Pergamon

Tetrahedron 56 (2000) 4811–4815

TETRAHEDRON

Boranophosphate Diesters as Stable Synthetic Analogues of 1-*O*-Glycosylphosphates

Davide Prosperi,[a] Luigi Panza,[b] Laura Poletti[a] and Luigi Lay[a,*]

[a]*Dipartimento di Chimica Organica e Industriale, Centro di Studio sulle Sostanze Organiche Naturali del CNR, Università degli Studi di Milano, via G. Venezian, 21-20133 Milan, Italy*

[b]*Dipartimento di Scienze Chimiche, Alimentari, Farmaceutiche e Farmacologiche, Facoltà di Farmacia, Università degli Studi del Piemonte Orientale, Viale Ferrucci, 33-28100 Novara, Italy*

Received 22 February 2000; revised 17 April 2000; accepted 8 May 2000

Abstract—Different 1-*O*-glycosylboranophosphate diesters were synthesised as stable analogues of 1-*O*-glycosylphosphates, using a one-pot procedure based on the anomeric *H*-phosphonate of 2,3,4-tri-*O*-benzyl-α-L-rhamnopyranose as a common precursor.

The phosphodiester bridge is a connection which often occurs in several important biomolecules, such as oligonucleotides, glycophospholipids (e.g. GPI anchors) and some bacterial capsular polysaccharides. During recent years many efforts directed towards the design and the synthesis of analogues of this motif have been made. Examples include phosphorothioate,[1] methylphosphonate[2] and boranophosphate[3] bridges, where the nonbridging oxygen is replaced by S^-, CH_3 or BH_3^- groups, respectively. These analogues are mainly used in the synthesis of modified oligonucleotides in order to improve some of their antisense and/or antigenic properties.[4]

In capsular polysaccharides of bacteria, such as *Streptococcus pneumoniae* type 19F and 19A or *Neisseria meningitidis* type A, the connection of the phosphodiester bridge to the anomeric position[5] gives rise to problems of chemical stability. The instability due to this structural motif makes the polysaccharide-based vaccines containing this linkage labile. It thus makes it difficult to store and to manipulate related fragments in the formulation of glycoconjugate vaccines.[6,7] For this reason, it is highly desirable to have access to analogues of the phosphodiester system endowed with better chemical stability, which could be incorporated into a saccharidic chain in order to obtain stable oligomers. These, after conjugation with a proper protein carrier, should elicit antibodies cross reacting with the natural capsular polysaccharides. In a program aimed at synthesising analogues of 1-*O*-glycosylphosphates, we envisaged the boranophosphate as an attractive candidate for obtaining a stable synthetic analogue of the intersaccharidic phosphodiester bridges. In fact, it is isosteric and isoelectronic with the methylphosphonate group. Another advantage of this group is that it carries a net negative charge, like the phosphate and the phosphorothioate groups, so that its oligomers are highly water soluble. Finally, as high stability of the boranophosphate internucleotide linkage towards hydrolysis and phosphodiesterase degradation has already been demonstrated,[8] a good linkage stability at the anomeric position can be predicted. In this paper, we wish to present the synthesis of various anomeric boranophosphate interglycosidic linkages.

To the best of our knowledge, only one example of an anomeric boranophosphate bridge has been reported.[9] In this case, the sugar was linked through the boranophosphate bridge to a simple decyl group. Our aim was to broaden the scope of this reaction in order to achieve a protocol which might be applicable to more complex and more biologically relevant saccharidic systems.

To obtain the boranophosphates, we decided to use the efficient and flexible *H*-phosphonate approach.[10] As the rhamnose-1-*O*-phosphate is involved in the phosphodiester bridge of the capsular polysaccharide of *Streptococcus pneumoniae* type 19F and 19A, we chose to prepare a number of boranophosphate derivatives of 2,3,4-tri-*O*-benzyl-α-L-rhamnopyranose **1**.

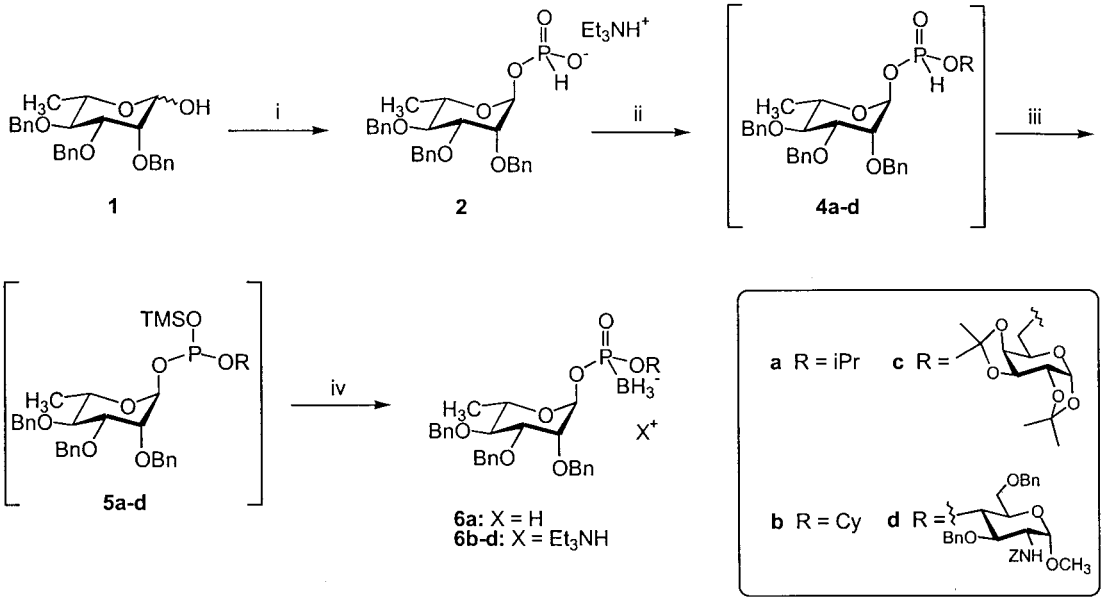
Results and Discussion

The anomeric *H*-phosphonate **2**[11] of the 2,3,4-tri-*O*-benzyl-α-L-rhamnopyranose (**1**) was obtained in 95% yield as a pure α isomer. In literature procedures,[12] the *H*-phosphonate diester intermediate is isolated and then converted into the

Keywords: borinating agents; glycosidic linkage; phosphodiester bridge.

* Corresponding author. Tel.: +39-02-2367613; fax: +39-02-2664952; e-mail: llay@mailserver.unimi.it

0040–4020/00/$ - see front matter
PII: S0040-4020(00)00386-0



Scheme 1. Reagents and conditions: (i) imidazole, PCl_3, TEA, dry CH_3CN, 0°C; (ii) ROH (**3a–d**), PivCl, py, THF, 30 min; (iii) BSTFA, 15 min; (iv) borane complex, then aqueous (**6a**) or Et_3NH^+ HCO_3^- (**6b–d**) work-up.

boranophosphate bridge. However, our *H*-phosphonate diesters proved to be very labile, probably because they involve the anomeric position. In fact, flash chromatography on silica gel invariably led to almost complete decomposition of the product. Therefore, we performed the whole sequence in one-pot, thus avoiding the isolation of the intermediates **4a–d** (Scheme 1). *H*-phosphonate diesters **4a–d** were obtained through the formation of the mixed anhydride with pivaloyl chloride and pyridine in anhydrous THF, followed by the condensation with simple alcohols or, more interestingly, with another saccharidic unit (see Table 1). Rapid formation of the silyl phosphite intermediates **5a–d** was then obtained by addition of *N,O*-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) to the reaction mixture. Finally, the borane complex (see Table 1) was added; aqueous work up resulted in the loss of the trimethylsilyl group and clean formation of the boranophosphate diesters **6a–d** which, after flash chromatography, were recovered in 70–95% yields (Table 1). In each case, a mixture of diastereoisomers differing in the configuration at the phosphorus atom was obtained. The diastereoisomeric ratio of compounds **6c** and **6d** was determined by HPLC on a reverse phase analytical column, where they appeared as two single sharp peaks, corresponding to a diastereoisomeric ratio of 55:45 in both cases. On the contrary, the HPLC profile of the glycosyl alkyl boranophosphates **6a** and **6b** showed one broad peak, and any attempt to obtain a better separation failed. However, the diastereoisomeric ratio of **6a** and **6b** was determined from ^{13}C NMR spectra.

Table 1. Experimental conditions

Entry	Product	ROH	BH_3 complex	Time	Yield (%)
1	**6a**	**3a**	DIPEA	3 h	95
2	**6b**	**3b**	DIPEA	3 h	90
3	**6c**	**3c**	CPy/DMS	10 min	70/67
4	**6d**	**3d**	Cpy	10 min	74

It is noteworthy that the reaction conditions strongly influence the experimental results. First, rigorously anhydrous conditions are required. Furthermore, the volume of THF must be calculated to give a 0.1 M borane concentration. A large decrease of the yields (up to 30%) was consistently observed by performing the reaction at lower concentrations.

The nature of the borane complex also plays a crucial role in determining the reaction outcome; using a mild borinating agent, such as borane–diisopropylethylamine complex (BH_3·DIPEA), excellent yields with the simpler alcohols **3a,b** were achieved, whereas with more reactive complexes, such as borane–dimethylsulfide (BH_3·DMS) or borane–2-chloropyridine (BH_3·CPy),[13] extensive reduction of **1** occurred. In contrast, the boranophosphates **6c,d** were only obtained in good yields using stronger borinating agents, as expected from more hindered phosphites joining the two sugar units. The best results have been obtained using the BH_3·CPy complex (entries 3 and 4 in Table 1).

Boranophosphates **6a–d** were fully characterised by NMR spectroscopy. In the ^1H NMR spectra, the signals corresponding to the anomeric protons of the rhamnopyranose residue in compounds **6a–d** appeared, as expected, as a broad doublet centred around 5.70 ppm ($J_{H,P}$=8.3–9.4 Hz). The structure of compounds **6a–d** was also proved by the corresponding signals in the ^{13}C NMR spectra as the presence of a diastereoisomeric mixture led to rather complicated spectra, in which many peaks are split. In particular, the anomeric carbon of the rhamnose unit invariably showed two peaks. Moreover, in the case of compounds **6c,d** the same splitting was observed for C-6. The presence of the boranophosphate moiety is clearly confirmed by ^{31}P and ^{11}B NMR spectroscopies. From literature data, ^{31}P NMR spectra of boranophosphates have a diagnostic chemical shift (between 90 and 100 ppm), compared with phosphate diesters (~0 ppm) and phosphites

Table 2. ^{31}P (relative to H_3PO_4) and ^{11}B NMR (relative to BF_3) chemical shifts of the boranophosphates **6a–d**

Product	^{31}P (ppm)	^{11}B (ppm)
6a	95	−38.4
6b	94	−38.3
6c	98	−38.5
6d	92.5	−37.1

(~145 ppm). ^{11}B NMR spectra show a similarly diagnostic broad peak centred around −40 ppm.[8,14]

The ^{31}P and ^{11}B NMR data observed for the synthesised compounds were in agreement with the expected chemical shifts (see Table 2); in fact, a unique complex broad signal ranging from 92.5 to 98 ppm (relative to H_3PO_4) appears in ^{31}P NMR spectra. The ^{11}B NMR spectra confirmed the presence of the boranophosphate, showing for compounds **6c,d** a broad signal centred between −37 and −39 ppm (relative to BF_3). The ^{11}B NMR spectra of **6a,b** were better resolved, allowing a doublet to be seen for each compound with a ^{31}P–^{11}B coupling constant of 140 and 130 Hz, respectively.

In conclusion, the synthesis of various anomeric interglycosidic boranophosphate bridges has been presented. The conversion of the anomeric *H*-phosphonate bridge into the corresponding boranophosphate is straightforward and seems to be of general application, either using simple alcohols or, more biologically relevant, monosaccharides. Further, in a qualitative preliminary assessment of its stability towards hydrolysis, boranophosphate **6a** did not show any sign of decomposition after stirring for five days at 40°C (pH 2/4; solvent MeOH/H_2O 1:1), unlike its corresponding phosphate.

Work to extend the use of this methodology to the synthesis of analogues of more complex phosphate bridged oligosaccharides is now in progress.

Experimental

Materials and methods

All reactions were performed under nitrogen atmosphere. Reagents and dry solvents were added via oven-dried syringes through septa. Acetonitrile was dried with activated 4 Å molecular sieves and distilled from calcium hydride. tetrahydrofuran was freshly distilled from sodium/benzophenone. Flash chromatography was performed on BDH silica gel 40–63 μm particle size. Thin-layer chromatography (TLC) was performed on Merck silica gel 60 F_{254} plates, and visualised by charring either with a mixture of 96% sulfuric acid (50 mL), methanol (450 mL) and H_2O (450 mL) or with a solution of 21 g of $(NH_4)_6Mo_7O_{24}$, 1 g of $CeSO_4$ and 31 mL of 96% H_2SO_4 in 500 mL of H_2O followed by heating. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker AC 200 or AC 300 instrument, while ^{31}P NMR was recorded on a ™Varian XL 200 mod. Gemini and ^{11}B NMR on a Bruker DRX 300 Avance. The aromatic signals are omitted. Specific rotations ($[\alpha]_D$) were determined on a Perkin–Elmer 241 polarimeter at 20°C. IR spectra were performed on a Perkin–Elmer 681 Infrared Spectrophotometer. The diastereoisomeric ratio for each of the resulting boranophosphate derivatives (**4a–d**) was determined by analytical HPLC on a Varian 9050 instrument (UV) with a 30 mm LiChroCART Purosphere® STAR RP18 (3 μm) column. Elemental analyses were performed using the Carlo Erba elemental analyser 1108.

Triethylammonium 2,3,4-tri-*O*-benzyl-α-L-rhamnopyranosyl hydrogenphosphonate (2). Imidazole (878 mg, 12.89 mmol) was dissolved in dry CH_3CN (12 mL) at room temperature in an inert atmosphere. The solution was cooled at 0°C and PCl_3 (0.345 mL, 3.96 mmol) was added, followed by dry triethylamine (1.925 mL, 13.81 mmol). After 15 min a solution of 2,3,4-tri-*O*-benzyl-L-rhamnopyranose[15] **1** (400 mg, 0.92 mmol) in dry CH_3CN (12 mL) was added dropwise during 20 min at 0°C. When the addition was completed, the mixture was stirred for 10 min at room temperature (TLC CH_2Cl_2/MeOH 9:1) and then quenched with 1 M triethylammonium hydrogencarbonate (TEAB) (pH 8, 5.2 mL), and the clear solution was stirred for 10 min. CH_2Cl_2 (60 mL) was added to the mixture and the solution washed with 1 M TEAB (2×25 mL), dried with Na_2SO_4, filtered through cotton and evaporated, affording the product **2** as an amorphous mass (550 mg, 95%) after flash chromatography filtration (CH_2Cl_2/MeOH 9:1). $[\alpha]_D$=−13.0 (*c* 1.4, $CHCl_3$, 20°C), IR ($CHCl_3$) ν_{max} 3680, 3618, 2391, 1210, 953, 925, 872, 757 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 6.93 (d, 1H, $J_{H,P}$=630 Hz, *H*-P), 5.67 (br. d, 1H, *J*=8.2 Hz, H-1), 4.93 (d, 1H, *J*=11.1 Hz, C*H*HPh), 4.74 (s, 2H, CH_2Ph), 4.63 (d, 1H, *J*=11.1 Hz, CH*H*Ph), 4.57 (s, 2H, CH_2Ph), 3.85–4.05 (m, 3H, H-2, H-3, H-5), 3.63 (t, 1H, $J_{4,5}$=$J_{3,4}$=9.3 Hz, H-4), 3.48 (s, 1H, NH) 3.03 (q, 6H, *J*=7.3 Hz, NCH_2CH$_3$), 1.37 (t, 9H, *J*=7.3, NCH$_2$CH_3), 1.36 (s, 3H, 3H-6). Anal. Calcd for $C_{33}H_{46}NO_7P$: C, 66.09; H, 7.73; N, 2.34. Found: C, 66.31; H, 7.59; N, 2.30.

General procedure for the preparation of 2,3,4-tri-*O*-benzyl-α-L-rhamnopyranosyl boranophosphates (6a–d)

Compound **2** (530 mg, 0.83 mmol) and the alcohol **3a–d** (0.83 mmol) were dissolved in dry THF (4 mL) in an inert atmosphere. Pyridine (190 μL, 2.24 mmol) and, subsequently, PivCl (255 μL, 2.07 mmol) were added with stirring at room temperature. The formation of the *H*-phosphonate diester proceeded rapidly (TLC EtOAc/*n*-Hex 1:1). After 30 min, *N,O*-bis(trimethylsilyl)-trifluoroacetamide (BSTFA, 660 μL, 2.49 mmol) was added. After 10 min, the boronation reaction was performed with the addition of the BH_3-complex (4.98 mmol). When the boronation was completed, the reaction was quenched by addition of a 1 M solution of TEAB (5 mL) resulting in loss of the trimethylsilyl group and furnishing the triethylammonium salt of the boranophosphate diester (TLC CH_2Cl_2/MeOH 9:1). dichloromethane (50 ml) was added and the organic layer was washed with 1 M TEAB (2×20 ml), dried with Na_2SO_4, filtered and evaporated at reduced pressure. The crude mixture was then purified by silica gel flash-chromatography to remove the excess of pyridine, PivCl and borane complex, providing the boranophosphate diester in good yields.

Isopropyl (2,3,4-tri-*O*-benzyl-α-L-rhamnopyranosyl boranophosphate H^+ form) (6a). Compound **3a** (64 μL) was submitted to the general procedure described above (except for the final work-up performed with water instead of 1 M TEAB), affording the product **6a** as a colourless glass (440 mg, 95%) with the employment of borane·DIPEA complex as a borinating agent. Diastereoisomeric ratio: 55/45 (from ^{13}C NMR spectrum). $[\alpha]_D=-25.8$ (*c* 0.5, $CHCl_3$, 20°C), IR ($CHCl_3$) ν_{max} 3680, 3618, 2391, 1212, 925, 873, 757 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 5.62 (br. d, 1H, $J_{1,P}$=9.2 Hz, H-1), 4.91 (d, 1H, *J*=11.0 Hz, C*H*HPh), 4.72 (s, 2H, CH_2Ph), 4.66 (d, 1H, *J*=11.7 Hz, C*H*HPh), 4.59 (d, 1H, *J*=11.0 Hz, CH*H*Ph), 4.55 (d, 1H, 11.7 Hz, CH*H*Ph), 4.21–4.48 (m, 1H, C*H*$(CH_3)_2$), 3.68–4.11 (m, 3H, H-2, H-3, H-5), 3.61 (t, 1H, $J_{4,5}$=$J_{3,4}$=9.5 Hz, H-4), 2.88 (br. s, 3H, BH_3), 1.27 (d, 3H, *J*=6.4 Hz, (C*H*$_3$)CH(CH_3)), 1.20 (d, 3H, *J*=6.4 Hz, (CH_3)CH(C*H*$_3$)), 1.05 (d, 3H, $J_{5,6}$=5.9 Hz, CH_3). ^{13}C NMR (75.46 MHz, $CDCl_3$) δ 92.71, 91.62 (2 d, C-1, 2 diast.), 80.01, 79.27, 75.73 (3 d), 75.32, 72.70, 71.63 (3 t, CH_2Ph) 69.84 (d), 69.30, 68.75 (2 d, C-1′, 2 diast.), 24.03 (q, 2 CH_3 ^{i}Pr) 17.78 (q, CH_3). ^{31}P NMR (80.96 MHz, $CDCl_3$) δ 95.0 ppm (br. m). ^{11}B NMR (96.25 MHz, $CDCl_3$) δ −38.4 ppm (br. d, $J_{P,B}$=140 Hz). Anal. Calcd for $C_{30}H_{40}BO_7P$: C, 64.99; H, 7.27. Found: C, 65.09; H, 7.05.

Cyclohexyl (2,3,4-tri-*O*-benzyl-α-L-rhamnopyranosyl boranophosphate) triethylammonium salt (6b). Compound **3b** (88 μL) was submitted to the general procedure described above, affording the product 6b as a colourless syrup (520 mg, 90%) with the employment of borane·DIPEA complex as a borinating agent. Diastereoisomeric ratio: 52/48 (from ^{13}C NMR spectrum). $[\alpha]_D=-2.3$ (*c* 1.1, $CHCl_3$, 20°C), IR ($CHCl_3$) ν_{max} 3677, 3618, 2391, 1211, 923, 871, 750 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 5.62 (br. d, 1H, $J_{1,P}$=9.4 Hz, H-1), 4.96 (d, 1H, *J*=11.0 Hz, C*H*HPh), 4.78 (s, 2H, CH_2Ph), 4.63 (d, 1H, *J*=11.0 Hz, C*H*HPh), 4.52 (s, 1H, CH_2Ph), 3.90–4.20 (m, 4H, H-2, H-3, H-5, CH Cy), 3.62 (t, 1H, $J_{4,5}$=$J_{3,4}$=9.4 Hz, H-4), 2.94 (q, 6H, *J*=7.5 Hz, NC*H*$_2$$CH_3$) 1.10–1.90 (br. m, 16H, BH_3, 3H-6, 10H Cy), 1.27 (t, 9H, *J*=7.5 Hz, NCH_2C*H*$_3$). ^{13}C NMR (75.46 MHz, $CDCl_3$) δ 90.63, 90.50 (2 d, C-1, 2 diast.), 78.71, 77.74, 73.94 (3 d), 73.36 (t, CH_2Ph), 71.21, 70.79 (2 d, C-1′, 2 diast.), 70.58, 69.91 (2 t, CH_2Ph), 67.06 (d), 43.54 (t, N*C*H_2CH_3), 32.64, 32.34, 23.73, 22.47, 22.34 (5 t, Cy), 16.33 (q, CH_3), 6.74 (q, NCH_2*C*H_3). ^{31}P NMR (80.96 MHz, $CDCl_3$) δ 94.0 ppm (br. m). ^{11}B NMR (96.25 MHz, $CDCl_3$) δ −38. 3 ppm (br. d, $J_{P,B}$=130 Hz). Anal. Calcd for $C_{39}H_{59}BNO_7P$: C, 67.33; H, 8.55; N, 2.01. Found: C, 67.39; H, 8.53; N, 1.99.

1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranoside 6-(2,3,4-tri-*O*-benzyl-α-L-rhamnopyranosyl boranophosphate) triethylammonium salt (6c). Compound **3c** (216 mg) was submitted to the general procedure described, affording the product **6c** as a white solid in 70 or 67% yield employing borane·CPy or borane·DMS complexes, respectively. Mp: 69–71°C. The diastereoisomeric ratio was determined to be 55/45 by HPLC (50:50 CH_3CN:34 mM phosphate buffer at 25°C) and confirmed by NMR analysis. $[\alpha]_D=-33.3$ (*c* 0.9, $CHCl_3$, 20°C), IR ($CHCl_3$) ν_{max} 3678, 3618, 2391, 1210, 922, 873, 759 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.68 (br. dd, 1H, $J_{1,P}$=8.7 Hz, H-1), 5.48 (d, 1H, $J_{1,2}$=4.8 Hz, H-1) 4.90 (d, 1H, *J*=11.0 Hz, C*H*HPh), 4.71 (s, 2H, CH_2Ph), 4.58 (d, 1H, *J*=11.0 Hz, CH*H*Ph), 4.55 (s, 1H, CH_2Ph), 3.82–4.70 (m, 8H, H-2, H-3, H-5, H-2′, H-3′, H-5′, 2H-6′), 3.61 (t, 1H, $J_{4,5}$=$J_{3,4}$=9.3 Hz, H-4), 3.02 (q, 6H, *J*=7.4 Hz, NC*H*$_2$$CH_3$), 2.60 (br. s, 3H, BH_3), 1.20–1.60 (m, 24H, 3H-6, 4 CH_3, NCH_2C*H*$_3$). ^{13}C NMR (50.32 MHz, $CDCl_3$) δ 109.39, 108.94, 108.82 (3 s), 96.17 (d, C-1′), 92.24, 92.06 (2 d, C-1, 2 diast.), 79.94, 79.33, 75.81 (3 d), 75.08, 72,67, 71.95 (3 t, CH_2Ph), 70.58, 69.61, 69.43, 67.93, 67.27 (5 d), 64.28, 63.01 (2 t, C-6′, 2 diast.), 45.41 (t, N*C*H_2CH_3), 25.91, 24.77, 24.51, 24.16 (4 q, CH_3 ^{i}Propylidene), 17.79 (q, CH_3), 8.41 (q, NCH_2*C*H_3). ^{31}P NMR (80.96 MHz, $CDCl_3$) δ 98.0 ppm (br. m). ^{11}B NMR (96.25 MHz, $CDCl_3$) δ −38.5 ppm (br. s). Anal. Calcd for $C_{45}H_{67}BNO_{12}P$: C, 63.16; H, 7.89; N, 1.64. Found: C, 62.95; H, 7.98; N, 1.70.

Methyl *N*-benzyloxycarbonyl-2-deoxy-3,6-di-*O*-benzyl-glucopyranoside 4-(2,3,4-tri-*O*-benzyl-α-L-rhamnopyranosyl boranophosphate) triethylammonium salt (6d). Compound **3d** (421 mg) was submitted to the general procedure described, affording the product 6d as a white glass in 74% yield employing borane·CPy complex as borinating agent. The diastereoisomeric ratio was determined to be 55/45 by HPLC (50:50 CH_3CN:34 mM phosphate buffer at 25°C) and confirmed by NMR analysis. $[\alpha]_D=+45.3$ (*c* 0.9, $CHCl_3$, 20°C), IR ($CHCl_3$) ν_{max} 3678, 3618, 2389, 1707, 1225, 925, 872, 759 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.74 (br. dd, $J_{1,P}$=8.3, H-1, 1 diast.), 5.71 (dd, $J_{1,P}$=7.1 Hz, $J_{1,2}$=1.7 Hz, H-1, 1 diast.), 4.85–5.20 (m, 4H, 2 CH_2Ph), 4.45–4.80 (m, 8H, 4 CH_2Ph), 3.75–4.15 and 3.55–3.75 (2 m, 8H and 2H, respectively), 3.37 (s, 3H, OCH_3), 2.80 (br. q, 9H, *J*=7.3 Hz, 3 NC*H*$_2$$CH_3$, BH_3), 1.10–1.40 (m, 12H, CH_3, NCH_2C*H*$_3$). ^{13}C NMR (75.46 MHz, $CDCl_3$) δ 156.19, 156.09 (2 s, CO, 2 diast.), 98.57 (d, C-1′), 93.30, 93.12 (2 d, C-1, 2 diast.), 80.36, 80.15, 80.00, 79.69, 79.49, 75.84 (6 d, 2 diast.), 75.26, 75.06, 73.80, 73.42, 73.25, 72.60 (6 t, 4 CH_2Ph, 2 diast.), 72.22 (d), 71.58 (t, CH_2Ph), 71.40 (d), 69.99, 69.66 (2 d, C-4′, 2 diast.), 69.05 (d), 66.80 (t, CH_2Ph), 55.04 (q, CH_3O), 54.24 (d, CHN), 18.24, 18.16 (2 q, CH_3, 2 diast.), 8.49 (q, NCH_2*C*H_3). ^{31}P NMR (80.96 MHz, $CDCl_3$) δ 92.5 ppm (br. m). ^{11}B NMR (96.25 MHz, $CDCl_3$) δ −37.1 ppm (br. s). Anal. Calcd for $C_{62}H_{80}BN_2O_{13}P$: C, 67.51; H, 7.31; N, 2.54. Found: C, 67.46; H, 7.28; N, 2.63.

Acknowledgements

We gratefully acknowledge financial support from MURST and CNR, Centro di Studio sulle Sostanze Organiche Naturali.

References

1. Eckstein, F. *Acc. Chem. Res.* **1979**, *12*, 204.
2. Furdon, P. J.; Dominski, Z.; Kole, R. *Nucleic Acid Res.* **1989**, *17*, 9193.
3. Sood, A.; Shaw, B. R.; Spielvogel, B. F. *J. Am. Chem. Soc.* **1990**, *112*, 9000.
4. Sergueev, D. S.; Shaw, B. R. *J. Am. Chem. Soc.* **1998**, *120*, 9417.

5. Jennings, H. J. In *Advances in Carbohydrate Chemistry and Biochemistry*, Tipson, R. S., Horton, D., Eds.; Academic Press: New York, 1983; Vol. 41.
6. Bishop, C. T.; Jennings, H. J. *The Polysaccharides*; Aspinall, G. O., Ed.; Academic Press: New York, 1982.
7. Arndt, B.; Porro, M. *Immunobiology of Proteins and Peptides VI*; Atassi, M. Z., Ed.; Plenum Press: New York, 1991.
8. Shaw, B. R.; Madison, J.; Sood, A.; Spielvogel, B. F. *Protocols for Oligonucleotides and Analogues*; Agrawal, S., Ed.; Humana Press: Totowa, 1993, pp 225–243.
9. Ross, A. J.; Higson, A. P.; Ferguson, M. A. J.; Nikolaev, A. V. *Tetrahedron Lett.* **1999**, *40*, 6695.
10. Sergueev, D.; Hasan, A.; Ramaswamy, M.; Shaw, B. R. *Nucleosides Nucleotides* **1997**, *16*, 1533.
11. For a general procedure of preparation of anomeric *H*-phosphonates, see: Nikolaev, A. V.; Ivanova, I. A.; Shibaev, V. N.; Kochetkov N. K. *Carbohydr. Res.* **1990**, *204*, 65.
12. Higson, A. P.; Sierzchala, A.; Brummel, H.; Zhao, Z.; Caruthers, M. H. *Tetrahedron Lett.* **1998**, *39*, 3899.
13. Borane-2-chloropyridine complex (BH_3·CPy) was freshly prepared according to: Foret, C. J.; Korzekwa K. R.; Martin, D. R. *J. Inorg. Nucl. Chem.* **1980**, *42*, 1223.
14. Li, H.; Hardin, C.; Shaw, B. R. *J. Am. Chem. Soc.* **1996**, *118*, 6606.
15. Banaszeck, A.; Cornet, X. B.; Zamojski, A. *Carbohydr. Res.* **1985**, *144*, 342.