A Flavin Receptor. Strong Binding Ability of a Melamine Derivative bearing a Guanidinium Ion for 6-Azaflavin: Five Hydrogen Bonds Formed in Chloroform

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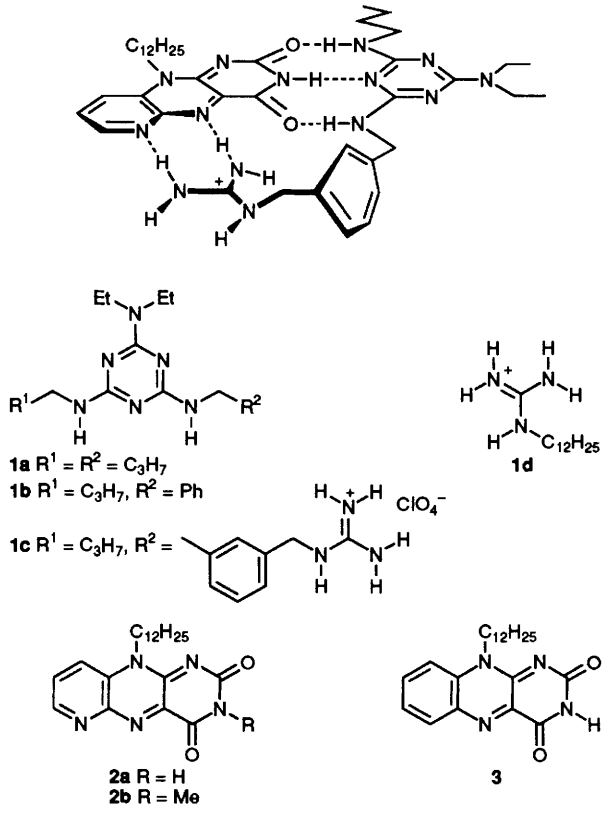
A melamine derivative bearing a guanidinium ion strongly binds 6-azaflavin *via* five hydrogen bonds with a binding constant of 140000 mol^{-1} dm^3 in $CHCl_3$.

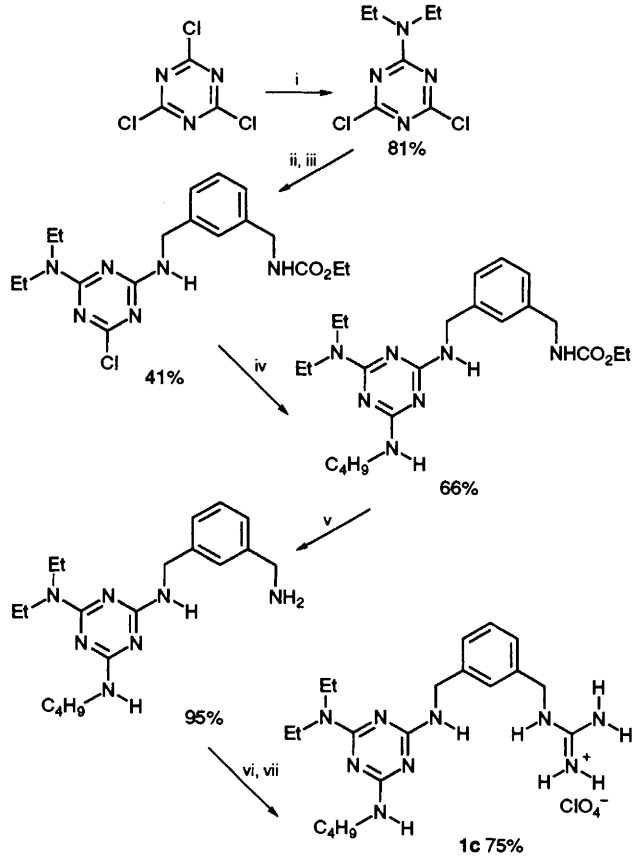
Molecular assemblies using hydrogen bonds have attracted considerable attention from the viewpoint of molecular recognition.[1] Flavin coenzymes exhibit diverse functions with apoproteins, in which flavins are tightly bound *via* noncovalent bonds such as hydrogen bonds.[2] The hydrogen bonding not only holds the flavin at an appropriate position in the proteins, but also regulates the reactivities of the flavin.[3] Therefore, it would be of primary importance to exploit a flavin receptor using hydrogen bonds to construct artificial flavoenzymes. We have previously reported that *N,N'*-diacyl-2,6-diaminopyridine derivatives act as flavin receptors *via* a triple hydrogen bond at C(2)=O, N(3)–H, and C(4)=O of the isoalloxazine ring in $CHCl_3$.[4] However, the binding strength is weak, with binding constants (K/mol^{-1} dm^3) in the order of 10^2 due to three alternating hydrogen bonds, as proposed by Jorgensen.[5] Additional and more effective hydrogen bonds are required to increase the binding strength. The stabilities of hydrogen-bonded complexes depend on the arrangement of the hydrogen donor (D) and acceptor (A) groups; a DDA·AAD complex is much more stable than an alternating DAD·ADA complex.[6] Hence, we have designed a melamine derivative **1c** bearing a guanidinium ion[7] which is well organized for binding 6-azaflavin **2a** as an oxidation active flavin mimic *via* five hydrogen bonds of type DAD·ADA and DD·AA. The melamine moiety of **1c** was chosen instead of an *N*-acyl aminopyridine moiety, because of its strong binding ability to flavins, because an *N*-benzoyl-2-aminopyridine moiety causes a significant decrease of the binding constants to isoalloxazine **3** (K = 21, and *ca.* 0 mol^{-1} dm^3 in *N*-benzoyl-*N'*-hexanoyl-2,6-diaminopyridine and *N,N'*-dibenzoyl-2,6-diaminopyridine, respectively), presumably due to steric hindrance.[4b]†

Receptors **1a** and **1b** were synthesized by stepwise substitution of cyanuric chloride with the corresponding amines.[8] Receptor **1c** was synthesized according to a route outlined in Scheme 1.

Complex formation of **1c** and **2a**,[9]‡ was confirmed by ^1H NMR ($CDCl_3$) (Fig. 1). The NMR spectrum of a 1 : 1 mixture of **1c** and **2a** indicates that all the N–H protons of **1c** shift downfield,§ whereas the melamine N–H protons do not shift for **2b**. The absorption spectrum of **2a** was found to change, passing through isosbestic points, upon addition of **1c** in $CHCl_3$ (Fig. 2). No spectral change was observed for **2b** under the same conditions. The Job plot obtained at λ_{max} 489 nm (ε 6100 mol^{-1} dm^3 cm^{-1}) showed a maximum at a mole fraction of 0.5, indicating 1 : 1 complex formation.

The binding constants and corresponding free energy changes for complexation of the receptors with **2a** and **3** are listed in Table 1. The binding constants increase, as expected, with increasing number of hydrogen bonds. In contrast to 2,6-diaminopyridine derivatives, the phenyl group of the





Scheme 1 *Reagents and conditions*: i, Et_2NH, Na_2CO_3, dioxane–H_2O, 0 °C; ii, *m*-$C_6H_4(CH_2NH_2)_2$, Na_2CO_3, CH_2Cl_2–H_2O, room temp.; iii, $ClCO_2Et$, K_2CO_3, THF, reflux; iv, *n*-$C_4H_9NH_2$, K_2CO_3, dioxane, reflux; v, KOH, EtOH–H_2O, reflux; vi, $EtSC(NH_2)=N^+H_2Br^-$, abs. EtOH, reflux; vii, $NaClO_4$, H_2O, room temp.

Table 1 Binding constants (K) and free energy changes ($-\Delta G$) determined by spectroscopic titration

	2a		**3**	
Receptor	K/ dm^3 mol^{-1}	$-\Delta G$/ kcal mol^{-1}	K/ dm^3 mol^{-1}	$-\Delta G$/ kcal mol^{-1}
1a	140 ± 1[a]	2.88	150 ± 15[b]	2.97
1b	150 ± 6[a]	2.92	140 ± 2[b]	2.88
1c	140 000 ± 20 000[c]	6.90	2000 ± 100[a]	4.43
1d	100 ± 15[a]	2.68	17 ± 1[a]	1.65

[a] Fluorescence spectroscopy; [flavin] = 1.0×10^{-5} mol dm^{-3}, $CHCl_3$, 20 °C. [b] ^{1}H NMR spectroscopy; [flavin] = 2.5×10^{-3} mol dm^{-3}, $CDCl_3$, 25 °C. [c] UV–VIS spectroscopy; [flavin] = 5.0×10^{-5} mol dm^{-3}, $CHCl_3$, 20 °C.

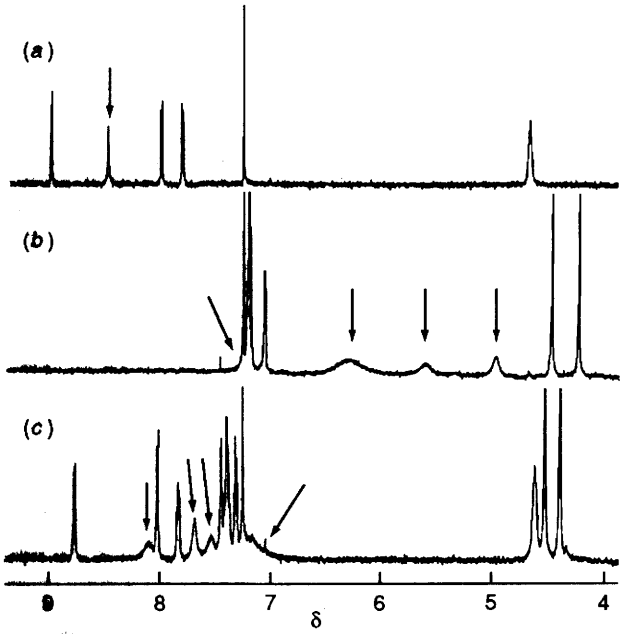


Fig. 1 ^{1}H NMR spectra (500 MHz) of (*a*) **2a** (*b*) **1c** (*c*) 1 : 1 mixture of **1c** and **2a**. The NH protons are shown by arrows. [**1c**] = [**2a**] = 2.5×10^{-3} mol dm^{-3}, $CDCl_3$, 25 °C.

melamine derivative **1b** does not decrease the binding constants (**1a** *vs.* **1b**). The larger K value of **1c** (2000 mol^{-1} dm^3) compared to those of **1a** and **1b** suggests that **1c** binds **3** *via* the triple hydrogen bond of the melamine moiety and complementary hydrogen bonding at the N(5)-position of **3** by the guanidinium ion (a total of four hydrogen bonds). The guanidinium ion **1d** binds **2a** with a K of 100 mol^{-1} dm^3, comparable to those of **1a** and **1b** (140–150 mol^{-1} dm^3), despite forming only two hydrogen bonds. This may be due to the formation of a doubly hydrogen-bonded complex of type DD·AA at the N(5)- and N(6)-positions of **2a**. The free energy change of formation of **1c·2a** ($-\Delta G° = 6.9$ kcal mol^{-1}) is larger than that of the sum of **1b·2a** and **1d·2a** (2.9 + 2.7 = 5.6 kcal mol^{-1}) by 1.3 kcal mol^{-1} (1 cal = 4.184 J). This suggests that the triple hydrogen bonding of the melamine moiety of **1c** facilitates the formation of the next hydrogen bonds of the intramolecular guanidinium ion, due to preorganization. All the data described here indicate that the remarkably large binding constant of **1c–2a** is due to a quintuple hydrogen bond, as shown above. Examination of CPK models indicates that the hydrogen-bonded complex (**1c–2a**) can be constructed quite smoothly by rotating the xylyl ring of **1c**. It would be of interest to examine the effect of the receptor on the reactivities of the flavin mimic in $CHCl_3$.

In conclusion, we have demonstrated that a melamine derivative bearing a guanidinium ion strongly binds 6-aza-flavin, which is an oxidation active flavin mimic, *via* a quintuple hydrogen bond in chloroform. We believe that this strong flavin receptor has many potential applications, since functional groups can be easily introduced into the melamine nucleus.

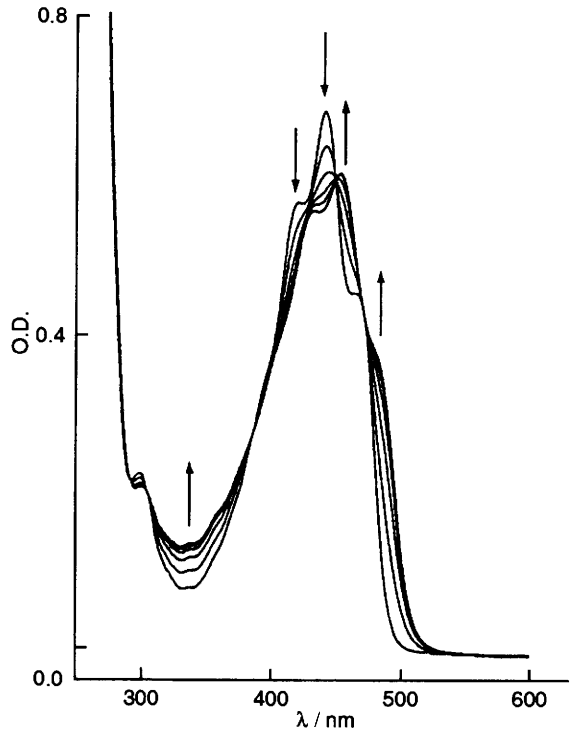


Fig. 2 Spectral changes of **2a** by addition of **1c**. [**1c**] = 0–1.5×10^{-4} mol dm^{-3}, [**2a**] = 5.0×10^{-5} mol dm^{-3}, $CHCl_3$, 25 °C.

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Footnotes

† PM3 calculation for *N*-benzoyl-2,6-diaminopyridine suggests that a planar conformation is the most stable.

‡ **1c**: mp 128–130 °C. **2a**: mp >250 °C (decomp.). Satisfactory elemental analyses were obtained for both compounds.

§ The NH proton of 6-azaflavin could not be assigned because of broadening.

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