Concise syntheses of bacteriohopanetetrol and its glucosamine derivative†

Weidong Pan,[acd] Yongmin Zhang,[a] Guangyi Liang,[cd] Stéphane P. Vincent*[ab] and Pierre Sinaÿ*[a]

Received (in Cambridge, UK) 4th April 2005, Accepted 16th May 2005
First published as an Advance Article on the web 9th June 2005
DOI: 10.1039/b504558d

This study describes the syntheses of bacteriohopanetetrol and its glucosamine derivative through a key direct coupling of a ribose derivative to the hopane skeleton.

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 solfataricus*,[1] and bacteriohopane derivatives **1** and **2** (Fig. 1), isolated from *Zymomonas mobilis*.[2–4] Ether **1** and glycoside **2** are not only major components of this eubacterium, they also share the same stereochemical pattern. To investigate their biosynthetic relationship, we performed a preliminary study where *Z. mobilis* was grown on a culture medium containing deuterated *N*-acetyl-D-glucosamine (GlcNAc).[5] To our delight, selective labelling of the cyclopentanic ether and the glycoside of **1** and **2**, respectively, has been observed. This experiment clearly showed that these two molecules share the same biogenetic pathway derived from GlcNAc. Moreover, the similarities of the isotope abundance found in labelled hopanoids suggested a product to precursor relationship between **1** and **2**. These intriguing results led us to hypothesize that a glycoside could be the precursor of an ether through a so far unknown enzymatic rearrangement.

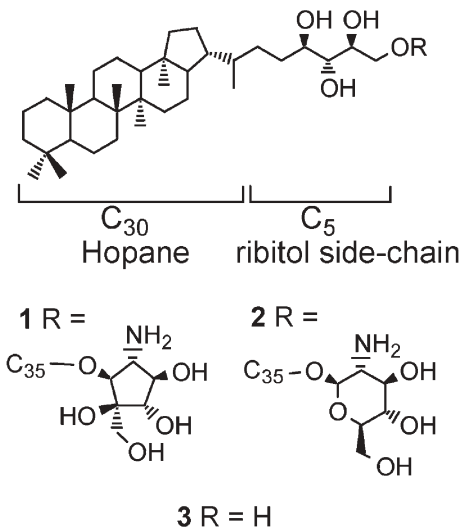
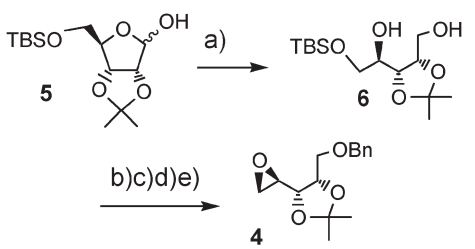


Fig. 1

In order to demonstrate or rule out this mechanistic assumption, we now need to show that glycoside **2** can be transformed into **1** by *Z. mobilis* enzymatic machinery. Unfortunately, this molecule has never been synthesized and is difficult to produce from natural sources. Glycoside **2** was first isolated as its peracetate[3,6] but due to its amphiphilic nature, its deacetylation proved unsuccessful. Later on, a procedure based on solid-phase extraction followed by semi-preparative HPLC purification of **2** was described, but no chemical characterisation was provided.[7] Moreover, this method does not allow the access to labelled analogs of **2** that represent valuable tools for biochemical investigations. A chemical synthesis of this challenging molecule was thus envisioned. Retrosynthetically, we chose to follow a biomimetic sequence: ligation of a D-ribose derivative (C_5 unit) to the terpenic hopane skeleton (C_{30} unit) followed by a glycosylation.

Two synthetic preparations of hopanetetrol **3** have been reported to date: a non-stereoselective synthesis allowing the preparation of the eight diastereomers of **3**,[8] and more recently a stereoselective version relying on a $C_{30} + C_2$ coupling reaction followed by an asymmetric construction of the D-ribitol substructure.[9] In these two cases the direct coupling of the hopane skeleton to a D-ribose derivative was abandoned due to poor yields (<10%). However, we decided to reinvestigate such an approach in the hope of disclosing a more convergent synthesis.

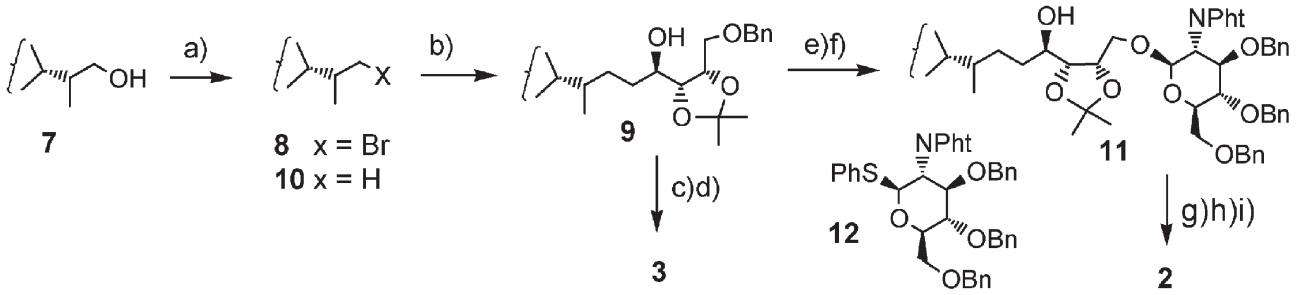
As a key step, we chose to activate the hopane C_{30} unit as an organocopper derivative and couple it to epoxide **4**. The synthesis of **4** started from known lactol **5**, easily prepared from D-ribose (Scheme 1).[10,11] Alcohol **6** was then benzylated and epoxide **4** was generated through a three step sequence: desilylation, regioselective tosylation and epoxide formation. Following this strategy, the key



Scheme 1 *Reagents and conditions:* a) $NaBH_4$ (quant.); b) BnBr, NaH (69%); c) TBAF (quant.); d) TsCl (79%); e) BuLi (quant.).

† Electronic supplementary information (ESI) available: analytical data of molecules **2** and **11**, preparation of thioglycoside **12** and coupling procedure of bromide **8** with epoxide **4**. See http://www.rsc.org/suppdata/cc/b5/b504558d/index.sht

*Stephane.Vincent@fundp.ac.be (Stéphane P. Vincent)
sinay@ens.fr (Pierre Sinaÿ)



Scheme 2 *Reagents and conditions:* a) PPh_3, Br_2 (95%); b) (Thienyl)CuCNLi, add NaphtLi, then **8**, then **4** (55%); c) H_2, $Pd(OH)_2$ (quant.); d) CSA (91%); e) H_2, $Pd(OH)_2/C$ (quant.); f) **12**, NIS, TfOH, CH_2Cl_2 (77%); g) CSA (82%); h) H_2NNH_2, 80 °C (92%); i) H_2, Pd black THF–AcOH–H_2O (quant.).

epoxide **4** could be prepared in multigram quantities in 43% overall yield from D-ribose.

The C_{30} primary alcohol **7** was produced from a natural hopanone following the procedure developed by Rohmer and Duvold.[9] Bromide **8** was then prepared in sufficient amount to allow an extensive methodological study (Scheme 2). The first attempts at metal–halogen exchange followed by nucleophilic attack on epoxide **4** or on various model epoxides or aldehydes were very disappointing, the expected product **9** being isolated only in trace amounts. The usual techniques optimized to generate organocopper derivatives from **8** were screened[12] but the main product was always that resulting from protonation (compound **10**), showing that the activation of the halogenide occurred but not the nucleophilic attack. For instance, we attempted, as a first approach, to generate organocuprates through a transient organolithium derivative. We screened different lithium–halogen exchange reactions using metallic lithium, lithium naphthalenide and LiDBB (lithium di-*tert*-butylbiphenylide) followed by addition of CuCN or CuI. These experiments only lead to the protonation product **10**. This poor reactivity might be attributable to the severe steric hindrance of position C-30 due to the convex shape of this pentacycle. However we reasoned that the metallated hopane derivative could also be unstable. Therefore we sought conditions allowing the fastest formation of the organocopper derivative in order to expose it to epoxide **4** as quickly as possible. With this purpose in mind, we took advantage of the procedure established by Rieke *et al*., who used 2-thienyl lithium organocuprate and lithium naphthalenide to generate the so-called zero-valent organocopper derivatives, directly from primary halogenides.[13] Application of this procedure allowed us to isolate the desired hopanetetrol derivative **9** in moderate yield (<25%). After optimization, we now have in hand a procedure giving a reproducible and satisfactory 55% yield. In particular, two simultaneous modifications have been found to play a critical role in determining the efficiency of the coupling procedure: the relative proportions of all reactants and the order of addition of the required lithium naphthalenide (NaphtLi). Typically, the initial procedure developed with simple bromides uses large excesses of NaphtLi (32 equivalents compared to bromide), lithium 2-thienyl-cyanocuprates (30 eq.) and epoxide (30 eq.).[13] We first limited the excess of epoxide **4** to 2.8 equivalents and quickly realized that reducing the excess of NaphtLi and thienylcyanocuprates to 4.4 equivalents significantly decreased the amount of the protonation side-product **10** without affecting the coupling kinetics.† Surprisingly, the yields were also improved by the way the intermediate thienyl-based activated copper was generated: reactions were systematically cleaner if NaphtLi was added into a solution of thienylcyanocuprate instead of the published reverse addition. Moreover, the yields were always better when the reaction was carried out with bromide **8** compared with the corresponding iodide. In summary, the key parameter allowing the successful coupling of the C_{30} hopane skeleton to the C_5 ribitol side chain was indeed the quickest formation of an organocopper derivative directly from a primary bromide, thus confirming our intuitive assumption that the metallated C-30 position is unstable.

Standard deprotections yielded bacteriohopanetetrol **3** in 3 steps and 50% overall yield from **7**. In all respects, analytical data of synthetic tetrol **3** and its peracetate corresponded to the natural ones.[9] This approach is the most efficient preparation of natural tetrol **3** and allowed us to complete the synthesis of the even more challenging glucosamine derivative **2**. Indeed, the quantitative removal of the benzyl group generated a diol that could be directly involved in a glycosylation (Scheme 2). As expected, the coupling of thioglycoside **12**† proceeded regio- and stereoselectively to give **11** in 77% yield. Given the amphiphilic character of the target molecule, the deprotection strategy was carefully chosen, the hydrogenolysis step being performed as the very last step. Therefore the acetonide and the phthalimido groups were removed first, using classical conditions. After screening both the proper catalyst and the proper solvent system, we finally found hydrogenolysis conditions yielding the final pure glycoside **2** in a quantitative yield. For such a reaction, a ternary solvent system composed of THF, AcOH and water was absolutely required to observe a total and clean hydrogenolysis. The structure of glycolipid **2** was confirmed by its peracetylation and comparison with the analytical data of the natural molecule's peracetate.†[4,6]

In conclusion, this work describes a new biomimetic strategy to efficiently construct bacteriohopanetetrol and its glucosamine derivative. This chemical synthesis gives access not only to the first full characterisation of natural glycoside **2** but also to interesting tools to study the biosynthesis of ether **1**.

This project was funded by the "*Association Franco-Chinoise pour la Recherche Scientifique et Technique*" (AFCRST, program PRA B02-07), the French embassy in China (PhD grant to W. P.) and the CNRS (Centre National de la Recherche Scientifique).

Weidong Pan,[acd] Yongmin Zhang,[a] Guangyi Liang,[cd] Stéphane P. Vincent*[ab] and Pierre Sinaÿ*[a]
[a]*Ecole Normale Supérieure, Département de Chimie, UMR 8642 du CNRS, 24 rue Lhomond, 75231 Paris Cedex 05, France. E-mail: sinay@ens.fr; Fax: +33 (0)1 44 32 33 97*
[b]*University of Namur, Laboratoire de Chimie Bio-Organique, rue de*

Bruxelles 61, 5000 Namur, Belgium.
E-mail: Stephane.Vincent@fundp.ac.be; Fax: +32 (0)81724517
[c]*The Key Laboratory of Chemistry for Natural Products of Guizhou Province and Chinese Academy of Sciences, Guiyang 550002, P. R. China. E-mail: guangyi_liang@21cn.com*
[d]*Guizhou University, The Center for Research and Development of Fine Chemicals, Guiyang 550025, P. R. China*

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