# Prokaryotic Triterpenoids. (22*R*,32*R*)-34,35-Dinorbacteriohopane-32,33-diols from *Acetobacter aceti* ssp. *xylinum*: New Bacteriohopane Derivatives with Shortened Side-Chain

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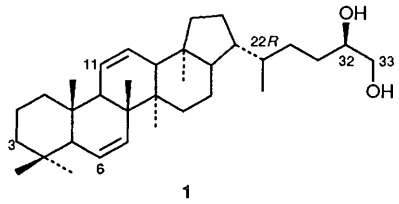
Five new $C_{33}$-pentacyclic triterpenoids, members of the hopanoid family, differ from all known biohopanoids in that their shortened side-chain bears only two vicinal hydroxy groups. They were isolated from *Acetobacter aceti* ssp, *xylinum* and were identified by spectroscopic methods. The absolute configuration at C-32 was shown to be (32*R*) by comparison of an appropriate derivative of the bacterial hopanoids with the diacetates of (22*R*,32*R*)- **11** (22*R*,32*S*)-34,35-dinorbacteriohopane-32,33-diol **12**, respectively prepared by asymmetric hemisynthesis.

Triterpenoids of the hopane series are bacterial equivalents of the sterols in eukaryotes, and act as membrane stabilizers.[1] They are considered to be the precursors of ubiquitous molecular fossils found in the organic matter of sediments.[2] Among the numerous prokaryotic taxa capable of synthesizing hopanoids, *Acetobacter aceti* ssp. *xylinum* was the first bacterium from which $C_{35}$-bacteriohopanetetraols with an extended side-chain were reported.[3,4] The *Acetobacter* species show the most complicated variation patterns of modification of the pentacyclic triterpene skeleton: introduction of double bonds at C-6 and/or C-11,[4,5] presence of an extra methyl group at C-3β,[4,6] and existence of two stereoisomers at C-22.[4] All these structural variations have been determined on the primary alcohols obtained after periodic acid oxidation of the *vic*-tetraols, followed by $NaBH_4$ reduction. In an attempt to isolate native bacteriohopanepolyols with intact side-chains from *Acetobacter aceti* ssp. *xylinum*, we found a new series of $C_{33}$-diols with a shortened side-chain.

34,35-*Dinorbacteriohopane*-32,33-*diols from* Acetobacter aceti *ssp.* xylinum.—Owing to their amphiphilic character, native hopanoids are poorly soluble in most organic solvents. To avoid low recovery yields in chromatographic separations the new diols, **1**–**5**, were isolated as diacetates after acetylation of the crude $CHCl_3$–MeOH extract of the bacteria. All structural variations on the triterpenic skeleton could be identified by comparison of the $^1H$ NMR and MS data with those of already known bacteriohopane derivatives.[4–6] Examination of the molecular ions, the fragments corresponding to the loss of the side-chain, and the ring C fragmentation confirmed that three supplementary carbon atoms, as well as two acetoxy groups, were grafted on the side-chain of the hopane skeleton. From the $^1H$ NMR spectra it was obvious that the new compounds were *vic*-diols. Two singlets in the region $\delta_H$ 2 corresponded to two methyl signals from acetoxy groups. The diastereotopic, geminal protons of the terminal C-33 methylene group appeared as two doublets of doublets, corresponding to the coupling of one proton with the other ($J$ 12.0 Hz) and with the C-32 proton adjacent to the secondary acetoxy group. From these spectroscopic data we concluded that the new hopanoids were derived from 34,35-dinorbacteriohopane-32,33-diols. In order to confirm these structures and to determine the configuration of the chiral centre at C-32, the diacetate of unsaturated diol **2** was hydrogenated in the presence of Adams' catalyst, and the saturated hydrogenation product was compared with synthetic reference material.

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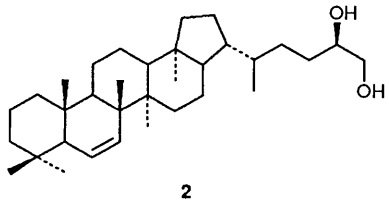


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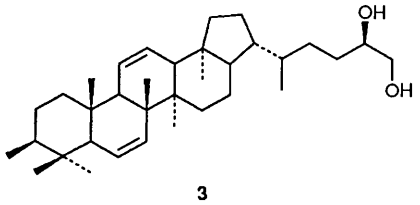


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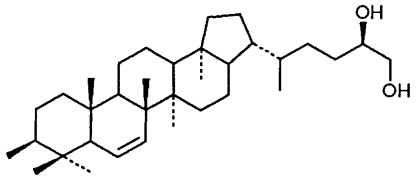


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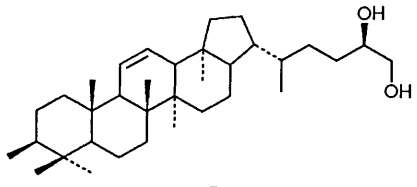


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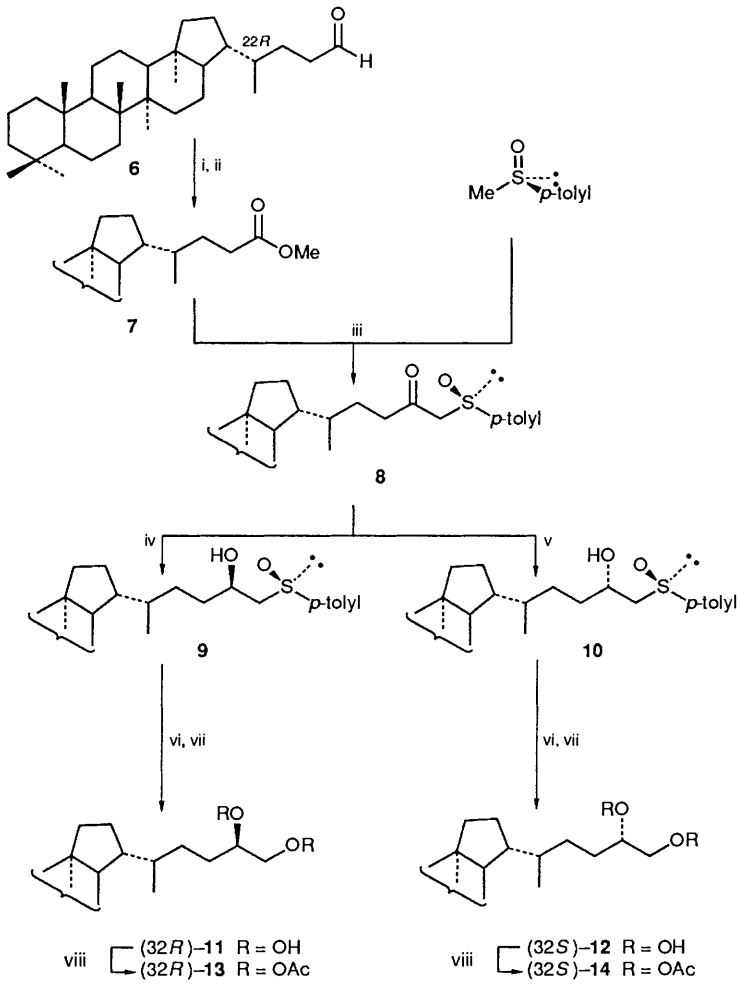


**Scheme 1** Synthesis of the 34,35-dinorbacteriohopanediols **11** and **12** and their respective diacetates, **13** and **14**. *Reagents and conditions:* i, $Br_2$, $NaHCO_3$, THF, water; ii, $CH_2N_2$, $Et_2O$; iii, $LiNPr^i_2$, THF, − 78 °C; iv, $ZnCl_2$, DIBAL, THF, −78 °C; v, DIBAL, THF, $Et_2O$, hexane, 100 °C; vi, TFAA, *sym*-collidine, $CH_2Cl_2$, 0 °C, Pummerer rearrangement; vii, $NaBH_4$, EtOH; viii, $Ac_2O$, pyridine.

*Synthesis of* (22R,32R)- **11** *and* (22R,32S)-34,35-*Dinorbacteriohopane*-32,33-*diol* **12** *Scheme.*—Aminobacteriohopanetriol, accessible from an industrial fermentation residue, was cleaved by using periodic acid as described previously.[5] Bromine oxidation of the corresponding aldehyde **6** of (22*R*)-configuration [7,8] and methylation of the resulting acid with diazomethane afforded methyl ester **7** in 58% overall yield. One-pot transformation of aminobacteriohopanetriol by periodic acid cleavage, followed by direct bromine oxidation in the presence of MeOH, according to the method of Lichtenthaler *et al.*,[7] was not employed as the presence of MeOH in the reaction mixture yielded *ca.* 50% of the easily formed dimethyl acetal from the aldehyde **6**, which therefore is withdrawn from the oxidation process. As aldehyde **6** and ester **7** show the same polarity on TLC, and since the intermediate acid could not be purified, traces of non-oxidized aldehyde **6** were removed by crystallization. Coupling between methyl ester **7** and (*R*)-(+)-methyl *p*-tolyl sulphoxide,[9] as well as both DIBAL reductions of the resulting β-keto sulphoxide **8**, were effected as described by Solladié *et al.*[10]

The reduction of the β-keto sulphoxide **8** with diisobutylaluminium hydride (DIBAL) in the presence of $ZnCl_2$ was as satisfying as expected. The $^1H$ NMR spectrum before chromatography showed only one diastereoisomer, the diastereoselectivity therefore being higher than 95% [diastereoisomeric excess (de) > 90%]. The *R*-configuration of the hydroxy-bearing C-32 carbon in the alcohol product **9** could be deduced from the reaction mechanism described by Solladié and co-workers.[11,12]

Reduction of the β-keto sulphoxide **8** with DIBAL at −100 °C led to an 80:20 mixture (de 60%) of the diastereoisomers **9** and **10**, with (32*S*)-**10**, as expected, as main product (at −78 °C the de was only 40%). The des were calculated from $^1H$ NMR integration of the 33-$H^b$ signal of the ABX-system. The presence of the bulky, chiral hopanoid skeleton might in this case significantly affect the reduction in the opposite manner to that induced by the chiral sulphoxide group and is probably responsible for the low de that Yang and co-workers recently reported for the reduction of a β-keto sulphoxide bearing a bulky, chiral camphor residue on the sulphur atom of the sulphoxide group. Even though the reduction with DIBAL in the presence of $ZnCl_2$ led to 100% asymmetric induction, the reduction with DIBAL only afforded a de of 50%.[13]

Conversion of both β-hydroxy sulphoxides **9** and **10** into the diacetates of the final diol products **11** and **12** was carried out by Pummerer rearrangement,[14,15] followed by direct reduction and acetylation with an overall yield of 65%. Even though we started from diastereoisomerically pure (32*R*)-β-hydroxy sulphoxide **9**, the de of the end-product **11** of (32*R*)-configuration was only 72%, owing to racemization of the unisolated α-hydroxy aldehyde intermediate *via* the corresponding enol. The extent of racemization could be restricted in the preparation of the (32*S*)-isomer **12**, which was obtained with a 52% de, compared with a de of 60% for the starting (32*S*)-sulphoxide **10**.

The 8% loss of de observed in this case was much lower than that for the formation of the (32*R*)-diol **11** (up to 28%). This might arise from the replacement of 78% ethanol by the usual 96% ethanol. Direct reduction of the α,β-bis(trifluoroacetyl) *p*-tolyl sulphide intermediate leads to the diols **11** and **12**, while prior hydrolysis leads to the α-hydroxy aldehydes,[16] which are capable of racemization.

The two end-products **11** and **12** could not be separated by TLC either as diacetates or as free diols. Pure analytical samples were, however, obtained by reversed-phase HPLC, despite incomplete resolution of the peaks.

*Absolute Configuration of the Side-chain of Bacterial* 34,35-*Dinorbacteriohopane*-32,33-*diols*.—The diacetate of the $\Delta^6$-unsaturated compound **2**, isolated from *Acetobacter aceti* ssp. *xylinum*, was hydrogenated in the presence of Adams' catalyst. Separation of unchanged starting material by reversed-phase HPLC gave the saturated product (77% yield), identical with the diacetate **13** of the synthetic (32*R*)-compound **11**.

The absolute configuration of the modified natural product was determined as 32*R* by comparison of the four slight, but characteristic differences, observed in the $^1$H NMR spectra of the (22*R*,32*R*)-compound **11** and the (22*R*,32*S*)-compound **12**. (a) The methyl singlets from the acetoxy groups are separated by 0.004 ppm in the case of the diacetate with (32*R*)-configuration, compound **13**, while they are coincidental in the spectrum of the (32*S*)-isomer **14**. (b) The 33-H$^b$ doublet of doublets of the (32*R*)-compound **13** shows a 33-H$^b$/32-H coupling constant of 3.5 Hz, compared with 3.0 Hz for the corresponding constant of the (32*S*)-diastereoisomer **14**. (c) The 32-H multiplet is located at δ 5.03 for the (32*R*)-compound **13** and at δ 5.01 for the (32*S*)-compound **14**. (d) The 22-methyl doublet is clearly resolved in the case of the (32*R*)-configuration (**13**, δ 0.92, *J* 6.5 Hz). In the spectrum of the (32*S*)-diastereoisomer **14**, only the upfield part of the doublet can be observed, the downfield part and the singlets of the 8β- and the 14α-methyl groups overlapping one another.

Results of the comparison (MS, HPLC, $^1$H NMR) of the saturated diacetate, obtained by hydrogenation from the natural $\Delta^6$-diol **2**, with the two synthesized diastereoisomeric diacetates **13** and **14** were in full accord with a (22*R*,32*R*)-configuration for all new 34,35-dinorbacteriohopanediols isolated from *Acetobacter aceti* ssp. *xylinum*.

The significance of these new $C_{33}$-diols is not obvious. We know that the supplementary $C_5$-polyoxygenated side-chain of the $C_{35}$-bacteriohopane derivatives arises from a D-pentose unit *via* the non-oxidative pentose phosphate pathway.[17] Does the side-chain of the $C_{33}$-diols arise from a similar, parallel pathway, involving the introduction of a $C_3$-unit instead of a $C_5$-unit? Or are these compounds catabolites of the bacteriohopanetetraols? Indeed, all five diols possess double bonds, which are introduced in the triterpenic skeleton after its formation and might represent the first steps of a hopanoid degradation sequence. Concerning the formation of the shortened side-chain, a bacteriohopane derivative possessing a 32-oxo-33,34,35-triol side-chain has already been isolated from the ethanol-producing bacterium *Zymomonas mobilis*[18] and might be a potential precursor. A retro-aldol reaction would cleave the carbon–carbon bond between C-33 and C-34 and would lead to the framework of the new diols. These diols have not, however, been detected in either *Acetobacter pasteurianus* ssp. *pasteurianus* NCIB 6249 or in *Zymomonas mobilis* ATCC 29191. The new data point towards the still completely unknown catabolism of biohopanoids.

## Experimental

Most of the analytical methods have been described in a previous paper (ref. 19). $^1$H NMR spectra were recorded on a Bruker AC 250 spectrometer, the operating frequency being 250 MHz. As the chemical shifts of methyl groups grafted on the pentacyclic hopanoid skeleton are strongly temperature dependent, they were cited with three significant figures only for the spectra of the natural compounds **1**–**4**, as well as these of the synthetic end-products **13** and **14** and the hydrogenation product of **2**, that were recorded under thermostatic conditions (300 K). The assignments of $^1$H NMR signals marked with an asterisk can be reversed. *J*-Values are given in Hz. Optical rotations were measured on a Schmidt and Haensch Polartronic Universal apparatus at 589 nm with a 1.2 cm$^3$ cell and 10 cm optical pathway. Flash chromatography was carried out according to the method of Still *et al.*,[20] using Merck Kieselgel 60 (230–400 mesh ASTM). HPLC was carried out on a Waters 510 apparatus, equipped with a Spectra Physics SP 6040 differential refractometer and an analytical column (4.6 mm × 25 cm) or a preparative column (21.2 mm × 25 cm) containing a Du Pont Zorbax ODS reversed phase, with MeOH as eluant. Organic phases separated from water were dried over anhydrous $Na_2SO_4$, and solvents were removed under reduced pressure (water-bath, 35 °C). Light petroleum refers to the fraction boiling in the range 40–60 °C.

*Isolation of the Diacetates of Compounds* **1**–**5** *from* Acetobacter aceti *ssp.* xylinum.—Freeze-dried cells (5 g, from Hoffmann-La Roche, Basel, strain R-2277) were extracted under reflux with $CHCl_3$–MeOH (2:1; 3 × 90 cm$^3$). After evaporation of the solvents the extract was acetylated at room temperature with acetic anhydride–pyridine (1:1; 6 cm$^3$). Flash chromatography with toluene–ethyl acetate (96:4) yielded a crude fraction containing the diacetates of compounds **1**–**5** with a slightly lower polarity than that of diplopterol. Further purification by preparative TLC (PLC) with cyclohexane–ethyl acetate (9:1; $R_f$ 0.34) gave a mixture of the diacetates of compounds **1**–**5** (3 mg), which were separated by reversed-phase HPLC to yield, in order of increasing retention time, the diacetates of compounds **1** (15%), **2** (34%), **3** (43%), **4** (5%) and **5** (3%).

*Diacetate of* (22R,32R)-34,35-*Dinorbacteriohopa*-6,11-*diene*-32,33-*diol* **1**. M.p. 159–160 °C; δ($CDCl_3$) 0.762 (3 H, s, Me), 0.840 (3 H, s, Me), 0.902 (3 H, s, Me), 0.928 (6 H, 2 s, 2 × Me), 0.94 (3 H, d, *J* 6.5, 22-Me), 1.045 (3 H, s, Me), 2.059 (3 H, s, 33-OAc), 2.065 (3 H, s, 32-OAc), 2.60 (1 H, br s, 9α-H), 4.03 (1 H, dd, $J_{gem}$ 12.0, $J_{vic}$ 6.5, 33-H$^a$), 4.22 (1 H, dd, $J_{gem}$ 12.0, $J_{vic}$ 3.5, 33-H$^b$), 5.05 (1 H, m, 32-H), 5.54 (1 H, ddd, $J_{11,12}$ 10.5, *J* 3.0, *J* 2.0, 11-H*), 5.59 (1 H, dd, $J_{6,7}$ 11.0, $J_{5\alpha,6}$ 2.0, 6-H), 5.63 (1 H, d, $J_{6,7}$ 11.0, 7-H) and 5.79 (1 H, ddd, $J_{11,12}$ 11.0, *J* 3.0, *J* 2.0, 12-H*); *m*/*z* 566 (M$^+$, 100%), 551 (M$^+$ − Me, 4), 506 (M$^+$ − AcOH, 2), 491 (M$^+$ − Me − AcOH, 2), 376 (retro-Diels–Alder after isomerization of $\Delta^{11}$ to $\Delta^9$, 4),[5] 365 (M$^+$ − side-chain, 9), 349 (ring C cleavage, 9),[21] 216 (retro-Diels–Alder after isomerization of $\Delta^{11}$ to $\Delta^9$, 54),[5] 189 (ring C cleavage, 51)[21] and 119 (characteristic fragment for hopanoids with $\Delta^6$-double bond, 72).[5]

*Diacetate of* (22R,32R)-34,35-*Dinorbacteriohop*-6-*ene*-32,33-*diol* **2**. M.p. 144–145 °C; δ($CDCl_3$) 0.677 (3 H, s, Me), 0.817 (6 H, 2 s, 2 × Me), 0.881 (3 H, s, Me), 0.92 (3 H, d, *J* 6.5, 22-Me), 0.947 (3 H, s, Me), 1.060 (3 H, s, Me), 2.057 (3 H, s, 32-OAc), 2.060 (3 H, s, 33-OAc), 4.03 (1 H, dd, $J_{gem}$ 12.0, $J_{vic}$ 6.5, 33-H$^a$), 4.21 (1 H, dd, $J_{gem}$ 12.0, $J_{vic}$ 3.5, 33-H$^b$), 5.03 (1 H, m, 32-H), 5.48 (1 H, dd, $J_{6,7}$ 10.5, $J_{5\alpha,6}$ 3.0, 6-H) and 5.61 (1 H, dd, $J_{6,7}$ 10.5, $J_{5\alpha,7}$ 2.0, 7-H); *m*/*z* 568 (M$^+$, 43%), 553 (M$^+$ − Me, 2), 508 (M$^+$ − AcOH, 1), 493 (M$^+$ − Me − AcOH, 6), 367 (M$^+$ − side-chain, 2), 349 (ring C cleavage, 13),[21] 189 (ring C cleavage, 100)[21] and 119 (characteristic fragment for hopanoids with $\Delta^6$-double bond, 61).[5]

*Diacetate of* (22R,32R)-3β-*Methyl*-34,35-*dinorbacteriohopa*-

6,11-*diene*-32,33-*diol* **3**. M.p. 139–140 °C; δ($CDCl_3$) 0.682 (3 H, s, Me), 0.762 (3 H, s, Me), 0.84 (3 H, d, *J* 6.5, 3β-Me), 0.893 (3 H, s, Me), 0.915 (6 H, 2 s, 2 × Me), 0.94 (3 H, d, *J* 6.5, 22-Me), 1.043 (3 H, s, Me), 2.057 (3 H, s, 32-OAc), 2.063 (3 H, s, 33-OAc), 2.58 (1 H, br s, 9α-H), 4.03 (1 H, dd, $J_{gem}$ 12.0, $J_{vic}$ 6.5, 33-H$^a$), 4.21 (1 H, dd, $J_{gem}$ 12.0, $J_{vic}$ 3.5, 33-H$^b$), 5.05 (1 H, m, 32-H), 5.54 (1 H, ddd, $J_{11,12}$ 10.5, *J* 3.0, *J* 2.0, 11-H*), 5.60 (1 H, dd, $J_{6,7}$ 10.5, $J_{5\alpha,6}$ 2.5, 6-H), 5.67 (1 H, dd, $J_{6,7}$ 10.5, $J_{5\alpha,7}$ 1.0, 7-H) and 5.79 (1 H, dt, $J_{11,12}$ 10.5, *J* 2.5, 12-H*); *m/z* 580 ($M^+$, 100%), 565 ($M^+$ − Me, 3), 520 ($M^+$ − AcOH, 1), 505 ($M^+$ − Me − AcOH, 2), 379 ($M^+$ − side-chain, 7), 376 (retro-Diels–Alder after isomerization of $\Delta^{11}$ to $\Delta^9$, 5),[5] 349 (ring C cleavage, 10),[21] 230 (retro-Diels–Alder after isomerization of $\Delta^{11}$ to $\Delta^9$, 59),[5] 203 (ring C cleavage, 59)[21] and 119 (characteristic fragment for hopanoids with $\Delta^6$ double bond, 87).[5]

*Diacetate of* (22R,32R)-3β-*Methyl*-34,35-*dinorbacteriohop*-6-*ene*-32,33-*diol* **4**. M.p. 137–138 °C; δ($CDCl_3$) 0.659 (3 H, s, Me), 0.677 (3 H, s, Me), 0.784 (3 H, s, Me), 0.83 (3 H, d, *J* 6.5, 3β-Me), 0.893 (3 H, s, Me), 0.93 (3 H, d, *J* 6.5, 22-Me), 0.944 (3 H, s, Me), 1.057 (3 H, s, Me), 2.057 (3 H, s, 32-OAc), 2.061 (3 H, s, 33-OAc), 4.03 (1 H, dd, $J_{gem}$ 12.0, $J_{vic}$ 6.5, 33-H$^a$), 4.21 (1 H, dd, $J_{gem}$ 12.0, $J_{vic}$ 3.5, 33-H$^b$), 5.03 (1 H, m, 32-H), 5.49 (1 H, dd, $J_{6,7}$ 10.5, $J_{5\alpha,6}$ 3.0, 6-H) and 5.64 (1 H, dd, $J_{6,7}$ 10.5, $J_{5\alpha,7}$ 1.5, 7-H); *m/z* 582 ($M^+$, 41%), 567 ($M^+$ − Me, 2), 522 ($M^+$ − AcOH, 1), 507 ($M^+$ − Me − AcOH, 6), 381 ($M^+$ − side-chain, 3), 349 (ring C cleavage, 15),[21] 203 (ring C cleavage, 87)[21] and 119 (characteristic fragment for hopanoids with $\Delta^6$ double bond, 100).[5]

*Diacetate of* (22R,32R)-3β-*Methyl*-34,35-*dinorbacteriohop*-11-*ene*-32,33-*diol* **5**. δ($CDCl_3$) 0.66 (3 H, s, Me), 0.78 (3 H, s, Me), 0.84 (3 H, d, *J* 7, 3β-Me), 0.85 (3 H, s, Me), 0.87 (6 H, 2 s, 2 × Me), 0.88 (3 H, s, Me), 0.94 (3 H, d, *J* 6.5, 22-Me), 2.05 (3 H, s, 32-OAc), 2.06 (3 H, s, 33-OAc), 4.04 (1 H, dd, $J_{gem}$ 12.0, $J_{vic}$ 6.5, 33-H$^a$), 4.22 (1 H, dd, $J_{gem}$ 12.0, $J_{vic}$ 3.5, 33-H$^b$), 5.04 (1 H, m, 32-H), 5.56 (1 H, d, $J_{11,12}$ 10.0, 11-H*) and 5.65 (1 H, d, $J_{11,12}$ 10.0, 12-H*); *m/z* 582 ($M^+$, 55%), 567 ($M^+$ − Me, 10), 522 ($M^+$ − AcOH, 1), 507 ($M^+$ − Me − AcOH, 3), 381 ($M^+$ − side-chain, 13), 376 (retro-Diels–Alder after isomerization of $\Delta^{11}$ to $\Delta^9$, 100),[5] 349 (ring C cleavage, 42),[21] 316 (376 − AcOH, 19), 232 (retro-Diels–Alder after isomerization of $\Delta^{11}$ to $\Delta^9$, 70)[5] and 205 (ring C cleavage, 33).[21]

*Synthesis of the Diacetates* **13** *and* **14** *of* (22R,32R)- **11** *and* (22R,32S)-34,35-*Dinorbacteriohopane*-32,33-*diol* **12**.—*Methyl* (22R)-33,34,35-*trinorbacteriohopan*-32-*oate* **7**. A mixture of bromine (0.6 $cm^3$, 11.8 mmol, 5.6 mol equiv.), water (1.5 $cm^3$) and $NaHCO_3$ (1 g, 12 mmol) was added to (22R)-33,34,35-trinorbacteriohopan-32-al **6** (1 g, 2.2 mmol) and $NaHCO_3$ (1 g) in tetrahydrofuran (THF) (10 $cm^3$). The reaction was shown to be complete by TLC after 90 min. After destruction of excess of bromine by solid $Na_2S_2O_3$ and addition of water, the acid was extracted with $Et_2O$ from the acidified aqueous phase. Methylation with excess of ethereal diazomethane and flash chromatography with light petroleum–$Et_2O$ (12:1) yielded ester **7** (618 mg, 58%); TLC (toluene) $R_f$ 0.5; m.p. 183–184 °C; $[\alpha]_D$ +58° (*c* 0.0082, $CHCl_3$) after two crystallizations from $CH_2Cl_2$–MeOH; δ(250 MHz; $CDCl_3$) 0.70 (3 H, s, 18α-Me), 0.79 (3 H, s, 4β-Me), 0.82 (3 H, s, 10β-Me), 0.85 (3 H, s, 4α-Me), 0.93 (3 H, d, *J* 8.5, 22-Me), 0.95 (6 H, s, 8β- and 14α-Me), 2.20 (1 H, ddd, $J_{gem}$ 15.5, $J_{vic}$ 9.0, $J_{vic}$ 6.5, 31-H$^a$), 2.34 (1 H, ddd, $J_{gem}$ 15.5, $J_{vic}$ 10.0, $J_{vic}$ 5.5, 31-H$^b$) and 3.65 (3 H, s, OMe); GLC–MS *m/z* 484 ($M^+$, 7%), 469 ($M^+$ − Me, 8), 453 ($M^+$ − OMe, 2), 369 ($M^+$ − side-chain, 16), 263 (ring C cleavage, 100)[21] and 191 (ring C cleavage, 58).[21]

(22R)-33-[(R)-(+)-p-*Tolylsulphinyl*]-34,35-*dinorbacteriohopan*-32-*one* **8**. A butyllithium solution in hexane (1.3 mol $dm^{-3}$; 385 $mm^3$, 0.5 mmol) was slowly added to a solution of diisopropylamine (71 $mm^3$, 0.5 mmol, 3 mol equiv. with regard to **7**) in THF (1.5 $cm^3$) at 0 °C. After 25 min the reaction mixture was cooled to −30 °C, and a solution of (*R*)-(+)-methyl *p*-tolyl sulphoxide[9] (70 mg, 0.45 mmol, 2.7 mol equiv.) in THF (1.5 $cm^3$) was added dropwise. After 45 min the temperature had reached −10 °C. The reaction mixture was then cooled to −78 °C and a solution of methyl ester **7** (81 mg, 0.17 mmol) in THF (2 $cm^3$) was added to the solution. After 1 h, only the reaction product **8** could be detected by TLC with $Et_2O$–light petroleum (4:1; $R_f$ 0.28). The mixture was stirred for 1 h at 0 °C before the addition of saturated aq. $NH_4Cl$. Extraction with $CH_2Cl_2$ and flash chromatography with $Et_2O$–light petroleum (2:1) afforded β-keto sulphoxide **8** (69.6 mg, 68%); m.p. 159–160 °C; $[\alpha]_D$ +113° (*c* 0.0089, $CHCl_3$); δ($C_6D_6$) 0.73 (3 H, s, Me), 0.88 (3 H, d, *J* 6.0, 22-Me), 0.88 (6 H, 2 s, 2 × Me), 0.93 (3 H, s, Me), 0.93 (3 H, s, Me), 1.00 (3 H, s, Me), 1.97 (3 H, s, Ar*Me*), 2.21 (1 H, ddd, $J_{gem}$ 18.0, $J_{vic}$ 8.5, $J_{vic}$ 7.0, 31-H$^a$), 2.31 (1 H, ddd, $J_{gem}$ 18.0, $J_{vic}$ 5.5, $J_{vic}$ 9.0, 31-H$^b$), AB-system (2 H, $\delta_A$ 3.26, $\delta_B$ 3.35, $J_{AB}$ 13.0, 33-$H_2$) and multiplet of AA′-XX′-type (4 H, centred at δ 6.88 and δ 7.39, *p*-disubstituted aromatic ring); δ($CDCl_3$) 0.67 (3 H, s, Me), 0.79 (3 H, s, 4β-Me), 0.81 (3 H, s, 10β-Me), 0.84 (3 H, s, 4α-Me), 0.84 (d, *J* 6.0, 22-Me), 0.94 (6 H, 2 s, 8β- and 14α-Me), 2.31 (1 H, ddd, $J_{gem}$ 18.0, $J_{vic}$ 9.5, $J_{vic}$ 6.0, 31-H$^a$), 2.42 (3 H, s, Ar*Me*), 2.48 (1 H, ddd, $\delta_{gem}$ 18.0, $J_{vic}$ 9.5, $J_{vic}$ 5.0, 31-H$^b$), AB-system (2 H, $\delta_A$ 3.75, $_B$ 3.88, $J_{AB}$ 13.5, 33-$H_2$) and multiplet of AA′BB′-type (4 H, centred at δ 7.34 and δ 7.54, *p*-disubstituted aromatic ring). As the β-keto sulphoxide **8** was slowly degraded in acidic $CDCl_3$ in the absence of a stabilizer, we measured the $^1H$ NMR spectra of all sulphoxide compounds in deuteriobenzene ($C_6D_6$).

(22R,32R)-33-[(R)-(+)-(p-*Tolylsulphinyl*)]-34,35-*dinorbacteriohopan*-32-*ol* **9**. A solution of β-keto sulphoxide **8** (52 mg, 0.086 mmol) in THF (1.5 $cm^3$) was stirred at room temp. with a $ZnCl_2$–THF solution (0.3 mol $dm^{-3}$; 400 $mm^3$, 0.12 mmol, 1.4 mol equiv.) for 40 min. The solution was cooled to −78 °C and was added dropwise to a DIBAL–hexane solution (1 mol $dm^{-3}$; 1 $mm^3$, 0.18 mmol, 2.1 mol equiv.) in THF (1 $cm^3$). After 2 h the reaction was quenched by addition of MeOH to yield compound **9**, $R_f$ [light petroleum–ethyl acetate (1:1)] 0.25. The solvent was removed and the residue was mixed with 5% aq. NaOH before extraction with $CH_2Cl_2$. Separation by flash chromatography yielded β-hydroxy sulphoxide **9** (45.5 mg, 87%); m.p. 218–219 °C; $[\alpha]_D$ +128° (*c* 0.0063, $CHCl_3$). The $^1H$ NMR spectrum before chromatography showed the presence of only one diastereoisomer, the stereoselectivity therefore being higher than 95% (de > 90%); δ($C_6D_6$) 0.78 (3 H, s, Me), 0.88 (6 H, 2 s, 2 × Me), 0.93 (3 H, s, Me), 1.00 (3 H, s, Me), 1.01 (3 H, s, Me), 1.02 (3 H, d, *J* 6.5, 22-Me), 1.97 (3 H, s, Ar*Me*), 2.33 (1 H, highfield part of ABX-system, $J_{gem}$ 13.0, $J_{vic}$ 2.0, 33-H$^a$), 2.75 (1 H, lowfield part of a ABX-system, $J_{gem}$ 13.0, $J_{vic}$ 9.5, 33-H$^b$), 4.28 (1 H, br s, OH), 4.31 (1 H, m, 32-H) and a multiplet of AA′XX′-type (4 H, centred at δ 6.87 and δ 7.37, *p*-disubstituted aromatic ring).

(22R,32S)-33-[(R)-(+)-(p-*Tolylsulphinyl*)]-34,35-*dinorbacteriohopan*-32-*ol* **10**. A DIBAL–hexane solution (1 mol $dm^{-3}$; 140 $mm^3$, 0.14 mmol, 1.3 mol equiv.) was added to a solution of β-keto sulphoxide **8** (63.8 mg, 0.105 mmol) in THF–$Et_2O$–hexane [2 $cm^3$; (4:1:1)] at −100 °C. After 135 min MeOH was added. Work-up was carried out as described for compound **9** and yielded both diastereoisomers **9** and **10** (56.8 mg, 89%), with 80% being the 32*S*-isomer **10** (de 60%), as calculated from $^1H$ NMR integration of the 33-$H_2$ ABX-system of the product before purification; δ($C_6D_6$) 0.67 (3 H, s, Me), 0.88 (6 H, 2 s, 2 × Me), 0.93 (3 H, s, Me), 0.93 (3 H, d, *J* 6.0, 22-Me), 0.97 (3 H, s, Me), 0.99 (3 H, s, Me), 1.95 (3 H, s, Ar*Me*), 2.25 (0.8 H, highfield part of ABX-system of 32*S*-isomer **10**, $J_{gem}$ 13.5, $J_{vic}$ 1.5, 33-H$^a$), 2.28 (0.2 H, highfield part of ABX-system of 32*R*-isomer **9**, $J_{gem}$ 13.0, $J_{vic}$ 2.0, 33-H$^a$), 2.74 (0.2 H, lowfield part of

ABX-system of 32*R*-isomer **9**, $J_{gem}$ 13.0, $J_{vic}$ 9.5, 33-H$^b$), 2.83 (0.8 H, lowfield part of ABX-system of 32*S*-isomer **10**, $J_{gem}$ 13.5, $J_{vic}$ 9.5, 33-H$^b$), 4.18 (1 H, m, 32-H), 4.31 (1 H, d, *J* 2.5, OH) and a multiplet of AA'XX'-type (4 H, centred at δ 6.85 and δ 7.34, *p*-disubstituted aromatic ring).

*Diacetate* **13** *of* (22R,32R)-34,35-*dinorbacteriohopane*-32,33-*diol* **11**. A solution of trifluoroacetic anhydride (TFAA) in $CH_2Cl_2$ (2.36 mol dm$^{-3}$; 250 mm$^3$, 0.59 mmol, 21 mol equiv.) was added to a mixture of (32*R*)-β-hydroxy sulphoxide **9** (17 mg, 0.028 mmol) and 2,4,6-trimethylpyridine (*sym*-collidine) (150 mm$^3$, 1.13 mmol, 40 mol equiv.) in THF (0.8 cm$^3$) at 0 °C. After 45 min the reaction mixture was evaporated to dryness and the residue was stirred for 5 h with an excess of sodium borohydride in ethanol–water [1 cm$^3$ (9:1)]. Quenching with aq. $KH_2PO_4$ (5 cm$^3$) and extraction (4 × $CH_2Cl_2$) afforded the diol **11**, which was acetylated overnight with excess of acetic anhydride–pyridine (1:1) at room temp. PLC with cyclohexane–ethyl acetate [(9:1), $R_f$ 0.28] yielded a diastereoisomeric mixture (10.6 mg, 66%), consisting of 86% acetate **13** and 14% acetate **14** [de 72%, calculated from $^1$H NMR integration of the double doublet of 33-H$^b$ before the isolation of compound **13** by HPLC]. Even though the chromatogram peaks were not fully resolved, an analytically pure sample of the slightly more polar, main diastereoisomer **13** could be obtained by reversed-phase HPLC, m.p. 189–190 °C; $[\alpha]_D$ +38° (*c* 0.0027, $CHCl_3$); δ($CDCl_3$) 0.688 (3 H, s, 18α-Me), 0.789 (3 H, s, 4β-Me), 0.811 (3 H, s, 10β-Me), 0.843 (3 H, s, 4α-Me), 0.92 (3 H, d, *J* 6.5, 22-Me), 0.944 (6 H, 2 s, 8β- and 14α-Me), 2.057 (3 H, s, 32-OAc), 2.061 (3 H, s, 33-OAc), 4.03 (1 H, dd, $J_{gem}$ 12.0, $J_{vic}$ 6.5, 33-H$^a$), 4.21 (1 H, dd, $J_{gem}$ 12.0, $J_{vic}$ 3.5, 33-H$^b$) and 5.03 (1 H, m, 32-H); *m/z* 570 (M$^+$, 19%), 555 (M$^+$ − Me, 7), 510 (M$^+$ − AcOH, 2), 369 (M$^+$ − side-chain, 25), 349 (ring C cleavage, 100)[21] and 191 (ring C cleavage, 72).[21]

*Diacetate* **14** *of* (22R,32S)-34,35-*dinorbacteriohopane*-32,33-*diol* **12**. The diastereoisomeric mixture (22*R*,32*S*)-**10** (80%) and (22*R*,32*R*)-**9** (20%) (12.2 mg, 0.02 mmol) was treated in THF with 2,4,6-trimethylpyridine and TFAA for 60 min, as described for the formation compound **13**. After evaporation to dryness the residue was stirred with an excess of sodium borohydride in ethanol (96%), without addition of water, for 21 h. Work-up and acetylation were carried out as for compound **13**. This yielded a diastereoisomeric mixture (7.3 mg, 64%) of acetates **14** (76%) and **13** (24%) [de 52%, calculated as for the formation of **13**). As described for compound **13**, an aliquot of pure compound could be isolated by HPLC, m.p. 189–190 °C; $[\alpha]_D$ +52° (*c* 0.0019, $CHCl_3$); δ($CDCl_3$) 0.692 (3 H, s, 18α-Me), 0.791 (3 H, s, 4β-Me), 0.814 (3 H, s, 10β-Me), 0.845 (3 H, s, 4α-Me), 0.93 (3 H, d, *J* 6.5, 22-Me), 0.947 (6 H, 2 s, 8β- and 14α-Me), 2.060 (6 H, 2 s, 32- and 33-OAc), 4.03 (1 H, dd, $J_{gem}$ 12.0, $J_{vic}$ 6.5, 33-H$^a$), 4.23 (1 H, dd, $J_{gem}$ 12.0, $J_{vic}$ 3.0, 33-H$^b$) and 5.01 (1 H, m, 32-H).

*Hydrogenation of the Diacetate of* (22R,32R)-34,35-*Dinorbacteriohop*-6-*ene*-32,33-*diol* **2**.—A mixture of the diacetate of the natural compound **2** (2 mg) and $PtO_2$ (Adams' catalyst, 20 mg) in ethyl acetate (0.7 cm$^3$) was stirred under hydrogen at room temperature for 35 h (catalyst and solvent were replaced twice). After filtration the residue was acetylated with an excess of acetic anhydride–pyridine (1:1) at room temp. overnight. PLC with cyclohexane–ethyl acetate [(9:1) $R_f$ 0.27] yielded a mixture of starting material (23% recovery) and hydrogenated compound **13** (77%), which was separated by reversed-phase HPLC, δ($CDCl_3$) 0.690 (3 H, s, 18α-Me), 0.790 (3 H, s 4β-Me), 0.814 (3 H, s, 10β-Me), 0.845 (3 H, s, 4α-Me), 0.92 (3 H, d, *J* 6.5, 22-Me), 0.946 (6 H, 2 s, 8β- and 14α-Me), 2.055 (3 H, s, 32-OAc), 2.059 (3 H, s, 33-OAc), 4.03 (1 H, dd, $J_{gem}$ 12.0, $J_{vic}$ 6.5, 33-H$^a$), 4.21 (1 H, dd, $J_{gem}$ 12.0, $J_{vic}$ 3.5, 33-H$^b$) and 5.03 (1 H, m, 32-H); *m/z* 570 (M$^+$, 25%), 555 (M$^+$ − Me, 9), 510 (M$^+$ − AcOH, 2), 495 (M$^+$ − Me − AcOH, 1), 369 (M$^+$ − side-chain, 22), 349 (ring C cleavage, 100)[21] and 191 (ring C cleavage, 65).[21]

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