Design, synthesis and DNA/RNA binding studies of nucleic acids comprising stereoregular and acyclic polycarbamate backbone: polycarbamate nucleic acids (PCNA)†

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The designed, chiral, acyclic polycarbamate nucleic acids (PCNA) exhibited sequence and orientation specific binding to nucleic acids. Complexes of PCNA with DNA were as stable as PNA:DNA complexes and those with RNA were as stable as natural DNA:RNA complexes.

Introduction

Complete replacement of sugar-phosphate backbone by achiral, acyclic and uncharged scaffold in aminoethylglycyl peptide nucleic acids (aegPNA) has been extensively studied in the last two decades since its discovery in 1991.[1] The strong and sequence specific binding to both DNA and RNA along with its unique strand invasion properties has proved to be extremely beneficial to both chemists and biologists working towards the development of ultimate antisense oligonucleotides.[2] Extensive work has been done to improve the properties of PNA and a large number of structurally interesting PNA analogues have been synthesized over the last decade. The balance between rigidity and flexibility has been achieved by having an extended backbone with constrained ring structures[3] but so far no PNA analogue has been found to be better than PNA itself from an applications perspective.

The carbamate linkages were earlier utilized to form DNA mimics by a few select groups as early as in 1974[4] but were not further exploited in the development of antisense oligomers. The carbamate linker being shorter than the phosphate linker probably destabilized the complexes with DNA or RNA. To ease the constraint of the linker group, acyclic carbamate linked dimer blocks were synthesized from sugar precursors and were incorporated into oligomers replacing a dimer sugar-phosphate block. This led to large destabilization of the duplexes probably because of conformational freedom of the modified backbone in an otherwise unmodified DNA.[5] The cytosine-carbamate hexamer joining morpholino-nucleosides formed highly stable complexes with DNA but failed to recognize complementary RNA sequences.[6] We, in our laboratory, synthesized pyrrolidinyl carbamate oligonucleotides, but too much flexibility in the linker group led to destabilization of the complexes.[7] In this paper we introduce a fresh approach to make the DNA mimics with an uncharged polycarbamate backbone which is structurally closely related to PNA. The chiral centre corresponds to C-4′ in natural nucleic acids and the individual nucleobases are attached through a secondary amide linker to 2,3-diamino-1-propanol. The linking of individual monomers is *via* a carbamate linkage to form polycarbamate nucleic acids (PCNA) (Fig. 1).

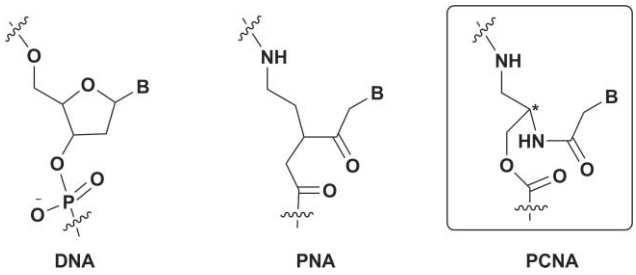

Fig. 1 Proposed PCNA compared with DNA and PNA structures.

The simple variation in the structure of PNA leading to PCNA allows the presence of a chiral center in the monomer unit and conserves six atom repeating backbone. A secondary amide function to attach the nucleobase may circumvent the likelihood of *cis*/*trans* isomers present in tertiary amide linker groups in PNA. This may render the present backbone conformationally more flexible than PNA. The additional hydrogen bonding sites in the PCNA backbone might render it more water soluble as compared with PNA. The hydroxy group is activated as a *p*-nitrophenyl carbonate and the oligomerization may be effected by the formation of carbamate linker groups.

The synthesis of thymine, cytosine, guanine and adenine containing activated monomer blocks is accomplished from naturally occurring chiral amino acid, L-serine, that would yield stereoregular polypyrimidine and mixed purine/pyrimidine PCNA sequences using solid phase synthesis methodology. We also present our results of their binding studies to complementary DNA/RNA sequences.

Results and Discussion

Synthesis of monomer units

The simple organic transformations to get the desired monomers are outlined in Scheme 1. The free hydroxy group in protected L-serine derivative **1** was converted to TBDMS ether. The reduction of the ester group using $NaBH_4$ in MeOH gave alcohol **2**. The alcohol **2** was then converted into azide *via* mesylate followed

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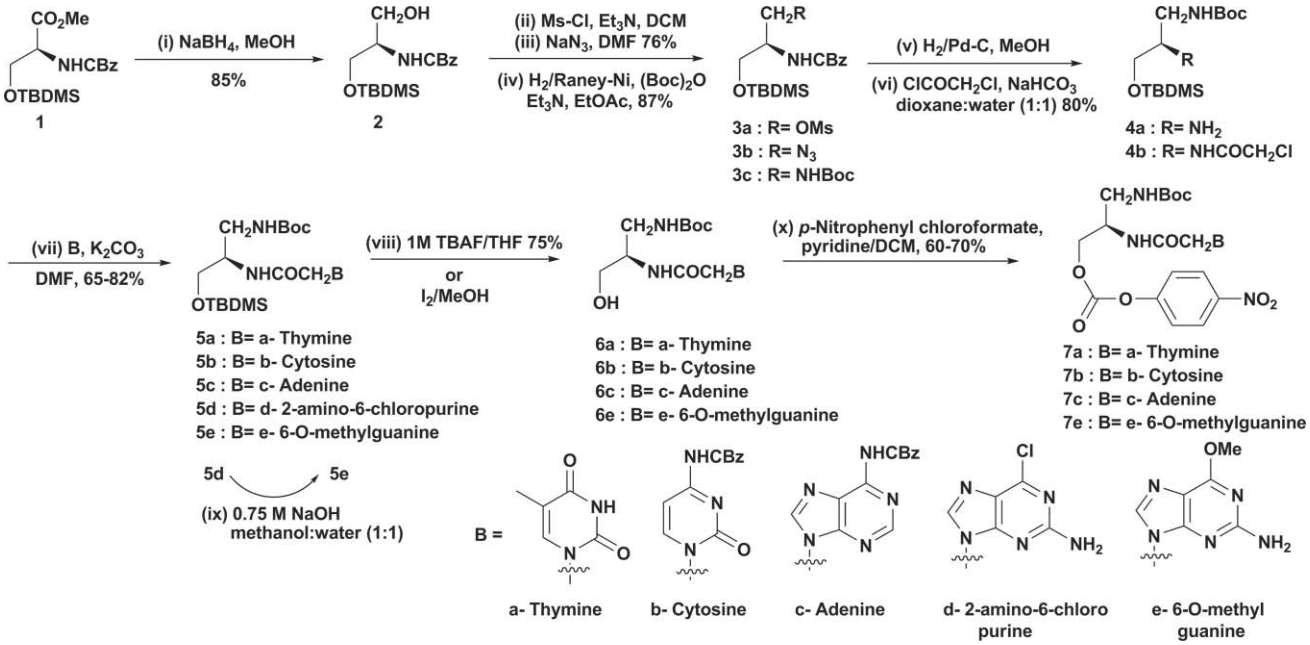
† Electronic supplementary information (ESI) available: ^1H, ^{13}C NMR and mass spectra data for all new compounds, HPLC and MALDI-TOF of PCNA sequences **1**–**4**, UV Job's Plot and UV-T_m studies of all the complexes. See DOI: 10.1039/c003405n

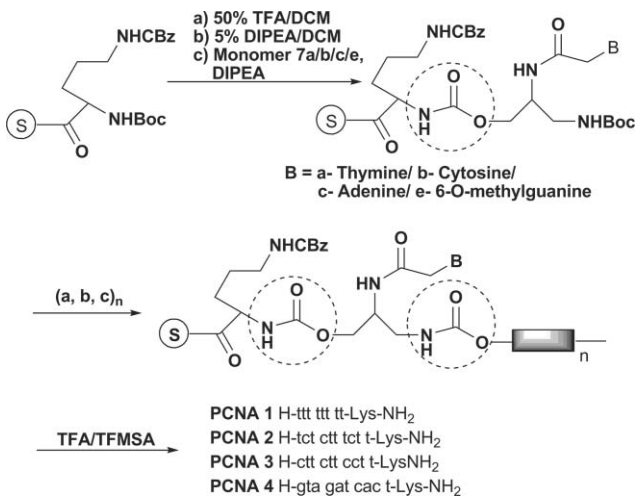


Scheme 1 Synthesis of monomer units to form PCNA oligomers.

by reduction over Ra–Ni/H_2 and *in situ* Boc protection of the primary amino group to get **3c**. Compound **3c** was subjected to hydrogenation over Pd–C to remove the CBz protection and the free amino group was acetylated using chloroacetyl chloride/$NaHCO_3$ in dioxane–water to get **4b**. The individual nucleobases thymine, N^6-CBz-adenine, N^4-CBz-cytosine and 2-amino-6-chloropurine were reacted with **4b** in the presence of K_2CO_3 to get individual protected monomer units **5a–d**. The exocyclic amino group of cytosine and adenine was protected as CBz but in the case of 2-amino-6-chloropurine and thymine, the base protection was not necessary. The 2-amino-6-chloropurine derivative **5d** was converted to **5e** with 0.75 M NaOH in 1 : 1 methanol–water. The enol ether in 6-OMe guanine derivative is hydrolysed in acidic medium and gets deprotected to give guanine derivative at the end of solid phase synthesis during TFA–TFMSA treatment.[8] **5e** was therefore carried forward in further steps as protected guanine derivative. Individual compounds **5a–c** and **5e** were deprotected using TBAF in dry THF or I_2/MeOH to get intermediate protected monomer units **6a–c** and **6e**. Individual monomers **6a–c** and **6e** were then activated using *p*-nitrophenyloxycarbonyl chloride to get **7a–c** and **7e** in good overall yield. All the new compounds were adequately characterized by ^{1}H, ^{13}C NMR and LC-MS mass spectroscopic analysis.

Synthesis of PCNA oligomers

The synthesis of PCNA oligomers was carried out in a sequential manner using MBHA resin to which L-lysine is attached as a first amino acid. The sequences were planned so that we could evaluate the feasibility of synthesis with t_8 oligomer (**PCNA1**), polypyrimidine sequences containing mixed t and c monomers, to evaluate parallel/antiparallel triplex binding (**PCNA2** and **PCNA3**), and a mixed purine–pyrimidine sequence (**PCNA4**) to evaluate the duplex binding as well as parallel/antiparallel binding selectivity. The Boc-protection of α-amino group of the lysine attached to the solid support was deprotected using 50% TFA in DCM. The resin was washed with DCM and was treated with 5% DIPEA in DCM. The activated monomers **7a–c** and **7e** (3–5 equivalents) were dissolved in 200 μL of dry DMF and the solution was added to the resin under dry conditions. After 2 h the completion of the coupling reaction was confirmed by negative Kaiser test.[9] The cycles including these steps (Scheme 2, a, b and c) were then continued until completion of the oligomer synthesis. After each coupling step (Scheme 2, c), the excess monomer could be recovered as no other reagent is used for coupling. The resin bound oligomers were subjected to treatment with TFA/TFMSA[4] to yield completely deprotected carbamate oligomers with a C-terminal amide group. The oligomers were precipitated using ether and were further purified by RP-HPLC. The purity of the oligomers was rechecked by analytical RP-HPLC. The complete



Scheme 2 Solid phase synthesis of PCNA, small letters a, t, g and c denote PCNA monomer units.

Table 1 PCNA sequences

Seq. code	PCNA Sequence[a]	Ret. time/min	Calculated/Found MW (mol. formula)
PCNA1	H-ttt ttt tt- Lys NH_2	10.0	2401.43/2402.74 ($C_{94}H_{127}O_{41}N_{35}+H^+$)
PCNA2	H-tct ctt tct t- Lys NH_2	9.8	2922.56/2921.79 ($C_{113}H_{155}O_{48}N_{46}$)
PCNA3	H-ctt ctt cct t- Lys NH_2	9.2	2905.18/2906.03 ($C_{112}H_{151}O_{47}N_{47}+H^+$)
PCNA4	H-gta gat cac t- Lys NH_2	8.9	3012.46/3021.6 ($C_{114}H_{147}O_{41}N_{60}+Li^+$)

[a] t, c, g, a are PCNA monomers.

Table 2 Comparative UV-T_m (°C) data for PCNA, PNA and DNA complexes with cDNA and RNA sequences[a]

Sequences	UV-T_m/°C		
	PCNA1 H-tttttttt-Lys	**DNA** 5′TTTTTTTT3′	**PNA1** H-*TTTTTTTT*-Lys
DNA1: 5′GCAAAAAAAACG3′	50.1	nd[11,b]	45.0[12,c]
RNA1: 5′GCAAAAAAAACG3′	30.1, (32.9)[d], (37.7)[e]	20[11]	
	PCNA2 H-tctctttctt-Lys	**DNA14** 5′TCTCTTTCTT3′	
DNA2: 5′AAGAAAGAGA3′	54.3	26.0	
RNA2: 5′AAGAAAGAGA3′	31.6	27.6	
	PCNA3 H-cttcttcctt-Lys	**DNA15** 5′CTTCTTCCTT3′	**PNA3** H-*CTTCTTCCTT*-Lys
DNA3: 5′AAGGAAGAAG3′	51.4	29.1	52.0[13,c]
RNA3: 5′AAGGAAGAAG3′	31.9, (34.6)[d], (39.4)[e]	30.9	
	PCNA4 H-gtagatcact-Lys	**DNA16** 5′GTAGATCACT3′	**PNA4** H-*GTAGATCACT*-Lys
DNA4: 5′AGTGATCTAC3′	53.4	31.6	52.0[14]
RNA4: 5′AGUGAUCUAC3′	30.4	32.0	

[a] 10 mM Sodium phosphate buffer and 10 mM NaCl, 1 μM PCNA and hyperchromicity 8–12%. All values are an average of at least 3 experiments and accurate within ±0.5 °C. [b] nd not detected, [c] please refer to ESI page 42, [d] 10 mM sodium phosphate buffer and 10 mM NaCl, 1.66 μM PCNA, [e] 10 mM sodium phosphate buffer and 10 mM NaCl, 2.5 μM PCNA.

deprotection of adenine, guanine and cytosine residues and structural integrity of the oligomers was established by MALDI-TOF mass spectral analysis (Table 1).

UV-T_m studies

The polypyrimidine sequences **PCNA1**, **PCNA2** and **PCNA3** were subjected to UV-Job's plot[10] with **DNA1** (5′GCAAAAAAAACG 3′), **DNA2** (5′AAGAAAGAGA 3′) and **RNA3** (5′AAGGAAGAAG 3′) respectively. The stoichiometry of binding was found to be 2 : 1 in each case. To find the strength of binding, UV-T_m experiments were carried out using the annealed samples of DNA:PCNA and RNA:PCNA in 1 : 2 stoichiometry. The parallel DNA sequences used were the same as **DNA2** and **DNA3**, but in the reverse direction. The single mismatch discrimination was studied using corresponding DNA sequences with a single mismatch in the central position. The mixed purine–pyrimidine sequence with either PCNA or DNA backbone **PCNA4** or **DNA16** (5′GTAGATCACT 3′) was used in 1 : 1 stoichiometry with **DNA4**/**RNA4** (5′AGTGATCTAC 3′/5′ AGUGAUCUAC 3′) for the melting studies. The results are presented in Table 2.

The results indicate that sequences with PCNA backbone (N-terminus of PCNA corresponding to 5′-terminus of DNA) bind strongly with cDNA in antiparallel orientation. The binding of PCNA sequences **PCNA1**, **PCNA3** and **PCNA4** with antiparallel DNA sequences at 1 μM concentration was found to be comparable with the binding of known PNA sequences earlier reported in the literature[12–14] (Table 2, column 4, also see ESI†). We confirm this strong DNA:PCNA binding by gel shift assay where the triplex mobility was found to be similar to aegPNA:DNA triplex (please refer to ESI†). The mixed purine–pyrimidine sequence **PCNA4** was also found to be highly stable duplex (**DNA4**:**PCNA4)** and the T_m was found to be 53.4 °C.

The most interesting results are those for RNA binding. The observed UV-T_m for PCNA:RNA triplex were much less at 1 μM concentration, compared to the PCNA:DNA triplexes. To confirm the binding of PCNA with complementary RNA, we studied the UV-T_m for **RNA1**:**PCNA1** and **RNA3**:**PCNA3** at higher strand concentrations (1.66 μM and 2.5 μM, Table 2). As expected, we observed concentration dependent increase in the triplex melting. The mixed purine–pyrimidine duplex **PCNA4**:**RNA4** was also less stable than the **PCNA4**:**DNA4** duplex (Table 2) but the T_m was comparable with natural **DNA16**:**DNA4** or **DNA16**:**RNA4** duplexes at high salt concentrations.

The effect of salt concentration on the stability of these duplexes was then studied. The change in T_m was found to be comparable (ΔT_m +0.1–3.7 °C) to that of PNA:DNA/RNA complexes[15] (please refer to ESI†). In parallel cDNA orientation (*p*), the binding of PCNA (**PCNA2:DNA9**, **PCNA3:DNA13**, **PCNA4:DNA12**) is reduced considerably (Table 3) in contrast to achiral PNA sequences.[14] The mismatch studies were carried out to study the sequence specific binding of PCNA to DNA. The complexes with mismatched base pair show reduced T_m with broad transitions (Table 3), and ΔT_m was found to be the least for a G:T mismatch as found in the case of the mismatched DNA:DNA duplexes.[16]

Table 3 UV-T_m data for PCNA complexes with mismatched DNA and parallel cDNA sequences[a]

Sequences	UV-T_m/°C
	PCNA1
DNA5: 5′ GCAAA**G**AAAACG 3′ (**G**:T) mismatch	43.6[c]
DNA6: 5′GCAAA**T**AAAACG 3′ (**T**:T) mismatch	42.4[c]
	PCNA2
DNA7: 5′ AAGA**G**AGAGA 3′ (**G**:T) mismatch	49.7[c]
DNA8: 5′ AAGA**C**AGAGA 3′ (**C**:T) mismatch	45.4[c]
DNA9: 5′ AGAGAAAGAA 3′(*p*)[b]	24.2[c]
	PCNA3
DNA10: 5′ AAGG**T**AGAA G 3′ (**T**:T) mismatch	43.4[c]
DNA13: 5′ GAAGAAGGA A 3′(*p*)[b]	22.0[c]
	PCNA4
DNA11: 5′ AGTG**T**TCTA C 3′ (**T**:T) mismatch	48.3[c]
DNA12: 5′ CATC**T**AGTG A 3′(*p*)[b]	nd[d]

[a] 10 mM Sodium phosphate buffer and 10 mM NaCl, 1 μM PCNA, [b] *p* parallel orientation, [c] Broad transitions with 3–6% hyperchromicity, [d] nd no detectable transition.

The RNA targeting with uncharged or positively charged oligomers with very high binding affinities sometimes leads to unwanted non-targeted affinities and hence toxicity in cellular assays.[17] The modest stability of such sequences comprising novel PCNA backbone may find application in antisense therapeutics.

Conclusions

In summary, a novel, uncharged, chiral backbone of PCNA presented in this paper could be quite attractive for its applications in therapeutics. The possibility of recovery of the excess monomer used in the synthesis allows the use of large excess of the same during the coupling step to improve yield at each coupling step in addition to reducing the cost of synthesis for therapeutic applications.

Experimental

General

All the reagents were purchased from Sigma–Aldrich and used without purification. DMF and pyridine were dried over KOH and 4 Å molecular sieves. THF was passed over basic alumina and dried by distillation over sodium. Ethanol was dried over Mg/Iodine. TLCs were run on Merck 5554 silica 60 aluminium sheets. All reactions were monitored by TLC and usual work-up implies sequential washing of the organic extract with water and brine followed by drying over anhydrous sodium sulfate and evaporation under vacuum. Column chromatography was performed for purification of compounds on silica gel (100–200 mesh, LOBA Chemie). TLCs were performed using dichloromethane–methanol or petroleum ether-EtOAc solvent systems for most compounds. Compounds were visualized with UV light and/or by spraying with ninhydrin reagent subsequent to Boc-deprotection (exposing to HCl vapours) and heating. ^{1}H (200 MHz) and ^{13}C (50 MHz) NMR spectra were recorded on a Bruker ACF 200 spectrometer fitted with an Aspect 3000 computer and all the chemical shifts (δ (ppm)) are referred to internal TMS for ^{1}H and chloroform-*d*, DMSO-d_6 for ^{13}C NMR. ^{1}H NMR data are reported in the order of chemical shift, multiplicity (s, singlet; d, doublet; t, triplet; br, broad; br s, broad singlet; m, multiplet and/or multiple resonance), number of protons. Optical rotations were measured on a JASCO DIP-181 polarimeter. Mass spectra were recorded on a Finnigan–Matt mass spectrometer, while MALDI-TOF spectra were obtained from a KRATOS PCKompact instrument. UV experiments were performed on a Perkin Elmer λ35 UV–VIS spectrophotometer fitted with a peltier temperature programmer and a Julabo water circulator. The DNA oligomers were synthesized on CPG solid support using a Pharmacia GA plus DNA synthesizer by β-cyanoethyl phosphoramidite chemistry followed by ammonia treatment and their purities checked by HPLC prior to the use. The RNA oligomers were procured from Sigma–Aldrich Bangalore.

***R*-1-*O*-*tert*-butyldimethylsilyl-2-benzyloxycarbonylamino-1,3-propanediol (2).** A solution of *O*-*tert*-butyldimethylsilyl-2-*N*-benzyloxycarbonylamino-L-serine methyl ester **1** (20 g, 54.4 mmol) in methanol (200 mL) was cooled to 0 °C in an ice bath. Solid $NaBH_4$ (12.4 g, 32.64 mol) was added in portions for a period of 30 min. The reaction mixture was stirred for a period of 3 h and finally quenched using NH_4Cl solution till pH is neutral. Methanol was removed under reduced pressure and the residue was extracted into EtOAc (4 × 100 mL), washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4 and solvent was removed under reduced pressure. The residue was purified by column chromatography (20% EtOAc in petroleum ether) affording compound **2** (15.6 g, 85%) as colourless oil. $[\alpha]_D^{20}$ + 8.0° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) *v*max cm^{-1}: 3436, 3106, 2954,1712, 1510; ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.04 (s, 6H, Si–(C$\underline{H}_3$)$_2$), 0.87 (s, 9H, Si–C(C$\underline{H}_3$)$_3$), 3.61–3.87 (m, 5H 1-C$\underline{H}_2$, 2-C$\underline{H}$, 3-C$\underline{H}_2$), 5.10 (s, 2H, NHCOOC$\underline{H}_2$Ph), 5.41 (br, 1H, carbamate NH), 7.35 (s, 5H, Ph); ^{13}C NMR (50 MHz, $CDCl_3$) δ (ppm) −5.6 (Si–(C$\underline{H}_3$)$_2$),18.6 (Si–*C*–(CH$_3$)$_3$), 25.9 (Si–C–(C$\underline{H}_3$)$_3$), 53.3 (2-C$\underline{H}$), 62.9 (3-C$\underline{H}_2$), 63.6 (1-C$\underline{H}_2$), 67.2 (NHCOC$\underline{H}_2$Ph), 127.1 (Ph), 127.6 (Ph), 128.9 (Ph), 136.1 (Ph), 155.6 (NHC$\underline{O}$O); EI-MS calcd for $C_{17}H_{29}NO_4Si$ (M^+): 339.19; found 340.47 (M^++1), 362.35 (M^++Na).

***R*-1-*O*-*tert*-butyldimethylsilyl-2-benzyloxycarbonylamino-3-*O*-mesyl-1,3-propane-diol (3a).** To a solution of alcohol **2** (4 g, 11.7 mmol) in dry DCM (40 mL) triethylamine (4.8 mL, 35.1 mmol) was added dropwise at 0 °C under nitrogen followed by dropwise addition of methanesulfonyl chloride (1.82 mL, 23.4 mmol) over a period of 15 min. The mixture was stirred for a period of 2 h. The reaction mixture was checked for completion of the reaction by TLC and was extracted in DCM (2 × 75 mL), washed with 5% $KHSO_4$ sol. and dried over anhydrous Na_2SO_4. The crude mixture was used for the next reaction without any further purification.

***R*-1-*O*-*tert*-butyldimethylsilyl-2-benzyloxycarbonylamino-3-*azido*-1-propanol (3b).** A stirred mixture of mesylate **3a** (6 g, 13.8 mmol) and NaN_3 (10.78 g, 165.8 mmol) was heated to 65 °C for a period of 5 h. After cooling, the solvent was removed under reduced pressure at low temperature and the residue was extracted into EtOAc (4 × 75 mL). The combined organic layer was washed with water and brine and dried over anhydrous Na_2SO_4. The residue was further purified by column chromatography (10% EtOAc in petroleum ether) to afford a colorless oil of azide **3b** (3.82 g, 76%). $[\alpha]_D^{20}$ – 4.0° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) *v*max cm^{-1}:

3334, 2954, 2929, 2102, 1724, 1255; ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.05 (s, 6H, Si–(C$\underline{H}_3$)$_2$), 0.88 (s, 9H, Si–C(C$\underline{H}_3$)$_3$), 3.48–3.87 (m, 5H, 1-C$\underline{H}_2$, 2-CH, 3-C$\underline{H}_2$), 5.0–5.10 (br s, 3H, NHCOOC$\underline{H}_2$Ph, carbamate NH), 7.35 (s, 5H, Ph); ^{13}C NMR (50 MHz, $CDCl_3$) δ (ppm) −5.6 (Si–(C$\underline{H}_3$)$_2$), 18.1 (Si–*C*–(CH$_3$)$_3$), 25.7 (Si–C–(C$\underline{H}_3$)$_3$), 50.9 (3-C$\underline{H}_2$), 51.5 (2-C$\underline{H}$), 61.7 (1-C$\underline{H}_2$), 66.8 (NHCOC$\underline{H}_2$Ph), 128.0 (Ph), 128.4 (Ph), 128.7 (Ph), 136.1, 155.0 (NHC$\underline{O}$O-); EI-MS calcd for $C_{17}H_{28}N_4O_3Si$ (M^+): 364.19; found 365.36 (M^++1), 387.26 (M^++Na).

***R*-1-*O*-*tert*-butyldimethylsilyl-2-benzyloxycarbonylamino-3-*tert*-butyloxycarbonyl-amino-1-propanol (3c).** To a solution of azide **3b** (6 g, 16.4 mmol) in dry EtOAc (25 mL) placed in hydrogenation flask was added di-*tert*-butyl dicarbonate (4.9 mL, 21.4 mmol) and RANEY®-nickel (2 mL). The mixture was hydrogenated in Parr apparatus at room temperature (35–40 psi) for 3 h. The slurry was filtered through celite and the filtrate was evaporated under reduced pressure. The residue was purified by column chromatography (15% EtOAc in petroleum ether) to afford compound **3c** (6.26 g, 87%) as colorless oil. $[\alpha]_D^{20}$ +22.0 (*c* 1.0, $CHCl_3$);IR ($CHCl_3$) *v*max cm^{-1}: 3348, 3012, 2954, 1704, 1514, 837; ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.03 (s, 6H, Si–(C$\underline{H}_3$)$_2$), 0.88 (s, 9H, Si–C(C$\underline{H}_3$)$_3$), 1.41 (s, 9H, *t*Boc), 3.27–3.43 (m, 2H, 1-C$\underline{H}_2$), 3.56–3.83 (m, 3H, 3-C$\underline{H}_2$, 2-CH), 4.98 (br, 1H, NH), 5.09 (s, 2H, NHCOOC$\underline{H}_2$Ph), 5.32 (br, 1H, NH), 7.35 (s, 5H, Ph); ^{13}C NMR (50 MHz, $CDCl_3$) δ (ppm) −5.5 (Si–(C$\underline{H}_3$)$_2$), 18.2 (Si–*C*–(CH$_3$)$_3$), 25.0 (Si–C–(C$\underline{H}_3$)$_3$), 28.3 (NHCOO–C–(C$\underline{H}_3$)$_3$), 42.5 (3-C$\underline{H}_2$), 52.5 (1-C$\underline{H}$), 63.6 (2-C$\underline{H}_2$), 66.7 (NHCOOC$\underline{H}_2$Ph), 79.5 (NHCOO*C*(CH$_3$)$_3$), 128.0 (Ph), 128.5 (Ph), 136.5 (Ph), 156.6 (NHC$\underline{O}$O-); EI-MS calcd for $C_{22}H_{38}N_2O_5Si$ (M^+): 438.25; found 439.01 (M^++1), 460.86 (M^++Na).

***R*-1-*O*-*tert*-butyldimethylsilyl-2-amino-3-*tert*-butyloxycarbonyl amino-1-propanol (4a).** To a solution of compound **3c** (5 g, 11.4 mmol) in methanol (15 mL) placed in hydrogenation flask was added Pd–C (0.5 g, 10 mol%). The mixture was hydrogenated in Parr apparatus (70 psi) at room temperature for 5 h. The slurry was filtered through celite and filtrate was evaporated under reduced pressure. This crude compound was used for the next reaction without further purification.

***R*-1-*O*-*tert*-butyldimethylsilyl-2-chloroacetylamino-3-*N*-*tert*-butyloxycarbonylamino-1-propanol (4b).** To a stirred solution of the amine **4a** (3.47 g, 11.4 mmol) in 1,4-dioxane (10 mL) was added 10% Na_2CO_3 (8.45 g, 79.8 mmol) in water (85 mL) and 1,4-dioxane (85 mL). The mixture was cooled to 0 °C, and chloroacetyl chloride (4.53 mL, 57.0 mmol) was added in two portions. After 30 min dioxane was removed under reduced pressure and the residue was extracted in EtOAc (2 x100 mL). The organic layer was dried over anhydrous Na_2SO_4 and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography (30% EtOAc in petroleum ether) affording the chloro compound **4b** (2.4 g, 80%) as colourless oil. $[\alpha]_D^{20}$ +18.0 (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) *v*max cm^{-1}: 3411, 3016, 2954, 1704, 1670, 1525, 1255; ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.06 (s, 6H, Si–(C$\underline{H}_3$)$_2$), 0.89 (s, 9H, Si–C(C$\underline{H}_3$)$_3$), 1.42 (s, 9H, *t*Boc), 3.29–3.45 (m, 2H, 3-C$\underline{H}_2$), 3.56–3.83 (m, 2H, 1-C$\underline{H}_2$), 4.01 (m, 1H, 2-CH), 4.02 (s, 2H, NHCOC$\underline{H}_2$Cl), 5.02 (br, 1H, carbamate NH); ^{13}C NMR (50 MHz, $CDCl_3$) δ (ppm) −5.5 (Si–(C$\underline{H}_3$)$_2$), 18.2 (Si–*C*–(CH$_3$)$_3$), 25.7 (Si–C–(C$\underline{H}_3$)$_3$), 28.3 (NHCOO–C–(C$\underline{H}_3$)$_3$), 42.1 (3-C$\underline{H}_2$), 42.5 (NHCOOC$\underline{H}_2$Ph), 51.4 (2-C$\underline{H}$), 62.9 (1-C$\underline{H}_2$), 79.6 (NHCOO*C*(CH$_3$)$_3$), 156.6 (NHC$\underline{O}$O-), 166.2 (NHC$\underline{O}$CH$_2$); EI-MS calcd for $C_{16}H_{33}ClN_2O_4Si$ (M^+): 380.19; found 381.31 (M^++1), 403.16 (M^++Na).

***R*-1-*O*-*tert*-butyldimethylsilyl-2-(*N*1-thyminylacetylamino)-3-*tert*-butyloxycarbonylamino-1-propanol (5a).** A mixture of compound **4b** (3 g, 7.89 mmol), thymine (0.99 g, 7.89 mmol) and anhydrous K_2CO_3 (1.19 g, 8.67 mmol) in dry DMF (40 mL) was stirred at room temperature for 12 h under nitrogen. The solvent was removed under reduced pressure and the residue was extracted into EtOAc (2 × 100 mL), washed with water, brine and dried over anhydrous Na_2SO_4. The solvent was evaporated and crude compound was purified by column chromatography (70% EtOAc in petroleum ether) to afford protected thymine monomer as white foam **5a** (2.96 g, 80%). $[\alpha]_D^{20}$ +18. 0° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) *v*max cm^{-1}: 3018, 1693, 1215, 757; ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.04 (s, 6H, Si–(C$\underline{H}_3$)$_2$), 0.87 (s, 9H, Si–C(C$\underline{H}_3$)$_3$), 1.42 (s, 9H, *t*Boc), 1.91 (s, 3H, thy-C$\underline{H}_3$), 3.22–3.33 (m, 2H, 3-CH$_2$), 3.5–3.76 (m, 2H, 1-CH$_2$), 3.91–4.03 (m, 1H, 2-CH), 4.27–4.30 (m, 2H, NHCOC$\underline{H}_2$-thy), 5.0 (br, 1H, NH), 7.02 (s, 1H, thy-C$\underline{H}$=C–CH$_3$), 8.0 (br, 1H, thy-NH); ^{13}C NMR (50 MHz, $CDCl_3$) δ (ppm) −5.4 (Si–(C$\underline{H}_3$)$_2$), 12.3 (thy-C$\underline{H}_3$), 18.1 (Si–*C*–(CH$_3$)$_3$), 25.8 (Si–C–(C$\underline{H}_3$)$_3$), 28.3 (NHCOO–C–(C$\underline{H}_3$)$_3$), 41.9 (3-C$\underline{H}_2$), 50.3 (NHCOC$\underline{H}_2$-thy), 52.0 (2-C$\underline{H}$), 62.6 (1-C$\underline{H}_2$), 79.7 (NHCOO*C*(CH$_3$)$_3$), 110.9 (thy-CH=*C*–CH$_3$), 140.9 (thy-C$\underline{H}$=C–CH$_3$), 151.1 (thy-2-C$\underline{O}$), 157.1 (NHC$\underline{O}$O-), 164.3 (thy-4-C$\underline{O}$), 166.6 (NHC$\underline{O}$CH$_2$-thy); EI-MS calcd for $C_{21}H_{38}N_4O_6Si$ (M^+): 470.26; found 470.89 (M^+), 492.71 (M^++Na).

***R*-1-*O*-*tert*-butyldimethylsilyl-2-[*N*1-(*N*^{4}-benzyloxycarbonyl-cytosinyl)-acetylamino]-3-*tert*-butyloxycarbonylamino-1-propanol (5b).** A mixture of compound **4b** (2.5 g, 6.57 mmol), *N*^{4}-benzyloxycarbonyl-cytosine (1.611 g, 6.57 mmol) and anhydrous K_2CO_3 (0.99 g, 7.23 mmol) in dry DMF (30 mL) was stirred at room temperature for 12 h under nitrogen. The solvent was removed under reduced pressure and the residue was extracted to EtOAc (2 × 100 mL) and dried over anhydrous Na_2SO_4. The solvent was evaporated and the crude compound was purified by column chromatography (75% EtOAc in petroleum ether) to afford a white foam of cytosine monomer **5b** (3.05 g, 79%). $[\alpha]_D^{20}$ +28. 0° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) *v*max cm^{-1}: 3341, 3018, 1689, 1215, 757; ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.03 (s, 6H, Si–(C$\underline{H}_3$)$_2$), 0.87 (s, 9H, Si–C(C$\underline{H}_3$)$_3$), 1.39–1.45 (d, 9H, rotamers *t*Boc), 3.1–4.25 (m, 6H, 1-CH$_2$, 3-CH$_2$, NHCOC$\underline{H}_2$-cyt), 4.49 (br, 1H, 2-CH), 5.20 (s, 2H, NHCOC$\underline{H}_2$Ph), 7.28–7.34 (br s, 6H, Ph, cyt-5-CH), 7.61–7.65 (d, 1H, cyt-6-CH, ΔJ = 7.43 Hz), 8.65 (br,1H, NH); ^{13}C NMR (50 MHz, $CDCl_3$) δ (ppm) −5.5 (Si–(C$\underline{H}_3$)$_2$), 18.4 (Si–*C*–(CH$_3$)$_3$), 25.8 (Si–C–(C$\underline{H}_3$)$_3$), 28.3 (NHCOO–C–(C$\underline{H}_3$)$_3$), 41.7 (3-C$\underline{H}_2$), 50.4 (2-C$\underline{H}$), 62.8 (NHCOC$\underline{H}_2$-cyt), 67.3 (1-C$\underline{H}_2$), 80.1 (NHCOO*C*(CH$_3$)$_3$), 95.8 (cyt-5-C$\underline{H}$), 127.6 (Ph), 128.1 (Ph), 128.5 (Ph), 135.3 (Ph), 149.6 (cyt-6-C$\underline{H}$), 153.1 (NHC$\underline{O}$OCH$_2$Ph), 156.3 (NHC$\underline{O}$OC(CH$_3$)$_3$), 157.8 (NHC$\underline{O}$OCH$_2$Ph), 166.7 (NHC$\underline{O}$CH$_2$-cyt); EI-MS calcd for $C_{28}H_{45}N_5O_7Si$ (M^+): 591.31; found 590.6543 (M^+), 612.6613 (M^++Na).

***R*-1-*O*-*tert*-butyldimethylsilyl-2-[*N*9-(*N*^{6}-benzyloxycarbonyl-adeninyl)-acetylamino]-3-*tert*-butyloxycarbonylamino-1-propanol (5c).** A mixture of compound **4b** (1.8 g, 4.73 mmol), *N*^{6}- benzyloxycarbonyladenine (1.274 g, 4.73 mmol) and

anhydrous K_2CO_3 (0.719 g, 5.2 mmol) in dry DMF (25 mL) under nitrogen was stirred at room temperature for 12 h. The solvent was removed under reduced pressure and the residue was extracted to EtOAc (2 × 100 mL), washed with water, brine and dried over anhydrous Na_2SO_4. The solvent was evaporated and the crude compound was purified by column chromatography (80% EtOAc in petroleum ether) to afford a white solid of protected monomer **5c** (1.8 g, 65%). $[\alpha]_D^{20}$ +8.0 (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) *v*max cm^{-1}: 3339, 3018, 1690, 1215, 757; ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.01 (s, 6H, Si–(C$\underline{H}_3$)$_2$), 0.83 (s, 9H, Si–C(C$\underline{H}_3$)$_3$), 1.37 (s, 9H, *t*Boc), 3.28–3.75 (m, 4H, 1-CH_2, 3-CH_2), 3.96 (m, 1H, 2-CH), 4.86 (s, 2H, NHCOC$\underline{H}_2$-ade), 7.35–7.5 (m, 5H, Ph), 8.05 (s, 1H, ade-8-CH), 8.19 (s, 1H, NH), 8.75 (s, 1H, ade-2-CH); ^{13}C NMR (50 MHz, $CDCl_3$) δ (ppm) –5.5 (Si–(C$\underline{H}_3$)$_2$), 18.4 (Si–*C*–$(CH_3)_3$), 25.8 (Si–C–(C$\underline{H}_3$)$_3$), 28.3 (NHCOO–C–(C$\underline{H}_3$)$_3$), 41.7 (3-CH_2), 50.4 (NHCOC$\underline{H}_2$-ade), 52.5 (2-C$\underline{H}$), 62.8 (1-C$\underline{H}_2$), 67.3 (NHCOOC$\underline{H}_2$Ph), 80.1 (NHCOO*C*$(CH_3)_3$), 128.2 (Ph), 128.5 (Ph), 128.3 (Ph), 139.4 (Ph), 140.1 (ade-8-C$\underline{H}$), 149.7 (ade-4-C$\underline{H}$), 153.0 (ade-2-C), 156.3 (NHC$\underline{O}$O$(CH_3)_3$), 157.8 (ade-6-C), 163.6 (NHC$\underline{O}$OCH$_2$Ph), 166.7 (NHC$\underline{O}$CH$_2$-ade); EI-MS calcd for $C_{29}H_{43}N_7O_6Si$ (M^+): 613.30; found 612.4265 (M^+), 634.2770 (M^++Na).

***R*-1-*O*-*tert*-butyldimethylsilyl-2-[*N*9-(2-amino-6-chloropurinyl)-acetylamino]-3-*tert*-butyloxycarbonylamino-1-propanol (5d).** A mixture of compound **4b** (3 g, 7.89 mmol), 2-amino-6-chloropurine (1.33 g, 7.89 mmol) and anhydrous K_2CO_3 (1.19 g, 8.67 mmol) in dry DMF (40 mL) was stirred at room temperature for 12 h under nitrogen. The solvent was removed under reduced pressure and the residue was extracted to EtOAc (2 × 100 mL), washed with water, brine and dried over anhydrous Na_2SO_4. The solvent was evaporated and the crude compound was purified by column chromatography (75% EtOAc in petroleum ether) to afford a white solid of monomer **5d** (3.3 g, 82%). $[\alpha]_D^{20}$ –8.0° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) *v*max cm^{-1}: 3411, 3016, 2950, 1704, 1525, 1255, 757; ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.02 (s, 6H, Si–(C$\underline{H}_3$)$_2$), 0.79 (s, 9H, Si–C(C$\underline{H}_3$)$_3$), 1.40 (s, 9H, *t*Boc), 3.26–3.72 (m, 4H, 1-CH_2, 3-CH_2), 3.97–4.08 (br, 1H, 2-CH), 4.7 (m, 2H, NHCOC$\underline{H}_2$-am), 4.97 (br, 1H, carbamate NH), 5.34 (br, 2H, am-2-C-N$\underline{H}_2$), 7.81 (s, 1H, am-8-C); ^{13}C NMR (50 MHz, $CDCl_3$) δ (ppm) –5.6 (Si–(C$\underline{H}_3$)$_2$), 18.0 (Si–*C*–$(CH_3)_3$), 25.6 (Si–C–(C$\underline{H}_3$)$_3$), 28.3 (NHCOO–C–(C$\underline{H}_3$)$_3$), 41.7 (3-C$\underline{H}_2$), 46.3 (NHCOC$\underline{H}_2$-am), 51.9 (2-C$\underline{H}$), 62.6 (1-C$\underline{H}_2$), 79.7 (NHCOO*C*$(CH_3)_3$), 124.3 (am-5-C), 142.9 (am-8-C), 151.1 (am-4-C), 153.8 (am-6-C), 159.5 (NHC$\underline{O}$OC$(CH_3)_3$), 162.6 (am-2-C), 166.1 (NHC$\underline{O}$CH$_2$-am); EI-MS calcd for $C_{21}H_{36}ClN_7O_4Si$ (M^+): 513.23; found 514.5155 (M^++1), 516.5043 (M^++2), 536.5173 (M^++Na), 538.5051 (M^++2+Na).

***R*-1-*O*-*tert*-butyldimethylsilyl-2-[*N*9-(2-amino-6-methoxy-purinyl)-acetylamino]-3-*tert*-butyloxycarbonylamino-1-propanol (5e).** Compound **5d** (2 g) was taken in methanol (10 mL) and 0.75 N NaOH (10 mL) was added. The reaction mixture was allowed to stir for 3.5 h. Reaction mixture was directly extracted into EtOAc (2 × 100 mL), washed with water, brine and dried over anhydrous Na_2SO_4. The solvent was evaporated and the crude compound was purified by column chromatography (80% EtOAc in petroleum ether) to afford colourless flakes of compound **5e** (1.58 g, 80%). $[\alpha]_D^{20}$ –12. 0° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) *v*max cm^{-1}: 3408, 3016, 2955, 1704, 1525, 757; ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.05 (s, 6H, Si–(C$\underline{H}_3$)$_2$), 0.76 (s, 9H, Si–C(C$\underline{H}_3$)$_3$), 1.41 (s, 9H, *t*Boc), 3.28–3.67 (m, 5H, 1-CH_2, 2-CH, 3-CH_2), 4.04 (s, 3H, O$\underline{Me}$), 4.66–4.68 (br, 2H, NHCOC$\underline{H}_2$-pu), 5.14 (br, 2H, pu-2-C–N$\underline{H}_2$), 7.61 (s, 1H, pu-8-CH); ^{13}C NMR (50 MHz, $CDCl_3$) δ (ppm) –5.6 (Si–(C$\underline{H}_3$)$_2$), 18.0 (Si–*C*–$(CH_3)_3$), 25.6 (Si–C–(C$\underline{H}_3$)$_3$), 28.3 (NHCOO–C–(C$\underline{H}_3$)$_3$), 41.8 (3-C$\underline{H}_2$), 46.8 (NHCOC$\underline{H}_2$-pu), 51.6 (2-C$\underline{H}$), 54.0 (pu-O$\underline{Me}$), 62.7 (1-C$\underline{H}_2$), 79.7 (NHCOO*C*$(CH_3)_3$), 114.9 (pu-5-C), 139.5 (pu-8-C), 153.5 (pu-4-C), 156.7 (NHC$\underline{O}$OC$(CH_3)_3$), 159.7 (pu-2-C), 161.6 (pu-6-C), 166.6 (NHC$\underline{O}$CH$_2$-pu), 174.61; EI-MS calcd for $C_{22}H_{39}N_7O_5Si$ (M^+): 509.28; found 510.5521 (M^++1), 532.5439 (M^++Na).

***R*-2-(*N*1-thyminylacetylamino)-3-*tert*-butyloxycarbonyl amino-1-propanol (6a).** A solution of **5a** (1.5 g, 3.189 mmol) in dry THF (10 mL) was cooled to 0 °C and TBAF (2.1 mL of 1M TBAF, 2.12 mmol) was added slowly. The reaction was allowed to stir for 2.5 h. THF was removed and the residue was extracted into EtOAc (2 × 100 mL), dried over anhydrous Na_2SO_4. The solvent was evaporated and the crude compound was purified by column chromatography (90% EtOAc in petroleum ether) to afford a white solid **6a** (0.84 g, 75%). $[\alpha]_D^{20}$ –6.0 (*c* 1.0, MeOH); IR ($CHCl_3$) *v*max cm^{-1}: 3427, 3106, 2954, 1712, 1510, 1215, 757; ^{1}H-NMR (200 MHz, $CDCl_3$) δ (ppm) 1.43 (s, 9H, *t*Boc), 1.89 (s, 3H, thy-CH_3), 3.86–4.26 (m, 6H, 1-CH_2, 3-CH_2, NHCOC$\underline{H}_2$-thy), 5.30 (br, 1H, 2-CH), 7.05 (s, 1H, thy-6-CH), 8.14 (br,1H, thy-NH); ^{13}C-NMR (50 MHz, $CDCl_3$) δ (ppm) 12.4 (thy-C$\underline{H}_3$), 28.9 (NHCOO–C– (C$\underline{H}_3$)$_3$), 40.9 (3-C$\underline{H}_2$), 49.9 (2-CH), 52.1 (NHCOC$\underline{H}_2$-thy), 61.5 (1-C$\underline{H}_2$), 78.5 (NHCOO*C*$(CH_3)_3$), 107.9 (thy-HC=*C*-CH_3), 142.3 (thy-H$\underline{C}$=C-CH_3), 151.7 (thy-2-CO), 155.9 (NHC$\underline{O}$OC$(CH_3)_3$), 165.1 (thy-4-CO), 166.7 (NHC$\underline{O}$CH$_2$-thy); EI-MS calcd for $C_{15}H_{24}N_4O_6$ (M^+): 356.17; found 379.20 (M^++Na).

***R*-2-[*N*1-(*N*^{4}-benzyloxycarbonyl-cytosinyl)-acetylamino]-3-*tert*-butyloxycarbonylamino-1-propanol (6b).** Protected cytosine derivative **5b** (1 g, 1.69 mmol) was taken in dry methanol (12 mL) and solid I_2 (179 mg, 3.39 mmol) was added. After stirring the reaction mixture for 6–8 h, it was quenched by adding $Na_2S_2O_3$ till the brown solution becomes colorless. Methanol was removed and the residue extracted into EtOAc (2 × 100 mL), dried over anhydrous Na_2SO_4. The solvent was evaporated and the crude compound was purified by column chromatography (EtOAc to 5% EtOAc in methanol) to afford a colourless solid of compound **6b** (0.6 g, 75%). $[\alpha]_D^{20}$ –6.0 (*c* 1.0, MeOH); IR ($CHCl_3$) *v*max cm^{-1}: 3432, 3110, 2958, 1710, 1510, 1215, 757; ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) 1.38 (s, 9H, *t*Boc), 3.4–3.9 (m, 6H, 1-CH_2, 2-CH, 3-CH_2), 4.55 (s, 2H, NHCOC$\underline{H}_2$-cyt), 5.2 (s, 2H, cyt-NHCOC$\underline{H}_2$-Ph), 7.17 (br, 1H, NH), 7.3 (br s, 5H, Ph), 7.9 (m, 2H, cyt-5-CH, 6-CH); ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm) 28.1 (NHCOO–C– (C$\underline{H}_3$)$_3$), 40.5 (3-C$\underline{H}_2$), 51.2 (NHCOC$\underline{H}_2$-cyt), 51.4 (2-C$\underline{H}$), 60.7 (1-C$\underline{H}_2$), 66.4 (NHCOOC$\underline{H}_2$Ph), 77.8 (NHCOO*C*$(CH_3)_3$), 93.7 (cyt-5-C), 127.9 (Ph), 128.3 (Ph), 128.4 (Ph), 135.9 (Ph), 150.8 (cyt-6-C), 153.0 (NHC$\underline{O}$OC$(CH_3)_3$), 154.9 (cyt-2-C), 155.8 (NHC$\underline{O}$OCH$_2$Ph), 162.9 (cyt-4-C); EI-MS calcd for $C_{22}H_{31}N_5O_7$ (M^+): 477.22; found 476.24 (M^+), 498.21 (M^++Na).

***R*-2-[*N*1-(*N*^{6}-benzyloxycarbonyladeninyl)-acetylamino]-3-*tert*-butyloxycarbonylamino-1-propanol (6c).** A solution of protected adenine compound **5c** (1 g, 1.63 mmol) in dry THF (10 mL) was cooled to 0 °C and TBAF (1.6 mL of 1M TBAF, 1.63 mmol) was

added slowly. The reaction was allowed to stir for 2.5 h. THF was removed and the residue was extracted into EtOAc (2 × 100 mL), dried over anhydrous Na_2SO_4. The solvent was evaporated and the crude compound was used for the next step without further purification.

***R*-2-[*N*9-(2-amino-6-methoxypurinyl)-acetylamino]-3-*tert*-butyloxycarbonylamino-1-propanol (6e).** A solution of silyl compound **5e** (1 g, 1.96 mmol) in dry THF (10 mL) was cooled to 0 °C and TBAF (1.9 mL of 1 M TBAF, 1.96 mmol) was added slowly. The reaction was allowed to stir for 2.5 h. THF was removed and the residue was extracted into EtOAc (2 × 100 mL), dried over anhydrous Na_2SO_4. The solvent was evaporated and the crude compound was purified by column chromatography (EtOAc to 5% EtOAc in methanol) to afford a white solid of monomer **6e** (0.54 g, 70%). $[\alpha]_D^{20}$ −8.0 (*c* 1.0, MeOH); IR ($CHCl_3$) *ν*max cm^{-1}: 3434, 3116, 2964, 1712, 1504, 1215, 757; ^1H NMR (200 MHz, $CDCl_3$ + DMSO-d_6) δ (ppm) 0.88 (s, 9H, *t*Boc), 2.45–2.70 (m, 4H, 1-CH_2, 3-CH_2), 3.26 (m, 1H, 2-CH), 3.46 (s, 3H, OMe), 4.19 (s, 2H, NHCOCH_2-pu), 5.87 (s, 2H, pu-2-C–NH_2), 6.1 (br, 1H, NH), 7.57 (s, 1H, pu-8-CH); ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm) 28.1 (NHCOO–C–(CH_3)$_3$), 40.5 (3-CH_2), 44.7 (NHCOCH_2-pu), 51.4 ((2-CH), 53.1 (OMe), 60.7 (1-CH_2), 77.8 (NHCOO*C*(CH_3)$_3$), 113.27 (pu-6-C), 140.6 (pu-8-C), 154.3 (pu-1-C), 155.8 (NHCOOC(CH_3)$_3$), 159.7 (pu-3-C), 160.5 (pu-5-C), 166.3 (NHCOCH_3-pu); EI-MS calcd for $C_{16}H_{25}N_7O_5$ (M^+): 395.19; found 396.2658 (M^++1), 418.2670 (M^++Na).

***R*-1-*O*-*p*-nitrophenoxycarbonyl-2-(*N*1-thyminylacetylamino)-3-*tert*-butyloxycarbonylamino-1-propanol (7a).** Compound **6a** (1 g, 2.807 mmol) was taken in dry DCM (12 mL) and cooled to 0 °C in an ice bath. Dry pyridine (0.68 mL, 8.42 mmol) was added and allowed to stir for 10 min. *p*-Nitrophenyl chloroformate (1.41 g, 7.019 mmol) dissolved in DCM (3 mL) was added and the reaction allowed to stir at room temperature for 2 h. After completion, the reaction mixture was extracted into DCM (2 × 50 mL), washed with water, brine and dried over anhydrous Na_2SO_4. The solvent was evaporated and the crude compound was purified by column chromatography (80% EtOAc in petroleum ether) to afford pale yellow foam of activated monomer **7a** (1.02 g, 70%). IR ($CHCl_3$) *ν*max cm^{-1}: 3305, 3018, 1770, 1681, 1525, 1215; ^1H NMR (200 MHz, $CDCl_3$) δ (ppm) 1.41 (s, 9H, *t*Boc), 1.87 (s, 3H, thy-CH_3), 3.37 (m, 2H, 3-CH_2), 4.32 (m, 4H, 1-CH_2, NHCOCH_2-thy), 5.23 (br,1H, 2-CH), 7.06 (s, 1H, thy-CH=C–CH_3), 7.35–7.4 (d, 2H, Ph-*ortho* to CO, ΔJ = 9.13 Hz), 8.24–8.28 (d, 2H, Ph-*meta* to CO, ΔJ = 9.08 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ (ppm) 12.17, 28.26, 36.56, 50.59, 53.18, 74.93, 79.95, 110.91, 122.0, 125.20, 141.21, 145.32, 151.40, 151.85, 155.36, 156.46, 164.62, 167.47; EI-MS calcd for $C_{22}H_{27}N_5O_{10}$ (M^+): 521.18; found 544.2196 (M^++Na).

***R*-1-*O*-*p*-nitrophenoxycarbonyl-2-[*N*1-(*N*^{4}-benzyloxycarbonyl-cytosinyl)-acetylamino]-3-*tert*-butyloxycarbonyl amino-1-propanol (7b).** Compound **6b** (1 g, 2.104 mmol) was taken in dry DCM (10 mL) and cooled to 0 °C in an ice bath. Dry pyridine (0.51 mL, 6.312 mmol) was added and allowed to stir for 10–15 min. *p*-Nitrophenyl chloroformate (1.05 g, 5.26 mmol) dissolved in DCM (3 mL) was added and the reaction was allowed to stir at room temperature for 2 h. After completion, the reaction mixture was extracted into DCM (2 × 50 mL), washed with water, brine and dried over anhydrous Na_2SO_4. The solvent was evaporated and the crude compound was purified by column chromatography (85% EtOAc in petroleum ether) to afford pale yellow foam of monomer **7b** (0.93 g, 70%). IR ($CHCl_3$) *ν*max: 3261, 3018, 1760, 1693, 1504, 1215, 757 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ (ppm) 1.46 (s, 9H, *t*Boc), 3.25–3.49 (m, 2H, 3-CH_2), 4.20–4.67 (m, 5H, 1-CH_2, 2-CH, NHCOCH_2-cyt), 5.19 (s, 2H, NHCOOCH_2Ph), 7.33–7.37 (br s,7H, Ph, Ph-*ortho* to CO), 8.20–8.25 (d, 2H, Ph-*meta* to CO, ΔJ = 9.09 Hz), 7.64–7.84 (m, 2H), 9.0 (br,1H, NH); EI-MS calcd for $C_{30}H_{32}N_8O_{10}$ (M^+): 642.23; found 663.23 (M^++Na).

***R*-1-*O*-*p*-nitrophenoxycarbonyl-2-[*N*1-(*N*^{6}-benzyloxycarbonyl-adeninyl)-acetylamino]-3-*tert*-butyloxycarbonyl amino-1-propanol (7c).** Compound **6c** (0.25 g, 0.5 mmol) was taken in dry DCM (5 mL) and cooled to 0 °C in an ice bath. Dry pyridine (0.12 mL, 1.50 mmol) was added and allowed to stir for 10 min. *p*-Nitrophenyl chloroformate (0.25 g, 1.25 mmol) dissolved in DCM (2 mL) was added and the reaction stirred at room temperature for 2 h. After completion, the reaction mixture was extracted into DCM (2 × 50 mL), washed with water, brine and dried over anhydrous Na_2SO_4. The solvent was evaporated and the crude mixture was purified by column chromatography (80% EtOAc in petroleum ether) to afford pale yellow foam of activated monomer **7c** (0.19 g, 60%). IR ($CHCl_3$) *ν*max cm^{-1}: 3257,3018,1760,1658,1214,757; ^1H NMR (200 MHz, $CDCl_3$) δ (ppm) 1.40 (s, 9H, *t*Boc), 3.30–3.41 (m, 2H, 3-CH_2), 4.21–4.30 (m, 3H, 2-CH, NHCOCH_2-ade), 4.9 (m, 2H, 1-CH_2), 5.29 (s, 2H, NHCOOCH_2-Ph), 7.35–7.41 (br s, 7H, Ph, Ph-*ortho* to CO), 8.03 (s, 1H, ade-8-CH), 8.12 (s, 1H, ade-2-CH), 8.23–8.28 (d, 2H, Ph-*meta* to CO, ΔJ = 9.21 Hz), 8.71 (br, 1H, ade-6-C–NH); EI-MS calcd for $C_{30}H_{32}N_8O_{10}$ (M^+): 664.22; found 664.9990 (M^++1).

***R*-1-*O*-*p*-nitrophenoxycarbonyl-2-[*N*9-(2-amino-6-methoxypurinyl)-acetylamino]-3-*tert*-butyloxycarbonylamino-1-propanol (7e).** Compound **6e** (0.5 g, 1.26 mmol) was dissolved in dry DCM (6 mL) and cooled to 0 °C in an ice bath. Dry pyridine (0.307 mL, 3.79 mmol) was added and allowed to stir for 10 min. *p*-Nitrophenyl chloroformate (0.635 g, 3.163 mmol) dissolved in DCM (3 mL) was added and the reaction allowed to stir at room temperature for 2 h. After completion, the reaction mixture was extracted into DCM (2 × 50 mL), washed with water, brine and dried over anhydrous Na_2SO_4. The solvent was evaporated and the crude compound was purified by column chromatography (90% EtOAc in petroleum ether) to afford pale yellow foam of activated monomer **7e** (0.45 g, 65%). IR ($CHCl_3$) *ν*max cm^{-1}: 3347, 3257, 3018,1760, 1658, 1214, 757; ^1H NMR (200 MHz, $CDCl_3$) δ (ppm) 1.42 (s, 9H, *t*Boc), 3.28–3.46 (m, 2H, 3-CH_2), 4.03 (s, 3H, OMe), 4.31 (br s, 3H, 2-CH, NHCOCH_2-pu), 4.89 (br, 2H, ade-2-C-NH_2), 5.03 (br, 1H, 2-CH), 7.33–7.38 (d, 2H, Ph-*ortho* to CO, ΔJ = 9.14 Hz), 7.63 (br,1H, NH), 7.85 (br,1H, pu-8-CH), 8.26–8.3 (d, 2H, Ph-*meta* to CO, ΔJ = 9.16 Hz); EI-MS calcd for $C_{23}H_{28}N_8O_9$ (M^+): 560.20; found 561.0808 (M^++1), 583.0296 (M^++Na).

Synthesis, cleavage from the solid support and purification of the PCNA oligomers

The PCNA oligomers were synthesized manually by solid phase peptide synthesis using the Boc-protection strategy. The solid support used was Merrifield resin that was derivatized with L-lysine (0.27 mmol g^{-1} resin) as the spacer amino acid. Synthesis

involved repetitive cycles, each comprising (i) deprotection of the *N*-protecting Boc-group using 50% trifluoroacetic acid (TFA) in CH_2Cl_2, (ii) neutralization of the TFA salt formed with diisopropylamine (5% solution in CH_2Cl_2, v/v) and (iii) coupling of the free amine with *p*-nitrophenyl carbonate activated monomer units **7a–c**,**e** (3 to 4 equivalents) in DMF or NMP as the solvent. The deprotection of the *N*-Boc protecting group and the coupling reaction were monitored by Kaiser's test.[8] Since the coupling efficiencies were found to be >98%, capping of the unreacted amino groups was not found necessary.

The PCNA oligomers were cleaved from the solid support using the TFA–TFMSA method to yield completely deprotected oligomers with free amide at their carboxy termini.[18] The resin-bound PCNA oligomer (10 mg) was stirred in an ice-bath with thioanisole (20 μL) and 1,2-ethanedithiol (8 μL) for 10 min. TFA (120 μL) was then added and the stirring was continued for another 10 min. TFMSA (16 μL) was added while cooling the reaction in an ice-bath and stirring was continued for 1 h 15 min. The reaction mixture was filtered through a sintered funnel, the residue washed with TFA (3 × 2 mL) and the combined filtrate and washings were evaporated under vacuum. The residual pellet was precipitated by adding cold ether. The precipitate was then re-dissolved in 200 μL of deionized water to obtain the crude PCNA oligomer. (In the case of oligomer **PCNA4**, the exocyclic methyl protection of guanine was found to be cleaved with TFMSA–TFA treatment). This was purified by HPLC-(RP-C18 analytical column 25 × 0.2 cm. 5 μm) with gradient elution: A to 100% B in 20 min. A = 0.1% TFA in H_2O:ACN (95 : 5), B = 0.1% TFA in H_2O:ACN (50 : 50) with flow rate 1.5 mL min^{-1} (Linear gradient from A to B in 20 min). The purity of the PCNA oligomer was checked by analytical RP-HPLC on a C18 column and found to be >90% (Supplementary Information†).

UV-Melting Studies

The concentration of the PCNA oligomers was calculated on the basis of the absorption at 260 nm, assuming the molar extinction coefficients of the nucleobases to be as in DNA, *i.e.*, T, 8.8 cm^2 μmol^{-1}; C, 7.3 cm^2 μmol^{-1}; G, 11.7 cm^2 μmol^{-1} and A, 15.4 cm^2 μmol^{-1}. The PCNA oligomers (**PCNA1–PCNA4**) and the relevant complementary DNA/RNA oligonucleotide were mixed together in a 1 : 1 molar ratio for mixed purine/pyrimidine sequence and 2 : 1 for polypyrimidine sequence in 10 mM sodium phosphate buffer and 10 mM NaCl, 0.01 mM EDTA, (pH 7.3) to get a final strand concentration of 1 μM. The samples were annealed by heating at 85 °C for 2 min, followed by slow cooling to room temperature, kept at room temperature for ~30 min and then, refrigerated overnight. The samples were heated at a rate of 0.5 °C rise per minute and the absorbance at 260 nm was recorded at every minute. The percent hyperchromicity at 260 nm was plotted as a function of temperature and the melting temperature was deduced from the peak in the first derivative plots.

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