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Synthesis and kinetic evaluation of 4-deoxy-4-phosphonomethyl-D-erythronate, the first hydrolytically stable and potent competitive inhibitor of ribose-5-phosphate isomerase

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Abstract—4-deoxy-4-Phosphonomethyl-D-erythronate, an isosteric and hydrolytically stable analogue of the known ribose-5-phosphate isomerase inhibitor 4-deoxy-4-phospho-D-erythronate, was obtained by a 14-step synthesis from D-arabinose through a highly improved synthesis of the precursor 5-deoxy-5-phosphonomethyl-D-arabinose. The title compound appears as the first stable and potent competitive inhibitor of the enzyme catalyzed isomerization of ribose-5-phosphate to D-ribulose-5-phosphate ($K_i = 74\,\mu M$, $K_m/K_i = 100$), exhibiting only a 3-fold weaker inhibitory activity than its phosphate analogue.
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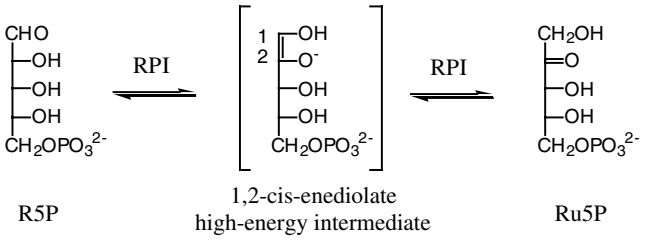
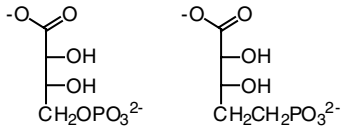
Ribose-5-phosphate isomerase (RPI, EC 5.3.1.6), an aldose–ketose isomerase involved in the pentose phosphate pathway, catalyzes the reversible isomerization reaction between D-ribose 5-phosphate (R5P) and D-ribulose 5-phosphate (Ru5P) (Scheme 1).[1] The reaction is thought to proceed through a proton transfer mechanism and to involve a 1,2-*cis*-enediolate high-energy intermediate. In course of our search for high-energy intermediate analogue inhibitors of this reaction, we have recently reported 4-deoxy-4-phospho-D-erythronohydroxamic acid as a new and strong competitive inhibitor of spinach RPI,[2] with inhibitory properties similar to that previously reported for the known RPI inhibitor 4-deoxy-4-phospho-D-erythronate **1** (Scheme 2).[1] Because of the known sensitivity of the phosphate group towards chemical and biological hydrolysis,[3] 4-deoxy-4-phosphonomethyl-D-erythronate **2** (Scheme 2) was designed as an isosteric and stable analogue of **1**. In phosphorylated molecules of biological interest, replacement of the labile C–O–P bond by a C–C–P bond[4–10] greatly enhances the stability of such molecules towards the action of digestive phosphatases, as well as their potential bioactivity as inhibitors or regulators of metabolic processes.[11] Because enzymes of the pentose phosphate pathway (and glycolysis) of some pathogenic organisms like *Trypanosoma brucei* have been considered crucial for their survival and development,[12] design of potent and stable RPI inhibitors might lead



Scheme 1. Isomerization reaction catalyzed by D-ribose-5-phosphate isomerases.



1 **2**

Scheme 2. 4-deoxy-4-Phospho-D-erythronate (**1**) and 4-deoxy-4-phosphonomethyl-D-erythronate (**2**) as mimics of the 1,2-*cis*-enediolate high-energy intermediate species of the RPI-catalyzed isomerization reaction.

Keywords: Enzyme inhibitor; Phosphonate; Ribose-5-phosphate isomerase.

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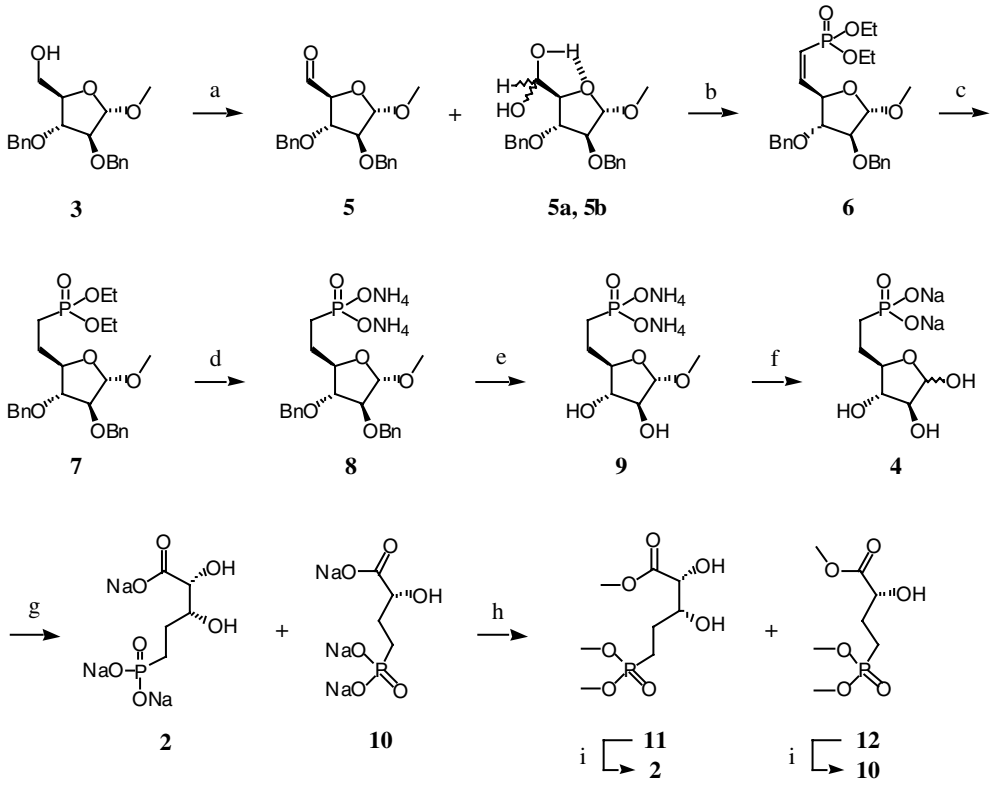
to molecules of therapeutic interest. Thus, we report in this study the synthesis of **2** as the first isosteric and stable reaction intermediate analogue of the R5P to Ru5P isomerization reaction catalyzed by spinach RPI. A comparison of its inhibitory properties with that of **1** is also reported.

Our synthetic strategy to obtain 4-deoxy-4-phosphonomethyl-D-erythronate **2** (Scheme 2) started from methyl 2,3-di-*O*-benzyl-α-D-arabinofuranoside **3**[13,14] (Scheme 3), which was prepared in five steps from D-arabinose using reported procedures.[15–19] Conversion of **3** to the known 5-deoxy-5-phosphonomethyl-D-arabinose **4**[5,14,15] (Scheme 3) was then achieved in six steps in 59% yield according to a highly improved procedure (lit.[14] 18%, lit.[15] 11%), leading thereafter to the title compound by simple oxidative cleavage of the phosphonomethyl precursor as depicted in Scheme 3. Indeed, oxidation of **3** under Mofatt conditions[13,14,20] led, after aqueous work-up, to a mixture of methyl 2,3-di-*O*-benzyl-5-dehydro-α-D-arabinofuranoside **5**[13,14] and its hydrated forms **5a** and **5b** (Scheme 3) in approximately equal proportion.[21] It should be noted that formation of the hydrated forms of **5** may have led to wrong conclusions about stability and purity of the product of this reaction, which indeed does not necessitate purification by silica gel chromatography as reported.[15] By-product dicyclohexylurea formed during reaction was simply eliminated by addition of diethyl ether. Following the reported Horner–Emmons condensation of **5** with the tetraethylmethylenediphosphonate carbanion using sodium hydride in diethyl ether,[13,14,22] the fully protected methyl 2,3-di-*O*-benzyl-5-deoxy-5-diethylphosphonomethylene-α-D-arabinofuranoside **6**[13,14] was isolated in 61% yield (two steps). Hydrogenation of the double bound was achieved as described with PtO_2 (10 bar) to give methyl 2,3-di-*O*-benzyl-5-deoxy-5-diethylphosphonomethyl-α-D-arabinofuranoside **7** in quantitative yield.[13,14] Thereafter, our strategy for the efficient deprotection of **7** started with phosphonate ethyl ester hydrolysis, using TMSBr in CH_2Cl_2 followed by NH_3/H_2O/MeOH hydrolysis, giving methyl 2,3-di-*O*-benzyl-5-deoxy-5-phosphonomethyl-α-D-arabinofuranoside **8** as the bis(ammonium) salt. After removal of NH_4Br by trituration in $CHCl_3$, **8**[23] was obtained in quantitative yield. It was then hydrogenolyzed (15 bar) over 10% Pd/C to afford methyl 5-deoxy-5-phosphonomethyl-α-D-arabinofuranoside **9**, bis(ammonium) salt[24] in quantitative yield. Following elution (H_2O) on Dowex® 50X4-400 (H^+ form), concentration and heating under argon for 3 h in water,[15,18] the reaction mixture was concentrated, eluted (H_2O) on Dowex® 50X4-400 (Na^+ form) and freeze-dried to give pure 5-deoxy-5-phosphonomethyl-α-D-arabinose **4**[5,14,15]



Scheme 3. Reagents and conditions: (a) i. DCC (5.3 equiv), DMSO, pyridine, TFA, 10 h, 25 °C; ii. $(CO_2H)_2$, MeOH, 0 °C; iii. Et_2O, filtration; (b) i. NaH (2 equiv), $CH_2[PO(OEt)_2)_2]$ (1.5 equiv), Et_2O, 2 h; ii. **5**, **5a** and **5b** mixture, 2 h, 61% (from **3**); (c) H_2, 10 bar, PtO_2, CH_2Cl_2, 12 h, 100%; (d) i. TMSBr (9 equiv), CH_2Cl_2, 24 h, 25 °C; ii. NH_3/H_2/MeOH, 30 min, 100%; iii. $CHCl_3$, filtration; (e) H_2, 15 bar, Pd/C, CH_2Cl_2/MeOH (10/1), 12 h, 100%; (f) i. Dowex® 50X4-400 (H^+ form), concentration; ii. H_2O, reflux under argon, 3 h; iii. Dowex® 50X4-400 (Na^+ form), freeze drying, 97%; (g) i. NaOH 0.5 M in water (3 equiv), 48 h, 25 °C; ii. HCl, pH 1.5; iii. $Ba(OH)_2$, pH 8.5; iv. Dowex® 50X4-400 (H^+ form); (h) i. CH_2N_2, Et_2O, MeOH; ii. Silica gel chromatography (MeOH/AcOEt, 2/8), 54% (**11**) and 29% (**12**) (from **4**); (i) TMSBr (4 equiv), CH_2Cl_2, 1 h, 0 °C; ii. NH_3/H_2O/MeOH, 30 min; iii. $Ba(OH)_2$; iv. Dowex® 50X4-400 (H^+ form); v. Dowex® 50X4-400 (Na^+ form), freeze drying, 95%.

as the disodium salt in 97% yield. This later deprotection step of methyl 5-deoxy-5-dihydrogenophosphonomethyl-α-D-arabinofuranoside to give compound **4** was achieved very mildly by simple heating in water. Our methodology differs from the reported one, which mentioned the use of a cation-exchange resin (Dowex® 50, H^+ form) in boiling water for the deprotection of the acid form of **9**.[15] Indeed, hydrolysis of methyl 5-deoxy-5-dihydrogenophosphate-α-D-arabinofuranoside to 5-deoxy-5-dihydrogenophosphate-D-arabinose was also reported to be achieved upon heating in water for 2 h under argon without any degradation of the product.[18] From our point of view, this facilitated hydrolysis of the protected anomeric group is most likely due to intramolecular catalytic assistance by the phosphonic (or phosphoric) acid group. Indeed, hydrolysis of the methylated anomeric hydroxyl group prior to phosphonate deprotection was reported to require much more drastic conditions (H_2SO_4 2 M, AcOH),[13,14] which led to compound **4** in much lower overall yield. In contrast to what we had reported for the synthesis of 5-deoxy-5-phosphate-D-arabinonate from 6-deoxy-6-phosphate-D-fructose[25] or 6-deoxy-6-phosphate-D-glucose,[26] oxidative cleavage (NaOH 0.5 M, molecular oxygen, 2 days) of compound **4** gave not only the expected 4-deoxy-4-phosphonomethyl-D-erythronate **2**, but also the unexpected 3-deoxy-3-phosphonomethyl-D-glycerate **10** in a 2:1 ratio (Scheme 3). Although it is known from the reaction mechanism of aldose (or ketose) oxidative cleavage proposed by Hendriks et al. that different types of enediolate intermediates (which lead to different products) may be formed,[27] formation of the two products surprised us. Indeed, oxidative cleavage of 5-deoxy-5-phosphate-D-arabinose (or 5-deoxy-5-phosphate-D-ribose) gave us only the expected product 4-deoxy-4-phosphate-D-erythronate **1** (results not shown). Nevertheless, the two products **2** and **10** could be separated according to the following treatment. Upon conversion to their respective methyl esters **11** and **12** (Scheme 3) using diazomethane,[28] and separation by silica gel chromatography (methanol/ethyl acetate=2/8), pure methyl 4-deoxy-4-dimethylphosphonomethyl-D-erythronate **11**[29] ($R_f = 0.26$) and 3-deoxy-3-dimethylphosphonomethyl-D-glycerate **12**[30] ($R_f = 0.36$) were separated and obtained, respectively, in 54% and 29% yield from **4**. Hydrolysis of the ester **11** was accomplished using TMSBr in CH_2Cl_2 followed by NH_3/H_2O/MeOH hydrolysis, which gave compound **2** as its bis(ammonium) salt. Upon addition of barium hydroxide, compound **2** precipitated as its barium salt and could be collected upon filtration. It was successively eluted (H_2O) on Dowex® 50X4-400 (H^+ form) and Dowex® 50X4-400 (Na^+ form). Following lyophilization, pure **2** as the disodium salt[31] was recovered in 95% yield. The same procedure was used on the ester **12** to give compound **10** as its disodium salt[32] in 95% yield.

The new compounds **2** and **10** were both evaluated against spinach RPI (Aldrich) as potential inhibitors of the R5P to Ru5P isomerization reaction (Scheme 1). Enzymatic activities were determined by following the change in ultraviolet absorbance that accompanies conversion of R5P to Ru5P,[33] ($\lambda = 282$ nm, $\epsilon = 58.6\ M^{-1}\,cm^{-1}$) at 25 °C in 50 mM Tris·HCl buffer (pH 7.5). Apparent Michaëlis constant (K_m) and inhibition constant (K_i) were determined (Fig. 1) from double reciprocal plots of the initial reaction velocity versus R5P concentration obtained at various concentrations of inhibitor **2** (Lineweaver–Burk graphical representation) with 0.5 U/mL of RPI (and replots of apparent K_m/V_{max} values vs inhibitor concentration). IC_{50} determinations were achieved for compound **10** using a 3.2 mM R5P concentration. As expected from its structure, compound **10** is not a good mimic of the enediolate high-energy intermediate (IC_{50}=5 mM). With a K_i value of 74 μM and a K_m/K_i ratio of 100 (K_m R5P=7.5 mM), compound **2** behaves as a new potent and competitive inhibitor of the isomerization reaction of R5P to Ru5P catalyzed by spinach RPI. It is remarkable to note that this K_i value we determined for the phosphonomethyl inhibitor **2** is only about 3-fold higher than the K_i value previously reported for its phosphate analogue **1** ($K_i = 28$ μM, $K_m/K_i = 270$),[2] the best known RPI inhibitor, which corresponds to a 0.6 kcal/mol decrease in the binding affinity of **2** versus **1** for the enzyme active site. Such a small value is unlikely to correspond to the loss of a hydrogen bond (typically in the range of 3–6 kcal/mol)[34] reflecting the change in the pK_{a2} value from phosphate to methylphosphonate (7.5–6.5). It is however consistent with the loss of a weak interaction between uncharged functions (typically 0.5–1.8 kcal/mol) and thus indicates that such a

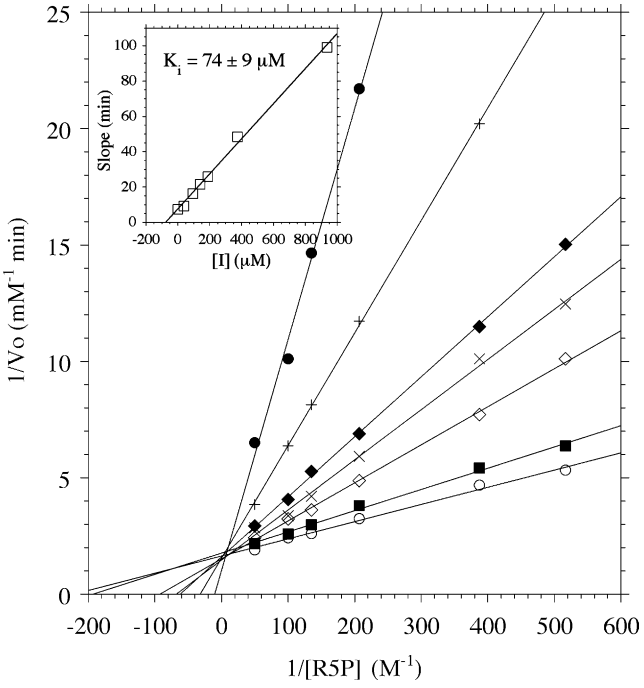


Figure 1. Inhibition of spinach RPI (50 mM Tris·HCl buffer, 25 °C, pH 7.5). Double reciprocal plot of initial reaction velocity versus R5P concentration obtained at various concentration of inhibitor **2** versus R5P concentration (Lineweaver–Burk graphical representation): ○, no inhibitor; ■, [I]=37 μM; ◇, [I]=93 μM; ×, [I]=140 μM; +, [I]=187 μM; ◆, [I]=374 μM; ●, [I]=935 μM. Lines drawn obtained from nonlinear least squares fit to the observed data using Michaelis–Menten equation for competitive inhibition.

replacement of the oxygen atom for CH_2 does not impair significantly strong inhibition of spinach RPI. Consequently, it does not seem useful in the case of RPI to design monofluoromethylphosphonate analogues of **1**, as reported for the case of glucose-6-phosphate dehydrogenase inhibition by good phosphate surrogates.[8] Comparison to the case of the fructose-6-phosphate to glucose-6-phosphate isomerization catalyzed by another aldose–ketose isomerase, namely phosphoglucose isomerase (PGI), is quite interesting. Indeed, it has been shown that such an oxygen for CH_2 replacement totally destroy the inhibition character of known strong PGI inhibitors.[35,36] In the case of the R5P to Ru5P isomerization catalyzed by spinach RPI, our study shows that this is not the case. Therefore, synthesis of the new potent and hydrolytically stable competitive inhibitor **2** appears very promising for the future development of stable and species-specific RPI inhibitors of therapeutic interest.

Acknowledgements

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21. Selected data for compound **5** (aldehyde form): ^{13}C NMR (50 MHz, $CDCl_3$) $\delta = 200.7$ (C-5), 137.9–137.2 (C-11), 128.6–128.0 (C-8, C-9, C-10), 108.4 (C-1), 86.8 (C-4), 84.7 (C-2), 82.7 (C-3), 72.2–71.8 (C-7), 55.4 (C-6); for compounds **5a** and **5b** (hydrated forms of **5**): ^{13}C NMR (50 MHz, $CDCl_3$) $\delta = 137.9$–137.2 (C-11^{ab}), 128.6–128.0 (C-8^{ab}, C-9^{ab}, C-10^{ab}), 107.8 and 107.5 (C-1^a, C-1^b), 97.3 and 97.2 (C-5^b, C-5^a), 87.4 and 87.2 (C-4^b, C-4^a), 85.7 and 84.1 (C-2^b, C-2^a), 83.4 and 83.8 (C-3^b, C-3^a), 72.2–71.8 (C-7^{ab}), 55.2 and 55.0 (C-6^b, C-6^a).
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23. Selected data for compound **8**: ^{13}C NMR (90 MHz, CD_3OD) $\delta = 138.7$–138.4 (C-12, C-12′), 129.4 (C-10, C-10′), 129.2 (C-11, C-11′), 128.9 (C-9, C-9′), 107.8 (C-1), 88.6 (C-2), 88.0 (C-3), 82.8 (d, C-4, $J = 18$ Hz), 73.1 and 72.8 (C-8, C-8′), 55.0 (C-7), 28.7 (C-5), 26.7 (d, C-6, $J = 135$ Hz). ^{31}P NMR (CD_3OD) $\delta = 26.3$.
24. Selected data for compound **9**: ^{13}C NMR (50 MHz, CD_3OD) $\delta = 109.1$ (C-1^α), 102.9 (C-1^β), 84.5 (d, C-4^α, $J = 18$ Hz), 82.9 (d, C-4^β, $J = 18$ Hz), 82.3 (C-2^α), 80.8 (C-3^α), 78.7 (C-2^β), 77.5 (C-3^β), 55.8 (C-7^β), 55.6 (C-7^α), 29.5 (C-5^β), 27.7 (d, C-5^α, $J = 3$ Hz), 24.7 (d, C-6^α, C-6^β, $J = 135$ Hz). ^{31}P NMR (CD_3OD) $\delta = 29.2$ (P^α), 28.9 (P^β).
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29. Selected data for compound **11**: $[\alpha]_D^{28}$ +10.1 (*c* 0.97, CH_3OH). IR ν_{max} (cm^{-1}) (KBr): 3394 (OH), 1743 (CO), 1034 (OC, PO). ^{1}H NMR (250 MHz, $CDCl_3$) $\delta = 4.21$–4.19 (d, 1H, H-1, $J = 4$ Hz), 3.92–3.82 (m, 1H, H-2), 3.79 (s, 3H, H-6), 3.72 (d, 6H, H-5, $J = 11$ Hz), 2.03–1.75 (m, 4H, H-3, H-4). ^{13}C NMR (90 MHz, $CDCl_3$) $\delta = 173.2$ (C-1), 74.6 (C-2), 73.1 (d, C-3, $J = 16$ Hz), 52.8 (d, C-6, $J = 6$ Hz), 52.5 (C-7), 25.0 (d, C-4, $J = 3$ Hz), 20.8 (d, C-5, $J = 142$ Hz). ^{31}P NMR ($CDCl_3$) $\delta = 35.9$. MS (positive-ion electrospray): *m*/*z* (%) 197 [M−2OCH$_3$+3H$^+$] (6), 257 [M+H$^+$] (13), 279 [M+Na$^+$] (100). HRMS (positive-ion electrospray): calcd for $C_8H_{17}O_7PNa$ (M+Na$^+$) 279.0610, found 279.0608.
30. Selected data for compound **12**: $[\alpha]_D^{28}$ +2.8 (*c* 1.20, CH_3OH). IR ν_{max} (cm^{-1}) (KBr): 3416 (OH), 1739 (CO), 1028 (OC, PO). ^{1}H NMR (250 MHz, $CDCl_3$) $\delta = 4.26$–4.20 (m, 1H, H-1), 3.76 (d, 6H, H-4, $J = 7$ Hz), 3.70 (s, 3H, H-5), 2.20–1.84 (m, 4H, H-2, H-3). ^{13}C NMR (90 MHz, $CDCl_3$) $\delta = 174.8$ (C-1), 70.3 (d, C-2, $J = 17$ Hz), 52.8 (d, C-5, $J = 7$ Hz), 52.7 (C-6), 27.5 (d, C-3, $J = 4$ Hz), 20.4 (d, C-4, $J = 142$ Hz). ^{31}P NMR ($CDCl_3$) $\delta = 34.8$. MS (positive-ion electrospray): *m*/*z* (%) 167 [M−2OCH$_3$ + 3H$^+$] (17), 227 [M+H$^+$] (10), 249 [M+Na$^+$] (77), 293 (100). HRMS (positive-ion electrospray): calcd for $C_7H_{15}O_6PNa$ (M+Na$^+$) 249.0504, found 249.0506.
31. Selected data for compound **2**: $[\alpha]_D^{28}$ +8.5 (*c* 1.09, H_2O). IR ν_{max} (cm^{-1}) (KBr): 3406 (OH), 1682 (CO), 1027 (OC, PO). ^{1}H NMR (250 MHz, D_2O) $\delta = 4.23$ (d, 1H, H-1, $J = 4$ Hz), 3.90–3.80 (m, 1H, H-2), 1.81–1.54 (m, 4H, H-3, H-4). ^{13}C NMR (50 MHz, D_2O) $\delta = 176.9$ (C-1), 74.8 (C-2), 74.0 (d, C-3, $J = 18$ Hz), 26.3 (C-4), 25.0 (d, C-5, $J = 133$ Hz). ^{31}P NMR (D_2O) $\delta = 28.9$.

32. Selected data for compound **10**: $[\alpha]_D^{28}$ +6.6 (*c* 1.04, H_2O). IR ν_{max} (cm^{-1}) (KBr): 3402 (OH), 1676 (CO), 1056 (OC, PO). ^1H NMR (250 MHz, D_2O) $\delta = 4.21$–4.16 (m, 1H, H-1), 21.0–1.50 (m, 4H, H-2, H-3). ^{13}C NMR (50 MHz, D_2O) $\delta = 179.8$ (C-1), 71.4 (d, C-2, $J = 18$ Hz), 27.8 (d, C-3, $J = 4$ Hz), 22.8 (d, C-4, $J = 137$ Hz). ^{31}P NMR (D_2O) $\delta = 27.7$.
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