Hydrophobic vitamin B_{12}. Part 12.† Preparation, characterization and enantioselective alkylation of strapped hydrophobic vitamin B_{12}

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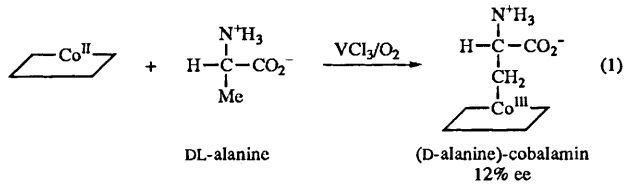
Cyanocobalamin has been modified to afford a strapped hydrophobic vitamin B_{12} by introducing a 1,3-phenylenediacetyl moiety into the peripheral site of the corrin's B ring. The modified complex was characterized by various spectroscopic methods and cyclic voltammetry in comparison with the corresponding data for a simple hydrophobic vitamin B_{12} without a strapping moiety. The alkylation of hydrophobic vitamin B_{12} derivatives with racemic 3-bromo-2-methylpropionic esters at the β-axial site was carried out in methanol, and extents of the enantioselectivity were examined by ^1H NMR spectroscopy. The strapped hydrophobic vitamin B_{12} and a simple hydrophobic vitamin B_{12} were found to react with (*S*)-3-bromo-2-methylpropionates more readily than the corresponding *R*-enantiomers; the highest *S*-selectivity (75% ee) was observed with the strapped hydrophobic vitamin B_{12}. The *S*-enantioselectivity was discussed from a stereochemical viewpoint based on the conformational search for the alkylated hydrophobic vitamin B_{12} by means of molecular mechanics and dynamics calculations.

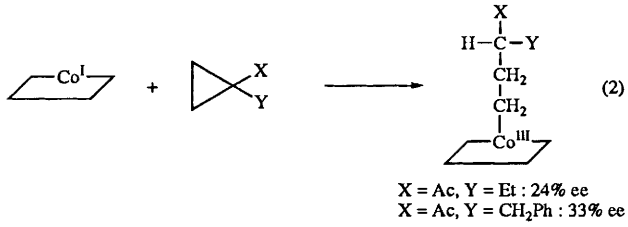
An important aspect of catalysis performed by vitamin B_{12}-dependent enzymes is chiral recognition toward substrate species. As regards our artificial holoenzyme composed of a hydrophobic vitamin B_{12} and a synthetic bilayer membrane,[1-5] the latter does not perform effective chiral recognition toward substrates even though the membrane lipid involves a chiral amino acid residue as a structural component. Under such circumstances, it becomes necessary to clarify the chiral recognition of substrate species by a hydrophobic vitamin B_{12} during the course of alkylation of the latter complex. Schrauzer *et al.* investigated the asymmetric alkylation of cob(II)alamin with DL-alanine in the presence of V^{3+} and oxygen radicals, and obtained the alanine-bound cobalamin in a 12% ee of the D-alanine-bound complex [eqn. (1)].[6] Ogoshi *et al.* studied the alkylation of cob(I)alamin with prochiral 1-acetyl-1-alkylcyclopropanes that induces an asymmetric centre in the resulting alkyl ligands [eqn. (2)] and found a 24% ee for X = Ac and Y = Et, and a 33% ee for X = Ac and Y = CH_2Ph.[7] Golding and co-workers carried out the alkylation of cob(I)-alamin with *tert*-butyloxirane and methyloxirane [eqn. (3)], affording preferentially (*R*)-alkyl-bound products in 62 and 50% ee, respectively.[8,9] Scheffold *et al.* reported an asymmetric isomerization of 1,2-epoxycyclopentane to (*R*)-cyclopent-2-enol *via* formation of the corresponding alkylated cobalamin, *ca.* 60% ee.[10]

It seems that the structural framework of the corrin moiety does not confer high enantioselectivity in non-enzymatic reactions. Therefore, the corrin moiety needs to be modified so as to provide an asymmetric reaction site on the β-axial site for the alkylation. We have previously investigated the enantioselective alkylation of hydrophobic vitamin B_{12} derivatives, which bear a chiral binaphthyl moiety, with various racemic 3-bromo-2-methylpropionic esters in methanol, and *S*-enantioselectivity as high as 65% ee was observed regardless of the chiral nature of the binaphthyl moiety.[11,12] In the present article, we report on the higher *S*-enantiomer selectivity achieved with a novel hydrophobic vitamin B_{12} modified by introducing a 1,3-phenylenediacetyl moiety into the peripheral site around the corrin's B ring.

† Part 11: see ref. 12.



(1)



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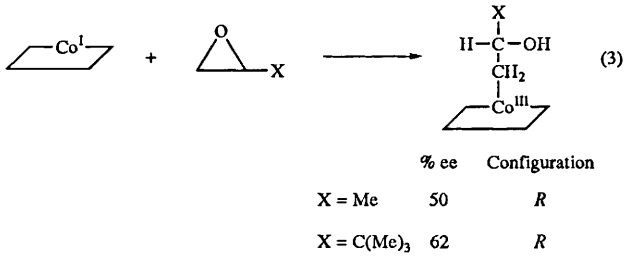

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Experimental

General analyses and measurements

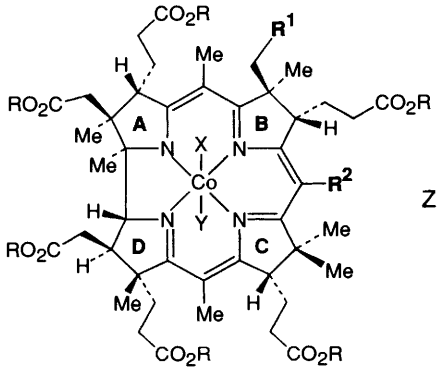
Elemental analyses were performed at the Microanalysis Centre of Kyushu University. IR spectra were taken on a JASCO IR-810 infrared spectrophotometer, while electronic absorption spectra were recorded on a Hitachi 220A or a Hitachi 340 spectrophotometer. Circular dichroism (CD) spectra were recorded on a JASCO J-500C spectropolarimeter. Mass spectroscopic analysis was performed on a Hitachi M-2500 spectrometer for SIMS, and on a JEOL JMS-AX505H

spectrometer for both FD and FAB techniques. ^{1}H NMR spectra were taken on a Hitachi R-1500 and a Bruker AC-250P spectrometer, and both ^{1}H and ^{13}C NMR spectra on a Bruker AMX-500 spectrometer installed at the Centre of Advanced Instrumental Analysis of Kyushu University. Assignments of NMR signals for the cobalt complexes were made by means of the 2D-NMR technique (H–H and C–H COSY). Cyclic voltammograms were obtained on an apparatus composed of a Hokuto Denko HA-501 potentiostat/galvanostat and a Hokuto Denko HB-104 function generator. HPLC analysis was performed on a Tosoh apparatus assembled in combination with a CCPM multi-pump, a MX-8010 mixing unit, an IF-8011 parallel-interface unit, and a CO-8011 column oven. Eluting fractions were monitored by a Tosoh UV-8010 spectrophotometer equipped with a Tosoh SC-8010 personal computer for data processing.

Materials

3-Bromo-2-methylpropionic acid, methyl 3-bromo-2-methylpropionate, and cyclohexyl 3-bromo-2-methylpropionate were synthesized in accordance with procedures reported previously.[12] All the bromides were confirmed to be sufficiently pure by GLC before use. Cyanocobalamin was donated by the Nippon Oil Company (Tokyo, Japan). Preparation of heptamethyl cobyrinate perchlorate, [Cob(II)7C$_1$ester]ClO$_4$, has been described previously.[13] Strapped hydrophobic vitamin B$_{12}$ derivatives were prepared by following the reaction steps shown in Scheme 1.

Simple hydrophobic vitamin B_{12}



Z

(CN)$_2$Cob(III)7C$_1$ester:
X = Y = CN, Z = none, R = Me, R^1 = CO$_2$Me, R^2 = H

[Cob(II)7C$_1$ester]ClO$_4$:
X = Y = none, Z = ClO$_4^-$, R = Me, R^1 = CO$_2$Me, R^2 = H

Cob(I)7C$_1$ester:
X = Y = Z = none, R = Me, R^1 = CO$_2$Me, R^2 = H

(Cob(II)7C$_3$ester]ClO$_4$:
X = Y = none, Z = ClO$_4^-$, R = Pr, R^1 = CO$_2$Pr, R^2 = H

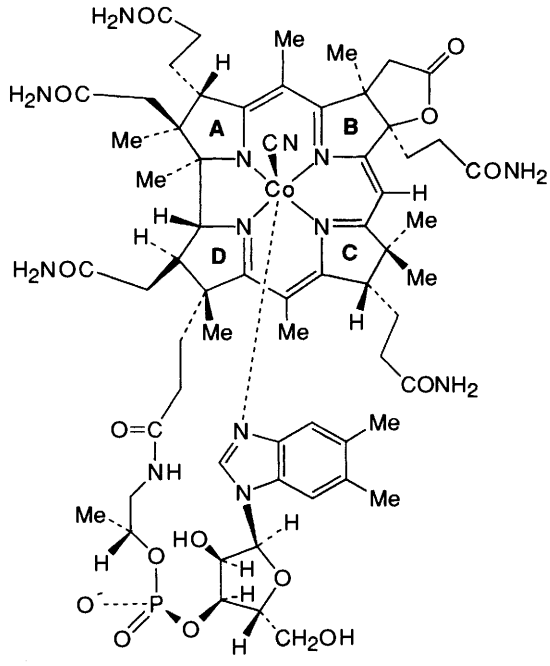
Cob(I)7C$_3$ester:
X = Y = Z = none, R = Pr, R^1 = CO$_2$Pr, R^2 = H

Cyanocobalamin-c,8-lactone (1)

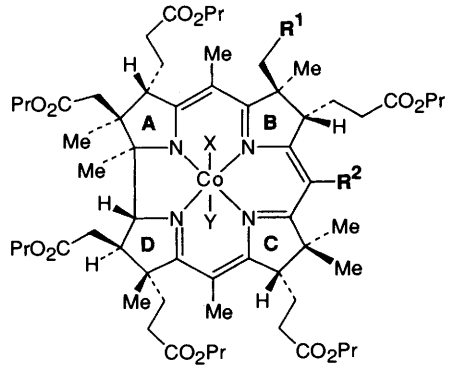
This compound was prepared by reaction of cyanocobalamin with *N*-bromosuccinimide (NBS) in aqueous acetic acid according to a reported procedure:[12,14] yield 68%.

Hexapropyl Coα,Coβ-Dicyano-7^1-de(carboxymethyl)-7^1,7^2-dihydro-7^2-oxofuro[3,2-*g*]cobyrinate (2)

Compound **1** (4.8 g, 3.6 × 10^{-3} mol) in dry propanol (400 cm^3) was mixed with cold conc. sulfuric acid (40 cm^3) and dry propanol (200 cm^3). The solution was heated at 80 °C for 120 h in the dark. The reaction mixture was concentrated to *ca.* 150



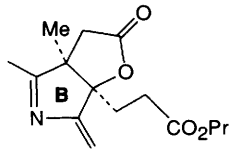
1



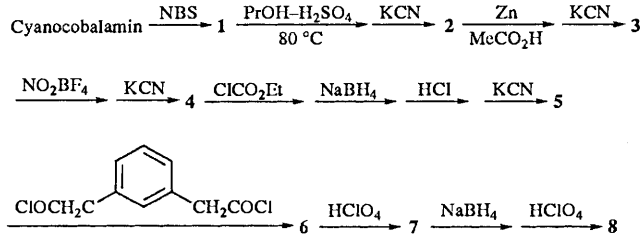
2 : X = Y = CN,

3 : X = Y = CN, R^1 = CO$_2^-$ K$^+$, R^2 = H

4 : X = Y = CN, R^1 = CO$_2^-$ K$^+$, R^2 = NO$_2$



5 : X = Y = CN, R^1 = CH$_2$OH, R^2 = NH$_2$



Scheme 1

cm^3, diluted with cold water (300 cm^3), and quickly neutralised with sodium carbonate powder. An aqueous solution (20 cm^3) of potassium cyanide (5.0 g, 7.7 × 10^{-2} mol) was added, after which the reaction mixture was extracted with carbon tetrachloride (150 cm^3 × 3). The extract was dried over sodium sulfate and evaporated to dryness at room temperature. The residue was purified by TLC on silica gel (Kieselgel 60, Merck) with dichloromethane–methanol (93:7 v/v) containing 0.05% (w/w) potassium cyanide as the eluent. The purple fraction, coming out second, was collected and extracted with methanol, and the methanol extract was evaporated to dryness *in vacuo* at room temperature. The residue was dissolved in dichloromethane, and the filtrate was evaporated *in vacuo* at room temperature to afford a purple solid: yield 3.9 g (68%); $\lambda_{max}(CH_3OH)$/nm 275 (ε 9.1 × 10^3 dm^3 mol^{-1} cm^{-1}), 314 (8.3 × 10^3), 366 (1.8 × 10^4), 414 (8.3 × 10^3), 545 (7.9 × 10^3) and 586 (8.9 × 10^3); ν_{max}(KBr)/cm^{-1} 2120 (C≡N), 1790 (lactone C=O) and 1730 (ester C=O). Analysis by HPLC showed that the desired compound was eluted with a retention time of 3.5 min at 30 °C: TSK-GEL Silica-60, a column of 4.6 × 250 mm; dichloromethane–methanol (97:3, v/v) at an elution rate of 1 cm^3 min^{-1}; monitoring at λ = 360 nm; purity of the product 100%.

Potassium hexapropyl Coα,Coβ-dicyano-7[1]-carboxylato-cobyrinate (3)

An acetic acid solution (100 cm^3) of compound **2** (1.23 g, 1.0 × 10^{-3} mol) was deoxygenated by bubbling nitrogen gas through it for 30 min, and treated with zinc powder (5.0 g) with vigorous stirring for 10 min at room temperature. After the residual zinc powder had been removed by filtration and distilled water (100 cm^3) added to the filtrate, the resulting cobalt complex was extracted with dichloromethane (100 cm^3). Then, the dichloromethane extract was mixed with 100 cm^3 of aqueous potassium cyanide (3.0 g, 4.6 × 10^{-2} mol), and the organic layer was separated and evaporated to dryness at room temperature. The residue was purified by TLC on silica gel (Kieselgel 60, Merck) with dichloromethane–methanol (93:7 v/v) containing 0.05% (w/w) potassium cyanide as the eluent. The purple-red fraction, coming out second, was collected and extracted with methanol, and the methanol extract was evaporated to dryness *in vacuo* at room temperature. The residue was dissolved in dichloromethane and filtered, and the filtrate was evaporated to dryness *in vacuo* at room temperature. The residue was dissolved in dichloromethane and filtered, and the filtrate was evaporated to dryness *in vacuo* at room temperature. The residue was dissolved in benzene, and the product was recovered as a red powder by reprecipitation with hexane: yield 829 mg (67%) (Found: C, 60.9; H, 7.4; N, 6.3. $C_{65}H_{94}CoKN_6O_{14}$ requires C, 60.92; H, 7.39; N, 6.56%); ν_{max}(KBr)/cm^{-1} 2130 (C≡N) and 1735 (ester C=O). Analysis by HPLC showed that the desired compound was eluted with a retention time of 7.3 min at 30 °C: TSK-GEL Silica-60, a column of 4.6 × 250 mm; dichloromethane–methanol (85:15 v/v) at an elution rate of 1 cm^3 min^{-1}; monitoring at λ = 360 nm; purity of the product 97%.

Potassium hexapropyl Coα,Coβ-dicyano-7[1]-carboxylato-10-nitrocobyrinate (4)

Compound **3** (187 mg, 1.5 × 10^{-4} mol) in dry dichloromethane (50 cm^3) was mixed with nitronium tetrafluoroborate (25 mg, 1.6 × 10^{-4} mol) and stirred vigorously for 5 h at room temperature. After the dichloromethane solution had been mixed with 100 cm^3 of aqueous potassium cyanide (3.0 g, 4.6 × 10^{-2} mol), the organic layer was separated and evaporated to dryness *in vacuo* at room temperature. The residue was purified by TLC on silica gel (Kieselgel 60, Merck) with dichloromethane–methanol (93:7 v/v), containing 0.05% (w/w) potassium cyanide, as the eluent. The purple-red fraction, coming out second, was collected and extracted with methanol, and the methanol extract was evaporated to dryness *in vacuo* at room temperature. The residue was dissolved in dichloromethane and filtered, and the filtrate was evaporated *in vacuo* at room temperature. The residue was then dissolved in benzene, and the product was recovered as a red powder by reprecipitation with hexane: yield 80 mg (41%) (Found: C, 58.65; H, 7.2; N, 7.25. $C_{65}H_{93}CoKN_7O_{16}$ requires C, 58.85; H, 7.07; N, 7.39%); 500 MHz ^{1}H NMR signal of a hydrogen at the C-10 position (δ_H 5.5) of the corrin ring was confirmed to be missing; ν_{max}(KBr)/cm^{-1} 1560 (NO_2, asym.) and 1370 (NO_2, sym.). Analysis by HPLC showed that the desired compound was eluted with a retention time of 5.8 min at 30 °C: TSK-GEL Silica-60, a column of 4.6 × 250 mm; dichloromethane–methanol (85:15 v/v) at an elution rate of 1 cm^3 min^{-1}; monitoring at λ = 360 nm; purity of the product 97%.

Hexapropyl Coα,Coβ-dicyano-7[1]-decarboxy-7[1]-hydroxymethyl-10-aminocobyrinate (5)

A dry benzene–dioxane (100 cm^3, 3:1 v/v) solution of compound **4** (1.0 g, 8.2 × 10^{-4} mol) was deoxygenated by bubbling nitrogen gas through it for 30 min, and cooled to −5 °C. Triethylamine (0.35 cm^3) and ethyl chloroformate (0.25 cm^3, 3.7 × 10^{-3} mol) were added to the solution under anaerobic conditions. The resulting solution was stirred for 30 min, and then warmed to room temperature. Sodium tetrahydroborate (2 g) in dry methanol–dioxane (1:1 v/v, 150 cm^3) was added to the solution, after which the resulting solution was stirred for 10 min at room temperature. Dichloromethane (100 cm^3) was added to the solution, and the excess of sodium tetrahydroborate was decomposed by careful addition of aqueous hydrochloric acid (1 mol dm^{-3}, 100 cm^3) to the mixture. The dichloromethane solution was mixed with 100 cm^3 of aqueous potassium cyanide (3.0 g, 4.6 × 10^{-2} mol), and the organic layer was dried over sodium sulfate. The organic layer was evaporated to dryness *in vacuo* at room temperature and purified by TLC on silica gel (Kieselgel 60, Merck) with dichloromethane–methanol (93:7 v/v) as the eluent. The second blue fraction was collected and extracted with methanol, and the methanol extract was evaporated to dryness *in vacuo* at room temperature. The residue was dissolved in dichloromethane and filtered, and the filtrate was evaporated to dryness *in vacuo* at room temperature to afford a blue solid: yield 317 mg (32%) (Found: C, 62.05; H, 7.9; N, 7.6. $C_{65}H_{98}CoN_7O_{13}\cdot H_2O$ requires C, 61.84; H, 7.98; N, 7.77%); IR signals due to NO_2 (asym and sym) were confirmed to be missing. Analysis by HPLC showed that the desired compound was eluted with a retention time of 3.0 min at 30 °C: TSK-GEL Silica-60, a column of 4.6 × 25 mm; dichloromethane–methanol (85:15 v/v) at an elution rate of 1 cm^3 min^{-1}; monitoring at λ = 360 nm; purity of the product 99%.

Strapped hydrophobic vitamin B_{12} with two cyano groups, $(CN)_2$Cob(III)(c,10-PDA)6C_3ester (6)

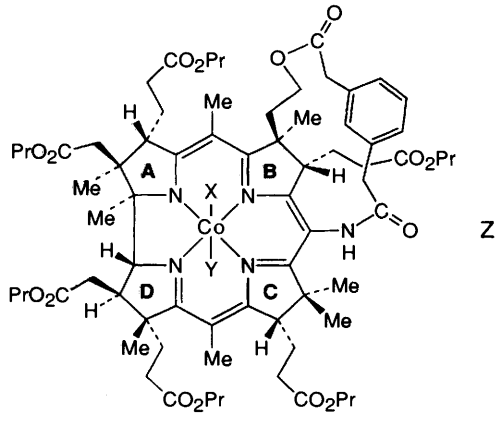
Compound **5** (100 mg, 8.04 × 10^{-5} mol) in dry dichloromethane (50 cm^3) was mixed with 1,3-phenylenediacetyl chloride (22.3 mg, 9.65 × 10^{-4} mol) in dichloromethane (50 cm^3) under anaerobic conditions, and the mixture was stirred for 12 h at room temperature. The dichloromethane solution was mixed with 100 cm^3 of aqueous potassium cyanide (3.0 g, 4.6 × 10^{-2} mol), after which the organic layer was evaporated to dryness *in vacuo* at room temperature. The residue was purified by TLC on silica gel (Kieselgel 60, Merck) with dichloromethane–methanol (93:7 v/v) containing 0.05% (w/w) potassium cyanide as the eluent. The first purple fraction was collected and extracted with methanol, and the methanol extract was evaporated to dryness *in vacuo* at room temperature. The residue was

dissolved in dichloromethane and filtered, and the filtrate was evaporated *in vacuo* at room temperature. The residue was dissolved in benzene, and the product was recovered as a purple powder by reprecipitation with hexane: yield 44 mg (39%) (Found: C, 63.4; H, 7.35; N, 6.85. $C_{75}H_{104}CoN_7O_{15}\cdot H_2O$ requires C, 63.41; H, 7.52; N, 6.96%); $\lambda_{max}(CH_3OH)$/nm, 284 (ε 5.1 × 10^3 dm^3 mol^{-1} cm^{-1}), 318 (4.7 × 10^3), 372 (1.8 × 10^4), 554 (4.4 × 10^3) and 592 (5.1 × 10^3); ν_{max}(KBr)/cm^{-1} 2120 (C≡N), 1735 (ester C=O), 1680 (amide C=O), 710 and 780 (Ar–H); $\Delta\varepsilon(CH_3OH)$/deg cm^2 dmol^{-1} −10.0 (254.0 nm), +6.0 (281.0 nm), −7.0 (310.0 nm), −11.7 (349.0 nm), −12.7 (366.0 nm), −20.8 (395.0 nm) and −2.91 (586.0 nm); *m/z* (SIMS) 1376 ([*M* − 26]$^+$; calc. *M* for [$C_{75}H_{104}CoN_7O_{15}$ − CN] 1376). Analysis by HPLC showed that the desired compound was eluted with a retention time of 4.0 min at 30 °C; TSK-GEL Silica-60, a column of 4.6 × 250 mm; dichloromethane–methanol (97:3 v/v) at an elution rate of 1 cm^3 min^{-1}; monitoring at λ = 360 nm; purity of the product 100%.

Strapped hydrophobic vitamin B_{12} with a single cyano group, [(CN)(H_2O)Cob(III)(c,10-PDA)6C_3ester]ClO_4 (7)

A purple solution of **6** (80 mg, 5.7 × 10^{-5} mol) dissolved in dichloromethane (50 cm^3) was mixed with 30% (w/w) aqueous perchloric acid (30 cm^3). The orange dichloromethane layer was washed with distilled water (100 cm^3 × 2), dried over sodium sulfate, and evaporated to dryness *in vacuo* at room temperature. The residue was dissolved in benzene, and the product was recovered as an orange powder by reprecipitation with hexane: yield 80 mg (93%) (Found: C, 59.2; H, 7.1; N, 5.6. $C_{74}H_{106}ClCoN_6O_{20}$ requires C, 59.49; H, 7.15; N, 5.62%); $\lambda_{max}(CH_3OH)$/nm 280 (ε 7.95 × 10^3 dm^3 mol^{-1} cm^{-1}), 355 (1.88 × 10^4), 498 (5.60 × 10^3) and 530 (5.12 × 10^3); ν_{max}(KBr)/cm^{-1} 2130 (C≡N), 1735 (ester C=O) and 1120 and 630 (ClO_4^-); $\Delta\varepsilon(CH_3OH)$/deg cm^2 dmol^{-1} +4.3 (283.6 nm), −2.7 (305.6 nm), +3.6 (354.4 nm), +11.3 (432.4 nm), −6.9 (489.2 nm) and +3.2 (546.8 nm).

Strapped hydrophobic vitamin B_{12}



$(CN)_2$Cob(III)(c,10-PDA)6C_3ester (**6**):
X = Y = CN, Z = none

[(CN)(H_2O)Cob(III)(c,10-PDA)6C_3ester]ClO_4 (**7**):
X = CN, Y = H_2O, Z = ClO_4^-

[Cob(II)(c,10-PDA)6C_3ester]ClO_4 (**8**):
X = Y = none, Z = ClO_4^-

Cob(I)(c,10-PDA)6C_3ester:
X = Y = Z = none

Strapped hydrophobic vitamin B_{12} with divalent cobalt, [Cob(II)(c,10-PDA)6C_3ester]ClO_4 (8)

A methanol solution (100 cm^3) of compound **7** (80 mg, 6.7 × 10^{-5} mol) was deoxygenated by bubbling nitrogen gas through it for 30 min at room temperature, and sodium tetrahydroborate (100 mg, 2.6 × 10^{-3} mol) was added to the deoxygenated solution with vigorous stirring under a nitrogen atmosphere. When the solution turned dark green, 60% (w/w) aqueous perchloric acid (3 cm^3) was added carefully to it in order to decompose the excess of sodium tetrahydroborate and to convert the Co^I species into the corresponding Co^{II} species. The resulting cobalt complex was extracted with dichloromethane (25 cm^3 × 2). The extract was washed with distilled water, dried over sodium sulfate, and evaporated to dryness at room temperature. The residue was dissolved in benzene, and the product was recovered as a brown powder by reprecipitation with hexane: yield 65 mg (79%) (Found: C, 59.55; H, 7.2; N, 4.85. $C_{73}H_{104}ClCoN_5O_{19}\cdot H_2O$ requires C, 59.73; H, 7.28; N, 4.77%); $\lambda_{max}(CH_3OH)$/nm 273 (ε 1.67 × 10^4 dm^3 mol^{-1} cm^{-1}), 318 (2.79 × 10^4) and 472 (1.03 × 10^4); $\Delta\varepsilon(CH_3OH)$/deg cm^2 dmol^{-1} +12.7 (314.0 nm), −0.1 (356.4 nm), +1.5 (368.8 nm), −3.0 (430.0 nm), +2.5 (464.8 nm) and −0.8 (509.9 nm); *m/z* (FD and FAB) 1349 ([*M* − 99]$^+$; calc. *M* for [$C_{73}H_{104}ClCoN_5O_{19}$ − $^{35}ClO_4^-$] 1349).

^{13}C NMR assignments were performed for **1**–**6** with reference to those reported previously,[15] and are available as supplementary data [Sup. No. 57085 (7 pp.)].†

Cyclic voltammetry

An electrochemical cell similar to that reported previously[16] was used and equipped with platinum wire of 0.5 mm diameter as a working electrode. A saturated calomel electrode (SCE) served as a reference electrode and was separated from a bulk electrolyte solution by a salt bridge which was prepared with 1,2:4,5-di-*O*-benzylidene-D-glucitol[17] and an organic solvent containing tetrabutylammonium perchlorate (TBAP, 5.0 × 10^{-2} mol dm^{-3}). Acetonitrile, acetone, *N*,*N*-dimethylformamide (DMF) and dimethyl sulfoxide (DMSO) were used as media for cyclic voltammetry. An organic solution containing a hydrophobic vitamin B_{12} derivative and TBAP was deoxygenated by bubbling argon gas through it for 1 h prior to each measurement, and the cell interior was maintained under an argon atmosphere throughout each measurement. All the measurements were carried out at 20 ± 2 °C, and the scan rates were varied in the range 50–500 mV s^{-1}. Half-wave potentials ($E_{\frac{1}{2}}$) and anodic and cathodic currents were evaluated according to the method reported previously.[18]

Enantioselective alkylation of hydrophobic vitamin B_{12} derivatives

Alkylated complexes were prepared by reaction of the hydrophobic vitamin B_{12} derivatives in the Co^I state with various alkyl bromides in methanol, and identified by electronic and ^{1}H NMR spectroscopy as described previously.[12] The enantioselective alkylation for the hydrophobic vitamin B_{12} derivatives was examined by 500 MHz ^{1}H NMR spectroscopy, (+)-methyl (*R*)-3-bromo-2-methylpropionate and (−)-methyl (*S*)-3-bromo-2-methylpropionate (both from Aldrich, Milwaukee, WI) being adopted as reference substrates. The enantioselectivity in alkylation was evaluated from ^{1}H NMR signal areas with the aid of NMR1™ software (New Methods Research, Inc., E. Syracuse, NY) on a DEC 5000/200PX workstation. Preparation of the heptamethyl cobyrinate with an (*R*)-2-(methoxycarbonyl)propyl moiety is described below.

A methanol solution (50 cm^3) of [Cob(II)7C_1ester]ClO_4 (50 mg, 4.4 × 10^{-5} mol) was deoxygenated by bubbling nitrogen gas through it for 30 min, and sodium tetrahydroborate (100 mg, 2.6 × 10^{-3} mol) was added to the deoxygenated solution

† For details of the British Library supplementary publications scheme see 'Instructions for Authors (1995),' *J. Chem. Soc., Perkin Trans. 2*, 1995, Issue 1.

with vigorous stirring under a nitrogen atmosphere. The following operations were carried out in the dark. When the solution turned dark green, (+)-methyl (*R*)-3-bromo-2-methylpropionate (500 mg, 2.8×10^{-3} mol) was added to it. The resulting solution was stirred for 7 min at room temperature, and 60% (w/w) aqueous perchloric acid (3 cm^3) was added carefully to it in order to decompose the excess of sodium tetrahydroborate. The resulting mixture was extracted with dichloromethane (25 $cm^3 \times 3$), and the extract was washed with distilled water. After being dried over sodium sulfate, the extract was evaporated to dryness at room temperature. The product was purified by gel-filtration chromatography on a column of Sephadex LH-20 with methanol as the eluent in the dark. The first brown fraction was collected and evaporated to dryness at room temperature under reduced pressure. The residue was dissolved in benzene, and the product was recovered as a brown powder by reprecipitation with hexane: yield 40 mg (61%) (Found: C, 54.65; H, 6.65; N, 4.55. $C_{57}H_{84}ClCoN_4O_{21}$ requires C, 54.52; H, 6.74; N, 4.46%); $\lambda_{max}(CH_2Cl_2)$/nm 264 (ε 2.22×10^4 dm^3 mol^{-1} cm^{-1}), 302 (2.24×10^4), 378 (7.86×10^3) and 456 (8.51×10^3); ν_{max}(KBr)/cm^{-1} 1730 (ester C=O), and 1100 and 620 (ClO_4^-).

Other alkylated complexes were obtained by an identical procedure and respective yields and elemental analysis data are given as follows. Heptamethyl cobyrinate with an (*S*)-2-(methoxycarbonyl)propyl moiety, yield 74% (Found: C, 54.6; H, 6.55; N, 4.55. $C_{57}H_{84}ClCoN_4O_{21}$ requires C, 54.52; H, 6.74; N, 4.46%). Heptamethyl cobyrinate with an (*R*)-2-(cyclohexyloxycarbonyl)propyl moiety, yield 85% (Found: C, 56.15; H, 6.65; N, 4.55. $C_{62}H_{92}ClCoN_4O_{21}$ requires C, 56.52; H, 7.00; N, 4.23%). Heptamethyl cobyrinate with an (*S*)-2-(cyclohexyloxycarbonyl)propyl moiety, yield 88% (Found: C, 56.8; H, 6.75; N, 4.45. $C_{62}H_{92}ClCoN_4O_{21}$ requires C, 56.52; H, 7.00; N, 4.23%). Strapped hydrophobic vitamin B_{12} with an (*R*)-2-(methoxycarbonyl)propyl moiety, yield 78% (Found: C, 59.3; H, 7.1; N, 4.1. $C_{78}H_{113}ClCoN_5O_{22}$ requires C, 59.70; H, 7.39; 4.46%). Strapped hydrophobic vitamin B_{12} with an (*S*)-2-(methoxycarbonyl)propyl moiety, yield 70% (Found: C, 59.4; H, 7.05; N, 4.25. $C_{78}H_{113}ClCoN_5O_{22}$ requires C, 59.70; H, 7.39; N, 4.46%). Strapped hydrophobic vitamin B_{12} with an (*R*)-2-(cyclohexyloxycarbonyl)propyl moiety, yield 72% (Found: C, 60.55; H, 7.3; N, 4.0. $C_{83}H_{121}ClCoN_5O_{22}$ requires C, 60.89; H, 7.57; N, 4.28%). Strapped hydrophobic vitamin B_{12} with an (*S*)-2-(cyclohexyloxycarbonyl)propyl moiety, yield 65% (Found: C, 60.5; H, 7.3; N, 3.95. $C_{83}H_{121}ClCoN_5O_{22}$ requires C, 60.89; H, 7.57; N, 4.28%).

The strapped hydrophobic vitamin B_{12} [Cob(III)(c,10-PDA)-6C_3ester] with an axial ligand gave a complicated ^{1}H NMR spectrum relative to that of heptamethyl cobyrinate [Cob(III)7C_1ester] with an identical axial ligand. When an (*R*)-2-(methoxycarbonyl)propyl moiety was adopted as an axial ligand, for example, the strapped B_{12} derivative gave two doublet peaks (δ_H 0.32 and 0.52) assignable to Co-$CH_2CH(CH_3)CO_2CH_3$ and two multiplet peaks (δ_H −0.17 and −0.28) assignable to Co-$CH_2CH(CH_3)CO_2CH_3$, while the heptamethyl cobyrinate derivative gave only one doublet peak (δ_H 0.26) for the former and one multiplet peak (δ_H −0.68) for the latter. A ^{1}H NMR pattern for heptapropyl cobyrinate [Cob(III)7C_3ester] with an identical axial ligand was similar to that observed for the strapped B_{12} derivative. In order to clarify the origin of such specific signal-splitting behaviour as observed when a hydrophobic vitamin B_{12} has bulky peripheral substituents relative to those of heptamethyl cobyrinate, the following three possibilities were examined. (1) If rotational isomers are generated as a result of steric hindrance between an axial ligand and the peripheral substituents, the ^{1}H NMR feature must be temperature-dependent. However, the NMR spectrum remained unchanged over the temperature range 20–60 °C. (2) An axial ligand *trans* to the β-alkyl moiety would exert an electronic effect on the rotational freedom of the β-alkyl moiety. The specific ^{1}H NMR signals indicated above remained unchanged even when deuteriated pyridine was added as an α-axial ligand. (3) If both α- and β-alkylated complexes are present, such a state of affairs would give an apparent signal splitting. Heptamethyl cobyrinate in the Co^{II} state was reduced to the Co^I species with zinc in acetic acid and the resulting complex underwent reaction with methyl iodide to afford the corresponding α- and β-methylated complexes in a molar ratio of 9:91; singlet peaks were observed for α-methyl (δ_H −0.20) and β-methyl (δ_H −0.14) in $CDCl_3$.[19] Heptapropyl cobyrinate was treated in a similar manner to obtain the α- and β-methylated complexes; a singlet peak for α-methyl (δ_H −0.21) and two singlet peaks for β-methyl (δ_H −0.10 and −0.12; relative intensity, 4:6) were observed in $CDCl_3$. The result indicates that the signal-splitting is specific to the β-axial ligand. Consequently, the above three possibilities were discounted. Since the proton resonance due to the β-methyl group coordinated to heptapropyl cobyrinate consists of two peaks, the splitting of NMR signals assignable to the β-axial ligand is specific to modified corrinoid complexes bearing bulky peripheral substituents and is not derived from a conformational effect caused by the axial ligand. However, the origin is not clear at present.

We adopted two experimental strategies to synthesize alkylated complexes. (1) The reductive alkylation of a hydrophobic vitamin B_{12} was carried out in zinc–acetic acid with an alkyl halide. Brown *et al.* reported that this synthetic procedure was useful for the preparation of a pair of α- and β-alkylated complexes.[20] (2) Sodium tetrahydroborate was adopted as the reducing reagent in place of zinc–acetic acid. Brown *et al.* found out that α-alkylated corrinoids were generally dealkylated rapidly with tetrahydroborate while β-diastereoisomers remained unchanged. As a result, reductive alkylation with tetrahydroborate leads exclusively to the formation of β-alkylated corrinoids regardless of the nature of alkylating reagents.[20]

In light of the above strategies, we carried out the reductive alkylation of heptamethyl cobyrinate in zinc–acetic acid with two reference substrates, (+)-methyl (*R*)-3-bromo-2-methylpropionate and (−)-methyl (*S*)-3-bromo-2-methylpropionate. The major proton signals for the 2-(methoxycarbonyl)propyl moieties placed at the β-axial site of Cob(III)7C_1ester in $CDCl_3$ were assigned as follows: δ_H −0.68 [1 H, m, Co-$CH_2CH(CH_3)CO_2CH_3$], 0.26 [3 H, d, *J* 6.9, Co-$CH_2CH(CH_3)CO_2CH_3$] and 3.35 [3 H, s, Co-$CH_2CH(CH_3)CO_2CH_3$] for the *R*-configuration; δ_H −0.50 [1 H, m, Co-$CH_2CH(CH_3)CO_2CH_3$], 0.43 [3 H, d, *J* 7.3, Co-$CH_2CH(CH_3)CO_2CH_3$] and 3.40 [3 H, s, Co-$CH_2CH(CH_3)CO_2CH_3$] for the *S*-configuration. The major proton signals for the same alkyl ligand placed at the α-axial site of Cob(III)7C_1ester were assigned as follows: δ_H −0.62 [1 H, m, Co-$CH_2CH(CH_3)CO_2CH_3$], 0.31 [3 H, d, *J* 6.8, Co-$CH_2CH(CH_3)CO_2CH_3$] and 3.38 [1 H, m, Co-$CH_2CH(CH_3)CO_2CH_3$] for the *R*-configuration; δ_H −0.46 [1 H, m, Co-$CH_2CH(CH_3)CO_2CH_3$], 0.51 [3 H, d, *J* 7.1, Co-$CH_2CH(CH_3)CO_2CH_3$] and 3.42 [3 H, s, Co-$CH_2CH(CH_3)CO_2CH_3$] for the *S*-configuration. The molar ratio of the α-diastereoisomer to the β-diastereoisomer was found to be 0.8:99.2. On the other hand, the reductive alkylation with sodium tetrahydroborate afforded exclusively the β-diastereoisomer in the manner observed by Brown *et al.*[20]

Conformational search for alkylated hydrophobic vitamin B_{12} derivatives

Lowest energy conformations of the strapped hydrophobic vitamin B_{12} derivatives, having either (*R*)- or (*S*)-2-(cyclohexyloxycarbonyl)propyl moiety as a β-axial ligand, in the gas phase were examined on the basis of molecular mechanics

(BIOGRAF, MM2, and MMP2)[21] and molecular dynamics (BIOGRAF, AMBER, and CHARMM)[22] calculations as well as by a Monte Carlo conformational search[23] on an IRIS- 4D/220GTX workstation (Silicon Graphics). The stable conformation evaluated by molecular mechanics and dynamics calculations is dependent on the starting conformation. In the case of the alkylated vitamin B_{12} derivative, an energy barrier between conformations of local minimum energies is considered to be extremely high. As a result, it is quite hard to pass over such an energy barrier so as to attain a conformation with a global minimum energy by normal molecular dynamics manipulation. In this regard, X-ray structural data[24] for heptamethyl dicyanocobyrinate, $(CN)_2$Cob(III)7C_1ester, was used as the initial structural provision for the strapped hydrophobic vitamin B_{12}. The strapping group, the peripheral propyl groups and the cyclohexyl 2-methylpropionate moiety were introduced into the hydrophobic vitamin B_{12} on the basis of the interactive input method of BIOGRAF. Since Co^{III} in a complex generally assumes hexacoordination, the α-axial ligand of the alkylated hydrophobic vitamin B_{12} must be a water molecule in the absence of a more efficient coordinating ligand. The computations were carried out in order to save time for finding the conformation with a global minimum energy in accordance with the following computational sequence. (1) Conformational energies for the peripheral propyl groups were calculated by molecular mechanics and dynamics calculations, while the other bonds were fixed. (2) In order to determine the stable conformation for the (*R*)- or (*S*)-2-(cyclohexyloxycarbonyl)propyl moiety bound to the nuclear cobalt as the β-axial ligand, the molecular mechanics calculation was carried out by rotating forcibly the Co–C^{α} and C^{α}–C^{β} bonds by one degree intervals (total 129 600 steps). After the molecular mechanics calculation had been performed, the conformation of the local minimum energy was obtained for the β-axial ligand. In order to obtain the conformation with global minimum energy for the axial ligand, the rest of the complex structure was fixed during the molecular dynamics calculation as well as during the Monte Carlo conformational search. (3) For the strapped hydrophobic vitamin B_{12} with a β-axial ligand, stable conformations of the 2-(cyclohexyloxycarbonyl)propyl moiety and the strapping moiety were searched by molecular mechanics and dynamics calculations, while the rest of complex structure remained fixed. This manipulation was skipped for the simple hydrophobic vitamin B_{12} derivative. (4) Stable conformations of the 2-(cyclohexyloxycarbonyl)propyl moiety and the peripheral ester groups (in the case of the strapped hydrophobic vitamin B_{12} derivative, the strapping moiety was included in the calculations) were examined on the basis of molecular mechanics and dynamics calculations, while the remaining portions of the complex were fixed. (5) As the final stage, the conformations with global minimum energies for the strapped and simple hydrophobic vitamin B_{12} with axial ligands were searched by removing restriction of all atoms involved in the complexes except Co–N, Co–C^{α} and Co–α-H_2O bonds on the bases of molecular mechanics and dynamics calculations. The total molecular energy (E_{total}) is expressed as an energy sum of bonded and non-bonded interactions, and an enantiomer complex with a lower energy value is regarded to be more stable relative to the other [see eqn. (4)]. The bonded

$$E_{total} = E_b + E_\theta + E_\varphi + E_i + E_{vdw} + E_{el} + E_{hb} \quad (4)$$

interactions consist of bond stretching (E_b), bond angle bending (E_θ), dihedral angle torsion (E_φ) and inversion (E_i) terms, while the non-bonded interactions are composed of van der Waals (E_{vdw}), electrostatic (E_{el}) and hydrogen bonding (E_{hb}) terms.

Results and discussion

Redox behaviour of strapped hydrophobic vitamin B_{12}

Redox behaviour of the strapped hydrophobic vitamin B_{12} in various organic solvents, such as acetonitrile, acetone, DMF and DMSO, was examined by means of cyclic voltammetry. We identified the complex species formed during each redox process by the controlled-potential electrolysis as described previously.[25] The Co^{III}/Co^{II} and Co^{II}/Co^{I} redox potentials in various media are summarized in Table 1.

The Co^{III}/Co^{II} and Co^{II}/Co^{I} redox potentials for the strapped hydrophobic vitamin B_{12} are comparable to those for the simple hydrophobic vitamin B_{12}, [Cob(II)7C_3ester]ClO_4, in all the solvents used here. Since the liquid junction potential is subject to change by the nature of solvent systems, the redox potentials observed in various solvents cannot be compared directly with each other. Though liquid junction potentials between electrolyte solutions in various solvents have been extensively investigated, the most conventional and reliable means of eliminating the difference in liquid junction potential between various media is to adopt the observed redox potentials for the ferrocene/ferrocenium ion couple in various media as references. Because the true redox potential for the ferrocene/ferrocenium ion couple is considered to remain constant regardless of the nature of the organic medium,[26] the redox potentials corrected for the difference in liquid junction potential in the light of observed redox potentials for ferrocene/ferrocenium ion are shown in Table 1. Redox potentials for Co^{II}/Co^{I} and Co^{III}/Co^{II} of hydrophobic vitamin B_{12} derivatives are considered to be essentially unchanged in various organic solvents. This means that the electronic state of the nuclear cobalt atom remains primarily unchanged even after the strapping modification of the simple hydrophobic vitamin B_{12} is carried out. Consequently, the strapped hydrophobic vitamin B_{12} is able to simulate catalytic functions of the natural vitamin B_{12} in various microenvironments.

Enantioselective alkylation of hydrophobic vitamin B_{12} derivatives

The enantioselective alkylation of Cob(I)7C_1ester and the strapped hydrophobic vitamin B_{12} was examined by 500 MHz ^{1}H NMR spectroscopy as described in the Experimental section; (+)-methyl (*R*)-3-bromo-2-methylpropionate and (−)-methyl (*S*)-3-bromo-2-methylpropionate being adopted as reference substrates. The proton signals for the 2-(methoxycarbonyl)propyl moiety placed at the β-axial site of cob(III)7C_1ester in $CDCl_3$ were assigned as follows: δ_H −0.68 [1 H, m, Co-$CH_2CH(CH_3)CO_2CH_3$], 0.26 [3 H, d, *J* 7.3, Co-$CH_2CH(CH_3)CO_2CH_3$] and 3.35 [3 H, s, Co-$CH_2CH(CH_3)CO_2CH_3$] for the *R*-configuration; δ_H −0.50 [1 H, m, Co-$CH_2CH(CH_3)CO_2CH_3$], 0.43 [3 H, d, *J* 6.9, Co-$CH_2CH(CH_3)CO_2CH_3$] and 3.40 [3 H, s, Co-$CH_2CH(CH_3)CO_2CH_3$] for the *S*-configuration. For the 2-(cyclohexyloxycarbonyl)propyl moiety coordinated to the same hydrophobic vitamin B_{12}, the following assignments were made (in $CDCl_3$): δ_H −0.62 [1 H, m, Co-$CH_2CH(CH_3)CO_2$-cyclohexyl] and 0.35 [3 H, d, *J* 7.2, Co-$CH_2CH(CH_3)CO_2$-cyclohexyl] for the *R*-configuration; δ_H −0.42 [1 H, m, Co-$CH_2CH(CH_3)CO_2$-cyclohexyl] and 0.44 [3 H, d, *J* 7.1, Co-$CH_2CH(CH_3)CO_2$-cyclohexyl] for the *S*-configuration. The proton signals for the 2-(methoxycarbonyl)-propyl moiety placed at the β-axial site of the strapped hydrophobic vitamin B_{12} were assigned as follows: $\delta_H(CDCl_3)$ −0.28, −0.17 [1 H, m, Co-$CH_2CH(CH_3)CO_2CH_3$], 0.32, 0.52 [3 H, d, *J* 7.0 and 7.1, Co-$CH_2CH(CH_3)CO_2CH_3$] and 3.44, 3.48 [3 H, s, Co-$CH_2CH(CH_3)CO_2CH_3$] for the *R*-configuration; $\delta_H(CDCl_3)$ −0.37, −0.32 [1 H, m, Co-$CH_2CH(CH_3)CO_2CH_3$], 0.46, 0.54 [3 H, d, *J* 7.0 each, Co-$CH_2CH(CH_3)CO_2CH_3$] and 3.42, 3.49 [3 H, s, Co-CH_2CH-

Table 1 Redox potentials for strapped and simple hydrophobic B_{12} derivatives in non-aqueous media[a]

Medium[b]	DN[c]	E_T^{Nd}	Complex[e]	$E_{\frac{1}{2}}$ vs. SCE/V		$E_{\frac{1}{2}}$ vs. (Fc^+/Fc)/V[f]	
				Co^{III}/Co^{II}	Co^{II}/Co^{I}	Co^{III}/Co^{II}	Co^{II}/Co^{I}
CH_3CN	14.1	0.460	Strapped B_{12}	+0.58	−0.53	+0.12	−0.99
			Cob(II)7C_3ester	+0.54	−0.57	+0.08	−1.03
MeCOMe	17.0	0.355	Strapped B_{12}	+0.87	−0.47	+0.35	−0.99
			Cob(II)7C_3ester	+0.82	−0.47	+0.30	−0.99
DMF	26.6	0.404	Strapped B_{12}	+0.41	−0.61	−0.03	−1.05
			Cob(II)7C_3ester	+0.43	−0.62	−0.01	−1.06
DMSO	29.8	0.444	Strapped B_{12}	+0.41	−0.65	+0.00	−1.06
			Cob(II)7C_3ester	+0.28	−0.66	−0.13	−1.07

[a] Measured at 20 ± 2 °C; scan rate, 100 mV s^{-1}. [b] Abbreviations: DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide. [c] Donor number of a solvent; refer to ref. 27. [d] Solvent polarity parameter; refer to ref. 28. [e] Abbreviations: strapped B_{12}, [Cob(II)(c,10-PDA)6C_3ester]ClO_4; Cob(II)7C_3ester, [Cob(II)7C_3ester]ClO_4. [f] Abbreviations: Fc, ferrocene; Fc^+, ferrocenium ion.

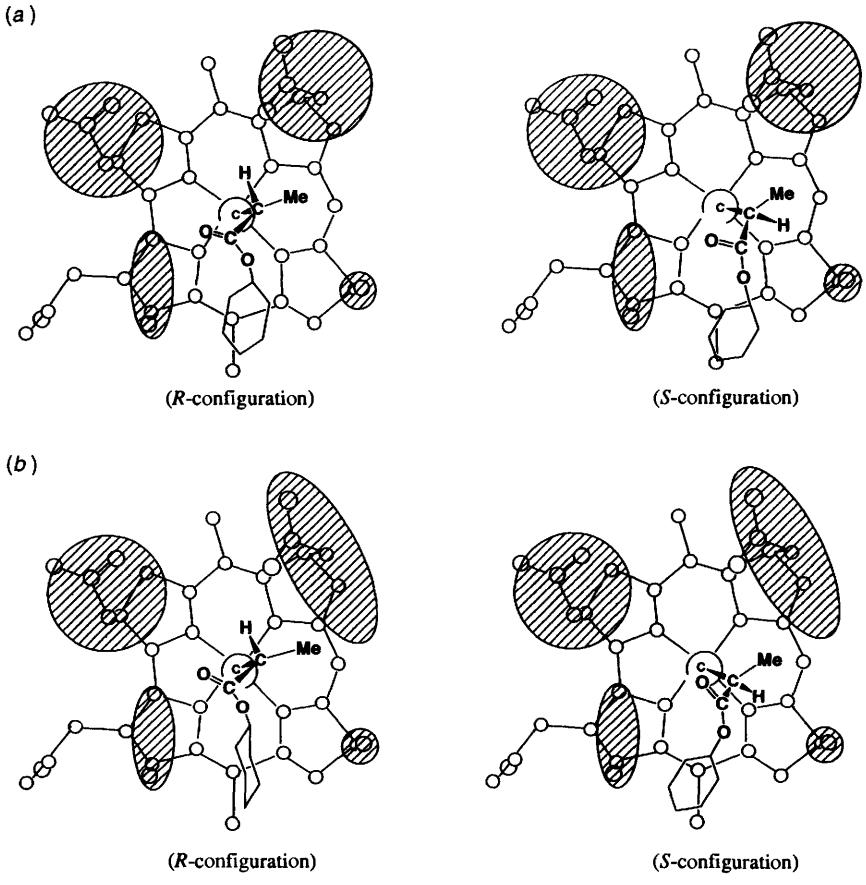
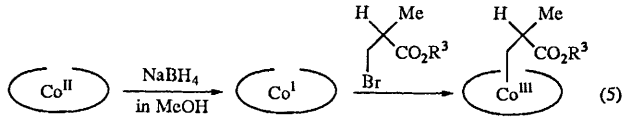


Fig. 1 Schematic representations (top views) for configurations of (*R*)- and (*S*)-2-(cyclohexyloxycarbonyl)propyl moieties bound to the β-axial site of: (*a*) simple hydrophobic vitamin B_{12} (heptamethyl cobyrinate); (*b*) strapped hydrophobic vitamin B_{12}

$(CH_3)CO_2CH_3$] for the *S*-configuration. For the 2-(cyclohexyloxycarbonyl)propyl moiety bound to the same hydrophobic vitamin B_{12}, the following assignments were made (in $CDCl_3$): δ_H −0.59, −0.48 [1 H, m, Co-$CH_2CH(CH_3)CO_2$-cyclohexyl] and 0.39, 0.48 [3 H, d, *J* 7.0 each, Co-$CH_2CH(CH_3)CO_2$-cyclohexyl] for the *R*-configuration; δ_H −0.40, −0.29 [1 H, m, Co-$CH_2CH(CH_3)CO_2$-cyclohexyl] and 0.43, 0.52 [3 H, d, *J* 7.4 each, Co-$CH_2CH(CH_3)CO_2$-cyclohexyl] for the *S*-configuration. The results indicate that the proton signals for the alkyl moiety with *R*-configuration appear in upper field ranges relative to those for an identical alkyl moiety with *S*-configuration. The enantioselectivity in alkylation was evaluated from the ^1H NMR signal areas.

When the racemic 3-bromo-2-methylpropionic ester was used, the *S*-enantiomer in the alkylation has an advantage over



(5)

that of the corresponding *R*-enantiomer in terms of reactivity. The enantioselectivity data for the formation of the alkylated complexes are listed in Table 2 [see eqn. (5)]. The highest enantioselectivity was attained for the reaction of the strapped hydrophobic vitamin B_{12} with a 3-bromo-2-methylpropionate bearing a cyclohexyl ester group, 75% ee. This is the highest enantioselectivity in alkylation so far observed among reactions with vitamin B_{12} and related compounds.

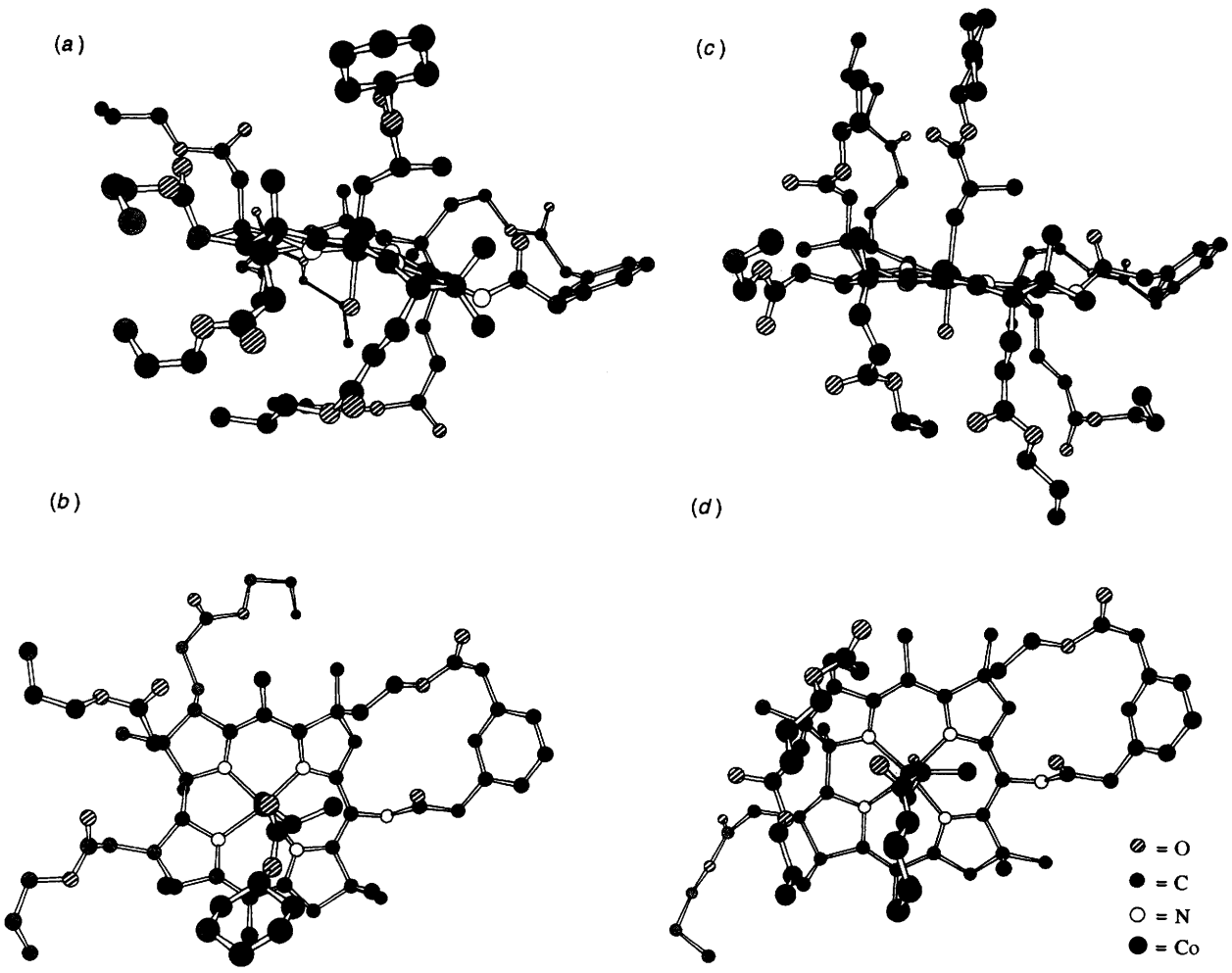


Fig. 2 Side and top views of low-energy conformations for alkylated strapped hydrophobic vitamin B_{12} derivatives having (*R*)- and (*S*)-2-(cyclohexyloxycarbonyl)propyl groups as β-axial ligands, as evaluated on the basis of molecular mechanics and dynamics calculations in the gas phase: (*a*) and (*b*), side and top views with the (*S*)-2-(cyclohexyloxycarbonyl)propyl group as an axial ligand, respectively; (*c*) and (*d*), side and top views with the (*R*)-2-(cyclohexyloxycarbonyl)propyl moiety as an axial ligand, respectively. Side views of the alkylated complexes are shown without hydrogen atoms, while top views of the complexes are given without hydrogen atoms and α-site peripheral substituents of the corrin ring.

Table 2 Enantioselectivity evaluated by ^1H NMR spectroscopy for alkylated complexes derived from hydrophobic B_{12} derivatives and racemic 3-bromo-2-methylpropionic ester in methanol at room temperature[a]

R^3 in alkyl bromide[b]	Vitamin B_{12} derivative[c]	Yield (%)[d]	Selectivity[e]	
			% ee	Configuration
CH_3	Cob(I)7C_1ester	67	34	*S*
	Strapped B_{12}	75	41	*S*
cyclohexyl	Cob(I)7C_1ester	86	55	*S*
	Strapped B_{12}	69	75	*S*

[a] Each reaction was carried out with a 1:5 molar ratio of a vitamin B_{12} derivative to alkyl bromide; vitamin B_{12} derivative, 50 mg. [b] Refer to eqn. (5). [c] Abbreviation: strapped B_{12}, Cob(I)(c,10-PDA)-6C_3ester. [d] Isolated yield, as purified by gel-filtration chromatography on a column of Sephadex LH-20 with methanol as the eluent in the dark; crude yield, above 80%. [e] Accurate and reproducible to within the numbers indicated here as confirmed by repeated experiments.

Conformational analysis for strapped vitamin B_{12} with an axial ligand

We evaluated stereochemical arrangements of *R*- and *S*-alkyl moieties bound to the strapped vitamin B_{12} on the basis of molecular mechanics and dynamics calculations as well as by a Monte Carlo conformational search.

We examined stereochemical arrangements of (*R*)- and (*S*)-2-(cyclohexyloxycarbonyl)propyl moieties bound to the nuclear cobalt as β-axial ligands for the simple and strapped hydrophobic vitamin B_{12} complexes in the light of X-ray crystal structures obtained for $(CN)_2$Cob(III)7C_1ester[24] as well as for adenosylcobalamin and 2,3-dihydroxypropylcobalamin.[29] Fig. 1 illustrates schematic representations of the alkylated vitamin B_{12} derivatives as viewed from the β-site of the corrin framework. The molecular structure of the cobalamin framework, as clarified for those cobalamin derivatives, was applied directly to these representations. The steric factors generated around rings A, B, C and D of the present B_{12} derivatives are shown by shaded circles to indicate qualitatively the effective bulkiness toward the axial ligand bound to the nuclear cobalt. The steric bulkiness of the peripheral substituent placed around ring B in the strapped hydrophobic vitamin B_{12} seems to be much larger than that in the simple hydrophobic vitamin B_{12}. The main controlling force for determining lowest energy conformations for the alkylated vitamin B_{12} derivatives is attributed to the magnitude of steric hindrance between the axial ligand and the equatorial ligand of the cobalt atom.

Top and side views of the low energy conformations for the strapped hydrophobic vitamin B_{12} derivatives, having the chiral

2-(cyclohexyloxycarbonyl)propyl group as a β-axial ligand, in the gas phase are illustrated in Fig. 2. The E_{total} values for the low energy conformations of the simple hydrophobic vitamin B_{12} (heptamethyl cobyrinate) with *R*- and *S*-axial ligands are 90.2 and 87.4 kJ mol^{-1}, respectively; E_b = 16.6 and 14.8 kJ mol^{-1}, E_θ = 26.1 and 25.3 kJ mol^{-1}, E_φ = 18.1 and 19.5 kJ mol^{-1}, E_i = 0.38 and 0.29 kJ mol^{-1}, E_{vdw} = 31.0 and 28.8 kJ mol^{-1}, E_{el} = −1.16 and −1.23 kJ mol^{-1}, E_{hb} = −0.85 and −0.008 kJ mol^{-1}, respectively, while the E_{total} values for the low energy conformations of the strapped hydrophobic vitamin B_{12} with the *R*- and *S*-alkyl moieties are 111.6 and 107.7 kJ mol^{-1}, respectively; E_b = 20.9 and 18.2 kJ mol^{-1}, E_θ = 32.9 and 31.2 kJ mol^{-1}, E_φ = 20.9 and 24.0 kJ mol^{-1}, E_i = 0.48 and 0.36 kJ mol^{-1}, E_{vdw} = 38.9 and 35.5 kJ mol^{-1}, E_{el} = −1.42 and −1.51 kJ mol^{-1}, E_{hb} = −1.04 and −0.005 kJ mol^{-1}, respectively. The alkylated complex with the *S*-axial ligand is low in energy relative to that with the *R*-ligand. This tendency is consistent with the result obtained by the alkylation studies. The peripheral substituent of the strapped hydrophobic vitamin B_{12} placed around ring B is considered to generate an effective steric effect toward an alkyl moiety bound to the nuclear cobalt, compared with the simple hydrophobic vitamin B_{12}. According to the lowest energy conformation for the above alkylated complex, the most bulky group, a cyclohexyl ester group, placed on the β-carbon atom of the alkyl moiety is directed toward a space intervening between rings C and D. The methyl group on the β-carbon of (*S*)- and (*R*)-2-(cyclohexyloxycarbonyl)propyl moieties bound to the nuclear cobalt is directed toward the strapping peripheral moiety of the corrin ring as illustrated in Figs. 1 and 2. According to the conformational analysis for the strapped hydrophobic vitamin B_{12} with the axial ligand, the stereochemical configuration of the *R*-enantiomer shown in Figs. 2(*c*) and 2(*d*) seems to be energetically less favourable than that of the *S*-enantiomer shown in Figs. 2(*a*) and 2(*b*), because a steric interaction is expected to be pronounced between the β-methyl group of the axial ligand and the strapping moiety of hydrophobic vitamin B_{12}. In addition, a difference in E_{total} between the strapped hydrophobic vitamin B_{12} with the *S*-axial ligand and that with the *R*-axial ligand (3.9 kJ mol^{-1}) is greater than the corresponding value (2.8 kJ mol^{-1}) for the simple hydrophobic vitamin B_{12}. This means that the *S*-selectivity of the strapped vitamin B_{12} is greater than that of the simple hydrophobic vitamin B_{12} by 1 kJ mol^{-1}.

The alkylation reaction must take place *via* nucleophilic attack of the supernucleophilic Co^I species on the alkyl bromide [30] in a specific chiral cage around the β-axial site of the corrin ring. Under such stereochemical conditions, the chiral selectivity in the transition state can be rationalised in the light of the above computational results obtained for the final alkylated complexes. In other words, the main driving force for the *S*-enantioselectivity in the alkylation of the strapped hydrophobic vitamin B_{12} comes from a significant steric effect caused by the strapping peripheral moiety placed around the corrin's B ring.

In conclusion, the enantioselectivity in alkylation of the strapped hydrophobic vitamin B_{12} is superior to that of the simple hydrophobic vitamin B_{12}. This means that the peripheral substituent of the strapped hydrophobic vitamin B_{12} provides an effective stereospecific microenvironment on the β-axial site of the corrin ring for recognition of the *S*-enantiomer. This effect is also supported by the molecular mechanics and dynamics calculations. To the best of our knowledge, the present finding can be cited as the highest enantioselectivity in alkylation of vitamin B_{12} model compounds. We believe that the present results provide a useful guide towards designing a functionalized and artificial vitamin B_{12} enzyme capable of performing high enantioselective reactivity.

Acknowledgements

The present work was supported by a Special Distinguished Grant for Scientific Research No. 02102006 from the Ministry of Education, Science and Culture of Japan and a grant from the Tokuyama Science Foundation.

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Paper 4/04654D
Received 29*th July* 1994
Accepted 1*st February* 1995