Synthesis of Boron-Containing ADP and GDP Analogues: Nucleoside 5′-(P^{α}-Boranodiphosphates)

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New 5′-(P^{α}-boronated) analogues of the naturally occurring nucleoside diphosphates ADP and GDP were synthesized in good yields, *i.e.*, adenosine 5′-(P^{α}-boranodiphosphate) (ADPαB; **5a**) and guanosine 5′-(P^{α}-boranodiphosphate) (GDPαB; **5b**). Their diastereoisomers were successfully separated by reversed-phase HPLC, and chemical structures were established *via* spectroscopic methods. The isoelectronic substitution of borane (BH_3) for one of the non-bridging O-atoms in phosphate diesters should impart an increase in lipophilicity and change in polarity in ADPαB and GDPαB. The boronated nucleoside diphosphates could be employed for investigations of the stereochemical course and metal requirements of enzymatic reactions involving ADP and GDP, and as carriers of ^{10}B in boron neutron-capture therapy (BNCT) for the treatment of cancer.

1. Introduction. – Analogues of naturally occurring nucleotides like cyclic-AMP, ADP, and ATP have a firm place in the array of tools biochemists use to unravel enzyme functions and mechanisms [1]. For example, substrate analogues have been constructed as reversible and irreversible inhibitors, transition-state analogues, and spectroscopic probes. A variety of nucleotides with modifications in the base, sugar, or phosphate residues have been synthesized. Of the latter, the most studied phosphate-modified nucleotides (*Fig.*) are nucleoside phosphorothioates [1] in which a non-bridging O-atom of a phosphate group is replaced by an S-atom. Nucleoside phosphorothioates have found wide applications in biochemistry and molecular biology [1–3]. Another type of modified nucleotide, in which one of the non-bridging O-atoms of a phosphate group is replaced with BH_3, are the *P*-boronated nucleotides (designated nucleoside boranophosphate or borane phosphonates) [2–18].

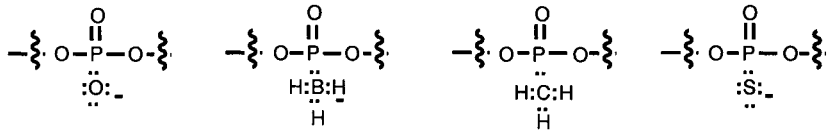


Figure. *Phosphate-modified nucleotides*

The borane moiety (BH_3) in the boranophosphate is isoelectronic with the O-atom in a phosphodiester. At neutral pH, the *P*-boronated nucleotides carry the same net negative charge as phosphodiesters and phosphorothioates (see *Fig.*), thus making boranophosphates soluble in aqueous solutions. However, distribution of charge

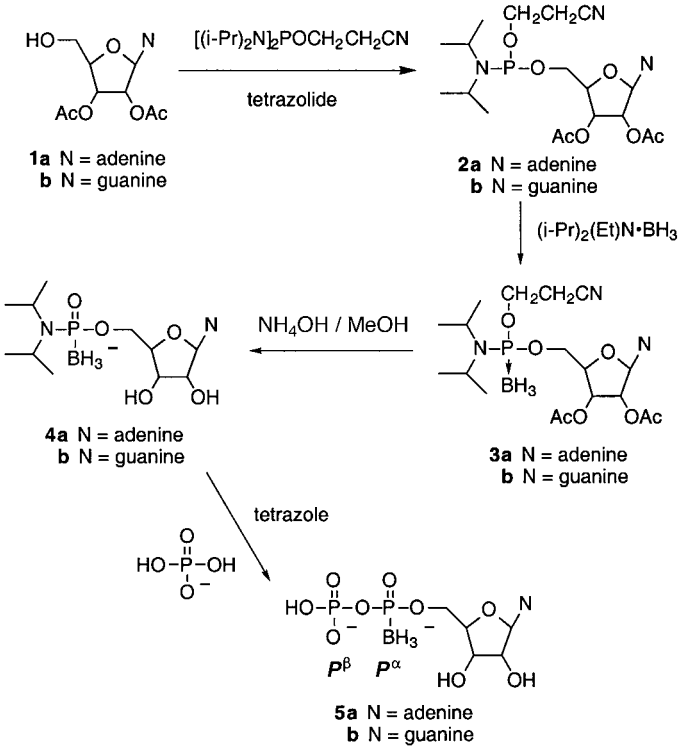
density and consequently the polarity of the modified P-moiety would differ as would the metal binding and selectivity. Furthermore, since the BH_3 group in boranophosphates is isoelectronic as well as isosteric with the CH_3^+ group, it might be expected that *P*-boronated nucleotides would also exhibit some desirable properties of the nucleoside methylphosphonates [19], such as increased lipophilicity and resistance to nucleases [10][14]. Additionally, *P*-boronated nucleotides may offer a unique advantage over other modified congeners because the former can be used for boron neutron-capture therapy (BNCT) [20], a radiation therapy that can selectively destroy cells that have preferentially taken up boron.

The synthesis and properties of some P^{α}-boranonucleotides and oligonucleoside boranophosphates have been reported [4–18]. For example, the nucleoside triphosphate analogues, nucleoside 5′-P^{α}-boranotriphosphates (dNTPαB and NTPαB), are good substrates for DNA and RNA polymerases [7][9]. Yet, after incorporation, the boranophosphate linkages in DNA or RNA are more resistant to endo- and exonucleases than normal phosphodiesters [6][10]. Here, we investigated methods to synthesize new diphosphate analogues of ADP and GDP. Substitution of BH_3 for the phosphate O-atom introduces a center of chirality into the nucleotide, so that a P^{α}-boronated nucleoside diphosphate (NDPαB) exists as a pair of diastereoisomers. The two isomers could exhibit quite different behavior toward a particular enzyme. For example, nucleoside 5′-(P^{α}-thiodiphosphates) (NDPαS) and nucleoside 5′-(P^{β}-thiodiphosphates) (NDPβS) have been widely used in stereochemical studies of phosphotransferases and NDP-dependent synthetases [1–3]. In the presence of acetate kinase and Mg^{II}, the [*P*(*R*)] isomer of ATPβS is obtained from β-prochiral ADPβS, while the [*P*(*S*)] isomer of ATPβS is synthesized predominantly in the presence of pyruvate kinase and Cd^{II} [21–23]. Nucleoside *P*-boranodiphosphates should show interesting behavior and special properties that differ from those of their biologically functional analogues, the closely related nucleoside diphosphates (NDP) and nucleoside P^{α}-thiodiphosphates (NDPαS). In this paper, we report the synthesis of two diphosphate analogues: adenosine 5′-(P^{α}-boranodiphosphate) (ADPαB) and guanosine 5′-(P^{α}-boranodiphosphate) (GDPαB), and the isolation of their diastereoisomers.

2. Synthesis and Discussion. – The phosphoramidite approach (*Scheme 1*) for the preparation of nucleoside 5′-(P^{α}-boranodiphosphates) (NDPαB; N = A, G) is a modification of our previous procedure for the synthesis of thymidine 5′-(P^{α}-boranotriphosphate) [5]. Phosphitylation of 2′,3′-di-*O*-acetylribonucleoside **1** by 2-cyanoethyl tetraisopropylphosphorodiamidite in the presence of diisopropylammonium 1*H*-tetrazolide [24] gave phosphoramidite **2**. Intermediate **2** was treated *in situ* with excess borane-*N*,*N*-diisopropylethylamine complex to yield the borane-phosphoramidite complex **3**. Subsequent treatment of **3** with a mixture of ammonium hydroxide and MeOH gave compound **4**, which was identified by a typical broad peak centered at 94 ppm in the ^{31}P-NMR spectrum of the reaction mixture. After removing solvents, compound **4** was precipitated from dry AcOEt (further purification of compound **4** by ion-exchange chromatography (*QA-52* cellulose, HCO_3^-) resulted in lower yields due to some loss of the diisopropylamino group during the chromatography, as recorded afterward in ^{31}P- and ^{1}H-NMR spectra). The precipitated compound **4** was treated with

excess tetrabutylammonium dihydrogen phosphate and 1*H*-tetrazole at 55° to yield the two diastereoisomers of nucleoside 5′-(*P*$^{\alpha}$-boranodiphosphate) **5**. They were identified by the appearance of ^{31}P-NMR signals in the crude reaction mixture at *ca.* −8 ppm (*d*) for P(β) and 84 ppm (broad peak) for P(α). The crude mixture was purified by ion-exchange chromatography to give compound **5** as the ammonium salt with 32–36% overall yields (from **1** to **5**). Successful separation of the two diastereoisomers of compound **5** was achieved by reversed-phase HPLC. The chemical structures of the two diastereoisomers were characterized by ^{31}P-NMR, ^{1}H-NMR, MS, and HR-MS.

Scheme 1. *Synthesis of Nucleoside 5′-(*P$^{\alpha}$*-Boranodiphosphates) ADPαB* (**5a**) *and GDPαB* (**5b**)



In our synthetic approach, base-unprotected nucleosides were chosen as starting materials. In the first step, we used 2-cyanoethyl tetraisopropylphosphorodiamidite ((^{i}Pr$_2$N)$_2$P(OCH$_2$CH$_2$CN)) as the preferred phosphitylating reagent. We avoided previously used 2-cyanoethyl diisopropylphosphoramidochloridite (^{i}Pr$_2$N-P(OCH$_2$CH$_2$CN)Cl) [5], which reacted with the unprotected NH$_2$ group in the nucleobases used here. With tetrazolide (*i.e.*, diisopropylammonium 1*H*-tetrazolide) as catalyst, only one of the two diisopropylamino groups of (^{i}Pr$_2$N)$_2$P(OCH$_2$CH$_2$CN) will be substituted by the free 5′-OH group of the nucleoside. By contrast, with 1*H*-tetrazole as catalyst, both diisopropylamino groups reacted with 5′-OH, resulting in the formation of undesirable by-products such as dinucleoside phosphite triester and a

lower overall yield of NDPαB. The key step for the synthesis of NDPαB **5** was the phosphorylation of compound **4**. Catalyzed by 1*H*-tetrazole, the phosphorylation of intermediate **4** by tetrabutylammonium dihydrogen phosphate occurred intermolecularly to produce the desired NDPαB **5** as the major product. We did not observe any by-products arising from the intramolecular cyclization of the diisopropylamino group with either the 2′- or 3′-OH group. The presence of a borane group replacing a non-bridging O-atom at P(α) in NDPαB **5** produced a pair of diastereoisomers that could be resolved by HPLC. Like phosphorothioate NDPαS, the two diastereoisomers of NDPαB are anticipated to have different substrate properties towards nucleosidyl transferases and hydrolases, and should be useful for investigating the roles of phosphate and metal ions in biological processes to elucidate the stereochemical and metal requirements of the enzymatic reactions involving nucleoside diphosphates.

Based on our previous studies showing that boranophosphates are more lipophilic than natural phosphates [14], the introduction of BH_3 at P(α) or P(β) of nucleoside diphosphates is likely to enhance their lipophilicity and assist in their crossing the plasma membrane while maintaining the negative charge of the phosphate. The BH_3 group in an NDPαB is expected to form neither classical H-bonds nor coordinate metal ions as well as the O-atom in the parental ADP and GDP. Thus, the novel combination of high lipophilicity, reasonable water solubility, and nuclease resistance could be extremely useful for drug design [14].

In summary, a simple and efficient synthetic approach to the synthesis of nucleoside 5′-(P^{α}-boranodiphosphates) (ADPαB and GDPαB) was developed. The individual diastereoisomers were separated by reversed-phase HPLC and characterized by spectroscopic methods. Further investigations of the potential applications of nucleoside 5′-(P^{α}-boranodiphosphates) as mechanistic probes for enzymatic reactions involving natural nucleoside diphosphates, as substrates for enzymatic synthesis of boranophosphate polyribonucleotides [25], and as carriers of ^{10}B in boron neutron-capture therapy (BNCT) [20] for the treatment of cancer are under consideration.

Experimental Part

General. All solvents and reagents were of anal. grade and used without further purification unless otherwise indicated. ^1H-Tetrazolide [24] was prepared from 1*H*-tetrazole purchased from *ChemGenes*, and 2′,3′-di-*O*-acetyladenosine, 2′,3′-di-*O*-acetylguanosine, and borane-*N*,*N*-diisopropylethylamine complex were purchased from *Aldrich*. Ion-exchange chromatography: column packed with *QA-52* cellulose (HCO_3^-) from *Whatman*; linear gradient of 0.05M and 0.2M ammonium hydrogen carbonate (pH 9.6) as eluent. Reversed phase HPLC: *Waters* dual pump system in combination with a *Waters-600E* system controller, a *991* photodiode array UV detector, and a *NEC-Powermate-386* computer; the diastereoisomer mixture of NDPαB was loaded on a *DeltaPackC18* reversed-phase column and eluted with buffers containing 100 mM (Et_3NH)OAc (made from Et_3N and AcOH) (pH 6.8) and MeOH; t_R in min. ^1H- and ^{31}P-NMR Spectra: *Varian Inova-400* spectrometer at 400.0 and 161.9 MHz, resp., δ in ppm downfield from the internal $SiMe_4$ (=0 ppm) standard, *J* in Hz (^{31}P with broad-band decoupling); spectra of intermediates were recorded after addition of $CDCl_3$ or (D_6)DMSO to the sample from the reaction soln.

*Adenosine 5′-(*P$^{\alpha}$*-Boranodiphosphate)* (**5a**) *and Guanosine 5′-(*P$^{\alpha}$*-Boranodiphosphate)* (**5b**): *General Procedure*. To a soln. of 2′,3′-di-*O*-acetyladenosine (**1a**; 1 mmol) or 2′,3′-di-*O*-acetylguanosine (**1b**; 1 mmol) in anh. dimethylformamide (DMF; 1.6 ml for **1a**; 4.0 ml for **1b**), 2-cyanoethyl tetraisopropylphosphorodiamidite (1.1 mmol, 0.36 ml) and diisopropylammonium 1*H*-tetrazolide (1 mmol, 171 mg) were added under Ar. The reaction was continued for 30 min (for **1a**) or 4 h (for **1b**) at r.t., after which excess borane-*N*,*N*-diisopropylethylamine complex (0.8 ml) was added *in situ*, and the boronation was allowed to proceed for

2 h. After evaporation, the residue was extracted with AcOEt (40.0 ml) and H_2O (8.0 ml, 2×4.0 ml), the combined org. layer evaporated, and the residue treated with NH_4OH/MeOH 1:1 (σ/σ) (5 ml) at r.t. for 4 h. The solvents were removed under vacuum, and dry AcOEt was added. Without purification, the precipitate **4a** or **4b** (*ca.* 0.5 mmol) was dissolved in anh. DMF (2.0 ml). Excess tetrabutylammonium dihydrogen phosphate (0.8 mmol, 272 mg) and 1*H*-tetrazole (2 mmol, 140 mg) were added, and the reaction was continued for 30 min at 55°. The mixture was extracted with H_2O (10.0 ml) and AcOEt (20.0 ml), the org. layer washed with H_2O (2×3.0 ml), the combined aq. layer concentrated, and the residue purified by ion-exchange column chromatography (15×300 mm LC column, *QA-52* cellulose, linear gradient of 0.005 and 0.2M ammonium hydrogen carbonate buffer (pH 9.56; 700 ml each)). The desired fractions were concentrated, and excess salts were removed by repeated lyophilization with deionized H_2O to yield the ammonium salt of adenosine 5′-(P^{α}-boranodiphosphate) (**5a**) and guanosine 5′-(P^{α}-boranodiphosphate) (**5b**). The two diastereoisomers of **5a** or **5b**, resp., were separated by ion-pair chromatography (reversed-phase column *Waters DeltaPakC18*, 3.9×300 mm, 15μ, 100 Å; isocratic conditions: 92% 100 mM $(Et_3NH)OAc$ (pH 6.8) and 8% MeOH as buffers for **5a**: 94% $(Et_3NH)OA$ (pH 6.8) and 6% MeOH as buffers for **5b**). After HPLC purification, the solvents were evaporated. The buffer components, $(Et_3NH)OAc$ and MeOH, were removed by repeated lyophilization.

Adenosine 5′-(P^{α}*-Boranodiphosphate)* (ADPαB; **5a**). Overall yield (from **1a** to **5a**), 36%. ^{31}P-NMR (D_2O): 80–84 (br., P(α)); −4.76, −4.93 (*d*, P(β)).

ADPαB, Isomer I: t_R 11.81. ^{1}H-NMR (D_2O): 8.43 (*s*, H−C(8)); 8.08 (*s*, H−C(2)); 5.97 (unresolved *m*, H−C(1′)); 4.44 (unresolved *m*, H−C(3′)); 4.21 (unresolved *m*, H−C(4′)); 4.09, 3.98 (2*m*, 2 H−C(5′)); 0.40, 0.15 (2 br., BH_3). ^{31}P-NMR (D_2O): 80–84 (br., P(α)); −7.09, −7.31 (*d*, $J = 35.13$, P(β)). FAB-MS: 424.06 (M^-). HR-MS: 424.0599 ($C_{10}H_{17}BN_5O_9P_2^-$, M^-; calc. 424.0593).

ADPαB, Isomer II: t_R 18.72. ^{1}H-NMR (D_2O): 8.41 (*s*, H−C(8)); 8.08 (*s*, H−C(2)); 5.97 (unresolved *m*, H−C(1′)); 4.36 (unresolved *m*, H−C(3′)); 4.22 (unresolved *m*, H−C(4′)); 4.09, 3.99 (2*m*, 2 H−C(5′)); 0.38, 0.12 (2br., BH_3). ^{31}P-NMR (D_2O): 80–84 (br., P(α)); −7.71, −7.90 (*d*, $J = 30.44$, P(β)). FAB-MS: 424.06 (M^-). HR-MS: 424.0588 ($C_{10}H_{17}BN_5O_9P_2^-$, M^-; calc. 424.0593).

Guanosine 5′-(P^{α}*-Boranodiphosphate*) (GDPαB; **5b**). Overall yield (from **1b** to **5b**), 32%. ^{31}P-NMR (D_2O): 80–84 (br., P(α)); −4.83 (*m*, P(β)).

GDPαB, Isomer I: t_R 7.94. ^{1}H-NMR (D_2O): 8.01 (*s*, H−C(8)); 5.76 (*d*, $J = 6.0$, H−C(1′)); 4.56 (*m*, H−C(2′)); 4.38 (*dd*, $J = 3.2$, 4.8, H−C(3′)); 4.17 (*d*, $J = 2.8$, H−C(4′)); 4.02–3.97 (*m*, 2 H−C(5′)); 0.44, 0.12 (2br., BH_3). ^{31}P-NMR (D_2O): 82–86 (br., P(α)); −8.96, −9.14 (*d*, $J = 29.14$, P(β)). FAB-MS: 440.09 (M^-). HR-MS: 440.0543 ($C_{10}H_{17}BN_5O_{10}P_2^-$, M^-; calc. 440.0544).

GDPαB, Isomer II: t_R 13.06. ^{1}H-NMR (D_2O): 8.00 (*s*, H−C(8)); 5.76 (*d*, $J = 6.0$, H−C(1′)); 4.57 (unresolved *m*, H−C(2′)); 4.32 (*dd*, $J = 3.2$, 5.2, H−C(3′)); 4.17 (*d*, $J = 2.8$, H−C(4′)); 4.05–3.97 (2*m*, 2 H−C(5′)); 0.41, 0.13 (2br., BH_3). ^{31}P-NMR (D_2O): 82–86 (br., P(α)); −8.97, −9.15 (*d*, $J = 29.14$, P(β)). FAB-MS: 440.07 (M^-). HR-MS: 440.0542 ($C_{10}H_{17}BN_5O_{10}P_2^-$, M^-; calc. 440.0544).

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