Molecular Design of Boronic Acid-Based Dye Receptors for Nucleosides

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Chromophric receptors for nucleosides were designed utilizing the boronic acid–diol interaction which effectively operates in aqueous solution. Receptors consist of a boronic acid moiety and a dimethylaminophenylazo moiety. The boronic acid moiety forms a covalently-bonded ester linkage with the 2,3-diol group in nucleosides in a reversible manner and the anionic charge generated on the boron atom is stabilized intramolecularly by the pyridinium cation. The azo moiety can overlap with the heteroaromatic ring moiety in nucleosides to stabilize the complex. The complexation constants could be conveniently determined by the absorption spectral change. Comparison of the complexation constants with those of 2-deoxyadenosine without 2-OH and 1,4-anhydroerythritol without the heteroaromatic ring established that the complex formation is primarily due to the boronic acid–diol (2,3-diol) interaction and secondarily facilitated by the hydrophobic and/or π–π stacking interactions.

Recognition of neutral organic molecules by synthetic receptors has been of interest to many chemists. Most of such known synthetic molecular receptors utilize hydrogen-bonding interactions in order to recognize and bind with guest molecules.[1] However, these interactions are less effective in aqueous media where the guest species is well solvated by water molecules. On the other hand, covalent interactions found in the binding between boronic acid and saccharides in aqueous media are stronger than such hydrogen-bonding interactions. Phenylboronic acid complexation with saccharides *via* two covalent bonds creates five- or six-membered rings. The usefulness of the boronic acid moiety as an artificial saccharide receptor has been demonstrated in saccharide recognition in rigid matrices,[2,3] CD and absorption spectroscopic detection of saccharides[4—6] and also recently in fluorescence detection of saccharides.[7—11] Taking these lines of potential usefulness of boronic acids for saccharide sensing into consideration, we here designed compound **1** for the spectroscopic detection of nucleosides (Chart 1). As nucleosides commonly possess a ribose moiety, the *cis*-2,3-diol group should be useful as a central recognition site for the interaction with boronic acids.[12,13] Although a few successful examples for the recognition of nucleotides with a cooperative action of the electrostatic interaction and the π–π stacking interaction have been reported by the Lehn's group,[14] Grotjohn and Czarnik[15] demonstrated a good example for the application of a boronic acid-ribose complexation to liquid-membrane transport of nucleosides. The boronic acid–diol complexation intensifies the acidity of boronic acid and creates an anionic charge on the boron atom.[6—11] This anionic charge development is facilitated by an intramolecular cationic charge.[16,17] Hence, a pyridinium group is introduced into the ortho position to the boronic acid. Examination of CPK molecular models suggests that when the boronic acid in **1** forms a complex with the *cis*-diol in adenosine, the adenine moiety can enjoy the stacking interaction with the dimethylaminophenylazo moiety (as in Fig. 1). Since this chromophoric moiety has a pair of push (dimethylamino) and pull (pyridinio) groups, the stacking interaction should induce some spectroscopic change. Examination of CPK molecular models predicts, on the other hand, that in compound **2** (Chart 2) the adenine moiety in the complexed adenosine cannot enjoy such a stacking interaction with the dimethylaminophenylazo moiety. We here report that compound **1**

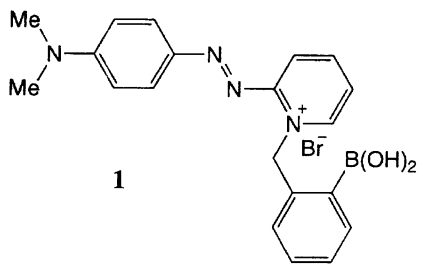

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Chart 1.

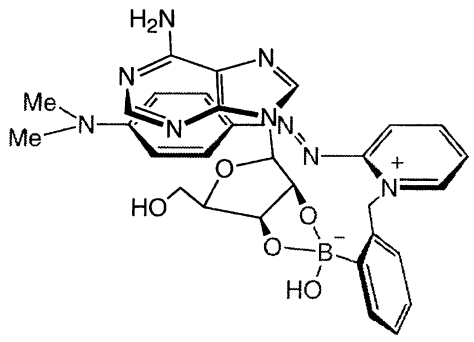

Fig. 1. Expected structure of a **1**–adenosine complex which can enjoy a stacking interaction between the dimethylaminophenylazo moiety and adenine moiety.

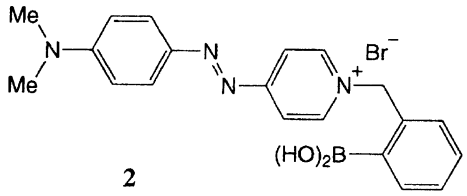

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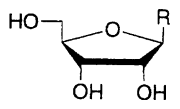
Chart 2.

has an ideal structure for discriminating among nucleosides through multi-point interactions (Chart 3).

Results and Discussion

Solvent Effect and pH Dependence. To confirm that **1** exists discretely in aqueous solution, we measured the absorption spectra in various water–methanol mixtures (water/methanol = 0/1, 1/1, 5/1, 10/1, 20/1, 30/1, and 300/1 v/v). To accurately detect even a slight spectra change, we used a dual-wavelength spectrophotometer (Shimadzu UV-3000) which enabled us to reproducibly measure the absorbance change of 0.001. At [**1**] = 1.00×10^{-5} mol dm^{-3}, the absorption spectra were scarcely change in water–methanol mixtures although the λ_{max} (561 nm in water/methanol = 0 : 1 v/v) shifted to slightly longer wavelength with increasing water concentration (λ_{max} 566 nm in water/methanol = 300 : 1 v/v). At [**1**] = (1.00—7.00) $\times 10^{-5}$ mol dm^{-3} (at pH 8.0 and 10.5) in water/methanol = 300/1 v/v a plot of [**1**] vs. absorbance satisfied the Lambert–Beer's law. These lines of evidence establish that **1** does not aggregate under these measurement conditions.

To find the optimum pH for the measurements we determined pK_a of **1** in the absence and the presence of saccharides. A typical spectral change is shown in Fig. 2. We see in Fig. 2 that with increasing medium pH the λ_{max} gradually shifts to shorter wavelength and the extinction coefficient decreases. At the high pH region, the $B(OH)_2$ group



nucleosides

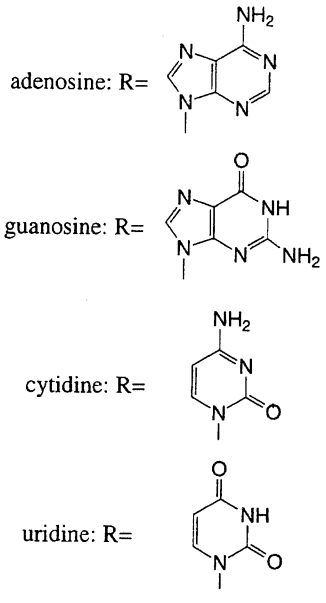


Chart 3.

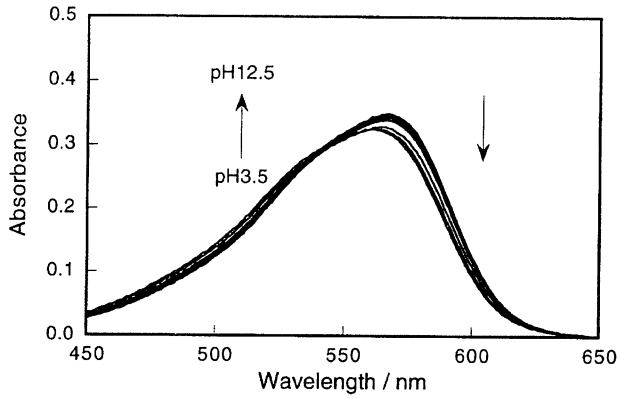


Fig. 2. Absorption spectra of **1** (1.00×10^{-5} mol dm^{-3}) in the absence of saccharide: 25 °C, 0.3 vol% methanol. The measurement conditions are recorded in Experimental Section.

is dissociated to the $B^-(OH)_3$ group. Hence, the electron-withdrawing nature of the pyridinium group is somewhat weakened by intramolecular charge neutralization. This effect partially suppresses the intramolecular charge transfer from the dimethylamino group to the pyridinium group and induces the observed hypsochromic shift.

We chose 1,4-anhydroerythritol (**3**) (Chart 4) as a saccharide to determine the pK_a, because the structure is very close to the furanose form of D-ribose. The absorption spectra changed gradually with increasing **3** concentration but became unaffected above 30 mmol dm^{-3}. We thus chose 100 mmol dm^{-3} as a standard concentration. Plots of ΔA_{580} vs. pH are shown in Fig. 3. This data indicates that the pK_a shifts to lower pH region with increasing **3** concentration. The result is in line with the previous finding that saccharide complexation generally lowers the acidity the of boronic acids.[6—11] From analysis of the titration curves, the pK_a values were determined to be 8.5 in the absence of **3** and 7.8 and 6.2 in the presence of 1.0 and 100 mmol dm^{-3} of **3**, respectively. Hereafter, we carried out the measurements either at pH 8.0, where most free $B(OH)_2$ is not dissociated while saccharide complex is dissociated, or at pH 10.5, where both $B(OH)_2$ and saccharide complex are dissociated.

Determination of Complexation Constants. The absorption spectra of **1** and **2** were measured at different nucleoside concentrations. As shown in Fig. 4, the λ_{max} for **1** shifted to longer wavelengths (from 558 to 568 nm) in the presence of nucleosides. The similar longer wavelength shift was also observed for **2** (from 563 to 570 nm). Plots of ΔA_{580} vs. [nucleoside] for **1** are shown in Fig. 5 (at pH 10.5) and Fig. 6 (at pH 8.0) and those for **2** are shown in Fig. 7 (at pH 10.5). These plots were analyzed by Benesi–Hildebrand equation

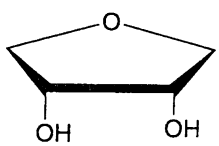


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Chart 4.

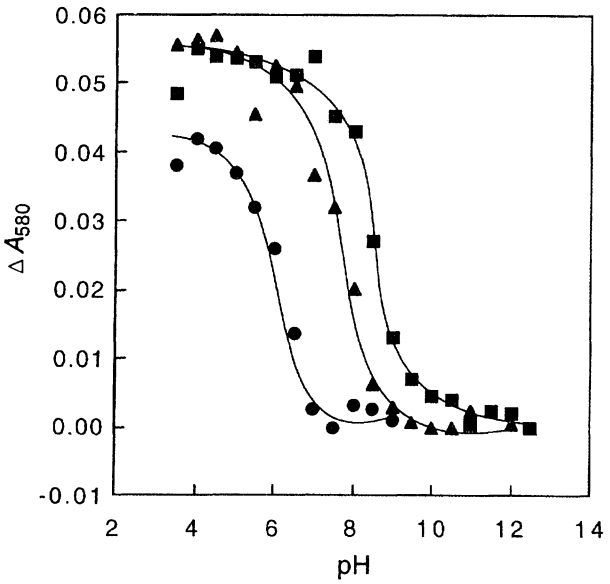


Fig. 3. Plots of ΔA_{580} vs. pH: 25 °C, 0.3 vol% methanol, [**1**] = 1.00×10^{-5} mol dm^{-3}, [**3**] = 0 (■), 1.0 (▲), and 100 (●) mmol dm^{-3}.

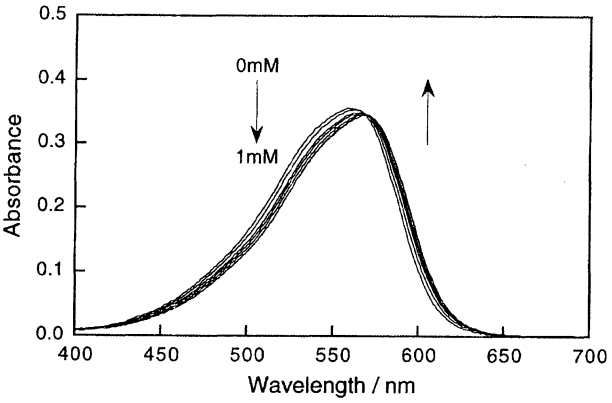


Fig. 4. Absorption spectral change in **1** (1.00×10^{-6} mol dm^{-3}) induced by added adenosine (($1.00—100)$\times10^{-5}$ mol dm^{-3}): 25 °C, pH 10.5 with 50 mmol dm^{-3} carbonate, 0.03 vol% methanol.

for the formation of a 1 : 1 complex. They showed the correlation coefficients higher than 0.99. The complexation constants (K_c) thus determined are summarized in Table 1.

In Table 1, the K_c values for **1** at pH 8.0 appear in the order of adenosine > guanosine > cytidine > uridine: that is, the larger is the area of the heteroaromatic ring, the greater is the K_c. This view is also supported by the finding reported by Mollica and Connors[18,19] that the association tendency of heterocyclic compounds in water is correlated with the overlapping area between heterocyclic compounds and guest molecules. The result indicates that not only the boronic acid–diol interaction but also the hydrophobic and/or π–π stacking interactions contributes to the stabilization of the complexes. On the other hand, the K_c values for **1** at pH 10.5 appear in the order of adenosine > cytidine > guanosine > uridine and are generally greater than those at pH 8.0. The increase in the K_c is readily explicable with the assistance of

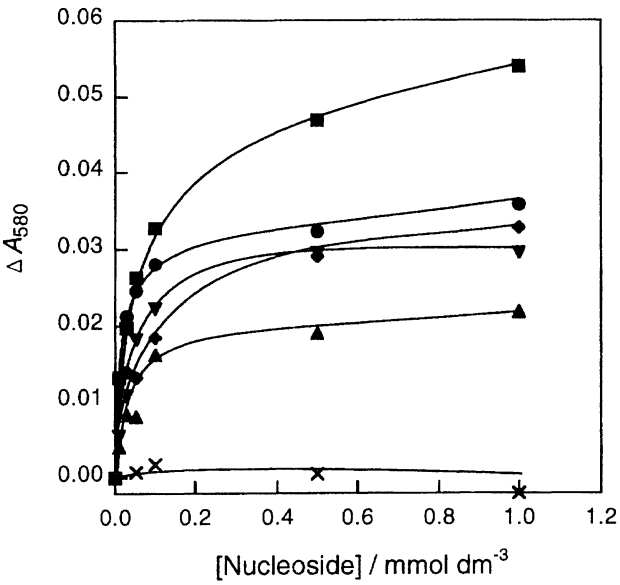


Fig. 5. Plots of ΔA_{580} vs. [nucleoside] for **1** at pH 10.5, adenosine (●), guanosine (■), cytidine (▲), uridine (◆), AMP (▼), and 2-deoxyadenosine (×).The measurement conditions are recorded in a caption to Fig. 4.

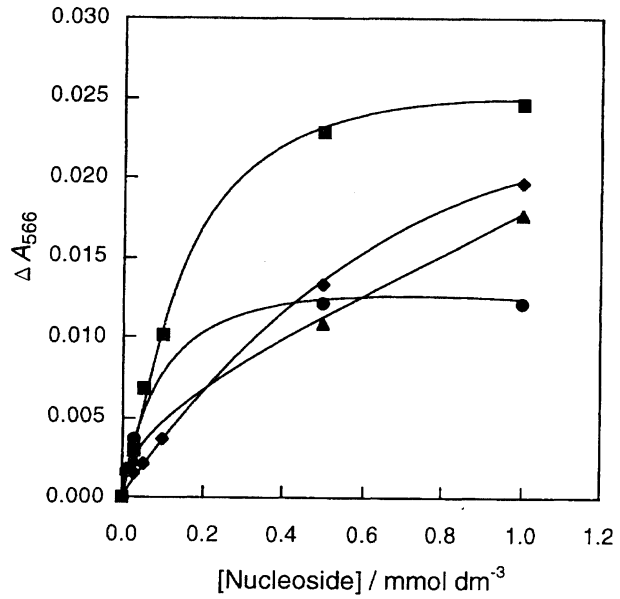


Fig. 6. Plots of ΔA_{566} vs. [nucleoside] for **1** at pH 8.0, adenosine (●), guanosine (■), cytidine (▲), uridine (◆).

OH^-, which changes unstable sp^2-hybridized boronate esters R—B(O)(O) to stable sp^3-hybridized ones R—(HO)B$^-$(O)(O) [6—11] Then, why dose the order of guanosine and cytidine reverse between pH 8.0 and 10.5? It is known that guanosine and uridine have an acidic proton which is dissociated at physiological pH region (pK_a 9.42 for guanosine and 9.38 for uridine).[20] Conceivably, the K_c values at pH 10.5 are primarily enhanced by the contribution of OH^- which stabilizes the boronic acid–diol interaction, but the additional stabilization effect arising from the stacking interaction is lessened in

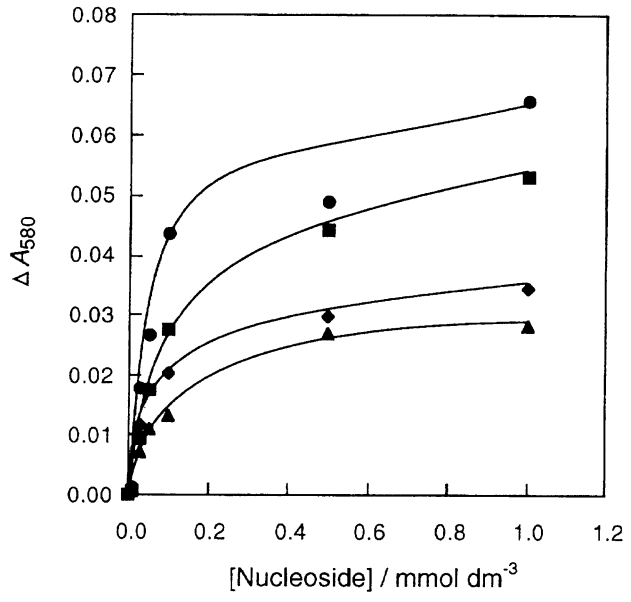


Fig. 7. Plots of ΔA_{580} vs. [nucleoside] for **2** at pH 10.5, adenosine (●), guanosine (■), cytidine (▲), uridine (◆).

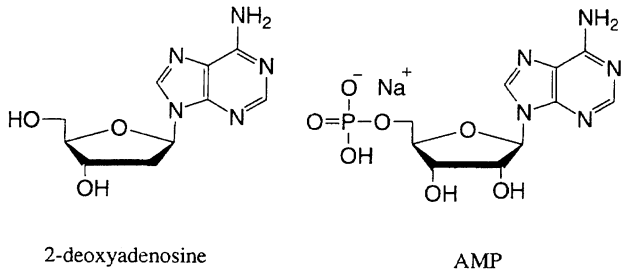
Table 1. Complexation Constants (10^{-4} K_c/dm^3 mol^{-1})[a)]

Guest	**1** at pH 10.5	**1** at pH 8.0	**2** at pH 10.5
Adenosine	6.16	1.26	1.01
Guanosine	2.01	0.62	0.55
Cytidine	2.30	0.30	0.97
Uridine	1.25	0.14	0.70
AMP	2.04		
2-Deoxyadenosine	ND[b)]		
3	0.78		

a) [**1**] = [**2**] = 1.00× 10^{-6} mol dm^{-3}, 25 °C, 0.03 vol% methanol. The pH was adjusted with 50 mmol dm^{-3} carbonate buffer for 10.5 and 50 mmol dm^{-3} phosphate buffer for 8.0. b) The spectral change was not detected.

guanosine and uridine because of the conversion of the hydrophobic neutral purine moiety to the less hydrophobic anionic one. In the association with theophylline, Mollica and Connors[17)] found that conversion of the neutral theophylline molecule to its anion reduces its association tendency with methyl *trans*-cinnamate (from K_c = 24 to 12 dm^3 mol^{-1}).

In order to specify which OH groups among the three OH groups in D-ribofuranoside are used for complexation with boronic acid, we compared the data with those of AMP and 2-deoxyadenosine (Chart 5). In AMP, 5-OH is phosphorylated, so that 2,3-diol is the sole, remaining complexation site. In 2-deoxyadenosine, 2-OH is depleted, so that boronic acid should form a complex with 3,5-diol. As show in Fig. 5 and Table 1, the K_c for AMP is approximately one-third of that for adenosine. The decrease is fully rationalized by the enhanced hydrophilicity caused by the phosphate group. In contrast, 2-deoxyadenosine did not induce any spectral change, indicating that 2-OH plays a crucial role in the complexation. These findings clearly support the view that *boronic acid forms a covalently-bonded complex with 2,3-diol.*



2-deoxyadenosine AMP

Chart 5.

Next, we compared the data with those of **2** and **3** in order to estimated the contribution of the hydrophobic and/or π–π stacking interactions. The K_c values for **2** were determined as those for **1** (see Fig. 7). On the other hand, **3** has no chromophoric group useful for the K_c determination. We thus employed a substitution method for the determination of the K_c. Firstly, a solution containing **1** (1.00 × 10^{-6} mol dm^{-3}) and adenosine (1.00 mmol dm^{-3}) was prepared at pH 10.5 and 25 °C. Judging from the K_c value **1** should exist as an adenosine complex under these conditions. To this solution was added **3** (1.00—500 mmol dm^{-3}). From the decrease in ΔA_{580}, the K_c for **3** could be computed assuming a 1 : 1 substitution between adenosine and **3** (Fig. 8). The results are recorded in Table 1. Based on the examination of Table 1, one can raise several interesting insights into the binding manner. As mentioned in the Introduction, in the **1**·nucleoside complexes the nucleoside base can overlap with the dimethylaminophenylazo moiety, whereas in the **2**·nucleoside complexes it cannot. The difference is well reflected by the K_c values at pH 10.5. The K_c values for **1** are greater than those for **2** by 6.1, 3.6, 2.3, and 1.8 fold, respectively, for adenosine, guanosine, cytidine, and uridine. It is also reasonable that the augmentation of adenine and guanine bearing the greater heteroaromatic ring area is greater than that of cytosine and uracil. More straightforward evidence for the contribution of the hydrophobic and/or π–π stacking interactions can be obtained from the K_c for **3**: It is smaller by 1.6—7.9 fold than those of four nucleosides.

The foregoing findings support the view that the association between **1** and nucleosides is primarily due to the boronic

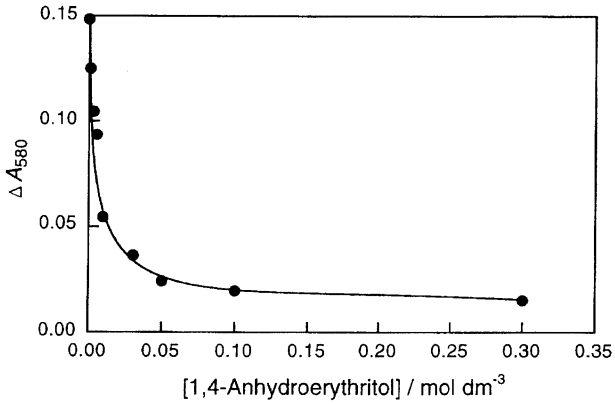


Fig. 8. Decrease in ΔA_{580} induced by added **3**.

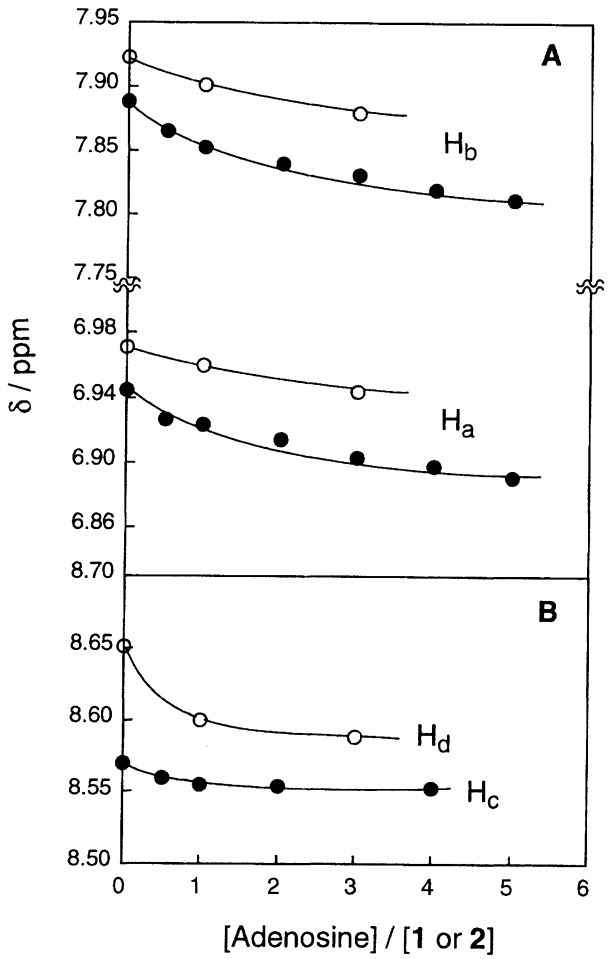
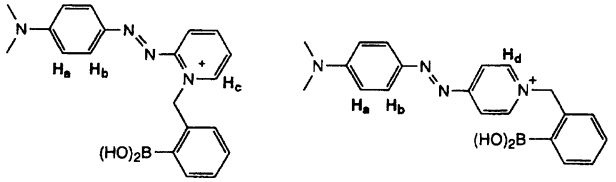


Fig. 9. Chemical shift changes induced by added adenosine (25°C, 400 MHz, D_2O:DMSO-d_6 = 9:1v/v, pD 10.9 with NaOD, [**1** or **2**]=1.00× 10^{-3} mol dm^{-3}): (A) azophenyl protons (H_a and H_b) for **1** (●) and **2** (○), (B) pyridinium 2-H protons (H_c and H_d) for **1** (●) and **2** (○).



acid–diol (2,3-diol) interaction and secondarily stabilized by the hydrophobic and/or π–π stacking interactions only when the complex can take such an orientation that two aromatic rings overlap with each other.

^{1}H NMR Spectral Studies. To further corroborate that the dimethylaminophenylazo moiety in **1** overlaps with the heteroaromatic moiety in nucleosides, we measured the ^{1}H NMR spectra of the adenosine complexes (25 °C, 400 MHz, D_2O : DMSO − d_6 = 9 : 1 v/v, pD 10.9 with NaOD, [**1** or **2**] = 1.00× 10^{-3} mol dm^{-3}). As shown in Fig. 9A, the azophenyl protons (H_a and H_b) shift to higher magnetic field with increasing adenosine concentration and the shift for **1** occurs more conspicuously than that for **2**. As shown in Fig. 9B, on the other hand, the 2-H protons in the pyridinium ring (H_c and H_d) also shift to higher magnetic field, but the shift for **2** occurs more conspicuously than that for **1**. These spectral data consistently support the view that in the **1**·adenosine complex the adenine ring mainly overlaps with the azophenyl moiety, whereas in the **2**·adenosine complex it mainly overlaps with the pyridinium ring. This view is in good agreement with that proposed on the basis of CPK molecular model examination (Fig. 1).

Conclusions. The present study has demonstrated that pre-organization of a boronic acid function as a *cis*-diol binder and a hydrophobic plane as a purine base binder creates an excellent nucleoside receptor like **1**. In particular, when the hydrophobic plane is chromophoric, one can readily monitor the complexation process by a spectroscopic method. In the present system, we could achieve the large complexation constants (ca. 10^4 dm^3 mol^{-1}) as well as the satisfactory difference in the complexation constants among nucleosides. However, the spectral change induced by the added nucleosides is relatively small. We are now trying to design new boronic-acid-based receptors which have a chromogenic or fluorescent group, expecting that the larger and more sensitive spectral changes can be induced by nucleosides.

Experimental

Compound 1. 2-(4-Dimethylaminophenylazo)pyridine was purchased from Tokyo Kasei. This compound (300 mg, 1.32 mmol) and 2-bromomethylphenylboronic acid (1.41 g, 6.60 mmol) were dissolved in DMF (40 ml). The mixture was stirred at room temperature for 10 h. The solution was evaporated to dryness and the residue was purified by reprecipitation from ethanol to hexane: Purple powder, mp 195.9—197.0 °C, yield 15 %; ^{1}H NMR (DMSO-d_6) δ_H = 3.27 (s, 6H), 6.33 (s, 2H), 6.85 (d, 1H), 6.99 (d, 2H), 7.32 (m, 2H), 7.88 (m, 4H), 8.16 (d, 1H), 8.45 (t, 1H), 8.83 (d, 1H); IR (KBr) 3600—3200 (O–H), 1600 (C=C), 1320 (B–O), 1440 cm^{-1} (N=N). Found: C, 53.21; H, 4.94 ; N, 12.09 %. Calcd for $C_{20}H_{22}BBrN_4O_2 \cdot 0.6H_2O$: C, 53.10; H, 5.13; N, 12.39 %.

4-(4-Dimethylaminophenylazo)pyridine.[21] 4-(4-Dimethylaminophenylazo)pyridine was obtained by coupling diazotized 4-aminopyridine with *N*,*N*-dimethylaniline in phosphoric acid. A solution of 2.0 g (21.2 mmol) of 4-aminopyridine in 10 ml of 85 % phosphoric acid and 5 ml of concentrated nitric acid were mixed at 0 °C. Sodium nitrite (1.47 g, 0.02 mol) and 25 g of ice were then added successively. The solution was poured into 20 ml of 30 % phosphoric acid containing 2.58 g (21.2 mmol) of *N*,*N*-dimethylaniline. After the reaction, the solution was neutralized with sodium carbonate, and the precipitate was filtered off and recrystallized from a methanol–water solution. The crystals were orange, mp 207.5—208.9 °C (lit,[19] mp 207—209 °C), yield 35 %; ^{1}H NMR ($CDCl_3$) δ_H = 3.12 (s, 6H), 6.75 (d, 2H), 7.64 (d, 2H), 7.91 (d, 2H), 8.70 (d, 2H); IR (KBr) 1600 (C=C), 1580 (C=N), 1360 cm^{-1} (C–N). Found: C, 68.83; H, 6.24; N, 24.62 %. Calcd for $C_{13}H_{14}N_4$: C, 68.99; H, 6.25; N, 24.72 %.

Compound 2. This compound was synthesized from 4-(4-dimethylaminophenylazo)pyridine and 2-bromomethylphenylboronic acid in a manner similar to that described for **1**: Mp 213.1—214.9 °C, yield 68 %; ^{1}H NMR (DMSO-d_6) δ_H = 3.23 (s, 6H), 5.97 (s, 2H), 6.99 (d, 2H), 7.31 (d, 3H), 7.81 (d, 1H), 7.93 (d, 2H), 8.12 (d, 2H), 8.47 (bs, 2H), 8.91 (d, 2H); IR (KBr) 3500—3100 (O–H), 1340 cm^{-1} (B–O). Found: C, 54.13; H, 5.19; N, 12.37 %. Calcd

for $C_{20}H_{22}BBrN_4O_2 \cdot 0.1H_2O$: C, 54.22; H, 5.06; N, 12.65 %.

Absorption Spectroscopic Measurements. To measure the absorbance with high precision we used a dual-wavelength spectrophotometer (Shimadzu UV-3000). In phototitration of **1** the medium pH was controlled with 50 mmol dm^{-3} acetate buffer for pH 3.5—5.5, 50 mmol dm^{-3} phosphate buffer for pH 6.8—8.0, 50 mmol dm^{-3} carbonate buffer for pH 8.5—10.5, and 50 mmol dm^{-3} phosphate buffer for pH 11.0—12.5.

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