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A mixed stationary phase containing two versatile cyclodextrin-based selectors for the simultaneous gas chromatographic enantioseparation of racemic alkanes and racemic α-amino acid derivatives☆

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ABSTRACT

In an effort to simultaneously enantioseparate racemic unfunctionalized alkanes and racemic α-amino acid derivatives by gas chromatography (GC) in forthcoming experiments related to the search for extraterrestrial homochirality, the two versatile modified cyclodextrin (CD) selectors octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin (Lipodex G) and heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin were dissolved in a polysiloxane and the mixed binary chiral selector system was coated onto a 50 m × 0.25 mm i.d. fused silica capillary column. Whereas the former CD selector enantioseparates racemic unfunctionalized alkanes the latter CD selector preferentially resolves *N*-(*O*,*S*)-trifluoroacetyl-α-amino acid alkyl esters. With both CD selectors employed as mixed binary chiral selector system present in one chiral stationary phase (CSP), the simultaneous gas chromatographic enantioseparation of racemic alkanes and of racemic derivatized α-amino acids is achieved in a single temperature-programmed run. Also for other classes of racemic compounds, the scope of enantioseparation could be extended as compared to the conventional use of the single CD selectors in GC.

1. Introduction

The phenomenon of chirality is of eminent importance for the creation of life on Earth. Almost all building blocks of biopolymers, such as α-amino acids and sugars, are chiral and, as a consequence, they exhibit the phenomenon of mirror image incongruence. A unique signature of life is the enantioenrichment of chiral building blocks toward complete homochirality. Indeed, in all self-replicating systems (viruses, bacteria, plants, animals, humans) only one enantiomeric form is found in nature, *i.e.*, D-configurated sugars and L-configurated α-amino acids (save for D-amino acids in bacterial cell walls). Even on the verge of the third millennium, two basic questions are not yet answered: (i) how was homochirality created on primordial Earth and (ii) why were L-amino acids and D-sugars selected in lieu of their enantiomeric counterparts. Enantiomeric bias may have occurred on Earth itself or may have its origin from outer space. In astrobiology, the stereochemical analysis of chiral alkanes and of α-amino acids, as potential biomarkers, is of particular interest in a search for extraterrestrial homochirality. Thus, refined tools for outer space enantioseparations such as enantioselective GC columns coated with commercially available Chirasil-Val [1], Chirasil-Dex [2,3] and octakis(2,6-di-*O*-pentyl-3-*O*-trifluoroacetyl)-γ-cyclodextrin (G-TA) [4], respectively, are integrated in the COSAC experiment as part of the payload of the Rosetta mission of ESA launched in 2004 and presently heading toward the comet 67P/Churyumov-Gerasimenko [5,6]. The existence of an ocean of hydrocarbons on Titan [7–10] has been confirmed by the Cassini-Huygens mission in 2005. The enantioseparation of chiral hydrocarbons on Titan is of interest in a search for extraterrestrial homochirality in the environment of Saturn in order to answer the question whether chiral alkanes in space exist as racemates or are enantioenriched. In the present work an effort is undertaken to *simultaneously* enantioseparate simple chiral alkanes and α-amino acid derivatives on a *single* enantioselective capillary column by GC subject to future applications in space experiments.

The diamide selector Chirasil-Val represents the classical CSP for the simultaneous enantioseparation of all proteinogenic α-amino acids as their *N*(*O*,*S*)-trifluoroacetyl (TFA) alkyl esters [1]. The complete temperature-programmed enantioseparation can be achieved in 30 min. However, proline and aspartic acid sometimes are not base-line resolved [1] and the chiral selector is prone to racemization at elevated temperatures [11]. Moreover, Chirasil-Val is unsuitable for classes of chiral com-

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pounds devoid of hydrogen-bonding functionalities since at least two hydrogen-bonds are usually required for efficient chiral recognition [12,13]. Advantageous is the availability of Chirasil-Val in the D- and L-form, thereby offering the possibility to reverse the elution order of the enantiomers. Gas chromatographic CSPs based on modified cyclodextrins [14] are only available in the *all*-D-form but they are configurationally stable at high temperature since racemization (reversal of *all* stereogenic centers) is precluded *per se* and on-column epimerization has not yet been observed. The GC enantioseparation of *N*(*O*,*S*)-TFA-α-amino acid alkyl esters on Chirasil-Val (alkyl = *i*-propyl) and on Chirasil-γ-Dex (poly(dimethylsiloxane)-linked octakis(3-*O*-butanoyl-2,6-di-*O*-pentyl)-γ-cyclodextrin (Lipodex E) [15]) (alkyl = ethyl) derivatives have been compared previously and the coupling of columns coated with Chirasil-γ-Dex and either Chirasil-D-Val or Chirasil-L-Val in a two-dimensional approach has been advocated [16]. In order to combine the individual enantioselectivities of different selectors, different mixtures of valine-type selectors and modified cyclodextrin selectors in one CSP have recently been realized in a single column format [17–20]. In the absence of cooperative effects, the enantioselectivity obtained on a mixed binary chiral selector system is always smaller than that of a single chiral selector system containing the more enantioselective selector. Therefore, at a first glance it may be unfavourable to combine different selectors in one CSP as inferred by Pirkle and Welch [21]. Yet for practical purposes, the combination of chiral selectors with complementary enantioselectivity toward enantiomers of very different classes of racemic compounds in one CSP may result in a broader spectrum of enantioselectivities as those provided by either of the single-selector CSP. A comprehensive quantitative analysis of mixed chiral selector systems including the treatment of matched–mismatched enantioselectivities has been advanced recently [22].

Octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin (Lipodex G) [23] exhibits an excellent enantioselectivity for unfunctionalized alkanes [24] and it shows an enantioselectivity pattern for derivatized α-amino acids complementary to that of the chiral selector heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin [25]. The aim of the present work is the simultaneous enantioseparation of a multitude of unfunctionalized C7–C8 alkanes possessing one stereogenic center and derivatized α-amino acids through the use of a *mixed binary chiral selector system* containing octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin (Lipodex G) [23] and heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin [25] in the polysiloxane PS 086 with the further option of the simultaneous enantioseparation of other classes of chiral compounds such as lactones, diols, secondary alcohols, ketones and terpenes.

2. Materials and methods

2.1. Materials

The chiral unfunctionalized alkanes were purchased from Lancaster (Frankfurt a. M., Germany), Fluka (Buchs, Switzerland) or TCI (Zwijndrecht, Belgium), respectively.

N-(*O*,*S*)-Trifluoroacetyl alkyl esters of α-amino acids were prepared via the general procedure [16]. During derivatizating asparagine is converted into aspartic acid and glutamine is converted into glutamic acid. Arginine, histidine and glycine were not included in the mixture of the α-amino acids analysed, because arginine can only be eluted on deactivated glass capillaries (unpublished results) and for the enantioseparation of histidine an additional step of derivatization is required. The smallest α-amino acid glycine is achiral.

The chiral selector octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin (Lipodex G) [23] was obtained from Cyclolab Ltd. (Budapest, Hungary). Heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin was synthesized as described by Takeo et al. [26].

Fused silica capillaries (0.25 mm i.d.) were purchased from Ziemer (Mannheim, Germany).

Polysiloxane PS 086 (poly(15%-diphenyl–85%-dimethylsiloxane)) and PS 268 (poly(0.1–0.3%-methylvinyl–15%-diphenyl–85%-dimethylsiloxane)) were obtained from Chrompack (Middleburg, The Netherlands).

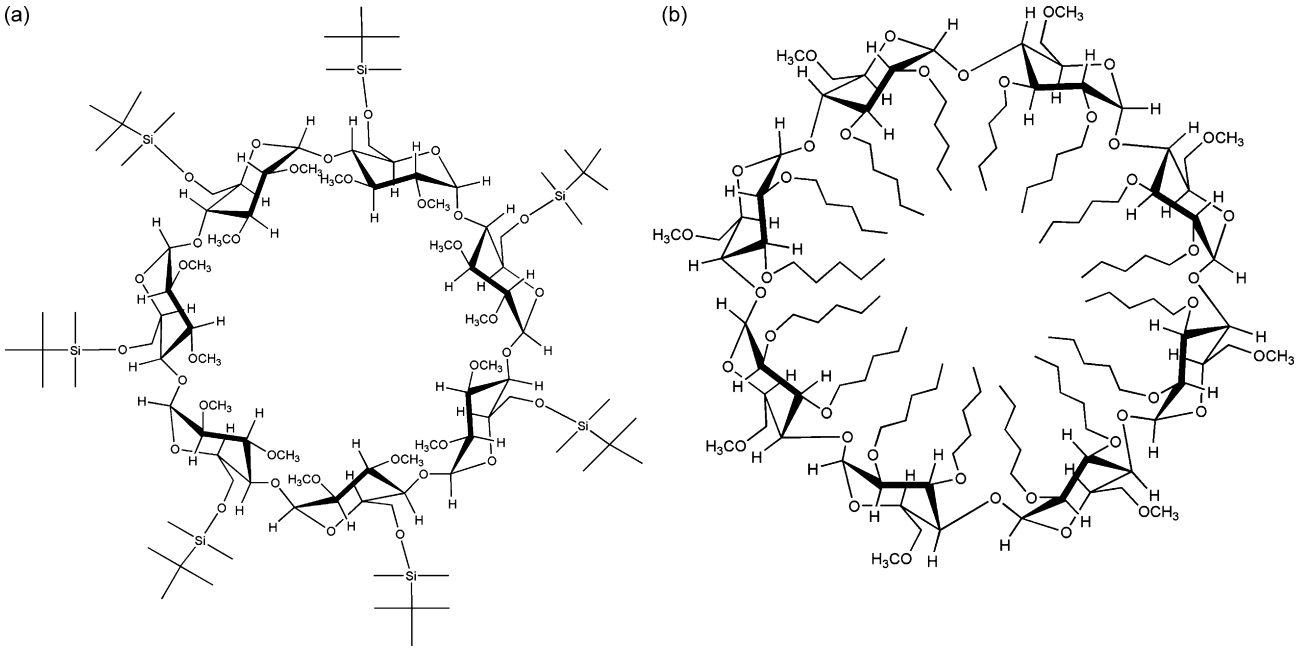

Fig. 1. Structure of the two modified cyclodextrins applied in the mixed binary chiral selector system (diluted in polysiloxane PS 086) for enantioselective gas chromatography.

Table 1
Temperature, retention factor k_2, enantioseparation factor α and enantioresolution factor R_s for members of different classes of racemic compounds on the mixed binary chiral selector system

Compound	Temperature (°C)	Retention factor (k_2[a])	Separation factor (α)	Resolution (R_s)
α-Amino acids				
Alanine-TFA-Et	80	9.6	1.162	14.2
Valine-TFA-Et	80	13.8	1.066	5.7
Norvaline-TFA-Et	80	26.1	1.288	39.0
2-Aminobutyric acid-TFA-Et	80	13.6	1.159	14.2
Norleucine-TFA-Et	80	29.1	1.018	10.4
Isoleucine-TFA-Et	90	13.0	1.034	3.3
Isoleucine-allo-TFA-Et	90	14.1	1.045	4.5
Leucine-TFA-Et	90	16.4	1.071	6.9
Aspartic acid-TFA-Et	90	46.4	1.003	2.9
Glutamic acid-TFA-Et	120	30.5	1.018	8.5
Proline-TFA-Et	100	24.0	1.018	3.9
Phenylalanine-TFA-Et	120	38.3	1.019	2.0
Threonine-TFA-Et	80	11.8	1.068	4.6
Serine-TFA-Et	100	8.4	1.072	5.5
Cysteine-TFA-Et	100	21.4	1.121	11.7
Methionine-TFA-Et	120	21.8	1.022	2.4
Tyrosine-TFA-Et	140	28.6	1.034	2.1
Ornithine-TFA-Et	140	31.7	1.025	2.5
Lysine-TFA-Et	140	44.6	1.012	2.7
Tryptophan-TFA-Et	160	17.4	1.058	2.2
Alkanes				
2,3-Dimethylpentane	15	5.5	1.026[b]	1.2
3-Methylhexane	15	6.9	1.040[b]	2.0
2,2,3-Trimethylpentane	27	10.0	1.037[b]	2.6
2,4-Dimethylhexane	27	13.2	1.235[b]	19.2
2,3-Dimethylhexane	27	12.9	1.040[b]	4.2
3-Methylheptane	27	14.1	1.026[b]	3.1
3,4-Dimethylhexane	40	12.8	1.119[b]	5.5
2,3-Dimethylheptane	27	25.1	1.041[b]	5.2
2,4-Dimethylheptane	27	24.3	1.269[b]	28.7
trans-1,2-Dimethylcyclohexane	35	7.6	1.054	2.4
Lactones				
γ-Valerolactone	100	4.2	1.055	3.6
γ-Hexalactone	100	8.1	1.052[c]	1.9
γ-Heptalactone	100	15.9	1.090[c]	3.9
γ-Octalactone	120	25.7	1.042[c]	3.5
γ-Nonalactone	130	33.2	1.021[c]	3.4
γ-Decalactone	140	39.4	1.013[c]	1.9
Diols				
trans-1,2-Dimethylcyclohexanediol	110	9.4	1.055	2.9
1,2-Propanediol	50	4.4	1.020	1.1
1,4-Pentanediol	50	26.2	1.054	3.9
2,3-Butanediol	50	3.9 8.5	1.038 2.179 (meso-e_2)	1.5 38.9
1,3-Butanediol	40	20.1	1.016	1.2
2-Methyl-2,4-pentanediol	50	11.2	1.025	1.5
Secondary alcohols				
1-Hepten-3-ol	70	11.2	1.017	2.4
1-(Pentafluorophenyl)-ethanol	100	11.0	1.094	6.5
1-(4-Methylphenyl)-ethanol	130	6.0	1.021	1.7
2-Methyl-2-hepten-6-ol	110	3.1	1.031	1.9
2-Methyl-3-pentanol	100	5.2	1.068	5.3
2,2-Dimethylhexanol	80	8.2	1.073	5.6
3-Octanol	80	12.7	1.031	2.0
2-Octanol	80	13.0	1.023	1.8
2-Nonanol	80	25.9	1.018	1.3
1-(2-Bromophenyl)-ethanol	130	28.0	1.391	19.7
1-(3-Bromophenyl)-ethanol	130	25.0	1.016	1.5
1-(2-Methylphenyl)-ethanol	120	12.0	1.106	7.5
1-(3-Methylphenyl)-ethanol	130	10.0	1.010	1.0
Others				
2-Bromopentane	50	5.6	1.086	5.7
α-Pinene	50	16.7	1.021	1.4
Camphor	90	15.2	1.020	5.3
Linalool	100	7.4	1.018	1.6
Limonene	60	23.4	1.023	2.0
Carvone	60	23.4	1.021	2.1
3,5-Dimethyl-2-cyclohexen-1-one	100	10.4	1.071	5.9
3-Methylcyclohexanone	80	7.7	1.023	1.5

Table 1 (*Continued*)

Compound	Temperature (°C)	Retention factor (k_2[a])	Separation factor (α)	Resolution (R_s)
2-Ethylhexanoic acid	110	21.2	1.029	2.9
Propyl mandelate	120	19.6	1.052	5.4
Methyl 2-chloropropionate	100	2.1	1.075	2.8

Column: 50 m × 0.25 mm i.d. fused silica capillary coated with 33% (w/w) octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin (Lipodex G) and 19% (w/w) heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin in 48% (w/w) polysiloxane PS 086, film thickness 0.25 μm. The gas chromatograms were recorded isothermally at 90 kPa dihydrogen on a 50 m × 0.25 mm i.d. fused silica capillary, film thickness 0.25 μm, if not otherwise stated.

[a] Second eluted enantiomer.

[b] The gas chromatograms were recorded at 45 kPa dihydrogen.

[c] The gas chromatograms were recorded at 50 kPa dihydrogen on a 20 m × 0.25 mm i.d. fused silica capillary.

2.2. Methods

2.2.1. Instrumentation

The gas chromatographic measurements with the mixed binary chiral selector system were carried out on a Thermo Finnigan Trace gas chromatograph (Egelsbach, Germany) equipped with a flame ionization detector operated at 250 °C. Dihydrogen was used as carrier gas. A splitting ratio of 1:50 was employed at the injector which was operated at 250 °C. An autosampler (Thermo Finnigan AS2000) and the software Chrom-card 32-bit (2.0) were used for automation and data acquisition, respectively.

The gas chromatographic measurements with the single selectors were carried out as described previously [24].

2.2.2. Preparation of capillary columns

The pretreatment of the *de novo* fused silica capillaries was performed by heating them in a slow stream of dinitrogen at 250 °C for 15 h followed by coating the inner surface with the chiral stationary phase (CSP) containing single selectors or the mixed binary chiral selector system in polysiloxane using the static method to yield a film thickness of 0.25 μm [27]. The columns coated with the CSP were conditioned in a stream of dihydrogen by gradual heating to 180 °C and maintaining this temperature for 15 h.

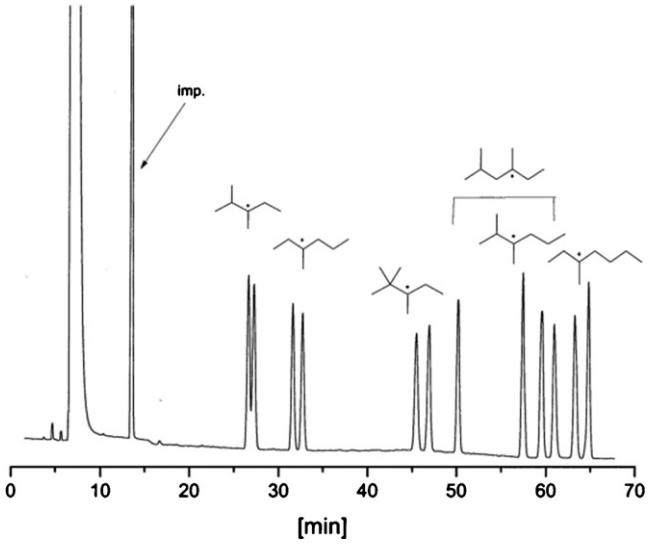

Fig. 2. Gas chromatographic enantioseparation of six racemic unfunctionalized aliphatic hydrocarbons (C7 and C8) on the mixed binary chiral selector system containing 33% (w/w) octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin (Lipodex G) and 19% (w/w) heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin in 48% (w/w) polysiloxane PS 086. Column: 50 m × 0.25 mm i.d. fused silica capillary, film thickness 0.25 μm, carrier gas H_2: 45 kPa, temperature program: 15 °C for 45 min, then 27 °C.

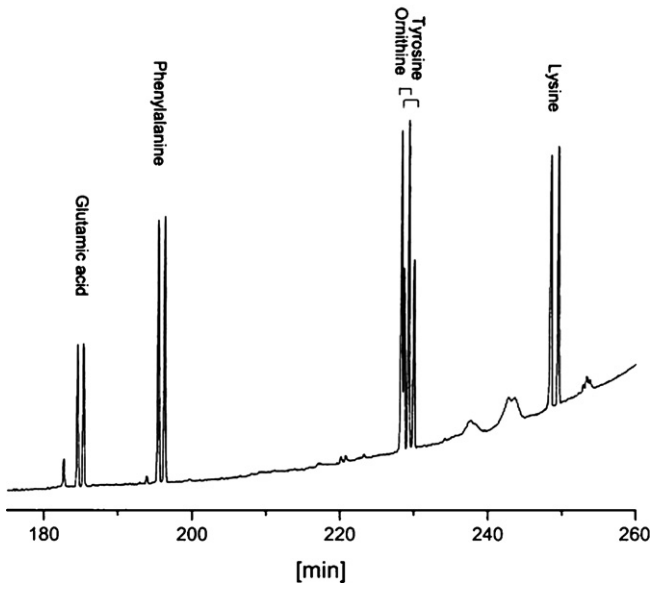

Fig. 3. Gas chromatographic enantioseparation of 17 racemic α-amino acids as *N*-(*O*,*S*)-trifluoroacetyl ethyl esters on the mixed binary chiral selector system containing 33% (w/w) octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin (Lipodex G) and 19% (w/w) heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin in 48% (w/w) polysiloxane PS 086. Column: 50 m × 0.25 mm i.d. fused silica capillary, film thickness 0.25 μm, carrier gas H_2: 100 kPa, temperature program: 70 °C for 60 min, with a rate of 0.5 °C/min up to 170 °C.

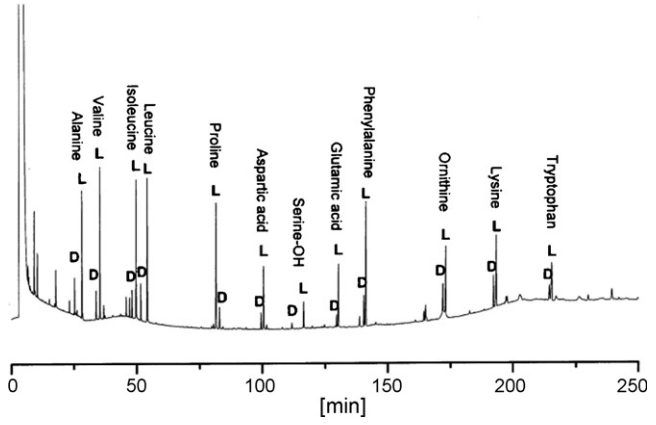

Fig. 4. Enantiomeric elution order of 12 racemic α-amino acids as *N*-(*O*,*S*)-trifluoroacetyl ethyl esters (the L-enantiomer is present in excess) on the mixed binary chiral selector system containing 33% (w/w) octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin (Lipodex G) and 19% (w/w) heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin in 48% (w/w) polysiloxane PS 086. Column: 50 m × 0.25 mm i.d. fused silica capillary, film thickness 0.25 μm, carrier gas H_2: 90 kPa, temperature program: 70 °C, with a rate of 0.5 °C/min up to 170 °C.

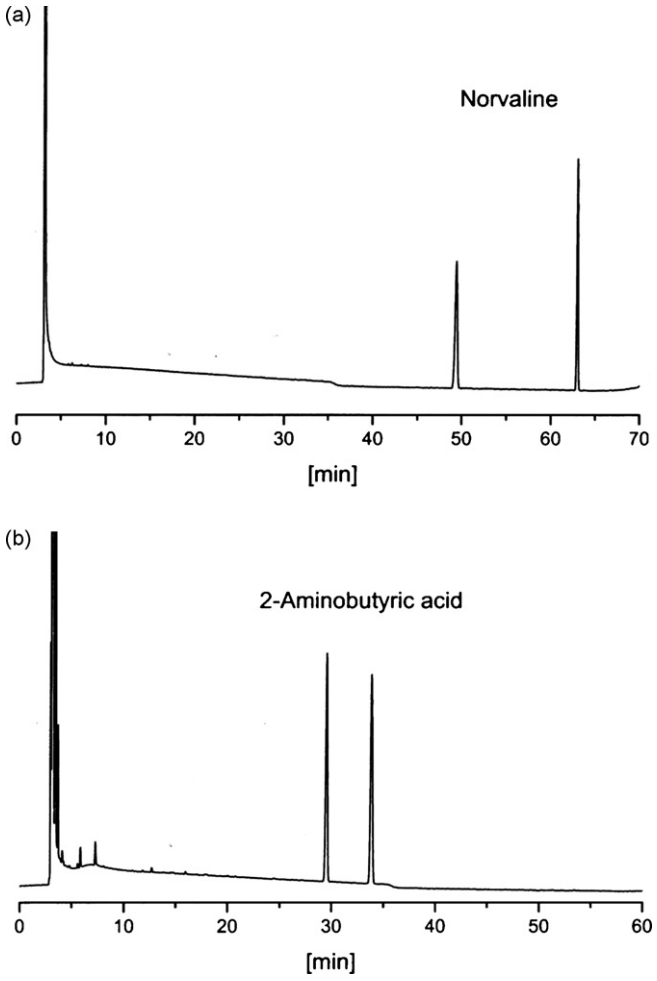

Fig. 5. Gas chromatographic enantioseparation of the racemic α-amino acids norvaline (top) and 2-aminobutyric acid (bottom) as *N*-trifluoroacetyl ethyl esters on the mixed binary chiral selector system containing 33% (w/w) octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin (Lipodex G) and 19% (w/w) heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin in 48% (w/w) polysiloxane PS 086. Column: 50 m × 0.25 mm i.d. fused silica capillary, film thickness 0.25 μm, carrier gas H_2: 90 kPa, temperature: 80 °C isothermal.

(i) The mixed chiral selector system of 19% (w/w) heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin and 33% (w/w) octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin (Lipodex G) diluted in 48% (w/w) polysiloxane PS 086:

5.3 mg of octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin (Lipodex G) [23], 3.1 mg of heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin [26] and 7.7 mg PS 086 were dissolved in 4 ml diethylether. After about 5 min shaking and filtration of the solution, the mixture was used for coating a 50 m × 0.25 mm i.d. capillary by the static method to yield a 0.25 μm film thickness of the CSP [27].

(ii) The single chiral selector system of 35% (w/w) octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin (Lipodex G) diluted in polysiloxane PS 268:

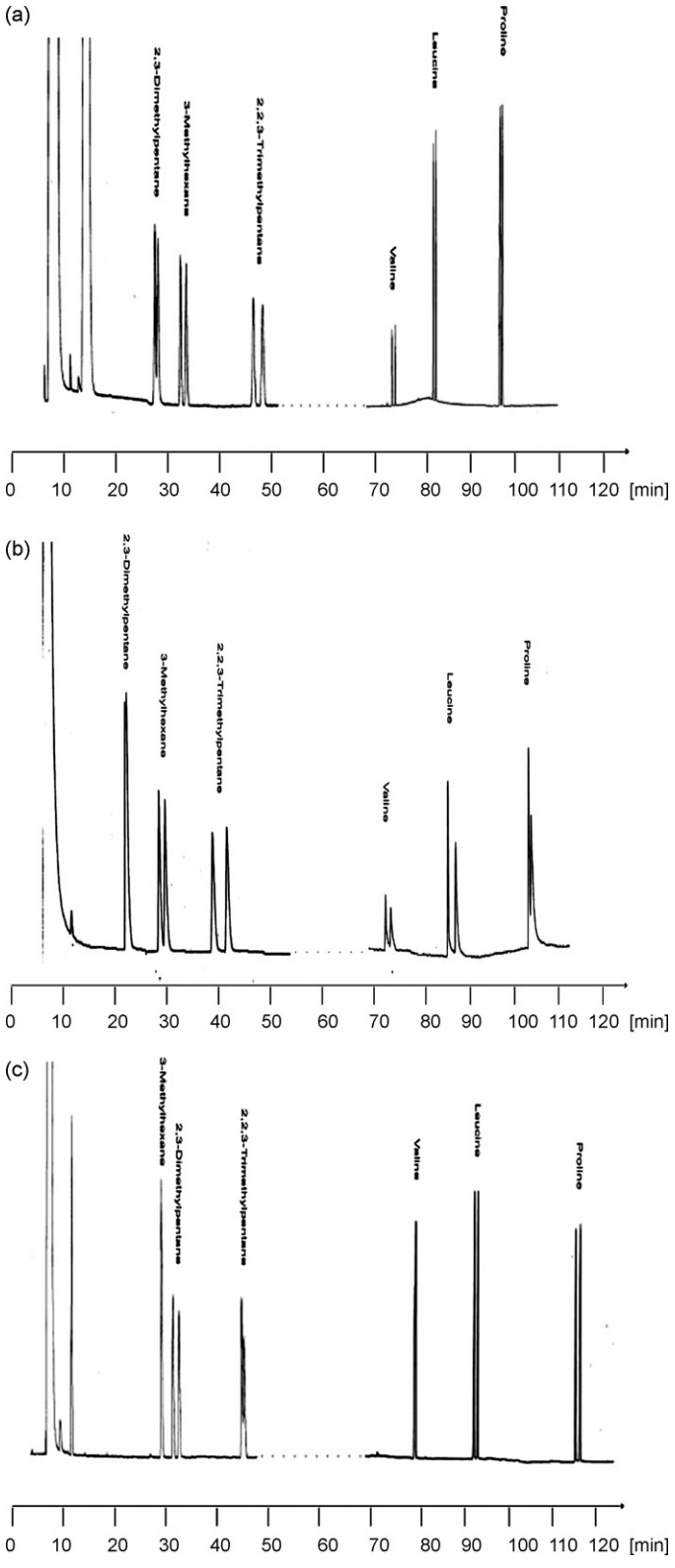

Fig. 6. Gas chromatographic enantioseparation of the three racemic alkanes 2,3-dimethylpentane, 3-methylhexane and 2,2,3-trimethylpentane (0–50 min) and the three racemic α-amino acids valine, leucine and proline as *N*-trifluoroacetyl *i*-propyl esters (70–120 min) on the mixed binary chiral selector system containing 33% (w/w) octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin (Lipodex G) and 19% (w/w) heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin in 48% (w/w) polysiloxane PS 086, column: 50 m × 0.25 mm i.d. fused silica capillary, film thickness 0.25 μm (top). Partial enantioseparation on 35% (w/w) octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin (Lipodex G) in PS 268, column: 50 m × 0.25 mm i.d. fused silica capillary, film thickness 0.25 μm (middle). Partial enantioseparation on 20% (w/w) heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin in PS 268, column: 50 m × 0.25 mm i.d. fused silica capillary, film thickness 0.25 μm (bottom). Alkanes (0–50 min): carrier gas H_2: 50 kPa, temperature program: 15 °C for 45 min, then 27 °C; α-Amino acids (70–120 min): carrier gas H_2: 100 kPa, temperature program: 80 °C for 20 min, then with a rate of 2 °C up to 120 °C; for all three columns.

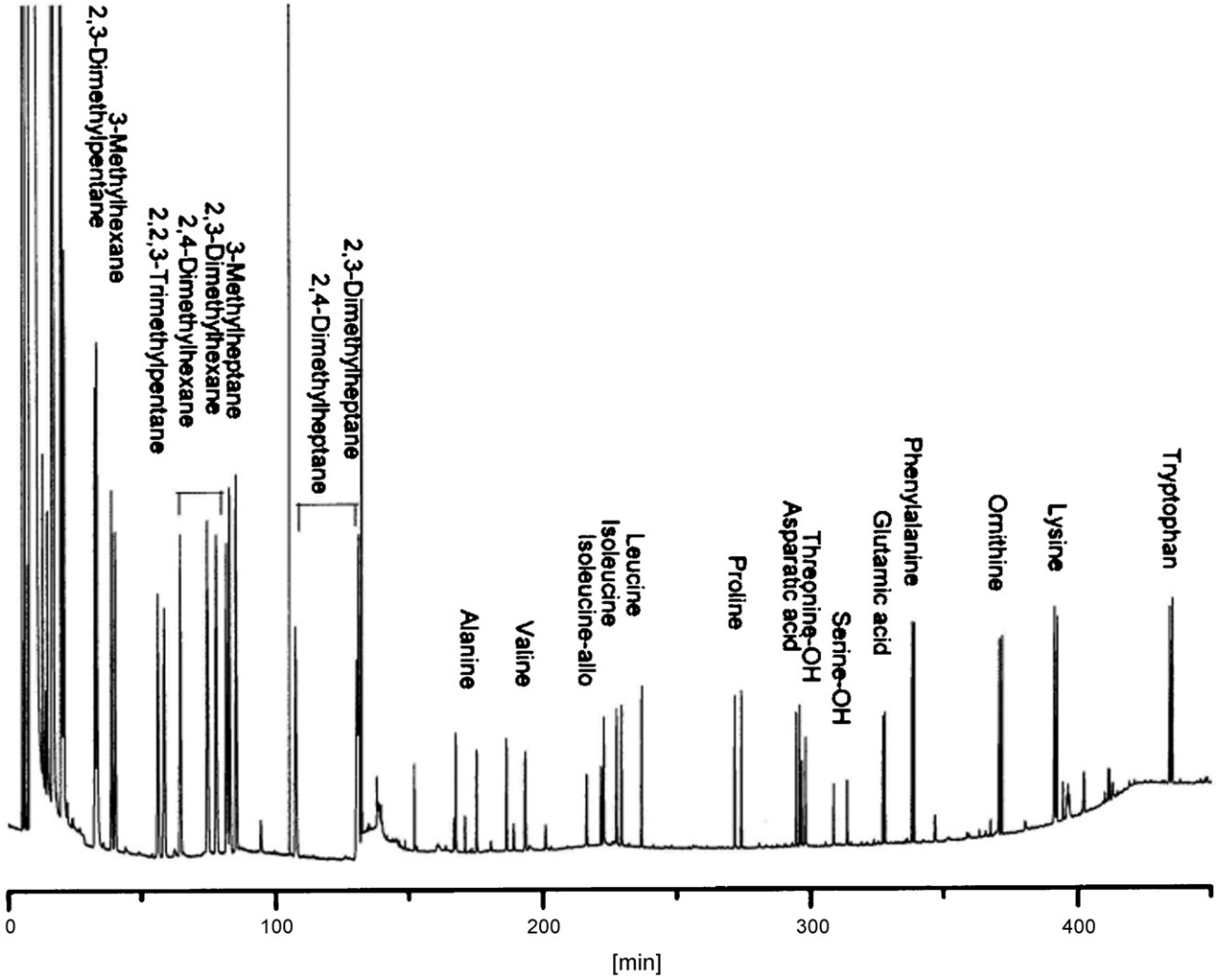

Fig. 7. Simultaneous gas chromatographic enantioseparation of eight racemic unfunctionalized aliphatic hydrocarbons and fourteen racemic α-amino acids as *N*-(*O*,*S*)-trifluoroacetyl ethyl esters on the mixed binary chiral selector system containing 33% (w/w) octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin (Lipodex G) and 19% (w/w) heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin in 48% (w/w) polysiloxane PS 086. Column: 50 m × 0.25 mm i.d. fused silica capillary, film thickness 0.25 μm, carrier gas H_2: 45 kPa for 130 min, then 90 kPa for 300 min. Temperature program: 15 °C for 45 min, then 27 °C for 85 min, then 70 °C with a rate of 0.5 °C/min up to 170 °C.

5.3 mg of octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin (Lipodex G) [23] and 10.7 mg PS 268 were dissolved in 4 ml diethylether. After about 5 min shaking and filtration of the solution, the mixture was used for coating a 50 m × 0.25 mm i.d. capillary by the static method to yield a 0.25 μm film thickness of the CSP [27].

(iii) The single chiral selector system of 20% (w/w) heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin diluted in polysiloxane PS 268:

3.1 mg of heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin [26] and 13.0 mg PS 268 were dissolved in 4 ml diethylether. After about 5 min shaking and filtration of the solution, the mixture was used for coating a 50 m × 0.25 mm i.d. capillary by the static method to yield a 0.25 μm film thickness of the CSP [27].

In this work, the maximum operating temperature of all investigated systems did not exceed 180 °C.

3. Results and discussion

The option to mix different cyclodextrin (CD) selectors in one chiral stationary phase (CSP) has been proposed previously [14] in the course of a discussion of the advantages to dilute modified CDs in a polysiloxane matrix for the separation of enantiomers by gas chromatography [28]. Subsequently, in a number of papers the use of mixed binary CD selector systems for the enantioseparation of different classes of chiral compounds has been described [29–35]. In the present work, a comprehensive enantioselectivity spectrum toward racemic unfunctionalized alkanes and racemic derivatized α-amino acids as well as to other classes of racemic compounds such as lactones, diols, secondary alcohols, ketones and terpenes has been realized by diluting the two versatile CD selectors depicted in Fig. 1, *i.e.*, octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin (Lipodex G) [23] (33%, w/w) and heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin [25] (19%, w/w) in the polysiloxane PS 086 (48%, w/w) (Table 1).

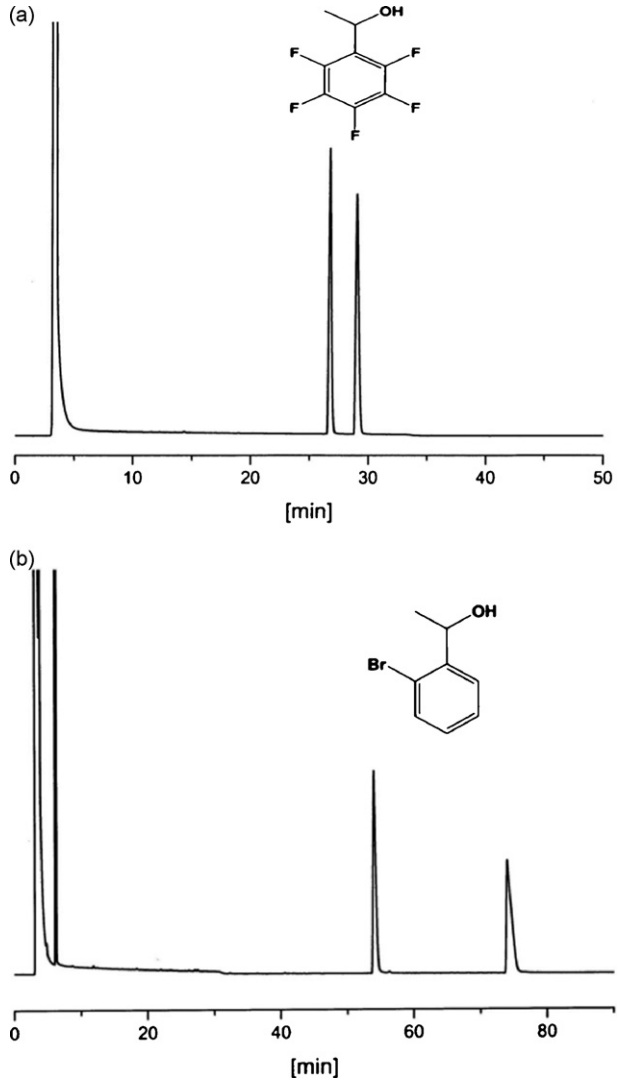

Fig. 8. Gas chromatographic enantioseparation of two racemic secondary alcohols on the mixed binary chiral selector system containing 33% (w/w) octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin (Lipodex G) and 19% (w/w) heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin in 48% (w/w) polysiloxane PS 086. Column: 50 m × 0.25 mm i.d. fused silica capillary, film thickness 0.25 μm, carrier gas H_2: 90 kPa. 1-(Pentafluorophenyl)-ethanol at 100 °C isothermal (top) and 1-(2-bromophenyl)-ethanol at 130 °C isothermal (bottom).

On the mixed binary chiral selector system the simultaneous gas chromatographic enantioseparation of all unfunctionalized C7 and C8 alkanes containing one stereogenic center, *i.e.*, C7: 2,3-dimethylpentane (=ethyl-methyl-*i*-propyl-methane), 3-methylhexane (=ethyl-methyl-propyl-methane); C8: 2,2,3-trimethylpentane, 2,4-dimethylhexane, 2,3-dimethylhexane and 3-methylheptane (3,4-dimethylhexane containing two stereogenic centers [24] was excluded from the study), has been achieved (Fig. 2).

On the single selector octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin (Lipodex G) [23] no enantioseparation of 2,3-dimethylpentane could be obtained, whereas the other C7 and C8 chiral alkanes are enantioseparated on this selector. For example, for 2,4-dimethylhexane an enantioseparation factor α as high as 1.52 has been obtained at 30 °C [23]. On the contrary, 2,3-dimethylpentane is well enantioseparated on heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin [25]. On the mixed binary

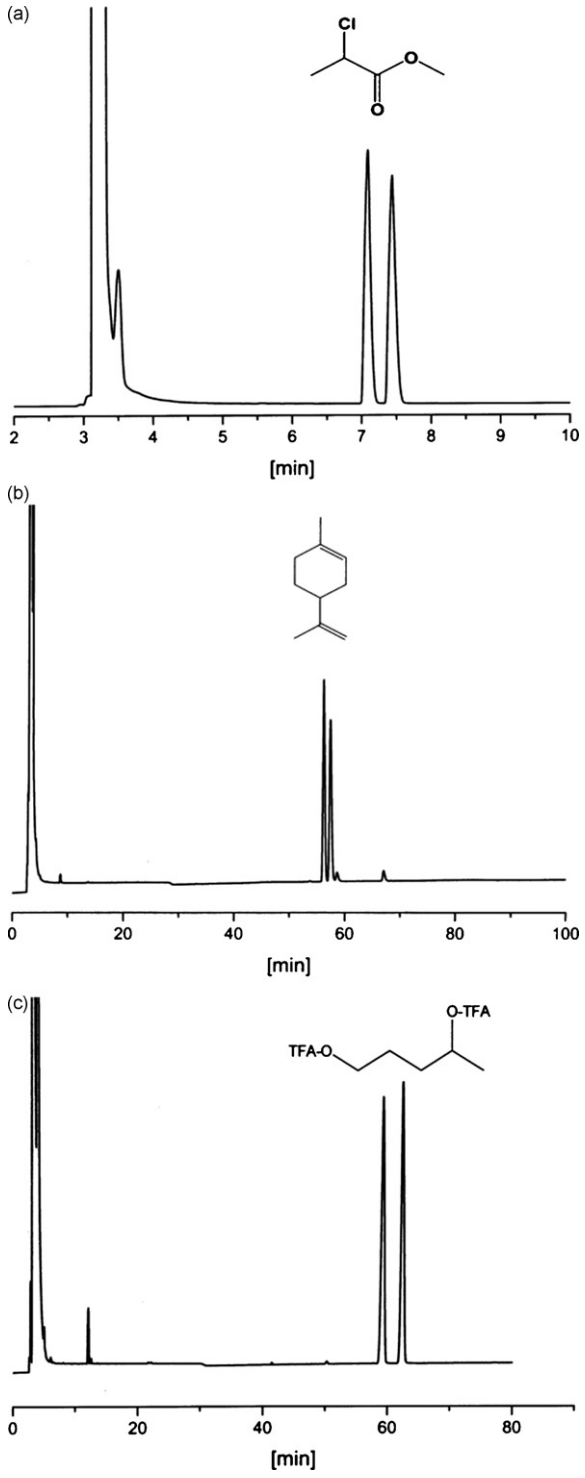

Fig. 9. Gas chromatographic enantioseparation of various racemic compounds on the mixed binary chiral selector system containing 33% (w/w) octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin (Lipodex G) and 19% (w/w) heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin in 48% (w/w) polysiloxane PS 086. Column: 50 m × 0.25 mm i.d. fused silica capillary, film thickness 0.25 μm, carrier gas H_2: 90 kPa. Methyl 2-chloropropionate at 100 °C isothermal (top); limonene (dipentene) at 60 °C isothermal (middle); 1,4-pentanediol as trifluoroacetyl derivative at 50 °C isothermal (bottom).

chiral selector system containing octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin (Lipodex G) and heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin all chiral C7 and C8 alkanes containing one stereogenic center are quantitatively resolved. This achievement is reminiscent to another mixed binary chiral selector system containing Chirasil-β-Dex and octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin (Lipodex G) [24]. However, on this complementary mixed binary chiral selector system, a simultaneous enantioseparation of most of the α-amino acid derivatives has not been possible as Chirasil-β-Dex is a poor chiral selector for this class of compounds (unpublished results).

In contrast, seventeen *N*-(*O*,*S*)-TFA-α-amino acid ethyl esters are baseline enantioseparated on the present mixed binary chiral selector system containing octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin (Lipodex G) and heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin (Fig. 3). The elutions of tyrosine and ornithine overlap. As ornithine does not represent a proteinogenic amino acid, its enantiomeric analysis may not be required in practice. The enantioselectivity and efficiency of the mixed binary chiral selector system comprising of two modified cyclodextrins (β and γ) toward α-amino acids is complementary to Chirasil-Val [1]. In addition, a quantitative and straightforward enantioseparation is obtained for the critical amino acids proline and aspartic acid. As depicted in Fig. 4, except for proline, all other L-amino acids are eluted as the second peak. A disadvantage of the novel mixed binary chiral selector system employing a 50 m × 0.25 mm i.d. capillary column is the extended analysis time which is much longer than the time required on Chirasil-Val [1,13]. The gas chromatographic enantioseparation of the racemic α-amino acids norvaline and 2-aminobutyric acid (as *N*-trifluoroacetyl ethyl esters) displays a very high enantioselectivity on the mixed binary chiral selector system containing octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin (Lipodex G) and heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin (Fig. 5). The enantioseparation of norvaline is characterized by the rare phenomenon that the first eluted enantiomer displays a larger peak width as compared to the second eluted enantiomer (Fig. 5). Peak broadening of the first eluted peak has been observed previously in complexation gas chromatography [36]. No rationalization for this seemingly kinetic effect is available at present.

In order to prove that indeed a mixed binary chiral selector system is required for the simultaneous enantioseparation of chiral hydrocarbons and α-amino acid derivatives a comparison of representative members from both classes of compounds with the single selectors octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin (Lipodex G) [23] and heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin [25] and the mixed binary system has been undertaken. As shown in Fig. 6 (top) the sequential gas chromatographic enantioseparation of the three racemic unfunctionalized alkanes 2,3-dimethylpentane, 3-methylhexane and 2,2,3-trimethylpentane as well as the three racemic α-amino acids valine, leucine and proline as *N*-trifluoroacetyl *i*-propyl esters is only possible on the mixed binary chiral selector system containing 33% (w/w) of octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin (Lipodex G) and 19% (w/w) of heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin in PS 086, whereas on octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin (Lipodex G) (35% (w/w) in PS 268) two alkanes, valine and leucine are resolved and on heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin (20% (w/w) in PS 268) only 2,3-dimethylpentane, leucine and proline are resolved.

For intended space experiments the simultaneous enantioseparation of unfunctionalized C7 and C8 alkanes and of α-amino acid derivatives in *one* gas chromatographic run is warranted. As shown in Fig. 7 this challenge has been met by use of the mixed binary chiral selector system containing octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin (Lipodex G) and heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin in PS 086 albeit the analysis time is exceedingly long. Yet if the full separation window is not required as only a limited number of racemates of interest may be considered, a miniaturization toward shorter columns would represent a straightforward option [37].

The mixed binary chiral selector system offers also the possibility to enantioseparate other racemic compounds such as lactones, diols, secondary alcohols, ketones and terpenes (Table 1). Some representative examples are shown in Figs. 8 and 9.

4. Conclusion

Since no reliable mechanistic details in enantioselective GC employing modified cyclodextrins are available, the present mixed binary chiral selector system has been selected by intuition rather then by rational planning.

The analytical tool of a simultaneous enantioseparation of chiral alkanes and derivatized α-amino acids by enantioselective gas chromatography in a one-column-set-up is now available for space experiments such as the COSAC probe [6], the Chirons of Titan [8] and as a viable alternative to the lab-on-a-chip microcapillary electrophoresis (CE) approach for chiral analysis in the UREY experiments of the Exo-Mars campaigns [38]. However, a subsequent challenge constitutes the miniaturization of the enantioseparation system [37]. Furthermore, comprehensive two-dimensional chromatographic techniques (GC × GC) may be required to pre-separate achiral members of compounds prior to the separation of the enantiomers and refined chemometric approaches may be needed to decode complex gas chromatograms [39].

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