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Solid–solid synthesis of a hydrophobic vitamin B_{12} having a benzo-18-crown-6 moiety at the C10 position of the corrin ring

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Abstract—A new hydrophobic vitamin B_{12} having a benzo-18-crown-6 moiety at the C-10 position of the corrin ring was synthesized by solid-state condensation reaction. The proton NMR titration study in acetonitrile exhibits a potassium ion binding behavior of the hydrophobic vitamin B_{12} at the benzo-18-crown-6 moiety.

Among vitamin B_{12}-dependent enzymatic reactions, isomerization reactions, which lead to intramolecular 1,2-migration of various functional groups, have been attracting much attention because of the interesting nature of these reactions from the viewpoint of catalytic organic chemistry.[1] Therefore, several model reactions have been developed and investigated to elucidate the enzymatic reaction mechanism in detail.[2] Substitution of the peripheral amide moieties of naturally occurring vitamin B_{12} into ester groups provides hydrophobicity to vitamin B_{12} derivatives, and that allows us to undertake various enzymatic simulations in organic media.[3] Recent progress of X-ray crystallography has revealed details of the three-dimensional structures of proteins including vitamin B_{12}-dependent enzymes. The structures of diol dehydratase and glycerol dehydratase, enzymes that catalyze the adenosylcobalamin-dependent conversion of 1,2-diols to the corresponding aldehydes, were determined by Toraya et al. and the existence of the potassium ion is directly exhibited as an essential co-factor.[4,5] The potassium ion is hepta-coordinated by the two hydroxyls of the substrate and five oxygen atoms from the active-site residues. The theoretical study suggested that substrates and reaction intermediates would always be kept bound to the potassium ion until the release of product aldehyde from the active site of the enzyme.[6] These findings prompted us to develop a more finely-tuned model compound for vitamin B_{12}-dependent enzyme which simulates a diol dehydratase-dependent reaction. In this study, synthesis and characterization of a new vitamin B_{12} derivative (**1**) which has a benzo-18-crown-6 moiety as the potassium ion binding site are reported.

Hydrophobic vitamin B_{12} complex, $(CN)_2$Cob(III)7C_1 ester, was synthesized by a previously reported method[7,8] and was nitrated at the C10 position of the corrin ring with nitronium tetrafluoroborate. The nitrated product, $(CN)_2$Cob(III)(10-NO_2)7C_1ester,[†] was reduced with sodium tetrahydroborate in dry methanol under anaerobic conditions to yield an amino-derivative, $(CN)_2$Cob(III)(10-NH_2)7C_1ester (**2**)[‡] as we previously reported.[9] The target molecule **1** was synthesized by a condensation reaction of **2** with 4-formyl-benzo-

Keywords: hydrophobic vitamin B_{12}; benzo-18-crown-6; solid-state reaction; potassium ion binding.

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[†] $(CN)_2$Cob(III)(10-NO_2)7C_1 ester: Yield, 91%; TOF MS (MALDI, m/z): $[M-CN+1]^+$, 1108.6; UV–vis (in CH_2Cl_2): [λ_{max}/nm(ε/M^{-1} cm^{-1})], 279 (10800), 314 (9060), 361 (21500), 504 (6240), 538 (7600), 572 (5900); CD (in CH_3OH): [λ_{max}/nm($\Delta\varepsilon$)], 292 (−0.82), 319 (3.40), 352 (3.38), 399 (4.90), 443 (−1.85), 513 (1.48); IR, ν/cm^{-1}: 2150 (C≡N str.), 1730 (C=O str.), 1570, 1370 (NO_2 str.). Anal. calcd for $C_{54}H_{72}N_7Co_1O_{16}\cdot H_2O$: C, 56.29; H, 6.47; N, 8.51. Found C, 56.08; H, 6.32; N, 8.43%.

[‡] $(CN)_2$Cob(III)(10-NH_2)7C_1ester (**2**): Yield, 76%; HR MS(FAB, m/z): calcd for $C_{54}H_{74}N_7Co_1O_{14}$: $[M]^+$, 1103.4626. Found: $[M]^+$, 1103.4614; UV–vis (in CH_2Cl_2): [λ_{max}/nm (ε/M^{-1} cm^{-1})], 312 (21330), 378 (52220), 616 (15800); CD (in CH_3OH): [λ_{max}/nm ($\Delta\varepsilon$)], 311 (−5.55), 358 (−4.73), 372 (−4.98), 405 (19.7), 614 (1.75); IR, ν/cm^{-1}: 2130 (C≡N str.), 1735 (C=O str.), 1630 (NH_2 str.). Anal. calcd for $C_{54}H_{74}N_7CoO_{14}\cdot 3/2H_2O$: C, 57.34; H, 6.86; N, 8.67. Found C, 57.24; H, 6.63; N, 8.58%.

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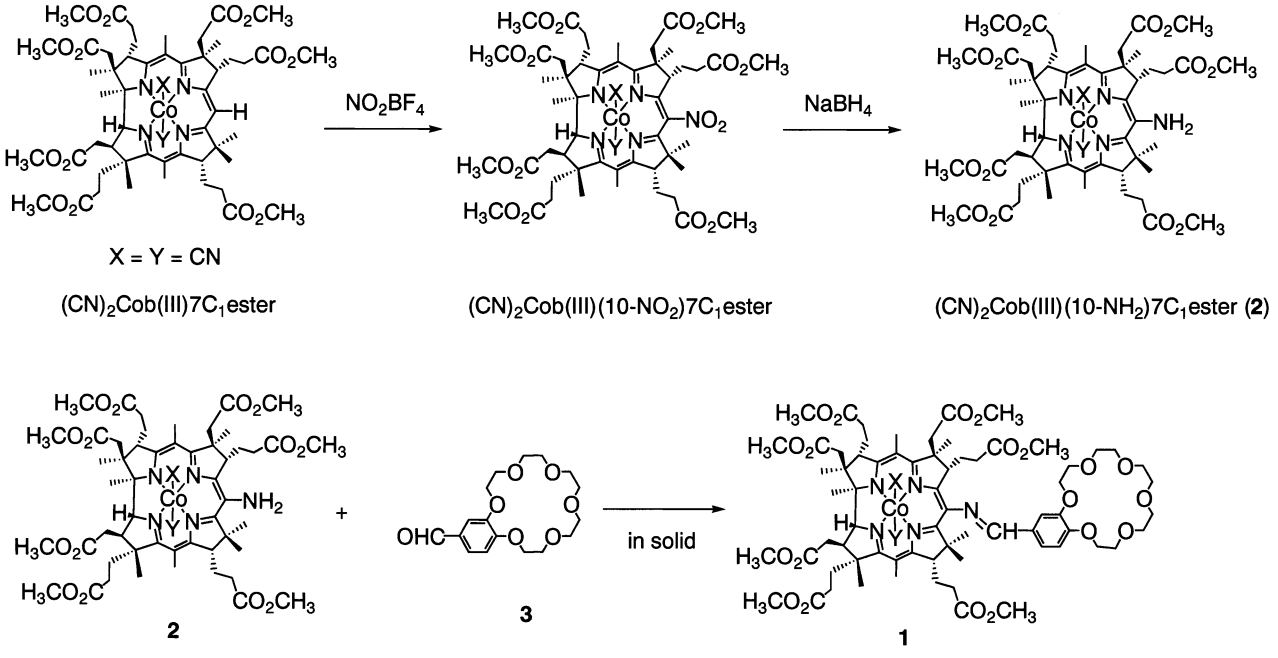
18-crown-6 (**3**)[10§] shown in Scheme 1. Various reaction conditions for the synthesis of **1** are summarized in Table 1. To a dry methanol solution of **2** (0.22 mM) was added 33 mol equivalents of **3** and the solution was refluxed for 12 h under a nitrogen atmosphere as shown by entry 1 in Table 1. The desired product **1** was not obtained under these conditions. An unsuccessful synthesis of **1** under more concentrated reaction conditions is shown by entry 2 in Table 1. In contrast to these reaction conditions, 9.08×10^{-6} mol of **2** and one equivalent mol of **3** were dissolved in dichloromethane, and the solvent was evaporated to dryness, and then the homogeneous mixture of **2** and **3** was stood for 24 h at room temperature under nitrogen atmosphere to form the condensation product **1** in 15% yield as shown by entry 3 in Table 1. Under the optimized reaction conditions as shown by entry 5 in Table 1, **1** was obtained in 56% isolated yield after GPC purification using three connected columns, JAIGEL-1H,2H, and 2.5 H as $CHCl_3$ elute. Modification of naturally occurring vitamin B_{12} including the C10 position of the corrin ring has been reported by several groups,[1,11] while the utility of the solid-state reaction is reported for the first time in this study. It is reported by Toda and Tanaka that some organic reactions can occur by mixing a powdered reactant and a reagent in the absence of a solvent, and the reaction products can be obtained efficiently.[12] In fact, condensation reactions of anilines and aromatic aldehydes to azomethines were found to proceed very efficiently in the absence of a solvent.[13] The solid-state organic reaction reduces pollution and is operationally simple so that it would be an attractive method from the viewpoint of green chemistry.

Compound **1** was characterized by UV–vis, ^{1}H NMR and HR mass spectroscopies as well as elemental analysis.¶ The ^{1}H NMR spectroscopy reveals six singlets corresponding to the seven methyl esters with 21-protons at δ 3.47, 3.52, 3.61, 3.63, 3.69 (superimposed two methyl protons), and 3.75, respectively. The aromatic protons ascribed to benzo-18-crown-6 appear as a pair of doublets (δ 6.95 and 7.36) and a singlet (δ 7.55). In addition, an imino proton appeared at 8.25 ppm clearly exhibits the progress of schiff-base condensation.

Electronic and circular dichroism(CD) spectra of **1** are shown in Figure 1 and are comparable to those of the $(CN)_2$Cob(III)7C_1 ester[14] except for a remarkable bathochromic shift of the α and β bands and a slight



Scheme 1.

§ **3** was synthesized by a Duff reaction of benzo-18-crown-6 with hexamethylenetetramine and trifluoroacetic acid reported by F. Wada et al.[8] Yield, 64%; ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 3.65 [s, 4H, –C$\underline{H}_2$–], 3.68 [m, 4H, –C$\underline{H}_2$–], 3.72 [m, 4H, –C$\underline{H}_2$–], 3.90 [m, 4H, –C$\underline{H}_2$–], 4.18 [m, 4H, –C$\underline{H}_2$–], 6.91 [d, 1H, Ph], 7.34 [d, 1H, Ph], 7.39 [dd, 1H, Ph], 9.79 [s, 1H, aldehyde]; TOF MS(MALDI, m/z): [M+Na]$^+$, 363.6.

¶ **1**: Yield, 56%; ^{1}H NMR(CD_2Cl_2, 500 MHz): δ = 3.47 [s, 3H, –OC$\underline{H}_3$], 3.52 [s, 3H, –OC$\underline{H}_3$], 3.61 [s, 3H, –OC$\underline{H}_3$], 3.63 [s, 3H, –OC$\underline{H}_3$], 3.66 [m, 4H, –C$\underline{H}_2$C$\underline{H}_2$–], 3.69 [s, 6H, –OC$\underline{H}_3$], 3.70 [m, 4H, –C$\underline{H}_2$C$\underline{H}_2$–], 3.75 [s, 3H, –OC$\underline{H}_3$], 3.89 [m, 4H, –C$\underline{H}_2$C$\underline{H}_2$–], 4.21 [m, 2H, –C$\underline{H}_2$–], 4.27 [m, 2H, –C$\underline{H}_2$–], 6.95 [d, 1H, Ph], 7.36 [d, 1H, Ph], 7.55 [s, 1H, Ph], 8.25 [s, 1H, imine]; HR MS (FAB, m/z): calcd for $C_{71}H_{96}N_7Co_1O_{20}$: [M]$^+$, 1425.6042. Found: [M]$^+$, 1425.6049; UV–vis (in CH_2Cl_2): [λ_{max}/nm(ε/M^{-1} cm^{-1})], 279 (14200), 307 (9350), 381 (37000), 595 (23900), 641 (24900); CD (in CH_3OH): [λ_{max}/nm ($\Delta\varepsilon$)], 273 (7.98), 311 (–12.2), 376 (–15.9), 409 (22.9), 635 (–5.34); IR, ν/cm^{-1}: 2122 (C≡N str.), 1732 (C=O str.), 1626 (C=N str.). Anal. calcd for $C_{71}H_{96}N_7CoO_{20}\cdot H_2O$: C, 59.03; H, 6.84; N, 6.79. Found C, 58.71; H, 6.74; N, 6.67%.

Table 1. Synthesis of **1** under various reaction conditions

Entry	Condition	Temperature	Molar ratio of **3**/**2**	Reaction time (h)	Yield (%)[a]
1[b]	In solution	Reflux	33	12	–
2[c]	In solution	Reflux	1.6	24	–
3[d]	In solid	Room temp.	1.0	24	15
4[e]	In solid	Room temp.	10	48	19
5[f]	In solid	Room temp.	10	168	56

[a] Yields are isolated yields based on the amount of **2**.
[b] Solvent, dry MeOH; [**2**]=0.22 mM; [**3**]=7.35 mM, under N_2.
[c] Solvent, dry MeOH; [**2**]=4.5 mM; [**3**]=7.35 mM, under N_2.
[d] [**2**]=9.08×10^{-6} mol; [**3**]=9.08×10^{-6} mol, under N_2.
[e] [**2**]=3.63×10^{-5} mol; [**3**]=3.63×10^{-4} mol, under N_2.
[f] [**2**]=3.63×10^{-5} mol; [**3**]=3.63×10^{-4} mol, under N_2.

bathochromic shift of the γ band. Similar bathochromic shift is observed in the complex with an electron-withdrawing substituent such as Cl and Br at the C10 position of the corrin ring.[11] The CD spectral patterns of **1** and $(CN)_2$Cob(III)7C_1 ester are similar to each other, so that the substitution of the imino benzo-crown unit at the C10 position of the corrin ring caused no significant structural change.

The binding property of **1** toward the potassium ion was evaluated using ^{1}H NMR spectroscopy. A comparison of the ^{1}H NMR spectra (CD_3CN, 298 K) of free **1** and **1** in the presence of KPF_6 reveals significant chemical shift differences in the resonances associated with the oxamethylene protons and the aromatic protons of the benzo-18-crown-6 unit of **1** as shown in Figure 2, indicating that the potassium ion is complexed within the benzo-18-crown-6 unit of **1**. Addition of increasing amounts of KPF_6 to a solution of **1** induced complexation which was followed by observing changes in the chemical shifts, and an excess potassium ion over 1.0 mol equivalent did not cause any further changes in the ^{1}H NMR spectrum of **1**, which confirms a 1/1 stoichiometry for the complexation of the potassium ion by **1**. While the binding for **1** was at the upper limits of determination by NMR methods, the log K_a value was estimated as over 5 since that of benzo-18-crown-6 in CH_3CN at 298 K was determined to 5.3 by conductance study.[15]‖

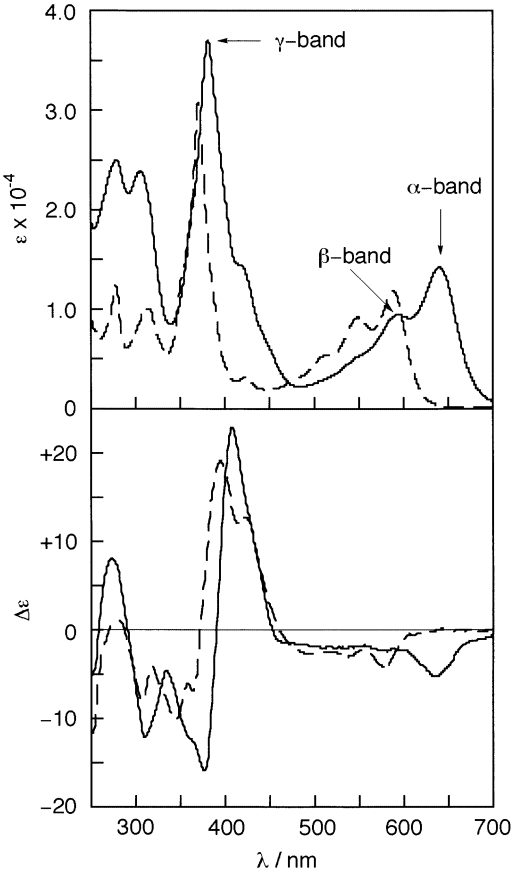


Figure 1. Electronic (in CH_2Cl_2) and CD (in MeOH) spectra of **1** (solid line) and $(CN)_2$Cob(III)7C_1ester (broken line) at 293 K.

In summary, a new hydrophobic vitamin B_{12} having the benzo-18-crown-6 unit was successfully synthesized by an unusual solid mixing method. In this complex, a potassium ion can be fixed at the near of cobalt center. This model complex is expected to elucidate the role of potassium ion in vitamin B_{12}-dependent enzymatic reactions. The functional simulation of diol dehydratase using 1 is now in progress in our laboratory.

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‖ The proton NMR titration was carried out for benzo-18-crown-6 under the same conditions for **1**, and a similar chemical shift was observed.

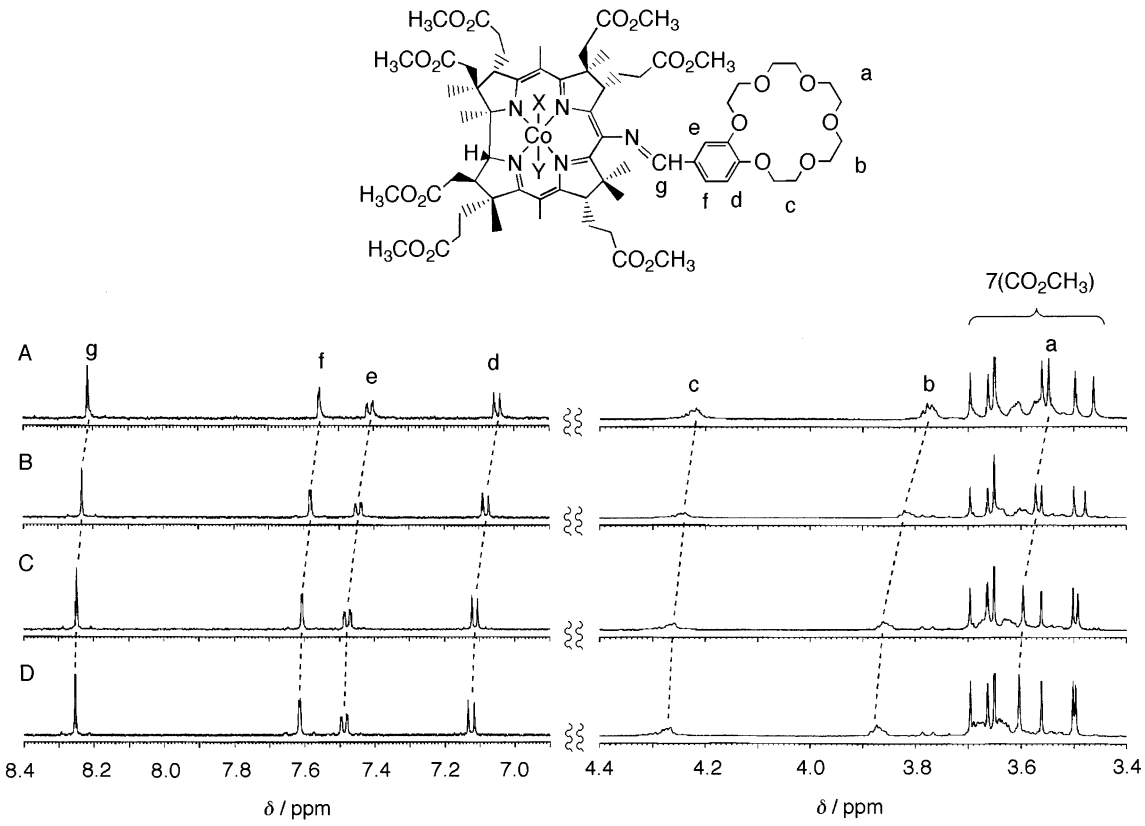

Figure 2. Partial ^{1}H NMR spectra on titration of **1** with K^+ in CD_3CN at 298 K. [**1**] = 1.22 mM, (A) **1** only; (B) **1** and 0.3 equiv. of K^+; (C) **1** and 0.6 equiv. of K^+; (D) **1** and 1.0 equiv. of K^+.

References

1. *Vitamin B_{12} and B_{12}-Proteins*, Kräutler, B.; Arigoni, D.; Golding, B. T., Eds.; Wiley-VCH, 1998.
2. Toscano, P. J.; Marzilli, L. G. *Prog. Inorg. Chem.* **1984**, *31*, 105.
3. Hisaeda, Y.; Nishioka, T.; Inoue, Y.; Asada, K.; Hayashi, T. *Coord. Chem. Rev.* **2000**, *198*, 21.
4. Shibata, N.; Masuda, J.; Tobimatsu, T.; Toraya, T.; Suto, K.; Morimoto, Y.; Yasuoka, N. *Structure* **1999**, *7*, 997.
5. Yamanishi, M.; Yunoki, M.; Tobimatsu, T.; Sato, H.; Matsui, J.; Dokiya, A.; Iuchi, Y.; Oe, K.; Suto, K.; Shibata, N.; Morimoto, Y.; Yasuoka, N.; Toraya, T. *Eur. J. Biochem.* **2002**, *269*, 4484.
6. Eda, M.; Kamachi, T.; Yoshizawa, K.; Toraya, T. *Bull. Chem. Soc. Jpn.* **2002**, *75*, 1469.
7. Werthemann, L.; Keese, R.; Eschenmoser, A. Unpublished results; see Werthemann, L. Dissertation, ETH Zürich (Nr. 4097), Juris Druck und Verlag, Zürich, 1968, 7.
8. Murakami, Y.; Hisaeda, Y.; Kajihara, A. *Bull. Chem. Soc. Jpn.* **1983**, *56*, 3642.
9. Ohno, T.; Ogawa, A.; Hisaeda, Y.; Murakami, Y. *J. Chem. Soc., Perkin Trans. 2* **1994**, 2271.
10. Wada, F.; Hirayama, H.; Namiki, H.; Kikukawa, K.; Matsuda, T. *Bull. Chem. Soc. Jpn.* **1980**, *53*, 1473.
11. Pratt, J. M. In *Chemistry and Biochemistry of B_{12}*; Wiley-Interscience, Banerjee, R., Ed.; 1999; Chapter 5.
12. Tanaka, K.; Toda, F. *Chem. Rev.* **2000**, *100*, 1025.
13. Schmeyers, J.; Toda, F.; Boy, J.; Kaupp, G. *J. Chem. Soc., Perkin Trans. 2* **1998**, *2*, 989.
14. Murakami, Y.; Hisaeda, Y.; Ohno, T. *Bull. Chem. Soc. Jpn.* **1984**, *57*, 2091.
15. Takeda, Y.; Ohyagi, Y.; Akabori, S. *Bull. Chem. Soc. Jpn.* **1984**, *57*, 3381.