A new model for the coenzyme B_{12} catalysed methylmalonyl–succinyl rearrangement incorporating peripheral A–T base pairing

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A novel B_{12} derivative 4 containing a peripheral thymine group is alkylated under reductive conditions with 2-bromo- methyl-2-methylmonothiomalonate 8 bearing the complementary adenine; photolysis in chloroform–acetonitrile gives the rearranged succinate 12 and dimethylmalonate 13 (2:1), whereas in MeOH–H_2O only the dimethylmalonate 13 is formed.

We have been interested in developing models for the methylmalonyl–succinyl rearrangement catalysed by the coenzyme B_{12} dependent methylmalonyl CoA mutase.[1] Although this rearrangement is an essential process in human metabolism, the electronic nature of the 1,2-migration of the thioester and the role of the Co in the rearrangement step have not been established in an irrefutable way. It could be an anionic or radical process or it could involve fragmentation and readdition of the thioester moiety.[1,2]

In order to understand better the role of the cobalt in the rearrangement step, we devised supramolecular structures that mimic the holoenzyme–substrate complex and keep the intermediate radical in the vicinity of the Co.

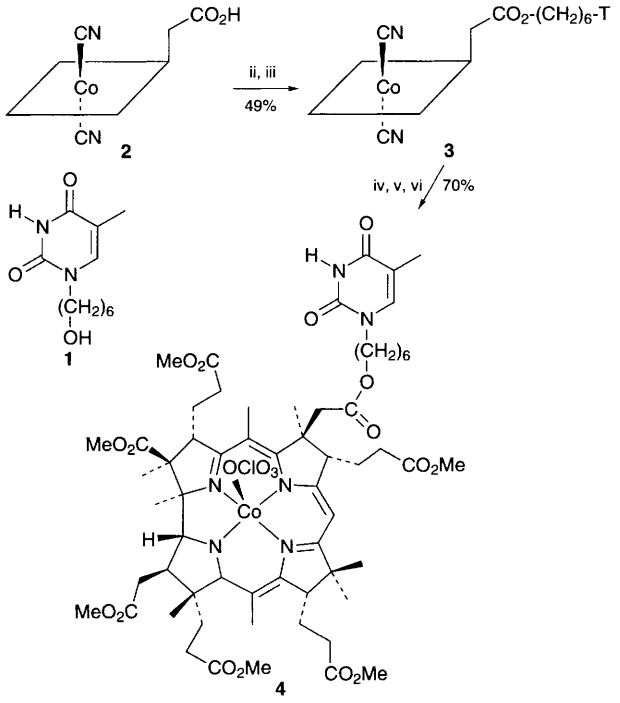
We have already reported on our model where molecular recognition and association of the B_{12} complex and the substrate takes place *via* hydrophobic interactions.[3] We have now extended this concept to other non-covalent associations.

In our new model for the B_{12} catalysed methylmalonyl–succinyl rearrangement recognition and association takes place through hydrogen bonding[4,5] of the nucleic acid bases adenine and thymine introduced into the substrate and into the B_{12} derivative respectively.

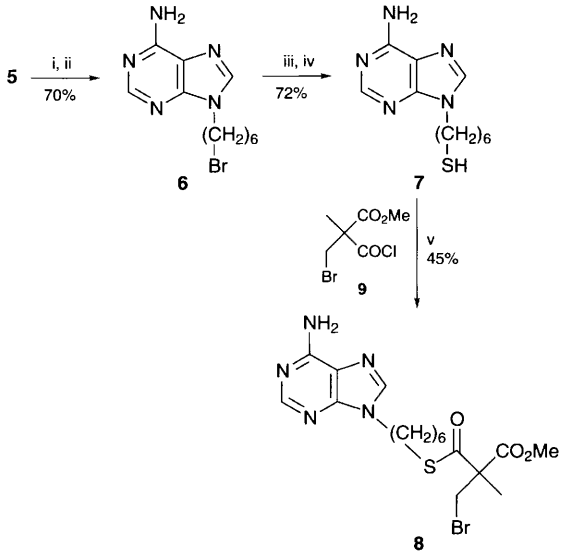
Here we report the syntheses of the new B_{12} derivatives **3** and **4** and the complementary bromide **8**. Our preliminary results concerning the association of **4** and **8** and the effect of base pairing in the rearrangement of the thioester moiety in **8** are also presented.

Compounds **3** and **4** were prepared as described in Scheme 1. 6-Bromohexylthymine[6] was converted into 6-hydroxyhexylthymine **1** in 61% yield by treatment with pyridine in DMF with 15% H_2O. Esterification of the cobester-c-monoacid **2**[7] with **1** gave the dicyano cobester **3** (T = thymine) in 49% yield after chromatography (methylene chloride–ethyl acetate–methanol, 9:9:2). The latter was reduced to the Co^{II} perchlorate **4** by known procedures[8] in 77% yield. Bromide **8** was prepared in 3 steps from adenine as shown in Scheme 2. Adenine **5** was alkylated with 6 equiv. of 1,6-dibromohexane to afford the 6-bromohexyladenine **6** in 70% yield. Bromide **6** was then converted into the corresponding thiol **7** by treatment first with thiourea and subsequently with sodium hydroxide[9] in 72% yield. Reaction of the acid chloride **9** with the thiol **7** produced the desired compound **8** in 45% yield after isolation by reversed phase HPLC (methanol–water 1.5:1). Compounds **1**, **3**, **4**, **6**, **7** and **8** gave ^1H, ^{11}C NMR, MS and HRMS data consistent with the proposed structures.

The association constant between **4** and **8** was determined by NMR titration using the dilution method.[10] A value of 71 $dm^3\ mol^{-1}$ in $CDCl_3$ was obtained which is comparable to the association constant value found for 6-bromohexylthymine and **6** under similar conditions (100 $dm^3\ mol^{-1}$).[11]



Scheme 1 *Reagents and conditions*: β,β,β-trichloro-*tert*-butyl chloroformate, Et_3N, CH_2Cl_2, −10 to 20 °C, 1 h; ii, **1**, CH_2Cl_2, 20 °C, 16 h; iii, 30% $HClO_4$, CH_2Cl_2; iv, $NaBH_4$, MeOH–H_2O (4:1); v, 30% $HClO_4$



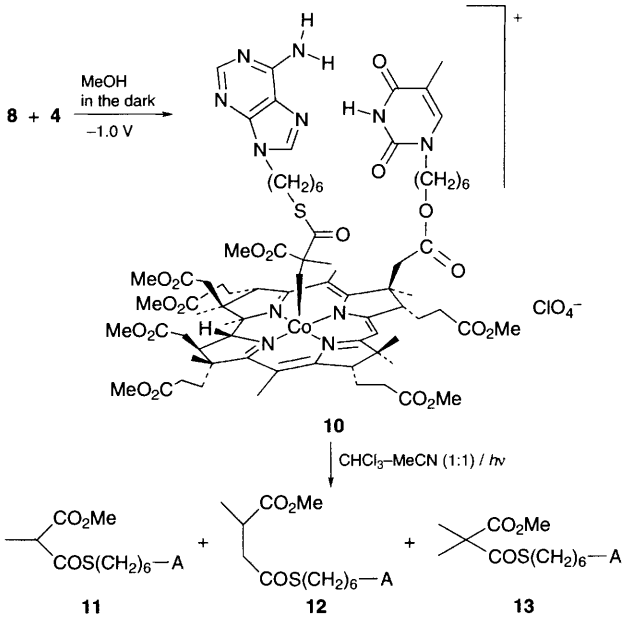
Scheme 2 *Reagents and conditions*: i, NaH, DMF, 20 °C, 1 h; ii, $Br(CH_2)_6Br$ (6 equiv.), 16 h, 20 °C; iii, $(H_2N)_2C{=}S$, 94% EtOH, 5 h, 20 °C; iv, NaOH–H_2O, 0.5 h, reflux; v, NEt_3, CH_2Cl_2, 20 °C, 16 h

The redox potential for the Co^{II}–Co^{I} interconversion in complex **4** was determined by CV to be −0.75 V for reduction and −0.65 V for oxidation in methanol. The redox behaviour of complex **4** proved however to be solvent dependent; whereas in methanol the redox reaction shows reversibility, in acetonitrile only 50% reversibility could be achieved. Results comparable to the methanol experiment could be obtained by adding a protic solvent like methanol or acetic acid to the acetonitrile solution of **4**.

Although at this point we can not explain these CV results, they are suggestive of an intramolecular interaction between the Co^{I} formed by reduction and the H–N function in the thymine moiety.

When **8** was treated with 5 mol% of **4** in acetonitrile at −0.85 V (*vs.* SCE) and irradiated with a 150 W lamp,[3] none of the expected products (**11**, **12**, **13**) could be detected by HPLC analysis (bromide **8** being recovered). When the reaction was run in methanol–water 4 : 1, reduction product **13** was identified together with the disulfide $[Ad(CH_2)_6S]_2$ by HPLC analysis and no starting bromide **8** was present after 18 h at room temperature. These results indicate that the alkylation of Co^{I} by bromide **8** does not take place in acetonitrile but does occur in methanol–water. The effect of the adenine and thymine interaction in the rearrangement of the thioester moiety was demonstrated as shown in Scheme 3. The complex **4** was electrochemically reduced to the corresponding Co^{I} complex in methanol and the latter was alkylated with **8** in the dark to give complex **10**. This complex was dissolved in chloroform–acetonitrile (*ca.* 1 : 1) after removal of methanol and irradiated with a 150 W lamp for 2 h at room temperature. The products obtained by photolysis were isolated by HPLC and corresponded to a mixture of **11**, **12** and **13** in a ratio of 1 : 2 : 1†

Our preliminary results have shown that a high percentage of rearrangement is observed with complex **4** and bromide **8** in chloroform–acetonitrile whereas no rearrangement takes place in methanol–water. This results indicate that hydrogen bonding between the adenine and thymine increases the amount of 1,2-migration of the thioester compared to reduction. Our present model also favours rearrangement to a higher degree than the previously reported model with hydrophobic interactions or models where peripheral association between the reacting species are not present.



Scheme 3

Footnotes

† *Experimental procedure* for the reaction shown in Scheme 3: To 25 ml of a 0.1 mol dm^{-3} $LiClO_4$ degassed methanolic solution in the electrochemical cell were added 10 μmol of complex **4** in 1 ml of methanol. The Co^{II} perchlorate **4** was reduced at −1.5 V for 2 h. To the resulting Co^{I} complex 10–30 μmol of **8** were added in the dark and the reaction mixture was stirred under Ar for 2 h under a potential of −0.8 V in the dark. The solvent was replaced by acetonitrile–chloroform 1 : 1 (15 ml) and the complex **10** was irradiated with a 150 W lamp at a distance of 40 cm under Ar for 2 h. The reaction products were isolated by reversed phase HPLC [Lichrospher 60 RP-Select B, methanol–water 1.5 : 1, **11** (t_R 8 min), **12** (t_R 10 min), **13** (t_R 12 min)]. The isolated compounds were identified by ^1H and ^{13}C NMR, MS and HRMS.

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