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Highly selective adenine recognition by a macrocyclic host molecule employing multiple hydrogen bonding and π–π stacking interactions

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Abstract—A new macrocyclic host, which contains a 2,6-bis(oxazol-2-yl)pyridine unit and a 2,7-dialkoxynaphthalene unit tethered by the appropriate length of alkyl side chains is prepared. This host undergoes highly selective complex formation with an adenine nucleobase, accompanied by a fluorescence response in $CHCl_3$ by a combination of multiple hydrogen bonding and π–π stacking interactions.

The specific recognition of adenine nucleotides and nucleosides is very important in the regulation of various functions in biological systems.[1,2] Since the late 1980's, as model studies have been developed to understand such systems, many host molecules that recognize a target nucleobase by multiple hydrogen bonding and a combination of other modes of interactions have been reported.[3] For adenine nucleobase, Hamilton,[4a] Rebek,[4b–d] and Zimmerman[4e,f] developed excellent synthetic host molecules[4] recognizing adenine units by a combination of multiple hydrogen bonding and π–π stacking interactions. However, the development of synthetic host molecules that specifically recognize adenine over all other nucleobases has been insufficient even in non-polar organic solvents and remains as an unsolved problem.[4]

We recently reported on a new host molecule (host **1a**) that is capable of selectively recognizing a lipophilized adenosine derivative in $CHCl_3$, in a highly selective manner ($K_s = 1.2 \times 10^4$ M^{-1}, greater than 100-fold over all other nucleobases).[5] As shown in Figure 1A, the structure of **1a** contains 5-6-5-membered heteroaromatic rings with two carbamoyl NH sites, and provides the

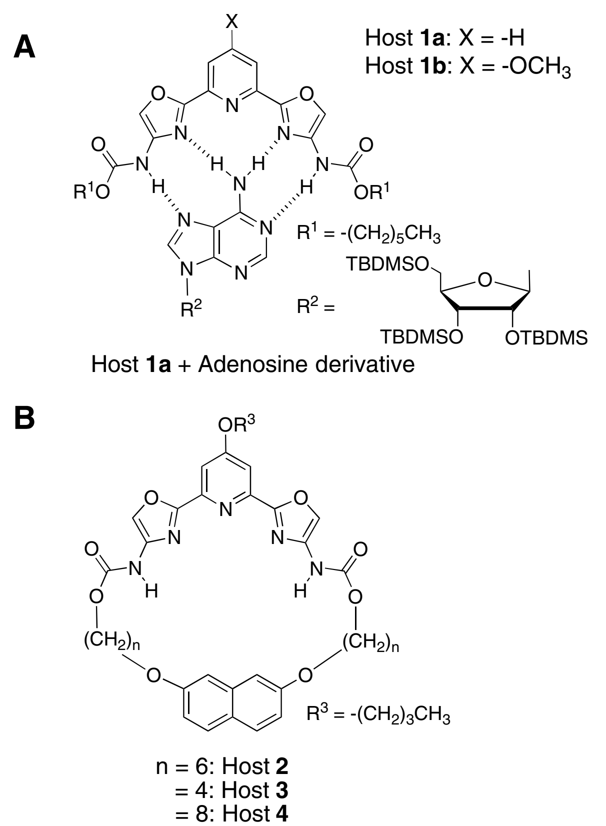

Figure 1. (A) Complexation between host **1** and adenosine derivative by multiple hydrogen bonding. (B) Molecular structure of host **2**–**4**.

Keywords: Macrocyclic host; π–π Stacking interactions; Multiple hydrogen bonding; High adenine selectivity; Fluorescence response.
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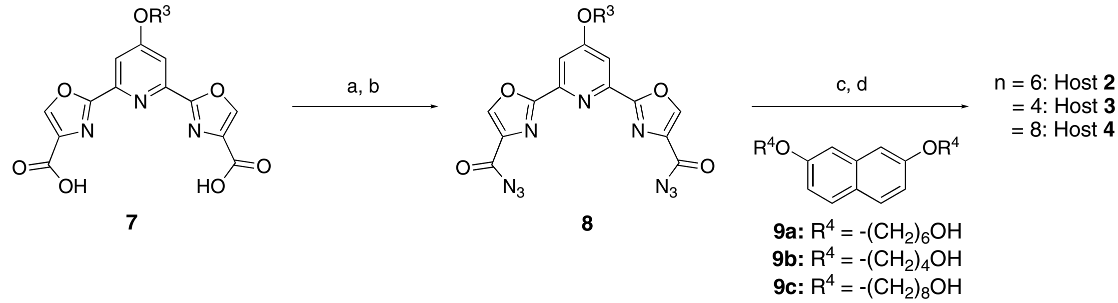

Scheme 1. Synthesis of macrocyclic host molecules **2**–**4**. Reagents and conditions: (a) $SOCl_2$, reflux; (b) NaN_3, acetone–H_2O, 0 °C to rt, 78% (two steps); (c) $CHCl_3$, reflux; (d) **9a**, **9b** or **9c**, $CHCl_3$, reflux, 25–37% (two steps).

correct orientation of complementary hydrogen bonding sites for adenine nucleobase, which exploits both Watson–Crick and Hoogsteen-type interactions.

Our further interest is to increase the stability constant by a combination of a π–π stacking site to the rigid structure of host **1a** without decreasing the high adenine selectivity. We wish to report herein on a new macrocyclic synthetic host molecule **2** in which both multiple hydrogen bonding and π–π stacking interactions are operative. In the macrocyclic host **2**, a 2,7-dialkoxynaphthalene[4a] as the π–π stacking site and a 2,6-bis(oxazol-2-yl)pyridine unit with two carbamoyl NH sites[5] (host **1a**) as the multiple hydrogen bonding sites is tethered with an appropriate length of the alkyl side chains ($n = 6$). The ability of host **2** to complex with an adenosine derivative is about 2.5-fold greater than that of host **1a** and the adenine selectivity over all other nucleobases also improved. The macrocyclic host molecule **2** was synthesized as shown in Scheme 1. Diacid **7** prepared in four steps from 4-butoxypyridine-2,6-dicarboxylic acid[6] (see: Supplementary data) was converted to the corresponding diazide **8** in 78% yield, which was heated under reflux to effect a Curtius rearrangement. The resulting diisocyanate was treated with diol **9a** to give the macrocyclic host molecule **2** in 35% yield.[7] As the guest molecules, the *tert*-butyldimethylsilyl protected nucleoside derivatives (**5A**: adenine, **5G**: guanine, **5C**: cytosine, **5U**: uracil, **6T**: thymine) are shown in Figure 2.

The complexation ability of host **2** was monitored by ^1H NMR spectroscopy in $CDCl_3$ using adenosine derivative (**5A**) as a guest (Fig. 3). In the presence of **5A** (1 equiv), a significant down field shift for the carbamoyl NH protons[8] of host **2** (H_c, $\Delta\delta$ +4.46 and +4.14 ppm) was observed, consistent with complexation by hydrogen bonding. In addition, the upfield shifts of naphthalene-1,8- (H_d, $\Delta\delta$ −0.36 ppm), 3,6- (H_e, $\Delta\delta$ −0.24 ppm), and 4,5- (H_f, $\Delta\delta$ −0.27 ppm) and adenine-2- (H_g, $\Delta\delta$ −0.10 ppm), and 8- (H_h, $\Delta\delta$ −0.36 ppm) proton resonances were observed, indicating π–π stacking interactions of two aromatic rings. The stoichiometry of the host-guest complex between host **2** and **5A** was confirmed to be 1:1 by a Job's plot.[9] Furthermore, NOESY cross-peaks were observed between host **2** and **5A** (H_c–H_g, H_c–H_h, probably H_c–H_d,[10] see Supplementary data). These results strongly indicate that host–guest complexation involved a combination of multiple hydrogen bonding and π–π stacking interactions, as shown in Figure 4.

Upon the addition of **5A** in $CHCl_3$, **2** showed a fluorescence response using 325 nm as the excitation wavelength.[11] Thus, as shown in Figure 5A, the fluorescence intensity ($\lambda_{max} = 368$ nm) of **2** was quenched by the addition of **5A** with no change in the fluorescence maximum.[5,12] From the change in fluorescence intensity, the stability constant of the 1:1 complex between **2** and **5A** was estimated to be $3.1 \times 10^4\ M^{-1}$ by the Benesi–Hildebrand method (Table 1).[13] This K_s value was of the same magnitude as $K_s = 2.7 \times 10^4\ M^{-1}$ (uncertainties = 11%) determined by UV–vis titration in $CHCl_3$ at 20 °C.[14] This result suggested that the K_s value determined by the fluorescent response reflected the complexation ability in the ground state. In contrast, other nucleoside derivatives (**5G**, **5C**, **5U**, **6T**) did not induce an appreciable fluorescence change in the concentration range of 80–320 μM (Fig. 5B). The stability constants of 1:1 complexes, determined from the fluorescence decrease under higher concentrations of guests, were $1.5 \times 10^2\ M^{-1}$ for **5C**, $3 \times 10^1\ M^{-1}$ for **5G**, $<3 \times 10^1\ M^{-1}$ for **5U** and **6T**, respectively (Table 1).[13] Therefore, the adenine selectivity of host **2** was about 200-fold over all other nucleobases. To compare the appropriate length of the alkyl side chains related to cavities of the macrocyclic host molecules, host **3** ($n = 4$) and **4** ($n = 8$) were also obtained in 37% and 25% yield from **8**, respectively.[15] The 1:1 stability constants for host **3** ($\lambda_{max} = 370$ nm) and host **4** ($\lambda_{max} = 371$ nm) to **5A** were determined to be 2.0×10^4, and $8.5 \times 10^3\ M^{-1}$, respectively by fluorescence titrations (Table 1).[13] These results show that host **2** has the appropriate length of alkyl side chains ($n = 6$) required

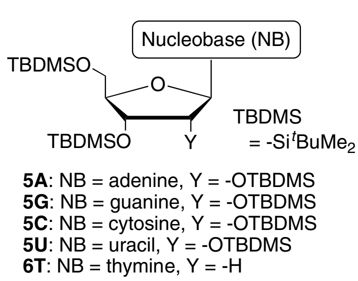

Figure 2. The structure of *tert*-butyldimethylsilyl protected nucleoside derivatives.

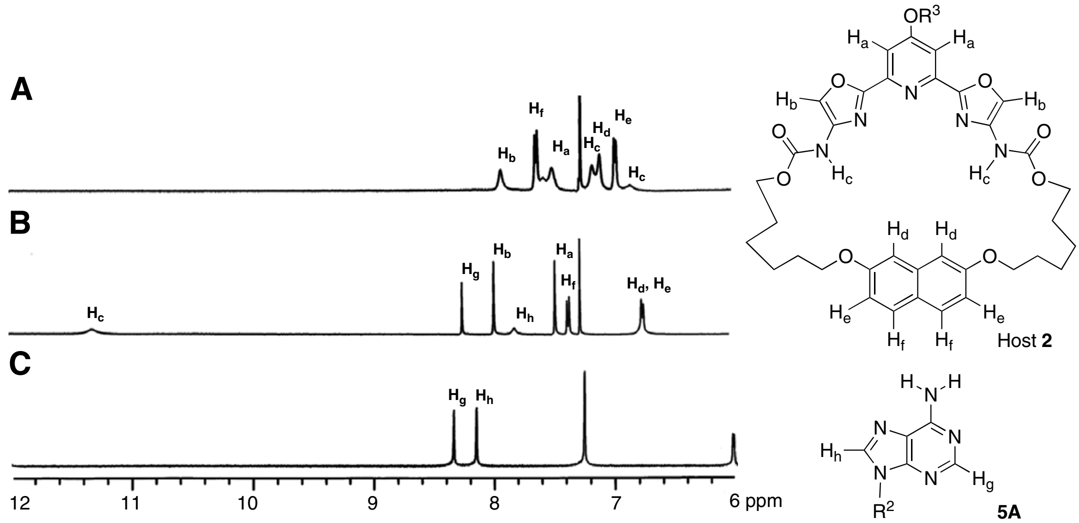

Figure 3. ^{1}H NMR spectra of host **2** and/or guest **5A** in $CDCl_3$, measured at 25 °C with TMS as the external standard: (A) [**2**] = 10.0 mM; (B) [**2**] = 10.0 mM, [**5A**] = 10.0 mM; (C) [**5A**] = 10.0 mM.

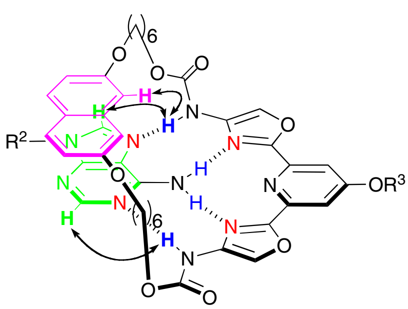

Figure 4. The proposed complexation mode of host **2** and **5A** by multiple hydrogen bonding and π–π stacking interactions and the key NOE contacts.

to recognize **5A** effectively. On the other hand, the stability constant of host **2** to 9-hexyladenine without sugar residue was determined to be $8.6 \times 10^4\ M^{-1}$ ($CHCl_3$, 20 °C),[14,16] about 25-fold higher than Hamilton's macrocyclic host[17] with a similar complexation mode for 9-butyladenine ($3.2 \times 10^3\ M^{-1}$, $CDCl_3$, 25 °C). Further, the stability constant of host **2** to **5A** was almost three-fold lower than that to 9-hexyladenine, which could be due to steric hindrance between the naphthalene unit of host **2** and the sugar residue of **5A**.[4d,18]

In conclusion, the new macrocyclic host **2**, which contains the 2,6-bis(oxazol-2-yl)pyridine unit and the 2,7-dialkoxynaphthalene unit tethered by the appropriate length of alkyl side chains is prepared. Host **2** undergoes highly selective complex formation with adenosine derivative **5A**, accompanied by the fluorescence response in $CHCl_3$ by the combination of multiple hydrogen bonding and π–π stacking interactions. Compared with host **1a**, host **2** shows improvements in both the stability constant for **5A** and adenine selectivity over all other nucleobases. Further studies for the developments of optimized macrocyclic host molecules and applications

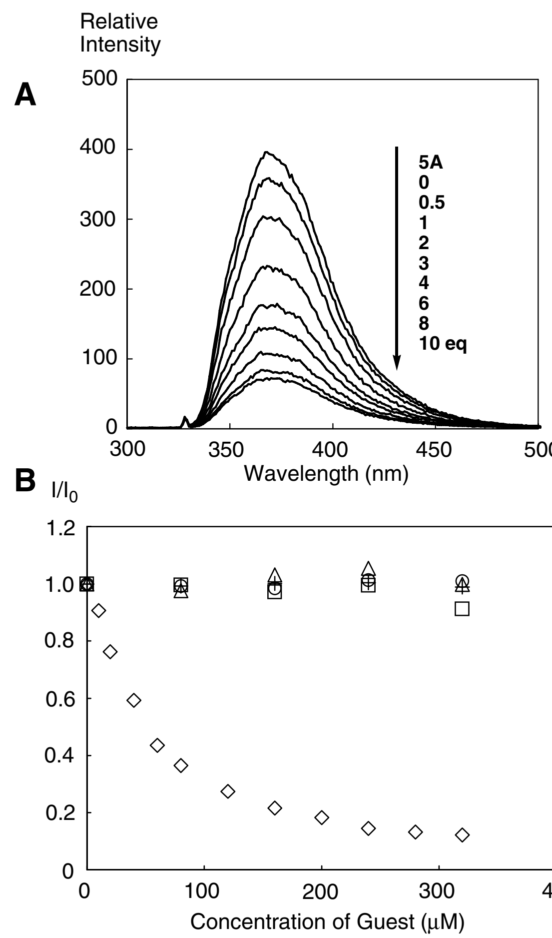

Figure 5. (A) Guest-induced quenching of the fluorescence of host **2** with increasing concentration of **5A**. (B) Plot of the ratios of fluorescence intensity at 368 nm of host **2** in absence (I_0) and in the presence (I) of nucleoside guests. The condition for (A): [**2**] = 20 μM, [**5A**] = 10–200 μM. (B): [**2**] = 20 μM, [**5A**] = 10–320 μM, [**5G**, **5C**, **5U**, **6T**] = 80–320 μM; ◊, **5A**; △, **5G**; □, **5C**; +, **5U**; ○, **6T**. For both (A) and (B): λ_{ex} = 325 nm; solvent, $CHCl_3$; temperature, 20 °C.

Table 1. Stability constants of the macrocyclic host molecules **2**–**4** for 1:1 complexes with a series of guests in $CHCl_3$ at 20 °C

Hosts	Guests	K_s (M^{-1})
2	**5A**	3.1×10^4 [a]
2	**5C**	1.5×10^2 [b]
2	**5G**	3×10^1
2	**5U**	$<3\times10^1$
2	**6T**	$<3\times10^1$
3	**5A**	2.0×10^4 [a]
4	**5A**	8.5×10^3 [a]
2	9-Hexyladenine	8.6×10^4 [c]

The stability constants are typically the average of two experiments:
[a] The value agreed within 15%.
[b] The value agreed within 20%.
[c] The value agreed within 6%.

for chemical sensing by potentiometric response[5,19] and fluorescence detection[12a] of adenine nucleotides on a membrane/water interface are currently in progress in our laboratory.

Acknowledgements

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Supplementary data

Experimental details describing the synthesis, characterization of all new compounds, and the spectroscopic measurements associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2006.11.106.

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7. Synthesis of compound **8** is as follows: a mixture of **7** (0.451 g, 1.21 mmol) and $SOCl_2$ (20 mL) was refluxed for 20 hours. $SOCl_2$ was removed under the reduced pressure, and the white solid was dried. Acyl chloride was used without further purification. To the solution of acyl chloride in acetone (20 mL), sodium azide (0.239 g, 3.68 mmol) in water (15 mL) was added dropwise during 0.5 h at 0 °C. The solution was stirred for additional 5 h at room temperature. Acetone was removed under reduced pressure at room temperature, and the precipitation of acyl azide was filtered off and dried to give **8** as a white solid (0.398 g, 78%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.01 (3H, t, $J = 7.3$ Hz) 1.53 (2H, m) 1.85 (2H, quint, $J = 6.4$ Hz), 4.19 (2H, t, $J = 6.4$ Hz), 7.92 (2H, s, Py*H*), 8.47 (2H, s, C*H* of oxazole) ppm. IR (KBr) ν_{max} 2150, 1705 cm^{-1}. MS (FAB): *m*/*z*: 424 $[M+H]^+$. Synthesis of macrocyclic host **2** is as follows: Under an inert nitrogen atmosphere, **8** (0.147 g, 0.347 mmol) dissolved in dry $CHCl_3$ (30 mL) was refluxed. After 10 h, diol **9a** (0.125 g, 0.347 mmol) dissolved in dry $CHCl_3$ (30 mL) was dropped for 2 h under the reflux condition and then stirred for additional 10 h. The solvent was removed under reduced pressure, then the product was purified by column chromatography (aluminum oxide, $CHCl_3$–hexane = 3:1) to give **2** as a white solid (0.088 g, 35%). Mp 173–174 °C (recrystallized from $CHCl_3$/hexane). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.00 (3H, t, $J = 7.3$ Hz) 1.49–1.58 (10H, m) 1.74–1.87 (10H, m) 4.09 (4H, br s), 4.15 (2H, t, $J = 6.4$ Hz), 4.27 (4H, br s), 6.85 (1H, br s, N*H*), 6.97 (2H, d, $J = 8.8$ Hz, Ar*H*), 7.10 (2H, br s, Ar*H*), 7.16 (1H, br s, N*H*) 7.50 (2H, br s, Py*H*) 7.63 (2H, d, $J = 8.8$ Hz, Ar*H*) 7.92 (2H, br s, C*H* of oxazole) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ 13.7, 19.1, 25.7, 26.6, 28.6, 29.4, 30.8, 66.7, 67.3, 68.7, 106.0, 108.6, 116.5, 124.2, 125.2, 129.0, 136.1, 138.9, 153.1, 157.3, 157.7, 166.9 ppm. IR (KBr) ν_{max} 3425, 1720 cm^{-1}. UV–vis ($CHCl_3$): λ_{max} (ε) = 312 nm (20,000 M^{-1} cm^{-1}). MS (FAB): *m*/*z*: 728 $[M+H]^+$. Anal. Calcd for $C_{39}H_{45}N_5O_9\cdot H_2O$: C, 62.81; H, 6.35; N, 9.39. Found: C, 62.94; H, 6.30; N, 9.36.
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10. Although the ^{1}H NMR signal of H_d and H_e of host **2** overlapped upon the addition of **5A** (see, Fig. 3B), it was reasonable that the NOESY cross-peak was observed between H_c and H_d, not H_c and H_e from the conformation of host **2**.[4a]
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14. The 1:1 stability constants were determined by the non-linear curve fitting method using Kaleida Graph program. See, Supplementary data.
15. Hosts **3** and **4** were obtained using the procedure analogous to that for host **2**.
16. 9-Alkyladenines are mainly used as the adenine guest molecules to investigate molecular recognition by a combination of hydrogen bonding and π–π stacking interactions in previous studies, see Ref. 4.
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