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Total Synthesis of Enantiopure β-D-Mannosyl Phosphomycoketides from *Mycobacterium tuberculosis*

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β-Mannosyl phosphomycoketides (MPMs) **1** and **2** are potent mycobacterial antigens for T cells that were isolated in 2000 from *Mycobacterium tuberculosis* and *Mycobacterium avium*, respectively (Figure 1).[1] Initial structural characterization did not include assignment of the stereochemistry of the highly unusual saturated oligoisoprenoid chain. However, the recent elucidation of the biosynthetic pathway of the side chain shows that all methyl-substituted stereogenic centers are introduced via the same mechanism through the iterative action of a polyketide synthase.[2]

In 2002, a synthesis of **2** was reported, confirming the overall molecular structure.[3] However, as a stereorandom alkyl chain was used, the stereochemistry of the lipid part remained unsolved. Since the side chain is the only structural feature that distinguishes foreign antigens **1** and **2** from endogenous β-mannosyl phosphodolichols, its fine structure must be the basis on which the immune system discriminates. Further insight into the relationship between structure and activity requires a general synthetic protocol to synthesize MPMs with lipid chains of any desired length and stereochemistry.

Our efforts toward a stereoselective total synthesis of **1** were inspired by the notion that it would be an ideal target to test our recently reported conjugate addition (CA) protocols. As outlined in the retrosynthetic scheme (Figure 1), we envisaged that **1** could be assembled by connection of four chiral building blocks (**3**−**6**). Fragments **4** and **5** can both be constructed starting from the same versatile saturated isoprenoid building block **8**. Recently, we disclosed an enantioselective protocol to access all diastereoisomers of **8** using Cu−phosphoramidite-catalyzed iterative CA of Me_2Zn to cycloocta-2,7-dienone, followed by oxidative ring opening.[4] To prepare fragment **3**, we planned to use the Cu-catalyzed 1,4-addition of MeMgBr to linear α,β-unsaturated thioesters as the key step.[5]

The strategy, as outlined above, is both highly convergent and general for the assembly of enantiopure oligoisoprenoid chains of any desired length and configuration. However, as the stereogenic centers are introduced at a relatively early stage, it is essential that the valuable linear fragments **3**−**5** are cross-coupled in an efficient manner. To this end, we examined several carbon−carbon bond-forming reactions (vide infra). Eventually, a Julia−Kocienski sequence proved to be the best method.[6]

Coupling of the resulting alkyl chain to mannose via a β-mannosyl phosphate linkage is a challenge in itself, considering the known difficulty of generating such linkages. Application of the elegant β-mannosylation protocol developed by Crich and Dudkin was in our case undesirable, as it would require 3 equiv of precious side chain.[3] We therefore decided to first install a β-phosphate moiety on a suitably protected mannopyranose (**6**) and then to couple to the aliphatic chain. Incidentally, this strategy also reduced the overall sequence with four steps and made the α-phosphate analogue accessible.

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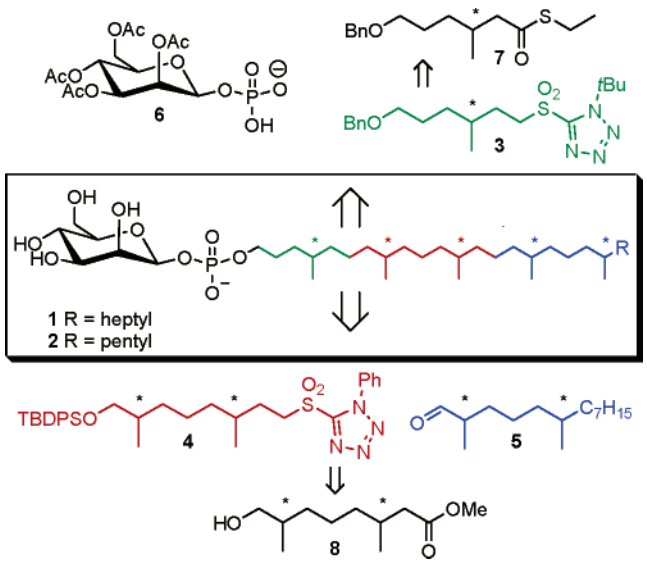
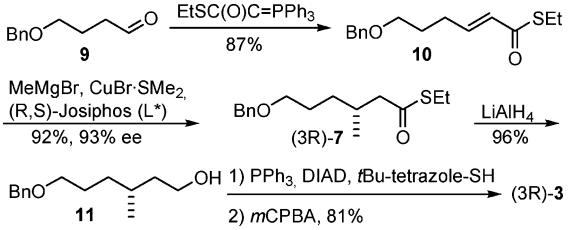


Figure 1. Structures of **1** and **2** and retrosynthetic analysis.

The construction of building block **3** started with a Wittig reaction of the readily accessible aldehyde **9** with $PPh_3CHCOSEt$, to afford α,β-unsaturated thioester **10** (Scheme 1).[7] CA of MeMgBr to *trans*-**10** in the presence of $CuBr{\cdot}SMe_2$ and chiral ligand L* resulted in the formation of (3*R*)-**7** with excellent selectivity (93% ee).[5] $LiAlH_4$ reduction of the thioester proceeded readily to provide the corresponding primary alcohol **11**. Finally, **11** was converted into sulfone (3*R*)-**3** via Mitsunobu reaction with 1-*tert*-butyltetrazole-5-thiol, followed by oxidation with *m*CPBA.[8]

Scheme 1. Catalytic Asymmetric Synthesis of Fragment (3*R*)-**3**



To prepare fragments **4** and **5** (Scheme 2), the hydroxyl moiety of (3*R*,7*S*)-**8**[4] was protected as a silyl ether, after which the methyl ester was reduced to afford the common precursor **12**. Sulfone (3*R*,7*S*)-**4** was subsequently obtained from **12** analogously to the synthesis of **3** from **11**. For the assembly of **5**, **12** was converted into the corresponding tosylate and then subjected to a Cu-catalyzed chain elongation reaction with *n*-pentylmagnesium bromide to give **13**. Finally, treatment of **13** with TBAF furnished the primary alcohol, which was oxidized with TPAP/NMO to give aldehyde (2*S*,6*S*)-**5**.[9]

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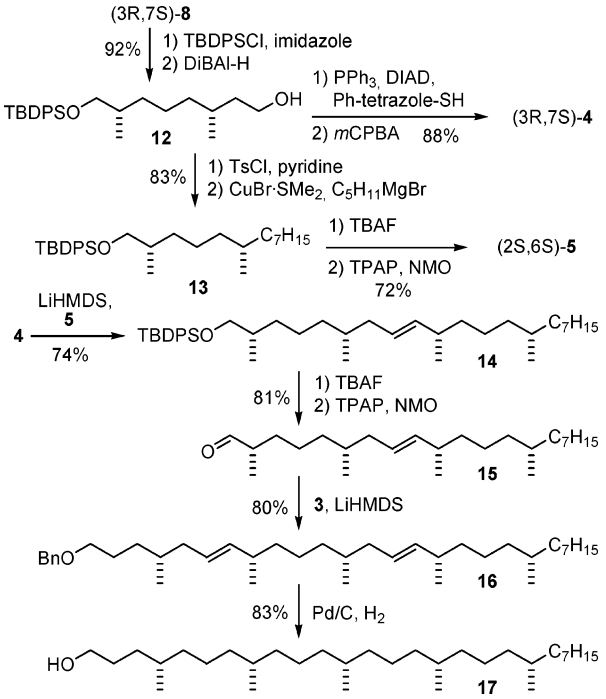
Scheme 2. Synthesis of Fragments **4** and **5** and Cross-Coupling

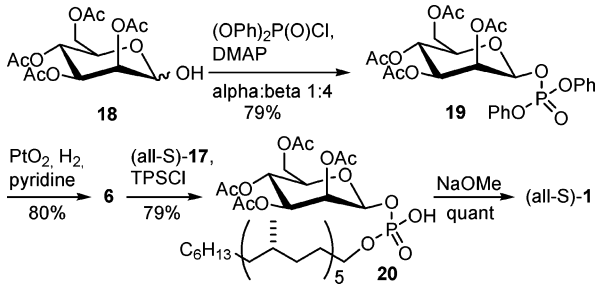


Coupling of **4** with a small excess of **5** (1.15 equiv) in the presence of LiHMDS (1.05 equiv) yielded diisoprenoid **14** (predominantly trans) in a rewarding 74%. The highest yields were obtained when **4** and **5** were mixed prior to addition of the base.[10] Similar procedures using Wittig or Horner−Wadsworth−Emmons analogues of **4** consistently gave inferior results. Direct formation of a C−C single bond by Cu-catalyzed cross-coupling of the corresponding alkyl halides either under Grignard conditions or in the presence of SmI_2 was highly unreliable and invariably gave low yields in our hands.[11] To make **14** suitable for coupling to fragment **3**, the primary alcohol was deprotected and oxidized to give aldehyde **15** (cf. conversion of **13** into **5**).

Julia−Kocienski coupling of **3** (1.2 equiv) to **15** using LiHMDS (1.1 equiv) as the base yielded **16** (predominantly trans) in a satisfying 80% yield. Exposure to hydrogen over Pd/C simultaneously deprotected the primary alcohol and hydrogenated the double bonds to give saturated isoprenoid (*all-S*)-**17**.[12]

Assembly of fragment **6** (Scheme 3) started with the reaction of hemiacetal **18** with limiting diphenyl chlorophosphate in the presence of DMAP to give diphenyl β-mannosyl phosphate **19**.[13]

Scheme 3. Synthesis of Fragment **6** and Coupling to (*all-S*)-**17**



Hydrogenation over Adams' catalyst, followed by quenching with pyridine, afforded pyridinium β-mannopyranosyl phosphate salt **6**.

We were pleased to find that coupling of a 2-fold excess of readily available **6** to (*all-S*)-**17** (1.0 equiv) in the presence of 2,4,6-triisopropylbenzenesulfonyl chloride (3.0 equiv) proceeded well to give **20** in a gratifying 79% yield.[14] Finally, deacetylation using NaOMe gave the target molecule (*all-S*)-**1** in quantitative yield.[15]

Biological evaluation of (*all-S*)-**1** revealed that its antigenic potency for T cells is identical to that of the natural product within the margin of error, while stereorandom **2** is significantly less potent.[16] The latter result implies that the stereochemistry of the lipid part has an unexpectedly strong influence on T cell response.

In summary, we disclose the first (general) procedure for the total synthesis of enantiopure MPMs. To this end, a fully catalytic method to synthesize saturated oligoisoprenoids was developed. In addition, an alternative approach for the formation of the β-mannosyl phosphate linkage was shown to be successful. The synthetic viability of this new protocol is demonstrated by the concise total synthesis of (*all-S*)-**1** with an overall yield of 6.7% and a longest linear sequence of 18 steps.

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Supporting Information Available: Detailed experimental procedures and spectroscopic (^{1}H and ^{13}C NMR) and analytical data of all reaction products. This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) The anomeric configuration of (*all-S*)-**1** was unequivocally determined by NOE correlation between the anomeric hydrogen and H's 3 and 5 of the sugar moiety and by a cross-ring fragmentation (*m*/*z* 587.5) and a dehydration (*m*/*z* 689.5) in the low-energy ESI CID spectrum. Additional support was given by comparison of the chemical shift of the anomeric proton of **1** with that of the anomeric proton of the independently synthesized (*all-S*)-α-**1** analogue as well as by comparison of the $^1J_{CH}$ coupling of both anomers (α = 169 Hz, β = 159 Hz).
(16) Bioassays were conducted at the Brigham and Women's Hospital, Harvard Medical School. Details will be reported in due course.

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