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 D₂-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 method5 in which 2,5-dimethyl-p-xylyldiamine was treated with 2,5-dimethoxyterephthaloyl chloride in a high dilute condition6 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 x 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 λmax = 384 nm were recorded after excitation at λex = 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 Kd = 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 x 10⁻⁴ M for β

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

Figure 2. UV-vis and fluorescence spectra of H in CHCl₃ at 298 K.
and $5.38 \times 10^{-4}$ M for $\alpha$-anomer), the binding affinity of $H$ to $\beta$-galactose is three times lower than that of $\beta$-D-glucose ($1.31 \times 10^{-3}$ M for $\beta$-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 ($1.72 \times 10^{-4}$ M) than $\beta$-D-glucose. Enhancement in the binding affinity for nucleosides probably results from the presence of $\pi$-surface and H-bonding donors and acceptors in the guests.

It is assumed that $\pi-\pi$ 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 $\pi$-basic guest 1,4-dimethoxybenzene was c.a. hundred times enhanced ($5.26 \times 10^{-4}$ M). The binding affinity of a $\pi$-acidic guest dimethylterephthalate, however, was too small to determine.

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 $\pi-\pi$ 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-dimethoxylterephthalic 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


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**Table 1. Dissociation constants between $H$ and guests$^a$**

<table>
<thead>
<tr>
<th>entry</th>
<th>guest structure</th>
<th>name</th>
<th>$K_d$ (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image1" alt="Structure" /></td>
<td>$\beta$-D-Glucose</td>
<td>$3.99(\pm 0.70) \times 10^{-4}$</td>
</tr>
<tr>
<td>2</td>
<td><img src="image2" alt="Structure" /></td>
<td>$\alpha$-D-Glucose</td>
<td>$5.38(\pm 3.22) \times 10^{-4}$</td>
</tr>
<tr>
<td>3</td>
<td><img src="image3" alt="Structure" /></td>
<td>$\beta$-D-Galactose</td>
<td>$1.31(\pm 0.58) \times 10^{-3}$</td>
</tr>
<tr>
<td>4</td>
<td><img src="image4" alt="Structure" /></td>
<td>Thymidine</td>
<td>$6.44(\pm 5.88) \times 10^{-4}$</td>
</tr>
<tr>
<td>5</td>
<td><img src="image5" alt="Structure" /></td>
<td>Uridine</td>
<td>$1.72(\pm 0.23) \times 10^{-4}$</td>
</tr>
</tbody>
</table>

$^a$ Fluorescence titration of constant host concentration (2.0 $\mu$M) in CHCl$_3$ at 298 K. Fluorescence intensity at $\lambda_{em} = 384$ nm ($\lambda_{ex} = 331$ nm) was monitored after each addition of guest.

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**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 eq.) 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$_2$Cl$_2$: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 in 2,5-dimethoxybenzene), 7.06 (s, 4H of ArH in p-xylylene), 4.51 (d, $J$ = 6.3 Hz, 8H of ArCH$_2$N), 3.90 (s, 12H of OCH$_3$), 2.29 (s, 12H of ArCH$_3$).

UV-vis (CHCl$_3$): $\varepsilon_{331 \text{nm}}$ = 3997 M$^{-1}$cm$^{-1}$, Fluorescence (CHCl$_3$): $\lambda_{em}$ = 384 nm ($\lambda_{ex}$ = 331 nm) in 2.0 µM

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

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**References**