A [2]rotaxane-based ^{1}H NMR spectroscopic probe for the simultaneous identification of physiologically important metal ions in solution†

Nai-Chia Chen,[a] Po-Yi Huang,[a] Chien-Chen Lai,[b] Yi-Hung Liu,[a] Yu Wang,[a] Shie-Ming Peng[a] and Sheng-Hsien Chiu*[a]

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We describe a [2]rotaxane molecule that exhibits distinct signals in its ^{1}H NMR spectra upon the complexation of physiologically important Li^+, Na^+, Mg^{2+} and Ca^{2+} ions; thus, the identification of these metal ions in solution is possible from the analysis of a single ^{1}H NMR spectrum of a single molecular sensor.

One area of research that is attracting a great deal of attention from synthetic chemists is the preparation of molecular sensors that bind specifically to a particular physiologically important metal ion, such as Li^+, Na^+, K^+, Mg^{2+} or Ca^{2+},[1] with subsequent changes in some measurable physical properties (*e.g.*, fluorescence).[2] Analyzing biological samples that contain these metal ions in such a manner would, however, require the use of mixture of molecular sensors. One possible method for which a single molecular sensor could be used for the simultaneous determination of physiologically important metal ions in solution is ^{1}H NMR spectroscopy—but only if the spectra of the metal ions bound to the receptor feature distinct signals for each type of complex.[3] Toward this goal, we became interested in using crown ether-like macrocycles to recognize 2,2′-bipyridine (BP) derivatives[4] because the resulting rotaxanes would have a 2,2′-bipyridine motif—a good binder for transition metal ions—in their threadlike component and oligo(ethylene glycol) chains—good binders for alkali metal ions—in their macrocyclic component; together, these units may collaborate orthogonally to introduce specific metal ion recognition properties. Herein, we describe a new molecular recognition system incorporating 2,2′-bipyridinium ($BP-H_2$) ions and crown ether-like macrocycles. The ^{1}H NMR spectra of the corresponding "neutral" [2]rotaxane recorded in the presence of a mixture of the physiologically important Li^+, Na^+, Mg^{2+} and Ca^{2+} ions feature distinct signals for each of the complexes, with the chemical shifts of pertinent probe protons identifying the chemical nature of each metal ion. That is to say, the identification of these metal ions in solution is possible from the analysis of a single ^{1}H NMR spectrum of a single molecular sensor.

Previously, we reported[5] that bis-*p*-xylyl[26]crown-6 (BPX26C6, Fig. 1) is capable of forming relatively strong complexes in solution with both dibenzylammonium (DBA^+) and 4,4′-bipyridinium ions through noncovalent interactions with the ethylene glycol chains and the π-electron-rich aromatic rings.[6] Thus, it seemed reasonable to us that BPX26C6 would also complex with dicationic 2,2′-bipyridinium ions ($BP-H_2^{2+}$), stabilized potentially through π-stacking and hydrogen bonding interactions (Scheme 1).

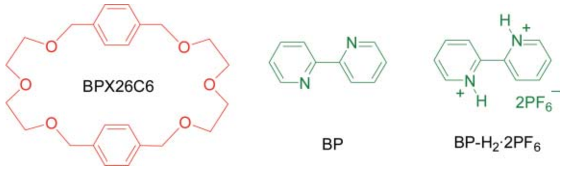
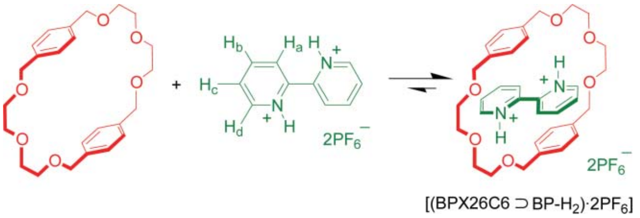


Fig. 1



Scheme 1 Formation of the pseudorotaxane complex.

The ^{1}H NMR spectrum of an equimolar mixture (40 mM) of BPX26C6 and 2,2′-bipyridinium bis(hexafluorophosphate) ($BP-H_2·2PF_6$)[7] in CD_3CN displays significant changes in the chemical shifts of the protons of the complex relative to those of its free components (Fig. 2). The observation of time-averaged signals in this spectrum suggests that the exchange rates for the complexation of BPX26C6 and $BP-H_2·2PF_6$ are fast under these conditions.[8] In the spectrum of the complex, the separation of the originally overlapping signals (at δ 3.62) for the protons of the ethylene glycol units of macroring BPX26C6 into two separate multiplets—shifted downfield to δ 3.64 and 3.73, respectively—in the presence of $BP-H_2·2PF_6$ suggests that hydrogen bonding probably occurs between the host and guest. The upfield shifts in the signals of the aromatic protons of both components imply the existence of interactions between the electronically complementary aromatic rings of BPX26C6 and the $BP-H_2^{2+}$ ion. A Job plot based on the ^{1}H NMR spectroscopic data obtained in CD_3CN provided conclusive evidence for 1 : 1 complexation (see the ESI†). Through ^{1}H NMR spectroscopic dilution experiments, we determined the association constants (K_a) for the complexation

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[a] *Department of Chemistry, National Taiwan University, Taipei, Taiwan, 10617, R.O.C. E-mail: shchiu@ntu.edu.tw; Fax: +886 2 33661677; Tel: +886 2 33661675*
[b] *Institute of Molecular Biology, National Chung Hsing University and Department of Medical Genetics, China Medical University Hospital, Taichung, Taiwan, R.O.C*

† Electronic supplementary information (ESI) available: Experimental procedures for the preparation of the [2]rotaxanes, their characterization data and complexation behaviour. See DOI: 10.1039/b706461f

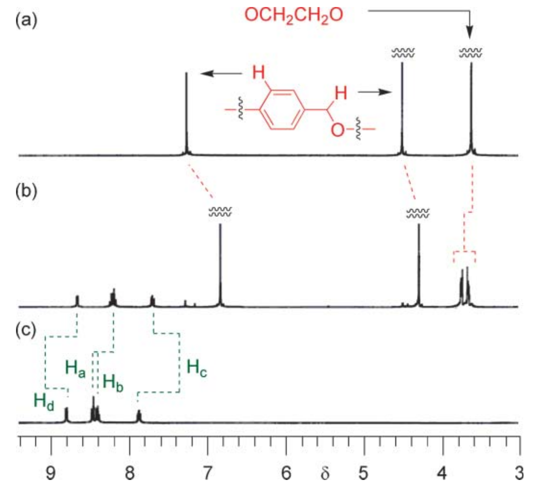


Fig. 2 Partial ^{1}H NMR spectra (400 MHz, CD_3CN, 298 K) of (a) BPX26C6, (b) an equimolar mixture (40 mM) of BPX26C6 and BP-H_2·$2PF_6$ and (c) BP-H_2·$2PF_6$.

of BPX26C6 with BP-H_2·$2PF_6$ to be 67 and 260 M^{-1} in CD_3CN and $CD_3CN/CDCl_3$ (1 : 1), respectively,[8] suggesting that hydrogen bonding may play a more important role than does π-stacking in this new molecular recognition system.

Adding 2 equiv. of triethylamine (Et_3N) to an equimolar (40 mM) mixture of BPX26C6 and BP-H_2·$2PF_6$ in CD_3CN led to the appearance in the ^{1}H NMR spectrum (Fig. 3(b)) of the characteristic absorptions of the free macrocycle, indicating that dissociation accompanies deprotonation of the complex (BPX26C6$\supset$BP-H_2)·$2PF_6$. Subsequent addition of an excess of trifluoroacetic acid (TFA) to the solution resulted in a ^{1}H NMR spectrum (Fig. 3(c)) similar to that of the original solution of macrocycle BPX26C6 and BP-H_2·$2PF_6$ (Fig. 3(a)); *i.e.*, the original [(BPX26C6$\supset$BP-H_2)]$^{2+}$ complex had regenerated. Thus, this supramolecular system behaves as an acid/base-controllable molecular switch in solution as a result of its threading (association) and dethreading (dissociation) processes being reversibly controllable through the sequential addition of excess amounts of Et_3N and TFA.[9]

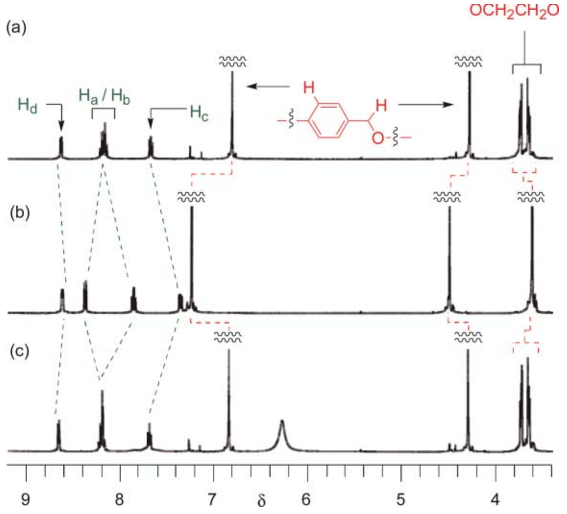
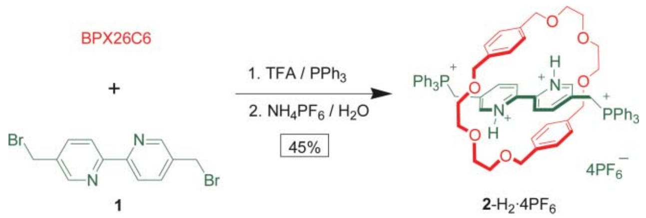


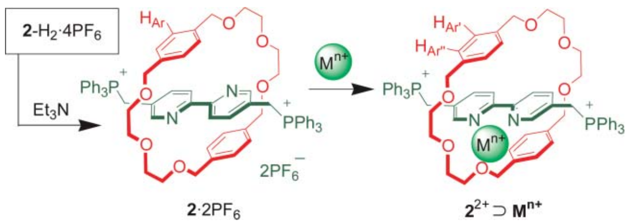
Fig. 3 Partial ^{1}H NMR spectra (400 MHz, CD_3CN, 298 K) of (a) an equimolar mixture (40 mM) of BPX26C6 and BP-H_2·$2PF_6$, (b) the mixture obtained after adding Et_3N (2 equiv.) to the solution in (a), and (c) the mixture obtained after adding TFA (4 equiv.) to the solution in (b).

To prove unambiguously the existence of the pseudorotaxane (BPX26C6$\supset$BP-H_2)$^{2+}$ in solution, we synthesized a corresponding [2]rotaxane from this molecular recognition system. Thus, the addition of triphenylphosphine (300 mM) to a solution of the dibromide **1**[10] (100 mM), BPX26C6 (150 mM) and TFA (400 mM) in CH_3NO_2 led to the isolation of the corresponding [2]rotaxane **2**-H_2·$4PF_6$ in 45% yield after ion exchange (NH_4PF_6/H_2O) and column chromatography (Scheme 2).[11]



Scheme 2 Synthesis of the [2]rotaxane.

Addition of Et_3N to a CH_3CN solution of the [2]rotaxane **2**-H_2·$4PF_6$ deprotonated the bipyridinium center; subsequent crystallization provided the "neutral" rotaxane **2**·$2PF_6$ (Scheme 3). The signal of the aromatic protons of the interlocked BPX26C6 moiety in the deprotonated [2]rotaxane **2**·$2PF_6$ appears in the ^{1}H NMR spectrum as a singlet at δ 6.45 (H_{Ar}; Scheme 3 and Fig. 4(a)); after complexation with Li^+, Na^+, Mg^{2+} or Ca^{2+} ions, however, this signal shifts upfield and appears as an AB quartet ($H_{Ar'}$ and $H_{Ar''}$; Scheme 3 and Fig. 4(b)–(e)), implying that circumrotation of the BPX26C6 unit about the dumbbell-shaped moiety had slowed, or stopped, through coordination of the metal ion to the bidentate BP unit and to the oxygen atoms of one of the diethylene glycol loops of the BPX26C6 moiety. The solid-state structure of the ($\mathbf{2}\supset$Na)$^{3+}$ complex[12] (Fig. 5) confirms this proposed complexation geometry, with an acetonitrile molecule positioned as the sixth ligating group. In contrast, the complexation of K^+ ions by **2**·$2PF_6$ under similar conditions resulted in time-average signals in the corresponding ^{1}H NMR spectrum (Fig. 4(f)); *i.e.*, this system exhibits relatively rapid complexation and decomplexation kinetics, possibly because of a steric mismatch between the relatively large K^+ ion and the pocket formed by the BP and diethylene glycol units of the interlocked molecule.[13]



Scheme 3 Binding metal ions to the "neutral" [2]rotaxane.

Because of the slow exchange kinetics of the complexation process manifested in the ^{1}H NMR spectra and the unique chemical shifts of the aromatic protons of the interlocked BPX26C6 macrocycle when the [2]rotaxane **2**·$2PF_6$ formed complexes with the various metal ions, we were curious to determine whether this rotaxane could be used to identify the

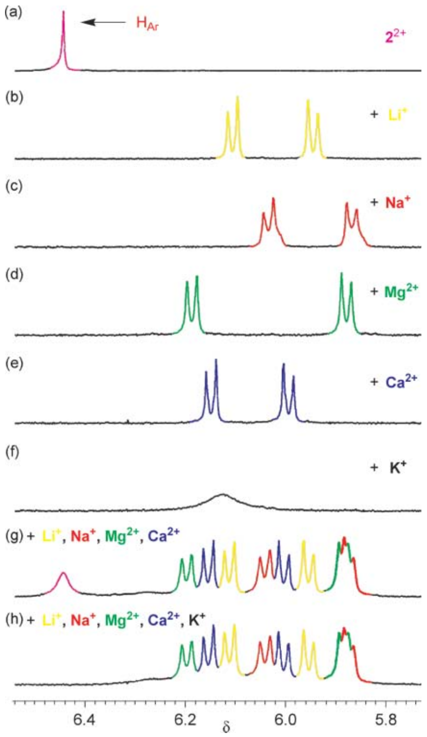


Fig. 4 Partial ^1H NMR spectra (400 MHz, CD_3CN, 298 K): (a) $\mathbf{2}^{2+}$; (b)–(f) equimolar mixtures of $\mathbf{2}^{2+}$ with Li^+, Na^+, Mg^{2+}, Ca^{2+} and K^+, respectively (4 mM); (g) $\mathbf{2}^{2+}$ (20 mM) mixed with *ca.* 0.2 equiv. of Li^+, Na^+, Mg^{2+} and Ca^{2+}; (h) *ca.* 0.2 equiv. of K^+ added to the solution in (g). For solubility reason, K^+ ions were added as the hexafluorophosphate salt; other metal ions were added in the form of perchlorate salts.

components of a mixture of Li^+, Na^+, Mg^{2+} and Ca^{2+} ions in solution. Gratifyingly, the ^1H NMR spectrum obtained after mixing a CD_3CN solution of these four metal ions with a CD_3CN solution of $\mathbf{2}\cdot 2PF_6$ clearly displayed the signals for each of the four different metalated [2]rotaxane complexes in addition to excess free $\mathbf{2}\cdot 2PF_6$ (Fig. 4(g)).[14] Under the same conditions, we observed no signal for free $\mathbf{2}\cdot 2PF_6$ in the ^1H NMR spectrum when K^+ accompanied the other four metal ions in the solution (Fig. 4(h)). Thus, the [2]rotaxane $\mathbf{2}\cdot 2PF_6$ might serve as a single-spectrum ^1H NMR-based sensor for biological samples that contain predominantly Li^+, Na^+, K^+, Mg^{2+} and Ca^{2+} ions.

We have demonstrated that the macrocycle BPX26C6 can form a [2]pseudorotaxane-like complex with the protonated 2,2′-bipyridinium ion in solution. The [2]rotaxane synthesized from this supramolecular recognition system possesses the ability to form complexes with physiologically important Li^+, Na^+, K^+, Mg^{2+} and Ca^{2+} ions, which can be identified uniquely from a single ^1H NMR spectrum of their mixture. In addition to use as an analytical sensor, we believe that suitably designed [2]rotaxanes containing such molecular recognition sites will have the potential to behave as interlocked organometallic catalysts or as interlocked building blocks for the construction of acid/base-controllable molecular architectures or aggregates; such applications are currently under investigation in our laboratory.

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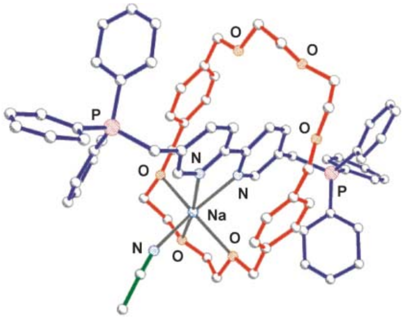

Fig. 5 Ball-and-stick representation of the solid state structure of the sodiated [2]rotaxane $[(\mathbf{2}\supset Na)\cdot MeCN]^{3+}$.

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12 *Crystal data* for $[(\mathbf{2}\supset Na)\cdot MeCN\cdot 0.5H_2O\cdot 0.5MeOH][PF_6]_3$: $[C_{74.5}H_{78}O_7N_3P_2Na][PF_6]_3$; M_r = 1647.24, triclinic, space group $P\bar{1}$, a = 10.2597(2); b = 14.8941(2); c = 26.3207(4) Å; V = 3885.98(11) $Å^3$; D_c = 1.408 g cm^{-3}, μ(Mo-Kα) = 0.221 mm^{-1}; T = 200(2) K; colorless plates; 13 666 independent measured reflections; F^2 refinement; R_1 = 0.1196; wR_2 = 0.3203. CCDC 640451. For crystallographic data in CIF or other electronic format see DOI: 10.1039/b706461f.
13 Complexation of the more-sizable Cs^+ ions to the [2]rotaxane $\mathbf{2}\cdot 2PF_6$ under the same conditions also appeared to exhibit fast exchange kinetics in the corresponding ^1H NMR spectrum.
14 No color change detectable to the naked eye was observed under these conditions.