline{C}H_2OH$], 71.4 [$\underline{C}HOH$], 80.1 [Boc $\underline{C}$] and 157.4 [Boc $\underline{C}O$].

***N-tert*-butoxycarbonylaminoacetaldehyde (17) [51]**

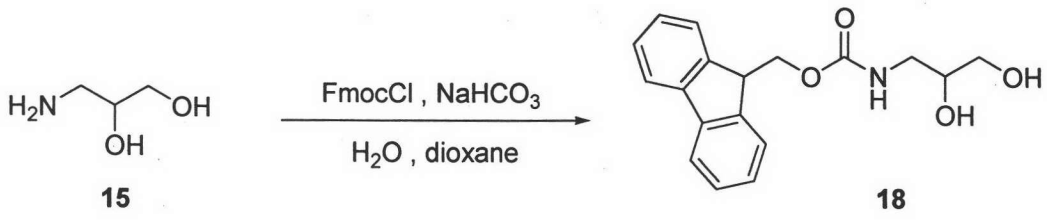


16 **17**

To *N*-Boc-amino-1,2-propanediol (**16**) (1.8122 g, 10 mmol) in water (10 mL) was added $NaIO_4$ (2.5582 g, 12 mmol) at room temperature with stirring. After 3 h the mixture was filtered, extracted with DCM (4 x 40 mL) and the combined organic phases were diried ($MgSO_4$) and concentrated to yield the Boc-aminoacetaldehyde (**17**) (1.0666 g, 67 %) as an oil.

δ_H (400 MHz, $CDCl_3$) 1.48 [9H, s, Boc $C\underline{H}_3$], 4.10 [2H, d, J = 5.2 Hz, $C\underline{H}_2CHO$], 5.27 [1H, s br, $N\underline{H}$] and 9.68 [1H, s, $C\underline{H}O$], δ_C (400 MHz, $CDCl_3$) 28.3 [Boc $\underline{C}H_3$], 51.4 [$\underline{C}H_2CHO$], 80.2 [Boc $\underline{C}$], and 197.2 [$\underline{C}HO$ aldehyde].

N-fluoren-9-ylmethoxycarbonylamino-1,2-propanediol (18) [50]

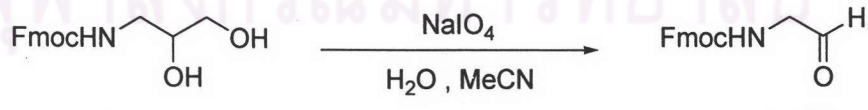


15 **18**

A mixture of 3-amino-1,2-propanediol (**15**) (0.4556 g, 5 mmol) , FmocCl (1.2935 g, 5 mmol) and $NaHCO_3$ (0.4201 g, 5 mmol) were dissolved in 1:1 dioxane : H_2O (10 mL). The reaction mixture was stirred at ambient temperature overnight. The dioxane was removed by rotary evaporation under reduced pressure. The residue was diluted with 20 mL of water and extracted with dichloromethane (3 x 30 mL) The combined organic extract was dried over magnesium sulfate and evaporated under reduced pressure to give the Fmoc-amino-1,2-propanediol (**18**) as white solid (2.3935 g, 90 %).

mp. = 128.4-130.0 °C, δ_H (400 MHz, $CDCl_3$) 2.60, 2.74 [1 x 1H, 2 x s, 2 x O<u>H</u>], 3.35 [2H, m, C<u>H</u>$_2$NH], 3.59 [2H, dd, J = 12.0, 30.4 Hz, C<u>H</u>$_2$OH], 3.79 [1H, m, C<u>H</u>OH], 4.24 [1H, t, J = 6.8 Hz, Fmoc aliphatic C<u>H</u>], 4.50 [2H, d, J = 6.8 Hz, Fmoc aliphatic C<u>H</u>$_2$], 5.19 [1H, br s, NH] and 7.37-7.81 [8H, m, Fmoc aromatic C<u>H</u>], δ_C (400 MHz, $CDCl_3$) 43.5 [Fmoc aliphatic <u>C</u>H], 47.1 [<u>C</u>H$_2$NH], 63.8 [<u>C</u>H$_2$OH], 66.3 [<u>C</u>HOH], 70.9 [Fmoc aliphatic <u>C</u>H$_2$], 119.9-143.8 [Fmoc aromatic <u>C</u>H] and 157.2 [Fmoc <u>C</u>O].

N-fluoren-9-ylmethoxycarbonylaminoacetaldehyde (19) [51]



18 **19**

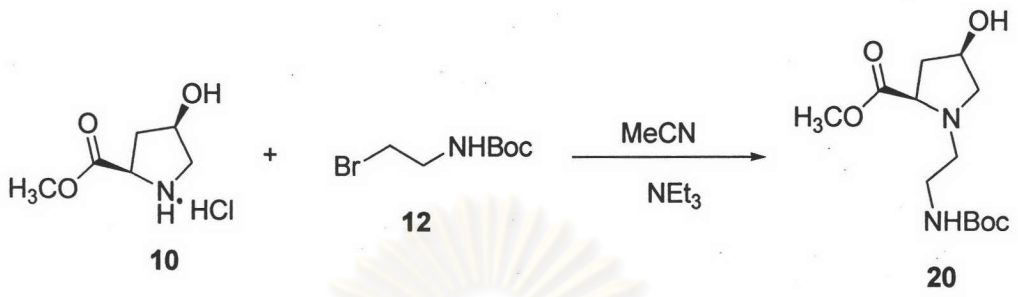
A mixture of Fmoc-amino-1,2-propanediol (**18**) (1.2534 g, 4 mmol) and $NaIO_4$ (1.0233 g, 4.8 mmol) were dissolved in a mixed solvent (4 mL of H_2O and 4mL of MeCN). The solution was allowed to stir for 3 h when TLC analysis revealed that reaction was completed. The acetonitrile was removed by rotary evaporation

under reduced pressure. The residue was diluted with 20 mL of water and extracted with dichloromethane (3 x 30 mL). The combined organic extract was dried over magnesium sulfate and evaporated to give the Fmoc-aminoacetaldehyde (**19**) as colorless fluffy solid (1.1224 g, 99 %).

mp. = 141.0-143.0 °C, δ_H (400 MHz, $CDCl_3$) 4.21 [2H, d, J = 19.2 Hz, C<u>H</u>$_2$NH], 4.27 [1H, t, J = 6.8 Hz, Fmoc aliphatic C<u>H</u>], 4.47 [2H, d, J = 6.8 Hz, Fmoc aliphatic C<u>H</u>$_2$], 5.48 [1H, br s, N<u>H</u>], 7.34-7.82 [8H, m, Fmoc aromatic C<u>H</u>] and 9.71 [1H, s, C<u>H</u>O aldehyde], δ_C (400 MHz, $CDCl_3$) 47.1 [Fmoc aliphatic <u>C</u>H], 51.7 [<u>C</u>H$_2$NH], 67.2 [Fmoc aliphatic <u>C</u>H2], 120.1-143.12 [Fmoc aromatic <u>C</u>H], 156.3 [Fmoc <u>C</u>O] and 196.6 [<u>C</u>HO aldehyde].

2.2.2 Synthesis of *aep*PNA monomer

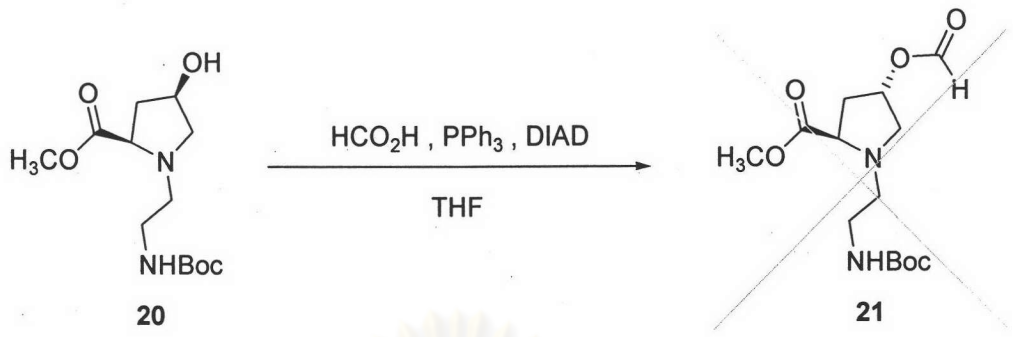
***N*-2-(*N-tert*-butoxycarbonylamino)ethyl-*cis*-4-hydroxy-D-proline methyl ester (20)**



To a mixture of *cis*-4-hydroxy-D-proline methyl ester (**10**) (1.9436 g, 10 mmol) and Boc-bromoethylamine (**12**) (2.1875 g, 10 mmol) in MeCN (10 mL) was added NEt_3 (2.8 mL, excess). The mixture was stirred at ambient temperature for 1 day, after which, TLC monitoring indicated complete reaction. The solvent was removed under pressure, the residue was dissolved in ethyl acetate and extracted twice with 5% HCl. The combined aqueous phase was added $NaHCO_3$ to pH 8 and extracted with ethyl acetate (3 x 30 mL). The combined organic extract was dried over magnesium sulfate and the solvent was removed under reduced pressure to give the product as colorless oil (1.5227 g, 58 %).

δ_H (400MHz, $CDCl_3$) 1.47 [9H, s, Boc C$\underline{H}_3$], 1.98 [1H, td, J_t = 1.6 and J_d = 14 Hz, 1x C$\underline{H}_2$(3')], 2.29-2.38 [1H, m, C$\underline{H}_2$(3')], 2.65[1H, dd, 4, 9.6 Hz, 1 x C$\underline{H}_2$(5')], 2.68-2.81 [2H, m, BocNHCH$_2$C$\underline{H}_2$N], 3.07-3.30 [3H, m, 2 x BocNHC$\underline{H}_2$CH$_2$N and1 x C$\underline{H}_2$(5')], 3.33 [1H, dd J = 4.0, 14.4 Hz, 1 x C$\underline{H}_2$(2')], 3.77 [3H, s, C$\underline{H}_3$O], 4.314 [1H, s, C$\underline{H}$(4')] and 5.24 [1H, s, BocN$\underline{H}$], δ_C (400MHz, $CDCl_3$) 28.4 [Boc $\underline{C}H_3$], 39.1 [$\underline{C}H_2$(3')], 52.3 [BocNHCH$_2\underline{C}$H$_2$N and $\underline{C}H_3$O], 53.2 [BocNHCH$_2\underline{C}$H$_2$N], 61.8 [$\underline{C}H_2$(5')], 64.2 [$\underline{C}$H(2')], 70.8 [$\underline{C}$H(4')] 79.0 [Boc $\underline{C}H_3$], 156.2 [Boc $\underline{C}$O] and 175.6 [ester $\underline{C}$O].

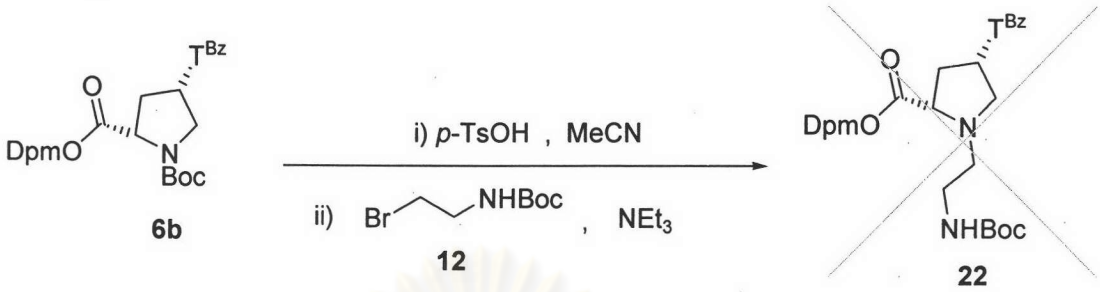
Attempted synthesis of *N*-2-(*N-tert*-butoxycarbonylamino)ethyl-*cis*-4-formyl-D-proline methyl ester (21)



20 **21**

In a dried 100 mL round bottom flask equipped with a magnetic bar, *N*-2-(*N-tert*-butoxycarbonylamino)ethyl-*cis*-4-hydroxy-D-proline methyl ester (**20**) (0.5767 g, 2 mmol), formic acid (90.6 μL, 2.4 mmol) and triphenylphosphine (0.6295 g, 2.4 mmol) were dissolved in dry THF (6 mL) and cooled down to 0 °C in an ice bath. The solution was stirred under nitrogen balloon and DIAD (464.5 μL, 2.4 mmol) was added drop wise within 15 min. The mixture was stirred at ambient temperature overnight. The solvent was evaporated and the residue was chromatographed on silica gel using ethyl acetate:hexanes (1:1) as eluent to give the colorless oil. It was characterized by ^{1}H-NMR spectroscopy. The signal of the aldehyde proton was not observed on the ^{1}H-NMR spectrum indicated that the desired product was not obtained.

Attempted synthesis of *N*-2-(*N-tert*-butoxycarbonylamino)ethyl-*cis*-4-(N^3-benzoylthymin-1-yl)-L-proline diphenylmethyl ester (20) by alkylation with Boc-bromoethylamine

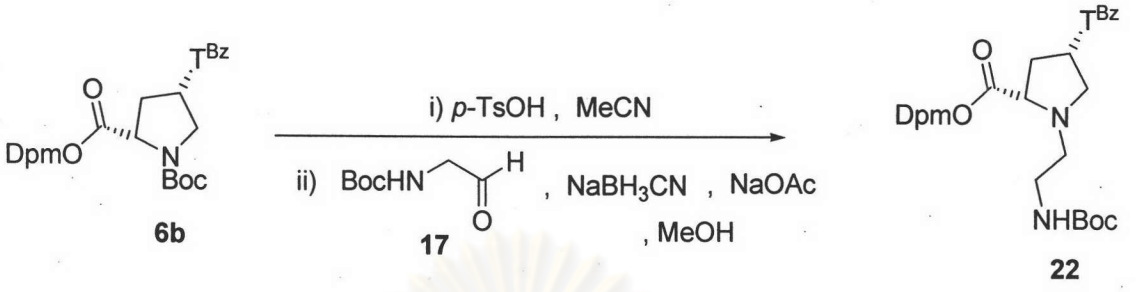


N-Boc-*cis*-4-(N^3-benzoylthymin-1-yl)-L-proline diphenylmethyl ester (**6b**) (0.3048 g, 0.5 mmol) and *p*-toluenesulfonic acid (0.2386 g, 1.25 mmol) were suspended in MeCN (5 mL). The suspension was allowed to stir at room temperature which gradually becomes homogeneous. After 1.5 h, a white precipitate reformed which indicated complete deprotection of the Boc-group. The result was confined by TLC analysis. *N-tert*-Butoxycarbonyl-bromoethylamine (**12**) (0.1121 g, 0.5 mmol) and triethylamine (969 µL, excess) were the added with stirring at room temperature. The TLC analysis revealed that reaction did not provide the desired product (**22**).

Attempted synthesis of *N*-2-(*N-tert*-butoxycarbonylamino)ethyl-*cis*-4-(N^3benzoylthymin-1-yl)-L-proline diphenylmethyl ester (20) by alkylation with Boc-aziridine

N-Boc-*cis*-4-(N^3-benzoylthymin-1-yl)-L-proline diphenylmethyl ester (**6c**) (0.3048 g, 0.5 mmol) was treated with *p*-toluenesulfonic acid (1.25 g, 0.2386 mmol) in MeCN (5 mL) for 2 h as described above. *N-tert*-Butoxycarbonyl-Aziridine (**14**) (0.0716 g, 0.5 mmol) and DIEA (685 µL, excess) were the added with stirring at room temperature. The TLC analysis revealed that reaction did not provide the desired product (**22**).

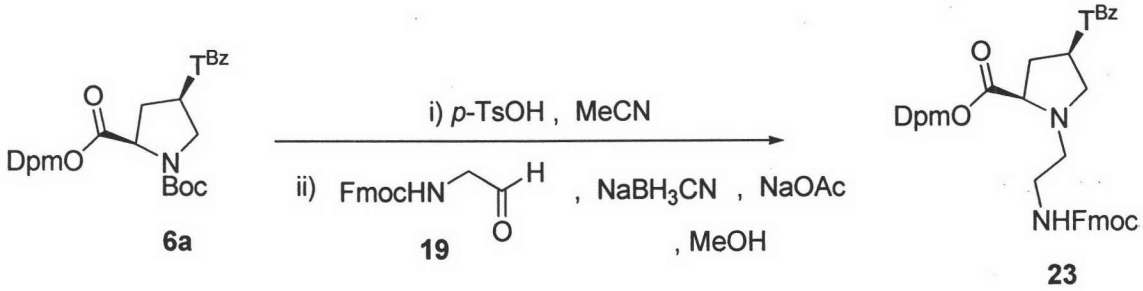
Attempted synthesis of *N*-2-(*N*-*tert*-butoxycarbonylamino)ethyl-*cis*-4-(N^3-benzoyl thymin-1-yl)-L-proline diphenylmethyl ester (22) by reductive alkylation with Boc-aminoaldehyde



N-Boc-*cis*-4-(N^3-benzoylthymin-1-yl)-L-proline diphenylmethyl ester (**6b**) (0.3048 g, 0.5 mmol) was treated with *p*-toluenesulfonic acid (0.1366 g, 1.25 mmol) in MeCN (2 mL) for 3 h as described above. The solvent was evaporated without heating. Then MeOH (1 mL), *N*-Boc-aminoacetaldehyde (**17**) (0.0956 g, 0.5 mmol), NaOAc (0.1 g, excess) and $NaBH_3CN$ (0.0314 g, 0.5 mmol) were added successively. The solution was stirred for a further one hour then the solvent was removed by rotary evaporation, diluted with ethyl acetate and extracted with 10% HCl. The organic layer was washed with aq $NaHCO_3$ and combined organic phase was dried over magnesium sulfate and evaporated under reduced pressure. The residue was purified by flash column chromatography eluting with hexanes:ethyl acetate (3:2) on silica gel to give clear viscous oil and was dried under vacuum to give the product (**22**) as an oil (0.0992 g, 30%).

δ_H (400 MHz, $CDCl_3$) 1.49 [9H, s, Boc C$\underline{H}_3$], 1.93 [3H, s, thymine C$\underline{H}_3$], 1.98 [1H, ddd, J = 2.0, 6.4, 14.4 Hz, 1x C$\underline{H}_2$(3')], 2.56-2.67 [1H, m, 1 x BocNHCH$_2$C$\underline{H}_2$N], 2.76-2.87 [2H, m, 1 x C$\underline{H}_2$(5') and 1 x BocNHCH$_2$C$\underline{H}_2$N], 2.92 [1H, ddd, J = 9.2, 9.2, 14.8, 1 x C$\underline{H}_2$(3')], 3.08-3.19 [1H, m, 1 x BocNHC$\underline{H}_2$CH$_2$N], 3.23-3.34 [2H, m, 1 x C$\underline{H}_2$(5') and 1 x FmocNHC$\underline{H}_2$CH$_2$N], 3.49 [1H, dd, J = 7.2, 8.8 Hz, C$\underline{H}$(2')], 5.17 [1H, br s, BocN$\underline{H}$], 5.26 [1H, t, J = 6.4 Hz, C$\underline{H}$(4')], 7.01 [1H, s, C$\underline{H}$Ph$_2$], 7.33-7.44 [10H, m, Dpm aromatic C$\underline{H}$], 7.51 [2H, t, J = 7.6 Hz, benzoyl *m*-C$\underline{H}$], 7.67 [1H, t, J = 7.6 Hz, benzoyl *p*-C$\underline{H}$], 7.94 [2H, d, J = 6 Hz, benzoyl *m*-C$\underline{H}$] and 8.02 [1H, s, thymine C$\underline{H}$(6)].

_N_-2-(_N_-fluoren-9-ylmethoxycarbonylamino)ethyl-_cis_-4-(N^3-benzoylthymin-1-yl)-D-proline diphenylmethyl ester (23)

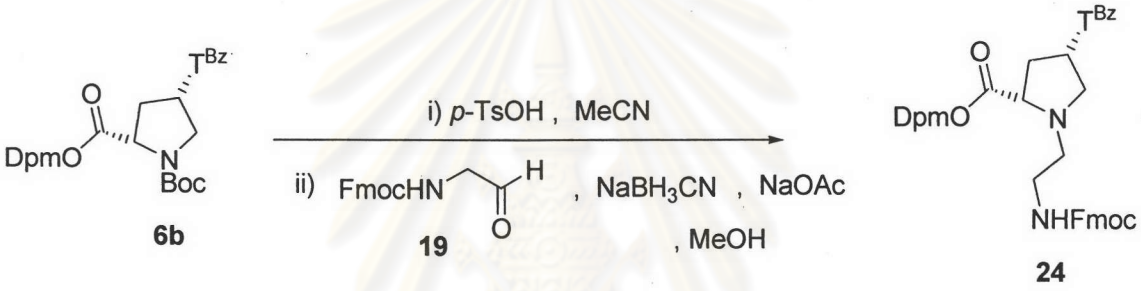


_N_-Boc-_cis_-4-(N^3-benzoylthymin-1-yl)-D-proline diphenylmethyl ester (**6a**) (0.6097 g, 1 mmol) was treated with _p_-toluenesulfonic acid (0.3278 g, 3 mmol) in MeCN (5 mL). The solvent was evaporated without heating. Then MeOH (2 mL), _N_-fluoren-9-ylmethoxycarbonylaminoacetaldehyde (**19**) (0.3376 g, 1.2 mmol), NaOAc (0.4102 g, excess) and $NaBH_3CN$ (0.0754 g, 1.2 mmol) were added successively. The solution was stirred for a further one hour then the solvent was removed by rotary evaporation, diluted with ethyl acetate and extracted with 10% HCl. The organic layer was washed with aq. $NaHCO_3$ and combined organic phase was dried over magnesium sulfate and evaporated under reduced pressure. The residue was purified by flash column chromatography eluting with hexanes:ethyl acetate (1:1) on silica gel to give clear viscous oil and was dried vacuum reduced to give the product (**23**) as white foam (0.6052 g, 76%).

δ_H (400 MHz, $CDCl_3$) 1.86 [3H, s, thymine C$\underline{H}_3$], 2.00 [1H, dd, J = 3.6, 13.6 Hz, 1x C$\underline{H}_2$(3')], 2.61-2.68 [1H, m, 1 x FmocNHCH$_2$C$\underline{H}_2$N], 2.77-2.88 [2H, m, 1 x C$\underline{H}_2$(5') and 1 x FmocNHCH$_2$C$\underline{H}_2$N], 2.92 [1H, ddd, J = 9.2, 9.2, 14.8, 1 x C$\underline{H}_2$(3')], 3.13-3.22 [1H, m, 1 x FmocNHC$\underline{H}_2$CH$_2$N], 3.31 [1H, d, J = 10.4 Hz, 1 x C$\underline{H}_2$(5')], 3.34-3.45 [1H, m, 1 x FmocNHC$\underline{H}_2$CH$_2$N], 3.49 [1H, t, J = 8 Hz, C$\underline{H}$(2')], 4.26 [1H, t, J = 6.8 Hz, Fmoc aliphatic C$\underline{H}$], 4.36 [1H, t, J = 7.2 Hz, 1 x Fmoc aliphatic C$\underline{H}_2$], 4.52 [1H, dd, J = 7.2, 10.4 Hz, 1 x Fmoc aliphatic C$\underline{H}_2$], 5.24-5.32 [1H, m, C$\underline{H}$(4')], 5.59 [1H, br s, Fmoc N$\underline{H}$], 7.00 [1H, s, C$\underline{H}$Ph$_2$], 7.24-7.48 [14H, m, 10 x Dpm aromatic C$\underline{H}$, 4 x Fmoc aromatic C$\underline{H}$], 7.51 [2H, t, J = 7.6 Hz, benzoyl C$\underline{H}$], 7.63-7.77 [3H, m, 2 x benzoyl C$\underline{H}$ and 1 x thymine C$\underline{H}$(6)], 7.82 [2H, d, J = 7.6 Hz, Fmoc aromatic C$\underline{H}$] and 7.92-8.00 [3H, m, 2 x Fmoc aromatic C$\underline{H}$ and 1 x benzoyl C$\underline{H}$], δ_C (400 MHz, $CDCl_3$) 12.7 [thymine C$\underline{H}_3$], 36.6 [C$\underline{H}_2$(3')], 39.5 [FmocNHCH$_2$C$\underline{H}_2$N], 47.3 [Fmoc

aliphatic CH], 52.8 [CH_2(5')], 53.3 [CH(2')], 58.2 [CH(4')], 65.0 [Fmoc aliphatic CH_2], 66.8 [Fmoc $NHCH_2CH_2N$], 78.2 [$CHPh_2$], 111.4 [thymine C(5)], 120.2, 125.3, 127.9 and 128.5 [Fmoc aromatic CH], 127.0, 127.2 and 128.8 [Dpm aromatic CH], 129.3 [benzoyl *o*-CH], 130.5 [benzoyl *m*-CH], 131.7 [benzoyl *p*-CH], 135.2 [benzoyl C], 138.0 [thymine CH(6)], 139.4 and 139.5 [Dpm aromatic C], 141.4, 143.9 and 144.0 [Fmoc aromatic C], 150.1 [thymine CO(2)], 156.7 [Fmoc CO], 162.9 [thymine CO(4)], 169.5 [benzoyl CO] and 172.6 [ester CO], MALDI-TOF M_{obs} ($M + Na^+$) = 797.64; M_{cal} ($M + Na^+$) = 797.30.

_N_-2-(_N_-fluoren-9-ylmethoxycarbonylamino)ethyl-_cis_-4-(N^3-benzoylthymin-1-yl)-L-proline diphenylmethyl ester (24)

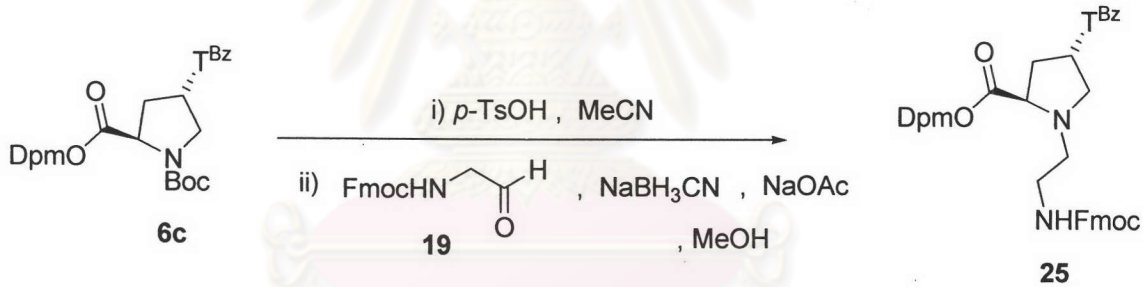


Synthesis of the titled compound (**24**) was accomplished in the same way as described for compound (**23**) above. Starting from *N*-Boc- *cis*-4-(N^3-benzoylthymin-1-yl)-L-proline diphenylmethyl ester (**6b**) (0.6097 g, 1 mmol) and *p*-toluenesulfonic acid (0.3278 g, 3 mmol) in MeCN (5 mL) followed by *N*-fluoren-9-ylmethoxy carbonylaminoacetaldehyde (**19**) (0.3371 g, 1.2 mmol), $NaBH_3CN$ (0.0754 g, 1.2 mmol) and NaOAc (0.4102 g, excess) in MeOH (3ml) afforded (**24**) (0.6840 g, 87%), as a white foam.

δ_H (400 MHz, $CDCl_3$) 1.87 [3H, s, thymine CH_3], 2.01 [1H, dd, J = 5.6, 14.4 Hz, 1x CH_2(3')], 2.61-2.68 [1H, m, 1 x $FmocNHCH_2CH_2N$], 2.78-2.88 [2H, m, 1 x CH_2(5') and 1 x $FmocNHCH_2CH_2N$], 2.93 [1H, ddd, J = 9.2, 9.2, 14.4, 1x CH_2(3')], , 3.13-3.22 [1H, m, 1 x $FmocNHCH_2CH_2N$], 3.32 [1H, d, J = 10.8 Hz, 1 x CH_2(5')], 3.35-3.45 [1H, m, 1 x $FmocNHCH_2CH_2N$], 3.50 [1H, t, J = 8.4 Hz, CH(2')], 4.27 [1H, t, J = 6.8 Hz, Fmoc aliphatic CH], 4.32-4.41 [1H, m, 1 x Fmoc aliphatic CH_2], 4.53 [1H, dd, J = 7.2, 10.4 Hz, 1 x Fmoc aliphatic CH_2], 5.25-5.33 [1H, m, CH(4')], 5.58 [1H, s, Fmoc NH], 7.04 [1H, s, $CHPh_2$], 7.32-7.49 [14H, m, 10 x Dpm aromatic CH and 4 x

Fmoc aromatic C<u>H</u>], 7.52 [2H, t, J = 7.6 Hz, benzoyl C<u>H</u>], 7.64-7.73 [3H, m, 2 x benzoyl C<u>H</u>, 1 x thymine C<u>H</u>(6)], 7.83 [2H, d, J = 7.6 Hz, Fmoc aromatic C<u>H</u>] and 7.94-8.02 [3H, m, 1 x benzoyl C<u>H</u>, 2 x Fmoc aromatic C<u>H</u>], δ_C (400 MHz, $CDCl_3$) 12.6 [thymine <u>C</u>H$_3$], 36.6 [<u>C</u>H$_2$(3')], 39.4 [FmocNHCH$_2$<u>C</u>H$_2$N], 47.3 [Fmoc aliphatic <u>C</u>H], 52.5 [<u>C</u>H$_2$(5')], 53.3 [<u>C</u>H(2')], 58.36 [<u>C</u>H(4')], 65.0 [Fmoc aliphatic <u>C</u>H$_2$], 66.7 [Fmoc NH<u>C</u>H$_2$CH$_2$N], 78.2 [<u>C</u>HPh$_2$], 111.5 [thymine <u>C</u>(5)], 120.1, 125.2, 127.8 and 128.5 [Fmoc aromatic <u>C</u>H], 126.9, 127.2 and 128.8 [Dpm aromatic <u>C</u>H], 129.2 [benzoyl *o*-<u>C</u>H], 130.5 [benzoyl *m*-<u>C</u>H], 131.7 [benzoyl *p*-<u>C</u>H], 135.1 [benzoyl <u>C</u>], 137.6 [thymine <u>C</u>H(6)], 139.3 and 139.4 [Dpm aromatic <u>C</u>], 141.4, 143.9 and 144.0 [Fmoc aromatic <u>C</u>], 150.0 [thymine <u>C</u>O(2)], 156.6 [Fmoc <u>C</u>O], 162.7 [thymine <u>C</u>O(4)], 169.3 [benzoyl <u>C</u>O] and 172.5 [ester <u>C</u>O], MALDI-TOF M_{obs} ($M + Na^+$) = 797.67 ; M_{cal} ($M + Na^+$) = 797.30.

_N_-2-(_N_-fluoren-9-ylmethoxycarbonylamino)ethyl-*trans*-4-(N^3-benzoylthymin-1-yl)-D-proline diphenylmethyl ester (25)

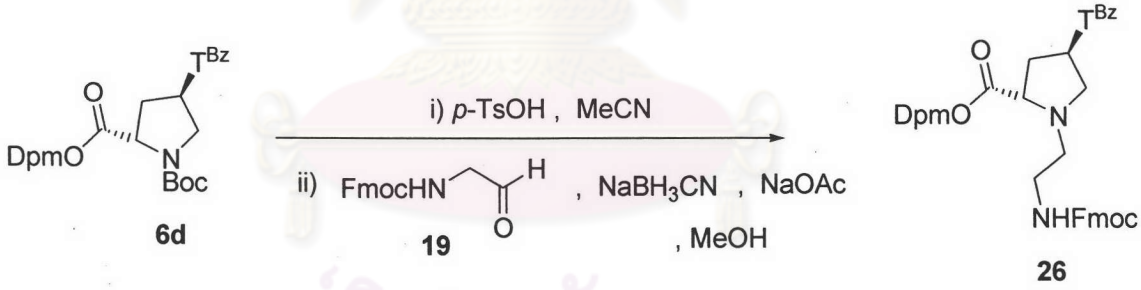


Synthesis of the titled compound (**25**) was accomplished in the same way as described for compound (**23**) above. Starting from *N*-Boc-*trans*-4-(N^3-benzoylthymin-1-yl)-D-proline diphenylmethyl ester (**6c**) (0.6097 g, 1 mmol) and *p*-toluenesulfonic acid (0.3278 g, 3 mmol) in MeCN (5 mL) followed by *N*-fluoren-9-ylmethoxy carbonylaminoacetaldehyde (**19**) (0.3371 g, 1.2 mmol), $NaBH_3CN$ (0.0754 g, 1.2 mmol)and NaOAc (0.4102 g, excess) in MeOH (3 mL) afforded (**25**) (0.3185 g, 40%), as a white foam.

δ_H (400 MHz, $CDCl_3$) 1.95 [3H, s, thymine C<u>H</u>$_3$], 2.21-2.32 [1H, m, 1x C<u>H</u>$_2$(3')], 2.57 [1H, ddd, J = 2.8, 9.2, 13.6 Hz, 1x C<u>H</u>$_2$(3')], 2.73 [2H, t, J = 4.8 Hz, FmocNHCH$_2$<u>C</u>H$_2$N], 2.97 [1H, dd, J = 3.2, 10.0 Hz, 1 x C<u>H</u>$_2$(5')], 3.30 [2H, t, J = 4.8 Hz, FmocNH<u>C</u>H$_2$CH$_2$N], 3.40 [1H, t, J = 9.2 Hz, 1 x C<u>H</u>$_2$(5')], 4.03 [1H, dd, J = 1.6,

6.8 Hz, CH(2')], 4.23 [1H, t, J = 7.2 Hz, Fmoc aliphatic CH], 4.28-4.45 [2H, m, Fmoc aliphatic CH_2], 5.25 [1H, br s, CH(4')], 5.48 [1H, br s, Fmoc NH], 6.95 [1H, s, $CHPh_2$], 7.27-7.49 [14H, m, 10 x Dpm aromatic CH, 4 x Fmoc aromatic CH], 7.53 [2H, t, J = 8.0 Hz, benzoyl CH], 7.57-7.72 [4H, m, 3 x benzoyl CH and 1 x thymine CH(6)], 7.81 [2H, d, J = 7.2 Hz, Fmoc aromatic CH] and 7.96 [2H, d, J = 7.2 Hz, Fmoc aromatic CH], δ_C (400 MHz, $CDCl_3$) 12.7 [thymine CH_3], 35.4 [CH_2(3')], 39.3 [FmocNHCH$_2$CH$_2$N], 47.3 [Fmoc aliphatic CH], 51.1 [CH_2(5')], 54.4 [CH(2')], 56.0 [CH(4')], 63.9 [Fmoc aliphatic CH_2], 66.6 [Fmoc NHCH$_2$CH$_2$N], 77.7 [CHPh2], 111.6 [thymine C(5)], 120.1, 125.1, 127.8 and 128.3 [Fmoc aromatic CH], 127.1, 127.2 and 128.7 [Dpm aromatic CH], 129.3 [benzoyl *o*-CH], 130.5 [benzoyl *m*-CH], 131.6 [benzoyl *p*-CH], 135.2 [benzoyl C], 137.7 [thymine CH(6)], 139.6 and 139.6 [Dpm aromatic C], 141.3 and 143.9 [Fmoc aromatic C], 149.8 [thymine CO(2)], 156.7 [Fmoc CO], 162.9 [thymine CO(4)], 169.3 [benzoyl CO] and 171.5 [ester CO], MALDI-TOF M_{obs} ($M + Na^+$) = 797.68; M_{cal} ($M + Na^+$) = 797.30.

N-2-(N-fluoren-9-ylmethoxycarbonylamino)ethyl-*trans*-4-(N^3-benzoylthymin-1-yl)-L-proline diphenylmethyl ester (26)

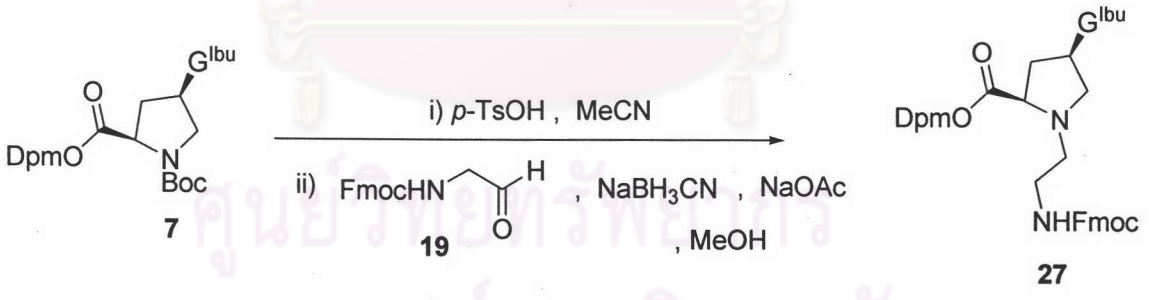


Synthesis of the titled compound (**26**) was accomplished in the same way as described for compound (**23**) above. Starting from N-Boc-*trans*-4-(N^3-benzoylthymin-1-yl)-L-proline diphenylmethyl ester (**6d**) (0.3296 g, 0.5 mmol) and *p*-toluenesulfonic acid (0.1774 g, 1.62 mmol) in MeCN (4 mL) followed by N-fluoren-9-ylmethoxy carbonylaminoacetaldehyde (**19**) (0.2250 g, 0.8 mmol), $NaBH_3CN$ (0.0503 g, 0.8 mmol) and NaOAc (0.2653 g, excess) in MeOH (2 mL) afforded (**26**) (0.1652 g, 43 %), as a white foam.

δ_H (400 MHz, $CDCl_3$) 1.94 [3H, s, thymine CH_3], 2.20-2.32 [1H, m, 1x CH_2(3')], 2.56 [1H, ddd, J = 2.8, 9.2, 13.2 Hz, 1x CH_2(3')], 2.73 [2H, t, J = 4.8 Hz,

FmocNHCH$_2$C$\underline{H}_2$N], 2.97 [1H, d, J = 7.6 Hz, 1 x C$\underline{H}_2$(5')], 3.22-3.34 [2H, m, FmocNHC$\underline{H}_2$CH$_2$N], 3.42 [1H, t, J = 9.2 Hz, 1 x C$\underline{H}_2$(5')], 4.05 [1H, dd, J = 2.0, 7.6 Hz, C$\underline{H}$(2')], 4.24 [1H, t, J = 6.8 Hz, Fmoc aliphatic C$\underline{H}$], 4.40-4.50 [2H, m, Fmoc aliphatic C$\underline{H}_2$], 5.26 [2H, br s, C$\underline{H}$(4') and Fmoc N$\underline{H}$], 6.95 [1H, s, C$\underline{H}$Ph$_2$], 7.23-7.47 [14H, m, 10 x Dpm aromatic C$\underline{H}$, 4 x Fmoc aromatic C$\underline{H}$], 7.51 [2H, t, J = 7.6 Hz, benzoyl C$\underline{H}$], 7.58-7.70 [4H, m, 3 x benzoyl C$\underline{H}$ and 1 x thymine C$\underline{H}$(6)], 7.80 [2H, d, J = 7.2 Hz, Fmoc aromatic C$\underline{H}$] and 7.96 [2H, d, J = 7.6 Hz, Fmoc aromatic C$\underline{H}$], δ_C (400 MHz, $CDCl_3$) 12.7 [thymine $\underline{C}H_3$], 35.5 [$\underline{C}H_2$(3')], 39.3 [FmocNHCH$_2\underline{C}$H$_2$N], 47.3 [Fmoc aliphatic $\underline{C}$H], 51.2 [$\underline{C}H_2$(5')], 54.3 [$\underline{C}$H(2')], 56.0 [$\underline{C}$H(4')], 63.9 [Fmoc aliphatic $\underline{C}H_2$], 66.6 [Fmoc NH$\underline{C}$H$_2$CH$_2$N], 77.6 [$\underline{C}$HPh$_2$], 111.7 [thymine $\underline{C}$(5)], 120.1, 125.0, 127.8 and 128.3 [Fmoc aromatic $\underline{C}$H], 127.1, 127.2 and 128.7 [Dpm aromatic $\underline{C}$H], 129.2 [benzoyl *o*-$\underline{C}$H], 130.5 [benzoyl *m*-$\underline{C}$H], 131.5 [benzoyl *p*-$\underline{C}$H], 135.2 [benzoyl $\underline{C}$], 137.5 [thymine $\underline{C}$H(6)], 139.5 and 139.6 [Dpm aromatic $\underline{C}$], 141.3 and 143.9 [Fmoc aromatic $\underline{C}$], 149.8 [thymine $\underline{C}$O(2)], 156.6 [Fmoc $\underline{C}$O], 162.8 [thymine $\underline{C}$O(4)], 169.2 [benzoyl $\underline{C}$O] and 171.5 [ester $\underline{C}$O], MALDI-TOF M_{obs} (M + Na^+) = 797.80; M_{cal} (M + Na^+) = 797.30.

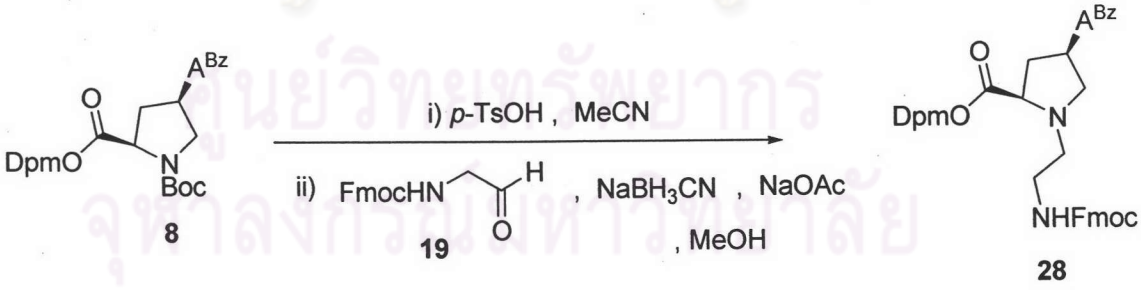
_N_-2-(_N_-fluoren-9-ylmethoxycarbonylamino)ethyl-_cis_-4-(N^2-isobutyrylguanin-9-yl)-D-proline diphenylmethyl ester (27)



Synthesis of the titled compound (**27**) was accomplished in the same way as described for compound (**23**) above. Starting from *N*-Boc- *cis*-4-(N^2-isobutaylguanin-9-yl)-D-proline diphenylmethyl ester (**7**) (0.3003 g, 0.5 mmol) and *p*-toluenesulfonic acid (0.2731 g, 2.5 mmol) in MeCN (5 mL) followed by *N*-fluoren-9-ylmethoxy carbonylaminoacetaldehyde (**19**) (0.1688 g, 0.6 mmol), $NaBH_3CN$ (0.0377 g, 0.6 mmol) and NaOAc (0.25 g, excess) in MeOH (2 mL) afforded (**27**) (0.2654 g, 69%), as a white foam.

δ_H (400 MHz, $CDCl_3$) 1.24 [6H, d, J = 6.0 Hz, $CH(C\underline{H}_3)_2$], 2.10-2.22 [1H, m, $C\underline{H}_2(3')$], 2.55-2.92 [6H, m, 1 x $C\underline{H}_2(3')$, 1 x $C\underline{H}(CH_3)_2$, 1 x $C\underline{H}_2(5')$, 2 x $FmocNHCH_2C\underline{H}_2N$ and 1 x $FmocNHC\underline{H}_2CH_2N$], 3.08-3.26 [1H, m, $FmocNHC\underline{H}_2CH_2N$], 3.26-3.40 [1H, m, $C\underline{H}_2(5')$], 3.46-3.09 [1H, m, $C\underline{H}_2(2')$], 4.23 [1H, t, J = 6.8 Hz, Fmoc aliphatic C$\underline{H}$], 4.35 [2H, d, J = 6.8 Hz, Fmoc aliphatic $C\underline{H}_2$], 5.00 [1H, s, $C\underline{H}_2(4')$], 5.72-5.82 [1H, m, FmocN$\underline{H}$], 6.92 [1H, s, $C\underline{H}Ph_2$], 7.21-7.44 [14H, m, 10 x Dpm aromatic C$\underline{H}$ and 4 x Fmoc aromatic C$\underline{H}$], 7.62 [2H, d, J = 7.2 Hz, Fmoc aromatic C$\underline{H}$], 7.75 [2H, d, J = 7.2 Hz, Fmoc aromatic C$\underline{H}$], 8.14 [1H, s, Guanine C$\underline{H}$(8)], 9.82 and 10.53 [1H, 2 x s, IbuN$\underline{H}$ rotamers], 12.12 and 12.46 [1H, 2 x s, Guanine N$\underline{H}$ rotamers], δ_C (400 MHz, $CDCl_3$) 19.1, 19.2 [$CH(\underline{C}H_3)_2$], 36.0 [$\underline{C}H(CH_3)_2$], 36.9 [$\underline{C}H_2(3')$], 39.6 [$FmocNHCH_2\underline{C}H_2N$], 47.2 [Fmoc aliphatic $\underline{C}H$], 51.9 [$\underline{C}H_2(5')$], 53.5 [$\underline{C}H(2')$], 59.1 [$\underline{C}H(4')$], 64.7 [Fmoc aliphatic $\underline{C}H_2$], 66.9 [Fmoc $NH\underline{C}H_2CH_2N$], 77.9 [$\underline{C}HPh_2$], 119.9, 125.2, 127.7 and 128.3 [Fmoc aromatic $\underline{C}H$], 126.9, 127.1 and 128.7 [Dpm aromatic $\underline{C}H$], 138.2 [Guanine $\underline{C}(5)$], 139.4 and 139.4 [Dpm aromatic $\underline{C}$], 141.2, 144.0 and 144.0 [Fmoc aromatic $\underline{C}$], 147.9 [Guanine $\underline{C}H(8)$], 148.5 [Guanine $\underline{C}(2)$], 156.0 [Guanine $\underline{C}O(6)$], 156.8 [Fmoc $\underline{C}O$], 172.4 [ester $\underline{C}O$] and 180.0 [$\underline{C}(O)CH(CH_3)_2$], MALDI-TOF M_{obs} ($M + H^+$) = 766.86 (H^+); M_{cal} ($M + H^+$) = 766.34.

N-2-(N-fluoren-9-ylmethoxycarbonylamino)ethyl-*cis*-4-(N^A-benzoyladenin-9-yl)-D-proline diphenylmethyl ester (28)

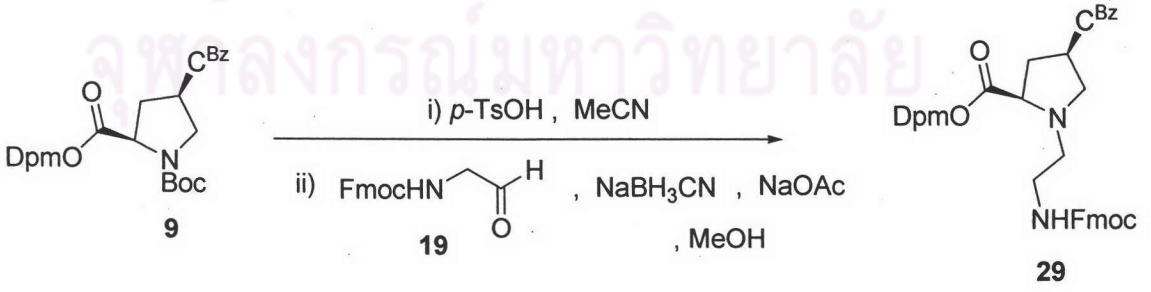


Synthesis of the titled compound (**28**) was accomplished in the same way as described for compound (**23**) above. Starting from N-Boc-*cis*-4-(N^A-benzoyladenin-9-yl)-D-proline diphenylmethyl ester (**8**) (0.4668 g, 0.75 mmol) and *p*-toluenesulfonic acid (0.4097 g, 3.75 mmol) in MeCN (5 mL) followed by N-fluoren-9-ylmethoxy carbonylaminoacetaldehyde (**19**) (0.2532 g, 0.9 mmol), NaOAc (0.3691 g, excess)

and $NaBH_3CN$ (0.0566 g, 0.9 mmol) in MeOH (3 mL) afforded (**28**) (0.3499 g, 60 %), as a white foam.

δ_H (400 MHz, $CDCl_3$) 2.26 [1H, dd, J = 4.8, 14.4 Hz, C$\underline{H}_2$(3')], 2.74-2.83 [1H, m, 1 x FmocNHCH$_2$C$\underline{H}_2$N], 2.85-2.94 [1H, m, 1 x FmocNHCH$_2$C$\underline{H}_2$N], 2.96-3.10 [2H, m, 1 x C$\underline{H}_2$(3') and 1 x C$\underline{H}_2$(5')], 3.12-3.32 [1H, m, FmocNHC$\underline{H}_2$CH$_2$N], 3.32-3.51 [2H, m, 1 x FmocNHC$\underline{H}_2$CH$_2$N and 1 x C$\underline{H}_2$(5')], 3.62 [1H, dd, J = 5.6, 10.4 Hz, C$\underline{H}$(2')], 4.29 [1H, t, J = 7.2 Hz, Fmoc aliphatic C$\underline{H}$], 4.32-4.44 [2H, m, Fmoc aliphatic C$\underline{H}_2$], 5.33-5.41 [1H, m, C$\underline{H}$(4')], 5.58-5.64 [1H, m, FmocN$\underline{H}$], 6.97 [1H, s, C$\underline{H}$Ph$_2$], 7.22-7.41 [14H, m, 10 x Dpm aromatic C$\underline{H}$ and 4 x Fmoc aromatic C$\underline{H}$], 7.50 [2H, t, J = 7.6 Hz, benzoyl C$\underline{H}$], 7.56-7.71 [3H, m, benzoyl C$\underline{H}$], 7.77 [2H, d, J = 7.2 Hz, Fmoc aromatic C$\underline{H}$], 8.02 [2H, d, J = 7.6 Hz, Fmoc aromatic C$\underline{H}$], 8.65 [1H, s, adenine C$\underline{H}$(2)], 8.82 [1H, s, adenine C$\underline{H}$(8)] and 9.36 [1H, s, BzN$\underline{H}$], δ_C (400 MHz, $CDCl_3$) 37.2 [$\underline{C}H_2$(3')], 39.5 [FmocNHCH$_2\underline{C}$H$_2$N], 47.3 [Fmoc aliphatic $\underline{C}$H], 52.1 [$\underline{C}H_2$(5')], 53.6 [$\underline{C}$H(2')], 59.1 [$\underline{C}$H(4')], 64.5 [Fmoc aliphatic $\underline{C}H_2$], 66.9 [Fmoc NH$\underline{C}$H$_2$CH$_2$N], 78.1 [$\underline{C}$HPh$_2$], 119.9, 125.4, 127.7 and 127.9 [Fmoc aromatic $\underline{C}$H] 126.9, 127.1 and 128.7 [Dpm aromatic $\underline{C}$H], 128.8 [benzoyl $\underline{C}$H] 132.7 [benzoyl $\underline{C}$], 133.8 [adenine $\underline{C}$(5)], 139.2 and 139.3 [Dpm aromatic $\underline{C}$], 141.3 [adenine $\underline{C}$H(8)], 142.2, 144.0 and 144.1 [Fmoc aromatic $\underline{C}$], 149.4 [adenine $\underline{C}$(6)], 151.5 [adenine $\underline{C}$H(2)], 152.4 [adenine $\underline{C}$(4)], 156.6 [Fmoc $\underline{C}$O], 164.7 [benzoyl $\underline{C}$O] and 172.5 [ester $\underline{C}$O], MALDI-TOF M_{obs} ($M + H^+$) = 784.53; M_{cal} ($M + H^+$) = 784.33.

_N_-2-(_N_-fluoren-9-ylmethoxycarbonylamino)ethyl-_cis_-4-(N^4-benzoylcytosin-1-yl)-D-proline diphenylmethyl ester (29)

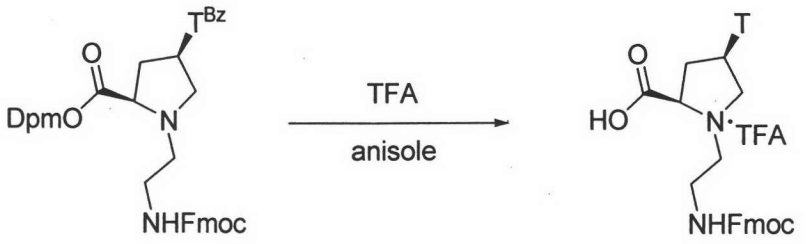


Synthesis of the titled compound (**29**) was accomplished in the same way as described for compound (**23**) above. Starting from _N_-Boc- _cis_-4-(N^4-benzoylcytosin-1-yl)-D-proline diphenylmethyl ester (**9**) (0.2870 g, 0.48 mmol) and _p_-toluenesulfonic

acid (0.2621 g, 2.4 mmol) in MeCN (5 mL) followed by *N*-fluoren-9-ylmethoxy carbonylaminoacetaldehyde (**19**) (0.1632 g, 0.58 mmol), $NaBH_3CN$ (0.0364 g, 0.58 mmol) and NaOAc (0.2362 g, excess) in MeOH (2 mL) afforded (**29**) (0.3231 g, 89 %), as a white foam.

δ_H (400 MHz, $CDCl_3$) 2.05 [1H, dd, J = 4.4, 15.2 Hz, 1x $C\underline{H}_2$(3')], 2.61-2.70 [1H, m, 1 x FmocNHCH$_2$C$\underline{H}_2$N], 2.78-2.91 [2H, m, 1 x $C\underline{H}_2$(5') and 1 x FmocNHCH$_2$C$\underline{H}_2$N], 2.98 [1H, ddd, J = 9.6, 9.6, 14.8, 1x $C\underline{H}_2$(3')], 3.09-3.25 [1H, m, 1 x FmocNHC$\underline{H}_2$CH$_2$N], 3.34 [1H, d, J = 10.8 Hz, 1 x $C\underline{H}_2$(5')], 3.38-3.47 [1H, m, 1 x FmocNHC$\underline{H}_2$CH$_2$N], 3.51 [1H, t, J = 8 Hz, C$\underline{H}$(2')], 4.28 [1H, t, J = 7.2 Hz, Fmoc aliphatic C$\underline{H}$], 4.42 [2H, t, J = 6.8 Hz, Fmoc aliphatic $C\underline{H}_2$], 5.43 [1H, s, C$\underline{H}$(4')], 6.96 [1H, s, C$\underline{H}$Ph$_2$], 7.18-7.72 [20H, m, 10 x Dpm aromatic C$\underline{H}$, 1 x cytosine C$\underline{H}$(3), 4 x Fmoc aromatic C$\underline{H}$, 2 x 3 x benzoyl C$\underline{H}$,], 7.79 [2H, d, J = 7.2 Hz, Fmoc aromatic C$\underline{H}$], 7.89-8.00 [2H, m, Fmoc aromatic C$\underline{H}$] and 8.43 [1H, d, J = 6.4 Hz, cytosine C$\underline{H}$(6)], δ_C (400 MHz, $CDCl_3$) 36.58 [$\underline{C}H_2$(3')], 39.39 [FmocNHCH$_2\underline{C}$H$_2$N], 47.24 [Fmoc aliphatic $\underline{C}$H], 53.68 [$\underline{C}H_2$(5')], 54.29 [$\underline{C}$H(2')], 58.57 [$\underline{C}$H(4')], 65.16 [Fmoc aliphatic $\underline{C}H_2$], 66.81 [Fmoc NH$\underline{C}$H$_2$CH$_2$N], 78.10 [$\underline{C}$HPh$_2$], 97.48 [cytosine $\underline{C}$H(5)], 119.97, 125.25, 127.68 and 128.33 [Fmoc aromatic $\underline{C}$H], 126.91, 127.14 [Dpm aromatic $\underline{C}$H], 128.75 [benzoyl $\underline{C}$H], 133.07 [benzoyl $\underline{C}$], 139.32 [Dpm aromatic $\underline{C}$], 141.34 and 144.09 [Fmoc aromatic $\underline{C}$], 146.81 [cytosine $\underline{C}$O(2)], 156.59 [Fmoc $\underline{C}$O], 161.93 [cytosine $\underline{C}$(4)] and 172.38 [ester $\underline{C}$O].

***N*-2-(*N*-fluoren-9-ylmethoxycarbonylamino)ethyl-*cis*-4-(thymin-1-yl)-D-proline (30)**

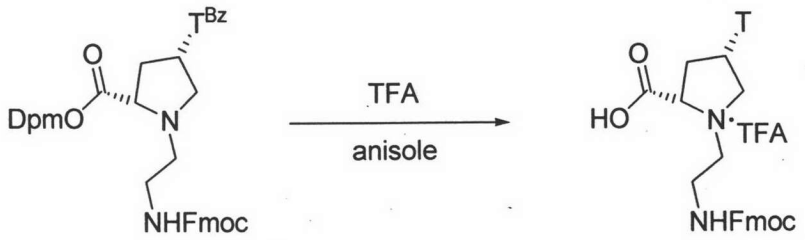


23 **30**

To a mixture of *N*-2-(*N*-fluoren-9-ylmethoxycarbonylamino)ethyl-*cis*-4-(N^3-benzoylthymin-1-yl)-D-proline diphenylmethyl ester (**23**) (0.280 g, 0.34 mmol), anisole (1 mL) and trifluoroacetic acid (3 mL) were stirred at ambient temperature overnight. The volatiles were removed by N_2 gas. Then diethyl ether (20 mL) was added to the residue to precipitate the required compound. The solid was collected by filtration and dried in vacuum to afford required product (**30**) as white solid (0.2545 g, 85 %).

δ_H (400 MHz, DMSO-d_6) 1.77 [3H, s, thymine C$\underline{H}_3$], 2.31-2.41 [1H, m, 1 x C$\underline{H}_2$(3')], 2.83 [1H, ddd, J = 9.2, 9.2, 17.2 Hz, 1 x C$\underline{H}_2$(3')], 3.16-3.25, 3.31-3.45 and 3.48-3.58 [4H, 3 x m, FmocNHC$\underline{H}_2$C$\underline{H}_2$N], 3.69 [1H, t, J = 12.4 Hz, 1 x C$\underline{H}_2$(5')], 3.95 [1H, dd, J = 3.2, 12.4 Hz, 1 x C$\underline{H}_2$(5')], 4.26 [1H, t, J = 6.8 Hz, Fmoc aliphatic C$\underline{H}$], 4.34-4.45 [2H, m, Fmoc aliphatic C$\underline{H}_2$], 4.54 [1H, t, J = 9.2 Hz, C$\underline{H}$(2')], 5.09-5.20 [1H, m, C$\underline{H}$(4')], 7.33 [2H, t, J = 7.2 Hz, Fmoc aromatic C$\underline{H}$], 7.41 [2H, t, J = 7.2 Hz, Fmoc aromatic C$\underline{H}$], 7.57 [1H, s, thymine C$\underline{H}$], 7.69 [2H, d, J = 7.2 Hz, Fmoc aromatic C$\underline{H}$] and 7.89 [2H, d, J = 7.2 Hz, Fmoc aromatic C$\underline{H}$], δ_C (400 MHz, DMSO-d_6) 12.7 [thymine C$\underline{H}_3$], 39.3 [$\underline{C}H_2$(3')], 40.6 [FmocNHCH$_2\underline{C}$H$_2$N], 47.2 [Fmoc aliphatic $\underline{C}$H], 53.3 [$\underline{C}H_2$(5')], 54.2 [$\underline{C}$H(2')], 57.5 [$\underline{C}$H(4')], 66.0 [Fmoc aliphatic $\underline{C}H_2$], 66.2 [FmocNH$\underline{C}$H$_2$CH$_2$N], 109.7 [thymine $\underline{C}$(5)], 120.6, 125.6, 127.6 and 128.1 [Fmoc aromatic $\underline{C}$H], 139.3 [thymine CH(6)], 141.2 and 144.3 [Fmoc aromatic $\underline{C}$], 151.6 [thymine $\underline{C}$O(2)], 156.7 [Fmoc $\underline{C}$O] and 164.3 [thymine $\underline{C}$O(4)], $[\alpha]^{25}_D$ = + 12.0 (c = 51 g/100 mL, DMF), MALDI-TOF M_{obs} (M + H^+) = 505.13; M_{cal} (M + H^+) = 505.03.

***N*-2-(*N*-fluoren-9-ylmethoxycarbonylamino)ethyl-*cis*-4-(thymin-1-yl)-L-proline (31)**

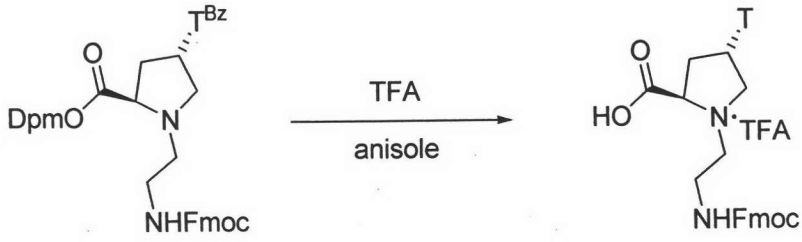


24 **31**

Synthesis of the titled compound (**31**) was accomplished in the same way as described for compound (**30**) above. Starting from *N*-2-(*N*-fluoren-9-ylmethoxy carbonylamino)ethyl-*cis*-4-(N^3-benzoylthymin-1-yl)-L-proline diphenylmethy ester (**24**) (0.6840 g, 0.9 mmol), anisole (1 mL) and trifluoroacetic acid (3 mL) afforded (**31**) (0.3711 g, 72%), as a white solid.

δ_H (400 MHz, DMSO-d_6) 1.76 [3H, s, thymine C$\underline{H}_3$], 2.31-2.41 [1H, m, 1 x C$\underline{H}_2$(3')], 2.83 [1H, ddd, J = 9.2, 9.2, 17.2 Hz, 1 x C$\underline{H}_2$(3')], 3.16-3.25, 3.29-3.45 and 3.46-3.58 [4H, 4 x m, FmocNHC$\underline{H}_2$C$\underline{H}_2$N], 3.69 [1H, t, J = 12.0 Hz, 1 x C$\underline{H}_2$(5')], 3.95 [1H, dd, J = 4, 12.4 Hz, 1 x C$\underline{H}_2$(5')], 4.25 [1H, t, J = 6.8 Hz, Fmoc aliphatic C$\underline{H}$], 4.34-4.45 [2H, m, Fmoc aliphatic C$\underline{H}_2$], 4.54 [1H, t, J = 8.8 Hz, C$\underline{H}$(2')], 5.09-5.21 [1H, m, C$\underline{H}$(4')], 7.33 [2H, t, J = 7.6 Hz, Fmoc aromatic C$\underline{H}$], 7.40 [2H, t, J = 7.6 Hz, Fmoc aromatic C$\underline{H}$], 7.57 [1H, s, thymine C$\underline{H}$], 7.68 [2H, d, J = 7.6 Hz, Fmoc aromatic C$\underline{H}$], 7.87 [2H, d, J = 7.6 Hz, Fmoc aromatic C$\underline{H}$] and 11.52 [1H, s, thymine N$\underline{H}$], δ_C (400 MHz, DMSO-d_6) 12.7 [thymine C$\underline{H}_3$], 39.3 [$\underline{C}H_2$(3')], 40.5 [FmocNHCH$_2\underline{C}$H$_2$N], 47.2 [Fmoc aliphatic $\underline{C}$H], 53.4 [$\underline{C}H_2$(5')], 54.3 [$\underline{C}$H(2')], 57.4 [$\underline{C}$H(4')], 66.0 [Fmoc aliphatic $\underline{C}H_2$], 66.3 [FmocNH$\underline{C}$H$_2$CH$_2$N], 109.8 [thymine $\underline{C}$(5)], 120.6, 125.6, 127.6 and 128.1 [Fmoc aromatic $\underline{C}$H], 139.2 [thymine CH(6)], 141.2 and 144.3 [Fmoc aromatic $\underline{C}$], 151.7 [thymine $\underline{C}$O(2)], 156.8 [Fmoc $\underline{C}$O] and 164.3 [thymine $\underline{C}$O(4)], $[\alpha]^{25}_D$ = -14.3 (c = 53 g/100 mL, DMF), MALDI-TOF M_{obs} ($M + H^+$) = 505.24; M_{cal} ($M + H^+$) = 505.21.

***N*-2-(*N*-fluoren-9-ylmethoxycarbonylamino)ethyl-*trans*-4-(thymin-1-yl)-D-proline (32)**

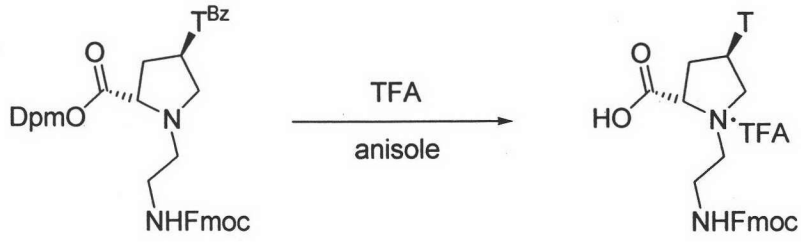


25 **32**

Synthesis of the titled compound (**32**) was accomplished in the same way as described for compound (**30**) above. Starting from *N*-2-(*N*-fluoren-9-ylmethoxy carbonylamino)ethyl-*trans*-4-(N^3-benzoylthymin-1-yl)-D-proline diphenylmethyl ester (**25**) (0.2102 g, 0.27 mmol), anisole (1 mL) and trifluoroacetic acid (3 mL) afforded (**32**) (0.1201 g, 72 %), as a white solid.

δ_H (400MHz, DMSO-d_6) 1.78 [3H, s, thymine C$\underline{H}_3$], 2.61-2.71 [1H, m, 1x C$\underline{H}_2$(3')], 3.20-3.30 [1H, m, 1 x C$\underline{H}_2$(3')], 3.30-3.50 [4H, 2 x m, FmocNHC$\underline{H}_2$C$\underline{H}_2$N], 3.53-3.63 [1H, m, 1 x C$\underline{H}_2$(5')], 3.95-4.04 [1H, m, 1 x C$\underline{H}_2$(5')], 4.25 [1H, t, J = 6.4 Hz, Fmoc aliphatic C$\underline{H}$], 4.40 [2H, d, J = 6.4 Hz, Fmoc aliphatic C$\underline{H}_2$], 4.77 [1H, t, J = 9.2 Hz, C$\underline{H}$(2')], 4.93-5.03 [1H, m, C$\underline{H}$(4')], 7.34 [2H, t, J = 7.6 Hz, Fmoc aromatic C$\underline{H}$], 7.42 [2H, t, J = 7.6 Hz, Fmoc aromatic C$\underline{H}$], 7.62 [1H, s, thymine C$\underline{H}$], 7.70 [2H, d, J = 7.6 Hz, Fmoc aromatic C$\underline{H}$], 7.90 [2H, d, J = 7.6 Hz, Fmoc aromatic C$\underline{H}$] and 11.42 [1H, s, thymine N$\underline{H}$], δ_C (400 MHz, DMSO-d_6) 12.6 [thymine $\underline{C}H_3$], 33.6 [$\underline{C}H_2$(3')], 38.6 [FmocNHCH$_2\underline{C}$H$_2$N], 47.2 [Fmoc aliphatic $\underline{C}$H], 52.9 [$\underline{C}H_2$(5')], 53.0 [$\underline{C}$H(2')], 55.6 [$\underline{C}$H(4')], 65.2 [Fmoc aliphatic $\underline{C}H_2$], 65.9 [Fmoc NH$\underline{C}$H$_2$CH$_2$N], 110.1 [thymine $\underline{C}$(5)], 120.6, 125.6, 127.5 and 128.1 [Fmoc aromatic $\underline{C}$H], 138.8 [thymine $\underline{C}$H(6)], 141.2 and 144.3 [Fmoc aromatic $\underline{C}$], 151.3 [thymine $\underline{C}$O(2)], 156.7 [Fmoc $\underline{C}$O], 164.3 [thymine $\underline{C}$O(4)] and 172.3 [$\underline{C}$OOH], $[\alpha]^{25}_D$ = +19.4 (c = 50 g/100 mL, DMF), MALDI-TOF M_{obs} (M + H^+) = 505.12; M_{cal} (M + H^+) = 505.21.

_N_-2-(_N_-fluoren-9-ylmethoxycarbonylamino)ethyl-_trans_-4-(thymin-1-yl)-L-proline (33)

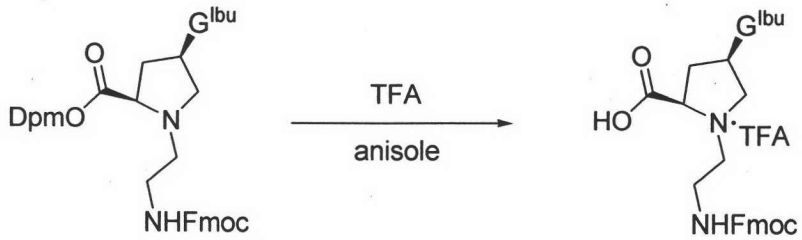


26 **33**

Synthesis of the titled compound (**33**) was accomplished in the same way as described for compound (**30**) above, Starting from *N*-2-(*N*-fluoren-9-ylmethoxy carbonylamino)ethyl-*trans*-4-(N^3-benzoylthymin-1-yl)-L-proline diphenylmethyl ester (**26**) (0.1308 g, 0.17 mmol), anisole (0.5 mL) and trifluoroacetic acid (2 mL) afforded (**33**) (0.0788 g, 75 %), as a white solid.

δ_H (400 MHz, DMSO-d_6) 1.77 [3H, s, thymine C$\underline{H}_3$], 2.60-2.71 [1H, m, 1x C$\underline{H}_2$(3')], 3.20-3.30 [1H, m, 1 x C$\underline{H}_2$(3')], 3.30-3.55 [4H, 2 x m, FmocNHC$\underline{H}_2$C$\underline{H}_2$N], 3.55-3.63 [1H, m, 1 x C$\underline{H}_2$(5')], 3.94-4.05 [1H, m, 1 x C$\underline{H}_2$(5')], 4.24 [1H, t, J = 6.4 Hz, Fmoc aliphatic C$\underline{H}$], 4.40 [2H, d, J = 6.8 Hz, Fmoc aliphatic C$\underline{H}_2$], 4.77 [1H, t, J = 9.6 Hz, C$\underline{H}$(2')], 4.90-5.02 [1H, m, C$\underline{H}$(4')], 7.32 [2H, t, J = 7.2 Hz, Fmoc aromatic C$\underline{H}$], 7.40 [2H, t, J = 7.2 Hz, Fmoc aromatic C$\underline{H}$], 7.60 [1H, s, thymine C$\underline{H}$], 7.68 [2H, d, J = 8.0 Hz, Fmoc aromatic C$\underline{H}$] and 7.87 [2H, d, J = 7.2 Hz, Fmoc aromatic C$\underline{H}$], δ_C (400 MHz, DMSO-d_6) 12.6 [thymine $\underline{C}H_3$], 33.6 [$\underline{C}H_2$(3')], 38.6 [FmocNHCH$_2\underline{C}$H$_2$N], 47.2 [Fmoc aliphatic $\underline{C}$H], 52.9 [$\underline{C}H_2$(5')], 53.0 [$\underline{C}$H(2')], 55.6 [$\underline{C}$H(4')], 65.2 [Fmoc aliphatic $\underline{C}H_2$], 65.9 [Fmoc NH$\underline{C}H_2$CH$_2$N], 110.1 [thymine $\underline{C}$(5)], 120.6, 125.6, 127.5 and 128.1 [Fmoc aromatic $\underline{C}$H], 138.8 [thymine $\underline{C}$H(6)], 141.2 and 144.3 [Fmoc aromatic $\underline{C}$], 151.3 [thymine $\underline{C}$O(2)], 156.7 [Fmoc $\underline{C}$O], 164.3 [thymine $\underline{C}$O(4)] and 172.3 [$\underline{C}$OOH], $[\alpha]^{25}_D$ = -22.6 (c = 50 g/100 mL, DMF), MALDI-TOF M_{obs} (M + H^+) = 505.00; M_{cal} (M + H^+) = 505.21.

***N*-2-(*N*-fluoren-9-ylmethoxycarbonylamino)ethyl-*cis*-4-(N^2-isobutyrylguanin-9-yl)-D-proline (34)**

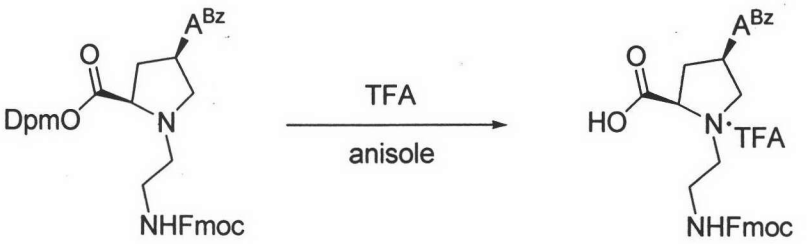


27 **34**

Synthesis of the titled compound (**34**) was accomplished in the same way as described for compound (**30**) above. Starting from *N*-2-(*N*-fluoren-9-ylmethoxy carbonylamino)ethyl-*cis*-4-(N^2-isobutyrylguanin-9-yl)-D-proline diphenylmethyl ester (**27**) (0.2149 g, 0.28 mmol), anisole (1 mL) and trifluoroacetic acid (3 mL) afforded (**34**) (0.1569 g, 77 %), as a white solid.

δ_H (400 MHz, DMSO-d_6) 1.14 [6H, d, J = 6.8 Hz, $CH(C\underline{H}_3)_2$], 2.09-2.25 [1H, m, $C\underline{H}_2(3')$], 2.51-2.73 [1H, m, $FmocNHCH_2C\underline{H}_2N$], 2.79 [1H, hep, J = 6.8 Hz, $C\underline{H}(CH_3)_2$], 2.90 [1H, ddd, J = 9.2, 9.2, 14.0Hz, $C\underline{H}_2(3')$], 2.98-3.29 [4H, 2 x m, 1 x $FmocNHCH_2C\underline{H}_2N$, 2 x $FmocNHC\underline{H}_2CH_2N$ and 1 x $C\underline{H}_2(5')$], 3.50-3.66 [2H, m, 1 x $C\underline{H}_2(5')$and 1 x $C\underline{H}_2(2')$], 4.20-4.43 [3H, m, 1 x Fmoc aliphatic C$\underline{H}$ and 2 x Fmoc aliphatic $C\underline{H}_2$], 5.04 [1H, s, $C\underline{H}_2(4')$], 7.28-7.48 [4H, m, Fmoc aromatic C$\underline{H}$], 7.70 [2H, d, J = 7.2 Hz, Fmoc aromatic C$\underline{H}$], 7.89 [2H, d, J = 7.2 Hz, Fmoc aromatic C$\underline{H}$], 8.26 [1H, s, Guanine C$\underline{H}$], 11.67 and 12.08 [1H, 2 x s, Guanine N$\underline{H}$ rotamers], δ_C (400 MHz, DMSO-d_6) 19.3 [$CH(\underline{C}H_3)_2$], 35.2 [$\underline{C}H(CH_3)_2$], 39.5 [$\underline{C}H_2(3')$], 40.4 [$FmocNHCH_2\underline{C}H_2N$], 47.2 [Fmoc aliphatic $\underline{C}H$], 52.1 [$\underline{C}H_2(5')$], 54.0 [$\underline{C}H(2')$], 58.7 [$\underline{C}H(4')$], 65.4 [Fmoc aliphatic $\underline{C}H_2$], 65.9 [Fmoc $NH\underline{C}H_2CH_2N$], 120.6, 125.6, 127.6 and 128.1 [Fmoc aromatic $\underline{C}H$], 138.5 [Guanine $\underline{C}(5)$], 141.2, 144.3 and 144.4 [Fmoc aromatic $\underline{C}$], 148.2 [Guanine $\underline{C}H(8)$], 148.7 [Guanine $\underline{C}(2)$], 155.4 [Guanine $\underline{C}O(6)$], 156.6 [Fmoc $\underline{C}O$] and 180.5 [$\underline{C}(O)CH(CH_3)_2$], $[\alpha]^{25}_D$ = -9.1 (c = 53 g/100 mL, DMF), MALDI-TOF M_{obs} ($M + H^+$) = 600.19; M_{cal} ($M + H^+$) = 600.26.

***N*-2-(*N*-fluoren-9-ylmethoxycarbonylamino)ethyl-*cis*-4-(N^4-benzoyladenin-9-yl)-D-proline (35)**

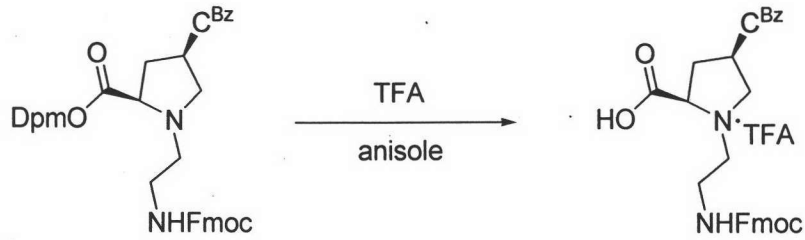


28 **35**

Synthesis of the titled compound (**35**) was accomplished in the same way as described for compound (**30**) above. Starting from *N*-2-(*N*-fluoren-9-ylmethoxy carbonyamino)ethyl-*cis*-4-(N^4-benzoyladenin-9-yl)-D-proline diphenylmethyl ester (**28**) (0.3499 g, 0.45 mmol), anisole (1 mL) and trifluoroacetic acid (3 mL) afforded (**35**) (0.2431 g, 75 %), as a white solid.

δ_H (400 MHz, DMSO-d_6) 2.35-2.42 [1H, m, C$\underline{H}_2$(3')], 2.78-2.90 [1H, m, C$\underline{H}_2$(3')], 2.91-3.09 and 3.10-3.42 [5H, 2 x m, 1 x C$\underline{H}_2$(5') and 4 x FmocNHC$\underline{H}_2$C$\underline{H}_2$N], 3.63-3.81 [1H, m, 1 x C$\underline{H}_2$(5')], 3.81-3.94 [1H, m, 1 x C$\underline{H}_2$(2')], 4.15-4.31 [3H, m, 2 x Fmoc aliphatic C$\underline{H}_2$ and 1 x Fmoc aliphatic C$\underline{H}$], 5.33-5.50 [1H, m, C$\underline{H}_2$(4')], 7.31-7.50 [4H, m, Fmoc aromatic C$\underline{H}$], 7.57 [2H, t, J = 7.6 Hz, benzoyl C$\underline{H}$], 7.52-7.74 [3H, m, benzoyl C$\underline{H}$], 7.89 [2H, d, J = 7.6 Hz, Fmoc aromatic C$\underline{H}$], 8.08 [2H, d, J = 7.6 Hz, Fmoc aromatic C$\underline{H}$], 8.72 [1H, s, adenine CH(2)] and 8.75 [1H, s, adenine CH(8)], δ_C (400 MHz, DMSO-d_6) 39.3 [$\underline{C}H_2$(3')], 40.4 [FmocNHCH$_2\underline{C}$H$_2$N], 47.1 [Fmoc aliphatic $\underline{C}$H], 52.2 [$\underline{C}H_2$(5')], 54.1 [$\underline{C}$H(2')], 58.4 [$\underline{C}$H(4')], 65.7 [Fmoc aliphatic $\underline{C}H_2$], 66.0 [Fmoc NH$\underline{C}$H$_2$CH$_2$N], 120.6, 125.7, 127.6 and 128.1 [Fmoc aromatic $\underline{C}$H], 129.0 [benzoyl $\underline{C}$H], 133.0 [benzoyl $\underline{C}$], 133.9 [adenine $\underline{C}$(5)], 141.2 [adenine $\underline{C}$H(8)], 141.2, 143.7 and 144.3 [Fmoc aromatic $\underline{C}$], 150.7 [adenine $\underline{C}$(6)], 151.6 [adenine $\underline{C}$H(2)], 152.3 [adenine $\underline{C}$(4)], 156.7 [Fmoc $\underline{C}$O] and 166.2 [benzoyl $\underline{C}$O], $[\alpha]^{25}_D$ = +18.1 (c = 53 g/100 mL, DMF), MALDI-TOF M_{obs} (M + H^+) = 618.19; M_{cal} (M + H^+) = 617.24.

***N*-2-(*N*-fluoren-9-ylmethoxycarbonylamino)ethyl-*cis*-4-(N^4-benzoylcytosin-1-yl)-D-proline (36)**

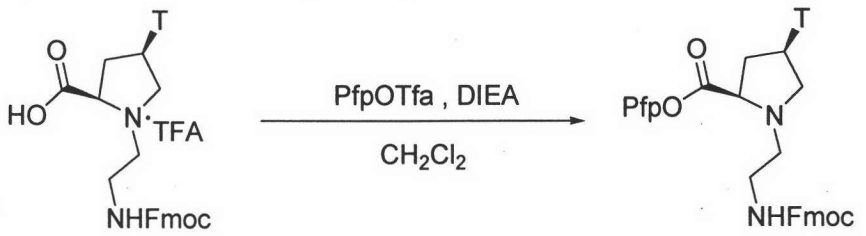


29 **36**

Synthesis of the titled compound (**36**) was accomplished in the same way as described for compound (**30**) above. Starting from *N*-2-(*N*-fluoren-9-ylmethoxy carbonylamino)ethyl-*cis*-4-(N^4-benzoylcytosin-1-yl)-D-proline diphenylmethyl ester (**29**) (0.3231 g, 0.43 mmol), anisole (1 mL) and trifluoroacetic acid (3 mL) afforded (**36**) (0.2226 g, 74 %), as a white solid.

δ_H (400 MHz, DMSO-d_6) 2.41-2.56 [1H, m, 1 x C$\underline{H}_2$(3') overlap with H_2O in DMSO], 2.91-3.03 [1H, m, 1 x C$\underline{H}_2$(3')], 3.19-3.28, 3.35-3.46 and 3.52-3.12 [4H, 3 x m, FmocNHC$\underline{H}_2$C$\underline{H}_2$N], 3.77 [1H, t, J = 12.0 Hz, 1 x C$\underline{H}_2$(5')], 4.14 [1H, d, J = 12.0 Hz, 1 x C$\underline{H}_2$(5')], 4.25-4.32 [1H, m, Fmoc aliphatic C$\underline{H}$], 4.34-4.41 [2H, m, Fmoc aliphatic C$\underline{H}_2$], 4.60 [1H, t, J = 9.2 Hz, C$\underline{H}$(2')], 5.07-5.18 [1H, m, C$\underline{H}$(4')], 7.28-7.39 [2H, m, Fmoc aromatic C$\underline{H}$], 7.39-7.47 [3H, m, 1 x cytosine C$\underline{H}$(3), 2 x Fmoc aromatic C$\underline{H}$], 7.50-7.75 [5H, m, benzoyl C$\underline{H}$], 7.90 [2H, d, J = 6.4 Hz, Fmoc aromatic C$\underline{H}$], 8.00 [2H, d, J = 7.6 Hz, Fmoc aromatic C$\underline{H}$] and 8.20 [1H, d, J = 5.6 Hz, cytosine C$\underline{H}$(6)], δ_C (400 MHz, DMSO-d_6) 39.3 [$\underline{C}H_2$(3')], 40.4 [FmocNHCH$_2\underline{C}$H$_2$N], 47.2 [Fmoc aliphatic $\underline{C}$H], 54.0 [$\underline{C}H_2$(5')], 55.8 [$\underline{C}$H(2')], 58.0 [$\underline{C}$H(4')], 65.7 [Fmoc aliphatic $\underline{C}H_2$ and Fmoc NH$\underline{C}$H$_2$CH$_2$N], 96.8 [cytosine $\underline{C}$H(5)], 120.6, 125.6, 127.5 and 128.1 [Fmoc aromatic $\underline{C}$H], 128.9 [benzoyl $\underline{C}$H], 133.2 [benzoyl $\underline{C}$], 133.7 [cytosine $\underline{C}$H(6)], 141.2 and 144.3 [Fmoc aromatic $\underline{C}$], 155.7 [cytosine $\underline{C}$O(2)], 156.7 [Fmoc $\underline{C}$O], 163.2 [cytosine $\underline{C}$(4)] and 167.9 [benzoyl $\underline{C}$O], $[\alpha]^{25}_D$ = +2.8 (c = 50 g/100 mL, DMF), MALDI-TOF M_{obs} ($M + H^+$) = 594.11; M_{cal} ($M + H^+$) = 594.24.

_N_-2-(_N_-fluoren-9-ylmethoxycarbonylamino)ethyl-_cis_-4-(thymin-1-yl)-D-proline pentafluorophenyl ester (37)



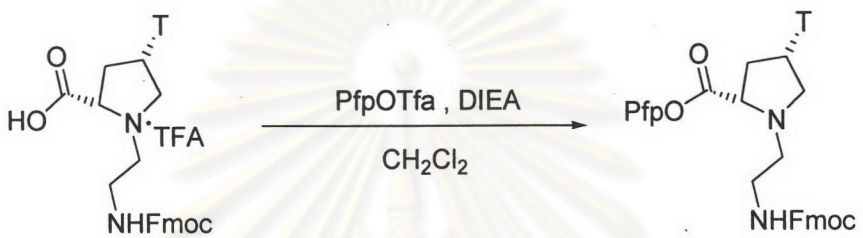
30 **37**

In a screwed cap test tube, a suspension of _N_-2-(_N_-fluoren-9-ylmethoxy carbonylamino)ethyl-_cis_-4-(thymin-1-yl)-D-proline (**30**) (92.7 mg, 0.15 mmol) and PfpOTfa (77.6 μL, 0.45 mmol) in dichloromethane (1 mL) was add DIEA (102.7 μL, 0.6 mmol). The resulted mixture was stirred for a further one hour. The reaction was completed as indicated by TLC analysis and diluted with dichloromethane and extracted with 2.5% HCl. The organic layer was washed with aq $NaHCO_3$ and combined organic phase were dried over magnesium sulfate and evaporated under reduced pressure. The residue was purified by flash column chromatography eluting with 100% ethyl acetate on silica gel to give a clear viscous oil (98.6 mg, 98 %). Scratching the oil with ice-cold hexane afford the product (**37**) as a white solid.

mp. = 93.8-95.0 °C, δ_H (400 MHz, $CDCl_3$) 1.89 [3H, s, thymine C$\underline{H}_3$], 2.17 [1H, d , J = 14.4 Hz, 1x C$\underline{H}_2$(3')], 2.75-2.83 [1H, m, 1 x FmocNHCH$_2$C$\underline{H}_2$N], 2.90-3.11 [3H, m, 1 x C$\underline{H}_2$(5'), 1 x FmocNHCH$_2$C$\underline{H}_2$N and 1x C$\underline{H}_2$(3')], 3.30-3.40 [2H, m, 1 x FmocNHC$\underline{H}_2$CH$_2$N and 1 x C$\underline{H}_2$(5')], 3.42-3.54 [1H, m, 1 x FmocNHC$\underline{H}_2$CH$_2$N], 3.76 [1H, t, J = 8.8 Hz, C$\underline{H}$(2')], 4.22 [1H, t, J = 6.8 Hz, Fmoc aliphatic C$\underline{H}$], 4.36 [1H, t, J = 6.8 Hz, 1 x Fmoc aliphatic C$\underline{H}_2$], 4.52 [1H, dd, J = 7.2, 10.4 Hz, 1 x Fmoc aliphatic C$\underline{H}_2$], 5.25-5.41 [2H, m, 1 x C$\underline{H}$(4') and 1 x Fmoc N$\underline{H}$], 7.29 [2H, m, Fmoc aromatic C$\underline{H}$ overlap with $CDCl_3$], 7.41 [2H, t, J = 7.6 Hz, Fmoc aromatic C$\underline{H}$], 7.59 [2H, t, J = 6.8 Hz, Fmoc aromatic C$\underline{H}$], 7.76-7.83 [3H, m, 2 x Fmoc aromatic C$\underline{H}$ and 1 x thymine C$\underline{H}$(6)] and 8.35 [1H, s, thymine N$\underline{H}$], δ_C (400 MHz, $CDCl_3$) 12.6 [thymine C$\underline{H}_3$], 36.8 [$\underline{C}$H$_2$(3')], 39.4 [FmocNHCH$_2\underline{C}$H$_2$N], 47.2 [Fmoc aliphatic $\underline{C}$H], 52.3 [$\underline{C}$H$_2$(5')], 53.7 [$\underline{C}$H(2')], 57.8 [$\underline{C}$H(4')], 64.4 [Fmoc aliphatic $\underline{C}$H$_2$], 66.8 [FmocNH$\underline{C}$H$_2$CH$_2$N], 111.9 [thymine $\underline{C}$(5)], 112.0, 125.0, 127.0 and 127.7 [Fmoc

aromatic CH], 136.7 [Pfp CF], 137.2 [thymine CH(6)], 138.5, 139.2 and 139.7 [Pfp CF], 141.3 [Fmoc aromatic C], 142.2 [Pfp CF], 143.9 [Fmoc aromatic C], 151.4 [thymine CO(2)], 156.6 [Fmoc CO], 164.2 [thymine CO(4)] and 169.8 [ester CO], $[\alpha]^{25}_{D}$ = -10.8 (c = 51 g/100 mL, $CHCl_3$), MALDI-TOF M_{obs} ($M + H^+$) = 670.96; M_{cal} ($M + H^+$) = 671.20.

_N_-2-(_N_-fluoren-9-ylmethoxycarbonylamino)ethyl-_cis_-4-(thymin-1-yl)-L-proline pentafluorophenyl ester (38)



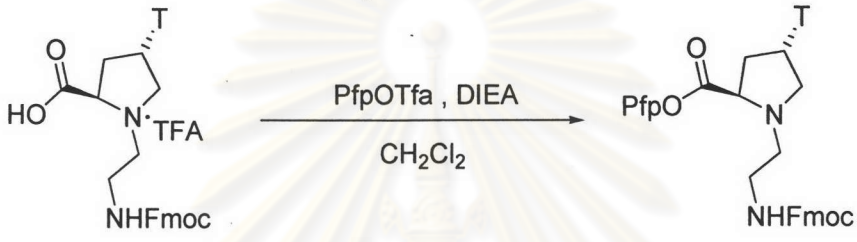
31 **38**

Synthesis of the titled compound (**38**) was accomplished in the same way as described for compound (**37**) above. Starting from *N*-2-(*N*-fluoren-9-ylmethoxy carbonylamino)ethyl-*cis*-4-(thymin-1-yl)-L-proline (**31**) (123.6 mg, 0.2 mmol), PfpOTfa (103.5 μL, 0.6 mmol) and DIEA (136.8 μL, 0.8 mol) in dichloromethane (2 mL) afford (**38**) (118.9 mg, 89 %), as a white solid.

mp. = 94.3-95.8 °C, δ_H (400MHz, $CDCl_3$) 1.75 [3H, s, thymine CH_3], 2.16 [1H, d , J = 16 Hz, 1x CH_2(3')], 2.72-2.85 [1H, m, 1 x FmocNHCH$_2$CH_2N], 2.90-3.12 [3H, m, 1 x CH_2(5'), 1 x FmocNHCH$_2$CH_2N and 1x CH_2(3')], 3.29-3.41 [2H, m, 1 x FmocNHCH_2CH$_2$N and 1 x CH_2(5')], 3.42-3.54 [1H, m, 1 x FmocNHCH_2CH$_2$N], 3.76 [1H, t, J = 7.6 Hz, CH(2')], 4.22 [1H, t, J = 7.2 Hz, Fmoc aliphatic CH], 4.31-4.41 [1H, m, 1 x Fmoc aliphatic CH_2], 4.52 [1H, dd, J = 7.2, 10.4 Hz, 1 x Fmoc aliphatic CH_2], 5.26-5.41 [2H, m, 1 x CH(4') and 1 x Fmoc NH], 7.30 [2H, m, Fmoc aromatic CH overlap with $CDCl_3$], 7.42 [2H, t, J = 7.6 Hz, Fmoc aromatic CH], 7.59 [2H, t, J = 7.2 Hz, Fmoc aromatic CH], 7.74-7.83 [3H, m, 2 x Fmoc aromatic CH and 1 x thymine CH(6)] and 8.89 [1H, s, thymine NH], δ_C (400MHz, $CDCl_3$) 12.6 [thymine CH_3], 36.3 [CH_2(3')], 39.1 [FmocNHCH$_2$CH$_2$N], 47.2 [Fmoc aliphatic CH], 52.8 [CH_2(5')], 53.9 [CH(2')], 57.8 [CH(4')], 64.6 [Fmoc aliphatic CH_2], 67.0

[FmocNHCH$_2$CH$_2$N], 112.0 [thymine C(5)], 112.0, 125.1, 127.0 and 127.7 [Fmoc aromatic CH], 137.7 [thymine CH(6)], 141.3 and 143.8 [Fmoc aromatic C], 151.3 [thymine CO(2)], 156.6 [Fmoc CO] and 163.7 [thymine CO(4)], $[\alpha]^{25}_D$ = +10.5 (c = 52 g/100 mL, $CHCl_3$), MALDI-TOF M_{obs} ($M + H^+$) = 671.07; M_{cal} ($M + H^+$) = 671.20.

_N_-2-(_N_-fluoren-9-ylmethoxycarbonylamino)ethyl-*trans*-4-(thymin-1-yl)-D-proline pentafluorophenyl ester (39)



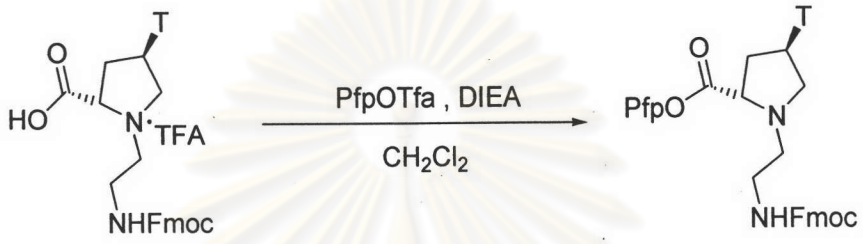
32 **39**

Synthesis of the titled compound (**39**) was accomplished in the same way as described for compound (**37**) above. Starting from *N*-2-(*N*-fluoren-9-ylmethoxy carbonylamino)ethyl-*cis*-4-(thymin-1-yl)-D-proline (**32**) (123.6 mg, 0.2 mmol), PfpOTfa (103.5 µL, 0.6 mmol) and DIEA (136.8 µL, 0.8 mmol) in dichloromethane (2 mL) afforded (**39**) (112.3 mg, 84 %), as a white foam.

mp. = 114.8-116.0 °C, δ_H (400 MHz, $CDCl_3$) 1.92 [3H, s, thymine CH$_3$], 2.31-2.49 [1H, m, 1x CH$_2$(3')], 2.71 [1H, t, *J* = 12.0 Hz, 1x CH$_2$(3')], 2.89-2.98 [2H, m, FmocNHCH$_2$CH$_2$N], 3.01-3.10 [1H, m, 1 x CH$_2$(5')], 3.32-3.50 [3H, m, 1 x CH$_2$(5') and 1 x FmocNHCH$_2$CH$_2$N], 4.06-4.18 [2H, m, 1 x Fmoc aliphatic CH and 1 x CH(2')], 4.46 [2H, d, *J* = 6.8 Hz, Fmoc aliphatic CH$_2$], 5.11-5.18 [2H, m, CH(4') and Fmoc NH], 7.27-7.33 [3H, m, 2 x Fmoc aromatic CH and 1 x thymine CH(6)], 7.42 [2H, t, *J* = 7.6 Hz, Fmoc aromatic CH], 7.61 [2H, d, *J* = 7.2 Hz, Fmoc aromatic CH], 7.78 [2H, d, *J* = 7.6 Hz, Fmoc aromatic CH] and 9.71 [1H, s, thymine NH(3)], δ_C (400 MHz, $CDCl_3$) 12.6 [thymine CH$_3$], 35.5 [CH$_2$(3')], 39.2 [FmocNHCH$_2$CH$_2$N], 47.3 [Fmoc aliphatic CH], 51.6 [CH$_2$(5')], 54.2 [CH(2')], 55.7 [CH(4')], 63.7 [Fmoc aliphatic CH$_2$], 66.8 [Fmoc NHCH$_2$CH$_2$N], 112.1 [thymine C(5)], 120.7, 125.0, 127.1 and 127.8 [Fmoc aromatic CH], 136.7 [Pfp CF], 137.5 [thymine CH(6)], 139.2 and

139.7 [Pfp CF], 141.4 [Fmoc aromatic C], 142.1 [Pfp CF], 143.9 [Fmoc aromatic C], 150.8 [thymine CO(2)], 156.7 [Fmoc CO], 163.8 [thymine CO(4)] and 168.3 [ester CO], $[\alpha]^{25}_{D}$ = +177 (c = 52 g/100 mL, $CHCl_3$), MALDI-TOF M_{obs} ($M + H^+$) = 671.14; M_{cal} ($M + H^+$) = 671.20.

_N_-2-(_N_-fluoren-9-ylmethoxycarbonylamino)ethyl-_trans_-4-(thymin-1-yl)-L-proline pentafluorophenyl ester (40)



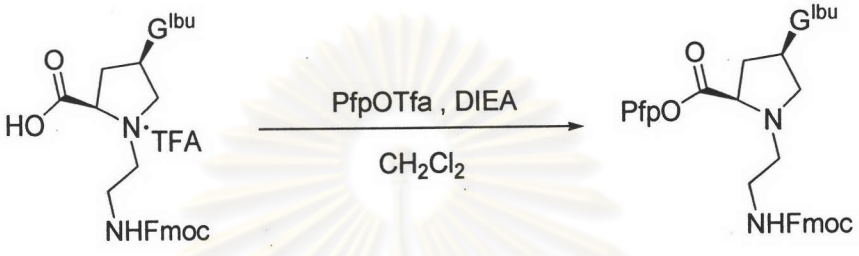
33 **40**

Synthesis of the titled compound (**40**) was accomplished in the same way as described for compound (**37**) above. Starting from *N*-2-(*N*-fluoren-9-ylmethoxy carbonylamino)ethyl-*trans*-4-(thymin-1-yl)-L-proline (**33**) (78.8 mg, 0.13 mmol), PfpOTfa (66 µL, 0.38 mmol) and DIEA (87.3 µL, 0.51 mol) in dichloromethane (1 mL) afforded (**40**) (71.3 mg, 83 %), as a white solid.

mp. = 115.0-116.1 °C, δ_H (400 MHz, $CDCl_3$) 1.80 [3H, s, thymine CH_3], 2.19-2.35 [1H, m, 1x CH_2(3')], 2.56 [1H, t, J = 11.6 Hz, 1x CH_2(3')], 2.70-2.88 [2H, m, FmocNHCH$_2$CH_2N], 2.90-2.99 [1H, m, 1 x CH_2(5')], 3.22-3.38 [3H, m, 1 x CH_2(5') and 1 x FmocNHCH_2CH$_2$N], 4.11 [1H, t, J = 6.8 Hz, Fmoc aliphatic CH], 4.18 [1H, d, J = 5.6 Hz, CH(2')], 4.33 [2H, d, J = 6.8 Hz, Fmoc aliphatic CH_2], 5.10-5.41 [2H, m, CH(4') and Fmoc NH], 7.14-7.22 [3H, m, 2 x Fmoc aromatic CH and 1 x thymine CH(6)], 7.29 [2H, t, J = 7.2 Hz, Fmoc aromatic CH], 7.48 [2H, d, J = 7.2 Hz, Fmoc aromatic CH], 7.66 [2H, d, J = 7.6 Hz, Fmoc aromatic CH] and 9.77 [1H, s, thymine NH(3)], δ_C (400 MHz, $CDCl_3$) 12.6 [thymine CH_3], 35.5 [CH_2(3')], 39.3 [FmocNHCH$_2$CH_2N], 47.2 [Fmoc aliphatic CH], 51.5 [CH_2(5')], 54.2 [CH(2')], 55.6 [CH(4')], 63.7 [Fmoc aliphatic CH_2], 66.8 [Fmoc NHCH_2CH$_2$N], 112.0 [thymine C(5)], 120.0, 125.0, 127.0 and 127.7 [Fmoc aromatic CH], 136.6 [Pfp CF], 137.6 [thymine CH(6)], 138.5, 139.2 and 139.7 [Pfp CF], 141.3 [Fmoc aromatic C], 142.2

[Pfp CF], 143.8 [Fmoc aromatic C], 151.0 [thymine CO(2)], 156.7 [Fmoc CO], 164.2 [thymine CO(4)] and 168.4 [ester CO], $[\alpha]^{25}_{D}$ = -19.0 (c = 52 g/100 mL, $CHCl_3$), MALDI-TOF M_{obs} ($M + H^+$) = 671.14; M_{cal}($M + H^+$) = 671.20.

_N_-2-(_N_-fluoren-9-ylmethoxycarbonylamino)ethyl-_cis_-4-(N^2-isobutyrylguanin-9-yl) -D-proline pentafluorophenyl ester (41)

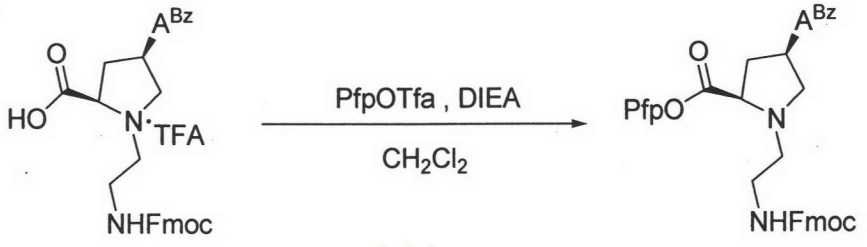


34 **41**

Synthesis of the titled compound (**41**) was accomplished in the same way as described for compound (**37**) above. Starting from _N_-2-(_N_-fluoren-9-ylmethoxy carbonylamino)ethyl-_cis_-4-(N^2-isobutyrylguanin-9-yl)-D-proline (**34**) (71.4 mg, 0.1 mmol), PfpOTfa (57 µL, 0.33 mmol) and DIEA (95 µL, 0.55 mol) in dichloromethane (2 mL) afford (**41**) (46.7 mg, 61 %), as a white solid.

δ_H (400 MHz, $CDCl_3$) 1.25 [6H, d, J = 6.8 Hz, $CH(CH_3)_2$], 2.35 [1H, d, J = 14.4 Hz, CH_2(3')], 2.72-2.90 [2H, m, 1 x $FmocNHCH_2CH_2N$ and 1 x $CH(CH_3)_2$], 2.90-3.12 [3H, m, 2 x $FmocNHCH_2CH_2N$ and 1 x CH_2(5')], 3.33-3.50 [2H, m, $FmocNHCH_2CH_2N$], 3.54 [1H, m, 1 x CH_2(5')] 3.76-3.88 [1 x CH_2(2')], 4.19 [1H, t, J = 7.2 Hz, Fmoc aliphatic CH], 4.29-4.41 [2H, m, Fmoc aliphatic CH_2], 5.17 [1H, s, CH_2(4')], 5.99 [1H, s, FmocNH], 7.18-7.44 [4H, m, Fmoc aromatic CH], 7.57 [2H, d, J = 7.6 Hz, Fmoc aromatic CH], 7.71 [2H, d, J = 7.6 Hz, Fmoc aromatic CH], 8.30 [1H, s, Guanine CH], 9.90 and 10.51 [1H, 2 x s, IbuNH rotamers], 12.17 and 12.48 [1H, 2 x s, Guanine NH rotamers].

_N_-2-(_N_-fluoren-9-ylmethoxycarbonylamino)ethyl-_cis_-4-(N^A-benzoyladenin-9-yl)-D-proline pentafluorophenyl ester (42)

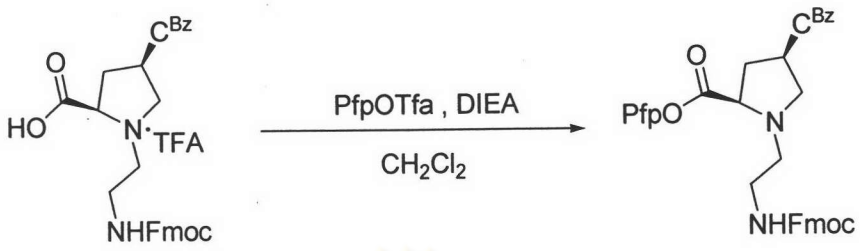


35 **42**

Synthesis of the titled compound (**42**) was accomplished in the same way as described for compound (**37**) above. Starting from *N*-2-(*N*-fluoren-9-ylmethoxy carbonylamino)ethyl-*cis*-4-(N^A-benzoyladenin-9-yl)-D-proline (**35**) (73.2 mg, 0.1 mmol), PfpOTfa (57 μL, 0.33 mmol) and DIEA (76 μL, 0.44 mol) in dichloromethane (1 mL) afford (**47**) (57.4 mg, 73 %), as a white solid.

mp. = 103.0-104.8 °C, δ_H (400 MHz, $CDCl_3$) 2.48 [1H, dd, *J* = 4.4, 15.2 Hz, C$\underline{H}_2$(3')], 2.80-2.94 [1H, m, 1 x FmocNHCH$_2$C$\underline{H}_2$N], 3.01-3.27 [3H, m, 1 x FmocNHCH$_2$C$\underline{H}_2$N, 1 x C$\underline{H}_2$(5') and 1 x C$\underline{H}_2$(3')], 3.32-3.45 [1H, m, FmocNHC$\underline{H}_2$CH$_2$N], 3.45-3.58 [1H, m, 1 x FmocNHC$\underline{H}_2$CH$_2$N] 3.64 [1H, d, *J* = 10.4 Hz, 1 x C$\underline{H}_2$(5')], 3.82-3.93 [1H, m, C$\underline{H}$(2')], 4.24 [1H, t, *J* = 7.2 Hz, Fmoc aliphatic C$\underline{H}$], 4.30-4.45 [2H, m, Fmoc aliphatic C$\underline{H}_2$], 5.38-5.54 [1H, m, C$\underline{H}$(4')], 7.19-7.41 [4H, m, Fmoc aromatic C$\underline{H}$], 7.51 [5H, m, *J* = 7.6 Hz, benzoyl C$\underline{H}$], 7.59 [3H, t, *J* = 7.6 Hz, benzoyl C$\underline{H}$], 7.74 [2H, d, *J* = 7.6 Hz, Fmoc aromatic C$\underline{H}$], 8.03 [2H, d, *J* = 7.6 Hz, Fmoc aromatic C$\underline{H}$], 8.67 [1H, s, adenine CH(2)] and 8.83 [1H, s, adenine CH(8)], δ_C (400 MHz, $CDCl_3$) 37.4 [$\underline{C}H_2$(3')], 39.5 [FmocNHCH$_2\underline{C}$H$_2$N], 47.2 [Fmoc aliphatic $\underline{C}$H], 52.2 [$\underline{C}H_2$(5')], 53.8 [$\underline{C}$H(2')], 58.8 [$\underline{C}$H(4')], 64.1 [Fmoc aliphatic $\underline{C}H_2$], 67.0 [Fmoc NH$\underline{C}$H$_2$CH$_2$N], 119.9, 125.1, 127.6 and 127.9 [Fmoc aromatic $\underline{C}$H], 128.8 [benzoyl $\underline{C}$H], 132.8 [benzoyl $\underline{C}$], 133.5 [adenine $\underline{C}$(5)], 136.7, 139.1, 139.3, 139.7 and 142.2 [Pfp $\underline{C}$F], 141.1 [adenine $\underline{C}$H(8)], 141.9, 143.9 and 144.0 [Fmoc aromatic $\underline{C}$], 149.5 [adenine $\underline{C}$(6)], 151.5 [adenine $\underline{C}$H(2)], 152.6 [adenine $\underline{C}$(4)], 156.7 [Fmoc $\underline{C}$O] and 169.6 [benzoyl $\underline{C}$O], $[\alpha]^{25}_D$ = -15.1 (c = 51 g/100 mL, $CHCl_3$), MALDI-TOF M_{obs} ($M + H^+$) = 784.37; M_{cal} ($M + H^+$) = 783.22.

_N_-2-(_N_-fluoren-9-ylmethoxycarbonylamino)ethyl-_cis_-4-(N^4-benzoylcytosin-1-yl)-D-proline pentafluorophenyl ester (43)



36 **43**

Synthesis of the titled compound (**43**) was accomplished in the same way as described for compound (**37**) above. Starting from *N*-2-(*N*-fluoren-9-ylmethoxy carbonylamino)ethyl-*cis*-4-(N^4-benzoylcytosin-1-yl)-D-proline (**36**) (106.2 mg, 0.15 mmol), PfpOTfa (103.6 μL, 0.6 mmol) and DIEA (154.2 μL, 0.9 mmol) in dichloromethane (1 ml) afford (**43**) (79.8 mg, 70 %), as a white solid.

mp. = 118.0-119.0 °C, δ_H (400 MHz, $CDCl_3$) 2.26 [1H, dd, J = 4.8, 14.8 Hz, 1x C$\underline{H}_2$(3')], 2.75-2.86 [1H, m, 1 x FmocNHCH$_2$C$\underline{H}_2$N], 2.93-3.09 [2H, m, 1 x C$\underline{H}_2$(5') and 1 x FmocNHCH$_2$C$\underline{H}_2$N], 3.16 [1H, ddd, J = 9.6, 9.6, 14.8, 1x C$\underline{H}_2$(3')], 3.30-3.48 [1H, m, 1 x FmocNHC$\underline{H}_2$CH$_2$N], 3.44-3.64 [2H, m, 1 x C$\underline{H}_2$(5') and 1 x FmocNHC$\underline{H}_2$CH$_2$N], 3.73-3.85 [1H, m, C$\underline{H}$(2')], 4.26 [1H, t, J = 6.8 Hz, Fmoc aliphatic C$\underline{H}$], 4.44 [2H, d, J = 6.8 Hz, Fmoc aliphatic C$\underline{H}_2$], 5.28-5.38 [1H, m, C$\underline{H}$(4')], 5.43-5.55 [1H, m, FmocN$\underline{H}$], 7.23-7.37 [2H, m, Fmoc aromatic C$\underline{H}$], 7.41 [2H, t, J = 7.2 Hz, Fmoc aromatic C$\underline{H}$], 7.46-7.70 [5H, m, benzoyl C$\underline{H}$,], 7.78 [2H, d, J = 7.2 Hz, Fmoc aromatic C$\underline{H}$], 7.95 [2H, d, J = 7.2 Hz, Fmoc aromatic C$\underline{H}$] and 8.47 [1H, d, J = 7.2 Hz, cytosine C$\underline{H}$(6)], δ_C (400 MHz, $CDCl_3$) 39.4 [$\underline{C}$H$_2$(3')], 41.4 [FmocNHCH$_2\underline{C}$H$_2$N], 47.2 [Fmoc aliphatic $\underline{C}$H], 54.2 [$\underline{C}$H$_2$(5')], 54.9 [$\underline{C}$H(2')], 58.0 [$\underline{C}$H(4')], 64.6 [Fmoc aliphatic $\underline{C}$H$_2$], 66.9 [Fmoc NH$\underline{C}$H$_2$CH$_2$N], 97.2 [cytosine $\underline{C}$H(5)], 120.0, 125.1, 127.0 and 127.7 [Fmoc aromatic $\underline{C}$H], 128.0 and 129.0 [benzoyl $\underline{C}$H], 132.6 [benzoyl $\underline{C}$], 133.4 [cytosine $\underline{C}$H(6)], 136.6, 138.5, 139.2, 139.6 and 142.1 [Pfp $\underline{C}$F], 141.3 and 143.8 [Fmoc aromatic $\underline{C}$], 147.7 [cytosine $\underline{C}$O(2)], 156.7 [Fmoc $\underline{C}$O], 161.6 [cytosine $\underline{C}$(4)] and 169.5 [benzoyl $\underline{C}$O], $[\alpha]^{25}_D$ = -32.1 (c = 50 g/100 mL, $CHCl_3$), MALDI-TOF M_{obs} ($M + H^+$) = 760.15; M_{cal} ($M + H^+$) = 760.22.

2.2.3 Synthesis of *aep*PNA Oligomer

(a) Preparation of the reaction pipette and apparatus for solid phase synthesis

All peptide syntheses were carried out using home-made peptide synthesis column from Pasteur pipette with fritted glass as described below. A new 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 about 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 al least 2 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 washing could be done 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**

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

(b) Solid phase peptide synthesis of CD-Ac-T_{10}-LysNH_2 (44)

Synthesis of this CD-Ac-T_{10}-LysNH_2 (**44**) was carried out on 1.5 μmol scale. The synthesis was divided step as follows.

i Removing Fmoc protecting group form the resin

The reaction pipette containing TentaGel S RAM Fmoc resin (6.3 mg, 1.5 μmol) was prepared as described above. The resin was treated 20% piperidine in DMF (piperidine 100 μL and DMF 400 μL) in a 1.5 mL eppendorf tube for 15 min at room temperature occasional agitation. After the specified period of time, the reagent was squeezed off and the reaction column was washed exhaustively with DMF.

ii Anchoring with the first amino acid (Lys) residue

Fmoc-L-Lysine was first attached to the free amino group on the RAM resin employing Fmoc-L-Lys(Boc)-OPfp. Fmoc-L-Lys(Boc)-OPfp (9.5 mg, 15 μmol) and HOAt (2.0 mg, 15 μmol) were dissolved in anhydrous DMF (35 μL) in a 1.5 mL eppendorf tube. The prepared resin was soaked in this solution with occasional agitation for 2 h at room temperature. 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 100 μL and DMF 400 μL) in a 1.5 mL eppendorf tube for 15 min at room temperature. 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 and then the UV-absorbance of dibenzofulvene-piperidine adduct at 254 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 >90 % for each step in order to give acceptable yield of the decamer *aep*PNA from the synthesis. If the overall efficiency had dropped bellows 50 %, the coupling must be stopped to save the valuable monomers.

vi Coulping with CD-*aep*PNA T monomer

The free amino group, generated from the deprotection step (**iii**) above, was further coupled with CD-*aep*PNA monomer. Fmoc-aminoethyl-D-Pro(*cis*-4-T)-OPfp (**37**) (4.0 mg, 6.0 μmol) and HOAt (0.8 mg, 6.0 μmol) were dissolved in 35 μL anhydrous DMF. The reaction pipette was treated with this solution for 2 h at room temperature with occasional agitation. After the specified period of time, the reagent was squeezed off and the reaction column was washed exhaustively with DMF.

v End capping

After coupling step, the free amino residue was capped with 10% Ac_2O/DIEA in anhydrous DMF (Ac_2O 10 μL, DIEA 10 μL and DMF 80 μL) in a 1.5 mL eppendorf tube to prevent formation of deletion sequences. The reaction pipette was occasionally agitated with this solution for 15 min at room temperature. 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 decamer.

vi Acetylation at C-teminal of CD-*aep*PNA T decamer

The synthesis cycle was repeated until the growing peptide chain was extended up to decamer. After final cleavage of Fmoc, the decamer *aep*PNA was treated with 10% Ac_2O/DIEA in anhydrous DMF (Ac_2O 10 μL, DIEA 10 μL and DMF 80 μL) in a 1.5 mL eppendorf tube. The reaction pipette was occasionally agitated with this solution for 15 min at room temperature. After the specified period

of time, the reagent was squeezed off and the reaction column was washed exhaustively with DMF.

vii Method for cleavage the decamer *aep*PNA from the resin

The decamer resin bound peptide was released from the resin by treatment with trifluoroacetic acid (1 mL) at room temperature 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 nitrogen stream (fume hood).The method was repeated again to ensure a complete cleavage of the peptide from the resin. The sticky residue was treated with diethyl ether to precipitate the crude PNA. The suspension was the centrifuged and decanted. The crude peptide was centrifugally washed with diethyl ether 3 times. Finally the crude peptide was air dried at room temperature and stored dried at -20 ^{o}C until use.

viii Purification and Identification

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 0.1% TFA in acetonitrile/water. For HPLC gradient system;
solvent A = 0.1% trifluoroacetic acid in acetonitrile
solvent B = 0.1% trifluoroacetic acid in milli Q water
First A:B (10:90) for 5 min then linear gradient to A:B (90:10) over a period of 30 min then hold on for 5 min before revert back to A:B (10:90). The major product (CD-Ac-T_{10}-LysNH_2 (**44**)) at retentions time 24.7 min was collected. After freeze drying, it was confirmed to be the desired CD-Ac-T_{10}-LysNH_2 (**44**) by MALDI-TOF mass spectrometry.

(c) Solid phase peptide synthesis of CL-Ac-T_{10}-LysNH_2 (45), TD-Ac-T_{10}-LysNH_2 (46) and TL-Ac-T_{10}-LysNH_2 (47)

Synthesis of these CL-Ac-T_{10}-LysNH_2 (**45**), TD-Ac-T_{10}-LysNH_2 (**46**) and TL-Ac-T_{10}-LysNH_2 (**47**) accomplished in the same way as described for CD-Ac-T_{10}-LysNH_2 (**44**) above. The experiment was showed in **Table 2.1**. In case of purification, the HPLC peak of CL-Ac-T_{10}-LysNH_2 (**45**), TD-Ac-T_{10}-LysNH_2 (**46**) and TL-Ac-T_{10}-LysNH_2 (**47**) appeared at t_R = 24.5, 24.7 and 24.2 min respectively.

Table 2.1 Synthesis of homothymine decamer *aep*PNA

sequence	scale (μmol)	resin weight (mg)	monomer	monomer weight (mg)	%efficiency (overall)
CD-Ac-T_{10}-LysNH_2 (**44**)	1.5	6.3	CD-T-Pfp (**36**)	4.0	66
CL-Ac-T_{10}-LysNH_2 (**45**)	1.5	6.3	CL-T-Pfp (**37**)	4.0	80
TD-Ac-T_{10}-LysNH_2 (**46**)	1.5	6.3	TD-T-Pfp (**38**)	4.0	72
TL-Ac-T_{10}-LysNH_2 (**47**)	1.0	4.2	TL-T-Pfp (**39**)	2.7	74

(d) Solid phase peptide synthesis of CD-Ac-A_{10}-LysNH_2 (48)

Synthesis of this CD-Ac-A_{10}-LysNH_2 (**48**) accomplished in the same way as described for CD-Ac-T_{10}-LysNH_2 (**44**) above. Starting from TentaGel S RAM Fmoc resin (6.3 mg, 1.5 μmol) and CD-A^{Bz}-Pfp monomer (**40**) (4.7 mg, 6.0 μmol) for each coupling cycle. Before cleavage CD-Ac-A_{10}-LysNH_2 (**48**) from resin, we must be deprotected nucleobase protecting groups (Bz,) treatment of the resin with aqueous ammonia/dioxane 1:1 at 60 °C for 6 h. In case of purification, the HPLC peak of CD-Ac-A_{10}-LysNH_2 (**48**) appeared at t_R = 23.6 mim.

(e) Solid phase peptide synthesis of CD-Ac-GTAGATCACT-LysNH$_2$ (49)

Synthesis of this CD-Ac-GTAGATCACT-LysNH$_2$ (**49**) accomplished in the same way as described for CD-Ac-T$_{10}$-LysNH$_2$ (**44**) above. Starting from TentaGel S RAM Fmoc resin (2.2 mg, 0.5 μmol) and monomer, CD-T-Pfp (**37**) (1.4 mg, 2.0 μmol), CD-C^{Bz}-Pfp (**43**) (1.6 mg, 2.0 μmol), CD-A^{Bz}-Pfp (**42**) (1.6 mg, 2.0 μmol), CD-C^{Bz}-Pfp (**43**) (1.6 mg, 2.0 μmol), CD-T-Pfp (**37**) (1.4 mg, 2.0 μmol), CD-A^{Bz}-Pfp (**42**) (1.6 mg, 2.0 μmol), CD-G^{Ibu}-Pfp (**41**) (1.5 mg, 2.0 μmol), CD-A^{Bz}-Pfp (**42**) (1.6 mg, 2.0 μmol), CD-T-Pfp (**37**) (1.4 mg, 2.0 μmol) and CD-G^{Ibu}-Pfp (**41**) (1.5 mg, 2.0 μmol) were added in each coupling cycle respectively. Before cleavage CD-Ac-GTA GATCACT-LysNH$_2$ (**49**) from resin, we must be deprotected nucleobase protecting groups (Bz, GIbu) treatment of the resin with aqueous ammonia/dioxane 1:1 at 60 °C for 6 h. In case of purification, the HPLC peak of CD-Ac-GTAGATCA CT-LysNH$_2$ (**49**) appeared at t_R = 23.6 mim.

2.2.4 Biophysical studies

(a) T_m experiments [52]

T_m experiments were performed on a CARY 100 Bio UV-Visible spectrophotometer (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 at least 30 min. The OD_{260} was recorded in steps from 20-90 °C (block temperature) with a temperature increment of 1 °C/min. The results were normalized by dividing the absorbance at each temperature by the initial absorbance. Analysis of the data was performed on a PC compatible computer using Microsoft Excel XP (Microsoft Corp.)

For example

Table 2.2 Data examples from UV analysis of CD-Ac-T_{10}-LysNH_2 (**44**) & Poly(dA)

entry	**CD-Ac-T_{10}-LysNH_2 (44) and poly(rA) 20.00-90.00 °C**			
	Temperature (°C)	**Absorbance**	**Correct temp* (°C)**	**Normalized Abs**
1	20.070	0.187	19.022	1.000
2	40.070	0.192	38.582	1.026
3	45.090	0.224	43.491	1.197
4	50.070	0.244	48.362	1.304
5	55.070	0.247	53.252	1.317
6	60.120	0.248	58.191	1.326
7	65.120	0.249	63.081	1.333
8	70.070	0.250	67.922	1.338
9	75.070	0.251	72.812	1.343
10	80.120	0.252	77.751	1.384
11	85.120	0.253	82.641	1.350
12	90.120	0.253	87.531	1.353

* 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 20-95 °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.

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

Normalized Abs. = Abs_{obs}/Abs_{init}

In entry 1; $T_{obs} = 20.07$ °C, $Abs_{init} = 0.187$, $Abs_{obs} = 0.187$;

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

Correct. Temp. = $(0.978 \times 20.070) - 0.6068$

= 19.02 °C

Normalized Abs. = Abs_{obs}/Abs_{init}

= 0.187/0.187

= 1.00

In entry 2; $T_{obs} = 40.07$ °C, $Abs_{init} = 0.187$, $Abs_{obs} = 0.192$;

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

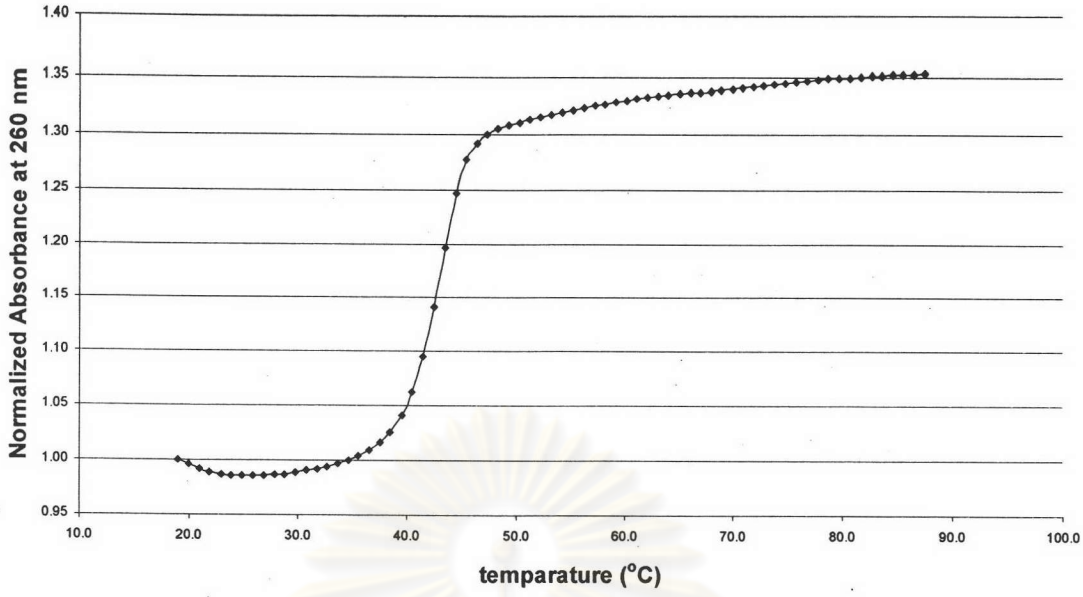
Correct. Temp. = $(0.978 \times 40.070) - 0.6068$

= 38.58 °C

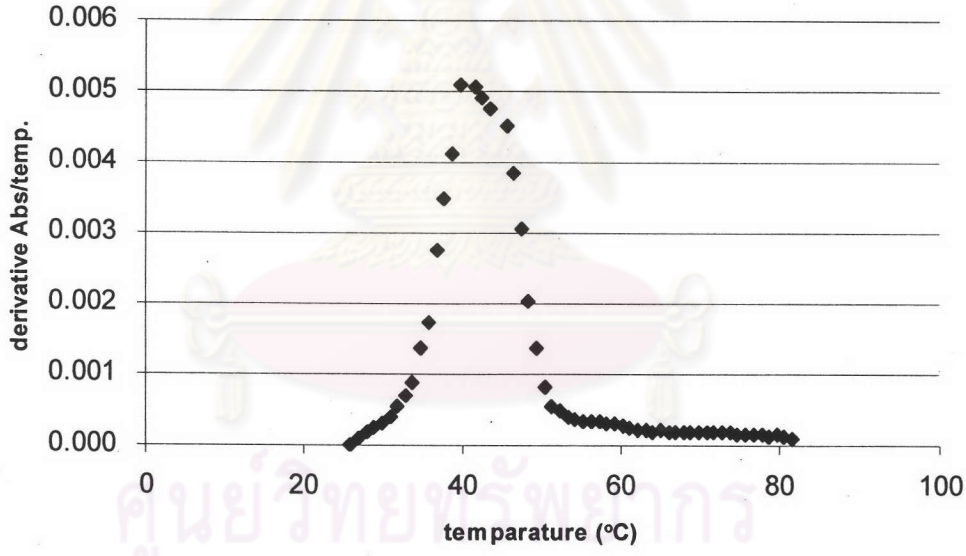
Normalized Abs. = Abs_{obs}/Abs_{init}

= 0.192/0.187

= 1.03



(a)



(b)

Figure 2.2 (**a**) Melting curve and (**b**) UV-Tm first derivative curves of CD-Ac-T_{10}-LysNH_2 (**44**) with poly(rA). Condition: 10 mM sodium phosphate buffer pH 7.0 1.0 μM ratio of T:A = 1:1

(b) UV-titration experiments [52,53]

The UV titration experiment was performed on a MILTON ROY spectronic 3000 array UV spectrophotometer at 25 °C. To a solution containing the CD-Ac-T_{10}-LysNH_2 (**44**) (2.39 μM) and 10 mM sodium phosphate buffer (20 mL) was added a 2-10 μL aliquot of a concentrated stock solution of dA_{50} (18.70 μM). 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 100 μL (corresponds to 1:4 T:A ratio) had been added. The ratio of the observed A_{260} and the calculated A_{260} were plotted against the mole ratio of T:A nucleotide and the stoichiometry was determined form the inflection point.

$$\text{Calcd. } OD_{260} = \frac{OD_{260}(T) \times V_T + OD_{260}(A) \times V_A}{V_T + V_A}$$

$$= \frac{0.21 \times 2 + 14.4 \times V_A\,(mL)}{2 + V_A\,(mL)}$$

$$\text{ratio of T:A} = \frac{\varepsilon_A \times OD_{260}(T) \times V_T}{\varepsilon_T \times OD_{260}(A) \times V_T}$$

$$= \frac{15.4 \times 0.21 \times 2}{8.8 \times 14.4 \times VA\,(mL)}$$