Dipeptides bearing nucleobases for the synthesis of novel peptide nucleic acids

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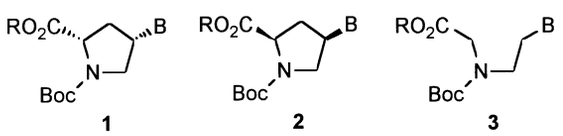
Three stereoisomers (*cis*-L, *trans*-D and *cis*-D) of *N*-Fmoc-glycyl-4-(thymin-1-yl)proline and their pentafluorophenyl esters have been prepared for use in the synthesis of novel peptide nucleic acids. In addition, *N*-Fmoc-glycyl-4-(N^6-benzoyladenin-9-yl)proline, *N*-Fmoc-glycyl-4-(N^4-benzoylcytosin-1-yl)proline and *N*-Fmoc-glycyl-4-(N^2-isobutyrylguanin-9-yl)proline and their pentafluorophenyl esters of the *cis*-D series have been synthesized.

Introduction

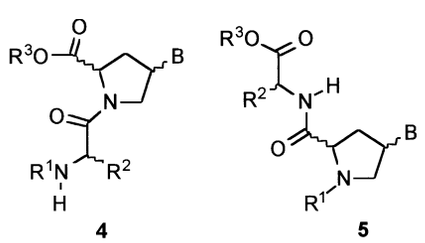
The design of two peptide nucleic acid systems, one with the conformationally restricted glycylproline backbone and the other with the conformationally more flexible glycyl-*N*-ethylglycine backbone, have been reported together with the preparation of the protected amino acids bearing nucleobases **1**–**3** for use in their synthesis.[1]



1 **2** **3**

A preliminary investigation of the solid-phase synthesis of the flexible peptide nucleic acid $[Gly\text{-}Eg(T)]_n$ from the thymine monomer (**3**, B = thymine, R = OH) revealed that the coupling was inefficient, especially that of the secondary amino group with Boc-glycine. This prompted us to explore the possibility of coupling the amino acids bearing nucleobases **1**–**3** with the spacer amino acid (*e.g.* glycine) to form dipeptides with the intention of performing the solid-phase synthesis using these dipeptide building blocks.

Although both possible dipeptides **4** and **5** were considered,



4 **5**

the dipeptide **4** appeared to be a better building block since the more difficult coupling (*i.e.* at the secondary amino group) could be achieved in solution and the product purified before the solid-phase synthesis was undertaken. Furthermore, if an optically active spacer amino acid was used instead of glycine, racemisation of the spacer amino acid during fragment coupling of the dipeptide of type **5** would be a serious problem, whereas such racemisation is minimised when the C-terminus of the activated fragment is proline[2] because *N*-acylproline could not be racemised by the oxazolone mechanism.

Owing to the mild conditions used for the deprotection of the *N*-Fmoc group, the Fmoc/OBu^t strategy in solid-phase peptide synthesis is favoured over the classical Boc/OBzl strategy.[3] Furthermore, most machine synthesizers capable of handling small-scale synthesis (50 μmol or less) can accommodate only the Fmoc/OBu^t strategy. For these reasons, it was decided to use the Fmoc instead of Boc as the N-protecting group.

There were two possible synthetic pathways to the target dipeptide **4**, the two amino acids may be coupled first and the nucleobase attached later by the Mitsunobu reaction or the nucleobase may be incorporated before the peptide coupling. The first approach has the advantage of being a more convergent approach. However, a preliminary investigation suggested that it is not satisfactory because of the extensive cleavage of the Fmoc group during the Mitsunobu reaction.[4] It also seemed likely that displacement of a tosyloxy group by a nucleobase would give similar premature cleavage of the Fmoc group since the reactions require basic conditions.

A temporary N-protecting group for the hydroxyproline was required, therefore, which is stable to the basic conditions of the Mitsunobu reaction but which can be removed, without disturbing the carboxy-protecting group, in order to allow coupling with Fmoc-glycine (or other amino acid) to give the Fmoc-dipeptide **4**. As the carboxy-protecting group must be selectively removed in the presence of the Fmoc group at the end of the synthesis, an acid-labile protecting group seemed appropriate. The combination of the acid labile Boc group and diphenylmethyl (Dpm)[5] ester is ideal because introduction and cleavage of both groups are simple and give high yields. The Dpm ester is fully compatible with the *N*-Fmoc group and a selective cleavage of a Boc group in the presence of a diphenylmethyl ester is possible.[6]

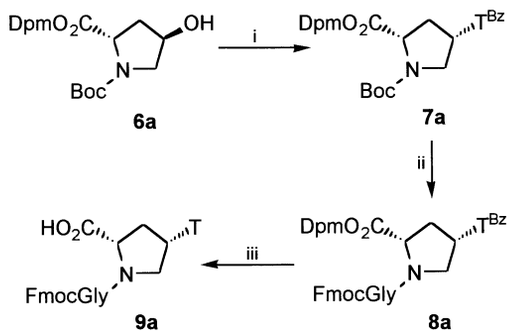
Results and discussion

Initial studies were undertaken with the cheap, commercially available *trans*-4-hydroxy-L-proline, which was protected as its *N*-Boc/Dpm ester derivative according to the method described by Tozuka and Takaya.[6b] The crystalline derivative **6a** was obtained in greater than 80% yield in two steps.

The Mitsunobu reaction on compound **6a** with N^3-benzoylthymine (BzT)[7] gave the thymine derivative **7a**, together with a less polar product, possibly the O^2-isomer or the elimination product. Fortunately, the thymine derivative **7a** is crystalline and after column chromatography and one recrystallisation, the pure material was obtained in 51% yield.

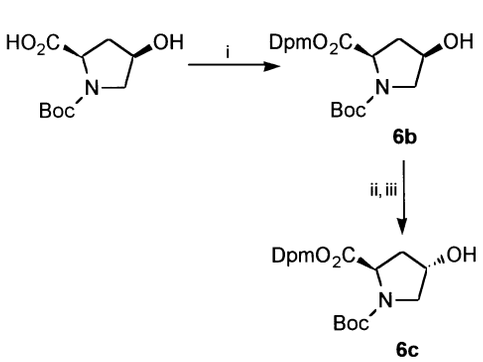
Deprotection of the *N*-Boc group of the protected thymine derivative **7a** was accomplished with methanolic HCl according to the literature.[6] The resulting amine salt was treated with Fmoc-glycine pentafluorophenyl ester in the presence of diisopropylethylamine (DIEA) to give the protected dipeptide **8a** in excellent yield. Treatment of compound **8a** with trifluoroacetic acid (TFA), either as a neat liquid or in the presence of phenol or anisole as a scavenger,[8] at room temperature for a few hours led to the formation of roughly equal amounts of two

products as shown by TLC and high-performance liquid chromatography (HPLC), which could not be separated by crystallisation. The unexpected product, which was more polar than the desired product, was identified as the debenzoylated thymine derivative **9a**. Since protection of thymine at N^3 was only required for selective alkylation at thymine-N^1, the debenzoylated thymine derivative **9a** was still suitable for the oligomer synthesis. However, attempts to remove the benzoyl group completely by prolonged treatment with TFA resulted in a complex mixture. HBr in acetic acid, which is commonly used for the removal of the benzyloxycarbonyl (Z) group,[9] appeared to give better results. Brief treatment of the mixture of products from TFA-cleavage with 10% HBr in acetic acid resulted in complete cleavage of the benzoyl group as shown by HPLC. The cleavage conditions have also been applied to the fully protected dipeptide **8a** without pre-treatment with TFA, with equal success. The synthesis of the Fmoc-dipeptide bearing thymine at the 4-position in the *cis*-L proline series is summarised in Scheme 1.



Scheme 1 *Reagents and conditions:* i, N^3-BzT, Ph_3P, DEAD in THF, room temp., overnight (51%); ii, (a) THF–satd. HCl in MeOH (1:1), room temp., 3 h; (b) FmocGlyOPfp, DIEA in 1,4-dioxane, room temp., overnight (99%); iii, 10% HBr in HOAc, room temp., 1 h (50%)

The protected *cis*- and *trans*-hydroxy-D-proline compounds **6b** and **6c** were required for the preparation of the *trans*- and *cis*-D-proline dipeptides bearing nucleobases. The reaction of *N*-Boc-*cis*-4-hydroxy-D-proline[10] with diphenyldiazomethane[11] gave the Dpm ester **6b** in 90% yield. Inversion of the 4-OH group in **6b** to give **6c** was effected by the Mitsunobu reaction in the presence of formic acid followed by treatment with methanolic ammonia as with the methyl ester.[1] By this route, *trans*-compound **6c** was prepared in multigram quantities from *cis*-stereoisomer **6b** in excellent yield (90%, 2 steps) (Scheme 2).



Scheme 2 *Reagents and conditions:* i, Ph_2CN_2, EtOAc, room temp., overnight (90%); ii, HCO_2H, Ph_3P, DEAD in THF, room temp., overnight (quant.); iii, aq. NH_3, MeOH, 1 h (90%)

The specific rotation of the product **6c** {$[\alpha]_D^{25}$ +53.0 (*c* 1.0, EtOH)} when compared with that of the *trans*-L isomer {$[\alpha]_D^{25}$ −54.3 (*c* 1.0, EtOH)} indicated that inversion was essentially complete.

Mitsunobu reaction on the protected hydroxyproline

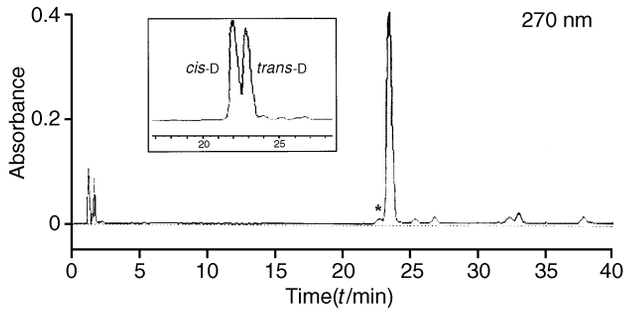
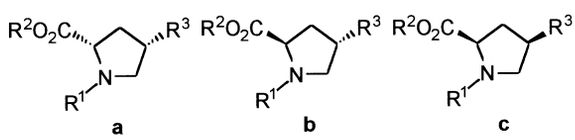


Fig. 1 Analytical reversed-phase chromatogram of *trans*-D-thymine Fmoc-dipeptide **9b**; Inset shows the HPLC chromatogram of a 1:1 mixture of *trans*-D (**9b**) and *cis*-D (**9c**) Fmoc-dipeptide under identical conditions (arbitrary scale). The peak marked by an asterisk is the diastereomeric impurity. HPLC conditions: C-18 Hypersil semipreparative reversed-phase HPLC column (3 μ particle size); linear gradient water–acetonitrile containing 0.1% TFA (75:25) for 5 min, then 10:90 over a period of 35 min; flow rate 1.0 ml min^{-1}.

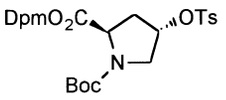
diastereomers **6b** and **6c** with N^3-benzoylthymine on a 20 mmol scale gave the products **7b** and **7c** in 33 and 36% yield, respectively. The Boc group in compounds **7b** and **7c** was removed with methanolic HCl and the products were treated with Fmoc-glycine pentafluorophenyl ester to give the protected dipeptides **8b** and **8c**. After treatment with 10% HBr in acetic acid the Fmoc-dipeptide acids **9b** and **9c** were obtained with concomitant cleavage of the N^3-benzoyl group. The intermediate protected dipeptide **8b** and the final product **9b** were not crystallised as readily as their diastereomers **8c** and **9c**. However, the purity of the crude Fmoc-dipeptides **9b** and **9c** was found to be satisfactory by HPLC (Fig. 1).



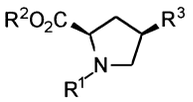
7 R^1 = Boc, R^2 = Dpm, R^3 = T^{Bz}
8 R^1 = FmocGly, R^2 = Dpm, R^3 = T^{Bz}
9 R^1 = FmocGly, R^2 = H, R^3 = T
10 R^1 = FmocGly, R^2 = Pfp, R^3 = T

The Fmoc-dipeptides **9a–c** were prepared in gram-quantities for solid-phase synthesis. Pentafluorophenyl esters of the diastereomeric thymine dipeptides (compounds **10a–c**) were all prepared by reactions of the free acids with pentafluorophenol in the presence of dicyclohexylcarbodiimide (DCCI) in dichloromethane.[12] These active esters were crystalline solids which were stable enough to permit purification by silica gel column chromatography and could be stored for several months at −20 °C without apparent decomposition according to ^{1}H NMR analysis. These were used for peptide synthesis and the results compared with direct coupling of the free acids.

Binding studies between the 10-mers derived from coupling of compounds **10a–c** and poly(dA) showed that the oligomer derived from isomer **10c** binds most strongly. The *cis*-D-proline series was selected therefore for further investigation. The protected *cis*-hydroxy-D-proline **6b** was converted into the crystalline *trans*-D-toluene-*p*-sulfonyl ester **11** in 68% yield by a Mitsunobu reaction with methyl toluene-*p*-sulfonate in the presence of triphenylphosphine and diethyl azodicarboxylate (DEAD).[10] Reaction of compound **11** and N^6-benzoyladenine in the presence of K_2CO_3 and a catalytic amount of 18-crown-6 in dimethylformamide (DMF) afforded the N^9-isomer of Boc-D-Pro(*cis*-4-BzA)-ODpm **12** in 42% yield. However, on scaling up, a small amount of another isomer (~5%) was also isolated. This was probably the N^7-isomer according to the



11



12 R^1 = Boc, R^2 = Dpm, R^3 = A^{Bz}
13 R^1 = FmocGly, R^2 = Dpm, R^3 = A^{Bz}
14 R^1 = FmocGly, R^2 = Pfp, R^3 = A^{Bz}
15 R^1 = Boc, R^2 = Dpm, R^3 = C^{Bz}
16 R^1 = FmocGly, R^2 = Dpm, R^3 = C^{Bz}
17 R^1 = FmocGly, R^2 = Pfp, R^3 = C^{Bz}
18 R^1 = Boc, R^2 = Dpm, R^3 = G^{Ibu}
19 R^1 = FmocGly, R^2 = Dpm, R^3 = G^{Ibu}
20 R^1 = FmocGly, R^2 = Pfp, R^3 = G^{Ibu}

upfield ^{13}C chemical shift of adenine C^5 (δ_C 115.0 and 114.6, rotamers) relative to the major product (δ_C 123.4).[13]

Deprotection of the Boc group in compound **12** was first attempted by methanolic HCl as described previously for the thymine derivatives; however, the selectivity was somewhat less than had been hoped. On the other hand, toluene-*p*-sulfonic acid (PTSA) in acetonitrile, which has been successfully applied to deprotect the *N*-Boc group during the synthesis of cephalosporin derivatives,[14] cleanly removed the Boc group without cleaving the Dpm ester. The product was treated with Fmoc-glycine pentafluorophenyl ester to give the Fmoc-dipeptide diphenylmethyl ester **13** in 85% yield. Deprotection of the Dpm ester with TFA in the presence of anisole gave the free acid, which was directly converted into the pentafluorophenyl ester **14** by treatment with pentafluorophenol in the presence of DCCI.[12] The N^6-benzoyl group on adenine remained intact throughout the reaction sequence. Attempted purification of the highly polar pentafluorophenyl ester **14** by column chromatography found only limited success. However, the crude product obtained after trituration and washing with hexane was shown by ^{1}H NMR spectroscopy to contain approximately 10% of dicyclohexylurea (DCU) as the only contaminant, and was used successfully for the solid-phase synthesis.

Reaction of the *trans*-D-toluene-*p*-sulfonyl ester **11** with N^4-benzoylcytosine in the presence of K_2CO_3/18-crown-6 in DMF gave the desired N^1-isomer, Boc-D-Pro(*cis*-4-N^1-BzC)-ODpm, **15** in 25% yield along with the less polar O^2-isomer in 41% yield, which could be readily separated by chromatography on silica gel. The identity of the two isomers was further confirmed by the characteristic downfield shift of the ^{13}C resonance of $C^{4'}$ of the O^2-isomer compared to the N^1-isomer as discussed earlier for the methyl ester derivatives.[1] Since N^4-benzoylcytosine was shown to be partially hydrolysed in hot 85% acetic acid to give uracil,[15] the stability of this group towards acids was tested before we attempted deprotection of the Boc group or the diphenylmethyl ester. The Boc-protected amino acid **15** was treated with TFA in the presence of anisole for 2 h. ^{1}H NMR analysis of the product showed that the Boc and Dpm groups were completely removed whereas the benzoyl group was stable, thus demonstrating that the deprotection conditions were satisfactory.

Removal of the Boc group of compound **15** and reaction of the product with Fmoc-glycine pentafluorophenyl ester as described for the adenine analogue gave the protected cytosine dipeptide **16** in 70% overall yield. The benzoylcytosine dipeptide and its pentafluorophenyl ester **17** were synthesized in essentially the same way as the thymine and adenine analogues.

The Mitsunobu reaction between N^2-isobutyryl-O^6-(4′-nitrophenylethyl)guanine[16] and the protected *trans*-4-hydroxy-D-proline **6c** was carried out in an analogous way to the methyl ester derivative.[1] The product, however, could not be isolated free from diethyl hydrazinedicarboxylate. Treatment with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in pyridine to remove the O^6-nitrophenylethyl protecting group followed by column chromatography gave the pure N^9-substituted isobutyrylguanine derivative **18** as a solid in 43% overall yield. Removal of the Boc group and reaction of the product with Fmoc-glycine pentafluorophenyl ester as before gave the protected guanine dipeptide **19** in 52% yield. Removal of the carboxy-protecting group and reaction of the product with pentafluorophenol and DCCI gave the isobutyrylguanine dipeptide and its pentafluorophenyl ester **20**.

Solid-phase synthesis of peptide nucleic acids from the dipeptide acids **9** and pentafluorophenyl esters **10**, **14**, **17** and **20** will be described elsewhere.

Experimental

Mps were recorded on a Kofler block apparatus and are quoted uncorrected. Specific rotations were measured on a Perkin-Elmer 241 polarimeter and $[\alpha]_D$-values are given in units of 10^{-1} deg cm^2 g^{-1}. IR spectra were recorded on a Perkin-Elmer 1750 Fourier Transform Infrared spectrometer. Elemental analyses were performed on a Carlo Erba CHN analyser model 1106.

Routine ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 200 spectrometer operating at 200 MHz (^{1}H) and 50.28 MHz (^{13}C). ^{13}C spectra were recorded in broad-band-decoupled mode and the chemical-shift assignment was assisted by a distortionless enhancement by polarisation transfer (DEPT) experiment performed on the Varian Gemini 200 spectrometer. Highfield ^{1}H NMR spectra were recorded on a Bruker AMX 500 spectrometer (500 MHz). ^{19}F NMR spectra were recorded on a Bruker AM 250 instrument at 235.35 MHz. ^{1}H and ^{13}C chemical shifts are quoted in ppm relative to tetramethylsilane and were internally referenced to the residual protonated solvent signal. ^{19}F chemical shifts were externally referenced to $CFCl_3$ in $CHCl_3$.

Chemical ionisation and fast-atom bombardment mass spectra were recorded on a VG 20-250 masslab and a VG Micromass ZAB-1F mass spectrometer. Electrospray mass spectra were recorded on a VG Biotech BioQ or VG Biotech Platform. Masses are quoted as *m/z*-values unless otherwise stated, only the molecular ions and major fragments being quoted.

Distilled water was used for all chemical experiments. Chemicals and solvents were obtained from Aldrich Chemical Company Ltd., Avocado Research Chemicals Ltd. and Lancaster Synthesis Ltd. and were purified according to the literature,[17] if necessary. *N*-Boc-*trans*-4-hydroxy-L-proline and Fmoc-glycine pentafluorophenyl ester were obtained from Calbiochem-Novabiochem Ltd. Toluene-*p*-sulfonyl chloride was purified by recrystallisation from light petroleum (distillation range 60–80 °C). DMF was peptide synthesis grade obtained from Rathburn Chemical Ltd. and was used without further purification except when strictly anhydrous conditions were required, where it was re-distilled from calcium hydride under reduced pressure. Acetonitrile was HPLC grade obtained from Rathburn and used without further purification. Tetrahydrofuran (THF) and 1,4-dioxane were distilled from sodium wire/benzophenone under argon and stored over 4 Å molecular sieves. Pyridine was distilled from calcium hydride and stored over 4 Å molecular sieves. Moisture-sensitive reactions were performed under argon in flame-dried glassware.

N-tert-Butoxycarbonyl-*trans*-4-hydroxy-L-proline diphenylmethyl ester 6a and *N-tert*-butoxycarbonyl-*cis*-4-hydroxy-D-proline diphenylmethyl ester 6b

To a solution of freshly prepared diphenyldiazomethane[11] (3.50 g, 18.0 mmol) in ethyl acetate (20 ml) was added a solution of *N*-Boc-*trans*-4-hydroxy-L-proline (3.25 g, 14.0 mmol) in ethyl

acetate (30 ml). Nitrogen gas was slowly evolved from the solution and the intense purple colour of diphenyldiazomethane was gradually discharged. The solution was stirred at room temperature overnight using a $CaCl_2$ guard tube. Evaporation off of the solvent followed by precipitation of the product from ethyl acetate–light petroleum (distillation range 40–60 °C) gave a solid, *N-tert*-butoxycarbonyl-*trans*-4-hydroxy-L-proline diphenylmethyl ester **6a** (5.10 g, 91%), mp 93–95 °C (lit.,[6b] 103–104 °C); δ_H(200 MHz; $CDCl_3$) 1.22 and 1.47 (9 H, 2 × s, Boc rotamers), 1.95–2.50 [3 H, br m, CH_2(3) and O*H*], 3.45–3.74 [2 H, br m, CH_2(5)], 4.40–4.65 [2 H, br m, CH(2) and CH(4)], 6.95 (1 H, br s, C*H*Ph_2), 7.25–7.55 (10 H, br m, phenyl CH); ν_{max}(KBr)/cm^{-1} 3491br (O–H), 1728s (C=O ester) and 1693s (C=O urethane); *m/z* (ES-MS) 436 (M + K^+, 55%), 420 (M + Na^+, 100), 415 (M + NH_4^+, 15) and 398 (M + H^+, 72); $[a]_D^{25}$ −54.3 (*c* 1.0, EtOH).

The *cis*-D-diastereoisomer **6b** was prepared similarly starting from *N*-Boc-*cis*-hydroxy-D-proline[10] (11.6 g, 50.0 mmol) and diphenyldiazomethane (11.3 g, 58.0 mmol) in ethyl acetate (150 ml). N-tert-*Butoxycarbonyl*-cis-4-*hydroxy*-D-*proline diphenylmethyl ester* **6b** was obtained as a solid after precipitation from ethyl acetate–light petroleum (40–60 °C) (17.9 g, 90%), mp 102–105 °C (Found: C, 69.5; H, 6.8; N, 3.3. $C_{23}H_{27}NO_5$ requires C, 69.5; H, 6.8; N, 3.5%); δ_H(200 MHz; $CDCl_3$) 1.26 and 1.30 (9 H, 2 × s, Boc rotamers), 2.08 and 2.35 [2 H, m, CH_2(3)], 2.88 and 3.09 (1 H, 2 × d, *J* 9.6, O*H* rotamers), 3.57–3.66 [2 H, br m, CH_2(5)], 4.32 [1 H, m, CH(4)], 4.42–4.58 [1 H, m, CH(2)], 6.91 and 6.99 (1 H, 2 × s, C*H*Ph_2 rotamers) and 7.25–7.48 (10 H, br m, Ph); δ_C(50.28 MHz; $CDCl_3$) 28.0 and 28.3 (Boc CH_3 rotamers), 37.6 and 38.6 [CH_2(3) rotamers], 55.4 and 55.9 [CH_2(5) rotamers], 58.0 and 58.1 [CH(2) rotamers], 70.1 and 71.2 [CH(4) rotamers], 78.1 and 78.6 (*C*HPh_2 rotamers), 80.4 and 80.6 (Boc C), 127.2–128.8 (phenyl CH rotamers), 139.6 and 139.8 (phenyl C rotamers), 154.1 (Boc CO) and 174.0 (ester CO rotamers); ν_{max}(KBr)/cm^{-1} 3466br (O–H), 1749s (C=O ester) and 1687s (C=O urethane); $[a]_D^{25}$ +41.2 (*c* 1.0, EtOH).

N-tert-Butoxycarbonyl-*trans*-4-hydroxy-D-proline diphenylmethyl ester 6c

N-Boc-*cis*-hydroxy-D-proline diphenylmethyl ester **6b** (0.20 g, 0.50 mmol), triphenylphosphine (0.160 g, 0.60 mmol) and formic acid (25 μl, 0.65 mmol) were dissolved in dry THF (10 ml) and the solution was cooled in an ice-bath. DEAD (100 μl, 0.60 mmol) was added dropwise. The reaction mixture was stirred under nitrogen at room temperature overnight. The solvent was evaporated off and the residue was chromatographed on silica gel with dichloromethane–acetone (20:1) as eluent to give the 4-formate ester (R_F 0.50) as an oil (0.248 g, quant.); δ_H(200 MHz; $CDCl_3$) 1.25 and 1.47 (9 H, 2 × s, Boc rotamers), 2.10–2.55 [2 H, br m, CH_2(3)], 3.57–3.80 [2 H, br m, CH_2(5)], 4.48–4.64 [1 H, m, CH(2)], 5.35–5.43 [1 H, br m, CH(4)], 6.91 and 6.95 (1 H, 2 × s, C*H*Ph_2 rotamers), 7.25–7.42 (10 H, br m, phenyl CH) and 8.03 [1 H, s, HC(O)].

The oil was taken up in methanol (10 ml), and conc. aq. ammonia (*d* 0.880; 0.5 ml) was added. TLC analysis indicated complete reaction after stirring at room temperature for 1 h. The solvent was removed under reduced pressure and the residue was chromatographed on a silica gel column with diethyl ether as eluent to give the product (R_F 0.30) as a foam (0.179 g, 90% from **6b**), which was further purified by reprecipitation from ethyl acetate–light petroleum (40–60 °C) to give N-tert-*butoxycarbonyl*-trans-4-*hydroxy*-D-*proline diphenylmethyl ester* **6c** as a solid, mp 105–108 °C (Found: C, 69.8; H, 6.9; N, 3.3. $C_{23}H_{27}NO_5$ requires C, 69.5; H, 6.8; N, 3.5%); δ_H(200 MHz; $CDCl_3$) 1.22 and 1.47 (9 H, 2 × s, Boc rotamers), 2.02 and 2.30 [2 H, 2 × br m, CH_2(3)], 2.70 and 2.82 (1 H, 2 × br d, *J* 3.1 and 3.4, OH rotamers), 3.45–3.70 [2 H, br m, CH_2(5)], 4.42–4.65 [2 H, br m, CH(2) and CH(4)], 6.90 and 6.95 (1 H, 2 × s, C*H*Ph_2) and 7.25–7.45 (10 H, br m, phenyl CH); δ_C(50.28 MHz; $CDCl_3$) 27.9 and 28.3 (Boc CH_3 rotamers), 38.1 and 38.9 [CH_2(3) rotamers], 54.7 [CH_2(5)], 57.8 and 58.1 [CH(2) rotamers], 69.2 and 70.0 [CH(4) rotamers], 77.2 and 77.6 (*C*HPh_2 rotamers), 80.6 (Boc C), 127.1–128.8 (phenyl CH rotamers), 140.0 and 140.2 (phenyl C rotamers), 154.4 (Boc CO) and 172.0 (ester CO); ν_{max}(KBr)/cm^{-1} 3491br (O–H), 1728s (C=O ester) and 1693s (C=O urethane); $[a]_D^{25}$ +53.0 (*c* 1.0, EtOH).

N-tert-Butoxycarbonyl-*trans*-4-(*p*-tolylsulfonyloxy)-D-proline diphenylmethyl ester 11

A solution of *N*-Boc-*cis*-4-hydroxy-D-proline diphenylmethyl ester **6b** (6.40 g, 16.1 mmol), triphenylphosphine (4.50 g, 16.8 mmol) and methyl toluene-*p*-sulfonate (3.04 g, 16.4 mmol) in THF was treated with DEAD (2.80 ml, 4.2 mmol) dropwise at −78 °C. The reaction was allowed to warm gradually to room temperature and was stirred overnight. Evaporation followed by column chromatography (SiO_2; diethyl ether; R_F 0.61) gave the crude product as an oil, which was reprecipitated from diethyl ether–light petroleum (40–60 °C) to give the essentially pure product as a solid (6.01 g, 68%). Recrystallisation from ethyl acetate–light petroleum (40–60 °C) gave N-tert-*butoxycarbonyl*-trans-4-(p-*tolylsulfonyloxy*)-D-*proline diphenylmethyl ester* **11** as crystals, mp 147–149 °C (Found: C, 65.2; H, 5.8; N, 2.4. $C_{30}H_{33}NO_7S$ requires C, 65.3; H, 6.0; N, 2.5%); δ_H(200 MHz; $CDCl_3$) 1.22 and 1.44 (9 H, 2 × s, Boc rotamers), 1.89–2.18 and 2.32–2.66 [2 H, m, CH_2(3)], 2.46 (3 H, s, tosyl CH_3), 3.54–3.74 [2 H, m, CH_2(5)], 4.50 [1 H, m, CH(2)], 4.98 [1 H, br m, CH(4)], 6.87 and 6.92 (1 H, 2 × s, C*H*Ph_2 rotamers), 7.25–7.38 (12 H, m, arom CH) and 7.77 (2 H, d, *J* 8.2, tosyl CH); δ_C(50.28 MHz; $CDCl_3$) 21.6 (tosyl CH_3), 27.9 and 28.2 (Boc CH_3 rotamers), 35.6 and 37.0 [CH_2(3) rotamers], 51.8 and 52.1 [CH_2(5) rotamers], 57.4 and 57.6 [CH(2) rotamers], 78.3 and 78.9 [CH(4) rotamers], 78.2 and 78.7 (*C*HPh_2 rotamers), 80.7 and 80.9 (Boc C rotamers), 127.0–130.3 (aromatic CH), 133.5, 139.9 and 145.3 (aromatic C), 154.6 and 154.8 (Boc CO rotamers) and 171.3 (ester CO rotamers); ν_{max}(KBr)/cm^{-1} 1742s (C=O ester), 1704 (C=O urethane), 1402s (−SO_2O−) and 1174s (−SO_2O−).

N-tert-Butoxycarbonyl-4-(N^3-benzoylthymin-1-yl)proline diphenylmethyl esters 7a–c

N-Boc-*trans*-4-hydroxy-L-proline diphenylmethyl ester **6a** (0.425 g, 1.07 mmol), triphenylphosphine (0.290 g, 1.10 mmol) and N^3-benzoylthymine (0.250 g, 1.09 mmol) were dissolved in dry THF (10 ml) and the solution was cooled to −15 °C. DEAD (180 μl, 1.10 mmol) was then added dropwise to the stirred mixture. The reaction mixture was stirred under argon at room temperature overnight. The solvent was evaporated off and the residue was chromatographed on silica gel with dichloromethane–acetone (20:1) as eluent to give N-tert-*butoxycarbonyl*-cis-4-(N[3]-*benzoylthymin*-1-*yl*)-L-*proline diphenylmethyl ester* **7a** (R_F 0.56), which was recrystallised from ethanol to give a fluffy solid (0.310 g, 51%), mp 183–185 °C (Found: C, 68.9; H, 5.5; N, 6.7. $C_{35}H_{35}N_3O_7$ requires C, 69.0; H, 5.8; N, 6.9%); δ_H(200 MHz; $CDCl_3$) 1.30 and 1.49 (9 H, 2 × br s, Boc rotamers), 1.82 (3 H, br s, thymine CH_3), 2.05 and 2.85 [2 H, br m, CH_2(3′)], 3.65 (1 H, br m) and 4.02 (1 H, dd, *J* 12.0 and 8.0) [CH_2(5′)], 4.54 [1 H, br m, CH(2′)], 5.26 [1 H, br m, CH(4′)], 6.94 (1 H, s, C*H*Ph_2), 7.12 and 7.18 [1 H, 2 × br s, CH(6) rotamers], 7.30–7.42 (10 H, br m, phenyl CH), 7.50 (2 H, t, *J* 7.0, benzoyl *m*-H), 7.67 (1 H, t, *J* 7.0, benzoyl *p*-H) and 7.92 (2 H, d, *J* 7.0, benzoyl *o*-H); δ_C(50.28 MHz; $CDCl_3$) 12.3 (thymine CH_3), 27.9 and 28.2 (Boc CH_3 rotamers), 34.9 and 35.3 [CH_2(3′) rotamers], 49.3 and 49.4 [CH_2(5′) rotamers], 52.1 and 52.5 [CH(4′) rotamers], 57.5 [CH(2′)], 78.1 and 78.4 (*C*HPh_2 rotamers), 81.3 (Boc C), 111.7 [C(5)], 126.9–130.6 (aromatic CH), 131.6 (benzoyl C), 135.3 (benzoyl *p*-CH), 136.0 and 136.2 [CH(6) rotamers], 139.4 (phenyl C), 150.1 [C(2)], 153.6 (Boc CO), 162.6 [C(4)], 169.1 (benzoyl CO) and 171.6 (ester CO); *m/z* (FAB) 632 (M + Na^+, 10%), 610 (M + H^+, 39), 554 ([M − C_4H_8 + H]$^+$, 27), 506 ([M − PhCO + H]$^+$, 18), 338

([M − PhCO − Ph_2CH]$^+$, 20), 266 (31), 231 (BzT + H^+, 52), 167 (Ph_2CH^+, 100), 105 ($PhCO^+$, 24) and 57 ($C_4H_9^+$, 28); ν_{max}(KBr)/cm^{-1} 1751, 1694 and 1659 (C=O); $[\alpha]_D^{25}$ −17.2 (*c* 1.0, DMF).

N-tert-*Butoxycarbonyl*-trans-4-(N^3-*benzoylthymin*-1-*yl*)-D-*proline diphenylmethyl ester* **7b** was similarly prepared starting from the *cis*-D-alcohol **6b** (8.0 g, 20 mmol). The product was obtained as a solid after column chromatography [SiO_2; dichloromethane–acetone (20:1)] and trituration with diethyl ether (4.20 g, 33%). Recrystallisation from ethyl acetate–hexane gave an analytically pure sample as crystals, mp 189–192 °C (Found: C, 68.9; H, 5.5; N, 6.7%); δ_H(200 MHz; $CDCl_3$) 1.30 and 1.47 (9 H, 2 × s, Boc rotamers), 1.80 (3 H, s, thymine CH_3), 2.40 and 2.58 [2 H, m, CH_2(3′)], 3.55–4.02 [2 H, m, CH_2(5′)], 4.53–4.64 [1 H, m, CH(2′)], 5.12 [1 H, m, CH(4′)], 6.90 and 6.94 (1 H, 2 × s, C*H*Ph_2 rotamers), 7.00 [1 H, s, CH(6) rotamers], 7.35–7.45 (10 H, br m, phenyl CH), 7.52 (2 H, t, *J* 7.2, benzoyl *m*-H), 7.69 (1 H, t, *J* 7.2, benzoyl *p*-H) and 7.94 (2 H, d, *J* 7.2, benzoyl *o*-H); δ_C(50.28 MHz; $CDCl_3$) 12.6 (thymine CH_3), 27.9 and 28.2 (Boc CH_3 rotamers), 33.4 and 35.1 [CH_2(3′) rotamers], 49.1 and 49.5 [CH_2(5′) rotamers], 53.8 and 54.4 [CH(4′) rotamers], 57.7 and 58.0 [CH(2′) rotamers], 77.9 and 78.2 (*C*HPh_2 rotamers), 81.2 and 81.3 (Boc C rotamers), 111.9 [C(5)], 127.0–130.6 (aromatic CH), 131.6 (benzoyl C), 135.3 (benzoyl *p*-CH), 136.2 [CH(6)], 139.4 and 139.7 (phenyl C rotamers), 149.9 [C(2)], 153.6 (Boc CO), 162.7 [C(4)], 169.1 (benzoyl CO) and 171.0 (ester CO); ν_{max}(KBr)/cm^{-1} 1739s (C=O), 1703s (C=O) and 1664s (C=O); $[\alpha]_D^{25}$ +11.3 (*c* 1.03, DMF).

N-tert-*Butoxycarbonyl*-cis-4-(N^3-*benzoylthymin*-1-*yl*)-D-*proline diphenylmethyl ester* **7c** was similarly prepared starting from the *trans*-D-alcohol **6c** (7.62 g, 19.2 mmol). The product was obtained as a crystalline solid after column chromatography [SiO_2; dichloromethane–acetone (20:1)] and recrystallisation from ethanol (4.20 g, 36%), mp 183–186 °C (Found: C, 69.1; H, 5.8; N, 6.8%); δ_H(200 MHz; $CDCl_3$) 1.30 and 1.49 (9 H, 2 × br s, Boc rotamers), 1.81 (3 H, br s, thymine CH_3), 2.04 and 2.86 [2 H, 2 × br m, CH_2(3′)], 3.66 (1 H, br m) and 4.02 (1 H, dd, *J* 12.0 and 8.0) [CH_2(5′)], 4.53 [1 H, br m, CH(2′)], 5.26 [1 H, br m, CH(4′)], 6.95 (1 H, s, C*H*Ph_2), 7.12 and 7.18 [1 H, 2 × br s, CH(6) rotamers], 7.30–7.44 (10 H, br m, phenyl CH), 7.50 (2 H, t, *J* 7.0, benzoyl *m*-H), 7.67 (1 H, t, *J* 7.0, benzoyl *p*-H), 7.92 (2 H, d, *J* 7.0, benzoyl *o*-H); ν_{max}(KBr)/cm^{-1} 1751s (C=O), 1699s (C=O) and 1661s (C=O); $[\alpha]_D^{25}$ +16.9 (*c* 1.03, DMF).

N-tert-Butoxycarbonyl-*cis*-4-(*N*^{6}-benzoyladenin-9-yl)-D-proline diphenylmethyl ester 12

A mixture of the *trans*-D-toluene-*p*-sulfonyl ester **11** (0.552 g, 1.00 mmol), *N*^{6}-benzoyladenine (0.595 g, 2.50 mmol), anhydrous K_2CO_3 (0.700 g, 5.00 mmol) and 18-crown-6 (0.100 g) in DMF (5 ml) was stirred under argon at 80 °C overnight. The reaction mixture was diluted with dichloromethane (20 ml) and washed with water, dried ($MgSO_4$) and evaporated to give the crude pruduct, which was purified by column chromatography (SiO_2; 2.5% methanol in dichloromethane; R_F 0.27) to give the product as a foam (0.260 g, 42%) which was spectroscopically pure. Further recrystallisation from ethanol–water gave analytically pure N-tert-*butoxycarbonyl*-cis-4-(N^6-*benzoyladenin*-9-*yl*)-D-*proline diphenylmethyl ester* **12** as needles, mp 115–119 °C (Found: C, 65.9; H, 5.5; N, 13.1. $C_{35}H_{34}N_6O_5\cdot H_2O$ requires C, 66.0; H, 5.7; N, 13.2%); δ_H(200 MHz; $CDCl_3$) 1.31 and 1.49 (9 H, 2 × s, Boc rotamers), 2.52 and 2.90 [2 H, 2 × br m, CH_2(3′)], 3.90–4.20 [2 H, br m, CH_2(5′)], 4.52 and 4.63 [1 H, 2 × br m, CH(2′) rotamers], 5.14 [1 H, br m, CH(4′)], 6.83 (1 H, s, C*H*Ph_2), 7.15–7.28 (10 H, m, phenyl CH), 7.35–7.60 (3 H, m, benzoyl *m*- and *p*-H), 7.95–8.05 [3 H, m, CH(8) and benzoyl *o*-H], 8.68 [1 H, s, CH(2)] and 9.39 (1 H, s, NH); δ_C(50.28 MHz; $CDCl_3$) 28.0 and 28.2 (Boc CH_3 rotamers), 34.5 and 35.7 [CH_2(3′) rotamers], 49.9 and 50.5 [CH_2(5′) rotamers], 52.3 and 52.8 [CH(4′) rotamers], 57.6 [CH(2′)], 77.8 (*C*HPh_2), 81.3 (Boc C), 123.4 [C(5)], 127.0–129.0 (aromatic CH), 132.9 (aromatic CH), 133.9 (aromatic C), 139.4 and 139.5 (aromatic C), 141.5 [CH(8)], 149.8 [C(4)], 152.0 [C(6)], 152.7 [CH(2)], 153.6 and 154.0 (Boc CO rotamers), 165.1 (benzoyl CO) and 170.9 (ester CO); *m/z* (ES-MS) 619 (M + H^+, 100%); ν_{max}(KBr)/cm^{-1} 1748 (C=O) and 1697s (C=O); λ_{max}($CHCl_3$)/nm 285 (ε/dm^3 mol^{-1} cm^{-1} 2.1 × 10^4); $[\alpha]_D^{25}$ +14.1 (*c* 0.63, $CHCl_3$).

N-tert-Butoxycarbonyl-*cis*-4-(*N*^{4}-benzoylcytosin-1-yl)-D-proline diphenylmethyl ester 15 and *N-tert*-butoxycarbonyl-*cis*-4-(4-benzoylaminopyrimidin-2-yloxy)-D-proline diphenylmethyl ester

A reaction mixture containing the *trans*-D-toluene-*p*-sulfonyl ester **11** (1.10 g, 2.00 mmol), *N*^{4}-benzoylcytosine (0.475 g, 2.20 mmol), anhydrous K_2CO_3 (0.300 g, 2.20 mmol) and 18-crown-6 (200 mg) in DMF (10 ml) was stirred at 70–80 °C under argon overnight. The suspension was diluted with dichloromethane (75 ml), filtered through Celite, and the organic phase was washed with water. Evaporation gave the crude product as an oil, which was purified by column chromatography (SiO_2; ethyl acetate). The more polar fractions (R_F 0.33) were combined and evaporated to give the N^1-isomer (0.299 g, 25%) as a foam. Recrystallisation from ethanol–water gave crystals of N-tert-*butoxycarbonyl*-cis-4-(N^4-*benzoylcytosin*-1-*yl*)-D-*proline diphenylmethyl ester* **15**, mp 133–135 °C (Found: C, 65.8; H, 6.5; N, 8.8. $C_{34}H_{34}N_4O_6\cdot C_2H_5OH\cdot H_2O$ requires C, 65.6; H, 6.4; N, 8.5%); δ_H(200 MHz; $CDCl_3$) 1.30 and 1.49 (9 H, 2 × s, Boc rotamers), 2.20 and 2.90 [2 H, br m, CH_2(3′)], 3.50–3.80 and 3.95–4.15 [2 H, 2 × br m, CH_2(5′)], 4.45–4.70 [1 H, br m, CH(2′)], 5.28 [1 H, br m, CH(4′)], 6.87 (1 H, s, C*H*Ph_2), 7.15–7.40 (10 H, m, phenyl CH), 7.40–7.75 [5 H, m, CH(5), CH(6) and benzoyl *m*- and *p*-H], 7.89 (2 H, d, *J* 7.4, benzoyl *o*-H) and 8.83 (1 H, br s, NH); δ_C(50.28 MHz; $CDCl_3$) 27.7 and 28.0 (Boc CH_3 rotamers), 34.4 and 36.0 [CH_2(3′) rotamers], 49.6 and 50.5 [CH_2(5′) rotamers], 54.3 and 54.9 [CH(4′) rotamers], 57.6 [CH(2′)], 78.0 and 78.3 (*C*HPh_2 rotamers), 81.3 (Boc C), 96.8 [CH(5)], 127.0–129.2 (aromatic CH), 133.4 (benzoyl C), 139.5 (aromatic C), 145.2 and 145.7 [CH(6) rotamers], 153.8 (Boc CO rotamers), 155.6 [C(2)], 162.0 [C(4)], 166.8 (benzoyl CO) and 171.3 (ester CO); *m/z* (ES-MS) 595 (M + H^+, 100%); ν_{max}(KBr)/cm^{-1} 1743 (C=O) and 1704s (C=O); λ_{max}($CHCl_3$)/nm 266 (ε/dm^3 mol^{-1} cm^{-1} 8.9 × 10^4) and 312 (3.4 × 10^4); $[\alpha]_D^{23}$ −13.6 (*c* 0.50, $CHCl_3$).

The less polar fractions (R_F 0.61) were combined and rechromatographed [SiO_2; dichloromethane–acetone (10:1)] to give the O^2-isomer as a foam (0.489 g, 41%), which was recrystallised from ethanol to give needles of N-tert-*butoxycarbonyl*-cis-4-(4-*benzoylaminopyrimidin*-2-*yloxy*)-D-*proline diphenylmethyl ester*, mp 145–147 °C (Found: C, 68.6; H, 5.5; N, 9.4. $C_{34}H_{34}N_4O_6$ requires C, 68.7; H, 5.8; N, 9.4%); δ_H(200 MHz; $CDCl_3$) 1.27 and 1.46 (9 H, 2 × s, Boc rotamers), 2.42 and 2.63 [2 H, br m, CH_2(3′)], 3.60–4.20 [2 H, br m, CH_2(5′)], 4.50 and 4.70 [1 H, 2 × m, CH(2′)], 5.40 [1 H, br m, CH(4′)], 6.91 and 6.98 (1 H, s, C*H*Ph_2), 7.15–7.32 (10 H, m, phenyl CH), 7.46–7.62 (3 H, m, benzoyl *m*- and *p*-H), 7.70–7.94 [3 H, m, benzoyl *o*-H and CH(5)], 8.37 [1 H, d, *J* 5.7, CH(6)] and 8.67 (1 H, br s, NH); δ_C(50.28 MHz; $CDCl_3$) 27.9 and 28.3 (Boc CH_3 rotamers), 35.1 and 36.0 [CH_2(3′) rotamers], 51.7 and 52.1 [CH_2(5′) rotamers], 57.6 and 57.9 [CH(2′) rotamers], 74.1 and 75.2 [CH(4′) rotamers], 77.3 and 77.5 (*C*HPh_2 rotamers), 80.2 and 80.4 (Boc C rotamers), 104.7 [CH(5)], 127.1–129.2 (aromatic CH), 133.1 and 133.4 (benzoyl C), 140.0–140.3 (aromatic C), 154.0 and 154.4 (Boc CO rotamers), 159.6 [C(2)], 160.5 [CH(6) rotamers], 163.8 [C(4)], 166.3 (benzoyl CO) and 170.9 and 171.1 (ester CO); *m/z* (ES-MS) 595 (M + H^+, 100%); ν_{max}(KBr)/cm^{-1} 1738s (C=O) and 1687s (C=O).

N-tert-Butoxycarbonyl-*cis*-4-(*N*^{2}-isobutyrylguanin-9-yl)-D-proline diphenylmethyl ester 18

To a stirred suspension of the *trans*-D-alcohol **6c** (0.400 g, 1.00 mmol), *N*^{2}-isobutyryl-*O*^{6}-(nitrophenylethyl)guanine (0.360 g,

1.00 mmol) and triphenylphosphine (0.294 g, 1.10 mmol) in anhydrous 1,4-dioxane (10 ml) at room temperature was slowly added DEAD (182 μl, 1.10 mmol) under argon. Another two aliquots of DEAD (91 μl, 0.55 mmol each) were added during a period of 36 h. The resulting clear yellow solution was evaporated and the residue was chromatographed (SiO_2; ethyl acetate; R_F 0.46) to give the O^6-nitrophenylethyl derivative as a foam (0.634 g, contaminated with diethyl hydrazinedicarboxylate). This was dissolved in dry pyridine (5 ml) containing DBU (300 μl, 2.00 mmol) and the solution was stirred at room temperature overnight under argon. The reaction mixture was diluted with dichloromethane, washed successively with 5% HCl and water, and then evaporated to dryness. The residue was purified by column chromatography [SiO_2; ethyl acetate–methanol (20:1)] to give the product as a foam (0.258 g, 43% from **6c**). Recrystallisation from ethyl acetate–light petroleum (40–60 °C) gave N-tert-*butoxycarbonyl*-cis-4-(N^2-*isobutyryl*-*guanin*-9-*yl*)-D-*proline diphenylmethyl ester* **18** as a crystalline solid, mp 140–145 °C (Found: C, 64.0; H, 5.7; N, 13.6. $C_{32}H_{36}N_6O_6$ requires C, 64.0; H, 6.0; N, 14.0%); δ_H(200 MHz; $CDCl_3$) 1.18–1.41 [15 H, m, Boc and $(CH_3)_2CH$], 2.30 (1 H, br m) and 2.75–2.95 (2 H, br m) [$CH_2(3')$ and $(CH_3)_2CH$], 3.78 and 4.05 [2 H, br m, $CH_2(5')$], 4.45–4.63 [1 H, 2 × m, CH(2′)], 4.90 [1 H, br m, CH(4′)], 6.77 and 6.80 (1 H, s, C*H*Ph$_2$), 7.15–7.30 (10 H, m, phenyl CH) and 7.60 and 7.66 [1 H, 2 × s, CH(8)]; δ_C(50.28 MHz; $CDCl_3$) 18.9 [$(CH_3)_2CH$], 27.9 and 28.2 (Boc CH_3 rotamers), 35.8 [$CH_2(3')$], 50.2 and 50.7 [$CH_2(5')$ rotamers], 51.9 and 52.3 [CH(4′) rotamers], 57.6 [CH(2′)], 60.4 [$(CH_3)_2CH$], 77.7 and 77.9 (*C*HPh$_2$ rotamers), 81.1 (Boc C), 121.1 [C(5)], 127.0–128.7 (aromatic CH), 137.2 [CH(8)], 139.5 (aromatic C), 148.2 and 149.1 [C(2)/C(6)], 153.6 and 154.2 (Boc CO rotamers), 156.1 [C(4)], 171.0 (ester CO) and 180.5 (amide CO); *m/z* (ES-MS) 601 (M + H$^+$, 100%); λ_{max}($CHCl_3$)/nm 255sh (ε/dm^3 mol^{-1} cm^{-1} 1.5 × 10^4) and 282 (1.2 × 10^4); $[\alpha]_D^{23}$ +37.8 (*c* 0.545, $CHCl_3$).

Procedure for selective deprotection of the *N*-Boc group in diphenylmethyl esters 7a, 7b and 7c and synthesis of *N*-Fmoc dipeptide diphenylmethyl esters 8a, 8b and 8c

The Boc-protected monomer (**7a**–**c**) was dissolved in THF (~10 ml mmol^{-1}), saturated methanolic HCl (~10 ml mmol^{-1}) was added, and the solution was stirred at room temperature for 3–12 h. The solvents were removed under reduced pressure. The residue was taken up in dry 1,4-dioxane and DIEA (~2 mol equiv. excess) was added until the solution was slightly basic (pH 8) when applied to a piece of moist pH paper. Fmoc-glycine pentafluorophenyl ester (1 mol equiv. excess) was then added and the solution was stirred at room temperature overnight. The reaction mixture was evaporated to dryness and the residue was purified by column chromatography [SiO_2; dichloromethane–acetone (10:1)].

N-[N-(*Fluoren*-9-*ylmethoxycarbonyl*)*glycyl*]-cis-4-(N^3-*benzoylthymin*-1-*yl*)-L-*proline diphenylmethyl ester* **8a** was obtained as a solid (99%, starting from 5.8 mmol of substrate **7a**) after column chromatography. Recrystallisation from ethanol gave fine needles, mp 201–204 °C (Found: C, 71.5; H, 5.0; N, 7.0. $C_{47}H_{40}N_4O_8$ requires C, 71.6; H, 5.1; N, 7.0%); δ_H(200 MHz; $CDCl_3$) 1.72 and 1.85 (3 H, 2 × s, thymine CH_3 rotamers), 2.10, 2.45, 2.72 and 2.95 [2 H, 4 × m, $CH_2(3')$ rotamers], 3.60–3.82 and 3.95–4.09 [4 H, br m, $CH_2(5')$ and Gly CH_2], 4.25 (1 H, t, *J* 7.1, Fmoc aliphatic CH), 4.40 (2 H, d, *J* 7.1, Fmoc CH_2), 4.77 [1 H, br m, CH(2′) rotamers], 5.15 and 5.35 [1 H, 2 × m, CH(4′) rotamers], 5.60 and 5.78 (1 H, 2 × br m, glycine NH rotamers), 6.86 and 6.93 (1 H, 2 × s, C*H*Ph$_2$ rotamers), 6.96 and 7.11 [1 H, 2 × s, CH(6) rotamers] and 7.25–7.95 (m, phenyl, Fmoc and benzoyl aromatic CH); δ_C(50.28 MHz; $CDCl_3$) 12.1 (thymine CH_3), 32.5 [$CH_2(3')$ rotamers], 43.5 (Gly CH_2), 47.0 (Fmoc aliphatic CH), 48.5 [$CH_2(5')$], 53.2 [CH(4′)], 57.8 [CH(2′)], 67.3 (Fmoc CH_2), 78.7 (*C*HPh$_2$), 112.3 [C(5)], 120.1 (Fmoc aromatic CH), 125.5–131.8 (aromatic CH), 135.8 [CH(6)], 139.4 and 139.6 (phenyl C rotamers), 141.7 and 144.0 (Fmoc aromatic C), 150.0 [C(2)], 156.2 (Fmoc CO), 162.4 [C(4)], 167.8 (benzoyl CO), 168.9 (peptide CO) and 170.8 (ester CO); *m/z* (FAB) 811 (M + Na$^+$, 21%), 789 (M + H$^+$, 5), 179 {[$(C_6H_4)_2C{=}CH_2$ + H]$^+$, 23}, 167 (Ph_2CH^+, 100) and 105 ($PhCO^+$, 22); ν_{max}(KBr)/cm^{-1} 1751s, 1737s, 1697 and 1657s (C=O); λ_{max}($CHCl_3$)/nm 260 (ε/dm^3 mol^{-1} cm^{-1} 3.1 × 10^4); $[\alpha]_D^{22}$ −41.2 (*c* 0.50, $CHCl_3$).

N-[N-(*Fluoren*-9-*ylmethoxycarbonyl*)*glycyl*]-trans-4-(N^3-*benzoylthymin*-1-*yl*)-D-*proline diphenylmethyl ester* **8b** was obtained as a solid (85%, starting from 5.3 mmol of substrate **7b**) after column chromatography. Recrystallisation from ethanol–water gave a solid, mp 125–128 °C (Found: C, 71.4; H, 5.0; N, 6.6%); δ_H(200 MHz; $CDCl_3$) 1.88 and 1.94 (3 H, 2 × s, thymine CH_3 rotamers), 2.30 and 2.60 [2 H, br m, $CH_2(3')$ rotamers], 3.70–4.10 [4 H, br m, $CH_2(5')$ and Gly CH_2], 4.22 (1 H, t, *J* 7.1, Fmoc aliphatic CH), 4.39 (2 H, d, *J* 7.0, Fmoc CH_2), 4.88 [1 H, br m, CH(2′)], 5.13 [1 H, br m, CH(4′)], 5.90 and 5.98 (1 H, 2 × br m, Gly NH), 6.91 and 6.94 (1 H, 2 × s, C*H*Ph$_2$ rotamers), 7.16 [1 H, s, CH(6)], 7.29–8.00 (m, phenyl, benzoyl and Fmoc aromatic CH); δ_C(50.28 MHz; $CDCl_3$) 12.4 (thymine CH_3), 32.0 [$CH_2(3')$ rotamers], 43.2 and 43.4 (Gly CH_2 rotamers), 47.0 (Fmoc aliphatic CH), 48.3 [$CH_2(5')$], 55.2 and 55.3 [CH(4′) rotamers], 57.9 [CH(2′)], 67.3 (Fmoc CH_2), 78.6 and 79.3 (*C*HPh$_2$ rotamers), 112.0 [C(5)], 120.2 (Fmoc aromatic CH), 125.4–131.6 (aromatic CH), 135.6 [CH(6)], 137.2 and 139.2 (phenyl C rotamers), 141.5 and 144.1 (Fmoc aromatic C), 150.0 [C(2)], 156.9 (Fmoc CO), 162.9 [C(4)], 168.4 (benzoyl CO), 169.4 (peptide CO) and 170.1 and 170.5 (ester CO); *m/z* (ES-MS) 806 (M + NH_4^+, 28%) and 789 (M + H$^+$, 100); ν_{max}(KBr)/cm^{-1} 1749, 1702 and 1660 (C=O).

N-[N-(*Fluoren*-9-*ylmethoxycarbonyl*)*glycyl*]-cis-4-(N^3-*benzoylthymin*-1-*yl*)-D-*proline diphenylmethyl ester* **8c** was obtained as a solid (98%, starting from 5.7 mmol of substrate **7c**) after column chromatography (R_F 0.30). Recrystallisation from ethanol–water gave needles, mp 201–203 °C (Found: C, 72.4; H, 4.9; N, 7.2%); δ_H(200 MHz; $CDCl_3$) 1.71 and 1.83 (3 H, 2 × s, thymine CH_3 rotamers), 2.06, 2.42, 2.78 and 2.92 [2 H, m, $CH_2(3')$ rotamers], 3.60–3.82 and 3.90–4.10 [4 H, br m, $CH_2(5')$ and Gly CH_2], 4.23 (1 H, t, *J* 7.0, Fmoc aliphatic CH), 4.39 (2 H, d, *J* 7.1, Fmoc CH_2), 4.73 [1 H, br m, CH(2′) rotamers], 5.14 and 5.42 [1 H, 2 × m, CH(4′) rotamers], 5.60 and 5.68 [1 H, 2 × br m, Gly NH rotamers], 6.87 and 6.91 (1 H, 2 × s, C*H*Ph$_2$ rotamers), 6.96 and 7.09 [1 H, 2 × s, CH(6) rotamers] and 7.21–7.92 (m, phenyl, Fmoc and benzoyl aromatic CH); *m/z* (ES-MS) 806 (M + NH_4^+, 98%) and 789 (M + H$^+$, 100); ν_{max}(KBr)/cm^{-1} 1751, 1737, 1697 and 1657 (C=O); λ_{max}($CHCl_3$)/nm 260 (ε/dm^3 mol^{-1} cm^{-1} 3.4 × 10^4); $[\alpha]_D^{22}$ +41.5 (*c* 0.50, $CHCl_3$).

Procedure for selective deprotection of the *N*-Boc group in diphenylmethyl esters 12, 15 and 18 and synthesis of *N*-Fmoc dipeptide diphenylmethyl esters 13, 16 and 19

The Boc-protected monomer (**12**, **15**, **18**) and PTSA monohydrate (5 mol equiv.) was dissolved in acetonitrile (~5 ml mmol^{-1}) and the resulting solution was stirred at room temperature overnight. The solvent was removed under reduced pressure and the residue was dissolved in DMF (~5–10 ml mmol^{-1}). DIEA (5 mol equiv. excess) was added until the solution was slightly basic (pH ~8) when applied to a piece of moist pH paper, followed by HOBt·H_2O (1.2 mol equiv.) and Fmoc-glycine pentafluorophenyl ester (1.2 mol equiv.) and the reaction mixture was stirred at room temperature overnight. The reaction mixture was diluted with dichloromethane and washed successively with saturated aq. $NaHCO_3$ and water. Evaporation gave the crude product, which was purified by column chromatography.

N-[N-(*Fluoren*-9-*ylmethoxycarbonyl*)*glycyl*]-cis-4-(N^6-*benzoyladenin*-9-*yl*)-D-*proline diphenylmethyl ester* **13** was obtained as a foam (85%, starting from 1.28 mmol of substrate **12**) after column chromatography (SiO_2; 10% methanol in ethyl

acetate), mp 130–133 °C (Found: C, 71.0; H, 4.8; N, 12.2. $C_{47}H_{39}N_7O_6$ requires C, 70.8; H, 4.9; N, 12.3%); δ_H(200 MHz; $CDCl_3$) 2.58 and 2.83 [2 H, 2 × m, CH_2(3′)], 3.78–4.40 [m, unresolved CH_2(5′), Gly CH_2, Fmoc aliphatic CH and CH_2], 4.78 [1 H, m, CH(2′)], 5.05 and 5.28 [1 H, 2 × m, CH(4′) rotamers], 6.13 (1 H, br t, Gly NH), 6.76 (1 H, 2 × s, C*H*Ph_2 rotamers), 7.20–7.75 (m, benzoyl *m*- and *p*-H, phenyl and Fmoc aromatic CH), 7.96–8.00 (2 H, d, *J* 7.1, benzoyl *o*-H), 8.23 [1 H, s, CH(8)], 8.70 [1 H, s, CH(2)] and 9.48 (1 H, br s, benzamide NH); δ_C(50.28 MHz; $CDCl_3$) 33.5 [CH_2(3′)], 43.3 (Gly CH_2), 47.0 (Fmoc aliphatic CH), 49.4 [CH_2(5′)], 53.2 [CH(4′)], 57.8 [CH(2′)], 67.1 (Fmoc CH_2), 78.6 (*C*HPh_2), 120.1 (Fmoc aromatic CH), 123.5 [C(5)], 125.2–128.9 and 133.0 (aromatic CH), 133.8, 139.4 and 141.4 (aromatic C), 142.0 [CH(8)], 144.1 (aromatic C), 150.0 [C(4)], 152.1 [C(6)], 152.7 [CH(2)], 156.9 (Fmoc CO), 165.4 (benzoyl CO), 168.6 (Gly CO) and 170.4 (ester CO); *m/z* (ES-MS) 798 (M + H^+, 100%); ν_{max}(KBr)/cm^{-1} 1718 (C=O) and 1668 (C=O); $[\alpha]_D^{22}$ +18.6 (*c* 0.21, $CHCl_3$).

N-[N-(*Fluoren*-9-*ylmethoxycarbonyl*)*glycyl*]-cis-4-(N^4-*benzoylcytosin*-1-*yl*)-D-*proline diphenylmethyl ester* **16** was obtained as a foam (70%, starting from 1.12 mmol of substrate **15**) after column chromatography [SiO_2; ethyl acetate–methanol (20:1)]. Recrystallisation from ethanol gave a solid, mp 131–133 °C (Found: C, 71.2; H, 4.9; N, 9.0. $C_{46}H_{39}N_5O_7$ requires C, 71.4; H, 5.1; N, 9.0%); δ_H(200 MHz; $CDCl_3$) 2.20 and 2.85 [2 H, 2 × m, CH_2(3′)], 3.61–4.40 [m, unresolved CH_2(5′), Gly CH_2, Fmoc aliphatic CH and CH_2], 4.76–4.81 [1 H, m, CH(2′) rotamers], 5.22 and 5.41 [1 H, 2 × m, CH(4′) rotamers], 6.03 and 6.12 (1 H, 2 × br t, Gly NH rotamers), 6.78 (1 H, br s, C*H*Ph_2), 7.05–8.00 [m, CH(5), CH(6), benzoyl, phenyl and Fmoc aromatic CH] and 9.32 (1 H, br s, benzamide NH); δ_C(50.28 MHz; $CDCl_3$) 33.2 [CH_2(3′)], 43.5 (Gly CH_2), 47.0 (Fmoc aliphatic CH), 49.2 [CH_2(5′)], 55.5 [CH(4′)], 57.8 [CH(2′)], 67.1 (Fmoc CH_2), 78.6 (*C*HPh_2), 97.3 [CH(5)], 120.1 (Fmoc aromatic CH), 125.4–129.1 and 133.1 (aromatic CH), 133.3, 139.4 and 141.1 (aromatic C), 144.1 (aromatic C), 145.6 [CH(6)], 155.8 [C(2)], 156.7 (Fmoc CO), 162.6 and 163.0 [C(4) rotamers], 167.1 (benzoyl CO), 168.6 (Gly CO) and 170.6 (ester CO); *m/z* (ES-MS) 774 (M + H^+, 100%); ν_{max}(KBr)/cm^{-1} 1750–1665br (C=O); $[\alpha]_D^{22}$ +20.9 (*c* 0.21, $CHCl_3$).

N-[N-(*Fluoren*-9-*ylmethoxycarbonyl*)*glycyl*]-cis-4-(N^2-*isobutyrylguanin*-9-*yl*)-D-*proline diphenylmethyl ester* **19** was obtained as a solid (52%, starting from 0.73 mmol of substrate **18**) after column chromatography (SiO_2; 10% methanol in ethyl acetate). Recrystallisation from ethyl acetate–light petroleum (40–60 °C) gave a crystalline solid, mp 145–150 °C (Found: C, 68.0; H, 5.2; N, 12.6. $C_{44}H_{41}N_7O_7$ requires C, 67.8; H, 5.3; N, 12.6%); δ_H(200 MHz; $CDCl_3$) 1.18 and 1.21 [6 H, d, *J* 6.7, (CH_3)$_2$CH], 2.31 (1 H, br m) and 2.61–2.79 (2 H, br m) [CH_2(3′) and (CH_3)$_2$C*H*], 3.89–4.20 [m, unresolved CH_2(5′), Gly CH_2 and Fmoc aliphatic CH], 4.37 (2 H, d, *J* 6.7, Fmoc CH_2), 4.63 [1 H, m, CH(2′)], 4.82 [1 H, m, CH(4′)], 6.10 (1 H, br m, Gly NH), 6.77 (1 H, s, C*H*Ph_2), 7.12–7.36 (m, phenyl and Fmoc aromatic CH), 7.49–7.57 [3 H, m, Fmoc aromatic CH and CH(8)], 7.69–7.73 (2 H, d, *J* 7.4, Fmoc aromatic CH) and 9.83 (1 H, br s, isobutyramide NH); δ_C(50.28 MHz; $CDCl_3$) 18.9 [(CH_3)$_2$CH], 35.7 [CH_2(3′)], 42.9 (Gly CH_2), 46.5 (Fmoc CH), 49.0 [CH_2(5′)], 52.9 [CH(4′)], 57.7 [CH(2′)], 66.9 (Fmoc CH_2), 78.3 (*C*HPh_2), 120.1 (Fmoc aromatic CH), 120.9 [C(5)], 125.2–128.8 (Fmoc aromatic CH), 137.5 [CH(8)], 139.4 and 139.6 (aromatic C), 144.0 (aromatic C), 148.3 and 148.8 [C(2)/C(6)], 155.8 [C(4)], 157.2 (Fmoc CO), 169.0 (Gly CO), 170.2 (ester CO) and 180.6 (isobutyramide CO); *m/z* (ES-MS) 780 (M + H^+, 100%); λ_{max}($CHCl_3$)/nm 270sh (ε/dm^3 mol^{-1} cm^{-1} 11.9 × 10^4); $[\alpha]_D^{23}$ +36.7 (*c* 0.645, $CHCl_3$).

N-[*N*-(Fluoren-9-ylmethoxycarbonyl)glycyl]-4-(thymin-1-yl)-prolines 9a–c

The protected dipeptide (**8a**, **8b** or **8c**) was treated with 10% HBr in acetic acid (5–10 ml $mmol^{-1}$) at room temperature for 1 h. The volatiles were evaporated under reduced pressure, the residue was triturated with diethyl ether and then washed with methanol–diethyl ether.

N-[N-(*Fluoren*-9-*ylmethoxycarbonyl*)*glycyl*]-cis-4-(*thymin*-1-*yl*)-L-*proline* **9a** was obtained as a solid (50%, starting from 2.5 mmol of substrate **8a**). Recrystallisation from ethanol–water gave a solid, mp >200 °C (Found: C, 62.5; H, 5.1; N, 10.6. $C_{27}H_{26}N_4O_7$ requires C, 62.5; H, 5.1; N, 10.8%); δ_H[200 MHz; (CD_3)$_2$SO] 1.75 (3 H, br s, thymine CH_3), 2.00–2.35 [2 H, br m, CH_2(3′) rotamers], 3.30–4.00 [br m, CH_2(5′) and Gly CH_2 obscured by the water signal], 4.15–4.30 (3 H, br m, Fmoc aliphatic CH and CH_2), 4.50–4.65 [1 H, br m, CH(2′) rotamers], 4.80–4.85 and 4.95–5.05 [1 H, br m, CH(4′) rotamers], 7.25–7.45 (4 H, m, Fmoc aromatic CH), 7.52 [1 H, br s, CH(6) rotamers], 7.70 and 7.85 (4 H, 2 × d, *J* 7.1, Fmoc aromatic CH); *m/z* (FAB) 541 (M + Na^+, 9%), 179 {[(C_6H_4)$_2$C=CH_2 + H]$^+$, 81}, 165 (32), 119 (30), 103 (44), 85 (83), 77 (32), 59 (85) and 47 (100); ν_{max}(KBr)/cm^{-1} 1731 (C=O), 1703s (C=O) and 1678 (C=O); $[\alpha]_D^{23}$ −4.13 (*c* 0.63, DMF).

N-[N-(*Fluoren*-9-*ylmethoxycarbonyl*)*glycyl*]-trans-4-(*thymin*-1-*yl*)-D-*proline* **9b** was obtained as a solid (57%, starting from 5.3 mmol of substrate **8b**), mp >200 °C; δ_H[200 MHz; (CD_3)$_2$SO] 1.70 (3 H, br s, thymine CH_3), 2.05–2.15 and 2.40–2.60 [br m, CH_2(3′) rotamers, obscured by the Me_2SO (DMSO) signal], 3.50–4.00 [br m, CH_2(5′) and Gly CH_2], 4.15–4.30 (3 H, br m, Fmoc aliphatic CH and CH_2), 4.35–4.45 and 4.75–4.85 [1 H, br m, CH(2′) rotamers], 4.90–5.00 and 5.05–5.10 [1 H, m, CH(4′) rotamers], 7.25–7.45 (4 H, m, Fmoc aromatic CH), 7.55 [1 H, br s, CH(6)], 7.68 (4 H, 2 × d, *J* 7.1, Fmoc aromatic CH); *m/z* (FAB) 519 (M + H^+, 6%), 179 {[(C_6H_4)$_2$C=CH_2 + H]$^+$, 34}, 85 (100), 59 (23) and 47 (32).

N-[N-(*Fluoren*-9-*ylmethoxycarbonyl*)*glycyl*]-cis-4-(*thymin*-1-*yl*)-D-*proline* **9c** was obtained as a solid (42%, starting from 6.8 mmol of substrate **8c**), mp >200 °C; δ_H[200 MHz; (CD_3)$_2$SO] 1.75 (3 H, br s, thymine CH_3), 2.11 and 2.52 [2 H, 2 × br m, CH_2(3′) rotamers], 3.50–4.00 [br m, CH_2(5′) and Gly CH_2], 4.18–4.30 [4 H, br m, CH(2′) and Fmoc aliphatic CH and CH_2], 4.73 and 4.98 [1 H, 2 × br m, CH(4′) rotamers], 7.28–7.41 (4 H, m, Fmoc aromatic CH), 7.51 (1 H, m, Gly NH), 7.54 [1 H, br s, CH(6) rotamers] and 7.72 and 7.88 (4 H, 2 × d, *J* 7.1, Fmoc aromatic CH); *m/z* (FAB) 541 (M + Na^+, 2%), 519 (M + H^+, 5), 179 (33), 103 (17), 85 (100), 77 (18), 59 (43) and 47 (45); $[\alpha]_D^{23}$ +4.26 (*c* 0.61, DMF).

N-[*N*-(Fluoren-9-ylmethoxycarbonyl)glycyl]-4-(thymin-1-yl)proline pentafluorophenyl esters 10a–c

A suspension of the Fmoc-dipeptide (**9a**, **9b** or **9c**) (1.0 mmol), pentafluorophenol (1.1 mmol) and DCCI (1.1 mmol) in dichloromethane (5 ml) was stirred at room temperature for 2–3 h. The precipitated DCU was filtered off and washed with dichloromethane. Evaporation of the filtrate followed by column chromatography (SiO_2; ethyl acetate) gave the product as a foam which in most cases could be made crystalline by trituration with diethyl ether–light petroleum, then being filtered and air dried.

N-[N-(*Fluoren*-9-*ylmethoxycarbonyl*)*glycyl*]-cis-4-(*thymin*-1-*yl*)-L-*proline pentafluorophenyl ester* **10a** was obtained from substrate **9a** as a solid (0.574 g, 84%), mp 124–126 °C; δ_H(200 MHz; $CDCl_3$) 1.94 (3 H, s, thymine CH_3), 2.26–2.42 and 2.85–3.00 [2 H, m, CH_2(3′)], 3.66–3.75 and 3.95–4.26 [m, unresolved CH_2(5′), Gly CH_2, Fmoc aliphatic CH], 4.38–4.42 (2 H, d, *J* 7.1, Fmoc CH_2), 4.85–4.93 [1 H, m, CH(2′)], 5.34–5.42 [1 H, m, CH(4′)], 5.80–5.82 (1 H, br t, Gly NH), 7.10 [1 H, s, CH(6)], 7.31–7.44, 7.60–7.63 and 7.75–7.79 (8 H, m, Fmoc aromatic CH) and 9.50 (1 H, s, thymine NH); δ_F(235.35 MHz; $CDCl_3$) −162.0 (dd, *J* 18.1 and 21.4) and −161.2 (t, *J* 19.6) (*m*-F major and minor rotamers), −157.0 (t, *J* 21.8) and −156.2 (t, *J* 21.7) (*p*-F major and minor rotamers) and −153.1 (d, *J* 18.5) and −152.8 (d, *J* 17.7) (*o*-F minor and major rotamers). The ratio of major:minor rotamers was ~15:1; *m/z* (ES-MS) 685.1

(M + H^+, 100%); ν_{max}(KBr)/cm^{-1} 1801 (C=O) and 1675br (C=O); $[\alpha]_D^{23}$ −15.9 (*c* 0.630, $CHCl_3$).

N-[N-(*Fluoren*-9-*ylmethoxycarbonyl*)*glycyl*]-trans-4-(*thymin*-1-*yl*)-D-*proline pentafluorophenyl ester* **10b** was obtained from substrate **9b** as a solid (0.400 g, 58%), mp 115–124 °C; δ_H(200 MHz; $CDCl_3$) 1.94 (3 H, s, thymine CH_3), 2.49–2.61 and 2.79–2.95 [2 H, m, CH_2(3′)], 3.79–4.13 [4 H, m, CH_2(5′) and Gly CH_2], 4.22 (1 H, t, *J* 6.7, Fmoc aliphatic CH), 4.37–4.41 (2 H, d, *J* 7.1, Fmoc CH_2), 5.04–5.18 [2 H, m, CH(2′) and CH(4′)], 5.78–5.82 (1 H, br t, Gly NH), 7.00 and 7.02 [1 H, 2 × s, CH(6) rotamers], 7.27–7.44, 7.58–7.61 and 7.74–7.78 (8 H, m, Fmoc aromatic CH) and 9.17 and 9.21 (1 H, 2 × s, thymine NH rotamers); δ_F(235.35 MHz; $CDCl_3$) −162.1 (t, *J* 19.3) and −161.4 (t, *J* 21.8) (*m*-F major and minor rotamers), −157.2 (t, *J* 22.5) and −156.3 (t, *J* 19.4) (*p*-F major and minor rotamers) and −153.3 (d, *J* 19.5) and −153.0 (d, *J* 20.0) (*o*-F minor and major rotamers); ν_{max}(KBr)/cm^{-1} 1797 (C=O) and 1679br (C=O); $[\alpha]_D^{23}$ +30.0 (*c* 0.73, $CHCl_3$).

N-[N-(*Fluoren*-9-*ylmethoxycarbonyl*)*glycyl*]-cis-4-(*thymin*-1-*yl*)-D-*proline pentafluorophenyl ester* **10c** was obtained from substrate **9c** as a solid (0.520 g, 76%), mp 122–126 °C; δ_H(200 MHz; $CDCl_3$) 1.94 (3 H, s, thymine CH_3), 2.26–2.42 and 2.85–3.00 [2 H, m, CH_2(3′)], 3.66–3.75 and 3.95–4.26 [5 H, m, CH_2(5′), Gly CH_2 and Fmoc aliphatic CH], 4.38–4.42 (2 H, d, *J* 7.1, Fmoc CH_2), 4.85–4.93 [1 H, m, CH(2′)], 5.34–5.42 [1 H, m, CH(4′)], 5.80–5.82 (1 H, br t, Gly NH), 7.10 [1 H, s, CH(6)], 7.31–7.44, 7.60–7.63 and 7.75–7.79 (8 H, m, Fmoc aromatic CH) and 9.50 (1 H, s, thymine NH); δ_F(235.35 MHz; $CDCl_3$) −162.0 (dd, *J* 18.1 and 21.4) and −161.2 (t, *J* 19.6) (*m*-F major and minor rotamers), −157.0 (t, *J* 21.8) and −156.2 (t, *J* 21.7) (*p*-F major and minor rotamers) and −153.1 (d, *J* 18.5) and −152.8 (d, *J* 17.7) (*o*-F minor and major rotamers). The ratio of major:minor rotamers was ~15:1; ν_{max}(KBr)/cm^{-1} 1800 (C=O) and 1683br (C=O); $[\alpha]_D^{23}$ +16.3 (*c* 0.645, $CHCl_3$).

Procedure for deprotection of diphenylmethyl esters and synthesis of Fmoc-dipeptide pentafluorophenyl esters 14, 17 and 20

The Fmoc dipeptide diphenylmethyl ester **13**, **16** or **19** was treated with TFA acid (~ 5–10 ml $mmol^{-1}$) containing anisole (50 μl ml^{-1} TFA) for 2–3 h. The volatiles were evaporated off under reduced pressure and the residue was triturated and washed with diethyl ether. The free acid was obtained as a solid in nearly quantitative yield after drying over NaOH pellets *in vacuo*. This was dissolved in 1:1 DMF–dichloromethane (5 ml $mmol^{-1}$) and pentafluorophenol (1.5 mol equiv.) and DCCI (1.5 mol equiv.) were added with stirring of the mixture at room temperature. The reaction mixture was stirred at room temperature for 1–3 h (monitored by TLC). The DCU precipitate was filtered off, and washed with dichloromethane. The combined organic phase was washed with water, dried ($MgSO_4$) and evaporated under reduced pressure. The residue was triturated with diethyl ether–light petroleum (40–60 °C) or reprecipitated from suitable solvents to give the product which contained a trace of DCU (~10%) as the only impurity according to ^{1}H NMR analysis, but which was pure enough for solid phase synthesis.

N-[N-(*Fluoren*-9-*ylmethoxycarbonyl*)*glycyl*]-cis-4-(N^6-*benzoyladenin*-9-*yl*)-D-*proline pentafluorophenyl ester* **14** was obtained as a solid (83%, starting from 0.5 mmol of substrate **13**), mp 125–130 °C; δ_H(200 MHz; $CDCl_3$) 2.86–3.02 and 3.10–3.24 [2 H, m, CH_2(3′)], 3.98–4.42 [7 H, m, CH_2(5′), Gly CH_2 and Fmoc aliphatic CH and CH_2], 4.97–5.06 [1 H, t, *J* 8.5, CH(2′)], 5.31–5.42 [1 H, m, CH(4′)], 5.70–5.74 (1 H, br t, *J* 3.8, Gly NH), 7.27–7.79 (11 H, m, Fmoc aromatic CH and benzoyl *m*- and *p*-H), 8.02–8.06 (2 H, d, *J* 6.7, benzoyl *o*-H), 8.13 [1 H, s, CH(8)], 8.80 [1 H, s, CH(2)] and 9.00 (1 H, br s, benzamide NH); *m/z* (ES-MS) 798 (M + H^+, 100%); ν_{max}(KBr)/cm^{-1} 1798 (C=O) and 1671br (C=O).

N-[N-*Fluoren*-9-*ylmethoxycarbonyl*)*glycyl*]-cis-4-(N^4-*benzoylcytosin*-1-*yl*)-D-*proline pentafluorophenyl ester* **17** was obtained as a solid (81%, starting from 0.17 mmol of substrate **16**), mp 133–137 °C; δ_H(200 MHz; $CDCl_3$) 2.46–2.60 and 2.90–3.12 [2 H, m, CH_2(3′)], 3.80–4.43 [7 H, m, CH_2(5′), Gly CH_2 and Fmoc aliphatic CH and CH_2], 4.93–5.01 [1 H, t, *J* 7.7, CH(2′)], 5.41–5.49 [1 H, m, CH(4′)], 5.72–5.78 (1 H, br t, *J* 4.5, Gly NH), 7.27–7.79 [m, CH(6), CH(5), Fmoc aromatic CH and benzoyl CH] and 7.90 (1 H, br s, benzamide NH); δ_F(235.35 MHz; $CDCl_3$) −162.0 (t, *J* 21.1) and −161.3 (t, *J* 19.1) (*m*-F major and minor rotamers), −157.2 (t, *J* 19.4) and −156.4 (t, *J* 19.4) (*p*-F major and minor rotamers) and −152.6 (d, *J* 20.0) (*o*-F); *m/z* (ES-MS) 774 (M + H^+, 100%); ν_{max}(KBr)/cm^{-1} 1797 (C=O) and 1669 (C=O).

N-[N-(*Fluoren*-9-*ylmethoxycarbonyl*)*glycyl*]-cis-4-(N^2-*isobutyrylguanin*-9-*yl*)-D-*proline pentafluorophenyl ester* **20** was obtained as a solid (63%, starting from 0.16 mmol of substrate **19**), mp 146–150 °C; δ_H(200 MHz; $CDCl_3$) 1.22–1.27 [6 H, 2 × d, *J* 6.9, $(CH_3)_2CH$], 2.60–2.82 and 2.97–3.12 [3 H, m, CH_2(3′) and $(CH_3)_2CH$], 4.08–4.30 [5 H, m, CH_2(5′), Gly CH_2 and Fmoc aliphatic CH], 4.37–4.41 (2 H, d, *J* 7.2, Fmoc CH_2), 4.85–4.94 [1 H, t, *J* 8.3, CH(2′)], 4.99–5.06 [1 H, m, CH(4′)], 5.79–5.84 (1 H, br t, *J* 4.5, Gly NH), 7.27–7.44 (4 H, m, Fmoc aromatic CH), 7.56–7.60 (2 H, d, *J* 7.4, Fmoc aromatic CH), 7.67 [1 H, s, CH(8)], 7.74–7.78 (2 H, d, *J* 7.4, Fmoc aromatic CH) and 8.95 (1 H, s, isobutyramide NH); *m/z* (ES-MS) 780 (M + H^+, 100%); ν_{max}(KBr)/cm^{-1} 1798 (C=O) and 1680br (C=O).

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