Amino acids bearing nucleobases for the synthesis of novel peptide nucleic acids

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All of the four nucleobases found in DNA have been incorporated in their protected form into the 4-position of *N*-*tert*-butoxycarbonyl-L-proline methyl ester with *cis*-stereochemistry. An efficient route for the synthesis of *N*-*tert*-butoxycarbonyl-*trans*-4-hydroxy-D-proline methyl ester has been developed from which the enantiomers may be synthesized. In addition an efficient synthesis of *N*-*tert*-butoxycarbonyl-*N*-(2-hydroxyethyl)glycine methyl ester has been achieved and its hydroxy group replaced with protected nucleobases using the Mitsunobu reaction

Introduction

Oligonucleotides are potentially useful for the regulation of genetic expression by binding with DNA or mRNA. The principle, which is known as the antisense principle,[1] provides a way to control protein synthesis at the nucleic acid level which should be much more effective than inhibition of enzymes. Furthermore, a highly specific inhibition can be achieved by a relatively short antisense oligonucleotide, whose sequence can be derived directly from the sequence of the target nucleic acids. However, for the antisense principle to be put into practice, the antisense oligonucleotides must be sufficiently stable under physiological conditions, able to pass through the cell membrane, and bind specifically and tightly to the target nucleic acids.[1] As natural oligonucleotides are readily degraded by nucleases *in vivo*, there is considerable interest in synthetic oligonucleotide analogues which are stable under physiological conditions. Recently, there has been interest in oligonucleotide analogues in which the sugar phosphate backbone is replaced by a peptide chain[2] after the success of the so-called peptide nucleic acids (PNA),[3,4] although such analogues were made much earlier.[5] PNAs were shown not only to retain the ability to recognise their complementary oligonucleotides, but also with a remarkably high affinity which is believed to be due to the uncharged nature of the peptide backbone.[4] These properties, in addition to their stability towards nucleases and their ease of synthesis make PNAs very attractive candidates for the antisense technology as well as other areas of research.[6] We report here the design and synthesis of amino acids bearing nucleobases with a view to their use for the synthesis of novel peptide nucleic acids.

The sugar phosphate backbone of a nucleic acid consists of a repeating unit of six atoms, configurationally and conformationally constrained by the D-ribose or 2′-deoxy-D-ribose ring. If this could be replaced by an isostructural dipeptide unit, the new backbone would be amenable to preparation by solid-phase peptide synthesis. Several molecular modelling investigations[2a,2b,7] indicated that the peptide backbone can adopt the helical B-form of DNA with a relatively low energy. Our preliminary model[8] suggested that a peptide chain consisting of an alternating sequence of a 'nucleo-amino acid' derived from proline and a 'spacer amino acid', which could be any amino acid, should be a suitable structural analogue of the ribose phosphate backbone of nucleic acids as shown in Fig. 1. The (2*R*,4*R*) ('*cis*-D') stereochemistry of proline was the obvious choice since this is analogous to the stereochemistry of deoxyribonucleotides. If the 3′-methylene group in the pyrrolidine ring is removed, the resulting analogue of DNA would not be expected to form as stable a duplex as the cyclic analogue, due to its greater conformational flexibility.[9] However, the lack of negative charge on the peptide backbone of both the 'rigid' and the 'flexible' peptide nucleic acids would be expected to allow a higher affinity for nucleic acids if the correct conformation were to be easily adopted. Moreover, these novel peptide nucleic acids can also be modified easily by replacing the glycine spacer with a range of amino acids to affect physical and biological properties such as solubility or cell permeability, both of which are important for therapeutic applications.

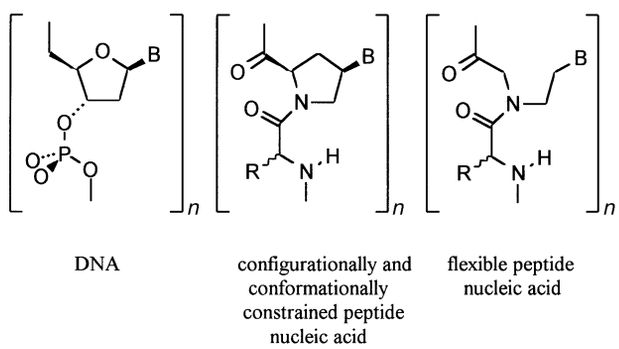
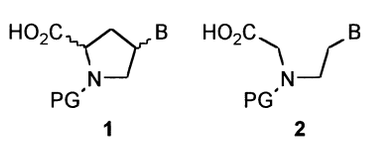


Fig. 1 Comparison of structure of the target peptide nucleic acids and DNA

The initial target molecules were *N*-Boc amino acids **1** and **2**.

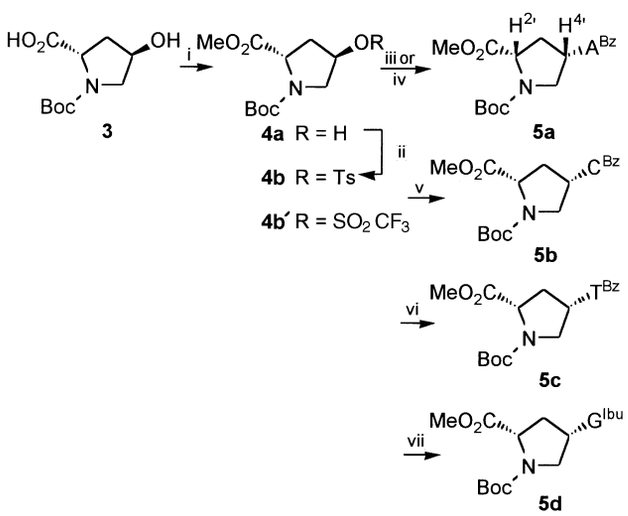


Although only the D-proline series would be able to mimic natural nucleotides, initially the more readily accessible and inexpensive L-proline series was investigated. Kaspersen and Pandit had reported the preparation of various pyrrolidine nucleosides by construction of the pyrimidine and purine rings from protected 4-aminoproline and 4-aminoprolinol, because reactions of 4-tosyl- or 4-chloro-proline derivatives with sodium salts of nucleobases or 2,4-dimethoxypyrimidines failed to give the desired products in acceptable yield.[10] The ring-construction method, although well documented for both pyrimidine[11] and purine[12] bases, requires a number of steps, uncommon reagents and includes harsh conditions. Our plan was to convert the hydroxy group of *N*-Boc-*trans*-4-hydroxyproline methyl ester into a good leaving group so that nucleophilic displacement with appropriately protected nucleobases would result in nucleo-amino acids with inverted configuration at the 4-position.

Results and discussion

The reaction of *N*-Boc-*trans*-4-hydroxy-L-proline **3** with diazomethane gave the methyl ester **4a** in near-quantitative yield. The ^{1}H NMR spectrum of this product showed two sets of broad signals for each proton, notably the two singlets of the Bu^t group at δ ~1.5. The effect is quite common in amides and urethanes and was previously observed in *N*-acetylproline and *N*-Boc-proline[13] and was attributed to the presence of two relatively stable conformers caused by restricted rotation around the C–N bond.[14] The methyl ester **4a** was then treated with trifluoromethanesulfonic anhydride in pyridine–dichloromethane or with toluene-*p*-sulfonyl chloride in anhydrous pyridine to give crystalline trifluoromethanesulfonate **4b**′ or toluene-*p*-sulfonate **4b** in 79 and 87% yield, respectively.

The trifluoromethanesulfonate **4b**′ was too unstable under the conditions required for the displacement reactions, but the toluene-*p*-sulfonate **4b** reacted smoothly with N^6-benzoyladenine[15] (N^6-BzA)–K_2CO_3 in dimethylformamide (DMF) in the presence of 18-crown-6 to give the product **5a** in 68% yield, and which was confirmed to be the N^9-isomer by ^{13}C NMR spectroscopy[16] after the *N*-Boc protecting group was removed in order to simplify the spectrum. A positive nuclear Overhauser effect (NOE) of H$^{4'}$ (1.5%) on irradiation at H$^{2'}$ was observed, indicating that inversion at C^4 had taken place, as expected, giving the *cis*-product **5a** (Scheme 1).



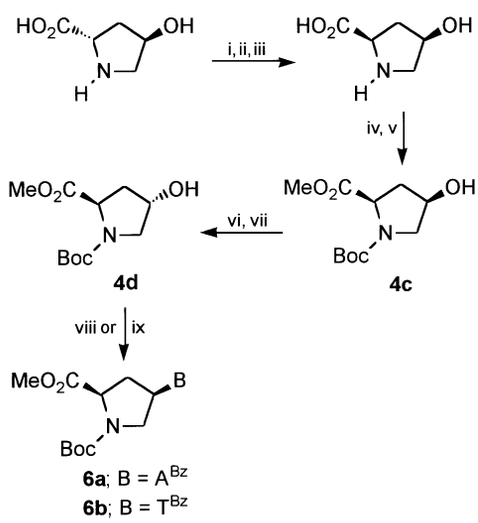
Scheme 1 *Reagents:* Boc = *tert*-butoxycarbonyl, Ts = *p*-tolylsulfonyl i, CH_2N_2; ii, TsCl, Py; iii, **4b**; N^6-BzA, K_2CO_3, 18-crown-6; iv, **4a**; N^6-BzA, Ph_3P, DEAD; v, **4b**; N^4-BzC, K_2CO_3, 18-crown-6; vi, **4a**; N^3-BzT, Ph_3P, DEAD; vii, **4a**; N^2-IbuG(ONpe), Ph_3P, DEAD; followed by DBU, Py

The analogous reaction with N^4-benzoylcytosine[17] (N^4-BzC) gave a mixture of two products—the N^1-isomer **5b** and the O^2-isomer in 31 and 32% yield, respectively. The two isomers could be separated readily by chromatography on silica gel. The identity of the O^2-isomer was shown by a characteristic downfield shift of the C$^{4'}$ resonance compared with that for the N^1-isomer.[18] A subsequent NOE experiment further confirmed the assignment as the NOE effect between H^6 and H^4 was observed only in the N^1-isomer. Furthermore, the *cis*-configuration in both compounds was confirmed by the presence of positive NOE between H$^{4'}$–H$^{2'}$ resonances.

Reaction of the toluene-*p*-sulfonate **4b** with N^2-isobutyrylguanine (N^2-IbuG) under similar conditions gave the undesired N^7-isomer as the major product, and reaction with thymine gave a mixture of elimination product and mono- and di-substituted thymines. These results suggested that protection of thymine at N^3 and guanine at O^6 might be necessary. As a result, N^3-benzoylthymine[19] and N^2-isobutyryl-O^6-(4-nitrophenylethyl)guanine[20] were synthesized and their reactions with compound **4b** attempted. However, the protecting groups used appeared to be labile under the reaction conditions and substantial cleavage was observed.

Recent studies[21,22] suggested that a direct displacement of hydroxy group with a nucleobase by the Mitsunobu reaction[23] is a very versatile method for the preparation of carbocyclic nucleosides. Reactions of compound **4a** with N^3-benzoylthymine and N^2-isobutyryl-O^6-(4-nitrophenylethyl)guanine in the presence of triphenylphosphine–diethyl azodicarboxylate (DEAD) were attempted and were found to give the desired products **5c** and **5d** {after removal of the O^6-nitrophenylethyl group by treatment with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in pyridine}[20] in 42 and 34% yield, respectively. The analogous reaction with N^6-benzoyladenine (N^6-BzA) was also attempted but the product **5a** was obtained in a lower yield (34%) than by the displacement reaction of the toluene-*p*-sulfonate. However, no product could be obtained from the reaction of compound **4a** and N^4-benzoylcytosine under similar conditions, probably because of the insolubility of the protected nucleobase in the reaction medium.

Having solved the chemistry in the L-hydroxyproline series we moved to the D-series. The required protected *trans*-4-hydroxy-D-proline was not commercially available but its synthesis starting from *cis*-4-hydroxy-D-proline by inversion of the 4-hydroxy group is well documented.[21,24] The *cis*-4-hydroxy-D-proline was synthesized by epimerisation of *trans*-4-hydroxy-L-proline by heating it with acetic anhydride followed by acid hydrolysis and fractional crystallisation of the epimeric hydroxyprolines (Scheme 2). The procedure used was essentially



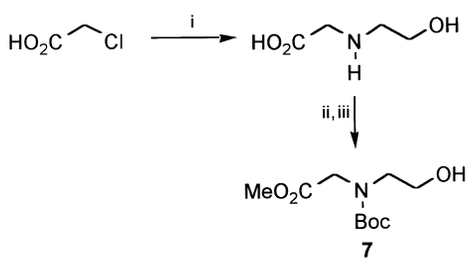
Scheme 2 *Reagents and conditions:* i, 1:2 Ac_2O–HOAc, reflux, 5.5 h; ii, 2 M HCl, reflux, 3 h (82%); iii, Et_3N–EtOH–water followed by recrystallisation (57%); iv, Boc_2O–Bu^{t}OH–aq. NaOH, room temp., overnight (quant.); v, CH_2N_2, 0 °C (quant.); vi, HCO_2H–DEAD–Ph_3P, THF, room temp., 5 h; vii, NH_3–MeOH, room temp., 2 h (78% from **4b**); viii, N^6-BzA, Ph_3P, DEAD (**6a**; 23%); ix, N^3-BzT, Ph_3P, DEAD (**6b**; 42%)

that developed by Greenstein and Winitz[24a] except that triethylamine was used for neutralising the epimeric hydrochloride salt instead of silver carbonate which considerably simplified the work-up and gave a comparable yield of the product. Protection of *cis*-4-hydroxy-D-proline as its *N*-Boc and methyl ester derivative to give compound **4c** was achieved in a similar fashion to the *trans*-L-isomer.[21] An early method of inversion of the 4-OH group involved conversion of the N,C-protected amino acid into the corresponding toluene-*p*-sulfonate followed by S_N2 displacement with acetate ion and hydrolysis.[24] We achieved considerable improvement following the method of Peterson and Vince[21] in which the alcohol **4c** was converted into the acetate with inverted configuration by Mitsunobu reaction in the presence of acetic acid. Subsequent

methanolysis with sodium methoxide–methanol gave the protected *trans*-4-hydroxy-D-proline **4d** in 68% overall yield. A modification using formic acid instead of acetic acid in the Mitsunobu reaction[25] gave a better yield (78%) of compound **4d** after removal of the formyl group by treatment with dilute ammonia in aq. methanol.[26] Mitsunobu reactions of compound **4d** with N^6-BzA and N^3-BzT gave the desired products **6a** and **6b** which were identical in all respects with the *cis*-L-isomers except for the sign of optical rotation.

Synthesis of the amino acids **2** required for the flexible PNAs (Fig. 1) was also investigated. The analogous route to the rigid analogue, starting from *N*-(2-hydroxyethyl)glycine, was studied. The amino acid is known in the literature,[27] but was not commercially available. The existing methods of synthesis of this simple compound include hydrolysis of the condensation product of hydroxyacetonitrile and ethanolamine,[28] oxidative dealkylation of *N*-(2-hydroxyethyl)iminodiacetic acid,[29] and oxirane ring opening with glycine and its derivatives.[30] All are either lengthy or inefficient. In fact, this hydroxy amino acid could be prepared easily from the reaction between chloroacetic acid and excess of ethanolamine in aqueous solution. Subsequent precipitation with ethanol followed by recrystallisation from ethanol–water gave the pure crystalline amino acid in 43% yield.

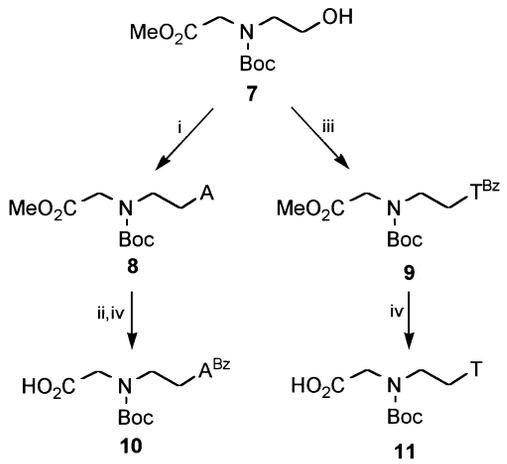
The N- and C-termini of the amino acid were protected in the same way as hydroxyproline to give the *N*-Boc-amino acid methyl ester Boc-Eg(OH)-OMe **7** in good overall yield (Scheme 3). Reactions of the alcohol **7** and BzA under Mitsunobu con-



Scheme 3 *Reagents and conditions:* i, $NH_2CH_2CH_2OH$ (2.5 mol equiv.) in water, room temp., overnight (43%); ii, Boc_2O, Bu^tOH, aq. NaOH, room temp., overnight (90%); iii, CH_2N_2, 0 °C (quant.)

ditions or *via* the toluene-*p*-sulfonate gave only a mixture of the N^7- and N^9-isomers, which could not be separated by column chromatography or crystallisation. Unprotected adenine, however, reacted with the alcohol **7** under Mitsunobu reaction conditions to give a single isomer in 27% yield, which was shown to be the desired N^9-isomer **8** by ^{13}C NMR spectroscopy.[16] The yield was not good but isolation of the product was far more convenient than for the benzoylated derivative due to the difference in polarity of the product and triphenylphosphine oxide. Protection of the exocyclic amino group by treatment with benzoyl chloride gave the dibenzoylated adenine derivative in quantitative yield. Treatment with aq. sodium hydroxide–acetone resulted in cleavage of the methyl ester and of one benzoate group to give the Boc-protected N^6-benzoyladenine derivative **10** in 60% yield (Scheme 4).

The alcohol **7** reacted with N^3-benzoylthymine under standard Mitsunobu conditions to give the product **9**, which could be purified by column chromatography followed by crystallisation from ethanol in 56% yield. Unlike the adenine analogue **8**, the protected thymine derivative **9** was relatively non-polar and eluted rapidly from the column. This property allowed a 20 mmol preparation of compound **9** to be carried out easily. The position of substitution in product **9** was confirmed to be N^1 of thymine by a ^{13}C–^1H heteronuclear multiple-bond correlation (HMBC) experiment, whereby long-range ^{13}C–^1H couplings over 2 and 3 bonds were detected and the connectivity of the molecule could be deduced from this information. The correla-



Scheme 4 *Reagents and conditions:* i, A–Ph_3P–DEAD in THF, room temp., overnight (27%); ii, BzCl, Py (quant.); iii, N^3-BzT–Ph_3P–DEAD in THF; iv, aq. NaOH–acetone 60% for **10**, 78% for **11** (from **7**)

tion between C^2/C^6–$H^{1'}$ and $C^{1'}$–H^6 and the lack of correlation between $C^4/H^{1'}$ were consistent with the proposed N^1-substitution and ruled out the possibilities of substitution at N^3, O^2 or O^4.

Since the acyclic thymidine analogue **9** could be synthesized from readily available starting materials and could be purified easily on a large scale, it was selected for the first solid phase peptide synthesis. The purpose was to establish suitable reaction conditions and to synthesize a model PNA for preliminary biological tests. Saponification of the methyl ester **9** by an equivalent excess of aq. NaOH–acetone led to substantial debenzoylation, giving mainly the free thymine acid **11**. Since protection of thymine was not necessary for the oligomer synthesis, the reaction was repeated with an excess of base and the reaction time was increased to give the debenzoylated derivative **11** as the sole product in very good yield (78% overall yield from hydroxy ester **7** if recrystallisation of the intermediate **9** was omitted) (Scheme 4). Recrystallisation once from ethanol–water gave pure material suitable for the peptide synthesis. Details of the synthesis of peptides and their biophysical properties will be discussed 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 10^{-1} deg cm^2 g^{-1}. IR spectra were recorded on a Perkin-Elmer 1750 Fourier Transform Infrared spectrometer. Elemental analyses were performed by Ms V. Lamburn on a Carlo Erba CHN analyser model 1106. Routine ^1H and ^{13}C NMR spectra were recorded on a Varian Gemini 200 spectrometer operating at 200 MHz (^1H) 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. ^{19}F NMR spectra were recorded on a Bruker AM 250 spectrometer at 235.35 MHz. High-field ^1H NMR spectra were recorded on a Bruker AMX 500 spectrometer (500 MHz). The ^1H/^{13}C HMBC experiment was performed on a Bruker AMX 500 spectrometer. ^1H 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 $CHCl_3$. *J*-Values are given in Hz. 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 other-

wise 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,[31] if necessary. *N*-Boc-*trans*-4-hydroxy-L-proline was obtained from Calbiochem-Novabiochem Ltd. Toluene-*p*-sulfonyl chloride was purified by recrystallisation from light petroleum (distillation range 60–80 °C). DMF (peptide synthesis grade) was obtained from Rathburn Chemical Ltd. and was used without further purification except when strictly anhydrous conditions were required, when it was re-distilled from calcium hydride under reduced pressure. 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-trifluoromethylsulfonyloxy-L-proline methyl ester 4b′

The alcohol **4a**[32] (0.24 g, 1.0 mmol) was dissolved in dry dichloromethane (10 ml) in a dry, round-bottomed flask fitted with a septum under argon. This solution was stirred and cooled in an ice–salt-bath at −10 °C. Anhydrous pyridine (100 μl, 1.25 mmol) was added *via* a microsyringe, followed by trifluoromethanesulfonic anhydride (210 μl, 1.25 mmol). A white precipitate immediately formed, while the solution turned yellow. After the reaction mixture had been stirred for 2 h, it was allowed to warm to ambient temperature and was stirred for another 1 h. The reaction mixture was evaporated to dryness, and the residue was extracted several times with 1:1 diethyl ether–hexane. The resulting light yellow solution was passed through a short silica gel column and the column was washed with more 1:1 diethyl ether–hexane. The solution obtained was evaporated to dryness to give the title compound as a solid (0.30 g, 79%), mp 53–55 °C (from diethyl ether–hexane); δ_H(200 MHz; $CDCl_3$) 1.35–1.60 (9 H, 2 × s, Boc rotamers), 2.25–2.70 [2 H, br m, CH_2(3)], 3.75 (3 H, s, Me), 3.80–4.05 [2 H, m, CH_2(5)], 4.35–4.65 [1 H, m, CH(2)] and 5.40–5.55 [1 H, m, CH(4)]; δ_F(235.35 MHz; $CDCl_3$) −76.86 and −76.90 (2 × s, CF_3SO_2 rotamers); *m/z* [CI(NH_3)] 395 ($M + NH_4^+$, 4%), 378 ($M + H^+$, 1), 339 ($M - C_4H_8 + NH_4^+$, 16), 278 ([M − Boc + 2 H]$^+$, 44), 128 {[$C_4H_6N(CO_2Me) + H$]$^+$, 100} and 68 ($C_4H_5N + H^+$, 58); ν_{max}(KBr)/cm^{-1} 1742 (C=O ester), 1687 (C=O urethane), 1410 (−SO_2O−) and 1142 (−SO_2O−).

N-tert-Butoxycarbonyl-*trans*-4-*p*-tolylsulfonyloxy-L-proline methyl ester 4b

A solution of *N*-Boc-*trans*-hydroxy-L-proline methyl ester **4a** (4.90 g, 20.0 mmol) in dry pyridine (10 ml) was cooled in an ice-bath. Recrystallised toluene-*p*-sulfonyl (tosyl) chloride (4.00 g, 21.0 mmol) was added with stirring of the mixture, which was continued until the reaction mixture became homogeneous. This solution was kept in a refrigerator at 0 °C for 48 h. The reaction mixture was poured into a solution of citric acid (40 g) in ice-cold water (250 ml) and extracted with diethyl ether (4 × 50 ml). The combined organic phase was dried ($MgSO_4$) and evaporated to give the crude product as a solid (6.93 g, 87%). Recrystallisation from diethyl ether–light petroleum (distillation range 40–60 °C) gave an analytical sample N-tert-*butoxycarbonyl*-trans-4-p-*tolylsulfonyloxy*-L-*proline methyl ester*, mp 78–79 °C (Found: C, 54.0; H, 6.1; N, 3.3. $C_{18}H_{25}NO_7S$ requires C, 54.1; H, 6.3; N, 3.5%); δ_H(200 MHz; $CDCl_3$) 1.39 and 1.42 (9 H, 2 × s, Boc rotamers), 2.00–2.60 [2 H, br m, CH_2(3)], 2.46 (3 H, s, tosyl CH_3), 3.50–4.00 [2 H, br m, CH_2(5)], 3.71 (3 H, s, Me ester), 4.35 [1 H, m, CH(2)], 5.03 [1 H, br m, CH(4)], 7.36 [2 H, d, *J* 7.9, tosyl H(3,5)] and 7.78 [2 H, d, *J* 7.9, tosyl H(2,6)]; δ_C(50.28 MHz; $CDCl_3$) 21.5 (tosyl CH_3), 28.0 (Boc CH_3), 37.1 and 35.9 [CH_2(3) rotamers], 51.8 and 52.3 [CH_2(5) rotamers], 51.2 (CO_2CH_3), 57.0 and 57.3 [CH(2) rotamers], 78.5 and 79.1 [CH(4) rotamers], 80.7 (Boc C), 127.9 and 130.3 (tosyl CH), 133.5 [tosyl C(4′)], 145.6 [tosyl C(1′)], 153.5 and 154.0 (Boc CO rotamers) and 172.9 and 173.1 (ester CO rotamers); *m/z* [CI(NH_3)] 400 ($M + H^+$, 1%), 361 ($M - C_4H_8 + NH_4^+$, 22), 300 ([M − Boc + 2 H]$^+$, 77), 128 {[$C_4H_6N(CO_2Me) + H$]$^+$, 48} and 68 ($C_4H_5N + H^+$, 100); ν_{max}(KBr)/cm^{-1} 1751s (C=O ester), 1695s (C=O Boc), 1407s (−SO_2O−), 1369s and 1180s (−SO_2O−); λ_{max}($CHCl_3$)/nm 242 (ε/dm^3 mol^{-1} cm^{-1}, 568), 258 (466), 264 (568), 268 (530) and 274 (470); $[\alpha]_D^{25}$ −37.4 (*c* 0.70, $CHCl_3$).

cis-4-Hydroxy-D-proline

A suspension of *trans*-4-hydroxy-L-proline (1.31 g, 10.0 mmol) in a mixture of acetic anhydride (10 ml) and glacial acetic acid (20 ml) was heated under reflux for 5.5 h. The dark solution was cooled, and evaporated under reduced pressure to give a thick oil. The oil was dissolved in 2 M HCl (25 ml) and the solution was refluxed for 3 h, then was decolourised with charcoal and filtered. The filtrate was concentrated under reduced pressure to give a light yellow oil. Trituration with diethyl ether gave a precipitate which was a mixture of epimeric hydrochloride salts. Recrystallisation from ethanol gave a crystalline solid (1.37 g, 82%), mp 145–149 °C. A portion of the hydrochloride salt (0.50 g, 3.0 mmol) was dissolved in water (2 ml), and triethylamine (1 ml) and absolute ethanol (40 ml) were added. The solution was stored at room temperature until crystallisation was complete. The crystals were collected by suction filtration and were recrystallised twice from water–ethanol to give pure *cis*-4-hydroxy-D-proline as needles (0.225 g, 57%), mp 250–254 °C (decomp.) [lit.,[24] mp 252–257 °C (decomp.)] (Found: C, 46.1; H, 7.2; N, 10.8. Calc. for $C_5H_9NO_3$: C, 45.8; H, 6.9; N, 10.7%); δ_H(200 MHz; D_2O) 2.00 (1 H, m) and 2.25 (1 H, ddd, *J* 14.3, 10.4 and 4.5) [CH_2(3)], 3.10 (1 H, ddd, *J* 12.4, 1.8 and 1.8) and 3.22 (1 H, dd, *J* 12.4 and 3.8) [CH_2(5)], 3.96 [1 H, dd, *J* 10.4 and 3.9, CH(2)] and 4.33 [1 H, m, CH(4)]; $[\alpha]_D^{23}$ +58.6 (*c* 2.0, H_2O) {lit.,[24] $[\alpha]_D^{25}$ +60.3 (*c* 2.0, H_2O)}.

N-tert-Butoxycarbonyl-*trans*-4-hydroxy-D-proline methyl ester 4d

DEAD (360 μl, 2.0 mmol) was added dropwise to a stirred solution of the *cis*-D-alcohol **4c**[21] (0.418 g, 1.71 mmol), triphenylphosphine (0.538 g, 2.0 mmol) and formic acid (75 μl, 2.0 mmol) in THF (10 ml) at −15 °C. The reaction mixture was warmed to room temperature and was stirred for 5 h. The clear solution was evaporated to dryness, the residue was treated with diethyl ether, and the mixture was filtered to remove triphenylphosphine oxide. The filtrate was evaporated to dryness and the residue chromatographed on a silica gel column with dichloromethane–acetone (20:1) as eluent. *N-tert*-Butoxycarbonyl-*trans*-4-formyloxy-D-proline methyl ester (R_f 0.52) was obtained as a clear oil (0.431 g). This was dissolved in methanol (20 ml) containing conc. aq. ammonia (250 μl) and the reaction mixture was stirred at room temperature for 2 h. The solvents were removed under reduced pressure and the residue was purified by column chromatography (SiO_2; ethyl acetate) to give *N-tert*-butoxycarbonyl-*trans*-4-hydroxy-D-proline methyl ester **4d** as an oil (0.310 g, 78% from **4c**) (Found: C, 53.6; H, 8.1; N, 5.7. Calc. for $C_{11}H_{19}NO_5$: C, 53.9; H, 7.8; N, 5.7%); δ_H(500 MHz; $CDCl_3$) 1.42 and 1.47 (9 H, 2 × s, Boc rotamers), 2.08 and 2.29 [2 H, m, CH_2(3)], 3.40–3.70 [2 H, m, CH_2(5)], 3.75 (3 H, s, Me ester), 4.42 [1 H, br m, CH(2)] and 4.52 [1 H, br m, CH(4)]; δ_C(50.28 MHz; $CDCl_3$) 28.1 and 28.2 (Boc CH_3 rotamers), 38.3 and 38.9 [CH_2(3) rotamers], 52.0 and 52.2 (CO_2CH_3), 54.6 [CH_2(5)], 57.5 and 57.9 [CH(2) rotamers], 69.2 and 69.9 [CH(4) rotamers], 80.3 and 80.5 (Boc C rotamers), 154.3 (Boc CO) and 174.1 (ester CO); *m/z* [CI(NH_3)] 246 ($M + H^+$, 18%), 207 ([$M - C_4H_8 + NH_4$]$^+$, 19), 190 ([$M - C_4H_8 + H$]$^+$, 100), 146 ([M − Boc + 2 H]$^+$, 53), 86 (69); ν_{max}(neat)/cm^{-1} 3439br (O–H),

1748s (C=O ester) and 1678s (C=O urethane); $[\alpha]_D^{24}$ +78.7 (*c* 0.625, MeOH).

***N*-*tert*-Butoxycarbonyl-*cis*-4-(*N*^{6}-benzoyladenin-9-yl)-L-proline methyl ester 5a and *N*-*tert*-butoxycarbonyl-*cis*-4-(*N*^{6}-benzoyladenin-9-yl)-D-proline methyl ester 6a (by Mitsunobu reaction)**

DEAD (2.0 ml, 12 mmol) was added dropwise to a cooled (ice-bath), stirred THF suspension (25 ml) of benzoyladenine (2.60 g, 11.0 mmol), the alcohol **4a** (2.45 g, 10.0 mmol) and triphenylphosphine (2.94 g, 11.0 mmol). The reaction mixture was warmed to room temperature and stirred for 48 h. Since the benzoyladenine was still incompletely dissolved, a further 0.5 mole equivalents of DEAD (1.0 ml, 6.0 mmol) and triphenylphosphine (1.47 g, 5.5 mmol) were added and stirring was continued for another 24 h. The clear orange solution was evaporated to dryness and the residue was chromatographed on silica gel with dichloromethane–acetone (3:1) as eluent. *N*-*tert*-Butoxycarbonyl-*cis*-4-(*N*^{6}-benzoyladenin-9-yl)-L-proline methyl ester **5a** (R_F 0.17) was obtained as a foam (1.58 g, 34%) (Found: C, 59.1; H, 5.7; N, 18.3. $C_{23}H_{26}N_6O_5$ requires C, 59.2; H, 5.6; N, 18.0%); δ_H(500 MHz; $CDCl_3$) 1.45 (9 H, br s, Boc), 2.58 and 3.00 [2 H, br m, CH_2(3′)], 3.72 (3 H, 2 × br s, CO_2CH_3 rotamers), 3.60–4.55 [3 H, br m, CH(2′) and CH_2(5′)], 5.27 [1 H, br m, CH(4′)], 7.53 (2 H, t, *J* 7.4, benzoyl *m*-CH), 7.65 (1 H, t, *J* 7.4, benzoyl *p*-CH), 8.03 (2 H, d, *J* 7.5, benzoyl *o*-CH), 8.21 [1 H, s, CH(8)], 8.81 [1 H, s, CH(2)] and 9.01 (1 H, br s, benzamide NH); δ_C(50.28 MHz; $CDCl_3$) 28.0 (Boc CH_3), 34.5 and 35.5 [CH_2(3′) rotamer], 50.0 and 50.5 [CH_2(5′) rotamer], 52.0 [CH(4′)], 52.5 (CH_3 ester), 57.5 [CH(2′)], 81.0 [Boc $(CH_3)_3C$], 123.5 [C(5)], 127.0 and 127.5 (benzoyl CH), 132.8 (benzoyl CH), 133.7 (benzoyl C), 141.5 [CH(8)], 150.0 [C(4)], 152.0 [C(6)], 152.5 [CH(2)], 153.5 (Boc CO), 165.5 (benzamide CO) and 172.5 (ester CO); *m/z* (FAB$^+$) 467 (M + H$^+$, 14%), 411 ([M − C_4H_8 + H]$^+$, 34), 240 ([BzA + H]$^+$, 18), 105 (PhCO$^+$, 47), 68 ([C_4H_5N + H]$^+$, 20) and 57 ($C_4H_9^+$, 100); ν_{max}(KBr)/cm^{-1} 3416br (N–H), 1750 and 1703s (C=O); λ_{max}(MeOH)/nm 279 (ε/dm^3 mol^{-1} cm^{-1} 1.6 × 10^4); $[\alpha]_D^{22}$ +14.2 (*c* 1.60, $CHCl_3$).

N-tert-*Butoxycarbonyl*-cis-4-(N^6-*benzoyladenin*-9-*yl*)-D-*proline methyl ester* **6a** was similarly prepared as a foam in 23% yield starting from the alcohol **4d** (1.0 mmol scale reaction) (Found: C, 58.9; H, 6.1; N, 17.4. $C_{23}H_{26}N_6O_5 \cdot 0.25H_2O$ requires C, 58.7; H, 5.7; N, 17.8%); δ_H(200 MHz; $CDCl_3$) 1.45 (9 H, br s, Boc), 2.56 and 3.00 [2 H, br m, CH_2(3′)], 3.72 (3 H, 2 × br s, CH_3 ester), 3.60–4.55 [3 H, br m, CH(2′) and CH_2(5′)], 5.27 [1 H, br m, CH(4′)], 7.53 (2 H, t, *J* 7.4, benzoyl *m*-CH), 7.65 (1 H, t, *J* 7.4, benzoyl *p*-CH), 8.03 (2 H, d, *J* 7.5, benzoyl *o*-CH), 8.21 [1 H, s, CH(8)], 8.81 [1 H, s, CH(2)] and 9.01 (1 H, br s, benzamide NH); δ_C(50.28 MHz; $CDCl_3$) 28.1 (Boc CH_3), 34.5 and 36.0 [CH_2(3′) rotamer], 50.1 and 50.8 [CH_2(5′) rotamer], 52.0 [CH(4′)], 52.1 (CH_3 ester), 57.5 [CH(2′)], 81.2 [Boc $(CH_3)_3C$], 123.1 [C(5)], 128.1 and 128.9 (benzoyl CH), 132.9 (benzoyl CH), 133.8 (benzoyl C), 141.4 [CH(8)], 149.9 [C(4)], 152.3 [C(6)], 152.8 [CH(2)], 153.5 (Boc CO), 165.2 (benzamide CO) and 172.6 (ester CO); *m/z* (ES MS) 467 (M + H$^+$, 100%) and 411 ([M − C_4H_8 + H]$^+$, 12); ν_{max}(KBr)/cm^{-1} 3257br (N–H), 1751 (C=O), 1701 (C=O); λ_{max}($CHCl_3$)/nm 285 (ε/dm^3 mol^{-1} cm^{-1} 1.65 × 10^4); $[\alpha]_D^{22}$ −11.6 (*c* 1.6, $CHCl_3$).

***N*-*tert*-Butoxycarbonyl-*cis*-4-(*N*^{6}-benzoyladenin-9-yl)-L-proline methyl ester 5a (by displacement of toluene-*p*-sulfonate 4b)**

A mixture of the toluene-*p*-sulfonate **4b** (0.400 g, 1.00 mmol), *N*^{6}-benzoyladenine (0.595 g, 2.50 mmol), anhydrous K_2CO_3 (0.350 mg, 2.50 mmol) and 18-crown-6 (100 mg) in DMF (5 ml) was stirred under argon at 80 °C overnight. Water (20 ml) was added to the reaction and the suspension was extracted with dichloromethane. The organic phase was washed with water, dried ($MgSO_4$) and evaporated to give the crude pruduct, which was purified by column chromatography (SiO_2; 5% methanol in dichloromethane) to give compound **5a** as a foam (0.316 g, 68%) which was identical with the material obtained from the Mitsunobu reaction.

***N*-*tert*-Butoxycarbonyl-*cis*-4-(*N*^{4}-benzoylcytosin-1-yl)-L-proline methyl ester 5b and *N*-*tert*-butoxycarbonyl-*cis*-4-[4-(benzoylamino)pyrimidin-2-yloxy]-L-proline methyl ester**

A suspension of the toluene-*p*-sulfonate **4b** (0.40 g, 1.0 mmol), *N*^{4}-benzoylcytosine (0.54 g, 2.5 mmol), anhydrous K_2CO_3 (0.70 g, 5.0 mmol) and 18-crown-6 (100 mg, cat.) in DMF (5 ml) was stirred at 70–80 °C under argon for 6.5 h. The reaction mixture was then diluted with dichloromethane (50 ml) and washed with water (4 × 50 ml). The organic layer was dried ($MgSO_4$), and filtered through Celite. The filtrate was evaporated to give an oil, which was chromatographed on silica gel with 5% methanol in ethyl acetate as eluent. The more polar fractions (R_F 0.39) were combined and evaporated to give *N*-*tert*-butoxycarbonyl-*cis*-4-(*N*^{4}-benzoylcytosin-1-yl)-L-proline methyl ester **5b** as a foam (134 mg, 31%). Recrystallisation from ethanol–water gave a *crystalline solid* mp 159–161 °C (Found: C, 59.5; H, 5.9; N, 12.6. $C_{22}H_{26}N_4O_6$ requires C, 59.7; H, 5.9; N, 12.7%); δ_H(200 MHz; $CDCl_3$) 1.39 (9 H, br m, Boc), 2.20 and 2.80 [2 H, 2 × m, CH_2(3′)], 3.69 and 3.96 [2 H, 2 × m, CH_2(5′)], 3.70 (3 H, br s, Me ester), 4.31–4.35 [1 H, 2 × m, CH(2′)], 5.23 [1 H, br m, CH(4′)], 7.35–7.58 [4 H, m, benzoyl CH and CH(5)], 7.86–7.94 [3 H, m, benzoyl CH and CH(6)] and 9.11 (1 H, br s, amide NH); δ_C(50.28 MHz; $CDCl_3$) 28.1 (Boc CH_3), 35.8 [CH_2(3′)], 49.5 and 50.2 [CH_2(5′) rotamers], 52.3 (ester CH_3), 54.4 and 54.9 [CH(4′) rotamers], 57.4 [CH(2′)], 81.2 (Boc C), 96.9 [CH(5)], 127.9 and 129.1 (benzoyl CH), 133.3 (benzoyl C), 145.8 and 146.0 [CH(6)], 153.6 (Boc CO), 155.8 [C(2)], 162.4 [C(4)], 167.1 (benzamide CO) and 172.9 (ester CO); *m/z* [CI(NH_3)] 443 (M + H$^+$, 100%), 387 ([M − C_4H_8 + H]$^+$, 10), 343 ([M − Boc + 2 H]$^+$, 12), 227 (14), 216 ([BzC + H]$^+$, 29), 127 ([M − Boc − BzC + H]$^+$, 95), 105 (PhCO$^+$, 51) and 68 (C_4H_5N + H$^+$, 52); ν_{max}(KBr)/cm^{-1} 1755 and 1683s (C=O); λ_{max}(MeOH)/nm 258 (ε/dm^3 mol^{-1} cm^{-1} 2.9 × 10^4) and 302 (1.3 × 10^4); $[\alpha]_D^{25}$ +33.8 (*c* 0.58, MeOH).

The less polar fractions (R_F 0.68) were combined and re-chromatographed on silica gel with dichloromethane–acetone 10:1 as eluent (R_F 0.40). *N*-*tert*-Butoxycarbonyl-*cis*-4-[4-(benzoylamino)pyrimidin-2-yloxy]-L-proline methyl ester was obtained as an oil (138 mg, 32%), δ_H(200 MHz; $CDCl_3$) 1.38 and 1.43 (9 H, 2 × s, Boc rotamers), 2.30 and 2.55 [2 H, 2 × m, CH_2(3′)], 3.63 and 3.88 [2 H, 2 × m, CH_2(5′)], 3.69 and 3.71 (3 H, 2 × s, Me ester), 4.33–4.53 [1 H, 2 × m, CH(2′) rotamers], 5.37 [1 H, m, CH(4′)], 7.43–7.60 (3 H, m, benzoyl *m*- and *p*-CH), 7.85–7.95 [3 H, m, benzoyl *o*-CH and CH(5)], 8.36–8.40 [1 H, m, CH(6)] and 8.78–8.80 (1 H, 2 × s, benzamide NH rotamers); δ_C(50.28 MHz; $CDCl_3$) 28.1 and 28.2 (Boc CH_3 rotamers), 35.2 and 36.0 [CH_2(3′) rotamers], 51.5 and 52.2 [CH_2(5′) rotamers], 52.3 (ester CH_3), 57.4 and 57.7 [CH(2′) rotamers], 74.1 and 75.2 [CH_2(4′) rotamers], 80.3 (Boc C), 104.6 [CH(5)], 127.6 and 129.1 (benzoyl CH), 133.1 (benzoyl CH), 133.4 (benzoyl C), 153.9 and 154.4 (Boc CO rotamers), 159.7 [C(2)], 160.7 and 160.8 [CH(6) rotamers], 163.9 [C(4)], 166.5 (benzamide CO) and 172.6 and 172.9 (ester CO rotamers); *m/z* [CI(NH_3)] 443 (M + H$^+$, 16%), 387 ([M − C_4H_8 + H]$^+$,15), 343 ([M − Boc + 2 H]$^+$, 14), 216 ([BzC + H]$^+$, 100), 127 ([M − Boc − BzC + H]$^+$, 47), 105 (PhCO$^+$, 86) and 68 (C_4H_5N + H$^+$, 56); ν_{max}(KBr)/cm^{-1} 1757 and 1698s (C=O); λ_{max}(MeOH)/nm 238 (ε/dm^3 mol^{-1} cm^{-1} 2.2 × 10^4) and 280 (2.7 × 10^4); $[\alpha]_D^{25}$ −49.5 (*c* 0.62, MeOH).

***N*-*tert*-Butoxycarbonyl-*cis*-4-(*N*^{3}-benzoylthymin-1-yl)-L-proline methyl ester 5c and *N*-*tert*-butoxycarbonyl-*cis*-4-(*N*^{3}-benzoylthymin-1-yl)-D-proline methyl ester 6b**

To a suspension of the alcohol **4a** (0.245 g, 1.0 mmol), *N*^{3}-benzoylthymine (0.230 g, 1.0 mmol) and triphenylphosphine (294 mg, 1.1 mmol) in dry THF (10 ml) was added DEAD (182 μl, 1.1 mmol) dropwise at −15 °C. The reaction mixture was

stirred at room temperature overnight. The clear solution was evaporated to dryness and the residue was purified by column chromatography (SiO_2; dichloromethane–acetone 20:1) to give *N-tert*-butoxycarbonyl-*cis*-4-(N^3-benzoylthymin-1-yl)-L-proline methyl ester **5c** (R_F 0.34) as a foam (0.193 g, 42%), δ_H(200 MHz; $CDCl_3$) 1.40 (9 H, br s, Boc), 1.90 (3 H, s, thymine CH_3) 2.10 and 2.71 [2 H, 2 × br m, $CH_2(3')$], 3.61 [1 H, m, $CH_aH_b(5')$], 3.72 (3 H, s, Me ester), 3.90 [1 H, dd, *J* 8.0 and 11.5, $CH_aH_b(5')$], 4.26 [1 H, m, CH(2′)], 5.18 [1 H, br m, CH(4′)], 7.37 [1 H, s, CH(6)], 7.44 (2 H, t, *J* 7.7, benzoyl *m*-H), 7.60 (1 H, t, *J* 7.4, benzoyl *p*-H) and 7.86 (2 H, d, *J* 7.1, benzoyl *o*-H); δ_C(50.28 MHz; $CDCl_3$) 12.4 (thymine CH_3), 28.1 (Boc CH_3), 35.1 and 33.8 [$CH_2(3')$ rotamers], 49.1 and 49.5 [$CH_2(5')$ rotamers], 52.4 [CH(4′) and ester CH_3], 57.3 [$CH_2(2')$ rotamers], 81.1 (Boc C), 111.6 [C(5)], 129.3 and 130.5 (2 × benzoyl CH), 131.6 (benzoyl C), 135.3 (benzoyl *p*-CH), 136.5 [CH(6)], 150.1 [C(2)], 153.1 (Boc CO), 162.7 [C(4)], 169.2 (CO amide) and 173.1 (ester CO); *m/z* [CI(NH_3)] 458 ($M + H^+$, 7%), 419 ($[M - C_4H_8 + NH_4]^+$, 8), 402 ($[M - C_4H_8 + H]^+$, 16), 358 ($[M - Boc + 2H]^+$, 69), 254 ($[M - Boc - PhCO + H]^+$, 53), 127 (T + H^+ and $[M - BzT - Boc + H]^+$, 100), 105 ($PhCO^+$, 100) and 68 ($C_4H_5N + H^+$, 72); ν_{max}(KBr)/cm^{-1} 1752s, 1701s and 1659s (C=O); λ_{max}(MeOH)/nm 253sh (ε/dm^3 mol^{-1} cm^{-1} 1.8×10^4); $[\alpha]_D^{25}$ +3.85 (*c* 0.52, MeOH).

N-tert-*Butoxycarbonyl*-cis-4-(N^3-*benzoylthymin*-1-*yl*)-D-*proline methyl ester* **6b** was similarly obtained as a foam in 42% yield starting from compound **4d** (0.95 mmol scale reaction), mp 84–86 °C (Found: C, 59.8; H, 6.0; N, 8.9. $C_{23}H_{27}N_3O_7 \cdot 0.25H_2O$ requires C, 59.8; H, 6.0; N, 9.1%); δ_H(200 MHz; $CDCl_3$) 1.43 (9 H, br s, Boc), 1.94 (3 H, s, thymine CH_3) 2.13 and 2.71 [2 H, 2 × br m, $CH_2(3')$], 3.58–3.66 [1 H, br m, $CH_aH_b(5')$], 3.75 (3 H, s, Me ester), 3.93 [1 H, dd, *J* 8.0 and 11.5, $CH_aH_b(5')$], 4.29–4.33 [1 H, br m, CH(2′)], 5.15–5.18 [1 H, br m, CH(4′)], 7.38 [1 H, s, CH(6)], 7.47 (2 H, t, *J* 7.7, benzoyl *m*-H), 7.63 (1 H, t, *J* 7.4, benzoyl *p*-H) and 7.89 (2 H, d, *J* 7.1, benzoyl *o*-H); δ_C(50.28 MHz; $CDCl_3$) 12.4 (thymine CH_3), 28.1 (Boc CH_3), 34.0 and 35.3 [$CH_2(3')$ rotamers], 49.1 and 49.5 [$CH_2(5')$ rotamers], 52.2 and 52.4 [CH(4′) and ester CH_3], 57.4 [$CH_2(2')$ rotamers], 81.2 (Boc C), 111.6 and 111.7 [C(5) rotamers], 129.4 and 130.6 (2 × benzoyl CH), 131.6 (benzoyl C), 135.3 (benzoyl *p*-CH), 136.4 [CH(6)], 150.2 [C(2)], 153.5 (Boc CO), 162.7 [C(4)], 169.2 (CO amide) and 173.2 (ester CO); *m/z* (APCI) 458 ($M + H^+$, 20%), 402 ($[M - C_4H_8 + H]^+$, 59), 358 ($[M - Boc + 2H]^+$, 98), 254 ($[M - Boc - PhCO + H]^+$, 100), 127 (T + H^+ and $[M - BzT - Boc + H]^+$, 9) and 105 ($PhCO^+$, 25); ν_{max}(KBr)/cm^{-1} 1750s, 1700s and 1660s (C=O); $[\alpha]_D^{21}$ −1.05 (*c* 1.05, MeOH).

N-tert-Butoxycarbonyl-*cis*-4-(N^2-isobutyrylguanin-7-yl)-L-proline methyl ester

A suspension of the toluene-*p*-sulfonate **4b** (0.40 g, 1.0 mmol), N^2-isobutyrylguanine (dried by azeotropic distillation with toluene) (0.55 g, 2.5 mmol), anhydrous K_2CO_3 (0.70 g, 5.0 mmol) and 18-crown-6 (100 mg) in DMF was stirred at 70 °C under argon for 48 h. The reaction mixture was worked up as usual to give a foam which was chromatographed on silica gel eluted first with ethyl acetate and then with 5% methanol in ethyl acetate. The less polar fractions (R_F 0.41, 10% methanol in ethyl acetate) were combined and evaporated to give the N^7-isomer (121 mg, 27%) as a foam which could be recrystallised from ethanol–water to give N-tert-*butoxycarbonyl*-cis-4-(N^2-*isobutyrylguanin*-7-*yl*)-L-*proline methyl ester* as shiny plates (monohydrate), mp >200 °C (Found: C, 51.3; H, 6.8; N, 17.9. $C_{20}H_{28}N_6O_6 \cdot H_2O$ requires C, 51.5; H, 6.5; N, 18.0%); δ_H(200 MHz; $CDCl_3$) 1.27 [6 H, d, *J* 7.0, $(CH_3)_2CH$], 1.45 (9 H, br s, Boc), 2.44 and 2.95 [2 H, 2 × m, $CH_2(3')$], 2.75 [1 H, m, $(CH_3)_2CH$], 3.70 (3 H, br s, Me ester), 3.90 and 4.14 [2 H, 2 × m, $CH_2(5')$], 4.44 [1 H, m, CH(2′)], 5.46 [1 H, br m, CH(4′)], 8.00 [1 H, s, CH(8)] and 9.10 (1 H, br s, amide NH); δ_C(50.28 MHz; $CDCl_3$) 18.9 [$(CH_3)_2CH$], 28.1 (Boc CH_3), 35.8 [$(CH_3)_2CH$], 37.2 and 36.5 [$CH_2(3')$ rotamers], 50.5 and 51.4 [$CH_2(5')$ rotamers], 52.2 (ester CH_3), 54.6 and 55.2 [CH(4′) rotamers], 57.3 and 57.5 [$CH_2(2')$ rotamers], 81.1 (Boc C), 111.9 [C(5)], 141.6 [CH(8)], 148.4 [C(2)/C(6)], 153.5 (Boc CO), 157.6 [C(6)/C(2)], 163.1 [C(4)], 172.6 (ester CO) and 180.6 (amide CO); *m/z* [CI(NH_3)] 449 ($M + H^+$, 6%), 349 ($[M - Boc + 2H]^+$, 5), 222 (IbuG + H^+, 100), 152 (G + H^+, 14), 127 ($[M - Boc - IbuG + H]^+$, 23) and 68 ($C_4H_5N + H^+$, 29); ν_{max}(KBr)/cm^{-1} 1758s and 1688s (C=O); λ_{max}(MeOH)/nm 264sh (ε/dm^3 mol^{-1} cm^{-1} 1.5×10^4); $[\alpha]_D^{25}$ +36.2 (*c* 0.16, MeOH).

N-tert-Butoxycarbonyl-*cis*-4-(*N*^{2}-isobutyrylguanin-9-yl)-L-proline methyl ester 5d

To a suspension of the alcohol **4a** (245 mg, 1.0 mmol), N^2-isobutyryl-O^6-[(4-nitrophenyl)ethyl]guanine (180 mg, 0.50 mmol) and triphenylphosphine (294 mg, 1.1 mmol) in dry 1,4-dioxane (10 ml) was added DEAD (182 μl, 1.1 mmol) dropwise at room temperature. The mixture was stirred at room temperature overnight. The clear solution was evaporated to dryness and the residue was purified by column chromatography (SiO_2; ethyl acetate) to give the *N-tert*-butoxycarbonyl-*cis*-4-(N^2-isobutyryl-O^6-nitrophenylethylguanin-9-yl)-L-proline methyl ester (R_F 0.39) which was contaminated with triphenylphosphine oxide. The impure material was dissolved in dry pyridine (5 ml), treated with DBU (150 μl, 1.0 mmol) and stirred at room temperature overnight. The reaction mixture was diluted with dichloromethane, and washed successively with 5% HCl and water. The organic phase was dried ($MgSO_4$) and evaporated to give the crude product. This was purified by column chromatography (SiO_2; ethyl acetate–methanol 10:1) to give the product as a foam (76 mg, 34% from **4a**). Crystallisation from ethanol–water gave N-tert-*butoxycarbonyl*-cis-4-(N^2-*isobutyrylguanin*-9-*yl*)-L-*proline methyl ester* **5d** as needles (dihydrate), mp 132–134 °C (Found: C, 49.6; H, 6.7; N, 17.4. $C_{20}H_{28}N_6O_6 \cdot 2H_2O$ requires C, 49.8; H, 6.9; N, 17.3%); δ_H(200 MHz; $CDCl_3$) 1.22 [6 H, d, *J* 6.9, $(CH_3)_2CH$], 1.39 (9 H, br s, Boc), 2.44 [1 H, br m, $CH_aH_b(3')$], 2.75–2.88 [2 H, m, $CH_aH_b(3')$ and $(CH_3)_2CH$], 3.69 (3 H, s, Me ester), 3.81 [1 H, dd, *J* 7.8 and 11.1, $CH_aH_b(5')$], 4.09 [1 H, m, $CH_aH_b(5')$], 4.37 [1 H, m, CH(2′)], 4.94 [1 H, br m, CH(4′)] and 7.77 [1 H, s, CH(8)]; δ_C(50.28 MHz; $CDCl_3$) 18.9 [$(CH_3)_2CH$], 28.1 (Boc CH_3), 35.6 and 34.8 [$CH_2(3')$ rotamers], 36.0 [$(CH_3)_2CH$], 50.0 and 50.3 [$CH_2(5')$ rotamers], 52.0 and 52.4 [CH(4′) rotamers], 52.5 (CH_3 ester), 57.4 and 57.5 [$CH_2(2')$ rotamers], 81.1 (Boc C), 121.2 [C(5)], 137.3 [CH(8)], 148.1 and 149.1 [C(2) and C(6)], 153.8 (Boc CO), 156.0 [C(4)], 172.1 (ester CO), 180.1 (amide CO); *m/z* [CI(NH_3)] 449 ($M + H^+$, 15%), 349 ($[M - Boc + 2H]^+$, 47), 222 (IbuG + H^+, 100), 152 (G + H^+, 28), 127 ($[M - IbuG - Boc + H]^+$, 18) and 68 ($C_4H_5N + H^+$, 55); ν_{max}(KBr)/cm^{-1} 1737s, 1708s and 1673s (C=O); λ_{max}(MeOH)/nm 260sh (ε/dm^3 mol^{-1} cm^{-1} 2.8×10^4) and 278 (2.0×10^4); $[\alpha]_D^{25}$ −2.43 (*c* 0.54, MeOH).

N-(2-Hydroxyethyl)glycine

To a swirled aqueous solution of ethanolamine (30.5 g, 0.500 mol in 25 ml) was gradually added solid chloroacetic acid (18.9 g, 0.200 mol). The reaction mixture was stored overnight at room temperature, then was evaporated to give a thick oil. Ethanol (95%; 2 l) was added with heating to dissolve the oil and the solution was kept in a refrigerator until precipitation was complete. The crude product was collected by filtration and was recrystallised from water–ethanol to give crystals (10.3 g, 43%), mp 186–188 °C [lit.,[27] 182–184 °C (decomp.)] (Found: C, 40.3; H, 8.0; N, 11.8. Calc. for $C_4H_9NO_3$: C, 40.3; H, 7.6; N, 11.8%); δ_H(200 MHz; D_2O) 2.99 (2 H, dd, *J* 5.3 and 5.0, CH_2CH_2NH), 3.43 (2 H, s, CH_2CO_2H) and 3.64 (2 H, dd, *J* 5.3 and 5.0, $HOCH_2CH_2$); δ_C(50.28 MHz; D_2O) 48.7 (CH_2CH_2NH and CH_2CO_2H), 56.4 ($HOCH_2CH_2$) and 171.4 (CO_2H); *m/z* [CI(NH_3)] 120 (100%, $M + H^+$).

***N*-*tert*-Butoxycarbonyl-*N*-(2-hydroxyethyl)glycine**

N-(2-Hydroxyethyl)glycine (1.19 g, 10.0 mmol) was added to stirred 10% aq. NaOH (6 ml). *tert*-Butyl alcohol (6 ml) was then added, followed by di-*tert*-butyl dicarbonate (2.82 g, 12.5 mmol) dropwise. The solution was stirred at room temperature overnight. The reaction mixture was evaporated to dryness and the residue was taken up in water and extracted several times with chloroform to remove any excess of di-*tert*-butyl dicarbonate. The solution was then acidified to pH 2 with 10% aq. $KHSO_4$ and was extracted with ethyl acetate (5 × 40 ml). The combined organic extracts were dried ($MgSO_4$) and evaporated to give the crude protected amino acid as a solid. Trituration with hexane and filtration gave the product as a solid (1.98 g, 90%). A portion of the sample was recrystallised from ethyl acetate–light petroleum (distillation range 40–60 °C) to give crystals of N-tert-*butoxycarbonyl*-N-(2-*hydroxyethyl*)*glycine*, mp 78–81 °C (Found: C, 49.2; H, 7.9; N, 6.3. $C_9H_{17}NO_5$ requires C, 49.3; H, 7.8; N, 6.4%); δ_H(200 MHz; $CDCl_3$) 1.43 and 1.50 (9 H, 2 × s, Boc rotamers), 3.47 [2 H, br m, CH_2CH_2N(Boc)] and 3.76 (2 H, br m, $HOCH_2CH_2$), 3.96 and 4.10 (2 H, 2 × s, CH_2CO_2H); δ_C(50.28 MHz; $CDCl_3$) 28.0 and 28.1 (Boc CH_3 rotamers), 50.4–51.0 (CH_2CO_2H rotamers), 51.5 and 52.0 [CH_2CH_2N(Boc) rotamers], 60.6 ($HOCH_2CH_2$), 81.3 (Boc C), 155.9 and 156.2 (Boc CO rotamers) and 174.6 and 174.9 (CO_2H rotamers); *m/z* [CI(NH_3)] 237 ($M + NH_4^+$, 18%), 220 ($M + H^+$, 100), 181 ([$M - C_4H_8 + NH_4$]$^+$, 70), 164 ([$M - C_4H_8 + H$]$^+$, 96), 120 ([M − Boc + 2 H]$^+$, 70), 88 ([M − Boc − $CH_2OH + H$]$^+$, 25), 74 ([M − Boc − $CH_2CH_2OH + H$]$^+$, 20) and 57 (12); ν_{max}(KBr)/cm^{-1} 3218 (O–H) and 1693s (C=O).

***N*-*tert*-Butoxycarbonyl-*N*-(2-hydroxyethyl)glycine methyl ester 7**

N-Boc-*N*-(2-hydroxyethyl)glycine (1.98 g, 9.04 mmol) was suspended in diethyl ether (~15 ml). Diazomethane (diluted with nitrogen)[33] was bubbled through the ice-cold solution until the yellow colour persisted. Excess of diazomethane was destroyed by addition of a few drops of glacial acetic acid. The resulting clear solution was evaporated under reduced pressure to give the methyl ester **7** as a clear oil (2.15 g, quant.). The crude product was used for the next step without further purification: δ_H(200 MHz; $CDCl_3$) 1.42 and 1.47 (9 H, 2 × s, Boc rotamers), 3.47 [2 H, br m, CH_2CH_2N(Boc)], 3.73 (2 H, br m, $HOCH_2CH_2$), 3.78 (3 H, s, Me ester) and 3.95 and 3.98 (2 H, 2 × s, CH_2CO_2H); δ_C(50.28 MHz; $CDCl_3$) 28.0 and 28.1 (Boc CH_3 rotamers), 50.3, 50.8, 51.9, 52.2 and 52.3 [CH_2CO_2, CH_2CH_2N(Boc) and ester CH_3 rotamers], 60.9 and 61.1 ($HOCH_2CH_2$ rotamers), 80.7 (Boc C rotamers), 155.7 and 155.9 (Boc CO rotamers), 172.5 and 172.9 (benzoyl CO).

***N*-*tert*-Butoxycarbonyl-*N*-[2-(adenin-9-yl)ethyl]glycine methyl ester 8**

DEAD (0.18 ml, 1.2 mmol) was added dropwise to a stirred cooled (ice-bath) THF suspension (15 ml) of adenine (0.171 g, 1.27 mmol), methyl ester **7** (0.235 g, 10.0 mmol) and triphenylphosphine (0.290 g, 1.20 mmol). The reaction mixture was warmed to room temperature and stirred overnight. The cloudy suspension was evaporated to dryness and the residue was chromatographed on silica gel with acetone–methanol (10 : 1) as eluent. The product was obtained as a solid (0.093 g, 27%) which was recrystallised from ethanol to give N-tert-*butoxycarbonyl*-N-[2-(*adenin*-9-*yl*)*ethyl*]*glycine methyl ester* **8** as a crystalline solid, mp 173–175 °C (Found: C, 51.5; H, 5.9; N, 23.9. $C_{15}H_{22}N_6O_4$ requires C, 51.4; H, 6.3; N, 24.0%); δ_H(200 MHz; $CDCl_3$) 1.10 and 1.30 (9 H, 2 × s, Boc rotamers), 3.55–3.90 [7 H, br m, CH_2CH_2N(Boc), Me ester and CH_2CO_2H], 4.33 (2 H, m, ACH_2CH_2), 6.40 (2 H, 2 × s, NH_2), 7.82 and 8.00 [1 H, 2 × s, CH(8)] and 8.30 [1 H, s, CH(2)]; δ_C(50.28 MHz; $CDCl_3$) 27.7 and 27.9 (Boc CH_3 rotamers), 42.3 and 42.7 (CH_2CO_2 rotamers), 48.6 and 48.7 [CH_2CH_2N(Boc) rotamers], 49.8 and 50.6 (ACH_2CH_2), 52.1 and 52.2 (ester CH_3 rotamers), 81.1 (Boc C), 119.7 [C(5)], 141.3 and 141.4 [CH(8)], 150.3 [C(4)], 153.1 [C(2)], 155.3 and 155.9 [C(6) and Boc CO], 170.6 and 171.0 (ester CO rotamers); *m/z* [CI(NH_3)] 351 ($M + H^+$, 100%), 251 ([M − Boc + 2 H]$^+$, 15), 136 ($A + H^+$, 16) and 115 ([M − Boc − A]$^+$, 8); ν_{max}(KBr)/cm^{-1} 1754s (C=O ester) and 1696s (C=O urethane).

***N*-*tert*-Butoxycarbonyl-*N*-[2-(*N*^{6},*N*^{6}-dibenzoyladenin-9-yl)-ethyl]glycine methyl ester and *N*-*tert*-butoxycarbonyl-*N*-[2-(*N*^{6}-benzoyladenin-9-yl)ethyl]glycine 10**

The adenine derivative **8** (0.315 g, 0.898 mmol) was dissolved in dry pyridine (8 ml) and the solution was cooled in an ice-bath. Benzoyl chloride (520 μl, 4.50 mmol) was then added portionwise with stirring of the mixture, which was then stirred at 4 °C overnight and diluted with dichloromethane (50 ml). The solution was washed successively with 10% aq. HCl, saturated aq. $NaHCO_3$ and water. Evaporation followed by column chromatography of the residue (SiO_2; EtOAc) gave the dibenzoylated product as an oil (0.533 g, quant.); δ_H(200 MHz; $CDCl_3$) 1.17 and 1.34 (9 H, 2 × s, Boc rotamers), 3.67 [5 H, m, Me ester and CH_2CH_2N(Boc)], 3.72 and 3.86 (2 H, 2 × s, CH_2CO_2Me rotamers), 4.31–4.44 (2 H, m, ACH_2CH_2), 7.29 (4 H, t, *J* 7.1, benzoyl *m*-CH), 7.42 (2 H, t, *J* 7.1, benzoyl *p*-CH), 7.83 (4 H, d, *J* 7.4, benzoyl *o*-CH), 8.23 and 8.32 [1 H, 2 × s, CH(8) rotamers] and 8.61 and 8.63 [1 H, 2 × s, CH(2) rotamers]; δ_C(50.28 MHz; $CDCl_3$) 27.8 and 28.0 (Boc CH_3 rotamers), 42.7 and 42.9 (CH_2CO_2 rotamers), 48.3 [CH_2CH_2N(Boc)], 49.5 and 50.6 (ACH_2CH_2), 52.2 (ester CH_3), 81.1 and 81.2 (Boc C rotamers), 127.5 [C(5)], 128.3–134.4 (benzoyl CH), 146.2 [CH(8)], 151.8 [C(4)], 153.7 [C(2)], 155.2 (Boc CO), 169.0 [C(6)], 170.7 and 171.0 (benzoyl CO rotamers) and 172.6 (ester CO); *m/z* [CI(NH_3)] 581 ($M + Na^+$, 6%), 559 ($M + H^+$, 100), 503 ([$M - C_4H_8 + H$]$^+$, 48), 459 ([M − Boc + 2 H]$^+$, 7), 455 ([M − Bz + 2 H]$^+$, 17), 437 (46), 399 ([$M - C_4H_8$ − Bz + 2 H]$^+$, 13), 355 ([M − Bz − Boc + 3 H]$^+$, 30) and 240 (BzA + H$^+$, 11).

The dibenzoyladenine methyl ester (0.211 g, 0.380 mmol) was dissolved in acetone (2 ml), aq. NaOH (2.0 M; 2 ml) was added and the reaction mixture was stirred for 3 h at room temperature. The solvents were evaporated off, the residue was taken up in water (10 ml) and the pH was adjusted to 2 with aq. $KHSO_4$. The product was obtained as a solid (0.100 g, 60%) after extraction into ethyl acetate and trituration with diethyl ether. Recrystallisation from ethanol–water gave *compound* **10** as a crystalline solid, mp 185–188 °C (decomp.) (Found: C, 56.3; H, 5.3; N, 19.0. $C_{21}H_{24}N_6O_5 \cdot 0.5H_2O$ requires C, 56.1; H, 5.6; N, 18.7%); δ_H(200 MHz; NaOD/D_2O) 0.62 and 0.90 (9 H, 2 × br s, Boc rotamers), 3.46 [2 H, br m, CH_2CH_2N(Boc)], 3.42 and 3.60 (2 H, 2 × s, CH_2CO_2H rotamers), 4.16 (2 H, br m, $BzACH_2CH_2$), 7.23–7.26 (3 H, br m, benzoyl *m*- and *p*-CH), 7.69–7.72 (2 H, br m, benzoyl *o*-CH), 7.91 and 7.94 [CH(8) rotamers] and 8.30 [CH(2)]; *m/z* (negative ion ES-MS) 439 ([M − H]$^-$, 100%); ν_{max}(KBr)/cm^{-1} 3327 (O–H) and 1693br (C=O).

***N*-*tert*-Butoxycarbonyl-*N*-[2-(*N*^{3}-benzoylthymin-1-yl)ethyl]-glycine methyl ester 9**

DEAD (1.80 ml, 11.0 mmol) was added dropwise to a stirred cooled (ice-bath) suspension of *N*^{3}-benzoylthymine (1.85 g, 8.0 mmol), the alcohol **7** (2.1 g, 9.0 mmol) and triphenylphosphine (2.90 g, 11.0 mmol) in THF (25 ml). The reaction mixture was warmed to room temperature and was stirred overnight. The light yellow solution was evaporated to dryness and the residue was purified by column chromatography (SiO_2; dichloromethane–acetone 15 : 1). The product **9** (R_F 0.33) was obtained as a solid (4.67 g) which may be used for the next step without further purification. Recrystallisation from ethanol gave N-tert-*butoxycarbonyl*-N-[2-(N^3-*benzoylthymin*-1-*yl*)*ethyl*]*glycine methyl ester* **9** as crystals (2.26 g, 56%), mp 177–179 °C (Found: C, 59.2; H, 6.0; N, 9.3. $C_{22}H_{27}N_3O_7$ requires C, 59.3; H, 6.1; N, 9.4%); δ_H(200 MHz; $CDCl_3$) 1.43 and 1.48 (9 H, 2 × s, Boc rotamers), 1.93 and 1.98 (3 H, 2 × s, thymine CH_3 rotamers),

3.60 [2 H, br m, CH_2CH_2N(Boc)], 3.78 (3 H, s, Me ester), 3.85–4.05 [4 H, br m, CH_2CH_2N(Boc) and CH_2CO_2Me], 7.28 [1 H, s, CH(6)], 7.35–7.70 (3 H, br m, benzoyl *m*- and *p*-CH) and 7.85–7.95 and 8.05–8.15 (2 H, br m, benzoyl *o*-CH rotamers); δ_C(50.28 MHz; $CDCl_3$) 12.2 (thymine CH_3), 28.0 and 28.1 (Boc CH_3 rotamers), 47.1 and 47.3 [CH_2CH_2N(Boc) rotamers], 47.6 and 48.3 (BzTCH_2CH_2 rotamers), 49.5 and 50.9 (CH_2CO_2Me rotamers), 52.2 (ester CH_3), 81.0 and 81.5 (Boc C), 109.7 [C(5)], 129.2 and 129.4 (benzoyl *m*-CH), 130.6 and 131.0 benzoyl *o*-CH), 131.7 and 131.9 (benzoyl C rotamers), 135.1 and 135.3 (benzoyl *p*-CH rotamers), 141.3 and 141.9 [CH(6)], 150.3 [C(2)], 155.4 (Boc CO), 163.9 [C(4)], 169.9 (benzoyl CO) and 171.0 and 171.2 (ester CO); *m/z* [CI(NH_3)] 446 ($M + H^+$, 25%), 390 ([$M - C_4H_8 + H]^+$, 97), 346 ([M − Boc + 2 H]$^+$, 26), 286 ([$M - C_4H_8$ − PhCO + 2 H]$^+$, 22), 242 ([M − Boc − PhCO + 3 H]$^+$, 68), 115 ([M − BzT − Boc]$^+$, 52), 105 ($PhCO^+$, 100) and 102 (54); ν_{max}(KBr)/cm^{-1} 1751s (C=O), 1696s (C=O) and 1639s (C=O).

***N*-*tert*-Butoxycarbonyl-*N*-[2-(thymin-1-yl)ethyl]glycine 11**

To a solution of the methyl ester **9** (50 mg, 0.11 mmol) in acetone (2 ml) was added aq. NaOH (0.2 M; 2 ml). The solution was left overnight in a refrigerator, then was evaporated to dryness. The residue was taken up in water (10 ml) and acidified to pH 2 with 10% aq. $KHSO_4$. The acid was extracted with ethyl acetate. Evaporation to almost dryness followed by addition of diethyl ether gave a solid, which was collected by suction filtration and washed several times with diethyl ether. Recrystallisation from ethanol–water gave N-tert-*butoxycarbonyl*-N-[2-(*thymin*-1-*yl*)*ethyl*]*glycine* **11** as a solid (30 mg, 83%), mp 202–205 °C (decomp.) (Found: C, 51.1; H, 6.4; N, 12.8. $C_{14}H_{21}N_3O_6$ requires C, 51.4; H, 6.5; N, 12.8%); δ_H(200 MHz; D_2O/NaOD) 1.05 and 1.10 (9 H, 2 × s, Boc rotamers), 1.55 (3 H, s, thymine CH_3), 3.32 [2 H, br m, CH_2CH_2N(Boc)], 3.40–3.45 and 3.50–3.60 [4 H, br m, CH_2CH_2N(Boc) and CH_2CO_2Me] and 6.95 [1 H, s, CH(6)]; *m/z* (negative ion ES-MS) 326 ([$M - H]^-$, 100%); ν_{max}(KBr)/cm^{-1} 1753s, 1732s and 1675s (C=O).

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