JOC*Note*

Chiral Resolution through Precipitation of Diastereomeric Capsules in the Form of 2:1 β-Cyclodextrin−Guest Complexes

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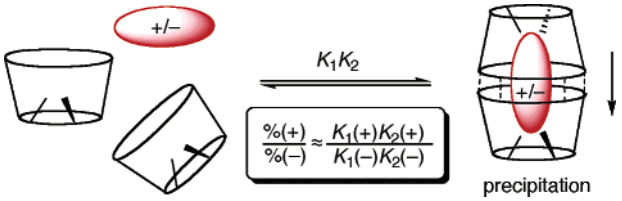

Preferential precipitation of one enantiomer from a racemic mixture of a camphanate ester of 2,3-diazabicyclo[2.2.2]oct-2-ene was induced by the formation of diasteromeric 2:1 β-cyclodextrin−guest complexes. The precipitate was enriched with the (−)-enantiomer and the supernatant solution with the (+)-form of a camphanate ester, which was quantitatively analyzed in terms of differential binding constants and intrinsic solubilities of the 2:1 complexes. The enantiomeric excess in the precipitate was determined as 30 ± 3% by induced circular dichroism.

Cyclodextrins (CDs) are natural macrocyclic oligosaccharide hosts that can enclose small guest molecules in their hydrophobic cavity.[1,2] Their inherent chirality, in particular, has led to applications as chiral selectors,[3] as stationary phases in chromotographic separations,[2] or as NMR shift reagents.[4] All of these analytical applications are based on the fact that the inclusion complexes between CDs and enantiomeric guests are intermolecular but diastereomeric in nature, with the associated differences in physical properties.

In contrast to the wealth of applications of CDs to enantioseparation on an analytical scale, mostly chromatography and electrophoresis, there are few reports on the use of CDs for the classical chiral resolution on a preparative scale, i.e., through precipitation, although the diasteromeric complexes are expected to vary in solubility and crystallization behavior. One underlying problem is that CDs have been traditionally used to *enhance* the solubility of hydrophobic guests, e.g., drugs.[5] The desired host-induced precipitation of the guest is therefore not applicable, but instead a guest-induced precipitation of the host[6] has to be chosen as the route for chiral resolution. This has been documented by Cramer and Dietsche in their pioneering study, which afforded an optical activation of 3−12% by precipitation from saturated β-CD solutions with a 5−10-fold *excess of racemic guest*.[7] Methodologically, this less common approach to chiral resolution is not economical, since the (less precious) chiral resolving agent is precipitated with an excess of racemate rather than added to precipitate a dilute racemate. The Cramer method was later employed for partial resolution of chiral phosphinates,[8] sulfinyl compounds,[9] and fenoprofen.[10] The latter X-ray crystallographic study, which revealed enantiomeric enrichment of fenoprofen in 1:1 complexes of β-CD during differential crystal growth on a microscopic scale, has been considered as the only exception of an appreciable partial resolution of a racemate by natural CDs.[11]

Since CD-assisted stereodifferentiation in the solid state from racemate solutions has proven to be difficult for *natural* CDs, alternative methods have involved synthetic (per)methylated CDs, which are generally considered to be better host molecules for chiral discrimination purposes than natural CDs due to their higher flexibility.[11−14] Nevertheless, even with the structurally modified CDs, the achieved ee in the precipitates was found to be less than 30%, unless repeated precipitation of enantiomerically enriched fractions was pursued,[13,14] or unless the guest was selected to be in a racemization equilibrium,[11,12] which presents an impressive yet very special case. In some cases, the enantioseparation relied on differential crystal growth kinetics[15] rather than on intrinsic solubility differences,[14] which further limits routine use.

In short, the use of CDs for chiral resolution through precipitation has been limited to examples employing either an excess of racemate or synthetic CD derivatives. In this Note, we document and quantitatively analyze a differential precipitation of chiral guests that employs unmodified β-CD and exploits the low solubility[16] of higher order (2:1) complexes, in which two host molecules encapsulate a single bifunctional chiral guest.

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SCHEME 1

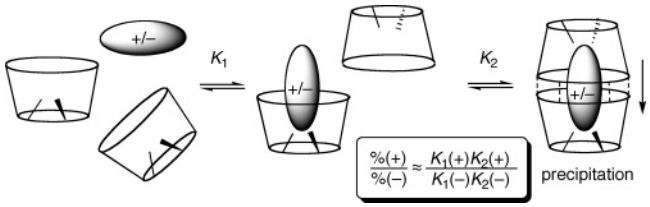
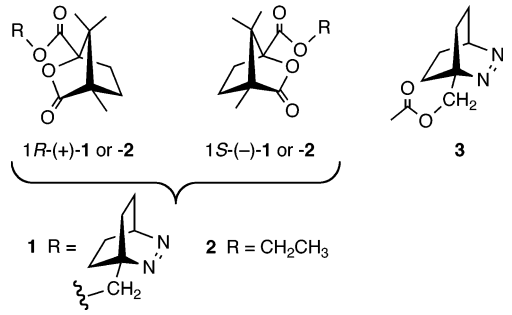


CHART 1



Results and Discussion

The general concept which we introduce in this Note relies on the formation of precipitating capsules self-assembled from a chiral guest molecule and two chiral host molecules (Scheme 1). The interior of the capsule is decisive for chiral recognition, while the exterior shell imposes a low solubility, which is distinct from previous applications of capsules in chiral recognition.[19] The presently explored method allows for a chiral resolution of racemate solutions solely on account of differential binding constants, while the solubility of the precipitating species may be identical. In other words, the chiral guest serves as a template for the formation of a capsule with low solubility. The precipitating capsules are formed with apparent equilibrium constants K_1K_2, the product of the microscopic binding constants. It is therefore expected, for precipitations on a preparative scale, that the enantiomeric ratio in the precipitate resembles the ratio of the apparent equilibrium constants, which leads to a chiral resolution by preferential encapsulation.

As chiral guest molecules we have selected camphanate esters of the water-soluble azoalkane 2,3-diazabicyclo-[2.2.2]oct-2-ene (DBO), which has recently been established as a versatile nonaromatic probe for CD complexation. It is accessible to a range of optical spectroscopies, including near-UV absorption and fluorescence, and has the potential to afford information on the solution structures of CD complexes[20,21] and the kinetics of complexation.[18,22] The probe binds strongest with β-CD, which was therefore selected as the chiral host. The camphanate moiety served as a chiral auxiliary, and the esters **1** were prepared from the hydroxymethyl derivative of DBO and camphanic acid chloride in enantiopure as well as racemic form. For reference purposes, we also prepared the individual components, i.e., the enantiopure ethyl camphanates **2** as well as the achiral acetate **3**. Precipitation from saturated β-CD solutions (14 mM) was observed when the concentration exceeded 0.38 mM for the (+)-enantiomer and 0.30 mM for the (−)-enantiomer of **1**, suggesting a lower solubility of the (−)-form in the presence of β-CD. The precipitates were characterized by NMR spectroscopy. Figure 1 shows the ^{1}H NMR spectrum of the precipitate obtained by mixing 2 mM (+)-**1** and 12 mM β-CD, redissolved in D_2O with DMF as an internal standard. The integration of the peaks corresponding to β-CD protons between 3.5 and 3.6 ppm (14 H, H-2 and H-4) and the upfield-shifted *gem*-dimethyl (1.08 ppm, 6H) and bridgehead methyl group (0.96 ppm, 3 H) of the guest revealed the precipitate as a ternary 2:1 β-CD·**1** complex.

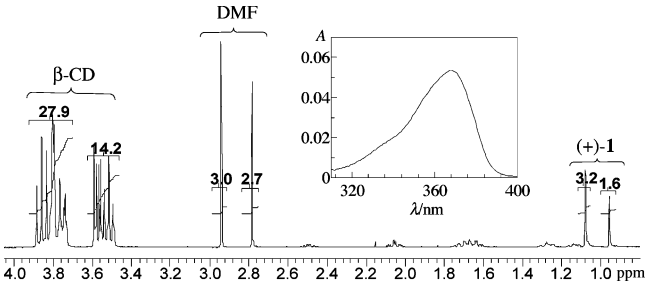
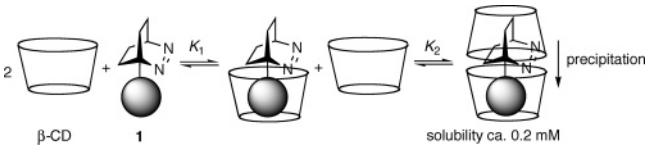


FIGURE 1. ^{1}H NMR spectrum of the precipitate obtained from (+)-**1** redissolved in D_2O containing 2 mM DMF; the inset shows the corresponding UV absorption spectrum of the same solution. Both experiments corroborate that the precipitate contains 1 mM **1** and 2 mM β-CD; see text.

SCHEME 2. Sequential Host−Guest Complexation of 1



The proportion of guest in the precipitate was independently established spectrophotometrically by measuring the absorbance of the redissolved precipitate in the near-UV range, where the DBO chromophore has a weak but characteristic absorption band (ϵ ca. 50 M^{-1} cm^{-1}).[22] For this purpose, a weighed amount of precipitate was redissolved, and from the UV absorbance (see inset of Figure 1) the host:guest ratio could be determined as 2:1.

On the basis of the NMR and UV evidence, we concluded that the precipitation was a consequence of the low solubility of the 2:1 inclusion complex. Presumably, the hydroxyl groups of the upper rim, which are important for solubilizing β-CD in water,[23] are involved in promoting the binding between the two CD units of the 2:1 complex to form a capsule (Scheme 2), thereby reducing its solubility. Since both moieties of the enantiopure guest (DBO and camphanate) have an intrinsic

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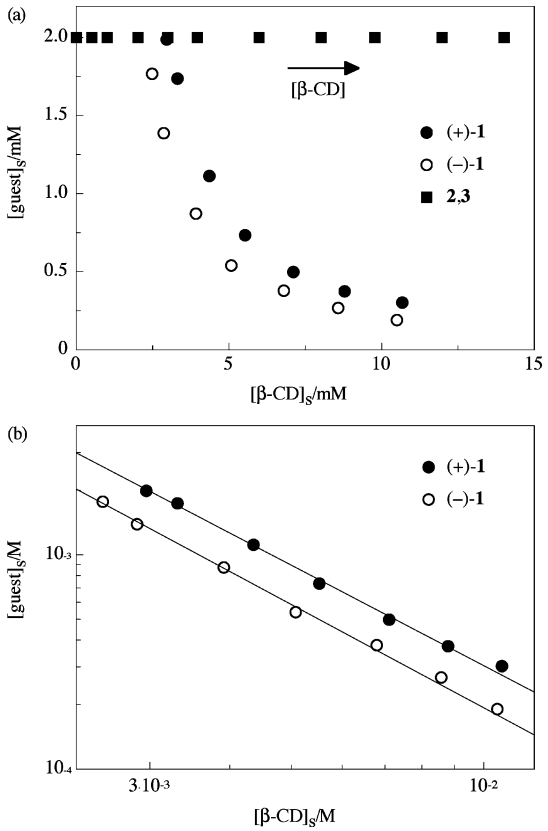

FIGURE 2. (a) Plot of the concentration of guest vs β-CD, both determined by ^{1}H NMR in the supernatant solution upon addition of varying amounts of β-CD to 2 mM guest solutions. (b) Corresponding double-logarithmic plot in the precipitation range for (+)/(−)-**1**.

TABLE 1. Binding Constants and Solubility Parameters for Enantiomers 1 in the Presence of β-CD in D_2O

guest	K_1 (M^{-1})	K_2 (M^{-1})	K_1K_2 (M^{-2})	K_{sp} (10^{-6} M^2)	S (mM)[a]	$[G]_{max}$ (mM)[b]
(+)-	1200 ± 180	130 ± 10	156 000	1.7 ± 0.3	0.22 ± 0.03	0.38
(−)-	1590 ± 190	210 ± 20	333 900	1.0 ± 0.2	0.21 ± 0.04	0.30

[a] Solubility of 2:1 complex. [b] Maximum (experimental) concentration of **1** not inducing precipitation in a saturated (14 mM) β-CD solution.

binding affinity with β-CD, quite weak for DBO ($K = 320 \pm 10$ M^{-1} for **3**) and weaker for the (+)-form of **2** (1090 ± 50 M^{-1}) than for the (−)-enantiomer (1320 ± 50 M^{-1}), we can schematically view the complexation process as an initial formation of a 1:1 complex with a binding constant K_1, in which the camphanate moiety preferentially enters the CD cavity (Scheme 2). This is followed by the formation of a 2:1 complex, in which the second moiety (DBO) is complexed by a second CD with a binding constant K_2, eventually followed by precipitation when the solubility limit is exceeded.

To quantify the amount of precipitation, we performed solubility measurements by NMR titrations, i.e., we successively added β-CD to 2 mM guest solutions, and determined the concentrations of guest and host retained in the supernatant. The resulting concentrations are plotted in Figure 2, which reveals the lower solubility of the (−)-enantiomer (Table 1). The double-logarithmic plots are linear ($r^2 > 0.99$, Figure 2b), and the slopes are larger than 1 (ca. 1.6), which is in line with the precipitation of a 2:1 complex.[16] In control experiments, no change in the solubility of reference compounds **2** and **3** was observed, suggesting that the individual moieties form soluble 1:1 complexes (complexation-induced shifts) but do not tend to form 2:1 complexes (also confirmed by Job plot analysis) and, therefore, do not precipitate, consistent with Scheme 2. The lower solubility of (−)-**1** could be principally due to two reasons: (i) a differential intrinsic solubility of the diastereomeric 2:1 complexes or (ii) different proportions of 2:1 complex in equilibrium resulting from different binding constants K_1 or K_2 or both. To discriminate between these two possibilities, accurate determinations of the microscopic binding constants as well as the intrinsic solubility needed to be performed.

The quantitative analysis of titration plots for sequential 1:1 and 2:1 host−guest binding phenomena is nontrivial even in cases where one concentration (either guest or host) can be kept constant. A closed analytical solution is not available,[21] such that algorithms to solve the related cubic equation are required. In the present case, the analysis became more intricate since both concentrations vary due to the interference of precipitation. We have therefore expanded the algorithm based on the cubic equation by the explicit consideration of two variables, cf. Supporting Information. This allowed the fitting of the complexation-induced shifts from the NMR titrations to afford the equilibrium constants K_1 and K_2. The solubility of 2:1 complex (S) was obtained in the same fitting procedure from the relationship $S = K_2[\beta\text{-CD}\cdot\mathbf{1}][\mathbf{1}]$. All data are listed in Table 1. Also included in Table 1 is the highest concentration of guest, which did not induce precipitation from a saturated β-CD solution, $[G]_{max}$. The values for K_1 and K_2 have been independently reproduced (within 10% error) by induced circular dichroism and isothermal microcalorimetry titrations in a lower concentration range where no precipitation interfered.

Note that the absolute solubility of the 2:1 complexes (S) is the same for both enantiomers, within error. The lower solubility of (−)-**1** is, however, reflected in the solubility products (K_{SP}), with $K_{sp} = [\beta\text{-CD}\cdot\mathbf{1}][\mathbf{1}] = S/K_2$, such that its higher tendency for precipitation can be traced back to differential values of K_2; in addition, the higher K_1 value also favors precipitation of (−)-**1**, since it increases the 1:1 complex concentration that enters the expression for K_{sp} as well. The product K_1K_2 is therefore decisive for chiral discrimination and reveals a factor of 2 difference (Table 1).

To illustrate the consequences of the differential solubility of the enantiomers on a preparative scale, a precipitation was performed from a 2.0 mM racemic solution of **1** (10 mL) by addition of 5.0 mM β-CD. The circular dichroism spectra of the redissolved precipitate and the supernatant solution (Figure 3) immediately indicated that the solution had been enriched with the (+)-enantiomer and the precipitate with the (−)-form, in line with the projected solubility data (note that the circular dichroism spectra are not symmetric due to the presence of β-CD, which contributes an *induced* circular dichroism.[18,20,21,24]

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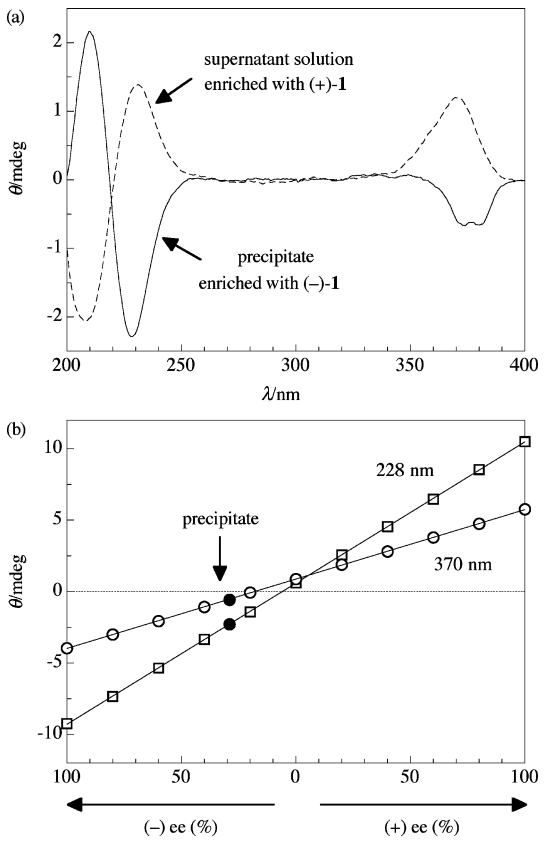

FIGURE 3. Circular dichroism analysis of the precipitation process induced by adding 5.0 mM β-CD to a 2.0 mM racemic solution of **1**. (a) Spectra of the supernatant solution after precipitation (- -) and the solution of redissolved (adjusted to 1 mM guest content) precipitate (—) in D_2O. (b) Determination of ee in the precipitate from calibration lines.

The enantiomeric excess in the precipitate (ee) was representatively quantified by measuring reference circular dichroism intensities[25] of samples with the same concentration of **1**, but different ee, in the presence of twice the concentration of β-CD to correct for the induced circular dichroism. Linear correlation lines ($r^2 > 0.99$, Figure 3b) were obtained at two different wavelengths (DBO absorption at 370 nm and camphanate absorption at 228 nm), which allowed a determination of the ee in the obtained precipitate as $30 \pm 3\%$. While moderate, this value is actually comparable to the optical purity achieved in preparative crystal growth experiments with modified CDs[13,14] and larger than the ee achieved in the original precipitation experiments by Cramer and Dietsche, which were conducted in the presence of a large excess of guest.[7] More importantly, the experimental enantiomeric ratio (2:1) is in excellent agreement with that expected (see above) from the ratio of the apparent equilibrium constants (K_1K_2 values in Table 1). This establishes the present method as a rational approach to chiral resolution, although the absolute variation of the binding constants and therefore the chiral resolution is relatively small for the presently selected pair of enantiomers.

The advantages of the present 2:1 precipitation method, while remote from broad applicability, are that (i) natural CDs are employed, (ii) the guest to be enantiomerically enriched can be used as a limiting reagent, and (iii) the enantiomeric enrichment is predictable on the basis of the binding constants (K_1K_2 product). In terms of the general concept described in the outset, we have formed capsules of limited solubility, namely, 2:1 CD complexes. We attribute the lower solubility of the capsules to the formation of intermolecular hydrogen bonds between the two hosts, which are thus no longer available to promote solubilization through hydrogen bonding with water, as is possible for the free CD and 1:1 CD complexes. A similar approach may therefore be applicable to other hydrogen-bonded capsules.[19]

In summary, we have analyzed in detail an intricate equilibrium involving the formation of 1:1 and 2:1 CD complexes with an enantiomeric guest displaying a concomitant precipitation of the 2:1 complex. The differential binding constants for 1:1 as well as 2:1 complex formation result in a lower solubility of one enantiomer in the presence of β-cyclodextrin. This can be exploited to achieve a chiral resolution by precipitation of the diastereomeric 2:1 complexes, as evidenced by circular dichroism spectroscopy.

Experimental Section

Materials. The commercial materials 1*R*-(+)- and 1*S*-(−)-camphanic acid chloride (>98%), δ-cyclodextrin (>98%), acetyl chloride (>99%), and silica gel 60 were used as received. 1-Hydroxymethyl-2,3-diazabicyclo[2.2.2]oct-2-ene was synthesized according to a literature procedure.[17,18] D_2O (>99%) was used as solvent for all spectroscopic measurements.

General Procedure for Esterification of Alcohols. The respective acid chloride (2 mmol) in 3 mL of anhydrous pyridine was added dropwise to a stirred solution of the corresponding alcohol in 3 mL of anhydrous pyridine (1.5 mmol) containing a catalytic amount of 4-(dimethylamino)pyridine at 0 °C under a dry nitrogen atmosphere. The mixture was stirred for 2 h and then extracted and washed three times, first with 10 mL of a 10% sodium hydrogen carbonate solution and then twice with 10 mL saturated aqueous NaCl. The organic phase was dried and concentrated under reduced pressure. Subsequent purification by silica gel chromatography (98:2 CH_2Cl_2/MeOH) followed by 3-fold recrystallization from *n*-hexane provided the purified ester. The compound characterization data for (+)-**1** are given below; those for (−)-**1**, **2**, and **3** are given in Supporting Information.

(+)-1. Yield 68%, mp 86−87 °C; UV (H_2O) $\lambda_{max} = 366$ nm, $\epsilon = 57$ M^{-1} cm^{-1}; circular dichroism (D_2O) $\lambda_{max} = 370$ nm, $\Delta\epsilon = 0.16$ M^{-1} cm^{-1}; $\lambda_{max} = 226$ nm, $\Delta\epsilon = 0.30$ M^{-1} cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.99 (s, 3H), 1.07 (s, 3H), 1.12 (s, 3H), 1.15−1.42 (m, 4H), 1.56−1.74 (m, 5H), 1.88−1.98 (m, 1H), 2.02−2.11 (m, 1H), 2.42−2.52 (m, 1H), 4.78 (dd, 2H, *J* 6.36, 11.43), 5.18 (bs, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 9.9, 17.0, 21.5, 23.6, 29.1, 30.9, 31.0, 54.5, 55.0, 61.8, 65.6, 69.8, 91.4, 167.6, 178.4; FAB-MS *m/z* 321 $(M - H)^+$. Anal. Calcd for $C_{17}H_{24}N_2O_4$: C, 63.73; H, 7.55; N, 8.74; O, 19.97. Found: C, 63.82; H, 7.63; N, 8.70; O, 19.95.

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Supporting Information Available: Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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