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Note

Synthesis of phosphate derivatives related to the glycosidase inhibitor salacinol

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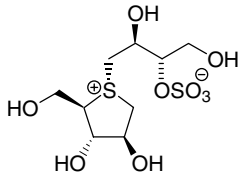
Abstract—The syntheses of polyhydroxylated imino- and anhydro thio-alditol compounds related to the naturally occurring glycosidase inhibitor, salacinol, containing a phosphate group in the side chain are described. The compounds lack hydroxyl groups on the acyclic side chain and are prototypes of the exact salacinol analogue. The synthetic strategy relies on the Mitsunobu reaction of *N*- and *S*-hydroxyalkyl derivatives of 2,3,5-tri-*O*-benzyl-1,4-dideoxy-1,4-imino-D-arabinitol and 1,4-anhydro-2,3,5-tri-*O*-benzyl-1-thio-D-arabinitol with dibenzyl phosphate to yield the corresponding protected heteroalditol phosphates. Screening of these compounds against recombinant human maltase glucoamylase (MGA), a critical intestinal glucosidase involved in the processing of oligosaccharides of glucose into glucose itself, shows that they are not effective inhibitors of MGA and demonstrates the importance of the hydroxyl and/or sulfate substituents present on the side chain for effective inhibition. The attempted synthesis of the exact analogue of salacinol by opening of cyclic phosphates is also described.

Keywords: Glycosidase inhibitors; Salacinol-related compounds; Synthesis; Phosphate derivatives; Maltase glucoamylase

Glycosidases play an important role in the metabolism of carbohydrates and in the processing of specific oligosaccharide structures on glycoproteins; the latter play important roles in intercellular recognition processes and their modification has been implicated in disease states such as cancer.[1–7] Inhibitors of glycosidases[8] have been attractive target compounds for synthetic chemists and biochemists, not only because they serve as useful biological tools for studying the biological functions of oligosaccharides,[9] but also because they have great potential as drugs to treat a variety of carbohydrate-mediated diseases.[10]

Alkaloids mimicking the structures of monosaccharides are now believed to be widespread in plants and microorganisms, and these sugar mimics inhibit glycosidases because of their structural resemblance to the sugar moiety. It has been hypothesized that this strong binding is the outcome of electrostatic interactions of the positively charged, protonated nitrogen atom with carboxylate residues in the enzyme active-site.[11] The lead glycosidase inhibitors bearing a permanent positive charge in the form of cyclic sulfonium ions, and presumably also interacting with active-site carboxylate residues, are perhaps the naturally occurring compounds salacinol (**1**) and kotalanol (**2**).[12,13]



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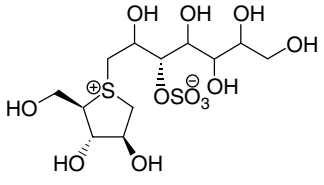

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The replacement of the carboxylic acid functional group in biologically important molecules by phosphoric acid continues to attract much interest in bioorganic and medicinal chemistry.[14] Much of the progress in this field has been associated with the phosphorus analogues of amino acids. The tetrahedral configuration, owing to

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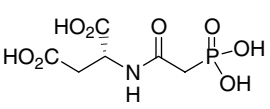
the presence of the phosphorus atom, allows these compounds to serve as stable analogues of the unstable tetrahedral intermediates formed in enzymatic processes.



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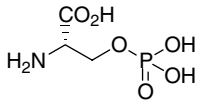

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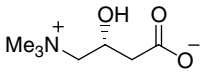

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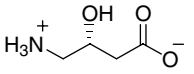

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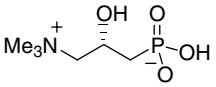

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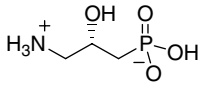

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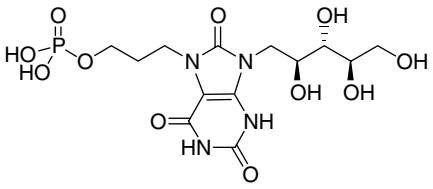

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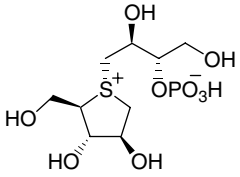

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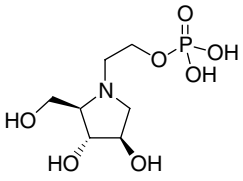

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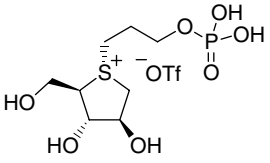

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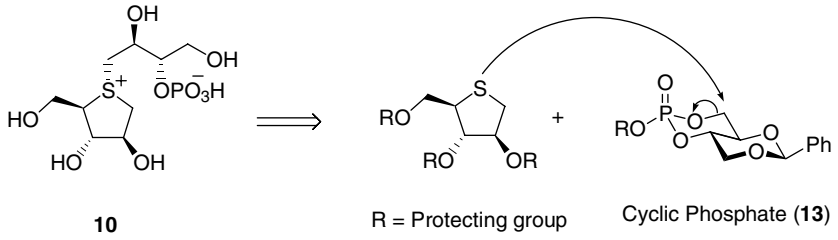
Many of these compounds act as enzyme inhibitors. Others are very interesting because they can help elucidate the functions of biologically active compounds in living organisms. For example, *N*-(phosphonoacetyl)-L-aspartate (**3**) and *O*-phosphate serine (**4**) were shown to be inhibitors of the carbonic anhydrase enzyme.[15] The involvement of carnitine (**5**) and γ-amino-β-hydroxybutyric acid (**6**) in the biology of mammalian cells and some important aspects of medicinal treatment has led to the development of their pharmacologically potent phosphate analogues (**7**, **8**).[16] The purinetrione bearing an alkyl phosphate (**9**) was tested and found to be an inhibitor of lumazine synthase.[17]

The structural modification of known inhibitors represents a promising approach in the search for new glycosidase inhibitors. It is of interest to synthesize phosphate derivatives of known glycosidase inhibitors containing other internal anions such as sulfates and carboxylates and study their inhibitory activities. Hence, we designed the phosphate analogues (**10**–**12**) of salacinol (**1**) as potential glycosidase inhibitors. These compounds will also serve to test the significance of sulfate and/or hydroxyl groups on inhibition of glycosidases.

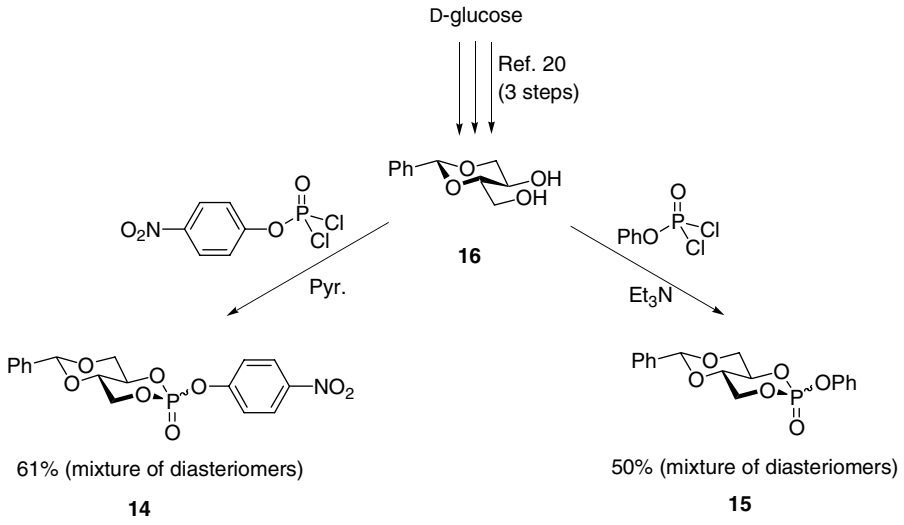
Compounds **10**–**12** could be synthesized by alkylating the anhydro-alditol derivatives at the ring heteroatom. The alkylation of a protected 1,4-anhydro-1-thio-D-arabinitol with the cyclic phosphate (**13**) derived from L-erythritol would afford compound **10** (Scheme 1). This potential reaction is patterned after our earlier syntheses of salacinol (**1**) and its analogues by opening of cyclic sulfates.[18–30]

In order to check the general reactivity of cyclic phosphates, the cyclic phosphates (**14**, **15**) derived from D-erythritol were synthesized, in turn, from the less expensive D-glucose using a similar protocol as for the synthesis of the corresponding cyclic sulfate (Scheme 2).[20] The diol (**16**) was prepared in three steps from D-glucose.[20] Subsequent treatment of the diol (**16**) with either *p*-nitrophenyl phosphorodichloridate or phenyl dichlorophosphate gave the D-cyclic phosphates **14** and **15**, respectively.

1,4-Anhydro-2,3,5-tri-*O*-benzyl-1-thio-D-arabinitol (**17**) was prepared from commercially available L-xylose in



Scheme 1.



Scheme 2.

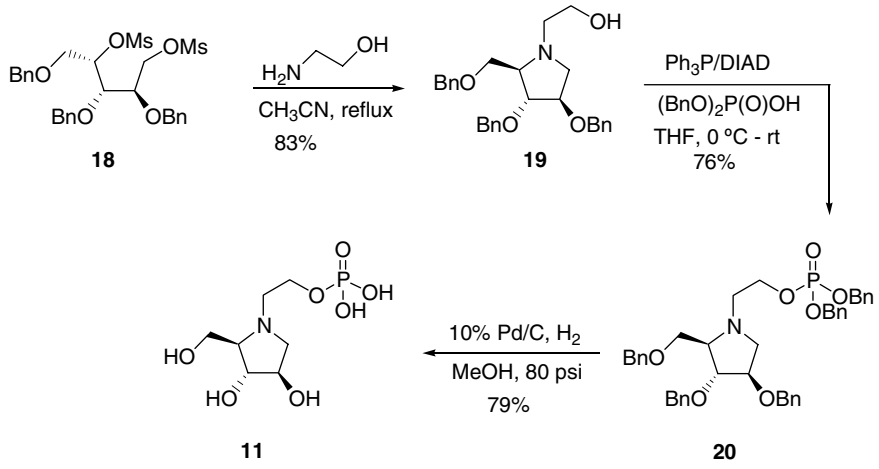
six steps using a procedure developed by Satoh et al.[31] Various conditions were tried to couple the D-cyclic phosphates (**14**, **15**) with either compound **17** or tetrahydrothiophene, but the reactions did not proceed as planned (Table 1). However, the reaction did proceed with thiourea as a nucleophile.[32] The principal difference between thiourea and the cyclic thioether appears to be the greater steric hindrance in the approach of the cyclic thioether. In addition, the sulfur atom in thiourea has a partial negative charge which makes it an even better nucleophile. Hence, it appears that the opening of the cyclic phosphates with the less nucleophilic thioether is not as facile as compared to their sulfate counterparts; thus, the syntheses using cyclic phosphates were abandoned.

We focused, therefore, on the synthesis of simpler prototype compounds related to salacinol (**1**) but containing a phosphate anion. Compounds **11** and **12** were synthesized by the Mitsunobu reaction of *N*- and *S*-hydroxyalkyl derivatives of heteroalditols with dibenzyl phosphate, followed by catalytic hydrogenolysis. The required dimesylated compound (**18**) was prepared from commercially available L-xylose in five steps following a literature procedure.[31] *N*-(2-Hydroxyethyl)-2,3,5-tri-

Table 1. Attempts at coupling **17** or tetrahydrothiophene with the D-cyclic phosphates (**14**, **15**)

Reactant 1	Reactant 2	Reaction conditions	Result
17	**15**	HFIP[a]/K_2CO_3, 2 days, 75 °C	Reaction did not proceed (starting materials were recovered)
17	**15**	Acetone, 2 days, 75 °C	
Tetrahydrothiophene	**15**	HFIP[a]/K_2CO_3, 4 days, 80 °C	
Tetrahydrothiophene	**15**	K_2CO_3, 5 days, 80 °C	
17	**14**	HFIP[a]/K_2CO_3, 4 days, 85 °C	
17	**14**	DMSO–CH_3CN (2:3) K_2CO_3, 5 days, 100 °C	
17	**14**	CH_3CN/K_2CO_3, 7 days, 105 °C	
Tetrahydrothiophene	**14**	HFIP[a]/K_2CO_3, 4 days, 80 °C	
Tetrahydrothiophene	**14**	DMF, 3 days, 98 °C	

[a] HFIP refers to 1,1,1,3,3,3-hexafluoroisopropanol.

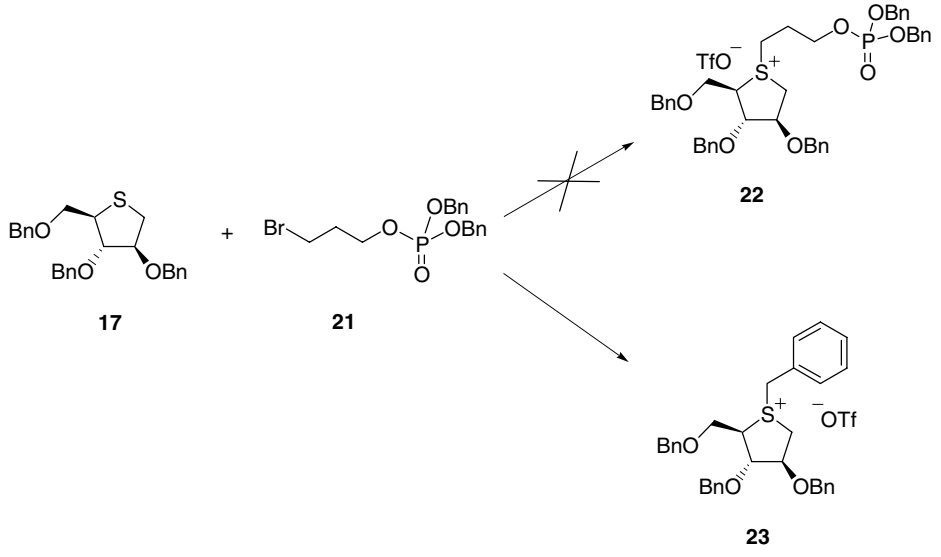


Scheme 3.

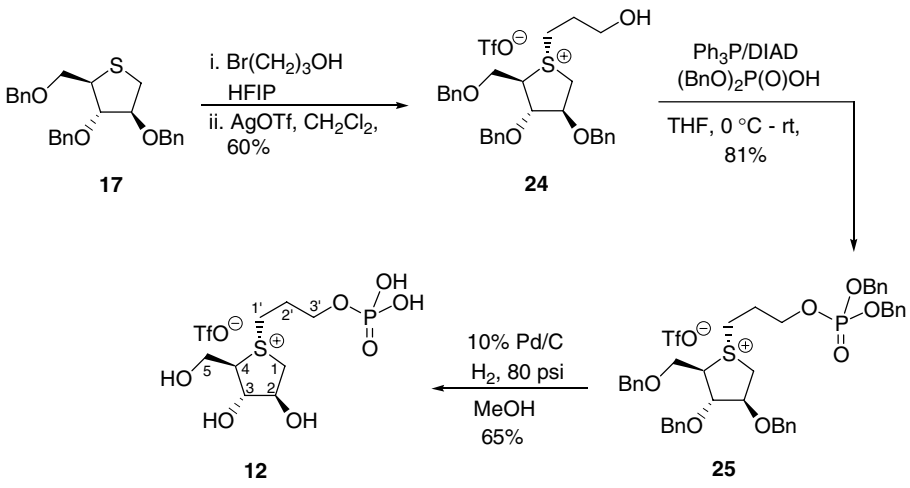
O-benzyl-1,4-dideoxy-1,4-imino-D-arabinitol (**19**) was synthesized in 83% yield from the reaction of dimesylate (**18**) with ethanolamine (Scheme 3). Treatment of compound **19** with dibenzyl phosphate under Mitsunobu reaction conditions furnished the corresponding phosphorylated derivative of *N*-(2-hydroxyethyl)-1,4-dideoxy-1,4-imino-D-arabinitol (**20**) in 76% yield. Finally, hydrogenolysis of **20** using 10% Pd on carbon afforded the desired product (**11**) as a white solid in 79% yield.

Initially, we anticipated that sulfonium salt **22** could be synthesized directly by coupling compound **17** and 3-bromopropyldibenzyl phosphate (**21**). After numerous attempts under a variety of reaction conditions, we failed to synthesize compound **22** directly since the nucleophilic sulfur atom attacked the benzyl group on the phosphate moiety to furnish the salt, 1,4-anhydro-2,3,5-tri-*O*-benzyl-1-[benzyl-episulfoniumylidene]-D-arabinitol triflate (**23**), as shown in Scheme 4.

The desired sulfonium salt **12** was synthesized from compound **17** as shown in Scheme 5. Reaction of **17** with 3-bromo-1-propanol in 1,1,1,3,3,3-hexafluoroisopropanol (HFIP), followed by treatment of the reaction mixture with silver triflate, afforded compound **24** in 60% yield. The stereochemistry at the sulfonium-ion center was assigned with the aid of a NOESY experiment which showed a correlation between H-4 and H-1′, suggesting that the propanol side chain and the C-4 substituent were *trans* to each other. Preparation of phosphate **25** from **24** was critical from a synthetic point of view as the mode of addition of reactants was found to be a determining factor for the efficient synthesis of **25**. Initially, a mixture of triphenylphosphine, compound **24**, and dibenzyl phosphate in anhydrous THF at 0 °C was stirred for 5–10 min, and then diisopropylazodicarboxylate (DIAD) was added dropwise, in accordance with the literature procedure.[33] However, the



Scheme 4.



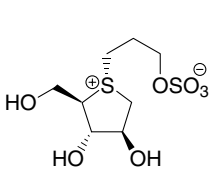
Scheme 5.

desired product was not obtained. We suspected that triphenylphosphine must have reacted with the sulfonium salt. A second set of conditions that involved initial stirring of triphenylphosphine and DIAD in dry THF for 10 min followed by the gradual addition of dibenzyl phosphate, then compound **24**, resulted in the consumption of 50% of the substrate. Finally, stirring triphenylphosphine and DIAD in dry THF at 0 °C for a short time (2–3 min), followed by the immediate addition of dibenzyl phosphate, followed, in turn, by the addition of a solution of **24** in anhydrous THF dropwise at 0 °C, with subsequent stirring at room temperature for 6 h furnished the desired product **25** in 81% yield. Debenzylation was carried out by hydrogenolysis using 10% Pd on carbon to obtain the thioalditol phosphate (**12**) as a viscous oil in 65% yield. The stereochemistry at the sulfonium center was assigned with the aid of a NOESY experiment, which showed a correlation between H-4 and H-1′, suggesting that the side chain and the C-4 substituent were *trans* to each other.

As a final point of interest, we comment on the screening of the compounds synthesized in this study against recombinant human maltase glucoamylase (MGA), a critical intestinal glucosidase involved in the processing of oligosaccharides of glucose into glucose itself. Compounds **11** and **12** were not effective inhibitors of MGA, whereas salacinol **1** inhibited this enzyme with a K_i value of 0.19 μM.[34] Hence, it appears that hydroxyl and/or sulfate substituents present on the side chain are critical for activity.

It is of interest to note that when a phosphate group was replaced by a sulfate in *myo*-inositol 1,4,5-trisphosphate, the resulting compound had no affinity for the IP_3 receptor or could not induce platelet aggregation.[35] A similar loss of biological activity was observed when an essential sulfate group in the antithrombin III binding motif of heparin at the non-reducing glucosamine was replaced by a phosphate group.[36] The same group later looked at a similar replacement in inhibitors of *myo*-inositol-1-phosphatase and suggested that the key differences between sulfate and phosphate groups are that they are present as mono-anionic and di-anionic entities, respectively.[37] The difference in the anionic charges of sulfate and phosphate groups might be one of the factors accounting for the low activities of compounds **11** and **12** against MGA.

Recently, Muraoka et al. reported the synthesis and inhibitory activity of compound **26**, which lacks a hydroxyl group at C-2′ and a hydroxymethyl moiety at C-3′, against intestinal α-glucosidases.[38] Compound **26** was not an effective inhibitor of this enzyme. This suggests that both the hydroxyl group at C-2′ and the hydroxymethyl group at C-3′ are essential for α-glucosidase inhibitory activity. In addition, Tanabe et al. concluded that the internal sulfate counterion is not necessary for activity since the de-*O*-sulfonated analogues **27** and **28** showed almost equal inhibitory activities against intestinal α-glucosidases when compared to salacinol (**1**).[39] Thus, it is likely that an exact phosphate analogue of salacinol (**1**) will be required in order to provide a definite answer as to whether the presence of a phosphate moiety or the lack of the hydroxyl group at C-2′ and hydroxymethyl moiety at C-3′ is the cause of the low activities of compounds **11** and **12** against MGA.



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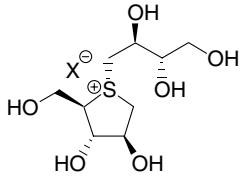

27: X=CH_3OSO_3
28: X=Cl

1. Experimental

1.1. General methods

Optical rotations were measured at 23 °C and reported in deg dm^{-1} g^{-1} cm^3. ^1H and ^{13}C NMR spectra were recorded with frequencies of 500 and 125 MHz, respectively. All assignments were confirmed with the aid of two-dimensional ^1H, ^1H (gCOSY) and ^1H, ^{13}C (gHMQC) experiments using standard Varian pulse programs. Processing of the spectra was performed with MestRec software. 1D-NOESY experiments were recorded at 295 K on a 500 MHz spectrometer. For each 1D-NOESY spectrum, 512 scans were acquired with a Q3 Gaussian Cascade pulse. A mixing time of 500 ms was used in the 1D-NOESY experiments. Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectra were obtained using 2,5-dihydroxybenzoic acid as a matrix. Analytical thin-layer chromatography (TLC) was performed on aluminum plates precoated with silica gel 60F-254 as the adsorbent. The developed plates were air-dried, exposed to UV light and/or sprayed with a solution containing 1% $Ce(SO_4)_2$ and 1.5% molybdic acid in 10% aqueous H_2SO_4, and heated. Column chromatography was performed with silica gel 60 (230–400 mesh).

1.2. *p*-Nitrophenyl-2,4-*O*-benzylidene-D-erythritol-1,3-cyclic phosphate (14)

A solution of diol (**16**) (1.0 g, 4.8 mmol) and Et_3N (2.5 mL, 4 equiv) in dry CH_2Cl_2 (15 mL) was added dropwise to a solution of *p*-nitrophenyl phosphorodichloridate (1.3 g, 1.1 equiv) in dry CH_2Cl_2 (15 mL) at 0 °C under N_2. The mixture was stirred at 0 °C for 90 min and then diluted with CH_2Cl_2 (20 mL). The mixture was washed with H_2O (3×10 mL), dried over anhydrous Na_2SO_4, and concentrated. The product was purified by flash chromatography (CH_2Cl_2–MeOH, 30:1 + 0.1% Et_3N) to give **14** as a mixture of diastereomers. Most of the more polar isomer **14a** was selectively recrystallized from hexanes and EtOAc giving faint pink crystals (0.63 g, 34%). The filtrate was recrystallized using hexanes and EtOAc to afford a mixture of **14a** and **14b** as a white solid (0.51 g, 27%).

Compound **14a**: $[\alpha]_D$ +60.0 (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.30–8.26 (2H, m, NO_2-Ar_{meta}), 7.35–7.48 (7H, m, Ar), 5.64 (1H, s, C*H*Ph), 4.58 (1H, ddd, $J_{1eq,2} = 5.0$ Hz, $J_{1ax,1eq} = 10.3$ Hz, $J_{1eq,P} = 23.7$ Hz, H-1eq), 4.52 (1H, dddd, $J_{3,P} = 0.9$ Hz, $J_{3,4eq} = 5.1$ Hz, $J_{3,4ax} = J_{2,3} = 9.2$ Hz, H-3), 4.45 (1H, dd, $J_{4ax,4eq} = 10.7$ Hz, H-4eq), 4.43 (1H, ddd, $J_{1ax,P} = 1.0$ Hz, $J_{1ax,2} = 10.7$ Hz H-1ax), 4.21 (1H, ddd, H-2), 3.56 (1H, dd, H-4ax); ^{13}C NMR ($CDCl_3$): δ 135.71–120.10 (12C, Ar), 102.35 (*C*HPh), 72.68 (d, 1C, $J_{2,P} = 6.3$ Hz, C-2), 72.46 (d, 1C, $J_{3,P} = 6.9$ Hz, C-3), 69.80 (d, 1C, $J_{1,P} = 8.7$ Hz, C-1), 68.40 (d, 1C, $J_{4,P} = 12.2$ Hz, C-4). Anal. Calcd for $C_{17}H_{16}NO_8P$: C, 51.92; H, 4.10. Found: C, 51.84; H, 4.30.

Compound **14b**: ^1H NMR ($CDCl_3$): δ 8.29–8.26 (2H, m, NO_2-Ar_{meta}), 7.46–7.36 (7H, m, Ar), 5.60 (1H, s, C*H*Ph), 4.65 (1H, dddd, $J_{3,P} = 0.9$ Hz, $J_{3,4eq} = 5.1$ Hz, $J_{3,4ax} = J_{2,3} = 10.1$ Hz, H-3), 4.57–4.47 (2H, m, H-1ax, H-1eq), 4.51 (1H, dd, $J_{4ax,4eq} = 9.9$ Hz, H-4eq), 4.20 (1H, ddd, $J_{1ax,2} = 9.7$ Hz, $J_{1eq,2} = 2.1$ Hz H-2), 3.86 (1H, dd, H-4ax); ^{13}C NMR ($CDCl_3$): δ 129.71–120.10 (12C, Ar), 102.22 (*C*HPh), 72.65 (d, 1C, $J_{2,P} = 7.9$ Hz, C-2), 70.51 (d, 1C, $J_{3,P} = 4.8$ Hz, C-3), 69.34 (d, 1C, $J_{1,P} = 7.7$ Hz, C-1), 68.59 (d, 1C, $J_{4,P} = 11.8$ Hz, C-4).

For the mixture of diastereomers: Anal. Calcd for $C_{17}H_{16}NO_8P$: C, 51.92; H, 4.10. Found: C, 51.85; H, 4.25.

1.3. Phenyl-2,4-*O*-benzylidene-D-erythritol-1,3-cyclic phosphate (15)

Phenyl dichlorophosphate (0.4 mL, 2.7 mmol) was added to a stirred solution of diol **16** (0.5 g, 2.4 mmol) in pyridine (5 mL). The mixture was stirred at ambient temperature for 18 h. The reaction was quenched with H_2O (1.5 mL) and the solvent was removed under high vacuum. The product was purified by flash chromatography (hexanes–EtOAc, 2:1) to give **15** as a mixture of diastereomers (0.4 g, 50%).

Compound **15a**: ^1H NMR ($CDCl_3$): δ 7.55–7.20 (10H, m, Ph), 5.65 (1H, s, C*H*Ph), 4.58–4.42 (3H, m, H-1eq, H-3, H-4eq), 4.43 (1H, dd, $J_{1ax,2} = J_{1ax,1eq} = 10.4$ Hz, H-1ax), 4.17 (1H, ddd, $J_{1eq,2} = 5.1$ Hz, $J_{2,3} = 9.8$ Hz, H-2), 3.93 (1H, dd, $J_{4ax,4eq} = J_{3,4ax} = 10.4$ Hz, H-4ax); ^{13}C NMR ($CDCl_3$): δ 135.98–128.75 (12C, Ph), 102.28 (*C*HPh), 72.90 (d, 1C, $J_{2,P} = 7.2$ Hz, C-2), 72.15 (d, 1C, $J_{3,P} = 6.4$ Hz, C-3), 69.38 (d, 1C, $J_{1,P} = 8.8$ Hz, C-1), 68.58 (d, 1C, $J_{4,P} = 12.1$ Hz, C-4).

Compound **15b**: ^1H NMR ($CDCl_3$): δ 7.55–7.20 (10H, m, Ph), 5.60 (1H, s, C*H*Ph), 4.60 (1H, dddd, $J_{3,P} = 0.9$ Hz, $J_{3,4eq} = 5.1$ Hz, $J_{3,4ax} = J_{2,3} = 9.7$ Hz, H-3) 4.58–4.47 (3H, m, H-1ax, H-1eq, H-4eq), 4.08 (1H, ddd, $J_{1eq,2} = 6.1$ Hz, $J_{1ax,2} = 9.8$ Hz, H-2), 3.80 (1H, dd, $J_{4ax,4eq} = 10.5$ Hz, H-4ax); ^{13}C NMR ($CDCl_3$): δ 130.01–128.75 (12C, Ph), 102.14 (*C*HPh), 73.03 (d, 1C, $J_{2,P} = 6.4$ Hz, C-2), 70.01 (d, 1C, $J_{3,P} = 5.6$ Hz, C-3), 68.92 (d, 1C, $J_{1,P} = 7.2$ Hz, C-1), 68.70 (d, 1C, $J_{4,P} = 11.3$ Hz, C-4).

For the mixture of diastereomers: Anal. Calcd for $C_{17}H_{17}O_6P$: C, 58.63; H, 4.92. Found: C, 58.31; H, 5.01.

1.4. *N*-(2-Hydroxyethyl)-2,3,5-tri-*O*-benzyl-1,4-dideoxy-1,4-imino-D-arabinitol (19)

Ethanolamine (2.1 mL, 34.6 mmol) was added to a stirred solution of dimesylate **18** (2.0 g, 3.5 mmol) in aceto-

nitrile (15 mL) and the mixture was heated to reflux for 20 h under N_2. The reaction mixture was concentrated in vacuum and the residue was dissolved in EtOAc (30 mL) and washed with water (3×15 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated. Purification by column chromatography (EtOAc–hexanes, 7:3) gave compound **19** as a colorless oil (1.28 g, 83%). $[\alpha]_D^{23}$ +1.0 (*c* 1.1, MeOH); ^{1}H NMR ($CDCl_3$): δ 7.31 (15H, m, $3 \times$ Ph), 4.50 (6H, ddd, $J = 12.0$ Hz, $3 \times CH_2Ph$), 3.99 (1H, dd, $J_{2,3} = 1.6$ Hz, $J_{2,1} = 3.7$ Hz, H-2), 3.89 (1H, br d, $J_{3,4} = 2.5$ Hz, H-3), 3.65–3.52 (2×2H, m, H-2′ and 5-H), 3.28 (1H, br d, $J_{1,2} = 3.6$ Hz, H-1a), 3.10–3.06 (1H, m, H-1′a), 2.91 (1H, br d, $J_{4,5} = 3.6$ Hz, H-4), 2.69 (1H, dd, $J_{1,2} = 5.3$ Hz, $J_{1a,1b} = 10.6$ Hz, H-1b), 2.61 (1H, d, $J_{1'a,1'b} = 12.6$ Hz, H-1′b); ^{13}C NMR ($CDCl_3$): δ 138.39, 138.32, 138.25 ($3 \times C_{ipso}$), 128.6–127.8 (15C, 3×5, Ph), 85.2 (C-3), 82.0 (C-2), 71.68, 71.39, 71.04 ($3 \times CH_2Ph$), 69.3 (C-4), 60.0 (C-2′), 57.5 (C-1), 57.3 (C-1′). MALDI-TOF-MS: *m*/*z* 448.04 $[M+H]^+$. Anal. Calcd for $C_{28}H_{33}NO_4$: C, 75.14; H, 7.43; N, 3.13. Found: C, 75.44; H, 7.10; N, 3.40.

1.5. *N*-(2-Dibenzylphosphorylethyl)-2,3,5-tri-*O*-benzyl-1,4-dideoxy-1,4-imino-D-arabinitol (20)

To a well-stirred mixture of triphenylphosphine (0.9 g, 3.4 mmol) and diisopropyl azodicarboxylate (DIAD) (0.65 mL, 3.4 mmol) in anhydrous THF (5 mL) at 0 °C, dibenzyl phosphate (0.94 g, 3.4 mmol) was added. After stirring the reaction mixture for 5 min at 0 °C, a solution of *N*-hydroxyethyl-2,3,5-tri-*O*-benzyl-1,4-dideoxy-1,4-imino-D-arabinitol (**19**) (1.0 g, 2.2 mmol) in THF (5 mL) was added dropwise at 0 °C under N_2 and the reaction mixture was stirred for 3 h at room temperature. After completion of the reaction, THF was removed in vacuum and the residue was purified by column chromatography (EtOAc–hexanes, 4:6) to give compound **20** as a colorless oil (1.2 g, 76%). $[\alpha]_D^{23}$ +26.0 (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$): δ 7.25–7.17 (25H, m, $5 \times$ Ph), 5.07–4.92 (m, 4H, $2 \times POCH_2Ph$), 4.42–4.29 (6H, m, $3 \times OCH_2Ph$), 4.10–3.96 (2H, m, H-2′), 3.82 (1H, d, $J_{2,3} = 5.0$ Hz, H-2), 3.73 (1H, d, $J_{3,4} = 3.7$ Hz, H-3), 3.42 (2H, ddd, $J_{5,4} = 6.0$ Hz, $J_{5a,5b} = 9.7$ Hz, H-5), 3.16 (1H, d, $J_{1a,1b} = 10.5$ Hz, H-1a), 3.07 (1H, td, $J_{1',2'} = 6.3$ Hz, $J_{1'a,1'b} = 12.9$ Hz, H-1′a), 2.72 (1H, dd, $J_{4,5} = 5.7$ Hz, H-4), 2.57 (2H, m, H-1b, H-1′b); ^{13}C NMR ($CDCl_3$): δ 138.53, 138.36, 138.31, 136.22, 138.18 ($5 \times C_{ipso}$), 128.7–127.7 ($5 \times$ Ph), 85.3 (C-3), 81.8 (C-2), 73.4–71.5 (CH_2Ph), 69.44 (d, $J_{C,P} = 5.4$ Hz, $POCH_2Ph$), 68.1 (d, $^2J_{C,P} = 5.7$ Hz, C-2′), 58.0 (C-1), 54.84 (d, $^3J_{C,P} = 6.6$ Hz, C-1′). MALDI-TOF-MS: *m*/*z* 708.3 $[M+H]^+$. Anal. Calcd for $C_{42}H_{46}NO_7P$: C, 71.27; H, 6.55; N, 1.98. Found: C, 71.09; H, 6.50; N, 1.92.

1.6. *N*-(2-Phosphorylethyl)-1,4-dideoxy-1,4-imino-D-arabinitol (11)

A solution of compound **20** (1.2 g, 1.7 mmol) in MeOH (5 mL) containing 10% palladium on carbon (0.5 g) was stirred under 80 psi of hydrogen at room temperature for 10 h. The mixture was diluted with 15% aqueous MeOH, filtered, and the solvent was removed in vacuum to give a white solid. The compound was purified further by column chromatography (H_2O–MeOH–H_2O, 10:3:1) to give **11** as a white solid (0.35 g, 79%). Mp 178–180 °C; $[\alpha]_D^{23}$ +2.1 (*c* 1.1, H_2O); ^{1}H NMR (D_2O): δ 4.36 (1H, td, $J_{2,3} = 2.0$ Hz, $J_{2,1} = 4.3$ Hz, H-2), 4.17 (2H, td, $J_{2',1'} = 4.8$ Hz, H-2′), 4.12 (1H, t, $J_{3,4} = 2.7$ Hz, H-3), 3.99 (2H, dq, $J_{5,4} = 6.1$ Hz, $J_{5a,5b} = 12.6$ Hz, H-5), 3.82 (1H, dd, $J_{1',2'} = 4.6$ Hz, H-1′a), 3.78 (1H, dd, $J_{1,2} = 5.0$ Hz, H-1a) 3.62 (1H, dd, $J_{1,2} = 5.0$ Hz, H-1b), 3.60 (1H, m, H-4), 3.51 (td, 1H, $J_{1',2'} = 4.5$ Hz, $J_{1'a,1'b} = 13.8$ Hz, H-1′b); ^{13}C NMR (D_2O): δ 75.8 (C-3), 75.6 (C-4), 73.8 (C-2), 60.2 (d, $^2J_{C-2',P} = 4.7$ Hz, C-2′), 59.4 (C-1), 58.3 (C-5), 57.01 (d, $^3J_{C-1',P} = 3.9$ Hz, C-1′). MALDI-TOF-MS: *m*/*z* 258.2 $[M+H]^+$. Anal. Calcd for $C_7H_{16}NO_7P$: C, 32.69; H, 6.27; N, 5.45. Found: C, 32.35; H, 6.16; N, 5.20.

1.7. 1,4-Anhydro-2,3,5-tri-*O*-benzyl-1-[3-hydroxypropyl-(*R*)-episulfoniumylidene]-D-arabinitol triflate (24)

The benzyl protected 4-thio-D-arabinitol (**17**) (2.0 g, 4.8 mmol) and 3-bromo-1-propanol (0.43 mL, 4.8 mmol) were dissolved in 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) (2 mL) and the mixture was stirred in a sealed tube at 92 °C for 26 h (prolonged heating of the reaction mixture resulted in the formation of side products). HFIP was removed and the residue was dissolved in CH_2Cl_2. Silver triflate (1.2 g, 4.8 mmol) was added and the mixture was stirred at ambient temperature for 2 h. The solvent was removed and the resulting residue was purified by column chromatography ($CHCl_3$–MeOH, 20:1) to give compound **24** as a colorless oil (1.82 g, 60%). $[\alpha]_D^{23}$ −6.0 (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$): δ 7.36–7.17 (15 H, m, $3 \times$ Ph), 4.60–4.47 (6H, m, $2 \times CH_2Ph$, *H*aCHPh, H-2), 4.40 (1H, d, $J_{A,B} = 11.5$ Hz, H_bCHPh 5.2 Hz, H-2), 4.22–4.18 (2H, m, H-2, H-1a), 3.97–3.90 (2H, ddd, $J_{5,4} = 5.0$ Hz, $J_{5a,5b} = 10$ Hz 5-H), 3.77 (1H, dd, $J_{3',2'} = 6.0$ Hz, H-3a′), 3.72–3.65 (3H, m, H-3′b, H-4, H-1′a), 3.622 (1H, dd, $J_{1,2} = 3.5$ Hz, $J_{1a,1b} = 13.0$ Hz, 1-Hb) 3.48 (1H, td, $J_{1'2'} = 6.5$ Hz, $J_{1'a,1'b} = 12.5$ Hz, H-1′b), 2.12–1.99 (2H, m, H-2′); ^{13}C NMR ($CDCl_3$): δ 136.94, 136.24, 136.11 ($3 \times C_{ipso}$), 129.0–128.1 ($3 \times$ Ph), 128.3-119.6 (CF_3 triflate), 83.0 (C-3), 82.5 (C-2), 73.9 (CH_2Ph), 72.6 (CH_2Ph), 72.1 (CH_2Ph), 67.1 (C-3′), 66.5 (C-5), 59.5 (C-4), 47.5 (C-1), 44.3 (C-1′), 28.7 (C-2′). MALDI-TOF-MS: *m*/*z* 479.03 $[M-Otf]^+$. Anal. Calcd for

$C_{30}H_{35}F_3O_7S_2$: C, 57.31; H, 5.61. Found: C, 57.23; H, 5.59.

1.8. 1,4-Anhydro-2,3,5-tri-*O*-benzyl-1-[3-dibenzylphosphorylpropyl-(*R*)-episulfoniumylidene]-D-arabinitol triflate (25)

A mixture of triphenylphosphine (1.5 g, 2.4 mmol) and diisopropyl azodicarboxylate (DIAD, 0.46 mL, 5.2 mmol) in anhydrous THF (5 mL) was stirred at 0 °C for 3 min and then dibenzyl phosphate (1.46 g, 5.2 mmol) was added. After stirring the reaction mixture for an additional 4 min at 0 °C, a solution of **24** (1.48 g, 2.4 mmol) in THF (5 mL) was added dropwise at 0 °C under N_2, and the reaction mixture was stirred for 3 h at room temperature. After completion of the reaction, THF was removed in vacuum and the residue was purified by column chromatography ($CHCl_3$–MeOH, 50:1) to give compound **25** as a colorless oil (1.7 g, 81%). $[\alpha]_D^{23}$ +3.0 (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$): δ 7.36–7.17 (15H, m, 3 × Ph), 4.94–4.89 (4H, m, 2 × POCH_2Ph), 4.60–4.47 (6H, m, 2 × CH_2Ph, 1 × H_aCHPh, H-2), 4.31 (1H, d, $J_{A,B} = 12.0$ Hz, H_bCHPh), 4.18 (1H, d, $J_{1a,1b} = 16.0$ Hz, H-1a), 4.06 (1H, br s, H-3), 4.03–3.98 (1H, m, H-3′a), 3.95 (3H, m, H3′b, H-5a, H-4), 3.65–3.50 (2H, m, H-5b, H-1′a), 3.48–3.30 (2H, m, H-1′b, H-1b), 2.12–1.99 (2H, m, H-2′); ^{13}C NMR ($CDCl_3$): δ 136.94, 136.24, 136.11 (3 × C_{ipso}), 129.0–128.1 (3 × Ph), 128.3–119.6 (*C*F_3 triflate), 83.0 (C-3), 82.5 (C-2), 73.9 (CH_2Ph), 72.6 (CH_2Ph), 72.1 (CH_2Ph), 69.93 (d, $J_{C,P} = 5.75$ Hz, PO*C*H_2Ph) 66.9 (C-4), 66.5 (C-5), 65.07 (d, $^2J_{C,P} = 5.8$ Hz, C-3′), 47.5 (C-1) 42.3 (C-1′), 26.9 (d, $^3J_{C,P} = 6.1$ Hz, C-2′). MALDI-TOF-MS: *m*/*z* 739.08 $[M-Otf]^+$. Anal. Calcd for $C_{44}H_{48}F_3O_{10}PS_2$: C, 59.45; H, 5.44. Found: C, 59.11; H, 5.43.

1.9. 1,4-Anhydro-1-[3-phosphorylpropyl-(*R*)-episulfoniumylidene]-D-arabinitol triflate (12)

A solution of compound **25** (1.0 g, 1.1 mmol) in MeOH (5 mL) containing 10% palladium on carbon (0.5 g) was stirred under 80 psi of hydrogen at room temperature for 16 h. The mixture was filtered, and the solvent was removed in vacuum to give the thio-alditol phosphate derivative as a viscous oil. The compound was purified further by column chromatography (H_2O–MeOH–H_2O, 10:3:1) to give **12** (0.25 g, 65%). $[\alpha]_D^{23}$ −2.0 (*c* 1.1, MeOH); ^{1}H NMR (D_2O): δ 4.70 (1H, ddd, $J_{2,3} = 3.5$ Hz, $J_{2,1b} = 7.0$ Hz, H-2), 4.39 (1H, dd, $J_{3,4} = 3.0$ Hz, H-3), 4.09 (1H, dd, $J_{5,4} = 4.5$ Hz, H-5a), 4.03 (2H, ddd, $J_{3',2'} = 6.0$ Hz, H-3′), 3.97–3.94 (1H, m, H-4), 3.87 (1H, dd, $J_{5,4} = 4.4$ Hz, H-5b), 3.89–3.76 (2H, m, H-1), 3.67–3.56 (2H, m, H-3′), 2.25–2.16 (2H, m, H-2′); ^{13}C NMR (D_2O): δ 119.7 (q, $J_{CF} = OCF_3$), 77.7 (C-3), 77.0 (C-2), 69.8 (C-4), 63.5 (d, C-3′ $^2J_{C-3',P} = 5.0$ Hz), 59.3 (C-5), 46.1 (C-1), 42.3 (C-1′), 26.4 (d, C-2′, $^3J_{C-2',P} = 4.5$ Hz). MALDI-TOF-MS: *m*/*z* 289.2 $[M-Otf]^+$. Anal. Calcd for $C_9H_{18}F_3O_{10}PS_2$: C, 24.66; H, 4.14. Found: C, 24.32; H, 3.98.

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References

1. Dwek, R. A. *Chem. Rev.* **1996**, *96*, 683–720.
2. Varki, A.; Cummings, R.; Esko, J.; Freeze, H.; Hart, G.; Marth, J. *Essentials of Glycobiology*; Cold Spring Harbor Laboratory Press: NY, 1999.
3. Jacob, G. S. *Curr. Opin. Struct. Biol.* **1995**, *5*, 605–611.
4. Holman, R. R.; Cull, C. A.; Turner, R. C. *Diabetes Care* **1999**, *22*, 960–964.
5. Asano, N. *Glycobiology* **2003**, *13*, 93–104.
6. Vasella, A.; Davies, G. J.; Bohm, M. *Curr. Opin. Chem. Biol.* **2002**, *6*, 619–629.
7. Heightman, T. D.; Vasella, A. T. *Angew. Chem., Int. Ed.* **1999**, *38*, 750–770.
8. de Melo, E. B.; Gomes, A. D.; Carvalho, I. *Tetrahedron* **2006**, *62*, 10277–10302.
9. Elbein, A. D. *Annu. Rev. Biochem.* **1987**, *56*, 497–534.
10. Hughes, A. B.; Rudge, A. J. *Nat. Prod. Rep.* **1994**, *11*, 135–162.
11. Stutz, A. E. *Iminosugars as Glycosidase Inhibitors:Nojirimycin and Beyond*; Wiley-VCH, Weinheim: NY, 1999.
12. Yoshikawa, M.; Murakami, T.; Shimada, H.; Matsuda, H.; Yamahara, J.; Tanabe, G.; Muraoka, O. *Tetrahedron Lett.* **1997**, *38*, 8367–8370.
13. Yoshikawa, M.; Murakami, T.; Yashiro, K.; Matsuda, H. *Chem. Pharm. Bull.* **1998**, *46*, 1339–1340.
14. Kafarski, P.; Lejczak, B. *Phosphorus, Sulfur, Silicon Relat. Elem.* **1991**, *63*, 193–215.
15. Winum, J. Y.; Innocenti, A.; Gagnard, V.; Montero, J. L.; Scozzafava, A.; Vullo, D.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1683–1686.
16. Tadeusiak, E. J. *Bioorg. Chem.* **2004**, *32*, 473–482.
17. Cushman, M.; Sambaiah, T.; Jin, G. Y.; Illarionov, B.; Fischer, M.; Bacher, A. *J. Org. Chem.* **2004**, *69*, 601–612.
18. Ghavami, A.; Johnston, B. D.; Jensen, M. T.; Svensson, B.; Pinto, B. M. *J. Am. Chem. Soc.* **2001**, *123*, 6268–6271.
19. Ghavami, A.; Johnston, B. D.; Maddess, M. D.; Chinapoo, S. M.; Jensen, M. T.; Svensson, B.; Pinto, B. M. *Can. J. Chem.* **2002**, *80*, 937–942.
20. Ghavami, A.; Johnston, B. D.; Pinto, B. M. *J. Org. Chem.* **2001**, *66*, 2312–2317.
21. Ghavami, A.; Sadalapure, K. S.; Johnston, B. D.; Lobera, M.; Snider, B. B.; Pinto, B. M. *Synlett* **2003**, 1259–1262.
22. Johnston, B. D.; Ghavami, A.; Jensen, M. T.; Svensson, B.; Pinto, B. M. *J. Am. Chem. Soc.* **2002**, *124*, 8245–8250.
23. Kumar, N. S.; Pinto, B. M. *Carbohydr. Res.* **2005**, *340*, 2612–2619.

24. Szczepina, M. G.; Johnston, B. D.; Yuan, Y.; Svensson, B.; Pinto, B. M. *J. Am. Chem. Soc.* **2004**, *126*, 12458–12469.
25. Nasi, R.; Pinto, B. M. *Carbohydr. Res.* **2006**, *341*, 2305–2311.
26. Nasi, R.; Sim, L.; Rose, D. R.; Pinto, B. M. *J. Org. Chem.* **2007**, *72*, 180–186.
27. Johnston, B. D.; Jensen, H. H.; Pinto, B. M. *J. Org. Chem.* **2006**, *71*, 1111–1118.
28. Liu, H.; Pinto, B. M. *J. Org. Chem.* **2005**, *70*, 753–755.
29. Liu, H.; Pinto, B. M. *Can. J. Chem.* **2006**, *84*, 497–505.
30. Liu, H.; Sim, L.; Rose, D. R.; Pinto, B. M. *J. Org. Chem.* **2006**, *71*, 3007–3013.
31. Satoh, H.; Yoshimura, Y.; Sakata, S.; Miura, S.; Machida, H.; Matsuda, A. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 989–992.
32. Kumar, N. S.; Pinto, B. M., unpublished.
33. Girotra, N. N.; Biftu, T.; Ponpipom, M. M.; Acton, J. J.; Alberts, A. W.; Bach, T. N.; Ball, R. G.; Bugianesi, R. L.; Parsons, W. H.; Chabala, J. C.; Davies, P.; Doebber, T. W.; Doherty, J.; Graham, D. W.; Hwang, S. B.; Kuo, C. H.; Lam, M. H.; Luell, S.; Macintyre, D. E.; Meurer, R.; Roberts, C. D.; Sahoo, S. P.; Wu, M. S. *J. Med. Chem.* **1992**, *35*, 3474–3482.
34. Rossi, E. J.; Sim, L.; Kuntz, D. A.; Hahn, D.; Johnston, B. D.; Ghavami, A.; Szczepina, M. G.; Kumar, N. S.; Sterchi, E. E.; Nichols, B. L.; Pinto, B. M.; Rose, D. R. *FEBS J.* **2006**, *273*, 2673–2683.
35. Westerduin, P.; Willems, H. M. A.; van Boeckel, C. A. A. *Carbohydr. Res.* **1992**, *234*, 131–140.
36. Vos, J. N.; Westerduin, P.; van Boeckel, C. A. A. *Bioorg. Med. Chem. Lett.* **1991**, *1*, 143–146.
37. van Steijn, A. M. P.; Willems, H. A. M.; de Boer, T.; Geurt, J. L. T.; van Boeckel, C. A. A. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 469–474.
38. Muraoka, O.; Yoshikai, K.; Takahashi, H.; Minematsu, T.; Lu, G.; Tanabe, G.; Wang, T.; Matsuda, H.; Yoshikawa, M. *Bioorg. Med. Chem.* **2006**, *14*, 500–509.
39. Tanabe, G.; Yoshikai, K.; Hatanaka, T.; Yamamoto, M.; Shao, Y.; Minematsu, T.; Muraoka, O.; Wang, T.; Matsuda, H.; Yoshikawa, M. *Bioorg. Med. Chem.* **2007**, *15*, 3926–3937.