HETEROCYCLES, Vol.. 60, No. 6, 2003, pp. 1441 - 1445
Received, 3rd March, 2003, Accepted, 17 April, 2003, Published online, 21st April, 2003

# DESIGN AND SYNTHESIS OF A NOVEL CYCLOPHANE AS HOST FOR BIOLOGICALLY RELEVANT PHOSPHATES

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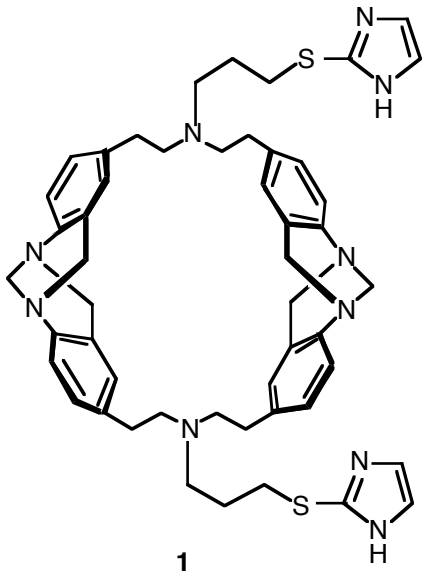
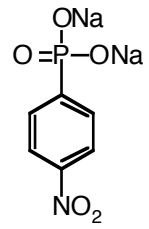
**Abstract** – A novel water-soluble cyclophane bearing 4-dimethylamino-pyridinium groups on the alkyl chains as blanches of 6*H*,12*H*-5,11-methanodibenzo-[*b*,*f*][1,5]diazocine skeleton was synthesized in order to investigate the ability as macrocyclic enzyme models to incorporate biologically relevant phosphates.

The design and synthesis of macrocyclic ring systems play an important role in host-guest chemistry.[1] Cyclophane, which forms inclusion complexes with nucleotides, has been subjected of intensive synthetic studies[2] since nucleotides play a central role in the cellular process of metabolism and replication.[3] We have recently designed and synthesized a water-soluble cyclophane having 6*H*,12*H*-5,11-methano-dibenzo[*b*,*f*][1,5]diazocine[4] skeleton, represented by host (**1**) shown below, as host compounds to trap organic guests such as 4-nitrophenyl phosphate (NPP) and 4-nitrophenol (NP) by hydrophobicity of their aromatic ring groups and electrostatic interactions of mercaptoimidazole groups in acidic solution (0.1M KCl-DCl buffer solution at pD1.4) (Figure 1). It found that cyclophane (**1**) works as host that form complexes selectively with aromatic phosphates as guests and NPP has stronger interactions with 2-mercaptoimidazole groups than NP. Although host (**1**) is dissolved in acidic aqueous solution, it does not dissolve in neutral aqueous solution. The acidic condition (pD 1.4) required to solubilize the host should limit its application. Therefore it is highly desirable to make it soluble in neutral aqueous solution. We expected that introduction of 4-dimethylaminopyridinium groups will be able to bind to the phosphate group by electrostatic interaction and also lead to remedy the solubility of cyclophane. In this report we wish to report the modification of host (**1**) by introducing 4-dimethylaminopyridinium group on the alkyl bridge as blanches of 6*H*,12*H*-5,11-methanodibenzo[*b*,*f*][1,5]diazocine skeleton in order to characterize this artificial host as a receptor for biologically relevant phosphates in their natural environment. The modified host (**3**) was synthesized as shown in Scheme 1.

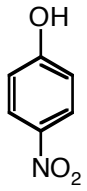
The modified host (**3**) was soluble in neutral aqueous solution (pD 6.8). The structure of the cyclophane (**3**) was confirmed by $^1$H NMR spectra, MS spectra, and elemental analysis.



**1**

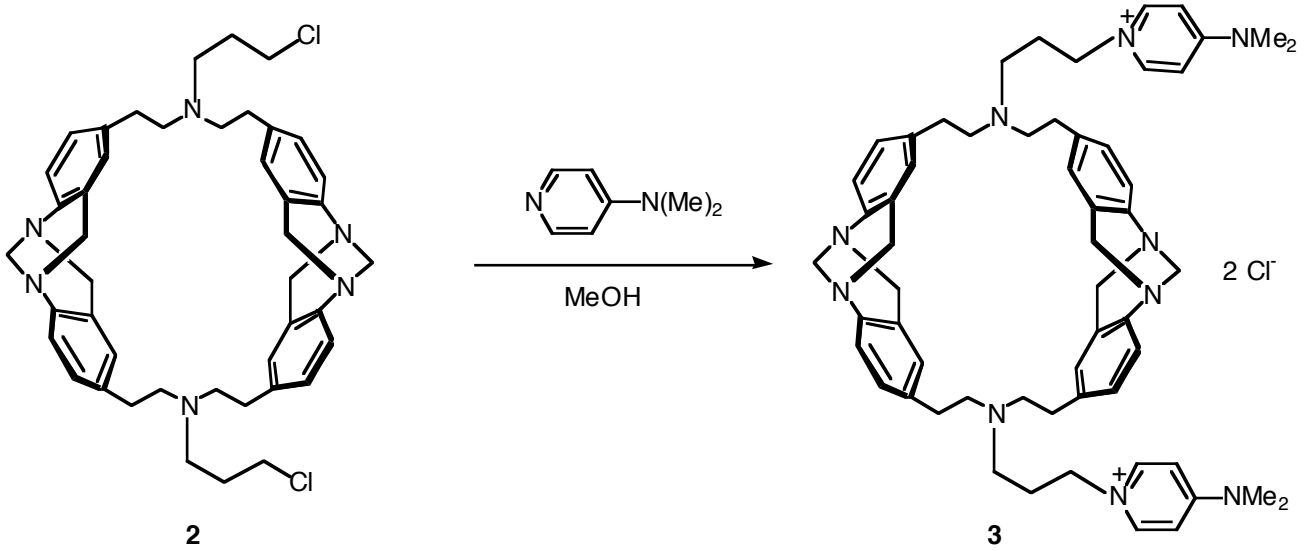


**NPP**



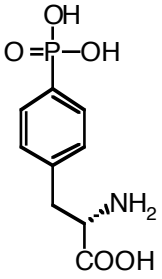
**NP**

**Figure 1.**

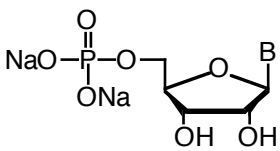


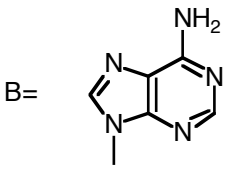
**2** **3**

**Scheme 1.**

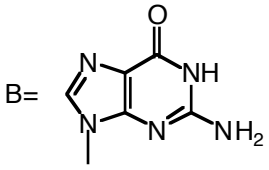


**PPT**

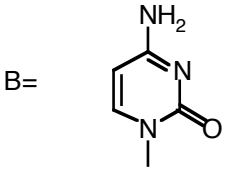




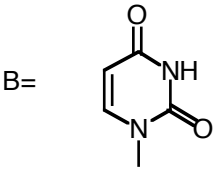
**AMP**



**GMP**



**CMP**



**UMP**

**Figure 2.**

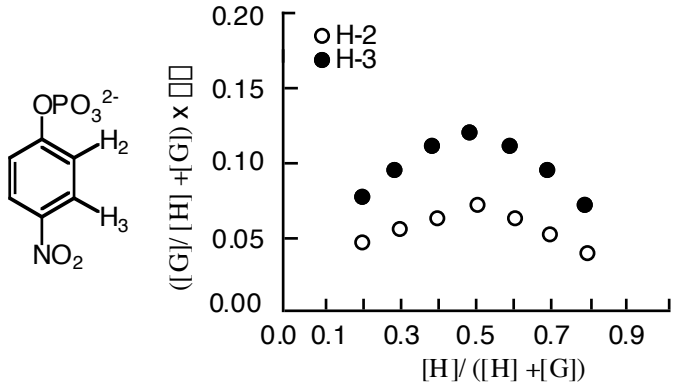



**Figure 3.** Job plots for the formation of a complex between host (**3**) and NPP in 0.1 M phosphate buffer (pD 6.8) at 303 K.

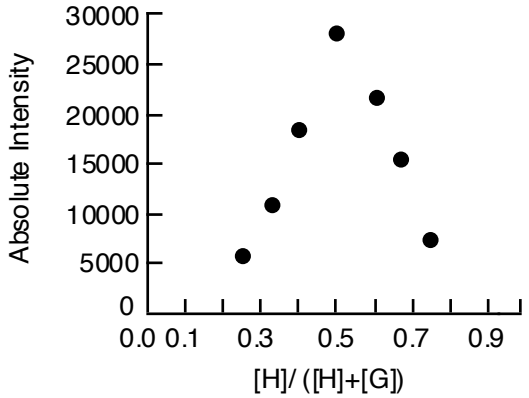



**Figure 4.** Job plot for the formation of a complex between host (**3**) and AMP in 0.02 M triethylamine acetate buffer (pH 7.0).

Examinations on the complex formation of **3** with NPP, *O*-phospho-L-tyrosine (PPT), guanosine 5'-monophosphate disodium salt (GMP), adenosine 5'-monophosphate disodium salt (AMP), cytidine 5'-monophosphate disodium salt (CMP), and uridine 5'-monophosphate disodium salt (UMP) were made by $^1$H NMR spectrometry in 0.1 M phosphate buffer solution at pD 6.8 below the critical micelle concentration (CMC) of **3**.[5] Marked upfield shifts of the aromatic protons of NPP were observed. Signals of the protons (□□) [6] at C-2 and C-3 are shifted upfield in the magnitudes of 0.4 and 0.8 ppm, respectively. The phenomena can be ascribable to a strong intermolecular shielding effect due to the aromatic rings of **3**, and the 1:1 inclusion complex was confirmed by Job's method of continuous variations (Figure 3).[7] Dissociation constant (Kd) was calculated on the basis of non-linear curve fitting procedure with the least squares method using the host-induced upfield shifts of the guest proton signals. The Kd value of the complex was 0.0076 M. Upon the complexation of **3** with PPT, small upfield shifts of the aromatic protons of PPT at C-2 (0.018 ppm) and C-3 (0.013 ppm) were observed (Kd = 0.0017). On the other hand, in the case of using GMP as a guest, signals of the protons (□□) at C-8 and C-1' are shifted upfield in the magnitudes of 0.076 and 0.08 ppm, respectively. The Kd value was 0.0024 M and in the case of using CMP and UMP as guests, those chemical shift changes (□□) at C-5, C-6 and C-1' were also observed (CMP: 0.043, 0.04 and 0.044 ppm) and (UMP: 0.062, 0.051, and 0.062 ppm), respectively and those Kd values were 0.0093 M and 0.0088 M, respectively. However, in the case of using of AMP as a guest under same conditions, the signal of proton at C-1' of AMP exhibited upfield shifts with broadning and the signals of aromatic protons were not detected in the presence of host (**3**). Therefore, it was impossible to confirm a complex formation AMP and **3**, and then MS (FAB) was measured using glycerol as a matrix in order to confirm the complex formation between **3** and AMP.[8] Ion peak for [(**3**-Cl-HCl)+(AMP-2Na+2H)]$^+$ was observed at 1254 (*m*/*z*) and molecular ion peaks of higher MS could not be detected. The MS (FAB) measurement suggests that **3** forms the 1:1 complex with AMP. The 1:1 complex stoichiometry for the complex between **3** and AMP was also confirmed by using Job's method of continuous variations using MS (FAB)[8] (Figure 4). The study described above clearly shows that inclusion and discrimination of hydrophobic guests in water by quaternary ammonium cyclophane is controlled mainly by the proper fit between the hydrophobic cavity of the host and the hydrophobic moiety of the guest. Further investigations on the molecular recognition properties of the novel water-soluble cyclophane (**3**) as synthetic receptor for the application of biomimetic studies are

under examination.

**EXPERIMENTAL**

Melting points were determined using a Yanagimoto Melting point Apparatus Yanaco MP and uncorrected. $^{1}$H- and $^{13}$C-NMR spectra were recorded on a JEOL JNM-LA400 spectrometer containing tetramethylsilane as standard. MS spectra were taken on a JEOL JMS-GCmate instrument. NPP, AMP, GMP, CMP, and UMP used in these experiments were purchased as disodium salts and PPT was used as free acid.

**Cyclophane 3**

A mixture of **2**[4] (*meso*-isomer 30 mg, 0.04 mmol) and 4-dimethylamiopyridine (11 mg, 0.09 mmol) in MeOH (1 mL) was refluxed for 24 h under $N_2$ atmosphere. After removal of the solvent under reduced pressure, the solid residue was triturated with EtOAc, and insoluble solid was collected by filtration to give a colorless solid 39 mg (100%). mp > 300°C. $^{1}$H NMR ($D_2O$ 0.1 M phosphate buffer (pD 6.8)) □: 1.75-1.88 (m, 4H), 2.04-2.11 (m, 4H), 2.18-2.25 (m, 4H), 2.65 (br s, 12H), 2.90 (s, 12H), 3.75-3.82 (m, 4H), 3.87 (d, 4H, *J* =17.1 Hz), 4.10 (s, 4H), 4.38 (d, 4H, *J* =17.1 Hz), 6.43 (s, 4H), 6.50 (d, 4H, *J* =7.6 Hz), 6.77 (d, 4H, *J* =8.3 Hz), 6.93 (d, 4H, *J* =8.3 Hz), 7.58 (d, 4H, *J* =7.6 Hz). MS (FAB) (*m/z*) 1001 $[M+Na]^+$. HRMS (FAB) (*m/z*) Calcd for $C_{58}H_{72}N_{10}Cl_2$: 1001.5216 $(M+Na)^+$, 1003.5186 $(M+Na+^{37}Cl)^+$, 1005.5156 $(M+Na+^{37}Cl_2)^+$. Found 1001.5220 $(M+Na)^+$, 1003.5189 $(M+Na+^{37}Cl)^+$, 1005.5159 $(M+Na+^{37}Cl_2)^+$.

Anal. Calcd for $C_{58}H_{72}N_{10}Cl_2$: C, 71.07; H, 7.40; N, 14.29. Found: C, 71.01; H, 7.69; N, 14.21.

**Determination of Kd Values of the Complexes**

The Kd values of the host-guest complexes were determined by $^{1}$H NMR spectra using the host-induced upfield shifts of the guest proton signals in 0.1 M phosohate buffer (pD 6.8) at 303 K. The concentration of the guest was 2.5 x $10^{-3}$ M, while those of the host **3** ranges from 6.25 x $10^{-4}$ M to 7.5 x $10^{-3}$ M (5 points). The non-linear curve fitting procedure with the least squares method was applied.

**Job Plots by $^{1}$H NMR Spectrum**

Equimolar solutions (5 x $10^{-3}$ M) of host and guest were prepared (0.1 M phosphate buffer (pD 6.8)) and mixed in various amounts. $^{1}$H NMR spectra of the mixture were recorded at 303 K, and the chemical shifts were analyzed by Job's method for $^{1}$H NMR results.

**MS (FAB) measurment for the complex between host and AMP**

For the MS (FAB) experiment host (**3**) (5 x $10^{-3}$ M in 2 □L $H_2O$), AMP (5 x $10^{-3}$ M in 2 □L $H_2O$), and glycerol (2 □L) as matrix in 0.02 M triethylamine acetate buffer (pH 7.0) (4 □L) were mixed. Then 2 □L of the mixture was loaded. Xe was employed as a fast atom bombardment gas. Scanning was performed from *m/z* 100 to 1500 in 10 s and several scans were summed to obtain the final spectrum.

**Job Plots by MS (FAB)**

Equimolar solutions (5 x $10^{-3}$ M) of host and guest in 0.02 M triethylamine acetate solution (pH 7.0) were prepared and mixed in various amounts ([H]:[G]=1:3, 1:2, 1:1.5, 1:1, 1.5:1, 2:1, and 3:1). Glycerol was used as matrix. FAB-MS spectra of the mixture were recorded, and the absolute intensities of the host-guest complex were analyzed by Job's method for FAB-MS results.

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4. M. Miyake and C. S. Wilcox, *Heterocycles*, 2002, **57**, 515.
5. $^1$H NMR spectra of the solutions of **3** in 0.1 M phosphate buffer (pD 6.8) were measured. Chemical shifts of all the protons did not change in concentration range of **3** from 1.25 x $10^{-3}$ M to 4.0 x $10^{-2}$ M. Therefore, the critical micelle concentration (CMC) of **3** was found to be not less than 4.0 x $10^{-2}$ M and all experiments were carried out below 4.0 x $10^{-2}$ M.
6. □□= □(host + guest) – □(guest). The magnitudes of □□ values are dependent on the ratio of the host to the guest.
7. a) P. Job, *Compt. Rend*, 1925, **180**, 928. b) M. T. Blanda, J. H. Horner, M. Newcomb, *J. Org. Chem.,* 1989**, 54**, 4626. c) Job's method of others guests (PPT, GMP, CMP and UMP) also gave a maximum at 0.5.
8 a) We reported that MS (FAB) measurment is useful for confirmation of host-guest complex formation. K. Metori, Y. Kimura, and M. Miyake, *J. Mass Spectrom. Soc. Jpn.,* 2002, **50**, 301. b) The Kd value data for AMP by use of MS spectrometry is under investigation.