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Building a successful structural motif into sialylmimetics—cyclohexenephosphonate monoesters as pseudo-sialosides with promising inhibitory properties

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Dedicated, with respect and gratitude, to Professor Nathan Sharon on the occasion of his 80th birthday.

Abstract—A variable synthesis of a new class of sialylmimetics which provides access to pseudo-sialosides containing the successful cyclohexene motif in the sialic acid mimicking part has been developed. The D- and L-*xylo* cyclohexenephosphonate scaffolds allow attachment of selected aglycons or aglycon mimetics via mixed phosphonate diester strategies and some target compounds thus synthesized displayed promising inhibitory properties when tested with parasitic or bacterial sialidases.

1. Introduction

Sialic acids are a family of naturally occurring derivatives of 3-deoxy-D-*glycero*-D-*galacto*-2-nonulosonic acids abundant on cell surfaces in higher animals (Fig. 1A).[1] At their terminal position at the non-reducing end of oligosaccharide chains in cell wall glycoconjugates, sialic acids represent a crucial first encounter and receptor for attachment of exogenous, often pathogenic, agents such as viruses, bacteria or parasites and their respective lectins and toxins. In addition, important endogenous events such as a cell's metastatic potential are mediated by density as well as degree and kind of modification of their cell surface sialic acids.[2,3]

Consequently, a variety of these interactions have been targeted by small molecule inhibitor design and synthesis, with varying success, leading to nearly nanomolar inhibition of sialyltransferases or of the sialidase form influenza virus A and B but yet failing, for instance, to provide the same potency for sialic acid binding lectins or other microbial sialidases.[4,5]

This fact, the persistent influenza threat and newly emerged targets such as parasitic *trans*-sialidases,[6,7] paramyxoviral hemagglutinin-neuraminidases,[7–9] and other sialic acid modifying enzymes, prompted us to search for an approach which allows the incorporation of a successful structural motif, namely the cyclohexene ring as a transition state mimetic of glycoside hydrolysis, into more elaborated structures. The diabetes drug acarbose, containing the cyclohexene valienamine or the anti-influenza drug and neuraminidase inhibitor GS-4071, containing a L-*xylo*-configured cyclohexenecarboxylic acid as the bioactive sialylmimetic, may serve as examples (Fig. 1B).

2. Results and discussion

2.1. Inhibitor design and retrosynthetic approach

Replacement of the carboxylate in the L-*xylo*-configured cyclohexene scaffold of GS-4071 by a phosphonate leads to L-*xylo*-configured cyclohexenephosphonates which allow attachment of aglycon mimetics or natural aglycons of sialic acids such as galactoses thus leading to pseudo-disaccharides containing a carbocyclic sialylmimetic (Fig. 1C). In such cyclohexenephosphonate monoesters, both the negative charge under physiological conditions as well as the flattened half-chair conformation are

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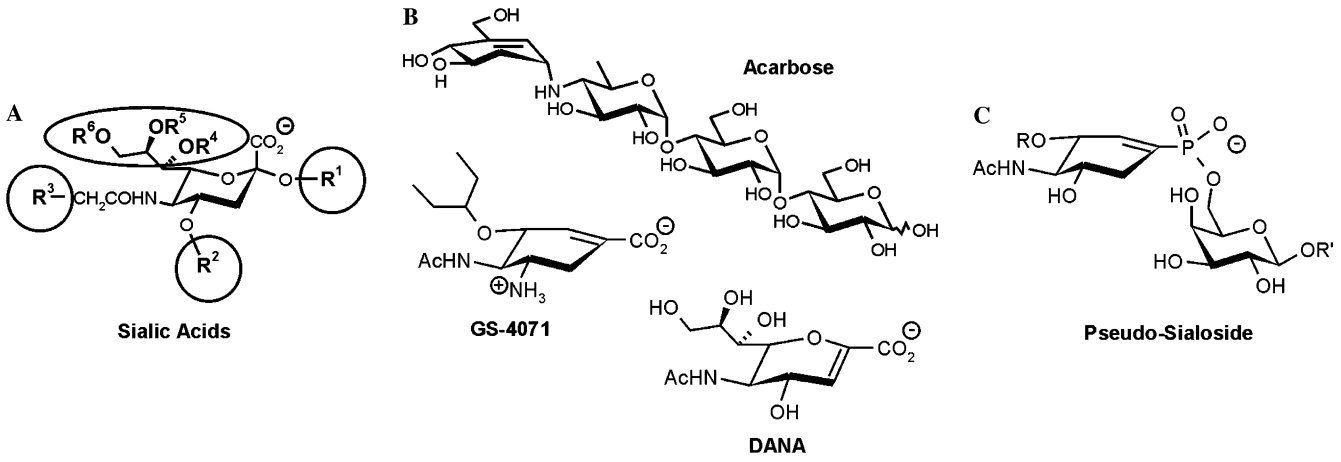


Figure 1. (A) *N*-Acetylneuraminic acid (Neu5Ac, R^1–R^6 = H) and locations of modifications leading to the structural variety in the sialic acid family, indicated by spheres and ellipsoids. (B) Drugs acarbose and GS-4071 and classical sialidase inhibitor DANA, which mimic carbohydrates by means of a cyclohexene moiety. (C) Example for a pseudo-sialoside based on a *xylo*-configured cyclohexenephosphonate monoester.

retained, properties at least difficult to achieve if a cyclohexenecarboxylic acid was to be functionalized. If required, simple hydrogenation then leads to chair conformations in the carbocycle, thus significantly increasing the number and type of accessible structures.

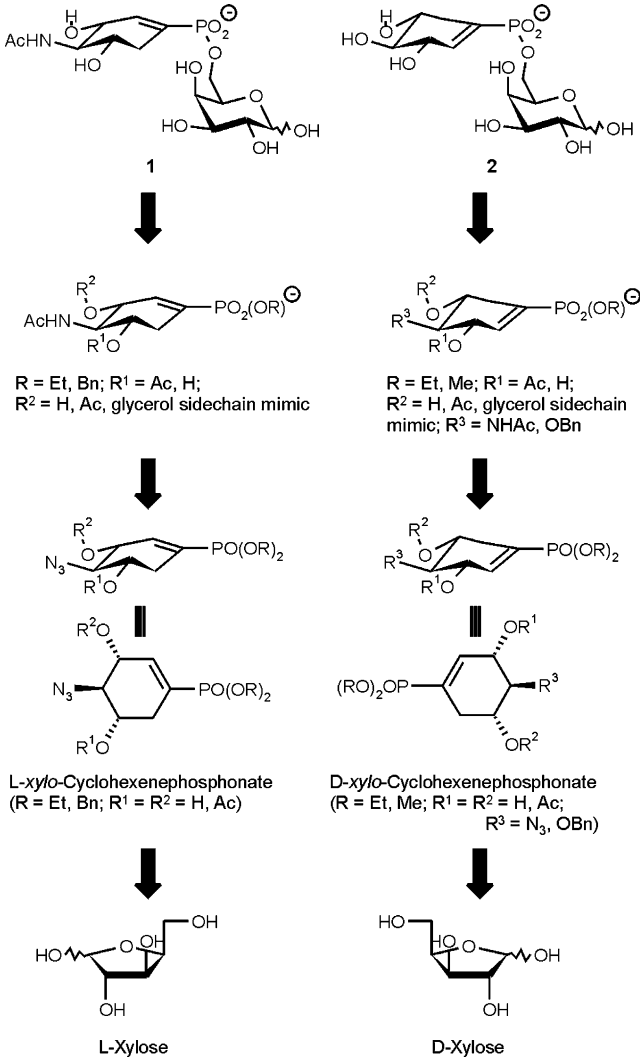
In our retrosynthetic approach, we deliberately included both the GS-4071-like double bond orientation and the orientation corresponding to the long-known transition-state analogous sialidase inhibitor DANA in order to ensure upmost variability (Scheme 1).

Starting from D- or L-xylose, respectively, the corresponding *xylo*-configured cyclohexenephosphonates with or without azide moiety at C-4 are obtained via Horner–Wadsworth–Emmons type cyclization of suitably modified xylofuranoses (Scheme 1).[10] This approach, previously reported by us for diethyl phosphonates,[11,12] is extended to dimethyl and dibenzyl phosphonates. These allow, following protection and partial saponification, synthesis of mixed diesters containing aglycon mimetics through alkylation or Mitsunobu esterification which, after deprotection, furnish pseudo-sialosides such as **1** and **2** (Scheme 1).

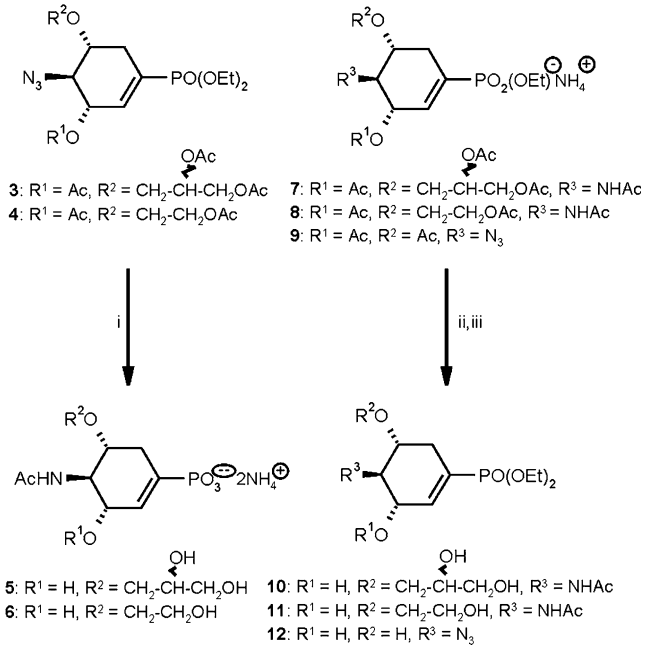
2.2. Syntheses

We have previously reported the conversion of diethyl phosphonates **3** and **4** to obtain cyclohexenephosphonates **5** and **6** carrying modified sidechains (Scheme 2).[13] In order to obtain the corresponding monoethyl esters, we partially saponified intermediates **7**–**9**,[13] with sodium hydroxide solution and obtained, after neutralization and purification on Biogel P2 columns, monoethyl esters **10**–**12** in literally quantitative yield (Scheme 2).

The synthesis of benzyl cyclohexenephosphonates started from 3-azido-3-deoxy-1,2-*O*-isopropylidene-5-*O*-trifluoromethanesulfonyl-L-xylofuranose,[11] which was reacted with the tetrabenzyl methylenediphosphonate anion to give the C-elongated xylofuranose diphosphonate **13**



Scheme 1. Retrosynthetic analysis of L-*xylo*-configured pseudo-sialoside **1** mimicking Neu5Ac and D-*xylo*-configured pseudo-sialoside **2** mimicking KDN.



Scheme 2. Synthesis of sidechain-functionalized cyclohexenephosphonates and their corresponding monoethyl esters. Reagents: (i) TMSBr/$CHCl_3$;[11,12] (ii) NaOH (0.4 M); (iii) Biogel P2, NH_4HCO_3 (0.1 M).

(Scheme 3). Removal of the isopropylidene group under acidic conditions liberated the aldehyde, which was cyclized with lithium bis(trimethylsilyl)amide as a base to yield dibenzyl cyclohexenephosphonate **14**, which was readily converted to **15**. Monobenzyl ester **16** was then obtained by partial cleavage with thiophenol/triethylamine, ready for alkylation with alkyl triflates. Deacetylation and purification by gel permeation chromatography yields **17**, which was included in inhibition assays. Mitsunobu condensation of **16** with suitable alcohols such as sugar hydroxy groups gave only marginal yields of desired mixed diesters. We therefore alkylated monoester **16** with octyl triflate to give an inseparable mixture of diastereomeric benzyl octyl phosphonates **18h,l**. Selective removal of the benzyl group with thiophenol/triethylamine followed by deacetylation and purification gave octyl phosphonate **19** in high yield. Introduction of a sugar moiety rather than a hydrophobic group was achieved by reacting monoester **16** with 1.2,3.4-di-*O*-isopropylidene-5-*O*-trifluoromethanesulfonyl-α-D-galactopyranoside **20**[14] as electrophile to give the diastereomeric mixed diesters **21h,l** in nearly quantitative yield, based on consumed phosphonate. The sequence of the final deprotection steps proved to be crucial, starting with debenzylation to give the monoester, which was deacetylated in the same step to furnish **22**. Isopropylidene cleavage with diluted trifluoroacetic acid gave fully deprotected pseudo-sialoside **1** (Scheme 3).

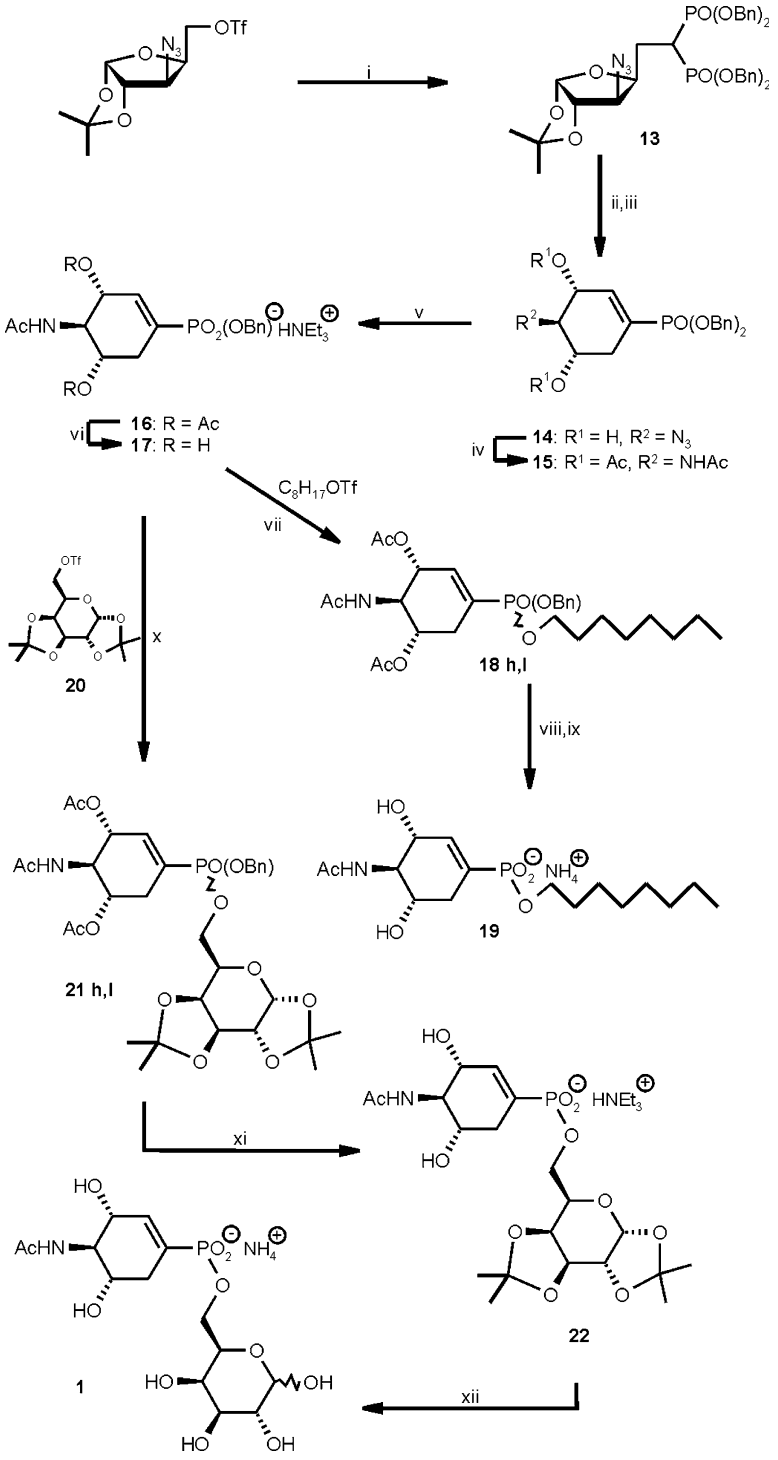
The dimethyl phosphonate methodology for the synthesis of cyclohexenephosphonate dimethyl esters was introduced in the D-*xylo* series (Scheme 4). 3-*O*-Benzyl-1,2-*O*-isopropylidene-D-xylofuranose-5-triflate[11] was substituted with the tetramethyl methylenediphosphonate anion to give xylofuranose diphosphonate **23**. Isopropylidene cleavage and cyclization gave D-*xylo*-configured dimethyl cyclohexenephosphonate **24**, which was readily acetylated to yield **25**. Thiophenol/triethylamine treatment of **25** gave monomethyl ester **26**, which could both be alkylated with sugar triflate **20** to give mixed diester **27h,l** and be condensed with 1.2,3.4-di-*O*-isopropylidene-α-D-galactopyranose under Mitsunobu conditions,[15,16] to yield the same compound. This reaction increases the range of accessible pseudo-sialosides significantly. The selective benzyl protection at C-4 in compounds **24**–**27** now offers selective access for modifications of all three hydroxy groups with the chemical differentiation of the hydroxy groups at positions 3 and 5 having been demonstrated by us previously. In this synthesis, however, the benzyl group had to be selectively removed in presence of the double bond by transfer hydrogenation with cyclohexadiene which, after acetylation, gave **28h,l** as an inseparable mixture of diastereomers. The three-step deprotection via protected monoesters **29** and **30** followed essentially the same methodology as in the benzyl series. Thus, fully deprotected pseudo-sialoside **2**, with the carbocycle being an analogue of 3-deoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid (KDN) rather than of its 5-deoxy-5-acetamido counterpart Neu5Ac (Scheme 4), was obtained.

2.3. Conformation of cyclohexenephosphonates

Sialidase inhibitor DANA (Fig. 1B) occupies a half-chair conformation leaving the acetamido substituent in a pseudo-equatorial position with the vicinal couplings of the corresponding pseudo-axial proton (H-5) being 8.9 and 10.9 Hz, respectively. This simulates the situation around the equatorial acetamide in sialic acids (10.4 and 10.9 Hz) (Fig. 1A).[17] Whenever examined, we find a similar setting for the C-4 substituent in D- and L-*xylo*-configured cyclohexenephosphonates, for instance in compound **17** (8.8 Hz, 10.8 Hz). Slightly lower values (~7 Hz and ~9 Hz) are typically found when the compounds are protected (see Section 4). A pseudo-equatorial position of the three substituents, especially the acetamide or hydroxy group at C-4, can therefore be assumed.

3. Sialidase inhibition

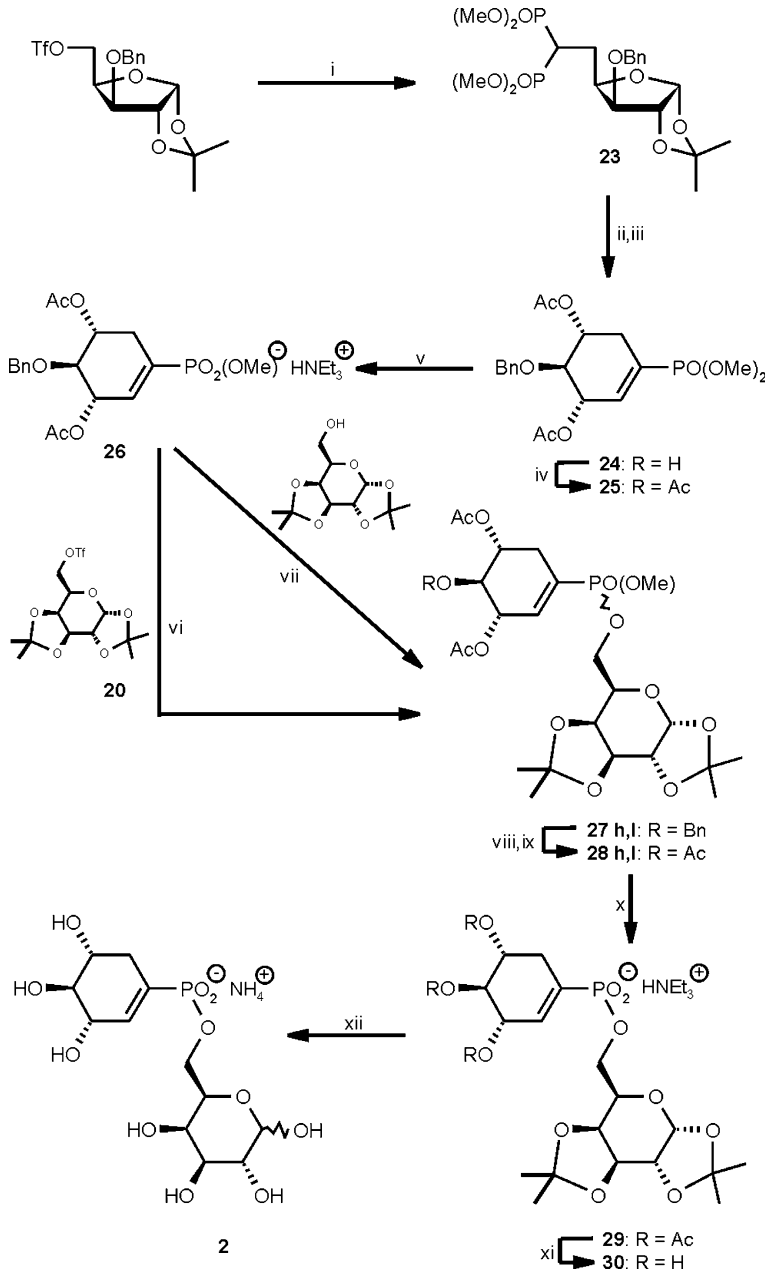
In order to demonstrate the usefulness of the structural concept, we have tested the inhibition of selected bacterial sialidases and a trypanosomal sialidase by our *xylo*-configured cyclohexenephosphonate monoesters. Compounds **1**, **17** and **19** all showed improved inhibition (0.2, 0.09 and 0.2 mM, respectively) of *Salmonella typhimurium* sialidase when compared to the parent cyclohexenephosphonate.[12,13,18] Moreover, although lacking an optimized side chain mimetic, monobenzyl phosphonate **17** exceeds benchmark sialidase inhibitor DANA in inhibitory potency by a factor of 4 and comes close to the so far best inhibitor of the latter enzyme, *iso*-carba-DANA (IC_{50} = 40 μM).[19] In addition, these monoesters display a significant preference for the *S. typhimurium* enzyme, with the enzyme from *Clostridium perfringens*, for instance, not being inhibited below 4 mM inhibitor concentration. Compound **2**, which



Scheme 3. Synthesis of pseudo-sialosides via dibenzyl phosphonates. Reagents: (i) $CH_2(PO_3Bn_2)_2$, $LiN(SiMe_3)_2$; (ii) H^+/H_2O; (iii) $LiN(SiMe_3)_2$; (iv) PMe_3/Ac_2O; (v) H_2/Pd/C; (vi) NaOH; (vii) $C_8H_{17}OTf$, DMF; (viii) H_2/Pd/C; (ix) NH_3/MeOH; (x) **20**, DMF; (xi) H_2/Pd/C then NH_3/MeOH; (xii) CF_3COOH (50%).

lacks the acetamide and is therefore expected to be inactive,[12] will be tested for inhibition of KDNases in due course (Table 1).

The *trans*-sialidase from *Trypanosoma cruzi* was tested in a fluorescence-based *trans*-sialidase assay as recently described by Schauer and co-workers[20] Interestingly, **1** was inactive but inhibition, albeit moderate, was found with unsubstituted monoethyl D-*xylo*-cyclohexenephosphonate,[12] and its derivative **11** (5.7 and 4.7 mM, respectively) carrying a hydroxyethyl moiety as the glycerol side chain mimetic. Very little inhibition (<20% at 8 mM) was detected with the mixture of diastereomers **10** and the 4-azido compound **12**.



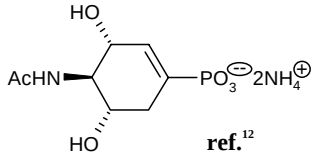
Scheme 4. Synthesis of pseudo-sialosides via dimethyl phosphonates. Reagents: (i) $CH_2(PO_3Me_2)_2$, $LiN(SiMe_3)_2$; (ii) H^+/H_2O; (iii) $LiN(SiMe_3)_2$; (iv) Ac_2O/pyridine; (v) PhSH/NEt_3; (vi) **20**, DMF; (vii) DIAD/Ph_3P; (viii) C_6H_8/EtOH; (ix) Ac_2O/pyridine; (x) PhSH/NEt_3; (xi) NaOH; (xii) CF_3COOH (50%).

The inhibition data obtained with monoethyl esters are highlighted by the fact that the parent cyclohexenephosphonate without ethyl ester group,[12] as well as the corresponding derivatives **5** and **6** with side chain mimetics displayed no detectable inhibition in the concentration range tested (Table 2).

Taking into account that it has been difficult to find donor substrate analogous inhibitors for *T. cruzi trans*-sialidase, with inhibitor DANA itself being only a weak inhibitor ($K_i > 14$ mM),[6,21] the data obtained indicate a beneficial effect of the monoethyl phosphonate group and a possible entry to such inhibitors. It is, however, too early to propose a structure–activity relationship before more substituents as well as both double bond regioisomers have been compared.

In conclusion, we have developed a synthetic approach towards carbocyclic sialylmimetics which allows exploration and mimicking of the full structural space displayed by sialoconjugates in physiological recognition events. The methodology can be applied in design and synthesis of inhibitors or inhibitor libraries of virtually any sialic acid binding lectin or enzyme, some of which are currently being under investigation in our laboratory.

Table 1. Inhibition of bacterial sialidases by cyclohexenephosphonate monoesters

Compound	*S.t.* sialidase IC_{50} (mM)	*C.p.* sialidase IC_{50} (mM)
 **ref.**[12]	0.3	2
1	0.2	n.i.
17	0.09	n.i.
19	0.2	n.i.
DANA	0.4	0.04

Inhibition of bacterial sialidases by cyclohexenephosphonate monoesters.
S.t.: *Salmonella typhimurium*.
C.p.: *Clostridium perfringens*.
Values are means of three independent experiments with a deviation of less than 20%. n.i.: non-inhibitory.

4. Experimental

4.1. General

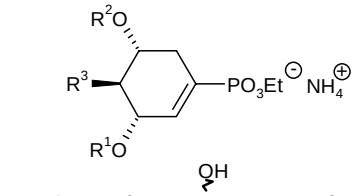
Reaction solvents were purchased anhydrous and used as received. Solvents for chromatography were distilled before use. Reactions were monitored by TLC using precoated silica gel 60 F_{254} plates. Compounds were detected by UV absorption and/or by staining with a molybdenum phosphate reagent (20 g ammonium molybdate and 0.4 g cerium(IV) sulfate in 400 mL of 10% aq sulfuric acid) and subsequent heating at 120 °C for 5 min. Silica gel 60 M (particle size 40–63 μm) from Macherey-Nagel, Düren, Germany, was used for flash chromatography. ^1H, ^{13}C and ^{31}P NMR spectra were recorded on a Bruker DRX 600 spectrometer at 600, 150.9 and 242.9 MHz, respectively. Chemical shifts in ^1H and ^{13}C NMR spectra were referenced to the residual proton resonance of the respective deuterated solvents, $CDCl_3$ (7.24 ppm), D_2O (4.63 ppm) and D_2O in CD_3OD (4.88 ppm). For ^{31}P NMR spectra H_3PO_4 was used as external standard (0 ppm). In some cases, ^{13}C chemical shifts were deduced from heteronuclear multiple quantum correlation (HMQC) spectra. In pseudo-disaccharidic systems, the cyclohexene ring is indicated by the suffix 'a', the sugar by the suffix 'b'. In cases where diastereomeric mixtures of mixed diesters were obtained, this is indicated by the suffixes h (higher moving) and l (lower moving) but no attempts of separation were made. HR-ESI-MS spectra were recorded on a Bruker Daltonics Apex III in positive mode with MeOH/H_2O as solvent. MALDI-MS spectra were recorded on a Bruker Biflex III spectrometer in positive, linear mode with a delayed extraction MALDI source or on a Kratos Analytic Kompact Maldi 2 using 3,5-dihydroxybenzoic acid (DHB), α-hydroxy-α-cyano-cinnamic acid (HCCA) or azidothymidine (ATT) as matrix. Gel permeation chromatography was carried out in the 1–5 mg scale on a XK 16/70 column (bed volume 130 mL), from Amersham packed with Biogel P2 fine (particle size 45–90 μm) and 0.1 M NH_4HCO_3 as buffer. Detection was achieved with a differential refractometer from Knauer, Berlin, Germany.

Compounds **3**–**9** were prepared as described previously.[11–13]

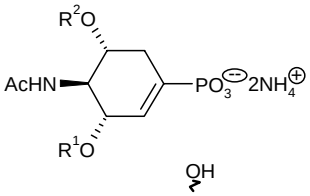
4.2. Ammonium [ethyl (3*S*,4*S*,5*R*)-4-acetamido-3-[(*R*,*S*)-2′,3′-dihydroxypropyloxy]-5-hydroxy-1-cyclohexene-phosphonate] (10h,l)

The diastereomeric mixture of protected diethylphosphonates **7h**,**l** (6 mg, 11.8 μmol) was dissolved in dioxane (1.5 mL) and NaOH solution (1.5 mL, 0.4 M) was added. The solution was stirred at room temperature for 24 h, diluted with H_2O (2 mL) and neutralized with Amberlite IR-120 (H^+). The resin was filtered off, the solvent was evaporated in vacuo and the residue was dissolved in 1 mL of aqueous $NaHCO_3$-solution (0.1 M).

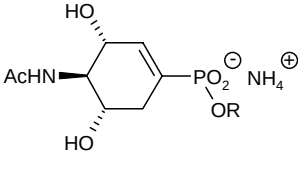
Table 2. Comparison of cyclohexenephosphonate monoethyl esters (column 1), the corresponding free phosphonates (column 2) and more elaborated monoalkyl esters (column 3) for inhibition of *Trypanosomal cruzi trans*-sialidase



10: R^1 = H, R^2 = CH_2-CH-CH_2OH, R^3 = NHAc
11: R^1 = H, R^2 = CH_2-CH_2OH, R^3 = NHAc
12: R^1 = H, R^2 = H, R^3 = N_3
ref[12]: R^1 = H, R^2 = H, R^3 = NHAc



5: R^1 = H, R^2 = CH_2-CH-CH_2OH
6: R^1 = H, R^2 = CH_2-CH_2OH
ref[12]: R^1 = H, R^2 = H, R^3 = NHAc



1: R = 6-Gal
17: R = benzyl
19: R = n-octyl

10: IC_{20} = >8 mM **11**: IC_{50} = 4.7 mM **12**: IC_{20} = >8 mM Ref. 12: IC_{50} = 5.7 mM	non-inhibitory in the concentration range tested	non-inhibitory in the concentration range tested

Inhibition of *Trypanosoma cruzi* TS by cyclohexenephosphonate monoesters. Values are means of three independent experiments with a deviation of less than 20%.

Gel permeation chromatography on a Biogel P2 column followed by lyophilization gave **10h,l** (3.7 mg, 10.6 mmol) in 90% yield. ^{1}H NMR (D_2O) δ 6.04 (d, 1H, $^3J_{H,P}$ = 19.6 Hz, H-2), 4.10 (d, 1H, $^3J_{3,4}$ = 11.2 Hz, H-3), 3.67–3.35 (m, 10H), 2.61 (ddd, 1H, J = 17.6, ~8 Hz, ~8 Hz, H-6a), 2.04 (m, 1H, H-6b), 1.88 (s, 3H, COCH_3), 1.07 (m, 3H, CH_2CH_3). ^{13}C NMR (D_2O) δ 137.2 (C-2) 76.4 (C-5) 70.9 (C-3) 70.8–62.8 (C-1′, C-2′, C-3′) 61.9 (CH_2CH_3) 56.9 (C-4) 31.1 (C-6) 22.3 (COCH$_3$). ^{31}P NMR (D_2O) δ 15.88 (s, PO_3Et^-). $C_{13}H_{24}NO_8P$ (acid form) (M 353.3) MALDI-MS (DHB) 376 $(M+Na)^+$ 392 $(M+K)^+$. HR-ESI-MS (*m*/*z*) $[M+Na]^+$ calcd for $C_{13}H_{24}NNaO_8P$: 376.1131. Found: 376.1135.

4.3. Ammonium [ethyl (3*S*,4*S*,5*R*)-4-acetamido-3-[2′hydroxyethyloxy]-5-hydroxy-1-cyclohexenephosphonate] (11)

Diethyl cyclohexenephosphonate **8** (4 mg, 9.2 μmol) was deprotected and purified as described for **7h,l**. After lyophilization, monoethyl ester **11** (2.5 mg, 7.8 μmol) was obtained in 85% yield. ^{1}H NMR (D_2O) δ 6.05 (d, 1H, $^3J_{H,P}$ = 19.5 Hz, H-2), 4.10 (d, 1H, H-3), 3.72 (dd, 1H, J = ~9 Hz, ~9 Hz, H-4), 3.67 (m, 2H, CH_2CH_3), 3.55–3.50 (m, 5H, H-5, H1′a, H-1′b, H-2′a, H-2′b), 2.59 (ddd, 1H, J = 17.4 Hz, ~8.5 Hz, ~8.5 Hz, H-6a), 2.04 (m, 1H, H-6b), 1.88 (s, 3H, COCH_3), 1.07 (t, 3H, CH_2CH_3). ^{13}C NMR (D_2O) δ 137.3 (C-2) 76.1 (C-5) 71.0 (C-3) 70.9 (C-1′), 61.5 (CH_2CH_3), 61.1 (C-2′), 31.3 (C-6), 22.3 (COCH$_3$) 16.0 (CH_2CH_3). ^{31}P NMR (D_2O) δ 15.90 (s, PO_3Et^-). $C_{12}H_{22}NO_7P$ (acid form) (M 323.3) MALDI-MS (DHB) 346 $(M+Na)^+$ 362 $(M+K)^+$. HR-ESI-MS (*m*/*z*) $[M+Na]^+$ calcd for $C_{12}H_{22}NNaO_7P$: 346.1026. Found: 346.1035.

4.4. Ammonium [ethyl (3*S*,4*S*,5*R*)-4-azido-3,5-dihydroxy-1-cyclohexenephosphonate] (12)

Diethyl 4-azido-cyclohexenephosphonate **9** (5 mg, 17.2 μmol) was deprotected and purified as described for **7h,l**. After lyophilization, monoethyl ester **12** (3.7 mg, 14.1 μmol) was obtained in 82% yield. ^{1}H NMR (D_2O) δ 6.02 (d, 1H, $^3J_{H,P}$ = 19.5 Hz, H-2), 4.08 (d, 1H, $^3J_{3,4}$ ~ 10 Hz, H-3), 3.70–3.65 (m, 3H, H-5, CH_2CH_3), 3.30 (dd, $^3J_{3,4}$, $^3J_{4,5}$ = 8.8 Hz, 10.6 Hz, H-4), 2.51 (m, 1H, J = 17.3 Hz, 6.9 Hz, ~7.1 Hz, H-6a), 2.07 (m, 1H, H-6b), 1.91 (s, 3H, COCH_3), 1.09 (m, 3H, CH_2CH_3). ^{13}C NMR (D_2O) δ 137.1 (C-2) 71.2 (C-5) 70.6 (C-4) 68.9 (C-5), 61.6 (CH_2CH_3), 33.6 (C-6), 22.0 (COCH$_3$) 16.0 (CH_2CH_3). ^{31}P NMR (D_2O) δ 15.53 (s, PO_3Et^-). $C_8H_{14}N_3O_5P$ (acid form) (M 263.2) MALDI-MS (DHB) 264 $(M+H)^+$ 286 $(M+Na)^+$ 302 $(M+K)^+$.

4.5. Tetrabenzyl [3-azido-1,2-*O*-isopropylidene-3,5,6-trideoxy-α-L-glucofuranos-6,6′-diyl]bisphosphonate (13)

To a solution of 3-azido-3-deoxy-1,2-*O*-isopropylidene-5-*O*-trifluoromethanesulfonyl-α-L-xylofuranose[12] (3.43 g, 9.88 mmol) in dry DMF (34 mL) under argon atmosphere at 0 °C was added, with stirring, a freshly prepared solution of the monosodium anion of tetrabenzyl methylenediphosphonate[22] (from NaH and tetrabenzyl methylenediphosphonate) (15.8 mmol) in dry DMF (30 mL). 100 μL of crown ether (15-crown-5) was added, the mixture was stirred at room temperature for 4 h and the reaction was quenched by addition of solid NH_4Cl. The solvent was evaporated in vacuo, the residue was dissolved in CH_2Cl_2 and extracted twice with saturated NaCl-solution. The organic layer was dried over $MgSO_4$, filtered and evaporated. The residue was chromatographed (toluene/EtOAc, 1:3) to give **13** (1.7 g, 2.31 mmol) in 24% yield. R_f = 0.14 (toluene/EtOAc, 1:10). $[\alpha]_D$ +5.3 (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 7.36–7.25 (m, 20H, 4C$_6H_5$) 5.80 (d, 1H, $^3J_{1,2}$ = 3.7 Hz, H-1) 5.13–5.01 (m, 8H, 4CH_2Ph) 4.62 (d, 1H, H-2) 4.58 (m, 1H, H-4) 3.68 (d, 1H, H-3) 2.75 (m, 1H, H-6) 2.37 (m, 1H, H-5a) 2.16 (m, 1H, H-5b) 1.37, 1.26 (2s, 6H, C(CH_3)$_2$). ^{13}C NMR ($CDCl_3$) δ 104.2 (C-1) 83.9 (C-2) 77.2 (C-4) 66.6 (C-3), 34.1 (C-6), 26.5, 26.4 (C(CH_3)$_2$) 25.5 (C-5). ^{31}P NMR ($CDCl_3$) δ 22.42, 22.05 (2s, 2PO_3Bn_2). $C_{37}H_{41}N_3O_9P_2$ (M 733.23) MALDI-MS (HCCA) 756 $(M+Na)^+$ 772 $(M+K)^+$.

4.6. Dibenzyl (3*R*,4*R*,5*S*)-4-azido-3,5-dihydroxy-1-cyclohexenephosphonate (14)

Bisphosphonate **13** (0.92 g, 1.25 mmol) was dissolved in dioxane/5%H_2SO_4 (60 mL) and the solution was stirred at 80 °C until TLC indicated the absence of starting material (ca. 2 h). Following neutralization with saturated $NaHCO_3$-solution the mixture was extracted with CH_2Cl_2, the organic layer was dried over $MgSO_4$, filtered and evaporated. The residue was chromatographed (EtOAc/toluene, 10:1, R_f = 0.5) to give the furanose as a colourless oil (480 mg, 0.69 mmol) in 55% yield. The product was used in the subsequent cyclization step without detailed characterization. The deisopropylidenated furanose obtained from **13** (130 mg, 0.19 mmol) was dissolved in dry dioxane (6 mL) and cooled to 0 °C. Lithium bis(trimethylsilyl)amide in hexane (1.5 M, 300 μL) was added and the solution was stirred for 4 h at room temperature. The reaction was neutralized with Amberlite IR-120 (H^+) and filtered. The filtrate was concentrated in vacuo and the residue was chromatographed (EtOAc/toluene, 1:5) to furnish **14** (50 mg, 0.12 mmol) as a colourless syrup in 64% yield. R_f = 0.4 (EtOAc/toluene, 10:1). $[\alpha]_D$ −26.8 (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 7.28–7.17 (m, 10H, 4C$_6H_5$) 6.43 (d, 1H, $^3J_{2,P}$ = 21.8 Hz, H-2) 4.99–4.92 (m, 4H, 2CH_2Ph) 4.05 (m, 1H, H-3) 3.51 (m, 1H, H-5) 3.25 (dd, 1H, H-4, ^3J = 9.9 Hz, ^3J = 8.1 Hz) 2.51 (m, 1H, H-6a) 2.05 (m, 1H, H-6b). ^{13}C NMR ($CDCl_3$) δ 142.7 (C-2) 71.7 (C-3) 69.3 (C-4) 68.1 (C-5) 32.2 (C-6). ^{31}P NMR ($CDCl_3$) δ 18.25 (s, PO_3Bn_2). $C_{20}H_{22}N_3O_5P$ (M 415.13) MALDI-MS (HCCA) 438 $(M+Na)^+$ 454 $(M+K)^+$.

4.7. Dibenzyl (3*R*,4*R*,5*S*)-4-acetamido-3,5-di-acetoxy-1-cyclohexenephosphonate (15)

Compound **14** (70 mg, 0.17 mmol), dissolved in dry THF (1 mL), was added dropwise to a solution of acetic anhydride (64 μL, 0.67 mmol) and trimethyl phosphine (1 mmol) in dry THF (2.5 mL). The mixture was stirred at room temperature until TLC (EtOAc/toluene, 10:1)

indicated the absence of starting material (ca. 24 h). The mixture was evaporated to dryness and the residue was chromatographed (EtOAc/toluene, 10:1 → EtOAc/MeOH, 10:1) to give **15** (53 mg, 0.11 mmol) in 61% yield. R_f = 0.21 (EtOAc/toluene, 10:1). $[\alpha]_D$ −49.5 (*c* 1, $CHCl_3$). ^1H NMR ($CDCl_3$) δ 7.38–7.26 (m, 10H, $4C_6H_5$) 6.38 (d, 1H, $^3J_{2,P}$ = 21.4 Hz, H-2) 5.49 (d, 1H, $^3J_{NH,4}$ = 9.8 Hz, NH) 5.39 (d, 1H, $^3J_{3,4}$ = 9.3 Hz, H-3) 5.06–5.00 (m, 4H, $2CH_2Ph$) 4.85 (m, 1H, H-5) 4.26 (ddd, 1H, $^3J_{4,5}$ = ~9 Hz, H-4) 2.59 (m, 1H, H-6a) 2.31 (m, 1H, H-6b). ^{13}C NMR ($CDCl_3$) δ 139.6 (C-2) 71.8 (C-3) 68.7 (C-5) 53.3 (C-4) 31.1 (C-6). ^{31}P NMR ($CDCl_3$) δ 15.04 (s, PO_3Bn_2). $C_{26}H_{30}NO_8P$ (M 515.50) MALDI-MS (HCCA) 539 $(M+Na)^+$ 555 $(M+K)^+$.

4.8. Triethylammonium [benzyl (3*R*,4*R*,5*S*)-4-acetamido-3,5-di-acetoxy-1-cyclohexenephosphonate] (16)

Dibenzyl ester **15** (100 mg, 0.19 mmol) was treated with thiophenol (139 µL, 1.36 mmol) and NEt_3 (376 µL, 2.66 mmol) in 2.25 mL THF for 48 h. The solvent was evaporated in vacuo and the residue was chromatographed (MeOH/EtOAc, 1:4, 1% NEt_3) to give **16** (100 mg) in nearly quantitative yield. R_f = 0.16 (EtOAc/MeOH, 4:1). $[\alpha]_D$ −36.8 (*c* 1, $CHCl_3$). ^1H NMR (CD_3OD) δ 7.38–7.26 (m, 5H, C_6H_5) 6.21 (d, 1H, $^3J_{2,P}$ = 18.8 Hz, H-2) 5.45 (1H, H-3) 4.96 (m, 1H, H-5) 4.82, 4.81 (2d, 2H, CH_2Ph) 4.16 (dd, 1H, H-4) 3.19 (q, 6H, $N(CH_2CH_3)_3$) 2.76 (m, 1H, H-6a) 2.28 (m, 1H, H-6b) 2.02, 1.98, 1.87 (3s, 9H, $3COCH_3$) 1.29 (t, 9H, $N(CH_2CH_3)_3$). ^{13}C NMR (CD_3OD) δ 134.0 (C-2) 73.3 (C-3) 70.4 (C-5) 67.2 (CH_2Ph) 53.7 (C-4) 47.6 ($N(CH_2CH_3)_3$) 31.9 (C-6). ^{31}P NMR ($CDCl_3$) δ 12.55 (s, PO_3Bn^-). $C_{19}H_{24}NO_8P$ (free acid) (M 425.37) MALDI-MS (DHB) 448 $(M+Na)^+$ 464 $(M+K)^+$ 486 $(M+Na+K-H)^+$.

4.9. Ammonium [benzyl (3*R*,4*R*,5*S*)-4-acetamido-3,5-di-hydroxy-1-cyclohexenephosphonate] (17)

Monoester **16** (100 mg, 0.24 mmol) was dissolved in H_2O/dioxane (20 mL), concentrated ammonia (25%, 2 mL) was added and the mixture was stirred at room temperature for 4 d. Following lyophilization inhibitor **17** was purified by gel permeation chromatography on a Biogel P2 column (0.1 M NH_4HCO_3) to yield **17** (80 mg), in almost quantitative yield. $[\alpha]_D$ −21.7 (*c* 1, D_2O). ^1H NMR (CD_3OD) δ 7.38–7.23 (m, 5H, C_6H_5) 6.31 (d, 1H, $^3J_{2,P}$ = 19.2 Hz, H-2) 4.82, 4.81 (2d, 2H, CH_2Ph) 4.09 (d, 1H, H-3) 3.74 (dd, 1H, ^3J = ~9 Hz, ~10.5 Hz, H-4) 3.56 (m, 1H, H-5) 2.69 (m, 1H, H-6a) 2.20 (m, 1H, H-6b) 2.00 (s, 3H, $COCH_3$). ^{13}C NMR (CD_3OD) δ 138.7 (C-2) 72.7 (C-3) 69.3 (C-5) 67.0 (CH_2Ph) 60.2 (C-4) 35.3 (C-6). ^{31}P NMR ($CDCl_3$) δ 14.68 (s, PO_3Bn^-). $C_{15}H_{20}NO_6P$ (free acid) (M 341.27) MALDI-MS (DHB) 364 $(M+Na)^+$ 386 $(M+2Na-H)^+$. HR-ESI-MS (*m*/*z*) $[M+Na]^+$ calcd for $C_{15}H_{20}NNaO_6P$: 364.0920. Found: 364.0924.

4.10. Benzyl 1-octyl (3*R*,4*R*,5*S*)-4-acetamido-3,5-di-acetoxy-1-cyclohexenephosphonate (18h,l)

Monoester **16** (20 mg, 0.04 mmol) was dissolved in dry DMF (0.4 mL), octyl triflate[23] (42 mg, 0.16 mmol) was added, the mixture was stirred at room temperature for 12 h and another 20 mg (0.04 mmol in 0.4 mL dry DMF) octyl triflate was added. After 12 h of stirring, the solvent was evaporated in vacuo and the residue was chromatographed (EtOAc/toluene, 10:1) to give mixed diesters **18h**,**l** (32 mg, 0.06 mmol) in 75% yield. Unreacted starting material (monoester **16**) could be recovered by elution with EtOAc/MeOH, 4:1, 1% NEt_3. R_f = 0.28 (EtOAc/toluene, 10:1). ^1H NMR ($CDCl_3$) δ 7.40–7.36 (m, 5H, C_6H_5) 6.40, 6.34 (2d, 1H, $^3J_{2,P}$ = 21.4 Hz, H-2(h,l)) 5.48, 5.40 (2m, 1H, H-3(h,l)) 5.09–5.00 (m, 3H, H-5, CH_2Ph) 4.15 (m, 1H, H-4) 4.00 (m, 2H, H-1′a, H-1′b) 2.67 (m, 1H, H-6a) 2.24 (m, 1H, H-6b) 2.05, 2.03, 1.90 (3s, 9H, $3COCH_3$) 1.63 (m, 2H, H-2′), 1.33–1.28 (m, 12H, H-3′–H-7′) 0.88 (s, 3H, H-8′). ^{13}C NMR ($CDCl_3$) δ 72.6 (C-3) 69.5 (C-5) 67.7 (C-1′) 53.3 (C-4) 31.1 (C-2′) 30.9 (C-6) 22.5 ($NHCOCH_3$) 20.4 ($COCH_3$). ^{31}P NMR ($CDCl_3$) δ 18.47, 18.45 (2s, $2PO_3BnOct$). $C_{27}H_{40}NO_8P$ (M 537.25) MALDI-MS (DHB) 560 $(M+Na)^+$ 576 $(M+K)^+$.

4.11. Ammonium [1-octyl (3*R*,4*R*,5*S*)-4-acetamido-3,5-dihydroxy-1-cyclohexenephosphonate] (19)

Mixed diester **18h**,**l** (15 mg, 0.028 mmol) in MeOH (2 mL) containing Pd/C (10%, 5 mg) was debenzylated under a hydrogen atmosphere for 5 min (TLC-monitoring). The mixture was filtered through Celite, concentrated ammonia was added (25%, 2 mL) and the mixture was stirred overnight at room temperature. Following evaporation to dryness the residue was purified on a Biogel P2 column (0.1 M NH_4HCO_3) and repeatedly lyophilized to give **19** (11 mg, 0.028 mmol) in literally quantitative yield. ^1H NMR (D_2O) 6.09 (d, 1H, $^3J_{2,P}$ = 19.4 Hz, H-2) 4.12 (m, 1H, H-3) 3.73–3.56 (m, 4H, H-4, H-5, H-1′a, H-1′b) 2.54 (m, 1H, H-6a) 2.12 (m, 1H, H-6b) 1.93 (s, 3H, $COCH_3$) 1.47 (m, 2H, H-2′), 1.36–1.13 (m, 12H, H-3′–H-7′) 0.72 (s, 3H, H-8′). ^{13}C NMR ($CDCl_3$) δ 136.8 (C-1) 70.2 (C-3) 69.0–57.5 (C-5, C-4, C-1′) 32.7 (C-6) 30.5–21.4 (C-2′–C-7′) 21.5 ($COCH_3$) 12.7 (C-8′). ^{31}P NMR ($CDCl_3$) δ 15.81 (s, PO_3BnOct). $C_{16}H_{30}NO_8P$ (M 363.39) MALDI-MS (DHB) 364 $(M+H)^+$ 386 $(M+Na)^+$. HR-ESI-MS (*m*/*z*) $[M+Na]^+$ calcd for $C_{12}H_{30}NaNO_6P$: 386.1702. Found: 386.1713.

4.12. 1,2:3,4-Di-*O*-isopropylidene-6-*O*-trifluormethanesulfonyl-α-D-galactopyranose (20)

Triflate **20** was synthesized according to literature procedures.[14]

4.13. Benzyl 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranos-6-yl [(3*R*,4*R*,5*S*)-4-acetamido-3,5-di-acetoxy-1-cyclohexenephosphonate] (21h,l)

Monobenzyl ester **16** (39 mg, 0.074 mmol) and galactose triflate **20** (95.3 mg, 0.24 mmol) were dissolved in dry DMF (0.7 mL) and stirred for 12 h. More triflate (39 mg, 0.074 mmol) was added and, after additional 12 h of stirring, the solvent was evaporated and the residue was chromatographed (EtOAc/toluene, 10:1) to

give **21h,l** (52 mg, 0.078 mmol) in 56% yield. Unreacted monoester could be recovered by elution of the column with MeOH/EtOAc, 1:4, 1%NEt_3. R_f = 0.22 (EtOAc/toluene, 10:1). ^{1}H NMR (CD_3OD) δ 7.43–7.36 (m, C_6H_5) 6.40 (2d, $^3J_{2a,P}$ = 21.5 Hz, H-2a(h,l)) 5.52–5.48 (m, 2H, H-3a(h,l), H-1b) 5.14–5.06 (m, 2.5H, H-5a(h), CH_2Ph) 4.97 (m, 0.5H, H-5a(l)) 4.64 (1H, H-3b) 4.38–4.36 (m, 1.5H, H-4a(l), H-2b) 4.25–4.03 (m, 4.5H, H-4a(h), H-4b, H-5b, H-6b, H-6b′) 2.74 (m, 0.5H, H-6a(h)) 2.64 (m, 0.5H, H-6a(l)) 2.46 (m, 0.5H, H-6a′(l)) 2.27 (m, 0.5H, H-6a′(h)) 2.05, 2.00, 1.98 (3s, 9H, 3COCH_3) 1.51–1.28 (12H, 2C(CH_3)$_2$). ^{13}C NMR ($CDCl_3$) δ 140.5, 140.4 (C-2a(h,l)) 97.3 (C-1b) 72.6 (C-3a(h,l)) 71.9 (C-3b) 71.7 (C-4b) 71.5 (C-2b) 70.0 (C-5a(l)) 69.4, 68.8 (C-5a(h), CH$_2$Ph) 68.6 (C-5b) 67.4, 66.5 (C-6b(h,l)) 53.2 (C-4a(h)) 52.9 (C-4a(l)) 30.7 (C-6a(h,l)). ^{31}P NMR ($CDCl_3$) δ 19.64, 18.41 (2s, 2 PO_3R_2(h,l)). $C_{31}H_{44}NO_{13}P$ (M 667.26) MALDI-MS (DHB) 690 $(M+Na)^+$ 706 $(M+K)^+$.

4.14. Triethylammonium [1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranos-6-yl (3*R*,4*R*,5*S*)-4-acetamido-3,5-dihydroxy-1-cyclohexenephosphonate] (22)

The mixture of diesters **21h,l** (20 mg, 0.03 mmol) was dissolved in MeOH (4 mL) containing Pd/C (10%, 8 mg) and debenzylated under a hydrogen atmosphere for 5 min (TLC-monitoring). The mixture was filtered through Celite, concentrated ammonia was added (25%, 2 mL) and the mixture was stirred overnight at room temperature. Repeated lyophilization from dioxane/H_2O gave **22** (17 mg, 0.03 mmol). ^{1}H NMR (D_2O) δ 6.13 (d, $^3J_{2a,P}$ = 19.7 Hz, H-2a) 5.54 (d, 1H, H-1b, $^3J_{1b,2b}$ = 4.8 Hz) 4.63 (1H, H-3b) 4.41 (1H, H-2b) 4.35 (1H, H-4b) 4.13 (m, 1H, H-3a) 3.97 (m, 1H, H-5b) 3.77–3.66 (m, 4H, H-4a, H-5a, H-6b, H-6b′) 2.54 (m, 1H, H-6a) 2.13 (m, 1H, H-6a′) 1.94 (s, 3H, COCH_3) 1.46–1.34 (12H, 2C(CH_3)$_2$). ^{13}C NMR (D_2O) δ 138.8 (C-2a) 97.0 (C-1b) 72.0 (C-3a) 71.4 (C-4b) 71.1 (C-2b) 68.9 (C-5a) 59.3 (C-4a) 34.5 (C-6a) 23.3 (COCH$_3$). ^{31}P NMR (D_2O) δ 16.19 (s, PO_3R^-). $C_{20}H_{32}NO_{11}P$ (M 493.45) MALDI-MS (DHB) 515.8 $(M+Na)^+$.

4.15. Ammonium [α,β-D-galactopyranos-6-yl (3*R*,4*R*,5*S*)-4-acetamido-3,5-dihydroxy-1-cyclohexenephosphonate] (1)

A solution of **22** (5 mg, 9.8 μmol) in trifluoroacetic acid (50%) was stirred for 2 h at room temperature and immediately lyophilized. Purification on a Biogel P2 column (0.1 M NH_4HCO_3) gave, after lyophilization, pseudo-sialoside **1** in 71% yield (3 mg, 7 μmol). ^{1}H NMR (D_2O) δ 6.12 (d, 1H, $^3J_{2a,P}$ = 19.7 Hz, H-2a) 5.12 (d, 0.4H, H-1b(α), $^3J_{1bα,2bα}$ = 3.8 Hz) 4.45 (d, 0.6H, H-1b(β), $^3J_{1bβ,2bβ}$ = 7.9 Hz) 4.12 (m, 1H, H-3a) 4.04 (0.4H, H-5b(α)) 3.87 (0.4H, H-4b(α)) 3.83 (0.6H, H-4b(β)) 3.77–3.66 (m, 6.4H) 3.50 (0.6H, H-3b(β)) 3.34 (dd, 0.6H, H-2b(β)) 2.54 (m, 1H, H-6a) 2.13 (m, 1H, H-6a′) 1.92 (s, 3H, COCH_3). ^{13}C NMR (D_2O) δ 139.0 (C-2a) 97.6 (C-1b(β)) 93.5 (C-1b(α)) 74.9 (C-4a) 73.8 (C-3b(β)) 72.9 (C-2b(β)) 71.9 (C-3a) 70.5 (C-5b(α)) 70.2 (C-4b(α),C-3b(α)) 69.5 (C-4b(β)) 68.9 (C-5a, C-2b(α)) 64.6, 64.3 (C-6b(α,β)) 59.2 (C-5b(β)) 34.3 (C-6a) 23.5 (COCH$_3$). ^{31}P NMR (D_2O) δ 16.28, 16.24 (2s, PO_3R^-(α,β)). $C_{14}H_{24}NO_{11}P$ (acid form) (M 413.32) MALDI-MS (DHB) 414.7 $(M+H)^+$ 436.7 $(M+Na)^+$ 452.7 $(M+K)^+$. FAB-MS (glycerol) 436 $(M+Na)^+$. HR-ESI-MS (*m*/*z*) $[M+Na]^+$ calcd for $C_{14}H_{24}NNaO_{11}P$: 436.0979. Found: 436.1047.

4.16. Tetramethyl [3-*O*-benzyl-5,6-dideoxy-1.2-*O*-isopropylidene-α-D-glucofuranos-6,6′-diyl]bisphosphonate (23)

To a solution of 3-*O*-benzyl-1,2-*O*-isopropylidene-5-*O*-trifluormethanesulfonyl-α-D-xylofuranose[11] (450 mg, 1.1 mmol) in dry DMF (7.5 mL) under argon atmosphere at 0 °C was added, with stirring, a freshly prepared solution of the monosodium anion of tetramethyl methylenediphosphonate[24] (from NaH and tetramethyl methylenediphosphonate) (1.75 mmol) in dry DMF (7.5 mL). The mixture was stirred at room temperature for 4 h and the reaction was quenched by addition of solid NH_4Cl (1 g). The solvent was evaporated in vacuo, the residue suspended in EtOAc/MeOH (10:1) and chromatographed to give **23** (290 mg, 0.59 mmol) in 54% yield. R_f = 0.59 (EtOAc/MeOH, 3:1). $[\alpha]_D$ −21.5 (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 7.30–7.19 (m, 5H, C_6H_5) 5.82 (d, 1H, $^3J_{1,2}$ = 3.7 Hz, H-1) 4.54 (d, 1H, H-2) 4.61 (d, CH_2Ph) 4.44–4.42 (m, 2H, H-4, CH_2Ph) 3.77–3.70 (m, 13H, H-3, 4OCH_3) 2.61 (m, 1H, H-6) 2.36 (m, 1H, H-5a) 2.05 (m, 1H, H-5b) 1.41, 1.24 (2s, 6H, C(CH_3)$_2$). ^{13}C NMR ($CDCl_3$) δ 104.7 (C-1) 82.6 (C-2) 81.9 (C-3) 77.5 (C-4) 71.9 (CH$_2$Ph), 53.6–53.1 (OCH$_3$), 32.3 (C-6), 27.0 C(CH$_3$)$_2$ 24.6 (C-5). ^{31}P NMR ($CDCl_3$) δ 27.66, 27.65 (2s, 2PO$_3$Me$_2$). $C_{20}H_{32}O_{10}P_2$ (M 494.41) MALDI-MS (DHB) 517 $(M+Na)^+$.

4.17. Dimethyl (3*S*,4*S*,5*R*)-4-benzyloxy-3,5-dihydroxy-1-cyclohexenephosphonate (24)

To a solution of bisphosphonate **23** (600 mg, 1.21 mmol) in dioxane/H_2O (1:1, 40 mM) was added toluenesulfonic acid monohydrate (100 mg) and the reaction mixture was stirred at 60 °C for 4 d. Following neutralization with $NaHCO_3$ (s) the solvent was evaporated in vacuo and the residue was chromatographed (EtOAc/MeOH, 3:1, R_f = 0.3–0.4) to give the furanose as a colourless oil (490 mg, 1.08 mmol) in 93% yield. The product was used in the subsequent cyclization step without detailed characterization. The de-isopropylidenated furanose obtained from **23** (140 mg, 0.31 mmol) was dissolved in dry dioxane (5 mL), 32 mg sodium methoxide (s) was added and the solution was stirred for 2 h at room temperature. Another 16 mg of NaOMe (s) was added and, following 2 h of stirring, the reaction was neutralized with Amberlite IR-120 (H^+) and filtered. The filtrate was concentrated in vacuo and the residue was chromatographed (EtOAc/MeOH, 10:1) to furnish **24** (45 mg, 0.14 mmol) in 45% yield. R_f = 0.59 (EtOAc/MeOH, 3:1). $[\alpha]_D$ −4.6 (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 7.38–7.30 (m, 5H, C_6H_5) 6.59 (d, 1H, $^3J_{2,P}$ = 21.7 Hz, H-2) 4.93, 4.78 (2d, 2H, CH_2Ph) 4.33 (br d, 1H, H-3) 3.91 (ddd, 1H, $^3J_{5,6a,b}$ = ~6 Hz, ~9 Hz, H-5) 3.75–3.71 (m, 6H, 2OCH_3) 3.51 (dd, 1H, $^3J_{4,3}$ = 6.6 Hz, $^3J_{4,5}$ = 8.9 Hz, H-4) 2.63 (m, 1H, H-6a) 2.24 (m, 1H, H-6b). ^{13}C NMR ($CDCl_3$) δ 143.7 (C-2) 83.4 (C-4)

74.3 (CH_2Ph) 71.7 (C-3) 68.3 (C-5) 52.9 (OCH_3), 31.6 (C-6). ^{31}P NMR ($CDCl_3$) δ 22.09 (s, PO_3Me_2). $C_{15}H_{21}O_6P$ (M 328.3) MALDI-MS (DHB) 329 $(M+H)^+$ 351 $(M+Na)^+$ 367 $(M+K)^+$.

4.18. Dimethyl (3*S*,4*S*,5*R*)-4-benzyloxy-3,5-di-acetoxy-1-cyclohexenephosphonate (25)

Compound **24** (68 mg, 0.21 mmol) was acetylated in pyridine/acetic anhydride (2:1, 2 mL) overnight and the solvent was evaporated in vacuo. Chromatography of the residue yielded **25** (85 mg, 0.21 mmol) in quantitative yield. R_f = 0.62 (EtOAc/MeOH, 10:1). $[\alpha]_D$ +40.1 (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 7.35–7.25 (m, 5H, C_6H_5) 6.46 (d, 1H, $^3J_{2,P}$ = 21.2 Hz, H-2) 5.51 (1H, H-3) 5.14 (ddd, 1H, $^3J_{5,6a,b}$ = ~8.5 Hz, ~6 Hz, H-5) 4.72, 4.68 (2d, 2H, CH_2Ph) 3.80 (dd, 1H, $^3J_{4,3}$ = 6.2 Hz, $^3J_{4,5}$ = 8.9 Hz, H-4) 3.74–3.69 (m, 6H, 2OCH_3) 2.77 (m, 1H, H-6a) 2.30 (m, 1H, H-6b). ^{13}C NMR ($CDCl_3$) δ 138.4 (C-2) 77.4 (C-4) 74.0 (CH_2Ph) 71.8 (C-3) 69.6 (C-5) 52.6 (OCH_3) 29.1 (C-6). ^{31}P NMR ($CDCl_3$) δ 20.77 (s, PO_3Me_2). $C_{19}H_{25}O_8P$ (M 412.38) MALDI-MS (DHB) 435 $(M+Na)^+$ 451 $(M+K)^+$.

4.19. Triethylammonium [methyl (3*S*,4*S*,5*R*)-4-benzyloxy-3,5-di-acetoxy-1-cyclohexenephosphonate] (26)

Dimethyl ester **25** (84 mg, 0.2 mmol) in THF (2 mL) was treated with thiophenol (168 μL) and triethylamine (420 μL) for 16 h at room temperature. Following evaporation of the solvent the residue was chromatographed (EtOAc/MeOH, 1:1, 1% NEt_3) to give **26** (90 mg, 0.18 mmol) in 90% yield. R_f = 0.4 (EtOAc/MeOH, 1:1, 1% NEt_3). $[\alpha]_D$ +47.9 (*c* 1, MeOH). ^{1}H NMR (CD_3OD) δ 7.34–7.25 (m, 5H, C_6H_5) 6.17 (d, 1H, $^3J_{2,P}$ = 18.7 Hz, H-2) 5.48 (m, 1H, H-3) 5.11 (ddd, 1H, $^3J_{5,6a,b}$ = ~6 Hz, ~9 Hz, H-5) 4.73, 4.68 (2d, 2H, CH_2Ph) 3.84 (dd, 1H, $^3J_{4,3}$ = 7.0 Hz, $^3J_{4,5}$ = 9.6 Hz, H-4) 3.48 (d, 3H, $^3J_{P,H}$ = 7.1 Hz, OCH_3) 3.17 (q, 6H, N(CH_2CH_3)$_3$) 2.77 (m, 1H, H-6a) 2.35 (m, 1H, H-6b) 2.02, 1.98 (2s, 6H, 2COCH_3) 1.29 (t, 9H, N(CH_2CH_3)$_3$). ^{13}C NMR (CD_3OD) δ 133.3 (C-2) 79.7 (C-4) 74.9 (CH_2Ph) 74.2 (C-3) 71.7 (C-5) 51.7 (OCH_3) 47.9 (N(CH_2CH_3)$_3$) 31.3 (C-6) 21. ^{31}P NMR ($CDCl_3$) δ 14.44 (s, PO_3Me^-). $C_{18}H_{23}O_8P$ (free acid) (M 398.35) MALDI-MS (DHB) 421 $(M+Na)^+$ 443 $(M+2Na-H)^+$.

4.20. Methyl 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranos-6-yl [(3*S*,4*S*,5*R*)-3-benzyloxy-3,5-di-acetoxy-1-cyclohexenephosphonate] (27h,l)

Via alkylation: monoester **26** (45 mg, 0.11 mmol) and triflate **20** (140 mg, 0.38 mmol) were stirred in 1 mL of dry DMF for 16 h. The solvent was evaporated in vacuo and the residue was chromatographed (toluene/EtOAc, 1:4) to give mixed diester (35 mg, 0.055 mmol) in 50% yield. Unreacted monoester could be quantitatively reisolated by eluting the column with EtOAc/MeOH (1:1, 1% NEt_3). The ^{31}P NMR spectra indicate the formation of both diastereomers in comparable amounts. *Via Mitsunobu condensation*: triethylammonium salt **26** was converted into the free acid by treatment with Amberlite IR-120 (H^+) in dioxane/H_2O (1:1), filtration and lyophilization. The acid (25 mg, 0.063 mmol) was dissolved in dry THF (1 mL), 1,2:3,4-di-*O* isopropylidene-α-D-galactopyranose[21] (33 mg, 0.126 mmol), triphenyl phosphine (33 mg, 0.126 mmol) and diisopropyl azodicarboxylate (25 μL, 0.126 mmol) were added and the mixture was stirred at 70 °C overnight. Evaporation of the solvent followed by chromatography (toluene/EtOAc, 1:1 → 1:10) gave **27h,l** (23 mg, 0.036 mmol) in 57% yield. R_f = 0.18 (toluene/EtOAc, 1:1). ^{1}H NMR ($CDCl_3$) δ 7.36–7.27 (m, C_6H_5) 6.48 (d, $^3J_{2a,P}$ = 21.2 Hz, H-2a) 5.54–5.53 (m, 2H, H-3a, H-1b) 5.16 (m, 1H, H-5a) 4.72–4.67 (m, 2H, CH_2Ph) 4.61 (m, 1H, H-3b) 4.31 (1H, H-2b) 4.22 (dd, 1H, H-4b) 4.18–4.15 (m, 2H, H-6b, H-6b′) 4.03 (m, 1H, H-5b) 3.78 (dd, 1H, H-4a) 3.76–3.74 (m, 3H, OCH_3) 2.80 (m, 1H, H-6a) 2.34 (m, 1H, H-6a′)2.04–2.00 (6H, 2COCH_3) 1.55–1.32 (12H, 2C(CH_3)$_2$). ^{13}C NMR ($CDCl_3$) δ 138.6 (C-2a) 96.7 (C-1b) 78.1 (C-4a) 74.3 (CH_2Ph) 72.6 (C-3a) 70.9 (C-4b, C-3b) 70.6 (C-2b) 70.1 (C-5a) 67.4 (C-5b) 65.6 (C-6b) 53.0 (OCH_3) 29.6 (C-6a). ^{31}P NMR ($CDCl_3$) δ 20.28, 19.58 (2s, 2PO_3R_2(h,l)). $C_{30}H_{41}O_{13}P$ (M 640.63) MALDI-MS (DHB) 663 $(M+Na)^+$ 679 $(M+K)^+$.

4.21. Methyl 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranos-6-yl [(3*S*,4*S*,5*R*)-3,4,5-tri-acetoxy-1-cyclohexenephosphonate] (28h,l)

Benzyl ether **27h,l** (24 mg, 37,5 μmol) was hydrogenated with cyclohexadiene (0.75 mL) and Pd/C (10 mg) in EtOH (1.5 mL) for 3 d at 55 °C. The mixture was filtered through Celite, the solvent was evaporated in vacuo and the residue was acetylated in pyridine/acetic anhydride (2:1) overnight. Following evaporation of the solvent the residue was chromatographed (toluene/EtOAc, 1:4) to give **28h,l** (22 mg, 37 μmol) in 98% yield. R_f = 0.12 (toluene/EtOAc, 1:1). ^{1}H NMR ($CDCl_3$) δ 6.48 (d, $^3J_{2a,P}$ = 21.2 Hz, H-2a) 5.58 (1H, H-3a) 5.53 (d, 1H, H-1b, $^3J_{1b,2b}$ = 5 Hz) 5.28 (m, 1H, H-4a) 5.17 (m, 1H, H-5a) 4.60 (m, 1H, H-3b) 4.32 (1H, H-2b) 4.22 (dd, 1H, H-4b) 4.18–4.15 (m, 2H, H-6b, H-6b′) 4.01 (m, 1H, H-5b) 3.76–3.74 (m, 3H, OCH_3) 2.83 (m, 1H, H-6a) 2.41 (m, 1H, H-6a′) 2.08–2.00 (9H, 3COCH_3) 1.54–1.31 (12H, 2C(CH_3)$_2$). ^{13}C NMR ($CDCl_3$) δ 138.6 (C-2a) 96.4 (C-1b) 71.7 (C-4a) 71.5 (C-3a) 70.9 (C-3b) 70.8 (C-4b) 70.6 (C-2b) 68.3 (C-5a) 67.2 (C-5b) 64.9 (C-6b) 52.6 (OCH_3) 29.9 (C-6a). ^{31}P NMR ($CDCl_3$) δ 19.66, 18.91 (2s, 2PO_3R_2(h,l)). $C_{25}H_{37}O_{14}P$ (M 592.54) MALDI-MS (DHB) 593 $(M+H)^+$ 615 $(M+Na)^+$ 631 $(M+K)^+$.

4.22. Triethylammonium [1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranos-6-yl (3*S*,4*S*,5*R*)-3,4,5-tri-acetoxy-1-cyclohexenephosphonate] (29)

Compound **28h,l** (25 mg, 42.2 μmol) was demethylated by stirring with thiophenol (100 μL) and triethylamine (250 μL) in THF (1 mL) for 3 d at room temperature. Following evaporation to dryness the residue was chromatographed (EtOAc/MeOH, 1:1, 1% NEt_3) to give monoester **29** (24 mg, 35 μmol) in 80% yield. R_f = 0.5 (EtOAc/MeOH, 1:1, 1% NEt_3). ^{1}H NMR (D_2O) δ 6.07 (d, $^3J_{2a,P}$ = 19.1 Hz, H-2a) 5.53 (1H, H-3a) 5.52 (d, 1H, H-1b) 5.16 (m, 1H, H-4a) 5.15 (m, 1H, H-5a) 4.66

(m, 1H, H-3b) 4.40 (1H, H-2b) 4.32 (m, 1H, H-4b) 3.94 (m, 1H, H-5b) 3.77–3.68 (m, 2H, H-6b, H-6b′) 3.06 (q, 6H, $N(CH_2CH_3)_3$) 2.68 (m, 1H, H-6a) 2.34 (m, 1H, H-6a′) 1.99–1.94 (9H, $3COCH_3$) 1.44–1.25 (12H, $2C(CH_3)_2$) 1.13 (t, 9H, $N(CH_2CH_3)_3$). ^{13}C NMR (D_2O) δ 132.2 (C-2a) 95.1 (C-1b) 72.3 (C-4a) 71.4 (C-3a) 69.6 (C-4b) 69.2 (C-3b, C-2b) 68.5 (C-5a) 66.4 (C-5b) 62.4 (C-6b) 45.9 ($N(CH_2CH_3)$) 29.2 (C-6a). ^{31}P NMR (D_2O) δ 14.56 (s, PO_3R^-). $C_{24}H_{35}O_{14}P$ (acid form) (M 578.52) MALDI-MS (HCCA) 579 $(M+H)^+$ 601 $(M+Na)^+$ 617 $(M+K)^+$ 623 $(M+2Na-H)^+$.

4.23. Triethylammonium [1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranos-6-yl (3*S*,4*S*,5*R*)-3,4,5-trihydroxy-1-cyclohexenephosphonate] (30)

Compound **29** (10 mg, 14.7 μmol) was deacetylated by stirring in aqueous ammonia (10%) for 3 h and repeated lyophilization from NH_4HCO_3 (0.1 M). Yield: 90% (6 mg, 13.2 μmol). ^1H NMR (D_2O) δ 6.06 (d, $^3J_{2a,P}$ = 19.6 Hz, H-2a) 5.51 (d, 1H, H-1b, $^3J_{1b,2b}$ = 5.0 Hz) 4.65 (m, 1H, H-3b) 4.40 (1H, H-2b) 4.32 (br d, 1H, $^3J_{4b,5b}$ = 8.2 Hz, H-4b) 4.07 (m, 1H, H-3a) 3.95 (m, 1H, H-5b) 3.77–3.67 (m, 2H, H-6b, H-6b′) 3.62 (m, 1H, H-5a) 3.34 (m, 1H, $^3J_{4a,3a}$ 8.2 Hz, $^3J_{4a,5a}$ = 10.2 Hz, = H-4a) 2.51 (m, 1H, H-6a) 2.04 (m, 1H, H-6a′) 1.45–1.26 (12H, $2C(CH_3)_2$). ^{13}C NMR (D_2O) δ 137.3 (C-2a) 95.1 (C-1b) 76.2 (C-4a) 71.9 (C-3a) 69.7 (C-4b) 69.4 (C-3b, C-2b) 68.5 (C-5a) 66.9 (C-5b) 62.3 (C-6b) 32.3 (C-6a). ^{31}P NMR (D_2O) δ 16.35 (s, PO_3R^-). $C_{18}H_{29}O_{11}P$ (acid form) (M 452.40) MALDI-MS (HCCA) 453 $(M+H)^+$ 475 $(M+Na)^+$ 491 $(M+K)^+$ 497 $(M+2Na-H)^+$ 513 $(M+Na+K-H)^+$.

4.24. Ammonium [α,β-D-galactopyranos-6-yl (3*S*,4*S*,5*R*)-3,4,5-trihydroxy-1-cyclohexenephosphonate] (2)

A solution of **30** (5 mg, 10.6 μmol) in aqueous trifluoroacetic acid (50%) was stirred for 3 h and lyophilized. The product was purified by gel permeation chromatography on a Biogel P2 column (0.1 M NH_4HCO_3) and subsequently lyophilized to give **2** in 72% yield (3 mg, 7.7 μmol). ^1H NMR (D_2O) δ 6.05 (d, 1H, $^3J_{2a,P}$ = 19.7 Hz, H-2a) 5.10 (d, 0.3H, H-1b(α), $^3J_{1b\alpha,2b\alpha}$ = 3.7 Hz) 4.44 (d, 0.7H, H-1b(β), $^3J_{1b\beta,2b\beta}$ = 7.9 Hz) 4.08 (m, 1H, H-3b(α,β)) 4.02 (0.3H, H-5b(α)) 3.87 (0.3H, H-4b(α)) 3.81 (0.7H, H-4b(β)) 3.75–3.64 (m, 3.3H), 3.50 (0.7H, H-3b(β)) 3.34–3.33 (m, 1.7H, H-4b(α,β), H-2b(β)) 2.52 (m, 1H, H-6a) 2.03 (m, 1H, H-6a′). ^{13}C NMR (D_2O) δ 139.2 (C-2a) 97.5 (C-1b(β)) 93.6 (C-1b(α)) 77.9, 75.0, 73.7, 73.1, 70.4, 70.2, 69.6 (C-3a, -4a, -5a, -2b, -3b, -4b, -5b(α,β)) 64.6 (C-6b(α,β)) 34.3 (C-6a). ^{31}P NMR (D_2O) δ 16.38, 16.35 (2s, PO_3R^-(α,β)). $C_{12}H_{21}O_{11}P$ (acid form) (M 372.27) MALDI-MS (DHB) 395 $(M+Na)^+$. HR-ESI-MS (*m*/*z*) $[M+Na]^+$ calcd for $C_{12}H_{21}NaO_{11}P$: 395.0713. Found: 395.0783. $[M+2Na-H]^+$ calcd for $C_{12}H_{20}Na_2O_{11}P$: 417.0533. Found: 417.0573.

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