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Journal of Chromatography A, 840 (1999) 145–150

JOURNAL OF CHROMATOGRAPHY A

Short communication

Enantiomer separation of amino acid derivatives on a new polymeric chiral resorc[4]arene stationary phase by capillary gas chromatography

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Received 8 December 1998; received in revised form 10 February 1999; accepted 11 February 1999

Abstract

A new resorc[4]arene basket-type selector containing chiral diamide groups was synthesized and bonded to a dimethylpolysiloxane backbone. Fifteen proteinogenic amino acids could be separated into their enantiomers as *N*(*O*,*S*)-trifluoroacetylmethyl esters. The new stationary phase exhibited excellent column efficiency above 100°C and thermal stability up to 200°C.

Keywords: Enantiomer separation; Chiral stationary phases; Calixarene stationary phases; Amino acids

1. Introduction

The enantiomeric purity of amino acids and the amount of racemization in peptide synthesis and peptide hydrolysis can easily be determined by the use of capillary gas chromatography (GC) on several hydrogen-bonding chiral stationary phases (CSPs) [1]. In 1966, Gil-Av et al. reported the first successful enantiomer separation of derivatized amino acids on a chiral amino acid derivative [2]. Later, Feibush developed CSPs containing diamide moieties which proved to be even more useful because of their high enantioselectivity and the reduced polarity [3] leading to shorter retention times. A remarkable improvement was achieved by coupling L-valine-*tert*.-butylamide to a dimethylpolysiloxane by Frank et al. in 1977 [4]. This polymeric CSP (Chirasil-Val) exhibits high thermal stability up to 200°C and allows the complete analysis of all proteinogenic amino acids within 30 min with a consistent elution order (on L-Chirasil-Val all derivatized D-amino acid enantiomers are eluted first). Both, L-Chirasil-Val and D-Chirasil-Val are commercially available.

A different approach to the enantiomer separation of amino acid derivatives by GC consists of the use of modified cyclodextrins. The separation of most proteinogenic amino acids as *N*-trifluoroacetyl alkyl esters was described by König et al. on octakis(3-*O*-butanoyl-2,6-*O*-di-*n*-pentyl)-γ-cyclodextrin (Lipodex E) [5]. Again, a consistent elution order was observed (on Lipodex E all derivatized D-amino acid enantiomers are eluted first, however, with the exception of Pro and Thr for which the L-enantiomer is eluted first).

Following the success of modified cyclodextrins as selective stationary phases in various modes of

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0021-9673/99/$ – see front matter
PII: S0021-9673(99)00224-1

chromatography [6], calixarenes are nowadays considered as interesting synthetic selectors in GC because of their high thermal stability and their unique cavity-type supramolecular shape [7,8]. Previously, the poor solubility of calixarenes in polysiloxanes restricted their use to gas–solid chromatography [9]. The solubility problem can be alleviated by the introduction of long alkyl chains into the molecules [10,11] or by chemically linking calixarenes to polysiloxanes [12].

The separation of enantiomers on chiral calixarenes has been reported in electrokinetic chromatography (EKC). Thus, several atropisomeric binaphthyl compounds have been resolved by addition of (*N*-L-valinoacyl)calix[4]arene [13]. In liquid chromatography (LC) a new chiral calixarene derivative functionalized with (−)-ephedrine and chemically bonded to silica has been used for enantiomer separation of *S*-(+)-1-phenyl-2,2,2-trifluoroethanol [14].

We now synthesized a resorc[4]arene basket-type compound containing four long ω-unsaturated alkyl chains, starting from resorcinol and 1-undecenal with an overall yield of 42% (cf. Fig. 1) [15]. Afterwards, chiral L-valine-*tert*.-butylamide moieties were attached to the eight hydroxy groups, giving rise to a cyclic arrangement of the chiral centers within the cyclic compound **1**. The chiral resorc[4]arene **1** is (i) diluted in a polysiloxane or (ii) chemically linked via the ω-unsaturated alkyl chains to a poly(dimethylmethylhydro)siloxane via platinum-catalyzed hydrosilylation [16] to yield chemically bonded Chirasil-Calix (cf. Fig. 1). Both CPS were compared for the enantiomer separation of *N*-TFA (trifluoroacetyl) amino acid methyl esters by gas–liquid chromatography.

2. Experimental

GC was carried out on a Carlo Erba 5300 Mega series instrument with hydrogen as carrier gas. Fused silica capillaries (0.25 mm I.D.) were obtained on stock from Microquartz, USA. The capillaries were divided into 20 m specimens and pretreated by heating the columns at 260°C with a slow stream of hydrogen for two days. The columns were coated with 0.25% Chirasil-Calix in diethyl ether by the static method yielding a film thickness of 0.25 μm and were subsequently conditioned at 100°C, 130°C and 160°C each for 4 h and finally at 190°C for 16 h.

The amino- and the carboxy groups have been derivatized as *N*(*O*,*S*)-TFA amino acid methyl esters as follows: 10 mg of racemic amino acid was heated in 1 ml 4 *M* hydrochloric acid for 1 h at 40°C in methanol (2 ml). After drying, the residue was dissolved in 1 ml dichloromethane and treated with trifluoroacetic acid anhydride (0.25 ml) for 1 h at room temperature. Trifluoroacetic acid was removed by blowing a small stream of nitrogen over the samples. The residue was dissolved in 1 ml of dichloromethane.

The synthesis and characterization of Chirasil-Calix will be described in detail elsewhere.

3. Results and discussion

The chiral resorc[4]arene **1** was first diluted in polysiloxanes (OV-1701 and PS-86) in amounts of 15 and 30% (w/w), respectively, but the performance of the columns was poor and no enantioselectivity toward *N*-TFA amino acid methyl esters was noted. Therefore, approximately 15% (w/w) of **1** was linked via the ω-unsaturated alkyl chains to a dimethylpolysiloxane containing about 10% Si–H entities by platinum-catalyzed hydrosilylation to give a Chirasil-Calix type CSP (cf. Experimental). Table 1 reports data for the separation of 17 D,L *N*(*O*,*S*)-TFA amino acid methyl esters . Fig. 2 shows a chromatogram for the simultaneous enantiomer separation of several proteinogenic D,L *N*(*O*,*S*)-TFA amino acid methyl esters. Fig. 3 shows a chromatogram of the more volatile amino acid derivatives including *N*-TFA-2-aminobutanoic acid methyl ester. In contrast to Chirasil-Val, Pro is separated with a satisfactory resolution factor while for Asp and Trp only a partial separation was achieved. While Tyr and Orn show similar separation factors on Chirasil-Val, Orn is much better separated than Tyr on Chirasil-Calix. It is remarkable and unexpected, that for amino acids containing an aromatic system, separation factors were not increased but even reduced compared to those of other amino acids. This can clearly be seen when comparing the closely eluted pairs of Glu/Phe and Tyr/Orn. Therefore, the anticipated participation of the supramolecular cavity

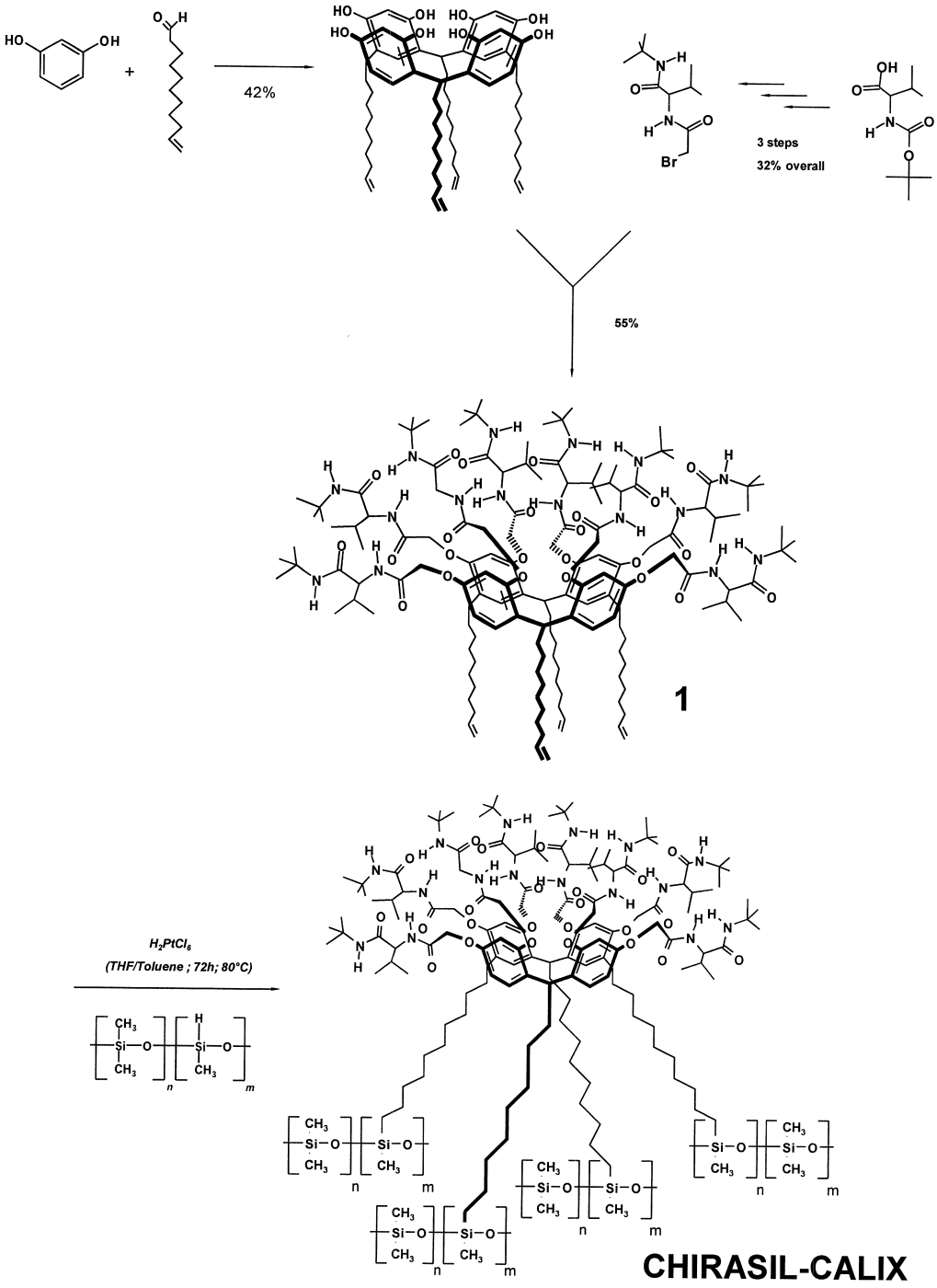

Fig. 1. Synthesis of **1** and structure of Chirasil-Calix (n:m=9:1).

Table 1
Gas chromatographic parameters of the enantiomer separation of *N*(*O*,*S*)-TFA-D,L-amino acid methyl esters (single isothermal measurements at increasing temperatures)[a]

	T (°C)	t'_{R1} (min)	t'_{R2} (min)	$\alpha_{L/D}$	R_s	k_1
Ala	75	4.19	4.59	1.096	2.21	4.60
Thr	80	7.52	7.96	1.059	1.87	7.84
Ser	80	12.71	13.56	1.067	1.49	11.68
Abu	85	3.33	3.59	1.080	2.12	4.32
Asp	85	18.11	18.61	1.027	0.96	18.69
Val	85	7.86	8.55	1.088	3.27	7.85
a-Ile	90	7.38	7.75	1.050	2.01	7.44
Ile	90	6.95	7.38	1.062	2.39	7.06
Leu	90	8.62	9.51	1.102	3.97	8.58
Pro	90	13.96	14.55	1.042	2.14	13.29
Cys	105	9.76	10.17	1.042	1.70	10.49
Phe	110	26.81	27.77	1.036	2.18	24.78
Glu	120	11.01	11.52	1.047	2.21	10.23
Met	125	7.90	8.25	1.045	1.74	7.90
Tyr	130	26.72	27.49	1.029	2.12	24.59
Orn	150	11.62	12.33	1.062	3.16	12.63
Lys	150	17.52	18.27	1.043	2.28	18.03
Trp	165	19.43	19.92	1.025	0.92	20.24

[a] Column: 20 m×0.25 mm fused-silica coated with Chirasil-Calix (film thickness 0.25 μm). Split 1:100, carrier gas 0.45 bar H_2. Breakthrough time t_M=1.01 min.

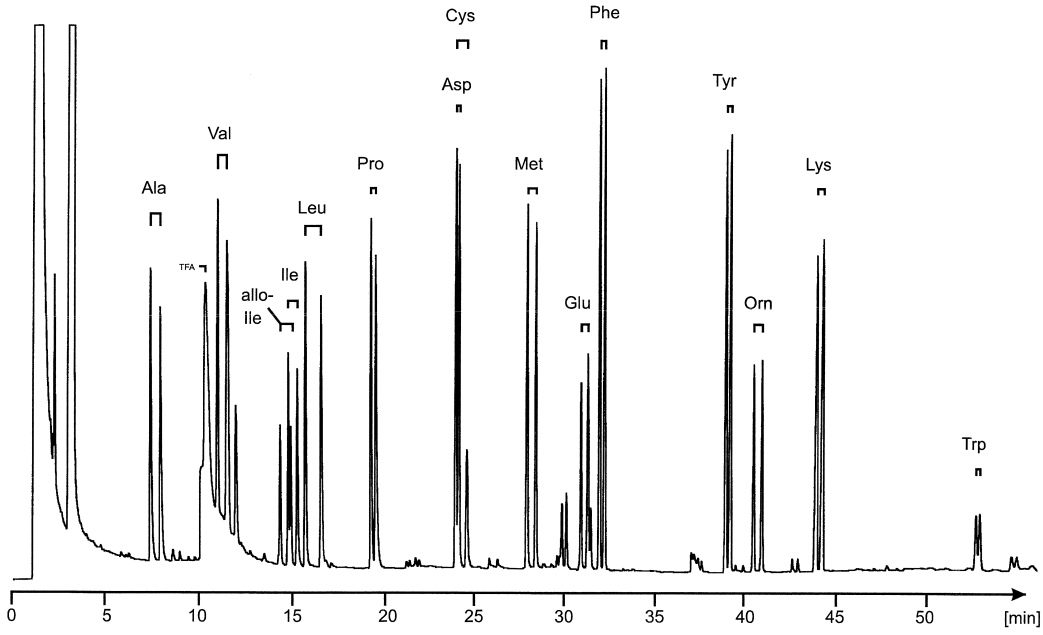

Fig. 2. Gas chromatographic enantiomer separation of 15 *N*(*O*,*S*)-TFA-D,L-amino acid methyl esters on a 20 m×0.25 mm fused-silica column coated with Chirasil-Calix (film thickness 0.25 μm). Temperature program: 70°C (4 min); 2.5°C/min; 120°C (5 min); 3.0°C/min; 180°C (10 min). Split 1:100; 0.45 bar H_2. The sample still contains a small amount of trifluoroacetic acid from the derivatization reaction.

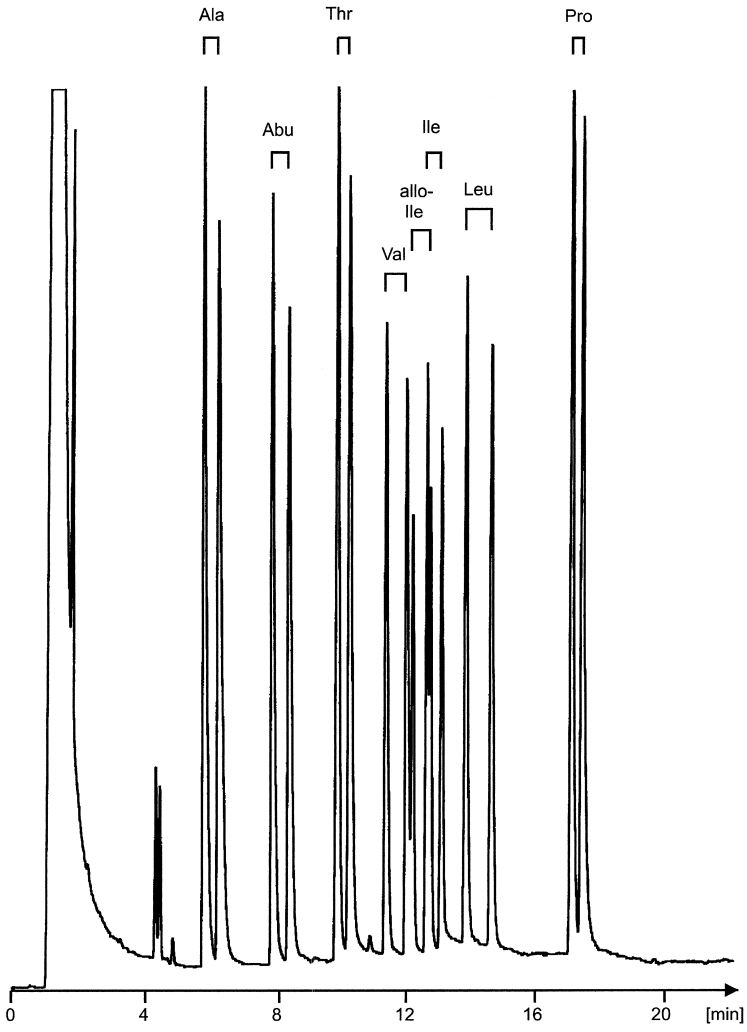

Fig. 3. Gas chromatographic enantiomer separation of *N*(*O*)-TFA-D,L-amino acid methyl esters on a 20 m×0.25 mm fused-silica column coated with Chirasil-Calix (film thickness 0.25 μm). Temperature program: 70°C (3 min); 2.5°C/min; 120°C (10 min). Split 1:100; 0.45 bar H_2. The first peak of each amino acid represents the D-enantiomer.

in **1** for molecular recognition could not be established and remains in doubt at present unless further investigations are performed. For Trp several structural factors may become important as, on the one hand, the aromatic system might be suitable for π–π interaction with the resorc[4]arene. On the other hand, the bulky indol group might cause serious problems for the formation of diastereomeric complexes because the close juxtaposition of the diamide groups within the cyclic system in **1** may hinder the approach of bulky selectands to the chiral centers. Moreover, it is very likely that single L-valine-*tert.*-butylamide groups strongly interact with each other via hydrogen bonding. The congestions of chiral selectors close to each other may indeed impair chiral recognition. Nevertheless the new type of amino acid selector may shed additional light on the mode of chiral recognition via hydrogen-bonding [1]. The preliminary results presented here will be complemented subsequently by systematic studies on the

role of the temperature on enantioselectivity and on the improvement of the separation factors by variations of the structure of **1**.

4. Note added in proof

This paper was presented at the 10th International Symposium on chiral discrimination, August 30–September 3, 1998, Vienna (abstract book p. 62). Meanwhile another group used a chiral thiacalixarene selector for gas chromatographic enantiomer separation [17].

Acknowledgements

The authors thank Deutsche Forschungsgemeinschaft and Fonds der Chemischen Industrie for support. We thank G.J. Nicholson for valuable advice and helpful discussion.

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