Model studies for the thiol-mediated methyl transfer to corrinoids

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The thiol-dependent methylation of heptamethyl cob(II)yrinate **8r** with methyl iodide and methyl tosylate was explored under a variety of conditions. The interaction of the heptamethyl cob(II)yrinate with a variety of thiols was monitored prior to the addition of the methylating agent, and the formation of the Co(I) complex was only apparent in the reaction with hexane thiol. Nevertheless, thiol-mediated methylation of the Co(II) complex **8r** takes place with methyl iodide under most conditions. The Co-methylation with methyl tosylate showed a different reactivity, was inhibited by pyridine or *N*-methylimidazole, and was strongly dependent on the the acidity of the thiol used. Mechanistic aspects are discussed.

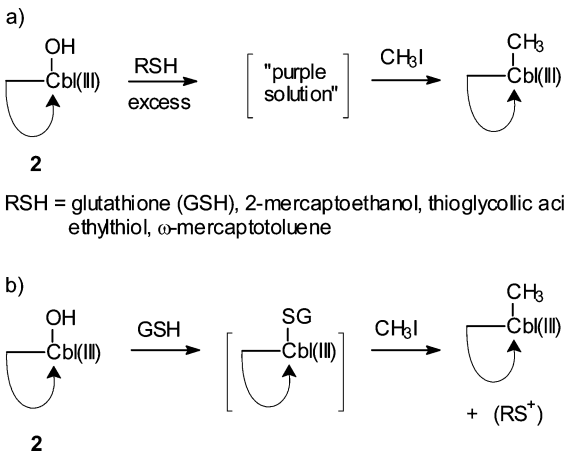
Introduction

The transfer of methyl groups from different methylated compounds to corrinoids is a process widely used in Nature.[1] Cobalamin serves as the cofactor in the cobalamin-dependent methionine synthase and mediates the transfer of a methyl group from CH_3-H4 folate to homocysteine.[2] Corrinoids are the key cofactors for methyl transfer in acetogenic prokaryotes and methanogenic Archaea, where a variety of one-carbon compounds can serve as methyl donors to enzyme-bound corrinoid cofactors.[3,4]

Mechanistic studies for the cobalamin-dependent methionine synthase have shown the methyl transfer to occur with overall retention of configuration.[5] This result is consistent with two successive S_N2-type transfers of the methyl group to and from cobalamin cofactor, but other pathways are discussed.[1] Overall retention of configuration has also been observed for the methyl transfer from CH_3-H4 folate to acetyl CoA in the acetyl CoA synthase.[6]

In vivo, methyl cobalamin **3** and coenzyme B_{12}, 5′-adenosylcobalamin **4**, are formed from vitamin B_{12}, cyanocobalamin **1**, by removing the cyano group, subsequent reduction of glutathionylcobalamin formed as an intermediate and alkylation with the the appropriate methyl agent or ATP, respectively (Fig. 1).[7] The interaction of glutathione with cobalamin may be related to other glutathione reductases.[8] *In vitro*, a stable glutathionylcobalamin complex can be prepared from glutathione and aquacobalamin **2**.[9] Other thiols like cysteine, homocysteine or mercaptoethanol give rather unstable thiolatocobalamins which decompose by initial formation of thiyl radicals and cob(II)alamin. It is apparent that thiols play a dual role in their interaction with Co complexes and may function as complexing agents and as reducing agents.

In the past several research groups have investigated the interaction of cobalamin with thiols and subsequent Co alkylations.[10] Dolphin and Johnson reported the reaction of cobalamin with an excess of thiols and alkylation with CH_3I to give methyl cobalamin (Scheme 1a).[11,12] Furthermore, they clearly stated that B_{12r}, cob(II)alamin, does not react with methyl iodide, ethyl iodide, methyl tosylate or 2′,3′-isopropylidene-5′-toluene-*p*-sulfonyladensosine under a variety of conditions, even at temperatures up to 100 °C. Independently, Wagner and Bernhauer prepared methyl cobalamin by reaction of cobalamin with glutathione and methyl iodide and formulated glutathionylcobalamin as an intermediate (Scheme 1b).[13,14]



Scheme 1 a) Formation of methyl cobalamin;[11,12] b) formation of methyl cobalamin **3** with glutathionyl cobalamin as intermediate.[13,14]

Subsequently, Schrauzer and Windgassen studied the chemistry of several mercaptocobaloximes (bis(dimethylglyoximato)cobalt complexes containing Co–S bonds) as model systems for biochemical methyl transfer reactions (Scheme 2).[15]

They found that methylcobaltoxime is formed to a limited extent when hydroxyaquo-cob(III)altoxime is treated with excess methyl mercaptan and methyl iodide at pH ≈ 7. At pH > 7 methyl thioether is formed, whereas this methylation is very slow at pH < 7. It was also shown that B_{12s} (cob(I)alamin) reacts with CH_3I at a very high rate and thus is considered a supernucleophile.[16]

In further explorations, Rafiq and Golding investigated the thiol-induced methylation of cobaltoxime *via* a stepwise reaction

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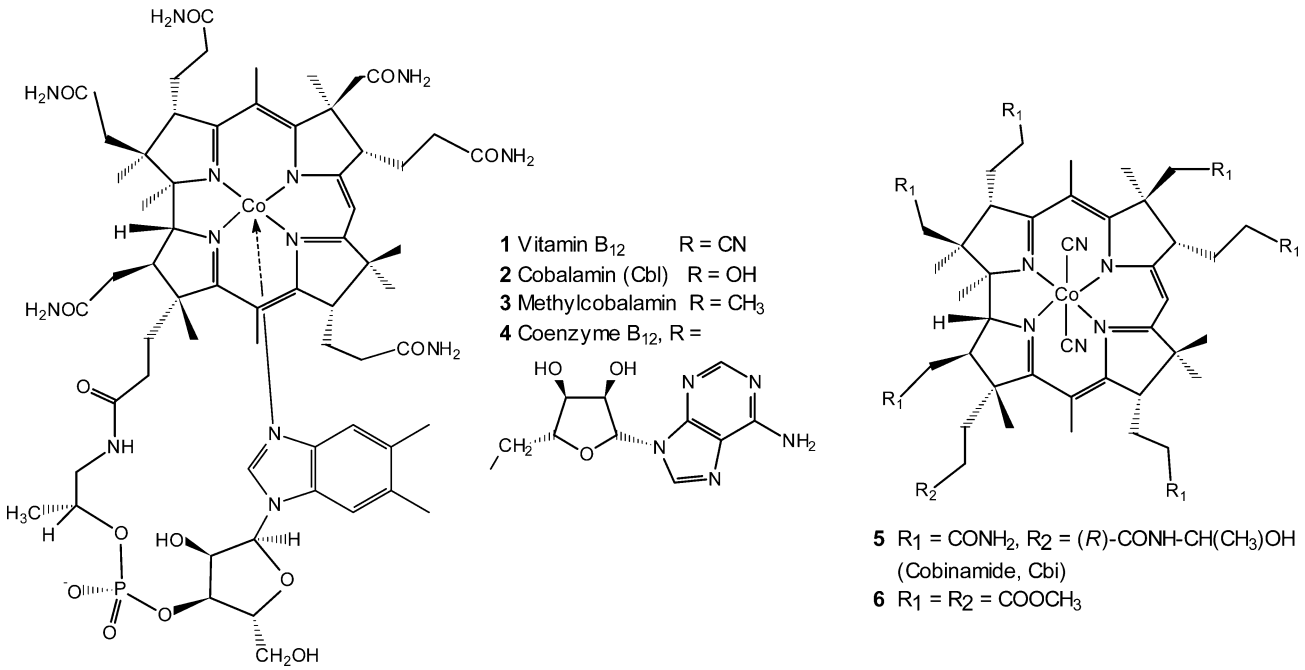
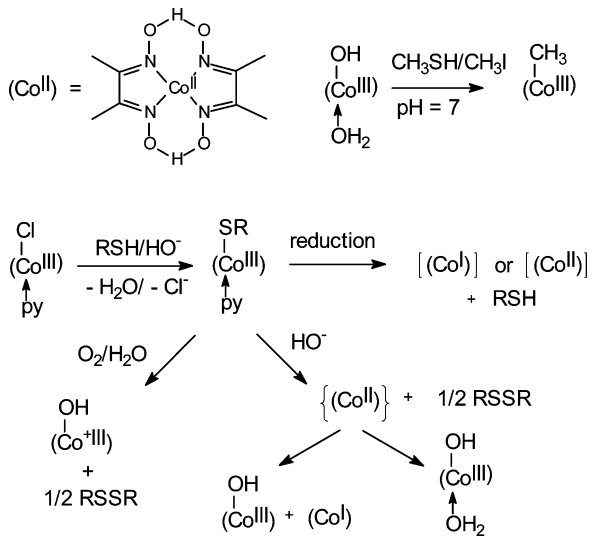
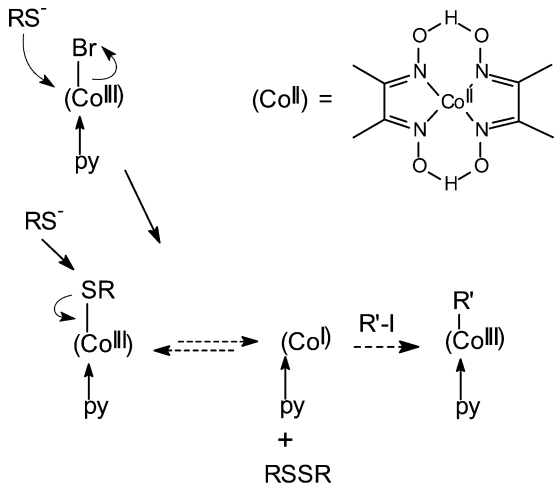


Fig. 1 Vitamin B_{12} and some derivatives.



Scheme 2 Reactions of cobaloximes with thiols.[15]



Scheme 3 Proposed mechanism for the thiol-induced alkylation of cobaloxime.[17]

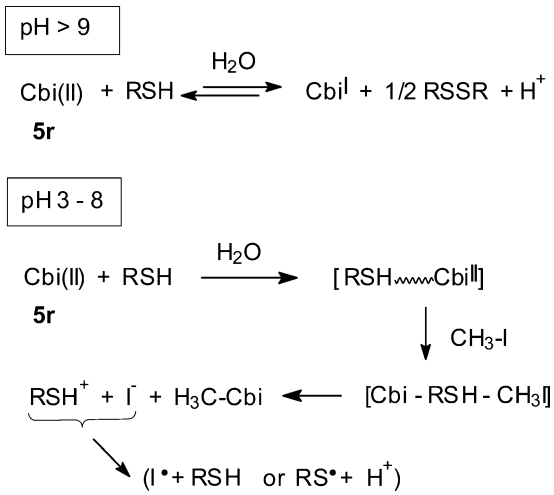
mode (Scheme 3).[17] However, when 2-methoxyphenylmercaptocobaloxime was incubated with 2-methoxythiophenol and methyl iodide, neither the formation of the disulfide nor the formation of methylcobaloxime was observed.

Pratt and co-workers found in their detailed kinetic studies that cob(II)inamide (Cbi^{II} **5r**, derived from Cbi **5**) in solvents with pH > 9 is reduced by thiols to Co(I), while only small changes of the Co(II) spectrum are observed in the range pH 3–8.[18,19] The addition of methyl iodide to the mixture of cobinamide and the thiol dithiothreitol converted the Co(II) complex to methylcobinamide in the range pH 3–8 independently of the Cbi concentration (Scheme 4).

A mechanism for the formation of methylcobinamide was proposed which involved a three-component complex [RSH–Co(II)–CH_3I] ('Pratt complex'). This mechanistic proposition establishes a third pathway, to be distinguished from the alkylation of Co(I) and the methylation of Co(II) (Cbl, Cbi) with methyl iodide. According to Blaser and Halpern, the methylation of cob(II)alamin is second order in Co(II) concentration.[20]

A few years ago, we experimentally established a model for cobalamin-dependent methionine synthase.[21] Conditions for the complete cycle were described involving the transfer of a methyl group from dimethylaniline to hexane thiol with a corrinoid as methyl transfer catalyst and Zn as reducing agent. In the first half-reaction, methyl heptamethyl cobyrinate was obtained by reaction of dimethylaniline with heptamethyl cob(II)yrinate in the presence of Zn/AcOH.

Motivated by these results, we have now investigated additional aspects of the thiol-mediated methylation of corrinoids which

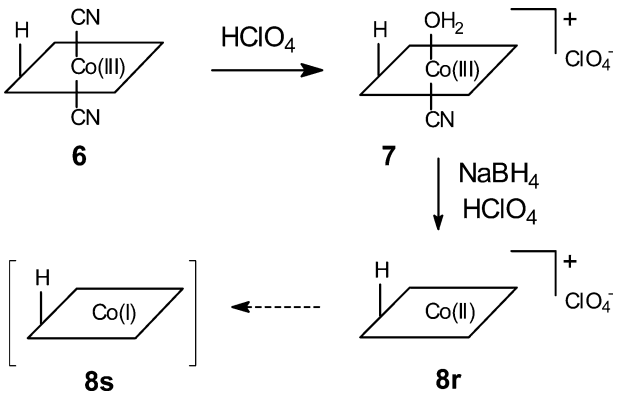


Scheme 4 Thiol-mediated reactions of cobinamide.[18,19]

we considered relevant for the successful reaction. Of specific interest were the methylation of heptamethyl cob(II)yrinate and cobinamide with CH_3I and CH_3OTs induced by a variety of thiols as reducing agents, the pH and solvent dependency, and the impact of (interaction with) additives.

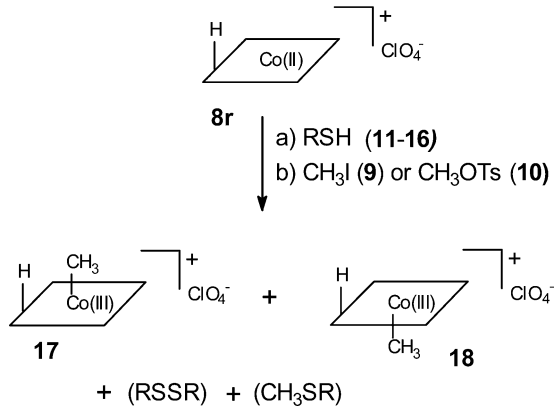
Results and discussion

The Co(III) and Co(II) corrinoids used for this investigation were prepared from heptamethyl dicyanocob(III)yrinate **6** following published procedures (Scheme 5).[21,22] Acid-induced ligand exchange led to the aquo-cyanocob(III)yrinate **7**, which upon reduction with $NaBH_4$ and treatment with $HClO_4$ gave the perchlorato heptamethyl cob(II)yrinate **8r**.



Scheme 5 Formation of aquo-cyanocob(III)yrinate **7** and cob(II)yrinate **8r**.

The reactions of heptamethyl cob(II)yrinate **8r** with thiols and subsequent methylation with methyl iodide **9** and methyl *p*-toluenesulfonate **10**, respectively, were of central interest (Scheme 6). Thiols covering a wide range of acidities were used as reducing agents under a variety of conditions. The alkylation reactions were studied in methanol, to which bases like triethylamine or pyridine were added in some experiments. In addition, the methylation reactions were studied in aqueous buffers at different



Scheme 6 Thiol-mediated methylations of cob(II)yrinate **8r**.

pH values. Also, some reactions of the heptamethylcob(III)yrinate **7** were explored.

The reactions were followed by UV–Vis measurements, and the methylation products **17**/**18** were detected by TLC with authentic methyl cobyrinates **17**/**18** as references.[23,24] The product yields are based on TLC evaluations.

Schrauzer had shown the rates of alkylation of B_{12s} with n-alkylchlorides ($C_nH_{2n+1}Cl$, n = 1–4) in methanol at room temperature to be very similar to the relative rates of the reactions of iodide ion with the same alkyl chlorides in acetone at 50 °C.[16] Therefore, the alkylation reactions of B_{12s} with methyl iodide **9** were considered to follow a typical S_N2 reaction mechanism. However, alkyl iodides are more prone than alkyl chlorides to undergo nucleophilic substitutions *via* outer sphere, dissociative single-electron transfer reactions,[25] particularly with supernucleophiles like B_{12s} and heptamethyl cob(I)yrinate **8s**. Methyl tosylate **10** on the other hand should strictly follow a typical S_N2 mechanism rather than an electron-transfer-initiated substitution pathway.

A variety of thiols with pK_a values in the range 2.7–10.7 were investigated as reducing agents of **8r** required for the methylation reaction (Fig. 2).

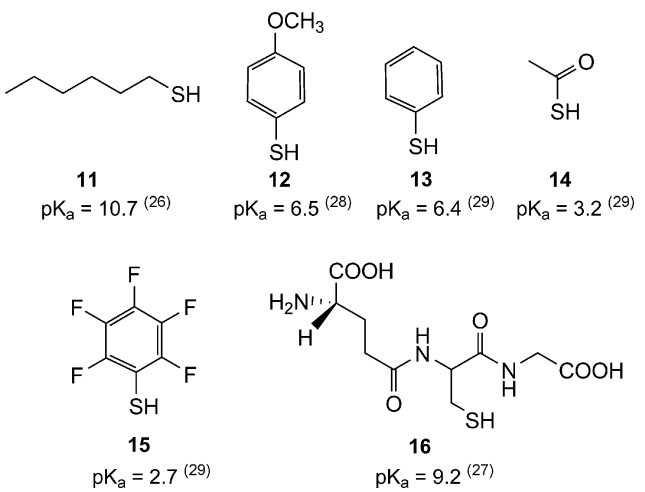


Fig. 2 Thiols used for reactions with cob(II)yrinate **8r**.

When heptamethyl cob(II)yrinate **8r** was treated in methanol with triethylamine and an excess of hexane thiol **11** (pK_a = 10.7), the colour of the solution turned immediately from brown to clear

dark green, evidence for the formation of a high concentration of the cob(I)yrinate **8s** (Scheme 5). Glutathione **16** ($pK_a = 9.2$) led also to the formation of the Co(I) complex, but at a lower concentration. Addition of phenyl thiol **13** to a methanolic solution of **8r** did not lead to the green colour of **8s**. According to the UV/Vis spectrum, the absorption pattern for **13** is more complex, showing plateaus but not the minima in the range for Co(I) **8s** (Fig. 3).

The absorption spectra obtained when the Co(II) complex **8r** was treated with 4-methoxythiophenol **12** in methanol containing aqueous buffer of pH 3.5 or 9 are rather unstructured (Fig. 4) and do not show the formation of the Co(I) complex **8s** (Fig. 3). They are very similar to that recorded for the interaction of **8r** with **12** in methanol (Fig. 5).

The conditions for the thiol-dependent methylation of Cob(II)ester **8r** with CH_3I **9** and CH_3OTs **10** were explored in greater detail (Table 1). The reaction of **8r** readily takes place in methanol containing triethylamine both with methyl iodide **9** and methyltosylate **10**, with the methylation being complete within 1 min (entries 1 and 2).

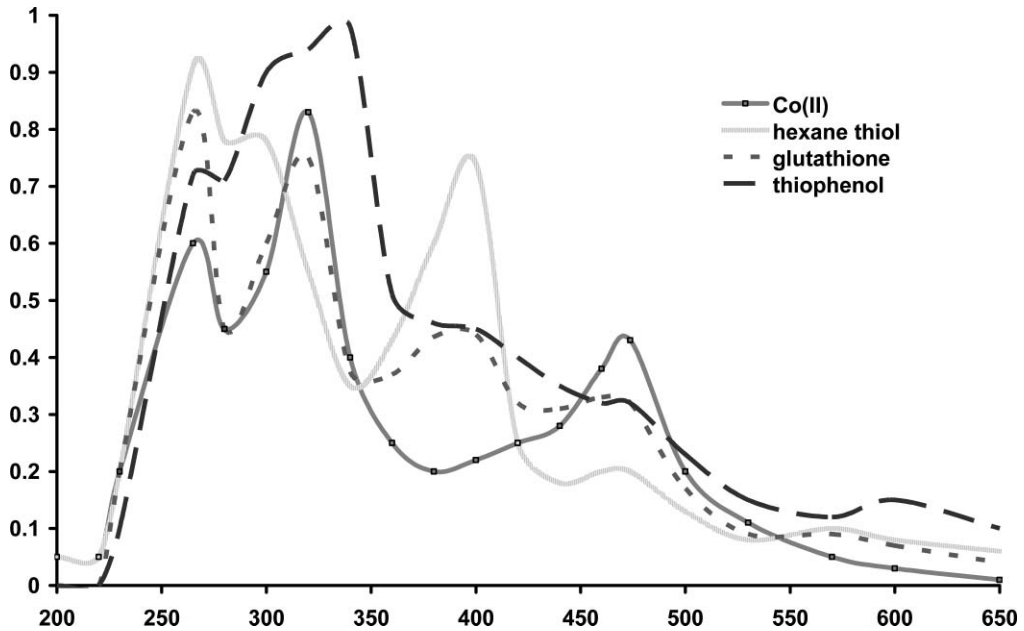


Fig. 3 UV–Vis spectra (absorbance *vs.* wavelength) of the interactions of heptamethyl cob(II)yrinate **8r** with the thiols **11**, **13** and **16** in methanol.

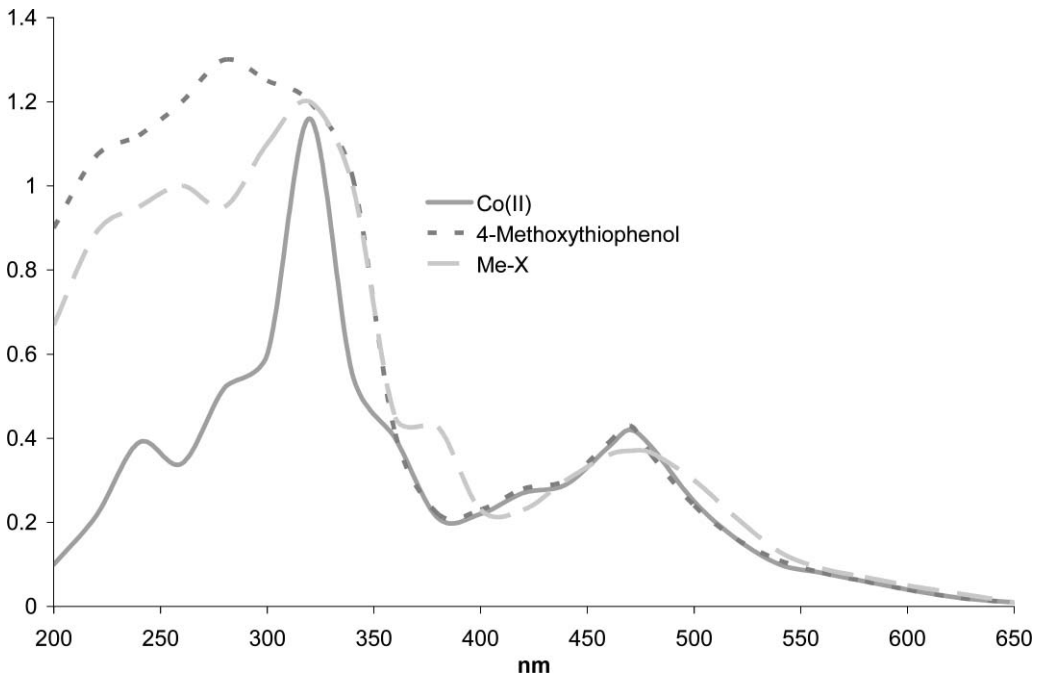


Fig. 4 UV–Vis spectrum (absorbance *vs.* wavelength) of the interaction of **8r** with the thiol **12** and methyl tosylate **10** in methanol with pH 3.5 aqueous buffer.

Table 1 Methylation of cob(II)yrinate **8r** with CH_3I **9** and CH_3OTs **10** induced by hexane thiol **11** and glutathione **16**

Entry	Solvent	Methylating agent	Time/min	Yield of **17**/**18** (%)[a]
1	CH_3OH (+ H_2O)[b] + 0.2 mL Et_3N	CH_3I	1 (10)[b]	100 (100)[b]
2	CH_3OH + 0.2 mL Et_3N	CH_3OTs	1 (10)[b]	100 (100)[b]
3	Toluene + 0.2 mL Et_3N	CH_3OTs	1	100
4	CH_3OH + 0.2 mL pyridine	CH_3I	10	100
5	CH_3OH + 0.2 mL pyridine	CH_3OTs	60	0
6	CH_3OH–H_2O + 0.2 mL pyridine	CH_3OTs	60	0
7	CH_3OH + 0.2 mL CH_3-imidazole	CH_3I	30	100
8	CH_3OH + 0.2 mL CH_3-imidazole	CH_3OTs	60	0
9	CH_3OH + pH 9 buffer	CH_3I	1 (30)[b]	100 (100)[b]
10	CH_3OH + pH 9 buffer	CH_3OTs	1 (120)[b]	100 (60)[b]
11	CH_3OH + pH 7.5 buffer	CH_3I	5	100
12	CH_3OH + pH 7.5 buffer	CH_3OTs	5	100
13	CH_3OH + pH 6.5 buffer	CH_3I	1	100
14	CH_3OH + pH 6.5 buffer	CH_3OTs	1	100
15	CH_3OH + pH 3.5 buffer	CH_3I	1 (60)[b]	100 (95)[b]
16	CH_3OH + pH 3.5 buffer	CH_3OTs	1 (60)[b]	100 (15)[b]
17	CH_3OH	CH_3I	120	0
18	CH_3OH	CH_3OTs	120	0
19	CH_3OH–H_2O (1 : 1)	CH_3I	30 (60)[b]	100 (40)[b]
20	CH_3OH–H_2O (1 : 1)	CH_3OTs	30 (60)[b]	40 (10)[b]

[a] Estimated (*cf.* text and Experimental section). [b] Data for the reaction of **8r** with glutathione **16**.

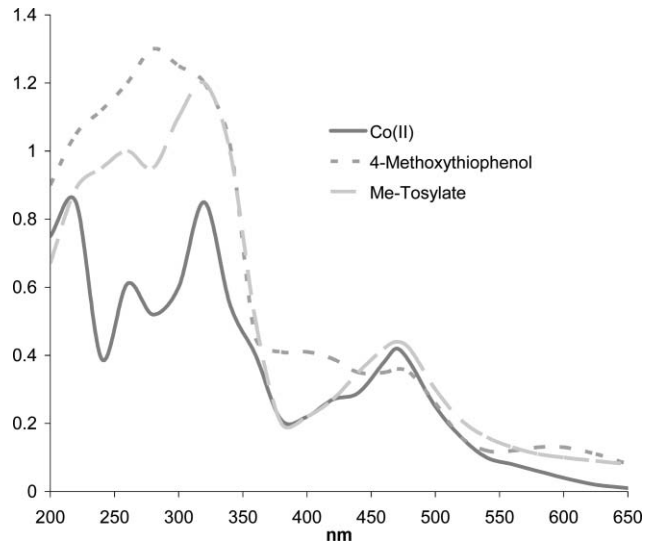


Fig. 5 UV–Vis spectrum (absorbance *vs.* wavelength) of the interaction of **8r** with the thiol **12** and methyl tosylate **10** in methanol with pH 9 aqueous buffer.

Upon addition of methyl iodide **9** or methyl tosylate **10** to the Co(I) complex **8s**, obtained from **8r** by reaction with hexane thiol **11** or glutathione **16**, the methylcobyrinates **17**/**18** were formed immediately.

The absence of any alkylation reaction in pure methanol is surprising (Table 1, entries 17 and 18). We interpret this observation in terms of an increased pK_a value of hexane thiol **11** in methanol, which reduces the dissociation to such an extent that no thiolate anions are formed, this most likely being required for reduction of **8r** and formation of the Co(I) complex **8s**. This interpretation is based on results well known for acetic acid: the pK_a value of acetic acid in water is 4.7 and increases to *ca.* 9 in ethanol, whereas the change in the pK_a value of pyridinium acetate in water (pK_a = 4.8) to that in ethanol is only 1.2 pK_a units.[30] Addition of water to the methanolic solution enhances the dissociation of hexane thiol and induces the alkylation of **8r**, albeit at a lower rate (Table 1, entries 19 and 20).

Buffered solutions

The methylation of **8r** mediated by hexane thiol **11** also proceeds rapidly in buffered aqueous solution with methyl iodide **9** and methyl tosylate **10** respectively (Table 1, entries 9–16). The reactions were run in a 1 : 1 mixture of methanol and aqueous buffer (see Experimental section). The pH of the mixtures differs only to a small extent from the pH in H_2O.[31] Although it is not completely clear whether the Co(I) complex is formed under acidic conditions, it is to be expected that the Co(I) complex **8s** is protonated and oxidized to Co(II) or Co(III), which is again reduced by excess thiol. The decay rate of Co(I) depends strongly on the pH of the solution. We have previously shown that when a solution of heptamethyl cob(I)yrinate **8s**, generated by cathodic reduction of **8r** in methanol, is treated with an excess of dilute H_2SO_4, the Co(I) complex **8s** is oxidized to Co(II) within a very short time (5–10 s), while it is oxidized within *ca.* 7 min when pyH^+ Cl^- in water is added. In a methanol–water mixture the oxidation Co(I) (**8s**) → Co(II) (**8r**) takes more than 25 min.[32]

Additives

In view of the extensive discussion of the base on/off problem in vitamin B_{12} and its congeners, it was of interest to explore the impact of bases with a potential for coordination to the Co center on the methylation of **8r**. In addition, it is known that pyridine, imidazole or benzimidazole interact with cob(II)alamin (derived from **2**) and cob(II)inamide (derived from **5**).[33] In contrast to the reaction of **8r** with an excess of **11** in methanol with triethylamine as additive, the addition of pyridine or *N*-methylimidazole suppresses the methylation with methyl tosylate **10** completely (Table 1, entries 4–8). Surprisingly, the formation of **17**/**18** with

methyl iodide as alkylating agent still proceeds efficiently (but seemingly more slowly) under the same conditions.

These observations support the hypothesis proposed above that the methylation of **8r** with methyl iodide **9** follows a pathway different from that with methyl tosylate even in the presence of pyridine and *N*-methylmidazole.

Mole equivalents

The methylation of **8r** in the presence of 1 or 2 mole equivalents of hexane thiol **11** was carried out in MeOH–Et_3N, as well as in MeOH–H_2O containing pH 3.5 buffer. No methylation product was detected when 1 mole equivalent of **11** was added, while the activation of **8r** with 2 mole equivalents of **11** resulted in quantitative methylation.

In a control experiment, a degassed solution of **8r** in MeOH–H_2O containing pH 3.5 buffer was stirred with a 10-fold excess of **11** for 15 h in the absence of O_2. Subsequent addition of methyl tosylate **10** did not give any methylated product **17/18**. GC analysis showed that **11** was quantitatively transformed into dihexyldisulfide (C_6H_{13}–S–S–C_6H_{13}).

Methylation of 8r in the presence of glutathione 16

The methylations of **8r** in the presence of glutathione **16** show that methyl iodide as well as methyltosylate **10** are efficient agents for its activation under a variety of conditions (Table 1). In comparison with hexane thiol **11**, the reactions induced by glutathione **16** seem to be somewhat slower (Table 1, entries in parentheses).

Methylation of 8r in the presence of 4-methoxythiophenol 12

The results for the methylation of **8r** in the presence of 4-methoxythiophenol **12** are shown in Table 2. It is apparent that the reaction with methyl iodide **9** is rapid and complete within a short time, whereas the methylation with methyl tosylate **10** is slower, incomplete (entry 6) or fails entirely (entry 2). In a further experiment, a methanolic solution of **8r** containing triethylamine was treated with methyl tosylate **10** for 20 min. As expected, no methylation was apparent, but when a small excess of hexane thiol **11** was added, the methylation products **17/18** where fully formed within 5 min.

It should be noted that the alkylation with both methylating agents proceeds well in pure methanol. In view of the pK_a value of **12** being lower by 4 pK_a units than that of hexane thiol **9**, it is plausible that 4-methoxythiophenolate ions are still formed in pure methanol (see above). Our results suggest that the formation of thiolate ions is required for activation of the Co(II) complex **8r**.

Similar results are obtained with thiophenol **13** (Table 3). It is unclear why the methylation of **8r** with methyl tosylate **10** does not proceed in methanol in the presence of triethylamine neither with 4-methoxythiophenol **12** (Table 2) nor with thiophenol **13** (Table 3) as reducing agent.

Table 2 Methylation reactions of **8r** with CH_3I **9** and CH_3OTs **10** induced by 4-methoxythiophenol **12**

Entry	Solvent	Methylating agent	Time/min	Yield of **17/18** (%)[a]
1	CH_3OH + 0.2 mL Et_3N	CH_3I	1	100
2	CH_3OH + 0.2 mL Et_3N	CH_3OTs	60	0
3	CH_3OH + 0.2 mL pyridine	CH_3I	10	100
4	CH_3OH + 0.2 mL CH_3-imidazole	CH_3I	5	100
5	CH_3OH + pH 9 buffer	CH_3I	1	100
6	CH_3OH + pH 9 buffer	CH_3OTs	60	75
7	CH_3OH + pH 6.5 buffer	CH_3I	1	100
8	CH_3OH + pH 6.5 buffer	CH_3OTs	1	100
9	CH_3OH + pH 3.5 buffer	CH_3I	1	100
10	CH_3OH + pH 3.5 buffer	CH_3OTs	30	100
11	CH_3OH	CH_3I	30	100
12	CH_3OH	CH_3OTs	60	100

[a] Estimated (*cf.* text).

Table 3 Methylation reactions of **8r** with CH_3I **9** and CH_3OTs **10** induced by thiophenol **13** and thioacetic acid **14**

Entry	Solvent	Methylating agent	Thiol	Time/min	Yield of **17/18** (%)[a]
1	CH_3OH + 0.2 mL Et_3N	CH_3I	**13**	1	100
2	CH_3OH + 0.2 mL Et_3N	CH_3OTs	**13**	60	0
3	CH_3OH + buffer pH 3.5	CH_3I	**13**	5	100
4	CH_3OH + buffer pH 3.5	CH_3OTs	**13**	15	95
5	CH_3OH	CH_3I	**13**	30	100
6	CH_3OH	CH_3OTs	**13**	30	90
7	CH_3OH + 0.2 mL Et_3N	CH_3I	**14**	35	60
8	CH_3OH + 0.2 mL Et_3N	CH_3OTs	**14**	60	0
9	CH_3OH + buffer pH 3.5	CH_3I	**14**	30	30
10	CH_3OH + buffer pH 3.5	CH_3OTs	**14**	90	Trace
11	CH_3OH	CH_3I	**14**	15	40
12	CH_3OH	CH_3OTs	**14**	60	15

[a] Estimated (*cf.* text).

Methylation of 8r in the presence of thioacetic acid 14

The methylations of **8r** with **9** and **10** in the presence of thioacetic acid **14** are similar to those obtained with 4-methoxythiophenol **12** and thiophenol **13** (Table 3). Extended reaction times did not lead to additional formation of alkylation product **17/18**.

Methylation of 8r in the presence of pentafluorothiophenol 15

The methylations of **8r** with methyl iodide **9** and methyl tosylate **10** in the presence of pentafluorothiophenol **15** as inducing agent, attempted in methanol with and without triethylamine, are shown in Table 4. Only traces of the methylated product **17/18** could be detected with **9** as alkylating agent. A small amount of the methylated product **17/18** was observed in the reaction of **8r** with **10** in pure methanol, whereas no product was detected in methanol containing triethylamine.

Attempted methylation of 8r with tertiary methylamines in the presence of hexane thiol 11

In view of our successful transfer reactions from dimethylaniline to hexane thiol *via* methyl cob(III)yrinate **17/18** in the presence of Zn and acetic acid,[20] the reactions of **8r** with dimethylaniline, *N*-methylimidazole and *N*,*N*-dimethylglycine induced by hexane thiol **11** in acidic buffer were explored. When dimethylaniline or *N*-methylimidazole were incubated with **8r** in methanol containing a pH 3.5 buffer for 3 h, no methyl cobyrinate **17/18** was observed. Similarly, interaction of **8r** in methanol containing an aqueous buffer and hexane thiol with *N*,*N*-dimethylglycine did not give any methyl cobyrinate at pH 2.5 (19 h), 3.5 (19 h), 4.5 (5.5 h), 5.5 (6 h) or 6.5 (6 h).

Methylation of heptamethyl cob(III)yrinate 7

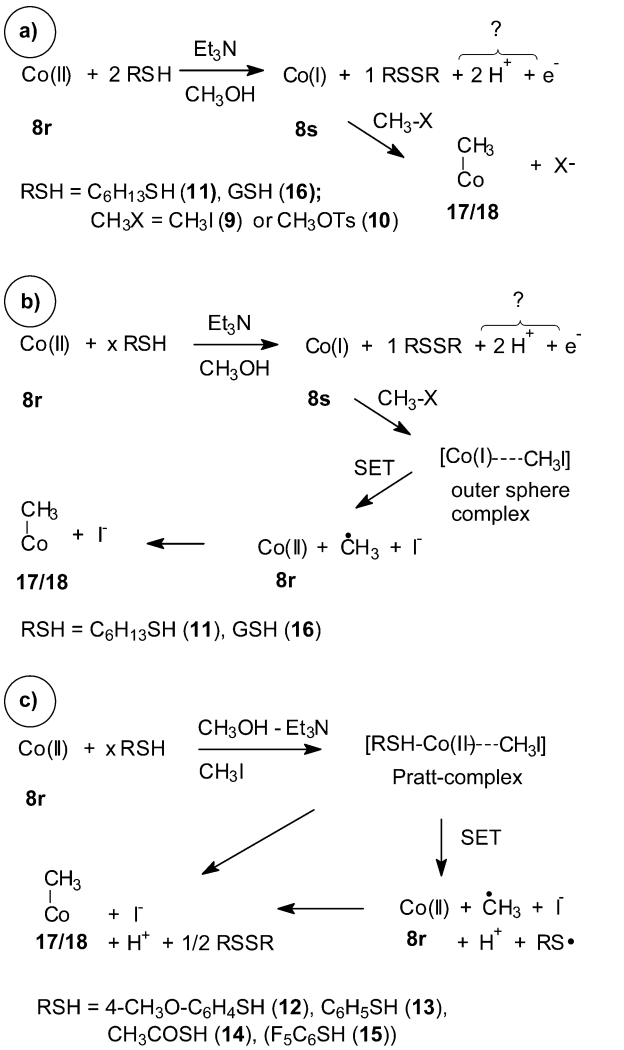
Since the Co(III) complex **7** is the precursor of heptamethyl cob(II)yrinate **8r** under reducing conditions with a variety of reducing agents (including thiols), it was of interest to explore the reaction of **7** with thiols and the methylating agents **9** and **10** under a variety of conditions. With a few exceptions, the results are very similar to those obtained with **8r** (Table 4).

Under acidic conditions the methylation of **7** is not observed with hexane thiol **11** as activating agent and alkylation with methyltosylate **10** (entry 5). Also, methylation is not detectable when **7** is treated with hexane thiol **11** in methanol containing triethylamine (entry 7). Methylation is negligible or absent when **7** is treated with the thioacetic acid **14** and pentafluorothiophenol **15** (Table 4, entries 13–15).

Mechanistic aspects

The earlier mechanistic discussions for the methylation of corrinoids included the nucleophilic substitution of Co(I) as a supernucleophile,[15,16] and the direct methylation of Co(II) (requiring the reaction to be second order in Co(II) concentration).[20] In the specific case of a thiol-induced reaction of the Co complex cobinamide **5** with methyl iodide in the pH range 3–8, Pratt reported evidence for a third pathway, involving association of the RSH–Co(II) adduct with methyl iodide, the formation of an [RSH–Co–CH_3I] complex ('Pratt complex') as a key intermediate, and the immediate formation of methyl cobinamide.[18]

In typical nucleophilic substitution reactions, methyl iodide **9** and methyl tosylate **10** show S_N2 kinetics. In the reaction of the rather 'soft' azide ions with methyl iodide **9** and methyl tosylate **10** in methanol, tosylate is a slightly better leaving group than iodide. However, alkyl iodides may undergo nucleophilic substitution reactions *via* a mechanism involving dissociative single electron transfer, particularly with supernucleophiles.[25]



Scheme 7 Possible mechanistic pathways for the thiol-mediated methylation of cob(II)yrinate **8r**.

Our results show the methylation of heptamethyl cob(II)yrinate **8r** induced by thiols is strongly dependent on the pK_a of thiol, the methylating agent and on the solvent. Coordinating bases like pyridine and *N*-methylimidazole suppress the methylation with methyl tosylate, but not that with methyl iodide.

The detection of the Co(I) complex **8s** in the interaction of **8r** with hexane thiol **11** (or glutathione **16**) in methanol containing triethylamine, and its subsequent methylation with methyl iodide **9** as well as with methyl tosylate **10**, are compatible with a nucleophilic substitution reaction (Scheme 7a). However, for the reaction with methyl iodide **9**, an alternative pathway *via* a single electron transfer cannot be ruled out on the basis of the present results (Scheme 7b). The reaction with glutathione **16**, with a

Table 4 Data for the methylation reactions of the Co(III) complex **7** with CH_3I **9** and CH_3OTs **10** in the presence of thiols **11–16**

Entry	Solvent	Thiol	Methylating agent	Time/min	Yield of **17/18** (%)[a]
1	CH_3OH + 0.2 mL Et_3N	**11**	CH_3I	5	100
2	CH_3OH + 0.2 mL Et_3N	**11**	CH_3OTs	5	100
3	toluene + 0.2 mL Et_3N	**11**	CH_3OTs	5	100
4	CH_3OH + buffer pH 3.5	**11**	CH_3I	60	70
5	CH_3OH + buffer pH 3.5	**11**	CH_3OTs	90	0
6	CH_3OH + 0.2 mL Et_3N	**12**	CH_3I	5	100
7	CH_3OH + 0.2 mL Et_3N	**12**	CH_3OTs	60	0
8	CH_3OH + buffer pH 3.5	**12**	CH_3I	5	100
9	CH_3OH + buffer pH 3.5	**12**	CH_3OTs	10	100
10	CH_3OH + 0.2 mL Et_3N	**13**	CH_3I	10	100
11	CH_3OH + buffer pH 3.5	**13**	CH_3I	5	100
12	CH_3OH + buffer pH 3.5	**13**	CH_3OTs	5	100
13	CH_3OH + 0.2 mL Et_3N	**14**	CH_3I	60	0
14	CH_3OH + buffer pH 3.5	**14**	CH_3I	60	10
15	CH_3OH + 0.2 mL Et_3N	**15**	CH_3I	60	0
16	CH_3OH + 0.2 mL Et_3N	**16**	CH_3I	5	100
17	CH_3OH + 0.2 mL Et_3N	**16**	CH_3OTs	5	100
18	CH_3OH + buffer pH 3.5	**16**	CH_3I	20	100
19	CH_3OH + buffer pH 3.5	**16**	CH_3OTs	60	10

[a] Estimated (*cf.* text).

very similar pK_a value, should follow analogous pathways for the methylations with methyl tosylate and methyl iodide.

The Co(I) complex **8s** was not formed to a UV-detectable amount when **8r** was treated with the more acidic thiols **12–14** in methanol containing triethylamine. Nevertheless, methylation proceeded efficiently with methyl iodide **9**, but was completely absent in the reaction with methyl tosylate **10** (Table 2, entry 2; Table 3, entries 2 and 8). Since nucleophilic substitutions with alkyl tosylates strictly follow a one-step S_N2-type reaction with the Co(I) complex **8s** as supernucleophile, the methylation reaction with methyl iodide must follow a different pathway. The reaction could be initiated by the formation of a Pratt-type intermediate (Scheme 7c) which directly forms the methylation product **17/18**.[18] Alternatively, the Pratt complex could undergo an SET reaction, leading to a methyl radical which reacts with the Co(II) complex **8r** to give the Co-methyl product **17/18**. The fact that pyridine and *N*-methylimidazole as bases and hexane thiol **9** as inducing agent lead to the methylation of **8r** in methanol with methyl iodide **9** but not with methyl tosylate **10** suggests that these bases prevent the formation of the Co(I) complex **8s** but not the formation of a Pratt-type intermediate.

When the Co(II) complex **8r** was treated in a methanolic solution containing buffer (with pH 3.5, 6.5 or 9) with 4-methoxythiophenol **12**, the methylation proceeded with methyl iodide **9** as well as with methyl tosylate **10** (Table 2, entries 5–10). The conditions are very similar to those described by Pratt for the methylation of cobinamide **5** with methyl iodide, and suggest a similar pathway for its reaction (Scheme 7c). The mechanistic pathway for the methylation with methyl tosylate **10** is less clear. The presence of the Co(I) complex **8s** could not be detected in the rather unstructured UV–Vis spectra when 4-methoxythiophenol **12** was added to an acidic solution of **8r** (Fig. 2 and 3). However, the formation of a small amount of the Co(I) complex **8s** under these 'steady state' conditions (due to the large excess of **12**) cannot be excluded.

The methylation of **8r** is also induced by thiophenol **13**, and proceeds in a methanolic solution containing pH 3.5 buffer with methyl iodide **9** and methyl tosylate **10**, respectively (Table 3, entries 3 and 4). With an excess of thioacetic acid **14**, the methylation of **8r** proceeds in methanol containing pH 3.5 buffer only with methyl iodide **9**, whereas with methyl tosylate **10** only a trace of **17/18** could be detected (Table 3, entries 9 and 10). Pentafluorothiophenol **15** did not show any methylation in methanol containing pH 3.5 buffer.

Concluding remarks

We have explored the reactivity of the Co(II) complex heptamethyl cob(II)yrinate **8r** with respect to its methylation with methyl iodide **9** and methyl tosylate **10** induced by a variety of thiols. UV-Vis spectroscopy of the interaction of the Co(II) complex with thiols was used for detection of the Co(I) complex **8s**, formulated as an intermediate for alkylation processes. The methylation depends strongly on the acidity of the thiol, and the addition of bases and the solvent. Whereas **8r** is readily methylated in methanol containing triethylamine with both methylating reagents **9** and **10**, methylation with methyl tosylate is suppressed when pyridine or *N*-methylimidazole are used instead of triethylamine. When the thiols **12–15** are used for induction of the methylation of **8r**, only methyl iodide **9** leads to the Co-methyl product **17/18**. These results suggest that methyl tosylate leads to methylation *via* a conventional S_N2 pathway only when the cob(I)yrinate **8s** is formed. Apparently, methyl iodide may react *via* additional reaction channels which include outer-sphere single-electron transfer and the intermediate formation of the complex between the thiol–Co(II) adduct and methyl iodide (the Pratt pathway).

Experimental

Materials and methods

The reactions were carried out with reagents and solvents of 'puriss.' or 'purum' grade under Ar. Hexane thiol (>97%), 4-methoxythiophenol (>98%), thiophenol (>99%), glutathione

(>97%), dimethylaniline (>99.5%), *N*,*N*-dimethylglycine (>99%), *N*-methylimidazole (>99%), triethylamine (>98%), methyl *p*-tosylate (>97%), methyl iodide (>99%) (Fluka), thioacetic acid (>97%), pentafluorothiophenol (>97%) (Aldrich) and pyridine (>99%) (Siegfried) were checked for purity by GC and/or NMR, and used as received. Solutions were degassed by sonication under reduced pressure and bubbling Ar through the solution.

Column chromatography (CC): distilled commercial-grade solvents; silica gel (40–63 μm) from Fluka. TLC: Merck-F-254 precoated sheets; visualisation by phosphomolybdic acid hydrate–ethanol (5%) or by UV. CC and TLC: CH_2Cl_2–THF–Et_2O = 2 : 1 : 3 as eluent, unless otherwise indicated. GC: Hewlett Packard HP-5890 instrument; HP-5 ultra capillary column (length 10 m, i.d. 0.2 mm), 5% phenylpolysiloxane; temperature program 40–270 °C (6 °C min^{-1}). UV–Vis: Hewlett-Packard 8451A diode array spectrophotometer; λ_{max} (log ε) in nm. NMR: Bruker-AC-300 (^{1}H, 300 MHz; ^{13}C, 75 MHz) and Bruker-AC-500 (^{1}H, 500 MHz;, ^{13}C, 125 MHz) spectrometers; δ in ppm (internal lock: $CDCl_3$ (δ_H: 7.27, δ_C: 77.00)); *J* in Hz. MS: Varian MAT-CH-7A instrument, 70 eV. FB-MS: Fison Autospec-Q; acceleration voltage 8 kV; ionisation Cs^+ (32 keV); matrix dithiotreitol (DTT)/dithioerythriol (DTE); values in *m*/*z* (%). ESI–MS: Fison Instrument VG Platform II; positive ion measurements at 3.5 kV; negative ion measurements at 2.5 kV; values in *m*/*z* (%) in the solvents given.

Heptamethyl Coα,Coβ-dicyano-cob(III)yrinate (6). Prepared from 20 g (14.76 mmol) cyanocobalamin (vitamin B_{12}) **1**, giving 11.4 g (71%) **6**.[22,23]

Heptamethyl aquocyanocob(III)yrinate perchlorate (7). Prepared from 500 mg (0.46 mmol) **6** as reported, giving 515 mg (95%) **7**.[22,23]

Heptamethyl Coβ-perchlorato-cob(II)yrinate (8r). Prepared from 500 mg (0.46 mmol) **7**, giving 453 mg (87%) **8r**.[22]

Heptamethyl Coα,β-methyl-cob(III)yrinate perchlorate (17/18). Prepared as a mixture of the stereoisomeric Co-methyl complexes **17/18** (6 : 1), as described in ref. 23,24.

Interactions of 7 or 8r with thiols 11–16 and the methylating reagents 9 or 10

General procedure. All experiments were carried out under an Ar atmosphere and green light. The reaction were performed in a special UV-cell (25 ml) and monitored *via* UV–Vis and TLC. The reference compounds **17/18** were prepared as a mixture according to published procedures and their R_f values established with CH_2Cl_2–THF–Et_2O = 2 : 1 : 3 as eluent.

In a typical experiment, heptamethyl cob(II)yrinate **8r** (5 mg, 4.4 μmol) was dissolved in degassed CH_3OH (or methanolic buffer) (15 mL) and the UV–Vis spectrum measured. The appropriate thiol (0.71–0.73 mmol) was added and the UV–Vis spectrum recorded. After addition of the base (200 μL) and CH_3I (50 μL, 0.803 mmol, *d* = 2.28) or methyl tosylate (0.16 g, 0.86 mmol), the reaction mixture was spotted under normalized conditions (capillary) onto a TLC plate after the reaction time given in the tables, and eluted with CH_2Cl_2–THF–Et_2O = 2 : 1 : 3, with methyl cobyrinate **17/18** as reference. The yellow spots of **17/18** were detected in the case of 100% formation of the methylated product **17/18**. Incomplete formation of **17/18** was qualitatively evaluated by comparison of the sizes of the yellow spots and red spots.

The reactions in buffered solution were run in a 1 : 1 mixture of methanol (or ethanol) and water containing buffer according to the specifications given below.

- pH 3.5: 50 mL 0.1 M potassium hydrogen phthalate + 8.2 mL 0.1 M HCl
- pH 6.5: 50 mL 0.1 M potassium dihydrogenphosphate + 13.9 mL 0.1 M NaOH
- pH 7.5: 50 mL 0.1 M potassium dihydrogenphosphate + 41.1 mL 0.1 M NaOH
- pH 9: 50 mL 0.025 M borax + 4.6 mL 0.1 M HCl

Reaction of 8r in methanol–Et_3N with 12 and subsequent addition of 11

A solution of **8r** (5 mg, 0.0044 mmol) in methanol (15 mL) and Et_3N (0.20 mL) was degassed for 15 min, then **12** (20 μL, 0.1636 mmol) was added. The colour changed and **10** (100 mg, 0.537 mmol) was added. No change in the UV spectrum was observed, and after 10 min, **11** (20 μL, 0.142 mmol) was added. The Co-methyl product **17/18** was formed immediately in 100% yield.

Isolation of the Co-methylated products 17/18 from 8r

All experiments were carried out under an argon atmosphere and green light. The reactions were monitored by TLC. A solution of **8r** (0.0176 mmol) in CH_3OH (10 mL) was degassed for 15 min, a 100-fold excess of thiol was added, followed after 5 min by a 200-fold excess of the methylating agent (**9** or **10**). The reaction was allowed to stir until no more methylated compounds were formed. For work-up, the reaction-mixture was treated with 10% $HClO_4$ (20 mL) and extracted with CH_2Cl_2 (20 mL). The product was purified by CC on $NaClO_4$-impregnated silica gel with ether as solvent for the non-corrinoid fraction, and CH_2Cl_2–CH_3OH = 2 : 1 for the corrinoid fraction. The methylated product **17/18** was identified by ^{1}H-NMR spectroscopy and was identical to the material described in ref. 24.

Reaction of 8r in methanol–Et_3N with 1 mole equivalent of 11

A solution of **8r** (20 mg, 0.0176 mmol) in methanol (15 mL) and Et_3N (0.25 mL) was degassed for 15 min, then **10** (250 mg, 1.342 mmol) was added. A degassed solution of **11** (2.4 μL, 0.0176 mmol) in methanol (10 mL) was added dropwise over 1 h, and the solution was stirred for 30 min. After work-up (extraction with 10% $HClO_4$ (30 mL) and CH_2Cl_2 (30 mL), evaporation of the solvent, and CC), neither TLC nor ^{1}H-NMR spectroscopy showed any product **17/18**.

Reaction of 8r in methanol–Et_3N with 2 mole equivalents of 11

A solution of **8r** (20 mg, 0.0176 mmol) in methanol (15 mL) and 0.2 mL Et_3N was degassed for 15 min, then **10** (250 mg, 1.342 mmol) was added. A degassed solution of **11** (5 μL, 0.0176 mmol) in methanol (1 mL) was added, and the solution was stirred for 30 min. After work-up (extraction with 10% $HClO_4$ (30 mL) and CH_2Cl_2 (30 mL), evaporation of the solvent, and CC), TLC and ^{1}H-NMR spectroscopy showed 100% of **17/18**.

Reaction of 8r in pH 3.5 buffer with 1 mole equivalent of 11

A solution of **8r** (20 mg, 0.0176 mmol) in pH 3.5 buffer (15 mL) was degassed for 15 min, then **10** (250 mg, 1.342 mmol) was added. A degassed solution of **11** (2.4 μL, 0.0176 mmol) in methanol (1 mL) was added, and the solution was stirred for 30 min. After work-up (extraction with CH_2Cl_2 (30 mL), evaporation of the solvent, and CC), neither TLC nor ^{1}H-NMR spectroscopy showed any product **17/18**.

Reaction of 8r in pH 3.5 buffer with 2 mole equivalents of 11

A solution of **8r** (20 mg, 0.0176 mmol) in pH 3.5 buffer (15 mL) was degassed for 15 min, then **10** (250 mg, 1.342 mmol) was added. A degassed solution of **11** (5 μL, 0.0367 mmol) in methanol (1 mL) was added and the solution stirred for 30 min. After work-up (extraction with CH_2Cl_2 (30 mL), evaporation of the solvent, and CC), TLC and ^{1}H-NMR spectroscopy showed 100% of **17/18**.

Reaction of 8r in pH 3.5 buffer with 10 mole equivalents of 11

A solution of **8r** (20 mg, 0.0176 mmol) in pH 3.5 buffer (15 mL) was degassed for 15 min, then **8** (25 μL, 0.176 mmol) was added and the solution was stirred for 15 h. In the next step, **10** (250 mg, 1.342 mmol) was added and the solution was allowed to stir for 1 h. After work-up (extraction with CH_2Cl_2 (30 mL), evaporation of solvent and CC), TLC and ^{1}H-NMR spectroscopy showed no **17/18**. $C_6H_{13}SSC_6H_{13}$ was detected as the only sulfur compound by GC analysis.

Reaction of 8r with 11 in different buffer solutions and N-CH_3-compounds as a methyl source

A solution of **8r** (20 mg, 0.0176 mmol) in buffer solution (10 mL, 50% ethanol and 50% water) was degassed for 15 min, then **11** (250 μL, 1.76 mmol) was added. After 5 min, the methyl amine (*N*,*N*-dimethylaniline, *N*-methylimidazole, *N*,*N*-dimethylglycine) was added (100-fold excess relative to Co) and the solution was stirred at rt for 3–19 h. After work-up (extraction with 10% $HClO_4$ (20 mL) and CH_2Cl_2 (20 mL), followed by CC, with ether as solvent for the non-corrinoid fraction, and methanol + 0.1% $NaClO_4$ for the corrinoid fraction), TLC and NMR spectroscopy showed that **17/18** was not formed.

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