α-PNA: A Novel Peptide Nucleic Acid Analogue of DNA

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Peptide nucleic acid (PNA) analogues of DNA have attracted interest as potential pharmacological regulators of gene expression since they have the capacity to invade duplex DNA forming Watson−Crick base paired PNA:DNA heteroduplexes. Unfortunately, strand invasion is limited to homopurine and homopyrimidine sequences and there is the need to explore further PNA analogues for the purpose of expanding the strand invasion alphabet. Accordingly, we have designed a true peptide mimic of DNA (designated α-PNA) involving novel L-α-amino acids, with side chains comprising the four DNA bases attached via an ethylene linkage, interspaced with glycine. The four base-containing amino acids have been synthesized from *N*-Boc-L-homoserine, via alkylation of the appropriate base with the key intermediate (*S*)-2-(*N*-Boc-amino)-4-bromobutyric acid methyl ester followed by hydrolysis. These amino acids have been incorporated into α-PNA oligomers using both solution and solid phase methods.

Introduction

Selective manipulation of gene expression will make a major impact on the therapy of human disease and much research is now focused on the design and synthesis of ligands that bind to DNA in a sequence specific manner so as to inhibit transcription. Nielsen *et al.*[1,2] have developed polyamide nucleic acid (PNA) mimics of DNA in which the entire deoxyribose−phosphate backbone has been exchanged with a structurally homomorphous uncharged polyamide backbone composed of *N*-(2-aminoethyl)glycine units (Figure 1). These compounds contain the same number of backbone bonds between the bases (i.e. 6) and the same number of bonds from the backbone to the base (i.e. 3) as in DNA.[3] PNA binds with high affinity and sequence specificity to both complementary RNA and DNA, and a number of template functions are inhibited on forming PNA/DNA and PNA/RNA complexes.[4−6] Thus PNA offers both antisense and antigene strategies for regulating gene expression. Homopyrimidine PNAs have been found to invade double-stranded DNA by displacing the noncomplementary strand to form $(PNA)_2$/DNA triplexes and a displaced strand analogous to a D-loop.[1,4,6,7] Homopurine PNAs also invade double-stranded DNA but fail to form triplexes, and their invasion complexes are less stable.[8]

Strand invasion is of great importance because, in principle, it provides a general solution to the molecular recognition problem since duplex formation is governed by the universal Watson−Crick hydrogen bonding scheme. Unfortunately, strand displacement for mixed purine−pyrimidine PNAs remains elusive and thus, to date, PNA binding to duplex DNA is confined to the same recognition alphabet as oligonucleotide triple helices.[9] One approach to extending the strand invasion alphabet to mixed sequences is to enhance the intrinsic stability of PNA/DNA heteroduplexes. However, the PNA backbone itself is not readily amenable to facile chemical modifications which would allow systematic exploration of structural features that may promote higher helix-to-coil transition temperatures. Principal among these must be electrostatic charge density, hydrophobicity, and backbone rigidity and topology. The importance of charge density on duplex stability has recently been illustrated in the case of oligonucleotides.[10−12]

The concept of peptide-based DNA surrogates is itself not new. De Konig and Pandit[13] prepared nucleopeptides of the type $[HNCH((CH_2)_4B)CO]_n$ and $[HNCH((CH_2)_4B)CONHCHRCO]_n$ but found that the polyuridine derivative, in the former configuration, complexed only weakly with polyadenylic acid. Buttrey *et al.*[14] made polymers of D-, L-, and DL-β-(thymin-1-yl)alanine and similarly found no interaction with polyadenylic acid. Weller and co-workers[15] reported the synthesis of nucleopolyamides of the form $[NHCH(CH_2B)CH_2CH_2CO]_n$, Garner *et al.*[16] described the preparation of nucleotide amino acids derived from serine and their subsequent incorporation into a peptide structure with glycine spacers, and Lewis[17] reported the synthesis of peptides containing nucleotide amino acids derived from β-alanine interspaced with

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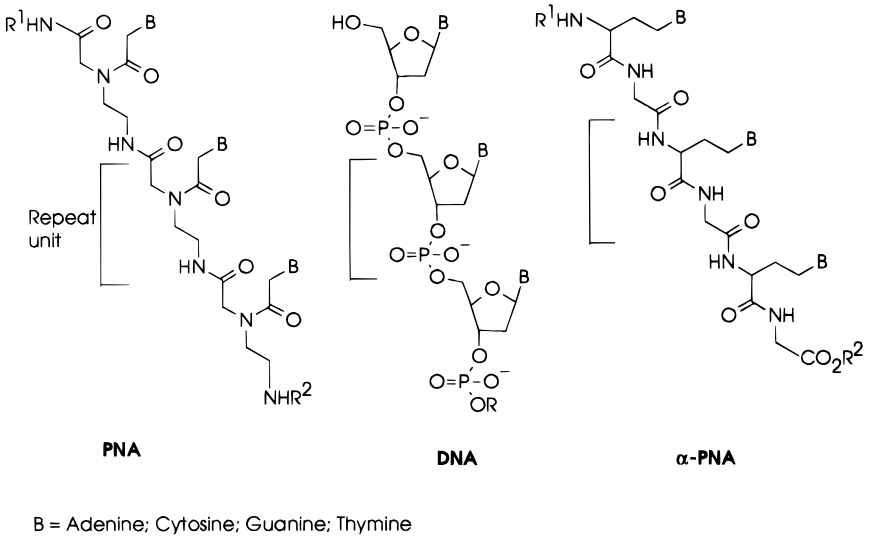
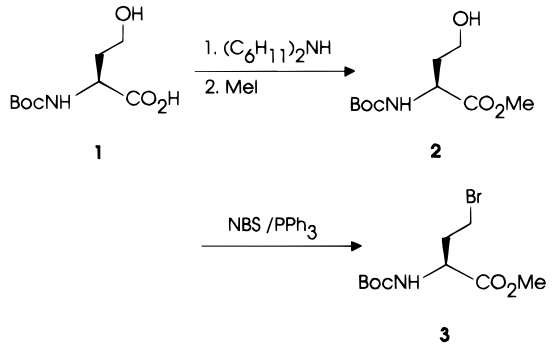


Figure 1. Comparison of DNA, PNA, and α-PNA structures.

Scheme 1. Synthesis of (*S*)-2-(*N*-Boc-amino)-4-bromobutyric Acid Methyl Ester



proline. No DNA binding studies have been reported for any of these compounds, and they all have different interbase and base-to-backbone bonding topologies compared to nucleic acids.

We have designed a true peptide nucleic acid analogue of DNA, designated α-PNA, where the base amino acid moieties are derived from homoserine and in which the base amino acids are interspaced with glycine (Figure 1). This results in the bases being separated from, and positioned along, the backbone by the same numbers of bonds as found in DNA. Since the synthesis of α-PNA is compatible with solid phase peptide chemistry it will be relatively straightforward to modify the backbone electrostatic charge, hydrophobicity, and ridigity by replacing glycine residues with appropriate amino acids. We have briefly reported a molecular model of an α-PNA: DNA duplex and progress in the synthesis toward α-PNA.[18,19] Here, we describe in detail the synthesis of the thymine, cytosine, adenine, and guanine base-containing amino acids from L-homoserine and their subsequent incorporation into a glycine-spaced tetrapeptide α-PNA using solution phase techniques. In addition, we outline a solid phase strategy for the preparation of α-PNA using the 20-mer H-αCLysαTGlyαCGlyαTLysα-CGlyαCGlyαTLysαTGlyαTGlyαTLys-NH_2[20] as an example.

During the course of our work, Lenzi *et al.*[21,22] presented a preliminary report on the preparation of an α-PNA in which the base-amino acids are derived from D-glutamic acid.[21] This results in an α-PNA of opposite chirality (i.e. D-) to that described here.

Results and Discussion

Synthesis of Key Intermediate (*S*)-2-(*N*-Boc-amino)-4-bromobutyric Acid Methyl Ester (3). (*S*)-2-(*N*-Boc-amino)-4-bromobutyric acid methyl ester (**3**) (Scheme 1) is the key intermediate in the alkylation of all four nucleotide bases in our synthetic pathways, giving rise to the base-containing amino acids. This compound was prepared from *N*-Boc-L-homoserine (**1**) in three steps as shown in Scheme 1. Firstly, the carboxylic acid group in **1** was protected as a methyl ester; this involved the initial formation of the corresponding dicyclohexylammonium salt followed by treatment of the salt with methyl iodide. Compound **2** was given in an overall yield of 75%. The methyl ester **2** was then converted into the desired bromo derivative **3** according to the method developed by Tius *et al.*[23] for transformation of primary hydroxyl groups into their corresponding bromides. Thus, a solution of **2** in dichloromethane (DCM) was added to the resulting complex formed on reacting triphenylphosphine with NBS. After purification by flash chromatography, **3** was afforded in a 75% yield.

Derivatization of the Pyrimidine Bases. Alkylation of thymine **4** with **3** was achieved by heating a mixture of **3**, thymine, and anhydrous potassium carbonate in anhydrous DMSO at 80 °C for 22 h (Scheme 2). Following an aqueous workup and purification by flash

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(20) We employ the following nomenclature: For the oligomer H-αTLysαCGlyαAGlyαGLys-NH_2, H denotes the free N-terminal amino group; αT, αC, αA, and αG the thymine, cytosine, adenine, and guanine base-containing amino acids; Gly and Lys refer to glycine and lysine, respectively, and NH_2 indicates a C-terminal amide. Additionally we propose the same designation to be used for the base-containing amino acids i.e. Boc-αT-OH for (*S*)-2-(*N*-Boc-amino)-4-(thymin-1-yl-)butyric acid.
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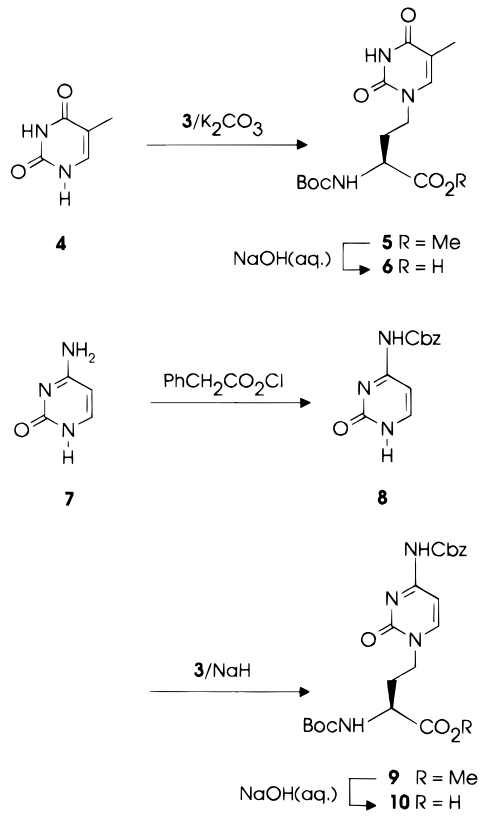
Scheme 2. Synthesis of Pyrimidine Monomers



chromatography, **5** was obtained in a 71% yield. Finally, the methyl ester was removed by hydrolysis, giving the thymine monomer **6** in a 92% yield (Scheme 2).

For the synthesis of the cytosine monomer, introduction of a protecting group for the N^4-amino group of cytosine was necessary in order to prevent chain extension from this position in the later peptide-coupling steps. A Cbz group was selected for this purpose since it had also been found to render the intermediates sufficiently soluble for chemical manipulation.[24] Thus, the exocyclic amino group of cytosine **7** was protected prior to alkylation by treatment with benzyl chloroformate to give N^4-Cbz-cytosine **8**[24] (Scheme 2). The subsequent alkylation of **8** with **3** was accomplished by firstly generating the anion, using sodium hydride in anhydrous DMF, followed by addition of a solution of **3** in anhydrous DMF (Scheme 2). After an aqueous workup and purification by flash chromatography, **9** was afforded in a 61% yield. Lastly, the methyl ester was hydrolyzed to give the Cbz-protected cytosine monomer **10** in an 86% yield (Scheme 2).

Preliminary studies to determine whether racemization occurs during the preparation of these amino acids have been undertaken. The cytosine monomer **10** was coupled to L-alanine *tert*-butyl ester via *in situ* formation of the *N*-hydroxysuccinimide activated ester (as described below for coupling the base-containing amino acids to glycine ethyl ester) to give the corresponding dipeptide. This would have yielded a mixture of diastereoisomers if racemization had occurred anywhere in the synthesis. However, both NMR and TLC analysis showed only one detectable product, and hence these results suggest that little or no racemization has been caused during the introduction of the nucleotide base.

Derivatization of the Purine Bases. For the synthesis of the adenine monomer, the N^6-amino group of adenine likewise needs to be protected, and once again a Cbz-protecting group was chosen. However here, unlike for the cytosine monomer, protection of the exocyclic amino group was performed after alkylation of adenine **11** with **3** in order to ensure that substitution occurred mainly at the N-9 position. Although alkylation of adenine is notoriously nonregiospecific, it has been reported that alkylation of sodium adenide in DMF gives rise primarily to N-9 substituted products;[25] therefore alkylation of adenine **11** with **3** was carried out by firstly generating sodium adenide *in situ* in anhydrous DMF, followed by addition of a solution of **3** in anhydrous DMF (Scheme 3). This procedure yielded only one isolable compound, which was the desired adenine monomer **12** (79% yield). N-9 alkylation was confirmed by comparison of the ^{1}H and ^{13}C NMR spectra of **12** with reported spectral data for N-7 and N-9 alkylated derivatives of adenine.[26,27] From the ^{1}H NMR spectra of N-7 and N-9 substituted adenines, it has been shown that $\Delta\delta$ values between H_2 and H_8 signals in N-7 isomers are much greater than those for N-9 isomers (cf. N^7-(1,1-dimethylpropargyl)adenine δ 8.66 (H_2) and 7.76 (H_8) i.e. $\Delta\delta$ 0.90;[26] N^9-(1,1-dimethylpropargyl)adenine δ 8.21 (H_2) and 8.11 (H_8) i.e. $\Delta\delta$ 0.10[26]) and the NH_2 signals in N-9 isomers are shifted upfield from those in N-7 isomers (cf. N^9-(dimethyl)propargyladenine δ 7.24 (NH_2);[26] N^7-(dimethyl)propargyladenine δ 8.09 and 7.99 (NH_2)[26]). The shifts observed for H_2, H_8, and NH_2 in the ^{1}H NMR spectrum of **12** were in agreement with that of N-9 substitution (δ 8.12 (H_2), 8.02 (H_8) and 7.19 (NH_2). On examining the ^{13}C NMR spectra of N-7 and N-9 substituted adenines, it has been found that the C5 signal in N-9 isomers is shifted downfield from that in N-7 isomers (cf. 7-methyladenine δ 111.7;[27] 9-methyladenine δ 118.7[27]) and that the C4 signal is shifted upfield (cf. 7-methyladenine δ 159.8;[27] 9-methyladenine δ 149.9[27]). The corresponding signals in the ^{13}C NMR spectrum of **12** were more consistent with N-9 substitution than with N-7 substitution (δ 123.08 (C5) and 153.81 (C4)).

Rapoport *et al*. have reported on the Cbz-protection of trimethylsilyl-protected adenine[28,29] and found that treatment with benzyl chloroformate under all the usual conditions did not give clean or efficient acylation of the N^6-amino group. However, when 1-(benzyloxycarbonyl)-3-ethylimidazolium tetrafluoroborate [$PhCH_2OCOIm^+Et\cdot BF_4^-$, "Rapoport's reagent"] was used instead, acylation was markedly improved. A similar observation was reported by Nielsen *et al*.[24] for the preparation of their adenine monomer. Thus, the exocyclic amino group in **12** was protected by treatment with a 6 molar excess of freshly prepared Rapoport's reagent in DCM (Scheme 3). After purification, **13** was obtained in an 82% yield. Final removal of the methyl ester by hydrolysis gave the Cbz-protected adenine monomer **14** in a 72% yield (Scheme 3).

As opposed to adenine, guanine cannot be alkylated to give only N-9 substituted products. However, since

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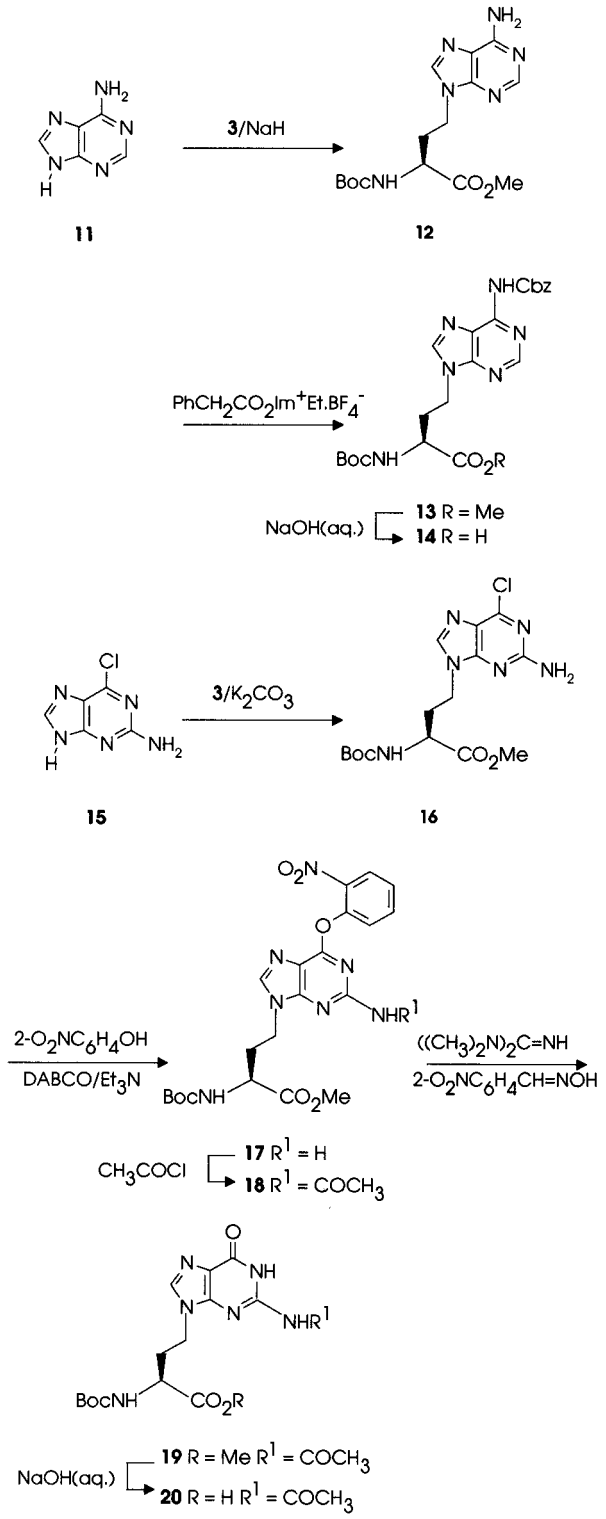
Scheme 3. Synthesis of Purine Monomers



2-amino-6-chloropurine (**15**) gives almost exclusively N-9 alkylation,[30] it is generally employed instead as the starting material for the synthesis of guanine derivatives. Consequently, the guanine monomer **20** was prepared from **15** as shown in Scheme 3. Firstly, alkylation of **15** with **3** was achieved by stirring a mixture of **15**, **3**, and anhydrous potassium carbonate in anhydrous DMF at rt. After workup and purification by flash chromatography, the desired 2-amino-6-chloropurine monomer **16** was given in a 90% yield. The ^{1}H and ^{13}C NMR spectra obtained for **16** proved to be consistent with published data for N-9 alkylated 2-aminopurines,[31,32] thus supporting that substitution had occurred at the required N-9 position.

The next step in the synthetic pathway involves replacement of the 6-chloro group with an oxygen functionality, which would yield the corresponding guanine carbonyl moiety at a later stage. The simplest and most obvious approach is to exchange the chloro group for an alkoxy group, which would liberate guanine on subsequent deprotection. However, the use of sodium alkoxide is not desirable in our case, as it could lead to racemization of the amino acid. Therefore, based on work by Reese *et al.*,[33,34] we chose to replace the chloro group with a 2-nitrophenoxy group instead. This was accomplished using the procedure developed by Sproat *et al.*;[35] a solution of **16** in 1,2-dichloroethane was treated with a solution of 2-nitrophenol, DABCO, and triethylamine in 1,2-dichloroethane at rt. Following workup and purification by flash chromatography, **17** was afforded in quantitative yield.

Protection of the N^2-amino group of **17** was then investigated, since there is a risk that it could interfere in the later peptide-coupling steps. Initially, protection with a Cbz group was tried, using benzyl chloroformate and "Rapoport's reagent", but it proved to be unsuccessful with decomposition of **17** occurring under the reaction conditions. Therefore, we protected the amine with an acetyl group. This was readily achieved by reacting **17** with acetyl chloride in anhydrous pyridine. After workup and purification by flash chromatography, **18** was obtained in a 94% yield.

Subsequently, the 2-nitrophenoxy group in **18** was removed according to the method described by Reese *et al.*[34] We chose to cleave this group at this stage, prior to performing the solution phase oligomerization described below, since it was anticipated that this group would be unlikely to withstand either the basic conditions used for ester hydrolysis or the acidic conditions associated with Boc removal. Thus, a solution of **18** and 2-nitrobenzaldoxime in anhydrous acetonitrile was treated with a solution of 1,1,3,3-tetramethylguanidine in anhydrous acetonitrile. After purification using flash chromatography, **19** was given in a 78% yield. Finally, the methyl ester was cleaved by hydrolysis to afford the N^2-acetylguanine monomer **20** in a 44% yield.

Solution Phase Oligomerization. Using solution phase techniques, the nucleotide base analogues of *N*-Boc-L-homoserine (i.e. thymine monomer **6**, N^4-Cbz-cytosine monomer **10**, N^6-Cbz-adenine monomer **14**, and N^2-acetylguanine monomer **20**) have been coupled to glycine ethyl ester, and the resulting dipeptides have been dimerized to give the corresponding tetrapeptides as outlined in Scheme 4. Thus, firstly these compounds were converted *in situ* into their respective *N*-hydroxysuccinimide activated esters using *N*-hydroxysuccinimide and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hy-

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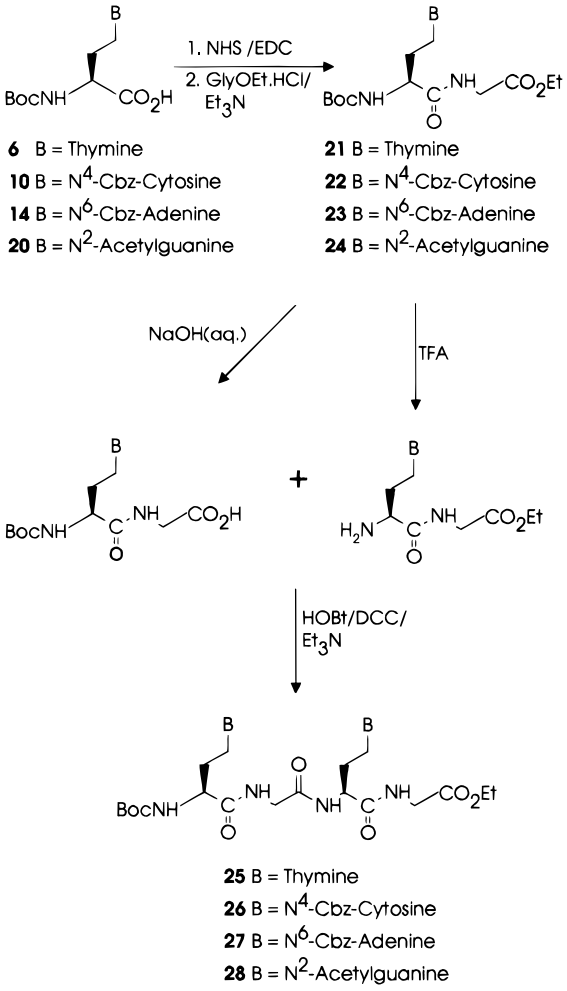
Scheme 4. Solution Phase Oligomerization



6 B = Thymine
10 B = N^4-Cbz-Cytosine
14 B = N^6-Cbz-Adenine
20 B = N^2-Acetylguanine

21 B = Thymine
22 B = N^4-Cbz-Cytosine
23 B = N^6-Cbz-Adenine
24 B = N^2-Acetylguanine

25 B = Thymine
26 B = N^4-Cbz-Cytosine
27 B = N^6-Cbz-Adenine
28 B = N^2-Acetylguanine

drochloride (EDC) in anhydrous DMF. Subsequently, glycine ethyl ester hydrochloride and triethylamine were added. After workup and purification by flash chromatography, dipeptides **21**, **22**, **23**, and **24** were obtained in 87%, 86%, 99%, and 33% yields respectively.

Dimerization of dipeptides **21**−**24** was then investigated. Before two dipeptides could be dimerized, one dipeptide required deprotection of the carboxylic acid group while the other needed deprotection of the amino function. The ethyl esters were removed by hydrolysis, under similar conditions to those which had been used to hydrolyze the methyl esters of **5**, **9**, **13**, and **19**. The corresponding carboxylic acid derivatives were afforded in yields ranging from 74% to 91%. The *N*-Boc groups were cleaved using a 50% solution of TFA in DCM. The trifluoroacetic acid salts of the free amino derivatives were isolated from the reaction mixture by precipitation with diethyl ether, in yields ranging from 64% to 88%.

Dimerization of these compounds was performed using *N*-hydroxybenzotriazole (HOBt) and DCC in anhydrous DMF. After purification by flash chromatography, tetrapeptides **25**, **26**, **27**, and **28** were given in 61%, 59%, 24% and 10% yields. These yields have not been optimized. Only one diastereoisomer has been observed for each of these tetrapeptides thereby reinforcing our previous finding that little or no racemization has occurred during the synthesis of the base-containing amino acids and in the subsequent peptide-coupling steps.

The low yields obtained for the guanine peptides are probably a result of two factors. Firstly, both the guanine

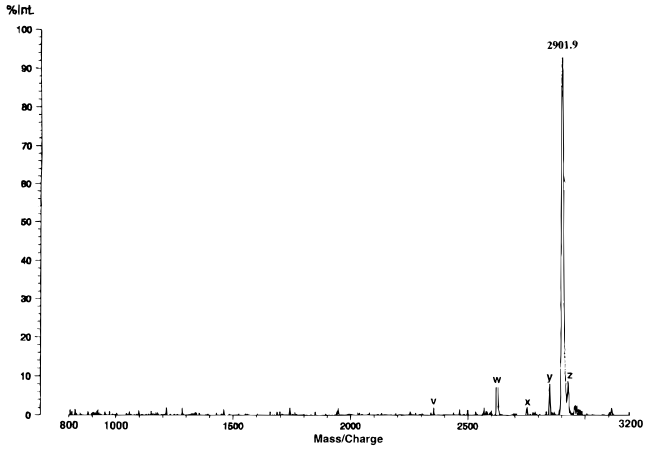


Figure 2. MALDI-TOF mass spectrum of crude H-αCLysαTGlyαCGlyαTLysαCGlyαCGlyαTLysαTGlyαTGlyαTLys-NH_2.[20] The matrix used was 3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid).

precursors and resulting peptides are poorly soluble in organic media, and secondly the unprotected 6-oxo function can cause problems when using carbodiimide reagents.[36] Thus, it may be better to use a suitably protected O^6-guanine monomer for solution phase oligomerzation. However, as our current main objective is to prepare longer oligomers of α-PNA for biochemical evaluation using the solid phase strategy described below, we have yet to explore this further. For the solid phase approach, which uses a uronium salt as the coupling reagent, the guanine monomer **20** is unlikely to need further protection.

Solid Phase Oligomerization. The preparation of longer α-PNA oligomers employing solid phase peptide synthesis techniques has been explored using the 20-mer H-αCLysαTGlyαCGlyαTLysαCGlyαCGlyαTLysαTGlyαTGlyαTLys-NH_2[20] as a model. Lysine was incorporated at the C-terminus and at three other positions within the oligomer in order to suppress self aggregation and to increase solubility in aqueous media. This α-PNA oligomer was assembled manually in a stepwise fashion using a similar protocol to that described by Christensen *et al.*[37] for the solid phase synthesis of PNA. A N^1-Boc-N^5-(2-chloro-Cbz)-L-lysine derivatized 4-methylbenzhydrylamine (Boc-Lys(2-Cl-Cbz)-MBHA) resin was used. Amino acids **6**, **10**, *N*-Boc-glycine (Boc-Gly-OH), and N^1-Boc-N^5-(2-chloro-Cbz)-L-lysine (Boc-Lys(2-Cl-Cbz)-OH) were coupled using 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) as the activating reagent in the presence of a slight excess of *N*,*N*-diisopropylethylamine (DIEA). In all cases only single couplings were required. Once all the amino acids had been coupled, the α-PNA was cleaved from the solid support using trifluoromethanesulfonic acid (TFMSA) under identical conditions to those reported by Christensen *et al.*[37]

The identity and purity of the crude material was determined by MALDI-TOF mass spectrometry (Figure 2). The principal mass peak at 2901.9 Da corresponds to the protonated form of the desired α-PNA oligomer (i.e. $[M + H]^+$) and the one at 2926.0 Da (Figure 2: peak z]

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(37) Christensen, L.; Fitzpatrick, R.; Gildea, B.; Petersen, K. H.; Hansen, H. F.; Koch, T.; Egholm, M.; Buchardt, O.; Nielsen, P. E.; Coull, J.; Berg, R. *J. Pept. Sci.* **1995**, *3*, 175−183.

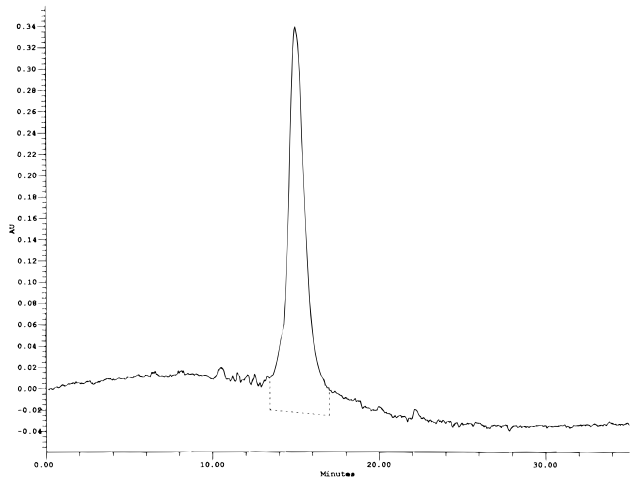

Figure 3. Analytical reverse-phase HPLC of crude H-αCLys-αTGlyαCGlyαTLysαCGlyαCGlyαTLysαTGlyαTGlyαTLys-NH_2[20] on a Deltapak C_{18}-reverse phase column at 55 °C. Buffer A was 0.1% TFA in water, and buffer B was 0.1% TFA in acetonitrile. A linear gradient of 0−50% buffer B over 30 min at a flow rate of 1 mL/min was used. The eluents were monitored at 260 nm.

to the sodium salt of the oligomer (i.e. $[M + Na]^+$). The mass spectrum also shows evidence for some capped failure sequences and one deletion fragment [Figure 2: peaks v, w, x and y]. Peak v at 2355 Da can be assigned to the capped failure sequence Ac-αCGlyαTLysαCGly-αCGlyαTLysαTGlyαTGlyαTLys-NH_2,[20] peak w at 2621 Da to the sequence Ac-αTGlyαCGlyαTLysαCGlyαCGly-αTLysαTGlyαTGlyαTLys-NH_2[20] and peak x at 2749 Da to the capped failure sequence Ac-LysαTGlyαCGlyαTLys-αCGlyαCGlyαTLysαTGlyαTGlyαTLys-NH_2.[20] Thus, product v appears to lack the N-terminal αCLysαTGly, product w the αCLys and product x the αC. Peak y at 2847 Da has a molecular weight that corresponds to a full length base sequence minus a glycine residue and may therefore be a glycine deletion fragment. No other impurities are discernible in the mass spectrum from which we estimate the purity of the desired oligomer to be 80%. The homogeneity of the crude α-PNA oligomer was also assessed using analytical reverse-phase HPLC which, at 260 nm, showed only a single peak at 14.97 min (Figure 3).

Conclusion

We have synthesized all four nucleotide base-containing amino acids from *N*-Boc-L-homoserine via the key intermediate (*S*)-2-(*N*-Boc-amino)-4-bromobutyric acid methyl ester by nucleophilic substitution of the bromine with the desired nucleotide base. Apart from the thymine derivative, all other base-containing amino acids are prepared with their exocyclic functionalities protected in order to prevent interference in the later peptide-coupling steps and to render intermediates sufficiently soluble for chemical manipulation. Using solution phase techniques, the base-containing amino acids have been coupled to glycine ethyl ester, and the resulting dipeptides have been further dimerized to give tetrapeptides, which are analogous to dinucleotides, thereby illustrating the feasibility of preparing short oligo-α-PNAs by these methods. We have also demonstrated that it is possible to synthesize longer oligomers with a high degree of integrity and purity using standard solid phase peptide synthesis protocols.

Experimental Section

General Procedures. All anhydrous organic solvents were either purchased from Aldrich Chemical Company or dried according to the procedures described by Perrin *et al.*[38] *N*-Boc-L-homoserine was purchased from Sigma Chemical Company. Thin-layer chromatography (TLC) was performed on precoated plates (Merck TLC aluminum sheets silica $60F_{254}$, Art. No. 5554). Products were visualized either by UV light or by spraying the plate with a 7% phosphomolybdic acid solution in methanol (w/v) followed by heating. Flash chromatography refers to the method of Still *et al.*[39] and was carried out using Silica Gel 60 (Merck particle size 0.040−0.063 mm). Analytical HPLC was performed at 55 °C on a Deltapak C_{18}-reverse phase column (0.49 × 15 cm, 10 μm) using a Waters 490 HPLC system. Buffer A was 0.1% TFA in water, and buffer B was 0.1% TFA in acetonitrile. A linear gradient of 0−50% buffer B over 30 min at a flow rate of 1 mL/min was used. ^1H and ^{13}C NMR spectra were measured on JEOL GX270 and Varian UNITY 500 NMR spectrometers. ^1H and ^{13}C chemical shifts were recorded in ppm relative to tetramethylsilane (TMS) or deuterated DMSO. *J* values are given in hertz. Mixtures of two rotamers were observed for some of the products containing amide bonds in the ratio of 2:1 unless otherwise indicated. As a consequence, several of the NMR signals for these products were doubled in the rotamer ratio as indicated by ma for major and mi for minor. Microanalysis was performed by University College Dublin Chemical Service unit. Mass spectra were either recorded by University College Dublin Mass Spectrometry Service or University College London Mass Spectrometry Service. MALDI-TOF spectra were recorded curtesy of The Panum Institute, Copenhagen N, Denmark. The matrix used was 3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid). Prior to each measurement, the spectrometer was calibrated using a mixture of four Nielsen type PNAs of appropriate known molecular weights. Experimental errors of the MALDI-TOF mass peaks are estimated to be ±0.1%.

Dicyclohexylammonium Salt of *N*-(*tert*-Butoxycarbonyl)-L-homoserine. *N*-Boc-L-homoserine (1.00 g, 4.56 mmol) was dissolved in ethanol (10 mL), and dicyclohexylamine was added dropwise, with stirring, to the organic solution until it became basic. Subsequently, the solvent was removed *in vacuo*, and the resulting white precipitate was resuspended in diethyl ether. The white solid was collected by suction filtration and dried in a vacuum desiccator over phosphorus pentoxide (1.55 g, 85%).

***N*-(*tert*-Butoxycarbonyl)-L-homoserine Methyl Ester (2).** Methyl iodide (638 μL, 1.45 g, 10.25 mmol) was added dropwise to a stirred suspension of the dicyclohexylammonium salt of *N*-Boc-L-homoserine (3.44 g, 8.60 mmol) in anhydrous DMF (60 mL) at rt. The reaction mixture was then left to stir overnight. Subsequently, the reaction was evaporated to dryness *in vacuo* and the residue coevaporated with toluene (3 × 30 mL). Water (55 mL) was added to the residue and the resulting aqueous solution extracted with ethyl acetate (6 × 68 mL). The combined organic extracts were washed with water (3 × 80 mL) and brine (80 mL) before being dried over $MgSO_4$. Filtration followed by solvent evaporation gave a crude viscous yellow oil which was purified by flash chromatography using diethyl ether as the eluting solvent. Compound **2** was obtained as a colorless viscous oil (1.30 g, 65%): R_f 0.28 [diethyl ether]; ^1H NMR ($CDCl_3$) δ 1.45 (s, 9H), 1.65 (m, 1H), 2.13 (m, 1H), 3.28 (br s, exchangeable, 1H), 3.70 (br m, 2H), 3.77 (s, 3H), 4.49 (m, 1H), 5.42 (br d, 1H); ^{13}C NMR ($CDCl_3$) δ 28.68, 36.54, 50.96, 52.97, 58.68, 80.92, 156.85, 173.77; MS[EI] *m*/*z* (%) 234 $[(M + H)^+]$ (<1), 174 (5), 160 (3), 118 (38), 102 (11).

(*S*)-2-[*N*-(*tert*-Butoxycarbonyl)amino]-4-bromobutyric Acid Methyl Ester (3). A solution of triphenylphosphine (3.78 g, 14.40 mmol, 2.8 equiv) in anhydrous DCM (10.64 mL) was added dropwise to a stirred suspension of NBS (2.75 g,

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15.42 mmol, 3 equiv) in anhydrous DCM (24 mL) at rt. The resulting reddish brown solution was left to stir for a further 5 min. Anhydrous pyridine (499 μL, 6.17 mmol, 1.2 equiv) followed by a solution of **2** (1.20 g, 5.14 mmol, 1 equiv) in anhydrous DCM (10.64 mL) were then added, and the reaction was left to stir at rt overnight. Subsequently, the reaction mixture was evaporated to dryness *in vacuo* and the residue coevaporated with toluene (3 × 20 mL). The dark brown oily residue was triturated with diethyl ether (10 mL) and the ethereal extract applied to a flash column in which the eluting solvent was 60:40 diethyl ether:hexane. The residue was then triturated twice with the above eluting solvent (2 × 10 mL) before running the column. Compound **3** was obtained as a colorless viscous oil which solidified on standing (1.14 g, 75%): R_f 0.54 [60:40, diethyl ether:hexane]; ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 9H), 2.22 (m, 1H), 2.40 (br m, 1H), 3.44 (t, 2H, J = 7.03), 3.77 (s, 3H), 4.43 (br m, 1H), 5.18 (br d, 1H); MS[EI] *m*/*z* (%) 296 [M^+] (10), 236 (11), 196 (8), 180 (17), 136 (78). Anal. Calcd for $C_{10}H_{18}NO_4Br$: C, 40.56; H, 6.12; N, 4.73; Br, 26.98. Found: C, 40.60; H, 6.16; N, 4.60; Br, 26.56.

(*S*)-2-[*N*-(*tert*-Butoxycarbonyl)amino]-4-(thymin-1-yl)butyric Acid Methyl Ester (5). A stirred mixture of **3** (1.20 g, 4.06 mmol, 1 equiv), thymine (1.79 g, 14.21 mmol, 3.5 equiv), and anhydrous K_2CO_3 (1.68 g, 12.18 mmol, 3 equiv) in anhydrous DMSO (14 mL) was heated at 80 °C for 22.5 h under nitrogen. Subsequently, water (100 mL) was added to the reaction and the resulting aqueous solution extracted with chloroform (6 × 60 mL). The combined organic extracts were washed with water (3 × 60 mL) and brine (60 mL) and dried over $MgSO_4$. Filtration followed by solvent evaporation gave a crude viscous yellow oil which was purified by flash chromatography using ethyl acetate as the eluting solvent. Compound **5** was obtained as a foamy white solid (0.98 g, 71%): R_f 0.37 (ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 9H), 1.92 (s, 3H), 2.02−2.24 (series of m, 2H), 3.68 (s, 1H), 3.73 (s, 3H), 4.06−4.40 (series of m, 2H), 5.40 (br m, 1H), 7.07 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 12.24, 28.20, 31.51, 45.32, 50.96, 52.61, 80.34, 110.74, 140.76, 151.01, 155.57, 164.54, 172.00; MS[EI] *m*/*z* (%) 341 [M^+] (1), 282 (23), 226 (14), 181 (16), 153 (47), 140 (19). Anal. Calcd for $C_{15}H_{23}N_3O_6$: C, 52.79; H, 6.74; N, 12.32. Found: C, 52.49; H, 6.92; N, 12.03.

(*S*)-2-[*N*-(*tert*-Butoxycarbonyl)amino]4-[N^4-(benzyloxycarbonyl)cytosin-1-yl]butyric Acid Methyl Ester (9). Sodium hydride (60% disp, 0.079 g, 1.98 mmol, 1.1 equiv) was added to a vigorously stirred suspension of N^4-Cbz-cytosine **8**[24] (0.49 g, 1.98 mmol, 1.1 equiv) in anhydrous DMF (5 mL) at rt. After hydrogen production had ceased, a solution of **3** (0.53 g, 1.80 mmol, 1 equiv) in anhydrous DMF (5 mL) was added, and the reaction mixture was left to stir overnight. Subsequently, water (25 mL) was added to the reaction and the aqueous solution extracted with chloroform (6 × 25 mL). The combined organic extracts were dried over $MgSO_4$. Filtration followed by solvent evaporation afforded a crude product which was purified by flash chromatography using ethyl acetate as the eluting solvent. Compound **9** was obtained as a white foam (0.50 g, 61%): R_f 0.24 (ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 9H), 2.10 (br m, 1H), 2.32 (br m, 1H), 3.70 (s, 3H), 3.83 (br m, 1H), 4.11 (br m, 1H), 4.31 (br m, 1H), 5.21 (s, 2H), 5.56 (br s, 1H), 7.20 (br m, 1H), 7.38 (s, 5H), 7.77 (d, 1H, J = 7.31); ^{13}C NMR ($CDCl_3$) δ 28.26, 31.75, 47.78, 51.00, 52.68, 67.96, 80.36, 94.94, 127.75, 128.34, 128.70, 134.97, 149.32, 155.72, 162.38, 172.31; MS[EI] *m*/*z* (%) 460 [M^+] (3), 429 (30), 404 (29), 387 (17), 343 (20). Anal. Calcd for $C_{22}H_{28}N_4O_7 \cdot {}^1/_2H_2O$: C, 56.29; H, 6.18; N, 11.94; C/N, 4.71. Found: C, 56.05; H, 6.16; N, 11.68; C/N, 4.80.

(*S*)-2-[*N*-(*tert*-Butoxycarbonyl)amino]-4-[adenin-9-yl-]butyric Acid Methyl Ester (12). Sodium hydride (60% disp, 0.079 g, 1.98 mmol, 1.1 equiv) was added to a vigorously stirred suspension of adenine (0.24 g, 1.80 mmol, 1.1 equiv) in anhydrous DMF (4.43 mL) at rt and the reaction left to stir for 2 h. A solution of **3** (0.53 g, 1.80 mmol, 1 equiv) in anhydrous DMF (5.32 mL) was then added and the reaction mixture left to stir overnight. Subsequently, the reaction was concentrated *in vacuo* and the residue coevaporated with toluene (3 × 10 mL). The crude product was purified by flash chromatography using 90:10 ethyl acetate:methanol as the eluting solvent. Compound **12** was obtained as a pale yellow solid (0.50 g, 79%): R_f 0.20 [90:10, ethyl acetate:methanol]; ^{1}H NMR (d_6-DMSO) δ 1.38 (s, 9H), 2.07 (m, 1H), 2.27 (m, 1H), 3.55 (s, 3H,), 3.87 (m, 1H), 4.19 (t, 2H), 7.19 (s, exchangeable, 2H), 7.43 (d, 1H), 8.02 (s, 1H), 8.12 (s, 1H); ^{13}C NMR (d_6-DMSO) δ 32.49, 34.86, 44.31, 55.25, 56.46, 82.86, 123.08, 145.12, 153.81, 156.76, 159.84, 160.30, 176.74; MS[EI] *m*/*z* (%) 350 [M^+] (14), 294 (10), 277 (21), 250 (20), 235 (14), 189 (17), 162 (24). Anal. Calcd for $C_{15}H_{22}N_6O_4 \cdot {}^1/_2CH_3OH$: C, 50.82; H, 6.56; N, 22.95; C/N, 2.21. Found: C, 50.87; H, 6.33; N, 23.02; C/N, 2.21.

(*S*)-2-[*N*-(*tert*-Butoxycarbonyl)amino]-4-[N^6-(benzyloxycarbonyl)adenin-9-yl]butyric Acid Methyl Ester (13). A 1 M solution of triethyloxonium tetrafluoroborate in DCM (3.66 mL, 3.66 mmol, 6 equiv) was added dropwise to a stirred solution of *N*-(benzyloxycarbonyl)imidazole (0.74 g, 3.66 mmol, 6 equiv) in anhydrous DCM (0.64 mL) at 0 °C under nitrogen. The reaction was then left to stir for 1 h at this temperature and for a further 2 h at rt. Subsequently, **12** (0.21 g, 0.61 mmol, 1 equiv) was added to the reaction mixture at rt and the reaction left to stir overnight. The reaction was quenched by addition of a saturated solution of $NaHCO_3$ (6 mL) and the organic layer separated. The aqueous layer was then further extracted with DCM (6 mL), and the combined organic extracts were dried over $MgSO_4$. Filtration followed by solvent evaporation gave a crude viscous oil which was purified by flash chromatography using 96:4 ethyl acetate:methanol as the eluting solvent. Compound **13** was obtained as a white foam (0.24 g, 82%): R_f 0.27 [96:4; ethyl acetate:methanol]; ^{1}H NMR (d_6-DMSO) δ 1.38 (s, 9H), 2.13 (m, 1H), 2.33 (m, 1H), 3.54 (s, 3H), 3.91 (m, 1H), 4.31 (t, 2H), 5.20 (s, 2H), 7.32−7.48 (series of m, 6H), 8.37 (s, 1H), 8.60 (s, 1H), 10.67 (s, 1H); ^{13}C NMR (d_6-DMSO) δ 32.46, 34.64, 44.64, 55.24, 56.28, 70.53, 82.84, 127.62, 132.16, 132.30, 140.70, 148.59, 153.74, 155.79, 156.44, 159.78, 176.62; MS[EI] *m*/*z* (%) 484 [M^+] (<1), 376 (3), 320 (30), 217 (34), 175 (57). Anal. Calcd for $C_{23}H_{28}N_6O_6 \cdot {}^1/_2CH_3OH$: C, 56.40; H, 6.00; N, 16.80; C/N, 3.36. Found: C, 56.71; H, 5.94; N, 16.65; C/N, 3.41.

(*S*)-2-[*N*-(*tert*-Butoxycarbonyl)amino]-4-[2-amino-6-chloropurin-9-yl]butyric Acid Methyl Ester (16). A mixture of 2-amino-6-chloropurine (0.24 g, 1.42 mmol, 1 equiv) and anhydrous K_2CO_3 (0.20 g, 1.42 mmol, 1 equiv) were suspended in anhydrous DMF (4 mL). Compound **3** (0.42 g, 1.42 mmol, 1 equiv) was then added with stirring at rt and the reaction left overnight. Subsequently, the reaction was evaporated to dryness *in vacuo* and the residue redissolved in chloroform (20 mL). The organic solution was washed with water (3 × 10 mL) and dried over $MgSO_4$. Filtration followed by solvent evaporation gave a crude viscous oil which was purified by flash chromatography using ethyl acetate as the eluting solvent. Compound **16** was afforded as a white foam (0.49 g, 90%): R_f 0.37 (ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 1.46 (s, 9H), 2.27 (m, 1H), 2.41 (m, 1H), 3.66 (s, 3H), 4.20 (m, 2H), 4.40 (br s, 1H), 5.25 (s, exchangeable, 2H), 5.56 (br d, 1H), 7.85 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 28.28, 32.47, 40.32, 51.17, 52.69, 80.51, 125.28, 142.63, 151.39, 153.73, 155.54, 159.02, 172.04; MS[EI] *m*/*z* (%) 384 [M^+] (7), 311 (17), 284 (20), 223 (17), 196 (21). Anal. Calcd for $C_{15}H_{21}N_6O_4Cl \cdot {}^3/_4CH_3OH$: C, 46.27; H, 5.62; N, 20.55; Cl, 8.68; C/N, 2.25. Found: C, 46.38; H, 5.86; N, 20.56; Cl, 8.78; C/N, 2.26.

(*S*)-2-[*N*-(*tert*-Butoxycarbonyl)amino]-4-[2-amino-6-(2-nitrophenoxy)purin-9-yl]butyric Acid Methyl Ester (17). A solution of 2-nitrophenol (0.33 g, 2.34 mmol, 3 equiv), DABCO (0.09 g, 0.78 mmol, 1 equiv), and triethylamine (330 μL, 2.34 mmol, 3 equiv) in anhydrous 1,2-dichloroethane (750 μL) was added to a stirred slurry of **16** (0.30 g, 0.78 mmol, 1 equiv) in anhydrous 1,2-dichloroethane (3 mL) at rt. The reaction was left to stir overnight. Subsequently, the reaction was diluted with DCM (6 mL) and the organic solution washed with a saturated solution of $NaHCO_3$ (9 mL). The aqueous layer was re-extracted with DCM (6 mL), and the combined organic extracts were dried over $MgSO_4$. Filtration followed by solvent evaporation gave a crude product which was purified by flash chromatography using ethyl acetate as the eluting solvent. Compound **17** was obtained as a yellow foam in quantitative yield: R_f 0.29 (ethyl acetate); ^{1}H NMR ($CDCl_3$)

δ 1.47 (s, 9H), 2.26−2.48 (series of m, 2H), 3.65 (s, 3H), 4.20 (m, 2H), 4.40 (br s, 1H), 4.82 (br s, exchangeable, 2H), 5.80 (br d, 1H), 7.40−7.46 (m, 2H), 7.66−7.76 (series of m, 3H), 8.09−8.12 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 28.25, 32.35, 40.00, 51.19, 52.58, 80.40, 114.80, 125.34, 125.51, 126.10, 134.56, 140.90, 142.47, 145.46, 155.22, 155.80, 158.65, 159.10, 172.03; MS[EI] *m*/*z* (%) 487 [M^+] (21), 387 (10), 328 (9), 286 (100), 226 (22).

(*S*)-2-[*N*-(*tert*-Butoxycarbonyl)amino]-4-[2-(acetylamino)-6-(2-nitrophenoxy)purin-9-yl]butyric Acid Methyl Ester (18). Acetyl chloride (290 μL, 4.08 mmol, 5 equiv) was carefully added dropwise to a stirred solution of **17** (0.40 g, 0.82 mmol, 1 equiv) in anhydrous pyridine (4 mL) at 0 °C. The reaction was allowed to warm slowly to rt before being left to stir overnight. Subsequently, the reaction was poured onto ice (15 mL) and the resulting aqueous solution extracted with ethyl acetate (6 × 12 mL). The combined organic extracts were dried over $MgSO_4$. Filtration followed by solvent evaporation gave a crude viscous orange oil which was purified by flash chromatography using 95:5 ethyl acetate:methanol as the eluting solvent. Compound **18** was obtained as an orange foam (0.41 g, 94%): R_f 0.30 (95:5, ethyl acetate:methanol); ^{1}H NMR ($CDCl_3$) δ 1.47 (s, 9H), 2.22 (s, 3H), 2.29−2.47 (series of m, 2H), 3.67 (s, 3H), 4.32 (br m, 3H), 5.63 (br s, 1H), 7.41−7.52 (series of m, 2H), 7.71−7.78 (series of m, 1H), 7.82 (br s, 1H), 8.01 (br s, 1H), 8.15−8.19 (series of m, 1H); ^{13}C NMR ($CDCl_3$) δ 24.71, 28.23, 32.50, 40.60, 51.11, 52.64, 80.42, 117.49, 125.45, 125.80, 126.70, 134.88, 142.17, 143.23, 145.44, 151.50, 154.46, 159.14, 171.88; MS[EI] *m*/*z* (%) 529 [M^+] (21), 456 (19), 370 (16), 352 (33), 328 (73), 315 (100).

(*S*)-2-[*N*-(*tert*-Butoxycarbonyl)amino]-4-[N^2-acetylguanin-9-yl]butyric Acid Methyl Ester (19). A solution of 1,1,3,3-tetramethylguanidine (306 μL, 2.44 mmol, 9 equiv) in anhydrous acetonitrile (2.71 mL) was added to a stirred solution of **18** (0.14 g, 0.27 mmol, 1 equiv) and 2-nitrobenzaldoxime (0.45 g, 2.71 mmol, 10 equiv) in anhydrous acetonitrile (2.71 mL) at rt under nitrogen. The reaction was left to stir overnight. Subsequently, the reaction mixture was evaporated to dryness *in vacuo* and the resulting residue purified using flash chromatography with 90:10 DCM:methanol as the eluting solvent. Compound **18** was afforded as a beige powder (0.087 g, 78%): R_f 0.33 (90:10, DCM:methanol); ^{1}H NMR (d_6-DMSO) δ 1.37 (s, 9H), 2.05 (br series of m, 1H), 2.17 (s, 3H), 2.26 (br series of m, 1H), 3.59 (s, 3H), 3.92 (br series of m, 1H), 4.11 (br series of m, 2H), 7.43 (d, 1H), 7.88 (br m, 1H), 11.71 (br m, 1H), 12.02 (s, 1H); ^{13}C NMR (d_6-DMSO) δ 29.00, 33.36, 35.79, 45.09, 56.22, 57.25, 83.84, 125.38, 144.91, 152.91, 153.60, 160.13, 160.74, 177.33, 178.74; MS[EI] *m*/*z* (%) 408 [M^+] (16), 335 (12), 308 (15), 249 (9), 207 (74). Anal. Calcd for $C_{17}H_{24}N_6O_6 \cdot {}^3/_4CH_3OH$: C, 49.30; H, 6.29; N, 19.43; C/N, 2.54. Found: C, 49.57; H, 6.16; N, 19.03; C/N, 2.60.

Base-Containing Amino Acids 6, 10, 14, and 20. Standard Protocol. A 0.67 M(aq) solution of sodium hydroxide (2.5 equiv) was added to a stirred solution of **5**, **9**, **13**, or **19** (1 equiv) in dioxane (1.75 mL/mmol) at rt. The reaction was left for 0.5 h before being diluted with water (6.65 mL/mmol). The aqueous solution was washed with DCM (3 × 6.65 mL/mmol) and the pH adjusted to 3.0 with a 2 M (aq) solution of citric acid.

(*S*)-2-[*N*-(*tert*-Butoxycarbonyl)amino]-4-(thymin-1-yl)butyric Acid [Boc-αT-OH^{20}] (6). The above procedure was followed using **5** (0.98 g, 2.87 mmol). After acidification, the aqueous layer was extracted with ethyl acetate (6 × 20 mL), and the combined organic extracts were dried over $MgSO_4$. Filtration followed by solvent evaporation and coevaporation with toluene (3 × 20 mL) gave **6** as a glassy white solid which was dried further in a vacuum desiccator over phosphorus pentoxide (0.86 g, 92%): ^{1}H NMR (d_6-DMSO) δ 1.39 (s, 9H), 1.74 (ma) and 1.77 (mi) (s, 3H), 1.74−2.12 (br m, 2H), 3.68 (br m, 1H), 3.83 (br m, 2H), 7.18−7.24 (series of m, 1H), 7.31 (mi) and 7.42 (ma) (s, 1H), 10.90 (mi) and 11.25 (ma) (s, exchangeable, 1H); MS[CI] *m*/*z* (%) 328 [$(M + H)^+$] (2), 303 (9), 289 (8), 284 (39), 272 (8), 245 (11), 228 (30). Anal. Calcd for $C_{14}H_{21}N_3O_6 \cdot CH_3OH$: C, 50.14; H, 6.96; N, 11.70; C/N, 4.29. Found: C, 49.83; H, 6.61; N, 11.56; C/N, 4.31.

(*S*)-2-[*N*-(*tert*-Butoxycarbonyl)amino]-4-[N^4-(benzyloxycarbonyl)cytosin-1-yl]butyric Acid [Boc-αC (Cbz)-OH^{20}] (10). The above procedure was followed using **9** (0.80 g, 1.74 mmol). After acidification, **10** precipitated from solution as a white solid and was collected by suction filtration. It was washed thoroughly with water and once with diethyl ether before being dried in a vacuum desiccator over phosphorus pentoxide (0.67 g, 86%): ^{1}H NMR (d_6-DMSO) δ 1.36 (s, 9H), 1.73−2.15 (br d, 2H), 3.80 (br s, 3H), 5.16 (s, 2H), 6.95 (d, 1H, J = 7.31), 7.23 (d, 1H, J = 7.88), 7.37 (m, 5H), 7.94 (d, 1H, J = 7.31); MS[EI] *m*/*z* (%) 446 [M^+] (1), 354 (1), 320 (4), 229 (6), 214 (4). Anal. Calcd for $C_{21}H_{26}N_4O_7 \cdot {}^1/_4H_2O$: C, 55.94; H, 5.88; N, 12.43; C/N, 4.50. Found: C, 55.89; H, 5.76; N, 12.22; C/N, 4.57.

(*S*)-2-[*N*-(*tert*-Butoxycarbonyl)amino]-4-[N^6-(benzyloxycarbonyl)adenin-9-yl]butyric Acid [Boc-αA (Cbz)-OH^{20}] (14). The above procedure was followed using **13** (0.20 g; 0.41 mmol). After acidification, the aqueous layer was extracted with ethyl acetate (6 × 4 mL). The combined organic extracts were washed with brine (5 mL) before being dried over $MgSO_4$. Filtration followed by solvent evaporation and coevaporation with toluene (3 x 10 mL) gave **14** as a white foam, which was dried further in a vacuum desiccator over phosphorous pentoxide (0.14 g, 72%). It was evident from the microanalysis that the product contained some salt but the C/N ratio corresponded well with the calculated value: ^{1}H NMR (d_6-DMSO) δ 1.40 (s, 9H), 2.09−2.50 (br d, 2H), 3.82 (br m, 1H), 4.32 (br m, 2H), 5.22 (s, 2H), 7.28−7.48 (series of m, 6H), 8.38 (s, 1H), 8.62 (s, 1H), 10.70 (br s, exchangeable, 1H); MS[EI] *m*/*z* (%) 470 [M^+] (<1), 447 (<1), 411 (<1), 392 (<1), 387 (<1), 370 (<1). Anal. Calcd for $C_{22}H_{26}N_6O_6$: C, 56.16; H, 5.57; N, 17.86; C/N, 3.14. Found: C, 55.89; H, 5.27; N, 16.55; C/N, 3.38.

(*S*)-2-[*N*-(*tert*-Butoxycarbonyl)amino]-4-(N^2-acetylguanin-9-yl)butyric Acid [Boc-αG (Ac)-OH^{20}] (20). The above procedure was followed using **19** (0.23 g, 0.58 mmol). After acidification, **20** precipitated from solution as a beige solid and was collected by suction filtration. It was washed thoroughly with water and once with diethyl ether before being dried in a vacuum desiccator over phosphorus pentoxide (0.10 g, 44%): ^{1}H NMR (d_6-DMSO) δ 1.39 (s, 9H), 1.89−2.36 (br d, 1H), 2.17 (s, 3H), 3.82 (br m, 1H), 4.12 (br m, 2H), 7.30 (d, 1H, J = 8.72), 7.90 (s, 1H), 11.75 (s, 1H), 12.03 (s, 1H); MS[FAB] *m*/*z* (%) 395 [$(M+1)^+$] (100). Anal. Calcd for $C_{16}H_{22}N_6O_6 \cdot 1{}^1/_4H_2O$: C, 46.10; H, 5.88; N, 20.17; C/N, 2.29. Found: C, 46.46; H, 5.77; N, 19.96; C/N, 2.33.

Solution Phase Synthesis of Dipeptides 21−24. Standard Protocol. EDC (1 equiv) was added to a stirred solution of **6**, **10**, **14**, or **20** (1 equiv) and *N*-hydroxysuccinimide (1 equiv) in anhydrous DMF (10 mL/g) at rt. The reaction was left to stir for 1 h whereupon a further quantity of EDC (0.5 equiv) was added. The reaction was then left for another 0.5 h before glycine ethyl ester hydrochloride (5 equiv) and triethylamine (7 equiv) were added. The reaction was left to stir at rt overnight. Subsequently, water (27 mL/mmol) was added to the reaction mixture and the resulting aqueous solution extracted with ethyl acetate (6 × 40 mL/mmol). The combined organic extracts were dried over $MgSO_4$. Filtration followed by solvent evaporation afforded a crude product.

Dipeptide 21. The above procedure was followed using **6** (0.86 g, 2.63 mmol). After workup, the crude product obtained was purified using flash chromatography with 96:4 ethyl acetate:methanol as the eluting solvent. The dipeptide **21** was given as a glassy white solid (0.94 g, 87%): R_f 0.29 (96:4, ethyl acetate:methanol]; ^{1}H NMR (d_6-DMSO) δ 1.16 (t, 3H, J = 7.03), 1.38 (s, 9H), 1.60−2.07 (series of m, 2H), 1.72 (ma) and 1.76 (mi) (s, 3H), 3.60−4.00 (series of m, 5H), 4.05 (mi) and 4.06 (ma) (q, 2H, J = 7.03), 6.90 (mi) and 7.10 (ma) (d, 1H), 7.30 (mi) and 7.35 (ma) (s, 1H), 8.27 (mi) and 8.32 (ma) (br t, 1H); ^{13}C NMR (d_6-DMSO) δ 11.93 (ma) and 12.37 (mi), 13.92, 28.08, 30.04 (mi) and 30.73 (ma), 40.59 (mi) and 40.64 (ma), 44.32, 51.39 (ma) and 52.40 (mi), 60.33, 78.06 (mi) and 78.17 (ma), 107.09 (mi) and 108.23 (ma), 136.19 (mi) and 141.35 (ma), 150.70 (ma) and 150.80 (mi), 155.14, 163.60 (mi) and 164.24 (ma), 169.54 (mi) and 169.63 (ma), 172.05; MS[EI] *m*/*z* (%) 413 [M^+] (6), 226 (15), 182 (16), 127 (13). Anal. Calcd for $C_{18}H_{28}$-

$N_4O_7 \cdot {}^3/_4CH_3OH$: C, 51.61; H, 7.11; N, 12.84; C/N, 4.02. Found: C, 51.59; H, 6.80; N, 12.78; C/N, 4.04.

Dipeptide 22. The above procedure was followed using **10** (0.67 g, 1.49 mmol). After workup, the crude product obtained was purified using flash chromatography with 96:4 ethyl acetate:methanol as the eluting solvent. The dipeptide **22** was given as a cream powder (0.68 g, 86%): R_f 0.29 [96:4, ethyl acetate:methanol]; ^{1}H NMR (d_6-DMSO) δ 1.16 (t, 3H, J = 7.03), 1.37 (s, 9H), 1.73−2.10 (br series of m, 2H), 3.70−4.00 (series of m, 5H), 4.06 (q, 2H, J = 7.03), 5.17 (s, 2H), 6.96 (br d, 1H, J = 7.03), 7.09 (d, 1H, J = 8.16), 7.36−7.40 (m, 5H), 7.92 (d, 1H, J = 7.60), 8.34 (br t, 1H); ^{13}C NMR (d_6-DMSO) δ 14.03, 28.17, 30.74, 40.73, 46.87, 51.66, 60.44, 66.44, 78.32, 94.01, 127.94, 128.16, 128.49, 136.00, 149.86, 153.20, 154.94, 155.24, 162.75, 169.73, 172.07; MS[EI] *m*/*z* (%) 487 [(M − Et + H)$^+$] (1), 280 (6), 267 (7), 221 (7), 138 (43), 125 (94). Anal. Calcd for $C_{25}H_{33}N_5O_8$: C, 56.50; H, 6.21; N, 13.18. Found: C, 56.27; H, 6.23; N, 12.96.

Dipeptide 23. The above procedure was followed using **14** (0.14 g, 0.29 mmol). After workup, the crude product obtained was purified using flash chromatography with 92:8 ethyl acetate:methanol as the eluting solvent. The dipeptide **23** was given as a cream powder (0.16 g, 99%): R_f 0.27 [92:8, ethyl acetate:methanol]; ^{1}H NMR (d_6-DMSO) δ 1.14 (t, 3H, J = 7.03), 1.37 (s, 9H), 1.94−2.36 (series of m, 2H), 3.66−4.40 (series of m, 5H), 4.05 (q, 2H, J = 7.03), 5.20 (s, 2H), 7.16 (d, 1H, J = 8.16), 7.34−7.46 (m, 5H), 8.32 (br s, 2H), 8.59 (s, 1H), 10.66 (br s, 1H); ^{13}C NMR (d_6-DMSO) δ 18.05, 32.21, 35.65, 44.25, 44.73, 55.50, 64.56, 70.20, 82.35, 127.32, 131.87, 132.02, 132.44, 140.42, 148.25, 153.40, 155.43, 156.03, 159.30, 165.65, 173.73, 176.01; MS[FAB] *m*/*z* (%) 556 [(M + 1)$^+$] (100).

Dipeptide 24. The above procedure was followed using **20** (0.11 g, 0.27 mmol). After workup, the crude product obtained was purified by flash chromatography using 90:10 DCM: methanol as the eluting solvent. The dipeptide **24** was given as a cream powder (0.042 g, 33%); R_f 0.31 (90:10, DCM: methanol); ^{1}H NMR (d_6-DMSO) δ 1.15 (t, 3H, J = 7.03), 1.37 (s, 9H), 1.89−2.34 (series of m, 2H), 2.16 (s, 3H), 3.64−4.00 (series of m, 5H), 4.06 (mi) and 4.07 (ma) (q, 2H, J = 7.03), 7.16 (d, 1H, J = 8.16), 7.86 (s, 1H), 8.37 (br s, 1H), 11.72 (br t, 1H), 12.02 (br s, 1H); ^{13}C NMR (d_6-DMSO) δ 18.00, 27.73, 32.13, 35.70 (mi) and 39.76 (ma), 44.57 (ma) and 45.41 (mi), 44.71, 55.44, 64.37 (mi) and 64.46 (ma), 82.42, 124.15, 143.64, 151.59, 152.63, 158.93 (ma) and 159.25 (mi), 161.79, 173.70 (ma) and 173.91 (mi), 175.32 (ma) and 175.87 (mi), 177.47; MS[FAB] *m*/*z* (%) 480 [(M + 1)$^+$] (100).

Solution Phase Synthesis of Tetrapeptides 25−28. (i) Removal of the Boc Protecting Groups. TFA (0.5 mL/100 mg) was added dropwise to a stirred solution of **21**, **22**, **23**, or **24** in anhydrous DCM (0.5 mL/100 mg) at rt. The reaction was left to stir for 4 h. The reaction was then saturated with diethyl ether until a precipitate formed; this was the TFA salt of the free amino dipeptide. The ethereal solution was decanted off from the precipitate, and the precipitate was triturated twice with further quantities of diethyl ether before being dried in a vacuum desiccator over phosphorus pentoxide.

TFA Salt of the Thymine Derivative. The above procedure was applied to dipeptide **21** (0.11 g, 0.26 mmol). The desired compound was given as a white solid (0.093 g, 85%).

TFA Salt of the Cytosine Derivative. The above procedure was applied to dipeptide **22** (0.20 g, 0.37 mmol). The desired compound was afforded as a cream solid (0.14 g, 69%).

TFA Salt of the Adenine Derivative. The above procedure was applied to dipeptide **23** (0.10 g, 0.19 mmol). The desired compound was obtained as a cream solid (0.093g, 88%).

TFA Salt of the Guanine Derivative. The above procedure was applied to dipeptide **24** (0.12 g, 0.26 mmol) except that the reaction was left for 5.5 h before workup. The desired compound was afforded as a cream solid (0.082 g, 64%).

(ii) Hydrolysis of the Ethyl Esters. A 0.67 M (aq) solution of sodium hydroxide (2.5 equiv) was added to a stirred solution of **21**, **22**, **23**, or **24** (1 equiv) in dioxane (1.75 mL/mmol) at rt. The reaction was left for 0.5 h before being diluted with water (6.65 mL/mmol). The aqueous solution was washed with DCM (3 × 6.65 mL/mmol) and the pH adjusted to 3.0 with a 2 M (aq) solution of citric acid. The aqueous solution was extracted with ethyl acetate (7 × 10 mL/mmol), and the combined organic extracts were washed with brine (10 mL/mmol) before being dried over $MgSO_4$. Filtration followed by solvent evaporation gave the free carboxylic acid dipeptide which was dried in a vacuum desiccator over phosphorus pentoxide.

Thymine Derivative. The above procedure was followed for dipeptide **21** (0.12 g, 0.29 mmol). The desired compound was obtained as a pale yellow powder (0.10 g, 91%).

Cytosine Derivative. The above procedure was followed for dipeptide **22** (0.06 g, 0.10 mmol). The desired compound was given as a white powder (0.05 g, 90%).

Adenine Derivative. The above procedure was followed for dipeptide **23** (0.10 g, 0.19 mmol). The desired compound was afforded as a white powder (0.08 g, 81%).

Guanine Derivative. The above procedure was followed for dipeptide **24** (0.11 g, 0.23 mmol). The desired compound was obtained as a glassy white solid (0.076 g, 74%).

(iii) Formation of the Tetrapeptides. The appropriate free amino dipetide (1 equiv), free carboxylic acid dipeptide (1 equiv), and HOBt (1.2 equiv) were dissolved in the relevant amount of anhydrous DMF so that the reaction was 0.2 M in both the amino and acid components. Subsequently, triethylamine (2 equiv) was added to the stirred mixture and the reaction cooled in an ice−salt bath to ca. −10 °C. DCC (1.1 equiv) was added and the reaction left to stir at this temperature for 1 h. The reaction was then allowed to warm slowly to rt over the next hour before being left to stir overnight. The precipitate, which formed during the course of the reaction, was removed by filtration and the filtrate concentrated *in vacuo*. The crude residue was finally coevaporated with toluene (3 × 10 mL/100 mg).

Tetrapeptide 25. The above procedure was used to couple the free carboxylic acid derivative of dipeptide **21** (0.05 g, 0.13 mmol) and the TFA salt of the free amino acid analogue of dipeptide **21** (0.055 g, 0.13 mmol). After workup the crude residue was purified using flash chromatography with 90:10 DCM:methanol as the eluting solvent. The tetrapeptide **25** was obtained as a pale yellow waxy solid (0.053 g, 61%): R_f 0.23 (90:10, DCM:methanol); ^{1}H NMR (d_6-DMSO) δ 1.145 (mi) and 1.15 (ma) (t, 3H, J = 7.07), 1.37 (s, 9H), 1.66−2.11 (series of m, 4H), 1.71 (s, 3H), 1.74 (s, 3H), 3.54-4.32 (series of m, 10H), 4.05 (br q, 2H, J = 7.07), 7.25 (br s, 1H), 7.30 (br m, 1H), 7.37 (br s, 1H), 8.00−8.50 (br series of m, 3H), 10.89 (mi) and 11.21 (ma) (br s, 2H); MS[FAB] *m*/*z* (%) 679 [(M + 1)$^+$] (1).

Tetrapeptide 26. The above procedure was used to couple the free carboxylic acid derivative of dipeptide **22** (0.13 g, 0.25 mmol) and the TFA salt of the free amino acid analogue of dipeptide **22** (0.14 g, 0.25mmol). After workup the crude residue was purified using flash chromatography with 90:10, DCM:methanol as the eluting solvent. The tetrapeptide **26** was obtained as a pale yellow powder (0.14 g, 59%): R_f 0.36 (90:10, DCM:methanol); ^{1}H NMR (d_6-DMSO) δ 1.15 (t, 3H, J = 7.03), 1.36 (s, 9H), 1.69−2.26 (series of m, 4H), 3.64−4.32 (series of m, 10H), 4.06 (q, 2H, J = 7.03), 5.15 (s, 4H), 6.92 (d, 1H, J = 7.31), 6.97 (d, 1H, J = 7.03), 7.17 (br m, 1H), 7.37 (m, 10H), 7.88 (d, 1H, J = 7.59), 7.97 (d, 1H, J = 7.31), 8.12−8.54 (br series of m, 3H), 10.73 (br s, 2H); MS[FAB] *m*/*z* (%) 917 [(M + 1)$^+$] (39).

Tetrapeptide 27. The above procedure was used to couple the free carboxylic acid derivative of dipeptide **23** (0.086 g, 0.16 mmol) and the TFA salt of the free amino acid analogue of dipeptide **23** (0.093 g, 0.16 mmol). After workup the crude residue was purified using flash chromatography with 85:15 DCM:methanol as the eluting solvent. The tetrapeptide **27** was obtained as a cream powder (0.038 g, 24%): R_f 0.47 (85:15, DCM:methanol); ^{1}H NMR (d_6-DMSO) δ 1.14 (t, 3H, J = 7.08), 1.35 (s, 9H), 2.06 (series of m, 2H), 2.36 (series of m, 2H), 3.70−4.38 (series of m, 10H), 4.05 (q, 2H, J = 7.08), 5.21 (s, 4H), 7.25 (br d, 1H, J = 7.82), 7.30−7.46 (series of m, 10H), 8.20−8.49 (br series of m, 3H), 8.38 (br s, 2H), 8.60 (br s, 2H), 10.66 (br s, 2H); MS[FAB] *m*/*z* (%) 965 [(M + 1)$^+$] (4).

Tetrapeptide 28. The above general procedure was used to couple the free carboxylic acid derivative of dipeptide **24** (0.062 g, 0.14 mmol) and the TFA salt of the free amino acid

analogue of dipeptide **24** (0.068 g, 0.14 mmol). After workup the crude residue was purified using flash chromatography with 75:25, DCM:methanol as the eluting solvent. The tetrapeptide **28** was obtained as a beige powder (0.011 g, 10%): R_f 0.68 (75:25, DCM:methanol); ^{1}H NMR (d_6-DMSO) δ 1.15 (t, 3H, J = 7.03), 1.37 (s, 9H), 1.89−2.34 (series of m, 4H), 2.16 (s, 6H), 3.64−4.32 (series of m, 10H), 4.06 (q, 2H, J = 7.03), 7.16 (d, 1H, J = 8.16), 7.86 (s, 2H), 8.12−8.50 (br series of m, 3H), 11.72 (br t , 2H), 12.02 (br s, 2H); MS[FAB] *m*/*z* (%) 814 [$(M + 2)^+$] (19).

Solid Phase Oligomerization. Stepwise Assembly of H-αCLysαTGlyαCGlyαTLysαCGlyαCGlyαTLysαTGly-αTGlyαTLys-NH$_2$.[20] The protected α-PNA was assembled manually on a Boc-Lys (2-Cl-Cbz) derivatized MBHA resin in a stepwise manner. The synthesis was initiated on 50 mg (dry weight) Boc-Lys (2-Cl-Cbz)-MBHA resin, which was preswollen overnight in DMF. The procedure is as follows: (1) Boc deprotection with TFA/*m*-cresol (95:5, v/v), 2 × 4 min. (2) Washing with DMF/DCM (1:1, v/v), 3 × 20 s; washing with pyridine, 2 × 20 s. (3) Coupling using a 0.1 M solution of Boc-amino acid (i.e. **6**, **10**, Boc-Lys (2-Cl-Cbz)-OH or Boc-Gly-OH) in DMF/pyridine (3:1) containing HBTU (0.95 equiv) and DIEA (1.1 equiv). The mixture was preactivated for 1 min prior to coupling, and coupling was allowed to proceed for 20 min at rt. (4) Washing with DMF, 2 × 20 s. (5) Capping of unreacted amino groups using Ac_2O/DMF/collidine (1:8:1, v/v/v), 1 × 5 min. (6) Washing with DMF, 3 × 20 s; washing with 5% piperidine in DMF, 1 × 4 min; washing with DMF/DCM (1:1, v/v), 3 × 20 s. Steps 1−6 were repeated until the desired sequence was obtained. All the couplings were monitored using Kaiser tests[40] and all were negative. After the final coupling, the resin was dried under vacuum, and the α-PNA oligomer was concomitantly deprotected and cleaved from the resin using the TFMSA procedure described by Christensen *et al.*:[37] yield 10.5 mg (crude: purity > 80% by MS analysis); MALDI-TOF MS: calcd average mass 2904.08, found 2901.9 $[M + H]^+$, 2926.0 $[M + Na]^+$. Analytical reverse phase HPLC recorded a single peak at 14.97 min (λ_{max} = 260 nm).

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