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Synthesis of Tetrakis(multifluoro-4-pyridyl)porphin Derivatives as Acetylcholinesterase Inhibitors

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Abstract—New tetrakis(multifluoro-4-pyridyl)porphin derivatives (**2**–**4**) and water soluble porphyrin (**5**) were synthesized to investigate their interactions with acetylcholinesterase from electric eel. These compounds have been found to be the potent reversible inhibitors of the enzyme with K_i values of μM range. In addition, porphyrin (**5**) showed broad spectrum of anticancer activities.

The porphyrins play important roles in divergent field of research, including catalysis, solar energy conversion, spectroscopy and the development of organic material.[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 Alzheimer's Disease (AD) in connection with acetylcholinesterase (AChE) inhibitors.[9] AChE is a significant enzyme in the central and peripheral nervous systems for the transmission of nerve impulses across nerve–nerve and neuromuscular synapses.[10] Among the various approaches to cholinergic enhancement, inhibition of the acetylcholine (ACh) 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.[11] Currently, several synthetic meso-porphyrin derivatives (**1a**, **1b**) which have difluorophenyl substituents on the periphery of the porphin skeleton turned out to be potent AChE inhibitors.[9]

Recently, Sugimoto et al. proposed a model of AChE active cites.[12] According to this model, there are four different active sites in the enzyme; hydrogen bonding site, negatively charged site and two different hydrophobic sites. These porphyrins might be fit to this model. The peripheral aryl groups in the porphyrins can bind to the hydrophobic region 2. The partial positive charge on the porphyrin skeleton developed by the electronegative fluorine atoms on the aromatic rings can interact with the negatively charged site in the enzyme.[9] In case of water soluble porphyrin salts, the positive charge on the peripheral pyridinium salt can also interact with this enzyme active site.[12] The precise sculpturing of the porphyrin environments to be a promising inhibitor seems that the peripheral tetra-aryl in the porphyrin should have at least a fluorine substituent at either 2 or 6-position of them.

We designed new porphyrin compounds (**2**–**4**) and water soluble porphyrin (**5**) as potential AChE inhibitors. As shown in Figure 1, porphyrins (**2**–**5**) have four multifluoropyridyl functionalities to fulfill the above assumption. Particularly porphyrin (**5**) could be the more useful inhibitor in view of its solubility in aqueous solution. In addition, it is interesting to examine how the number of fluorine substituents present on the aromatic rings affects the interaction with AChE.

Herein we describe the synthesis and preliminary inhibition data of porphyrins (**2**–**5**). Electric eel AChE was used to determine inhibition constant, K_i on the enzyme assay. All the synthetic compounds were isolated either by flash chromatography or recrystallization. Porphyrin

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(**2**) was successfully prepared in two steps from 2,3,5,6-tetrafluoropyridine-4-carbonitrile (**6**) in overall 3.2% yield (Scheme 1). The highly volatile aldehyde (**7**) can be obtained by the reduction of (**6**) with diisobutylaluminum hydride (DIBAH) at low temperature.[13] 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.[14] Typical reaction between equimolar amount of the aldehyde (**7**) and pyrrole in refluxing propionic acid afforded reasonable yield (7%) of the porphyrin (**2**). The synthesis of porphyrin (**3**) was achieved in six steps (overall 5% yield). The hydride reduction of the aldehyde (**7**) and the selective displacement of 6-fluorine with hydrazine produced the 6-hydrazinopyridine-4-carbinol (**9**).[15] The sequential removal of hydrazine by copper sulfate and Swern oxidation[16] of the subsequent alcohol (**10**) gave the aldehyde (**11**). The condensation reaction between equimolar amount of the aldehyde (**11**) and pyrrole in the presence of BF_3.etherate and the subsequent oxidation with *p*-chloranil led to produce the corresponding porphyrin (**3**). The optimized condition typically gave 29% of porphyrin (**3**). All the intermediates (**8**–**11**) in this synthesis were also new compounds.

The porphyrins (**2**, **3**) were not basic enough to form ionic, water-soluble porphyrins. Porphyrin (**4**) was

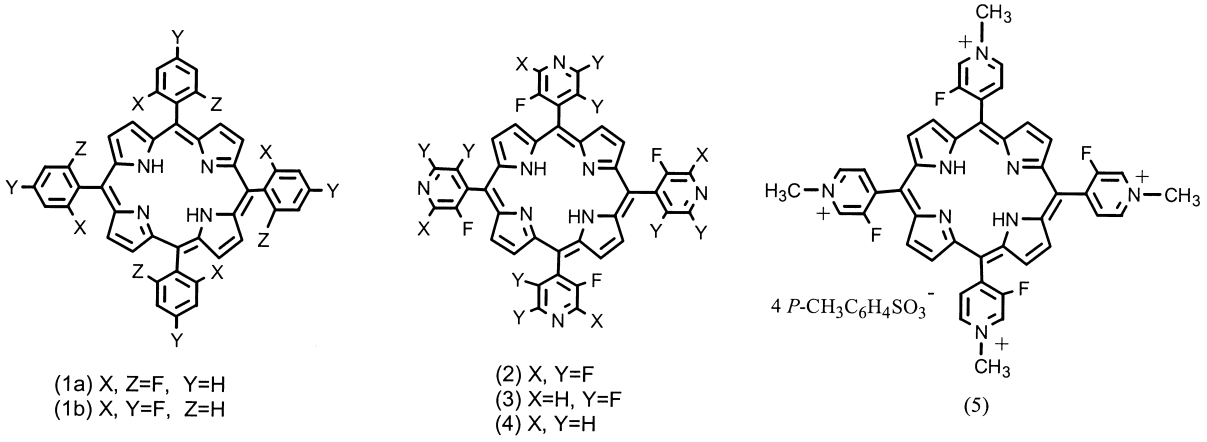
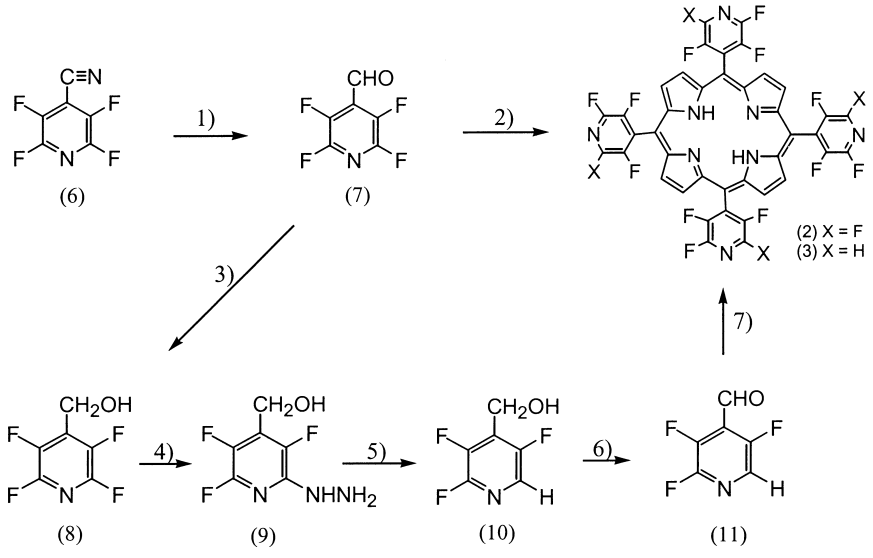
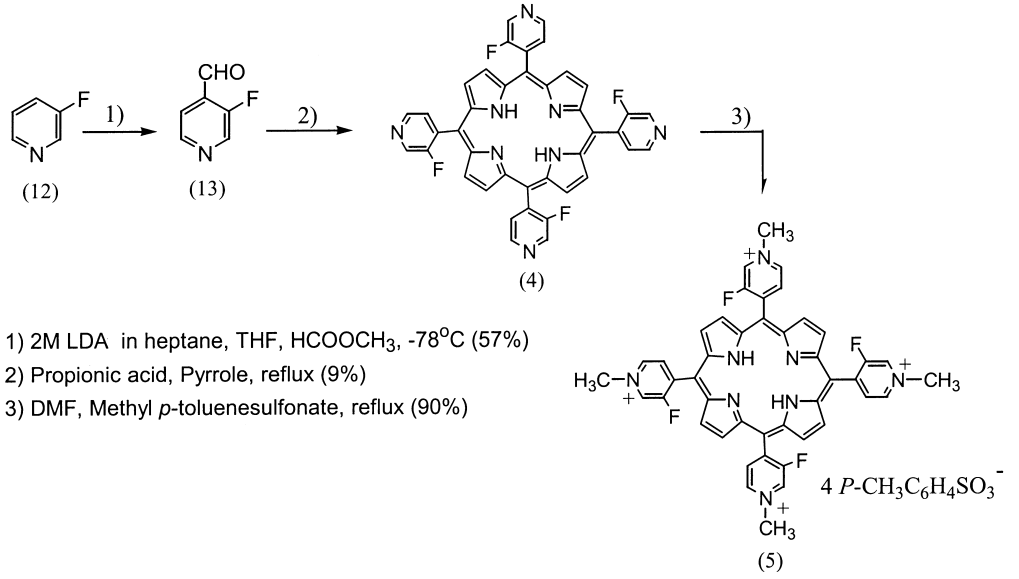


Figure 1.



Scheme 1.



(12) (13) (4) (5)

Scheme 2.

synthesized in two steps from 3-fluoropyridine (**12**) in overall 5% yield (Scheme 2). Lithiation of the pyridine (**12**) and the sequential reaction with methyl formate introduced the formyl group exclusively at C-4 position of the pyridine.[17] The typical reaction of the aldehyde (**13**) with pyrrole in propionic acid generated 9% of porphyrin (**4**). This method was usually best to produce porphyrin (**4**). It is remarkable that sufficiently basic porphyrin (**4**) was extracted with dichloromethane in concentrated HCl solution. Methylation of porphyrin (**4**) with methyl *p*-toluene sulfonate afforded the excellent yield (90%) of the water soluble porphyrin (**5**).[18] Porphyrins (**4**, **5**) are also new compounds. The structures of porphyrins (**2**–**5**) were confirmed by spectroscopic analyses.

The inhibition constants, K_I, of the porphyrin inhibitors determined by K_m/V_{max} versus inhibitor concentration replot are shown in Table 1. The inhibition constants of the pentafluorophenylporphin (F_{20}TPP) and fluoropyridylporphins (**2**–**5**) are in μM range. All the inhibitors (**2**–**5**) having fluorine atoms are reversible competitive inhibitors since they have the increased K_m values and little effect on the V_{max} values in these inhibition reactions. In the case of the porphyrins containing hydroxyl groups, they are either reversible noncompetitive inhibitors or reversible mixed type inhibitors for AChE. The fluoropyridylporphins are more potent inhibitors than pentafluorophenylporphin. Neither 2-hydroxyphenyl nor 3-hydroxyphenyl group gives much difference in the potency of the porphyrin inhibitors. The 2,5-dihydroxyphenyl group increased the inhibition potency of the porphyrin by 10-fold compared to 3-hydroxyphenyl group. All these results suggest that the compounds should have 2 or 6-fluoroaryl or 2-hydroxyaryl substitution at the periphery of the porphyrin to be the good porphyrin-based AChE inhibitors. Similar porphyrin compounds without fluorine or hydroxyl group are found to be ineffective in the AChE inhibition, which suggests that the presence of these groups in the inhibitors might be effective in the process of the enzyme inhibition.

Table 1. The inhibition constants of the porphyrin inhibitors[a]

Inhibitors	K_i (μ M)[b]	(K_m/K_i)[c]
F_{20}TPP[9]	15.2±4.30	9.0
(**1a**)[9]	0.005±0.001	26,000
(**1b**)[9]	0.012±0.002	12,000
(**2**)	7.29±1.24	19.2
(**3**)	2.85±0.42	49.1
(**4**)	5.08±0.8	27.6
(**5**)	3.06±0.4	45.8
2-FTPP[20]	50.9±2.5	2.7
3-FTPP[20]	9.7±0.6	14.3
2-(OH)TPP[20]	18.3±0.7	7.6
3-(OH)TPP[20]	29.6±1.2	4.7
2,5-$(OH)_2$TPP[20]	2.5±0.1	55.6

[a]Timecourses for the AChE-catalyzed hydrolysis of ATCh were followed by monitoring the formation of thioanion of nitrobenzoic acid at 412 nm by Ellman's coupled enzyme assay. Reactions were run in duplicate.
[b]K_i is determined from the K_m/V_{max} versus inhibitor concentration replot.
[c]K_m of acetylthiocholine (ATCh) for AChE is 0.14 mM. Porphyrins (**2**–**5**) contain fluoropyridyl groups and the others have fluorophenyl groups in their structures.

K_m/K_i Ratio roughly estimates the relative binding affinity of an inhibitor to the enzyme compared to the substrate.[9] Replacing carbon with nitrogen in phenyl groups of porphyrin mostly increased the binding affinity by 2–5-fold except the porphyrins (**1a**, **1b**). We have also reported the porphyrins (**1a**, **1b**) were much more potent inhibitors of AChE than ordinary inhibitors containing quaternary ammonium salt functionalities.[9] However these compounds (**1a**, **1b**) are rarely soluble in water. Therefore it had difficulties in controlling the concentration of inhibitors when animal test was carried out. Water soluble porphyrin (**5**) is more useful AchE inhibitors in this sense.

Moreover porphyrin (**5**) showed broad spectrum of anticancer activities; based on our preliminary studies,[19]

porphyrin (**5**) had potent antiangiogenic activity in vivo (more than 60% inhibition at 10 μg does per egg) on chorioallantoic membrane (CAM) of growing chick embryos.

It also exhibited good anticancer activity ex vivo on HL-60 leukemia cell, B16 BL6 melanoma cell and in vitro antimutational activities in Ames tests (data not shown here). Broad anticancer activities of porphyrin (**5**) are presumably considered to depend on the fluorine substituent and its proper position on the pyridine ring. Considering all the results mentioned in this study, synthetic manipulation of its derivatives could be another issue in the field of chemotherapeutics. The detailed biological tests of the water soluble porphyrin (**5**) and its derivatization is recently in progress.

Acknowledgements

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