Total Synthesis of the Phenolic Glycolipid Mycoside B and the Glycosylated p-Hydroxybenzoic Acid Methyl Ester HBAD-I, Virulence Markers of *Mycobacterium tuberculosis*


**Keywords:** Natural products / Carbohydrates / Glycolipids / Asymmetric synthesis / Total synthesis

The phenolic glycolipid mycoside B, present in *Mycobacterium tuberculosis* and hypervirulent strains of *Mycobacterium bovis*, has been synthesized for the first time. Multiple methyl groups were introduced by the extensive use of catalytic asymmetric 1,4-addition reactions, asymmetric hydrogenation of a β-keto ester afforded the basis for the central 1,3-diol moiety, and introduction of the 2-O-methyl-α-L-rhamnoside unit was achieved by stereoselective glycosylation with p-iodophenol and subsequent Sonogashira coupling, providing a basis for the generation of analogues. In addition, the related monosaccharide HBAD-I, present in the same species, has been efficiently synthesized for the first time by selective methylation of the hydroxy group at C-2 of a rhamnoside.

**Introduction**

Tuberculosis (TB) is today still one of the most important infectious diseases. Although antibiotics and a vaccine (Bacillus Calmette–Guérin, BCG) are available, the number of cases reveals that the disease continues to be a global problem: 8 million casualties and 1.5 million deaths every year.[1] The synergy with HIV and the advent of new multidrug-resistant and hypervirulent strains poses additional threats.[2] *Mycobacterium tuberculosis* (*M. tuberculosis*) is the most prominent cause of TB, but the related species, *Mycobacterium bovis* (*M. bovis*), can also cause the disease. The disclosure of the full genome sequence of *M. tuberculosis* H37Rv[3] has allowed scientists to identify the enzymes involved in the biosynthesis and transport of secondary metabolites that are important in host–pathogen interactions. Furthermore, knockout experiments have shed light on the specific role of a number of these compounds. The most prominent representatives are the complex methyl-branched lipids with long alkyl chains, often glycosylated.[4] This is also the case in the phenolic glycolipids (PGLs), which are suspected of being involved in the hypervirulence of specific mycobacterial strains.[5]

Monoglycosylated mycoside B (Figure 1, 1) is the major PGL in *M. bovis*.[6] This compound is also found as a minor PGL in some specific strains of *M. tuberculosis*[6c] in which triglycosyl PGL-tb1 is the predominant species.[7] PGLs are among the most specific virulence factors and permit the strain causing an infection to be deduced and discrimination between recent infection and vaccination. Furthermore, it has been shown that not all *Mycobacterium bovis* BCG strains used for vaccination are able to produce mycoside B and phthiocerol dimycocerosates (PDIMs), providing a correlation between the ability to produce these compounds and the risk of complications after vaccination observed in clinical studies.[8] In this regard, an enzyme-linked immunosorbent assay (ELISA) based on PGLs has recently shown potential for the diagnosis of TB in HIV-infected patients.[9] In addition, a lipidomic platform has been established for the chemotaxonomic analysis of *M. tuberculosis*.[10]

The variation in the structures of the different mycobacterial PGLs mainly resides in the glycosyl terminus. Differences in occurrence are found between species of the genus, but also occur within strains of the same species. For example, minor PGLs, different to mycoside B and PGL-tb1, have been found in some strains of *M. tuberculosis*, which has biological implications.[11] Because access to pure, chemically synthesized PGLs is crucial for reliable immunological studies, a synthetic strategy to readily obtain with minimal changes the different PGLs is desirable. We recently communicated the first total synthesis of trisaccharide PGL-tb1.[12] The key elements of the strategy are the modularity, the high control over the stereoselectivity, and the introduction of the glycosyl moiety (the most variable part of the PGLs) by a late-stage Sonogashira reaction. Here, a full account of this work is presented combined with a second application of this strategy to the synthesis of mycoside B.
Aryl rhamnoside HBAD-I (2, Figure 1) is clearly closely related to mycoside B. The compound has been detected in the culture medium of all reference strains of *M. tuberculosis* and *M. bovis*.\(^{[13]}\) According to Daffé and co-workers, \(p\)-hydroxybenzoic acid is the precursor of both 1 and 2. However, it is remarkable that 2 and its acid form are not precursors of 1. Thus, compound 2 is a final metabolite of both mycobacteria. Concerning the biosynthesis of these compounds, Daffé and co-workers proposed that the Pks15/1 gene is responsible for the elongation of the \(p\)-hydroxybenzoic acid to form a \(p\)-hydroxyphenylalkanoate.\(^{[13,14]}\) The latter compound is transformed by the gene PpsA-E into the phenolphthiocerol unit. Daffé and co-workers disclosed that mutations in pks15/1 are responsible for the lack of phenolic glycolipids in PGL-deficient *M. tuberculosis* strains. Glycosylation is achieved by the use of Rv2962c encoded enzymes in the final step of the biosynthesis of both 1 and 2.

Note, the secretion of methyl benzoate 2 and its analogue triglycosyl methyl benzoate appears to be highly relevant. Studies with knockout bacteria have shown that the inhibited production of some or all \(p\)-HBADs is inter-related to an increased secretion of pro-inflammatory cytokines.\(^{[15]}\) In other words, \(p\)-HBADs seem to attenuate the immune response of the host. Given the immunological interest and the connection of 2 to mycoside B, we decided to synthesize 2 as well.
**Results and Discussion**

The dissimilar nature of the different parts of mycoside B calls for a strongly convergent synthetic strategy. The retrosynthesis we devised for mycoside B (1) parallels the one used in the synthesis of PGL-tb1 and divides this complex molecule into three main building blocks: rhamnoside 3, mycocerosic acid (4), and diol 5, as summarized in Scheme 1.

The first of these units, 2-O-methyl-α-L-rhamnoside 3, appropriately benzylated (see above), is equipped with a p-iodophenol unit. This functionalization allows the carbohydrate to be installed through a Sonogashira coupling reaction instead of by glycosylation. In our view, this should simplify the connection of the carbohydrate to the lipid chain. No less important, the α-anomeric center at the anomeric center could exclusively be ensured at an earlier stage of the synthesis.

The synthesis of building block 5 faces two main challenges. One is the selective generation of the different stereocenters, there being a significant distance between them. To solve this problem we relied on a one-pot 1,4-addition/alkylation reaction by using 2-cycloheptenone (6) as the starting material. The aforementioned approach provides the first two stereocenters on the right hand side of the molecule and bridges the gap between these and the anti-1,3-diol unit, in turn prepared by asymmetric Ru(II)-catalyzed hydrogenation of a β-keto ester followed by alkylation and subsequent reduction.

The other challenge encountered in 5 is the 16-carbon spacer that has to connect the 1,3-diol moiety with the aromatic ring in rhamnoside 3. It was found that such an α,ω-difunctionalized alkyl chain suitable for a Sonogashira reaction was readily accessible from commercially available alkynol 7.

The third main block, mycocerosic acid (4), is one of several methyl-branched fatty acids from *M. tuberculosis* that we have synthesized over the years by using an iterative protocol comprising catalytic asymmetric conjugate addition, reduction, and Horner–Wadsworth–Emmons olefination. Conveniently, esterification of the 1,3-diol moiety was planned for the last-but-one step of our synthesis, due to the precious nature of the all-R-tetramethyl-branched acid 4. Finally, concomitant reduction of the triple bond and hydrogenolysis of the benzyl protecting groups should deliver mycoside B.

In parallel with the synthesis of PDIM-A and PGL-tb1, the synthesis of mycoside B commenced with building block 6 (Scheme 2). Thus, 2-cycloheptenone (6) was transformed into 9 in a one-pot catalytic asymmetric 1,4-addition of Me2Zn followed by in situ alkylation with iodoethane. Use of a catalyst prepared in situ from CuII triflate and a chiral phosphoramidite ligand (Feringa’s ligand) afforded 9 in an *er* of 97.5:2.5. The *dr* of the alkylation is >20:1, considerably higher than generally observed in the corresponding six-membered-ring systems. We have also noted these excellent diastereoselectivities previously in eight-membered-ring systems. A regioselective and stereospecific Baeyer–Villiger reaction served to insert the oxygen at the stereogenic center bearing the ethyl substituent. Despite extensive experimentation, the best result obtained with the Baeyer–Villiger reaction was a moderate 60% yield, achieved by using *m*CPBA. Undoubtedly this outcome is due to the increase in ring strain incurred on progressing from a seven- to an eight-membered ring, which renders the C-to-O shift in the reaction unfavorable. Versatile solutions to this problem are scarce, and also enzymatic Baeyer–Villiger reactions are invariably carried out on six-membered-ring systems or substrates for which ring strain is relieved. An accompanying problem is that more reactive congeners of *m*CPBA raise safety concerns. After methanalysis of 10, methyl ester 11 was O-methylated to give ester 12, thereby completing the construction of the right-hand side of 5.

Ther reduction of 12 with DIBAL–H gave directly aldehyde 13, which was smoothly transformed into intermediate β-keto ester 14 by using ethyl diazoacetate and 5 mol-% of NbCl5 as catalyst. This opened the door for the anticipated asymmetric hydrogenation. By using 1 mol-% of (R)-(RuCl(tol-BINAP))2(μ-Cl)3[NH3Me2] and a hydrogen pressure of 20 bar, we were rewarded with β-hydroxy ester 15 in good yield and with an excellent stereoselectivity.
Total Synthesis of Mycoside B and HBAD-I

Scheme 2. Reagents and conditions: a) Cu(OTf)2 (0.5 mol-%), (S,R,R)-phosphoramidite (1 mol-%), Me2Zn, toluene, –25 °C; then EtI, HMPA, 0 °C, 83%, >20:1 dr trans: cis, 97.5:2.5 er (for trans); b) mCPBA, CH2Cl2, reflux, 60%; c) K2CO3, MeOH, room temp., 90%; d) NaH, MeI, DMF, room temp., 92%; e) DIBAL-H, Et2O, –84 °C, 86%; f) ethyl diazoacetate, NbCl5 (5 mol-%), CH2Cl2, 86%; g) (R)-([RuCl(tol-BINAP)]2(μ-Cl))3[NH2Me2] (1 mol-%), 20 bar H2, EtOH, room temp., 76%, dr >99:1; h) AlMe3, MeNH(OMe) · HCl, THF, 73%. HMPA = hexamethylphosphoramide, mCPBA = m-chloroperbenzoic acid, DIBAL-H = diisobutylaluminium hydride.

Scheme 3. Reagents and conditions: a) NaH, 1,3-diaminopropane, 70 °C, 61%; b) nBuLi, TMSCl, THF, –40 °C to room temp., 92%; c) pTsCl, pyridine, CHCl3, room temp., 87%; d) NaI, acetone, room temp., 86%.

The addition of 20, after the formation of the corresponding alkyl Li reagent, to Weinreb amide 16 turned out not to be trivial (Scheme 4). First, a stepwise procedure with initial deprotonation of the hydroxy group in 16 was investigated. The deprotonation is important as it avoids elimination to the corresponding unsaturated amide. However, tBuLi could be used for this purpose as long as the amount of base did not exceed 1 equiv., otherwise the excess reagent formed a tert-butyl ketone by nucleophilic attack on the amide. At the same time, tBuLi was used to lithiate 20 by Li/halogen exchange. For this reason, the added amount of tBuLi for the exchange had to be carefully controlled to prevent any excess leading to the undesired formation of the inseparable tert-butyl ketone. Optimization showed that a ratio of 20/tBuLi of 1:1.6 for the lithiation avoided that problem. For reasons of simplification and ro-
bustness, we finally decided to discard the stepwise process and use an excess of lithiated 20 for both deprotonation and alkylation. After extensive optimization, we were very pleased to see the exclusive formation of the desired β-hydroxy ketone 21 in 81% yield.

Having both defined the third stereocenter and installed the spacer, the 1,3-diol in the required anti configuration had to be prepared from the β-hydroxycarbonyl moiety. Initially we applied the method reported recently by Phansavath and co-workers by diastereoselective hydrogenation with catalytic RuCl₃ and PPh₃. Although this provided the desired anti-diol, it also unfortunately resulted in concomitant reduction of the triple bond. Fortunately, the methodology of Evans and Chapman using NH₄BH(OAc)₃ gave 22 in good yield. The solvent for the reaction had to be adapted to secure sufficient solubility. With a view to the subsequent Sonogashira reaction, the TMS group had to be removed. Stirring 22 in MeOH/K₂CO₃ completed this step to give the central building block 5 in quantitative yield.

At this point we turned our attention to the synthesis of rhamnoside 3. The major challenge was to selectively methyate the hydroxy group at C-2. This was achieved by a strategy reported by Fürstner and Müller (Scheme 5). Peracetylation of L-rhamnose was followed by the formation of rhamnosyl bromide and subsequent reflux in ethanol with sym-collidine as an acid scavenger to provide orthoester 25. One-pot acetate hydrolysis and benzylzation of the hydroxy groups at C-3 and C-4 provided 26, which, upon acidic hydrolysis, afforded a mixture of acetates 27 at C-1 and C-2. Separation was not necessary because under the conditions used for the formation of trichloroacetimidate 28, both acetates rapidly interconvert, but only the rhamnoside with the acetate at C-2 is transformed into 28.
Although the formation of 28 has previously been achieved with Cs₂CO₃ as a catalyst, we were not satisfied with the reported yield. The use of NaH as base greatly improved the yield and gave 28 as an 87:13 mixture of α and β anomers in 86% yield.[31]

The next step was the glycosylation of 28 with 4-iodophenol. A high α selectivity was ensured by participation of the acetyl group at C-2, which afforded 29 in 90% yield. Although we chose to use pure 28α for the glycosylation, 28β can also be used in this step with a similar result. Finally, the 2-acetyl group was methanolyzed and the resulting hydroxy function methylated to give 3 in high yield.

With both 5 and 3 in hand we proceeded with the Sonogashira reaction, a key step in our synthesis (Scheme 6). Pleasingly, the use of catalytic [PdCl₂(PPh₃)₂] and CuI gave 31 in 91% yield.

The synthesis of mycocerosic acid (4) in 12% yield over 15 steps has previously been reported by our group.[17c] Our methodology for the stereoselective synthesis of 1,3-methyl arrays was based on an iterative protocol. The first step involves a highly enantioselective Cu-catalyzed conjugate addition of MeMgBr to an α,β-unsaturated thioester (starting with achiral compound 8). The reduction of the thioester to its aldehyde followed by olefination elongates the chain and leads to a new α,β-unsaturated thioester. For this synthesis of mycocerosic acid we applied the refinements developed in the synthesis of mycolipenic acid, using DI-BAL-H for the reduction (replacing the Pd/C/Et₃SiH system) and Horner–Wadsworth–Emmons instead of Wittig olefinations.[17d,17e]

Esterification of 31 with mycocerosic acid (4) was accomplished by employing EDC and DMAP in a moderate but acceptable 60% yield. As planned, the triple bond in ester 32 was completely reduced with Pd/C and 1 bar of H₂ with simultaneous removal of both benzyl protecting groups in the final step. In this way, mycoside B was prepared in 14 steps in an overall yield of 4.3% (linear sequence starting from cycloheptenone 6). Full analysis of the resulting compound by NMR spectroscopy, MS, and optical rotation showed 1 to be identical to natural mycoside B.[6]

With the experience gained in the synthesis of building block 3, a synthetic route to p-HBAD-I (2) was planned. Most likely, methoxycarbonylation of 3 followed by debenzylation would provide 2. However, orthogonal protection with benzyl groups, as in the synthesis of mycoside B, was not required in the synthesis of p-HBAD-I. Therefore we opted for an alternative and more efficient synthesis.

Again, methylation at O-2 was the key and for this, selective protection of 3-OH and 4-OH was required. Unfortunately, methods that simultaneously protect two hydroxy groups in l-rhamnose-like acetals and orthoesters lead to cis-fused five-membered rings involving O-2 and O-3. Protection of diequatorial trans-1,2-diols with 1,2-diacetals according to Ley and co-workers, however, seemed to be a perfect alternative to protect 3-OH and 4-OH.[32] To achieve this, Frost modification of Ley’s methodology using 2,2,3,3-tetramethoxybutane was most convenient in terms of cost, ease of preparation, and selectivity.[33]

Therefore peracetylated l-rhamnose 23 was subjected to BF₃·OEt₂-mediated glycosylation with methyl p-hydroxybenzoate (Scheme 7). Upon removal of the acetyl groups, triol 34 was successfully protected at O-3 and O-4 with 2,2,3,3-tetramethoxybutane, and O-2 was subsequently methylated. Both the protection and methylation steps were carried out in a convenient one-pot procedure in a moderate 43% yield.[34] Treatment with TFA/water afforded the desired p-HBAD-I (2) in 50% yield. In retrospect, this
route, comprising only five steps, could function as an alternative to the synthesis of building block 3, requiring only benzylation at O-3 and O-4. Increasing the yield of the sequence 34→35→3 requires attention, however.

Conclusions

A first synthesis of mycoside B (1) has been achieved. The approach provides full stereocontrol; Cu-catalyzed asymmetric conjugate addition of MeMgBr and Me2Zn introduces the methyl groups into the mycocerosic acid and the phthiocerol chain and RuII-catalyzed stereoselective hydrogenation followed by anti reduction defines the stereochemistry of the 1,3-diol. In the final stages of the synthesis, Sonogashira cross-coupling proved to be a versatile tool for connecting the glycosyl moiety. Taken together, this demonstrates the applicability of our approach to the synthesis of PGL-tb1 and mycoside B and therefore could be applied to the synthesis of other PGLs found in mycobacteria. In addition, p-HBAD-I (2) has been synthesized for the first time. Ley-type protection provides the desired compound in only five steps from rhamnose, and this should elicit further studies on the role of this compound in the attenuation of the host immune response.

Experimental Section

All reactions were performed by using oven- or flame-dried glassware and dry solvents. Solvents were distilled prior to use: MTBE, Et2O, and THF over Na/benzophenone, and CH2Cl2 over CaH2. All other reagents were purchased from Sigma–Aldrich, Acros, TCI Europe, Alfa Aesar, Merck, and Apollo Scientific, and used without further purification unless noted otherwise. Grignard and organolithium reagents were titrated by using s-BuOH and catalytic amounts of 1,10-phenanthroline. All moisture-sensitive reactions were performed under nitrogen.

1H and 13C NMR spectra were recorded with a Varian AMX400 or 400-MR spectrometer (400 and 100.59 MHz, respectively) using CDCl3 or CD3OD as solvent, unless stated otherwise. Chemical shifts are reported in ppm with the solvent resonance as the internal standard (CHCl3: δ = 7.26 ppm for 1H, δ = 77.0 ppm for 13C; CD3OD: δ = 3.31 ppm for 1H). Data are reported as follows: chemical shift (δ), multiplicity (s = singlet, d = doublet, dd = double doublet, tt = triple doublet, t = triplet, q = quartet, br. = broad, m = multiplet), coupling constants J, and integration. Due to the (multiple) long alkyl chains in some of the compounds, we were not able to resolve all the individual 13C signals in the spectra. High-resolution mass spectra were recorded with a Thermo Scientific LTQ Orbitrap. Optical rotations were measured with a Propol automatic polarimeter (sodium D line, λ = 589 nm). HPLC analysis was performed with a Chiralcel AD 250 × 4.6 mm column with an ELS detector (ELSD).

Flash chromatography was performed by using SiliCycle SiliaFlash P60 (230–400 mesh), as obtained from Screening Devices, or by automated column chromatography using a Reveleris flash system purchased from Grace Davison Discovery Sciences. TLC analysis was performed on Merck silica gel 60/Kieselguhr F254 (0.25 mm). Compounds were visualized by using either Seebach’s reagent [a mixture of phosphomolybdic acid (25 g), cerium(IV) sulfate (7.5 g), H2O (500 mL), and H2SO4 (25 mL)] or a KMnO4 stain [K2CO3 (40 g), KMnO4 (40 g), K2CO3 (6 g), H2O (600 mL), and 10% NaOH (5 mL)].

(6S,7R)-7-Methoxy-6-methylnonanal (13): For the complete synthesis of building block 12 from 2-cyclohepten-1-one (6), see ref[18] Diisobutylaluminium hydride (1 m in CH2Cl2, 4.33 mL, 4.33 mmol) was added dropwise to a stirred solution of methyl (6S,7R)-7-methoxy-6-methylnonanoate (12)[18] (780 mg, 3.61 mmol) in Et2O (10 mL) at –84 °C under nitrogen. After completion of the reaction (20 min), as indicated by TLC (pentane/Et2O, 85:15), a few drops of MeOH were added to quench the reaction and the solution was warmed to room temp. A saturated solution of Rochelle’s salt (3 mL) was added and the mixture was stirred for 30 min. Then the reaction mixture was diluted with Et2O (40 mL), the layers were separated and the organic layer was washed with brine (5 mL) and dried (Na2SO4). The solvent was removed under reduced pressure.
and the product was purified by flash chromatography to give aldehyde 13 (560 mg, 3.01 mmol, 89% yield) as a colorless oil together with a small amount of the related alcohol (18 mg, 3% yield). The spectroscopic data were in agreement with a previous report.\[18\]

**Ethyl (8S,9R)-9-Methoxy-8-methyl-3-oxoundecanoate (14):** NbCl₅ (38.9 mg, 0.144 mmol) was added to a stirred solution of aldehyde 13 (536 mg, 2.88 mmol) in CH₂Cl₂ (30 mL) under nitrogen. After 5 min, ethyl diazoacetate (0.527 mL, 4.32 mmol) was added dropwise to the solution, taking care with the gas evolution. After completion of the reaction (2 h), as indicated by TLC (pentane/Et₂O, 3:7, 4 h), the reaction was quenched by adding 20% Rochelle's salt aqueous solution (5 mL) and the mixture was stirred for 30 min. Then most of the THF was removed under reduced pressure and the product was purified by flash chromatography to give 14 (677 mg, 2.49 mmol, 86% yield) as a colorless oil. [δ]_{D}^{29}= -48.6 (c = 1.0, CHCl₃). [1H NMR (400 MHz, CDCl₃): δ = 4.01 (m, 1 H), 3.77 (s, 3 H), 3.68 (s, 3 H), 3.33 (s, 3 H), 3.19 (s, 3 H), 2.86 (ddd, J = 7.6, 5.0, 4.0 Hz, 1 H), 2.66 (d, J = 16.6 Hz, 1 H), 2.43 (dd, J = 17.0, 9.7 Hz, 1 H), 1.69 (d, J = 6.6 Hz, 1 H), 1.61–1.17 (m, 12 H), 1.17–1.04 (m, 1 H), 0.90 (t, J = 7.4 Hz, 3 H), 0.82 (d, J = 6.8 Hz, 3 H) ppm. 13C NMR (101 MHz, CDCl₃): δ = 173.6 (s), 86.4 (d), 67.6 (d), 61.0 (q), 57.1 (q), 38.0 (t), 36.4 (t), 34.5 (d), 32.4 (t), 31.5 (q), 27.3 (t), 25.6 (t), 22.1 (t), 14.5 (q), 9.8 (q) ppm. HRMS (ESI⁺): calcd. for C₁₅H₂₃O₄Na [M + Na]⁺ 290.2331; found 290.2314.]

**Hexadec-15-yn-1-ol (17):** NaH (60% in mineral oil, 6.41 g, 147 mmol) was washed with pentane and carefully added to a flask containing 1,3-diaminopropane (70 mL, highly hygroscopic). The mixture was heated at 50 °C until it became clear (approx. 1 h). Then the mixture was cooled to room temp. and alkynyl 7 (5.0 g, 21 mmol) was added. The mixture was then heated to 69 °C and stirred for 24 h. It was then poured into a beaker containing ice/water and extracted with Et₂O (3 × 75 mL). The combined organic extracts were washed with 0.1 M HCl (30 mL), brine, and dried (Na₂SO₄). The solvent was removed under reduced pressure and the product purified by flash chromatography to give 17 (3.05 g, 12.8 mmol, 61% yield) as a white solid. The spectroscopic data were in agreement with the literature.\[19\] [1H NMR (400 MHz, CDCl₃): δ = 3.60 (t, J = 6.7 Hz, 2 H), 2.15 (dt, J = 2.6, J = 7.1 Hz, 2 H), 1.91 (t, J = 2.6 Hz, 1 H), 1.67 (s, 1 H), 1.52 (m, 4 H), 1.38–1.24 (m, 20 H) ppm. 13C NMR (101 MHz, CDCl₃): δ = 84.6 (s), 67.9 (d), 62.8 (t), 32.6 (t), 29.56 (t), 29.54 (t), 29.52 (t), 29.43 (t), 29.37 (t), 29.03 (t), 28.6 (t), 28.4 (t), 25.6 (t), 18.3 (t) ppm.]

**16-(Trimethylsilyl)hexadec-15-yn-1-ol (18):** n-BuLi (1.6 M, 22 mL, 5.57 mL, 5.73 mL, 1.17 mL, 0.31 mL) was added to a solution of alkynol 17 (2.72 g, 11.4 mmol) in THF (150 mL) at –45 °C. The mixture was stirred for 3 h, allowing the reaction to slowly warm to room temp. during this time. TMSCl (3.2 mL, 5.11 mmol) was then added and the cloudy mixture became clear in a few minutes. The reaction was monitored by monitoring small samples of the reaction mixture with Et₂O/aq. NH₄Cl and analyzing the organic layer by TLC (pentane/EtOAc, 9:1). When full conversion was observed (TLC, ca. 1.5 h), aq. HCl (2 M, 5 mL) was added and the mixture was stirred for 30 min. Then most of the THF was removed under reduced pressure, water was added (60 mL), and the mixture was extracted with EtOAc (3 × 20 mL). The solvent was removed under reduced pressure and the mixture was purified by flash chromatography to give 18 (3.25 g, 10.58 mmol, 92% yield) as a colorless oil. [1H NMR (400 MHz, CDCl₃): δ = 3.62 (t, J = 6.7 Hz, 2 H), 2.19 (t, J = 7.2 Hz, 2 H), 1.58–1.44 (m, 5 H), 1.29 (m, 20 H), 0.13 (s, 9 H) ppm. 13C NMR (101 MHz, CDCl₃): δ = 107.8 (s), 84.2 (s), 63.0 (t), 32.8 (t), 29.6 (t), 29.58 (t), 29.56 (t), 29.45 (t), 29.41 (t), 28.76 (t), 28.60 (t), 25.7 (t), 19.8 (t), 0.2 (q) ppm. HRMS (ESI⁺): calcd. for C₁₉H₃₈O₅SiNa [M + Na]⁺ 333.2584; found 333.2581.]

**16-(Trimethylsilyl)hexadec-15-yn-4-Toluene-4-sulfonylchloride (19):** Alkyne 18 (2.3 g, 7.4 mmol) and pyridine (1.8 mL, 22 mmol) were dissolved in CHCl₃ (10 mL) and the mixture stirred at 0 °C. Then, p-toluene-sulfonyl)chloride (2.82 g, 14.5 mmol) was added and the mixture was stirred until full conversion was observed by TLC (5 h). Subsequently, the mixture was diluted with Et₂O (75 mL) and washed...
with water (10 mL),aq. HCl (2 mL, 10 mL), aq. NaHCO₃ (10 mL), and brine (10 mL). The organic extract was dried (MgSO₄), the solvent removed under reduced pressure, and the product purified by flash chromatography to give 19 (3.0 g, 6.5 mmol, 87% yield) as a colorless solid. ¹H NMR (400 MHz, CDCl₃): δ = 7.78 (d, J = 8.3 Hz, 2 H), 7.33 (d, J = 8.0 Hz, 2 H), 4.01 (t, J = 6.5 Hz, 2 H), 2.44 (s, 3 H), 2.20 (t, J = 7.2 Hz, 2 H), 1.62 (dt, J = 6.6, J = 13.5 Hz, 2 H), 1.50 (dt, J = 6.6, J = 13.5 Hz, 2 H), 1.40–1.01 (m, 20 H), 0.14 (s, 9 H) ppm. ¹³C NMR (101 MHz, CDCl₃): δ = 144.5 (s), 133.2 (s), 129.7 (d), 127.8 (d), 107.7 (s), 84.2 (s), 70.7 (d), 29.58 (d), 29.56 (d), 29.54 (d), 29.45 (d), 29.35 (d), 29.04 (d), 28.89 (d), 28.78 (d), 28.75 (d), 28.59 (d), 25.29 (d), 21.59 (d), 19.8 (d), 0.2 (q) ppm. HRMS (ESI⁺): calcd. for C₇₆H₇₄O₅SNa [M + Na]⁺ 487.2678; found 487.2686.

(16-Iodohexadec-1-ynyl)trimethylsilane (20): Tosylate 19 (1.71 g, 3.68 mmol) and sodium iodide (1.82 g, 12.1 mmol) were dissolved in Et₂O (20 mL) was added. The mixture was washed with aq. Na₂S₂O₃ (10%, 10 mL), water (10 mL), and brine (10 mL). Then the mixture was diluted with EtOAc (50 mL), most of the acetone was removed under reduced pressure and the product purified by flash chromatography to give compound 20 (1.33 g, 3.16 mmol, 86% yield) as a colorless oil. ¹H NMR (400 MHz, CDCl₃): δ = 212.6 (s), 108.7 (s), 86.6 (d), 84.2 (s), 67.6 (d), 57.3 (q), 48.9 (s), 43.7 (t), 36.4 (t), 34.8 (d), 32.6 (t), 29.59 (d), 29.55 (d), 29.54 (d), 29.44 (t), 29.41 (t), 29.34 (t), 29.13 (t), 29.0 (t), 28.8 (t), 28.6 (t), 27.4 (t), 25.8 (t), 23.6 (t), 22.3 (t), 19.8 (t), 14.8 (q), 10.0 (q), 0.1 (q) ppm. HRMS (ESI⁺): calcd. for C₂₆H₄₄O₃SiNa [M + Na]⁺ 443.1607; found 443.1609.

(3R,4S,9R,11R)-9-Hydroxy-3-methoxy-4-methyl-heptacos-26-yn-11-one (21): tBuLi (1.7 M, 3.58 mL, 6.08 mmol) was added to a stirred solution of iodoalkyne 22 (484 mg, 0.926 mmol, 81% yield) as a colorless oil. ¹H NMR (400 MHz, CDCl₃): δ = 4.60 (m, 1 H), 3.31 (s, J = 0.6 Hz, 3 H), 3.07 (s, 1 H), 2.85 (d(d, J = 7.7, 5.2, 4.2 Hz, 1 H), 2.57 (d, J = 17.4, 2.6 Hz, 1 H), 2.46 (dd, J = 17.4, 8.4 Hz, 1 H), 2.25 (t, J = 7.4 Hz, 2 H), 2.19 (t, J = 7.1 Hz, 1 H), 1.72–1.62 (m, 1 H), 1.60–1.32 (m, 13 H), 1.31–1.15 (m, 20 H), 1.13–1.01 (m, 1 H), 0.89 (t, J = 7.4 Hz, 3 H), 0.81 (d, J = 6.7 Hz, 3 H), 0.12 (s, 9 H) ppm. ¹³C NMR (101 MHz, CDCl₃): δ = 212.6 (s), 107.7 (s), 86.6 (d), 84.2 (s), 67.6 (d), 57.3 (q), 48.9 (s), 43.7 (t), 36.4 (t), 34.8 (d), 32.6 (t), 29.59 (d), 29.55 (d), 29.54 (d), 29.44 (t), 29.41 (t), 29.34 (t), 29.13 (t), 29.0 (t), 28.8 (t), 28.6 (t), 27.4 (t), 25.8 (t), 23.6 (t), 22.3 (t), 19.8 (t), 14.8 (q), 10.0 (q), 0.1 (q) ppm. HRMS (ESI⁺): calcd. for C₃₃H₅₄O₅SiNa [M + Na]⁺ 545.4366; found 545.4355.
mixture was stirred at 0 °C. Then Cl₂CCN (20.5 mL, 205 mmol, 7 equiv.) was added and the mixture stirred until consumption of the starting material (1.5 h). Silica gel (ca. 500 mg) was added to quench the reaction, and the mixture was filtered and concentrated under reduced pressure. The product was purified by flash chromatography to give compound 28a (11.7 g, 22.1 mmol, 75% yield) and its anomeric 28b (1.7 g, 3.2 mmol, 10.9% yield). The spectroscopic data are in agreement with those of previous reports for these compounds.³⁰

28a: ¹H NMR (400 MHz, CDCl₃): δ = 8.67 (s, 1 H), 7.40–7.29 (m, 10 H), 6.20 (d, J = 1.9 Hz, 1 H), 5.51 (dd, J = 3.3, 2.0 Hz, 1 H), 4.95 (d, J = 10.8 Hz, 1 H), 4.75 (d, J = 11.2 Hz, 1 H), 4.66 (d, J = 10.8 Hz, 1 H), 4.60 (d, J = 11.2 Hz, 1 H), 4.02 (dd, J = 9.5, 3.4 Hz, 1 H), 3.97 (dq, J = 9.8, 6.3 Hz, 1 H), 3.55 (t, J = 9.6 Hz, 1 H), 2.21 (s, 3 H), 1.38 (d, J = 6.2 Hz, 3 H) ppm. ¹³C NMR (101 MHz, CDCl₃): δ = 170.0 (s), 160.1 (s), 138.1 (s), 137.5 (s), 128.4 (d), 128.3 (d), 128.1 (d), 127.85 (d), 95.2 (d), 79.3 (d), 77.2 (d), 75.6 (t), 72.0 (t), 70.7 (d), 67.6 (d), 21.0 (g), 18.0 (q) ppm.

28b: ¹H NMR (400 MHz, CDCl₃): δ = 8.66 (s, 1 H), 7.51–7.18 (m, 10 H), 5.90 (d, J = 2.9 Hz, 2 H), 4.96 (d, J = 10.9 Hz, 1 H), 4.79 (d, J = 11.2 Hz, 1 H), 4.66 (d, J = 10.9 Hz, 1 H), 4.55 (d, J = 11.2 Hz, 1 H), 3.77 (dd, J = 8.7, 3.1 Hz, 1 H), 3.68–3.46 (m, 2 H), 2.22 (s, 3 H), 1.44 (d, J = 5.8 Hz, 3 H) ppm. ¹³C NMR (101 MHz, CDCl₃): δ = 170.23 (s), 160.17 (s), 138.07 (s), 137.35 (s), 128.39 (d), 128.32 (d), 128.07 (d), 129.75 (d), 127.82 (d), 95.04 (d), 79.49 (d), 79.01 (d), 75.35 (t), 72.79 (d), 71.49 (t), 66.47 (d), 20.85 (q), 17.79 (q) ppm.

The four compounds interconvert under these conditions and can converge into a single product. Similarly, both 28a and 28b can be used in the next step to give a very similar result.

*p-Iodophenyl 2-O-Acetyl-3,4-di-O-benzyl-a-L-rhamnopyranoside (29):* A solution of 28a (4.80 g, 9.04 mmol) in CH₂Cl₂ (15 mL) was added to a suspension of 4-iodophenol (3.78 g, 17.2 mmol) in CH₂Cl₂ (40 mL) at −45 °C. Then TMSOTf (163 μL, 0.9 mmol) was added and the mixture stirred while slowly warming to 20 °C. When 28a had been completely consumed (1.5 h), pyridine (1 mL) was added to quench the reaction. The mixture was filtered and the solvents removed under reduced pressure. The residue was diluted in EtOAc (300 mL) and washed with a 3 m NaOH solution (2 × 30 mL), water (20 mL), NH₄Cl (20 mL), and brine (20 mL). A stock solution of the catalyst was prepared by mixing PPh₃ (7.0 mg, 26.8 mol), CuI (10.2 mg, 53.6 mol) in freshly distilled Et₃N (8.9 mL) and stirring this solution at 40 °C for 15 min. Then this solution was added to a solution of 28a (58.2 mg, 128 mol) in freshly distilled EtOAc (8.8 mL) and stirring this solution at 0 °C until completion (1 h), quenched with a saturated solution of NH₄Cl (20 mL), and diluted with EtO (30 mL). The organic layer was washed with NH₄Cl (20 mL) and brine (20 mL), dried (Na₂SO₄), the solvent removed under reduced pressure, and the residue purified by flash chromatography to give compound 3 (2.0 g, 5.71 mmol, 95% yield) as a colorless oil. [α]D = −101.2 (c = 1.0, CHCl₃).

*p-Iodophenyl 3,4-Di-O-benzyl-a-L-rhamnopyranoside (3):* NaH (55% dispersion in mineral oil, 527 mg, 12.1 mmol) was added at 0 °C to a solution of 3 (3.30 g, 6.04 mmol) and methyl iodide (1.50 mL, 24.2 mmol) in DMF (30 mL). The mixture was stirred at 0 °C until completion (1 h), quenched with a saturated solution of NH₄Cl (20 mL), and diluted with EtO (30 mL). The organic layer was washed with NH₄Cl (20 mL) and brine (20 mL), dried (Na₂SO₄), the solvent removed under reduced pressure, and the residue purified by flash chromatography to give compound 3 (3.20 g, 5.71 mmol, 95% yield) as a colorless oil. [α]D = −101.2 (c = 1.0, CHCl₃).
in CDCl3 (0.6 mL) and the mixture was stirred overnight. After methylheptacos-1-ynyl[32]H2 was attached to the apparatus and the reaction was stirred for 17 mg, 0.32 mmol) was added to a solution of compound 33 (1.83 g, 12.04 mmol) in CH2Cl2 (20 mL). The mixture was stirred at room temp. until complete conversion was observed by TLC (2 h). The reaction was quenched by the addition of Amberlite H+ (10 g) and the mixture stirred for 5 min. The mixture was filtered, the solvent removed under reduced pressure, and the crude was purified by flash chromatography to give compound 34 (903 mg, 3.03 mmol, 94% yield) as a white solid, m.p. 44–46 °C. [α]D22 = –19.8 (c = 1.0, CHCl3). 1H NMR (400 MHz, CDCl3): δ = 1.8 Hz, 2 H), 1.98–0.79 (m, 176 H) ppm. 13C NMR (101 MHz, CDCl3): δ = 166.7 (s), 159.5 (s), 131.5 (s), 115.8 (s), 95.3 (d), 70.7 (d), 69.4 (d), 68.7 (d), 67.4 (d), 51.9 (q), 20.8 (q), 20.71 (q), 17.4 (q) ppm. HRMS (ESI†): calcld. for C20H24O10Na [M + Na]+ 1833.5673; found 1833.5672.

4-Methoxybenzoylphenyl α-L-Rhamnose (34): Sodium methoxide (17 mg, 0.32 mmol) was added to a solution of diester 32 (8.0 mg, 4.4 μmol) in a mixture of EtOAc (0.8 mL) and EtOH (0.2 mL) under nitrogen. A balloon filled with palladium on carbon (10% dry basis Degussa type B-5, 0.02 mmol) and DMAP (7.0 mg, 57 μmol) in a mixture of EtOAc (0.8 mL) and EtOH (0.2 mL) under nitrogen. The mixture was stirred until TLC analysis showed complete consumption (2 h). The reaction was quenched by adding HCl (1 M in EtOH solution, 0.2 mL), diluted with toluene, and the solvents partially removed under a nitrogen stream. The crude was purified by flash chromatography (pentane/EtO) from 2:1 to 1:1) to give compound 32 (8.0 mg, 4.4 μmol, 62% yield). [α]D22 = –35.0 (c = 0.4, CHCl3). 1H NMR (400 MHz, CDCl3): δ = 7.42 (d, J = 6.6 Hz, 2 H), 7.38–7.27 (m, 10 H), 6.93 (d, J = 8.7 Hz, 2 H), 5.51 (d, J = 1.8 Hz, 1 H), 4.95 (d, J = 10.8 Hz, 1 H), 4.8 (s, 2 H), 4.78 (s, 2 H), 4.64 (d, J = 10.8 Hz, 1 H), 4.04 (dd, J = 9.3, 3.1 Hz, 1 H), 3.78–3.65 (m, 2 H), 3.59 (t, J = 9.5, 8.2 Hz, 1 H), 3.56 (s, 3 H), 3.36 (s, 3 H), 3.28 (dt, J = 9.0, 4.6 Hz, 1 H), 2.65–2.43 (m, 2 H), 2.37 (t, J = 7.1 Hz, 2 H), 1.79–0.97 (m, 133 H), 0.96–0.77 (19 H) ppm. 13C NMR (101 MHz, CDCl3): δ = 176.04 (s), 175.97 (s), 155.5 (s), 138.4 (s), 138.3 (s), 132.8 (d), 128.42 (d), 128.36 (d), 128.0 (d), 127.9 (d), 127.74 (d), 127.66 (d), 117.9 (s), 116.1 (d), 95.4 (d), 89.3 (s), 86.7 (d), 80.3 (d), 80.0 (s), 79.6 (d), 78.0 (d), 75.5 (t), 72.5 (t), 70.3 (d), 68.8 (d), 59.6 (q), 57.4 (q), 45.3 (t), 41.0 (t), 37.7 (d), 36.6 (t), 34.8 (t), 32.62 (t), 31.93 (t), 31.87 (t), 31.69 (t), 30.99 (t), 29.94 (t), 29.79 (t), 29.72 (t), 29.66 (t), 29.58 (t), 29.55 (t), 29.36 (t), 29.34 (t), 28.05 (d), 27.49 (d), 27.21 (d), 26.97 (t), 25.56 (t), 25.16 (t), 22.69 (d), 22.35 (t), 20.76 (q), 20.46 (q), 20.42 (q), 20.39 (q), 18.43 (q), 17.56 (q), 14.72 (q), 14.12 (q), 10.10 (q) ppm. HRMS (ESI†): calcld. for C70H80O22Na [M + Na]+ 1875.9606; found 1875.9604.
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(70 mL) and washed with water (15 mL) and brine (15 mL). The organic phase was dried (Na2SO4) and the solvent removed under reduced pressure. The crude was purified by flash chromatography to give compound 35 (246 mg, 0.58 mmol, 43% yield) as a white solid containing 10% of a minor diastereomer (in the dioxolane ring), m.p. 176–178 °C. [α]D 22 = –254.2 (c = 1.0, CHCl3). 1H NMR (400 MHz, CDCl3): δ = 7.98 (d, J = 9.0 Hz, 2 H), 7.06 (d, J = 8.9 Hz, 2 H), 5.60 (d, J = 1.6 Hz, 1 H), 4.20–4.15 (m, 1 H), 3.87 (s, 3 H), 3.83–3.72 (m, 2 H), 3.65 (dd, J = 7.3 Hz, 3 H), 3.67 (dd, J = 1.0, CHCl3). 13C NMR (101 MHz, CDCl3): δ = 166.67 (s), 159.8 (s), 131.5 (d), 123.9 (s), 115.7 (d), 100.0 (s), 99.6 (s), 95.7 (d), 78.3 (d), 68.35 (d), 68.05 (d), 68.00 (d), 59.4 (q), 51.9 ppm. HRMS (ESI): calcd. for C29H36O10Na [M + Na]+ 335.1101; found 335.1107.

4-(Methoxycarbonyl)phenyl 2-


For some examples of the application of the zipper reaction in hazardous properties of chemical substances...