# Synthesis of a deuterated analogue of bacteriohopanetetrol–glucosamine, a probe of complex hopanoid biosynthesis

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This study reports the straightforward preparation of a deuterated biohopanoid that will be used to probe a new biosynthetic pathway. This multistep hemisynthesis required a stereoselective glycosylation, a regioselective deuteration as well as a properly defined strategy for the final deprotections and the purification of the amphiphilic final glycoside.

In contrast with most other common functionalities present in biomolecules, there is no general biochemical reaction explaining the formation of natural ethers. Surprisingly, some living organisms produce polyhydroxylated ethers along with their corresponding glycosides. This is the case for calditol, found in the archaean *Sulfolobus solfactaricus*,[1] and bacteriohopane derivatives **1** and **2** (Fig. 1), isolated from *Zymomonas mobilis*.[2–4] Triterpenoids of the hopane series are widespread amongst eubacteria. Composite hopanoids are the major constituents of the lipids of *Z. mobilis*, by far the most abundant being bacteriohopanetetrol derivatives **1** and **2**. We thus recently developed synthetic methods to construct biohopanoids to investigate both their biosynthesis and their biological function which are poorly understood.

A preliminary biosynthetic investigation led us to hypothesize that a glycoside such as **2** could be the precursor of ether **1** through a so far unknown enzymatic ring contraction (Scheme 1).[5] As a matter of fact we are also investigating the mechanism of the mycobacterial galactofuranose–pyranose interconversion, which is also a ring contraction.[6–10] At first sight, the process depicted in Scheme 1 appears analogous to the well-established inositol–synthase mechanism,[11] with, however, a very significant difference: the putative mechanism implied in the biosynthesis of cyclitol **1** from glycoside **2** would involve an *endo* opening of the sugar ring giving rise to an oxonium intermediate whereas the inositol synthase pathway involves the formation of an intermediate aldehyde leading to an intramolecular aldol process.[11]

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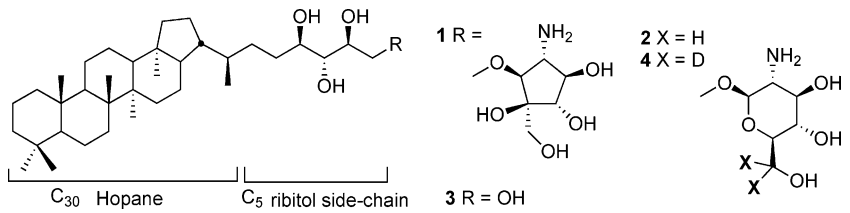
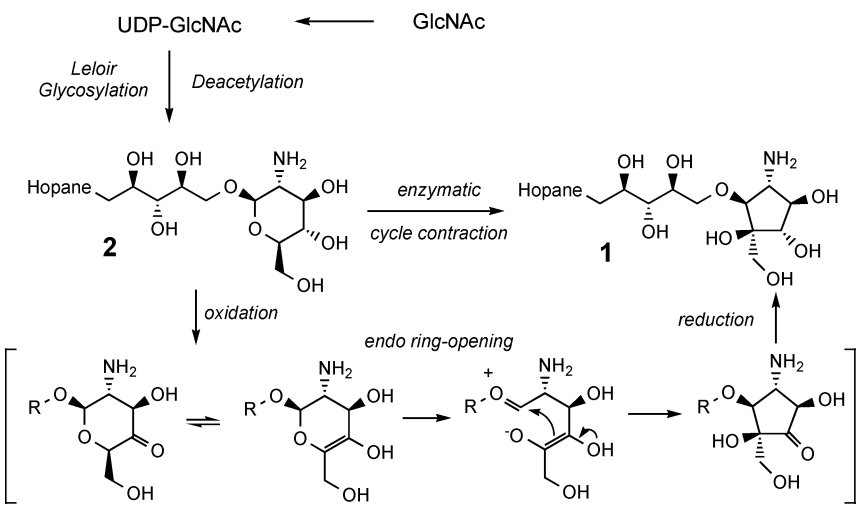



**Fig. 1** Natural hopanoids (**1–3**) isolated from *Z. mobilis* and deuterated analog **4**.




**Scheme 1** Putative biosynthesis of bacteriohopane–carbapseudopentose **1**.

The isotopic labeling of a molecule used in feeding experiments is exploited as a tracer of a specific biosynthetic pathway. In the case of the pentacyclic hopane skeleton of natural molecules **1–3**, feeding experiments with $^{13}C$- and deuterium-labelled molecules led Rohmer *et al.* to discover the long overlooked non-mevalonate pathway for isoprenoid biosynthesis.[4,12–15] More recently, a preliminary feeding experiment using deuterated *N*-acetyl-D-glucosamine (6,6′-$D_2$-GlcNAc) proved that glycoside **2** and ether **1** share the same biosynthetic pathway derived from GlcNAc.[5] Moreover, the similar isotope abundance found in the glycon moieties of hopanoids **1** and **2**, after feeding experiments, suggested a precursor to product relationship between glycoside **2** and cyclopentitol **1**.

In order to demonstrate or rule out this biosynthetic assumption, we now need to show that glycoside **2** can be transformed into ether **1** by *Z. mobilis* enzymatic machinery. Therefore, the synthesis of deuterated glycoside **4** is required to unambiguously demonstrate that **2** is the direct biosynthetic precursor of **1** (Fig. 1).

Recently, we have reported a concise strategy centered on the copper activation of the $C_{30}$ hopane moiety for the synthesis of bacteriohopanetetrol **3** and its glycosylated derivative **2**.[16] Then, we developed a more general approach based on a LiDBB lithiation of phenyl sulfide **5** (Scheme 2) for the synthesis of a wide range of biohopanoids including bacteriohopanepentols bearing an additional hydroxyl group at position C-31.[17,18]

The synthesis of deuterated analog **4** thus started from phenyl sulfide **5** (Scheme 2), easily obtained in a few steps from the commercially available Dammar resin.[17,19] Diol **6** was then prepared by the new coupling procedure we recently developed.[17] The properly protected glucosamine donor **7** was prepared by a quantitative acetylation of the known[20] primary alcohol. The acetate group was chosen to allow its selective deprotection, after the glycosylation step.

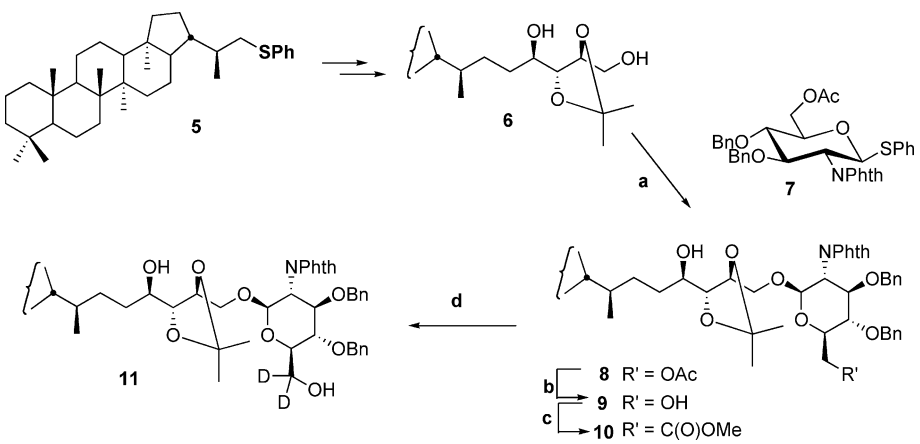
The regio- and stereoselective coupling of diol **6** and phenyl sulfide **7** was conducted in the presence of NIS and a catalytic amount of TfOH in $CH_2Cl_2$ at a temperature ranging from −78 °C to −30 °C. The reaction proceeded smoothly and furnished the desired β-D-glucosaminylhopanetetrol derivative **8** as the main product in 84% yield (Scheme 2). The $^1H$ and $^{13}C$ NMR spectra of **8** were in complete agreement with the assigned structure, especially the expected β-configuration.

The 6′-acetate selective deprotection was readily achieved by carefully treating glycoside **8** with MeONa in a mixed solvent of THF–MeOH at room temperature. Under these conditions, the phthalimido group was not deprotected and the desired 6′-hydroxy glycoside **9** was obtained in 94% yield. Intermediate **9** could then undergo an oxidation followed by a $LiAlD_4$ reduction to introduce the two deuterium atoms in the target molecule **11**. Many methods are available for the conversion of primary hydroxy groups into carboxylic acids. However, most of them are not appropriate for highly functionalized structures such as compound **9** bearing acid and base sensitive protective groups. Moreover, we wanted to achieve the regioselective oxidation of the glucosamine primary alcohol in presence of the secondary alcohol of the ribitol side-chain. After the screening of the best oxidation reagents, we found that the TEMPO oxidation procedure developed by Davis and Flitsch gave, from far, the best yields.[21] From the known procedure, we only modified the esterification condition, using an excess amount of iodomethane in presence of sodium bicarbonate and tetrabutylammonium chloride. The desired ester **10** was thus obtained in 69% yield for two steps (Scheme 2).

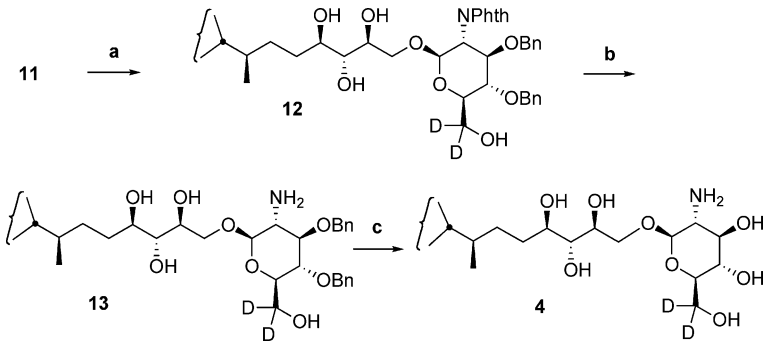
As expected, the following reduction was much more difficult to optimize. The use of $LiAlD_4$ was essential for the complete bisdeuteration of ester **10**, since $NaBD_4$ is generally not efficient for the reduction of carboxylic esters. Not surprisingly, the main side-reaction was the removal of the phthalimido group. The desired deuterated alcohol **11** was then isolated in a moderate but reproducible yield.

At this stage, the bisdeuteration was easily confirmed by the comparison of $^1H$, $^{13}C$ NMR and mass spectra of derivative **11** with those of the corresponding non-deuterated molecule **9**. A small multiplet was observed for the carbon C-6′ of the glucosamine subunit due to the coupling between the carbon and the two deuterium atoms. In addition, the expected α- and β-shifts were also observed for the C-6′ ($\Delta\delta = 0.4$ ppm, see Experimental section) and C-5′ atoms ($\Delta\delta = 0.2$ ppm), respectively.

The sequence of deprotections of **11** was similar to that we had developed for the non-labelled one (Scheme 3).[16] Thus, the acetonide group in **11** was removed using hydrochloric acid (1% in




**Scheme 2** *Reagents and conditions*: (a) NIS, TfOH, MS 4 Å, $CH_2Cl_2$, −78 to −30 °C, 84%; (b) MeONa, THF–MeOH, rt, 94%; (c) TEMPO, NaOCl, $NaHCO_3$, KBr, $Bu_4NCl$, $CH_2Cl_2$, 0 °C; then MeI, $NaHCO_3$, $Bu_4NCl$, rt, 69%; d) $LiAlD_4$ THF, −40 to −20 °C, 36%.




**Scheme 3** *Reagents and conditions*: (a) HCl (1%), MeOH–THF, rt, 83%; (b) $H_2N–NH_2·H_2O$, EtOH–$H_2O$, 80 °C, 88%; (c) Pd black, $H_2$, THF–AcOH–$H_2O$, rt.

MeOH) at room temperature yielding bisdeuterated glycoside **12** in 83% yield. The hydrazine promoted phthalimido deprotection afforded the desired amine **13**. The purification of this product proved more difficult than its tri-*O*-benzyl analogue[16] due to its higher polarity which complicated the required silica gel chromatography. Given the glycolipidic nature of molecule **13**, purification using standard reversed phase preparative HPLC or ion exchange chromatography was not appropriate. A successful purification was finally achieved in two steps: a silica gel chromatography using a polar solvent system ($CH_2Cl_2$–MeOH, 20 : 1), followed by a size-exclusion chromatography ($CH_2Cl_2$–$CH_3OH$, 2 : 1) to afford pure **13** in 88% yield. After screening catalyst and solvent systems, the final bisdeuterated glycoside **4** was obtained after the hydrogenolysis of **13** in the presence of palladium black under a hydrogen atmosphere in 93% yield.

The structure of **4** was confirmed by comparing all analytical data with its non-deuterated analogue **2**,[16] and also by the comparison of the $^1$H-NMR of the peracetates of **4** and **2**.[22,23]

In conclusion, we have developed a straightforward preparation of deuterated glycoside **4** that will be used as a biosynthetic probe for the discovery of a new enzymatic reaction. This multistep synthesis required a stereoselective glycosylation, a regioselective deuteration as well as a properly defined strategy for the final deprotections and the purification of the amphiphilic glycoside **4**.

Feeding experiments using deuterated molecule **4** are in progress and the results will be reported in due course.

## Experimental

### Phenyl 6-*O*-acetyl-3,4-di-*O*-benzyl-1,2-dideoxy-2-phthalimido-1-thio-β-D-glucopyranoside 7

Phenyl 3,4-di-*O*-benzyl-1,2-dideoxy-2-phthalimido-1-thio-β-D-glucopyranoside[13] (1.84 g, 3.16 mmol) was acetylated (pyridine–$Ac_2O$, 2 : 1, v/v, 30 mL) at room temperature for 1 h. The excess of $Ac_2O$ was decomposed by pouring the reaction mixture into ice–water. The resulting mixture was extracted three times with $CH_2Cl_2$. The combined extracts were washed with brine, dried ($MgSO_4$) and filtered through cotton. The solvents were concentrated to dryness under reduced pressure. The crude acetate was chromatographed on silica gel (cyclohexane–EtOAc, 3 : 1) to yield **7** as a light yellow syrup (2.04 g, quant., $R_f$ 0.32, cyclohexane–EtOAc, 3 : 1). $[\alpha]_D^{25}$ +92 (*c* 1.00, $CHCl_3$). $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 6.92–7.73 (19H, m, ArH), 5.58 (1H, d, $J_{1,2}$ = 10.6 Hz, 1-H), 4.47–4.94 (4H, m, $BnCH_2$), 4.50 (1H, dd, $J_{5,6b}$ = 2.1 Hz, $J_{gem}$ = 11.9 Hz, 6-$H_b$), 4.46 (1H, dd, $J_{2,3}$ = 10.3 Hz, $J_{3,4}$ = 8.6 Hz, 3-H), 3.44 (1H, t, $J$ = 10.3 Hz, 2-H), 3.43 (1H, dd, $J_{5,6a}$ = 5.2 Hz, $J_{gem}$ = 11.9 Hz, 6-$H_a$), 3.81 (1H, ddd, $J_{5,6b}$ = 2.1 Hz, $J_{5,6a}$ = 5.2 Hz, $J_{4,5}$ = 9.9 Hz, 5-H), 3.71 (1H, $J_{3,4}$ = 8.6 Hz, $J_{4,5}$ = 9.9 Hz, 4-H), 2.12 (3H, s, OAc). $^{13}$C NMR (101 MHz): $\delta$ = 170.5 ($CH_3C$=O), 133.8, 133.7, 132.6, 128.7, 128.5, 128.0, 127.8, 127.4, 123.4, 123.3, 83.1 (C-1), 80.4 (C-3), 79.1 (C-4), 77.0 (C-5), 75.01 and 75.00 ($BnCH_2$), 62.9 (C-6), 54.7 (C-2), 20.8 ($CH_3$C=O). HRMS (DCI): *m/z* 641.2319 [M + $NH_4^+$], calcd for $C_{36}H_{37}O_7N_2S$: 641.2321.

### Acetate 8

A suspension of diol **6** (53.0 mg, 0.09 μmol), glycoside **7** (78.9 mg, 126 μmol) and molecular sieves (4 Å, 30.0 mg) in anhydrous $CH_2Cl_2$ (3 mL) under an atmosphere of argon was stirred at room temperature for 15 min, then NIS (51.0 mg, 0.225 mmol) was added at room temperature, followed by a slow addition of TfOH (0.40 μL, 0.0045 μmol) at −78 °C. The reaction temperature was allowed to warm up to −30 °C gradually within *ca.* 3 h and the reaction was monitored by TLC during that period, until diol **6** could not be detected any more. The reaction mixture was quenched with $Et_3N$ at −20 °C before it was diluted with $CH_2Cl_2$. Then the organic phase was washed successively with saturated aqueous $Na_2S_2O_3$, brine, and dried over $MgSO_4$, filtered through cotton and the filtrates were evaporated under reduced pressure. The resulting crude product was chromatographed on silica gel (toluene–EtOAc, 5 : 1) to yield **8** as a light yellow syrup (83.3 mg, 84%, $R_f$ 0.47, toluene–EtOAc, 5 : 1). $[\alpha]_D^{19}$ +19 (*c* 0.35, $CHCl_3$). $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 6.92–7.71 (14H, m, ArH), 5.29 (1H, d, $J_{1',2'}$ = 8.5 Hz, 1′-H), 4.45–4.93 (4H, m, $BnCH_2$), 4.46 (1H, m, 6′-$H_b$), 4.39 (1H, dd, $J_{2',3'}$ = 10.7 Hz, $J_{3',4'}$ = 8.0 Hz, 3′-H), 4.30 (1H, dd, $J_{5',6'a}$ = 3.9 Hz, $J_{gem}$ = 11.8 Hz, 6′-$H_a$), 4.26 (1H, m, 34-H), 4.22 (1H, dd, $J_{1',2'}$ = 8.5 Hz, $J_{2',3'}$ = 10.7 Hz, 2′-H), 3.83 (2H, m, 33-H and 35-$H_b$), 3.74 (2H, m, 5′-H and 4′-H), 3.63 (1H, t, $J_{34,35a}$ = $J_{gem}$ = 9.6 Hz, 35-$H_a$), 3.35 (1H, m, 32-H), 2.55 (1H, brs, $J$ = 3.0 Hz, 32-OH), 2.13 (3H, s, AcO), 1.28 and 1.26 (6H, 2 s, acetal-Me), 1.00 and 0.98 (6H, 2 s, 8β- and 14α-Me), 0.89 (3H, s, 4α-Me), 0.86 (3H, s, 10β-Me), 0.84 (3H, s, 4β-Me), 0.82 (3H, d, $J_{22,29}$ = 6.4 Hz, 22-Me), 0.71 (3H, s, 18α-Me), 1.11–1.80 (29H, m, hopane), 0.72–0.97 (3H, m, hopane); $^{13}$C NMR (101 MHz): $\delta$ = 170.6 ($CH_3C$=O), 137.5, 133.3, 128.5, 128.0, 127.8, 127.4, 123.3, 108.2 (acetal-*C*), 97.9 (C-1′), 80.3 (C-33), 79.1 (C-3′), 79.0 (C-4′),

75.02 and 74.95 ($BnCH_2$), 74.8 (C-34), 73.1 (C-5′), 69.0 (C-32), 67.4 (C-35), 62.5 (C-6′), 56.1 (C-5), 55.3 (C-2′), 54.4 (C-17), 50.4 (C-9), 49.2 (C-13), 45.6 (C-21), 44.3 (C-18), 42.0 (C-3), 41.7 (C-14), 41.6 (C-8), 41.5 (C-19), 40.3 (C-1), 37.3 (C-10), 36.7 (C-22), 33.6 (C-15), 33.4 (C-24), 33.22 and 33.19 (C-4 and C-7), 30.3 (C-30), 30.2 (C-20), 28.0 (acetal-Me), 27.6 (C-31), 25.4 (acetal-Me), 23.9 (C-12), 22.8 (C-16), 21.6 (C-23), 20.9 (C-11), 20.8 ($CH_3C{=}O$), 20.1 (C-29), 18.6 (C-2 and C-6), 16.5 and 16.4 (C-26 and C-27), 15.9 (C-25 and C-28). HRMS (FAB): $m/z$ 1122.6663 [M + $Na^+$], calcd for $C_{66}H_{91}O_{10}NNa$: 1122.6646.

**Alcohol 9**

To a solution of compound **8** (79.0 mg, 72 μmol) in anhydrous THF (2 mL) under an atmosphere of argon was added a freshly prepared solution of MeONa in MeOH (0.03 M, 4 mL) at room temperature. The resulting mixture was then stirred for 70 min. The reaction mixture was neutralized (pH = 7) by adding Amberlyst A-120 resin ($H^+$ form) before it was filtered through cotton. The filtrates were evaporated under reduced pressure and the residue was chromatographed on silica gel (cyclohexane–acetone, 7 : 1) affording the desired product **9** as a white amorphous solid (71.0 mg, 94%, $R_f$ 0.28, cyclohexane–EtOAc, 2 : 1). $[\alpha]_D^{18}$ +52 ($c$ 1.0, $CHCl_3$). $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 6.91–7.70 (14H, m, ArH), 5.32 (1H, d, $J_{1',2'}$ = 8.6 Hz, 1′-H), 4.45–4.92 (4H, m, $BnCH_2$), 4.38 (1H, dd, $J_{2',3'}$ = 10.7 Hz, $J_{3',4'}$ = 8.7 Hz, 3′-H), 4.27 (1H, m, 34-H), 4.18 (1H, dd, $J_{1',2'}$ = 8.6 Hz, $J_{2',3'}$ = 10.7 Hz, 2′-H), 3.97 (1H, dd, $J_{5',6'b}$ = 2.1 Hz, $J_{gem}$ = 12.1 Hz, 6′-$H_b$), 3.76–3.85 (4H, m, 33-H, 6′-$H_a$, 35-$H_b$, 4′-H), 3.67 (1H, t, $J_{34,35a} = J_{gem}$ = 9.6 Hz, 35-$H_a$), 3.61 (1H, m, 5′-H), 3.36 (1H, m, 32-H), 2.58 (1H, brs, 32-OH), 2.19 (1H, brs, 6′-OH), 1.29 and 1.26 (6H, 2 s, acetal-Me), 0.99 and 0.98 (6H, 2 s, 8β- and 14α-Me), 0.89 (3H, s, 4α-Me), 0.86 (3H, s, 10β-Me), 0.84 (3H, s, 4β-Me), 0.82 (3H, d, $J_{22,29}$ = 6.4 Hz, 22-Me), 0.71 (3H, s, 18α-Me), 1.10–1.79 (29H, m, hopane), 0.72–0.97 (3H, m, hopane); $^{13}$C NMR (101 MHz): $\delta$ = 137.7, 133.7, 128.5, 128.0, 127.8, 127.4, 123.3, 108.4 (acetal-$C$), 98.1 (C-1′), 80.3 (C-33), 79.0 (C-4′), 78.8 (C-3′), 75.5 (C-5′), 75.02 (Bn$CH_2$), 74.96 (C-34), 74.8 (Bn$CH_2$), 69.1 (C-32), 67.7 (C-35), 61.5 (C-6′), 56.1 (C-5), 55.5 (C-2′), 54.4 (C-17), 50.4 (C-9), 49.2 (C-13), 45.7 (C-21), 44.4 (C-18), 42.1 (C-3), 41.7 (C-14), 41.61 (C-8), 41.56 (C-19), 40.3 (C-1), 37.3 (C-10), 36.7 (C-22), 33.6 (C-15), 33.4 (C-24), 33.23 and 33.21 (C-4 and C-7), 30.4 (C-30), 30.2 (C-20), 28.0 (acetal-Me), 27.6 (C-31), 25.4 (acetal-Me), 23.9 (C-12), 22.8 (C-16), 21.6 (C-23), 20.9 (C-11), 20.1 (C-29), 18.7 (C-2 and C-6), 16.5 and 16.4 (C-26 and C-27), 15.9 (C-25 and C-28). HRMS (FAB): $m/z$ 1080.6553 [M + $Na^+$], calcd for $C_{66}H_{91}O_{10}NNa$: 1080.6541.

**Ester 10**

To a solution of **9** (35.0 mg, 33 μmol) in $CH_2Cl_2$ (5 mL) was added TEMPO (1.0 mg, 7 μmol) at 0 °C, followed by a mixture of saturated aqueous $NaHCO_3$ (1 mL), KBr (5.0 mg) and $Bu_4NCl$ (6.0 mg). The resulting mixture was stirred vigorously for 20 min at 0 °C before a mixture of saturated aq. NaCl, saturated aq. $NaHCO_3$ and 10% aq. NaOCl (2 : 1 : 2, v/v/v, 1.5 mL) was added dropwise within 1 h at 0 °C. Then, to this yellowish mixture was added successively $NaHCO_3$ (20.0 mg), $Bu_4NCl$ (20.0 mg) and MeI (1 mL) at 0 °C. The reaction mixture was allowed to warm up gradiently to room temperature and the stirring was continued overnight. The resulting dark red mixture was extracted three times with $CH_2Cl_2$, and the combined extracts were washed with brine, dried over $MgSO_4$ and filtered through cotton. The resulting filtrates were concentrated to dryness and the residue was chromatographed on silica gel (cyclohexane–EtOAc, 4 : 1) to afford **10** as a syrup (24.6 mg, 69%, $R_f$ 0.49, cyclohexane–EtOAc, 2 : 1). $[\alpha]_D^{19}$ +40 ($c$ 0.80, $CHCl_3$). $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 6.89–7.71 (14H, m, ArH), 5.31 (1H, d, $J_{1',2'}$ = 8.4 Hz, 1′-H), 4.44–4.85 (4H, m, $BnCH_2$), 4.37 (1H, dd, $J_{2',3'}$ = 10.6 Hz, $J_{3',4'}$ = 8.7 Hz, 3′-H), 4.26 (1H, dd, $J_{1',2'}$ = 8.4 Hz, $J_{2',3'}$ = 10.6 Hz, 2′-H), 4.25 (1H, m, 34-H), 4.15 (1H, d, $J_{4',5'}$ = 9.7 Hz, 5′-H), 3.99 (1H, dd, $J_{3',4'}$ = 8.7 Hz, $J_{4',5'}$ = 9.7 Hz, 4′-H), 3.87 (1H, dd, $J_{34,35b}$ = 3.6 Hz, $J_{gem}$ = 9.6 Hz, 35-$H_b$), 3.81 (1H, m, 33-H), 3.80 (3H, s, OMe), 3.62 (1H, dd, $J_{34,35a} = J_{gem}$ = 9.6 Hz, 35-$H_a$), 3.29 (1H, m, 32-H), 2.39 (1H, brs, 32-OH), 1.16 and 1.13 (6H, 2 s, acetal-Me), 0.87 (6H, 2 s, 8β- and 14α-Me), 0.78 (3H, s, 4α-Me), 0.75 (3H, s, 10β-Me), 0.72 (3H, s, 4β-Me), 0.68 (3H, d, $J_{22,29}$ = 6.4 Hz, 22-Me), 0.59 (3H, s, 18α-Me), 1.02–1.75 (29H, m, hopane), 0.50–0.85 (3H, m, hopane); $^{13}$C NMR (101 MHz): $\delta$ = 168.4 (ester-$C{=}O$), 137.4, 133.6, 131.6, 128.4, 128.0, 127.9, 127.4, 123.3, 108.2 (acetal-$C$), 98.1 (C-1′), 81.0 (C-4′), 80.3 (C-33), 78.1 (C-3′), 75.0, 74.8, 74.7 (C-34), 74.3 (C-5′), 69.1 (C-32), 67.4 (C-35), 56.1 (C-5), 55.0 (C-2′), 54.4 (C-17), 52.6 (OMe), 50.4 (C-9), 49.2 (C-13), 45.5 (C-21), 44.3 (C-18), 42.1 (C-3), 41.7 (C-14), 41.6 (C-8), 41.5 (C-19), 40.2 (C-1), 37.3 (C-10), 36.6 (C-22), 33.6 (C-15), 33.3 (C-24), 33.20 and 33.17 (C-4 and C-7), 30.2 (C-30), 30.1 (C-20), 28.0 (acetal-Me), 27.5 (C-31), 25.3 (acetal-Me), 23.9 (C-12), 22.8 (C-16), 21.5 (C-23), 20.9 (C-11), 20.0 (C-29), 18.6 (C-2 and C-6), 16.5 and 16.4 (C-26 and C-27), 15.8 (C-25 and C-28). HRMS (FAB): $m/z$ 1108.6479 [M + $Na^+$], calcd for $C_{67}H_{91}O_{11}NNa$: 1108.6490.

**Bisdeuterated alcohol 11**

To a solution of **10** (95.0 mg, 87 μmol) in freshly distilled THF (8 mL) under an atmosphere of dry argon was added $LiAlD_4$ (5.8 mg, 138 μmol) portionwise in four amounts at a temperature varying from −40 °C to −20 °C until the starting material could not be detected any more by TLC. Excess $LiAlD_4$ was reacted with EtOAc (2 mL) at −78 °C before the reaction mixture was concentrated to dryness *in vacuo*, the residue was purified by column chromatography (cyclohexane–EtOAc, 4 : 1) yielding **11** as a white amorphous solid (33.4 mg, 36%, $R_f$ 0.28, cyclohexane–EtOAc, 2 : 1). $[\alpha]_D^{18}$ +59 ($c$ 1.25, $CHCl_3$). $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 6.91–7.70 (14H, m, ArH), 5.32 (1H, d, $J_{1',2'}$ = 8.5 Hz, 1′-H), 4.45–4.92 (4H, m, $BnCH_2$), 4.38 (1H, dd, $J_{2',3'}$ = 10.7 Hz, $J_{3',4'}$ = 8.7 Hz, 3′-H), 4.27 (1H, m, 34-H), 4.18 (1H, dd, $J_{1',2'}$ = 8.5 Hz, $J_{2',3'}$ = 10.7 Hz, 2′-H), 3.81 (3H, m, 33-H, 35-$H_b$, 4′-H), 3.67 (1H, t, $J_{34,35a} = J_{gem}$ = 9.6 Hz, 35-$H_a$), 3.60 (1H, d, $J_{4',5'}$ = 9.8 Hz, 5′-H), 3.35 (1H, m, 32-H), 2.55 (1H, brd, 32-OH), 2.08 (1H, s, 6′-OH), 1.29 and 1.26 (6H, 2 s, acetal-Me), 0.99 and 0.98 (6H, 2 s, 8β- and 14α-Me), 0.89 (3H, s, 4α-Me), 0.86 (3H, s, 10β-Me), 0.84 (3H, s, 4β-Me), 0.82 (3H, d, $J_{22,29}$ = 6.4 Hz, 22-Me), 0.71 (3H, s, 18α-Me), 1.10–1.79 (29H, m, hopane), 0.72–0.97 (3H, m, hopane); $^{13}$C NMR (101 MHz): $\delta$ = 137.7, 133.7, 128.5, 128.0, 127.8, 127.4, 123.3, 108.4 (acetal-$C$), 98.1 (C-1′), 80.4 (C-33), 79.0 (C-4′), 78.8 (C-3′), 75.3 (C-5′, β-shift), 75.02 (Bn$CH_2$), 74.97 (C-34), 74.85 (Bn$CH_2$), 69.1 (C-32), 67.7 (C-35), 60.9 (m, C-6′$D_2$, α-shift), 56.1 (C-5), 55.5 (C-2′), 54.4 (C-17), 50.4 (C-9), 49.3 (C-13), 45.7 (C-21), 44.4 (C-18), 42.1 (C-3), 41.8 (C-14), 41.63 (C-8),

41.57 (C-19), 40.3 (C-1), 37.4 (C-10), 36.7 (C-22), 33.6 (C-15), 33.4 (C-24), 33.24 and 33.21 (C-4 and C-7), 30.4 (C-30), 30.2 (C-20), 28.1 (acetal-Me), 27.6 (C-31), 25.4 (acetal-Me), 23.9 (C-12), 22.8 (C-16), 21.6 (C-23), 20.9 (C-11), 20.1 (C-29), 18.7 (C-2 and C-6), 16.5 and 16.4 (C-26 and C-27), 15.9 (C-25 and C-28). HRMS (FAB): *m/z* 1082.6656 [M + $Na^+$], calcd for $C_{66}H_{89}O_{10}D_2NNa$: 1082.6666.

**Bisdeuterated tetrol 12**

The diol **11** (6.5 mg, 6 μmol) was dissolved in THF–MeOH (1 : 1, v/v, 2 mL) containing aqueous HCl (37%, 12 μL) at 0 °C. The resulting mixture was stirred overnight vigorously at room temperature. Then, it was neutralized with solid $NaHCO_3$, filtered through cotton and concentrated to dryness. The residue was chromatographed on silica gel ($CH_2Cl_2$–MeOH, 30 : 1) providing **12** as a white solid (5.2 mg, 83%, $R_f$ 0.18, $CH_2Cl_2$–MeOH, 20 : 1). $[\alpha]_D^{18}$ +29 (*c* 0.61, $CHCl_3$). $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 6.91–7.72 (14H, m, ArH), 5.25 (1H, d, $J_{1',2'}$ = 8.5 Hz, 1′-H), 4.47–4.93 (4H, m, $ArCH_2$), 4.43 (1H, dd, $J_{2',3'}$ = 10.7 Hz, $J_{3',4'}$ = 8.5 Hz, 3′-H), 4.18 (1H, dd, $J_{1',2'}$ = 8.5 Hz, $J_{2',3'}$ = 10.7 Hz, 2′-H), 3.88 (2H, m, 35-$H_a$ and 35-$H_b$), 3.74 (2H, m, 34-H and 4′-H), 3.63 (2H, m, 32-H and 5′-H), 3.52 (1H, m, 33-H), 3.22 (1H, brd, 34-OH), 2.97 (1H, brd, *J* = 4.8, 33-OH), 2.72 (1H, s, 6′-OH), 2.47 (1H, brd, 32-OH), 0.98 (6H, 2 s, 8β- and 14α-Me), 0.92 (3H, d, $J_{22,29}$ = 6.3 Hz, 22-Me), 0.89 (3H, s, 4α-Me), 0.85 (3H, s, 10β-Me), 0.83 (3H, s, 4β-Me), 0.72 (3H, s, 18α-Me), 1.10–1.85 (29H, m, hopane), 0.72–0.95 (3H, m, hopane); $^{13}$C NMR (101 MHz): $\delta$ = 137.8, 137.7, 133.8, 131.5, 128.6, 128.1, 128.0, 127.8, 127.4, 123.4, 98.8 (C-1′), 79.3 (C-4′), 79.0 (C-3′), 75.5 (C-5′), 75.1 (Bn$CH_2$), 74.9 (Bn$CH_2$), 73.9 (C-33), 73.3 (C-32), 71.7 (C-35), 71.6 (C-34), 56.1 (C-5), 55.8 (C-2′), 54.5 (C-17), 50.4 (C-9), 49.3 (C-13), 46.1 (C-21), 44.3 (C-18), 42.1 (C-3), 41.8 (C-14), 41.7 (C-8), 41.6 (C-19), 40.3 (C-1), 37.4 (C-10), 36.9 (C-22), 33.7 (C-15), 33.4 (C-24), 33.3 and 33.2 (C-4 and C-7), 31.4 (C-30), 28.9 (C-20), 27.7 (C-31), 23.9 (C-12), 22.8 (C-16), 21.6 (C-23), 20.9 (C-11), 20.0 (C-29), 18.7 (C-2 and C-6), 16.6 and 16.5 (C-26 and C-27), 15.9 (C-25 and C-28). HRMS (FAB): *m/z* 1042.6343 [M + $Na^+$], calcd for $C_{63}H_{85}O_{10}D_2NNa$: 1042.6353.

**Bisdeuterated amine 13**

To a suspension of **12** (5.2 mg, 5 μmol) in EtOH (2 mL) and $H_2O$ (3 drops) under an atmosphere of argon was added hydrazine monohydrate (100%, 120 μL), the reaction mixture was refluxed overnight at 80 °C. The resulting mixture was concentrated to dryness and the crude product was chromatographed on silica gel ($CH_2Cl_2$–MeOH–$Et_3N$, 20 : 1 : 0.02) followed by a gel filtration on a size-exclusion Sephadex LH-20 chromatography ($CH_2Cl_2$–$CH_3OH$, 2 : 1) to afford **13** as a colorless solid (4.0 mg, 88%, $R_f$ 0.31, $CH_2Cl_2$–MeOH, 10 : 1). $[\alpha]_D^{19}$ +37 (*c* 0.96, $CHCl_3$). $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 7.32–7.39 (10H, m, ArH), 4.69–5.01 (4H, m, $ArCH_2$), 4.47 (1H, brd, 1′-H), 4.08 (1H, m, 35-$H_b$), 3.90 (2H, m, 35-$H_a$ and 34-H), 3.65 (4H, m, 32-H, 33-H and 4′-H), 3.56 (1H, m, 3′-H), 3.44 (1H, d, $J_{4',5'}$ = 9.5 Hz, 5′-H), 2.93 (1H, m, 2′-H), 0.99 and 0.98 (6H, 2 s, 8β- and 14α-Me), 0.96 (3H, d, $J_{22,29}$ = 6.3 Hz, 22-Me), 0.89 (3H, s, 4α-Me), 0.85 (3H, s, 10β-Me), 0.83 (3H, s, 4β-Me), 0.73 (3H, s, 18α-Me), 1.10–1.88 (29H, m, hopane), 0.72–0.95 (3H, m, hopane). $^{13}$C NMR (101 MHz): $\delta$ = 137.8, 137.6, 128.7, 128.6, 128.5, 128.3, 128.2, 128.0, 127.9, 103.3 (C-1′), 84.2 (C-3′), 78.2 (C-4′), 75.7 (C-5′), 75.3 (Bn$CH_2$), 75.0 (Bn$CH_2$), 74.0 (C-32), 73.5 (C-33), 71.8 (C-34), 71.6 (C-35), 60.6 (m, C-6′), 56.4 (C-2′), 56.1 (C-5), 54.5 (C-17), 50.5 (C-9), 49.2 (C-13), 46.2 (C-21), 44.4 (C-18), 42.1 (C-3), 41.8 (C-14), 41.7 (C-8), 41.6 (C-19), 40.3 (C-1), 37.4 (C-10), 36.9 (C-22), 33.7 (C-15), 33.4 (C-24), 33.3 and 33.2 (C-4 and C-7), 31.7 (C-30), 29.0 (C-20), 27.8 (C-31), 24.0 (C-12), 22.9 (C-16), 21.6 (C-23), 20.9 (C-11), 20.1 (C-29), 18.7 (C-2 and C-6), 16.6 and 16.5 (C-26 and C-27), 15.9 (C-25 and C-28). HRMS (FAB): *m/z* 912.6284 [M + $Na^+$], calcd for $C_{55}H_{83}O_8D_2NNa$: 912.6298.

**Bisdeuterated 35-*O*-β-D-glucosaminylbacteriohopanetetrol 4**

A solution of **13** (14.8 mg, 17 μmol) in THF–AcOH–$H_2O$ (4 mL, 16 : 8 : 3, v/v/v) was vigorously stirred overnight under an atmosphere of hydrogen in presence of Pd black (20 mg) at room temperature. The catalyst was filtered off by passing the reaction mixture through a short plug of Celite®, and the solvents were evaporated under reduced pressure yielding the deprotected bisdeuterated analogue **4** as a pale solid (11.0 mg, 93%, $R_f$ 0.53, EtOAc–*i*-PrOH–$H_2O$, 4.5 : 3 : 2). $[\alpha]_D^{22}$ +5 (*c* 0.52, pyridine). $^1$H NMR (400 MHz, pyridine-$D_5$): $\delta$ = 4.92 (1H, d, $J_{1',2'}$ = 8.0 Hz, 1′-H), 4.68 (1H, m, 34-H), 4.67 (1H, m, 35-$H_b$), 4.58 (1H, m, 35-$H_a$), 4.42 (1H, m, 32-H), 4.30 (1H, m, 33-H), 4.21 (1H, dd, $J_{3',4'}$ = 9.1 Hz, $J_{4',5'}$ = 9.6 Hz, 4′-H), 4.03 (1H, dd, $J_{2',3'}$ = 9.4 Hz, $J_{3',4'}$ = 9.1 Hz, 3′-H), 3.88 (1H, d, $J_{4',5'}$ = 9.6 Hz, 5′-H), 3.32 (1H, m, 2′-H), 1.06 (3H, d, $J_{22,29}$ = 5.7 Hz, 22-Me), 0.95 and 0.94 (6H, 2 s, 8β- and 14α-Me), 0.86 (3H, s, 4α-Me), 0.80 (6H, 2 s, 10β-Me and 4β-Me), 0.69 (3H, s, 18α-Me), 1.08–2.20 (29H, m, hopane), 0.70–0.91 (3H, m, hopane). $^{13}$C NMR (101 MHz): $\delta$ = 105.2 (C-1′), 78.3 (C-5′, β-shift), 77.7 (C-3′), 75.4 (C-33), 73.5 (C-32), 73.3 (C-34), 73.1 (C-35), 71.4 (C-4′, γ-shift), 58.4 (C-2′), 56.1 (C-5), 54.4 (C-17), 50.3 (C-9), 49.3 (C-13), 46.5 (C-21), 44.2 (C-18), 41.9 (C-3), 41.6 (C-14), 41.5 (C-8 and C-19), 40.1 (C-1), 37.2 (C-10), 37.0 (C-22), 33.6 (C-15), 33.2 (C-24), 33.0 (C-4 and C-7), 32.1 (C-30), 29.8 (C-20), 27.7 (C-31), 23.9 (C-12), 22.6 (C-16), 21.5 (C-23), 21.1 (C-11), 20.2 (C-29), 18.7 (C-2 and C-6), 16.54 and 16.45 (C-26 and C-27), 15.8 (C-25 and C-28). HRMS (FAB): *m/z* 732.5379 [M + $Na^+$], calcd for $C_{41}H_{71}D_2O_8NNa$: 732.5359.

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## References

1 Y. Blériot, E. Untersteller, B. Fritz and P. Sinaÿ, *Chem.–Eur. J.*, 2002, **8**, 240–246.
2 G. Flesch and M. Rohmer, *Biochem. J.*, 1989, **262**, 673–675.
3 J.-M. Renoux and M. Rohmer, *Eur. J. Biochem.*, 1985, **151**, 405–410.
4 M. Rohmer, M. Knani, P. Simonin, B. Sutter and H. Sahm, *Biochem. J.*, 1993, **295**, 517–524.
5 S. P. Vincent, P. Sinaÿ and M. Rohmer, *Chem. Commun.*, 2003, 782–783.
6 A. Caravano, H. Dohi, P. Sinaÿ and S. P. Vincent, *Chem.–Eur. J.*, 2006, **11**, 3114–3123.
7 A. Caravano, D. Mengin-Lecreulx, J.-M. Brondello, S. P. Vincent and P. Sinaÿ, *Chem.–Eur. J.*, 2003, **9**, 5888–5898.
8 A. Caravano, P. Sinaÿ and S. P. Vincent, *Bioorg. Med. Chem. Lett.*, 2006, **16**, 1123–1125.
9 A. Caravano, S. P. Vincent and P. Sinaÿ, *Chem. Commun.*, 2004, 1216–1217.

 

10 W. Pan, C. Ansiaux and S. P. Vincent, *Tetrahedron Lett.*, 2007, **48**, 4353–4356.
11 F. Tian, M. E. Migaud and J. W. Frost, *J. Am. Chem. Soc.*, 1999, **121**, 5795–5796.
12 G. Flesch and M. Rohmer, *Eur. J. Biochem.*, 1988, **175**, 405–411.
13 M. Rohmer, *Nat. Prod. Rep.*, 1999, **16**, 656–574.
14 M. Rohmer, *Pure Appl. Chem.*, 1993, **65**, 1293–1298.
15 O. Meyer, C. Grosdemange-Billiard, D. Tritsch and M. Rohmer, *Org. Biomol. Chem.*, 2003, **1**, 4367–4372.
16 W. Pan, Y. Zhang, G. Liang, S. P. Vincent and P. Sinaÿ, *Chem. Commun.*, 2005, 3445–3447.
17 W. Pan, C. Sun, Y. Zhang, G. Liang, P. Sinaÿ and S. P. Vincent, *Chem.–Eur. J.*, 2007, **13**, 1471–1480.
18 W. Pan, Y. Zhang, G. Liang, M. Rohmer, P. Sinaÿ and S. P. Vincent, *Chem. Biodiversity*, 2007, **4**, 2182–2189.
19 W. J. Dunstan, H. Fazakerley, T. G. Halsall and E. R. H. Jones, *Croat. Chim. Acta*, 1957, 29.
20 S. Takahashi and H. Kuzuhara, *Chem. Lett.*, 1994, 2119–2122.
21 N. J. Davis and S. L. Flitsch, *Tetrahedron Lett.*, 1993, **34**, 1181–1184.
22 T. Duvold, *Chemistry and biochemistry of bacterial isoprenoids*, PhD Thesis, 1997, Université Louis Pasteur, Strasbourg, France.
23 T. Duvold and M. Rohmer, *Tetrahedron*, 1999, **55**, 9847–9858.