Synthesis of 2,6-Diamidopyridine Derivatives and their Functions as Flavin Receptors in Chloroform

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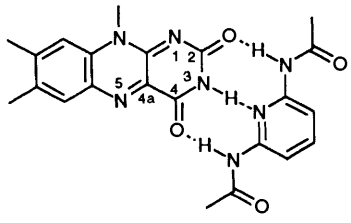
It has been found that 2,6-diamidopyridine derivatives act as flavin receptors by a triple hydrogen bond towards a uracil moiety of an isoalloxazine ring in $CHCl_3$. The association constants were determined by ^1H NMR (in $CDCl_3$) and fluorescence (in $CHCl_3$) spectroscopies; the largest is *ca.* 10^3 mol^{-1} dm^3. The triple hydrogen bond toward C(2)=O, N(3)–H and C(4)=O of the isoalloxazine ring was found to enhance slightly the oxidation activity in $CHCl_3$.

For the construction of totally synthetic catalysts that exhibit enzyme-like functions (artificial enzymes), the following are of primary importance; (*i*) a highly active catalytic group; (*ii*) that the catalyst has a substrate-binding site and (*iii*) that the functional groups are arranged properly.[1] Many model systems employing functionalized micelles, macrocycles, membranes and polymers have been reported so far.[2] In such systems, arrangement of the functional group is achieved by covalent and/or non-covalent bonds such as hydrophobic and electrostatic interactions and hydrogen bonds.

To construct an artificial flavoenzyme, we have successfully exploited remarkably high oxidation-active flavin mimics by the chemical modification of an isoalloxazine ring.[3] Among them benzodipteridine (BDP), which shows *ca.* 10^7-fold rate enhancement for the oxidations proceeding *via* C(4a)-attack, is quite useful for studies of flavin-mediated oxidations in model systems.[3c,e,f] Thus, our next subject is the incorporation of functionality into the oxidation-active flavin mimic by covalent and/or non-covalent bonds. A successful example for the covalent functionalization is a D-lactate dehydrogenase model system; BDP having a bipyridin-6-ylmethyl moiety at the N(3)-position oxidizes α-hydroxy acids to α-keto acids in the presence of Zn^{2+} and a base in an organic solvent such as Bu^tOH.[4] It is found that Zn^{2+} bound into the bipyridine moiety not only improves the oxidation activity of BDP but also acts as a substrate-binding site and activates the substrate.

For the non-covalent functionalization, one may consider a flavin receptor bearing functional groups. The functionalized flavin receptor binds a flavin to form a molecular complex in which the functional groups would be arranged near the reaction site by non-covalent bonds. Thus we focused our attention on the exploitation of a flavin receptor. Meanwhile, functions of biological molecules generally appear through specific interactions with another molecule and a hydrogen bond is one of the significant factors in such interactions. Many receptors using hydrogen bonds have been reported from a molecular recognition viewpoint.[5] For example, Feibush *et al.* reported that barbiturates, glutamides and hydantoins, possessing a structural similarity to uracil and thymine skeletons, are able to interact with 2,6-diamidopyridine derivatives by the triple hydrogen bond in a solute–stationary phase of HPLC.[6] Hamilton *et al.* have shown that a macrocyclic receptor containing 2,6-diamidopyridine and naphthalene components (**1h**) forms a molecular complex with a thymine derivative by the triple hydrogen bond and π–π stacking.[5a,7] These facts suggest that 2,6-diamidopyridine derivatives are able to act as a flavin receptor by the triple hydrogen bond as shown in Scheme 1, since an isoalloxazine ring possesses a uracil moiety within the molecule.

In this paper, we describe the synthesis of 2,6-diamidopyridine derivatives, association constants with flavin models in chloroform, potentiality as a flavin carrier and the effect of the receptors on the oxidation activity of flavin models in $CHCl_3$.[8] The compounds employed are shown in Scheme 2 [receptors (**1**) and flavin model compounds (**2**–**5**)].



Scheme 1

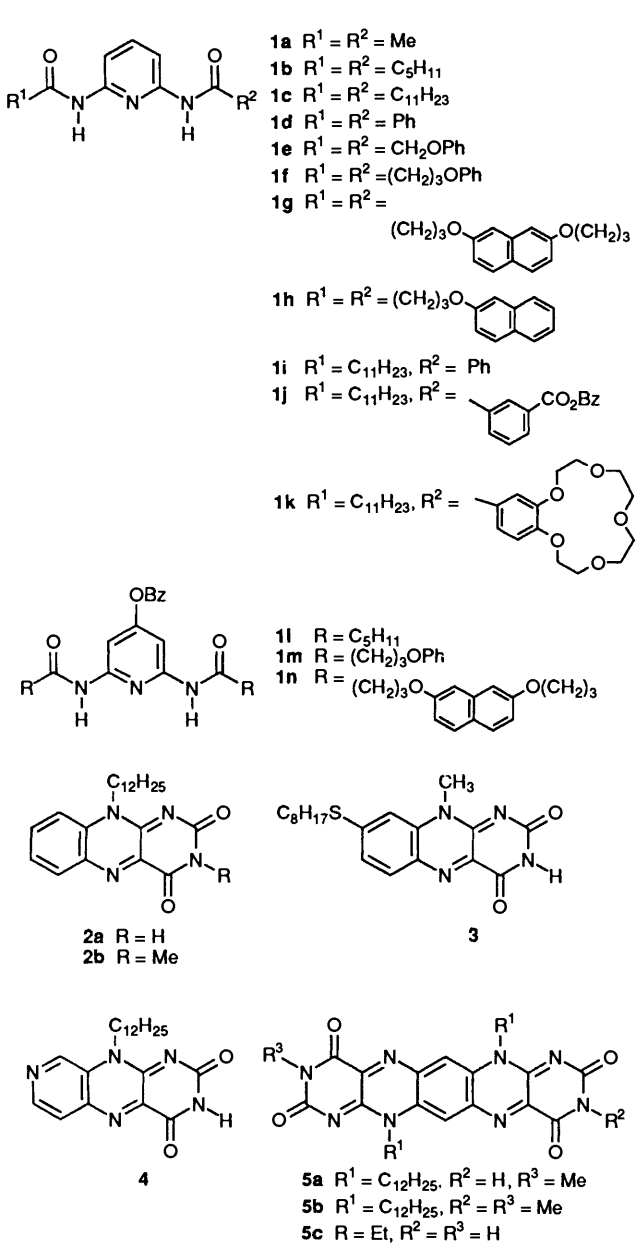
Results and Discussion

Synthesis.—Receptors (**1a**–**1k**) were synthesized from 2,6-diaminopyridine and the corresponding acid chlorides in a similar manner to the literature.[7b] 4-Substituted 2,6-diamidopyridine derivatives (**1l**–**1n**) were synthesized by a similar procedure described by Feibush *et al.*[6] Flavins **2a** and **2b**,[9] **3**,[10] **4**[3a] and **5a**–**5c**[3d,e] were synthesized according to literature methods. Flavin **5a** was synthesized as for **5b** and **5c** except for the stepwise condensation of *N*,*N*′-didodecyl-*p*-phenylene diamine with 6-chloro-3-methyluracil and 6-chlorouracil as shown in Scheme 3.

^1H *NMR Study.*—Formation of the triple hydrogen bond as shown in Scheme 1 was examined by ^1H NMR spectroscopy in $CDCl_3$. The ^1H NMR spectra of **1f**, **2a** and their 1 : 1 mixture are shown in Fig. 1. As can be seen in Fig. 1(*c*), both the N–H protons of **1f** and **2a** showed downfield shifts. In the case of **2b**, however, such a chemical shift of the N–H of the receptor was not observed. This indicates clearly that **1f** and **2a** form a molecular complex by the triple hydrogen bond as shown in Scheme 1. In this complex, slight upfield shifts on the phenyl protons of **1f** were also observed, suggesting the existence of π–π stacking between the phenyl ring of **1f** and **2a**. Similar upfield shifts were also observed for the aromatic protons of **1g**, **1h**, **1m** and **1n**.

The stoichiometry of the complex formation was determined by a Job plot, which has a maximum at a mole fraction of *ca.* 0.5 (Fig. 2). This indicates explicitly a 1 : 1 complex formation as shown in Scheme 1.

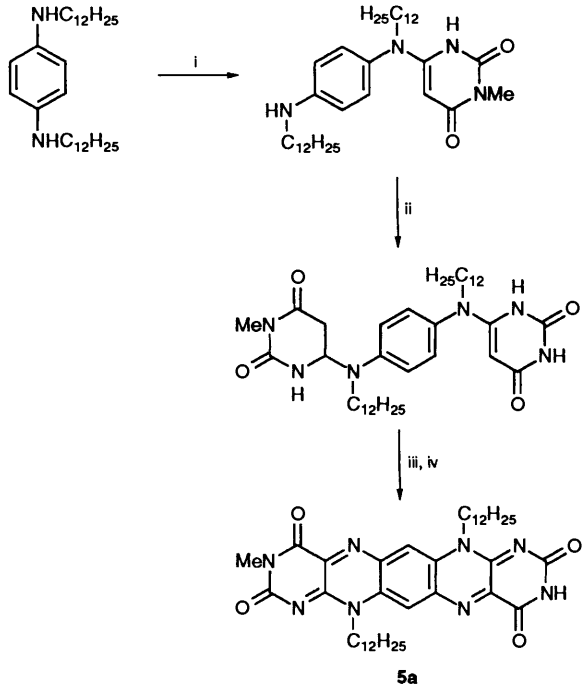
Determination of Association Constants.—The association constants were determined by ^1H NMR spectroscopy in $CDCl_3$. Plots of the changes of the chemical shifts of the flavin N(3)–H



Scheme 2 1, Receptors; 2–5 flavin model compounds

resonances as a function of the receptor concentration gave a titration curve which allowed us to calculate the association constants by the non-linear least-squares fitting of the titration curve. A typical ^{1}H NMR titration curve of the N(3)–H of **2a** with **1b** are shown in Fig. 3.

Some association constants were also determined by fluorescence spectroscopy in $CHCl_3$.[11] The results are summarized in Table 1 together with the chemical shifts of N–H of the receptors. Association constants determined by each method show good agreement. It was confirmed that the association constants of **2b** with **1b** or **1h** are less than 20 mol^{-1} dm^3, indicating that dynamic fluorescence quenching of flavins by receptors is quite small. Inspection of Table 1 indicates that the *K* values are dependent on the structure of the receptors, and seem to be related to the chemical shifts of the N–H of the receptors. The more acidic N–Hs with downfield chemical shifts form stronger hydrogen bonds, resulting in larger *K* values. However, **2a** does not form the complexes with **1d** and **1e** and weakly form complexes with the receptors **1i**, **1j** and **1k**. These results may be explained by steric hindrance of the *ortho* hydrogens of the phenyl group of the receptors in the complex



Scheme 3 *Reagents:* i, 6-chloro-3-methyluracil, *N*,*N*-diethylaniline, BuOH; ii, 6-chlorouracil, *N*,*N*-diethylaniline; iii, $NaNO_3$, H_2SO_4, AcOH; iv, $Na_2S_2O_4$, DMF–H_2O

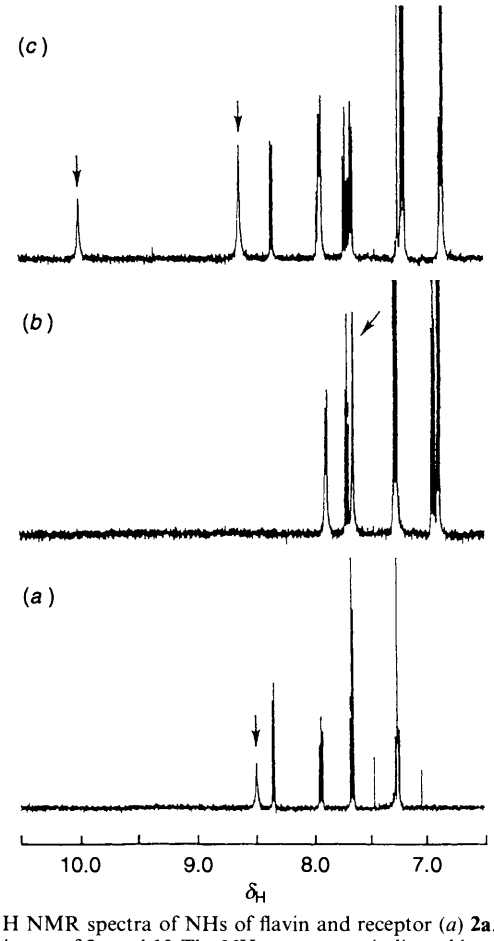


Fig. 1 ^{1}H NMR spectra of NHs of flavin and receptor (*a*) **2a**, (*b*) **1f**, (*c*) 1 : 1 mixture of **2a** and **1f**. The NH protons are indicated by arrows.

formation, since the amide bonds of the receptors require coplanarity of the phenyl group. No complex formation of **1e** may be explained by formation of an intramolecular five-membered hydrogen bond between the ether oxygen and the

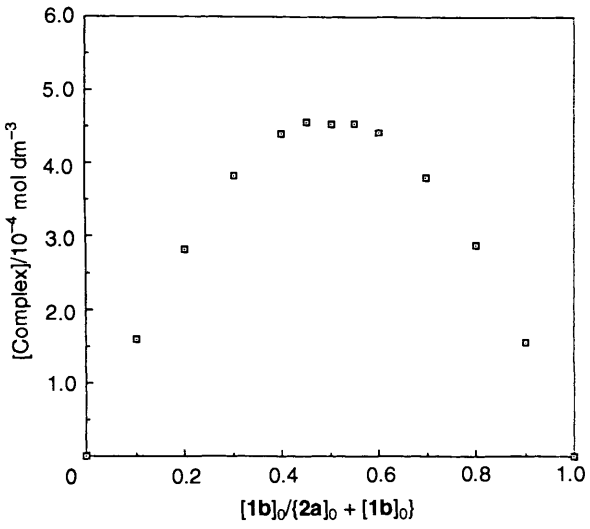


Fig. 2 Job plot for **2a** and **1b**. [**2a**] + [**1b**] = 5.0 × 10^{-3} mol dm^{-3} in $CDCl_3$, 25 °C

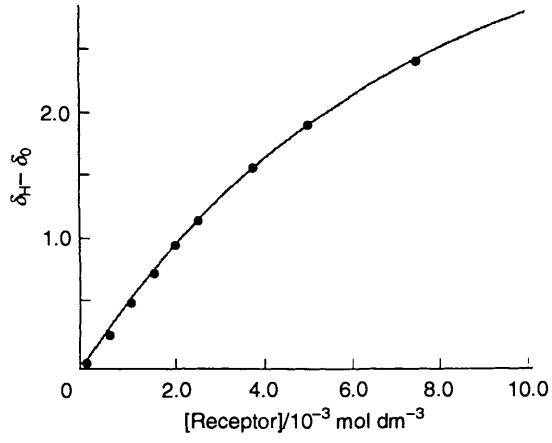


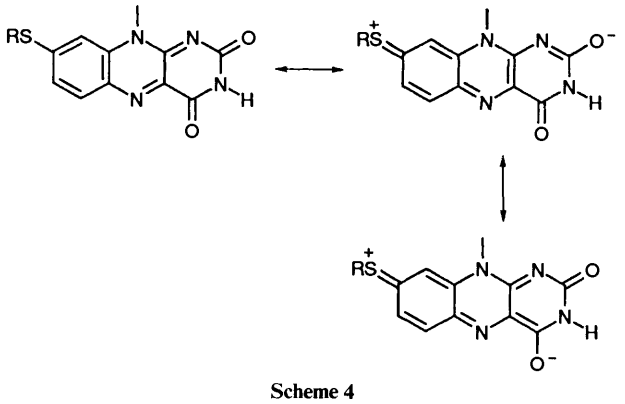
Fig. 3 Plot of chemical shifts of NH proton of **2a** *vs.* [**1b**] in $CDCl_3$ at 25 °C. [**2a**] = 2.5 × 10^{-3} mol dm^{-3}, the theoretical line was obtained by the curve fitting method.

Table 1 Association constants with flavin **2a**

Receptors	NH (receptor) ppm	K/mol^{-1} dm^3	
1a	7.53	510 ± 30[a]	(410 ± 30)[b]
1b	7.50	150 ± 10	(180 ± 10)
1c	7.50	160 ± 30	(170 ± 10)
1d	8.29	~0	
1e	8.73	~0	
1f	7.65	290 ± 20	(320 ± 0)
1g	7.60	800 ± 80	(1060 ± 10)
1h	7.74	820 ± 120	(810 ± 80)
1i	7.57, 8.28	*ca.* 10 ± 0	
1j	7.55, 8.16	*ca.* 20 ± 10	
1k	7.57, 8.28	*ca.* 30 ± 20	
1l	7.49	210 ± 20	(230 ± 0)
1m	7.64	390 ± 0	(390 ± 20)
1n	7.70	770 ± 10	(940 ± 40)

[a] In $CDCl_3$ at 25 °C. The uncertainty is expressed by standard deviation. [b] Association constants determined by fluorescence spectroscopy are given in parentheses; in $CHCl_3$ at 20 °C.

amide NH hydrogen, which disturbs the intermolecular hydrogen bond formation with flavin. The larger K values of **1g** and **1h** suggest the contribution of π–π stacking between the



Scheme 4

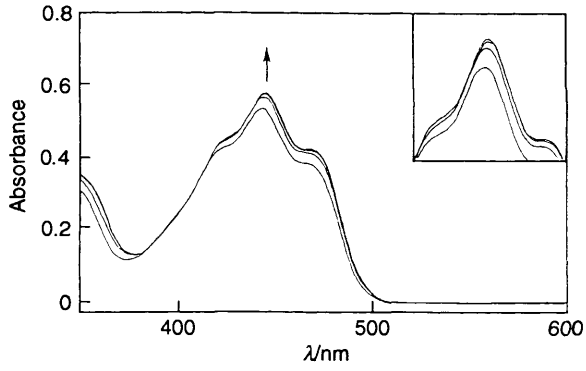


Fig. 4 Spectral change of **2a** by addition of **1a** in $CHCl_3$ at 25 °C. [**2a**] = 1.0 × 10^{-5} mol dm^{-3}, [**1a**] = 0 – 1.38 × 10^{-2} mol dm^{-3}.

naphthalene ring and the isoalloxazine ring. The association constants obtained from fluorescence spectroscopy are in fairly good agreement with those from the ^{1}H NMR spectra (**1a**, **1b**, **1f**, **1g**, **1h**, **1l** and **1m**).

The association constants are also dependent on the structure of the flavins (Table 2). The electron densities on the carbonyl oxygens at the C(2)- and C(4)-positions of **3** are expected to be increased by the 8-octylmercapto group owing to the resonance structures as shown in Scheme 4. In fact, the association constants of **3** are larger than those of **2a**, **4** and **5a**. However, for electron-deficient flavins (**4** and **5a**),[12] no noticeable decrease in the K values was observed.

Effect of Receptors on the Electronic Absorption Spectra of Flavins.—The absorption spectra of flavins are known to be changed by hydrogen bonding at the hetero atoms of the isoalloxazine ring.[13] The absorption band of **2a** (λ_{max} 441 nm) slightly shifted to λ_{max} 443 nm with the increase of the optical density upon the addition of **1a** (Fig. 4). This observation is in agreement with the result of *ab initio* calculations of the hydrogen-bonded isoalloxazine at the C(2)=O, N(3)–H and C(4)=O.[14] Similar spectral changes were also observed for **3**, **4** and **5a** in the presence of **1b**, **1c** or **1l**. No spectral change was observed for **2b** in the presence of the receptors. These results also suggest formation of the triple hydrogen bond as shown in Scheme 1. On the other hand, when the receptors having the naphthalene component (**1g**, **1h** and **1n**) were employed, the spectral changes of the flavins (**2a**, **3**, **4** and **5a**) were found to be slightly different from those with **1a**. The absorption spectra of **2a** in the presence of **1g** and **1h** are shown in Fig. 5. Fig. 5 shows that the spectral shape is broadened in the presence of **1g** or **1h** with slight shift to longer wavelength, probably due to π–π stacking between the flavin and the naphthalene ring(s). Furthermore, the optical density of **2a** at λ_{max} 443 nm was found to increase in the presence of the macrocyclic receptors (**1h** and

Table 2 Association constants of flavins and receptors[a]

Flavins	K/mol^{-1} dm^3				
	1b	**1h**	**1l**	**1m**	**1n**
2a	150 ± 20	820 ± 120	210 ± 20	390 ± 30	550 ± 70
3	270 ± 30	1300 ± 140	510 ± 40	1300 ± 250	1300 ± 230
4	86 ± 10	630 ± 90	280 ± 10	170 ± 20	820 ± 280
5a	90 ± 20	630 ± 60	150 ± 10	430 ± 100	1400 ± 200

[a] In $CDCl_3$, at 25 °C; the uncertainty is expressed by standard deviation.

Table 3 Extraction of **5c** by receptors

Receptor	Extractability (%)
None	< 1.5
1b	3.2
1f	5.1
1g	24
1h	16
1l	4.6

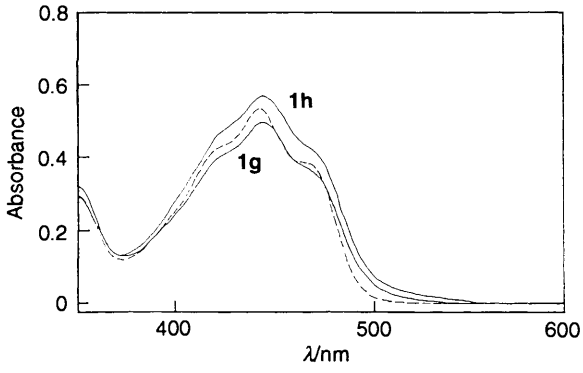


Fig. 5 Spectral change of **2a** by addition of **1g** or **1h** in $CHCl_3$ at 25 °C. A dotted line shows the spectrum of **2a** without the receptor. [**2a**] = 1.0 × 10^{-5} mol dm^{-3}, [**1g**] = [**1h**] = 7.2 × 10^{-3} mol dm^{-3}.

1n), and to decrease with the non-ring receptor (**1g**). The same trend was observed for the other flavins (**3**, **4** and **5a**). However, the reason is not clear at present, although it suggests a different π–π stacking form for **1g** and **1h**.

Flavin Extraction by the Receptor.—The molecular complex of a water-soluble flavin with the receptor is considered to be strongly hydrophobic. If so, the receptor would act as a carrier of water-soluble flavins from an aqueous layer into an organic layer. This possibility was examined by employing a flavin extraction experiment in a two-phase system of H_2O–$CHCl_3$. A solution of $CHCl_3$ and H_2O containing **5c** was stirred vigorously and the concentration of **5c** in the $CHCl_3$ layer was determined spectrophotometrically (542 nm). The extractability was calculated by eqn. (1), the results are shown in Table 3. In

$$\text{Extractability}(\%) = \frac{[\text{Flavin}]_{org}}{[\text{Flavin}]_0 - [\text{Flavin}]_{org}} \times 100 \quad (1)$$

the absence of the receptors, **5c** was found to be scarcely extracted into the $CHCl_3$ layer (< 1.5%). In the presence of the receptors (250 molar excess over **5c**), however, **5c** was found to be extracted into the $CHCl_3$ layer. It is notable that **1g** and **1h** extract **5c** more effectively compared with the other receptors. Extraction equilibrium constant (K_e) of **1g** and **1h** with **5c** can be determined by the following equations [eqns. (2)–(4)].[15] Thus

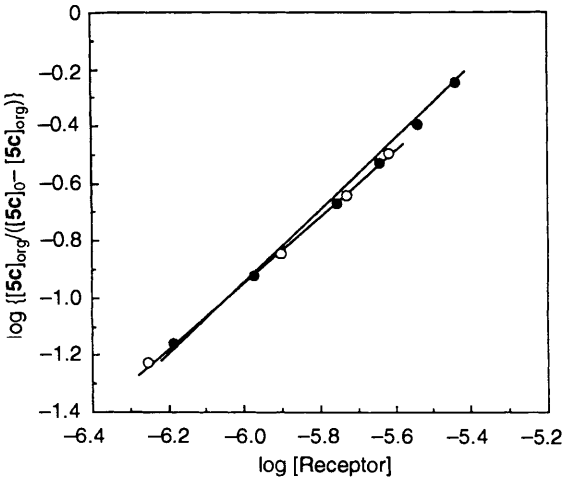


Fig. 6 Plots of eqn. (6) for **1g** or **1h**. ●, **1g**; ○, **1h**

$$[\text{Flavin}]_{aq} \overset{K_1}{\rightleftharpoons} [\text{Flavin}]_{org} \quad (2)$$

$$[\text{Flavin}]_{org} + n[\text{Receptor}]_{org} \overset{K_2}{\rightleftharpoons} [\text{Complex}] \quad (3)$$

$$[\text{Flavin}]_{aq} + n[\text{Receptor}]_{org} \overset{K_e}{\rightleftharpoons} [\text{Complex}] \quad (4)$$

the overall binding of the receptor with the flavin in the solvent extraction can be represented as shown in eqn. (5), where

$$K_e = K_1K_2 = \frac{[\text{Complex}]}{[\text{Flavin}]_{aq}[\text{Receptor}]^n_{org}} = \frac{[\text{Flavin}]_{org}}{([\text{Flavin}]_0 - [\text{Flavin}]_{org})[\text{Receptor}]^n_{org}} \quad (5)$$

$[\text{Flavin}]_{aq}$ and $[\text{Flavin}]_{org}$ denote the concentrations of the flavin in aqueous and organic layers, respectively. Since $[\text{Flavin}]_{aq} + [\text{Complex}] \gg [\text{Flavin}]_{org}$ can be assumed on the basis of insolubility of **5c** in the organic layer, eqn. (5) is represented as eqns. (6) and (7). The n value was determined to

$$\log \frac{[\text{Flavin}]_{org}}{[\text{Flavin}]_0 - [\text{Flavin}]_{org}} = n \log [\text{Receptor}]_{org} + \log K_e \quad (6)$$

be 1.0 from the slope in a plot of log $[\mathbf{5c}]_{org}/([\mathbf{5c}]_0 - [\mathbf{5c}]_{org})$ *vs.* log [receptor] (Fig. 6). The unity of the n value indicates that **5c** is extracted *via* the formation of a 1 : 1 complex, although **5c** has two binding sites. K_e values were also calculated to be 6.3 and 5.9 mol^{-1} dm^3 for **1g** and **1h**, respectively.

Effect of Receptors on Oxidation-activity of Flavin.—It is known that flavin coenzymes bind to specific sites of

Table 4 Effect of receptors on the oxidation of $PhNHNH_2$ and BNAH by **5** in $CHCl_3$[a]

Receptor	$10^3 k_{obs}/s^{-1}$ (rel. rate)					
	$PhNHNH_2$			BNAH		
	5a	**5b**	**5c**[b]	**5a**	**5b**	**5c**[b]
None	5.71 (1.0)	4.48 (1.0)	15.0 (1.0)	3.03 (1.0)	1.22 (1.0)	20.3 (1.0)
1a	7.63 (1.3)	3.58 (0.80)	61.1 (4.1)	4.15 (1.3)	0.993 (0.76)	27.8 (1.3)
1b	7.34 (1.3)	4.59 (1.0)	25.0 (1.7)	3.28 (1.1)	1.21 (1.0)	21.0 (1.0)
1c	8.41 (1.5)	3.77 (0.84)	27.7 (1.8)	2.84 (0.94)	1.17 (0.96)	19.6 (0.96)
1f	10.3 (1.8)	4.41 (0.98)	46.1 (3.1)	3.48 (1.1)	1.01 (0.83)	21.5 (1.1)
1h	11.0 (1.9)	4.16 (0.93)	22.6 (1.5)	3.77 (1.2)	1.08 (0.88)	31.4 (1.5)
1l	—	—	28.6 (1.9)	3.04 (1.0)	1.15 (0.94)	21.7 (1.1)
1m	9.37 (1.6)	3.67 (0.82)	49.1 (3.3)	3.40 (1.1)	1.02 (0.84)	23.2 (1.1)
1n	7.35 (1.3)	2.80 (0.63)	22.1 (1.5)	3.45 (1.1)	1.24 (1.0)	37.7 (1.8)

[a] [**5**] = 1.0×10^{-5} mol dm^{-3}, [$PhNHNH_2$] = 1.83×10^{-3} mol dm^{-3}, [BNAH] = 8.00×10^{-4} mol dm^{-3}, [Receptor] = 5.00×10^{-3} mol dm^{-3}, N_2, 25 °C. The data are the average values of at least two runs; relative error is less than 10%. [b] 2% (v/v) DMF.

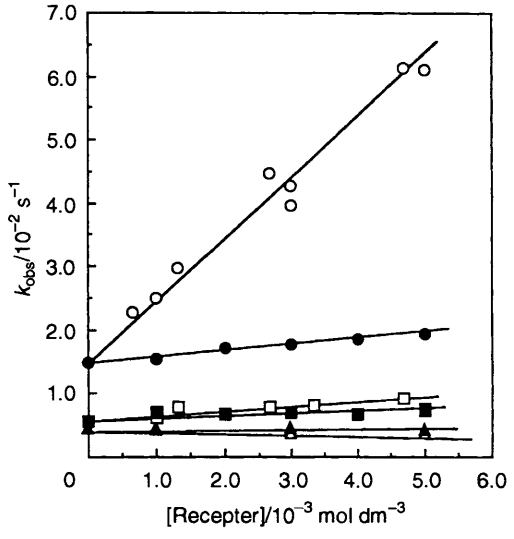


Fig. 7 Concentration effect of receptors on the oxidation of $PhNHNH_2$ by **5**. With **1a**: □, **5a**; △, **5b**; ○, **5c** (2% DMF). With **1c**: ■, **5a**; ▲, **5b**; ●, **5c** (2% DMF). [**5**] = 1.0×10^{-5} mol dm^{-3}, [$PhNHNH_2$] = 1.83×10^{-3} mol dm^{-3}, N_2, 25 °C

apoproteins, in which hydrogen bondings towards heteroatoms of an isoalloxazine ring play an important role at active sites of flavoproteins.[16] In model systems, it is well established that the hydrogen bonding at the N(5)-position of an isoalloxazine ring facilitates the reactions proceeding *via* C(4a)-attack by stabilizing the negative charge generated on the N(5)-atom.[17] Meanwhile Nishimoto *et al.* have suggested, on the basis of quantum mechanical considerations, that hydrogen bonds occurring at C(2)=O, N(3)–H and C(4)=O of an isoalloxazine ring are one of the important factors to regulate the catalytic activity of flavoproteins.[14] It is of interest, therefore, to examine the effect of the receptor on the reactivity of flavins.

Effects of the receptors on the reactivity of flavins were kinetically examined for oxidations of *N*-benzyl-1,4-dihydronicotinamide (BNAH)[18] and phenylhydrazine[19] by **5** in $CHCl_3$ under anaerobic conditions. It should be noted that these oxidations occur smoothly in $CHCl_3$ only when the oxidation-active flavin mimic is used. Pseudo-first-order rate constants were determined by following the absorption increase of the reduced **5** at 640 nm.[3a] The concentration effect of the receptors (**1a** and **1h**) on the rate of the oxidation of $PhNHNH_2$ by **5** are shown in Fig. 7.

Fig. 7 shows clearly that the rates increase with increase of [receptor] in the case of **5c**, and **1a** is much more effective than **1h**.[20] This may be owing to steric hindrance of the naphthalene ring of **1h** for nucleophilic attack of $PhNHNH_2$ at the C(4a)-position.[19] In the case of **5a** possessing one triply hydrogen-bonded site, the rate enhancement is smaller than **5c**, probably because $PhNHNH_2$ is able to attack another C(4a)-position of the non-hydrogen-bonded part of **5a**. Flavin model **5** could be considered to possess two isoalloxazine rings fused to a common benzo moiety, resulting in two reaction sites. For **5b**, no rate enhancement is observed for both the reactions because there is no binding site for the receptors. The rate constants and relative rates at [receptor] = 5×10^{-3} mol dm^{-3} are listed in Table 4.[21] The effect of the hydrogen bonds on the rates of the oxidation of BNAH is smaller than that on the rates of $PhNHNH_2$ probably because of the difference of the reaction mechanisms. The oxidation of BNAH proceeds *via* a hydride transfer (or its equivalents) to the N(5)-position[18] which is a little distant from the hydrogen bonding sites compared with the C(4a)-position for $PhNHNH_2$. It does not always follow that the receptors possessing larger K values further increase the reactivity of the complexed flavin. For example, **1h** and **1n** do not show larger rate-accelerations despite their larger K values among the receptors as shown in Table 4. This suggests the existence of at least two roles for the receptors; (*i*) a rate-accelerating effect due to the hydrogen bonding, and (*ii*) a rate-retarding effect due to steric hindrance of the substituents of the receptors. We consider that this information is quite important for the design of sophisticated flavin receptors. From these observations, it would be concluded that the hydrogen bonding to carbonyl oxygens at the C(2)- and C(4)-positions of an isoalloxazine ring activates slightly its reactivity, but it depends on the reactions. Meanwhile Fukuzumi *et al.* reported that a flavin–metal complex, in which metal ions such as Mg^{2+} and Zn^{2+} interact with the C(2)=O group, exhibits a high activity towards photooxidation of benzyl alcohol in MeCN.[22] This high reactivity could be explained by the stronger electron-seeking abilities of the metal-complexed species and protonated ones owing to the full positive charges of metal ions and protons. Shinkai *et al.* reported that **1h** decreases the rate of photooxidation of 1,4-butanedithiol to one third, whereas **1a** shows no effect.[8b] This may also be explained by steric hindrance owing to the naphthalene ring of **1h**, although the reasons are not mentioned.

In conclusion, the present study has demonstrated that 2,6-diamidopyridine derivatives act as flavin receptors *via* a triple hydrogen bond at the C(2)=O, N(3)–H and C(4)=O atoms of the isoalloxazine ring and act as a flavin carrier of a water-soluble flavin into a chloroform layer from a water layer. The triply hydrogen-bonded flavin shows a slightly increased

reactivity and the degree of its magnitude is dependent on the reaction.

Experimental

^{1}H NMR spectra were recorded on a JEOL JNM-PMX60si (60 MHz), Varian Gemini-200 (200 MHz) or a JEOL JNM-A500 (500 MHz) instrument with chemical shifts from tetramethylsilane. Electronic absorption spectra were measured on a JASCO Ubest-560 spectrophotometer. Fluorescence spectra were measured on a Hitachi 850 fluorescence spectrophotometer. Melting points are uncorrected. Flash column chromatography was performed by using Wakogel C200 (silica gel, 70–150 μm, Wako). Elemental analyses were performed at the Microanalytical Laboratory, Gunma University.

The receptors were synthesized from 2,6-diaminopyridine derivatives and the corresponding acid chlorides according to the literature.[7b]

Synthesis of N,N'-*Pyridine*-2,6-*diylbis*(*alkanamide*)*s* **1a**–**1e**.—To a stirred solution of 2,6-diaminopyridine (1 equiv.) and triethylamine (2 equiv.) in dry tetrahydrofuran (THF) was added a solution of the corresponding acid chloride (2 equiv.) in dry THF with cooling (ice-bath) and the mixture was stirred overnight at room temp. The reaction mixture was concentrated *in vacuo* and dissolved in CH_2Cl_2. The organic layer was washed with water and dried over $MgSO_4$. After filtration of the $MgSO_4$, the solvent was distilled off *in vacuo*. Crude products formed were purified by recrystallization.

1a; Yield 86%; m.p. 200 °C ($CHCl_3$) (lit.[6a] m.p. 205–206 °C); δ_H(500 MHz, $CDCl_3$) 2.20 (6 H, s, CH_3), 7.53 (2 H, br s, NH), 7.70 (1 H, t, J = 8.5 Hz, py-H_4) and 7.89 (2 H, d, J 8.5 Hz, py-$H_{3,5}$).

1b; Yield 82%, m.p. 256–257 °C (THF–hexane); δ_H(500 MHz, $CDCl_3$) 0.91 (6 H, t, J 7.5 Hz, CH_3), 1.3–1.4 [8 H, m $(CH_2)_2CH_3$], 1.7–1.8 [4 H, m, $CH_2(CH_2)_2CH_3$], 2.37 (4 H, t, J 8.0 Hz, $COCH_2$), 7.50 (2 H, br s, NH), 7.70 (1 H, t, J 8.5 Hz, py-H_4) and 7.90 (2 H, d, J 8.5 Hz, py-$H_{3,5}$) (Calc. for $C_{17}H_{27}N_3O_2$: C, 66.85; H, 8.91; N, 13.76. Found: C, 66.6; H, 8.8; N, 13.6%).

1c; Yield 74%, m.p. 110–111 °C ($CHCl_3$–hexane); δ_H(500 MHz, $CDCl_3$) 0.88 (6 H, t, J 7.5 Hz, CH_3), 1.2–1.4 [32 H, m, $(CH_2)_8CH_3$], 1.6–1.8 [4 H, m, $CH_2(CH_2)_8CH_3$], 2.36 (4 H, t, J 7.5 Hz, $COCH_2$), 7.50 (2 H, br s, NH), 7.70 (1 H, t, J 8.0 Hz, py-H_4) and 7.90 (2 H, d, J 8.0 Hz, py-$H_{3,5}$) (Calc. for $C_{29}H_{51}N_3O_2 \cdot H_2O$: C, 73.25; H, 10.85; N, 8.84. Found: C, 74.1; H, 11.0; N, 8.9%).

1d; Yield 76%, m.p. 176–177 °C (THF–hexane) (lit.[23] m.p. 176 °C), δ_H(500 MHz, $CDCl_3$) 7.50–7.55 (4 H, m, Ph-H_m), 7.56–7.62 (2 H, m, Ph–H_p), 7.84 (1 H, t, J 8.5 Hz, py-H_4), 7.90–7.94 (4 H, m, Ph–H_o), 8.14 (2 H, t, J 8.0 Hz, py-$H_{3,5}$) and 8.29 (2 H, br s, NH).

1e; Yield 82%, m.p. 148–149 °C (THF–hexane), δ_H(500 MHz, $CDCl_3$) 4.63 (4 H, s, $COCH_2O$), 7.01–7.10 (6 H, m, Ph–$H_{o,p}$), 7.34–7.39 (4 H, m, Ph–H_m), 7.78 (1 H, t, J 8.0 Hz, py-H_4), 8.04 (2 H, d, J 8.0 Hz, py-$H_{3,5}$) and 8.73 (2 H, br s, NH) (Calc. for $C_{21}H_{19}N_3O_4$: C, 66.8; H, 5.07; N, 11.1. Found: C, 66.55; H, 5.1; N, 11.1%).

Synthesis of **1f**–**1h**.—These compounds were also prepared from 2,6-diaminopyridine and the corresponding acid chlorides. The acid chlorides were obtained by chlorination of 4-phenoxybutyric acid and 4-(2-naphthyloxy)butyric acid with $SOCl_2$ in benzene or 2,7-bis(3-carboxypropoxy)naphthalene with oxalyl chloride in CH_2Cl_2. These compounds were used without further purification. The acids were obtained from alkaline hydrolysis of the corresponding ethyl esters which were prepared from ethyl 4-bromobutyrate and phenol and the naphthol derivatives in acetone in the presence of K_2CO_3.[7b] 4-Phenoxybutyric acid: m.p. 62–64 °C (EtOH–H_2O) (lit.,[24] m.p. 62–63 °C); δ_H(500 MHz, $CDCl_3$) 2.09–2.16 (2 H, m, OCH_2CH_2), 2.59 (2 H, t, J 7.3 Hz, CH_2CO), 4.03 (2 H, t, J 6.1 Hz, OCH_2), 6.87–6.91 (2 H, m, Ph–H_o), 6.92–6.97 (1 H, m, Ph–H_p) and 7.25–7.30 (2 H, m, Ph–H_m). 4-(2-Naphthyloxy)butyric acid: m.p. 123–124 °C (EtOH–H_2O) (lit.,[25] m.p. 124 °C); δ_H(500 MHz, $CDCl_3$) 2.16–2.23 (2 H, m, OCH_2CH_2), 2.64 (2 H, t, J 7.3 Hz, CH_2CO), 4.15 (2 H, t, J 6.1 Hz, OCH_2), 7.11–7.15 (2 H, m, naph-$H_{1,3}$), 7.33 (1 H, t, J 7.0 Hz, naph-H_6), 7.43 (t, J 7.9 Hz, naph-H_7) and 7.69–7.77 (3 H, m, naph-$H_{4,5,8}$).

2,7-Bis(3-carboxypropoxy)naphthalene was prepared as described in the literature.[7a] Yield 74%. M.p. 154–155 °C (ethyl acetate); δ_H(500 MHz, $CDCl_3$: [$^2H_{10}$]Me_2SO = 10:1) 2.11–2.17 (4 H, m, OCH_2CH_2), 2.53 (4 H, t, J 7.3 Hz, CH_2CO), 4.12 (4 H, t, J 6.1 Hz, OCH_2), 6.97 (2 H, d, J 8.8 Hz, naph-$H_{4,5}$), 7.04 (2 H, s, naph-$H_{1,8}$) and 7.63 (2 H, d, J 8.8 Hz, naph-$H_{3,6}$).

1f; 4-Phenoxybutyroyl chloride was obtained by refluxing 4-phenoxybutyric acid (3.0 g, 16.6 mmol) and thionyl chloride (9.8 g, 83 mmol) in benzene (50 cm^3) for 4 h. After the excess $SOCl_2$ and the solvent were evaporated *in vacuo*, the residue was dried in a vacuum desiccator. A methylene dichloride solution (20 cm^3) containing the acid chloride was added dropwise into a mixture of 2,6-diaminopyridine (0.82 g, 7.5 mmol) and Et_3N (2.3 cm^3, 16 mmol) in CH_2Cl_2 (50 cm^3) on an ice-bath and the reaction mixture was stirred overnight at room temp. The organic layer was washed twice with water (50 cm^3) and then dried over $MgSO_4$. After filtration of $MgSO_4$, CH_2Cl_2 was evaporated *in vacuo* to give a white solid which was purified by recrystallization from THF–hexane. Yield 2.5 g (76%), m.p. 87–88 °C, δ_H(500 MHz, $CDCl_3$) 2.18–2.25 (4 H, m, $COCH_2CH_2$), 2.61 (4 H, t, J 7.0 Hz, $COCH_2$), 4.06 (4 H, t, J 6.0 Hz, OCH_2), 6.90–6.92 (4 H, m, Ph–$H_{o,p}$), 6.93–6.97 (2 H, m, Ph–H_m), 7.65 (2 H, br s, NH), 7.70 (1 H, t, J 8.0 Hz, py-H_4) and 7.89 (2 H, d, J 8.0 Hz, py-$H_{3,5}$) (Calc. for $C_{25}H_{27}N_3O_4 \cdot 1/3H_2O$: C, 68.3); H, 6.3; N, 9.69. Found: C, 68.5; H, 6.4; N, 9.6%).

1g was prepared from 4-(2-naphthyloxy)butyric acid as described above. Yield 74%; m.p. 151–152 °C (THF–hexane); δ_H(500 MHz, $CDCl_3$) 2.16–2.23 (2 H, m, OCH_2CH_2), 2.64 (2 H, t, J 7.3 Hz, CH_2CO), 4.15 (2 H, t, J 6.1 Hz, OCH_2), 7.11–7.15 (2 H, m, naph-$H_{1,3}$), 7.33 (1 H, t, J 7.0 Hz, naph-H_6), 7.43 (t, J 7.9 Hz, naph-H_7) and 7.69–7.77 (3 H, m, naph-$H_{4,5,8}$) (Calc. for $C_{33}H_{31}N_3O_4$: C, 74.28; H, 5.86; N, 7.87. Found: C, 74.0; H, 5.9; N, 7.5%).

1h was prepared according to the essentially same procedure of Hamilton.[7a] Namely 2,7-bis(3-carboxypropoxy)naphthalene (5.0 g, 15 mmol) was chlorinated as described above. Into 1 dm^3 of dry CH_2Cl_2, a mixture of 2,6-diaminopyridine (1.6 g, 14.7 mmol) and Et_3N (4.2 cm^3, 30 mmol) in dry CH_2Cl_2 (200 cm^3) and the diacid chloride in dry CH_2Cl_2 (200 cm^3) were added dropwise simultaneously over a period of 1 h at room temp. under vigorous stirring and the stirring was continued overnight. The solvent was evaporated to 300 cm^3 and the organic layer was washed with water (150 cm^3 × 2), dried over $MgSO_4$, and evaporated to dryness. The residue was purified by flash column chromatography (CH_2Cl_2–acetone = 20:1). Yield 0.45 g (7.4%); m.p. 202–203 °C (THF–hexane). δ_H(500 MHz, $CDCl_3$) 2.20–2.30 (4 H, m, $COCH_2CH_2$), 2.50–2.54 (4 H, m, $COCH_2$), 4.27 (4 H, t, J 6.5 Hz, OCH_2), 7.03–7.05 (2 H, dd, J 2.5, 8.5 Hz, naph-$H_{3,6}$), 7.11 (2 H, d, J 2.5 Hz, naph-$H_{1,8}$), 7.71 (2 H, d, J 8.5 Hz, naph-$H_{4,5}$), 7.74 (2 H, br s, NH), 7.78 (1 H, t, J 8.0 Hz, py-H_4) and 7.97 (2 H, d, J 8.0 Hz, py-$H_{3,5}$) (Calc. for $C_{23}H_{23}N_3O_4$: C, 68.13; H, 5.72; N, 10.36. Found: C, 68.5; H, 5.8; N, 10.5%).

Syntheses of **1i**–**1k**.—These compounds were prepared by a stepwise acylation of 2,6-diaminopyridine. 6-Amino-2-dodecanoylaminopyridine was synthesized as follows. To a stirred solution of 2,6-diaminopyridine 10.0 g (91.6 mmol) and

triethylamine 13 cm^3 (93 mmol) in dry THF (100 cm^3) was added a solution of dodecanoyl chloride 21 cm^3 (91 mmol) in dry THF (100 cm^3) with cooling (ice-bath) and stirred overnight at room temp. The reaction mixture was concentrated *in vacuo* and dissolved in CH_2Cl_2 (300 cm^3). The organic layer was washed with water (100 cm^3 × 3), dried over $MgSO_4$ and evaporated to dryness. The crude product was purified by recrystallization from EtOH to give white crystals. Yield 10.0 g (38%); m.p. 108–109 °C; δ_H(60 MHz, $CDCl_3$) 0.7–2.0 [21 H, m, $(CH_2)_9CH_3$], 2.32 (2 H, t, *J* 6.8 Hz, $COCH_2$), 2.16 (2 H, br s, NH_2), 6.23 (1 H, dd, *J* 2.0, 6.4 Hz, py-H_5), 7.4–7.7 (2 H, m, py-$H_{3,4}$) and 7.84 (1 H, br s, NH). The amine was allowed to react with the corresponding acid chloride in the same manner as described above.

1i; Yield 70%; m.p. 110–111 °C (THF–hexane). δ_H(500 MHz, $CDCl_3$) 0.88 (6 H, t, *J* 7.0 Hz, CH_3), 1.2–1.4 [16 H, m, $(CH_2)_8CH_3$], 1.71–1.77 (4 H, m, $COCH_2CH_2$), 2.39 (4 H, t, *J* 8.0 Hz, $COCH_2$), 7.50–7.54 (2 H, m, Ph-H_m), 7.56–7.59 (2 H, m, Ph-H_p, N*H*$COC_{11}H_{23}$), 7.77 (1 H, t, *J* 8.0 Hz, py-H_4), 7.88–7.92 (4 H, m, Ph-H_o), 7.96 (1 H, d, *J* 8.0 Hz, py-H_3), 8.07 (1 H, d, *J* 8.0 Hz, py-H_5) and 8.24 (1 H, br s, N*H*COPh) (Calc. for $C_{24}H_{33}N_3O_2$: C, 72.88; H, 8.41; N, 10.6. Found: C, 72.95; H, 8.3; N, 10.6%).

1j; Yield 75%; m.p. 115–116 °C (EtOH); δ_H(500 MHz, $CDCl_3$) 0.88 (6 H, t, *J* 7.3 Hz, CH_3), 1.2–1.4 (16 H, m, $(CH_2)_8CH_3$), 1.71–1.77 (4 H, m, $COCH_2CH_2$), 2.39 (4 H, t, *J* 7.6 Hz, $COCH_2$), 5.42 (2 H, s, CH_2Ph), 7.34–7.48 (5 H, m, OPh), 7.57 (1 H, br s, N*H*$COC_{11}H_{23}$), 7.61 (1 H, t, *J* 7.6 Hz, isophthal-H_5), 7.77 (1 H, t, *J* 8.0 Hz, py-H_4), 7.97 (1 H, d, *J* 8.2 Hz, py-H_3), 8.05 (1 H, d, *J* 8.0 Hz, py-H_5), 8.14–8.29 (2 H, m, isophthal-$H_{4,6}$), 8.28 (1 H, br s, N*H*COPh) and 8.55 (1 H, s, isophthal-H_2) (Calc. for $C_{32}H_{39}N_3O_4$: C, 66.8; H, 5.07; N, 11.1. Found: C, 66.55; H, 5.2; N, 11.0%).

1k was purified by flash column chromatography (CH_2Cl_2–MeOH = 10:1). Yield 43%; m.p. 162–165 °C (THF); δ_H(500 MHz, $CDCl_3$) 0.88 (6 H, t, *J* 7.0 Hz, CH_3), 1.2–1.4 (16 H, m, $(CH_2)_8CH_3$), 1.71–1.77 (4 H, m, $COCH_2CH_2$), 2.38 (4 H, t, *J* 8.0 Hz, $COCH_2$), 3.76–3.78, 3.91–3.94, 4.18–4.24 (16 H, m, polyether protons), 6.90 (1 H, d, *J* 8.2 Hz, crown aromatic proton), 7.41 (1 H, dd, *J* 2.1, 8.2 Hz, crown aromatic proton), 7.48 (1 H, d, *J* 2.1 Hz, crown aromatic proton), 7.55 (1 H, br s, N*H*$COC_{11}H_{23}$), 7.77 (1 H, t, *J* 8.0 Hz, py-H_4), 7.93 (1 H, d, *J* 8.2 Hz, py-H_3), 8.04 (1 H, d, *J* 8.2 Hz, py-H_5) and 8.16 (1 H, br s, N*H*COPh) (Calc. for $C_{32}H_{47}N_3O_7$: C, 65.6; H, 8.09; N, 7.17. Found: C, 65.35; H, 8.0; N, 7.0%).

1l, **1m** and **1n** were synthesized in the same manner described above by employing 2,6-diamino-4-benzyloxypyridine.[26]

1l was purified by flash column chromatography (CH_2Cl_2–ethyl acetate = 50:1) and recrystallization from hexane to yield white crystals. Yield 82%; mp. 67–68 °C (hexane); δ_H(500 MHz, $CDCl_3$) 0.91 (6 H, t, *J* 6.5 Hz, CH_3), 1.33–1.38 (8 H, m, $CH_2CH_2CH_3$), 1.70–1.74 (4 H, m, $COCH_2CH_2$), 2.35 (4 H, t, *J* 8.0 Hz, $COCH_2$), 5.14 (2 H, s, OCH_2Ph), 7.25–7.45 (5 H, m, Ph), 7.49 (2 H, s, NH) and 7.65 (2 H, s, py). (Calc. for $C_{24}H_{33}N_3O_3 \cdot 1/3H_2O$: C, 69.04; H, 8.13; N, 10.06. Found: C, 69.3; H, 8.1; N, 9.7%).

1m was purified by flash column chromatography (CH_2Cl_2–acetone = 30:1). Yield 77%; m.p. 107–109 °C (THF–hexane); δ_H(500 MHz, $CDCl_3$) 2.18–2.24 (4 H, m, $COCH_2CH_2$), 2.59 (4 H, t, *J* 7.5 Hz, $COCH_2$), 4.06 (4 H, t, *J* 6.5 Hz, OCH_2), 5.14 (2 H, s, OCH_2Ph), 6.89–6.93 (6 H, m, OPh-$H_{o,p}$), 6.92–6.97 (4 H, m, OPh-H_m), 7.28–7.44 (5 H, m, Ph), 7.64 (2 H, s, NH), 7.64 (2 H, s, py) and 1.84–1.86, 3.73–3.76 (THF) (Calc. for $C_{30}H_{29}N_3O_5 \cdot 1.0$ THF: C, 69.96; H, 6.39; N, 7.20. Found: C, 69.9; H, 6.5; N, 7.2%).

1n was purified by flash column chromatography (CH_2Cl_2–acetone = 20:1). Yield 7.4%; m.p. 194–195 °C (THF–hexane). δ_H(500 MHz, $CDCl_3$) 2.23–2.27 (4 H, m, $COCH_2CH_2$), 2.48–2.52 (4 H, m, $COCH_2$), 4.26 (4 H, t, *J* 5.8 Hz, OCH_2), 5.19 (2 H, s, OCH_2Ph), 7.03 (2 H, dd, *J* 2.4, 8.8 Hz, naph-$H_{3,6}$), 7.10 (2 H, d, *J* 2.4 Hz, naph-$H_{1,8}$), 7.34–7.48 (5 H, m, Ph), 7.70 (2 H, d, *J* 8.8 Hz, naph-$H_{4,5}$), 7.70 (2 H, s, NH), 7.72 (2 H, s, py) and 1.84–1.86, 3.73–3.76 (THF) (Calc. for $C_{32}H_{33}N_3O_5 \cdot 1.0$ THF: C, 70.68; H, 6.76; N, 6.87. Found: C, 70.2; H, 6.8; N, 6.9%).

Syntheses of Flavins.—Flavin models were synthesized according to the literature; **2a**; m.p. 262–263 °C (EtOH) (lit.,[9b] m.p. 261–263 °C), δ_H(500 MHz, $CDCl_3$) 0.88 (3 H, t, *J* 6.5 Hz, CH_3), 1.23–1.60 [14 H, m, $(CH_2)_8CH_3$], 1.85–1.89 (2 H, m, NCH_2CH_2), 4.69–4.73 (2 H, m, NCH_2), 7.64–7.69 (2 H, m, $H_{7,9}$), 7.93–7.96 (1 H, m, H_8), 8.35 (1 H, d, *J* = 7.5 Hz, H_6) and 8.49 (1 H, br s, NH).

2b; m.p. 178–179 °C (EtOH) (lit.[9b] m.p. 177–179 °C), δ_H(500 MHz, $CDCl_3$) 0.88 [3 H, t, *J* 6.5 Hz, $(CH_2)_{11}CH_3$], 1.23–1.60 [14 H, m, $(CH_2)_8CH_3$], 1.85–1.89 (2 H, m, NCH_2CH_2), 3.54 (3 H, s, NCH_3), 4.69–4.73 (2 H, m, NCH_2), 7.62–7.65 (2 H, m, $H_{7,9}$), 7.89–7.92 (1 H, m, H_8) and (1 H, d, *J* 7.5 Hz, H_6).

8-Octylmercapto-10-methylisoalloxazine **3** was prepared from 8-chloro-10-methylisoalloxazine[10] 0.43 g (1.6 mmol) and octylmercaptan 0.40 cm^3 (2.3 mmol) in the presence of triethylamine 0.40 ml (2.9 mmol) in *N*,*N*-dimethylformamide (DMF) (20 cm^3).[27] After the reaction mixture was refluxed overnight, the solvent was removed *in vacuo*. The residue was dissolved in chloroform (50 cm^3) and washed with water (50 cm^3). The organic layer was dried over $MgSO_4$ and the solvent was evaporated to dryness. The crude product was purified by flash column chromatography (diethyl ether–acetone = 7.3) and recrystallized from EtOH. Yield 128 mg (21%). M.p. > *ca.* 230 °C (decomp.) (EtOH); δ_H(500 MHz, $CDCl_3$) 0.89 (3 H, t, *J* 7.0 Hz CH_2CH_3), 1.24–1.27 [8 H, m $(CH_2)_4CH_3$], 1.50–1.57 [2 H, m, $S(CH_2)_2CH_2$], 1.78–1.84 (2 H, m, SCH_2CH_2), 3.13 (2H, t, *J* 7.0 Hz, SCH_2), 4.10 (3 H, s, NCH_3), 7.31 (1 H, d, *J* 2.0 Hz, flavin-H_7), 7.46 (1 H, dd, *J* 2.0, 9.0 Hz, flavin-H_9), 8.14 (1 H, d, *J* 8.5 Hz, flavin-H_6) and 8.37 (1 H, br s, NH) (Calc. for $C_{19}H_{24}N_4O_2S \cdot H_2O$: C, 59.82; H, 6.61; N, 14.69. Found: C, 59.8; H, 6.35; N, 15.0%).

10-Dodecyl-8-azaisoalloxazine **4** was prepared from 4-amino-3-dodecylaminopyridine and alloxan according to the synthetic method of 3,10-dimethyl-8-azaisoalloxazine.[3a] A mixture of 3-bromo-4-nitropyridine-*N*-oxide (5.0 g, 23 mmol),[3a] dodecylamine (5.7 g, 31 mmol) and K_2CO_3 (3.3 g, 24 mmol) was stirred for 24 h at 80 °C in DMF (30 cm^3). After cooling, 150 cm^3 of HCl (3 mol dm^{-3}) was added to the reaction mixture and the resulting precipitate was obtained by filtration. Yield 4.46 g (60%); m.p. 96–97 °C (EtOH); δ_H(60 MHz, $CDCl_3$) 0.88 (3 H, t, *J* = 6.0 Hz, CH_3), 1.1–2.1 [20 H, m,$(CH_2)_{10}CH_3$], 3.35 (2 H, q, *J* 6.0 Hz, $NHCH_2$) and 7.4–8.2 (3 H, m, Ar). The *N*-oxide was used without further purification. 3-Dodecylamino-4-nitropyridine *N*-oxide 1.7 g (5.3 mmol) was reduced to the diamine by hydrogenation in the presence of 10% Pd/C (0.1 g) in AcOH (40 cm^3). After filtration of Pd/C, condensation of the amine and alloxan monohydrate (0.89 g, 5.56 mmol) in the presence of H_3BO_3 (0.70 g, 11.3 mmol) was conducted (24 h at room temp.).[3a] After the solvent was removed *in vacuo*, the residue was dissolved in chloroform (100 cm^3) and washed with water (100 cm^3). The organic layer was dried over $MgSO_4$, and the solvent was evaporated to dryness. The crude product was purified by recrystallization from EtOH to yield an orange powder. Yield 0.91 g (45%); m.p. > *ca.* 240 °C (decomp.); δ_H(500 MHz, $CDCl_3$) 0.89 (3 H, t, *J* 7.0 Hz, CH_3), 1.27–1.83 [20 H, m, $(CH_2)_{10}CH_3$], 4.47 (2 H, m, NCH_2), 8.08 (1 H, d, *J* 7.0 Hz, flavin-H_6), 8.21–8.22 (1 H, m, flavin-H_7), 8.49 (1 H, d, *J* 1.5 Hz, flavin-H_9) and 8.53 (1H, br s, NH) (Calc. for $C_{21}H_{27}N_5O_2 \cdot H_2O$: C, 63.14; H, 7.32; N, 17.53. Found: C, 62.8; H, 7.3; N, 17.5%).

Benzodipteridine derivatives **5** were synthesized from *N*,*N*′-

dialkyl-*p*-phenylenediamines and 6-chlorouracil or 6-chloro-3-methyluracil according to the known procedures.[3d,e] Compound **5a** was prepared by the stepwise condensation of *N,N'*-didodecyl-*p*-phenylenediamine with 6-chloro-3-methyluracil and 6-chlorouracil. *N,N'*-Didodecyl-*N*-(3-methyluracil-6-yl)-*p*-phenylenediamine was prepared according to the literature procedures.[3d,e] Yield 73%; m.p. 88–89 °C (EtOH) (lit.,[3d] m.p. 89 °C). Similarly this compound (2.6 g, 4.6 mmol) was allowed to react with 6-chlorouracil (1.0 g, 6.8 mmol) in *N,N'*-diethylaniline (1.6 cm^3) at 180 °C for 2 h under N_2. After cooling, methanol (20 cm^3) was added to the reaction mixture. The resulting precipitate was filtered and washed with water. This compound was used without purification. To a mixture of *N,N'*-didodecyl-*N*-(3-methyluracil-6-yl)-*N'*-uracil-6'-yl-*p*-phenylenediamine (0.46 g, *ca.* 0.68 mmol), sodium nitrate (0.66 g, 6.8 mmol) in acetic acid (5 cm^3), conc. H_2SO_4 (0.2 cm^3) was added at 90 °C and stirred for 30 min. After cooling, diethyl ether was added to the reaction mixture. The resulting precipitate was collected by filtration and washed with water. Deoxygenation of the di-*N*-oxide was conducted by stirring the mixture of $Na_2S_2O_4$ (0.76 g, 3.6 mmol) in H_2O (6 cm^3)–DMF (6 cm^3) for 24 h at room temp. After addition of water (100 cm^3), the reaction mixture was extracted with $CHCl_3$ (100 cm^3). The organic layer was dried over $MgSO_4$ and evaporated to dryness. The crude product was purified by recrystallization from EtOH to yield a dark-purple powder. Yield 0.18 g (20%); m.p. > *ca.* 270 °C (decomp); δ_H(500 MHz, $CDCl_3$) 0.88 [6 H, t, *J* 6.5 Hz, $(CH_2)_{11}CH_3$], 1.25–1.89 [40 H, m, $(CH_2)_{10}CH_3$], 3.57 (3 H, s, NCH_3), 4.68 (4 H, m, NCH_2), 8.49, 8.53 (2 H, s, Ar) and 8.56 (1 H, s, NH) (Calc. for $C_{39}H_{52}N_8O_4 \cdot 0.5\ H_2O$: C, 66.36; H, 7.57; N, 15.87. Found: C, 66.6; H, 8.0; N, 15.8%).

5b was prepared according to the literature;[3d] **5c** was synthesized as for **5b**. *N,N'*-Diethyl-*N,N'*-bis(uracil-6-yl)-*p*-phenylenediamine; yield 77%, m.p. > 300 °C (AcOH) (Calc. for $C_{18}H_{20}N_6O_4 \cdot 0.5$ AcOH: C, 55.07; H, 5.35; N, 20.28. Found: C, 55.5; H, 5.25; N, 20.4%). Cyclization of this compound by $NaNO_3$ gave the di-*N*-oxide, yield 32%, m.p. > 300 °C (AcOH). Deoxygenation of the di-*N*-oxide gave **5c**. Yield 59%, m.p. > 300 °C (DMF) (Calc. for $C_{18}H_{14}N_8O_4 \cdot 0.25H_2O$: C, 52.62; H, 3.56; N, 27.2. Found: C, 52.5; H, 3.5; N, 27.6%).

NMR Titration.—To a $CHCl_3$ solution of flavin (270 mm^3, 5.0×10^{-3} mol dm^{-3}) in an NMR tube (5 mm diameter) was added an appropriate amount (54, 108, 162, 216, 270, 405, 540 and 810 mm^3) of a $CHCl_3$ solution of the receptor (5.0×10^{-3} mol dm^{-3}) and $CHCl_3$ in the NMR tube was removed completely *in vacuo*. After addition of $CDCl_3$ (540 mm^3), the chemical shifts of N(3)–H proton of the flavins were recorded at 25 ± 1 °C. The association constants were determined by the nonlinear least-regression curve fitting of the titration curve. The association constants were calculated in the range 0.2–0.8 of the complexation ratio.[7]

Job Plot.—$CHCl_3$ solutions of **2a** (5.0×10^{-3} mol dm^{-3} in $CHCl_3$) and **1b** (5.0×10^{-3} mol dm^{-3} in $CHCl_3$) were prepared in the NMR tubes in the following ratios; 540:0, 486:54, 432:108, 378:162, 324:216, 297:243 and 270:270 (mm^3:mm^3). The chemical shifts of the N(3)–H of **2a** in $CDCl_3$ were recorded in a similar manner as described above.

Fluorescence Spectrum.—A $CHCl_3$ solution of **2a** (1.0×10^{-5} mol dm^{-3}) was titrated with the receptors (0–5.0×10^{-3} mol dm^{-3}) by using stock solutions (0.100 mol dm^{-3} in $CHCl_3$). The concentration of stock solution of **1a** or **1g**, **1h** was 5.0 or 3.00×10^{-2} mol dm^{-3}, respectively, because of a solubility problem, and the titration range was 0–2.5 or 0–1.5×10^{-3} mol dm^{-3}. The emission spectrum of **2a** was recorded at the excitation wavelength (440 nm) at 20 ± 1 °C. The association constants were calculated by monitoring the emission decrease at 530 nm according to the literature.[11] The data were obtained from duplicate experiments. Association constants between **2b** and **1b** or **1h** were determined to be less than 10 or 20 mol^{-1} dm^3 corresponding to dynamic quenching of the flavin by the receptors.

Extraction Experiment.—A capped sample tube (30 cm^3) containing **5c** (2.0×10^{-5} mol dm^{-3}) in distilled H_2O (3 cm^3) and $CHCl_3$ (3 cm^3) was stirred vigorously for 2 h at 25 ± 1 °C and centrifuged for 5 min to make the solution clear. The concentration of **5c** extracted into the $CHCl_3$ layer was determined spectrophotometrically by using λ_{max} 542 nm of **5a** (ε 2.2×10^4 cm^{-1} mol^{-1} dm^3 in $CHCl_3$), since **5c** was scarcely soluble in $CHCl_3$. The data were obtained from duplicate experiments.

Rate Measurement.—Kinetic measurements were performed similarly to those described previously.[3e] In a Thunberg cuvette, 30 mm^3 of **5a**, **5b** or 60 mm^3 of **5c** stock solution (**5a**, **5b**; 1.0×10^{-3} mol dm^{-3} in $CHCl_3$, **5c**; 5.0×10^{-4} mol dm^{-3} in DMF) and an appropriate amount of the receptors (**1a**; 5.00×10^{-2} mol dm^{-3}, **1h**; 3.00×10^{-2} mol dm^{-3}, other receptors; 0.100 mol dm^{-3}) were added into the cell part with $CHCl_3$, and 60 mm^3 of the BNAH (4.00×10^{-2} mol dm^{-3} in $CHCl_3$) or 50 mm^3 of the $PhNHNH_2$ (0.110 mol dm^{-3} in $CHCl_3$) was placed in the upper part of the cuvette. In all cases, the total volume of the contents in the cuvette was adjusted to 3 cm^3 by adding $CHCl_3$ into the cell part. Then both the solutions were bubbled with $CHCl_3$-prehumidified O_2-free N_2,[28] obtained by passing through vanadous sulfate solution, H_2O, paraffin, NaOH pellets, and $CHCl_3$ for 15 min (since the rates are sensitive to a moisture of N_2, a $CHCl_3$-bubbling bottle for prehumidification must be freshly prepared prior to experiments). The reaction was initiated by mixing. The pseudo-first-order rate constants were determined by following the absorption increase of the reduced **5** at 660 nm.

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