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Confirmation of the Connectivity of 4,8,12,16,20-Pentamethylpentacosylphoshoryl β-D-Mannopyranoside, an Unusual β-Mannosyl Phosphoisoprenoid from *Mycobacterium avium*, through Synthesis

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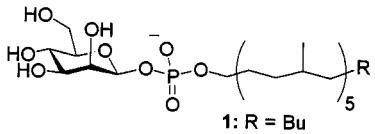
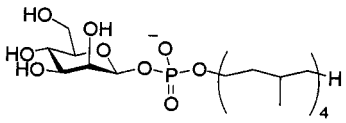
Abstract: The synthesis of the title glycolipid is reported. Comparison of the electrospray and high-energy collision-induced dissociation mass spectra of the synthetic material with those reported for the isolate confirm the structure of this unusual antigenic substance with its modified isoprenoid chain.

Introduction

Two unusual β-mannosyl phosphoisoprenoids were recently isolated from *Mycobacterium avium* and *Mycobacterium tubercolosis* on the basis of their recognition by a CD1 c-restricted, mycobacterial specific T-cell line.[1] They were assigned the structures **1** and **2**, respectively, on the basis of degradation and mass spectrometric studies.[1] It was suggested[1] that **1** and **2** follow a general mechanism for lipid antigen presentation by CD1[2] in which the isoprenoid chain is bound within a hydrophobic groove of the CD1 protein[3] and the exposed hydrophilic component is recognized by specific T-cell receptors. The T-cell response was shown to be inversely proportional to the length of the phosphoisoprenoid chain, preferring a C_{35} derived system over one of C_{55} and not recognizing a C_{95} dolichol-based β-mannosyl phosphoisoprenoid.[1] Additionally, it was found that full saturation of the aliphatic chain was required and that a glucose, as opposed to a mannose, headgroup was not recognized.[1] Biosynthetically, **1** and **2** are interesting because of their modified isoprenoid chains, which deviate from the isoprene rule by incorporation of additional methylene groups, at both ends of the chain. Chemically, they are of interest because of the considerable challenge presented by the synthesis of the β-mannosyl phosphate, surely one of the more significant obstacles presented by nature given the already considerable difficulty of synthesizing β-mannosides themselves.[4,5] Indeed, the synthesis of all but the simplest β-mannosyl phosphates had previously eluded other workers in the field.[6,7] Numerous factors therefore combined to make **1** and **2** viable and challenging targets with which to test the β-mannosylation protocols developed in this laboratory.[8,9] Accomplishment of this endeavor would also point the way toward syntheses of other biologically relevant β-mannosyl phosphates such as the partly saturated β-mannosyl heptaprenyl phosphate from *Mycobacterium smegmatis*,[10] that serves as a carrier of mycolic acid, and the β-mannosyl decaprenyl phosphate from the same organism,[11] thought to be an intermediate in α-1→6 linked manno-oligosaccharide biosynthesis. Here, we report full details of our synthesis[12] of a dihydrophytyl model for **1** and the successful synthesis of **1** itself, including that of its unusual, modified isoprenoid chain in the form of a stereorandom mixture of isomers.



1: R = Bu
2: R = C_6H_{13}



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Results and Discussion

The synthetic effort required for the preparation of the isoprenoid chain of **1** and the obviously difficult nature of any

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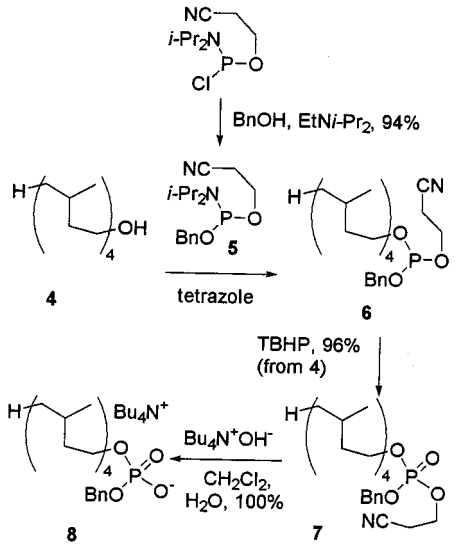
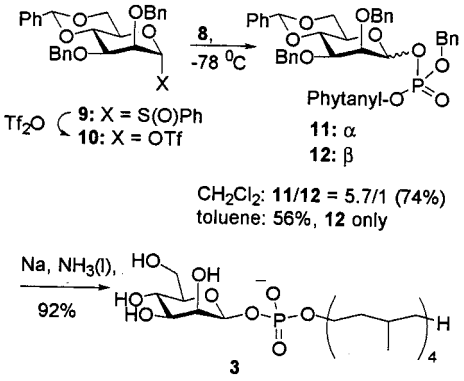
Scheme 1



synthesis of β-mannosyl phosphates dictated that we begin our study with a model investigation. Accordingly, the phytanyl β-mannosyl phosphate **3** was selected as a first target. Toward this end commercial phytol, a mixture of isomers, was reduced over Adam's catalyst to give phytanol **4** quantitatively. Benzyl 2-cyanoethyl *N*,*N*-diisopropylphosphoramidite **5** was prepared in 94% yield from commercial 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite and benzyl alcohol in the presence of Hunig's base and coupled with **4** in the presence of tetrazole giving **6**, which was immediately oxidized with *tert*-butyl hydroperoxide to provide **7** in 96% overall yield from **4** (Scheme 1). Treatment of **7** with tetrabutylammonium hydroxide in a dichloromethane/water biphasic system then afforded the salt **8** quantitatively (Scheme 1).

The α-mannosyl sulfoxide **9**,[8] a single diastereomer,[13] was converted at −78 °C to the triflate **10**,[14] by treatment of a dichloromethane solution with triflic anhydride in the presence of 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP). Two equivalents of salt **8** were added and the resulting mixture was stirred for several hours before quenching, extraction, and chromatographic separation. In this manner, the α- and β-mannosyl phosphates **11** and **12** were isolated in 63 and 11% yields, respectively (Scheme 2). Both anomers were approximately 1/1 mixtures of diastereomers at phosphorus and, in **12**, these could be separated even if the configuration was not assigned. The anomeric configuration of **11** and **12** was assigned on the basis of the chemical shifts of the mannose H5 signals, especially that of the β-anomer **12** (δ 3.41 and 3.44 for the two diastereomers at P), which is diagnostic of configuration in 4,6-*O*-benzylidene protected mannopyranosides.[8] Although the diastereoselectivity of this process was disappointing, the isolation of **12**, following chromatography on silica gel, was viewed as very encouraging given that Schmidt and co-workers had reported that a related dibenzyl β-mannosyl phosphate

Scheme 2



underwent rapid isomerization to the α-anomer simply on standing in deuteriochloroform and could not be isolated.[7] We reasoned that the poor diastereoselectivity observed was a function of the low nucleophilicity of **8** and that this might be countermanded by moving to a less polar solvent. This would enhance the stability of the triflate **10** and so suppress α-selective, dissociative mechanisms in favor of β-selective, associative ones. This argument holds whether the nucleophilic displacement of triflate from **10** is a true S_N2 reaction or, as is quite possible, proceeds via attack on a contact ion pair that is in dynamic equilibrium with **10**. In the event, in toluene at −78 °C, the reaction was completely selective and afforded only **12** in 56% isolated yield (Scheme 2) with the mass balance consisting mainly of 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-D-mannopyranose, that is, the hydrolysis product of **10**. We first attempted deprotection of **12** by hydrogenolysis but, under all conditions assayed, noted substantial hydrolysis and the formation of D-mannopyranose. We therefore turned to the Birch reduction and found that exposure of **12** to sodium in liquid ammonia, followed by quenching with ammonium chloride, minimized this problem and enabled the isolation of **3**, as its sodium salt, in 92% yield. Importantly, no anomerization was observed during the deprotection process and **3** was isolated in the form of a single diastereomer. Its anomeric configuration was confirmed by the NOE correlation of its anomeric hydrogen to both H3 and H5 as well as by its anomeric $^1J_{CH}$ coupling[15] of 157.6 Hz.[12]

With the essential methodology established, we turned to the preparation of the branched C_{30} alcohol **13** required for the synthesis of **1**. As the structure of **1** had been deduced purely from mass spectral considerations, no information was available regarding the relative or absolute stereochemistry of the five stereogenic centers in the isoprenoid chain.[1,16] In the absence of any stereochemical information or of a rational hypothesis predicting a particular stereoisomer, a stereorandom synthesis was designed with a view to expediency and the verification of

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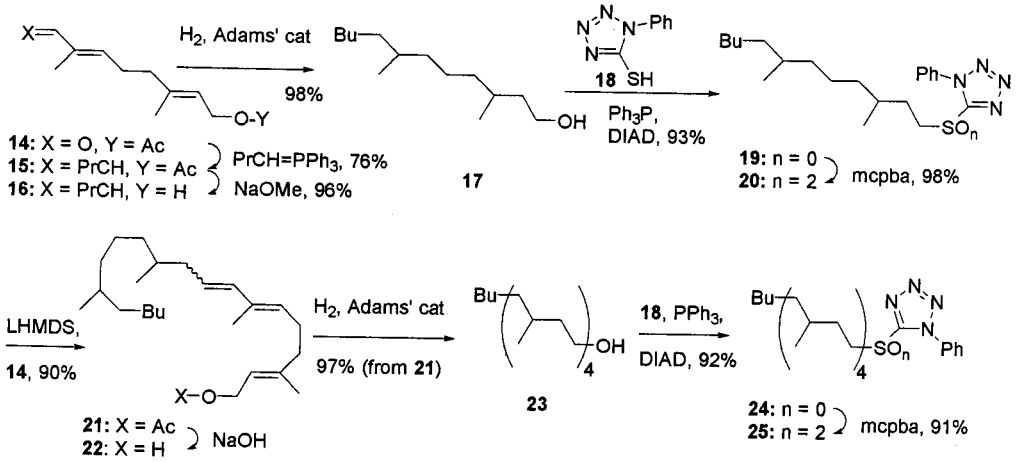
(16) The original report[1] on the isolation, structural elucidation, and activity of **1** and **2** did not consider, being based solely on mass spectrometric evidence, the relative and absolute stereochemistry of the five stereogenic centers in their isoprenoid chains. It was further reported[1] that a semisynthetic fully saturated C_{35} β-mannosyl phosphoisoprenoid, obtained by transfer of mannose from GDP mannose to a pure, naturally derived saturated polyprenol with a mannosyl transferase, had a comparable effect on the proliferation of human CD1 c-restricted T-cell lines to that of **1** and **2**. Given that the exact location of methylation (the first methyl of this model compound being one methylene unit closer to the phosphate than in **1** and **2**) does not have a significant effect on activity, it is unlikely that the absolute and relative stereochemistry of the chain in **1** and **2** has a major influence on the activity of these compounds.

Scheme 3

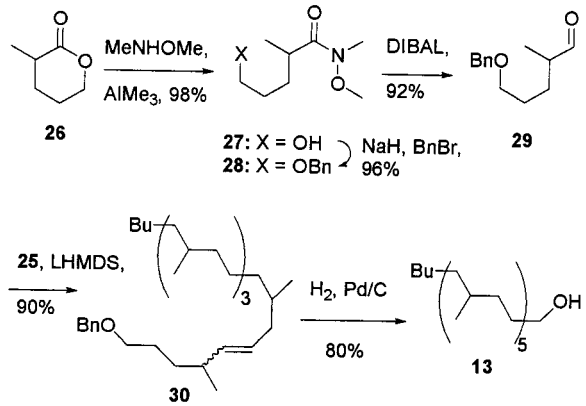


the excursions from the isoprene rule at both ends of the chain. To this end, geranyl acetate was converted to the distal aldehydo ester **14** by the regioselective allylic hydroxylation.[17,18] Standard Wittig olefination installed the butyl group and gave ester **15** in 76% yield. Saponification and then hydrogenation gave alcohols **16** and then **17** in 96% and 98% yields, respectively. Mitsunobu reaction[19] of **17** with 1-phenyltetrazole-5-thiol **18** as nucleophile afforded the sulfide **19** in 93% yield which, on treatment with mcpba, gave 98% of the sulfone **20**. Coupling of **20** with a second aliquot of aldehyde, following deprotonation with lithium hexamethyldisilazide, resulted in the formation of ester **21** in 90% yield. This modified Julia sequence[20−22] was far superior in our hands to any alternative Wittig sequence. Saponification and hydrogenation subsequently gave **22** and then **23** in 97% overall yield. The conversion of **23** to the sulfone **25** via sulfide **24** was conducted analogously to the preparation of **20** from **17** (Scheme 3).

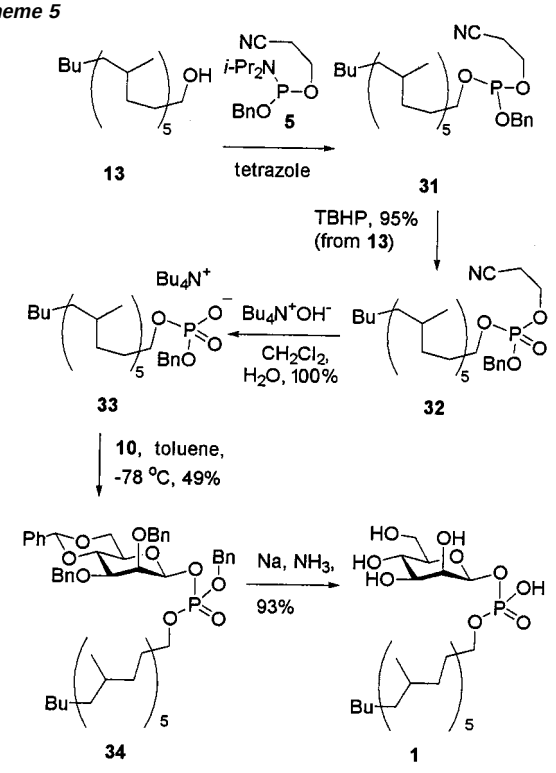
The final six-carbon segment of **13** was obtained by conversion of 2-methyl-δ-valerolactone (**26**)[23] to the Weinreb amide[24] **27**, benzylation to give **28** and, finally, DIBAL reduction[25] giving the aldehyde **29** (Scheme 4). Coupling of **29** with sulfone **25** gave the C_{30} ether **30**, which on exposure to hydrogen over palladium on carbon underwent hydrogenation and hydrogenolysis to afford **13** (Scheme 4).

Completion of the synthesis of **1** closely mimicked the protocol established for the model **3**. Thus, alcohol **13** was converted to the phosphate **32**, via the phosphite **31**. Treatment with tetrabutylammonium hydroxide gave the salt **33** which was coupled in a highly β-selective manner with the mannosyl triflate **10** in toluene at −78 °C to give **34** in 49% yield, with no indication of the α-anomer (Scheme 5). As with the model, the mass balance in this coupling was provided by the pyranose arising from the hydrolysis of triflate **10**. Also in common with

Scheme 4



Scheme 5



the model, the stereochemistry of **34** was initially determined from the chemical shift of the mannose H5 resonance and subsequently confirmed by NOE measurements and by the

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magnitude of the anomeric $^1J_{CH}$ coupling constant. Finally, Birch reduction of **34** gave the target molecule **1** in 93% isolated yield with no detectable anomerization (Scheme 5). The anomeric stereochemistry of **1** was fully confirmed by the NOE correlation between the anomeric hydrogen and H's 3 and 5 of the sugar moiety.

The anomeric configuration of **1** and **2** and the unusual nature of their modified isoprenoid chains were both originally characterized by mass spectral fragmentation patterns.[1] In particular, in the negative ion spectrum a cross-ring fragmentation of the carbohydrate moiety, known to be characteristic of *cis*-1,2-glycosyl phosphates,[26] established the β-mannoside stereochemistry, whereas a high-energy collision-induced remote fragmentation pattern[27] located the methylation sites on the lipid. Mass spectrometric investigation of synthetic **1**, using the same array of techniques, reproduced the published spectra with a high degree of fidelity. Thus, two cross-ring fragmentations (*m*/*z* 559.6 in the low-energy ESI CID spectrum and *m*/*z* 546 in the high-energy FAB CID spectrum) and a dehydration (*m*/*z* 661.3 in the low-energy ESI CID spectrum) originally used to assign the β-mannosyl phosphate configuration were nicely reproduced. More importantly, given the unusual modified nature of the isoprenoid chain, the remote fragmentation pattern of the isoprene chain in the high-energy FAB CID spectrum was a good match with the original. A feature of this spectrum is the series of consecutive losses of 14 mass units with a jump at every fifth position, indicating a methyl group on every fourth carbon in an otherwise unbranched aliphatic chain. The sequence of ions at *m*/*z* 662, 649, 635, 621, and 607 distinguish the remote end of the chain, with its unbranched five carbon chain, from that of a regular isoprenoid. The sequence *m*/*z* 327, 299, and 285, with the skip between 327 and 299, distinguish the functionalized end of the chain from that of a regular C_{30} isoprenyl ester for which at peak at *m*/*z* 313 with a skip to *m*/*z* 285 would have been expected. The connectivity, if not the sterochemistry, of the isoprenoid chain and the anomeric stereochemistry of this highly unusual glycolipid is therefore confirmed.

The protected β-mannosyl phosphates **12** and **34** described here are configurationally stable and may be isolated by silica gel chromatography, whereas Schmidt found closely related per-*O*-benzyl-β-mannosyl phopshates to be unstable and very rapidly equilibrated to the α-anomers in deuteriochloroform solution.[7] This dichotomy, we believe, is another demonstration of the torsionally disarming power[28] of the 4,6-*O*-benzylidene protecting group. In β-mannosylation by the sulfoxide method, this protecting group is essential and functions by destabilizing the anomeric oxacarbenium ion with respect to the covalent glycosyl triflate.[14] As anomerization of the glycosyl phosphate proceeds via the same oxacarbenium ion, the configurational stability of **12** and **34** is readily understood.

In conclusion, we have described a synthesis of the β-mannosyl phosphoisoprenoid **1** from *Mycobacterium avium* and, by comparison of its mass spectral fragmentation patterns with those of the original isolate, confirm the gross structure and connectivity of this substance.

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Supporting Information Available: Full experimental details together with copies of ^{1}H and ^{13}C NMR spectra for compounds **1**, **3**, **7**, **8**, **11**−**13**, **15**, **19**−**21**, **24**, **25**, **29**, **32**−**34** and the mass spectra of synthetic **1** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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