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A concise synthesis of isoguanine 2'-deoxyriboside and its adenine-like triplex formation when incorporated into DNA

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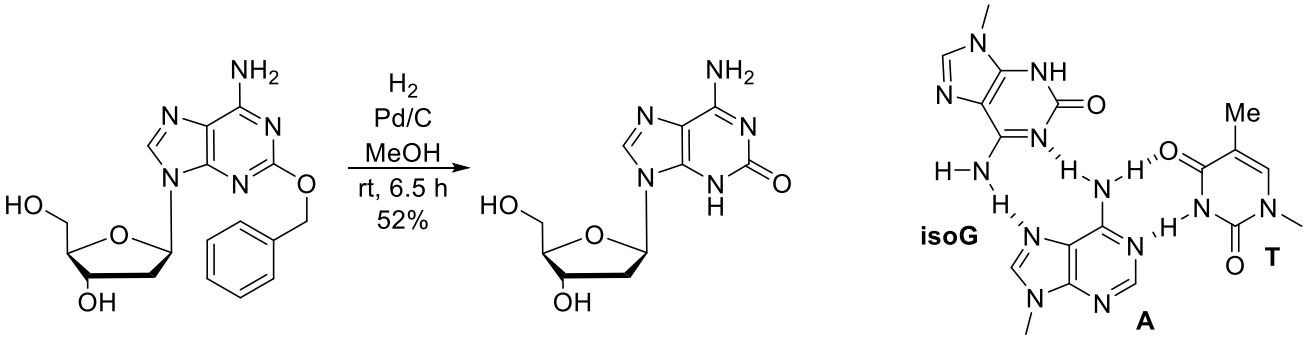
Abstract

A concise synthesis of 2'-deoxyisoguanosine is achieved whereby 2,6-dichloropurine is glycosylated using the Hoffer sugar to give a pair of beta-configured nucleoside N9/N7 regioisomers that are aminated using methanolic ammonia with concomitant deprotection of the sugar. Following chromatographic separation, pure 2-chloro-2'-deoxyadenosine was isolated as a single isomer. Displacement of the C2 chlorine atom using sodium benzyloxide, followed by hydrogenolysis of the benzyl group, gives 2'-deoxyisoguanosine. Isoguanine was incorporated into DNA by solid supported synthesis using the suitably protected 2-allyloxy-2'-deoxyadenosine phosphoramidite with the allyl group being removed post-oligomerisation under Noyori conditions. DNA melting studies showed isoguanine to exhibit adenine-like triplex formation.



Keywords: Isoguanine, 2'-deoxyisoguanosine, adenine, Hoffer sugar, glycosylation, phosphoramidite, triplex

Introduction

Although not a DNA base, isoguanine (**isoG**) occurs naturally and various methods have been reported for the preparation of derivatives[1-4] including its riboside,[5] 2'-deoxyriboside[6-9] and other analogues.[10,11] Isoguanine has been widely studied and when incorporated into nucleic acids,[12-23] has been shown to form stable base pair[7,24-28] (Figure 1 left) and triplex motifs[29] (Figure 1 middle) as its N1-H tautomer. These experimental observations are supported by theoretical calculations.[7,30,31] Strong, Watson-Crick purine-purine base pairing is one of various defining features of 2',3'-dideoxyhexose nucleic acid duplexes whereby isoguanine adopts the N3-H tautomeric form[32] (Figure 1 right). In pyranosyl-RNA duplexes, isoguanine adopts either N1-H or N3-H tautomeric forms.[33]

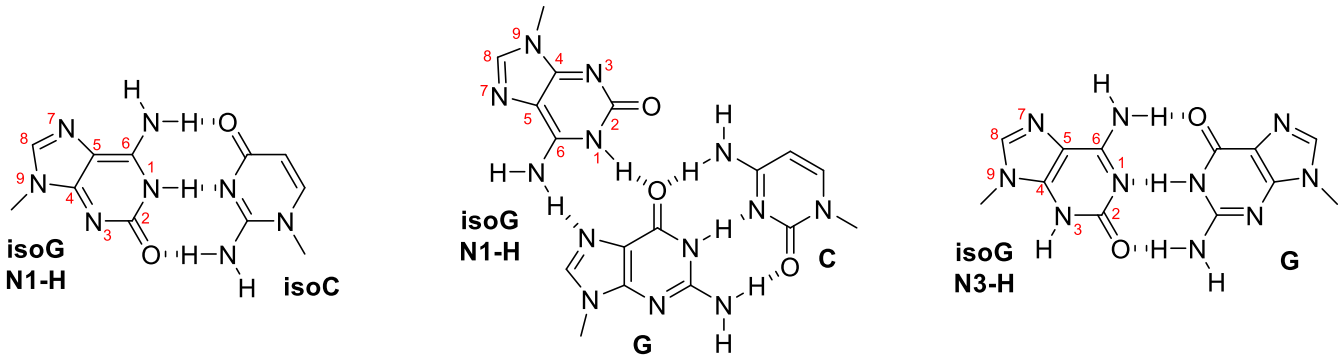


Figure 1. Representative motifs in oligonucleotides where the N1-H tautomer of isoguanine (**isoG**) is targeted to isocytosine[7] (**isoC**), to a guanine.cytosine (**G**.**C**) base pair[29] and as the N3-H tautomer, targeted to guanine[32] (**G**).

We sought to examine whether isoguanine could exhibit adenine (**A**)-like triplex formation in its N3-H tautomeric form when incorporated into DNA, thus extending the triplex-froming capabilities of this intriguing purine heterocycle (Figure 2 right). To accompany the incorporation of isoguanine into DNA, we considered 2-benzyloxyadenine (**B**) and 2-allyloxyadenine (**L**) as further representations of the adenine hydrogen bonding pattern (Figure 2 left). At the nucleoside level, hydrogenolysis of the 2-benzyloxy group[34] would give a concise route to 2'-deoxyisoguanosine from non-nucleoside precursors. At the DNA level, removal of the 2-allyloxy group using Noyori conditions[35] would give isoguanine[29] (Figure 2 right) for the study of its triplex-forming properties and also allow comparison between **A**, **isoG**, **B** and **L**.

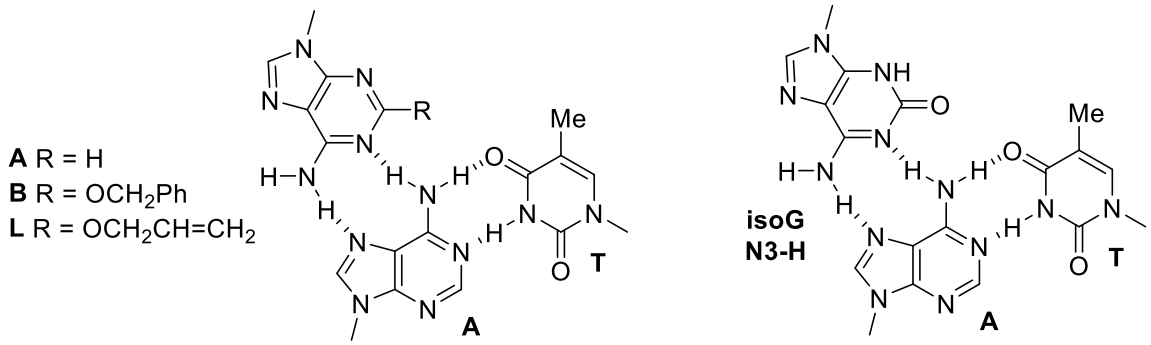
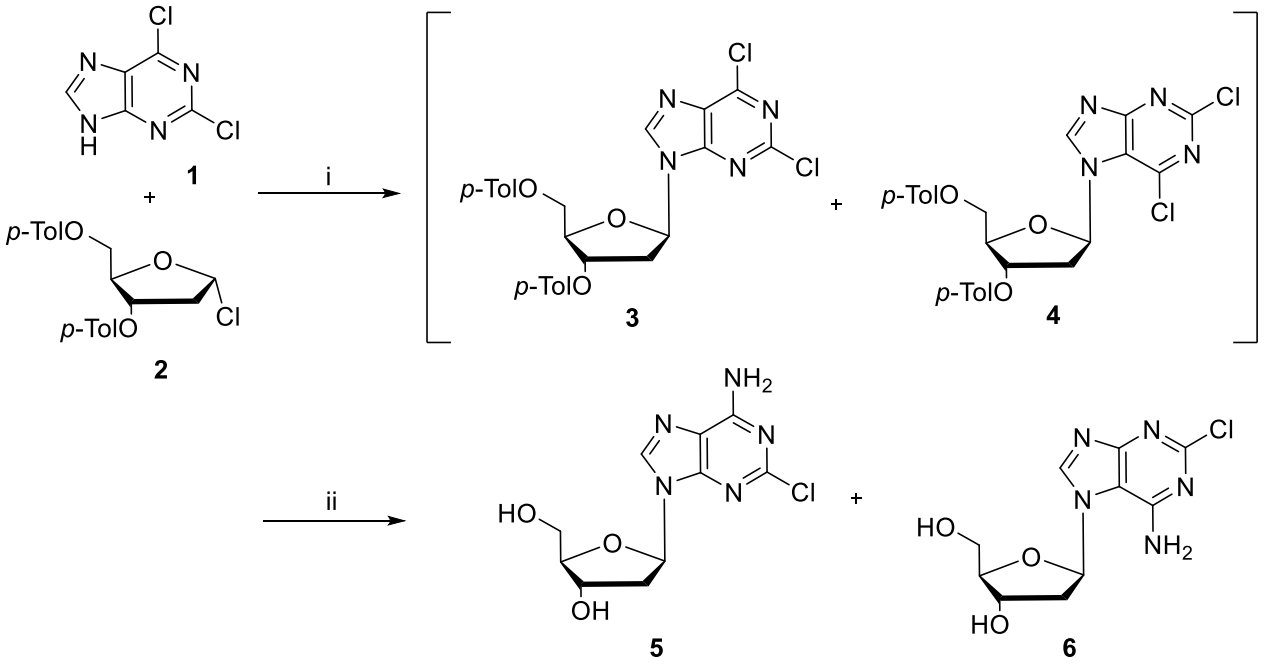


Figure 2. Triplex DNA motifs involving adenine (**A**) and the adenine-like 2-benzyloxyadenine (**B**), 2-allyloxyadenine (**L**) (left) and N3-H tautomer of isoguanine (**isoG**) (right).

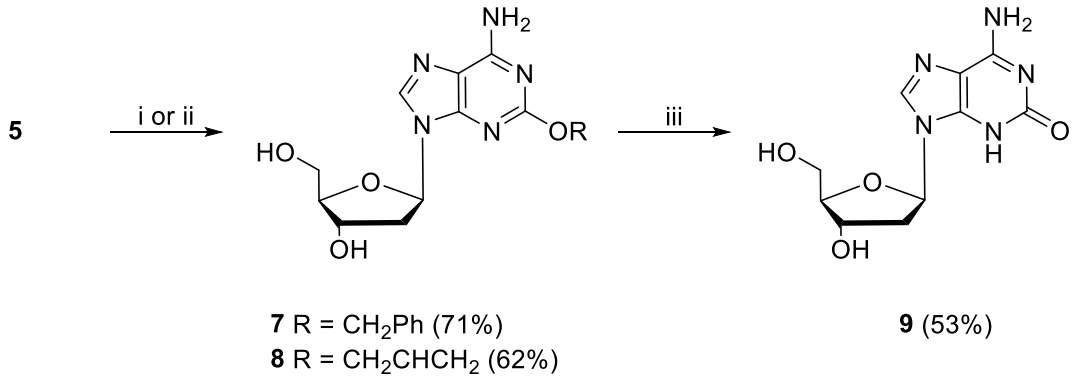
Results and Discussion

As the starting point for synthesis of the nucleoside intermediate **5**,[36] we selected the well-known and widely used[6,29,37-41] Hoffer sugar **2** (2-deoxy-3,5-di-*O*-(*p*-toluoyl)-α-D-*erythro*-pentofuranosyl chloride).[42] Recently, we reported an improved synthesis of the Hoffer sugar that was achieved without need for distillation or chromatographic separation of intermediates, or the use of gaseous HCl.[43] The sodium salt of 2,6-dichloropurine (**1**) was generated, *in situ*, using NaH in MeCN.[41] Addition of Hoffer's sugar **2**[43] to the reaction mixture afforded the nucleoside regioisomers **3** (N9) and **4** (N7), as a 4:1 mixture (Scheme 1). Chromatographic separation of the regioisomers **3** and **4** was possible[41,44] but in our hands it proved to be tricky and time consuming. Thus the mixture of regioisomers **3** and **4** was treated with methanolic ammonia solution (6 h) with heating (100 °C) to afford 2-chloro-2'-deoxyadenosine isomers **5** and **6** that were readily separated by flash chromatography (Scheme 1). The analytical and spectroscopic properties of the regioisomers **5** and **6** were in agreement with the literature values, thus confirming their identities.[45] Despite good regioisomeric control, the isolated yield (14% over two steps) for nucleoside **5** is a drawback. This might be overcome by using a pre-formed metal salt of 2,6-dichloropurine. For example, glycosylation of the pre-formed cesium salt of 4(5)-nitroimidazole using the Hoffer sugar (**2**), gave the corresponding 2'-deoxyriboside with good regioselectivity but in much better yield (68%).[43]

Preparation of 2-benzyloxy-2'-deoxyadenosine (**7**) was achieved by adding nucleoside **5** to solution of benzylalkoxide that was generated by mixing sodium hydride (50% dispersion in paraffin oil) and benzyl alcohol with heating (80 °C). Continued heating for 12 h at 85 °C afforded 2-benzyloxy-2'-deoxyadenosine (**7**) following chromatographic purification (71%) (Scheme 2). The benzyl group was removed from nucleoside **7** by catalytic hydrogenolysis (6.5 h) to give 2'-deoxyisoguanosine (**9**) in 53% yield thus providing a concise, four-step route to **9** from non-nucleoside precursors. Analytical and spectroscopic properties were in agreement with those repored for **9** made from 2'-deoxyguanosine as nucleoside starting material.[8]

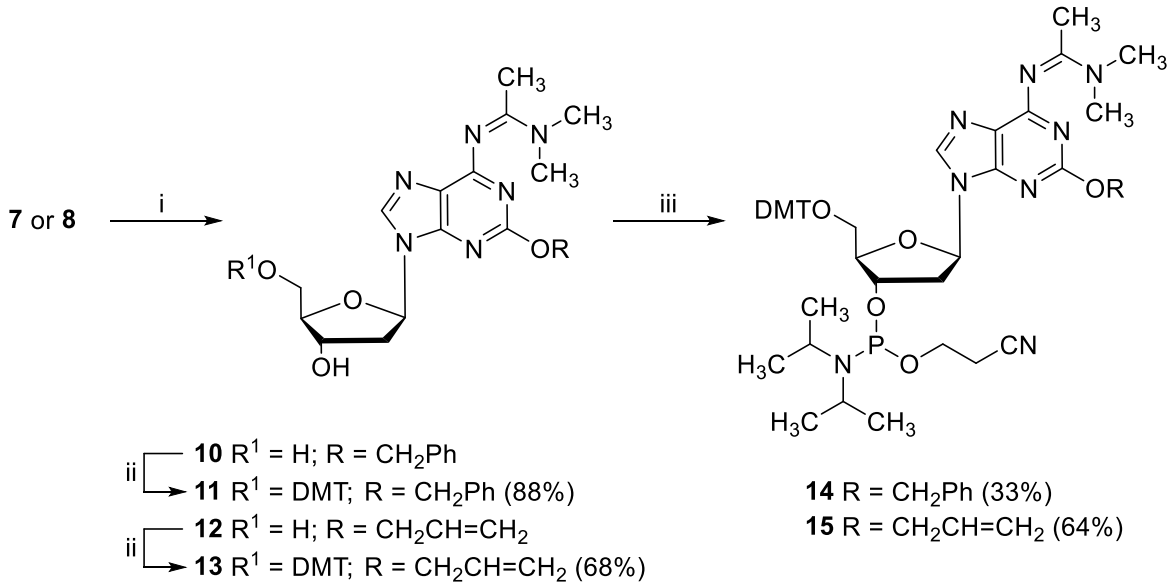


Scheme 1. Reagents and conditions: i, NaH, MeCN, rt, 24 h ; ii, MeOH-NH_3 (satd.), 100 °C, 6 h. Product ratios **3** to **4** (80:20), and **5** to **6** (82:18).



Scheme 2. Reagents and conditions: i, sodium benzylalkoxide, 85 °C, 12 h; ii, sodium allylalkoxide, 88 °C, 12 h; iii, H_2, Pd-C, MeOH, rt, 6.5 h.

2-Allyloxy-2'-deoxyadenosine (**8**) was prepared in a similar fashion to the 2-benzyloxy derivative **7** using allyl alcohol (Scheme 2). Separately, the amino groups in nucleosides **7** and **8** were protected by reaction with *N,N*-dimethylacetamide dimethyl acetal in anhydrous methanol (Scheme 3). The volatile reagents were removed under vacuum and the crude products **10** and **12** used in the next step without further purification. Separately, the 5'-hydroxyl functions of **10** and **12** were protected using 4,4'-dimethoxytrityl chloride affording **11** (88%) and **13** (68%) over 2 steps. Phosphitylation at the 3'-hydroxyl functions of **11** and **13** using 2-cyanoethyl-*N,N*-diisopropyl chlorophosphoramidite afforded the DNA monomers **14** and **15** as mixtures of diastereoisomers. Following initial chromatographic purification, the phosphoramidites **14** and **15** were precipiated, separately, into hexane to remove residual non-phosphoramidite impurity from compound **14** although in the ^{31}P NMR spectrum of compound **15**, a trace of impurity persisted (Supplementary Material **S14**).



Scheme 3. Reagents and conditions: i, *N,N*-dimethylacetamide dimethyl acetal, MeOH, 40 °C, 26 h; ii, DMTCl, pyridine, rt, 12 h or 110 min ; iii, 2-cyanoethyl-*N,N*-diisopropyl chlorophosphoramidite, THF, rt, 40 min or 1 h.

The DNA sequences **S1** to **S7** were prepared by solid supported synthesis on a 1 µmol scale using phosphoramidites including **14** to insert the 2-benzyloxyadenine (**B**) into **S6**, and **15** to insert 2-allyloxyadenine (**L**) and isoguanine (**I**) into **S4** and **S5**. Cleavage and deprotection (16 h) using conc. NH_3 (aq) at 55 °C afforded the crude oilgomers that were purified by reversed phase HPLC and analysed by negative ion electrospray mass spectrometry in weakly basic ammonia solution (Table 1)**.**

Table 1. HPLC retention times and the found and calculated masses of prepared DNA sequences **S1** to **S7**

Entry	*Sequence*[a]	t_R/*min*[c]	*Mass Found*[d]	*Mass Calculated*
S1	3'-d(TTTTCTTTTCCCCCCT)	13.8	4698.8	4697.8
S2	5'-d(AAAAGAAAAGGGGGGA)	12.6	5061.4	5058.9
S3	3'-d(AAAAGAA**A**AGGGGGGA)	12.4	5060.7	5058.9
S4	3'-d(AAAAGAA**I**AGGGGGGA)[b]	12.7	5076.9	5074.9
S5	3'-d(AAAAGAA**L**AGGGGGGA)	12.6	5119.7	5115.0
S6	3'-d(AAAAGAA**B**AGGGGGGA)	14.2	5168.0	5165.0
S7	3'-d(AAAAGAA**G**AGGGGGGA)	12.7	5075.8	5074.9

[a] **A** = adenine, **I** = isoguanine, **L** = 2-benzyloxyadenine, **B** = 2-allyloxyadenine, **G** = guanine. [b] Removal of the 2-*O*-allyl protecting group to give **I** was achieved post-oligomerisation using Noyori conditions.[35] [c] Gradient elution 260 nm) using a C18 Reversed Phase Column (250 x 4.6 mm) at a flow rate of 1 mL.min^{-1} where solvent system A was mixed with solvent system B. Solvent system A was composed of 1 M aqueous triethylammonium acetate (TEAA, 10%) and MeCN (2%) at pH 7.0, and solvent system B was composed of 1 M aqueous TEAA (10%) and MeCN (80%) at pH 7.0. [d] The molecular masses and masses of mono-sodium adducts (not shown) were measured from the deconvoluted mass-to-charge (*m/z*) ratios of the multiply-charged anions from electrospray mass spectrometry and were consistently within ±0.08% of their calculated values.

The thermal denaturation of the DNA triplexes formed from sequences **S3** to **S7** targeted to duplex **S2**.**S1** is summarised in Table 2. Notable was the sensitivity of the triplex to mismatch where the presence of X = **G** in sequence **S7** was significantly destabilising to the triplex structure. Triplexes formed from **S3** (X = **A**) and **S4** (X = **I**) targeted to **S2**.**S1** were identical in stability demonstrating adenine-like triplex formation (Figure 2 left) by isoguanine, with the likelihood of isoguanine adopting the N3-H tautomer (Figure 2 right). The 2-benzyloxy (**B**) and 2-allyloxy (**L**) derivatives of adenine were accommodated in triplex structures **S5*****S2**.**S1** and **S6*****S2**.**S1** (Figure 2 left).

Spermine is known to stabilise DNA triplexes and its addition was equally stabilising to both the adenine- and isoguanine-containing triplexes **S3*****S2**.**S1** and **S4*****S2**.**S1** (Table 2).

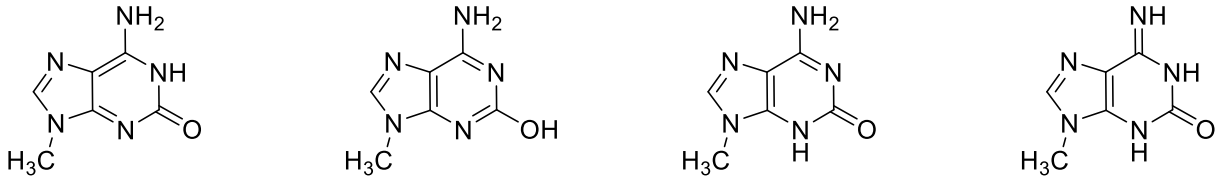
To examine the preferred or dominant modes of tautomerization, we performed semi-empirical (AM1) calculations on tautomers of *N*9-methylisoguanine as representative structures. The similarity between the calculated heats of formation around 53 kcal.mol^{-1} suggested that isoguanine exists in more than one stable tautomer including the N3-H form ascribed to its adenine-like triplex forming behaviour (Figure 2 right) and to its characteristic duplex-forming behaviour in 2',3'-dideoxyhexose DNA[32] (Figure 1, right).

Table 2. The thermal stability of triplexes containing X = **A** compared with X = **I**, **L**, **B** and **G**

3′-dTTTTCTTTTCCCCCCT-5′ **S1**
5′-dAAAAGAAAAGGGGGGA-3′ **S2**
3′-dAAAAGAAXAGGGGGGA-5′ **S3-S7**

Triplex	*TFOs (**S3-S7**)*[a]	T_m/°C[b]	T_m/°C[c]
S3*S2.S1	X = **A**	15 ±1	59.0 ±0.5
S4*S2.S1	X = **I**	15 ±1	59.0 ±0.5
S3*S2.S1	X = **A**	23 ±1[d]	59.0 ±0.5
S4*S2.S1	X = **I**	23 ±1[d]	59.0 ±0.5
S5*S2.S1	X = **L**	16 ±1	59.0 ±0.5
S6*S2.S1	X = **B**	15 ±1	59.0 ±0.5
S7*S2.S1	X = **G**	< 5	59.0 ±0.5

[a] **A** = adenine, **I** = isoguanine, **L** = 2-benzyloxyadenine, **B** = 2-allyloxyadenine, **G** = guanine. [b] Triplex **S3** to **S7*S1.S2** melting temperature. [c] Duplex **S2.S1** melting temperature. [d] 0.5 mM spermine added. Denaturation was performed with 1.5 mM of each sequence in 0.1 M NaCl, 0.01 M $MgCl_2$, 25 mM Tris.HCl buffer at pH 7.2 at detection wavelength 260 nm.



16 53.6 **17** 53.5 **18** 51.1 **19** 56.5

Figure 3. The calculated (AM1) heats of formation ΔH_f (kcal.mol^{-1}) for N9-methylated isoguanine tautomers **16** N1-H, **17** O2-H, **18** N3-H, and **19** 6-imino-2-oxo.

Conclusions

We have established a concise synthesis of isoguanine 2'-deoxyriboside from non-nucleoside starting materials 2,6-dichloropurine **1** and the Hoffer sugar **2**. Isoguanine was incorporated into DNA and shown to participate in adenine-like triplex formation, most likely in its N3-H tautomeric form.

The DNA melting studies and semi-empirical heats of formation calculated for representative N9-methylated isoguanine tautomers, support the viability of isoguanine as its N3-H tautomer in DNA triplex formation thus furthering the elucidation of the molecular recognition characteristics of this intriguing purine heterocycle.

Experimental Section

General. NMR spectra were recorded on a Bruker AC-250 spectrometer with ^{1}H (250.1 MHz) spectra referenced to TMS, ^{13}C spectra (62.9 MHz) referenced to $CDCl_3$ or $(CD_3)_2SO$, and ^{31}P spectra (101.3 MHz) referenced to 85% H_3PO_4 (aq). Mass spectrometric analysis were carried out at EPSRC (Swansea) in EI+ or CI+ mode using a VG Quatro II or FAB+ mode using a VG AutoSpec instrument or at Aston University in electrospray ionisation (ES) mode with a Hewlett-Packard HP 5989B MS Engine apparatus using a HP 59987A API-electrospray LC/MS interface. Electron impact ionisation (EI) mass spectra (70eV) were recorded on a AEI MS1Z mass spectrometer. Infrared spectra were recorded using a Mattson Galaxy 2020 FT-IR Spectrophotometer. Ultraviolet spectra were recorded using a Unicam PU8730 Spectrophotometer. Melting points were measured on a Gallenkamp Electrothermal Digital apparatus and are uncorrected. Flash column chromatography was performed using Sorbsil C60 silica using the method described by Still, Kahn and Mitra.[46] TLC was carried out on pre-coated Merck 60 F254 aluminium-backed plates and visualized using UV (254 and 366 nm) and vanillin reagent; vanillin (6.0 g), ethanol (250 mL) and conc. sulfuric acid (2 mL). Oligonculeotides were prepared on a Beckman Oligo 1000 DNA synthesiser following the manufacturer's protocol using commercially available reagents (LINK Technologies). Purification of oligonucleotides was performed by semi-preparative reverded phase HPLC. Thermal UV analysis of oligonucleotides was conducted using a Varian Cary 1E UV-Visible Spectrophotometer with a 12 sample heating block.

6-Amino-2-chloro-9-(2'-deoxy-β-D-*erythro*-pentofuranosyl)-purine (5) and 6-amino-2-chloro-7-(2'-deoxy-β-D-*erythro*-pentofuranosyl)-purine (6). A mixture of 2,6-dichloropurine (**1**) (5.00 g, 26.45 mmol) and sodium hydride (1.13 g, 28.25 mmol, 60% dispersion in oil) in anhydrous MeCN (100 mL) was stirred (90 min) at rt under Ar. Hoffer's sugar[43] **2** (10.28 g, 26.46 mmol) was added in four equal portions during 30 min. After 24 h, the reaction mixture was filtered through Celite. Evaporation of the solvent gave a crude mixture of nucleosides **3** and **4** (7.82 g) in a 4:1 ratio by ^{1}H NMR. A saturated solution of methanolic NH_3 (70 mL) was added to a portion (3.02 g) of the crude mixture of nucleosides **3** and **4**, and the mixture was heated (6 h) in a bomb at 100 °C. The product solution was evaporated concentrated. The residue was purified by flash chromatography, eluting with CH_2Cl_2-MeOH (9:1) to give N9 isomer **5** (0.40 g, 14% over 2 steps); mp >300 °C (lit.[45] mp >300 °C); TLC [MeOH-CH_2Cl_2 (1:4)]: R_f 0.38; ^{1}NMR [$(CD_3)_2SO$]: δ 2.20-2.30 (m, 1 H, 2'-CH_2), 2.60-2.70 (m, 1 H, 2'-CH_2), 3.40-3.60 (m, 2 H, 5'-CH_2), 4.38 (m, 1 H, 3'-CH), 4.99 (t, 1 H, *J* 5.8 Hz, 5'-OH), 5.34 (d, 1 H, *J* 4.2 Hz, 3'-OH), 6.26 (t, 1H, *J* 6.4 Hz, 1'-CH), 7.86 (br s, 2 H, NH_2), 8.34 ppm (s, 1 H, 8-CH). Further elution gave the N7 isomer **6** (0.10 g, 3% over 2 steps); mp >300 °C (lit.[45] mp >300 °C); TLC [MeOH-CH_2Cl_2 (1:4)]: R_f 0.24; ^{1}H NMR [$(CD_3)_2SO$]: δ 2.28-2.33 (m, 2 H, 2'-CH_2), 3.42 (m, 2 H, 5'-CH_2), 3.90 (m, 1 H, 4'-CH), 4.39 (m, 1 H, 3'-CH), 5.19 (t, 1 H, *J* 4.6 Hz, 5'-OH), 5.41 (d, 1 H, *J* 4.5 Hz, 3'-OH), 6.30 (t, 1 H, 6.3 Hz, 1'-CH), 7.49 (br s, 2 H, NH_2), 8.56 ppm (s, 1 H, 8-CH); ^{13}C NMR [$(CD_3)_2SO$]: δ 41.2 (2'-C), 60.4 (5'-C), 69.3 (3'-C), 85.7 (1'-C), 88.0 (4'-C), 109.6 (5-C), 144.8 (8-C), 152.7 (6-C or 2-C), 153.2 (6-C or 2-C), 162.2 ppm (4-C).

6-Amino-2-benzyloxy-9-(2'-deoxy-β-D-*erythro*-pentofuranosyl)-purine (7). Sodium as a 50% dispersion in paraffin oil (190 mg, 8.26 mmol) was added to anhydrous benzyl alcohol (15 mL) under Ar and the mixture was heated at 80 °C for 45 min to give a clear, colourless solution. The nucleoside **5** (450 mg, 1.58 mmol) was then added and the solution was heated at 85 °C during 12 h. Silica (10 g) and MeCN (15 mL) were then added to the cooled, deep-red, reaction mixture and the solvent evaported. The resulting gel was added to a column of silica gel and the unreacted benzyl alcohol was eluted firstly using CH_2Cl_2 and then CH_2Cl_2-MeOH (95:5). Further elution with 10% MeOH-CH_2Cl_2 afforded the title compound **7** (400 mg, 71%) as a yellow solid; mp 206-209 °C; TLC [MeOH-CH_2Cl_2 (1:4)]: R_f 0.52; IR (cm^{-1}) 3396, 3132, 2937, 1653, 1592; ^{1}H NMR [$(CD_3)_2SO$]: δ

2.22 (m, 1 H, 2'-CH_2), 2.71 (m, 1 H, 2'-CH_2), 3.50-3.58 (m, 2 H, 5'-CH_2), 3.85 (m, 1 H, 4'-CH), 4.41 (m, 1 H, 3'-CH), 5.07 (t, 1 H, *J* 5.6 Hz, 5'-OH), 5.31-5.33 (m, 3 H, CH_2 and 3'-OH), 6.26 (t, 1 H, *J* 6.4 Hz, 1'-CH), 7.31-7.47 (m, 7 H, 5 x CH (Ar), NH_2), 8.16 ppm (s, 1 H, 8-CH); ^{13}C NMR [$(CD_3)_2SO$]: δ 39.2 (2'-CH_2), 62.1 (5'-CH_2), 67.9 (CH_2), 71.2 (3'-CH), 83.7 (1'-CH), 88.0 (4'-CH), 116.0 (5-C), 127.9 (CH (Ar)), 128.0 (CH (Ar)), 128.5 (CH (Ar)), 137.6 (C (Bn)), 138.6 (8-CH), 150.8 (4-C), 157.0 (6-C) and 161.2 ppm (2-C). Mass spectrum (ES^+): *m/z* (I_r) 358 (M + H, 100%), 113 (35%); Calcd for $C_{17}H_{19}N_5O_4$: 410.008. Found 410.009; Calcd for $C_{17}H_{19}N_5O_4$: C, 57.1%; H, 5.3%; N, 19.6%. Found 57.2%; H, 5.3%; N, 19.5%.

6-Amino-2-allyloxy-9-(2'-deoxy-β-D-*erythro*-pentofuranosyl)-purine (8). Sodium (640 mg, 27.83 mmol) as a 50% dispersion in paraffin oil, was added to allyl alcohol (14 mL) in three portions during 5 min at 0 °C under Ar. After 1 h, the nucleoside **5** (0.76 g, 2.65 mmol) was added and the mixture was heated at 88 °C during 12 h after which time the mixture was concentrated to give a red oil which was purified by flash chromatography by gradient elution using CH_2Cl_2 containing MeOH (0 to 20%) to afford the title compound **8** (0.50 g, 62%) as a cream coloured solid.; mp 170-173 °C; TLC [MeOH-CH_2Cl_2 (1:4)]: R_f 0.47; IR (cm^{-1}) 3371, 3336, 2942, 2874, 1662, 1610; ^{1}H NMR [$(CD_3)_2SO$]: δ 2.22 (m, 1 H, 2'-CH_2), 2.71 (m, 1 H, 2'-CH_2), 3.53 (m, 2 H, 5'-CH_2), 3.84 (m, 1 H, 4'-CH), 4.38 (m, 1 H, 3'-CH), 4.74 (m, 2 H, CH_2O), 5.04 (br s, 1 H, 5'-OH), 5.17-5.38 (m, 3 H, CH_2, 3'-OH), 6.03 (m, 1 H, CH), 6.22 (t, 1 H *J* 6.9 Hz, 1'-CH), 7.32 (br s, 2 H, NH_2), 8.13 ppm (s, 1 H, 8-CH); ^{13}C NMR [$(CD_3)_2SO$]: δ 40.0 (2'-C), 62.0 (5'-C), 66.8 (CH_2O), 71.0 (3'-C), 83.5 (1'-C), 87.8 (4'-C), 115.7 (5-C), 116.9 (CH_2), 134.0 (CH), 138.4 (8-C), 150.7 (4-C), 156.9 (6-C), 160.9 ppm (2-C). Mass spectrum (ES^+): *m/z* (I_r) 308 (M + H, 100%); Calcd for $C_{13}H_{17}N_5O_4$: (M + H) 308.136. Found 308.135; Calcd for $C_{13}H_{17}N_5O_4$: C, 51.0%; H, 5.2%, N, 22.9%. Found 50.7%; H, 5.5%; N, 22.5%.

2'-Deoxyisoguanosine (9). To nucleoside **7** (270 mg, 0.75 mmol) dissolved in MeOH (70 mL) was added 5% Pd on C (220 mg) and the mixture was hydrogenolysed (6.5 h) at rt using a hydrogen baloon. The product mixture was filtered through Celite after which activated charcoal (1 g) was added and the mixture was boiled for 10 min. The charcoal was removed by filtration before concentration of the product mixture, affording the title compound **9** (110 mg, 53%) as an off-white solid; decomposed >230 °C (lit.[8] decomposed >230 °C); ^{1}H NMR [$(CD_3)_2SO$]: δ 2.14 (m, 1 H, 2'-CH_2), 2.49 (m, 1 H, 2'-CH_2), 3.53 (m, 2 H, 5'-CH_2), 3.83 (m, 1 H, 4'-CH), 4.33 (m, 1 H, 3'-CH), 5.27 (br s, 1 H, 3'-OH), 5.60 (br s, 1 H, 5'-OH), 6.09 (t, 1 H, *J* 7.9 Hz, 1'-CH), 7.70 (br s, 2 H, NH_2), 7.94 (s, 1 H, 8-CH), 10.70 ppm (br s, 1 H, NH); ^{13}C NMR [$(CD_3)_2SO$]: δ 39.3 (2'-C), 62.0 (5'-C), 71.1 (3'-C), 83.8 (1'-C), 88.0 (4'-C), 109.6 (5-C), 137.7 (8-C), 152.5 (6-C), 156.1 ppm (2-C).

6-Amino-2-benzyloxy-6-*N*-[1-(dimethylamino)-ethylidene]-9-(2'-deoxy-5'-*O*-(4,4'-dimethoxytrityl)-β-D-e*rythro*-pentofuranosyl)-purine (11). Nucleoside **7** (550 g, 1.54 mmmol) was coevaporated with anhydrous pyridine (3 x 10 mL), dissolved in anhydrous MeOH (3 mL) and placed under Ar. To this was added *N*,*N*-dimethylacetamide dimethyl acetal (0.68 mL, 4.65 mmol) and the mixture was heated at 40 °C. After 26 h, H_2O (0.3 mL) was added and the mixture stirred for a further 10 min. The mixture was concentrated, the residue co-evaporated with anhydrous pyridine (3 x 3 mL) and dissolved in pyridine (15 mL) under Ar. DMTCl (640 mg, 1.89 mmol) was added and the solution stirred (12 h) after which time MeOH (0.5 mL) was added and stirring continuted (10 min). The mixture was concentrated and purified by flash chromatography eluting firstly with EtOAc-Et_3N (100:1) and then MeOH-EtOAc-Et_3N (5:95:1, then 10:90:1) affording a yellow foam. This was precipitated from CH_2Cl_2 (3 mL) into cold hexane-Et_2O (2:1, 700 mL). The product was dissolved in CH_2Cl_2 (10 mL) and dried under high vacuum at 40 °C to give the title compound **11** (990 mg, 88% yield over 2 steps) as a white solid; mp 102-105 °C; TLC [MeOH-EtOAc-Et_3N (10:90:1)]: R_f 0.37; IR (cm^{-1}) 3413, 2929, 1606, 1564; ^{1}H NMR [$(CD_3)_2SO$]: δ 2.01 (s, 3 H, CH_3C), 2.27 (m, 1 H, 2'-CH_2), 2.81 (m, 1 H, 2'-CH_2), 3.08 (s, 8 H, 5'-CH_2, $(CH_3)_2N$), 3.68 (s, 6 H, 2 x CH_3O), 3.96 (m, 1 H, 4'-CH), 4.45 (m, 1 H, 3'-CH), 5.17 (m, 2 H, CH_2), 5.34 (m, 1 H, 3'-OH), 6.32 (m, 1 H, 1'-CH), 6.68-7.35 (m, 18 H, 13 x CH (Ar), 5 x CH (Ar)), 8.13 ppm (s, 1 H, 8-CH); ^{13}C NMR [$(CD_3)_2SO$]: δ

17.2 (*C*H_3C), 37.1, 38.2, ((CH_3)$_2$N), 38.4 (2'-CH_2), 54.96, 54.99 (2 x CH_3O), 64.5 (5'-CH_2), 68.1 (CH_2), 70.8 (3'-CH_2), 83.5 (1'-CH), 85.4 (Ar_3C), 86.0 (4'-CH), 113.1 (CH), 122.3 (5-C), 126.6 (CH), 127.7 (CH (Ar)), 127.8 (CH (Ar)), 128.3 (CH (Ar)), 129.65 (CH (Ar)), 129.72 (CH (Ar)), 135.5 (C), 135.7 (C), 137.3 (C (Ar)), 140.2 (8-CH), 145.0 (C (Ph)), 152.0 (4-C), 157.97 (*C*OCH_3), 158.01 (*C*OCH_3), 160.7, 160.9, 161.3 ppm (C-2, C-6, C=N); Mass spectrum (FAB^+): *m/z* (I_r) 729 (M + H, 50%), 303 (100%); Calcd for $C_{42}H_{44}N_6O_6$: (M + H) 729.340. Found 729.343; Calcd for $C_{42}H_{44}N_6O_6$: C, 69.2%; H, 6.0%; N, 11.5%. Found C, 68.7%; H, 6.0%; N, 11.5%.

6-Amino-2-allyloxy-6-*N*-[1-(dimethylamino)-ethylidene]-9-(2'-deoxy-5'-*O*-(4,4'-dimethoxytrityl)-β-D-*erythro*-pentofuranosyl)-purine (13). The nucleoside **8** (890 mg, 2.90 mmol) was co-evaporated with anhydrous pyridine (3 x 10 mL) and dissolved in anhydrous MeOH (2 mL) under Ar. To this, *N*,*N*-dimethylacetamide dimethylacetal (1.7 mL, 11.63 mmol) was added and the mixture stirred (47 h) at rt before water (0.3 mL) was added and stirring continued (10 min). The product mixture was concentrated, the residue co-evaporated with anhydrous pyridine (3 x 3 mL) and dissolved in pyridine (40 mL) under Ar. DMTCl (1.08 g, 3.19 mmol) was added in two portions during 20 min and the solution stirred (110 min) at rt after which MeOH (0.05 mL) was added and stirring continued (10 min). The product mixture was concentrated and the residue purified by flash chromatography, eluting with MeOH-EtOAc-Et_3N (5:95:1 and then 10:90:1) affording a yellow foam which was precipitated from CH_2Cl_2 (3 mL) into hexane-Et_2O (2:1, 750 mL). The product was dissolved in CH_2Cl_2 (3 mL) and dried under high vacuum to give the title compound **13** (1.33 g, 68% yield over 2 steps) as a white solid; mp 98-101 °C; TLC [MeOH-EtOAc-Et_3N (10:90:1)]: R_f 0.26; IR (cm^{-1}) 3265, 2929, 1564, 1505; ^{1}H NMR [(CD_3)$_2$SO]: δ 2.04 (s, 3 H, CCH_3), 2.32 (m, 1 H, 2'-CH_2), 2.87 (m, 1 H, 2'-CH_2), 3.00-3.20 (m, 8 H, N(CH_3)$_2$ and 5'-CH_2), 3.69 (s, 3 H, CH_3O), 3.70 (s, 3 H, CH_3O), 3.98 (m, 1 H, 4'-CH), 4.49 (m, 1 H, 3'-CH), 4.69 (m, 2 H, CH_2O), 5.16-5.41 (m, 3 H, CH_2 and 3'-OH), 6.00 (m, 1 H, CH), 6.73-7.34 (m, 13 H, 13 x CH (Ar)), 8.14 ppm (s, 1 H, 8-CH); ^{13}C NMR [(CD_3)$_2$SO]: δ 17.4 (CH_3C), 38.2, 38.4 (CH_3)$_2$N), 38.6 (2'-CH_2), 55.1 (CH_3O), 55.2 (CH_3O), 64.6 (5'-CH_2), 67.2 (CH_2), 71.0 (3'-CH), 83.5 (1'-CH), 85.6 (Ar_3C), 86.0 (4'-CH), 113.2 (CH (Ar)), 113.2 (CH (Ar)), 117.1 (CH_2), 122.4 (5-C), 126.8 (CH (Ar)), 127.9 (CH (Ar)), 129.8 (CH (Ar)), 129.9 (CH (Ar)), 134.1 (CH), 135.7 (C), 135.8 (C), 140.2 (8-CH), 145.1 (C (Ph)), 152.2 (C-4), 158.2 (2 x *C*OCH_3), 160.8, 161.1, 161.4 ppm (C2, C6, C=N); Mass spectrum (FAB^+): *m/z* (I_r) 679 (M + H, 55%), 303 (100%); Calcd for $C_{38}H_{42}N_6O_6$: (M + H) 679.324. Found 679.326; Calcd for $C_{38}H_{42}N_6O_6$: C, 67.3%; H, 6.2%; N, 12.4%. Found C, 66.8%; H, 6.0%; N, 12.3%.

6-Amino-2-benzyloxy-6-*N*-[1-(dimethylamino)-ethylidene]-9-(2'-deoxy-5'-*O*-(4,4'-dimethoxytrityl)-D-*erythro*-pentofuranosyl)-purine-3'-*O*-(2-cyanoethyl)-*N*,*N*-diisopropylphosphoramidite (14). Nucleoside **11** (200 mg, 0.28 mmol) was dried by coevaporation with anhydrous pyridine (3 x 5 mL) and suspended in anhydrous THF (5 mL) under Ar. DIPEA (0.19 mL, 1.09 mmol) followed by 2-cyanoethyl-*N,N*-diisopropyl chlorophosphoramidite (0.09 mL, 0.40 mmol) were then added with stirring. After 40 min a white precipitate had formed and the product mixture was concentrated and the residue purified by flash chromatography eluting with EtOAc-CH_2Cl_2-Et_3N (50:50:1) affording an oil which was precipitated five times from CH_2Cl_2 (3 mL) into cold hexane (300 mL). The product was dissolved in CH_2Cl_2 (3 mL) and dried under high vacuum at 30 °C giving the title compound **14** (0.09 g, 33%) as a colourless viscous oil; mp 66-69 °C; TLC [EtOAc-CH_2Cl_2-Et_3N (50:50:1)]: R_f 0.28; IR (cm^{-1}): 3551, 3473, 2962, 2961, 1606, 1570; ^{1}H NMR [(CD_3)$_2$SO]: δ 0.95-1.23 (m, 12 H, 4 x CH_3), 2.03 (s, 3 H, CH_3C), 2.62 (t, 1 H *J* 5.8 Hz, CH_2CN), 2.74 (t, 1 H *J* 5.8 Hz, CH_2CN), 2.95-3.25 (m, 8 H, (CH_3)$_2$N, 2'-CH_2), 3.40-3.80 (m, 12 H, 2 x CH_3O, $POCH_2$, 2 x NCH, 5'-CH_2), 4.10 (m, 1 H, 4'-CH), 4.82 (m, 1 H, 3'-CH), 5.11-5.24 (m, 2 H, CH_2), 6.31 (m, 1 H, 1'-CH), 6.68-7.35 (m, 18 H, 13 x CH (Ar), 5 x CH (Ph)), 8.16, 8.17 ppm (2 s, 1 H, 8-CH); ^{13}C NMR [(CD_3)$_2$SO]: δ 17.2 (*C*H_3C), 19.8 (*C*H_2CN), 24.1, 24.2 (4 x CH_3), 37.0, 37.1 (2'-CH_2), 37.6, 38.1 ((CH_3)$_2$N), 42.5, 42.7 (2 x NCH), 54.9 (2 x CH_3O (Ar)), 58.2, 58.3, 58.4, 58.6 (PO*C*H_2CH_2CN), 63.8 (5'-CH_2), 68.1, 68.2 (CH_2 (Ph)), 73.3, 73.6 (3'-CH), 83.6 (1'-CH), 84.6, 84.8 (4'-CH), 85.4 (Ar_3C), 113.0 (CH (Ar)) 118.9, 119.0 (CN), 122.3 (5-C), 126.6, 127.6, 127.7, 128.3, 129.6 (CH (Ar)), 135.4 (2 x C (Ar)), 137.15, 137.22 (C (Ph)), 140.5

(8-CH), 144.8 (C (Ph)), 151.8 (4-C), 158.0 (2 x *C*OCH_3), 160.7, 160.9, 161.4 ppm (2-C, 6-C, C=N); ^{31}P NMR [$(CD_3)_2SO$]: δ 147.8, 148.5 ppm; Mass spectrum (APCI$^+$): *m/z* (I_r) 929 (M + H, 38%), 303 (100%); Calcd for $C_{51}H_{61}N_8O_7P$: (M + Na) 951.430. Found 951.429. Calcd for $C_{51}H_{61}N_8O_7P$: C, 66.0%; H, 6.6%; N, 12.1%; P, 3.3%. Found C, 65.7%; H, 6.5%; N, 12.0; P, 3.2%.

6-Amino-2-allyloxy-6-*N*-[1-(dimethylamino)-ethylidene]-9-(2'-deoxy-5'-*O*-(4,4'-dimethoxytrityl)-β-D-*erythro*-pentofuranosyl)-purine-3'-*O*-(2-cyanoethyl)-*N*,*N*-diisopropylphosphoramidite (15). The nucleoside **13** (22 mg, 0.32 mmol) was coevaporated with anhydrous pyridine (3 x 5 mL) and suspended in anhydrous THF (4 mL) under Ar. To this solution was added DIPEA (0.22 mL, 1.26 mmol) followed by 2-cyanoethyl-*N,N*-diisopropyl chlorophosphoramidite (0.11 mL, 0.49 mmol). After stirring (1 h) a white precipitate had formed and the product mixture was concentrated and the residue purified by flash chromatography eluting with EtOAc-CH_2Cl_2-Et_3N (50:50:1) affording an oil which was precipitated from CH_2Cl_2 (3 mL) into cold hexane (300 mL). The product was dissolved in CH_2Cl_2 (3 mL) and dried under high vacuum at 30 °C giving the phosphoramidite **15** (0.18 g, 64%) as a colourless viscous oil; mp 59-62 °C; TLC [CH_2Cl_2-EtOAc-Et_3N (50:50:1)]: R_f 0.13**;** IR (cm^{-1}): 3411, 3056, 2968, 1606, 1568; ^{1}H NMR [$(CD_3)_2SO$]: δ 0.96-1.19 (m, 12 H, 4 x CH_3), 2.03 (s, 3 H, CH_3), 2.63 (t, 1 H *J* 5.9 Hz, CH_2CN), 2.75 (t, 1 H *J* 5.8 Hz, CH_2CN), 2.95-3.25 (m, 8 H, $(CH_3)_2N$, 2'-CH_2), 3.40-3.80 (m, 10 H, 2 x CH_3O, $POCH_2$, 2 x NCH, 5'-CH_2), 4.1 (m, 1 H, 4'-CH), 4.62-4.80 (m, 3 H, CH_2O, 3'-CH), 5.16-5.32 (m, 2 H, CH_2), 5.75 (m, 1 H, CH), 6.31 (m, 1 H, 1'-CH), 6.70-7.30 (m, 13 H, 13 x CH (Ar)), 8.13, 8.14 ppm (2 s, 1 H, 8-CH); ^{13}C NMR [$(CD_3)_2SO$]: δ 17.1 (*C*H_3C), 19.4 (*C*H_2CN), 19.76 (*C*H_2CN), 19.8 (*C*H_2CN), 19.9 (*C*H_2CN), 24.1, 24.18, 24.23, 24.27, 24.29, 24.34, 24.39 (4 x CH_3), 36.9, 37.0 (2'-CH_2), 37.4, 38.1 ($(CH_3)_2N$), 42.5, 42.7 (2 x NCH), 54.92, 54.95, 54.96 (2 x CH_3O), 58.1, 58.3, 58.4, 58.5 (PO*C*H_2CH_2CN), 63.6 (5'-CH_2), 67.1 (CH_2O), 72.9, 73.1, 73.3, 73.6, (3'-CH), 83.5 (1'-CH), 84.4, 84.5, 84.7 (4'-CH), 85.5 (Ar_3C), 113.0 (CH (Ar)), 116.9, 117.0 (CH_2), 118.7, 119.0 (CN), 122.2, 122.3 (5-C), 126.6, 127.6, 127.63, 129.5, 129.6, 129.64 (CH (Ar)), 133.3, 133.8, 133.81 (CH), 135.37, 135.43, 135.45 (2 x C (Ar)), 140.2, 140.4 (8-CH), 144.8 (PhC), 151.7, 151.8 (4-C), 157.96, 157.97, 158.0 (2 x *C*OCH_3), 160.5, 160.9, 161.3 ppm (2-C, 6-C, C=N); ^{31}P NMR [$(CD_3)_2SO$]: δ 147.9, 148.6 ppm; Mass spectrum (APCI$^+$): *m/z* (I_r) 879 (M + H, 50%), 303 (100%); Calcd for $C_{47}H_{59}N_8O_8P$: (M + Na) 901.414. Found 901.410.

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Supplementary Material

Copies of ^{1}H, ^{13}C and ^{31}P NMR spectra are provided as supplementary material.

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