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The Co(I) induced methylmalonyl-succinyl rearrangement in a model for the coenzyme B_{12} dependent methylmalonyl-CoA mutase

Fangping Sun and Tamis Darbre*

Department of Chemistry and Biochemistry, University of Bern, Freiestrasse 3, CH-3012 Bern, Switzerland

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The rearrangement of 2-bromomethyl-2-methylmonothiomalonates to succinyl derivatives was found to take place in quantitative yields in the presence of one molar equivalent of Co(I) generated by the reduction of heptamethyl Co(II)yrinate perchlorate with $NaBH_4$ or electrochemically. The chiral thiomalonate gave racemic succinate.

Introduction

The mechanism of the carbon-skeleton rearrangement catalysed by the coenzyme B_{12} dependent methylmalonyl succinyl mutase has been the subject of much interest and numerous studies.[1,2] In this rearrangement, a thioester group migrates intramolecularly to an adjacent carbon. In spite of efforts to understand the reaction, the mechanism of the elementary steps remains unclear. Although radicals have been shown to be formed during the enzymatic process,[3] the actual electronic nature of the rearranging species has not been determined in an irrefutable way.

Model systems have been developed for the methylmalonyl CoA–succinyl-CoA rearrangement in order to investigate the electronic features of the thioester migration step. Although model studies have shown that radicals are competent intermediates,[4] radical rearrangement could only be obtained in low yield (1–9%) and under high temperatures. A large number of model systems have been published where anionic species are involved in the rearrangement.[5–7] The presence of anionic intermediates in a model has been nicely shown by Dowd and co-workers. In their model system, a cyclopropyl probe was designed to reveal a reaction pathway *via* intermediate radicals. However, cleavage of the cyclopropane ring was not observed and so the intermediacy of radicals was discounted.[6a,b] Schrauzer also reported that reductive cleavage of alkylated cobalamins induces the methylmalonyl succinyl rearrangement.[7]

We developed models that generate an organic radical in the vicinity of the Co(II) of the corrinoid. Rearrangements have been observed in these systems although the radical nature of the rearranging species could not be demonstrated and experiments in deuterated solvents indicate rather an anionic intermediate.[8]

Although the formation of Co–C bonds has not been demonstrated in the enzymatic process, alkylcobalamins and alkyl cobesters have been part of model systems for the rearrangements. Therefore it is necessary to understand well the mechanism of the C–Co bond formation, as well as the mechanism of the C–Co bond cleavage, in order to build relevant models.

Here we report that the interaction of halides bearing the methylmalonyl skeleton with one molar equivalent of Cob(I)-ester gave very fast and quantitative rearrangement to succinates. The stereochemical course of the rearrangement, an issue that had not been addressed in model systems, was investigated with a chiral bromide.

Results and discussion

Alkylated B_{12} and B_{12} derivatives have been prepared by the reaction of Co(I) with a large excess (from 5 to 50 molar equivalents) of alkyl halides.[8,9] We have now found out that when the molar ratio of Co(I) to halide is 1 : 1, no alkylated product is obtained, but a very fast rearrangement takes place with the halides bearing a thioester group.

Thus, when the heptamethyl Cob(II)ester perchlorate (**1**)[8a] was reduced to the Cob(I)ester with $NaBH_4$ in MeOH, and was then treated with one molar equiv. of the halides **2a**–**2f** at RT for 15 min, the rearranged products **3a**–**3f** were obtained in more than 90% isolated yield (Scheme 1). No other product could be detected and the formation of alkylated complexes could not be observed, even when the reaction was run in the dark.[10] As shown in Scheme 1, bromides (as well as the iodide **2f**[11]), bearing different substituents in the thioester moiety, undergo rearrangement in the presence of heptamethyl Cob(I)-ester. The quantitative yields of succinates as well as the very fast rate of the reaction, under the present conditions, contrast to the previous results where rearrangement and reduction are obtained.[5,7–9]

We could also show that only the thioester and not the carbomethoxy group migrates under these conditions, since the bromide **4** gave only the reduced products **5** together with **6**[12] (in a 1.5 : 1 ratio) and no succinate could be detected, in this case, by GC analysis (Scheme 2). The high yields of products formed by thioester migration also depend on the solvent. When MeOH is replaced by acetonitrile, the bromide **2c** afforded a mixture of **7** and **3c** (1 : 1 ratio) (Scheme 2). In this case, alkylated cobester was also absent.[10]

The alkylated complexes were, however, isolated when the Cob(II)ester was reduced with excess $NaBH_4$ as above and then treated with 3–5 equivalents of the bromides. Thus, complex **8** (Scheme 2) was obtained, as a mixture of two diastereoisomers, when the Cob(II)ester was reduced with $NaBH_4$ and treated with 5 molar equiv. of **2c**. Besides the complex **8**, unchanged **2c** and the products **9**,**7** and **3c** were observed in a ratio of 14 : 1 : 3 : 10 (Scheme 2).

When the reaction of **2b** was carried out with hydroxycobalamin instead of cobester, the succinate **3b** was obtained in a yield of 49% (unchanged **2b** was recovered). The cobester and the cobalamin show the same reactivity when the ratio of Co(I) : bromide is kept at 1 : 1.

The stereochemical course of the reaction was studied with the chiral bromide *S*-**2e**[13] in the reaction shown in Scheme 3. Analysis by chiral GC of succinate **3e**, obtained from (*S*)-**2e**, showed the product to be a racemic mixture. The chirality of the bromide is therefore lost during the rearrangement step indicating that an achiral intermediate (radical or enol ester) is involved and that the intermediate formed is not bound or in close proximity of the corrin

The products obtained in deuterated solvents are shown in Scheme 4 and Table 1: in the first reaction, the ratio of **1** : **2c** :

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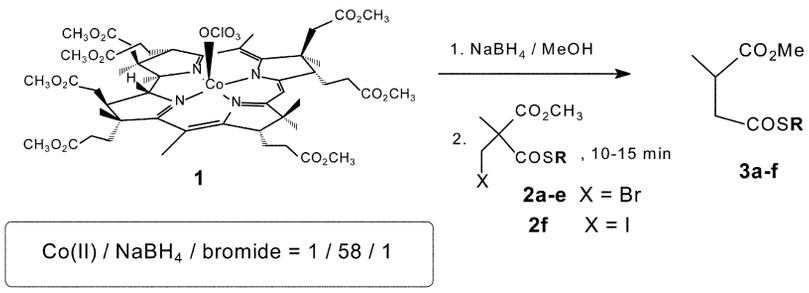
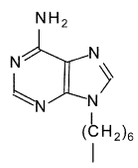
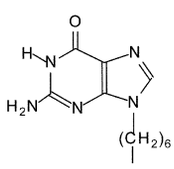


Substrate	R	Product	Yield[a] (%)
2a		**3a**	96
2b	$(CH_2)_{17}CH_3$	**3b**	95
2c	$(CH_2)_{11}CH_3$	**3c**	99
2d	$(CH_2)_5CH_3$	**3d**	91
2e	CH_2CH_3	**3e**	[b]
2f		**3f**	92

Scheme 1 (a) Isolated yields; (b) **3e** was characterized by GC with an authentic sample[8a] and was the only product observed.

Table 1 Deuterium incorporation in the reduced and the rearranged products

Solvent	**7/7-D**	**3c-D**
	$D_0 : D_1$	$D_0 : D_1$
CD_3OD	21.2 : 100	9.2 : 100
CH_3OD	52.3 : 100	8.1 : 100

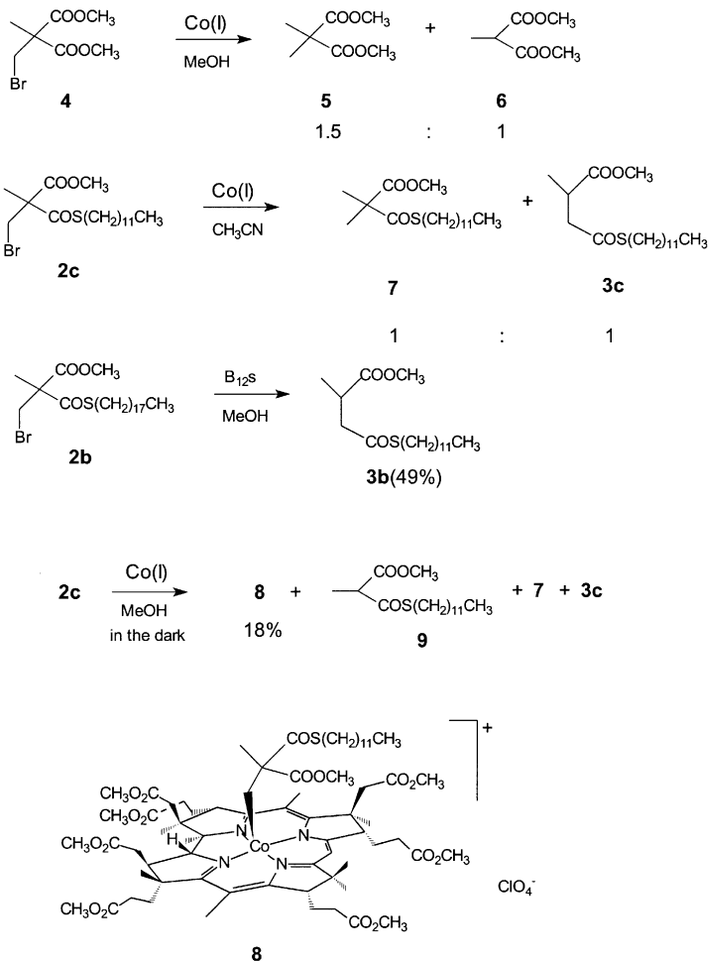
The relative intensity was measured with the peaks $D_0 = m/z$ 129.0 and $D_1 = m/z$ 130.0.

$NaBH_4$ is kept at 1 : 1 : 58 and the solvent is deuterated methanol (CH_3OD). In this case, only deuterated succinate (**3c-D** was obtained. ^{1}H- and ^{2}H-NMR data of **3c-D** support the structure shown in Scheme 4 (see Experimental). When the reaction is run with a **1** : **2c** : $NaBH_4$ ratio of 1 : 5 : 58, three products are obtained, deuterated and undeuterated dimethylmalonate (**7** and **7-D**) and deuterated succinate (**3c-D**). The data in Table 1 reveal two significant features. Firstly, one deuterium is incorporated into the rearrangement product (**3c-D**) whereas undeuterated **3c** is not detected with CD_3OD or CH_3OD as solvent, indicating that the succinate anion is formed, which abstracts a D^+. Secondly, for the reduction product, the main product is deuterated **7-D** with one deuterium incorporated. However, the amount of undeuterated **7** is much higher when CH_3OD rather than CD_3OD is used as the solvent. These results suggest that the interaction of Co(I) with the bromide produces both a methylmalonyl radical and a methylmalonyl anion. Subsequent abstraction of a hydrogen atom or a proton from the solvent leads to the reduced product **7**. However, only the anionic succinate is formed, since in CH_3OD, only D^+ abstraction to form **3c-D** is observed.

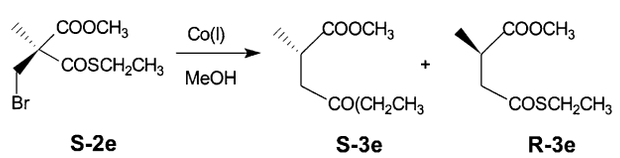
In order to establish the function of Co(I) in the rearrangement, the contribution of the reductive cleavage induced by the reducing agent[5,7] should be excluded. Cob(I)ester was then generated electrochemically and the effect of the Co(I) concentration in the reaction with halides was studied in the absence of $NaBH_4$. Reduction of **1** with a potential of −1.2 V for 1–2 hours under Argon, produced the green (sometimes dark brown) Co(I) complex solution. After the potential was shut down, one molar equivalent of **2b** was added in the dark, whereupon the color changed quickly to red–orange. After 20 min, no alkylated complexes were observed by TLC. GC and GC-MS analysis showed only rearranged **10** and bromide **4** (Scheme 5). The latter products can be considered to be formed by methanolysis of the corresponding thioesters. Reactions performed with 5 molar equivalents of cobesters, under the same conditions, have previously been shown to give alkylated B_{12} complex in 20–30% yields.[8a,9,13] When the dimethyl ester **4** was the substrate, only reduced **6** and the fragmentation product **5** were observed. The electrochemical experiments corroborate the role of Co(I) in the rearrangement observed, since no excess of reducing agent was present.

The following conclusions can be drawn from the experiments performed: 1) the rearrangement is induced by the high concentration of Co(I); 2) our results on deuterium incorporation indicate the formation of carbanionic species in the rearrangement process; 3) the reaction with *S*-**2e** shows that chirality is lost during migration of the thioester moiety.

The following question arises from our results: is the Co–C bond formed and subsequently cleaved or is the excess of Co(I) preventing the formation of the Co–C bond? The possible pathways are shown in Scheme 6. Considering the neopentyl character of bromides **2a**–**f**, it is conceivable that the radical **11** is initially formed by interaction of the halide with Co(I) *via*



Scheme 2 Co(I) was prepared by reduction of heptamethyl Cob(II)ester **1** with $NaBH_4$. B_{12s} was prepared by reduction of hydroxocobalamin with $NaBH_4$.



Scheme 3

dissociative electron transfer,[6,14] instead of the alternative S_N2 process to form a Co–C bond directly.[15] The radical pair could then collapse to form the alkylated cobester. The radical could also escape the solvent cage and abstract a hydrogen atom from the solvent to form the reduced product. Another possibility to be envisioned is an electron transfer from Co(I).[15–17] to the radical **11** to form a carbanion **12** that immediately rearranges to the succinyl anion.[18,19] The latter abstracts a proton from the solvent to give the rearranged product.

Although formation of the Co–C bond is expected, we did not detect the alkylated product. For the electron transfer process, the redox potential of Co(I)/Co(II) (~−0.7 V)[8a] is lower than that for and R˙/R- (~−1.4 V).[20] However, complexation of the radical with the metal could substantially lower the radical redox potential and allow the electron transfer to take place.[21] The very fast and irreversible rearrangement of a carbanion with p*K*a ~50 to a carbanion with p*K*a ~20[22] would be the diving force for the process. The reaction mixture remains green indicating a persistent Co(I).

If the rearrangement was derived from an alkylated complex, some stereoselectivity would be expected with the enantiomeric pure bromide *S*-**2e**. The fact that the product obtained is racemic, favors the hypothesis of radical or anion as intermediate.

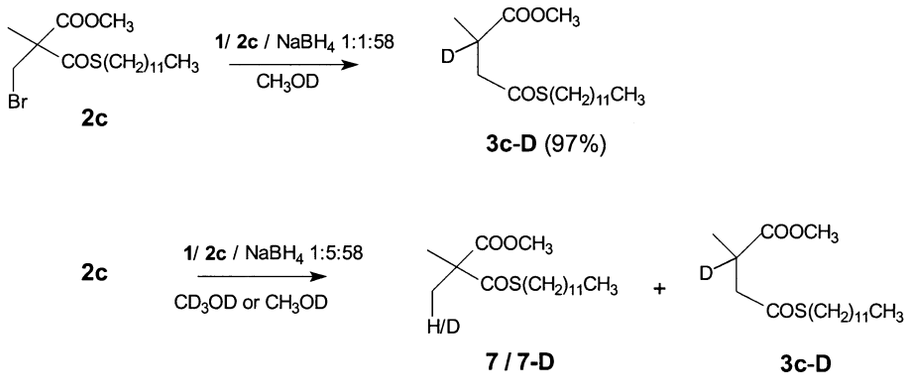
Our results show that the thioester migration *via* anionic intermediates can be induced by Cob(I)ester and that formation of alkylated complexes requires a large excess of alkylating agents with respect to the concentration of Co(I) corrinoid. We have also shown that a chiral bromide gave racemic rearranged products.

It should be noted that our experiments were conducted under reductive conditions and an excess of Co(I); conditions that are not similar to the biological environment. The enzymatic reaction could, therefore, follow a different mechanism, that should involve radicals instead of anions.

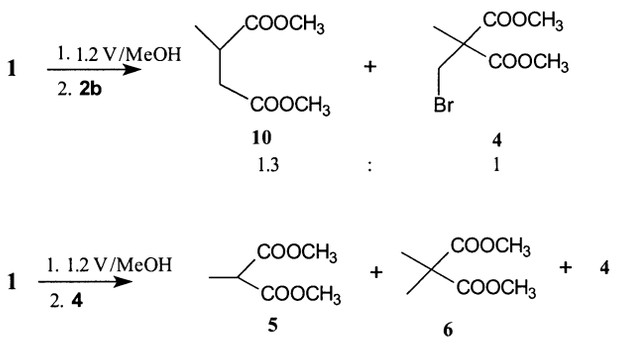
Experimental

General

Reagents were purchased from Fluka Chemie AG. Solvents for chemical reaction and chromatography were distilled prior to use. Methanol, dichloromethane and acetonitrile: Romil, super purity; solvents were degassed in an ultrasound bath under Argon for 15 min and were then cooled down to room temperature. Column chromatography (CC) and Flash chromatography (FC): silica gel 60 (40–60 μm) from Baker (analyzed reagents). Thin layer chromatography (TLC): reactions monitored on Alugram® Sil G/UV_{254} from Macherey-Nagel, detection with a Camag-53000 UV lamp (λ 254 nm) or an aq. $KMnO_4$ soln. UV/VIS: Hewlett-Packard-8451-A diode-array spectrophotometer; λ_{max} (log ε) in nm. IR: Perkin-Elmer 1600 FTIR; KBr discs or $CHCl_3$ soln. in 0.2 mm path NaCl cells; in cm^{-1}. NMR: Bruker-AC-300 (^{1}H, 300 MHz; ^{13}C, 75 MHz) and Bruker-AC-500 (^{1}H, 500 MHz; ^{13}C, 125 MHz); δ in ppm rel. to $CDCl_3$ (δ (H) 7.27, δ (C) 77.00) or CD_3OD (δ (H) 4.84, δ (C)



Scheme 4



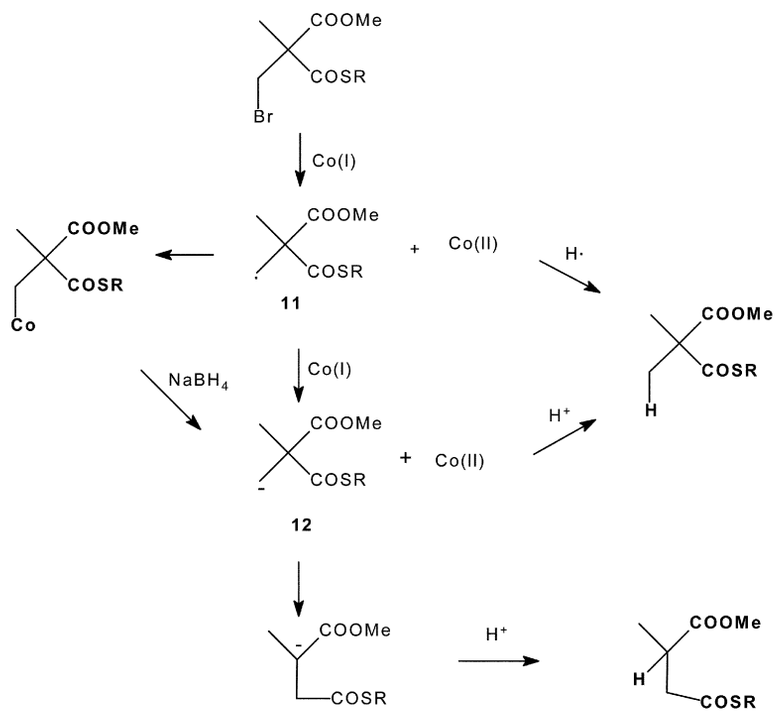
Scheme 5 Reaction of Co(I)ester generated by electrochemical reduction of **1** with thiomalonate **2b** and malonate **4**.

51.53) in Hz; ^{13}C multiplicities from DEPT spectra. Mass spectra: EI-MS Varian MAT-CH-7A, 70 eV; in *m/z* (%). LSI MS: Fisions Micromass AutoSpec Q, acceleration voltage 8 kV, ionisation Cs^+ (32 KeV); matrix: 1,3-dithiothreitol (DTT)–1,3-dithioerythrol (DTE) 5 : 1; in *m/z* (%). GC: Hewlett Packard HP-5890 with a flame ionisation detector and He as a carrier gas; capillary column 20 m × 0.3 mm, HB Ultra 2, diameter of column (df) 0.33 μm, temperature 1: 40 °C, temperature 2: 270 °C, rate: 6 °C min^{-1}; for the chiral GC 50% Diglyb in PS086 as stationary phase; GC-MS: Carlo Erba Mega GC, directly coupled to a Micromass Autospec Q EI (70). Electrochemical reaction: Potentiostat AMEL 553, H-Type electrochemical cell as described,[23] reference electrode SCE Metrohm 6.0724.000, working electrode carbon felt connected to a Pt wire, auxiliary electrode Pt foil. The bromide **2a**[8b] and bromides **2b–e**[8a] and **2f**[11] were prepared as described.

General experimental procedure for the reaction of Cobester heptamethyl ester 1 with halides

$NaBH_4$ (42 mg, 1.1 mmol) was added to heptamethyl Co(II) ester[24] (**1**, 22 mg, 0.019 mmol) in 8 ml degassed MeOH under Ar. The color of the solution changed immediately from brown to dark green. After 3 min, **2a**–**2f** (0.019 mmol) in 1 ml degassed MeOH were added dropwise; the green color remained. After stirring for 15 min, the reaction mixture was worked up by adding 10 ml of 5% $NaClO_4$–3% $HClO_4$ solution (cooled at 0 °C), extracting with CH_2Cl_2, filtering through cotton and evaporating the solvent. The products **3a**–**f** were isolated by FC (hexane–ether 8 : 1 or Sephadex LH-20, MeOH) and characterized by ^1H and ^{13}C NMR, MS and comparison with authentic samples (**3a**,[8b] **3b–e**[8a]).

***O*-Methyl-*S*-(1-9*H*-guanine-6-hexyl)-3-methylmonothiosuccinate (3f).** R_f (CH_2Cl_2–MeOH 10 : 1): 0.31. IR (KBr): 3418, 3150, 2935, 2857, 1734, 1690, 1629, 1576, 1541,



Scheme 6 Possible pathways for the formation of succinates from bromomalonates in the presence of Co(I) and $NaBH_4$.

1384, 1178, 1012, 782, 682, 636 cm^{-1}. ^1H NMR (300 MHz, CD_3OD): 1.13 (d, J = 7.0 Hz, 3H), 1.20–1.43 (m, 4H), 1.44–1.60 (m, 2H), 1.70–1.88 (m, 2H), 2.60–2.75 (m, 1H), 2.78–2.97 (m, 4H), 3.62 (s, 3H), 4.01 (t, J = 7.2 Hz, 2H), 7.68 (s, 1H). ^{13}C NMR (75 MHz, CD_3OD): 19.88 (q), 29.82 (t), 31.85 (t), 32.26 (t), 33.38 (t), 33.60 (t), 40.18 (d), 47.24 (t), 50.52 (t), 55.25 (q), 142.50 (d), 157.80 (s), 179.97 (s), 201.97 (s). EI-MS: 395 (6, M^+), 363 (63), 330 (60), 316 (30), 267 (50), 248 (60), 234 (56), 220 (37), 152 (39), 113 (70), 70 (60), 42 (100), 32 (50), 28 (97). HR-EI-MS: Calcd for $C_{17}H_{25}N_5O_4S$: 395.1627. Found: 395.1636. LSI-MS: 396 (100, $[M+1]^+$), 268 (15).

Reaction of dimethyl methylbromomethylmalonate 4 with heptamethyl cobester

Following the general procedure, a solution of **4**[13] (6.4 mg, 0.027 mmol) in 1.4 ml MeOH was dropped to the green Co(I) solution, which was prepared by reduction of **1** (30.4 mg, 0.027 mmol) in 10.5 ml MeOH with $NaBH_4$ (58.7 mg, 1.6 mmol), the color changed to brown. After work-up, the reaction mixture was concentrated and characterized by GC and GC-MS by comparison with reference compounds.[8a] GC-analysis determined the ratio **5** (t_R = 5.67 min) : **6** (t_R = 6.09) was 1 : 1.5.

Reaction of *S*-dodecyl *O*-methyl 2-(bromomethyl)-2-methylmonothiomalonate 2c with heptamethyl cobester in CH_3CN

A solution of **2c** (7.8 mg, 0.019 mmol) in 1 ml CH_3CN was added dropwise to the green Co(I) solution, which was prepared by reduction of **1** (21.9 mg, 0.019 mmol) in 7.8 ml CH_3CN with $NaBH_4$ (42.3 mg, 1.1 mmol) for 5 min; the color changed to light brown then again to green. After stirring for 30 min, the reaction mixture was worked up and the products were characterized by GC and GC-MS by comparison with reference compounds. GC analysis determined the ratio **7** (t_R = 30.73 min) : **3c** (t_R = 32.16 min) to be 1 : 1.

Reaction of *S*-dodecyl *O*-methyl 2-(bromomethyl)-2-methylmonothiomalonate 2c (5 molar equivalents) with heptamethyl cobester

According to the general procedure, a solution of **2c** (38.9 mg, 0.095 mmol) in 1 ml MeOH was added to the green Co(I) solution, which was prepared by reduction of **1** (21.9 mg, 0.019 mmol) in 7.8 ml MeOH with $NaBH_4$ (42.3 mg, 1.1 mmol), the color changed to orange. After work up, the reaction mixture was submitted to FC ($NaClO_4$ impregnated silica gel,[8a] hexane–ether 8 : 1) to yield two fractions: the colored fraction was submitted to CC ($NaClO_4$ impregnated silica gel, CH_2Cl_2–MeOH 40 : 1) and the alkylated complex **8** was isolated in 18% yield as a mixture of two diastereoisomers. The colorless fraction was characterized by GC by comparison with reference compounds. The ratio **9** (t_R = 30.53 min) : **7** (t_R = 30.83 min) : **3c** (t_R = 32.36 min) : **2c** (t_R = 35.19 min) was 1 : 3 : 10 : 14.

8 R_f (CH_2Cl_2–MeOH 15 : 1): 0.65. UV/VIS ($c = 5.5 \times 10^{-5}$ M, CH_2Cl_2): $\lambda_{max}(\varepsilon)$ 234 (sh, 1.06), 266 (sh, 0.91), 298 (sh, 0.87), 424 (br, 0.37). IR ($CHCl_3$): 3030m, 2954m, 2930m, 2854w, 1732s, 1600w, 1570w, 1490m, 1470w, 1438m, 1350w, 1180m, 1150m, 1130m, 1100m, 990w, 626w. ^1H NMR (300 MHz, $CDCl_3$): −0.07 (s, 1.8H), 0.22 (s, 1.2H), 0.34 (d, J = 6.6 Hz, 0.6H), 0.65 (d, J = 7.0 Hz, 0.4H), 0.75–2.98 (m, superimposed 1.18 (s), 1.23 (s), 1.25 (s), 1.28 (s), 1.32 (s), 1.41 (s), 1.60 (s), 1.65 (s), 1.67 (s), 1.69 (s), 1.82 (s), 2.37 (s), 2.45 (s), 2.49 (s), total 75H), 3.30–3.87 (m, superimposed 3.36 (s), 3.45 (s), 3.59 (s), 3.60 (s), 3.64 (s), 3.65 (s), 3.69 (s), 3.71 (s), 3.72 (s), 3.73 (s), 3.77 (s), 3.79 (s), 3.80 (s), total 26H), 3.87–3.98 (m, 1H), 4.01–4.11 (m, 1H), 4.65 (d, J = 9.6 Hz)/4.71 (d, J = 9.9 Hz) (total 1H), 6.99 (s)/7.02 (s) (total 1H); ^{13}C-NMR (75 MHz, $CDCl_3$): 13.97 (q), 16.16 (q), 16.20 (q), 16.32 (q), 16.42 (q), 16.86 (q), 16.89 (q), 18.50 (q), 18.54 (q), 19.13 (q), 19.37 (q), 19.71 (q), 19.73 (q), 21.59 (q), 21.91 (q), 22.51 (t), 22.53 (t), 24.33 (q), 25.34 (t), 25.35 (t), 25.39 (t), 26.33 (t); 26.35 (t), 26.53 (t), 26.70 (t), 27.07 (t), 28.56 (t), 28.58 (t), 28.63 (t), 28.73 (t), 28.81 (t), 28.84 (t), 28.87 (t), 28.90 (t), 28.91 (t), 29.07 (t), 29.15 (t), 29.17 (t), 29.26 (t), 29.32 (t), 29.38 (t), 29.40 (t), 29.44 (t), 29.45 (t), 29.53 (t), 31.44 (t), 31.52 (t), 31.64 (t), 31.73 (t), 31.75 (t), 31.86 (t), 31.95, 32.10, 32.24 (t), 33.18 (t), 39.64 (q), 39.68 (q), 41.75 (t), 41.90 (t), 42.05 (t), 42.40 (t), 45.36 (s), 45.50 (s), 46.89 (s), 46.97 (s), 47.59 (t), 49.84 (s), 50.04 (s), 51.59 (q), 51.61 (q), 51.69 (q), 51.73 (q), 51.82 (q), 51.86 (q), 51.93 (q), 52.03 (q), 52.06 (q), 52.29 (q), 52.41 (q), 52.43 (q), 52.58, 52.66, 52.98, 53.15, 53.26, 53.72, 54.82, 55.27, 55.62, 55.71, 59.24 (s), 59.27 (s), 64.52 (s), 65.05 (s), 74.73 (d), 74.84 (d), 77.23 (d), 87.38 (s), 87.49 (s), 98.50 (d), 106.91 (s), 107.06 (s), 109.05 (s), 163.12 (s), 163.78 (s), 165.67 (s), 166.30 (s), 167.20 (s), 168.62 (s), 170.30 (s), 170.73 (s), 171.40 (s), 171.45 (s), 171.56 (s), 171.60 (s), 172.61 (s), 172.66 (s), 172.78 (s), 172.85 (s), 173.40 (s), 173.43 (s), 173.68 (s), 174.41 (s), 176.51 (s), 176.65 (s), 176.84 (s), 177.01 (s), 177.35 (s), 177.60 (s), 192.55 (s), 193.75 (s). LSI-MS : 1367 (6, $[M - ClO_4^-]^+$), 1190, 1038 (100, $[M - ClO_4^- - (CH_3)(CH_2)C(CO_2CH_3)(COS(CH_2)_{11}CH_3)]^+$, 964 (6), 877 (7), 318 (4), 129 (8).

Reaction of *O*-methyl *S*-octadecyl 2-(bromomethyl)-2-methylmonothiomalonate 2b with hydroxocobalamin

A solution of **2b** (32.6 mg, 0.066 mmol) in 3.5 ml MeOH was added to the green Co(I) solution, which was produced by reduction of hydroxocobalamin (91.3 mg, 0.066 mmol) in 3 ml MeOH with $NaBH_4$ (144.8 mg, 3.8 mmol), the color changed gradually to orange and remained until work-up. After CC (hexane/ether 20:1), **3b** (11.2 mg, 49%) was isolated and **2b** (5.1 mg) was recovered

Reaction of (*S*)-*O*-methyl *S*-ethyl 2-(bromomethyl)-2-methylmonothiomalonate *S*-2a with heptamethyl cobester

According to the general procedure: a solution of *S*-**2e** (5.1 mg, 0.019 mmol) was added to the Co(I) solution (from reduction of **1** (21.9 mg, 0.019 mmol) with $NaBH_4$ (42.3 mg, 1.1 mmol)) in 7.8 ml of MeOH. The products *R*-**3e** and *S*-**3e** were characterized by a chiral GC-column by comparison with independently prepared *S*-**3e**.[19] The retention times *S*-**3e** (t_R = 49.67 min) : *R*-**3e** (t_R = 50.68 min). The product obtained yielded a 1 : 1 mixture of *S*-**3e** and *R*-**3e**.

Reaction of *S*-dodecyl *O*-methyl 2-(bromomethyl)-2-methylmonothiomalonate 2c with heptamethyl cobester in CD_3OD or CH_3OD

Refer to the general procedure. A solution of **2c** (38.9 mg, 0.095 mmol) in 1 ml CD_3OD or CH_3OD was added to the green Co(I) solution, which was prepared by reduction of **1** (21.9 mg, 0.019 mmol) in 7.8 ml of the corresponding deuterated solvents with $NaBH_4$ (42.3 mg, 1.1 mmol), the color changed to orange and then gradually back to green. After work up, the reaction mixture was characterized by GC and GC-MS by comparison with reference compounds.**7**/**7-D** (t_R = 30.58 min) and **3c-D** (t_R = 32.14 min) were obtained. From the reaction with 1 molar equivalent of **1**, **3c-D** was isolated after FC (silica gel, hexane–ether 8 : 1) in 97% yield. R_f (hexane–ether 20 : 1): 0.37. ^1H NMR (300 MHz, $CDCl_3$): 0.89 (t, J = 6.6 Hz, 3H), 1.21 (s, 3H), 1.21–1.41 (m, 18H), 1.50–1.64 (m, 2H), 2.65 (d, J = 16.2 Hz, 1H), 2.88 (t, J = 7.4 Hz, 2H), 3.00 (d, J = 16.2 Hz, 1H), 3.70 (s, 3H). ^2H NMR (76.776 MHz, CCl_4): 5.83 (s), reference $CDCl_3$: 7.24. ^{13}C-NMR (300 MHz, $CDCl_3$): 14.10 (q), 16.69 (q), 22.68 (t), 28.81 (t), 28.97 (t), 29.10 (t), 29.33 (t), 29.47 (t), 29.50 (t), 29.56 (t), 29.61 (t), 29.62 (t), 31.90 (t), 46.78 (t), 51.93 (q), 174.87 (s), 197.63 (s). EI-MS: 300 (28), 144 (5), 130 (100), 102 (23), 60 (21), 43 (7).

Reaction of *O*-methyl *S*-octadecyl 2-(bromomethyl)-2-methylmonothiomalonate 2b with cobester heptamethylester reduced electrochemically

The cathode compartment (*ca.* 20 ml) and the anode compartment (*ca.* 5 ml) of the electrochemical cell were filled with

degassed 0.1 M methanolic $LiClO_4$ solution. Residual oxygen was reduced at −1.0 V for 30 min under Ar and **1** (21.9 mg, 0.019 mmol) dissolved in 1 ml degassed MeOH was added. The mixture was reduced at −1.0–−1.2 V for 1–2 h. The color changed slowly to green or dark brown. After the potential was cut down, **2b** (9.4 mg, 0.019 mmol) dissolved in 1 ml degassed methanol was added in the dark. The color changed quickly to red–orange. After 20 min, the reaction solution was taken out and concentrated to 1/3 of the original volume, water was added, the solution was extracted with CH_2Cl_2 (3×), the combined organic phase was dried through cotton then concentrated. After CC (hexane–ether 8 : 1), the products were characterized by GC and GC-MS by comparison with reference compounds. **10** (t_R = 7.98 min) : **4** (t_R = 12.08 min) 1.3 : 1.0.

Reaction of dimethyl methylbromomethylmalonate 4 with heptamethyl cobester reduced electrochemically

Same procedure as above, with **1** (21.9 mg, 0.019 mmol) and **4** (4.5 mg, 0.019 mmol). FC: silica gel, hexane–ether 8 : 1. The products were characterized by GC and GC-MS by comparison with reference compounds. GC analysis provided the ratio: **5** (t_R = 5.66 min) : **6** (t_R = 6.07 min) : **4** (t_R = 12.04 min) 1.0 : 2.3 : 2.8.

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