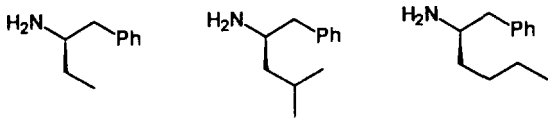

Figure 2. Structures of various optically pure primary amines.

Thus, we have developed a simple, practical and enantioselective method for the synthesis of a chiral primary amine using L- or D-amino acid as a starting material.

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5. R_f Value of **7** and **8** was 0.35 and 0.20 in 30% ethyl acetate in n-hexane, respectively.
   7 : ^{1}H NMR ($CDCl_3$) δ 0.81 (t, 3H), 1.25 (m, 2H), 1.34 (m, 2H), 2.44 (m, 1H), 2.68 (dd, 1H), 2.66 (m, 2H), 2.77 (dd, 1H), 3.16 (dd, 1H), 4.03 (m, 1H), 4.41 (m, 1H), 4.82 (s, 1H), 4.92 (m, 2H), 5.42 (m, 1H), 5.51 (dd, 1H), 6.54 (d, 1H), 7.07-7.31 (m, 10H).
   8 : ^{1}H NMR ($CDCl_3$) δ 0.80 (t, 3H), 1.16-1.29 (m, 3H), 1.39 (m, 1H), 2.60-2.81 (m, 6H), 4.03 (m, 1H), 4.28 (m, 1H), 4.71 (s, 1H), 4.94 (m, 2H), 5.40 (m, 1H), 5.49 (dd, 1H), 5.63 (d, 1H), 7.03-7.28 (m, 10H).
6. $[\alpha]_D = -30.6$ (c=0.05, CH_2Cl_2).
   ^{1}H NMR ($CDCl_3$) δ 0.89 (t, 3H), 1.16 (s, 2H), 1.41 (m, 2H), 1.50 (m, 2H), 2.48 (m, 2H), 2.81 (m, 1H), 3.01 (m, 1H), 7.18-7.33 (m, 5H).

Assignment of Heme Proton Signals of Cytochrome c_3 of *Desulfovibrio vulgaris* Miyazaki F by ^{1}H NMR

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Cytochrome c_3 (cyt c_3) isolated from a sulfate-reducing bacterium, possesses four *c*-type heme groups per molecule. It is involved in the electron-transport system in the bacteria, as a partner of hydrogenase. Since the crystal structure of cyt c_3 from *Desulfovibrio vulgaris* Miyazaki F (*Dv*MF) is available at 0.18 nm resolution,[1] the relationship between its structure and redox behavior can be discussed in detail. The final goal is to elucidate the structural factors which determine the redox potentials of each of the four hemes. In this study, the total assignment of heme methyl and propionate signals was carried out.

*Dv*MF was cultured in medium C.[2] Cyt c_3 was purified according to the procedure reported previously.[3] In NMR experiments, a trace amount of hydrogenase was add to a 1.3 mM cyt c_3 solution (molar ratio, *ca.* 0.001) as a redox catalyst. The hydrogenase was purified from *Dv*MF cells according to the reported method.[4] Partial reduction (referred to as the intermediate redox stage hereafter) of a cyt c_3 solution was achieved by controlling the ratio of hydrogen and argon gases in an NMR tube. ^{1}H NMR spectra were obtained on a Bruker AM 400 NMR spectrometers at 30 ℃. Chemical shifts are presented in parts per million relative to an internal standard of 2,2-dimethyl-2-silpentane-5-sulfonate (DSS). Saturation transfer experiments were carried out for various intermediate redox stages in order to assign heme methyl resonances in the five macroscopic oxidation states. Sixteen free induction decays (FID) were accumulated alternately under on-resonance and off-resonance irradiation for 1s. Nuclear Overhauser effect (NOE) experiments were performed with typically 0.1s preirradiation and accumulation of 8000 transients. One thousand transients were accumulated for each FID. Two-dimensional (2D) TOCSY(HOHAHA) spectra were measured at 30 ℃ with a data size of 512x2048, spectral width of 8064 Hz and mixing time of 26.6 ms. 2D NOESY spectra were measured with the same data size, spectra width of 12820 Hz and mixing time 60 ms.

The NMR spectra of *Dv*MF cyt c_3 in various redox stages were discussed previously.[5,6] The assignment of 13 heme methyl signals (designated as A-M) has been performed.[3,7] Among them, signals J (13.46 ppm) and L (10.30 ppm) were classified to each heme groups on the basis of the first and second reduction fractions, R^1 and R^{II},[5] (the term of electron distribution probability was used in the reference). Unfortunately, they did not include the major reduction step ($R^I > 0.5$). It was shown for cyt c_3 from *D. vulgaris* Hildenborough (*Dv*H) that the reduction behavior of signal J is unusual.[8] Since J was the key signal in the heme assignment,[7] our assignment for hemes 2 and 3 was questioned.[8] Our assignment for hemes 1 and 4 was consistent signals A, H, I, K (heme 4) and B, F, G, M (heme 1) are on the firm basis. To make the correct assignment, we have carried out the total assignment of the heme methyl and propionate signals.

Saturation transfer experiments have been carried out again for signal J at p^{2}H 7.0 to determine the chemical shifts of heme methyl J in all reduction steps (macroscopic oxidation states). As shown in Figure 1, signal J could be identified in the five macroscopic oxidation states.

The chemical shifts were 13.46, 13.86, 11.45, 12.10 and about 4.7 ppm for the fully oxidized (S_0), one-electron reduced (S_1), two-electron reduced (S_2), three-electron reduced (S_3) and fully reduced (S_4) states, respectively. The chemical shift for the fully reduced state was also confirmed in the assignment of the heme protons of ferrocytochrome c_3 (unpublished data). The unusual behavior of signal J was similar

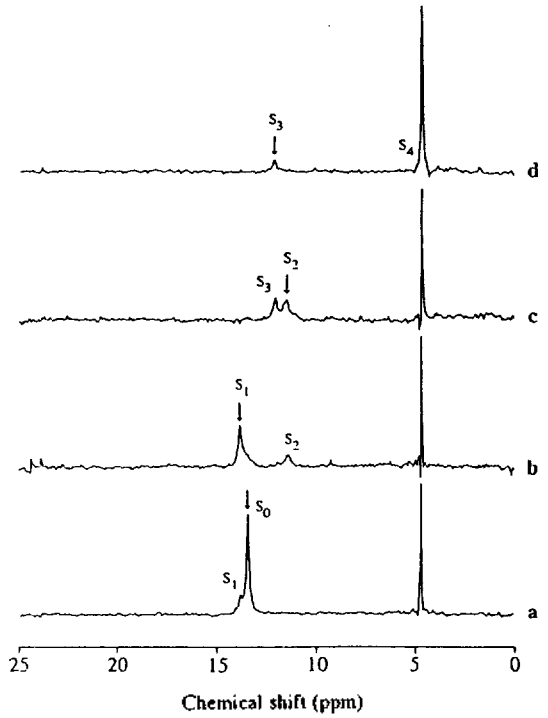

Figure 1. Saturation transfer NMR difference spectra of *Dv*MF cyt c_3 at p^2H 7.0 and 30 ℃. The irradiated position are indicated with arrows. S_0, S_1, S_2, S_3 and S_4 stand for the fully oxidized, one-electron reduced, two-electron reduced, three-electron reduced and fully reduced states, respectively.

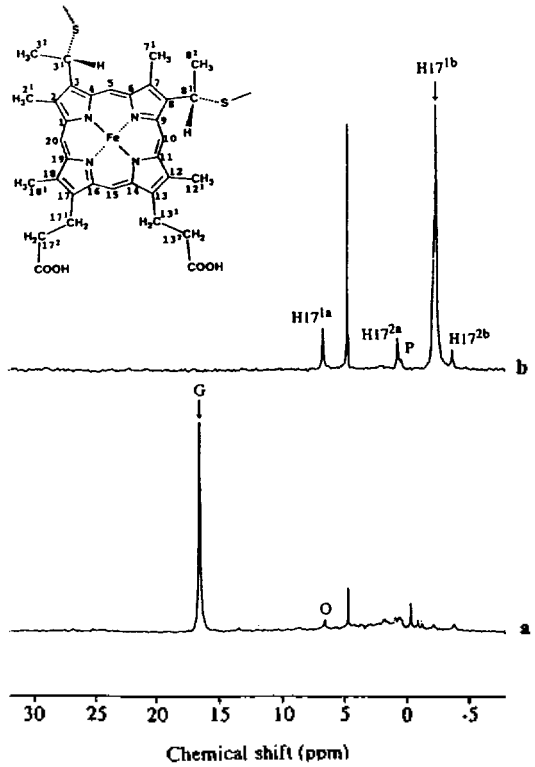

Figure 2. NOE difference spectra of *Dv*MF ferricytochrome c_3 at p^2H 7.1 and 30 ℃. Heme methyl signal G (a) and an α-CH_2 signal of a propionate (b) were irradiated. O and P are the labels for the heme methyl signals. IUPAC-IUB nomenclature of *c*-type heme is presented on the top.

to that found for *Dv*H cyt c_3. Since signal J was mainly reduced in the fourth step, J should be classified to heme 3 (the heme numbers indicating the order of bonding to the primary sequence) as indicated by Park *et al.*[9] J was assigned to the methyl protons at C-2 of heme 3 on the basis of interheme NOE[3,5] (the IUPAC-IUB nomenclature was also used for the heme carbons and protons in this paper).

We have shown that heme methyl groups at C-2 and C-7 are transferred from the methyl group of methionine in *Dv*MF cells. By specific deuteration, a heme methyl signal at 6.44 (signal O) was ascribed to the heme methyl group either at C-2 or C-7 of heme 2 or 3.[10] Furthermore, interheme NOE was observed for a pair of signal G and O as shown in Figure 2a.

The strongest interheme NOE signal was observed for the pair of signals I and J.[5] According to the crystal structure, the interproton distance between C-12^1 of heme 1 (signal G) and C-2^1 of heme 2 is 0.312 nm. It is the second shortest in all interheme methyl proton distance. Now, signal O can be ascribed to the heme methyl group at C-2 of heme 2.

The proton signals of six propionate groups were identified.[3] Assignment of β-CH_2 of the propionate at C-13 of heme 1 was completed by the 2D NMR experiments at different p^2H. Since the assignments were connected with those of heme methyl signals, the heme number of two propionate groups should be revised. In addition to them, two sets of ABXY connectivity have been identified in a 2D TOCSY spectrum. They were confirmed also by 1D NOE difference spectra. One of them, irradiating at proton of the propionate,

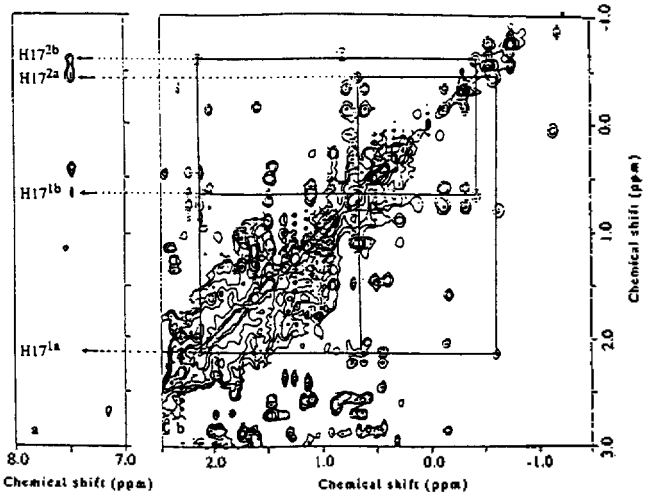

Figure 3. Parts of 2D-TOCSY and NOESY spectra of ferricytochrome c_3 at p^2H 7.1 and 30 ℃. Connectivities in the TOCSY spectrum are shown by solid lines and the correlation with the NOESY cross peaks are shown by broken lines.

is shown in Figure 2b. A weak signal at 0.42 ppm can be assigned to a heme methyl group (signal P). Since this signal also appears in the NOE difference spectrum on irradiation at signal J,[3] this can be assigned to the heme methyl group at C-18 of heme 3, which is the second nearest one from

Table 1. Resonance assignments of heme methyl and propionate protons of *Dv*MF ferricytochrome c_3 at p^2H 7.0 and 30 ℃

Heme number	Position	Chemical shift/ppm
	2^1CH_3	18.92 (F)
	7^1CH_3	9.60 (M)
	12^1CH_3	18.07 (G)
1	18^1CH_3	29.27 (B)
h2′	13^1CH_2	0.41, −3.92
	13^2CH_2	1.42, −2.20
	17^1CH_2	5.79, 4.46
	17^2CH_2	2.65, 2.41
	2^1CH_3	6.44 (O)
	7^1CH_3	20.21 (D)
	12^1CH_3	20.49 (C)
2	18^1CH_3	7.51 (N)
h3′	13^1CH_2	11.36, 4.67
	13^2CH_2	0.67, −0.63
	17^1CH_2	2.22, 0.71
	17^2CH_2	−0.35, −0.57
	2^1CH_3	13.46 (J)
	7^1CH_3	10.30 (L)
	12^1CH_3	19.91 (E)
3	18^1CH_3	0.42 (P)
h4′	13^1CH_2	17.67, 16.05
	13^2CH_2	0.08, −1.18
	17^1CH_2	6.71, −2.32
	17^2CH_2	0.80, −3.60
	2^1CH_3	17.47 (H)
	7^1CH_3	10.64 (K)
	12^1CH_3	16.51 (I)
4	18^1CH_3	30.46 (A)
h1′	13^1CH_2	−0.23, −3.76
	13^2CH_2	0.20, 0.60
	17^1CH_2	9.62, 6.12
	17^2CH_2	3.62, 3.35

( ), labels of the heme methyl signals in the text and hi′, the heme numbering according to the order of the major reduction.

2-CH_3 of heme 3 (J). The TOCSY connectivity of the last propionate is shown in Figure 3.

From NOESY cross peaks, the heme methyl group in the proximity (signal N) could be identified (Figure 3). On irradiation at signal N, an NOE signal was observed at signal O (the spectrum is not shown). Since the second nearest heme methyl group from 2-CH_3 of heme 2 (O) is that at C-18 of heme 2, signal N can be assigned to it. This was confirmed by an NOESY cross between the β proton of 17-propionate of heme 2 and His67 C_4H, the interproton distance of which is 0.326 nm according to the crystal structure. Now, signal L is the only one left and should be ascribed to 7-CH_3 of heme 3. The assignment could be carried out consistently just by moving signal J from h3′ to h4′. The assignments of heme methyl groups and propionate groups were summarized in Table 1.

The heme assignment was revised for hemes 2 and 3 (sequential heme number). Our next target is to elucidate the structural factors which determine the redox potentials of each of the four hemes on the basis of the assignments established in this work.

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Intramolecular Hydrodimerization of Activated Dienes Mediated by Magnesium in Methanol

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It has been reported that the electrochemical hydrodimerization of α,β-unsaturated ketones, esters, and nitriles proceeds *via* anion radicals in aprotic media in the absence of metal cations, and allyl radicals in protic media, respectively.[1] As previously noted for sequential C-C bond formation *via* one electron transfer, methods for intramolecular β-coupling reaction of activated olefins have been limited to electrochemical hydrodimerization and n-Bu_3SnH.[2] Recently, it has been reported that intramolecular cyclization of activated dienes with magnesium metal in methanol at room temperature proceeds smoothly.[3] we proposed that reactions proceed *via* allyl radical intermediate resulting from one electron transfer to activated olefins followed by protonation of anion radicals in the presnece of proton donor, methanol.[4]

Here we report that intramolecular hydrodimerization of