SYNTHESIS OF A FLAVIN RECEPTOR FOR REGULATION OF ION RECOGNITION BY ASSEMBLING COMPONENTS NECESSARY FOR A BINDING SITE

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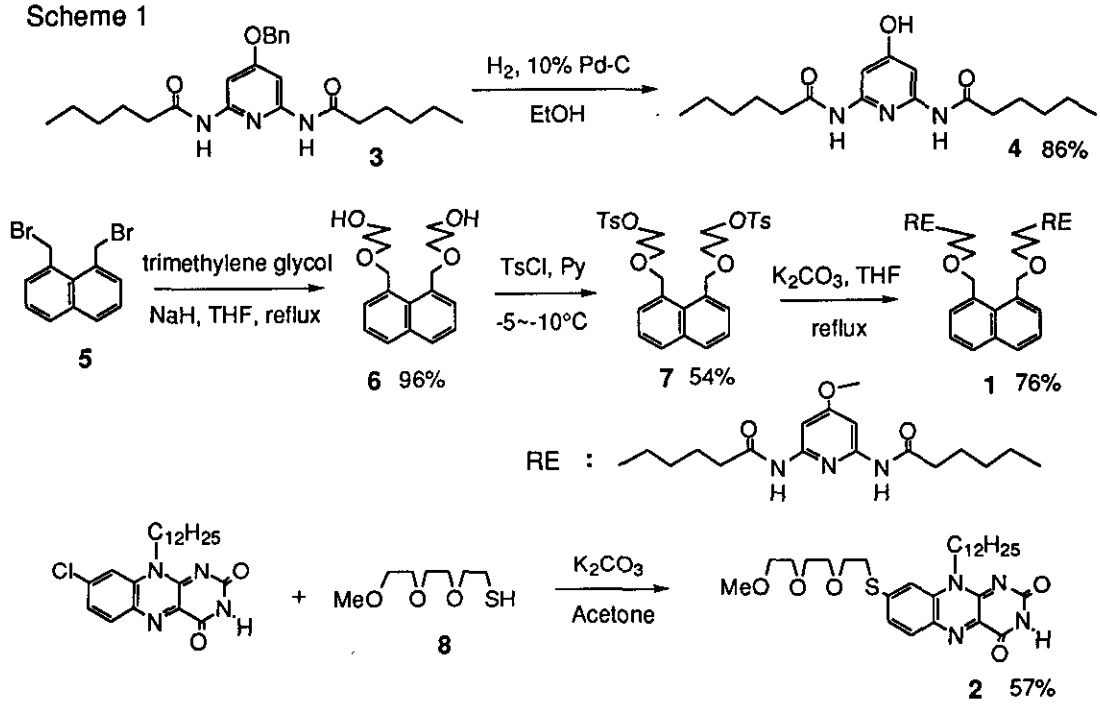
Abstract - A flavin receptor with two 2, 6-diacylaminopyridine moieties as the binding sites was synthesized. Upon complexation between the host and flavins bearing a polyether chain, the complex exhibits higher extractability toward K^+ than the host or the guest alone.

In host-guest chemistry, regulation of the binding ability has recently attracted much attention. Cooperativity, allostery[1] and self-assembling[2] have been employed for the regulation strategies in artificial recognition systems. In biological systems specific functions are sometimes induced by assembling several proteins with a protein called molecular chaperone which helps to constitute the assembled protein system but does not contribute the function directly.[3] This remote controlling methodology must be very important and efficient to regulate diverse functions of artificial molecules. In addition we think that this way will create a new concept in the artificial systems, *evolution of host molecules*, i.e., conversion of a host as the first generation to the second generation having different or more sophisticated functions at molecular level. This process may proceed over many generations of hosts. We, hence, planned application of this interesting regulation mechanism of molecular functions to artificial recognition systems. Here we report a new receptor (**1**, a host as the first generation) bearing two 2, 6-diacylaminopyridine moieties at the peri positions of a naphthalene nucleus. We also designed a guest (**2**) containing a polyether chain at the 8 position of the isoalloxiazine ring, because i) the pyridine moieties bind flavins[4] (the first guest) as well as uracyl derivatives[5] by a triple hydrogen bond, and ii) the supramolecule thus obtained (the second host) is expected to recognize an alkali metal ion (the second guest) due to assembling the polyether moieties in close proximity.

Scheme 1



The synthetic route to receptor (**1**) was shown in Scheme 1. Hydrogenolysis of 2, 6-bis(hexanoylamino)-4-benzyloxypyridine (**3**)[4] in EtOH in the presence of 10% Pd-C as a catalyst gave 2, 6-bis(hexanoylamino)-4-hydroxypyridine (**4**) in 86% yield. 1, 8-Bis(bromomethyl)naphthalene (**5**)[6] was treated with excess amount of trimethylene glycol and NaH in THF at reflux temperature overnight to yield diol (**6**) in 96%. Tosylation of **6** was carried out carefully by TsCl in pyridine at -5~-10°C to afford **7**. The receptor was obtained in 76% by the reaction of **4** with **7** and K_2CO_3 in THF at reflux temperature overnight.[7] A polyether side chain was introduced into the flavin nucleus by treatment of 8-chloroflavin[8] with mercaptan (**8**) and K_2CO_3 in acetone to give **2**[7] in 57% yield. The number of oxygen atoms in the side chain is critical to regulate ion recognition in this system. Generation of a new ion recognition site is expected upon complexation of **1** and **2**, but free **2** would exhibit very low affinity to alkali metal ions. Because i) coordination of three oxygen atoms in ethylene glycol oligomers is insufficient for effective binding to alkali metal ions, and ii) the two polyether chains assembled in close proximity probably provide a much better ion-recognition site consisting of six oxygen atoms.[9]

Interaction due to triple hydrogen bonding between **1** and **2** was supported by downfield shifts of ^{1}H nmr ($CDCl_3$) resonances assigned to the amide protons of **1** and **2**, as seen in other flavin receptors.[4] Titration using the resonance of **1** suggested that one diacylaminopyridine moiety binds one flavin molecule.

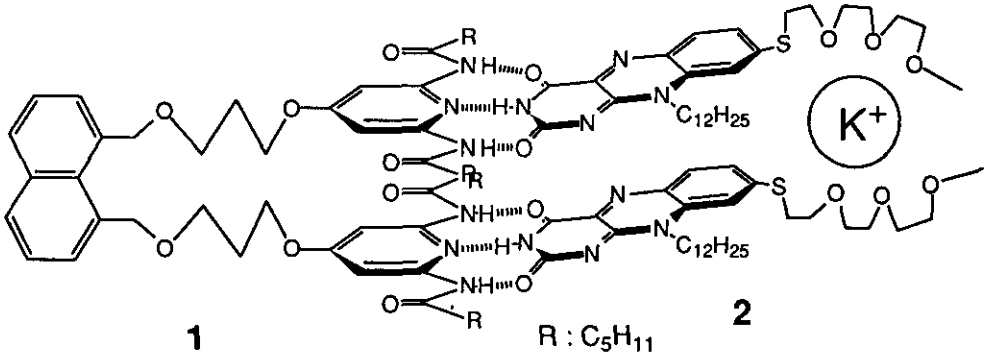
Table 1 Solvent Extraction of Potassium Ion

Carrier	Extractability(%)
1	2 ± 1
2	0
1 + **2**	15 ± 3
3	2 ± 1
2 + **3**	2 ± 1

Extractability(%) = {(5.0 x 10^{-5} - $[Pic^-]_{aq}$) / 5.0 x 10^{-5}} x 100
aq. phase : [PicK] = 5.0x10^{-5} M
org. phase(1,2-dichloroethane) : [**1**] = 1.25x10^{-3} M,
[**2**] = 6.00x10^{-3} M, [**3**] = 2.5x10^{-3} M, stirred for 2 h at 25 °C.

Solvent extraction experiment (H_2O - 1,2-dichloroethane) was performed to clarify an effect of molecular assembling between **1** and **2** on binding ability toward potassium picrate. The amounts of picrate anion extracted into the organic phase were determined by spectrophotometrical measurements of decrease of picrate anion in the aqueous phase. Extractability thus estimated indicated that receptor (**1**) and guest (**2**) show very low values, if any (**1**, 2 ± 1 %, 1.25 x 10^{-3} M; **2**, 0 %, 6 x 10^{-3} M, Table 1). In contrast, a mixture of **1** (1.25 x 10^{-3} M) and **2** (6 x 10^{-3} M) exhibits a much higher value (15 ± 3 %). The ^1H nmr titration curve implied that 51 % of binding moieties of **1** are occupied by the imido moiety of **2** under these conditions.[10] Obviously, **1** and **2** remaining free should not contribute the enhancement of extractability (see Table 1). Compound (**3**) as a receptor exhibits *ca.* 2 % of extractability for K^+. In addition, a mixture of **2** and **3** (**2**, 6 x 10^{-3} M; **3**, 2.5 x 10^{-3} M) does not enhance the value at all. Therefore, the considerable increase of binding ability of a mixture of **1** and **2** is ascribed to formation of a new binding site for K^+ because of assembling the two short polyether chains of **2** in close proximity. A plausible structure suggested from the nmr measurements and the solvent extraction is depicted in Figure 1, although more detailed study is necessary to clarify the fine structure.

Figure 1 A Plausible Structure of a Supramolecule generated from **1**, **2** and K^+



1 **2**

From the results obtained here, **1** is considered the first generation of host and the supramolecule is considered the second generation of host which has completely different binding affinity. At best this system is one example of evolution of molecular functions in artificial systems. Assembling and orienting components[11] necessary for construction of a host will be also a useful method for all-or-non type regulation of molecular functions. Detailed analysis of the structure of the supramolecule and study of the catalytic activity on oxidation reactions mediated by flavins especially for cationic substrates are currently undergoing in our laboratory.

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7. **1**: Colorless oil. ^{1}H Nmr (500MHz, $CDCl_3$) δ (ppm); 0.91 (t, 6H, J = 7.3 Hz), 1.3-1.8 (m, 12H), 2.0-2.1 (m, 4H), 2.34 (t, 4H, J = 7.7 Hz), 3.64 (t, 4H, J = 6.4 Hz), 4.08 (t, 4H, J = 5.8 Hz), 4.97 (s, 4H), 7.38 (t, 2H, J = 7.7 Hz), 7.49 (s, 4H), 7.52 (d, 2H, J = 6.1 H), 7.60 (br s, 4H), 7.78 (d, 2H, J = 8.3 Hz). ^{13}C Nmr (125MHz, $CDCl_3$) δ (ppm); 13.91, 22.41, 25.02, 29.41, 31.36, 37.76, 65.20, 65.96, 73.73, 96.26, 124.88, 130.36, 131.07, 131.32, 133.72, 135.78, 150.57, 168.85,

171.71. Ir (KBr); 3294, 2955, 2929, 2870, 1614, 1582, 1547, 1536, 1513, 1503, 1441, 1207, 1162 cm^{-1}. Anal. Calcd for $C_{52}H_{74}N_6O_8 \cdot H_2O$: C; 67.22, H; 8.24, N; 9.04. Found: C; 67.60, H; 8.19, N; 8.68.

2: Yellow powder. mp 146-7°C (EtOH). ^1H Nmr (200MHz, $CDCl_3$) δ (ppm); 0.88 (t, 6H, J = 6.4 Hz), 1.2-1.7 (m, 18H), 1.7-2.0 (m, 2H), 3.3-3.9 (m, 15H), 4.6-4.8 (m, 2H), 7.42 (d, 1H, J = 1.7 Hz), 7.53 (dd, 1H, J = 9, 1.7 Hz), 8.10 (d, 1H, J = 9 Hz), 8.44 (s, 1H, NH). ^{13}C Nmr (50MHz, $CDCl_3$) δ (ppm); 14.17, 22.76, 26.98, 27.06, 29.46, 29.66, 29.71, 32.01, 32.31, 45.44, 59.22, 69.75, 70.79, 70.85, 71.01, 72.13, 110.91, 125.17, 133.42, 133.56, 134.46, 150.95, 151.43, 155.79, 160.02. Ir (KBr); 3150, 3101, 3024, 2922, 2852, 1714, 1653, 1560, 1525, 1467, 1444, 1423, 1394, 1351, 1282, 1197, 1131, 1084 cm^{-1}. Uv-vis; 475.5 (log ε; 4.41), 463.5 (4.35), 450.0 (4.43), 271.5 (4.46), 257.5 nm (4.53). Hrms calcd for $C_{29}H_{44}O_5N_4S$: 560.3032. Found: 560.3060. Anal. Calcd for $C_{29}H_{44}N_4O_5S \cdot 1/3\ H_2O$: C; 61.46, H; 7.94, N; 9.89. Found: C; 61.37, H; 7.91, N; 9.82.

8 The 8-chloroflavin was obtained by treating 6-docecylaminouracil with excess *p*-chloronitrosobenzene in acetic anhydride - acetic acid (4:1, v/v) at 110°C overnight in 52 % yield by a similar method employed for preparation of *N*-methylisoalloxazine analogue. See, F. Yoneda, Y. Sakuma, M. Ichiba, and K. Shinomura, *J. Am. Chem. Soc.*, 1976, **98**, 830.

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10 The chemical shift of the amide protons of the pyridine moieties in the complex of **2** and **3** was calculated to be 10.16 ppm from a titration curve using non-linear-least-squares-curve fitting. The value was used as a chemical shift for the complex of **1** and **2** to estimate a fraction of the pyridine moieties of **1** bound to **2**, because an accurate value of the complex was not obtained from a titration curve for **1** and **2**.

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