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Highly Selective Recognition of Adenine Nucleobases by Synthetic Hosts with a Linked Five-Six-Five-Membered Triheteroaromatic Structure and the Application to Potentiometric Sensing of the Adenine Nucleotide

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Abstract: A new structure for an adenine-selective host molecule, featuring the pertinent link of five-six-five-membered heteroaromatic rings and two carbamoyl NH sites, was developed. This structure provides a correctly oriented array of complementary hydrogen bonding sites for the adenine nucleobase, which exploits both Watson–Crick and Hoogsteen-type interactions. The complexation with adenine nucleobases by multiple hydrogen bonding was supported by ^{1}H NMR spectroscopy. This type of host displayed high selectivity in complexation, with an accompanying fluorescent response to lipophilized adenosine in $CHCl_3$. Furthermore, a remarkably selective potentiometric response was attained for adenosine 5′-monophosphate over 5′-GMP, 5′-CMP, and 5′-UMP by using an ion-selective electrode with a PVC-supported solvent polymeric membrane. This indicates recognition of water-soluble nucleotide guests through the membrane–water interface. These findings are expected to form a reliable basis for the development of artificial sensing systems for mononucleotides in biological systems.

Keywords: fluorescence spectroscopy · host–guest systems · hydrogen bonds · membrane potential · nucleobases

Introduction

Precise recognition of nucleobases is essential for biologically significant functions such as genome duplication, protein synthesis, and signal transduction.[1] Several types of synthetic motifs capable of monomeric complexation with nucleobases, either by multiple hydrogen bonding or by combination of different modes of interactions, have been reported.[2] Development of synthetic hosts for selective complexation with target nucleobases has been extensively investigated from the late 1980's, focusing on complementary hydrogen bonding in relatively nonpolar organic solvents.[3] More recently, these investigations have been extended to complexation in polar solvents or water by utilizing coordination, ion pairing and/or π–π stacking interactions in addition to multiple hydrogen bonding.[4] Recently, synthetic hydrogen-bonding motifs have been applied for the recognition of single nucleotide polymorphism (SNP), either for a given DNA duplex containing a bulged base[5a] or a mismatched base pair,[5b] or for a DNA duplex reconstituted with an oligonucleotide containing an abasic site.[5c] Such motifs have also been employed for ion-selective electrodes mounted with solvent polymeric membranes, which display potentiometric responses to nucleotide guests.[6,7] However, despite their potential applicability to the regulation or detection of nucleobase-bearing biomolecules, the complexation selectivity, particularly the selectivity to *adenine over all other nucleobases*, is still insufficient and remains as an unsolved, challenging problem. This is primarily because the adenine nucleobase is not furnished with three neighboring

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hydrogen-bonding/coordinating sites in contrast with the other nucleobases (vide infra).

We report here a new structure for an adenine-selective host molecule (**1**, **2**) based on the 2,6-bis(oxazol-2-yl)pyridine unit. This host structure, featuring the pertinent linking of five-six-five-membered heteroaromatic rings and two carbamoyl NH sites, provides a correctly oriented array of complementary hydrogen-bonding sites for an adenine nucleobase (Figure 1a). This type of host displayed a fluorescent response accompanying the highly selective complexation to lipophilized adenosine in $CHCl_3$. Furthermore, a remarkably selective potentiometric response was displayed to adenosine 5′-monophosphate by recognition through the membrane–water interface of a solvent polymeric membrane. The host molecules (**1**, **2**), the reference molecules (**3**, **4**) for comparison with hosts **1** and **2**, and the lipophilic nucleoside guests (**5A**, **5G**, **5C**, **5U**, **6T**) used in the present study are shown in Figure 1b.

Results and Discussion

Design and synthesis: Complexation selectivity to a target nucleobase over all other nucleobases is quite important for seeking substantial application to biological systems. So far, high selectivity in this sense has been achieved, even in highly polar solvents, for thymine[4g] (in water) and cytosine[4f] (in DMSO); both of these nucleobases contain three neighboring hydrogen-bonding/coordinating sites. On the other hand, complexation selectivity to adenine has so far been examined by Hamilton et al. [K_s(A) = 3.2×10^3 M^{-1} in $CDCl_3$],[3a] Zimmerman et al. [K_s(A) = 1.55×10^4 M^{-1} in $CDCl_3$ at 25 °C; the ratio of K_s: A/G = 5.5, A/C = 73, A/U = 120],[3b] Ogoshi et al. [K_s(A) = 5.0×10^3 M^{-1} in CH_2Cl_2 at 15 °C; the ratio of K_s: A/G = 3.8, A/C = 17; negligible complexation with dT],[3c] Vögtle et al. [K_s(A) = 4.1×10^4 M^{-1} in $CDCl_3$; the ratio of K_s: A/U = 2.9; negligible complexation with G or C],[3d] Rebek et al. [K_s(A) = 2.2×10^2 M^{-1} in $CDCl_3$; the ratio of K_s: A/C > 22][3e] and Rebek et al. [K_s(A) = 5.77×10^2 M^{-1} in CD_3OD at ca. 22 °C; the ratio of K_s: A/G = 8.0].[3f] In contrast to thymine and cytosine, high selectivity over *all* other nucleobases has not been reported for the adenine nucleobase, even in relatively nonpolar organic solvents.

To afford a highly complementary structure for the adenine nucleobase, we designed and synthesized a new planar structure based on a linked five-six-five-membered triheteroaromatic core appended with two carbamoyl NH sites. As the first step, we thoroughly examined complexation selectivity in a nonpolar organic solvent ($CHCl_3$) using lipophilized nucleoside guests. This structure provides a correctly oriented array of complementary hydrogen bonding sites for the adenine nucleobase (Figure 1a), as supported by the optimized structure of a model complex (vide infra).

For the synthesis of hosts **1** and **2**, and reference molecule **4**, a Curtius rearrangement[8] was employed as a key step to directly link a carbamoyl NH group to each of the oxazole rings. Thus, for the synthesis of host **1** (Scheme 1),[9a] bis-hydroxyamide **7**,[10] derived from pyridine-2,6-dicarboxylic acid and L-serine methyl ester, was cyclized by diethylaminosulfur trifluoride (DAST)[11] to give bis(oxazolin-2-yl)pyridine

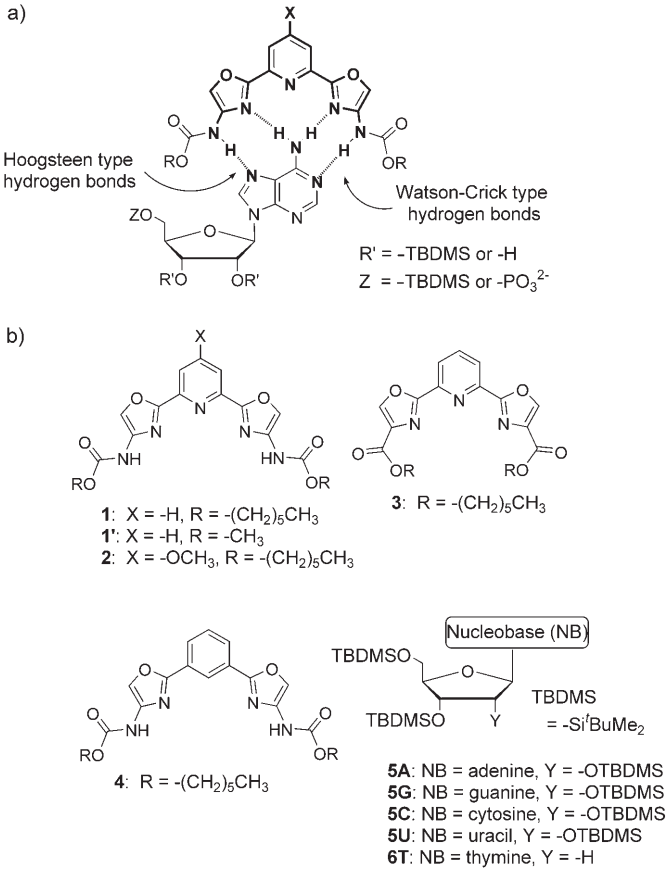
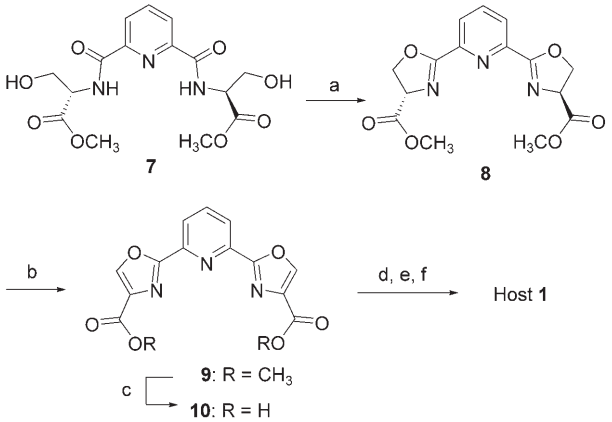
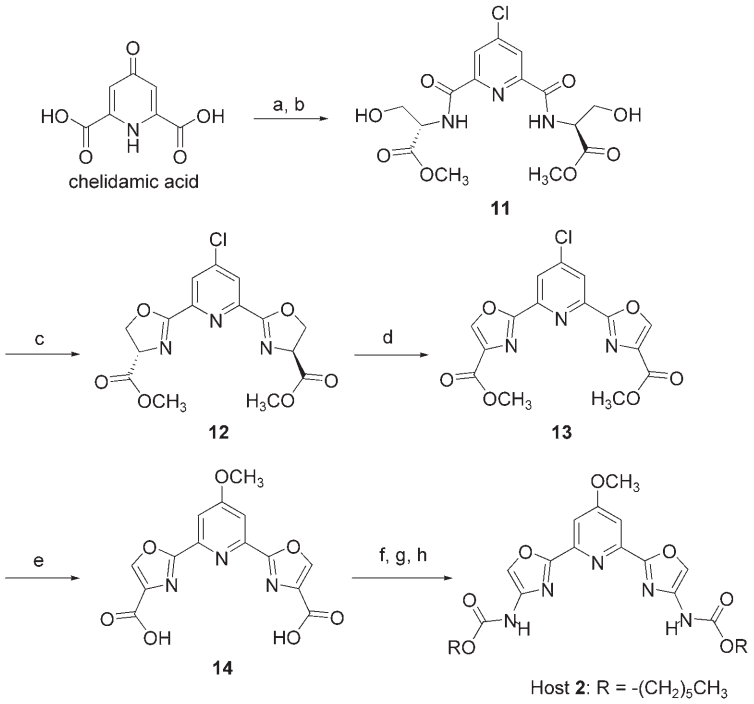


Figure 1. a) Complexation between host **1** or **2** and adenine by complementary hydrogen bonding. b) The structures of hosts (**1**, **2**), references (**3**, **4**) and lipophilic nucleoside guests (**5A**, **5G**, **5C**, **5U**, **6T**).



Scheme 1. Synthesis of host **1**: a) Et_2NSF_3, CH_2Cl_2, −78 °C, 4.5 h; then K_2CO_3, −78 °C to room temperature, overnight; 61%; b) $BrCCl_3$, DBU, CH_2Cl_2, 0 °C, overnight, 88%; c) KOH, H_2O, overnight, quant.; d) $SOCl_2$, reflux, overnight; e) NaN_3, acetone–H_2O, 0 °C, 1 h →RT, 3 h; f) reflux, 3 h; then 1-hexanol, $CHCl_3$, reflux, overnight; 62% from **10**.

8, which was oxidized with bromotrichloromethane[12] to bis(oxazol-2-yl)pyridine **9** and then hydrolyzed to **10**. Diacid **10** was converted to the corresponding diazide, which was heated at reflux to effect the Curtius rearrangement. The resulting diisocyanate was treated with 1-hexanol to give host **1** (62% from **10**). The treatment of diacid **10** with diphenylphosphoryl azide (DPPA)[13] gave host **1** with lower yield (41% from **10**) but also afforded reference **3**[9b] as a side product (22% from **10**). Reference compound **4**[9b] was synthesized by using the same route as for host **1**, starting from isophthalic acid. The synthesis of host **2**[9a] was carried out in a similar way as for host **1**, but starting from chelidamic acid and converting not only the side substituents but also the *para* substituent of the pyridine ring (Scheme 2).



Scheme 2. Synthesis of host **2**: a) $SOCl_2$, reflux, 5 days; b) L-Ser-OMe·HCl, Et_3N, $CHCl_3$, RT, 1 days, 37% from **11**; c) Et_2NSF_3, CH_2Cl_2, −78°C, 5 h; then K_2CO_3, −78°C to room temperature, overnight; 91%; d) $BrCCl_3$, DBU, CH_2Cl_2, 0°C, overnight, 95%; e) KOH, MeOH–H_2O, 60°C, 13 h, 64%; f) $SOCl_2$, reflux, overnight; g) NaN_3, acetone–H_2O, 0°C, 1 h →RT, 3 h; h) $CHCl_3$, reflux, 3 h; then 1-hexanol, $CHCl_3$, reflux, overnight; 57% from **14**.

Complexation with adenine by multiple hydrogen bonding (^{1}H NMR study): The complexation ability of host **1** was monitored first by ^{1}H NMR spectroscopy in $CDCl_3$ using a lipophilic derivative of adenosine (**5A**) as a guest. In the presence of guest **5A** (0→1.0→2.0 equiv), a significant downfield shift ($\Delta\delta = 4.29$ ppm) was observed for the amide NH protons on the side chains of host **1** (Figure 2a→2b→2c). In addition, the NH_2 signal of guest **5A** disappeared (probably by broadening) in the presence of host **1** (Figure 2d vs. Figure 2b). These ^{1}H NMR spectral changes strongly support host–guest complexation by multiple hydrogen bonding as represented in Figure 1a. Such spectral changes were not observed[14] under the same conditions for the NH_2 signal of guest **5A** upon addition of the reference compound **3**, which has no carbamoyl NH groups for hydrogen bonding with the purine nitrogen atoms. The stoichiometry of the host–guest complex between **1** and **5A** was confirmed as 1:1 by a Job plot.[14,15a,b]

Selectivity in complexation and fluorescence response to adenine: The 2,6-bis(oxazol-2-yl)pyridine unit of host **1** affords a linked triheteroaromatic fluorophore, and, upon complexation with guest **5A** in $CHCl_3$, host **1** showed a fluorescent response. Thus, as shown in Figure 3a, the fluorescence from host **1** was quenched by addition of guest **5A** with no change in the fluorescence maximum ($\lambda_{max} = 380$ nm). Again, for reference compound **3**, such a guest-induced fluorescence change was negligible under the same conditions.[14] From the change in the fluorescence intensity, the stability constant (K_s) of the 1:1 complex between **1** and **5A** was determined to be $(12400 \pm 600)\ M^{-1}$ ($CHCl_3$, 20°C) by a Benesi–Hildebrand analysis.[15b,c] This K_s value was of the same magnitude as that determined by the UV/Vis titration ($K_s = (12300 \pm 1600)\ M^{-1}$ in $CHCl_3$, 20°C).[14,16] In contrast, other nucleoside guests did not induce an appreciable fluorescence change in the concentration range of 80–320 μM (Figure 3b). The stability constants of the 1:1 complexes, determined from fluorescence, decrease at much higher guest concentrations. As listed in Table 1, host **1** attained a high complexation selectivity (greater than 100-fold) for adenine over all other nucleobases. Although the adenine selectivity was lower than that of host **1**, host **2,** with the *para*-methoxy group, also displayed highly selective complexation and a fluorescent response to the adenosine guest about 60-fold over all other nucleobases (Table 1).

Compared to the synthetic hosts reported so far (vide supra),[3a–f] the complexation with adenine by hosts **1** and **2** in the present study is comparable or somewhat weaker. However, from another viewpoint, the adenine-complexation ability of hosts **1** and **2** may be recognized as excellent in the sense that the complexation by the present hosts, unlike most of the other hosts, should stem only from multiple hydrogen bonding by neutral groups and would not involve π–π stacking and/or electrostatic interactions.[17] Furthermore,

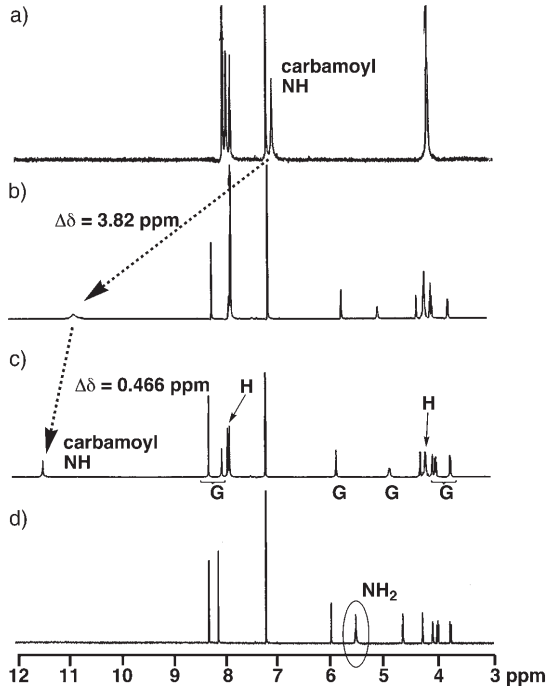


Figure 2. ^{1}H NMR spectra (500 MHz) of host **1** and/or guest **5A** in $CDCl_3$, measured at 25 °C with TMS as the external standard: a) [host **1**]$_0$ = 10.0 mM; b) [host **1**]$_0$ = 10.0 mM, [guest **5A**]$_0$ = 10.0 mM; c) [host **1**]$_0$ = 10.0 mM, [guest **5A**]$_0$ = 20.0 mM; d) [guest **5A**]$_0$ = 10.0 mM.

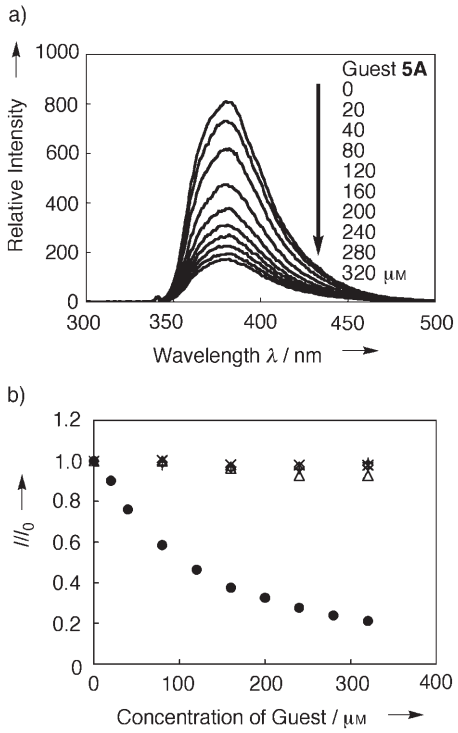


Figure 3. a) Guest-induced quenching of the fluorescence of host **1** with increasing concentration of adenosine guest **5A**. b) Comparison of the fluorescence intensity at 380 nm in the presence of a series of nucleoside guests ●, **5A**; ◇, **5G**; △, **5C**; ×, **5U**; +, **6T**. The conditions for a): [**1**] = 20 μM, [**5A**] = 20–320 μM. The conditions for b): [**1**] = 20 μM, [**5A**] = 20–320 μM, [**5G**, **5C**, **5U** or **6T**] = 80–320 μM. For both a) and b): λ_{ex} = 336 nm; solvent: $CHCl_3$; temperature: 20 °C.

Table 1. Stability constants for 1:1 complexes with a series of nucleoside guests (K_s) in $CHCl_3$ at 20 °C.[14]

Guest	K_s [M^{-1}] Host **1**	Host **2**
5A	12400 ± 600	10500 ± 400
5G	37 ± 10	62 ± 8
5C	104 ± 6	163 ± 40
5U	< 10	< 10
6T	27 ± 11	27 ± 16

the high complexation selectivity for the adenine nucleobase has been displayed *over all other nucleobases*, which is the first true example for adenine selectivity.

Optimized structures for the five-six-five-membered triheteroaromatic host: The high adenine selectivity of hosts **1** and **2**, together with the lack of selectivity towards reference compound **3**, clearly shows the significance of the two carbamoyl NH groups attached directly to the 2,6-bis(oxazol-2-yl)pyridine unit. This is supported by the optimized structure of the model host–guest complex between **1′** and 9-methyladenine (Figure 4), obtained by the nonempirical molecular orbital calculation with the hybrid density functional method (B3LYP/6–31G*).[18] This model complex shows that, unlike the six-six-six-membered counterpart, the five-six-five-membered triheteroaromatic structure provides a highly complementary structure for adenine nucleobases. In the model host–guest complex, four definite hydrogen bonds, formed by both Watson–Crick and Hoogsteen-type interactions (N−H···N distance = 2.06, 1.90, 2.04, and 1.97 Å from left to right in Figure 4), were observed,[19] which suggest the formation of four hydrogen bonds with an adenine nucleobase in the complex with host **1** or **2**.

On the other hand, hydrogen bonding was not observed between the pyridine nitrogen atom of the host and the NH_2 hydrogen atoms of the guest (N···H−N distance = 2.92 and 2.69 Å), suggesting that the pyridine nitrogen atom of host **1** does not contribute as a hydrogen-bonding acceptor. However, the pyridine structure still plays an important role for adenine selectivity by affording a suitable space to accommodate the adenine NH_2 group without steric hindrance. This is supported by the experimental fact that the complexation and fluorescent response for guest **5A** with reference compound **4** with a benzene ring [$K_s = (159 \pm 22)$ M^{-1} ($CHCl_3$, 20 °C)][20] was much weaker than that with host **1** with a pyridine ring [$K_s = (12400 \pm 600)$ M^{-1} ($CHCl_3$, 20 °C)].

The high complexation selectivity of host **1** or **2** to adenine nucleobase over guanine and uracil (thymine) can be explained by the mismatched hydrogen-bonding sites of these guests. On the other hand, although these hosts could form at least three hydrogen bonds with cytosine in a similar manner as with adenine, the carbonyl oxygen atom of the former could cause electrostatic repulsion to the carbamoyl oxygen atom of the host (Figure 5). This may be an additional advantage of this type of host structure for adenine selectivity.

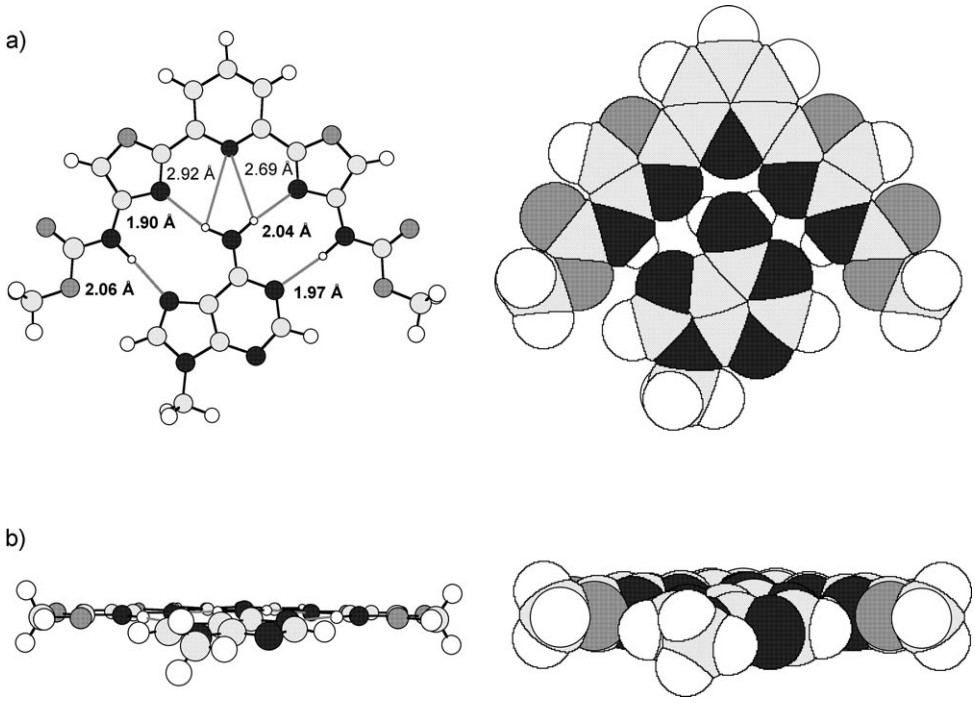


Figure 4. a) Top view and b) lateral view for the optimized structure of the model complex of host **1**′ and 9-methyladenine, obtained by the nonempirical molecular orbital calculation with the hybrid density-functional method (B3LYP/6–31G*).[18]

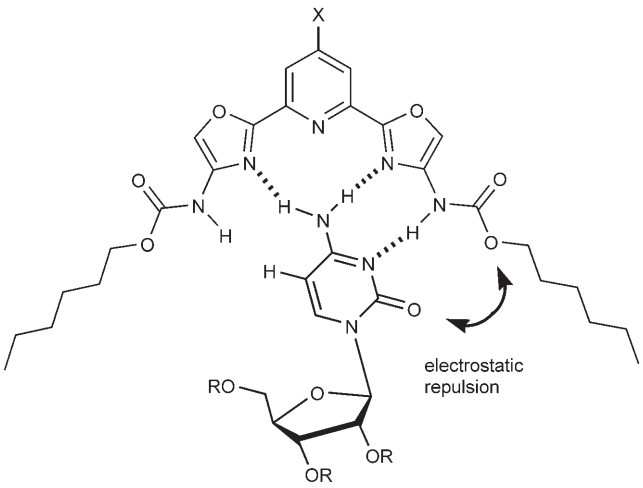


Figure 5. Presumed interactions between host **1** (X = H) or **2** (X = OCH_3) and cytosine (R = Si*t*BuMe$_2$).

Potentiometric selectivity to adenosine 5′-monophosphate (5′-AMP): Selective recognition of water-soluble adenine guests with consequent membrane-potential changes induced by synthetic hosts has been even more difficult than the recognition of lipophilized adenine derivatives in organic solvents. Guest-induced changes in membrane potential displayed by hosts embedded in membranes are widely estimated by solvent polymeric membranes supported with poly(vinyl chloride) (PVC). So far, potentiometric recognition of guanosine nucleotides (5′-GMP, 5′-GTP) by PVC-supported solvent polymeric membranes has been attained by using synthetic hosts having a cytosine group capable of forming three neighboring hydrogen bonds.[7] Relative magnitudes of potentiometric responses to 5′-GMP over 5′-AMP, estimated as potentiometric selectivity coefficients ($K^{pot}_{5'\text{-GMP},5'\text{-AMP}}$), have been reported as 0.42 for the alkylated cytosine[7b] and 0.045 for the cytosine-appended Co^{II} porphyrin.[7d] On the other hand, potentiometric recognition of 5′-AMP over 5′-GMP has so far been attempted with a thymine derivative having a branched alkyl chain, but was not successful. This is probably because of a less-selective complexation ability due to the formation of only two neighboring hydrogen bonds with both adenine and guanine nucleobases.[7b]

We applied the adenine-selective host **2** to potentiometric recognition of 5′-AMP over other nucleoside 5′-monophosphates. Only host **2** was used for the membrane potential experiments because of poor solubility of host **1** in the membrane solvent (2-nitrophenyl octyl ether). Thus, the membrane potentials (ΔEMF)[21] with increasing concentration of each nucleotide guest were measured for ion-selective electrodes fitted with PVC-supported solvent polymeric membranes containing host **2**[22] and the blank membrane without a host. Comparing the magnitudes of membrane potential change to the negative direction (anionic potential change) induced by each nucleotide guest, the membrane containing host **2** showed a potentiometric response, which was by far greatest for 5′-AMP than for other nucleoside 5′-monophosphates, irrespective of the guest concentration (Figure 6a). In contrast, the blank membrane without host **2** showed low selectivity, reflecting only the lipophilicity of the guests (Figure 6b). The host-induced changes in membrane potential [ΔEMF(Figure 6a) − ΔEMF(Figure 6b)] were approximately −40 mV for 5′-AMP and ~−10 mV for the other nucleotide guests. The potentiometric selectivity coefficient for 5′-AMP over 5′-GMP was remarkable ($K^{pot}_{5'\text{-AMP},5'\text{-GMP}} = 0.041$). This is, to the best of our knowledge, the first example of an artificial host capable of displaying a selective potentiometric response to 5′-AMP. Interestingly, by invoking correctly oriented multiple hydrogen bonds, host **2** is capable of achieving potentiometric selectivity for 5′-AMP over 5′-GMP, which is as remarkable as the potentiometric selectivity for 5′-GMP over 5′-AMP ($K^{pot}_{5'\text{-GMP},5'\text{-AMP}} = 0.045$)[7d] by a host employing metal coordination in addition to three neighboring hydrogen bonds.

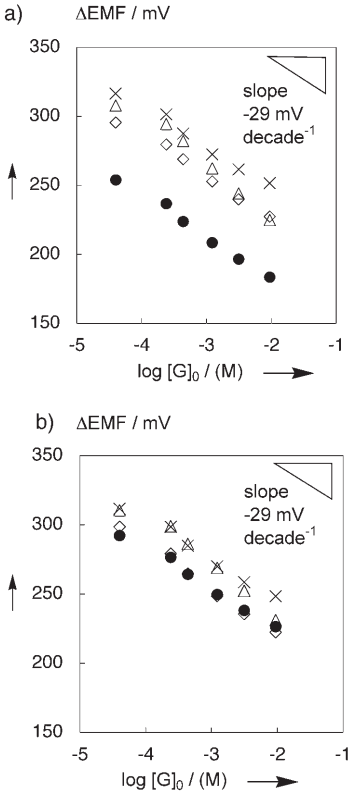


Figure 6. Membrane potentials (ΔEMF) with increasing concentration of each of the nucleoside 5′-monophosphate guests, measured at room temperature (ca. 25 °C) with a) PVC-supported solvent polymeric membrane containing host **2** and b) blank membrane without a host. ●: 5′-AMP; ◇: 5′-GMP; △: 5′-CMP; ×: 5′-UMP.

Conclusion

The results reported herein demonstrate that the linked five-six-five-membered triheteroaromatic core, attached directly with two carbamoyl NH sites, provides a multiply hydrogen-bonding structure with high complementarity for adenine nucleobases. By this host structure, high complexation selectivity was attained in $CHCl_3$ for lipophilized the adenine nucleobase over *all* other nucleobases for the first time. Furthermore, remarkable potentiometric selectivity was attained for adenosine 5′-monophosphate (5′-AMP) over 5′-GMP, 5′-CMP, and 5′-UMP by recognition through the membrane/water interface of PVC-supported solvent polymeric membranes. These findings are expected to be a reliable basis for the development of artificial sensing systems for mononucleotides in biological systems.

Experimental Section

General information: Melting points were measured with a Yanaco MP-500 V melting point apparatus and are uncorrected. Infrared spectra were recorded on JASCO FT-IR-5300 or IR Report-100 spectrometers. Fluorescence spectra were recorded on a Shimadzu RF-5300(PC) spectrometer. UV/Vis spectra were recorded on a JASCO UV-550. Mass spectra were recorded on Bruker APEX III, JASCO QUATTRO II or JEOL AX-505 spectrometers. ^{1}H NMR spectra were recorded on JEOL GSX400, LAMBDA500, or ALPHA500 spectrometers. ^{13}C NMR spectra were recorded on JEOL ALPHA500 or LAMBDA500 spectrometers with complete proton decoupling and DEPT modes. Chemical shifts are reported in δ values in ppm downfield from tetramethylsilane as the internal standard.

Solvents for extraction and chromatography were of reagent grade. Chloroform and 1-hexanol were distilled over calcium hydride. Dichloromethane (CH_2Cl_2) was distilled over phosphorus(V) oxide. *N,N*-Dimethylformamide (DMF) was dried with and distilled over barium oxide under reduced pressure. Triethylamine (Et_3N), 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU) was distilled over potassium hydroxide.

Synthesis of hosts 1 and 2[9b]

*Dimethyl (S,S)-2,2′-(pyridine-2,6-diyl)bis(4,5-dihydrooxazole-4-carboxylate) (**8**)*: This compound was prepared by employing the reported procedure[11] with some modifications. Diethylaminosulfur trifluoride (DAST, 13.22 g, 85.5 mmol) was added drop wise over a period of 30 min under a N_2 atmosphere to a stirred solution of **7** (8.31 g, 22.5 mmol) in $CHCl_3$ (225 mL), precooled to −78 °C. After the reaction was stirred at −78 °C for 4 h, K_2CO_3 was added. The reaction mixture was allowed to warm to RT and stirred for an additional 25 h. After H_2O (100 mL) was added, the mixture was extracted with $CHCl_3$ (3×100 mL), and the combined organic layers were washed with brine (1×50 mL), dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (silica gel, $CHCl_3$/MeOH 30:1) to give **8** as a white solid (5.68 g, 76 %): m.p. 154–156 °C (recrystallized from EtOH); ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 3.82 (s, 6H; CH_3), 4.71 (dd, ^2J(H,H) = 10.7, ^3J(H,H) = 9.0 Hz, 2H; one of CH_2), 4.80 (dd, ^3J(H,H) = 8.3, 9.0 Hz, 2H; CH), 5.02 (dd, ^2J(H,H) = 10.7, ^3J(H,H) = 8.3 Hz, 2H; one of CH_2), 7.91 (t, ^3J(H,H) = 8.1 Hz, 1H; PyH(*para*)), 8.28 ppm (d, ^3J(H,H) = 8.1 Hz, 2H; PyH(*meta*)); ^{13}C NMR (125 MHz, $CDCl_3$, TMS): δ = 52.8, 68.7, 70.4, 126.7, 137.6, 146.2, 164.9, 171.1 ppm; IR (KBr): $\tilde{\nu}_{max}$ = 1744 and 1726 cm^{-1} (ν(C=O), ester); MS (EI, 70 eV): *m*/*z* (%): 333 (27) [M^+], 275 (58), 274 (100), 246 (77), 186 (38); HRMS calcd (*m*/*z*) for $C_{15}H_{15}N_3O_6$ [M^+]: 333.0961; found: 333.0967.

*Dimethyl 2,2′-(pyridine-2,6-diyl)bis(oxazole-4-carboxylate) (**9**)*: This compound was prepared by employing the reported procedure[12] with some modifications. Bromotrichloromethane (17.25 g, 84.7 mmol) was added to a stirred solution of **8** (5.65 g, 17.0 mmol) and DBU (6.70 g, 42.4 mmol) in CH_2Cl_2 (250 mL), precooled at 0 °C, over a period of 1 min, and was stirred at 0 °C for 20 h. The resulting precipitate was collected by filtration to give **9**. The filtrate was evaporated and purified by column chromatography (silica gel, $CHCl_3$) to give an additional amount of **9**. The combined white solid (total 3.95 g, 71 %) was recrystallized from AcOEt/EtOH to give an analytical sample: mp 287–288 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 3.98 (s, 6H; CH_3), 8.03 (t, ^3J(H, H) = 8.3 Hz, 1H; PyH(*para*)), 8.42 (d, ^3J(H,H) = 8.3 Hz, 2H; PyH(*meta*)), 8.44 ppm (s, 2H; CH of oxazole); ^{13}C NMR (125 MHz, $CDCl_3$, TMS): δ = 52.5, 124.5, 134.7, 138.4, 145.3, 145.5, 160.2 ppm; IR (KBr): $\tilde{\nu}_{max}$ = 1746 and 1723 cm^{-1} (ν(C=O), ester); MS (EI, 70 eV): *m*/*z* (%): 329 (100) [M^+], 298 (45), 231 (48), 203 (79), 198 (62); elemental analyses calcd (%) for $C_{15}H_{11}N_3O_6$: C 54.72, H 3.37, N 12.76; found: C 54.42, H 3.49; N 12.95.

*2,2′-(Pyridine-2,6-diyl)bis(oxazole-4-carboxylic acid) (**10**)*: Compound **9** (1.26 g, 3.8 mmol) was added to a stirred solution of potassium hydroxide (1.43 g, 25 mmol) in H_2O (50 mL), and the whole was stirred at RT for 20 h. The reaction mixture was acidified with 5 % HCl, and the resulting precipitate was collected by filtration to give **10** as a white solid (1.126 g, quantitative): m.p. 296 °C (decomp); ^{1}H NMR (400 MHz, [D_6]DMSO, TMS): δ = 8.23 (t, ^3J(H,H) = 9.0 Hz, 1H; PyH(*para*)), 8.31 (d, ^3J(H,H) = 9.0 Hz, 2H; PyH(*meta*)), 8.97 ppm (s, 2H; CH of oxazole); ^{13}C NMR (125 MHz, [D_6]DMSO, TMS): δ = 124.0, 134.8, 139.5, 145.1, 146.5, 159.6, 161.8 ppm; IR (KBr): $\tilde{\nu}_{max}$ = 3750–3250 (ν(OH), carboxy), 1719 (ν(C=O), carboxy) cm^{-1}; MS (EI, 70 eV): *m*/*z* (%): 301 (78) [M^+], 257 (70), 189 (100), 145 (70); HRMS calcd (*m*/*z*) for $C_{13}H_7N_3O_6$ [M^+]: 301.0335; found: 301.0337; elemental analyses calcd (%) for $C_{13}H_7N_3O_6$: C 51.84, H 2.34, N 13.95; found: C 51.70, H 2.41, N 13.71.

*Dihexyl 2,2′-(Pyridine-2,6-diyl)bis(oxazole-4-carbamate) (**1**)*: This compound was prepared from diacid **10**, which was first converted to the cor-

responding diazide and then subjected to a Curtius rearrangement followed by treatment with 1-hexanol. A mixture of **10** (150 mg, 0.50 mmol) and $SOCl_2$ (9.0 mL, 0.12 mol) was heated at reflux for 19 h. The reaction mixture was evaporated and dried in vacuo to give the diacid chloride. To a stirred solution of the diacid chloride in dry acetone (9 mL), precooled at 0 °C, was added a solution of sodium azide (162 mg, 2.5 mmol) in H_2O (1 mL) during a period of 1 h. The whole was stirred for 3 h at room temperature. The resulting precipitate was collected by filtration and washed with H_2O to give the diazide as a white solid. A stirred suspension of the diazide in $CHCl_3$ (8 mL) was heated at reflux for 2 h under a N_2 atmosphere. 1-Hexanol (8 mL) was added to the resulting solution, and the mixture was heated at reflux for 14 h. The reaction mixture was diluted with $CHCl_3$ (40 mL), and washed successively with 5% aq citric acid (20 mL), saturated aqueous $NaHCO_3$ (20 mL) and brine (20 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered and evaporated. The residue was purified by column chromatography (alumina, $CHCl_3$/hexane 1:1) to give **1** as a white solid (156 mg, 62%), which was recrystallized from EtOH to give a white powder: mp 196–198 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): $\delta = 0.90$ (t, ^3J(H, H) = 7.0 Hz, 6H; CH_3), 1.3–1.4 (br m, 12H in total; $(CH_2)_3CH_3$), 1.7 (m, 4H; OCH_2CH_2), 4.19 (br t, ^3J(H,H) = 6.6 Hz, 4H; OCH_2), 7.16 (br s, 2H;NH), 7.93 (t, ^3J(H,H) = 7.6 Hz, 1H; PyH(*para*)), 8.01 (s, 2H; CH of oxazole), 8.08 ppm (d, ^3J(H,H) = 7.6 Hz, 2H; PyH(*meta*)); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 14.0$, 22.5, 25.4, 28.8, 31.4, 66.2, 122.6, 126.0, 138.0, 138.7, 145.9, 153.1, 157.1 ppm; IR (KBr): $\tilde{\nu}_{max} = 3308$ (ν(NH), carbamoyl), 1709 (ν(C=O), carbamoyl) cm^{-1}; UV/Vis ($CHCl_3$): λ_{max} (ε) = 326 nm (19000 $M^{-1}cm^{-1}$); HRMS (EI, 70 eV) calcd (m/z) for $C_{25}H_{33}N_5O_6$ [M^+]: 499.2431; found: 499.2417; elemental analyses calcd (%) for $C_{25}H_{33}N_5O_6$: C 60.11, H 6.66, N 14.02; found: C 59.96, H 6.47, N 14.03.

*(S,S)-N,N′-(4-Chloropyridine-2,6-diyldicarbonyl)bis-L-serine dimethyl ester (**11**)*: This compound was prepared by employing the reported procedure[23] with some modifications. Chelidamic acid (9.71 g, 53.1 mmol), moistened with a small amount of DMF (0.170 g, 2.3 mmol), was mixed with $SOCl_2$ (125 mL, 4.4 mol) and heated at reflux for five days. The mixture was evaporated, coevaporated with anhydrous toluene and dried in vacuo to give the diacid chloride. This material was used for the next step without further purification. To a suspension of L-serine methyl ester hydrochloride (18.67 g, 120 mmol) in $CHCl_3$ (250 mL), precooled at 0 °C, were successively added Et_3N (65 mL, 430 mmol) in one portion and a solution of the diacid chloride in $CHCl_3$ (100 mL, drop wise over a period of 30 min under N_2 atmosphere). The whole was stirred at room temperature for one day and concentrated to a small volume. The residue was treated with AcOEt–MeOH and the resulting white solid was filtered off; these operations were repeated twice. The filtrate was evaporated and the residue was purified by column chromatography (silica gel, $CHCl_3$/MeOH 60:1) to give **11** as a white solid (7.38 g, 37%): m.p. 179 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): $\delta = 3.65$ (s, 2H; OH), 3.84 (s, 6H; CH_3), 4.11 (dd, ^2J(H, H) = 11.7, ^3J(H,H) = 3.9 Hz, 2H; one of CH_2), 4.21 (dd, ^2J(H,H) = 11.7, ^3J(H,H) = 3.9 Hz, 2H; one of CH_2), 4.84 (dd, ^3J(H,H) = 3.9, 7.8 Hz, 2H; CH), 8.19 (s, 2H; PyH), 8.80 ppm (d, ^3J(H,H) = 7.8 Hz, 2H; NH); IR (KBr): $\tilde{\nu}_{max} = 3350$ (ν(OH), br), 1735 (ν(C=O), ester), 1655 (ν(C=O), amide) cm^{-1}; MS (FAB): *m/z*: 404 [M+H]$^+$; elemental analyses calcd (%) for $C_{15}H_{18}ClN_3O_8$: C 44.62, H 4.49, N 10.41; found: C 44.22, H 4.44, N 10.28.

*Dimethyl (S,S)-2,2′-(4-chloropyridine-2,6-diyl)bis(4,5-dihydrooxazole-4-carboxylate) (**12**)*: This compound was obtained from **11** (1.210 g, 3.00 mmol) by a similar protocol as for the cyclization of bis-hydroxyamide **7** to **8**. After purification by column chromatography (silica gel, $CHCl_3$/MeOH 60:1), the desired product **12** was obtained as a yellow solid (1.00 g, 91%): m.p. 97–99 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): $\delta = 3.83$ (s, 6H; OCH_3), 4.72 (dd, ^2J(H,H) = 10.7, ^3J(H,H) = 9.0 Hz, 2H; one of CH_2), 4.81 (dd, ^3J(H,H) = 8.3, 9.0 Hz, 2H; CH), 5.02 (dd, ^2J(H,H) = 10.7, ^3J(H,H) = 8.3 Hz, 2H; one of CH_2), 8.30 ppm (s, 2H; PyH); IR (KBr): $\tilde{\nu}_{max} = 1740$ cm^{-1} (ν(C=O), ester); HRMS (ESI) calcd (m/z) for $C_{15}H_{14}{}^{35}ClN_3O_6$ [M+Na]$^+$: 390.0462; found: 390.0463.

*Dimethyl 2,2′-(4-chloropyridine-2,6-diyl)bis(oxazole-4-carboxylate) (**13**)*: This compound was obtained from bis(oxazolin-2-yl)pyridine **12** (974 mg, 2.65 mmol) by a similar protocol as for the oxidation of **8** to **9**. After purification by column chromatography (silica gel, $CHCl_3$), the desired product **13** was obtained as a yellow solid (931 mg, 95%): m.p. 211 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): $\delta = 3.99$ (s, 6H; OCH_3), 8.448 and 8.454 ppm (two s, each 2H; PyH and oxazolyl CH); IR (KBr): $\tilde{\nu}_{max}$ = 1735 cm^{-1} (ν(C=O), ester); HRMS (ESI) calcd (*m/z*) for $C_{15}H_{10}{}^{35}ClN_3O_6$ [M+H]$^+$: 364.0318; found: 364.0331.

*2,2′-(4-Methoxypyridine-2,6-diyl)bis(oxazole-4-carboxylic acid) (**14**)*: Compound **13** (625 mg, 1.72 mmol) was added to a stirred solution of potassium hydroxide (0.90 g, 14 mmol) in a mixture of H_2O (22 mL) and MeOH (17 mL). The mixture was heated at 60 °C for 13 h. The reaction mixture was acidified with 5% HCl, and the resulting precipitate was collected by filtration to give the methoxy diacid **14** as a white solid (360 mg, 64%): m.p. 168–169 °C; ^{1}H NMR (400 MHz, [D_6]DMSO, TMS): $\delta = 4.06$ (s, 3H; OCH_3), 7.77 (s, 2H; PyH), 8.96 ppm (s, 2H; CH of oxazole); IR (KBr): $\tilde{\nu}_{max} = 3410$ (ν(OH), br, carboxy), 1710 (ν(C=O), carboxy) cm^{-1}; HRMS (ESI) calcd (*m/z*)for $C_{14}H_9N_3O_7$ [M+Na]$^+$: 354.0333; found: 354.0320.

*Dihexyl 2,2′-(4-methoxypyridine-2,6-diyl)bis(oxazole-4-carbamate) (**2**)*: This compound was obtained from methoxy diacid **14** (90 mg, 0.27 mmol) by a Curtius rearrangement–carbamate ester formation with a similar protocol as for the sequence of reactions from **10** to **1**. After purification by column chromatography (alumina, $CHCl_3$/hexane 1:1), the desired product **2** was obtained as a white solid (90 mg, 57%): m.p. 126–127 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): $\delta = 0.90$ (t, ^3J(H,H) = 6.8 Hz, 6H; $(CH_2)_3CH_3$), 1.3–1.4 (m, 12H in total; $(CH_2)_3CH_3$), 1.7 (br m, 4H; OCH_2CH_2), 4.00 (s, 3H; OCH_3), 4.19 (br t, ^3J(H,H) = 6.6 Hz, 4H; OCH_2), 6.95 (br s, 2H; NH), 7.60 (s, 2H; PyH), 8.00 ppm (s, 2H; CH of oxazole); ^{13}C NMR (125 MHz, $CDCl_3$, TMS): $\delta = 13.9$, 22.4, 25.3, 28.7, 31.3, 55.7, 66.0, 108.7, 125.7, 138.4, 147.3, 152.9, 157.0, 167.0 ppm; IR (KBr): $\tilde{\nu}_{max} = 3300$ (ν(NH), carbamoyl), 1710 (ν(C=O), carbamoyl) cm^{-1}; HRMS (ESI) calcd (*m/z*) for $C_{26}H_{35}N_5O_7$ [M+Na]$^+$: 552.2443; found: 552.2429; elemental analyses calcd (%) for $C_{26}H_{35}N_5O_7$: C 58.97, H 6.66, N 13.22; found: C 58.85, H 6.69, N 13.26.

Fluorescence spectroscopic measurements for host–guest complexation

Measurements of fluorescence intensity or spectra were conducted on a Shimadzu RF-5300 spectrometer at 20 °C, using $CHCl_3$ of fluorescence spectrophotometric grade purchased from Kanto Chemical(Tokyo, Japan; catalog code 07278–3B).

Determination of the stability constants of the host–guest complexes: Stability constants of 1:1 complexes (K_s [M^{-1}]) were determined by the Benesi–Hildebrand[15a,c] analysis, measuring the decrease of fluorescence intensity with increasing concentration of the guest with a constant concentration of the host.[14] The conditions: [**1**] = 20 μm, [**5A**] = 240–580 μm, [**5G**, **5C**, **5U**, or **5T**] = 4.0–16.0 mm, [**2**] = 20 μm, [**5A**] = 240–580 μm, [**5C**] = 0.80–1.6 mm, [**5G**, **5U**, or **5T**] = 4.0–16.0 mm, [**4**] = 20 μm, [**5A**] = 2.0–12.0 mm. Fluorescence emission at 380 nm (host **1**), 371 nm (host **2**) or 376 nm (reference **4**) was measured in $CHCl_3$ at 20 °C with excitation at 336 nm (host **1** with all guests), 326 nm (host **2** with all guests) or 312 nm (reference **4** with guest **5A**).

Potentiometric measurements[24]

Solvent polymeric membranes: Solvent polymeric membranes with poly(vinyl chloride) (PVC) matrix were prepared according to the literature[25] with slight modifications. Membranes of about 0.1-mm thickness were obtained by pouring a solution of approximately 300 mg of membrane components, dissolved in THF (ca. 3 mL), into a flat Petri dish (34 mm i.d.). The solvent was allowed to evaporate off at room temperature for two days. From the resulting solvent polymeric membrane, several disks of 7-mm diameter were punched and soaked in a 1.0×10^{-2} M KCl solution overnight as a conditioning process. The composition of solvent polymeric membrane containing host **2** and that of the blank membrane without a host were host/NPOE/PVC/TDDMACl = 0.7:70.6:28.5:0.4 and NPOE/PVC/TDDMACl = 70.6:28.5:0.4 (weight ratio), respectively. The molar ratio of TDDMACl relative to host **2** was 50 mol%. NPOE = 2-nitrophenyl octyl ether; TDDMACl = tridodecylmethylammonium chloride (Cl).

Electrode system: Each membrane was fixed on a liquid membrane type ion-selective electrode body type IS 561 (Willi Möller AG, Zürich, Swit-

zerland). A 1.0×10^{-2} M KCl solution was used as an internal solution. The reference electrode was a double-junction type based on an Ag/AgCl electrode (DKK·TOA, Tokyo, Japan) containing a saturated aqueous KCl solution in the inner compartment and a 1 M AcOLi solution in the outer compartment. The electrode cell for the potential measurements was as follows: Ag | AgCl | satd aq KCl ⋮ 1 M AcOLi ⋮ guest solution | membrane | 1.0×10^{-2} M KCl | AgCl | Ag.

Potential measurements: Membrane potentials were measured for unbuffered aqueous solutions of guests. All measurements were carried out with gentle stirring at room temperature (ca. 25 °C) with a pH–mV meter model HM-60 V (TOA Electronics, Tokyo, Japan). Deionized and purified water (specific resistance, 18.2 MΩ cm) was obtained with a Milli-Q Labo system. The sample solutions of each guest were prepared by adding an aliquot (10, 20, 50, 200, 500, 1800 μL) of 1.0×10^{-1} M guest solution to 25 mL of water. Membrane potentials for each guest anion were measured in the order of increasing lipophilicity of the guest (5′-CMP, 5′-UMP, 5′-AMP, 5′-GMP). The potential was measured for 1–5 min. During this period of time, the potential drift was around 1 mV min^{-1} in most cases. The potential measurement for each guest was repeated two or three times. By the separate solution method,[26] according to the conventional Nicolsky–Eisenman equation, the potentiometric selectivity coefficient[27] ($K_{5'\text{-AMP},5'\text{-GMP}}^{pot}$) was determined for 5′-AMP and 5′-GMP as the primary and interfering ions, respectively, with the concentration range of $1.0\times10^{-3.4}$ to $1.0\times10^{-2.0}$ M.

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[16] The experimental results suggest that the K_s values determined by the fluorescent responses mainly reflect the complexation ability in the ground state. Therefore, for the present host **1**, the fluorescence method can be used to determine the K_s values for firm discussions on host–guest complexation ability and selectivity displayed under ordinary conditions.

[17] Another example of a host with only neutral hydrogen-bonding sites, which exhibits similar complexation ability for 9-ethyladenine, has been reported ($K_s=2.4\times10^3$ M^{-1} in $CDCl_3$). a) Complexation ability for adenine nucleobase can be improved by employing acidic hydrogen-bonding groups ($K_s=4.5\times10^4$ M^{-1} in $CDCl_3$). b) K. S. Jeong, T. Tjivikua, A. Muehldorf, G. Deslongchamps, M. Famulok, J. Rebek, Jr., *J. Am. Chem. Soc.* **1991**, *113*, 201–209; c) J. C. Adrian, Jr., C. S. Wilcox, *J. Am. Chem. Soc.* **1989**, *111*, 8055–8057.

[18] The calculation was carried out with the GAUSSIAN-98 program on the IBM 7038–6M2 pSeries650 computer system of the Library and Information Processing Center of Nagoya City University. GAUSSIAN-98: Gaussian 98, Revision A.11.3, produced by M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, V. G. Zakrzewski, J. A. Montgomery, Jr., R. E. Stratmann, J. C. Burant, S. Dapprich, J. M. Millam, A. D. Daniels, K. N. Kudin, M. C. Strain, O. Farkas, J. Tomasi, V. Barone, M. Cossi, R. Cammi, B. Mennucci, C. Pomelli, C. Adamo, S. Clifford, J. Ochterski, G. A. Petersson, P. Y. Ayala, Q. Cui, K. Morokuma, N. Rega, P. Salvador, J. J. Dannenberg, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. Cioslowski, J. V. Ortiz, A. G. Baboul, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. Gomperts, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, J. L. Andres, C. Gonzalez, M. Head-Gordon, E. S. Replogle, J. A. Pople, Gaussian, Inc., Pittsburgh (USA), **2002**.

[19] These values agree well with those calculated by a similar method for the 2:1 complex of *N*-((*E*)-(methylimino)methyl)formamide and 9-ethyladenine. See: I. Alkorta, J. Elguero, S. Goswami, R. Mukherjee, *J. Chem. Soc. Perkin Trans. 2* **2002**, 894–898.
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