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A Convenient Route to *N*-[2-(Fmoc)aminoethyl]glycine Esters and PNA Oligomerization Using a Bis-*N*-Boc Nucleobase Protecting Group Strategy

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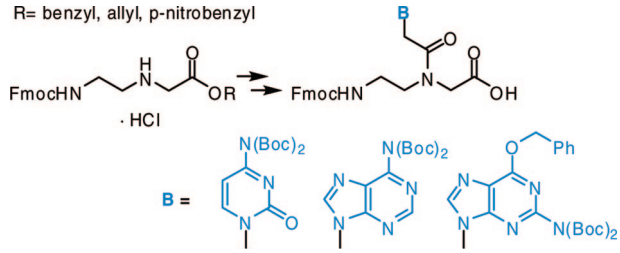
Received January 24, 2008



A simple and practical synthesis of the benzyl, allyl, and 4-nitrobenzyl esters of *N*-[2-(Fmoc)aminoethyl]glycine is described starting from the known *N*-(2-aminoethyl)glycine. These esters are stored as stable hydrochloride salts and were used in the synthesis of peptide nucleic acid monomers possessing bis-*N*-Boc-protected nucleobase moieties on the exocyclic amino groups of ethyl cytosin-1-ylacetate, ethyl adenin-9-ylacetate and ethyl (O^6-benzylguanin-9-yl)acetate. Upon ester hydrolysis, the corresponding nucleobase acetic acids were coupled to *N*-[2-(Fmoc)aminoethyl]glycine benzyl ester or to *N*-[2-(Fmoc)aminoethyl]glycine allyl ester in order to retain the O^6 benzyl ether protecting group of guanine. The Fmoc/bis-*N*-Boc-protected monomers were successfully used in the Fmoc-mediated solid-phase peptide synthesis of mixed sequence 10-mer PNA oligomers and are shown to be a viable alternative to the currently most widely used Fmoc/Bhoc-protected peptide nucleic acid monomers.

Introduction

Peptide nucleic acid (PNA) is an achiral and uncharged nucleic acid analogue constructed from a polyamide backbone composed of *N*-(2-aminoethyl)glycine to which the nucleobases are attached by a methylene carbonyl group.[1] PNA oligomers hybridize to cDNA and cRNA, obeying Watson−Crick base-pairing rules, with very high affinity and sequence specificity and are resistant to nucleases and proteases. Because of these remarkable properties, PNA has received much attention. PNA oligomers are intensively pursued and applied as probes in clinical diagnostics[2,3] and in potential therapeutic applications as antisense/antigene agents.[4] Due to the modular nature of PNA, many backbone-modified PNA analogues have been synthesized.[5,6] PNA has also stimulated the synthesis of numerous non-natural nucleobases.[7] All of these endeavors depend on accessible methods of PNA monomer synthesis and especially so for the synthesis of PNA bearing additional functional groups.

As part of our current research, we are interested in the synthesis of nucleobase modified PNA by modifying the non-Watson/Crick base-pairing face of pyrimidines with charged, hydrophilic, luminescent, and potential helix-stabilizing substituents.[8] The synthesis of such modified nucleobase monomers relies on the assembly of a protected nucleobase acetic acid derivative with a suitably protected *N*-(2-aminoethyl) glycine backbone. Appending charged or hydrophilic groups onto the nucleobase is sometimes difficult to carry out due to the required orthogonal protection. Synthetic routes toward *N*-[2-(Boc)aminoethyl]glycine esters have been extensively investigated since the introduction of Boc/Cbz oligomerization of PNA, giving an assortment of synthetic methods

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10.1021/jo800195j CCC: $40.75
Published on Web 04/16/2008

toward such molecules.[9–11] Conversely, synthetic routes toward *N*-[2-(Fmoc)aminoethyl]glycine esters are less well documented since the first report on Fmoc-mediated synthesis of PNA by Thomson et al. in 1995.[12] Currently there are three Fmoc backbones in use: methyl ester, allyl ester and *tert*-butyl ester of *N*-[2-(Fmoc)aminoethyl]glycine. The methyl ester has not been extensively used since partial removal of the Fmoc group is a hazard during its hydrolysis. Use of the methyl ester then requires tedious optimization of the hydrolysis conditions[13,14] or the subsequent addition of excess Fmoc-OSu to reintroduce the Fmoc group.[9] The orthogonal allyl ester has previously been synthesized by esterification of *N*-[2-(Fmoc)aminoethyl]glycine[15] or *N*-(2-aminoethyl)glycine dihydrochloride,[13] but both routes are inefficient giving the product in an overall low yield. Likewise, the most widely used *tert*-butyl ester is synthesized in low yield (54%) by reacting *N*-(2-aminoethyl)glycine *tert*-butyl ester with Fmoc-OSu.[12] Furthermore, the most convenient removal of the *tert*-butyl ester is by acidolysis and therefore limited to nucleobases and other compounds that contain no acid labile groups. Since several common amino protecting groups (Boc, Mmt, Bhoc) are acid labile, the removal of the *tert*-butyl ester is accomplished with unwanted removal of the amino protecting groups or in low yield as illustrated in the recent literature.[16,17] Therefore, a convenient synthetic route to *N*-[2-(Fmoc)aminoethyl]glycine esters would advance the synthesis of molecules that were previously difficult to synthesize and allow for modified nucleobases or reporter groups to be more easily introduced into PNA by Fmoc-mediated oligomerization.

The solid-phase peptide synthesis of PNA was first accomplished by Boc/Cbz-protected building blocks where the Boc group is used for the protection of the amino group of the backbone and the Cbz for protection of the exocyclic amino groups of the nucleobases cytosine and adenine, while guanine was protected with the O^6-benzyl group.[18] In part due to the harsh conditions required to remove the Cbz groups, most commonly HF or TFMSA, other protecting group strategies were investigated to enable the synthesis of PNA-peptide or PNA−oligonucleotide conjugates which demanded milder deprotection conditions. The Mmt/acyl[19] and Fmoc/acyl[20] monomers were prepared and evaluated for the preparation of PNA−oligonucleotide conjugates, while the Fmoc/Cbz-,[12] Fmoc/Mmt-,[21] and Fmoc/Bhoc-protected monomers were used for the synthesis of PNA−peptide conjugates.

Solid-phase peptide synthesis employing Fmoc chemistry usually involves the use of commercially available amino acids, such as lysine or ornithine, which have their side-chain amino groups Boc-protected. PNA is often appended with lysine at the N- or C-terminus in order to improve aqueous solubility and reduce self-aggregation. Although the side-chain amino group of lysine is protected with the Boc group, a strategy for the oligomerization of PNA using Boc protection of the nucleobases has not yet been reported. Only a brief description of Fmoc/Boc monomer synthesis has appeared in the literature, but it does neither include detailed experimental procedures nor demonstrate the oligomerization of the monomers.[22]Although the Boc group has been used intermittently in nucleobase and nucleoside chemistry and for many years, there are only a few examples that are directly related to the PNA-based work reported herein. A bis-*N*-Boc-protected adenine PNA monomer was first utilized by Condom and co-workers for the solution-phase synthesis of a PNA dimer; however, this report contained only a brief description of experimental conditions which were disclosed later.[23a,b] Much later, the same group communicated the use of a bis-*N*-Boc-protected cytosine PNA acetic acid derivative for the solution-phase synthesis of a PNA dimer, but without disclosing the experimental details.[23c] More recent work from Condom's group has used Cbz-protected cytosine.[23d] Some relevant work on the use of the Boc-protecting group for purines was reported by Garner and co-workers, presumably in support of their research into α-helical peptide nucleic acids (α-PNA), wherein bis-*N*-Boc adenine and mono-*N*-Boc guanine were prepared. These nucleobase derivatives were not alkylated at N9 or used further for the synthesis of PNA-like monomers.[24] Finally, Hultin and Sikchi quite recently reported an elegantly simple solventless approach to bis-*N*-Boc protected nucleosides which also indicated the feasibility of our approach reported herein.[25]

To date, there exists no attempt at the synthesis of Fmoc/bis-*N*-Boc PNA monomers for the oligomerization of PNA. We have decided to pursue the bis-*N*-Boc protection of the adenine, guanine, and cytosine for the following reasons: (i) Fmoc/Bhoc monomers are expensive and have limited commercial sources,[26] (ii) introduction of the Bhoc group onto the nucleobases requires expensive reagents such as carbonyldiimidazole and triphosgene, (iii) the Bhoc group is unstable toward hydrogenation or mild acidolysis rendering the Fmoc monomer synthesis difficult, (iv) both the bis-*N*-Boc protecting group and Boc group may be removed under neutral conditions or in a single acidolysis step which is compatible with a convenient single deprotection/cleavage postsynthetic step, (vi) bis-*N*-Boc-protected monomers

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are highly lipophilic and do not possess any amide functionality which is expected to improve monomer solubility and reduce self-aggregation of these residues during oligomerization.

Synthesis of PNA oligomers by alternative terminal amino protecting groups has also received attention. Protective group strategies that have recently appeared include: Dde/Mmt,[27] NVOC/acyl,[28] and azide/Bhoc.[29] Of late, the synthesis of PNA oligomers using novel self-activated PNA monomers has been reported. Although this strategy allows the synthesis of PNA oligomers in excellent purity, it requires 20 equiv of the Bhoc protected cytosine, guanine, and adenine monomers for each coupling and extended coupling times.[30] This is a severe drawback since the incorporation of custom-made nucleobases or reporter groups would waste a significant amount of the monomer.

Results and Discussion

Synthesis of Fmoc-protected PNA monomers containing natural and non-natural nucleobases may be divided into the synthesis of the protected nucleobase-substituted acetic acids and *N*-[2-(Fmoc)aminoethyl]glycine esters. First, we desired to devise a practical route to 2-aminoethylglycine derivatives that would ultimately possess orthogonal ω-amino-, carboxyl-, and nucleobase-protecting groups. For convenience, this scheme was to result in PNA monomers compatible with standard Fmoc-based oligomerization chemistry. Thus, the Fmoc group and acid-labile nucleobase protecting groups were already specified, and we focused on carboxyl protecting groups that could be removed under neutral or other mild conditions.

Our initial attempt at the synthesis of benzyl *N*-[2-(Fmoc)aminoethyl]glycinate involved making *N*-(2-aminoethyl)glycine benzyl ester, which would be reacted with Fmoc-OSu to form the desired product. Following the procedure of Thomson et al. for the synthesis of *tert*-butyl *N*-(2-aminoethyl)glycinate,[12] we attempted to alkylate an excess ethylenediamine with benzyl bromoacetate. Unfortunately, upon aqueous workup, no benzyl *N*-(2-aminoethyl)glycinate was isolated. Presumably, 1 equiv of ethylenediamine reacts with benzyl bromoacetate to form the desired product, which in the presence of excess ethylenediamine can further undergo cyclization resulting in lactam formation. Alternatively, the disubstituted water-soluble *N*-(2-aminoethyl)-2-(2-aminoethylamino)acetamide could form in the presence of excess ethylenediamine. As an alternative approach, the esterification of *N*-(2-aminoethyl)glycine[31] was pursued. Previously, we have described the esterification of *N*-(2-aminoethyl)glycine in an excess of allyl alcohol saturated with $HCl_{(g)}$ to give the allyl ester in 98% yield.[13] Unfortunately, esterification with benzyl alcohol saturated with $HCl_{(g)}$ failed to give the desired benzyl ester and only *N*-(2-aminoethyl)glycine dihydrochloride was isolated. It is suspected that the benzyl ester failed to form due to the poor solubility of *N*-(2-aminoethyl)glycine dihydrochloride in benzyl alcohol coupled with the inability to remove water from the reaction mixture. Classically, in peptide chemistry, this problem was remedied by replacing HCl with *p*-toluenesulfonic acid and the removal of water is achieved by azeotropic distillation using benzene or toluene,[32] and this is the approach that we have adopted.

Our route to *N*-[2-(Fmoc)aminoethyl]glycine esters began with the alkylation of ethylenediamine by chloroacetic acid to give *N*-(2-aminoethyl)glycine,[31] which was isolated by precipitation from a DMSO/ethanol/ether mixture.[33] Treatment of *N*-(2-aminoethyl)glycine with *p*-toluenesulfonic acid under reflux gave the *p*-toluenesulfonate salt of *N*-(2-aminoethyl)glycine after solvent removal under reduced pressure and precipitation with ether. Compound **1** may be easily synthesized on a large scale (ca. 75 g); in contrast to *N*-(2-aminoethyl)glycine, it is not hygroscopic and stable to storage for extended periods. This material allows for the synthesis of *N*-[2-(Fmoc)aminoethyl]glycine esters with benzyl alcohol, 4-nitrobenzyl alcohol, and allyl alcohol, compounds **2**, **3**, and **4**, respectively, in excellent yield and without the need for an exogenous acid. As reported by Thompson et al., the introduction of the Fmoc group can proceed with low yield (53%) onto *tert*-butyl *N*-(2-aminoethyl)glycinate.[12] Likewise, our previous attempts at introducing the Fmoc group onto allyl *N*-(2-aminoethyl)glycinate dihydrochloride gave a low yield (63%).[13] This low yield may be due to the low solubility of aminoethylglycine ester dihydrochloride, lactam formation, or extending the reaction time to longer than 12 h may cause Fmoc removal in the presence of Hünig's base. We reasoned that introduction of the Fmoc group onto the primary amine of **2** in the presence of an appropriate base with an excess of Fmoc-OSu under concentrated conditions would drive the reaction to completion at the primary amino group and any di-Fmoc product could be removed in the workup. Instead of neutralizing compound **2** followed by dropwise addition of Fmoc-OSu, which could lead to lactam formation, we decided to take advantage of the appreciable solubility of **2** in organic solvents and pursue in situ neutralization of **2** in the presence of 1.1 equivalents of Fmoc-OSu. Initially, introduction of the Fmoc group onto **2** with Fmoc-OSu was performed in a dioxane/water mixture with the dropwise addition of 4.0 equiv of sodium bicarbonate to give the desired product in a modest yield of 66%. The yield improved significantly by using 3.3 equiv of Hünig's base (*N*,*N*-diisopropylethylamine) instead of sodium bicarbonate and performing the reaction in THF. The reaction was arbitrarily decided to be complete within 1.5 h giving compound **5** in 85% yield. Workup involved filtering through a short plug of silica to remove any polar impurities and isolating **5** as the hydrochloride salt by the dropwise addition of HCl in ether. The last step may be substituted for column chromatography to remove any unreacted Fmoc-OSu. Likewise, **6** is isolated in good yield, thereby improving on previous procedures for the synthesis of the allyl backbone, which required the synthesis of *tert*-butyl *N*-[2-(Fmoc)aminoethyl]-glycinate followed by acidolysis and re-esterification.[15] The 4-nitrobenzyl ester, as contained in **7**, may be removed by hydrogenation or mildly acidic reducing conditions (Zn/acetic acid).[34] This protecting group could find use in the rare case that a compound or non-natural nucleobase contains protecting groups susceptible to hydrogenation, acidolysis, and deallylation. Compounds **1**−**4** were characterized by ^1H and ^{13}C NMR but resisted characterization by mass spectrometry. Protecting the

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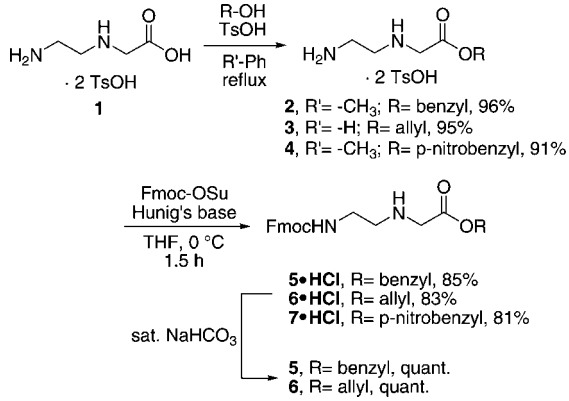
SCHEME 1. Synthesis of Fmoc Backbones



amino group of **2**, **3**, and **4** gave the desired Fmoc backbones **5**, **6**, and **7**, respectively, which were amenable to mass spectroscopic characterization, Scheme 1.

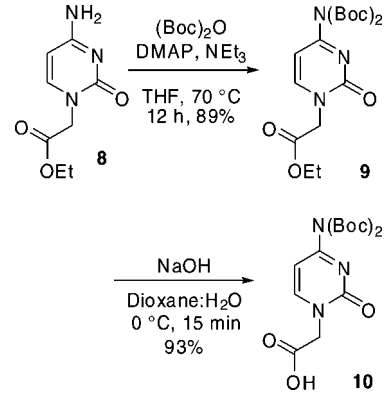
We now routinely use these backbones, particularly *N*-[2-(Fmoc)aminoethyl]glycine benzyl ester, in the synthesis of novel nucleobases containing appended amino groups protected by Boc groups. Catalytic hydrogenation proceeds readily and in very good yield without any detectable loss of the Fmoc group. The Boc group which is used commonly as an amino protecting group in peptide and heterocyclic chemistry is stable to hydrogenation and is usually removed with acid (TFA or HCl) giving *t*-BuOH or isobutylene and CO_2 as the byproduct.[34] Furthermore, the bis-*N*-Boc group may be selectively converted to the mono-*N*-Boc derivative, and if so desired, the remaining Boc group may be removed without the use of acid under neutral conditions.[34,35]

While we decided to pursue the synthesis of bis-*N*-Boc-protected cytosine, adenine, and guanine for reasons already outlined, our main objective in this paper was to investigate whether the introduction of this lipophilic protecting group would occur readily on the exocyclic amino groups of the nucleobases and if the bis-*N*-Boc protected nucleobases are compatible with Fmoc oligomerization, thereby providing a useful alternative to Bhoc protection.

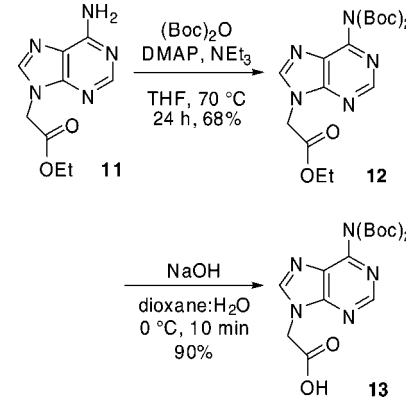
Alkylation of cytosine and adenine with ethyl bromoacetate was performed prior to bis-*N*-Boc protection of the exocyclic amino group due to the increased organic solubility of the alkylated nuclobases and to lower the amount of $(Boc)_2O$ needed for protection. The alkyation of cytosine was performed in the same fashion as described for the synthesis of methyl cytosin-1-ylacetate,[36] using NaH and ethyl bromoacetate to yield ethyl cytosin-1-ylacetate **8**. Compound **8** was reacted with a catalytic amount of DMAP, 2.2 equiv of $(Boc)_2O$, and triethylamine under reflux to give **9** in excellent yield (89%). The ethyl ester was hydrolyzed using 8 equiv of NaOH in a dioxane:water mixture to give **10** in near-quantitative yield (Scheme 2).

The introduction of bis-*N*-Boc onto *N*9-alkylated adenine has been previously reported in the liquid-phase synthesis of a PNA dimer[23a] or in the synthesis of lipophilic PNA containing a 1,3-diyne group.[37] In both cases the bis-*N*-Boc protecting group was introduced using 3 equiv of $(Boc)_2O$ and 3 equiv DMAP

SCHEME 2. Synthesis of Bis-*N*-Boc-Protected Cytosine



SCHEME 3. Synthesis of Bis-*N*-Boc-Protected Adenine



in DMF or THF to give the bis-*N*-Boc-protected compounds in 57% and 62% yield, respectively. Under these conditions, we obtained **12** in 66% yield. In order to reduce the amount of $(Boc)_2O$ and DMAP, ethyl adenin-9-ylacetate **11**[19] was refluxed with a catalytic amount of DMAP, 2.2 equiv of $(Boc)_2O$, and triethylamine to give **12** in 68% yield. Compound **12** was hydrolyzed to give **13** in 90% yield (Scheme 3).

With the uncomplicated synthesis of compounds **10** and **13** established, we turned our attention to the Boc-protection of guanine, which was expected to be the most challenging nucleobase to derivatize. Direct alkylation of guanine, which itself has very poor organic solubility, is known to give a complex reaction mixture including both N9- and N7-alkylated regioisomers.[12] To circumvent the low solubility and poor regioselectivity, other derivatives such as 2-amino-6-chloropurine, N^2-acetyl-O^6-diphenylcarbamoylguanine,[38] and N^2-isobutyrylguanine have been used.[28] Synthesis of **14** was performed as described in the literature by reacting N^2-acetyl-O^6-diphenylcarbamoylguanine with ethyl bromoacetate in the presence of Hünig's base.[21] Compound **14** contains the dipenylcarbamoyl protecting group (Dpc) which can be removed by either TFA[21] or NH_3(aq)/MeOH.[38] Removing the acetyl group in **14** and reacting the exocyclic amino group with $(Boc)_2O$ would give our desired bis-*N*-Boc protected guanine. Unfortunately, our attempts to remove the acetyl group by treatment with EtOH/NEt_3 at reflux failed, and only starting material was recovered. We therefore decided to remove the Dpc group by use of TFA and subsequent reflux of the intermediate N^2-acetyl-9-ethylcar-

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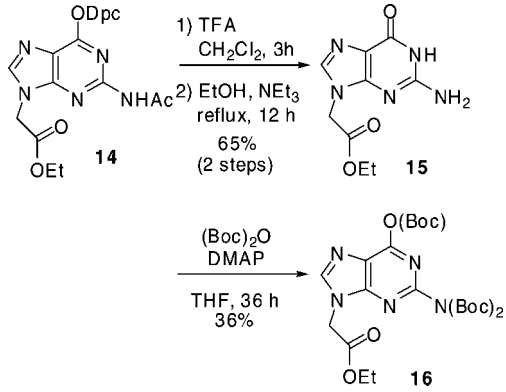
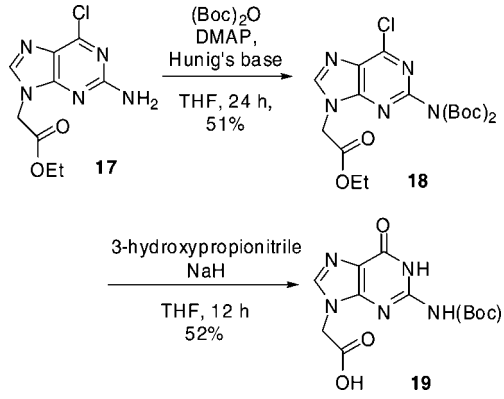
SCHEME 4. Synthesis of Compound 16



SCHEME 5. Synthesis of Compound 19

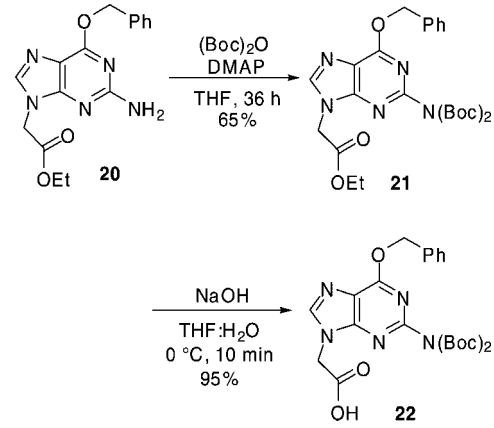


boxymethylguanine in EtOH/NEt_3 gave **15** in 65% yield. This illustrates that under identical conditions, removal of the acetyl group proceeds easily in the lactam structure of guanine but not in the lactim form. Next, **15** was reacted with an excess of $(Boc)_2O$ in an attempt to form N^2,N^2-(bis-Boc)-9-ethylcarboxymethylguanine; unfortunately, only compound **16** was isolated (Scheme 4).

Although compound **16** was considered a good candidate as a lipophilic protected guanine, it was isolated in a disappointing yield of 36%. All attempts to improve the yield for the synthesis of **16** were unsuccessful. To overcome the failure of introducing the tris-Boc groups, compound **17**[36] was reacted with $(Boc)_2O$ in the presence of DMAP to give **18** in 51% yield. Refluxing the reaction did not improve the yield possibly due to the consumption of DMAP by its reaction with 6-Cl group of **17** giving the 6-dimethylaminopyridinium derivative.[39] It is known that 6-chloropurines react with 3-hydroxypropionitrile to give the guanine derivative.[40] Unfortunately, converting **18** to N^2,N^2-(bis-Boc)-9-carboxymethylguanine resulted in displacement of one of the Boc groups leading to compound **19** (Scheme 5), which has poor organic solubility.

Exhausting the routes to bis-*N*-Boc-protected guanine by way of compounds **14** and **17**, we decided to work with guanine in the lactim form in order to focus on Boc protection at the exocyclic amino group. We reasoned that guanine protected at the 6-position with a benzyl group would be a feasible approach. The first report of PNA oligomerization used this protecting group strategy,[18] the benzyl ether may be removed by either

SCHEME 6. Bis-*N*-Boc Protection of 20



hydrogenation or acidolysis with TFA. Although reaction of ethyl [2-amino-6-(benzyloxy)purin-9-yl]acetate **20** with benzyloxycarbonyl chloride or "Rapoport's reagent" was unsuccessful, treatment of **20** with the capping reagent used in the oligomerization (acetic anhydride/pyridine/CH_2Cl_2 1:2:2) for 1.5 h led to 23% of the acylated product.[18] The protection of the exocyclic amino group has been also investigated by Koch and co-workers who treated [2-amino-6-(benzyloxy)purin-9-yl]acetic acid with isobutyryl chloride to give the product in 30%.[10] The protection was improved by avoiding the use of the carboxylic acid derivative and treating compound **20** with isobutyryl chloride in pyridine to give ethyl [N^2-(isobutyryl)-O^6-(benzyl)guanin-9-yl]acetate in 80% yield. Recently the high yielding synthesis of O^6-benzyl guanine has been described.[41] We have decided not to start with this reagent because its alkylation with ethyl bomoacetate will most likely proceed with poor regioselectivity for N9/N7. Therefore, **20** was synthesized using 2-amino-6-chloropurine as described in the literature.[10] Compound **20** was bis-*N*-Boc-protected in 65% yield using 2.2 equiv of $(Boc)_2O$ and DMAP to give **21**, which upon hydrolysis gave **22** in 95% (Scheme 6).

The attachment of compounds **10** and **13** onto *N*-[2-(Fmoc)aminoethyl]glycine benzyl ester and compound **22** onto allyl *N*-[2-(Fmoc)aminoethyl]glycinate was accomplished by neutralizing the hydrochloride salts to give the free bases **5** and **6** prior to coupling. Coupling of **5** or **6** to the backbone was accomplished using EDC, DCC/NHS, or EDC/HOBt. Removal of the benzyl ester of **23** was accomplished using Pd/C and 1,4-cyclohexadiene[42,43] to give monomer **26** in 89%. Removal of the benzyl ester from **24** using Pd/C and H_2 gave the desired monomer **27** in 94% (Scheme 7).

Compound **22** was coupled to the allyl protected backbone **6** in order to retain the O^6 benzyl ether as a lipophilic group. Removal of the allyl group in **25** was achieved using a catalytic amount of $Pd(PPh_3)_4$ and N-methylmorpholine as the scavenger to give **28** in 84% (Scheme 8). Alternatively, in order to avoid the use of the allyl ester, **22** may be coupled to **5** and hydrogenation of both the benzyl ester and O^6 benzyl ether would give the bis-*N*-Boc-protected guanine monomer.

In order to investigate whether the monomers were compatible with Fmoc-mediated oligomerization and if the removal of the protecting groups occurred readily, the newly synthesized

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(41) Barth, C.; Seitz, O.; Kunz, H. *Z. Naturforsch.* **2004**, *59b*, 802–806.
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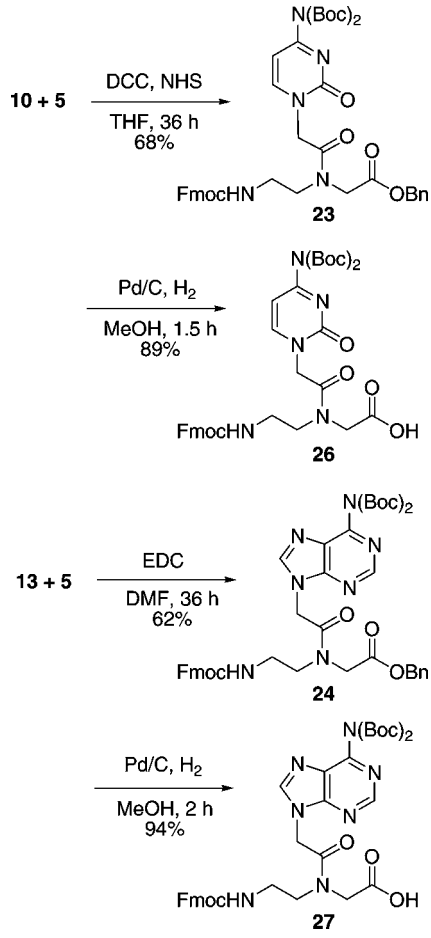
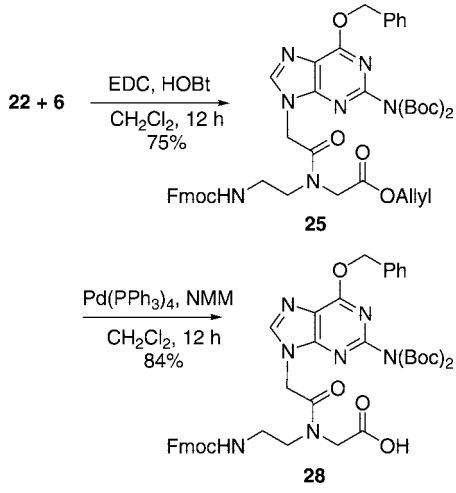
(43) Johnson, D.C., II; Widlanski, T. S. *Org. Lett.* **2004**, *6*, 4643–4646.

SCHEME 7. Monomer Synthesis: Coupling of 10 and 13 with 5



SCHEME 8. Monomer Synthesis: Coupling of 22 onto 6



monomers were incorporated into a 10-mer PNA sequence. Oligomers were synthesized under identical conditions whether using either Fmoc/Bhoc- or Fmoc/bis-*N*-Boc-based monomers and employed standard conditions.[44] The consecutive incorporation of monomers **26**, **27**, and **28** was performed in order to judge if the monomers were compatible with commercially Bhoc-protected monomers (Table 1).

Sequence 3 was synthesized using all three bis-*N*-Boc protected monomers **26**, **27** and **28** to give the crude oligomer.

TABLE 1. ESI-TOF MS Analysis of Oligomers

entry	sequence[a] (N → C)	expected (Da)	found (Da)
1	**H-GTA GAT CAC** T-Lys-NH_2	2854.8	2854.75
2	**H-GTA GAT CAC** T-Lys-NH_2	2854.8	2854.88
3	**H-GTA GAT CAC** T-Lys-NH_2	2854.8	2854.01

[a] PNA sequences possess a free N-terminal amino group and C-terminal amide. Bold type indicates use of bis-*N*-Boc-protected monomers.

The monomers performed equivalently to the conventional Fmoc/Bhoc monomers during the machine-assisted synthesis. The crude oligomers were analyzed and subsequently purified by RP-HPLC. The identity of the oligomers was confirmed by ESI-MS. Analysis of the crude product mixture indicated that the desired oligomer represented >90% of the material (by HPLC area, Figure S1, Supporting Information), which leads to an average cycle (deprotection and coupling) yield of >99% per monomer.

Conclusion

We have described a convenient method for the synthesis of *N*-[2-(Fmoc)aminoethyl]glycine esters (benzyl, allyl, 4-nitrobenzyl) starting with inexpensive *N*-(2-aminoethyl)glycine. The syntheses of both *N*-[2-(Fmoc)aminoethyl]glycine benzyl ester and *N*-[2-(Fmoc)aminoethyl]glycine 4-nitrobenzyl ester have not yet been described in the literature, and we have improved the synthesis of allyl *N*-[2-(Fmoc)aminoethyl]glycinate. Both esterification of **1** and introduction of the Fmoc group onto **2**−**4** proceeds in very good yield. These backbones will find use in the synthesis of modified reporter groups or modified nucleobases that were previously difficult to make or altogether not attempted due to the lack of orthogonal protecting groups present in the backbone and nucleobase. Introduction of the bis-*N*-Boc as a nucleobase protecting group strategy proceeds easily with alkylated cytosine and in excellent yield. Likewise protection of *N*9-alkylated adenine is performed in good yield. The protection of guanine proved to be most challenging but was accomplished with the aid of the O^6 benzyl ether protecting group to give a fully protected guanine derivative. Removal of the benzyl ester in **23** and **24** is achieved with Pd/C and H_2 or Pd/C and 1,4-cyclohexadiene. Monomers **26**, **27**, and **28** were incorporated into a PNA oligomer and are compatible with commercially available Bhoc-protected monomers or may substitute completely for them with no modification to the standard automated cycles and conditions.

During solid-phase PNA oligomer synthesis, aggregation of growing polyamide chains is a commonly encountered problem which leads to inaccessibility of the terminal Fmoc group to piperidine deprotection. Besides extended deprotection times that are sometimes required, the on-resin aggregation may result in the formation of truncated oligomers as impurities that complicate the purification of the final product or even to complete failure of the synthesis.[45] It is also known that the Fmoc/Bhoc-protected cytosine and guanine monomers have limited organic solubility and often require sonication for full

(44) PNA synthesis followed the manufacturer's guidelines for small-scale synthesis (5 μmol) employing FastMoc cycles on an Applied Biosystems 433A peptide synthesizer. This protocol uses 5 equiv of monomer. The preactivation time for the monomers was 120 s. ABI 433A Peptide Synthesis 3 mL reaction vessel User's Manual, 2001, Chapter 4.

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solubilization. The bis-*N*-Boc protecting group strategy may alleviate these solubility issues and reduce aggregation while increasing solvation in the growing oligomer as indicated by the trouble-free syntheses performed already; however, this aspect will be evaluated more fully elsewhere.

With an ever increasing interest in PNA applications, new methods and inexpensive reagents for the synthesis of PNAs continue to be of great interest in the scientific community in order to allow the flourishing synthesis and evaluation of variously modified PNA in medicinal and diagnostic applications. This report describes useful improvements to standard PNA synthesis to support such endeavors.

Experimental Section

***N*-(2-Aminoethyl)glycine·2TsOH (1).** A suspension of *N*-(2-aminoethyl)glycine (19.0 g, 161 mmol) and *p*-toluenesulfonic acid (68.0 g, 358 mmol) in toluene (400 mL) was refluxed for 3 h. The solvent was removed in vacuo, and ether (300 mL) was added. After the mixture was kept at −20 °C for 2 h, the precipitate was collected by filtration and rinsed with diethyl ether (100 mL) to give **1** as a white solid (74.0 g, 99%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.98 (br s, 3 H), 7.53 (d, 4H, J = 8.0), 7.14 (d, 4H, J = 8.0), 3.96 (s, 2H), 3.24 (m, 2H) 3.18 (m, 2H) 2.28 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.2, 144.8, 138.5, 128.5, 125.6, 47.4, 43.9, 35.3, 21.0.

Benzyl *N*-(2-Aminoethyl)glycinate·2TsOH (2). Benzyl alcohol (60 mL) was added to a suspension of **1** (14.9 g, 32.0 mmol) and *p*-toluenesulfonic acid (2.5 g, 13.1 mmol) in toluene (250 mL). The suspension was refluxed (oil bath, 135 °C) for 6 h, and then the reaction mixture was concentrated in vacuo to approximately 100 mL. Ether (250 mL) was added to the reaction mixture, and the resultant suspension was stored at −20 °C for 12 h. The precipitate was collected by filtration and rinsed with diethyl ether (150 mL) to give **2** as an off-white solid (19.3 g, 96%): ^{1}H NMR (400 MHz, D_2O) δ 7.61 (d, 4H, J = 7.4), 7.33 (br s, 5H, J = 8.0), 7.2 (d, 4H, J = 7.8), 5.14 (s, 2H), 3.99 (s, 2H), 3.43 (m, 2H) 3.39 (m, 2H) 2.26 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.7, 144.6, 138.7, 135.2, 128.6, 128.5, 128.4, 125.7, 67.2, 47.3, 44.1, 35.3, 21.0.

Allyl *N*-(2-Aminoethyl)glycinate·2TsOH (3). Allyl alcohol (150 mL) was added to a suspension of **1** (11.7 g, 25.0 mmol) and *p*-toluenesulfonic acid (1.0 g, 5.3 mmol) in benzene (150 mL). The suspension was refluxed (oil bath, 110 °C) for 12 h. The solution was concentrated to an oil, diethyl ether (200 mL) was added, and the solution was sonicated for 30 min to initiate precipitation. At this point, the suspension was stored at −20 °C for 12 h. The precipitate was collected by filtration and rinsed with diethyl ether (100 mL) to give **3** as an off-white solid (12.0 g, 96%): ^{1}H NMR (400 MHz, D_2O) δ 7.72 (d, 4H, J = 7.8), 7.38 (d, 4H, J = 8.2), 5.97 (ddd, 1H, J = 16.4, 10.2, 5.9) 5.38 (m, 2H), 4.76 (d, 2H, J = 5.9), 4.13 (s, 2H), 3.54 (m, 2H) 3.47 (m, 2H) 2.40 (s, 6H); ^{13}C NMR (100 MHz, D_2O) δ 166.3, 142.0, 139.7, 130.6, 129.3, 125.3, 119.3, 67.3, 47.5, 44.1, 35.4, 20.5.

4-Nitrobenzyl *N*-(2-aminoethyl)glycinate·2TsOH (4). To 4-nitrobenzyl alcohol (7.0 g, 45.7 mmol), **1** (2.5 g, 5.4 mmol), and *p*-toluenesulfonic acid (0.25 g, 1.3 mmol) was added toluene (50 mL). The suspension was then refluxed (oil bath, 135 °C) for 12 h. The solution was concentrated in vacuo, the solid residue was triturated with warm acetone (50 mL) and cooled to 0 °C for 12 h, and the white solid was collected by filtration to give **4** as a white solid (2.97 g, 91%): ^{1}H NMR (400 MHz, D_2O) δ 8.19 (d, 2H, J = 6.8), 7.63 (d, 4H, J = 8.2), 7.56 (d, 2H, J = 7.0), 7.3 (d, 4H, J = 7.8), 5.35 (s, 2H), 4.16 (s, 2H), 3.48 (m, 2H) 3.41 (m, 2H) 2.34 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.6, 147.3, 144.6, 142.9, 138.5, 128.8, 128.4, 125.6, 123.6, 65.8, 47.2, 44.1, 35.3, 20.9.

Benzyl *N*-[2-(Fluorenylmethoxycarbonyl)aminoethyl]glycinate Hydrochloride (5·HCl). To **2** (11.1 g, 20.0 mmol) and Fmoc-OSu (7.5 g, 22.2 mmol) was added THF (50 mL), and then the resultant suspension was cooled to 0 °C and a solution of Hünig's base (11 mL, 63.0 mmol) in THF (15 mL) was added dropwise over 15 min. The reaction was stirred at 0 °C for 1 h, removed from the ice bath, and stirred for a further 15 min. The reaction was poured into a separatory funnel containing EtOAc (200 mL) and saturated $NaHCO_3$ (50 mL). The organic layer was washed with saturated $NaHCO_3$ (50 mL), water (50 mL), and brine (2 × 50 mL), dried over Na_2SO_4, and concentrated in vacuo. The organic layer was dissolved in a minimum amount of EtOAc and filtered through silica gel (5.0 × 10 cm bed) eluting with a gradient of (EtOAc to EtOAc/acetone 6:4). The solvent was removed in vacuo, the crude oil was dissolved in ether (50 mL) and cooled to 0 °C, and $HCl_{(g)}$ in ether (20 mL, 2 M) was added dropwise. The flask was kept at −20 °C for 2 h, after which the precipitate was collected by filtration and rinsed with ether (25 mL) to give **5** as a white solid (7.96 g, 85%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.40 (br s, 2H), 7.90 (d, 2H, J = 7.6), 7.69 (d, 2H, J = 7.4), 7.57 (t, 1H, J = 5.4), 7.42 (m, 5H), 7.33 (m, 3H), 5.25 (s, 2H), 4.33 (d, 2H, J = 6.6), 4.23 (t, 1H, J = 6.6), 4.07 (s, 2H), 3.33 (m, 2H), 3.03 (t, 2H, J = 6.1); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.6, 156.3, 143.8, 140.7, 135.2, 128.5, 128.3, 128.2, 127.7, 127.1, 125.2, 120.1, 66.9, 65.7, 46.7, 46.6, 36.7; HRMS (EI) m/z calcd for $C_{26}H_{26}N_2O_4$ [M^+] 430.1893, found 430.1895. Compound **5**·HCl may be neutralized and extracted into an organic solvent (CH_2Cl_2 or EtOAc) by partitioning against a solution of saturated aqueous $NaHCO_3$. The organic solvent was dried over anhydrous Na_2SO_4 and evaporated to give a quantitative yield of the free base **5**.

Allyl *N*-[2-(Fluorenylmethoxycarbonyl)aminoethyl]glycinate Hydrochloride (6·HCl). The reaction was performed as described for **5** using 10.0 mmol of **3**. After removal of the solvent, the residue was redissolved in a minimum amount of CH_2Cl_2 and filtered through silica gel (2.5 × 5 cm bed) eluting with a gradient of (CH_2Cl_2 to CH_2Cl_2/acetone 5:5). The solvent was removed, the crude oil was dissolved in diethyl ether (50 mL) and cooled to 0 °C, and $HCl_{(g)}$ in ether (10 mL, 2 M) was added dropwise. The flask was kept at −20 °C for 2 h, after which time the precipitate was collected by filtration and rinsed with diethyl ether to give **6**·HCl as a white solid (3.46 g, 83%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.40 (br s, 2H), 7.90 (d, 2H, J = 7.4), 7.70 (d, 2H, J = 7.4), 7.57 (t, 1H, J = 5.7), 7.42 (t, 2H, J = 7.4), 7.34 (t, 2H, J = 7.4), 5.94 (ddt, 1H, J = 17.0, J = 10.8, J = 5.5), 5.38 (dd, 1H, J = 17.2, J = 1.4) 5.27 (dd, 1H, J = 10.6, J = 1.2), 4.7 (d, 2H, J = 5.5), 4.34 (d, 2H, J = 6.8), 4.23 (t, 1H, J = 7.0), 4.06 (s, 2H), 3.35 (m, 2H), 3.02 (t, 2H, J = 6.0); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.3, 156.3, 143.8, 140.8, 131.7, 127.7, 127.1, 125.3, 120.1, 118.5, 67.8, 46.7, 46.6, 36.7; HRMS (EI) m/z calcd for $C_{22}H_{24}N_2O_4$ [M^+] 380.1736, found 380.1743. Compound **6**·HCl may be extracted into CH_2Cl_2 or EtOAc from aqueous $NaHCO_3$ to give the free base **6**.

4-Nitrobenzyl *N*-[2-(Fluorenylmethoxycarbonyl)aminoethyl]-glycinate Hydrochloride (7·HCl). The reaction was performed as described for **5** using 5.0 mmol of **4** to give 2.09 g (81%) of **7** as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.52 (br s, 2H), 8.25 (d, 2H, J = 8.6), 7.90 (d, 2H, J = 7.4), 7.70 (m, 4H), 7.59 (t, 1H, J = 5.7), 7.42 (t, 3H, J = 7.4), 7.33 (t, 3H, J = 7.4), 5.40 (s, 2H), 4.33 (d, 2H, J = 6.8), 4.22 (t, 1H, J = 6.8), 4.14 (s, 2H), 3.35 (m, 2H), 3.05 (t, 2H, J = 5.5); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.5, 156.4, 147.2, 143.9, 143.0, 140.8, 128.7, 127.7, 127.2, 125.3, 123.6, 120.2, 65.8, 65.6, 46.7, 46.6, 37.0; HRMS (ESI) m/z calcd for $C_{26}H_{26}N_3O_6$ [MH^+] 476.1822, found 476.1824. Compound **7**·HCl may be extracted into CH_2Cl_2 or EtOAc from aqueous $NaHCO_3$ to give the free base **7**.

Ethyl [N^4,N^4-Bis(*tert*-butoxycarbonyl)cytosin-1-yl]acetate (9). To ethyl cytosin-1-yl acetate (**8**, 3.5 g, 17.7 mmol), Boc_2O (8.6 g, 39.5 mmol), and DMAP (215 mg, 1.76 mmol) was added THF (100 mL) followed by NEt_3 (4.9 mL, 35.0 mmol), and the reaction mixture was heated at 60 °C for 12 h under N_2. The orange solution

was concentrated in vacuo, and the residue was purified by FCC on silica gel eluting with a gradient of hexanes/EtOAc (7:3 to 2:8). The desired fractions were concentrated to give a light yellow oil, which was coevaporated with hexanes (2 × 25 mL) and dried under vacuum to give **9** as an off-white solid (6.3g, 87%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 (d, 1H, J = 7.6), 7.10 (d, 1H, J = 7.0), 4.55 (s, 2H), 4.23 (q, 2H, J = 7.0), 1.55 (s, 18H) 1.28 (t, 3H, J = 7.0); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.1, 162.6, 154.7, 149.2, 149.0, 96.2, 84.6, 61.5, 50.8, 27.4, 13.8; HRMS (EI) *m/z* calcd for $C_{18}H_{27}N_3O_7$ [M^+] 397.1849, found 397.1860.

[*N*^{4},*N*^{4}-Bis(*tert*-butoxycarbonyl)cytosin-1-yl]acetic Acid (10). A suspension of compound **9** (1.5 g, 3.8 mmol) in dioxane/water (40 mL/12 mL) and water (12 mL) was placed in an ice bath, followed by the dropwise addition of NaOH (2.5 M, 12 mL) over 1 min. The suspension was removed from the ice bath and stirred until TLC analysis indicated complete consumption of the starting material (15 min). The reaction mixture was poured into a separatory funnel containing $KHSO_4$ (1.0 M, 75 mL) and EtOAc (75 mL). The aqueous layer was further extracted with EtOAc (4 × 75 mL) and the combined organic fractions were dried over Na_2SO_4 and concentrated in vacuo. The colorless oil was coevaporated with hexanes (2 × 25 mL), and dried under vacuum to give **10** as a white solid (1.3 g, 93% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.2 (br s, 1H) 8.14 (d, 1H, J = 7.6), 6.83 (d, 1H, J = 7.2), 4.56 (s, 2H), 1.49 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.8, 153.5, 152.2, 150.4, 150.3, 145.2, 128.4, 83.7, 62.4, 44.4, 27.8, 14.1; HRMS (ESI) *m/z* calcd for $C_{16}H_{24}N_3O_7$ [MH^+] 370.1614, found 370.1620.

Ethyl [*N*^{6},*N*^{6}-Bis(*tert*-butoxycarbonyl)adenin-9-yl]acetate (12). Method A. A suspension of ethyl adenin-9-ylacetate **11** (3.3 g, 15.0 mmol) in THF (100 mL) was cooled to approximately 0 °C, in an ice bath, followed by the addition of Boc_2O (9.8 g, 45.0 mmol) and DMAP (5.5 g, 45.0 mmol). The reaction mixture was purged with N_2, removed from the ice bath, and stirred for 24 h under N_2 after which time TLC analysis indicated complete consumption of the starting material. The solution was concentrated in vacuo (approximately 20 mL), and the residue was extracted with CH_2Cl_2 (200 mL) and consecutively washed with 1 M NaH_2PO_4 (pH = 4.0, adjusted with H_3PO_4, 2 × 50 mL), saturated solution of $NaHCO_3$ (50 mL), and brine (50 mL). The organic layer was separated, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by FCC on silica gel eluting with a gradient of (CH_2Cl_2/hexanes 8:2 to CH_2Cl_2:acetone 8:2). The fractions containing the desired product were concentrated to give a light yellow oil which was coevaporated with hexanes (2 × 25 mL) and placed under vacuum to give 4.3 g (68% yield) of **12** as an off-white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.80 (s, 1H), 8.12 (s, 1H), 5.01 (s, 2H), 4.21 (q, 2H, J = 7.2), 1.48 (s, 18H) 1.23 (t, 3H, J = 7.2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.1, 162.6, 154.7, 149.2, 149.0, 96.2, 84.6, 61.5, 50.8, 27.4, 13.8; HRMS (EI) *m/z* calcd for $C_{19}H_{27}N_5O_6$ [M^+] 421.1961, found 421.1944.

Method B. To a suspension of ethyl adenin-9-ylacetate **11** (1.8 g, 8.15 mmol) in THF (50 mL) was added Boc_2O (3.9 g, 17.9 mmol) and DMAP (100 mg, 0.82 mmol) followed by NEt_3 (2.2 mL, 15.7 mmol). The reaction mixture was refluxed for 12 h under N_2. The reaction was concentrated in vacuo, and the residue was purified by FCC on silica gel eluting with a gradient of (hexanes/EtOAc 7:3 to 5:5), the fractions were concentrated to give 2.27 g (66%) of **12** as an off-white solid.

[*N*^{6},*N*^{6}-Bis(*tert*-butoxycarbonyl)adenin-9-yl]acetic Acid (13). To **12** (3.47 g, 8.23 mmol) was added dioxane (65 mL) and water (25 mL), the solution was then placed in an ice bath, followed by the dropwise addition of NaOH (2.5 M, 25 mL) over 1 min. The reaction mixture was removed from the ice bath and stirred until TLC indicated complete consumption of the starting material (10 min). The reaction mixture was poured into a separatory funnel containing $KHSO_4$ (1.0 M, 150 mL) and EtOAc (150 mL). The aqueous layer was further extracted with EtOAc (3 × 100 mL), and the combined organic fractions were dried over Na_2SO_4 and concentrated in vacuo. The colorless oil was coevaporated with hexanes (2 × 25 mL) and placed in vacuo to give 2.9 g (90%) of **13** as a off-white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.46 (br s, 1H) 8.84 (s, 1H), 8.61 (s, 1H), 5.15 (s, 2H), 1.38 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.8, 153.4, 152.4, 150.1, 150.0, 146.7, 127.6, 84.4, 44.6, 27.9; HRMS (EI) *m/z* calcd for $C_{17}H_{23}N_5O_6$ [M^+] 393.1648, found 393.1646.

Ethyl Guanin-9-ylacetate (15). A suspension of **14** (3.5 g, 7.38 mmol) in CH_2Cl_2 (20 mL) was cooled to 0 °C, followed by dropwise addition of TFA (10 mL) over 10 min. The reaction was taken off the ice bath and stirred for a further 3 h, after which time the solvent was removed in vacuo, followed by coevaporation with CH_2Cl_2 (3 × 25). To the residue were added EtOH (150 mL) and NEt_3 (4.2 mL, 30 mmol), and the suspension was refluxed for 12 h. The solvent was reduced to approximately 25 mL and placed at −20 °C for 12 h. A precipitate formed that was collected by filtration and washed with ethanol and diethyl ether to give **15** as a white solid (1.34 g, 65%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.73 (s, 1H), 6.54 (br s, 1H), 4.86 (s, 2H), 4.14 (q, 2H, J = 7.0), 1.19 (t, 3H, J = 7.0); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.0, 156.9, 153.8, 151.5, 138.0, 116.1, 61.3, 43.9, 14.0; HRMS (EI) *m/z* calcd for $C_9H_{11}N_5O_3$ [M^+] 237.0862, found 237.0871.

Ethyl [*N*^{2},*N*^{2}-Bis(*tert*-butoxycarbonyl)-*O*^{6}-(*tert*-butoxycarbonyl)purin-9-yl]acetate (16). A suspension of **15** (577 mg, 2.43 mmol), DMAP (980 mg, 8.02 mmol), and Boc_2O (2.12 g, 9.71 mmol) in THF (7 mL) was stirred for 36 h under N_2. The solvent was removed in vacuo, the residue was extracted with EtOAc (50 mL), and the organic layer was subsequently washed with water (25 mL) and brine (25 mL) and then separated and dried over Na_2SO_4. After removal of the solvent, the organic residue was subjected to FCC eluting with a gradient of (hexanes/EtOAc 9:1 to 8:2). The combined fractions were dried to give **16** as a yellow solid (472 mg, 36%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (s, 1H), 4.95 (s, 2H), 4.23 (q, 2H, J = 7.0), 1.72 (s, 9H), 1.39 (s, 18H), 1.28 (t, 3H, J = 7.0); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.8, 161.0, 152.6, 151.3, 150.6, 142.8, 120.4, 83.8, 82.8, 82.1, 44.3, 28.3, 27.8, 14.0; LRMS (ESI) *m/z* calcd for $C_{24}H_{35}N_5O_9$ [M^+] 537.2, found 438.1 [MH^+ − Boc].

Ethyl [*N*^{2},*N*^{2}-(Bis(*tert*-butoxycarbonyl)amino)-6-chloropurin-9-yl]acetate (18). To a suspension of **17** (2.0 g, 7.82 mmol), DMAP (98.0 mg, 0.8 mmol), and Boc_2O (5.15 g, 23.5 mmol) in THF (25 mL) was added Hünig's base (4.1 mL, 23.5 mmol) dropwise, and the suspension was stirred for 36 h under N_2. The solvent was removed in vacuo, and the residue was extracted with EtOAc (150 mL), washed with water (50 mL) and brine (50 mL), dried over Na_2SO_4, and reduced in vacuo. The residue was subjected to FCC eluting with (hexanes/EtOAc 8:2). The solvent was removed from the combined fractions to give **18** as a yellow solid (1.82 g, 51%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (s, 1H), 5.02 (s, 1H), 4.26 (q, 2H, J = 7.2), 1.42 (s, 18 H), 1.29 (t, 3H, J = 7.2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.3, 152.8, 151.9, 150.9, 150.3, 146.8, 129.5, 83.5, 62.3, 44.6, 27.7, 14.0; HRMS (ESI) *m/z* calcd for $C_{19}H_{26}ClN_5O_6Na$ [M^+] 478.1469, found 478.1446.

[*N*^{2}-(*tert*-Butoxycarbonyl)guanin-9-yl]acetic Acid (19). A solution of 3-hydroxypropionitrile (0.65 mL, 9.5 mmol) in THF (10 mL) was cooled to −78 °C, and NaH (375 mg, 9.5 mmol) was added under N_2. The reaction was allowed to warm to 0 °C and stirred for 1 h, at which point **18** (1.0 g, 2.33 mmol) was added in one portion. The reaction was stirred at 0 °C for 3 h and for a further 8 h at room temperature. The solvent was evaporated in vacuo, and to the residue was added 10 mL of water and the pH adjusted to 8.0 by the addition of $KHSO_4$. The aqueous phase was washed with EtOAc (2 × 25 mL) which was discarded, and then the aqueous phase was acidified to pH = 2.0 with $KHSO_4$. The aqueous phase was extracted with EtOAc (4 × 150 mL), which was subsequently separated and dried over Na_2SO_4 and reduced in vacuo. The residue was triturated with equal volumes of diethyl ether/hexanes (50 mL) and collected by filtration to give **19** as an off-white solid (370 mg, 52%): ^{1}H

NMR (400 MHz, DMSO-d_6) δ 13.26 (s, 1H), 11.39 (s,1H), 11.12 (s, 1H), 7.91 (s, 1H), 4.85 (s, 2H), 1.48 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.1, 155.2, 153.9, 151.5, 149.5, 147.8, 140.2, 119.1, 82.6, 44.4, 27.8; HRMS (ESI) *m*/*z* calcd for $C_{12}H_{16}N_5O_5$ [M^+] 310.1151, found 310.1157.

Ethyl [N^2,N^2-Bis(*tert*-butoxycarbonyl)-O^6-benzylguanin-9-yl]acetate (21). A suspension of **20** (1.0 g, 3.0 mmol), DMAP (600 mg, 4.9 mmol), and Boc_2O (1.5 g, 6.9 mmol) in THF (7 mL) was stirred for 36 h under N_2. The solvent was removed, the residue was extracted with EtOAc (150 mL) and washed with water (50 mL) and brine (50 mL), and the organic phase was dried over Na_2SO_4. The solvent was reduced in vacuo and the residue subjected to FCC using EtOAc/hexanes (8:2) as the eluent. The combined fractions were dried to give **21** as a yellow solid (1.03 g, 65%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (s, 1H), 7.51 (m, 2H), 7.35 (m, 3H), 5.64 (s, 2H), 4.97 (s, 2H), 4.24 (q, 2H, J = 7.0), 1.40 (s, 18 H), 1.28 (t, 3H, J = 7.0); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.6, 160.7, 153.1, 151.7, 150.6, 143.6, 135.7, 128.3, 128.1, 128.0, 119.3, 82.83, 68.6, 61.9, 44.2, 27.6, 13.9; HRMS (EI) *m*/*z* calcd for $C_{26}H_{33}N_5O_7$ [M^+] 527.2380, found 527.2384.

[N^2,N^2-Bis(*tert*-butoxycarbonyl)-O^6-benzylguanin-9-yl]acetic Acid (22). A solution of **21** (860 mg, 1.6 mmol) in THF/water (10 mL/12 mL) was placed in an ice bath followed by the dropwise addition of NaOH (2.5 M, 5 mL) over 1 min. The resultant suspension was removed from the ice bath and stirred until TLC indicated complete consumption of the starting material (10 min). The reaction mixture was transferred into a separatory funnel containing $KHSO_4$ (2.5 M, 10 mL) and EtOAc (100 mL). The aqueous layer was further extracted with EtOAc (3 × 50 mL), and the combined organic fractions were dried over Na_2SO_4 and concentrated in vacuo. The yellow oil was coevaporated with hexanes (2 × 25 mL) and placed in vacuo to give 750 mg (94%) of **22** as a yellow solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (s, 1H), 7.47 (m, 2H), 7.34 (m, 3H), 5.59 (s, 2H), 4.90 (s, 2H), 1.37 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.8, 160.7, 152.9, 152.3, 151.0, 144.4, 135.8, 128.6, 128.4, 128.2, 118.0, 83.6, 68.9, 44.6, 27.9; HRMS (ESI) *m*/*z* calcd for $C_{24}H_{30}N_5O_7$ [MH^+] 500.2145, found 500.2144.

Benzyl *N*-[2-(Fluorenylmethoxycarbonyl)aminoethyl]-*N*-[(N^4,N^4-bis(*tert*-butoxycarbonyl)cytosin-1-yl)acetyl]glycinate (23). To an ice-cooled suspension of **10** (443 mg, 1.2 mmol) in THF (7 mL) were added DCC (250 mg, 1.2 mmol) and NHS (135 mg, 1.2 mmol). The reaction was stirred at 0 °C for 15 min and at room temperature for 1.5 h, followed by the dropwise addition of **5** (473 mg, 1.1 mmol) in THF (5 mL). The reaction was stirred for 36 h, the formed DCU was filtered, and the product was extracted with EtOAc (100 mL) and washed with $NaHCO_3$ (2 × 50 mL) and brine (50 mL). The residue was subjected to FCC using EtOAc/MeOH as the eluent. The combined fractions were reduced in vacuo to give (585 mg, 68%) of **23** as a white solid. Compound **23** exists in solution as a pair of slowly exchanging rotamers; the signals due to the major (ma.) and minor (mi.) rotamers are designated: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.0 (m, 1H), 7.89 (d, 2H, J = 7.6), 7.69 (m, 2H), 7.40−7.29 (m, 10H), 6.84 (m, 1H), 5.21 (s, 0.6 H, mi.), 5.12 (s, 1.4 H, ma.), 4.88 (s, 1.4 H, ma.), 4.71 (s, 0.6 H, mi.), 4.42−4.23 (m, 4H), 4.14 (s, 1H), 3.48−3.10 (m, 5H), 1.49 (s, 18 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.2, 167.2, 162.6, 156.7, 154.9, 149.4, 143.8, 141.1, 135.2, 128.5, 128.2, 128.0, 127.5, 127.0, 125.0, 119.8, 96.0, 84.7, 67.0, 66.7, 49.6, 48.8, 48.5, 47.0, 27.5; HRMS (ESI) *m*/*z* calcd for $C_{42}H_{48}N_5O_{10}$ [MH^+] 782.3401, found 782.3378.

Benzyl *N*-[2-(Fluorenylmethoxycarbonyl)aminoethyl]-*N*-[(N^6,N^6-bis(*tert*-butoxycarbonyl)adenin9-yl)acetyl]glycinate (24). To an ice-cooled suspension of **13** (775 mg, 2.0 mmol) and **5** (820 mg, 1.9 mmol) in DMF (7 mL) was added EDC (775 mg, 4.0 mmol), and the reaction was stirred on ice for 0.5 h and at room temperature for 12 h. The reaction was poured into $NaHCO_3$ (50 mL) and extracted into CH_2Cl_2 (150 mL). The organic phase was washed sequentially with aqueous $NaHCO_3$ (2 × 50 mL) and brine (50 mL). After the organic phase was dried and the solvent removed, the residue was subjected to FCC using CH_2Cl_2/acetone as the eluent. The combined fractions were reduced in vacuo to give 1.0 g (62%) of **24** as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.8 (s, 0.4H, mi.), 8.74 (s, 0.6H, ma.), 8.45 (m, 1H), 7.88 (m, 2H), 7.68 (m, 2H), 7.33 (m, 10H), 5.42−5.11 (m, 3H), 4.53−4.21 (m, 4H), 4.16 (s, 1H), 3.60−3.13 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.3, 156.8, 153.5, 151.9, 150.4, 150.3, 150.1, 146.1, 146.0, 143.8, 143.7, 141.3, 134.9, 128.8, 128.7, 128.6, 128.3, 127.8, 127.0, 125.0, 124.9, 120.0, 83.8, 83.7, 67.4, 49.0, 48.7, 47.3, 27.8; HRMS (ESI) *m*/*z* calcd for $C_{43}H_{48}N_7O_9$ [MH^+] 806.3514, found 806.3500.

Allyl *N*-[2-(Fluorenylmethoxycarbonyl)aminoethyl]-*N*-[N^2,N^2-bis(*tert*-butoxycarbonyl)-O^6-benzylguanin-9-yl)acetyl]glycinate (25). To an ice-cooled suspension of **22** (700 mg, 1.4 mmol) and **6** (533 mg, 1.4 mmol) in CH_2Cl_2 (5 mL) were added EDC (403 mg, 2.1 mmol) and HOBt hydrate (54 mg, 0.35 mmol), and the reaction was stirred on ice for 0.5 h and at room temperature for 12 h. The reaction was diluted with CH_2Cl_2 (10 mL) and $NaHCO_3$ (25 mL) and then extracted with CH_2Cl_2 (100 mL) and brine (50 mL). The residue was subjected to FCC using a gradient of EtOAc/hexanes (7:3) to EtOAc/CH_2Cl_2 (8:2) as the eluent. The combined fractions were reduced in vacuo to give **25** as a pale yellow solid (905 mg, 75%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.27 (s, 1H), 7.89 (d, 2H, J = 7.0), 7.67 (t, 2H, J = 7.2), 7.51−7.29 (m, 10H), 5.88 (m, 1H), 5.60 (s, 2H), 5.40−5.16 (m, 4H), 4.70−4.36 (m, 4H), 4.32−4.30 (m, 2H), 4.12 (s, 1H), 3.54−3.10 (m, 4H), 1.34 (s, 7H, mi.), 1.32 (s, 11H, ma.); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.1, 160.8, 153.1, 151.6, 151.0, 144.3, 143.8, 143.7, 135.8, 131.3, 128.4, 128.3, 128.2, 127.7, 127.0, 124.9, 120.0, 119.9, 119.0, 83.2, 68.7, 66.1, 49.0, 48.9, 47.2, 27.8; HRMS (ESI) *m*/*z* calcd for $C_{46}H_{52}N_7O_{10}$ [MH^+] 862.3776, found 862.3734.

***N*-[2-(Fluorenylmethoxycarbonyl)aminoethyl]-*N*-[(N^4,N^4-bis(*tert*-butoxycarbonyl)cytosin-1-yl)acetyl]glycine (26).** Compound **23** (536 mg, 0.686 mmol) dissolved in EtOH (20 mL) was purged with N_2, and 10% Pd/C (600 mg) was added, followed by cyclohexadiene (1.0 mL, 10.6 mmol). The reaction was stirred for 1.5 h and filtered through a short pad of Celite, and the solvent was removed in vacuo. The residue was coevaporated with hexanes (4 × 25 mL) to give the title compound in 89% yield as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) (two rotamers) δ 7.93 (m, 1H), 7.83 (d, 2H, J = 7.4), 7.62 (m, 2H), 7.37−7.25 (m, 5H), 6.75 (m, 1H), 4.89−4.57 (m, 2H), 4.29−4.14 (m, 4H), 3.94 (s, 1H), 3.41−3.22 (m, 5H), 1.44 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.8, 167.0, 162.7, 162.5, 156.8, 155.7, 149.2, 143.7, 140.9, 127.5, 127.0, 128.9, 125.2, 119.7, 85.0, 84.8, 66.7, 46.8, 27.5; HRMS (ESI) *m*/*z* calcd for $C_{35}H_{41}N_5O_{10}Na$ [M^+] 714.2751, found 714.2742.

***N*-[2-(Fluorenylmethoxycarbonyl)aminoethyl]-*N*-[(N^6,N^6-bis(*tert*-butoxycarbonyl)adenin-9-yl)acetyl]glycine (27).** Compound **24** (720 mg, 0.89 mmol) was dissolved in MeOH (50 mL) and purged with N_2, and then Pd/C (200 mg) was added and the solution was saturated with H_2 by slow bubbling over 2 h. The reaction was filtered through a short pad of Celite, and the solvent was removed in vacuo. The residue was coevaporated with hexanes (4 × 50 mL) to give the title compound in 94% as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) (two rotamers) δ 10.03 (s, 1H), 8.78−8.48 (m, 2H), 8.28 (s,1H), 7.89−7.87 (m, 3H), 7.69−7.29 (m, 11H), 7.18 (br m, 1H), 5.39−5.14 (m, 3H), 4.37−4.22 (m, 4H), 3.99 (s, 1H), 3.54−3.09 (m, 5H), 1.48 (s, 8H) 1.37 (s, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.9, 153.5, 151.9, 150.5, 150.3, 149.7, 143.6, 141.1, 127.6, 127.0, 125.0, 119.8, 84.0, 47.0, 40.7, 27.7. HRMS (ESI) *m*/*z* calcd for $C_{36}H_{41}N_7O_9Na$ [M^+] 738.2863, found 738.2849.

***N*-[2-(Fluorenylmethoxycarbonyl)aminoethyl]-*N*-[N^2,N^2-bis(*tert*-butoxycarbonyl)-O^6-benzylguanin-9-yl)acetyl]glycine (28).** Compound **25** (850 mg, 1.0 mmol) was dissolved in a degassed solution of chloroform/acetic acid/*N*-methylmorpholine (25 mL, 1.5 mL, 0.75 mL), the solution was purged with N_2, and $Pd(PPh_3)_4$ (115 mg, 0.1 mmol) was added. The solution was deoxygenated with freeze−pump−thaw degassing and stirred under N_2 for 12 h.

The solvent was removed in vacuo and the residue was extracted with CH_2Cl_2 (100 mL) and $KHSO_4$ (1 M, 2 × 25 mL), dried over Na_2SO_4, and reduced in vacuo. The residue was subjected to FCC using CH_2Cl_2/MeOH as the eluent. The combined fractions were reduced in vacuo to give **28** as an off-white solid (691 mg, 84%): ^{1}H NMR (400 MHz, DMSO-d_6) (two rotamers) δ 8.28 (s, 1H), 7.89 (d, 2H, $J = 7.4$), 7.67 (t, 2H, $J = 7.0$), 7.49 (d, 2H, $J = 7.0$), 7.46−7.29 (m, 8H), 5.61 (s, 2H), 5.32 (s, 1H), 5.14 (s, 1H), 4.37−4.20 (m, 4H), 4.01 (s, 1H), 3.54−3.09 (m, 5H), 1.32 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.1, 166.1, 160.3, 156.6, 156.5, 152.8, 151.6, 150.6, 144.6, 143.6, 143.4, 140.9, 135.5, 131.8, 128.2, 127.9, 127.4, 126.8, 124.9, 124.8, 119.7, 118.2, 83.1, 68.5, 66.4, 48.8, 27.6; HRMS (ESI) *m*/*z*: calcd for $C_{43}H_{48}N_7O_{10}Na$ [MH^+] 845.3360, found 845.3359.

Acknowledgment. We thank the Natural Sciences and Engineering Research Council of Canada (NSERC) for funding this work through their Discovery Grants program. Ms. Paula Pittock of the UWO Biological Mass Spectrometry Laboratory is thanked for expert collection of mass spectral data for the polyamides.

Supporting Information Available: General experimental conditions and copies of ^{1}H and ^{13}C NMR spectra for compounds **1**−**7**, **9**, **10**, **12**, **13**, **15**, **16**, **18**, **19**, and **21**−**28** and an HPLC spectrum for sequence **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO800195J