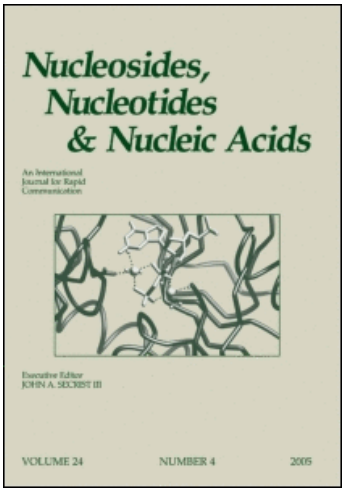

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Synthesis and Triplex Forming Properties of an Acyclic N^7-Glycosylated Guanine Nucleoside

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SYNTHESIS AND TRIPLEX FORMING PROPERTIES OF AN ACYCLIC N^7-GLYCOSYLATED GUANINE NUCLEOSIDE

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ABSTRACT

A chiral acyclic nucleoside, one in which the ribose carbohydrate has been replaced with a glycerol-based linker, is prepared by glycosylating guanine at the N^7-nitrogen. The stereochemically pure derivative is converted to a DMT-protected phosphoramidite for incorporation into DNA sequences. Sequence containing the acyclic N^7-dG nucleoside are capable of forming DNA triplexes in which it is likely that the N^1-H and N^2-amino groups of the N^7-dG are involved in recognition of the guanine base in G-C base pairs.

A variety of nucleoside analogues have been incorporated into DNA sequences with the goal of generalizing the formation of DNA triplexes at all possible duplex DNA target sites. Among such nucleoside derivatives, N^7-glycosylated guanine has been shown[1-3] to be effective in forming base triplets with dG-dC base pair targets at those target sites containing blocks of contiguous dG-dC base pairs, but is less effective with alternating dG-dC target sites. The structural details of these DNA triplexes, and DNA triplexes in general, remain at present unclear although fragments of a triple helical structure have been observed crystalographically.[4] The third strand of nearly all DNA triplexes contains a deoxyribofuranose polymer, but the role of this rigid deoxyribose ring structure is unknown. A nucleoside analogue that retains the heterocyclic portion of the molecule, essential for base-base recognition, but lacks the rigid conformational features of the ribose/deoxyribose sugar, can be obtained by replacing the sugar residue with a simple linker. Such analogues could provide increased flexibility in the formation of DNA triplexes, and may contribute favorably to overall triplex stability. The N^7-glycosylated purine derivative appeared to be a recognition element that might benefit from such elaboration.

The synthesis of a number of such acyclic nucleosides (containing simple conformationally flexible linkers replacing the ribose or 2'-deoxyribose residue) have been

described using a variety of acyclic moieties.[5-24] Many of these analogue (monomeric) nucleosides function as effective antivirals – for example, the guanine derivative 9-[(2-hydroxyethoxy)-methyl]guanine (DHPG), has been approved for clinical use against cytomegalovirus,[25] and both DHPC and acylovir are active inhibitors of herpes simplex virus type 1.[5] In addition to being antiviral agents, some acyclic nucleosides analogues have been incorporated into DNA sequences for examination as potential hybridization probes,[26-29] but the decrease in observed double-stranded helix stability when such derivatives are present has largely diminished further interest in these analogues.

In the present work we describe the synthesis of a nucleoside analogue of dG, 7-{[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl}guanine, containing an acyclic linker attached to the N^7-nitrogen. This analogue is converted to the fully protected phosphoramidite and incorporated into DNA strands with a single stereogenic center for studies with DNA triplexes.

EXPERIMENTAL

Materials: HPLC grade solvents were obtained from Fisher Scientific (Fair Lawn, NJ), other reagents were from Aldrich Chemical Co. (Milwaukee, WI). 5'-Dimethoxytrityl nucleoside phosphoramidite monomers as well as all ancillary reagents for nucleic acid synthesis were obtained from Cruachem through Fisher Scientific or from Applied Biosystems, Inc. (Foster City, CA). Oligonucleotides were synthesized using nucleoside phosphoramidite derivatives and an Applied Biosystems 381A DNA synthesizer. High-performance liquid chromatography (HPLC) was carried out on ODS-Hypersil column (0.46 x 25 cm, Shandon Southern, England), using a Beckman HPLC system. ^{1}H NMR spectra were obtained at 300 or 400 MHz on Varian XL-300 or 400 multinuclear spectrometers. ^{31}P NMR spectra were obtained at 121 MHz on the Varian XL-400. Absorption spectra were recorded by a Perkin-Elmer Lambda 3B UV/Vis spectrophotometer. Mass spectra were obtained from the Mass Spectrometry Laboratory, School of Chemical Sciences, University of Illinois, Urbana, IL.

Methods:

(*R*)-2,2-dimethyl-1,3-dioxolane-4-benzoxymethyl (2)

To 5g (37.8 mmol) of (R)-2,2-dimethyl-1,3-dioxolane-4-methanol (**1**) dissolved in 150 ml of anhydrous THF was added 1.8 g (45.0 mmol) of 60% NaH in mineral oil. After 10 min, 10 ml (84.1 mmol) of benzyl bromide was added portionwise. The mixture was stirred for 3 h at ambient temperature and then 200 ml methylene chloride was added to the reaction mixture. The solid residue was removed by filtration through celite and the filtrate

was evaporated to dryness. Column chromatography (silica gel) with gradient methylene chloride in hexane yielded 8.06 g (96%) of the product **2**.

R_f (dichloromethane): 0.22

^1H NMR ($CDCl_3$): δ = 1.36 (s, 3H, CH_3), 1.44 (s, 3H, CH_3), 3.52 (dd, 2H, CH_2), 3.75, 4.05 (dd, 2H, CH_2), 4.31 (m, 1H, CH), 4.57 (dd, 2H, CH_2), 7.34 (s, 5H, Ar-H) ppm. ^{13}C ($CDCl_3$): δ = 25.4, 26.8, 66.9, 71.1, 73.5, 74.7, 109.4, 127.7, 128.4 ppm.

(*S*)-3-Benzyloxy-1,2-propanediol (3)

To 8 g (36.0 mmol) of (R)-2,2-dimethyl-1,3-dioxolane-4-benzoxymethyl dissolved in 200 ml of 50% methanol in methylene chloride and was added 5 ml of conc. HCl and the solution stirred for 10 min. A solution of saturated sodium bicarbonate was added to neutralize the excess HCl and the organic phase was separated and dried over sodium sulfate. After removal of solvent 6.6 g (~99%) of the desired product **3** was obtained.

R_f (dichloromethane): 0.1

^1H NMR ($CDCl_3$): δ = 2.15 (t, 1H, OH), 2.65 (d, 1H, OH), 3.56 (m, 2H, CH_2), 3.68 (m, 2H, CH_2), 3.90 (m, 1H, CH), 4.56 (s, 2H, CH_2), 7.32 (m, 5H, ArH) ppm.

Otherwise identical with the commercially available material.

(*R*)-1-benzyloxy-3-O-t-butyldiphenylsilylpropanol (4)

To 5 g (27.4 mmol) of (S)-3-Benzyloxy-1.2-propanediol (**3**) dissolved in 200 ml of anhydrous methylene chloride was added 0.25 g 4-dimethylaminopyridine and 6 ml of triethylamine followed by dropwise addition of 10 ml (37.7 mmol) of t-butyldiphenyl chlorosilane while stirring under an argon atmosphere. The reaction mixture stirred overnight at ambient temperature and the mixture was then washed with saturated sodium bicarbonate solution. The organic phase was separated, dried over sodium sulfate and evaporated to dryness. Column chromatography (silica gel) with gradient methanol in dichloromethane yielded 10.4 g (90.1%) of desired product as an oil.

R_f (dichloromethane/methanol 98.5/1.5) = 0.325

UV (methanol): λ max = 206, 252, 259, 264, 270 nm, λ min = 239, 256, 262, 268 nm.

HRMS (FAB) for $C_{26}H_{32}O_3SiNa$ (M + Na) calculated 443.2018 found 443.2016

^1H NMR ($CDCl_3$): δ = 1.06 (s, 9H, 3 x CH_3), 2.48 (d, 1H, OH), 3.57 (m, 2H, CH_2), 3.73 (d, 2H, CH_2), 3.92 (m, 1H, CH), 4.53 (s, 2H, CH_2), 7.25-7.75 (15H, ArH) ppm.

^{13}C NMR ($CDCl_3$): δ = 19.3, 26.8, 64.7, 70.8, 70.9, 73.4, 127.7, 127.8, 128.4, 129.8, 133.12, 135.6 ppm.

(*R*)-1-benzyloxy-2-chloromethoxy-3-O-t-butyldiphenylsilylpropanol (5)

To 4.21 g (10 mmol) of (R)-1-benzyloxy-3-O-t-butyldiphenylsilylpropanol (**4**) dissolved in 50 ml of anhydrous methylene chloride was added 1.39 g of paraform-

aldehyde. The mixture was cooled to -4 °C and HCl gas was passed through the solution until all solid material had disappeared. The mixture was sealed and stored overnight at 4 °C. The reaction mixture dried over sodium sulfate and evaporated to dryness at low temperature (10 °C). The crude product (**5**) obtained was partially characterized:.

Rf (dichloromethane/methanol 98.5/1.5) = 0.35

^{1}H NMR ($CDCl_3$): δ = 1.04 (s, 9H, 3 x CH_3), 3.65 (m, 2H, CH_2), 3.79 (d, 2H, CH_2), 4.05 (m, 1H, CH), 4.53 (s, 2H, CH_2), 5.61 (dd, 2H, CH_2), 7.25-7.75 (15H, ArH) ppm.

This material was used directly in the next reaction:

(*R*)-7-{[2-O-t-butyldiphenylsilyl-1-(benzyloxymethyl)ethoxy]-methyl}-N-acetylguanine (7), (R)-9-{[2-O-t-butyldiphenylsilyl-1-(benzyloxymethyl)-ethoxy]-methyl}-N-acetylguanine (8)

To 4 g (20.7 mmol) of oven dried (100°C) N-acetylguanine[30] suspended in 150 ml of anhydrous acetonitrile was added 11 ml of N,O-bis(trimethylsilyl)acetamide. The mixture was stirred at ambient temperature under argon for 30 min at which point the solution turned clear. The mixture was cooled in an ice-water bath and the crude (R)-1-benzyloxy-2-chloromethoxy-3-O-t-butyldiphenylsilylpropanol (**5**) from the previous step was added. To this cooled mixture was added tetrabutylammonium iodide (0.18 g, 0.5 mmol) and the reaction stirred at ambient temperature for a day and refluxed overnight. The reaction was quenched with 40 ml methanol and 10 ml water and stirred for one hour at ambient temperature. The solvent was removed to yield an orange solid. The N^7 and N^9 products were not resolvable at this stage by silica gel chromatography but represented the two major products and could be resolved from other impurities to yield 3.20 g (24%) of a mixture of **7** and **8**.

R_f (5% methanol/dichloromethane): 0.575 (**7**) and 0.525 (**8**)

Preparative tlc permitted the resolution of small quantities of each product for NMR characterization:

Compound **7**:

^{1}H NMR ($CDCl_3$): δ = 1.04 (s, 9H, CH_3), 2.38 (s, 3H, CH_3), 3.70 (d, 2H, CH_2), 3.65 (m, 1H, CH), 3.95 (s, 2H, CH_2), 4.81, 4.87 (dd, 2H, CH_2), 5.78 (m, 2H, CH_2), 7.38-7.66 (15H, ArH), 7.84 (s, 1H, C8H), 11.18 (bs, 1H, NH) and 12.41 (bs, 1H, NH) ppm.

^{13}C NMR ($CDCl_3$): δ = 20.2, 25.7, 27.9, 63.6, 65.2, 76.7, 81.4, 113.0, 128.9, 131.0, 133.9, 134.1, 136.6, 144.9, 149.3, 154.4, 158.6, 174.4 ppm.

Compound **8**:

^{1}H NMR ($CDCl_3$): δ = 1.0 (s, 9H, CH_3), 2.1 (s, 3H, CH_3), 3.48, 3.53 (dd, 2H, CH_2), 3.66 (d, 1H, CH), 4.4 (s, 2H, CH_2), 4.67 (d, 2H, CH_2), 5.54 (m, 1H, CH), 7.1-7.6 (15H, ArH), 7.71 (s, 1H, C8H), 8.60 (bs, 1H, NH), 11.96 (bs, 1H, NH) ppm.

^{13}C NMR ($CDCl_3$): δ = 23.8, 27.3, 28.9, 31.4, 67.1, 68.6, 77.6, 84.7, 96.3, 125.5, 132.5, 134.7, 137.5, 140.4, 143.9, 153.4, 160.34, 178.1 ppm.

(*R*)-7-{[2-O-t-butyldiphenylsilyl-1-(hydroxymethyl)ethoxy]-methyl}-N-acetylguanine (9)

To 2.3 g (3.68 mmol) of a mixture of **7** and **8** dissolved in 120 ml of ethanol and 80 ml of cyclohexane was added 2.5 g 20% $Pd(OH)_2/C$. The reaction refluxed for 24 hrs (75°C), was then cooled and the reaction mixture filtered through celite. The solvents were removed, and the residue purified by silica gel chromatography (a gradient of methanol in dichloromethane) to yield 0.40 g (20%) of the N^7 glycosylated product (**9**) resolved from 0.37 g (16 %) of the N^9-glycosylated product (**9a**).

Compound **9**:

Rf (5% methanol/dichloromethane): 0.17

HRMS (FAB) for $C_{27}H_{34}O_5N_5Si$ ($M + H^+$) calculated: 536.2329, found: 536.2329

^1H NMR ($CDCl_3$): δ = 1.04 (s, 9H, $3CH_3$), 1.08 (s, 1H, OH), 2.38 (s, 3H, CH_3), 3.70 (m, 5H, $2CH_2$ and CH), 5.8 (dd, 2H, CH_2), 7.3-7.6 (Ar), 7.84 (s, 1H, C_8H), 11.05 (bs, 1H, NH), 12.42 (bs, 1H, NH) ppm.

^{13}C NMR ($CDCl_3$): δ = 1.5, 17.6, 25.2, 28.1, 60.9, 62.4, 74.1, 78.7, 126.3, 128.4, 133.0, 133.9, 142.3, 146.4, 171.4 ppm.

Compound **9a**:

Rf (5% methanol/dichloromethane): 0.12

HRMS (FAB) for $C_{27}H_{34}O_5N_5Si$ ($M + H^+$) calculated: 536.2329, found: 536.2329

^1H NMR ($CDCl_3$): δ = 0.99 (s, 9H, $3CH_3$), 1.98 (s, 3H, CH_3), 2.46 (s, 1H, OH), 3.60-3.75 (m, 5H, $2CH_2$ and CH), 5.40 (dd, 2H, CH_2), 7.26 (s, 1H, C_8H), 7.30-7.50 (ArH), 11.68 (bs, 1H, NH), 12.23 (bs, 1H, NH) ppm.

^{13}C NMR ($CDCl_3$): δ = 22.9, 25.1, 27.7, 63.4, 65.0, 74.0, 81.2, 122.0, 128.9, 131.0, 133.9, 134.0, 136.5, 140.4, 149.9, 156.8, 174.8 ppm.

(*R*)-7-{[2-O-t-butyldiphenylsilyl-1-O-(4,4'-dimethoxytritylmethyl)ethoxy]-methyl}-N-acetylguanine (10)

To 395 mg (0.74 mmol) of **9** coevaporated from anhydrous pyridine (x3) and then dissolved in 2 ml of anhydrous pyridine was added 375 mg (1.11 mmol, 1.5 equiv) of dimethoxytrityl chloride. The reaction stirred at ambient temperature for 2 h at which point it was quenched with methanol, the solvents removed and the residue coevaporated from toluene (3x) to yield a yellow foam. Purification of the product by silica gel chromatography using a gradient of methanol in 0.5% triethylamine/dichloromethane yielded 378 mg (61%) of **10**.

Rf (5% methanol/dichloromethane): 0.36
HRMS (FAB) for $C_{48}H_{52}O_7N_5Si$ (M + H^+) calculated 838.0457 found: 838.0460
^1H NMR ($CDCl_3$): δ = 0.95 (s, 9H, 3 x CH_3), 2.33 (s, 3H, CH_3), 3.16 (m, 2H, 2 x CH_2), 3.64 (d, 2H, CH_2), 3.74 (s, 6H, $2CH_3$), 4.08 (m, 1H, CH), 7.16-7.59 (ArH), 7.91 (s, 1H, C8H), 11.10 (bs, 1H, NH), 12.30 (bs, 1H, NH) ppm.
^{13}C NMR ($CDCl_3$): δ = 19.7, 25.8, 56.6, 64.9, 65.0, 76.6, 79.5, 86.8, 112.9, 114.4, 126.6, 127.6, 128.0, 129.1, 129.6, 129.9, 131.2, 131.4, 133.6, 135.1, 135.5, 143.1, 145.1, 145.3, 157.5, 159.0, 174.3 ppm.

(*S*)-1-{[2-O-4,4'-dimethoxytrityl-1-(hydroxymethyl)ethoxy]methyl}-N-acetylguanine (11)

To 448 mg (0.54 mmol) of **10**, dissolved in 50 ml of anhydrous THF was added 1.8 ml of 1M nBu4NF in THF. The reaction stirred overnight at ambient temperature. The reaction was stopped by the addition of a few grams of silica gel, the solvent was removed and the solid material was added to the top of a silica gel column. The product was resolved by chromatography using a gradient of methanol in 0.5% triethylamine/dichloromethane to yield 309 mg (96%) of **11**,.
R_f (5% methanol/dichloromethane): 0.14
HRMS (FAB) for $C_{32}H_{34}O_7N_5$ (M + H^+) calculated 600.2458 found: 600.2470
^1H NMR ($CDCl_3$): δ = 1.25 (bs, 1H, OH), 2.35 (s, 3H, CH_3), 3.20 (m, 2H, CH_2), 3.64 (m, 2H, CH_2), 3.76 (s, 6H, $2CH_3$), 3.98 (m, 1H, CH), 4.85-4.92 (dd, 2H, CH_2), 6.7 (d, Ar), 7.19-7.39 (ArH), 7.97 (s, 1H, C8H), 11.5 (bs, 1H, NH), 12.41 (bs, 1H, NH).
^{13}C NMR ($CDCl_3$): δ = 26.4, 56.2, 63.8, 64.8, 76.9, 80.7, 87.4, 112.7, 114.2, 127.9, 128.9, 129.2, 131.1, 136.9, 145.5, 145.8, 149.0, 154.6, 158.4, 159.6, 174.6 ppm.

(*R*)-1-{[2-O-(2-cyanoethoxy)-diisopropylaminophosphino-1-O-(4,4'-dimethoxytritylmethyl)ethoxy]methyl}thymine (12)

To 296 mg (0.50 mmol) of **11** dissolved in 1 ml of freshly distilled anhydrous dichloromethane was added 0.35 ml (2.0 mmol, 4 equiv) of anhydrous diisopropylethylamine and 0.33 ml (1.49 mmol, 3 equiv) of 2-cyanoethyl N,N-diisopropylchlorophosphoramidite. The reaction stirred under argon at ambient temperature for 1 h and then quenched with methanol. The mixture was washed with saturated sodium bicarbonate, the organic phase separated and dried over magnesium sulfate. The organic fraction was evaporated to dryness and the residue was precipitated into hexane to yield 324 mg (85%) of **12**.
R_f (5% methanol/dichloromethane): 0.37
^{31}P NMR ($CDCl_3$): δ = 147.64, 147.64 ppm.

DNA Synthesis

The 15-mers containing acyclic N^7-dG derivative as well as the native 25-mers were prepared by solid phase DNA synthesis under standard conditions.[31] The analogue could be incorporated into DNA strands with coupling efficiencies that were comparable to those of common nucleoside phosphoramidites.

Deprotection of the assembled oligonucleotides was performed under the commonly used conditions of conc. ammonia at 50 °C overnight (12h). Purification of the oligonucleotides was accomplished by HPLC (trityl on) using 50 mM triethylammonium acetate (pH 7.0) and a gradient of acetonitrile (20 - 65% over 40 min). The DMT-protected 15 mer oligonucleotides had retention times of about 20 min. The collected oligonucleotides were reduced in volume, detritylated with 80% aqueous acetic acid (30 min, 0 °C), desalted (Sephadex G-10), and stored at -20 °C. The resulting oligonucleotides were analyzed by HPLC (4.6 x 250 mm column of ODS-Hypersil) using triethylammonium acetate (pH 7.0) and a gradient of acetonitrile (20 - 70% over 120 min). The 15-mer oligonucleotides had retention times of about 28 min and eluted as single peaks.

Nucleoside Analysis

Oligomers containing the acyclic N^7-dG analogue were effectively digested into monomeric units only with a combination of spleen phosphodiesterase, S1 nuclease, and snake venom phosphodiesterase/calf intestinal alkaline phosphatase: a 10 μL reaction mixture containing 0.5 A_{260} unit of oligomer in 200 mM sodium chloride/5 mM $MgCl_2$/0.1 mM $ZnSO_4$/25 mM sodium acetate, pH 5.9, 1 unit of Spleen phosphodiesterase and 1 unit of S1 nuclease was incubated overnight at 37°C. To this mixture was added 5 μL of 0.1 M Tris-HCl, pH 8.0, and 1 unit of calf intestinal alkaline phosphatase and, 1 unit of snake venom phosphodiesterase and the reaction incubated for an additional 60 min at ambient temperature. An aliquot of this mixture was analyzed by HPLC (4.6 x 250 mm column of ODS-Hypersil, 50 mM triethylammonium acetate pH 7.0) and resulted in the elution of the acyclic N^7-dG derivative with a retention time of 9.1 min (0 - 70% acetonitrile over 2 h).

Thermal Denaturation Studies

Thermal denaturation studies were performed in 25 mM PIPES pH 6.4 or pH 7.0, 25 mM HEPES, pH 7.5 or pH 8.0 and 10 mM magnesium chloride and 50 mM sodium chloride at strand ratios of 1:1:1 at a concentration of 1 μM). Absorbance and temperature values were measured with an AVIV 14DS UV/Visible spectrophotometer equipped with digital temperature control. The temperature of the cell compartment was increased in 0.5 °C steps (from 0 to 85 °C) and when thermal equilibrium was reached, temperature and

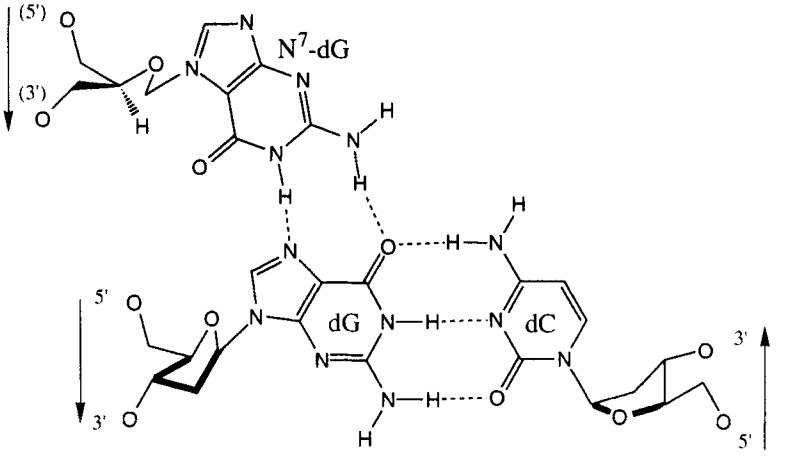


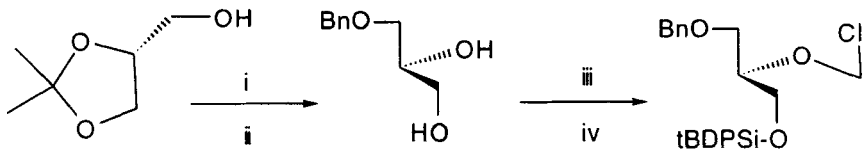
Figure 1. Illustration of the base triplet involving the acyclic derivative of N^7-dG and a dG-dC base pair target. Arrows denote the 5'→3' polarities of the strands.

absorbance data were collected. T_m values were determined both from first-order derivatives and by graphical analysis of the Absorbance vs Temperature plots - if these values were not in agreement (± 1 °C), then the graphical analysis was used as the first choice.

RESULTS and DISCUSSION

The acyclic linker chosen for this study is that which can be viewed as a ring-opened carbohydrate resulting from the elimination of the C-2' carbon. This change results in a chiral acyclic linker that can adopt the same conformation as the more rigid deoxyribose residue in the formation of base triplets involving N^7-dG and dG-dC base pairs (Figure 1). Nucleoside analogues containing this linker have been prepared by glycosylation of the appropriate heterocycle with 2-O-(acetoxymethyl)-1,3-di-O-benzylgycerol.[19,20,26,32] This approach generates a racemic mixture of glycosylation products when the *meso* acyclic nucleoside is deprotected and converted to the DMT-protected phosphoramidite derivative necessary for incorporation into DNA by chemical synthesis protocols. Maintaining the integrity of the C4'-chiral center in such derivatives can be accomplished by using the mono-benzyl derivative, (S)-3-benzyloxy-1,2-propanediol (see **3**, Scheme 1),[26] but the high cost of this material makes that route less attractive. The use of a pivaloyl protecting group in other work resulted in protecting group migration[33] during the chloromethylation procedure, further complicating the synthetic route.

An improved synthesis was achieved by starting with the more affordable (*R*)-(-)-2,2-dimethyl-1,3-dioxolane-4-methanol (**1**, Scheme 1), which after benzylation and



1 3 5

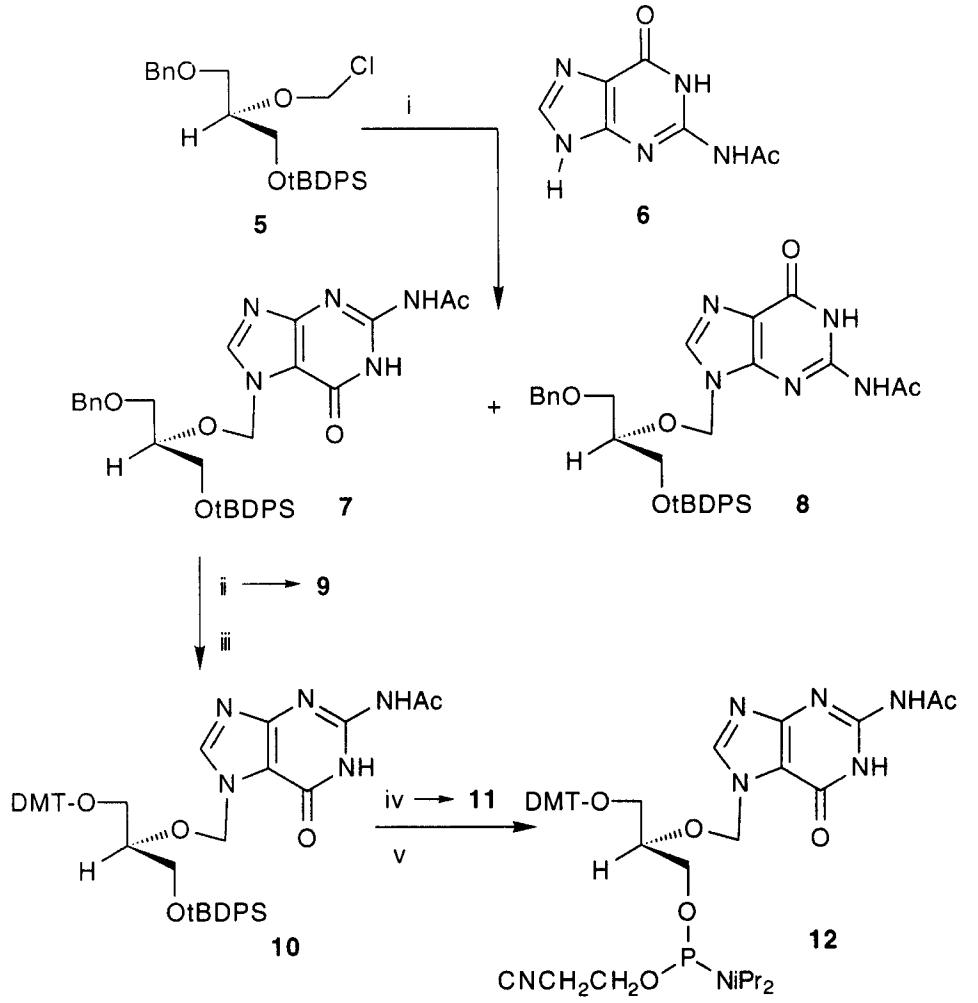
(i) NaH, Bn-Br, (ii) HCl, CH_3OH, (iii) tBDPSi-Cl, DMAP, (iv) $(CH_2O)_n$, HCl.

Scheme 1

hydrolysis produces the commercially available **3**. The remaining primary hydroxyl group was then protected as the *t*-butyldiphenylsilyl ether prior to chloromethylation. No migration or loss of the *t*-butyldiphenylsilyl group was observed in this step. Glycosylation of N-acetyl guanine produced both the N^7- and N^9-glycosylated nucleosides (**7** and **8**, Scheme 2) in roughly equivalent yields. Identification of the two products could be achieved by comparison of NMR data with related compounds. Separation of the two regioisomers was most efficiently accomplished after removal of the benzyl group, producing **9** from **7** (and the corresponding regioisomer from **8**). The desired isomer **9** was converted to the DMT-protected compound **10** and subsequently to the phosphoramidite **12** using standard procedures.

The analogue **12** could be used for incorporation of the acyclic N^7-dG analogue into 15-mer DNA sequences using standard procedures with yields that approximated those of native nucleosides. After purification of the oligonucleotides by HPLC, enzyme digestion of a small quantity, followed by HPLC resolution of the monomers confirmed the presence of the acyclic N^7-dG monomer.

We examined the formation of DNA triplexes of 15 residues containing the polypurine target sequence 5'-...GAGAG...3' or 5'-...GGGGG...3' (Table 1). A third strand composed of thymine (T) and 5-methylcytosine residues (M) at alternating positions forms very stable triplexes with duplex targets containing alternating A-T and G-C base pairs. At pH 6.4 in the presence of the polyamine spermine, a T_m of 55 °C was observed for this complex (entry b, Table 1). By comparison, a third strand containing five adjacent M residues formed triplexes of questionable stability near neutral pH, and with poor hyperchromicity even at pH 6.4 (entries c and d, Table 1). Presumably the presence of adjacent charged M+ residues, necessary to target adjacent G-C base pairs, leads to charge-charge repulsion and destabilization of the complex. The acyclic N^7-G analogue (aN^7-G) provides bidentate hydrogen bonding to target G-C base pairs without the need for protonation (see Figure 1). However, simply substituting aN^7-G for M in order to target the sequence of alternating A-T and G-C base (entries e and f, Table 1) resulted in a loss of



(i) tetrabutylammoniaum iodide, (ii) $Pd(OH)_2/C$, cyclohexene (iii) DMT-Cl/pyridine, (iv) nBu_4NF, (v) $iPr_2NP(Cl)OCH_2CH_2CN$

Scheme 2

Table 1. Effect of the Acyclic N^7-G Analogue on the T_m Values for Triplexes Containing Sequences Rich in G-C Base Pairs[a]

```
5'        TTTMTTTT W X W X W TT          3'
5'  GCGCGAAAGAAAA G Y G Y G AA CCCGG    3'
3'  CGCGCTTTCTTTT C Z C Z C TT GGGCC    5'
```

						pH Value			
entry	**W[b]**	**X[b]**	**Y**	**Z**	**[Spermine]**	**6.4**	**7.0**	**7.5**	**8.0**
a	M	T	A	T		43	35	33	29
b	M	T	A	T	0.5 mM	55	43	38	27
c	M	M	G	C	-	11	-[c]	-	-
d	M	M	G	C	0.5 mM	18	-	-	-
e	aN^7-G	T	A	T	-	12	11	-	-
f	aN^7-G	T	A	T	0.5 mM	15	13	14	15
g	aN^7-G	aN^7-G	G	C	-	21	17	17	17
h	aN^7-G	aN^7-G	G	C	0.5 mM	24	18	17	16

a. Buffer consists of 50 mM NaCl, 10 mM $MgCl_2$, and 25 mM PIPES (pHs 6.4 and 7.0) and 25 mM HEPES (pHs 7.5 and 8.0).
b. "a" denotes the nucleoside derivative containing the acyclic carbohydrate linker, M = m^5C.
c. "-" no triplex transition observed.

complex stability. This observation can most likely be explained in that base stacking effects for the alternating purine and pyrimidine sequence may be less than optimal. By comparison, a sequence composed of five adjacent aN^7-G residues was much more effective than that containing M residues in targeting the sequence containing five adjacent G-C base pairs (entries g and h, Table 1). In fact the observations for the stability of triplexes containing contiguous and non-contiguous G-C base pairs is reversed when aN7-G is used in place of M. The triplexes containing five adjacent aN^7-G residues (entries g and h, Table 1) exhibit T_m values that are significantly higher than the triplex containing alternating G-C base pairs (entries e and f, Table 1). Similar observations have been made for the N^7-dG nucleoside[1-3] in which complexes containing adjacent residues are found to be more stable than those containing isolated residues. This observation likely reflects improved base stacking effects between adjacent N^7-glycosylated guanines.

CONCLUSIONS

The described derivative can be prepared in a relatively straightforward synthetic pathway from a common linker derivative containing a single stereogenic center - one

which corresponds to that of the C4' in native nucleosides. In our hand the acyclic nucleosides (aN^7-G and aN^9-G) can be resolved more easily than the corresponding nucleoside derivatives which simplifies somewhat the synthetic procedures. The aN^7-G derivative represents a useful addition to the repertoire of nucleoside analogues available for the formation of DNA triplexes.

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