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Synthesis and Antituberculosis Activity of *C*-Phosphonate Analogues of Decaprenolphosphoarabinose, a Key Intermediate in the Biosynthesis of Mycobacterial Arabinogalactan and Lipoarabinomannan

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Received July 26, 2002

The cell wall complex in mycobacteria, including the human pathogen *Mycobacterium tuberculosis*, is comprised in large part of two polysaccharides that contain a significant number of arabinofuranose residues. Both polysaccharides are assembled by a family of arabinosyltransferases that use decaprenolphosphoarabinose (**3**) as the donor species. In this paper, we describe the synthesis of a panel of *C*-phosphonate analogues of **3**, which were designed to inhibit these arabinosyltransferases and thus block the biosynthesis of mycobacterial cell wall polysaccharides. A number of routes were explored for the preparation of the targets. The successful approach involved the synthesis of a protected *C*-phosphonate allyl ester **16**, which was then coupled to an alkene via an olefin cross metathesis reaction. Subsequent reduction of the alkene with diimide and deprotection afforded the targets. Screening of these compounds in vitro against *Mycobacterium tuberculosis* revealed that one of the compounds, **15f**, possessed antituberculosis activity, with an MIC value of 3.13 μg/mL.

Introduction

Mycobacteria, including the human pathogens *Mycobacterium tuberculosis* and *Mycobacterium leprae*, possess an extraordinarily thick and complicated cell wall structure that provides the organism with a great deal of protection from its environment.[1] The viability of the organism is dependent upon its ability to synthesize an intact cell wall and, consequently, inhibitors of the enzymes that are involved in the biosynthesis of various cell wall components are potential antimycobacterial agents.[2] Indeed, some of the antibiotics currently used to treat tuberculosis (e.g., ethambutol and isoniazid) act by blocking cell wall assembly.[3]

Key structural components of the mycobacterial cell wall are two polysaccharides, arabinogalactan (AG) and lipoarabinomannan (LAM), which are unusual in that all of the arabinose and galactose residues exist in the furanose ring form. Glycoconjugates that contain galactofuranose and arabinofuranose are xenobiotic to mammals and, hence, the enzymes that are involved in the biosynthesis of these glycans are ideal targets for drug action.[4] Over the past few years, we have carried out synthetic and conformational investigations directed ultimately at the development of inhibitors of the glycosyltransferases that assemble the arabinan portions of AG and LAM.[5]

Our interest in mycobacterial arabinosyltransferases (AraT's) originated from studies that validated these enzymes as suitable targets for drug action. Previous reports[6] have demonstrated that ethambutol, an antibiotic used to treat tuberculosis, acts by inhibiting the AraT's involved in AG and LAM biosynthesis.

The structure of the arabinan in AG and LAM is essentially identical. The glycan core consists of a linear α-(1→5) linked chain of arabinofuranose residues, with periodic α-(1→3)-linked branch points from which additional α-(1→5) linked chains are attached. Attached to this core arabinan, at the nonreducing termini of each linear chain, is the hexasaccharide **1** (Chart 1). The biosynthesis of this arabinan is postulated to involve a family of AraT's that produce β-(1→2), α-(1→3), and α-(1→5) arabinofuranosyl linkages. A prototypical AraT-catalyzed reaction (Figure 1) involves the coupling of an oligosaccharide acceptor (e.g., **2**), with decaprenolphosphoarabinose (DPA, **3**)[7] to afford an elongated oligosaccharide, **4**.[8]

(1) (a) Lowary, T. L. Mycobacterial Cell Wall Components. In *Glycoscience: Chemistry and Chemical Biology*; Fraser-Reid, B., Tatsuta, K., Thiem, J., Eds.; Springer-Verlag: Berlin, Germany, 2001; pp 2005−2080. (b) Brennan, P. J.; Nikaido, H. *Annu. Rev. Biochem.* **1995**, *64*, 29.

(2) Barry, C. E., III *Biochem. Pharmacol.* **1997**, *54*, 1165.

(3) (a) Rastogi, N.; David, H. L. *Res. Microbiol.* **1993**, *144*, 133. (b) Blanchard, J. S. *Annu. Rev. Biochem.* **1996**, *65*, 215−239. (c) Young, D. B.; Duncan, K. *Annu. Rev. Microbiol.* **1995**, *49*, 641−673.

(4) (a) Besra, G. S.; Brennan, P. J. *J. Pharm. Pharmacol.* **1997**, *49* (Suppl. 1), 25. (b) Crick, D. C.; Mahapatra, S.; Brennan, P. J. *Glycobiology* **2001**, *11*, 107R.

(5) (a) Ayers, J. D.; Lowary, T. L.; Morehouse, C. B.; Besra, G. S. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 437. (b) Subramaniam, V.; Lowary, T. L. *Tetrahedron* **1999**, *55*, 5965. (c) Callam, C. S.; Lowary, T. L. *J. Org. Chem.* **2001**, *67*, 8961. (d) Yin, H.; D'Souza, F. W.; Lowary, T. L. *J. Org. Chem.* **2002**, *67*, 892. (e) McGurk, P.; Chang, G. X.; Lowary, T. L.; McNeil, M.; Field, R. A. *Tetrahedron Lett.* **2001**, *42*, 2231.

(6) (a) Mikusová, K.; Slayden, R. A.; Besra, G. S.; Brennan, P. J. *Antimicrob. Agents Chemother.* **1995**, *39*, 2484. (b) Deng, L.; Mikusová, K.; Robuck, K. G.; Scherman, M.; Brennan, P. J.; McNeil, M. R. *Antimicrob. Agents Chemother.* **1995**, *39*, 694. (c) Khoo, K.-H.; Douglas, E.; Azadi, P.; Inamine, J. M.; Besra, G. S.; Mikusová, K.; Brennan, P. J.; Chatterjee, D. *J. Biol. Chem.* **1996**, *271*, 28682−28690.

10.1021/jo026247r CCC: $22.00
Published on Web 11/08/2002

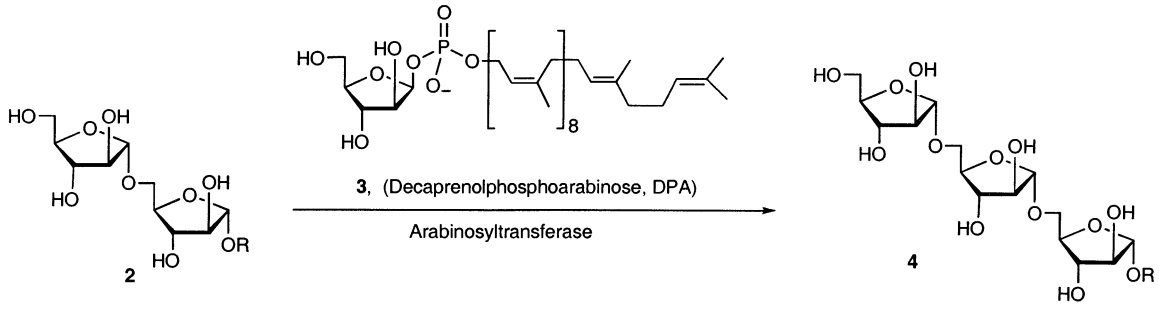
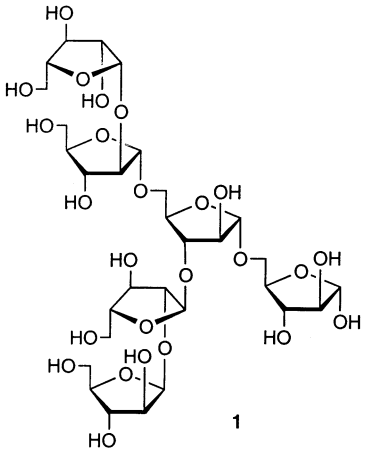


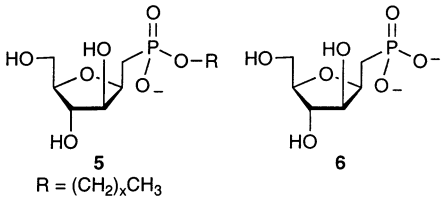
FIGURE 1. Prototypical arabinosyltransferase-catalyzed reaction.

CHART 1



In designing potential inhibitors of these AraT's, logical first choices are analogues of the two substrates recognized by the enzyme. The majority of our previous work in this area has focused on synthesizing analogues of the acceptor oligosaccharides.[5a−d] This approach has also been used widely in developing inhibitors that are specific for particular human glycosyltransferases.[9] Donor analogues have been less widely studied because a single glycosyl donor is usually a substrate for a number of glycosyltransferases, and these compounds are therefore expected to lack specificity for a particular enzyme. In bacterial systems, this lack of specificity with regard to the donor can be exploited. Because **3** is a substrate for more than one AraT, analogues of this compound would be expected to block a number of biosynthetic steps and, in turn, be especially potent antimycobacterials.

CHART 2



We describe here the synthesis of a panel of *C*-phosphonate-based DPA analogues. It was our expectation that replacement of the glycosidic oxygen in **3** with a methylene group would provide compounds (**5**, Chart 2) that would be bound by the AraT's, but that could not turn over. These *C*-phosphonates would therefore block the assembly of mycobacterial arabinan and thus prevent growth of the organism. Our goal was not to prepare compounds that contained the long isoprenoid chain present in **3**. Rather, we endeavored to synthesize molecules in which simple long-chain linear alkyl groups replace this lipid.

Results and Discussion

Initial Approaches. Although a number of *C*-phosphonate analogues of glycosyl phosphates (e.g., **6**, Chart 2) have been synthesized,[10] far fewer reports have described the preparation of the corresponding phosphonate ester derivatives (e.g., **5**).[11] We initially explored the possibility of synthesizing these compounds from iodide **7** (Figure 2A) via a route that involves the formation of an *H*-phosphonate,[12] esterification with the appropriate alcohol,[13] oxidation of the *H*-phosphonate,[14] and subsequent deprotection. Thus (Scheme 1), **7** was prepared[15] and then treated with bis(trimethylsilyl)phosphonite.[12] However, despite a number of attempts under different reaction conditions, only unreacted **7** was isolated following the reaction. We therefore explored an approach in which the carbon−phosphorus bond was installed via an Michaelis−Arbuzov reaction[16] (Figure 2B). The long-

(7) Biosynthesized from 5-phosphoribose pyrophosphate (pRpp): (a) Scherman, M. S.; Kalbe-Bournonville, L.; Bush, D.; Xin, Y.; Deng, L.; McNeil, M. *J. Biol. Chem*. **1996**, *271*, 29652. (b) Scherman, M.; Weston, A.; Duncan, K.; Whittington, A.; Upton, R.; Deng, L.; Comber, R.; Friedrich, J. E.; McNeil, M. *J. Bacteriol.* **1995**, *177*, 7125.

(8) Incorporation of arabinose into AG has been shown through the use of radiolabeled **3** and mycobacterial membrane extracts. In addition, incubation of small oligosaccharide substrates with **3**, in the presence of a mycobacterial membrane preparation, led to the formation of an oligosaccharide with additional β-(1→2) and α-(1→5) linkages (Lee, R. E.; Brennan, P. J.; Besra, G. S. *Glycobiology* **1997**, *7*, 1121). The lack of α-(1→3) linkages was attributed to either instability or absence of α-(1→3) AraT activity in the membrane preparation, or the possibility that this enzyme recognizes oligosaccharide substrates larger than those investigated. It is also conceivable that another activated donor (e.g., a sugar nucleotide) is used by this AraT. The presence of UDP-Ara*f* in mycobacteria has been reported (Singh, S.; Hogan S. E. *Microbios* **1994**, *77*, 217), but incorporation of this donor into arabinan has not been demonstrated.

(9) Qian, X.; Palcic, M. M. *Carbohydr. Chem. Biol.* **2000**, *3*, 293.

(10) Nicotra, F. Synthesis of Glycosyl Phosphate Mimics. In *Carbohydrate Mimics*; Chapleur, Y., Ed.; Wiley-VCH: Weinheim, Germany, 1998.

(11) (a) Brooks, G.; Edwards, P. D.; Hatto, J. D. I.; Smale, T. C. Southgate, R. *Tetrahedron* **1995**, *29*, 7999. (b) Qiao, L.; Vederas, J. C. *J. Org. Chem*. **1993**, *58*, 3480.

(12) (a) Boyd, E. A.; Regan, A. C.; James, K. *Tetrahedron Lett*. **1994**, *35*, 4223. (b) Boyd, E. A.; Regan, A. C.; James, K. *Tetrahedron Lett.* **1992**, *33*, 813.

(13) Malachowski, W. P.; Coward, J. K. *J. Org. Chem*. **1994**, *59*, 7625.

(14) Lindh, I.; Stawinski, J. *J. Org. Chem*. **1989**, *54*, 1338.

(15) Freeman, F.; Robarge, K. D. *Carbohydr. Res.* **1987**, *171*, 1.

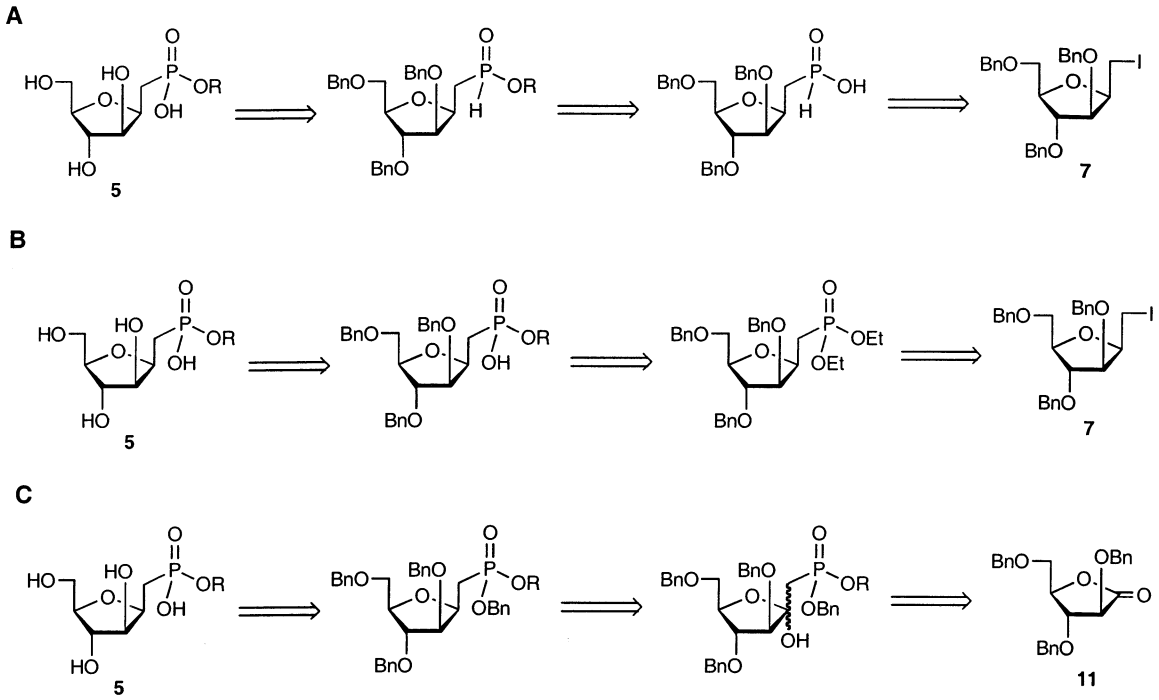
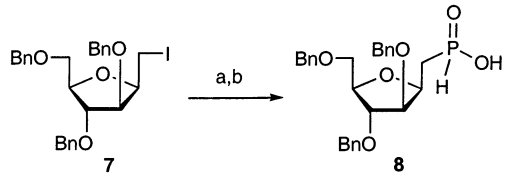


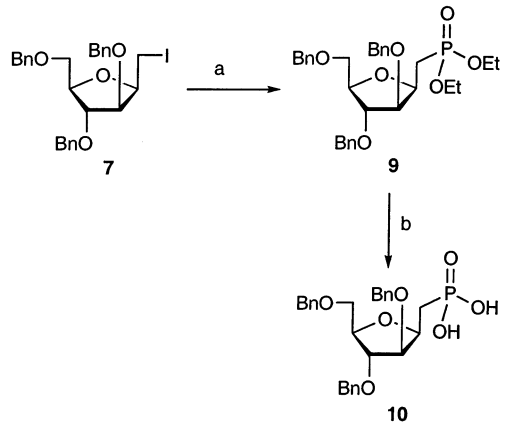
FIGURE 2. Retrosynthesis of **5**.

SCHEME 1[a]



[a] Conditions: (a) $(TMSO)_2PH$, CH_2Cl_2, 40 °C; (b) H_2O.

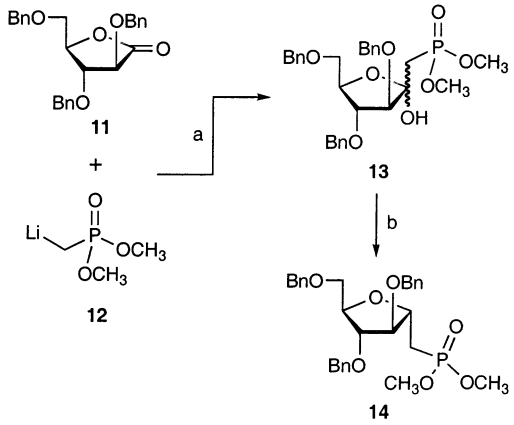
SCHEME 2[a]



[a] Conditions: (a) $(EtO)_3P$, 156 °C, 91%; (b) TMSBr or TMSI.

chain alkyl moiety was to be introduced via an esterification reaction following the selective cleavage of the ethyl groups. With this route in mind (Scheme 2), reaction of **7** with triethyl phosphite at 156 °C (reflux) provided the diethyl phosphonate derivative **9**[17] in 91% yield. Next, the conversion of **9** to **10** via the selective

SCHEME 3[a]



[a] Conditions: (a) THF, −78 °C, 93%; (b) TMSOTf, Et_3SiH, CH_2Cl_2, 95%.

deprotection of the ethyl groups using TMSI and TMSBr was explored. We were unsuccessful, however, in producing **10** in good yield as the cleavage of the ethyl groups was also accompanied by loss of one or more of the benzyl groups. It was extremely difficult to separate the reaction products and we therefore abandoned this approach.

We next explored a route starting from lactone **11**[18] (Figure 2C). To assess the viability of this approach (Scheme 3), **11** was reacted with **12**[19] to provide the adduct **13**[20] in 93% yield. Deoxygenation of **13** with triethylsilane and trimethylsilyltrifluoromethanesul-

(16) Bhattacharya, A. K.; Thyagarajan, G. *Chem. Rev.* **1981**, *81*, 415.

(17) Maryanoff, B. E.; Nortey, S. O.; Inners, R. R.; Campbell, S. A.; Reitz, A. B.; Liotta, D. *Carbohydr. Res.* **1987**, *171*, 259.

(18) Csuk, R.; Doerr, P. *J. Carbohydr. Chem.* **1995**, *14*, 35.

(19) Teulade, M. P.; Savignac, P.; Aboujaoude, E. E.; Collignon, N. *J. Organomet. Chem.* **1986**, *312*, 283.

(20) Nicotra, F.; Panza, L.; Russo, G.; Senaldi, A.; Burlini, N.; Tortora, P. *J. Chem. Soc., Chem. Commun.* **1990**, 1396.

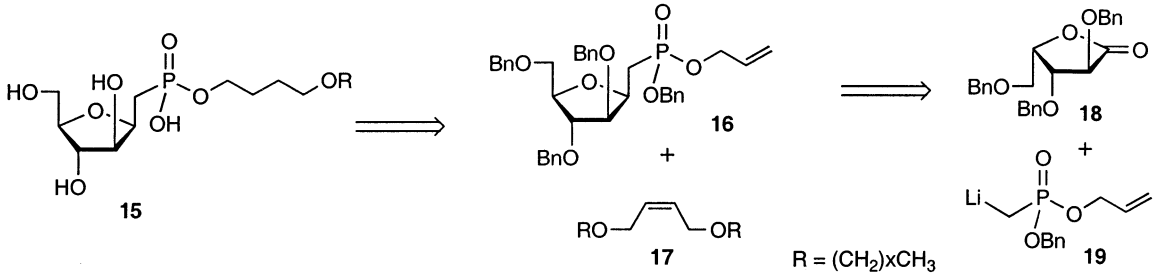
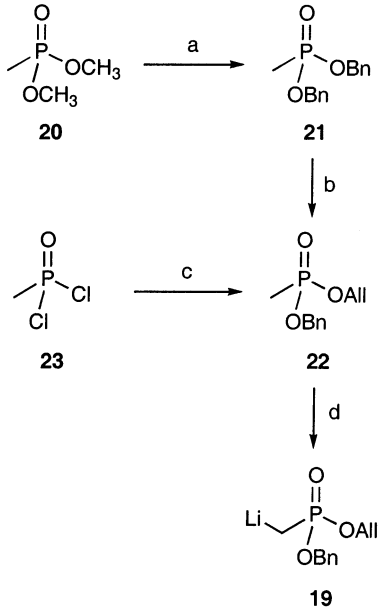


FIGURE 3. Retrosynthesis of **15**.

SCHEME 4[a]



[a] Conditions: (a) BnOAc, *t*-BuOK, THF, rt, 90%; (b) AllOAc, *t*-BuOK, THF, rt, 47%; (c) BnOH, pyridine, then AllOH 0 °C, 33%; (d) *n*-BuLi, THF, −78 °C.

fonate as the Lewis acid[21] provided a deoxygenated product in nearly quantitative yield. Unfortunately, the product produced from this reaction was shown to be **14**, which possessed the incorrect stereochemistry at the "anomeric" center.[22]

Having been unsuccessful in the synthesis of **5** via what we considered to be the most direct routes, a number of other approaches were explored, also without success. Therefore, we slightly modified the structure of the targets and developed a successful route for their synthesis. The retrosynthesis for this approach is provided in Figure 3. The modified targets (**15**) differ from **5** in that an oxygen atom is present in the long alkyl chain. We anticipated that this modification would not significantly influence the inhibitory potential of **15** relative to **5**. In this approach, the long-chain alkyl group was to be introduced via an olefin cross metathesis reaction[23] of **16** and **17**. Subsequent reduction of the

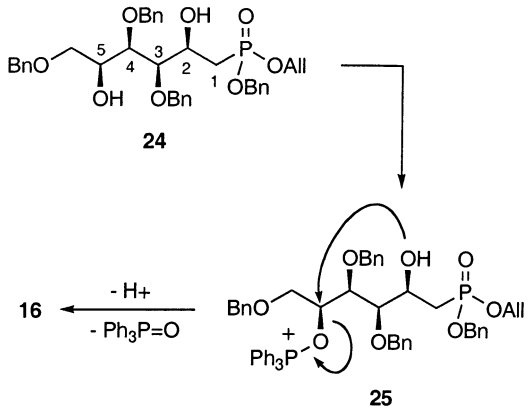


FIGURE 4. Proposed formation of **16** from **24** via Mitsunobu cyclization.

double bond and deprotection would afford the targets. Phosphonate **16** can be accessed from **19** and the protected L-xylose derivative **18**.[24]

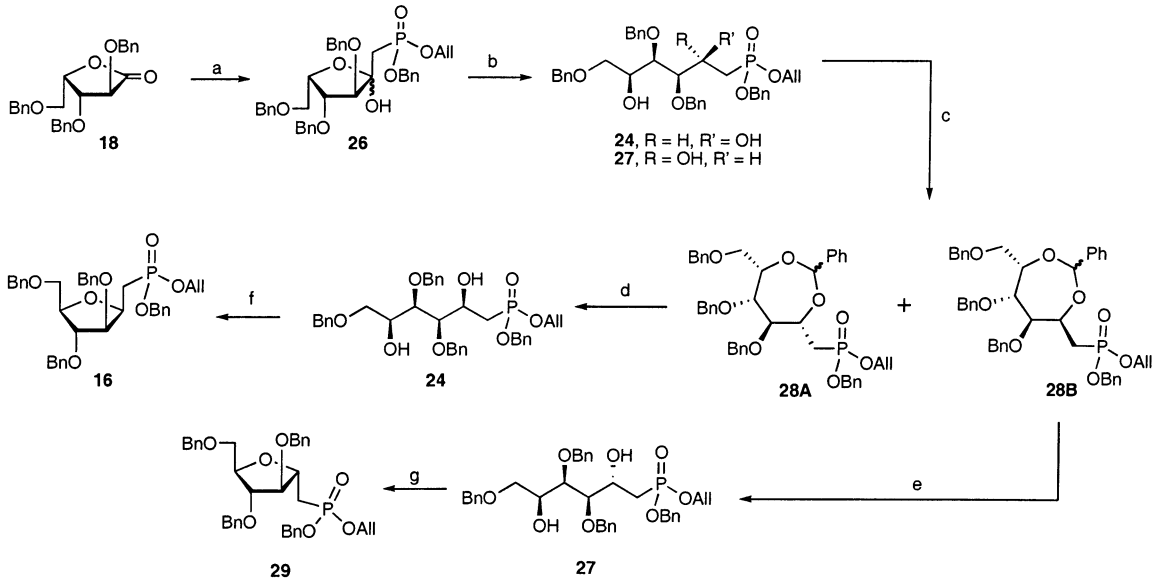
Preparation of Phosphonate 19. For the synthesis of **19** we employed methodology developed by Gagne and co-workers (Scheme 4).[25] Dimethyl methylphosphonate (**20**) was converted to the corresponding dibenzyl ester **21**[26] in 90% yield by reaction with benzyl acetate and potassium *tert*-butoxide. Subsequent transesterification of **21** with allyl acetate and potassium *tert*-butoxide under controlled conditions afforded **22** in 47% yield. The synthesis of **22** from the commercially available dichloride **23** was also possible, but the yield of the product was lower than the route from **20**. Lithiation of **22** with *n*-BuLi in THF afforded **19**.

Synthesis of 16 from 18 and 19. On the basis of previous investigations,[27] we envisioned that *C*-phosphonate **16** could be obtained via a Mitsunobu reaction of acyclic diol **24** (Figure 4). In this earlier study, diols of this general type were shown to undergo Mitsunobu cyclization with inversion of the stereochemistry at C-5, presumably via formation of a phosphonium ion such as **25**.

The synthesis of **16** is illustrated in Scheme 5. Reaction of the L-xylose derivative **18**[24] with **19** afforded a 90% yield of ketose **26**, which was subsequently reduced, in 87% yield, with sodium borohydride. This reaction was only marginally stereoselective affording the two possible diastereomeric products (**24** and **27**) as a 2:1 mixture, which was impossible to separate. Other reducing agents

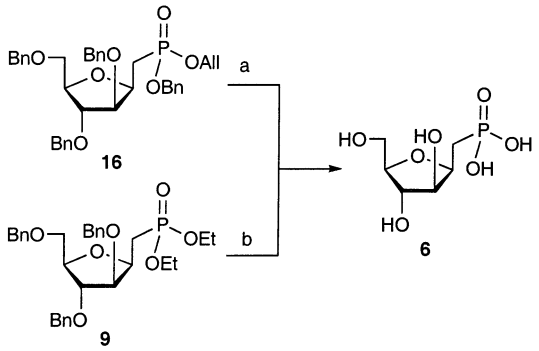
(21) Dondoni, A.; Marra, A.; Pasti, C. *Tetrahedron: Asymmetry* **2000**, *11*, 305.
(22) The stereochemistry was proven by comparison of the ^{1}H, ^{13}C, and ^{31}P spectral data previously reported for **14**: McClard, R. W.; Tsimikas, S.; Schriver, K. E. *Arch. Biochem. Biophys.* **1986**, *245*, 282.
(23) Blackwell, H. E.; O'Leary, D. J.; Chatterjee, A. K.; Washenfelder, R. A.; Bussmann, D. A.; Grubbs, R. H. *J. Am. Chem. Soc.* **2000** *122*, 58.
(24) Meng, Q.; Hesse, M. *Helv. Chim. Acta* **1991**, *74*, 445.
(25) Kissling, R. M.; Gagne, M. R. *J. Org. Chem.* **1999**, *64*, 1585.
(26) Campbell, M. M.; Carruthers, N. I.; Mickel, S. J. *Tetrahedron* **1982**, *38*, 2513.
(27) Perksy, R.; Albeck, A. *J. Org. Chem.* **2000**, *65*, 3775.

SCHEME 5[a]



[a] Conditions: (a) **19**, THF, −78 °C, 90%; (b) $NaBH_4$, THF, rt, 87%; (c) benzaldehyde dimethyl acetal, *p*-TsOH, CH_3CN, 75% from **26** (combined **28A** and **28B**); (d) 80% AcOH, 50 °C, 93%; (e) 80% AcOH, 50 °C, 81%; (f) Ph_3P, DIAD, THF, rt, 90%; (g) Ph_3P, DIAD, THF, rt, 72%.

SCHEME 6[a]

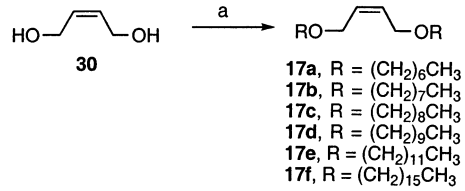


[a] Conditions: (a) H_2, Pd/C, AcOH; then TMSBr, CH_2Cl_2, rt, 81%; (b) H_2, Pd/C, AcOH; then TMSBr, CH_2Cl_2, rt, 75%.

were explored to carry out this reduction (e.g., L-Selectride and (+)-diisopinocamphenylborane), but with no improvement in stereoselectivity. To separate the diastereomers, the mixture was treated with benzaldehyde dimethyl acetal and *p*-TsOH, which provided all four possible diastereomeric benzylidene derivatives **28A** and **28B**. These were separated by chromatography and the acetal protecting group was cleaved under acidic conditions to provide **24** and **27** as pure compounds. Each of the diols was cyclized upon treatment with triphenylphosphine and diisopropyl-azodicarboxylate (DIAD) affording **16** and its stereoisomer **29**, in 90 and 72% yields, respectively. The stereochemistry of **16** was proven by complete deprotection, which afforded a product (**6**) that was identical with that obtained by total deprotection of **9** (Scheme 6).

Synthesis of 17 and Cross Metathesis Reactions. The synthesis of the targets required as a key step an olefin cross metathesis reaction between **16** and a panel of alkenes with the general structure **17**. The synthesis of these alkenes was achieved by alkylation of *cis*-2-buten-1,4-diol (**30**) under standard conditions (Scheme 7). Each was then reacted with **16** in the presence 20 mol % of the Grubbs catalyst (**31**,[28] Scheme 8) in refluxing dichloromethane, which provided the cross metathesis products **32** in 51−66% yield as a mixture of cis/trans isomers. Subsequent reduction of the alkene was achieved by reaction with diimide to afford the products **33** in 74−95% yield. We also explored the use of $(Ph_3P)_3RhCl/H_2$ and Raney Nickel/H_2 to carry out this reduction, but found the diimide reaction gave better yields of the products.[29] The final targets **15** were obtained by cleavage of the benzyl groups by hydrogenation. Following chromatography on Iatrobeads,[30] the products were obtained in low to modest yield (20−68%). We are unsure as to why the yield of the deprotection step is relatively poor in some cases. TLC of the crude reaction mixtures indicated that in some cases a significant amount of very polar material was formed in addition to the desired product. Previous work with *C*-phosphonate analogues of arabinofuranosyl pyrophosphates (e.g., **32**, Figure 5)

SCHEME 7[a]



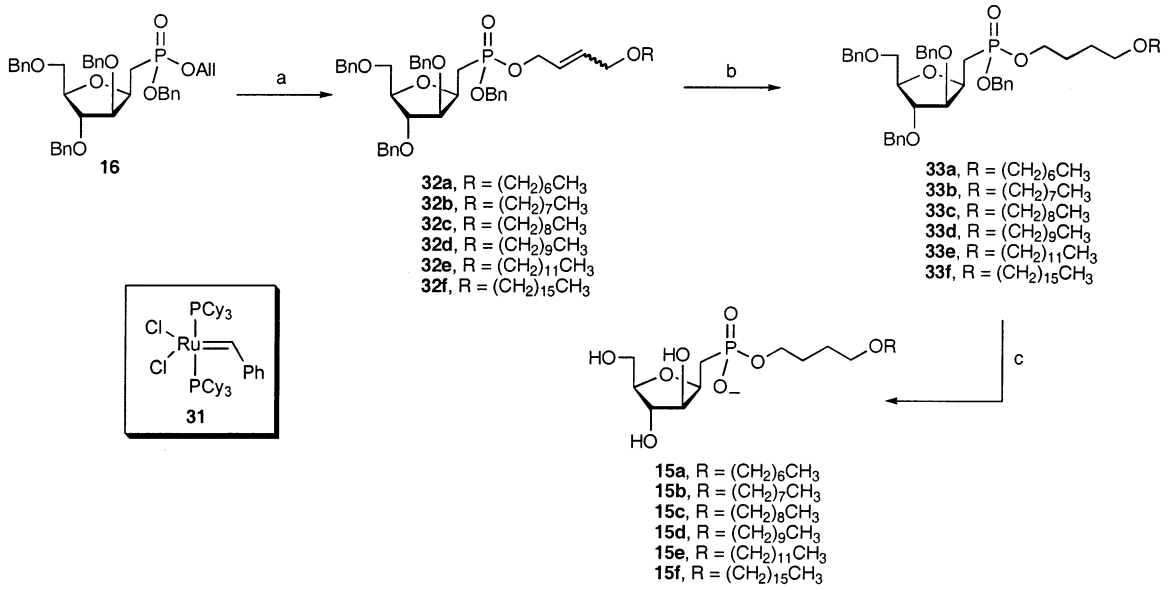
[a] Conditions: (a) NaH, RI, DMF, rt.

(28) Schwab, P.; France, M. B.; Ziller, J. W.; Grubbs, R. H. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2039.

(29) We attempted simultaneous reduction of the alkene and hydrogenolysis of the benzyl ethers upon reaction with H_2, Pd/C, but were unsuccessful. Under these conditions, decomposition of the substrate was observed, which we attribute to the formation of a palladium−π-allyl complex from the allyl phosphonate.

(30) Iatrobeads refers to a beaded silica gel 6RS-8060, which is manufactured by Iatron Laboratories (Tokyo).

SCHEME 8[a]



[a] Conditions: (a) **31**, **17**, CH_2Cl_2, 40 °C, 51−66%; (b) KOC(O)N=NC(O)OK, AcOH, CH_3OH, 40 °C, 74−95%; (c) H_2, Pd/C, CH_3OH, 20−68%.

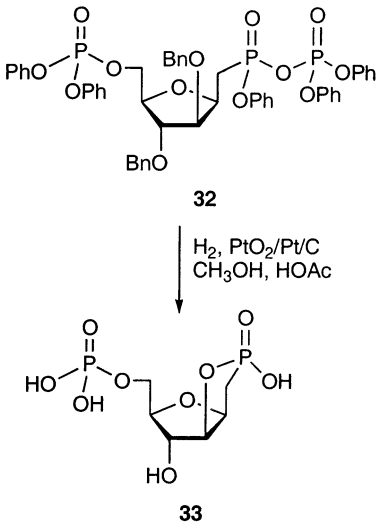


FIGURE 5. Formation of phostone **33** from **32** (see ref 32).

demonstrated[31] the formation of phostone **33**. The formation of a compound analogous to **33** is also possible here, although we could not detect the presence of such a species in the eluants obtained by washing the Iatrobead column with 3:1 dichloromethane:methanol.

Screening of Final Compounds as Antituberculosis Agents. Compounds **15a**−**f** were tested in vitro for the ability to prevent growth of *Mycobacterium tuberculosis* strain H_{37}Rv (ATCC 27294) using the Alamar Blue Microplate assay.[32] Only one of the analogues, **15f**, possessed activity, and was shown to have an MIC of 3.13 μg/mL. It appears, therefore, that a fairly lengthy alkyl chain is required for compounds of this type to possess antituberculosis activity.

(31) McClard, R. W.; Witte, J. F. *Bioorg. Chem.* **1990**, *18*, 165.
(32) Collins, L. A.; Franzblau, S. G. *Antimicrob. Agents. Chemother.* **1997**, *41*, 1004.

In conclusion, we report here the synthesis of six *C*-phosphonate analogues of decaprenolphosphoarabinose, **3**. These compounds were synthesized via a route that had as a key step an olefin cross metathesis between allyl *C*-phosphonate **16** and an alkene **17**. One of the six compounds was shown to prevent growth of mycobacteria in an in vitro assay. Screening of the compounds as inhibitors of mycobacterial arabinosyltransferases is currently in progress.

Experimental Section

General. Solvents were distilled from the appropriate drying agents before use. Unless stated otherwise, all reactions were carried out at room temperature under a positive pressure of argon and were monitored by TLC on silica gel 60 F_{254}. Spots were detected under UV light or by charring with 10% H_2SO_4 in ethanol. Solvents were evaporated under reduced pressure and below 40 °C (bath). Organic solutions of crude products were dried over anhydrous Na_2SO_4. Unless otherwise indicated, column chromatography was performed on silica gel 60 (40−60 μM). The ratio between silica gel and crude product ranged from 100 to 50:1 (w/w). Optical rotations were measured at 21 ± 2 °C. ^{1}H NMR spectra were recorded at 250, 400, or 500 MHz, and chemical shifts are referenced to either TMS (0.0, $CDCl_3$) or CD_3OH (4.78, CD_3OD). ^{13}C NMR spectra were recorded at 62.5, 100, or 125 MHz and chemical shifts are referenced to $CDCl_3$ (77.00, $CDCl_3$) or CH_3OH (49.00, CD_3OD). ^{31}P NMR spectra were recorded at 101, 162, or 202 MHz and chemical shifts are referenced to external phosphoric acid (0.0, $CDCl_3$, CD_3OD). Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA. Electrospray mass spectra were recorded on samples suspended in mixtures of THF and CH_3OH with added trifluoroacetic acid or NaCl. Analytical data for all new compounds (^{1}H NMR, ^{13}C NMR, HRMS, elemental analysis, $[\alpha]_D$) are provided in the Supporting Information.

2,5-Anhydro-D-glucityl Phosphonic Acid (6). Phosphonate **9**[17] (2.7 g, 4.87 mmol) was dissolved in glacial HOAc (10 mL). Palladium (10% on activated carbon, 500 mg) was added and the reaction mixture was stirred under hydrogen overnight at atmospheric pressure. The solid was then filtered off and

the filtrate was concentrated to a clear residue that was purified by chromatography (9:1, CH_2Cl_2:CH_3OH) affording 1-diethyl-2,5-anhydroglucityl phosphonate (1.13 g, 83%): R_f 0.33 (9:1, CH_2Cl_2:CH_3OH). The diethyl phosphonate from above was dissolved in CH_2Cl_2 (5 mL). Bromotrimethylsilane (1.7 mL, 12.9 mmol) was added and the reaction mixture stirred overnight. Methanol (1 mL) was added to the orange reaction mixture and the solvent was evaporated to give a crude oil. The oil was purified by ion-exchange chromatography (AG 1-X8 resin) and was eluted with a 0.2 M solution of triethylammonium bicarbonate. After evaporation of the eluant, the clear oil was dissolved in CH_3OH (5 mL) and treated with Amberlite H^+ resin. The resin was then filtered to afford **6** (853 mg, 91%) as a clear oil. Compound **6** was also prepared (in 81% yield) by subjecting phosphonate **16** to these reaction conditions.

1-(Butyl-4′-*O*-heptyl)-2,5-anhydro-D-glucityl Phosphonate (15a). Phosphonate **33a** (95 mg, 0.125 mmol) was dissolved in a mixture of CH_3OH and AcOH (5 mL:10 μL). Palladium (10% on activated carbon, 22 mg) was added and the reaction mixture was stirred under hydrogen overnight at atmospheric pressure. After filtering off the solid and evaporating the solvent, the residual oil was purified by chromatography on Iatrobeads (CH_2Cl_2 → 3:1, CH_2Cl_2:CH_3OH). The pure product was then redissolved in water and then lyophilized to give **15a** (13 mg, 26%) as an off-white solid.

1-(Butyl-4′-*O*-octyl)-2,5-anhydro-D-glucityl Phosphonate (15b). Phosphonate **33b** (132 mg, 0.171 mmol) was hydrogenated as described for **33a**. Purification of the product was done as outlined for **15a** to provide **15b** (33 mg, 47%) as an off-white solid.

1-(Butyl-4′-*O*-nonyl)-2,5-anhydro-D-glucityl Phosphonate (15c). Phosphonate **33c** (190 mg, 0.254 mmol) was hydrogenated as described for **33a**. Purification of the product was done as outlined for **15a** to provide **15c** (56 mg, 54%) as an off-white solid.

1-(Butyl-4′-*O*-decyl)-2,5-anhydro-D-glucityl Phosphonate (15d). Phosphonate **33d** (99 mg, 0.124 mmol) was hydrogenated as described for **33a**. Purification of the product was done as outlined for **15a** to provide **15d (**11 mg, 20%) as an off-white solid.

1-(Butyl-4′-*O*-dodecyl)-2,5-anhydro-D-glucityl Phosphonate (15e). Phosphonate **33e** (132 mg, 0.159 mmol) was hydrogenated as described for **33a**. Purification of the product was done as outlined for **15a** to provide **15e (**51 mg, 68%) as an off-white solid.

1-(Butyl-4′-*O*-hexadecyl)-2,5-anhydro-D-glucityl Phosphonate (15f). Phosphonate **33f** (122 mg, 0.138 mmol) was hydrogenated as described for **33a**. Purification of the product was done as outlined for **15a** (except that a gradient of CH_2Cl_2 → CH_2Cl_2:CH_3OH, 5:1 was used in the chromatography) to provide **15f (**38 mg, 53%) as an off-white solid.

1-(Allylbenzyl)-3,4,6-tri-*O*-benzyl-2,5-anhydro-D-glucityl Phosphonate (16). Acyclic phosphonate **24** (638 mg, 0.987 mmol) was dissolved in THF (12 mL) and stirred before Ph_3P (311 mg, 1.19 mmol) and diisopropylazodicarboxylate (0.39 mL, 1.97 mmol) were added. The reaction mixture was stirred for 2 h and then the solvent was evaporated to give a clear yellow residue that was purified by chromatography (2:1, hexane:EtOAc) to yield **16** (547 mg, 90%) as a clear oil.

***cis*-2-Butenyl-1,4-diheptyl Ether (17a).** Sodium hydride (874 mg, 36.4 mmol) was added to a solution of *cis*-2-buten-1,4-diol (**30**, 803 mg, 9.1 mmol) in DMF (20 mL). After ~10 min, 1-iodoheptane (6.0 mL, 36.4 mmol) was added and the reaction mixture was stirred for 1 h. Water (20 mL) was added to dissolve the salt precipitate and the solution was diluted with hexane (75 mL). The organic layer was separated, washed with brine (20 mL), and dried over Na_2SO_4. After the solvent was evaporated, the yellowish residue was purified by chromatography (hexane → 6:1, hexane:EtOAc) to afford *cis*-2-butenyl-1,4-diheptyl ether (2.48 g, 96%) as a clear liquid.

***cis*-2-Butenyl-1,4-dioctyl Ether (17b).** The preparation of **17b** was achieved by the reaction of **30** (500 mg, 5.67 mmol) and 1-iodooctane (4.1 g, 17.01 mmol) as described for the preparation of **17a**. The product was purified by chromatography (8:1, hexane:EtOAc) to afford **17b** (1.63 g, 92%) as a clear oil.

***cis*-2-Butenyl-1,4-dinonyl Ether (17c).** The preparation of **17c** was achieved by the reaction of **30** (500 mg, 5.67 mmol) and 1-iodononane (4.3 g, 17.01 mmol) as described for the preparation of **17a**. The product was purified by chromatography (9:1, hexane:EtOAc) to afford **17c** (1.60 mg, 83%) as a clear oil.

***cis*-2-Butenyl-1,4-didecyl Ether (17d).** The preparation of **17d** was achieved by the reaction of **30** (535 mg, 6.07 mmol) and 1-iododecane (6.5 g, 6.07 mmol) as described for the preparation of **17a**. The product was purified by chromatography (10:1, hexane:EtOAc) to afford **17d** (2.0 g, 91%) as a clear oil.

***cis*-2-Butenyl-1,4-didodecyl Ether (17e).** The preparation of **17e** was achieved by the reaction of **30** (500 mg, 5.67 mmol) and 1-iodododecane (6.72 g, 22.68 mmol) as described for the preparation of **17a**. The product was purified by chromatography (12:1, hexane:EtOAc) to afford **17e** (2.39 g, 99%) as a clear oil.

***cis*-2-Butenyl-1,4-dihexadecyl Ether (17f).** The preparation of **17f** was achieved by the reaction of **30** (500 mg, 5.67 mmol) and 1-iodohexadecane (8.0 g, 22.68 mmol) as described for the preparation of **17f**. The product was purified by chromatography (12:1, hexane:EtOAc) to afford **17e** (1.6 g, 52%) as a clear oil.

Allylbenzyl Methylphosphonate (22). THF (40 mL) was added to a stirring solution of **21**[25] (36.7 g, 0.133 mol) and allyl acetate (28.7 mL, 0.266 mol). A 1 M solution of potassium *tert*-butoxide (20 mL) was prepared and then added in 5-mL aliquots approximately every 15 min. The reaction mixture was then neutralized with AcOH and diluted with EtOAc (100 mL). The organic layer was washed with water (100 mL) followed by brine (100 mL) and then dried over Na_2SO_4. Evaporation of the solvent provided a thick yellow liquid. The product was distilled at 135 °C (1 mmHg) affording **22** (14.1 g, 47%) as a clear liquid.

1-(Allylbenzyl)-3,4,6-tri-*O*-benzyl-D-glucityl Phosphonate (24). Benzylidene **28** (559 mg, 0.761 mmol) was dissolved in an 80:20 mixture of AcOH/H_2O (20 mL) and heated at 50 °C overnight. After being cooled to room temperature, the reaction mixture was diluted with CH_2Cl_2 (30 mL) and the product was extracted from the aqueous layer. The organic layer was dried over Na_2SO_4 and the solvent was evaporated. The residue was purified by chromatography (1:3, hexane:EtOAc) to give **24** (456 mg, 93%) as a clear oil.

1-(Allylbenzylphosphinyl)-1-deoxy-3,4,6-tri-*O*-(benzyl)-D-fructofuranose (26). Phosphonate **22** (1.5 g, 6.7 mmol) was dissolved in 5 mL of THF and the mixture was stirred at −78 °C. *n*-BuLi (11.7 mL of a 1.6 M solution in hexane) was added and, after 5 min, a solution of lactone **18**[24] (4.9 g, 11.7 mmol) in THF (10 mL) was added in one aliquot. After 1 h, the solution was removed from the dry ice bath and stirred for another 30 min. The reaction mixture was then neutralized with AcOH and diluted with EtOAc (100 mL). The organic layer was washed with water (75 mL) followed by brine (75 mL) and then dried over Na_2SO_4. The solvent was evaporated and the residue was purified by chromatography (1:1, hexane:EtOAc) to give **26** (6.8 g, 90%) as a clear oil.

1-(Allylbenzyl)-3,4,6-tri-*O*-benzyl-D-mannityl Phosphonate (27). Benzylidene **29** (336 mg, 0.457 mmol) was dissolved in an 80:20 mixture of AcOH/H_2O (10 mL) and heated at 40 °C for 3 h. After being cooled to room temperature, the reaction mixture was diluted with CH_2Cl_2 (30 mL) and the product was extracted from the aqueous layer. The organic layer was dried over Na_2SO_4 and the solvent was evaporated. The residue was purified by chromatography (1:3, hexane:EtOAc) to give **27** (240 mg, 81%) as a clear oil.

1-(Allylbenzyl)-3,4,6-tri-*O*-benzyl-2,5-*O*-benzylidene-D-glucityl Phosphonate (28A) and 1-(Allylbenzyl)-3,4,6-tri-*O*-benzyl-2,5-*O*-benzylidene-D-mannityl Phosphonate (28B). Sodium borohydride (963 mg, 25.5 mmol) was added to a solution of lactol **26** (13.7 g, 21.2 mmol) in THF (200 mL) and the reaction mixture was stirred for 2 h. Upon completion, the reaction mixture was neutralized with AcOH, diluted with EtOAc (100 mL), and washed with water (100 mL) followed by brine (100 mL). The organic layer was dried over Na_2SO_4 and the solvent was evaporated affording a crude reduction product (14.6 g, 87%), which was a 2:1 mixture of **24** and **27**. This crude material was used in the next step without any further purification. Benzaldehyde dimethyl acetal (9.5 mL, 63.6 mmol) and *p*-toluenesulfonic acid (193 mg, 1.01 mmol) were added to a solution of the crude diol in CH_3CN (60 mL) and the mixture stirred overnight. The solvent was evaporated and the residue, which was a mixture of 4 diastereomers, was purified by chromatography (hexane:EtOAc, 1:1) to afford the major diasteromers **28A** (5.9 g, 38% over both steps) and **28B** (5.8 g, 37% over both steps) as clear oils. It was possible to obtain more of these products by resubjecting the minor diastereomers and mixed fractions from the column to the reaction.

1-(Allylbenzyl)-3,4,6-tri-*O*-benzyl-2,5-anhydro-D-mannityl Phosphonate (29). Acyclic phosphonate **27** (240 mg, 0.371 mmol) was dissolved in THF (5 mL) and stirred. Triphenylphosphine (107 mg, 0.408 mmol) was added, followed by diisopropyl azodicarboxylate (0.15 mL, 0.742 mmol), and the reaction mixture was stirred for 2 h. The solvent was then evaporated to give a clear yellow residue that was purified by chromatography (1:1, hexane:EtOAc) to afford **29** (168 mg, 72%) as a clear oil.

1-[Benzyl((*E*)-2′-butenyl-4′-*O*-heptyl)]-3,4,6-tri-*O*-benzyl-2,5-anhydro-D-glucityl Phosphonate (32a). Alkene **17a** (366 mg, 1.29 mmol) was added to a solution of **16** (269 mg, 0.43 mmol) in dry CH_2Cl_2 (10 mL). The Grubbs' catalyst **31**[28] (70 mg, 20 mol %) was added and the reaction mixture was heated at reflux for 3 h. After the mixture was cooled to room temperature, $Pb(OAc)_4$ (114 mg, 0.26 mmol) was added and the mixture was stirred overnight. The solvent was evaporated and the black residue was purified by chromatography (2:1, hexane:EtOAc) to give **32a** (179 mg, 55%) as a clear oil. The product was a mixture of cis and trans isomers.

1-[Benzyl((*E*)-2′-butenyl-4′-*O*-octyl)]-3,4,6-tri-*O*-benzyl-2,5-anhydro-D-glucityl Phosphonate (32b). The preparation of **32b** was achieved by the reaction of **16** (210 mg, 0.334 mmol) and **17b** (313 mg, 1.00 mmol) as described for the preparation of **32a**. The product was purified by chromatography (2:1, hexane:EtOAc) to afford **32b** (163 mg, 63%) as a clear oil. The product was a mixture of cis and trans isomers.

1-[Benzyl((*E*)-2′-butenyl-4′-*O*-nonyl)]-3,4,6-tri-*O*-benzyl-2,5-anhydro-D-glucityl Phosphonate (32c). The preparation of **32c** was achieved by the reaction of **16** (235 mg, 0.373 mmol) and **17c** (508 mg, 1.49 mmol) as described for the preparation of **32a**. The product was purified by chromatography (2:1, hexane:EtOAc) to afford **32c** (168 mg, 57%) as a clear oil. The product was a mixture of cis and trans isomers.

1-[Benzyl((*E*)-2′-butenyl-4′-*O*-decyl)]-3,4,6-tri-*O*-benzyl-2,5-anhydro-D-glucityl Phosphonate (32d). The preparation of **32d** was achieved by the reaction of **16** (268 mg, 0.426 mmol) and **17d** (628 mg, 1.70 mmol) as described for the preparation of **32a**. The product was purified by chromatography (2:1, hexane:EtOAc) to afford **32c** (174 mg, 51%) as a clear oil. The product was a mixture of cis and trans isomers.

1-[Benzyl((*E*)-2′-butenyl-4′-*O*-dodecyl)]-3,4,6-tri-*O*-benzyl-2,5-anhydro-D-glucityl Phosphonate (32e). The preparation of **32e** was achieved by the reaction of **16** (237 mg, 0.377 mmol) and **17e** (640 mg, 1.51 mmol) as described for the preparation of **32a**. The product was purified by chromatography (2:1, hexane:EtOAc) to afford **32c** (205 mg, 66%) as a clear oil. The product was a mixture of cis and trans isomers.

1-[Benzyl((*E*)-2′-butenyl-4′-*O*-hexadecyl)]-3,4,6-tri-*O*-benzyl-2,5-anhydro-D-glucityl Phosphonate (32f). The preparation of **32f** was achieved by the reaction of **16** (218 mg, 0.347 mmol) and **17f** (746 mg, 1.39 mmol) as described for the preparation of **32a**. The product was purified by chromatography (2:1, hexane:EtOAc) to afford **32c** (188 mg, 62%) as a clear oil. The product was a mixture of cis and trans isomers.

1-[Benzyl(butyl-4′-*O*-heptyl)]-3,4,6-tri-*O*-benzyl-2,5-anhydro-D-glucityl Phosphonate (33a). Dipotassium azodicarboxylate (DAPA, 20 mg, 0.123 mmol) was added to a solution of **32a** (149 mg, 0.197 mmol) in methanol (10 mL). Glacial HOAc (10 μL) was added and the reaction mixture was heated to 40 °C open to the atmosphere. As the yellow color of the solution started to fade, more DAPA (20 mg) and HOAc (10 μL) were added. This addition procedure was repeated several times over the course of 10 h, and the reaction progress was monitored by NMR. Upon completion of the reaction, the reaction mixture was cooled and then a saturated aqueous solution of $NaHCO_3$ (15 mL) was added. The product was then extracted into CH_2Cl_2 (3 × 25 mL) and the organic layer dried over Na_2SO_4. After evaporation of the solvent, the clear residue was purified by chromatography (2:1, hexane:EtOAc) to give **33a** (130 mg, 87%) as a clear oil.

1-[Benzyl(butyl-4′-*O*-octyl)]-3,4,6-tri-*O*-benzyl-2,5-anhydro-D-glucityl Phosphonate (33b). Alkene **32b** (149 mg, 0.193 mmol) was converted to **33b** as described for the preparation of **33a**. Purification by chromatography (2:1, hexane:EtOAc) afforded the product (132 mg, 89%) as a clear oil.

1-[Benzyl(butyl-4′-*O*-nonyl)]-3,4,6-tri-*O*-benzyl-2,5-anhydro-D-glucityl Phosphonate (33c). Alkene **32c** (135 mg, 0.172 mmol) was converted to **33c** as described for the preparation of **33a**. Purification by chromatography (2:1, hexane:EtOAc) afforded the product (100 mg, 74%) as a clear oil.

1-[Benzyl(butyl-4′-*O*-decyl)]-3,4,6-tri-*O*-benzyl-2,5-anhydro-D-glucityl Phosphonate (33d). Alkene **32d** (143 mg, 0.179 mmol) was converted to **33d** as described for the preparation of **33a**. Purification by chromatography (2:1, hexane:EtOAc) afforded the product (122 mg, 85%) as a clear oil.

1-[Benzyl(butyl-4′-*O*-dodecyl)]-3,4,6-tri-*O*-benzyl-2,5-anhydro-D-glucityl Phosphonate (33e). Alkene **32e** (175 mg, 0.212 mmol) was converted to **33e** as described for the preparation of **33a**. Purification by chromatography (2:1, hexane:EtOAc) affords the product (150 mg, 86%) as a clear oil.

1-[Benzyl(butyl-4′-*O*-hexadecyl)]-3,4,6-tri-*O*-benzyl-2,5-anhydro-D-glucityl Phosphonate (33f). Alkene **32f** (158 mg, 0.179 mmol) was converted to **33f** as described for the preparation of **33a**. Purification by chromatography (2:1, hexane:EtOAc) afforded the product (150 mg, 95%) as a clear oil.

Measurement of Antituberculosis Activity of 15a–f. Measurement of the antituberculosis activity of the target compounds was carried out as previously reported using the fluorescence-based Alamar Blue Microplate assay.[32] All compounds were initially tested against *Mycobacterium tuberculosis* strain $H_{37}Rv$ (ATCC 27294) at a concentration of 6.25 μg/mL. At this concentration, only **15f** inhibited the growth of the bacteria. The MIC for **15f** was determined by testing this compound at lower concentrations of the compound; the MIC is defined as the lowest concentration producing a 90% reduction in fluorescence relative to controls.

Acknowledgment. The National Institutes of Health (AI44045-01) supported this work. C.A.C. is a recipient of a GAANN fellowship from the U.S. Department of

Education. Antimycobacterial data were provided by the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) through a research and development contract with the U.S. National Institute of Allergy and Infectious Diseases.

Supporting Information Available: ^{1}H, ^{13}C, and ^{31}P NMR spectra for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

JO026247R