Base-boronated dinucleotides: synthesis and effect of N7-cyanoborane substitution on the base protons

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ABSTRACT
Boron-modified nucleic acids comprise a new set of DNA mimics that have potential biological and therapeutic applications. A series of nine dinucleotides containing N7-cyanoborane-2'-deoxyguanosine (7b dG) at the 3', 5' or both positions of the phosphodiester linkage have been synthesized using solution phase phosphoramidite chemistry. Fmoc was used as the 5'-protecting group because of incompatibility of the cyanoborane moiety with 5'-DMT cations generated during the deprotection step. The presence of the cyanoborane group was confirmed on the basis of comparison of the D2O exchange kinetics of the H-8 proton of 7b dG in the dinucleotides shifted 0.35–0.80 p.p.m. downfield relative to that of unmodified dG. A comparison of the D2O exchange kinetics of the H-8 proton at 60°C showed that H-8 of 7b dG is very labile relative to unmodified dG, indicating that the N7-cyanoborane substitution increases the acidity of the H-8 proton of 7b dG. These studies illustrate the feasibility of synthesizing boron-containing oligonucleotides which are modified at the N7-guanine to block Hoogsteen pairing in the DNA major groove.

INTRODUCTION
Recent advances in the field of nucleic acid chemistry have drawn attention to the synthesis of oligonucleotides with modified backbones (1–11). A number of these modified oligonucleotides have resulted in compounds with potentially useful therapeutic properties, i.e., targeting cellular DNA or RNA and interfering with gene expression via antisense and anti-malarial activity in mammalian cell lines, and anti-inflammatory and hypolipidemic activities in mice (30,31). The N7-boronated-2'-deoxyguanosine (7b dG) is of particular interest because, like the N7-deaza analogue, it does not prevent Watson–Crick base pairing (32), but rather blocks Hoogsteen base pairing. Furthermore, the 5'-triphosphate-7b dG is an excellent substrate for DNA polymerases including the thermostable Vent® and Taq® polymerases, and it is incorporated within an M13mp2 DNA duplex efficiently (33,34). The unique properties exhibited by 7b dG and the ability to enzymatically prepare long DNA polymers of good stability (34) have led us to explore the chemical syntheses of oligonucleotides containing a cyanoborane moiety on the heterocyclic base.

A series of boron-modified d(7b dG)X, d(Xp7b dG) and d(7b dG)X deoxydinucleotides have been chemically synthesized, wherein 7b dG is N7-cyanoborane-dG, and X is either a dA, dC, dG or dT. The structural properties of all nine dinucleotides containing 7b dG have been studied using 1H and 31P NMR. It was of interest to examine the effect of the cyanoborane moiety on the base stacking interactions and the sugar–base backbone conformation, as well as the influence of sequence. For comparison, the corresponding unmodified deoxydinucleotides d(GpX) and d(XpG) were also included in this study. We report here, for the first time, the syntheses of all nine possible combinations of dinucleotides containing N7-cyanoborane 2'-deoxyguanosine and the effect of cyanoborane substitution on the base protons of dinucleotides.

MATERIALS AND METHODS

General
All solvents, chemicals and reagents were of analytical grade and used without further purification unless otherwise indicated. The 3-O-(acetyl)-2'-deoxyinosine monomers (11, 12 and 14) required for coupling reactions were purchased from ChemGenes Corporation, Waltham, MA, and monomer 13 was purchased from Sigma Chemical Co. UV spectra were recorded on a Milton Roy Spectronic 3000 Array spectrometer. Baker analyzed silica gel (60–200 mesh) was used for flash column chromatography. Thin layer chromatography (TLC) was performed using 250 µ layers of silica gel GF precoated glass plates (Analtech, Inc.). Spots

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on the TLC plates were detected by visualization under short wave UV light or by heating the chromatogram at 100°C after spraying with 5% sulfuric acid in methanol. 1H NMR spectra of protected nucleosides were recorded on a Varian-300 spectrometer and reported in p.p.m. downfield from the internal tetramethylsilane (TMS=0) standard. The signals are expressed as s (singlet), d (doublet), t (triplet), m (multiplet) or br (broad). The presence of exchangeable protons was confirmed by treatment with deuterium oxide followed by reintegrating the NMR spectrum.

**NMR experiments**

All 1H, 31P and 11B spectra of dinucleotides were collected using a reverse-detect probe on a Varian Unity-500 MHz NMR spectrometer. All 2H chemical shifts were measured relative to TSP (3-trimethylsilyl-propionate-2,3,3,3-d4 sodium salt) as internal reference. The field-frequency lock was provided by deuteron oxide in the solvent. Spectra were measured at 30 ± 0.2 and 60°C for deuterium exchange experiments. Bulk susceptibility corrections have not been made for any of the 1H NMR data.

11B NMR spectra were acquired at 160 MHz and the 1H chemical shifts were referenced externally to a solution of diethylboron trifluoride Et2O·BF3. 31P NMR spectra were acquired at 202 MHz and the chemical shifts were referenced externally to a solution of 85% H3PO4. 11B signals of BH2CN in the boronated dinucleotides were not observed even in the 1H-decoupling modes under the conditions used, possibly due to the 11B broadening effect and asymmetric electronic environments of 11B in this molecule (35). Thus, the 1H chemical shifts of N7-cyano-borane-containing dinucleotides are not reported in this paper.

**NMR samples**

Purified unmodified dinucleotides were obtained from Sigma Chemical Co. [d(CpG), d(ApG) and d(GpG)], Clonter Laboratories, Inc., Palo Alto, CA [d(TpG)] and ChemGenes Corporation [dGpG]). Weighed dinucleotides for NMR experiments were dissolved in 100 mM NaCl, 0.1 mM EDTA, 10 mM potassium phosphate, pH 7.4. The samples were brought into D2O by lyophilizing the solution once or twice with 99.996% D2O to a final concentration of 3 mM of dinucleotide. A 5 mm NMR sample tube (Wilmad) was used.

**Kinetics of GH-8 proton exchange with D2O**

In order to examine the ability of GH-8 base protons to exchange with D2O, we monitored the GH-8 1H NMR spectra at different time intervals while incubating an equimolar mixture of d′(7b GpG) and d′(7b CpG) in D2O at 60°C over 2 h. The deuterium substitution rates were measured by following changes in intensities of the respective 1H NMR signals with time and the data was analyzed according to pseudo-first order kinetics.

**General procedure for the synthesis of 5′-O-(Fmoc)-3′-phosphoramidite 2′-O-deoxynucleosides (6–9)**

5′-O-(Fmoc)-2′-O-deoxynucleoside (1 mmol) was dissolved in anhydrous THF (10 ml) under argon atmosphere. N,N-diisopropylethylamine (4 equiv.) was added in the solution followed by 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (2.5 equiv.) under anhydrous conditions. The mixture was stirred at room temperature under argon atmosphere for 30 min. The TLC (solvent: TEA:MeOH:CH2Cl2:1:5:94 v/v/v) showed complete disappearance of the starting material and formation of a less polar compound. The reaction was quenched by the addition of methanol (0.1 ml). The solvents and excess of reagents were removed under reduced pressure. The residue was dissolved in a solution of 2% triethylamine in ethyl acetate (100 ml) and washed with saturated sodium bicarbonate solution (2×50 ml), saturated sodium chloride solution (2×50 ml) followed by water (50 ml). All washings were combined and re-extracted with 2% triethylamine in ethyl acetate solution (50 ml). The organic phase was dried (Na2SO4) and filtered. The solvent was removed under reduced pressure, and the resulting residue was then taken up in ethyl acetate and precipitated with cold hexane. The solid was collected by filtration and dried under vacuum to afford 72–90% (depending upon the nucleoside) of the corresponding 5′-Fmoc-deoxynucleoside 3′-phosphoramidite. The purity and homogeneity of the compound was checked with 31P and 1H NMR.

5′-O-(Fmoc)-thymidine 3′-O-(N,N-diisopropylaminophosphoramidite) (6)

Compound 6 was prepared in 74% yield following the general procedure using 5′-O-(Fmoc)-thymidine 1 (464 mg, 1 mmol), N,N-diisopropylethylamine (0.69 ml, 4 mmol) and 2-cyanoethyl N,N-diisopropyl chlorophosphite (0.456 ml, 2 mmol) in anhydrous THF (8.0 ml). 1H NMR (CDCl3): δ 8.07 (s, 1H, H-5′), 7.72 (d, J = 7.5 Hz, 2H, Ar-H), 7.74 (d, J = 7.5 Hz, 2H, Ar-H), 7.54 (t, J = 7.5 Hz, 2H, Ar-H), 7.34 (m, 4H, Ar-H), 7.26 (m, 2H, Ar-H), 6.85 (s, 1H, H-6′), 5.58–4.32 (m, 5H, H-3′, H-4′, CH2OCH2CN), 4.20 (m, 2H, H-5′), 3.8–3.51 (3xH, 4H, 2×CH2→H, OCH2CH2CN), 1.29–1.17 (m, 12H, 2×CH2OCH2CN). 31P NMR (CDCl3): δ 149.97, 149.82 p.p.m.

N6-Benzoyl-5′-O-(Fmoc)-2′-O-deoxyadenosine 3′-O-(N,N-diisopropylaminophosphoramidite) (7)

Compound 7 was prepared in 87.7% yield following the general procedure using compound 2 (575 mg, 1 mmol), N,N-diisopropylethylamine (0.69 ml) and 2-cyanoethyl N,N-diisopropyl chlorophosphite (0.46 ml, 2 mmol) in anhydrous THF (30 ml). 1H NMR (CDCl3): δ 8.94 (s, 1H, NH), 8.82 and 8.81 (2×s, 1H, H-8), 8.27 and 8.27 (2×s, 1H, H-2), 7.97 (d, J = 7.2 Hz, 2H, Ar-H), 7.75 (d, J = 7.5 Hz, 2H, Ar-H), 7.56 (m, 3H, Ar-H), 7.50 (t, J = 7.5 Hz, 2H, Ar-H), 7.38 (t, J = 7.2 Hz, 2H, Ar-H), 7.32 (t, J = 7.2 Hz, 2H, Ar-H), 6.53 (q, J = 6.0 Hz, 1H, H-1′), 4.82 (m, 1H, H-3′), 4.47–4.39 (m, 3H, H-4′, H-5′), the methylene protons were assigned as in compound 6. 31P NMR (CDCl3): δ 149.92, 149.77 p.p.m.

N4-Ibu′-5′-O-(Fmoc)-2′-O-deoxycytidine 3′-O-(N,N-diisopropylaminophosphoramidite) (8)

Compound 8 was prepared in 72% yield following the general procedure using compound 3 (519 mg, 1 mmol), N,N-diisopropylethylamine (0.69 ml) and 2-cyanoethyl N,N-diisopropyl chlorophosphite (0.46 ml, 2 mmol) in anhydrous THF (10 ml). 1H NMR (CDCl3): δ 8.09 and 8.03 (2×d, J = 7.5 and 12.6 Hz, 2H, H-5′, H-6′), 7.77 (d, J = 7.2 Hz, 2H, Ar-H), 7.56 (m, 2H, Ar-H), 7.41 (m, 2H, Ar-H), 7.39 (m, 2H, Ar-H), 6.28 (q, J = 7.5 Hz, 1H, H-1′), the
methylene protons were assigned as in compound 6; $^{31}$P NMR (CDCl$_3$): $\delta$ 150.13, 149.71 p.p.m.

**N$_3$-Ibu-5′-O-((Fmoc)-2′-O-deoxyguanosine-3′-O-(N,N-diisopropylamino)-phosphorodiamidite (9)**

Compound 9 was prepared in 82% yield following the general procedure using compound 4 (559 mg, 1 mmol), N,N-diisopropylethylamine (0.69 ml) and 2-azaacenaphthene-1,4-dione (0.46 ml, 2 mmol) in anhydrous THF. TLC (solvent, CDCl$_3$): $\delta$ 9.02 and 9.01 (2x, 1H, H-8), 7.77 (t, $J$ = 8.2 Hz, 2H, Ar-H), 7.59 (m, 2H, Ar-H), 7.43–7.38 (m, 2H, Ar-H), 7.37–7.28 (m, 2H, Ar-H), 2.60 (m, 1H, H-1′), 4.83–4.55 (m, 2H, H-3′, H-4′), 4.50–4.38 (m, 5H, H-5′), 3.94–3.63 (3m, 4H, 2×OCO), 2.88 and 2.78 (m, 2H, OCOCH$_2$CH), the methylene protons were assigned as in compound 6; $^{31}$P NMR (CDCl$_3$): $\delta$ 149.52, 149.27 p.p.m.

**N$_2$-Ibu-N$_7$-cyanoboranyl-5′-O-((Fmoc)-2′-O-deoxyguanosine (5)**

5′-O-((Fmoc)-N$_2$-IbuG (838.5 mg, 1.5 mmol) was dissolved in THF (18 ml) and triphenylphosphine cyanoborane (1.5 g, 5.0 mmol) was added. The mixture was heated at 85–90°C for 2 h. TLC (solvent, MeOH:CH$_2$Cl$_2$:5.95 v/v) showed that ≈50% of the starting material had been converted to a less polar compound. Solvent was removed under reduced pressure and the resulting residue was purified on silica gel column. Elution of the column with 2–3% methanol in methylene chloride gave 550 mg (61.3% yield, 93% based on recovered starting material) followed by unreacted starting material (228 mg). FAB-MS (M+H): 599.22 (calculated 599.39). $^{1}$H NMR (DMSO-d$_6$): $\delta$ 12.29 (s, 1H, NH), 11.85 (s, 1H, NHCO), 8.98 (s, 1H, H-8), 7.87 (d, $J$ = 7.5 Hz, 2H, Ar-H), 7.59 (t, $J$ = 6.9 Hz, 2H, Ar-H), 7.42 (m, 2H, Ar-H), 7.30–7.27 (m, 2H, Ar-H), 6.23 (t, $J$ = 6.6 and 6.1 Hz, H-1′), 5.54 (d, $J$ = 4.2 Hz, 1H, H-4′), 4.51–4.01 (m, 7H, H-3′, H-4′, H-5′, CH$_2$COO), 2.82–2.34 (m, 5H, H-2′, BH$_2$, CH), 1.12 (d, $J$ = 6.8 Hz, 6H, 2×CH$_3$).

**N$_2$-Ibu-N$_7$-cyanoboranyl-5′-O-((Fmoc)-2′-O-deoxyguanosine-3′-O-(N,N-diisopropylphosphorodiamidite (10)**

Compound 5 (490 mg, 0.82 mmol) was dissolved in anhydrous THF (6 ml, Aldrich Sure/Seal™) under argon atmosphere. The mixture was stirred at room temperature until a clear solution was obtained (∼15 min). N,N-Diisopropylethylamine (0.6 ml, 3.5 mmol) and cyanoethyl N,N-diisopropyl chlorophosphite (0.4 ml, 1.75 mmol) were added under argon atmosphere. The TLC (solvent, TEA:MeOH:DCM:EtOAc, 0.5:6.5:45:48 v/v/v/v) showed complete disappearance of the starting material. A mixture of oxidizing solution (0.1 M iodine in THF:lutidine:water, 7:2:1 v/v/v) was added until iodine color persisted (∼3.0 ml). After 1 h stirring at room temperature the TLC (solvent, TEA:MeOH:DCM:EtOAc, 0.5:6:5:45 v/v/v/v) showed complete conversion of the non-oxidized compound to a more polar product. A saturated solution of sodium thiosulfate was then added to the reaction mixture to quench the reaction and the solvent was removed under reduced pressure. The resulting residue was dissolved in dichloromethane (50 ml) and washed with a saturated solution of sodium bicarbonate (30 ml). The solution was cooled and the solvent was removed under reduced pressure. The resulting residue was dissolved in dichloromethane (50 ml) and washed with a saturated solution of sodium bicarbonate (50 ml). The aqueous washings were combined and re-extracted with dichloromethane (50 ml). The organic phase was dried over sodium sulfate, filtered, and solvent was removed under reduced pressure. The residue was dissolved in ethyl acetate and added into cold hexane. The separated solid was removed via filtration and dried under vacuum to yield compound 10 (534 mg, 81.8%). $^{1}$H NMR (CDCl$_3$): $\delta$ 12.23 (br, s, 1H, NH), 9.59 (bs, s, 1H, NHCO), 8.32, 8.299 (2×s, 1H, H-8). 7.751 (d, $J$ = 7.2 Hz, 2H, Ar-H), 7.59 (d, badly separated, 2H, Ar-H), 7.41–7.38 (m, 2H, Ar-H), 6.25 (m, 1H, H-1′), 4.84–4.25 (3m, 7H, H-3′, H-4′, H-5′, OCOCH$_2$CH), 3.94–3.63 (3m, 4H, 2×NH$_2$Me$_2$, OCH$_2$CH$_2$CN), 2.94–2.55 (2m, 7H, COCH$_2$Me$_2$, H-2′, OCH$_2$CH$_2$CN, BH$_3$), 1.22–1.19 (m, 18H, COCHMe$_2$, 2×NCH$_2$Me$_2$). $^{31}$P NMR (CDCl$_3$): $\delta$ 150.82, 149.51 p.p.m.
and solvent was removed under reduced pressure to yield pure compounds (19–22). In a similar manner, the compounds containing \( ^{7b}dG \) at either the 3'- or both (3' and 5') ends were prepared starting with monomers 23 and 6–10; Scheme 3. The physical properties and yields of all the nine dinucleotides containing boronated guanosine are reported in Tables 1 and 2.

RESULTS

Synthesis

All nine possible combinations of dinucleotides containing \( ^{7b}dG \) at either one or both 3'-, 5'- ends of a phosphodiester linkage were synthesized using solution phase phosphoramidite chemistry (Schemes 1–3). Initially, we envisioned the use of commercially available 5'-DMT-2'-deoxynucleosides as the viable intermediate and the N7-BH2CN precursor was readily prepared by treatment of 5'-DMT-2'-deoxyguanosine with Ph3PBH2CN (36). However, further experiments revealed that standard deprotection conditions (30 s, 20°C, 2.5 M dichloroacetic acid) for removal of DMT resulted in a complex mixture, including deboronation. Attempts to remove the 5'-DMT group selectively, without affecting deboronation, using different acids such as trichloroacetic acid or benzene sulfonic acid in various solvents (chloroform:methanol, 7:3 v/v, acetonitrile and tetrahydrofuran) were futile. It should be noted that the cyanoborane guanosine derivative is quite stable in acidic media. The cyanoborane instability is caused by the formation of an unstable adduct between the cyanoborane and DMT cation (36–38) generated during DMT deprotection. This led us to investigate an alternate route which would not involve use of the 5'-DMT protecting group.

Monomers required for the coupling reaction were synthesized via the treatment of commercially available 2'-deoxynucleosides with 9-fluorenylethoxycarbonyl chloride (Fmoc-Cl) in anhydrous pyridine following the procedure of Lehmann et al. (39) to yield the corresponding 5'-O-blocked nucleosides (1-4) in 35–62% yields (Scheme 1). The actual yields based on the recovered starting material were 58–80%. The purity and homogeneity of the compounds were checked by TLC, and the structures were confirmed on the basis of 1H and 31P NMR spectroscopy.
Table 1. Chemical shifts of $^{31}$P and base protons of d($^7$b GpX), d(Xp$^7$b G) and d($^7$b Gp$^7$b G) series in 100 mM NaCl, 0.1 mM EDTA, 10 mM phosphate, PH 7.4 at 30°C

<table>
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<tr>
<th>No./compd.</th>
<th>AH-2</th>
<th>AH-8</th>
<th>CH-5</th>
<th>CH-6</th>
<th>GH-8 (5')</th>
<th>GH-8 (3')</th>
<th>TH-6</th>
<th>T-CH$_3$</th>
<th>$^{31}$P$^b$</th>
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</thead>
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<tr>
<td>19; d($^7$b GpC)</td>
<td>-</td>
<td>-</td>
<td>5.84</td>
<td>7.82</td>
<td>8.55</td>
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<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>N d(GpC)</td>
<td>-</td>
<td>-</td>
<td>5.94</td>
<td>7.87</td>
<td>7.87</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>20; d($^7$b GpG)</td>
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<td>-</td>
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<td>8.00</td>
<td>-</td>
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<td>-3.65</td>
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<tr>
<td>N d(GpG)</td>
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<td>-</td>
<td>-</td>
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<td>8.02</td>
<td>7.78</td>
<td>-</td>
<td>-</td>
<td>-2.97</td>
</tr>
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<td>21; d($^7$b GpA)</td>
<td>8.10</td>
<td>8.38</td>
<td>-</td>
<td>-</td>
<td>8.39</td>
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<td>-</td>
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<td>7.65</td>
<td>1.74</td>
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<td>-0.91</td>
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<tr>
<td>29; d(Cp$^7$b G)</td>
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<td>-</td>
<td>8.58</td>
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<td>-</td>
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<tr>
<td>31; d(Tp$^7$b G)</td>
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<td>8.55</td>
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<td>7.78</td>
<td>-</td>
<td>-</td>
<td>-2.97</td>
</tr>
</tbody>
</table>

a Compounds in bold letter have at least one N7-boronated deoxyguanosine and those numbered N are the corresponding unmodified oligonucleotides.

b Referenced internally to the resonance from phosphate buffer.

Table 2. Physical data of dinucleotides containing N7-boronated-deoxyguanosine

<table>
<thead>
<tr>
<th>Compound</th>
<th>% yield (overall)</th>
<th>$R_f$</th>
<th>FAB-MS (m/e)</th>
<th>UV $\lambda_{max}$</th>
<th>H$_2$O</th>
<th>0.1 N HCl</th>
<th>0.1 N NaOH</th>
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</thead>
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<td>(M+Na)$^+$</td>
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<td></td>
<td></td>
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<tr>
<td>19; d($^7$b GpC)</td>
<td>39</td>
<td>0.61</td>
<td>y; 618.23</td>
<td>262.1</td>
<td>277.0</td>
<td>273.3</td>
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<tr>
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<td>31</td>
<td>0.59</td>
<td>y; 658.23</td>
<td>255.0</td>
<td>256.5</td>
<td>268.4</td>
<td></td>
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<tr>
<td>21; d($^7$b GpA)</td>
<td>37</td>
<td>0.63</td>
<td>y; 642.23</td>
<td>258.0</td>
<td>257.6</td>
<td>261.7</td>
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<tr>
<td>22; d($^7$b GpT)</td>
<td>18</td>
<td>0.62</td>
<td>y; 633.23</td>
<td>260.2</td>
<td>261.0</td>
<td>271.0</td>
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<td>y; 618.23</td>
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<td>277.3</td>
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<td>259.9</td>
<td>270.7</td>
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<td>y; 697.07</td>
<td>257.3</td>
<td>257.6</td>
<td>275.5</td>
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$^a$TLC solvent: isopropanol:ammonium hydroxide:water, 7:1.2 v/v/v; y = calculated values and z = found.

N7-Boronated monomers (5'- and 3'-fragments of dinucleotides)

The boronated monomer, N7-Ibu-5'-Fmoc-3-phosphoramidite (10), was prepared starting from compound 4 as outlined in Scheme 1. Cyanoboronation of N7-Ibu-2'-deoxyguanosine is not a suitable alternative since the loss to some extent of cyanoborane moiety occurs in pyridine. It was important, therefore, that all reactions involving use of pyridine were carried out prior to the boronation step. Thus, compound 4 on refluxing with triphenylphosphine cyanoborane (Ph$_3$P:BH$_2$CN) in THF for 2 h (heating the reaction mixture for longer time in some decomposing) gave the N7-cyanoborane derivative 5. In the $^1$H NMR spectrum of compound 5, the H-8 proton shifted downfield to 8.98 p.p.m. compared with unmodified guanine, and a broad peak for BH$_2$ at 2.84--2.34 p.p.m. merged with the H-2' proton was observed.
Treatment of compound 5 with 2-cyanoethyl N.N-diisopropylchlorophosphoramidite and diisopropylethylamine in anhydrous tetrahydrofuran gave the corresponding phosphoramidite (10; Scheme 1). $^{31}$P NMR showed phosphorus peaks at 150.82 and 149.51 p.p.m. 3'-O-(acetyl)-N2-1bu-7b-dG (23) was prepared in 65% yield starting with the compound 11 as described for 5, and the structure of compound 23 was confirmed by FAB-MS and NMR spectroscopy analogous to compound 5.

5'-Borilated dinucleotides d(7b-dGpX)

Dinucleotides containing a normal dG at the 3'-end and a boronated 7b-dG at the 5'-end were synthesized as shown in Scheme 2. N-protected-3'-O-(acetyl)-2'-deoxynuceloside (1 eqv.) was dissolved in acetonitrile and 1H-tetrazole (4.0 eqv.) was added to the solution under argon atmosphere. A solution of compound 10 (1.2 eqv.) in acetonitrile was added and the reaction mixture was stirred at room temperature for 30 min. A mixture of hydroxide at 50°C was quenched after 1 h with saturated sodium thiosulfate oxidizing solution (0.1 M iodine in THF:lutidine:water, 7:2:1 v/v/v) mixture was stirred at room temperature for 30 min. A mixture of 1H-5p-furan gave the corresponding phosphoramidite (Scheme 1).

3'-Borilated dinucleotides d(Xp7b-dG)

The synthesis of dinucleotides (29–33) containing 7b-dG at the 3'-end and either dG or 7b-dG at the 5'-ends were carried out as shown in Scheme 3. All the reaction conditions were similar to those described for Scheme 4 except that compound 23 was used as the boron-containing monomer and compounds 6–9 were used as the 5'-protected-2'-deoxynuceloside-3'-phosphoramidite. Compounds 23 and 10 were used to synthesize the dinucleotide 33 containing two 7b-dG. The structure of compounds 29–33 were confirmed on the basis of NMR ($^1$H and $^{31}$P) and FAB-MS (Tables 1 and 2). The molecular ion peak confirmed the presence of a cyanoborane moiety in the molecule.

$^1$H NMR spectral assignments

All NMR experiments were done in D$_2$O. Protons N2 and N1 of G, N4 of C, N6 of A and N3 of T are exchanged with D$_2$O at room temperature (data not included). Assignment of other protons of dinucleotides is divided into four parts: (i) non-exchangeable endocyclic base protons (H-2 and H-8 of A, H-5 and H-6 of C, H-8 of G and H-6 of T); (ii) cyanoborane protons; (iii) CH$_3$-protons of T; and (iv) sugar ring protons (H-1′, H-2′, H-3′, H-4′, H-5′). In this paper we focus primarily on the $^1$H NMR studies of the endocyclic base protons.

The assignment of proton resonances in unmodified dinucleotides, d(GpX) and d(XpG), was well documented (40). Since the $^1$H resonance of the modified bases occur in a pattern similar to that of unmodified dinucleotides, the assignments for $^1$H resonances in the boronated-dinucleotides d(7b-GpX), d(X7b-G) and d(7b-Gp7b-G) were tentatively made analogous to the unmodified dinucleotides, d(GpX), d(XpG), as shown in Table 1.

The $^1$H resonance of the BH$_2$CN moiety can easily be located. In this paper we focus primarily on the $^1$H NMR studies of the modified bases. The chemical shift of the modified base is comparable with the chemical shift of the corresponding unmodified base (Fig. 1), have values almost comparable with the corresponding unmodified dinucleotides, d(GpX) and d(XpG). In general, the chemical shift differences are greater than 0.5 p.p.m., but less than 1.0 p.p.m. The values are all positive, indicating that the presence of a BH$_2$CN moiety in the molecule causes an upfield shift of the corresponding proton resonance.

$^{31}$P NMR spectroscopy

$^{31}$P chemical shifts of all unmodified and boronated dinucleotides are reported in Table 1. The $^{31}$P chemical shifts have very similar values within a range of 2.0 p.p.m.
Chemical shift difference of GH-8 protons between boronated and unmodified deoxyguanosine in dinucleotides. Dinucleotide (∼3 mM) was prepared in 100 mM NaCl, 0.1 mM EDTA, 10 mM phosphate, pH 7.4 at 30°C in D2O. *Indicates modified or unmodified guanine.

Exchange of 7bGH-8 in d(7bGpG) with D2O

Figure 3. Exchange kinetics of GH-8 proton in d(7b GpG) and d(GpG) with D2O at 60°C.

31P chemical shifts provide information about the phosphate backbone

The 31P chemical shifts and the 1H, 31P coupling constants of oligonucleotides provide information about the phosphodiester backbone (41). The internucleotide linkage is defined by six torsion angles from one phosphate atom to the next along the DNA backbone. Theoretical studies have shown that the conformation of two of the six torsion angles (O3′-P-O5′-C5′ and C3′-O3′-P-O5′) appear to be most important in determining 31P chemical shifts (42,43). The small differences of 31P chemical shifts (<2 p.p.m.) between boron-modified and unmodified dinucleotides (Table 1) indicate that a cyanoborane moiety at N7-guanine in the d(7bGpX), d(Xp7bG) and d(7bGp7bG) series at 30°C did not induce large alterations in the phosphodiester backbone conformations, which is not surprising because cyanoborane is spatially located far from the phosphorus atom. However, care should be taken in interpreting the shifts observed in oligonucleotides, since it is uncertain to what degree the stacking and base interaction shifts are influenced by the neighboring bases (sequence effect) in dinucleotides. Detailed conformational studies by 1H NMR are in progress and will be published elsewhere.

Effect of BH2CN on the chemical shifts of non-exchangeable base protons in dinucleotides

BH2CN is an electron withdrawing group and is expected to cause a downfield shift on the neighboring protons. This is reflected (see Table 1 and Fig. 2) in the GH-8 protons of all the N7-boronated guanosines, situated either at the 5′- or 3′-end or at both the ends (3′ and 5′), such as d(7b GpX), d(Xp7bG) and d(7bGp7bG) respectively. The 7bGH-8 proton in these compounds shifted downfield by 0.35–0.8 p.p.m. relative to the corresponding GH-8 proton of unmodified residues; presumably these downfield shifts are through-bond effects. As expected, the BH2CN moiety has a relatively smaller effect (<0.1 p.p.m. in general) on the proton chemical shifts of the neighboring unmodified base in the boronated-dinucleotide (Table 1 and Fig. 1). The observed small chemical shift differences relative to unmodified dinucleotides must be due to base stacking and conformational changes caused by modification. It is noted that, in d(7bGpG), the BH2CN had a greater effect on the GH-8 chemical shifts of the neighboring unmodified guanosine residue compared with d(7bGpX) and d(Xp7bG), ∼0.2 p.p.m. and 0.1 p.p.m. respectively, where X is either a dA, a dC or a dT. The GH-8 proton shift of the unmodified residue of the d(7bGpG) dinucleotide may indicate different base stacking interactions as compared with the corresponding parent dinucleotide, d(GpG), due to the presence of a BH2CN moiety on the adjacent dinucleotide base.
GH-8 proton exchange ability in d(7bGpG)

The fact that BH2CN, an electron withdrawing group, will generate a partial positive charge on the neighboring carbon through inductive effects and will make 7bGH-8 more acidic compared with the unmodified base proton is supported by our findings. A more acidic GH-8 proton can explain our result of ∼10-fold faster deuterium exchange rate of GH-8 (5′-residue, 7bG) in the dinucleotide, d(7bGpG)1, compared with the unmodified guanosine (5′-residue) of d(GpG)1 (Fig. 3).

CONCLUSIONS

The N7b-dinucleotides were readily prepared using 5′-Fmoc-3′-phosphoramidite-2-deoxynucleosides in good yields (26–52%, including coupling, oxidation and deblocking steps). The solution phase synthesis employed for dinucleotides using 5′-Fmoc-3′-phosphoramidite-2-deoxynucleosides should be adaptable to the solid phase synthesis for oligonucleotides containing mixed sequences. The ability to readily prepare dinucleotides modified with cyano-borane at the N7b-position of guanosine that are stable to acid and base (44) facilitates further studies with these base-boronated compounds. 1H NMR at 60′ phase synthesis employed for dinucleotides using 5′-3′ borane at the N7b-position of guanosine that are stable to acid and acidic compared with unmodified GH-8 as evidenced by the 7bGH-8 proton can explain our result of ∼10-fold faster deuterium exchange rate of GH-8 (5′-residue, 7bG) in the dinucleotide, d(7bGpG)1, compared with the unmodified guanosine (5′-residue) of d(GpG)1 (Fig. 3).

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