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Extraordinary chiral discrimination in inclusion gas chromatography. Thermodynamics of enantioselectivity between a racemic perfluorodiether and a modified γ-cyclodextrin

Volker Schurig*, Roswitha Schmidt
Institute of Organic Chemistry, University of Tübingen, Auf der Morgenstelle 18, D-72076 Tübingen, Germany

Abstract

The enantiomers of the perfluorodiether ''compound B'' [2-(fluoromethoxy)-3-methoxy-1,1,1,3,3-pentafluoropropane], a decomposition product of the inhalational anesthetic sevoflurane [2-(fluoromethoxy)-1,1,1,3,3,3-hexafluoropropane], were separated by gas chromatography on octakis(3-*O*-butanoyl-2,6-di-*O*-*n*-pentyl)-γ-cyclodextrin (Lipodex E), dissolved in polysiloxane PS 255 (30% w/w), with an unexpectedly high separation factor of $\alpha = 10.6$ at 26 °C. Using the concept of the retention increment R', non-enantioselective and enantioselective contributions to retention were separated and thus reliable thermodynamic parameters of enantioselectivity, i.e. $-\Delta_{S,R}(\Delta G) = 5.7$ (0.05) kJ/mol at 303 K, $-\Delta_{S,R}(\Delta H) = 20.1$ (0.64) kJ/mol, $\Delta_{S,R}(\Delta S) = -47.4$ (2.0) J/K mol and $T_{\text{isoenant}} = 424$ (30) K or ~150 °C, were determined by temperature-dependent measurements. The enantiomeric bias represents the largest values ever measured in enantioselective gas chromatography. An equation is presented which allows calculation of the non-enantioselective contributions to retention from measurements at two arbitrary concentrations of Lipodex E in polysiloxane. Surprisingly, the enantioselectivity is greatly reduced when employing the β-cyclodextrin analogue and breaks down completely with the α-cyclodextrin analogue of Lipodex E.

Keywords: Enantioselective inclusion, GC; Retention increment; Enantioselectivity; Lipodex E; Perfluorodiether, chiral; Compound B

1. Introduction

According to Scheme 1, the perfluorodiether ''compound B'' [2-(fluoromethoxy)-3-methoxy-1,1,1,3,3-pentafluoropropane] represents a minor decomposition product of the inhalational anesthetic sevoflurane [2-(fluoromethoxy)-1,1,1,3,3,3-hexafluoropropane] [1]. It is formed when air is passed through soda lime via a closed rebreathing circuit in an effort to trap exhaled carbon dioxide during narcosis with sevoflurane [2,3]. The perfluorodiether ''compound B'' represents a chiral molecule. The gas-chromatographic separation of the enantiomers of ''compound B'' has been achieved on several modified cyclodextrins diluted in polysiloxanes [4–6]. A large separation factor α of 4.1 at 30 °C was obtained for ''compound B'' on the sterically congested cyclodextrin derivative heptakis(6-*O*-

*Corresponding author. Tel.: +49-7071-297-6257; fax: +49-7071-295-538.
E-mail address: volker.schurig@uni-tuebingen.de (V. Schurig).

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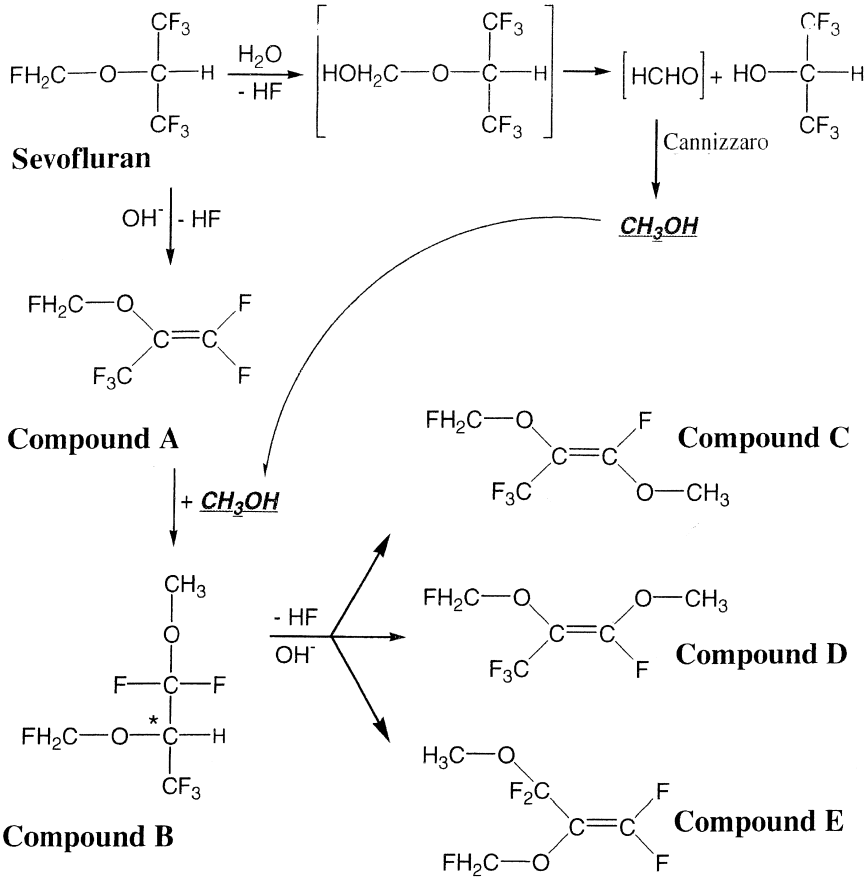
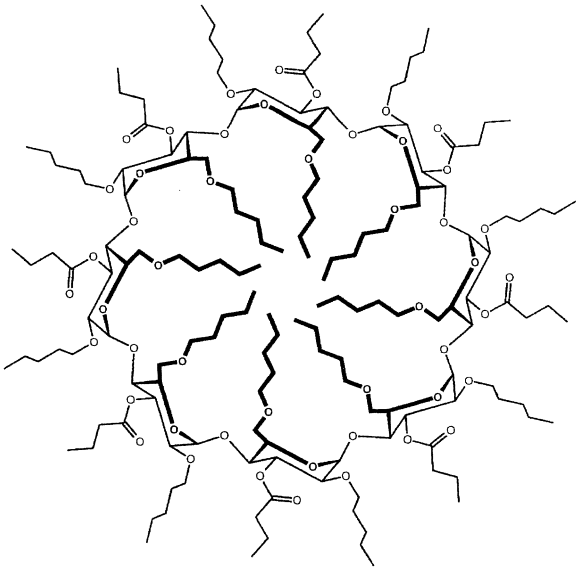


Scheme 1. Decomposition products of sevoflurane in an alkaline environment [2].

t-butyldimethylsilyl-2,3-di-*O*-acetyl)-β-cyclodextrin (6-TBDMS-2,3-ac-β-CD) dissolved in PS 86 (20% w/w) [4], and an even higher α value of 7.7 was obtained [5] on octakis(3-*O*-butanoyl-2,6-di-*O*-*n*-pentyl)-γ-cyclodextrin (Lipodex E, cf. Scheme 2) [7] dissolved in SE 54 (20% w/w), which can even be extended to $\alpha = 10$ at 26 °C (cf. Fig. 1). The results imply that one enantiomer undergoes a very strong molecular complexation, whereas the other does not.

Modified cyclodextrins comprise important chiral selectors for the gas-chromatographic separation of enantiomers [8–12], notably when diluted in polysiloxanes [13,14] or chemically linked to a polysiloxane matrix (Chirasil-Dex) [15,16]. Usually, only very low or modest α values of between 1.02–1.20 are observed for a host of different classes of racemic compounds. Low α values are indeed beneficial for fast enantiomeric analysis involving high-resolution capillary columns, but are detrimental to reliable mechanistic studies on enantioselectivity and are inappropriate for predictions of the elution order of enantiomers on modified cyclodextrins by molecular modelling studies [17,18]. Only rarely are values of $\alpha > 1.5$ encountered in inclusion gas chromatography, mainly for compounds containing halo atoms. Thus, König et al. separated methyl 2-chloropropanoate on heptakis(3-*O*-acetyl-2,6-di-*O*-*n*-pentyl)-β-cyclodextrin (Lipodex D) with $\alpha = 2$, corresponding to $-\Delta_{S,R}(\Delta G) = 0.5$ kcal/mol at 60 °C [19], and, subsequently, NMR studies and molecular calculations for this enantioselective system were presented [20,21]. Koen de Vries et al. separated methyl 2-chloropropanoate on octakis(3-*O*-butanoyl-2,6-di-*O*-*n*-pentyl)-γ-cyclodextrin (Lipodex E, cf. Scheme 2) [7] with $\alpha = 2.27$, corresponding to $-\Delta_{S,R}(\Delta G) = 0.56$ kcal/mol,



Scheme 2. Structure of octakis(3-*O*-butanoyl-2,6-di-*O*-*n*-pentyl)-γ-cyclodextrin (Lipodex E) [7].

$-\Delta_{S,R}(\Delta H) = 3.3$ kcal/mol and $\Delta_{S,R}(\Delta S) = 8$ cal/mol K at 70 °C [22]. Similar values were observed by Armstrong et al. for methyl 2-chloropropanoate with the related 3-trifluoroacetylated 2,6-di-*n*-pentylated β- and γ-cyclodextrins [23]. On octakis(3-*O*-butanoyl-2,6-di-*O*-*n*-pentyl)-γ-cyclodextrin (Lipodex E, cf. Scheme 2) [7] diluted in polysiloxane SE-54 or attached to a polydimethylsiloxane matrix (Chirasil-γ-Dex) [24], large α values in the region of 1.7 and 2.1 were observed for the inhalational anesthetics enflurane, isoflurane and desflurane, and the thermodynamic data $-\Delta_{S,R}(\Delta G)$, $-\Delta_{S,R}(\Delta H)$ and $\Delta_{S,R}(\Delta S)$ were determined [25].

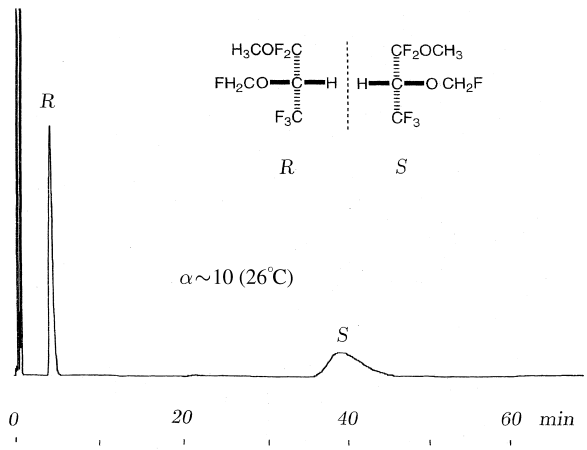


Fig. 1. Gas-chromatographic separation of the enantiomers of ''compound B''. 5 m×0.25 mm I.D. fused-silica capillary coated with 30% (w/w) Lipodex E in polysiloxane PS 255 ($d_f = 0.28$ μm), 0.12 bar hydrogen (over-pressure), 26 °C.

Large separation factors α are the prerequisite for the isolation of the enantiomers of these inhalational anesthetics on Lipodex E [26] and on undiluted per-trifluoroacetylated γ-cyclodextrin, a commercially available mixture of isomers and homologues [27–29], by chiral preparative GC, and of enflurane by a novel chiral simulated moving bed (SMB) GC approach [30]. This efficient enantioselective system has also been employed for a chiral sensor array relying on only one theoretical plate. Thus the enantiomers of enflurane were discriminated quantitatively by a quartz microbalance resonator coated with Lipodex E [31,32].

The observation of an exceptionally large enantioselective separation factor α of ''compound B'' on Lipodex E, uncommon in chiral GC, prompted us to determine concise thermodynamic data of chiral recognition via inclusion gas chromatography. The requirement to separate non-enantioselective (achiral) contributions to retention, arising from the solvent and being identical for enantiomers, and enantioselective (chiral) contributions to retention, arising from the chiral selector and being different for enantiomers in enantioselective chromatography, was solved via the concept of the retention increment (or chemical retention factor) R', which was previously derived in enantioselective complexation GC. Thus reliable thermodynamic data, $-\Delta_{S,R}(\Delta G)$, $-\Delta_{S,R}(\Delta H)$ and $\Delta_{S,R}(\Delta S)$, measured between 30 and 80 °C are now accessible.

2. Theoretical treatment

Enantioselectivity is defined as $-\Delta_{S,R}(\Delta G)$ and is frequently correlated with the chiral separation factor α according to:

$$-\Delta_{S,R}(\Delta G)\{=\}RT \ln \alpha = RT \ln(k'_S/k'_R) \quad (1)$$

where k' is the retention factor, R the gas constant and T the absolute temperature. The subscripts (S)

and (*R*) denote, within this work, the true *absolute configuration* of the enantiomers of ''compound B'' as determined by X-ray crystallography [4], whereby the (*S*)-enantiomer is gas-chromatographically eluted after the (*R*)-enantiomer on Lipodex E [5].

In enantioselective gas chromatography, the chiral selector is preferentially diluted in, or is chemically bonded to, an achiral stationary liquid (polysiloxane). Since the achiral matrix is incapable of discriminating between enantiomers and therefore produces the same retention factor k' for the stereoisomers, it is mandatory to separate non-enantioselective (achiral) and enantioselective (chiral) contributions to retention in chromatographic selector–selectand systems in an effort to quantify the true enantioselectivity $-\Delta_{S,R}(\Delta G)$ [33–36]. Indeed, it has been stated previously in enantioselective complexation GC that Eq. (1) does not appear to have a chemical meaning in the present case, since k' is the sum of two contributions to retention, i.e. firstly, the (identical) physical partition of the two enantiomers between the gaseous and liquid phases and, secondly, the (different) chemical diastereomeric equilibration between the enantiomers and the optically active metal chelate in the liquid phase [33]. Thus caution should be exercised when α is used as a criterion for $-\Delta_{S,R}(\Delta G)$ according to Eq. (1). In enantioselective complexation GC employing nonracemic metal coordination compounds as chiral stationary phases, the concept of the *retention increment* R' has been developed to quantitatively differentiate between the *physical* non-enantioselective contributions to retention arising from achiral gas–liquid partitioning and the *chemical* enantioselective contributions to retention arising from chiral molecular complexation, whereby only the latter contribution leads to the separation of enantiomers [33]. Thus, in a gas-chromatographic setup as shown in Fig. 2 employing a selector A diluted in a solvent S as stationary phase and a selectand B as a solute, the following equation for the retention increment R' of B has been derived [34–37]:

$$K\,a_{\mathrm{A}} = \frac{r - r^{\circ}}{r^{\circ}} = R' \qquad (2)$$

where r refers to the relative retention of the selectand B with respect to an inert reference standard B* in the complexation column (cf. Fig. 2, right) containing the selector A with activity a_{A} in solvent S, and r° refers to the relative retention of the selectand B with respect to the same reference standard B* in a reference column (cf. Fig. 2, left) containing the pure solvent S without the selector A.

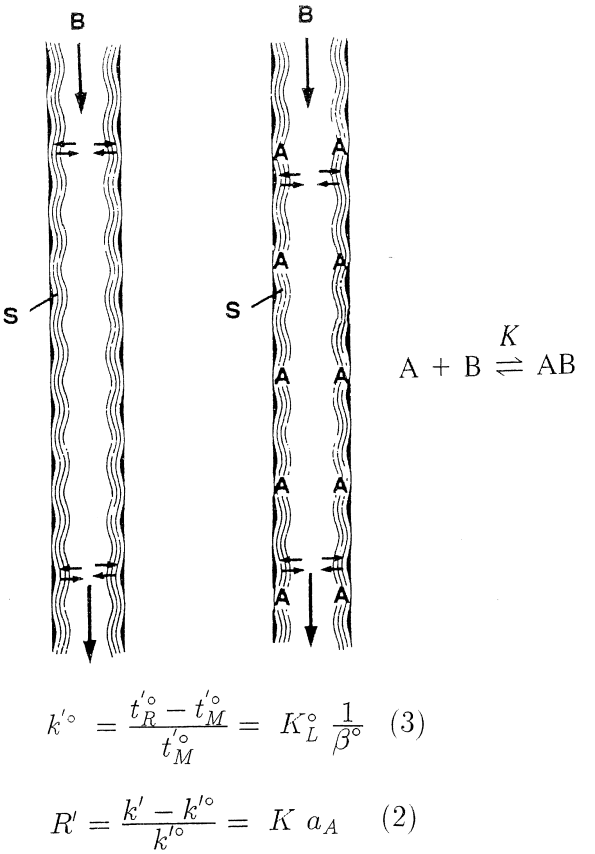


Fig. 2. The principle of complexation GC. Left: Reference column containing the pure solvent S. Right: Complexation column containing the selector A in the solvent S. Retention parameters given as retention factors in Eq. (2) refer to the selectand B.

The term R' is called the *retention increment* (previously called the retention increase [34]). R' is a quantitative measure of complexation between A and B in S and is proportional to the thermodynamic complexation constant K. According to Eq. (2), the retention increment R' is linearly related to a_{A} at a given temperature when a 1:1 molecular complex is formed between A and B. As the selectand B is employed at high dilution vis-a-vis the selector A, the occurrence of a 1:1 complexation equilibrium is

plausible. The formal similarity between the basic equation of chromatography [Eq. (3)], describing the chromatographic process in the reference column (cf. Fig. 2, left), and the derived equation of complexation chromatography [Eq. (2)], describing the chromatographic process in the complexation column (cf. Fig. 2, right), should be noted. Consequently, the retention increment R' may also be referred to as a *chemical retention factor* and r° may be related to a *physical hold-up time*. The essence of Eq. (2) is depicted graphically in Fig. 3, which also highlights the role of r° as physical hold-up time. Thus the complexation chromatogram has its origin at time r°. Originating from this point, the *chemically* mediated retention increments R'_S and R'_R of the enantiomers B_S and B_R increase linearly, but differently, as the activity a_A (concentration) of selector A increases.

The validity of Eq. (2) has previously been scrutinized by careful and extensive experiments [34]. Whereas the retention increment R' can be accurately measured, an error in the *absolute* value of the thermodynamic complexation constant K according to Eq. (2) may arise due to the incertitude of the activity a_A of the selector A in the solvent S. The unknown activity a_A can be substituted by the molarity M_A or, preferably, by the molality m_A, in very diluted solutions. The unit molality is independent of the temperature, and, for practical reasons, it is advantageous to add A to a weighed amount of S [34]. Fortunately, the unknown activity a_A of the selector A in the solvent S cancels when the *ratio* of the thermodynamic complexation constants of the enantiomers B_S and B_R, competing for the selector A, are compared.

From Eq. (2) the following basic equation describing enantioselectivity employing diluted chiral selectors can be obtained:

$$-\Delta_{S,R}(\Delta G) = -\Delta_{S,R}(\Delta H) + T\Delta_{S,R}(\Delta S) = RT\ln\left(\frac{K_S}{K_R}\right) = RT\ln\left(\frac{R'_S}{R'_R}\right) = RT\ln\left(\frac{r_S - r^\circ}{r_R - r^\circ}\right) \quad (4)$$

As the enantiomers B_S and B_R compete for the same selector A in S, the ratio K_S/K_R is directly related to the ratio of their retention increments R'_S/R'_R and is thus accessible from the relative retention data ($r_S -$

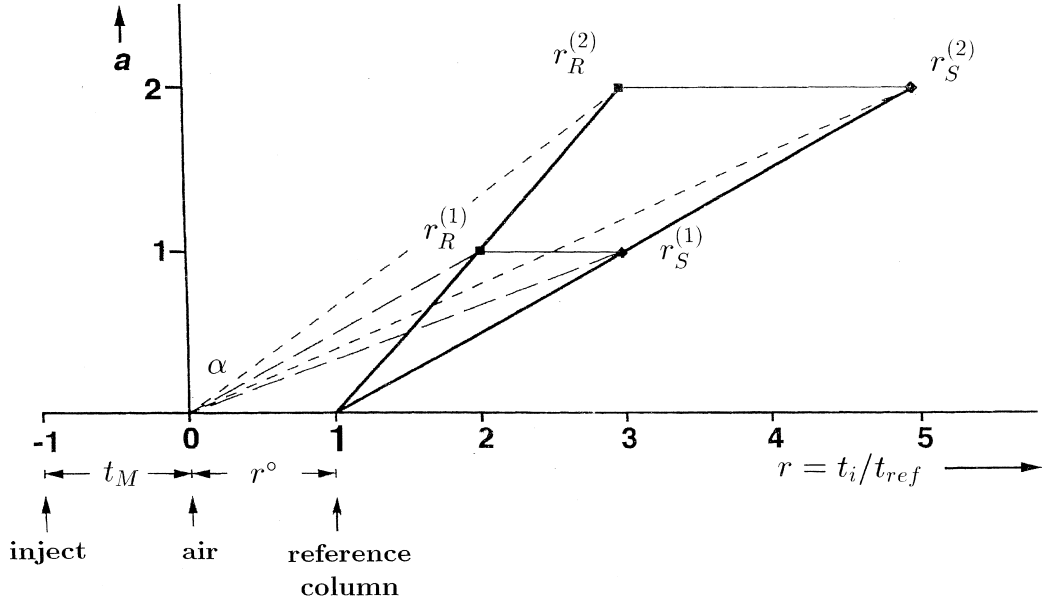


Fig. 3. Schematic representation of the distinction between (i) the non-enantioselective contribution to the relative retention of B, r°, and (ii) the enantioselective contribution to the relative retention of B_i, $r_i - r^\circ$ [$i = (S)$ and (R), denoting enantiomers of B], leading to constancy of the ratio R'_S/R'_R as required by Eq. (2). t_M and r° were arbitrarily set at unity, and the ratio R'_S/R'_R was arbitrarily set at 2 [39].

r°) and ($r_R - r^\circ$) according to Eq. (4). As outlined above, this ratio is independent of the activity of A in S, a_A, and thus from the concentration of the selector. The thermodynamic parameters $-\Delta_{S,R}(\Delta G)$, $-\Delta_{S,R}(\Delta H)$ and $\Delta_{S,R}(\Delta S)$ of enantioselectivity are thus obtained from Eq. (5) by van't Hoff plots when measurements are performed at different temperatures T according to:

$$R \ln \frac{R'_S}{R'_R} = \frac{-\Delta_{S,R}(\Delta G)}{T} = \frac{-\Delta_{S,R}(\Delta H)}{T} + \Delta_{S,R}(\Delta S) \quad (5)$$

As a thermodynamic quantity, $-\Delta_{S,R}(\Delta G)$ is strictly independent of the activity a_A of A in S and, hence, also from its concentration (molality). It has previously been verified in complexation GC that isothermal measurements at different concentrations of the selector A yielded the same value for $-\Delta_{S,R}(\Delta G)$ at a very high level of confidence [33,38]. Even concentration gradients of A in S do not affect $-\Delta_{S,R}(\Delta G)$ [such concentration gradients arise when columns containing S chemically bonded to the surface, e.g. CB-fused-silica capillary columns, are impregnated (doped) with the selector A by dynamic coating]. In GC, selectivity is customarily linked to the separation factor α. For practical reasons, this applies also for enantiomers according to:

$$\alpha = \frac{k'_S}{k'_R} = \frac{t'_S}{t'_R} = \frac{r_S}{r_R} \quad (6)$$

However, when the selector A is diluted in S, the separation factor α_{dil} becomes concentration-dependent [37]. By substituting r_S and r_R in Eq. (6) by Eq. (2), with r° being equal for enantiomers, a new expression for the separation factor α as a function of the activity a_A and the retention increment R' is obtained [37]:

$$\alpha_{dil} = \frac{K_S a_A + 1}{K_R a_A + 1} = \frac{R'_S + 1}{R'_R + 1} \quad (7)$$

Thus α_{dil} depends on the activity of the selector a_A and is thus rendered concentration-dependent. An optimum value may already be reached at low concentrations if chemical complexation is strong (i.e., large K, or $R' \gg 1$). Therefore, the separation factor α is an inappropriate term to describe enantioselectivity $-\Delta_{S,R}(\Delta G)$ for diluted selectors according to Eq. (1), because the thermodynamic quantity $-\Delta_{S,R}(\Delta G)$ must strictly be concentration-independent at a given temperature. As a matter of fact, the numerical value of α *underestimates* the chiral discrimination ability of A since the retention factor k' from which it is calculated is the sum of the non-enantioselective (physical) contribution to retention and the enantioselective (chemical) contribution to retention. It is only the ratio of the latter which leads to enantiomeric separation according to Eq. (4), i.e. $(r_S - r^\circ)/(r_R - r^\circ)$.

The validity of Eq. (4) has amply been corroborated by the gas-chromatographic separation of enantiomers using different diluted cyclodextrin selectors [37,39–42], even in systems exhibiting only low enantioselectivities and invoking modest retention increments R' where secondary equilibria such as the complexation of the reference standards (*n*-alkane) with the selector may render Eq. (2) inaccurate [43,44]. Therefore, an adjusted Eq. (2) has been considered, accounting for the complexation of the reference standard with modified cyclodextrins, producing another retention increment R'°, whereby R'° is typically in the range of 0.1–0.2, only [37]. Yet the data may even lead to negative values for R' when complexation between the selectand B and the selector A is very weak as with chiral hydrocarbons. In the present case, however, large retention increments R' are observed (cf. Table 1), rendering the competition of the reference standard with the selectand B negligible.

3. Experimental

3.1. Materials

"Compound B" [2-(fluoromethoxy)-3-methoxy-1,1,1,3,3-pentafluoropropane] was prepared according to a modified procedure originally described by Huang et al. [2]. 110 ml (0.6 mol) sodium methanolate in methanol (30% w/w) was placed into a 250 ml flask and the mixture was cooled in a water bath at 10–15 °C under an atmosphere of nitrogen. After slow addition of 26 ml (0.2 mol) of sevoflurane, obtained from a local clinic, within 90 min, the

Table 1
Relative retentions r, retention increments R' and enantioselectivity $-\Delta_{S,R}(\Delta G)$ of the complexation between ''compound B'' and two concentrations of octakis(3-*O*-butanoyl-2,6-di-*O*-*n*-pentyl)-γ-cyclodextrin (Lipodex E, cf. Scheme 2) [7] in polysiloxane PS 255 determined for four reference standards according to Eqs. (2) and (4) at 11 temperatures and comparison of calculated and experimental data for r° according to Eq. (9)

T (°C)	$r^{\circ}_{\text{found (calc)}}$	5% Chiral selector					10% Chiral selector				
		$r_{R(5\%)}$	$r_{S(5\%)}$	$R'_{R(5\%)}$	$R'_{S(5\%)}$	$-\Delta\Delta G_{(5\%)}$	$r_{R(10\%)}$	$r_{S(10\%)}$	$R'_{R(10\%)}$	$R'_{S(10\%)}$	$-\Delta\Delta G_{(10\%)}$
Reference: C_5											
30.0	4.69 (4.91)	11.16	66.21	1.38	13.12	5.7	16.69	120.41	2.56	24.67	5.7
35.0	4.39 (4.65)	8.95	43.00	1.04	8.80	5.5	13.73	85.66	2.13	18.51	5.5
39.9	4.13 (4.00)	7.70	33.56	0.86	7.13	5.5	10.96	59.61	1.65	13.43	5.5
44.6	3.90 (4.08)	6.54	21.94	0.68	4.63	5.1	8.97	39.61	1.30	9.16	5.2
50.5	3.63 (3.87)	5.67	15.62	0.56	3.30	4.8	7.31	26.35	1.01	6.25	4.9
55.2	3.44 (3.74)	5.09	11.95	0.48	2.47	4.5	6.27	19.14	0.82	4.56	4.7
60.1	3.26 (3.25)	4.40	8.85	0.35	1.72	4.4	5.53	14.36	0.70	3.41	4.4
65.1	3.09 (3.12)	4.01	7.09	0.30	1.30	4.1	4.85	10.84	0.57	2.51	4.2
70.0	2.93 (2.98)	3.66	5.80	0.25	0.98	3.9	4.27	8.34	0.46	1.85	4.0
75.0	2.79 (2.89)	3.44	4.95	0.24	0.78	3.4	3.95	6.87	0.42	1.46	3.6
80.0	2.65 (2.87)	3.24	4.32	0.22	0.63	3.1	3.55	5.54	0.34	1.09	3.4
Reference: C_6											
30.0	1.72 (1.76)	4.03	23.91	1.34	12.90	5.7	6.07	43.80	2.53	24.47	5.7
35.0	1.66 (1.73)	3.35	16.10	1.02	8.70	5.5	5.15	32.11	2.10	19.34	5.7
39.9	1.60 (1.51)	2.94	12.80	0.84	7.00	5.5	4.23	23.01	1.64	13.38	5.5
44.6	1.55 (1.59)	2.58	8.67	0.66	4.59	5.1	3.57	15.77	1.30	9.17	5.2
50.5	1.49 (1.53)	2.27	6.24	0.52	3.19	4.9	2.97	10.69	0.99	6.17	4.9
55.2	1.44 (1.48)	2.07	4.86	0.44	2.38	4.6	2.62	8.02	0.82	4.57	4.7
60.1	1.40 (1.43)	1.89	3.79	0.35	1.71	4.4	2.33	6.06	0.66	3.33	4.5
65.1	1.36 (1.37)	1.76	3.11	0.30	1.30	4.1	2.13	4.76	0.57	2.50	4.2
70.0	1.32 (1.34)	1.65	2.60	0.25	0.97	3.9	1.93	3.75	0.46	1.84	4.0
75.0	1.28 (1.31)	1.56	2.24	0.22	0.75	3.6	1.80	3.13	0.41	1.45	3.7
80.0	1.24 (1.30)	1.49	1.99	0.20	0.60	3.2	1.66	2.60	0.34	1.10	3.5
Reference: C_7											
30.0	0.64 (0.65)	1.49	8.83	1.33	12.80	5.7	2.25	16.25	2.52	24.39	5.7
35.0	0.63 (0.66)	1.28	6.13	1.03	8.73	5.5	1.97	12.26	2.13	18.46	5.5
39.9	0.63 (0.59)	1.15	5.01	0.83	6.95	5.5	1.66	9.04	1.64	13.35	5.5
44.6	0.62 (0.64)	1.04	3.49	0.68	4.63	5.1	1.44	6.37	1.32	9.27	5.2
50.5	0.62 (0.63)	0.94	2.58	0.52	3.16	4.9	1.24	4.45	1.00	6.18	4.9
55.2	0.61 (0.61)	0.87	2.04	0.43	2.34	4.6	1.12	3.42	0.84	4.61	4.6
60.1	0.60 (n.a.)	0.82	1.65	0.37	1.75	4.3	n.a.	n.a.	n.a.	n.a.	n.a.
65.1	0.60 (n.a.)	0.78	1.38	0.30	1.30	4.1	n.a.	n.a.	n.a.	n.a.	n.a.
70.0	0.59 (0.61)	0.75	1.19	0.27	1.02	3.8	0.80	1.72	0.49	1.92	3.9
75.0	0.59 (n.a.)	n.a.	n.a.	n.a.	n.a.	n.a.	0.84	1.45	0.42	1.46	3.6
80.0	0.58 (n.a.)	n.a.	n.a.	n.a.	n.a.	n.a.	0.79	1.24	0.36	1.14	3.4
Reference: C_8											
30.0	0.24 (0.25)	0.56	3.30	1.33	12.75	5.7	0.84	6.08	2.50	24.33	5.8
35.0	0.25 (0.25)	0.49	2.36	0.96	8.44	5.6	0.76	4.73	2.04	17.92	5.6
39.9	0.25 (0.24)	0.46	1.98	0.84	6.92	5.5	0.66	3.59	1.64	13.36	5.5
44.6	0.26 (0.25)	0.42	1.42	0.62	4.46	5.2	0.59	2.60	1.27	9.00	5.2
50.5	0.26 (0.26)	0.39	1.08	0.50	3.15	5.0	0.52	1.88	1.00	6.23	4.9
55.2	0.27 (0.25)	0.37	0.88	0.37	2.26	4.9	0.48	1.47	0.78	4.44	4.8
60.1	0.27 (0.23)	0.36	0.73	0.33	1.70	4.6	0.45	1.16	0.67	3.30	4.4
65.1	0.27 (0.27)	0.35	0.62	0.25	1.21	4.5	0.42	0.94	0.56	2.48	4.2
70.0	0.28 (0.28)	0.35	0.55	0.25	0.96	3.9	0.41	0.79	0.46	1.82	3.9
75.0	0.28 (0.29)	0.34	0.49	0.21	0.75	3.7	0.39	0.68	0.39	1.43	3.8
80.0	0.29 (0.30)	0.34	0.45	0.17	0.55	3.5	0.38	0.60	0.31	1.07	3.6

n.a. = data not available due to peak overlap of the first-eluted enantiomer with the reference standard *n*-heptane.

reaction mixture was stirred for 3 h and then refluxed for 2 h. The turbid mixture was hydrolyzed by pouring it into ice water. The upper part of the organic layer was separated. The intermediate layer, which did not separate well, was centrifuged in 10 ml tubes. The combined organic phases were washed twice with 20 ml of water. After drying over anhydrous sodium sulfate at 4 °C for 12 h, the crude product was distilled at 76–83 mbar. 9.3 g of ''compound B'' were obtained at 50–51 °C. Yield, 22%; purity (GC), 98%. ^{1}H-NMR (C_6D_6): 3.12 (s, 3H, –OCH_3), 3.84–3.91 (m, 1H, CH), 4,74 (d, 2H, $^{2}J_{HF}$ 54.4 Hz, –OCH_2F). ^{13}C-{^{1}H}-NMR (C_6D_6): 50.2 (t, $^{3}J_{CF}$ 7.4 Hz, –OCH_3), 75.0–76.6 (m, CH), 102.9 (d, $^{2}J_{CF}$ 224.5 Hz, –OCH_2F), 119.8 (s, –CF_2), 120.5 (s, –CF_3). ^{19}F-{^{1}H}-NMR (C_6D_6): −153 (t, 1F, –CH_2F), −83 (dd, 2F, –OCF_2), −74 (s, 3F, –CF_3). MS (EI): 193.1 ([M−F]$^+$, 29%), 163.0 ([M−CH$_2$FO]$^+$, 13%), 131.0 ([M−CH$_3$OCF$_2$]$^+$, 19%), 112.8 ([CF$_3$CHOCH$_3$]$^+$, 57%), 81.1 ([CH$_3$OCF$_2$]$^+$, 100%), 63.1 ([CH$_2$FOCH$_2$]$^+$, 36%), 51.3 ([CHF$_2$]$^+$, 54%). Elemental analysis. Calc.: C, 27.78; H, 2.85; F, 53.74. Found: C, 28.31; H, 2.77; F, 53.02.

The selector octakis(3-*O*-butanoyl-2,6-di-*O*-*n*-pentyl)-γ-cyclodextrin (Lipodex E, cf. Scheme 2) [7] was prepared by the procedure originally described in Ref. [7] and slightly modified in Ref. [24].

3.2. Thermodynamic measurements

A gas chromatograph HP 5890 A equipped with a FID (200 °C) and a split injector (200 °C, 1:100) was used. The oven temperature was checked by an external thermosensor.

For the determination of thermodynamic data, three columns were used. The reference column (30 m×0.25 mm I.D.) was coated by the static method with pure polysiloxane PS 255 ($d_f = 0.5$ μm). The two complexation columns (10 m×0.25 mm I.D.) were coated by the static method with ~5 and ~10% (w/w) Lipodex E in polysiloxane PS 255 ($d_f = 0.5$ μm).

''Compound B'', the corresponding reference standard, and methane were filled into head-space vials and the vapour was injected at a split ratio of approx. 1:100. The instrument was set at its highest sensitivity in order to avoid overloading conditions, which may result in peak tailing and reduction of retention for the strongly interacting second-eluted enantiomer. Thus the amount of injected ''compound B'' was minimized. Methane was always co-injected as a void-time marker to determine t_M. Each measurement was carried out three or four times. The mean of the retention data was used for the data of Table 1, whereby the adjusted retention time t'_R of ''compound B'' was related to that of the reference standards (*n*-pentane, *n*-hexane, *n*-heptane, *n*-octane, and ad libitum *n*-nonane) $t'_{R\,ref}$ to give relative retentions $r = t'_R/t'_{R\,ref}$.

4. Results and discussion

4.1. Thermodynamic parameters

Thermodynamic parameters of the enantioselective complexation between ''compound B'' and Lipodex E were determined by measurements of the adjusted retention times of ''compound B'' and the four reference standards on a reference column (30 m× 0.25 mm I.D.) coated with pure polysiloxane PS 255 and two complexation columns (each of 10 m×0.25 mm I.D.) coated with 5 and 10% (w/w) Lipodex E dissolved in PS 255. Typical gas chromatograms are shown in Fig. 4.

The precision of the data for $r°$, obtained on the reference column, is critical for the reliability of absolute values of the retention increment R'. These data were therefore acquired by interpolation of linear plots of ln $r°$ (obtained for the four reference standards) vs. $10^3/T$ according to Fig. 5. Precise data for $r°$ are also required to compare measured (found) and extrapolated (calc) data (cf. Table 1 and Section 4.4).

Table 1 summarizes the measured relative retentions $r°$, r_R and r_S, the calculated retention increments R'_S and R'_R and the calculated enantioselectivity $-\Delta_{S,R}(\Delta G)$. The four sets of data refer to the different reference standards *n*-pentane, *n*-hexane, *n*-heptane and *n*-octane. The data on the left were obtained on the complexation column containing ~5% Lipodex E in PS 255, whereas the data on the right were obtained on the complexation column containing ~10% Lipodex E in PS 255. The

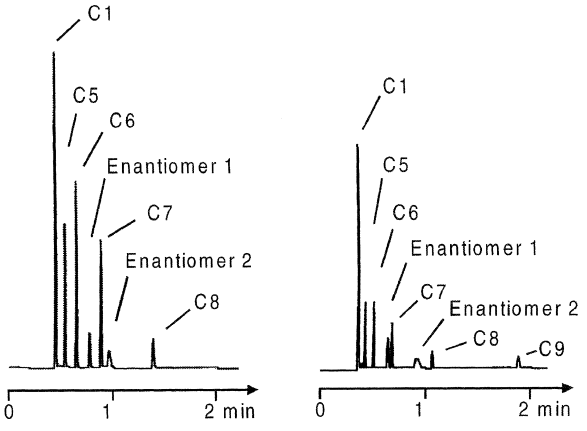


Fig. 4. Gas chromatograms of ''compound B'' and the reference standards *n*-pentane (C_5), *n*-hexane (C_6), *n*-heptane (C_7), *n*-octane (C_8) and *n*-nonane (C_9) with methane (C_1) as void-time marker at 70 °C. Carrier gas: 0.25 (left) and 0.28 bar (right) hydrogen (over-pressure), split 50 ml/min. Left: complexation column (10 m×0.25 mm I.D.) coated with 5% (w/w) Lipodex E in PS 255 (d_f: 0.5 μm). Right: complexation column (10 m×0.25 mm I.D.) coated with 10% (w/w) Lipodex E in PS 255 (d_f: 0.5 μm).

data merit the following comments. The retention increments R' should be strictly independent within experimental error of the nature of the reference standards, assuming their negligible complexation with Lipodex E. The small deviation for R' obtained with different reference standards on the left of Table 1 at low selector concentration is probably due to the inherent incertitude associated with the measurement of low retention times. It may also be caused by the finite complexation of the reference standards with Lipodex E [37]. However, since $-\Delta_{S,R}(\Delta G)$ is calculated from the logarithm of the ratio of the retention increments R'_S/R'_R the small deviations are essentially cancelled within the confidence level of this thermodynamic quantity. Thus highly precise data for the enantioselectivity $-\Delta_{S,R}(\Delta G)$ are obtained irrespective of the choice of the reference standard and the concentration of the selector in the solvent (5 vs. 10%). The experimental results clearly underline the validity of Eq. (2) and justify the simplifications and assumptions made for its derivation. This result commands special attention since, for the first time, very large differences in the relative retention times between the enantiomers are involved, which may have revealed inconsistencies of the entire approach. The results also clearly reinforce the need to rigorously separate achiral and chiral contributions to retention via the concept of the retention increment R'. It can be demonstrated from the data of Table 1 that the ratio of the

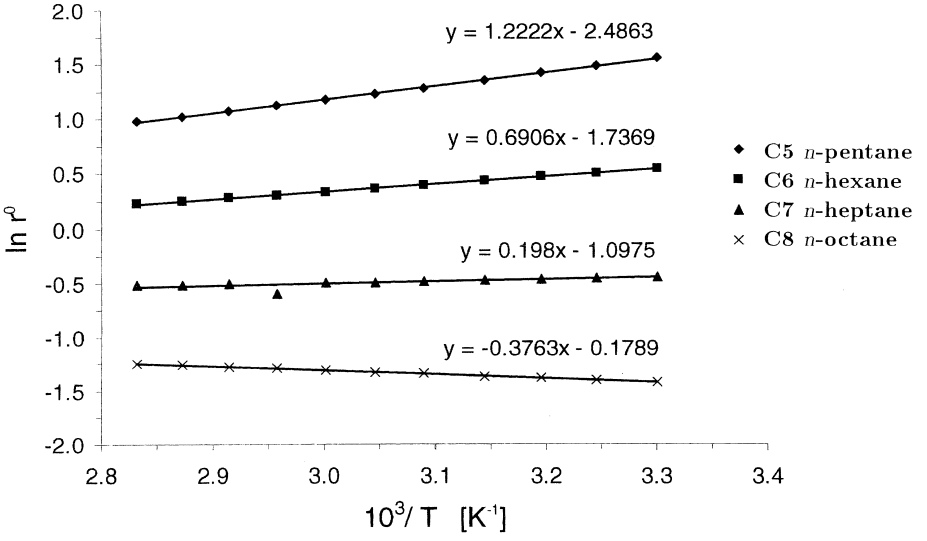


Fig. 5. Linear interpolation of ln $r°$ of ''compound B'' obtained with the reference standards C_5, C_6, C_7 and C_8 on a reference column (30 m×250 μm I.D.) coated with PS 255 (0.25 μm) vs. $10^3/T$ between 30 and 80 °C. Carrier gas: 0.25 bar hydrogen (over-pressure), split 50 ml/min.

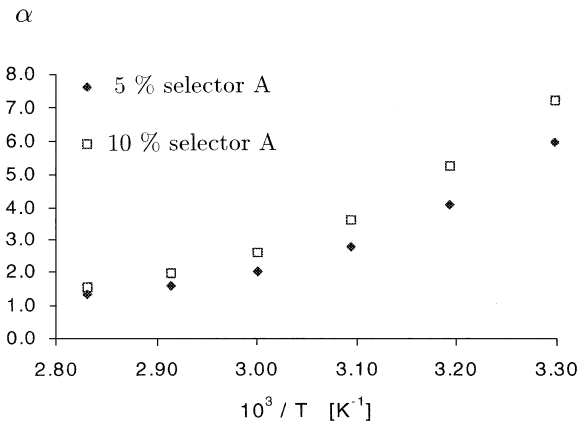


Fig. 6. Separation factors α of the enantiomers of ''compound B'' at different temperatures and concentrations of Lipodex E dissolved in PS 255 according to the data of Table 1.

retention increments R'_S/R'_R is independent of the selector concentration, whereas α values (r_S/r_R) calculated from data on the left (5%) and right (10%) (cf. Fig. 6) furnish different (concentration-dependent) values and, consequently, the use of Eq. (1) is clearly inappropriate. Thus it is demonstrated also for a highly efficient enantioselective system involving very large separation factors α, that only Eq. (2) gives reliable and concentration-independent results in inclusion gas chromatography employing selectors diluted in an achiral solvent or chemically linked to an achiral matrix.

The measured values for $-\Delta_{S,R}(\Delta G)$ up to 5.7 kJ/mol at 30 °C are very high indeed and are beyond the intrinsic error of molecular modelling calculations [45]. The present system therefore constitutes an interesting target for such studies. Important questions arise on the type of interaction which causes the high degree of noncovalent molecular complexation between the halodiether and the modified γ-cyclodextrin and on the steric differences responsible for the odd finding that this strong complexation is displayed only for one enantiomer! Since the halodiether possesses large dipoles and the cyclodextrin cavity may be endowed with a large and oriented dipole vector [46], enantiomeric discrimination may mainly be due to electrical forces. It is suggested to perform intermolecular NOE studies by NMR with single enantiomers of ''compound B'' in the presence of Lipodex E as carried out previously for enflurane and Lipodex E [25].

4.2. Enthalpic and entropic contributions to enantioselectivity

Concise data for the enantioselectivity $-\Delta_{S,R}(\Delta G)$ at 11 temperatures between 30 and 80 °C with intervals of 5 °C have been measured (cf. Table 1). These temperature-dependent measurements furnish the additional thermodynamic parameters $-\Delta_{S,R}(\Delta H)$ and $\Delta_{S,R}(\Delta S)$. In order to further increase the confidence of the data, the retention increase R', from which $-\Delta_{S,R}(\Delta G)$ is determined, was based on relative retentions r obtained for four reference standards, *n*-pentane, *n*-hexane, *n*-heptane and *n*-octane. The corresponding van't Hoff plots are shown in Fig. 7 with the complexation column containing 10% (w/w) concentration of the selector. The results are collected in Table 2, right. Additionally, the measurements were repeated with the complexation column containing only 5% (w/w) concentration of the selector. The results are collected in Table 2, left. From the host of data the following thermodynamic parameters of enantioselectivity between ''compound B'' and Lipodex E in PS 255 have been derived (standard deviation obtained from all data is listed in parentheses):

$$-\Delta_{S,R}(\Delta G) = 5.7\ (0.05)\ \text{kJ/mol}\ (303\ \text{K})$$

$$-\Delta_{S,R}(\Delta H) = 20.1\ (0.64)\ \text{kJ/mol}$$

$$\Delta_{S,R}(\Delta S) = -47.4\ (2.0)\ \text{J/K mol}$$

To our knowledge the enantioselective Gibbs free energy, enthalpy and entropy data represent the highest figures ever found for chiral gas chromatography.

4.3. Isoenantioselective temperature

As the van't Hoff plots are strictly linear (cf. Fig. 7) they traverse the line for $-\Delta_{S,R}(\Delta G)/T = 0$ at $1/T$ assuming the temperature-independence of $-\Delta_{S,R}(\Delta H)$ and $\Delta_{S,R}(\Delta S)$. An isoenantioselective temperature T_{isoenant} of 424 (30) K or ~150 °C is calculated according to

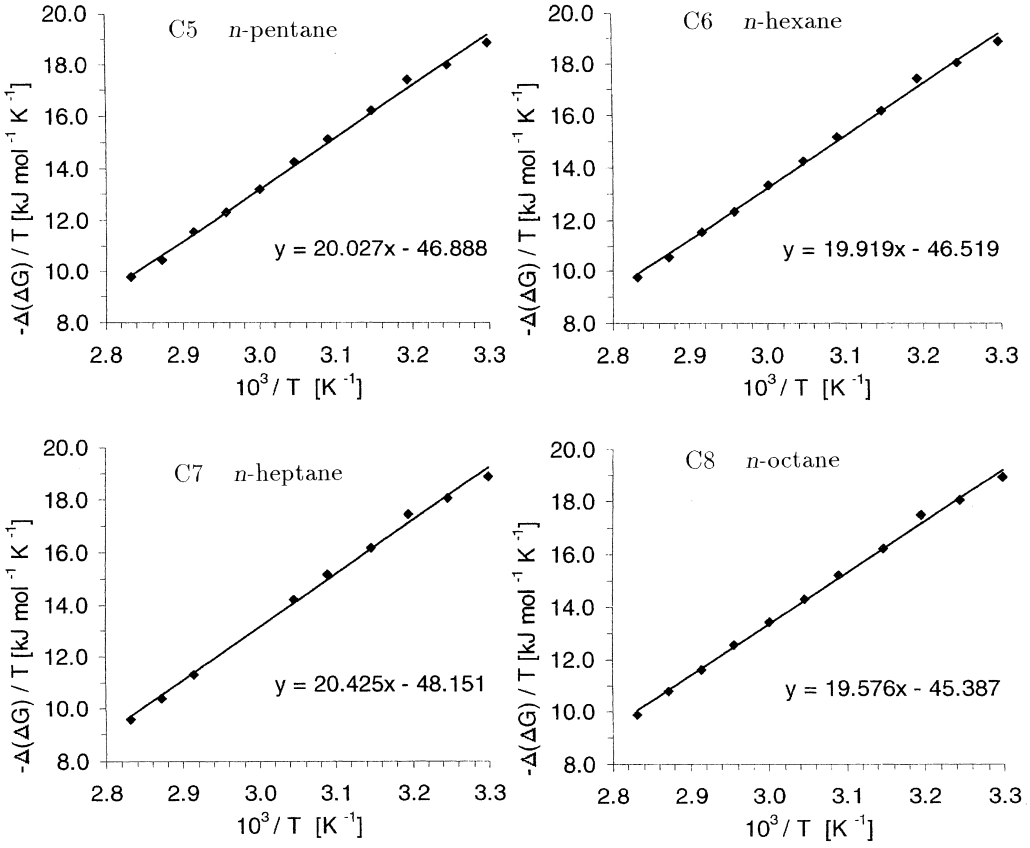


Fig. 7. Van't Hoff plots of $-\Delta_{S,R}(\Delta G)/T$ vs. $1/T$ with respect to four reference standards. Complexation column: 10% (w/w) Lipodex E in PS 255.

$$T_{\text{isoenant}} = \Delta_{S,R}(\Delta H)/\Delta_{S,R}(\Delta S) \text{ at } -\Delta_{S,R}(\Delta G) = 0 \quad (8)$$

As the enantioselectivity $-\Delta_{S,R}(\Delta G)$ is governed by an enthalpy term, $-\Delta_{S,R}(\Delta H)$, and an entropy term, $\Delta_{S,R}(\Delta S)$, both terms oppose each other in determining $-\Delta_{S,R}(\Delta G)$ for a 1:1 complexation process. The resulting enthalpy/entropy compensation is due to the fact that the more tightly bonded complex of one enantiomer ($-\Delta H_S > -\Delta H_R$) is also more ordered,

Table 2
Gibbs–Helmholtz parameters, $-\Delta_{S,R}(\Delta H)$ and $\Delta_{S,R}(\Delta S)$, of the enantioselective complexation between "compound B" and two concentrations of octakis(3-*O*-butanoyl-2,6-di-*O*-*n*-pentyl)-γ-cyclodextrin (Lipodex E, cf. Scheme 2) [7] in polysiloxane PS 255 determined for four reference standards according to Eq. (4) and Fig. 7

Reference standard	Lipodex E—5% in PS 225		Lipodex E—10% in PS 225	
	$-\Delta_{S,R}(\Delta H)$ (kJ/mol)	$\Delta_{S,R}(\Delta S)$ (J/mol K)	$-\Delta_{S,R}(\Delta H)$ (kJ/mol)	$\Delta_{S,R}(\Delta S)$ (J/mol K)
C_5 *n*-pentane	21.2	−50.7	20.0	−46.9
C_6 *n*-hexane	20.6	−48.6	19.9	−46.6
C_7 *n*-heptane	20.3	−47.9	20.4	−48.2
C_8 *n*-octane	19.1	−43.8	19.6	−46.4

i.e. $\Delta S_S < \Delta S_R$ (and vice versa for the other enantiomer). Since the entropy term increases with temperature T according to Eq. (4), the enantioselectivity $-\Delta_{S,R}(\Delta G)$ is rendered temperature-dependent and, at T_{isoenant}, the enantiomers cannot be separated [$-\Delta_{S,R}(\Delta G) = 0$ and $\alpha = 0$]. At T_{isoenant} the sign of enantioselectivity is changed when going from low to high temperatures. Although the change of the elution order of enantiomers on the same CSP with increasing temperature has been previously observed in enantioselective gas chromatography [35,36,42,47–49], the peak reversal could not be verified in the present work due to the rather high value of $T_{\text{isoenant}} \sim 150$ °C. Because of the extremely strong complexation, extensive peak broadening is observed for the second-eluted enantiomer of ''compound B'' with Lipodex E (cf. Fig. 1). Since this peak is still symmetrical, peak broadening does not result from overloading conditions which usually causes asymmetric peak shapes but is ascribed to finite chemical kinetics of the complexation between selector (host) and selectand (guest).

4.4. Extrapolation of r°

Since r° in Eq. (3) is identical for the enantiomers B_S and B_R (cf. Fig. 3), its value can be extrapolated as a consequence of Eq. (2) [38]:

$$r^\circ = \frac{r_S^{(1)} r_R^{(2)} - r_R^{(1)} r_S^{(2)}}{(r_S^{(1)} + r_R^{(2)}) - (r_R^{(1)} + r_S^{(2)})} \tag{9}$$

The derivation of Eq. (9) is immediately apparent when comprehending the principle of Fig. 3. According to Eq. (9) and Fig. 3, the retention of the enantiomers on the reference column containing only the solvent S, r°, can be extrapolated from two arbitrary sets of data of the relative retention, r_R and r_S, of the enantiomers B_S and B_R at two (unknown) activities a_A (or concentrations) 1 and 2 of the selector A in the solvent S obtained with two complexation columns. The validity of Eq. (9) has previously been verified by complexation gas chromatography [38]. In regard to the present work, Table 1 reports values for r° (in parentheses) calculated for four reference standards at 11 temperatures from the data of r listed also in Table 1. A reasonably good agreement between calculated and measured data is observed. Whereas for the acquisition of the absolute quantity R' a high precision of the data of r° would be required according to Eq. (2), for the relative ratio $\ln(R'_S/R'_R)$ according to Eq. (4) the error in r° will cancel and $-\Delta_{S,R}(\Delta G)$ will depend only on the difference $r - r^\circ$, making this quantity less sensitive to errors in r°.

Thus it follows that only two columns are required for the determination of precise data of enantioselectivity using diluted chiral selectors A in gas chromatography. Either a reference column and a complexation column are used or two complexation columns with different concentrations of the selector are employed. Significantly, for the latter approach, Eq. (9) can be used to estimate non-enantioselective contributions to retention when r° is not readily accessible by the inavailability of the solvent S to prepare a reference column, e.g. with polysiloxane-anchored chiral selectors (*Chirasil*-type stationary phases) in gas chromatography [15,16,24,50]. It is proposed to use this approach also in other enantioselective techniques (HPLC) where it is difficult to separate achiral and chiral contributions to enantioselectivity [51,52].

4.5. Comparison of α-, β- and γ-(3-O-butanoyl-2,6-di-O-n-pentyl)-cyclodextrins

The modified γ-cyclodextrin (Lipodex E) used in this work possesses a large cavity for inclusion of the small molecule of ''compound B''. It was therefore reasoned that the enantioselectivity in the present system may even be increased when the corresponding β- and α-congeners of Lipodex E are used as chiral stationary phases. Unexpectedly, the opposite was found. Whereas on heptakis(3-*O*-butanoyl-2,6-di-*O*-*n*-pentyl)-β-cyclodextrin the separation of the enantiomers of ''compound B'' still commences with a reduced separation factor α of 2.1, the separation totally collapsed on hexakis(3-*O*-butanoyl-2,6-di-*O*-*n*-pentyl)-α-cyclodextrin (cf. Table 3). This unusual finding in enantioselective inclusion GC clearly warrants a sound theoretical rationalization in the future. One tentative explanation for the versatility of Lipodex E for the separation of enantiomers in general [9] and of ''compound B'' in particular may be associated with self-inclusion of *n*-pentyl groups [53] into the cavity of

Table 3
Separation factors α of the enantiomers of ''compound B'' on hexakis(3-*O*-butanoyl-2,6-di-*O*-*n*-pentyl)-α-cyclodextrin, heptakis-(3-*O*-butanoyl-2,6-di-*O*-*n*-pentyl)-β-cyclodextrin and octakis(3-*O*-butanoyl-2,6-di-*O*-*n*-pentyl)-γ-cyclodextrin (Lipodex E, cf. Scheme 2) [7] at three temperatures [6]

T (°C)	α (α-CD)	α (β-CD)	α (γ-CD)
30	1.0	2.1	9.7
40	1.0	1.6	7.7
50	1.0	1.4	6.0

the selector (host) followed by competitive displacement by the selectand (guest). A clue to this proposal may again be obtained by NMR measurements [53] and molecular modelling studies. It is also of interest to test the corresponding δ-CD congener of Lipodex E.

5. Conclusion

The data obtained in this work represent the highest thermodynamic values ever measured in enantioselective gas chromatography. The experimental results will guide the elucidation of chiral discrimination by static NMR spectroscopic measurements and by molecular modelling studies. The extraordinary enantiomeric bias causes large differences in elution times of the enantiomers and allows the probing of the concept of the retention increment R' in separating non-enantioselective and enantioselective contributions to retention. The new method permits the quantitation of non-enantioselective contributions to retention by measurements at two arbitrary concentrations of a chiral selector present in an achiral environment.

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