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A Convenient Synthesis of 2'-Deoxyribonucleoside 5'-(α-*P*-Borano)triphosphates

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Abstract: A new class of nucleotides, with one of the two non-bridging oxygens at α-phosphorus replaced by a borane (BH_3) group, has shown potential applications in DNA sequencing. We have developed a convenient method for the synthesis of the 2'-deoxy-5'-(α-P-borano)triphosphates of adenosine, guanosine, cytidine, thymidine and uridine (**6a-e**) that is time and cost effective compared to the previously reported method. The appropriate base/sugar protected nucleoside **1** is phosphitylated with 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one to give the corresponding cyclic intermediate, 2-(2'-deoxyribonucleosidyl-5'-*O*-)-4*H*-1,3,2-benzodioxaphosphorin-4-one (**2**). The *in situ* reaction of **2** with pyrophosphate leads to P^2,P^3-dioxo-P^1-(2'-deoxyribonucleosidyl-5'-)cyclotriphosphite (**3**). Subsequent reaction of **3** with *N,N*-diisopropylethylamine-borane complex yields P^1-borano-(2'-deoxyribonucleosidyl-5')cyclotriphosphate (**4**), which upon hydrolysis under mild conditions gives the base/sugar protected 2'-deoxyribonucleoside-5'-(α-*P*-borano)triphosphate (**5**). Treatment of **5** with ammonia:methanol (2:1 v/v) yields the diastereoisomeric mixture of 2'-deoxyribonucleoside-5'-(α-*P*-borano)triphosphate (**6**). Separation of the two diastereoisomers of **6** is performed by reverse phase HPLC.

INTRODUCTION

Boron containing derivatives of biologically active compounds such as amino acids,[1-3] nucleosides[4,5] and nucleotides[6-9] continue to be of great interest due to their potential therapeutic activity and diagnostic applications. Numerous boron containing species show promising characteristics[10-14] such as anticancer,[10,11,14] hypolipidemic,[12] antiinflammatory[13] and antiosteoporotic[13] activity in model studies. The α-boronated triphosphates of 2'-deoxyribonucleosides constitute a particularly important class of compounds. They contain a borane group (BH_3) instead of one nonbridging oxygen in the α-phosphate moiety and due to this structural modification are unique among modified nucleotides: isoelectronic and isoionic with corresponding phosphates and thiophosphates, and isostructural with methylphosphonates. They are recognized to be good substrates for DNA polymerases and can be used for enzymatic synthesis of P-boronated oligonucleotides[15,16] with phosphodiester linkages replaced by boranophosphate diester bonds. Incorporation of boranophosphates into DNA results in an increased resistance to exo- and endonucleases[5,17] in comparison with a non-modified DNA. Due to these facts, 2'-deoxyribonucleoside α-boronated triphosphates can be applied in a direct PCR sequencing method.[17] Their applicability in DNA sequencing and enzymatic synthesis of backbone boronated oligonucleotides permits them to be considered as potential diagnostic and therapeutic agents.

The original method for synthesis of α-boronated 2'-deoxyribonucleoside triphosphates via a 'phosphoramidite' intermediate was reported from this laboratory, as illustrated by the example of α-*P*-boranotriphosphate of 2'-(deoxy)thymidine.[18] That method involved isolation of three intermediates and

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required two ion exchange column chromatographies and a reverse phase HPLC to separate the two diastereoisomers. In applying that method to α-boronated triphosphates of deoxyadenosine, deoxyguanosine, and deoxycytidine, we found in unpublished studies that the overall yields varied from 3% to 12%. We have developed and are presenting for the first time a convenient one-pot synthesis of these compounds. We have adapted the procedure employed by Ludwig and Eckstein[19] for the synthesis of α-thiotriphosphates, using 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one (salicyl phosphorochloridite) as the phosphitylating agent. The advantage of this method is that it requires only a single ion-exchange column chromatography and results in better yields compared to the earlier method. In this paper, we report the synthesis of 2'-deoxy-5'-(α-*P*-borano)triphosphates of adenosine, guanosine, cytidine, thymidine and uridine.

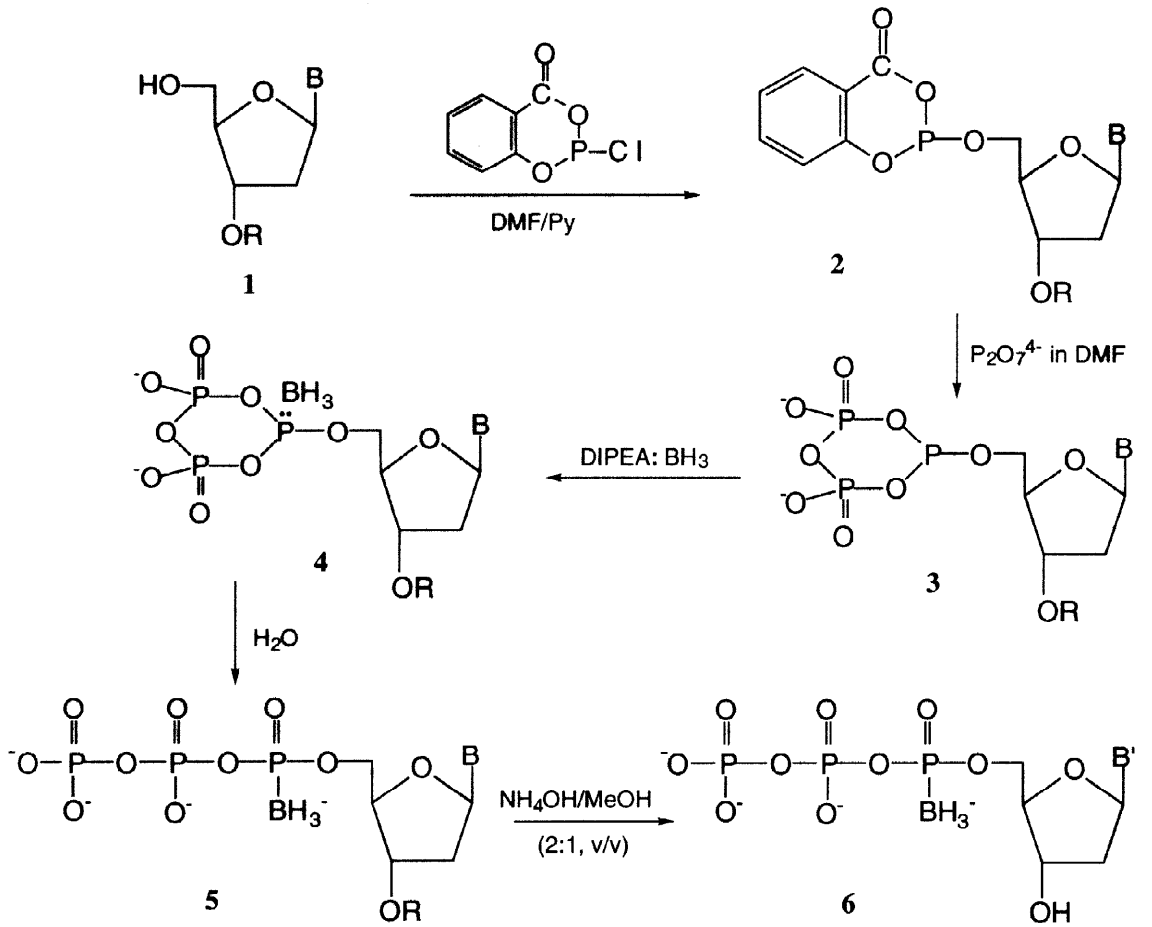
Synthesis of the α-boronated triphosphate of 2'-deoxyuridine was prompted by a medical interest in deoxyuridine triphosphate pyrophosphatase (dUTPase). This highly specific enzyme has been found among widely different species including humans and has proved to be essential for the viability of *Escherichia coli*[20] and *Saccharomyces cerevisiae*.[21] Its crucial role in pyrimidine metabolism and DNA replication depends on hydrolysis of dUTP to dUMP and pyrophosphate in order to prevent incorporation of uracil residues into DNA.[22,23] In light of these and other[24-30] observations, 2'-deoxy-5'-(α-*P*-borano)triphosphate of uridine may be an interesting and useful tool for structural and mechanistic studies of dUTPase and is the subject of ongoing investigations.

RESULTS AND DISCUSSION

The sequence of reactions for the synthesis of 2'-deoxyribonucleoside 5'-(α-*P*-borano)triphosphates is indicated in Scheme 1. The first synthetic step involves the reaction of salicyl phosphorochloridite with a protected nucleoside **1**, to give 2-(2'-deoxyribonucleosidyl-5'-*O*-)-4*H*-1,3,2-benzodioxaphosphorin-4-one (**2**). The two diastereoisomers of **2** are distinguishable by ^{31}P NMR and their chemical shifts are analogous to the values reported in the literature[19] (see Table I).

The reaction of **2** with pyrophosphate yields P^2,P^3-dioxo-P^1-(2'-deoxyribonucleosidyl-5'-)cyclotriphosphite (**3**). The structure of this compound has been confirmed by ^{31}P NMR spectrum in which the tervalent phosphorus atom is represented by a triplet at δ ~ 100 – 108 ppm and the two diastereotopic pentavalent phosphorus atoms are represented by two quartets[31a] appearing at δ ~ -19 ppm.

It has been shown[19] that compound **3** can be oxidized either by iodine/water or by elemental sulfur which leads finally to the triphosphate or α-thiotriphosphate, respectively. In both oxidation processes nucleophilic substitution can be assumed, with the tervalent phosphorus atom acting as a nucleophile, either with iodine (I^- as a leaving group)[32] or with rhombohedral sulfur S_8 (S_7^- as a leaving group during opening of a ring structure).[33] In the boronation process the tervalent phosphorus atom should react as a 'Lewis base' donor of electrons to electron-deficient BH_3. Any reaction of BH_3 with pentavalent phosphorus atoms seemed unlikely since it is assumed that a phosphorus atom in a tervalent state is more reactive than in a pentavalent state. Based on these observations we assumed that compound **3** also could be a good substrate in the boronation process, leading finally to α-boranotriphosphate.



	B	R	B'
a	A^{bz}	Bz	A
b	G^{ib}	Bz	G
c	C^{bz}	Bz	C
d	T	Ac	T
e	U	Ac	U

Scheme 1

Without isolation, the cyclotriphosphate **3** was subjected to boronation using different boronating agents. Pyridine-borane complex did not react with compound **3** at room temperature, and heating of the reaction mixture at 55°C for several hr led to decomposition of **3** as evidenced by a decrease in intensity of the triplet at δ ~106 ppm and appearance of numerous peaks at δ ~ -20.0 – 10.0 ppm. Using the more reactive *N,N*-diisopropylethylamine-borane complex resulted in coordination of BH_3 at the tervalent phosphorus atom; after 6 hr at room temperature all five derivatives **3(a-e)** underwent complete boronation to give P^1-borano-(2'-deoxy-ribonucleosidyl-5'-)cyclotriphosphates (**4 a-e**).

Table I. ^{31}P NMR Spectral Data of **2**

	DMSO-d_6/DMF	$CDCl_3$/DMF
2a	124.70; 125.08	125.01; 126.83
2b	125.37; 125.91	126.23; 127.04
2c	125.96; 126.28	127.28; 127.95
2d	125.69; 126.69	126.51; 127.18
2e	126.81; 127.16	127.37; 128.48

The formation of the boronated compounds **4** was confirmed by their ^{31}P NMR spectra that exhibited, in each case, a broad diagnostic signal at $\delta \sim 87 - 90$ ppm (corresponding to the phosphorus atom coordinated with the BH_3 group[18]) and signals located at $\delta \sim -23$ ppm (representing phosphate groups).[31b] While the signals of the phosphate groups in **3** differ slightly from those in **4** ($\Delta\delta \sim 5$ ppm), the corresponding signals for the tervalent phosphorus in **3** differ significantly in comparison with the boronated phosphorus atom in **4**, both in chemical shifts ($\Delta\delta \sim 15 - 20$ ppm) and multiplicity[34] (a triplet in **3** and a broad multiplet in **4**). The multiplicity and significant broadening of the signals arise from bonding between the phosphorus atom (spin I = 1/2) and the boron atom (naturally abundant boron consists of 80.4% ^{11}B, spin I = 3/2, and 19.6% ^{10}B, spin I = 3), indicating that the borane group is introduced in the desired position (See Table II).

The cyclic boronated product **4** was hydrolyzed to the base/sugar protected 2'-deoxyribonucleoside-5'-(α-*P*-borano)triphosphate **5**. While nucleophilic attack of water at either the boronated phosphorus atom or one of the two other equivalent phosphorus atoms would open the cyclic structure of **4**, we expected nucleophilic attack to occur at the phosphate moiety rather than at the phosphorus-bearing borane group. Our expectations were based on previous observations cited in the literature concerning other nucleotide analogs.[35,36] In order to investigate the direction of nucleophilic ring opening in **4** we performed ammonolysis. Treatment of compound **4c** with concentrated NH_4OH in methanol led to 2'-deoxycytidine 5'-(α-*P*-borano-γ-*P*-amino)triphosphate (**7c**)[37], consistent with nucleophilic attack of ammonia at phosphate. Compound **7c**, to our best knowledge, has not been previously described in the literature.

After hydrolytic ring opening, the reaction mixture containing nucleoside α-boranotriphosphate **5** was treated with concentrated ammonia in methanol for 24 – 48 hr at room temperature to remove protecting groups from base and sugar. Products **6a-e** were isolated by chromatography on Q Sepharose FF in overall 25 – 43% yield. Separation into pure diastereoisomers was accomplished using HPLC (see Experimental Part). Structure and homogeneity of the compounds were confirmed by spectral and chromatographic analytical methods.

In the ^1H NMR spectra of compounds **6a-e** we observe all the signals analogous to those characteristic for unmodified triphosphates and an additional broad multiplet for the BH_3 group. In the ^{31}P NMR spectra of

compounds **6a-e** we observe the chemical shifts for β and γ phosphorus atoms at δ ~ -20 – -21 and -5 – -8 ppm, respectively, which are typical for unmodified triphosphates as well as their analogs modified only at the α-position (e.g., α-thiotriphosphates). Modification of a nucleoside triphosphate at the α-position, however, results in a noticeable change of chemical shift for the α-phosphorus atom: from δ ~ -10 ppm for a normal triphosphate to δ ~ 40 ppm for α-thiotriphosphate, δ ~ 20 ppm for α-methylphosphonyl-β,γ-diphosphate[38] and, as we have found in our studies, δ ~ 82 – 87 ppm (broad multiplet) for α-boranotriphosphate.

The reasonable yields (30% on average) and the convenience of performing the whole synthesis in relatively short time makes this methodology especially attractive for synthesis of α-boronated triphosphates of 2'-deoxyribonucleosides.

Table II. ^{31}P NMR Spectral Data of **3** and **4**[31a,b]

	Chemical shifts [ppm] and observed coupling constants [Hz] ($CDCl_3$)		
	δ_{P2}; δ_{P3}	δ_{P1}	$^2J_{P1,P}$
3a	-18.44; -18.71 (d)	107.17 (t)	43.55
4a	-23.01 – -23.42 (m)	87 – 93 (br)	
3b	-18.77 – -19.74 (m)	100.38; 100.06 (2 d)	41.93
4b	-23.03 – -23.59 (m)	84 – 88 (br)	
3c	18.86, 18.92; 19.13, 19.19 (2d)	106.44 (t)	43.55
4c	-22.77, -23.05 (d)	87 – 91 (br)	
3d	-19.30 – -20.00 (m)	105.28 (t) (DMSO-d_6)	43.15
4d	-22.88; -23.17 (d)	86 – 89 (br) (DMSO-d_6)	
3e	-18.33; -18.60 (d)	108.47 (t)	43.47
4e	-21.56; -21.84 (d)	88 – 91 (br)	

MATERIALS AND METHODS

All solvents and reagents were of analytical grade and were used without further purification unless otherwise indicated. N^6,3'-*O*-Dibenzoyl-2'-deoxyadenosine (**1a**), N^2-isobutyryl-3'-*O*-benzoyl-2'-deoxyguanosine (**1b**), N^4,3'-*O*-dibenzoyl-2'-deoxycytidine (**1c**), and 3'-*O*-acetylthymidine (**1d**) were purchased from Sigma Chemical Co. 3'-*O*-Acetyl-2'-deoxyuridine[39] (**1e**) was synthesized from 5'-dimethoxytrityl-2'-

deoxyuridine purchased from Chem-Impex International. 2-Chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one, pyridine-borane complex, *N,N*-diisopropylethylamine-borane complex and tributylamine were purchased from Aldrich. UV spectra were recorded on a Milton Roy Spectronic 3000 Array spectrometer. Thin layer chromatography (TLC) was performed on silica gel 60F-254 25DC-Alufolien 20 × 20 cm aluminum plates (EM Industries, Inc.). Spots on TLC plates were detected by visualization under short wave UV light or by heating the plate at 100 °C after spraying with 5% sulfuric acid in methanol. ^{1}H NMR spectra were recorded on a Varian Inova-400 spectrometer at 400.0 MHz and reported in ppm downfield from the internal tetramethylsilane (TMS = 0) standard. The signals are expressed as s(singlet), d(doublet), t(triplet), q(quartet), m(multiplet), or br(broad). ^{31}P NMR spectra were recorded on a Varian Inova-400 spectrometer operating at 161.9 MHz with broad band decoupling. Spectra of intermediates were recorded after addition of chloroform-d_3 ($CDCl_3$) or dimethylsulphoxide-d_6 (DMSO-d_6) to the reaction solution. Mass spectra were recorded on a JEOL-JMS-SX-102 using FAB/MS at 3000 resolution. Xenon was used for FAB analysis as the fast atom and an accelerating voltage of 10 kV was used. Reverse phase HPLC was performed with a Waters dual pump system in combination with a Waters 600E system controller, a 991 photodiode array UV detector and NEC Powermate 386 computer, using a Delta Pack C18 reverse phase column and a mobile phase containing 6-11% methanol and 89 - 94% 100 mM triethylammonium acetate (TEAA), pH 6.8. Ion exchange chromatography was carried out on Q Sepharose FF (Pharmacia Biotech AB) using a linear gradient of 0.05 M and 0.5 M ammonium bicarbonate, pH 9.6.

EXPERIMENTAL PART

2'-Deoxyribonucleoside 5'-(α-*P*-borano)triphosphate (6).

General procedure: The protected nucleoside [N^6,3'-*O*-dibenzoyl-2'-deoxyadenosine (**1a**), N^2-isobutyryl-3'-*O*-benzoyl-2'-deoxyguanosine (**1b**), N^4,3'-*O*-dibenzoyl-2'-deoxycytidine (**1c**), 3'-*O*-acetylthymidine (**1d**)] or 3'-*O*-acetyl-2'-deoxyuridine (**1e**)], (0.25 mmole) was dried overnight over P_2O_5 under high vacuum at room temperature in a desiccator. The 25 mL reaction flask was filled with argon by introducing the gas into the desiccator and closed with a rubber septum. While maintaining an argon atmosphere in the reaction vessel, anhydrous pyridine (0.1 mL) was added followed by anhydrous dimethylformamide (DMF) (0.75 mL). A freshly prepared 1 M solution of 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one (0.25 mL) in DMF was injected through the septum into the flask while vigorously stirring the solution of the nucleoside. After 10 min an aliquot (0.15 mL) of the reaction solution was added to 0.50 mL of anhydrous $CDCl_3$ or DMSO-d_6 and was analyzed by ^{31}P NMR. The formation of two diastereoisomeric cyclic phosphites **2(a-e)** was observed as evidenced by appearance of two ^{31}P peaks at δ ~ 125 ppm (see Table I). A freshly prepared 0.5 M solution of bis(tri-n-butylammonium) pyrophosphate (0.75 mL) was then added, followed by 0.125 mL of anhydrous tri-n-butylamine. The progress of the reaction was monitored by phosphorus NMR. After 10 min, the ^{31}P NMR ($CDCl_3$) spectrum showed the appearance of new peaks as a triplet at δ ~ 106 ppm and disappearance of the peaks at δ ~125 ppm, indicating the formation of P^2,P^3-dioxo-P^1-(2'-deoxyribonucleosidyl-5'-*O*-)cyclotriphosphite (**3a-e**) (see Table II). The signals for the two diastereotopic phosphoryl groups were localized in the region of δ = - (18-19) ppm. *N,N*-diisopropylethylamine-borane complex (1 mL) was added and the reaction

mixture was stirred for 2-6 hr at room temperature. The completion of the boronation step was confirmed by disappearance of phosphorus signals at δ ~106 ppm and the appearance of a broad signal at δ ~ 87 – 90 ppm (see Table I). The reaction solution was diluted with deionized water (25 mL). After 0.5-1.0 hr stirring at room temperature the solvents were evaporated to dryness in vacuo. The residue was treated with ammonium hydroxide:methanol mixture/2:1 (37.5 mL) for 24-48 hr at room temperature. The reaction mixture was evaporated to dryness and the resulting residue was dissolved in water and extracted with dichloromethane. The water layer was evaporated in vacuo and the crude product was purified on ion exchange chromatography using Q Sepharose FF (column 1 × 10 cm). The column was eluted with a linear gradient of 350 mL each of 0.05 M and 0.5 M ammonium bicarbonate, pH 9.6. The pyrimidine derivatives **6c**, **6d** and **6e** were eluted between 0.35 M and 0.45 M buffer, and the purine derivatives **6a** and **6b** between 0.45 M and 0.50 M. The fractions containing desired products were combined and evaporated to smaller volume (~50 mL) and dried by lyophilization. The excess of ammonium bicarbonate was removed by repeated lyophilization with deionized water. The amount of isolated products was estimated on the basis of UV absorbance measurements at 260 nm. The ammonium salts of all five 2'-deoxynucleoside boranotriphosphates were obtained within the range of yields: 25-43%. The diastereoisomers were separated using reverse-phase HPLC with an isocratic gradient varying from 6-11% of methanol in 100 mM triethylammonium acetate buffer, pH 6.8 (See Table III).

2'-Deoxyadenosine 5'-(α-*P*-borano)triphosphate (6a). Compound **6a** was prepared in 25% yield following the general procedure by using N^6,3'-*O*-dibenzoyl-2'-deoxyadenosine **1a** (0.122 g, 0.25 mmol). **^{1}H NMR** (D_2O): δ = -0.2 – 0.7 (br, 3H, BH_3); 2.39 – 2.65 (2m, 2H, H-2'); 3.96 – 4.12 (2m, 3H, H-4', H-5'); 6.28 (t, $^3J_{HH}$= 6.8 Hz, 1H, H-1'); 7.99 (s, 1H, H-2); 8.29, 8.31 (2s, 2 isomers, 1H, H-8). **^{31}P NMR** (D_2O): δ = 82 – 87 (br, 1P, α-P), -20.41 (m, 1P, β-P), -5.39 (d, $^2J_{\gamma\beta}$= 19.75 Hz, 1P, γ-P). **MS (FAB)** found: *m/z* 488.1 (M^-); **HRMS** found: *m/z* 488.0321 (for ^{11}B); calcd for $C_{10}H_{18}BN_5O_{11}P_3$: 488.0309. **UV** ($H_2O$): λ_{max} = 260.6 nm.

2'-Deoxyguanosine 5'-(α-*P*-borano)triphosphate (6b). Compound **6b** was prepared in 30% yield following the general procedure by using N^2-isobutyryl-3'-*O*-benzoyl-2'-deoxyguanosine (**1b**) (0.126 g, 0.25 mmol). **^{1}H NMR** (D_2O): δ = -0.2 – 0.7 (br, 3H, BH_3); 2.30 – 2.64 (2m, 2H, H-2'); 3.97 – 4.07 (2m, 3H, H-4', H-5'); 4.55-4.62 (m, 1H, H-3'); 6.13 (t, $^3J_{HH}$= 6.8 Hz, 1H, H-1'); 7.96 (s, 1H, H-8). **^{31}P NMR** (D_2O): δ = 82 – 87 (br, 1P, α-P), -20.5 – -20.0 (m, 1P, β-P), -5.45 (d, $^2J_{\gamma\beta}$= 18.8 Hz, 1P, γ-P). **MS (FAB)** found: *m/z* : 504.1 (M^-); **HRMS** found: *m/z* 504.0273 (for ^{11}B); calcd for $C_{10}H_{18}BN_5O_{12}P_3$: 504.0258. **UV** ($H_2O$): λ_{max} = 252.8 nm.

2'-Deoxycytidine 5'-(α-*P*-borano)triphosphate (6c). Compound **6c** was prepared in 29% yield following the general procedure by using N^4,3'-*O*-dibenzoyl-2'-deoxycytidine (**1c**) (0.115 g, 0.25 mmol). **^{1}H NMR** (D_2O): δ = -0.2 – 0.7 (br, 3H, BH_3); 2.08 – 2.24 (2m, 2H, H-2'); 3.95 – 4.10 (m, 3H, H-4', H-5'); 4.40 – 4.55 (2m, 1H, H-3'); 5.95 (d, $^3J_{HH}$= 7.6 Hz, 1H, H-5); 6.14 (t, $^3J_{HH}$= 6.8 Hz, 1H, H-1'); 7.85, 7.86 (2d, 2 isomers, $^3J_{HH}$= 7.6 Hz, 1H, H-6). **^{31}P NMR** (D_2O): δ = 82 – 87 (br, 1P, α-P), -21.03, 21.21 (2 d, $^2J_{\beta\gamma}$= 18.3 Hz, 1P, β-P), -8.29 (m, 1P, γ-P). **MS (FAB)** found: *m/z* : 464.1 (M^-); **HRMS** found: *m/z* 464.0213 (for ^{11}B); calcd for $C_9H_{18}BN_3O_{12}P_3$: 464.0196. **UV** (H_2O): λ_{max} = 272.9 nm.

2'-Deoxythymidine 5'-(α-*P*-borano)triphosphate (6d). Compound **6d** was prepared in 43% yield following the general procedure by using 3'-*O*-acetylthymidine (**1d**) (0.119 g, 0.25 mmol).

^{1}H NMR (D_2O): δ = -0.2 – 0.7 (br, 3H, BH_3) 1.77 (2s, 3H, CH_3); 2.17 – 2.21 (m, 2H, H-2'); 3.95 – 4.15 (2m, 3H, H-4', H-5'); 4.47 – 4.57 (m, 1H, H-3'); 6.15 – 6.20 (unresolved, 1H, H-1'); 7.57, 7.59 (2s, 2 isomers, 1H, H-6). **^{31}P NMR** (D_2O): δ = 82 – 87 (br, 1P, α-P), -21.12, -21.30 (2d, $^2J_{\gamma\beta}$ = 19.9 Hz, 1P, β-P), -8.10 (d, $^2J_{\gamma\beta}$ = 20.7 Hz, 1P, γ-P). **MS (FAB)** found: *m/z* : 479.1 (M^-); **HRMS** found: *m/z* : 479.0197 (for ^{11}B) calcd for $C_{10}H_{19}BN_2O_{13}P_3$: 479.0193. **UV** (H_2O): λ_{max} = 268.6 nm.

2'-Deoxyuridine 5'-(α-*P*-borano)triphosphate (6e). Compound **6e** was prepared in 36% yield following the general procedure by using 3'-*O*-acetyl-2'-deoxyuridine[40] (**1e**) (0.079 g, 0.25 mmol). **^{1}H NMR** (D_2O): δ = -0.2 – 0.7 (br, 3H, BH_3); 2.15 – 2.17 (m, 2H, H-2'); 3.9 – 4.1 (m, 3H, H-4', H-5'); 4.39 – 4.48 (2m, 1H, H-3'); 5.72 (d, $^3J_{HH}$= 8.0 Hz, 1H, H-5); 6.12 (t, $^3J_{HH}$= 6.8 Hz, 1H, H-1'); 7.80, 7.81 (2d, 2 isomers, $^3J_{HH}$= 8.0 Hz, 1H, H-6). **^{31}P NMR** (D_2O): δ = 83 – 87 (bm, 1P, α-P), -20.97, -21.13 (2d, $^2J_{\gamma\beta}$= 20.07 Hz, 1P, β-P), -7.65 (m, 1P, γ-P). **MS (FAB)** found: *m/z* : 465.0 (M^-); **HRMS** found: *m/z* : 465.0017 (for ^{11}B); calcd for $C_9H_{17}BN_2O_{13}P_3$: 465.0037. **UV**(H_2O): λ_{max} = 265 nm.

Table III. Chromatographic Data of **6.**

Cmpd #	HPLC retention times and relative yield (MeOH/TEAA, pH 6.8)				Isocratic Conditions	TLC*, R
	Fast diastereoisomer		Slow diastereoisomer			
	min.	%	min.	%	% MeOH	
6a	13.54	54	17.37	46	11	0.11
6b	12.64	50	15.63	50	8	0.08
6c	9.61	49	12.74	51	6	0.09
6d	10.29	58	14.78	42	10	0.11
6e	12.45	52	18.20	48	6	0.14

* Developing system: iPrOH/NH_4OH/H_2O 11:7:2

Separation of diastereoisomers of 2'-deoxyribonucleoside 5'-(α-*P*-borano) triphosphates. The separation of diastereoisomers of each 2'-deoxyribonucleoside 5'-(α-P-borano)triphosphate was achieved by reverse phase HPLC using a Delta Pak C18 column (7.8 × 300 mm, 15 μ, 300 Å). The column was eluted with 100 mM TEAA, pH 6.8 and methanol under isocratic conditions (see Table III). After HPLC purification, the solvents were evaporated under high vacuum and the samples were lyophilized from deionized water.

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31. a. Two quartets representing diastereotopic pentavalent atoms have been observed only for compound **3d** (in DMSO-d_6) indicating the ABX type of the spectrum. In the case of **3a,c** and **e** the AB region of the spectrum (in $CDCl_3$) appears to be a doublet of two very closely spaced doublets (**3c**) or a doublet of two unsplit lines (characteristic for AA'X pattern). For **3b** an unresolved multiplet was observed.
    b. The multiplicity of signals representing diastereotopic phosphate groups in compounds **4** corresponds also with either ABX or AA'X pattern.
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37. Spectral characteristics of **7c**: **^{1}H NMR** (D_2O): δ = 0.25 (bm, 3H, BH_3); 2.15, 2.23 (2m, 2H, H-2'); 3.95-4.10 (m, 3H, H-4', H- 5'); 4.35-4.50 (m, 1H, H-3'); 5.96 (d, $^3J_{HH}$= 7.6 Hz, 1H, H-3); 6.16 (m, 1H, H-1'); 7.87 (d, $^3J_{HH}$=7.6 Hz, 1H, H-2). **^{31}P NMR** (D_2O): δ = 85.00 (bm, 1P, α-P), -20.87 (m, 1P, β-P), 0.64 (m, 1P, γ-P). **MS (FAB)** found: *m/z* 463.0 (M^-); calcd for $C_9H_{19}BN_4O_{11}P_3$: 463.0. **UV** (H_2O): λ_{max} = 272.2 nm.
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39. Spectral characteristics of 3'-*O*-acetyl-2'-deoxyuridine: **^{1}H NMR** (DMSO-d_6): δ =2.01 (s, 3H, $COCH_3$); 2.22 (m, 2H, H-2'); 3.56 (m, 2H, H-5'); 3.96 (m, 1H, H-4'); 5.17 (m, 2H, H-3', 5'-OH); 5.63 (d, $^3J_{HH}$= 8.0 Hz, 1H, H-5); 6.11 (t, $^3J_{HH}$=7.2 Hz, 1H, H-1'); 7.84 (d, $^3J_{HH}$= 8.0 Hz, 1H, H-6); 11.30 (s, 1H, NH); **MS (FAB):** found: *m/z* 271 ($[M+H]^+$); calcd for $C_{11}H_{14}N_2O_6$: 270.24.
40. **6e** can also be synthesized starting with 3'-*O*-benzoyl-2'-deoxyuridine.