Chiral Recognition Thermodynamics of β-Cyclodextrin: The Thermodynamic Origin of Enantioselectivity and the Enthalpy−Entropy Compensation Effect

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Abstract: The complex stability constant (K), standard free energy ($\Delta G°$), enthalpy ($\Delta H°$), and entropy ($\Delta S°$) for the 1:1 inclusion complexation of 43 enantiomeric pairs of chiral guests with β-cyclodextrin at 25 °C have been determined by microcalorimetry. The overall complexation thermodynamics are related to variations in the structure of the cyclic and acyclic guest, including its aromatic or aliphatic nature, the chain length, branching, flexibility, charge, and incorporated oxygen atom. The differences in the thermodynamic parameters due to the chirality are comprehensively discussed in terms of the stereochemistry, skeleton, chain length, and functional groups of the guest, and the mode of penetration upon inclusion complexation. The enthalpy−entropy compensation plot, using the differential thermodynamic parameters ($\Delta\Delta H°$ and $\Delta T\Delta S°$ at 298.15 K) for the chiral recognition equilibrium, gave an excellent straight line of unit slope, from which the isokinetic, or isoenantiodifferentiating, temperature was calculated as 25 °C for this chiral recognition system using a β-cyclodextrin host.

Introduction

The naturally occurring chiral α-, β-, and γ-cyclodextrins (CDs) are the first receptor molecules whose ability to bind organic molecules has been recognized and extensively studied by various experimental techniques.[1−6] A wide variety of compounds which can be included in natural α-, β-, and γ-CDs with different cavity sizes (top/bottom diameters of the cavity: 4.7/5.3, 6.0/6.5, and 7.5/8.3 Å respectively) have been subjected to systematic complexation studies. These cover almost every class of guest compound, including hydrocarbons,[7,8] aliphatic alcohols,[8−14] diols,[13,15] amines and acids,[16] cyclohexanes,[17] amino acids,[18−19] oligopeptides,[20] sugars,[21] phenols,[22−24] aromatic amines,[25] azo compounds,[26−29] naphthalene derivatives and other aromatic compounds,[30−33] and various pharmaceuticals.[34−39] In this context, it is rather surprising that only relatively limited efforts have been devoted to thermodynamic

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studies, not only to evaluate the complex stability constant, but also to determine the reaction enthalpy and entropy for the inclusion complexation of enantiomeric pairs with naturally occurring CDs. To date, reported studies of this sort include the complexation of α-CD with norleucine and norvaline,[40] 1-ferrocenylethanol,[41] phenylalanine, α-methylbenzylamine, mandelic acid, phenylfluoroethanol, and amphetamine.[42a] However, the last two studies were performed under conditions that allowed the coexistence of different species of guest and/or CD in the solution. The complexation thermodynamics of several carbohydrates with α- and *β*-CDs were also investigated,[21] as well as our own works concerning the complexation thermodynamics of α- and *β*-CDs with 2-alkanols[14] and ephedrines and pseudoephedrines.[25]

In this study, we have carried out microcalorimetric measurements to obtain accurate thermodynamic quantities for the inclusion complexation of 43 enantiomeric pairs of selected guests with *β*-CD in aqueous buffer solutions. On the basis of the thermodynamic parameters obtained for several families of structurally related chiral guests, the relationship between the guest's structure and the enantioselectivity has been elucidated, and the mechanisms and thermodynamic origin of the chiral recognition displayed by CDs is discussed.

Experimental Section

Materials. The host and guest compounds used in this study, their Chemical Abstracts registry number, empirical formula, formula weight, and supplier (A = Aldrich, B = Bachem, F = Fluka, L = Lancaster, S = Sigma, W = Wako) are as follows: α-cyclodextrin, 10016-2-3, $C_{36}H_{60}O_{30}$, 972.85, S; *β*-cyclodextrin, 68168-23-0, $C_{42}H_{70}O_{35}$, 1135.0, A; 1-propanol, 71-23-8, C_3H_8O, 60.10, W; 1-butanol, 71-36-3, $C_4H_{10}O$, 74.12, W; 1-pentanol, 71-41-0, $C_5H_{12}O$, 88.15, W; 1-hexanol, 111-27-3, $C_6H_{14}O$, 102.18, W; cyclohexanol, 108-93-0, $C_6H_{12}O$, 100.16, W; (*R*)-2-phenylbutyric acid, 938-79-4, $C_{10}H_{12}O_2$, 164.20, A; (*S*)-2-phenylbutyric acid, 4286-15-1, $C_{10}H_{12}O_2$, 164.20, A; (*R*)-camphanic acid, 67111-66-4, $C_{10}H_{14}O_4$, 198.22, A; (*S*)-camphanic acid, 13429-83-9, $C_{10}H_{14}O_4$, 198.22, A; (*R*)-1-cyclohexylethylamine, 5913-13-3, $C_8H_{17}N$, 127.23, F; (*S*)-1-cyclohexylethylamine, 17430-98-7, $C_8H_{17}N$, 127.23, F; (*R*)-mandelic acid, 611-71-2, $C_8H_8O_3$, 152.15, A; (*S*)-mandelic acid, 17199-29-0, $C_8H_8O_3$, 152.15, A; (*R*)-hexahydromandelic acid, 53585-93-6, $C_8H_{14}O_3$, 158.20, F; (*S*)-hexahydromandelic acid, 611475-31-8, $C_8H_{14}O_3$, 158.20, F; (*R*)-10-camphorsulfonic acid, 35963-20-3, $C_{10}H_{16}O_4S$, 232.30, F; (*S*)-10-camphorsulfonic acid, 3144-16-9, $C_{10}H_{16}O_4S$, 232.30, F; *O*,*O*′-dibenzoyl -D-tartaric acid, 17026-42-5, $C_{18}H_{14}O_8$, 358.31, W and F; *O*,*O*′-dibenzoyl-L-tartaric acid, 2743-38-6, $C_{18}H_{14}O_8$, 358.31, W and F; *N*-acetyl-D-phenylalanine, 10172-89-1, $C_{11}H_{13}NO_3$, 207.20, S; *N*-acetyl-L-phenylalanine, 2018-61-3, $C_{11}H_{13}NO_3$, 207.20, S; *N*-Cbz-D-alanine (Cbz = benzyloxycarbonyl), 26607-51-2, $C_{11}H_{13}NO_4$, 223.20, S; *N*-Cbz-L-alanine, 1142-20-7, $C_{11}H_{13}NO_4$, 223.20, S; (1*R*,2*R*,5*R*)-2-hydroxy-3-pinanone, 24047-72-1, $C_{10}H_{16}O_2$, 168.24, A; (1*S*,2*S*,5*S*)-2-hydroxy-3-pinanone, 1845-25-6, $C_{10}H_{16}O_2$, 168.24, A; (1*R*,2*R*,3*S*,5*R*)-2,3-pinanediol, 22422-34-0, $C_{10}H_{18}O_2$, 170.25, A; (1*S*,2*S*,3*R*,5*S*)-2,3-pinanediol, 18680-27-8, $C_{10}H_{18}O_2$, 170.25, A; (*R*)-α-methoxyphenylacetic acid, 3966-32-3, $C_9H_{10}O_3$, 166.18, F; (*S*)-α-methoxyphenylacetic acid, 26164-26-1, $C_9H_{10}O_3$, 166.18, F; (*R*)-α-methoxy-α-trifluoromethylphenylacetic acid, 20445-31-2, $C_{10}H_9F_3O_3$, 234.18, W and A; (*S*)-α-methoxy -α-trifluoromethylphenylacetic acid, 17257-71-5, $C_{10}H_9F_3O_3$, 234.18, W and A; (*R*)-1-aminoindan, 10277-74-4, $C_9H_{11}N$, 133.19, A; (*S*)-1-aminoindan, 61341-86-4, $C_9H_{11}N$, 133.19, A; (1*R*,2*S*)-*cis*-1-amino-2-indanol, 136030-00-7, $C_9H_{11}NO$, 149.19, A; (1*S*,2*R*)-*cis*-1-amino-2-indanol, 126456-43-7, $C_9H_{11}NO$, 149.19, A; (*R*)-*N*,*N*-dimethyl-1-ferrocenylethylamine, 31886-58-5, $C_{14}H_{19}FeN$, 257.15, F; (*S*)-*N*,*N*-dimethyl-1-ferrocenylethylamine, 31886-57-4, $C_{14}H_{19}FeN$, 257.15, F; (1*S*,2*R*)-2-amino-1,2-diphenylethanol, 23190-16-1, $C_{14}H_{15}NO$, 213.28, A; (1*R*,2*S*)-2-amino-1,2-diphenylethanol, 23364-44-5, $C_{14}H_{15}NO$, 213.28, A; (*R*)-α-methylbenzylamine, 3886-69-9, $C_8H_{11}N$, 121.18, F; (*S*)-α-methylbenzylamine, 2627-86-3, $C_8H_{11}N$, 121.18, F; *O*,*O*′-di-*p*-toluoyl-D-tartaric acid, 32634-68-7, $C_{20}H_{18}O_8$, 386.36, F; *O*,*O*′-di-*p*-toluoyl-L-tartaric acid, 32634-66-5, $C_{20}H_{18}O_8$, 386.36, F; (*R*)-2-phenylpropionic acid, 7782-26-5, $C_9H_{10}O_2$, 150.18, F; (*S*)-2-phenylpropionic acid, 7782-24-3, $C_9H_{10}O_2$, 150.18, F; (2*R*,3*R*)-3-benzyloxy-1,2,4-butanetriol, 84379-52-2, $C_{11}H_{16}O_4$, 212.50, F; (2*S*,3*S*)-3-benzyloxy-1,2,4-butanetriol, 84379-51-1, $C_{11}H_{16}O_4$, 212.50, F; (1*R*,2*R*)-*trans*-1,2-cyclohexanediol, 1072-86-2, $C_6H_{12}O_2$, 116.16, A; (1*S*,2*S*)-*trans*-1,2-cyclohexanediol, 57794-08-8, $C_6H_{12}O_2$, 116.16, A; 2,3-*O*-benzylidene-D-threitol, 58383-35-0, $C_{11}H_{14}O_4$, 210.23, F; 2,3-*O*-benzylidene-L-threitol, 35572-34-0, $C_{11}H_{14}O_4$, 210.23, F; (*R*)-1-phenyl-1,2-ethanediol, 16355-00-3, $C_8H_{10}O_2$, 138.17, F; (*S*)-1-phenyl-1,2-ethanediol, 25779-13-9, $C_8H_{10}O_2$, 138.17, F; (*R*)-benzyl glycidyl ether, 16495-13-9, $C_{10}H_{12}O_2$, 164.20, F; (*S*)-benzyl glycidyl ether, 14618-80-5, $C_{10}H_{12}O_2$, 164.20, F; D-phenylalanine methyl ester hydrochloride, 13033-84-6, $C_{10}H_{14}NO_2Cl$, 215.70, S; L-phenylalanine methyl ester hydrochloride, 7524-50-7, $C_{10}H_{14}NO_2Cl$, 215.70, S; (1*R*,3*S*)-camphoric acid, 124-83-4, $C_{10}H_{16}O_4$, 200.24, F; (1*S*,3*R*)-camphoric acid, 560-09-8, $C_{10}H_{16}O_4$, 200.24, F; *N*-*t*-Boc-D-alanine (*t*-Boc = *tert*-butoxycarbonyl), 7764-95-6, $C_8H_{15}NO_4$, 189.20, S; *N*-*t*-Boc-L-alanine, 15761-38-3, $C_8H_{15}NO_4$, 189.20, S; D-phenyllactic acid, 7326-19-4, $C_9H_{10}O_3$, 166.18, F; L-phenyllactic acid, 20312-36-1, $C_9H_{10}O_3$, 166.18, F; (*R*)-3-phenylbutyric acid, 772-14-5, $C_{10}H_{12}O_2$, 164.21, F; (*S*)-3-phenylbutyric acid, 772-15-6, $C_{10}H_{12}O_2$, 164.21, F; (±)-3-phenylbutyric acid, 4593-90-2, $C_{10}H_{12}O_2$, 164.21, F and W; *N*-acetyl-D-tryptophan, 2280-01-5, $C_{13}H_{14}N_2O_3$, 246.30, S; *N*-acetyl-L-tryptophan, 1218-34-4, $C_{13}H_{14}N_2O_3$, 246.30, S; *O*-benzyl-D-serine, 10433-52-0, $C_{10}H_{13}NO_3$, 195.20, S; *O*-benzyl-L-serine, 4726-96-9, $C_{10}H_{13}NO_3$, 195.20, S; *N*-Boc-D-alanine methyl ester, 91103-47-8, $C_9H_{17}NO_4$, 203.20, S; *N*-Boc-L-alanine methyl ester, 28875-17-4, $C_9H_{17}NO_4$, 203.20, A; (*R*)-camphorquinone-3-oxime, 22472-58-8, $C_{10}H_{15}NO_2$, 181.24, F; (*S*)-camphorquinone-3-oxime, 22472-58-8, $C_{10}H_{15}NO_2$, 181.24, F; (*R*)-3-bromo-2-methyl-1-propanol, 93381-28-3, C_4H_9BrO, 153.02, F; (*S*)-3-bromo-2-methyl-1-propanol, 98244-48-5, C_4H_9BrO, 153.02, F; *N*-*t*-Boc-D-serine, 6368-20-3, $C_8H_{15}NO_5$, 205.20, S; *N*-*t*-Boc-L-serine, 3262-72-4, $C_8H_{15}NO_5$, 205.20, S; *N*-acetyl-D-tyrosine, 537-55-3, $C_{11}H_{13}NO_4$, 223.20, B; *N*-acetyl-L-tyrosine, 537-55-3, $C_{11}H_{13}NO_4$, 223.20, S; Gly-D-Phe, 34258-14-5, $C_{11}H_{14}N_2O_3$, 222.20, B; Gly-L-Phe, 3321-03-7, $C_{11}H_{14}N_2O_3$, 222.20, B; (*R*)-3-bromo-8-camphorsulfonic acid, ammonium salt, 14575-84-9, $C_{10}H_{18}BrNO_4S$, 328.23, A; (*S*)-3-bromo-8-camphorsulfonic acid, ammonium salt, 55870-50-3, $C_{10}H_{18}BrNO_4S$, 328.23, A; D-phenylalanine amide, 5241-58-7, $C_9H_{12}N_20$, 164.20, B; L-phenylalanine amide, 5241-58-7, $C_9H_{12}N_20$, 164.20, B; (*R*)-methyl 3-bromo-2-methylpropionate, 110556-33-7, $C_5H_9BrO_2$, 181.03, F; (*S*)-methyl 3-bromo-2-methylpropionate, 98190-85-3, $C_5H_9BrO_2$, 181.03, F; (*R*)-methyl mandelate, 20698-91-3, $C_9H_{10}O_3$, 166.18, F; (*S*)-methyl mandelate, 21210-43-5, $C_9H_{10}O_3$, 166.18, F; (*R*)-propranolol hydrochloride, 13071-11-9, $C_{16}H_{22}NO_2Cl$, 295.81, F; (*S*)-propranolol hydrochloride, 4199-10-4, $C_{16}H_{22}NO_2Cl$, 295.81, F; *N*-Cbz-L-serine, 1142-20-7, $C_{11}H_{13}NO_4$, 223.20, S; *N*-Cbz-glycine, 1138-80-3, $C_{10}H_{11}NO_4$, 209.20, S; 3-ethoxy-1-propylamine, 6291-85-6, $C_5H_{13}NO$, 103.17, W; benzyloxyacetaldehyde dimethyl acetal, 127657-97-0, $C_{11}H_{16}O_3$, 196.24, L; cyclohexylacetic acid, 5292-21-7, C_8H_{14},O_2, 142.20, A; 3-phenylpropionic acid, 501-52-0, $C_9H_{10}O_2$, 150.18, A; 1-methyl-3-phenylpropylamine, 22374-89-6, $C_{10}H_{15}N$, 149.24, A; 4-phenylbutylamine, 13214-66-9, $C_{10}H_{15}N$, 149.24, A; 3-bromo-1-propanol, 627-18-9, C_3H_7OBr, 139.00, A. The guest's structures are illustrated in Charts 1−3.

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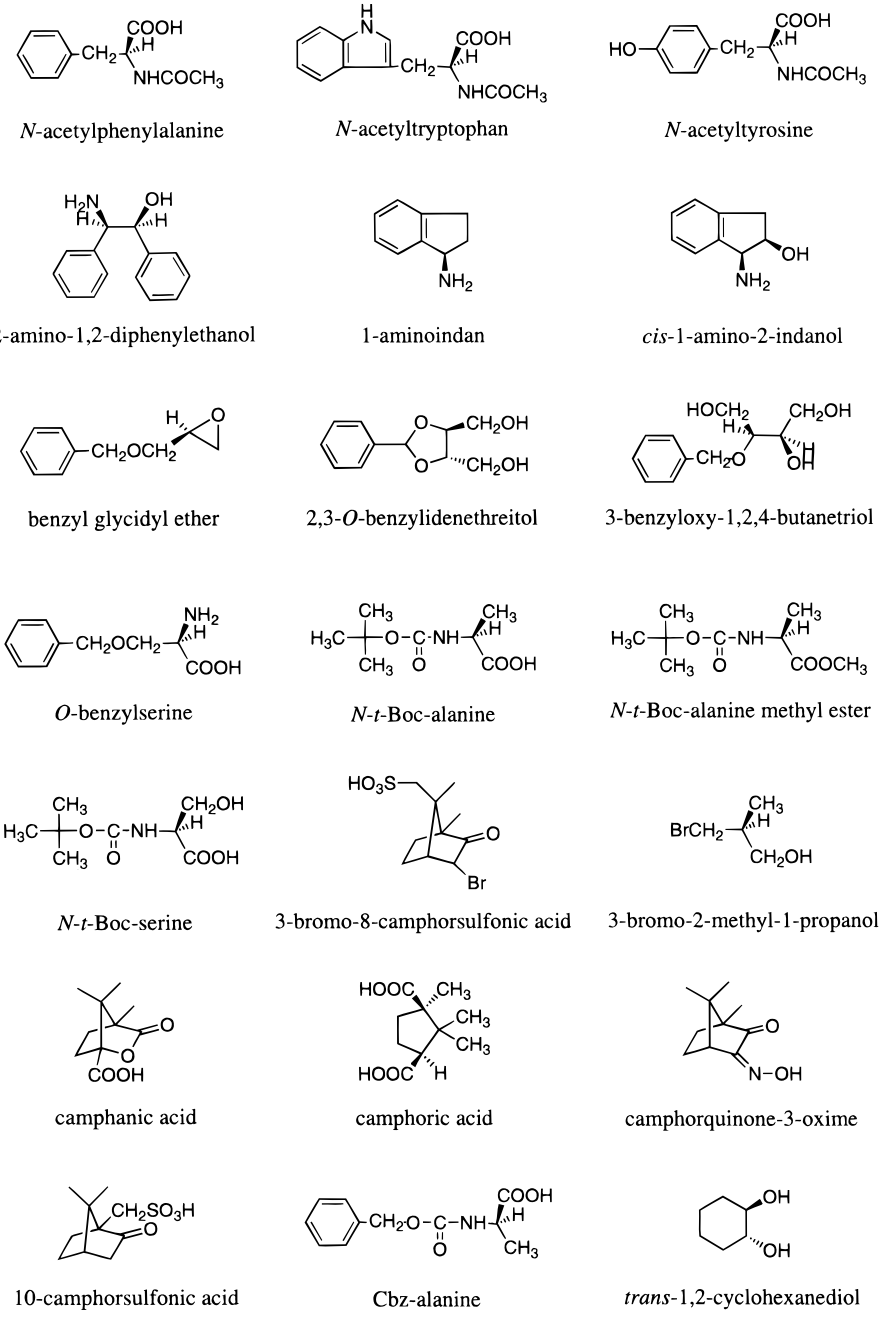
Chart 1



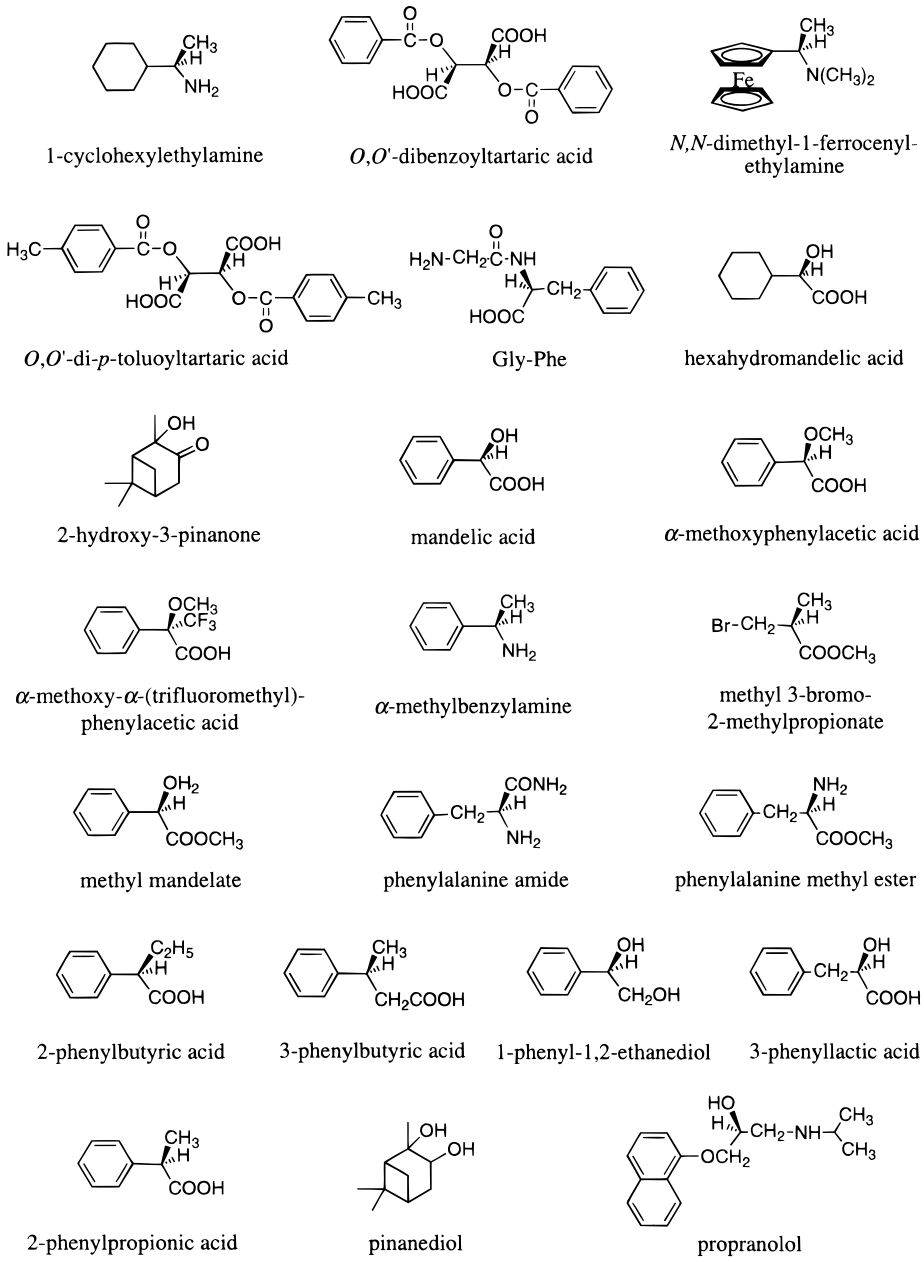
The highest purity commercially available samples were used in the microcalorimetric experiments without further purification. The vendors employed a variety of methods to determine the purities of the guests and to guarantee the enantiomeric purities of >98−99% (HPLC, LC, GC, optical rotation, titration, or elemental analysis). When the stated purity was less than 98% (but higher than 95%), the calorimetric measurements were repeated with different samples obtained from independent vendors. Even in such cases, the results of the independent runs were in satisfactory agreement.

The host cyclodextrins and some of the guest compounds contained water of hydration or crystallization, and appropriate corrections were made for this moisture content, based on values determined by the vendors using the Karl Fisher technique.[42b]

Microcalorimetric Measurements. An ITC instrument, purchased from Microcal Inc., MA, was used for all microcalorimetric experiments. Titration microcalorimetry allows us to determine simultaneously, enthalpy and equilibrium constant from a single titration curve.[14,17,25] The calorimetric and computational procedures for the ITC Microcal instrument are almost identical to those used on a Microcal Omega isothermal titration calorimeter, and these have been described previously.[14,17,25] Each microcalorimetric titration experiment consisted of 20 successive injections of a constant volume (5 μL/injection) of guest solution into the reaction cell (1.36 mL) charged with a CD solution in the same buffer; the concentrations of guest and CD in each run are indicated in the Tables. The heat of dilution of the guest solution when added to the buffer solution in the absence of CD was determined in each run, using the same number of injections and concentration of guest as in the titration experiments. The dilution enthalpies determined in these control experiments were subtracted from the enthalpies measured in the titration experiments. It should be emphasized that the enthalpies of dilution obtained in all runs were of the same order of magnitude as the enthalpies of dilution of simple electrolytes such as NaCl at the same concentration. Thus, it was concluded that there is no significant self-association of any guest in the experimental conditions used.

The Origin computer program (Microcal Inc.), which was used to calculate the equilibrium constant and standard molar enthalpy of reaction from the titration curve, gave a standard deviation based on

Chart 2



the scatter of the data points in a single titration curve. To check the accuracy of the calculated thermodynamic quantities, we carried out several independent titration runs with three different guest−CD combinations which had been examined previously.[16,25] Additional experimental data used to clarify the significance of uncertainties will be discussed below. The uncertainties in the observed thermodynamic quantities are 2 standard deviations of the mean value unless otherwise noted.

In the data analysis, the influence of removing some data points (up to 5 out of 20 points) from the initial and final parts of titration curve on the overall quality of the fit was routinely checked. In the initial stages of the titration experiment, the concentration of the CD in the reaction cell far exceeds the concentration of guest (G), and the occasionally observed systematic deviation of experimental points may be ascribed to the formation of more complicated species other than a stoichiometric 1:1 complex, e.g., 2:1 or higher-order CD_n:G ($n > 1$) complexes. During the final stages of the titration, the concentration of G is much higher than that of the CD, leading sometimes to 1:2 or higher-order CD:G_n ($n > 1$) complexes. When such systematic deviations were observed, the experiments were repeated using 2−3 times less concentrated guest and/or CD solutions in order to reduce the contribution of these more complicated host−guest complexes. Two typical examples will be discussed later in more detail. It should be emphasized that in addition to calculations based on the 1:1 stoichiometric complex formation, we also performed calculations assuming 1:*n* and *n*:1 binding models whenever such higher-order complexes were suspected. However, such calculations did not lead to any appreciable improvement of the overall fit, rendering these more complicated models irrelevant in this instance, and the assumption of a 1:1 model and a single binding site appears to be the only reasonable choice for all of the host−guest combinations examined.

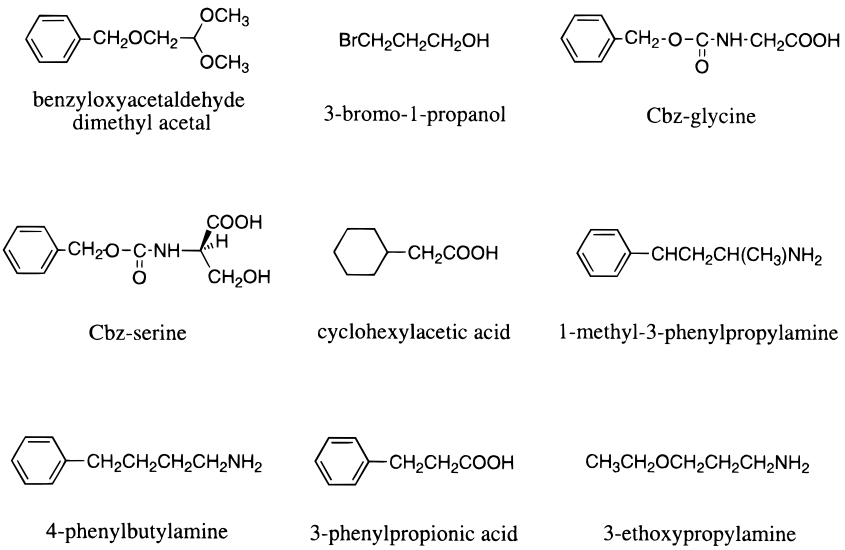
As described above, all equilibrium constants (K) and standard molar enthalpies ($\Delta H°$) reported are based on the 1:1 binding model with a single binding site

$$CD_{sln} + G_{sln} = CD{\cdot}G_{sln} \quad (1)$$

$$K = \gamma_{CD\cdot G}[CD{\cdot}G]/(\gamma_H[CD]{\cdot}\gamma_G[G]) \quad (2)$$

where γ is the activity coefficient of the relevant species. Non-ideality corrections were assumed to be unnecessary under the conditions employed. This approximation should hold reasonably well even when

Chart 3



a charged guest is involved, e.g., 1-methyl-3-phenylpropylammmonium, since the reaction is charge symmetric and the activity coefficients in the numerator and denominator should largely cancel at low and moderate ionic strengths.

The standard molar Gibbs free energies ($\Delta G°$) and entropies ($\Delta S°$) of reaction (listed in Tables 1 and 2) were calculated from the experimentally determined K and $\Delta H°$ values. In a few cases as indicated in the Tables, the thermodynamic quantities obtained are comparable to the uncertainty in the value and therefore considered to be approximate. In some cases the binding was too weak and/or the heat production was too small to be determined with the titration calorimeter.

Four different aqueous buffers were used in this study, these being a phosphate buffer at pH 6.9 [NaH_2PO_4 (0.025 mol kg^{-1}) + $NaHPO_4$ (0.025 mol kg^{-1})], a phosphate buffer at pH 6.1 [NaH_2PO_4 (0.025 mol kg^{-1}) + $NaHPO_4$ (0.025 mol kg^{-1}) + HCl], a glycine buffer at pH 10.0 [glycine (0.1 mol kg^{-1}) + NaOH], and an acetate buffer at pH 4.8 [sodium acetate (0.05 mol kg^{-1}) + acetic acid]. The main reason for using different buffers is to meet the variety of pH conditions which are necessary to keep the solution pH away from the pK_a of the particular guest species, i.e., $|pH - pK_a| > 2$. It has been demonstrated previously that the components of phosphate or glycine buffer do not interact with β-cyclodextrin.[25,42c] However, since acetic acid is known to interact appreciably with β-cyclodextrin,[25] the use of the acetate buffer was limited to cases where only this buffer could afford the required pH conditions. Acetate buffer also improves the very limited solubilities of certain guests, e.g., 2-amino-1,2-diphenylethanol, in comparison with those of non-interactive phosphate or glycine buffers. For camphorquinone-3-oxime, calorimetric measurements were performed at pH 4.8 and 6.9, since it is not clear if the requirement of $|pH - pK_a| > 2$ is met with this compound in neutral solution (pH 6.9). The charge of the guest species at the designated pH is indicated in parentheses in the tables.

The ITC Microcal instrument was periodically calibrated electrically using an internal electric heater. The instrument was also calibrated chemically by measurement of the neutralization enthalpy of the reaction of HCl with NaOH and the ionization enthalpy of TRIS buffer. These standard reactions gave excellent agreement (±1−2%) with the most reliable literature data.[43,44] Determination of the thermodynamic parameters of the complexation reaction of cyclohexanol with β-cyclodextrin was also shown to be in good agreement with our previous results.[45] A combined treatment of the previous and present thermodynamic parameters led to exactly the same heat capacity (−333 J mol^{-1} K^{-1}) as that reported previously (−332 ± 8 J mol^{-1} K^{-1}).[45]

Additional verification as to the reliability of the microcalorimeter and experimental procedures employed was obtained from the determination of the equilibrium constants and reaction enthalpies for the complexation of several alkanols (from propanol to hexanol) with α-cyclodextrin in water, all of which were consistent with the literature values.[9,46] Previously reported thermodynamic data, which were obtained by using a ThermoMetric 4-channel microcalorimetric system[9] and a Tronac 558 isothermal titration calorimeter,[46] allow us to make a thorough comparison for all alkanols under consideration. For all complexation reactions, log K and $\Delta H°$ values are in good agreement with those reported by Wadsö et al.[9] within an error of 1−4%. Similarly, our data are consistent with those reported for most alkanols by Fujiwara et al.[46] (i.e., within 1−2%) but show significant deviations from those for 1-propanol, for which we have no rationalization.

Good to excellent agreement of our experimental data with the results of two different microcalorimetric studies[9,46] as well as the neutralization enthalpy,[43] the ionization enthalpy of TRIS buffer,[44] and the thermodynamic parameters of the complexation reaction between β-cyclodextrin and cyclohexanol[45] give us confidence in the reliability of the thermodynamic quantities obtained in this study.

Results and Discussion

The complex stability constant (K), standard free energy ($\Delta G°$), enthalpy ($\Delta H°$), and entropy ($T\Delta S°$) for the 1:1 inclusion complexation of enantiomeric pairs of various compounds with β-cyclodextrin at $T = 298.15$ K are presented in Table 1. In Table 2, the thermodynamic parameters for the 1:1 inclusion complexation of single enantiomers, racemic and achiral compounds, with cyclodextrins at $T = 298.15$ K are listed. In total, more than 100 individual guest compounds including 43 enantiomeric pairs have been examined in this microcalorimetric study. The presentation of experimental results and discussion has been divided into two parts. In the first part, the relationship between the structure of the guest and complexation thermodynamics is discussed in terms of chain length, branching, flexibility, charge, and oxygen atom incorporated in cyclic and acyclic guest compounds. We then move on to consider the origin and mechanism of chiral recognition by cyclodextrin from the thermodynamic point of view.

Complexation Thermodynamics

Effect of Adding Methyl/Methylene Groups to the Aliphatic and Aromatic Compounds. It has been widely observed

(43) Chen, X.; Oscarson, J. L.; Gillespie, S. E.; Cao, H.; Izatt, R. M. *J. Sol. Chem.* **1994**, *23*, 747−768.
(44) Ojelund, G.; Wadso, I. *Acta Chim. Scand.* **1968**, *22*, 2691−2695.
(45) Ross, P. D.; Rekharsky, M. V. *Biophys. J.* **1996**, *71*, 2144−2154.
(46) Fujiwara, H.; Arakawa, H.; Murata, S.; Sasaki, Y.; *Bull. Chem. Soc. Jpn.* **1987**, *60*, 3891−3894.

Table 1. Complex Stability Constant (K), Standard Free Energy ($\Delta G°$), Enthalpy ($\Delta H°$), and Entropy Changes ($T\Delta S°$) for 1:1 Inclusion Complexation of Enantiomeric Pairs of Chiral Compounds with β-cyclodextrin at $T = 298.15$ K

guest (charge)	guest conc/mM	cyclodextrin conc/mM	pH	N[a]	K/M^{-1}	$\Delta G°$/ kJ mol^{-1}	$\Delta H°$/ kJ mol^{-1}	$T\Delta S°$/ kJ mol^{-1}
N-acetyl-D-phenylalanine (−1)	182	2.19	6.9[b]	2	60.7 ± 1.3	−10.18 ± 0.05	−8.14 ± 0.07	2.04 ± 0.08
N-acetyl-L-phenylalanine (−1)	171	2.19−3.08	6.9[b]	2	67.5 ± 1.4	−10.44 ± 0.05	−8.17 ± 0.08	2.27 ± 0.09
N-acetyl-D-tryptophan (−1)	192	2.19	6.9[b]	2	12.7 ± 0.5	−6.3 ± 0.1	−25.5 ± 0.6	−19.2 ± 0.6
N-acetyl-L-tryptophan (−1)	169	2.63	6.9[b]	2	17.1 ± 0.5	−7.04 ± 0.08	−23.8 ± 0.4	−16.8 ± 0.4
N-acetyl-D-tyrosine (−1)	100	1.43	6.9[b]	2	125 ± 2	−11.97 ± 0.04	−16.7 ± 0.3	−4.7 ± 0.3
N-acetyl-L-tyrosine (−1)	97−103	1.55−1.59	6.9[b]	3	130 ± 2	−12.07 ± 0.04	−17.1 ± 0.3	−5.0 ± 0.3
(1*R*,2*S*)-2-amino-1,2-diphenylethanol (+1)	188	2.19−2.24	4.8[e]	2	55 ± 3	−9.9 ± 0.1	−10.0 ± 0.2	−0.1 ± 0.2
(1*S*,2*R*)-2-amino-1,2-diphenylethanol (+1)	184	2.29	4.8[e]	2	46 ± 2	−9.5 ± 0.1	−10.0 ± 0.2	−0.5 ± 0.2
(*R*)-aminoindan (+1)	150	2.17	4.8[e]	2	*f*			
(*S*)-aminoindan (+1)	150	2.17	4.8[e]	2	*f*			
(*RS*)-cis−1-amino-2-indanol (+1)	240−320	3.00	4.8[e]	2	*f*			
(*SR*)-cis−1-amino-2-indanol (+1)	240−320	3.00	4.8[e]	2	*f*			
(*R*)-benzyl glycidyl ether (0)	26	1.23	6.9[b]	2	234 ± 12	−13.52 ± 0.15	−9.2 ± 0.2	4.3 ± 0.3
(*S*)-benzyl glycidyl ether (0)	25	1.20	6.9[b]	2	228 ± 10	−13.5 ± 0.1	−9.3 ± 0.2	4.2 ± 0.2
2,3-*O*-benzylidene-D-threitol (0)	111	1.54−1.94	6.9[b]	2	117 ± 2	−11.81 ± 0.04	−7.56 ± 0.08	4.25 ± 0.09
2,3-*O*-benzylidene-L-threitol (0)	109	1.77	6.9[b]	2	115 ± 2	−11.76 ± 0.04	−7.49 ± 0.07	4.27 ± 0.08
(2*R*,3*R*)-3-benzyloxy-1,2,4-butanetriol (0)	104	1.73	6.9[b]	2	83 ± 2	−10.95 ± 0.06	−8.07 ± 0.08	2.9 ± 0.1
(2*S*,3*S*)-3-benzyloxy-1,2,4-butanetriol (0)	106	1.06−1.82	6.9[b]	2	85 ± 2	−11.01 ± 0.06	−7.79 ± 0.07	3.2 ± 0.1
O-benzyl-D-serine (zwitterion)	49	1.77	6.9[b]	2	71 ± 4	−10.57 ± 0.15	−8.9 ± 0.4	1.7 ± 0.4
O-benzyl-L-serine (zwitterion)	50	1.94	6.9[b]	2	69 ± 3	−10.5 ± 0.1	−9.2 ± 0.3	1.3 ± 0.3
N-*t*-Boc-D-alanine (−1)	62	1.19	6.9[b]	2	392 ± 4	−14.80 ± 0.03	−9.7 ± 0.1	5.1 ± 0.1
N-*t*-Boc-L-alanine (−1)	57	0.95	6.9[b]	2	367 ± 4	−14.64 ± 0.03	−9.8 ± 0.1	4.8 ± 0.1
N-*t*-Boc-D-alanine methyl ester (0)	74	1.74	6.9[b]	2	659 ± 6	−16.09 ± 0.02	−13.82 ± 0.15	2.3 ± 0.2
N-*t*-Boc-L-alanine methyl ester (0)	72	1.72	6.9[b]	2	578 ± 4	−15.77 ± 0.02	−12.80 ± 0.15	3.0 ± 0.2
N-*t*-Boc-D-serine (−1)	104	1.47−1.56	6.9[b]	2	306 ± 2	−14.19 ± 0.02	−11.0 ± 0.1	3.2 ± 0.1
N-*t*-Boc-L-serine (−1)	102	1.56−1.62	6.9[b]	2	285 ± 2	−14.01 ± 0.02	−10.6 ± 0.1	3.4 ± 0.1
(*R*)−3-bromo-8-camphorsulfonic acid (−1)	31	0.45−0.50	6.9[b]	2	3760 ± 100	−20.41 ± 0.07	−30.1 ± 0.3	−9.7 ± 0.3
(*S*)−3-bromo-8-camphorsulfonic acid (−1)	29	0.45	6.9[b]	2	3640 ± 70	−20.32 ± 0.06	−29.6 ± 0.3	−9.3 ± 0.3
(*R*)−3-bromo-2-methyl-1propanol (0)	51	1.29−1.47	6.9[b]	2	142 ± 4	−12.29 ± 0.07	−9.3 ± 0.2	3.0 ± 0.2
(*S*)−3-bromo-2-methyl-1propanol (0)	51	1.29	6.9[b]	2	140 ± 4	−12.25 ± 0.07	−10.1 ± 0.2	2.2 ± 0.2
(*R*)−3-bromo-2-methylpropionic acid methyl ester (0)	28	1.07−2.16	6.9[b]	2	265 ± 25	−13.8 ± 0.2	−12.05 ± 0.15	1.8 ± 0.3
(*S*)−3-bromo-2-methylpropionic acid methyl ester (0)	27	1.07	6.9[b]	2	270 ± 20	−13.9 ± 0.2	−12.4 ± 0.2	1.5 ± 0.3
(*R*)-camphanic acid (−1)	90−131	1.07−185	6.9[b]	4	178 ± 2	−12.85 ± 0.03	−17.8 ± 0.2	−5.0 ± 0.2
(*S*)-camphanic acid (−1)	80−125	1.07−2.01	6.9[b]	4	207 ± 3	−13.22 ± 0.04	−17.7 ± 0.2	−4.5 ± 0.2
(1*R*,3*S*)-camphoric acid (−2)	214	1.99	6.9[b]	2	19 ± 1	−7.30 ± 0.15	−15.5 ± 0.6	−8.2 ± 0.6
(1*S*,3*R*)-camphoric acid (−2)	223	2.11	6.9[b]	2	24 ± 1	−7.9 ± 0.1	−8.3 ± 0.4	−0.4 ± 0.4
(*R*)-camphorquinone-3-oxime (0)	15−22	0.31−0.45	6.9[b]	3	2610 ± 40	−19.50 ± 0.04	−27.1 ± 0.2	−7.6 ± 0.2
(*R*)-camphorquinone-3-oxime (0)	16	0.33	4.8[e]	2	2450 ± 30	−19.35 ± 0.03	−27.0 ± 0.2	−7.7 ± 0.2
(*S*)-camphorquinone-3-oxime (0)	23	0.45	6.9[b]	2	2440 ± 40	−19.34 ± 0.04	−27.2 ± 0.2	−7.9 ± 0.2
(*S*)-camphorquinone-3-oxime (0)	16	0.32	4.8[e]	2	2340 ± 40	−19.23 ± 0.04	−27.1 ± 0.2	−7.9 ± 0.2
(*R*)-10-camphorsulfonic acid (−1)	103	1.12−1.82	6.9[b]	2	564 ± 10	−15.70 ± 0.05	−20.7 ± 0.2	−5.0 ± 0.2

Table 1 (Continued)

guest (charge)	guest conc/mM	cyclodextrin conc/mM	pH	N^a	K/M^{-1}	$\Delta G°$/ kJ mol^{-1}	$\Delta H°$/ kJ mol^{-1}	$T\Delta S°$/ kJ mol^{-1}
(*S*)-10-camphorsulfonic acid (−1)	76	1.93	6.9[b]	3	489 ± 10	−15.35 ± 0.05	−19.5 ± 0.2	−4.2 ± 0.2
N-Cbz-D-alanine (−1)	45−74	0.78−1.00	6.9[b]	2	149 ± 4	−12.40 ± 0.07	−8.9 ± 0.2	3.5 ± 0.2
N-Cbz-L-alanine (−1)	57	0.84	6.9[b]	2	147 ± 4	−12.37 ± 0.07	−10.0 ± 0.2	2.4 ± 0.2
(1*R*,2*R*)-*trans*-1,2-cyclohexanediol (0)	227	2.36	6.9[b]	2	85 ± 2	−11.01 ± 0.06	−3.98 ± 0.04	7.03 ± 0.07
(1*S*,2*S*)-*trans*-1,2-cyclohexanediol (0)	209	2.06−2.36	6.9[b]	2	86 ± 2	−11.04 ± 0.06	−4.21 ± 0.04	6.83 ± 0.07
(*R*)-1-cyclohexyl ethylamine (+1)	147−184	1.43−1.97	6.9[b]	3	329 ± 3	−14.37 ± 0.03	−7.85 ± 0.08	6.5 ± 0.1
(*S*)-1-cyclohexyl ethylamine (+1)	167−180	1.97−2.15	6.9[b]	4	328 ± 3	−14.36 ± 0.03	−7.87 ± 0.08	6.5 ± 0.1
O,*O*′-dibenzoyl-D-tartaric acid (−2)	189−202	2.18−3.77	6.9[b]	4	32 ± 2	−8.6 ± 0.2	−7.0 ± 0.8	1.6 ± 0.8
O,*O*′-dibenzoyl-L-tartaric acid (−2)	199−212	2.17−3.80	6.9[b]	5	20 ± 2	−7.4 ± 0.2	−4.9 ± 0.6	2.5 ± 0.6
(*R*)-*N*,*N*-dimethyl-1-ferrocenylethylamine (+1)	18−58	0.27−0.89	4.8[e]	4	5600 ± 300	−21.4 ± 0.2	−28.6 ± 0.5	−7.2 ± 0.5
(*S*)-*N*,*N*-dimethyl-1-ferrocenylethylamine (+1)	16−52	0.27−0.79	4.8[e]	4	6700 ± 500	−21.8 ± 0.2	−28.7 ± 0.4	−6.9 ± 0.5
O,*O*′-di-*p*-toluoyl-D-tartaric acid (−2)	80−174	0.86−1.86	6.9[b]	3	105 ± 6	−11.54 ± 0.1	−5.78 ± 0.15	5.8 ± 0.2
O,*O*′-di-*p*-toluoyl-L-tartaric acid (−2)	87	0.82−1.37	6.9[b]	3	94 ± 8	−11.3 ± 0.2	−4.59 ± 0.15	6.7 ± 0.3
Gly-D-Phe (zwitterion)	100	1.93	6.1[c]	2	47 ± 1	−9.54 ± 0.05	−7.93 ± 0.15	1.6 ± 0.2
Gly-L-Phe (zwitterion)	96	2.54	6.1[c]	2	54 ± 1	−9.89 ± 0.05	−8.59 ± 0.15	1.3 ± 0.2
(*R*)-hexahydromandelic acid (−1)	94−149	2.06−2.09	6.9[b]	4	648 ± 12	−16.05 ± 0.05	−5.61 ± 0.07	10.44 ± 0.08
(*S*)-hexahydromandelic acid (−1)	98−169	1.89−1.96	6.9[b]	4	603 ± 10	−15.87 ± 0.05	−5.36 ± 0.05	10.51 ± 0.07
(1*R*,2*R*,5*R*)-2-hydroxy-3-pinanone (0)	16−32	0.31−1.19	6.9[b]	4	2360 ± 90	−19.3 ± 0.1	−19.5 ± 0.2	−0.2 ± 0.2
(1*S*,2*S*,5*S*)-2-hydroxy-3-pinanone (0)	17−35	0.31−1.10	6.9[b]	4	2310 ± 50	−19.20 ± 0.05	−20.0 ± 0.2	−0.8 ± 0.2
(*R*)-mandelic acid (−1)	232	1.66	6.9[b]	2	11 ± 2	−5.9 ± 0.5	−4.9 ± 0.3	1.0 ± 0.6
(*S*)-mandelic acid (−1)	224	2.73	6.9[b]	2	9 ± 2	−5.4 ± 0.6	−4.6 ± 0.3	0.8 ± 0.7
(*R*)-mandelic acid methyl ester (0)	79	2.08−2.69	6.9[b]	2	67 ± 2	−10.42 ± 0.08	−7.8 ± 0.1	2.6 ± 0.1
(*R*)-mandelic acid methyl ester (0)	70	1.87	4.8[e]	2	60 ± 3	−10.15 ± 0.15	−8.2 ± 0.2	2.0 ± 0.2
(*S*)-mandelic acid methyl ester (0)	82	2.69	6.9[b]	2	72 ± 2	−10.60 ± 0.07	−8.2 ± 0.1	2.4 ± 0.1
(*S*)-mandelic acid methyl ester (0)	82	2.07	4.8[e]	2	66 ± 2	−10.39 ± 0.08	−8.44 ± 0.15	2.0 ± 0.2
(*R*)-α-methoxy phenylacetic acid (−1)	231	6.74	6.9[b]	2	11 ± 2	−5.9 ± 0.5	−4.4 ± 0.3	1.5 ± 0.6
(*S*)-α-methoxy phenylacetic acid (−1)	242	6.89	6.9[b]	2	10 ± 1	−5.7 ± 0.3	−5.1 ± 0.3	0.6 ± 0.4
(*R*)-α-methoxy-α-trifluoromethyl phenylacetic acid (−1)	106−114	1.93−2.08	6.9[b]	4	175 ± 2	−12.80 ± 0.03	−17.48 ± 0.15	−4.7 ± 0.2
(*S*)-α-methoxy-α-trifluoromethyl phenylacetic acid (−1)	102−106	152−2.08	6.9[b]	5	141 ± 2	−12.27 ± 0.04	−16.35 ± 0.15	−4.1 ± 0.2
(*R*)-α-methyl benzylamine (+1)	300	2.07	6.9[b]	1	*f*			
(*S*)-α-methyl benzylamine (+1)	300	2.07	6.9[b]	1	*f*			
D-phenylalanine amide (0)	142	1.87	10.0[d]	2	101 ± 1	−11.44 ± 0.03	−10.0 ± 0.1	1.4 ± 0.1
L-phenylalanine amide (0)	145	1.87−2.25	10.0[d]	2	109 ± 1	−11.63 ± 0.03	−10.6 ± 0.1	1.0 ± 0.1
D-phenylalanine methyl ester (+1)	202	2.32−4.52	4.8[e]	2	11 ± 2	−5.9 ± 0.5	−5.6 ± 0.8	0.3 ± 0.9
L-phenylalanine methyl ester (+1)	212	5.01	4.8[e]	2	12 ± 1	−6.2 ± 0.3	−5.0 ± 0.5	1.2 ± 0.6
(*R*)−2-phenylbutyric acid (−1)	204	1.82−1.92	6.9[b]	2	94 ± 2	−11.26 ± 0.06	−9.79 ± 0.15	1.5 ± 0.2
(*S*)−2-phenylbutyric acid (−1)	184−203	1.82−1.92	6.9[b]	3	95 ± 2	−11.29 ± 0.05	−9.91 ± 0.15	1.4 ± 0.2
(*R*)−3-phenylbutyric acid (−1)	113	1.80	6.9[b]	2	402 ± 4	−14.86 ± 0.03	−8.62 ± 0.09	6.24 ± 0.09
(*S*)−3-phenylbutyric acid (−1)	110	1.79	6.9[b]	2	430 ± 4	−15.03 ± 0.02	−8.68 ± 0.09	6.35 ± 0.09

Table 1 (Continued)

guest (charge)	guest conc/mM	cyclodextrin conc/mM	pH	N^a	K/M^{-1}	$\Delta G°$/ kJ mol^{-1}	$\Delta H°$/ kJ mol^{-1}	$T\Delta S°$/ kJ mol^{-1}
(*R*)−1-phenyl-1,2-ethanediol (0)	188	2.03	6.9[b]	2	62 ± 1	−10.23 ± 0.04	−7.54 ± 0.08	2.69 ± 0.09
(*S*)−1-phenyl-1,2-ethanediol (0)	181	2.03	6.9[b]	2	62.7 ± 1.5	−10.26 ± 0.06	−7.30 ± 0.07	2.96 ± 0.08
(*R*)-phenyllactic acid (−1)	195	2.45	6.9[b]	2	88 ± 1	−11.10 ± 0.03	−9.34 ± 0.08	1.8 ± 0.1
(*S*)-phenyllactic acid (−1)	225	2.59	6.9[b]	2	83 ± 1	−10.95 ± 0.03	−8.65 ± 0.08	2.3 ± 0.1
(*R*)−2-phenyl propionic acid (−1)	202	1.99	6.9[b]	2	34 ± 2	−8.74 ± 0.15	−8.81 ± 0.15	−0.1 ± 0.2
(*S*)−2-phenylpropionic acid (−1)	208	1.98	6.9[b]	2	36 ± 2	−8.88 ± 0.15	−8.69 ± 0.15	0.2 ± 0.2
(1*R*,2*R*,3*S*,5*R*)-pinanediol (0)	11−27	0.20−0.94	6.9[b]	4	6430 ± 120	−21.74 ± 0.05	−20.4 ± 0.2	1.3 ± 0.2
(1*S*,2*S*,3*R*,5*S*)-pinanediol (0)	12	0.21−0.22	6.9[b]	2	6360 ± 120	−21.71 ± 0.05	−20.3 ± 0.2	1.4 ± 0.2
(*R*)-propranolol (+1)	96	1.20−2.37	4.8[e]	2	115 ± 10	−11.8 ± 0.3	−21.2 ± 0.5	−9.4 ± 0.6
(*S*)-propranolol (+1)	91	1.20−1.39	4.8[e]	2	117 ± 10	−11.8 ± 0.3	−20.3 ± 0.5	−8.5 ± 0.6

[a] *N* is number of independent titration experiments performed. [b] Phosphate buffer [NaH_2PO_4 (0.025 mol kg^{-1}) + $NaHPO_4$ (0.025 mol kg^{-1})]. [c] Phosphate buffer [NaH_2PO_4 (0.025 mol kg^{-1}) + $NaHPO_4$ (0.025 mol kg^{-1}) + HCl]. [d] Glycine buffer [$C_2H_5NO_2$ (0.1 mol kg^{-1}) + NaOH]. [e] Acetate buffer [$NaC_2H_3O_2$ (0.05 mol kg^{-1}) + $C_2H_4O_2$]. [f] *K* and/or $\Delta H°$ for this reaction were too small to determine with titration microcalorimeter. The basis of the uncertainties is discussed in the text.

Table 2. Complex Stability Constant (*K*), Standard Free Energy ($\Delta G°$), Enthalpy ($\Delta H°$), and Entropy Changes ($T\Delta S°$) for 1:1 Inclusion Complexation of Single Enantiomers, Racemic, and Achiral Compounds with β-Cyclodextrin at $T = 298.15$ K

guest (charge)	guest conc/mM	cyclodextrin conc/mM	pH	N^a	K/M^{-1}	$\Delta G°$/ kJ mol^{-1}	$\Delta H°$/ kJ mol^{-1}	$T\Delta S°$/ kJ mol^{-1}
benzyloxyacetaldehyde dimethyl acetal (0)	20	1.06	6.9[b]	1	220 ± 25	−13.7 ± 0.3	−8.7 ± 0.5	5.0 ± 0.6
3-bromo-1-propanol (0)	197	2.08	6.9[b]	2	22 ± 1	−7.7 ± 0.1	−7.5 ± 0.2	0.2 ± 0.2
N-Cbz-glycine (−1)	62	0.95	6.9[b]	2	157 ± 4	−12.53 ± 0.06	−10.6 ± 0.3	1.9 ± 0.3
N-Cbz-L-serine (−1)	72	1.00	6.9[b]	1	109 ± 2	−11.63 ± 0.05	−9.83 ± 0.15	1.8 ± 0.2
cyclohexanol (0)	136	1.59	6.9[b]	2	701 ± 6	−16.24 ± 0.02	−6.3 ± 0.1	9.9 ± 0.1
cyclohexylacetic acid (−1)	113	1.54	6.9[b]	1	1270 ± 60	−17.7 ± 0.1	−4.93 ± 0.07	12.8 ± 0.1
1-methyl-3-phenylpropylamine (+1)	117	1.95	6.9[b]	2	188 ± 3	−12.98 ± 0.04	−8.64 ± 0.08	4.34 ± 0.09
4-phenyl butylamine (+1)	87	1.88	6.9[b]	2	405 ± 6	−14.88 ± 0.04	−10.4 ± 0.1	4.5 ± 0.1
(±)−3-phenyl butyric acid (−1)	112−124	2.01−2.03	6.9[b]	3	415 ± 5	−14.94 ± 0.03	−8.6 ± 0.1	6.3 ± 0.1
3-phenylpropionic acid (−1)	86−186	1.54−2.20	6.9[b]	4	162 ± 4	−12.61 ± 0.06	−6.9 ± 0.1	5.7 ± 0.1
3-ethoxy propylamine (+1)	94	1.11 (α-CD)[c]	6.9[b]	1	8 ± 4	−5 ± 2	−13 ± 6	−8 ± 6

[a] *N* is number of independent titration experiments performed. [b] Phosphate buffer [NaH_2PO_4 (0.025 mol kg^{-1}) + $NaHPO_4$ (0.025 mol kg^{-1})]. [c] α-Cyclodextrin was used as the host in this particular case. The basis of the uncertainties is discussed in the text.

that the free energy of complexation with cyclodextrins increases with the extension of the methylene chain in the guest molecule. In our recent review,[47] we demonstrated that the free energy of complexation is proportional to the number of methylene groups in a guest (N_C) and that the average increment of free energy per methylene ($d\Delta G°/dN_C$) is essentially the same for a variety of guest molecules upon complexation with both α-CD ($d\Delta G°/dN_C = -3.1$ kJ/mol) and β-CD ($d\Delta G°/dN_C = -2.8$ kJ/mol). It was also shown that the increased stability caused by the addition of a methylene group is predominantly enthalpic in origin, since these $d\Delta G°/dN_C$ values are very close to the unit increments of complexation enthalpy for α- and β-CDs ($d\Delta H°/dN_C = -3.3$ kJ/mol).

The effect of methyl-branching in aliphatic guests on complexation thermodynamics does not follow such a uniform trend as that observed for the linear extension of the methylene chain in the guest molecule.[11,13,14,17,47−49] The presence of a branched aliphatic chain in the guest increases the steric bulk, which in turn makes the guest's penetration into the small cavity of α-CD difficult. Thus, steric hindrance is probably the reason the pronounced enthalpy CH_2-increment that occurs upon α-methyl-branching of aliphatic alcohols does not lead to a significant increase in affinity, and we observe that the 1-propanol and 2-butanol pair, and the 1-butanol and 2-pentanol pair, etc. have similar affinities. In some examples, methyl-branching reveals thermodynamic behavior which is completely different from that observed upon extension of a straight methylene chain. For instance, the enhanced affinities observed for the complexation of α-CD with 3-methyl-1-butanol and 2-methyl-1-propanol, as compared with 1-butanol and 1-propanol, respectively,[48] are exclusively entropically driven, in contrast with 1-alkanols.

(47) Rekharsky, M. V.; Inoue, Y.; *Chem. Rev.* **1998**, *98*, 1875−1917.

(48) Spencer, J. N.; DeGarmo, J.; Paul, I. M.; He, Q.; Ke, X.; Wu, Z.; Yoder, C. H.; Chen, S.; Mihalick, J. E. *J. Solution Chem.* **1995**, *24*, 601−609.

(49) Barone, G.; Castronuovo, G.; Elia, V.; Muscetta, M. *Thermochim. Acta* **1985**, *85*, 443−446.

Indeed, positive entropy gains cancel or even outweigh unfavorable enthalpy changes.

As is the case with the inclusion of branched acyclic alkanols into α-CD, methyl cyclohexanols cannot be accommodated in the cavity of β-CD without steric hindrance. On the basis of geometrical reasoning, it is acceptable to assume that the introduction of a methyl group at the 2- or 3-position will result in more steric hindrance than if the methyl group was added to the 1- or 4-position. Experimental data confirm this simple rationale, since 2- and 3-methylcyclohexanols show the smallest enhancement of affinity.[16,45] In all cases, the enhancement of affinity is enthalpic in origin, and the enthalpic gain obtained by introducing a methyl is canceled out in part by the accompanying entropic loss, except for that of *trans*-4-methylcyclohexanol.

It is certain that there is enough space in the cavity of β-CD to accommodate methyl-branched acyclic alcohols without significant steric hindrance, and the observed reaction enthalpies for the complexation of 1- and 2- alkanol with β-CD are very small.[47] Under such circumstances, large uncertainties are expected for the thermodynamic parameters determined by microcalorimetry, and to examine the thermodynamic behavior of branched acyclic alcohols inside the cavity of β-CD with higher accuracy, we performed calorimetric experiments with 3-bromo-1-propanol (Table 2) and (R)- and (S)-3- bromo-2-methyl-1-propanol (Table 1), leads to a large affinity enhancement.

The above discussion indicates clearly that the enhancement of the complex stability observed for acyclic and cyclic alkanols due to methyl branching cannot be assigned to a single thermodynamic term. Indeed, increases in stability may be attributable exclusively to the entropy term, cf. 2-methyl-1-propanol vs 1-propanol and 3-methyl-1-butanol vs 1-butanol with α-CD, or to the enthalpy term, cf. 2-alkanols with α-CD and methylcyclohexanols with β-CD, or alternatively to a combination both enthalpy and entropy changes, cf. 3-bromo-1-propanol vs (*R*)- and (*S*)-3- bromo-2-methyl-1-propanol. Nevertheless, it is likely that simple geometrical rationale can explain all three of the above cases, if it is assumed that the enthalpy gain is obtained predominantly through van der Waals interactions of the additional methylene group with the walls of the CD cavity. As an example of the first-case scenario where the entropy term is dominant, both 2-methyl-1-propanol and 3-methyl-1-butanol are too bulky to penetrate completely into α-CD, and this leaves their main hydrophobic parts outside of the cavity. In such circumstances, less pronounced van der Waals interactions and a reduction in the exothermic enthalpy should be expected for methylated, as opposed to non-methylated guests. The increased complex stability for 3-methyl-1-butanol vs 1-butanol and 2-methyl-1-propanol vs 1-propanol is believed to originate from a change in solvation around the CD molecule. Here 3-methyl-1-butanol and 2-methyl-1-propanol induce a more extensive rearrangement and/or displacement of water molecules closest to the cavity, which involves changes in solvation of both host and guest, forming a more expanded hydrophobic cavity, and the enhanced affinity is attributable to the more positive entropy term. It should be emphasized that here and below we always discuss the differential solvation entropy (and the other thermodynamic parameters) which is affected by the solvation changes of both the guest and the host upon complexation. In the second case, the increased affinities of higher homologues of 2-alkanols to α-CD and of methylated cyclohexanols to β-CD are reasonably explained through the enthalpy term. Here, the additional methylene groups do not greatly interfere with the guest inclusion by α- or β-CD and therefore have good van der Waals interactions with the CD cavity. Such an inclusion mechanism renders the process enthalpically driven, as is the case with straight-chain guests. Since the movements of the methyl group of 2-alkanols and methylcyclohexanols are considered to be fairly restricted in the CD cavity due to the relative sizes of the guest/host, the entropy changes obtained upon complexation should be zero or negative under such circumstances. Finally, there are the intermediate cases, where both the enthalpy and entropy factors play an equally important role. A comparison of complexation of 3-bromo-1-propanol and 3-bromo-2-methyl-1-propanol with β-CD is an example of such a case. Here the methyl-branched aliphatic chain, located on the inside of the relatively large β-CD cavity, not only experiences van der Waals interactions with the walls of the cavity, leading to an enthalpic gain, but also induces the rearrangement of the water molecules in the CD cavity and/or loss of solvation of the guest, affording an entropic gain.

To discuss the complexation behavior of cyclodextrins from a more global point of view, we extended the range of guest molecules from alkanols to include amines and carboxylic acids. As described previously,[47] the complexation free energies ($|\Delta G°|$) reported for a series of alkanols with α-CD are consistently larger than those of corresponding amines and carboxylic acids which possess the same number of carbon atoms and are comparable to those for the next homologues.[9,10,14,16] Thus, alkanol guests always possess a one-carbon "advantage" over amines and acids. As discussed above, the increase in complex stability with increasing methylene chain length in aliphatic guests is predominantly enthalpic in origin. Hence, the complexation enthalpies ($\Delta H°$) show exactly the same trend as $\Delta G°$, and both thermodynamic values can be used as a measure of the "depth" of penetration of aliphatic guests.[47] This indicates that alkanols are included more deeply in the α-CD cavity by approximately one more methylene group than the corresponding amines and acids. Certainly, the number of the methylene groups involved in complex formation is essentially the same for corresponding amines and acids.

In cases involving β-CD, it is more difficult to make straightforward conclusions about the depth of penetration of the aliphatic chain, since there is enough space for a guest included in the cavity to take on a variety of conformations. This is demonstrated clearly by the more favorable entropy changes obtained upon complexation of the same guests with β-CD than with α-CD[16] and also by the less negative CH_2-increment of heat capacity for β-CD than for α-CD.[45] Certainly, there are several contributions, which may be responsible for the more favorable entropy in the case of β-CD, such as more favorable conformational entropy, rearrangement of the water molecules in the CD cavity and/or loss of guest solvation, and it is impossible to quantatively separate these contributions. It should also be noted that the CH_2-increments in enthalpy are not as uniform for β-CD complexation with aromatic amines and acids as those observed for α-CD complexation with aliphatic amines and acids.[47] Nevertheless, aromatic amines and acids that possess the same number of carbons afford very similar free energies of complexation with β-CD, as is the case with the complexation of the aliphatic amines and acids with α-CD. This is good thermodynamic evidence for the hypothesis that the number of methylene groups involved in complex formation with both α- and β-CD is essentially the same for the corresponding amines and acids. In addition to the literature data,[47] we obtained a binding constant for complexation of

4-phenylbutylammonium with β-CD ($K = 405 \pm 4$ M^{-1}, see Table 2) which is comparable to the value reported for 4-phenylbutanoate (437 ± 4 M^{-1}).[16]

An intriguing question arises from the above discussion: can the guest molecule be clearly divided into two parts on the basis of the degree of contribution of each part to the overall complexation thermodynamics? In other words, it is our desire to know what part of the guest molecule is actually interacting with the CD cavity and what part of the guest molecule remains in the bulk water after complexation without contributing to the overall complexation thermodynamics.

In our previous article, we showed that the *N*-methyl group does not contribute positively to the overall thermodynamics upon complexation of α-CD with aliphatic amines and that a methyl group introduced at the α-carbon of aliphatic amines also makes little contribution to the thermodynamics.[45] It seems reasonable to us to assume that only the part of molecule that undergoes environmental changes upon complexation will contribute to the overall thermodynamics. Thus, negligible contributions from the 1-methyl and *N*-methyl groups upon complexation with α-CD are rationalized as follows: (1) if the ammonium is surrounded by a hydration shell which is large enough (i.e., considerably more than two C−C bond lengths) to accommodate the 1- or *N*-methyl group and this hydration shell does not suffer significant changes upon complexation, or (2) if the radius of the shell is equal to or less than two C−C bond lengths, which is insufficient to accommodate the 1-methyl group in the shell, and therefore the 1-methyl group is left in the bulk water upon complexation with α-CD, which does not allow for the flexible inclusion of the 1-methyl group of the guest.

If the second model is correct and the hydration shell around the charged group is equal to or less than two C−C bond lengths, the 1-methyl group of the guest should be able to interact with the wider β-CD cavity and thus contribute to the overall thermodynamics. However, if the first model is correct, the 1-methyl group is located within the hydration shell of the charged group and cannot interact, even with β-CD. Comparison of the binding constants for 1-methyl-3-phenylpropylammonium ($K = 188$ M^{-1}) (Table 2) and 3-phenylpropylammonium ($K = 107$ M^{-1})[25] with β-CD gives convincing evidence in support of the second complexation model, since the additional methyl group results in an appreciable enhancement of affinity of almost 2-fold. The several-fold enhancement of binding affinities from phenylethylamine to ephedrines and pseudoephedrines can also be explained if the shell radius is assumed to be equal to or less than two C−C bond lengths.

The above observations indicate clearly that the hydration shell does not completely surround the 2(β)-carbon atom(s) of amines, but the exact boundary is not specified yet. However, the fact that *N*-methyl groups introduced to amine guests do not noticeably affect the complexation behavior of both α- and β-CDs in any situation implies that the hydration shell must absorb the 1(α)-carbon(s) of amine guests.[16,47] It is concluded, therefore, that the boundary of the hydration shell around the charged ammonium group lies somewhere around 2(β)-carbons of the charged guests. This shell size can explain why *N*-methyl groups have a negligible effect on overall thermodynamics, as well as explaining why the affinities for 1-methylbenzylammonium, 1-amino-2-indanol (cation +1), and aminoindan (cation +1) ions are several times lower than that for phenylethylammonium, since even the benzene ring of the former ions interacts with the hydration shell and it is not possible in these cases to determine the exact equilibrium constant by microcalorimetry (see Table 1).

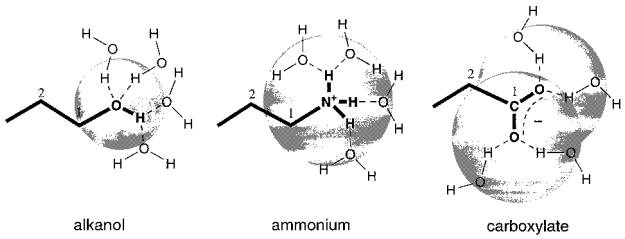


Figure 1. Schematic drawings of solvation shell for alkanol, ammonium, and carboxylate as evaluated from thermodynamic behavior upon complexation with cyclodextrins.

In the case of carboxylic acid guests, it is impossible to test the effects of 1-methylation. However, as described above, carboxylate guests show almost the same thermodynamic behavior as that of the corresponding amines with the same number of carbon atoms, with a one-carbon atom disadvantage over the corresponding alkanol guests upon complexation with CDs. On the basis of these observations, the boundary of the hydration shell for the carboxylate anion appears to be located somewhere around the 2(β)-carbons of the aliphatic chain, the same as for ammonium guests . Accordingly, it is not surprising that the equilibrium constant for 2-phenylpropionate with β-CD ($K = 34$, Table 1) is twice as large as that for phenylacetate ($K = 17$).[16]

The hydration shells that have been elucidated from the complexation thermodynamic behavior of aliphatic and aromatic guests with cyclodextrins are illustrated schematically for an alkanol, alkylammonium ion, and an alkanoate ion in Figure 1. It should be noted that the estimated hydration shell is purely based on, and completely compatible with, the observed complexation thermodynamic behavior; comparison with the relevant values evaluated, for example, by NMR/NOE study would be interesting. The charged ammonium and carboxyl groups should lead to a more tightly bound solvation shell around themselves as compared to the neutral hydroxyl group, and the weaker shell around the hydroxyl group results in higher complexation affinities for alkanols in comparison with those for the corresponding amines and acids, and it is certain that the absence of a tightly bound solvation shell in the case of alkanes leads to a further enhancement of affinity.[50] It is interesting to note that the removal of the oxygen atom from alkanols leads to an increase in affinity of about 1 order of magnitude for the corresponding alkane.[50] Similar thermodynamic behavior is also seen for the pentylammonium[16,45] and 3-ethoxypropylammonium pair in their reactions with α-cyclodextrin (see Table 2). Nevertheless, in some other cases, such as Cbz- or Boc-amino acid derivatives, camphanic acid, and benzyloxyacetaldehyde dimethyl acetal (see Tables 1 and 2), the presence of the ester-bridging oxygen does not appear to lead to such a large reduction of affinity.

It should be noted that thermodynamic behavior upon complexation with β-CD for the two pairs of methyl-branched aromatic acids, 2-phenylpropionate and phenylacetate, and 3-phenylbuturate and 3-phenylpropionate, is essentially the same as the behavior of the 3-bromo-1-propanol and 3-bromo-2-methyl-1-propanol pair, which is described above. In all three cases the methyl-branched aliphatic chain, located on inside of the relatively large β-CD cavity, not only experiences van der

(50) Inoue, Y.; Yamamoto, K.; Wada, T.; Everitt, S.; Gao, X.-M.; Hou, Z.-J.; Tong, L.-H.; Jiang, S.-K.; Wu, H.-M. *J. Chem. Soc., Perkin Trans. 2* **1998**, 1807.

Waals interactions with the cavity walls, yielding an enthalpic gain, but also induces the rearrangement of the water molecules in the CD cavity, affording an entropic gain. If we assume that the enthalpy gain is obtained predominantly through van der Waals interactions of the additional methylene group with the walls of the CD cavity, it is likely that the *p*-methyl group in molecule such as 4-methylphenethylamine and ditoluoyl tartaric acid appears to be sheltered by the benzene ring and is not able to fully interact with the cavity walls. Indeed, the *p*-methylation of both enantiomers of *O*,*O*′-dibenzoyltartaric acid, giving the corresponding di-*p*-toluoyltartaric acids, shows zero enhancement or even positive changes in $\Delta H°$ (Table 1). A comparison of phenylethylammonium and 4-methylphenylethylammonium also gives similar results; 4-methylphenylethylammonium gives a *K* value that is 3 times larger, but a $\Delta H°$ similar to that of phenylethylammonium. The enhanced affinity is attributable solely to the entropic gain, and this is probably the result of the rearrangement of water molecules, or a change of solvation inside the β-CD cavity. The same explanation may be applied to the phenylacetate and phenylpropionate pairs, etc., where only a change in $\Delta S°$ is observed.

Now we wish to compare changes in $\Delta G°$ and $\Delta H°$ upon inclusion complexation with CDs caused by the addition of a methylene group with values obtained for the transfer reaction of a methylene group from bulk water to nonpolar organic solvents. Interestingly, both processes give essentially the same $\Delta G°$ values of 3−4 kJ/mol per methylene unit,[9,51−54] while the $\Delta H°$ increment for the water to nonpolar solvent transfer process does not exceed −1.5 kJ/mol,[9,51−54] which is much smaller than that observed for complexation with CD.[47] This behavior appears to be reasonable, since inclusion into a CD cavity with its restricted size and shape induces much stronger van der Waals interactions, giving larger $\Delta H°$ increments, but greatly reduces the guest's freedom, giving less positive or negative entropic contributions than those observed for the transfer to fluid organic solvents in which the van der Waals interactions are less intimate, but where the guest molecules enjoy more freedom. It is also interesting to note that, when a steric factor or the geometry of the guest disturbs the complete set of van der Waals interactions of the additional methyl/methylene, the entropy term begins to play a crucial role in determining the overall thermodynamics; e.g., para-substitution of benzene ring, substitution adjacent to a benzene ring, or incomplete inclusion of the hydrophobic part of the guest. From the thermodynamic viewpoint, the lack of full van der Waals interactions with the CD cavity and the subsequent increase in freedom of the included guests make this type of complexation reaction more closely related to the processes that occur in the transfer reaction from bulk water to nonpolar organic solvents.

Effects of Adding Methyl/Methylene Groups to Saturated Polycyclic Guests. It was shown that the trend of thermodynamic parameters for the complexation of C_4−C_8 cycloalkanols with β-CD can be accounted for simply in terms of the relative size of the guest and β-CD.[16] In this study, we have examined more complex saturated cyclic guests, e.g., camphor derivatives and related saturated cyclic compounds which possess a variety of different substituents, these being camphanic acid, 10-camphorsulfonic acid, 2-hydroxy-3-pinanone, pinanediol, camphorquinone-3-oxime, and 3-bromo-8-camphorsulfonic acid. 10-camphorsulfonic acid and camphanic acid have some structural similarity, possessing the similar skeleton with ionizable acid moiety. The major difference is the 3-position, which bears a methylene group or an oxygen, respectively. As a result, 10-camphorsulfonic acid binds to β-CD 3 times more strongly, and has a $\Delta H°$ that is 2.3 kJ/mol more negative than that of camphanic acid. This enthalpic gain is almost comparable to the unit increment for the addition of an extra methylene group and therefore indicates that in this case the ester oxygen has little effect upon the complexation behavior.

Formally, it seems inappropriate to compare directly the thermodynamic behavior of 10-camphorsulfonic acid, 2-hydroxy-3-pinanone, and pinanediol. Nevertheless, since they share similar bicyclic terpenoid skeletons, it is interesting to discuss their respective complexation thermodynamics. All three molecules possess similar hydrocarbon structures suitable for inclusion into the β-CD cavity, but only 10-camphorsulfonic acid is anionic under the conditions employed (pH 6.9), which diminishes its complex stability by a factor of 4−13 as compared to results obtained for 2-hydroxy-3-pinanone and pinanediol. However, it should be emphasized that the $\Delta H°$ values obtained are remarkably consistent with one another ($\Delta H°$ from −19.5 to −20.7 kJ/mol, see Table 1), and the large deviations observed in the complex stability of more than 1 order of magnitude (i.e., from 489 to 6430 M^{-1}), are attributable solely to differences in $\Delta S°$. In these cases, the consistent $\Delta H°$ values indicate that the van der Waals interactions are quite similar for these terpenoid skeletons, while the degree of desolvation upon complexation substantially differs, depending on the nature of the attached hydrophilic group. Thus, the sulfonate anion is the most difficult to dehydrate and gives a more negative $\Delta S°$ value, whereas the neutral hydroxyketone and the 1,2-diol, to which waters of hydration are more weakly bound, dehydrate more richly, giving less negative or even positive entropies of complexation.

The complexation of camphorquinone-3-oxime gives one of the largest $\Delta H°$ values among the camphor and pinane derivatives studied, and its comparison with hydroxypinanone is interesting. The structural difference between the two guests involves the insertion of an imino nitrogen in the oxime guest. However, this causes significant changes in both $\Delta H°$ and $\Delta S°$, which ultimately compensate for one another, giving practically the same *K* values for both guests. The higher $\Delta H°$ value indicates that the oxime group is more hydrophobic than the hydroxyl group, leading to stronger van der Waals and hydrophobic interactions. However, the more negative $\Delta S°$ value suggests that the oxime is not readily dehydrated upon complexation or, as is more likely, is not originally so heavily hydrated, probably as a result of a six-membered ring, formed by an intramolecular hydrogen bond to the adjacent carbonyl group.

If the positional difference of the sufoxyl group is assumed to have no impact on the overall thermodynamics, the effect of introducing a bromine atom to the camphor skeleton may be demonstrated when the thermodynamic behavior of 3-bromo-8-camphorsulfonic acid is compared with that of 10-camphorsulfonic acid. The brominated camphorsufuric acid affords a binding constant that is 7 times higher than the reference compound, and this large enhancement is attributable predominantly to the increased enthalpic gain from −20 to −30 kJ/mol, which is canceled out in part by the accompanying entropic loss. The $\Delta H°$ increment of 10 kJ/mol for one bromine atom is 3 times larger than that observed for a methylene group (3.3 kJ/mol).[47] We may conclude that in this case one bromine atom

(51) Hallén, D.; Nilsson, S.-O.; Rotschild, W.; Wadsö, I. *J. Chem. Thermodyn.* **1986**, *18*, 429−442.
(52) Nilsson, S.-O.; Wadsö, I. *J. Chem. Thermodyn.* **1986**, *18*, 673−681.
(53) Nichols, N.; Sköld, R.; Spink, C.; Wadsö, I. *J. Chem. Thermodyn.* **1976**, *8*, 993−999.
(54) Konicek, J.; Wadsö, I. *Acta Chem. Scand.* **1971**, *25*, 1541−1551.

equates to three methylene groups from the point of view of the van der Waals interactions with β-CD.

When we take an overview of the thermodynamic behavior upon addition of methylene group to various guests, it is apparent that the increment in $\Delta G°$ per methylene group is more uniform than changes observed in $\Delta H°$ or $\Delta S°$. Quite frequently, large or irregular changes in $\Delta H°$ caused by the additional methylene unit are canceled out in part by compensatory changes in $\Delta S°$, giving the regular increments in $\Delta G°$, and vice versa. This widespread compensating effect may be considered as an example of Le Chatelier-Braun's principle, the physical meanings and origin of which should be seriously discussed.

Effect of Aliphatic Hydroxyl Group. It has been shown that the phenolic group in a guest such as tyramine or 3-(4-hydroxyphenyl)propionic acid forms hydrogen bonds to the inside of the β-CD cavity.[45] Both compounds have affinities that are 3 times higher than reference compounds that lack the OH group, i.e., phenylethylamine and 3-phenylpropionic acid, respectively. This affinity gain is almost exclusively enthalpic in origin. In the present study, the *N*-acetyl-tyrosine and *N*-acetyl-phenylalanine pair show similar thermodynamic behavior (Table 1).

In this context, it is interesting to examine the effects of adding an aliphatic OH group upon the complexation thermodynamics. For this purpose, we selected the following three pairs of guest compounds with and without an aliphatic OH, these being mandelic acid and phenylacetic acid, hexahydromandelic acid and cyclohexylacetic acid, and phenyllactic acid and 3-phenylpropionic acid. All of these pairs show clearly that the guest with an OH lowers the complex stability by a factor of 2. Thermodynamically, the decreased stabilities observed for guests with an added hydroxyl group originate mostly from the unfavorable contribution of the entropic term, except for the first pair, which both form very weakly bound complexes. In agreement with these observations, Cbz- and Boc-serine gave 1.3−1.4 times lower K values than the corresponding alanine derivatives, again as a result of the unfavorable entropic contribution (Tables 1 and 2).

Chiral Recognition Thermodynamics

Perhaps the most intriguing aspect of this study is the thermodynamics of chiral recognition by cyclodextrin, since previous studies concerning chiral guests are fairly limited in quantity and sometime in quality, which leads to a less comprehensive understanding of the chiral recognition behavior from the thermodynamic point of view. However, before beginning a detailed discussion, we will first discuss the accuracy of the thermodynamic data obtained or reported for the complexation of chiral guests with cyclodextrins.

Significant and Insignificant Differences in Thermodynamic Parameters. In general, the differences in thermodynamic parameters for the complexation of β-CD with antipodal guests are often very small, as can be seen from Table 1. If this is the case, the evaluation of uncertainties becomes very crucial when enantiodifferentiating ability is discussed according to the thermodynamic parameters. At this point, further discussion is confusing or meaningless if we do not determine the significance level, or threshold, of chiral recognition. We will therefore describe possible sources of error and the procedures for error assessment.

First, there are two possible sources of random errors in microcalorimetric experiments using the ITC Microcal instrument. One of the errors is associated with a random deviation of experimental points from calculated curve in each individual microcalorimetric titration experiment. These deviations are determined by baseline noise, the accuracy of the volume of each injection, the accuracy of calculations of the heat from each injection, and so on. Another kind of error emerges, not from the performance of the instrument itself, but from differences or erroneous preparations of the sample concentration, pH, and/or ionic strength. It has been shown that the first source of error dominates in the complexation reactions where K is in the order of several hundreds.[25] If one repeats titration microcalorimetric measurements for the same reaction several times, using a new solution of different concentration each time, and then the standard deviation (σ) of the mean is calculated from the data, it turns out that σ is comparable to or even smaller than the value given in each run by the Origin fitting program.[16] In the present study, we confirmed this fact once again, in many cases by using different chiral compounds and by repeating microcalorimetric measurements four to five times. When a guest shows a high affinity ($K > 1000$) and large heat effect, the experimental data points deviate only slightly from the fitted curve, affording a fairly small σ in each run. In contrast, the σ value arising from repeated measurements is not reduced and therefore often exceeds the σ value given by Origin in each independent run. Even for a moderately stable complex with a K value of several hundreds, the σ value arising from repeated experiments is in some cases 2−3 times larger than that given by Origin for each run. For this reason we have always employed a larger value of uncertainty (2σ) for the thermodynamic parameters listed in tables.

We must also take into account possible systematic errors originating from the instrument. MicroCal's Omega isothermal titration calorimeter used in the previous work[14,16,17,25] and the ITC Microcal titration calorimeter used in this study are well-established instruments, which have been examined by many independent researchers for systematic errors associated with electrical calibration, volume of injection, baseline stability, determination of produced heat, among other factors, and both instruments have been found to give satisfactory results. Although the temperature of the reaction cell of some older instruments displayed a tendency to increase by 0.3−0.5 K during a run (an error that can be significant if the enthalpy change is large and the equilibrium constant is strongly temperature-dependent), this unfavorable effect is almost completely eliminated in more modern instruments.

In fact, the major source of systematic errors lies in the inadequate application of the 1:1 model to more complicated systems, as we can illustrate using our own experimental data. In previous papers, we reported two separate sets of data for the complexation of 3-phenylpropionic and 3-phenylbutyric acid with β-CD. Slightly different values for 3-phenylpropionic acid were reported, these being (a) $K = 141 \pm 3\ M^{-1}$, $\Delta H° = -7.60 \pm 0.08$ kJ/mol[25] and (b) $K = 149 \pm 4\ M^{-1}$, $\Delta H° = -7.32 \pm 0.08$ kJ/mol,[45] and for 3-phenylbutyric acid values of (a) $K = 379 \pm 10\ M^{-1}$, $\Delta H° = -9.41 \pm 0.10$ kJ/mol[16] and (b) $K = 387 \pm 6\ M^{-1}$, $\Delta H° = -9.16 \pm 0.04$ kJ/mol were given.[45] In the present study, we repeated the microcalorimetric measurements with 3-phenylpropionic and 3-phenylbutyric acids using the same physicochemical conditions as before. A better fit of the experimental points was obtained using the new ITC Microcal instrument, allowing us to see small but systematic deviations of the points from the theoretical curve toward the end of experiments with both 3-phenylpropionic and 3-phenylbutyric acid. If one ignores this systematic deviation and executes Origin calculations using all of the experimental points, the calculations for 3-phenylpropionic acid, for example, give

the following values: $K = 151 \pm 5\ M^{-1}$, $\Delta H° = -7.11 \pm 0.15$ kJ/mol. It should be noted that, although this result is in good agreement with the previous study,[45] the quality of the fit can be improved (by more than 4 times in χ^2) by introducing just one extra parameter (n) to the fitting equation for the 1:1 model. We assigned the origin of this systematic deviation from the best fit line to the involvement of a complicated 1:n complex upon addition of an excess amount of guest to the CD solution. In fact, when only the first half of the data points in the same experiment are used in the calculation to reduce the final guest/host ratio, the Origin program gives somewhat different results which have a better fit: $K = 160 \pm 3\ M^{-1}$, $\Delta H° = -6.90 \pm 0.08$ kJ/mol. For further confirmation, we repeated the same experiments using host and guest solutions at half of the original concentration. In these subsequent runs the quality of the fit (χ^2) to the theoretical curve was not appreciably improved through the use of models more sophisticated than the 1:1 case. The newest, most accurate thermodynamic parameters (data set (c)) obtained from several runs are listed in Table 2 for the complexations of β-CD with 3-phenylpropionic and 3-phenylbutyric acid. The dilution test may be considered as a standard procedure when involvement of sophisticated complexes is suspected because the contribution from these can be reduced by lowering the concentrations of the guest or/and host. In this study, attempts were made to avoid the involvement of 1:2 or any other more sophisticated complexes in solution, and all experiments were performed at concentrations as low as possible to ensure the sole formation of the 1:1 species.

It should also be noted that there is an elusive correlation between K and $\Delta H°$ observed in the erroneous and correct data presented above: that for a larger equilibrium constant, a smaller heat effect is seen. Such a correlation between K and $\Delta H°$, or more generally, between two linearly correlated parameters, has been discussed in the literature from various points of view.[55−58] Here we will present a simple, illustrative explanation for the source of this $K-\Delta H°$ correlation. For the simplest 1:1 model, the square sum of the deviation as a function of K and $\Delta H°$, $\Sigma(\Delta Q_{exp} - \Delta Q_{cal})^2$, appears as an unsymmetrical three-dimensional well ($\Delta Q_{exp} - \Delta Q_{cal}$ is a difference between the observed experimental heat effect upon each injection and the theoretical curve).[59,60] The sides of this well become very steep if one tries to change one variable (for instance, K), keeping the others constant, and become even steeper if one tries to vary simultaneously two variables in the same direction. One can only alter the variables with the minimum increment of $\Sigma(\Delta Q_{exp} - \Delta Q_{cal})^2$ by decreasing one variable while simultaneously increasing another. Hence, if some perturbation takes place (in our case, the formation of a 1:2 species in addition to a 1:1 species) that affects the experimental data (K and/or $\Delta H°$), it is always most beneficial for K and $\Delta H°$ values to be adjusted in such a way as to minimize of changes in the $\Sigma(\Delta Q_{exp} - \Delta Q_{cal})^2$ value.

What is most important for us in the context of this paper is to know if or how differences in $\Delta G°$ and $\Delta H°$, caused by changing the number of methylene groups or the chirality of the guest, depend on the experimental data set employed, including the erroneous data discussed above. It is interesting, therefore, to examine the thermodynamic consequences of the extra methylene group in the complexation of 3-phenylbutyric acid or 3-phenylpropionic acid with β-CD. Despite there being three different data sets a−c with some degree of error in sets a and b, the increments in $\Delta G°$ and $\Delta H°$ caused by the additional methylene group are surprisingly similar in the above three cases, where $\Delta\Delta G°$ and $\Delta\Delta H°$ are (a) −2.45 and −1.81, (b) −2.36 and −1.84, and (c) −2.33 and −1.73 kJ/mol, giving $\Delta\Delta G°_{average} = -2.38 \pm 0.07$ and $\Delta\Delta H°_{average} = -1.79 \pm 0.07$ kJ/mol. It should be emphasized that, even if a double standard deviation of the mean (2σ) is as large as 0.5−0.7 kJ/mol for the original sets of $\Delta G°$ and $\Delta H°$ values, $\Delta\Delta G°$ and $\Delta\Delta H°$ are consistent within 0.07 kJ/mol, as a result of the $K - \Delta H°$ correlation.

Similar systematic deviations of experimental points from the theoretical curve for the 1:1 model that occur in the final stages of the run were observed in preliminary microcalorimetric experiments with D- and L-isomers of Cbz-alanine when guest concentrations in the range 170−130 mM were used. If one ignores these systematic deviations, and Origin calculations are carried out using all of the experimental points, the following values are obtained: $K = 135 \pm 5\ M^{-1}$ for Cbz-L-alanine and $K = 138 \pm 4\ M^{-1}$ for Cbz-D-alanine, and thus there is no chiral discrimination by β-CD. As can be seen from Table 1, the final K values, obtained at 2−3 times lower guest concentrations, deviate from the preliminary K values by 8−9%, and lie outside of the range of assigned uncertainty for the preliminary data. However, the same conclusion can be derived from more accurate data (Table 1). Thus, we wish to emphasize that the differences in the overall complexation thermodynamic parameters ($\Delta\Delta G°$ and $\Delta\Delta H°$) due to the addition of a methylene unit or the chirality are much smaller than the fluctuations in the original parameters ($\Delta G°$ and $\Delta H°$) themselves.

The Relationship Between Penetration Mode and Chiral Recognition. It is widely accepted that the most probable mode of a guest's interaction with CD involves the insertion of the more hydrophobic part of the guest into the CD cavity,[2,4−6,9,30,47,61,62] while the more polar, often charged group of the guest is exposed to the bulk water just outside the wider opening of the cavity and is derived from both thermodynamic and NMR studies.[4,5,9,62] Since naturally occurring CDs are chiral, it might be expected that a chiral guest can be recognized through different modes of penetration into the cavity.

When discussing the CD complexation of structurally related chiral guests, it is reasonable to assume that guests with the same absolute configuration should be preferred, unless the hydrophobicity order of the substituents around the asymmetric center that the CD cavity recognizes is different for each of two enantiomeric guests. It is also likely that, if one alters the position of the hydrophobic substituent around the asymmetric center, the antipodal guest should be preferred. Amino acids are perhaps the most suitable guests for validating this hypothesis, since a hydrophobic substituent is readily introduced at several different positions around an asymmetric center of the same absolute configuration.

Earlier we showed that zwitterionic phenylalanine has a very low K value of 3 M^{-1} with β-CD.[14] In this study we examined *O*-benzylserine which possesses a distance between the phenyl

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and charged NH_3^+ group that is increased by two C−O bond lengths compared to phenylalanine. This guest gives an appreciable K value of around 70 M^{-1} for both enantiomers, while showing no chiral recognition (Table 1). It appears that the asymmetric center, which is located away from the penetrating phenyl group in the broken water shell around the ammonium group, does not participate significantly in the inclusion complexation. *N*-Acetylation of phenylalanine greatly enhances its hydrophobicity to give higher K values of 61 and 68 M^{-1} for the D- and L-isomers, respectively. The chiral recognition observed may be attributed to the location of the asymmetric carbon atom at the boundary of the broken water shell around the carboxylate group. The enantioselectivity, as defined by the relative K value (K_L/K_D), is 1.11. Similarly, *N*-acetyl-L-tryptophan and *N*-acetyl-L-tyrosine were preferred with enantioselectivities of 1.35 and 1.04, respectively.

Dipeptides showed higher affinities toward β-CD than the component amino acids. Thus, the D- and L-isomers of glycidylphenylalanine afforded appreciable K values of 47 and 54 M^{-1}, respectively. Again the L-isomer is bound more strongly by β-CD than the D-isomer, with a K_L/K_D ratio of 1.15.

The methyl-esterification of phenylalanine did not greatly improve the affinity to CD ($K = 11-12$ M^{-1}), probably due to the charged NH_3^+ group, which is located close to the phenyl group, as is the case with the zwitterionic phenylalanine. The enantioselectivity does not seem high, although in this case the uncertainty is large. An attempt to study this guest in its neutral form at pH 10 was unsuccessful due to the relatively fast hydrolysis of phenylalanine methyl ester in the alkaline solution. Phenylalaninamide was then considered, since the amide group is more stable than the ester in alkaline solution. Upon complexation with β-CD at pH 10, uncharged D- and L-phenylalanine amide gave fairly high K values of 101 and 109 M^{-1}, respectively, with a K_L/K_D ratio of 1.08.

The consistent preference observed for a variety of modified L-amino acids examined clearly agrees with our theory that, as far as the degree of hydrophobicity of the substituents around the asymmetric center is conserved, modification of the amino or carboxyl groups of amino acids do not alter the enantioselectivity of the CD or the mode of guest penetration. Thus, the chiral recognition of amino acid derivatives by β-CD is well defined and is not affected by introducing less hydrophobic substituents. In contrast, the introduction of a Cbz or Boc group to alanine or serine at the amino terminus converts the hydrophobicity order by switching what was originally the most hydrophilic group (NH_3^+) to the most hydrophobic group ($PhCH_2OCON$ or *t*-BuOCON), while the absolute configuration at the α-carbon remains unchanged. In these amino acid derivatives, the penetrating hydrophobic group is attached not to the β-carbon, but to the amino nitrogen. In the case of *N*-Cbz-alanine, the enthalpy of complexation differs by ∼1 kJ/mol between two enantiomers, which is comparable with the effect of adding a methylene group, as shown by the comparison of *N*-Cbz-alanine with *N*-Cbz-glycine (Tables 1 and 2). Unfortunately this difference is entirely canceled out by the compensating entropic change, and no chiral recognition is observed. However, as anticipated, *N*-Boc-alanine, *N*-Boc-serine, and *N*-Boc-alanine methyl ester showed significant chiral recognition in favor of the D-isomer in all three cases, with K_D/K_L ratios of 1.07, 1.14, and 1.07, respectively. For *N*-Boc-alanine the chiral recognition is entropic in origin, while for *N*-Boc-serine and *N*-Boc-alanine methyl ester the recognition is an enthalpically driven process, although the enthalpic gains are partially canceled out by entropy changes.

Two more series of guest compounds remain to be discussed with respect to the chirality affinity relationship. Possessing substituents with similar hydrophobicities at the asymmetric center, hexahydromandelic acid, phenyllactic acid, and mandelic acid (which has large associated uncertainties) gave higher K values for the (*R*)-isomers upon complexation with β-CD; the K_R/K_S ratios are 1.07, 1.06, and ∼1.2, respectively. Other enantiomeric pairs of the guests are (1*R*,2*S*)- and (1*S*,2*R*)-2-amino-1,2-diphenylethanol and (1*R*,2*S*)- and (1*S*,2*R*)-ephedrine. Although the 2-phenyl group in 2-amino-1,2-diphenylethanol is replaced by a methyl group in ephedrine, these two pairs share the same backbone structure with respect to the stereochemistry and to the mode of penetration, with the 1-phenyl moiety acting as the penetrating group, and the amino group as the hydrophilic tail. The substituents around one of the asymmetric centers are identical for both pairs, while the second asymmetric center is located inside the destructured water shell formed around the ammonium group and does not, therefore, make significant contributions to the overall thermodynamics and chiral recognition. With the same absolute configuration, the (1*R*,2*S*)-isomers of both guests show a higher affinity toward β-CD than the antipodal (1*S*,2*R*)-isomer, with a K_{RS}/K_{SR} ratio of 1.11 for ephedrine[25] and 1.20 for 2-amino-1,2-diphenylethanol.

It is interesting to note that the *R*-configuration gives a higher affinity with β-CD for all guests which bear a hydroxyl group joined to the asymmetric center, an aromatic/aliphatic cycle for inclusion, a hydrogen atom, and a charged group for dissolution in water. This rationalizes the unexpected *S*-preference observed for mandelic acid methyl ester ($K_S/K_R = 1.07$), since the hydrophobicity order is most probably switched by esterification of the carboxyl group.

It should be emphasized that our current understanding of the thermodynamics of chiral recognition by β-CD is not comprehensive, and in general we cannot predict the preferred affinity based on the stereochemistry of the guest molecule. We have observed an appreciable chiral recognition for ephedrines, pseudoephedrines, 2-amino-1,2-diphenylethanol, hexahydromandelic acid, and phenyllactic acid, but it is not easy to explain why structurally related compounds, such as 1-phenyl-1,2-ethanediol, 2-phenylpropionic acid, 2-phenylbutyric acid, and propranolol, do not undergo enantioselective binding with β-CD.

Effect of Chiral Centers Bearing an Alkyl Group. Since the cavity of CD is hydrophobic and includes size-matched aliphatic/aromatic guests through van der Waals interactions, it is expected that the chiral center of guests bearing a hydrophobic alkyl group (i.e., methyl group) can undergo enantiodiscrimination, as shown above for *N*-Boc-alanine and *N*-Boc-alanine methyl ester. In our previous study,[14] we reported that none of a range of 2-alkanols examined were enantioselectively recognized by α-CD, exhibiting exactly the same thermodynamic parameters within experimental error. It is difficult to discuss the thermodynamics of chiral recognition of the 2-alkanols with β-CD, owing to small reaction enthalpies, which impose instrumental limitations on the accuracy of the microcalorimetric determinations. To overcome these limitations, we decided to study (*R*)- and (*S*)-3-bromo-2-methyl propanol as guests for β-CD, and here, quite large, negative enthalpies and moderate equilibrium constants were obtained. However, the appreciably different enthalpy and entropy changes for the (*R*)- and (*S*)-isomers appear to have canceled out one another, giving essentially no chiral recognition.

We may classify chiral guests that show no enantioselective binding into two categories according to the thermodynamic behavior described above, as follows: (1) $\Delta H^\circ_R = \Delta H^\circ_S$ and

$\Delta S°_R = \Delta S°_S$, where $\Delta G°_R = \Delta G°_S$ and (2) $\Delta H°_R \neq \Delta H°_S$ and $\Delta S°_R \neq \Delta S°_S$, but $\Delta G°_R = \Delta G°_S$. For the first category of guests that give the same $\Delta H°$ and $\Delta S°$ values for each enantiomer, it is reasonable to assume that the CD cavity cannot recognize the stereochemistry of guest because the asymmetric center of these guests is located close to the hydrophilic group. It is more difficult to rationalize the thermodynamic behavior of the second category of guests, yet interestingly, such an enthalpy−entropy canceling effect has been observed frequently in host−guest chemistry.[30,63,64] In our case, if one of the enantiomers can produce slightly stronger van der Waals interactions through a deeper penetration or closer contact with the CD cavity than with the other, the enthalpic gain can be canceled out easily by the entropic loss arising from the accompanying structural freezing in the complex.

The effect on the chiral recognition behavior of a methyl or alkyl group introduced to aromatic and aliphatic acids and amines was studied. The aromatic acids investigated were 2-phenylpropionic acid, 2-phenylbutyric acid, and 3-phenylbutyric acid. Whereas the first two guests did not show any chiral recognition upon complexation with β-CD, the third showed an appreciable enantioselectivity ($K_S/K_R = 1.07$), which was caused by a slight entropic gain for the (*S*)-isomer ($T\Delta\Delta S° = 0.11$ kJ/mol). Two enantiomer pairs of amines were studied: 1-cyclohexylethylamine and *N*,*N*-dimethyl-1-ferrocenylethylamine. Although the enantiomers of 1-cyclohexylethylamine showed no chiral discrimination, ferrocenylamine gave high binding constants with a K_S/K_R ratio of 1.20, which was driven solely by a favorable entropic contribution ($T\Delta\Delta S° = 0.3$ kJ/mol).

The results concerning relatively simple guests that have been described above lead us to conclude that the chiral recognition behavior of β-CD is most likely to occur when the distance between the hydrophilic group and asymmetric center of the guest is as large as possible. This is observed for 3-phenylbutyric acid, which is chirally discriminated by β-CD, as compared to 2-phenylpropionic acid and 2-phenylbutyric acid, which are not. In this example an additional separation of one C−C bond between the hydrophilic group and asymmetric center lies at the origin of the enantioselectivity. Other examples of the same sort can be found in the Table 1, for instance, zwitterions of L- and D-benzylserine are not recognized by β-CD, but *N*-acetyl-amino acids show appreciable chiral discrimination.

It is also reasonable to assume that chiral guests which possess rigid penetrating groups will show better chiral recognition, since a more flexible group will adjust its shape inside the cavity, giving minimal enantioselectivity. We can see typical examples of this behavior when we compare 1-cyclohexylethylamine and *N*,*N*-dimethyl-1-ferrocenylethylamine. In this case, the cyclohexyl group is obviously more flexible and less bulky in comparison to the ferrocenyl moiety. This means that each enantiomer of the former guest can more easily adjust its shape and position within in the β-CD cavity, minimizing the structural differences between the enantiomers, while the rigid ferrocenyl guest has little room to adjust its conformation upon complexation, resulting in good enantiodifferentiation. It is interesting to note that *N*,*N*-dimethyl-1-ferrocenylethylamine is the only known guest, with its chiral center next to the ammonium group, which is chirally recognized by β-CD. This may be attributed to the three alkyl groups attached to the nitrogen which probably make the hydration shell less strongly bound as compared to an $-NH_3^+$, thus facilitating the accessibility of the center toward enantiodifferentiating interactions.

To further justify the above argument, let us now consider the chiral recognition of more sterically related enantiomeric guest pairs, e.g., dibenzoyl and ditoluoyl tartaric acids. High levels of chiral recognition are observed for dibenzoyl tartaric acid ($K_D/K_L = 1.6$), a result which is entirely enthalpic in origin, whereas ditoluoyl tartaric acid shows a much smaller K_D/K_L value of 1.1. In this case, the structural difference is merely the methyl group at the para position. Interestingly, the presence of this methyl group increases the entropic gain ($T\Delta S°$) by exactly the same amount for both enantiomers (4.2 kJ/mol), but diminishes the enthalpic gain by a different extent for the D- and L-isomers (1.2 and 0.3 kJ/mol, respectively), thus enhancing the binding abilities, but vastly reducing enantioselectivity. The increased positional and rotational freedom of the penetrating group of the guest are probably responsible for this reduced enantioselectivity.

A comparison of the three aromatic amino acids, *N*-acetyltryptophan, *N*-acetylphenylalanine, and *N*-acetyltyrosine, gives us some understanding concerning the relationship between complex stability and chiral recognition. *N*-Acetyltryptophan, for which the indole moiety does not match to the shape and the size of β-CD cavity and is fairly restricted in its movements inside the cavity, gave the least stable complex with β-CD and the highest enantioselectivity of $K_L/K_D = 1.34$. The phenyl group of *N*-acetylphenylalanine led to a higher affinity toward β-CD, but the enantioselectivity decreased to $K_L/K_D = 1.11$. This enhancement of affinity is exclusively entropic in origin, which unfortunately diminishes the enantioselectivity. The formation of an additional hydrogen bond between the host and guest causes *N*-acetyltyrosine to interact more strongly with β-CD than *N*-acetylphenylalanine.[45] In this case, the affinity gain driven by the enthalpy and the enantioselectivity further decreases to $K_L/K_D = 1.04$. It is now apparent that for a series of guests, an enhancement of binding affinity often leads to a reduction in chiral recognition, irrespective of the driving force that results in the affinity enhancement. Indeed, if local weak interaction forces are not cooperative, chiral recognition tends to vanish when the host−guest affinity is enhanced. The above observations agree with the common sense reasoning that a high level of chiral recognition can only be achieved when the host molecule has a shape and location of specific functional groups that are complimentary to the structure of the guest.

The hydrophobicity of the trifluoromethyl group is much higher than that of methyl group, rendering the complex stability of α-methoxy-α-trifluoromethylphenylacetic acid approximately 15 times higher than that of α-methoxyphenylacetic acid, giving the two guests distinctly different reaction enthalpies ($\Delta\Delta H° = 11-13$ kJ/mol). The significant chiral recognition ($K_R/K_S = 1.24$) observed for α-methoxy-α-trifluoromethylphenylacetic acid is entirely enthalpic in origin, although the accuracy of our experimental data for α-methoxyphenylacetic acid is not high enough to discuss the existence/non-existence of significant chiral recognition.

At present it seems difficult for us to obtain general rules for the structural features that are responsible for entropy- or enthalpy-driven chiral recognition processes, even if one considers only a set of structurally related guest molecules. For instance, the complexation entropies of anionic (*R*)-camphor derivatives are always less favorable than those of the (*S*)-isomers, although the favored enantiomer varies from guest to guest, these being (*S*)-camphanic acid, (1*R*)-10-camphorsulfonic acid, and (*R*)-camphoric acids, with no recognition observed

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for 3-bromo-8-camphorsulfonic acid. It is also difficult to draw conclusions concerning the chiral recognition behavior of neutral camphor derivatives, since only camphorquinone-3-oxime is recognized by β-CD, whereas 2-hydroxy-3-pinanone and pinanediol are not. Furthermore, the chiral recognition of *N*-acetylphenylalanine is predominantly entropy-driven, whereas the structurally related Gly-Phe dipeptide and phenylalaninamide are charily recognized exclusively through the enthalpy term.

Effect of the Hydroxyl Group. As described for ephedrines and pseudoephedrines in our previous study,[25] appreciable chiral recognition was observed generally for chiral alcoholic guests in which the hydroxyl group is not the principle solubilizing group in aqueous media. Thus, both phenyllactic acid and hexahydromandelic acid give appreciable enantioselectivity upon complexation with β-CD. It is also noted that an aliphatic hydroxyl group at a non-asymmetric carbon has practically no impact on the magnitude of chiral recognition, as exemplified by *N*-Boc-alanine and *N*-Boc-serine. In contrast, chiral mono- and diol guests, i.e., *O*-benzylidenethreitol, 3-benzyloxy-1,2,4-butanetriol, 3-bromo-2-methyl-1-propanol, *trans*-1,2-cyclohexanediol, 2-hydroxypinanone, 1-phenyl-1,2-ethanediol, and pinanediol, do not show significant chiral recognition. Exactly the same thermodynamic parameters were obtained for both enantiomers of *O*-benzylidenethreitol, phenyl-1,2-ethanediol, and pinanediol, and although 3-benzyloxy-1,2,4-butanetriol, 3-bromo-2-methyl-1-propanol, *trans*-1,2-cyclohexanediol, and 2-hydroxypinanone afford significantly different $\Delta H°$ and $\Delta S°$ values for each enantiomer, no chiral recognition is achieved as a result of compensatory changes of the enthalpy and entropy terms. Similarly, the enantiomers of benzyl glycidyl ether gave exactly the same thermodynamic parameters.

When the most hydrophilic part of the guest is the hydroxyl group, it is intriguing that none of the chiral (cyclo)alkanols, diols, or triols that we examined in the present study or in previous work[14,25] are discriminated by α- and/or β-CDs upon complexation. This phenomenon may be related to the "structure-forming" nature of the hydroxyl group, as compared to the "structure-breaking" nature of the ammonium and carboxylate groups. Since the aliphatic hydroxyl group is smoothly accommodated by the hydrogen bond network of the bulk water, any conformational differences in the host−guest complex with CD are likely to be absorbed by a balance of the enthalpic gain arising from van der Waals interactions and the entropic loss caused by the rearrangement of the hydrogen bond network. However, this does not mean that by switching the major hydrophilic group from an aliphatic hydroxyl to some other hydrophilic group one can automatically obtain an appreciable chiral recognition, as illustrated by the enantiomers of 3-bromo-2-methylpropanol and 3-bromo-2-methylpropionic acid methyl ester.

Chiral Recognition and Enthalpy−Entropy Compensation. A compensatory enthalpy−entropy relationship has often been observed empirically in the kinetic and thermodynamic parameters determined for a wide variety of reactions and equilibria.[65−69] Much debate has focused on the basis of this extrathermodynamic relationship,[70−78] since no explicit relationship between the enthalpy and entropy changes can be derived logically from fundamental thermodynamics. Thermodynamic parameters are more reliable for validating the compensatory enthalpy−entropy relationship, although in the case of kinetic data the relationship can be a mathematical artifact rather than experimental fact.[82]

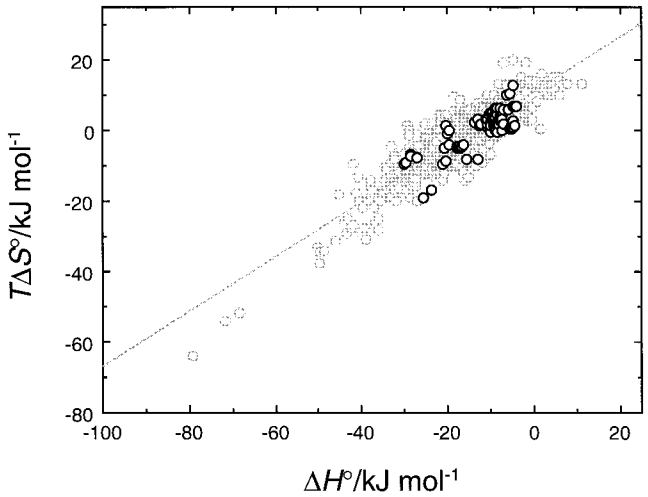

Figure 2. Enthalpy−entropy compensation plot for the inclusion complexation of various guests with native cyclodextrins obtained in the previous[47] (light circle) and present work (dark square).

In our previous studies,[30,63,64,79−81] we have demonstrated that diverse chemical and biological supramolecular systems, including cyclodextrins, can be analyzed consistently by using the slope (α) and intercept ($T\Delta S°_0$) of the $\Delta H° - T\Delta S°$ plot as quantitative measures of the conformation changes and the extent of desolvation, respectively. Recently we have examined in more detail the general validity of the compensatory enthalpy−entropy relationship in the complexation thermodynamics of cyclodextrins by using compiled data which has been reported for various types of guests.[16,17,25,47] The use of a very large amount of thermodynamic data is essential in evaluating quantitatively the enthalpy−entropy compensation effect, since a limited number of data may lead to a scattered plot and an erroneous analysis. The thermodynamic parameters obtained for the 46 enantiomeric pairs in the previous[14,25] and present studies do not meet this data size criterion at all. Hence, the thermodynamic parameters for the chiral guests in this study inevitably scatter over a fairly wide range in the conventional $\Delta H° - T\Delta S°$ plot, as shown in Figure 2 (black circles). However, these scattered data points fit well to the global $\Delta H° - T\Delta S°$ plot, which has been reported previously for the complexation of 1070 guest molecules with natural CDs (slope, $\alpha = 0.88$ and intercept, $T\Delta S°_0 = 12$ kJ/mol).[47]

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The general concept developed by Grunwald et al.[67a,67b] provides us with a reliable tool for the prediction of existence or non-existence of meaningful enthalpy−entropy compensation in a particular set of limited amount of thermodynamic data. The idea is based on the separation of overall complexation thermodynamic parameters into two terms: *nominal* and *environmental*. The nominal part (ΔG_{nom}, ΔH_{nom}, and ΔS_{nom}) states that solvated CD plus solvated G form a solvated CD·G complex, while the environmental part (ΔG_{env}, ΔH_{env}, and ΔS_{env}) is associated with water molecules which are involved in solvation/desolvation processes upon complexation. It was shown that only ΔG_{env} is equal to zero in dilute solution, and thus only ΔH_{env} and ΔS_{env} terms are subject to distinct enthalpy−entropy compensation.[67a,67b] Consequently, no meaningful enthalpy−entropy compensation was observed even in a series of very similar homologues which differ by one or several methylene groups, since ΔH_{nom} plays a predominant role in determination of the overall free energy of complexation of the homologues guests.[16] One of the possible ways to reduce contribution of the nominal part (ΔG_{nom}, ΔH_{nom}, and ΔS_{nom}) is the transfer reaction of the same guest (G) between α- and β-cyclodextrin:

$$[\alpha\text{-CD}\cdot\text{G}] + [\beta\text{-CD}] = [\alpha\text{-CD}] + [\beta\text{-CD}\cdot\text{G}]$$

Although good enthalpy−entropy compensation relationships were not obtained for the direct complexation reactions, the transfer reactions of cyclohexanes[17] and phenols[23] between α- and β-CD cavities led to satisfactory compensatory effect. Another way is to minimize the structural differences between the guests. Consequently, an excellent enthalpy−entropy compensation was observed for the complexation reactions of stereoisomers of ephedrines and pseudoephedrines with α-CD (slope $\alpha = 1.07 \pm 0.14$) and β-CD (slope $\alpha = 1.22 \pm 0.23$), probably because the structural variation in the guest is only at the chiral center.[25]

The enthalpy−entropy compensation effect for the differential thermodynamic parameters ($\Delta\Delta H°$ and $\Delta T\Delta S°$) for the chiral recognition can be defined by the following hypothetical exchange equilibrium between the (*R*)- and (*S*)-isomers of the same chiral guest:

$$[\beta\text{-CD}\cdot R] + [S] = [\beta\text{-CD}\cdot S] + [R]$$

In discussing the differential thermodynamic parameters for chiral recognition, it is essential to use only the data for enantiomeric pairs that exhibit statistically meaningful, well-established chiral recognition behavior, and which give distinctly different free energies of complexation ($\Delta G°$) for both enantiomers. This is because similar or identical $\Delta G°$ values for both enantiomers lead to a set of calculated $\Delta\Delta H°$ and $T\Delta\Delta S°$, that are automatically plotted on the "ideal" entropy-enthalpy compensation line, i.e., $T\Delta\Delta S° = \Delta\Delta H°$, simply by definition, regardless of their magnitude. Twenty-two enantiomer pairs which have been differentiated by β-CD beyond the level of uncertainty are collected in Table 3. The differential enthalpy changes ($\Delta\Delta H°$) were plotted against the differential entropy changes ($T\Delta\Delta S°$, $T = 298.15$ K) to give an excellent straight line with a slope equal to unity and with a very small intercept ($T\Delta\Delta S_0 = 0.4$ kJ/mol), as shown in Figure 3. In comparison to the widely scattered $\Delta H° - T\Delta S°$ plot (Figure 2) for the same sets of chiral guests, this excellent fit is quite impressive.

This result seems quite reasonable, since the differential thermodynamic parameters for the enantiomer pairs reflect only differences arising from the change in chirality. In this treatment dealing with the exchange equilibrium $[\beta\text{-CD}\cdot R] + [S] = [\beta\text{-CD}\cdot S] + [R]$, we can simplify the system, and offset all other structural features except for the chirality of the guest, thus reducing the contribution of the nominal part (ΔG_{nom}, ΔH_{nom}, and ΔS_{nom}) almost to zero. To reiterate, an excellent enthalpy−entropy compensation can be observed if the number of variables in the system can be made as small as possible. In this context, the complexation thermodynamics of various enantiomeric guests with the other chiral hosts should certainly be considered one of the most important subjects for future research in supramolecular chemistry.

Table 3. Differences of Reaction Enthalpies ($\Delta\Delta H°$) and Reaction Entropies ($\Delta T\Delta S°$) for the Complexation of Two Stereoisomers of Various Chiral Chemical Compounds with β-Cyclodextrin at 298.15 K

guest	$\Delta\Delta H°$/ kJ mol^{-1}	$\Delta T\Delta S°$/ kJ mol^{-1}	ref
N-acetyl-phenylalanine	−0.03	0.23	*a*
N-acetyl-tryptophan	1.7	2.4	*a*
N-acetyl-tyrosine	−0.4	−0.3	*a*
2-amino-1,2-diphenylethanol	0.0	0.4	*a*
N-*t*-Boc-alanine	0.1	0.3	*a*
N-*t*-Boc-alanine methyl ester	−1.02	−0.7	*a*
N-*t*-Boc-serine	−0.4	−0.2	*a*
camphanic acid	0.1	0.5	*a*
camphoric acid	7.2	7.8	*a*
camphorquinone-3-oxime	0.1	0.3	*a*
camphor-10-sulfonic acid	−1.2	−0.8	*a*
O,*O*′-dibenzoyl-tartaric acid	−2.1	−0.9	*a*
N,*N*-dimethyl-1-ferrocenyl ethylamine	−0.1	0.3	*a*
ephedrine	−0.99	−0.75	*b*
Gly-Phe	−0.66	−0.3	*a*
hexahydromandelic acid	−0.25	−0.07	*a*
α-methoxy-α-trifluoromethyl phenylacetic acid	−1.13	−0.6	*a*
phenylalanine amide	−0.6	−0.4	*a*
3-phenylbutyric acid	−0.06	0.11	*a*
phenyllactic acid	−0.69	−0.5	*a*
pseudoephedrine	−2.55	−1.74	*b*
O,*O*′-*p*-toluyl-tartaric acid	−1.11	−0.9	*a*

[a] This work; data extracted from Table 1. [b] Reference 25.

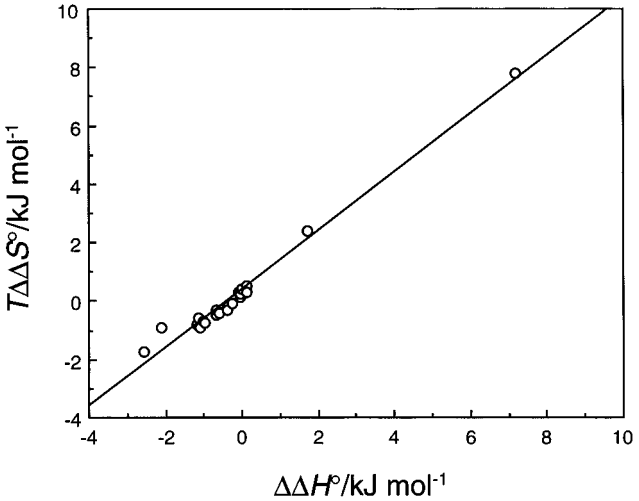

Figure 3. Plot of the differential entropy change against the differential enthalpy upon complexation of 20 enantiomeric pairs with β-cyclodextrin, which give statistically meaningful chiral recognition.

Conclusions

The new, accurate thermodynamic parameters obtained in this thermodynamic study have enabled us significantly improve our understanding of the relationship between the stereochemistry of the guest and the complexation thermodynamics of these

chiral molecules with cyclodextrin. The present study reveals clearly that there is no direct relationship or even general tendency between the thermodynamic parameters and chiral recognition by β-CD. Thus, appreciable chiral recognition was found with almost equal probability among the chiral guests examined, irrespective of the magnitudes of $\Delta G°$, $\Delta H°$, and $\Delta S°$. To understand the reasons for the presence or absence of chiral recognition, attention should be paid to the weak interactions involved in the complexation process.

Our knowledge of chiral recognition by β-CD is still far from comprehensive, and we cannot predict the magnitude of chiral recognition from a consideration of the structure of the guest. However, it is reasonable to emphasize the correlations which have been elaborated by this study:

(1) A direct correlation between the mode of penetration and chiral recognition by β-CD for aromatic amino acid derivatives (and for some other classes of organic chiral compounds) has been established.

(2) Several examples have been used to demonstrate that chiral guests with a less symmetrical, nonpolar penetrating group and chiral guests with a larger distance between chiral center and the most hydrophilic, often charged group, are more likely to exhibit chiral recognition.

(3) Almost any alterations made to the guest molecule that result in stronger binding with β-CD lead to a loss of chiral recognition, since in almost all cases the additional weak interactions involved in the complexation process result in non-complimentarity between the chiral guest and CD cavity.

(4) A much better enthalpy−entropy compensation effect can be obtained for various pairs of enantiomers by plotting the differential, rather than the original, thermodynamic parameters.

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Supporting Information Available: Table of thermodynamic date and references (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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