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Fmoc Mediated Synthesis of Peptide Nucleic Acids.

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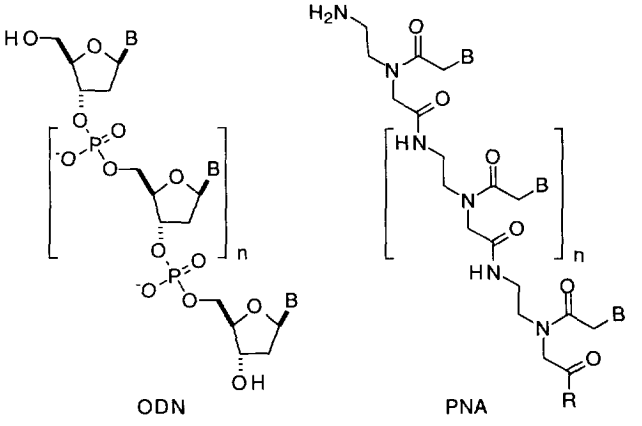
Abstract: The syntheses of the Fmoc-protected Peptide Nucleic Acid (PNA) monomer pentafluorophenyl esters of adenine (**26**), cytosine (**23**), guanine (**29**) and thymine (**20**), and their oligomerization are described. The Fmoc PNA backbone **1** is prepared as a stable hydrochloride salt. The base acetic acids of adenine (**4**) and cytosine (**3**) were prepared by Cbz protection of the exocyclic amino groups followed by alkylation with *t*-butylbromoacetate and subsequent acid hydrolysis of the *t*-butyl ester. Allylation of 6-chloro-2-aminopurine followed by acid hydrolysis, Cbz protection with *N*-(benzyloxycarbonyl)imidazole, ozonolytic cleavage, and oxidation afforded the Cbz-protected guanine acetic acid (**5**). The base acetic acids (**2**, **3**, **4** and **5**) were coupled to the backbone (**1**) with either EDC (**2** and **3**) or BOP reagent (**4** and **5**). Acid hydrolysis of the resulting *t*-butyl esters and transesterification afforded the corresponding pentafluorophenyl esters (**20**, **23**, **26** and **29**). Oligomerization is conducted on a 0.05 mmol scale with a mere 2 fold excess of monomer in each coupling cycle. The *N*-terminal Fmoc group is retained on the final oligomer, following HF cleavage and deprotection, providing a convenient lipophilic handle for HPLC purification.

The sequence specific binding of oligodeoxynucleotides (ODNs) to RNA or in the major groove of duplex DNA through triple helix formation provides a potentially powerful means of modulating gene expression.[1] The deoxyribose phosphate backbone of ODNs has been extensively modified in an attempt to increased *in vivo* stability and to promote cellular permeation.[2,3] In the recently reported peptide nucleic acids[4] (PNAs) the deoxyribose phosphate backbone of an ODN has been replaced by a (2-aminoethyl)glycine unit. These unique agents have been shown to bind in a sequence specific manner to complementary ssDNA and ssRNA via Watson-Crick hydrogen bonding[5] and represent a structurally disparate analog of ODNs. This binding is manifested in the ability of these agents to inhibit gene expression in vitro and in whole cell assays.[6]



Scheme 1

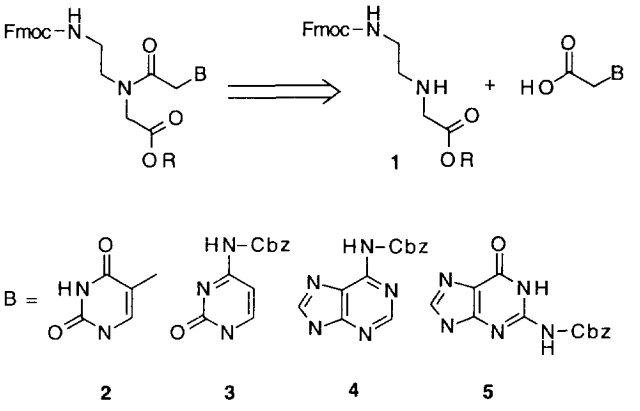
Synthetically, PNAs incorporate repetitive elements which are readily amenable to assembly via automated solid phase synthesis. Specifically, protecting group strategies, solid phase peptide synthesis protocols, deprotection methods and purification procedures developed for peptide synthesis may be directly applicable to the synthesis of PNAs and their analogs. In addition, the chemistry required to obtain structural diversity may be more obtainable in the PNAs compared to ODNs, due to the simpler achiral structure and the wealth of information on amino acid synthesis and oligomerization.

Previous reports on PNA synthesis have focused on Boc protection methods.[4] The use of 9-fluorenylmethoxycarbonyl (Fmoc) protection offers several advantages, including milder synthesis conditions, improved monomer solubility, high coupling efficiencies and facilitated purification of the final product.[7] In our hands, we have found the Fmoc strategy to be superior to Boc chemistry. Herein we describe the efficient synthesis of all four PNA monomers as Fmoc protected pentaflurophenyl esters, bearing Cbz protection of the exocyclic amino group of the nucleobases A, C and G.[8] In addition, the solid phase synthesis of PNAs in excellent yield and high purity, with economic use of monomers, is described.

RESULTS AND DISCUSSION

Synthesis of PNA Fmoc monomers.

Synthesis of the required monomers can be divided into the backbone (**1**) and the base acetic acids (**2**,**3**,**4**,**5**), as shown in Scheme 2. The synthesis of 1-carboxymethylthymine (**2**) was carried out as described in the literature.[9] For the synthesis of **3**,**4** and **5** we selected to protect the exocyclic amine with the benzyloxycarbonyl (Cbz) group. Protection at these sites not only blocks undesired side reactions in the oligomerization, but also increases the solubility of the monomers, especially the purines. This protecting group could then be removed post oligomerization by either treatment with strong acid (HF) or under hydrogenolysis conditions.[10]



Scheme 2

The syntheses of **3** and **4** are shown in Figure 1. After installing the Cbz group at the 4-amino position of cytosine under standard conditions, the *N*-1 position was alkylated with *t*-butyl bromoacetate under basic

conditions to give **8**. Removal of the *t*-butyl ester with anhydrous hydrochloric acid gave **3**. Protection of the exocyclic amine of adenine required harsher conditions. We initially employed Rapoport's reagent,[10] but a difficult chromatographic purification and the labor required to prepare the reagent lead us to explore simpler methods. Treatment of adenine with excess sodium hydride followed by benzyl chloroformate yielded **10** in moderate yield without the need for chromatography. While carbamate formation occurs at both *N*-1 and *N*-6, the *N*-1 carbamate is hydrolyzed under the workup conditions. Treatment of **10** with *t*-butyl bromoacetate under basic conditions gave a mixture of *N*-7 and *N*-9 alkylation products. The desired *N*-9 product (**11**) could be obtained free of the *N*-7 isomer by recrystallization. Removal of the *t*-butyl ester with TFA in CH_2Cl_2 gave **4** with concomitant loss of the Cbz group (ca. 10%). We subsequently found that loss of Cbz can be suppressed by employing the cation scavenger triethylsilane in the reaction mixture.[11]

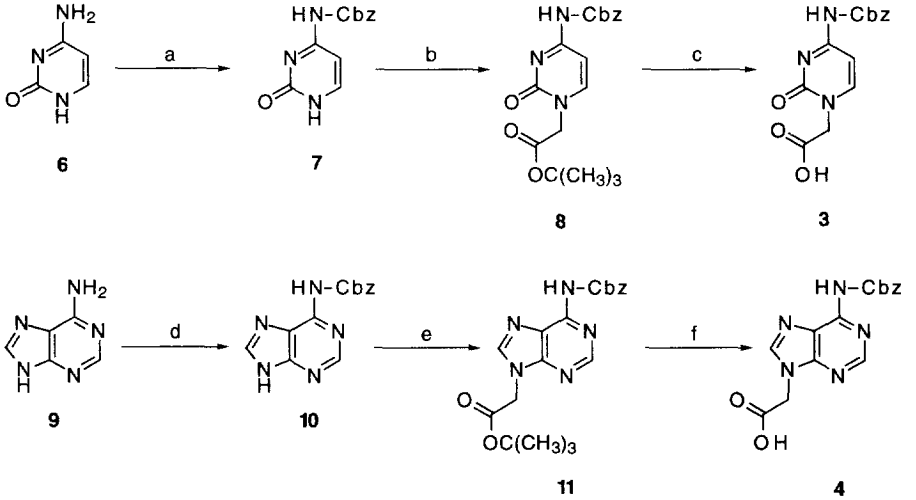


Figure 1. Reagents: (a) Cbz-Cl, DMAP, pyridine; (b) $BrCH_2CO_2C(CH_3)_3$, K_2CO_3, Cs_2CO_3, DMF; (c) HCl/1,4-dioxane, CH_2Cl_2; (d) NaH, Cbz-Cl, DMF; (e) $BrCH_2CO_2C(CH_3)_3$, K_2CO_3, Cs_2CO_3, DMF; (f) TFA, CH_2Cl_2, Et_3SiH.

Synthesis of the Cbz protected guanine acetic acid (**5**) proved to be the most challenging. Alkylation of guanine with methyl bromoacetate followed by attempted protection of the exocyclic amine under strong basic conditions gave complex reaction mixtures.[4a] To avoid this problem we installed a masked acetate unit at *N*-9, as shown in Figure 2. Alkylation of 2-amino-6-chloropurine with allylbromide gave a ca. 3 to 1 ratio of *N*-9 to *N*-7 products which were easily separated by flash chromatography.[12] The desired *N*-9 alkylation product (**13**) was converted to the guanine nucleus (**14**) by acid hydrolysis. With the allyl group at *N*-9, deprotonation of the exocyclic amine with potassium hydride/18-crown-6 and reaction with *N*-(benzyloxycarbonyl)imidazole[10] gave **15** in high yield. Ozonolysis of **15** followed by oxidation afforded the desired acid **5**.

With the base acetic acids (**2**,**3**,**4**,**5**) in hand we turned to the synthesis of the backbone unit (**1**). Compound **1** bears a secondary amine which could potentially remove the Fmoc group.[7] Our plan was to either convert this secondary amine to the amide shortly after its formation or to isolate **1** as a stable salt. The synthesis of **1** is outlined in Figure 3. Alkylation of excess ethylenediamine with *t*-butyl bromoacetate, followed by aqueous workup gave **17**. This material was taken on to the next step without further purification. The Fmoc group was

installed on the primary amine selectively, and **1** was purified by flash chromatography. A dilute ethyl acetate solution of **1** could be stored at -20 °C and used as needed, although loss of the Fmoc group does occur with time. A more attractive solution is to simply wash the crude reaction mixture with dilute aqueous hydrochloric acid and store at -20 °C overnight. This results in precipitation of the pure hydrochloride salt of **1** as a white powder. As the hydrochloride salt, **1** can be stored at -20 °C indefinitely.

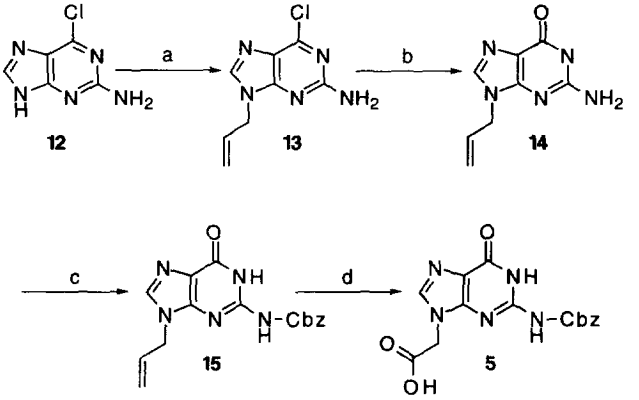
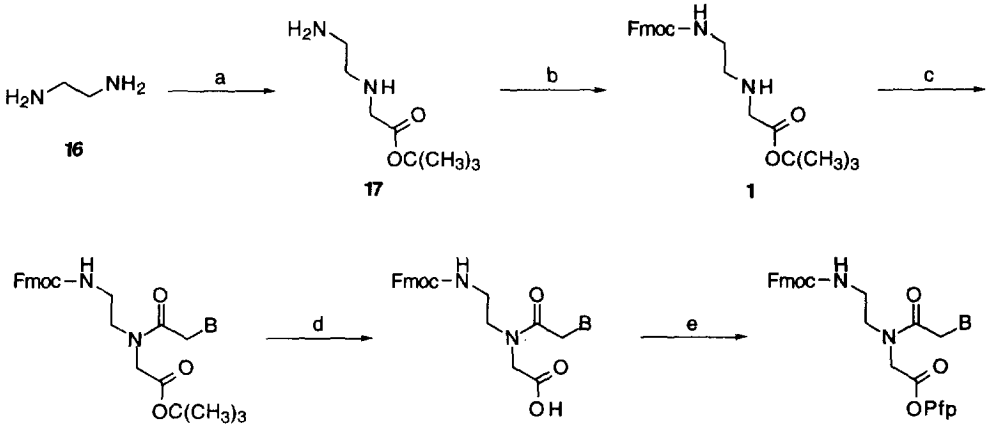


Figure 2. Reagents: (a) allylbromide, K_2CO_3, DMF; (b) aq. 1 N HCl, reflux; (c) Cbz-imidazole, 18-crown-6, KH, THF; (d) (*i*) O_3, CH_2Cl_2, MeOH; (*ii*) Me_2S; (*iii*) $NaClO_2$, NaH_2PO_4, H_2O, THF, $(CH_3)_3COH$, 2-methyl-2-butene.



18: B = T
21: B = C(Z)
24: B =A(Z)
27: B =G(Z)

19: B = T
22: B = C(Z)
25: B =A(Z)
28: B =G(Z)

20: B = T
23: B = C(Z)
26: B =A(Z)
29: B =G(Z)

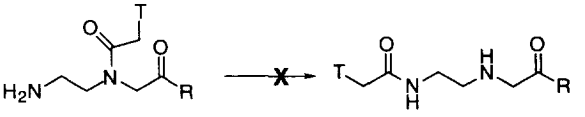
Figure 3. Reagents: (a) $BrCH_2CO_2C(CH_3)_3$, CH_2Cl_2; (b) N-(9-fluorenylmethoxycarbonyloxy)-succinimide, DIEA; (c) for **18** and **21**: **2** or **3**, EDC, DMF; for **24** and **27**: **4** or **5**, BOP reagent, HOBT, DIEA, DMF (d) HCl/1,4-dioxane or TFA, CH_2Cl_2, Et_3SiH (e) $CF_3CO_2C_6F_5$, pyridine, DMF.

Amide bond formation between the backbone amine **1** and the base acetic acids (**2**,**3**,**4**,**5**) can be carried out under several standard methods. We have found BOP reagent/HOBT for the purines and EDC for the pyrimidines to be the simplest and most effective reagents. As shown in Figure 3 coupling of the base acetic acid to the backbone was followed by removal of the *t*-butyl ester under standard conditions. Again for the adenine containing monomer the *t*-butyl ester was removed in the presence of triethylsilane. The free acid was converted to the pentafluorophenyl ester by transesterification with pentafluorophenyl trifluoroacetate and pyridine in DMF.[13] The resulting pentafluorophenyl esters are solids which can be stored indefinitely at -20 °C under anhydrous conditions.

Solid phase synthesis.

For the oligomerization of PNAs we desired methods that would *conserve monomers, give high yields, and allow for easy purification.* Given the requirement of preparing our own monomers, we could not accept the large excess (4 to 10 equivalents) of monomer consumed in typical solid phase peptide synthesis.[4a,7] To conserve monomer, the syntheses are conducted on a relatively small scale (0.05 mmol) and a mere two equivalents (0.1 mmol) of monomer are used with single couplings. The use of 3 or more equivalents of monomer did not give a discernible improvement in coupling yield.

The hydrofluoric acid cleavable MBHA (*p*-methyl-benzhydrylamine) resin or the TFA cleavable Rink amide MBHA resin were employed to give C-terminal amides upon cleavage. For C-terminal acids we employed *o*-chlorotrityl resin. Oligomer syntheses were performed by batch/vortex methods on an ABI 431A peptide synthesizer. A typical synthetic cycle consisted of a single 30 minute coupling of 2 equivalents of pentafluorophenyl ester monomer to the growing PNA chain, followed by capping of unreacted free amines with acetic anhydride, and deprotection of the Fmoc amino group with piperidine. The possibility of acyl migration of the base acetyl group to the primary amine after Fmoc removal was considered. Such a migration would result in a free secondary amine which could continue the chain upon coupling of the next monomer. Such an isomeric product would have the same mass as the desired product. We prepared the acyl migration product by a separate route and under the Fmoc deprotection conditions no acyl migration was observed (Scheme 3). Also, solution phase test reactions employing each of the monomers proved the bases to be unaffected by either the coupling or deprotection conditions. The product integrity is also supported by high field NMR structural assignment [5b] and x-ray crystallographic analysis.[14]

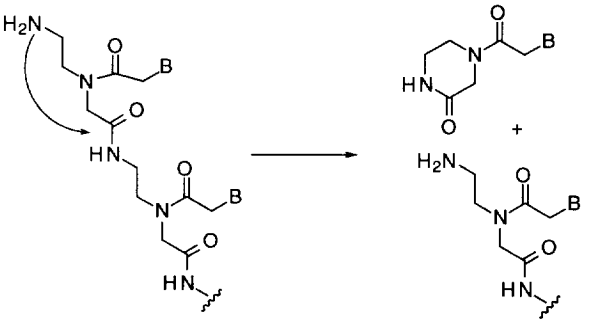


Scheme 3

Although a comprehensive evaluation of solvent effects has not been performed, test syntheses show 20% DMSO in *N*-methylpyrrolidinone to be a superior solvent system. This solvent has been shown to improve access of the reagents to the nascent polymer in standard peptide synthesis.[15] Coupling yields were determined by UV monitoring (301 nm) of the dibenzofulvene-piperidine adduct formed upon piperidine removal of the Fmoc group. This allows one to monitor the progress of the oligomerization without resin sampling. In general, coupling yields are in the 95 to 99% range for oligomers up to 20 residues in length. For example in the synthesis of the PNA GTACGTCTTCAC# (**30**),[8] which contains all four bases, the average coupling yield was 97%, which translates into a 70% crude yield. In this case, following purification, a 43%

isolated yield was realized. We have also used the monomers as free acids with HBTU/HOBT activation[16] in the coupling step. In general the average coupling yields with this activation were slightly less than the pentafluorophenyl ester method. After the last residue is coupled, the *N*-terminal Fmoc protecting group is retained as a purification handle (*vide infra*). The crude PNA was cleaved from the resin and the bases deprotected with neat hydrofluoric acid under standard conditions.[7]

A key aspect of Fmoc PNA synthesis is the relative ease of purification of the oligomer. With the *N*-terminal Fmoc group remaining, separation of the full length product from failure sequences by reverse phase (C_{18}) HPLC is trivial. The Fmoc group provides a lipophilic handle which is retained on the HPLC column to a greater extent than the *N*-acetyl capped failure sequences. Retention time differences of greater than ten minutes are easily obtained. This strategy is similar to the DMT-on/DMT-off purification method used in ODN synthesis.[17] After purification of the Fmoc-on PNA the Fmoc group can be removed by treatment with 10-20% aqueous piperidine at 0 °C, and the final product is purified by reverse phase HPLC. Higher piperidine concentrations and longer reaction times at room temperature can result in the formation of *N*-terminal deletion products. These products presumably arise by an intramolecular acyl migration to form the lactam as shown in Scheme 4.[18] If glycine is incorporated as the *N*-terminal residue the PNA is stable to these conditions, consistent with the mechanism shown in Scheme 4. Final products were analyzed for purity by two HPLC methods and mass determinations were performed by electrospray mass spectrometry. We have prepared over 100 different PNAs with this method, several with modifications to either the base portion or to the backbone.[19]



Scheme 4

In summary, the synthesis of Fmoc protected PNA monomers has been described, and methods for oligomerization outlined. This method is not only milder and more efficient than Boc methods, but also yields a lipophilic handle for easy purification of the PNA. The milder Fmoc synthesis conditions will allow for incorporation of a wide range of modifications to the standard PNA structure, which will be required to advance this interesting structure into a useful human therapeutic.

EXPERIMENTAL SECTION

General.

Unless otherwise noted, all reagents were obtained from commercial suppliers and used without further purification. The following abbreviations are employed: *N*-methylpyrrolidinone (NMP), 1-(3-

dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), *O*-benzotriazol-1-yl-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (BOP reagent), 1-hydroxybenzotriazole (HOBT), and 2-(1-*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU). Reactions involving air- and/or moisture-sensitive reagents were executed under an atmosphere of dry nitrogen. Flash chromatography was performed using Merck silica gel 60 (230-400 mesh ASTM). Melting points are uncorrected and (d) indicates decomposition. ^1H and ^{13}C NMR spectra were obtained in the solvents indicated at 300 MHz and 75 MHz, respectively, unless otherwise noted. Chemical shifts are reported in parts per million (ppm, δ) down field relative to the internal standard tetramethylsilane. Coupling constants are reported in hertz (Hz). Spectral patterns are designated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; and br, broad. Mass spectra were recorded obtained by fast atom bombardment (FAB) unless otherwise noted. Compounds were assayed for purity by analytical HPLC with a UV detector set at 254 nm using a C_{18} stationary phase and CH3CN/ aq. 0.1 % TFA gradient elution.

***tert*-Butyl N-[2-(N-9-fluorenylmethoxycarbonyl)aminoethyl] glycinate hydrochloride (1).** To a solution of **17** (78 g, 0.45 mol) in CH_2Cl_2 (3 L) was added diisopropylethylamine (75 mL, 0.43 mol) with mechanical stirring. A solution of N-(9-fluorenylmethoxycarbonyloxy)succinimide (145 g, 0.43 mol) in CH_2Cl_2 (800 mL) was added dropwise over 5 hours. The resulting solution was stirred ca. 12 h, and then washed with 1 N aq. HCl (5 x 500 mL), and brine (500 mL). The organic layer was dried (Na_2SO_4) and partially concentrated in vacuo (ca. 1 L). Cooling (ca.- 20 °C) overnight resulted in a precipitate which was collected by filtration and washed with CH_2Cl_2 until the filtrate was colorless. The solids were dried in vacuo (60 °C) to give the HCl salt of **1** (98 g, 53%) as a white solid. An analytically pure sample was obtained by dissolution in a minimum of warm acetone, precipitation with hexanes and drying in vacuo (60 °C) to give **1**•HCl as an amorphous white solid: ^1H NMR (d_6-DMSO) δ 9.22 (s, 2 H), 7.88 (d, *J* = 7 Hz, 2 H), 7.68 (d, *J* = 7 Hz, 2 H), 7.54 (t, *J* = 6 Hz, 1 H), 7.41 (t, *J* = 7 Hz, 2 H), 7.32 (t, *J* = 7 Hz, 2 H), 4.32 (d, *J* = 6 Hz, 2 H), 4.22 (t, *J* = 6 Hz, 1 H), 3.85 (s, 2 H), 3.32 (m, 2 H), 2.97 (t, *J* = 6 Hz, 2 H), 1.44 (s, 9 H); ^{13}C NMR (d_6-DMSO) δ 165.4, 156.0, 143.6, 140.5, 127.4, 126.9, 125.0, 119.9, 82.7, 65.4, 46.9, 46.4, 46.1, 36.3, 27.3; mass spectrum *m/z* 397 $(M+H)^+$. Anal. Calcd for $C_{23}H_{28}N_2O_4$•HCl: C, 63.81; H, 6.75; N, 6.47. Found C, 63.92; H, 6.85; N, 6.32.

The free base of **1** can be obtained in the following manner. The above HCl salt was dissolved in $CHCl_3$, washed with aq. saturated $NaHCO_3$, dried (Na_2SO_4), and concentrated in vacuo to give the free base of **1** as an oil: ^1H NMR ($CDCl_3$) δ 7.85 (d, *J* = 7 Hz, 2 H), 7.66 (d, *J* = 7 Hz, 2 H), 7.39 (t, *J* = 7 Hz, 2 H), 7.30 (t, *J* = 7 Hz, 2 H), 7.20 (t, *J* = 6 Hz, 1 H), 4.26 (d, *J* = 6 Hz, 2 H), 4.18 (t, *J* = 6 Hz, 1 H), 3.30 (s, 2 H), 3.03 (m, 2 H), 2.55 (t, *J* = 6 Hz, 2 H), 1.39 (s, 9 H); ^{13}C NMR ($CDCl_3$) δ 171.8, 156.6, 144.1, 141.3, 127.6, 127.0, 125.1, 119.9, 81.2, 66.3, 51.1, 48.5, 47.2, 40.6, 28.0.

1-(Carboxymethyl)-4-N-(benzyloxycarbonyl)cytosine (3). To a vigorously stirred suspension of **8** (10.50 g, 29.25 mmol) in anhyd CH_2Cl_2 (50 mL) was added 4 N HCl in 1,4-dioxane (100 mL). The reaction mixture was stirred ca. 16 h, and then partially concentrated in vacuo. Hexane (100 mL) was added and the thick suspension was filtered and air dried. The residue was exhaustively triturated with CH_2Cl_2 and dried in vacuo (60 °C) to give **3** (8.86 g, 100%) as a white solid: mp = 214 °C (d); ^1H NMR (d_6-DMSO) δ 8.02 (d, *J* = 7, 1 H), 7.38 (m, 5 H), 7.01 (d, *J* = 7, 1 H), 5.18 (s, 2 H), 4.51 (s, 2 H); ^{13}C NMR (d_6-DMSO) δ 169.4, 163.3, 154.9, 153.3, 150.9, 136.1, 128.7, 128.4, 128.2, 94.2, 66.8, 50.7; mass spectrum *m/z* 304.0935 ($C_{14}H_{13}N_3O_5$ +

H requires 304.0933). Anal. Calcd for $C_{14}H_{13}N_3O_5$: C, 55.45; H, 4.32; N, 13.66. Found: C, 55.53; H, 4.33; N, 13.89.

6-N-(Benzyloxycarbonyl)-9-(carboxymethyl)adenine (4). A solution of **11** (0.15 g, 0.39 mmol) in anhyd CH_2Cl_2 (3.1 mL) was treated with triethylsilane (0.60 mL), cooled to 0 °C and TFA (1.5 mL) was added slowly. After 5 min the reaction was allowed to warm to rt. After 7.5 h, HPLC showed the reaction to be complete. The mixture was evaporated to dryness in vacuo and any remaining volatiles removed by azeotroping with chloroform (3 x) to give **4** (0.13 g, 100%) as a white foam. An analytically pure sample of **4** was obtained by recrystallization from acetone: mp = 139-143 °C; ^{1}H NMR (d_6-DMSO) δ 10.68 (br s, 1 H), 8.60 (s, 1 H), 8.41 (s, 1 H), 7.29-7.45 (m, 5 H), 5.20 (s, 2 H), 5.06 (s, 2 H); ^{13}C NMR (d_6-DMSO) δ 168.8, 151.9, 151.3, 149.1, 144.5, 136.0, 128.1, 127.7, 127.5, 125.5, 122.7, 65.9, 43.9; mass spectrum *m/z* 328.1043 ($C_{15}H_{13}N_5O_4$ + H requires 328.1046). Anal. Calcd for $C_{15}H_{13}N_5O_4$: C, 55.05; H, 4.00; N, 21.40. Found: C, 54.93; H, 4.05; N, 21.47.

2-N-(Benzyloxycarbonyl)-9-(carboxymethyl)guanine (5). To a solution of **15** (8.4 g, 26 mmol) in CH_2Cl_2 (500 mL) and MeOH (50 mL) at -78 °C was introduced a stream of ozone until the solution turned brown/green. The excess ozone was removed by purging with N_2 and the reaction was quenched by the addition of methyl sulfide (5 mL). The reaction was maintained at -78 °C for 0.5 h, then allowed to warm slowly to rt (ca. 4 h). The mixture was concentrated in vacuo and the residue was suspended in THF (80 mL), *t*-butyl alcohol (160 mL), and 2-methyl-2-butene (26 mL). To the suspension was added an aq. solution (60 mL) of sodium chlorite (18.9 g, 209 mmol) and $NaH_2PO_4 \cdot H_2O$ (15.7 g, 114 mmol) dropwise. After 14 h at rt the mixture was concentrated in vacuo, and the residue was partitioned between 0.25 M aq. sodium hydroxide (600 mL) and ethyl acetate (300 mL). The aqueous layer was acidified to ca. pH 3 with concentrated HCl, and the resulting precipitate was filtered and dried to afford **5** (6.63 g, 75%) as a white powder. An analytically pure sample was obtained by precipitation from anhyd DMF with anhyd diethyl ether: mp = 214-216 °C; ^{1}H NMR (d_6-DMSO) δ 11.53 (s, 1 H), 11.37 (s, 1 H), 7.93 (s, 1 H), 7.45-7.35 (m, 5 H), 5.25 (s, 2 H), 4.87 (s, 2 H); ^{13}C (d_6-DMSO, 100 MHz) δ 169.5, 155.6, 149.7, 147.8, 140.7, 135.9, 128.9, 128.7, 128.4, 128.3, 119.7, 67.6, 44.8; mass spectrum, *m/z* 344.0995 ($C_{15}H_{13}N_5O_5$ + H requires 344.0995); Anal. Calcd for $C_{15}H_{13}N_5O_5$: C, 52.48; H, 3.82; N, 20.40. Found: C, 52.44; H, 3.91; N, 20.53.

4-*N*-(Benzyloxycarbonyl)cytosine (7). To a suspension of cytosine (10 g, 90 mmol) in anhyd pyridine (160 mL) was added DMAP (2.2 g, 18 mmol), followed by dropwise addition of benzyl chloroformate (28.3 mL, 198 mmol). The mixture was stirred for ca. 3 days, and then poured into ice water (350 mL) and stirred for 15 min. The resulting solid was collected by filtration and washed with water. Upon standing, additional material precipitated from the mother liquor which was also collected by filtration and washed with water. Drying in vacuo (50 °C) gave **7** (10.38 g, 43%) as a white solid: mp = >250 °C; ^{1}H NMR (d_6-DMSO) δ 11.1 (br s, 1 H), 7.80 (d, *J* = 7, 1 H), 7.37 (m, 5 H), 6.92 (d, *J* = 7, 1 H), 5.18 (s, 2 H); ^{13}C (d_6-DMSO, 100 MHz) d 163.6, 155.8, 153.4, 146.7, 136.0, 128.5, 128.1, 127.9, 93.5, 66.4; mass spectrum *m/z* 246 $(M+H)^+$. Anal. Calcd for $C_{12}H_{11}N_3O_3$: C, 58.77; H, 4.52; N, 17.13. Found: C, 58.89; H, 4.58; N, 16.97.

1-(*tert*-Butoxycarbonylmethyl)-4-N-(benzyloxycarbonyl)cytosine (8). To a suspension of **7** (30.0 g, 122.5 mmol) in anhyd DMF (500 mL) was added anhyd K_2CO_3 (16.9 g, 122.2 mmol) and anhyd Cs_2CO_3 (4.0 g, 12.2 mmol). After stirring 10 min, *tert*-butyl bromoacetate (20.8 mL, 129 mmol) was added dropwise, and the mixture was stirred for ca. 2 days. The resulting suspension was filtered and the filtrate concentrated in vacuo to give an oil. Dissolution of this oil in ethyl acetate and standing overnight at -20 ° C afforded a solid.

The solid was recrystallized from ethyl acetate/hexanes (2x) to afford **8** (25.85 g, 59%) as a white solid: mp = 168-169 °C; ^{1}H NMR ($CDCl_3$) δ 7.52 (s, 1 H), 7.50 (d, *J* = 7, 1 H), 7.36 (s, 5 H), 7.22 (d, *J* = 7, 1 H), 5.20 (s, 2 H), 4.50 (s, 2 H), 1.44 (s, 9 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 166.4, 162.7, 155.5, 152.2, 149.0, 134.9, 128.7, 128.3, 94.9, 83.3, 68.0, 51.3, 28.0; mass spectrum *m/z* 360 $(M+H)^+$. Anal. Calcd for $C_{18}H_{21}N_3O_5$: C, 60.16; H, 5.89; N, 11.69. Found: C, 59.95; H, 5.91; N, 11.81.

6-N-(Benzyloxycarbonyl)adenine (10). Sodium hydride (6.07 g, 152 mmol, 60% dispersion in oil) was washed with petroleum ether (3 x). After cooling in an ice bath, anhyd DMF (150 mL) was added, followed by adenine (5.00 g, 37.0 mmol) in small portions. The suspension was stirred vigorously for 3 min and then benzyl chloroformate (11.6 mL, 81.5 mmol) was added dropwise. After stirring for 4 h the reaction mixture was poured into ice water (300 mL) and the pH adjusted to 7 with 1 N aq. HCl. The light yellow precipitate was collected by filtration and washed with water, and ether to afford crude product (8.46 g). Recrystallization from methanol/chloroform afforded two crops of **10** (4.23 g, 44%) as a white solid: mp = 222 °C (d); ^{1}H NMR (d_6-DMSO) δ 12.4 (br s, 1 H), 11.0 (br s, 1 H), 8.6 (s, 1 H), 8.4 (s, 1 H), 7.5-7.3 (m, 5 H), 5.3 (s, 2 H); ^{13}C NMR (d_6-DMSO, 0.1% DCl, 100 MHz) δ 153.5, 152.5, 150.5, 147.0, 144.7, 144.6, 135.5, 129.0, 128.6, 113.4, 69.3; mass spectrum *m/z* 270 $(M+H)^+$. Anal. Calcd for $C_{13}H_{11}N_5O_2$: C, 57.99; H, 4.12; N, 26.01. Found: C, 57.82; H, 4.12; N, 25.96.

6-N-(Benzyloxycarbonyl)-9-(*tert*-butoxycarbonylmethyl)adenine (11). To a suspension of **10** (0.50 g, 1.9 mmol) in anhyd DMF (4 mL) was added anhyd Cs_2CO_3 (0.06 g, 0.19 mmol) and anhyd K_2CO_3 (0.26 g, 1.9 mmol). After stirring for 5 min, *tert*-butyl bromoacetate (0.33 mL, 2.1 mmol) was added dropwise. After stirring for ca.23 h, the mixture was evaporated to dryness in vacuo and the residue partitioned between ethyl acetate (35 mL) and water (10 mL). The organic phase was washed with water and brine, dried (Na_2SO_4) and concentrated in vacuo. The residue was crystallized from ethyl acetate/hexane to afford **11** (0.38 g, 53%) as a white solid: mp = 141-142 °C; ^{1}H NMR ($CDCl_3$) δ 8.78 (s, 1 H), 8.5 (br s, 1 H), 8.0 (s, 1 H), 7.5-7.3 (m, 5 H), 5.3 (s, 2 H), 4.86 (s, 2 H), 1.5 (s, 9 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 165.7, 153.0, 151.5, 151.0, 149.5, 143.3, 135.4, 128.7, 128.6, 128.5, 121.3, 83.8, 67.7, 44.8, 27.9; mass spectrum *m/z* 384 $(M+H)^+$. Anal. Calcd for $C_{19}H_{21}N_5O_4$: C, 59.52; H, 5.52; N, 18.27. Found: C, 59.61; H, 5.50; N, 18.34.

2-Amino-6-chloro-9-allylpurine (13). To a solution of 2-amino-6-chloropurine (10.0 g, 58.9 mmol) in DMF (100 mL) was added K_2CO_3 (24.3 g, 176 mmol). After 10 min, allyl bromide (5.35 mL, 61.8 mmol) was added in one portion. The resulting solution was vigorously stirred for 15 min, then warmed in a 50 °C oil bath for ca. 16 h. The reaction was concentrated in vacuo, and partitioned between ethyl acetate (750 mL) and water (200 mL). The organic phase was washed with brine, dried (Na_2SO_4) and concentrated in vacuo. Flash chromatography (ethyl acetate/ hexane, gradient elution) afforded **13** (7.35 g, 59%) as a white solid: mp = 148-151 °C; ^{1}H NMR ($CDCl_3$) δ 7.78 (s, 1 H), 6.01 (ddt, *J* = 17.1, 10.3, 5.6 Hz, 1 H), 5.41 (br s, 2 H), 5.32 (d, *J* = 10.3 Hz, 1 H), 5.20 (d, *J* = 17.1 Hz, 1 H), 4.70 (d, *J* = 5.7 Hz, 2 H); ^{13}C NMR ($CDCl_3$) δ 159.2, 153.6, 151.2, 142.1, 131.3, 125.0, 119.1, 45.6; mass spectrum *m/z* 210 $(M+H)^+$; Anal. Cald for $C_8H_8ClN_5$: C, 45.84; H, 3.85; N, 33.41. Found: C, 45.84; H, 3.89; N, 33.29.

9-Allylguanine (14). A solution of **13** (7.35 g, 35.0 mmol) in 1 N aq. HCl (200 mL) was warmed to a gentle reflux for 2 h. After cooling to rt, the pH of the mixture was adjusted to ca. 7 with solid NaOH, and then cooled in an ice bath for 0.5 h. The precipitate was colleceted by filtration and dried in vacuo to afford **14** (6.72 g, 100%) as a white solid: ^{1}H NMR (d_6-DMSO, 400 MHz) δ 10.57 (br s, 1 H), 7.64 (s, 1 H), 6.45 (br s, 2 H), 5.99 (ddt, *J* = 17.1, 10.4, 5.3 Hz, 1 H), 5.15 (d, *J* = 10.4 Hz, 1 H), 4.94 (d, *J* = 17.1 Hz, 1 H), 4.57 (d, *J* = 5.1

Hz, 2 H); ^{13}C NMR (d_6-DMSO, 100 MHz) δ 156.8, 153.6, 151.1, 137.3, 133.6, 116.9, 116.4, 44.6; mass spectrum *m/z* 192 $(M+H)^+$; Anal. Cald for $C_8H_9N_5O$: C, 50.26; H, 4.74; N, 36.63. Found: C, 50.11; H, 4.82; N, 36.47.

2-N-(Benzyloxycarbonyl)-9-allylguanine (15). To a solution of **14** (6.72 g, 35.1 mmol) in THF (75 mL) was added N-(benzyloxycarbonyl)imidazole[9] (8.52 g, 42.1 mmol) and 18-crown-6 (9.72 g, 36.8 mmol). After 15 min, potassium hydride (35% dispersion in oil, 4.83 g, 42.1 mmol) was added dropwise. The resulting solution was stirred at rt for 2 h then concentrated in vacuo. The residue was partitioned between ethyl acetate (750 mL) and a 1:1:1 mixture of water/brine/saturated aqueous NH_4Cl (200 mL). The aqueous layer was back extracted with ethyl acetate (100 mL) and the combined organics were washed with brine (100 mL) and dried (Na_2SO_4). The organics were concentrated in vacuo to ca. 300 mL and placed in a -20 °C freezer. After 20 h, the precipitate was collected by filtration. The mother liquors were concentrated onto 50 g of silica gel. Flash chromatography (hexane/ethyl acetate, gradient elution) provided more solids. The combined solids were recrystallized from acetone to afford **15** (10.4 g, 91%) as a white solid: mp = 171-173 °C; ^1H NMR ($CDCl_3$) δ 11.31 (br s, 1 H), 8.52 (br s, 1 H), 7.57 (s, 1 H), 7.36 (s, 5 H), 5.98-5.85 (ddt, *J* = 17.1, 10.4, 5.6 Hz, 1 H), 5.26 (d, *J* = 10.2 Hz, 1 H), 5.24 (s, 2 H), 5.12 (d, *J* = 17.1 Hz, 1 H), 4.57 (d, *J* = 5.6 Hz, 2 H); ^{13}C NMR ($CDCl_3$) δ 155.6, 153.4, 148.5, 146.5, 138.6, 134.3, 131.6,128.9, 128.7, 128.6, 120.6, 118.9, 68.6, 45.7; mass spectrum *m/z* 326 $(M+H)^+$. Anal. Calcd for $C_{16}H_{15}N_5O_3$: C, 59.07; H, 4.65; N, 21.53. Found: C, 59.17; H, 4.71; N, 21.59.

***tert*-Butyl N-(2-aminoethyl)glycinate (17).** To a vigorously stirred solution of ethylenediamine (300 mL, 4.5 mol) in CH_2Cl_2 (2 L) at 0 °C was added *tert*-butyl bromoacetate (82.8 mL, 0.51 mol) in CH_2Cl_2 (400 mL) over 5 h. The resulting mixture was allowed to warm slowly to rt (ca. 3 h), and then stirred overnight. The reaction mixture was washed with water (3 x 500 mL), and the combined aqueous wash was back-extracted with CH_2Cl_2 (500 mL). The combined organics were dried (Na_2SO_4) and filtered. This solution was used directly in the next step. An analytical sample was obtained by concentration of a 1 mL aliquot to dryness in vacuo (0.026 g/mL x 3000 mL = 78 g, ca. 87%): ^1H NMR ($CDCl_3$) d 3.27 (s, 2 H), 2.75 (t, *J* = 9 Hz, 2 H), 2.62 (t, *J* = 9 Hz, 2 H), 1.51 (s, 3 H), 1.42 (s, 9 H); mass spectrum *m/z* 175 $(M+H)^+$.

***tert*-Butyl N-[2-(N-9-fluorenylmethoxycarbonyl)aminoethyl]-N-[(thymin-1-yl)acetyl]glycinate (18).** To a solution of **1** (36.6 g, 91 mmol) in anhyd DMF (500 mL) was added the acid **2** (36.0 g, 196 mmol), and the mixture was stirred until most of the acid dissolved. EDC (36.0 g, 188 mmol) was added in two equal portions over 30 min, and the mixture was stirred overnight. The solution was concentrated in vacuo, and to the resulting thick oil was added water (1 L). The mixture was shaken vigorously for several minutes to precipitate a fine white solid. The solids were collected by filtration, stirred with water (1 L), filtered, washed with cold water, and dried in vacuo. Purification by flash chromatography (5% MeOH/CH_2Cl_2) gave **18** (42.41 g, 83%) as a white solid. An analytically pure sample of **18** was obtained by recrystallization from CH_2Cl_2/ethyl acetate/hexanes: mp = 167.5-168.5 °C; ^1H NMR ($CDCl_3$) (two rotomers) δ 8.56 (d, *J* = 12 Hz, 1 H), 7.75 (d, *J* = 7 Hz, 2 H), 7.60 (t, *J* = 7 Hz, 2 H), 7.40 (t, *J* = 7 Hz, 2 H), 7.31 (t, *J* = 7 Hz, 2 H), 6.98 (s, 0.4 H), 6.86 (s, 0.6 H), 6.03 (t, *J* = 5 Hz, 0.6 H), 5.44 (t, *J* = 5 Hz, 0.4 H), 4.51-4.34 (m, 3 H), 4.22 (t, *J* = 6 Hz, 1 H), 4.09 (s, 0.4 H), 3.95 (s, 0.6 H), 3.58-3.36 (m, 4 H), 1.89 and 1.87 (rotomer s, 3H), 1.50 and 1.47 (rotomer s, 9H); ^{13}C NMR ($CDCl_3$) δ 168.8, 163.8, 156.7, 150.7, 143.8, 141.1, 127.8, 127.1, 125.0, 120.0, 110.6, 83.8, 82.8, 66.7, 51.2, 49.9, 49.0, 47.6, 47.2, 39.3, 28.0; mass spectrum *m/z* 563 $(M+H)^+$. Anal. Calcd for $C_{30}H_{34}N_4O_7$: C, 64.04; H, 6.09; N, 9.96. Found; C, 64.07; H, 6.10; N, 9.98.

N-[2-(N-9-Fluorenylmethoxycarbonyl)aminoethyl]-N-[(thymin-1-yl)acetyl]glycine (19). To a suspension of **18** (25.0 g, 44.4 mmol) in anhyd CH_2Cl_2 (200 mL) was added 4 N HCl in 1,4-dioxane (300 mL) and the mixture was stirred overnight. The solid was collected by filtration, washed with hexane and dried in vacuo to give **19** (22.5 g, 100%) as a white solid: mp = 216-219 °C; ^{1}H NMR (d_6-DMSO) (two rotomers) δ 11.23 (s, 1 H), 7.9-7.2 (m, 10 H), 4.41 (s, 1.2 H), 4.61 (s, 0.8 H), 4.35-4.18 (m, 3 H), 3.95 (s, 2 H), 3.05-3.35 (m, 4 H), 1.970 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 171.2, 170.8, 168.0, 167.6, 164.8, 156.7, 156.5, 151.3, 144.2, 142.4, 141.1, 127.9, 127.4, 125.4, 120.4, 108.4, 65.7, 48.0, 47.0, 12.1; mass spectrum *m/z* 507.1882 ($C_{26}H_{26}N_4O_7$ + H requires 507.1880). Anal. Calcd for $C_{26}H_{26}N_4O_7$: C, 61.65; H, 5.17; N, 11.06. Found: C, 61.40; H, 5.19; N, 10.94.

Pentafluorophenyl N-[2-(N-9-fluorenylmethoxycarbonyl)aminoethyl]-N-[(thymin-1-yl)acetyl] glycinate (20). To a solution of **19** (5.00 g, 9.87 mmol) in DMF (40 mL) was added pyridine (1.00 mL, 12.4 mmol) and pentafluorophenyl trifluoroacetate (2.12 mL, 12.4 mmol). The mixture was stirred at rt for 3 h. The reaction was diluted with ethyl acetate (500 mL), washed with 0.1 N aq. HCl (3 x 500 mL), 5% aq. $NaHCO_3$ (3 x 500 mL), brine (200 mL), dried (Na_2SO_4) and concentrated. The resulting paste was suspended in ethyl acetate and filtered through a plug of silica gel eluting with ethyl acetate. Concentration of the filtrate, trituration of the resulting solid with ethyl acetate and drying in vacuo to give **20** (5.37 g, 81%) as a white solid. An analytically pure sample of **20** was obtained by recrystallization from acetone/ether: mp = 177.5-179 °C; ^{1}H NMR ($CDCl_3$) δ 9.03 (br s, 1 H), 7.75-7.26 (m, 8 H), 6.96 (s, 0.2 H) 6.90 (s, 0.8 H), 5.79 (br t, 0.8 H), 5.40 (br t, 0.2 H), 4.60-4.41 (m, 5 H), 4.22-4.15 (m, 2 H), 3.64 (br s, 2 H), 3.46 (br m, 2 H), 1.90 (s, 3 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 167.7, 165.8, 164.3, 156.9, 151.2, 143.8, 143.7, 141.2, 141.0, 127.7, 125.0, 119.9, 110.9, 66.8, 48.5, 48.3, 47.8, 47.2, 39.4, 12.3; mass spectrum, *m/z* 673.1716 ($C_{32}H_{25}F_5N_4O_7$ + H requires 673.1722). Anal. Calcd for $C_{32}H_{25}F_5N_4O_7$: C, 57.15; H, 3.75; N, 8.33. Found: C, 56.81; H, 3.82; N, 8.22.

***tert*-Butyl N-[2-(N-9-fluorenylmethoxycarbonyl)aminoethyl]-N-[[4-N-(benzyloxycarbonyl)cytosin-1-yl]acetyl]glycinate (21).** To a solution of **1** (15 g, 38 mmol) in anhyd DMF (115 mL) at 0 °C was added **3** (12.74 g, 42 mmol) follwed by EDC (16.10 g, 84 mmol) in one portion. The solution was allowed to warm to rt, and stirred ca. 12 h. The resulting mixture was poured into ice water (2 L) with vigorous stirring. The resulting precipitate was collected by filtration, washed with cold water (3 x 500 mL) and dried in vacuo. The resulting solid was dissolved in warm CH_2Cl_2 and precipitated with hexane. The solid was collected and dried in vacuo to give **21** (20.34 g, 79%) as an off-white powder. An analytically pure sample of **21** was obtained by recrystallization from CH_2Cl_2/MeOH/ethyl acetate: mp = 194-196 °C; ^{1}H NMR (d_6-DMSO) (two rotomers) δ 10.78 (br s, 1 H), 7.9-7.8 (m, 3 H), 7.65 (d, J = 7 Hz, 2 H), 7.5-7.2 (m, 10 H), 7.00 (br m, 1 H), 5.19 (s, 2 H), 4.80 (s, 1.5 H), 4.59 (s, 0.5 H), 4.36-4.17 (m, 3 H), 3.92 (s, 1.5 H), 3.84 (s, 0.5 H), 3.50-2.90 (m, 4 H), 1.47 (s, 4.5 H), 1.35 (s, 4.5 H); ^{13}C NMR (d_6-DMSO) (two rotomers) δ 163.1, 154.4, 153.2, 150.9, 143.9, 140.7, 139.8, 137.4, 136.0, 128.9, 128.5, 128.1, 127.9, 127.6, 127.2, 127.0, 125.1, 120.1, 120.0, 109.8, 93.8, 80.9, 66.5, 65.5, 49.7, 27.7; mass spectrum *m/z* 682 $(M+H)^+$. Anal. Calcd for $C_{37}H_{39}N_5O_8$: C, 65.19; H, 5.77; N, 10.27. Found: C, 64.95; H. 5.75; N, 10.23.

N-[2-(N-9-fluorenylmethoxycarbonyl)aminoethyl]-N-[[4-N-(benzyloxycarbonyl)cytosin-1-yl]acetyl]glycine (22). To a suspension of **21** (10.0 g, 14.7 mmol) in CH_2Cl_2 (20 mL) at 0 °C was added TFA (100 mL). The reaction was stirred for 30 min at 0 °C and then warmed to rt. Upon complete comsumption of **21** (HPLC) the reaction was concentrated to one fifth volume in vacuo and the resulting

residue was added dropwise to anhyd Et_2O (1 L) with stirring. The resulting solid was collected by filtration, and purified by flash chromatography (10% MeOH/CH_2Cl_2). The combined fractions were concentrated to one-fourth original volume, and hexane was added to precipitate the acid. The resulting solids were collected by filtration and dried in vacuo to give **22** (7.9 g, 86%) as an amorphous white solid: ^{1}H NMR (d_6-DMSO) (two rotomers) δ 10.78 (br s, 1 H), 7.9-7.8 (m, 3 H), 7.65 (d, *J* = 7 Hz, 2 H), 7.5-7.2 (m, 10 H), 7.00 (br m, 1 H), 5.19 (s, 2 H), 4.78 (s, 1 H), 4.60 (s, 1 H), 4.32-4.20 (m, 3 H), 4.02 (s, 1 H), 3.90 (s, 1 H), 3.45-3.08 (m, 5 H); ^{13}C NMR (d_6-DMSO, 100 MHz) (two rotomers) δ 171.0, 170.5, 167.5, 167.1, 163.2, 163.1, 156.4, 156.1, 155.0, 153.2, 150.9, 143.9, 140.7, 136.0, 128.5, 128.1, 127.9, 127.6, 127.1, 125.2, 120.1, 93.9, 66.5, 65.5, 49.6, 49.5, 47.8, 46.9, 46.7; mass spectrum *m/z* 626.2230 ($C_{33}H_{31}N_5O_8$ + H requires 626.2251). Anal. Calcd for $C_{33}H_{31}N_5O_8$: C, 63.35; H, 4.99; N, 11.19. Found: C, 63.13; H. 5.04; N, 11.24.

Pentafluorophenyl N-[2-(N-9-fluorenylmethoxycarbonyl)aminoethyl]-N-[[4-N-(benzyloxycarbonyl)cytosin-1-yl]acetyl]glycinate (23). To a suspension of **22** (10.0 g, 16 mmol) in anhyd DMF (65 mL) was added pyridine (1.5 mL, 18.2 mmol) and pentafluorophenyl trifluoroacetate (3.3 mL, 19.2 mmol). After stirring for 3 h, the resulting solution was poured into ice water (700 mL) with stirring. The resultant precipitate was collected, washed with water (2 x 100 mL) and diethyl ether (4 x 100 mL), and dried in vacuo to give **23** (10.3 g, 81%) as a white solid: mp = 196-198 °C; ^{1}H (d_6-DMSO) δ 7.91-7.86 (m, 3 H), 7.68 (d, *J* = 7 Hz, 2 H), 7.42-7.29 (m, 10 H), 7.01 (d, *J* = 7 Hz, 1 H), 5.18 (s, 2 H), 4.82 (s, 1 H), 4.63 (s, 1 H), 4.34-4.20 (m, 4 H), 4.00 (s, 1 H), 3.44 (t, *J* = 6 Hz, 1 H), 3.37-3.29 (m, 2 H), 3.11 (q, *J* = 5 Hz, 1 H); ^{13}C NMR (d_6-DMSO) δ 170.9, 170.6, 167.7, 167.2, 156.5, 155.1, 155.3, 151.0, 144.0, 140.8, 136.1, 128.6, 128.2, 128.0, 127.7, 127.2, 125.2, 120.2, 93.9, 66.5, 65.6, 65.5, 49.5, 47.7, 46.9, 46.7; mass spectrum, *m/z* 792.2088 ($C_{39}H_{30}F_5N_5O_8$ + H requires 792.2093). Anal. Calcd for $C_{39}H_{30}F_5N_5O_8$: C, 59.17; H, 3.82; N, 8.85. Found: C, 58.92; H, 3.89; N, 8.86.

***tert*-Butyl N-[2-(N-9-fluorenylmethoxycarbonyl)aminoethyl]-N-[[6-N-(benzyloxycarbonyl)adenin-9-yl]acetyl]glycinate (24).** To a solution of **1** (9.98 g, 25.2 mmol) in anhyd DMF (125 mL), was added a solution of **4** (8.24 g, 25.2 mmol) in anhyd DMF (50 mL), BOP reagent (12.8 g, 29.0 mmol), HOBT (3.91 g, 29.0 mmol) and diisopropylethylamine (8.77 mL, 50.4 mmol). After stirring at rt for 3.5 h the reaction was judged incomplete by HPLC and additional BOP reagent (2.78 g, 6.3 mmol), HOBT (0.85 g, 6.3 mmol), and diisopropylethylamine (2.00 mL, 11.3 mmol) were added and the mixture was stirred an additional 3 h. The mixture was concentrated in vacuo and the residue was partitioned between ethyl acetate (300 mL) and half saturated brine (300 mL), and the aqueous phase was extracted with ethyl acetate (4 x 300 mL). The combined organic extract was washed with 1 N aq. HCl (2 x 150 mL), sat. aq. $NaHCO_3$ (2 x 150 mL), brine (1 x 150 mL), dried ($MgSO_4$) and concentrated in vacuo. The resulting solid was purified by flash chromatography eluting with 95:5 CH_2Cl_2/CH_3OH to give **24** (13.95 g, 78%) as a brittle white foam: ^{1}H NMR (d_6-DMSO) δ 10.68 (s, 1 H),8.56 (d, *J* = 12.1 Hz, 1 H), 8.32 (br s, 1 H), 7.88 (s, 1 H), 7.86 (s, 1 H), 7.67 (m, 2 H), 7.52-7.28 (m, 9 H), 5.34 (s, 1 H), 5.22 (s, 2 H), 5.15 (s, 1 H), 4.39-4.20 (m, 4 H), 3.97 (s, 1 H), 3.55 (br t, 1 H), 3.39 (m, 1 H), 3.13 (br q, 1 H), 1.49 (s, 3 H), 1.36 (s, 6 H); ^{13}C NMR (d_6-DMSO) δ 168.5, 167.9, 167.0, 166.6, 156.4, 152.4, 152.2, 151.5, 149.4, 145.1, 143.8, 140.7, 136.4, 128.4, 128.0, 127.8, 127.6, 127.2, 125.1, 122.9, 120.1, 82.1, 81.0, 66.2, 65.5, 48.8, 47.1, 46.7, 27.6; mass spectrum *m/z* 706.2986 ($C_{38}H_{40}N_7O_7$ + H requires 706.2989); Anal. Calcd for $C_{38}H_{40}N_7O_7$: C, 64.58; H, 5.70; N, 13.87. Found: C, 64.29; H, 5.67; N, 13.78.

N-[2-(N-9-Fluorenylmethoxycarbonyl)aminoethyl]-N-[[6-N-(benzyloxycarbonyl)adenin-9-yl]acetyl]glycine (25). To **24** (14.4 g, 20.4 mmol) was added CH_2Cl_2 (160 mL) and triethylsilane (31 mL).

The mixture was cooled to 0 °C and TFA (78 mL) was added over 5 min. After 15 min the reaction was allowed to warm to rt. After 6 hr, the mixture was concentrated in vacuo to give a foam which was recrystallized from acetone/hexanes, and the resulting solid was dried in vacuo (85 °C) to afford **25** (11.74 g, 88%) as a white solid. An analytically pure sample of **25** was obtained by recrystallization from acetone/hexanes: mp = 198-200 °C; ^{1}H NMR (d_7-DMF) (two rotomers) δ 10.57 (br s, 1 H), 8.56 (s, 0.4 H), 8.50 (s, 0.6 H), 8.37 (s, 1 H), 7.92 (d, *J* = 8 Hz, 2 H), 7.72 (t, *J* = 8 Hz, 2 H), 7.59-7.30 (m, 10 H), 5.51 (s, 1.2 H), 5.35 (s, 0.8 H), 5.29 (s, 2 H), 4.56 (s, 0.8 H), 4.40 (d, *J* = 7 Hz, 1.2 H), 4.34 - 4.25 (m, 1.8 H), 4.19 (s, 1.2 H), 3.78 (t, *J* = 5 Hz, 1.2 H), 3.60 - 3.28 (m, 2.8 H); ^{13}C NMR (d_7-DMF) (two rotomers) δ 171.7, 171.1, 167.9, 167.3, 162.9, 157.5, 157.2, 153.2, 152.0, 149.8, 146.1, 144.8, 141.7, 137.1, 129.1, 128.6, 128.5, 128.3, 128.2, 127.7, 125.9, 122.1, 122.0, 120.7, 120.6, 67.4, 66.8, 66.7, 65.8, 50.0, 48.4, 48.2, 48.0, 47.7, 45.1, 44.8, 39.7, 39.0, 15.4; mass spectrum *m/z* 650 $(M+H)^+$. Anal. Calcd for $C_{34}H_{31}N_7O_7$: C, 62.86; H, 4.81; N, 15.09. Found: C, 62.89; H, 4.87; N, 15.17.

Pentafluorophenyl N-[2-(N-9-fluorenylmethoxycarbonyl)aminoethyl]-N-[[6-N-(benzyloxycarbonyl)adenin-9-yl]acetyl]glycinate (26). To a suspension of **25** (1.68 g, 2.60 mmol) in anhyd DMF (10 mL) was added pyridine (0.23 mL, 2.8 mmol) and pentafluorophenyl trifluoroacetate (0.53 mL, 3.1 mmol). After 2 hr, the reaction was diluted with ethyl acetate (250 mL) and washed with 0.1 N aq. HCl (3 x 100 mL), half sat. aq. $NaHCO_3$ (2 x 100 mL), brine (100 mL), dried (Na_2SO_4) and concentrated in vacuo to afford **26** (2.10 g, 99%) as a brittle white foam: ^{1}H NMR ($CDCl_3$, 400 MHz) (two rotomers) δ 8.63 (s, 1 H), 8.47 (s, 1 H), 7.91 (s, 1 H), 7.68 (d, *J* = 7.5 Hz, 2 H), 7.52 (d, *J* = 7.4 Hz, 2 H), 7.42 (d, *J* = 7.0 Hz, 2 H), 7.35 (m, 5 H), 7.23 (m, 4 H), 5.73 (br t, 1 H), 5.28 (s, 2 H),4.53 (d, *J* = 6.0 Hz, 2 H), 4.40 (s, 1 H), 4.17 (br t, 2 H), 3.71 (br s, 1 H), 3.50 (d, *J* = 6.0 Hz, 2 H); ^{13}C NMR ($CDCl_3$, 100 MHz)) (two rotomers) δ 166.7, 165.9, 156.8, 152.8, 151.1, 150.7, 149.2, 143.7, 143.5, 141.3, 135.3, 128.6, 128.5, 127.8, 127.0, 120.9, 120.0, 67.8, 66.6, 49.0, 48.4, 47.2, 43.5, 39.5, 30.9; mass spectrum, *m/z* 816.2217 ($C_{40}H_{30}F_5N_7O_7$ + H requires 816.2205).

***tert*-Butyl N-[2-(N-9-fluorenylmethoxycarbonyl)aminoethyl]-N-[[2-N-(benzyloxycarbonyl)guanin-9-yl]acetyl]glycinate (27).** To a solution of **1** (10.0 g, 25.3 mmol) in DMF (100 mL) was added **5** (8.66 g, 25.3 mmol). Upon complete dissolution, BOP reagent (13.96 g, 31.6 mmol), HOBT (4.26 g, 31.6 mmol) and diisopropylethylamine (4.90 g, 37.9 mmol) were added sequentially. The resulting solution was stirred at rt for 3.5 h. The crude reaction mixture was concentrated in vacuo and the residue was partitioned between ethyl acetate (200 mL) and brine (300 mL). The layers were separated and the aqueous layer was back extracted with ethyl acetate (3 x 150 mL). The combined organic extract was washed with 0.1 N aq. HCl (2 x 200 mL), sat. aq. $NaHCO_3$ (2 x 200 mL) and brine (1 x 300 mL). Following the brine wash, a solid formed in the organic layer, which was filtered and washed with ethyl acetate. The filtrate was stored at -20 °C for 16 h. The resulting solid was filtered and combined with the first filtration product to provide an off white solid. The crude solid was dissolved in MeOH and concentrated onto silica gel (100 g). Flash chromatography (MeOH/ethyl acetate gradient) provided **27** (9.97 g, 55%) as a white solid: mp = 151-153 °C; ^{1}H NMR (d_6-DMSO) (two rotomers) δ 11.38 (br s, 1 H), 7.84 (m, 3 H), 7.64 (d, *J* = 6 Hz, 2 H), 7.53 (br s, 1 H), 7.35-7.27 (m, 9 H), 5.21 (s, 2 H), 5.09 (s, 1 H), 4.91 (s, 1 H), 4.32-4.19 (m, 3 H), 3.99 (s, 1 H), 3.48-3.32 (m, 4 H), 3.09 (m, 1 H), 1.44 (s, 3 H), 1.40 (s, 6 H); ^{13}C NMR (d_6-DMSO) δ 168.9, 168.2, 167.3, 166.8, 156.5, 156.3, 155.5, 154.8, 149.6, 147.6, 143.9, 140.8, 140.6, 135.6, 129.0, 128.6, 128.4, 128.2, 127.7, 127.4, 127.1, 125.1, 121.5, 120.2, 119.2, 109.8, 82.1, 81.1, 67.1, 65.5, 50.1, 48.8, 47.1, 46.7, 43.8, 38.0, 27.7; mass spectrum, *m/z* 722.2940 ($C_{38}H_{39}N_7O_8$ + H requires 722.2938).

N-[2-(N-9-Fluorenylmethoxycarbonyl)aminoethyl]-N-[[2-N-(benzyloxycarbonyl)guanin-9-yl]acetyl]glycine (28). To a suspension of **27** (8.10 g, 11.2 mmol) in CH_2Cl_2 (40 mL) was added TFA (25 mL). The resulting suspension was stirred at rt for 15 h upon which all solids were dissolved. Acetonitrile (50 mL) was added, and the mixture was concentrated to half volume. Concentration from CH_3CN was repeated three times, then diethyl ether (75 mL) was added. The resulting solid was filtered and washed with Et_2O. This solid was dried in vacuo (40 °C) to provide **28** (7.45 g, 100%) as a white solid. An analytically pure sample was obtained by precipitation from methanol with diethyl ether to give an amorphous solid: ^1H NMR (d_6-DMSO) (two rotomers) δ 11.63 (s, 0.5 H), 11.51 (s, 0.5 H), 8.03 (d, J = 5 Hz, 2 H), 7.85 (d, J = 7 Hz, 2 H), 7.64 (t, J = 7 Hz, 2 H), 7.48-7.26 (m, 9 H), 5.22 (s, 2 H), 5.11 (s, 1 H), 4.96 (s, 1 H), 4.37-4.26 (m, 3 H), 4.20 (t, J = 6 Hz, 1 H), 4.01 (s, 1 H), 3.47 (m, 1 H), 3.33 (m, 2 H), 3.11 (q, J = 7 Hz, 1 H); ^{13}C NMR (d_6-DMSO) δ 171.0, 170.5, 167.0, 166.5, 156.5, 156.3, 154.8, 154.7, 149.4, 147.7, 143.9, 140.9, 140.8, 135.5, 128.6, 128.4, 128.2, 127.7, 127.1, 125.2, 125.1, 120.2, 118.1, 67.3, 65.5, 49.2, 47.9, 47.0, 46.8, 44.3, 44.1, 37.9; mass spectrum, *m/z* 666.2310 ($C_{34}H_{31}N_7O_8$ + H requires 666.2312); Anal. Calcd for $C_{34}H_{31}N_7O_8 \cdot H_2O$: C, 59.73; H, 4.87; N, 14.34. Found: C, 59.87; H, 4.82; N, 14.61.

Pentafluorophenyl N-[2-(N-9-fluorenylmethoxycarbonyl)aminoethyl]-N-[[2-N-(benzyloxycarbonyl)guanin-9-yl]acetyl]glycinate (29). To a solution of **28** (1.00 g, 1.54 mmol) in DMF (4 mL) was added pyridine (0.134 mL, 1.64 mmol) and pentafluorophenyl trifluoroacetate (0.30 mL, 1.78 mmol). The resulting solution was stirred at rt for 2 h. The mixture was poured into cold water and the resulting solid was filtered, washed with cold water, then diethyl ether to provide an off-white solid, which was purified by precipitation from anhyd DMF with anhyd diethyl ether to give **29** (0.99 g, 77%) as a white solid: mp = 210-212 °C; ^1H NMR (d_6-DMSO) (two rotomers) δ 11.35 (s, 0.6 H), 11.31 (s, 0.4 H), 7.85 (d, J = 7 Hz, 2 H), 7.81 (s, 1 H), 7.65 (d, J = 7 Hz, 2 H), 7.48 (t, J = 5 Hz, 1 H), 7.39-7.34 (m, 7 H), 7.28 (t, J = 7 Hz, 2 H), 5.21 (s, 2 H), 5.12 (s, 1.5 H), 5.02 (s, 0.5 H), 4.53 (s, 2 H), 4.37 (d, J = 6 Hz, 1.5 H), 4.29 (d, J = 4 Hz, 0.5 H), 4.20 (m, 1 H), 3.62 (m, 2 H), 3.36 (m, 2.5 H), 3.16 (m, 0.5 H); ^{13}C NMR (d_6-DMSO) δ 171.0, 170.5, 167.2, 166.6, 156.5, 156.2, 155.2, 154.7, 154.6, 149.6, 149.5, 147.5, 147.2, 143.9, 140.8, 140.5, 135.5, 128.6, 128.4, 128.2, 128.1, 127.7, 127.0, 125.2, 125.1, 120.2, 119.2, 67.2, 65.4, 49.2, 47.9, 46.9, 46.7, 43.8, 37.8; mass spectrum, *m/z* 832.2158 ($C_{40}H_{30}F_5N_7O_8$ + H requires 832.2154): Anal. Calcd for $C_{40}H_{30}F_5N_7O_8$: C, 57.77; H, 3.64; N, 11.79. Found: C, 57.56; H, 3.69; N, 11.85.

Oligomer synthesis. Oligomer synthesis were performed on an ABI 431A peptide synthesizer. The standard small scale (0.05 mmol) cycles provided by ABI were altered. In general a typical synthesis consisted of the following. The resin was swollen by several washes with 20% DMSO in *N*-methylpyrrolidinone (NMP), the Fmoc group of the resin or growing peptide chain was removed by treatment with 30% piperidine in 20% DMSO/NMP (3 x 5 min), and the resin was rinsed with 20% DMSO/NMP. The monomers **20**, **23**, and **26** (1 x 0.1 mmol) were loaded into cartridges as solids and transfer to the resin with 20% DMSO/NMP (1.5 mL), whereas **29** (1 x 0.1 mmol) was dissolved in NMP (0.5 mL) prior to loading on the machine and was transferred to the resin with 20% DMSO/NMP (1 mL). After transfer of the monomer to the resin, coupling proceeded for 30 min. The resin was rinsed with 20% DMSO/NMP, and then treated with the capping solution of 0.5 M acetic anhydride/0.5 M DIEA in NMP (1.5 mL) twice for 15 min. This cycle was repeated for each base. After the last coupling the Fmoc deprotection step was omitted, and the resin was rinsed with 20% DMSO/NMP, CH_2Cl_2, and dried under an N_2 stream.

The resin was transferred to a Kel-F tube and treated with anisole (ca. 0.5 mL) for 2-12 h. The tube was cooled in a N_2(l) bath, evacuated, and HF was distilled into the vessel (ca. 5 mL) (**caution HF is extremely hazardous**). This mixture was stirred at 0 °C for ca. 30-45 min, followed by removal of the HF in vacuo. The resulting residue was treated with TFA for ca. 10 min, filtered, and the filtrate diluted with ice cold Et_2O (ca. 10 volumes). Centrifugation gave a pellet which was purified by RP (C_{18}) HPLC (CH_3CN/aq. 0.1% TFA, gradient) to give the Fmoc-on PNA.

The purified Fmoc-on PNA was treated with 10-20% piperidine in water (ca. 5 mL) at 0 °C for 45 min. The mixture was filtered and the filtrate diluted with water and lyophilized. The resulting solid was purified by RP (C_{18}) HPLC (CH_3CN/aq. 0.1% TFA, gradient) to give the final PNA. The PNA was analyzed by electrospray MS (mass resolution of 1000).

PNA: GTACGTCTTCAC# (30). MBHA resin (208 mg, 0.24 meq/g, 0.05 mmol) was employed using the above standard conditions. After the oligomerization process the resin was treated with HF/anisole under the conditions outlined above, and RP (C_{18}) HPLC (CH_3CN/aq. 0.1% TFA, gradient) gave the Fmoc-on PNA GTACGTCTTCAC# (84 mg) as a white solid. Removal of the Fmoc group was performed as described above and RP (C_{18}) HPLC (CH_3CN/aq. 0.1% TFA, gradient) gave **30** (70 mg, 43%) as a white solid: mass spectrum (electrospray), *m/z* 3219.2 ($C_{128}H_{163}N_{65}O_{38}$ requires 3220.1). HPLC: 96%.

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   The monomers are similarly abbreviated, *eg.* Fmoc-A(Z)-OPfp for pentafluorophenyl *N*-[2-(*N*-9-fluorenylmethoxycarbonyl)aminoethyl]-*N*-[[6-N-(benzyloxycarbonyl)adenin-9-yl]acetyl]glycinate.
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