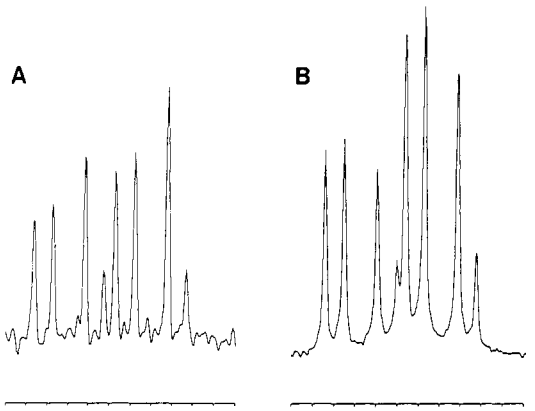
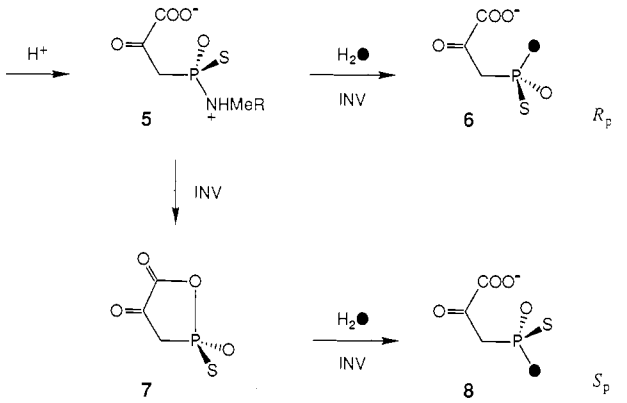


Figure 1. ^{31}P NMR spectra[15] of the axial methyl esters of the α-D-glucopyranoside cyclic 4,6-phosphates derived from stereochemical analysis of the α-D-glucopyranoside 6-[^{16}O,^{17}O,^{18}O]phosphates obtained from the phosphomutase reaction of (*R*)- and (*S*)-[^{16}O,^{17}O,^{18}O]-phosphonopyruvate (A and B, respectively).

Scheme II. Conversion of **5** to (*R*)-Thiophosphonopyruvate **6** with Inversion (as Assumed in Ref 3) or to (*S*)-Thiophosphonopyruvate **8** via **7** with Overall Retention



reaction proceeds with overall *retention* of the configuration at phosphorus.

In contrast to these results, a recent report[3] has suggested that the stereochemical course of the mutase reaction is inversion. This study used ^{18}O and sulfur to create the chirality at phosphorus, and the substrate was therefore the [^{18}O]phosphorothioate of phosphonopyruvate. While there have been occasional concerns that the use of phosphorothioates (as distinct from phosphates) could give misleading stereochemical outcomes, we do not believe this to be the cause of the discrepancy. The fact that phosphorothioate substrates have always been found to follow a stereochemical course identical with that of their all-oxy parents[10] argues against such an explanation.[11] It seems more likely that the synthesis of chiral thiophosphonopyruvate reported in ref 3 included a step in which an unnoticed inversion at phosphorus occurred. Thus the transformation of **5** (Scheme II) was presumed to go with inversion to **6**, yet the free neighboring carboxylate in **5** can displace the ammonium leaving group to give **7**, which then hydrolyzes to **8**. Such a well-precedented[13] double displacement reaction would thus give **8** with overall retention, instead of **6** with inversion, as was assumed by McQueney et al.[3]

We conclude, therefore, that the phosphomutase proceeds with overall retention and follows a mechanistic route that involves either an unremarkable phospho-enzyme intermediate or, conceivably, the intramolecular participation of the substrate's carboxylate group.[14]

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Boron-Containing Nucleic Acids: Synthesis of Cyanoborane Adducts of 2′-Deoxynucleosides

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Antiviral[1,2] and antitumor[2] activity associated with a wide variety of structurally divergent modified nucleosides has stimulated a great interest in the synthesis and biological activity of new classes of nucleic acid compounds. We have been interested in the synthesis[3-9] and activity[10-16] of boron-containing antime-

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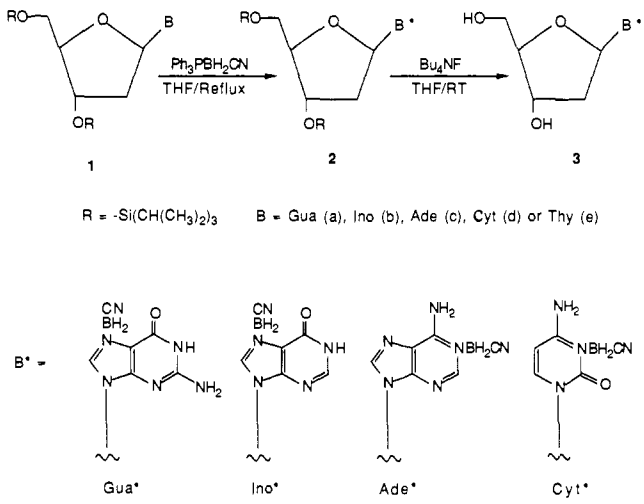
Scheme I



tabolites for a possible two-pronged attack on neoplastic tissues. In addition to the direct inhibition of tumor growth, the preferential localization of boron compounds in the neoplasm would allow the use of boron-10 neutron capture therapy (BNCT)[17] for the destruction of tumor cells. In this communication, we report the successful synthesis of a novel set of boronated 2′-deoxynucleosides and preliminary data on their physical, biochemical, and pharmacological properties.

Cyanoborane adducts of 2′-deoxynucleosides, specifically 2′-deoxyguanosine-N7-cyanoborane (**3a**), 2′-deoxyinosine-N7-cyanoborane (**3b**), 2′-deoxyadenosine-N1-cyanoborane (**3c**), and 2′-deoxycytidine-N3-cyanoborane (**3d**), were prepared by an exchange reaction of triphenylphosphine–cyanoborane[18] (Ph_3PBH_2CN) with 3′,5′-O-protected nucleosides according to Scheme I. The 3′- and 5′-hydroxy groups were protected as silyl ethers by reaction with excess chlorotriisopropylsilane[19,20] at room temperature in DMF in the presence of imidazole. In addition to the protection, the use of bulky silyl groups imparts an added important feature: it solubilizes these nucleosides in organic solvents,[19,20] thereby permitting the exchange reaction. The exchange was carried out in anhydrous THF at reflux temperature using 2 equiv of Ph_3PBH_2CN. No increase in the amount of product could be observed (by TLC) after 2–3 h, although substantial amounts of reactants were present. The major products were purified by flash chromatography. The yields obtained were 72% for the guanine derivative (**2a**), 59% for the adenine derivative (**2c**), 46% for the cytosine derivative (**2d**), and 26% for the hypoxanthine derivative (**2b**). While the first three adducts were readily purified, **2b** was obtained only in ca. 95% purity (by ^1H NMR). Attempts to prepare the boron-substituted thymidine derivative from **1e** by exchange reaction with Ph_3PBH_2CN gave no desired product.

Deprotection[21] of boronated nucleosides **2a–d** with Bu_4NF to give **3a–d** was complete within 0.5 h. Purification was achieved by flash chromatography, followed by crystallization from $MeOH/Et_2O$. Satisfactory (within ±0.25%) C, H, N analyses were obtained for the final compounds. The yields ranged from 44% for **3d** to 55% for **3b**.

The incomplete nature of the exchange reaction may be attributed to the establishment of an equilibrium as shown in eq 1. This was confirmed by the formation of **1a** and Ph_3PBH_2CN

$$\text{nucleoside} + Ph_3PBH_2CN \rightleftharpoons \text{nucleoside–}BH_2CN + Ph_3P \quad (1)$$

upon reaction of **2a** with Ph_3P in refluxing THF. The lack of reaction with **1e** is probably due to its low basicity and the absence of a less hindered two-coordinate nitrogen for thymidine. Thus, steric factors are indicated as an important component of the stability of the Lewis acid cyanoborane adducts with the various purine and pyrimidine bases.

The site of boron coordination was determined by ^{15}N NMR spectroscopy. On a JEOL FX90Q instrument, no peak was observed for the coordinated nitrogen (in both coupled and decoupled spectra) due to quadrupole broadening by boron. The absence of peaks for N7 of Gua* and Ino*, N1 of Ade*, and N3 of Cyt* indicated these nitrogens to be the site of BH_2CN coordination. When ^{15}N NMR spectra of **2a** and **2d** were obtained on a GE GN500 system, the above quadrupole effect was not observed. In this case, upfield shifts of 56.6 ppm for N7 of Gua* and 50.1 ppm for N3 of Cyt* confirmed the assignments. The shifts upon boronation are qualitatively similar to but lower than those observed upon protonation[22] (ca. 65–70 ppm). The coordination site in Gua* and Ino* is away from the sites required for Watson–Crick base pairing and should not affect pairing to a large extent. Variable-temperature ^1H NMR studies[23] on the Gua*·Cyt base pair indeed show the H bonding to be approximately as strong as in a normal Gua·Cyt base pair. The coordination at N1 in Ade* and N3 in Cyt*, however, should completely disrupt the base pairing, and if incorporated into DNA, these nucleosides may lead to inhibition of replication.

All compounds have been characterized by various spectroscopic techniques. Detailed interpretations of these data will be published separately. By HPLC, compounds **3a**, **3b**, and **3d** in aqueous medium (0.01 M TEAAc) are >94% stable over a period of 168 h. Compound **3b**, however, is >50% decomposed during this period. The good stability of **3a**, **3c**, and **3d** in aqueous medium makes these compounds suitable for pharmacological testing. In preliminary studies[24] on the antitumor activity, compounds **3c** and **3d** showed potent activity in T molt-3 and human colorectal adenocarcinoma screens.

In summary, we have prepared a set of novel boronated nucleosides. These compounds are hydrolytically stable, and preliminary results show that some of these modified nucleosides possess potent antitumor activity. It remains to be tested whether these compounds will preferentially localize in the neoplasm, making it susceptible to BNCT.

Further, it is useful to compare boronated nucleosides with alkylated nucleosides. Addition of BH_2R or CH_2R on the ring nitrogen yields adducts of similar geometry and size, yet alkylation results in a positively charged species, while boronation gives a neutral adduct. In the latter, cancellation of formal N^+ and B^- changes may yield adducts of greater stability. Thus, boronation may serve as a model for alkylation, providing insight into the biological action of alkylated nucleosides.

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Registry No. **1a**, 109460-89-1; **1b**, 123054-58-0; **1c**, 123054-59-1; **1d**, 109460-88-0; **1e**, 54925-58-5; **2a**, 123054-60-4; **2b**, 123054-61-5; **2c**, 123054-62-6; **2d**, 123054-63-7; **3a**, 123054-64-8; **3b**, 123073-91-6; **3c**, 123054-65-9; **3d**, 123054-66-0; Ph_3PBH_2CN, 67374-60-1.

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