Stereochemical Course of Methyl Transfer from Methanol to Methyl Coenzyme M in Cell-Free Extracts of *Methanosarcina barkeri*

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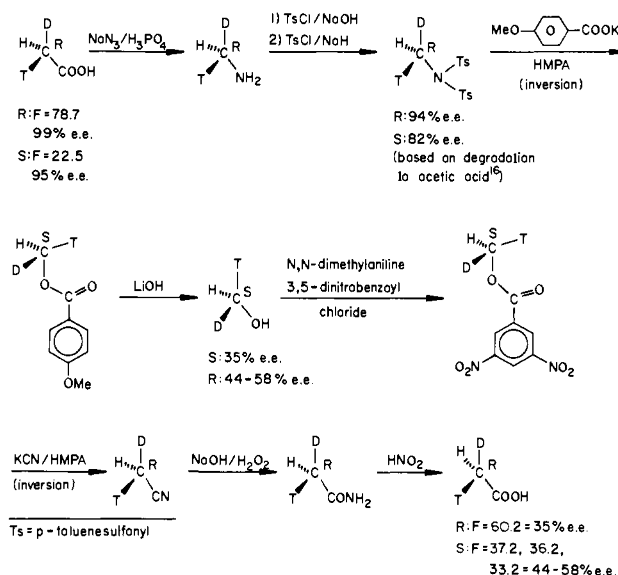
The majority of methanogens produce methane from CO_2 and H_2;[1,2] however, some species, such as *Methanosarcina barkeri*, can utilize methanol, methylamines, or acetic acid to produce methane and cellular carbon compounds.[3,4] The conversion of methanol to methane was originally thought to involve transfer of the methyl group to the cobalt of vitamin B_{12} followed by reduction of the resulting methylcobalamin.[5,6] However, more recent work, following the discovery of coenzyme M as a methyl carrier in methanogens,[7] points to the involvement of two methyltransferases, MT_1 and MT_2, which convert CH_3OH to CH_3-SCoM,[8] followed by reduction of the latter to methane by methylreductase.[9] MT_1 is a corrinoid enzyme which requires reductive activation,[10] and MT_2 can transfer the methyl group from a free or bound methylated corrin to coenzyme M. The conversion of CH_3OH to CH_3–SCoM, therefore, appears to involve two sequential transfers of the methyl group, each presumably occurring with inversion of configuration,[11] predicting that the overall reaction proceeds with net retention of methyl group configuration. Alternate reaction sequences or mechanisms, e.g., free-radical intermediates, might result in opposite stereochemistry or in significant degrees of racemization.

To probe the steric course of methyl coenzyme M formation we synthesized (*R*)- and (*S*)-[$^2H_1$,^3H]methanol from (*S*)- and (*R*)-[^2H,^3H]acetic acid[14] as shown in Scheme I. Samples of the methanol and the intermediate methylditosylimide were degraded to acetic acid[15,16] to determine their chiral purity by using the chirality analysis method of Cornforth et al.[17] and Arigoni and co-workers.[18] It is evident from the data in Scheme I that the conversion of methylditosylimide to methanol was accompanied by substantial racemization, probably due to displacement of the methyl group from methyl *p*-methoxybenzoate by the *p*-methoxybenzoate anion as a side reaction. Nevertheless the chiral purity of the methanol was sufficient for the following experiment.

Scheme I. Synthesis and Configurational Analysis of Chiral Methanol



Samples of the chiral methanol (R: $9.32 \cdot 10^6$ and $1.15 \cdot 10^7$ dpm; S: $2.1 \cdot 10^7$ dpm) were converted to methyl coenzyme M in a cell-free extract obtained from *Methanosarcina barkeri* strain 227 cells grown on methanol.[19,20] The extract was activated by preincubation with 7.5 mM ATP and 15 mM $MgCl_2$ in 20 mM TES buffer, pH 7.2, for 15 min at 37 °C under 40 psi hydrogen pressure[10] and then incubated with the chiral methanol (0.5–1 mM), 2 mM dithiothreitol, 18 mM coenzyme M, and 0.25 mM bromoethanesulfonic acid (to inhibit methyl reductase) for 5–6 h at 37 °C under 40 psi H_2. Labeled methyl coenzyme M was isolated from the reaction mixture, in 30–40% yield, by passage through an AG 50 W-X8 (H^+) column and subsequent TLC on cellulose plates (MeOH/1,3-dioxolane/NH_4OH/H_2O 3:6:1:1, R_f 0.72).[22] The samples plus nonlabeled carrier material were degraded, as shown in Scheme II,[12] to recover the methyl group as acetic acid for chirality analysis. This degradation procedure involves two S_N2 displacements at the methyl group, and hence the configuration of the acetic acid corresponds[23] directly to that of the methyl coenzyme M. As shown in Scheme II, methanol of 44–58% ee R configuration gave methyl coenzyme M which, upon degradation, produced acetic acid of 33–38% ee R configuration; the methyl coenzyme M from methanol of 35% ee S configuration gave acetic acid of 42% ee S configuration.

The results show clearly that the transformation of the methyl group of methanol into methyl coenzyme M proceeds with net retention of methyl group configuration and without significant racemization.[24] This is consistent with a mechanism proposed by Vogels and co-workers[8] in which the methyl group is transferred from methanol first to the cobalt of the corrinoid enzyme MT_1 and then to the sulfur of coenzyme M. This resembles the transfer

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(24) In our hands[25] the chirality analysis of acetic acid is reproducible to ±2 F values of ±7% ee.

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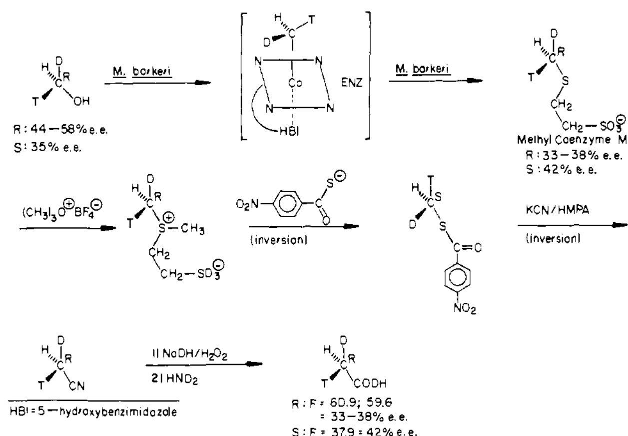
Scheme II. Steric Course of the Enzymatic Synthesis of Methyl Coenzyme M from Methanol and Configurational Analysis of Methyl Coenzyme M



of the methyl group of methyltetrahydrofolate to homocysteine, catalyzed by the B_{12}-dependent methionine synthase from *E. coli*, which we have demonstrated also occurs with net retention of methyl group configuration.[26] Both reactions pose the same question of how a relatively inert bond, the C–O bond of methanol in the present case or the C–N bond of methyltetrahydrofolate in the case of methionine synthase, is cleaved in the transfer of a methyl group.

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Low Temperatures Favor Kinetic 1,4- over 1,2-Addition of Organolithiums to α-Enones. The Crucial Role of Ion Pairing[1]

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We present here a straightfoward and theoretically satisfying model which explains and *predicts* the effects of reaction conditions and organolithium structure in promoting the kinetic 1,2- or 1,4-addition to enones.[2,3] The key feature of the model (Scheme I) is a rapid[4] equilibrium between contact ion pairs **1** and solvent-separated ion pairs **2**.[5]

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Scheme I

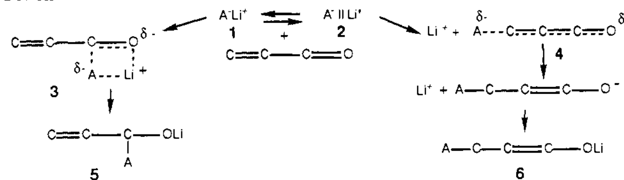

Table I. Conjugative vs 1,2-Addition of **7a–c** (Eq 1) and **10** (Eq 2) in THF

entry	RLi	temp, °C	yields[a] 1,4:1,2
1	**7a**	−100	56:20
2	**7a**	−23	39:32
3	**7a**	10	29:36
4	**7b**	−78	58:4
5	**7c**	−78	58:3
6	**10**	−100	62:35
7	**10**	−78	54:43
8	**10**	−78	59:37[b]
9	**10**	−50	51:47
10	**10**	−50	52:44[c]
11	**10**	0	35:61
12	**10**	−78	44:51[d]
13	**10**[e]	−78	24:47[d,f]
14	**10**	0	81:8[g]
15	**10**	0	31:57[h]

[a] Isolated in entries 4–14; additions to cyclohexenone were exceedingly clean. In entries 1–3, HPLC ratios were determined on isolated binary mixtures. [b] LiI (4 equiv) added. [c] Concentration (0.009 M) 20 times less than in other additions of **10**. [d] THF–pentane 20:3. [e] Potassium analogue.[32] [f] Bis(phenylthio)methane (15 %) recovered. [g] HMPA (4 equiv) present. [h] TMEDA (2 equiv present).

To a first approximation, the contact ion pairs (CIP, **1**) are assumed to undergo only 1,2-addition, which is believed to involve a four-center transition state **3**[6–8] and very low activation energy.[6] Conjugate attack of **1** on enones such as cyclohexenone, which cannot attain a cisoid configuration, requires rupture of the carbon–lithium bond without the energetic compensation arising from simultaneous formation of an oxygen–lithium bond.

Correspondingly, solvent-separated ion pairs (SSIP, **2**) are assumed to undergo only 1,4-addition. Attack of the anion of the SSIP **2** on the 4-position of the enone (directly or via electron transfer[3f]) is more likely than attack at the carbonyl carbon atom. In an early transition state, the position of attack should be determined by the relative magnitudes of the LUMO coefficients at the 4 and 2 carbon atoms[7] and possibly by electrostatic repulsion between the carbonyl oxygen atom and the anionic nucleophile;[9] in acrolein, the 4-carbon atom has the larger LUMO coefficient.[7,10] In a late transition state, the position of attack would be governed

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