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PAPER

Synthesis and oligomerization of Fmoc/Boc-protected PNA monomers of 2,6-diaminopurine, 2-aminopurine and thymine†

André H. St. Amant and Robert H. E. Hudson*

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A Boc-protecting group strategy for Fmoc-based PNA (peptide nucleic acid) oligomerization has been developed for thymine, 2,6-diaminopurine (**DAP**) and 2-aminopurine (**2AP**). The monomers may be used interchangeably with standard Fmoc PNA monomers. The **DAP** monomer was incorporated into a PNA and was found to selectively bind to **T** ($\Delta T_m \geq +6$ °C) in a complementary DNA strand. The **2AP** monomer showed excellent discrimination of **T** ($\Delta T_m \geq +12$ °C) over the other nucleobases. **2AP** also acted as a fluorescent probe of the PNA:DNA duplexes and displayed fluorescence quenching dependent on the opposite base.

Introduction

Peptide nucleic acid (PNA) is a nucleic acid analogue containing an *N*-(2-aminoethyl)glycine backbone.[1] It has the ability to form complementary base-pairing and has been investigated for diagnostic and therapeutic properties.[2] The automated synthesis of PNA began with the Boc/Cbz‡ protecting group strategy,[3] which was superseded by Fmoc/Bhoc‡ for the milder conditions and the lipophilic handle that the terminal Fmoc provides.[4] Recently, a Fmoc/Boc strategy has been investigated by our laboratory,[5] which provides several useful features such as: additional levels of orthogonality as the Bhoc protecting group is sensitive to hydrogenation while Boc is not; the per-Boc nucleobases have excellent solubility in a range of organic solvents; decreased self-aggregation due to a lack of H-bond donors; and compatibility with Fmoc/Bhoc monomers. The synthesis of guanine, adenine, and cytosine Fmoc/Boc protected PNA monomers has been reported,[5] but the synthesis of the corresponding protected thymine (**T**) was not undertaken at that time. Traditionally thymine has been left unprotected in PNA synthesis, but there are reports of low coupling for poly-**T** tracts that has been ascribed to on-resin aggregation.[6] As an alternative to the allyl ether-based protection of thymine, that has already been demonstrated,[6] we have extended the Boc protecting group strategy, which is expected to increase the organic solubility of growing poly-**T** tracts and reduce truncation products caused by self-aggregation.

2,6-Diaminopurine (**DAP**) is an adenine analogue (Fig. 1) that is used for its ability to form three hydrogen bonds to thymine, thus increasing the stability of the duplex.[7] The nucleobase can also be paired with a complementary sequence containing thiouracil to form duplex-invading PNAs.[8] Despite its usefulness, this unusual PNA is not commercially available, and this deficiency has motivated us to develop a straightforward synthesis of the protected monomer.

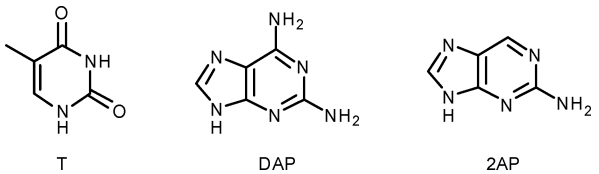


Fig. 1 Structure of the nucleobases used in this study: **T** (thymine), **DAP** (2,6-diaminopurine), and **2AP** (2-aminopurine).

The nucleobase 2-aminopurine (**2AP**) is an adenine analogue (Fig. 1) that possesses environmentally sensitive fluorescence that has been exploited in the study of nucleic acid structures and dynamics. The synthesis of an Fmoc-protected **2AP** PNA monomer with an unprotected exocyclic amine has been reported,[9] but the detailed experimental conditions were not provided. The binding-dependent fluorescence of **2AP** in PNA is of interest to our group and hence spurred the development of the synthesis of an Fmoc/Boc protected PNA monomer.

Results and discussion

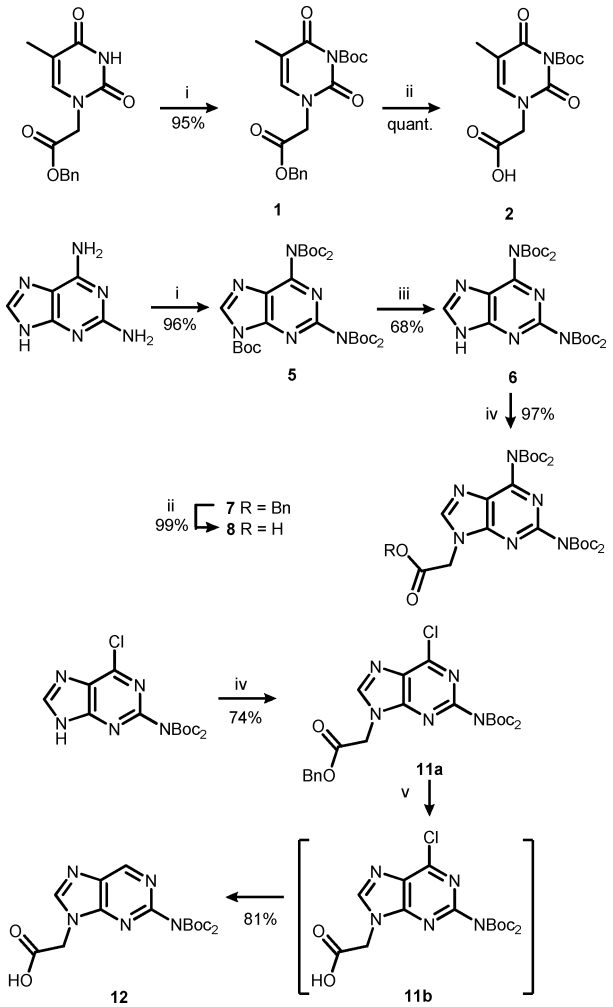
Synthesis of protected PNA monomers

N-Boc-thymin-1-yl acetic acid (**2**) was prepared through the *N*-Boc-protection of benzyl thyminyl-1-acetate[10] (Scheme 1) which furnished **1** in 95% yield under conditions similar to those previously reported by us for the Boc-protection of other nucleobases.[5] Subsequent hydrogenolysis of the benzyl ester using Pd/C produced the acid **2** in quantitative yield. The hydrogenation of the

Department of Chemistry, University of Western Ontario, 1151 Richmond St., London, Canada. E-mail: rhhudson@uwo.ca; Fax: +1-519-661-3022; Tel: +1-519-661-2166

† Electronic supplementary information (ESI) available: ^{1}H and ^{13}C-NMR spectra for compounds **1**–**14** and mass spec for PNAs, melt curves, and fluorescence studies. See DOI: 10.1039/c1ob06582c

‡ Abbreviations: Boc = *tert*-butyloxycarbonyl; Cbz = benzyloxycarbonyl; Fmoc = fluorenyloxycarbonyl; Bhoc = benzhydryloxycarbonyl.



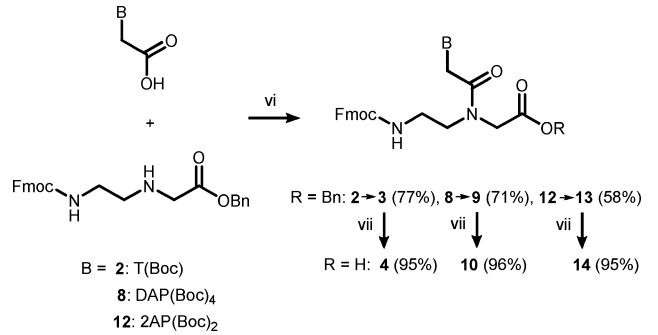
Scheme 1 Reagents and Conditions: (i) Boc_2O, DMAP, THF, rt, 18 h; (ii) H_2, Pd/C, acetone and/or MeOH, rt, 2 h; (iii) $NaHCO_3$, MeOH, 50 °C 1 h then rt, 18 h; (iv) benzyl bromoacetate, K_2CO_3, DMF, rt, 18 h; (v) HCOOH, Et_3N, Pd/C, acetone, reflux, 6 h.

pyrimidine ring for cytosine[11] and the bis-Boc protected cytosine has been noted, but was not observed in this instance for the *N*-protected thymine.

The coupling of **2** with the Fmoc/Bn PNA backbone using EDC/HOBt conditions produced **3** in 77% yield (Scheme 2). The monomer benzyl ester was hydrogenated to yield the PNA monomer **4** in 95% yield, again with no evidence of pyrimidine hydrogenation.

The commercially available 2,6-diaminopurine was penta-Boc protected, under conditions previously established for adenine, to produce **5** in 96% yield. A mono-Boc deprotection[12] was performed on the N^9 position of **5** using $NaHCO_3$ in MeOH to furnish **6** in 68% yield. The N^9 position of **6** was alkylated with benzyl bromoacetate to yield the benzyl ester **7** (97%), which was hydrogenated to acid **8** in high yield (99%) using Pd/C. The coupling of **8** with the Fmoc/Bn PNA backbone was performed in the same manner as the thymine analogue, giving the benzyl ester **9** (71%), which, after hydrogenation, yielded PNA monomer **10** in 96% yield (Scheme 2).

The synthesis of the **2AP** monomer presented much more of a challenge. We explored a variety of synthetic routes to yield



Scheme 2 Reagents and Conditions: (vi) EDC, HOBt, DCM, 0 °C to rt; (vii) H_2, Pd/C, acetone and/or MeOH, rt.

di(Boc)-2-aminopurin-9-yl acetic acid (**12**). Firstly, it was desirable to work with a 6-chloro-2-aminopurine derivative to ensure alkylation at the N^9 position; however, conditions for successful reductive dehalogenation were elusive. Both the benzyl and ethyl esters of 6-chloro-2-aminopurin-9-yl acetate were synthesized, but efficient hydrogenolysis of the chloro substituent was not possible. Use of a Parr apparatus for higher H_2 pressure (50 psi), stoichiometric amounts of Pd/C, and a variety of solvents were examined, but in all cases the 6-chloro group remained intact. Attempts at photochemical dechlorination (xenon lamp, 1 to 24 h) in THF only produced an intractable mixture. A substitution reaction using thiourea to produce the 6-thio derivative was high-yielding, but RANEY® Ni desulfurization was only effective in cases where the reactant was an ester and the nucleobase was unprotected. Subsequent installation of the Boc groups, on the free acid or esters of 2-aminopurin-9-yl acetate proved to be low-yielding.

The most successful pathway to acid **12** was from the known di(Boc)-2-amino-6-chloropurine,[13] which was alkylated at the N^9 position with benzyl bromoacetate to afford **11a** in 74% yield, with the major impurity the N^7 alkylated isomer. The ester **11a** was subjected to a simultaneous benzyl ester and 6-chloro hydrogenolysis with Pd/C in refluxing acetone using HCOOH/Et_3N as a hydrogen transfer agent.[14] The ester was observed to be cleaved much more quickly than the 6-chloro group, producing intermediate **11b**, which can be followed by HPLC. The reaction was continued until the complete disappearance of **11b**, thus yielding **12** in 81% yield (Scheme 1). The acid **12** was coupled with the Fmoc/Bn PNA backbone using EDC/HOBt to form the monomer benzyl ester **13** (58%), which was then hydrogenated using Pd/C to yield the PNA monomer **14** (95%, Scheme 2).

PNA synthesis

PNAs were synthesized on an Applied Biosystems 433A peptide synthesizer using FastMoc chemistry. The syntheses were performed on a 5 μmol scale using 5 equivalents of the monomers. The peptides were cleaved from the resin and isolated/purified according to literature procedures.[15]

The coupling performance of the thymine monomer **4** was indistinguishable from the commercially available **T** PNA monomer. Successful peptide syntheses have been performed using a mixture of **4** and commercial **T** monomer with no deleterious consequences. A peptide with a penta(**T**) tract has been synthesized using **4** with no evidence of premature truncation of the sequence

Table 1 PNA sequences and DNA complements

	Sequence*	T_m ≠ (°C)
PNA1	^{N}C-T-T-T-C-C-T-**DAP**-C-A-C-T-G-T-K^{C}	-
DNA1MMG	$^{3'}$G-A-A-A-G-G-A- G -G-T-G-A-C-A$^{5'}$	49.5
DNA1MMA	$^{3'}$G-A-A-A-G-G-A- A -G-T-G-A-C-A$^{5'}$	50.0
DNA1MMC	$^{3'}$G-A-A-A-G-G-A- C -G-T-G-A-C-A$^{5'}$	56.5
DNA1T	$^{3'}$G-A-A-A-G-G-A- T -G-T-G-A-C-A$^{5'}$	62.5
PNA2	^{N}G-T-T-G-C-**2AP**-C-T-G-G-A-K^{C}	-
DNA2MMG	$^{3'}$C-A-A-C-G- G -G-A-C-C-T$^{5'}$	45.5
DNA2MMA	$^{3'}$C-A-A-C-G- A -G-A-C-C-T$^{5'}$	46.0
DNA2MMC	$^{3'}$C-A-A-C-G- C -G-A-C-C-T$^{5'}$	51.0
DNA2T	$^{3'}$C-A-A-C-G- T -G-A-C-C-T$^{5'}$	62.5

*__DAP__ = 2,6-diaminopurine, __2AP__ = 2-aminopurine, K = *L*-lysine. Mismatched nucleobases (MM) are outlined. ≠ Oligos at 2 μM in a buffer containing 100 mM NaCl, 0.1 mM EDTA, and 10 mM Na_2PO_4 at pH 7.

(data not shown). The **DAP** monomer **9** was incorporated into **PNA1** and the **2AP** monomer **13** into **PNA2** (Table 1).

DNA binding studies

A PNA:DNA binding study was performed using **PNA1** to examine the effect of 2,6-diaminopurine on duplex stability. As expected, the **PNA1**:**DNA1T** duplex exhibited the highest melting temperature (62.5 °C), followed by the pyrimidine mismatch **PNA1**:**DNA1C** melting at 56.5 °C. Due to the increased steric bulk, the purine mismatches **PNA1**:**DNA1G** and **PNA1**:**DNA1A** showed greater decreases in helix stability with T_m values of 49.5 °C and 50 °C, respectively. Overall the **DAP** substitution showed a mismatch selectivity of +6 °C, in this particular sequence context, which is comparable with other **DAP** PNA:DNA studies.[7]

The binding study of **PNA2** with DNA showed a surprising selectivity for **T** of at least +12 °C. This is the first study of the stabilization effect of **2AP** in a PNA:DNA duplex, and demonstrates its promise as a replacement for A.

Fluorescence studies

Using **2AP**, duplex denaturation was also examined by variable-temperature fluorescence studies (Fig. 2). The results are comparable to those obtained by temperature-dependent UV-vis spectroscopy and indicate that the changes in the environment about the fluorescent **2AP** are representative of global melting, for this particular sequence.

The steady-state fluorescence excitation and emission spectra were measured for single stranded **PNA2** and **PNA2**:**DNA2** duplexes (Fig. 3). G has been shown to stack with **2AP**, creating a charge-transfer complex which quenches fluorescence.[16] However, in the **PNA2** sequence there is no adjacent intrastrand G. Rather, when base paired to a G mismatch, the degree of fluorescence is similar to the single strand which implies that **2AP** is extrahelical, a situation observed for this mismatch in DNA:DNA duplexes.[17]

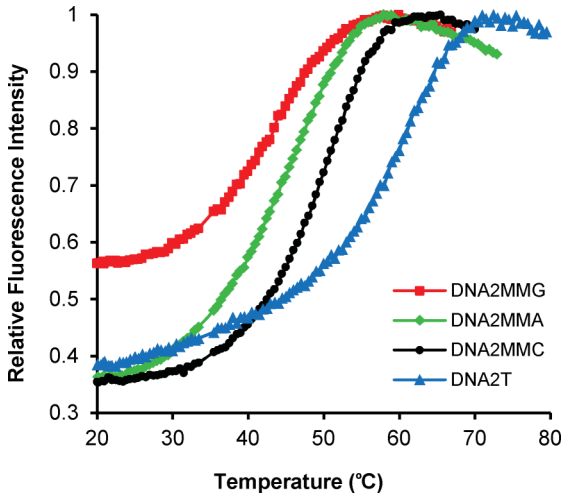


Fig. 2 Temperature-dependent fluorescence spectra for **PNA2** annealed with DNA strands containing a single mismatch, either guanine (MMG, ■), adenine (MMA, ♦), cytosine (MMC, ●) or the matched complementary strand containing thymine (**T**, ▲). Strand concentration is 2 μM in a buffer containing NaCl (100 mM), EDTA (0.1 mM), and Na_2PO_4 (10 mM, pH 7). Fluorescence excitation at λ = 312 nm and emission was measure at λ = 349 nm.

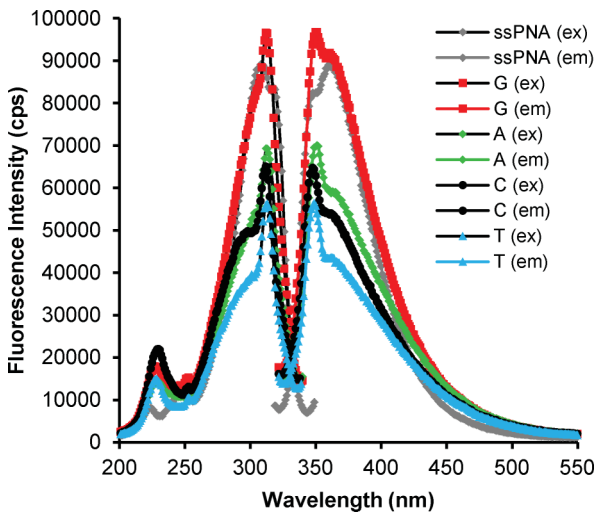


Fig. 3 Fluorescence profile of **PNA2** in single strand form and annealed to **DNA2B** at 2 μM, in a buffer containing NaCl (100 mM), EDTA (0.1 mM), and Na_2PO_4 (10 mM) at pH 7. Excitation spectra (ex) are presented in the range 200–330 nm and emission spectra (em) are presented in the range 330–550 nm.

The degree of fluorescence quenching parallels that of the duplex stability. The most stable duplex is the perfectly matched **PNA2**:**DNA2T**, which exhibits the greatest fluorescence quenching, likely because it forms a stable stacked base pair within the helix. The mismatched duplexes with **2AP** across from C or A likely possess bulged structures with correspondingly less stacking and thus exhibited less fluorescence quenching.

Conclusion

A facile synthesis of Fmoc/Boc protected PNA monomers has been established for thymine, 2,6-diaminopurine and

2-aminopurine, which is compatible with standard Fmoc-based oligomerization chemistry. In conjugation with our previous report,[5] there now exists a complete set of the natural nucleobases and two useful analogues as the Fmoc/Boc monomers. The per-Boc protected nucleobase monomers offers excellent organic solvent solubility and orthogonality to hydrogenolysis that the Bhoc- and Cbz-protected nucleobases do not.

The **T** PNA monomer was found to incorporate into PNAs under standard coupling conditions as well as the commercially available monomer with no evidence of truncation for a $\mathbf{T}_5$ tract. The **DAP** monomer was incorporated into a PNA, and in a binding study with DNA, it was found to selectively bind to **T** (+6 °C). The **2AP** monomer showed excellent discrimination of **T** (+12 °C) over the other nucleobases and also acted as a fluorescent probe of the PNA:DNA duplexes. A fully matched duplex was characterized by the greatest degree of fluorescence quenching whereas mismatches displayed less quenching. The severity of the mismatch, as judged by the degree of helix destabilization determined by the UV-vis measured T_m values (G > A > C), is qualitatively inversely correlated with the degree of quenching (C > A > G). These results strongly suggest that **2AP** is a base discriminating fluorophore in PNA:DNA duplexes, much in the same way as it is in dsDNA, and may find similar uses in the study of PNA:DNA structures and dynamics.

Experimental

General

All chemicals were obtained from commercial sources and were of ACS reagent grade or higher and were used without further purification. Solvents for solution-phase chemistry were dried by passing through columns of activated alumina. Flash column chromatography (FCC) was performed on Merck Kieselgel 60, 230–400 mesh. Thin layer chromatography (TLC) was performed on Merck Kieselgel 60 TLC plates. Chemical shifts (δ) are reported in parts per million (ppm), were measured from tetramethylsilane (0 ppm) and are referenced to the solvent $CDCl_3$ (7.26 ppm), DMSO-d_6 (2.49 ppm), D_2O (4.79 ppm) for ^{1}H NMR and $CDCl_3$ (77.0 ppm), DMSO-d_6 (39.5 ppm) for ^{13}C NMR. Multiplicities are described as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) and br s (broad singlet). Coupling constants (J) are reported in Hertz (Hz). Resonances due to restricted rotation around the amide bond (rotamers) are reported as major (ma.) and minor (mi.). High resolution mass spectra (HRMS) were obtained using electron impact (EI) or electrospray ionization (ESI).

Benzyl N^3-Boc-thymin-1-yl acetate (1). Benzyl thyminyl-1-acetate (3.0 g, 11 mmol), Boc_2O (4.8 g, 22 mmol), and DMAP (2.7 g, 22 mmol) were added to THF (30 mL) at 0 °C. After 0.5 h the solution was warmed to room temperature. After 18 h, the reaction mixture was concentrated *in vacuo*. The residue was dissolved in DCM (100 mL) and extracted with H_2O (3 × 100 mL). The organic layer was dried over anhydrous Na_2SO_4 and the solvent removed. The residue was purified by FCC (EtOAc : hexane = 2 : 3, v/v) to give **1** (3.9 g, 95% yield) as a light-yellow foam: ^{1}H NMR (400 MHz, $CDCl_3$) = 7.30–7.41 (m, 5 H), 6.90 (s, 1 H), 5.20 (s, 2 H), 4.47 (s, 2 H), 1.92 (s, 3 H), 1.56–1.62 (m, 9 H);^{13}C NMR (101 MHz, $CDCl_3$) = 167.1, 161.4, 148.8, 147.6, 139.7, 134.6, 128.5, 128.4, 128.2, 110.5, 86.6, 67.5, 48.6, 27.2, 12.1; HRMS (EI) calculated for $[C_{19}H_{22}N_2O_6]^+$: 374.1478, found 374.1484.

N^3-Boc-thyminyl-1-acetic acid (2). **1** (0.75 g, 2.0 mmol) was dissolved in minimal acetone : MeOH solution (1 : 1, v/v). Pd/C (80 mg) was added and the reaction flask was placed under an atmosphere of H_2. The suspension was stirred at room temperature for 2 h. The solvent was removed and the residue suspended in MeOH (100 mL). The suspension was filtered through Celite, and the solvent removed to yield **2** (0.57 g, quant. yield) as a glassy white solid: ^{1}H NMR (400 MHz, DMSO-d_6) = 7.66 (s, 1 H), 4.43 (s, 2 H), 1.81 (s, 3 H), 1.51 (s, 9 H); ^{13}C NMR (101 MHz, DMSO-d_6) = 169.8, 164.5, 151.1, 142.0, 141.8, 108.4, 49.1, 48.7, 27.7, 12.0; HRMS (EI) calculated for $[C_{11}H_{16}N_2O_6]^+$: 284.1008, found 284.1021.

N^3-Boc-thyminyl-1-acetate Fmoc/Bn PNA backbone (3). Fmoc/Bn PNA backbone (0.927 g, 2.13 mmol) and **2** (0.759 g, 2.67 mmol) were dissolved in DCM (10 mL) and cooled to 0 °C. EDC (0.497 g, 3.20 mmol) and HOBt hydrate (0.081 g, 0.53 mmol) were added. The solution was stirred for 0.5 h then allowed to warm to room temperature. After 18 h a saturated $NaHCO_3$ solution (100 mL) was added and extracted with DCM (2 × 100 mL). The organic layers were dried over Na_2SO_4 and the solvent removed. The residue was purified by FCC (EtOAc : hexane = 8 : 2 to 9 : 1) to yield **3** (1.14 g, 77% yield) as a white foam: ^{1}H NMR (400 MHz, DMSO-d_6) = 7.88 (d, J = 7.4 Hz, 2 H), 7.67 (d, J = 7.8 Hz, 2 H), 7.26–7.48 (m, 10 H), 5.21 (s, min.), 5.13 (s, maj.), 4.76 (s, 2 H), 4.58 (s, min.), 4.10–4.40 (m, 5 H), 2.99–3.61 (m, 5 H), 1.79 (s, 3 H), 1.48 (s, 9 H); ^{13}C NMR (101 MHz, $CDCl_3$) = 170.7, 169.0, 168.8, 167.2, 166.9, 161.4, 161.3, 156.4, 156.3, 148.7, 148.6, 147.7, 143.5, 143.4, 140.8, 140.5, 140.3, 134.7, 134.4, 128.2, 128.1, 128.1, 127.9, 127.8, 127.4, 127.3, 126.7, 124.7, 119.6, 109.7, 109.5, 86.3, 86.2, 67.4, 66.8, 66.4, 66.2, 60.0, 49.8, 48.5, 48.2, 47.9, 47.6, 47.5, 46.7, 38.8, 38.4, 27.0, 20.6, 13.8, 11.9; HRMS (EI) calculated for $[C_{38}H_{40}N_4O_9Na]^+$: 719.2693, found 719.2693.

N^3-Boc-thymin-1-yl acetate Fmoc PNA monomer (4). **3** (0.251 g, 0.36 mmol) was dissolved in the minimal amount of an acetone : MeOH solution (1 : 1, v/v). Pd/C (80 mg) was added and the reaction flask was placed under an atmosphere of H_2 at 0 °C. After 0.5 h the suspension was stirred at room temperature for a further 2 h. The solvent was removed and the residue suspended in MeOH (100 mL). The suspension was filtered through Celite, and the solvent removed to yield **4** (0.212 g, 95% yield) as an off-white powder: ^{1}H NMR (400 MHz, DMSO-d_6) = 12.79 (s, 1 H), 7.89 (d, J = 7.4 Hz, 2 H), 7.68 (d, J = 7.4 Hz, 2 H), 7.10–7.52 (m, 5 H), 4.74 (s, 1 H), 4.55 (s, 1 H), 4.14–4.39 (m, 3 H), 4.00 (s, 1 H), 3.19–3.47 (m, 5 H), 3.03–3.17 (m, 1 H), 1.79 (s, 3 H), 1.49 (s, 9 H);^{13}C NMR (151 MHz, DMSO-d_6) = 171.0, 170.6, 167.8, 167.4, 166.8, 164.5, 161.4, 156.5, 156.3, 151.1, 148.6, 148.1, 144.0, 142.1, 140.8, 127.7, 127.2, 125.2, 120.2, 108.3, 86.2, 65.7, 47.9, 47.1, 46.9, 31.4, 27.1, 12.0; HRMS (ESI) calculated for $[C_{31}H_{34}N_4O_9Na]^+$: 629.2223, found 629.2252.

Penta(Boc)-2,6-diaminopurine (5). 2,6-Diaminopurine (0.500 g, 3.33 mmol), Boc_2O (6.12 g, 26.6 mmol), and DMAP (0.061 g, 0.50 mmol) were added to THF (25 mL) at 0 °C. The solution was stirred for 5 min then warmed to room temperature. After 18 h the solvent was removed. The residue was dissolved in DCM (150 mL) and washed with H_2O (100 mL). The organic layer was

dried over Na_2SO_4 and the solvent was removed. The residue was purified by FCC (EtOAc : hexane = 1 : 2) to yield **5** (2.08 g, 96% yield) as a white foam: ^{1}H NMR (400 MHz, $CDCl_3$) = 8.52 (s, 1 H), 1.68 (s, 9 H), 1.43 (s, 18 H), 1.41 (s, 18 H); ^{13}C NMR (101 MHz, $CDCl_3$) = 153.7, 152.9, 151.6, 150.6, 149.7, 145.5, 143.8, 127.8, 87.3, 83.9, 83.2, 41.9, 27.8, 27.7, 27.6; HRMS (EI) calculated for $[C_{30}H_{46}N_6O_{10}]^+$: 650.3275, found 650.3262.

Tetra(Boc)-9*H*-2,6-diaminopurine (6). **5** (1.93 g, 2.97 mmol) was suspended in mixture of MeOH (50 mL) and saturated $NaHCO_3$ solution (25 mL). The turbid solution was heated to 50 °C for 1 h and then cooled to room temperature. After 18 h the MeOH was removed by rotary evaporation and the solution was diluted with H_2O (100 mL) and extracted with DCM (2 × 100 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was removed to yield **6** (1.12 g, 68% yield) as a white foam: ^{1}H NMR (400 MHz, $CDCl_3$) = 8.63 (s, 1 H), 5.05 (br s, 2 H), 1.36 (s, 18 H), 1.33 (s, 18 H);^{13}C NMR (101 MHz, $CDCl_3$) = 151.4, 150.8, 149.9, 145.3, 109.9, 84.3, 83.4, 27.7, 27.6; HRMS (EI) calculated for $[C_{25}H_{38}N_6O_8]^+$: 550.2751, found 550.2757.

Benzyl tetra(Boc)-2,6-diaminopurin-9-yl acetate (7). **6** (0.500 g, 0.908 mmol) and K_2CO_3 (0.138 g, 0.999 mmol) were added to DMF (3 mL) at 0 °C. Benzyl bromoacetate (0.16 mL, 1.0 mmol) was added dropwise over 5 min. The solution was stirred for 10 min then warmed to room temperature. After 18 h the reaction mixture was diluted with H_2O (100 mL) and extracted with ether (2 × 100 mL). The organic layers were combined, washed with water (50 mL), dried over Na_2SO_4, and the solvent removed to yield **6** (0.616 g, 97% yield) as a white foam: ^{1}H NMR (400 MHz, $CDCl_3$) = 8.14 (s, 1 H), 7.31–7.40 (m, 5 H), 5.21 (s, 2 H), 5.05 (s, 2 H), 1.40 (s, 18 H), 1.39 (s, 18 H); ^{13}C NMR (101 MHz, $CDCl_3$) = 176.0, 167.0, 154.2, 152.2, 151.0, 150.6, 149.8, 145.8, 128.7, 128.4, 126.7, 83.7, 83.2, 67.9, 52.9, 44.3, 44.1, 27.7, 27.6; HRMS (ESI) calculated for $[C_{34}H_{47}N_6O_{10}]^+$: 699.3348, found 699.2743.

Tetra(Boc)-2,6-diaminopurin-9-yl acetic acid (8). **7** (0.509 g, 0.728 mmol) was dissolved in MeOH (5 mL). Pd/C (60 mg) was added and the reaction vessel was placed under an atmosphere of H_2. The suspension was stirred for 2 h at room temperature. The suspension was filtered through Celite with MeOH (100 mL) and the solvent removed to yield **8** (0.439 g, 99% yield) as a glassy grey solid: ^{1}H NMR (400 MHz, DMSO-d_6) = 8.63 (s, 1 H), 5.05 (br s, 2 H), 1.35 (s, 18 H), 1.33 (s, 18 H); ^{13}C NMR (101 MHz, MeOH-d_4) = 200.7, 156.1, 153.2, 152.4, 151.5, 150.0, 85.6, 85.1, 28.2, 28.1; HRMS (ESI) calculated for $[C_{27}H_{41}N_6O_{10}]^+$: 609.2879, found 609.2688.

Tetra(Boc)-2,6-diaminopurin-9-yl acetate Fmoc/Bn PNA backbone (9). Fmoc/Bn PNA backbone (0.061 g, 0.180 mmol), **8** (0.164 g, 0.270 mmol), EDC (0.056 g, 0.36 mmol) and HOBt hydrate (0.006 g, 0.04 mmol) were added to DCM (5 mL) at 0 °C. The solution stirred for 20 min then warmed to room temperature. The reaction was monitored by TLC. Upon completion, the reaction mixture was diluted with DCM (100 mL) and washed against a mixture of a saturated $NaHCO_3$ solution (25 mL) and brine (50 mL). The organic layer was dried over Na_2SO_4, and the solvent removed. The residue was subjected to FCC (EtOAc : hexane = 2 : 1 to 1 : 0) to yield **9** (0.131 g, 71% yield) as a white powder: ^{1}H NMR (400 MHz, DMSO-d_6) = 8.49 (s, min.), 8.47 (s, maj.), 7.83–7.89 (m, 2 H), 7.66 (t, J = 6.3 Hz, 2 H), 7.25–7.49 (m, 9 H), 5.41 (br s, 1 H), 5.23 (br s, 2 H), 5.10 (s, 1 H), 4.50 (s, 1 H), 4.36 (d, J = 7.0 Hz, 1 H), 4.13–4.32 (m, 3 H), 3.50–3.60 (m, 1 H), 3.06–3.16 (m, 1 H), 1.28–1.37 (m, 36 H);^{13}C NMR (101 MHz, $CDCl_3$) = 154.6, 152.2, 151.1, 151.1, 150.2, 147.1, 144.0, 143.8, 141.5, 129.2, 129.1, 128.9, 128.8, 128.5, 128.0, 128.0, 127.3, 125.3, 125.1, 120.3, 84.0, 83.6, 67.6, 66.7, 49.4, 49.2, 47.4, 31.8, 28.0, 27.9, 22.9, 14.4; HRMS (ESI) calculated for $[C_{53}H_{65}N_8O_{13}]^+$: 1021.4671, found 1021.4711.

Tetra(Boc)-2,6-diaminopurin-9-yl acetate Fmoc PNA monomer (10). **9** (0.796 g, 0.78 mmol) was dissolved in MeOH (20 mL) and Pd/C (50 mg) was added. The reaction vessel was placed under an atmosphere of H_2. The suspension was stirred for 3 h at room temperature. The reaction was filtered through Celite with MeOH (100 mL) and the solvent removed to yield **10** (0.726 g, 96% yield) as a flaky white solid: ^{1}H NMR (400 MHz, acetone-d_6) = 8.31 (s, 1 H), 7.75–7.83 (m, 2 H), 7.63 (d, J = 7.4 Hz, 2 H), 7.22–7.39 (m, 4 H), 5.38 (s, 1 H), 5.26 (s, 1 H), 4.34–4.44 (m, 2 H), 4.09–4.29 (m, 3 H), 3.69 (t, J = 6.3 Hz, 1 H), 3.43–3.55 (m, 2 H), 3.25–3.33 (m, 1 H), 1.30–1.42 (m, 36 H); ^{13}C NMR (101 MHz, acetone-d6) = 171.0, 167.9, 167.3, 157.5, 155.8, 152.8, 151.7, 151.2, 151.0, 148.9, 145.1, 142.1, 128.6, 128.0, 128.0, 127.3, 126.2, 126.1, 120.9, 84.0, 83.5, 67.0, 48.1, 45.3, 40.0, 28.0, 27.9; HRMS (ESI) calculated for $[C_{46}H_{59}N_5O_{13}]^+$: 931.4196, found 931.3406.

Benzyl 6-chloro-2-diBoc-aminopurin-9-yl acetate (11a). 6-Chloro-2-diBoc-aminopurine (1.28 g, 3.45 mmol) and K_2CO_3 (0.524 g, 3.79 mmol) were suspended in DMF (15 mL) and cooled to 0 °C. Benzyl bromoacetate (0.60 mL, 3.8 mmol) was added dropwise over 10 min. The solution was stirred for 5 min and then warmed to room temperature. After 18 h the suspension was filtered through Celite with EtOAc (100 mL) then concentrated *in vacuo*. The oil obtained was suspended in H_2O (100 mL) and extracted with Et_2O (2 × 200 mL). The organic layers were combined, dried over Na_2SO_4 and the solvent removed. The residue was purified by FCC (EtOAc : Hex = 1 : 1) to yield **11** (1.32 g, 74% yield) as a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) = 8.19 (s, 1 H), 7.31–7.41 (m, 5 H), 5.22 (s, 2 H), 5.05 (s, 2 H), 1.42 (s, 18 H); ^{13}C NMR (101 MHz, $CDCl_3$) = 166.2, 152.7, 152.0, 151.1, 150.3, 146.4, 134.2, 129.4, 128.8, 128.6, 128.5, 83.6, 68.1, 44.4, 27.7; HRMS (ESI) calculated for $[C_{24}H_{29}ClN_5O_6]^+$: 518.1801, found 518.1806.

2-Di(Boc)-aminopurin-9-yl acetic acid (12). **11a** (0.304 g, 0.587 mmol) was dissolved in a mixture of acetone (4 mL), Et_3N (1.4 mL), and formic acid (0.2 mL). Pd/C (40 mg) was added and the reaction mixture was brought to reflux. The two products formed were tracked by HPLC. Two further equivalents of Pd/C (40 mg), Et_3N (1.4 mL), and formic acid (0.2 mL) were added after 2 h and 4 h. When only 1 product remained by HPLC, the mixture was filtered through Celite with acetone (100 mL) and the solvent removed. The residue was suspended in H_2O (25 mL) and extracted with DCM (3 × 50 mL). The organic fractions were combined and washed with a 1 M $KHSO_4$ solution (50 mL). The organic layer was dried over Na_2SO_4 and the solvent removed to yield **12** (0.186 g, 81% yield) as a glassy white solid: ^{1}H NMR (400 MHz, MeOH-d_4) = 9.10 (s, 1 H), 8.57 (s, 1 H), 5.17 (s, 2 H), 1.39 (s, 18 H); ^{13}C NMR (101 MHz, MeOH-d_4) = 170.0, 154.4, 153.9, 152.2, 150.3, 149.8, 133.3, 85.0, 45.3, 28.2; HRMS (ESI) calculated for $[C_{17}H_{24}N_5O_6]^+$: 394.1721, found 394.1727.

2-Di(Boc)-aminopurin-9-yl acetate Fmoc/Bn PNA (13). Fmoc/Bn PNA backbone (0.276 g, 0.81 mmol), **12** (0.264 g, 0.67 mmol), EDC (0.155 g, 1.00 mmol) and HOBt hydrate (0.026 g, 0.17 mmol) were added to DCM (5 mL) at 0 °C. The solution stirred for 40 min and then warmed to room temperature. After 2 days the reaction was diluted with DCM (150 mL), washed with a saturated $NaHCO_3$ solution (100 mL), H_2O (100 mL) and brine (100 mL). The organic layer was dried over Na_2SO_4 and the solvent removed. The residue was subjected to FCC (EtOAc : Hex = 4 : 1 to 1 : 0) to yield **13** (0.313 g, 58% yield) as a white foam: ^{1}H NMR (400 MHz, DMSO-d_6) = 9.17 (s, 1 H), 8.47 (d, 1 H), 7.87 (d, J = 7.2 Hz, 2 H), 7.66 (t, J = 7.5 Hz, 2 H), 7.22–7.52 (m, 9 H), 5.40 (br s, 1 H), 5.23 (br s, 1 H), 5.09 (s, 1 H), 4.50 (br s, 1 H), 4.10–4.40 (m, 4 H), 3.51–3.60 (m, 1 H), 3.07–3.15 (m, 1 H), 1.31 (s, 18 H); ^{13}C NMR (101 MHz, $CDCl_3$) = 169.3, 156.6, 152.7, 151.0, 149.3, 147.4, 146.4, 143.7, 143.5, 141.2, 128.8, 128.6, 128.6, 128.2, 127.7, 127.7, 127.0, 124.8, 120.0, 109.9, 83.3, 67.4, 49.2, 47.1, 29.6, 27.8, 27.3; HRMS (ESI) calculated for $[C_{43}H_{48}N_7O_9]^+$: 806.3514, found 806.3529.

2-Di(Boc)-aminopurin-9-yl acetate Fmoc PNA monomer (14). **13** (0.313 g, 0.389 mmol) was dissolved in MeOH (10 mL) and Pd/C (100 mg) was added. The reaction mixture was placed under an atmosphere of H_2. The suspension was stirred for 12 h at room temperature. The suspension was filtered through Celite with MeOH (100 mL) and the solvent removed to yield **14** (0.266 g, 95% yield) as an off-white solid: ^{1}H NMR (400 MHz, $CDCl_3$) = 8.99–9.21 (m, 2 H), 8.23–8.39 (m, maj.), 8.10 (br s, min.), 7.62–7.81 (m, 2 H), 7.44–7.62 (m, 2 H), 7.11–7.41 (m, 4 H), 6.83 (br s, maj.), 6.05 (br s, min.), 4.96–5.16 (m, 1 H), 4.85 (br s, 1 H), 4.29–4.42 (m, 1 H), 4.11–4.27 (m, 2 H), 4.01 (br s, 1 H), 3.77 (br s, 1 H), 3.51 (br s, 2 H), 3.26–3.42 (m, 1 H), 2.74 (br s, 2 H), 1.44 (s, 18 H); ^{13}C NMR (400 MHz, $CDCl_3$) = 171.6, 166.9, 166.3, 156.9, 152.7, 151.0, 148.9, 148.3, 143.6, 141.2, 131.4, 127.7, 127.1, 125.0, 124.3, 120.0, 83.6, 66.7, 50.6, 48.9, 47.1, 39.3, 27.8; HRMS (ESI) calculated for $[C_{36}H_{41}N_7O_9Na]^+$: 738.2863, found 738.2877.

Melting temperature (T_m) measurements

All T_m measurements of the PNA:DNA duplexes (2 μM concentration) were made in a 10 mM sodium phosphate buffer (pH 7.0) containing 100 mM NaCl and 0.1 mM EDTA. Absorbance *vs.* temperature profiles were measured using a Cary 300-Bio spectrophotometer using a cell with a 1 cm path length. The absorbance of the samples was monitored at 260 nm from 15 °C to 85 °C, at a rate of 0.5 °C min^{-1}.

Notes and References

1 P. Nielsen, M. Egholm, R. Berg and O. Buchardt, *Science*, 1991, **254**, 1497–1500.
2 E. Uhlmann, A. Peyman, G. Breipohl and D. W. Will, *Angew. Chem., Int. Ed.*, 1998, **37**, 2796–2823.
3 K. L. Dueholm, M. Egholm, C. Behrens, L. Christensen, H. F. Hansen, T. Vulpius, K. H. Petersen, R. H. Berg, P. E. Nielsen and O. Buchardt, *J. Org. Chem.*, 1994, **59**, 5767–5773.
4 S. A. Thomson, J. A. Josey, R. Cadilla, M. D. Gaul, C. Fred Hassman, M. J. Luzzio, A. J. Pipe, K. L. Reed, D. J. Ricca, R. W. Wiethe and S. A. Noble, *Tetrahedron*, 1995, **51**, 6179–6194.
5 F. Wojciechowski and R. H. E. Hudson, *J. Org. Chem.*, 2008, **73**, 3807–3816.
6 F. Altenbrunn and O. Seitz, *Org. Biomol. Chem.*, 2008, **6**, 2493–2498.
7 G. Haaima, H. F. Hansen, L. Christensen, O. Dahl and P. E. Nielsen, *Nucleic Acids Res.*, 1997, **25**, 4639–4643.
8 J. Lohse, O. Dahl and P. E. Nielsen, *Proc. Natl. Acad. Sci. U. S. A.*, 1999, **96**, 11804–11808.
9 B. P. Gangamani and V. A. Kumar, *Chem. Commun.*, 1997, 1913–1914.
10 S. Breeger, M. von Meltzer, U. Hennecke and T. Carell, *Chem.–Eur. J.*, 2006, **12**, 6469–6477.
11 X. Zhang, H. Xia, X. Dong, J. Jin, W.-D. Meng and F.-L. Qing, *J. Org. Chem.*, 2003, **68**, 9026–9033.
12 S. Dey and P. Garner, *J. Org. Chem.*, 2000, **65**, 7697–7699.
13 A. Porcheddu, G. Giacomelli, I. Piredda, M. Carta and G. Nieddu, *Eur. J. Org. Chem.*, 2008, 5786–5797.
14 A. Štimac and J. Kobe, *Synthesis*, 1990, **461**, 464.
15 R. H. Hudson, Y. Liu and F. Wojciechowski, *Can. J. Chem.*, 2007, **85**, 302–312.
16 M. A. O'Neill, C. Dohno and J. K. Barton, *J. Am. Chem. Soc.*, 2004, **126**, 1316–1317.
17 C. R. Guest, R. A. Hochstrasser, L. C. Sowers and D. P. Millar, *Biochemistry*, 1991, **30**, 3271–3279.