Molecular cleft possessing a cholic acid moiety as a podant and its conformation

Shigeo Kohmoto,* Katsuya Sakayori, Keiki Kishikawa and Makoto Yamamoto

Department of Materials Science, Faculty of Engineering, Chiba University, 1-33, Yayoi-cho, Inage-ku, Chiba 263-8522, Japan

Received (in Cambridge) 9th November 1998, Accepted 1st February 1999

A cleft type host molecule **4** possessing a cholic acid moiety as a podant was synthesized from the condensation of naphthalene-1,4,5,8-tetracarboxylic dianhydride with a 3α-aminocholanoate derivative. It was found that **4** could be associated with naphthalene-2,6-dimethanol **5** with the binding constant of 71–92 M^{-1} with a host–guest ratio of 1 : 1 from ^{1}H NMR spectrometric titration. Conformational analysis of **4** in the absence or the presence of **5** was carried out by variable temperature ^{1}H NMR spectroscopy. Without a guest molecule, **4** adopted two stable conformations at 213 K, however, a single different conformation was found in the presence of **5**. The cleft type conformation of **4** was induced by the inclusion of the guest molecule.

Considerable effort has been devoted to developing efficient host molecules for molecular recognition.[1] A large number of host molecules are designed to be cyclic in order to give rigid conformations for binding of guest molecules. Some devices are required in acyclic hosts to fix their conformations. The fixation of their conformation in a C-shape creates a cleft-like structure, which provides a concave area for binding of guest molecules. In order to create a molecular cleft, it is highly desirable to have a rigid podant and a spacer unit and also to restrict the rotation of a podant along the spacer–podant single bond. Owing to its rigidity and unique amphiphilicity, having a hydrophobic outer surface and a hydrophilic inner surface, cholic acid has been used in building molecules for molecular recognition.[2] The concept of linking two cholic acids to form an acyclic cleft was first reported by McKenna *et al.*,[3] and other examples have appeared since.[4] As a continuation of our study, the application of cholic acid (3α,7α,12α-trihydroxy-5β-cholan-24-oic acid) as a potent building block for host molecules,[5] we have been interested in the conformational regulation of an acyclic host possessing this building block. In this paper, we report the synthesis of a cholic acid based molecular cleft with a rigid spacer unit, naphthalenetetracarboximide, and its conformational analysis. An advantage of using the large aromatic imide moiety was demonstrated by Rebek *et al.*[6] in which the restricted rotation along its C–N single bond was crucial.[7]

Results and discussion

The cholic acid based molecular cleft **4** was synthesized as follows. Methyl 3α-azido-7α,12α-bis(formyloxy)cholanoate **1**[8] was reduced with zinc powder to give the 3α-amino derivative **2**. Crude **2** was heated with naphthalene-1,4,5,8-tetracarboxylic dianhydride at 120 °C in DMF for 2 h to give tetrakisformyloxy derivative **3** in 37% yield. Deprotection of the formyloxy groups was achieved by adding dropwise a saturated methanolic lithium hydroxide solution to a THF solution of **3** to afford **4** in 50% yield. The structure of **4** was confirmed by ^{1}H NMR (500 MHz) and HRMS (FAB). Since cholic acid has the L-type shape and the 3-imido group has the α-configuration, the molecule can take a concave structure. In this structure, the hydroxy groups at the positions 7α and 12α can be positioned as binding sites, orthogonal to the concave surface.

In order to survey the recognition of guest molecules with difunctional groups, an equivalent amount of guest molecule was added to **4** and the ^{1}H NMR spectrum of the mixture was measured at ambient temperature. Out of the compounds examined, the addition of naphthalene-2,6-dimethanol **5**, 1,6-dihydroxyhexa-2,4-diyne, *p*-xylylenediol, *p*-nitrophenol, and *N-cbz-L*-tryptophan caused the chemical shift change of the aromatic protons of **4**, suggesting an interaction between **4** and these guest molecules. Except for 1,6-dihydroxyhexa-2,4-diyne, upfield shifts of the aromatic protons were observed. Upfield shifts of 0.4, 0.05, 0.05, and 0.10 ppm were observed when one equivalent amount of **5**, *p*-xylylenediol, *p*-nitrophenol, and *N-cbz-L*-tryptophan were added, respectively. The results indicated that the aromatic protons of **4** were shielded magnetically by the aromatic rings of the guest molecules. In the case of 1,6-dihydroxyhexa-2,4-diyne, downfield shifts of the protons (0.01 ppm with one equivalent amount) were observed due to the deshielding effect of its triple bonds. From the observed shielding and deshielding effects, it can be deduced that guest molecules are associated in parallel to the diimide moiety of **4**. The binding constants for all the guest molecules except **5** could not be determined due to the large errors originated in the small changes in chemical shifts of both **4** and guest molecules. The host–guest ratio between **4** and **5** was determined to be 1 : 1 from the Job plot[9] (Fig. 1) using ^{1}H NMR spectroscopy since the maximum value was observed at the [**4**]/([**4**] + [**5**]) ratio of 0.5. The binding constant between **4** and **5** was determined by ^{1}H NMR spectrometric titration by adding the $CDCl_3$ solution of **4** to the $CDCl_3$ solution of **5**. Fig. 2 shows the induced chemical shift changes of the hydroxy protons of **5**. The bind-

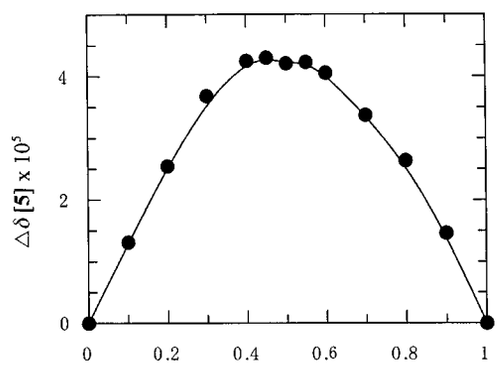


Fig. 1 Job plot and the simulated curve fit for titration of **4** with **5**.

ing constant of 91 ± 9 M^{-1} was obtained using non-linear least squares regression.[10] A similar value (72 ± 9 M^{-1}) was obtained from the induced chemical shift changes of aromatic protons of **6**. The binding ability of **4** is almost equivalent to the Rebek's cleft.[6] The conformational flexibility of **4** may be responsible for the relatively small binding constant.

In order to examine the conformational flexibility of **4** in the absence or the presence of **5**, the variable temperature (VT) ^{1}H NMR study of **4** was carried out. Fig. 3 shows the VT ^{1}H NMR study of **4** (the aromatic protons) in the absence of **5**. The sharp peak at 273 K was gradually broadened and split into two peaks (243 K). According to the MM2 calculation of **4**, this molecule can adopt two stable conformations, conformations A and B, as shown in Scheme 1. The conformation B is 1.7 kJ mol^{-1} more stable than the conformation A according to the calculation. These conformations seem to be appropriate since imide compounds which showed a restricted rotation about the C–N single bond, were known to adopt two stable conformations in which the imide ring would be positioned perpendicular to the *N*-substituents to avoid the steric repulsion between them. In the conformation A, there are two kinds of aromatic protons. Two protons inside the cavity are magnetically equivalent and two other protons outside the cavity are also magnetically equivalent. Therefore, the ^{1}H NMR spectrum corresponding to the conformation A should give two singlet peaks due to the above mentioned two kinds of protons. On other hand, the conformation B has two different types of aromatic protons. Due to the C_2 symmetric nature of this conformation, two protons across the naphthalenediimide skeleton are magnetically equivalent. In this situation, the aromatic protons corresponding to the conformation B should give two doublet peaks. Therefore, it is reasonable to consider that the observed ^{1}H NMR spectrum of **4** at 213 K is the mixture of two conformations A and B. From the coalescence temperature at 250 K, the rotational barrier[11] is determined to be 57.5 kJ mol^{-1}. The singlet peaks corresponding to the aromatic protons of conformer A might be overlapped with the doublet peaks of the aromatic protons of conformer B. When the spectral changes of **4** with varying amounts of **5** were examined at 213 K (Fig. 4), two doublet peaks appeared with J = 7.8 Hz (4 : 5, 5 : 1) which might correspond to the aromatic protons of conformer B together with a broad singlet located between the two doublets. The conformer A might be associated preferentially with **5**, which resulted in the formation of the above-mentioned broad singlet. At the end of the titration, all the peaks joined together to give a single singlet peak corresponding to the aromatic protons of the complex. A similar phenomenon was observed for the 18β-methyl protons too. The peak corresponding to **4** gradually changed to the peak corresponding to the complex and converted completely at the host–guest ratio of 1 : 8.

Similarly, the VT ^{1}H NMR study of **4** in the presence of excess (8 equiv.) **5** was carried out. At ambient temperature, the signal corresponding to the aromatic protons of **4** became very broad and it was hard to identify its chemical shift (Fig. 5), which suggested a rapid equilibrium between the host and the guest. However, it gradually became sharp with the lowering of the measured temperature and finally a sharp singlet peak was observed at 213 K. The results showed a nice contrast to those in the absence of the guest. The observation of a single singlet peak suggested that all four aromatic protons of **4** were in a magnetically equivalent environment. This indicated that a

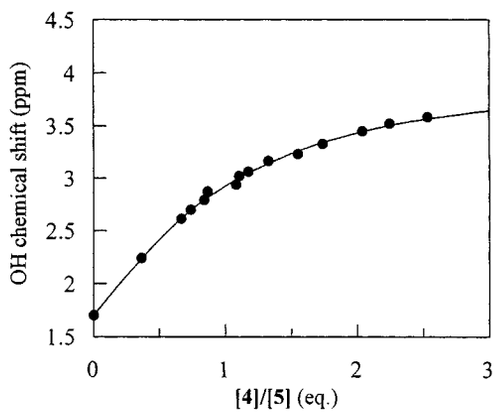


Fig. 2 ^{1}H NMR (500 MHz) titration data and the simulated curve fit for **4** binding **5** in $CDCl_3$, monitoring the hydroxy proton of **5**.

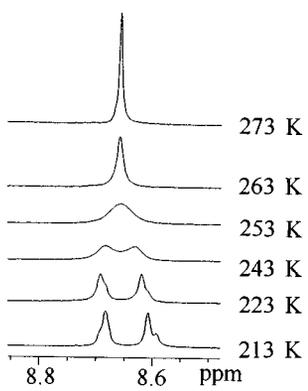
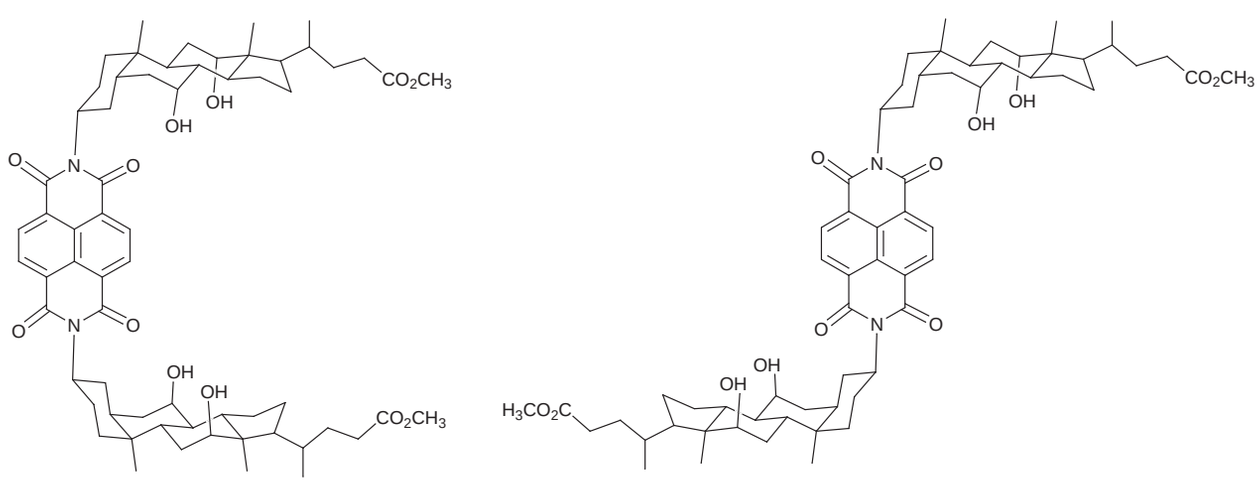
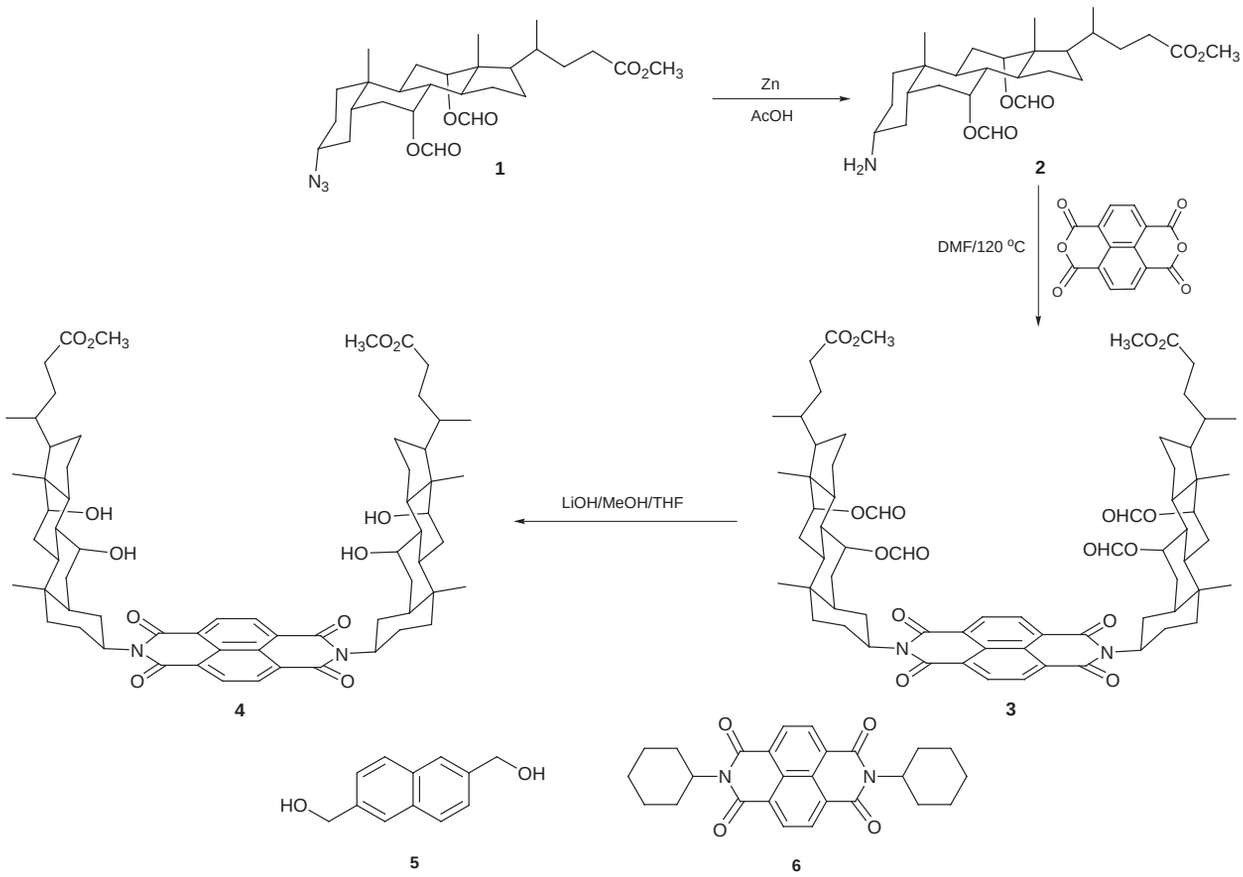


Fig. 3 VT ^{1}H NMR (400 MHz) spectra of the aromatic protons of **4** in $CDCl_3$.



Scheme 1



Scheme 2

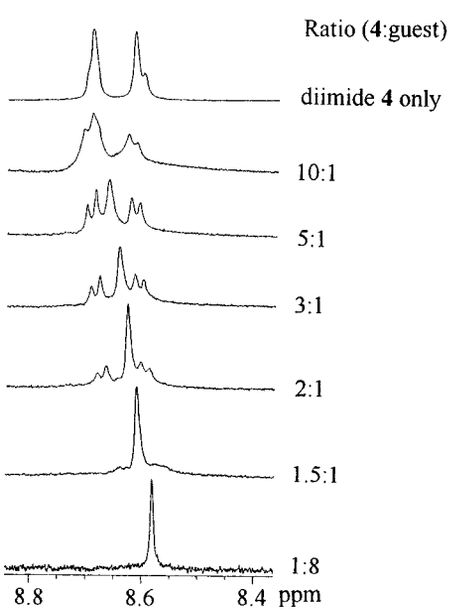


Fig. 4 ^{1}H NMR (500 MHz) spectra of the aromatic protons of **4** with varying amounts of **5** in $CDCl_3$ at 213 K.

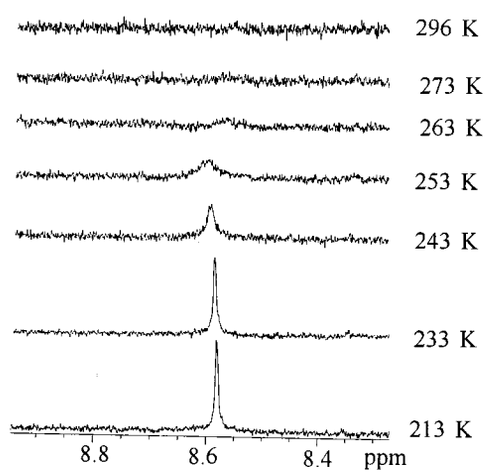


Fig. 5 VT ^{1}H NMR (500 MHz) spectra of the aromatic protons of **4** with 8 equiv. amount of **5** in $CDCl_3$.

unique fixed conformation of the associated complex between **4** and **5** existed with the host–guest ratio of 1 : 1. Such a conformation should be the cleft type conformation depicted as the structure of **4** in Scheme 2. This conformation is 33.6 kJ mol^{-1} less stable than the conformation B by MM2 calculation.

In order to demonstrate the significance of cholic acid moieties as the binding sites, a similar ^{1}H NMR study of the anhydride **6** possessing two cyclohexyl groups substituted at nitrogen atoms and the formyl derivative **3** was carried out with **5**. From the VT ^{1}H NMR study of **6** in $CDCl_3$, its rotational barrier was found to be 51.2 kJ mol^{-1} at the coalescence temperature (228 K). In contrast to **4**, **6** showed no chemical shift change upon addition of **5**. No change was also observed in the chemical shift of **5**, which indicated that **6** did not have the ability to recognize **4**. Thus the cholic acid moiety is required for association with **5**. The importance of the hydroxy groups in the cholic acid moiety for the recognition of **5** was confirmed by the titration experiment of the formyl derivative **3** with **5**. No meaningful binding (binding constant; 5 ± 13 M^{-1}) of **5** with **3** was observed. The large error was associated with the small shift change. These results suggested that the hydrogen bonding between the hydroxy groups of **4** and **5** would be the driving force for the formation of cleft type conformation.

As we demonstrated, the conformation of **4** was flexible in the absence of the guest molecule. Its podant part, the cholic acid moiety, was freely rotated at ambient temperature and it showed two stable conformations at 213 K, though these

two conformations were not suitable as a cleft. However, the addition of **5** at 213 K caused the fixation of the conformation of **4** as a cleft type. This kind of guest responsive conformational change of a host molecule is interesting to study dynamic process of self-assembling system.

Experimental

General

The melting points were determined on a Yanako MP-S3 melting-point apparatus or a Mac Science DSC 3100S and are uncorrected. IR spectra were obtained in a HITACHI I-2000 spectrometer. NMR spectra were recorded on JEOL LA-400 or LA-500 spectrometers in $CDCl_3$ with Me_4Si as an internal standard; *J* values are given in Hz. Mass spectra were obtained on a JEOL JMS-HX110. Reaction mixtures were concentrated on a rotary evaporator at 15–20 mmHg (1 mmHg = 133.322 Pa). Chromatographic separations were accomplished by flash column chromatography on silica gel (Fuji gel BW 200).

Preparation of tetrakisformyl dipodant 3

An acetic acid solution (20 cm^3) of 3α-azidocholanoate **1** (944.7 mg, 1.876 mmol) and zinc powder (4.000 g) was stirred for 24 h at room temperature. The progress of the reaction was monitored by tlc ($CHCl_3$: MeOH = 10 : 1). The reaction mixture was filtered by suction and the residue was washed with acetic acid (10 cm^3 × 2). A combined filtrate was evaporated and the residue was dissolved in chloroform (100 cm^3) and washed with saturated aqueous $NaHCO_3$ solution (100 cm^3). The organic layer was separated and washed with distilled water (100 cm^3). After evaporation of solvent, the crude 3α-aminocholanoate **2** thus obtained, was used for the preparation of **3** without further purification. To a dimethylformamide (DMF) solution of naphthalene-1,4,5,8-tetracarboxylic dianhydride was added dropwise a solution of crude **2** (927 mg, prepared as above mentioned) in 10 cm^3 of DMF during 0.5 h at 120 °C and stirred for an additional 2 h at this temperature. After evaporation of solvent, the residue was chromatographed on silica gel with $CHCl_3$–ethyl acetate (20 : 1) to give **3** (408 mg, 37%) as brown powder, mp 271.7 °C; ν_{max}(KBr)/cm^{-1} 2940, 1710, 1670, 1585, 1455, 1330, 1255, and 1180; δ_H(400 MHz; $CDCl_3$) 0.80 (6 H, s), 0.87 (6 H, d, *J* 6.3), 1.01 (6 H, s), 1.17–2.60 (46 H, m), 3.21 (2 H, q, *J* 12.9), 3.68 (6 H, s), 4.95 (2 H, br m), 5.11 (2 H, br s), 5.32 (2 H, br s), 8.09 (2 H, s), 8.34 (2 H, s) and 8.70 (4 H, s); HRMS (FAB) Calcd for $C_{68}H_{87}N_2O_{16}$: (MH^+), 1187.6056. Found: 1187.6044.

Preparation of tetraol dipodant 4

Deprotection of **3** was carried out by hydrolysis with LiOH. To a THF solution (15 cm^3) of **3** was added dropwise a saturated methanolic solution of LiOH under stirring. The progress of the reaction was monitored by tlc ($CHCl_3$–MeOH = 40 : 1). When the spot corresponding to **3** (R_f = 0.95) was completely (after the addition of 50 drops) converted to that of **4** (R_f = 0.2), distilled water (50 cm^3), brine (70 cm^3) and THF (100 cm^3) were added to the reaction mixture and an organic layer was separated. After evaporation of solvent, the residue was chromatographed on silica gel with $CHCl_3$–MeOH (20 : 1) to afford **4** (180 mg, 50%), mp 421.5 °C; ν_{max}(KBr)/cm^{-1} 3540, 2940, 1710, 1665, 1585, 1455, 1335, 1250, 1180 and 1095; δ_H(500 MHz; $CDCl_3$) 0.72 (6 H, s), 0.96 (6 H, s), 1.01 (6 H, d, *J* 6.1), 1.16–1.92 (38 H, m), 2.02 (4 H, m), 2.25 (2 H, m), 2.38 (2 H, m), 2.47 (2 H, m), 2.69 (2 H, m), 2.71 (2 H, q, *J* 12.0), 3.42 (2 H, q, *J* 13.0), 3.66 (6 H, s), 3.87 (2 H, br s), 4.02 (2 H, br s), 4.93 (2 H, br m) and 8.66 (4 H, s); HRMS (FAB) Calcd for $C_{64}H_{87}N_2O_{12}$: (MH^+), 1075.6259. Found: 1075.6204.

Preparation of dipodant 6

To a DMF solution (20 cm^3) of naphthalene-1,4,5,8-tetracarboxylic dianhydride (262.9 mg, 1.00 mmol) was added cyclohexylamine (0.229 cm^3, 2.00 mmol) quickly at 120 °C. The resulting mixture was continued to be heated for 15 h. After evaporation of solvent, chloroform (100 cm^3) was added to the residue. The chloroform layer was washed with aqueous 1 M HCl solution (50 cm^3) and then dried over anhydrous Na_2SO_4. After evaporation of solvent, the residue was chromatographed on silica gel with chloroform as eluent to give **6** (441 mg, 0.96 mmol, 96%) as pale yellow blocks, mp > 300 °C; ν_{max}(KBr)/cm^{-1} 2940, 1710, 1670, 1585, 1455, 1330, 1255, 1180 and 770; δ_H(400 MHz; $CDCl_3$) 1.33 (2 H, qt, *J* 13.3 and 3.4), 1.44 (2 H, qt, *J* 13.3 and 3.4), 1.65–1.80 (8 H, br m), 1.89 (4 H, br d, *J* 13.3), 2.31 (4 H, qd, *J* 12.0 and 3.6), 4.99 (2 H, tt, *J* 12.0 and 3.6) and 8.68 (4 H, s); MS (EI); 431 (M + 1), 430 (M^+, 22), 349 (95), 267 (31), 248 (21), 129 (14), 97 (27) and 57 (100); HRMS (FAB) Calcd for $C_{26}H_{26}N_2O_4$: (MH^+), 430.1893. Found: 430.1894.

^{1}H NMR study

^{1}H NMR spectroscopic titration for the determination of binding constant between **4** and **5** was carried out as follows. A measured amount of $CDCl_3$ solution of **4** (25 mmol dm^{-3}) was added to the $CDCl_3$ solution of **5** (2.0 mmol dm^{-3}, 0.50 cm^3), and after each addition the ^{1}H NMR spectrum of the mixture was recorded. The induced chemical shift value was plotted *vs.* the ratio of **4** to **5**. The binding constant was calculated using non-linear least square regression minimizing the value of $\Sigma(\delta_{obs} - \delta_{calc})$. For the variable temperature (VT) ^{1}H NMR study, the $CDCl_3$ solution (5.0 mml dm^{-3}, 0.50 cm^3) of **4** was used.

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Paper 8/08755E