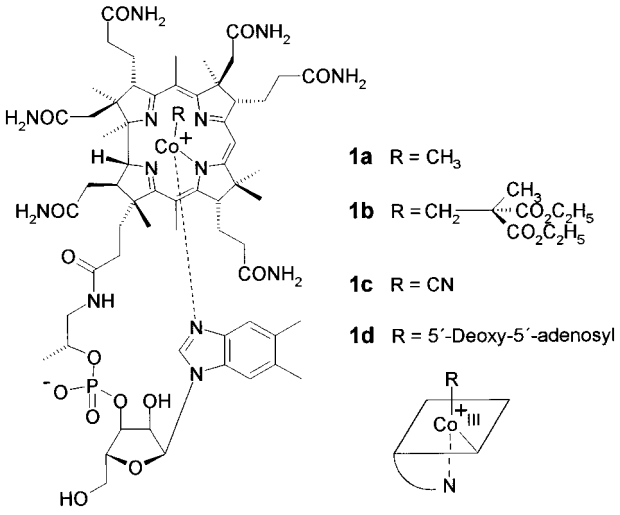
[37] M. Koza, H. Shobert, A. Tölle, F. Fujara, T. Hansen, *Nature* **1999**, *397*, 660 – 661.
[38] O. Mishima, H. E. Stanley, *Nature* **1998**, *396*, 329 – 335.
[39] P. Loubeyre, R. LeToullec, E. Wolanin, M. Hanfland, D. Hausermann, *Nature* **1999**, *397*, 503 – 506.
[40] J. K. Gregory, D. C. Clary, K. Liu, M. G. Brown, R. J. Saykally, *Science* **1997**, *275*, 814 – 817.
[41] M. Alcamí, O. Mó, M. Yáñez, *J. Am. Chem. Soc.* **1993**, *115*, 11074 – 11083.
[42] I. Alkorta, J. Elguero, M. Alcamí, O. Mó, M. Yáñez, unpublished results.
[43] D. J. Wales, *Science* **1996**, *271*, 925 – 929.
[44] D. J. Wales, *J. Am. Chem. Soc.* **1993**, *115*, 11180 – 11190.
[45] N. Pugliano, R. J. Saykally, *Science* **1992**, *257*, 1937 – 1940.

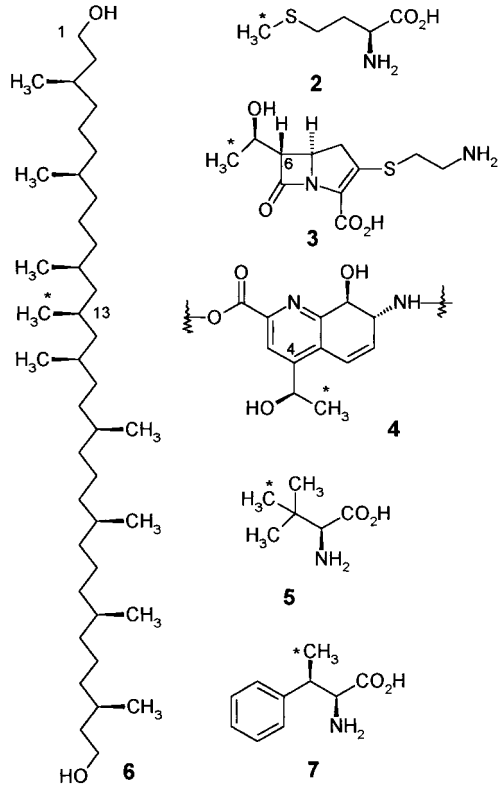
Unusual Alkylation Reactions in the Biosynthesis of Natural Products and Elucidation of Their Reaction Mechanisms

Martina Glasenapp-Breiling and Franz-Peter Montforts*

The vitamin B_{12} derivative methylcobalamin (**1a**) mediates numerous enzymatic methylation reactions in the biosynthesis of various classes of natural products. The reactions proceed with inversion of configuration with respect to the transferred methyl groups.[1] The mechanism of a S_N2 reaction was established by investigation of the stereochemical course using chiral methyl groups (CHDT), which were exclusively transferred to reactive nucleophilic centers .



Recently, there have been an increasing number of examples of unusual methylation reactions in which methyl groups originating from methionine **2** were transmitted intact, with overall retention of configuration, to saturated non-activated carbon atoms. At first Floss et al. observed such methylation processes in biosynthetic studies related to thienamycin **3** and thiostrepton. The 6″ methyl group of **3** as well as the 4″ methyl group of the quinaldine subunit **4** of thiostrepton were transferred from methionine with retention of configuration.[2] Also the methyl groups of the unusual amino acids *tert*-butylglycine (**5**) and β-methylphenylalanine (**7**) of the peptide antibiotic bottromycin occurring in *streptomyces bottropensis* originate from methionine .[3]



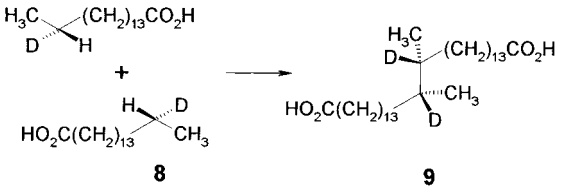
The stereochemical analysis demonstrates that the methyl groups themselves were transferred to valine and phenylalanine with overall retention of configuration and that the methylated carbon atoms experienced inversion. A similar methylation process that occurs with retention at the transmitted methyl group and with inversion at the methylated

[*] Prof. Dr. F.-P. Montforts, Dr. M. Glasenapp-Breiling
Institut für Organische Chemie
FB 2 (Biologie/Chemie) der Universität
Postfach 33 04 40, 28334 Bremen (Germany)
Fax: (+49) 421-218-3720
E-mail: mont@chemie.uni-bremen.de

  0570-0833/00/3904-0721 $ 17.50+.50/0

carbon center was observed in biosynthetic investigations of the lipid ethers caldarcheol and isocaldarcheol, which are found in the lipid membrane of archae bacteria.[4] In the case that the microorganisms were cultivated under stress conditions the normal lipid ethers form homocaldarcheol and homoisocaldarcheol by methylation. The additional methyl groups are located in the 13-position of the bisphytanol **6** which is the non-glyceridic alcohol component of the membrane forming lipids.

Dimerization of palmitic acid **8** leading to diabolic acid **9** in the eubacterium *butyrivibrio fibrisolvens* is closely related to these methylation processes (Scheme 1).[4] Diabolic acid **9**,

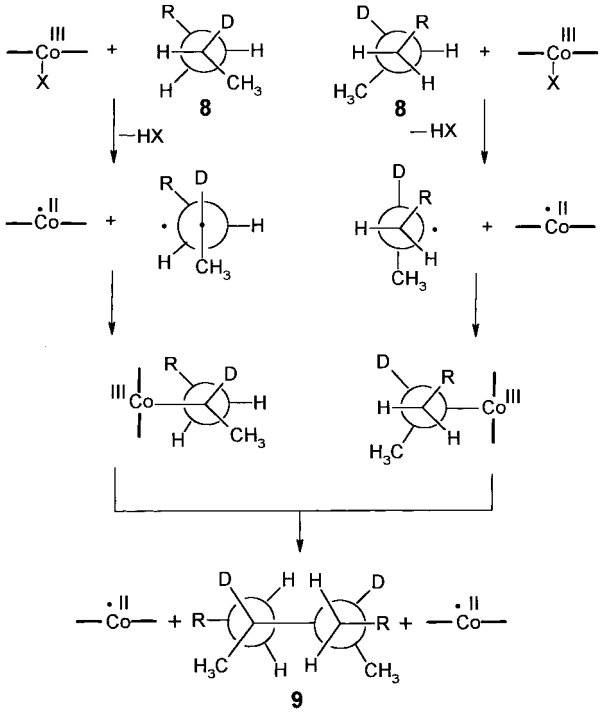


Scheme 1. Dimerization of palmitic acid **8** to diabolic acid **9**.

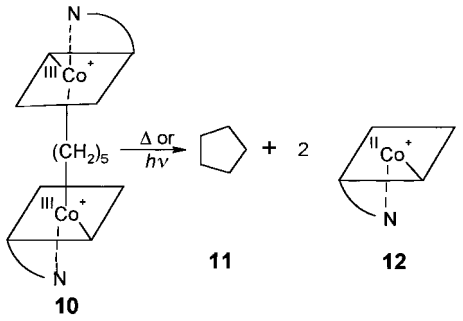
which acts as the key component of the bacterial lipids, is formed from **8** with inversion of configuration at both reacting carbon centers which are non-activated. Investigations using isotopically labeled compounds demonstrate that neither neighboring methyl or methylene groups participate in the formation of the central carbon–carbon bond. A common feature of the bond-forming reactions observed by Arigoni et al. is that they proceed under strictly anaerobic conditions a fact which excludes the participation of oxgygen-dependent cofactors related to cytochrome P_{450}.

Arigoni et al. proposed a radical reaction mechanism for the formation of diabolic acid **9**, in which, for instance, the coenzyme B_{12} (**1d**) could be involved. The participation of B_{12}-like coenzymes appears plausible in view of the occurrence of corrinoids in corresponding microorganisms (Scheme 2).[4] According to the proposed mechanism a radical intermediate is generated by homolysis of a Co–X bond in an alkylated cobalamin followed by hydrogen abstraction from the substrate **8**. The radical intermediate reacts with retention of configuration with the cobalt(II) complex to yield the corresponding alkylcobalamin. Two palmityl cobal amins could then dimerize in an enzymatically controlled stereoselective reaction giving diabolic acid **9**. The evidence of this mechanistic course is supported by a model reaction in which the biscobalamin derivative **10** bridged by a chain of five CH_2 units between the central metal atoms forms cyclopentane **11** on heating or on irradiation (Scheme 3).[5]

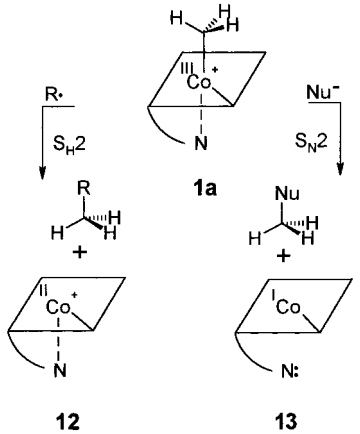
Normally, methylcobalamin (**1a**) methylates nucleophiles according to a S_N2 mechanism. The binding pair of electrons of the cobalt–carbon bond of **1a** is transferred to the central metal ion thus giving the cobalt(I) complex **13** (Scheme 4).[1, 6] Quite recently Kräutler et al. demonstrated in a thus far unprecedented model reaction the methylation of alkyl radicals by means of methylcobalamin (**1a**) according to a S_H2 reaction. The central cobalt ion is thereby reduced with bond fission to the Co^{II} complex **12**.[7]



Scheme 2. Proposed mechanism for the formation of **9**.



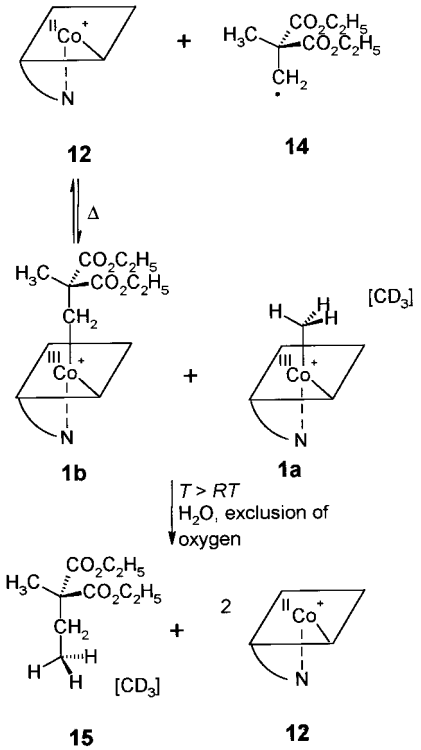
Scheme 3. Model reaction for the reaction process shown in Scheme 2.



Scheme 4. Reaction of methylcobalamin **1a** with radicals and nucleophiles.

To confirm the methylation of a carbon radical experimentally a mixture of methylcobalamin (**1a**) or its tris(deuteriomethyl) derivative and 2′,2′-bis(ethoxycarbonyl)propylcobalamin (**1b**) was heated with exclusion of oxygen and light for about 5 h at 70 °C.

  0570-0833/00/3904-0722 $ 17.50+.50/0



Scheme 5. Experimental proof of the methylation of carbon radicals.

Diethyl 2-ethylmalonate (**15**) or the corresponding deuterated derivative and diethyl 2,2-dimethylmalonate were obtained as reaction products in a 4.7:1 ratio with 70% total yield (Scheme 5). The thermolabile cobalamin **1b** decomposes homolytically even at room temperature to yield cobalamin **12** and the bis(ethoxycarbonyl)propyl radical **14** with a half-life of about 50 min, whereas methylcobalamin (**1a**) decomposes at 130 °C with a half-life of about 4 h. Accordingly, the formation of **15** from **1a** and **1b** can be attributed to a substitution of the cobalt corrin part of **1a** by the alkyl radical **14** generated from **1b.** The estimated homolytic dissociation energy ($\Delta H^\circ \approx -48$ kcal mol^{-1})[8] for the abstraction of the cobalt-bound methyl group from **1a** by the primary alkyl radical **14** reveals that this reaction step can be characterized as very exothermic. The reaction can successfully compete with the recombination of radical **14** with the Co^{II} corrin **12** to give **1b**. The enzymatic methyl transfer from **1a** to a carbon radical should proceed with inversion of configuration of the methyl groups when a chiral methyl group is applied. The transfer should show net-retention with regard to the configuration of methionine because prior to this methionine transmits its methyl group to the central metal atom with inversion.

[1] a) J. M. Wood in *B_{12}, Vol. 2* (Ed.: D. Dolphin), Wiley, New York, **1982**, pp. 151–164; b) R. T. Taylor in *B_{12}, Vol. 2* (Ed.: D. Dolphin), Wiley, New York, **1982**, pp. 307–355; c) B. Kräutler in *Vitamin B_{12} and B_{12} Proteins* (Eds.: B. Kräutler, D. Arigoni, B. T. Golding), Wiley-VCH, Weinheim, **1998**, pp. 3–43; d) R. G. Matthews, R. V. Banerjee, S. W. Ragsdale, *BioFactors* **1990**, 2, 147–152; e) H. G. Floss, M. Tsai, *Adv. Enzymol.* **1979**, *50*, 243; f) D. Arigoni, E. L. Eliel, *Topics in Stereochemistry, Vol. 4*, Wiley, New York, **1969**; g) H. G. Floss, R. G. Matthews, S. J. Benkovic, *J. Am. Chem. Soc.* **1986**, *108*, 3152–3153.

[2] a) D. R. Houck, K. Kobayashi, J. M. Williamson, H. G. Floss, *J. Am. Chem. Soc.* **1986**, *108*, 5365–5366; b) P. Zhou, D. O'Hagan, U. Mocek, Z. Zeng, L.-D. Yuen, T. Frenzel, C. J. Unkefer, J. M. Beale, H. G. Floss, *J. Am. Chem. Soc.* **1989**, *111*, 7274–7276.

[3] L. Kellenberg, D. Holmes, B. Martinoni, D. Arigoni, *Chimia* **1996**, *50*, 344.

[4] P. Galliker, O. Gräther, M. Rümmler, W. Fitz, D. Arigoni in *Vitamin B_{12} and B_{12} Proteins* (Eds.: B. Kräutler, D. Arigoni, B. T. Golding), Wiley-VCH, Weinheim, **1998**, pp. 447–458.

[5] B. Kräutler, T. Dérer, P. Liu, W. Mühlecker, M. Puchberger, K. Gruber, C. Kratky, *Angew. Chem.* **1995**, *107*, 66–69; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 84–89.

[6] a) B. Kräutler, Helv. *Chim. Acta* **1987**, *70*, 1268–1278; b) C. L. Drennan, M. M. Dixon, D. M. Hoover, J. T. Jarrett, C. W. Goulding, R. G. Matthews, M. L. Ludwig in *Vitamin B_{12} and B_{12} Proteins* (Eds.: B. Kräutler, D. Arigoni, B. T. Golding), Wiley-VCH, Weinheim, **1998**, pp. 133–155; c) E. Stupperich, R. Konnte, M. Lehle in *Vitamin B_{12} and B_{12} Proteins* (Eds.: B. Kräutler, D. Arigoni, B. T. Golding), Wiley-VCH, Weinheim, **1998**, pp. 179–197; d) B. T. Golding, R. J. Anderson, S. Ashwell, C. H. Edwards, I. Garnett, F. Kroll, W. Buckel in *Vitamin B_{12} and B_{12} Proteins* (Eds.: B. Kräutler, D. Arigoni, B. T. Golding), Wiley-VCH, Weinheim, **1998**, pp. 201–216.

[7] H. Mosimann, B. Kräutler, *Angew. Chem.* **1999**, *111*, 417–419; *Angew. Chem. Int. Ed.* **1999**, *38*, 393–395.

[8] a) B. D. Martin, R. G. Finke, *J.Am. Chem. Soc.* **1992**, *114*, 585–592; b) A. J. Gordon, R. A. Ford in *The Chemist's Companion*, Wiley, New York, **1972**.

  0570-0833/00/3904-0723 $ 17.50+.50/0