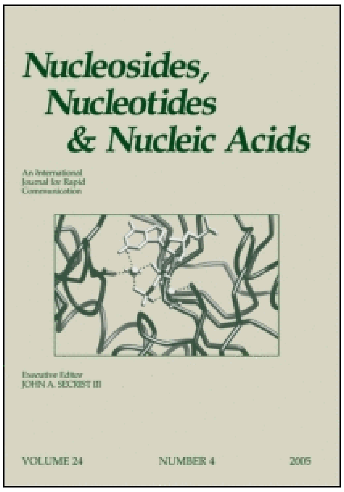

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Synthesis of Simple Adenosine Diphosphate Ribose Analogues

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SYNTHESIS OF SIMPLE ADENOSINE DIPHOSPHATE RIBOSE ANALOGUES

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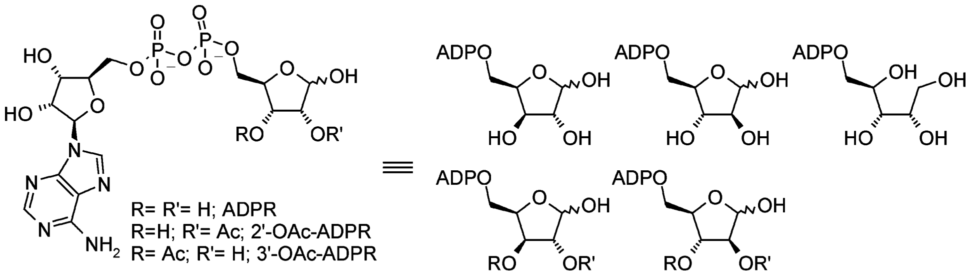

The synthesis of analogues of adenosine diphosphate ribose and acetylated adenosine diphosphate ribose, modified at the northern pentose, is reported. The stereochemistry at the acetylated centers was chosen to minimize acetyl migration and dictated the overall synthetic strategy.

Keywords Acetylated adenosine diphosphate ribose; acetyl migration

Adenosine diphosphate ribose (ADPR), a ubiquitous metabolite of nicotinamide adenine dinucleotide (NAD), recently has been described as a calcium channel activator[1,2] directly involved in cell death initiation.[3] Similarly, another NAD metabolite, *O*-acetylated ADPR (existing in solution as a mixture of 2$''$-OAc and 3$''$-OAc ADPR), which has been shown to directly regulate the same calcium channel,[4] has also been proposed to mediate cell survival. Additionally, *O*-acetylated ADPR has been shown to act as reporter of NAD-dependent protein deacetylase activities at remote sites[5]

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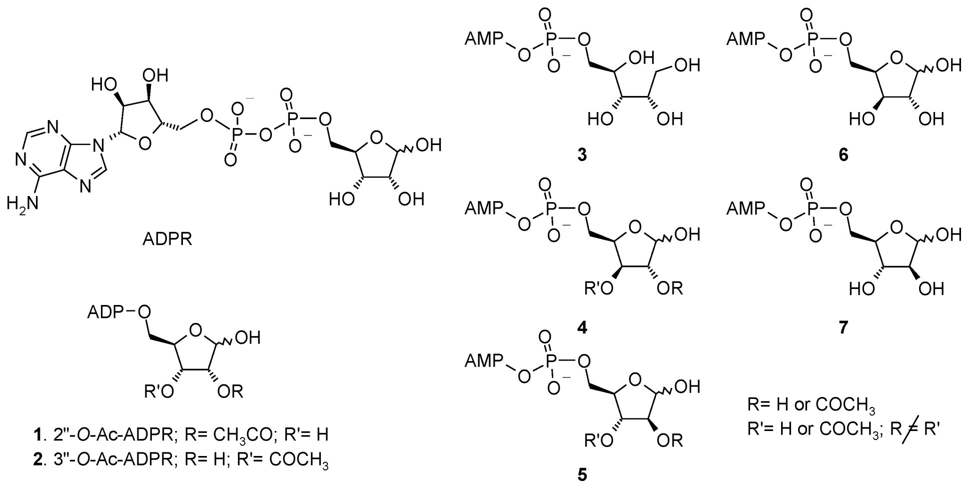
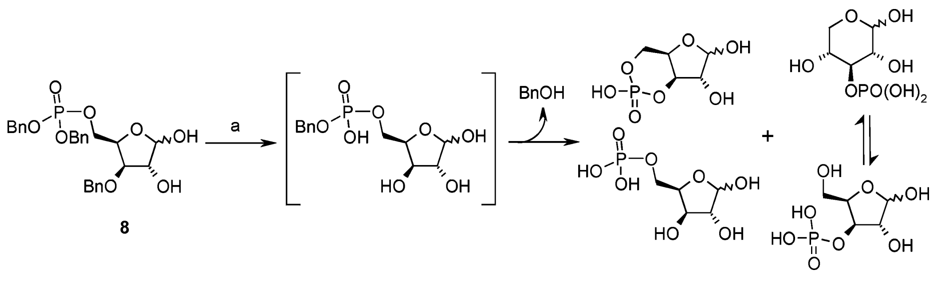


FIGURE 1 ADPR, acetylated ADPR and synthetic targets.

and has been implicated in chromatin silencing[6] and histone macroH2A binding.[7]

In all cases, more studies are needed to get a better understanding of the role of ADPR and of *O*-acetyl ADPR as potential second messengers as well as the mechanisms controlling their metabolisms. In order to investigate the biological targets of ADPR and acetylated ADPR as well as their metabolism, the chemical synthesis of ADPR-like molecules deemed the most appropriate approach, as illustrated by a recent report on the synthesis of non-hydrolysable analogues of *O*-acetyl ADPR.[8] We recently reported a study on acetate migration in partially protected furanoside systems and the products distribution observed under basic, neutral and acidic conditions depending on the stereochemistry at the C1, C2, and C3 carbon centers of the respective furanosides.[9] The modifications on ADPR reported here were structurally simple as they involved the reduction of the furanose ring to a ribitol moiety (**3**) and the inversions of configuration, either at the C2″ or at the C3″ position of the northern ribose, as shown by analogues (**4–7**) in Figure 1.

Adenosine diphosphate ribitol **3** was prepared by simple treatment at 0°C of ADPR in water with sodium borohydride. Attempts were made at synthesizing adenosine diphosphate xylose **6** from the partially protected 3-*O*-benzyl 5-*O*-(dibenzylphospho)-xylose **8** (Scheme 1). Unfortunately, the removal of the benzyl protecting groups under mild hydrogenolysis conditions resulted in phosphate ester migration between the C5 position and the C3 position via the formation of a cyclic phosphate triester (Scheme 1). Similarly when attempts were made at synthesizing a dibenzylphosphotriester from 3-*O*-benzoyl 1,2-*O*,*O*-isopropylidene ribose under basic conditions using tetrabenzylpyrophosphate, rapid migration of the benzoate

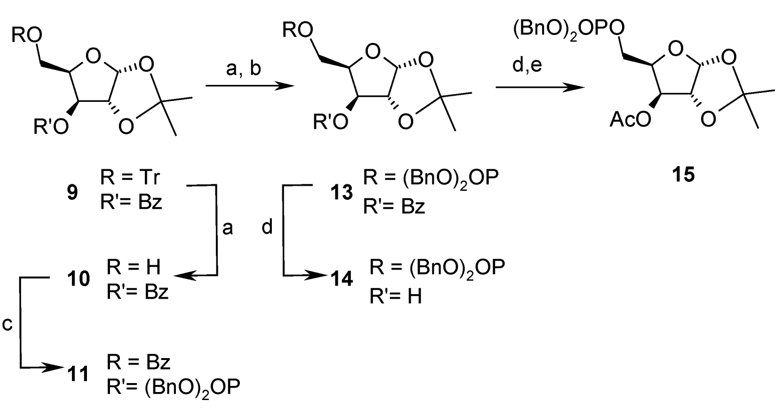


SCHEME 1 Phospho-ester migration occurring in the partially protected xylose intermediate. a) H_2; Pd/C, THF.

moiety and phosphorylation at the C-3 position took place. It therefore was concluded that adenosine diphosphate xylose **6** should be synthesized from the acetylated adenosine diphosphate xylose **4** by simple deacetylation as final synthetic step. A similarly divergent route was used for the preparation of ADP-arabinose **7** from **5**.

SYNTHESIS OF THE PHOSPHOSUGAR PRECURSORS TO 4–7

As had been often observed, the C-3 stereochemistry of a xylofuranose derivative facilitates acetyl migration from the C-3 hydroxyl to the C-5 primary hydroxyl either in acidic or basic conditions.[9] Consequently, in order to synthesize a 3-*O*-acetylated 5-phosphorylated xylose derivative, the acetyl moiety had to be introduced after the phosphorylation step. This requirement involved the temporary masking of the C-3 hydroxyl group prior to phosphorylation. The C-5 trityl furanose **9**, prepared from 1,2-*O*,*O*-isopropylidene 5-*O*-trityl xylose, was deprotected under acidic conditions, without benzoyl migration, to give the precursor **10** (Scheme 2). Under

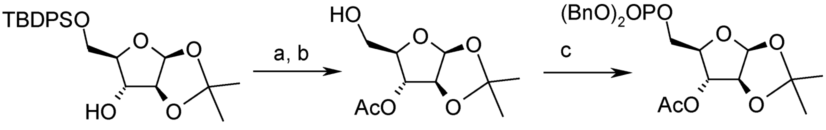


SCHEME 2 Synthesis of 3-*O*-acetyl-5-*O*-dibenzylphosphate-1,2-*O*,*O*-isopropylidene α-D-xylofuranoside. a) HCOOH, 1H-Dowex, CH_2Cl_2, 62%; b) i. $(BnO)_2PN(iPr)_2$ (**12**); 2,4-DNP, CH_2Cl_2, ii. *t*BuOOH, 60%; c) $(BnO)_4P_2O_3$, THF, *t*BuLi, 37%; d) CH_3OH, $NaOCH_3$, 96%; e) Ac_2O; Pyr, 100%.

basic phosphorylation conditions, a complex mixture of products was obtained where only one main compound was identified unambiguously as the phosphate triester derivative **11** (Scheme 2) and for which the benzoate ester migrated from the C-3 position to the C-5 primary alcohol. Phosphorylation under acidic conditions was then carried out on the carbohydrate derivative **10**. The dibenzylphosphoramidite reagent **12**, prepared in 56% yield from phosphorus trichloride, diisopropylamine and benzyl alcohol, was used with 2,4-DNP as activator.[10] The phosphite triester intermediate was then oxidized in situ with *tert*-butyl hydroperoxide to yield the dibenzylphosphate triester sugar **13**.

Protecting group inter-conversion at the C-3 hydroxyl was conducted under Zemplen conditions (catalytic sodium methoxide in methanol) to afford the crude phosphate triester **14**. Acetylation of the remaining alcohol using classic acetylation procedures afforded the phosphorylated xylose **15**, quantitatively.

The strategy developed for the synthesis of the 3-*O*-acetyl-5-*O*-dibenzylphosphate-1,2-*O*,*O*-isopropylidene-β-D-arabinofuranose **16** was similar to the one developed for the xylose intermediate **15**. However, with the arabinose series, it was possible to functionalize the C3 hydroxyl position with the required acetyl group before the phosphorylation step as the *anti* configuration of the C3 and C5 hydroxyls does not favor an intramolecular acyl migration.[9] 1,2-*O*-Isopropylidene-5-*O*-*tert*-butyldiphenylsilyl-β-D-arabinofuranose **17** (Scheme 3) was synthesized in 53% overall yield from D-arabinose.[11] Acetylation of the C3-hydroxyl was achieved using acetic anhydride in pyridine to give quantitatively the fully protected sugar **18**. TBAF-promoted cleavage of the silyl ether **18** afforded the desired arabinose **19** in 73% yield while the subsequent phosphorylation yielded the arabinofuranose **16** in 64% yield.

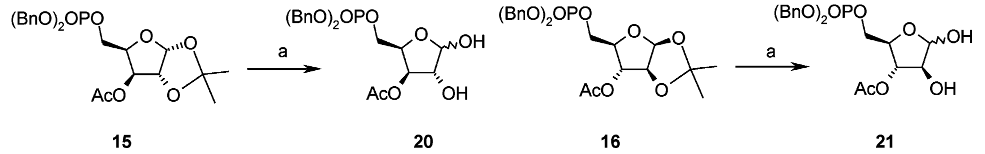


17 **19** **16**

SCHEME 3 Synthesis of 3-*O*-acetyl-5-*O*-dibenzylphosphate-1,2-*O*,*O*-isopropyledene β-D-arabinofuranoside. a) Ac_2O, pyr, quant; b) TBAF, THF, 73%; **12**, 2,4-DNP, CH_2Cl_2, 64%.

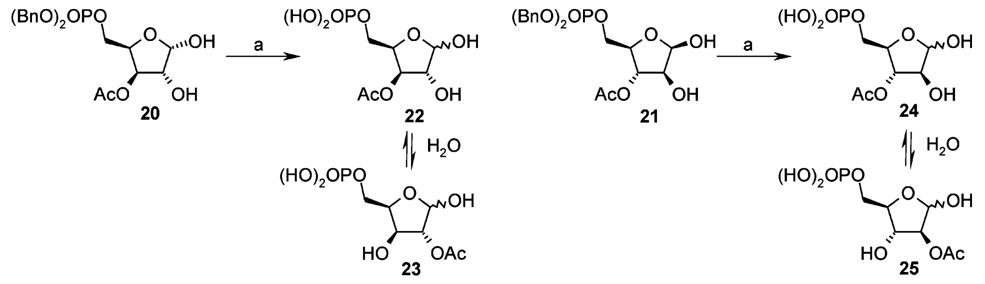
The acetonide deprotection of the two acetylated compounds **15** and **16** was carried out with aqueous TFA with a subsequent workup requiring ethyl acetate and an excess of pyridine. This sequence, necessary to prevent acetate migration and hydrolysis, provided the compounds **20** and **21** in 61% and 55% yield, respectively (Scheme 4).

Removal of the phosphate triester benzyl protecting groups was carried out on the xylofuranose derivative **20** using Pd/C as catalyst in THF under



SCHEME 4 Acetonide removal with no acetyl migration. a) TFA/H_2O EtOAc/Pyr; **20**, 61%; **21**, 55%.

balloon pressure of hydrogen at room temperature. The products of this hydrogenolysis were identified as the 3-*O*-acetyl derivative **22**(*α* and *β*) and the 2-*O*-acetyl **23** (*α* and *β*) (Scheme 5). Similar conditions were used on the arabinofuranose **21** to give a similar mixture of acetylated materials **24**(*α* and *β*) and **25**(*α* and *β*). This acetyl migration was thought to be generated by the acidity of the reaction condition due to the intrinsic acidity of the catalyst and the formation of the acidic phosphate monoester. Yet, the same mixture of acetylated compounds was obtained when $Pd(OH)_2$ was used as catalyst.



SCHEME 5 Hydrogenolysis and mixture of acetyl compounds. a) H_2, $Pd(OH)_2$; THF.

The reaction was then carefully monitored by TLC and stopped after total disappearance of the starting material, about 30 minutes. The ^{1}H-NMR and ^{13}C-NMR of the reaction mixtures were taken immediately after filtration of the catalyst and removal of the solvent. These analyses showed the unique presence of the expected phosphorylated and acetylated carbohydrate **22** as a mixture of *α*/*β* anomers. However, these compounds rearranged rapidly in aqueous solution to give the same distribution of acetylated products as observed previously indicating that the acetyl migration occurred after the removal of the benzyl protecting groups. Under the same conditions, arabinose **21** presented similar reactivity. In hindsight, the mixture of acetylated products can be explained by the fact that unlike the partially protected furanosides,[9] mutarotation equilibrium exists between the cyclic form and the open form of C1-deprotected-pentoses. Acetyl migrations occurred in arabinose and xylose when the hydroxyls at C2 and C3 were no longer sterically restricted in a trans-configuration imposed by the furanoside structure.

The monoacetylated furanosyl precursors **22/23** and **24/25** were used to access the acetylated ADP-xylose **4** and ADP-arabinose **5**. The condensation between the free acid phosphate monoesters **22/23** and **24/25** and adenosine monophosphomorpholidate (AMP-morpholidate) was carried out in anhydrous pyridine. Once the reaction reached equilibrium and pyridine evaporated, the unreacted monophosphates were removed using DSC-SAX pre-packed anion exchange column eluted first with water and then with a formic acid buffer (50 mM, pH 4). The fractions containing pyrophosphate products were identified by HPLC using an anion exchange SAX column (KH_2PO_4, 50 mM, 5% MeOH, pH 3.5) and were pooled together before being purified further by semi-preparative RP-C18-HPLC chromatography column eluted with an ammonium formate buffer (HCOOH, 50 mM, 5% MeOH, pH 6). The pure acetylated ADP-xylose **4** and acetylated ADP-arabinose **5** were isolated in 4% and 9% yields, respectively (Table 1).

Such moderate yields for the formation of the target compounds are not unusual for a pyrophosphate linkage[8] and can be explained by the poor solubility in anhydrous pyridine of the ester phosphate precursors leading to heterogeneous reaction conditions and formation of an important amount of side-products. However, prior modification of the phosphor-sugar counter ion to improve solubility proved difficult since exchange for a more lipophilic cation such as a triethylammonium or an octylammonium salt, under nucleophilic, protic conditions (methanol, triethylamine or octylamine) would have resulted in the rapid hydrolysis of the acetate moiety, already quite labile under neutral conditions.

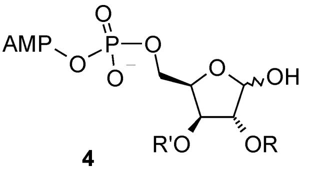
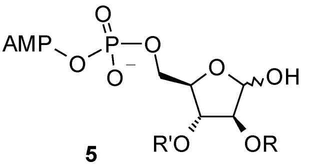
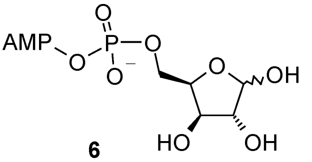
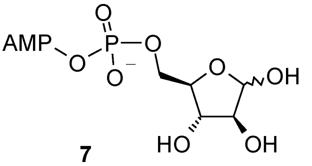
In conclusion, we have synthesized five analogues of ADPR, two of which are also analogues of acetylated ADPR. As for acetylated ADPR, the acetylated xylose and arabinose parents are present in solution as mixture of regioisomers due to the facile acyl migration of the acetyl group that can occur when the sugars are ring-opened.

EXPERIMENTAL SECTION

General Experimental Protocol

All reactions requiring anhydrous or inert conditions were carried out in oven dried glassware under a positive atmosphere of argon. ^1H, ^{13}C, and 2D (H-COSY, HMQC) NMR spectra were all recorded on Brüker avance DPX 300 and Brüker avance DPX 500. TMS (0 ppm, ^1H NMR), $CDCl_3$ (77 ppm, ^{13}C NMR), and HMPA (26.7 ppm, ^{31}P NMR) were used as internal references. The chemical shifts (δ) are reported in ppm (parts per millions). HPLC runs were performed on a Varian Workstation system with UV detector operating at 254 nm. UV quantification was carried out by using the absorbance of a solution of final compounds in water at their λ_{max} and found to be in all cases at 254 nm. The extinction coefficient used to

TABLE 1 Characterisation of compounds **5**-**7**

Compound	Retention time (minutes)	^{31}P NMR, ppm	Isolated yield (%)
 **4**	6.17	−10.1	4
 **5**	6.52	−9.9	9
 **6**	2.71	−10.0	2
 **7**	2.62	−10.0	7

HPLC conditions: RP-C18 analytical column Supelco, 4.6 × 150 mm, 5 mm, formic acid 50 mM, pH 6, 5%; MeOH, 1 ml/min, UV detector: 254 nm.

calculate the solution concentration was that of ADPR, that is, 14.3 10^3 M^{-1} cm^{-1}. By comparison to concentration obtained by phosphorus content assay, this extinction coefficient was consistent for compound **3** and was used for the subsequent evaluations. Reversed-phase chromatography was run on RP-C_{18} column (analytical column, 4.6 × 150 mm, 5 μm, Supelco; semi-preparative column, 10 × 250 mm, 10 μm, Phenomex luna) using ammonium formate buffer (50 mM, pH = 6.0, 5% MeOH) at a flow rate of 1 mL/min. Mass spectra were recorded on a VG Autospec spectrometer, Varian Workstation 1200 (ES). Optical rotations were measured on a Perkin-Elmer 341 polarimeter.

Synthetic Procedures

Adenosine-diphospho-ribitol 3

To a solution of commercially available ADPR (63 mg, 112.7 μmol) dissolved in mQ water (10 mL) was added $NaBH_4$ (5 mg, 132.1 mmol) at 0 C. The resulting solution was allowed to warm up at room temperature and kept under stirring for 14 hours. The reaction mixture was then neutralized with Dowex (H^+ form) and filtered. The resin was rinsed with mQ water (5 mL×3) and freeze-dried to afford **3** as a fluffy powder (79.6 μmol, 71% quantified by UV).

^1H NMR (D_2O) 8.49 (s, 1H, H_2); 8.21 (s, 1H, H_8); 6.13 (d, J = 6.0 Hz, 1H, $H_{1'}$); 4.68 (dd, J = 3.4 Hz, J = 6.3 Hz, 1H); 4.55–4.52 (m, 1H); 4.42–4.40 (m, 1H); 4.24–4.21 (m, 2H); 4.13–4.09 (m, 1H); 4.07–3.99 (m, 2H); 3.89–3.82 (m, 2H); 3.79–3.72 (m, 2H). ^{13}C NMR (D_2O) δ 156.0 (C_{Ade}); 153.2 (×2) (C_{Ade}); 149.4 (C_{Ade}); 140.7 (C_{Ade}); 119.0 ($C_{1'}$); 90.5, 85.9, 81.1, 72.5, 72.0, 71.4 (C_{ribo}); 67.4 (d, J = 4.8 Hz, $C_{5'}$ or $C_{5''}$); 66.6 (d, J = 5.3 Hz, $C_{5'}$ or $C_{5''}$); 62.8 (C_{CH2}). ^{31}P NMR (D_2O) δ -9.3(d, $J_{P\text{-}O\text{-}P}$ = 21.1 Hz); -9.9 (d, $J_{P\text{-}O\text{-}P}$ = 20.8 Hz).

HRMS *m/z* 562.0942 (calcd for $C_{15}H_{26}O_{14}N_5P_2([M+H]^+)$ 562.0952).

3-*O*-Benzyl-5-*O*-dibenzylphosphate-1,2-*O,O*-isopropylidene-α-D-xylofuranose. A solution of 3-*O*-benzyl-1,2-*O,O*-isopropylidene-α-D-xylofuranose[12] (180 mg, 0.64 mmol) in THF (20 mL) was treated at -78°C with *ter*-BuLi (415 μL of a 1.7 M solution in pentane, 0.71 mmol). This resulting mixture was stirred for 30 minutes and a solution of TBPP (380 mg, 0.71 mmol) in THF (10 mL) was added to the reaction still at −78°C. The reaction mixture was stirred for 1 hour at −78°C, before being allowed to warm up to room temperature. After 14 hours under stirring at room temperature, the reaction mixture was then diluted with Et_2O (20 mL) and NH_4Cl_{aq}. (30 mL) was then slowly added. The aqueous layer was extracted with Et_2O. The combined organic layers were then washed with $NH_4Cl_{(aq)}$, water and brine; dried, filtered, and concentrated under reduced pressure to afford the crude phosphorylated sugar. Purification by flash chromatography (gradient from 75/25 to 45/55 v/v petroleum ether/ethyl acetate) gave 3-*O*-benzyl-5-*O*-dibenzylphosphate-1,2-*O,O*-isopropylidene-α-D-xylofuranose (286 mg, 83%) as a colorless oil.

^1H NMR ($CDCl_3$) δ 7.36–7.28 (m, 15H, H_{Ar}); 5.93 (d, $J_{1,2}$ = 3.7 Hz, 1H, H_1); 5.05–5.03 (m, 4H, H_{CH2Bn}); 4.61 (AB, J_A = 11.8 Hz, 1H, H_{CH2Bn}); 4.59 (d, $J_{1,2}$ = 3.8 Hz, 1H, H_2); 4.45 (AB, J_A = 11.8 Hz, 1H, H_{CH2Bn}); 4.37 (ddd, $J_{3,4}$ = 3.3 Hz, $J_{4,5}$ = 6.0 Hz, $J_{4,5'}$ = 6.1 Hz, 1H, H_4); 4.27 (ABX, $J_{4,5}$ = 6.0 Hz, $J_{5,5'}$ = 10.3 Hz, 1H, H_5); 4.23 (ABX, $J_{4,5'}$ = 6.1 Hz, $J_{5,5'}$ = 10.3 Hz, 1H, $H_{5'}$); 3.92 (d, $J_{3,4}$ = 3.3 Hz, 1H, H_3); 1.46 (s, 3H, CH_{3Iso}); 1.31 (s, 3H, CH_{3Iso}). ^{13}C NMR ($CDCl_3$) δ 137.0, 135.7, 135.6, 128.5, 128.4, 128.3, 127.9, 127.8, 127.5 (C_{Ar}); 111.8 (C_{qIso}); 105.1 (C_1); 82.0 (C_2); 81.2 (C_3); 78.6 (d, $J_{P\text{-}O\text{-}C\text{-}C}$ = 8.5

Hz, C_4); 71.8 (C_{CH2Bn}); 69.3 (d, $J_{P\text{-}O\text{-}C} = 5.1$ Hz, C_{CH2Bn}); 69.2 (d, $J_{P\text{-}O\text{-}C} = 5.3$ Hz, C_{CH2Bn}); 65.0 (d, $J_{P\text{-}O\text{-}C} = 5.3$ Hz, C_5); 26.7 (C_{MeIso}); 26.2 (C_{MeIso}). ^{31}P NMR ($CDCl_3$) δ 0.2. HRMS *m/z* 541.5670 (calcd for $C_{29}H_{34}O_8P$ ($[M+H]^+$) 541.5633).

3-*O*-Benzyl-5-*O*-dibenzylphosphate-D-xylofuranose 8. 3-*O*-Benzyl-5-*O*-dibenzylphosphate-1,2-*O,O*-isopropylidene-α-D-xylofuranose (189 mg, 0.35 mmol) was treated with a mixture of TFA/H_2O (8 mL/2 mL) at 0°C. The resulting mixture was stirred for 90 minutes and was then diluted with EtOAc (25 mL). The resulting solution was carefully poured in a saturated solution of Na_2CO_3 (30 mL) at 0°C. The aqueous layer was extracted with EtOAc (20 mL × 4) and the combined organic fractions were dried, filtered, and concentrated under reduced pressure to yield the crude xylofuranose derivative. Purification by flash chromatography (gradient from 1/0 to 97/ v/v $CHCl_3$/ethanol) afforded the pure 3-*O*-benzyl-5-*O*-dibenzylphosphate-D-xylofuranose **8** (122 mg, 70%) as a mixture of anomers (α/β:7/3).

^{1}H NMR ($CDCl_3$) 7.31–7.27 (m, 15H, H_{Ar}); 5.46 (t, $J = 4.8$ Hz, 0.7H, $H_{1\alpha}$); 5.18 (d, $J = 9.6$ Hz, 0.3H, $H_{1\beta}$); 5.04–4.99 (m, 4H, H_{CH2Bn}); 4.66 (AB, $J_A = 12.0$ Hz, 0.7H, $H_{\alpha CH2Bn}$); 4.62 (AB, $J_A = 11.6$ Hz, 0.3H, $H_{\beta CH2Bn}$); 4.50 (AB, $J_A = 11.6$ Hz, 0.3H, $H_{\beta CH2Bn}$); 4.48 (AB, $J_A = 12.0$ Hz, 0.7H, $H_{\alpha CH2Bn}$); 4.44–4.40 (m, 1H, H_4); 4.35–4.29 (m, 0.3H, $H_{5\beta}$); 4.26–4.25 (m, 0.2H, $H_{2\beta}$); 4.24–4.20 (m, 0.3H, $H_{5'\beta}$); 4.18–4.15 (m, 2.1H, $H_{2\alpha}$, $H_{3\alpha}$, $H_{5\alpha}$,); 4.00–3.99 (m, 1 H, $H_{5'\alpha}$, $H_{3\beta}$). ^{13}C NMR ($CDCl_3$) δ 137.6, 137.0, 135.5, 135.6, 128.5, 128.4, 128.0, 127.9, 127.8, 127.7, 127.5 (C_{Ar}), 103.4 ($C_{1\beta}$); 96.2 ($C_{1\alpha}$); 83.1 ($C_{3\alpha}$); 82.5 ($C_{2\beta}$); 79.2 (d, $J_{P\text{-}O\text{-}C\text{-}C} = 7.1$ Hz, $C_{4\beta}$); 78.1 ($C_{3\beta}$); 76.6 (d, $J_{P\text{-}O\text{-}C\text{-}C} = 7.7$ Hz, $C_{4\alpha}$); 75.2 ($C_{2\alpha}$); 72.5 ($C_{CH2\beta Bn}$); 71.8 ($C_{CH2\alpha Bn}$); 69.6 ($C_{CH2\beta Bn}$); 69.5 ($C_{CH2\beta Bn}$); 69.5 ($C_{CH2\alpha Bn}$); 69.4 ($C_{CH2\alpha Bn}$); 67.4 (d, $J_{P\text{-}O\text{-}C} = 5.6$ Hz, $C_{5\beta}$); 66.8 (d, $J_{P\text{-}O\text{-}C} = 5.6$ Hz, $C_{5\alpha}$). ^{31}P NMR ($CDCl_3$) δ 0.2 (β), 0.0 (α).HRMS *m/z* 501.1701 (calcd for $C_{26}H_{30}O_8P$ ($[M+H]^+$) 501.1678).

3-*O*-Benzoyl-1,2-*O,O*-isopropylidene-5-*O*-trityl-α-D-xylofuranose 9.[13] To a solution of 1,2-*O,O*-isopropylidene-5-*O*-trityl-α-D-xylofuranose[12] (17.9 g, 41.4 mmol) in dry DCM/dry pyridine (50 mL/30 mL) was added dropwise at 0°C a solution of benzoyl chloride (5.8 mL, 49.9 mmol) in dry DCM (40 mL). The resulting solution was stirred overnight at room temperature. The reaction mixture was then concentrated under reduced pressure to afford a yellowish oil. This residue was partitioned between water (100 mL) and $CHCl_3$ (100 mL). The organic layer was then washed with $NaHCO_3$ (100 mL), water, brine, dried filtered, and concentrated under vacuum to afford the crude fully protected furanoside as a yellow oil. Purification by flash chromatography (gradient 1/0 to 8/2 v/v hexane/ethyl acetate) afforded 3-*O*-benzoyl-1,2-*O,O*-isopropylidene-5-*O*-trityl-α-D-xylofuranose **9** (21.3 g, 95%).

^{1}H NMR ($CDCl_3$) δ 7.83–7.81 (m, 2H, H_{Bz}); 7.57–7.54 (m, 1H, H_{Bz}); 7.40–7.37 (m, 8H, H_{Ar}); 7.19–7.14 (m, 9H, H_{Ar}); 5.95 (d, $J_{1,2} = 3.7$ Hz, 1H,

H_1); 5.66 (d, $J_{3,4}$ = 3.0 Hz, 1H, H_3); 4.68–4.66 (m, 1H, 1H, H_4); 4.64 (d, $J_{1,2}$ = 3.7 Hz, 1H, H_2); 3.58 (ABX, $J_{4,5}$ = 7.9 Hz, $J_{5,5'}$ = 9.1 Hz, 1H, H_5); 3.41 (ABX, $J_{4,5'}$ = 5.6 Hz, $J_{5,5'}$ = 9.1 Hz, 1H, $H_{5'}$); 1.61 (s, 3H, CH_{3Iso}); 1.34 (s, 3H, CH_{3Iso}). ^{13}C NMR ($CDCl_3$) δ 164.9 (C_{OBz}); 143.3 (C_{Tr}); 133.0, 129.6 (C_{Bz}); 128.4 (C_{Tr}); 128.2 (C_{Bz}); 127.6, 126.8 (C_{Tr}); 111.9 (C_{qIso}); 104.7 (C_1); 86.7 (C_{qTr}); 83.2 (C_2); 77.9 (C_4); 76.3 (C_3); 60.2 (C_5); 26.5 (C_{MeIso}); 26.0 (C_{MeIso}). HRMS *m/z* 559.2114 (calcd for $C_{34}H_{32}O_6Na([M+Na]^+)$ 559.2097). $[\alpha]_D{}^{20}$ = -42.9 (c = 0.3, MeOH)

3-*O*-Benzoyl-1,2-*O,O*-isopropylidene-α-D-xylofuranose 10.[13] 3-*O*-Benzoyl-1,2-*O,O*-isopropylidene-5-*O*-trityl-α-D-xylofuranose **9** (17.2 g, 32.1 mmol) was dissolved in DCM (100 mL). To this resulting solution was added Dowex (H^+ form) and formic acid (5 mL) and the suspension was then kept under stirring overnight at room temperature. The solvent was removed under vacuum to afforded the crude 3-*O*-benzoyl-1,2-*O*-isopropylidene-α-D-xylofuranoside as a yellow oil. Purification by flash chromatography (gradient 9/1 to 1/1 v/v hexane/ethyl acetate) afforded 2 fractions, the 3-*O*-benzoyl-1,2-*O,O*-isopropylidene-α-D-xylofuranose **10** (5.9 g, 62%) and the starting material **9** (3.9 g, 23%).

^1H NMR ($CDCl_3$) δ 8.03–7.99 (m, 2H, H_{Bz}); 7.62–7.57 (m, 1H, H_{Bz}); 7.48–7.43 (m, 2H, H_{Bz}); 6.02 (d, $J_{1,2}$ = 3.7 Hz, 1H, H_1); 5.45 (d, $J_{3,4}$ = 2.8 Hz, 1H, H_3); 4.72 (d, $J_{1,2}$ = 3.7 Hz, 1H, H_2); 4.51 (ddd, 1H, $J_{3,4}$ = 2.8 Hz, $J_{4,5}$ = $J_{4,5'}$ = 6.2 Hz, 1H, H_4); 3.89 (ABX, $J_{4,5}$ = 6.3 Hz, $J_{5,5'}$ = 11.9 Hz, 1H, H_5); 3.71 (ABX, $J_{4,5'}$ = 6.1 Hz, $J_{5,5'}$ = 11.9 Hz, 1H, $H_{5'}$); 1.55 (s, 3H, CH_{3Iso}); 1.34 (s, 3H, CH_{3Iso}). ^{13}C NMR ($CDCl_3$) δ 166.2 (C_{OBz}); 133.8, 129.9, 128.7 (C_{Bz}); 112.5 (C_{qIso}); 104.8 (C_1); 83.7 (C_2); 79.9 (C_4); 77.4 (C_3); 59.9 (C_5); 26.7 (C_{MeIso}); 26.4 (C_{MeIso}). $[\alpha]_D{}^{20}$ = -10.9 (c = 0.4, MeOH). HRMS *m/z* 295.3113 (calcd for $C_{15}H_{19}O_6$ ($[M+H]^+$) 295.3151).

5-*O*-Benzoyl-3-*O*-dibenzylphosphate-1,2-*O,O*-isopropylidene-α-D-xylofuranose 11. A solution of 3-*O*-benzoyl-1,2-*O*-isopropylidene-α-D-xylofuranose **10** (137 mg, 0.46 mmol) in THF (20 mL) was treated at -78°C with *t*-BuLi (328 μL of a 1.7 M solution in pentane, 0.56 mmol). This resulting mixture was stirred for 30 minute and a solution of TBPP (301 mg, 0.56 mmol) in THF (10 mL) was added to the reaction still at -78°C. The reaction mixture was stirred for 1 hour at -78°C, before being warmed up to room temperature. After 8 hours under stirring at room temperature, the reaction mixture was diluted with Et_2O (20 mL) and NH_4Cl_{aq}. (30 mL) was then slowly added. The aqueous layer was extracted with Et_2O. The combined organic layers were then washed with NH_4Cl_{aq}, water and brine; dried, filtered, and concentrated under reduced pressure to afford the crude phosphorylated sugar. Purification by flash chromatography (gradient from 75/25 to 45/55 v/v petroleum ether/ethyl acetate) gave 5-*O*-benzoyl-3-*O*-dibenzylphosphate-1,2-*O,O*-isopropylidene-α-D-xylofuranose **11** (94 mg, 37%) as a colorless oil.

^{1}H NMR (CDCl$_3$) δ 8.06–8.03 (m, 2H, H$_{Bz}$); 7.58–7.53 (m, 1H, H$_{Bz}$); 7.43–7.38 (m, 2H, H$_{Bz}$); 7.35–7.28 (m, 10H, H$_{Ar}$); 5.83 (d, $J_{1,2}$ = 3.7 Hz, 1H, H$_1$); 5.04–4.99 (m, 4H, H$_{CH2Bn}$); 4.87 (dd, $J_{3,4}$ = 2.3 Hz, $J_{3,4}$ = 7.8 Hz, 1H, H$_3$); 4.57 (d, $J_{1,2}$ = 3.7 Hz, 1H, H$_2$); 4.54–4.47 (m, 3H, H$_4$, H$_{5'}$, H$_5$); 1.48 (s, 3H, CH$_{3Iso}$); 1.26 (s, 3H, CH$_{3Iso}$). ^{13}C NMR (CDCl$_3$) δ 166.0 (C$_{OBz}$); 135.4 (d, $J_{P\text{-}O\text{-}C\text{-}C}$ = 6.5 Hz, C$_{Ar}$); 135.3 (d, $J_{P\text{-}O\text{-}C\text{-}C}$ = 6.6 Hz, C$_{Ar}$); 133.1, 129.8, 128.7, 128.6, 128.4, 128.0 (C$_{Ar}$); 112.3 (C$_{qIso}$); 104.8 (C$_1$); 83.2 (d, $J_{P\text{-}O\text{-}C}$ = 1.6 Hz, C$_2$); 79.8 (d, $J_{P\text{-}O\text{-}C}$ = 5.5 Hz, C$_3$); 77.2 (d, $J_{P\text{-}O\text{-}C\text{-}C}$ = 7.3 Hz, C$_4$); 69.9 (d, $J_{P\text{-}O\text{-}C}$ = 5.6 Hz, C$_{CH2Bn}$); 69.8 (d, $J_{P\text{-}O\text{-}C}$ = 5.9 Hz, C$_{CH2Bn}$); 61.6 (C$_5$); 26.6 (C$_{MeIso}$); 26.2 (C$_{MeIso}$). ^{31}P NMR (CDCl$_3$) δ -0.2.

3-*O*-Benzoyl-5-*O*-dibenzylphosphate-1,2-*O,O*-isopropylidene-α-D-xylofuranose 13. To a solution of 3-*O*-benzoyl-1,2-*O*-isopropylidene-α-D-xylofuranose **10** (2.9 g, 9.8 mmol) and dibenzylphosphoramidate (3.7 g, 10.8 mmol) in dry DCM (30 mL) was added dropwise a solution of 2,4-DNP (2.0 g, 10.8 mmol) in dry DCM (20 mL). This resulting mixture was stirred overnight at room temperature, then a solution of *ter*-BuOOH in toluene (6 ml of a 3 M solution) was added. The resulting solution was stirred for 20 minutes. After dilution with DCM (30 mL), the reaction mixture was washed with a sodium thiosulfate solution (50 mL), water (30 mL x 3), dried, filtered, and concentrated under vacuum to afford the crude fully protected phosphorylated precursor. Purification by flash chromatography (gradient 85/15 to 6/4 v/v hexane/ethyl acetate) yielded 3-*O*-benzoyl-5-*O*-dibenzylphosphate-1,2-*O,O*-isopropylidene-α-D-xylofuranose **13** (3.4 g, 63%) as a yellow oil.

^{1}H NMR (CDCl$_3$) δ 7.99–7.97 (m, 2H, H$_{Bz}$); 7.60–7.56 (m, 1H, H$_{Bz}$); 7.47–7.41 (m, 2H, H$_{Bz}$); 7.32–7.27 (m, 10H, H$_{Ar}$); 5.94 (d, $J_{1,2}$ = 3.7 Hz, 1H, H$_1$); 5.40 (d, $J_{3,4}$ = 3.1 Hz, 1H, H$_3$); 4.59 (d, $J_{1,2}$ = 3.7 Hz, 1H, H$_2$); 4.53 (ddd, 1H, $J_{3,4}$ = 3.1 Hz, $J_{4,5}$ = 6.1 Hz, $J_{4,5'}$ = 6.3 Hz, 1H, H$_4$); 4.24 (ABX, $J_{4,5}$ = 6.3 Hz, $J_{5,5'}$ = 10.8 Hz, 1H, H$_5$); 4.19 (ABX, $J_{4,5'}$ = 6.1 Hz, $J_{5,5'}$ = 10.8 Hz, 1H, H$_{5'}$); 1.49 (s, 3H, CH$_{3Iso}$); 1.28 (s, 3H, CH$_{3Iso}$). ^{13}C NMR (CDCl$_3$) δ 165.0 (C$_{OBz}$); 135.7 (d, $J_{P\text{-}O\text{-}C\text{-}C}$ = 6.1 Hz, C$_{Ar}$); 135.6 (d, $J_{P\text{-}O\text{-}C\text{-}C}$ = 6.2 Hz, C$_{Ar}$); 133.5, 129.7, 129.0, 128.6, 128.5,128.4, 127.9 (C$_{Ar}$); 112.4 (C$_{qIso}$); 105.0 (C$_1$); 83.3 (C$_2$); 77.6 (d, $J_{P\text{-}O\text{-}C\text{-}C}$ = 8.5 Hz, C$_4$); 76.4 (C$_3$); 69.4 (d, $J_{P\text{-}O\text{-}C}$ = 6.0 Hz, C$_{CH2Bn}$); 69.3 (d, $J_{P\text{-}O\text{-}C}$ = 5.8 Hz, C$_{CH2Bn}$); 64.5 (d, $J_{P\text{-}O\text{-}C}$ = 5.1 Hz, C$_5$); 26.7 (C$_{MeIso}$); 26.2 (C$_{MeIso}$). ^{31}P NMR (CDCl$_3$) δ -0.4. $[\alpha]_D^{20}$ = -7.6 (c = 1.1, MeOH). HRMS *m/z* 555.1788 (calcd for $C_{29}H_{32}O_9P([M+H]^+)$ 555.1784).

5-*O*-Dibenzylphosphate-1,2-*O,O*-isopropylidene-α-D-xylofuranose 14.[14] 3-*O*-Benzoyl-5-*O*-dibenzylphosphate-1,2-*O,O*-isopropylidene-α-D-xylofuranose **13** (3.2 g, 5.8 mmol) was dissolved in MeOH (50 ml). To the resulting solution was added MeONa (130 μL of a 25% wt solution, 0.62 mmol) and was left under stirring for 4 hours. The reaction mixture was then neutralized with Dowex (H$^+$ form), filtered. The resin was rinsed with MeOH (50 mL×3). The combined filtrates were then concentrated under reduced pressure to afford the crude deprotected carbohydrate derivative. Purification by flash chromatography (gradient from 1/0 to 97/3 v/v $CHCl_3$/ethanol) gave 5-*O*-dibenzylphosphate-1,2-*O*,*O*-isopropylidene-α-D-xylofuranose **14** (2.5 g, 96%) as a colorless oil.

^{1}H NMR ($CDCl_3$) 7.37–7.32 (m, 10H, H_{Ar}); 5.89 (d, $J_{1,2}$ = 3.5 Hz, 1H, H_1); 5.08–4.98 (m, 4H, H_{CH2Bn}); 4.56 (d, $J_{1,2}$ = 3.5 Hz, 1H, H_2); 4.29–4.23 (m, 1H, H_3, H_4, H_5);4.03 (ABX, $J_{4,5'}$ = 6.3 Hz, $J_{5,5'}$ = 12.4 Hz, 1H, $H_{5'}$); 1.49 (s, 3H, CH_{3Iso}); 1.31 (s, 3H, CH_{3Iso}). ^{13}C NMR ($CDCl_3$) δ 135.3 (d, $J_{P\text{-}O\text{-}C\text{-}C}$ = 6.3 Hz, C_{Ar}), 135.2 (d, $J_{P\text{-}O\text{-}C\text{-}C}$ = 6.8 Hz, C_{Ar}), 128.8, 128.8, 128.7, 128.6, 128.1, 128.0 (C_{Ar}); 111.8 (C_{qIso}); 105.0 (C_1); 85.0 (C_2); 78.8 (d, $J_{P\text{-}O\text{-}C\text{-}C}$ = 4.8 Hz,C_4); 73.6 (C_3); 70.0 (d, $J_{P\text{-}O\text{-}C}$ = 5.9 Hz, C_{CH2Bn}); 68.9(d, $J_{P\text{-}O\text{-}C}$ = 5.7 Hz, C_{CH2Bn}); 63.0 (d, $J_{P\text{-}O\text{-}C}$ = 4.9 Hz, C_5); 26.9 (C_{MeIso}); 26.2 (C_{MeIso}). ^{31}P NMR ($CDCl_3$) δ -0.6. HRMS *m/z* 451.1511 (calcd for $C_{22}H_{28}O_8P([M+H]^+)$ 451.1522).

3-*O*-Acetyl-5-*O*-dibenzylphosphate-1,2-*O*,*O*-isopropylidene-α-D-xylofuranose 15. To a solution of 5-*O*-dibenzylphosphate-1,2-*O*,*O*-isopropylidene-α-D-xylofuranose (2.3 g, 5.1 mmol) in pyridine (35 ml) was added acetic anhydride (0.6 ml, 5.6 mmol). The resulting solution was stirred for 3 hours at room temperature. Then the reaction mixture was concentrated under vacuum and azeotrope 3 times with toluene to afford 3-*O*-acetyl-5-*O*-dibenzylphosphate-1,2-*O*-isopropylidene-α-D-xylofuranose **15** (2.5 g, 100%) as a colorless oil.

^{1}H NMR ($CDCl_3$) 7.34–7.32 (m, 10H, H_{Ar}); 5.90 (d, $J_{1,2}$ = 3.7 Hz, 1H, H_1); 5.19 (d, $J_{3,4}$ = 3.1 Hz, 1H, H_3); 5.05–5.00 (m, 4H, H_{CH2Bn}); 4.49 (d, $J_{1,2}$ = 3.7 Hz, 1H, H_2); 4.44 (ddd, $J_{3,4}$ = 3.1 Hz, $J_{4,5}$ = $J_{4,5'}$ = 6.1 Hz, 1H, H_4); 4.16–4.13 (m, 2H, H_5, $H_{5'}$); 2.02 (s, 3H, CH_{3Ac}); 1.49 (s, 3H, CH_{3Iso}); 1.30 (s, 3H, CH_{3Iso}). ^{13}C NMR ($CDCl_3$) δ 169.6 (C_{OAc}); 135.8 (d, $J_{P\text{-}O\text{-}C\text{-}C}$ = 3.8 Hz, C_{Ar}), 135.7 (d, $J_{P\text{-}O\text{-}C\text{-}C}$ = 3.9 Hz, C_{Ar}), 128.5, 128.0 (C_{Ar}); 112.4 (C_{qIso}); 104.9 (C_1); 83.3 (C_2); 77.6 (d, $J_{P\text{-}O\text{-}C\text{-}C}$ = 4.8 Hz,C_4); 75.8 (C_3); 69.5(d, $J_{P\text{-}O\text{-}C}$ = 5.3 Hz, C_{CH2Bn}); 69.4(d, $J_{P\text{-}O\text{-}C}$ = 5.2 Hz, C_{CH2Bn}); 64.2 (d, $J_{P\text{-}O\text{-}C}$ = 5.0 Hz, C_5); 26.7 (C_{MeIso}); 26.2 (C_{MeIso}); 20.6 (C_{MeAc}). ^{31}P NMR ($CDCl_3$) δ -0.4. $[\alpha]_D^{20}$ = -8.5 (c = 0.8, MeOH). HRMS *m/z* 493.1662 (calcd for $C_{24}H_{30}O_9P([M+H]^+)$ 493.1627).

3-*O*-Acetyl-5-*O*-dibenzylphosphate-D-xylofuranose 20. 3-*O*-Acetyl-5-*O*-dibenzylphosphate-1,2-*O*-isopropylidene-α-D-xylofuranose (385 mg, 0.58 mmol) was treated with a mixture TFA/H_2O (8 ml/2 ml) at 0°C. The resulting solution was stirred for 25 minutes and was then diluted with EtOAc. Pyridine (15 mL) was then slowly added at 0°C. The resulting mixture was concentrated under vacuum to give a white residue which was dissolved with EtOAc(25 mL). This solution was washed with water (20 mL x 3) dried, filtered, and concentrated under vacuum to afford the deprotected furanose as an oil. Purification by flash chromatography (gradient from 1/0 to 97/3 v/v $CHCl_3$/ethanol) gave 3-*O*-acetyl-5-*O*-dibenzylphosphate-D-xylofuranose **20** (159 mg, 61%) as a α/β mixture (4/1).

^1H NMR ($CDCl_3$) 7.35–7.30 (m, 10H, H_{Ar}); 5.43 (t, J = 4.2 Hz, 0.8H, $H_{1\alpha}$); 5.29 (s, 0.2H, $H_{1\beta}$); 5.17 (dd, $J_{3,4}$ = 3.5 Hz, $J_{2,3}$ = 5.1 Hz, 0.8H, $H_{3\alpha}$); 5.12 (dd, $J_{3,4}$ = 2.5 Hz, $J_{2,3}$ = 6.1 Hz, 0.2H, $H_{3\beta}$); 5.07–4.98 (m, 4H, H_{CH2Bn}); 4.54–4.49 (m, 1H, H_4); 4.29–4.24 (m, 0.2H, $H_{5\beta}$); 4.15–4.16 (m, 0.2H, $H_{2\beta}$); 4.13–4.11 (m, 1H, $H_{2\alpha}$, $H_{5'\beta}$); 4.06–4.00 (m, 1.6H, $H_{5\alpha}$, $H_{5'\alpha}$); 2.03 (s, 0.6H, $CH_{3Ac\beta}$); 2.01 (s, 2.4H, $CH_{3Ac\alpha}$). ^{13}C NMR ($CDCl_3$) δ 170.6 (C_{OAc}); 135.7, 135.6, 135.5 (C_{Ar}), 128.5, 128.0 (C_{Ar}); 102.9 ($C_{1\beta}$); 95.9 ($C_{1\alpha}$); 80.3 ($C_{2\beta}$); 78.3 ($C_{3\alpha}$); 78.2 ($C_{3\beta}$); 77.7 (d, $J_{P\text{-}O\text{-}C\text{-}C}$ = 6.3 Hz, $C_{4\beta}$); 75.3 ($C_{2\alpha}$); 75.2 (d, $J_{P\text{-}O\text{-}C\text{-}C}$ = 6.3 Hz, $C_{4\alpha}$); 69.8(d, $J_{P\text{-}O\text{-}C}$ = 5.6 Hz, $C_{CH2Bn\beta}$); 69.6(d, $J_{P\text{-}O\text{-}C}$ = 5.3 Hz, $C_{CH2Bn\alpha}$); 69.5(d, $J_{P\text{-}O\text{-}C}$ = 5.1 Hz, $C_{CH2Bn\alpha}$); 67.0 (d, $J_{P\text{-}O\text{-}C}$ = 5.4 Hz, $C_{5\beta}$); 65.5 (d, $J_{P\text{-}O\text{-}C}$ = 5.5 Hz, $C_{5\alpha}$); 20.6 (C_{MeAc}). ^{31}P NMR ($CDCl_3$) δ 0.3 (β), -0.1 (α). HRMS *m/z* 453.1328 (calcd for $C_{21}H_{26}O_9P([M+H]^+)$ 453.1314).

3-*O*-Acetyl-5-*O*-phosphate-D-xylofuranose 22. 3-*O*-Acetyl-acetyl-5-*O*-dibenzylphosphate-D-ribofuranose (42 mg, 0.81 mmol) was diluted in THF (10 ml) at room temperature and added under argon to commercial $Pd(OH)_2$/C. The mixture was stirred at room temperature under H_2 pressure (balloon pressure) for 30 minutes. The catalyst was then filtered off (AUTOTOP syringe filters, Whatman) and the solvent removed under reduced pressure to yield quantitatively 3-*O*-acetyl-5-*O*-phosphate-D-ribofuranose as a mixture of α/β anomers (1/1) which rearranged in solution to yield a mixture of 3-*O*-acetyl-5-*O*-phosphate-D-ribofuranose **22** and 2-*O*-acetyl-5-*O*-phosphate-D-ribofuranose **23**.

^1H NMR (D_2O) 5.32 (d, J = 4.4 Hz, 0.5H, $H_{1\alpha}$); 5.17 (s, 0.5H, $H_{1\beta}$); 5.15 (dd, $J_{3,4}$ = 3.6 Hz, $J_{2,3}$ = 5.6 Hz, 0.5H, $H_{3\alpha}$); 5.08 (dd, $J_{3,4}$ = 1.9 Hz, $J_{2,3}$ = 5.2 Hz, 0.5H, $H_{3\beta}$); 4.48–4.42 (m, 1H, H_4); 4.03–4.01 (m, 0.5H, $H_{2\alpha}$); 4.00–3.95 (m, 1H, $H_{2\beta}$, $H_{5\beta}$); 3.91 (ABX, $J_{4,5}$ = 4.2 Hz, $J_{5,5'}$ = 11.2 Hz, 0.5H, $H_{5\alpha}$); 3.88 (ABX, $J_{4,5''}$ = 6.0 Hz, $J_{5,5'}$ = 10.5 Hz, 0.5H, $H_{5'\beta}$); 3.81 (ABX, $J_{4,5'}$ = 5.6 Hz, $J_{5,5'}$ = 11.3 Hz, 0.5H, $H_{5'\alpha}$); 2.03 (s, 1.5H, $CH_{3Ac\beta}$); 2.02 (s, 1.5H, $CH_{3Ac\alpha}$). ^{13}C NMR (D_2O) δ 173.9, 173.5 (C_{OAc}); 102.2 ($C_{1\beta}$); 95.7 ($C_{1\alpha}$); 79.1 ($C_{2\beta}$); 78.8 (d, $J_{P\text{-}O\text{-}C\text{-}C}$ = 8.9 Hz, $C_{4\beta}$); 77.6 (C_{3A}); 77.2 ($C_{3\beta}$); 75.3 (d, $J_{P\text{-}O\text{-}C\text{-}C}$ = 8.7 Hz, $C_{4\alpha}$); 74.2 ($C_{2\alpha}$); 65.0 (d, $J_{P\text{-}O\text{-}C}$ = 4.9 Hz, $C_{5\beta}$); 64.4 (d, $J_{P\text{-}O\text{-}C}$ = 4.9 Hz, $C_{5\alpha}$); 20.5 (C_{MeAc}). ^{31}P NMR ($CDCl_3$) δ 1.6 (br). HRMS *m/z* 271.0215 (calcd for $C_7H_{12}O_9P([M\text{-}H])$ 271.0219).

***O*-Acetyl-adenosine-diphospho-xylose 4.** The product of the hydrogenolysis of 3-*O*-acetyl-acetyl-5-*O*-dibenzylphosphate-D-ribofuranose (37 mg, 136.0 μmol) was made anhydrous by repeated dissolution in dry pyridine and evaporation of the solvent. After each evaporation step, argon was flushed into the rotary evaporator. AMP-morpholidate (4'-morpholine-*N*-*N*'-dicyclohexylcarboxamidinium salt; 106 mg, 149.6 μmol) was dissolved in dry pyridine, evaporated to dryness and the process was repeated three times with exclusion of moisture. Both components were finally dissolved in dry pyridine and combined. The reaction mixture was repeatedly sonicated and flushed with argon. A final amount of pyridine was added and the reaction mixture (5 mL total volume) was stirred for 2 days. The solvent was then removed under vacuum and the yellowish residue was dissolved in mQ water (0.5 mL) to be purified using pre-packed DSC-SAX column (6 mL/1 g) with water (10 mL) and then buffer (formic acid 5 mM, pH = 4.0, 10 mL). The fractions containing pyrophosphate products, identified by HPLC using anion exchange chromatography (SAX column, 200 × 4.5 mm, 5 μm; phosphate buffer, KH_2PO_4 50 mM, pH = 3.5, 5% MeOH) were freeze-dried to afford a white residue which was purified further using reversed-phase chromatography. The residue was dissolved in mQ water (0.5 mL) and purified by HPLC using a semi-preparative column with ammonium formate buffer (formic acid 50 mM, pH = 6.0, 5% MeOH) eluated at a flow rate of 2.5 mL/minute to yield adenosine-diphospho-xylose **6** (2.7 μmol quantified by UV, 2%), *O*-acetyl-adenosine-diphospho-xylose **4** (9.5 μmol quantified by UV, 7%).

^{1}H NMR (D_2O) 8.40 (s, 1H, H_2); 8.13 (s, 1H, H_8); 6.02 (d, J = 5.3 Hz, 1H, $H_{1'}$); 5.62 (m, 1H, $H_{1''}$); 4.41–4.39 (m, 2H); 4.27–4.26 (m, 2H); 4.17–4.00 (m, 6H); 1.92, 1.91, 1.90 (s, 3H, CH_{3Ac}). ^{31}P NMR (D_2O) δ -10.1 (br, P-O-P). HRMS *m/z* 600.0779 (calcd for $C_{17}H_{24}O_{14}N_5P_2$([M-H]) 600.0744).

Adenosine-Diphospho-Xylose 6

^{1}H NMR (D_2O) 8.41 (s, 1H, H_2); 8.17 (s, 1H, H_8); 6.05 (d, J = 6.0 Hz, 1H, $H_{1'}$); 5.10–5.06(m, 1H, $H_{1''}$); 4.58–4.55 (m, 2H); 4.47–4.42 (m, 2H); 4.31–4.29 (m, 2H); 4.14–4.01 (m, 4H). ^{31}P NMR (D_2O) δ -10.0 (br, P-O-P). HRMS *m/z* 558.0635 (calcd for $C_{15}H_{22}O_{14}N_5P_2$([M-H]) 558.0639).

3-*O*-Acetyl-5-*O*-*tert*-butyldiphenylsilyl-1,2-*O*,*O*-isopropylidene-β-D-arabinofuranose 18. A solution of 5-*O*-*tert*-butyldiphenylsilyl-1,2-*O*,*O*-isopropylidene-β-D-arabinofuranose[11] **17** (7.0 g, 16.3 mmol) in pyridine (50 ml) with acetic anhydride (1.8 ml, 19.6 mmol) was stirred overnight at room temperature. The reaction mixture was concentrated under vacuum and azeotroped 3 times with toluene to afford 3-*O*-acetyl-5-*O*-*tert*-butyldiphenylsilyl-1,2-*O*,*O*-isopropylidene-β-D-arabinofuranose **18** (7.5 g, 98%) as a colorless oil.

^{1}H NMR ($CDCl_3$) δ 7.70–7.67 (m, 4H, H_{Ar}); 7.44–7.37 (m, 6H, H_{Ar}); 5.89 (d, $J_{1,2}$ = 4.0 Hz, 1H, H_1); 5.34 (d, $J_{3,4}$ = 1.9 Hz, 1H, H_3); 4.56 (d, $J_{1,2}$ = 4.1 Hz, 1H, H_2); 4.22 (ddd, $J_{3,4}$ = 2.0 Hz, $J_{4,5'}$ = 5.8 Hz, $J_{4,5}$ = 7.9 Hz, 1H, H_4); 3.87 (ABX, $J_{4,5}$ = 8.1 Hz, $J_{5,5'}$ = 10.3 Hz, 1H, H_5); 3.82 (ABX, $J_{4,5'}$ = 5.8 Hz, $J_{5,5'}$ = 10.3 Hz, 1H, $H_{5'}$); 2.10 (C_{MeAc}); 1.32 (s, 3H, CH_{3Iso}); 1.28 (s, 3H, CH_{3Iso}); 1.07 (s, 9H, tBu). ^{13}C NMR ($CDCl_3$) δ 169.6(C_{OAc}); 135.6, 133.2, 129.6, 127.6 (C_{Ar}); 112.6 (C_{qIso}); 105.7 (C_1); 85.5 (C_4); 84.8 (C_2); 77.3 (C_3);

63.6 (C_5); 26.7 (C_{tBu}); 26.6 (C_{MeIso}); 25.9 (C_{MeIso}); 20.8 (C_{MeAc}); 19.1 (C_{Si}). HRMS *m/z* 493.2009 (calcd for $C_{26}H_{34}O_6NaSi([M+Na]^+)$ 493. 2022).

3-*O*-Acetyl-1,2-*O,O*-isopropylidene-β-D-arabinofuranose 19. 3-*O*-Acetyl-5-*O*-*tert*-butyldiphenylsilyl-1,2-*O,O*-isopropylidene-β-D-arabinofuranose **18** (5.6 g, 11.9 mmol) was dissolved in THF (100 mL). To this resulting solution was added TBAF (14.3 ml of 1M solution in THF) and was left under stirring at room temperature overnight. A saturated solution of NH_4Cl (50 mL) was then added. The aqueous layer was extracted with chloroform (3 × 50 mL) and the combined organic layers were washed with water, brine, dried, filtered, and concentrated under vacuum to afford an oil. Purification by flash chromatography (gradient from 8/2 to 1/1 v/v petroleum ether/ethyl acetate) afforded two fractions 3-*O*-acetyl-1,2-*O,O*-isopropylidene-β-D-arabinofuranose **19** (2.0 mg, 73%) and 3,5-bis-*O*-acetyl-1,2-*O,O*-isopropylidene-β-D-arabinofuranose (610 mg, 7%).

^{1}H NMR ($CDCl_3$) δ 5.93 (d, $J_{1,2}$ = 4.0 Hz, 1H, H_1); 5.03 (d, $J_{3,4}$ = 2.2 Hz, 1H, H_3); 4.63 (d, $J_{1,2}$ = 4.1 Hz, 1H, H_2); 4.17 (ddd, $J_{3,4}$ = 2.2 Hz, $J_{4,5'}$ = 5.2 Hz, $J_{4,5}$ = 7.3 Hz, 1H, H_4); 3.83 (ABX, $J_{4,5}$ = 7.4 Hz, $J_{5,5'}$ = 11.4 Hz, 1H, H_5); 3.82 (ABX, $J_{4,5'}$ = 4.7 Hz, $J_{5,5'}$ = 11.4 Hz, 1H, $H_{5'}$); 2.09 (s, 3H, C_{MeAc}); 1.55 (s, 3H, CH_{3Iso}); 1.32 (s, 3H, CH_{3Iso}). ^{13}C NMR ($CDCl_3$) δ 170.1(C_{OAc}); 112.9 (C_{qIso}); 105.6 (C_1); 86.5 (C_4); 84.8 (C_2); 77.7 (C_3); 62.7 (C_5); 26.7 (C_{MeIso}); 26.0 (C_{MeIso}); 20.8 (C_{MeOAc}). HRMS *m/z* 233.1034 (calcd for $C_{10}H_{17}O_6$ ($[M+H]^+$) 233.1025).

3,5-Bis-*O*-acetyl-1,2-*O,O*-isopropylidene-β-D-arabinofuranose. ^{1}H NMR ($CDCl_3$) δ 5.94 (d, $J_{1,2}$ = 3.8 Hz, 1H, H_1); 5.10 (d, $J_{3,4}$ = 1.9 Hz, 1H, H_3); 4.60 (d, $J_{1,2}$ = 3.8 Hz, 1H, H_2); 4.32–4.30 (m, 2H, H_4, H_5); 4.27–4.24 (m, 1H, $H_{5'}$); 2.10 (s, 6H, C_{MeAc}); 1.57 (s, 3H, CH_{3Iso}); 1.32 (s, 3H, CH_{3Iso}). ^{13}C NMR ($CDCl_3$) δ 170.6, 169.7(C_{OAc}); 113.1 (C_{qIso}); 105.9 (C_1); 84.4 (C_2); 83.0 (C_4); 77.4 (C_3); 63.8 (C_5); 26.7 (C_{MeIso}); 26.0 (C_{MeIso}); 20.8 (C_{MeAc}). HRMS *m/z* 275.2768 (calcd for $C_{12}H_{19}O_7$ ($[M+H]^+$) 275.2810).

3-*O*-Acetyl-5-*O*-dibenzylphosphate-1,2-*O,O*-isopropylidene-β-D-arabinofuranose 16. To a solution of 3-*O*-acetyl-1,2-*O*-isopropylidene-β-D-arabinofuranose **19** (0.59 g, 2.54 mmol) and dibenzylphosphoramidate **12** (1.2 g, 3.31 mmol) in dry DCM (20 mL) was added dropwise a solution of 2,4-DNP (0.61 g, 3.31 mmol) in dry DCM (20 mL). This resulting mixture was stirred for 3 hours at room temperature, and then a solution of *ter*-BuOOH in toluene (1.6 ml of a 3 M solution) was added and kept under stirring for 20 minutes. After dilution with DCM (20 mL), the reaction mixture was washed with a sodium thiosulfate solution (30 mL), water (30 mL × 3), dried, filtered, and concentrated under vacuum to afford the crude fully protected phosphorylated precursor. Purification by flash chromatography (gradient from 1/0 to 95/5 v/ $CHCl_3$/ethanol) yielded 3-*O*-acetyl-5-*O*-dibenzylphosphate-1,2-*O,O*-isopropylidene-β-D-arabinofuranose **16** (800 mg, 64%) as a yellow oil.

^{1}H NMR (CDCl$_3$) 7.30–7.27 (m, 10H, H$_{Ar}$); 5.88 (d, $J_{1,2}$ = 3.8 Hz, 1H, H$_1$); 5.08 (m, 1H, H$_3$); 5.05–5.01 (m, 4H, H$_{CH2Bn}$); 4.54 (d, $J_{1,2}$ = 3.8 Hz, 1H, H$_2$); 4.23–4.16 (m, 3H, H$_4$, H$_5$, H$_{5'}$); 2.00 (s, 3H, CH$_{3Ac}$); 1.44 (s, 3H, CH$_{3Iso}$); 1.23 (s, 3H, CH$_{3Iso}$). ^{13}C NMR (CDCl$_3$) δ 169.2 (C$_{OAc}$); 135.5, 135.4 (C$_{Ar}$); 128.2, 128.1 (C$_{Ar}$); 112.6 (C$_{qIso}$); 105.6 (C$_1$); 83.9 (C$_2$); 83.3 (d, $J_{P\text{-}O\text{-}C\text{-}C}$ = 8.3 Hz,C$_4$); 76.5 (C$_3$); 69.1(d, $J_{P\text{-}O\text{-}C}$ = 4.9 Hz, C$_{CH2Bn}$); 69.0(d, $J_{P\text{-}O\text{-}C}$ = 4.6 Hz, C$_{CH2Bn}$);66.3 (d, $J_{P\text{-}O\text{-}C}$ = 5.4 Hz, C$_5$); 26.3 (C$_{MeIso}$); 25.5 (C$_{MeIso}$); 20.3 (C$_{MeAc}$). ^{31}P NMR (CDCl$_3$) δ -0.1. HRMS *m/z* 493.1625 (calcd for $C_{24}H_{30}O_9P$([M+H]$^+$) 493.1627).

3-*O*-Acetyl-5-*O*-dibenzylphosphate-D-arabinofuranose 21. The same procedure as described for **20** was used for the synthesis of **21** (55%).

^{1}H NMR (CDCl$_3$) 7.34–7.30 (m, 10H, H$_{Ar}$); 5.43 (s, 0.4H, H$_{1\alpha}$); 5.39 (d, $J_{1,2}$ = 4.3 Hz, 0.6H, H$_{1\beta}$); 5.06–4.99 (m, 4H, H$_{CH2Bn}$); 4.96 (t, $J_{2,3} = J_{3,4}$ = 4.4 Hz, 0.6H, H$_{3\beta}$); 4.83 (dd, $J_{2,3}$ = 1.6 Hz, $J_{3,4}$ = 4.4 Hz, 0.4H, H$_{3\alpha}$); 4.33–4.20 (m, 2.4H, H$_{4\alpha}$, H$_{5\beta}$, H$_{5'\beta}$, H$_{5\alpha}$, H$_{5'\alpha}$); 4.18 (d, $J_{2,3}$ = 1.8 Hz, 0.4H, H$_{2\alpha}$); 4.17 (dd, $J_{1,2} = J_{2,3}$ = 4.4 Hz, 0.6H, H$_{2\beta}$); 4.00 (ddd, $J_{3,4}$ = 4.2 Hz, $J_{4,5}$ = 4.2 Hz, $J_{4,5'}$ = 7.2 Hz, 1H, H$_{4\beta}$); 2.07 (s, 1.8H, CH$_{3Ac\beta}$); 2.06 (s, 1.2H, CH$_{3Ac\alpha}$). ^{13}C NMR (CDCl$_3$) δ 171.0 (C$_{OAc\alpha}$); 170.8 (C$_{OAc\beta}$); 135.6, 135.5 (x2), 135.4 (C$_{Ar}$); 128.5, 128.0, 127.9 (C$_{Ar}$); 102.7 (C$_{1\alpha}$); 96.9 (C$_{1\beta}$); 81.0 (d, $J_{P\text{-}O\text{-}C\text{-}C}$ = 6.2 Hz,C$_{4\alpha}$); 80.3 (C$_{2\alpha}$); 78.2 (C$_{3\alpha}$); 79.8 (d, $J_{P\text{-}O\text{-}C\text{-}C}$ = 5.9 Hz,C$_{4\beta}$); 79.1 (C$_{3\beta}$); 75.8 (C$_{2\beta}$); 69.7(d, $J_{P\text{-}O\text{-}C}$ = 5.5 Hz, C$_{CH2Bn}$); 69.6(d, $J_{P\text{-}O\text{-}C}$ = 5.9 Hz, C$_{CH2Bn}$); 68.6 (d, $J_{P\text{-}O\text{-}C}$ = 5.7 Hz, C$_{5\beta}$); 67.2 (d, $J_{P\text{-}O\text{-}C}$ = 5.8 Hz, C$_{5\alpha}$); 20.7 (C$_{MeAc}$). ^{31}P NMR (CDCl$_3$) δ -0.4 (β), -0.5 (α). HRMS *m/z* 453.1315 (calcd for $C_{21}H_{26}O_9P$([M+H]$^+$) 453.1314).

3-*O*-Acetyl-5-*O*-phosphate-D-arabinofuranose 24. The same procedure as described for **22** was used for the synthesis of **24**.

^{1}H NMR (D$_2$O) 5.25 (s, 0.7H, H$_{1\alpha}$); 5.24 (d, $J_{1,2}$ = 3.8 Hz, 0.3H, H$_{1\beta}$); 5.00 (t, $J_{2,3} = J_{3,4}$ = 4.2 Hz, 0.3H, H$_{3\beta}$); 4.81 (dd, $J_{2,3}$ = 1.8 Hz, $J_{3,4}$ = 4.2 Hz, 0.7H, H$_{3\alpha}$); 4.32–4.29 (m, 0.7H, H$_{4\alpha}$); 4.19 (dd, $J_{1,2}$ = 3.8 Hz, $J_{2,3}$ = 4.2 Hz, 0.3H, H$_{2\beta}$); 4.06 (m, 0.7H, H$_{2\alpha}$); 4.04–3.90 (m, 2.3H, H$_{4\beta}$, H$_{5\beta}$, H$_{5'\beta}$, H$_{5\alpha}$, H$_{5'\alpha}$);2.01 (s, 0.9H, CH$_{3Ac\beta}$); 2.00 (s, 2.1H, CH$_{3Ac\alpha}$). ^{13}C NMR (D$_2$O) δ 173.3 (C$_{OAc}$); 103.9 (C$_{1\alpha}$); 98.0 (C$_{1\beta}$); 82.5 (d, $J_{P\text{-}O\text{-}C\text{-}C}$ = 8.3 Hz,C$_{4\alpha}$); 81.5 (C$_{2\alpha}$); 80.9 (C$_{3\alpha}$); 80.8 (d, $J_{P\text{-}O\text{-}C\text{-}C}$ = 8.2 Hz,C$_{4\beta}$); 79.9 (C$_{3\beta}$); 76.6 (C$_{2\beta}$); 69.3 (d, $J_{P\text{-}O\text{-}C}$ = 5.2 Hz, C$_{5\beta}$); 67.7 (d, $J_{P\text{-}O\text{-}C}$ = 5.1 Hz, C$_{5\alpha}$); 20.8 (C$_{MeAc}$). ^{31}P NMR (CDCl$_3$) δ 1.8 (br). HRMS *m/z* 271.0217 (calcd for $C_7H_{12}O_9P$([M-H]) 271.0219).

***O*-Acetyl-adenosine-diphospho-arabinose 5.** The same procedure as described for **4** and **6** was used for the synthesis of **5** (9.3 μmol; 9%) and **7** (2.3 μmol; 4%).

^{1}H NMR (D$_2$O) 8.53 (s, 1H, H$_2$); 8.08 (s, 1H, H$_8$); 6.10 (d, J = 5.9 Hz, 1H, H$_{1'}$); 5.41 (d, J = 4.5 Hz, 0.5H, H$_{1''}$); 5.30 (s, 0.2H, H$_{1''}$); 5.24 (s, 0.3H, H$_{1''}$); 4.54–4.48 (m, 2H); 4.40–4.33 (m, 2H); 4.19–4.16 (m, 2H); 4.14–3.94 (m, 4H); 2.09, 2.03, 2.02 (s, 3H, CH$_{3Ac}$). ^{31}P NMR (D$_2$O) δ -9.9 (br, P-O-P). HRMS *m/z* 600.0775 (calcd for $C_{17}H_{24}O_{14}N_5P_2$([M-H]) 600.0744).

Adenosine-diphospho-arabinose 7

^{1}H NMR (D_2O) 8.47 (s, 1H, H_2); 8.23 (s, 1H, H_8); 6.10 (d, $J = 5.9$ Hz, 1H, $H_{1'}$); 5.20 (d, $J = 4.6$ Hz, 0.5H, $H_{1''}$); 5.18 (s, 0.5H, $H_{1''}$); 4.58–4.56 (m, 2H); 4.40–4.33 (m, 2H); 4.36–4.33 (m, 2H); 4.16–3.99 (m, 4H). ^{31}P NMR (D_2O) δ -10.0 (br, P-O-P). HRMS *m/z* 558.0667 (calcd for $C_{15}H_{22}O_{14}N_5P_2$ ([M-H]) 558.0639).

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