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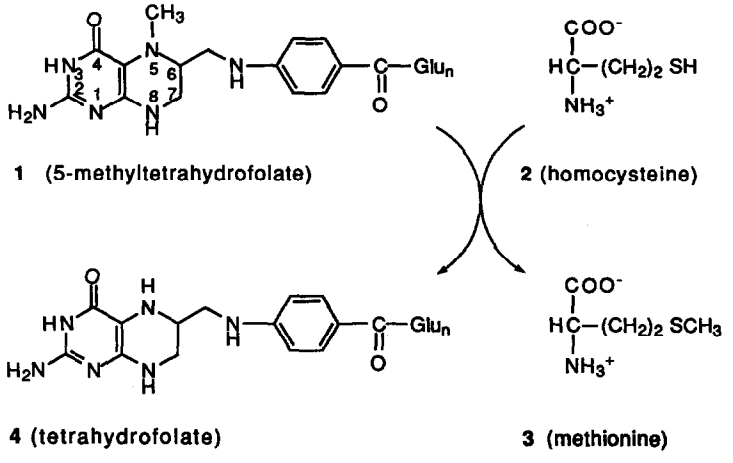
Dealkylation of Quaternary Ammonium Salts by Thiolate Anions: A Model of the Cobalamin-independent Methionine Synthase Reaction.

Ellen Hilhorst[1], Tjoe B.R.A. Chen, Atef S. Iskander and Upendra K. Pandit*

Organic Chemistry Laboratory, University of Amsterdam,
Nieuwe Achtergracht 129, 1018 WS Amsterdam, The Netherlands.

Abstract: The reactions of thiolate ions derived from thiophenol and homocysteine with substituted quaternary ammonium salts result in alkyl transfer from nitrogen to sulfur. A radical mechanism for this transalkylation, accounts for the reactivity pattern of the substrate salts. In a model study of the cobalamin-independent methionine synthase reaction, 5,5,6,7-tetramethyl-5,6,7,8-tetrahydropteridinium salt (**25**), which can be considered as a model for the natural coenzyme 5-CH_3H_4-folate (**1**), was allowed to react with the thiolate of homocysteine, whereupon the formation of methionine was observed in good yield. These results suggest that in the enzymatic process the N(5)-CH_3 bond may be activated for the methyl transfer step, by coordination of the N(5) with an electrophile or a proton at the active site.

The methionine synthase catalyzed reaction[2] involves the overall methyl transfer from the coenzyme 5-methyltetrahydrofolate (**1**) to the thiol group of the substrate homocysteine (**2**), generating methionine (**3**) and tetrahydrofolate (**4**) (Scheme 1).



Scheme 1

Two types of enzymes have been distinguished[2] for the catalysis of the conversion of **2** to **3**. These are: (i) the cobalamin-independent and (ii) the cobalamin-dependent methionine synthases. The cobalamin-

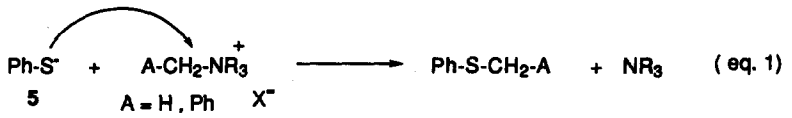
independent enzyme causes a direct transfer of the methyl group from nitrogen to sulfur, as shown in Scheme 1.

In case of the second enzyme, cobalamin functions as an intermediary methyl carrier; the initial reaction involving the methyl transfer from nitrogen to cobalt, generating methylcobalamine, and the latter subsequently donating its methyl substituent to the sulfur of homocysteine (2), to give the amino acid methionine (3). The present report deals with a model mechanistic study of the cobalamin-independent methionine synthase reaction. The results of the model studies on the cobalamin-dependent enzyme are described in a separate paper.

The cobalamin-independent methionine synthase reaction constitutes a nucleophilic displacement of a secondary amine, at the carbon of the methyl group, by the thiolate residue of homocysteine (2). Since the secondary amine anion, corresponding to the tetrahydropterin moiety of 4, is a poor leaving group, it would seem necessary that the N(5)-methyl bond is somehow activated[3], prior to the transfer of the methyl substituent to the thiolate ion. One mechanism of activation of the coenzyme is *via* a single or a two electron oxidation of the nitrogen at the 5-position. This could result in the formation of a radical or an iminium intermediate, respectively. It should be emphasized that the oxidative activation mechanism requires an additional redox active group in the holoenzyme system. Thus far, no redox (cofactor) system has been identified in association with the enzyme. However, the possibility of a redox active disulfide group, functioning as the electron acceptor, cannot be excluded. A second mechanism would involve the coordination of N(5) with an electrophilic species in the active site of the enzyme. Both modes of activation, namely oxidation and quaternization, have in common that they generate an electron-deficient center at N(5), causing it to become more susceptible to a nucleophilic displacement by the thiolate anion.

As a model of the methyl transfer process from N(5)-quaternized 5-CH_3H_4-folate 1 to homocysteine (2) we have examined the reaction of a number of substituted ammonium salts with the thiolate anions of thiophenol 5 and homocysteine (2) (Tables 1 and 2).

Examples of demethylation of trimethylanilinium salt (9)[4] and triethylmethyl ammonium chloride[5] and the debenzylation of several ammonium salts by thiophenolate ion, have been reported previously[5,6]. In case of a salt carrying both a methyl and a benzyl substituent, the latter is transferred exclusively[6]. It has been proposed[5,6] that both demethylation and debenzylation of the ammonium salts proceed *via* an S_N2 displacement reaction (eq. 1).



(eq. 1)

In the first phase of our systematic study, the ammonium salts 9-14 were employed as their tetrafluoro-borates and the reactions were performed with potassium thiophenolate in acetonitrile (343 K) in the presence of 18-crown-6 (1 eq.), 24 h. The yields of the transfer products were determined in the reaction mixtures by HPLC. The results are presented in Table 1.

The results of the reactions of salts 9 to 14 with potassium thiophenolate can, in the first instance, be rationalized on the basis of a nucleophilic displacement reaction. According to an S_N2 type mechanism, methyl transfer would be preferred on steric grounds. On the other hand, in a dissociative S_N1 process, the benzyl transfer would be favoured, due to electronic stabilization of the benzyl cation.

The results, presented in Table 1, suggest that electronic rather than steric effects play a dominant role during the reaction. Support for this line of reasoning is provided by the transfer of the benzhydryl substituent from salt 15. Despite the fact that this transfer is disfavoured due to steric hindrance of the bulky phenyl substituents, the formation of the benzhydryl transfer product proceeds in an impressively good yield (53%). These results are clearly not consistent with the S_N2 mechanism, proposed in the literature[5,6].

In order to investigate the possible operation of an S_N1 type process, the (substituted) benzyl group transfer from DABCO salts 17 and 18 to thiophenolate ion was studied. Monitoring of the reactions by

HPLC revealed that there was a very significant difference in the rates of formation of the product sulfides (**19** or **20**) from the two substrate ammonium salts. The *p*-nitrobenzyl group was observed to transfer much more rapidly at 343 K and even at 298 K, compared to the analogous transfer of the *p*-methoxybenzyl group at 298 K (Fig. 1). These results are contrary to the reactivity pattern expected on the basis of an S_N1 type mechanism, which would have favoured the dissociation of the *p*-methoxybenzyl cation in case of salt **18**.

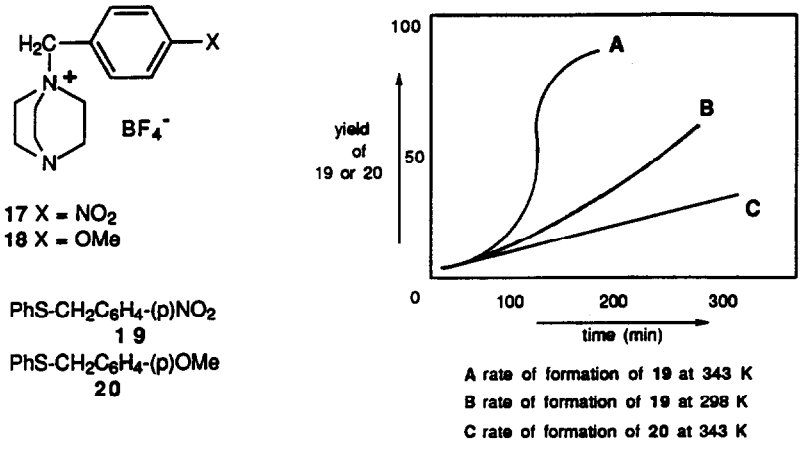
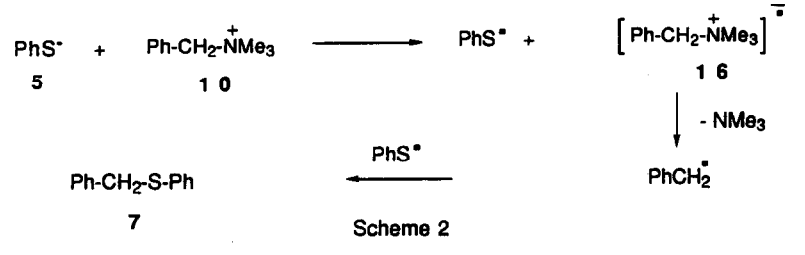


Fig 1

The results presented in Table 1, taken together with the observed difference between the rates of debenzylation of **17** and **18** can be best accommodated in a radical mechanism (Scheme 2) for the group transfer from the ammonium nitrogen to the sulfur of the thiolate ion. Such a mechanism involves an electron transfer from the thiolate ion to the ammonium salt, to give a labile radical intermediate **16** and a thiol radical. Intermediate **16** would dissociate to generate a tertiary amine and the more stable radical which would subsequently quench the thiol radical to yield the mixed sulfide product. In Scheme 2, the mechanism is illustrated for the reaction between salt **10** and phenyl thiolate **5**. It is obvious from this mechanism why a benzyl group is transferred in preference to a methyl substituent and that the salt **15** gives the benzhydryl phenyl sulfide in good yield.



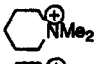
Scheme 2

The mechanism described in Scheme 2 is also consistent with the fact that salt **17** transfers its substituted benzyl group so much more effectively than salt **18**. The electron-withdrawing *p*-nitro group in **17** is expected to favour both electron acceptance from the thiolate ion[7] and decomposition of the resulting radical complex intermediate, into DABCO and the stabilized *p*-nitrobenzyl radical.

Having established that alkyl transfer from ammonium salts to thiophenolate is a general reaction, we turned our attention to the simulation of alkyl transfer, and especially methyl transfer, to the thiolate ion of homocysteine (**2**), the natural substrate of the methionine synthase reaction. In Table 2 the results of the reaction of homocysteine anion with ammonium salts **9**, **12**, **14**, **22** and **23** are presented.

Table 1

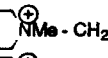
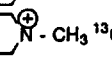
Dealkylation of ammonium tetrafluoroborates by reaction with $K^+ {}^-SPh$ [18-crown-6 (1 eq.), CH_3CN, 343K,24 h].

Salt	Transfer Product (yield)		
	PhS - Me(**6**)	PhS -CH_2Ph (**7**)	PhS -$CHPh_2$ (**8**)
9 $Ph\text{-}\overset{\oplus}{N}Me_3$	100 %		
10 $Ph\text{-}CH_2\text{-}\overset{\oplus}{N}Me_3$	3 %	97 %	
11 	23 %		
12 	4 %	87 %	
13 *[DABCO] - Me	4 %		
14 *[DABCO] - CH_2Ph		40 %	
15 *[DABCO] - $CHPh_2$			53 %

* [DABCO] = 1,4 diazabicyclo{2,2,2} octane

Table 2

Transfer of alkyl groups from ammonium salts to homocysteine [NaOH (2 eq.), EtOH, 343K ,24 h].

Salt	Transfer Product (yield)	
	Methionine (**3**)	S - Benzylhomocysteine (**21**)
9 $Ph\text{-}\overset{\oplus}{N}Me_3$	100 %	
22 $Ph\text{-}\overset{\oplus}{N}Me_2CH_2Ph$	not detected	67 %
14 *[DABCO] - CH_2Ph		20 %
12 	not detected	10 %
23 	trace**	

** HPLC / NMR

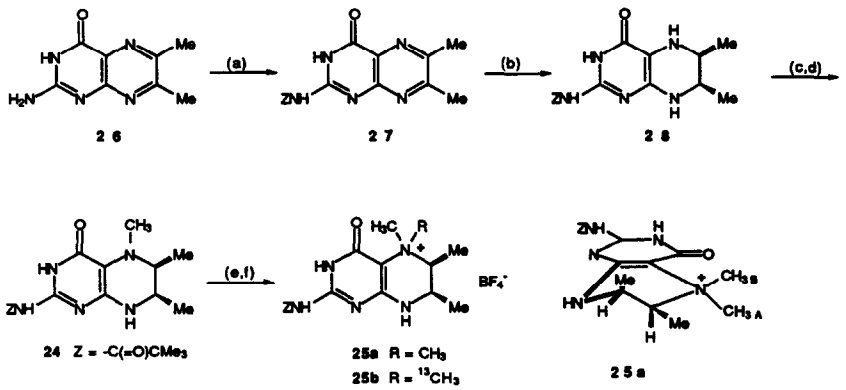
The transalkylation experiments involving homocysteine were performed in aqueous ethanol in the presence of two equivalents of sodium hydroxide. The use of ethanol as solvent was dictated by considerations of solubility of homocysteine, the acid being better soluble in ethanol than in acetonitrile.

The yields of the "transfer products" are based on NMR spectra of the reaction mixtures (*vide* experimental). It is especially noteworthy that no methionine could be detected in the reactions of salts **12**

and **22**, where a competition exists between methyl and benzyl transfer. Instead, the NMR spectra of the reaction mixtures, from these substrates, showed the formation of S-benzylhomocysteine (**21**) as the sole transfer product.

In case of the mono- $^{13}CH_3$-labelled salt **23**, the formation of unlabelled methionine (**3**) was poorly detectible *via* the -S-$\underline{CH_3}$ signal at 2.10 ppm. The transfer of $^{13}CH_3$ was, however, confirmed by the presence of a signal at 16.0 ppm in the ^{13}C-NMR spectrum of the reaction mixture. In general, the yields of the mixed thio ethers (alkyl transfer products) are lower when homocysteine, instead of thiophenol, is employed as an acceptor.

In designing a reaction which would faithfully model the process catalyzed by the cobalt-independent methionine synthase enzyme, the pteridine derivative **24** (Scheme 3) was chosen as a functional analogue of the cofactor 5-methyltetrahydrofolate (**1**). Furthermore, the feature representing the coordination of N(5) with an electrophile was mimicked by conversion of **24** into its methyl salts **25a,b**. The synthesis of these compounds is described in Scheme 3. It may be noted that the use of the 2-amino pivaloyl derivatives in this study was necessitated by the improved solubility of these compounds in organic solvents[8].



(a) pivalic anhyride, DMAP, 458 K; (b) H_2/PtO_2/HAc; (c) CH_3I/CH_2Cl_2; (d) Et_3N/CH_2Cl_2; (e) CH_3I or $^{13}CH_3I$; (f) $AgBF_4$

Scheme 3

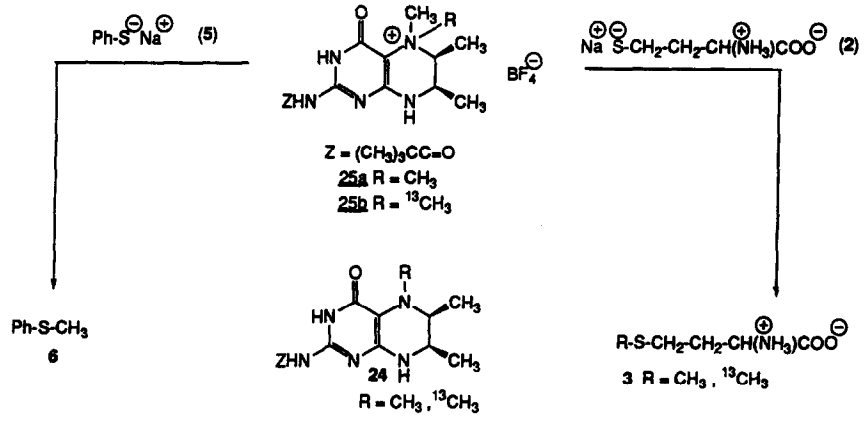
The conversions **26** → **27** → **28** → **24** were straightforward (*vide* experimental). Methylation of **24** with methyl iodide provided a salt (**25a**) in which the two methyl signals displayed different chemical shifts in the ^{1}H-NMR spectrum. The reason for this becomes apparent if one inspects the half-chair conformation[9] of **25a**. An analysis of the NOE-NMR experiments allowed the assignments of N^+Me_A and N^+Me_B in **25a**, at 3.55 ppm and 3.74 ppm, respectively. Irradiation of the methyl group at 3.55 ppm resulted in enhancement of the C(6)-H signal at 4.28 ppm, where as irradiation of the methyl signal at 3.74 ppm caused an enhancement of the C(6)-methyl at 1.36 ppm. Treatment of **24** with $^{13}CH_3I$ gave, as anticipated, a mixture of two isotopomers[10], corresponding to the structure represented by **25b**. The $^{13}CH_3$ groups in the isotopomers resonated at 57.7 ppm and 55.0 ppm. The reactions of **25a** and **25b** with thiolate ions of thiophenol (**5**) and homocysteine (**2**) are shown in Scheme 4.

Reaction of **25a** (1 equiv.) with freshly prepared potassium salt of **5** (2.2 equiv.), [18-crown-6 (1 equiv.), acetonitrile, 343 K, 24 h] resulted in a reaction mixture from which PhSMe **6** could be isolated and identified (^{1}H-NMR, 200 MHz, $CDCl_3$, δ 2.49, s, Ph-S-$\underline{CH_3}$). The residue showed the presence of the demethylated pterin **24**, which was attested by signals at δ 2.55 (s, N-$\underline{CH_3}$) and 0.77 [d, J 6.7 Hz, C(6)-Me or C(7)-Me)].

HPLC analysis of the reaction mixture showed the formation of the thioether **6** in 57% yield. It should be pointed out that **6** is a volatile product, so that its actual yield in the reaction is presumably higher than the observed value. These results reflect a very substantial amount of methyl transfer from the salt to the thiolate.

In a subsequent experiment, the transfer of the N(5)-methyl substituent of **25a** to the natural substrate homocysteine (**2**) was examined. Homocysteine (2 equiv.) was allowed to react with the pterin salt **25a** (1 equiv.) for 24 hours in aqueous ethanol, in the presence of sodium hydroxide (4 eq.) at 343 K. The reaction resulted in a mixture whose ^{1}H-NMR spectrum (200 MHz, 0.1 mol dm^{-3} NaOD/D_2O) showed clearly recognizable signals for (a) methionine (**3**) (δ 2.10, s, S-C$\underline{H}_3$), (b) pterin derivative **24** [0.75, d, J 6.7 Hz, C(6)-Me or C(7)-Me)] and (c) the disulfide corresponding to homocysteine. From the integration of these signals, a methyl transfer of 40% from the salt to the homocysteine anion could be estimated. It is noteworthy that the intensities of the signals for **24** and **3** gave a ratio of 1:1 for these two reaction products.

Further evidence in support of the methyl transfer was obtained by repeating the reaction of homocysteine (**2**) anion with the ^{13}C-labelled pterin salt **25b**. As expected, the ^{13}C-NMR spectrum revealed the presence of four labelled compounds in the reaction mixture. The signals at 57.7 and 55.0 ppm could be assigned to the two isotopomers of the starting material **25b**; the signal at 45.0 ppm originated from the labelled demethylated product **24** and finally methionine (**3**) could be recognized by the signal at 16.0 ppm (-S-$^{13}\underline{CH}_3$).



Scheme 4

The reaction of pterin salts **25a,b** with the thiolate anion of homocysteine constitutes a model for the cobalamin-independent methionine synthase reaction. This model suggests that the N-methyl bond in the natural coenzyme N(5)-CH_3-tetrahydrofolate (**1**) is activated by converting the N(5) into a charged electron deficient nitrogen. While in the model system such activation has been achieved by quaternization of the relevant nitrogen, in the enzymic process this could be attained either by N(5)-protonation or *via* its coordination with an electrophilic group in the active site pocket of the enzyme. A somewhat related suggestion, namely, that N(1)-coordination of the folate coenzyme with an electrophile should assist the release of the N(5)-methyl group, has been made by Armarego and Schou[11]. These investigators have shown that 1,3,5,6-tetramethyl-5,6,7,8-tetrahydropterinium chloride can be readily demethylated at the N(5)-position by treatment with ammonia or aqueous sodium hydroxide.

Experimental

Melting points (m.p.) have been determined with a Leitz-melting point microscope and are uncorrected. NMR measurements have been performed on Brucker WM-250 or AC-200 instruments. The chemical shifts are given in ppm downfield from tetramethylsilane (TMS). Coupling constants (J) are given in Hertz (Hz). Mass spectra were obtained on a Varian-Matt 711 mass spectrometer. Mass peaks are given in m/z. Flash chromatographic separations have been carried out according to the method of Still[12], using Janssen Chimica silica gel (0.0035-0.07 mm, pore diameter ca 6 nm). Elemental analysis were performed by the micro-analytical laboratory of Dornu and Kolbe in Mülheim a.d. Ruhr.

Analytical HPLC was performed using a Perkin Elmer Series 100 pump on a reversed-phase column (polygosil 60 C18; particle size 10 μm; 250 x 4 mm) and a holochrome variable detector.

In order to determine the concentration of transfer products in the reaction mixture (methyl phenyl sulphide **6**, benzyl phenyl sulphide **7** or benzhydryl phenyl sulphide **8**), standard curves were prepared by HPLC from stock solutions, using authentic samples of **6**, **7** and **8**, under the following conditions: detection wavelength 254 nm; flow 2.0 ml/min; the eluents varied from 90/10, 80/20 to 75/25 MeOH/H_2O.

Methyl phenyl sulphide **6** was purchased from Merck. ^{13}C-Methyl iodide (99% atom label) was obtained from Isotec Inc. Trimethylanilinium iodide was purchased from Buch SG.

Acetonitrile was obtained absolute by distilling from CaH_2, diethyl ether was distilled from sodium and both were stored over 4 Å mol-sieves.

Absolute ether was used as a solvent during the alkylation of the amines with the coresponding alkyl halides, in order to precipitate the quaternary ammonium salt.

Homocysteine (**2**) and methionine (**3**) were obtained from Fluka Chemical. HPLC-chromatography was used for the detection of methionine (**3**) and S-benzyl-homocysteine (**21**) (60 MeOH/40 H_2O/HCl pH = 2; flow 2.0 ml/min; λ = 220 nm). 6,7-dimethylpterin **26** was synthesized according to the method of Mager[13].

Benzyl phenyl sulfide (7).

A solution of potassium thiophenolate (600 mg, 4.0 mmol) in 40 ml of acetonitrile was allowed to react overnight with 480 μl benzyl bromide (4.0 mmol) at RT. After the solvent was evaporated *in vacuo*, 50 ml ether was added. The subsequently formed precipitate was removed by filtration. The filtrate was concentrated and the residue was purified by flash chromatography (PE/EA: 7/1), yield: 497 mg (61%) of compound **7**. ^{1}H-NMR (200 MHz, $CDCl_3$): 4.14 (s, 2H, Ph-S-CH_2-Ph), 7.19-7.36 (m, > 10H, Ar-H).

Bis[phenyl]methyl phenyl sulfide (8).

Bromodiphenylmethane (696 mg, 2.8 mmol) was allowed to react at RT for 6 h with 417 mg potassium thiophenolate (2.8 mmol), dissolved in 40 ml ether, by addition of an equimolar amount of 18-crown-6. After filtration of the reaction mixture, the ether was removed by evaporation *in vacuo*. Compound **8** was purified by flash chromatography (100% PE) of the residue. Yield: 25% (192 mg). ^{1}H-NMR (200 MHz, $CDCl_3$): 5.61 (s, 1H, Ph_2C<u>H</u>-S-Ph), 7.15-7.51 (s, 15H, Ar-H); MS (EI): 276 (M^+, 5), 200 (24), 167 [M($CHPh_2$), 100], 165 (20), 91 (50).

Benzyltrimethylammonium chloride (10).

To a solution of 500 mg of trimethylamine (8.48 mmol) in 2 ml H_2O, was added a solution of 1.07 g benzylchloride (8.48 mmol) in 10 ml of ether and the mixture was allowed to react at RT overnight. The resulting precipitate was filtered off and the solid residue was dissolved in 10 ml of toluene. The solvent was evaporated *in vacuo* and dried to give salt **10** in 94% yield (1.48 g). ^{1}H-NMR (200 MHz, CD_3CN): 3.07 (s, 9H, 3x CH_3), 4.62 (s, 2H, CH_2), 7.45 (m, 5H, Ar-H).

N,N-Dimethylpiperidinium iodide (11).

A solution of 1 g of N-methylpiperidine (10 mmol) in 10 ml of ether was allowed to react with 2 ml of methyl iodide at RT overnight. The salt **11** was isolated by filtration. Yield: 2.19 g (90%). m.p. > 290°C; ^{1}H-NMR (200 MHz, D_2O): 1.60-1.70 (m, 2H, C(4)H_2), 1.80-2.0 [br, 4H, C(3)H_2 and C(4)H_2], 3.10 [s, 6H, N(CH_3)$_2$], 3.34 [t, 4H, J = 6.0, C(2)H_2 and C(6)H_2].

N,N-Benzylmethylpiperidinium chloride (12).
To a solution of 3 ml of N-methylpiperidine (24.7 mmol) in 4 ml ether, benzyl chloride (4 ml, 34.8 mmol) was added and the reaction mixture was stirred overnight at RT. After addition of 50 ml of ether, the salt **12** was isolated by filtration. The white powder (0.74 g) was thoroughly washed with ether and dried *in vacuo*.. Yield: 13%. m.p.: 225°C (dec.); ^{1}H-NMR (200 MHz, D_2O): 1.55-1.82 [m, 2H, C(4)H_2], 1.92 [br, 4H, C(3)H_2 and C(5)H_2], 2.95 (s, 3H, N-CH_3), 3.25-3.45 [m, 4H, C(2)H_2 and C(6)H_2], 4.50 (s, 2H, Ar-CH_2), 7.54 (s, 5H, Ar-H).

4-Aza-1-methylazoniabicyclo[2.2.2]octane iodide (13).
A solution of 11.2 g of 1,4-diazabicyclo[2.2.2]octane (DABCO, 10 mmol) was allowed to react with 7.5 ml MeI (120 mmol) at RT overnight. The salt **13** was isolated by filtration and thoroughly washed with ether to remove the excess of methyl iodide. Yield: 93% (23.8 g). m.p.: 280-282°C (lit.[14] 285°C); ^{1}H-NMR (200 MHz, CD_3CN): 3.08 (s, 3H, N^+-CH_3); 3.21 (t, 6H, J = 7.8, N-CH_2), 3.43 (t, 6H, J = 7.8, N^+-CH_2). Anal. found: N 10.28; calc. for $C_7H_{15}N_2I.H_2O$: N 10.29.

4-Aza-1-benzylazoniabicyclo[2.2.2]octane chloride (14).
To a solution of 1.0 g of DABCO (8.9 mmol) in 15 ml ether, benzyl chloride (2 ml, 17.4 mmol) was added and the reaction mixture was stirred at RT overnight. The salt **14** was isolated by filtration and thoroughly washed with ether to remove the excess of benzyl chloride. Yield: 84% (1.8 g). m.p.: 240°C (dec.); ^{1}H-NMR (200 MHz, $CDCl_3$): 3.14 (t, 6H, J = 7.2, N-CH_2), 3.74 (t, 6H, J = 7.2, N^+-CH_2), 5.09 (s, 2H, Ar-CH_2), 7.35-7.41 (m, 3H, Ar-H), 7.61-7.65 (m, 2H, Ar-H). Anal. found: C 61.42; H 8.42; N 10.67; calc. for $C_{13}H_{19}N_2ClH_2O$: C 60.81; H 8.24; N 10.91.

4-Aza-1-bis(phenyl)methylazoniabicyclo[2.2.2]octane bromide (15).
DABCO (1.0 g, 8.9 mmol), dissolved in 15 ml ether, was allowed to react overnight with 2.22 g bromodiphenylmethane (8.9 mmol) at RT. The precipitate (**15**) was isolated by filtration and dried *in vacuo*. Yield: 94% (3.0 g). m.p.: 204-206°C; ^{1}H-NMR (200 MHz, $CDCl_3$): 3.14 (t, 6H, J = 7.3, N-CH_2), 3.80 (t, 6H, J = 7.3, N^+-CH_2, 7.07 [s, 1H, N^+-CH(Ph)$_2$], 7.41-7.48 (m, 6H, Ar-H), 7.98 (m, 4H, Ar-H).

4-Aza-1-*p*-nitrobenzylazoniabicyclo[2.2.2]octane bromide (17).
DABCO (0.5 g, 4.4 mmol), dissolved in 20 ml ether, was added to a solution of 950 mg *p*-nitrobenzyl bromide (4.4 mmol) in 20 ml ether. Immediately after addition a precipitate (**17**) was formed. After 20 h the salt was isolated by filtration, thoroughly washed with ether and dried *in vacuo*. Yield: 100% (1.45 g). m.p.: 225-227°C (dec.); ^{1}H-NMR (200MHz, CD_3CN): 3.03 (t, 6H, J = 7.5, N-CH_2), 3.43 (t, 6H, J = 7.5, N^+-CH_2), 4.81 (s, 2H, N^+-CH_2-Ar), 7.82 (d, 2H, J = 8.8, Ar-H), 8.26 (d, 2H, J = 8.8, Ar-H). Anal. found: N 11.77; calc. for $C_{13}H_{18}N_3O_2Br.H_2O$: N 12.12.

4-Aza-1-*p*-methoxybenzylazoniabicyclo[2.2.2]octane chloride (18).
DABCO (1.0 g, 8.9 mmol), dissolved in 15 ml ether, was allowed to react with 1.39 g *p*-methoxybenzyl chloride (8.9 mmol) at RT during 3 h. Compound **18** was isolated by filtration, thoroughly washed with ether and dried *in vacuo*. Yield: 73% (1.74 g). m.p.: 215-216°C; ^{1}H-NMR (200 MHz, $CDCl_3$): 3.13 (t, 6H, J = 7.0, N-CH_2), 3.67 (t, 6H, J = 7.1, N^+-CH_2), 3.77 (s, 3H, O-CH_3), 4.97 (s, 2H, N^+-CH_2-Ar), 6.86 (d, 2H, J = 8.5, Ar-H), 7.53 (d, 2H, J = 8.5, Ar-H). Anal. found: C 62.21; H 8.38; N 10.36; Cl 13.12; calc. for $C_{14}H_{21}N_2OCl$: C 62,58; H 7.94; N 10.36; Cl 13.10.

***p*-Nitrobenzyl phenyl sulfide (19).**
Potassium thiophenolate (2.6 mmol, 385 mg), dissolved in 40 ml ether by addition of an equimolar amount of 18-crown-6 (680 mg), was allowed to react overnight with 560 mg *p*-nitrobenzyl bromide (2.6 mmol) at RT. Ether (40 ml) was added and the precipitate was removed by filtration. The filtrate was concentrated *in vacuo*, after which the residue was purified by flash chromatography to yield 274 mg (43%) of **19**. ^{1}H-NMR (200 MHz, $CDCl_3$): 4.12 (s, 2H, Ar-CH_2-S), 7.24 (m, > 5H, Ar-H), 7.36 (d, 2H, J = 8.7, Ar-H), 8.09 (d, 2H, J = 8.7, Ar-H); MS (FABMS): 245 (M^+), 136.

p-Methoxybenzyl phenyl sulfide (20).
Potassium thiophenolate (429 mg, 2.9 mmol), dissolved in 40 ml ether by addition of an equimolar amount of 18-crown-6 (765 mg), was allowed to react overnight with 390 µl *p*-methoxybenzyl chloride (2.9 mmol) at RT. After addition of extra 40 ml ether, the precipitate was removed by filtration. The filtrate was concentrated *in vacuo*, followed by flash chromatography of the residue (PE/EA: 6/1, yielding 280 mg (42%) of **20**. ^{1}H-NMR (200 MHz, $CDCl_3$): 3.77 (s, 3H, O-CH_3, 4.06 (s, 2H, Ar-CH_2-S), 6.80 (d, 2H, J = 8.6, Ar-H), 7.18-7.36 (m, > 7H, Ar-H); MS (FABMS): 121 ($MeOC_6H_4CH_2^+$).

Transalkylation reaction between the quaternary ammonium salts and potassium thiophenolate.
The reactions of potassium thiophenolate with the quaternary ammonium salts (**9** to **15**) were performed according to the following general procedure: the freshly prepared potassium thiophenolate (2 to 3.5 mmol; 2.2 equivalents) was dissolved in 10 to 20 ml CH_3CN by addition of an equimolar amount of 18-crown-6. The counter ion (I^-, Br^- or Cl^-) of the original quaternary ammonium salt was exchanged for BF_4^- by dissolving this salt in CH_3CN, followed by addition of an equimolar amount of $AgBF_4$, upon which the silver halide immediately precipitated. After 30 minutes stirring, the precipitate was filtered off over highflow. The solvent of the filtrate was evaporated *in vacuo* in order to determine the amount of the BF_4^- salt. The latter was added as a solution in 10 to 20 ml of CH_3CN to the potassium thiophenolate solution. The reaction mixture was stirred at 343 K under nitrogen atmosphere. After 24 h the yield of transfer product, present in the reaction mixture, was determined by HPLC. Stock solutions of methyl phenyl sulfide **6**, benzyl phenyl sulfide **7** and benzhydryl phenyl sulfide **8** were prepared in order to determine the amount of transfer products present in the reaction mixture by standard curves. The sulfide (**6**, **7** or **8**) was isolated from the reaction mixture in order to identify it by ^{1}H-NMR (200 MHz, $CDCl_3$). For this, the solvent was evaporated *in vacuo*, followed by extraction of the residue with PE. The extract was concentrated again and the transfer product was isolated from the residue by flash chromatogaphy (100% PE).

Reactions of 17 or 18 with potassium thiophenolate at 343K/298K.
These experiments were performed according to the general procedure described for the reaction with the quaternary ammonium salts **9** to **15**. The reactions were monitored by HPLC chromatography (80/20 MeOH/H_2O; 2.0 ml/min.) by following the concentration of substituted the benzyl transfer product (**19** or **20**). N,N-dimethylaniline was used as an internal standard in the HPLC sample. After 24 h the reaction mixture was concentrated *in vacuo*. The residue was extracted with PE. After evaporation of the solvent *in vacuo*, the ^{1}H-NMR spectrum of the mixture was recorded. This allowed identification of the transfer products (**19** or **20**). Since the reaction of potassium thiophenolate with **17** was a very rapid reaction at 343 K, the latter was repeated at 298 K.

S-Benzylhomocysteine (21).
Homocysteine (200 mg, 1.5 mmol), dissolved in 10 ml acetonitrile by addition of 0.5 ml DBU (3.3 mmol), was allowed to react with 230 µl benzyl chloride (2.0 mmol). After 20 h the white precipitate (**21**), present in the reaction mixture, was isolated by filtration, washed with ether and dried. Yield: 262 mg (78%). m.p. 192-195°C (lit.[6] 190-191°C); ^{1}H-NMR (200 MHz, 0.1 M NaOD/D_2O): 1.66-1.92 (m, 2H, βCH_2), 2.47 (t, 2H, J = 7.9, γCH_2), 3.25 (dd, 1H, J = 7.4 and J = 5.5, αCH), 3.75 (s, 2H, S-CH_2-Ph), 7.26-7.38 (m, 5H, Ar-H).

^{13}C-labelled methionine .
Homocysteine (50 mg, 0.37 mmol), dissolved in 10 ml acetonitrile by addition of 200 µl DBU, was allowed to react with 80 µl ^{13}C-labelled methyl iodide. After addition of the latter, the solution immediately became turbid. After 23 h the precipitate was isolated by filtration and dried. Yield: 73% (40 mg). According to the NMR spectrum methionine was contaminated with homocysteine. ^{1}H-NMR (200 MHz, 0.1 M NaOD/D_2O). The signals of methionine, characteristically at 2.09 ppm, (S-CH_3), attested to the compound.^{13}C-NMR (0.1 M NaOD/D_2O, 62.89 MHz): 16.9 (S-$^{13}CH_3$). This in accordance with the reported value[15] of 15.0 ppm.

Benzyldimethylanilinium chloride (22).
N,N-dimethylaniline (4 ml, 31.6 mmol) was stirred with 54 ml benzyl chloride (34.8 mmol) at RT. After 3 days 50 ml ether was added and the salt **22** was isolated by filtration, thoroughly washed with ether and dried. Yield: 1.16 g of **22** (15%). m.p.: 168-171°C; ^{1}H-NMR (200 MHz, CD_3CN): 3.64 (s, 6H, N^+-CH_3), 5.21 (s, 2H, N^+-CH_2-Ph), 7.08-7.37 (m, 5H, PhH), 7.50-7.53 (m, 3H, BzH), 7.76-7.81 (m, 2H, BzH).

N,N-$^{13}CH_3$,$^{12}CH_3$-Piperidinium iodide (23).
The addition of 2 ml ^{13}C-methyl iodide to 1 ml N-methylpiperidine caused a vigorous reaction, immediately resulting in the quantitative conversion of the latter compound to **23**. The white powder (1.99 g) was thoroughly washed with ether and dried. Yield: 100%. ^{1}H-NMR (200 MHz, D_2O): 1.62-1.71 [m, 2H, C(4)H_2], 1.88 [br, 4H, C(3)H_2 and C(5)H_2], 3.11 (d, 3H, J = 143.6, N^+-$^{13}CH_3$), 3.11 (d, 3H, J = 3.7, N^+-$^{12}CH_3$), 3.33-3.38 [m, 4H, C(2)H_2 and C(6)H_2]; ^{13}C-NMR (50.32 MHz, APT, $CDCl_3$): 51.1 (N^+-$^{13}CH_3$); ^{13}C-NMR (50.32 MHz, APT, D_2O): 53.6, 53.5 and 53.4 (N^+-$^{13}CH_3$).

Reaction of homocysteine (2) with the quaternary ammonium salts 9, 12, 14, 22 and 23 in alkaline ethanol.
General procedure: The counter ion of the original ammonium salt (I^- or Cl^-) was exchanged for the non-nucleophilic tetrafluoroborate anion, before reacting the salt with homocysteine. For this, 0.5 mmol of the salt was dissolved in 15 ml EtOH, followed by the addition of an equimolar amount of silver tetrafluoroborate. The precipitate of silver halide was removed by filtration over high-flow. The filtrate was concentrated *in vacuo* in order to determine the yield of the tetrafluoroborate salt. The latter compound was dissolved in 15 ml of EtOH *prior* to addition to homocysteine. The solution of homocysteine was prepared by suspending 135 mg of homocysteine (1 mmol) in 10 ml EtOH. The thiolate anion was generated upon addition of 333 µl 6M NaOH (2 mmol). Water (3 ml) was added to homogenize the reaction mixture. The reaction mixture, consisting of homocysteine and the ammonium salt, was stirred at 343 K under nitrogen atmosphere. After 24 h the solvent was removed by evaporation *in vacuo*, followed by extraction of the residue by dichloromethane (15 ml). The precipitate was isolated by centrifugation (g = 2000). Identification of the homocysteine deriatives was accomplished by ^{1}H-NMR and by HPLC (60/40 MeOH/H_2O/HCl pH = 2, flow 1.5 ml/min.; λ = 220 nm). The yield of the benzyl- or methyl transfer was calculated from the NMR spectrum.

2-Pivaloyl-6,7-dimethylpterin (27).
The pivaloylation of **26** was performed according to a procedure described in the literature[8]. Compound **26** (0.5 g, 2.62 mmol) was stirred in 10 ml pivalic anhydride in the presence of a catalytic amount of DMAP, at 458 K. After 5 hours the pivalic anhydride was evaporated *in vacuo*. The residue was dissolved in 3 ml dichloromethane and passed through a pad of silica gel, by eluting with 2% MeOH/CH_2Cl_2. The dichloromethane was evaporated *in vacuo*, yielding 475 mg of **28** (100%). ^{1}H-NMR (200 MHz, $CDCl_3$): 1.10 (d, 3H, J = 6.4, CH_3), 1.14 (d, 3H, J = 6.4, CH_3), 1.28 [s, 9H, $(CH_3)_3C$], 3.33 [dq, 1H, J = 6.4 and J = 2.8, C(7)H], 3.56 [dq, 1H, J = 2.8 and J = 6.4, C(6)-H], 4.56 (br, 1H, NH), 8.34 (br, 1H, NH); MS (EI): 279 (M^+, 100), 264 (33), 180 (27), 57 (13).

5,6,7-Trimethyl-2-pivaloyl-tetrahydropterin 24.
Compound **28** (630 mg, 2.25 mmol) was suspended in 4 ml methyl iodide. After addition of 4 ml dichloromethane, the homogeneous reaction mixture was stirred at RT for three days. Subsequently, the solvents were evaporated *in vacuo*. Yield: 935 mg of **24** HI salt (99%).^{1}H-NMR (200 MHz, D_2O): 1.17 [d, 3H, J = 6.6, C(7)-CH_3], 1.26 [s, 9H, $(CH_3)_3C$], 1.30 [d, 3H, J = 6.5, C(6)-CH_3], 3.12 (s, 3H, N^+-CH_3), 3.73 [m, 1H, C(7)-H], 4.03 [m, 1H, C(6)-H].
The HI salt of **24**, (935 mg, 2.22 mmol), suspended in 10 ml dichloromethane, was deprotonated by addition of 340 µl triethylamine (2.44 mmol, 1.1 eq.). The dichloromethane solution was extracted with sat. NaCl (2 x 10 ml), followed by the concentration of the organic layer *in vacuo*, whereupon 475 mg (73%) of **24** was obtained. ^{1}H-NMR (200 MHz, $CDCl_3$): 0.81 (d, 3H, J = 6.7, C-CH_3), 1.19 (d, 3H, J = 6.7, C-CH_3), 1.28 [s, 9H, $(CH_3)_3C$], 2. 68 (s, 3H, N-CH_3), 2.78 [br, 1H, C(7)-H], 3.46 [m, 1H, C(6)-H], 4.62 (br, 1H, NH), 8.00 (br, 1H, NH); MS (EI); 293 (M^+, 100), 279 (14), 178 (35). Anal. found: C 56.26; H 8.17; N 22.83; calc. for

$C_{14}H_{23}O_2N_5.1/2\ H_2O$: C 55.61; H 8.00; N 23.16.

N,N-Dimethyl-6,7-dimethyl-2-pivaloyl-tetrahydropteridinium iodide (25a).
Compound **24** (500 mg, 1.7 mmol) was stirred in 10 ml methyl iodide at RT overnight. After evaporation of the methyl iodide *in vacuo*, the resulting powder was thoroughly dried. Yield: 710 mg of **25a** (96%). m.p.: 127-130°C (dec.); ^{1}H-NMR (200 MHz, D_2O): 1.27 [s, 9H, $C(CH_3)_3$], 1,36 (d, 3H, J = 6.4, C-CH_3), 3.55 (s, 3H, N^+-$\underline{Me}_A Me_B$), 3.74 (s, 3H, N^+-$Me_A\underline{Me}_B$), 3.82 [dq, 1H, J = 6.5 and J = 2.8, C(7)-H], 4.28 [dq, 1H, J = 6.6 and J = 2.8, C(6)-H]. Anal. found: C 37.18; H 6.09; N 13.53; I 24.63; cal. for $C_{15}H_{26}O_2N_5I.3H_2O$: C 36.81; H 6.59; N 14.31; I 25.93.

N,N-$^{13}CH_3$,CH_3-6,7-Dimethyl-2-pivaloyl-tetrahydropteridinium iodide (25b).
The salt was synthesized according to the procedure described for the unlabelled compound. ^{1}H-NMR (200 MHz, D_2O): 1.25 [(s, 9H, $C(CH_3)_3$], 1.34 (d, 3H, J = 6.4, C-CH_3), 1.36 (d, 3H, J = 6.3, C-CH_3), 3.53 (d, 2H, J = 2.9, $N^+\underline{Me}_A{}^{13}Me_B$), 3.53 (d, 1H, J = 145.8, N^+-$^{13}\underline{Me}_A Me_B$), 3.72 (d, 1H, J = 3.0, N^+-$Me_A{}^{13}\underline{Me}_B$), 3.72 (d, 2H, J = 146.8, N^+-$Me_A{}^{13}\underline{Me}_B$), 3.81 [m, 1H, C(7)-H], 4.25 [dq, 1H, J = 2.7 and J = 6.6, C(6)-H]; ^{13}C-NMR (50.32 MHz, D_2O): 58.0 and 55.0 ($^{13}Me_A$ and $^{13}Me_B$; ratio 1/2).

***General procedure of converting* 25a,b *iodides into the corresponding fluoroborates*.**
To a solution of the iodide salt **25a** (200 mg, 0.46 mmol) in 30 ml dry acetonitrile, silver tetrafluoroborate (87 mg, 0.46 mmol) was added, directly resulting in the formation of a precipitate of silver iodide. The reaction mixture was stirred for 15 minutes, followed by filtration over high-flow. The filtrate was concentrated *in vacuo*, yielding 181 mg (100%) of **25a** fluoroborate. Although the shape of the signals is broader, the ^{1}H-NMR spectrum of the fluoroborate is identical with the spectrum of **25a** iodide. ^{1}H-NMR (200 MHz, $CDCl_3$): 1.28 [s, 9H, $C(CH_3)_3$], 1.38 ([br, 6H, C(6)-CH_3 and C(7)-CH_3], 3.58 (s, 3H, N^+-$\underline{Me}_A Me_B$), 3.75 (s, 3H, N^+-$^{13}Me_A\underline{Me}_B$), 3.85[br, 1H, C(7)-H], 4.22 [br, 1H, C(6)-H].

Reaction of 25a with potassium thiophenolate.
According to the procedure described above, the iodide anion of 200 mg of **25a** was exchanged for the tetrafluoroborate anion. The resulting salt, dissolved in 15 ml dry acetonitrile, was allowed to react with a freshly prepared solution of potassium thiophenolate and 18-crown-6 in 15 ml acetonitrile. The reaction was performed at 343 K under nitrogen atmosphere. After 24 h, the yield of methyl transfer (57%) was determined by HPLC. For this, a standard plot was prepared under the following conditions: 80/20 MeOH/H_2O; 1.5 ml/min.; λ = 254 nm. In order to identify methyl phenyl sulfide **6** by ^{1}H-NMR, the solvent of the reaction mixture was evaporated *in vacuo*. The resulting residue was extracted with PE. The PE extract was concentrated *in vacuo*. The ^{1}H-NMR spectrum (200 MHz, $CDCl_3$) attested the formation of **6** [signal at 2.49 ppm (S-Me)]. The ^{1}H-NMR spectrum (200 MHz, $CDCl_3$) of the residue revealed the presence of the demethylated compound **24** by the doublet at 0.77 ppm C(6)-Me or C(7)-Me and the singlet at 2.55 ppm [N(5)-Me].

Reaction of 25a with homocysteine.
Homocysteine (203 mg, 1.5 mmol), dissolved in 30 ml EtOH by addition of 500 µl 6M NaOH (3 mmol) and 3 ml H_2O, was allowed to react with a solution of **25a** fluoroborate in 20 ml EtOH. The reaction mixture was stirred at 343 K under nitrogen atmosphere. After 24 hours the solvent was evaporated *in vacuo*. The ^{1}H-NMR (200 MHz, 0.1 M NaOD/D_2O) of the crude reaction mixture revealed the presence of methionine by the sharp signal at 2.10 ppm (S-Me). The demethylated pterin **24** could easily be recognized by the doublet at 0.75 ppm. The calculated methyl transfer to homocysteine amounted to 40%. The ratio of methionine **3** to the demethylated pterin **24** was 1:1, according to the ^{1}H-NMR spectrum.

Reaction of 25b with homocysteine.
The reaction was performed according to the procedure, previously described for the reaction of homocysteine with the unlabelled compound **25a**. The ^{1}H-NMR spectrum clearly revealed the signals of methionine **3** and the demethylated labelled pterin **24**. The presence of these compounds was confirmed by the signals in the ^{13}C-NMR spectrum. ^{13}C-NMR (50.32 MHz, 0.1 M. 16.0 ppm (S-$^{13}CH_3$), 45.0 (N-

$^{13}CH_3$), 57.7, 55.0 (N^+-$^{13}Me_A$, N^+-$^{13}Me_B$). The shift of 45.0 ppm, assigned to the labelled N(5)-methyl substituent in compound **24** is in agreement with the value of 48.1 ppm in D_2O for the non-pivaloylated N(5)-$^{13}CH_3$-6,7-dimethyltetrahydropterin.

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* To whom correspondence may be addressed.

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