

NMR Spectroscopy on the Complexation of 3,6-Anhydro-β-cyclodextrin with 2,6-Naphthalenedicarboxylate Ion

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The 2,6-naphthalenedicarboxylate ion (2,6-NDC) was included into the interior cavity of $3^A,6^A$-anhydro-β-cyclodextrin (**1**) in D_2O containing 0.1 mol dm^{-3} Na_2CO_3 and caused a shift in the ^{1}H NMR signals due to the C_3- and C_5-H's of **1** to different directions, depending on the positions of glucose units (anisotropic ring-current effect). The decrease in entropy accompanied by the complexation was much larger than that for the complexation of native β-cyclodextrin with 2,6-NDC. These results indicate that the molecular rotation of 2,6-NDC is retarded within the deformed cavity of **1**.

$3^A,6^A$-Anhydro-β-cyclodextrin (**1**) is a β-cyclodextrin (β-CD) derivative, in which one of the seven glucose units in β-CD is chemically converted to 3,6-anhydroglucose (the sugar unit A in Fig. 1).[1] The conformation of the 3,6-anhydroglucose unit in **1** is $^1C_4$, which has an axial 2-OH directed to the cavity center,[2] whereas those of the glucose units B to G in **1** are $^4C_1$. X-ray crystallography on a derivative of mono-*altro*-β-cyclodextrin has revealed that the $^1C_4$ conformation of the altrose part causes significant torsional changes in the glycosidic linkages to distort the overall frame of the macrocycle towards an ellipsoid.[3] Hence, the modification of β-CD to **1** should also cause the deformation of the hydrophobic cavity. It has been reported that deformed β-CD's show unique guest-inclusion properties. For example, the binding constant (K_a) for an inclusion complex of **1** with Methyl Orange is smaller than that for a β-CD–Methyl Orange complex, and thermodynamic parameters ΔH and ΔS for the former are also smaller than those for the latter.[2] The decrease in K_a accompanied by the cavity deformation is due to the decrease in ΔS which overcomes the decrease in ΔH. The result suggests that tighter guest-binding causes an excessive decrease in the degree of freedom of guest motion. Another example is an inclusion complex of mono-*altro*-β-CD with 2-naphthalenesulfonate.[4] Assignment of NMR signals for the system revealed that the host molecule in the complex is subject to an anisotropic ring-current effect, indicating that the rotation of the guest molecule is significantly restricted upon binding into the distorted cavity of mono-*altro*-β-CD. Thus, the inclusion properties of deformed β-CD's are very interesting. However, only a few works,[2,4] as far as we know, have been reported. The present work dealt with complexation of **1** with the 2,6-naphthalenedicarboxylate ion (2,6-NDC) in an alkaline solution. It has been reported that 2,6-NDC forms an inclusion complex with native β-CD, of which binding constant is larger than those of the other regioisomers of 2,6-NDC.[5] The main object of this work was to explore the effect of the deformed cavity of **1** on guest binding by means of one- (1D) and two-dimensional (2D) ^{1}H NMR spectroscopy.

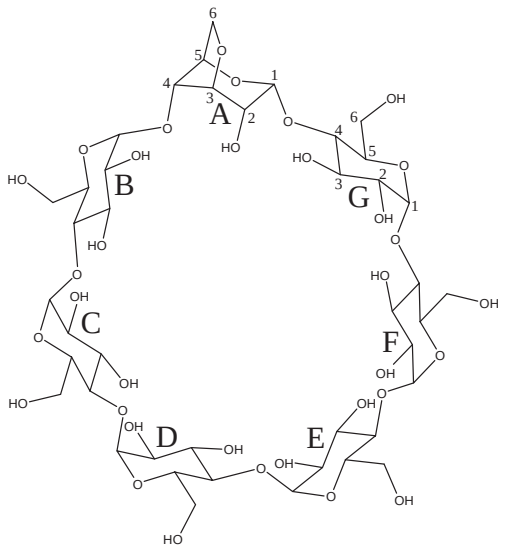

Fig. 1. Structure and numbering of **1**.

Results and Discussion

Assignment of ^{1}H NMR Signals. The 1D ^{1}H NMR spectra of **1** (10 mmol dm^{-3}) were recorded in the absence and in the presence of 2,6-NDC (30 mmol dm^{-3}) at 323 K, at which ^{1}H NMR signals due to C_1-H's were almost separated from one another (Fig. 2). The spectrum of **1** in the absence of 2,6-NDC was virtually identical to that reported by Fujita et al.,[1] who have assigned signals given by the 3,6-anhydroglucose unit of **1** as shown in Fig. 2a. On the other hand, to our knowledge, the assignment of the other signals involved in glucose units B to G has not been reported. In the present study, the assignment was attempted by combing the data from 2D COSY, 2D ROESY (rotating-frame nuclear Overhauser enhancement spectroscopy), and 1D HOHAHA (homonuclear Hartmann–Hahn spectroscopy) spectra, which have routinely been used for signal assignment.[6–8] However, the attempt was unsuccessful, since signals due to C_2- to C_6-H's in the glu-

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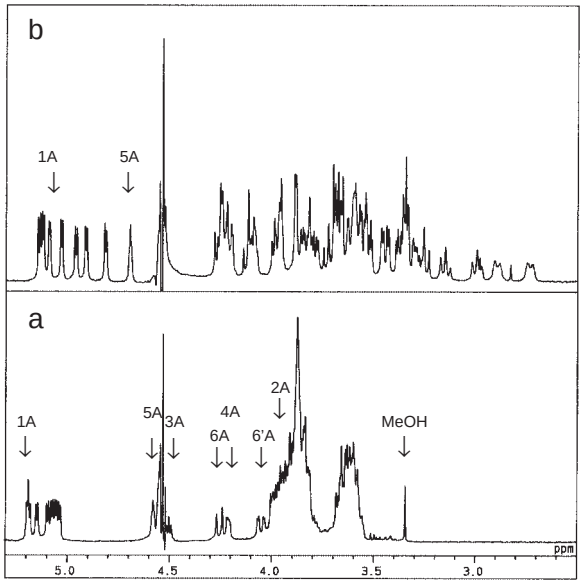

Fig. 2. ^{1}H NMR spectra of **1** (10 mmol dm^{-3}) in D_2O containing 0.1 mol dm^{-3} Na_2CO_3 at 323 K in the absence (a) and in the presence (b) of 2,6-NDC (30 mmol dm^{-3}). Capitals A to G in the figure refer to anhydroglucose or glucose units in **1**, and the numbers preceded by them represent the numbers of carbon atoms to which protons are attached as shown in Fig. 1. The letter MeOH refers to the signal of methanol used as internal reference.

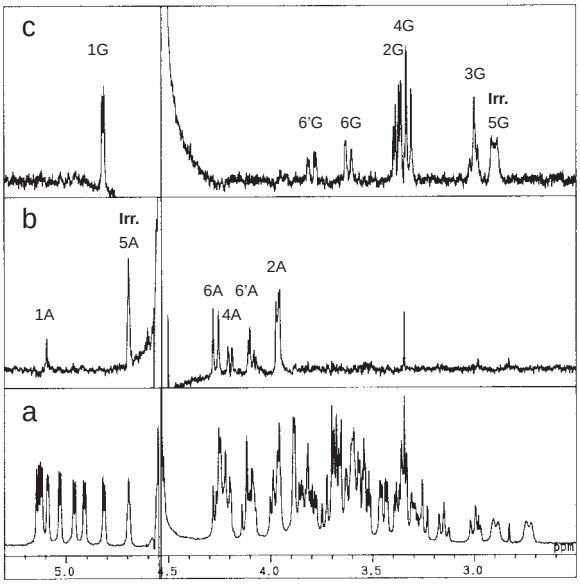

Fig. 3. 1D HOHAHA spectra of **1** (10 mmol dm^{-3}) in the presence of 2,6-NDC (30 mmol dm^{-3}) in 0.1 mol dm^{-3} Na_2CO_3/D_2O at 323 K. a: A normal 1D spectrum; b and c: 1D HOHAHA spectra obtained by irradiation at δ 4.692 [C_5-H(A) signal] and 2.894 [C_5-H(G) signal], respectively. The meanings of letters tagged to signals are the same as in Fig. 2.

cose units B to G were too crowded to be assigned. On the other hand, well-separated signals were obtained in 1D ^{1}H NMR spectra of **1** in the presence of 2,6-NDC at 323 K (Fig. 2b), and all of the signals of **1** were successfully assigned. A clue for the assignment was the signal at δ 4.692, which was attributed to the C_5-H(A) by simple comparison of the spectrum in Fig. 2a with that in Fig. 2b. Irradiation of the C_5-H(A) signal gave a 1D HOHAHA spectrum (Fig. 3b), which showed a signal due to C_1-H(A) at ca. δ 5.09, together with signals of the other H's involved in 3,6-anhydroglucose unit A. The signal due to C_3-H(A) was concealed behind the large signals due to HDO. Then, signals of H's involved in glucose units B to G were assigned by analyzing the 2D ROESY and 1D HOHAHA spectra. For example, a ROESY spectrum (Fig. 4) gave a cross-peak between C_1-H(A) and C_4-H(G), where the chemical shift of C_4-H(G) was assigned by 1D HOHAHA spectrum irradiated at δ 2.894 (Fig. 3c). The HOHAHA spectrum also showed the signal for C_1-H(G), together with those of the other H's involved in the glucose G. In a similar manner, signals of H's involved in the glucose unit F were assigned by combined analysis of ROESY and HOHAHA spectra on the basis of the C_1-H(G) signal. Thus, successive use of ROESY and HOHAHA spectra allowed us to assign all the signals of **1** in the presence of 2,6-NDC. The assigned positions for the C_3- and C_5-H's are roughly illustrated in Fig. 5b.

Next, the C_1-H signals given by **1** in the absence of 2,6-NDC were assigned by observing changes in the chemical shifts of C_1-H's of **1** (3.0 mmol dm^{-3}) with the addition of 2,6-NDC at 323 K (Fig. 6). The C_1-H signals of **1** at higher concentrations of 2,6-NDC were assigned as described above. We followed a change in δ for each C_1-H signal with decreasing 2,6-NDC concentration and assigned each doublet signals of **1** in the absence of 2,6-NDC as shown in Fig. 6. In order to confirm the validity of the assignment, changes ($\Delta\delta$) in δ's of C_1-H signals with 2,6-NDC concentrations were analyzed by the nonlinear least-squares curve-fitting method,[9] based on an assumption of 1:1 complexation between **1** and 2,6-NDC (Fig. 7). The calculated curves (solid lines) fitted well to the observed data, indicating that the assignment was valid. Then, we independently irradiated all the doublet signals due to C_1-

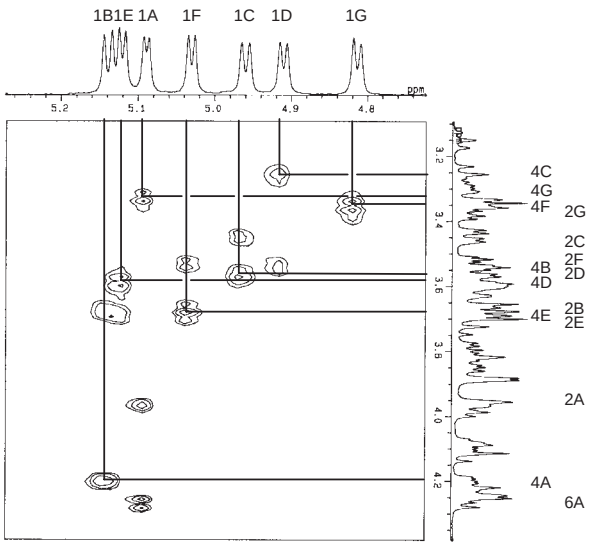

Fig. 4. 2D ROESY spectrum of **1** (10 mmol dm^{-3}) in the presence of 2,6-NDC (30 mmol dm^{-3}) in 0.1 mol dm^{-3} Na_2CO_3/D_2O at 323 K. The meanings of letters tagged to signals are the same as in Fig. 2.

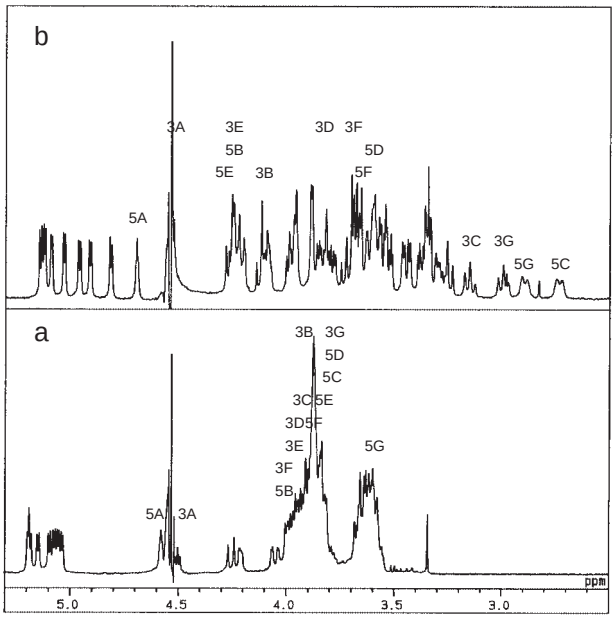

Fig. 5. The assigned positions for the C_3- and C_5-H's of **1** (10 mmol dm^{-3}) in 0.1 mol dm^{-3} Na_2CO_3/D_2O at 323 K in the absence (a) and in the presence (b) of 2,6-NDC (30 mmol dm^{-3}). The meanings of letters tagged to signals are the same as in Fig. 2.

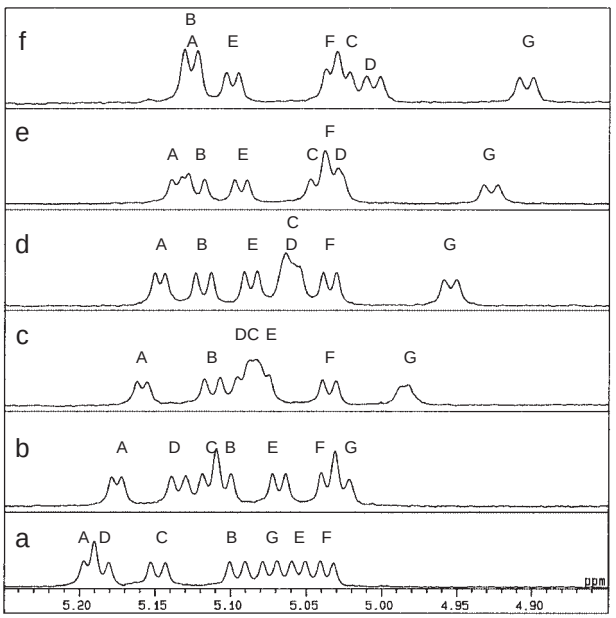

Fig. 6. Changes in ^{1}H NMR spectra for C_1-H's of **1** (3.0 mmol dm^{-3}) with the addition of 2,6-NDC in 0.1 mol dm^{-3} Na_2CO_3/D_2O at 323 K. [2,6-NDC]/mmol dm^{-3} = 0 (a), 2.0 (b), 4.0 (c), 6.0 (d), 8.0 (e), and 10.0 (f), respectively. The meanings of letters tagged to signals are the same as in Fig. 2.

H's (δ 5.194 to 5.036) given by **1** in the absence of 2,6-NDC at 323 K to obtain 1D HOHAHA spectra. Each HOHAHA spectrum obtained gave signals due to all the H's involved in the corresponding sugar unit and allowed us to assign almost all the signals of **1** in the absence of 2,6-NDC. The assigned

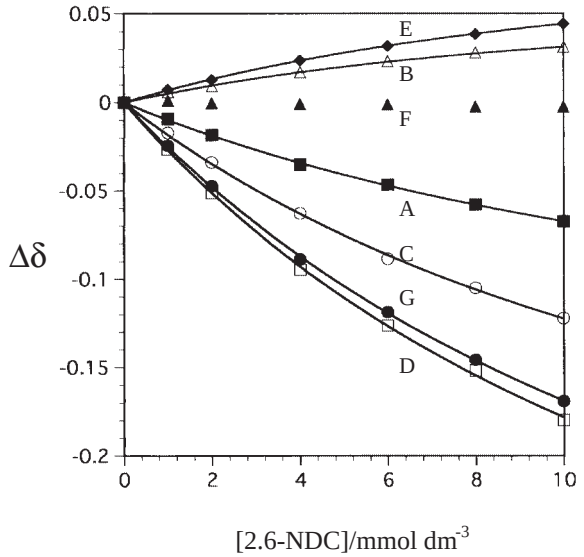

Fig. 7. $\Delta\delta$ for C_1-H's of **1** (3.0 mmol dm^{-3}) with the addition of 2,6-NDC in 0.1 mol dm^{-3} Na_2CO_3/D_2O at 323 K. The solid lines were obtained by a nonlinear least-squares curve-fitting analysis based on an assumption that **1** forms a 1:1 complex with 2,6-NDC. The meanings of letters tagged to signals are the same as in Fig. 2.

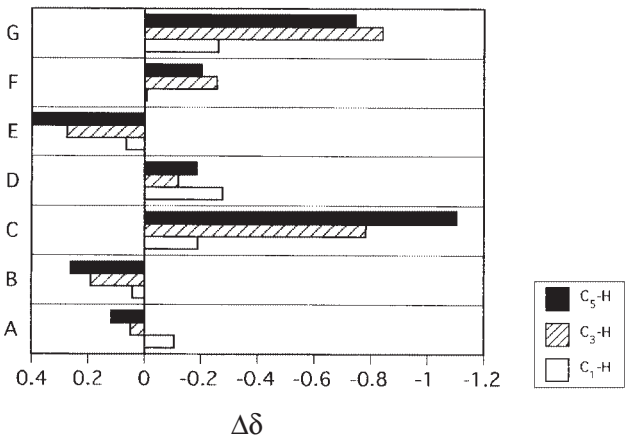

Fig. 8. $\Delta\delta$ for C_1-, C_3-, and C_5-H's of **1** (10 mmol dm^{-3}) with the addition of 30 mmol dm^{-3} 2,6-NDC in 0.1 mol dm^{-3} Na_2CO_3/D_2O at 323 K. The meanings of letters tagged to signals are the same as in Fig. 2.

positions for the C_3- and C_5-H's are roughly illustrated in Fig. 5a.

Anisotropic Ring-Current Effect. On the basis of signal assignment described above, we evaluated $\Delta\delta$ with the addition of 2,6-NDC for the C_1-, C_3-, and C_5-H's of **1** and illustrated in Fig. 8. The C_1-H is an anomeric proton, and its $\Delta\delta$ value reflects a change in macrocyclic conformation of a host molecule with complexation. The C_3- and C_5-H's are located within the cavity of **1** and susceptible to an effect of guest inclusion. The amplitude and direction of $\Delta\delta$ for these H's were significantly different from one another, depending on the constituent sugar units A to G. Thus, the signals of the C_3- and C_5-H's involved in sugar units A, B, and E of **1** showed lower-field shifts, whereas those involved in C, D, F, and G units showed

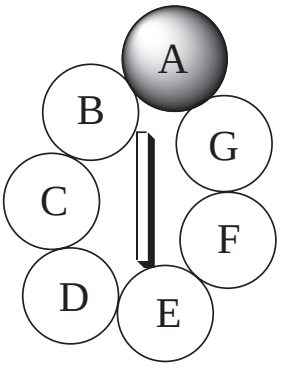

Fig. 9. Schematic illustration for a possible structure of an inclusion complex of **1** with 2,6-NDC.

higher-field shifts, upon the inclusion of 2,6-NDC. The largest $\Delta\delta$ for C_3- and C_5-H's were -0.84 (sugar G) and -1.10 (sugar C), respectively, which are comparable to -0.77 and -1.18 observed for a complex of mono-*altro*-β-CD with 2-naphthalenesulfonate.[4] In general, H's located in the lateral zone of an aromatic ring show lower-field shifts, and those located above or below the aromatic ring, higher-field shifts (anisotropic ring-current effect). Thus, the anisotropic effect of 2,6-NDC inclusion on the C_3- and C_5-H's of **1** suggests that the rotation of the guest molecule is retarded within the cavity of **1**, and that sugar units A, B, and E are at the lateral zone of the aromatic ring, whereas the C, D, F, and G units are above or below the aromatic ring. The distorted cavity of **1** will be responsible for the retardation of molecular rotation, as in the case of a mono-*altro*-β-CD-2-naphthalenesulfonate system.[4] A possible structure of an inclusion complex of **1** with 2,6-NDC is schematically illustrated in Fig. 9. A ROESY spectrum for a **1**-2,6-NDC system showed a cross-peak connecting the $C_{4,8}$-H's of 2,6-NDC to the C_5-H(B) and/or C_5-H(E), supporting the schematic structure. The C_1-H's were also subject to the anisotropic ring-current effect similarly to the C_3- and C_5-H's, though the C_1-H(A) shifted to higher-field, contrary to the C_3- and C_5-H(A)'s which shifted to lower-field. Not only the anisotropic ring-current effect but also a change in macrocyclic conformation upon complexation should be observed in the $\Delta\delta$ values of the C_1-H's.

Determination of Binding Constants and Thermodynamic Parameters. ^{1}H NMR spectra of **1** (3.08 mmol dm^{-3}) were recorded in D_2O containing 0.1 mol dm^{-3} Na_2CO_3 and various concentrations of 2,6-NDC at 298, 306, 314, and 323 K. On the basis of an assumption that **1** and 2,6-NDC form a 1:1 inclusion complex, $\Delta\delta$ of the C_1-H signals with 2,6-NDC concentrations were analyzed by the nonlinear least-squares curve-fitting method[9] (Fig. 7) to give a binding constant (K_a) for the complex (Table 1). The enthalpy change (ΔH) and entropy change (ΔS) accompanied by the complexation were calculated from the slope and intercept, respectively, of the straight line obtained by a least-squares analysis of a relationship between ln K_a and $1/T$, where T is temperature. For comparison, similar measurements were carried out for complexation of β-CD with 2,6-NDC. The K_a values for β-CD was about 2.4 and 3.3 times larger than those for **1** at 298 and 323 K, respectively, indicating that the deformation of the β-CD cavity is unfavorable for the binding of 2,6-NDC. On the other hand, the $-\Delta H$ value (29.0 kJ mol^{-1}) for **1** was larger than that (21.5 kJ mol^{-1}) for β-CD, that is, the complexation of 2,6-NDC with **1** is more exothermic than that with β-CD. This suggests that 2,6-NDC is bound to the deformed cavity of **1** more tightly than to the cavity of β-CD. However, the ΔS value (-52.2 J K^{-1} mol^{-1}) for **1** was remarkably smaller than that (-19.2 J K^{-1} mol^{-1}) for β-CD, indicating that the molecular motion of the guest is strongly retarded within the deformed cavity of **1**. The decrease in K_a with the cavity deformation is brought about by large decrease in ΔS which overcomes the decrease in ΔH. A similar result has been reported for the complexation of **1** with Methyl Orange.[2] A decrease in ΔH usually accompanies a decrease in ΔS (enthalpy–entropy compensation effect). However, in a series of normal β-CD complexes with various guests, a decrease in ΔH causes an increase in K_a, since a decrease in ΔH overcomes a decrease in ΔS.[10] Thus, it is reasonable to conclude that the large decrease in ΔS for the complexation of **1** with 2,6-NDC is caused by the retardation of molecular rotation of the guest within the deformed cavity of **1** as described in a previous paragraph.

Table 1. Binding Constants (K_a's/mol^{-1} dm^3) for the Complexation of **1** and β-CD with 2,6-NDC in D_2O Containing 0.1 mol dm^{-3} Na_2CO_3 at Various Temperatures[a)]

Temperature/K	3,6-Anhydro-β-CD (**1**)	β-CD
298	222	537
306	166	462
314	120	382
323	91	303
333	—	231

a) The ΔH and ΔS values determined were -29.0 kJ mol^{-1} and -52.2 J K^{-1} mol^{-1}, respectively, for **1** and -21.5 kJ mol^{-1} and -19.2 J K^{-1} mol^{-1}, respectively, for β-CD.

Experimental

Materials. Compound **1** was prepared according to the direction of Fujita et al.[1] 2,6-NDC and Na_2CO_3 were of reagent grade and commercially available from Wako Pure Chem. Ind., Ltd. The D_2O (Isotec) used for ^{1}H NMR measurements was the grade of 99.9 atom % D.

Apparatus. 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, 306, 314, or 323 K. Sample solutions contained about 3 or 10 mmol dm^{-3} **1** and various concentrations of 2,6-NDC in D_2O containing 0.1 mol dm^{-3} Na_2CO_3. Methanol (1.0 mmol dm^{-3}) was added to the sample solutions as an internal reference (δ 3.343[8]) of ^{1}H NMR measurements. The ROESY spectra of the host and its inclusion complex with 2,6-NDC were acquired with a mixing time of 500 ms and 512 × 256 data points, followed by zero-filling. The 1D HOHAHA spectra were obtained with a mixing time of 150 ms.

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