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Solid-phase synthesis of positively charged deoxynucleic guanidine (DNG) oligonucleotide incorporating 7-deazaguanine bases

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Abstract—DNG nucleotides represent a positively charged DNA analog in which the negatively charged phosphodiester linkages of DNA are replaced by positively charged guanidinium linkages. We report herein the synthesis of 3′-end, middle, and 5′-end monomers required for the synthesis of a DNG sequence in which the natural guanine base is replaced by 7-deazaguanine (c^7G). 7-Deazaguanine nucleobase was chosen because of their unique glycoside bond stability and their ability to prevent G-quartet formation. A facile and high yield two-step synthesis of xylo-7-deazaguanine **7**, a key intermediate for introducing 3′-amino functionality, is carried out under Mitsunobu conditions. Subsequently, the 3′-Fmoc-protected thiourea monomers **13** and **19** were prepared from **7** via their corresponding 3′-amino-7-deazaguanines **11** and **18**, respectively. The smooth coupling of these thiourea monomers with monomethoxytrityl (MMTr)-protected 3′-end monomer **25**, prepared from **5**, occurred on solid phase in 3′ → 5′ direction. The resultant trimeric HO-c^7Ggc^{7}Ggc^{7}G-OH (**1**) has been designed to be included into DNA using standard DNA synthesis technology. The combination of C-c^7G base pairing and electrostatic association of phosphodiester and guanidinium backbone allows the small synthesized DNG trimer **1** to form 1:1 complex with DNA-C pentamer.

1. Introduction

The artificial control of gene expression by synthetic oligonucleotides, either at translational (antisense) or at a transcriptional (antigene) level, is a long-standing dream in human therapy and biotechnology. There has been steady progress in antisense technology, which is evident from the fact that one antisense drug, Fomivirsen, is currently on the market to treat cytomegalovirus (CMV) retinitis, while several others are at different stages of clinical trial for treatment of cancer/and or viral diseases.[1,2] In contrast, there is no antigene oligodeoxynucleotide (ODN) in clinical trials, although targeting the gene dsDNA is a more logical approach as there are only two copies of genes in the eukaryotes that are to be targeted forming a triple helix, instead of thousands of copies of m-RNA in the antisense approach. It has been demonstrated that the best triplex forming oligonucleotides (TFOs) are those that contain polypurine stretches, especially G-rich oligonucleotides.[3] However, the major concern with G-rich TFOs is that they form G-quartets, especially in the presence of monovalent cations via inter- or intrastrand H-bondings (Fig. 1a). This potentially restricts the effectiveness of purine-rich TFOs as antigene agents.[4,5] G-rich oligonucleotides have also been shown to have a strong antiproliferative activity against a number of cancer cell lines.[6] In view of these findings, it is important to design potential therapeutic TFOs that do not form G-quartets. Among other modifications, the replacement of guanosine N-7 with carbon creates 7-deazaguanosine (c^7G). c^7G nucleosides have gained extensive attention because some of them, such as queuosine[7] and cadeguomycin,[8–10] exhibits a broad spectrum of biological and chemotherapeutic activities. It has been demonstrated that the removal of this H-bond acceptor (N-7) eliminates the ability of ODNs to form G-quartets by removing Hoogsteen H-bonds between the 2-amino and N-7 nitrogen,[11,12] while retaining the H-bond donor and acceptor pattern required for the formation of c^7G-C duplexes[13,14] and c^7G:G-C triplets[15] (Fig. 1b). However, the binding study of these duplexes[13,14] and triplexes[15] of the ODN substituting c^7G in place of dG showed a substantial decrease in binding affinity compared to dG, due to altered π-electron system of pyrrolo[2,3-*d*]pyrimidine nucleobase which affects base stacking and hydrogen bonding.

Keywords: Deoxynucleic guanidine (DNG); 7-Deazaguanine; Triplex forming oligonucleotide (TFO); G-quatrets.
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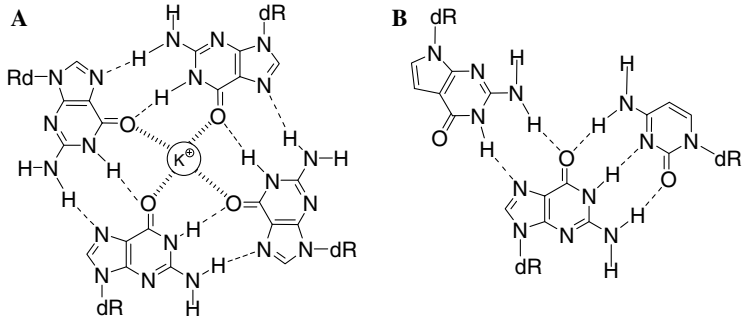

Figure 1. (A). Structure of a G-tetrad showing Hoogsteen H-bonds involving N-7 of G; (B) reverse Hoogsteen triplet interactions of 7-deazaguanine with G–C base pair, dR signifies 2′-deoxyribose.

To overcome the challenges in designing an ideal antisense/antigene ODN having not only high binding affinity but also maintaining base pair fidelity, resistance to degradation by nucleases, and improved cell permeability, several approaches have been reported. These include backbone modification, for example, phosphorothiolate,[16] phosphonates,[17] carbamates,[18] methylene methylimino,[19] and locked nucleic acids;[20] or replacing the entire sugar phosphodiester backbone such as in the case of peptide nucleic acid (PNA),[21] phosphonic ester nucleic acid (PHONA),[22] or nucleic acid analog peptide (NAAP).[23] Recently it has been shown that the introduction of positively charged groups at multiple sites in the backbone,[24–27] sugar[28,29] or base[30,31] greatly enhances the duplex and triplex stability.[32] Our investigation in this area has resulted in the discovery of positively charged guanidinium linkages replacing the negatively charged phosphodiester linkages.[33] As expected, these deoxynucleic guanidines (DNGs) bind strongly to the complementary DNA/RNA sequences with high affinity and fidelity because the repulsive electrostatic effects of natural duplex DNA are replaced by electrostatic attraction. DNG linkages have been shown to be resistant to nucleases.[34] It is also possible that the positive charge of the DNG backbone may improve the cell permeability through electrostatic attraction of the negatively charged phosphate groups on the cell surface. Our earlier studies have demonstrated that thimidinyl DNG oligomers have a high affinity for and bind only with complementary poly(dA) in a 2:1 stoichiometry without forming any complexes with poly(dG), poly(dC) or poly(dT).[33c] Similarly, adenyl DNG[33b] and cytidinyl DNG[35] bind with poly(dT) and poly(dG) in 1:2 and 1:1 stoichiometry, respectively, with high affinity. Indeed, poly(dC) DNG–poly(dG) DNA duplex is 1000 time more stable than poly(dC–dG) duplex.[35] We have also reported a DNG chimera having mixed thymidinyl and guanyl bases.[36] However, we were unable to prepare DNG with only guanyl bases. Therefore, we chose to incorporate c^7G bases in place of guanine because of their unique glycosidic bond stability,[37,38] stability toward enzymatic hydrolysis,[39] and most importantly, their ability to prevent G-quartet formation.

A logical choice was to combine one of our best backbone modifications (i.e. DNG) with an appropriate modified guanine base (i.e. c^7G) to create a ODN which can exhibit higher affinity toward single or double stranded DNA, as well as general improved antisense/antigene properties. Herein, we describe the preparation of monomer building blocks and solid-phase synthesis of DNG oligomer **1** incorporating c^7G bases (Fig. 2). A convenient and new method for the synthesis of xylo-7-deazaguanine is also presented.

2. Results and discussion

2.1. Synthesis of monomers

The xylo-7-deazaguanine **7**, required to prepare building blocks **13** and **19** for the solid-phase synthesis of positively charged DNG oligonucleotide with guanidinium linkages, was prepared as shown in Scheme 1. The protected 7-deazaguanine was prepared from 3′,5′-bis-*O*-(4-chlorobenzoyl-2′-deoxy-α-D-ribofuranosyl chloride (**2**) instead of Hoffer's α-chloro sugar synthon reported earlier,[40] as **2** is more stable and readily crystallizes from the solvent.[41] Treatment of the sodium salt of 2-amino-4-chloropyrrolo[2,3-*d*]pyrimidine[42] with **2** in acetonitrile underwent regio- and stereospecific glycosylation to afford exclusively the desired β-anomer of the protected 3′5′-bis-*O*-benzoyl-4-chloro-7-deazanucleoside **3** in high yield (78%). Hydrolysis of the benzoyl groups with simultaneous nucleophilic substitution of the 4-chloro group of **3** was initially carried out using sodium methoxide in refluxing methanol, affording **4** in 70% yield.

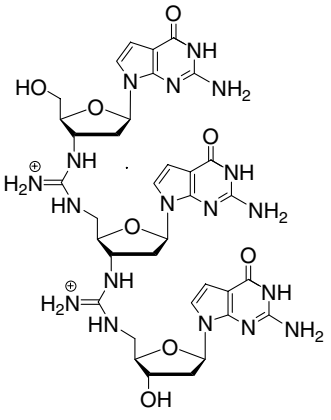

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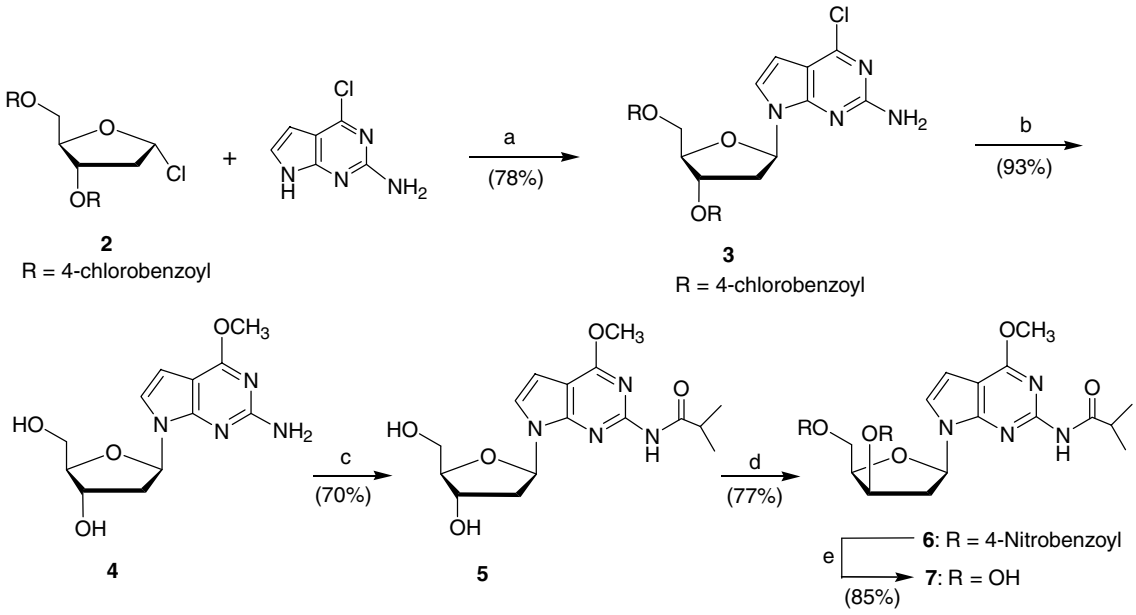
Figure 2. DNG containing c^7G bases.



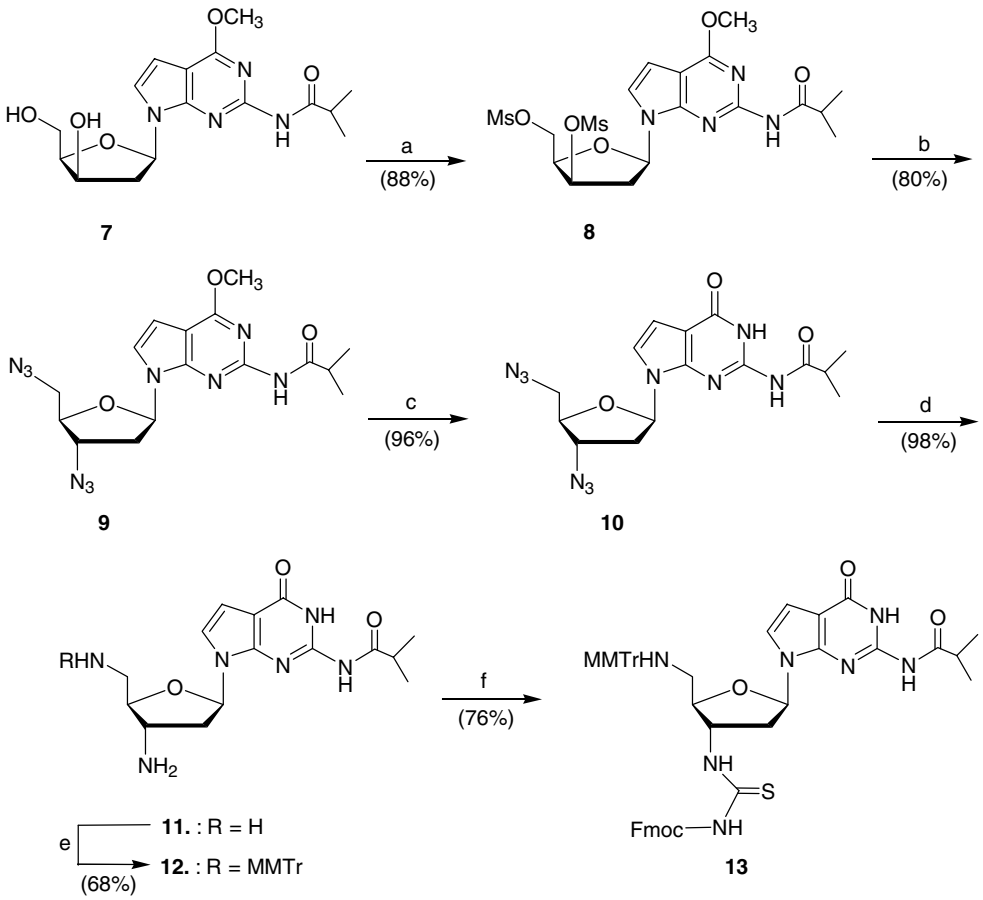
Scheme 1. Reagents and conditions: (a) NaH, CH_3CN, rt, 3 h; (b) Dowex 550 A (OH) anion exchange resin, MeOH, reflux, 2 h; (c) TMSCl, pyridine, rt, 0.5 h; $(^iPrCO)O_2$, pyridine, rt, 6 h; (d) 4-Nitrobenzoic acid, DIAD, PPh_3, THF, rt, 4 h; (e) NH_3/MeOH, rt, 1 h.

However, a clean and quantitative conversion occurred when **3** was refluxed with Dowex 550A (OH) anion-exchange resin in methanol for 2 h. To our knowledge, this is the first example of using this resin for one-pot simultaneous debenzoylation and substitution of 4-chloro group to introduce methoxy functionality. The amino function of **4** was masked as the corresponding isobutyric amide **5** in one step using a transient protection strategy[43] instead of using a two-step procedure reported earlier.[44] Attention was then focused on the stereospecific inversion of the 3′-hydroxy group to prepare xylo-isomer **7**, a key intermediate to introduce amino functionality at the 3′-position. In pyrimidine nucleosides, inversion of the stereochemistry at 3′-position can be easily achieved via anhydride formation resulting from the intramolecular attack of the pyrimidine C2 oxygen onto the 3′-carbon functionalized with a suitable leaving group.[45] Since there is no anhydride formation with purines, the strategy involved the reaction of **5** with 4-nitrobenzoic acid under Mitsunobu conditions.[46,47] The electron withdrawing *p*-nitro functionality of the benzoic acid not only leads to complete stereo selective inversion to afford exclusively the xylo-diester **6** in excellent yield (77%), but also its subsequent removal with ammonia was very clean. The overall yield of this two-step conversion for preparing xylo-isomer **7** from **5** was 68%. This provides a facile and high yielding two step route to prepare the xylo-intermediate from its corresponding threo-isomer, for introducing 3′-azido or amino functionality, in comparison to lengthy or low yielding routes available to prepare 2′,3′-dideoxy-3′-amino guanine.[48–51]

The elongating monomer, 3′,5′-protected diamino **13**, was prepared from **7** as shown in Scheme 2. Mesylation of **7** with MsCl in pyridine afforded 3′,5′-dimesylate **8** which underwent nucleophilic substitution with lithium azide in DMF at 90 °C, affording diazido derivative **9**. Our initial approach for removal of methoxy group of **9** using aqueous NaOH or TMSCl/NaI resulted in poor yields (65–70%) of **10**. However, a clean and quantitative conversion occurred by heating **9** with sodium thiocresolate in DMF at 90 °C for 1 h, instead of using carcinogenic phosphorus triamide solvent as previously reported.[52] Catalytic hydrogenation of diazide **10** with activated Pd/C yielded corresponding diamine **11** in quantitative yields. For regioselective tritylation of 5′-NH_2 function of diamine **11**, the best results were obtained by dropwise addition of a cooled solution of monomethoxytrityl chloride (MMTrCl) in CH_2Cl_2 during 1 h to a cooled solution of **11** and diethyl amine at −40 °C in CH_2Cl_2, to afford the desired 5′-*N*-tritylamino derivative **12** in 68% yield. A small amount of bis-tritylated derivative (<5%) formed, which was easily separated by silica gel column chromatography. Finally, the 3′-amino group was protected using fluorenylmethyloxycarbonyl isothiocyanate (Fmoc-NCS)[53] to afford the desired thiourea building block **13** in high yields.

The capping building block **19**, also synthesized from 2′-deoxy-3′xylo-7-deazaguanosine **7**, is shown in Scheme 3. Tritylation of 5′-OH with MMTrCl in pyridine followed by mesylation afforded the 5′-monomethoxytritylated-3′mesyl derivative **15**. Nucleophilic substitution of **15** with lithium azide occurred smoothly to afford the corresponding azide **16**. Deprotection of the methoxy group using sodium thiocresolate, followed by catalytic hydrogenation using Pd/C, and protection of the amino functionality with Fmoc-NCS (as discussed for preparing **13** from **10**) afforded the desired capping thiourea monomer **19**.

The loading monomer **25** was prepared from **5** as shown in Scheme 4. The regioselective 5′ mesylation



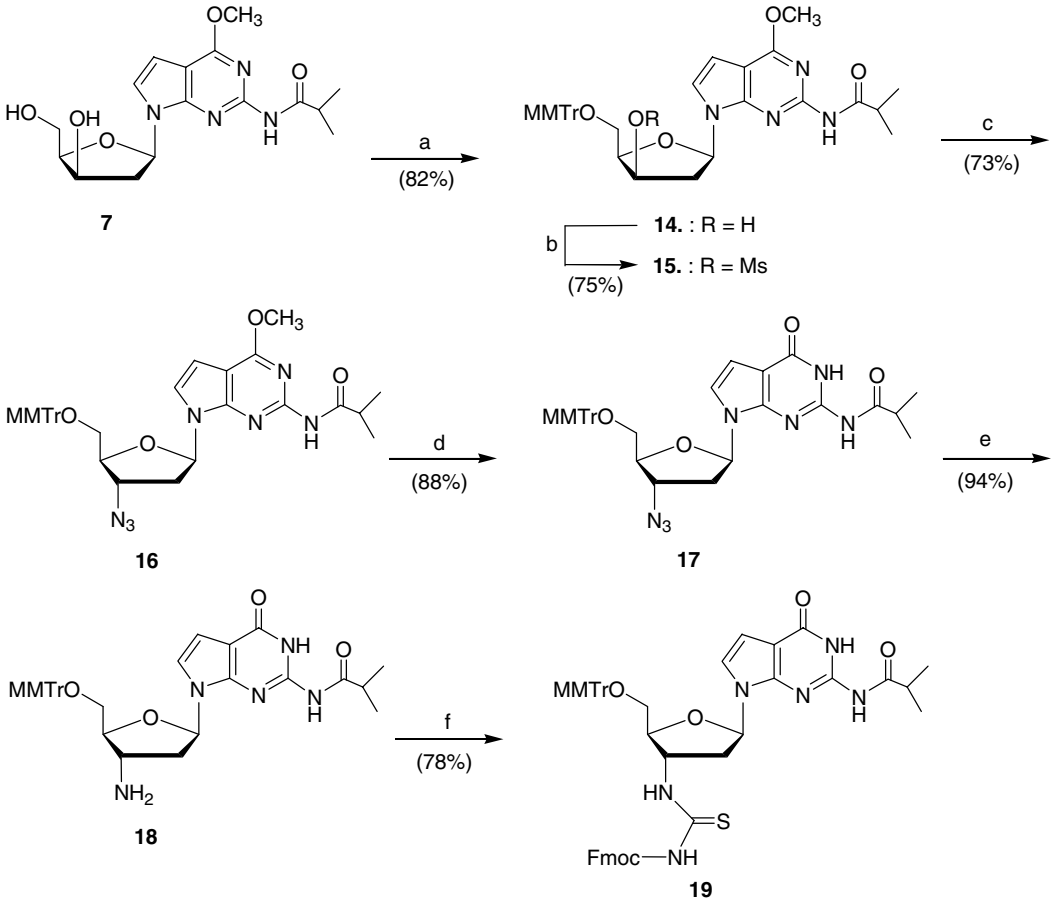
Scheme 2. Reagents and conditions: (a) MsCl, DMAP, pyridine, rt, 15 h; (b) LiN_3, DMF, 90 °C, 2 h; (c) sodium thiocresolate, DMF, 90 °C, 1 h; (d) H_2, Pd/C, EtOH, rt, 5 h; (e) MMTCl, Et_2NH, CH_2Cl_2, −40 °C to rt; (f) Fmoc-NCS, CH_2Cl_2, rt, 2 h.

of **5** was carried out using MsCl in pyridine at 0 °C. Subsequent conversion to azide **21** using lithium azide, followed by catalytic hydrogenation over Pd/C, gave corresponding 5′-amino derivative **22**. Tritylation of the amino function by dropwise addition of MMTrCl in pyridine at 0 °C followed by subsequent removal of the methoxy group with sodium thiocresolate occurred smoothly in high yield to give **24**. Esterification of the 3′-OH with succinic anhydride afforded the loading monomer **25**.

The triethylammonium salt of **25** underwent excellent coupling with the amino function of long-chain alkyl amino controlled pore glass (LCAA-CPG) in presence of 4-(4,6-dimethoxy-1,3,5-traiazin-2-yl)-4-methylmorpholinium chloride (DMTMM)[54] to afford monomer loaded CPG **26**. In comparison to the frequently used approach of activating the acid group by 4-nitrophenol using DCC and subsequent loading on CPG reported earlier for DNG synthesis[33] which is tedious, time-consuming, and often gives lower loading yields (25–30 μmol/g loading after first coupling), the present method is facile, clean, and gave excellent loading (51 μmol/g) on CPG within 1 h (Scheme 4). The loading yield was determined spectrophotometrically from the amount of MMT cation released upon treatment with 4% dichloroacetic acid (DCA) solution in CH_2Cl_2. After loading, the unprotected sites were capped with Ac_2O/TEA.

2.2. Solid-phase synthesis of DNG (1)

Solid-phase synthesis of DNG trimer was performed on CPG support from 3′ → 5′ direction, as in the standard DNA synthesis. A typical solid-phase synthesis is outlined in Scheme 5. Upon deblocking of the acid labile MMTr group, the 5′-amino functionality becomes available to couple with incoming precursor for the formation of a guanidinium linkage. The coupling reaction was accomplished in the presence of $HgCl_2$/TEA, whereby the 3′-Fmoc-protected thiourea of the incoming precursor **13** is converted into an activated carbodiimide intermediate via abstraction of the sulfur atom on thiourea by Hg (II). The carbodiimide intermediate then reacts with 5′-NH_2 of the unmasked CPG loaded monomer to provide a guanidinium linkage.[33d] The HgS precipitate formed during the reaction was removed by demercuration using 20% thiophenol in DMF. After coupling, the unreacted 5′-amino sites were blocked by capping, rendering them inert toward further chain elongation. The terminal 5′-MMTr was then deprotected and the coupling yield was determined to be 80% by UV analysis. The coupling cycle was then repeated using capping monomer **19**. After final coupling, the capping and deprotection steps were omitted to simplify the crude product purification. The resulting trimer 5′-c^{7}Ggc^{7}Ggc^{7}G*-3′ was removed from CPG using methanolic ammonia at 60 °C. The Fmoc-protect-



Scheme 3. Reagents and conditions: (a) MMTrCl, pyridine, rt, 18 h; (b) MsCl, pyridine, rt, 16 h; (c) LiN_3, DMF, 90 °C, 2 h; (d) sodium thiocresolate, DMF, 90 °C, 1 h; (e) H_2, Pd/C, EtOH, rt, 6 h; (f) Fmoc-NCS, CH_2Cl_2, rt, 1.5 h.

ing groups on guanidinium linkages and isobutyryl groups on exocyclic deazaguanine bases were also removed simultaneously to afford MMTr-protected DNG **29** (Scheme 5).

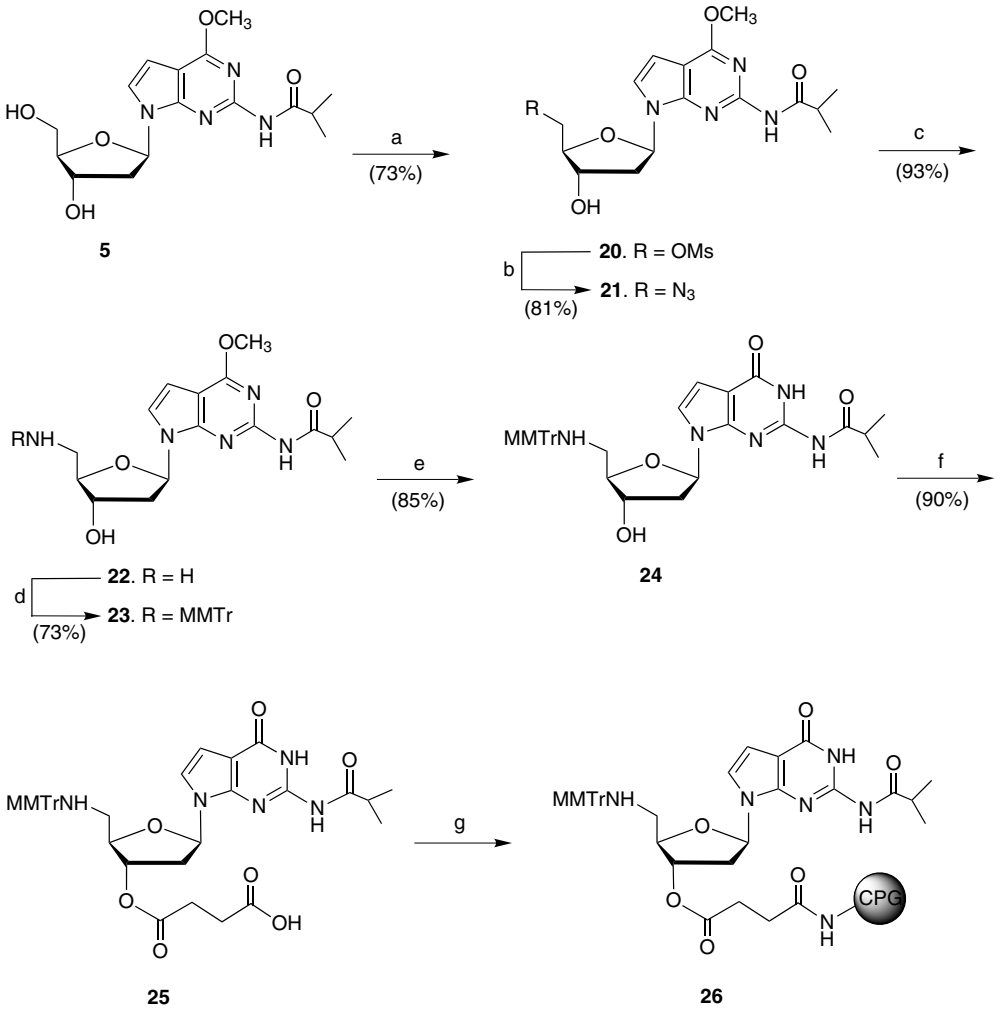
The crude DNG **29** was purified on reverse-phase HPLC (altech C_8 column) using 100 mM triethylammonium acetate (TEAA) buffer (pH 7.0) as solvent A with a gradient of 5% → 95% CH_3CN as solvent B in 40 min. The trityl group on the 5′-terminus of the oligomer **29** was then deprotected with 4% DCA in CH_2Cl_2 solution and precipitated with excess ether. The precipitated product was centrifuged, dried, and purified by reverse-phase HPLC to afford desired detritylated trimer **1**.

2.3. Binding studies of DNG 1

The binding stoichiometry of the trimeric DNG **1** with complimentary pentameric cytidine DNA (DNA-C_5) was determined by the method of continuous variation[55] to generate mixing curves of the absorbance versus mole fraction of DNG and DNA (Fig. 3). This method is based on the assumption that a decrease in absorbance is proportional to the number of base pairs hydrogen bonded between the interacting species. Increasing mole fraction of DNA-C_5 to DNG **1** lowered the UV absorbance at 260 nm. The inflection point at 50% DNA-C_5 indicates that DNG **1** binds to complimentary pentameric DNA-C_5 in 1:1 ratio, consistent with the formation of a Watson–Crick base paired duplex.

3. Conclusions

The first stepwise solid-phase synthesis of a dual modified ODN with both a positively charged guanidinium backbone and incorporating 7-deazaguanine bases has been accomplished. The trimeric DNG oligomer 3′-OH-c^{7}Ggc^{7}Ggc^{7}G-OH-5′ (**1**) was synthesized in 3′ → 5′ direction on CPG solid support, comparable with standard DNA solid-phase synthesis. The synthesis of orthogonally protected precursor deazaguanyl monomers and a novel facile method for the inversion of 3′-OH functionality to prepare xylo-7-deazaguanine using 4-nitrobenzoic acid under Mitsunobu conditions are described. The synthesized DNG trimer ODN binds to cytidinyl-pentamer in 1:1 ratio. The synthesis of monomers **13** and **19**, as well as DNG **1** having a free hydroxyl groups at both 3′ and 5′ ends described here can allow us to prepare chimera DNA-O-c^{7}Gg-(c^{7}Gg)$_n$-O-DNA or DNA-O-c^{7}Gg-Ag-Tg-Cg-G-O-DNA of desired length. The synthesis of these chimeras is currently underway to fully explore the Watson–Crick base pairing for duplex and triplex formation, and their antisense and antigene properties.



Scheme 4. Reagents and conditions: (a) MsCl, pyridine, 0 °C to rt, 14 h; (b) LiN_3, DMF, 90 °C, 2 h; (c) H_2, Pd/C, EtOH, rt, 5 h; (d) MMTrCl, pyridine, 0 °C to rt 18 h; (e) sodium thiocresolate, DMF, 90 °C, 1 h; (f) succinic anhydride, Et_3N, CH_2Cl_2, 3 h; (g) LCAA-CPG, DMTMM, MeOH, 1 h.

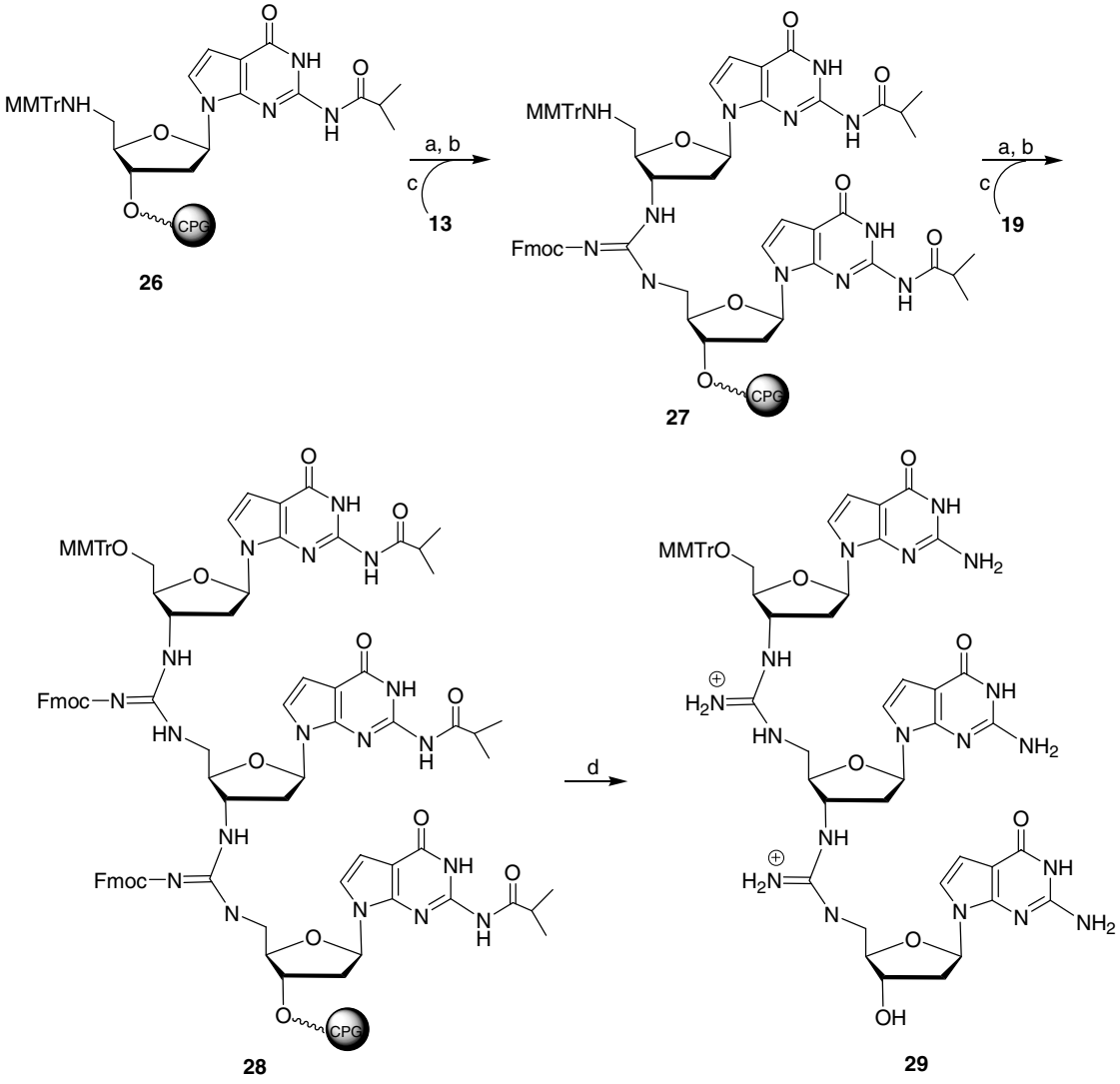
4. Experimental

4.1. Materials

All anhydrous solvents and reagents were purchased from Aldrich and used without further purification. HPLC grade triethylammonium acetate buffer was purchased from Fluka and LCAA-CPG (500 , 80–120 mesh size) was purchased from Sigma. All ^1H and ^{13}C spectra were recorded on 400 and 500 MHz Varian instruments as indicated, using $CDCl_3$ or DMSO-d_6 as solvents, and chemical shifts are reported in δ ppm. TLC was carried out on silica gel (Kieselgel 60 F_{254} glass-backed commercial plates) and visualized by UV light. All reactions were performed under nitrogen unless otherwise indicated. Hydrogenation was carried out with a par hydrogenator equipped with a 500 mL hydrogenation vessel. Reverse-phase HPLC was performed on a Hewlett Packard 1050 system equipped with a quaternary solvent delivery system, UV detector set at 260 nm, and an Altech macrosphere C8 RP semiprep column (10 × 250 mm).

4.2. Synthesis: 2-amino-4-chloro-7-[2′-deoxy-3′,5′-di-*O*-(4-chlorobenzoyl)-β-D-*erythro*-pentofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (3)

To a suspension of sodium hydride (60% emulsion in oil, 1.70 g, 42.5 mmol) in dry acetonitrile (100 mL) was added 2-amino-4-chloropyrrolo[2,3-*d*]pyrimidine (6.50 g, 38.6 mmol) at rt. After 1 h, 1-chloro-2′deoxy-3′5′-di-*O*-(4-chlorobenzoyl)-α-D-*erythro*-pentofuranose (**2**) (18.0 g, 46.4 mmol) was added to the reaction mixture and stirred further for 3 h. The reaction mixture was filtered and solvent was removed under vacuum. The residue was partitioned in CH_2Cl_2 (250 mL) and water (50 mL), and organic phase was dried (anhydrous Na_2SO_4). The residue obtained after removal of the solvent was chromatographed on silica gel (EtOAc/Hexanes 1:4) to afford **3** (15.5 g, 78% yield) as a white foam. ^1H NMR (400 MHz, $CDCl_3$): δ 2.63–2.69 (m, 1H, H-2′), 2.93–2.99 (m, 1H, H-2′), 4.55–4.63 (m, 2H, H-4′ and H-5′), 4.75–4.80 (m, 1H, H-5′), 5.09 (s, 2H, NH_2), 5.76 (m, 1H, H-3′), 6.41 (d, J = 3.6 Hz, 1H, H-5), 6.53–6.56 (dd, J = 6.0, 8.4 Hz, 1H, H-1′), 6.98 (d, J = 3.6 Hz, 1H, H-6),



Scheme 5. Reagents and conditions: (a) Capping: $(CH_3CO)_2O$, TEA, DMF, 10 min; (b) Deprotection: 4% DCA in CH_2Cl_2, 1 min; (c) Coupling: $HgCl_2$, TEA, DMF, 3 h, then 20% PhSH in DMF, 1 min; (d) NH_4OH, 60 °C, 12 h.

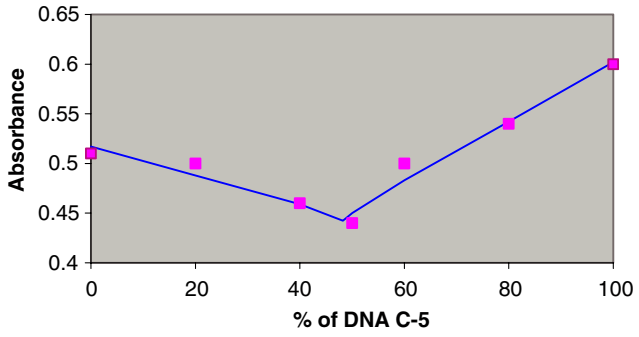


Figure 3. Job plot illustrating 1:1 binding of DNG **1** to DNA C-5. Total oligomer concentration was 4 μmol and buffer contained 100 mM [NaCl], 10 mM [$NaHPO_4$], adjusted to pH 7.1.

7.36–7.47 (m, 4H, Ar*H*), 7.92–8.02 (m, 4H, Ar*H*); ^{13}C NMR (500 MHz, $CDCl_3$): δ 36.8, 64.3, 95.4, 81.6, 84.2, 101.4, 111.3, 122.5, 127.6, 127.8, 128.7, 128.8, 128.9, 129.0, 130.9, 131.0, 131.1, 139.8, 140.1, 152.9, 153.5, 158.6, 165.0, 165.34; HRMS (ESI) *m/z* calcd for $C_{25}H_{19}N_4O_5Cl_3(M+H)^+$ 561.0493. Found 564.0520.

4.3. 2-Amino-7-(2′-deoxy-β-D-*erythro*-pentofuranosyl)-4-methoxy-7*H*-pyrrolo-[2,3-*d*]pyrimidine (4)[40]

Dowex 550A anion exchange resin (41.0 g) was added to a solution of **3** (10.2 g, 18.2 mmol) in anhydrous MeOH (100 mL) and the reaction mixture was heated to reflux for 2 h until all the starting material was consumed, as observed from TLC. The suspension was filtered, washed with hot MeOH and combined filtrates were concentrated under vacuum. The residue was triturated with ether to afford **4** (4.71 g, 93%) as white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.04–2.12 (m, 1H, H-2′), 2.37–2.42 (m, 1H, H-2′), 3.48–3.52 (m, 2H, H-5′). 3.72–3.77 (m, 1H, H-4′), 3.91 (s, 3H, O*CH*$_3$), 4.28–4.31 (m, 1H, H-3′), 4.95 (t, J = 5.4 Hz, 1H, O*H*), 5.23 (d, J = 3.6 Hz, 1H, O*H*), 6.23 (br s, 2H, N*H*$_2$), 6.26 (d, J = 4.0 Hz, 1H, H-5), 6.41 (dd, J = 6.0, 8.4 Hz, 1H, H-1′), 7.10 (d, J = 4.0 Hz, 1H, H-6); HRMS (ESI) *m/z* calcd for $C_{12}H_{16}N_4O_4$ $(M+H)^+$ 281.1244. Found 281.1249.

4.4. 7-(2′-deoxy-β-D-*erythro*-pentofuranosyl)-2-isobutyrylamino-4-methoxy-7*H*-pyrrolo[2,3-*d*]pyrimidine (5)[44]

TMSCl (7.53 g, 69.3 mmol) was added dropwise to a solution of **4** (2.48 g, 8.86 mmol) in anhydrous pyridine (50 mL). After stirring for 0.5 h, isobutyric anhydride (7.34 g, 46.4 mmol) was added to the reaction mixture and let stirred for 6 h at rt. The reaction was quenched by addition of water (40 mL) and NH_4OH (10 mL) and held for 0.5 h. The oil obtained after removal of the volatiles under vacuum was diluted with CH_2Cl_2 (150 mL) and washed with water (50 mL). The organic-phase dried (Na_2SO_4), concentrated under vacuum, and the residue was chromatographed over silica gel (MeOH/EtOAc 1:9) to afford **5** (2.17 g, 70% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.09 (d, J = 6.8 Hz, 6H, (CH_3)$_2$), 2.13–2.19 (m, 1H, H-2′), 2.45–2.52 (m, 1H, H-2′ partially merged with solvent peak), 2.85–2.92 (m, 1H, CH), 3.47–3.57 (m, 2H, H-5′), 3.76–3.82 (m, 1H, H-4′), 4.02 (s, 3H, OCH_3), 4.32–4.37 (m, 1H, H-3′), 4.91 (t, J = 5.2 Hz, 1H, OH), 5.28 (d, J = 3.6 Hz, 1H, OH), 6.48 (d, J = 3.6 Hz, 1H, H-5), 6.53 (dd, J = 6.0, 8.4 Hz, 1H, H-1′), 7.48 (d, J = 3.6 Hz, 1H, H-6), 10.24 (s, 1H, NH); ^{13}C NMR (500 MHz, DMSO-d_6): δ 19.4, 19.4, 34.2, 39.0–40.0 (one signal merged with solvent peaks), 53.5, 61.9, 71.0, 82.4, 87.2, 99.2, 101.2, 123.0, 151.6, 152.5, 162.4, 175.0; HRMS (ESI) m/z calcd for $C_{16}H_{22}N_4O_5$ $(M+Na)^+$ 373.1482. Found 373.1501.

4.5. 7-[2′-Deoxy-3′,5′-di-*O*-(4-nitrobenzoyloxy)-β-D-*threo*-pentofuranosyl]-2-isobutyrylamino-4-methoxy-7*H*-pyrrolo[2,3-*d*]pyrimidine (6)

To a solution of **5** (3.40 g, 9.71 mmol) in anhydrous THF (50 mL) were added PPh_3 (7.67 g, 29.2 mmol) and DIAD (5.9 g, 29.4 mmol) at rt. 4-Nitrobenzoic acid (4.90 g, 29.3 mmol) was added to the reaction mixture after 20 min and the reaction mixture was stirred further for 4 h. The solvent was removed under vacuum and the residue was chromatographed over silica gel (EtOAc/Hexanes 1:5) to afford **6** (4.76 g, 77%) as a yellow foam. ^{1}H NMR ($CDCl_3$): δ 1.27 (d, J = 6.8 Hz, 3H, CH_3), 1.29 (d, J = 6.8 Hz, 3H, CH_3), 2.92–3.03 (m, 1H, H-2′), 3.03–3.11 (m, 2H, CH and H-2′), 4.04 (s, 3H, OCH_3), 4.62–4.66 (m, 1H, H-4′), 4.74 (d, J = 5.6 Hz, 2H, H-5′), 5.92 (t, J = 4.0 Hz, 1H, H-3′), 6.52 (d, J = 3.6 Hz, 1H, H-5), 6.63 (dd, J = 3.2, 7.6 Hz, 1H, H-1′), 7.23 (d, J = 3.6 Hz, 1H, H-6), 7.75 (s, 1H NH), 8.08 (d, J = 8.4 Hz, 2H, ArH), 8.16 (d, J = 8.4 Hz, 2H, ArH), 8.26 (dd, J = 3.6, 8.4 Hz, 4H, ArH); ^{13}C NMR (500 MHz, $CDCl_3$): δ 19.5, 19.5, 36.2, 39.0, 54.0, 63.5, 74.3, 79.4, 83.5, 100.4, 102.5, 121.6, 123.7, 123.9, 128.3, 130.9, 131.0, 132.4, 134.5, 134.8, 150.8, 151.0, 151.6, 152.6, 163.5, 163.8, 164.4, 174.8; HRMS (ESI) m/z calcd for $C_{30}H_{28}N_6O_{11}$ $(M+H)^+$ 649.1888. Found 649.1917.

4.6. 7-[2′-Deoxy-β-D-*threo*-pentofuranosyl]-2-isobutyrylamino-4-methoxy-7*H*-pyrrolo[2,3-*d*]pyrimidine (7)

A suspension of **6** (4.4 g, 6.79 mmol) in methanolic ammonia (40 mL) was stirred for 1 h at rt. The homogeneous solution was concentrated under vacuum and the residue was chromatographed on silica gel (EtOAc/MeOH 1:5) to give desired product (2.0 g, 85%) as white foam. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.08 (d, J = 6.8 Hz, 6H, (CH_3)$_2$), 2.07–2.12 (m, 1H, H-2′), 2.71–2.78 (m, 1H, H-2′), 2.85–2.90 (m, 1H, CH), 3.56–3.62 (m, 1H, H-5′), 3.68–3.74 (m, 1H, H-5′), 3.82–3.86 (m, 1H, H-4′), 4.02 (s, 3H, OCH_3), 4.30–4.34 (m, 1H, H-3′), 4.63 (t, J = 6.0 Hz, 1H, OH), 5.33 (d, J = 4.0 Hz, 1H, OH), 6.42 (dd, J = 2.8, 8.4 Hz, 1H, H-1′), 6.46 (d, J = 3.6 Hz, 1H, H-5), 7.64 (d, J = 3.6 Hz, 1H, H-6), 10.20 (s, 1H, NH); ^{13}C NMR (500 MHz, $CDCl_3$): δ 19.9, 19.9, 34.8, 41.4, 54.1, 60.5, 69.6, 82.1, 85.0, 99.4, 101.6, 124.9, 152.0, 152.8, 163.0, 175.8; HRMS (ESI) m/z calcd for $C_{16}H_{22}N_4O_5$ $(M+Na)^+$ 373.1482. Found 373.1467.

4.7. 7-[2′-Deoxy-3′,5′-di-*O*-mesyl-β-D-*threo*-pentofuranosyl]-2-isobutyrylamino-4-methoxy-7*H*-pyrrolo[2,3-*d*]pyrimidine (8)

To a solution of **7** (2.15 g, 6.5 mmol) in anhydrous pyridine (30 mL) were added MsCl (2.40 mL, 3.52 g, 30.8 mmol) and DMAP (0.21 g, 1.71 mmol) at rt. The reaction mixture was quenched with MeOH after stirring for 15 h and concentrated. The residue was dissolved in CH_2Cl_2 (150 mL) and washed with water (2× 50 mL). The organic layer was dried (Na_2SO_4), concentrated under vacuum, and the residue was purified by silica gel chromatography (EtOAc/hexanes 1:5) to afford **8** as a white foam (2.72 g, 88%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.28 (d, J = 6.8 Hz, 6H, (CH_3)$_2$), 2.84–2.90 (m, 1 H, H-2′) 2.95–3.00 (m, 1H, H-2′), 3.05–3.08 (m, 4H, SO_2CH_3, and CH), 3.13 (s, 3H, SO_2CH_3), 4.07 (s, 3H, OCH_3), 4.37–4.42 (m, 1H, H-4′), 4.47–4.56 (m, 2H, H-5′) 5.39–5.42 (m, 1H, H-3′), 6.54 (d, J = 3.6 Hz, 1H, H-5), 6.64 (dd, J = 4.0, 8.0 Hz, 1H, H-1′), 7.21 (d, J = 3.6 Hz, 1H, H-6), 7.80 (br s, 1H, NH); ^{13}C NMR (500 MHz, $CDCl_3$): 19.5, 19.5, 35.8, 37.8, 38.8, 39.0, 54.1, 66.2, 78.2, 78.5, 82.5, 101.1, 102.3, 122.1, 151.8, 153.0, 163.6, 176.6; HRMS (ESI) m/z calcd for $C_{18}H_{26}N_4O_9S_2$ $(M+H)^+$ 507.1213. Found 507.1229.

4.8. 7-[3′,5′-Diazido-2′3′-dideoxy-β-D-*erythro*-pentofuranosyl]-2-isobutyrylamino-4-methoxy-7*H*-pyrrolo[2,3-*d*]pyrimidine (9)

Lithium azide (2.60 g, 54.1 mmol) was added to a solution of **8** (2.70 g, 5.34 mmol) in anhydrous DMF (20 mL). The reaction mixture was heated at 90 °C until all the starting material was consumed (2 h). The solvent was evaporated under vacuum and the residue was chromatographed over silica gel (EtOAc/hexanes 2:3) to afford **9** as a white foam (1.70 g, 80%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.28 (d, J = 6.8 Hz, 3H, CH_3), 1.30 (d, J = 6.8 Hz, 3H, CH_3), 2.44–2.51 (m, 1H, H-2′), 3.02–3.10 (m, 2H, H-2′, and CH), 3.60 (dd, J = 4.0, 13.2 Hz, 1H, H-5′), 3.69 (dd, J = 4.0, 13.2 Hz, 1H, H-5′), 3.98–4.02 (m, 1H, H-4′), 4.05 (s, 3H, OCH_3), 4.88 (br s, 1H, H-3′), 6.30 (dd, J = 5.6, 7.2 Hz, 1H, H-1′), 6.47 (d, J = 3.6 Hz, 1H, H-5), 7.03 (d, J = 3.6 Hz, 1H, H-6), 7.80 (br s, 1H, NH); ^{13}C NMR (500 MHz, $CDCl_3$): 19.5, 19.6, 36.2, 37.1, 52.3, 54.0, 61.5, 82.7, 85.1, 100.1, 103.1, 123.6, 151.3, 152.3,

163.6, 175.8; HRMS (ESI) *m/z* calcd for $C_{16}H_{20}N_{10}O_3$ $(M+H)^+$ 401.1792. Found 401.1807.

4.9. 7-[3′,5′-Diazido-2′3′-dideoxy-β-D-*erythro*-pentofuranosyl]-2-isobutyrylamino-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one (10)

Sodium thiocresolate (1.84 g, 12.6 mmol) was added to a solution of **9** (0.75 g, 1.87 mmol) in anhydrous DMF (15.0 mL) and the reaction mixture was heated at 90 °C for 1 h. The solvent was removed under vacuum and the residue was chromatographed over silica gel (EtOAc/hexanes 3:2) to give **10** as a yellow foam (0.70 g, 96%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.25 (d, $J = 6.8$ Hz, 3H, CH_3), 1.27 (d, $J = 6.8$ Hz, 3H, CH_3), 2.41–2.47 (m, 1H, H-2′), 2.58–2.71 (m, 2H, H-2′ and C*H*), 3.48 (dd, $J = 4.0$, 13.2 Hz, 1H, H-5′), 3.64 (dd, $J = 4.0$, 13.2 Hz, 1H, H-5′), 3.97–4.01 (m, 1H, H-4′), 4.31–4.36 (m, 1H, H-3′), 6.34 (t, $J = 6.4$ Hz, 1H, H-1′), 6.71 (d, $J = 3.6$ Hz, 1H, H-5), 6.95 (d, $J = 3.6$ Hz, 1H, H-6), 8.16 (br s, 1H, N*H*), 11.76 (br s, 1H, N*H*); ^{13}C NMR (500 MHz, $CDCl_3$): 19.2, 19.2, 36.7, 37.5, 52.3, 61.2, 82.2, 83.1, 104.9, 105.8, 118.8, 146.4, 147.6, 157.9, 178.4; HRMS (ESI) *m/z* calcd for $C_{15}H_{18}N_{10}O_3$ $(M+H)^+$ 387.1636. Found 387.1619.

4.10. 7-[3′,5′-Diamino-2′,3′-dideoxy-β-D-*erythro*-pentofuranosyl]-2-isobutyrylamino-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one (11)

To a solution of **10** (0.44 g, 1.14 mmol) in absolute EtOH (50 mL), 10% Pd/C (0.10 g) was added and the suspension was shaken under hydrogen gas (45 psi) for 5 h. The reaction mixture was filtered through a pad of Celite and washed with ethanol (2× 5 mL). Combined filtrate was concentrated under vacuum and the residue was evaporated twice with CH_2Cl_2 to afford **11** (0.39 g, 98%) as pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.11 (d, $J = 6.8$ Hz. 6H, (CH_3)$_2$), 2.11 (m, 1H, H-2′) 2.36 (m, 1H, H-2′), 2.76 (m, 3H, H-5′, and C*H*), 3.51 (m, 2H, H-4′, and H-3′), 6.34 (t, $J = 6.8$ Hz, 1H, H-1′), 6.48 (d, $J = 3.6$ Hz, 1H, H-5), 7.20 (d, $J = 3.6$ Hz, 1H, H-6), 8.26 (s, 1H, NH); ^{13}C NMR (500 MHz, DMSO-d_6): δ 18.9, 18.9, 34.7, 40.5, 43.8, 52.6, 81.9, 87.7, 102.9, 104.0, 119.6, 146.8, 147.3, 156.7, 180.0; HRMS (ESI) *m/z* calcd for $C_{15}H_{22}N_6O_3$ $(M+H)^+$ 335.1826. Found 335.1834.

4.11. 7-[3′-Amino-5′-*N*-(4-monomethoxytritylamino)-2′,3′-dideoxy-β-D-*erythro*-pentofuranosyl]-2-isobutyrylamino-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one (12)

A solution of MMTrCl (0.47 g, 1.52 mmol) in CH_2Cl_2 (10.0 mL) was added dropwise over 1 h to a cooled (−40 °C) suspension of **11** (0.50 g, 1.43 mmol) and diethylamine (0.3 mL, 2.88 mmol) in CH_2Cl_2 (35 mL). The reaction mixture was warmed to room temperature after 1 h and quenched with MeOH (5 mL). The solvent was removed under vacuum and the residue was chromatographed (MeOH/EtOAc 1:20) to afford **12** (0.59 g, 68%). ^{1}H NMR ($CDCl_3$): δ 1.18 (d, $J = 6.8$ Hz, 3H, CH_3), 1.20 (d, $J = 6.8$ Hz, 3H, CH_3), 2.10–2.17 (m, 1H, H-2′), 2.29–2.42 (m, 2H, H-2′ and C*H*), 2.49–2.59 (m, 2H, H-5′), 3.70–3.81 (m, 5H, H-3′, H-4′ and OCH_3), 6.27 (dd, $J = 4.8$, 6.8 Hz, 1H, H-1′), 6.60 (d, $J = 3.2$ Hz, 1H, H-5), 6.62 (d, $J = 3.2$ Hz, 1H, H-6), 6.77 (d, $J = 8.8$ Hz, 2H, Ar*H*), 7.17–7.31 (m, 10H, Ar*H*), 7.41 (d, $J = 8.8$ Hz, 2H, Ar*H*), 8.89 (br s, 2H, NH_2), 10.29, (br s, 1H, N*H*); ^{13}C NMR (500 MHz, $CDCl_3$): δ 19.1, 19.2, 36.6, 41.2, 45.9, 53.6, 55.4, 70.3, 82.6, 86.9, 104.3, 105.4, 113.3, 113.4, 119.0, 126.6, 128.1, 128.7, 130.0, 137.8, 146.0, 146.4, 147.5, 158.1, 158.1, 178.8. HRMS (ESI) *m/z* calcd for $C_{35}H_{38}N_6O_4$ $(M+H)^+$ 607.3027. Found 607.3054.

4.12. 7-[3′-*N*-(9-fluorenylmethoxycarbonylamino)-5′-*N*-(4-monomethoxytritylamino)-2′,3′-dideoxy-β-D-pentofuranosyl]-2-isobutyrylamino-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one (13)

A solution of Fmoc-NCS (0.29 g, 1.04 mmol) in CH_2Cl_2 (5 mL) was added to a solution of **12** (0.43 g 0.70 mmol) in anhydrous CH_2Cl_2 (50 mL) and stirred for 2 h at rt. The reaction mixture was concentrated and the crude product was purified by ether precipitation to give **13** (0.48 g, 76% yield) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.10 (d, $J = 7.2$ Hz, 3H, CH_3), 1.18 (d, $J = 7.2$ Hz, 3H, CH_3), 2.32–2.49 (m, 5H, H-2′, C*H*, and H-5′), 3.20–3.26 (m, 1H, H-3′), 3.74 (s, 3H, OCH_3), 4.02–4.06 (m, 1H, H-4′), 4.25 (t, $J = 6.4$ Hz, 1H, OCH$_2$C*H*), 4.54 (d, $J = 6.8$ Hz, 2H, OCH_2), 6.05 (dd, $J = 2.8$, 8.4 Hz, 1H, H-1′), 6.18–6.24 (m, 1H, N*H*), 6.34 (d, $J = 3.6$ Hz, 1H, H-5), 6.74 (d, $J = 8.8$ Hz, 2H, Ar*H*), 6.77 (d, $J = 3.6$ Hz, 1H, H-6), 7.10–7.57 (m, 18H, Ar*H*), 7.79 (d, $J = 8.0$ Hz, 2H, Ar*H*), 8.27 (br s, 1H, N*H*), 8.59 (s, 1H, N*H*), 9.85 (br s, 1H, N*H*), 11.70 (br s, 1H, N*H*); ^{13}C NMR (500 MHz, $CDCl_3$): δ 19.0, 19.1, 36.8, 37.2, 44.2, 46.6, 55.4, 56.4, 68.7, 70.0, 83.3, 85.1, 103.9, 106.6, 113.2, 120.5, 121.9, 124.9, 126.5, 127.5, 128.0, 128.2, 128.4, 128.6, 129.9, 137.9, 141.6, 142.8, 142.9, 145.8, 146.0, 146.1, 146.7, 152.8, 157.9, 158.0, 178.3, 179.7; HRMS (ESI) *m/z* calcd for $C_{51}H_{49}N_7O_6S$ $(M+Na)^+$ 910.3357. Found 910.3314.

4.13. 7-[2′-Deoxy-5′-*O*-(4-monomethoxytrityl)-β-D-*threo*-pentofuranosyl]-2-isobutyrylamino-4-methoxy-7*H*-pyrrolo[2,3-*d*]pyrimidine (14)

MMTrCl (1.27 g, 4.10 mmol) was added dropwise to a solution of **13** (1.25;g, 3.57 mmol) in anhydrous pyridine (50 mL). After 18 h, the reaction mixture was quenched with MeOH (5 mL) and concentrated under vacuum. The residue was purified by silica gel chromatography (EtOAc/hexanes 4:1) to afford **14** (1.82 g, 82%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.24 (d, $J = 6.8$ Hz, 3H, CH_3), 1.26 (d, $J = 6.8$ Hz, 3H, CH_3), 2.54–2.59 (m, 1H, H-2′) 2.72–2.80 (m, 1H, H-2′), 3.05 (br s, 1H, C*H*), 3.51–3.55 (m, 1H, H-5′), 3.58–3.62 (m, 1H, H-5′), 3.78 (s, 3H, MMTr-OCH_3), 4.0–4.06 (m, 1H, H-4′), 4.07 (s, 3H, OCH_3), 4.42–4.50 (m, 1H, H-3′), 4.92 (d, $J = 3.2$ Hz, 1H, O*H*), 6.19 (dd, $J = 3.2$, 9.2 Hz, 1H, H-1′), 6.43 (d, $J = 3.6$ Hz, 1H, H-5), 6.79 (d, $J = 8.8$ Hz, 2H, Ar*H*), 7.16–7.45 (m, 11H, H-6, and Ar*H*), 7.45 (d, $J = 7.2$ Hz, 2H, Ar*H*) 7.64 (s, 1H, N*H*). ^{13}C NMR (500 MHz, $CDCl_3$): δ 19.5, 19.6, 35.9, 40.6,

54.14, 55.40, 62.6, 71.5, 82.8, 85.3, 87.0, 99.2, 103.4, 113.4, 125.5, 126.4, 127.4, 128.0, 128.1, 128.6, 129.4, 130.6, 135.6, 144.5, 147.3, 151.1, 151.6, 158.7, 163.5, 176.2; HRMS (ESI) *m/z* calcd for $C_{36}H_{38}N_4O_6$ $(M+Na)^+$ 645.2683. Found 645.2665.

4.14. 7-[2′-Deoxy-3′-*O*-mesyl-5′-*O*-(4-monomethoxytrityl)-β-D-*threo*-pentofuranosyl]-2-isobutyryl-4-methoxy-7*H*-pyrrolo[2,3-*d*]pyrimidine (15)

MsCl (0.81 g, 7.05 mmol) was added to a mixture of **14** (1.75 g, 2.81 mmol) and DMAP (1.80 g, 14.1 mmol) in anhydrous pyridine at 0 °C. After 10 min, reaction mixture was allowed to warm to rt and stirred for 16 h. The reaction mixture was partitioned between water and CH_2Cl_2. Organic phase was washed with water, dried (Na_2SO_4), and concentrated. The residue was purified on silica gel (EtOAc/hexanes 3:2) to afford **15** (1.52 g, 75%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.27 (d, J = 6.8 Hz, 6H, $(CH_3)_2$), 2.70–2.75 (m, 2H, H-2′), 2.78 (s, 3H, SO_2CH_3), 3.22 (br s, 1H, C*H*), 3.29–3.33 (m, 1H, H-5′), 3.63–3.67 (m, 1H, H-5′), 3.80 (s, 3H, MMTr-OCH_3), 4.05 (s, 3H, OCH_3), 4.22–4.27 (m, 1H, H-4′), 5.41–5.44 (m, 1H, H-3′), 6.46 (d, J = 3.6 Hz, 1H, H-5), 6.52 (dd, J = 3.2, 8.8 Hz, 1H, H-1′), 6.84 (m, 2H, Ar*H*), 7.17 (d, J = 3.6 Hz, 1H, H-6), 7.22–7.32 (m, 10H, Ar*H*), 7.41 (d, J = 7.2 Hz, 2H, Ar*H*), 7.82 (s, 1H, N*H*); ^{13}C NMR (500 MHz, $CDCl_3$): δ 19.5, 19.6, 35.8, 38.7, 39.9, 54.1, 55.5, 60.9, 79.6, 80.7, 81.9, 87.3, 100.7, 102.2, 113.4, 122.4, 127.4, 128.0, 128.1, 128.2, 128.4, 128.5, 130.4, 135.1, 143.9, 144.0, 151.7, 153.0, 158.9, 163.5, 175.8; HRMS (ESI) *m/z* calcd $C_{37}H_{40}N_4O_8S$ $(M+H)^+$ 701.2639. Found 701.2625.

4.15. 7-[3′-Azido-5′-*O*-(4-monomethoxytrityl)-2′,3′-dideoxy–β-D-*erythro*-pentofuranosyl]-2-isobutyrylamino-4-methoxy-7*H*-pyrrolo[2,3-*d*]pyrimidine (16)

To a solution of **15** (1.40 g, 2.0 mmol) in anhydrous DMF (20 mL) was added lithium azide (0.5 g, 10.4 mmol) and the reaction mixture was heated at 90 °C until all starting material was consumed (2 h). The solvent was removed under vacuum and the residue was taken in CH_2Cl_2 and filtered. The filtrate was concentrated and the residue was chromatographed over silica gel (EtOAc/hexanes 2:5) to afford the desired product **16** (0.93 g, 73%) as a white foam. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.25 (d, J = 6.8 Hz, 6H, $(CH_3)_2$), 2.44–2.50 (m, 1H, H-2′), 2.72–2.80 (m, 1H, H-2′), 3.20 (m, 1H, C*H*), 3.32 (dd, J = 4.0, 10.4 Hz, 1H, H-5′), 3.39 (dd, J = 4.0, 10.4 Hz, 1H, H-5′), 3.79 (s, 3H, MMTr-OCH_3), 4.0–4.04 (m, 1H, H-4′), 4.07 (s, 3H, OCH_3), 4.51–4.56 (m, 1H, H-3′), 6.44 (d, J = 3.6 Hz, 1H, H-5), 6.48 (t, J = 6.4 Hz, 1H, H-1′), 6.80 (d, J = 8.8 Hz, 2H, Ar*H*), 7.09 (d, J = 3.6 Hz, 1H, H-6), 7.20–7.31 (m, 10H, Ar*H*), 7.41 (d, J = 7.2 Hz, 2H, Ar*H*), 7.73 (br s, 1H, N*H*). ^{13}C NMR (500 MHz, $CDCl_3$): 19.5, 19.5, 35.5, 37.8, 54.1, 55.4, 61.2, 63.5, 83.2, 83.7, 87.0, 100.2, 102.5, 113.4, 122.4, 127.2, 127.4, 128.0, 128.1, 128.5, 128.6, 129.4, 130.6, 135.3, 144.2, 144.3, 151.63, 152.6, 158.8, 163.5, 176.2; HRMS (ESI) *m/z* calcd for $C_{36}H_{37}N_7O_5$ $(M+H)^+$ 648.2928. Found 648.2936.

4.16. 7-[3′-Azido-2′,3′-dideoxy-5′-*O*-(4-monomethoxytrityl)-β-D-*erythro*-pentofuranosyl]-2-isobutyrylamino-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one (17)

Sodium thiocresolate (0.72 g, 4.98 mmol) was added to a solution of **16** (1.01 g, 1.54 mmol) in anhydrous DMF (5 mL) and the reaction mixture was heated at 90 °C until all starting material was consumed, as shown by TLC (1 h). The solvent was removed under vacuum and the residue was chromatographed over silica gel (EtOAc/hexanes 3:2) to give **17** as a white foam (0.90 g, 88%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.14 (d, J = 6.8 Hz, 3H, CH_3), 1.21 (d, J = 6.8 Hz, 3H, CH_3), 2.29–2.42 (m, 2H, H-2′ and C*H*), 2.67–2.74 (m, 1H, H-2′), 3.26 (dd, J = 3.6, 10.4 Hz, 1H, H-5′), 3.38 (dd, J = 3.6, 10.4 Hz, 1H, H-5′), 3.79 (s, 3H, MMTr-OCH_3), 4.05 (q, J = 4.0, 8.0 Hz, 1H, H-4′), 4.37–4.41 (m, 1H, H-3′), 6.30 (t, J = 7.2 Hz, 1H, H-1′), 6.62 (d, J = 3.2 Hz, 1H, H-5), 6.82 (d, J = 8.4 Hz, 2H, Ar*H*), 6.88 (d, J = 3.6 Hz, 1H, H-6), 7.17–7.33 (m, 10H, Ar*H*), 7.42 (d, J = 7.2 Hz, 2H, Ar*H*), 7.87 (br s, 1H, N*H*), 11.68 (br s, 1H, N*H*). ^{13}C NMR (500 MHz, $CDCl_3$): 19.1, 19.1, 36.7, 37.6, 55.5, 61.5, 63.6, 83.2, 83.5, 87.1, 104.4, 105.9, 113.4, 119.4, 127.4, 128.0, 128.1, 128.2, 128.5, 129.4, 130.6, 135.2, 144.1, 144.2, 146.2, 147.6, 158.0, 159.0, 178.4; HRMS (ESI) *m/z* calcd for $C_{35}H_{35}N_7O_5$ $(M+Na)^+$ 656.2591. Found 656.2586.

4.17. N^2-Isobutyryl-7-[3′-amino-2′3′-dideoxy-5′-*O*-(4-monomethoxytrityl)-β-D-*erythro*-pentofuranosyl]-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrmidin-4-one

To a solution of **17** (0.78 g, 1.2 mmol) in EtOH (125 mL) was added Pd/C (0.14 g, 10% wet), and the suspension was shaken over hydrogen gas (45 psi) for 6 h. The suspension was filtered through a pad of Celite and filtrate was concentrated to an oil. The residue was evaporated with CH_2Cl_2 to afford **18** as a pale yellow foam (0.70 g, 94%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.22 (dd, J = 4.8, 6.8 Hz, 6H, $CH_3)_2$), 2.17–2.22 (m, 1H, H-2′), 2.46–2.55 (m, 2H, H-2′ and C*H*), 3.30–3.38 (m, 2H, H-5′), 3.74–3.82 (m, 5H, H-3′, H-4′ and MMTr-OCH_3), 6.35 (dd, J = 4.4, 6.8 Hz, 1H, H-1′), 6.60 (d, J = 3.6 Hz, 1H, H-5), 6.80 (d, J = 8.8 Hz, 2H, Ar*H*), 6.86 (d, J = 3.6 Hz, 1H, H-6), 7.20–7.31 (m, 10H, Ar*H*), 7.41 (d, J = 7.2 Hz, 2H, Ar*H*), 8.18 (br s, 1H, N*H*), 11.84 (s, 1H, N*H*); ^{13}C NMR (500 MHz, $CDCl_3$): δ 19.2, 19.2, 36.7, 41.1, 53.0, 55.4, 64.2, 82.9, 86.1, 86.5, 104.1, 105.6, 113.3, 119.3, 127.3, 128.1, 128.6, 130.6, 135.4, 144.3, 146.1, 147.4, 158.0, 158.8, 178.4; HRMS (ESI) *m/z* calcd for $C_{35}H_{37}N_5O_5$ $(M+H)^+$ 608.2867. Found 608.2866.

4.18. 7-[2′Deoxy-3′-*N*-(9-fluorenylmethoxycarbonylamino)-5′-*O*-(4-monomethoxytrityl)-β-D-*erythro*-pentofuranosyl]-2-isobutyrylamino-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one (19)

A solution of Fmoc-NCS (0.24 g, 0.85 mmol) in CH_2Cl_2 (10 mL) was added to a solution of **18** (0.41 g, 0.68 mmol) in CH_2Cl_2 (50 mL), and stirred for 1.5 h. The reaction mixture was concentrated and the residue upon precipitation with ether afforded the desired product (0.47 g, 78%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.17 (d, J = 6.8 Hz, 3H,

CH_3), 1.20 (d, J = 6.8 Hz, 3H, CH_3), 2.31–2.44 (m, 3H, H-2′ and CH), 3.14–3.24 (m, 1H, H-3′), 3.32–3.42 (m, 2H, H-5′), 3.74 (s, 3H, OCH_3), 4.04–4.08 (m, 1H, H-4′), 4.23 (t, J = 6.8 Hz, 1H, OCH$_2$CH), 4.45–4.55 (m, 2H, OCH_2), 5.70–5.76 (m, 1H, NH), 6.12 (dd, J = 4.0, 7.6 Hz, 1H, H-1′), 6.60, (d, J = 3.2 Hz, 1H, H-5), 6.76 (d, J = 8.8 Hz, 2H, ArH), 6.84 (d, J = 3.2 Hz, 1H, H-6), 7.15–7.56 (m, 18H, ArH), 7.80 (d, J = 7.6 Hz, 2H, ArH), 8.12 (s, 1H, NH), 8.36 (s, 1H, NH), 9.88 (br s, 1H, NH), 11.64 (s, 1H, NH); ^{13}C NMR (500 MHz, $CDCl_3$): 19.1, 19.2, 36.8, 37.4, 46.6, 55.4, 57.2, 64.2, 68.69, 82.8, 84.8, 87.0, 104.0, 106.3, 113.2, 120.5, 121.3, 125.0, 127.2, 127.5, 128.0, 128.1, 128.4, 128.6, 130.6, 135.3, 141.5, 142.9, 144.2, 145.8, 146.9, 152.8, 158.0, 158.8, 178.2, 179.5; HRMS (ESI) m/z calcd for $C_{51}H_{48}N_6O_7S$ (M+Na)$^+$ 911.3197, found 911.3241.

4.19. 7-[2′-Deoxy-5′-*O*-mesyl-β-D-ery*thro*-pentofuranosyl)-2-isobutyrylamino-4-methoxy-7*H*-pyrrolo[2,3-*d*]pyrimidine (20)

MsCl (0.90 g, 7.82 mmol) was added dropwise to a cooled solution (0 °C) of **5** (2.50 g, 7.14 mmol) in anhydrous pyridine (50 mL). The reaction mixture was warmed to rt and let stirred for 14 h. The residue obtained after removing the solvent under vacuum was diluted with CH_2Cl_2 (150 mL), washed with water (40 mL), and dried (Na_2SO_4). The solvent was removed under vacuum and the residue was chromatographed over silica gel (EtOAc/hexanes 4:1—MeOH/EtOAc 1:20) to afford **20** as a white foam (2.22 g, 73% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.28 (d, J = 7.2 Hz, 6H, (CH_3)$_2$), 2.46–2.52 (m, 2H, H-2′), 2.82–2.89 (m, 1H, CH), 2,95 (s, 3H, OSO$_2$CH_3), 3.54 (br s, 1H, OH), 4.05 (s, 3H, OCH_3), 4.28–4.31 (m, 1H, H-3′), 4.50–4.54 (m, 2H, H-5′), 4.80–4.86 (m, 1H, H-4′), 6.49 (d, J = 3.6 Hz, 1H, H-5), 6.72 (t, J = 6.8 Hz, 1H, H-1′), 7.10 (d, J = 3.6 Hz, 1H, H-6), 7.89 (s, 1H, NH); ^{13}C NMR ($CDCl_3$,): δ 19.7,19.7, 36.6, 37.5, 39.7, 54.1, 70.2, 72.1, 84.3, 84.8, 100.3, 103.2, 123.2, 151.3, 152.7, 163.5, 176.0; HRMS (ESI) m/z calcd for $C_{17}H_{24}N_4O_7S$ (M+Na)$^+$ 451.1257. Found 451.1242.

4.20. 7-[5′-azido-2′-deoxy-β-D-*erythro*-pentofuranosyl]-2-isobutyrylamino-4-methoxy-7*H*-pyrrolo[2,3-*d*]pyrimidine (21)

Lithium azide (1.20 g, 20.8 mmol) was added to a solution of **20** (2.10 g, 4.90 mmol) in anhydrous DMF (20 mL) and the reaction mixture was heated at 90 °C for 2 h. The solvent was removed under vacuum and the residue was taken in CH_2Cl_2 (75 mL) and filtered. The filtrate was concentrated and the residue was purified by silica gel chromatography (MeOH/EA 1:9) to afford **21** as a white foam (1.54 g, 81%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.28 (d, J = 6.8 Hz, 6H, (CH_3)$_2$), 2.47–2.52 (m, 1H, H-2′), 2.60–2.67 (m, 1H, H-2′), 2.95 (br s, 1H, CH), 3.58–3.68 (m, 2H, H-5′), 4.03 (s, 3H, OCH_3), 4.20–4.24 (m, 1H, H-4′), 4.50 (br s, 1H, OH) 4.63–4.66 (m, 1H, H-3′), 6.50 (d, J = 3.6 Hz, 1H, H-5), 6.83 (t, J = 6.8 Hz, 1H, H-1′), 7.19 (d, J = 3.6 Hz, 1H, H-6), 8.01 (s, 1H, NH); ^{13}C NMR (500 MHz, $CDCl_3$,): δ 19.6, 19.6, 37.5, 40.3, 52.9, 54.0, 72.6, 83.9, 85.3, 100.2, 102.9, 122.6, 151.1, 152.7, 163.4, 176.1; HRMS (ESI) m/z calcd for $C_{16}H_{21}N_7O_4$ (M+H)$^+$ 376.1727. Found 376.1727.

4.21. 7-[5′-Amino-2′-deoxy-β-D-*erythro*-pentofuranosyl]-2-isobutyrylamino-4-methoxy-7*H*-pyrrolo[2,3-*d*]pyrimidine (22)

Pd/C (10% wet, 0.30 g) was added to a solution of **21** (1.25 g, 3.20 mmol) in anhydrous EtOH (20 mL) and hydrogenated at 45 psi for 5 h. The reaction mixture was filtered through a pad of Celite and washed with ethanol (25 mL). The solvent was removed from the combined filtrates and the residue was evaporated twice with CH_2Cl_2 to afford **22** as a yellow solid (1.08 g, 93%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.10 (d, J = 6.8 Hz, 6H, (CH_3)$_2$), 2.15–2.20 (m, 1H, H-2′), 2.49–2.56 (m, 1H, H-2′, partially merged with solvent peak), 2.64–2.74, (m, 2H, H-5′), 2.84–2.92 (m, 1H, CH), 3.68–3.74 (m, 1H, H-4′), 4.02 (s, 3H, OCH_3), 4.31–4.35 (m, 1H, H-3′), 5.30 (br s, 1H, OH), 6.48 (d, J = 3.6 Hz, 1H, H-5), 6.50 (t, J = 8.4 Hz, 1H, H-1′, partially merged with H-5 peak), 7.46 (d, J = 3.6 Hz, 1H, H-6), 10.24 (s, 1H, NH). ^{13}C NMR (500 MHz, DMSO-d_6): δ 19.4, 19.4, 34.3, 39.0–40.0 (one signal merged with solvent peaks), 44.1, 53.5, 71.2, 82.3, 87.8, 99.2, 101.2, 123.1, 151.6, 152.5, 162.5, 175.1; HRMS (ESI) m/z calcd for $C_{16}H_{23}N_5O_4$ (M+H)$^+$ 350.1822. Found 350.1826.

4.22. 7-[2′-Deoxy-5′-*N*-(4-monomethoxytritylamino)-β-D-*erythro*-pentofuranosyl]-2-isobutyrylamino-4-methoxy-7*H*-pyrrolo[2,3-*d*]pyrimidine (23)

MMTrCl (0.93 g, 3.01 mmol) was added dropwise to a cooled solution (0 °C) of **22** (1.01 g, 2.75 mmol) in anhydrous pyridine over 15 min. and reaction mixture was allowed to warm to rt and stirred for 18 h. The solvent was removed under vacuum and the residue was taken in CH_2Cl_2 (150 mL), washed with water (40 mL), dried (Na_2SO_4), and concentrated to an oil. The crude product was purified by silica gel chromatography (EtOAc/hexanes 4:1) to afford **23** as a yellow foam (1.27 g, 73%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.25 (d, J = 6.8 Hz, 6H, (CH_3)$_2$), 2.30–2.37 (m, 1H, H-2′), 2.40–2.50 (m, 2H, H-5′), 2.54–2.60 (m, 1H, H-2′), 2.85–2.90 (m, 1H, CH), 3.77 (s, 3H, MMTr-OCH_3), 4.05 (s, 3H, OCH_3), 4.08–4.14 (m, 1H, H-4′), 4.58–4.62 (m, 1H, H-3′), 6.43 (d, J = 3.6 Hz, 1H, H-5), 6.64 (t, J = 6.8 Hz, 1H, H-1′), 6.73 (d, J = 3.6 Hz, 1H, H-6), 6.79 (d, J = 8.8 Hz, 2H, ArH), 7.31 (m, 11H, ArH a nd NH), 7.43 (d, J = 8.0 Hz, 2H, ArH), 7.80 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 19.5, 19.6, 35.8, 40.5, 46.3, 54.0, 55.4, 70.3, 73.5, 83.2, 86.1, 100.3, 102.5, 113.3, 122.0, 126.5, 128.1, 128.7, 130.0, 140.0, 146,1, 151.5, 152.8, 158.1, 163.5; HRMS (ESI) m/z calcd for $C_{36}H_{39}N_5O_5$ (M+H)$^+$ 622.3023, found 622.3028.

4.23. 7-[2′-Deoxy-5′-*N*-(4-monomethoxytritylamino)-β-D-*erythro*-pentofuranosyl]-2-isobutyrylamino-3,7-dihydro-4*H*-pyrrolo[2,3- *d*]pyrimidin-4-one (24)

To a solution of **23** (0.70 g, 1.1 mmol) in anhydrous DMF (10.0 mL) was added sodium thiocresolate (0.80 g, 5.48 mmol), and reaction mixture was heated

at 90 °C for 1 h. The solvent was removed under vacuum and the residue was chromatographed over silica gel (MeOH/EtOAc 1:20) to afford the desired product (0.68 g, 85%). ^{1}H NMR ($CDCl_3$): δ 1.18 (d, J = 6.8 Hz, 6H, (CH_3)$_2$), 2.25–2.35 (m, 2H, H-5′), 2.40–2.46 (m, 1H, H-2′), 2.51–2.56 (m, 1H, H-2′), 2.68 (br s, 1H, C*H*), 3.77 (s, 3H, MMTr–OCH_3), 4.10–4.14 (m, 1H, H-3′), 4.40–4.46 (m, 1H, H-4′), 5.58 (br s, 1H, O*H*), 6.39 (t, J = 6.8 Hz, 1H, H-1′), 6.49 (d, J = 3.6 Hz, 1H, H-5), 6.60 (d, J = 3.6 Hz, 1H, H-6), 6.80 (d, J = 8.8 Hz, 2H, Ar*H*), 7.25–7.34 (m, 11H, Ar*H*, and N*H*), 7.40 (d, J = 8.4 Hz, 2H, Ar*H*) 8.44 (s, 1H, N*H*), 11.80 (br s, 1H, N*H*); ^{13}C NMR (500 MHz, $CDCl_3$): 19.1, 19.2, 36.6, 40.3, 46.6, 55.4, 70.3, 73.6, 83.0, 86.5, 104.6, 105.5, 113.4, 118.7, 126.7, 128.1, 128.6, 130.0, 137.7, 145.8, 145.9, 146.4, 147.9, 158.1, 158.2, 178.9; HRMS (ESI) *m*/*z* calcd for $C_{35}H_{37}N_5O_5$ $(M+H)^+$ 608.2867, found 608.2884.

4.24. 7-[2′-Deoxy-5′-*N*-(4-monomethoxytritylamino)-3′*O*-succinyl-β-D-*erythro*-pentofuranosyl]-2-isobutyryl-amino-3,7-dihydro-4*H*-pyrrolo[2,3- *d*]pyrimidin-4-one (25)

Succinic anhydride (0.062 g, 0.62 mmol) was added to a mixture of **24** (0.25 g, 0.42 mmol) and triethylamine (0.18 mL, 1.29 mmol) in anhydrous CH_2Cl_2 (10 mL) and stirred for 3 h. The reaction mixture was concentrated under vacuum and the residue was diluted with CH_2Cl_2 (100 mL) and washed with water. The organic phase was dried (Na_2SO_4), and concentrated to a white foam. It was re-dissolved in CH_2Cl_2 and the product was precipitated out with excess hexanes to afford **25** as a white solid (0.26 g, 90%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.19 (d, J = 6.8 Hz, 6H, (CH_3)$_2$), 2.24-2.81 (m, 9H, H-2′, H-5′, C*H*, CO (CH_2)$_2$), 3.76 (s, 3H, OCH_3), 4.23–4.27 (m, 1H, H-4′), 5.24–5.30 (m, 1H, H-3′), 6.20 (dd, J = 4.4, 8.4 Hz, 1H, H-1′), 6.51 (d, J = 3.6 Hz, 1H, H-5), 6.58(d, J = 3.6 Hz, 1H, H-6), 6.78 (d, J = 8.8 Hz, 2H, Ar*H*), 7.41 (m, 11H, Ar*H*, and N*H*), 7.43 (d, J = 7.6 Hz, 2H, Ar*H*), 9.60 (s, 1H, N*H*), 11.92 (br s, 1H, N*H*). ^{13}C NMR (500 MHz, $CDCl_3$): δ 19.1, 19.2, 30.0, 30.1, 36.4, 37.6, 46.2, 55.4, 70.3, 76.4, 83.5, 83.6, 104.5, 105.4, 113.4, 113.5, 118.7, 126.7, 127.4, 128.0, 128.1, 128.6, 129.4, 129.9, 137.6, 145.8, 147.0, 147.3, 158.0, 158.2, 172.2, 176.5, 179.4; HRMS (ESI) *m*/*z* calcd for $C_{39}H_{41}N_5O_8$ $(M-H)^-$ 706.2882. Found 706.2878.

5. Solid-phase synthesis of 5′-MMTr-protected DNG (29)

5.1. Loading

Triethylammonium salt of 5′-monomethoxytritylamino-3′-succinate **25** (0.075 g, 0.107 mmol) was loaded onto commercially available LCAA-CPG (0.50 g) by shaking with DMTMM (0.030 g, 0.108 mmol) min MeOH for 1 h. The suspension was then filtered; the beads were washed with copious amounts of MeOH, CH_2Cl_2, and dried. The efficiency of loading was determined to be 51.36 μmol/g by treating an aliquot of 7-deazaguanine loaded CPG **26** with a solution of 4% DCA in CH_2Cl_2 and assaying the trityl cation released by UV spectroscopy. The unreacted amino groups on the CPG were capped with acetic anhydride and triethyl amine to prevent side reactions. The dzaG loaded CPG was stored at 4 °C.

5.2. Deblocking

An aliquot of 100 mg (5 μmol scale) of **26** was placed in a screw-capped vial with a coarse frit and stopcock, and treated with a deblock solution (4% DCA solution in CH_2Cl_2) while collecting the solution into a 25 mL volumetric flask as it drips through the frit by gravity. The beads were treated with the deblock solution until no more yellow coloration was apparent. The beads were then washed thoroughly with CH_2Cl_2 and neutralized with 1% TEA in DMF. The filtrate was collected in the volumetric flask and assayed for the released monomethoxytrityl cation to determine the loading yield.

5.3. Coupling

A solution of fully protected **13** (5 equiv in 1 mL DMF) was added to the deblocked resin followed by simultaneous addition of $HgCl_2$ (10 equiv in 0.5 mL DMF) and TEA (10 equiv in 0.5 mL DMF) to the reaction vessel, upon which a cloudy yellow-white precipitate was formed. The reaction mixture was agitated for 3 h, the supernatant was filtered. The beads were washed with 20% thiophenol in DMF until the beads were clear, and washed with copious amounts of DMF to afford **27**. The coupling was repeated two more times to optimize the coupling yields.

5.4. Capping

The unreacted sites on CPG were capped by the addition of a solution of acetic anhydride (1 mL, 100 mM in DMF) and TEA (1 mL, 200 mM in DMF). The reaction mixture was agitated for 10 min, filtered, washed thoroughly with DMF, CH_2Cl_2, and then dried under high vacuum.

5.5. Elongation

The coupling yield of the reaction was calculated to be 80% from the trityl release assay. The coupling was repeated with monomer **19** to give the desired protected DNG trimer **28** on the LCAA-CPG. The capping and deblocking steps were omitted to allow the MMTr group to remain on the 5′-terminus of the DNG.

5.6. Cleavage and deprotection

The CPG was transferred to a pressure-resistant vial, and methanolic ammonia solution (5 mL) was added. The vial was sealed and heated at 60 °C for 12 h. The isobutyryl and Fmoc-protecting groups were concurrently cleaved under these conditions. After cooling, the volatiles were removed by vacuum centrifugation, yielding a white residue containing the crude MMTr-protected DNG trimer **29**.

6. HPLC purification of *29* and *1*

The crude trityl-on product **29** was purified on reverse-phase HPLC using 5% → 95% gradient of acetonitrile in 100 mM TEAA buffer (pH 7.0), and characterized by ESI ESI/TOF+ analysis which exhibited peaks at *m/z* 1117.48 (M+H) and 559.24 (M+2H), calcd 1117.47 (M+H) and 559.24 (M+2H) for $C_{55}H_{60}N_{18}O_9$.

To the trityl-on DNG **29** was added 3% DCA in CH_2Cl_2 (1 mL) and agitated for 1 min. Excess of ether was added to precipitate trityl-off DNG **1**. The solvents were decanted after centrifugation, and the product was dried and purified by RP-HPLC revealing a single peak. ESI/TOF+ analysis of the final detritylated trimer **1** exhibited the expected peak at *m/z* 845.35 (M+H) and 423.14 (M+2H); calcd 845.35 (M+) and 423.17 (M+2H) for $C_{35}H_{44}N_{18}O_8$.

7. Stoichiometry of the binding

Job plot analysis[55] was performed to measure the stoichiometry of association. To accommodate the difference in strand lengths of DNG **1** and DNA-C_5 oligomer, the concentration of nucleotide solutions was determined by using the extinction coefficients (per mole of the nucleotide). The absorbance at 260 nm was measured for samples containing a constant concentration of 4 μM oligonucleotides, varying between 0 and 100% DNA-C_5 with DNG **1** making up the remainder. All experiments were conducted in buffer containing 100 mM [NaCl] and 10 mM $NaHPO_4$ adjusted to pH 7.1. The solutions were heated to 90 °C for 5 min. and allowed to cool to rt slowly before being stored at 4 °C overnight. The inflection point at 50% of DNA-C_5 in the plot indicates the DNG **1** binds to pentameric cytidine strand with 1:1 stoichiometry to form a Watson–Crick base paired duplex.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2006.05.074.

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