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# Synthesis of C-glycosidically linked ADP *glycero*-β-D-*manno*-heptose analogues

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**Abstract**—C-Glycosides of L-*glycero*-D-*manno*- and D-*glycero*-D-*manno*-heptose containing either (*S*)- or (*R*)-2-hydroxypropyl aglycons are easily accessible compounds via condensation of reducing heptoses with pentane-2,4-dione. 2′,3′-Di-*O*-acetyl adenosine was transformed into the corresponding 5′-*O*-cyanoethyl *N*,*N*-diisopropylaminophosphoramidite derivative, which was coupled in fair yields to the O-acetylated diastereoisomeric C-glycosidic alcohols. Oxidation of the phosphite triesters followed by deprotection furnished four ADP-heptose analogues, wherein the heptosyl phosphate moiety had been replaced by a three carbon-skeleton. The compounds serving as substrate analogues will be used for co-crystallization experiments with ADP heptosyl transferases.


## 1. Introduction

Lipopolysaccharides (LPS) are structurally complex amphipathic glycolipids which are essential constituents of the outer membrane of Gram-negative bacteria.[1] In general, LPS are composed of lipid A and a core region containing 3-deoxy-D-*manno*-oct-2-ulosonic acid (Kdo), heptose of the L-*glycero*-D-*manno*- and—less frequently—heptose of the D-*glycero*-D-*manno*- configuration.[2] Since these higher-carbon sugars do not occur in mammalian systems, the inhibition of their biosynthesis may be regarded as an attractive target for the design of novel antibiotics. The biosynthesis of the nucleotide-activated heptoses of the D-*glycero*- and L-*glycero*-D-*manno*- configurations has recently been fully elucidated.[3,4] Heptoses are transferred to the inner core of enterobacterial LPS via glycosyl transferases acting upon the corresponding ADP-linked sugars.[5,6] The L-configured ADP heptosyl substrate results from an epimerase reaction, inverting the stereochemistry at carbon 6 of the precursor ADP D-*glycero*-β-D-*manno*-heptose.[7,8] (Fig. 1). In contrast to the α-anomeric GDP heptose counterpart, ADP heptopyranoses of the β-anomeric configuration are labile compounds due to neighbouring group participation of the axial 2-OH group inducing the formation of a cyclic 1,2-heptosyl phosphate and release of AMP.[9,10] To overcome the inherent

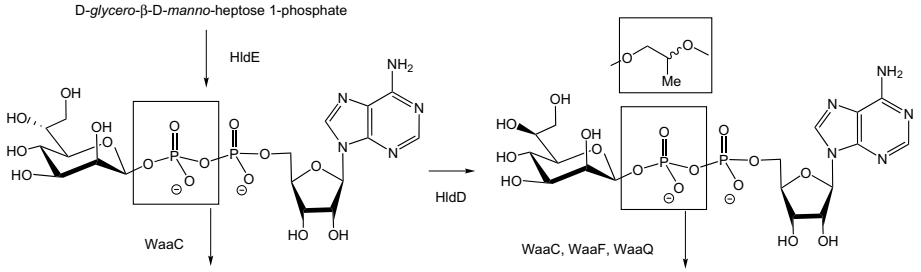


**Figure 1.** Structure of ADP-heptoses and enzymes involved in heptose activation and heptosyl transfer reactions. HldE: Heptose 1-phosphate adenylyltransferase; HldD: ADP D-*glycero*-β-D-*manno*-heptose epimerase; WaaC, WaaF, WaaQ: inner core ADP-heptosyl transferases. Boxes illustrate the replacement of the heptosyl phosphate entity by a 2-hydroxypropyl residue.

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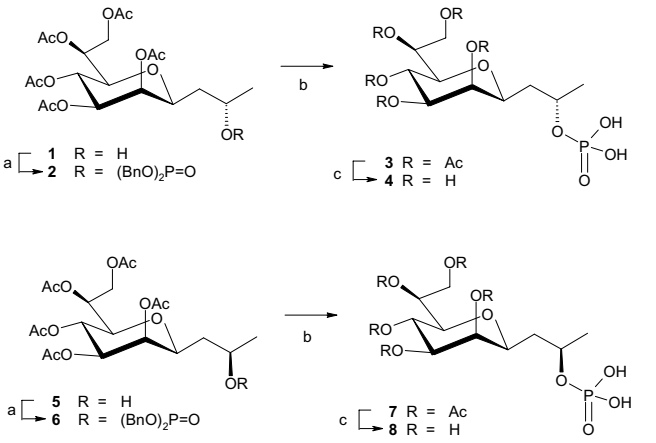
hydrolytic lability of the ADP heptoses, we have started to synthesize stable C-glycosides in the β-anomeric configuration to be used for the synthesis of substrate analogues. Previously, we have reported on the straightforward conversion of reducing heptoses into β-2-carbonylalkyl glycosides, which were elaborated into (*S*)- and (*R*)-configured 2-hydroxypropyl glycosides.[11] Herein, we report on the further transformation into the corresponding sugar nucleotide analogues.

## 2. Results and discussion

The chemical synthesis of C-glycosides has considerably expanded over the past decade.[12] In addition, numerous modifications of the diphosphate backbone in nucleotide-linked sugars have been introduced, in order to generate glycosyl transferase inhibitors. While most of these analogues have been derived from anomeric C-phosphonates, as well as C-glycosidic hydroxymethyl or hydroxyethyl phosphates,[13,14] noncharged phosphate surrogates have also been described.[15] Thus, we set out to replace the anomeric phosphate moiety by a noncharged, hydrolytically stable, three-carbon skeleton, while keeping the second, 5′-*O*-phosphate unit intact (Fig. 1). This group may thus be regarded as a mimic of the diphosphate bridge and, although lacking the charged moiety of the glycosyl phosphate, could provide a proper formal distance to fit into the binding site of the glycosyl transferases.[14a]

The previously described 1,3-dideoxy-L-*lyxo*-L-*manno*-decitol derivative **1**, as well as diastereoisomer **5**, are readily available via reaction of the unprotected sugar with pentane-2,4-dione, subsequent enrichment of the β-anomeric form, followed by acetylation and reduction of the keto group.[11,16] In order to test for potential interference of the bulky methyl terminus in the binding, both diastereoisomers were prepared. Reaction of **1** and **5** with dibenzyl-*N*,*N*-diisopropylphosphoramidite/1*H*-tetrazole and subsequent oxidation with *tert*-BuOOH furnished the phosphate triester derivatives **2** and **6** in 71% and 77% yield, respectively (Scheme 1). Deprotection was accomplished in two steps via hydrogenolysis of the benzyl groups with 10% Pd–carbon, followed by removal of the acetyl groups with ammonia in aqueous MeOH to give the phosphates **4** and **8** in 88% and 86% yield for two steps, respectively. Both phosphate derivatives **4** and **8** serve as L-*glycero*-D-*manno* heptose 1-phosphate analogues.

For the assembly of the phosphodiester linkage of the target ADP-Hep analogues, the phosphoramidite approach was applied. Synthons **1**, **5**, **14** and **17** were coupled with the base-unprotected 5′-*O*-(2-cyanoethyl-*N*,*N*-diisopropyl)phosphoramidite of di-*O*-acetyladenosine **9** in the presence of 1*H*-tetrazole as a weak acid catalyst. Phosphoramidite **9** was prepared by reaction of 2′,3′-di-*O*-acetyladenosine with 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphoramidite, catalyzed by diisopropylammonium tetrazolide and was used as a crude mixture (60–70% purity of **9**) after partial purification by flash chromatography on silica gel.[17] The resulting phosphite triesters were subsequently oxidized with *tert*-BuOOH to afford phosphate triesters **10**, **12**, **15** and **18**, respectively (Schemes 2 and 3).



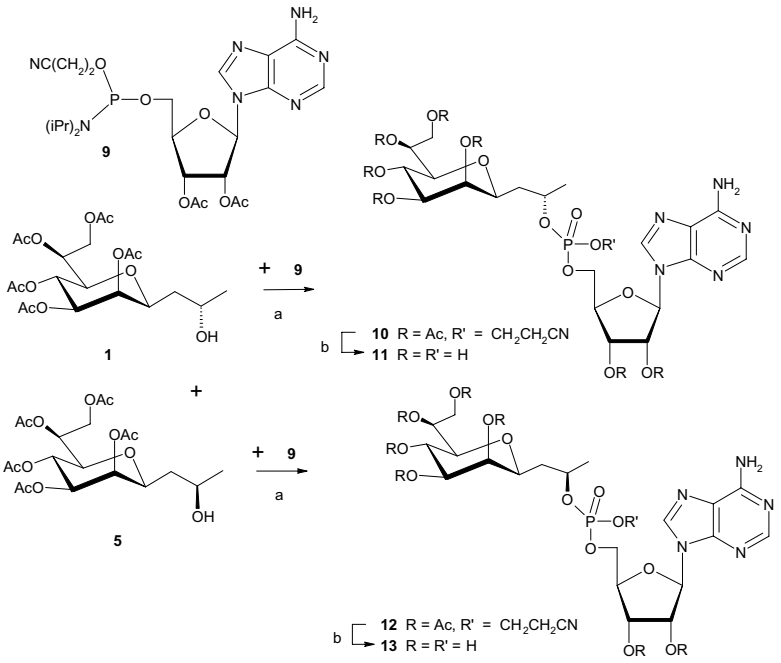

**Scheme 1.** Synthesis of L-*glycero*-D-*manno*-heptopyranosyl phosphate analogues. Reagents and conditions: (a) *i*-$Pr_2NP(OBn)_2$, 1*H*-tetrazole, $MeCN–CH_2Cl_2$, then *t*-BuOOH, 71% for **2**, 77% for **6**; (b) $H_2$/Pd–C, MeOH, 92% for **3**, 92% for **7**; (c) 7:3:1 MeOH–water–25% aq $NH_4OH$, 96% for **4**, 93% for **8**.

Starting from the L-*glycero*-β-D-*manno*-heptosyl analogues **1** and **5** (Scheme 2), an intermediate purification (prior to oxidation with *tert*-BuOOH) of the higher-running phosphite triesters (TLC mobility with respect to the products of the side reactions of crude **9**) was performed. Subsequent oxidation with *tert*-BuOOH afforded the phosphate triesters **10** and **12** as diastereomeric mixtures on phosphorus in 87% and 83% yield, respectively.

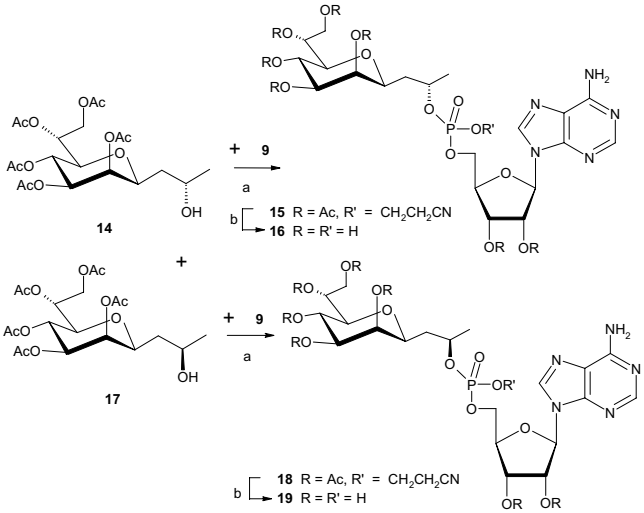
Subsequent deprotection of **10** and **12** with $Et_3N$–MeOH–water and purification on silica gel afforded the target ADP-Hep analogues **11** and **13** in 84% and 72% yield (based on starting *C*-heptosyl analogues **1** and **5**).

Phosphate triesters **15** and **18**, obtained from D-*glycero*-β-D-*manno*-precursors **14** and **17** (Scheme 3), could not be isolated in chemically pure form, because of the presence of several by-products with similar $R_f$ values, resulting from side reactions of the crude mixture of base-unprotected **9**, which, apart from unreactive H-phosphonates contained traces of diacetyladenosine. The products were finally isolated by silica gel chromatography in 30% yield (with respect to starting *C*-heptosyl analogues **14** and **17**) as phosphodiesters **16** and **19** after treatment with triethylamine in aqueous MeOH, which removed both acetyl and 2-cyanoethyl protecting groups.

The $^1H$ and $^{13}C$ NMR spectra of all four target compounds **11**, **13**, **16** and **19** could be fully assigned, since the chemical shifts of the ribose and heptosyl moiety were well separated (Fig. 2). Comparison of the chemical shifts of the ADP β-heptoses shows that the adenosine moiety presents no significant differences, whereas the heptosyl moiety is influenced by the presence of the aglycone rather than by the diphosphate bridge. In addition, the heteronuclear $J_{C,P}$ coupling constants observed for carbon C-2 ($J_{C,P} = 5.7$ Hz for **11**, $J_{C,P} = 5.8$ Hz for **12** and **15**, $J_{C,P} = 5.6$ Hz for **19**)




**Scheme 2.** Synthesis of ADP L-*glycero*-D-*manno*-heptopyranosyl analogues. Reagents and conditions: (a) 1*H*-tetrazole, MeCN–$CH_2Cl_2$, then *t*-BuOOH, 87% for **10**, 83% for **12**; (b) MeOH–$H_2O$–$Et_3N$, 7:3:1, 97% for **11**, 87% for **13**.




**Scheme 3.** Synthesis of ADP D-*glycero*-D-*manno*-heptopyranosyl analogues. Reagents and conditions: (a) 1*H*-tetrazole, MeCN–$CH_2Cl_2$, then *t*-BuOOH, 60% for **15**, 60% for **18**; (b) MeOH–$Et_3N$–$H_2O$, 53% for **16**, 52% for **19**.

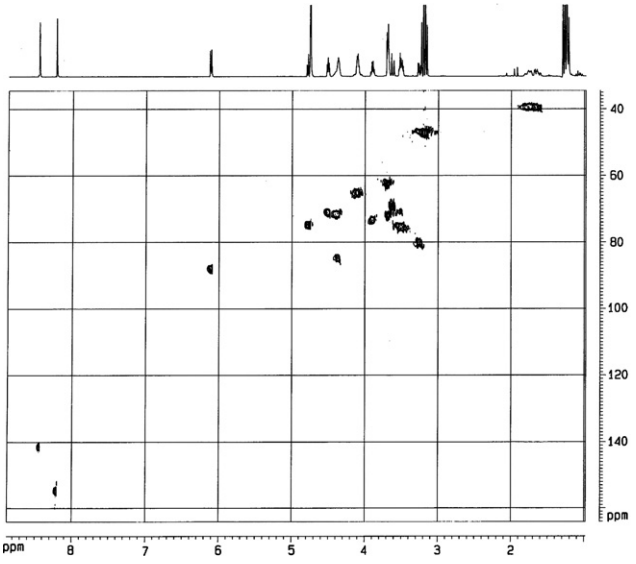



**Figure 2.** 300 MHz HSQC spectrum of compound **19**.

and C-$5_{Rib}$ ($J_{C,P} = 5.3$ Hz for **11** and **15**, $J_{C,P} = 5.2$ Hz for **13** and $J_{C,P} = 4.8$ Hz for **19**) as well as the $^{31}P$ chemical shifts ($\delta$ 0.77 for **11**, −0.76 for **13**, 0.04 for **16** and 0.06 for **19**) substantiate the structural assignments as phosphodiester derivatives. Finally, the values recorded for the specific rotation of target compounds **11**, **13**, **16** and **19** are in agreement with the previously made stereochemical assignments of C-2 of the 2-hydroxypropyl entity.[11]

The analogues are being used for co-crystallization experiments and inhibition studies with ADP D-*glycero*-β-D-*manno*-heptose epimerase as well as ADP heptosyl transferases. The results of the crystallographic studies will be reported in due course.

## 3. Conclusion

The coupling of easily available fully acetylated C-glycosidic alcohols of *glycero*-D-*manno*-heptose with 2-cyanoethyl-protected adenosine 5′-*O*-phosphoramidite provides a straightforward access to sugar nucleotide analogues.

## 4. Experimental

### 4.1. General methods

Column chromatography was performed on silica gel 60 (230–400 mesh, Merck). Reactions were monitored by TLC on silica gel 60 $F_{254}$ precoated glass plates (Merck) or on silica gel 60 $F_{254}$ HPTLC precoated glass plates with 2.5 cm concentration zone (Merck); spots were visualized by spraying with anisaldehyde–$H_2SO_4$; phosphorus-containing compounds were additionally detected with a molybdate solution [0.02 M solution of ammonium cerium(IV)sulfate dihydrate and ammonium molybdate(VI)tetra-hydrate in aqueous $H_2SO_4$], adenosine-containing compounds were detected by examination under UV light. Di-*O*-acetyl adenosine was purchased from SIGMA. Triethylamine and $CH_2Cl_2$ were dried by refluxing with $CaH_2$ (5 g/L) for 16 h, distilled and stored under argon. Toluene and acetonitrile were distilled from phosphorus pentaoxide and redistilled from $CaH_2$. The liquids were stored over molecular sieves 0.4 nm. DMF was stirred with $CaH_2$ (5 g/L) for 16 h at 20 °C, distilled under reduced pressure and stored over molecular sieves 0.3 nm. Concentration of solutions was performed at reduced pressure at temperatures <25 °C. Optical rotations were measured with a Perkin–Elmer 243 B polarimeter. $[\alpha]_D^{20}$-Values are given in units of $10^{-1}$ deg cm$^3$ g$^{-1}$. NMR spectra were recorded at 297 K in $CDCl_3$ (unless stated otherwise) with a Bruker DPX 300 spectrometer ($^1$H at 300.13 MHz, $^{13}$C at 75.47 MHz and $^{31}$P at 121.50 MHz) using standard Bruker NMR software. $^1$H NMR spectra were referenced to tetramethylsilane or 2,2-dimethyl-2-silapentane-5-sulfonic acid. $^{13}$C NMR spectra were referenced to chloroform ($\delta$ 77.00) or external 1,4-dioxane ($\delta$ 67.40). $^{31}$P NMR spectra were referenced externally to 85% aq $H_3PO_4$ ($\delta$ 0.0). Elemental analyses were provided by Dr. J. Theiner, Mikroanalytisches Laboratorium, Institut für Physikalische Chemie, Universität Wien. TOF-ES-MS spectra were recorded on a Waters Micromass Q-TOF Ultima Global instrument.

**4.1.1. 5,6,7,9,10-Penta-*O*-acetyl-4,8-anhydro-2-*O*-[bis(benzyloxy)]phosphoryl]-1,3-dideoxy-L-*lyxo*-L-*manno*-decitol 2.** A solution of **1** (50 mg, 0.11 mmol) and dibenzyl-*N*,*N*-diisopropylphosphoramidite (0.09 mL, 0.26 mmol) was repeatedly evaporated with dry toluene (3 × 10 mL) and then dried under reduced pressure for 5 h. Then the flask was charged with $CH_2Cl_2$ (3 mL), a solution of 1*H*-tetrazole (30 mg, 0.43 mmol) in dry $CH_3CN$ (1 mL) was added and the mixture was stirred at rt for 1 h under Ar. Monitoring of the reaction by TLC showed the formation of intermediate diastereomeric phosphite triesters ($R_f$ 0.54 and 0.56; 1:1, *n*-hexane/EtOAc). The reaction mixture was cooled to −20 °C and a solution of *tert*-BuOOH (40 μL of an 80% solution in di-*tert*-butyl peroxide) in $CH_2Cl_2$ (2 mL) was gradually added (~30 min). The reaction mixture was warmed to rt and stirred for 12 h. The solvent was concentrated, the residue redissolved in $CH_2Cl_2$ (50 mL) and washed sequentially with saturated aq $NaHCO_3$, water and brine. The organic phase was dried over $Na_2SO_4$ and concentrated. The residue was purified by chromatography on silica gel (1:4→1:1 EtOAc/*n*-hexane) to give **2** (55 mg, 71%) as a colourless syrup; $[\alpha]_D^{20} = -31$ (*c* 0.8, $CHCl_3$); $^1$H NMR: $\delta$ 7.40–7.30 (m, 10H, Ph), 5.31 (dd, 1H, $^3J_{5,6} = 3.5$, $^3J_{5,4} = 0.9$ Hz, H-5), 5.23 (t, 1H, $^3J_{7,8} = {}^3J_{7,6} = 10.0$ Hz, H-7), 5.22 (ddd, 1H, $^3J_{8,9} = 2.5$, $^3J_{9,10a} = 4.9$, $^3J_{9,10b} = 7.9$ Hz, H-9), 5.10–4.96 (m, 4H, 2 × C$H_2$Ph), 4.98 (dd, 1H, H-6), 4.63–4.50 (m, 1H, H-2), 4.29 (dd, 1H, $^2J_{10a,10b} = 11.5$ Hz, H-10a), 4.12 (dd, 1H, H-10b), 3.67 (m, 1H, H-4), 3.57 (dd, 1H, H-8), 2.14, 2.10, 2.02, 1.99, 1.96 (5s, each 3H, 5Ac), 1.98 (m, 1H, H-3a), 1.64–1.55 (m, H-3b), 1.27 (d, 3H, $^3J_{1,2} = 6.3$ Hz, $CH_3$); $^{13}$C NMR: $\delta$ 170.41, 170.35, 170.32, 169.86, 169.49 (5C, CO, Ac), 135.88, 135.79 (2C, Ph), 128.56, 128.51, 128.44, 128.03, 127.91 (10C, Ph), 76.52 (C-8), 74.18 (C-4), 72.90 (1C, $^3J_{2,P} = 5.8$ Hz, C-2), 72.31 (C-6), 69.75 (C-5), 69.31 (1C, $^2J_{C,P} = 5.7$ Hz, *C*$H_2$Ph), 69.21 (1C, $^2J_{C,P} = 5.7$ Hz, *C*$H_2$Ph), 66.99 (C-9), 64.67 (C-7), 62.39 (C-10), 37.66 (1C, $^3J_{C,P} = 5.9$ Hz, C-3), 21.13 (1C, $^3J_{C,P} = 2.3$ Hz, C-1), 20.70, 20.62, 20.56 and 20.51 (5C, 5Ac); $^{31}$P NMR ($CDCl_3$): $\delta$ −0.9; TOF-ES-MS: $m/z = 723.182$ [M+H$^+$]; calcd 723.24 [M+H$^+$].

**4.1.2. (4,8-Anhydro-1,3-dideoxy-L-*lyxo*-L-*manno*-decit-2-yl)phosphate 4 (ammonium salt).** A solution of **2** (55 mg, 0.076 mmol) in dry MeOH (20 mL) was hydrogenated at rt in the presence of 10% Pd/C (25 mg) for 12 h at atmospheric pressure. After the completion of the reaction, the catalyst was removed by filtration through a pad of Celite and washed with MeOH. The combined filtrates were neutralized by the addition of $Et_3N$ (15 μL) and concentrated. The residue was lyophilized from water (10 mL) to give the monotriethylammonium salt of 5,6,7,9,10-penta-*O*-acetyl-4,8-anhydro-1,3-dideoxy-L-*lyxo*-L-*manno*-decitol 2-phosphate **3** (45 mg, 92%) as an amorphous solid; $^1$H NMR (MeOD): $\delta$ 5.39 (d, 1H, $^3J_{5,6} = 3.5$ Hz, H-5), 5.25 (ddd, 1H, H-9), 5.22 (t, 1H, $^3J_{7,8} = {}^3J_{6,7} = 9.7$ Hz, H-7), 5.16 (dd, 1H, H-6), 4.40–4.31 (m, 1H, H-2), 4.35 (dd, 1H, $^3J_{10a,9} = 4.2$, $^2J_{10a,10b} = 11.5$ Hz, H-10a), 4.18 (dd, 1H, $^3J_{10b,9} = 8.4$ Hz, H-10b), 4.05–4.00 (m, 1H, H-4), 3.85 (dd, 1H, $^3J_{8,9} = 2.5$ Hz, H-8), 3.00 (q, ~6H, $CH_2$, $Et_3N$), 2.16, 2.06, 2.00, 1.97, 1.92 (5s, each 3H, 5Ac), 2.04–1.93 (m, 1H, H-3a), 1.64–1.56 (m, H-3b), 1.24 (t, ~9H, $CH_3$, $Et_3N$), 1.22 (d, 3H, $^3J_{1,2} = 7.2$ Hz, $CH_3$); $^{13}$C NMR (MeOD): $\delta$ 172.38, 172.24, 172.21, 171.49, 171.44 (5C, CO, Ac), 77.63 (C-8), 75.50 (C-4), 73.99 (C-6), 71.77 (C-5), 69.65 (1C, $^3J_{2,P} = 5.4$ Hz, C-2), 68.84 (C-9), 66.59 (C-7), 64.23 (C-10), 47.50 ($CH_2$, $Et_3N$), 39.40 (1C, $^3J_{C,P} = 5.3$ Hz, C-3), 22.00 (C-1), 20.85, 20.78, 20.67, 20.63, 20.56 (5C, 5Ac), 9.17 ($CH_3$, $Et_3N$); $^{31}$P NMR (MeOD): $\delta$ 1.69.

A solution of **3** (30 mg, 0.047 mmol) in 7:3:1 MeOH–water–aq 25% $NH_4OH$ (3 mL, pH 12) was stirred at rt for 12 h. The reaction mixture was diluted with water (20 mL), concentrated to 10 mL volume and lyophilized. The residue was purified on a prepacked Pd-10 column (Sephadex G-25, Amersham Pharmacia) by elution with water. The appropriate fractions were collected and lyophilized to give ammonium salt of **4** (15.5 mg, 96%) as a white fluffy solid; $[\alpha]_D^{20} = -29$ (*c* 0.7, $H_2O$); $^1$H NMR ($D_2O$): $\delta$ 4.30–4.17 (m, 1H, H-2), 3.85 (ddd, 1H, $^3J_{8,9} = 1.6$, $^3J_{10a,9} = 6.6$, $^3J_{10b,9} = 7.9$ Hz, H-9), 3.83 (d, 1H, $^3J_{5,6} = 3.2$ Hz, H-5), 3.68 (dd, 1H, $^3J_{6,7} = 9.6$, $^3J_{8,7} = 9.5$ Hz, H-

7), 3.65–3.56 (m, 4H, H-4, H-6, H-10a, H-10b), 3.18 (dd, 1H, H-8), 1.93–1.83 (m, 1H, H-3a), 1.66–1.58 (m, 1H, H-3b), 1.22 (d, 3H, $^3J_{1,2} = 6.3$ Hz, $CH_3$); $^{13}C$ NMR ($D_2O$): $\delta$ 78.63 (C-8), 75.47 (C-4), 74.89 (C-6), 71.46 (C-5), 69.43 (C-9), 69.12 (1C, $^3J_{2,P} = 4.9$ Hz, C-2), 66.87 (C-7), 63.36 (C-10), 37.99 (1C, $^3J_{C,P} = 4.7$ Hz, C-3), 21.27 (C-1, $^3J_{2,P} = 2.9$ Hz); $^{31}P$ NMR ($D_2O$): $\delta$ 2.74; TOF-ES-MS: $m/z = 333.079$ $[M+H]^+$; calcd 333.095 $[M+H]^+$.

**4.1.3. 5,6,7,9,10-Penta-*O*-acetyl-4,8-anhydro-2-*O*-[bis(benzyloxy)]phosphoryl]-1,3-dideoxy-L-*lyxo*-L-*gluco*-decitol 6.** Compound **6** as prepared from **5** (50 mg, 0.11 mmol) and dibenzyl-*N*,*N*-diisopropylphosphoramidite (0.09 mL, 0.26 mmol) in the presence of 1*H*-tetrazole (30 mg, 0.43 mmol) in the same manner as described for the synthesis of **2**. Yield for **6**: 60 mg, 77% (colourless syrup); $[\alpha]_D^{20} = -49$ (*c* 0.7, $CHCl_3$); $^1H$ NMR: $\delta$ 7.43–7.30 (m, 10H, Ph), 5.22 (dt, 1H, H-9), 5.18 (dd, 1H, $^3J_{5,6} = 3.6$, $^3J_{5,4} = 0.9$ Hz, H-5), 5.16 (t, 1H, $^3J_{7,8} = {}^3J_{7,6} = 10.0$ Hz, H-7), 5.14–4.97 (m, 4H, $2 \times CH_2$Ph), 4.87 (dd, 1H, H-6), 4.67–4.55 (m, 1H, H-2), 4.48 (dd, 1H, $^2J_{10a,10b} = 12.2$, $^3J_{10a,9} = 3.1$ Hz, H-10a), 4.12 (dd, 1H, $^3J_{10b,9} = 8.8$ Hz, H-10b), 3.67 (br d, 1H, H-4), 3.36 (dd, 1H, $^3J_{8,9} = 2.5$ Hz, H-8), 2.16, 2.09, 2.02, 1.99 and 1.96 (5s, each 3H, 5Ac), 1.80–1.70 (m, 1H, H-3a), 1.50–1.41 (m, H-3b), 1.26 (d, 3H, $^3J_{1,2} = 6.3$ Hz, $CH_3$); $^{13}C$ NMR: $\delta$ 170.63, 170.43, 169.96, 169.55 (5C, CO, Ac), 135.88 (2C, Ph), 128.70, 128.65, 128.59, 128.56, 128.01 (10C, Ph), 76.58 (C-8), 73.31 (C-4), 72.43 (C-6), 72.23 (1C, $^3J_{2,P} = 5.8$ Hz, C-2), 70.31 (C-5), 69.38 (1C, $CH_2$Ph), 69.21 (1C, $CH_2$Ph), 67.64 (C-9), 64.66 (C-7), 63.61 (C-10), 38.74 (1C, $^3J_{C,P} = 6.2$ Hz, C-3), 22.13 (1C, $^3J_{C,P} = 2.6$ Hz, C-1), 20.78, 20.60, 20.55 (5C, 5Ac); $^{31}P$ NMR ($CDCl_3$): $\delta$ 0.06. Anal. Calcd for $C_{34}H_{43}O_{15}P$ (722.67): C, 56.51; H, 6.00. Found: C, 56.54; H, 6.23.

**4.1.4. (4,8-Anhydro-1,3-dideoxy-L-*lyxo*-L-*gluco*-decit-2-yl)-phosphate 8 (ammonium salt).** A solution of **6** (55 mg, 0.076 mmol) in dry MeOH (20 mL) was hydrogenated at rt in the presence of 10% Pd/C (25 mg) for 12 h at atmospheric pressure. The catalyst was removed by filtration through a pad of Celite, and washed with MeOH ($3 \times 5$ mL). The combined filtrates were neutralized by the addition of $Et_3N$ (15 μL) and concentrated. The residue was lyophilized from water (10 mL) to give the monotriethylammonium salt of 5,6,7,9,10-penta-*O*-acetyl-4,8-anhydro-1,3-dideoxy-L-*lyxo*-L-*gluco*-decitol 2-phosphate **7** (45 mg, 92%) as an amorphous solid; $^1H$ NMR (MeOD): $\delta$ 5.29 (m, 1H, H-9), 5.26 (d, 1H, $^3J_{5,6} = 3.4$ Hz, H-5), 5.21 (dd, 1H, $^3J_{7,8} = 9.6$, $^3J_{6,7} = 10.1$ Hz, H-7), 5.12 (dd, 1H, H-6), 4.54 (dd, 1H, $^3J_{10a,9} = 2.8$, $^2J_{10a,10b} = 12.2$ Hz, H-10a), 4.44–4.33 (m, 1H, H-2), 4.36 (dd, 1H, $^3J_{10b,9} = 9.1$ Hz, H-10b), 4.09 (br d, 1H, H-4), 3.85 (dd, 1H, $^3J_{8,9} = 2.0$ Hz, H-8), 3.18 (q, ~6H, $CH_2$, $Et_3N$), 2.16, 2.06, 1.97, 1.96 and 1.92 (5s, each 3H, 5Ac), 1.76–1.65 (m, 1H, H-3a), 1.50–1.40 (m, H-3b), 1.31 (t, ~9H, $CH_3$, $Et_3N$), 1.29 (d, 3H, $^3J_{1,2} = 7.5$ Hz, $CH_3$); $^{13}C$ NMR (MeOD): $\delta$ 172.64, 172.40, 172.26, 171.55, 171.48 (5C, CO, Ac), 77.38 (C-8), 74.97 (C-4), 74.12 (C-6), 72.52 (C-5), 69.70 (1C, $^3J_{2,P} = 5.2$ Hz, C-2), 69.35 (C-9), 66.49 (C-7), 65.31 (C-10), 47.52 ($CH_2$, $Et_3N$), 41.01 (1C, $^3J_{C,P} = 6.0$ Hz, C-3), 23.06 (C-1), 20.83, 20.72, 20.67 and 20.55 (5C, 5Ac), 9.12 ($CH_3$, $Et_3N$); $^{31}P$ NMR (MeOD): $\delta$ 1.03.

A solution of **7** (50 mg, 0.078 mmol) in 7:3:1 MeOH–water–aq 25% $NH_4OH$ (3 mL, pH 12) was stirred at rt for 12 h. The reaction mixture was diluted with water (20 mL), concentrated to 10 mL volume and lyophilized. The residue was purified on a prepacked PD-10 column (Sephadex G-25, Amersham Pharmacia) by elution with water. The appropriate fractions were collected and lyophilized to give **8** (25 mg, 93%) as white fluffy solid; $[\alpha]_D^{20} = -50$ (*c* 0.7, $H_2O$); $^1H$ NMR ($D_2O$): $\delta$ 4.34–4.22 (m, 1H, H-2), 3.84 (ddd, 1H, $^3J_{8,9} = 1.3$, $^3J_{10a,9} = 5.8$ Hz, H-9), 3.72 (dd, 1H, $^3J_{5,6} = 3.5$ Hz, H-5), 3.65 (t, 1H, $^3J_{7,8} = {}^3J_{7,6} = 9.3$ Hz, H-7), 3.68–3.57 (m, 3H, H-4, H-6, H-10a), 3.55 (dd, 1H, $^2J_{10a,10b} = 11.4$, $^3J_{9,10b} = 6.7$ Hz, H-10b), 3.24 (dd, 1H, H-8), 1.75–1.65 (m, 1H, H-3a), 1.58–1.50 (m, 1H, H-3b), 1.18 (d, 3H, $^3J_{1,2} = 6.2$ Hz, H-1); $^{13}C$ NMR ($D_2O$): $\delta$ 77.58 (C-8), 74.64, 74.59 (C-4, C-6), 71.75 (C-5), 69.52 (1C, $^3J_{2,P} = 5.4$ Hz, C-2), 68.74 (C-9), 66.51 (C-7), 62.20 (C-10), 39.11 (1C, $^3J_{C,P} = 6.2$ Hz, C-3), 21.94 (C-1); $^{31}P$ NMR ($D_2O$): $\delta$ 1.85; TOF-ES-MS: $m/z = 333.086$ $[M+H]^+$; calcd 333.095 $[M+H]^+$.

**4.1.5. 2-Cyanoethyl-[5′-*O*-(2′,3′-di-*O*-acetyl)-adenosine]-*N*,*N*-diisopropylaminophosphine 9.** 2′,3′-Di-*O*-acetyladenosine (350 mg, 0.99 mmol) was dried by repeated co-evaporations with toluene ($3 \times 10$ mL), then under diminished pressure and dissolved in DMF/$CH_2Cl_2$ (1:1, v/v, 4 mL) under Ar. A solution of 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphoramidite (500 μL, 1.57 mmol) in $CH_2Cl_2$ (2 mL) along with a solution of diisopropylammonium tetrazolide (100 mg, 0.59 mmol) in dry DMF (2 mL) was added to this solution. The mixture was stirred for 4 h under Ar. Solvents were removed, the residue was redissolved in ethyl acetate (100 mL) and washed respectively with water and brine. The organic phase was dried over $Na_2SO_4$ and concentrated. The residue was purified by flash chromatography on silica gel (20:1:0.2 $CH_2Cl_2$/MeOH/$Et_3N$). Appropriate fractions were concentrated, dried by repeated co-evaporations with toluene ($3 \times 10$ mL) to give a crude diastereomeric mixture of **9** (500 mg, purity ~70%). $R_f$ 0.64 and 0.62 ($CH_2Cl_2$/MeOH/$Et_3N$, 20:1:0.2); $^{31}P$ NMR ($CDCl_3$): $\delta$ 150.28 and 149.77.

**4.1.6. Adenosine 5′-(4,8-anhydro-1,3-dideoxy-L-*lyxo*-L-*manno*-decit-2-yl)phosphate (triethylammonium salt) 11.** A mixture of **1** (50 mg, 0.11 mmol) and crude **9** (300 mg, ~60% purity, 0.32 mmol) was dried by repeated co-evaporations with toluene ($3 \times 10$ mL) and then under diminished pressure for 12 h. The mixture was dissolved in $CH_3CN$–$CH_2Cl_2$ (1:1, 2 mL) and a solution of 1*H*-tetrazole (28 mg, 0.4 mmol) was added under Ar. The mixture was stirred for 1 h, diluted with $CH_2Cl_2$ (20 mL) and washed sequentially with saturated aq $NaHCO_3$, water and brine. The organic phase was dried over $Na_2SO_4$ and concentrated. The residue was purified by flash chromatography on silica gel (40:1→20:1 $CH_2Cl_2$–$CH_3OH$) to give the intermediate phosphite triester [$R_f$ 0.62 (25:1 $CHCl_3$/MeOH)]. The appropriate fractions were collected and concentrated, the residue was transformed into the flask and dried under

diminished pressure for 5 h. The flask was charged with $CH_2Cl_2$ (2 mL), cooled to −15 °C and a solution of *t*-BuOOH (30 μL of 80% solution in di-*tert*-butyl peroxide) in $CH_2Cl_2$ (2 mL) was gradually added (~30 min). The reaction mixture was warmed to rt and stirred for 2 h. The solvent was concentrated, the residue dissolved in $CH_2Cl_2$ (50 mL) and washed sequentially with saturated aq $NaHCO_3$, water and brine. The organic phase was dried over $Na_2SO_4$, concentrated and the residue purified by flash chromatography on silica gel (20:1→10:1 $CH_2Cl_2$–$CH_3OH$) to give 2′,3′-di-*O*-acetyl-adenosine 5′-(5,6,7,9,10-penta-*O*-acetyl-4,8-anhydro-1,3-dideoxy-L-*lyxo*-L-*manno*-decit-2-yl) 2-cyanoethylphosphate **10** as a diastereomeric mixture on phosphorus as a syrup (73 mg, 87%). $^1$H NMR: δ 8.36 and 8.35 (s, 1H, H-$8_{Ade}$), 8.13 and 8.12 (s, 1H, H-$2_{Ade}$), 6.30 and 6.28 (d, 1H, $^3J_{1,2} = 6.0$ Hz, H-$1_{Rib}$), 5.95 and 5.90 (br s, 2H, NH), 5.85 and 5.81 (t, $^3J_{2,3} = 5.7$ Hz, H-$2_{Rib}$), 5.69 and 5.686 (m, 1H, H-$3_{Rib}$), 5.395 (br d, 1H, $^3J_{5,6} = 3.5$ Hz, H-5), 5.27 (t, 1H, $^3J_{7,8} = {}^3J_{7,6} = 9.7$ Hz, H-7), 5.30–5.23 (m, 1H, H-9), 5.16 and 5.00 (dd, 1H, H-6), 4.69–4.55 (m, 1H, H-2), 4.44–4.36 (m, 4H, H-10a, H-$5a_{Rib}$, H-$5b_{Rib}$, H-$4_{Rib}$), 4.32–4.22 (m, 2H, $OCH_2$), 4.17–4.08 (dd, 1H, H-10b), 3.89 (m, 1H, H-4), 3.76 and 3.64 (dd, 1H, $^3J_{8,9} = 2.0$ Hz, H-8), 2.82–2.75 (m, 2H, $CH_2CN$), 2.17, 2.15, 2.11, 2.10, 2.06, 2.05, 2.03, 2.01, 2.00 and 1.95 (10s, total 15H, 5Ac), 2.00 (m, 1H, H-3a), 1.83–1.71 (m, 1H, H-3b), 1.38 and 1.37 (d, 1H, $^3J_{1,2} = 6.2$ Hz, H-1); $^{13}$C NMR ($CDCl_3$): δ 170.71, 170.44, 170.25, 170.09, 169.58, 169.41, (5C, CO, Ac), 155.46 (C-$6_{Ade}$), 153.11 (C-$2_{Ade}$), 149.89 (C-$4_{Ade}$), 138.90 (C-$8_{Ade}$), 119.83 and 116.55 (C-$5_{Ade}$), 85.43 and 85.34 (C-$1_{Rib}$), 81.19 (C-$4_{Rib}$, $^3J_{4,P} = 7.6$ Hz), 77.15 (C-8), 74.08 (C-$2_{Rib}$), 73.89 and 73.80 (C-4), 73.35 and 73.16 (C-2), 72.51 and 72.42 (C-6), 70.58 (C-$3_{Rib}$), 69.74 and 69.58 (C-5), 67.19 and 67.07 (C-9), 66.67 (C-$5_{Rib}$), 64.75 and 64.67 (C-7), 62.88 and 62.72 (C-10), 62.78 and 62.48 ($OCH_2$, $^3J_{C,P} = 5.0$ Hz), 37.69 and 37.60 (C-3), 21.43 and 21.30 (C-1), 20.78, 20.62, 20.34, 19.76, 19.56, 19.45 (CO, Ac); $^{31}$P NMR ($CDCl_3$): δ −1.69 and −1.75. TOF-ES-MS: *m*/*z* = 929.294 $[M+H]^+$; calcd 929.281 $[M+H]^+$.

A solution of **10** (35 mg, 0.040 mmol) in 7:3:1 MeOH–water–$Et_3N$ (3 mL, pH 12) was stirred at rt for 4 h. The mixture was concentrated and the residue purified on silica gel (15:10:1:1, $CHCl_3$/MeOH/$H_2O$/$Et_3N$). Appropriate fractions were collected, concentrated, the residue redissolved in water (10 mL) and lyophilized to give **11** (25 mg, 97%) as a white fluffy solid. $[\alpha]_D^{20} = -34$ (*c* 0.8, $H_2O$); $^1$H NMR ($D_2O$): δ 8.39 (s, 1H, H-$8_{Ade}$), 8.14 (s, 1H, H-$2_{Ade}$), 6.03 (d, 1H, $^3J_{1,2} = 5.7$ Hz, H-$1_{Rib}$), 4.68 (unresolved signal, H-$2_{Rib}$), 4.43 (t, 1H, $^3J_{3,4} = 4.0$, $^3J_{2,3} = 4.8$ Hz, H-$3_{Rib}$), 4.29–4.22 (m, 2H, H-2, H-$4_{Rib}$), 4.02–3.99 (m, 2H, H-$5a_{Rib}$, H-$5b_{Rib}$), 3.79 (br t, 1H, H-9), 3.74 (br d, 1H, $^3J_{5,6} = 3.7$ Hz, H-5), 3.65 (t, 1H, $^3J_{7,8} = {}^3J_{7,6} = 9.6$ Hz, H-7), 3.55–3.43 (m, 4H, H-4, H-6, H-10a, H-10b), 3.10 (q, ~6H, $CH_2$, $Et_3N$), 3.02 (dd, 1H, $^3J_{8,9} = \sim 2.0$ Hz, H-8), 1.90–1.80 (m, 1H, H-3a), 1.66–1.57 (m, 1H, H-3b), 1.83 (t, ~9H, $CH_3$, $Et_3N$), 1.10 (d, 3H, $^3J_{1,2} = 6.2$ Hz, H-1). $^{13}$C NMR ($D_2O$): δ 155.24 (C-$6_{Ade}$), 152.48 (C-$2_{Ade}$), 149.02 (C-$4_{Ade}$), 139.60 (C-$8_{Ade}$), 118.52 (C-$5_{Ade}$), 86.98 (C-$1_{Rib}$), 83.90 (C-$4_{Rib}$, $^3J_{4,P} = 8.7$ Hz), 78.35 (C-8), 74.75, 74.46 (C-4, C-6), 74.28 (C-$2_{Rib}$), 71.05 (C-2, $^3J_{2,P} = 5.7$ Hz), 70.95 (C-5), 70.43 (C-$3_{Rib}$), 68.98 (C-9), 66.33 (C-7), 64.64 (C-$5_{Rib}$, $^2J_{5,P} = 5.3$ Hz), 63.05 (C-10), 46.67 ($CH_2$, $Et_3N$), 37.89 (C-3, $^3J_{3,P} = 5.5$ Hz), 20.64 (C-1, $^3J_{1,P} = 2.9$ Hz), 8.21 ($CH_3$, $Et_3N$); $^{31}$P NMR ($D_2O$): δ 0.77; TOF-ES-MS: *m*/*z* = 582.133 $[M+H]^+$; calcd 582.181 $[M+H]^+$.

**4.1.7. Adenosine 5′-(4,8-anhydro-1,3-dideoxy-L-*lyxo*-L-*gluco*-decit-2-yl)phosphate (triethylammonium salt) 13.** First, 2′,3′-di-*O*-acetyl-adenosine 5′-(5,6,7,9,10-penta-*O*-acetyl-4,8-anhydro-1,3-dideoxy-L-*lyxo*-L-*gluco*-decit-2-yl) 2-cyanoethylphosphate **12** was prepared from **5** (50 mg, 0.11 mmol) by treatment with crude **9** (300 mg, ~60% purity, 0.32 mmol) in the presence of 1*H*-tetrazole (28 mg, 0.4 mmol) in the same manner as described for the synthesis of **10**. Yield for **12**: 70 mg, 83% (syrup). $^1$H NMR: δ 8.34 (s, 1H, H-$8_{Ade}$), 8.17 (s, 1H, H-$2_{Ade}$), 6.35 and 6.33 (d, 1H, $^3J_{1,2} = 6.4$ Hz, H-$1_{Rib}$), 6.13 (br s, 2H, NH), 5.80 and 5.78 (t, $^3J_{2,3} = 5.7$ Hz, H-$2_{Rib}$), 5.69 and 5.68 (m, 1H, H-$3_{Rib}$), 5.50 (br d, 1H, H-5), 5.39 and 5.34 (dt, 1H, $^3J_{9,8} = 9.0$ Hz, H-9), 5.27 (t, 1H, $^3J_{7,8} = {}^3J_{7,6} = 9.7$ Hz, H-7), 5.22 and 5.19 (dd, 1H, $^3J_{6,5} = 3.5$ Hz, H-6), 4.77–4.66 (m, 1H, H-2), 4.59 and 4.53 (dd, 1H, $^3J_{10,9} = 3.1$, $^2J_{10a,10b} = 12.1$ Hz, H-10a), 4.44–4.22 (m, 6H, $OCH_2$, H-4, H-$5a_{Rib}$, H-$5b_{Rib}$, H-$4_{Rib}$), 4.25 and 4.19 (dd, 1H, $^3J_{10b,9} = 8.8$ Hz, H-10b), 4.03 and 3.96 (br d, 1H, H-4), 3.92–3.85 (m, 1H, H-8), 2.87–2.71 (m, 2H, $CH_2CN$), 2.20, 2.19, 2.16, 2.15, 2.05, 2.02, 2.01, 2.00 and 1.95 (9s, total 15H, 5Ac), 1.90–1.80 (m, 1H, H-3a), 1.70–1.57 (m, 1H, H-3b), 1.38 (d, 1H, $^3J_{1,2} = 6.3$ Hz, H-1); $^{13}$C NMR: δ 171.02, 170.67, 170.42, 170.23, 170.18, 169.79, 169.72, 169.33 (5C, CO, Ac), 155.58 (C-$6_{Ade}$), 153.00 (C-$2_{Ade}$), 150.06 (C-$4_{Ade}$), 119.77 and 116.80 (C-$5_{Ade}$), 85.10 (C-$1_{Rib}$), 81.60 (C-$4_{Rib}$, $^3J_{4,P} = 7.3$ Hz), 76.86 and 76.77 (C-8), 73.88 and 73.80 (C-4), 73.51 and 73.27 (C-2, C-$2_{Rib}$), 72.76 and 72.53 (C-6), 71.03 and 70.79 (C-$3_{Rib}$), 70.54 and 70.44 (C-5), 67.80 and 67.60 (C-9), 67.30 and 67.02 (C-$5_{Rib}$, $^3J_{C,P} = 6.6$ Hz), 65.03 and 64.89 (C-7), 64.36 and 63.82 (C-10), 62.78 and 62.48 ($OCH_2$, $^3J_{C,P} = 4.9$ Hz), 38.39 (C-3), 22.28 and 22.08 (C-1), 20.88, 20.84, 20.76, 20.70, 20.64, 20.57, 20.35, and 20.30 (5C, 5Ac), 19.90, 19.80, 19.73 and 19.64 ($CH_2CN$); $^{31}$P NMR ($CDCl_3$): δ −1.37 and −1.72. TOF-ES-MS: *m*/*z* = 929.291; calcd 929.281 $[M+H]^+$. A solution of **12** (50 mg, 0.054 mmol) in 7:3:1 MeOH–water–$Et_3N$ (3 mL, pH 12) was stirred at rt for 20 h. The mixture was concentrated and the residue purified on silica gel (15:10:1:1, $CHCl_3$/MeOH/$H_2O$/$Et_3N$). The appropriate fractions were collected and concentrated. The residue was redissolved in water (10 mL) and lyophilized to give **13** (32 mg, 87%) as a white fluffy solid. $[\alpha]_D^{20} = -45$ (*c* 0.7, $H_2O$); $^1$H NMR ($D_2O$): δ 8.36 (s, 1H, H-$8_{Ade}$), 8.13 (s, 1H, H-$2_{Ade}$), 6.02 (d, 1H, $^3J_{1,2} = 5.5$ Hz, H-$1_{Rib}$), 4.65 (m, 1H, $^3J_{2,3} = 5.1$ Hz, H-$2_{Rib}$), 4.42 (dd, 1H, $^3J_{3,4} = 4.1$ Hz, H-$3_{Rib}$), 4.35–4.23 (m, 2H, H-$4_{Rib}$, H-2), 4.07–3.95 (m, 2H, H-$5a_{Rib}$, H-$5b_{Rib}$), 3.81 (ddd, 1H, $^3J_{9,8} = 1.5$, $^3J_{9,10a} = 6.1$, $^3J_{9,10b} = 7.6$ Hz, H-9), 3.64 (t, 1H, $^3J_{7,6} = {}^3J_{7,8} = 9.8$ Hz, H-7), 3.60 (dd, 1H, $^2J_{10a,10b} = 11.5$ Hz, H-10a), 3.58 (dd, 1H, $^3J_{5,6} = 3.4$, $^3J_{4,5} < 1.0$ Hz, H-5), 3.53 (dd, 1H, H-10b), 3.54 (dd, 1H, H-10a), 3.43 (dd, 1H, H-6), 3.43–3.39 (m, 1H, H-4), 3.11 (q, 6H, $CH_2$, $Et_3N$), 3.09 (dd, 1H, H-8), 1.75–1.65 (m, 1H, H-3a), 1.52 (ddd, 1H,

$^{2}J_{3a,3b} = 14.6$ Hz, H-3b), 1.18 (t, 9H, $CH_3$, $Et_3N$), 1.13 (d, 3H, $^{3}J_{1,2} = 6.3$ Hz, H-1). $^{13}C$ NMR ($D_2O$): $\delta$ 155.47 (C-$6_{Ade}$), 152.82 (C-$2_{Ade}$), 149.02 (C-$4_{Ade}$), 139.80 (C-$8_{Ade}$), 118.55 (C-$5_{Ade}$), 87.08 (C-$1_{Rib}$), 83.80 (C-$4_{Rib}$, $^{3}J_{4,P} = 8.9$ Hz), 77.89 (C-8), 74.69, 74.55 (C-4, C-6), 74.06 (C-$2_{Rib}$), 71.73 (C-5), 71.27 (C-2, $^{2}J_{2,P} = 5.8$ Hz), 70.39 (C-$3_{Rib}$), 68.84 (C-9), 66.35 (C-7), 64.58 (C-$5_{Rib}$, $^{2}J_{5,P} = 5.2$ Hz), 62.51 (C-10), 46.67 ($CH_2$, $Et_3N$), 39.00 (C-3, $^{3}J_{3,P} = 6.6$ Hz), 21.76 (C-1, $^{3}J_{1,P} = 1.9$ Hz), 8.21 ($CH_3$, $Et_3N$); $^{31}P$ NMR ($D_2O$): $\delta$ −0.76; TOF-ES-MS: $m/z = 582.143$ $[M+H]^+$; calcd 582.181 $[M+H]^+$.

**4.1.8. Adenosine 5′-(4,8-anhydro-1,3-dideoxy-D-*ribo*-L-*manno*-decit-2-yl)phosphate (triethylammonium salt) 16.** The mixture containing crude **9** (200 mg, ~70% purity, 0.42 mmol) and **14** (41 mg, 0.088 mmol) was repeatedly dried by co-evaporation with dry toluene (3 × 5 mL) and then kept under reduced pressure for 5 h. The mixture was dissolved in dry $CH_2Cl_2$ (2 mL) and a solution of 1*H*-tetrazole (32 mg, 0.44 mmol) in dry $CH_3CN$ (1 mL) was added and the mixture was stirred at rt for 30 min under Ar. The reaction was monitored by TLC showing the formation of intermediate diastereomeric phosphite triesters ($R_f$ 0.54, 9:1 $CHCl_3$/MeOH), that was also substantiated by $^{31}P$ NMR ($\delta$ 141.52 and 140.66). The reaction mixture was cooled to −10 °C and a solution of *tert*-BuOOH (22 μL of 80% solution in di-*tert*-butyl peroxide) in $CH_2Cl_2$ (2 mL) was gradually added over 20 min. The reaction mixture was warmed to rt and stirred for 12 h. The solvent was concentrated, the residue dissolved in 1:1 diethyl ether/ethyl acetate (30 mL) and washed sequentially with saturated aq $NaHCO_3$, water and brine. The organic phase was dried ($Na_2SO_4$) and concentrated. The residue was purified by flash chromatography on silica gel (14:1 $CHCl_3$/MeOH) affording crude triphosphate (diastereomeric mixture on phosphorus) **15** (49 mg, ~60%) as a syrup. $R_f$ 0.40; $^{1}H$ NMR: $\delta$ 8.36 and 8.35 (s, 1H, H-$8_{Ade}$), 8.18 and 8.15 (s, 1H, H-$2_{Ade}$), 6.33 (d, 1H, $^{3}J_{1,2} = 6.0$ Hz, H-$1_{Rib}$), 6.14 and 6.11 (br s, 2H, NH), 5.79 and 5.78 (t, $^{3}J_{2,3} = 5.7$ Hz, H-$2_{Rib}$), 5.69 and 5.67 (m, 1H, H-$3_{Rib}$), 5.50 and 5.37 (br d, 1H, $^{3}J_{5,6} = 3.5$ Hz, H-5), 5.24 (t, 1H, $^{3}J_{7,8} = {}^{3}J_{7,6} = 9.7$ Hz, H-7), 5.24–5.12 (m, 1H, H-9), 5.14 and 4.97 (dd, 1H, H-6), 4.77–4.67 (m, 1H, H-2), 4.44–4.17 (m, 7H, H-10a, H-10b, H-$5a_{Rib}$, H-$5b_{Rib}$, H-$4_{Rib}$, $OCH_2$), 3.92–3.86 (m, H, H-4), 3.79 and 3.76 (dd, 1H, $^{3}J_{8,9} = 2.0$ Hz, H-8), 2.84–2.77 (m, 2H, $CH_2CN$), 2.17, 2.16, 2.15, 2.09, 2.07, 2.05, 1.97 and 1.96 (8s, total 15H, 5Ac), 2.00 (m, 1H, H-3a), 1.76–1.61 (m, 1H, H-3b), 1.38 (d, 1H, $^{3}J_{1,2} = 6.2$ Hz, H-1); $^{31}P$ NMR ($CDCl_3$): $\delta$ −1.65 and −1.88.

A solution of **15** (38 mg, 0.041 mmol) in 7:3:1 MeOH–water–$Et_3N$ (2 mL, pH 12) was stirred at room temperature for 3 h. The reaction mixture was diluted with water (20 mL), concentrated to 10 mL volume and lyophilized. The residue was purified on silica gel (15:10:1:1, $CHCl_3$/MeOH/$H_2O$/$Et_3N$) to give **16** (15 mg, 53%) as a white solid. $R_f$ 0.35 (15:10:1:1 $CHCl_3$/MeOH/$H_2O$/$Et_3N$); $[\alpha]_D^{20} = -19.8$ (*c* 0.8, $H_2O$); $^{1}H$ NMR ($D_2O$): $\delta$ 8.47 (s, 1H, H-$8_{Ade}$), 8.23 (s, 1H, H-$2_{Ade}$), 6.13 (d, 1H, $^{3}J_{1,2} = 6.1$ Hz, H-$1_{Rib}$), 4.76 (m, $^{3}J_{2,3} = 5.7$ Hz, H-$2_{Rib}$), 4.52 (dd, 1H, $^{3}J_{3,4} = 3.7$, $^{3}J_{3,2} = 4.9$ Hz, H-$3_{Rib}$), 4.37 (m, 1H, H-$4_{Rib}$), 4.34 (m, 1H, H-2), 4.08 (m, 1H, H-$5_{Rib}$), 3.88 (m, 1H, H-9), 3.85 (m, 1H, H-5), 3.65 (dd, 1H, $^{3}J_{10a,9} = 6.1$, $^{2}J_{10a,10b} = 12.0$ Hz, H-10a), 3.71 (dd, 1H, $^{3}J_{7,6} = {}^{3}J_{7,8} = 9.5$ Hz, H-7), 3.56 (dd, 1H, $^{3}J_{10b,9} = 3.2$ Hz, H-10b), 3.57 (m, 2H, H-4, H-6), 3.17 (dd, 1H, $^{3}J_{8,9} = 3.2$ Hz, H-8), 3.17 (q, ~6H, $CH_2$, $Et_3N$), 1.96–1.85 (m, 1H, H-3a), 1.76–1.67 (m, H-3b), 1.27 (t, ~9H, $CH_3$, $Et_3N$), 1.18 (d, 1H, $^{3}J_{1,2} = 6.2$ Hz, H-1). $^{13}C$ NMR ($D_2O$): $\delta$ 156.26 (C-$6_{Ade}$), 153.59 (C-$2_{Ade}$), 149.84 (C-$4_{Ade}$), 140.48 (C-$8_{Ade}$), 119.28 (C-$5_{Ade}$), 87.63 (C-$1_{Rib}$), 84.69 (C-$4_{Rib}$, $^{3}J_{4,P} = 8.7$ Hz), 80.64 (C-8), 75.85, 75.15 (C-4, C-6), 74.93 (C-$2_{Rib}$), 73.21 (C-9), 71.71 (C-2, $^{3}J_{2,P} = 5.8$ Hz), 71.22, 71.04 (C-$3_{Rib}$, C-5), 68.71 (C-7), 65.43 (C-$5_{Rib}$, $^{2}J_{5,P} = 5.3$ Hz), 62.71 (C-10), 47.42 ($CH_2$, $Et_3N$), 38.61 (C-3, $^{3}J_{3,P} = 6.1$ Hz), 21.63 (C-1, $^{3}J_{1,P} = 2.5$ Hz), 8.96 ($CH_3$, $Et_3N$); $^{31}P$ NMR ($D_2O$): $\delta$ 0.04; TOF-ES-MS: $m/z = 582.180$ $[M+H]^+$; calcd 582.181 $[M+H]^+$.

**4.1.9. Adenosine 5′-(4,8-anhydro-1,3-dideoxy-D-*ribo*-L-*gluco*-decit-2-yl)phosphate (triethylammonium salt) 19.** First, intermediate triphosphate **18** was prepared from **17** (33 mg, 0.071 mmol) and **9** (170 mg, ~70% purity, 0.36 mmol) in the presence of 1*H*-tetrazole (28 mg, 0.4 mmol) in the same manner as described for the synthesis of **16**. Yield for **18**: 40 mg (~60%). $R_f$ 0.40; $^{1}H$ NMR: $\delta$ 8.36 and 8.35 (s, 1H, H-$8_{Ade}$), 8.16 and 8.13 (s, 1H, H-$2_{Ade}$), 6.32 and 6.31 (d, 1H, $^{3}J_{1,2} = 6.4$ Hz, H-$1_{Rib}$), 5.95 (br s, 2H, NH), 5.80 and 5.79 (t, $^{3}J_{2,3} = 5.7$ Hz, H-$2_{Rib}$), 5.69 and 5.68 (m, 1H, H-$3_{Rib}$), 5.50 and 5.37 (br d, 1H, H-5), 5.24 and 5.21 (dt, 1H, $^{3}J_{9,8} = 10.0$ Hz, H-9), 5.20–4.95 (m, 2H, H-6, H-7), 4.77–4.69 (m, 1H, H-2), 4.59 and 4.53 (dd, 1H, $^{3}J_{10,9} = 3.1$, $^{2}J_{10a,10b} = 12.1$ Hz, H-10a), 4.43–4.17 (m, 7H, $OCH_2$, H-4, H-10b, H-$5a_{Rib}$, H-$5b_{Rib}$, H-$4_{Rib}$), 4.00 and 3.96 (br d, 1H, H-4), 3.92–3.77 (m, 1H, H-8), 2.83–2.77 (m, 2H, $CH_2CN$), 2.17, 2.16, 2.09, 2.08, 2.06, 2.04, 1.99, 1.97 and 1.96 (9s, 15H, 5Ac), 1.90–1.80 (m, 1H, H-3a), 1.77–1.59 (m, 1H, H-3b), 1.39 and 1.38 (d, 1H, $^{3}J_{1,2} = 6.3$ Hz, H-1); $^{31}P$ NMR ($CDCl_3$): $\delta$ −1.74 and −2.02. A solution of **18** (40 mg, 0.044 mmol) in 7:3:1 MeOH–water–$Et_3N$ (2 mL, pH 12) was stirred at rt for 3 h. The reaction mixture was diluted with water (20 mL), concentrated to 10 mL volume and lyophilized. The residue was purified on silica gel (15:10:1:1 $CHCl_3$/MeOH/$H_2O$/$Et_3N$) to give **19** (16 mg, 52%) as a white solid. $R_f$ 0.35 (15:10:1:1 $CHCl_3$/MeOH/$H_2O$/$Et_3N$); $[\alpha]_D^{20} = -31$ (*c* 0.8, $H_2O$); $^{1}H$ NMR ($D_2O$): $\delta$ 8.46 (s, 1H, H-$8_{Ade}$), 8.23 (s, 1H, H-$2_{Ade}$), 6.12 (d, 1H, $^{3}J_{1,2} = 5.5$ Hz, H-$1_{Rib}$), 4.78 (m, $^{3}J_{2,3} = 5.3$ Hz, H-$2_{Rib}$), 4.51 (t, 1H, $^{3}J_{3,4} = {}^{3}J_{3,2} = 4.5$ Hz, H-$3_{Rib}$), 4.40 (m, 1H, H-2), 4.37 (m, 1H, H-$4_{Rib}$), 4.10 (m, 2H, H-$5a_{Rib}$, H-$5b_{Rib}$), 3.89 (m, 1H, H-9), 3.68–3.66 (m, 2H, H-5, H-10), 3.64 (t, 1H, $^{3}J_{7,6} = {}^{3}J_{7,8} = 9.6$ Hz, H-7), 3.51 (m, 2H, H-4, H-6), 3.24 (dd, 1H, $^{3}J_{8,9} = 3.9$ Hz, H-8), 3.19 (q, ~6H, $CH_2$, $Et_3N$), 1.82–1.72 (m, 1H, H-3a), 1.69–1.60 (m, 1H, H-3b), 1.27 (t, ~9H, $CH_3$, $Et_3N$), 1.23 (d, 1H, $^{3}J_{1,2} = 6.5$ Hz, H-1). $^{13}C$ NMR ($D_2O$): $\delta$ 156.28 (C-$6_{Ade}$), 153.62 (C-$2_{Ade}$), 149.77 (C-$4_{Ade}$), 140.52 (C-$8_{Ade}$), 119.32 (C-$5_{Ade}$), 87.85 (C-$1_{Rib}$), 84.53 (C-$4_{Rib}$, $^{3}J_{4,P} = 8.8$ Hz), 80.16 (C-8), 75.73, 75.16 (C-4, C-6), 74.85 (C-$2_{Rib}$), 73.39 (C-9), 72.06, (C-5), 71.93 (C-2, $^{2}J_{2,P} = 5.6$ Hz), 71.11 (C-$3_{Rib}$), 69.13 (C-7), 65.33 (C-$5_{Rib}$, $^{2}J_{5,P} = 4.8$ Hz), 62.71 (C-10), 47.42

($CH_2$, $Et_3N$), 39.61 (C-3, $^3J_{3,P} = 6.6$ Hz), 22.47 (C-1, $^3J_{1,P} = 2.0$ Hz), 8.96 ($CH_3$, $Et_3N$); $^{31}P$ NMR ($D_2O$): $\delta$ 0.06; TOF-ES-MS: $m/z = 582.180$ $[M+H]^+$; calcd 582.178 $[M+H]^+$.

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## References

1. Raetz, C. R. H.; Whitfield, C. *Annu. Rev. Biochem.* **2002**, *71*, 635–700.
2. Holst, O. In *Endotoxin in Health and Disease*; Brade, H., Opal, S. M., Vogel, S. N., Morrison, D. C., Eds.; Marcel Dekker: New York, Basel, USA, 1999; pp 115–154, and references cited therein.
3. Kneidinger, B.; Marolda, C. L.; Graninger, M.; Zamyatina, A.; McArthur, F.; Kosma, P.; Valvano, M. A.; Messner, P. *J. Bacteriol.* **2002**, *184*, 363–369.
4. Valvano, M. A.; Messner, P.; Kosma, P. *Microbiology* **2002**, *148*, 1979–1989.
5. Eidels, L.; Osborn, M. J. *Proc. Natl. Acad. Sci. U.S.A.* **1971**, *68*, 1673–1677.
6. Kocsis, B.; Kontrohr, T. *J. Biol. Chem.* **1984**, *259*, 11858–11860.
7. Ding, L.; Seto, B. L.; Ahmed, S. A.; Coleman, W. G., Jr. *J. Biol. Chem.* **1994**, *269*, 24384–24390.
8. Gronow, S.; Oertelt, C.; Ervelä, E.; Zamyatina, A.; Kosma, P.; Skurnik, M.; Holst, O. *J. Endotoxin Res.* **2001**, *7*, 263–270.
9. (a) Zamyatina, A.; Gronow, S.; Oertelt, C.; Puchberger, M.; Brade, H.; Kosma, P. *Angew. Chem., Int. Ed.* **2000**, *39*, 4150–4153; (b) Zamyatina, A.; Gronow, S.; Puchberger, M.; Graziani, A.; Hofinger, A.; Kosma, P. *Carbohydr. Res.* **2003**, *338*, 2571–2589.
10. (a) Read, J. A.; Ahmed, R. A.; Tanner, M. E. *Org. Lett.* **2005**, *7*, 2457–2460; (b) Morrison, J. P.; Read, J. A.; Coleman, W. G., Jr.; Tanner, M. E. *Biochemistry* **2005**, *44*, 5907–5915.
11. Graziani, A.; Amer, H.; Zamyatina, A.; Hofinger, A.; Kosma, P. *Tetrahedron: Asymmetry* **2005**, *16*, 167–175.
12. (a) Du, Y.; Linhardt, R. J. *Tetrahedron* **1998**, *54*, 9913–9959; (b) Taillefumier, C.; Chapleur, Y. *Chem. Rev.* **2004**, *104*, 263–292; (c) Postema, M. *C-Glycosides Synthesis*; CRC Press: Boca Raton, USA, 1995; (d) Levy, D.; Tang, C. *The Chemistry of C-Glycosides*; Pergamon Press: Oxford, UK, 1995; (e) Beau, J.-M.; Vauzeilles, B.; Skydstrup, T. In *Carbohydrates in Chemistry and Biology*; Ernst, B., Hart, G. W., Sinay, P., Eds.; The Chemistry of Saccharides; Wiley-VCH: New York, 2000; pp 495–530; (f) Dondoni, A.; Marra, A. *Chem. Rev.* **2000**, *100*, 4395–4422.
13. (a) McGurk, P. M.; Chang, G. X.; Lowary, T. L.; McNeil, M.; Field, R. A. *Tetrahedron Lett.* **2001**, *42*, 2231–2234; (b) Compain, P.; Martin, O. R. *Bioorg. Med. Chem.* **2001**, *9*, 3077–3092.
14. (a) Stolz, F.; Reiner, M.; Blume, A.; Reutter, W.; Schmidt, R. R. *J. Org. Chem.* **2004**, *69*, 665–679; (b) Chang, R.; Vo, T.-T.; Finney, N. S. *Carbohydr. Res.* **2006**, *341*, 1998–2004; (c) Schäfer, A.; Thiem, J. *J. Org. Chem.* **2000**, *65*, 24–29; (d) Luengo, J. I.; Gleason, J. G. *Tetrahedron* **1992**, *33*, 6911–6914.
15. (a) Carchon, G.; Chrétien, F.; Delaanoy, P.; Verbert, A.; Chapleur, Y. *Tetrahedron Lett.* **2001**, *42*, 8821–8824; (b) Grugier, J.; Xie, J.; Duarte, I.; Valéry, J.-M. *J. Org. Chem.* **2000**, *65*, 979–984.
16. (a) Bragnier, N.; Scherrmann, M.-C. *Synthesis* **2005**, 814–818; (b) Rodriguez, F.; Canac, Y.; Lubineau, A. *Chem. Commun.* **2000**, 2049–2050; (c) Riemann, I.; Papadopoulos, M. A.; Knorst, M.; Fessner, W.-D. *Aust. J. Chem.* **2002**, *55*, 147–154; (d) Wang, Z.; Shao, H.; Lacroix, E.; Wu, S.-H.; Jennings, H.; Zou, W. *J. Org. Chem.* **2003**, *68*, 8097–8105.
17. (a) Barone, A. D.; Tang, J.-Y.; Caruthers, M. H. *Nucl. Acid Res.* **1984**, *12*, 4051–4061; (b) Nielsen, J.; Taagaard, M.; Marugg, J. E.; van Boom, J. H.; Dahl, O. *Nucl. Acid Res.* **1986**, *14*, 7391–7404.