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Synthesis of alkylcarbonate analogs of *O*-acetyl-ADP-ribose†

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The non-hydrolyzable alkylcarbonate analogs of *O*-acetyl-ADP-ribose have been synthesized from the phosphorylated ribose derivatives after coupling with AMP morpholidate promoted by mechanical grinding. The analogs were assessed for their ability to inhibit the human sirtuin homolog SIRT1.

Introduction

O-Acetyl-adenosine diphosphoribose (*O*AADPR) is the most common metabolite generated by nicotinamide adenine dinucleotide (NAD^+)-dependent histone deacetylases called sirtuins. Sirtuins play a key role in gene silencing and the regulation of metabolism and lifespan extension.[1] Among histone deacetylases, they utilize a unique mechanism for protein deacetylation by coupling it with a stoichiometric consumption of NAD^+.[2] The product of this enzymatic reaction, 2′-*O*-acetyl-ADP-ribose, isomerizes rapidly to form a 1 : 1 mixture of 2′-*O*-acetyl and 3′-*O*-acetyl-ADP-ribose regioisomers.[3]

The role of *O*AADPR in biological processes remains largely unknown. It is hypothesized that *O*AADPR may serve as a second messenger, in a similar manner to structurally related molecules such as ADPR. From a few studies already implemented, *O*AADPR appears to positively regulate the assembly of the Sir complex[4] and the opening of TRPM2 (transient receptor potential channel 2) calcium channel.[5] In addition, it caused a delay in oocyte and blastomere maturation.[6] *O*AADPR is also known to bind the macro domain of H2A1.1 protein, which has been involved in transcriptional regulation.[7]

Further studies of *O*AADPR targets are restricted by its limited availability and susceptibility to enzymatic hydrolysis. *O*AADPR is a substrate for NUDIX hydrolases, which hydrolyse the pyrophosphate linkage,[7] and for a range of esterases, such as ARH hydrolases and macrodomain proteins, hydrolysing the acetyl functionality to generate ADP-riboside.[8]

In order to overcome the extreme instability of the acetyl moiety, a range of alkylcarbonate analogs of *O*AADPR were synthesized. The alkylcarbonate functionality was selected due to its enhanced stability when compared to esters and its lower susceptibility to acyl migrations, which have been reported for acetate esters on carbohydrates.[9] Methyl, ethyl and isobutylcarbonates were chosen as structural mimics not only of the acetyl moiety of *O*AADPR, but also of the more recently confirmed products of protein deacylations, which are the propionyl and butyryl derivatives of ADP-riboses.[10] The synthesized derivatives were then assessed for their ability to inhibit the SIRT1 sirtuin homolog.

Results and discussion

Synthetic strategy

The synthesis of alkylcarbonate *O*AADPR analogs started from 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranoside (**1**) which was reacted with benzyl alcohol under Lewis acid catalysis to afford 1-*O*-benzyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranoside (**2**).

1-*O*-Benzyl-β-D-ribofuranoside (**3**) was obtained after deprotection of benzoyl groups in **2** under Zemplén conditions. The free primary position on this ribofuranoside **3** was then regioselectively protected with a *tert*-butyldimethylsilyl group (TBDMS) to give the ribofuranoside **4** (Scheme 1).

Bis(alkylcarbonates) **5a–c** were prepared from the corresponding chloroformates under basic catalysis utilizing DMAP

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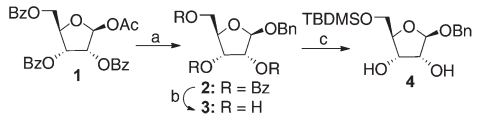
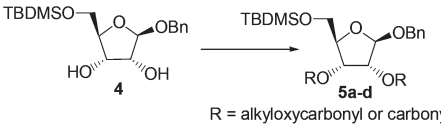
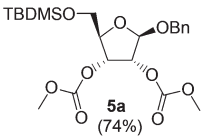
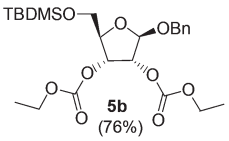
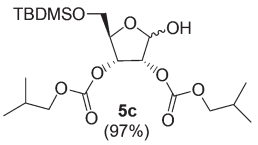
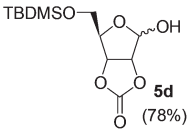
†Electronic supplementary information (ESI) available: Copies of ^{1}H-NMR and ^{13}C-NMR spectra of all new compounds. See DOI: 10.1039/c3ob41016a



Scheme 1 (a) BnOH, $SnCl_4$, DCM, 0 °C, 2 h, quant.; (b) MeONa–MeOH, 12 h, 100%; (c) TBDMSCl, DMAP, pyridine, DCM, 0 °C, 18 h, 75%.

Table 1 Synthesis of alkylcarbonate derivatives **5a–d**

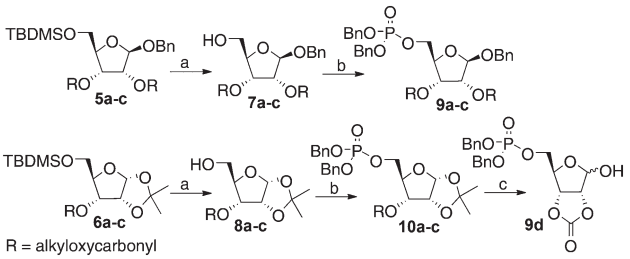


Entry	Reagent	Product (product yield)[a]
1	MeOCOCl	 **5a** (74%)
2	EtOCOCl	 **5b** (76%)
3	$(CH_3)_2CHCH_2COCl$	 **5c** (97%)
4	$(Bu)_2SnO$ TBABr MeOCOCl	 **5d** (78%)

[a] Yield after column chromatography.

and pyridine (Table 1, entries 1–3).[11] Monosubstituted derivatives could not be prepared in this way as an excess of chloroformate reagent was necessary to ensure any product formation. Other attempts to obtain monosubstituted products through either the stannylene acetal methodology (Table 1, entry 4)[12] or using 3-*O*-alkylcarbonate-1,2,5-*O*-protected ribosides **6a–c** (Scheme 2)[11] were unsuccessful due to rapid carbonate migration with a concomitant methoxy group cleavage which readily afforded the 2,3-*O*-cyclic carbonate derivatives **5d** and **9d**. The deprotection of the TBDMS group in **5a–c** and **6a–c** was accomplished using a rather unusual reagent, cerium ammonium nitrite (CAN), which has been found to avoid acetyl migration in furanosides (Scheme 2).[9] Similarly, this reagent prevented the tendency of alkylcarbonates to migrate from secondary to primary positions within the ribofuranoside scaffold.[11]

The resulting carbohydrate derivatives **7a–c** and **8a–c** were suitable for their direct phosphorylation with bis(benzyloxy)-



Scheme 2 (a) CAN, MeCN–H_2O (9/1), 3 h, 86–96%; (b) (i) $(BnO)_2PN(iPr)_2$, 2,4-DNP, DCM, 12 h, (ii) *t*BuOOH, 2 h, 68–96%; (c) TFA–H_2O (4/1), 0 °C, 15 min, 67%.

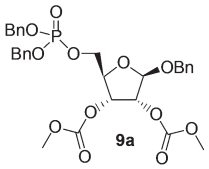
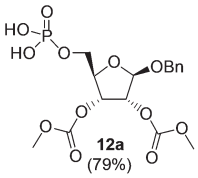
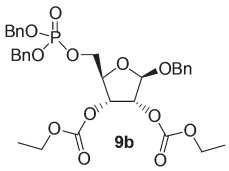
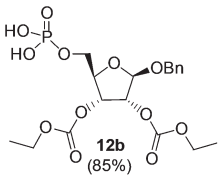
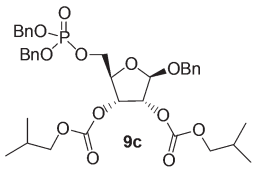
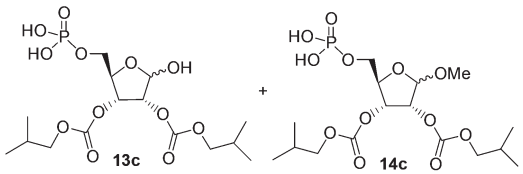
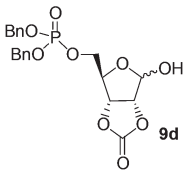
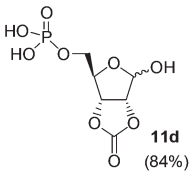
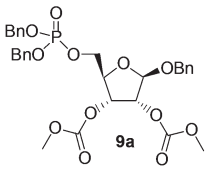
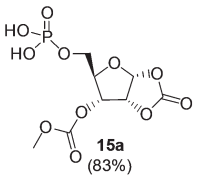
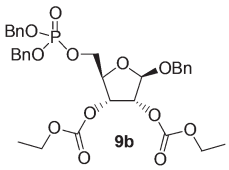
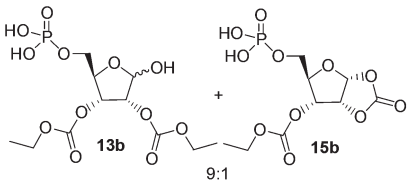
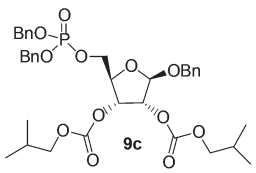
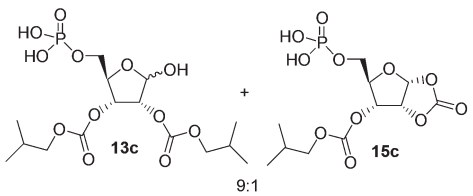
N,*N*-diisopropyl phosphoramidite reagent prepared from phosphorus trichloride.[13] This benzyl protected phosphorus reagent was selected due to the possibility of concomitant removal of all benzyls from the carbohydrate derivatives **9a–c** in one step, affording the free phosphate derivatives for subsequent coupling with AMP morpholidate.

The phosphorylation step proceeded smoothly to give all the phosphorylated derivatives **9a–c** and **10a–c** in high yields (Scheme 2). However, the removal of the protecting groups proved to be challenging. Firstly, the isopropylidene group deprotection in derivatives **10a–c** was accomplished using TFA–H_2O (4/1). This optimized method prevented the cleavage of the alkylcarbonate moiety that occurs under acidic conditions; however, it did not forestall its migration with concomitant alkoxy group cleavage. Therefore, from all the derivatives **10a–c**, only the 2,3-*O*-cyclic carbonate product **9d** was formed. Such a migration could be explained due to similar electronic and steric properties of 2-OH and 3-OH groups in ribosides and because a more structurally rigid conformation of riboside derivatives is generated.

Secondly, the removal of benzyl groups in derivatives **9a–d** was carried out. The reaction was performed in an autoclave under 10 bars hydrogen pressure in various solvents, as using only balloon pressure was found to be insufficient (Table 2). When THF was utilized to hydrogenolyse derivatives **9a–b**, the anomeric benzyl group was not deprotected even after prolonged reaction times and products **12a–b** were formed. The use of methanol to hydrogenate derivative **9c**, on the other hand, deprotected the anomeric benzyl group, but a partial methylation of this position occurred to give a 1 : 1 mixture of **13c** : **14c** as revealed by HPLC-MS (Table 2). The use of a THF–water mixture finally afforded the desired products **11d** and **13a–c**. However, the dialkylcarbonate products **13a–c** were highly unstable and a spontaneous migration of 2-*O*-alkylcarbonate moiety occurred to form 1,2-*O*-cyclic carbonate derivatives **15a–c** (Table 2). This is probably caused by the high reactivity of the anomeric position under either acidic or basic conditions; therefore once this position is deprotected, it is susceptible to the attack of the adjacent alkylcarbonate moiety to form the stable cyclic carbonate.

For the final step of the pyrophosphate formation, the phosphomorpholidate methodology developed by Moffatt and Khorana (1959) was employed.[14] This methodology for the

Table 2 Hydrogenation of disubstituted phosphate derivatives **9a–d** on 10% Pd/C at 10 bars hydrogen pressure

Substrate	Solvent	Reaction time	Product (product yield)[a], product ratio[b]
 **9a**	THF	24 or 48 h	 **12a** (79%)
 **9b**	THF	24 or 48 h	 **12b** (85%)
 **9c**	MeOH	48 h	 **13c** + **14c** 1:1 (87%)
 **9d**	THF–H_2O	48 h	 **11d** (84%)
 **9a**	THF–H_2O	48 h	 **15a** (83%)
 **9b**	THF–H_2O	48 h	 **13b** + **15b** 9:1
 **9c**	THF–H_2O	48 h	 **13c** + **15c** 9:1

[a] Isolated product yield without purification. [b] According to HPLC-MS.

synthesis of unsymmetrical pyrophosphates, based on the activation of one of the two coupling substrates, prevents the formation of symmetrical diphosphate side-products, even though the strictly anhydrous conditions and long reaction times negatively influence the yield.

Recently, a further improvement of the phosphomorpholidate methodology was reported by Ravalico *et al.* (2011),[15] who performed the reaction in a ball mill without the use of a solvent, but in the presence of 6 equivalents of water. This novel, environmentally friendly procedure significantly improved the yield of the reaction.

Thus the phosphate derivatives **11d**, **12b**, and **15a–c** were coupled with AMP morpholidate in a ball mill in the presence of 1*H*-tetrazole and hexahydrate of magnesium chloride according to the reported procedure.[15] The corresponding pyrophosphate analogs of *O*AADPR **13d** and **16a–c** and 1″-*O*-benzyl-2″,3″-*O*-diethylcarbonate pyrophosphate **17b** were generated in 45–68% yields (Table 3).

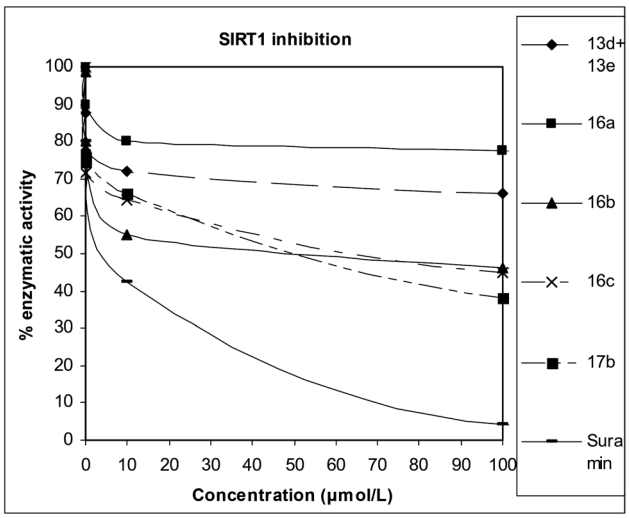
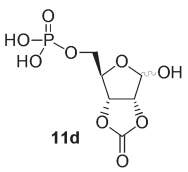
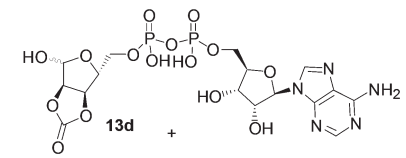
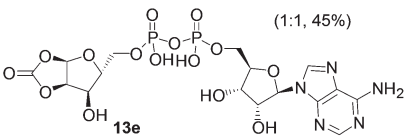
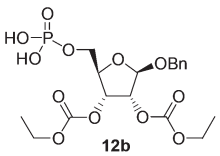
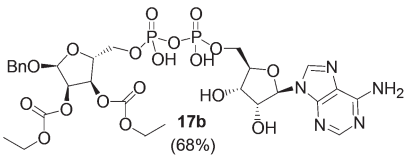
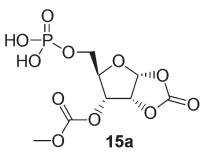
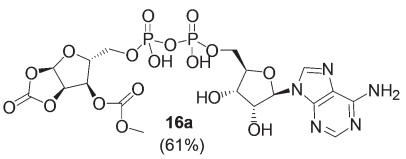
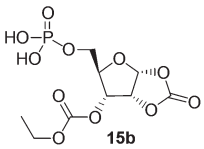
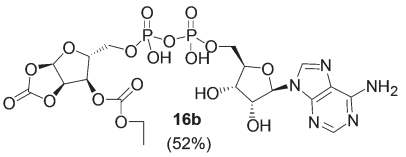
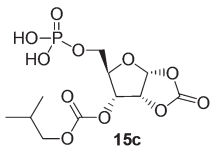
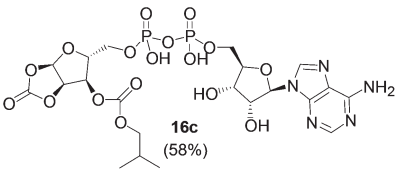


Fig. 1 Inhibitory activity of compounds **13d** + **13e**, **16a–c** and **17b**.

Table 3 Ball mill coupling: AMP morpholidate, $MgCl_2 \cdot 6H_2O$, 1*H*-tetrazole, water

Reactant	Product (product yield)[a]
 **11d**	 **13d** + (1:1, 45%)  **13e**
 **12b**	 **17b** (68%)
 **15a**	 **16a** (61%)
 **15b**	 **16b** (52%)
 **15c**	 **16c** (58%)

[a] Yield after preparative HPLC.

Inhibitory activity of the prepared pyrophosphates

The inhibitory activity of pyrophosphates **13d** + **13e**, **16a–c** and **17b** towards the human sirtuin homolog, SIRT1, was tested using a commercially available assay kit. The prepared pyrophosphates as structural mimics of the sirtuin enzymatic product, *O*AADPR, were expected to act as nonselective competitive inhibitors of sirtuins. Therefore, the SIRT1 assay has been chosen to test their inhibitory activity as it is the most commonly used assay and offers a broad spectrum of compounds for the comparison of activities. The best inhibitory activity was displayed by compounds **16b**, **16c** and **17b**, which was quite surprising as these compounds possess a longer alkyl chain and in the case of compound **17b** also the benzyl protecting group on 1″-OH. The IC_{50} values of these three compounds were 53 μM for compound **17b**, 56 μM for compound **16b** and 65 μM for compound **16c** (Fig. 1). Even though the inhibitory activity of these compounds was in the μM range, it is still about one order of magnitude higher than the potency of the known sirtuin inhibitor, Suramin, which was used as a control in these experiments and shown to have an IC_{50} value of 5.8 μM under these conditions. From these results, we can conclude that the rigidity of the northern ribose moiety caused by the presence of cyclic carbonate prevents the compounds from better binding to the binding site of the enzyme, and that the best alkyl group fitting into the binding pocket is ethyl.

Conclusions

3″-*O*-Methyl, ethyl and isobutylcarbonate-1″,2″-*O*-cyclic carbonate-ADP-ribose derivatives **16a–c** and 2″,3″-*O*-cyclic carbonate-ADP-ribose derivative **13d** were synthesized and their inhibitory activity against the human sirtuin homolog, SIRT1, was assessed and compared with the inhibitory activity of a prepared 1″-*O*-benzyl-2″,3″-*O*-ethylcarbonate-ADP-ribose derivative **17b** and a known SIRT1 inhibitor, Suramin. The best inhibitory activity was displayed by compound **17b** followed by **16b** and **16c**. The IC_{50} values of these three compounds were 53 μM (**17b**), 56 μM (**16b**) and 65 μM (**16c**). Even though the inhibitory activity of these compounds was in the μM range, it was still one order of magnitude higher than the activity of Suramin (IC_{50} = 5.8 μM). From these results, it seems that the rigidity of the northern ribose moiety caused by the presence of cyclic carbonate prevents the compounds from better binding to the binding site of the enzyme.

Experimental

General

All reactions requiring anhydrous or inert conditions were carried out under a positive atmosphere of argon in oven-dried glassware. Solutions or liquids were introduced in round bottom flasks using oven dried syringes through rubber septa. All reactions were stirred magnetically using Teflon-coated

stirring bars. If needed, reactions were warmed up using an electrically heated silicon oil bath, and the stated temperature corresponds to the temperature of the bath. When reactions required cooling to −78 °C, a dry ice/acetone bath was used. Organic solutions obtained after aqueous work-up were dried over $MgSO_4$. The removal of solvents was accomplished using a rotary evaporator at water aspirator pressure.

Chemicals were purchased from Sigma-Aldrich Chemical Company. Technical grade solvents for extractions and chromatography were purchased from Penta Chemicals s.r.o., Czech Republic. Solvents used in reactions were distilled from appropriate drying agents and stored under argon over activated Linde 4 Å molecular sieves. Column and flash chromatography was carried out using Merck Silica (60–200 μm) or Merck neutral Alumina 90 (63–200 μm). Analytical TLC was performed with Merck Silica gel 60 F_{254} plates. Visualisation was accomplished by UV-light (254 nm) and staining with a vanillin solution, followed by heating. IR spectra were recorded using a Nicolet 6700 FT-IR spectrometer. ^{1}H, ^{13}C and 2D (H-COSY, HMQC) NMR spectra were all recorded using Bruker DPX 400 MHz. Trimethylsilane (0 ppm, ^{1}H NMR) and $CDCl_3$ (77 ppm, ^{13}C NMR) were used as internal references. The chemical shifts (δ) are reported in ppm (parts per millions) and the coupling constants (J values) are recorded in Hz (hertz). The hydrogen and carbon assignments were done according to 2D experiments (^{1}H–^{1}H COSY and ^{1}H–^{13}C HMQC). Mass spectra were recorded using an LTQ Orbitrap XL spectrometer. Optical rotations were measured using a Perkin-Elmer 341 polarimeter at 589 nm. Melting points were recorded using a Büchi Melting Point apparatus B-540. The elementary analyses were performed using a Perkin-Elmer 2400 Series II CHNS/O analyzer.

SIRT1 enzyme inhibitory assays

For the evaluation of the inhibitory activity on SIRT1 enzyme, a commercially available assay kit from Enzo Life Sciences was used. For the reaction were used: 1 U per well of SIRT1 enzyme (1 U = 1 pmol min^{-1} at 37 °C), 25 μM NAD^+ (K_m = 558 μM, V_{max} = 1863 AFU min^{-1}), 25 μM *Fluor de Lys*® peptide substrate (K_m = 64 μM, V_{max} = 1107 AFU min^{-1}), reaction time 45 min at 37 °C, then 200 μM *Fluor de Lys*® developer and 40 μM NAD were added. The results were measured after 15 min of incubation at 37 °C using a Tecan microplate fluorimeter at an excitation wavelength of 360 nm and detection of emitted light at 460 nm. The test was repeated twice, and the compounds were assessed in triplicate. No slow-onset inhibition was observed for any of the tested compounds.

Synthesis

2,3,5-Tri-*O*-benzoyl-1-*O*-benzyl-β-D-ribofuranoside 2.[16] Dry BnOH (266 μL, 2.57 mmol) was added to a solution of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranoside **1** (1.00 g, 1.98 mmol) in dry DCM at 0 °C, followed by addition of $SnCl_4$ (232 μL, 1.98 mmol). The reaction mixture was stirred at 0 °C for 2 hours and then it was allowed to warm up to room temperature while stirring overnight. The reaction was quenched with a saturated solution of $NaHCO_3$ (80 mL), the aqueous layer was extracted with DCM (3 × 50 mL), and the combined organic layers were washed with water and brine, dried, filtered and evaporated.

Purification by chromatography (7/3, v/v, hexane–EtOAc) gave 1-*O*-benzyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranoside **2** (1.10 g, quant.) as a white solid.

m.p.: 68–69 °C; $[\alpha]_D^{20}$ = −16.1° (*c* 1.27, $CHCl_3$); Anal. calcd for $C_{20}H_{21}O_9P$: C 71.73, H 5.11; found: C 71.64, H 5.50%; ^{1}H NMR ($CDCl_3$): δ 8.03 (4H, tddd, J = 15.5, 8.4, 3.2, 1.2 Hz, Ar), 7.88 (2H, tdd, J = 8.4, 3.2, 1.2 Hz, Ar), 7.57 (1H, tt, J = 7.9, 1.3 Hz, Ar), 7.50 (2H, tt, J = 7.8, 1.3 Hz, Ar), 7.42 (2H, t, J = 7.8 Hz, Ar), 7.33–7.30 (9H, m, Ar), 5.93 (1H, dd, J = 6.8, 4.7 Hz, H-3), 5.77 (1H, d, J = 4.7 Hz, H-2), 5.34 (1H, s, H-1), 4.82 (1H, d, J = 11.7 Hz, CH_2Ph), 4.77–4.71 (2H, m, H-4, H-5), 4.60 (1H, d, J = 11.7 Hz, CH_2Ph), 4.56 (1H, dd, J = 11.8, 5.0 Hz, H-5); ^{13}C NMR ($CDCl_3$): δ 166.2 (CO), 165.3 (CO), 165.2 (CO), 136.8 (Ar), 133.5 (Ar), 133.4 (Ar), 133.1 (Ar), 129.8 (Ar), 129.8 (Ar), 129.7 (Ar), 128.5 (Ar), 128.3 (Ar), 128.0 (Ar), 127.9 (Ar), 104.5 (C-1), 79.1 (C-4), 75.6 (C-2), 72.4 (C-3), 69.7 (CH_2Ph), 64.7 (C-5); HRMS *m*/*z* 575.1691 (calc. for $C_{33}H_{28}O_8Na$ ([M + Na]$^+$): 575.1682).

1-*O*-Benzyl-β-D-ribofuranoside 3. 2,3,5-Tri-*O*-benzoyl-1-*O*-benzyl-β-D-ribofuranoside **2** (400 mg, 0.75 mmol) was dissolved in MeOH and a catalytic amount of a MeONa–MeOH solution (25% w.t., 0.1 mL) was added. The reaction mixture was stirred overnight. The reaction was quenched with DOWEX H^+ resin until neutral pH, filtered and evaporated.

The residue was dissolved in water (20 mL) and washed with hexane (3 × 10 mL) and the aqueous layer was freeze-dried to give pure 1-*O*-benzyl-β-D-ribofuranoside **3** (184 mg, quant.) as a white solid.

m.p.: 95–96 °C; $[\alpha]_D^{20}$ = −62.8° (*c* 1.34, H_2O); Anal. calcd for $C_{20}H_{21}O_9P$: C 59.99, H 6.71; found: C 59.26, H 6.41%; ^{1}H NMR (D_2O): δ 7.42–7.35 (5H, m, Ar), 5.08 (1H, s, H-1), 4.78–4.75 (1H, m, CH_2Ph), 4.53 (1H, d, J = 11.2 Hz, CH_2Ph), 4.16 (1H, dd, J = 6.7, 4.6 Hz, H-3), 4.03 (1H, br d, J = 4.6 Hz, H-2), 4.00 (1H, dd, J = 6.7, 3.3 Hz, H-4), 3.77 (1H, dd, J = 12.3, 3.3 Hz, H-5a), 3.59 (1H, dd, J = 12.3, 6.7 Hz, H-5b); ^{13}C NMR (D_2O): δ 137.0 (Ar), 129.2 (Ar), 129.0 (Ar), 128.8 (Ar), 106.6 (C-1), 83.2 (C-4), 74.7 (C-2), 71.3 (C-3), 70.2 (CH_2Ph), 63.3 (C-5); HRMS *m*/*z* 239.0879 (calc. for $C_{12}H_{17}O_5$ ([M + H]$^+$): 239.0919).

1-*O*-Benzyl-5-*O*-*tert*-butyldimethylsilyl-β-D-ribofuranoside 4. A solution of 1-*O*-benzyl-β-D-ribofuranoside **3** (300 mg, 1.24 mmol) and DMAP (31.0 mg, 0.025 mmol) in dry pyridine (20 mL) was cooled down to 0 °C under an argon atmosphere. To this solution was added a solution of TBDMSCl in dry DCM (10 mL) in three portions by a syringe over 6 h at 0 °C. The reaction mixture was stirred overnight and allowed to warm up to room temperature. The reaction mixture was then evaporated and the residue was dissolved in DCM, washed with a solution of $CuSO_4$, $NaHCO_3$ and brine, dried and evaporated.

Purification by flash chromatography (gradient from 7/1 to 4/1, v/v, hexane–EtOAc) gave 1-*O*-benzyl-5-*O*-*tert*-butyldimethylsilyl-β-D-ribofuranoside **4** (307 mg, 85%) as a colorless oil. By column chromatography using a gradient from 100/0 to 95/5, v/v, $CHCl_3$–EtOH, α- and β-anomers could be separated: α-anomer: 18 mg (5%), β-anomer: 271 mg (75%).

α-Anomer: $[\alpha]_D^{20}$ = +68.5° (*c* 0.36, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.38–7.30 (5H, m, Ar), 5.12 (1H, d, *J* = 4.4 Hz, H-1), 4.85 (1H, d, *J* = 11.8 Hz, CH_2Ph), 4.58 (1H, d, *J* = 11.8 Hz, CH_2Ph), 4.15–4.10 (2H, m, H-2, H-4), 4.00 (1H, ddd, *J* = 8.6, 6.2, 2.4 Hz, H-3), 3.77 (1H, dd, *J* = 11.2, 3.2 Hz, H-5a), 3.72 (1H, dd, *J* = 11.2, 3.2 Hz, H-5b), 2.94 (1H, d, *J* = 9.8 Hz, OH), 2.61 (1H, d, *J* = 8.7 Hz, OH), 0.89 (9H, s, SiC(CH_3)$_3$), 0.06 (3H, s, Si(CH_3)$_2$), 0.05 (3H, s, Si-(CH_3)$_2$); ^{13}C NMR ($CDCl_3$): δ 137.2 (Ar), 128.5 (Ar), 127.9 (Ar), 101.1 (C-1), 85.9 (C-4), 72.1 (C-2), 71.3 (C-3), 69.6 (*C*H_2Ph), 63.4 (C-5), 25.9 (SiC(*C*H_3)$_3$), 18.3 (Si*C*(CH$_3$)$_3$), −5.4 (Si(*C*H_3)$_2$), −5.5 (Si(*C*H_3)$_2$); HRMS *m*/*z* 377.17543 (calc. for $C_{18}H_{30}O_5NaSi$ ([M + Na]$^+$): 377.17547).

β-Anomer: $[\alpha]_D^{20}$ = −64.2° (*c* 0.34, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.36–7.28 (5H, m, Ar), 5.03 (1H, s, H-1), 4.73 (1H, d, *J* = 11.8 Hz, CH_2Ph), 4.48 (1H, d, *J* = 11.8 Hz, CH_2Ph), 4.31 (1H, td, *J* = 5.5, 4.7 Hz, H-3), 4.12 (1H, br d, *J* = 4.0 Hz, H-2), 4.00 (1H, dt, *J* = 6.5, 5.2 Hz, H-4), 3.85 (1H, dd, *J* = 10.2, 5.2 Hz, H-5a), 3.69 (1H, dd, *J* = 10.2, 6.5 Hz, H-5b), 2.71 (1H, br s, OH), 2.45 (1H, d, *J* = 4.3 Hz, OH), 0.90 (9H, s, SiC(CH_3)$_3$), 0.09 (6H, s, Si-(CH_3)$_2$); ^{13}C NMR ($CDCl_3$): δ 137.5 (Ar), 128.4 (Ar), 127.9 (Ar), 127.7 (Ar), 106.3 (C-1), 83.1 (C-4), 75.4 (C-2), 73.3 (C-3), 69.3 (*C*H_2Ph), 65.1 (C-5), 25.9 (SiC(*C*H_3)$_3$), 18.4 (Si*C*(CH$_3$)$_3$), −5.4 (Si-(*C*H_3)$_2$), −5.4 (Si(*C*H_3)$_2$); HRMS *m*/*z* 377.17543 (calc. for $C_{18}H_{30}O_5NaSi$ ([M + Na]$^+$): 377.17547).

General procedure for the synthesis of bis(alkylcarbonate) ribofuranosides 5a–c

To a solution of 1-*O*-benzyl-5-*O*-*tert*-butyldimethylsilyl-β-D-ribofuranoside **4** (1 × *n*), DMAP (0.2 × *n*) and pyridine (2.2 × *n*) in DCM was added alkyl chloroformate (2.2 × *n*) at 0 °C. The reaction mixture was stirred overnight and allowed to warm up to room temperature, and then it was diluted with DCM, washed with a solution of $CuSO_4$, $NaHCO_3$, water and brine, dried and evaporated.

1-*O*-Benzyl-2,3-*O*-bis(methylcarbonate)-5-*O*-*tert*-butyldimethylsilyl-β-D-ribofuranoside 5a. For the reaction, 378 mg (1.07 mmol) of ribofuranoside **4** and methyl chloroformate reagent was used.

Purification by flash chromatography (gradient from 99/1 to 9/1, v/v, hexane–EtOAc) gave 1-*O*-benzyl-2,3-*O*-bis(methylcarbonate)-5-*O*-*tert*-butyldimethylsilyl-β-D-ribofuranoside **5a** (372 mg, 74%) as a colorless oil.

$[\alpha]_D^{20}$ = −41.3° (*c* 0.31, $CHCl_3$); IR: ν_{max}/cm^{-1} ($CHCl_3$): 3034, 2957, 2929, 2857, 1760 (CO), 1444, 1290, 1134, 1099, 999, 838, 699; ^1H NMR ($CDCl_3$): δ 7.37–7.29 (5H, m, Ar), 5.34 (1H, dd, *J* = 5.9, 4.9 Hz, H-3), 5.20 (1H, dd, *J* = 4.6, 1.1 Hz, H-2), 5.15 (1H, d, *J* = 1.1 Hz, H-1), 4.77 (1H, d, *J* = 12.0 Hz, CH_2Ph), 4.53 (1H, d, *J* = 12.0 Hz, CH_2Ph), 4.22 (1H, *J* = 10.7, 4.9 Hz, H-4), 3.79–3.76 (8H, m, H-5, CH_3), 0.88 (9H, s, SiC(CH_3)$_3$), 0.06 (3H, s, Si(CH_3)$_2$), 0.06 (3H, s, Si(CH_3)$_2$); ^{13}C NMR ($CDCl_3$): δ 154.7 (CO), 137.0 (Ar), 128.4 (Ar), 127.9 (Ar), 127.8 (Ar), 103.7 (C-1), 80.9 (C-4), 78.0 (C-2), 75.4 (C-3), 69.5 (*C*H_2Ph), 63.8 (C-5), 55.2 (*C*H_3), 55.1 (*C*H_3), 25.8 (SiC(*C*H_3)$_3$), 18.2 (Si*C*(CH$_3$)$_3$), −5.5 (Si(*C*H_3)$_2$), −5.5 (Si(*C*H_3)$_2$); HRMS *m*/*z* 493.18634 (calc. for $C_{22}H_{34}O_9NaSi$ ([M + Na]$^+$): 493.18643).

1-*O*-Benzyl-2,3-*O*-bis(ethylcarbonate)-5-*O*-*tert*-butyldimethylsilyl-β-D-ribofuranoside 5b. For the reaction, 270 mg (0.76 mmol) of ribofuranoside **4** and ethyl chloroformate reagent was used.

Purification by flash chromatography (isocratic 9/1, v/v, hexane–EtOAc) gave 1-*O*-benzyl-2,3-*O*-bis(ethylcarbonate)-5-*O*-*tert*-butyldimethylsilyl-β-D-ribofuranoside **5b** (287 mg, 76%) as a colorless oil.

$[\alpha]_D^{20}$ = −34.5° (*c* 0.67, $CHCl_3$); IR: ν_{max}($CHCl_3$): 3029, 2956, 2929, 2858, 1756 (CO), 1464, 1373, 1282, 1094, 1007, 950, 838, 699; ^1H NMR ($CDCl_3$): δ 7.40–7.27 (5H, m, Ar), 5.33 (1H, dd, *J* = 6.2, 4.8 Hz, H-3), 5.19 (1H, dd, *J* = 4.8, 1.5 Hz, H-2), 5.15 (1H, d, *J* = 1.5 Hz, H-1), 4.77 (1H, d, *J* = 11.9 Hz, CH_2Ph), 4.53 (1H, d, *J* = 11.9 Hz, CH_2Ph), 4.25–4.17 (5H, m, H-4, CH_2CH$_3$), 3.78 (2H, dd, *J* = 4.4, 3.9 Hz, H-5), 1.30 (6H, t, *J* = 7.1 Hz, CH$_2$CH_3), 0.88 (9H, s, SiC(CH_3)$_3$), 0.06 (3H, s, Si(CH_3)$_2$), 0.05 (3H, s, Si-(CH_3)$_2$); ^{13}C NMR ($CDCl_3$): δ 154.1 (CO), 154.0 (CO), 137.0 (Ar), 128.4 (Ar), 127.9 (Ar), 127.8 (Ar), 103.7 (C-1), 80.9 (C-4), 77.8 (C-2), 75.1 (C-3), 69.4 (*C*H_2Ph), 64.5 (*C*H_2CH$_3$), 63.8 (C-5), 25.8 (SiC(*C*H_3)$_3$), 18.2 (Si*C*(CH$_3$)$_3$), 14.3 (CH$_2$*C*H_3), 14.2 (CH$_2$*C*H_3), −5.5 (Si(*C*H_3)$_2$), −5.5 (Si(*C*H_3)$_2$); HRMS *m*/*z* 521.21766 (calc. for $C_{24}H_{38}O_9NaSi$ ([M + Na]$^+$): 521.21773).

1-*O*-Benzyl-2,3-*O*-bis(isobutylcarbonate)-5-*O*-*tert*-butyldimethylsilyl-β-D-ribofuranoside 5c. For the reaction, 1.04 g (2.93 mmol) of ribofuranoside **4** and isobutyl chloroformate reagent was used.

Purification by flash chromatography (isocratic 95/5, v/v, hexane–EtOAc) gave 1-*O*-benzyl-2,3-*O*-bis(isobutylcarbonate)-5-*O*-*tert*-butyldimethylsilyl-β-D-ribofuranoside **5c** (1.57 g, 97%) as a white solid.

m.p.: 65–66 °C; $[\alpha]_D^{20}$ = −30.8° (*c* 0.35, $CHCl_3$); IR: ν_{max}/cm^{-1} ($CHCl_3$): 3035, 2958, 2929, 2857, 1754 (CO), 1471, 1381, 1346, 1283, 1132, 1101, 1050, 998, 839, 698; ^1H NMR ($CDCl_3$): δ 7.37–7.27 (5H, m, Ar), 5.33 (1H, dd, *J* = 6.2, 4.9 Hz, H-3), 5.20 (1H, dd, *J* = 4.9, 1.6 Hz, H-2), 5.16 (1H, d, *J* = 1.6 Hz, H-1), 4.77 (1H, d, *J* = 11.9 Hz, CH_2Ph), 4.53 (1H, d, *J* = 11.9 Hz, CH_2Ph), 4.25 (1H, td, *J* = 6.2, 4.9 Hz, H-4), 3.95 (2H, ddd, *J* = 10.3, 6.7, 4.8 Hz, CH_2CH(CH$_3$)$_2$), 3.89 (2H, ddd, *J* = 10.3, 6.7, 1.9 Hz, CH_2CH(CH$_3$)$_2$), 3.79 (1H, dd, *J* = 10.8, 4.7 Hz, H-5a), 3.75 (1H, dd, *J* = 10.8, 4.9 Hz, H-5b), 1.95 (2H, doublet of heptuplet, *J* = 6.7, 1.1 Hz, CH$_2$C*H*(CH$_3$)$_2$), 0.95–0.92 (12H, m, CH$_2$CH(CH_3)$_2$), 0.88 (9H, s, SiC(CH_3)$_3$), 0.06 (3H, s, Si(CH_3)$_2$), 0.05 (3H, s, Si-(CH_3)$_2$); ^{13}C NMR ($CDCl_3$): δ 154.3 (CO), 154.2 (CO), 137.0 (Ar), 128.4 (Ar), 127.9 (Ar), 103.7 (C-1), 80.9 (C-4), 77.8 (C-2), 75.2 (C-3), 74.6 (*C*H$_2$CH(CH$_3$)$_2$), 74.5 (*C*H$_2$CH(CH$_3$)$_2$), 69.4 (*C*H_2Ph), 63.8 (C-5), 27.7 (CH$_2$*C*H(CH$_3$)$_2$), 27.7 (CH$_2$*C*H(CH$_3$)$_2$), 25.8 (SiC(*C*H_3)$_3$), 18.8 (CH$_2$CH(*C*H$_3$)$_2$), 18.8 (CH$_2$*C*H(CH$_3$)$_2$), 18.8 (CH$_2$*C*H(CH$_3$)$_2$), 18.2 (Si*C*(CH$_3$)$_3$), −5.5 (Si(*C*H_3)$_2$), −5.5 (Si(*C*H_3)$_2$); HRMS *m*/*z* 577.28017 (calc. for $C_{28}H_{46}O_9NaSi$ ([M + Na]$^+$): 577.28033).

1-*O*-Benzyl-2,3-*O*-carbonate-5-*O*-*tert*-butyldimethylsilyl-β-D-ribofuranoside 5d. A solution of 1-*O*-benzyl-5-*O*-*tert*-butyldimethylsilyl-β-D-ribofuranoside **4** (1.00 g, 2.80 mmol) and dibutyltin oxide (0.77 g, 3.10 mmol) in toluene (50 mL) was refluxed under Dean Stark overnight. The reaction mixture was then cooled down to room temperature and tetra-

n-butylammonium bromide (1.00 g, 3.10 mmol) was added. The reaction mixture was cooled down to 0 °C and stirred for 10 min before methyl chloroformate was added (the same product was also obtained when ethyl chloroformate or isobutyl chloroformate was used as the reagent). The reaction mixture was stirred for 2 h, and then it was diluted with chloroform, washed with a solution of $NaHCO_3$, water and brine, dried and evaporated.

Purification by flash chromatography (gradient 97/3 to 93/7, v/v, hexane–EtOAc) gave 1-*O*-benzyl-2,3-*O*-carbonate-5-*O*-*tert*-butyldimethylsilyl-β-D-ribofuranoside **5d** (840 mg, 78%) as a colorless oil.

$[\alpha]_D^{20} = -98.1°$ (*c* 0.32, $CHCl_3$); IR: ν_{max}/cm^{-1} ($CHCl_3$): 3033, 2956, 2931, 2859, 1809 (CO), 1472, 1372, 1259, 1161, 1089, 1012, 839, 699; ^1H NMR ($CDCl_3$): δ 7.39–7.27 (5H, m, Ar), 5.30 (1H, s, H-1), 5.16 (1H, d, J = 6.8 Hz, H-3), 5.04 (1H, d, J = 6.8 Hz, H-2), 4.68 (1H, d, J = 11.5 Hz, CH_2Ph), 4.49 (1H, d, J = 11.5 Hz, CH_2Ph), 4.41 (1H, dd, J = 10.0, 5.5 Hz, H-4), 3.72 (1H, dd, J = 10.2, 5.5 Hz, H-5a), 3.61 (1H, t, J = 10.2 Hz, H-5b), 0.91 (9H, s, SiC(CH_3)$_3$), 0.06 (3H, s, Si(CH_3)$_2$), 0.05 (3H, s, Si(CH_3)$_2$); ^{13}C NMR ($CDCl_3$): δ 155.3 (CO), 136.1 (Ar), 128.6 (Ar), 128.3 (Ar), 128.2 (Ar), 105.7 (C-1), 86.2 (C-4), 83.6 (C-2), 81.0 (C-3), 69.8 (*C*H$_2$Ph), 62.4 (C-5), 25.7 (SiC(*C*H$_3$)$_3$), 18.1 (Si*C*(CH$_3$)$_3$), −5.4 (Si(*C*H$_3$)$_2$), −5.5 (Si(*C*H$_3$)$_2$); HRMS *m*/*z* 403.15466 (calc. for $C_{19}H_{28}O_6NaSi$ ([M + Na]$^+$): 403.15474).

General procedure for the synthesis of alkylcarbonate ribofuranosides 6a–c[11]

To a solution of 1,2-*O*-isopropylidene-5-*O*-*tert*-butyldimethylsilyl-β-D-ribofuranoside (1 × *n*; prepared as reported previously[10]), DMAP (0.2 × *n*) and pyridine (1.2 × *n*) in DCM was added alkyl chloroformate (1.2 × *n*) at 0 °C. The reaction mixture was stirred overnight and allowed to warm up to room temperature, and then it was diluted with DCM, washed with a solution of $CuSO_4$, $NaHCO_3$, water and brine, dried and evaporated.

5-*O*-*tert*-Butyldimethylsilyl-1,2-*O*-isopropylidene-3-*O*-methylcarbonate-α-D-ribofuranoside 6a. For the reaction was used 1.00 g (3.30 mmol) of 5-*O*-*tert*-butyldimethylsilyl-1,2-*O*-isopropylidene-α-D-ribofuranoside and methyl chloroformate reagent.

Purification by silica gel column chromatography (gradient from 85/15 to 65/45, v/v, hexane–EtOAc) afforded 5-*O*-*tert*-butyldimethylsilyl-1,2-*O*-isopropylidene-3-*O*-methylcarbonate-α-D-ribofuranoside **6a** (1.00 g, 83%) as a white solid.

The spectroscopic data corresponded to those previously reported.[11]

5-*O*-*tert*-Butyldimethylsilyl-1,2-*O*-isopropylidene-3-*O*-ethylcarbonate-α-D-ribofuranoside 6b. For the reaction was used 510 mg (1.70 mmol) of 5-*O*-*tert*-butyldimethylsilyl-1,2-*O*-isopropylidene-α-D-ribofuranoside and ethyl chloroformate reagent.

Purification by silica gel column chromatography (gradient from 95/5 to 9/1, v/v, hexane–EtOAc) afforded 5-*O*-*tert*-butyldimethylsilyl-1,2-*O*-isopropylidene-3-*O*-ethylcarbonate-α-D-ribofuranoside **6b** (460 mg, 75%) as a colorless oil.

The spectroscopic data corresponded to those previously reported.[11]

5-*O*-*tert*-Butyldimethylsilyl-1,2-*O*-isopropylidene-3-*O*-isobutylcarbonate-α-D-ribofuranoside 6c. For the reaction was used 330 mg (1.08 mmol) of 5-*O*-*tert*-butyldimethylsilyl-1,2-*O*-isopropylidene-α-D-ribofuranoside and isobutyl chloroformate reagent.

Purification by silica gel column chromatography (gradient from 95/5 to 9/1, v/v, hexane–EtOAc) afforded 5-*O*-*tert*-butyldimethylsilyl-1,2-*O*-isopropylidene-3-*O*-isobutylcarbonate-α-D-ribofuranoside **6c** (0.41 g, 94%) as a colorless oil.

The spectroscopic data corresponded to those previously reported.[11]

General procedure for TBDMS deprotection with CAN[9]

Ribofuranosides **5a–c** and **6a–c** (1 × *n*) were dissolved in AcCN–H_2O (9/1, v/v) and CAN (1.1 × *n*) was added. The reaction mixture was stirred for 3 h. Then it was diluted with chloroform and washed with a solution of $NaHCO_3$. The aqueous layer was extracted with $CHCl_3$ (3×) and the combined organic fractions were dried, filtered and evaporated.

1-*O*-Benzyl-2,3-*O*-bis(methylcarbonate)-β-D-ribofuranoside 7a. For the reaction, 246 mg (0.52 mmol) of ribofuranoside **5a** was used.

Purification by flash chromatography (isocratic 98/2, v/v, $CHCl_3$–EtOH) gave 1-*O*-benzyl-2,3-*O*-bis(methylcarbonate)-β-D-ribofuranoside **7a** (172 mg, 91%) as a colorless oil.

$[\alpha]_D^{20} = -37.4°$ (*c* 0.61, $CHCl_3$); IR: ν_{max}/cm^{-1} ($CHCl_3$): 3029, 2959, 2929, 2857, 1761 (CO), 1444, 1289, 1150, 1092, 950, 699; ^1H NMR ($CDCl_3$): δ 7.40–7.30 (5H, m, Ar), 5.35 (1H, dd, J = 6.5, 4.9 Hz, H-3), 5.21 (1H, d, J = 4.9 Hz, H-2), 5.19 (1H, s, H-1), 4.77 (1H, d, J = 11.8 Hz, CH_2Ph), 4.60 (1H, d, J = 11.8 Hz, CH_2Ph), 4.30 (1H, td, J = 6.5, 3.4 Hz, H-4), 3.86–3.80 (7H, m, H-5a, CH_3), 3.67 (1H, ddd, J = 12.3, 8.7, 3.8 Hz, H-5b), 2.04 (1H, dd, J = 8.7, 4.5 Hz, OH); ^{13}C NMR ($CDCl_3$): δ 154.8 (CO), 154.7 (CO), 136.6 (Ar), 128.6 (Ar), 128.2 (Ar), 104.3 (C-1), 81.7 (C-4), 78.3 (C-2), 74.2 (C-3), 70.7 (*C*H$_2$Ph), 62.4 (C-5), 55.3 (*C*H$_3$), 55.3 (*C*H$_3$); HRMS *m*/*z* 379.09991 (calc. for $C_{16}H_{20}O_9Na$ ([M + Na]$^+$): 379.09995).

1-*O*-Benzyl-2,3-*O*-bis(ethylcarbonate)-β-D-ribofuranoside 7b. For the reaction, 287 mg (0.58 mmol) of ribofuranoside **5b** was used.

Purification by flash chromatography (isocratic 98/2, v/v, $CHCl_3$–EtOH) gave 1-*O*-benzyl-2,3-*O*-bis(ethylcarbonate)-β-D-ribofuranoside **7b** (190 mg, 86%) as a colorless oil.

$[\alpha]_D^{20} = -32.0°$ (*c* 0.34, $CHCl_3$); IR: ν_{max}/cm^{-1} ($CHCl_3$): 3029, 2955, 2928, 2856, 1756 (CO), 1374, 1281, 1093, 942, 880, 699; ^1H NMR ($CDCl_3$): δ 7.38–7.29 (5H, m, Ar), 5.34 (1H, t, J = 5.7 Hz, H-3), 5.22 (1H, d, J = 4.8 Hz, H-2), 5.19 (1H, s, H-1), 4.76 (1H, d, J = 11.8 Hz, CH_2Ph), 4.60 (1H, d, J = 11.8 Hz, CH_2Ph), 4.30 (1H, pentuplet, J = 3.7 Hz, H-4), 4.24–4.20 (4H, m, CH_2CH$_3$), 3.83 (1H, td, J = 12.2, 3.7 Hz, H-5a), 3.67 (1H, ddd, J = 12.2, 8.6, 3.7 Hz, H-5b), 2.04 (1H, dd, J = 8.6, 4.6 Hz, OH), 1.31 (6H, m, CH$_2$CH_3); ^{13}C NMR ($CDCl_3$): δ 154.2 (CO), 154.1 (CO), 136.6 (Ar), 128.6 (Ar), 128.1 (Ar), 128.0 (Ar), 104.3 (C-1), 81.7 (C-4), 78.1 (C-2), 74.0 (C-3), 70.4 (*C*H$_2$Ph),

64.7 (CH_2CH_3), 64.7 (CH_2CH_3), 62.5 (C-5), 14.1 (CH_2CH_3); HRMS *m*/*z* 407.13121 (calc. for $C_{18}H_{24}O_9Na$ ($[M + Na]^+$): 407.13125).

1-*O*-Benzyl-2,3-*O*-bis(isobutylcarbonate)-β-D-ribofuranoside 7c. For the reaction, 1.57 g (2.83 mmol) of ribofuranoside **5c** was used.

Purification by flash chromatography (isocratic 98/2, v/v, $CHCl_3$–EtOH) gave 1-*O*-benzyl-2,3-*O*-bis(isobutylcarbonate)-β-D-ribofuranoside **7c** (1.07 g, 86%) as a colorless oil.

$[\alpha]_D^{20} = -35.4°$ (*c* 0.53, $CHCl_3$); IR: ν_{max}/cm^{-1} ($CHCl_3$): 3034, 2962, 2930, 2877, 1755 (CO), 1456, 1381, 1282, 1091, 1045, 1011, 971, 699; ^{1}H NMR ($CDCl_3$): δ 7.38–7.29 (5H, m, Ar), 5.34 (1H, dd, *J* = 6.4, 4.9 Hz, H-3), 5.22 (1H, dd, *J* = 4.9, 0.8 Hz, H-2), 5.19 (1H, d, *J* = 0.8 Hz, H-1), 4.77 (1H, d, *J* = 11.8 Hz, CH_2Ph), 4.61 (1H, d, *J* = 11.8 Hz, CH_2Ph), 4.32 (1H, ddd, *J* = 6.8, 3.6, 3.2 Hz, H-4), 3.96 (2H, dd, *J* = 10.4, 6.8 Hz, CH_2CH(CH_3)$_2$), 3.89 (2H, ddd, *J* = 10.4, 6.8, 1.9 Hz, CH_2CH(CH_3)$_2$), 3.83 (1H, ddd, *J* = 12.2, 3.8, 3.6 Hz, H-5a), 3.67 (1H, ddd, *J* = 12.2, 8.6, 3.8 Hz, H-5b), 2.07 (1H, dd, *J* = 8.6, 3.6 Hz, 5-OH), 1.98 (2H, doublet of pentuplet, *J* = 6.7, 4.2 Hz, $CH_2CH(CH_3)_2$), 0.94 (12H, d, *J* = 6.7 Hz, $CH_2CH(CH_3)_2$); ^{13}C NMR ($CDCl_3$): δ 154.4 (CO), 154.3 (CO), 136.7 (Ar), 128.6 (Ar), 128.1 (Ar), 128.0 (Ar), 104.4 (C-1), 81.8 (C-4), 78.1 (C-2), 74.7 ($CH_2CH(CH_3)_2$), 74.7 ($CH_2CH(CH_3)_2$), 74.1 (C-3), 70.4 (CH_2Ph), 62.5 (C-5), 27.7 ($CH_2CH(CH_3)_2$), 18.8 ($CH_2CH(CH_3)_2$); HRMS *m*/*z* 463.19380 (calc. for $C_{22}H_{32}O_9Na$ ($[M + Na]^+$): 463.19385).

1,2-*O*-Isopropylidene-3-*O*-methylcarbonate-α-D-ribofuranoside 8a. For the reaction was used 210 mg (0.58 mmol) of 5-*O*-*tert*-butyldimethylsilyl-1,2-*O*-isopropylidene-3-*O*-methylcarbonate-α-D-ribofuranoside **6a**.

Purification by silica gel column chromatography (gradient from 6/4 to 1/1, v/v, hexane–EtOAc) gave 1,2-*O*-isopropylidene-3-*O*-methylcarbonate-α-D-ribofuranoside **8a** (137 mg, 95%) as a white solid.

The spectroscopic data corresponded to those previously reported.[11]

1,2-*O*-Isopropylidene-3-*O*-ethylcarbonate-α-D-ribofuranoside 8b. For the reaction was used 148 mg (0.39 mmol) of 5-*O*-*tert*-butyldimethylsilyl-1,2-*O*-isopropylidene-3-*O*-ethylcarbonate-α-D-ribofuranoside **6b**.

Purification by silica gel column chromatography (gradient from 100/0 to 99/1, v/v, DCM–EtOH) afforded 1,2-*O*-isopropylidene-3-*O*-ethylcarbonate-α-D-ribofuranoside **8b** (99.0 mg, 96%) as a colorless oil.

The spectroscopic data corresponded to those previously reported.[11]

1,2-*O*-Isopropylidene-3-*O*-isobutylcarbonate-α-D-ribofuranoside 8c. For the reaction was used 70.0 mg (0.17 mmol) of 5-*O*-*tert*-butyldimethylsilyl-1,2-*O*-isopropylidene-3-*O*-isobutylcarbonate-α-D-ribofuranoside **6c**.

The purification by silica gel column chromatography (gradient from 100/0 to 99/1, v/v, DCM–EtOH) afforded 1,2-*O*-isopropylidene-3-*O*-ethylcarbonate-α-D-ribofuranoside **8c** (48.0 mg, 96%) as a colorless oil.

The spectroscopic data corresponded to those previously reported.[11]

Bisbenzyloxy-diisopropylamino phosphine.[13] A solution of freshly distilled diisopropylamine (16 mL, 114.6 mmol) in dry hexane (40 mL) was added dropwise to a solution of PCl_3 (5 mL, 57.3 mmol) in dry hexane (40 mL) at 0 °C. The reaction mixture was allowed to warm up to room temperature while stirring overnight. A solution of dry BnOH (12 mL, 114.6 mmol) and dry Et_3N (16 mL, 114.6 mmol) in dry Et_2O (50 mL) was then added and stirring was continued for 1 hour. The reaction mixture was then filtered and washed with Et_2O. The filtrate was washed with a solution of $NaHCO_3$, water and brine, dried and evaporated. The residue obtained was dissolved in hexane (100 mL), washed with AcCN (3 × 80 mL), and the hexane layer was dried and evaporated to afford bisbenzyloxy-diisopropylamino phosphine (2.9 g, 15%) as a colourless oil.

The spectroscopic data corresponded to those previously reported.

General procedure for phosphorylation[17]

A solution of 2,4-DNP (1.1 × *n*) in dry DCM was added dropwise to a solution of furanoside (1 × *n*) and dibenzylphosphoramidate (1.1 × *n*) in dry DCM. The resulting reaction mixture was stirred overnight at room temperature, and then *t*BuOOH (2 × *n*, 5.5 M in decane) was added and stirring was continued for another 20 minutes. Afterwards, the reaction mixture was diluted with DCM, washed with a saturated solution of $Na_2S_2O_3$ and water (3×), dried, filtered and evaporated.

5-*O*-Dibenzylphosphate-1-*O*-benzyl-2,3-*O*-bis(methylcarbonate)-β-D-ribofuranoside 9a. For the reaction, 132 mg (0.41 mmol) of ribofuranoside **7a** was used.

Purification by flash chromatography (gradient, from 3/1 to 2/1, v/v, hexane–EtOAc) gave 5-*O*-dibenzylphosphate-1-*O*-benzyl-2,3-*O*-bis(methylcarbonate)-β-D-ribofuranoside **9a** (200 mg, 88%) as a colorless oil.

$[\alpha]_D^{20} = -27.4°$ (*c* 0.29, $CHCl_3$); IR: ν_{max}/cm^{-1} ($CHCl_3$): 3035, 2959, 2927, 2856, 1762 (CO), 1498, 1456, 1378, 1289, 1097, 1001, 951, 882, 698; ^{1}H NMR ($CDCl_3$): δ 7.36–7.28 (15H, m, Ar), 5.30 (1H, dd, *J* = 6.7, 5.0 Hz, H-3), 5.19 (1H, d, *J* = 4.6 Hz, H-2), 5.12 (1H, s, H-1), 5.03 (4H, d, *J* = 7.6, 5.8 Hz, CH_2Ph), 4.74 (1H, d, *J* = 11.8 Hz, CH_2Ph), 4.47 (1H, d, *J* = 11.8 Hz, CH_2Ph), 4.33 (1H, dd, *J* = 10.8, 5.0 Hz, H-4), 4.23–4.13 (2H, m, H-5), 3.79 (1H, s, CH_3), 3.77 (1H, s, CH_3); ^{13}C NMR ($CDCl_3$): δ 154.7 (CO), 154.5 (CO), 128.5 (Ar), 128.4 (Ar), 128.0 (Ar), 127.9 (Ar), 103.7 (C-1), 78.6 (d, *J* 8.3 Hz, C-4), 77.6 (C-2), 74.5 (C-3), 69.5 (CH_2Ph), 69.5 (CH_2Ph), 69.4 (CH_2Ph), 67.2 (d, *J* 5.5 Hz, C-5), 55.3 (CH_3), 55.3 (CH_3); ^{31}P NMR ($CDCl_3$): δ −1.10; HRMS *m*/*z* 639.16026 (calc. for $C_{30}H_{33}O_{12}NaP$ ($[M + Na]^+$): 639.16018).

5-*O*-Dibenzylphosphate-1-*O*-benzyl-2,3-*O*-bis(ethylcarbonate)-β-D-ribofuranoside 9b. For the reaction, 190 mg (0.49 mmol) of ribofuranoside **7b** was used.

Purification by flash chromatography (gradient, from 3/1 to 7/3, v/v, hexane–EtOAc) gave 5-*O*-dibenzylphosphate-1-*O*-benzyl-2,3-*O*-bis(ethylcarbonate)-β-D-ribofuranoside **9b** (306 mg, 96%) as a colorless oil.

$[\alpha]_D^{20} = -22.2°$ (*c* 0.33, $CHCl_3$); IR: ν_{max}/cm^{-1} ($CHCl_3$): 3035, 2955, 2927, 2855, 1757 (CO), 1498, 1456, 1374, 1280, 1094,

1001, 882, 697; ^{1}H NMR ($CDCl_3$): δ 7.36–7.28 (15H, m, Ar), 5.30 (1H, dd, J = 7.0, 4.7 Hz, H-3), 5.20 (1H, d, J = 4.7 Hz, H-2), 5.13 (1H, s, H-1), 5.03 (4H, d, J = 7.8, 5.6 Hz, CH_2Ph), 4.73 (1H, d, J = 11.8 Hz, CH_2Ph), 4.46 (1H, d, J = 11.8 Hz, CH_2Ph), 4.35 (1H, bdd, J = 11.2, 4.7 Hz, H-4), 4.20–4.13 (4H, m, H-5, CH_2CH$_3$), 4.13–4.06 (2H, m, CH_2CH$_3$), 1.34–1.27 (6H, m, CH$_2$CH_3); ^{13}C NMR ($CDCl_3$): δ 154.0 (CO), 153.9 (CO), 136.6 (Ar), 128.7 (Ar), 128.5 (Ar), 128.4 (Ar), 128.0 (Ar), 127.9 (Ar), 103.7 (C-1), 78.7 (d, J = 8.0 Hz, C-4), 77.4 (C-2), 74.3 (C-3), 69.5 (*C*H$_2$Ph), 69.4 (*C*H$_2$Ph), 67.3 (dd, J = 5.4, 5.2 Hz, C-5), 64.7 (*C*H$_2$CH$_3$), 64.7 (*C*H$_2$CH$_3$), 14.1 (CH$_2$*C*H$_3$); ^{31}P NMR ($CDCl_3$): δ –1.11; HRMS *m*/*z* 639.16026 (calc. for $C_{30}H_{33}O_{12}NaP$ ([M + Na]$^+$): 639.16018).

5-*O*-Dibenzylphosphate-1-*O*-benzyl-2,3-*O*-bis(isobutylcarbonate)-β-D-ribofuranoside 9c. For the reaction, 1.07 g (2.43 mmol) of ribofuranoside **7c** was used.

Purification by flash chromatography (gradient, from 3/1 to 7/3, v/v, hexane–EtOAc) gave 5-*O*-dibenzylphosphate-1-*O*-benzyl-2,3-*O*-bis(isobutylcarbonate)-β-D-ribofuranoside **9c** (1.51 g, 89%) as a colorless oil.

$[\alpha]_D^{20}$ = −21.4° (*c* 0.72, $CHCl_3$); IR: ν_{max}/cm^{-1} ($CHCl_3$): 3035, 2929, 2856, 1756 (CO), 1498, 1471, 1456, 1381, 1280, 1034, 1001, 971, 919, 697; ^{1}H NMR ($CDCl_3$): δ 7.37–7.28 (15H, m, Ar), 5.29 (1H, dd, J = 7.0, 4.8 Hz, H-3), 5.20 (1H, d, J = 4.8 Hz, H-2), 5.14 (1H, s, H-1), 5.03 (4H, d, J = 8.0, 5.3 Hz, CH_2Ph), 4.74 (1H, d, J = 11.8 Hz, CH_2Ph), 4.47 (1H, d, J = 11.8 Hz, CH_2Ph), 4.39–4.34 (1H, m, H–4), 4.21 (1H, dd, J = 10.3, 6.2, 2.7 Hz, H-5a), 4.14 (1H, dd, J = 10.3, 6.2 Hz, H-5b), 3.95 (2H, ddd, J = 10.3, 6.7, 2.7 Hz, CH_2CH(CH$_3$)$_2$), 3.87 (2H, dt, J = 10.3, 6.7 Hz, CH_2CH(CH$_3$)$_2$), 1.96 (2H, doublet of hexuplet, J = 6.7, 2.3 Hz, CH$_2$C*H*(CH$_3$)$_2$), 0.94 (6H, dd, J = 6.7, 0.7 Hz, CH_2CH(CH$_3$)$_2$), 0.93 (6H, d, J = 6.7 Hz, CH_2CH(CH$_3$)$_2$); ^{13}C NMR ($CDCl_3$): δ 154.2 (CO), 154.1 (CO), 136.6 (Ar), 128.5 (Ar), 128.4 (Ar), 128.0 (Ar), 127.9 (Ar), 103.8 (C-1), 78.7 (d, J = 8.2 Hz, C-4), 77.4 (C-2), 74.7 (*C*H$_2$CH(CH$_3$)$_2$), 74.7 (*C*H$_2$CH(CH$_3$)$_2$), 74.4 (C-3), 69.5 (*C*H$_2$Ph), 69.4 (*C*H$_2$Ph), 67.2 (d, J = 5.5 Hz, C-5), 27.7 (CH$_2$*C*H(CH$_3$)$_2$), 27.7 (CH$_2$*C*H(CH$_3$)$_2$), 18.8 (CH$_2$CH(*C*H$_3$)$_2$), 18.8 (CH$_2$CH(*C*H$_3$)$_2$); ^{31}P NMR ($CDCl_3$): δ –1.11; HRMS *m*/*z* 723.25411 (calc. for $C_{36}H_{45}O_{12}NaP$ ([M + Na]$^+$): 723.25408).

5-*O*-Dibenzylphosphate-1,2-*O*-isopropylidene-3-*O*-methylcarbonate-α-D-ribofuranoside 10a. For the reaction, 1.20 g (4.83 mmol) of ribofuranoside **8a** was used.

Purification by chromatography (gradient from 6/4 to 4/6, v/v, hexane–EtOAc) yielded 1,2-*O*-isopropylidene-5-*O*-dibenzylphosphate-3-*O*-methylcarbonate-α-D-ribofuranoside **10a** (2.0 g, 81.0%) as a yellow oil.

$[\alpha]_D^{20}$ = +48.1° (*c* 0.09, $CHCl_3$); IR: ν_{max}/cm^{-1} ($CHCl_3$): 3030, 2959, 1756 (CO), 1498, 1456, 1444, 1385, 1376, 1277, 1114, 1014, 874, 697; ^{1}H NMR ($CDCl_3$): δ 7.41–7.30 (10H, m, Ar), 5.73 (1H, d, J = 3.7 Hz, H-1), 5.05 (1H, d, J = 8.1 Hz, CH_2Ph), 5.04 (1H, dd, J = 8.1, 4.2 Hz, CH_2Ph), 4.77 (1H, t, J = 4.2 Hz, H-2), 4.67 (1H, dd, J = 8.8, 4.8 Hz, H-3), 4.30 (1H, ddd, J = 11.6, 6.1, 2.4 Hz, H-5), 4.27–4.23 (1H, m, H-4), 4.14–4.06 (1H, m, H-5), 3.80 (3H, s, CH_3), 1.53 (3H, s, C(CH_3)$_2$), 1.33 (3H, s, C(CH_3)$_2$); ^{13}C NMR ($CDCl_3$): δ 154.6 (CO), 135.7 (d, J = 6.8 Hz, Ar), 128.6 (Ar), 128.5 (Ar), 128.0 (Ar), 127.9 (Ar), 113.5 (*C*(CH$_3$)$_2$), 104.2 (C-1), 77.2 (C-2), 76.1 (d, J = 7.6 Hz, C-4), 74.2 (C-3), 69.4 (dd, J = 5.4, 3.0 Hz, *C*H$_2$Ph), 65.0 (d, J = 5.2 Hz, C-5), 55.2 (*C*H$_3$), 26.6 (C(*C*H$_3$)$_2$); ^{31}P NMR ($CDCl_3$): δ 0.26; HRMS *m*/*z* 493.1178 (calc. for $C_{23}H_{26}O_{10}P$ ([M − CH$_3$]$^+$): 493.1264).

5-*O*-Dibenzylphosphate-1,2-*O*-isopropylidene-3-*O*-ethylcarbonate-α-D-ribofuranoside 10b. For the reaction, 65.0 mg (0.25 mmol) of ribofuranoside **8b** was used.

Purification by flash chromatography (gradient 100/0 to 98/2, v/v, DCM–MeOH) yielded 5-*O*-dibenzylphosphate-1,2-*O*-isopropylidene-3-*O*-ethylcarbonate-α-D-ribofuranoside **10b** (88.0 mg, 68%) as a yellow oil.

$[\alpha]_D^{20}$ = +56.0° (*c* 0.43, $CHCl_3$); IR: ν_{max}/cm^{-1} ($CHCl_3$): 3034, 2928 2856, 1751 (CO), 1456, 1376, 1268, 1115, 1015, 873, 697; ^{1}H NMR ($CDCl_3$): δ 7.35–7.33 (10H, m, Ar), 5.74 (1H, d, J = 3.7 Hz, H-1), 5.07–5.04 (4H, m, CH_2Ph), 4.77 (1H, dd, J = 4.4, 4.0 Hz, H-2), 4.68 (1H, dd, J = 8.8, 4.8 Hz, H-3), 4.30 (1H, ddd, J = 11.6, 6.2, 2.4 Hz, H-5a), 4.28–4.25 (1H, m, H-4), 4.21 (2H, q, J = 7.2 Hz, CH_2CH$_3$), 4.09 (1H, ddd, J = 11.6, 6.6, 3.6 Hz, H-5b), 1.54 (3H, s, C(CH_3)$_2$), 1.34 (3H, s, C(CH_3)$_2$), 1.32 (3H, t, J = 7.2 Hz, CH$_2$CH_3); ^{13}C NMR ($CDCl_3$): δ 154.0 (CO), 135.7 (Ar), 128.6 (Ar), 128.5 (Ar), 128.0 (Ar), 127.9 (Ar), 113.5 (*C*(CH$_3$)$_2$), 104.2 (C-1), 77.2 (C-2), 76.1 (d, J = 6.2 Hz, C-4), 74.0 (C-3), 69.4 (dd, J = 5.4, 3.0 Hz, *C*H$_2$Ph), 65.0 (d, J = 5.4 Hz, C-5), 64.7 (*C*H$_2$CH$_3$), 26.6 (C(*C*H$_3$)$_2$), 26.6 (C(*C*H$_3$)$_2$), 14.1 (CH$_2$*C*H$_3$); ^{31}P NMR ($CDCl_3$): δ –1.02; HRMS *m*/*z* 545.15443 (calc. for $C_{25}H_{31}O_{10}NaP$ ([M + Na]$^+$): 545.15470).

5-*O*-Dibenzylphosphate-1,2-*O*-isopropylidene-3-*O*-isobutylcarbonate-α-D-ribofuranoside 10c. For the reaction, 48.0 mg (165 μmol) of ribofuranoside **8c** was used.

Purification by flash chromatography (gradient 8/2 to 7/3, v/v, hexane–EtOAc) yielded 5-*O*-dibenzylphosphate-1,2-*O*-isopropylidene-3-*O*-isobutylcarbonate-α-D-ribofuranoside **10c** (70.0 mg, 77%) as a yellow oil.

$[\alpha]_D^{20}$ = +43.0° (*c* 1.25, $CHCl_3$); IR: ν_{max}/cm^{-1} ($CHCl_3$): 3032, 2964, 2877, 1747 (CO), 1498, 1456, 1385, 1377, 1277, 1168, 1134, 1096, 1014, 918, 697; ^{1}H NMR ($CDCl_3$): δ 7.37–7.35 (10H, m, Ar), 5.74 (1H, d, J = 3.6 Hz, H-1), 5.11–5.08 (4H, m, CH_2Ph), 4.64 (1H, t, J = 4.1 Hz, H-2), 4.46 (1H, ddd, J = 9.0, 6.8, 4.6 Hz, H-3), 4.41 (1H, dd, J = 12.1, 2.2 Hz, H-5a), 4.23 (1H, ddd, J = 9.0, 4.5, 2.3 Hz, H-4), 4.16 (1H, dd, J = 12.0, 4.5 Hz, H-5b), 3.93–3.85 (2H, m, CH_2CH(CH$_3$)$_2$), 1.96 (1H, d, J = 6.7 Hz, CH$_2$C*H*(CH$_3$)$_2$), 1.55 (3H, s, C(CH_3)$_2$), 1.30 (3H, s, C(CH_3)$_2$), 0.92 (6H, d, J = 6.7 Hz, CH$_2$CH(CH_3)$_2$); ^{13}C NMR ($CDCl_3$): δ155.6 (CO), 128.7 (Ar), 128.6 (Ar), 128.0 (Ar), 127.9 (Ar), 110.0 (*C*(CH$_3$)$_2$), 103.9 (C-1), 77.7 (C-2), 75.8 (d, J = 10.3 Hz, C-4), 74.5 (C-3), 74.3 (*C*H$_2$CH(CH$_3$)$_2$), 69.7 (m, *C*H$_2$Ph), 64.9 (m, C-5), 27.7 (CH$_2$*C*H(CH$_3$)$_2$), 26.7 (C(*C*H$_3$)$_2$), 26.5 (C(*C*H$_3$)$_2$), 18.9 (CH$_2$CH-(*C*H$_3$)$_2$); ^{31}P NMR ($CDCl_3$): δ –1.97; HRMS *m*/*z* 573.18585 (calc. for $C_{27}H_{35}O_{10}NaP$ ([M + Na]$^+$): 573.18600).

Deprotection of the isopropylidene group[12]

Furanoside (1 × *n*) was treated with a mixture of TFA–H_2O (4/1, 10 mL) at 0 °C for 2.5 hours. The reaction mixture was then slowly poured into a saturated solution of Na_2CO_3. The aqueous layer was extracted with DCM (3 × 40 mL) and the combined organic layers were washed with brine, dried, filtered and evaporated.

5-*O*-Dibenzylphosphate-2,3-*O*-carbonate-β-D-ribofuranose 9d. For the reaction, 500 mg (0.98 mmol) of ribofuranoside **10a** was used.

Purification by flash chromatography (gradient from 1/0 to 98/2, v/v, $CHCl_3$–EtOH) gave 5-*O*-dibenzylphosphate-2,3-*O*-carbonate-β-D-ribofuranose **9d** (275 mg, 67%) as a white solid. When ribofuranosides **10b** and **10c** were used as the starting material, the same product was obtained.

$[\alpha]_D^{20}$ = −12.7° (*c* 0.14, $CHCl_3$); Anal. calcd for $C_{20}H_{21}O_9P$: C 55.05, H 4.85; found: C 55.08, H 4.82%; IR: ν_{max}/cm^{-1} ($CHCl_3$): 3068, 3035, 2959, 2856, 1762 (CO), 1498, 1456, 1378, 1289, 1150, 1097, 951, 882, 698; ^{1}H NMR ($CDCl_3$): δ 7.39–7.32 (10H, m, Ar), 5.60 (1H, d, *J* = 2.2 Hz, H-1), 5.10 (2H, d, *J* = 8.6 Hz, C*H*$_2$Ph), 5.03 (2H, dd, *J* = 8.6, 5.7 Hz, C*H*$_2$Ph), 4.94 (2H, s, H-2, H-3), 4.49 (1H, dd, *J* = 8.5, 5.5 Hz, H-4), 4.26 (1H, dd, *J* = 11.3, 8.5 Hz, H-5a), 3.98 (1H, dt, *J* = 11.3, 5.5 Hz, H-5b); ^{13}C NMR ($CDCl_3$): δ 153.5 (CO), 135.3 (Ar), 128.9 (Ar), 128.7 (Ar), 128.1 (Ar), 101.8 (C-1), 84.0 (C-2), 83.9 (d, *J* 6.0 Hz, C-4), 80.0 (C-3), 70.0 (m, *C*H$_2$Ph), 67.2 (d, *J* = 5.4 Hz, C-5); ^{31}P NMR ($CDCl_3$): δ 1.19; HRMS *m*/*z* 436.0961 (calc. for $C_{20}H_{21}O_9P$ ($[M + H]^+$): 436.0923).

Removal of benzyl groups

Furanoside (1 × *n*) was dissolved in an appropriate solvent, poured into a hydrogenation tube to which 10% Pd/C (20 mg) was added. The hydrogenation was carried out in an autoclave under 10 bars of hydrogen pressure for 24 h. The reaction mixture was then filtered through celite, washed with MeOH and evaporated.

5-*O*-Phosphate-1-*O*-benzyl-2,3-*O*-bis(methylcarbonate)-β-D-ribofuranoside 12a. For the reaction, 387 mg (0.63 mmol) of ribofuranoside **9a** in THF was used.

The spectroscopic analyses revealed the product to be 5-*O*-phosphate-1-*O*-benzyl-2,3-*O*-bis(methylcarbonate)-β-D-ribofuranoside **12a** (217 mg, 79%).

$[\alpha]_D^{20}$ = −18.5° (*c* 0.71, MeOH); IR: ν_{max}/cm^{-1} (KBr): 3436 (NH), 2958, 2926, 1812, 1760 (CO), 1632 (CN), 1445, 1289, 1265, 1081, 946, 788; ^{1}H NMR (D_2O): δ 7.34–7.25 (5H, m, Ar), 5.27–5.24 (1H, m, H-3), 5.11–5.10 (2H, m, H-1, H-2), 4.77 (1H, d, *J* = 11.7 Hz, CH_2Ph), 4.51 (1H, d, *J* = 11.7 Hz, CH_2Ph), 4.28 (1H, br s, H-4), 4.20–3.97 (2H, m, H-5), 3.73 (6H, s, CH_3); ^{13}C NMR (D_2O): δ 156.4 (CO), 156.3 (CO), 138.6 (Ar), 129.6 (Ar), 129.5 (Ar), 129.1 (Ar), 105.2 (C-1), 80.8 (d, *J* = 6.9 Hz, C-4), 79.2 (C-2), 76.5 (C-3), 70.7 (CH_2Ph), 67.5 (m, C-5), 56.0 (*C*H$_3$); ^{31}P NMR (D_2O): δ 1.00; HRMS *m*/*z* 435.06981 (calc. for $C_{16}H_{20}O_{12}P$ ($[M - H]^+$): 435.06979).

5-*O*-Phosphate-1-*O*-benzyl-2,3-*O*-bis(ethylcarbonate)-β-D-ribofuranoside 12b. For the reaction, 245 mg (0.38 mmol) of ribofuranoside **9b** in THF was used.

The spectroscopic analyses revealed the product to be 5-*O*-phosphate-1-*O*-benzyl-2,3-*O*-bis(ethylcarbonate)-β-D-ribofuranoside **12b** (150 mg, 85%).

$[\alpha]_D^{20}$ = −103.4° (*c* 0.19, MeOH); ^{1}H NMR (MeOD): δ 7.27–7.17 (5H, m, Ar), 5.18 (1H, dd, *J* = 6.6, 4.5 Hz, H-3), 5.05–5.04 (2H, m, H-1, H-2), 4.71 (1H, d, *J* = 11.6 Hz, CH_2Ph), 4.43 (1H, d, *J* = 11.6 Hz, CH_2Ph), 4.22 (1H, q, *J* = 5.2 Hz, H-4), 4.13–4.03 (5H, m, C*H*$_2$CH$_3$, H-5a), 4.00–3.95 (1H, m, H-5b), 1.17 (6H, t, *J* = 7.1 Hz, CH$_2$C*H*$_3$); ^{13}C NMR (MeOD): δ 155.7 (CO), 155.6 (CO), 138.5 (Ar), 129.5 (Ar), 129.4 (Ar), 129.0 (Ar), 105.3 (C-1), 80.6 (d, *J* = 8.5 Hz, C-4), 78.9 (C-2), 76.2 (C-3), 70.7 (CH_2Ph), 67.6 (d, *J* = 4.2 Hz, C-5), 65.9 (*C*H$_2$CH$_3$), 14.6 (CH$_2$*C*H$_3$); ^{31}P NMR (D_2O): δ 1.17; HRMS *m*/*z* 463.10117 (calc. for $C_{18}H_{24}O_{12}P$ ($[M - H]^+$): 463.10109).

5-*O*-Phosphate-2,3-*O*-bis(methylcarbonate)-β-D-ribofuranose 13a. For the reaction, 250 mg (0.41 mmol) of ribofuranoside **9a** in a THF–mQ H_2O (1/2, v/v, 15 mL) mixture was used.

The spectroscopic analyses revealed the product to be 5-*O*-phosphate-1,2-*O*-carbonate-3-*O*-methylcarbonate-β-D-ribofuranoside **15a** (105 mg, 83%) as a colorless oil.

$[\alpha]_D^{20}$ = +46.8° (*c* 0.21, MeOH); ^{1}H NMR (D_2O): δ 6.38 (1H, d, *J* = 4.8 Hz, H-1), 5.51 (1H, t, *J* = 5.2 Hz, H-2), 5.13 (1H, dd, *J* = 7.8, 5.2 Hz, H-3), 4.52 (1H, d, *J* = 7.8 Hz, H-4), 4.18 (1H, dd, *J* = 12.0, 5.1 Hz, H-5a), 4.07–4.00 (1H, m, H-5b), 3.85 (3H, s, CH_3); ^{13}C NMR (D_2O): δ 155.1 (CO), 154.8 (CO), 103.7 (C-1), 78.7 (d, *J* = 7.5 Hz, C-4), 77.8 (C-2), 73.0 (C-3), 62.7 (d, *J* = 2.2 Hz, C-5), 56.0 (*C*H$_3$); ^{31}P NMR (D_2O): δ −0.22; HRMS *m*/*z* 312.99668 (calc. for $C_8H_{10}O_{11}P$ ($[M - H]^+$): 312.99662).

5-*O*-Phosphate-2,3-*O*-bis(ethylcarbonate)-β-D-ribofuranose 13b. For the reaction, 300 mg (0.47 mmol) of furanoside **9b** in a THF–mQ H_2O (1/1, v/v, 15 mL) mixture was used.

The HPLC-MS analysis revealed the product to be a 9 : 1 mixture of 5-*O*-phosphate-2,3-*O*-bis(ethylcarbonate)-D-ribofuranose **13b** in α-(A) and β-(B) form, and 5-*O*-phosphate-1,2-*O*-carbonate-3-*O*-ethylcarbonate-β-D-ribofuranoside **15b** (C) (150 mg, 86%), which upon storage underwent a migration towards product **15b**. When the NMR was measured, the mixture ratio was 4 : 1 (A + B : C).

^{1}H NMR (D_2O): δ 6.40 (0.5H, d, *J* = 4.9 Hz, H-1C), 5.66 (1H, d, *J* = 4.5 Hz, H-1B), 5.53 (0.5H, t, *J* = 4.9 Hz, H-2C), 5.46 (1H, d, *J* = 2.7 Hz, H-1A), 5.30 (1H, t, *J* = 4.7 Hz, H-3A), 5.29 (1H, q, *J* = 3.0 Hz, H-3B), 5.15–5.10 (2.5H, m, H-2A, H-2B, H-3C), 4.56 (0.5H, br s, H-4C), 4.51 (1H, br s, H-4B), 4.35 (1H, dd, *J* = 8.6, 4.7 Hz, H-4A), 4.30–4.22 (10H, m, C*H*$_2$CH$_3$, H-5Aa), 4.10–4.00 (4H, m, H-5Ab, H-5B, H-5C), 1.31–1.28 (13.5H, m, CH$_2$*C*H$_3$); ^{13}C NMR (D_2O): δ 155.1 (CO), 154.8 (CO), 154.5 (CO), 154.4 (CO), 154.1 (CO), 103.8 (C-1C), 98.9 (C-1A), 94.5 (H-1B), 80.4 (d, *J* = 8.3 Hz, C-4B), 79.2 (m, H-4A, H-4C), 78.1 (C-3C), 77.9 (C-2C), 74.4 (C-3A), 73.8 (C-3B), 73.7 (C-2A), 72.9 (C-2B), 66.2 (*C*H$_2$CH$_3$), 66.0 (*C*H$_2$CH$_3$), 66.0 (*C*H$_2$CH$_3$), 65.9 (*C*H$_2$CH$_3$), 65.8 (*C*H$_2$CH$_3$), 64.8 (d, *J* = 4.9 Hz, C-5A), 64.5 (d, *J* = 4.3 Hz, C-5B), 62.7 (d, *J* = 4.3 Hz, C-5C), 13.4 (CH$_2$*C*H$_3$), 13.4 (CH$_2$*C*H$_3$), 13.3 (CH$_2$*C*H$_3$); ^{31}P NMR (D_2O): δ 0.21; **15b**: HRMS *m*/*z* 327.01233 (calc. for $C_9H_{12}O_{11}P$ ($[M - H]^+$): 327.01227).

5-*O*-Phosphate-1-*O*-methyl-2,3-*O*-bis(isobutylcarbonate)-β-D-ribofuranose 13c. For the reaction, 225 mg (0.32 mmol) of ribofuranoside **9c** in MeOH was used.

The HPLC-MS analysis revealed the product to be a 1 : 1 mixture of 5-*O*-phosphate-1-*O*-methyl-2,3-*O*-bis(isobutylcarbonate)-β-D-ribofuranose **13c** and 5-*O*-phosphate-2,3-*O*-bis(isobutylcarbonate)-β-D-ribofuranoside **14c** (120 mg, 87%).

13c: HRMS *m*/*z* 429.11678 (calc. for $C_{15}H_{26}O_{12}P$ ($[M - H]^+$): 429.11674).

14c: HRMS *m*/*z* 443.13227 (calc. for $C_{16}H_{28}O_{12}P$ ($[M - H]^+$): 433.13239).

5-*O*-Phosphate-2,3-*O*-bis(isobutylcarbonate)-β-D-ribofuranose 13c

For the reaction, 470 mg (0.67 mmol) of ribofuranoside **9c** in a THF–mQ H_2O (3/2, v/v, 15 mL) mixture was used.

The HPLC-MS analysis revealed the product to be a 9 : 1 mixture of 5-*O*-phosphate-2,3-*O*-bis(isobutylcarbonate)-β-D-ribofuranose **13c** and 5-*O*-phosphate-1,2-*O*-carbonate-3-*O*-isobutylcarbonate-β-D-ribofuranoside **15c** (280 mg, 97%), which underwent a migration towards product **15c**.

13c: HRMS *m*/*z* 429.11678 (calc. for $C_{15}H_{26}O_{12}P$ ($[M - H]^+$): 429.11674).

15c: $[\alpha]_D^{20}$ = +30.1° (*c* 0.33, MeOH); IR: ν_{max}/cm^{-1} (KBr): 3437, 2925, 2855, 1820, 1755 (CO), 1470, 1383, 1258, 1178, 1095, 1008, 962, 787; ^{1}H NMR (D_2O): δ 6.35 (1H, d, *J* = 4.9 Hz, H-1), 5.49 (1H, t, *J* = 5.3 Hz, H-2), 5.10 (1H, dd, *J* = 7.5, 5.4 Hz, H-3), 4.51 (1H, d, *J* = 7.5 Hz, H-4), 4.16 (1H, ddd, *J* = 12.2, 5.6, 2.2 Hz, H-5a), 4.05–3.90 (3H, m, H-5b, C*H*$_2$CH(CH$_3$)$_2$), 1.95 (1H, m, CH$_2$C*H*(CH$_3$)$_2$), 0.83 (6H, d, *J* = 6.7 Hz, CH$_2$CH(C*H*$_3$)$_2$); ^{13}C NMR (D_2O): δ 155.1 (CO), 154.3 (CO), 103.8 (C-1), 79.1 (d, *J* = 7.9 Hz, C-4), 77.8 (C-2), 75.8 (*C*H$_2$CH(CH$_3$)$_2$), 72.3 (C-3), 62.8 (d, *J* = 4.0 Hz, C-5), 27.2 (CH$_2$*C*H(CH$_3$)$_2$), 17.9 (CH$_2$CH(*C*H$_3$)$_2$); ^{31}P NMR (D_2O): δ 0.01; HRMS *m*/*z* 355.04372 (calc. for $C_{11}H_{16}O_{11}P$ ($[M - H]^+$): 355.04357).

5-*O*-Phosphate-2,3-*O*-carbonate-β-D-ribofuranose 11d. For the reaction, 190 mg (0.44 mmol) of ribofuranoside **9d** was used.

HPLC-MS of the crude product revealed pure 5-*O*-phosphate-2,3-*O*-carbonate-β-D-ribofuranose **11d** as a mixture of α- and β-anomers in a 3 : 7 ratio in 84% yield (94 mg).

^{1}H NMR (D_2O): δ 6.14 (0.3H, br s, H-1α), 5.59 (0.3H, br s, H-2α), 5.46 (0.7H, s, H-1β), 5.38 (0.7H, d, *J* 6.1 Hz, H-2β), 5.17 (0.3H, br s, H-3α), 5.06 (0.7H, d, *J* = 6.1 Hz, H-3β), 4.44 (0.7H, br s, H-4β), 4.24–4.17 (0.3H, m, H-4α), 4.08–3.97 (2H, m, H-5); ^{13}C NMR (D_2O): δ 102.8 (C-1), 86.1 (C-4, C-2), 82.9 (C-3), 66.10 (C-5); ^{31}P NMR (D_2O): δ 0.80; HRMS *m*/*z* 254.99111 (calc. for $C_6H_8O_9P$ ($[M - H]^+$): 254.99114).

Coupling of 5-phosphate-ribofuranoside derivatives with AMP morpholidate (ball mill technique)[15]

5-Phosphate ribofuranoside (1 × *n*), AMP morpholidate (1.1 × *n*), 1*H*-tetrazole (2 × *n*), $MgCl_2 \cdot 6H_2O$ (1.5 × *n*) and water (6 × *n*) were put together into a ball milling jar of 25 mL inner volume. A 1.5 cm steel ball was added inside, the jar was closed and set to a ball milling machine to move at 30 Hz frequency for 90 min. Once finished, the jar was left to cool down to room temperature, and then the product was dissolved in mQ water and freeze-dried.

1′,2′-*O*-Carbonate-3′-*O*-methylcarbonate-ADP-ribofuranoside 16a. For the reaction, 27.0 mg (86.0 μmol) of ribofuranoside **15a** was used.

The purification by reversed phase HPLC on a C18 column (10 × 250 mm, A: ammonium formate 10 mM, B: AcCN, gradient 0–13% B in 40 min) revealed pure diphosphate derivative **16a** eluted at 25 min in 61% yield (34 mg).

^{1}H NMR (D_2O): δ 8.54 (1H, s, H-8), 8.16 (1H, s, H-2), 6.28 (1H, d, *J* = 4.9 Hz, H-1″), 6.13 (1H, d, *J* = 5.7 Hz, H-1′), 5.44 (1H, t, *J* = 5.3 Hz, H-2″), 5.03 (1H, dd, *J* = 7.4, 5.6 Hz, H-3″), 4.75 (1H, dd, *J* = 5.7, 5.0 Hz, H-2′), 4.52 (1H, dd, *J* = 5.0, 3.8 Hz, H-3′), 4.43–4.37 (2H, m, H-4′, H-4″), 4.21 (3H, br s, H-5′, H-5″a), 4.06 (1H, ddd, *J* = 12.1, 5.8, 3.4 Hz, H-5″b); ^{13}C NMR (D_2O): δ 154.7 (CO), 151.7 (CO), 148.1 (C-2), 140.3 (C-6), 135.1 (C-4), 131.7 (C-8), 103.8 (C-1″), 87.1 (C-1′), 83.9 (d, *J* = 2.4 Hz, C-4′), 79.1 (m, C-4″), 77.8 (C-2″), 74.4 (C-2′), 73.2 (C-3″), 70.3 (C-3′), 65.2 (m, C-5″), 63.7 (m, C-5′), 56.0 (CH$_3$); ^{31}P NMR (D_2O): δ −11.40; HRMS *m*/*z* 642.04870 (calc. for $C_{18}H_{22}O_{17}N_5P_2$ ($[M - H]^+$): 642.04914).

1′,2′-*O*-Carbonate-3′-*O*-ethylcarbonate-ADP-ribofuranoside 16b. For the reaction, 16.0 mg (47.9 μmol) of ribofuranoside **15b** was used.

The purification by reversed phase HPLC on a C18 column (10 × 250 mm, A: ammonium formate 10 mM, B: AcCN, gradient 0–13% B in 20 min, 13–30% B in 10 min, 30–50% B in 10 min, run time 40 min) revealed pure diphosphate derivative **16b** eluted at 15 min in 52% yield (16 mg).

^{1}H NMR (D_2O): δ 8.43 (1H, s, H-8), 8.18 (1H, s, H-2), 6.19 (1H, d, *J* = 4.9 Hz, H–1″), 6.04 (1H, d, *J* = 5.4 Hz, H-1′), 5.35 (1H, t, *J* = 5.4 Hz, H-2″), 4.93 (1H, dd, *J* = 6.9, 5.8 Hz, H-3″), H-2′under the water signal, 4.42 (1H, dd, *J* = 4.9, 3.3 Hz, H-3′), 4.35–4.31 (1H, m, H-4″), 4.30–4.26 (1H, m, H-4′), 4.15–4.06 (5H, m, H-5′, H-5″a, C*H*$_2$CH$_3$), 4.00–3.93 (1H, m, H-5″b), 1.15 (3H, t, *J* = 7.2 Hz, CH$_2$C*H*$_3$); ^{13}C NMR (D_2O): δ 154.0 (CO), 150.3 (CO), 145.5 (C-2), 145.1 (C-6), 143.9 (C-4), 140.6 (C-8), 103.8 (C-1″), 87.2 (C-1′), 83.9 (d, *J* = 4.3 Hz, C-4′), 79.1 (d, *J* = 5.1 Hz, C-4″), 77.8 (C-2″), 74.4 (C-2′), 73.0 (C-3″), 70.2 (C-3′), 66.1 (*C*H$_2$CH$_3$), 65.1 (d, *J* = 2.5 Hz, C-5′), 63.7 (d, *J* = 4.5 Hz, C-5″), 13.2 (CH$_2$*C*H$_3$); ^{31}P NMR (D_2O): δ −11.48; HRMS *m*/*z* 656.06396 (calc. for $C_{19}H_{24}O_{17}N_5P_2$ ($[M - H]^+$): 656.06479).

1′,2′-*O*-Carbonate-3′-*O*-isobutylcarbonate-ADP-ribofuranoside 16c. For the reaction, 32.0 mg (90.0 μmol) of ribofuranoside **15c** was used.

The purification by reversed phase HPLC on a C18 column (10 × 250 mm, A: ammonium formate 10 mM, B: AcCN, gradient 0–13% B in 20 min, 13–30% B in 10 min, 30–50% B in 10 min, run time 40 min) revealed pure diphosphate derivative **16c** eluted at 18 min in 58% yield (36 mg).

^{1}H NMR (D_2O): δ 8.56 (1H, s, H-8), 8.30 (1H, s, H-2), 6.30 (1H, d, *J* = 4.9 Hz, H-1″), 6.14 (1H, d, *J* = 5.6 Hz, H-1′), 5.46 (1H, t, *J* = 5.3 Hz, H-2″), 5.05 (1H, dd, *J* = 6.7, 5.7 Hz, H-3″), 4.75 (1H, t, *J* = 5.0 Hz, H-2′), 4.52 (1H, dd, *J* = 5.0, 3.9 Hz, H-3′), 4.46 (1H, td, *J* = 6.7, 3.4 Hz, H-4″), 4.38 (1H, bd, *J* = 2.7 Hz, H-4′), 4.27–4.20 (3H, m, H-5′, H-5a″), 4.08 (1H, ddd, *J* = 11.9, 6.1, 3.4 Hz, H-5b″), 3.96 (1H, dd, *J* = 10.2, 6.7 Hz, C*H*$_2$CH(CH$_3$)$_2$), 3.89 (1H, dd, *J* = 10.2, 6.7 Hz, C*H*$_2$CH(CH$_3$)$_2$), 1.89 (2H, heptuplet, *J* = 6.7 Hz, CH$_2$C*H*(CH$_3$)$_2$), 0.87 (6H, d, *J* = 6.7 Hz, CH$_2$CH-(C*H*$_3$)$_2$); ^{13}C NMR (D_2O): δ 156.0 (CO), 154.1 (CO), 150.9 (C-2), 140.5 (C-8), 103.9 (C-1″), 87.2 (C-1′), 83.9 (d, *J* 3.4 Hz, C-4′), 79.5 (d, *J* 4.2 Hz, C-4″), 77.8 (C-2″), 75.7 (*C*H$_2$CH(CH$_3$)$_2$), 74.4 (C-2′), 73.1 (C-3″), 70.3 (C-3′), 65.2 (d, *J* 2.2 Hz, C-5′), 63.9 (t, *J* 3.5 Hz,

C-5″), 27.2 ($CH_2CH(CH_3)_2$), 17.9 ($CH_2CH(CH_3)_2$), 17.9 ($CH_2CH(CH_3)_2$); ^{31}P NMR (D_2O): δ −11.38; HRMS *m*/*z* 684.09537 (calc. for $C_{21}H_{28}O_{17}N_5P_2$ ($[M - H]^+$): 684.09609).

2′,3′-*O*-Carbonate-ADP-ribose 13d. For the reaction was used 48.0 mg (185 μmol) of ribofuranoside **11d**.

The purification by reversed phase HPLC on a C18 column (10 × 250 mm, A: ammonium formate 10 mM, B: AcCN, gradient 0–13% B in 40 min) revealed pure diphosphate derivative **13d** eluted at 17 min in 45% yield (50.5 mg).

The product **13d** exists as an equilibrium mixture of α- and β-anomeric forms (S2 + S3) together with the 1′,2′-*O*-carbonate/ADP/ribofuranoside **13e** (S1) form arising from carbonate migration in a 1 : 1 : 1 ratio.

^{1}H NMR (D_2O): δ 8.53 (1H, d, *J* = 2.4 Hz, H-8), 8.28 (1H, d, *J* = 2.4 Hz, H-2), 6.20 (0.3H, d, *J* = 4.8 Hz, H-1″, S1), 6.15 (1H, d, *J* = 5.7 Hz, H-1′), 5.71 (0.4H, d, *J* = 4.0 Hz, H-1″, S2), 5.57 (0.3H, s, H-1″, S3), 5.43 (0.3H, br d, *J* = 7.3 Hz, H-3″, S3), 5.32 (0.4H, dd, *J* = 7.3, 1.4 Hz, H-3″, S2), 5.24–5.18 (0.7H, m, H–2″, S2, H-2″, S1), 5.15 (0.4H, d, *J* = 6.8 Hz, H-2″, S3), H-2′ and H-3″, S1 under water signal, 4.56–4.50 (1.6H, m, H-3′, H-4″, S2, S3), 4.42–4.37 (1H, m, H-4′), 4.28 (0.4H, d, *J* 4.6 Hz, H-4″, S1), 4.25–4.21 (2H, m, H-5′), 4.12–4.03 (2H, m, H-5″, S1, S2, S3); ^{13}C NMR (D_2O): δ 155.1 (CO), 152.1 (C-2), 149.1 (CO), 140.0 (C-8), 103.2 (C-1″, S1), 101.1 (C-1″, S3), 96.5 (C-1″, S2), 86.9 (C-1′), 84.8 (C-2″, S3), 83.8 (C-4′), 83.7 (C-4″, S3), 81.8 (C-3″, S3), 81.4 (C-3″, S2), 79.9 (C-4″, S2), 79.8 (C-2″, S1), 79.6 (C-4″, S1), 79.0 (C-2″, S2), 74.2 (C-2′), 70.3 (C-3′), 68.7 (C-3″, S1), 66.2 (d, *J* = 4.7 Hz, C-5″, S3), 65.4 (d, *J* = 4.2 Hz, C-5″, S2), 65.2 (t, *J* = 2.9 Hz, C-5′), 63.2 (d, *J* = 3.3 Hz, C-5″, S1); ^{31}P NMR (D_2O): δ −10.80; HRMS *m*/*z* 586.05823 (calc. for $C_{16}H_{22}O_{15}N_5P_2$ ($[M + H]^+$): 586.05821).

1′-*O*-Benzyl-2′,3′-*O*-bis(ethylcarbonate)-ADP-ribofuranoside 17b. For the reaction, 40.0 mg (86.1 μmol) of ribofuranoside **12b** was used.

The purification by reversed phase HPLC on a C18 column (10 × 250 mm, A: ammonium formate 10 mM, B: AcCN, gradient 0–13% B in 20 min, 13–30% B in 10 min, 30–50% B in 10 min, run time 40 min) revealed pure diphosphate derivative **17b** eluted at 35 min in 68% yield (46 mg).

^{1}H NMR (D_2O): δ 8.52 (1H, s, H-8), 8.21 (1H, s, H-2), 7.30–7.23 (5H, m, Ar), 6.06 (1H, d, *J* = 5.4 Hz, H-1′), 5.24 (1H, dd, *J* = 6.1, 4.7 Hz, H-3″), 5.17 (1H, d, *J* = 1.2 Hz, H-1″), 5.11 (1H, dd, *J* = 4.7, 1.2 Hz, H-2″), 4.67–4.64 (2H, m, H-2′, CH_2Ph), 4.48 (1H, dd, *J* = 4.8, 4.2 Hz, H-3′), 4.43 (1H, d, *J* = 11.6 Hz, CH_2Ph), 4.37–4.33 (2H, m, H-4′, H-4″), 4.27–4.13 (7H, m, H-5′, H-5″a, CH_2CH_3), 4.08–4.00 (1H, m, H-5″b), 1.23 (6H, m, CH_2CH_3); ^{13}C NMR (D_2O): δ 154.2 (CO), 149.4 (CO), 149.1 (C-2), 141.9 (C-6), 140.7 (C-4), 140.6 (C-8), 136.0 (Ar), 128.5 (Ar), 128.4 (Ar), 128.2 (Ar), 103.5 (C-1″), 87.4 (C-1′), 83.8 (d, *J* = 2.7 Hz, C-4′), 79.3 (d, *J* = 2.1 Hz, C-4″), 77.3 (C-2″), 74.6 (C-2′), 74.5 (C-3″), 70.2 (C-3′), 69.7 (CH_2CH_3), 66.0 (m, C-5′, CH_2Ph), 65.0 (m, C-5″), 13.3 (CH_2CH_3); ^{31}P NMR (D_2O): δ −11.28; HRMS *m*/*z* 792.15351 (calc. for $C_{28}H_{36}O_{18}N_5P_2$ ($[M - H]^+$): 792.15361).

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