Synthesis of Fluorine Analogues of Protoporphyrin Potentially Useful for Diagnosis and Therapy of Tumors1)

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With the aim of obtaining a porphyrin derivative useful for diagnosis and therapy of cancer, fluorine analogues of protoporphyrin, in which the vinyl group(s) were replaced by difluorovinyl group(s), were synthesized by the reaction of the formylporphyrins with sodium chlorodifluoroacetate in the presence of triphenylphosphine. Some improvements in the reported procedures for the synthesis of formylporphyrins are also described. Preliminary results of biological tests of the products showed that $8^2,8^2$-difluoroprotoporphyrin accumulates to gastric cancer more selectively than other fluorine analogues and that $3^2,3^2,8^2,8^2$-tetrafluoroprotoporphyrin is taken up by rat hepatoma cells more readily than the others.

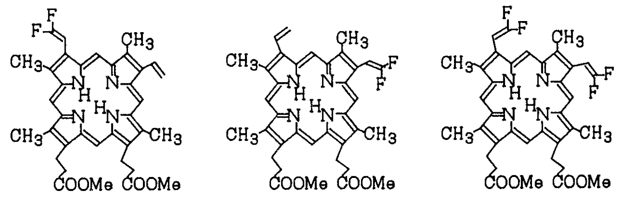
Keywords fluorine; porphyrin; protoporphyrin; photodynamic therapy; formyl; difluorovinyl; deuteroporphyrin; tumor cell; triphenylphosphine; chlorodifluoroacetate

It is well known that some porphyrin derivatives are localized to tumor tissue, and recently photoradiation therapy using lasers has been suggested to have clinical value.2) In the early stage of our work, hematoporphyrin derivatives (HPD) attracted our attention. Although the structure of the active component has been proposed to be a dimer of hematoporphyrin,3) HPD has generally been (and is still) used as a mixture. We thought that if we could synthesize a porphyrin derivative that localized specifically to a certain tissue or certain cancer cells, it would be potentially useful for diagnosis and therapy of cancer. For this purpose, we have synthesized methanol adducts of protoporphyrin by treating the dimethyl ester of protoporphyrin with hydrogen chloride gas in methanol. Since methanol was eliminated easily from these adducts, it was very difficult to obtain these methanol adducts in pure forms, but we were able to isolate three esters, dimethyl 3-(methoxyethyl)-8-vinyldeuteroporphyrin (MVD), dimethyl 3-vinyl-8-(methoxyethyl)deuteroporphyrin (VMD) and dimethyl 3,8-bis(methoxyethyl)deuteroporphyrin (MMD), using column chromatography and preparative thin layer chromatography, and determine their structures unambiguously by two dimensional nuclear magnetic spectroscopy.4) These esters were hydrolyzed with sodium hydroxide, as shown in Chart 1.

We found that one of the adducts, MVD, was taken up by human gastric cancer selectively, but not by human hepatocellular carcinoma.5) Compounds, VMD and MMD, were slightly taken up by both cancer cells.

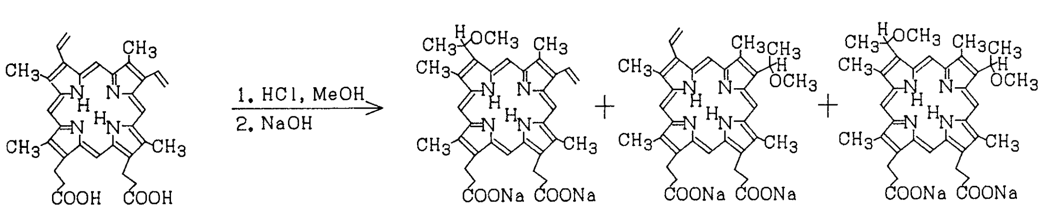
This experiment showed that a derivative of porphyrin could be a specific sensitizer for certain tumor cells. Therefore, we set out to synthesize other porphyrin derivatives, with the aim of finding compounds which would localize specifically to a certain tumor and so could be useful for diagnosis and therapy of that cancer.

Now, we should like to report the synthesis of three fluorine analogues of protoporphyrin. Protoporphyrin has two vinyl groups. We thought that these two vinyl groups might be biologically significant, so we chose the three fluorine analogues (**1**—**3**) of protoporphyrin shown in Fig. 1 as our targets. The reasons for our choice were as follows. First, if one of these compounds is localized to a certain tumor, it could be used as a photosensitizer in the same manner as other porphyrin derivatives. Second, a difluorovinyl group might react with biologically important constituents such as a membrane components or an enzyme of cells at which the compound is localized and manifest some anti-tumor activity without irradiation. Finally, the content of fluorine in biological cells is usually so low as to be negligible. Thus, the localization of the fluorine analogue of porphyrin could be followed not only by laser photoirradiation, but also by fluorine-nuclear magnetic resonance (F-NMR) imaging, if the sensitivity of F-NMR can be made sufficient. This would open a new field of



3-FPPD (1) 8-FPPD (2) 3,8-FPPD (3)

Fig. 1. Fluorine Analogues of Protoporphyrin



MVD VMD MMD

Chart 1

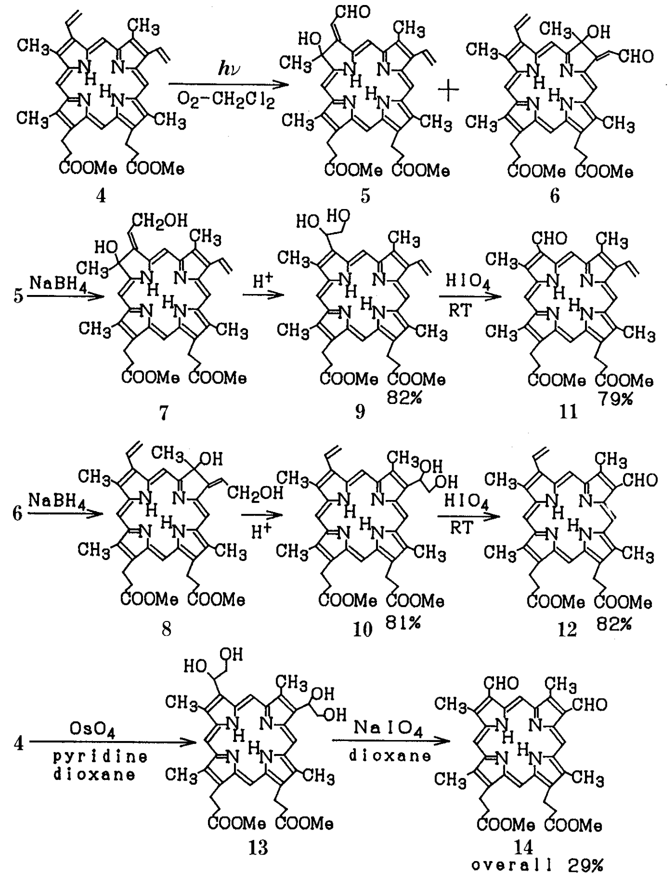


Chart 2

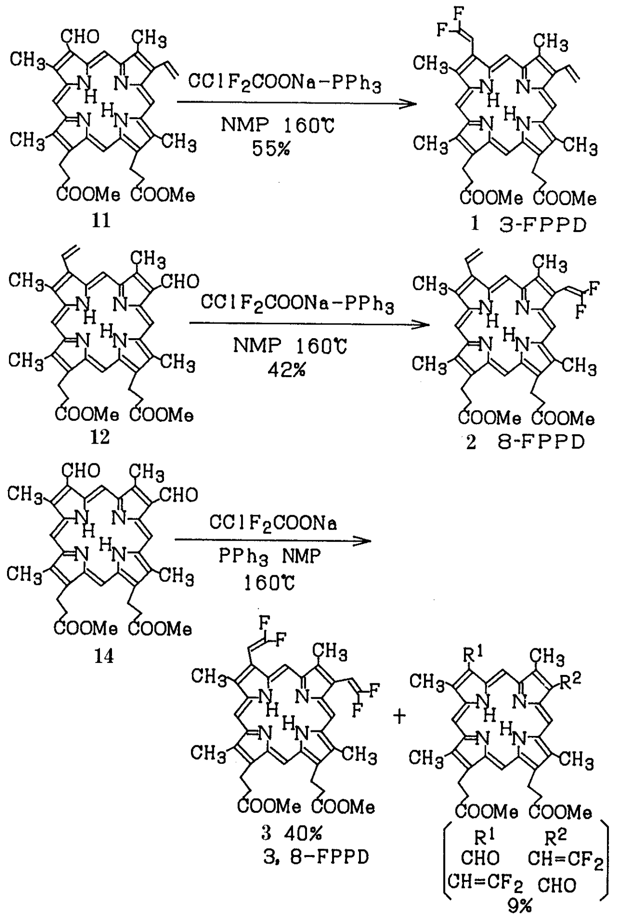


Chart 3

application of NMR in diagnostics.

Our synthetic route is shown in Charts 2 and 3.

Photooxidation of protoporphyrin dimethyl ester (**4**) was carried out according to the literature[6] with a small modification. Thus, **4** was irradiated with an ordinary white light lamp in methylene chloride in an atmosphere of oxygen. First, the singlet oxygen added to vinyl and pyrrole ring double bonds in a Diels–Alder manner, and spontaneous rearrangement occurred to give two isomeric hydroxy aldehyde compounds (**5** and **6**), which were separated by silica-gel column chromatography. The yields of **5** and **6** were 33 and 30%, respectively with 16% recovery of **4**.

One of the 2-hydroxy-3-methylene compound, **5**, was reduced with sodium borohydride at room temperature instead of under heating on a steam bath as stated in the literature,[6] since large amounts of tarry substances were formed at high temperature. The reaction mixture was acidified, and a rearrangement of the primary product (**7**) occurred spontaneously to give a glycol compound (**9**) in a much higher yield than in the case of reduction at the high temperature reported in the literature. Although compound **9**, which was obtained after acidification of the reaction mixture followed by extraction with methylene chloride, had been reported to be too unstable to be purified by column chromatography, it was isolated in 82% yield after purification on an SiO_2 column. Oxidation of **9** with periodic acid gave spirographisporphyrin dimethyl ester (**11**) in 79% yield. Improvement of the yield was accomplished by using dioxane as a solvent in the oxidation step. Namely, this solvent dissolves the starting material and the reagent much effectively, and gives a much higher yield of **11** than that in the reported two phase reaction using methylene chloride–benzene and water as solvents.[6]

Similarly, the other product (**6**) of photooxidation was converted to another glycol compound (**10**) and thence to isospirographisporphyrin dimethyl ester (**12**) in comparable yields to those above.

Treatment of **4** with osmium tetroxide gave the bis(1,2-diol) compound (**13**), which was further oxidized with sodium periodate to the diformyl compound (**14**) in an overall yield of 29%.[7] Dioxane was a good solvent in these reactions, too.

The next step was conversion of a formyl group to a difluorovinyl group. For this purpose, the formyl compounds were treated with triphenylphosphonium difluoromethylide, which was generated by the thermolysis of sodium chlorodifluoroacetate in the presence of triphenylphosphine in an aprotic solvent such as diglyme or dimethylformamide.[8] However, porphyrin derivatives do not dissolve in these solvents, and the yield was very poor. We carried out the reaction of **11** with sodium chlorodifluoroacetate in the presence of triphenylphosphine in *N*-methylpyrrolidone and obtained 3^2,3^2-difluoroprotoporphyrin dimethyl ester (**1**) in 55% yield with 26% recovery of the starting material. Compound **1** was obtained as dark red crystals of mp 203.5—205.5 °C. Its structure was supported by examination of the spectral data, especially by the coupling of the signals of one of the vinylic protons and a fluorine atom in the NMR.

Table I. Localization of Fluorinated Protoporphyrins in Cancer Cells and Organs (Relative Intensity of Fluorescence at 630 nm)

	Cancer (MKN-45)	Liver	Stomach
3-FPPN	106	66	80
8-FPPN	1600	140	1033
3,8-FPPN	40	186	400

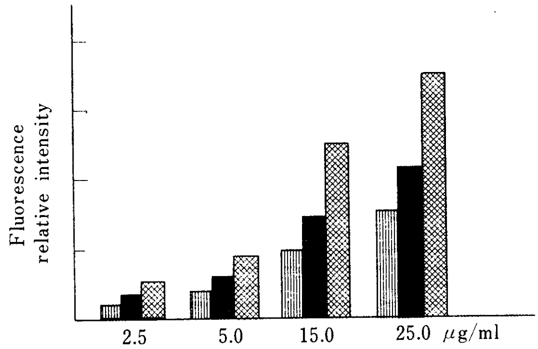


Fig. 2. Uptake of F-Protoporphyrins by Rat Hepatoma Cells
▥, 3-FPPN; ■, 8-FPPN; ▨, 3,8-FPPN.

Compound **12** reacted similarly to give the 8-difluorovinyl compound (**2**) in 42% yield with 16% recovery of the starting material.

The dialdehyde (**14**) was condensed with phosphonium difluoromethylide (generated from triphenylphosphine and sodium chlorodifluoroacetate) to give the bis(difluorovinyl) compound (**3**) in 40% yield. In this reaction, a mixture of mono(difluorovinyl)aldehydes was obtained in 9% yield.

Next, accumulation of these compounds in organs and cancer cells was examined. First, the dimethyl esters (**1**, **2** and **3**) were hydrolyzed with sodium hydroxide to sodium salts, which were conveniently designated as 3-FPPN, 8-FPPN and 3,8-FPPN, respectively. These salts were intraabdominally injected into nude mice transplanted with human gastric cancer (MKN-45). After 24 h, the cancer cell nest and organs were extirpated from the mice. Protoporphyrin derivatives were extracted with diisopropylamine–methanol and measured by fluorescence analysis.[9] 8-FPPN showed remarkably high values in cancer and stomach, as shown in Table I. This suggests that 8-FPPN is localized specifically to stomach cancer.

Next, the uptake of FPPN's by rat ascite hepatoma cells (JTC-16) was examined. The sodium salts were added to the culture media and incubated for 48 h. The cells were washed with buffer solution and extracted with diisopropylamine–methanol. The uptake was determined by measuring the intensity of fluorescence. Interestingly, 3,8-FPPN was taken up most efficiently in this case, as shown in Fig. 2.

These results indicate that one porphyrin is taken up specifically by one cancer and another porphyrin by the other cancer. We are now investigating the biological behavior of FPPN's more extensively, and synthesizing other porphyrin derivatives.

Experimental

Synthesis of the formyl compounds was carried out according to the literature[6,7] with some modifications, as described below. ^{1}H-NMR spectra were obtained on JNM-FX90Q and JNM-GX400 spectrometers. ^{19}F-NMR spectra were recorded on a JNM-FX90Q spectrometer, using fluorotrichloromethane as an internal standard.

Photooxidation of Protoporphyrin Dimethyl Ester (4) This reaction was carried out with a white light lamp in an atmosphere of oxygen instead of air. This modification made the irradiation time shorter and reduced the formation of tarry substances as compared to the reported oxidation in air. The products were separated by column chromatography as reported. The first fraction yielded **4**, the second fraction **6** and the last **5**.

Spirographisporphyrin Dimethyl Ester (11) A solution of $NaBH_4$ (1.01 g) in MeOH (30 ml) was added to a solution of **5** (500 mg) in CH_2Cl_2 (20 ml) at room temperature under stirring. Stirring was continued for 30 min, then the mixture was acidified with 2% HCl to pH 3. The mixture was extracted with CH_2Cl_2 and purified on an SiO_2 column in CH_2Cl_2–AcOEt (7 : 3) to give the diol compound (**9**, 412 mg, 82%).

A solution of **9** (202 mg) in 1,4-dioxane (50 ml) was treated with $HIO_4 \cdot 2H_2O$ (418 mg), the mixture was stirred at room temperature for 30 min, then was poured onto ice-water and extracted with CH_2Cl_2. The organic layer was washed with H_2O and dried over $MgSO_4$. After evaporation on the solvent, the residue was purified on an SiO_2 column in CH_2Cl_2–AcOEt (98 : 2) to give **11** (160 mg, 79%). Elution with CH_2Cl_2–AcOEt (7 : 3) gave **9** (19 mg, 9%).

Isospirographisporphyrin Dimethyl Ester (12) Treatment of **6** (500 mg) with $NaBH_4$ followed by acidification with HCl in the same manner as above gave the diol compound (**10**, 404 mg, 81%).

Compound **10** (171 mg) was oxidized with $HIO_4 \cdot H_2O$ (359 mg) as above to give **12** (133 mg, 82%) with recovery of **10** (33 mg, 19%).

3^2,3^2-Difluoroprotoporphyrin Dimethyl Ester (1) Compound **11** (70 mg) and triphenylphosphine (746 mg) were dried under vacuum and dissolved in *N*-methylpyrrolidone (NMP, 10 ml) under an atmosphere of argon. The mixture was heated at 160 °C, and a solution of sodium chlorodifluoroacetate (436 mg) in NMP (7 ml) was added at this temperature under stirring in 15 min. The mixture was stirred at this temperature for a further 30 min. The mixture was cooled with ice, then was poured into ice-water, and extracted with CH_2Cl_2. The organic layer was washed with H_2O and dried over $MgSO_4$. After evaporation of the solvent, the residue was purified on an SiO_2 column in CH_2Cl_2–AcOEt (98 : 2 to 95 : 5) to give dark red crystals (**1**, 41 mg, 55%) with recovery of **11** (18 mg, 26%) from a later effluent fraction. **1**: Dark red crystals, mp 203.5—205.5 °C. MS *m/z*: 626 (M^+). High-resolution MS Calcd for $C_{36}H_{36}F_2N_4O_4$: 626.2703. Found: 626.2702. IR (KBr) cm^{-1}: 3324 (N–H), 1738 (C=O), 1198, 1176, (C–F). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 9.99 (1H, s), 9.89 (1H, s), 9.87 (1H, s), 9.66 (1H, s), 8.18 (1H, dd, J=17, 11 Hz), 6.47 (1H, d, $J_{H\text{-}F}$=25 Hz), 6.33 (1H, dd, J=17, 1.4 Hz), 6.17 (1H, dd, J=11, 1.4 Hz), 4.45 (4H, m), 3.66 (3H, s), 3.65 (3H, s), 3.55 (6H, s), 3.52 (3H, s), 3.46 (3H, d, J=2.4 Hz), 3.23 (4H, m), −4.09 (2H, br). ^{19}F-NMR ($CDCl_3$): −82.14 (1F, dd, $J_{H\text{-}F}$=25 Hz, $J_{F\text{-}F}$=26 Hz), −83.76 (1F, d, J=26 Hz).

8^2,8^2-Difluoroprotoporphyrin Dimethyl Ester (2) A similar reaction of **12** (70 mg) with triphenylphosphine (786 mg) and sodium chlorodifluoroacetate (456 mg) in NMP (17 ml) gave **2** (31 mg, 42%) with recovery of **12** (11 mg, 16%). **2**: Dark red crystals, mp 204.0—205.5 °C. MS *m/z*: 626 (M^+). High-resolution MS Calcd for $C_{36}H_{36}F_2N_4O_4$: 626.2703. Found: 626.2702. IR (KBr) cm^{-1}: 3324 (N–H), 1738 (C=O), 1194, 1170 (C–F). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 9.98 (1H, s), 9.87 (1H, s), 9.85 (1H, s), 9.63 (1H, s), 8.17 (1H, dd, J=16, 11 Hz), 6.44 (1H, d, $J_{H\text{-}F}$=25 Hz), 6.32 (1H, d, J=16 Hz), 6.16 (1H, d, J=11 Hz), 4.31 (4H, m), 3.66 (3H, s), 3.65 (3H, s), 3.55 (3H, s), 3.54 (3H, s), 3.51 (3H, s), 3.45 (3H, d, J=2.4 Hz), 3.23 (4H, m), −4.10 (2H, br). ^{19}F-NMR ($CDCl_3$): −82.02 (1F, dd, $J_{H\text{-}F}$=25 Hz, $J_{F\text{-}F}$=26 Hz), −83.64 (1F, d, J=26 Hz).

3^2,3^2-8^2,8^2-Tetrafluoroprotoporphyrin Dimethyl Ester (3) A similar reaction of diformyl compound (**14**, 40 mg) with triphenylphosphine (788 mg) and sodium chlorodifluoroacetate (455 mg) in NMP (17 ml) gave **3** (18 mg, 40%) with a mixture of difluorospirographisporphyrin dimethyl ester and difluoroisospirographisporphyrin dimethyl ester (4 mg, 9%). **3**: Dark red crystals, mp 212—219.5 °C. MS *m/z*: 662 (M^+). High-resolution MS Calcd for $C_{36}H_{34}F_4N_4O_4$: 662.2523. Found: 662.2530. IR (KBr) cm^{-1}: 3324 (N–H), 1736 (C=O), 1198, 1172 (C–F). ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 9.92 (2H, s), 9.76 (2H, s), 6.55 (2H, d, $J_{H\text{-}F}$=26 Hz), 4.33 (4H, dd, J=7, 7 Hz), 3.66 (6H, s), 3.54 (6H, s), 3.48 (6H, s), 3.24 (4H, dd, J=7, 7 Hz). ^{19}F-NMR ($CDCl_3$): −81.86 (2F, dd, $J_{H\text{-}F}$=26 Hz, $J_{F\text{-}F}$=26 Hz), −83.38 (2F, d, J=26 Hz).

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References and Notes

1) A part of this work was presented at the 3rd International Symposium

on Photodynamic Therapy, London, July 1988.

2) Concerning the chemistry and biochemistry of HpD, see "Advances in Experimental Medicine and Biology," Vol. 160, "Porphyrin Photosensitization," ed. by D. Kessel and T. J. Dougherty, Plenum Press, New York, 1983.

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9) This method of extraction of porphyrins is very efficient; the details will be published elsewhere.