Notes

Synthesis of New Tetrakis(multifluoro-4-pyridyl)porphyrin Derivatives as the Electric Eel Acetylcholinesterase Inhibitors

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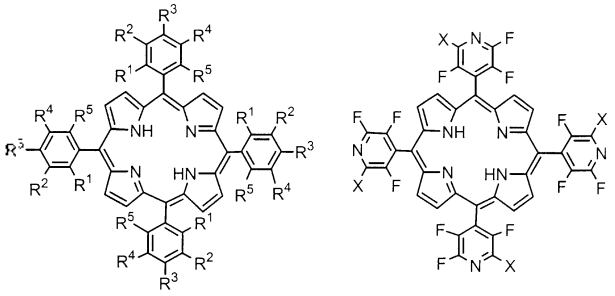
The porphyrins play important roles in divergent field of research, including catalysis, solar energy conversion, spectroscopy and the development of organic materials.[1] Furthermore, these compound have been well known to show selective affinity to tumor cells[2,3] and applied for photodynamic cancer therapy[4-7] as a photosensitizer and even for antiviral treatment.[8] Another recent topic relating with porphyrins is the studies on the treatment for Alzheimers Disease (AD) in connection with acetylcholinesterase (AChE) inhibitors.[9] Among the various approaches to cholinergic enhancement, inhibition of the degrading enzyme, AChE is presently the most promising in terms of providing candidate drugs for alleviating the symptoms of AD. Porphyrin inhibitors of AChE are especially valuable in the sense that they usually have low cytotoxicity and possibility to reach to the central nervous system through the blood brain barrier.[10] Currently, several synthetic meso-porphyrin derivatives (1a, 1b) which have difluorophenyl substituents on the periphery of the porphyrin skeleton turned out to be the potent AChE inhibitors.[9] The precise sculpturing of the porphyrin environments to be a promising inhibitor seems that the peripheral tetra-aryl groups in the porphyrin should have at least a fluorine substituent at 2 or 6-position of them.

We designed new porphyrin compounds (2, 3) as potential AChE inhibitors, before we deal with both metalloporphyrin and water-soluble porphyrin chemistry on this field. As shown in Figure 1, porphyrins (2, 3) have four multifluoropyridyl functionalities such as 2,3,5,6-tetrafluoro-4-pyridyl and 2,3,5-trifluoro-4-pyridyl units to fulfill the above assumption. In addition, it is interesting to examine how the number of fluorine substituents present on the aromatic rings affects the interaction with AChE.



(1a) R^1=R^5=F R^2=R^3=R^4=H
(1b) R^1=R^3=F R^2=R^4=R^5=H

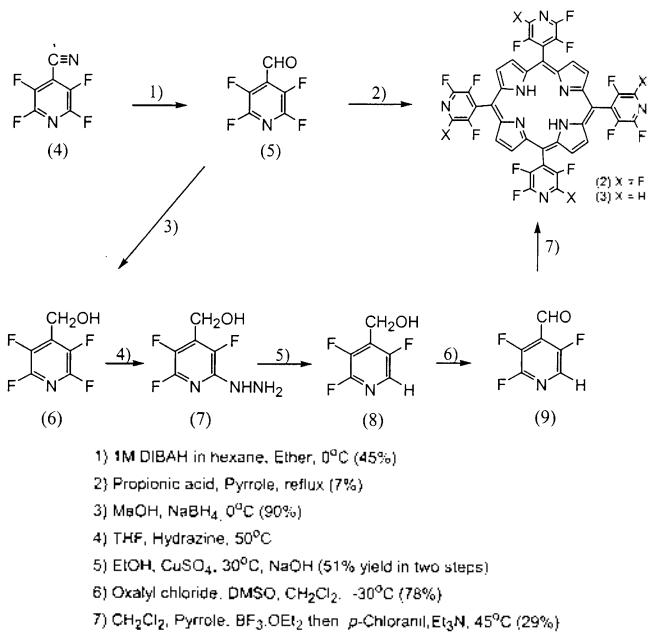
(2) X = F
(3) X = H

Figure 1

Results and Discussion

The synthesis of new porphyrins (2, 3) was carried out as shown in Scheme 1.

Porphyrin (2) was successfully prepared in two steps from 2,3,5,6-tetrafluoropyridine-4-carbonitrile (4) in overall 3% yield. The highly volatile aldehyde (5) can be obtained by several ways; 1) ozonolysis of tetrafluoro-4-propenylpyridine[11] 2) reduction of tetrafluoropyridine-4-carbonitrile with Raney nickel in aqueous formic acid (Staskun's method)[12,13] 3) reaction between 4-bromotetrafluoropyridine and *N*-methylformanilide in the presence of *n*-butyllithium.[14] The



Scheme 1

first method involves one more step and does not show satisfying yield. The second method has difficulties in controlling the reaction. The third method is less cost-efficient as more expensive pyridine derivative is required as a starting material. However, our own method includes the reaction between diisobutylaluminum hydride and terafluoropyridine-4-carbonitrile at low temperature.[15] This reaction produced 45% of the aldehyde (5) which is the equivalent yield of the second method. The formation of porphyrin is known to depend on a variety of factors such as oxidant, catalyst, solvent and reaction concentration. Macrocyclization to form porphyrin (2) seems not to be affected by these factors. Typical reaction between equimolar amount of the aldehyde (5) and pyrrole in refluxing propionic acid afforded the reasonable yield (7%) of the porphyrin (2). The attempted reactions applying Lewis acid catalyst (BF_3 etherate) and oxidants (DDQ or *p*-chloranil)[16] did not improve the yield. The synthesis of porphyrin (3) was achieved in six steps (overall 5% yield). The hydride reduction of the aldehyde (5) and the selective displacement of 6-fluorine with hydrazine produced the 6-hydrazinopyridine-4-carbinol (7).[17] Halopyridines do normally undergo nucleophilic aromatic substitution at C-2 and 4 position instead of C-3 position.[17] Nucleophilic aromatic substitution of compound (7) occurred exclusively at C-2 position. The compound (7) was rather unstable and extensive decomposition was observed during chromatographic purification. The sequential removal of hydrazine by copper sulfate[17] and Swern oxidation[18] of the subsequent alcohol (8) gave the aldehyde (9). Porphyrin forming reaction of the aldehyde (9) with pyrrole was conducted in the same manner as the synthesis of porphyrin (2), but it resulted in complex mixture of porphyrin (3). The high level of impurities produced presents purification problems in this case. However the condensation reaction of equimolar amount of the aldehyde (9) and pyrrole in the presence of acid catalyst (BF_3 etherate) and the subsequent oxidation with *p*-chloranil led to produce the corresponding porphyrin (3).[16] The optimized condition typically gave 29% of porphyrin (3). To the best of our knowledge, all the intermediates (6-9) in this synthesis were also new compounds. The structures of porphyrins (2, 3) were confirmed by spectroscopic analyses. The pyridine functionalities in the porphyrins (2, 3) are rarely basic due to a number of electronegative fluorine atoms. Therefore the porphyrins (2, 3) were not enough to form ionic, water soluble porphyrins.

The porphyrins (2, 3) were assayed as an inhibitor of electric eel acetylcholinestrase (AChE). They showed potent and specific inhibition of AChE [K_i (2): 7.3 μM, (3): 2.8 μM]. This result supports that our anticipation could be acceptable. Present studies support that the compounds should have 2 or 6-fluoroaryl substitution at the periphery of the porphyrin in order to be a good porphyrin-based AchE inhibitors.

Experimental Section

General Directions. Melting points of all the compounds were recorded on a Electrothermal 9100 without correction. Infrared spectra were obtained using a Midac PRS-INT FT-IR spectrophotometer. Infrared spectra of samples were normally run as solutions in chloroform. ^1H and ^{13}C NMR spectra were recorded with Bruker DPX-300 or Bruker AMX-500 NMR spectrometer. All Chemical shifts are reported on the δ scale using residual solvent as internal standard. Mass spectra were recorded on VG70-VSEQ or Jeol JMS-AX 505WA mass spectrometer using fast atomic bombardment (FAB) or electron impact technique. Ultraviolet spectra were recorded on a HewLett Packard 8452A Diode Array spectrophotometer. Fluorescence spectra were recorded on an ISS Spectrophotometer. TLC analyses were carried out on glass-plates coated with Merck silica gel $60F_{254}$. Compounds were visualized with UV light. Flash chromatography was carried out using Merck Kiesegel 60 flash silica gel. All the solvents and chemicals were directly used without further purification.

Synthesis. 2,3,5,6-Tetrafluoropyridine-4-carbaldehyde (5) Diisobutylaluminum hydride (DIBAH) (1.0 M in hexane, 17.9 mL, 18.0 mmol) was slowly added to a solution of 2,3,5,6-tetrafluoropyridine-4-carbonitrile (3.00 g, 17.0 mmol) in ether (30 mL) at -20 °C under nitrogen. The reaction mixture was stirred at -20 °C for 14 h. The reaction mixture was quenched with 18% hydrochloric acid (20 mL), diluted with dichloromethane (30 mL) and washed with water (2 × 60 mL); the organic layer was dried over sodium sulfate and the solvent removed to give, after purification by flash column chromatography(dichloromethane), the desired product (1.37 g, 45%) (Lit.[14] 45%) as a colorless oil. ν_{max} ($CHCl_3$): 1728 (C=O) cm^{-1}.

5,10,15,20-Tetrakis(2,3,5,6-tetrafluoro-4-pyridyl)-21*H*, 23*H*-porphyrin (2) Pyrrole (0.40 mL, 5.60 mmol) was slowly dropped to a boiling solution of 2,3,5,6-tetrafluoropyridine-4-carbaldehyde (1.00 g, 5.60 mmol) in propionic acid (20 mL). The reaction mixture was refluxed for 1 h. The solvent was removed under reduced pressure and the residue partitioned between dichloromethane (20 mL) and 1M aqueous NaOH solution (20 mL). The organic layer was washed with water (20 mL) and dried over magnesium sulfate. The dichloromethane was evaporated to give, after purification by flash column chromatography (ethyl ether : hexane, 1 : 3), the porphyrin (90.0 mg, 7%) as a violet crystal, mp: 350 °C above (dec.). λ_{max} (CH_2Cl_2, loge): 412 (4.71), 506 (3.66), 584 (3.18), 638 (2.57) nm. ^1H NMR ($CDCl_3$): δ 8.92 (s, 8H, CH), -3.00 (brs, 2H, NH). m/e (FAB, *m*-nitrobenzyl alcohol): 907 ($M+H^+$, 100%), 618 (15%), 507 (18%), 408 (20%). The fluorescence profile of compound (2) has two bands at 640 nm and 704 nm in acetone solution.

2,3,5,6,-Tetrafluoropyridine-4-carbinol (6) Sodium borohydride (230 mg, 6.00 mmol) was slowly added to a solution of (5) (900 mg, 5.00 mmol) in methanol (5 mL) at 0 °C. The reaction mixture was stirred for 1 h. The reaction mixture was quenched with water (1 mL), diluted with dichloromethane (15 mL) and dried over sodium sulfate. The solvents were removed to give, after purification by flash column chromatography (dichloromethane), the carbinol

(820 mg, 90%) as a pale yellow oil. ν_{max} ($CHCl_3$): 3422 (OH) cm^{-1}. ^1H NMR ($CDCl_3$): δ 4.86 (d, 2H, CH_2, J_{CH_2OH}, 6.0 Hz), 3.13 (t, 1H, OH).

2,3,5-Trifluoropyridine-4-carbinol (8) Hydrazine monohydrate (1.72 mL, 17.7 mmol) was added to a solution of 2,3,5,6-Tetrafluoropyridine-4-carbinol (800 mg, 4.42 mmol) in THF (5 mL). The reaction mixture was stirred at 45 °C for 4 h. The solvent was removed and the residue washed with water (2 × 2 mL), dried to give the crude intermediate (850 mg) as a yellow solid. Compound (7) was directly used to the next step without further purification; to a solution of the crude mixture (850 mg, 4.42 mmol) in ethanol (15 mL) was slowly added a solution of copper sulfate (2.20 g, 8.81 mmol) in water (15 mL). The reaction mixture was stirred at 30 °C for 12 h. 0.6 M Aqueous sodium hydroxide (15 mL) was added at r.t. to the solution and the reaction mixture was stirred for another 5 min. The solution was diluted with dichloromethane (40 mL) and the solvent was dried over sodium sulfate, evaporated to give, after purification by flash column chromatography (chloroform : methanol, 13 : 1), the alcohol (370 mg, 51%) as a yellow oil. ν_{max} ($CHCl_3$): 3426 (OH) cm^{-1}. m/e (GC/MS): 163 (M^+, 100%), 142 (60%), 134 (30%), 114 (35%). ^1H NMR ($CDCl_3$): δ 7.83 (s, 1H, ArH), 4.79 (s, 2H, CH_2), 2.11 (s, 1H, OH).

2,3,5-Trifluoropyridine-4-carbaldehyde (9) DMSO (993 μL, 14.0 mmol) dissolved in dry dichloromethane (4 mL) was slowly dropped to a solution of oxalyl chloride (628 μL, 7.20 mmol) in dry dichloromethane (7 mL) at -30 °C under nitrogen. Stirring was continued at -30 °C for 5 min and another 15 min after dropwise addition of solution of 2,3,5-trifluoropyridine-4-carbinol (580 mg, 3.56 mmol) in dry dichloromethane (5 mL). Triethylamine (2.5 mL, 18.0 mmol) was added at -30 °C to the reaction mixture and stirring was continued for additional 5min. The cooling bath was removed and water (10 mL) was added at r.t.. The organic layer was dried over sodium sulfate, evaporated to give, after purification by flash column chromatography (dichloromethane), the aldehyde (450 mg, 78%) as a pale yellow oil.

ν_{max} ($CHCl_3$): 1716 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$): δ 10.31 (s, 1H, CHO), 8.00 (s, 1H, ArH).

5,10,15,20-tetrakis(2,3,5-trifluoro-4-pyridyl)-21*H*,23*H*-porphyrin (3) BF_3 · etherate (2.5 M solution in dichloromethane, 0.25 mL, 0.62 mmol) was added to a solution of 2,3,5-trifluoropyridine-4-carbaldehyde (500 mg, 3.10 mmol) and pyrrole (0.22 mL, 3.10 mmol) in dry dichloromethane (300 mL). The reaction mixture was stirred at r.t. under nitrogen for 1 h and refluxed for 1 h after rapid addition of *p*-chloranil (610 mg, 2.48 mmol) into the solution. The solvent removal and purification by flash column chromatography (dichloromethane) gave the desired porphyrin (190 mg, 29%) as a pupple crystal. mp: 300×C above (sublimed). λ_{max} (CH_2Cl_2, logε): 412 (4.53), 506 (3.65), 584 (3.19), 656 (2.98) nm. ^1H NMR ($CDCl_3$): δ 8.87 (s, 8H, CH), 8.38 (s, 4H, Pyridyl-H), -2.97 (brs, 2H, NH). m/e (FAB, *m*-nitrobenzyl alcohol): 835 ($M+H^+$, 100%), 391 (57%) 364 (44%), 355 (73%). The fluorescence spectrum of porphyrin (3) shows two bands at 641nm and 707nm in acetone solution.

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