A concise synthesis of α-D-ribofuranosyl alkylphosphonates — Putative substrate intermediates for the carbon–phosphorous lyase system[1]

Yan Luo and David L. Zechel

Abstract: Carbon–phosphorous lyase is a multienzyme system found in many species of bacteria that is distinguished by its ability to hydrolyze a broad array of unactivated alkylphosphonates. α-D-Ribofuranosyl alkylphosphonates are potential metabolic intermediates generated by the carbon–phosphorous lyase pathway. Here we describe a facile synthesis of α-D-ribofuranosyl alkylphosphonates using β-D-ribofuranosyl trichloracetimidate as a glycosyl donor.

Key words: carbon–phosphorous lyase, *phn* operon, phnN, phosphonates, glycosyl trichloroacetimidate donor, α-D-ribofuranosyl ethylphosphonate.

Résumé : La lyase carbone–phosphore est un système multienzyme qu'on retrouve dans plusieurs espèces et qui se distingue par sa facilité à hydrolyser une grande variété d'alkylphosphonates qui ne sont pas activés. Les alkylphosphonates de α-D-ribofuranosyle sont des intermédiaires métaboliques potentiels générés par la voie réactionnelle de la lyase carbone–phosphore. Dans ce travail, on décrit une méthode facile de synthèse d'alkylphosphonates de α-D-ribofuranosyle utilizant le trichloroacétimidate de β-D-ribofuranosyle comme donneur de glycosyle.

Mots clés : lyase carbone–phosphore, *phn* opéron, phnN, phosphonates, trichloroacétimidate de glycosyle comme donneur, éthylphosphonate de α-D-ribofuranosyle.

Introduction

Phosphate is a fundamental functional group in biology as exemplified by its many structural, signalling, and energy storage roles. Organophosphonates are characterized by a highly stable carbon–phosphorous (CP) bond that is comparable in energy to a carbon–carbon bond (1). In addition to other reduced forms of phosphate such as phosphite and hypophosphite, organophosphonates are significant contributors to the global phosphate pool. They may well have served as the original source of water soluble phosphorous for the assembly of biological precursors on prebiotic earth, as inorganic phosphate would have been largely inaccessible as the mineral apatite (2). Although anthropogenic generation of organophosphonates is considerable (1), natural production is also substantial. The biosynthesis of the majority of organophosphonate natural products begins with the rearrangement of phosphenolpyruvate to phosphonopyruvate by phosphoenolpyruvate mutase (3). From this precursor, *Streptomyces* species biosynthesize a number of bioactive phosphonates, including the antibiotic fosfomycin and the herbicide bialaphos (4). 2-Amino-3-phosphonopropionic acid and 2-aminoethylphosphonic acid are the most widely distributed biogenic organophosphonates, occurring as constituents of lipids, proteins, and polysaccharides in virtually all organisms, including man (5). Due to the global abundance of organophosphonates, bacteria have also evolved a number of strategies to cleave the CP bond to produce inorganic phosphate when the latter is scarce in the environment (1, 6). At present, four distinct CP bond-cleaving activities are known. Three of these activities, comprised of phosphonoacetaldehyde hydrolase (7), phosphonopyruvate hydrolase, (8) and phosphonoacetate hydrolase (9), share a β-carbonyl group as a feature of their respective substrates (**1** in Scheme 1). A mechanism for the cleavage of the CP bond in these substrates can be envisioned as direct nucleophilic attack by water or an enzyme residue on phosphorous, displacing a carbanion to form an enzyme stabilized enolate. In the case of phosphonoacetaldehyde hydrolase, the substrate β-carbonyl is activated as a Schiff base (**2**) followed by attack of a carboxylate nucleophile to form an acyl phosphate intermediate (**3**); hydrolysis forms acetaldehyde (**4**) and inorganic phosphate (7).

Unlike phosphonoacetaldehyde hydrolase, CP-lyase is a multienzyme system that is distinguished by its ability to cleave a broad array of "unactivated" alkylphosphonates (**5**) yielding alkanes (**7**) and inorganic phosphate as products (Scheme 2) (10). In *Escherichia coli*, the *phn* operon encoding CP-lyase consists of 14 genes (*phnCDEFGHIJKLMNOP*) (11, 12); mutational and sequence analysis of the *phn* operon in whole cell metabolic studies has shown that *phnCDE* encodes a phosphonate transport system and *phnG–phnP* en-

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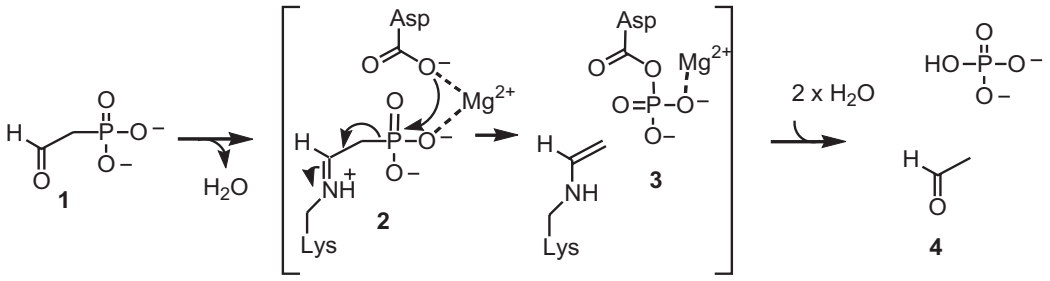
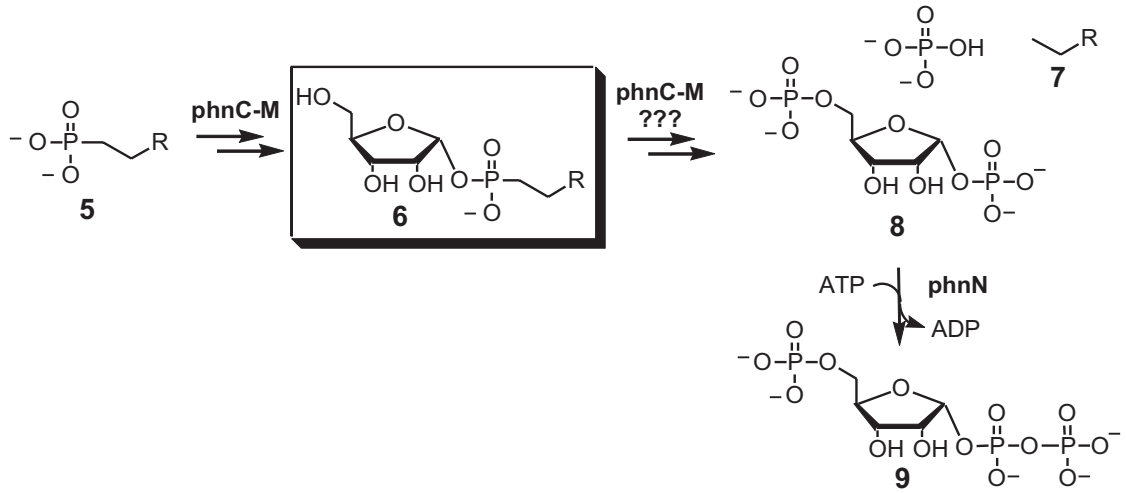
Scheme 1. Cleavage of an "activated" carbon–phosphorous bond by phosphonoacetaldehyde hydrolase (7).



Scheme 2. A hypothetical reaction pathway depicting the cleavage of "unactivated" alkylphosphonates by CP-lyase utilizing **6** as an intermediate. The subsequent phosphorylation reaction by phnN produces a precursor (**9**) for NADH biosynthesis (14).



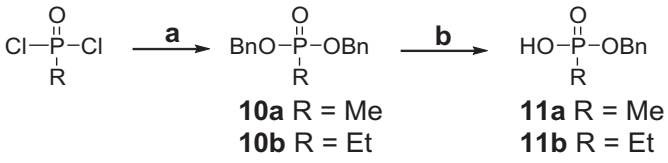
codes enzymes that are required for CP bond cleavage and transcription regulation (13). The sequences of several of these enzymes are unique, yielding no insight via homology into their possible function. An in vitro reconstruction of CP-lyase activity has not yet been achieved, further hampering functional assignments. This is primarily due to the difficulty in expressing several of these enzymes, some of which appear to be associated with the membrane of bacterial cells (13). The only in vitro reaction assigned to a *phn* protein is that of phnN, which was recently shown to be an ATP-dependent kinase, phosphorylating 5-phospho-α-D-ribofuranosyl phosphate (**8**) to yield 5-phospho-α-D-ribofuranosyl diphosphate (PRPP, **9**), a precursor in the biosynthesis of the essential cofactor NAD (14). Intriguingly, α-D-ribofuranosyl ethylphosphonate (**6**) was observed to reach significant concentrations in the culture medium when *E. coli* with a mutated, yet partly functional *phn* operon subsisted on ethylphosphonate. Free phosphate was not produced. By contrast, a mutant strain of *E. coli* with an inoperative *phn* operon did not produce **6** when fed ethylphosphonate, indicating that the some of the CP-lyase enzymes were likely involved in its biosynthesis (15). The substrate specificity of phnN and the biosynthesis of **6** by CP-lyase enzymes strongly suggest that a *phn* enzyme can cleave the CP bond of **6** (or a derivative) and in doing so support NAD biosynthesis. The chemical mechanism of CP-lyase is of great interest, not only for its ability to cleave the remarkably stable carbon–phosphorous bond, but also for the development of environmental remediation catalysts that will degrade phosphonates, such as nerve agents and herbicides. As part of our current program in characterizing the functions and reactions of individual CP-lyase enzymes, we have synthesized **6** and other phosphonate analogues as putative CP-lyase substrate intermediates.

Results and discussion

A major challenge facing the stereoselective synthesis of α-D-ribofuranosyl phosphates is the reactivity and 1,2-cis arrangement of the glycosidic linkage (the product itself is a glycosyl donor (16)). The diphosphate **8** has been synthesized enzymatically (17) by direct condensation of the per-O-acetylated sugar with phosphoric acid (18, 19) or by reaction of the ribofuranosyl bromide with dibenzyl phosphate (20, 21). A synthetically useful enzymatic route to **6** is not yet possible, and the current synthetic methods suffer from low yields or the use of toxic and difficult to handle reagents (e.g., HBr gas). We report that β-D-ribofuranosyl trichloroacetimidate serves as a high yielding and stereoselective donor for the synthesis of **6**.

The required glycosyl acceptors (**11a** and **11b**) were conveniently prepared in two steps (Scheme 3). The corresponding phosphonochloridates were reacted with benzyl alcohol to yield dibenzyl methylphosphonate (**10a**) and dibenzyl ethylphosphonate (**10b**) (22). Refluxing with DABCO in anhydrous toluene afforded mono-protected **11a** and **11b** quan-

Scheme 3. Reagents and conditions: (*a*) BnOH, toluene–pyridine, 0 °C to RT, 5 h; (*b*) DABCO, toluene, reflux, 3 h.



titatively (23). The trichloroacetimidate glycosyl donor **12** was prepared from methyl 2,3,5-tri-*O*-benzyl-α-D-ribofuranoside after solvolysis in 5% aq. HCl – dioxane and treatment of the free anomeric hydroxyl with trichloroacetonitrile and potassium carbonate in dry dichloromethane (Scheme 4). Phosphonic acids **11a** and **11b** were reacted with the donor **12** in dry dichloromethane in the presence of 4 Å molecular sieves to produce a 2:1 α/β mixture of the ribofuranosyl alkylphosphonates **14** and **15** in 60% yield. Reaction with dibenzyl phosphoric acid produced a similar mixture of the corresponding ribofuranosyl phosphate (**13**). An attempt to increase the α/β ratio by reducing the reaction temperature to –20 °C was unsuccessful. However, the β anomers readily isomerized to the thermodynamically stable α anomer in the presence of $BF_3{\cdot}Et_2O$ and 2-chloropyridine. The β anomer was notably unstable, as we observed that **13**β decomposed overnight at room temperature. The stereochemistry of the α anomer was assigned based on NOESY experiments: the signal corresponding to H-1 (δ 6.09) exhibits an NOE with both H-2 and H-3, indicating that H-1, H-2, and H-3 are on the same side of the ribofuranose ring. Compounds **13**α, **14**α, and **15**α were deprotected by catalytic hydrogenolysis to afford the desired α-D-ribofuranosyl phosphate (**16**) and alkylphosphonates (**17** and **18**) in quantitative yield. These were subsequently converted to the triethylammonium salts.

The putative substrate intermediates (**17** and **18**) are very likely to be crucial for assigning the functions of CP-lyase proteins as they are all, or in part, produced in a soluble form suitable for in vitro studies. The stereoselective and high yielding synthetic route described here is considerably more facile than previously reported methods. We can now readily synthesize a wide array of putative CP-lyase intermediates that can also include radiolabels, chromaphores, or fluorophores. Of particular interest is the substrate specificity of the CP-lyase enzyme that ultimately cleaves the CP bond: Does this enzyme cleave simple alkylphosphonates or only glycosylated derivatives like **6** or both? Likewise, which CP-lyase enzyme is responsible for forming **6**? As the ultimate CP bond cleaving mechanism is suspected to be radical based (10), we can also readily incorporate radical stabilizing functionalities (thio, allyl, etc.) into **17** and **18** to serve as radical probes. Our current success in producing soluble CP-lyase proteins will benefit from access to these substrates and will be the topic of future reports.

Experimental

General

Solvents were distilled under dry nitrogen by standard methods (THF from Na/benzophenone and CH_2Cl_2 from CaH_2) prior to use. All commercially available reagents were used without further purification. All reactions were performed under a dry nitrogen atmosphere. ^{1}H NMR spectra was recorded on a 400 MHz Bruker spectrometer using $CDCl_3$ (δ 7.26 ppm), MeOD (δ 3.35 ppm), and D_2O (δ 4.81 ppm) as internal references. ^{13}C NMR and ^{31}P NMR spectra were recorded at 100.6 and 162.0 MHz, respectively. ^{31}P NMR spectra are reported relative to phosphate. Mass spectra were determined by electrospray ionization (ESI) or fast atom bombardment (FAB) mass spectroscopy. Analytical TLC was perfomed on Silicycle F_{254} silica gel plates. Visualization was performed under UV light or by staining with ceric ammonium molybdate. Flash chromatography was performed using Silicycle (40–63 μm) silica gel.

Synthesis

Dibenzyl ethylphosphonate (10b)

A suspension of ethylphosphonylchloridate (1 mL, 9.4 mmol) was stirred rapidly in dry toluene (5 mL) at 0 °C. A mixture of dry benzyl alcohol (2 mL, 18.8 mmol) and dry pyridine (1.5 mL, 18.8 mmol) was added over 90 min while the temperature was maintained. After the addition was complete, the reaction was allowed to reach room temperature and stirred for a further 3 h. The solid product, dibenzyl ethylphosphonate (**10b**), was removed by filtration and washed with toluene, 2 mol/L NaOH, water, then dried over Na_2SO_4 and concentrated in vacuo. Crude **10b** was used in the next reaction without further purification. Data for **10b**: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.4–7.3 (m, 10H, aromatic), 5.08 (m, 2H, OCH_2Ph), 4.98 (m, 2H, OCH_2Ph), 1.76 (m, 2H, CH_2), 1.22 (m, 3H, CH_3). ^{31}P NMR ($CDCl_3$) δ: 35, 37.

Benzyl ethylphosphonic acid (11b)

To a stirred solution of crude **10b** (3.0 g, 10 mmol) in dry toluene (20 mL) was added DABCO (1.12 g, 10 mmol) under a nitrogen atmosphere. The reaction mixture was refluxed for 3 h before the solvent was removed in vacuo and the residue was redissolved in 5% aq. HCl. The aqueous layer was extracted with ethyl acetate, dried over Na_2SO_4, then evaporated under reduced pressure to yield benzyl ethylphosphonic acid (**11b**) (1.87 g, 99% calcd. from ethylphosphonylchloridate). Data for **11b**: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.07 (m, 2H, OCH_2Ph), 1.8 (m, 2H, CH_2), 1.2 (m, 3H, CH_3). ^{31}P NMR ($CDCl_3$) δ: 36.

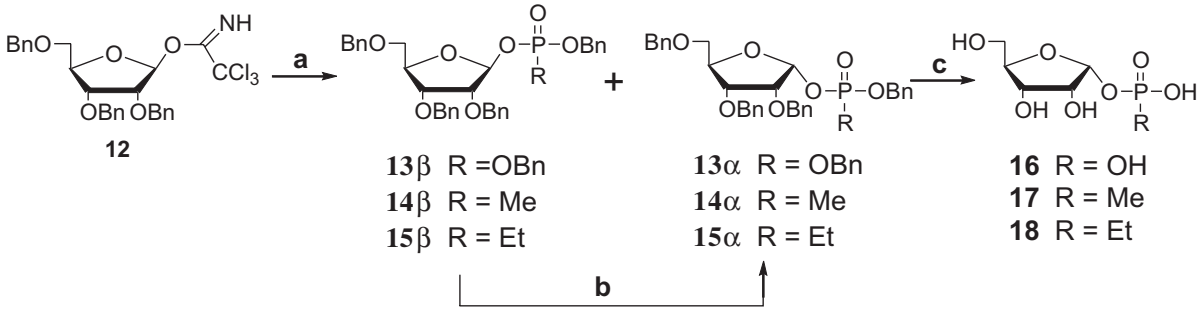
Dibenzyl methylphosphonate (10a)

The procedure for the preparation of **10b** was followed. Data for **10a**: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.4–7.3 (m, 10H, aromatic), 5.08 (m, 2H, OCH_2Ph), 4.98 (m, 2H, OCH_2Ph), 1.5 (d, J = 17.6 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ: 67.16 (OCH_2Ph), 67.09 (OCH_2Ph), 12.42, 10.99 (CH_3). ^{31}P NMR ($CDCl_3$) δ: 31.

Benzyl methylphosphonic acid (11a)

The procedure for the preparation of **11b** was followed. Data for **11a**: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.3–7.4 (m, 5H, aromatic), 5.07 (m, 2H, OCH_2Ph), 1.55 (d, J = 17.9 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ: 66.59 (OCH_2Ph), 12.39, 11.14 (CH_3).

Scheme 4. Reagents and conditions: (*a*) phosphonic acid, dichloromethane, 0 °C to RT, 3 h; (*b*) $BF_3{\cdot}Et_2O$, 2-chloropyridine, RT, 2 h; (*c*) Pd/C (10%), H_2, 12 h.



*2,3,5-Tri-*O*-benzyl-*D*-ribofuranosyl benzyl ethylphosphonate (15)*

A solution of 2,3,5-tri-*O*-benzyl-D-ribofuranose (1 g, 2.5 mmol), potassium carbonate (396 mg, 2.87 mmol), and trichloroacetonitrile (4 mL, 40 mmol) in anhydrous dichloromethane (20 mL) was stirred overnight at room temperature. The mixture was filtered through Celite and concentrated in vacuo. Flash chromatography using ethyl acetate – hexanes (1:2) yielded pure 2,3,5-tri-*O*-benzyl-β-D-ribofuranosyl trichloracetimidate (**12**). Data for **12**: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.5 (s, 1H, NH), 7.4–7.3 (m, 15H, aromatic), 6.35 (s, 1H, H1), 4.8–4.4 (m, 7H, OCH_2Ph, H2), 4.15 (m, 2H, H3, H4), 3.7 (dd, 2H, H5a, H5b). ^{13}C NMR ($CDCl_3$) δ: 160.77 (C=NH), 103.43 (C1), 82.15 (C4), 78.48 (C2), 77.22 (C3), 73.25, 72.54, 72.18 (CH_2Ph), 70.19 (C5). To an ice-cold solution of **12** (698 mg, 1.24 mmol) in anhydrous dichloromethane was added **11b** (527 mg, 2.6 mmol) in the presence of 4 Å molecular sieves. The mixture was stirred for 3 h whereupon TLC indicated complete conversion of **12**. The mixture was filtered through Celite and concentrated in vacuo to yield an anomeric mixture of **15** (450 mg, 60%, α/β = 2:1). The anomers were resolved by flash chromatography (ethyl acetate – hexanes, 1:3 to 1:1). The β anomer **15**β (100 mg, 0.17 mmol) was subsequently dissolved in anhydrous dichloromethane (5 mL) followed by $BF_3{\cdot}Et_2O$ (10 μL, 0.087 mmol) and 2-chloropyridine (16 μL, 0.174 mmol). The mixture was stirred at room temperature for 1.5 h then neutralized with triethylamine and evaporated in vacuo. The crude product was purified by flash chromatography (ethyl acetate – hexane, 1:1) to yield the α anomer **15**α (75 mg, 75%). Data for **15**α: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.1–7.4 (m, 20H, aromatic), 6.09 (dd, $J_{1,2}$ = 4.4 Hz, $J_{1,P}$ = 4.6 Hz, 1H, H1), 5.0–5.1 (m, 2H, $POCH_2Ph$), 4.8–4.4 (m, 6H, OCH_2Ph), 4.38 (m, 1H, H4), 3.9 (d, 2H, H2, H3), 3.5 (dd, 2H, H5), 1.9 (m, 2H, CH_2), 1.2 (m, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ: 97 (C1), 84.3 (C4), 78.97 (C2), 75.54 (C3), 73.53, 72.56, 72.63 (OCH_2Ph), 69.92 (C5), 66.20 ($POCH_2Ph$), 18.89 (CH_2), 6.44 (CH_3). ^{31}P NMR ($CDCl_3$) δ: 34. ESI-MS *m*/*z*: 603.22 (50%, $[M + H]^+$), 295.12 (100%, $[M - C_{16}H_{20}O_4P - H]^+$). HR-MS *m*/*z* calcd. for $C_{35}H_{40}O_7P$ $[M + H]^+$: 603.25116; found: 603.21992. Data for **15**β: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.1–7.4 (m, 20H, aromatic), 5.88 (dd, $J_{1,2}$ = 5.9 Hz, $J_{1,P}$ = 6.1 Hz, 1H, H1), 4.9–5.1 (m, 2H, $POCH_2Ph$), 4.5–4.7 (m, 6H, OCH_2Ph), 4.4 (m, 1H, H4), 4.15 (m, 1H, H3), 3.9 (m, 1H, H2), 3.7 (dd, 1H, H5a), 3.55 (dd, 1H, H5b), 1.9 (m, 2H, CH_2), 1.2 (m, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ: 100.63 (C1), 81.54 (C4), 80.01 (C2), 77.03 (C3), 73.16, 72.54, 72.27 (OCH_2Ph), 72.19 (C5), 66.8 ($POCH_2Ph$). ^{31}P NMR ($CDCl_3$) δ: 34.

*2,3,5-Tri-*O*-benzyl-*D*-ribofuranosyl dibenzylphosphate (13)*

The procedure for **15** was followed. Data for **13**α: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.34–7.2 (m, 25H, aromatic), 6.1 (t, $J_{1,2}$ = 1.5 Hz, $J_{1,P}$ = 2.1 Hz, 1H, H1), 5.08 (m, 4H, $P(OCH_2Ph)_2$), 4.7 (m, 2H, OCH_2Ph), 4.5–4.6 (m, 4H, OCH_2Ph), 4.4 (m, 1H, H4), 4.02 (d, 2H, H2,H3), 3.5 (d, 2H, H5). ^{13}C NMR ($CDCl_3$) δ: 99.1, 99.06 (C1), 8.4.64 (C4), 79.05 (C2), 75.44 (C3), 73.55, 72.68, 72.63 (OCH_2Ph), 69.79 (C5), 69.31, 69.25 ($P(OCH_2Ph)_2$). ^{31}P NMR ($CDCl_3$) δ: –2.51. Data for **13**β: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.34–7.2 (m, 25H, aromatic), 5.86 (d, *J* = 4.9 Hz, 1H, H1), 5.0–5.1 (m, 4H, $P(OCH_2Ph)_2$), 4.7–4.5 (m, 6H, OCH_2Ph), 4.4 (m, 1H, H4), 4.1 (m, 1H, H3), 3.9 (m, 1H, H2), 3.6–3.7 (dd, 2H, H5). ^{13}C NMR ($CDCl_3$) δ: 102.65 (C1), 81.08 (C4), 79.13 (C2), 77.71 (C3), 73.18, 72.58, 72.32 (OCH_2Ph), 70.4 (C5), 69.33, 69.29 ($P(OCH_2Ph)_2$). ^{31}P NMR ($CDCl_3$) δ: –3.1.

*2,3,5-Tri-*O*-benzyl-*D*-ribofuranosyl benzyl methylphosphonate (14)*

The procedure for **15** was followed. Data for **14**α: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.4–7.3 (m, 20H, aromatic), 6.09 (dd, 1H, $J_{1,2}$ = 1.6 Hz, $J_{1,P}$ = 3.8 Hz, H1), 4.9–5.1 (m, 2H, $POCH_2Ph$), 4.7 (d, 2H, OCH_2Ph), 4.6 (m, 2H, OCH_2Ph), 4.5 (d, 2H, OCH_2Ph), 4.3 (m, 1H, H4), 3.9 (m, 2H, H2, H3), 3.4 (m, 2H, H5). ^{13}C NMR ($CDCl_3$) δ: 97.37 (C1), 84.13 (C4), 78.63 (C2), 75.27 (C3), 73.52 (OCH_2Ph), 72.73 (OCH_2Ph), 72.49 (OCH_2Ph), 69.81 (C5), 67.15 ($POCH_2Ph$). ^{31}P NMR ($CDCl_3$) δ: 32, 31. Data for **14**β: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.4–7.3 (m, 20H, aromatic), 5.87 (d, 1H, *J* = 5.8 Hz, H1), 5.0 (m, 2H, $POCH_2Ph$), 4.7 (d, 2H, OCH_2Ph), 4.6 (d, 2H, OCH_2Ph), 4.5 (m, 2H, OCH_2Ph), 4.1 (m, 1H, H4), 3.9 (m, 2H, H2, H3), 3.59 (m, 2H, H5). ^{13}C NMR ($CDCl_3$) δ: 100.75 (C1), 80.07 (C4), 79.99 (C2), 76.65 (C3), 73.17 (OCH_2Ph), 72.58 (OCH_2Ph), 72.26 (OCH_2Ph), 69.84 (C5), 66.95 ($POCH_2Ph$). ^{31}P NMR ($CDCl_3$) δ: 30, 31.

α-D-Ribofuranosyl ethylphosphonate (18)

To a solution of compound **15**α (200 mg, 0.33 mmol) in MeOH (10 mL) was added 10% Pd/C. The reaction mixture was stirred under H_2 overnight, filtered through Celite, and concentrated to afford **18** (82 mg, 100%). The crude product was purified through an Amberlite IR-120 ion exchange column, titrated with triethylamine, then dried in vacuo to obtain the triethylammonium salt. Data for **18**: ^{1}H NMR

(400 MHz, D_2O) δ: 4.78 (s, 1H, H1), 4.03 (m, 1H, H3), 3.9–3.8 (m, 2H, H2, H4), 3.7 (dd, 1H, H5a), 3.5 (dd, 1H, H5b), 1.3 (m, 2H, CH_2), 0.9 (m, 3H, CH_3). ^{13}C NMR (D_2O) δ: 107.82 (C1), 82.69 (C2), 74.07 (C4), 70.64 (C3), 62.62 (C5), 21 (CH_2), 7 (CH_3). ^{31}P NMR (D_2O) δ: 25.89.

α-D-Ribofuranosyl phosphate (16)

The procedure for **18** was followed. Data for **16**: ^1H NMR (400 MHz, D_2O) δ: 4.77 (s, 1H, H1), 4.02 (m, 1H, H2), 3.8–3.9 (m, 2H, H3, H4), 3.67 (dd, 1H, H5a), 3.5 (m, 1H, H5b). ^{13}C NMR ($CDCl_3$) δ: 107.82 (C1), 82.7 (C2), 74.08 (C4), 69.57 (C3), 61.40 (C5). ^{31}P NMR ($CDCl_3$) δ: 0.11.

α-D-Ribofuranosyl methylphosphonate (17)

The procedure for **18** was followed. Data for **17**: ^1H NMR (400 MHz, MeOD) δ: 4.76 (s, 1H, H1), 4.04 (m, 1H, H3), 3.96 (m, 1H, H2), 3.89 (d, 1H, H4), 3.74 (dd, 1H, H5a), 3.55 (dd, 1H, H5b), 1.3 (s, 3H, CH_3). ^{13}C NMR (MeOD) δ: 108.44 (C1), 83.44 (C2), 74.77 (C4), 71.27 (C3), 63.61 (C5), 15 (CH_3). ^{31}P NMR (MeOD) δ: 30.24.

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