Diastereoisomer-Selective Inclusion Complexation of Cinchona Alkaloids with a Modified β-Cyclodextrin: Fluorescent Behavior Enhanced by Chiral-Tether Binding

Short Communication

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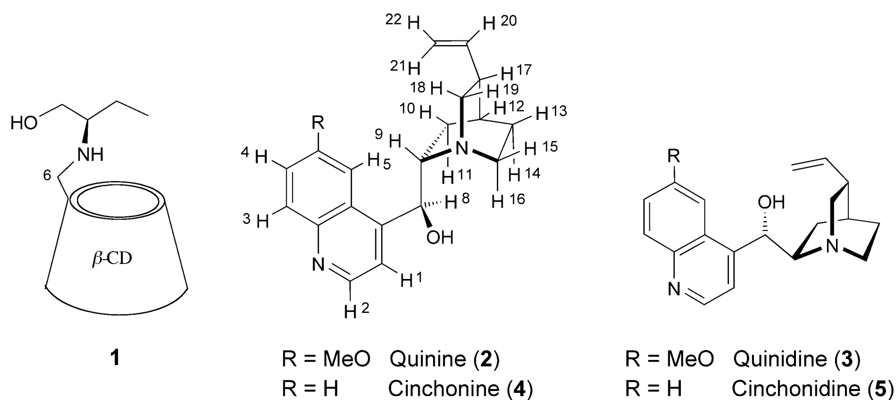
The molecular 1:1 complexation of cinchona alkaloids by mono(6-deoxy-6-{[(*R*)-1-(hydroxymethyl)propyl]amino})-β-cyclodextrin (**1**) in aqueous solution has been investigated by 2D-NMR, fluorescence titration, and fluorescence-lifetime experiments. Generally, with **1** as the host, in contrast to β-cyclodextrin proper, strong binding of quinine (**2**; $K_a = 84200$ M^{-1}) and quinidine (**3**; $K_a = 27300$ M^{-1}) at pH 6.8 was observed, as monitored by an increase in fluorescence intensity, with a fair degree of diastereoisomer discrimination (*ca.* 3:1). To rationalize these results, two possible cooperative complexation modes, including specific H-bonding interactions to the chiral tether of the cyclodextrin portion, are proposed.

Introduction. – The cinchona alkaloids are, from a commercial point of view, the most important alkaloid family. They are widely used in pharmaceutical industry, in soft drinks, as chiral auxiliaries (enantiomer separation and asymmetric syntheses), as chemical inhibitors, and as photosensitizer for biological systems [1–6]. Cyclodextrins (CDs) and their derivatives are excellent receptors, some of which exhibit chiral or enantioselective recognition upon complexation with a variety of chiral guests [7][8], and have been successfully applied to biomimetic chemistry [9], as well as in separation techniques [10]. Hence, a detailed understanding of the interactions between CDs and cinchona alkaloids in both chemical and biological systems are of general interest. To the best of our knowledge, diastereoisomer-selective binding of chiral alkaloids by modified β-CDs with chiral tethers have not been reported so far. Herein, we present our results on the binding behavior of mono(6-deoxy-6-{[(*R*)-1-(hydroxymethyl)propyl]amino})-β-cyclodextrin (**1**) [11] toward quinine (**2**) and quinidine (**3**) in aqueous buffer solution by means of both 2D-NMR and fluorescence experiments, including fluorescence-lifetime measurements.

Results and Discussion. – *Fluorescence-Titration Experiments.* We observed significant fluorescence-intensity enhancements of quinine (**2**) and quinidine (**3**) upon complexation with **1** (see *Fig. 1*), but little increase in the fluorescence of cinchonine (**4**) and cinchonidine (**5**), which entirely contrasts the fluorescence quenching of cinchona-alkaloid sulfates upon addition of (−)-(*R*)-2-aminobutan-1-ol in control experiments. Different results had been obtained with both native β-CD and bridged bis(β-CD), as reported previously [12] . This opposite spectral behavior (increase in fluorescence)



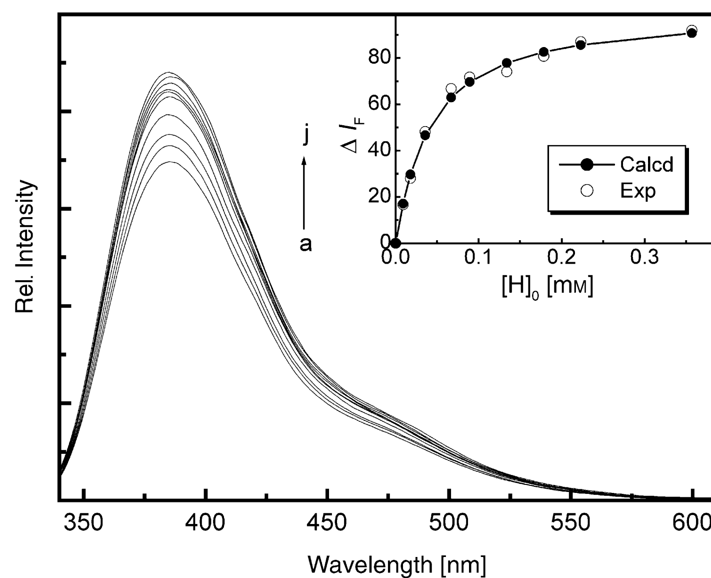


Fig. 1. *Fluorescence spectral changes* (curves *a–j*) of *quinidine* (**3**; 8 μM) *upon addition of the host* **1** (0–400 μM) *in aqueous phosphate buffer* (pH 6.8). Inset: calculated *vs.* experimental data points for nonlinear least-squares analysis of ΔI_F (differential fluorescence intensities) as a function of the host concentration $[H]_0$.

indicated a cooperative mode of binding, in which the chiral tether, the cavity of β-CD, and parts of the cinchona-alkaloidal guest were involved (see below).

To quantitatively investigate the complexation strength of **1** towards cinchona alkaloids, fluorometric titrations were performed at 25° in buffer solution at different pH values. As can be seen from *Fig. 1*, the fluorenscence intensity of quinidine gradually increased with increasing concentration of **1**. From the titration curves, the inclusion-complex association constant (K_a) was calculated by a non-linear least-

squares method [13]. As shown in the *Table*, the K_a values for the complexation of quinine and quinidine at pH 6.8 were determined to be 84200 and 27300 M^{-1}, respectively, which corresponds to a diastereoisomer selectivity of *ca.* 3:1. Unfortunately, the spectral changes were too small to allow the calculation of the corresponding K_a values of the inclusion complexes with natural β-CD.

Table. *Association Constants* (K_a) *and* Gibbs *Free-Energy Changes* ($-\Delta G^\circ$) *for the 1:1 Inclusion Complexes between Cinchona Alkaloids and Cyclodextrin* **1** *in Aqueous Buffer Solution.* The K_a values were determined by fluorometric titration at 25°, followed by non-linear least-squares curve fitting.

Guest	K_a [M^{-1}]	$-\Delta G^\circ$ [kJ · mol^{-1}]	pH[a])
Quinine (**2**)	[b])	–	2.0
	84200 ± 300	28.11	6.8
	2680 ± 70	19.57	10.5
Quinidine (**3**)	[b])	–	2.0
	27300 ± 200	25.33	6.8
	970 ± 30	17.05	10.5

[a]) Aq. HCl buffer (pH 2.0), phosphate buffer (pH 6.8), and $NaHCO_3$ buffer (pH 10.5) were used. [b]) The spectral changes were too small to allow the calculation of K_a.

Compared to β-CD proper, the enhanced binding ability of **1** towards quinine/quinidine may be attributed to stronger *Van der Waals*, hydrophobic, and additional H-bonding interactions between the host and the guests due to the additional chiral [1-(hydroxymethyl)propyl]amino substituent, which replaces one of the CD's primary OH functions in 6-position, potentially leading to cooperative binding by the chiral tether and the CD cavity. Interestingly, at pH 10.5, the K_a values decreased remarkably to 2680 and 970 M^{-1} for quinine and quinidine, respectively. Even more so, at pH 2, no binding was observed.

Binding Mode. To elucidate the structural features responsible for the remarkable complexation selectivity and the enhanced fluorescence intensity of quinine/quinidine in the complex with **1** relative to β-CD, 2D ^{1}H-NMR experiments were performed in neutral D_2O at 25°. The NOESY spectrum of **1** displayd no NOE cross-peaks between the H-atoms of the chiral tether and those at C(3), C(5), and C(6) of the β-CD moiety, suggesting that the 1-[(hydroxymethyl)propyl]amino group in **1** protrudes from the CD cavity.

In the NOESY spectra of the 1:1 complexes quinine · **1** (*Fig. 2*) and quinidine · **1** (*Fig. 3*), no NOE cross-peaks were observed between H–C(3) and H–C(5) of **1** and the H-atoms of the quinoline ring, suggesting that the quinoline moiety of the guests does *not* reside in the cavity of the host, which is in excellent agreement with the results obtained by fluorescence-lifetime experiments. However, the spectrum of quinine · **1** exhibited clear NOE cross-peaks between the 3-ethenyl-1-azabicyclo[2.2.2]octane unit and the H–C(5) and CH_2(6) H-atoms. For example, in *Fig. 2*, *A* and *B* represent the NOE cross-peaks between H–C(5) of the host (**1**), and H(20) and H(17) of the guest (quinine)[1]). Regions *C*, *D*, and *E* represent the NOEs between H(10), H(11), and H(15)–H(17) of quinine, and the CH_2(6) group of **1**. These cross-peaks demonstrate

[1]) Trivial atom numbering; see chemical formulae of **2** and **3**.

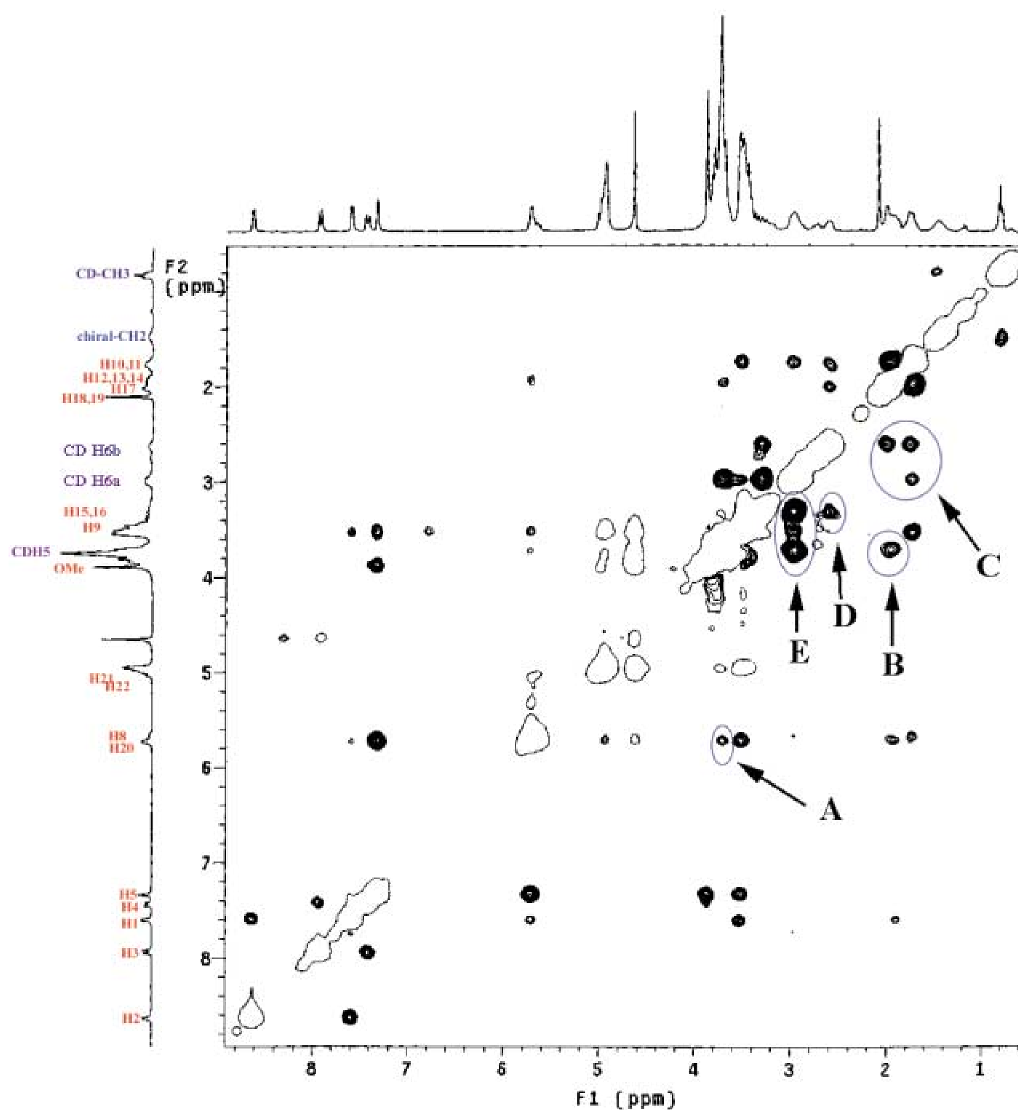


Fig. 2. *^{1}H-NMR NOESY Spectrum* (300 MHz) *of a 1:1 mixture of* **1** *and quinine* (**2**) (10^{-3} M each) *in D_2O at 298 K after a mixing time of 0.6 s.* Red signals belong to the guest, blue ones to the host. Important cross-peaks are marked.

that the azabicyclo[2.2.2]octane moiety moiety is, at least partly, accommodated in the cavity of the host, entering from the primary side of **1**.

In the case of the complex quinidine · **1**, cross-peaks were observed between the H-atoms of the 3-ethenyl-1-azabicyclo[2.2.2]octane moiety next to the quinoline group and the CH_2 H-atoms of the chiral tether (signals *G* in *Fig. 3*), and also between those of the 3-ethenyl-1-azabicyclo[2.2.2]octane moiety pointing away from the quinoline

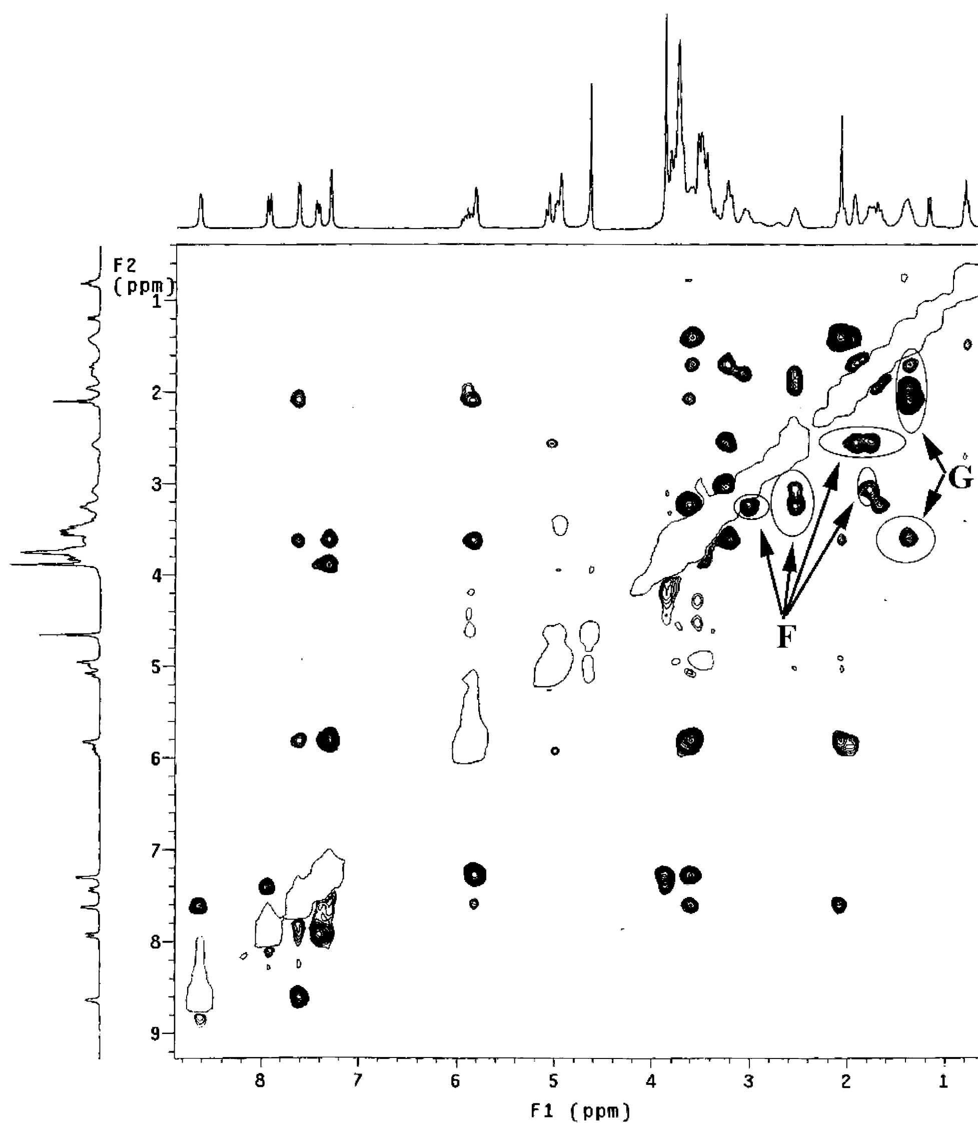


Fig. 3. *^{1}H-NMR NOESY Spectrum* (300 MHz) *of a 1:1 mixture of* **1** *and quinidine* (**3**) (10^{-3} M each) *in D_2O at 298 K after a mixing time of 0.6 s.*

group and H_a–C(6a) and/or H_b–C(6b) (signals *F* in *Fig. 3*). We, thus, infer that the quinidine molecule is positioned on top of the CD cavity of **1** (see below).

Interestingly, cinchonine (**4**) and cinchonidine (**5**), which lack the quinoline MeO group (R = H), did not show any significant fluorescent changes upon addition of **1**. This indicates that the complexation of quinine/quinidine relative to cinchonine/ cinchonidine profits from enhanced *Van der Waals* and hydrophobic interactions

between host and guest, but also from additional H-bonding interactions between the OH group of the chiral tether and the MeO group of the quinoline nucleus. Based on these observations, we propose the structures **A** and **B** shown in *Fig. 4* for the quinine · **1** and quinidine · **1** complexes, respectively[2]). These binding modes were supported by a molecular-modeling study performed with the HyperChem (v. 6.01) and the WebLab-ViewerPro-3.7 software (*Fig. 5*).

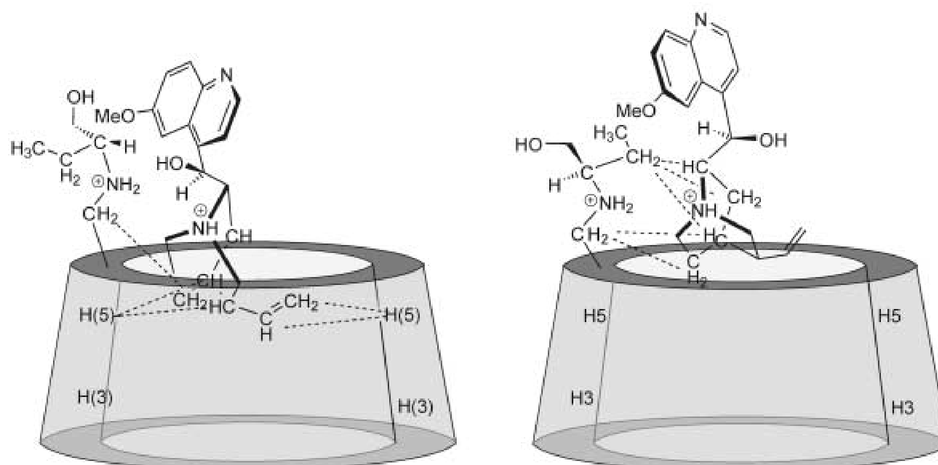


Fig. 4. *Plausible molecular structures of the inclusion complexes* **A** *and* **B** *between the host* **1** *and* a) *quinine, and* b) *quinidine*, *resp.* See also *Fig. 5*.

The immobilization of quinine and quinidine upon complexation reduces their conformational freedom, which nicely explains the observed enhancement in fluorescence intensity (reduced degree of internal conversion of the excited states). In quinine · **1**, the complex stability probably arises from *a*) the inclusion of the 3-ethenyl-1-azabicyclo[2.2.2]octane moiety into the cavity of **1**; *b*) possible H-bonding between the OH group of the chiral tether and the MeO group of the guest; and *c*) possible H-bonding between the suitably oriented (*S*)-configured OH group at C(8) of the guests with the NH group of **1**. For the complex quinidine · **1**, the position of the 3-ethenyl-1-azabicyclo[2.2.2]octane moiety and the configurations at C(8) and C(9) of the guest, as well as the (*R*)-configuration at C(1) of the tether, hamper the formation of these H-bonds. This would rationalize the observed differences in complexation strength for quinine over quinidine. Finally, the observed pH sensitivity (poor or no binding under basic and acidic conditions, respectively) may be due to H^+- or OH^--promoted destruction of the optimum complex geometries between host and guests, a feature that can be readily exploited to control the binding strength of these systems.

[2]) At neutral pH, the quinoline N-atom in **1** and the N-atoms of quinine and quinidine [6b] are protonated.

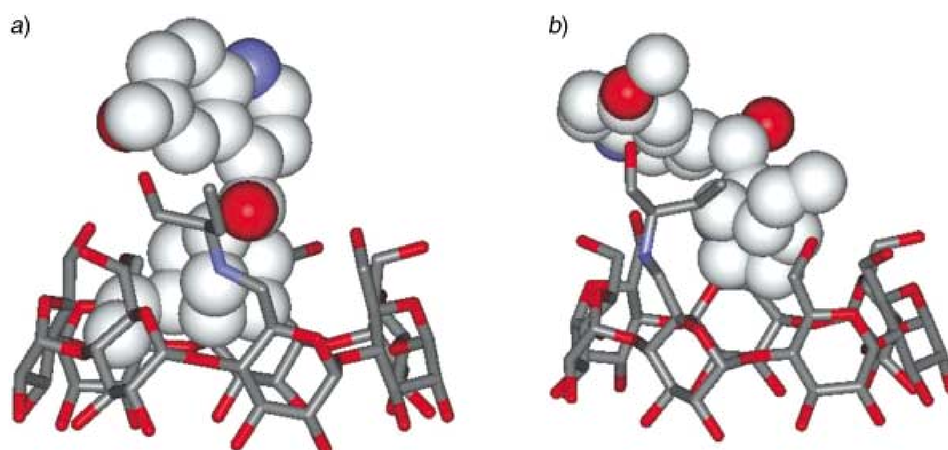


Fig. 5. *Molecular modeling of the inclusion complexes* **A** *and* **B** *between* a) **1** *and quinine, and* b) **1** *and quinidine.* The HyperChem (v. 6.01 for Windows) and WebLab-ViewerPro-3.7 software packages were used, respectively.

Conclusions. – The diastereoselective recognition of cinchona alkaloids by the chirally-modified β-CD **1**, relative to natural β-CD, resulted in an unusually strong binding, with K_a values rising from essentially zero to *ca*. 84000 (quinine) and 27000 M^{-1} (quinidine) at pH 6.8, as monitored by fluorescence titration and 2D-NMR experiments. These results are interesting in terms of quinine/quinidine-promoted diastereoisomer separation of pharmaceutically active (bio)molecules in aqueous solution. We proposed a cooperative binding mode, with steric, H-bonding, and hydrophobic interactions being crucial for inclusion complexation. A more-detailed understanding of the supramolecular properties of modified cyclodextrins, which are excellent models for mimicking substrate-specific interaction of enzymes, would certainly be helpful. Our results have revealed the potential of chiral-tether-modified β-CDs as stereo-discriminating hosts that hold promise for future applications in 'chirotechnology'.

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