SYNTHESIS OF D-GALACTOPYRANOSYLPHOSPHONIC AND (D-GALACTOPYRANOSYLMETHYL)PHOSPHONIC ACIDS AS INTERMEDIATES OF INHIBITORS OF GALACTOSYLTRANSFERASES

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Employing the Michaelis–Arbuzov reaction of 1-*O*-acetyl-2,3,4,6-tetra-*O*-benzyl-D-galactopyranose with triethyl phosphite and trimethylsilyl trifluoromethanesulfonate, α- and β-D-galactopyranosylphosphonic acids were prepared in 33 and 28% yields, respectively. (α-D-Galactopyranosylmethyl)phosphonic acid was synthesized by a five-step route from 2,3,4,6-tetra-*O*-benzyl-D-galactopyranose in 52% overall yield. When tested against bovine α-1,3- and β-1,4-galactosyltransferases, all three compounds showed, at best, very poor inhibitions. Both enzymes were inhibited more effectively by β-D-galactopyranosylphosphonic acid (IC_{50} = 17 mmol l^{-1} at 13.5 μmol l^{-1} of UDP-Gal for β4GalT).

Keywords: Carbohydrates; UDP-Gal; Michaelis–Arbuzov reaction; Phosphonates; Galactosyltransferases; *C*-Glycosides.

Interactions of lectin-type receptors with a carbohydrate epitope of glycoconjugates form the basis of cell recognition processes in biological systems. Complex oligosaccharides are synthesized in the endoplasmatic reticulum and Golgi complex by the sequential transfer of monosaccharide residue from an activated donor to an appropriate acceptor[1]. Thus the selective inhibition of enzymes participating in the biosynthesis of oligosaccharide chains is of great importance as it may lead to the development of new therapeutic agents. Galactosyltransferases catalysing the transfer of D-galactose from uridine-5′-diphospho-α-D-galactose (UDP-Gal) can be classified mechanistically as either inverting or retaining, depending on the relative anomeric configuration of their substrate and product. Inverting

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β-1,4-galactosyltransferase (β4GalT) utilizes *N*-acetylglucosamine and all *N*-acetylglucosamine-terminated oligosaccharides as the acceptor while retaining α-1,3-galactosyltransferase (α3GalT) creates a new glycosidic bond to *N*-acetyllactosamine. The mechanism of action is now rather well documented for inverting glycosyltransferases, and it is assumed to proceed via a single nucleophilic SN2 displacement[2,3]. However, the mechanism of retaining glycosyltransferases remains unclear. Recently, it has been assumed to involve a double nucleophilic displacement with the formation of a covalent glycosyl-enzyme intermediate. In this case, the participation of two catalytic residues has been widely accepted although the catalysis of only one base species was also reported[4]. As the existence of a covalent intermediate has not been evidenced so far, a single, front-side displacement SN*i* reaction can also be taken into account.

In particular, we are interested in the development of α3GalT inhibitors. Unlike most other mammals, humans and Old-World primates do not possess α3GalT activity, which is relevant for a hyperacute vascular rejection observed in pig-to-human xenotransplantations. A strategy to this point could be a pretreatment of pigs using the α3GalT specific inhibitor[5]. Different approaches have been used to design galactosyltransferase inhibitors; however, only a limited success has been achieved, in particular for α3GalT. The known synthetic inhibitors of GalT (Fig. 1) include analogues of both the transition state[6–8] and sugar nucleotide[9–12] although simple methylene diphosphonates were also identified[7] as new inhibitors of β4GalT. Iminocyclitol **1** (Fig. 1) was shown to be a noncompetitive inhibitor of α3GalT with a K_i of 75 (ref.[7]) or 70 μmol l^{-1} (ref.[8]) against UDP-Gal but it was also a very potent inhibitor of β-glycosidase[8]. If the simple motif of iminoalditol **1** was introduced into a more complex structure **2** (Fig. 1), the inhibition potency of **2** against α3GalT increased markedly giving K_i of 4.4 μmol l^{-1} whilst its activity against β-glycosidase was suppressed. The authors suggested that both **1** and **2** interact not only with the free enzyme but also with the enzyme–substrate complex[8]. This conclusion is important since conjugate **2** actually mimics the glycosyl donor and binding of such inhibitors requires generally the presence of a nucleotide moiety[11]. Thus, phosphono analogues **3**, **4** and **5** were found to be efficient inhibitors of β4GalT with a K_i of 165 (ref.[9]), 97 (ref.[10]) and 62 μmol l^{-1} (ref.[13]), respectively. More recently, inhibition activity of 2-(galactosylethyl)phosphonophosphate **6** was evaluated[14] towards bovine milk β4GalT displaying IC_{50} of 40 μmol l^{-1}. The finding that simple phosphonates[7] or (2,6-anhydro-1,3-dideoxy-D-*lyxo*-hept-2-enit-1-yl)phosphonate[15] are inhibitors of β4GalT prompted us to focus on α- and β-D-galactopyranosylphosphonic acids (**7** and **8**) and

(α-D-galactopyranosylmethyl)phosphonic acid (**9**) as structure scaffolds for the construction of UDP-Gal analogues with a modified diphosphate linkage.

Herein, we would like to report the synthesis of the required starting compounds **7**–**9**, which were also tested for their ability to inhibit either α3GalT or β4GalT.

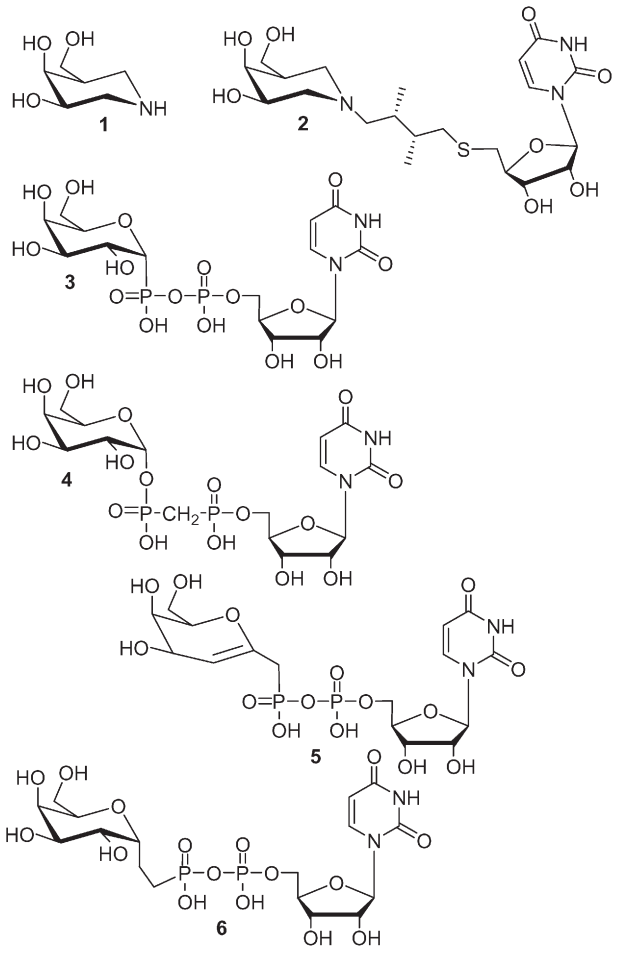
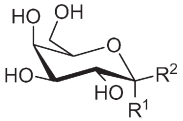


FIG. 1
Potent inhibitors of α3GalT or β4GalT

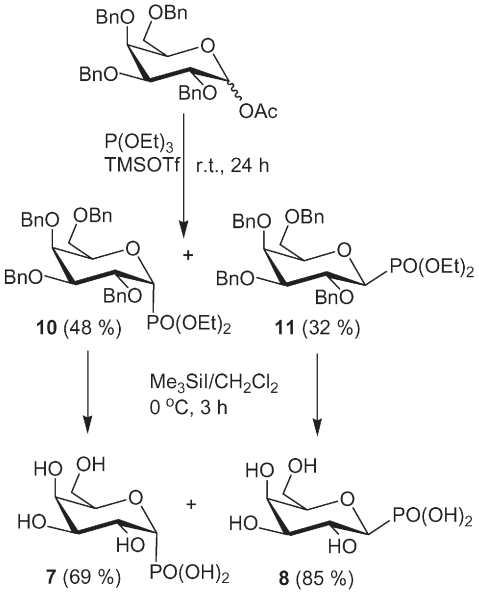
RESULTS AND DISCUSSION

Employing a Michaelis–Arbuzov reaction of 1-*O*-acetyl-2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranose with trimethyl phosphite, both galactopyranosylphosphonic acids **7** and **8** were already synthesized in the form of their per-*O*-benzyl dimethyl esters[16]. Free α-phosphonic acid **7** was obtained from the reaction of 1-*O*-acetyl-2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranose with tris(trimethylsilyl) phosphite followed by hydrolysis and



7, $R^1 = PO(OH)_2$, $R_2 = H$
8, $R^1 = H$, $R^2 = PO(OH)_2$
9, $R^1 = CH_2PO(OH)_2$, $R^2 = H$

catalytic hydrogenation[9]. In our hands, treatment of a mixture of anomers of 1-*O*-acetyl-2,3,4,6-tetra-*O*-benzyl-D-galactopyranose (α/β = 2) with triethyl phosphite and trimethylsilyl trifluoromethanesulfonate (TMSOTf) according to the described procedure[17] gave corresponding diethyl phosphonates **10** and **11** in excellent 80% yield (Scheme 1), which was higher than 58% reported for trimethyl phosphite[16]. It should be noted that the



SCHEME 1

yield decreased to 20% if triisopropyl phosphite was used instead of triethyl phosphite. According to the previous finding that the ratio of products arising from the Michaelis–Arbuzov reaction does not depend substantially on the anomeric configuration of an incoming glycosyl acetate[15], phosphonates **10** and **11** were obtained in the ratio α/β = 1.5 and were easily separated by silica gel chromatography. Finally, the protecting benzyl and ester groups were removed in one step using an excess of iodotrimethylsilane and the phosphonic acids **7** and **8** were purified by the precipitation from methanol by addition of diethyl ether[18]. The overall yields of **7** and **8** were 33 and 28%, respectively. The structure and stereochemistry of phosphonates **10** and **11** was confirmed by NMR spectrometry and by comparison of the data with those published for corresponding dimethyl esters[16]. According to the generally accepted rules for phosphonates[16,17,19], chemical shift of the anomeric axial proton (3.65 ppm for **11**) occurred at a higher field than the equatorial one (4.42 ppm for **10**). In ^{13}C NMR, the C-1 signals of **10** and **11** were found at 69.20 ppm with J(C-1,P) = 157.2 Hz and 75.16 ppm with J(C-1,P) = 171.0 Hz, respectively. The signal at a higher field with smaller coupling constant indicates the axial orientation of the P-substituent. The assignment of anomeric configurations of galactopyranosylphosphonic acids **7** and **8** was based on the same analysis; α-anomer **7** with axial phosphonic group showed the H-1 signal at 4.24 and C-1 signal at 73.15 ppm with J(C-1,P) = 151.1 Hz, while the respective NMR signals of β-anomer **8** were at 3.41 (H-1) and 76.70 ppm (C-1) with J(C-1,P) = 162.7 Hz. ^{13}C NMR data of phosphonic acid **7** were in accord with those previously described[9] if experimental error was taken into account, but ^{1}H chemical shifts were different. Therefore NOE contacts for α-anomer **7** were also measured (Fig. 2) and C-1 configuration was verified undoubtedly.

Two alternative routes were elaborated for the synthesis of novel (α-D-galactopyranosylmethyl)phosphonate **12**. The more straightforward strategy was the one step Horner–Emmons/Michael reaction[20] of sodium

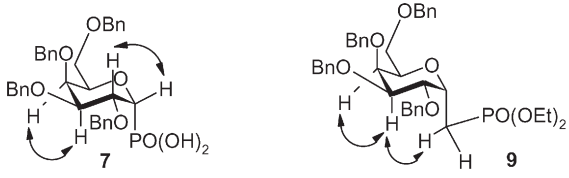
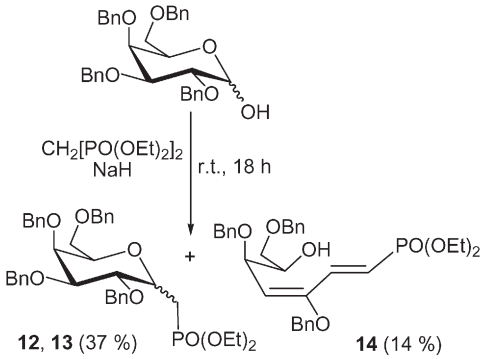


FIG. 2
NOE contacts in free acids **7** and **9**

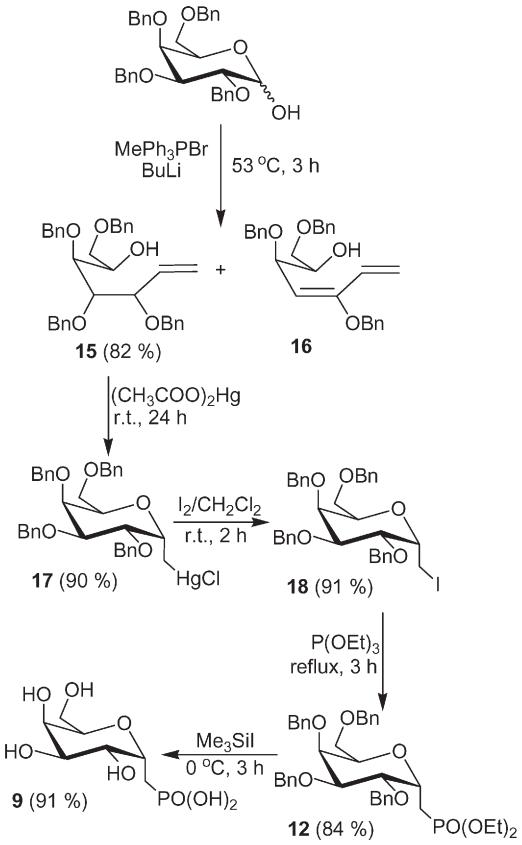
tetraethyl methylenediphosphonate with 2,3,4,5-tetra-*O*-benzyl-D-galactopyranose. The reaction, however, yielded a mixture of anomers of (galactopyranosylmethyl)phosphonates **12** and **13** accompanied by a considerable amount of the elimination product **14** even if the experimental conditions were optimized (Scheme 2). The best result was achieved with NaH in tetrahydrofuran at room temperature; the desired phosphonates **12** and **13** were isolated in 37% yield and the conjugated vinylphosphonate **14** in 12%



SCHEME 2

yield. If tetraisopropyl methylenediphosphonate was used instead of tetraethyl methylenediphosphonate, the reaction exclusively gave the elimination product under several conditions. The observation that 3-benzyloxy group can be easily cleaved during this reaction was already made when 2,3,4,-tetra-*O*-benzyl-α-D-glucopyranosyl bromide[21], 2,3,4-tri-*O*-benzyl-L-rhamnose[18,22] or 2,3,4-tri-*O*-benzyl-L-fucose[18] was investigated. Moreover, both phosphonates **12** and **13** were isolated as a mixture only and all attempts to separate them effectively failed. Thus, we decided to investigate the more general procedure involved the Michaelis–Arbuzov reaction as a key step in the reaction sequence (Scheme 3)[20]. In the first step, the Wittig reaction of 2,3,4,5-tetra-*O*-benzyl-D-galactopyranose with methyltriphenylphosphonium bromide afforded[21,23] galactoenitol **15** in excellent yield 81% if the reaction was performed in THF with butyllithium at 53 °C for 3 h. Under these optimised conditions, the formation[23] of an undesired side product, diene **16**, arising again due to low stability of an intermediate in alkaline solution, was not observed. However, the formation of diene **16** was dependent on the conditions used; the reaction performed at 60 °C gave 15% of **16** accompanied by the yield of the target product **15** decreasing to 26%. Mercury-mediated cyclization[23] of galactoenitol **15** provided stereoselectively the mercury derivative **17** isolated after chromatography

in a very high yield of 89%. Then, chlorine in **17** was substituted with iodine in a Finkelstein-like reaction and iodide **18** was obtained in 92% yield. The reaction of triethyl phosphite with iodide **18** led smoothly to α-phosphonate **12** under the conditions described above in 84% yield. The protecting groups in phosphonate **12** were removed in one step using an excess of iodotrimethylsilane. Free phosphonic acid **9** was isolated by precipitation from methanol by addition of diethyl ether[21]. Thus the overall yield of **9** prepared by this five-step procedure was 52% on a gram scale. The new compounds **9**, **12** and **15**–**18** were identified in the usual manner (optical rotation, MS, ^{1}H, ^{13}C and ^{31}P NMR; see Experimental) and the C-1 configuration in **9** was verified by NOE (Fig. 2).



SCHEME 3

Compounds **7**–**9** were tested as inhibitors of bovine α-1,3- and β-1,4-galactosyltransferases. α-Galactosylphosphonic acid **7** showed almost no inhibition against α3GalT; it slightly inhibited only β4GalT. β-Galactosylphosphonic acid **8** and (α-galactosylmethyl)phosphonic acid **9** were very poor inhibitors of both enzymes acting mostly in a noncompetitive mode. Of the phosphonic acids evaluated, β-galactosylphosphonic acid **8** displayed the highest activity against both enzymes (for β4GalT: IC_{50} = 17 mmol l^{-1} at 13.5 μmol l^{-1} of UDP-Gal).

We elaborated an efficient synthesis of phosphonic acids **7**–**9** in reasonable overall yields. These compounds will be converted into phosphono analogues of UDP-Gal as the nucleotide moiety was proved to be an important structural motif, which can significantly contribute to the binding of inhibitor in the active site of enzyme.

EXPERIMENTAL

Optical rotations were measured on a Jasco Model DIP-370 polarimeter at 25 °C and are given in 10^{-1} deg cm^2 g^{-1}. NMR spectra were recorded on a Bruker Avance DRX-500 (500.1 MHz for ^{1}H, 125,7 MHz for ^{13}C and 202.2 MHz for ^{31}P). Chemical shifts are expressed in parts per million downfield from Me_4Si or phosphoric acid for ^{31}P spectra. ^{1}H NMR chemical shifts (δ, ppm) and coupling constants (*J*, Hz) were obtained by first-order analysis of the spectra and decoupling experiments. ^{13}C NMR spectra were recorded using APT pulse sequence. Reactions were followed on TLC on silica gel (10–40 μm, Merck) or on cellulose. Column chromatography was carried out on silica gel (100–160 μm, Merck) and chromatographic systems are described in the text. Mass spectra were recorded on a Q-TOF Micro spectrometer (Waters-Micromass) with electrospray ionisation in negative mode. Elemental analysis was performed on a CHN-Perkin–Elmer-2400. Solvents were evaporated at 40 °C/2 kPa and compounds were dried at 13 Pa. The usual work-up refers to dilution with water, extraction with an organic solvent (diethyl ether or $CHCl_3$), washing the extract with water to neutral reaction, drying (anhydrous Na_2SO_4) and evaporation of the solvent. 2,3,4,5-Tetra-*O*-benzyl-D-galactopyranose was prepared from methyl β-D-galactopyranoside (Sigma–Aldrich, Czech Republic) by alkylation with benzyl chloride in the presence of KOH and subsequent acid hydrolysis (AcOH–H_2SO_4) of the glycosidic group[24]. Finally, acetylation with Ac_2O/pyridine (1:2 v/v) gave 1-*O*-acetyl-2,3,4,6-tetra-*O*-benzyl-D-galactopyranose (α/β = 2).

Enzyme Preparation

The genes of the bovine β4GalT (Δ114-402) and α3GalT (Δ25-368) were cloned individually into pVT-Bac vector. The co-transfection of Sf9 cells with the transfer vectors and the BaculoGold (BaculoGold, Becton Dickinson France) linear DNA[25] was done according to the manufacturer's instructions. Recombinant proteins are secreted from baculovirus-infected cells in the culture medium.

Inhibition Studies

The assay used to assess the inhibitory potential of compounds is based on the transfer of radiolabeled (^{14}C) galactose from the donor UDP-Gal (Amersham, 300 mCi/mmol) to the acceptor molecule. The unincorporated label was separated from the radiolabeled product Gal(^{14}C)-acceptor by adsorption onto AG 1X8 Resine (formate). The ratio of incorporated to total added radioactivity is proportional to the enzyme activity.

β4GalT Activity

Assays contained 10 mM D-galactose, 1 mM ATP, 10 mM $MnCl_2$, 50 mM cacodylate buffer (pH 7), 3.5 μM UDP-(^{14}C)Gal (106 000 cpm), UDP-Gal (1–200 μM), GlcNAc acceptor (25 mM), inhibitor (0.5–50 mM) and 5 μl of protein solution in a total assay volume of 0.05 ml. In other series of assays, the concentration of UDP-Gal was fixed (200 μM) and the concentrations of GlcNAc acceptor were varied (5–40 mM). Assays were performed at 37 °C for 10–30 min. Reactions were stopped by addition of 1 ml of a mixedbed resin AG 1X8 slurry (1:4 w/v in water). Samples were vortexed, centrifuged, and washed three times with 600 μl of water. Supernatants (4 × 600 μl) were pooled, and the radioactivity was measured by scintillation counting. Parallel control reactions were performed in the absence of acceptor. Each initial rate was obtained at a consumption of UDP-Gal lower than 20%.

α3GalT Activity

Assays contained 1 mM ATP, 10 mM $MnCl_2$, 50 mM cacodylate buffer (pH 7), 3.5 μM UDP-(^{14}C)Gal (106 000 cpm), UDP-Gal (1–200 μM), *N*-acetyllactosamine as acceptor (25 mM), inhibitor (0.5–50 mM) and 5 μl of protein solution in a total assay volume of 0.05 ml. In other series of assays, the concentration of UDP-Gal was fixed (200 μM) and the concentration of lactose acceptor was varied (5–40 mM). Reactions were then processed as indicated above for the β4GalT activity.

Diethyl (2,3,4,6-Tetra-*O*-benzyl-α- and β-D-galactopyranosyl)phosphonates (**10** and **11**)

Under argon, TMSOTf (1.1 ml, 6.0 mmol) was added dropwise during 10 min to a mixture of 1-*O*-acetyl-2,3,4,6-tetra-*O*-benzyl-D-galactopyranose (3.49 g, 6.0 mmol) and $P(OEt)_3$ (1.2 ml, 6.6 mmol) in CH_2Cl_2 (20 ml) at 0 °C. The solution was left to warm to room temperature and stirred until the starting material had disappeared (24 h). The reaction was quenched with water, diluted with AcOEt, the organic layer was extracted with saturated aqueous $NaHCO_3$, saturated aqueous NaCl and H_2O, dried over anhydrous Na_2SO_4 and concentrated. Chromatography of the residue on silica gel in the system AcOEt–CH_2Cl_2–hexane (1:1:1) afforded compound **10** (1.88 g, 2.9 mmol, 48%), $[\alpha]_D$ +51.7 (*c* 6.4, $CHCl_3$) and the β-anomer **11** (1.31 g, 2.0 mmol, 32%), $[\alpha]_D$ +34.3 (*c* 2.6, $CHCl_3$).

Compound **10**: ^{1}H NMR (500 MHz, $CDCl_3$): 7.31–7.24 m, 20 H (H-arom); 4.75–4.45 m, 8 H (C**H**$_2$Ph); 4.42 dd, 1 H, *J*(1,2) = 4.5, *J*(1,P) = 12.9 (H-1); 4.35–4.31 m, 1 H (H-5); 4.20–4.01 m, 7 H (CH_2O, H-2, H-3, H-4); 3.81 dd, 1 H, *J*(6′,5) = 7.7, *J*(gem) = 9.6 (H-6′); 3.58 dd, 1 H, *J*(6,5) = 4.2, *J*(gem) = 10.7 (H-6); 1.23–1.18 m, 6 H (CH_3). ^{13}C NMR: 138.36, 138.25, 138.15, 137.96 (quaternary C-arom); 127.47–128.33 (CH-arom); 75.29 (C-3); 75.26 (C-2); 75.20 (C-5); 73.83 (C-4); 73.83, 73.42, 73.11, 72.97 (CH_2Ph); 69.20 d, *J*(C,P) = 157.2 (C-1); 67.35 (C-6); 62.93 d, *J*(C,P) = 6.4 (CH_2O); 62.06 d, *J*(C,P) = 6.4 (CH_2O); 16.33 d, *J*(C,P) = 5.8 (CH_3);

16.25 d, *J*(C,P) = 6.3 (CH_3). ^{31}P NMR: 21.71 (*J*(2,P) = 25.0). For $C_{38}H_{45}O_8P$ (660.8) calculated: 69.08% C, 6.86% H; found: 69.19% C, 6.98% H.

Compound **11**: ^{1}H NMR: 7.34–7.25 m, 20 H (H-arom); 4.96 d, 1 H, *J*(gem) = 11.6 (CH_2Ph); 4.93 d, 1 H, *J*(gem) = 10.3 (CH_2Ph); 4.86 d, 1 H, *J*(gem) = 10.2 (CH_2Ph); 4.74 d, 1 H, *J*(gem) = 11.7 (CH_2Ph); 4.70 d, 1 H, *J*(gem) = 11.7 (CH_2Ph); 4.58 d, 1 H, *J*(gem) = 11.5 (CH_2Ph); 4.45 d, 1 H, *J*(gem) = 11.9 (CH_2Ph); 4.41 d, 1 H, *J*(gem) = 11.8 (CH_2Ph); 4.23 ddd, 1 H, 4.96 d, 1 H, *J*(2,1) = *J*(2,3) = *J*(2,1) = *J*(2,P) = 10.0 (H-2); 4.19–4.07 m, 4 H (CH_2O); 3.97 bs, 1 H (H-4); 3.65 dd, 1 H, *J*(1,2) = *J*(1,P) = 9.8 (H-1); 3.62–3.52 m, 4 H (H-3, H-6′, H-6, H-5); 1.26–1.19 m, 6 H (CH_3). ^{13}C NMR: 138.69, 138.43, 138.14, 137.76 (quaternary C-arom); 128.41–127.42 (CH-arom); 84.66 d, *J*(C,P) = 17.6 (C-3 or C-5); 78.78 d, *J*(C,P) = 16.6 (C-3 or C-5); 75.34 (C-2); 75.19 (CH_2Ph); 75.16 d, *J*(C,P) = 171.0 (C-1); 74.44 (CH_2Ph); 73.54 (C-4); 73.47, 72.44 (CH_2Ph); 68.64 (C-6); 63.17 d, *J*(C,P) = 6.4 (OCH_2); 62.42 d, *J*(C,P) = 6.4 (OCH_2); 16.20 d, *J*(C,P) = 6.4 (CH_3); 16.31 d, *J*(C,P) = 6.4 (CH_3). ^{31}P NMR: 20.41. For $C_{38}H_{45}O_8P$ (660.8) calculated: 69.08%C, 6.86% H; found: 69.25% C, 7.02% H.

α-D-Galactopyranosylphosphonic Acid (**7**)

Under argon, a solution of **10** (1.81 g, 2.7 mmol) in CH_2Cl_2 (10 ml) was cooled to 0 °C and Me_3SiI (2.5 ml, 18.4 mmol) was added. After 3 h, the solvent was removed under diminished pressure; the brown residue was dissolved in a few ml of MeOH and precipitated by addition of diethyl ether affording hygroscopic amorphous **7** (0.45 g, 1.84 mmol, 69%), $[\alpha]_D$ +58.1 (*c* 0.4, H_2O). ^{1}H NMR (D_2O): 3.63 dd, 1 H, *J*(5,6a) = 4.1, *J*(6a,6b) = 12.0 (H-6a); 3.75 dd, 1 H, *J*(5,6b) = 7.9 (H-6b); 3.98 bs, 1 H (H-4); 4.01–4.04 m, 1 H (H-5); 4.06 bs, 1 H (H-3); 4.11 m, 1 H (H-2); 4.24 dd, *J*(1,2) = 6.2, *J*(1,P) = 11.0 (H-1). ^{13}C NMR: 61.57 (C-6); 68.52 (C-2); 69.19 (C-4); 71.02 (C-3); 73.13 d, *J*(C,P) = 151.1 (C-1); 77.30 d, *J*(C,P) = 3.0 (C-5); ^{31}P NMR: 19.16. HR-MS: for $C_6H_{12}O_8P$ $(M - H)^-$ calculated: 243.0210, found: 243.0350.

β-D-Galactopyranosylphosphonic Acid (**8**)

Treatment of **11** (0.97 g, 1.5 mmol) in CH_2Cl_2 (8 ml) with Me_3SiI (1.8 ml, 13.2 mmol), as described for **7**, afforded hygroscopic amorphous **8** (0.31 g, 1.27 mmol, 85%), $[\alpha]_D$ –75.8 (*c* 0.5, H_2O). ^{1}H NMR (D_2O): 3.88 d, 1 H, *J*(4,3) = 3.1 (H-4); 3.81 ddd, 1 H, *J*(2,3) = *J*(1,2) = *J*(2,P) = 9.8 (H-2); 3.71 dd, 1 H, *J*(6′,5) = 8.4, *J*(gem) = 11.6 (H-6′); 3.63–3.54 m, 3 H (H-3, H-5, H-6); 3.41 dd, 1 H, *J*(1,2) = *J*(1,P) = 9.8 (H-1). ^{13}C NMR: 81.48 d, *J*(C,P) = 14.5 (C-5); 76.70 d, *J*(C,P) = 162.7 (C-1); 75.23 d, *J*(C,P) = 16.2 (C-3); 70.28 (C-4); 68.58 (C-2); 62.70 (C-6). ^{31}P NMR: 17.77. HR-MS: for $C_6H_{12}O_8P$ $(M - H)^-$ calculated 243.0210, found: 243.0532. MS-ESI: 243 $(M - H)^-$, 225 $(M - H - H_2O)^-$.

Diethyl [(2,3,4,6-Tetra-*O*-benzyl-α- and β-D-galactopyranosyl)methyl]-phosphonates (**12** and **13**)

To a suspension of NaH (1 g, 41.7 mmol) in THF (50 ml), at 0 °C under argon, $CH_2[PO(OEt)_2]_2$ (7 ml, 28.2 mmol) was slowly added. The clear solution obtained was stirred at 0 °C for 30 min. Then a solution of 2,3,4,6-tetra-*O*-benzyl-D-galactopyranose (1.60 g, 2.9 mmol) in THF (40 ml) was added. The solution was warm to room temperature and stirred for 18 h. The mixture was then washed with saturated aqueous NH_4Cl and the water layer extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4 and concen-

trated. The residue was chromatographed on a column of silica gel in the system petroleum ether–AcOEt (1:4). Phosphonates **12** and **13** (0.74 g, 37%) were obtained as an inseparable mixture followed by diethyl [(1*E*,3*E*,5*R*,6*R*)-3,5,7-tris(benzyloxy)-6-hydroxyhept-1,3-dien-1-yl]phosphonate (**14**) (0.20 g, 0.4 mmol, 14%). ^{1}H NMR (500 MHz, $CDCl_3$): 7.30–7.25 m, 6 H (H-arom); 6.95 dd, 1 H, *J*(6,7) = 17.1, *J*(6,P) = 21.8 (H-6); 6.02 dd, 1 H, *J*(7,6) = 17.4, *J*(7,P) = 18.5 (H-7); 5.45 d, 1 H, *J*(4,3) = 9.6 (H-4); 4.70 s, 2 H (C**H**$_2$Ph); 4.52–4.46 m, 3 H (C**H**$_2$Ph); 4.42 dd, 1 H, *J*(3,4) = 9.6, *J*(3,2) = 5.4 (H-3); 4.24 d, 1 H *J*(gem) = 11.7 (C**H**$_2$Ph); 4.14–4.05 m, 4 H (OCH_2); 3.70 dd, 1 H, *J*(2,3) = 10.4, *J*(2,1) = 5.2 (H-2); 3.47 d, 2 H, *J*(1,2) = 5.2 (H-1, H-1′); 1.35 t, 6 H, *J*(CH_3CH_2) = 7.0 (CH_3). ^{13}C NMR: 154.86 d, *J*(5,P) = 23.9 (C-5); 143.43 d, *J*(6,P) = 6.9 (C-6); 137.94 (2 × quaternary C-arom); 136.47 (quaternary C-arom); 128.56–127.57 (CH-arom); 122.78 (C-4); 116.21 d, *J*(7,P) = 190.7 (C-7); 74.35, 73.50 (CH_2Ph); 73.29 (C-3); 72.97 (C-2); 70.70 (CH_2Ph, C-1); 62.00 d, *J*(H,P) = 5.4 (OCH_2); 16.37 d, *J*(H,P) = 5.6 (CH_3). ^{31}P NMR: 18.87. For $C_{32}H_{39}O_7P$ (566.6) calculated: 67.83% C, 6.94% H; found: 68.00% C, 7.02% H.

3,4,5,7-Tetra-*O*-benzyl-1,2-dideoxy-D-*galacto*-hept-1-enitol (**15**)

Method A. To a suspension of $MePh_3PBr$ (2.34 g, 6.5 mmol) in THF (15 ml), under argon, butyllithium (1.6 M in hexane, 4.9 ml, 7.8 mmol) was added dropwise. After 30 min of vigorous stirring, a solution of 2,3,4,6-tetra-*O*-benzyl-D-galactopyranose (1.23 g, 2.3 mmol) in THF (12 ml) was added; the mixture was heated at 60 °C and stirred for 3 h. The organic layer was then washed several times with saturated aqueous Na_2CO_3 and the combined aqueous layers were extracted with diethyl ether. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated. The resulting syrup was subjected to silica gel chromatography in system petroleum ether–AcOEt (4:1) to afford the main product **15** (0.32 g, 0.6 mmol, 26%), $[\alpha]_D$ –6.8 (*c* 1.1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): 7.34–7.17 m, 20 H (H-arom); 5.87 ddd, 1 H, *J*(2,3) = 7.9 (H-2); 5.87 d, 1 H, *J*(1′,2) = 17.4 (H-1′); 5.29 d, 1 H, *J*(1,2) = 10.4 (H-1); 4.75 s, 2 H (C**H**$_2$Ph); 4.64 d, 1 H, *J*(gem) = 11.8 (C**H**$_2$Ph); 4.47 d, 1 H, *J*(gem) = 11.9 (C**H**$_2$Ph); 4.44–4.32 m, 4 H (C**H**$_2$Ph); 4.12 dd, 1 H, *J*(6,7) = *J*(6,7′) = 6.0 (H-6); 4.08 dd, 1 H, *J*(3,4) = 4.1 (H-3); 3.82–3.78 m, 2 H (H-4, H-5); 3.58–3.46 m, 2 H (H-7′, H-7); 3.09 s, 1 H (OH). ^{13}C NMR: 138.23, 138.17, 138.12, 138.01 (quaternary C-arom); 135.70 (C-2); 128.26–127.51 (CH-arom); 119.08 (C-1); 82.13 (C-4 or C-5); 80.75 (C-3); 76.63 (C-4 or C-5); 75.16, 73.11 (2 × CH_2Ph); 73.06 (C-7); 71.17, 70.30 (2 × CH_2Ph); 69.67 (C-6). For $C_{35}H_{38}O_5$ (538.7) calculated: 78.04% C, 7.11% H; found: 77.82.25% C, 6.98% H.

Then the elimination product (2*R*,3*S*,4*E*)-1,3,5-tris(benzyloxy)hepta-4,6-dien-2-ol (**16**) was isolated (0.16 g, 0.3 mmol, 15%), $[\alpha]_D$ –5.8 (*c* 0.5, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): 7.36–7.26 m, 15 H (H-arom); 6.24 dd, 1 H (H-6); 5.58 d, 1 H, *J*(7′,6) = 17.4 (H-7′); 5.28 d, 1 H, *J*(7,6) = 10.9 (H-7); 5.12 d, 1 H, *J*(4,3) = 9.7 (H-4); 4.78 d, 1 H, *J*(gem) = 11.3 (C**H**$_2$Ph); 4.74 d, 1 H, *J*(gem) = 11.3 (C**H**$_2$Ph); 4.54–4.48 m, 3 H (C**H**$_2$Ph); 4.42 dd, 1 H, *J*(3,2) = 6.0 (H-3); 4.27 d, 1 H, *J*(gem) = 11.7 (C**H**$_2$Ph); 3.73–3.69 m, 1 H (H-2); 3.51–3.45 m, 2 H (H-1′, H-1); 2.72 s, 1 H (OH). ^{13}C NMR: 156.79 (C-5); 138.34, 138.12, 137.10 (quaternary C-arom); 131.84 (C-6); 128.50–127.51 (CH-arom); 116.46 (C-7); 115.10 (C-4); 73.47 (CH_2Ph); 73.43 (C-3); 73.40 (CH_2Ph); 73.28 (C-2); 70.99 (C-1); 70.25 (CH_2Ph). For $C_{28}H_{30}O_4$ (430.6) calculated: 78.11% C, 7.02% H; found: 77.95% C, 7.22% H.

Method B. Using $MePh_3PBr$ (6.18 g, 17.3 mmol) in THF (45 ml), butyllithium (1.6 M in hexane, 13 ml, 20.8 mmol) and a solution of 2,3,4,6-tetra-*O*-benzyl-D-galactopyranose (3.32 g,

6.1 mmol) in THF (15 ml) and keeping reaction temperature in the procedure described above at 53 °C, compound **15** was prepared in 82% yield (2.69 g, 5.0 mmol).

2,6-Anhydro-1,3,4,5-tetra-*O*-benzyl-7-(chlorohydrargyrio)-7-deoxy-D-*glycero*-L-*galacto*-heptitol (**17**)

A solution of **15** (2.69 g, 5.0 mmol) in THF (20 ml) was stirred with mercuric acetate (1.9 g, 6.0 mmol) until the starting material disappeared (24 h). Then aqueous KCl (*c* 0.34 g/ml, 8 ml) was added and the mixture was stirred for 2 h. After the usual work-up, the resulting syrup was purified by chromatography on silica gel in petroleum ether–AcOEt (3:1) to afford **17** (3.44 g, 4.5 mmol, 90%), $[\alpha]_D$ +29.3 (*c* 1.2, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): 7.37–7.30 m, 20 H (H-arom); 4.76 d, 1 H, *J*(gem) = 11.8 (C**H**$_2$Ph); 4.68–4.48 m, 7 H (C**H**$_2$Ph); 4.38–4.32 m, 1 H (H-2); 4.19 bs, 1 H (H-6); 4.00–3.92 m, 2 H (H-5, H-7′); 3.80–3.73 m, 1 H (H-4); 3.67 dd, 1 H, *J*(7,6) = 3.2, *J*(gem) = 10.6 (H-7); 3.60–3.54 m, 1 H (H-3); 2.02 dd, 1 H, *J*(1′,2) = 7.5, *J*(gem) = 11.9 (H-1′); 1.55 dd, 1 H, *J*(1′,2) = 3.1 (H-1). ^{13}C NMR: 138.26, 138.20 (2×); 137.06 (quaternary C-arom); 128.86–127.54 (CH-arom); 76.32 (C-3); 75.13 (C-4); 73.83 (C-5); 73.43 (CH_2Ph); 73.20 (C-6); 73.09 (2×); 72.65 (CH_2Ph); 68.20 (C-2); 66.71 (C-7); 28.85 (C-1). For $C_{35}H_{37}ClHgO_5$ (773.7) calculated: 54.33% C, 4.82% H; found: 54.25% C, 4.14% H.

2,6-Anhydro-1,3,4,5-tetra-*O*-benzyl-7-deoxy-7-iodo-D-*glycero*-L-*galacto*-heptitol (**18**)

Compound **17** (2.61 g, 3.4 mmol) was dissolved in an iodine solution (0.86 g, 3.4 mmol) in CH_2Cl_2 (30 ml). The mixture was stirred for 2 h, washed with 10% aqueous Na_2SO_3 and then 5% aqueous KI, and worked up as usual to afford **18** as oil (2.06 g, 3.1 mmol, 91%). ^{1}H NMR (500 MHz, $CDCl_3$): 7.36–7.26 m, 20 H (H-arom); 4.70–4.52 m, 8 H (C**H**$_2$Ph); 4.13–4.08 m, 1 H (H-6); 4.07–4.04 m, 1 H (H-2); 4.02 dd, 1 H, *J*(5,4) = 2.8, *J*(5,6) = 3.8 (H-5); 3.94 dd, 1 H, *J*(3,4) = 6.4, *J*(3,2) = 3.6 (H-3); 3.91 dd, 1 H, *J*(1′,2) = 7.3, *J*(gem) = 10.6 (H-1′); 3.73 dd, 1 H, *J*(1,2) = 4.5 (H-1); 3.71 dd, 1 H (H-4); 3.40 dd, 1 H, *J*(7′,6) = 6.2, *J*(gem) = 10.4 (H-7′); 3.31 dd, 1 H, *J*(7,6) = 8.7 (H-7). ^{13}C NMR: 138.32, 138.28, 138.23, 137.84 (quaternary C-arom); 128.44–127.50 (CH-arom); 75.85, 75.77 (C-4, C-3); 73.70 (C-5); 73.48, 73.26 (CH_2Ph); 73.13 (C-2); 72.92 (2 × CH_2Ph); 71.63 (C-6); 66.83 (C-1); 2.72 (C-7). For $C_{35}H_{37}IO_5$ (664.6) calculated: 63.26% C, 5.61% H; found: 63.35% C, 5.72% H.

Diethyl [(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)methyl]phosphonate (**12**)

Under argon, the iodo derivative **18** (2.06 g, 3.1 mmol) was dissolved in $P(OEt)_3$ (4 ml, 23.0 mmol). The mixture was heated under reflux for 3 h. The excess of reagent was removed under reduced pressure and the residue was chromatographed on silica gel in petroleum ether–AcOEt (1:1) to afford **12** (1.75 g, 2.6 mmol, 84%), $[\alpha]_D$ +45.2 (*c* 0.5, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): 7.30–7.25 m, 20 H (H-arom); 4.69 d, 1 H, *J*(gem) = 11.8 (C**H**$_2$Ph); 4.67 d, 1 H, *J*(gem) = 12.1 (C**H**$_2$Ph); 4.61–4.43 m, 7 H (C**H**$_2$Ph, H-1); 4.08–4.02 m, 5 H (CH_2O, H-5); 4.01 dd, *J*(2,3) = 2.8, *J*(2,1) = 3.1 (H-2); 3.91–3.86 m, 1 H (H-4); 3.85–3.80 m, 1 H (H-6′); 3.71 dd, 1 H, *J*(6,5) = 5.3, *J*(gem) = 10.1 (H-6); 3.63 dd, 1 H, *J*(3,4) = 7.02 (H-3); 2.17–2.14 m, 1 H (C**H**$_2$P); 2.12–2.10 m, 1 H (C**H**$_2$P); 1.27 t, 1 H, *J*(CH_3,CH_2) = 7.1 (CH_3); 1.22 t, 1 H, *J*(CH_3,CH_2) = 7.1 (CH_3). ^{13}C NMR: 138.43, 138.35, 138.27, 138.07 (quaternary C-arom); 128.34–127.45 (CH-arom); 76.28, 76.20 (C-3, C-4); 73.70 (C-2, C-5); 73.28, 73.21, 73.11, 72.75 (C**H**$_2$Ph); 67.20 (C-1); 67.15 (C-6); 61.67 d, *J*(C,P) = 6.4 (CH_2O); 61.58 d,

J(C,P) = 6.4 (CH_2O); 24.79 d, J(C,P) = 142.5 ($\mathbf{C}H_2P$); 16.37 d, J(C,P) = 6.3 (CH_3). ^{31}P NMR: 29.72. For $C_{39}H_{47}O_8P$ (674.8) calculated: 69.48% C, 7.02% H; found: 69.68% C, 6.95% H.

(α-D-Galactopyranosylmethyl)phosphonic Acid (**9**)

A solution of **12** (1.47 g, 2.2 mmol) in CH_2Cl_2 (15 ml) under argon was cooled to 0 °C and Me_3SiI (1.7 ml, 12.5 mmol) was added. After 3 h the solvent was removed under reduced pressure, the brown residue was dissolved in a few ml of MeOH and precipitation by addition of ethyl methyl ketone afforded amorphous compound **9** (0.52 g, 2.0 mmol, 91%), $[\alpha]_D$ +86.9 (*c* 0.2, H_2O). ^{1}H NMR (500 MHz, D_2O): 4.30–4.22 m, 1 H (H-1); 3.87–3.78 m, 2 H (H-2, H-4); 3.70 dd, J(5,6) = J(5,4) = 5.1 (H-5); 3.60–3.55 m, 2 H (H-6′, H-3); 3.51 dd, 1 H, J(gem) = 11.5 (H-6); 2.15 dd, 1 H, J(gem) = 16.0, J(H,P) = 28.8 ($C\mathbf{H}_2P$); 1.95 dd, 1 H J(H,P) = 16.8 ($C\mathbf{H}_2P$). ^{13}C NMR: 72.93 (C-5); 72.39 (C-1); 70.48 (C-3); 69.67 (C-4); 68.71 d, J(C,P) = 12.3 (C-2); 61.65 (C-6); 23.85 d, J(C,P) = 138.2 ($\mathbf{C}H_2P$). ^{31}P NMR: 28.39. HR-MS: calculated for $C_7H_{14}O_8P$ (M – H): 257.0427, found: 257.0676.

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