Nucleoside Recognition by a Fluorescent Macrolactam

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Molecular recognition of nucleosides or nucleotides is attracting a great deal of interest due to their genetic functions in living organisms.[1] Hydrophilic nature of nucleosides and nucleotides allows only a conformationally well defined receptor to form a hydrogen-bonded, electrostatic or hydrophobic complex with nucleosides or nucleotides in water.[2] Recently, an anthracene derivative was reported to show a higher affinity toward GTP over ATP owing to cooperative interactions of hydrogen bonding and electrostatic interactions between an imidazolium moiety and a phosphate unit.[3]

We have developed various sugar receptors with hydrogen-bonding acceptors and donors.[4] Herein, we report a novel D_2-symmetric fluorescent macrolactam. This host possesses not only an aromatic cavity for π-π interaction, but also hydrogen-bonding donors/acceptors in the peripheral site of the macrolactam for effective nucleoside recognition.

Macrolactam host was synthesized via the typical acid chloride coupling method[5] in which 2,5-dimethyl-*p*-xylyldiamine was treated with 2,5-dimethoxyterephthaloyl chloride in a high dilute condition[6] to afford the desired 2:2 macrocyclization product (**H**). The calculated structure shows that the host has a large cavity with dimension of 10.5 Å × 6.9 Å (Fig. 1. left). The global minimum structure clearly indicates that π-π stacking interaction exists between the dimethoxy aryl groups of **H** and the uracil base of uridine with aromatic-aromatic surface distances of 3.56 and 3.55 Å, and one intermolecular H-bonding interaction also exists between the carbonyl group of **H** and 2'-OH group of uridine (Fig. 1. right).[7]

Owing to the characteristic fluorescence property of **H**,[8] fluorescence titration was carried out in chloroform. Fluorescence emission intensities at λ_{max} = 384 nm were recorded after excitation at λ_{ex} = 331 nm (Fig. 2). Fluorescence intensities of the host-guest complex increase upon addition of sugars or nucleosides presumably due to the restricted rotation of **H**.[9] The resulting fluorescence enhancements at 384 nm are shown in the inset of Figure 3. The binding stoichiometry between **H** and guests was also confirmed to be 1:1 by Job's plot (Fig. 4).[10]

Curve fitting of the host signals to a 1:1 binding isotherm gives apparent dissociation constants of up to $K_d = 10^{-4}$ M, which are summarized in Table 1.

While the dissociation constants between **H** and anomers of D-glucose were found to be similar (3.99×10^{-4} M for β

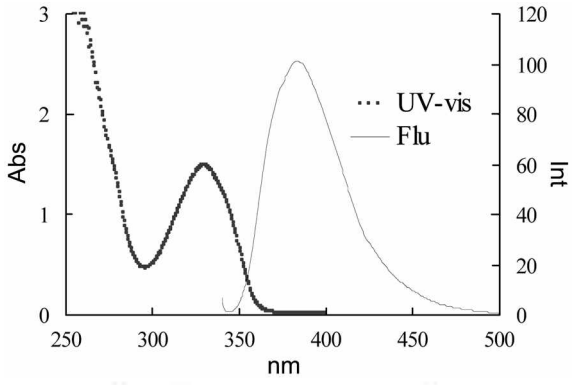


Figure 2. UV-vis and fluorescence spectra of **H** in $CHCl_3$ at 298 K.

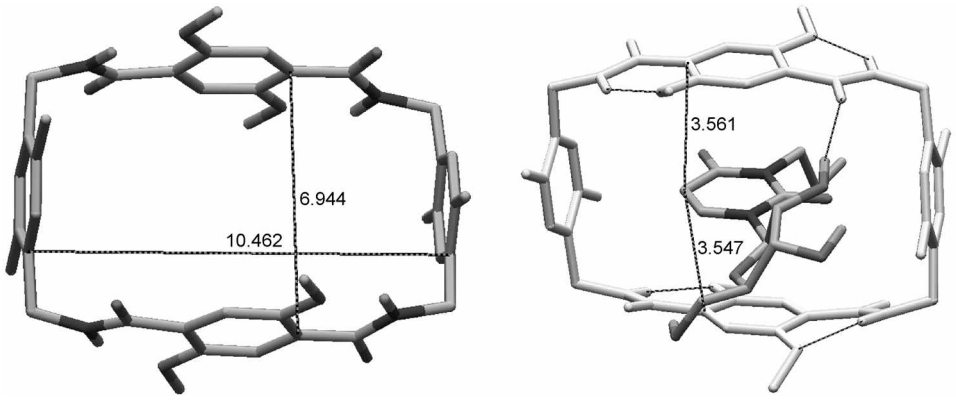


Figure 1. Global minimum structures of **H** (left) and its uridine complex (right).

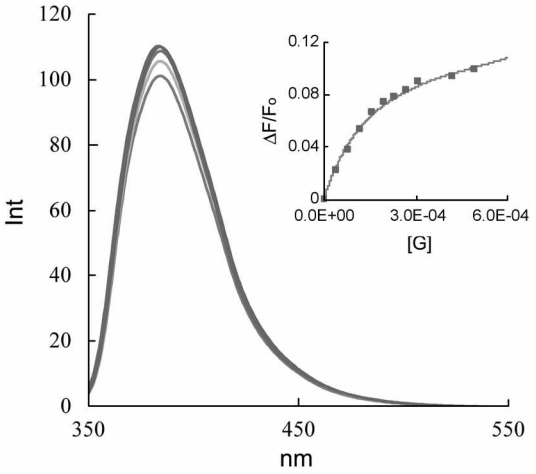


Figure 3. Fluorescence titration of **H** and uridine in $CHCl_3$ at 298 K. [**H**] = 2.0 μM.

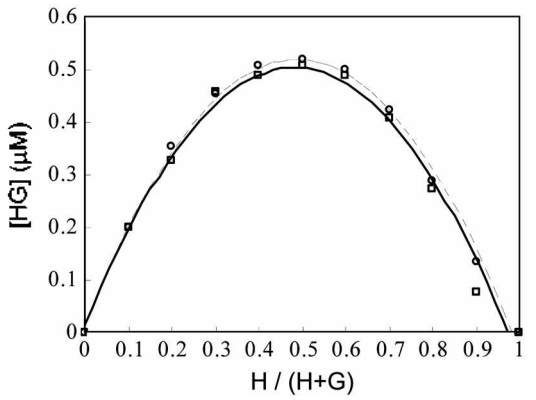


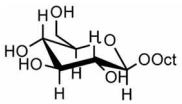
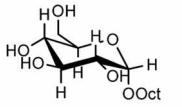
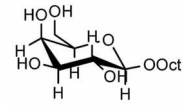
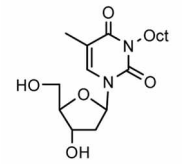
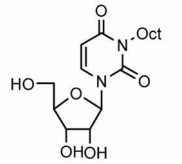
Figure 4. Job's plot between **H** and D-glucopyranosides at 298 K. [H] + [G] = 2.0 μM, each in 2.0 mL. Rectangular and circle represent β-glucose and uridine, respectively.

and 5.38×10^{-4} M for α anomer), the binding affinity of **H** to β-galactose is three times lower than that of β-D-glucose (1.31×10^{-3} M for β-D-galactose). This diastereoselectivity for sugars plausibly results from the slight energetic difference in the intermolecular H-bonding patterns due to the varying degree of steric interaction between sugars and **H**. This indicates that geometrical complementarities of H-bonding partners are crucial in hydrogen bond-based molecular recognition system.

It is noticeable that nucleosides, deoxythymidine (d-Thy) and uridine (Uri) show the comparable binding affinities although they have fewer number of hydroxyl groups compared with the pyranosides. Uridine shows much higher binding affinity (1.72×10^{-4} M) than β-D-glucose. Enhancement in the binding affinity for nucleosides probably results from the presence of π-surface and H-bonding donors and acceptors in the guests.

It is assumed that π-π stacking interaction between **H** and nucleosides plays an important role in host-guest binding. We have chosen several commercially available aromatic guests to test this assumption. While benzene is weakly bound to **H** ($K_d = 4.05 \times 10^{-2}$ M), the binding affinity of a π basic guest 1,4-dimethoxybenzene was *c.a.* hundred times enhanced (5.26×10^{-4} M). The binding affinity of a π acidic guest dimethylterephthalate, however, was too small to determine.

Table 1. Dissociation constants between **H** and guests[a]

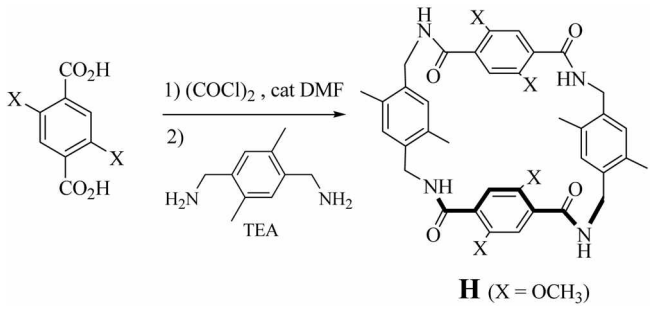
entry	guest structure	name	K_d (M)
1		β-D-Glucose	$3.99(\pm 0.70) \times 10^{-4}$
2		α-D-Glucose	$5.38(\pm 3.22) \times 10^{-4}$
3		β-D-Galactose	$1.31(\pm 0.58) \times 10^{-3}$
4		Thymidine	$6.44(\pm 5.88) \times 10^{-4}$
5		Uridine	$1.72(\pm 0.23) \times 10^{-4}$

[a]Fluorescence titration of constant host concentration (2.0 μM) in $CHCl_3$ at 298 K. Fluorescence intensity at λ_{em} = 384 nm (λ_{ex} = 331 nm) was monitored after each addition of guest.

In conclusion, we have developed a novel fluorescent macrolactam as an artificial receptor for nucleosides. The receptor has shown high diastereoselectivity for sugars and even higher affinities for nucleosides due to the intermolecular π-π stacking interaction as well as H-bonds between the macrolactam and sugars/nucleosides.

Experimental

Acid chloride synthesis. To a solution of 400 mg (1.77 mmol) of 2,5-dimethoxyterephthalic acid in 20 mL of dichloromethane was added cat. amount of DMF and 2.0 mL of 2 M oxalic acid chloride in dichloromethane (2 eq. ex., 4.0 mmol). Resulting white suspension was stirred at rt



Scheme 1. Synthetic scheme of macrolactam.

under nitrogen for 5 hrs to afford a yellow clear solution. All volatiles were removed under the reduced pressure, dried in vacuum.

Cyclization. To a solution of p-xylyl diamine (1 eq. 1.77 mmol) and TEA (2 eq ex.) in 500 mL of dichloromethane was dropwise added a solution of above crude 2,5-dimethoxyterephthaloyl chloride in 50 mL of dichloromethane at 0 °C under nitrogen over a period of 2 hrs. Resulting yellow solution was stirred for additional 24 hrs under nitrogen. All volatiles are removed under reduced pressure and purified by column chromatography. Column chromatography on silica gel (CH_2Cl_2:MeOH = 10:1, R_f = 0.48) gave a greenish mixture. Additional column chromatography on silica gel (EtOAc, R_f = 0.30) gave the desired product, **H** as a white solid in a 4.2% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): 8.09 (t, J = 6.3 Hz, 4H of NH), 7.68 (s, 4H of ArH_a in 2,5-dimethoxybenzene), 7.06 (s, 4H of ArH in *p*-xylylene), 4.51 (d, J = 6.3 Hz, 8H of $ArCH_2N$), 3.90 (s, 12H of OCH_3), 2.29 (s, 12H of $ArCH_3$).

UV-vis ($CHCl_3$): ε_{331nm} = 3997 $M^{-1}cm^{-1}$, Fluorescence ($CHCl_3$): λ_{em} = 384 nm (λ_{ex} = 331 nm) in 2.0 μM

Mass (FAB$^+$, m-NBA): *m/z* 709 ([M+H], 50%)

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