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Synthesis of L-xylose derived cyclohexenephosphonates—versatile precursors of sialidase inhibitor libraries

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Abstract—L-*xylo* Configured cyclohexenephosphonates **1** and **2** have been synthesized as scaffolds for sialidase inhibitor libraries. These compounds were obtained by chain elongation and cyclization of suitably protected L-xylose. An unexpected phosphorylation was observed, however, the resulting phosphate could be removed in a final enzymatic step. Optimization of the reaction conditions avoided the undesired phosphorylation and opened the way to a purely chemical synthesis. © 2001 Elsevier Science Ltd. All rights reserved.

1. Introduction

Sialic acids have been shown to play a significant role in a wide variety of physiological recognition events, both normal and pathogenic.[1] This has led to an intensive study of the role played by sialidases, enzymes that cleave terminal sialic acids from glycan chains, during microbial infection. Rational design of inhibitors, based on X-ray crystal structure data, in particular targeting the sialidase from influenza virus, has led to spectacular enhancements in affinity and, as a result, in the development of new anti-influenza drugs.[2–5] Although sialidases from micro-organisms other than viruses, e.g. from bacteria[6] or protozoa[7], have been identified as potential targets for drug design, similar success in inhibition of the latter enzymes is still lacking. This may be due to the fact that bacteria possess a much larger number of modes of interaction with the host cell glycocalix. These include special adhesion molecules such as *E. coli* fimbriae and also the carbohydrate-binding sites within the sialidase molecule. In recent years, several such sites have been detected by X-ray crystallography and sequence alignment methods, and the protein domains they are part of have been termed 'lectin-like' domains or 'sialidase wings'.[8]

Our interest in the bifunctionality of these enzymes inspired us to synthesize potential sialidase inhibitors which allow attachment of a spacer molecule or an aglycon mimic while at the same time retaining (a) the half-chair conformation that mimicks the sialosyl cation transition-state and (b) the negative charge, which has been shown to be important for optimal recognition.

1.1. Inhibitor design and synthetic approach

As target structures we have chosen *xylo*-configured cyclohexenephosphonates of type **A** that resemble the influenza drug GS 4104,[4,5] which is itself an improved carbocyclic analog of the well known inhibitor Neu5Ac2en (Scheme 1).[9,10] Access to a high variety of structures is possible, as the synthetic approach includes the possibility of selective modification of all functional groups.

In the case of the influenza virus sialidase, the most effective inhibition was achieved by cyclohexene systems having the double bond in a position shown in Scheme 1 for GS 4104 and **A**.[4] As this is, however, not always the case for other sialidases, access to double bond regioisomers was included in our strategy to furnish compounds with a double bond position resembling that of Neu5Ac2en. The synthetic approach is outlined in Scheme 2.

Herein we report the synthesis of L-*xylo* substituted cyclohexenephosphonates **1** and **2** as highly versatile precursors of sialidase inhibitors and libraries thereof (Scheme 2). As shown in Scheme 2, a simple shift of the synthetic methodology from L-xylose to D-xylose will lead to the enantiomeric cyclohexenephosphonates, thus giving access to regioisomers having the double bond in the Neu5Ac2en-orientation.

1.2. Retrosynthesis

Starting from L-xylose, we employ well established methodologies[11–13] to obtain suitably functionalized and protected L-*xylo*-furanosyl-5-triflates. Nucleophilic substitution with tetraethyl methylenediphosphonate anions leads to chain elongated diphosphonates which, after protecting group modifications, can be cyclized in an intramolecular Knoevenagel-type condensation to yield the

Keywords: sialidase inhibitors; sialic acid; cyclohexenephosphonic acid; alkaline phosphatase.

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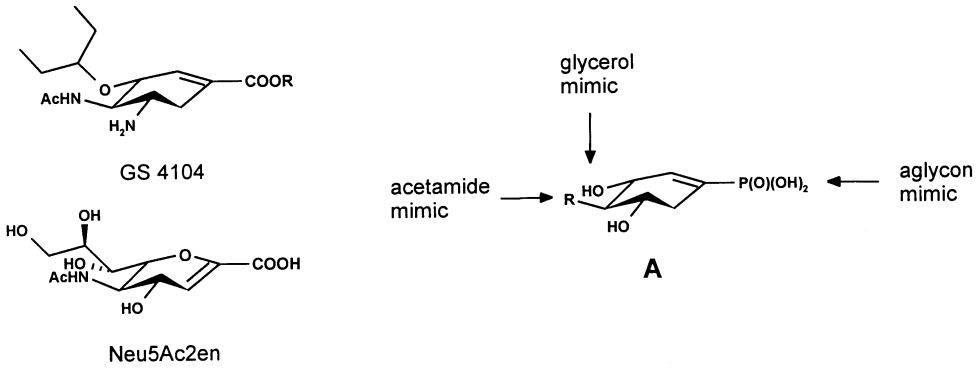
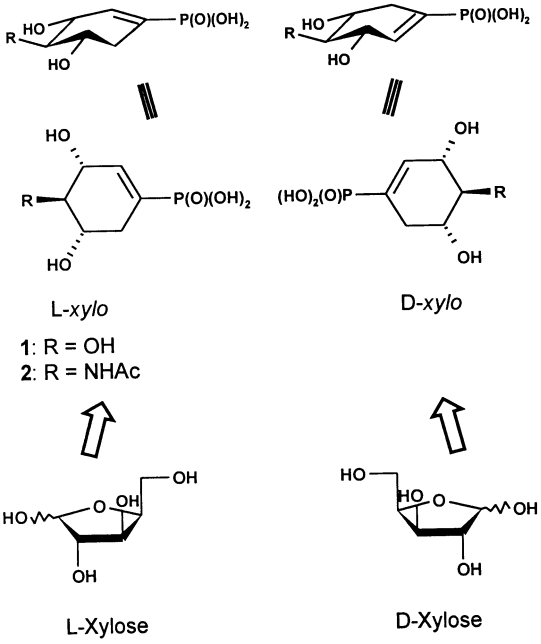
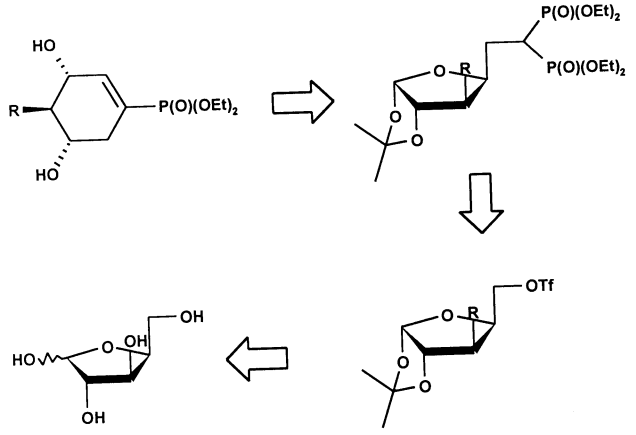


Scheme 1. Prodrug GS 4104 (R=Et) and Neu5Ac2en as lead compounds for the cyclohexenephosphonates **A** envisaged in our synthesis.



Scheme 2. Effective approach towards the synthesis of *xylo*-configured cyclohexenephosphonic acids with regioisomeric double bonds.

corresponding cyclohexenephosphonates (Scheme 3). This approach has been introduced by both Fleet et al.,[14] and Vasella and coworkers[15] in their syntheses of (−) shikimic acid and shikimic acid analogs.[15,16]



Scheme 3. Retrosynthetic concept, inspired by syntheses of shikimic acid and analogs.[14–16]

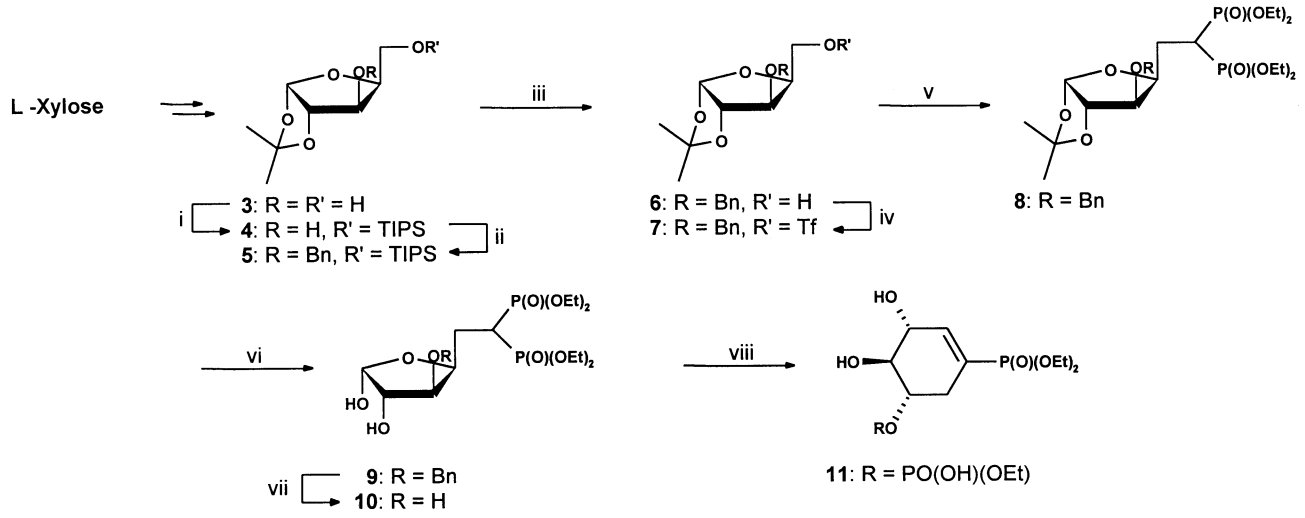
2. Results and discussion

A well known route was applied to the preparation of the suitably protected and activated L-xylofuranose derivative. In brief, 1,2-*O*-isopropylidene-L-xylofuranose **3**, which is readily available from L-xylose in high yield was silylated selectively in the primary position to give the corresponding 5-*O*-triisopropylsilyl(TIPS)-xylofuranose **4**. Benzylation of the 3-position to give **5**, followed by selective cleavage of the silyl group with TBAF yields 5-unprotected xylofuranose derivative **6** with all yields greater than 90%. Reaction of **6** with triflic anhydride and pyridine yields the 5-*O*-triflate **7**, which, after quick purification, was reacted with deprotonated methylene tetraethyldiphosphonate in DMF to give C_1-elongated diphosphonate **8** in 51% yield (Scheme 4).

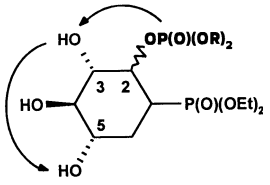
Acidic cleavage of the isopropylidene moiety followed by hydrogenolytic cleavage of the benzyl ether gave **9** (89%) and **10** (qu), which should be ideally suited for intramolecular condensation to form the cyclohexene system. Upon treatment of **10** with sodium ethanoate in ethanol, the expected condensation took place instantly, but quite unexpectedly, the cyclohexenephosphonate formed (**11**) still contained a monoethylphosphate moiety at position 5, rather than having eliminated the phosphate to bulk (Schemes 4 and 5).

A similar effect was not reported in either the syntheses of shikimic acid by Fleet et al.[14] and Vasella et al.[15] or in the synthesis of its phosphonate analog.[15,16] However, in these cases the 3- and 4-positions were blocked by protecting groups and the stereochemistry at C-4 and C-5 was different. It may be speculated that the 3-OH of the newly formed cyclohexenephosphonate system plays some role in the intramolecular transesterification (Scheme 5), allowing transfer to the 5-position via subsequent 5- and 6-membered rings.

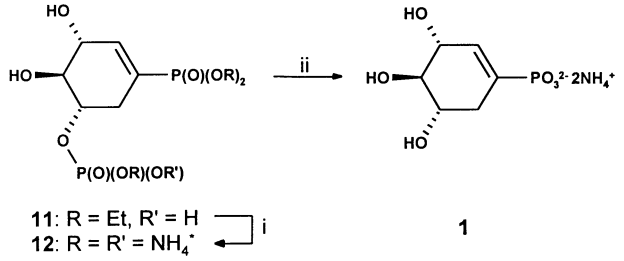
Although cyclohexenephosphonates such as **11**, once the esters are cleaved, may be of interest for investigation of other biological processes, we focused, of course, on the removal of the phosphate group (Scheme 6). Cleavage of a phosphate diester containing an ethyl group in presence of a diethylphosphonate is not trivial. We therefore treated compound **11** with TMSBr in $CHCl_3$, followed by hydrolysis, which led to cleavage of all three ethyl ester



Scheme 4. (i) TIPSCl, imidazole, CH_2Cl_2, (91%); (ii) NaH, BnBr, DMF, (92%); (iii) TBAF, THF, (98%); (iv) Tf_2O, pyridine, CH_2Cl_2, (96%); (v) $CH_2(P(O)(OEt)_2)_2$, NaH, DMF, (51%); (vi) TsOH, dioxane, H_2O, (89%); (vii) H_2, Pd/C, dioxane, H_2O, (qu); (viii) NaOEt, EtOH, (52%).



Scheme 5. Possible triester intermediate (R=Et) of the Knoevenagel-type condensation. If the desired elimination of the phosphate group is followed by an undesired transesterification, formation of the described 5-phosphate (**11**) seems possible. It may, however, be speculated that this reaction occurs rather through successive 5- and 6-membered ring formation than through a 7-membered intermediate.



Scheme 6. (i) TMSBr, $CHCl_3$, (qu); (ii) alkaline phosphatase, Biogel P2.

groups, thus giving phosphonate-phosphate **12** (Scheme 6). Fortunately, alkaline phosphatase from calf intestine, a readily available enzyme with broad substrate specificity,[17] was successful in cleaving the phosphate of **12** to form the first target compound **1** in high yield (Scheme 6).

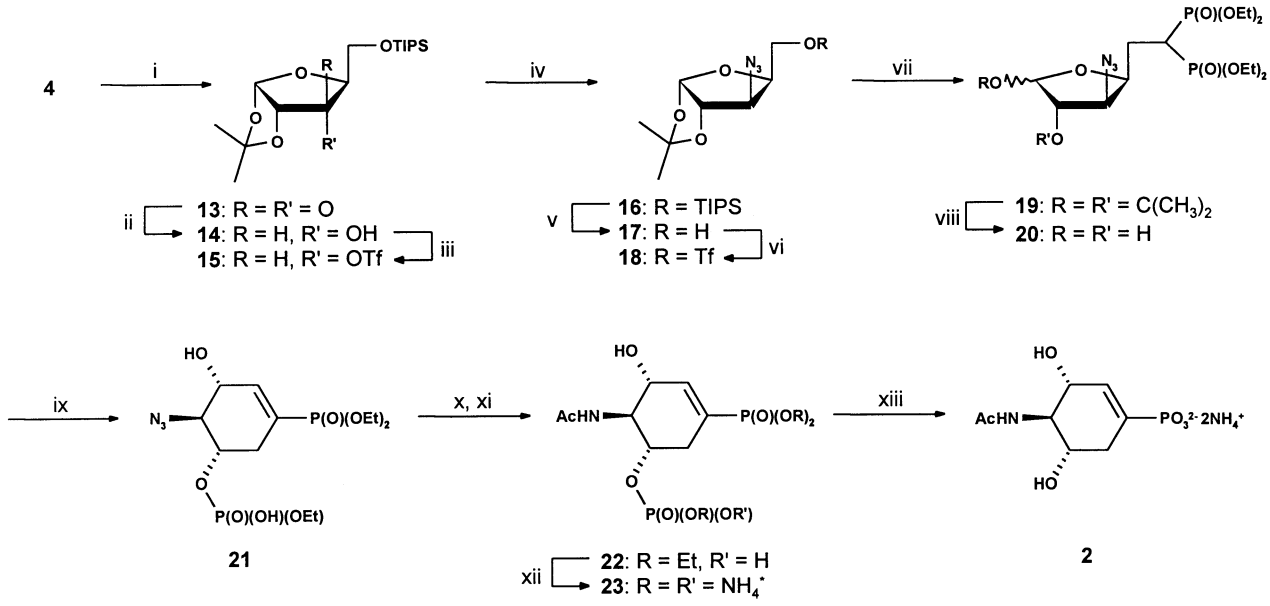
2.1. Introduction of acetamide

Our first approach to introduce an acetamido group into cyclohexenephosphonates to obtain target compound **2** included the introduction of an azide moiety at an early stage for two reasons. Firstly, azide reduction after cyclohexene fomation allows the acylation of the free amine group with a large variety of side chains and secondly, early introduction into the well investigated L-xylofuranose system avoids the need for protecting and activating group manipulations in the lesser known cyclohexenephosphonate systems.

Perhaps the most straightforward way to obtain 3-azido xylofuranoses, described recently by Horenstein et al.[11] for D-xylose and well known for the closely related glucofuranose, is to twice invert the absolute configuration at position 3.

Swern oxidation of the L-configurated alcohol **4** gave the ketone **13** in 95% yield, which was stereospecifically reduced to L-ribofuranose **14** (93%) (Scheme 7). Reaction with triflic anhydride and pyridine gave triflate **15** which could be readily converted to an azide by nucleophilic substitution to form L-xylo azide **16** in 82% yield (Scheme 7). Cleavage of the silyl ether to form **17** and conversion of the alcohol into the triflate **18** allowed reaction with tetraethyl methylenediphosphonate anion to form chain-elongated diphosphonate **19** in reasonable yield. In this case, it proved to be beneficial to use lithium-bis-trimethylsilylamide as the base (Scheme 7). Cleavage of the isopropylidene group with toluenesulfonic acid gave hemiacetal **20**, which was cyclized under the same conditions as described in the synthesis of **1**, to give the azido-cyclohexenephosphonate **21** which again contained a monoethylphosphate moiety in position 5 (Scheme 7). This result confirms that the 4-hydroxyl group is not participating in the transesterification.

Fortunately, conversion to the corresponding deprotected acetamido-cyclohexenephosphonate proved to be straightforward. Reduction of the azide in **21** with hydrogen sulfide and immediate acetylation under Schotten–Baumann conditions gave acetamide **22**. Again, all three ethyl ester groups were cleaved with TMSBr in $CHCl_3$ to yield phosphonate-phosphate **23** and the remaining phosphate in **23** was removed quantitatively with alkaline phosphatase. Thus, target compound **2** was obtained in good yield after purification by gel chromatography (Scheme 7).



Scheme 7. (i) $(COCl)_2$, DMSO, (95%); (ii) $NaBH_4$, EtOH, (93%); (iii) Tf_2O, pyridine, CH_2Cl_2; (iv) NaN_3, EtOH, (82%); (v) TBAF, THF, (99%); (vi) Tf_2O, pyridine, CH_2Cl_2, (87%); (vii) $CH_2(P(O)(OEt)_2)_2$, $LiN(SiMe_3)_2$, DMF, (38%); (viii) IR-120 (H^+), dioxane, H_2O, (51%); (ix) NaOEt, EtOH, (77%); (x) H_2S, pyridine, H_2O, (qu); (xi) Ac_2O, MeOH, $NaHCO_3$, (67%); (xii) TMSBr, $CHCl_3$, (qu); (xiii) alkaline phosphatase, Biogel P2.

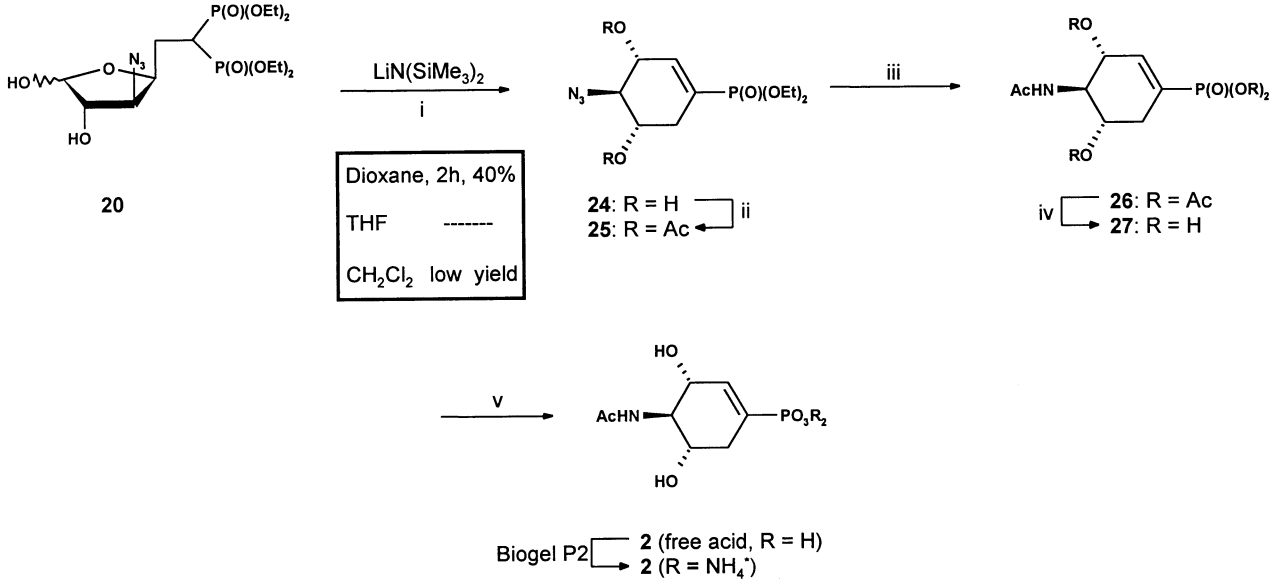
2.2. Position of phosphate

The position of the phosphate moiety in the cyclohexene system was confirmed to be position C-5 by a combination of spin echo diffusion (SED), ^{13}C NMR and ^{1}H NMR spectroscopy. In the NMR spectra a considerable upfield shift of C-5 and H-5 is observed upon removal of the phosphate group (see compounds **12** and **1**, or **23** and **2**, respectively).

2.3. Avoiding phosphate migration

As modifications and purifications of the highly polar phosphoric acid derivatives **21**, **22** and **23** proved to be somewhat difficult, further investigation of the ring closure reaction was required (Scheme 8). Phosphate migration could be avoided by the use of lithium-bis-trimethylsilylamide in dioxane as solvent and the desired azido-cyclohexenephosphonate **24** was obtained, albeit in only 40% yield.

The reaction proved to be highly dependent on the solvent used, e.g. cyclization in THF furnished only side products while CH_2Cl_2 slowed down the reaction considerably and led to lower yields (Scheme 8). For analytical purposes, azide **24** was acetylated to give **25**. Reduction of **24** followed by peracetylation readily gave the acetamide **26**, the acetyl groups of which could be selectively removed with sodium ethanolate in ethanol to yield **27**. Final cleavage of the phosphonate diester with trimethylsilyl bromide in $CHCl_3$ followed by hydrolyis in water furnished



Scheme 8. (i) $LiN(SiMe_3)_2$, dioxane, (40%); (ii) Ac_2O, pyridine, (qu); (iii) H_2S, pyridine, H_2O then Ac_2O, pyridine, (53%); (iv) NaOEt, EtOH, (qu); (v) TMSBr, $CHCl_3$, (qu).

the free acid of **2** which was purified and converted into the ammonium salt by gel chromatography (Scheme 8).

3. Conclusions

L-*xylo* Configured cyclohexenephosphonates, both with and without acetamido group in the 4-position, were synthesized from L-xylose. At first, an unexpected transesterification occurred and the problem could be solved by addition of one enzymatic step. Optimization of the reaction conditions avoided phosphate migration and allowed the synthesis of the target compounds in a purely chemical synthesis. The cyclohexenephosphonates obtained are highly functionalizable scaffolds for inhibitors of sialic acid recognizing proteins, which allow attachment of aglycon mimics through a phosphonate monoester. In addition, the synthetic methodology can be transferred to the D-series with the regioisomeric double bond position. Physical data of the D-series, alongside with some exemplary modifications towards fully capable inhibitors will be published by us shortly.

4. Experimental

4.1. General

^{1}H, ^{13}C and ^{31}P NMR spectra were recorded on a Bruker DRX-600 and a Bruker AC-250 spectrometer, taking the chemical shift of deuterated solvent as standard, except for ^{31}P spectra, where 85% phosphoric acid was used (0 ppm). ^{13}C chemical shifts were deduced from HMQC-(heteronuclear multiple quantum correlation) spectra. MALDI-MS were recorded on a Kratos Analytic Kompact Maldi 2, using DHB: 3,5-dihydroxybenzoic acid HCCA: α-Hydroxy-α-cyano-cinnamic acid or ATT: azidothymidine as matrix. FAB-MS was recorded on a modified Finnigan MAT 312/AMD-5000. Reactions were monitored with plastic plates coated with silica gel $60F_{254}$. Solvents for flash chromatography (EE: ethyl acetate, Tol: toluene) were distilled before use. Chemicals: TIPSCl: triisopropylsilyl chloride, TMSBr: bromotrimethylsilane, TBAF: tetrabutylammonium fluoride.

4.1.1. 1,2-*O*-Isopropylidene-α-L-xylofuranose (3). L-Xylose (25 g, 0.167 mmole) is suspended in 400 ml of dry acetone, the mixture is cooled to 0°C and 10 ml of concentrated sulfuric acid (96%) was added in portions. The mixture is stirred until TLC indicates the absence of starting material (~3 h), neutralized with sodium hydroxide and sodium bicarbonate, filtered and evaporated. The crude product is dissolved in MeOH (200 ml) and hydrochloric acid (0.2%, 200 ml) is added. The solution is stirred overnight at room temperature, neutralized with triethyl amine and evaporated. Flash chromatography (EE) furnished **3** (25.5 g, 80%).

4.1.2. 1,2-*O*-Isopropylidene-5-*O*-triisopropylsilyl-α-L-xylofuranose (4). Compound **3** (10.85 g, 0.057 mmol) and TIPSCl (14.5 ml, 0.068 mmol) are dissolved in dry CH_2Cl_2 (300 ml) and the solution is cooled to 0°C. Imidazole (8.54 g, 0.125 mmol) is added and the mixture is stirred at room temperature for 24 h. Following extraction with saturated NH_4Cl solution the organic layer is dried ($MgSO_4$) and evaporated. Flash chromatography (Tol/EE 4:1) gave **4** (17.91 g, 91%) as a colourless oil. R_f=0.7 (Tol/EE 1:1); $[\alpha]_D^{20}$=7.7° (c=1, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): δ=5.95 (d, 1H, H-1, J_{1-2}=3.7 Hz), 4.51–4.48 (m, 2H, H-2, OH), 4.33 (m, 1H, H-3), 4.20 (m, 2H, H-5, H-5′), 4.11 (m, 1H, H-4), 1.46 (s, 3H, C(CH_3)$_2$), 1.30 (s, 3H, C(CH_3)$_2$), 1.09–1.01 (m, 21H, 3Si (CH(CH_3)$_2$).

4.1.3. 3-*O*-Benzyl-1,2-*O*-isopropylidene-5-*O*-triisopropylsilyl-α-L-xylofuranose (5). Compound **4** (15 g, 43.3 mmol) and benzyl bromide (6.7 ml, 65.3 mmol) are dissolved in dry DMF (250 ml), the solution is cooled to 0°C and NaH (1.56 g, 64.9 mmol) is added in portions. The mixture is stirred at room temperature until TLC indicates the absence of starting material (~ 60 min). MeOH (5 ml) is added, the solvent is evaporated and the residue is taken up in EE (200 ml). After extraction with water the organic layer is dried ($MgSO_4$), evaporated and the residue is chromatographed to give **5** (17.3 g, 92%) as a colourless oil. R_f=0.73 (Tol/EE 9:1); $[\alpha]_D^{20}$=33.3° (c=1, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): δ=7.36–7.21 (m, 5H, C_6H_5), 5.89 (d, 1H, H-1, J_{1-2}=3.8 Hz), 4.66, 4.58 (2d, 2H, CH_2Ph, J=11.9 Hz), 4.57 (d, 1H, H-2), 4.25 (ddd, 1H, H-4), 4.02–3.88 (m, 3H, H-3, H-5, H-5′), 1.48 (s, 3H, C(CH_3)$_2$), 1.30 (s, 3H, C(CH_3)$_2$), 1.19–0.09 (m, 21H, 3Si (CH(CH_3)$_2$).

4.1.4. 3-*O*-Benzyl-1,2-*O*-isopropylidene-α-L-xylofuranose (6). Compound **5** (17.3 g, 39.6 mmol) is dissolved in dry THF (200 ml), cooled to 0°C and TBAF (1 M in THF, 44 ml, 44 mmol) is added. After stirring for 1 h at room temperature the solvent is evaporated and the residue is chromatographed (Tol/EE 3:1) to give **6** (10.9 g, 98%). Physical data correspond to those reported in the literature for the D-enantiomer[18] with opposite optical rotation.

4.1.5. 3-*O*-Benzyl-1,2-*O*-isopropylidene-5-*O*-trifluoromethanesulfonyl-α-L-xylofuranose 7 and tetraethyl [3-*O*-benzyl-5,6-dideoxy-1,2-*O*-isopropylidene-α-L-xylofuranose-6,6′-diyl]bisphosphonate (8). Alcohol **6** (3 g, 10.7 mmol) and pyridine (1.92 ml) are dissolved in dry CH_2Cl_2 (60 ml) and cooled to −30°C. Trifluoromethanesulfonic acid anhydride (1.92 ml, 13.9 mmol) is added in portions during 30 min and the mixture is stirred for additional 30 min. MeOH (2 ml) is added, the mixture is brought to room temperature and extracted twice with KH_2PO_4 solution (1 M, 30 ml). The organic layer is dried ($MgSO_4$) and evaporated. The resulting triflate **7** (4.22 g, 96%) is used without further purification.

NaH (367 mg, 15.3 mmol) is suspended in dry DMF (60 ml) and cooled to 0°C. Tetraethyl methylenediphosphonate (4 ml, 16.3 mmol) is added within 20 min and the mixture is stirred for 1 h at room temperature. Triflate **7** (4.2 g, 10.2 mmol), dissolved in dry DMF (30 ml), is added and the mixture is stirred until TLC indicates the absence of triflate. The reaction is quenched with KH_2PO_4-solution (1 M, 200 ml), the aqueous layer is thoroughly extracted with $CHCl_3$, dried ($MgSO_4$) and evaporated. The residue is chromatographed (Tol/EE 20:1) to give diphosphonate **8** (2.87 g, 49% from alcohol **6**). R_f=0.19 (Tol/EE 30:1); $[\alpha]_D^{20}$=24.2 (c=1, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$):

δ=7.35–7.25 (m, 5H, C_6H_5), 5.89 (d, 1H, H-1, J_{1-2}=3.9 Hz), 4.68, 4.49 (2d, 2H, CH_2Ph, J=11.8 Hz), 4.61 (d, 1H, H-2), 4.57 (m, 1H, H-4), 4.20–4.13 (m, 8H, 4CH_2CH_3), 3.85 (d, 1H, H-3, J_{3-4}=3.3 Hz), 2.57 (m, 1H, H-6), 2.43 (m, 1H, H-5), 2.16 (m, 1H, H-5′), 1.48 (s, 3H, C(CH_3)$_2$), 1.36–1.29 (m, 15H, C(CH_3)$_2$, 4CH_2CH_3). ^{13}C NMR (150.9 MHz, $CDCl_3$): δ=104.5 (C-1), 82.5 (C-2), 81.9 (C-3), 77.9 (C-4), 71.7 (CH_2Ph), 62.8–62.3 (4 CH_2CH_3), 33.3 (C-6), 26.5 (C-5), 16.3 (CH_2CH_3). ^{31}P NMR (242.9 MHz, $CDCl_3$): δ=24.5, 24.4 (2s, 2P, 2P(O)(OEt)$_2$). $C_{24}H_{40}P_2O_{10}$ (M 550.52). MALDI-MS (pos. mode, DHB): 588.6 (M+K^+), 572.7 (M+Na^+).

4.1.6. Tetraethyl [3-*O*-benzyl-5,6-dideoxy-α,β-L-xylofuranose-6,6′-diyl]bisphosphonate (9). Diphosphonate **8** (500 mg, 0.91 mmol) is dissolved in dioxane/water (1:1, 20 ml), toluenesulfonic acid monohydrate (170 mg) is added and the mixture is stirred at 80°C until TLC indicates absence of starting material (~ 20 h). Following neutralization with saturated NH_4HCO_3 solution the mixture is concentrated to approximately half its volume and extracted with $CHCl_3$. The organic layer is dried ($MgSO_4$), evaporated and the residue is chromatographed to give **9** (α,β-mixture, 413 mg, 89%) as a colourless oil. R_f=0.18 (Tol/EE 10:1); ^{1}H NMR (600 MHz, DMSO-d_6): δ=7.37–7.27 (m, 10H, $C_6H_5^{\alpha,\beta}$), 6.22, 6.13, 5.39, 5.05 (4d, 4H, 2OH^{α}, 2OH^{β}), 4.66, 4.62, 4.47, 4.44 (4d, 4H, 4CH_2Ph, J=12 Hz), 4.40, 4.32 (2m, 2H, H-4$^{\alpha}$, H-4$^{\beta}$), 4.03–3.95 (m, 16H, 4$CH_2CH_3^{\alpha,\beta}$), 3.97, 3.91 (2m, 2H, H-2$^{\alpha}$, H-2$^{\beta}$), 3.85, 3.73 (2m, 2H, H-3$^{\alpha}$, H-3$^{\beta}$), 2.45 (m, 2H, H-6$^{\alpha}$, H-6$^{\beta}$), 2.03 (m, 4H, H-5$^{\alpha}$, H-5′$^{\alpha}$, H-5$^{\beta}$, H-5′$^{\beta}$), 1.23–1.17 (m, 24H, 4$CH_2CH_3^{\alpha,\beta}$). ^{13}C NMR (150.9 MHz, $CDCl_3$): δ=102.3, 95.1 (C-1$^{\alpha}$, C-1$^{\beta}$), 83.3, 82.7 (C-3$^{\alpha}$, C-3$^{\beta}$), 79.4, 74.8 (C-2$^{\alpha}$, C-2$^{\beta}$), 73.9, 73.8 (C-4$^{\alpha}$, C-4$^{\beta}$). ^{31}P NMR (242.9 MHz, $CDCl_3$): δ=25.34, 25.31 (2s, 2P, 2P(O)(OEt)$_2^{\alpha,\beta}$), 25.21 (2P, 2P(O)(OEt)$_2^{\alpha,\beta}$). $C_{21}H_{36}P_2O_{10}$ (M 510.52). MALDI-MS (pos. mode, DHB): 549.0 (M+K^+), 533.2 (M+Na^+).

4.1.7. Tetraethyl [5,6-dideoxy-α,β-L-xylofuranose-6,6′-diyl]bisphosphonate (10). The mixture of anomeric diphosphonates **9** (100 mg, 0.2 mmol) in dioxane/water (1.1, 8 ml) is hydrogenated overnight over palladium–charcoal (10%, 10 mg) at room temperature. The mixture is filtered through Celite and evaporated to give **10** (α,β-mixture, 82 mg, qu) as a colourless oil of excellent purity. R_f=0.5 (EE/MeOH/H_2O 70:30:3); ^{1}H NMR (250 MHz, MeOH-d_4): δ=5.32, 5.03 (1d, 1bs, 2H, H-1$^{\alpha}$, H-1$^{\beta}$), 4.18, 4.06, 3.94 (3m, 22H, H-2$^{\alpha}$, H-2$^{\beta}$, H-3$^{\alpha}$, H-3$^{\beta}$, H-4$^{\alpha}$, H-4$^{\beta}$, 4$CH_2CH_3^{\alpha}$, 4$CH_2CH_3^{\beta}$), 2.76 (m, 2H, H-6$^{\alpha}$, H-6$^{\beta}$), 2.32–2.04 (m, 4H, H-5$^{\alpha}$, H-5′$^{\alpha}$, H-5$^{\beta}$, H-5′$^{\beta}$), 1.38–1.32 (m, 24H, 4$CH_2CH_3^{\alpha,\beta}$). $C_{14}H_{30}P_2O_{10}$ (M 420.39). MALDI-MS (pos. mode, DHB): 443.3 (M+Na^+).

4.1.8. Ethyl [diethyl (3*R*,4*R*,5*S*)-3,4,5-trihydroxy-1-cyclohexenephosphonate-5-yl]phosphoric acid (11). The anomeric mixture of compound **10** (800 mg, 1.90 mmol) is stirred in a solution of NaOEt in EtOH (0.1 M) until TLC indicates the absence of starting material (~1 h). The solution is neutralized with Amberlite IR-120 (H^+-form), filtered and evaporated. The residue is chromatographed (EE/MeOH 1:1) to give cyclohexenephosphonate **11** (260 mg, 52%) as a colourless foam. R_f=0.37 (EE/MeOH 1:1); $[\alpha]_D^{20}$=−11.4 (c=1, MeOH); ^{1}H NMR (600 MHz, DMSO-d_6): δ=6.24 (bd, 1H, H-2, J_{P-2}=21 Hz), 4.10–3.90 (m, 6H, H-3, H-5, 2CH_2CH_3), 3.71 (m, 2H, CH_2CH_3), 3.31 (m, 1H, H-4), 2.38, 2.04 (2m, 2H, H-6, H-6′), 1.22, 1.09 (2m, 9H, 3CH_2CH_3). ^{13}C NMR (150.9 MHz, DMSO-d_6): δ=144.0 (C-2), 124.0 (C-1), 75.7 (C-4), 72.5 (C-3), 71.2 (C-5), 31.7 (C-6). ^{31}P NMR (242.9 MHz, DMSO-d_6): δ=19.23 (1s, 1P, P(O)(OEt)$_2$), 2.21 (bs, 1P, OP(OH)(OEt). $C_{12}H_{24}P_2O_9$ (M 374.089). MALDI-MS (pos. mode, DHB): 396.9 (M+Na)$^+$, 418.9 (M−H+2Na)$^+$. FAB-MS (pos. mode, NBA): 397 (M+Na)$^+$, 419 (M−H+2Na)$^+$.

4.1.9. Ammonium [(3*R*,4*R*,5*S*)-3,4,5-trihydroxy-1-cyclohexenephosphonate-5-yl]phosphate (12). Compound **11** (50 mg, 0.19 mmol) is dissolved in $CHCl_3$ (5 ml) and a solution of TMSBr in $CHCl_3$ (10%, v/v, 0.5 ml) is added. The mixture is stirred for 24 h, water (2 ml) is added and the mixture is concentrated in vacuo and the crude product is purified by gel filtration (Biogel P2, 0.1 M NH_4HCO_3) to give **12**. $[\alpha]_D^{20}$=−2 (c=0.5, H_2O/MeOH 1:1). ^{1}H NMR (600 MHz, D_2O): δ=5.98 (ddd, 1H, H-2, J_{P-2}=19.4 Hz, J_{2-3}=2.5 Hz, J_{2-6}=2.5 Hz), 4.14 (m, 1H, H-3), 4.07 (m, 1H, H-5), 3.49 (dd, 1H, H-4, J_{4-3}=8.2 Hz, J_{4-5}=9.7 Hz), 2.68 (m, 1H, H-6), 2.20 (m, 1H, H-6′). ^{13}C NMR (150.9 MHz, D_2O): δ=134.4 (C-2), 76.4 (C-4), 73.2 (C-5), 73.0 (C-3), 32.7 (C-6). ^{31}P NMR (242.9 MHz, D_2O): δ=12.6 (1s, 1P, PO_3^{2-}), 3.6 (bs, OPO_3^{2-}).

4.1.10. Ammonium (3*R*,4*R*,5*S*)-3,4,5-trihydroxy-1-cyclohexenephosphonate (1). The ammonium salt of phosphate monoester **12** (30 mg, ~ 0.1 mmol) is dissolved in buffer (1 ml, Tris HCl, 0.1 M, pH 8.6, 0.25 M $MgCl_2$), and alkaline phosphatase from calf intestine (50 U) is added. The mixture is incubated for 4 h at 37°C, the precipitate formed is filtered off through a 0.45 μm filter and the product purified by gel filtration (Biogel P2, 0.1 M NH_4HCO_3) to give **1**. ^{1}H NMR (600 MHz, D_2O): δ=5.88 (dd, 1H, H-2, $J_{P-2=}$18.4 Hz), 4.06 (ddd, 1H, H-3, J_{3-4}=8 Hz), 3.62 (m, 1H, H-5, J_{5-4}=10.2 Hz), 3.35 (dd, 1H, H-4), 2.56 (m, 1H, H-6), 2.07 (m, 1H, H-6′). ^{13}C NMR (150.9 MHz, D_2O): δ=132.3 (C-2), 77.4 (C-4), 73.2 (C-3), 69.9 (C-5), 33.8 (C-6). ^{31}P NMR (242.9 MHz, D_2O): δ=11.9 (1s, 1P, PO_3^{2-}). $C_6H_{11}PO_6$ (free acid) (M 210.03). FAB-MS (pos. Mode, glycerol): 421 (2M+H)$^+$, 233 (M+Na)$^+$, 228 (M+NH_4)$^+$, 211 (M+H)$^+$, 193 (M−H_2O+H)$^+$.

4.1.11. 1,2-*O*-Isopropylidene-5-*O*-triisopropylsilyl-α-L-erythro-pentos-3-ulose (13). Alcohol **4** (12.06 g, 34.8 mmol) is oxidized as described in the literature for the comparable TBDMS-protected compound.[11] Flash chromatography of the crude product gave carbonyl compound **13** (11.4 g, 95%). R_f=0.5–0.7 (Tol/EE 9:1); $[\alpha]_D^{20}$=−98.8 (c=1, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): δ=6.14 (d, 1H, H-1, J_{1-2}=4.5 Hz), 4.34 (m, 1H, H-4), 4.28 (dd, 1H, H-2, J_{2-4}=1 Hz), 3.97 (dd, 1H, J_{5-5}=10.6 Hz, J_{5-4}=1.8 Hz), 3.93 (dd, 1H, H-5′, J_{5-4}=2.1 Hz), 1.44, 1.43 (2s, 6H, C(CH_3)$_2$), 1.33–0.96 (m, 21H, 3CH(CH_3)$_2$). Anal. Calcd for $C_{17}H_{32}O_5Si$ (M 344.56): C, 59.26; H, 9.37. Found: C, 59.36; H, 9.51.

4.1.12. 1,2-*O*-Isopropylidene-5-*O*-triisopropylsilyl-α-L-ribofuranose (14). Carbonyl compound **13** (8.7 g, 25.25 mmol) is reduced with $NaBH_4$ as described[11,19] for

comparable D-xylofuranose derivatives. Flash chromatography of the crude product gave compound **14** (8.1 g, 93%). R_f=0.45 (Tol/EE 9:1); $[\alpha]_D^{20}$=−31.1 (c=1, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): δ=5.79 (d, 1H, H-1, J_{1-2}=3.9 Hz), 4.56 (dd, 1H, H-2, J_{2-3}=5 Hz), 4.06 (ddd, 1H, H-1, J_{3-OH}=9.2 Hz), 3.93–3.79 (m, 3H, H-4, H-5, H-5′), 2.39 (d, 1H, OH), 1.55, 1.35 (2s, 6H, C(CH_3)$_2$), 1.29–1.07 (m, 21H, 3CH(CH_3)$_2$). Anal. Calcd for $C_{17}H_{34}O_5Si$ (M 346.56)×0.5 H_2O: C, 57.37; H, 9.84. Found: C, 57.34; H, 9.70

4.1.13. 1,2-*O*-Isopropylidene-3-*O*-trifluoromethanesulfonyl-5-*O*-triisopropylsilyl-α-L-ribofuranose (15) and 3-azido-3-deoxy-1,2-*O*-isopropylidene-5-*O*-triisopropylsilyl-α-L-xylofuranose (16). Pyridine (3.8 ml, 47.2 mmol) and trifluoromethanesulfonic acid anhydride (5.06 ml, 30.7 mmol) are dissolved in dry CH_2Cl_2 (50 ml), the mixture is cooled to −20°C and ribose **14** (8.18 g, 23.6 mmol), dissolved in dry CH_2Cl_2 (50 ml) is added dropwise during 10 min. The mixture is stirred until TLC indicates complete conversion of **14** into the triflate (R_f=0.95, Tol/EE 9:1). The mixture is extracted with saturated $NaHCO_3$ solution, KH_2PO_4 solution (1 M) and NaCl solution (50 ml each), dried ($MgSO_4$) and concentrated to give the triflate **15** (11.03 g) as a yellow oil.

The triflate (11.03 g) is dissolved in EtOH (120 ml), sodium azide (7.5 g, 115 mmol) is added and the suspension is refluxed at 90°C for 40 h. Sodium azide (7.5 g, 115 mmol) is added and after refluxing for additional 24 h TLC indicates the absence of starting material. The mixture is concentrated in vacuo to about half its volume, NH_4Cl solution (50 ml) is added and the mixture is extracted twice with ethyl acetate (100 ml). The organic layer is washed with NaCl solution, dried ($MgSO_4$) and concentrated in vacuo. The resulting oil is chromatographed (Tol) to give azide **16** (7.22 g, 19.35 mmol, 82%) as a colourless oil. R_f=0.58 (Tol/EE 20:1); $[\alpha]_D^{20}$=33.7 (c=1, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): δ=5.83 (d, 1H, H-1, J_{1-2}=3.7 Hz), 4.59 (d, 1H, H-2), 4.30 (ddd, 1H, H-4), 4.04 (d, 1H, H-3, J_{3-4}=3.2 Hz), 3.97 (dd, 1H, H-5, J_{5-4}=5.2 Hz, J_{5-5}=9.8 Hz), 3.85 (dd, 1H, H-5′, J_{5-4}=8.8 Hz), 1.50, 1.30 (2s, 6H, C(CH_3)$_2$), 1.13–0.96 (m, 21H, 3CH(CH_3)$_2$). IR ($CHCl_3$): ν=2108.25 cm^{-1} (N_3). Anal. Calcd for $C_{17}H_{33}O_4N_3Si$ (M 371.60): C, 54.99; H, 8.96; N, 11.33. Found: C, 55.58; H, 8.87; N, 11.34.

4.1.14. 3-Azido-3-deoxy-1,2-*O*-isopropylidene-α-L-xylofuranose (17). Azide **16** (7.2 g, 19.3 mmol) is dissolved in dry THF (150 ml), cooled to 0°C and TBAF (1 M in THF, 21.2 ml, 21.2 mmol) is added. After stirring for 1 h at room temperature the solvent is evaporated and the residue is chromatographed (Tol/EE 4:1) to give **17** (4.12 g, 99%). R_f=0.27 (Tol/EE 4:1); $[\alpha]_D^{20}$=47 (c=1, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$):): δ=5.89 (d, 1H, H-1, J_{1-2}=3.6 Hz), 4.65 (d, 1H, H-2), 4.34 (m, 1H, H-4), 3.99 (d, 1H, H-3, J_{3-4}=3.3 Hz), 3.88, 3.77 (m, 2H, H-5, H-5′), 2.14 (bs, 1H, OH), 1.49, 1.30 (2s, 6H, C(CH_3)$_2$).

4.1.15. 3-Azido-3-deoxy-1,2-*O*-isopropylidene-5-*O*-trifluormethanesulfonyl-α-L-xylofuranose (18) and tetraethyl [3-azido-1,2-*O*-isopropylidene-3,5,6-trideoxy-α-L-xylofuranose-6,6′-diyl]bisphosphonate (19). Alcohol **17** (1 g, 4.61 mmol) and pyridine (0.74 ml, 9.7 mmol) are dissolved in dry CH_2Cl_2 (20 ml) and cooled to −30°C. Trifluoromethanesulfonic acid anhydride (0.94 ml, 5.53 mmol), dissolved in dry CH_2Cl_2 (10 ml) is added dropwise. The mixture is stirred for 30 min and then poured into saturated $NaHCO_3$ solution (30 ml). $CHCl_2$ (30 ml) is added, the organic layer is washed twice with KH_2PO_4 solution (1 M, 25 ml) and NaCl solution (25 ml), dried ($MgSO_4$) and evaporated. The crude triflate is passed through a short column (silica gel, CH_2Cl_2) to give the triflate **18** (1.4 g, 4 mmol, 87%) as a colourless oil.

The triflate (395 mg, 1.13 mmol) is dissolved in dry DMF (2 ml) and 1.6 equiv. of lithiated tetraethyl methylenediphosphonate, prepared from tetraethyl methylenediphosphonate and lithium-bis-trimethylsilylamide (1 M in THF) are added (4 ml DMF). The mixture is stirred at rt for 4 h and quenched with saturated NH_4Cl solution, extracted with CH_2Cl_2 and the organic layer is evaporated to dryness. The residue is chromatographed (Tol/EE 25:1) to give diphosphonate **19** (210 mg, 38%) as a slightly yellow oil. R_f=0.2 (EE/MeOH 20:1); $[\alpha]_D^{20}$=26 (c=1, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ=5.85 (d, 1H, H-1, J_{1-2}=3.8 Hz), 4.67 (d, 1H, H-2), 4.61 (m, 1H, H-4), 4.20–4.14 (m, 8H, 4CH_2CH$_3$), 3.85 (d, 1H, H-3, J_{3-4}=3.3 Hz), 2.55 (m, 1H, H-6), 2.31, 2.16 (2m, 2H, H-5, H-5′), 1.49 (s, 3H, 1CH_3), 1.34–1.30 (m, 15H, 1CH_3, 4CH$_2$CH_3). ^{13}C NMR (150.9 MHz, $CDCl_3$): δ=104.2 (C-1), 83.9 (C-2), 77.3 (C-4), 66.5 (C-3), 33.4 (C-6), 25.4 (C-5). ^{31}P NMR (242.9 MHz, $CDCl_3$): δ=23.98, 23.83 (2s, 2P, 2P(O)(OEt)$_2$). Anal. Calcd for $C_{17}H_{33}N_3O_9P_2$ (M 485.41): C, 42.06; H, 6.85; N, 8.66. Found: C, 42.36; H, 6.61; N, 8.55. MALDI-MS (pos. mode, DHB): 486.5 (M+H)$^+$, 508.6 (M+Na)$^+$.

4.1.16. Tetraethyl [3-azido-3,5,6-trideoxy-α,β-L-xylofuranose-6,6′-diyl]bisphosphonate (20). Diphosphonate **19** (250 mg, 0.56 mmol) is dissolved in dioxane/water (1:1, 20 ml), Amberlite IR-120 (H^+-form) is added and the mixture is stirred at 80°C until TLC indicates absence of starting material (~ 20 h). The resin is filtered off, the solvent is evaporated and the residue is chromatographed to give **20** (α,β-mixture, 118 mg, 51%) as a colourless oil. R_f=0.36–0.42 (EE/MeOH 4:1); ^{1}H NMR (major anomer, 600 MHz, $CDCl_3$): δ=5.43 (d, 1H, H-1, J_{1-2}=4.3 Hz), 4.60 (m, 1H, H-4), 4.25–4.14 (m, m, 5H, H-2, 2CH_2CH$_3$), 3.97 (m, 1H, H-3), 2.72 (m, 1H, H-6), 2.17 – 2.03 (m, 2H, H-5, H-5′), 1.35–1.23 (m, 6H, 2CH$_2$CH_3). ^{13}C NMR (150.9 MHz, $CDCl_3$): δ=95.3 (C-1), 76.8 (C-2), 74.8 (C-4), 68.2 (C-3), 32.7 (C-6), 26.4 (C-5). ^{31}P NMR (242.9 MHz, $CDCl_3$): δ=24.50, 24.36 (2s, 2P, 2P(O)(OEt)$_2$). Anal. Calcd for $C_{14}H_{29}N_3O_9P_2$ (M 445.35): C, 37.76; H, 6.56; N, 9.44. Found: C, 37.74; H, 6.57; N, 8.59. MALDI-MS (pos. mode, DHB): 446.1 (M+H)$^+$, 468.2 (M+Na)$^+$.

4.1.17. Ethyl [diethyl (3*R*,4*R*,5*S*)-4-azido-3,5-dihydroxy-1-cyclohexenephosphonate-5-yl]phosphoric acid (21). The anomeric mixture of compound **20** (123 mg, 0.27 mmol) is stirred in a solution of NaOEt in EtOH (0.1 M, 10 ml) until TLC indicates the absence of starting material (~ 1 h). The solution is neutralized with Amberlite IR-120 (H^+-form), filtered and evaporated. The residue is

chromatographed (EE/MeOH 2:1) to give cyclohexenephosphonate **21** (60 mg, 77%) as a colourless foam. R_f=0.55 (EE/MeOH 1:1); $[\alpha]_D^{20}$=−24.2 (c=1, MeOH); ^{1}H NMR (600 MHz, MeOH-d_4): δ=6.45 (bd, 1H, H-2, $J_{P\text{-}2}$=21 Hz), 4.19 (m, 1H, H-5), 4.11–3.89 (m, 7H, H-3, 3CH_2CH$_3$), 3.46 (dd, 1H, H-4, J_{4-3}=8.5 Hz, J_{4-5}=10.1 Hz), 2.96 (m, 1H, H-6), 2.32 (m, 1H, H-6′), 1.35–1.21 (m, 9H, 3CH$_2$CH_3). ^{13}C NMR (150.9 MHz, MeOH-d_4): δ=144.2 (C-2), 72.9 (C-5), 71.2 (C-3), 69.8 (C-4), 32.7 (C-6). ^{31}P NMR (242.9 MHz, MeOH-d_4): δ=18.98 (s, 1P, P(O)(OEt)$_2$), 1.01 (s, 1P, OP(O)(OH)-(OEt)). $C_{12}H_{23}N_3P_2O_8$ (M 399.096). FAB-MS (pos. mode, NBA): 422 (M+Na)$^+$, 444 (M−H+2Na)$^+$.

4.1.18. Ethyl [diethyl (3*R*,4*R*,5*S*)-4-acetamido-3,5-dihydroxy-1-cyclohexenephosphonate-5-yl]phosphate sodium salt (22). Azido compound **21** (30 mg, 0.1 mmol) is dissolved in pyridine/water (4:1, 4 ml), the solution is saturated with H_2S and stirred at rt for 72 h. The solvent is evaporated, the residue is dissolved in MeOH (20 ml) and acetic anhydride (10 equiv.) and $NaHCO_3$ (15 equiv.) are added. The mixture is stirred overnight, evaporated and the residue is chromatographed (EE/MeOH 1:1) to give acetamide **22** (21 mg, 67%). R_f=0.45 (EE/MeOH 1:1); $[\alpha]_D^{20}$=−33.4 (c=1, MeOH); ^{1}H NMR (600 MHz, MeOH-d_4): δ=6.51 (bd, 1H, H-2, $J_{P\text{-}2}$=21.6 Hz), 4.25–4.18 (m, 2H, H-3, H-5), 4.12–4.07 (m, 4H, 2CH_2CH$_3$), 3.93–3.88 (m, 3H, H-4, CH_2CH$_3$), 2.86 (m, 1H, H-6), 2.35 (m, 1H, H-6′), 2.02 (s, 1H, C(O)CH_3), 1.35–1.31 (m, 6H, 2CH$_2$CH_3), 1.26–1.23 (bt, 3H, CH$_2$CH_3). ^{13}C NMR (150.9 MHz, MeOH-d_4): δ=145.2 (C-2), 72.7 (C-3), 71.3 (C-5), 58.2 (C-4), 33.4 (C-6), 22.9 (C(O)CH$_3$). ^{31}P NMR (242.9 MHz, MeOH-d_4): δ=19.43 (s, 1P, P(O)(OEt)$_2$), 2.31 (s, 1P, OP(O)(OH)(OEt)). $C_{14}H_{27}NP_2O_9$ (free acid) M (415.116). MALDI-MS (pos. mode, DHB): 437.7 (M+Na)$^+$, 459.7 (M−H+2Na)$^+$. MALDI-MS (neg. mode, ATT): 414.5 (M−H)$^-$.

4.1.19. Ammonium [(3*R*,4*R*,5*S*)-4-acetamido-3,5-dihydroxy-1-cyclohexenephosphonate-5-yl]phosphate (23). Acetamide **22** (20 mg, 0.067 mmol) is dissolved in dry $CHCl_3$ (3 ml) containing 5% (v/v) TMSBr. The mixture is stirred for 48 h at rt, evaporated, the residue is taken up in water (3 ml), stirred for 2 h and lyophilized to give the acid (16 mg, qu) in excellent purity. The product is passed through a short column of Biogel P2 (0.1 M NH_4HCO_3) and again lyophilized to give **23** (15 mg, 85%). $[\alpha]_D^{20}$=−24 (c=1, H_2O); ^{1}H NMR (600 MHz, D_2O): δ=6.22 (d, 1H, H-2, $J_{P\text{-}2}$=20.6 Hz), 4.23–4.16 (m, 2H, H-3, H-5), 3.85 (dd, 1H, H-4, J_{4-3}=9.7 Hz, J_{4-5}=10.1 Hz), 2.74 (m, 1H, H-6), 2.35 (m, 1H, H-6′), 1.94 (s, 3H, C(O)CH_3). ^{13}C NMR (150.9 MHz, D_2O): δ=138.2 (C-2), 72.7 (C-5), 70.5 (C-3), 56.7 (C-4), 32.7 (C-6). ^{31}P NMR (242.9 MHz, D_2O): δ=15.20 ((s, 1P, PO3^{2-}), 1.22 (s, 1P, OPO3^{2-}).

4.1.20. Diethyl (3*R*,4*R*,5*S*)-4-azido-3,5-dihydroxy-1-cyclohexenephosphonate (24). Diphosphonate **20** (100 mg, 0.22 mmol) is dissolved in dry dioxane (5 ml) and lithum-bis-trimethylsilylamide (1M in hexane, 0.22 ml) is added. The mixture is stirred at rt for 2 h, neutralized with Amberlite IR-120 (H^+-form), filtered and evaporated. The residue is chromatgraphed (EE/MeOH 10:1) to give cyclohexenephosphonate **24** (25 mg, 40%). R_f=0.5 (EE/MeOH 6:1); $[\alpha]_D^{20}$=−17 (c=1, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ=6.56 (bd, 1H, H-2, $J_{P\text{-}2}$=21.6 Hz), 4.24 (m, 1H, H-3), 4.11–4.04 (m, 4H, 2CH_2CH$_3$), 3.77 (m, 1H, H-5), 3.52 (dd, 1H, H-4, J_{4-3}=J_{4-5}=8.2 Hz), 2.66 (m, 1H, H-6) 2.28 (m, 1H, H-6′), 1.35–1.31 (m, 6H, 2CH$_2$CH_3). ^{13}C NMR (150.9 MHz, $CDCl_3$): δ=142.3 (C-2), 126.2 (d, C-1, $J_{P\text{-}1}$=184 Hz), 71.1 (C-3), 69.2 (C-4), 68.1 (C-5), 32.1 (C-6). ^{31}P NMR (242.9 MHz, $CDCl_3$): δ=17.88 (s, 1P, P(O)(OEt)$_2$). $C_{10}H_{18}N_3O_5P$ (M 291.24). MALDI-MS (pos. mode, DHB): 313.8 (M+Na)$^+$, 291.7 (M+H)$^+$.

4.1.21. Diethyl (3*R*,4*R*,5*S*)-3,5-di-acetoxy-4-azido-1-cyclohexenephosphonate (25). Compound **24** (20 mg, 0.069 mmol) is stirred overnight in acetic anhydride/pyridine (1:1, 3 ml), the solvent is evaporated and the residue is chromatographed (Tol/EE 1:2) to give **25** (24 mg, 90%). R_f=0.2 (Tol/EE 1:1); $[\alpha]_D^{20}$=−64 (c=0.5, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): δ=6.40 (bd, 1H, H-2, $J_{P\text{-}2}$=21.2 Hz), 5.37 (m, 1H, H-3), 5.00 (ddd, 1H, H-5, J=6.1, 8.8, 9.8 Hz), 4.15–3.99 (m, 4H, 2CH_2CH$_3$), 3.79 (dd, 1H, H-4, J=10.6, 8.8 Hz), 2.81 (m, 1H, H-6), 2.24 (m, 1H, H-6′), 2.11 (2s, 6H, 2COCH$_3$), 1.38–1.20 (m, 6H, 2CH$_2$CH_3). $C_{14}H_{22}N_3O_7P$ (M 375.32) MALDI-MS (pos. mode, DHB): 414.2 (M+K)$^+$. Anal. Calcd for $C_{14}H_{22}N_3O_7P$ (M 375.32): C, 44.80; H, 5.91; N, 11.20. Found: C, 44.93; H, 5.99; N, 10.76.

4.1.22. Diethyl (3*R*,4*R*,5*S*)-4-acetamido-3,5-di-acetoxy-1-cyclohexenephosphonate (26). Azide **24** (50 mg, 0.17 mmol) is dissolved in a mixture of pyridine/water (1:1, 5 ml), the solution is saturated with hydrogen sulfide and stirred at rt for 48 h. The solvent is evaporated and the residue is stirred overnight in a mixture of acetic anhydride/pyridine (1:1, 5 ml). Following evaporation of the solvent the residue is chromatographed (Tol/EE 10:1) to give **26** (35 mg, 53%). R_f=0.4 (EE/MeOH 10:1); $[\alpha]_D^{20}$=−54 (c=1, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ=6.42 (bd, 1H, H-2, $J_{P\text{-}2}$=21.1 Hz), 5.55 (d, 1H, NH, $J_{NH\text{-}4}$=9.7 Hz), 5.51 (m, 1H, H-3), 5.05 (m, 1H, H-5) 4.36 (ddd, 1H, H-4, J=10.3, 10.4 Hz), 4.10–4.05 (m, 4H, 2CH_2CH$_3$), 2.71 (m, 1H, H-6), 2.44 (m, 1H, H-6′), 2.06, 2.04, 1.91 (3s, 9H, 3COCH$_3$), 1.33–1.30 (m, 6H, 2CH$_2$CH_3). ^{13}C NMR (150.9 MHz, $CDCl_3$): δ=138.9 (C-2), 128.8 (d, C-1, $J_{P\text{-}1}$=186 Hz), 72.8 (C-3), 68.9 (C-5), 62.3 (C-4), 30.8 (C-6). ^{31}P NMR (242.9 MHz, $CDCl_3$): δ=16.45 (s, 1P, P(O)(OEt)$_2$). $C_{16}H_{26}NO_8P$ (M 391.14). MALDI-MS (pos. mode, DHB): 414.1 (M+Na)$^+$, 392.1 (M+H)$^+$.

4.1.23. Diethyl (3*R*,4*R*,5*S*) 4-acetamido-3,5-dihydroxy-1-cyclohexenephosphonate (27). Acetylated phosphonate **26** is treated with a catalytical amount of sodium ethanolate in ethanol, neutralized with Amberlite IR-120 (H^+-form), the mixture filtered and the solvent is evaporated to give **27** in excellent purity. $[\alpha]_D^{20}$=−22 (c=0.5, MeOH).); ^{1}H NMR (250 MHz, $CDCl_3$): δ=6.45 (bd, 1H, H-2, $J_{P\text{-}2}$=21.4 Hz), 4.22 (m, 1H, H-3), 4.09–4.01 (m, 4H, 2CH_2CH$_3$), 3.75–3.65 (m, 2H, H-4, H-5), 2.63 (m, 1H, H-6), 2.25 (m, 1H, H-6′), 2.05 (s, 3H, COCH$_3$), 1.34–1.27 (m, 6H, 2CH$_2$CH_3). $C_{12}H_{22}NO_6P$ (M 307.14) MALDI-MS (pos. mode, DHB): 330.7 (M+Na)$^+$, 308.7 (M+H)$^+$.

4.1.24. Ammonium (3*R*,4*R*,5*S*)-4-acetamido-3,5-dihydroxy-1-cyclohexenephosphonate (2). From **23**:

The ammonium salt of phosphate monoester **23** (15 mg, ~0.1 mmol) is dissolved in buffer (1 ml, Tris HCl, 0.1 M, pH 8.6, 0.25 M $MgCl_2$), and alkaline phosphatase from calf intestine (50 U) is added. The mixture is incubated for 4 h at 37°C, the precipitate formed is filtered off through a 0.45 μm filter and the product is purified by gel filtration (Biogel P2, 0.1 M NH_4HCO_3) to give **2** (8 mg, 55%).

From **26**:

Diester **26** (20 mg, 0.07 mmol) is dissolved in dry $CHCl_3$ (2 ml) containing 10% (v/v) TMSBr. The mixture is stirred for 48 h at rt, evaporated, the residue is taken up in water (3 ml), stirred for 2 h and lyophilized to give **2** (free acid, 16 mg, qu) in excellent purity. The acid can be converted into the ammonium salt by lyophilization from a 0.1 M NH_4HCO_3 solution. $[\alpha]_D^{20}=-14$ (c=0.5, H_2O). ^{1}H NMR (600 MHz, D_2O): δ=5.88 (d, 1H, H-2, $J_{P\text{-}2}$=17.8 Hz), 4.07 (m, 1H, H-3), 3.68–3.59 (m, 2H, H-4, H-5), 2.59 (m, 1H, H-6), 2.14 (m, 1H, H-6′), 1.93 (s, 3H, C(O)CH_3). ^{13}C NMR (150.9 MHz, D_2O): δ=132.9 (C-2), 73.4 (C-3), 70.4 (C-5), 60.6 (C-4), 36.2 (C-6). ^{31}P NMR (242.9 MHz, D_2O): δ=14.26 (s, 1P, PO_3^{2-}). $C_8H_{14}NPO_6$ (free acid) (M 251.056). MALDI-MS (pos. mode, HCCA): 274.6 $(M+Na)^+$. FAB-MS (pos. mode, glycerol): 503 $(2M+H)^+$, 344 $(M+glycerol+H)^+$, 274 $(M+Na)^+$, 252 $(M+H)^+$, 234 $(M-H_2O+H)^+$.

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