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Headline Articles

Anisotropic Ring Current Effect of *p*-Nitrophenolate Ion Inclusion on the ^{1}H NMR Signals of the Pyridinio Derivatives of α-Cyclodextrin

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The *p*-nitrophenolate ion (pNP$^-$) was strongly bound to the mono- and dipyridinio derivatives of α-cyclodextrin (α-CD) in an aqueous 0.1 mol dm^{-3} Na_2CO_3 solution, and shifted the ^{1}H NMR signals due to the α-CD C_5-H's to different directions, depending on the position of α-D-glucopyranose (anisotropic ring-current effect). In contrast, the iodide ion and *p*-hydroxybenzoate ion showed no such effect upon complexation. Furthermore, the C_5-H's of a dipyridinio derivative of β-CD was not subject to such an anisotropic ring-current effect of pNP$^-$. These results indicate that the molecular rotation of pNP$^-$ is significantly retarded within the cavities of the pyridinio derivatives of α-CD by electrostatic and van der Waals interactions. Thus, electrostatic interactions are available for controlling the molecular motion or orientation of an ionic guest compound in a CD cavity and for the design of an enzyme-mimic modified CD.

Cyclodextrin (CD) is a cyclic oligomer composed of six (α-CD), seven (β-CD), eight (γ-CD), or more α-D-glucopyranose (GP) units. CD provides a hydrophobic interior cavity into which a variety of molecules and ions can be trapped to form inclusion complexes in an aqueous solution. The structures and dynamics of CD complexes with guests have been extensively examined by means of high-resolution NMR spectroscopy.[1,2] It is well known that the complexation of CD with an aromatic molecule causes significantly higher-field shifts in the ^{1}H NMR chemical shifts (δ) of the C_5- and/or C_3-H's,[3–5] which locate in the interior cavity of CD. This is due to a ring-current effect of the included aromatic guest. In such a case, each of the C_3-H's or C_5-H's on the different GP residues of CD generally receives an identical shielding contribution from the included guest because of various averaging factors. The most effective one is that the molecular rotation of a guest within the CD cavity, together with the association-dissociation process of CD complexation, is rapid on the NMR time-scale basis.[5] The authors recently found in a preliminary experiment[6] that the negatively charged *p*-nitrophenolate ion (pNP$^-$) is strongly bound to a positively charged host, bis[6^A,6^D-(1-pyridinio)-6^A,6^D-deoxy]-α-CD (A,D-α-CDpy$_2$), in an aqueous 0.1 mol dm^{-3} Na_2CO_3 solution, and shifts the ^{1}H NMR signals due to the C_5-H's of A,D-α-CDpy$_2$ to different directions, depending on the position of GP. This anisotropic ring-current effect of pNP$^-$ suggests that the molecular rotation of the anionic guest is retarded in the CD cavity by electrostatic interactions with the cationic host. There have been few reports on the regulation of molecular rotation of a guest within a CD cavity.[7–9] In the present study, we examined the effects of the complexation of a few anionic guests, including pNP$^-$, on ^{1}H NMR signals of A,D-α-CDpy$_2$. Furthermore, we also examined the effects of pNP$^-$ on ^{1}H NMR signals of the other pyridinio derivatives of CD's including mono[6-(1-pyridinio)-6-deoxy]-α-CD (α-CDpy), bis[6^A,6^B-(1-pyridinio)-6^A,6^B-deoxy]-α-CD (A,B-α-CDpy$_2$), bis[6^A,6^C-(1-pyridinio)-6^A,6^C-deoxy]-α-CD (A,C-α-CDpy$_2$), and bis-[6^A,6^D-(1-pyridinio)-6^A,6^D-deoxy]-β-CD (A,D-β-CDpy$_2$), as illustrated in Fig. 1, to elucidate the effects of the number and position of the pyridinio group(s) and the cavity size of CD on the molecular rotation of pNP$^-$ in the CD cavity.

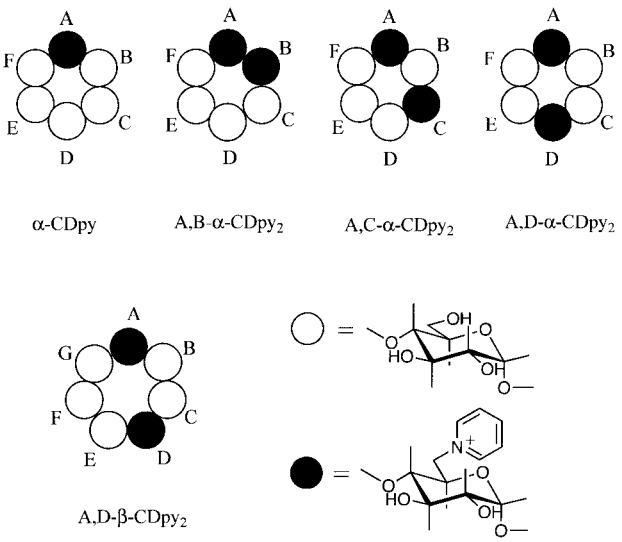

Fig. 1. The pyridinio derivatives of α- and β-CD's examined.

Experimental

Materials. The α- and β-CD were supplied by Nihon Shokuhin Kako Co., Ltd. and Bio Research Corporation of Yokohama, Ltd, respectively. They were dried overnight in vacuo at 110 °C. Reagent-grade pyridine was dried over CaH_2 and distilled in the presence of CaH_2 before use. Reagent-grade *p*-nitrophenol (*p*NP), *p*-hydroxybenzoic acid (*p*HBA), and KI were purchased from Wako Pure Chemical Industries Ltd. and used without further purification. The HCO_3^- salts of the pyridinio derivatives of α- and β-CD's were prepared by refluxing solutions of the corresponding 6-*O*-arylsulfonated CD's in dry pyridine, followed by elution of the products from a column of CM-cellulose (50 × 550 mm, Serva) with aqueous NH_4HCO_3, as previously described.[10] The D_2O (Isotec) used for ^{1}H NMR measurements was the grade of 99.9 atm% D.

Apparatus. The UV/Vis spectra of *p*NP–CD systems were recorded using a Shimadzu UV-2100 UV/Vis spectrophotometer equipped with a temperature-controlled cell holder. The ^{1}H NMR spectra were recorded on a JEOL Model JNM-A400 FT NMR spectrometer (400 MHz) with a sample tube of 5.0 mm diameter at 298 K. Sample solutions contained about 10 mmol dm^{-3} guest and/or host in D_2O containing 0.1 mol dm^{-3} Na_2CO_3, unless otherwise noted. A trace amount of methanol (0.4 mmol dm^{-3}) was added to the sample solutions as an internal reference ($\delta = 3.343$[11]) of ^{1}H NMR measurements. The phase-sensitive ROESY (rotating-frame nuclear Overhauser enhancement spectroscopy) spectra of the hosts and their inclusion complexes with *p*NP$^-$ were acquired with a mixing time of 500 ms and 512 × 256 data points, followed by zero-filling. The one-dimensional (1D) HOHAHA (homonuclear Hartmann–Hahn spectroscopy) spectra were obtained with a mixing time of 150 ms.

Spectrophotometric Determination of Binding Constants for *p*NP Complexes with α-CD's. The binding constants (K_a) for the complexation of *p*NP with native α-CD and its pyridinio derivatives were determined by UV/Vis spectroscopy in 0.1 mol dm^{-3} Na_2CO_3 (pH 11.3) and in 0.1 mol dm^{-3} KH_2PO_4–Na_2HPO_4 buffer (pH 5.6) at 298 K. The concentration of *p*NP was 0.05 mmol dm^{-3}. The complexation was observed at 421 nm for sample solutions at pH 11.3 and at 413 nm for those at pH 5.6. The K_a values for the host–guest complexes were determined by a nonlinear least-squares curve-fitting analysis of changes in the absorbance of *p*NP with the host concentration, based upon an assumption of 1:1 complexation. The thus-calculated curves were well-fitted to the observed data with correlation coefficients greater than 0.999.

Assignments of ^{1}H NMR Signals of A,D-α-CDpy$_2$. The ^{1}H NMR signals of A,D-α-CDpy$_2$ were assigned by combined measurements of 2D COSY, 2D ROESY, and 1D HOHAHA spectra,[1,2,12] together with a measurement of the 1D spectra during inversion-recovery with a pulse sequence of π–τ–$\pi/2$ (τ: pulse interval). A,D-α-CDpy$_2$ has a two-fold symmetry axis, and its ^{1}H NMR spectrum is so simple that we can clearly show the process of the assignment. Figure 2-a shows the 1D ^{1}H NMR spectrum of A,D-α-CDpy$_2$ in 0.1 mol dm^{-3} Na_2CO_3/D_2O. The HDO signal was decoupled by irradiation to give clear signals close to the HDO signal. Some of the observed signals have already been assigned, as shown in the figure.[13] The α-, β-, and γ-H's of the pyridinio groups give a doublet at $\delta = 8.928$ and triplets at $\delta = 8.158$ and 8.688, respectively. Three doublets at $\delta = 5.0$–5.2 are due to the anomeric C_1-H's. A pair of double doublet (dd) signals at $\delta = 5.265$ ($J = 13.66$ and 1.95 Hz) and

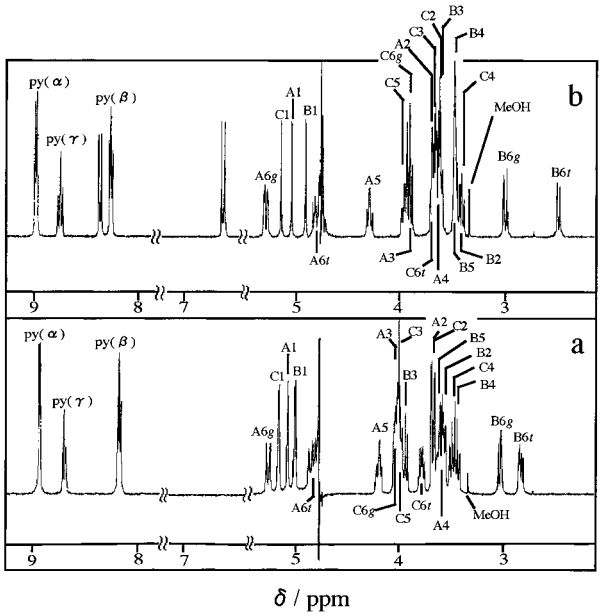

Fig. 2. ^{1}H NMR spectra of A,D-α-CDpy$_2$ in 0.1 mol dm^{-3} Na_2CO_3/D_2O at 298 K in the absence (a) and in the presence (b) of *p*NP. Capitals A, B, and C in the figure refer to GP(A,D), GP(B,E), and GP(C,F), respectively, and the numbers following them represent the numbers of carbon atoms to which protons are attached in GP. The Greek letters α, β, γ represent the positions of protons in the pyridinio group. The letter MeOH refers to the signal of methanol used as an internal reference.

$\delta = 4.844$ ($J = 13.66$ and 8.78 Hz) are due to the diastereotopic geminal C_6-H's of substituted GP moieties referred to GP(A,D). A pair of dd signals at $\delta = 3.040$ ($J = 12.45$ and 1.71 Hz) and $\delta = 2.838$ ($J = 12.45$ and 4.88 Hz) are due to the geminal C_6-H's of GP(B,E). The geminal C_6-H's of native α-CD give a pair of dd signals at $\delta = 3.91$ and 3.86.[14] The large lower-field shifts of the C_6-H signals for GP(A,D) are due to a deshielding effect of the positively charged pyridinio groups directly bound to the C_6-carbons. The remarkable higher-field shifts of the C_6-H signals for GP(B,E) are attributed to strong shielding by the ring current of the adjacent pyridinio groups in GP(A,D).[13] The other signals have never been assigned.

Figure 3 illustrates the 1D HOHAHA spectra together with a part of the normal 1D spectrum (Fig. 3-a) of A,D-α-CDpy$_2$. The doublet signals due to the C_1-H's at $\delta = 5.165$, 5.078, and 5.009 were independently irradiated to give the spectra shown in Figs. 3-b, -c, and -d, respectively. The irradiation of the doublet signals at $\delta = 5.078$ and 5.009 gave pairs of the dd signals due to the C_6-H's of GP(A,D) and GP(B,E), respectively, indicating that the irradiated signals are due to the C_1-H's of GP(A,D) and GP(B,E), respectively. Thus, the remaining signal at $\delta = 5.165$ is due to the C_1-H's of GP(C,F). Each HOHAHA spectrum also showed signals due to the other protons involved in the same GP. These signals were easily assigned, as shown in Fig. 3, by their multiplicity and by consulting the 2D COSY spectrum. Only the δ values for C_3-, C_5-, and one of C_6-H's for GP(C,F) remained to be determined, since their signals were overlapped with one another at around $\delta = 4.0$.

It is known that the longitudinal relaxation time (T_1) for the C_6-H's of α-CD is 0.35 s, which is much smaller than those for other

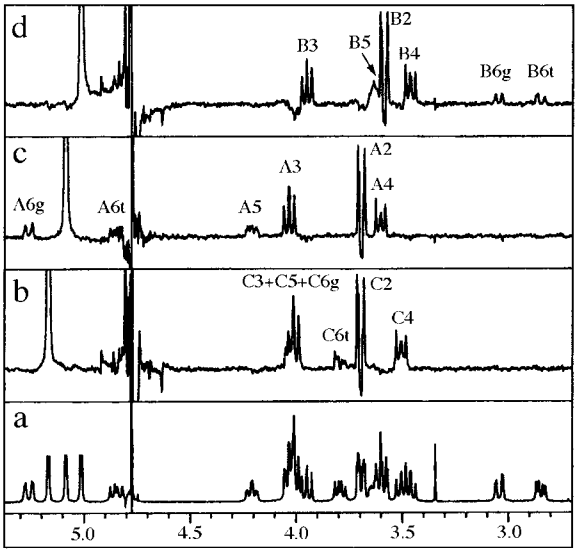

Fig. 3. The 1D HOHAHA spectra of A,D-α-CDpy$_2$ in 0.1 mol dm^{-3} Na_2CO_3/D_2O at 298 K. a: a part of a normal 1D spectrum; b, c, and d: 1D HOHAHA spectra obtained by irradiation at $\delta = 5.165$, 5.078, and 5.009, respectively. The meanings of letters tagged to signals are the same as in Fig. 2.

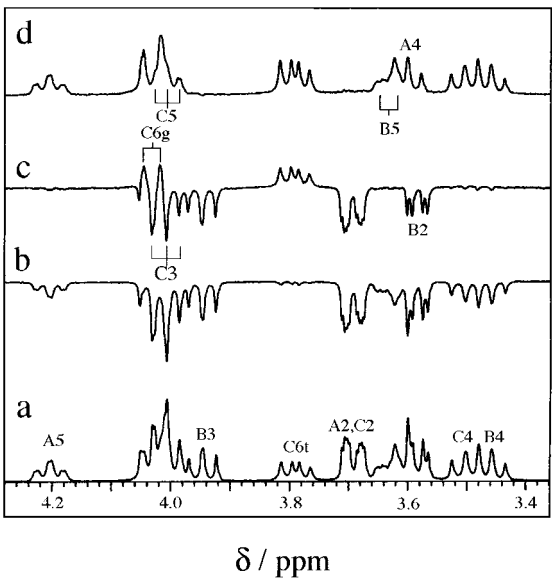

Fig. 4. A part of normal ^{1}H NMR spectrum (a) of A,D-α-CDpy$_2$ and those during the inversion-recovery at $\tau = 0.20$ (b), 0.37 (c), and 0.69 (d) s. The meanings of letters tagged to signals are the same as in Fig. 2.

protons, such as C_2-H ($T_1 = 1.07$ s), C_3-H ($T_1 = 1.03$ s), C_4-H ($T_1 = 0.64$ s), and C_5-H ($T_1 = 0.46$ s), in the presence of dissolved oxygen.[14] Figure 4 illustrates a part of the ^{1}H NMR spectra of A,D-α-CDpy$_2$ measured during the inversion-recovery process with a pulse sequence of π–τ–$\pi/2$: Figure 4-a is a part of the normal ^{1}H NMR spectrum, and Figs. 4-b, -c, and -d show the spectra measured at $\tau = 0.20$, 0.37, and 0.69 s, respectively. At $\tau = 0.20$ s, signals for C_6-H's almost disappeared, and the other

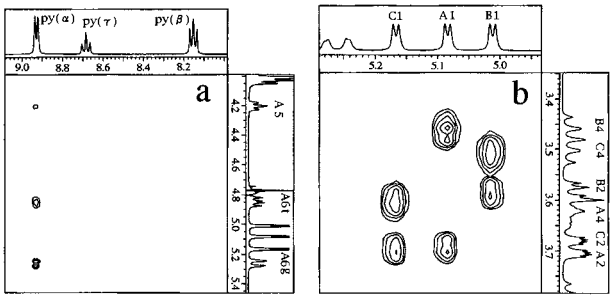

Fig. 5. 2D ROESY spectra of A,D-α-CDpy$_2$ in 0.1 mol dm^{-3} Na_2CO_3/D_2O at 298 K. The meanings of letters tagged to signals are the same as in Fig. 2.

signals were still inverse. At $\tau = 0.37$ s, the signals for C_4- and C_5-H's disappeared, those for C_2- and C_3-H's were still inverse, and those for C_6-H's had already recovered with significant intensities. At $\tau = 0.69$ s, the signals for C_2- and C_3-H's disappeared, and those for the other protons recovered. Thus, the overlapped signals for the C_3-, C_5-, and C_6-H's of GP(C,E) were separately assigned, as shown in Fig. 4.

The 2D ROESY spectrum of A,D-α-CDpy$_2$ was available for the confirmation of the signal assignment described above. Figure 5-a illustrates a part of the ROESY spectrum, which apparently gave cross-peaks between the α-H ($\delta = 8.928$) of the pyridinio group and C_5- ($\delta = 4.202$) and C_6-H's ($\delta = 5.265$ and 4.844) of GP(A,D). Figure 5-b shows another part of the ROESY spectrum. The C_1-H of GP(A,D) ($\delta = 5.078$) gave cross-peaks not only with the C_2-H of the same GP ($\delta = 3.683$), but also with the C_4-H of the adjacent GP(B,E) ($\delta = 3.452$). In the same manner, the C_1-H's of GP(B,E) ($\delta = 5.009$) and GP(C,F) ($\delta = 5.165$) gave cross-peaks with C_4-H's of the adjacent GP(C,F) ($\delta = 3.497$) and GP(A,D) ($\delta = 3.596$), respectively. These data indicate that the above assignment is valid. The result of the peak assignment for all of the protons of GP's is shown in Fig. 2-a. The addition of a virtually equimolar amount of *p*NP to a solution of A,D-α-CDpy$_2$ in 0.1 mol dm^{-3} Na_2CO_3/D_2O resulted in a significant change in the ^{1}H NMR spectrum of the host due to its complexation with pNP$^-$ (Fig. 2-b). The obtained signals were assigned in a similar manner as described above.

Assignment of the ^{1}H NMR Signals for the Other Pyridinio Derivatives of CD's. The hosts A,B- and A,C-α-CDpy$_2$ are unsymmetrical molecules, and gave more complex signals (Fig. 6) than A,D-α-CDpy$_2$. Two pyridinio groups for each host were different in circumstances from each other, and gave ^{1}H NMR signals at different positions. There was no predetermined data for judging which signals were attributed to either pyridinio group. Thus, we could not depend on these signals for the peak assignment. A clue to signal assignment of A,B-α-CDpy$_2$ was a pair of dd signals at $\delta = 2.944$ and 2.640, which have already been assigned to be due to the geminal C_6-H's of GP(C).[13] Similarly, a clue to signal assignment of A,C-α-CDpy$_2$ was a pair of dd signals at $\delta = 3.017$ and 2.737, which have already been assigned to be due to the geminal C_6-H's of GP(D).[13] Fortunately, these hosts gave six separated C_1-H signals, though being partly crowded. Thus, all of the C_1-H signals were irradiated one by one to give six 1D HOHAHA spectra. Among the obtained spectra for each host, there was only one which gave rise to a pair of dd signals described above, and thus we could assign the irradiated signal due to the C_1-H of GP(C) for A,B-α-CDpy$_2$ or that of GP(D) for A,C-α-CDpy$_2$. Once one of the irradiated signals

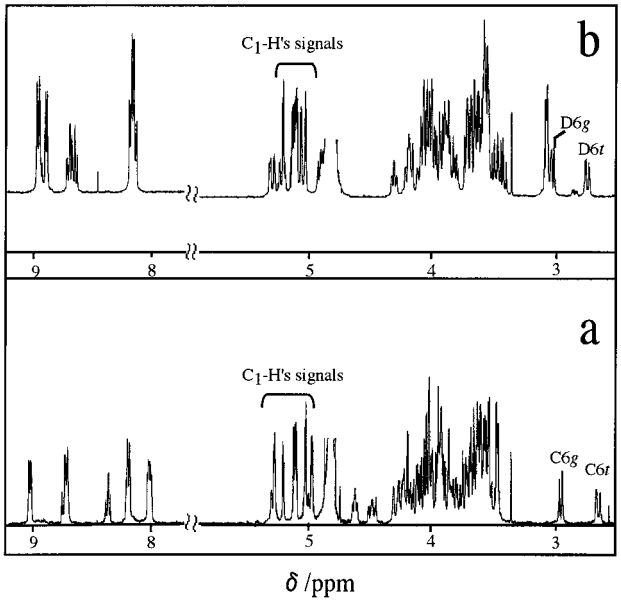

Fig. 6. ^{1}H NMR spectra of A,B-α-CDpy$_2$ (a) and A,C-α-CDpy$_2$ (b) in 0.1 mol dm^{-3} Na_2CO_3/D_2O at 298 K in the absence of *p*NP.

was assigned, it was possible to assign the C_1-H signal due to the adjacent GP by the combined use of 2D ROESY and 1D HOHAHA spectra. When ^{1}H NMR signals were too crowded to assign, they were separated by measuring spectra during inversion-recovery, as described above. Thus, we could determine the δ values for all of the protons of these hosts. The monopyridinio derivative α-CDpy also gave 6 separated C_1-H signals and a pair of dd signals due to the geminal C_6-H's of GP(B),[15] though the ^{1}H NMR spectrum was also complex. Thus, it was possible to assign the signals for all of the protons involved in the host in a similar manner as described above. The assignment of ^{1}H NMR signals of A,D-β-CDpy$_2$ was the most difficult among the hosts examined, since the circumstances of the GP(A) and GP(D) pyridinio groups are similar, but different, and thus the signals due to the pyridinio groups, as well as those due to the C_6-H's of GP(B) and GP(E), overlapped, though they did not completely coincide with each other. Furthermore, its seven C_1-H's gave only five doublet signals in the peak-area ratio of 1:1:2:1:2 from lower- to higher-field. The irradiation of these signals by the 1D HOHAHA method and the combined use of ROESY, COSY, and inverse-recovery measurement suggested that these doublet signals are due to the C_1-H's of GP(G), GP(C), GP(A) and GP(F) (overlapped), GP(D), and GP(B) and GP(E) (overlapped) from lower- to higher-field. The chemical shifts of the other protons were also assigned in a similar manner as described above.

Results and Discussion

Binding Constants for *p*NP Complexes with α-CD's. Table 1 lists the binding constants (K_a) for the complexation of *p*NP with native α-CD and its pyridinio derivatives determined by UV/Vis spectroscopy at pH 11.3 and 5.6. The pK_a value of *p*NP is 6.89.[16] The K_a values at pH 11.3 were much larger than those at pH 5.6, indicating that the binding of the ionized *p*NP (*p*NP$^-$) to α-CD's is much stronger than that of the neutral *p*NP. Furthermore, the negatively charged *p*NP$^-$ was more strongly bound to the positively charged pyridinio derivatives than to native α-CD. Among the hosts examined, A,D-α-CDpy$_2$ gave the largest K_a value of 11,800 mol^{-1} dm^3, which was ca. 6-times that of the native α-CD. On the other hand, the K_a values for the complexes of the neutral *p*NP with the pyridinio derivatives were only 2.6-times, at most, that with native α-CD. These results suggest that electrostatic interactions between the pyridinio groups of the hosts and *p*NP$^-$ effectively contribute to the stabilization of complexes.

Table 1. Binding Constants (K_a) for the Inclusion Complexes of *p*NP with α-CD and its Pyridinio Derivatives in 0.1 mol dm^{-3} Na_2CO_3 (pH 11.3) and KH_2PO_4–Na_2HPO_4 Buffer (pH 5.6) at 298 K

Host	K_a/mol^{-1} dm^3	
	pH 11.3	pH 5.6
α-CD	2050	210
α-CDpy	4190	210
A,B-α-CDpy$_2$	5280	230
A,C-α-CDpy$_2$	8650	360
A,D-α-CDpy$_2$	11800	550
A,D-β-CDpy$_2$	1710	—

Effects of *p*NP$^-$ and Other Anionic Guests Inclusion on the ^{1}H NMR Signals of A,D-α-CDpy$_2$. The inclusion of *p*NP$^-$ within the cavity of A,D-α-CDpy$_2$ resulted in a significant change in the ^{1}H NMR spectrum of the host (Fig. 2-b). Figure 7 illustrates the changes ($\Delta\delta$) in δ for all of the protons of GP's with the inclusion of *p*NP$^-$ in 0.1 mol dm^{-3} Na_2CO_3. It is clear that the amplitude and direction of $\Delta\delta$ are significantly different from one another, depending on the position of GP. The C_5-H signals showed the most characteristic behavior: The signal due to GP(A,D) showed a lower-field shift, whereas those of GP(B,E) and GP(C,F) showed large and small higher-field shifts, respectively, with the inclusion of *p*NP$^-$. Although not so apparent as the C_5-H, the C_3-H also showed anisotropy. The C_3-H of GP(A,D) showed a signifi-

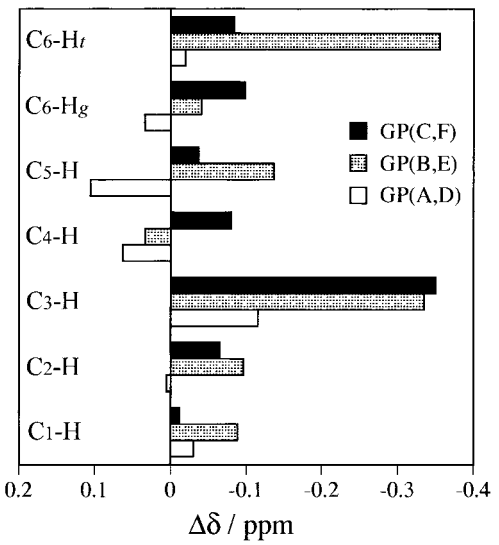

Fig. 7. Changes ($\Delta\delta$) in δ for the protons involved in A,D-α-CDpy$_2$ (7.3 mmol dm^{-3}) with the addition of *p*NP (7.0 mmol dm^{-3}) in 0.1 mol dm^{-3} Na_2CO_3/D_2O at 298 K.

cantly smaller higher-field shift than those of G(B,E) and G(C,F). It is apparent that the included pNP$^-$ affects differently on GP's depending on their positions. The ROESY spectrum of an A,D-α-CDpy$_2$-pNP$^-$ system gave clear cross-peaks between m-H of pNP$^-$ and the C$_5$-H of GP(A,D), together with those between the m- and o-H's of pNP$^-$ and the C$_3$-H of GP(A,D), indicating that pNP$^-$ deeply penetrates into the CD cavity in such a manner that the nitro group locates in the vicinity of the pyridinio groups of A,D-α-CDpy$_2$. For a comparison, we examined the effect of pNP$^-$ inclusion on the chemical shifts of native α-CD protons. The addition of 10 mmol dm^{-3} pNP$^-$ to a solution of 10 mmol dm^{-3} α-CD in 0.1 mol dm^{-3} Na_2CO_3 caused higher-field shifts of $\Delta\delta = -0.231$ for C$_3$-H and -0.001 for C$_5$-H. No difference was found in $\Delta\delta$ between the corresponding protons of different GP's, suggesting that electrostatic interactions between the pyridinio groups of A,D-α-CDpy$_2$ and the nitro groups of pNP$^-$ are responsible for the diamagnetic anisotropy in an A,D-α-CDpy$_2$-pNP$^-$ system.

In order to discriminate whether the anisotropic effect of pNP$^-$ is specific or not, we examined the effects of a few other anionic guests on the δ values of the A,D-α-CDpy$_2$ protons. Figure 8 illustrates $\Delta\delta$ for the ^{1}H NMR signals of A,D-α-CDpy$_2$ induced by the addition of 25 mmol dm^{-3} KI to 8 mmol dm^{-3} A,D-α-CDpy$_2$ in D_2O at 298 K. It is known that the iodide ion is strongly bound to A,D-α-CDpy$_2$ ($K_a = 1320$ mol^{-1} dm^3 in H_2O at 298 K[17]). If an included anionic guest affects the electron density of the host molecule in a different manner from one GP to another, even the iodide ion will show diamagnetic anisotropy. Actually, virtually no difference in $\Delta\delta$ for both C$_3$- and C$_5$-H's was found between GP's upon the addition of KI. This result suggests that the diamagnetic anisotropy observed for the pNP$^-$ is not always general for anionic guests. Thus, we assumed that the diamagnetic anisotropy is brought about by a retardation of the molecular rotation of pNP$^-$ within the cavity of A,D-α-CDpy$_2$. If the benzene ring of pNP$^-$ is forced to direct to the GP(A,D), the C$_5$-H of GP(A,D) locates in the lateral zone of the benzene ring and shifts to a lower-field, whereas those of GP(B,E) and GP(C,F) locate above or below the benzene ring and shift to a higher-field. This interpretation was supported by the ROESY spectrum. The cross-peak between the m-H of pNP$^-$ and the C$_5$-H of GP(A,D) is obviously higher than those between the m-H and the C$_5$-H's of GP(B,E) and GP(C,F).

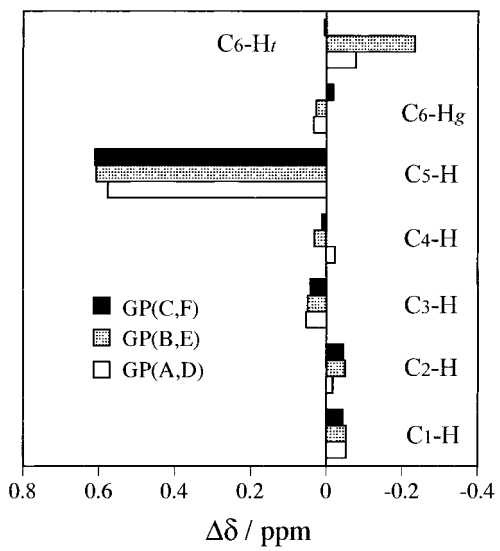

Fig. 8. Changes ($\Delta\delta$) in δ for the protons involved in A,D-α-CDpy$_2$ (8 mmol dm^{-3}) with the addition of KI (25 mmol dm^{-3}) in D_2O at 298 K.

The next question is how the molecular rotation of pNP$^-$ is retarded within the cavity of A,D-α-CDpy$_2$. A possible interpretation is that the electrostatic interactions between the nitro group of pNP$^-$ and the pyridinio groups of A,D-α-CDpy$_2$ are so strong that the oxygen atoms of the nitro group are fixed to the direction of the pyridinio groups. The internal rotation of the C–N bond linking the benzene ring to the nitro group in pNP$^-$ will be retarded to a considerable extent by a rigid quinonoid form with the C=N bond, one of the resonance structures of pNP$^-$, as shown in Fig. 9. Thus, once the nitro group is trapped by the pyridinio groups of A,D-α-CDpy$_2$, the benzene ring is also forced to be directed to the GP(A,D) of A,D-α-CDpy$_2$. Another possible interpretation is that the molecular rotation of pNP$^-$ is sterically hindered in the cavity of A,D-α-CDpy$_2$. If the electrostatic repulsive interactions between the pyridinio groups of GP(A,D) are strong enough to distort the cavity conformation to ellipsoidal, it would be difficult for the included pNP$^-$ to rotate freely in the cavity. In order to judge which is the case, we examined the effect of p-hydroxybenzoic acid (pHBA) on the chemical shifts of A,D-α-CDpy$_2$. Figure 10 shows $\Delta\delta$ for A,D-α-CDpy$_2$ induced by the addition of 10 mmol dm^{-3} pHBA to 10 mmol dm^{-3} A,D-α-CDpy$_2$ in D_2O containing 0.1 mol dm^{-3} KH_2PO_4–NaOH (pD 7.4) at 298 K. At this pD, the carboxyl group of pHBA dissociates to give a monoanionic species (pHBA$^-$). As illustrated in Fig. 10, the C$_3$-H's showed a higher-field shift, and the C$_5$-H's showed a lower-field shift. Interestingly, only a slight anisotropy was found in $\Delta\delta$ for the C$_5$- and C$_3$-H's, despite the molecular structure of the pHBA$^-$ anion being very similar to that of pNP$^-$. This indicates that the effect of the steric hindrance on the molecular rotation of a benzene ring is minor, if any. Thus, it is more probable that the molecular rotation of pNP$^-$ is retarded and oriented to a definite direction by strong electrostatic interactions between the nitro

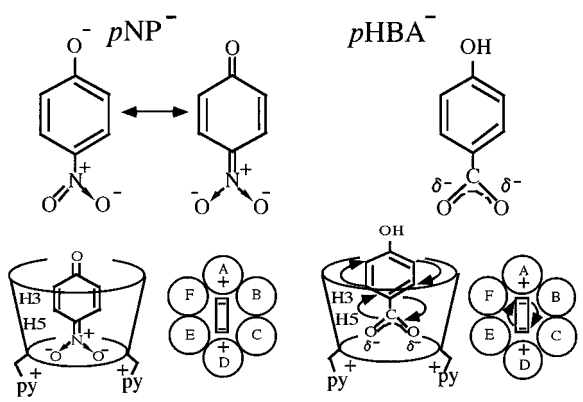

Fig. 9. Molecular structures (upper side) of pNP in 0.1 mol dm^{-3} Na_2CO_3 and pHBA in 0.1 mol dm^{-3} KH_2PO_4–NaOH (pD 7.4), and possible structures (lower side) of their inclusion complexes with A,D-α-CDpy$_2$.

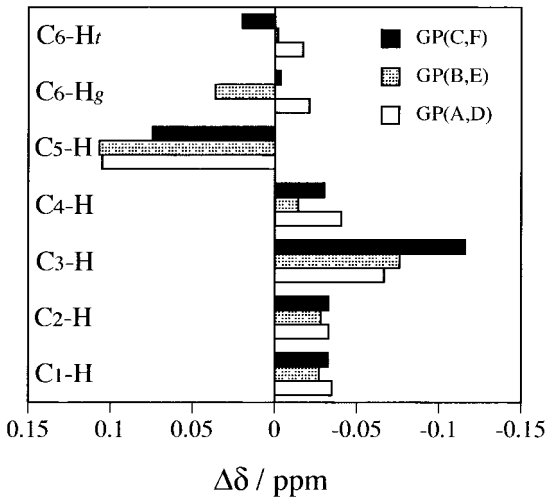

Fig. 10. Changes ($\Delta\delta$) in δ for the protons involved in A,D-α-$CDpy_2$ (10 mmol dm^{-3}) with the addition of *p*HBA (10 mmol dm^{-3}) in 0.1 mol dm^{-3} KH_2PO_4–NaOH (pD 7.4) at 298 K.

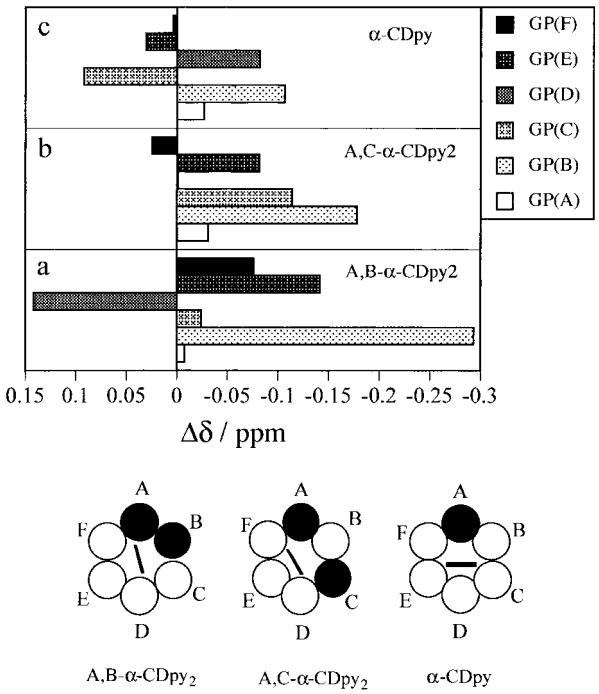

Fig. 11. Changes ($\Delta\delta$) in δ for the C_5-H's of A,B-α-$CDpy_2$ (a), A,C-α-$CDpy_2$ (b), and α-CDpy (c) with the addition of 10 mmol dm^{-3} *p*NP in 0.1 mol dm^{-3} Na_2CO_3 at 298 K. Concentrations of the hosts were ca. 10 mmol dm^{-3}.

group of *p*NP$^-$ and the pyridinio groups of the host (Fig. 9). In the case of *p*HBA$^-$, its carboxylate group is connected to the benzene ring by a freely rotating C–C bond, and the benzene ring can freely rotate in the CD cavity, even when the carboxylate group is trapped by the pyridinio groups of A,D-α-$CDpy_2$ (Fig. 9). It is interesting that electrostatic interactions are available for controlling the molecular motion or the orientation of an ionic guest in the CD cavity and, then, for the design of enzyme-mimic modified CD. Tabushi, et al. have also reached a similar conclusion through measurements of the deuterium relaxation times for charged host and guest systems.[7,8]

We also measured the longitudinal relaxation time (T_1) to confirm the above interpretation. The T_1 values of *p*NP$^-$ protons were determined by means of the inversion-recovery method for degassed 0.1 mol dm^{-3} Na_2CO_3/D_2O solutions of 16.4 mmol dm^{-3} *p*NP in both the absence and presence of equimolar A,D-α-$CDpy_2$ at 298 K. Complexation of *p*NP$^-$ with A,D-α-$CDpy_2$ caused a marked decrease in T_1 of the *m*-H of *p*NP$^-$ from 2.49 s to 0.85 s. This effective relaxation in the magnetic energy of *m*-H after complexation also suggests that the *m*-H is very close to the C_5-H of GP(A,D). The T_1 of the *o*-H was also decreased, though to a less extent, from 3.67 s to 1.53 s. The thermal fluctuation will be more vigorous at the *o*-H side of *p*NP$^-$ than at the *m*-H side.

Effects of *p*NP$^-$ Inclusion on the ^{1}H NMR Signals for the Other Pyridinio Derivatives of CD's. The addition of *p*NP$^-$ to solutions of the other pyridinio derivatives of α-CD also caused significant anisotropic changes in ^{1}H NMR chemical shifts of the C_5-H's, indicating that the molecular rotation of *p*NP$^-$ is also retarded within the cavities of these hosts. Figure 11 illustrates the $\Delta\delta$ values of the C_5-H's for A,B-α-$CDpy_2$, A,C-α-$CDpy_2$, and α-CDpy. In A,B-α-$CDpy_2$, only the C_5-H of GP(D) showed a lower-field shift. The other C_5-H's showed higher-field shifts, though the $\Delta\delta$ values for the C_5-H's of GP(A) and GP(C) were small. In A,C-α-$CDpy_2$, only the C_5-H of GP(F) showed a lower-field shift. The other C_5-H's showed higher-field shifts, though the $\Delta\delta$ values for the C_5-H's of GP(A) and GP(D) were small. In α-CDpy, the C_5-H's of GP(C), GP(E), and GP(F) showed lower-field shifts, and the other C_5-H's, higher-field shifts. Possible inclusion structures for these host-*p*NP$^-$ systems were shown as vertical sectional views in the figures, which were estimated based on an assumption that the C_5-H's located in the lateral zone of the benzene ring show lower-field shifts, and that those located above or below the benzene ring, higher-field shifts in a similar manner as in the case of A,D-α-$CDpy_2$. It is interesting that the benzene ring of *p*NP$^-$ is oriented in parallel with a line running through GP(A) and GP(C) in A,C-α-$CDpy_2$, indicating that the nitro group of *p*NP$^-$ interacts with both of the pyridinio groups of G(A) and G(C). However, in A,B-α-$CDpy_2$, the benzene ring of *p*NP$^-$ is not in parallel with a line running through GP(A) and GP(B). Upon illustrating these inclusion structures, we assumed that the macrocyclic conformations of the CD's are not distorted. In fact, however, the macrocycles will be distorted, especially in the case of A,B-α-$CDpy_2$, and the orientation of *p*NP$^-$ will be affected by the distortion.

In sharp contrast to the pyridinio derivatives of α-CD, the C_5-H's of a β-CD analog A,D-β-$CDpy_2$ showed no clear anisotropic shift (Fig. 12). All of the C_5-H's showed higher-field shifts, though the magnitudes were somewhat different from one another, indicating that the molecular rotation of *p*NP$^-$ is not strongly retarded in the cavity of A,D-β-$CDpy_2$. The size of the A,D-β-$CDpy_2$ cavity will be too large to retard any molecular rotation. In the case of the pyridinio derivatives of α-CD, the sizes of the cavities are so small that their C_5-H's are in close contact with the guest ion, and retard the molecular rotation by strong van der Waals interactions. Thus, it is

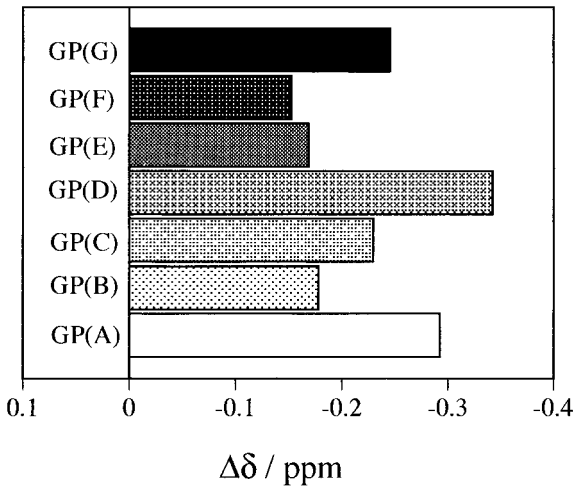

Fig. 12. Changes ($\Delta\delta$) in δ for the C_5-H's of A,D-β-$CDpy_2$ (10 mmol dm^{-3}) with the addition of 10 mmol dm^{-3} *p*NP in 0.1 mol dm^{-3} Na_2CO_3 at 298 K.

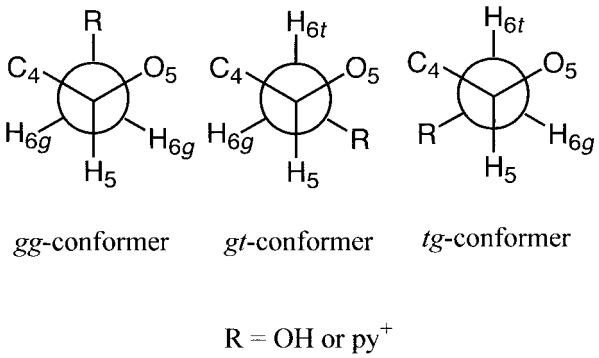

Fig. 13. Conformers about the C_5–C_6 bonds of the pyridinio derivatives of α-CD.

regarded that not only electrostatic interactions, but also van der Waals interactions, play an important role in retarding the molecular rotation of *p*NP^- within the cavities of the α-CD derivatives.

Effect of *p*NP^- Inclusion on Conformer Distribution around the C_5–C_6 Bond of the Dipyridinio Derivatives of α-CD. The conformation about the C_5–C_6 bond of GP has been discussed in terms of the relative contributions from the three staggered conformers of *gauche–gauche* (*gg*), *gauche–trans* (*gt*), and *trans–gauche* (*tg*), as shown in Fig. 13.[1,2,5,18–20] The residue R in the figure is directed to the outside of the CD cavity in the conformer *gg* and to the inside in conformers *gt* and *tg*. The time-averaged fractional populations (P_{gg}, P_{gt}, and P_{tg}) of these conformers can be estimated by measuring the two spin-spin coupling constants, $J_{5,6a}$ and $J_{5,6b}$, between the C_5-H and two C_6-H's.[18] Table 2 shows the populations calculated for A,D-, A,C-, and A,B-α-$CDpy_2$ in the absence and in the presence of *p*NP^-. For A,D-α-$CDpy_2$ without *p*NP^-, a conformer *gt* was the major component for GP(A,D), indicating that the pyridinio groups of GP(A,D) are mainly directed to the inside of the CD cavity. A conformer *gt* was also dominant for GP(C,F), but a conformer *gg* for GP(B,E). The population of a conformer *tg* was very small, if any, similarly to the other cases.[1,2,5,19,20] Upon adding *p*NP^-, the P_{gt} value increased and the P_{gg} value decreased for GP(A,D), suggesting that the pyridinio groups of GP(A,D) are attracted by electrostatic interactions to the inside of the CD cavity within which *p*NP^- is included. In contrast, the P_{gg} value for GP(B,E) significantly increased with *p*NP^- inclusion, suggesting that the OH groups of GP(B,E) are forced to be directed to the outside of the CD cavity. The electrostatic repulsion between the OH groups and the included *p*NP^- may be responsible for the change. The changes in P_{gg} and P_{gt} for GP(C,F) with *p*NP^- inclusion were slight. It is evident that the induced-fit type of inclusion occurs in the complexation of A,D-α-$CDpy_2$ with *p*NP^- in a similar manner as in an enzyme. In A,C-α-$CDpy_2$ without *p*NP^-, the P_{gt} values for GP(A) and GP(C) were similar to that for GP(A,D) of A,D-α-$CDpy_2$ without *p*NP^-. The pyridinio groups locate far from each other, and the circumstances around the C_5–C_6 bonds would be similar to those of A,D-α-$CDpy_2$. The effect of the *p*NP^- addition on P_{gt} was also similar to the case of A,D-α-$CDpy_2$. In A,B-α-$CDpy_2$ without *p*NP^-, the P_{gt} values for GP(A) and GP(B) were considerably smaller and larger, respectively, than that for GP(A,D) of A,D-α-$CDpy_2$, suggesting that the electrostatic repulsion between the pyridinio groups of adjacent GP(A) and GP(B) forces the pyridinio group of GP(A) to move to the outside of the CD cavity, and that of GP(B) to the inside of the CD cavity. Upon adding *p*NP^-, the P_{gt} values for GP(A) and GP(B) approached each other, indicating that the repulsive interactions are reduced by attractive interactions between the pyridinio groups and *p*NP^-. This result suggests that the induced-fit type of inclusion also occurs in the complexation of A,B-α-$CDpy_2$ with *p*NP^-.

Table 2. Fractional Populations of Conformers for the C_5–C_6 Bonds of A,D-, A,C-, and A,B-α-$CDpy_2$ in the Absence and in the Presence of *p*NP^-

GP	[*p*NP^-]/mmol dm^{-3}	P_{gg}	P_{gt}	P_{tg}
	[A,D-α-$CDpy_2$] = 7.3 mmol dm^{-3}			
A,D	0.0	0.23	0.76	0.01
A,D	7.0	0.12	0.88	0.00
B,E	0.0	0.65	0.35	0.00
B,E	7.0	0.93	0.07	0.00
C,F	0.0	0.37	0.63	0.00
C,F	7.0	0.40	0.60	0.00
	[A,C-α-$CDpy_2$] = 10.0 mmol dm^{-3}			
A	0.0	0.23	0.73	0.04
A	10.0	0.21	0.78	0.01
C	0.0	0.16	0.78	0.06
C	10.0	0.08	0.88	0.04
	[A,B-α-$CDpy_2$] = 11.0 mmol dm^{-3}			
A	0.0	0.41	0.58	0.01
A	10.0	0.35	0.65	0.00
B	0.0	0.13	0.81	0.06
B	10.0	0.25	0.75	0.00

Conclusions

The *p*NP^- was strongly bound to A,D-α-$CDpy_2$ in an aqueous 0.1 mol dm^{-3} Na_2CO_3 solution. An anisotropic ring-cur-

rent effect of pNP$^-$ on the ^{1}H NMR chemical shifts was observed for the pyridinio derivatives of α-CD, such as A,D-α-CDpy$_2$, whereas the iodide ion and pHBA$^-$ showed no such effect. Furthermore, a dipyridinio derivative of β-CD was not subject to such an anisotropic ring-current effect of pNP$^-$. These results suggest that the molecular rotation of pNP$^-$ is retarded within the cavity of the pyridinio derivatives of α-CD by electrostatic interactions, together with van der Waals interactions. It is evident that electrostatic interactions are available for controlling the molecular motion or orientation of an ionic guest compound in the CD cavity and, thus, for the design of an enzyme-mimic modified CD.

The conformer distribution around the C_5–C_6 bonds of GP's in A,D-α-CDpy$_2$ was also affected by pNP$^-$ inclusion. The pyridinio groups were attracted by the included pNP$^-$ to the inside of the CD cavity, and the population of the *gt* conformer in GP(A,D) increased. This fact indicates that the induced-fit type of inclusion occurs in the complexation of A,D-α-CDpy$_2$ in a similar manner as in enzyme. Similar changes in the conformer distribution were also observed for the complexation of A,B-α-CDpy$_2$ and A,C-α-CDpy$_2$ with pNP$^-$.

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