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Novel Synthetic Routes to PNA Monomers and PNA-DNA Linker Molecules

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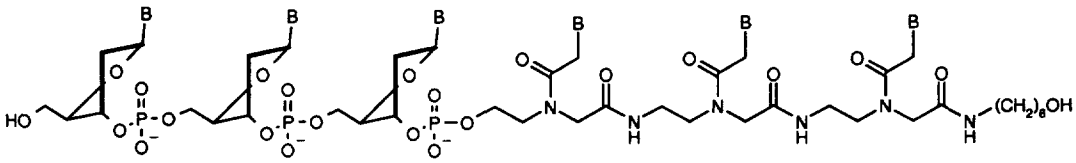
Abstract: Novel methods for the preparation of monomethoxytrityl (Mmt) protected aminoethylglycine building blocks and dimethoxytrityl (Dmt) protected hydroxyethylglycine derivatives useful for the synthesis of polyamide nucleic acids (PNAs) and PNA/DNA chimeras are described. The protecting group strategy employed for PNA monomer synthesis produces easily isolable intermediates, minimizes chromatographic purification, and is suitable for large-scale monomer synthesis.

Interest in Polyamide (or Peptide) Nucleic Acids (PNAs) has grown exponentially since they were first reported by Nielsen *et al.*[1] in 1991. PNAs are oligonucleotide analogues composed of oligomers of nucleobase-derivatized *N*-(2-aminoethyl)glycine, which recognize and bind strongly to specific DNA or RNA sequences. PNA oligomers have a number of properties which make them potentially extremely useful as antisense therapeutics and as diagnostic tools.[2]

The first synthetic strategy reported for PNA synthesis was Merrifield solid-phase synthesis using a Boc/benzyloxycarbonyl protecting group strategy.[3,4,5] The repeated treatment with TFA required for Boc-deprotection, and the harsh HF or TFMSA treatment required for cleavage from the resin and deprotection render this strategy incompatible with the synthesis of many types of modified PNAs. The synthesis of PNAs using Fmoc/ benzyloxycarbonyl[6] and Fmoc/ Mmt[7] protected PNA monomers has also been reported. However, all of these methods have serious drawbacks in terms of monomer solubility and preparation, harsh reaction conditions, and side-reactions either during monomer synthesis and/or PNA oligomer synthesis. More recently we described a novel protecting-group strategy for the synthesis of PNAs utilising the Mmt group as an N-terminal temporary protecting group and base-labile acyl protecting groups for the exocyclic amino functions of the nucleobases[8]. The Mmt group can be removed under mild acidic conditions (3% trichloroacetic acid), and the nucleobase protecting groups are removed at the end of synthesis using conc. aqueous ammonia. This highly efficient and flexible strategy allows the automated synthesis of PNAs[8] using equipment and conditions very similar to those employed in standard DNA synthesis. It also enables the preparation of chimeric DNA-PNA oligomers[9,10] with interesting properties (Figure 1). The linkers between the PNA part and the DNA part of the chimeric oligomers are Dmt-*O*-protected nucleobase-derivatized hydroxyethylglycine derivatives.



B=adenine, guanine, cytosine or thymine.

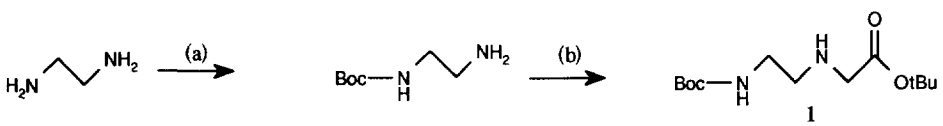
Figure 1: DNA-PNA chimeric oligomer with a hydroxyethylglycine-based linker.

Here we report a new synthetic route to all four Mmt-protected PNA monomers, which is more efficient than the previously reported procedure[8]. The main advantage of the new procedure is the introduction of the very acid labile Mmt protecting group in the final step, which simplifies the handling of the intermediates and minimises the use of chromatographic purification steps. It is thus especially suited to the large scale preparation of PNA building blocks. We also report for the first time experimental procedures for the synthesis of suitably protected hydroxyethylglycine PNA-DNA linker derivatives of adenine, guanine, thymine and cytosine by a novel route.

RESULTS AND DISCUSSION

Mmt-Protected PNA Monomer Synthesis

The key intermediate in our new synthetic route to PNA building blocks is *N*-(*tert*-butyloxycarbonyl-aminoethyl)glycine *tert*-butyl ester **1**. A very simple and effective method to prepare this compound on a large scale is shown in Scheme 1. Thus diaminoethane was mono-Boc-protected using *tert*-butyl dicarbonate in dichloromethane[11], to give Boc-diaminoethane. Following removal of excess diaminoethane by aqueous extraction, the crude intermediate was directly alkylated using *tert*-butyl chloroacetate and triethylamine in dichloromethane. Removal of polar impurities by passage through a short silica gel column gave **1** in excellent purity from readily available and inexpensive starting materials.



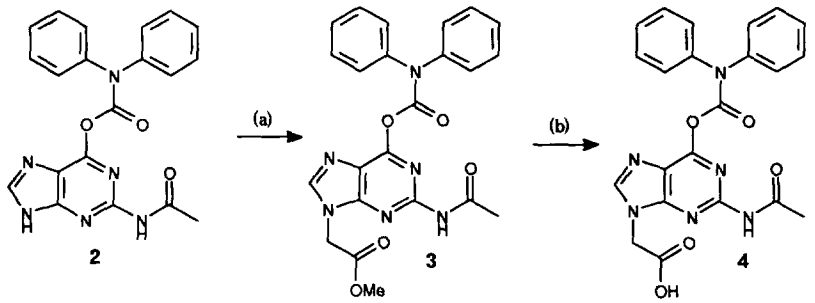
Reagents and Conditions: (a) *tert*-Butyl dicarbonate, CH_2Cl_2; (b) *tert*-Butyl chloroacetate, KI, NEt_3, CH_2Cl_2.

Scheme 1: Synthesis of of N-(*tert*-butyloxycarbonylaminoethyl)glycine *tert*-butyl ester.

N1-carboxymethylthymine was synthesized according to the procedure of Kosynkina *et al.*[12]. *N6*-(4-methoxybenzoyl)-9-(carboxymethyl)adenine and *N2*-(isobutyryl)-9-(carboxymethyl)guanine were synthesized as described previously[8]. 1-(Carboxymethyl)-*N4*-(4-methoxybenzoyl)cytosine was synthesized analogously to 1-(carboxymethyl)-*N4*-(4-*tert*-butylbenzoyl)cytosine described previously[8], but using anisoyl chloride instead of 4-*tert*-butylbenzoyl chloride.

Direct alkylation of *N2*-acyl-guanine results in poor regioselectivity at the N7 and N9 positions. Therefore, *N2*-acetyl-*O4*-diphenylcarbamoyl-9-carboxymethylguanine **4** was synthesized by alkylation of *N2*-

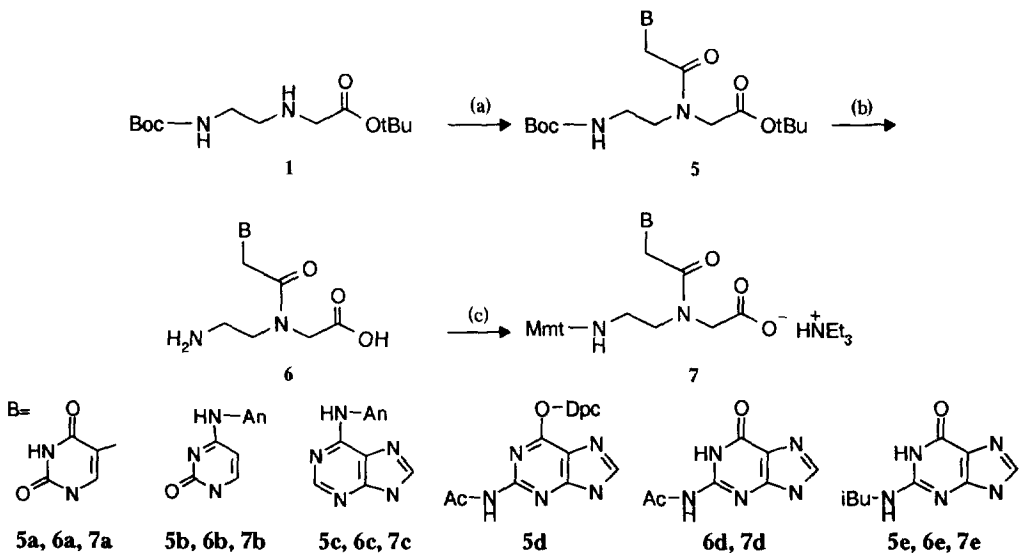
acetyl-*O4*-diphenylcarbamoylguanine[13] **2** with methyl bromoacetate in DMF in the presence of diisopropylethylamine to give **3**, followed by saponification of the methyl ester using sodium hydroxide in a dioxan/ water/ methanol mixture (Scheme 2). The alkylation of the doubly-protected guanine derivative was highly selective for the N9 position of the guanine, and no chromatographic purification of **3** or **4** was necessary.



Reagents and Conditions: (a) Methyl bromoacetate/ DMF/ DIEA. (b) NaOH/ MeOH/ dioxan/ H_2O.

Scheme 2: Synthesis of *N2*-acetyl-*O4*-diphenylcarbamoyl-9-carboxymethylguanine.

The carboxymethylated nucleobases were coupled to backbone synthon **1** using the peptide coupling agent O-[(cyano(ethoxycarbonyl)methylene)amino]-1,1,3,3-tetramethyluronium tetrafluoroborate (TOTU)[14] in DMF in the presence of a tertiary amine, to give **5a-e** in 64-91% yield (Scheme 3).



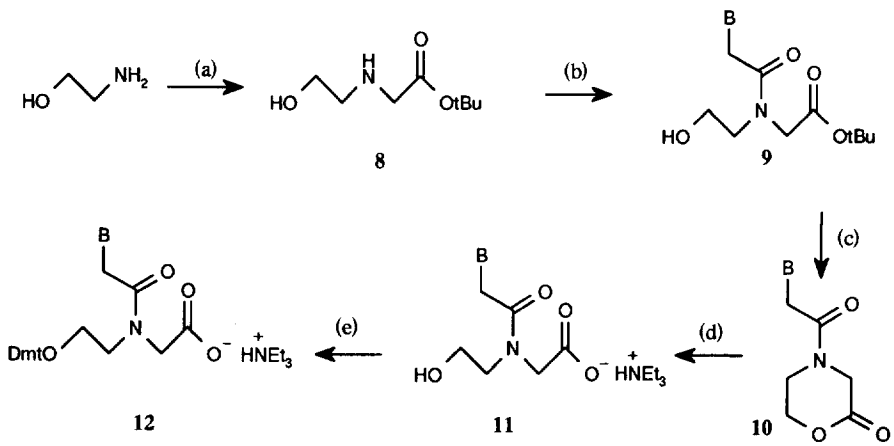
Reagents and Conditions: (a) B-CH_2-COOH + TOTU, NEt_3, DMF; or propylphosphonic anhydride, EtOAc, NEt_3 (b) TFA, CH_2Cl_2; (c) Mmt-Cl, NEt_3, DMF.

Scheme 3: Synthesis of Mmt-protected PNA monomers.

For the large-scale synthesis of **5a** and **5b** the inexpensive peptide coupling reagent propylphosphonic anhydride[15] was used as an alternative to TOTU. All compounds were very easily isolated from the reaction mixture by precipitation during workup and no chromatographic purification was necessary. This is a major advantage over our previously described procedure[8], which utilized Mmt-protected aminoethylglycine methyl ester intermediates, especially for large scale monomer preparation. In the next step the Boc and *tert*-butyl groups were removed simultaneously by treatment with trifluoroacetic acid (TFA) in dichloromethane. During this step the diphenylcarbamoyl protecting group of **5d** was also removed, and the *N2*-acetyl protecting group remained intact as desired. Simple trituration with diethyl ether gave the deprotected aminoethylglycine derivatives **6** in almost quantitative yield. Reaction with (4-methoxyphenyl)-diphenylmethyl chloride (Mmt-Cl) in DMF / triethylamine gave, after trituration with diethyl ether, the fully protected PNA monomers **7** in good yield, and without a single chromatographic purification step. The monomers can then be used in PNA synthesis as described previously[8].

PNA-DNA Linker Synthesis

We and others recently described the synthesis and properties of chimeric DNA-PNA oligomers using the Mmt synthesis strategy[9,10]. The linkers between the PNA part and the DNA part of the chimeric oligomers are Dmt-protected nucleobase derivatized hydroxyethylglycine. The synthesis of the thymine hydroxyethylglycine derivative has been reported previously by Petersen *et al.* using an eight step low yielding synthetic route[16]. We employed a completely different, and simpler, method for the synthesis of the linker molecules, making use of a similar synthetic strategy to that described above for the aminoethylglycine PNA monomers.



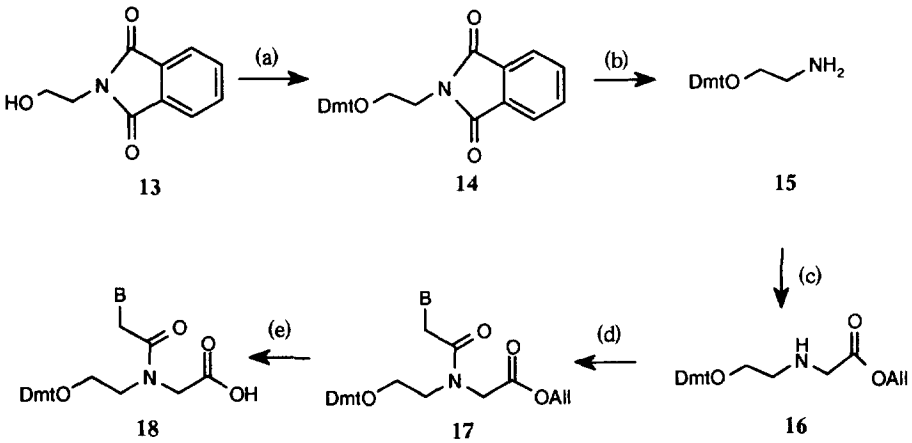
9a, 10a, 11a, 12a: B=thymine; **9b, 10b, 11b, 12b:** B=*N4*-anisoyl cytosine; **9c, 10c, 11c, 12c:** B=*N6*-anisoyl adenine.

Reagents and Conditions: (a) *tert*-Butyl bromoacetate, DMF; DIEA. (b) B-CH_2COOH, TOTU, DMF, NEt_3. (c) TFA:H_2O/ 95:5, CH_2Cl_2. (d) NEt_3, H_2O, dioxan. (e) Dmt-Cl, pyridine or DMF, NEt_3.

Scheme 4: Synthesis of Dmt-protected PNA-DNA linkers.

Thus 2-aminoethanol was monoalkylated using *tert*-butyl bromoacetate in DMF/DIEA to give *N*-(2-hydroxyethyl)glycine *tert*-butyl ester **8**. The carboxymethylated nucleobases were coupled to **8** using TOTU in DMF / NEt_3, to give **9** (Scheme 4). The *tert*-butyl groups were removed by treatment with TFA in dichloromethane. During this acid treament various amounts of lactones **10** were formed. Removal of the TFA *in vacuo* and treatment with triethylamine in dioxan/water converted the lactones to the desired hydroxyacid triethylammonium salts **11**. These were then treated with Dmt-Cl in pyridine, or DMF / NEt_3, to give the PNA-DNA linkers **12** which were then used in the synthesis of DNA-PNA chimeras as described previously[9].

For the synthesis of the Dmt-protected guanine PNA-DNA linker **18** we used as an alternative strategy the procedure outlined in Scheme 5. The hydroxyl function of commercially available *N*-hydroxyethylphthalimide **13** was protected by treatment with Dmt-Cl in pyridine / NEt_3 to give **14** in 70% yield. Removal of the phthalimido protection with hydrazine hydrate gave **15** which then was alkylated with allyl chloroacetate to give **16**. *N2*-isobutyryl-9-carboxymethylguanine[8] was coupled to this backbone synthon using diisopropylcarbodiimide/HOObt. After chromatographic purification of **17** the allyl group was removed by treatment with tetrakis(triphenylphosphine)palladium(0) and triphenylphosphine to give the guanine PNA-DNA linker **18**.



Reagents and Conditions: (a). Dmt-Cl, pyridine, NEt_3; (b) Hydrazine hydrate, MeOH; (c) Allyl chloroacetate, DMF, DIEA; (d) B-CH_2COOH, DIPCD, HOObt, DMF, NEM (B=*N2*-(isobutyryl)-guanine); (e).$(PPh_3)_4Pd(0)$, PPh_3, $Et_2NH_2CO_3$, CH_2Cl_2.

Scheme 5: Synthesis of Dmt-protected guanine PNA-DNA linker.

In conclusion, we have described a new synthetic route to Mmt protected PNA monomers, and have demonstrated that this route can be used for the large-scale synthesis of PNA monomers. The unprotected intermediate **6** can in principle also be used to prepare a variety of other PNA monomers by introduction of other suitable N-protecting groups. We have also described new synthetic routes to the thymine, cytosine, guanine and adenine hydroxyethylglycine PNA-DNA linkers used for the solid-phase synthesis of PNA-DNA chimeric oligomers.

EXPERIMENTAL

General

The following abbreviations are employed: (4-methoxyphenyl)diphenylmethyl (Mmt); *N,N'*-diisopropylcarbodiimide (DIPCD); di-(4-methoxyphenyl)phenylmethyl (Dmt); *tert*-butyloxycarbonyl (Boc); 4-methoxybenzoyl (An); *N,N*-diphenylcarbamoyl (Dpc); *O*-[(cyano(ethoxycarbonyl)methylene)amino]-1,1,3,3-tetramethyluronium-tetrafluoroborate (TOTU); trifluoromethanesulfonic acid (TFMSA); trifluoroacetic acid (TFA); 4-ethylmorpholine (NEM); *N,N*-dimethylformamide (DMF); *N,N* -diisopropylethylamine (DIEA); 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine (HOObt).

Reagents were obtained from Sigma-Aldrich-Fluka (Neu-Ulm, Germany). Flash chromatography was performed using Merck silica gel 60 (230-400mesh ASTM). TLC was carried out on Merck DC Kieselgel 60 F-254 glass plates. ^{1}H NMR spectra were recorded in the solvents indicated at 270 MHz. Chemical shifts (δ) are reported in parts per million down field relative to the internal TMS standard. Mass spectra were recorded using either Fast Atom Bombardment (FAB), Electrospray (ES) or Direct Chemical (DCI) ionisation.

N-(*tert*-Butyloxycarbonylaminoethyl)glycine *tert*-butyl ester (1)

1,2-diaminoethane (4.80l; 72.0mol) was dissolved in dichloromethane (18l). With stirring and cooling a solution of di-*tert*-butyl dicarbonate (1.97kg; 9.03mol) in dichloromethane (4.5l) was added dropwise over 2h. The mixture was stirred for a further 20 h. Water (9l) was added and the resulting mixture was agitated vigorously. Following phase separation the aqueous phase was extracted twice with dichloromethane (4.5l). The combined organic phases were washed with half-saturated $NaCl_{(aq)}$, dried (Na_2SO_4) and filtered. The organic phase was concentrated to a volume of approx. 9 l and NEt_3 (1.24l; 8.9mol) and potassium iodide (5.00g; 30mmol) were added. A solution of *tert*-butyl chloroacetate (1.24l; 8.7mol) in dichloromethane (4.5l) was then added dropwise with stirring and the mixture was stirred at 40°C for 50h. Water (5l) was added and the mixture was agitated vigorously. The organic phase was separated, dried (Na_2SO_4), filtered and the solvent was removed *in vacuo*. The resulting crude product was chromatographically purified on silica gel eluting with EtOAc/methanol 95:5. Yield:1.03kg (41% from di-*tert*-butyl dicarbonate). R_f =0.46 (EtOAc); MS(ES$^+$) 275.2 $(M+H)^+$; ^{1}H NMR ($CDCl_3$) δ 5.05(1H, br s, NH); 3.28(2H, s, CH_2CO); 3.20 (2H, dt, CH2); 2.74(2H, t, CH2), 1.46/1.44(18H, 2xs, Boc + OtBu).

N2-Acetyl-*O4*-diphenylcarbamoyl-9-methoxycarbonylmethyl guanine(3)

N2-acetyl-*O4*-diphenylcarbamoylguanine **2** (15.52g; 40mmol) (prepared as described[13]) and DIEA (13.60ml; 79mmol) were added to anhydrous DMF (200ml) and the suspension was heated briefly until a clear solution formed. Methyl bromoacetate (4.04ml; 44mmol) was added and the solution was stirred overnight. The solvent was removed *in vacuo* and the residue was dissolved in methanol (200ml). This solution was added slowly to water (600ml) with vigorous stirring, whereupon the product precipitated. The resulting crude product was filtered off, washed with water and redissolved in methanol The solution was concentrated to dryness, and the residue was triturated with EtOAc. The resulting product was filtered off, washed with EtOAc and ether and dried *in vacuo*. Further product was obtained from the mother liquor after concentration and trituration with EtOAc. Yield: 13.20g (71%). R_f= 0.79 (2-butanone/pyridine/H_2O/AcOH 70:15:15:2); MS(ES$^+$) 461.3$(M+H)^+$; ^{1}H NMR (d6-DMSO) δ 10.72 (1H, s, NH); 8.42 (1H, 1, H-8); 7.85-7.25 (10H, m, Ar); 5.17 (2H, s, CH_2); 3.72(3H, s, OCH_3); 2.18(3H,s, CH_3-CO).

***N2*-Acetyl-*O4*-diphenylcarbamoyl-9-carboxymethylguanine (4)**

Compound **3** (7.54g;16.4mmol) was dissolved in a mixture of methanol (20ml), dioxan (80ml) and water (40ml) and treated with 1N NaOH (18ml) at pH 13. The solution was acidified to pH 6 by the addition of 1M $HCl_{(aq)}$ and the solvent was evaporated *in vacuo* to a volume of approximately 50 ml. Water (400ml) was added to the solution and the pH was adjusted to 3 by the addition of 1M $HCl_{(aq)}$, whereupon the product precipitated. The precipitate was filtered off, washed with water and dried *in vacuo*. Yield: 5.92g (81%). R_f =0.48 (2-butanone/pyridine/H_2O/AcOH 70:15:15:2); MS(ES^+) 447.2 $(M+H)^+$; ^1H NMR (d6-DMSO) δ 10.72 (1H, s, NH); 8.42 (1H, s, H-8); 7.85-7.25 (10H, m, Ar); 5.02 (2H, s, CH_2); 2.18(3H, s, CH_3-CO).

***N*-(*tert*-Butyloxycarbonylaminoethyl)-*N*-((1-thyminyl)acetyl)glycine *tert*-butyl ester (5a)**

Compound **1** (202.5g; 0.738mol) was dissolved in DMF (1.6l) and *N1*-carboxymethylthymine (135.0g; 0.733mol), propylphosphonic anhydride (50% solution in EtOAc) (770.6ml; 0.733mol) and DIEA (251.0ml; 1.46mol) were added. The mixture was stirred at room temperature for 5h and then poured into a stirred mixture of ice-water (9l) and saturated $NaHCO_3$ solution (1.2l). The product precipitated and was collected after standing overnight by filtration. The crude product was dissolved in hot EtOAc (2l) and precipitated by the addition of methyl-*tert*.butyl ether (2l) and cooling to 20°C. The product was filtered off and dried *in vacuo*. Yield: 279.0g (86%). R_f =0.55 (CH_2Cl_2:MeOH:NEt_3/ 100:10:1); MS(ES^+) 441.3 $(M+H)^+$; ^1H NMR (d6-DMSO) δ 11.25 (1H, 2xs(rotamers), H-3); 7.31/7.25 (1H, 2xd(rotamers), H-6); 6.95-6.65(1H, m, NH); 4.64/4.46 (2H, 2xs(rotamers), CH_2); 4.17/3.92(2H, 2xs(rotamers), CH_2); 3.45-2.95(m, CH_2+H_2O); 1.75(3H, br s(rotamers), T-CH_3); 1.45/1.40 (18H, 2xs(rotamers), Boc + OtBu)

***N*-(2-Aminoethyl)-*N*-((1-thyminyl)acetyl)glycine (6a)**

Compound **5a** (11.0g; 25 mmol) was dissolved in a mixture of dichloromethane (75ml) and trifluoroacetic acid (50ml). A clear solution was formed which was stirred at room temperature for 2h. The reaction mixture was dried *in vacuo* and residual trifluoroacetic acid was removed by co-evaporation twice with toluene. The residue was triturated with diethyl ether and filtered. The precipitated crude product was used directly for the next step in the synthesis. Yield: 13.3g of an amorphous solid. R_f =0.14 (2-butanone: H_2O:pyridine:AcOH /70:15:15:2); MS(ES^+) 285.2 $(M+H)^+$; ^1H NMR (d6-DMSO) δ 11.35/11.28 (1H, 2xs(rotamers), H-3); 7.39/7.27 (1H, 2xd(rotamers), H-6); 4.68/4.50 (2H, 2xs(rotamers), CH_2); 4.25/4.02(2H, 2xs(rotamers), CH_2); 3.65-2.95(4H, m, CH_2); 1.75(3H, br s(rotamers), T-CH_3).

***N*-(2-(4-Methoxyphenyl)diphenylmethylamino)ethyl-*N*-((1-thyminyl)acetyl) glycine (7a)**

Crude **6a** (13.3g) was dissolved in DMF (250ml) and NEt_3 (18.8ml; 135mmol) was added, followed by Mmt-Cl (11.3g; 45mmol) in 5 portions. The mixture was stirred overnight, after which the solvent was evaporated *in vacuo*. The residue was taken up in dichloromethane (300ml), and washed three times with water (30ml). The organic phase was dried (Na_2SO_4), filtered and evaporated *in vacuo*. The residue was triturated with diethyl ether. The precipitated product was filtered off, washed with ether, and dried *in vacuo*. Yield: 14.1g (84% from **5a**). R_f =0.25 (CH_2Cl_2:MeOH:NEt_3/100:10:1); MS(FAB, MeOH/NBA/LiCl) 569.5 $(M+Li)^+$; ^1H NMR (d6-DMSO) δ 11.27/11.22 (1H, 2xs,(rotamers) H-3); 7.45-6.80 (15H, m, Mmt+H-6); 4.80/4.45 (2H, 2xs(rotamers), CH_2); 3.90/3.82(2H, 2xs(rotamers), CH_2); 3.72(3H, s, Mmt-OCH_3); 3.50-3.0(m, CH_2+H_2O); 2.72(6H, q, NEt_3); 2.22-2.02(2H, m(rotamers), CH_2); 1.75(3H, br s(rotamers), T-CH_3); 1.05(9H, t, NEt_3).

***N*-(*tert*-Butyloxycarbonylaminoethyl)-*N*-(1-(*N4*-(4-methoxybenzoyl)cytosyl)acetyl)glycine *tert*-butyl ester (5b)**

Compound **1** (127g; 0.418mol), and *N4*-(4-methoxybenzoyl)-*N1*-carboxymethylcytosine (127g; 0.418 mol) were suspended in EtOAc (1.5l) and NEt_3 (115ml; 0.836mol) was added. A 50% solution of propylphosphonic anhydride in EtOAc (400ml; approx. 0.628 mol) was added within 2 min. The mixture was adjusted to pH 8 by the addition of NEt_3 (65ml; 0.469 mol) and stirred for a further 2.5h. The precipitate was filtered off and washed with EtOAc (500ml) and water (1l). Further product precipitated from the mother liquor. The combined precipitates were stirred with water (500ml), then filtered off, washed with water (500ml) and dried *in vacuo*. Yield: 196.6g (84%). R_f =0.53 (CH_2Cl_2:MeOH: NEt_3/100:10:1); MS(ES^+) 560.3 $(M+H)^+$; ^1H NMR (d6-DMSO) δ 11.02 (1H, s, NH); 8.02 (2H, d, An); 7.98/7.92 (1H, 2xd(rotamers), H-6); 7.32 (1H, 2xd(rotamers), H-5); 7.05 (2H, d, An); 6.98-6.70 (1H, m, NH); 4.85/4.67 (2H, 2xs(rotamers), CH_2); 4.23/3.97 (2H, 2xs(rotamers), CH_2); 3.83(3H, s, An-OCH_3); 3.50-2.95(m, CH_2+H_2O); 1.48/1.40 (18H, 2xs(rotamers), Boc + OtBu).

***N*-(2-Aminoethyl)-*N*-(1-(*N4*-(4-methoxybenzoyl)cytosyl)acetyl)glycine (6b)**

Compound **5b** (11.20g; 20mmol) was dissolved in a mixture of dichloromethane (75ml) and trifluoroacetic acid (50ml). A clear solution was formed which was stirred at room temperature for 2h. The reaction mixture was then concentrated and the residue was triturated with diethyl ether. The precipitated crude product was used directly for the next step of the synthesis. Yield: 13.08g of an amorphous solid. R_f =0.34 (2-butanone:H_2O:pyridine:AcOH /70:15:15:2); MS(ES^+) 404.3 $(M+H)^+$; ^1H NMR (d6-DMSO) δ 8.02 (2H, d, An); 7.94 (1H, d, H-6); 7.35/7.31 (1H, 2xd(rotamers), H-5); 7.04 (2H, d, An); 4.88/4.71 (2H, 2xs(rotamers), CH_2); 4.30/4.05(2H, 2xs(rotamers), CH_2); 3.83(3H, s, An-OCH_3); 3.70-2.97(4H, m, CH_2).

***N*-(2-(4-Methoxyphenyl)diphenylmethylamino)ethyl-*N*-(1-(*N4*-(4-methoxybenzoyl)cytosyl)acetyl)glycine (7b)**

Crude **6b** (13.0g) from the previous reaction was dissolved in DMF (250ml). Triethylamine (20ml; 134 mmol) was added, followed by Mmt-Cl (11.7g; 48mmol) in 3 portions. The mixture was stirred for 2h, then additional NEt_3 (6.7ml; 48mmol) was added. The mixture was stirred overnight, after which the solution was evaporated *in vacuo*. The residue was taken up in dichloromethane (200ml), and this solution was washed three times with water (30ml). The organic phase was dried (Na_2SO_4), filtered, concentrated to approx. 40 ml, and added with stirring to diethyl ether(200ml). The precipitated product was filtered off, washed with ether, and dried *in vacuo*. Yield 10.9g (70% from **5b**). R_f =0.62 (2-butanone:H_2O: pyridine:AcOH/70:15:15:2); MS(FAB, MeOH/NBA) 676.4 $(M+H)^+$; ^1H NMR (d6-DMSO) δ 11.0 (1H, brs, NH); 8.02 (2H, d, An); 7.95 (1H, 2xd(rotamers), H-6); 7.50-6.80 (17H, m, Mmt+H-5+An); 5.02/4.65 (2H, 2xs(rotamers), CH_2); 4.02-3.87 (5H, m(rotamers), CH_2 + An-OCH_3); 3.75 (3H, s, Mmt-OCH_3); 3.70-3.0 (m, CH_2+H_2O); 2.22-2.02 (2H, m(rotamers), CH_2); 2.70(6H, q, NEt_3); 1.05(9H, t, NEt_3).

***N*-(*tert*-Butyloxycarbonylaminoethyl)-*N*-(9-(*N6*-(4-methoxybenzoyl)adenosyl)acetyl)glycine *tert*-butyl ester (5c)**

Compound **1** (300.0g; 1.094mol) was dissolved in DMF (2.8l). To this solution was added *N6*-(4-methoxybenzoyl)-*N9*-carboxymethyladenine (358.2g; 1.094mol), TOTU (360.5g; 2mol) and DIEA (373.9ml; 2.184mol). The mixture was stirred at room temperature for 2h and then added dropwise to a stirred, ice-cooled

solution of $NaHCO_3$ (214.3g) in water (2.2l), whereupon the product precipitated out. Methyl *tert*-butyl ether (2l) was then added. This mixture was left to stand for 16h. The precipitate was filtered off and washed thoroughly, in succession, with water (2x2l) and methyl *tert*-butyl ether (2x2l); the precipitate was dried *in vacuo*. Yield: 437.7g (68%). R_f =0.74 (CH_2Cl_2:MeOH/10:1 + 1% NEt_3); MS(FAB, MeOH/NBA) 584.2 $(M+H)^+$; ^1H NMR (d6-DMSO) δ 11.02 (1H, s, NH); 8.67 (1H, s, H-8); 8.32 (1H, s, H-2); 8.05 (2H, d, An); 7.08 (2H, d, An); 7.02-6.75 (1H, m, NH); 5.35/5.15 (2H, 2xs(rotamers), CH_2); 4.35/3.96 (2H, 2xs(rotamers), CH_2); 3.86 (3H, s, An-OCH_3); 3.60-2.95 (m, CH_2+H_2O); 1.52/1.40 (18H, 2xs(rotamers), Boc + OtBu).

_N_-(2-Aminoethyl)-_N_-(9-(_N6_-(4-methoxybenzoyl)adenosyl)acetyl)glycine (6c)

Compound **5c** (10.0g; 17 mmol) was dissolved in a mixture of dichloromethane (75ml) and trifluoroacetic acid (75ml). A clear solution was formed which was stirred at room temperature for 2h. The reaction mixture was then concentrated, coevaporated twice with toluene and the residue was triturated with diethyl ether. The precipitated crude product was used directly for the next step of the synthesis. Yield: 11.8g of an amorphous solid. R_f =0.09 (CH_2Cl_2:MeOH:NEt_3/100:10:1); MS(FAB, NBA) 428.2 $(M+H)^+$; ^1H NMR (d6-DMSO) δ 8.71/8.70 (1H, 2xs(rotamers), H-8); 8.38/8.32 (1H, 2xs(rotamers), H-2); 8.05 (2H, d, An); 7.08 (2H, d, An); 5.42/5.21 (2H, 2xs(rotamers), CH_2); 4.40/4.05(2H, 2xs(rotamers), CH_2); 3.86(3H, s, An-OCH_3); 3.77-2.98(m, CH_2+H_2O).

_N_-(2-(4-Methoxyphenyl)diphenylmethylamino)ethyl-_N_-(9-(_N6_-(4-methoxybenzoyl)adenosyl)acetyl glycine (7c)

Crude **6c** (11.8g) from the previous reaction was dissolved in DMF (250ml). Triethylamine (11.6ml; 84 mmol) was added, followed by Mmt-Cl (10.5g; 34 mmol) in 3 portions. The mixture was stirred overnight, after which a little undissolved material was removed by filtration and the filtrate was evaporated *in vacuo*. The residue was taken up in dichloromethane(200ml), and washed with water (3x30ml). The organic phase was dried (Na_2SO_4), filtered, concentrated *in vacuo* and the residue triturated with diethyl ether. The precipitated product was filtered off, washed with ether and dried *in vacuo*. Yield: 12.4g (90% from **5c**). R_f =0.59 (2-butanone:H_2O:pyridine:AcOH/70:15:15:2); MS(ES^+) 700.3 $(M+H)^+$; ^1H NMR (d6-DMSO) δ 11.0 (1H, brs, NH); 8.70/8.65 (1H, 2xs(rotamers), H-8); 8.35/8.30 (1H, 2xs(rotamers), H-2); 8.05 (2H, dd, An); 7.50-6.80 (16H, m, Mmt+An); 5.55/5.15 (2H, 2xs(rotamers), CH_2); 4.10/3.90(2H, 2xs(rotamers), CH_2); 3.87(3H, s, An-OCH_3); 3.75(3H, s, Mmt-OCH_3); 3.72-2.98(m, CH_2+H_2O); 2.72(6H, q, NEt_3); 2.22-2.02(2H, m(rotamers), CH_2); 1.05(9H, t, NEt_3).

_N_-(_tert_-Butyloxycarbonylaminoethyl)-_N_-((9-(_N2_-acetyl-_O6_-diphenylcarbamoyl)guanosyl)acetyl)glycine _tert_-butyl ester (5d)

Compound **1** (5.50g; 20mmol) was dissolved in DMF (100ml). To this solution were added *N2*-acetyl-*O6*-diphenylcarbamoyl-9-carboxymethylguanine (8.92g; 20mmol), TOTU (6.56g; 20mmol) and DIEA (6.8ml; 40mmol). The mixture was stirred at room temperature for 16h and then evaporated *in vacuo*. The residue was taken up in EtOAc (150ml), and washed with sat. $NaHCO_3$ solution (3x15ml), 1M $KHSO_4$ solution (3x15ml), and water (15ml). The organic phase was dried (Na_2SO_4), filtered and the filtrate was concentrated to about 30 ml. This solution was added dropwise to diisopropyl ether (400ml) with stirring whereupon the product precipitated. The mixture was stirred for 1h and the product was filtered off, washed with diisopropyl ether and

dried *in vacuo*. Yield: 12.8g (91%). R_f = 0.80 (CH_2Cl_2:MeOH:NEt_3/100:10:1); MS(FAB, NBA/MeOH) 703.3 $(M+H)^+$; ^1H NMR (d6-DMSO) δ 10.65 (1H, br s (rotamers), NH); 8.32 (1H, br s(rotamers), H-8); 7.85-7.25 (10H, m, Ar); 7.05-6.65(1H, m, NH); 5.25/5.08 (2H, 2xs(rotamers), CH_2); 4.35/3.98 (2H, 2xs(rotamers), CH_2); 3.40-2.95(m, CH_2+H_2O); 2.20(3H,s, CH_3-CO); 1.50/1.38 (18H, 2xs(rotamers), Boc+OtBu).

***N*-(2-Aminoethyl)-*N*-((9-(*N2*-acetyl)guanosyl)acetyl)glycine (6d)**

Compound **5d** (10.0g; 14mmol) was dissolved in a mixture of dichloromethane (65ml) and trifluoroacetic acid (65ml). A clear solution was formed which was stirred at room temperature for 2h. The reaction mixture was then concentrated, coevaporated twice with toluene and the residue was triturated with diethyl ether. The precipitated crude product was used directly for the next step of the synthesis. Yield: 8.1g of an amorphous solid. R_f = 0.15 (2-butanone:H_2O:pyridine:AcOH/70:15:15:2); MS(FAB, NBA/MeOH) 352.1 $(M+H)^+$; ^1H NMR (d6-DMSO) δ 12.08/11.65 (1H, 2xs(rotamers), NH); 7.85/7.81 (1H, 2xs(rotamers), H-8); 5.15/4.95 (2H, 2xs(rotamers), CH_2); 4.37/4.05 (2H, 2xs(rotamers), CH_2); 4.12-2.93 (m, CH_2+H_2O); 2.18 (3H, s, CH_3-CO).

***N*-(2-(4-Methoxyphenyl)diphenylmethylamino)ethyl-*N*-(9-(*N2*-acetyl-guanosyl) acetyl)glycine (7d)**

Crude **6d** (8.10g) from the previous reaction was dissolved in DMF (130ml). Triethylamine (6.12ml; 64mmol) was added, followed by Mmt-Cl (6.18g; 20mmol) in 3 portions. The mixture was stirred overnight, after which a little undissolved material was removed by filtration and the filtrate was evaporated *in vacuo*. The residue was taken up in EtOAc (30ml), and washed three times with sat. $NaHCO_3$ solution and water. The organic phase was dried (Na_2SO_4), filtered, and evaporated *in vacuo*. The residue was triturated with ether. The precipitated product was filtered off, washed with ether, and dried *in vacuo*. Yield: 2.58g (25% from **5d**). R_f = 0.14 (CH_2Cl_2:MeOH:NEt_3/100:10:1); MS(ES^+) 624.3 $(M+H)^+$; ^1H NMR (d6-DMSO) δ 12.1 (1H, brs, NH); 8.05/7.80 (1H, 2xs(rotamers), H-8); 7.45-6.85 (14H, m, Mmt); 5.15/4.95 (2H, 2xs(rotamers), CH_2); 3.98/3.85 (2H, 2xs(rotamers), CH_2); 3.70 (3H, s, Mmt-OCH_3); 3.55-3.10 (m, CH_2+H_2O); 2.75 (6H, q, NEt_3); 2.18 (3H,s, CH_3-CO); 2.17-2.02 (2H, m(rotamers), CH_2); 1.05 (9H, t, NEt_3).

***N*-(*tert*-Butyloxycarbonylaminoethyl)-*N*-(9-(*N2*-isobutyryl-guanosyl) acetyl)glycine *tert*-butyl ester (5e)**

Compound **1** (16.5g; 60mmol) was dissolved in DMF (300ml), and *N2*-isobutyryl-9-carboxymethylguanine (13.3g; 60mmol), TOTU (19.5g; 60mmol) and DIEA (20.4ml; 120mmol) were added. The mixture was stirred at room temperature for 16h and evaporated *in vacuo*. The residue was taken up in EtOAc (300ml), washed with sat. $NaHCO_3$ solution (3x30ml), 1M $KHSO_4$ solution (3x30ml), and water (3x30ml). The organic phase was dried (Na_2SO_4), filtered, and evaporated *in vacuo*. The residue was triturated with ether and cooled. The precipitated product was filtered off, washed with cold ether and dried. Yield: 20.7g (64%). R_f =0.75 (CH_2Cl_2:MeOH NEt_3/100:7,5:1); MS(FAB, NBA/MeOH) 536.3 $(M+H)^+$; ^1H NMR (d6-DMSO) δ 12.08/11.55 (1H, 2xs(rotamers), NH); 8.08/7.85 (1H, 2xs(rotamers), H-8); 7.02-6.60(1H, m, NH); 5.38/5.18/5.11/4.98 (2H, 4xs(rotamers), CH_2); 4.32/4.28/3.98/3.94(2H, 4xs(rotamers), CH_2); 3.52-2.73(m, CH_2+CH-iBu+H_2O); 1.50/1.38 (18H, 2xs(rotamers), Boc + OtBu); 1.10(6H, d, iBu).

***N*-(2-Aminoethyl)-*N*-(9-(*N2*-isobutyryl-guanosyl) acetyl)glycine (6e)**

Compound **5e** (10.0g; 19mmol) was dissolved in a mixture of dichloromethane (65ml) and trifluoroacetic acid (65ml). A clear solution was formed which was stirred at room temperature for 2h. The solvent was evaporated *in vacuo*, coevaporated twice with toluene and the residue was triturated with diethyl ether. The precipitated crude product was used directly for the next step of the synthesis. Yield: 10.1g of an amorphous solid. R_f =0.16 (2-butanone:H_2O:pyridine:AcOH/70:15:15:2); MS(ES^+) 380.1 $(M+H)^+$; ^1H NMR (d6-DMSO) δ 12.08/11.65 (1H, 2xs(rotamers), H-3); 8.10/7.85 (1H, 2xd(rotamers), H-8); 5.42/5.22/5.16/4.98 (2H, 4xs(rotamers), CH_2); 4.35/4.05(2H, 2xs(rotamers), CH_2); 3.72-3.52(2H, m, CH_2); 3.22-2.90(2H, m, CH_2); 2.75(1H, sept, CH-iBu); 1.10(6H, d, iBu).

***N*-(2-(4-Methoxyphenyl)diphenylmethylamino)ethyl-*N*-(9-(*N2*-isobutyryl-guanosyl) acetyl)glycine (7e)**

Crude **6e** (10.10g) from the previous reaction was dissolved in DMF (350ml), and NEt_3 (15.0ml; 100mmol) was added, followed by Mmt-Cl (8.20g; 26mmol). The mixture was stirred overnight and concentrated *in vacuo*. The residue was taken up in dichloromethane (400ml) and washed with water (100ml). The organic phase was evaporated to dryness, taken up in dichloromethane (20ml) containing 1% NEt_3 and evaporated again. The residue was triturated with ether and the precipitated product was filtered off, washed with ether and dried *in vacuo*. Yield: 10.65g (74% from **5e**). R_f =0.17 (CH_2Cl_2:MeOH:EtOAc:NEt_3/100:30:20:1); MS(ES^+) 652.2 $(M+H)^+$; ^1H NMR (d6-DMSO) δ 12.1 (1H, brs, NH); 8.05/7.80 (1H, 2xs(rotamers), H-8); 7.45-6.85 (14H, m, Mmt); 5.20/4.95 (2H, 2xs(rotamers), CH_2); 3.98/3.85 (2H, 2xs(rotamers), CH_2); 3.70(3H, s, Mmt-OCH_3); 3.55-3.10 (m, CH_2+H_2O); 2.82-2.70 (7H, m, NEt_3 + iBu); 2.22-2.02 (2H, m(rotamers), CH_2); 1.15-1.02 (15H, m, NEt_3 + iBu).

***N*-(2-Hydroxyethyl)glycine *tert*-butyl ester (8)**

2-Aminoethanol (30.2ml; 0.5mol) was dissolved in DMF (200ml). DIEA (17.0ml; 0.1mol) was added, followed by *tert*-butyl bromoacetate(14.8ml; 0.1mol). The mixture was stirred at room temperature for 24h and the solvent was evaporated *in vacuo*. The residue was taken up in water (100ml), and this solution was saturated with NaCl and extracted with EtOAc (3x100ml). The organic phase was washed with a small quantity of sat. $NaCl_{(aq)}$, dried (Na_2SO_4), filtered, and evaporated *in vacuo*. Yield: 11.96g (68%) colorless oil. R_f =0.51 (2-butanone:H_2O:pyridine:AcOH/70:15:15:2); MS(ES^+) 176.2 $(M+H)^+$; ^1H NMR ($CDCl_3$) δ 3.62 (2H, dt, HO-CH_2); 3.33 (2H, s, CH_2CO); 2.78 (2H, dt, CH_2NH); 2.15-1.95, (m, NH +OH +H_2O); 1.48(9H, s, OtBu).

***N*-(2-Hydroxyethyl)-*N*-((1-thyminyl)acetyl)glycine *tert*-butyl ester (9a)**

Compound **8** (8.9g; 53mmol) was dissolved in DMF(100ml), and *N1*-carboxymethylthymine (8.8g; 48mmol), TOTU (17.4g; 53mmol) and NEt_3 (19.4ml; 106mmol) were added. The mixture was stirred at room temperature for 3h and the solvent was removed *in vacuo*. The residue was treated with EtOAc, whereupon the product began to precipitate. The mixture was then left to stand at 4°C for 16h, and the precipitated product was filtered off, washed with EtOAc and dried *in vacuo*. Yield: 11.8g (71%). R_f =0.75 (2-butanone:H_2O:pyridine:AcOH/70:15:15:2); MS(ES^+) 342.2 $(M+H)^+$; ^1H NMR (d6-DMSO) δ 11.25 (1H, br s(rotamers), H-3); 7.31/7.26 (1H, 2xs(rotamers), H-6); 4.70/4.45 (2H, 2xs(rotamers), CH_2); 4.08/3.98(2H, 2xs(rotamers), CH_2); 3.68-3.20(m, CH_2+H_2O+OH); 1.75/1.72(3H, 2xs(rotamers), T-CH_3); 1.48/1.38(9H, 2xs(rotamers), OtBu).

***N*-(2-Hydroxyethyl)-*N*-((1-thyminyl)acetyl)glycine (11a)**

Compound **9a** (9.47g; 28mmol) was suspended in dichloromethane (150ml), and TFA:water / 95:5 (100 ml) was added. The resulting clear solution was stirred at room temperature for 3h. The reaction mixture was then added dropwise to thoroughly stirred methyl *tert*-butyl ether (1l) at 0°C. The precipitated crude product (also containing lactone **10a**) was dissolved in a mixture of dioxan (170ml), water (170ml) and NEt_3 (6.4ml), and stirred at room temperature for 2h, whereby the lactone hydrolysed. The mixture was evaporated to dryness and the residue was dried *in vacuo*. Yield: 10.51g amorphous solid which was used for the following reaction directly. R_f =0.12 (CH_2Cl_2:MeOH:EtOAc/10:2:1 and 1% NEt_3); MS(ES^+) 286.2 $(M+H)^+$; ^1H NMR (d6-DMSO) δ 11.25 (1H, br s(rotamers), H-3); 7.30/7.25 (1H, 2xs(rotamers), H-6); 4.70/4.45 (2H, 2xs(rotamers), CH_2); 4.08/3.98(2H, 2xs(rotamers), CH_2); 3.65-3.10(m, CH_2+H_2O+OH); 3.05(6H, q, NEt_3); 1.75/1.72(3H, 2xs(rotamers), T-CH_3); 1.08(9H, t, NEt_3).

***N*-(2-(Di-(4-methoxyphenyl)phenylmethoxy)ethyl)-*N*-((1-thyminyl)acetyl) glycine (12a)**

Crude **11a** (1.00g; 2.6mmol) was dissolved in DMF (10ml). Triethylamine (1.4ml; 10mmol) and a solution of Dmt-Cl (1.80g; 5.2mmol) in dichloromethane (10ml) were added. The reaction was stirred at room temperature for 16 h. The solvent was removed *in vacuo* and the residue was taken up in dichloromethane; This solution was washed with water and the organic phase was dried (Na_2SO_4), filtered, and evaporated *in vacuo*. The product was purified by column chromatography on silica gel using dichloromethane/methanol/ EtOAc 15:1:1 containing 1% NEt_3 as the eluent. The fractions containing the product were combined and dried *in vacuo*. Yield 0.96g (53%) white foam. R_f =0.29 (CH_2Cl_2:MeOH:EtOAc/10:2:1 and 1% NEt_3); MS(FAB, MeOH/NBA) 587.3 $(M)^+$; ^1H NMR (d6-DMSO) δ 11.25 (1H, br s(rotamers), H-3); 7.42-7.18 (9H, m, Dmt + H-5); 7.02 (1H, br s, H-6); 6.90 (4H, m, Dmt); 4.62/4.46 (2H, 2xs(rotamers), CH_2); 3.95/3.80 (2H, 2xs(rotamers), CH_2); 3.76 (6H, s, Dmt-OCH_3); 3.55-2.95 (m, CH_2+H_2O); 2.73 (6H, q, NEt_3); 1.75/1.68 (3H, 2xs(rotamers), T-CH_3); 1.05 (9H, t, NEt_3).

***N*-(2-Hydroxyethyl)-*N*-(1-(*N4*-(4-methoxybenzoyl)cytosyl)acetyl)glycine *tert*-butyl ester (9b)**

Compound **8** (1.8g; 11mmol) was dissolved in DMF (100ml). To this solution were added *N4*-(4-methoxybenzoyl)-*N1*-carboxymethylcytosine (3.0g; 10 mmol), TOTU (3.6g; 11mmol) and NEt_3 (3.0ml; 22mmol). The reaction mixture was stirred at room temperature for 4h and the solvent was removed *in vacuo*. The residue was taken up in EtOAc, and washed twice with sat. $NaHCO_3$ solution, whereupon the product started to precipitate in the EtOAc phase. The precipitated product was filtered off, washed with EtOAc and dried *in vacuo*. Yield: 3.06g (66%). R_f =0.81 (2-butanone: H_2O: pyridine: AcOH/70:15:15:2); MS(FAB, MeOH/NBA) 461.3 $(M+H)^+$; ^1H NMR (d6-DMSO) δ 8.05 (2H, d, An); 7.93/7.88 (1H, 2xd(rotamers), H-6); 7.22 (1H, br d(rotamers), H-5); 7.04 (2H, d, An); 4.90/4.65 (2H, 2xs(rotamers), CH_2); 4.28/4.02 (2H, 2xs(rotamers), CH_2); 3.83 (3H, s, An-OCH_3); 3.68-3.20 (m, CH_2+H_2O+OH); 1.48/1.38 (9H, 2xs(rotamers), OtBu).

***N*-(2-Hydroxyethyl)-*N*-(1-(*N4*-(4-methoxybenzoyl)cytosyl)acetyl)glycine (11b)**

Compound **9b** (1.5g; 3.4mmol) was dissolved in a mixture of dichloromethane (30ml); TFA (20ml) and anisole (2.5ml). The clear solution was stirred at room temperature for 5h and added dropwise to thoroughly stirred methyl *tert*-butyl ether (500ml) at 0°C, whereupon the product precipitated. The precipitated crude product (also containing lactone **10b**) was dissolved in a mixture of dioxan (25ml), water (25ml) and NEt_3 (0.44ml). This

solution was stirred at room temperature for 3h, whereby the lactone was hydrolysed. The mixture was then evaporated to dryness and the residue was purified by column chromatography on silica gel using dichloromethane/methanol/EtOAc 10:3:2 containing 1% NEt_3 as the eluent. Yield: 0.92g (53%) amorphous solid. R_f=0.16 (CH_2Cl_2:MeOH: EtOAc/10:3:2 and 1% NEt_3); MS(ES^+): 405.2 $(M+H)^+$; ^1H NMR (d6-DMSO) δ 8.05 (2H, d, An); 7.96/7.92 (1H, 2d(rotamers), H-6); 7.28/7.25 (1H, 2d(rotamers), H-5); 7.04, (2H, d, An); 4.90/4.65 (2H, 2xs(rotamers), CH_2); 4.25/4.05(2H, 2xs(rotamers), CH_2), 3.83 (3H, s, An-OCH_3); 3.65-3.10 (m, CH_2+H_2O+OH); 2.80 (6H, q, NEt_3); 1.08 (9H, t, NEt_3).

_N_-(2-(Di-(4-methoxyphenyl)phenylmethyloxy)ethyl)-_N_-(1-(_N4_-(4-methoxybenzoyl)cytosyl)acetyl)glycine (12b)

Compound **11b** (0.92g; 1.8mmol) was dissolved in DMF (10ml). Triethylamine (1.0ml; 7.2mmol) and a solution of Dmt-Cl (1.2g; 3.6mmol) in dichloromethane (10ml) were added and the mixture was stirred at room temperature for 16h. The solvent was removed *in vacuo*, the residue was taken up in dichloromethane and washed with water. The organic phase was dried (Na_2SO_4), filtered, and evaporated *in vacuo*. The resulting crude product was purified by column chromatography on silica gel using dichloromethane:methanol:EtOAc/ 15:1:1 containing 1% NEt_3 as the eluent. The fractions containing the product were pooled and dried *in vacuo*. Yield: 0.90g (61%) foam. R_f =0.24 (CH_2Cl_2:MeOH: EtOAc/10:3:2 + 1% NEt_3); MS(FAB, MeOH/NBA) 707.3 $(M+H)^+$; ^1H NMR (d6-DMSO) δ 8.05 (2H, d, An); 7.90/7.80 (1H, 2xd(rotamers), H-6); 7.42-7.18 (10H, m, Dmt + H-5); 7.04 (2H, d, An); 6.90 (4H, m, Dmt); 4.82/4.70 (2H, 2xs(rotamers), CH_2); 4.18/3.90 (2H, 2xs(rotamers), CH_2); 3.84 (3H, s, An-OCH_3); 3.75 (6H, s, Dmt-OCH_3); 3.58-2.85 (m, CH_2+H_2O); 2.98 (6H, q, NEt_3); 1.18 (9H, t, NEt_3).

_N_-(2-Hydroxyethyl)-_N_-(9-(_N6_-(4-methoxybenzoyl)adenosyl)acetyl)glycine *tert*-butyl ester (9c)

Compound **8** (0.53g; 3.1mmol) was dissolved in DMF (10ml), and *N6*-(4-methoxybenzoyl)-*N9*-carboxymethyladenine (1.0g; 3.1mmol), TOTU (1.0g; 3.1mmol) and NEM (0.77ml; 4.5mmol) were added. The mixture was stirred at room temperature overnight and the solvent was evaporated *in vacuo*. The residue was taken up in EtOAc, and washed twice with sat. $NaHCO_3$ solution and water. The aqueous phase was washed three times with EtOAc and the combined organic phase was dried (Na_2SO_4), filtered, and evaporated *in vacuo*. The resulting crude product was purified by column chromatography on silica gel using dichloromethane:ethanol/5:1 as the eluent. The fractions containing the product were pooled and dried *in vacuo*. Yield: 1.33g (88%). R_f = 0.63 (n-butanol:H_2O:AcOH /3:1:1); MS(FAB, MeOH/NBA) 485.3 $(M+H)^+$; ^1H NMR ($CDCl_3$) δ 9.00 (1H, brs, NH); 8.75 (1H, s, H-8); 8.18/8.10 (1H, 2xs(rotamers), H-2); 8.00 (2H, d, An); 7.00 (2H, d, An); 5.35/5.05 (2H, 2xs(rotamers), CH_2); 4.27/4.00 (2H, 2xs(rotamers), CH_2); 3.89 (3H, s, An-OCH_3); 3.80 (2H, m, CH_2); 3.65 (2H, m, CH_2); 1.45 (9H, 2xs(rotamers), tBu).

_N_-(2-Hydroxyethyl)-_N_-(9-(_N6_-(4-methoxybenzoyl)adenosyl)acetyl)glycine (11c)

Compound **9c** (1.3g; 2.7mmol) was dissolved in TFA:water/95:5 (15ml). The reaction was stirred at room temperature for 1h and was added dropwise to thoroughly stirred diethyl ether (250ml) at 0°C, whereupon the product precipitated. The precipitate was filtered off, and dried *in vacuo*. The crude product (also containing lactone **10c**) was dissolved in a mixture of dioxan (15ml), water (15ml) and NEt_3 (0.4ml), and stirred at room temperature for 2h, whereby the lactone was hydrolysed. The mixture was evaporated to dryness and the residue

was co-evaporated 3 times with pyridine. The residue was used directly for the next reaction. R_f = 0.37 (n-butanol:H_2O: AcOH water:acetic acid/3:1:1); MS(FAB, NBA/MeOH/LiCl) 435.2 $(M+Li)^+$; ^1H NMR (d6-DMSO) δ 11.00 (1H, brs, NH); 8.70 (1H, s, H-8); 8.35 (1H, 2xs(rotamers), H-2); 8.05 (2H, d, An); 7.05, (2H, d, An); 5.40/5.20 (2H, 2xs(rotamers), CH_2); 4.40/4.05 (2H, 2xs(rotamers), CH_2); 3.85(3H, s, An-OCH_3); 3.75/3.60 (2xm, CH_2+H_2O); 3.55/3.40 (2xm, CH_2+H_2O).

***N*-(2-(Di-(4-methoxyphenyl)phenylmethyloxy)ethyl)-*N*-(9-(*N6*-(4-methoxybenzoyl)adenosyl)acetyl)-glycine (12c)**

Crude **11c** obtained in the previous reaction was dissolved in pyridine (10ml), Dmt-Cl (1.70g; 5.0mmol) was added and the mixture was stirred at room temperature for 16h. The solvent was removed *in vacuo* and the residue was taken up in dichloromethane. This solution was washed with 5% aqueous citric acid and sat. $NaCl_{(aq)}$. The organic phase was dried (Na_2SO_4), filtered, and evaporated *in vacuo*. The resulting crude product was purified by column chromatography on silica gel using dichloromethane with a gradient of 1-10% methanol, 1% NEt_3 as the eluent. The fractions containing the product were pooled and concentrated. The residue was dissolved in dichloromethane (5ml), and added dropwise to rapidly stirred diethyl ether (100ml), whereupon the product precipitated. Yield: 0.58g (26% from **9c**) white powder. R_f =0.70 (n-butanol:H_2O:AcOH/3:1:1); MS(FAB, MeOH/NBA) 731.3 $(M+H)^+$; ^1H NMR (d6-DMSO) δ 11.00 (1H, s, NH); 8.65/8.60 (1H, 2xs(rotamers, H-8); 8.25/8.15 (1H, 2xs(rotamers), H-2); 8.05 (2H, d, An); 7.50-7.20 (9H, m, Dmt); 7.05 (2H, d, An); 6.85 (4H, m, Dmt); 5.40/5.20 (2H, 2xs(rotamers), CH_2); 4.40/3.95 (2H, 2xs(rotamers), CH_2); 3.85 (3H, s, An-OCH_3); 3.75 (6H, s, Dmt-OCH_3); 3.65/3.45 (2H, 2xm(rotamers), CH_2); 3.30-3.10 (m, CH_2+H_2O).

***N*-(2-(Di-(4-methoxyphenyl)-phenylmethoxy)ethyl)phthalimide (14)**

N-(2-Hydroxyethyl)phthalimide **13** (19.1g; 0.1mol) was dissolved in anhydrous pyridine (300 ml). Triethylamine (55.7ml; 0.4mol) and Dmt-Cl (33.9g; 0.1mol) were added and the mixture was stirred for 16h at room temperature. The solvent was evaporated *in vacuo*, the residue was dissolved in EtOAc, and washed with saturated aqueous $NaHCO_3$, water and saturated aqueous NaCl. The organic phase was dried (Na_2SO_4), filtered and evaporated *in vacuo*. The residue was purified by column chromatography on silica gel using n-heptane:EtOAc:NEt_3 /80:19:1 as the eluent. The product-containing fractions were combined and concentrated *in vacuo* to give **14** as a white solid in 34.8g (70%) yield. R_f =0.72 (n-heptane:EtOAc/10:1); MS(FAB/NBA/LiCl) 500.2 $(M+Li)^+$, 493 M^+; ^1H NMR (d6-DMSO) δ 7.85 (4H, m, phthaloyl); 7.20 (9H, m, Dmt); 6.75 (4H, m, Dmt); 3.80 (2H, t, CH_2); 3.70 (6H, s, Dmt-OCH_3); 3.20 (2H, t, CH_2).

2-(Di-(4-methoxyphenyl)-phenylmethoxy)ethylamine (15)

Compound **14** (32.1g; 0.065mol) was suspended in methanol (300 ml). Hydrazine hydrate (34.0ml; 0.70mol) was added dropwise to give a clear solution. After 1h a white precipitate formed. The mixture was stirred for 3h at room temperature. The precipitate was removed by filtration and the solvent was evaporated *in vacuo*. The residue was dissolved in dichloromethane:EtOAc:methanol (10:2:1) containing 1% NEt_3, filtered and purified by column chromatography on silica gel using CH_2Cl_2:EtOAc:MeOH (10:2:1) containing 1% NEt_3 as the eluent. The product-containing fractions were combined and concentrated *in vacuo* to give **15** as a white solid in 22.2g (94.1%) yield. R_f = 0.30 (CH_2Cl_2: EtOAc:MeOH /10:2:1); MS(FAB/NBA/LiCl) 370.2 $(M+Li)^+$; ^1H NMR (d6-

DMSO) δ 7.25 (9H, m, Dmt); 6.85 (4H, m, Dmt); 3.75 (6H, s, Dmt-OCH_3); 2.90 (2H, t, CH_2); 2.70 (2H, t, CH_2).

***N*-(2-(Di-(4-methoxyphenyl)phenylmethoxy)ethyl)glycine allyl ester (16)**

Compound **15** (5.0g; 14mmol) was dissolved in DMF (50ml). DIEA (4.76ml; 28mmol) and allyl chloroacetate (1.6ml; 14mmol) were added and the mixture was stirred for 16h at room temperature. Further DIEA (4.76ml) and allyl chloroacetate (1.6ml) were added and the reaction was stirred for a further 5h. The solvent was evaporated *in vacuo*. The residue was purified by column chromatography on silica gel using n-heptane: EtOAc /2:1 containing 1% NEt_3 as the eluent. The product-containing fractions were combined and concentrated *in vacuo* to give **16** as a white solid in 4.2g (63.6%) yield. R_f = 0.25 (n-heptane: EtOAc/2:1); MS(FAB/NBA/LiCl) 468.3 $(M+Li)^+$; ^{1}H NMR (d6-DMSO) δ 7.30 (9H, m, Dmt); 6.90 (4H, m, Dmt); 5.90 (1H, m, Allyl); 5.25 (2H, m, Allyl); 4.60 (2H, m, Allyl); 3.75 (6H, s, Dmt-OCH_3); 3.40 (2H, s, CH_2CO); 3.00 (2H, t, CH_2); 2.75 (2H, t, CH_2).

***N*-2-(Di-(4-methoxyphenyl)phenylmethoxy))ethyl)-*N*-((9-(*N2*-isobutyryl)guanosyl)acetyl)glycine allyl ester (17)**

Compound **16** (3.7g; 8.1mmol) was dissolved in DMF (60ml). N-Ethylmorpholine (4.1ml; 32mmol), HOObt (1.3g; 8.1mmol), *N2*-(isobutyryl)-9-(carboxymethyl)guanine (2.3g; 89mmol) and DIPCD (1.4ml; 89mmol) were added and the mixture was heated at 55°C for 4h, then stirred for 16h at room temperature. The solvent was evaporated *in vacuo*. The residue was purified by column chromatography on silica gel using CH_2Cl_2: EtOAc /5:1+1%NEt_3 as the eluent. The product-containing fractions were combined and concentrated *in vacuo* to give **17** as a pale yellow foam in 2.8g (45%) yield. R_f = 0.40 (CH_2Cl_2: EtOAc:MeOH /10:2:1); MS(FAB/NBA) 723.3 $(M+H)^+$; ^{1}H NMR (d6-DMSO) δ 8.05-7.90 (1H, 2xs(rotamers), H-8); 7.30 (9H, m, Dmt); 6.85 (4H, m, Dmt); 5.90 (1H, m, Allyl); 5.25 (4H, m, Allyl+CH_2); 4.60-4.00 (4H, m, Allyl+CH_2); 3.75 (6H, 2xs(rotamers), Dmt-OCH_3); 3.55 (2H, m, CH_2); 3.30-3.10 (2H, m, CH_2) 2.75 (1H, septet, iBu); 1.15 (6H, d, iBu).

***N*-(2-(Di-(4-methoxyphenyl)phenylmethoxy))ethyl-*N*-(9-(*N2*-isobutyryl)guanosyl)acetyl)glycine (18)**

Compound **17** (1.70g; 2.35mmol) and diethylammonium hydrogencarbonate (3.20g; 2.35mmol) were dissolved in CH_2Cl_2 (250ml). A solution of tetrakis(triphenylphosphine)palladium(0) (1.35g; 1.18mmol) and triphenylphosphine (0.184g; 0.7mmol) in CH_2Cl_2 (130ml) was added to the reaction solution, then stirred for 16h at room temperature. The solvent was evaporated *in vacuo*. The residue was purified by column chromatography on silica gel using EtOAc:methanol/3:2+1%NEt_3 as the eluent. The product-containing fractions were combined and concentrated *in vacuo* to give **18** as a pale yellow foam in 1.07g (67%) yield. R_f =0.17 (EtOAc: MeOH/3:2); MS(FAB/NBA) 683.3 $(M+H)^+$; ^{1}H NMR (d6-DMSO) δ 8.00-7.90 (1H, 2xs(rotamers), H-8); 7.20 (9H, m, Dmt); 6.85 (4H, m, Dmt); 5.25/5.15 (2H, 2xs(rotamers), CH_2); 3.95-3.85 (2H, m, CH_2); 3.75 (6H, 2xs(rotamers), Dmt-OCH_3); 3.55-3.00 (m, CH_2+H_2O); 2.60 (6H, q, NEt_3); 1.15 (6H, d, iBu); 1.00 (9H, t, NEt_3).

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