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On the total synthesis of (*S*)-methanophenazine and the formal synthesis of (*R*)-methanophenazine from a common precursor

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Abstract

Methanophenazine is a new cofactor from methanogenic archaea. (*S*)-Methanophenazine has been synthesized from three building blocks, i.e. 2-hydroxy-phenazine, ethyl (*R*)-3-methylglutarate and (*E*,*E*)-farnesyl acetone; a stereodivergent approach from ethyl (*R*)-3-methylglutarate warrants the formal synthesis of (*R*)-methanophenazine.

Keywords: electron transfer; natural products; nitrogen heterocycles; phenazines; terpenoids.

Methanogenesis is the terminal step in the degradation of complex organic compounds and is essential for the mineralization of organic material. The formation of methane from simple compounds such as hydrogen and carbon dioxide, formic acid, methanol, methylamines or acetic acid, is the characteristic feature of the strictly anaerobic methanogenic archaea. The metabolic pathways leading to the generation of methane are unique and involve a number of enzymes and cofactors that only occur in methanogens. Methyl-S-CoM, the central intermediate of methanogenesis, is reductively demethylated to yield methane by methyl CoM reductase.[1] Both electrons for this process come from CoB–SH to yield the heterodisulfide CoB–S–S–CoM.[2] The energy-conserving step in the metabolism of methylotrophic methanogens is the reduction of CoB–S–S–CoM with H_2 or $F_{420}H_2$.[3] The H_2:hetero-disulfide oxidoreductase- and the $F_{420}H_2$:hetero-disulfide oxidoreductase systems are responsible for the reductive regeneration of HS–CoM and HS–CoB from CoB–S–S–CoM. Both the membrane-bound hydrogenase and the $F_{420}H_2$ dehydrogenase are key enzymes of these electron transport systems. The electrons are transferred to the heterodisulfide reductase and finally to CoB–S–S–CoM. The most interesting question relates to the nature of the electron carriers mediating the electron transfer.[4]

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† Dedicated to Professor Dr. Horst Kunz on the occasion of his 60th birthday.

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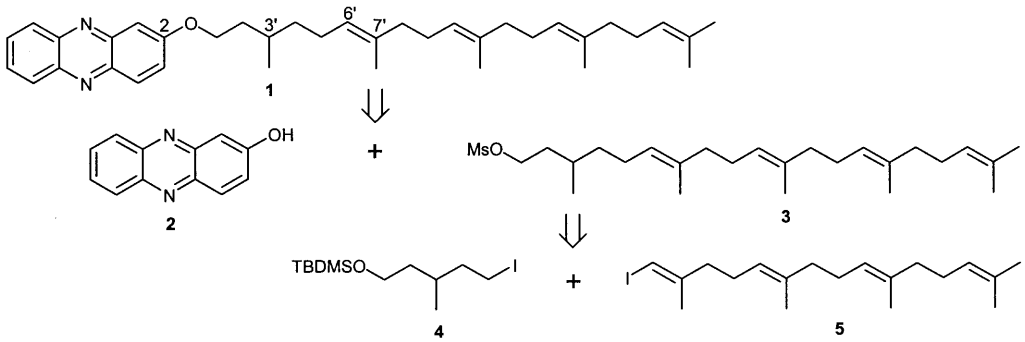
Recently methanophenazine (**1**) has been isolated from the cytoplasmic membranes of *Methanosarcina mazei* Gö1,[5] and racemic methanophenazine (*rac*-**1**) was shown to function as an electron carrier in the enzyme catalyzed heterodisulfide reduction with either H_2 or $F_{420}H_2$.[6] Most importantly, these results indicate that the role of the new cofactor in the energy metabolism of methanogenic archaea corresponds to that of ubiquinone in bacteria and mitochondria. The absolute configuration of the new natural product could not be elucidated by analytical or spectroscopic methods. An alternative relies on chemical degradation. As only small amounts of methanophenazine are available from the natural source it will be necessary to carry out the degradation first with synthetic material. This is why we designed a route that can easily be tailored to (*S*)- and (*R*)-methanophenazine and is flexible enough to synthesize a number of methanophenazine derivatives for degradation and biological studies. Here we report on both the first total synthesis of (*S*)-methanophenazine [(*S*)-**1**] and the formal synthesis of (*R*)-methanophenazine [(*R*)-**1**] from a common enantiomerically pure precursor.

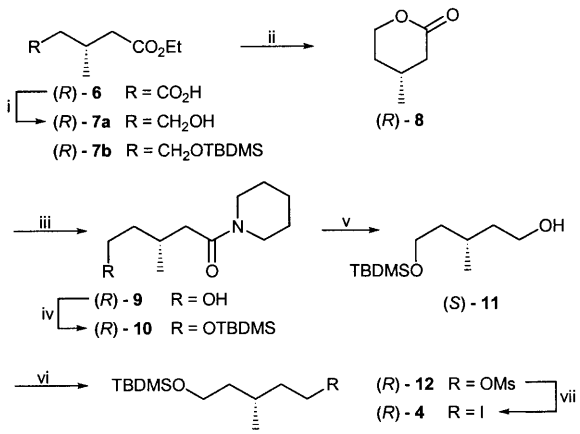
The retrosynthetic analysis results in 2-hydroxy-phenazine (**2**) and the terpenoid unit **3** to be linked by etherification. **3** was divided into the alkyl iodide **4** and the (*E*)-vinyl iodide **5** to be coupled by a Pd(0) catalyzed cross coupling reaction in order to ensure the correct geometry of the C-6′,C-7′ double bond of the side chain (Scheme 1).



Scheme 1.

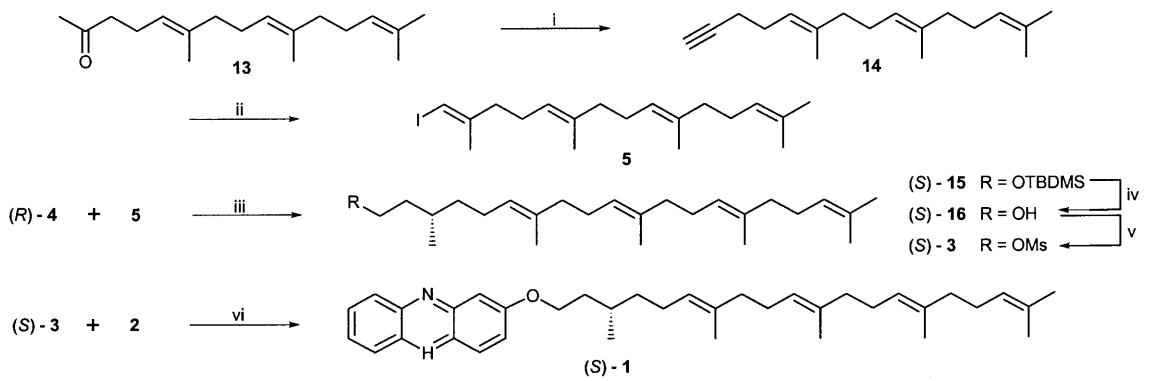
Particular attention has been paid to develop a stereodivergent approach for (*R*)-**4** and (*S*)-**4** from a common precursor. Ethyl (*R*)-3-methylglutarate [(*R*)-**6**] was found to be suitable for the synthesis of both enantiomerically pure bisfunctionalized C_6-building blocks. For the synthesis of enantiomerically pure (*S*)-methanophenazine [(*S*)-**1**] we had to prepare the monoprotected diol (*S*)-**11**. In accordance with earlier reports[7] the direct conversion of (*R*)-**6** into (*R*)-**7b** via chemoselective reduction of the carboxyl group with $BH_3{\cdot}Me_2S$ and silylation of the resulting hydroxy group could not be achieved. In all experiments mixtures of varying compositions of (*R*)-**7b** and the lactone (*R*)-**8** were isolated. This is why we had to develop a new protocol for the transformation of (*R*)-**6** into (*S*)-**11**. First the lactone (*R*)-**8** was prepared in 92% yield by chemoselective reduction of (*R*)-**6** with $BH_3{\cdot}Me_2S$, followed by cyclization of the resulting 5-hydroxy ester (*R*)-**7a** under basic conditions (Scheme 2).[8] We found that the lactone could be opened reliably with piperidine to afford the acyclic 5-hydroxy-amide (*R*)-**9** with 97% yield. After silylation of the hydroxyl group with TBDMSCl/NEt_3 the amide function of (*R*)-**10** was reduced selectively with lithium triethylborohydride[9] to the corresponding protected alcohol (*S*)-**11** in enantiomerically pure form.[10] Finally (*S*)-**11** was transformed into the iodide (*R*)-**4** via

mesylate (*R*)-**12**. To summarize, a reliable and practical synthesis of (*R*)-**4** in seven steps with a total yield of 75% was developed providing the material in gram quantities.



Scheme 2. (i) $BH_3{\cdot}SMe_2$ (1.2 equiv.), THF, 36 h, rt; (ii) 2 M aq. KOH, 10% MeOH, 8 h, 6N HCl, rt, 92% (2 steps); (iii) piperidine (8.0 equiv.), sealed tube, 12 h, 70°C, 97%; (iv) TBDMSCl (1.1 equiv.), NEt_3 (1.3 equiv.), CH_2Cl_2, 4 h, 0°C, 98%; (v) $LiBHEt_3$ (2.5 equiv.), THF, 2 h, 0°C to rt, 92%; (vi) MsCl (1.1 equiv.), NEt_3 (1.3 equiv.), THF, 15 min, 0°C; (vii) LiI (1.4 equiv.), THF, 2 h, 80°C, 93% (2 steps)

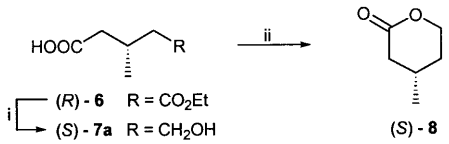
Preparation of **5** started with the transformation of (*E*,*E*)-farnesyl acetone (**13**) into the terminal alkyne **14** (Scheme 3).[11] Carboalumination of **14** with Me_3Al and Cp_2ZrCl_2 followed by a stereospecific quench of the resulting aluminium species with I_2[12] afforded the (*E*)-vinyl iodide **5** in 74% yield.[13] Due to the low reactivity the cross-coupling between **4** and **5** this was difficult to achieve. However, the reaction of **4** with anhydrous $ZnCl_2$ and *tert*-BuLi provided the



Scheme 3. (i) **13** (1.0 equiv.), LiTMP (1.05 equiv.), THF, 1 h, −78°C, then $ClP{=}O(EtO)_2$ (1.05 equiv.), 3 h, −78°C to rt, transfer to LiTMP (2.25 equiv.), 3 h, −78°C to rt, then H_2O, 75%; (ii) **14** (1.0 equiv.), Cp_2ZrCl_2 (0.25 equiv.), Me_3Al (3.0 equiv.), CH_2ClCH_2Cl, 15 h, 0°C to rt, I_2 (1.2 equiv.), THF, 1 h, −40 to 0°C, 74%; (iii) (*R*)-**4** (1.5 equiv.), $ZnCl_2$ (1.5 equiv.), Et_2O, 1 h, rt, then *tert*-BuLi (4.5 equiv.), 2 h, −90°C to rt, transfer to **5** (1.0 equiv.), $Pd[P(Ph)_3]_4$ (cat.), 8 h, rt, 65%; (iv) TBAF (3.0 equiv.), THF, 30 min, 70°C, 98%; (v) (*S*)-**16** (1.0 equiv.), MsCl (1.1 equiv.), NEt_3 (1.3 equiv.), THF, 20 min, 0°C; (vi) (*S*)-**3** (1.0 equiv.), **2** (1.2 equiv.), KOH (1.2 equiv.), Aliquat 336 (cat.), toluene, 2 h, 110°C, 87% (2 steps)

corresponding organozinc derivative. This species underwent a clean Pd(0) catalyzed sp^3–sp^2 coupling with the (*E*)-vinyl iodide **5** to afford (*S*)-**15** in stereoisomeric pure form[14] with 65% yield. Using $PdCl_2$(dppf) and $PdCl_2[P(Ph)_3]_2$ as catalysts instead of $Pd[P(Ph)_3]_4$ lower yields of (*S*)-**15** were isolated. With enantio- and diastereomerically pure (*S*)-**15** in hand the last three steps of the total synthesis of (*S*)-methanophenazine [(*S*)-**1**] were performed with 85% yield. Cleavage of the TBDMS ether with TBAF provided the alcohol (*S*)-**16**,[15] which itself delivered the mesylate (*S*)-**3** upon treatment with $MsCl/Et_3N$. Etherification of 2-hydroxy-phenazine (**2**) and (*S*)-**3** with Aliquat as a phase transfer catalyst[16] finally yielded (*S*)-methanophenazine [(*S*)-**1**]. The spectral data for the synthetic methanophenazine are identical to those of the natural product.[17]

As the lactone (*S*)-**8** can also be prepared from (*R*)-**6** by reduction of its ester group with $LiBH_4$ and subsequent lactonization of the resulting 5-hydroxy carboxylic acid (*S*)-**7a** with 84% yield[18] (Scheme 4), our approach will also allow the synthesis of (*R*)-methanophenazine [(*R*)-**1**].



Scheme 4. (i) $LiBH_4$ (1.5 equiv.), MeOH (1.5 equiv.), DME, 2 h, 84°C; (ii) 2 M aq. NaOH, 12 h, rt, 6N HCl, 84% (2 steps)

In conclusion, we have developed a highly convergent route to enantiomerically pure (*S*)-methanophenazine [(*S*)-**1**]. By the modular building block strategy numerous methanophenazine derivatives will be accessible. With (*S*)-**1** in hand further studies will be directed to its chemical degradation and finally the determination of the absolute configuration of natural methanophenazine as well as to studies related to the electron transport and the energy conservation in methanogenic archaea.

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