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C.P.–M.A.S. ^{13}C-N.M.R. STUDY OF SOME SOLID-STATE INCLUSION COMPLEXES OF CYCLOMALTO-OLIGOSACCHARIDES WITH PARA-SUBSTITUTED BENZENES

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ABSTRACT

Cross polarisation–magic-angle sample spinning ^{13}C-n.m.r. spectra have been measured in the solid state for *p*-nitrophenol, *p*-iodophenol, and their inclusion complexes with cyclomaltohexaose, cyclomaltoheptaose, and methylated cyclomaltohexaose. Analysis of the line-shapes of the resonances and the dipolar-dephasing experiments indicate that the guest molecules undergo motion in the host cavities, whereas the host molecules are almost static. The mode and rate of guest motion depend on the size of the cavity.

INTRODUCTION

High-resolution, high-power, dipolar-decoupling, cross-polarisation (c.p.), and magic-angle sample spinning (m.a.s.) ^{13}C-n.m.r. spectroscopy is a useful method for the analysis of the structure and molecular dynamics of cyclomalto-oligosaccharides (cyclodextrins) and their inclusion complexes in the solid state[1]. Characterisation of these inclusion complexes is important, since they are utilised in drugs, foods, *etc.* in the solid form[2]. We have analysed[3,4] the line-shapes of c.p.–m.a.s. ^{13}C-n.m.r. spectra of some substituted benzenes included in cyclomaltohexaose (α-CD), cyclomaltoheptaose (β-CD), and hexakis (2,3,6-tri-*O*-methyl)-cyclomaltohexaose (α-TMCD), and demonstrated that the guest molecules undergo molecular motion even in the solid state.

We now report an analysis of the time scale of motion of *p*-nitrophenol (PNP) included in the cavities of α-CD, β-CD, and α-TMCD by using the ^{13}C dipolar-dephasing technique[5] and new ^{13}C-n.m.r. data on the solid-state inclusion complexes of α-CD and α-TMCD with *p*-iodoaniline (PIA). The molecular structure of the crystalline α-CD–[6] and α-TMCD–PNP[7] and α-CD–[8] and α-TMCD–PIA inclusion-complexes[9] have been characterised by X-ray diffraction.

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EXPERIMENTAL

Materials. — α-CD, β-CD, PNP, and PIA were commercial materials and were recrystallised from water. α-TMCD was synthesised[16] from α-CD and recrystallised several times from hot water. Each solid-state inclusion-complex was obtained[6-9] from an aqueous solution containing cyclomalto-oligosaccharide and guest in a 1:1 molar ratio.

Methods. — High-resolution c.p.–m.a.s. ^{13}C-n.m.r. spectra were recorded with JEOL JNM FX-200 and GX-270 spectrometers operated at 50.0 and 67.5 MHz, respectively. The details of the n.m.r. operations have been reported[3,4].

RESULTS AND DISCUSSION

PNP inclusion-complexes. — Fig. 1 shows the PNP region of c.p.–m.a.s. ^{13}C-n.m.r. spectra of crystalline PNP and PNP complexed with α-CD, β-CD, and α-TMCD[3,4]. There are several striking difference between these spectra. Only for free PNP was the resonance of the carbon (C-4′) bonded to nitrogen split into an asymmetric doublet, because of the ^{14}N–^{13}C dipolar coupling which cannot be completely suppressed by m.a.s.[11,12]. The non-equivalence of PNP C-2′,6′, which leads to line-broadening of their resonances, also appeared only in the spectrum of free PNP. These observations indicate that the free PNP in the crystalline state is almost static, a conclusion supported by the appearance of large spinning side-bands in the spectrum of free PNP[4]. Further, unlike the spectra of inclusion complexes, the low signal-to-noise ratio of the spectrum of free PNP even after 6000 accumulations, which was about ten times more than for observing the spectra of inclusion complexes, indicates also the lack of motion with a moderate rate which can reduce the proton relaxation time. Saturation of the proton spin system due to inefficient relaxation reduces the c.p. efficiency, and consequently lowers the signal-to-noise ratio of the spectrum.

Features contrasting with those of free PNP were observed in the spectra of the inclusion complexes, *i.e.*, absence of line-splitting due to the non-equivalence of C-2′,6′ and the ^{14}N–^{13}C dipolar coupling, absence of the spinning side-bands (although these were not removed artificially), and notable improvement of the signal-to-noise ratio even after smaller accumulation times. These observations indicate that the PNP molecule included in the CD cavity is undergoing molecular motion.

According to the theory of Rothwell and Waugh[13], the spin–spin relaxation time T_2 (the inverse of the line-width) for a ^{13}C spin dipolar coupled to a proton spin under conditions of random rotational motion and proton-spin r.f. decoupling is expressed as

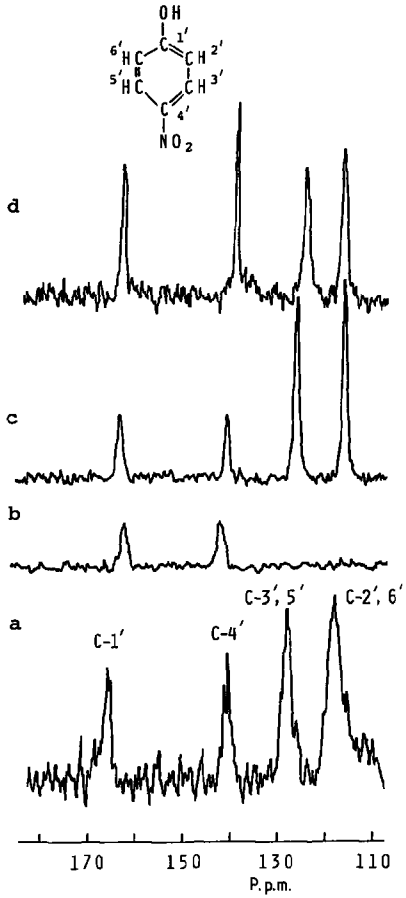

Fig. 1. C.p–m.a.s. ^{13}C-n.m.r. spectra (50 MHz; contact time, 2 ms) of (*a*) PNP (6000 scans; repetition time, 10 s); (*b*) PNP in the α-CD–PNP inclusion-complex (700 scans; repetition time, 5 s); (*c*) PNP in the β-CD–PNP inclusion-complex (620 scans; repetition time, 5 s); and (*d*) PNP in the α-TMCD–PNP inclusion-complex (500 scans; repetition time, 5 s).

$$\frac{1}{T_2} = K\frac{\tau_c}{1 + \omega_1^2\tau_c^2}, \tag{1}$$

where

$$K = \frac{4\gamma_C^2\gamma_H^2\hbar^2 I(I+1)}{15r^6}, \tag{2}$$

I is the ^{1}H spin, γ_C and γ_H are the gyromagnetic ratios for ^{13}C and ^{1}H, τ_c is the correlation time of motion, ω_1 is the frequency of the proton decoupling, and r is the internuclear ^{13}C–^{1}H distance. There are three interesting regions in the $1/T_2$ *vs.* τ_c plot expressed by equation *1* (ref. 13). The first is the long-correlation limit, where $\omega_1\tau_c \geqslant 1$ and equation *1* reduces to

$$\frac{1}{T_2} = K \frac{1}{\omega_1^2 \tau_c}, \tag{3}$$

and the line-width is narrowed with increasing τ_c by the efficient ^{13}C–^{1}H dipolar decoupling.

In the second region, where τ_c is equal or nearly equal to $1/\omega_1$, the line-width is the broadest. The third is the short-correlation limit, where $\omega_1 \tau_c \ll 1$ and equation *1* reduces to

$$\frac{1}{T_2} = K \tau_c, \tag{4}$$

and the line-width is narrowed by the rapid motional averaging. As is evident from the theory, due to the dependence of line-width on r^{-6}, the influence of the decoupling field is more significant for the resonance of a carbon directly bonded to a proton(s). Thus, the selective disappearance of the resonances of protonated carbons, namely C-2′,3′,5′,6′ of PNP, upon complexation with α-CD arises[3,4] from the restricted motion of the PNP molecule with a rate close or equal to the nutation rate of the proton-decoupling field. In this situation, the correlation time for the motion is $\sim 3 \times 10^{-5}$ s.

The preferred motion of the PNP molecule in the α-CD cavity may be that restricted about the axis which spans C-1′, C-4′, and the nitrogen atom. This supposition is supported by the structure of complexes in the solid state[6] as well as in solution[14], in which the C-1′–C-4′ axis of the included PNP molecule nearly coincides with the axis of the α-CD cavity. Such rotational motion would not affect the line-widths of the resonances of C-1′ and C-4′. It also explains why the resonance of C-4′ in the spectrum of the α-CD complex is clearly broader than that in the spectra of other complexes, because the ^{13}C–^{14}N dipolar broadening is not effectively suppressed by rotational motion about the C–N bond[15].

In the spectrum of the α-TMCD complex, the resonances of protonated carbons of PNP appeared and the resonances of C-1′ and C-4′ become sharper. These results indicate the presence of motion of the PNP molecule other than rotation about the C-1′–C-4′ axis, which reduces the line-widths of the resonances of C-1′ as well as those of the other carbons. This conclusion is supported by the structural analysis in the solid state[7]; *i.e.*, in the α-TMCD cavity, the PNP molecule is more loosely packed than in the α-CD cavity and the PNP C-1′–C-4′ axis is not parallel to the axis of the α-TMCD cavity. The mean line-width of the resonances of C-2′, C-6′, and C-3′,5′ is ~80 Hz which corresponds, according to the theory of Rothwell and Waugh[13], to a τ_c value of $\sim 9 \times 10^{-7}$ s.

The protonated carbon atoms of PNP show sharper resonances in the spectrum of the β-CD complex. In this situation, the mean line-width of the resonances of C-2′,6′ and C-3′,5′ is 50 Hz, which corresponds to the τ_c value of $\sim 7 \times 10^{-7}$ s (short correlation-time side) or $\sim 8 \times 10^{-4}$ s (long correlation-time side).

In order to investigate the molecular motion more quantitatively, c.p.–m.a.s. ^{13}C-n.m.r. dipolar dephasing spectra were obtained for the β-CD and α-TMCD complexes by using the Opella–Frey delay-without-decoupling pulse sequence[5]. In this sequence, a delay time or a dipolar-dephasing period, during which ^{1}H decoupling is removed and the ^{13}C magnetisation precesses in the nearby ^{1}H dipolar field so that the ^{13}C resonances are attenuated, is inserted just before detecting the ^{13}C free-induction decay. The attenuation rate depends on the strength of ^{13}C–^{1}H dipolar interaction, and so the presence of faster molecular motion usually weakens more significantly the ^{13}C–^{1}H interaction and results in a slower attenuation rate.

Fig. 2 shows a series of dipolar dephasing spectra of the α-TMCD–PNP inclusion-complex. After a delay time of 40 μs (Fig. 2b), which is a typical dephasing period for almost full elimination of the resonances of the methine and methylene carbons from the ^{13}C spectra of ordinary, rigid solid-state compounds[5], the intensities of the corresponding resonances of α-TMCD were reduced significantly as compared with those in the normal spectra (Fig. 2a). The resonances for the protonated carbons of PNP as well as those of α-TMCD, except the methyl resonances, disappeared after a dipolar-dephasing period of 70 μs. These results indicate that the macrocyclic ring and the side-chain methylene group of α-TMCD are almost rigid, whereas the PNP molecule and all the methyl groups of α-TMCD undergo molecular motion. The methyl resonances of α-TMCD remained even

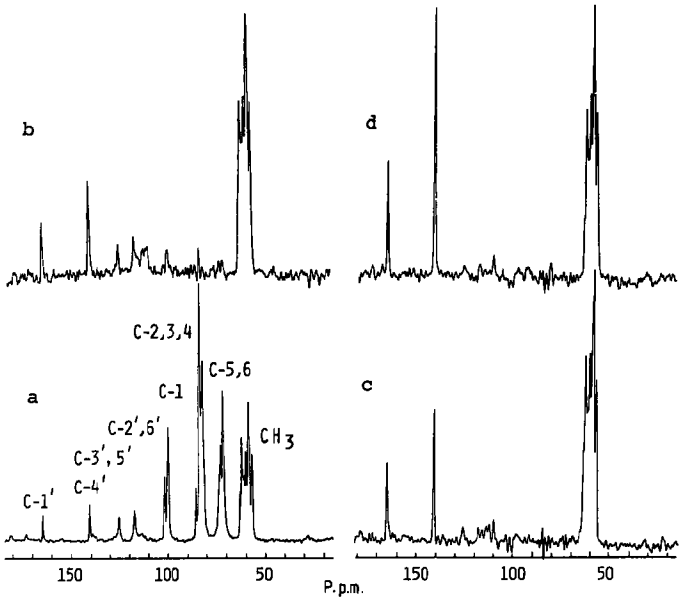

Fig. 2. C.p.–m.a.s. ^{13}C-n.m.r. spectra (67.5 MHz; contact time, 2 ms; repetition time, 5 s) of the α-TMCD–PNP inclusion-complex. (*a*) Normal spectrum (840 scans), and (*b*)–(*d*) dipolar-dephased spectra after delay periods without proton decoupling prior to data acquisition: 40 μs (*b*; 307 scans), 70 μs (*c*; 1510 scans), and 200 μs (*d*; 11,004 scans).

after a dipolar-dephasing period of 200 μs, and the details of the spectral pattern (Fig. 2c) differed from those in the normal spectrum (Fig. 2a). Thus, the methyl groups undergo rapid motion but the degree of mobility is not the same for all methyl groups.

Fig. 3. shows the dipolar-dephasing spectra of the β-CD–PNP complex. The resonances of β-CD disappeared in the early period of dipolar dephasing. All the PNP resonances remained even after a dipolar-dephasing period of 200 μs and no notable change of the relative intensity of resonances was found between the normal (Fig. 3a) and the dipolar-dephased spectra (Figs. 3b and 3c). These results indicate that the PNP molecule in the rigid cavity of β-CD is undergoing motion more rapidly than in that of α-TMCD. Hence, a likely τ_c value between those deduced from the line-width for the PNP motion in the β-CD cavity is $\sim 7 \times 10^{-7}$ s.

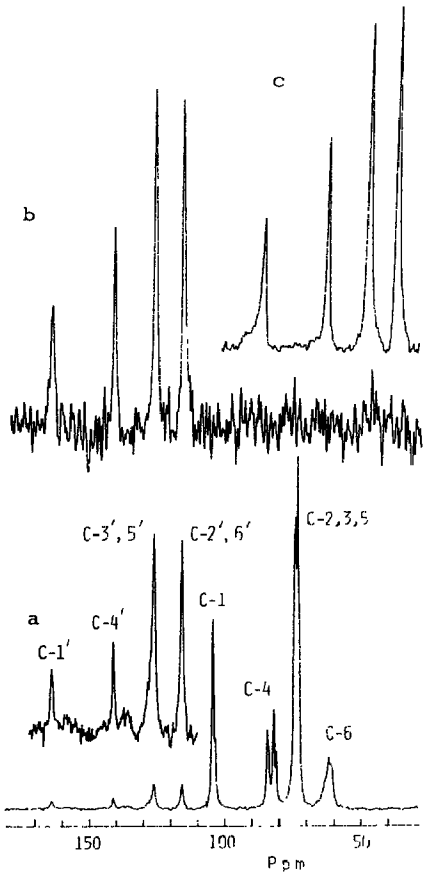

Fig. 3. C.p.–m.a.s. ^{13}C-n.m.r. spectra (67.5 MHz; contact time, 2 ms; repetition time, 5 s) of the β-CD–PNP inclusion-complex: (*a*) normal spectrum (351 scans), and (*b*) and (*c*) dipolar dephased spectra after delay periods, without proton decoupling prior to data acquisition, of 100 μs (*b*; 204 scans) and 200 μs (*c*; shown only PNP region; 13,164 scans).

Thus, if it is assumed that the mode of molecular motion of PNP is the same irrespective of the size or structure of the cavity, it can be concluded that the rate of PNP motion is largest in the β-CD complex, intermediate in the α-TMCD complex, and smallest in the α-CD complex. This conclusion is reasonable, since this order is parallel to that of the size of the cavity. According to CPK molecular models, β-CD has the largest size of cavity. X-Ray crystallography has shown that the size of the secondary-hydroxyl side of the cavity is widened by methylation of all the hydroxyl groups[7,9]. In the solid-state α-CD–PNP inclusion-complex[6], several intermolecular hydrogen–hydrogen contacts shorter than the ideal Van der Waals contact are found between the inside of α-CD and the PNP benzene ring (the shortest is ~2.0 Å), leading to tight packing of the PNP molecule in the cavity. In the α-TMCD–PNP complex[7], all the intermolecular hydrogen–hydrogen distances are >2.35 Å, indicating that the PNP benzene ring has weaker Van der Waals contacts with the inside of the α-TMCD cavity than with that of the α-CD cavity.

The rate of guest motion depends on the geometry of the complex, and such factors as the host–guest orientation and the position of the guest in the cavity must be taken into account in quantitative considerations. In the crystalline inclusion-complexes of α-CD and α-TMCD with PNP, an inverted relationship with respect to the orientation of the guest PNP molecule has been observed by X-ray diffraction[6,7]. In the α-CD–PNP complex[6], the nitrophenyl group is located in the cavity and the phenolic hydroxyl groups protrudes from the secondary-hydroxyl side and interacts with the hydroxyl groups of neighbouring CD molecules. However, in the α-TMCD–PNP complex[7], the hydroxyphenyl group is included within the cavity. There are no crystallographic data for the β-CD–PNP inclusion-complex. The c.p.–m.a.s. ^{13}C chemical shift data suggest[3] that, in the solid state, PNP is included into the β-CD cavity in the same manner as for α-CD, namely, with the nitro group in the lead from the secondary-hydroxyl side of the cavity. These structural differences must also influence strongly the molecular dynamics.

PIA inclusion-complexes. — Fig. 4 shows the c.p.–m.a.s. ^{13}C-n.m.r. spectrum of crystalline PIA. The Opella–Frey pulse-sequence was used again as a means of differentiating between protonated and non-protonated carbons. The spectrum shown in Fig. 1b, which was obtained after a dipolar-dephasing period of 40 μs, includes two resonances assigned to non-protonated carbons. One of these shows splitting in the normal spectrum of Fig. 4a due to the ^{14}N–^{13}C coupling. Thus, the resonances of two non-protonated carbons are assigned as shown in Fig. 4. Since the intensity of the resonance of C-4′ is high compared to that for the resonance of C-1′, it is possible that the resonance of protonated carbons also contributed. Other resonances appeared in the region 135–145 p.p.m. and are accordingly assigned to the protonated carbons. These resonances disappeared during a typical dipolar-dephasing period of 40 μs. The spinning side-bands appeared clearly in the PIA spectrum. The appearance of ^{14}N–^{13}C splitting and spinning side-bands and the result of the dipolar-dephasing experiment indicate that the PIA molecule is almost static in the crystalline state.

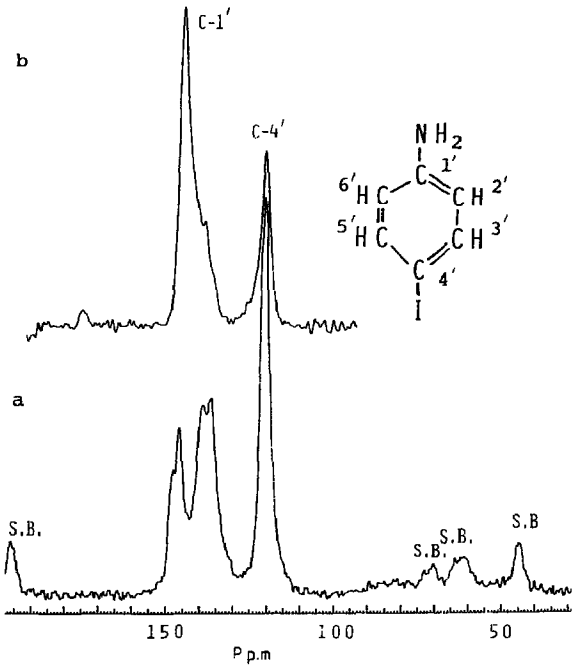

Fig. 4. C.p.–m.a.s. ^{13}C-n.m.r. spectra (50 MHz; repetition time, 5 s) of (*a*) PIA (1500 scans: contact time, 2 ms) and (*b*) PIA non-protonated carbons (11,081 scans; contact time, 0.8 ms; delay period without proton decoupling prior to data acquisition, 40 μs). Side-bands are indicated by "S.B.".

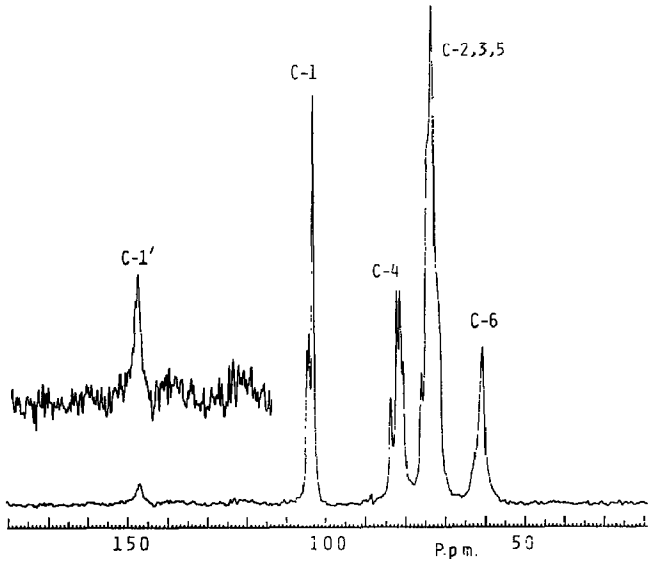

Fig. 5. C.p.–m.a.s. ^{13}C-n.m.r. spectrum (50 MHz; contact time, 2 ms; repetition time, 5 s) of the α-CD–PIA inclusion-complex (2000 scans).

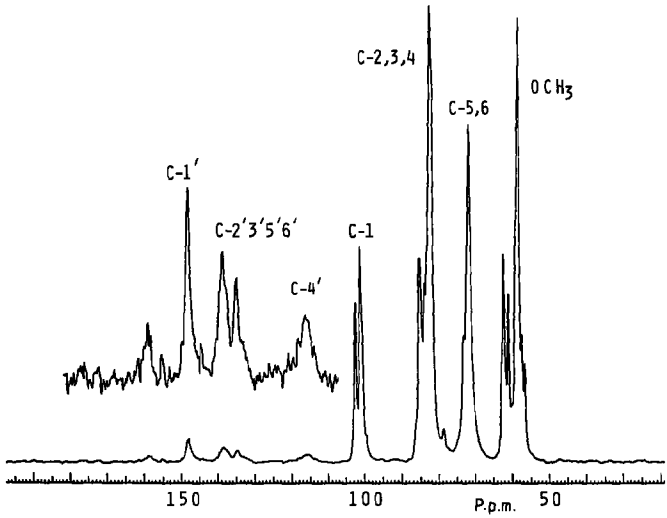

Fig. 6. C.p.–m.a.s. ^{13}C-n.m.r. spectrum (50 MHz; contact time, 2 ms; repetition time, 5 s) of the α-TMCD–PIA inclusion-complex (2700 scans).

Figs. 5 and 6 show the c.p.–m.a.s. ^{13}C-n.m.r. spectra of α-CD–PIA and α-TMCD–PIA complexes, respectively. In the spectrum of the α-CD complex, all the PIA resonances, except that of C-1′, were broadened and virtually disappeared, whereas all those of the α-CD appeared clearly. For the α-TMCD complex, all the resonances of PIA were discernible as well as those of α-TMCD, although some of the resonances of PIA were broadened. The ^{14}N–^{13}C dipolar splitting of the C-1′ resonance of PIA collapsed to a singlet upon complexation with α-CD and α-TMCD. The distinct spinning side-bands could be detected in the spectra of the α-CD and α-TMCD complexes. All these results correspond well with those observed for the PNP complexes, *i.e.*, the PIA molecule included in the α-CD and α-TMCD cavities undergoes molecular motion.

X-Ray studies[8,9] have shown that the PIA molecule is inserted into the α-CD and α-TMCD cavities from the O-2,3 side with the iodine atom in the lead. However, the geometries of inclusion are different, namely, the iodine atom and about half of the benzene ring are located in the α-TMCD cavity, whereas the whole iodophenyl group is included in the α-CD cavity. The shortest intermolecular distance in the α-TMCD–PIA complex is 3.87 Å, which is found[9] between the glycosidic oxygen and C-5′, whereas that in the α-CD–PIA complex is 3.25 Å between the glycosidic oxygen and C-6′. These results explain why the PIA molecule has a higher mobility in the α-TMCD cavity than in the α-CD cavity.

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