Synthesis of Peptide Nucleic Acid Monomers Containing the Four Natural Nucleobases: Thymine, Cytosine, Adenine, and Guanine and Their Oligomerization

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The preparation of mixed-sequence PNAs (PNAs containing the four natural nucleobases; thymine, cytosine, adenine, and guanine) is described. The PNA monomers containing thymine, Cbz-protected cytosine, or adenine or benzyl-protected guanine were prepared via convergent syntheses. Subsequent to introduction of the carboxymethyl linker at N-1 of the pyrimidines or N-9 of the purines and suitable protection of exocyclic groups, the nucleobase derivatives were coupled to the Boc-protected backbone esters **10a** or **10b** and finally hydrolyzed affording the monomers **12a–d**. The exocyclic amino groups of cytosine and adenine were protected with a benzyloxycarbonyl group. The exocyclic amino group of guanine was left unprotected whereas O^6 was protected as the benzyl ether. Two mixed-sequence 10-mers, each with five purines incorporated, and a mixed-sequence 15-mer containing seven purines were assembled essentially following standard solid phase peptide synthesis protocols.

DNA analogues are of considerable interest in medicinal chemistry and molecular biology, mainly due to their possible use as therapeutic agents[1] and their potential applications in diagnostics and as biomolecular tools. We have recently described a new type of DNA-analogues termed peptide nucleic acids (PNAs), in which the nucleobases are attached *via* a linker to a neutral and achiral backbone consisting of *N*-(2-aminoethyl)glycine units.[2,3]

PNA is a very potent DNA mimic capable of hybridizing to complementary DNA, RNA,[4] or PNA[5] and exhibits sequence discrimination equal to or better than that of DNA.[4] Furthermore, the resultant duplexes show higher thermal stability than the corresponding DNA duplexes at physiological ionic strength.[4] Triplexes of unprecedented high thermal stability are formed with homopurine DNA (or RNA) targets and *two* complementary homopyrimidine *PNA* strands (*i.e.* PNA·DNA·PNA or PNA·RNA·PNA).[3,5] Due to the high stability of such triplexes, PNA binds to homopurine targets in double stranded DNA by a novel mechanism, strand displacement,[2,6,7] and not by conventional triple helix formation.

In vitro experiments have supported the obvious potential of PNA as an antisense or antigene drug, since binding of PNA to mRNA inhibits translation[8] whereas binding to double-stranded DNA inhibits transcription[8,9] as well as binding of proteins recognizing the same DNA target.[8,10] Furthermore, recent results have shown that

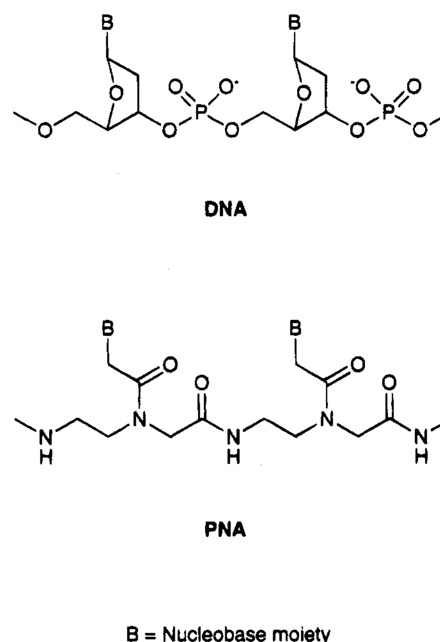

Figure 1.

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(1) Uhlmann, E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 544.
(2) Nielsen, P. E.; Egholm, M.; Berg, R. H.; Buchardt, O. *Science* **1991**, *254*, 1497.
(3) Egholm, M.; Buchardt, O.; Nielsen, P. E.; Berg, R. H. *J. Am. Chem. Soc.* **1992**, *114*, 1895.
(4) Egholm, M.; Buchardt, O.; Christensen, L.; Behrens, C.; Freier, S. M.; Driver, D. A.; Berg, R. H.; Kim, S. K.; Nordén, B.; Nielsen, P. E. *Nature* **1993**, *365*, 566.
(5) Egholm, M.; Nielsen, P. E.; Buchardt, O.; Berg, R. H. *J. Am. Chem. Soc.* **1992**, *114*, 9677.
(6) Cherny, D. Y.; Belotserkovskii, B. P.; Frank-Kamenetskii, M. D.; Egholm, M.; Buchardt, O.; Berg, R. H.; Nielsen, P. E. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 1667.
(7) Nielsen, P. E.; Egholm, M.; Ole Buchardt *J. Mol. Recognition*, in press.
(8) Hanvey, J. C.; Peffer, N. C.; Bisi, J. E.; Thomson, S. A.; Cadilla, R.; Josey, J. A.; Ricca, D. J.; Hassman, C. F.; Bonham, M. A.; Au, K. G.; Carter, S. G.; Bruckenstein D. A.; Boyd, A. L.; Noble S. A.; Babiss, L. E. *Science* **1992**, *258*, 1481.
(9) Nielsen, P. E.; Egholm. M.; Berg, R. H.; Buchardt, O. *Anti Cancer Drug Des.* **1993**, *8*, 53.

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$(PNA)_2$·dsDNA strand displacement complexes can function as efficient promoters for prokaryotic and eukaryotic RNA polymerases[11] thereby indicating the possibility of developing PNA into novel specific gene-activating drugs. Finally, it has been shown that PNA is biologically stable in various biological fluids including human serum and eukaryotic cell extracts.[12]

Since PNA is formally a pseudopeptide with the potential of carrying genetic information (*via* the nucleobase code in a manner identical to DNA or RNA) and forming helical duplexes between complementary PNAs,[13] it is theoretically possible that PNA-like molecules could have played a role at the origin of life during the evolution of the genetic material which eventually resulted in the DNA and RNA molecules we know today.[14]

These properties of PNA make this oligomer of significant interest in many disciplines of chemistry, molecular biology, and medicine. In this paper we describe the syntheses of the monomers containing thymine, cytosine, adenine, and guanine and the oligomer syntheses of the two 10-mers with the sequences H-GTA GAT CAC T-Lys-NH_2 and H-AGT GAT CTA C-Lys-NH_2 and the 15-mer with the sequence H-TGT ACG TCA CAA CTA-NH_2.[15]

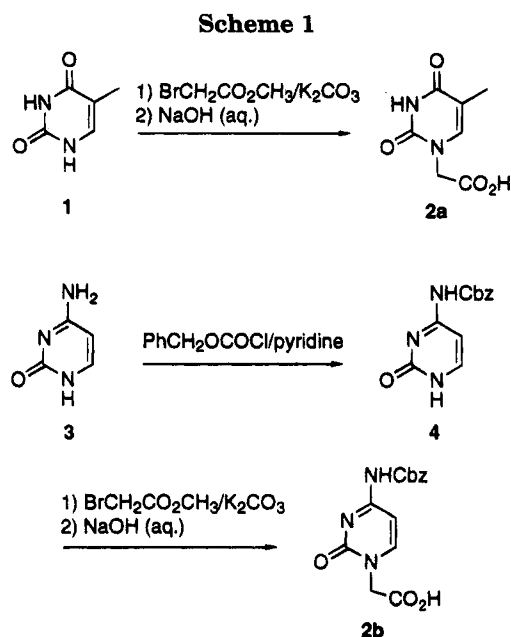
Results and Discussion

Derivatization of the Pyrimidines. We initially chose the Boc/Cbz protection strategy, as it seemed the most suitable for this type of reagents. The previously reported synthesis[3] of the thymine monomer involved alkylation of thymine (**1**) with methyl bromoacetate followed by hydrolysis of the resultant methyl ester to give thymin-1-ylacetic acid (**2a**, Scheme 1).

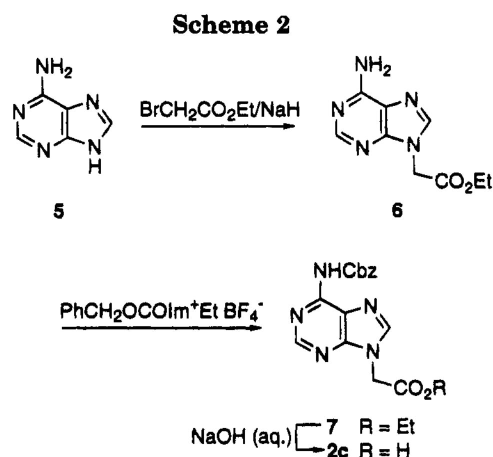
For the synthesis of the cytosine monomer,[5] introduction of a protection group at the N^4-amino group of cytosine was necessary to prevent chain extension from this position or acetylation during the capping procedure (*vide infra*). Protection of the cytosine monomer with the Cbz group also served to render the intermediates sufficiently soluble for chemical manipulations. Thus, cytosine (**3**) was reacted with benzyloxycarbonyl chloride to give N^4-(benzyloxycarbonyl)cytosine (**4**) and, subsequently, the carboxymethyl group was introduced by the procedure used for alkylation of thymine (Scheme 1).

Derivatization of the Purines. As Cbz protection proved to be suitable for the protection of the exocyclic amino group of cytosine, the same group was used for the protection of the exocyclic amino group of adenine. Adenine (**5**) could be alkylated with ethyl bromoacetate in DMF by first generating the anion with sodium hydride (Scheme 2). This procedure yielded only one isolable compound which was identified as the expected

Scheme 1



Scheme 2



ethyl adenin-9-ylacetate (**6**). Alkylation at the 9-position was verified by X-ray crystallography.[16]

6 gave rise to complex mixtures when reacted with benzyloxycarbonyl chloride under a variety of conditions. This is consistent with reports by Rapoport *et al.* on the Cbz-protection of trimethylsilyl-protected adenosine.[17,18] They found that *N*-(benzyloxycarbonyl)-*N'*-ethylimida-

(10) Nielsen, P. E.; Egholm. M.; Berg, R. H.; Buchardt, O. *Nucleic Acids Res.* **1993**, *21*, 197.
(11) Møllegaard, N. E.; Buchardt, O.; Egholm, M.; Nielsen, P. E. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 3892.
(12) Demidov, V.; Potaman, V. N.; Frank-Kamenetskii, M. D.; Buchardt, O.; Egholm, M.; Nielsen, P. E. *Biochem. Pharmacol.* In press.
(13) Wittung, P.; Nielsen, P. E.; Buchardt, O.; Egholm, M.; Nordén, B. *Nature* **1994**, *368*, 561.
(14) Nielsen, P. E. *Origins Life* **1993**, *23*, 323.
(15) We employ the following nomenclature: In the oligomer H-TCA G-Lys-NH_2, H denotes the free N-terminal amino group; T, C, A, and G the T-acetyl, C-acetyl, A-acetyl, and G-acetyl *N*-(2-aminoethyl)-glycinyl units, respectively, and Lys-NH_2 the C-terminal lysine amide. Additionally, we propose that the same designations be used for the monomer building blocks, *i.e.*, Boc-T-OH for *N*-(2-Boc-aminoethyl)-*N*-(thymin-1-ylacetyl)glycine, etc.

(16) Flensburg, C.; Egholm, M. *Acta Crystallogr. Sect. C 50*, in press.
(17) Watkins, B. E.; Kiely, J. S.; Rapoport, H. *J. Am. Chem. Soc.* **1982**, *104*, 5702.
(18) Watkins, B. E.; Rapoport, H. *J. Org. Chem.* **1982**, *47*, 4471.

zolium tetrafluoroborate ($PhCH_2OCOIm^+Et$, BF_4^-, "Rapoport's reagent") rather than benzyloxycarbonyl chloride gave a very clean and efficient acylation of the 6-amino group. When ethyl adenin-9-ylacetate (**6**) reacted with a 4 molar excess of this reagent for 24 h, we could isolate the warranted ethyl (N^6-Cbz-adenin-9-yl)acetate (**7**) in good yield, and subsequent hydrolysis of the ethyl ester also proceeded in good yield (Scheme 2).

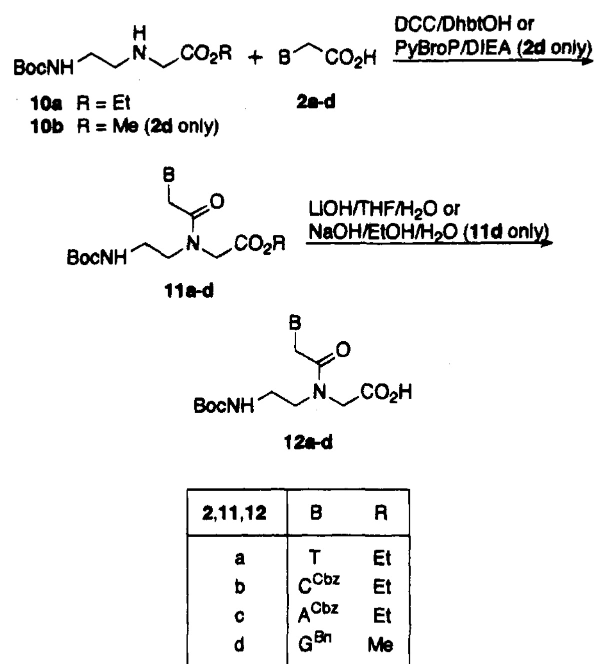
Guanine cannot be alkylated to give only 9-alkylated products and, consequently, 2-amino-6-chloropurine (**8**) is frequently used as the starting material.[19] Alkylation of **8** with bromoacetic acid afforded predominantly (2-amino-6-chloropurin-9-yl)acetic acid (**9**) but some of the 7-substituted product could also be isolated. Attempts to let **9** react with either benzyloxycarbonyl chloride or "Rapoport's reagent" failed. No reaction took place. Since this particular amino group could not be acylated with these reagents, it would appear that it needs no protection at all. However, a lipophilic group is needed to render the intermediates sufficiently soluble for carrying out subsequent reactions. Thus, the chloro group of **9** was exchanged for a benzyloxy group. The resultant (2-amino-6-(benzyloxy)purin-9-yl)acetic acid (**2d**, Scheme 2) is soluble in a range of organic solvents.

To test whether employment of the monomer of **2d** in the oligomer synthesis would lead to the incorporation of guanine, ethyl (2-amino-6-(benzyloxy)purin-9-yl)acetate (prepared from ethyl bromoacetate and 2-amino-6-(benzyloxy)purine, in turn prepared from 2-amino-6-chloropurine) was treated with 50% TFA in dichloromethane (DCM) to mimic the conditions used for removal of the Boc groups under the oligomer synthesis. NMR and MS showed unambiguously that the *O*-benzyl group was cleaved. In addition, treatment with HF under the conditions used for cleavage of the oligomer from the resin led to the same result (determined by HPLC analysis), *i.e.*, quantitative formation of ethyl guanin-9-ylacetate.

Attempts to acylate the amino group of ethyl (2-amino-6-(benzyloxy)purin-9-yl)acetate with benzyloxycarbonyl chloride or "Rapoport's reagent" were also unsuccessful in a wide range of solvent systems. However, there was the risk that the 2-amino group could be acetylated during the capping step of the oligomer synthesis cycle. Treatment of ethyl (2-amino-6-(benzyloxy)purin-9-yl)acetate with acetic anhydride/pyridine/DCM 1:2:2 for 1.5 h converted 23% of the starting material to product according to HPLC. It was characterized as ethyl (2-(acetylamino)-6-(benzyloxy)purin-9-yl)acetate by extending the reaction time to 48 h and identifying the product. Thus, the amino group of the guanine monomer would be partially acetylated during the capping procedure. At present this problem is circumvented by omitting the capping after the introduction of the first guanine residue.

Monomer Synthesis. For the attachment to the backbone moiety ethyl *N*-(2-Boc-aminoethyl)glycinate (**10a**)[20] was used rather than *N*-(2-Boc-aminoethyl)glycine as previously reported,[3] as this improved the workup and purification. Thymin-1-yl-, (N^4-Cbz-cytosin-1-yl)-, or (N^6-Cbz-adenin-9-yl)acetic acid (**2a**, **2b**, or **2c**, respectively)

Scheme 3



2,11,12	B	R
a	T	Et
b	C^{Cbz}	Et
c	A^{Cbz}	Et
d	G^{Bn}	Me

were activated with DhbtOH (3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine) and DCC, *in situ*, for the reaction with **10a**, and the resultant monomer ethyl esters (**11a**, **11b**, or **11c**) were hydrolyzed with LiOH in water/THF to afford the pure monomers (**12a**, **12b**, or **12c**, respectively, Scheme 3). **11a** and **11b** were not isolated or characterized prior to the hydrolysis.

The best procedure for the reaction of **2d** with the backbone moiety was found to be condensation, mediated by bromotris(pyrrolidino)phosphonium hexafluorophosphate (PyBrop), with the methyl ester **10b** rather than the ethyl ester **10a** of *N*-(2-Boc-aminoethyl)glycine. Without isolation and characterization of **11d** the ester function was hydrolyzed in aqueous sodium hydroxide (**12d**, Scheme 3).

Oligomer Synthesis. The assembly of the oligomers essentially followed standard solid phase peptide synthesis (SPPS) protocols. A 4-methylbenzhydrylamine (MBHA) resin was used. For the synthesis of homothymine oligomers, the thymine monomers were activated as pentafluorophenyl esters.[3] However, the Cbz-cytosine monomer pentafluorophenyl ester was incorporated poorly giving only a 50% coupling yield.[5] Utilizing an *in situ* DCC-coupling protocol resulted in quantitative couplings of both the thymine (**12a**) and the Cbz-cytosine (**12b**) monomers. Cleavage from the solid support was accomplished with hydrogen fluoride under conditions normally used in peptide chemistry. The concomitant complete removal of the Cbz-group from the product by the HF-treatment was confirmed by MS (FAB) analysis of the crude product. No indications of lack of deprotection or alkylation by the released benzyl or *tert*-butyl cations could be detected.[5] Crude $H-T_4CT_5-Lys-NH_2$ was pure according to reverse phase HPLC and could be used directly for hybridization studies.[5] The C-terminal lysine amide was originally included in order to suppress self-association of PNAs containing only thymine residues and has been used in later experiments to facilitate comparison.

The Cbz-adenine monomer (**12c**) was incorporated only partially when the *in situ* DCC coupling protocol was

(19) Geen, G. R.; Grinter, T. J.; Kincey, P. M.; Jarvest, R. L. *Tetrahedron* **1990**, *46*, 6903.
(20) Dueholm, K. L.; Egholm, M.; Buchardt, O. *Org. Prep. Proc. Int.* **1993**, *25*, 457.

tested in the oligomer synthesis, and even a double coupling protocol was not sufficient to obtain a quantitative reaction. However, by employing *N,N'*-diisopropylcarbodiimide (DIPCDI) as the coupling reagent a nearly quantitative yield could be obtained with a double coupling protocol.[21] The synthesis of the 10-mer H-T_4-AT_5-$LysNH_2$ was accomplished using single couplings for the incorporation of the thymine monomers and a double coupling for the incorporation of the Cbz-adenine monomer. The resultant oligomer was pure by reverse phase HPLC, and MS (FAB) confirmed the identity.[21]

The Bn-guanine monomer (**12d**) was incorporated quantitatively by using the DIPCDI coupling protocol described above. Leaving out the capping after the incorporation of the Bn-guanine residue allowed the oligomer H-T_4GT_5-$LysNH_2$ to be isolated.[21]

A mixed sequence 15-mer containing all four bases was designed for the purpose of studying PNA/DNA-duplex formation.[4] The sequence H-TGT ACG TCA CAA CTA-NH_2 was synthesized by following the above protocol employing DIPCDI couplings and with no capping after incorporation of the first Bn-guanine residue. The oligomer was cleaved from the support following the general procedure affording a crude product which was more than 80% pure according to reverse phase HPLC (λ = 260 nm). The identity of the product was confirmed by FAB mass spectrometry. Essentially the same protocol was used to synthesize two mixed-sequence 10-mers; H-GTA GAT CAC T-Lys-NH_2 and H-AGT GAT CTA C-Lys-NH_2.

Progress is currently being made toward improving the oligomer[22] and monomer syntheses and will be reported in due time.

Conclusion. This work demonstrates that all four natural DNA nucleobases may be incorporated into PNA applying standard peptide synthesis protocols, including common protections schemes from peptide chemistry.

Experimental Section

Reagents and solvents were obtained from commercial sources and used without further purification unless indicated. Na_2SO_4 or $MgSO_4$ were employed as drying agents for organic solutions. NMR spectra were recorded in DMSO-d_6 unless indicated. Products containing amide bonds (**11c** and **12a–d**) were isolated as mixtures of two rotamers, the ratios of which were 2:1 unless indicated. Several of the NMR signals of these products were doubled in the rotamer ratio as indicated by ma. for major and mi. for minor. For the monomers **12b–d** only the NMR signals characteristic of the base moiety are provided.

A gradient composed of A (0.1% TFA in water) and B (0.1% TFA in 10% H_2O/90% acetonitrile) was used for analytical and preparative HPLC: Time 0, 0% B. Time 2 min, 0% B. Time 30 min, 50% B. Time 35 min, 50% B. Time 37 min, 0% B (flow, analytical: 1 mL/min, flow preparative: 10 mL/min).

Monomer Synthesis. Thymin-1-ylacetic Acid (2a). This procedure is different from the one in the literature,[23] but it is easier, gives higher yields and leaves no unreacted thymine in the product. To a suspension of **1** (50.0 g, 397 mmol) and K_2CO_3 (54.8 g, 397 mmol) in dry DMF (1.2 L) was added methyl bromoacetate (37.5 mL, 397 mmol), and the mixture was stirred vigorously overnight under N_2. The mixture was filtered and evaporated to dryness, *in vacuo*. The solid residue was cooled to 0 °C, treated with water (375 mL) and 4 M HCl (aqueous, 16 mL), and stirred for 30 min. The precipitate was collected by filtration and washed with water (3 × 200 mL). The precipitate was treated with water (400 mL) and 2 M NaOH (aqueous, 200 mL) and boiled for 10 min. The mixture was cooled to 0 °C, treated with 4 M HCl (aqueous, 135 mL), and stirred for 30 min. The title compound was collected by filtration, washed with water (3 × 200 mL), and dried over P_2O_5 yielding 45.3 g (62%): ^1H NMR δ 11.33 (s, 1H), 7.49 (s, 1H), 4.38 (s, 2H), 1.76 (s, 3H). Anal. Calcd for $C_7H_8N_2O_4$: C, 45.66; H, 4.38; N, 15.21. Found: C, 45.68; H, 4.36; N, 15.22.

***N*^{4}-(Benzyloxycarbonyl)cytosine (4).** Benzyloxycarbonyl chloride (52 mL, 0.36 mol) was added dropwise over a period of ca. 1 h to a suspension of cytosine (**3**, 20.0 g, 0.18 mol) in dry pyridine (1000 mL) at 0 °C under N_2. The mixture was stirred overnight and, subsequently, the pyridine suspension was evaporated to dryness, *in vacuo*. Water (200 mL) was added and the pH was adjusted to 1 with 4 M HCl (aqueous). The resultant white precipitate was filtered off, washed with water, and partially dried. The wet precipitate was boiled with absolute EtOH (500 mL) for 10 min, cooled to 10 °C, filtered, washed thoroughly with ether and dried, *in vacuo*: yield 24.7 g (56%); mp > 250 °C. No NMR spectra were recorded, since it was impossible to dissolve the product in a suitable solvent. Anal. Calcd for $C_{12}H_{11}N_3O_3$: C, 58.77; H, 4.52; N, 17.13. Found: C, 58.59; H, 4.55; N, 17.17.

(*N*^{4}-(Benzyloxycarbonyl)cytosin-1-yl)acetic Acid (2b). To a suspension of **4** (20.3 g, 82.6 mmol) and K_2CO_3 (11.4 g, 82.6 mmol) in DMF (230 mL) was added methyl bromoacetate (7.7 mL, 82.6 mmol), and the mixture was stirred vigorously overnight under N_2. The mixture was filtered and evaporated to dryness, *in vacuo*. The solid residue was treated with water (80 mL) and 4 M HCl (aqueous, 3.1 mL), stirred for 15 min at 0 °C, filtered, and washed with water (2 × 20 mL). The precipitate was treated with water (120 mL) and 2 M NaOH (aqueous, 60 mL) and stirred for 30 min. The mixture was cooled to 0 °C and filtered, and the title compound was precipitated by the addition of 4 M HCl (aqueous, 35 mL). Crude **2b** was recrystallized from MeOH (1000 mL) and washed thoroughly with ether yielding 7.76 g (31%). An additional 2.25 g (9%) of **2b** could be isolated from the mother liquor by reducing it to a volume of 200 mL and cooling it to 0 °C: mp 266–274 °C; ^1H NMR δ 8.02 (d, 1H, J = 7 Hz), 7.39 (s, 5H), 7.01 (d, 1H, J = 7 Hz), 5.19 (s, 2H), 4.52 (s, 2H). Anal. Calcd for $C_{14}H_{13}N_3O_5$: C, 55.45; H, 4.32; N, 13.86. Found: C, 55.41; H, 4.23; N, 14.04.

Ethyl adenin-9-ylacetate (6). Adenine (**5**, 100 g, 0.7 mol) was suspended in dry DMF (1.5 L) and NaH (33 g, 0.8 mol, Aldrich, 19.923-0, washed with hexane) was added portionwise with mechanical stirring. The reaction mixture was stirred for 2 h at rt. Subsequently, ethyl bromoacetate (150 mL, 1.4 mol) was added dropwise during 3 h at rt. After an additional 2 h of stirring the solvent was removed by evaporation, *in vacuo*. The remaining oil was shaken with water (1 L) resulting in crystallization. The crystals were filtered off and washed with water, followed by recrystallization from EtOH to give 113 g (73%) of white crystalline product: mp 227–228 °C. The position of the alkylation was verified by X-ray crystallography:[16] ^1H NMR δ 8.22 (s, 1H), 8.20 (s, 1H), 7.38 (s, 2H), 5.15 (s, 2H), 4.24 (q, 2H, J = 7 Hz), 1.28 (t, 3 H, J = 7 Hz); ^{13}C NMR δ 168.0, 156.0, 152.7, 149.8, 141.3, 118.3, 61.4, 44.0, 14.1; MS (FAB) m/z 222 $(M + H)^+$. Anal. Calcd for $C_9H_{11}N_5O_2$: C, 48.70; H, 4.91; N, 31.42. Found: C, 48.78; H, 4.91; N, 31.37.

Ethyl (*N*^{6}-(benzyloxycarbonyl)adenin-9-yl)acetate (7). **6** (3.40 g, 15.4 mmol) was dissolved in dry DMF (50 mL) by gentle heating and then cooled to rt. The mixture was added to a solution of *N*-(benzyloxycarbonyl)-*N'*-ethylimidazolium tetrafluoroborate (62 mmol) in DCM (50 mL), prepared as described by Watkins *et al.*,[17] over a period of 15 min at 0 °C. Hereby some precipitation was observed. The cooling was discontinued and the solution was stirred overnight. Subsequently, the reaction mixture was treated with saturated aqueous $NaHCO_3$ (100 mL). After stirring for 10 min the phases were separated and the organic phase was washed successively with 1 volume of water, dilute aqueous $KHSO_4$ (2 × 50 mL), and finally brine (1 × 50 mL). The solution was dried and evaporated to dryness, *in vacuo*, affording 11 g of

(21) Egholm, M.; Christensen, L.; Behrens, C.; Berg, R. H.; Nielsen, P. E.; Buchardt, O. *J. Chem. Soc., Chem. Commun.* **1993**, 800.
(22) Christensen, L. *et al.* Manuscript in preparation.
(23) Rabinowitz, J. L.; Gurin, S. *J. Am. Chem. Soc.* **1953**, *75*, 5758.

an oily residue. This material was dissolved in DCM (25 mL), cooled to 0 °C, and treated with petroleum ether (50 mL). The precipitate was isolated by filtration, redissolved in DCM, and precipitated once more with petroleum ether to give 3.45 g (63%) of the title compound: mp 132–135 °C; ^{1}H NMR ($CDCl_3$) δ 8.77 (s, 1H), 7.99 (s, 1H), 7.45–7.26 (m, 5H), 5.31 (s, 2H), 4.96 (s, 2H), 4.27 (q, 2H, J = 7 Hz), 1.30 (t, 3H, J = 7 Hz); MS (FAB) m/z 356 (M + H)$^+$, 312 (M + H − CO_2)$^+$; HRMS exact mass 356.1342 (M + H)$^+$, calcd for $C_{17}H_{18}N_5O_4$ 356.1359. Anal. Calcd for $C_{17}H_{17}N_5O_4$: C, 57.46; H, 4.82; N, 19.71; C/N, 2.92. Found: C, 56.95; H, 4.71; N, 19.35; C/N, 2.94.

(N^6-(Benzyloxycarbonyl)adenin-9-yl)acetic Acid (2c). **7** (3.20 g, 9.01 mmol) was mixed with MeOH (50 mL) and cooled to 0 °C. 2 M NaOH (aqueous, 50 mL) was added, whereby the ester quickly dissolved. After 30 min of stirring at 0 °C the alkaline solution was washed with DCM (2 × 50 mL). The aqueous solution was brought to pH 1.0 with 4 M HCl (aqueous) at 0 °C, whereby the title compound precipitated. After filtration, washing with water and drying, the yield was 3.08 g (104%). The product contained salt which is also evident from the elemental analysis, but the C/N ratio found corresponded well with the calculated value. According to HPLC (215 nm, 260 nm) the product contained minor impurities, all less than 2%: ^{1}H NMR δ 8.70 (s, 2H), 7.50–7.35 (m, 5H), 5.27 (s, 2H), 5.15 (s, 2H). ^{13}C NMR δ 168.8, 152.5, 151.4, 148.8, 145.1, 128.5, 128.2, 128.0, 66.8, 44.8; MS (FAB) m/z 328 (M + H)$^+$, 284 (M + H − CO_2)$^+$; HRMS exact mass 328.1029 (M + H)$^+$, calcd for $C_{15}H_{14}N_5O_4$ 328.1046. Anal. Calcd for $C_{15}H_{13}N_5O_4$: C, 55.05; H, 4.00; N, 21.40; C/N, 2.56. Found: C, 46.32; H, 4.24; N, 18.10; C/N, 2.57.

(2-Amino-6-chloropurin-9-yl)acetic Acid (9). To a suspension of 2-amino-6-chloropurine (**8**, 5.02 g, 29.6 mmol) and K_2CO_3 (12.91 g, 93.5 mmol) in DMF (50 mL) was added bromoacetic acid (4.70 g, 33.8 mmol) and the mixture was stirred vigorously for 20 h under N_2. Water (150 mL) was added and the suspension was filtered through Celite giving a clear yellow solution. The solution was acidified to pH 3 with 4 M HCl (aqueous). The precipitate was isolated by filtration and dried, *in vacuo*, over P_2O_5: yield 3.02 g (45%); ^{1}H NMR δ 8.13 (s, 1H), 6.96 (s, 2H), 4.91 (s, 2H); ^{13}C NMR δ 169.1, 160.0, 154.4, 149.5, 143.7, 123.1, 44.3; HRMS exact mass 227.0202 (M)$^+$, calcd for $C_7H_6ClN_5O_4$ 227.0210. Anal. Calcd for $C_7H_6ClN_5O_2 \cdot 0.25H_2O$: C, 36.22; H, 2.82; N, 30.17; C/N, 1.20. Found: C, 36.48; H, 2.84; N, 30.58; C/N, 1.19.

(2-Amino-6-(benzyloxy)purin-9-yl)acetic Acid (2d). Sodium (2.0 g, 87.0 mmol) was dissolved in benzyl alcohol (20 mL) and heated to 130 °C for 2 h. After cooling to 0 °C a solution of **9** (4.05 g, 18.0 mmol) in DMF (85 mL) was slowly added, and the resultant suspension was stirred overnight at rt. 1 M NaOH (aqueous, 100 mL) was added, and the clear solution obtained was washed with EtOAc (3 × 100 mL). The water phase was then acidified to pH 3 with 4 M HCl (aqueous). The precipitate was taken up in EtOAc (200 mL) and the water phase was extracted with EtOAc (2 × 100 mL). The combined organic phases were washed with brine (2 × 75 mL), dried, and evaporated, *in vacuo*. The residue was recrystallized from EtOH (300 mL): yield 2.76 g (52%); mp 159–165 °C; ^{1}H NMR δ 7.84 (s, 1H), 7.52–7.31 (m, 5H), 6.50 (s, 2H), 5.51 (s, 2H), 4.82 (s, 2H); ^{13}C NMR δ 169.3, 160.0, 159.8, 154.7, 140.4, 136.7, 128.4, 128.3, 128.0, 113.3, 66.9, 43.9; MS (EI) m/z 299 (M)$^+$, 241 (M − CH_2COOH)$^+$, 91 ($PhCH_2$)$^+$. Anal. Calcd for $C_{14}H_{13}N_5O_3$: C, 56.18; H, 4.38; N, 23.40. Found: C, 55.97; H, 4.32; N, 23.10.

Ethyl *N*-((N^6-(Benzyloxycarbonyl)adenin-9-yl)acetyl)-*N*-(2-Boc-aminoethyl)glycinate (11c). This compound was prepared analogously to **12a** (*vide infra*) from **10a** (2.00 g, 8.12 mmol), DhbtOH (1.46 g, 8.93 mmol), **2c** (2.92 g, 8.93 mmol), and DCC (2.01 g, 9.74 mmol) in DMF (15 mL) and DCM (15 mL). The ice bath was removed after 2.5 h and the stirring was continued for another 1.5 h at rt. The DCU (*N,N'*-dicyclohexylurea) was removed by filtration and was washed with DMF (1 × 15 mL) and with DCM (2 × 15 mL). To the combined filtrates was added more DCM (100 mL) and the solution was washed successively with dilute aqueous $NaHCO_3$ (2 × 100 mL), dilute aqueous $KHSO_4$ (2 × 100 mL), and finally brine (1 × 100 mL). The organic phase was evaporated to dryness, *in vacuo*, affording 3.28 g of a yellowish oil. The oil was dissolved in absolute EtOH (50 mL), charcoal was added, and after stirring for 5 min the solution was filtered. Water (30 mL) was added to the filtrate and the mixture was stirred overnight. The resultant white precipitate was isolated by filtration, washed with water, and dried to afford 1.16 g (26%) of product with a purity higher than 98% determined by HPLC (λ = 260 nm). Addition of water to the mother liquor afforded another 0.53 g (12%) with a purity of approximately 95%: mp 91–92 °C dec; rotamer ratio 3:1; ^{1}H NMR ($CDCl_3$) δ 8.74 (s, 1H), 8.18 (br, 1H), 8.10 (mi.) and 8.04 (ma.) (s, 1H), 7.46–7.34 (m, 5H), 5.63 (br, 1H), 5.30 (s, 2H), 5.16 (ma.) and 5.00 (mi.) (s, 2H), 4.29 (mi.) and 4.06 (ma.) (s, 2H), 4.20 (q, 2H, J = 7 Hz), 3.67–3.29 (m, 4H), 1.42 (s, 9H), 1.27 (t, 3H, J = 7 Hz). The spectrum showed traces of EtOH and DCU. HRMS exact mass 556.2538 (M + H)$^+$, calcd for $C_{26}H_{34}N_7O_7$ 556.2520. Anal. Calcd for $C_{26}H_{33}N_7O_7$,H_2O: C, 54.44; H, 6.15; N, 17.09. Found: C, 55.01; H, 6.85; N, 16.47.

***N*-(2-Boc-Aminoethyl)-*N*-(thymin-1-ylacetyl)glycine (12a).** Ethyl *N*-(2-Boc-aminoethyl)glycinate (**10a**, 13.5 g, 54.8 mmol), DhbtOH (9.84 g, 60.3 mmol), and thymin-1-ylacetic acid (**2a**, 11.1 g, 60.3 mmol) were dissolved in DMF (210 mL) and DCM (210 mL) was added. The solution was cooled to 0 °C and DCC (13.6 g, 65.8 mmol) was added. The ice bath was removed after 1 h and the stirring was continued for another 2 h at rt. The precipitated DCU was removed by filtration and washed with DCM (2 × 75 mL). To the combined filtrates was added more DCM (650 mL) and the solution was washed successively with dilute aqueous $NaHCO_3$ (3 × 500 mL), dilute aqueous $KHSO_4$ (2 × 500 mL), and finally brine (1 × 500 mL). The precipitate in the organic phase was removed by filtration, whereupon the organic phase was dried and evaporated to dryness, *in vacuo*. The oily residue was dissolved in DCM (150 mL) and filtered, and the title compound was precipitated by the addition of petroleum ether (350 mL) at 0 °C. The precipitate was redissolved in DCM and precipitated once more with petroleum ether. This afforded 16.0 g of **11a** which was more than 99% pure according to HPLC (260 nm). **11a** (16.0 g, 38.8 mmol) was suspended in THF (194 mL) and 1 M LiOH (aqueous, 116 mL) was added. The mixture was stirred for 45 min at rt and was then filtered to remove residual DCU. Water (40 mL) was added to the solution and it was washed with DCM (300 mL). Additional water (30 mL) was added and the solution was washed once more with DCM (150 mL). The aqueous solution was cooled to 0 °C and the pH was adjusted to 2 by the dropwise addition of 1 M HCl (aqueous, 110 mL). The title compound was extracted with EtOAc (9 × 200 mL). The combined extracts were dried and then evaporated to dryness, *in vacuo*. The residue was evaporated once from MeOH and afforded Boc-T-OH as a colorless glassy solid after drying overnight:[15] yield 9.57 g (45%); mp 122–123 °C dec; ^{1}H NMR δ 11.26 (m, 1 H), 7.30 (ma.) and 7.26 (mi.) (s, 1 H), 6.90 (ma.) and 6.70 (mi.) (m, 1 H), 4.64 (ma.) and 4.47 (mi.) (s, 2 H), 4.18 (mi.) and 3.97 (ma.) (s, 2 H), 3.39 (ma.) and 3.30 (mi.) (m, 2 H and H_2O), 3.18 (ma.) and 3.05 (mi.) (m, 2 H and H_2O), 1.76 (s, 3H), 1.38 (ma.) and 1.37 (mi.) (s, 9 H); ^{13}C NMR δ 170.8 (mi.) and 170.4 (ma.), 167.6 (mi.) and 167.2 (ma.), 164.4, 155.8, 151.0, 142.0, 108.2, 78.1 (ma.) and 78.0 (mi.), 49.2, 47.8 (mi.) and 47.6 (ma.), 47.0 (mi.) and 46.9 (ma.), 38.2 (ma.) and 37.7 (mi.), 28.3 (mi.) and 28.2 (ma.), 12.0. Anal. Calcd for $C_{16}H_{24}N_4O_7$, 0.25 H_2O: C, 49.42; H, 6.35; N, 14.41. Found: C, 49.29; H, 6.52; N, 14.11.

***N*-((N^4-(Benzyloxycarbonyl)cytosin-1-yl)acetyl)-*N*-(2-Boc-aminoethyl)glycine (12b).** **10a** (5.00 g, 20.3 mmol), DhbtOH (3.64 g, 22.3 mmol), and (N^4-(benzyloxycarbonyl)-cytosin-1-yl)acetic acid (**2b**, 6.77 g, 22.3 mmol) were suspended in DMF (100 mL), and DCM (100 mL) was added. The solution was cooled to 0 °C and DCC (5.03 g, 24.4 mmol) was added. The ice bath was removed after 2 h and the stirring was continued for 1 h at rt, whereupon the reaction mixture was evaporated to dryness, *in vacuo*. The residue was suspended in ether (100 mL) and stirred vigorously for 30 min. The solid material was removed by filtration and the ether washing procedure was repeated twice. The residue resulting from evaporation of the ether was stirred vigorously for 15 min with

aqueous $NaHCO_3$ (approximately 4% solution, 100 mL), filtered, and washed with water. This procedure was repeated once which after drying leaves 17.0 g of yellowish solid material. The solid was boiled in dioxane (200 mL) and filtered while hot. After the filtrate had cooled, water (200 mL) was added. The precipitated material was isolated by filtration, washed with water, and finally dried leaving 14.50 g of a mixture of **11b** and DCU (according to HPLC at 260 nm where DCU is not detected, this product had a purity higher than 99%). This mixture was suspended in THF (100 mL) and cooled to 0 °C, and 1 M LiOH (aqueous, 61 mL) was added. After stirring for 15 min the mixture was filtered, and the filtrate was washed with DCM (2 × 150 mL). The alkaline aqueous solution was then cooled to 0 °C and the pH was adjusted to 2.0 with 1 M HCl (aqueous). Crude title compound was isolated by filtration and washed once with water giving 11.3 g of white powder after drying. This material was suspended in DCM (300 mL), and petroleum ether (300 mL) was added. Filtration and washing afforded 7.1 g (69%) of Boc-C(Cbz)-OH:[15] mp 181–182 °C dec; HPLC (λ = 260 nm) showed a purity of 99% and a minor impurity (ca. 1%), most likely Boc-C-OH:[15] ^{1}H NMR δ 7.89 (ma.) and 7.87 (mi.) (d, 1 H, J = 7 Hz), 7.43–7.34 (m, 5 H), 7.01 (ma.) and 7.00 (mi.) (d, 1 H, J = 7 Hz), 5.19 (s, 2 H); ^{13}C NMR δ 163.1, 154.9, 153.2, 150.7, 136.0, 128.5, 128.1, 127.9, 93.9, 66.5; HRMS exact mass 504.2086 (M + H)$^+$, calcd for $C_{23}H_{30}N_5O_8$ 504.2094. Anal. Calcd for $C_{23}H_{29}N_5O_8$: C, 54.87; H, 5.81; N, 13.91; C/N, 3.94. Found: C, 54.16; H, 5.76; N, 13.65; C/N, 3.97.

***N*-((*N*^{6}-(Benzyloxycarbonyl)adenin-9-yl)acetyl)-*N*-(2-Boc-aminoethyl)glycine (12c).** The ethyl ester **11c** (1.48 g, 2.66 mmol) was suspended in THF (13 mL). This suspension was cooled to 0 °C and 1 M LiOH (aqueous, 8 mL) was added. After 15 min of stirring the reaction mixture was filtered, extra water (25 mL) was added and the solution was washed with DCM (2 × 25 mL). The aqueous solution was adjusted to pH 2.0 with 1 M HCl (aqueous). The precipitate was isolated by filtration and washed with water affording 0.82 g of crude product. The compound was reprecipitated twice from DCM/petroleum ether yielding 0.77 g (55%) of Boc-A(Cbz)-OH:[15] mp 118–119 °C dec; ^{1}H NMR δ 10.65 (br, 1 H), 8.60 (s, 1 H), 8.32 (s, 1 H), 7.48–7.34 (m, 5 H), 5.23 (s, 2 H); ^{13}C NMR δ 152.4, 152.2, 151.5, 149.4, 145.2 (mi.) and 145.1 (ma.), 136.4, 128.4, 128.0, 127.8, 123.0 (ma.) and 122.9 (mi.), 66.3; HRMS exact mass 528.2219 (M + H)$^+$, calcd for $C_{24}H_{30}N_7O_7$ 528.2207. Anal. Calcd for $C_{24}H_{29}N_7O_7$, H_2O: C, 52.84; H, 5.73; N, 17.97; C/N, 2.94. Found: C, 53.32; H, 5.71; N, 17.68; C/N, 3.02.

***N*-((2-Amino-6-(benzyloxy)purin-9-yl)acetyl)-*N*-(2-Boc-aminoethyl)glycine (12d).** **2d** (0.50 g, 1.67 mmol), methyl *N*-(2-Boc-aminoethyl)glycinate (**10b**, 0.65 g, 2.80 mmol), diisopropylethylamine (DIEA, 0.54 g, 4.19 mmol), and bromotris(pyrrolidino)phosphonium hexafluorophosphate (PyBroP, 0.80 g, 1.71 mmol) were stirred in DMF (2 mL) for 4 h. The clear solution was poured into ice-cooled 1 M $NaHCO_3$ (aqueous, 40 mL) and extracted with EtOAc (3 × 40 mL). The combined extracts were washed with 1 M $KHSO_4$ (aqueous, 2 × 40 mL), 1 M $NaHCO_3$ (aqueous, 1 × 40 mL), and brine (1 × 60 mL). After drying and evaporation, *in vacuo*, the solid residue was recrystallized from EtOAc/hexane (2:1, 20 mL) to give **11d** in a yield of 0.54 g (63%) (MS (FAB) m/z 514 (M + H)$^+$). The ester **11d** (0.51 g, 1.00 mmol) was dissolved in EtOH/water (1:2, 30 mL) containing concd NaOH (aqueous, 1 mL). After stirring for 2 h the solution was filtered and acidified to pH 3 by the addition of 4 M HCl (aqueous). Boc-G(Bn)-OH was isolated by filtration:[15] yield 0.37 g (74%, hydrolysis only, 47% based on **2d**); mp 134–136 °C dec. The purity determined by HPLC (λ = 260 nm) was higher than 99%. The product contained salt which is also evident from the elemental analysis, but the C/N ratio found corresponded well with the calculated value: ^{1}H NMR δ 7.73 (s, 1 H), 7.52–7.33 (m, 5 H), 6.65 (mi.) and 6.42 (ma.) (br, 2 H), 5.51 (s, 2 H); ^{13}C NMR δ 159.8, 159.5, 154.7, 140.5 (mi.) and 140.4 (ma.), 136.6, 128.2, 128.0, 127.9, 113.0, 66.8; HRMS exact mass 500.2290 (M + H)$^+$, calcd for $C_{23}H_{30}N_7O_6$ 500.2258. Anal. Calcd for $C_{23}H_{29}N_7O_6$: C, 55.30; H, 5.85; N, 19.63; C/N, 2.82. Found: C, 54.05; H, 5.75; N, 18.68; C/N, 2.89.

Acetylation of the Guanine Residue under Capping Conditions. Ethyl (2-amino-6-(benzyloxy)purin-9-yl)acetate (1.00 g, 3.1 mmol) was dissolved in a mixture of Ac_2O, pyridine, and DCM (1:2:2) and stirred at rt. HPLC analysis (λ = 260 nm) of the reaction showed after 1.5 h a peak (of 23%) eluting later. After 7 h this peak had increased to 43%. The compound giving rise to this peak was identified by letting the reaction mixture stir for 48 h. The reaction mixture was evaporated to dryness, *in vacuo*, and the residue recrystallized from water/EtOH to yield 0.83 g (72%) of ethyl (2-(acetylamino)-6-(benzyloxy)purin-9-yl)acetate: ^{1}H NMR δ 10.46 (br, 1H), 8.23 (s, 1H), 7.69–7.34 (m, 5H), 5.62 (s, 2H), 5.09 (s, 2H), 4.18 (q, 2H), 2.25 (s, 3H), 1.22 (t, 3H).

TFA-Mediated Debenzylation of Ethyl (2-Amino-6-(benzyloxy)purin-9-yl)acetate. The ethyl ester of **2d** (0.50 g, 1.5 mmol) was stirred for 1 h at rt with 50% TFA in DCM (10 mL). The reaction mixture was evaporated under reduced pressure. The residue was washed with 2 M $NaHCO_3$ (aqueous), filtered off, and washed with water and ether yielding 0.33 g (93%) of ethyl guanin-9-ylacetate: ^{1}H NMR δ 8.51 (s, 1H), 5.16 (s, 2H), 4.37 (q, 2H, J = 7.1 Hz), 1.39 (t, 3H, J = 7.1 Hz); ^{13}C NMR δ 169.4, 157.5, 155.9, 152.3, 140.2, 64.3, 46.2, 15.0; MS (EI) m/z 237 (M)$^+$.

Oligomer Synthesis. General Protocol (100 mg of resin).[15] All oligomer syntheses were initiated on a preswollen resin (overnight in DCM). (1) Boc-Deprotection with TFA/DCM (1:1, v/v), 2 mL, 1 × 2 min and 1 × 30 min. (2) Washing with DCM, 2 mL, 4 × 20 s; washing with DMF, 2 mL, 2 × 20 s; washing with DCM, 2 mL, 2 × 20 s. (3) Neutralization with DIEA/DCM (1:19, v/v), 2 mL, 2 × 3 min. (4) Washing with DCM, 2 mL, 4 × 20 s, and drain. (5) A few beads were taken out for a qualitative ninhydrin analysis (Kaiser test).[24] (6) Addition of 4 equiv of DIPCDI (or DCC) and 4 equiv of Boc-protected monomer dissolved in DCM/DMF (1:1, v/v) giving a final concentration of PNA-monomer of 0.1 M. The coupling reaction was allowed to proceed for 1 h with shaking at rt. (7) Washing with DMF, 2 mL, 2 × 20 s and 1 × 2 min; washing with DCM, 2 mL, 4 × 1 min. (8) Neutralization with DIEA/DCM (1:19, v/v), 2 mL, 1 × 2 min. (9) Washing with DCM, 2 mL, 4 × 20 s. (10) A few beads were taken out for a Kaiser test. Step 6 was repeated in case of a positive Kaiser test. (11) Blocking (*capping*) of unreacted amino groups by acetylation with a 25 mL mixture of Ac_2O/pyridine/DCM (1:1:2, v/v/v) for 5 min (except after the last cycle). This step was only performed prior to the incorporation of Boc-G(Bn)-OH monomers. (12) Washing with DCM, 2 mL, 6 × 1 min. Steps 1–12 were repeated until the desired sequence was obtained.

Stepwise Assembly of H-GTA GAT CAC T-Lys-NH_2.[15] The protected PNA was assembled on a Boc-Lys(ClCbz) modified MBHA resin (ClCbz = 2-chlorobenzyloxycarbonyl) with a substitution of approximately 0.30 mmol/g (determined by a quantitative ninhydrin reaction). The synthesis was initiated on 150.4 mg (dry weight) of Boc-Lys(ClCbz)-MBHA resin. The incorporation of the monomers followed the general protocol [DIPCDI (24 μL), Boc-C(Cbz)-OH (75.5 mg), Boc-G(Bn)-OH (74.9 mg), and Boc-T-OH (57.7 mg) in 1.5 mL of DCM/DMF (1:1, v/v)]. However, for the incorporation of Boc-A(Cbz)-OH step 6 was modified: (6) Addition of 4 equiv of DIPCDI (24 μL, 0.15 mmol) and 4 equiv of Boc-A(Cbz)-OH (79.1 mg, 0.15 mmol) dissolved in 1.5 mL of DCM/DMF (1:1, v/v). The coupling reaction was allowed to proceed for 1 × 15 min and 1 × 60 min (recoupling). Coupling reactions were monitored by Kaiser tests all of which were negative (straw-yellow color with no coloration of the beads, indicating nearly 100% coupling yield) except for G-7 (double and triple coupling overnight) and T-9 (double coupling overnight). The PNA-oligomer was cleaved from the support with HF (2.5% thioanisole) for 1 h at 0 °C. The PNA was purified by preparative reverse phase HPLC: yield 38.2 mg (crude), 21.0 mg (purified to >95% pure according to HPLC at 215 nm); MS (FAB) m/z 2854.29 (M + H)$^+$ (calcd 2854.19).

Stepwise Assembly of H-AGT GAT CTA C-Lys-NH_2.[15] This PNA was assembled analogously to H-GTA GAT CAC

(24) Sarin, V. K.; Kent, S. B. H.; Tam, J. P.; Merrifield, R. B. *Anal. Biochem.* **1981**, *117*, 147.

T-Lys-NH_2 (*vide supra*). The synthesis was initiated on 138.5 mg (dry weight) of Boc-Lys(ClCbz)-MBHA resin. All Kaiser tests were negative except for A-6 (double coupling overnight) and A-10 (double coupling overnight): yield 30.8 mg (crude: purity > 80%); MS (FAB) m/z 2854.38 $(M + H)^+$ (calcd 2854.19).

Stepwise Assembly of H-TGT ACG TCA CAA CTA-NH_2.[15] The synthesis was initiated on 200 mg (dry weight) of Boc-A(Cbz) modified MBHA resin with a substitution of approximately 0.30 mmol/g (determined by a quantitative ninhydrin reaction). The incorporation of the monomers followed the general protocol [DIPCDI (18.9 μL), Boc-C(Cbz)-OH (60.4 mg), Boc-G(Bn)-OH (63.3 mg), and Boc-T-OH (46.1 mg) in 1.2 mL of DCM/DMF]. However, for the incorporation of Boc-A(Cbz)-OH step 6 was modified: (6) Addition of 2 equiv of DIPCDI (18.9 μL, 0.12 mmol) and 2 equiv of Boc-A(Cbz)-OH (63.3 mg, 0.12 mmol) dissolved in 1.5 mL of DCM/DMF (1:1, v/v). The coupling reaction was allowed to proceed for 1 × 15 min and 1 × 60 min (recoupling). Coupling reactions were monitored by Kaiser tests and were allowed to proceed for 2–12 h. The PNA-oligomer was cleaved from the support with HF (2.5% thioanisole) for 1 h at 0 °C: yield 41.4 mg (crude: purity > 80%); MS (FAB) m/z 4044.8 $(M + H)^+$ (calcd 4044.6).

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Supplementary Material Available: Copies of ^1H and ^{13}C NMR spectra of **12a–d**, HPLC diagrams of the crude and purified PNA-oligomers, and MS (FAB) spectra thereof (15 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.