SYNTHESIS OF FLUORINE ANALOGS OF HEMATOPORPHYRIN[1]

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This paper is dedicated to Professor Edward C. Taylor on the occasion of his 70th birthday.

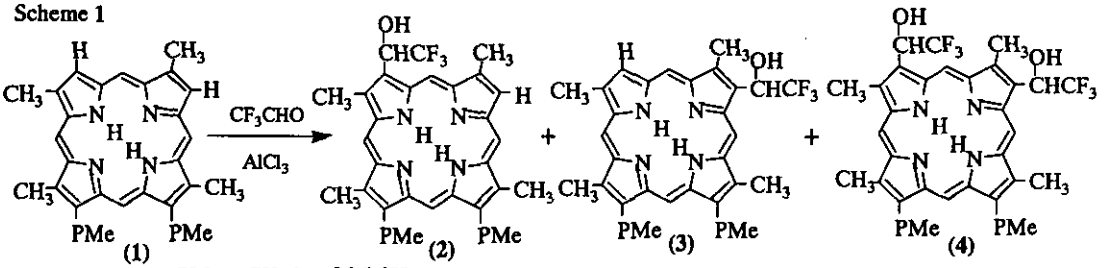
Abstract -- With the aim of obtaining a porphyrin derivative useful for diagnosis and therapy of cancer, fluorine analogs of hematoporphyrin, which had trifluorohydroxyethyl group(s) in the place of hydroxyethyl groups, were synthesized by the reaction of deuteroporphyrin dimethyl ester with trifluoroacetaldehyde in the presence of aluminum chloride. Preliminary results of biological tests of the products showed that the hexafluoro analog of hematoporphyrin accumulates to Human liver cancer cells more selectively than other fluorine analogs.

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 derivative (HPD) attracted our attention. Although the structure of the active component of HPD has been proposed to be a dimmer of hematoporphy-

rin,[3] it 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 tumor tissue or certain cancer cells, it would be potentially useful for diagnosis and therapy of cancer. For this purpose, we have synthesized fluorine analogs of protoporphyrin, some of which were taken up by some tumor cells preferentially.[4] Now, we would like to report synthesis of fluorine analogs of hematoporphyrin and results of the preliminary test of their uptake by human liver cancer cells.

First, we tried the reaction of deuteroporphyrin dimethyl ester (1) with trifluoroacetaldehyde ethyl hemiacetal in the presence of a Lewis acid.[5] Aluminum chloride was found to be the best catalyst among the Lewis acids examined. Introduction of a 2,2,2-trifluoro-1-hydroxyethyl group to 3- or 8- position of the porphyrin ring was accomplished by this catalyst. However, the yields of this reaction were very low, and the separation of isomers was difficult. To improve the yields of the products, free trifluoroacetaldehyde, generated by the reaction of the hemiacetal with concentrated sulfuric acid, was used for the reaction. The products were separated by column chromatography to give 3-(2,2,2-trifluoro-1-hydroxyethyl)deuteroporphyrin dimethyl ester (**2**), 8-(2,2,2-trifluoro-1-hydroxyethyl)deuteroporphyrin dimethyl ester (**3**) and 3,8-bis(2,2,2-trifluoro-1-hydroxyethyl)deuteroporphyrin dimethyl ester (**4**) in the yields of 36 %, 27 % and 6 %, respectively, with recovery of the starting material (13 %). The structures of **2** and **3** were estimated based on the two-dimensional nuclear Overhauser effects collected on 400 MHz proton nmr. (See Scheme 1)

Scheme 1



The esters (**2** to **4**) were hydrolyzed by sodium hydroxide, and the each sodium salt (**2'**, **3'** or **4'**) was subjected to the preliminary test of uptake by human liver cancer cells.

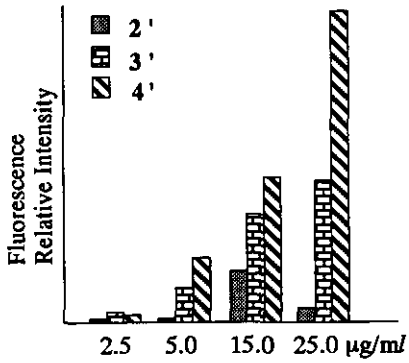


Figure 1. Uptake of F-hematoporphyrins by JTC-16 (Human liver cancer cell).

The sodium salts were added to the culture media of JTC-16 cells and incubated for 48 h. The cells were washed with buffer solution and extracted with diisopropylamine-methanol. The uptake was measured by fluorescence analysis.[6] The salt (**4'**) was found to be taken up more readily than other salts, as shown in Figure 1. This result shows that a certain porphyrin would be localized to a certain cancer more selectively.

In conclusion, we could obtain fluorine analogs of hematoporphyrin by the Friedel-Crafts reaction of deuteroporphyrin with trifluoroacetaldehyde, where aluminum chloride was found to be the best catalyst. Here, one derivative (**4'**) was taken up more readily by a human liver cancer cells. This result suggests that some porphyrin is localized selectively to a certain cancer. If we can obtain fluorine derivative of this type, it will be useful for diagnosis of cancer, since fluorine compounds could be traced by fluorine nmr CT scan.

EXPERIMENTAL

CF_3CHO, generated from $CF_3CH(OH)OEt$ (8 ml, 56 mmol) and conc. H_2SO_4 (8 ml), was added to a suspension of $AlCl_3$ (4.965 g, 37 mmol) in CH_2Cl_2 (24 ml) at -78°C. To this mixture, **1** (2.00 g, 3.7 mmol) was added in portions at room temperature, then the mixture was warmed at 50°C for 4 h. The mixture was poured onto ice water, then extracted with CH_2Cl_2. The CH_2Cl_2 layer was washed with water, then dried over $MgSO_4$, and concentrated under vacuum. The residue was separated by column chromatography (SiO_2, 5 % to 30 % AcOEt - CH_2Cl_2) to give 3-(2,2,2-trifluoro-1-hydroxyethyl)deuteroporphyrin dimethyl ester (**2**, 0.847 g, 36 %), 8-(2,2,2-trifluoro-1-hydroxyethyl)deuteroporphyrin dimethyl ester (**3**, 0.638 g. 27 %) and 3,8-bis(2,2,2-trifluoro-1-hydroxyethyl)deuteroporphyrin dimethyl ester (**4**, 164 mg, 6 %) with recovery of **1** (0.263 g, 13 %). **2**: mp 222-224°C. Red crystals. Ms: m/z 636 (M^+), High Resolution Mass (HRms): $C_{34}H_{35}N_4F_3O_5$ Calcd

636.2559. Found 636.2563. ^{1}H-Nmr (acetone-d_6) δ : 10.89 (5-H), 10.76 (15-H), 10.61 (20-H), 10.40 (10-H), 9.10 (1H, s, 8-H), 6.66 (1H, q, J=7.5 Hz, -CH(OH)-), [4.39 (2H, t, J=7.3 Hz), 4.37 (2H, t, J=7.5 Hz)] (13'-, 17'-CH_2-), 3.65 (3H, s, 7-CH_3), 3.63 (3H, s, 2-CH_3), 3.61 (3H, s, 18-CH_3), [3.57 (3H, s), 3.56 (3H, s)] ($COOCH_3$), 3.51 (3H, s, 12-CH_3), [3.25 (2H, t, J=7.3 Hz), 3.24 (2H, t, J=7.5 Hz)] (13"-, 17"-CH_2-). ^{19}F-Nmr ($CDCl_3$) ppm (from $CFCl_3$): 76.48 (d, J=7.5 Hz). **3**: mp 223-226°C. Red crystals. Ms: m/z 636 (M^+), HRms: $C_{34}H_{35}N_4F_3O_5$ Calcd 636.2559, Found 636.2567. ^{1}H-Nmr (acetone-d_6) δ : 10.74 (10-H), 10.30 (5-H), 10.29 (15-H), 10.21 (20-H), 9.29 (1H, s, 3-H), 6.99 (1H, m, -CH(OH)-), 6.66 (1H, d, J=4.9 Hz, -OH), [4.48 (2H, t, J=7.8 Hz), 4.37 (2H, t, J=7.8 Hz)] (13'-, 17'-CH_2-), 3.78 (3H, s, 7-CH_3), 3.75 (3H, d, J=1.0 Hz, 2-CH_3), 3.69 (3H, s, 12-CH_3), [3.62 (3H, s), 3.61 (3H, s)] ($COOCH_3$), 3.60 (3H, s, 18-CH_3), [3.35 (2H, t, J=7.8 Hz), 3.30 (2H, t, J=7.8 Hz)] (13"-, 17"-CH_2-), -3.78 (2H, bs, NH). ^{19}F-Nmr (acetone-d_6) ppm: 75.39 (d, J=7.3 Hz). **4**: mp 130°C, Red crystals. Ms: m/z 734 (M^+), HRms: $C_{36}H_{36}N_4F_6O_6$ Calcd 734.2538, Found 734.2530. ^{1}H-Nmr (acetone-d_6) δ : [10.85, 10.73, 10.30, 10.28] (4H, all s, meso-H), 7.03 (2H, m, -CH(OH)-), [6.77 (1H, d, J=5.4 Hz), 6.75 (1H, d, J=5.4 Hz)] (OH), [4.43 (2H, t, J=7.5 Hz), 4.41 (2H, t, J=7.5 Hz)] (13'-, 17'-CH_2-), 3.83, (3H, s), 3.81 (3H, s), 3.66 (3H,s), 3.64 (3H, s), 3.62 (3H, s), 3.61 (3H, s), [3.33 (2H, t, J=7.5 Hz), 3.31 (2H, t, J=7.5 Hz)] (13"-, 17"-CH_2-), -3.64 (2H, bs, NH). ^{19}F-Nmr (acetone-d_6) ppm: 75.51 (d, J=7.3 Hz).

REFERENCES AND NOTES

1. Part of this work was presented at 109 Annual Meeting of Pharmaceutical Society of Japan, 1989, Nagoya.
2. Concerning the chemistry and biochemistry of HPD, see "Advances in Experimental Medicine and Biology", **160**, "Porphyrin Photosensitization", ed. by D. Kessel and T. J. Dougherty, Plenum Press, New York, 1983.
3. D. Kessel, Biochem. Pharmacology, 1984, **33**, 1389.
4. A. Ando, T. Shinada, S. Kinoshita, N. Arimura, M. Koyama, T. Nagai, T. Miki, I. Kumadaki, and H. Sato, Chem. Pharm. Bull., 1990, **38**, 2175.
5. A. Guy, A. Lobgeois, and M. Lemaire, J. Fluorine Chem., 1986, **32**, 361.
6. This method of extraction of porphyrins is very efficient; the details will be published elsewhere.

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