Synthesis and conformational analysis of an α-cyclodextrin [2]-rotaxane

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An α-cyclodextrin [2]-rotaxane has been prepared in 10% yield, by threading α-cyclodextrin (α-CD) with (*E*)-4,4′-diaminostilbene in aqueous solution and capping the included guest through reaction with 2,4,6-trinitrobenzene-1-sulfonate. 1D ^{1}H NMR spectroscopy and DQCOSY and ROESY experiments show that the α-CD rotates freely around the axle of the rotaxane, but is localised over the olefinic moiety of the stilbene. The pK_a values of the α-CD [2]-rotaxane were found to be 9.3 and 9.6, which are attributable to deprotonations of the (*E*)-4,4′-bis(2,4,6-trinitrophenylamino)stilbene moiety. NMR experiments show that these deprotonations do not perturb the conformation of the rotaxane.

Introduction

Rotaxanes are mechanically interlocked supramolecular assemblies in which a ring component is threaded by a linear chain bearing bulky capping groups to prevent dethreading.[1] Their preparation is facilitated by preassociation of the ring and chain components prior to capping.[2] In this regard cyclodextrins (CDs) (cyclic oligomers of α-1,4-linked D-(+)-glucopyranose) have found favour as ring components owing to their propensity to form host–guest or inclusion complexes with hydrophobic molecules in aqueous solution.[3–5] This has allowed the assembly of a number of CD [2]-rotaxanes,[5,6] with those comprising an axle where the chain is covalently linked to the capping groups[7–9] being the more resistant to dissociation and dethreading. Generally these rotaxanes have not been subjected to rigorous conformational analysis, although NOE experiments have been used to confirm the encapsulation of the axle within the CD annulus of several rotaxanes,[8] and Wenz *et al.*[9] have used NMR spectroscopy to determine the preferred conformation of a rotaxane of a modified β-CD as being that shown in Fig. 1. We now report the synthesis of the new rotaxane **8**, of (*E*)-4,4′-bis(2,4,6-trinitrophenylamino)stilbene with α-CD. NMR experiments show that the CD rotates freely around the axle of this rotaxane, but is localised over the olefinic moiety of the stilbene.

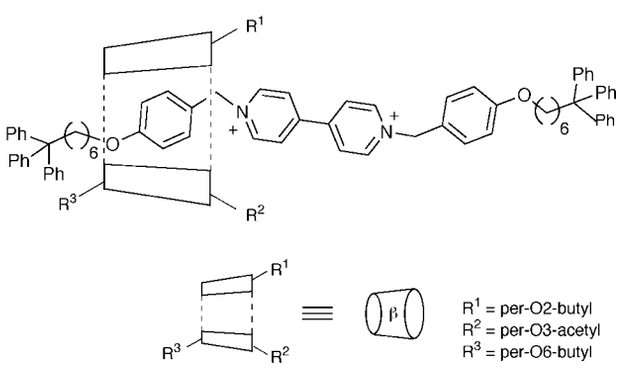


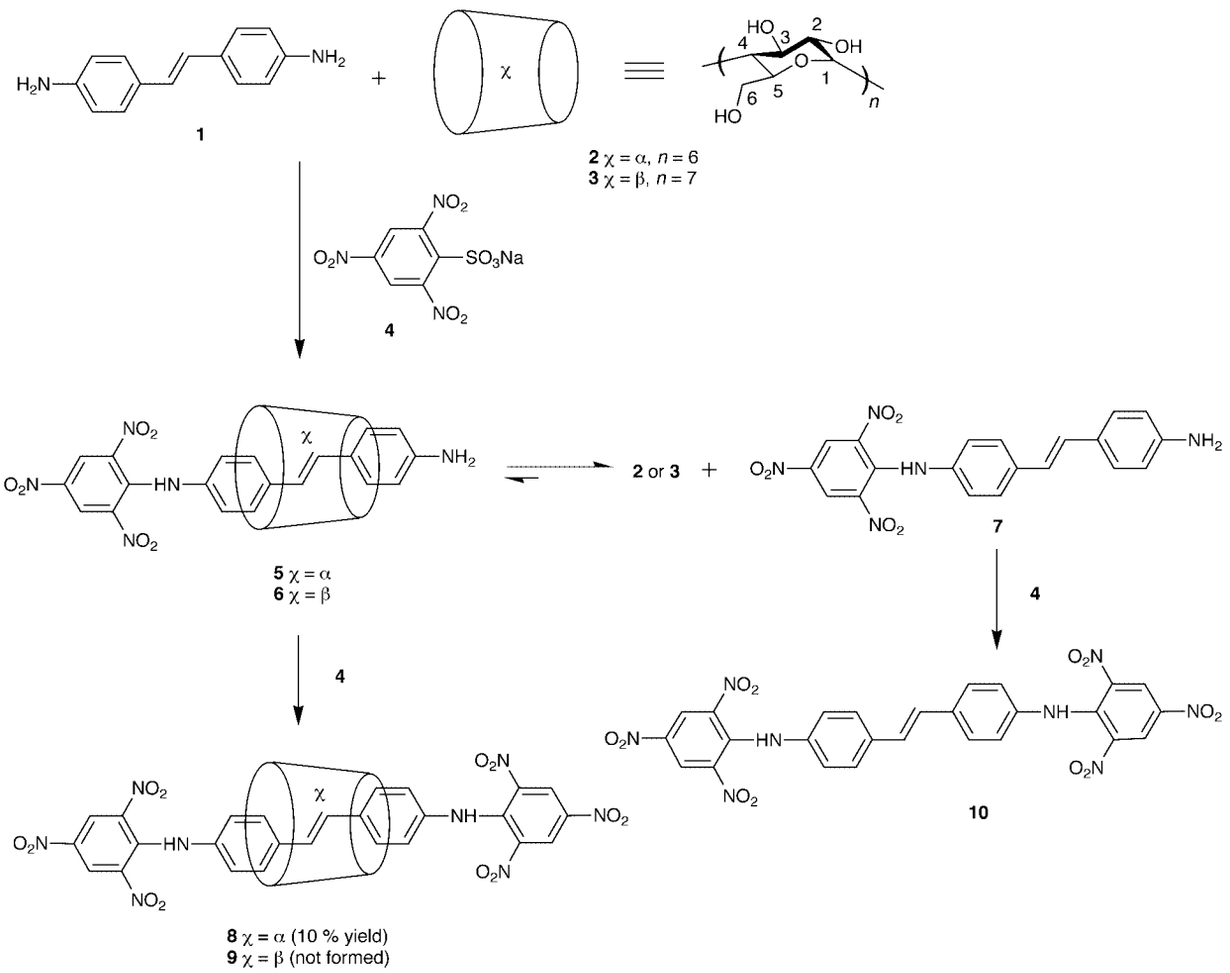
Fig. 1 The conformation of a β-CD rotaxane determined by Wenz *et al.*[9] using NOE experiments.

Results and discussion

In order to assemble a rotaxane, (*E*)-4,4′-diaminostilbene **1** was selected as the chain component and the sodium salt of 2,4,6-trinitrobenzene-1-sulfonate (TNBS) **4** was chosen as the capping agent. For spectroscopic comparison with a corresponding rotaxane, the disubstituted stilbene **10** was prepared by treating the diamine **1** with two equivalents of TNBS **4**. An attempt was made to synthesise [(*E*)-4,4′-bis(2,4,6-trinitrophenylamino)stilbene]–[β-CD]-[rotaxane] **9** (Scheme 1) by first treating a basic aqueous solution of β-CD **3** (2.5 mM) with 0.2 molar equivalents of (*E*)-4,4′-diaminostilbene **1**. After stirring the mixture until the initial emulsion clarified, indicating that the stilbene **1** had included within the CD **3**, 0.4 molar equivalents of TNBS **4** were added. However, the only new material isolated following this procedure was the disubstituted stilbene **10**. The procedure described above is referred to as the *threading approach*[7–9,10] to rotaxane synthesis. An alternative approach, known as the *slippage method*,[11] is to preassemble the capped axle and then use vigorous conditions to force the ring component over the cap, and assemble the rotaxane. This was attempted by sonicating a suspension of the dicapped stilbene **10** in a saturated aqueous solution of β-CD **3** for 2 h, but there was no evidence for the formation of the rotaxane **9** under these conditions.

Although the threading approach proved unsuitable for the synthesis of the β-CD [2]-rotaxane **9**, a similar procedure using α-CD **2** instead of β-CD **3**, but at a higher concentration, was successful. Treating an aqueous solution of the CD **2** (50 mM) and the diamine **1** with TNBS **4** afforded the rotaxane **8** in 10% yield (Scheme 1). It was possible to use the much higher concentration of α-CD **2** since it is more soluble than β-CD **3** in aqueous solutions. Presumably this facilitates rotaxane production since formation of the corresponding intermediate inclusion complexes **5** and **6** is concentration dependent.

The α-CD [2]-rotaxane **8** was separated from the reaction mixture through chromatography and was readily identified on the basis of its physical properties and spectroscopic characteristics. Thin layer chromatographic analysis showed a single component different to both α-CD **2** and the disubstituted stilbene **10**, yet showing both the characteristic ultraviolet absorbance of a stilbene and the pink colouration of a CD on



Scheme 1

exposure to acidic 1,3-dihydroxynaphthalene. The rotaxane **8** is much less soluble than α-CD **2** in aqueous solution and considerably more soluble than either the disubstituted stilbene **10** or α-CD **2** in methanol. The 1D ^{1}H NMR spectrum of the rotaxane **8** in CD_3OD (Fig. 2) shows resonances for both a CD and an (*E*)-4,4′-bis(2,4,6-trinitrophenylamino)stilbene moiety. The interaction of these components is apparent from the complexity of the signals associated with the stilbene. Whereas the NMR spectrum of the disubstituted stilbene **10** (recorded in d_6-DMSO due to the low solubility of this compound in methanol) is consistent with the two-fold symmetry, comprising one singlet for the olefinic protons, an aryl proton singlet and two aryl proton doublets, the stilbene moiety of the rotaxane **8** gives rise to two olefinic proton doublets (δ 7.18 and 7.08, J_{trans} 16.5), four aryl proton doublets and two aryl proton singlets, reflecting the asymmetry induced by the CD.

The conformation of the α-CD [2]-rotaxane **8** is as shown in Fig. 3, and was elucidated with the aid of 2D NMR spectroscopy. This approach has been employed extensively to investigate CD inclusion complexes.[12] Firstly, it was necessary to fully assign each resonance in the 1D ^{1}H NMR spectrum (Fig. 2). However, integration of the region associated with the CD annulus (Fig. 2(c)) revealed that a number of resonances were overlapping. To accurately assign these resonances a DQCOSY NMR spectrum was recorded. Fig. 4 shows an enlargement of this spectrum in the region of the CD resonances with the relevant cross-peaks and assignments illustrated. The signal associated with the C1 anomeric protons of the CD annulus is readily assigned, since it is known that these protons resonate at lower field than the C2–C6 protons.[3] A ^{1}H–^{1}H correlation between these protons and those giving rise to the signal at δ 3.46 manifests itself as a cross-peak, and allows this resonance to be assigned as being associated with the CD C2 protons. In a similar manner, a cross-peak between the C2 proton signal and the resonance at δ 3.86 permits this to be assigned to the CD C3 protons. The signals at δ 3.74 and 3.63 are assigned as being associated with the CD $C6^A$ and $C6^B$ protons, on the basis of an intense cross-peak which arises due to their geminal relationship. This is confirmed from the 1D ^{1}H NMR spectrum (Fig. 2(c)), which shows the expected coupling pattern for each of the C6 proton signals. Cross peaks between both the $C6^A$ and $C6^B$ proton resonances and the signal at δ 3.86 reveal that the latter region also consists of resonances associated with the CD C5 protons. This is confirmed from the value of the integral in the 1D ^{1}H NMR spectrum (Fig. 2(c)), which shows that this region is composed of two overlapping signals. The remaining resonance is consequently assigned as belonging to the CD C4 protons since it correlates to signals associated with both the C3 and C5 protons, and it possesses the expected coupling pattern in the 1D ^{1}H NMR spectrum (Fig. 2(c)).

The DQCOSY technique does not allow an unambiguous assignment of the doublet resonances associated with the protons of the stilbene moiety. The existence of cross-peaks only allows each doublet proton signal to be paired with that of its nearest neighbouring proton (Fig. 5). Thus, a definitive assignment was achieved by recording a ROESY NMR spectrum (Fig. 6). The relevant nuclear Overhauser effect (NOE)

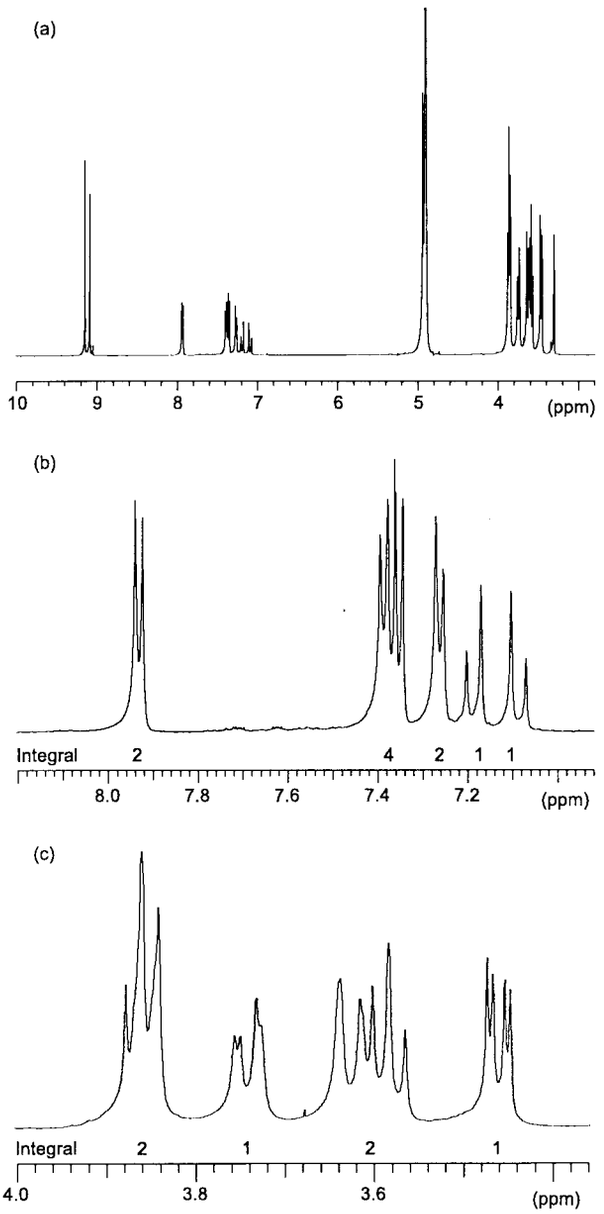


Fig. 2 ^{1}H NMR spectrum (500 MHz, CD_3OD) of the α-CD rotaxane **8** with expansions.

interactions and assignments are indicated. Since NOEs are observed between the alkene proton resonances at δ 7.18 and 7.08 and the aryl proton signals at δ 7.93 and 7.39, these aryl protons are in close proximity to the alkene protons. Therefore, if the protons of the stilbene moiety are numbered as shown in Fig. 3, these aryl proton signals are due to H(2) and H(5). Then from the ^{1}H–^{1}H correlations between the signals at δ 7.93 and 7.39, with those at δ 7.35 and 7.26, in the DQCOSY NMR spectrum (Fig. 5), the latter are assigned to H(1) and H(6) of the stilbene, respectively. The assignment of the signal at δ 7.08 to the alkene proton H(4) is based upon a more intense NOE to H(2) than H(5), since H(4) is closer through-space to H(2). In an analogous fashion, the signal at δ 7.18 is assigned to H(3) since the NOE with H(5) is greater than that with H(2), and H(3) is closer through-space to H(5).

The conformation of the α-CD [2]-rotaxane **8** is then apparent by analysis of the NOEs between the CD annulus and the stilbene moiety in the ROESY spectrum (Fig. 7). NOEs between the C3 and/or C5 protons of the CD annulus and H(1), H(2), H(3), H(4) and H(5) of the stilbene moiety are evident. This, together with the absence of interactions between the CD

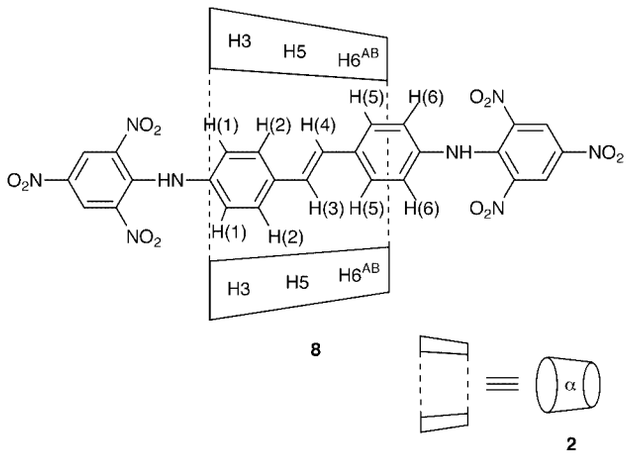


Fig. 3 The conformation of the α-CD rotaxane **8**.

C3 and/or C5 protons and H(6) of the stilbene, reveals that the CD is localised over the olefinic moiety of the stilbene. Another feature of the ROESY spectrum is that the CD C6^A and C6^B protons only display NOEs with H(5) of the stilbene. This reveals the location of the end of the CD delineated by the primary hydroxy groups (Fig. 3). While the α-CD is localised along the axle it must be rotating freely around the axle since all six glucopyranose rings of the CD give identical ^{1}H NMR signals.

The α-CD [2]-rotaxane **8** possesses two secondary amine groups, which are acidic because they are located adjacent to strongly electron-withdrawing 2,4,6-trinitrophenyl groups. Their pK_a values were determined from a pH potentiometric titration,[13] and found to be 9.3 and 9.6. It was not feasible to determine the pK_a values of the disubstituted stilbene **10** for comparison, due to the limited solubility of this compound in protic solvents. The ultraviolet/visible spectra of the rotaxane **8** and its dianion were recorded at pH 3.1 and 12.8, respectively. Deprotonation of the rotaxane **8** increases both the wavelength of maximum absorption (λ_{max}) and the molar extinction coefficient at that wavelength (ε_{max}), from 422 nm and 34 370, to 478 nm and 48 000.

The NMR experiments described above were repeated using a solution of the rotaxane **8** in CD_3OD, that had been made basic with CD_3ONa. Again all the resonances in the spectra were fully assigned. The pattern of NOEs in the ROESY spectrum recorded under these conditions is identical to that recorded for the neutral solution, indicating that the conformation of the α-CD [2]-rotaxane **8** is not affected by deprotonation. The chemical shifts of individual proton resonances for the stilbene moiety move upfield as a result of deprotonation, by between 0.18–0.36 ppm.

Experimental

General

^{1}H NMR spectra were recorded at 500 MHz, and ^{13}C NMR spectra were recorded at 75.5 MHz. Spectra were recorded using either d_6-DMSO or CD_3OD as both the solvent and internal reference. *J* values are given in Hz. Rotating frame ^{1}H–^{1}H nuclear Overhauser effect spectroscopy (ROESY) was performed with a mixing time of 250 ms. Electrospray ionisation mass spectra were measured at 120 eV in the negative ion mode. Ultraviolet/visible spectra were recorded on a Shimadzu UV-2101PC spectrophotometer. Microanalyses were performed by the Australian National University Microanalytical Service. High performance liquid chromatography (HPLC) involved analysis with a differential refractometer operating at 254 nm, and was conducted using a YMC ODS-AQ 250 × 20 mm column, eluting at 12 cm^3 min^{-1} with methanol–water (30%

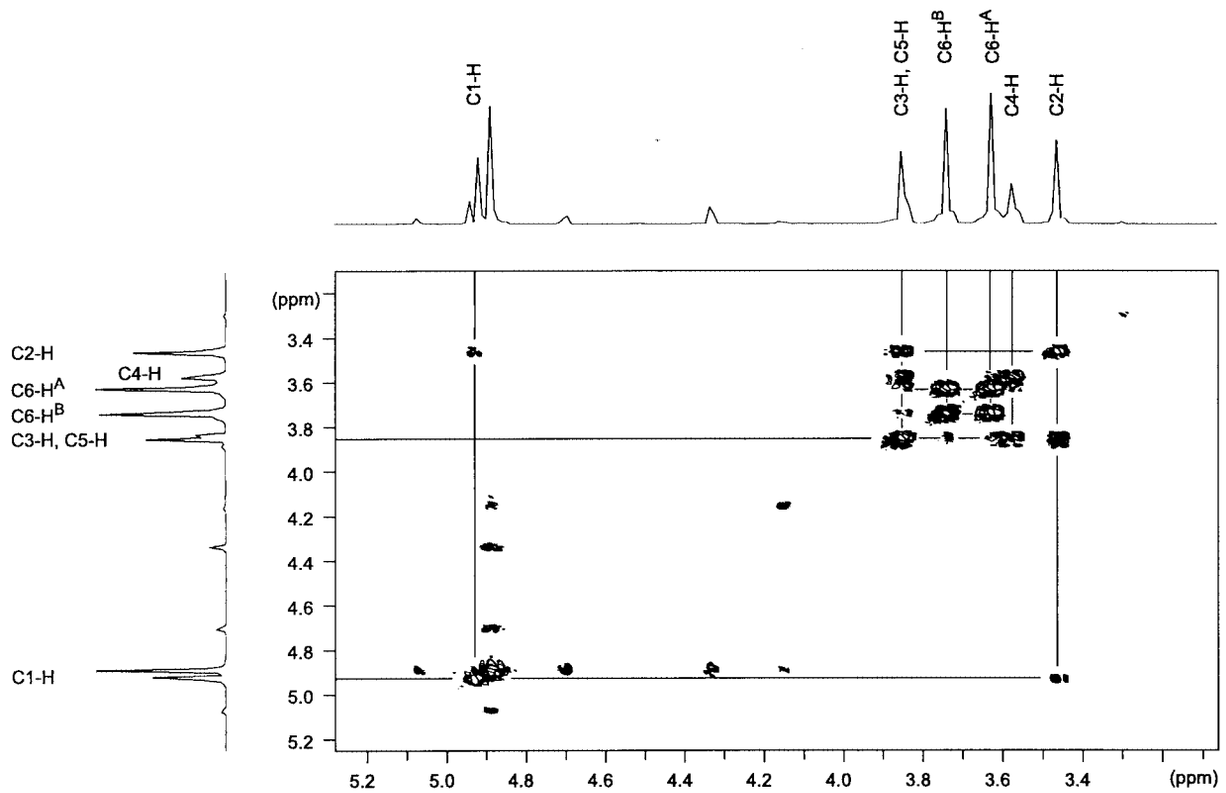


Fig. 4 The region of the DQCOSY spectrum (CD_3OD) associated with the CD annulus of the α-CD [2]-rotaxane **8**.

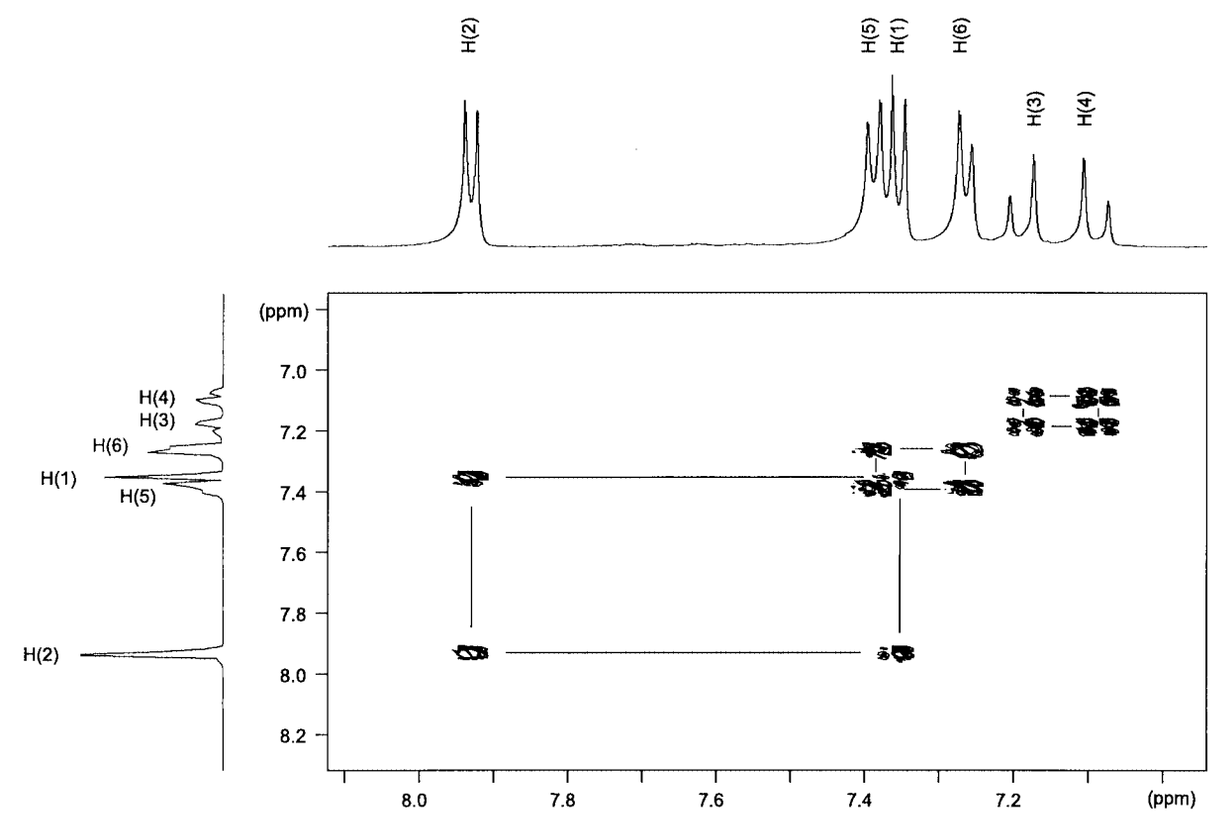


Fig. 5 The region of the DQCOSY spectrum (CD_3OD) associated with the stilbene moiety of the α-CD [2]-rotaxane **8**.

v/v). Thin layer chromatography was performed on alumina plates coated with Merck silica gel 60 F_{254}. Flash column chromatography[14] was carried out on Merck silica gel 60 (230–440 mesh). Melting points were determined on a hot-stage apparatus and are uncorrected. The pH potentiometric titration profile was determined using the method previously reported.[13] The pK_a values were calculated for the best fit of the variation of pH with added volume of NaOH titrant using the program SUPERQUAD.[15] Water was purified using a Waters™ Millipore filtration system. α-CD **2** and β-CD **3**

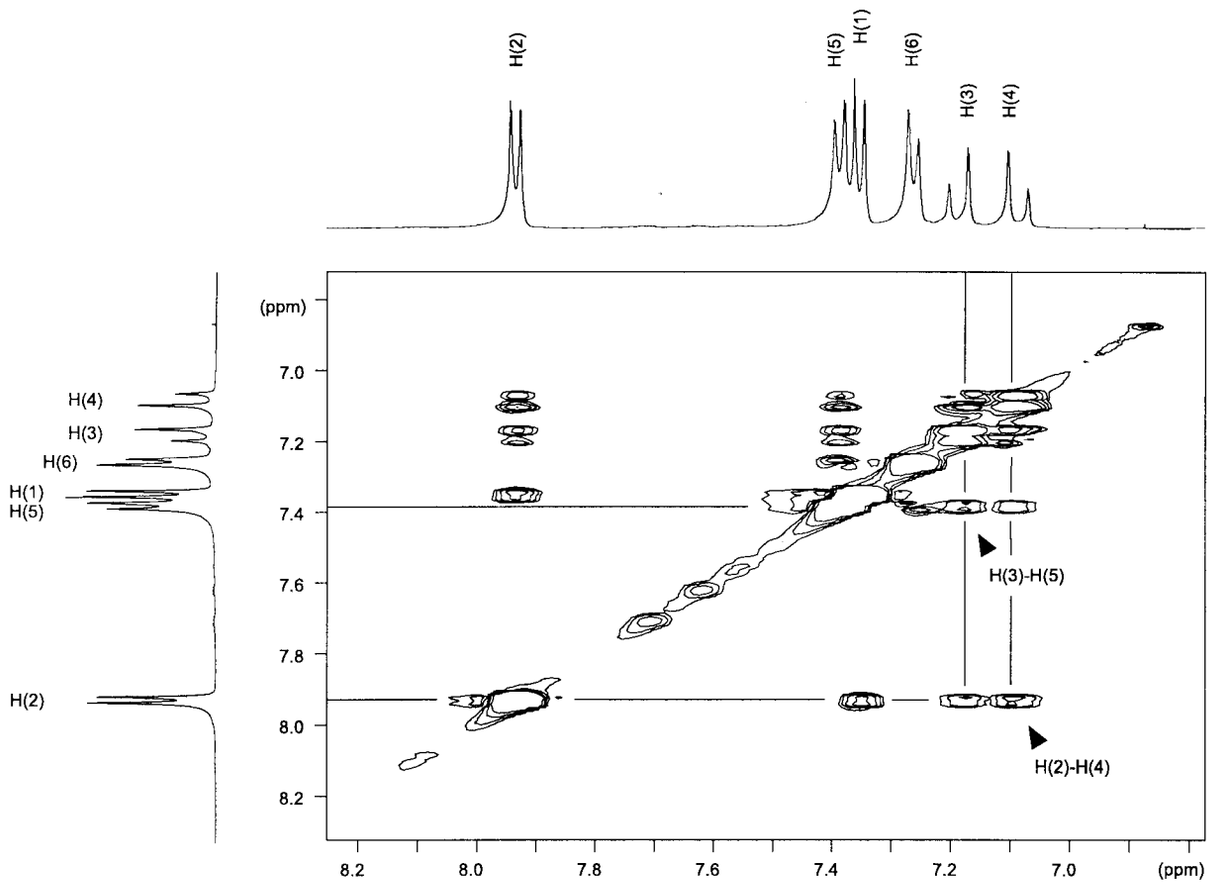


Fig. 6 The region of the ROESY spectrum (CD_3OD) associated with the stilbene moiety of the α-CD [2]-rotaxane **8**.

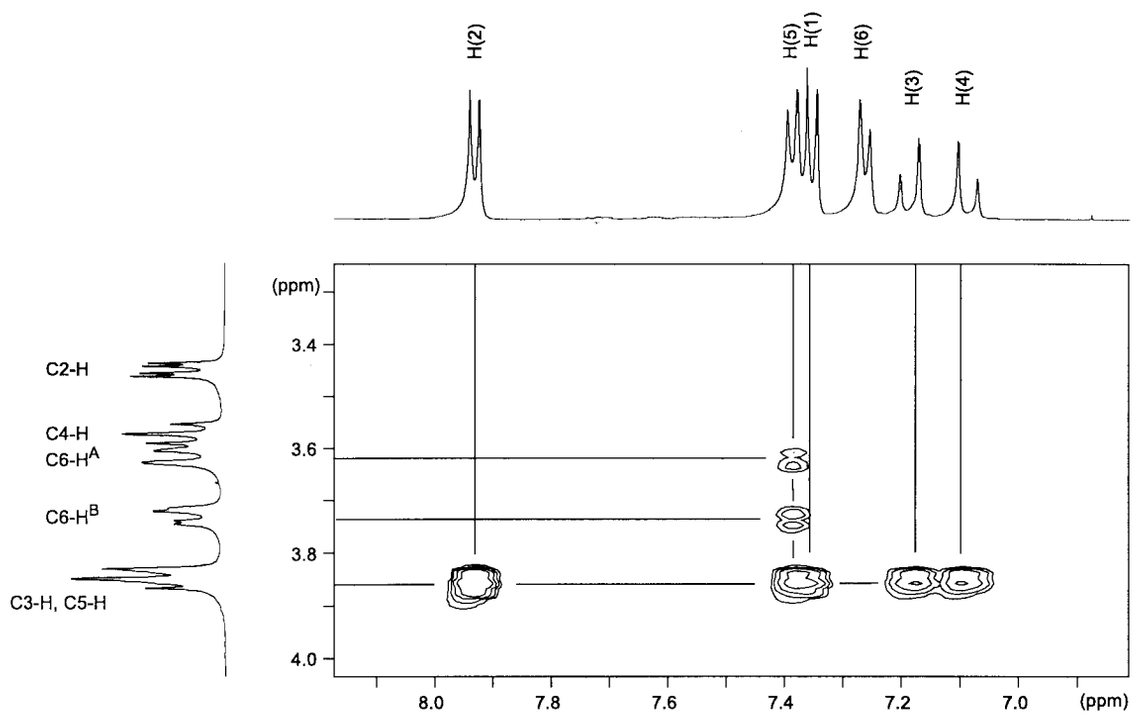


Fig. 7 The region of the ROESY spectrum (CD_3OD) showing the NOEs between the stilbene moiety and the CD annulus of the α-CD [2]-rotaxane **8**.

were gifts from Nihon Shokuhin Kako Co., Japan, and were recrystallised from water and dried *in vacuo* over P_2O_5 to constant weight. Commercially available compounds were used as received.

[(*E*)-4,4′-Bis(2,4,6-trinitrophenylamino)stilbene]–[α-CD]-[rotaxane] 8

The dihydrochloride salt of (*E*)-4,4′-diaminostilbene **1** (566 mg, 2.0 mmol) was added to a stirred aqueous solution (200 cm^3) of

α-CD **2** (9.73 g, 10.0 mmol) and the mixture was adjusted to pH 10 through the addition of $NaHCO_3$. Following stirring for 2 h at 25 °C, the dihydrate of 2,4,6-trinitrobenzene-1-sulfonic acid sodium salt **4** (1.40 g, 4.0 mmol) was added and the mixture was stirred at 25 °C for an additional 20 h. The resultant solution was washed with ethyl acetate (5 × 100 cm^3) and concentrated *in vacuo*. Flash chromatography of the residue, eluting with methanol, gave a powder, which recrystallised from water to yield the title compound **8** (311 mg, 10%) as a red solid, mp 285 °C dec. (Found: C, 45.8; H, 5.1; N, 6.8. Calc. for $C_{62}H_{76}N_8O_{42}\cdot 1H_2O$: C, 45.9; H, 4.8; N, 6.9%); δ_H (CD_3OD) 9.15 (2H, s, trinitrophenyl H), 9.09 (2H, s, trinitrophenyl H), 7.93 (2H, d, *J* 7.5, stilbene H(2)), 7.38 (2H, d, *J* 8.0, stilbene H(5)), 7.36 (2H, d, *J* 7.5, stilbene H(1)), 7.26 (2H, d, *J* 8.0, stilbene H(6)), 7.18 (1H, d, J_{trans} 16.5, stilbene H(3)), 7.08 (1H, d, J_{trans} 16.5, stilbene H(4)), 4.94 (6H, s, CD C1-H), 3.87–3.84 (12H, m, CD C3-H and C5-H), 3.74 (6H, dd, *J* 3.5 and 12.5, CD C6-H^B), 3.63 (6H, apparent d, *J* 11.5, CD C6-H^A), 3.58 (6H, apparent t, *J* 9.5, CD C4-H), 3.46 (6H, dd, *J* 3.0 and 10.0, CD C2-H); δ_H (CD_3OD–CD_3ONa) 8.58 (2H, s, trinitrophenyl H), 8.50 (2H, s, trinitrophenyl H), 7.75 (2H, d, *J* 8.0, stilbene H(2)), 7.13 (2H, d, *J* 7.5, stilbene H(5)), 7.00 (1H, d, J_{trans} 16.5, stilbene H(3)), 6.93 (1H, d, J_{trans} 16.5, stilbene H(4)), 6.90 (2H, d, *J* 8.0, stilbene H(1)), 6.78 (2H, d, *J* 7.5, stilbene H(6)), 4.98 (6H, s, CD C1-H), 3.92–3.86 (12H, m, CD C3-H and C5-H), 3.76 (6H, dd, *J* 3.5 and 12.5, CD C6-H^B), 3.64 (6H, apparent d, *J* 11.5, CD C6-H^A), 3.59 (6H, apparent t, *J* 9.5, CD C4-H), 3.42 (6H, dd, *J* 3.0 and 10.0, C2-H); δ_C (CD_3OD) 140.5, 140.3, 139.9, 139.8, 139.7, 139.5, 137.7, 137.2, 136.0 and 134.9 (all quaternary), 129.8, 129.4, 128.3, 128.4, 128.1, 123.1, 123.0 and 122.9 (all methine), 104.0 (CD C1), 83.2 (CD C4), 75.0 (CD C3), 73.9–73.6 (CD C5 and C2), 61.4 (CD C6); *m/z* 1627 (M^- + Na, 100%); TLC R_f 0.80 (methanol); HPLC t_R 12 min.

(*E*)-4,4′-Bis(2,4,6-trinitrophenylamino)stilbene 10

A solution of the dihydrate of 2,4,6-trinitrobenzene-1-sulfonic acid sodium salt **4** (65 mg, 0.22 mmol) in water–acetone (3:2 v/v, 2 cm^3) was added to a stirred solution of the dihydrochloride salt of (*E*)-4,4′-diaminostilbene **1** (28 mg, 0.10 mmol) in water–acetone (3:2 v/v, 40 cm^3) containing $NaHCO_3$ (0.01 mol dm^{-3}). The mixture was allowed to stir at 25 °C for 24 h after which it was concentrated *in vacuo*. The resultant precipitate was collected by filtration, repeatedly washed with water, and then recrystallised from acetone to yield the title compound **10** (53 mg, 85%) as an orange solid, mp 305 °C dec. (Found: C, 49.3; H, 2.5; N, 17.1. Calc. for $C_{26}H_{16}N_8O_{12}$: C, 49.4; H, 2.55; N, 17.7%); δ_H (d_6-DMSO) 10.26 (2H, br s, NH), 8.91 (4H, s, trinitrophenyl H), 7.52 (4H, d, *J* 8.0, ArH), 7.18 (2H, s, CH=CH), 7.12 (4H, d, *J* 8.0, ArH); δ_C (d_6-DMSO) 139.4, 137.9, 135.1, 134.5 and 113.9 (all quaternary), 127.5, 127.3, 126.9 and 121.0 (all methine); *m/z* 630 (M^-, 100%); TLC R_f 0.65 ($CHCl_3$).

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