SYNTHESIS OF 1-FLUORO-1-DEMETHYLMESOPORPHYRIN-IX

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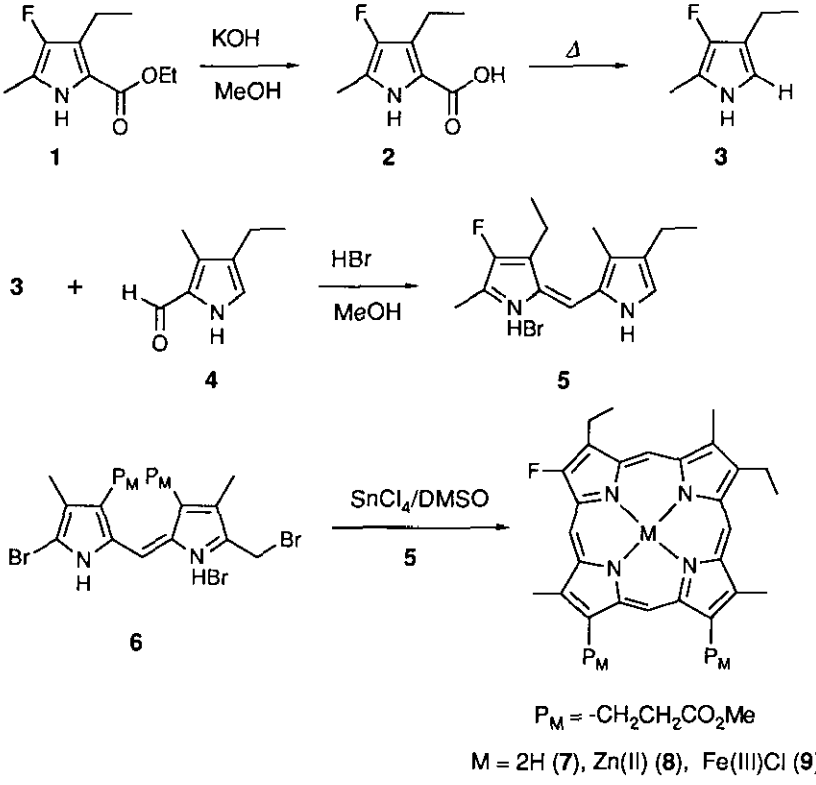
Abstract-Total syntheses of 1-fluoro-1-demethylmesoporphyrin-IX and its metal complexes are described. The iron(III) complex forms stable reconstituted myoglobin. Paramagnetic ^{19}F-nmr spectra of metalloporphyrins were measured for the high and low spin states of the iron(III) complexes.

Regio- and site-specific fluorination of biologically important organic compounds and proteins has provided sophisticated approach in order to give deep insight to understanding structure and function of enzymes. If ^{19}F nmr signal of the synthetic hemin containing fluorine at peripheral positions of porphyrin incorporated in the heme enzymes is observable for both diamagnetic and paramagnetic complexes, the present method using unnatural element as a nuclear probe may give us new information about heme enzyme. For this purpose, we have prepared pyrroles, porphyrins, and hemes directly linked with perfluoroalkyl groups and fluorine.[1] Recently Smith has reported 8-fluoromethyl analogues of protoporphyrin-IX and related derivatives.[2] We have reported that ^{19}F nmr spectra of perfluoroalkylporphyrin iron(III) complexes and reconstituted myoglobin reveal clear signals for the high and low spin states.[3] In particular, the ring-fluorinated hemes replaceable with naturally occurring prosthetic heme such as iron complex of protoporphyrin-IX have interested us in the reconstitution investigation of heme enzymes and hemeproteins. Here, we wish to report briefly synthesis of the ring-fluorinatedporphyrin,1-fluoro-2,4-diethyl-3,5,8-trimethyl-6,7-dimethoxycarbonylethylporphyrin, characteristic physico-chemical properties, and reconstituted myoglobin with the monofluorohemin and sperm whale apomyoglobin.

Porphyrin frame-work is divided to two kinds of dipyrromethenes on the basis of facile retro-synthetic consideration. Monofluoropyrrole (**1**)[4] was obtainable in 11.5% yield from photochemical reaction of tetrafluoroborate salt of ethyl 2-methyl-3-diazonium-4-ethylpyrrole-5-carboxylate according to our previous report.[1c] Hydrolysis of **1** (798 mg) with KOH (1.5 g) in CH_3OH (30 ml) containing H_2O (1 ml) at 75 °C for 12 h gave 2-methyl-3-fluoro-4-ethylpyrrole-5-carboxyric acid (**2**)[5] in 72.4% yield. Decarboxylation of **2** (221 mg) in a glass vessel of Kugelrohr at 180°C/3 mmHg under N_2 atmosphere afforded light green colored liquid **3** (133mg, 78.3%). The product was used immediately without further purification.[6] Condensation of

3 (133 mg) and 2-formyl-3-methyl-4-ethylpyrrole (**4**)[7] (143 mg) with 47% HBr (0.23 ml) in CH_3OH (1.1 ml) gave 3',5-dimethyl-3,4'-diethyl-4-fluoro-2,2'-dipyrromethene hydrobromide (**5**)[8] (268 mg, 78.3%). A mixture of pyrrolemethene (**5**) (129mg) and 3,3'-dimethoxycarbonylethyl-4,4'-dimethyl-5-bromo-5'-bromomethyl-2,2'-dipyrromethenehydrobromide (**6**)[9] (236 mg) was treated with anhydrous $SnCl_4$ in dry CH_2Cl_2 (95 ml). After concentration of reaction mixture to dryness, the residue was oxidized in DMSO (70 ml) containing pyridine (1.7 ml) at room temperature for 48 h. Usual work-up, chromatography on alumina gel with $CHCl_3$, and crystallization from $CHCl_3$-CH_3OH afforded 1-fluoro-2,4-diethyl-3,5,8-trimethyl-6,7-dimethoxycarbonylethylporphyrin (**7**)[10] as red purple crystals in 27.5% yield; mass spectrum m/z 598 (M^+); ^{19}F nmr ($CDCl_3$, $CFCl_3$); singlet at -147 ppm. The Zn(II) complex (**8**) was prepared by refluxing chloroform solution of free base (**7**) containing $Zn(OCOCH_3)_2$ (45 %).[11]



The Fe(III) complex (**9**) was prepared by heating acetic acid solution of free base (**7**) containing $FeSO_4 \cdot 7H_2O$, NaCl and pyridine (74%).[12] Hydrolysis of dimethyl ester (**9**) with aq. KOH and CH_3OH gave the hemin (**10**) in 56.6% yield. Reconstitution with sperm whale apo-myoglobin and **10** in a 0.1 M potassium phosphate buffer solution (pH 7.0) brought about formation of stable unnatural myoglobin. The iron(III) complexes at both high (S = 5/2) and low spin states (S =1/2) can be identified by ^{19}F nmr measurement. Two sharp singlets were observed for the high spin complex at 151 ppm and the low spin state at -103 ppm, respectively.

The reconstituted myoglobin with the monofluorohemin in bufferized solution containing excess molar amount of KCN revealed a sharp signal of ^{19}F nucleus at -125 ppm, assignable to the low spin state. Axial chloro ligand of the prosthetic heme is probably replaced by CN^- ligand. Therefore, the spin states of particular iron(III) complex are readily identified by chemical shift of ^{19}F signal. Difference of paramagnetic ^{19}F chemical shifts between the high and low spin states is much larger than that of the paramagnetic proton chemical shifts for both spin states. Visible spectra of the free base and its metal complexes are almost similar to those of mesoporphyrin-IX.

Table I. Half-Wave Potentials of Porphyrin Free Bases and Their Metal Complexes[a]

Compound	M	ring oxidation $E_{1/2}$ (V vs. SCE)			ring reduction $E_{1/2}$ (V vs. SCE)	
		second	first		first	second
7	2H	1.34	1.01		-1.30	
	Zn(II)	1.09	0.74		-1.49	
	Fe(III)Cl	1.44	1.13		-0.88	-1.16
				Fe(III)/Fe(II) -0.31		
Mesoporphyrin-IX dimethyl ester	2H	1.34	0.91		-1.37	-1.74
	Zn(II)	1.03	0.69		-1.55	
	Fe(III)Cl	1.37	1.03			
				Fe(III)/Fe(II) -0.37		

[a]Measured in CH_2Cl_2 (0.1 M TBPA)

Cyclic voltammetric measurements of the free base and metal complexes were accomplished in CH_2Cl_2 with tetra-n-butylammonium perchlorate as a supporting electrolyte. Table I lists redox potentials of the free base and metalloporphyrins with those of mesoporphyrin as a reference. Redox potentials of monofluoroporphyrin and metal complexes show slight anodic shifts comparing with those of the reference compounds. Present trend is readily explicable on the basis of electronic effect of fluorine substituent for aromatic compounds. Since electronic effect of fluorine on the π-electron structure of porphyrin is very small, the ring-fluorinated hemin is useful as a mimicking prosthetic heme in the reconstitution studies of various heme enzymes. Further work is now under way to investigate interaction between apo-enzyme and ring-fluorinated heme and reaction behavior of enzymes.

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2. K. M. Snow and K. M. Smith, *J. Org. Chem.*,1989, **54**, 3270.

3. H. Toi, M. Homma, A. Suzuki, and H. Ogoshi, *J. Chem. Soc., Chem. Commun.*, 1985, 1791.
4. Compound **1**; mp 83-85 °C; ms m/z 199 (M^+): ir (KBr) 3270, 1660, 1190 cm^{-1}; ^1H nmr ($CDCl_3$, TMS) δ 8.35 (br s, 1H, NH), 4.25 (q, J=7 Hz, 2H, $OC\underline{H}_2CH_3$), 2.70 (q, J=7 Hz, 2H, $C\underline{H}_2CH_3$), 2.10 (s, 3H, CH_3), 1.30 (t, J=8 Hz, 3H, $OCH_2C\underline{H}_3$), 1.10 (t, J=8 Hz, 3H, $CH_2C\underline{H}_3$) ppm; ^{19}F nmr ($CDCl_3$, $CFCl_3$) -171.5 ppm.
5. Compound **2**; ms m/z 171 (M^+); ir (KBr) 3350, 1660 cm^{-1}.
6. Pyrrole **3** is very air sensitive at room temperature. It was manipulated under N_2 atmosphere.
7. (a) A. R. Battersby, G. L. Hodgson, M. Ihara, E. McDonald, and J. Sanders, *J. Chem. Soc., Perkin Trans. I*, 1973, 2923. (b) M. F. Hudson, and K. M. Smith, *Synthesis*, 1976, 464.
8. Compound **5**; ms m/z 246 (M^+); ir (KBr) 3100, 1630 1185cm^{-1} ,^1H nmr ($CDCl_3$, TMS) δ 13.22 (br s, 2H, NH), 7.68 (s, 1H, CH), 7.12 (s, 1H, -H), 2.71 (q, J=7 Hz, 2H, $C\underline{H}_2CH_3$), 2.70 (s, 3H, CH_3), 2.48 (q, J=7 Hz, 2H, $C\underline{H}_2CH_3$), 2.30 (s, 3H, CH_3), 1.30 (t, J=7 Hz, 3H,$CH_2C\underline{H}_3$), 1.20 (t, J=7 Hz, 3H, $CH_2C\underline{H}_3$).
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10. Compound **7**; ms m/z 598 (M^+), ir (KBr), 3300, 1738, 1200cm^{-1}; ^1H nmr ($CDCl_3$, TMS), δ 10.08, 10.01 (s, meso-4H), 4.49, 4.36 (t, J=7 Hz, 4H, two $C\underline{H}_2CH_2CO_2CH_3$), 4.15,4.00 (q, J=7 Hz, 4H, two $C\underline{H}_2CH_3$), 3.71, 3.68, 3.64, 3.62, 3.60 (s, 15H, five CH_3), 3.30, 3.29 (t, J=7 Hz, 4H, two $CH_2C\underline{H}_2CO_2CH_3$), 1.95 (t, J=7 Hz, 3H, $CH_2C\underline{H}_3$), 1.90 (t, J=7 Hz, 3H, $CH_2C\underline{H}_3$), -3.93 (br s, 2H, two NH); vis ($CHCl_3$) λ_{max} (log ε) 398(5.29), 497(4.25), 533(4.11), 564(4.06), 617(3.93) nm.
11. Compound **8**; ms m/z 661 (M^+); ir (KBr) 1735, 1185 cm^{-1}; ^1H nmr ($CDCl_3$, TMS) δ 9.85, 9.80, 9.75 (s, meso-4H), 4.22 (m, 4H, two $C\underline{H}_2CH_2CO_2CH_3$), 4.00, 3.99 (q, J=7 Hz, 4H, two $C\underline{H}_2CH_3$), 3.67 (s, 6H, two OCH_3), 3.59, 3.52, 3.50 (s, 9H, three ring-CH_3), 3.22, 3.20 (t, J=6 Hz, 4H, two $CH_2C\underline{H}_2CO_2CH_3$), 1.90 (t, J=7 Hz, 3H, $CH_2C\underline{H}_3$), 1.80 (t, J=7 Hz, 3H, $CH_2C\underline{H}_3$); ^{19}F nmr ($CDCl_3$,$CFCl_3$) -148.5 ppm; vis ($CHCl_3$) λ_{max} (log ε) 400(5.45), 532(4.25), 568(4.29) nm.
12. Compound **9**; ms m/z 652 (M^+-Cl); ir (KBr) 1735, 1140 cm^{-1}; vis ($CHCl_3$) λ_{max} (log ε) 376(4.90), 504(4.03), 532(4.20), 575(3.84), 632(3.97) nm.

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