DOI: 10.1002/ejoc.200800891

A Practical and Efficient Approach to PNA Monomers Compatible with Fmoc-Mediated Solid-Phase Synthesis Protocols

Andrea Porcheddu,*[a] Giampaolo Giacomelli,[a] Ivana Piredda,[a] Mariolino Carta,[a] and Giammario Nieddu[a]

Dedicated to the memory of Dr. Charles Mioskowski

Keywords: Peptide nucleic acids / Guanine / Protecting groups / Solid-phase synthesis

A straightforward synthesis of orthogonally protected PNA monomers is described. Protected aminoethylglycine (Aeg) monomers were efficiently prepared by reductive amination of *N*-Fmoc-glycinaldehyde with glycine methyl ester and the subsequent acylation of the free amine with *N*-bis-Boc-protected nucleobase acetic acids. The exocyclic amine group of the nucleobases, including the notoriously difficult-to-protect guanine nucleobase, was protected with a bis-Boc carbamate group; this increased the solubility of the nucleobases in the most common organic solvents. The current protocol allows all Aeg monomers to be prepared on both the micro- and macroscale, which avoids or minimizes the use of toxic reagents or solvents, and moreover, cheap starting materials are used.

Introduction

Natural oligonucleotides (DNA, RNA) have limited potential as therapeutic agents, and main reason is their poor in vivo stability as a result of the susceptibility of the phosphodiester backbone to undergo enzymatic cleavage by cellular nucleases. To overcome this general problem in the antisense field, there have been significant advances in the discovery and development of analogues bearing structural features that improve the pharmacological properties of natural oligonucleotides. Peptide nucleic acids (PNAs) are DNA/RNA mimics in which the sugar–phosphate backbone of natural nucleic acid has been replaced by an uncharged pseudopeptide skeleton composed of *N*-(2-aminoethyl)glycine units (Figure 1).[1] The nucleobases A, C, G and T are linked to this achiral skeleton through a two-atom carboxymethyl spacer. PNAs have very flexible structures, but they are still able to bind complementary DNA and RNA strands with high specificity and selectivity.[2] PNA–RNA and PNA–DNA hybrids are more stable than the corresponding nucleic acid complexes. The increased stability of PNA–DNA and PNA–RNA duplex in comparison to DNA–DNA (RNA) duplex is mainly attributed to the lack of electrostatic repulsion between the two strands. At the same time, the change in ionic strength has little effect on the stability of PNA–DNA duplexes.[3] They also possess high chemical and biological stability due to the unnatural amide backbone that is not recognized by nucleases or proteases and thus not degraded inside a living cell.[4] These properties of PNAs make these oligomers of significant interest in many disciplines of chemistry, molecular biology and medicine.[5] A ubiquitous requirement in the field of PNAs research[6] is the preparation of monomers for subsequent oligomerization. A PNA monomer consists of *N*-protected (2-aminoethyl)glycine to which a protected nucleobase is attached (Figure 1). These two protecting groups have to be orthogonal, that is, Pg^2 must be stable under the conditions used to remove Pg^1.

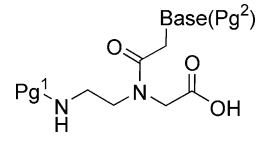


Figure 1. General structures of a PNA monomer.

In the literature, during these last years several combinations of protecting groups were reported for PNA synthesis.[7] PNA oligomers can be prepared by following standard solid-phase synthesis protocols for peptides by using, for example, a (methylbenzhydryl)amine polystyrene resin as the solid support.[8] Previous reports on PNA synthesis have focused on Boc/Cbz protection methods (Pg^1 = Boc and

[a] Dipartimento di Chimica, Università degli Studi di Sassari, Via Vienna 2, 07100 Sassari, Italy
Fax: +39-079-229559
E-mail: anpo@uniss.it
Supporting information for this article is available on the WWW under http://www.eurjoc.org or from the author.

Pg^2 = Cbz, Figure 1), giving an assortment of synthetic procedures. The repeated use of TFA (Boc group deprotection) and the harsh treatments (HF or TFMSA) necessary to remove the Cbz protecting group makes this procedure incompatible with several modified PNAs, in particular with PNA–DNA chimeras, which are sensitive to strong acids.[9] Moreover, the use of hazardous acids often are not commercially embraced in view of safety concerns for the operators and the corrosive effect on automation equipment and lines.

With the advent of Nielsen's PNAs and related amide-linked oligonucleotide surrogates, there is a continuous need for protecting groups that can be removed under acidic or neutral conditions that are compatible with 9-fluorenylmethoxycarbonyl-mediated (Fmoc) solid-phase synthesis protocols. The use of an Fmoc group for the protection of the primary amine in the backbone offers several advantages, including milder synthesis conditions, improved monomer solubility, high coupling efficiencies and facilitated purification of the final products.[10]

Another current limitation on the synthesis of PNA synthons is the formation of the side-chain nucleobase protecting group. Although Fmoc/Bhoc PNA monomers[11] are nowadays the most used for routine PNA synthesis, they are costly and of limited variety.[12] Moreover, the employment of the Bhoc group for protection of the exocyclic amino groups (Pg^2) onto the nucleobases requires toxic reagents such as triphosgene, which renders the Fmoc monomer synthesis easier said than done.[13]

Although there are several examples of nucleobases with acid-labile protecting groups,[14] strangely, the *tert*-butoxycarbonyl group (Boc), which is the most popular acid-labile protecting group for amines, has received little attention in the literature for nucleobase protection.[15] The Boc protecting group has the additional advantage that its acidolytic removal is less sensitive to steric factors and it can also be removed under neutral conditions.[16] Boc protection was envisaged as a highly attractive strategy, as this protecting group is orthogonal to Fmoc-based solid-phase peptide synthesis (SPPS) protocol, and in contrast to the known acid-labile monomethoxytrityl (Mmt) protecting group, it can sustain mildly acidic conditions. Thus, with the appropriate choice of resin/linker, on-resin deprotection and modification of a specific amine group would also be possible.

In 2002, Sugiyama et al. reported the first example of the synthesis of Fmoc PNA monomers, whose nucleobases were orthogonally protected with a Boc group.[17] Unfortunately, in this note the authors do not provide any exhaustive experimental procedure or investigate whether the final monomers are compatible with a solid-phase PNA oligomer synthesis. Nonetheless, the N^2-Boc protected carboxymethylguanine was found to possess very poor solubility in common solvents for peptide coupling. Although this problem was overcome by employing a trimethylsily ether protecting group at the guanine O^6 position, this last methodology is laborious and requires toxic reagents such as OsO_4 and $NaClO_2$.

Such problems may be overcome by using two Boc (bis-Boc) protecting groups, as the complete protection of the exocylic amine functionality increase the lipophilicity of bases, which makes them more soluble in the most common solvents for organic synthesis. Moreover, it generally known that the bis-Boc protecting group often avoids side reactions or unwanted cyclizations.[18] In 1996, Condom et al. reported, for the first time, the preparation of a bis-*N*-Boc-protected adenine PNA acetic acid derivative for the solution-phase synthesis of a PNA adenine–guanine dimer.[19] However, the paper contained only a summary description of experimental conditions, which were disclosed later.[20] In 1997, Garner and Dey reported in detail the synthesis of gram quantities of Boc-protected purines from inexpensive starting materials without the need for elaborate purification steps.[21] In 2006, Hultin et al.[22] synthesized the bis-*N*-Boc protection of the heterocyclic amino group of nucleosides and derivatives and showed that this bis-carbamate is a very useful N-protecting group for the nucleobase portions. These last seminal papers encouraged us to develop a general strategy to prepare Fmoc/bis-*N*-Boc Aeg–PNA (Aeg = aminoethylglycine) monomers for the following oligomerization of PNAs.

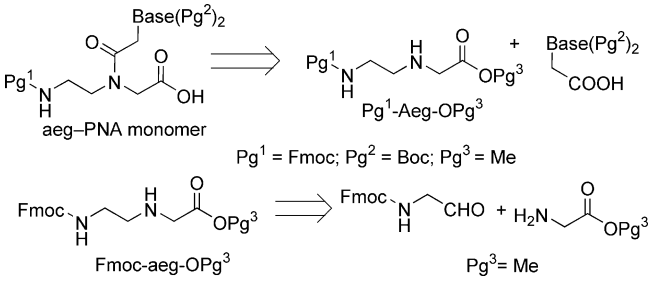
During the preparation of this manuscript, the beneficial effect of the protection of nucleobases with a bis-Boc group in the Fmoc-mediated solid-phase peptide synthesis of PNAs (Fmoc/bis-Boc strategy) was successfully described by Hudson and Wojciechowski.[23] At the same time, a paper on the scope and orthogonality of PNA synthesis was published by Winssinger et al.[24,25]

Results and Discussion

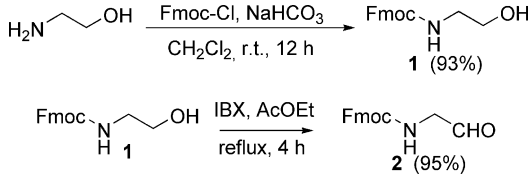
The preparation of the monomers can be divided into the synthesis of a suitably protected *N*-2-aminoethylglycine (Pg^1-Aeg-OPg^3) and the nucleobase acetic acid derivatives (Scheme 1). In such monomers, the Fmoc group was introduced to protect the 2-ethylamino moiety in the growing PNA backbone, whereas the bis-Boc group was chosen as the protecting group for the exocyclic amino functions of the A, G and C bases. In the exploration for a useful protecting group (Pg^3) for the carboxylic function of Pg^1-Aeg-OH, we found methyl ester to be ideal in terms of deactivation of COOH towards nucleophiles, ease of deprotection and compatibility with our Fmoc/Boc strategy. The key intermediate in our new synthetic route to PNA building blocks is *N*-Fmoc-aminoethylglycine methyl ester (Scheme 1). We chose the methyl ester,[26] as it has less propensity than the allyl ester to give 2-oxopiperazine during the carbamylation reaction as observed by Seitz and Kohler[27] and confirmed by Hudson et al.[28]

A very simple and efficient approach to prepare this compound on large scale is shown in Scheme 2. The Fmoc-protected backbone (Fmoc-Aeg-OH) could be prepared by reductive amination of *N*-Fmoc-glycinaldehyde with glycine methyl ester.

First, we protected the amino group of ethanolamine by using Fmoc-Cl, following a classical procedure,[29] and then

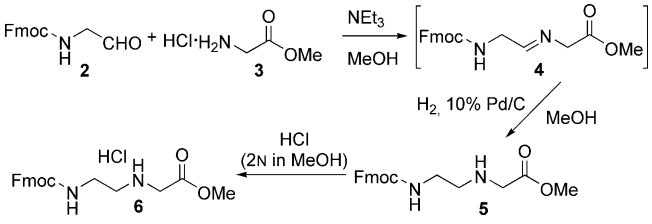


Scheme 1. Retrosynthetic scheme for PNA monomers.



Scheme 2. Synthesis of *N*-Fmoc-glycinaldehyde.

we oxidized[30] its alcoholic function to the corresponding aldehyde with IBX.[31] This procedure gave *N*-Fmoc-protected glycine **2** (Scheme 2) in high yield and purity. This material was taken on to the next step without further purification. Aldehyde **2** was treated with glycine methyl ester (**3**) in methanol, and intermediate imine **4** was efficiently transformed into amine **5** with 10% Pd/C. *N*-Fmoc protected monomer backbone **5** was obtained as a pale yellow oil in 75% yield after purification by flash chromatography (Scheme 3).



Scheme 3. Synthesis of *N*-(2-Fmoc-aminoethyl)glycine methyl ester.

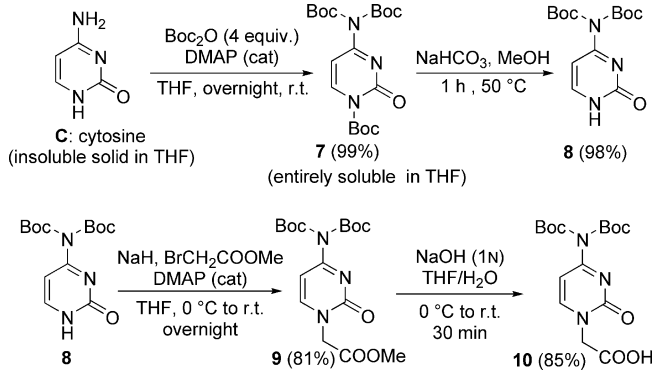
One of main drawbacks of *N*-(2-Fmoc-aminoethyl)glycine methyl ester (**5**) is that once obtained it can be stored only for a few days at low temperature.[32] A more attractive method involved transforming the crude materials into the corresponding stable hydrochloride salt **6** by treatment of Fmoc-Aeg-OMe (**5**) with dry methanolic HCl[33] (2 N) at 0 °C, which could be stored at –20 °C indefinitely.[34] Our aim was to either convert this secondary amine (Fmoc-Aeg-OMe) into the amide shortly after its synthesis or to isolate **5** as stable salt **6**.

As known from the literature, and well remarked by Hudson et al.,[23] the introduction of the Fmoc group on the primary amine of the 2-aminoethylglycine moiety is never trivial and often proceeds in low yield. Because our procedure to prepare Fmoc-submonomer **5** is straightforward and inexpensive, no further attempts at optimization of the reaction yields were taken into consideration. Our goal was to develop a simple and reproducible procedure to overcome many of the practical problems observed during the synthesis of PNA monomers.

Second, we focused our attention on the synthesis of bis-Boc protected nucleobase acetic acids by avoiding or minimizing the use of toxic reagents or solvents such as dimethylformamide[35] (DMF).[36]

The suitably protected nucleobases were prepared according to minor modifications of Garner's procedure,[21] which allowed these intermediates to be synthesized in gram quantities by starting from inexpensive starting materials and without the need for elaborate purification steps.

Cytosine (**C**) was suspended in dry THF and treated with an excess amount of Boc_2O (4 equiv.) in the presence of a catalytic amount of 4-*N*,*N*-(dimethylamino)pyridine (DMAP). This mixture was stirred at room temperature until complete solubilization/disappearance of starting nucleobase **C** (overnight). The excess amount of THF was removed under vacuum to give a yellow oil, which was redissolved in AcOEt. The organic solvent was first washed with HCl (1 N) to give an oil containing tris-Boc-protected cytosine **7** and *tert*-butyl alcohol (Scheme 4). This mixture, after treatment with hexane, afforded desired and pure compound **7** in quantitative yield. Tris-Boc cytosine **7** can be easily converted into bis-Boc cytosine **8** almost quantitatively by treatment with aq. $NaHCO_3$ in MeOH at reflux (until disappearance of starting material by TLC). Longer reaction times led to the formation of significant quantities of cytosine.

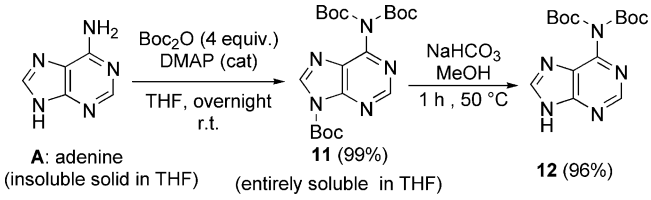


Scheme 4. Synthesis of bis-Boc citosylacetic acid.

The alkylation of cytosine derivative **8** was performed by using NaH and methyl bromoacetate in THF to give, after purification by flash chromatography, methyl cytosin-1-yl-acetate **9** in high yield (Scheme 4). Finally, cleavage of the methyl ester group under basic conditions (NaOH 1 N in THF) gave the corresponding cytosylacetic acid derivative **10** as a white solid in high yield (85%, Scheme 4). Different from the procedure of Hudson, we prefer to introduce the protecting group of the nucleobase in the first step, just before the alkylation step.[37] This simple shrewdness not only

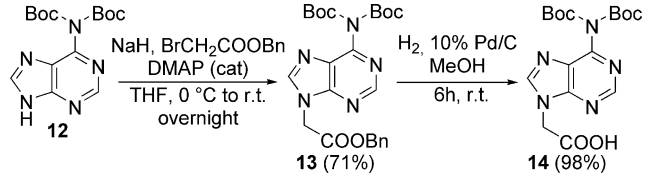
renders the intermediates sufficiently soluble for chemical manipulations, but also makes the whole synthesis of PNA monomers easier.

The next step in the synthesis of the PNA components is the formation of the N^9-acetic acid derivatives of adenine and guanine. The introduction of bis-*N*-Boc onto N^9-alkylated adenine was performed by following the protocol already described for cytosine.[21] Under these conditions, we obtained bis-*N*-Boc-protected compound **12** (96%, Scheme 5).



Scheme 5. Synthesis of bis-Boc adenine.

Afterward, bis-*N*-Boc adenine **12** was alkylated with the use of benzyl bromoacetate in THF by first generating the anion with sodium hydride (Scheme 6). By using benzyl bromoacetate instead of methyl bromoacetate it was possible to recover the corresponding N^9-alkylated adenine **13** in higher yield and purity. This procedure yielded only the expected ethyl adenin-9-ylacetate **13**, as was unambiguously confirmed by NMR spectroscopic analysis.[38] Smooth and clean cleavage of the benzyl ester by treatment with a catalytic amount of Pd/C quantitatively afforded the desired bis-*N*-Boc protected adenylacetic acid **14**.



Scheme 6. Synthesis of bis-Boc-adenylacetic acid.

Put out by the synthesis of compounds **10** and **14**, we focused our attention on the preparation of bis-Boc-protected guanine monomer (Figure 2). It is evident from the literature that guanine (**G**) is insoluble in almost all solvents (water included), and it undergoes direct alkylation to give a complex reaction mixture including both N^9- and N^7-alkylated regioisomers.

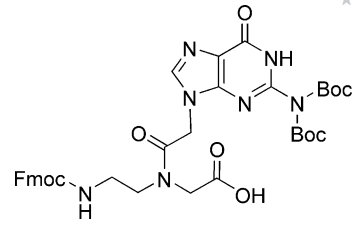


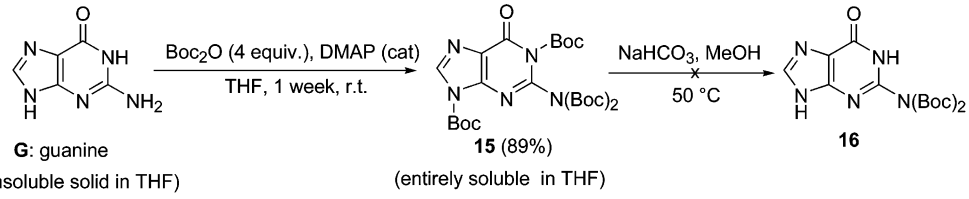
Figure 2. Bis-Boc-protected guanine monomer.

The development of an easy and successful synthesis of guanine monomer represents here our more arduous objective, as the most part of published procedures fail just in the preparation of this monomer. In contrast, guanine quadruplexes are gaining increasing attention due to their suspected roles in regulating gene expression at the transcriptional and translational levels. In a pioneering work, Armitage et al.[39] demonstrated that PNAs are capable of forming a hybrid quadruplex with DNA, a structure composed of PNA_2–DNA_2. PNA_2–DNA_2 quadruplex may potentially act as a repressor in diverse gene expression regulatory pathways, including oncogenes and genes involved in neuropathologies.[40]

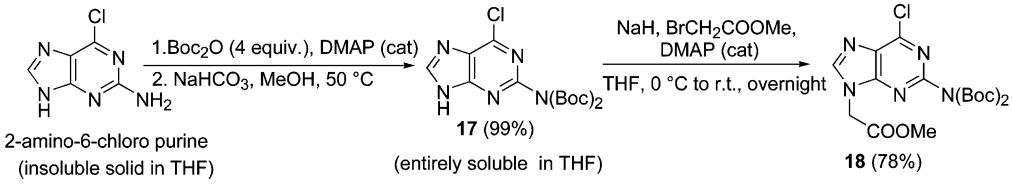
At first, we tried to solubilize the guanine following a modified procedure of Garner, so we performed the di-Boc protection of **G** in THF. After a week, we unexpectedly observed that most of the starting base was solubilized and protected as N^1, N^2, $N^{2'}$, N^9-tetraBoc guanine **15** (Scheme 7). Unfortunately, after several attempts we were not able to convert **15** into desired bis-Boc-protected base **16**; under all conditions a complicated mixture of inseparable compounds was obtained.

To overcome the low solubility and poor regioselectivity, we employed 2-amino-6-chloropurine, which is frequently used as a guanine analogue.[41] Bis-Boc protected guanine synthon **18** was easily prepared in 75% overall yield by starting from 2-amino-6-chloropurine after (i) bis-Boc-protection of the exocyclic amine by using Boc_2O/DMAP in THF and (ii) alkylation of **17** with methyl bromoacetate (Scheme 8). The synthesis was performed by following the same methodology as that above for cytosine and adenine.[42]

It is known that suitably protected 2-amino-6-halopurine compounds may be converted into protected guanine compounds by several methods involving attack at the 6-halo group by oxygen nucleophiles.[43] Remarkably, the alkoxide of 3-hydroxypropionitrile can convert a 6-halo group into a



Scheme 7. Tentative synthesis of bis-Boc-guanine.



Scheme 8. Synthesis of bis-Boc-6-chloropurine intermediate.

6-carbonyl group and simultaneously hydrolyze the acetate ester group. Two equivalents of alkoxide of 3-hydroxypropionitrile are consumed in the conversion of the 6-halo group into the 6-carbonyl group, whereas an additional equivalent of alkoxide is required to hydrolyze the acetate methyl ester. Unfortunately, by following this procedure we are able to recover poorly soluble mono-Boc protected guaninylacetic acid **19** in only very low yields[23] (<5%).[44]

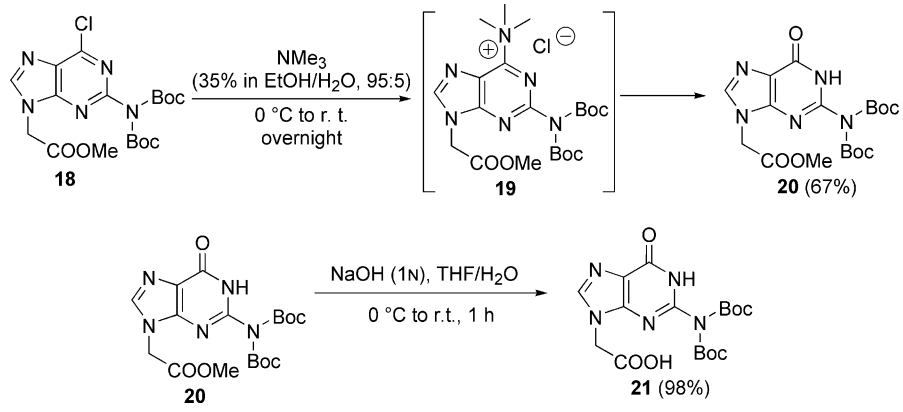
Alternatively, the displacement of the halogen with benzyloxide (with the alcohol as the solvent) from 2-amino-6-chloropurine could be an interesting synthetic route for the conversion of the 6-chloro group into the 6-oxo function after hydrogenation or acidolysis with TFA. Unfortunately, this approach was not compatible with our strategy, as it requires two further steps, exchange of halogen with benzyloxide and then benzyl ether removal, and would not solve the problem of a regioselective subsequent alkylation at N^9.[45] Moreover, these strong basic conditions, together with the high temperature (65 °C for 16 h) needed to exchange the 6-halo group for a benzyloxy group, could easily remove the bis-Boc protecting group in the nucleobases.[46]

It is known from the literature that 6-chloropurines react with trimethylamine to afford 6-trimethylammonium species, which are susceptible to nucleophilic displacement of the trimethylammonium group.[47] Therefore, we applied this method to our substrate. Compound **18** was treated with 10 equiv. of NMe_3 (35% in EtOH[48]) at 0 °C overnight to give an insoluble solid. Strangely and unexpectedly, the precipitated solid collected by simple suction filtration was not the expected ammonium salt **19** but desired final guanine **20** (Scheme 9).[49] Removal of the methyl ester from **20** by using NaOH (1 N) in THF at 0 °C for 1 h gave desired protected guaninylacetic acid **21** in near quantitative yield (Scheme 9).

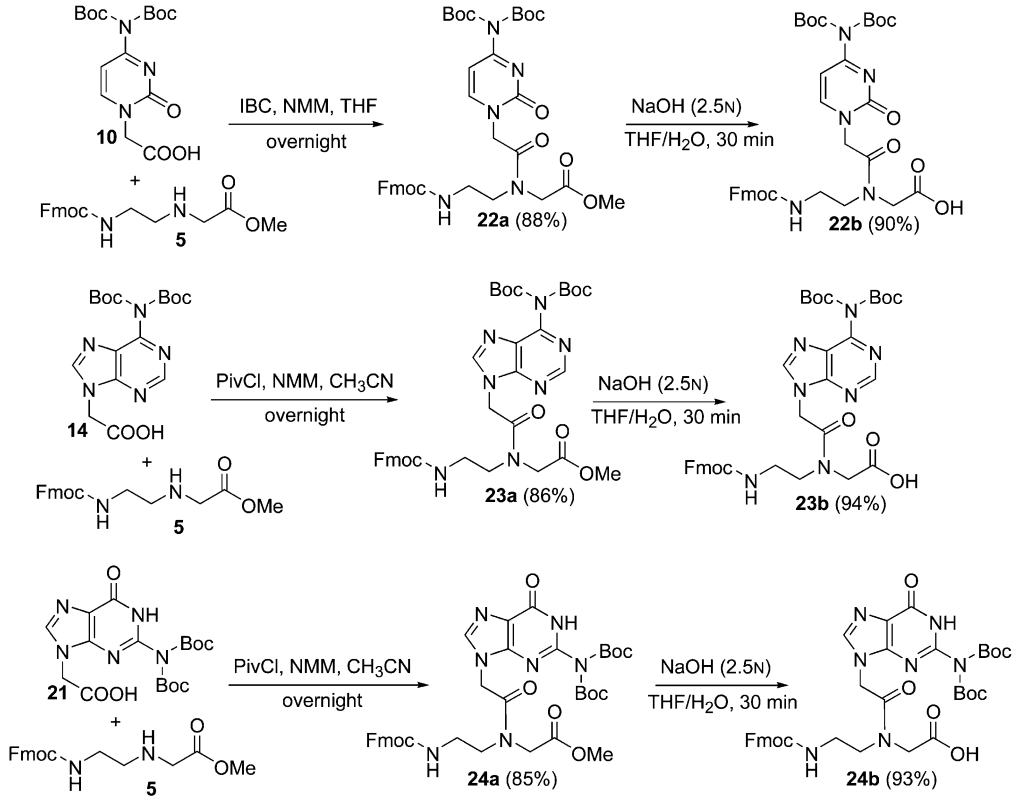
Unlike the procedure employed by Winssinger, our approach allows bis-Boc-guaninylacetic acid **21** to be synthesized by avoiding expensive and toxic reagents such as triphosgene, which is necessary for the protection of the exocyclic amine group of guanine.[50] Moreover, as is well highlighted by Hudson et al., all bis-*N*-Boc-protected monomers (in particular guanine residues) are highly lipophilic, and this chemical property improves their solubility and reduces self-aggregation of these residues during oligomerization.

In the last step of our strategy, we planned the condensing reaction (assembling) between *N*-2-Fmoc-aminoethyl glycine ester **5** or **6** and the bis-*N*-Boc protected nucleobase acetic acid units **10**, **14** and **21** by exploring several coupling reagents. The coupling could be accomplished by a number of methods. Examples of coupling reagents used to facilitate this transformation include, but are not limited to, carbodiimides such as dicyclohexylcarmodiimide (DCC), phosphonium salts such as benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) and uronium salts such as HBTU and HATU. Following activation, the side chain moiety is reacted with the amino-protected backbone to form a PNA synthon. In general, the use of HBTU and HATU, when applied to the synthesis of PNA monomers, enhances the coupling yields and shortens coupling times.

In place of the above-cited coupling reagents, we preferred to activate the carboxylic acid by use of a mixed anhydride. The mixed anhydride has an economical advantage



Scheme 9. Synthesis of bis-Boc-guaninylacetic acid.



Scheme 10. Synthesis of monomers.

over the more expensive HBTU and HATU reagents, and it allowed us to prepare the final monomer on a macroscale too.[51]

Examples of sterically hindered acid chlorides and chloroformates used to form "mixed carboxylic anhydrides" and "mixed carbonic anhydrides", respectively, include pivaloyl chloride (PivCl) and isobutylchloroformate (IBC).[52]

After several coupling tests, our results show that cytosylacetic acid **10** could couple to amine **5** to afford **22a** in good yield (88%) by using IBC as the coupling reagent, whereas the other acids gave poor results under these conditions (Scheme 10). On the contrary, acid **14** and **21** could be effectively coupled to amine **5** to give **23a** and **24a** (86 and 85% yield, respectively) through formation of the pivaloyl mixed anhydride.

In detail, the coupling reagent is added to a cooled THF solution of bis-Boc protected nucleobases (**10**, **14** or **21**) in the presence of a useful nonnucleophilic base such as *N*-methylmorpholine (NMM). After stirring at low temperature (0 °C for PvCl and –10 °C for IBC) for a sufficient time to permit the formation of mixed anhydride, a THF solution of Fmoc-protected backbone **5** and NMM was then added to the cooled solution. The hydrolysis of the resulting methyl esters **22a**–**24a** was carried out with NaOH (2.5 N) in THF and proceeded smoothly (30 min) to afford the corresponding PNA monomers **22b**–**24b** as white solids in almost quantitative yields (>90%).

A 12mer PNA sequence containing the four natural nucleobases was synthesized to check whether the monomers were compatible with Fmoc-mediated oligomerization and if the elimination of the protecting groups occurred readily. The sequence was synthesized by using all three bis-*N*-Boc protected monomers **22b**, **23b** and **24b** to give the crude oligomer H-GAGTACGCGCAT-Lys-NH_2 under standard conditions.[53] The monomers were linked just as well as the conventional Fmoc/Bhoc monomers during the machine-assisted process. The crude oligomers were analyzed and subsequently purified by RP-HPLC by using a C18 column and a linear gradient of acetonitrile. The identity of the oligomers was confirmed by ESI-MS. Analysis of the crude product mixture indicated that the desired oligomer represented >95% of the material (by HPLC area). Thus, the new monomers were demonstrated to be valid building blocks for PNA oligomerization.

Conclusions

In brief, a simple and practical synthesis of orthogonally protected PNA monomers is described. Protected Aeg monomers were efficiently prepared by reductive amination of *N*-Fmoc-glycinaldehyde with glycine methyl ester and the subsequent acylation of free amine with *N*-bis-Boc-protected nucleobases acetic acids. The exocyclic amine group

of the nucleobases, including guanine, which is notoriously difficult to handle, was protected with a bis-Boc carbamate group; this increased the solubility of the nucleobases in the most common organic solvents. The present protocol allows all Aeg monomers to be prepared on both the micro- and macroscale, which avoids or minimizes the use of toxic reagents or solvents, and moreover, cheap starting materials are used.

Experimental Section

General Remarks: Starting materials, reagents and dry solvents were purchased from commercial suppliers and used without further purification. Flash-column chromatography was performed on Merck Kieselgel 60 (230–400 mesh). Thin-layer chromatography (TLC) was performed on Merck Kieselgel 60 TLC plates. ^{1}H and ^{13}C NMR spectra were recorded in the solvents indicated at 300 and 75 MHz, respectively, unless otherwise noted. Chemical shifts are reported in parts per million (ppm, δ), are measured relative to tetramethylsilane (0.0 ppm) and referenced to the solvent $CDCl_3$ (7.26 ppm), [D_6]DMSO (2.49 ppm) and D_2O (4.79 ppm) for ^{1}H NMR and $CDCl_3$ (77.0 ppm) and [D_6]DMSO (39.5 ppm) for ^{13}C NMR. Data are reported as follows: chemical shifts, multiplicity (s = singlet, d= doublet, t = triplet, q = quartet, br. = broad, br. s = broad singlet, m = multiplet, p = pseudo), coupling constants *J* (Hz), relative integration value. Melting points were determined in open capillary tubes in circulating oil apparatus and are not corrected. High-resolution mass spectra (HRMS) were obtained by using electron impact (EI) or electrospray (ESI). All the new compounds gave satisfactory elemental analysis.

(9*H*-Fluoren-9-yl)methyl 2-Hydroxyethylcarbamate (1): To a solution of ethanolamine (1.00 g, 16.37 mmol) in CH_2Cl_2 (50 mL) was added saturated Na_2CO_3 aq. (100 mL) with mechanical stirring. A solution of 9-fluorenylmethyl chloroformate (Fmoc-Cl, 2.12 g, 8.20 mmol) in CH_2Cl_2 (15 mL) was added dropwise over 15 min. The reaction was briskly stirred overnight at room temperature. The mixture was poured into a separatory funnel, the two layers were separated and the aqueous layer was extracted again with CH_2Cl_2 (3 × 30 mL). The combined organic layer was washed with HCl aq. (1 N, 3 × 30 mL) and brine (1 × 30 mL), dried with anhydrous sodium sulfate, filtered and then evaporated to give a white solid. The resulting crystalline white solid was dried well in vacuo. Yield: 2.17 g (93%). R_f = 0.21 (hexane/AcOEt, 1:1). M.p. 143–145 °C. ^{1}H NMR ($CDCl_3$): δ = 7.76 (d, *J* = 7.2 Hz, 2 H), 7.58 (d, *J* = 7.2 Hz, 2 H), 7.42–7.25 (m, 5 H), 5.17 (s, 1 H), 4.43 (d, *J* = 6.7 Hz, 2 H), 4.21 (t, *J* = 7.1 Hz, 1 H), 3.70 (m, 2 H), 3.37–3.31 (m, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 155.3, 144.3, 141.6, 128.0, 127.2, 125.1, 120.1, 68.1, 58.3, 49.0, 43.8 ppm.

(9*H*-Fluoren-9-yl)methyl 2-Oxoethylcarbamate (2): To a solution of alcohol **1** (0.45 g, 1.59 mmol) dissolved in ethyl acetate (25 mL) was added IBX (1.35 g, 4.82 mmol). The resulting suspension was immersed in an oil bath set to 80 °C and stirred vigorously open to the atmosphere. After 4 h (TLC monitoring), the reaction was cooled to room temperature and filtered through a medium glass frit. The filter cake was washed with ethyl acetate (3 × 5 mL), and the combined filtrates were dried (Na_2SO_4) and then concentrated to afford Fmoc-glycinaldehyde **2**[54] (0.43 g, 96%, >95% pure by ^{1}H NMR) as a pale yellow solid. R_f = 0.3 (hexane/AcOEt, 3:7). M.p. 118°–120 °C. ^{1}H NMR ($CDCl_3$): δ = 9.61 (s, 1 H), 7.75 (d, *J* = 7.5 Hz, 3H, NH), 7.58 (d, *J* = 7.5 Hz, 2 H), 7.41–7.27 (m, 4 H), 4.41 (d, *J* = 6.9 Hz, 2 H), 4.21 (t, *J* = 6.8 Hz, 1 H), 4.11 (d, *J* = 5.0 Hz, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 196.4, 156.1, 143.6, 141.2, 127.7, 127.0, 124.9, 119.9, 67.1, 51.5, 47.0 ppm.

Methyl 2-(2-{[(9*H*-Fluoren-9-yl)methoxy]carbonylamino}ethylamino)-acetate (5): A solution of **2** (0.53 g, 1.9 mmol) in MeOH (30 mL) was added to a solution of glycine methyl ester hydrochloride (0.24 g, 1.9 mmol) and NEt_3 (0.26 mL, 1.9 mmol) in methanol (50 mL). The mixture was cooled to 0 °C under an atmosphere of nitrogen for 10 min and 10% Pd/C (0.01 g) was added with vigorous stirring. The reaction mixture was hydrogenated at atmospheric pressure and room temperature uptake had ceased (overnight). The catalyst was filtered through a pad of Celite, and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography (hexane/AcOEt, 1:4) to give backbone **5**[14] (0.50 g, 75%) as pale yellow oil. R_f = 0.26 (hexane/AcOEt, 1:4). ^{1}H NMR ($CDCl_3$): δ = 7.71 (d, *J* = 7.3 Hz, 2 H), 7.57 (d, *J* = 7.2 Hz, 2 H), 7.37–7.24 (m, 5 H), 4.39 (d, *J* = 6.6 Hz, 2 H), 4.17 (t, *J* = 6.7 Hz, 1 H), 3.65 (s, 3 H), 3.31 (s, 2 H), 3.21 (m, 2 H), 2.66 (t, *J* = 5.7 Hz, 2 H), 1.90 (s, 1 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 172.6, 156.3, 143.7, 141.0, 127.3, 126.7, 124.7, 119.6, 66.1, 51.5, 49.9, 48.3, 47.0, 40.3 ppm.

***N*-(2-{[(9*H*-Fluoren-9-yl)methoxy]carbonylamino}ethyl)-2-methoxy-2-oxoethanaminium (6):** To a precooled solution of **5** (2.83 g, 8.0 mmol) in dry dichloromethane (20 mL) was added HCl (2 N in MeOH, 5 mL, 10 mmol), whereupon the product precipitated out of the solution immediately. The mixture was allowed to stand at 4 °C overnight in a closed flask. The precipitate was filtered off with suction and dried for at least 5–6 h under high vacuum to give **6**[55] (3.06 g, 98%) as a white solid. M.p. 99–101 °C. R_f = 0.50 (*n*-BuOH/AcOH/H_2O, 3:1:1). ^{1}H NMR (CD_3OD): δ = 7.77 (d, *J* = 7.5 Hz, 2 H), 7.63 (d, *J* = 7.5 Hz, 2 H), 7.37 (t, *J* = 7.5 Hz, 2 H), 7.29 (t, *J* = 7.5 Hz, 2 H), 4.38 (d, *J* = 6.3 Hz, 2 H), 4.19 (t, *J* = 6.3 Hz, 1 H), 4.00 (s, 2 H), 3.81 (s, 3 H), 3.45 (t, *J* = 5.5 Hz, 2 H), 3.20 (d, *J* = 5.5 Hz, 2 H) ppm. ^{13}C NMR (CD_3OD): δ = 168.0, 159.4, 145.1, 142.6, 128.8, 128.1, 126.1, 120.9, 68.1, 53.5, 49.2, 48.2, 48.1, 38.1 ppm. IR (KBr): $\tilde{\nu}$ = 3465, 2958, 1749, 1531, 1453, 1250, 742, 588 cm^{-1}.

***tert*-Butyl 4-[Bis(*tert*-butoxycarbonyl)amino]-2-oxopyrimidine-1(2*H*)-carboxylate (7):** To a 100-mL Ar-flushed flask equipped with a magnetic stir bar and containing cytosine (1.0 g, 9.0 mmol) and DMAP (0.100 g, 0.9 mmol) was added dropwise dry THF (50 mL). To the stirred suspension was added Boc_2O (7.85 g, 36 mmol) under an Ar atmosphere. The reaction mixture was stirred overnight at room temperature (TLC analysis indicated the presence of a single product and starting solid cytosine was entirely solubilized). The excess amount of THF was evaporated, and the crude product was dissolved in AcOEt (300 mL), washed with HCl aq. (1 N, 1 × 15 mL) and brine (2 × 15 mL), dried with Na_2SO_4 and concentrated in vacuo to give **7** as a pale yellow oil[56] (3.67 g, 99%). R_f = 0.45 (hexane/AcOEt, 7:3). ^{1}H NMR ($CDCl_3$): δ = 7.97 (d, *J* = 7.9 Hz, 1 H), 7.07 (d, *J* = 7.8 Hz, 1 H), 1.60 (s, 9 H), 1.56 (s, 18 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 162.3, 160.0, 150.1, 149.0, 143.3, 96.8, 85.2, 27.5 ppm. HRMS (EI): calcd. for $C_{19}H_{29}N_3O_7$ $[M]^+$ 411.2006; found 411.1907.

Bis-Boc-Cytosine (8): To a solution of **7** (8.08 g, 19.64 mmol) dissolved in MeOH (290 mL) was added saturated $NaHCO_3$ aq. (90 mL). The turbid solution was stirred at 50 °C for 1 h, at which point clean conversion to bis-Boc protected cytosine was observed by TLC. After evaporation of MeOH, water (130 mL) was added to the suspension, and the aqueous layer was extracted with CH_2Cl_2 (3 × 100 mL). The organic layer was dried with Na_2SO_4, filtered and evaporated to give pure **8** (5.99 g, 99%) as a white solid. R_f = 0.65 (AcOEt). M.p. 320 °C (decomp.). ^{1}H NMR ($CDCl_3$): δ = 7.68

(d, J = 7.1 Hz, 1 H), 7.26 (s, 1 H), 7.12 (d, J = 7.1 Hz, 1 H), 1.56 (s, 18 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 163.6, 158.4, 149.4, 145.6, 96.7, 84.9, 27.6 ppm. HRMS (ESI): calcd. for $C_{14}H_{22}N_3O_5$ [M + H]$^+$ 312.1554; found 311.1548.

Methyl 2-{4-[Bis(*tert*-butoxycarbonyl)amino]-2-oxopyrimidin-1(2*H*)-yl}acetate (9): Sodium hydride (0.33 g, 13.9 mmol) was added to dry THF (150 mL) in a dry 500-mL round-bottom flask under an argon atmosphere. The reaction mixture was stirred at 0 °C for a period of 15–20 min and a solution of bis-Boc cytosine (3.94 g, 12.6 mmol) in dry THF (150 mL) was then added dropwise. To the rapidly stirred mixture was then adjoined dropwise (over 15–20 min) methyl bromoacetate (1.32 mL, 13.90 mmol) and DMAP (0.02 g, 0.16 mmol). After complete addition, the reaction mixture was stirred overnight whilst warming to room temperature. The reaction was then quenched by the addition of H_2O (0.5 mL, 27.8 mmol). The solvent (THF) was removed, and the residue was then redissolved in CH_2Cl_2 (500 mL) and washed with water (3 × 100 mL). After evaporation of THF, a light-brown oil was obtained, which was purified by flash chromatography on silica gel (AcOEt) to give **9** (4.20 g, 86%) as a pale-orange oil. R_f = 0.70 (AcOEt). ^{1}H NMR ($CDCl_3$): δ = 7.58 (d, J = 7.4 Hz, 1 H), 7.09 (d, J = 7.3 Hz, 1 H), 4.59 (s, 2 H), 3.76 (s, 3 H), 1.55 (s, 18 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 198.7, 167.6, 162.7, 149.3, 148.1, 96.4, 84.8, 52.6, 29.5, 27.5 ppm. HRMS (ESI): calcd. for $C_{17}H_{26}N_3O_7$ [M + H]$^+$ 384.1765; found 384.1751.

2-{4-[Bis(*tert*-butoxycarbonyl)amino]-2-oxopyrimidin-1(2*H*)-yl}acetic Acid (10): To a solution of **9** (4.7 g, 12.3 mmol) in THF (40 mL) cooled to 0 °C in an ice bath was added dropwise NaOH aq. (1 N, 13.5 mL, 13.5 mmol). The reaction was then allowed to stir for about 30 min until TLC indicated complete consumption of the starting material (the reaction was continuously monitored by TLC) and then quenched by the addition of H_2O (40 mL) and $KHSO_4$ (portionwise) to pH 2–3. The reaction mixture was poured into a separatory funnel containing CH_2Cl_2 (100 mL). The aqueous layer was further extracted with AcOEt (3 × 50 mL). The combined organic fraction was dried with $MgSO_4$, and the solvent was evaporated to dryness in vacuo. The colourless oil was first coevaporated with CH_2Cl_2 (3 × 15 mL) and then concentrated under high vacuum for at least 3 h (to an unchanged weight) to give **10** (3.86 g, 85%) as an off-white solid. R_f = 0.1 (hexane/AcOEt, 6:4). M.p. 127°–130 °C. ^{1}H NMR ([D$_6$]DMSO): δ = 9.82 (s, 1 H), 8.13 (d, J = 7.2 Hz, 1 H), 6.83 (d, J = 7.2 Hz, 1 H); 4.56 (s, 2 H), 1.49 (s, 18 H) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 169.1, 161.9, 154.2, 151.2, 149.2, 95.3, 84.4, 54.8, 27.2 ppm. HRMS (EI): calcd. for $C_{16}H_{24}N_3O_7$ [M + H]$^+$ 370.1614; found 370.1610.

***tert*-Butyl 6-[Bis(*tert*-butoxycarbonyl)amino]-9*H*-purine-9-carboxylate (11):** To a 100-mL Ar-flushed flask equipped with a magnetic stir bar and containing adenine (1.00 g, 7.4 mmol) and DMAP (0.08 g, 0.74 mmol) was added dry THF (50 mL). To the stirred suspension was added Boc_2O (6.46 g, 29.6 mmol) under an Ar atmosphere. The reaction mixture was stirred overnight at room temperature, at which point TLC analysis indicated the presence of a single product. The excess amount of THF was removed to give **11** (3.22 g, 99%) as a yellow oil. R_f = 0.45 (hexane/AcOEt, 7:3). M.p. 54–55 °C. ^{1}H NMR ($CDCl_3$): δ = 9.02 (s, 1 H), 8.53 (s, 1 H), 1.72 (s, 9 H), 1.44 (s, 18 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 156.4, 153.6, 152.1, 150.8, 149.6, 142.9, 129.2, 87.1, 83.5, 27.3 ppm.

Bis-Boc-Adenine (12): To a solution of **11** (3.22 g, 7.4 mmol) dissolved in MeOH (80 mL) was added saturated $NaHCO_3$ aq. (40 mL). The turbid solution was stirred at 50 °C for 1 h, at which point clean conversion to bis-Boc protected adenine was observed by TLC. After evaporation of MeOH, water (50 mL) was added to the suspension, and the aqueous layer was extracted with CH_2Cl_2 (3 × 100 mL). The organic layer was dried with Na_2SO_4, filtered and evaporated to give pure **12** (2.38 g, 96%) a as a white solid. R_f = 0.45 (AcOEt). M.p. 148–149 °C. ^{1}H NMR ($CDCl_3$): δ = 8.87 (s, 1 H), 8.47 (s, 1 H), 1.50 (s, 18 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 151.9, 151.6, 150.1, 149.1, 144.3, 129.3, 84.5, 27.6 ppm.

Benzyl 2-{6-[Bis(*tert*-butoxycarbonyl)amino]-9*H*-purin-9-yl}acetate (13): To a solution of **12** (1.12 g, 3.34 mmol) in dry THF (60 mL) cooled in an ice bath for 15 min was added NaH (0.15 g, 5.0 mmol) under an argon atmosphere whilst stirring. To the rapidly stirred mixture was then added dropwise (over 10 min) benzyl bromoacetate (0.64 mL, 4.01 mmol) and DMAP (0.01 g, 0.08 mmol). After complete addition, the reaction was stirred overnight whilst warming to room temperature. The reaction mixture was then quenched by the addition of H_2O (0.18 mL, 10.0 mmol). The solvent (THF) was removed, and the residue was then redissolved in CH_2Cl_2 (200 mL) and washed with H_2O (3 × 50 mL). After evaporation of THF, a light-yellow oil was obtained, which was purified by flash chromatography on silica gel (hexane/AcOEt, 6:4) to give **13** (1.147 g, 71%) as a pale-yellow oil. R_f = 0.26 (hexane/AcOEt, 6:4). ^{1}H NMR ($CDCl_3$): δ = 8.83 (s, 1 H), 8.18 (s, 1 H), 7.33 (ps, 5 H), 5.20 (s, 2 H), 5.11 (s, 2 H), 1.41 (s, 18 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 166.4, 153.1, 151.8, 151.3, 149.9, 145.0, 134.2, 128.3, 128.1, 128.0, 127.8, 83.3, 67.6, 60.0, 44.0, 27.3 ppm. HRMS (ESI): calcd. for $C_{24}H_{29}N_5O_6$ [M]$^+$ 483.2118; found 483.2131.

2-{6-[Bis(*tert*-butoxycarbonyl)amino]-9*H*-purin-9-yl}acetic Acid (14): To a suspension of **13** (0.71 g, 1.47 mmol) in methanol (40 mL) was added 10% Pd/C (0.05 g) under an argon atmosphere, and the mixture was hydrogenated until the substrate disappeared (by TLC, about 8–12 h). The reaction mixture was filtered thought a pad of Celite, and the solvent was evaporated to give pure **14** (0.57 g, 98%) as a white solid. R_f = 0.1 (hexane/AcOEt, 6:4). M.p. 155–156 °C. ^{1}H NMR ($CDCl_3$): δ = 8.89 (s, 1 H); 8.47 (s, 1 H); 5.10 (s, 2 H); 1.40 (s, 18 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 168.5, 153.1, 152.2, 149.9, 149.8, 146.4, 127.2, 84.2, 44.4, 27.7 ppm. HRMS (ESI): calcd. for $C_{17}H_{23}N_5O_6$ [M]$^+$ 393.1648; found 393.1644.

***tert*-Butyl 2-[Bis(*tert*-butoxycarbonyl)amino]-6-chloro-9*H*-purine-9-carboxylate (17a):** To a 100-mL Ar-flushed flask equipped with a magnetic stir bar and containing 2-amino-6-chloropurine (1.0 g, 5.9 mmol) and DMAP (0.07 g, 0.59 mmol) was added dropwise dry THF (50 mL). To the stirred suspension was added Boc_2O (5.15 g, 23.6 mmol) under an Ar atmosphere. The reaction mixture was stirred overnight at room temperature (TLC analysis indicated the presence of a single product and starting solid 2-mino-6-chloropurine was entirely solubilized). The excess amount of THF was removed, and the crude product was dissolved in AcOEt (300 mL), washed with HCl aq. (1 N, 1 × 15 mL) and brine (2 × 15 mL), dried with Na_2SO_4 and concentrated in vacuo to give **17a** as a white foam (2.77 g, 99%). R_f = 0.50 (hexane/AcOEt, 6:4). M.p. 51–52 °C. ^{1}H NMR ($CDCl_3$): δ = 8.58 (s, 1 H); 1.69 (s, 9 H); 1.47 (s, 18 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 153.0, 152.7, 151.2, 149.9, 148.7, 144.3, 139.6, 87.4, 83.2, 27.2 ppm.

Bis-Boc-2-amino-6-chloropurine (17b): To a solution of **17a** (2.8 g, 6.0 mmol) in MeOH (100 mL) was added saturated $NaHCO_3$ aq. (50 mL). The turbid solution was stirred at 50 °C for 1 h, at which point clean conversion to bis-Boc protected adenine was observed by TLC. After evaporation of MeOH, H_2O (40 mL) was added to the suspension, and the aqueous layer was extracted with CH_2Cl_2 (3 × 80 mL). The organic layer was dried with Na_2SO_4, filtered and evaporated to give pure **17b** (2.20 g, 99%) as a waxy solid. R_f = 0.60 (AcOEt). ^{1}H NMR ($CDCl_3$): δ = 8.41 (s, 1 H); 1.49 (s, 18 H)

ppm. ^{13}C NMR ($CDCl_3$): δ = 155.7, 150.6, 150.3, 148.0, 147.2, 127.5, 83.5, 27.3 ppm.

Methyl 2-{2-[Bis(*tert*-butoxycarbonyl)amino]-6-chloro-9*H*-purin-9-yl}acetate (18): Sodium hydride (0.2 g, 4.9 mmol) was added to dry THF (100 mL) in a dry 500-mL round-bottom flask under an argon atmosphere. The reaction mixture was stirred at 0 °C for a period of 10–15 min, and a solution of bis-Boc 2-amino-6-chlorpurine (1.64 g, 4.43 mmol) in dry THF (100 mL) was added dropwise. To the rapidly stirring mixture was then adjoined dropwise (over 10 to 15 min) methyl bromoacetate (0.46 mL, 4.9 mmol) and DMAP (0.01 g, 0.08 mmol). After complete addition, the reaction was stirred overnight whilst warming to room temperature. The reaction mixture was then quenched by the addition of H_2O (0.18 mL, 9.8 mmol). The solvent (THF) was removed, and the residue was then redissolved in CH_2Cl_2 (300 mL) and washed with water (3 × 100 mL). After evaporation of THF, a light-brown oil was obtained, which was purified by flash chromatography on silica gel (AcOEt/hexane, 7:3) to give **18** (1.53 g, 78%) as a pale-yellow oil. R_f = 0.44 (AcOEt/hexane, 7:3). ^1H NMR ($CDCl_3$): δ = 8.31 (s, 1 H), 5.10 (s, 2 H), 3.82 (s, 3 H), 1.43 (s, 18 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 166.7, 152.6, 151.8, 150.9, 150.2, 146.6, 129.3, 83.5, 52.9, 44.3, 27.6 ppm. HRMS (ESI): calcd. for $C_{18}H_{24}ClN_5O_6$ $[M]^+$ 441.1415; found 441.1418.

Methyl 2-{2-[Bis(*tert*-butoxycarbonyl)amino]-6-oxo-1*H*-purin-9(6*H*)-yl}acetate (20): To a stirred ethanolic solution of NMe_3 (35% in EtOH/H_2O, 95:5; 12 mL, 2 mmol) was portionwise added **18** (1.07 g, 2.42 mmol), and the reaction mixture was allowed to stir at room temperature overnight.[57] The mixture was centrifuged, and the crude precipitate was filtered off and washed with anhydrous Et_2O to give **20** as a white solid, which was further dried under high vacuum (2 mbar) for at least 3 h (0.69 g, 67%). M.p. 180–182 °C (decomp.). ^1H NMR ($CDCl_3$): δ = 7.81 (s, 1 H), 4.95 (s, 2 H), 3.76 (s, 3 H), 1.45 (s, 18 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 167.6, 155.1, 152.4, 151.6, 151.2, 139.0, 117.8, 82.3, 52.5, 43.7, 27.7 ppm. HRMS (ESI): calcd. for $C_{18}H_{26}N_5O_7$ $[M + H]^+$ 424.1827; found 424.1830.

2-{2-[Bis(*tert*-butoxycarbonyl)amino]-6-oxo-1*H*-purin-9(6*H*)-yl}acetic Acid (21): To a solution of **20** (1.69 g, 4.0 mmol) in THF (25 mL) cooled to 0 °C in an ice bath was added dropwise NaOH aq. (1 N, 4.4 mL, 4.4 mmol). The reaction mixture was then allowed to stir for about 30 min until TLC indicated complete consumption of the starting material (the reaction was continuously monitored by TLC) and then quenched by the addition of H_2O (22 mL) and $KHSO_4$ (portionwise) to pH 3. The reaction mixture was poured into a separatory funnel containing CH_2Cl_2 (100 mL). The aqueous layer was further extracted with AcOEt (3 × 50 mL). The combined organic fraction was dried with $MgSO_4$, and the solvent was evaporated to dryness in vacuo. The colourless oil was first coevaporated with CH_2Cl_2 (3 × 15 mL) and then concentrated under high vacuum for at least 3 h (to an unchanged weight) to give **21** (1.60 g, 98%) as an off-white solid. ^1H NMR ($CDCl_3$): δ = 7.93 (s, 1 H), 4.91 (s, 2 H), 1.43 (s, 18 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 168.9, 156.9, 155.1, 151.2, 151.4, 140.1, 117.4, 82.8, 44.2, 27.8 ppm. HRMS (ESI): calcd. for $C_{17}H_{24}N_5O_7$ $[M + H]^+$ 410.1670; found 410.1668.

Methyl 2-[*N*-(2-{[(9*H*-Fluoren-9-yl)methoxy]carbonylamino}ethyl)-2-{4-[bis(*tert*-butoxycarbonyl)amino]-2-oxopyrimidin-1(2*H*)-yl}acetamido]acetate (22a): Under an atmosphere of argon at –15 °C, isobutylchloroformate (IBC, 0.28 mL, 2.14 mmol) was dosed to a solution of **10** (0.79 g, 2.14 mmol) in dry THF (60 mL). Subsequently, *N*-methyl morpholine (NMM, 0.44 mL, 4.28 mmol) in dry THF (3 mL) was added dropwise at such a rate that the temperature remained –15 °C. Stirring was continued for an additional 15 min. A reaction temperature between –10 and –15 °C is recommended, where the reaction occurs immediately upon addition of NMM, yet prevents the mixed anhydride from decomposing too rapidly. Compound **5** (0.83 g, 2.35 mmol), meanwhile, was dissolved in dry THF (15 mL) and cooled to 0 °C. This latter solution was upon completion of the activation added at a rate that maintained the temperature around –15 °C (about 10 min). Stirring was subsequently continued for 30 min at –10 °C. The reaction mixture was warmed to room temperature and further stirred at this temperature overnight. After removal of the solvent, the organic residue was redissolved in AcOEt (250 mL) and then washed with HCl aq. (1 N, 3 × 50 mL) and saturated $NaHCO_3$ aq. (3 × 50 mL). The organic phase was then dried with Na_2SO_4, and the solvent was evaporated under vacuum. The crude reaction product was purified by flash chromatography (AcOEt). The combined fractions were dried to give **22a** as a pale-yellow oil (1.32 g, 88%). ^1H NMR ($CDCl_3$): δ = 7.73 (d, J = 7.5 Hz, 2 H), 7.60 (t, J = 7.4 Hz, 2 H), 7.44–7.27 (m, 5 H), 7.06 (t, J = 7.3 Hz, 1 H), 6.16 (t, J = 7.4 Hz, 1 H), 4.58 (s, 2 H), 4.40 (d, J = 7.2 Hz, 2 H), 4.19 (t, J = 7.5 Hz, 1 H), 4.03 (s, 2 H), 3.71 (s, 3 H), 3.59–3.34 (m, 4 H), 1.53 (s, 18 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 170.3, 167.5, 162.9, 156.9, 155.3, 155.2, 149.6, 149.4, 144.1, 141.4, 127.9, 127.3, 126.9, 125.3, 120.1, 96.3, 85.1, 66.9, 53.0, 52.6, 49.7, 49.1, 47.3, 39.5, 27.8 ppm. HRMS (ESI): calcd. for $C_{36}H_{44}N_5O_{10}$ $[M]^+$ 706.3083; found 706.3095.

2-[*N*-(2-{[(9*H*-Fluoren-9-yl)methoxy]carbonylamino}ethyl)-2-{4-[bis(*tert*-butoxycarbonyl)amino]-2-oxopyrimidin-1(2*H*)-yl}acetamido]-acetic Acid (22b): Methyl ester **22a** (0.71 g, 1 mmol) dissolved in THF (2 mL) and cooled to 0 °C was subjected to hydrolysis with NaOH aq. (2.5 N, 4 mL, 10 equiv.). The mixture was stirred at 0 °C for about 30 min, at which point the TLC showed complete disappearance of the starting ester. Cold H_2O (10 mL) was added to the reaction mixture, which was first extracted with Et_2O (3 × 5 mL) and then acidified to pH 3 with $KHSO_4$ (at 0 °C, the flask was placed in an ice bath). The aqueous solution was extracted with AcOEt (3 × 25 mL), and the combined organic layer was washed with brine (2 × 25 mL). The organic solution was dried with $MgSO_4$ overnight, and the solvent was then evaporated to dryness in vacuo to give the desired final monomer **22b** (0.622 g, 90%) as an off-white solid. M.p. 110–112 °C. ^1H NMR ($CDCl_3$): δ = 7.73 (d, J = 7.7 Hz, 2 H), 7.60–7.48 (m, 3 H), 7.37–7.23 (m, 5 H), 7.14–7.07 (m, 1 H), 6.40 (t, J = 7.3 Hz, 1 H), 4.67 (s, 2 H), 4.33 (d, J = 7.4 Hz, 2 H), 4.21–4.17 (m, 1 H), 4.00 (s, 2 H), 3.52–3.38 (m, 4 H), 1.51 (s, 18 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 171.1, 166.9, 162.8, 156.9, 156.3, 149.6, 149.2, 143.8, 141.1, 127.5, 127.0, 126.3, 125.1, 119.8, 97.0, 85.3, 66.9, 53.3, 52.9, 48.5, 47.0, 38.9, 27.5 ppm. HRMS (ESI): calcd. for $C_{35}H_{41}N_5O_{10}Na$ $[M]^+$ 714.2751; found 714.2756.

Methyl 2-[*N*-(2-{[(9*H*-Fluoren-9-yl)methoxy]carbonylamino}ethyl)-2-{6-[bis(*tert*-butoxycarbonyl)amino]-9*H*-purin-9-yl}acetamido]-acetate (23a): To a solution of **14** (0.98 g, 2.5 mmol) in dry acetonitrile (80 mL) was added in one portion freshly distilled *N*-methylmorpholine (NMM, 0.52 mL, 5 mmol) at room temperature, and the resulting mixture was stirred for 10 min. The mixture was cooled to 0 °C, and pivaloyl chloride (PivCl, 0.33 mL, 2.5 mmol) was added dropwise under an argon atmosphere. The reaction mixture was further stirred for 30 min at 0 °C. Then, **5** (1 g, 2.8 mmol) was added under vigorous stirring to the starting reaction mixture, and the resulting mixture was stirred overnight at room temperature. The solution was diluted with ethyl acetate (300 mL) and then washed with brine (2 × 50 mL). The aqueous layer was back extracted with ethyl acetate (4 × 50 mL). The combined organic layer

was washed with HCl aq. (1 N, 3 × 50 mL), brine (1 × 50 mL), saturated $NaHCO_3$ aq. (3 × 50 mL) and brine (1 × 50 mL) and then dried with $MgSO_4$, and the solvent was evaporated to dryness in vacuo. The crude material was purified by flash chromatography (AcOEt) to give desired monomer **23a** (1.57 g, 86%) as a pale-yellow solid. R_f = 0.16 (AcOEt). M.p. 84°–85 °C. ^{1}H NMR ($CDCl_3$): δ = 8.17 (s, 1 H), 8.05 (s, 1 H), 7.76–7.69 (m, 3 H), 7.60–7.56 (m, 2 H), 7.38–7.26 (m, 4 H), 5.06 (s, 2 H), 4.52 (d, J = 6.7 Hz, 2 H), 4.21 (t, J = 6.1 Hz, 1 H), 4.04 (s, 2 H), 3.72 (s, 3 H), 3.63–3.58 (m, 2 H), 4.45–3.41 (m, 2 H), 1.41 (s, 18 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 170.2, 169.2, 156.5, 153.3, 151.8, 150.4, 150.0, 143.7, 143.5, 141.2, 127.6, 126.9, 125.0, 119.9, 87.9, 67.1, 50.1, 49.2, 47.1, 39.0, 38.8, 28.2 ppm. HRMS (ESI): calcd. for $C_{37}H_{44}N_7O_9$ $[M + H]^+$ 730.7862; found 730.7853.

2-[*N*-(2-{[(9*H*-Fluoren-9-yl)methoxy]carbonylamino}ethyl)-2-{6-[bis(*tert*-butoxycarbonyl)amino]-9*H*-purin-9-yl}acetamido]acetic Acid (23b): Methyl ester **23a** (0.729 g, 1 mmol) dissolved in THF (2 mL) cooled to 0 °C was subjected to hydrolysis with NaOH aq. (2.5 N, 4 mL, 10 equiv.). The mixture was stirred at 0 °C for about 30 min, at which point the TLC showed complete disappearance of the starting ester. Cold H_2O (10 mL) was added to the reaction mixture, which was first extracted with Et_2O (3 × 5 mL) and then acidified to pH 3 with $KHSO_4$ (at 0 °C, the flask was placed in an ice bath). The aqueous solution was extracted with AcOEt (3 × 25 mL), and the combined organic layer was further washed with brine (2 × 25 mL). The organic solution was dried with $MgSO_4$ overnight, and the solvent was evaporated to dryness in vacuo to give the desired final monomer **23b** (0.672 g, 94%) as an off-white solid. M.p. 103–104 °C. ^{1}H NMR ($CDCl_3$): δ = 8.27 (s, 1 H), 8.16 (s, 1 H), 7.74–7.65 (m, 3 H), 7.58–7.48 (m, 2 H), 7.36–7.23 (m, 4 H), 5.14 (s, 2 H), 4.43 (d, J = 7.4 Hz, 2 H), 4.20–4.17 (m, 1 H), 4.01 (s, 2 H), 3.51–3.35 (m, 4 H), 1.38 (s, 18 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 176.4, 171.4, 157.2, 153.5, 152.1, 150.1, 149.8, 143.7, 143.6, 141.1, 127.6, 127.0, 125.4, 125.0, 119.8, 83.9, 66.8, 48.8, 48.3, 47.0, 39.5, 38.7, 27.6 ppm. HRMS (ESI): calcd. for $C_{36}H_{41}N_7O_9Na$ $[M + Na]^+$ 738.2863; found 738.2850.

Methyl 2-[*N*-(2-{[(9*H*-Fluoren-9-yl)methoxy]carbonylamino}ethyl)-2-{2-[bis(*tert*-butoxycarbonyl)amino]-6-oxo-1*H*-purin-9(6*H*)-yl}acetamido]acetate (24a): To a solution of **21** (1.29 g, 3.15 mmol) in dry acetonitrile (100 mL) was added in one portion *N*-M ethylmorpholine (NMM, 0.65 mL, 6.3 mmol) at room temperature, and the resulting mixture was stirred for 10 min. The mixture was cooled to 0 °C, and pivaloyl chloride (PivCl, 0.42 mL, 3.15 mmol) was added dropwise under an argon atmosphere. The reaction mixture was further stirred for 30 min at 0 °C. Then, **5** (1.23 g, 3.45 mmol) was added under vigorous stirring to the starting reaction mixture, and the resulting mixture was stirred overnight at room temperature. The solution was diluted with ethyl acetate (350 mL) and then washed with brine (2 × 50 mL). The aqueous layer was back extracted with AcOEt (4 × 50 mL). The combined organic layer was washed with HCl aq. (1 N, 3 × 50 mL), brine (1 × 50 mL), saturated $NaHCO_3$ aq. (3 × 50 mL) and brine (1 × 50 mL) and then dried with $MgSO_4$, and the solvent was evaporated to dryness in vacuo. The crude material was purified by flash chromatography (AcOEt) to give the desired monomer **24a** (2.0 g, 85%) as a pale-yellow oil. ^{1}H NMR ($CDCl_3$): δ = 7.87 (s, 1 H), 7.73 (t, J = 7.1 Hz, 3 H), 7.55 (t, J = 6.9 Hz, 2 H), 7.38–7.24 (m, 4 H), 4.96 (s, 2 H), 4.32 (d, J = 6.9 Hz, 2 H), 4.17–4.11 (m, 1 H), 4.05 (s, 2 H), 3.72 (s, 3 H), 3.61–3.29 (m, 4 H), 1.46 (s, 18 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 169.7, 167.1, 157.7, 156.6, 155.1, 152.2, 151.5, 143.6, 141.1, 139.5, 127.5, 126.9, 125.8, 124.9, 119.8, 117.7, 82.7, 66.6, 52.3, 49.2, 48.6, 47.0, 38.8, 29.5, 27.8 ppm. HRMS (ESI): calcd. for $C_{37}H_{44}N_7O_{10}$ $[M + H]^+$ 746.3144; found 746.3130.

2-[*N*-(2-{[(9*H*-Fluoren-9-yl)methoxy]carbonylamino}ethyl)-2-{2-[bis(*tert*-butoxycarbonyl)amino]-6-oxo-1*H*-purin-9(6*H*)-yl}acetamido]acetic Acid (24b): Methyl ester **24a** (0.75 g, 1 mmol) dissolved in THF (2 mL) and cooled to 0 °C was subjected to hydrolysis with NaOH aq. (2.5 N, 4 mL, 10 equiv.). The mixture was stirred at 0 °C for about 30 min, at which point the TLC showed complete disappearance of the starting ester. Cold H_2O (10 mL) was added to the reaction mixture, which was first extracted with Et_2O (3 × 5 mL) and then acidified to pH 3 with $KHSO_4$ (at 0 °C, the flask was placed in an ice bath). The aqueous solution was extracted with AcOEt (3 × 25 mL), and the combined organic layer was further washed with brine (2 × 25 mL). The organic solution was dried with $MgSO_4$ overnight, and the solvent was then evaporated to dryness in vacuo to give the desired final monomer **24b** (0.680 g, 93%) as an off-white solid. M.p. 255 °C (decomp.). ^{1}H NMR ($CDCl_3$): δ = 7.99 (s, 1 H), 7.69 (t, J = 7.4 Hz, 3 H), 7.53 (t, J = 7.1 Hz, 2 H), 7.36–7.22 (m, 5 H), 5.06 (s, 2 H), 4.30 (t, J = 6.8 Hz, 2 H), 4.17–4.12 (m, 1 H), 4.05 (s, 2 H), 3.55–3.34 (m, 4 H), 1.42 (s, 18 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 173.1, 167.5, 156.5, 155.1, 152.1, 151.4, 143.8, 143.6, 141.0, 140.2, 127.5, 126.8, 125.0, 124.7, 119.7, 117.7, 82.4, 66.6, 53.3, 49.3, 46.2, 38.2, 29.4, 27.7 ppm. HRMS (ESI): calcd. for $C_{36}H_{41}N_7O_{10}$ $[M + H]^+$ 731.2915; found 731.2920.

Supporting Information (see footnote on the first page of this article): ^{1}H and ^{13}C NMR spectra for compounds **1**–**24b**; HPLC spectrum for the PNA sequence.

Acknowledgments

This work was financially supported by the University of Sassari, Fondazione Banco di Sardegna and MIUR (Rome) within the project PRIN 2005: "Structure and Activity Studies of DNA Quadruplexes through the Exploitation of Synthetic Oligonucleotides and Analogues". For a closer examination, see: http://www.ricercaitaliana.it/prin/unita_op_en-2005030447_004.htm.

[1] P. E. Nielsen, M. Egholm, R. H. Berg, O. Buchardt, *Science* **1991**, *254*, 1497.
[2] M. Egholm, O. Buchardt, L. Christensen, C. Behrens, S. M. Freier, D. A. Driver, R. H. Berg, S. K. Kim, B. Norden, P. E. Nielsen, *Nature* **1993**, *365*, 566.
[3] M. Egholm, O. Buchardt, P. E. Nielsen, R. H. Berg, *J. Am. Chem. Soc.* **1992**, *114*, 1895.
[4] O. Buchardt, M. Egholm, R. H. Berg, P. E. Nielsen, *Trends Biotechnol.* **1993**, *11*, 384.
[5] a) P. E. Nielsen, *Curr. Opin. Biotechnol.* **2001**, *12*, 16; b) M. Leijon, M. Mousavi-Jazi, M. Kubista, *Mol. Aspects Med.* **2006**, *27*,160.
[6] For more about the application, properties and standard syntheses of PNA, see the following review: P. E. Nielsen, *Acc. Chem. Res.* **1999**, *32*, 624 and the references cited therein.
[7] a) A. Porcheddu, G. Giacomelli, *Curr. Med. Chem.* **2005**, *12*, 2561; b) K. L. Dueholm, M. Egholm, C. Behrens, L. Christensen, H. F. Hassen, T. Vulpius, *J. Org. Chem.* **1994**, *59*, 5767; c) L. Christensen, R. Fitzpatrick, B. Gildea, K. H. Petersen, H. F. Hansen, T. Koch, M. Egholm, O. Buchardt, P. E. Nielsen, J. Coull, R. Berg, *J. Pept. Sci.* **1995**, *1*, 175; d) T. Koch, H. F. Hansen, P. Andresen, T. Larsen, H. G. Batz, K. Ottensen, H. Orum, *J. Pept. Res.* **1997**, *49*, 80; e) S. A. Thomson, J. A. Josey, R. Cadilla, M. D. Gaul, C. F. Hassman, M. J. Luzzio, A. J. Pipe, K. L. Reed, D. J. Ricca, R. W. Wiethe, S. A. Noble, *Tetrahedron* **1995**, *51*, 6179; f) D. W. Will, G. Breipohl, D. Langner,

J. Knolle, E. Uhlmann, *Tetrahedron* **1995**, *51*, 12069; g) A. C. Van der Laan, N. J. Meeuwenoord, E. Kuyl-Yeheskiely, R. S. Oosting, R. Brands, J. H. van Boom, *Recl. Trav. Chim. Pays-Bas* **1995**, *114*, 295; h) A. C. Van der Laan, R. Brill, R. G. Kuimelis, E. Kuyl-Yeheskiely, E. Uhlmann, J. H. van Boom, A. Andrus, R. Vinayak, *Tetrahedron Lett.* **1997**, *38*, 2249; i) A. C. Van der Laan, P. Havenaar, R. S. Oosting, E. Kuyl-yeheskiely, E. Uhlmann, J. H. van Boom, *Bioorg. Med. Chem. Lett.* **1998**, *8*, 663.

[8] a) See ref.[7c]; b) J. C. Norton, J. H. Waggenspack, E. Varnum, D. R. Corey, *Bioorg. Med. Chem.* **1995**, *3*, 437; c) G. Haaima, A. Lohse, O. Buchardt, P. E. Nielsen, *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1939; d) K. Dueholm, M. Egholm, C. Behrens, L. Christensen, H. F. Hansen, T. Vulpius, K. H. Petersen, R. H. Berg, P. E. Nielsen, O. Buchardt, *J. Org. Chem.* **1994**, *59*, 5767.

[9] Y. Wu, J.-C. Xu, *Tetrahedron* **2001**, *57*, 8107.

[10] S. A. Thomson, J. A. Josey, R. Cadilla, M. D. Gaul, C. F. Hassman, M. J. Luzzio, A. J. Pipe, K. L. Reed, D. J. Ricca, R. W. Wiethe, S. A. Noble, *Tetrahedron* **1995**, *51*, 6179.

[11] Fmoc/Bhoc PNA monomers are commercially available from ASM Research Chemicals: www.asm-research-chemicals.com/index.

[12] The modular nature of PNA is particularly amenable to structural variation, and this has led to the synthesis of a wide variety of modified PNAs.

[13] For a more detailed comparison between *N*-Bhoc and *N*-bis-Boc nucleobase protective groups see Hudson's paper, page 3809 (last paragraph).[23]

[14] G. Breiphol, J. Knolle, D. Langner, G. O'Malley, E. Uhlmann, *Bioorg. Med. Chem. Lett.* **1996**, *6*, 665.

[15] Protecting group for the exocyclic amino functions on the nucleobases.

[16] a) Ceric ammonium nitrate (CAN): J. R. Hwu, M. L. Jain, S.-C. Tsay, H. Hakimelahi, *Tetrahedron Lett.* **1996**, *37*, 2035; b) microwave irradiation: J. G. Siro, J. Martın, J. L. Garcia, M. J. Remuinan, J. J. Vaquero, *Synlett* **1998**, 147; c) NaI/acetone: J. Ham, K. Choi, J. Ko, H. Lee, M. Jung, *Protein Pept. Lett.* **1998**, *5*, 257.

[17] T. Sugiyama, A. Kittaka, Y. Takemoto, H. Takayama, R. Kuroda, *Nucleic Acids Res. Suppl.* **2002**, *2*, 145.

[18] a) B. C. J. Van Esseveldt, F. L. Van Delft, J. M. M. Smits, R. Gelder, H. E. Schoemaker, F. P. J. T. Rutjes, *Adv. Synth. Catal.* **2004**, *346*, 823; b) S. B. Vogensen, J. R. Greenwood, A. R. Varming, L. Brehm, D. S. Pickering, B. Nielsen, T. Liljefors, R. P. Clausen, T. N. Johansen, P. Krogsgaard-Larsen, *Org. Biomol. Chem.* **2004**, *2*, 206; c) D. J. Hamilton, A. Sutherland, *Tetrahedron Lett.* **2004**, *45*, 5739; d) J. L. Roberts, C. Chan, *Tetrahedron Lett.* **2002**, *43*, 7679.

[19] A. Farese, N. Patino, R. Condom, S. Dalleu, R. Duedj, *Tetrahedron Lett.* **1996**, *37*, 1413.

[20] A. Farese, S. Pairot, N. Patino, V. Ravily, R. Condom, A. Aumelas, R. Guedj, *Nucleosides Nucleotides* **1997**, *16*, 1893.

[21] S. Dey, P. Garner, *J. Org. Chem.* **2000**, *65*, 7697.

[22] S. A. Sikchi, P. G. Hultin, *J. Org. Chem.* **2006**, *71*, 5888.

[23] F. Wojciechowski, R. H. E. Hudson, *J. Org. Chem.* **2008**, *73*, 3807.

[24] S. Pothukanuri, Z. Pianowski, N. Winssinger, *Eur. J. Org. Chem.* **2008**, 3141.

[25] Winssinger et. al. investigated six types of protecting groups for the terminal nitrogen atom of the Aeg residue (Alloc, Teoc, 4-N_3Cbz, Fmoc, 4-OTBSCbz and Azoc) and five protecting groups on the nucleobases (Cl-Bhoc, FBhoc, Teoc, 4-OMeCbz and Boc). The authors observed that the most suitable combination of protecting groups was mono-Boc-protected nucleobases with either Azoc or Fmoc protecting groups for the terminal nitrogen atom.

[26] R. H. E. Hudson, Y. Liu, F. Wojciechowski, *Can. J. Chem.* **2007**, *85*, 302.

[27] O. Seitz, O. Köhler, *Chem. Eur. J.* **2001**, *7*, 3914.

[28] R. H. E. Hudson, M. Goncharenko, A. P. Wallman, F. Wojciechowski, *Synlett* **2005**, *9*, 1442.

[29] T. W. Green, P. G. M. Wuts, *Protective Groups in Organic Synthesis*, Wiley-Interscience, New York, **1999**, pp. 518–525.

[30] The reaction on macroscale (>2 g) was successfully carried out by using the less-expensive oxidizing reagent TCCA and TEMPO (cat.), as described by the same authors in a previous paper: L. De Luca, G. Giacomelli, A. Porcheddu, *Org. Lett.* **2001**, *3*, 3041.

[31] a) D. B. Dess, J. C. Martin, *J. Org. Chem.* **1983**, *48*, 4155; b) J. D. More, N. S. Finney, *Org. Lett.* **2002**, *4*, 3001.

[32] Up to 1–2 d under an atmosphere of argon at –20 °C. A dilute ethyl acetate solution of **6** could be stored at –20 °C for a few days and used as needed, although loss of the Fmoc group does occur with time.[10]

[33] T. Stafforst, U. Diederichsen, *Eur. J. Org. Chem.* **2007**, 681.

[34] Working on macroscale (5–10 g), 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 **6** as a white powder.

[35] DMF has been linked to cancer in humans, and it is thought to cause birth defects. In some sectors of industry women are banned from working with DMF. For a DMF chronic toxicity summary, see: http://www.oehha.org/air/chronic_rels/pdf/68122.pdf.

[36] The oral toxicity of tetrahydrofuran used in many steps of this procedure as an alternative to DMF is relatively low. The rat oral LD_{50} is 3 g/kg: E. T. Kimura, D. M. Ebert, P. W. Dodge, *Toxycol. Appl. Pharmacol.* **1971**, *19*, 699. Tetrahydrofuran is not mutagenic in a variety of in vitro and in vivo mutagenic assays: K. Mortelmans, S. Haworth, T. Lawlor, W. Speck, B. Tainer, E. Zeiger, *Environmental Mutagenesis* **1986**, *8* (Suppl, 7), 1. NTP, **1996**. Toxicology and Carcinogenesis Studies of Tetrahydrofuran (CAS No. 109–99–9) in F344/N Rats and B6C3F1 Mice. National Toxicology Program, Technical Report Series No. 475. NIH Publication No. 96–3965. US Department of Health and Human Services, Public Health Service, National Institutes of Health. Research Triangle Park, NC. The no-observed-adverse-effect level of tetrahydrofuran administered in the drinking water of rats is 9000 ppm for fertility and reproductive performance, and 3000 ppm for systemic parental and developmental toxicity. J. Hellwiga, C. Gembardta, S. Jastib, *Food Chem. Toxicol.* **2002**, *40*, 1515.

[37] The synthesis of thyminylacetic acid, which does not require an additional base protecting group, can be prepared either by alkylation of thymine with halogenoacetic acid esters followed by saponification or by direct alkylation with bromoacetic acid. This latter method is better because of its simplicity.

[38] a) J. Kjellberg, M. Liljenberg, N. G. Johansson, *Tetrahedron Lett.* **1986**, *27*, 877–880; b) J. Kjellberg, N. G. Johansson, *Tetrahedron* **1986**, *42*, 6541–6544.

[39] B. Datta, M. E. Bier, S. Roy, B. A. Armitage, *J. Am. Chem. Soc.* **2005**, *127*, 4199. For further details, see: http://www.chem.cmu.edu/groups/army/index_files/Pub.html.

[40] V. Foubister, *Drug Discovery Today* **2003**, *8*, 520.

[41] G. R. Geen, T. J. Grinter, P. M. Kincey, R. L. Jarvest, *Tetrahedron* **1990**, *46*, 6903.

[42] The alkylation reaction was found to be regioselective for N-9, as no N-7 isomer was detected by ^{1}H and ^{13}C NMR spectroscopy.

[43] a) M. Ashwell, C. Bleasdale, B. T. Golding, I. K. O'Neill, *J. Chem. Soc., Chem. Commun.* **1990**, 955; b) R. P. Hodge, C. K. Brush, C. M. Harris, T. M. Harris, *J. Org. Chem.* **1991**, *56*, 1553.

[44] This procedure was conducted in duplicate and did not always give reproducible results.

[45] From our experience, most displacement reactions with alkoxide from 2-amino-6-chloropurine are rather elaborate and give the desired guanine in low yields. Hudson and Wojcie-

chowski (a team with a deep experience in PNA synthesis) in their seminal paper suggest the procedure outline by Kunz (C. Barth, O. Seitz, H. Z. Kunz, *Naturforsch.* **2004**, *59b*, 802) to climb this obstacle. Honestly, we have no direct experience with this last procedure.

[46] After several attempts and two years of unsuccessful research we still were at a dead end.

[47] Trimethylammonium is a better leaving group than chloride in the addition–elimination mechanism leading to displacement of the 6-substituent by a nucleophile.

[48] The ethanolic solution of NMe_3 is not dry and contains 5% H_2O (EtOH/H_2O, 95:5).

[49] The structure of the collected solid was confirmed by detailed spectroscopic, mass and elemental analysis. The same reaction was conducted in duplicate and with increasing quantities of the substrate (up to 2 g); we never observed the presence of a 6-dimethylamino byproduct. For a closer examination, see the NMR spectroscopic analysis in the Supporting Information.

[50] In the protocol outlined by Winssinger to prepare *N*-Boc-protected guanine, the exocyclic nitrogen atom was converted into an isocyanate, which was then treated with *t*BuOH. Unlike other nucleobases, the lower reactivity of 6-amino-2-chloropurine necessitated the use of triphosgene for isocyanate formation.

[51] This procedure was successfully applied for final quantities of monomers up to 5 g.

[52] These mixed anhydrides overcome some waste problems, as the only side products are, respectively, pivalic acids (it could be recovered and recycled) or CO_2 and isobutyl alcohol (two volatile compounds). Moreover, IBC and pivaloyl chloride are cheap and couple easily to the acid. These last points of view are not marginal if our goal is the preparation of all monomers on macroscale.

[53] Used protocols were based on those provided in: R. Casale, I. S. Jensen, M. Egholm, "Synthesis of PNA Oligomers by Fmoc Chemistry" in *Peptide Nucleic Acids: Protocols and Applications* (Eds.: P. E. Nielsen), 2nd ed., Garland Science, **2003**.

[54] J. D. More, N. S. Finney, *Org. Lett.* **2002**, *4*, 3001.

[55] For the preparation and full characterization of HCl·Fmoc-Aeg-OMe, see the very interesting paper of Stafforst and Diederichsen in ref.[33]

[56] The residue was put under high vacuum for at least 2–3 h to remove all traces of *t*BuOH.

[57] An off-white solid started to precipitate just after 30 min.

Received: September 11, 2008
Published Online: October 29, 2008

