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# Alkyl Transfer from Quaternary Ammonium Salts to Cobalt (I): Model for the Cobalamin-Dependent Methionine Synthase Reaction

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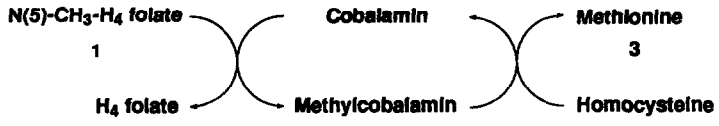
*Abstract:* The reaction of cobaloxime(I) with diverse quaternary ammonium salts leads, in general, to a group transfer from nitrogen to cobalt. The behaviour of the salts in these transalkylations is consistent with an $S_N2$ mechanism, involving Co(I) as a nucleophile. In a model study of the cobalamin-dependent methionine synthase reaction, 5-$^{13}CH_3$-methyl labelled 5,5,6,7-tetramethyl-5,6,7,8-tetrahydropteridinium salt (23) -a model of the natural coenzyme 5-$CH_3H_4$-folate (1)- was allowed to react with cobaloxime(I) and cobalamin(I). In each case the formation of the methyl transfer product, namely, methylcobaloxime and methylcobalamin, respectively, was shown by $^{13}$C-NMR spectroscopy.

Methionine synthases catalyze the overall methyl transfer from the coenzyme N(5)-methyltetrahydrofolate **1** (Scheme 1) to the thiolate of homocysteine **2**, to result in the formation of methionine **3** and tetrahydrofolate **4**[1]. The enzymes found in micro-organisms and mammals utilize cofactor **1** as a reagent and, additionally, require cobalamin as the prosthetic group. The cobalamin or vitamin $B_{12}$ functions as an intermediate methyl carrier in this biological transmethylation reaction (Scheme I).




Scheme I

The overall transformation catalyzed by cobalamin-dependent methionine synthase consists of two half reactions: (i) transfer of the methyl moiety from the folate coenzyme **1** to the cobalt atom of cobalamin and (ii) donation of the $CH_3$-group of methylcobalamin to the thiol residue of homocysteine (2).

The methyl transfer from methylcobalamin to homocysteine, the second half reaction, has been previously demonstrated[2] in absence of the enzyme. It is now generally assumed that a nucleophilic displacement of the methyl moiety of methylcobalamin by the thiolate of homocysteine is involved in the cobalamin-dependent methionine synthesis[3,4].

To date a nonenzymatic precedent of the first half reaction has been lacking. The displacement of

the methyl group of 5-methyltetrahydrofolate **1** by cobalamin(I) is commonly shown in the text books as a simple $S_N2$ reaction. However, although cobalamin(I) is a super nucleophile, a nucleophilic displacement of a tertiary amine at the α-carbon constitute an unprecedented chemical process[5]. To make this plausible, it is assumed that the N(5)-position of the coenzyme is suitably activated prior to the transfer of its methyl substituent. This activation could be achieved by oxidation of N(5), either *via* loss of one or two electrons or by its coordination with an electrophile/proton at the enzymic site[5].

Attempted alkyl transfers from tertiary amines, including 5-methyltetrahydrofolate, and from a number of quaternary ammonium salts, to cobalamin or cobaloxime, have not been successful[2]. In a more recent study[6], however, the transfer of a benzyl substituent from benzyltrimethylammonium iode and a methyl group from N,N-dimethylpiperidinium iodide, to cobaloxime(I), have been described. It is also claimed that a methyl transfer is observed from an ammonium species[6] which is generated *in situ* by adding tertiary amines to acetylenic esters. In view of the goal of developing a precedent for the methyl transfer from nitrogen to cobalt, in cobalamin-dependent methionine synthase, we initially investigated the reaction of a range of ammonium salts, with cobaloxime(I) [Co(I)(dmgH)2pyr] **5**. Since the behaviour of cobaloximes is known to be very similar to that of the natural cobalamin, they are highly suited for model studies of vitamin $B_{12}$ dependent reactions[7,8,9].

In our study, the ammonium salts **6-18** (Table I) were allowed to react with cobaloxime(I) under strictly anaerobic conditions. Cobaloxime(I) was generated by base-mediated disproportionation of cobaloxime(II)[10]. At room temperature (4-16 hours), after stirring, the Co-alkylated products were isolated and identified by comparison (NMR) with authentic samples. The results of these reactions are described in Table I.

Table I: Transfer of alkyl groups from quaternary ammonium salts to Co(I)[dmgH]2pyr (**5**).

| Ammonium Salt | | Group Transferred (R) | RCo(dmgH)2pyr yield |
|---|---|---|---|
| **6** | $PhN^+Me_3I^-$ | Me | 47% |
| **7** | $PhCH_2N^+Me_3Cl^-$ | $PhCH_2$ | 58% |
| **8** | *p*-$MeOPhCH_2N^+Me_3Cl^-$ | *p*-$MeOPhCH_2$ | 41% |
| **9** | *p*-$MeOPhCH_2N^+Me_2CH_2PhCl^-$ | *p*-$MeOPhCH_2$ | 47% (**22**) |
| | | $PhCH_2$ | 20% (**20**) |
| **10** | *p*-$NO_2PhCH_2N^+Me_3Cl^-$ | - | - |
| **11** | *p*-$NO_2PhCH_2N^+Me_2CH_2PhBr^-$ | - | - |
| **12** | $[DABCO]^+MeCl^-$ | - | - |
| **13** | $[DABCO]^+CH_2PhCl^-$ | $PhCH_2$ | 54% |
| **14** | $[DABCO]^+CH_2Ph$(*p*-MeO)$Cl^-$ | *p*-$MeOPhCH_2$ | 52% |
| **15** | $[DABCO]^+CH_2Ph$(*p*-$NO_2$)$Cl^-$ | - | - |
| **16** | $[DABCO]^+CHPh_2Br$- | - | - |
| **17** | $(CH_2)_5N^+MeCH_2PhBF_4^-$ | $PhCH_2$ | 47% |
| **18** | $(CH_2)_5N^+Me_2I^-$ | - | - |

* DABCO = 1,4-diazabicyclo[2.2.2]octane

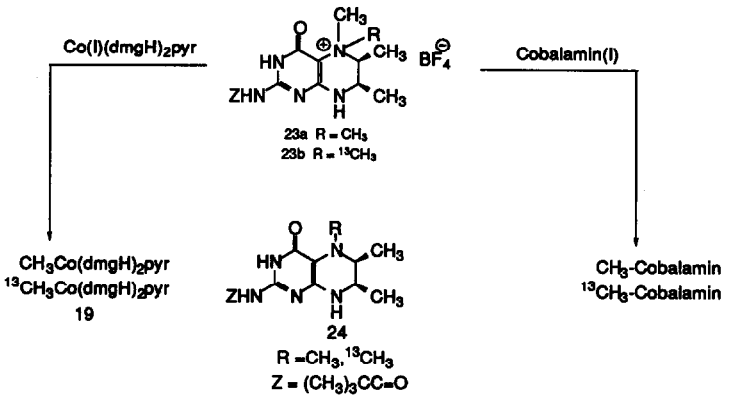
The results presented in Table I show the following trends. A methyl group is effectively transferred from the trimethylanilinium salt **6** to the cobalt atom of **5**. It is obvious from the reactions of **7, 12, 13, 17** and **18** that the transfer of a benzyl substituent is preferred to that of an analogously located methyl group. In these experiments the presence of methyl(pyridine)cobaloxime (**19**) could not be observed in the $^1$H-NMR spectra of the reaction mixtures. The reaction of compound **9** with **5** resulted in the formation of two transfer products viz. *p*-methoxybenzylcobaloxime **22** and benzylcobaloxime **20** in the ratio 2.3 to 1. This suggests that the transfer of the *p*-methoxybenzyl group is favoured in comparison with the transfer of the unsubstituted benzyl moiety. The lack of reactivity of the benzhydryl salt **16** is highly significant. It stresses the salient role of steric features, implying the operation of a mechanism in which the process is strongly inhibited by bulky groups. The result also tells us that mechanisms involving a carbenium ion ($Ph_2CH^+$) or a radical ($Ph_2CH\cdot$) transfer are inconsistent with the experimental observations, since such mechanisms would be expected to give a facile benzhydryl transfer from **16**. It should, however, be noted that, except in the case of the *p*-nitrobenzyl salts (**10, 11** and **15**), where no transfer is observed, the results can be best rationalized on the basis of a nucleophilic displacement mechanism ($S_N2$). In first instance, the behaviour of these salts is somewhat puzzling. The electron-withdrawing nitro substituent (in **10, 11** and **15**) would make the benzyl carbon more prone to attack by the nucleophilic Co(I). However, no product formation corresponding to this process is experimentally observed. An explanation of the exceptional behaviour of the nitrobenzyl salts may be sought in the high acidity of the benzylic protons -due to the adjacent positively charged nitrogen atom and the presence of the electron withdrawing *p*-nitrophenyl substituent. This relative acidic character is revealed when one compares the chemical shifts (in $CDCl_3$) of the benzylic hydrogen atoms of the salts **13** ($\delta$ 5.09, $PhC\underline{H}_2$), **14** ($\delta$ 4.97, *p*-$MeOPhC\underline{H}_2$) and **15** ($\delta$ 5.38, *p*-$NO_2PhC\underline{H}_2$). Although Co(I) of cobaloxime is an extremely powerful nucleophile (n = 13.8)[11,12], it has also the possibility of acting as a base towards the acidic benzyl protons of the *p*-nitro-benzyl salts. The transfer of a proton to Co(I) results in the formation of cobaloximehydride, which is known to decompose into cobaloxime(II) and molecular hydrogen. .

In order to mimic the cobalamin-dependent methionine synthase reaction, we have developed a model of the natural coenzyme 5-methyltetrahydrofolate (**1**) in which the MeN(5)-position is activated by coordination with a methyl group. In this model (**23a**)[13], one of the N-methyl substituents was labelled with $^{13}$C, to give salt **23b**[13]. In the latter system the fate of the methyl transfer to the Co(I) of either cobaloxime(I) or cobalamin (I) could be followed by $^{13}$C-NMR without isolation of the reaction products. The syntheses of the labelled and unlabelled pteridinium salts have been described previously[13].

Reactions (Scheme II) of cobaloxime(I) **5** at 293K or 313K, with pteridinium salts **23a** or **23b**, resulted in reaction mixtures in which the formation of the Co-$^{12}CH_3$ and Co-$^{13}CH_3$ derivatives of **5** could be readily identified by NMR spectroscopy. In the case of the reaction with the unlabelled salt **23a** the $^1$H-NMR spectrum revealed a small signal at 0.81 ppm. The latter signal could be assigned to the cobalt-bounded methyl substituent in methylcobaloxime **19**.

Performance of the reaction at the elevated temperature of 313K, resulted in a slightly higher yield of the methyl transfer product as evidenced by a stronger signal at 0.81 ppm in the NMR spectrum.

An analogous experiment with the labelled compound **23b** (313K) resulted in the formation of a mixture of the labelled and unlabelled methylcobaloxime. Once again, the signal at 0.81 ppm could be assigned to Co-$^{12}CH_3$. The signal of the Co-$^{13}CH_3$ substituent was observed to split into a doublet (0.47 ppm and 1.15 ppm) with a coupling constant of 139.3 Hz.




Scheme II

Finally, the reaction of natural vitamin $B_{12}$, cobalamin(I) with the labelled cofactor model **23b**, was carried out in the anticipation that the identification of the transfer product, namely, methylcobalamin would be facilitated by $^{13}$C-NMR spectrometry. The reaction was performed in a thoroughly degassed solution at pH 9.3. At the latter pH, cobalamin(I) is reasonably stable and as such it is expected to have an enhanced opportunity to react with **23b**, to give the transfer product methylcobalamin. Addition of salt **23b** to a solution of cob(I)alamin resulted in an immediate change of colour from black [Co(I) to red [Co(III)], indicating that even at pH 9.3, the cobalamin(I) system is not entirely stable. To analyse the reaction mixture, the solution was frozen after 90 min and concentrated by lyophilization overnight. The $^{13}$C-NMR spectrum of the reaction mixture was highly significant; it clearly revealed the presence of a large amount of the unreacted pteridinium salt **23b** (signals at 58.7 and 55.8 ppm), traces of demethylated pterin **24** (signal at 47.0 ppm) and, most importantly, methylcobalamin (signal at 10.6 ppm). The spectra thus established, albeit in low conversion, the transfer of a methyl substituent from N(5) of an activated pterin model system, to the cobalt atom of vitamin $B_{12}$.

***Experimental***

Melting points (m.p.) have been determined with a Leitz-melting point microscope and are uncorrected. Decomposition points of alkyl(pyridine)cobaloximes will not be mentioned since this temperature range generally varies with the conditions and is therefore no criterion of purity[7]. The experiments with cobaloxime (I) and cobalamine(I) are performed under strictly anaerobic conditions. NMR measurements have been performed on a Brucker AC-200 instrument. The chemical shifts are given in ppm downfield from tetramethylsilane (TMS). Coupling constants (J) are given in Hertz (Hz). Elemental analyses were performed by the micro-analytical laboratory of Dornu and Kolbe in Mülheim a.d. Ruhr. Absolute ether (distilled from sodium) was used as a solvent during the alkylation of the amines with the corresponding alkyl halides, in order to precipitate the quaternary ammonium salt. Trimethylanilinium iodide was purchased from Buchs S.G. The syntheses of the ammonium salts **7**, **12**, **13**, **14**, **15**, **16**, **17**, **18** and **23a,b** have been described previously[13].

Prof. Dr. R. Scheffold is gratefully acknowledged for his gift of methylcobalamin, which has been used as a reference for NMR analyses.

$^{13}$C-Methyl iodide (99% atom label) was purchased from Isotec Inc.

**Synthesis of alkyl(pyridine)cobaloximes 19, 20, 21 and 22.**

These experiments were performed under strictly anaerobic conditions. Pyridinecobaloxime(II) was synthesized according to Brown and coworkers[10]. Cobaloxime(I) was generated by the disproportionation method in strongly alkaline solution[10]. The alkyl halide, dissolved in a minimum amount of methanol, was added *via* a dropping funnel. The alkyl(pyridine)cobaloxime was isolated *via* the conventional method[10].

**Methyl(pyridine)cobaloxime 19.**

The experiment was performed at 16 mmol scale. Upon addition of pure methyl iodide (200 µl, 10 mmol), the colour of the reaction mixture immediately changed from black to red. Methyl(pyridine)cobaloxime **19** was isolated in 60% yield (228 mg). The spectrum was consistent with that reported in the literature[6,7].
$^1$H-NMR (200 MHz, $CDCl_3$): 0.81 (s, 3H, Co-$CH_3$), 2.12 (s, 12H, dmgH), 7,32 (t, 2H, J = 6.9, pyr-H), 7.72 (t, 1H, J = 7.6, pyr-H), 8.59 (d, 2H, J = 5.0, pyr-H).

**Benzyl(pyridine)cobaloxime 20.**

This experiment was performed at 1 mmol scale. Two equivalents of benzyl chloride (1 mmol, 115 µl), dissolved in 1 ml MeOH, were added to the cobaloxime(I) solution. Benzylcobaloxime **20** was isolated in 74% yield (170 mg). The $^1$H-NMR spectrum was identical with the one reported in the literature[6].
$^1$H-NMR (200 MHz, $CDCl_3$): 1.94 (s, 12H, dmgH), 2.84 (s, 2H, Co-$CH_2$-Ph), 6.98 (m, 5H, Ar-H), 7.30 (m, 2H, pyr-H), 7.68 (m, 1H, pyr-H), 8.54 (d, 2H, J = 5.0, pyr-H).

***p*-Nitrobenzyl(pyridine)cobaloxime 21.**

This experiment was performed at 1.25 mmol scale. *p*-Nitrobenzyl chloride (107 mg, 0.63 mmmol), dissolved in 1 ml MeOH, was allowed to react with cobaloxime(I). *p*-Nitrobenzylcobaloxime **21** was isolated in 32% yield (101 mg). The $^1$H-NMR spectral data were consistent with the literature[14]. Compound **21** was contaminated with *p*-nitrobenzyl chloride according to $^1$H-NMR.
$^1$H-NMR (200 MHz, $CDCl_3$): 2.0 (s, 12H, dmgH), 2.73 (s, 2H, Co-$CH_2$-Ph), 7.03 (d, 2H, J = 8.7, Ar-H), 7.31 (m, 2H, pyr-H), 7.66 (m, 1H, pyr-H), 7.86 (d, 2H, J = 8.7, Ar-H), 8.47 (m, 2H, pyr-H).

***p*-Methoxybenzyl(pyridine)cobaloxime 22.**

This experiment was performed at 1.25 mmol scale. One equivalent of *p*-methoxybenzyl chloride (98 mg, 0.63 mmol), dissolved in 1 ml MeOH, was allowed to react with cobaloxime(I). *p*-Methoxybenzyl-(pyridine)-cobaloxime **22** was isolated in 78% yield (239 mg).
$^1$H-NMR (200 MHz, $CDCl_3$): 1.95 (s, 12H, dmgH), 2.85 (s, 2H, Co-$CH_2$-Ph), 3.72 (s, 3H, O-$CH_3$), 6.58 (d, 2H, J = 8.4, Ar-H), 6.92 (d, 2H, J = 8.4, Ar-H), 7.31 (m, 2H, pyr-H), 7.64 (t, 1H, J = 7.6, pyr-H), 8.53 (d, 2H, J = 4.9, pyr-H).

***p*-Methoxybenzyltrimethylammonium chloride 8.**

A solution of trimethylamine (0.5 g, 8.48 mmol) in 2 ml $H_2O$ and 3 ml MeOH, was allowed to react at RT with *p*-methoxybenzyl chloride (1.33 g, 8.48 mmol), dissolved in 15 ml MeOH, for 48 hr. After evaporation of the solvents, toluene was added in order to remove $H_2O$ by evaporation. The resulting residue was dried, yielding 1.73 g (95%) of compound **8**.

$^1$H-NMR (200 MHz, $CD_3CN$): 3.11 (s, 9H, $N^+(CH_3)_3$), 3.95 (s, 3H, O-$CH_3$), 4.41 (s, 2H, $N^+CH_2$), 7.12 (d, 2H, J = 8.8, Ar-H), 7.4 (d, 2H, J = 8.8, Ar-H).

**Benzyl-*p*-methoxybenzyldimethylammonium chloride 9.**

A solution of *p*-methoxybenzyl chloride (2.3 g, 14.7 mmol) in 10 ml ether, was allowed to react overnight with N,N-dimethylbenzylamine (1.8 g, 13.3 mmol), dissolved in 10 ml ether, at RT. The resulting precipitate was filtered off and dried, yielding 3.22 g of **9** (83%).

$^1$H-NMR (200 MHz, $CDCl_3$): 3.07 (s, 6H, $N^+(CH_3)_2$), 3.78 (s, 3H, O-$CH_3$), 5.06 (s, 4H, $N^+(CH_2)_2$), 6.86 (d, 2H, J = 8.7, Ar-H), 7.38 (m, 3H, Ph-H), 7.57 (d, 2H, J = 8.7, Ar-H), 7.61 (m, 2H, Ph-H).

***p*-Nitrobenzyltrimethylammonium chloride 10.**

A solution of trimethylamine (0.5 g, 8.48 mmol) in 2 ml $H_2O$ and 3 ml MeOH, was allowed to react at RT with *p*-nitrobenzyl chloride (1.45 g, 8.48 mmol), dissolved in methanol (5 ml), for 48 hr. Compound **10** was isolated by lyophilization overnight. The resulting residue was washed with ether and dried, yielding 1.85 g of **10** (95%).

$^1$H-NMR (200 MHz, $D_2O$): 3.17 (s, 9H, $N^+(CH_3)_3$), 4.65 (s, 2H, $N^+CH_2$), 7.81 (d, 2H, J = 8.7, Ar-H), 8.37 (d, 2H, J = 8.7, Ar-H).

**Benzyl-*p*-nitrobenzyltrimethylammonium bromide 11.**

*p*-Nitrobenzyl bromide (1.6 g, 7.4 mmol), dissolved in 25 ml ether, was stirred for 48 h with a solution of N,N-dimethylbenzylamine (1.0 g, 7.4 mmol) in 5 ml ether. The resulting precipitate was isolated by filtration, washed with ether and dried, yielding 1.68 g of **11** (65%).

$^1$H-NMR (200 MHz, $CDCl_3$): 3.19 (s, 6H, $N^+(CH_3)_3$), 5.21(s, 2H, $N^+CH_2Ph$), 5.54( 2, 2H, $N^+CH_2Ar$), 7.37 (m, 3H, Ph-H), 7.63 (m, 2H, Ph-H), 8.04 (d, 2H, J = 8.7, Ar-H), 8.18 (d, 2H, Ar-H).

**General procedure for the reaction of cobaloxime(I) with quaternary ammonium salts.**

Pyridinecobaloxime(I) was prepared by disproportionation, according to the previously described method[10]. The experiment was performed starting from 1.25 mmol cobalt chloride. The quaternary ammonium salt (1 eq.), dissolved in a minimum amount of methanol (1 or 2 ml), was allowed to react with 1 eq. of cobaloxime(I) under strictly anaerobic conditions during several hours at RT. After reaction, MeOH was evaporated by a vigorous stream of nitrogen gas. The resulting residue was washed with ice-water in air, in order to precipitate the alkylcobaloxime. If no precipitate was formed upon washing with ice-water, the water layer was extracted with dichloromethane (2 x 10 ml). The combined organic layers were concentrated *in vacuo* and thoroughly dried. The isolated alkyl(pyridine)cobaloxime was identified by $^1$H-NMR (200 MHz, $CDCl_3$). The results and yields of these transfer experiments are listed in Table 1.

**Synthesis of $^{13}$C-methylcobaloxime.**

$^{13}$C-methylcobaloxime was synthesized according to the procedure described for the synthesis of unlabelled methylcobaloxime **19**, using $^{13}$C-methyl iodide.

$^1$H-NMR (200 MHz, $CDCl_3$): 0.81 (d, 3H, J = 139.3, Co-$^{13}CH_3$), 2.12 (s, 12H, dmgH), 7.32 (t, 2H, J = 6.9, pyr-H), 7.72 (t, 1H, J = 7.6, pyr-H), 8.59 (d, 2H, J = 5.0, pyr-H); $^{13}$C-NMR (50.3 MHz, $CDCl_3$): 9.8 (Co-$^{13}CH_3$).

**Reaction of 23a with cobaloxime(I) at 293 K.**

The solution of pyridinatocobaloxime (II) in 10 ml MeOH, was prepared by addition of: (a) 80 µl pyridine (1 mmol), (b) 232 mg dimethylglyoxime (2 mmol) and (c) 238 mg $CoCl_2.6H_2O$ (1 mmol). Cobaloxime(I) was generated by the *disproportionation method*[10]. Therefore, 160 mg NaOH (4 mmol), dissolved in 1 ml water, was added to the cobaloxime(II) solution. Upon addition of the base, an immediate change of colour, from brown to black, was observed. The black colour indicated the presence of cobaloxime(I).

A solution of **23a** (178 mg, 0.45 mmol) in 3 ml MeOH was added to the former solution. The reaction mixture was stirred at RT. After 20 hours, the solvent was evaporated by a stream of nitrogen gas. The residue was stirred with ice-water, blowing a stream of air over the solution in order to oxidize unreaction cobaloxime. After 30 min the water layer was extracted with dichloromethane (2x10 ml). The combined organic layers were concentrated *in vacuo*, yielding only 6 mg of an impure solid compound. The $^1$H-NMR spectrum (200 MHz, $CDCl_3$) revealed a small signal at 0.81 ppm, which could be assigned to the cobalt-bounded methyl substituent.

**Reaction of 23a with cobaloxime(I) at 313 K.**

The reaction was repeated at the elevated temperature of 313 K according to the described procedure. Concentration of the dichloromethane extract yielded 13 mg impure solid compound. The intensity of the signal at 0.81 ppm, which could be assigned to the cobalt-bounded methyl substituent, was slightly stronger than after the reaction performed at 293 K.

**Reaction of 23b with cobaloxime(I) at 313 K.**

The reaction is performed according to the procedure, described for the reaction with **23a** at 313 K. Concentration of the dichloromethane fraction yielded 10 mg of an impure solid compound. The $^1$H-NMR spectrum revealed the signals at 0.81 ppm (s, Co-$^{12}CH_3$) and 0.47 ppm. The latter signal belongs to a doublet, which could be assigned to $^{13}$C-labelled methylcobaloxime (J = 193.3 Hz). The signal at 1.15 coincided with other signals and could not clearly be observed.

**Reaction of 23b with cobalamin(I).**

This experiment was performed under exclusion of day light, in aluminium foil shielded glassware. All the solvents were thoroughly degassed before using. Cyanocobalamin (136 mg, 0.1 mmol) was dissolved in 5 ml $H_2O$. The pH was adjusted to 9.3 by addition of 0.1 M NaOH. Sodium borohydride (15 mg), dissolved in 1 ml $H_2O$ (pH = 9.3), was allowed to reduce cyanocobalamin to cobalamin (I), in the presence of a catalytic amount of $CoCl_2$. After 0.5 h, five drops of acetone were added in order to destroy the excess of sodium borohydride. The quaternary pteridinium salt **23b** (105 mg, 0.26 mmol) was dissolved in a mixture of ethanol/aqueous sodium hydroxide (4/1; pH = 9.3). Upon addition of the latter solution to the cobalamin(I) solution, the colour of the reaction mixture immediately changed to red (i.e. the colour of cobalamin(III). After stirring the reaction mixture for another 30 minutes, the solution was frozen in liquid nitrogen and dried by lyophilization overnight. The resulting residue was dissolved in $D_2O$ and a $^{13}$C-NMR spectrum was recorded.

$^{13}$C-NMR (62.89 MHz, $D_2O$): 58.7 and 55.8 ppm (both N(5)-methyl substituents in unreacted **23b**), 47.0 (*N*-Me in **24**), 10.6 (Me-Co).

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