Lehrstuhl für Allgemeine Lebensmitteltechnologie
der Technischen Universität München

Modified cyclodextrins as chiral stationary phases for capillary gas chromatographic separation of enantiomers

Eisuke TAKAHISA

Vollständiger Abdruck der von der Fakultät Wissenschaftszentrum
Weihenstephan für Ernährung, Landnutzung und Umwelt
der Technischen Universität München
zur Erlangung des akademischen Grades eines

Doktors der Naturwissenschaften
(Dr. rer. nat.)

genehmigten Dissertation

Vorsitzender:	Univ.-Prof. Dr. Wilfried Schwab
Prüfer der Dissertation:	1. Univ.-Prof. Dr. Karl-Heinz Engel
	2. Univ.-Prof. Dr. Peter Schieberle

Die Dissertation wurde am 19.08.2005 bei der Technischen Universität München eingereicht und durch die Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt am 09.09.2005 angenommen.

I would like to thank Prof. Dr. Karl-Heinz Engel for his guidance in accomplishing this work and offering me this exiting opportunity. Many thanks also go to Dr. Hans-Georg Schmarr for his support both in terms of scientific and hands-on knowledge on column preparation and chromatography.

I am indebted to Dr. Masashi Ishikawa, vice-present of T. Hasegawa Co. Ltd., Dr. Yoshikazu Takagi, former director of the technical research center, and Dr. Akira Fujita, present director of the technical research center, for initiating this fascinating chance of studying at the Technical University of Munich. I am grateful to all my colleagues at the T. Hasegawa Co., Ltd., especially Dr. Tsuneo Kawanobe, Dr. Yasuhiro Warita and those of the Synthetic division in helping me in providing the essential chemical materials and analyte stocks.
I am grateful to T. Hasegawa Co., Ltd (Tokyo, Japan) for generous financial support of this work.

I also would like to thank Dr. Carsten Wattenbach for kind sharing of the NMR instruments and for some of the most influential samples in this work. The kind help of Dr. Dorothée Klein and Prof. Dr. Wilfried Schwab in acquisition of mass spectra of cyclodextrin derivatives is greatly acknowledged.

My thanks go to all the members of the Lehrstuhl für Allgemeine Lebensmitteltechnologie, especially to Dr. Marta Dregus for support in everyday trouble-solving and helping me greatly in aspects of German translation. Miss Natalie Bosse is thanked for her assistance in building up a chiral test sample library. Thanks for support in GC work provided by Dr. Ludwig Adam, and thanks for the comfortable but still stimulating and exciting laboratory environment; Dr. Andreas Miller, Dr. Francisco Moreano, Dr. Hidehiko Wakabayashi, Mrs. Motoko Wakabyashi, Prof. Dr. Etsuko Sugawara, Mr. Tobbias Müller and all others.

Finally, I would like to thank my parents and all my friends who have helped me always in encouraging me through this fascinating and challenging period. I have just named a very few here, but many thanks go to all of them.

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1. Introduction

Chirality is a ubiquitous phenomenon in chemistry. Its importance is due to the fact that the biological properties of enantiomers may differ significantly. One of the outstanding examples in the area of pharmaceuticals is the sedative thalidomide, of which one enantiomer turned out to be teratogenic (Blaschke et al., 1979). Other examples are the influence of configuration on the efficacy of agrochemicals (Kurihara et al., 1997), on pheromone activities (Miller et al., 1989) or on taste (Belitz and Grosch, 1999).

Chirality is also an important property of many flavor and aroma compounds. The classical example is carvone of which the (*R*)-enantiomer exhibits the typical scent of spearmint whereas the (*S*)-antipode is a character impact compound for caraway aroma (Leitereg et al., 1971; Russell and Hills, 1971). Meanwhile many examples of enantiomers differing in odor quality or potency have been reported (Brenna et al., 2003). The determination of naturally occurring enantiomeric compositions of flavor and fragrances is also important in terms of elucidation of biogenetic pathways (Hiltunen and Laakso, 1995; Fuchs et al., 1999) and authentication (Casabianca and Graff, 1994; Tateo et al., 1997; Ruiz del Castillo et al., 2003).

The analysis of chiral flavor and aroma compounds is a challenging task because they mostly occur only at trace levels. Therefore, conventional techniques such as polarimetry or NMR which require purified materials in sufficient amounts are hardly applicable. Accordingly, gas chromatography, which is the method of choice for analysis of volatiles, has also been made suitable for the determination of enantiomeric compositions. First approaches had been based on the conversion of enantiomers into diastereoisomeric derivatives (Casanova and Corey, 1961; Gil-Av and Nurok, 1962). The breakthrough was achieved by the development of chiral stationary phases (Gil-Av et al., 1966). Three major types of chiral selectors have been employed: (i) peptide derivatives (Frank et al., 1977), (ii) metal complexes (Schurig, 1977) and (iii) cyclodextrin derivatives (Juvancz et al., 1987; Alexander et al., 1988).

Cyclodextrins are the most popular chiral stationary phases presently used in gas chromatographic analysis (Schurig and Nowotny, 1990; Schurig, 1994; Schurig, 2001). The importance of cyclodextrins and their derivatives has

continuously increased from the late 1980s; more than 60% of all gas chromatographic separations of enantiomers reported in the period from 1978 to 1997 (a total of 1640 publications) have been accomplished on this type of chiral stationary phase (Juvancz and Szejtli, 1998). A particular area of applications are enantiodifferentiations of chiral flavor and fragrance compounds (Bicchi et al., 1999b; Werkhoff et al., 2002).

To improve the gas chromatographic performance, the free hydroxy groups have been subjected to various types of derivatizations (Schurig, 2001). Blocking the 6-hydroxy position of the glucose unit with a bulky silyl group and subsequent modification of the 2,3-hydroxy groups by acylation or alkylation resulted in useful chiral stationary phases (Schmarr et al., 1991b; Dietrich et al., 1992c).

Taking into account that the glycosidic bonds are essential structural elements in the cyclodextrin torus, the objective of this study was to incorporate this feature also in the side-chains at positions 2 and 3 of the glucose units and to investigate their influence on the separation of enantiomers. Octakis(2,3-di-*O*-methoxymethyl-6-*O*-tert-butyldimethylsilyl)-γ-cyclodextrin was synthesized as first representative of this new class of cyclodextrin derivatives. To elucidate the influence of the size of the cyclodextrin torus, the acetal moiety was also introduced into the corresponding α- and β-analogs. In addition, the impact of the alkoxymethyl side chain on the enantiodifferentiations was investigated by introducing the elongated ethoxymethyl moiety, the polar (2-methoxyethoxy)methyl group and the apolar and bulky (2-trimethylsilylethoxy)methyl group.

Although a wide spectrum of substituents has been introduced at positions 2 and 3 of the glucose units in cyclodextrins to improve their gas chromatographic performance, the aspect of inserting an additional chiral center has merely been considered. Permethyl-*O*-(*S*)-2-hydroxypropyl-cyclodextrins and the analogous (*R*)-2-hydroxypropyl derivatives are the only examples reported (Armstrong et al., 1990). Therefore, an additional objective was to study properties of cyclodextrins derivatized with (*R*)- and (*S*)-2-methylbutyryl moieties, respectively. The impact of the configurations at the asymmetric centers in the side chains on the degree of enantioseparation and on the order of elution of enantiomers should be investigated.

2. Background

2.1 Separation of enantiomers by capillary gas chromatography using chiral stationary phases

Three major principles have been applied to separate enantiomers on chiral stationary phases: (i) hydrogen bonding on chiral amino acid derivatives, (ii) coordination on chiral metal complexes, and (iii) host-guest interactions with cyclodextrin derivatives.

Chiral stationary phases based on amino acid derivatives

Direct gas chromatographic separations of enantiomers using a chiral stationary phase was first demonstrated in 1966 (Gil-Av et al., 1966). In this pioneering work *N*-trifluoroacetyl-L-isoleucine lauryl ester (Figure 2.1.1) was used as chiral selector.

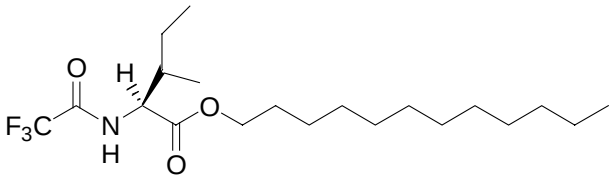


Figure 2.1.1 *N*-TFA-L-isoleucine lauryl ester, used as chiral selector in capillary GC (Gil-Av et al., 1966)

Following this first attempt, various efforts have been made to improve the performance of enantioseparation. For instance, the amino acid ester was replaced by a dipeptide ester (Gil-Av and Feibush, 1967). It turned out that the additional amide moiety rather than the amino acid was essential for the resolution of enantiomers due to the formation of an additional anchoring hydrogen bond. Therefore, the second amino acid group was replaced by a bulky amino moiety. In addition, the TFA group at the *N*-terminal end was replaced by long chain fatty acid ester, e.g. lauric acid ester (Feibush, 1971).

The strategies described suffered from racemizations of the amino acid moieties upon long-term usage and from their instable chemical characteristics, particularly at higher temperatures. The latter challenge was met by linking the chiral selector to polysiloxane polymers. Thus, the *N*-terminal end of the amino

group was attached to a backbone polysiloxane via an amide bond to yield a more stable stationary phase (Frank et al., 1977; Frank et al., 1978). This type of chiral stationary phase has been broadly applied (Schurig, 1994; Schurig, 2001). One of the most widely used representatives is the so-called Chirasil-val, a chiral stationary phase in which (L)-valine *tert*-butyl amide is covalently bound to a polysiloxane polymer; the key structural elements are depicted in Figure 2.1.2.

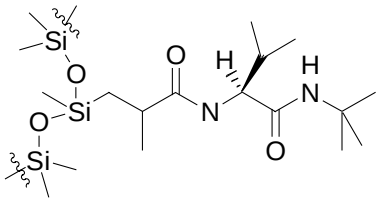


Figure 2.1.2 Structure of polysiloxane (L)-valine tert-butyl amide (Chirasil-val)

Chiral stationary phases based on metal complexes

A different approach based on a metal coordination mechanism as chiral discriminative power was introduced in 1977 (Schurig, 1977). Dicarbonyl rhodium(I)-3-trifluoroacetyl-1(*R*)-camphorate as shown in Figure 2.1.3 was employed as chiral selector.

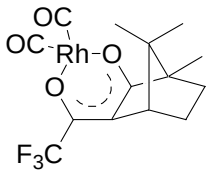
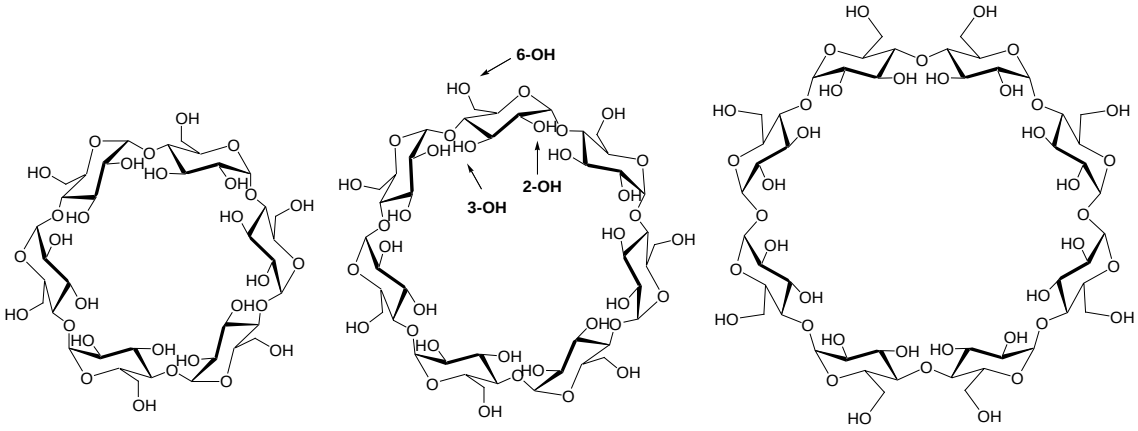


Figure 2.1.3 Dicarbonyl rhodium(I)-3-trifluoroacetyl-1(*R*)-camphorate used as chiral selector in capillary GC

Enantioseparation on this derivative is based on coordinating forces between the analyte and the stationary phase. Therefore, it is possible to analyze relatively inert molecules such as unsaturated hydrocarbons. This type of enantioseparation mechanism offered a valuable and complementary alternative to the hydrogen bonding-type chiral selectors. Examples for separations and detailed characteristics of coordination-type chiral stationary phases have been reviewed extensively (Schurig, 2001; Schurig, 2002).

2.2 Cyclodextrins in gas chromatography

Cyclodextrins are a class of cyclic saccharide oligomers in which D-glucopyranoses are attached via α-1,4-glucosidic bonds. They were originally isolated from starch in 1891 by Villiers and characterized as cyclic oligosaccharides by Schardinger in 1903 (Szejtli, 1998). Three major types of cyclodextrins (α, β and γ) differing in the number of glucose units (6,7 or 8) are commercially available (Figure 2.2.1).

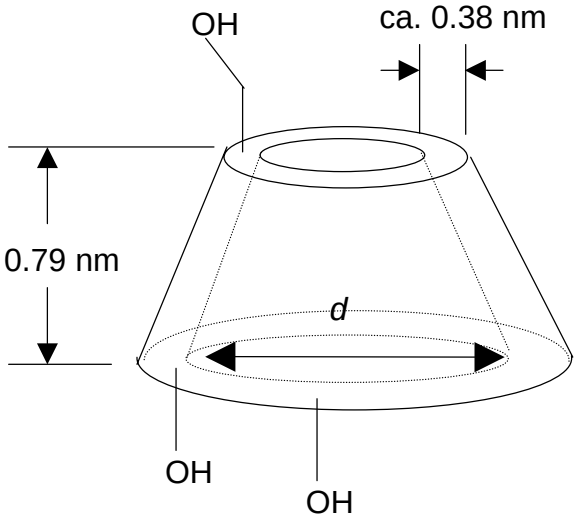


α-Cyclodextrin **β-Cyclodextrin** **γ-Cyclodextrin**

Figure 2.2.1 Structures of cyclodextrins

These macrocyclic molecules are characterized by their unique abilities to include compounds in their cavities. As shown in Figure 2.2.2, they may be considered as conically shaped tori which differ in diameters.

The interior of the CD tori is hydrophobic whereas their outside is hydrophilic. The three hydroxy groups present in each of the glucopyranose units are aligned on the rims of the cavity opening. The secondary alcohols in positions 2- and 3- are on the rim of the wider opening; the primary 6-OH group is positioned on the narrower opening.



	d (nm)
α	0.57
β	0.78
γ	0.95

Figure 2.2.2. Schematic model of cyclodextrins

Underivatized cyclodextrins

The first application of cyclodextrins as chiral stationary phase for capillary gas chromatographic separation of enantiomers was reported in 1983 (Koscielski et al., 1983). The enantiomeric resolution of α- and β-pinene was achieved using underivatized α- and β-cyclodextrin which had been dissolved in a water/formamide mixture, deposited onto celite® and packed in a 2 m long column. This remarkable work has followed the former research on resolving xylene regioisomers and ethylbenzene utilizing the same setup (Sybilska and Koscielski, 1983). Although the separation factors α were relatively high (α= 1.73, for α-pinene on α-cyclodextrin), the separation suffered from low column efficiency, and the column had a relatively short lifetime.

Alkylated and acylated cyclodextrins

The three hydroxy groups present at each of glucose units of cyclodextrins are accessible to derivatizations, such as acylations or alkylations. Acylated β-cyclodextrin was employed as gas chromatographic stationary phase as early as 1961 (Sand and Shlenk, 1961), not for the separation of enantiomers but of fatty acids, and proved to be superior in terms of temperature stability compared to polyester phases. Permethylated cyclodextrin was introduced for gas chromatographic separations in the course of studies on the mechanism of the

inclusion of hydrocarbons in cyclodextrins (Reggiani et al., 1979). It was either applied in pure form or dissolved in silicone oil as stationary phase in packed columns. A major breakthrough was achieved by the use of permethylated β-cyclodextrin in capillary GC. Due to the greatly enhanced separation efficiency, it was possible to separate regioisomers of aromatic compounds (Juvancz et al., 1987) and eventually chiral compounds (Alexander et al., 1988).

In addition to permethylation, perpentylation of cyclodextrins has been performed, particularly to improve the melting behavior. An early successful attempt in this respect is the introduction of 2,3,6-*n*-pentylated cyclodextrin phases, such as 2,3,6-per-*n*-pentyl-α- and β-cyclodextrin, also known as Lipodex® A and Lipodex® C, respectively. These *n*-pentyl type derivatives are liquid at room temperature, thus being advantageous in terms of lowest possible operating temperature (Koenig et al., 1988b; Koenig et al., 1989b; Koenig et al., 1989c).

Regioselective derivatization

The three hydroxy groups at the glucose units of the cyclodextrin differ in reactivity due to different acidities of the protons and due to differences in sterical hindrance. The 2-hydroxy group is the most acidic and the 6-OH and 3-OH group follow in respective order. However, due to steric hindrance, the 2-OH group is not necessarily the most reactive position in the molecule. This forms the basis for a broad spectrum of regioselective alkylations and acylations which have been applied to modify the properties of cyclodextrins as chiral stationary phases (Khan et al., 1998).

One example of this approach is the synthesis of 2,6-*n*-pentyl-cyclodextrin as intermediate and the subsequent acylation of the remaining 3-OH position resulting in 2,6-di-*O*-*n*-pentyl-3-*O*-butyryl-γ-CD (commercial name: Lipodex® E) (Koenig et al., 1989a) . Other examples are 2,6-dialkylated-3-acylated-cyclodextrin phases such as 2,6-*n*-pentyl-3-acetyl-α-cyclodextrin (Lipodex® B), 2,6-n-pentyl-3-acetyl- β-cyclodextrin (Lipodex® D) and 2,6-*n*-pentyl-3-methyl-γ-cylcodextrin (Lipodex® G) which are also commercially available (Koenig et al., 1988c; Koenig et al., 1988d). The acylation strategy was extended towards the introduction of fluorinated acyl side chains, for example in

2,6-*n*-pentyl-3-trifluoroacetyl-α-, β- and γ-cyclodextrin belonging to the so-called Chiraldex TA series (Berthod et al., 1990; Li et al., 1990; Koen de Vries et al., 1992). A switching from 2,6-*n*-pentyl side chains to 2,6-methyl side chains was tested and the resulting 2,6-methyl-3-heptafluorobutanoyl-β-cyclodextrin showed good enantioseparation properties (Schurig and Jung, 1990). Although these organofluorine-type CD generally exhibit unique separation characteristics, their use is hampered by the fact that the side chains are vulnerable towards hydrolysis or decomposition at high temperature (Betts, 1995).
It is noteworthy that acylations at the 3-OH position have positive impacts on the enantioseparation performance whereas such modifications at the 6-OH position had a strong negative effect on enantioseparations (Schmarr et al., 1991a).

6-O-Silylated cyclodextrins

A major improvement in separation power was achieved when the 6-OH group was silylated rather than alkylated or acylated. The first pioneering work in introducing a tert-butyldimethylsilyl (TBDMS) group onto cyclodextrin was accomplished by using *N*-TBDMS-*N*-trifluoracetamide for silylation (Aichholz et al., 1990). Although this CD derivative is a complex mixture of regioisomers, it turned out to be a very useful chiral stationary phase when diluted in PS-086 silicone.
Following this attempt, 2,3-*O*-diacetyl-6-TBDMS-γ-CD was the first representative of silylated cyclodextrins with a clearly defined structure (Schmarr et al., 1991b). It was assumed at that time that an introduction of a very bulky protection group like the TBDMS at the 6-OH position of the CD torus would have a negative impact on the host-guest interaction of the analyte with the cyclodextrin cavity. However, the TBDMS phase turned out to be one of the most effective cyclodextrin chiral stationary phases and nowadays many variations of this class of cyclodextrins have been made commercially available. Especially the 2,3-methoxy-6-TBDMS-β-CD and the 2,3-acetyl-6-TBDMS-β- and γ-CD are versatile chiral stationary phases which are widely applied especially for enantioselective analysis of flavor and fragrance compounds (Bicchi et al., 1999b). The mechanism of the enhancement of performance is not totally clear but the bulky substituents influencing the conformation of the cyclodextrin may be one of the reasons (however this process is fast and is not observable at slow

time frames such as NMR experiments). Another explanation might be that the introduction of a highly hydrophobic substituent increases the solubility of such cyclodextrin phases in the diluting achiral stationary phases thus enhancing the peak efficiency required for sufficient baseline resolution even with small separation factors.

Besides the above-mentioned two pioneering phases (2,3-methoxy-6-TBDMS-β-CD and 2,3-acetyl-6-TBDMS-γ-CD), other analogs of this family such as 2,3-ethyl-6-TBDMS-β-CD, 2,3-*n*-butyryl-6-TBDMS-γ-CD and 2,3-propionyl-6-TBDMS-γ-CD are known as useful phases. For TBDMS-type stationary phases β-CD gives good results compared to its γ-analog especially in the case where the 2,3-rim is substituted by an ether group, and the γ-CD results in good separation performance in comparison with its β-analog if the 2,3-hydroxy rim is substituted by an acyl group. An overview of acylated/alkylated cyclodextrins developed in the last decade is given in Table 2.2.1.

A unique derivatization of cyclodextrins was reported by Armstrong (Armstrong et al., 1990). 2-Hydroxypropyl moieties are attached by reaction of the cyclodextrin under aqueous alkaline conditions with propylene oxide and subsequent methylation. This procedure is not selective and the hydroxyl groups are randomly substituted. After methylation of the intermediate 2-hydroxypropylated cyclodextrin, the methyl groups will substitute either the remaining free hydroxy groups or the hydroxy groups at the propyl side chains. Therefore, the material obtained is a mixture of different types of isomers; their distribution could be demonstrated by mass spectrometric analysis (Armstrong et al., 1990). Despite of this ambiguity as regards the structure, this phase has been shown to be useful for enantioseparations.

Table 2.2.1 Overview on alkylated and acylated 6-TBDMS-cyclodextrins

	α	β	γ
		Alkylated CD	
Methyl	(Schmarr et al., 1991b)[a)] 7[b)]	(Dietrich et al., 1992c) 76	(Dietrich et al., 1992a) 13
Ethyl	(Kim et al., 1997c) 1	(Bicchi et al., 1996) 8	(Bicchi et al., 1996) 3
Propyl	-[c)]	(Kim et al., 1997b) 1	-
Butyl	-	-	(Maas et al., 1995) 2
n-Pentyl	(Koenig et al., 1990) 2	(Miranda et al., 1998) 5	(Maas et al., 1995) 1
		Acylated CD	
Acetyl	(Schmarr et al., 1991b) 5	(Dietrich et al., 1992b) 53	(Schmarr et al., 1991b) 12
Propionyl	-	-	(Beck et al., 2000a) 1
Butyryl	-	(Abe et al., 1994) 3	(Abe et al., 1994) 1

(a) First report in literature, (b) number of applications reported, (c) type of derivatization not described.

Cyclodextrins diluted in silicones

In the early stage of developments, the use of undiluted cyclodextrins as chiral stationary phases was considered necessary to obtain maximum separation performance. The ability of stationary phases to maintain their liquid states is crucial to obtain sufficient separation efficiency, i.e. , number of plates per unit length. Permethyl-β-cyclodextrin in pure form has been employed to study thermally induced phase transitions (Venema and Tolsma, 1989). It was concluded that high resolution is only possible above the glass transition temperature (T_g = 76 °C) of the cyclodextrin derivative. By using differential scanning calorimetry (DSC), it was shown that a cyclodextrin phase once heated above 200 °C and cooled downed again behaves as a super-cooled liquid. However, even in this state the diffusion of the analyte molecule is not sufficient to result in satisfactory column efficiency. Therefore, several cyclodextrin derivatives have been designed to maintain the liquid phase behavior at room temperature by attaching longer side chains like *n*-pentyl on the cyclodextrin torus (Koenig et al., 1988a).

An important discovery in that respect was the fact that the separation power of diluted phases comes close to its maximum at relatively low concentrations (ca 20%-30% w/w), and therefore the use of undiluted phases is not that essential (Jung and Schurig, 1993). Diluting the cyclodextrin derivative in polysiloxane was first introduced in 1988 (Schurig and Nowotny, 1988). This significantly extended the spectrum of cyclodextrin derivatives suitable for use as stationary phases. Nowadays the dilution of cyclodextrin derivatives in achiral silicones is standard practice (Schurig, 2001). It is also assumed to be advantageous in terms of a providing a protective environment for the cyclodextrin and there are reports on faster degradation of the cyclodextrins when being used without dilution (Miranda et al., 1998).

The selection of the diluting silicone has a strong impact on the separation performance. In order to achieve higher separation factors, the diluting phase should be as apolar as possible. For instance, in the case of 2,3-di-O-*n*-propionyl-6-TBDMS-γ-CD, the use of PS264 (10% phenyl, 90%

dimethylsiloxane) instead of OV-1701vi (7% cyanopropyl, 93% dimethylsiloxane) resulted in such an enhancement of separation factors that only 33% (w/w) of cyclodextrin was needed to almost exceed the performance of the same phase diluted in 50% (w/w) concentration in OV-1701vi (Beck et al., 2000b). It is generally accepted that using polar polysiloxane as diluting phase will reduce the significance of the chiral interaction (originating from the polar cyclodextrin derivative) between the analyte and the CD derivative. However, this advantage is accompanied by a certain decrease in column efficiency. When a less polar siloxane is used to dilute cyclodextrin derivatives, the affinity between the polar cyclodextrin and the apolar siloxane is not sufficient at low temperature range, resulting in drastic reduction of the efficiency (i.e., number of plates per unit length of column) (Dietrich et al., 1995). Therefore, for a polar cyclodextrin phase, a polar diluting phase such as OV-1701vi is advantageous compared to apolar phases (e.g. PS264, SE54) according to the wider temperature range in which the column can be operated.

3. Materials and Methods

3.1. Materials

3.1.1. Chemicals

Acetone-d_6	Aldrich 15,179-3
Acetyl chloride	Fluka 00990
Alkane standards C_8-C_{20}	Fluka 04070
tert-Butyldimethylchlorosilane	Merck 8.18642.0025
Butyryl chloride	Fluka 19310
Calcium chloride (Granular)	Merck 1.02379.1000
Calcium hydride	Fluka 21170
Chloroform-*d*3	Merck 1.03420.0100
Chloromethylmethylether	Aldrich 10,033-1
β-Cyclodextrin	Wako 039-10642
γ-Cyclodextrin	Tokyo Kasei C0777
Deuterated water	Merck 113366
Dichloromethane	Riedel de Haën 24233
Diethylether	Condea Chemie 33/1155
N,*N*-Diisopropylethylamine	Aldrich 38,764-9
N,*N*-Dimethylaminopyridine	Fluka 39405
Dimethylphenylsilane	Fluka 41410
Ethanol (95%)	Riedel de Haën 24102
Ethoxymethylchloride	Aldrich 14,267-0
Ethyl acetate	Riedel de Haën 27227
Grob test mixture I	Fluka 86499
Hexane	Prolabo 24 574.460
Hexanoyl chloride	Fluka 21590
Hydrochloric acid	Acros 124620025
Imidazole	Fluka 56756
Magnesium sulfate	Fluka 63136
Methanol	Prolabo 20 903.368
(2-Methyoxyethoxy)methylchloride	Fluka 64735
2-Methoxy-2-methylpropane	Oxeno 80033270
2-Methylbutanoic acid	Fluka 66130
2-Methylbutanol	Fluka 65990

(*S*)-2-Methylbutanoic acid	T. Hasegawa Co., Ltd.
(*R*)-2-Methylbutanoic acid	T. Hasegawa Co., Ltd.
3-Methyl-2-pentanone	Aldrich (Europe) M6700-1
Molecular sieves 4Å	Carl Roth 8471.2
(*R*)-5-Octanolide	Fluka 74876
(*S*)-5-Decanolide	Fluka 30624
n-Pentane	J.T. Baker 8685
(*R*)-1-Phenylethanol	Sigma Aldrich P4277
Phosphorous pentoxide (with indicator)	Merck 1.00543.0500
Phosphomolybdic acid hydrate	Fluka 76560
1-Propanol	Riedel de Haën 24135
Pyridine	Fluka 82702
Silica gel 60 (40-63 μm)	Merck 1.09385.1000
Silicone OV1701-vi	Supelco 21281
Silicone SE54	Machery Nagel GE SE54
Sodium borohydride	Merck 806372
Thionyl chloride	Fluka 88950
Toluene	Carl Roth 7115.2
Triethylamine	Fluka 90342
3,3,5-Trimethylcyclohexanone	Fluka 92405
(2-Trimethylsilylethoxy)methylchloride	Fluka 92749

Solvents

Solvents were purified before use according to established methods (Armarego and Chai, 2003). Dimethylformamide, triethylamine and dichloromethane were refluxed with calcium hydride for 3 hours and then distilled under reduced pressure. Pyridine was refluxed with potassium hydroxide pellets for 3 hours and then distilled at atmospheric pressure.

3.1.2. Chemicals for analyses

Chemicals used as analytical specimen were obtained from Aldrich (Milwaukee, WI, USA), Fluka (Buchs, Switzerland), Frey and Lau (Henstedt-Ulzburg, Germany), Merck (Hohenbrunn, Germany) and T. Hasegawa (Tokyo, Japan) and were used without further purification.

Others

Thin layer chromatography was carried out using silica gel pre-coated plastic sheets; Polygram® Sil G/UV254 with UV indicator from Machery-Nagel (Düren, Germany).

3.1.3. Fused silica columns

Fused silica column material was obtained from Microquartz München (München, Germany) and Polymicrotechnologies (Phoenix, AZ, USA).

3.2. Instruments

3.2.1. NMR

NMR spectra were recorded on a Bruker AC 250 spectrometer (^{1}H 250.133 MHz, ^{13}C 62.896 MHz) with an ASPECT 3000 workstation running DISR94 program. High temperature experiments were achieved utilizing Bruker B-VT2000 temperature regulation unit (temperature drift < ±1 °C). The chemical shift values for both ^{1}H and ^{13}C spectra were recorded in part per million and chloroforom-*d*3 was used as solvent and internal standard (7.26 ppm and 77.1 ppm, respectively). In some cases (indicated therein), acetone-d_6 was used as solvent and internal chemical shift standard (2.05 ppm and 30.8 ppm, respectively) in order to avoid overlapping of resonance near 77 ppm in ^{13}C NMR experiments.

3.2.2. Mass spectrometry

Mass spectrometry data were obtained after direct introduction of the derivatized CD (methanol solution) into an Esquire 3000+ (Bruker) instrument. Electrospray ionization was used to ionize the cyclodextrin molecule in positive mode, with source voltage of 4.0 kV, nebulizer gas flow of 5.0 L/min (operating at 69 kPa) and drying temperature of 300 °C.

3.2.3. Gas chromatography

Gas chromatograms were recorded on Carlo Erba Strumentazione Fractovap series models 4130 and 4160 equipped with flame ionization detectors. The

chromatograms were processed by the Chromcard system from Thermoquest (Milan, Italy). Hydrogen was used as carrier gas at an inlet pressure of 100 kPa, and the analytes were introduced via split injection method with a split ratio of 30:1. The injector and the detector temperatures were 220 °C and 230 °C, respectively. The injection volume of the samples was 1 μL and the concentrations of the compounds tested were 0.2 μg/mL in diethyl ether.

3.2.4. Special equipments

Drying apparatus

A glass drying oven (bulb-to-bulb distillation apparatus) B-580 GKR from Büchi (Flawil, Switzerland) and a rotation drying flask with sintered glass filter attachment (Nr. 37143) were used to dry the intermediate cyclodextrin derivative.

Coating equipment

A water bath for column coating was set up by combining a heating stirrer (ETS-D4 fuzzy: IKA Labortechnik, Germany) with a slate plate and a glass water bath (ca 6 L volume) filled with distilled water. Pressure-regulated vacuum source was provided by a diaphragm pump MZ2C / 2.4 (Vacuubrand, Wertheim) with pressure regulator along with a 1.5 L glass cylinder as vacuum damper.

Oven for column dehydration

A thermostat-controlled oven from Fractovap Model GH (Carlo Erba Strumentazione, Milan, Italy) was used to dehydrate rinsed fused silica column material.

High temperature flame source

A butane-propane-oxygen flame hand torch Roxy plus 3100 (Rothenberger Werkzeuge GmbH, Kelkheim) with a fine needle outlet was utilized to efficiently melt and manipulate pure fused silica.

3.3. Syntheses

6-O-tert-butyldimethylsilyl-cyclodextrin

Octakis(6-*O*-TBDMS)-γ-cyclodextrin, heptakis(6-*O*-TBDMS)-β-cyclodextrin and hexakis(6-*O*-TBDMS)-α-cyclodextrin were synthesized according to Fuegedi (Fuegedi, 1989). The purification procedure was slightly modified by replacing

the chloroform-methanol gradient and the dichloromethane-methanol-water (80:19:1) mixture which were used during the open column preparative chromatography by MTBE-methanol-water (72:8:3). The reaction was monitored by thin layer chromatography using acidic phosphomolybdic acid hydrate solution as visualization reagent.

2,3-Di-O-methoxymethyl-6-O-tert-butyldimethylsilyl-γ-cyclodextrin

Octakis(6-*O*-TBDMS)-γ-Cyclodextrin was heated at 100 °C under high vacuum (0.001 mmHg) overnight using a bulb-to-bulb distillation apparatus. The obtained dry octakis(6-*O*-TBDMS)-γ-cyclodextrin (214mg) was dissolved in dry dichloromethane (10 mL). Diisopropylethylamine (3.6 g) was added at room temperature and stirred. The clear solution was cooled to 0 °C with an ice-water bath and methoxymethylchloride (1.62 g) was added drop-wise. After stirring at 0 °C for 15 min, the solution was allowed to warm up to room temperature and then stirred overnight at 40 °C. After TLC analysis showed completion of the reaction, the reaction mixture was poured into water/MTBE mixture and extracted with MTBE. The organic phase was washed with 1N HCl aq., water, sodium bicarbonate solution, saturated sodium chloride solution and dried over anhydrous magnesium sulfate, concentrated and purified by column chromatography (silica gel 60, toluene : ethanol = 9 : 1, v/v) to yield 186 mg of the titled compound as fine white powder (isolated yield 66%.). The structure was checked by means of NMR (^{1}H, ^{13}C, DEPT 135 and double quantum filtered COSY) and MS.

2,3-Di-O-methoxymethyl-6-O-tert-butyldimethylsilyl-β-cyclodextrin

Heptakis(6-*O*-TBDMS)-β-cyclodextrin was heated at 100 °C under high vacuum (0.001 mmHg) overnight using a bulb-to-bulb distillation apparatus. The obtained dry heptakis(6-*O*-TBDMS)-β-cyclodextrin (206mg) was dissolved in dry dichloromethane (10 mL). Diisopropylethylamine (3.5 g) was added at room temperature and stirred. The clear solution was cooled to 0 °C with an ice-water bath and methoxymethylchloride (1.59 g) was added drop-wise. After stirring at 0 °C for 15 min, the solution was allowed to warm up to room temperature and then stirred overnight at 40 °C. After TLC analysis showed completion of the reaction, the reaction mixture was poured into water/MTBE mixture and

extracted with MTBE. The organic phase was washed with 1N HCl aq., water, sodium bicarbonate solution, saturated sodium chloride solution and dried over anhydrous magnesium sulfate, concentrated and purified by column chromatography (silica gel 60, toluene : ethanol = 93 : 7, v/v) to yield 174 mg of the titled compound as fine white powder (isolated yield 64%). The structure was checked by means of NMR (^{1}H, ^{13}C and DEPT 135) and MS.

2,3-Di-O-methoxymethyl-6-O-tert-butyldimethylsilyl)-α-cyclodextrin

Hexakis(6-*O*-TBDMS)-α-cyclodextrin was heated at 100 °C under high vacuum (0.001 mmHg) overnight using a bulb-to-bulb distillation apparatus. The obtained dry hexakis(6-*O*-TBDMS)-α-cyclodextrin (228 mg) was dissolved in dry dichloromethane (3 mL). Diisopropylethylamine (2.77 g) was added at room temperature and stirred. The clear solution was cooled to 0 °C with an ice-water bath and methoxymethylchloride (2.39 g) was added drop-wise. After stirring at 0 °C for 15 min, the solution was allowed to warm up to room temperature and then stirred overnight at 40 °C. After TLC analysis showed completion of the reaction, the reaction mixture was poured into water/MTBE mixture and extracted with MTBE. The organic phase was washed with 1N HCl aq., water, sodium bicarbonate solution, saturated sodium chloride solution and dried over anhydrous magnesium sulfate, concentrated and purified by column chromatography (silica gel 60, toluene : ethanol = 93 : 7, v/v) to yield 282 mg of the titled compound as fine white powder (isolated yield 94%). The structure was checked by means of NMR (^{1}H, ^{13}C).

2,3-Di-O-ethoxymethyl-6-O-tert-butyldimethylsilyl)-γ-cyclodextrin

Octakis(6-*O*-TBDMS)-γ-cyclodextrin was heated at 100 °C under high vacuum (0.001 mmHg) overnight using a bulb-to-bulb distillation apparatus. The obtained dry octakis(6-*O*-TBDMS)-γ-cyclodextrin (228 mg) was dissolved in dry dichloromethane (3 mL). Diisopropylethylamine (2.77 g) was added at room temperature and stirred. The clear solution was cooled to 0 °C with an ice-water bath and methoxymethylchloride (2.39 g) was added drop-wise. After stirring at 0 °C for 15 min, the solution was allowed to warm up to room temperature and then stirred overnight at 40 °C. After TLC analysis showed completion of the reaction, the reaction mixture was poured into water/MTBE mixture and

extracted with MTBE. The organic phase was washed with 1N HCl aq., water, sodium bicarbonate solution, saturated sodium chloride solution, dried over anhydrous magnesium sulfate, concentrated and purified by column chromatography (silica gel 60, toluene : ethanol = 93 : 7, v/v) to yield 282 mg of the titled compound as fine white powder (isolated yield 94%). The structure was checked by means of NMR (^{1}H, ^{13}C).

^{1}H NMR (250 MHz, acetone-d_6)
0.12 (*s*; 48H; Si(C**H**$_3$)$_2$-^{t}Bu); 0.94 (*s*; 72H; SiMe$_2$C(C**H**$_3$)$_3$); 1.18 (*t*, *J* = 7.0 Hz, 24H, -O-CH$_2$-O-CH$_2$C**H**$_3$); 1.19 (*t*, *J* = 7.0 Hz, 24H, -OCH$_2$OCH$_2$C**H**$_3$); 3.40 (*dd*, *J*= 3.3 Hz, 11.0 Hz, 8H, H$_2$); 3.53-3.62 (*m*, 8H, -OCH$_2$OC**H**$_2$CH$_3$); 3.65-3.68 (*m*, 16H, H$_{6a}$ + H$_{6b}$); 3.75-3.86 (*m*, 24H, -OCH$_2$OC**H**$_2$CH$_3$); 3.89-3.97 (*m*, 16H, H$_3$ + H$_4$); 4.36 (*d*, *J* = 11.0 Hz, 8H, H$_5$); 4.79 (*d*, *J* = 7.0 Hz, 8H, -OC**H**$_2$OCH$_2$CH$_3$); 4.82 (*d*, *J* = 7.0 Hz, 8H, -OC**H**$_2$OCH$_2$CH$_3$); 4.91 (*d*, *J* = 7.0 Hz, 8H, -OC**H**$_2$OCH$_2$CH$_3$); 5.01 (*d*, *J* = 7.0 Hz, 8H, -OC**H**$_2$OCH$_2$CH$_3$); 5.35 (*d*, J = 3.3 Hz, 8H, H$_1$).

^{13}C NMR (62.5 MHz, acetone-d_6)
-3.6 (Si(**C**H$_3$)$_2$C(CH$_3$)$_3$), -3.2 (Si(**C**H$_3$)$_2$C(CH$_3$)$_3$), 16.5 (-OCH$_2$OCH$_2$**C**H$_3$), 16.6 (-OCH$_2$OCH$_2$**C**H$_3$), 20.0 (Si(CH$_3$)$_2$**C**(CH$_3$)$_3$), 27.5 (Si(CH$_3$)$_2$C(**C**H$_3$)$_3$), 64.3 (C6), 65.1×2 (-OCH$_2$O**C**H$_2$CH$_3$), 74.0, 79.0, 79.5, 79.9 (C2, C3, C4, C5), 97.5 (-O**C**H$_2$OCH$_2$CH$_3$), 99.3 (-O**C**H$_2$OCH$_2$CH$_3$), 100.7 (C1).

2,3-Di-O-(2-methoxyethoxy)methyl-6-O-tert-butyldimethylsilyl)-γ-cyclodextrin
Octakis(6-*O*-TBDMS)-γ-cyclodextrin was heated at 100 °C under high vacuum (0.001 mmHg) overnight using a bulb-to-bulb distillation apparatus. The obtained dry octakis(6-*O*-TBDMS)-γ-cyclodextrin (311 mg) was dissolved in dry dichloromethane (10 mL). Diisopropylethylamine (1.75 g) was added at room temperature and stirred. The clear solution was cooled to 0 °C with an ice-water bath and (2-methoxyethoxy)methylchloride (1.16 g) was added drop-wise. After stirring at 0 °C for 15 min, the solution was allowed to warm up to room temperature and then stirred for two days at 50 °C. After TLC analysis showed completion of the reaction, the reaction mixture was poured into water/MTBE mixture and extracted with MTBE. The organic phase was washed with 1N HCl aq., water, sodium bicarbonate solution, saturated sodium chloride solution and

dried over anhydrous magnesium sulfate, filtered and concentrated to yield a viscous brown oil. The majority of this crude oil, the degradation product of (2-methoxyethoxy)methylchloride was removed using a bulb-to-bulb distillation apparatus under high vacuum. The residual brown resin was purified by column chromatography (silica gel 60, MTBE : methanol : water = 72 : 8 : 3, v/v) to yield 203 mg of the titled compound as colorless solid (isolated yield 40%). The structure was checked by means of NMR (^{1}H, ^{13}C).

^{1}H NMR (250 MHz, acetone-d_6)
0.15 (*s*; 48H; Si(C**H**$_3$)$_2$-^{t}Bu); 0.98 (*s*; 72H; SiMe$_2$C(C**H**$_3$)$_3$); 3.34 (s, 24H, -OCH$_2$OCH$_2$CH$_2$OC**H**$_3$); 3.36 (s, 24H, -OCH$_2$OCH$_2$CH$_2$OC**H**$_3$); 3.50 (*dd*, *J*= 3.0 Hz, 9.5 Hz, 8H, H$_2$); 3.56 (*t*, *J* = 5.0 Hz, 16H, -OCH$_2$OCH$_2$C**H**$_2$OCH$_3$); 3.57 (*t*, *J* = 5.0 Hz, 16H, -OCH$_2$OCH$_2$C**H**$_2$OCH$_3$); 3.68-3.78 (*m*, 16H, H$_{6a}$ + H$_{6b}$); 3.79-3.88 (*m*, 32H, -OCH$_2$OC**H**$_2$CH$_2$OCH$_3$); 3.89-4.02 (*m*, 16H, H$_3$ + H$_4$); 4.34 (*d*, *J* = 11.5 Hz, 8H, H$_5$); 4.85 (*d*, *J* = 6.6 Hz, 8H, -OC**H**$_2$OCH$_2$CH$_2$OCH$_3$); 4.91 (*d*, *J* = 6.6 Hz, 8H, -OC**H**$_2$OCH$_2$CH$_2$OCH$_3$); 5.36 (*d*, J = 3.3 Hz, 8H, H$_1$).

^{13}C NMR (62.5 MHz, acetone-d_6)
-3.3 (Si(**C**H$_3$)$_2$C(CH$_3$)$_3$), -3.0 (Si(**C**H$_3$)$_2$C(CH$_3$)$_3$), 20.2 (Si(CH$_3$)$_2$**C**(CH$_3$)$_3$), 27.8 (Si(CH$_3$)$_2$C(**C**H$_3$)$_3$), 60.0 (-OCH$_2$OCH$_2$CH$_2$O**C**H$_3$), 60.1 (-OCH$_2$OCH$_2$CH$_2$O**C**H$_3$), 64.7 (C6), 69.6 (-OCH$_2$OCH$_2$**C**H$_2$OCH$_3$), 69.7 (-OCH$_2$OCH$_2$**C**H$_2$OCH$_3$), 74.00 (-OCH$_2$O**C**H$_2$CH$_2$OCH$_3$), 74.04 (-OCH$_2$O**C**H$_2$CH$_2$OCH$_3$), 74.7, 79.54, 74.57. 80.5 (C2, C3, C4, C5), 98.2 (-O**C**H$_2$OCH$_2$CH$_2$OCH$_3$), 100.1 (-O**C**H$_2$OCH$_2$CH$_2$OCH$_3$), 100.2 (C1).

2,3-Di-O-(2-trimethylsilylethoxy)methyl-6-O-tert-butyldimethylsilyl)-γ-cyclodextrin
Octakis(6-*O*-TBDMS)-γ-cyclodextrin was heated at 100 °C under high vacuum (0.001 mmHg) overnight using a bulb-to-bulb distillation apparatus. The obtained dry octakis(6-*O*-TBDMS)-γ-cyclodextrin (196 mg) was dissolved in dry dichloromethane (10 mL). Diisopropylethylamine (2.19 g) was added at room temperature and stirred. The clear solution was cooled to 0 °C with an ice-water bath and (2-trimethylsilylethoxy)methylchloride (1.18 g) was added drop-wise. After stirring at 0 °C for 15 min, the solution was allowed to warm up to room temperature, stirred overnight at room temperature, and further stirred at 50 °C

overnight. After TLC analysis showed completion of the reaction, the reaction mixture was poured into water/MTBE mixture and extracted with MTBE. The organic phase was washed with 1N HCl aq., water, sodium bicarbonate solution, saturated sodium chloride solution and dried over anhydrous magnesium sulfate, filtered and concentrated to yield a viscous yellow oil. The majority of this crude oil, the degradation product of (2-trimethylethoxy)methylchloride was removed using a bulb-to-bulb distillation apparatus under high vacuum. The residual brown resin (397 mg) was purified by column chromatography (silica gel 60, gradient elution; toluene only to toluene : MTBE = 9:1) to yield 355 mg of primary rectified material. This was further purified using 1% (v/v) MTBE in Toluene to yield the titled compound as colorless solid (171 mg, isolated yield 45%). The structure was checked by means of NMR (^{1}H, ^{13}C).

^{1}H NMR (250 MHz, acetone-d_6)
0.07×2 (s, 144H, -$OCH_2OCH_2CH_2Si(C\mathbf{H}_3)_3$); 0.13 (*s*; 48H; $Si(C\mathbf{H}_3)_2$-^{t}Bu); 0.95 (*s*; 72H; $SiMe_2C(C\mathbf{H}_3)_3$); 0.99×2 (s, 32H, -$OCH_2OCH_2C\mathbf{H}_2Si(CH_3)_3$); 3.40 (*d*, *J*= 6.8 Hz, 8H, H_2); 3.52-3.69 (*m*, 16H, H_{6a} + H_{6b}); 3.77-3.95 (*m*, 48H, -$OCH_2OC\mathbf{H}_2CH_2Si(CH_3)_3$ + H_3 + H_4); 4.56 (*d*, *J* = 11.5 Hz, 8H, H_5); 4.75 (*d*, *J* = 6.5 Hz, 8H, -$OC\mathbf{H}_2OCH_2CH_2Si(CH_3)_3$); 4.86 (*d*, *J* = 6.5 Hz, 8H, -$OC\mathbf{H}_2OCH_2CH_2Si(CH_3)_3$); 4.90 (*d*, *J* = 6.5 Hz, 8H, -$OC\mathbf{H}_2OCH_2CH_2Si(CH_3)_3$); 5.03 (*d*, *J* = 6.5 Hz, 8H, -$OC\mathbf{H}_2OCH_2CH_2Si(CH_3)_3$); 5.33 (*d*, J = 3.3 Hz, 8H, H_1).

^{13}C NMR (62.5 MHz, acetone-d_6)
-3.5 ($Si(\mathbf{C}H_3)_2C(CH_3)_3$), -3.0 ($Si(\mathbf{C}H_3)_2C(CH_3)_3$), 0.1 (-$OCH_2OCH_2CH_2Si(\mathbf{C}H_3)_3$), 0.2 (-$OCH_2OCH_2CH_2Si(\mathbf{C}H_3)_3$), 19.5 ($SiMe_2\mathbf{C}(CH_3)_3$), 19.97 (-$OCH_2OCH_2$-$\mathbf{C}H_2Si(CH_3)_3$), 20.04 (-$OCH_2OCH_2\mathbf{C}H_2Si(CH_3)_3$), 64.3 (C6), 66.8 (-$OCH_2O\mathbf{C}H_2$-$CH_2Si(CH_3)_3$), 67.0 (-$OCH_2O\mathbf{C}H_2CH_2Si(CH_3)_3$), 74.2, 78.6, 79.6, 80.7 (C2, C3, C4, C5), 97.0 (-$O\mathbf{C}H_2OCH_2CH_2Si(CH_3)_3$), 99.3 (-$O\mathbf{C}H_2OCH_2CH_2Si(CH_3)_3$), 101.3 (C1).

2-Methylbutanoic acid anhydrides

Synthesis of 2-methylbutanoic acid anhydride was accomplished by condensation of 2-methylbutyryl chloride and 2-methylbutanoic acid, in analogy

to the procedure described for heptanoic acid anhydride (Allen et al., 1955). The enantiomeric excesses of the (*S*)- and (*R*)-2-methylbutanoic acid were 99% and 97%, respectively (checked by using a column [i.d. 0.25 mm, length 30m] coated with 2,3-MOM-6-TBDMS-α-CD dissolved in OV-1701vi [film thickness: 0.25 μm]). The structures of the anhydrides were confirmed via ^{1}H and ^{13}C NMR.

(S)-2-Methylbutanoic acid anhydride

(Step 1) A 50 mL one neck flask equipped with a magnetic stirring bar and a 50 mL dropping funnel (with a pressure-equalizing side tubing) was thoroughly dried under high vacuum and cooled in a desiccator. A drying tube stuffed with anhydrous calcium chloride (granulated) and a glass wool stopper were placed on top of the dropping funnel. After addition of thionyl chloride (21.4 g), (S)-2-methylbutanoic acid (15.3 g) was added drop-wise at room temperature (note: endothermic reaction) and stirred for additional 2 hrs. Then the dropping funnel was replaced by a reflux condenser and the reaction mixture was refluxed at 100 °C for 2 hrs. The resulting pale yellow oil was distilled under slightly reduced pressure (ca 150 mmHg) and fractionated to yield (*S*)-2-methylbutyryl chloride as colorless oil (16.72 g, 92%).

(Step 2) A 200 mL flask equipped with a magnetic stirring bar and a 50 mL dropping funnel (with a pressure-equalizing side tubing) was thoroughly dried under high vacuum and cooled in a desiccator. Toluene (previously dried over molecular sieves 4Å overnight, 35 mL) and pyridine (21.6 g) were placed into the flask and the mixture was stirred. (*S*)-2-methylbutyryl chloride (16.5 g) was placed into the dropping funnel and a drying tube as described in step 1 was fitted. (*S*)-2-Methylbutyryl chloride was added drop-wise into the solution of pyridine and toluene the under efficient stirring to yield a cloudy yellow suspension. After the addition was complete, (*S*)-2-methylbutanoic acid was added drop-wise over a 50 min time period under vigorous stirring and efficient cooling so that the reaction temperature would not exceed 40 °C. After the addition was complete, the ice bath was removed and the reaction mixture was stirred for additional 30 min at room temperature. The brown slurry obtained was rapidly suction-filtered using a Kiriyama funnel and the precipitate was rinsed with dry toluene. The filtrate (ca 125 mL) was concentrated under vacuum using a rotary evaporator and 23.6 g of the crude anhydride was obtained. Upon

distillation under high vacuum (42 °C / 0.03 mmHg), 21.7 g of (S)-2-methylbutyric acid anhydride was obtained as clear oil with a warm, fruity scent. The structure of the prepared material was checked using ^{1}H and ^{13}C NMR.

(R)-2-Methylbutanoic acid anhydride

A 10 mL flask and a type 29/32 glass taper seal joint were thoroughly dried using a heat-gun under high vacuum and cooled in a desiccator. The joint was stuffed with granulated anhydrous calcium chloride to substitute a drying tube. 480 mg of thionyl chloride was placed into the flask and (*R*)-2-methylbutyric acid (613 mg) was added drop-wise under sufficient shaking. The resulting clear solution was stirred at room temperature for 7 hours: the reaction progress was monitored by checking the remaining acid via NMR using dry chloroform-d_3 as solvent. After the reaction was complete, the composition of the crude mixture was analyzed using NMR. A mixture of dry toluene (1.4 g) and dry pyridine (718 mg) was added, resulting in a turbid yellow solution. (*R*)-2-methylbutanoic acid (458 mg) was slowly added to this solution and heated at 60 °C for 1 hour to yield a white waxy paste, which was diluted in dry toluene, filtered through a pad of glass wool stuffed in a pipette and concentrated under reduced pressure using a rotary evaporator. Final residues of solvent were removed under high vacuum at room temperature. NMR analysis revealed that the desired (*R*)-2-methylbutyric acid anhydride amounted to approximately 89% (w/w) of the material obtained (yield: 93% yield).

Preparation of 2-methylbutyryl type cyclodextrin phases

2,3-Di-O-[(S)-2-methylbutyryl-6-O-tert-butyldimethylsilyl]-γ-cyclodextrin

Octakis-(6-TBDMS)-γ-cyclodextrin was heated at 100 °C under high vacuum (0.001 mmHg) overnight using a bulb-to-bulb distillation apparatus. The obtained dry heptakis-(6-TBDMS)-γ-cyclodextrin (383 mg) was dissolved in dry triethylamine (8.5 g). 4-Dimethylaminopyridine (178 mg) was added at room temperature and stirred. The clear solution was cooled to 0 °C with an ice-water bath and (*S*)-2-methylbutyryic anhydride (1773 mg) was added drop-wise. After stirring at 0 °C for 15 min, the solution was stirred at room

temperature for 3 days, until TLC analysis showed completion of the reaction. The reaction mixture was poured into a water/MTBE mixture, extracted with MTBE, dried over anhydrous magnesium sulfate, concentrated and purified by column chromatography (silica gel Merck 60; polarity gradient elution; hexane only – hexane : ethyl acetate = 10:1) to yield 283 mg of the titled compound as fine white powder (yield: 46%). The structure was confirmed by means of NMR and MS (data given in Chapter 4.5).

2,3-Di-O-[(R)-2-methylbutyryl-6-O-tert-butyldimethylsilyl]-γ-cyclodextrin

Octakis-(6-TBDMS)-γ-Cyclodextrin was heated at 100 °C under high vacuum (0.001 mmHg) overnight using a bulb-to-bulb distillation apparatus. The obtained dry Heptakis-(6-TBDMS)-γ-Cyclodextrin (193 mg) was dissolved in dry pyridine (4.05 g). 4-Dimethylaminopyridine (188 mg) and imidazole (105 mg) were added at room temperature and stirred. (*R*)-2-methylbutyryic anhydride (781 mg) was added to the white suspension and stirred overnight at 60 °C. After TLC analysis showed completion of the reaction, the mixture was poured into water/MTBE mixture and extracted with MTBE. The organic phase was rinsed with 0.5 N HCl aq (40 mL), water (10 mL), saturated sodium bicarbonate solution (30 mL), dried over anhydrous magnesium sulfate, concentrated and purified by column chromatography (silica gel Merck 60, polarity gradation eluting; hexane : ethyl acetate = 100:1 – 17:1) to yield 165 mg (yield 53%) of the titled compound as fine white powder. The structure was confirmed by means of NMR and MS (data given in Chapter 4.5).

Methyl branched secondary alcohols

3-Methyl-2-pentanol and 3,3,5-trimethylcyclohexanol were prepared by reduction of the corresponding ketones using sodium borohydride. The syn/anti ratios of the resulting alcohols were determined on the basis NMR data obtained from the National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Japan (SDBS, URL: http://www.aist.go.jp/RIODB/SDBS/). Alcohols were analyzed in presence of D_2O to enhance resolution.

3-Methyl-2-pentanol

3-Methyl-2-pentanone (7.34 g) and methanol (50 mL) were stirred in a 250 mL

flask. Sodium borohydride (1.74 g) was added portion-wise while maintaining the reaction temperature below room temperature with an ice-water bath. After the addition of sodium borohydride was complete, the ice-water bath was removed, the reaction mixture was allowed to come up to room temperature and was stirred overnight. After completion of the reaction (confirmed by GC analysis), 1N HCl was added slowly to the reaction mixture under water bath-cooling. The reaction mixture was then concentrated to approximately 20 mL utilizing a rotary evaporator and the residue was taken up in MTBE (50 mL). The organic phase was washed with 1N HCl (20 mL), saturated sodium bicarbonate solution and saturated sodium chloride solution, and then dried over anhydrous magnesium sulfate filtered and carefully concentrated using a rotary evaporator to yield a pale yellow oil as crude product (6.5 g). This was further purified using a bulb-to-bulb distillation apparatus to yield a clear oil product (2.82 g, isolated yield 38%)

3,3,5-Trimethylcyclohexanol

Sodium borohydride (1.7 g) was added portion-wise over 20 min to a solution of 3,3,5-trimethylcyclohexanone (10 g) in methanol (50 mL) in a 250 mL flask under efficient stirring. The reaction temperature was kept below 40 °C by water bath-cooling. After the addition of sodium borohydride was complete, the reaction was continued for additional 30 min and then quenched carefully by addition of 1N HCl aq. (10 mL). The reaction mixture was concentrated to approximately 20 mL using a rotary evaporator and then taken up in MTBE (100 mL). The organic phase was washed successively with water (50 mL), 1 N HCl (50 mL), water (50 mL), 5% sodium bicarbonate solution and saturated sodium chloride solution, dried with anhydrous magnesium sulfate, suction-filtered and concentrated. Additional purification using bulb-to-bulb distillation apparatus yielded 3,3,5-trimethylcyclohexanol as colorless oil (7.83 g, 77% isolated yield).

2-Alkyl esters

Syntheses of 2-alkyl esters were carried out starting from the corresponding acyl chlorides using 4-dimethylaminopyridine as catalyst. The structures of the synthesized materials were confirmed using ^{1}H and ^{13}C NMR.

2-Heptyl acetate

2-Heptanol (2.32 g) was dissolved in pyridine (15 mL) in a 50 mL flask. 4-Dimethylaminopyridine (250mg) was added at room temperature using ultrasonic until clear solution was obtained. The flask was immersed in an ice-water bath and acetyl chloride (1.73 g) was added drop-wise over a period of 20 minutes. After the addition was complete, the reaction mixture (yellow suspension) was allowed to come up to room temperature and stirred for additional 3 hrs. Methanol was slowly added to quench excessive amounts of acetyl chloride and the mixture was stirred for 30 min. Then the reaction mixture was poured into a stirred suspension of MTBE and water (60 mL each). The organic layer was separated and washed with 2N HCl (30 mL, twice), water (30 mL), saturated sodium bicarbonate solution and saturated sodium chloride solution, dried over anhydrous magnesium sulfate, filtered and concentrated under reduced pressure to 2.91 g of a pale yellow oil. This was distilled (120 °C / 17 mmHg) to yield the pure product as colorless oil (2.18 g, 69% isolated yield).

Other 2-alkyl esters were synthesized according to a similar procedure as described for 2-heptyl acetate. The isolated yields of the products were as follows: 2-nonyl acetate (83%), 2-pentyl butanoate (73%), 2-heptyl butanoate (87%), 2-nonyl butanoate (82%), 2-pentyl hexanoate (77%), 2-heptyl hexanoate (82%), 2-nonyl hexanoate (91%).

4-Methylhexanol

Preparation of 4-methyhexanol was accomplished by hydroboration and subsequent oxidation of 4-methyl-1-hexene following the procedure given elsewhere (Dregus et al., 2003).

2-Methylbutyl esters and n-propyl 2-methylbutanoate

The compounds were prepared in analogy to the synthesis of the 2-heptyl acetates. (*rac*)-2-Methylbutyryl chloride used for the synthesis of *n*-propyl-2-methylbutanoate was prepared in a same manner as described for (S)-2-methylbutyryl chloride (colorless oil, isolated yield 97%). The isolated yields were: 2-methylbutyl acetate (15 %), 2-methylbutyl butanoate (54 %) and n-propyl 2-methylbutanoate (84%).

3.4. Preparation of the capillary columns

The cyclodextrin derivative synthesized was diluted in polysiloxane OV-1701vi (0.11 mol/kg) and used as GC stationary phase. Untreated fused-silica capillary column (i.d. 0.25 mm, length 30 m) was rinsed with 2% HCl aq, dried statically under vacuum at 240 °C (2hrs), dried dynamically at 240 °C (2 hrs) and deactivated using phenyldimethylsilane at 380 °C (reaction time: 10 hrs). Residual silicone waste material was removed by consecutive rinsing with toluene, methanol and diethyl ether. The deactivated fused-silica column was coated with the above-described phase by means of the static coating method according to Grob (Grob, 1986). A mixture of *n*-pentane and dichloromethane (1:1, v/v) was used as solvent in the coating procedure. The column was coated in stationary phase thickness of 0.25 μm. After coating was completed, the column was mounted on a GC oven and conditioned as follows: 40 °C (initial temperature, 15 min hold), then ramp at rate of 2 °C/min to 210 °C (final temperature, held for 4 hrs). The column thus prepared was tested by injecting 1 μL of Grob-I test mixture (Grob, 1986).

3.5 Test of the stability of the stationary phase

Diethyl ether (30 mL) was shaken thoroughly (5 min) with water (20 mL) in a separation funnel and the aqueous layer was discarded. One μL of the water-saturated diethyl ether was injected at 5 minutes intervals into the GC column (140 °C isothermal) coated with 2,3-MOM-6-TBDMS-γ-CD as stationary phase (total: 3225 injections). At the beginning of the experiment and after every 100 injections, the performance of the column was checked by injecting 1 μL of Grob test mixture I as well as 1 μL of a diethyl ether solution containing 2-pentanol, 2-pentanthiol, limonene, 2-methylbutyl acetate, 2-methylbutanal diethylacetal, 5-methyl-3-heptanone, 1-phenylethanol, 2-methylhexanoic acid and γ-hexalactone (0.2 μL/mL each).

4 Results and Discussion

4.1 2,3-*O*-MOM-6-*O*-TBDMS-γ-cyclodextrin

4.1.1 Synthesis

Octakis(2,3-di-*O*-methoxymethyl-6-*O*-*tert*-butyldimethylsilyl)-γ-cyclodextrin (2,3-MOM-6-TBDMS-γ-CD) was synthesized by reaction of octakis (6-*O*-*tert*-butyldimethylsilyl)-γ-cyclodextrin with methoxymethylchloride (MOM-Cl) as shown in Figure 4.1.1.

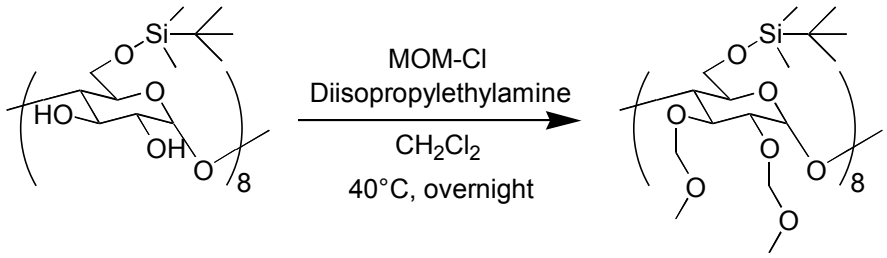


Figure 4.1.1 Synthesis of 2,3-MOM-6-TBDMS-γ-CD

Various silyl groups have been shown to be suitable to block the 6-position of CDs; one successful example is the *t*-hexyldimethylsilyl (THDMS) moiety. However the experiences gained with this group are limited to a rather small spectrum of substituents (methyl, ethyl and acetyl) at the 2,3-positions of the glucose units (Kim et al., 1997a; Kim et al., 1997b; Bicchi et al., 1999; Bicchi et al., 2002; Bicchi et al., 2003). On the other hand, the general versatility of the *t*-butyldimethylsilyl (TBDMS) group has been demonstrated in combination with a much wider spectrum of substituents at positions 2,3 and was therefore selected in the present approach.

The use of mixed acetals obtained by proton-catalyzed addition of 2-methoxypropene for protection of hydroxy groups in cyclodextrins has recently been described (Liptak et al., 2002). In this approach methoxymethylchloride was applied as acetalization reagent to introduce the methoxymethyl (MOM) moiety at the 2,3-hydroxyl rim of γ-cyclodextrin. The MOM group is widely used in organic chemistry for protection of alcohols. The introduction of this moiety using diisopropylethylamine as proton scavenger is well documented (Greene and Wuts, 1999). The reaction proceeded efficiently under homogeneous conditions, did not require extensive purification to remove by-products, and resulted in sufficient and reproducible yield (see chapter 3.3).

4.1.2 Structural characterization

^{1}H NMR and ^{13}C NMR as well as MS data were used to confirm the structure of 2,3-MOM-6-TBDMS-γ-CD. The NMR signals (Table 4.1.2) represent a set of resonance which corresponds to one glucose unit, indicating the eight-fold symmetry of the prepared material. Starting from the proton signal (H_1) at 5.27 ppm, it was possible to trace the coupling constants and proton integrations using conventional ^{1}H NMR; these data were confirmed by dqf-COSY experiments. Due to the influence of the chiral centers at C2 and C3 of the glucose units, geminal coupling was observed for the methylene protons of the newly introduced methoxymethyl groups (-OC**H**$_2$OCH$_3$). The chemical shifts and the coupling constants are in accordance with values previously reported for other MOM-protected chiral secondary alcohols (Friesen and Vanderwal, 1996). The ^{13}C NMR spectrum (Table 4.1.2) also confirmed the eight-fold symmetry of the synthesized cyclodextrin derivative. The resonance patterns were in accordance with the postulated structure. DEPT measurements were performed to differentiate the three peaks crowded around 100 ppm (99.5+100.6 (-O**C**H$_2$OCH$_3$), 100.7 (C1)). The resonance of C2-C5 could not be distinguished because the molar concentration required for CH COSY experiments could not be achieved.

Table 4.1.2 ^{1}H NMR and ^{13}C NMR data for 2,3-MOM-6-TBDMS-γ-CD

^{1}H NMR
0.02 (*s*; 48H; Si(C**H**$_3$)$_2$); 0.87 (*s*; 72H; Si(C**H**$_3$)$_3$); 3.36 (*dd*; *J* = 2.5 Hz, 8.5 Hz; 8H; H_2); 3.39 (s; 24H; -OCH$_2$OC**H**$_3$); 3.44 (s; 24H; -OCH$_2$OC**H**$_3$); 3.64 (*d*; *J* = 10.0 Hz; 16H; H_{6a+6b}); 3.87 (*t*; *J* = 8.5 Hz; 8H; H_4); 3.94 (*t*; *J* = 8.5 Hz; 8H; H_3); 4.25 (*d*; *J* = 11.3 Hz; 8H; H_5); 4.69 (*d*; *J* = 6.3 Hz; 8H; -OC**H**$_2$OCH$_3$); 4.79 (*d*; *J* = 6.5 Hz; 8H; -OC**H**$_2$OCH$_3$); 4.82 (*d*; *J* = 6.5 Hz; 8H; -OC**H**$_2$OCH$_3$); 4.99 (*d*; *J* = 6.5 Hz; 8H; -OC**H**$_2$OCH$_3$); 5.27 (*d*; *J* = 2.5 Hz; 8H; H_1).
^{13}C NMR
-3.6 (Si(**C**H$_3$)$_2$C(CH$_3$)$_3$), -3.2 (Si(**C**H$_3$)$_2$C(CH$_3$)$_3$), 20.0 (Si(CH$_3$)$_2$**C**(CH$_3$)$_3$), 27.5 (Si(CH$_3$)$_2$C(**C**H$_3$)$_3$), 57.0 (-OCH$_2$O**C**H$_3$), 57.2 (-OCH$_2$O**C**H$_3$), 64.3 (C6), 74.0, 78.6, 79.7, 80.1 (C2, C3, C4, C5), 99.5+100.6 (-O**C**H$_2$OCH$_3$), 100.7 (C1).

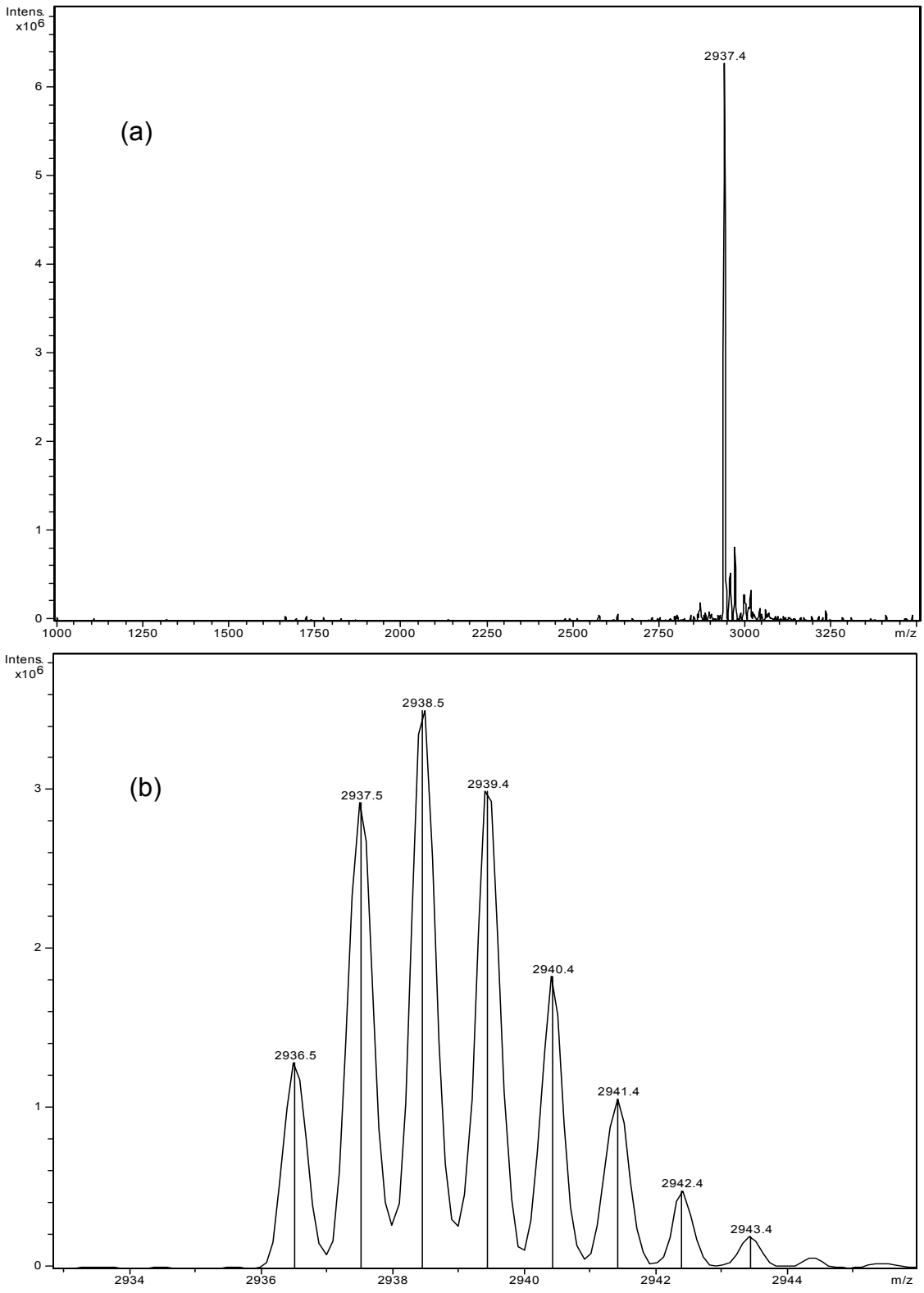


Figure 4.1.2 MS spectra of 2,3-MOM-6-TBDMS-γ-CD:

(a) wide range scan from m/z 1000 to 3500

(b) detailed scan of M+Na+H peak showing isotopic distribution

(for conditions see Materials and Methods)

To get additional confirmation of a complete derivatization of the cyclodextrin molecule, direct MS analysis utilizing Electrospray Ionization (ESI) was conducted. Despite the relatively harsh ionization method, it was possible to detect an adduct of the molecular ion (Fig. 4.1.2a); more detailed scanning (Fig. 4.1.2b) revealed a major mass of m/z = 2938.5 [M+Na+H], which is in accordance with the calculated isotopic distribution. This indicates that electrospray ionization did not result in a cleavage of the methoxymethyl moiety and confirms the stability of this type of CD derivative.

4.1.3 Coating and general performance

The polarity of the newly synthesized CD phase was estimated on the basis of its TLC behavior. Using toluene/ethanol, 90/10 (v/v) as developing solution, the rf value (0.41) determined for 2,3-MOM-6-TBDMS-γ-CD was in the same order of magnitude as that determined for 2,3-di-*O*-acetyl-6-*O*-TBDMS-γ-CD (0.37). Taking into account the reported influence of the polarity of the diluting stationary phase on column efficiency (Kim et al., 1997c), OV-1701vi was selected as polysiloxane solvent. Dissolving CDs in this moderately polar polysiloxane has already been described in 1988 (Schurig and Nowotny, 1988).

The column was prepared by coating a fused silica capillary with 33% 2,3-MOM-6-TBDMS-γ-CD in OV-1701vi (film thickness: 0.25 μm). Its general performance was tested using the Grob test mixture I (Figure 4.1.3).

The column showed very good performance for all compound classes, except for the acid contained in the mixture. A decreased peak height and tailing was observed for 2-ethylhexanoic acid under the chromatographic conditions of this test. Nevertheless, the use of more suitable parameters (e.g. isothermal runs) allowed the enantioseparation of free acids on this chiral stationary phase (see examples in Table 4.1.5.1).

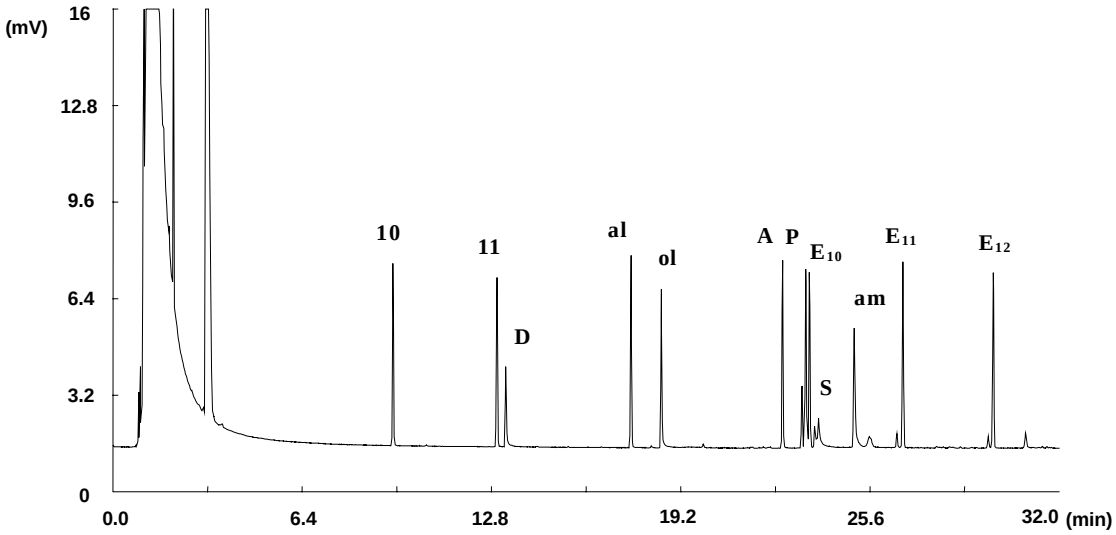


Figure 4.1.3 Grob test chromatogram of a 2,3-MOM-6-TBDMS-γ-CD (0.11 mol/kg OV-1701vi) column. Temperature programming: 40 °C initial (2 min hold) then ramp at 4.0 °C/min rate.
10: *n*-decane; 11: undecane; D: (-)-2,3-butanediol; al: 1-nonanal; ol: 1-octanol; A: 2,6-dimethylaniline; P: 2,6-dimethylphenol; E10: methyl decanoate; S: 2-ethylhexanoic acid; am: dicyclohexyl-amine; E11: methyl undecanoate; E12: methyl dodecanoate.

4.1.4 Stability

Repeated heating of the column up to 230 °C, keeping the column temperature at 220 °C for a period of over 12 hours as well as repeated injection of solutions of free alkanoic acids did not affect the column performance. In an additional stability test, water-saturated diethyl ether was injected at 5 minutes intervals (total: 3225 injections). At the beginning of the experiment and after every 100 injections, the performance of the column was checked by injecting Grob test mixture as well as a mixture containing chiral representatives of different compound classes. The repeated injection of water-saturated diethyl ether resulted in significantly reduced peak heights for the two acids tested (2-ethylhexanoic acid and 2-methylhexanoic acid). For all other compounds contained in the Grob test mixture and in the test mixture of chiral compounds the performance of the column in terms of retention times, peak heights and separation factors was not affected. Considering the structure of the side-chain as a mixed acetal of formaldehyde, this stability of the column was rather unexpected. It may be explained by the stabilizing effect of the diluting

polysiloxane (Miranda et al., 1998).

4.1.5 Characteristics of enantioseparation

The potential of 2,3-MOM-6-TBDMS-γ-CD to separate enantiomers was tested using a broad spectrum of chiral compounds from different classes most of them being used as flavoring and fragrance materials. A total of 125 compounds were investigated. The separation factors α, the resolutions R_s and the retention factors *k* are listed in Tables 4.1.5.1 - 4.1.5.6.

Methyl branched compounds

As demonstrated for 2-methyl branched compounds (Table 4.1.5.1), the use of 2,3-MOM-6-TBDMS-γ-CD as chiral stationary phase is suitable for enantiodifferentiation of volatiles containing various functional groups (alcohol, aldehyde, ketone, acid, ester and acetal). The good enantioseparations observed for the esters of 2-methyl branched acids (with an optimum resolution for ethyl 2-methylbutanoate) are remarkable, because esters have been reported to be more poorly separated than the corresponding alcohol compounds on TBDMS-type cyclodextrin stationary phases (Maas et al., 1996). Beside 2-methylbutyrates, the esters of 2-methylbutanol such as 2-methylbutyl acetate and 2-methylbutyl butanoate could be baseline-separated into their enantiomers. The latter compound is known to be resolved rather difficultly into enantiomers on TBDMS-CD-type phases (Beck et al., 2000a).

A separation factor of 1.69 was observed for 5-methyl-2-hepten-4-one, the so-called filbertone, a key aroma compound found in hazelnuts (Guentert et al., 1990). The α value obtained for 3-methyl-2-pentanone (1.65) is in the same order of magnitude. A replacement of the ketone function in this compound by an aldehyde moiety (2-methylbutanal) resulted in a drastic reduction of the separation efficiency. Comparably, the separation factor was decreased significantly by reduction of the ketone to the corresponding secondary alcohol 3-methyl-2-pentanol (Table 4.1.5.2).

An exceptionally high separation factor of 1.60 was also determined for the

cyclic ketone 3,3,5-trimethylcyclohexanone. The essential role of the carbonyl group for enantiodifferentiation was confirmed by the lowered separation factors determined for 3,3,5-trimethylcyclohexanol (Table 4.1.5.2) obtained by reduction of 3,3,5-trimethylcyclohexanone. When comparing the corresponding chromatograms (Figure 4.1.5.1) it is noteworthy that the second eluted enantiomer of the ketone (Figure 4.1.5.1a) is retained stronger than any of the four alcohol stereoisomers (Figure 4.1.5.1b), indicating the high affinity of the ketone enantiomer towards the CSP.

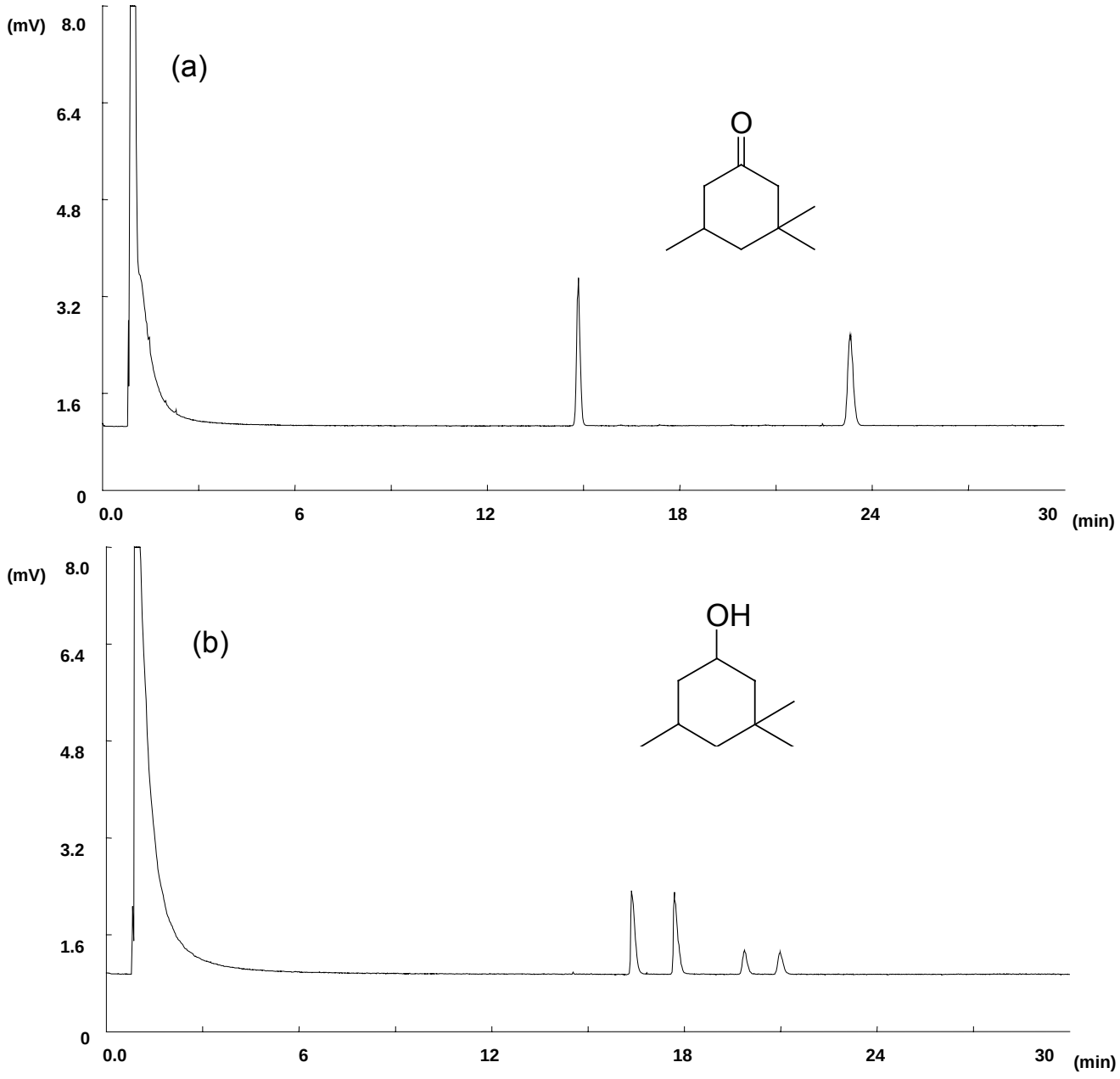
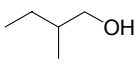
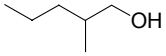
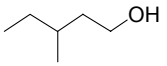
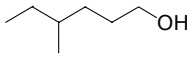
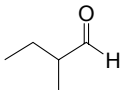
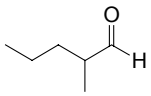
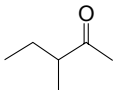
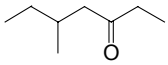
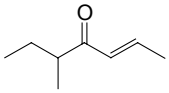
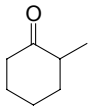
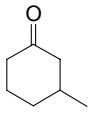
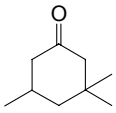
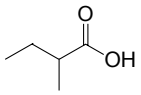
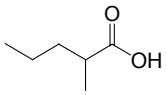
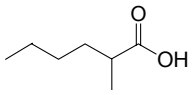
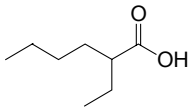
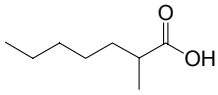
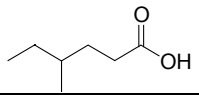
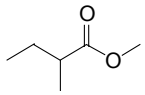
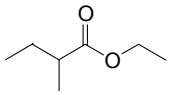
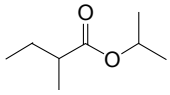
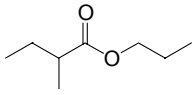
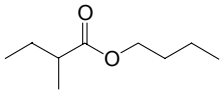
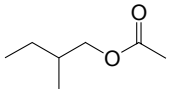
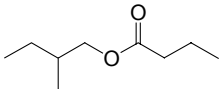
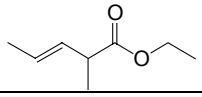
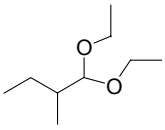


Figure 4.1.5.1 Separation of (a) 3,3,5-trimethylcyclohexanone and (b) 3,3,5-trimethylcyclohexanol on 2,3-MOM-6-TBDMS-γ-CD (70 °C).

Table 4.1.5.1 Separation of the enantiomers of methyl branched compounds

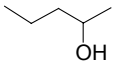
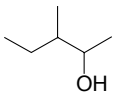
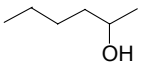
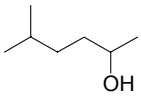
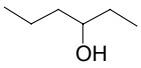
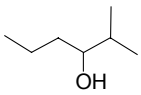
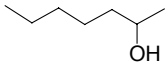
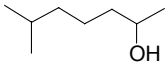
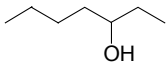
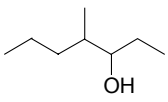
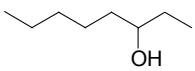
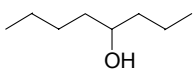
		T (°C)	k	α	R_S
alcohols					
2-Methylbutanol		40	11.74	1.02	1.17
2-Methylpentanol		55	13.33	1.07	3.88
3-Methylpentanol		50	21.68	1.19	11.43
4-Methylhexanol		65	21.08	1.03	1.69
aldehydes					
2-Methylbutanal		40	3.33	1.05	1.84
2-Methylpentanal		40	13.06	1.12	6.20
ketones					
3-Methyl-2-pentanone		40	7.89	1.65	25.89
5-Methyl-3-heptanone		65	13.77	1.29	17.15
5-Methyl-2-hepten-4-one		70	13.56	1.69	37.42
2-Methylcyclohexanone		60	15.16	1.12	10.11
3-Methylcyclohexanone		60	16.83	1.02	1.37
3,3,5-Trimethylcyclohexanone		70	18.02	1.60	33.21
2-Methylcyclopentanone		40	20.27	1.11	6.53

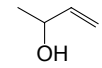
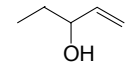
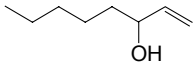
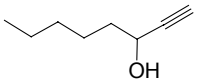
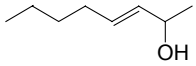
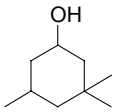
		T (°C)	k	α	R_S
acids					
2-Methylbutanoic acid		70	17.93	1.01	0.94
2-Methylpentanoic acid		80	22.60	1.08	6.29
2-Methylhexanoic acid		90	25.88	1.09	6.47
2-Ethylhexanoic acid		100	20.40	1.04	2.52
2-Methylheptanoic acid		100	27.69	1.05	4.70
4-Methylhexanoic acid		110	11.73	1.05	4.27
esters					
Methyl 2-methylbutanoate		40	7.43	1.12	6.07
Ethyl 2-methylbutanoate		40	16.31	1.17	10.1
iso-Propyl 2-methylbutanoate		40	19.08	1.11	7.05
Propyl 2-methylbutanoate		50	18.25	1.10	6.17
Butyl 2-methylbutanoate		60	20.44	1.04	2.97
2-Methylbutyl acetate		40	21.06	1.07	4.59
2-Methylbutyl butanoate		65	17.31	1.03	2.28
Ethyl 2-methyl-3-pentenoate		50	19.53	1.06	3.94
acetal					
2-Methylbutanal diethyl acetal		65	6.07	1.07	3.57

Secondary alcohols

For secondary alcohols (Table 4.1.5.2) a comparison of the α values for 2-hexanol/3-hexanol and 2-heptanol/3-heptanol demonstrates that there is no consistent influence of the position of the hydroxy group on the separation efficiency. As shown for 3-octanol, 1-octen-3-ol and 1-octyn-3-ol, the insertion of a double bond improves the separation whereas a triple bond has a negative impact on the separation of enantiomers. An analogous effect has been observed for the C4 homologues: 3-buten-2-ol is separated better than 2-butanol whereas no separation could be achieved for 3-butyn-2-ol (not listed in the Table). The α-values obtained for 2-hexanol/5-methyl-2-hexanol and 2-heptanol/6-methyl-2-heptanol demonstrate that the insertion of a methyl group at a position distant from the chiral center bearing the hydroxy group results in a decrease of the separation efficiency. On the other hand insertion of a methyl group in adjacent position to the chiral center (2-butanol/3-methyl-2-butanol and 3-hexanol/2-methyl-3-hexanol) improves the separation. If the presence of such an adjacent methyl group results in an additional chiral center (2-pentanol/3-methyl-2-pentanol and 3-heptanol/4-methyl-3-heptanol), the pair of *trans* enantiomers was resolved better.

Table 4.1.5.2 Separation of the enantiomers of secondary alcohols

		T (°C)	*k*	α	R_S
2-Butanol[(a)]		40	3.79	1.05	2.11
3-Methyl-2-butanol		30	8.90	1.08	4.59
2-Pentanol		40	11.70	1.10	5.63
threo-3-Methyl-2-pentanol		40	16.40	1.07	3.89
erythro-3-Methyl-2-pentanol		40	16.91	1.21	11.0
2-Hexanol		50	17.06	1.11	7.31
5-Methyl-2-hexanol		60	10.69	1.08	5.31
3-Hexanol		50	13.53	1.06	3.47
2-Methyl-3-hexanol		60	9.85	1.12	7.05
2-Heptanol		60	22.76	1.05	3.96
6-Methyl-2-heptanol		65	16.47	1.02	1.68
3-Heptanol		60	13.49	1.08	5.02
erythro-4-Methyl-3-heptanol		70	13.22	1.39	22.10
threo-4-Methyl-3-heptanol		70	14.36	1.03	1.77
3-Octanol		70	17.78	1.04	2.90
4-Octanol		70	16.27	1.04	2.62

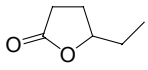
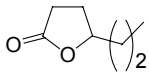
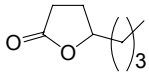
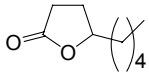
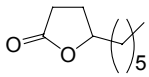
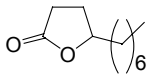
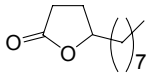
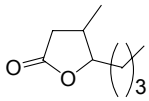
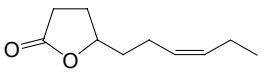
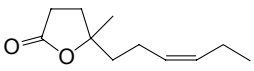
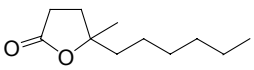
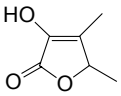
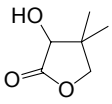
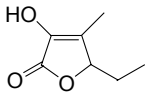
		T (°C)	k	α	R_S
3-Buten-2-ol[(a)]		30	6.92	1.10	4.92
1-Penten-3-ol		40	10.65	1.09	4.81
1-Octen-3-ol		70	16.71	1.09	6.05
1-Octyn-3-ol		80	18.13	1.03	2.24
3-Octen-2-ol		70	22.83	1.04	3.43
trans-3,3,5-Trimethyl-cyclohexanol		70	19.96	1.09	5.66
cis-3,3,5-Trimethyl-cyclohexanol		70	24.47	1.06	3.97

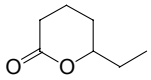
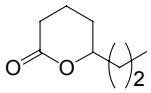
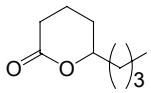
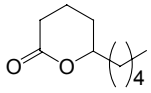
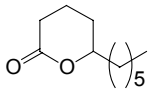
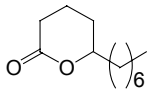
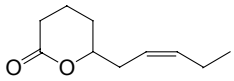
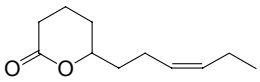
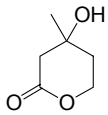
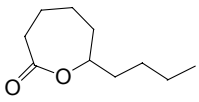
(a) Analysis performed at 50 kPa inlet pressure.

Lactones

The enantiomers of the homologous series of both γ-lactones and δ-lactones, important flavor compounds, could be separated (Table 4.1.5.3). Optimum resolutions were obtained for the homologues C6 and C7 (γ-lactones) and C8 (δ-lactones). Representatives containing alkyl chains of 2 to 3 carbons attached to the ring are preferentially resolved. The enantiomers of a lactone with a larger ring system (ε-decalactone) and of γ-lactones exhibiting a branched ring structure (e.g. whiskey lactones) or other additional functional groups (e.g. sotolone and 5-ethyl-3-hydroxy-4-methyl-2(5H)-furanone) were also well separated. The α values obtained for γ-octalactone and the whiskey lactones demonstrate that the creation of an additional chiral center by a methyl substituent adjacent to the carbon bearing the alkyl chain results in improved separation of one pair of enantiomers (trans-whiskey lactone) and a worse separation for the other pair (cis-whiskey lactone), comparable to the data described for the secondary alcohols (Table 4.1.5.2).

Table 4.1.5.3 Separation of the enantiomers of lactones

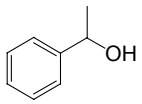
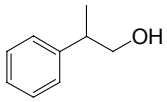
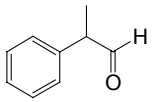
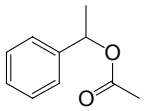
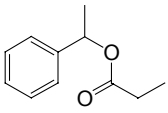
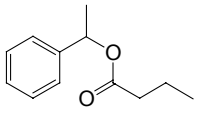
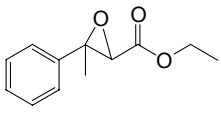
		T (°C)	*k*	α	R_S
γ-Lactones					
gamma-Hexalactone		120	6.28	1.15	9.59
gamma-Heptalactone		130	6.31	1.14	9.16
gamma-Octalactone		140	6.17	1.07	5.13
gamma-Nonalactone		150	6.60	1.03	2.38
gamma-Decalactone		160	7.07	1.02	1.26
gamma-Undecalactone		170	7.72	1.01	1.00
gamma-Dodecalactone		170	11.85	1.01	1.13
trans- Whiskey lactone *cis*-		130 130	8.97 11.08	1.20 1.04	13.80 3.12
(*Z*)-Dec-7-en-4-olide		150	15.93	1.03	1.99
4-Methyl-(*Z*)-dec-7-en-4-olide		145	11.24	1.04	2.97
4-Methyl-4-decanolide		140	14.26	1.06	4.96
Sotolone		120	11.01	1.15	9.35
Pantolactone		110	10.72	1.03	2.00
5-Ethyl-3-hydroxy-4-methyl-2(5H)-furanone		130	10.86	1.28	16.91

δ-lactones					
delta-Heptalactone		120	11.94	1.04	2.86
delta-Octalactone		130	10.93	1.13	8.91
delta-Nonalactone		140	10.72	1.05	3.74
delta-Decalactone		150	11.02	1.02	1.69
delta-Undecalactone		150	17.50	1.02	1.77
delta-Dodecalactone		150	27.89	1.02	1.90
(*Z*)-Dec-7-en-5-olide		140	17.62	1.04	3.04
(*Z*)-Undec-7-en-5-olide		150	16.09	1.03	1.95
Mevalonic acid lactone		140	21.82	1.02	1.78
ε-lactone					
epsilon-decalactone		150	8.33	1.11	7.99

Aromatic compounds

A broad spectrum of aromatic compound classes including the ethyl methylphenylglycidates could be separated (Table 4.1.5.4). For the 1-phenylethyl esters a significant impact of the length of the alkyl chain of the acid moiety was observed.

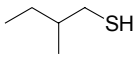
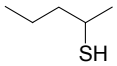
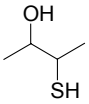
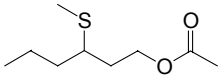
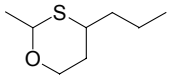
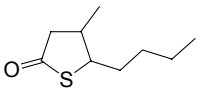
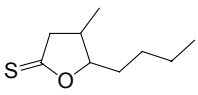
Table 4.1.5.4 Separation of the enantiomers of aromatic compounds

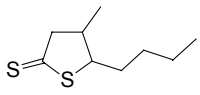
		T (°C)	*k*	α	R_S
1-Phenylethanol		100	14.79	1.14	11.08
Hydratropalcohol		110	12.75	1.09	6.36
Hydratropaldehyde		90	23.26	1.04	3.56
1-Phenylethyl acetate		90	23.88	1.06	4.56
1-Phenylethyl propionate		100	22.79	1.09	7.75
1-Phenylethyl butyrate		110	17.44	1.02	1.79
cis-Ethyl methylphenylglycidate		120	20.39	1.02	1.68
trans-Ethyl methylphenylglycidate		130	23.45	1.06	4.90

S*ulfur-containing compounds*

Enantiomers of sulfur-containing compounds from different classes could be separated (Table 4.1.5.5). A comparison of the α values obtained for 2-pentanethiol/2-pentanol and 2-methylbutanethiol/2-methylbutanol demonstrates that the replacement of the hydroxy group by a thiol group had no significant impact on the separation of the enantiomers. For the sulfur-containing whiskey lactone-derivatives (5-butyldihydro-4-methyl-2(3H)-thiophenone; 5-butyldihydro-4-methyl-2(3H)-furanthione; 5-butyldihydro-4-methyl-3(3H)-thiophenthione) the improved separation observed for the *trans*-configured stereoisomers (Table 4.1.5.3) remained unchanged independent from the insertion of sulfur at various positions of the lactone ring.

Table 4.1.5.5 Separation of the enantiomers of sulfur-containing compounds

		T (°C)	*k*	α	R_S
2-Methylbutanethiol		30	14.28	1.06	3.44
2-Pentanthiol		40	8.15	1.10	5.31
erythro-2-Mercapto-3-butanol		55	19.43	1.20	12.28
threo-2-Mercapto-3-butanol		55	21.94	1.05	3.12
3-Methylthio-1-hexyl acetate		105	19.72	1.02	1.45
cis-2-Methyl-4-propyl-1,3-oxathiane		90	14.10	1.19	13.68
trans-2-Methyl-4-propyl-1,3-oxathiane		90	17.70	1.21	14.20
trans-5-Butyldihydro-4-methyl-2(3H)-thiophenone		130	11.71	1.15	10.89
cis-5-Butyldihydro-4-methyl-2(3H)-thiophenone		130	13.76	1.09	6.59
trans-5-Butyldihydro-4-methyl-2(3H)-furanthione		130	18.06	1.07	5.67
cis-5-Butyldihydro-4-methyl-2(3H)-furanthione		130	21.28	1.02	1.34

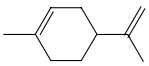
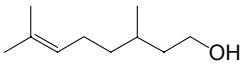
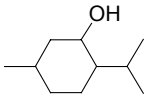
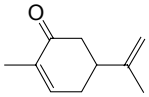
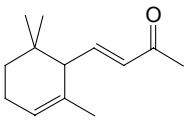
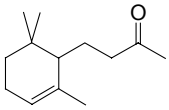
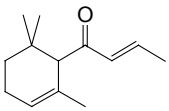
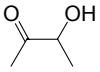
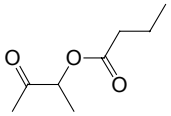
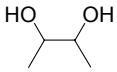
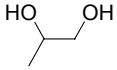
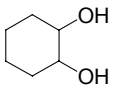
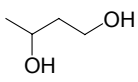
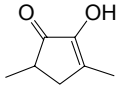
trans-5-Butylhydro-4-methyl-2(3H)-thiophenthione		140	17.69	1.09	6.87
cis-5-Butylhydro-4-methyl-2(3H)-thiophenthione		140	20.80	1.04	3.55

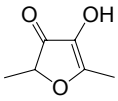
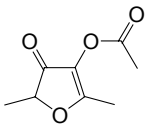
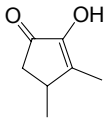
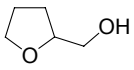
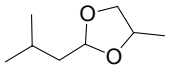
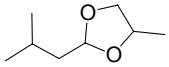
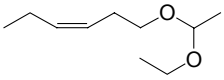
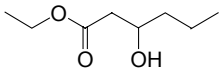
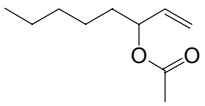
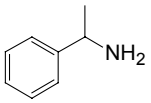
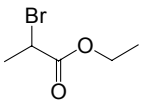
Compounds from miscellaneous structural classes

The potential of 2,3-di-MOM-6-TBDMS-γ-CD to separate enantiomers of monoterpenes was demonstrated for different structural classes (the hydrocarbon limonene, the acyclic and cyclic alcohols citronellol and menthol, and the cyclic ketone carvone; Table 4.1.5.6). The separation of the C13-norisoprenoid compounds α-ionone, dihydro-α-ionone and α-damascone and of the cyclic propylene glycol acetals are other examples for the usefulness of this chiral stationary phase for separation of important flavor substances.

The highest separation factor among the compounds tested was found for acetoin. The α value of 1.81 decreased drastically by either esterification of the hydroxy moiety (acetoin *n*-butanoate) or by reduction of the keto group (2,3-butanediol). This apparent importance of the hydroxycarbonyl structure for enantioseparation was also confirmed for the cyclic enols 3,5-dimethyl-2-hydroxy-2-cyclopentenone and 2,5-dimethyl-4-hydroxy-3(2H)-furanone. Shifting of the methyl group (3,4-dimethyl-2-hydroxy-2-cyclopentenone) or esterification of the hydroxy group (2,5-dimethyl-4-acetyl-3(2H)-furanone) resulted in significant decrease of the α value.

Table 4.1.5.6 Separation of compounds from miscellaneous structural classes

		T (°C)	k	α	R_S
Limonene		50	19.39	1.06	3.85
Citronellol		100	21.62	1.02	1.79
Menthol		100	13.24	1.04	3.27
Carvone		110	8.82	1.04	2.76
α-Ionone		110	25.01	1.02	2.08
Dihydro-α-ionone		120	14.15	1.04	2.69
α-Damascone		110	18.83	1.02	1.490
Acetoin		50	12.44	1.81	36.73
Acetoin *n*-butanoate		80	22.92	1.18	15.10
threo-2,3-Butanediol		70	10.32	1.10	5.85
Propylene glycol		65	11.70	1.05	2.50
trans-1,2-Cyclohexanediol		95	17.82	1.03	1.93
1,3-Butanediol		80	15.19	1.05	3.77
3,5-Dimethyl-2-hydroxy-2-cyclopentenone(Coronol®)		100	12.10	1.37	22.01

		T (°C)	k	α	R_S
2,5-Dimethyl-4-hydroxy-3(2H)-furanone (Furaneol®)		110	12.35	1.31	24.79
2,5-Dimethyl-4-acetyl-3(2 H)-furanone (Acetyl furaneol)		110	19.16	1.02	2.00
3,4-Dimethyl-2-hydroxy-2-cyclopetenone (Methyl Corylone®)		90	16.39	1.11	7.35
2-Methyltetrahydro-furan-3-one		40	7.43	1.09	5.77
Tetrahydrofurfuryl alcohol		60	18.94	1.04	2.50
cis-4-Methyl-2-(2-methyl propyl)-1,3-dioxolane		55	10.67	1.09	5.22
trans-4-Methyl-2-(2-methyl propyl)-1,3-dioxolane		55	12.78	1.07	4.15
Acetaldehyde ethyl *cis*-3-hexenyl acetal		70	16.03	1.08	5.56
Ethyl 3-hydroxyhexanoate		100	12.56	1.03	2.26
1-Octen-3-yl acetate		70	19.78	1.22	17.50
1-Phenylethylamine		100	7.74	1.03	1.95
2-Bromobutane[(a)]		40	4.83	1.08	3.63
2-Iodobutane		40	7.93	1.04	2.01
Ethyl 2-bromopropionate		70	15.30	1.26	14.97

(a) Analysis performed at 50 kPa inlet pressure.

The results obtained for 2,3-MOM-6-TBDMS-γ-CD demonstrate that the acetalization of cyclodextrins is a useful approach to obtain modified cyclodextrins suitable for gas chromatographic enantioseparations. 2,3-MOM-6-TBDMS-γ-CD proved to be a CSP suitable for enantioseparation of a very broad spectrum of volatiles comprising various functional groups. Only a few compound classes turned out to be not accessible to enantiodifferentiation on this phase: tertiary alcohols (e.g. linalool, α-terpineol) and their esters, bicyclic compounds (e.g. camphene, camphor, borneol, fenchol), and less volatile esters (e.g. hexyl 2-methylbutanoate, β-phenylethyl 2-methylbutanoate, benzyl 2-methylbutanoate).

An extraordinary feature of 2,3-MOM-6-TBDMS-γ-CD are the high separation factors α exhibited for the hydroxyketone (acetoin), for cyclic enolones (3,5-dimethyl-2-hydroxy-2-cyclopentenone and 2,5-dimethyl-4-hydroxy-3(2H)-furanone), for acyclic methyl branched ketones (3-methyl-2-pentanone and 5-methyl-2-hepten-4-one), and for the cyclic ketone 3,3,5-trimethyl-cyclohexanone. The gas chromatographic separation exemplarily shown in Figure 4.1.5.2 demonstrates the suitability of 2,3-MOM-6-TBDMS-γ-CD for this type of compound classes.

So far, α values higher than 1.5 have been mainly reported for compounds containing halo-atoms such as 2-halopropanoates and fluoroethers (Koenig et al., 1988c; Berthod et al., 1992; Koen de Vries et al., 1992; Grosenick and Schurig, 1997). An impressive example is a separation factor of 10 observed on Lipodex E for 2-(fluoromethoxy)-3-methoxy-1,1,1,3,3-pentafluoropropane, a minor decomposition product of the inhalational anesthetic sevoflurane (Schurig and Schmidt, 2003).

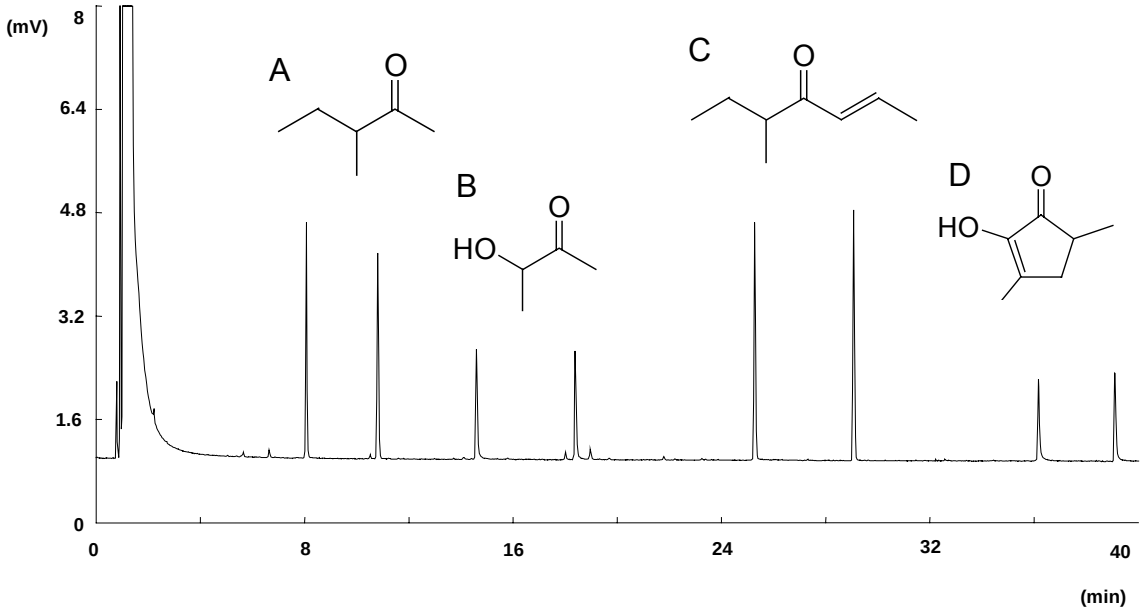


Figure 4.1.5.2 Separation of: (A) 3-methyl-2-pentanone; (B) acetoin; (C) 5-methyl-2-hepten-4-one (filbertone); (D) 2-hydroxy-3,5-dimethyl-2-cyclopentenone (coronol®). Temperature programming: 30 °C (initial, 2 min hold) then ramp at 2 °C/min rate.

4.2 2,3-O-MOM-6-O-TBDMS-β-cyclodextrin

4.2.1 Synthesis

Heptakis(2,3-di-*O*-methoxymethyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin (2,3-MOM-6-TBDMS-β-CD; Figure 4.2.1) was obtained by reaction of heptakis(6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin with methoxymethylchloride (MOM-Cl). In analogy to the synthesis of the 2,3-MOM-6-TBDMS-γ-CD, the reaction proceeded efficiently and resulted in sufficient and reproducible yield (see chapter 3.3).

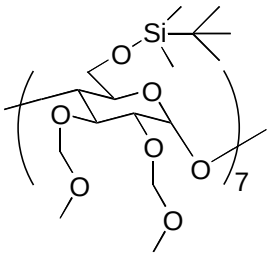


Figure 4.2.1 Structure of 2,3-MOM-6-TBDMS-β-CD

4.2.2 Structural characterization

The structure of 2,3-MOM-6-TBDMS-β-CD was confirmed by means of NMR (Table 4.2.2).

The ^{1}H NMR pattern observed was analogous to that for 2,3-MOM-γ-CD. The slight downfield shifts of around 0.03 ppm observed for the protons $H_{1,}$ $H_{2,}$ H_4 and H_5 may be explained by the different sizes of the CD rings. Differences were also observed for the methylene protons in the MOM side chains compared to the γ-CD derivative: for one of the methylene protons (4.75 ppm), the geminal coupling constant was increased from 6.3 to 7.0Hz and its chemical shift was drifted downwards (0.06 ppm).

^{13}C NMR signals were essentially identical to those observed for 2,3-MOM-6-TBDMS-γ-CD.

Table 4.2.2 ^{1}H NMR and ^{13}C NMR data for 2,3-MOM-6-TBDMS-β-CD

^{1}H NMR
0.02 (*s*; 42H; Si(C**H**$_3$)$_2$); 0.95 (*s*; 63H; Si(C**H**$_3$)$_3$); 3.38-3.42 (*m*; 7H; H$_2$); 3.39 (s; 21H; -OCH$_2$OC**H**$_3$); 3.44 (s; 21H; -OCH$_2$OC**H**$_3$); 3.62-3.80 (*m*; 14H; H$_6$); 3.88-4.02 (*m*; 14H; H$_3$+H$_4$); 4.33 (*d*; *J* = 11.8 Hz; 7H; H$_5$); 4.75 (*d*; *J* = 7.0 Hz; 7H; -OC**H**$_2$OCH$_3$); 4.79 (*d*; *J* = 7.0 Hz; 7H; -OC**H**$_2$OCH$_3$); 4.83 (*d*; *J* = 6.3 Hz; 7H; -OC**H**$_2$OCH$_3$); 5.02 (*d*; *J* = 6.3 Hz; 7H; -OC**H**$_2$OCH$_3$); 5.30 (*d*; *J* = 3.0 Hz; 7H; H$_1$).
^{13}C NMR
-3.6 (Si(**C**H$_3$)$_2$C(CH$_3$)$_3$), -3.3 (Si(**C**H$_3$)$_2$C(CH$_3$)$_3$), 20.0 (Si(CH$_3$)$_2$**C**(CH$_3$)$_3$), 27.5 (Si(CH$_3$)$_2$C(**C**H$_3$)$_3$), 57.12 (-OCH$_2$O**C**H$_3$), 57.14 (-OCH$_2$O**C**H$_3$), 64.3 (C6), 74.0, 78.7, 79.6, 80.0 (C2, C3, C4, C5), 99.5+100.6 (-O**C**H$_2$OCH$_3$), 101.0 (C1).

Additionally, MS analysis was performed to assure full derivatization of the 2,3-hydroxyl groups (Figure 4.2.2). The peak with m/z = 2573 corresponds to M(2749)+Na+H and confirms the expected structure. Further MS-MS analysis revealed fragments in 364 m/z intervals representing the consecutive cleavage of the MOM-derivatized glucose units. The fact that the α–1,4 glycosidic bonds of the CD torus rather than the glycosidic bonds in the MOM side chains are cleaved is a strong indication of the stability of the newly synthesized CD derivative.

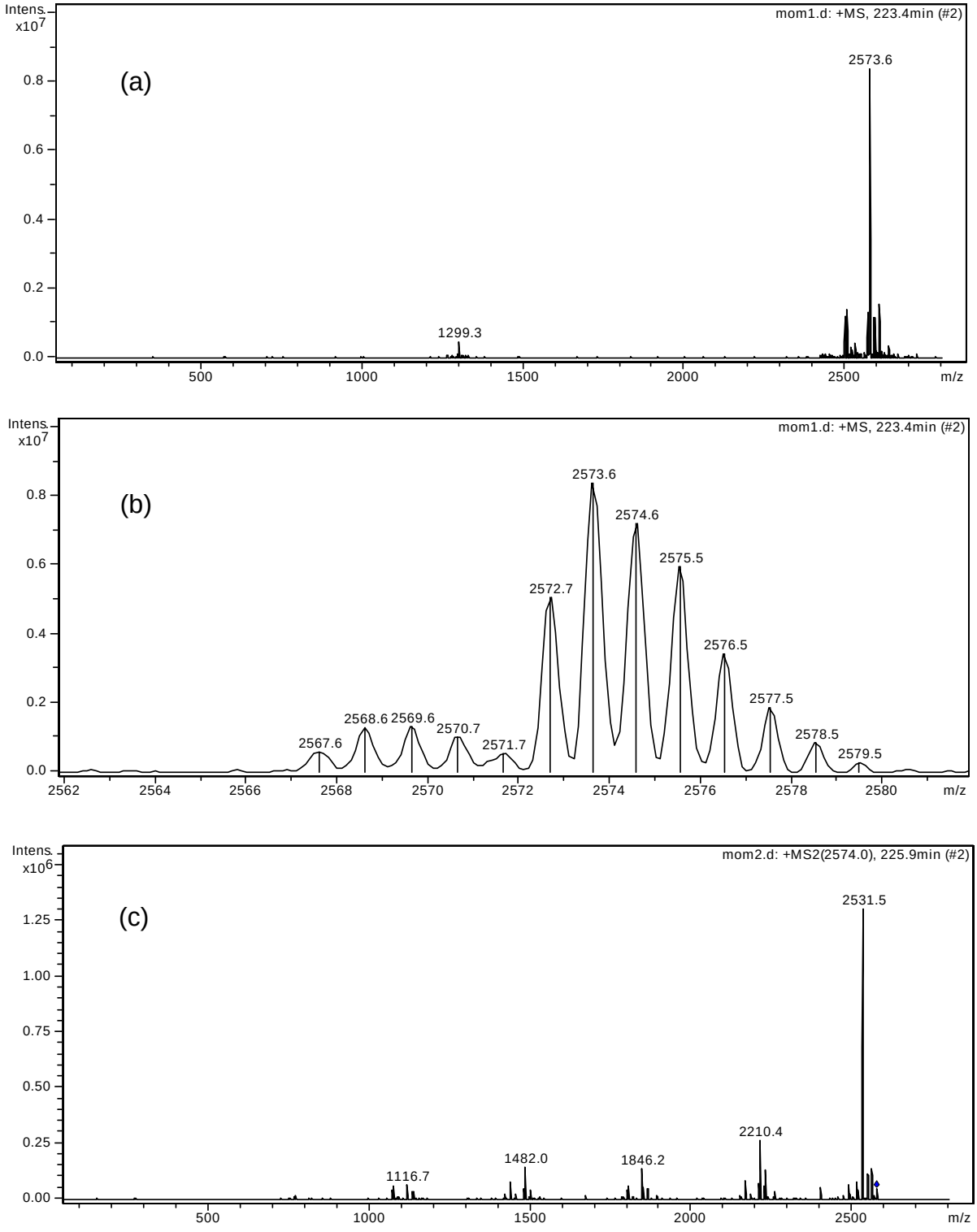


Figure 4.2.2 MS spectra of 2,3-MOM-6-TBDMS-β-CD in ESI positive mode: (a) wide range scan; (b) isotopic distribution; (c) MS/MS spectra (MS1 = 2574.0; fragmentation amplitude 3.75).
(for conditions see Material and Methods)

4.2.3 Coating and general performance

The column was prepared by statically coating a fused silica capillary with 28% w/w 2,3-MOM-6-TBDMS-β-CD in OV-1701vi (film thickness: 0.25 μm). Its general performance was tested using the Grob test mixture I. Except for a tailing observed for the acid, the column exhibited excellent performance for all compound classes contained in the mixture (Figure 4.2.3.).

For 2,3-MOM-6-TBDMS-γ-CD the stability of the acetal groups present as side chains has been demonstrated by repeated injection of water-containing samples. 2,3-MOM-6-TBDMS-β-CD also proved to be stable under harsh conditions (e.g., heating at 220 °C for 12 hours, injection of free alkanoic acids or temperature programming up to 230 °C); a column used daily for 10 months showed no decrease in performance.

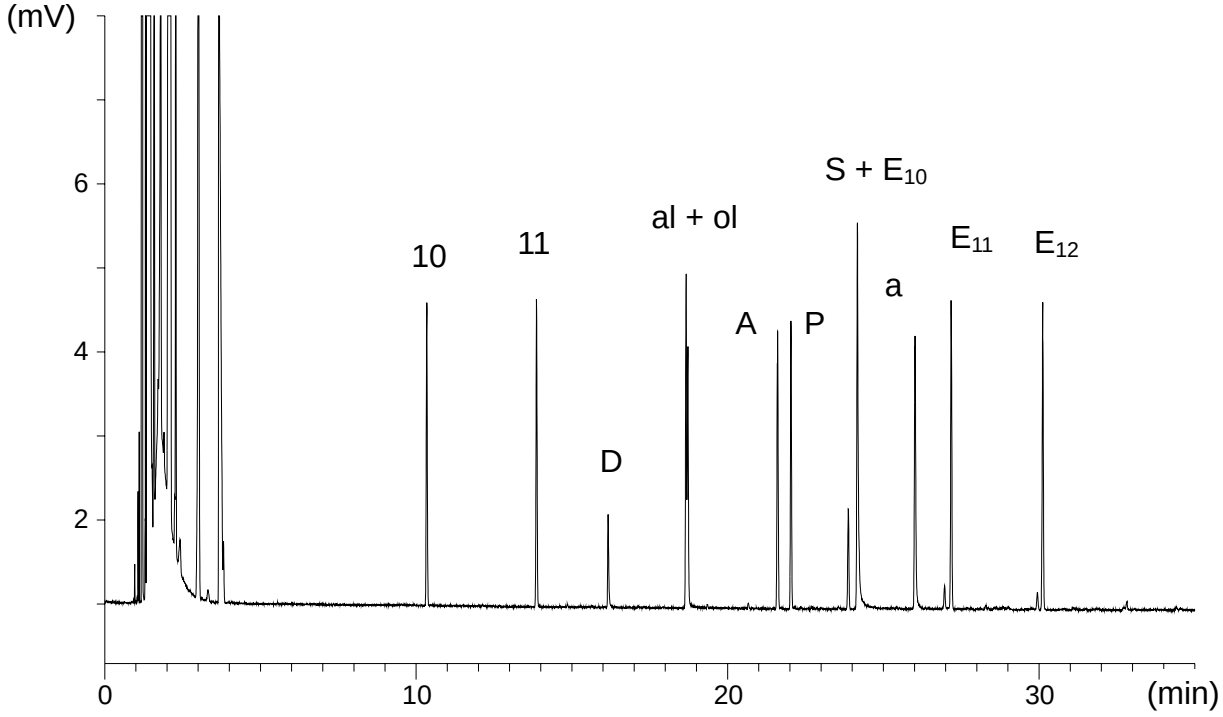


Figure 4.2.3 Grob test chromatogram of a 2,3-MOM-6-TBDMS-β-CD (0.11M OV-1701vi) column. Temperature programming: 40 °C initial (2 min hold) then ramp at 4.0 °C/min rate.
10: *n*-decane; 11: undecane; D: (-)-2,3-butanediol; al: 1-nonanal; ol: 1-octanol; A: 2,6-dimethylaniline; P: 2,6-dimethylphenol; E10: methyl decanoate; S: 2-ethylhexanoic acid; am: dicyclohexylamine; E11: methyl undecanoate; E12: methyl dodecanoate.

4.2.4 Separation characteristics

The properties of the stationary phase were assessed by testing enantioseparations of various flavor compounds representing different chemical classes. Table 4.2.4 shows data for compounds the enantiomers of which had been separated on 2,3-MOM-6-TBDMS-γ-CD (see Tables 4.1.5.1 - 4.1.5.6) and which could also be enantiodifferentiated on the β-CD analog.

Methyl branched compounds

Among the methyl branched compounds representatives of alcohols, ketones and esters could be separated into their enantiomers. However, neither methyl branched aldehydes and their acetals nor 2-methyl branched acids could be resolved satisfactorily. The ketones 3-methyl-2-pentanone, 5-methyl-2-hepten-4-one (Filbertone®) and 3,3,5-trimethylcyclohexanone which exhibited good resolutions on 2,3-MOM-6-TBDMS-γ-CD (α= 1.65, 1.69 and 1.60, respectively) were also well separated on 2,3-MOM-6-TBDMS-β-CD, but with slightly lower separation factors.

Secondary alcohols

2,3-MOM-TBDMS-β-CD turned out to be not very suitable for the separation of enantiomers of secondary alcohols. Investigations of saturated and unsaturated representatives showed that 2-methyl-3-hexanol, 2-heptanol, 3-buten-2-ol and 3-octen-2-ol could be moderately resolved into their enantiomers; however, 2-butanol, 2-pentanol, 2-hexanol, 5-methylhexanol, 1-penten-3-ol and 1-octen-3-ol could not be separated.

Lactones

Except for δ-heptalactone, the enantiomers of δ-lactones could not be separated on 2,3-MOM-6-TBDMS-β-CD. The separation factors determined for γ-lactones are in the same range as those observed on 2,3-MOM-6-TBDMS-γ-CD. For sotolone, a γ-lactone possessing an enol-structure in the ring, the separation (α= 1.49) was significantly better on 2,3-MOM-6-TBDMS-β-CD than on the corresponding γ-CD analog (α= 1.15).

Aromatic compounds

In the class of aromatic compounds it is interesting to note that the separation factor for 1-phenylethanol on 2,3-MOM-6-TBDMS-β-CD is lower than on 2,3-MOM-6-TBDMS-γ-CD (α= 1.05 vs 1.14), whereas the corresponding acetate is resolved much better on the β-CD derivative (α= 1.22 vs 1.06).

Sulfur-containing compounds

Representative sulfur-containing compounds were tested and the performance was comparable to 2,3-MOM-6-TBDMS-γ-CD but slightly inferior in terms of separation factors.

Compounds from miscellaneous structural classes

For acetoin, the hydroxy ketone for which a high α-value of 1.81 has been determined on 2,3-MOM-6-TBDMS-γ-CD, the separation factor observed on the β-CD derivative was also in the upper range (α= 1.46). On the other hand, the pronounced enantioseparations observed for cyclic pentenolones on 2,3-MOM-6-TBDMS-γ-CD could not be confirmed on 2,3-MOM-6-TBDMS-β-CD; for example, 3,5-dimethyl-2-hydroxy-2-cyclopentenone (Coronol®) was not separated at all into its enantiomers. The tertiary monoterpene alcohol linalool which had not been separated on 2,3-MOM-6-TBDMS-γ-CD showed a sufficiently high separation factor on 2,3-MOM-6-TBDMS-β-CD.

Apart from a few exceptions, the overall conclusion to be drawn from the comparison of separation characteristics as summarized in Table 4.2.4 is that 2,3-MOM-6-TBDMS-β-CD is a useful stationary phase for gas chromatographic separation of enantiomers of compounds from various chemical classes. However, compared to 2,3-MOM-6-TBDMS-γ-CD the spectrum of compounds for which enantiomers can be separated is more limited and the enantioseparations achieved are generally less pronounced.

Table 4.2.4 Separation characteristics of 2,3-MOM-6-TBDMS-β-CD.

Compound	T (°C)	k_1	α	R_S
Methyl branched compounds				
alcohols				
2-Methylbutanol	40	28.90	1.04	2.03
2-Methylpentanol	55	18.01	1.02	1.43
ketones				
3-Methyl-2-pentanone	40	17.80	1.50	22.07
5-Methyl-2-hepten-4-one	65	16.48	1.55	28.93
2-Methylcyclohexanone	70	15.62	1.03	1.90
3-Methylcyclohexanone	75	15.08	1.02	1.18
3,3,5-Trimethylcyclohexanone	80	16.54	1.43	25.55
2-Methylcyclopentanone	60	13.50	1.08	3.48
esters				
Methyl 2-methylbutanoate	40	10.73	1.09	4.68
Ethyl 2-methylbutanoate	40	15.16	1.10	5.78
Propyl 2-methylbutanoate	60	16.90	1.04	2.34
Butyl 2-methylbutanoate	60	21.90	1.02	1.56
Secondary alcohols				
2-Methyl-3-hexanol	60	15.07	1.07	4.13
2-Heptanol	60	22.72	1.03	1.63
3-Buten-2-ol	30	12.21	1.04	1.67
3-Octen-2-ol	65	35.34	1.02	1.31

Lactones				
gamma-Pentalactone	100	9.65	1.28	15.19
gamma-Hexalactone	110	8.98	1.15	10.20
gamma-Heptalactone	120	9.09	1.10	6.79
gamma-Octalactone	130	9.83	1.05	3.61
gamma-Nonalactone	140	10.60	1.04	2.82
gamma-Decalactone	150	11.24	1.03	1.99
gamma-Undecalactone	160	11.74	1.02	1.40
gamma-Dodecalactone	170	12.40	1.01	1.10
trans-Whiskey lactone	120	16.42	1.08	5.99
cis-Whiskey lactone	120	21.10	1.01	1.07
Sotolone	125	11.19	1.49	29.58
delta-Heptalactone	120	11.82	1.02	1.38
epsilon-Decalactone	140	13.98	1.03	2.37
Aromatics				
1-Phenylethanol	100	11.85	1.05	3.30
Hydratropalcohol	110	11.53	1.03	2.28
1-Phenylethyl acetate	90	19.48	1.22	15.48
1-Phenylethyl propanoate	100	19.79	1.02	1.26
(*E*)-Ethyl methylphenylglycidate	140	16.23	1.02	1.54
Sulfur-containing compounds				
2-Pentanethiol	40	9.79	1.06	3.12
threo-2-Mercapto-3-butanol	70	24.73	1.02	1.11

cis-2-Methyl-4-propyl-1,3-oxathiane	85	16.55	1.03	2.13
trans-2-Methyl-4-propyl-1,3-oxathiane	85	21.74	1.05	3.95
Miscellaneous				
Limonene	50	25.10	1.03	2.05
Linalool	80	13.70	1.02	1.42
Acetoin	70	12.38	1.46	19.65
Acetoin *n*-butanoate	80	20.08	1.11	7.33
3,4-Dimethyl-2-hydroxy-2-cylclo-pentenone (Methyl Corylone®)	90	23.68	1.27	19.85
2-Methyltetrahydrofuran-3-one	60	12.10	1.04	2.84
Ethyl 3-hydroxyhexanoate	80	11.83	1.06	4.33
1-Octen-3-yl acetate	80	13.47	1.32	19.62

4.2.5 Separation of 2-alkyl esters

An additional class of flavoring compounds which was included in the set of substances screened to test the potential of 2,3-MOM-6-TBDMS-β-CD are esters of secondary alcohols. Esters of 2-alkanols are known as important flavor compounds and are targets of interest also because of their differences in odor perception depending on the configuration (Mosandl and Deger, 1987). For 2-pentyl acetate a high separation factor α of 4.31 (K_1 = 20.61 at 35 °C isothermal) was found. As discussed in 4.1.5, so far, α-values in that order of magnitude have been mainly reported for compounds containing halo atoms. Based on this result, a homologous series of esters of secondary alcohols varying in chain lengths were investigated. A comparison of the separation data determined on 2,3-MOM-6-TBDMS-β-CD and 2,3-MOM-6-TBDMS-γ-CD is given in Table 4.2.5. On both stationary phases the separation factors decreased

which increasing chain lengths of the acyl moieties (from acetate to hexanoate) and of the alcohol moieties (from 2-pentanol to 2-nonanol). The suitability for enantiodifferentiation of the acetates of secondary alcohols was especially pronounced for 2,3-MOM-6-TBDMS-β-CD. On 2,3-MOM-6-TBDMS-γ-CD the decreases in separation factors upon elongation of the acid chain length were not so drastic; consequentially, α-values determined for the butanoates and hexanoates are higher on the 2,3-MOM-6-TBDMS-γ-CD than on the β-CD derivative.

It is interesting to note that in contrast to the good enantioseparations observed for the esters of secondary alcohols on 2,3-MOM-6-TBDMS-β-CD the corresponding free alcohols 2-pentanol and 2-nonanol could not be separated and the α-value observed for 2-heptanol was rather low. The above-described differences in separation factors for 1-phenylethanol and 1-phenylethyl acetate on 2,3-MOM-6-TBDMS-β-CD are in agreement with these observations.

Table 4.2.5 Comparison of the separations of 2-alkyl esters on 2,3-MOM-6-TBDMS-β-CD and 2,3-MOM-6-TBDMS-β-CD.

	2,3-MOM-6-TBDMS-β-CD			2,3-MOM-6-TBDMS-γ-CD		
	α	k_1	*T* (°C)	α	k_1	*T* (°C)
2-Pentyl acetate	3.80	15.1	40	2.44	13.1	35
2-Pentyl butanoate	1.09	12.3	65	1.68	13.0	60
2-Pentyl hexanoate	1.03	14.3	90	1.14	14.6	85
2-Heptyl acetate	1.72	13.4	70	1.30	15.8	60
2-Heptyl butanoate	1.03	13.9	90	1.15	14.1	85
2-Heptyl hexanoate	1.01	16.8	110	1.03	14.2	110
2-Nonyl acetate	1.25	13.8	95	1.10	14.0	90
2-Nonyl butanoate	1.01	13.5	115	1.05	14.1	110
2-Nonyl hexanoate	(a)	14.0	135	1.01	15.9	130

(a) No resolution

4.2.5.1 Thermodynamic parameters

To get some understanding of the phenomena underlying the enantioseparation of 2-pentyl acetate on 2,3-MOM-6-TBDMS-β-CD, thermodynamic parameters were determined. Since the cyclodextrin derivative is used as stationary phase after dilution in OV-1701vi silicone, a method introduced by Schurig (Schurig and Jung, 1990) is applicable. According to this procedure, thermodynamic data of chiral recognition ($\Delta_{R,S}$(ΔG), $\Delta_{R,S}$(ΔH) and $\Delta_{R,S}$(ΔS)) can be determined by measuring the retention increases *R*' of the enantiomers on the cyclodextrin dissolved in the solvent (i.e., OV-1701vi) in comparison to a reference column coated only with the dissolving achiral phase.

The following equations were used:

$$R'_{(E1)} = (r_{(E1)}-r_0)/r_0 \quad \text{(Eq. 1a)}$$

$$R'_{(E2)} = (r_{(E2)}-r_0)/r_0 \quad \text{(Eq. 1b)}$$

$$R\ln(R'_{(E2)}/R'_{(E1)}) = -(\Delta_{E1,E2}(\Delta H^0)/T)+\Delta_{E1,E2}(\Delta S^0) \quad \text{(Eq. 2)}$$

Where:

r_0: ratio of net retentions of the analyte and a reference hydrocarbon on the achiral phase; $r_{(E1)}$, $r_{(E2)}$: ratios of net retentions of the analyte and a reference hydrocarbon on the chiral phase for the first and second eluted enantiomer; $R'_{(E1)}$, $R'_{(E2)}$: retention increases for the first and second eluted enantiomer; R: gas constant; T: absolute temperature (K); $\Delta_{E1,E2}(\Delta H^0)$: association enthalpy (J/mol); $\Delta_{E1,E2}(\Delta S^0)$: association entropy (J/mol*K).

In addition to 2-pentyl acetate, the procedure was also performed for γ-pentalactone. This compound was selected as comparator because it exhibits a moderately high α-value on 2,3-MOM-6-TBDMS-β-CD and its thermodynamic parameters have been determined on another CD derivative (Beck et al., 2000).

Retention increases for the enantiomers of γ-pentalactone obtained using reference standards (*n*-decane to *n*-pentadecane) at 85 °C are listed in Table 4.2.5.1. Additionally, retention increase data using the same hydrocarbon standards were elaborated in a temperature range from 85 °C to 115 °C at 5 °C temperature intervals. Average ratios $R'_{(E2)}/R'_{(E1)}$ were determined and the correlation between $R*\ln(R'_{(E2)}/R'_{(E1)})$ and $1/T$ is depicted in Figure 4.2.5.1a. On the basis of the linear regression ($R^2 > 0.99$), the thermodynamic parameters ($\Delta_{R,S}(\Delta H_0)$ = -2.95 kJ/mol, $\Delta_{R,S}(\Delta S_0)$ = -4.11 kJ/mol) as well as the isoenantioselective temperature (T_{iso} = 445 °C) could be determined.

The attempt to determine thermodynamic parameters for the separation of 2-pentyl acetate on 2,3-MOM-6-TBDMS-β-CD by the same approach is summarized in Table 4.2.5.1 Using *n*-octane as standard it was possible to estimate $\Delta\Delta G$ but the plot of $R\ln(R'_{(E2)}/R'_{(E1)})$ versus $1/T$ did not result in a linear relationship and the calculation of the thermodynamic parameters was not possible (Figure 4.2.5.1b).

Table 4.2.5.1 Relative retention data of γ-pentalactone and 2-pentyl acetate measured on 2,3-MOM-6-TBDMS-β-CD and a reference column coated with OV-1701vi only.

Standard	*T* (°C)	r_0	$r_{(E1)}$	$r_{(E2)}$	$R'_{(E1)}$	$R'_{(E2)}$	$\Delta\Delta G$ (kJ/mol)
γ-Pentalactone							
n-Decane	85	3.07	8.04	11.08	1.62	2.61	-1.42
n-Undecane	85	1.610	4.08	5.62	1.53	2.49	-1.44
n-Dodecane	85	0.834	2.04	2.81	1.45	2.37	-1.47
n-Tridecane	85	0.430	1.02	1.40	1.37	2.26	-1.50
n-Tetradecane	85	0.221	0.504	0.695	1.28	2.14	-1.53
n-Pentadecane	85	0.114	0.251	0.346	1.20	2.04	-1.57
2-Pentyl acetate							
n-Octane	35	2.849	3.20	13.77	0.12	3.83	-8.84
	40	2.728	3.01	11.43	0.10	3.19	-8.93
	45	2.635	2.85	9.84	0.08	2.56	-9.17
	50	2.522	2.68	7.76	0.06	2.08	-9.35
	55	2.440	2.55	6.38	0.04	1.61	-9.85
	60	2.341	2.41	5.38	0.03	1.30	-10.4
	65	2.247	2.27	4.45	0.01	0.98	-12.5
n-Nonane	35	1.154	1.22	5.24	0.05	3.54	-10.7
	40	1.144	1.19	4.51	0.04	2.94	-11.4
	45	1.136	1.16	3.82	-0.04	2.18	-
	50	1.126	1.13	3.28	-0.05	1.36	-
	55	1.120	1.12	2.80	-0.03	1.50	-
	60	1.111	1.10	2.45	-0.01	1.20	-
	65	1.104	1.08	2.12	-0.02	0.92	-

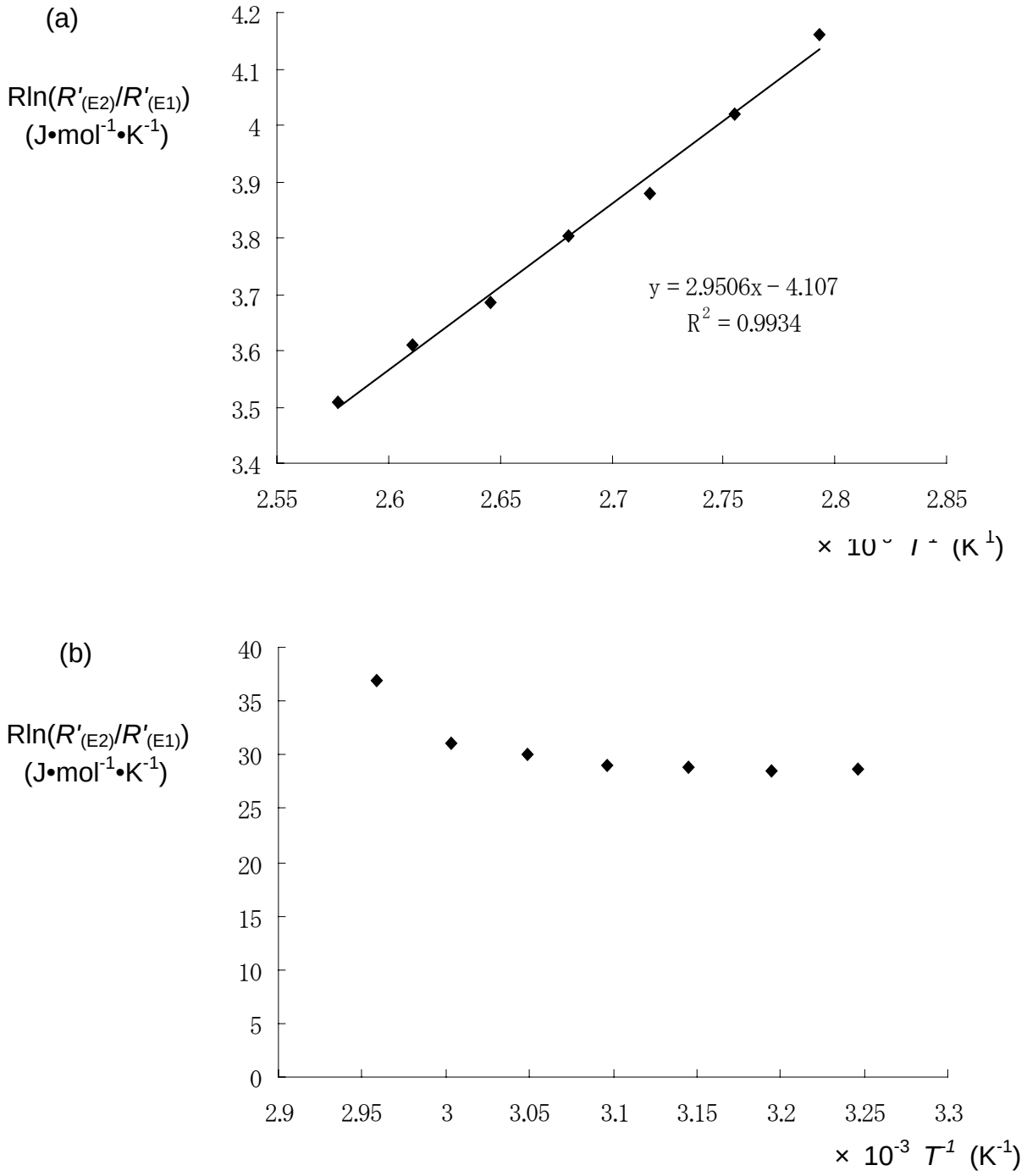


Figure 4.2.5.1 Plot of $R\ln(R'_{(E2)}/R'_{(E1)})$ versus T^{-1} for: (a) γ-pentalactone: Averaged $R\ln(R'_{(E2)}/R'_{(E1)})$ from datasets utilizing standards through C_{10}-C_{15} were used to plot the data. Estimated association enthalpy and association entropy was $\Delta_{E2,E1}(\Delta H_0)$ = -2.95 kJ/mol, $\Delta_{E2,E1}(\Delta S_0)$ = -4.11 J mol^{-1} K^{-1}, repectively, and T_{ISO} was calculated to be 445 °C; (b) 2-pentylacetate: Plot of $R\ln(R'_{(E2)}/R'_{(E1)})$ versus T^{-1} for 2-pentyl acetate. Datasets shown on Table 4.2.8 utilizing *n*-octane as standard were used to plot the data.

The retention increases *R*' for the enantiomers of γ-pentalactone range from 1.20-1.62 for the first and from 2.04-2.61 for the second eluted enantiomer (Table 4.2.8). These increments are lower than those calculated from data reported for the enantioseparation of γ-pentalactone on 2,3-*O*-*n*-propanoyl-6-*O*-TBDMS-γ-CD ($R'_{(E1)}$ 5.26 - 12.2 and $R'_{(E2)}$ 5.46 - 13.0) (Beck et al., 2000). That means, the interactions of the enantiomers with this CD derivative are stronger than those with 2,3-MOM-6-TBDMS-β-CD. However, the differences between the two enantiomers are more pronounced on 2,3-MOM-6-TBDMS-β-CD.

In contrast, for 2-pentyl acetate only the retention increases for the second eluted enantiomers are in the order of magnitude as reported for enantiodifferentiations on cyclodextrin stationary phases (Schurig and Jung, 1990; Bicchi et al., 1995; Buda et al., 1995; Schurig and Schmidt, 2003). For the first eluted enantiomer, however, the *R*' values are extremely low, indicating that the interactions of this enantiomer with the chiral selector are comparable to the interactions of the hydrocarbons used as references.

Different types of ratios of retention increases resulting in enantiodifferentiations have been reported (Schurig and Jung, 1990; Bicchi et al., 1995; Buda et al., 1995; Beck et al., 2000b). The phenomenon shown for the enantioseparation of 2-pentyl acetate on 2,3-MOM-6-TBDMS-β-CD, i.e., only one enantiomer is significantly retained whereas the other one shows a retention behavior comparable to the hydrocarbons used as references, has not yet been described. Analogous studies are in progress to find out whether this principle is of general relevance for enantioseparations on CD derivatives possessing acetal groups as side chains.

4.3 2,3-*O*-MOM-6-*O*-TBDMS-α-cyclodextrin

4.3.1 Synthesis and structural characterization

Hexakis(2,3-di-*O*-methoxymethyl-6-*O*-*tert*-butyldimethylsilyl)-α-cyclodextrin (2,3-MOM-6-TBDMS-α-CD; Figure 4.3.1) was obtained by reaction of hexakis(6-*O*-*tert*-butyldimethylsilyl)-α-cyclodextrin with methoxymethylchloride (MOM-Cl). In analogy to the synthesis of the 2,3-MOM-6-TBDMS-γ-CD, the reaction proceeded efficiently and resulted in very good yield (94%).

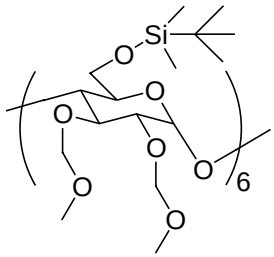


Figure 4.3.1 Structure of 2,3-MOM-6-TBDMS-α-CD

^{1}H and ^{13}C NMR data determined for the stationary phase are summarized in Table 4.3.1. The set of ^{1}H NMR data obtained for 2,3-MOM-6-TBDMS-α-CD is comparable to those described for the γ- and β-analogs. There were only differences in the coupling constants of the methylene protons on the MOM groups, two of which showed 7.3 Hz, which is higher than those measured for the β (6.3 Hz) and γ (6.5 Hz) CD derivative, respectively. In addition, the chemical shifts of the most downfield protons at the methylene moiety of the MOM (5.06 ppm) were different from those seen for the β (4.99 ppm) and γ (5.02) analog.

Table 4.3.1 ^{1}H NMR and ^{13}C NMR data for 2,3-MOM-6-TBDMS-α-CD

^{1}H NMR
0.11+0.12 (*s*; 36H; Si(C**H**$_3$)$_2$); 0.94 (*s*; 54H; Si(C**H**$_3$)$_3$); 3.31 (*dd*, *J* = 3.0 Hz, 10.0 Hz, H$_2$, 6H); 3.36 (s; 18H; -OCH$_2$OC**H**$_3$); 3.44 (s; 18H; -OCH$_2$OC**H**$_3$); 3.71-3.80 (*m*; 12H; H$_{6a,b}$); 3.87 (*t*; *J* = 9.0 Hz; 6H; H$_4$); 3.99 (*t*; *J* = 8.8 Hz; 6H; H$_3$); 4.28 (*dd*, *J* = 2.5 Hz, 11.5 Hz, H$_5$, 6H); 4.71 (*d*; *J* = 7.0 Hz; 6H; -OC**H**$_2$OCH$_3$); 4.76 (*t*; *J* = 7.3 Hz; 12H; -OC**H**$_2$OCH$_3$); 5.03 (*d*; *J* = 3.0 Hz; 6H; H$_1$); 5.06 (*d*; *J* = 7.3 Hz; 6H; -OC**H**$_2$OCH$_3$).
^{13}C NMR
-3.7 (Si(**C**H$_3$)$_2$C(CH$_3$)$_3$), -3.5 (Si(**C**H$_3$)$_2$C(CH$_3$)$_3$), 19.9 (Si(CH$_3$)$_2$**C**(CH$_3$)$_3$), 27.4 (Si(CH$_3$)$_2$C(**C**H$_3$)$_3$), 56.8 (-OCH$_2$O**C**H$_3$), 57.0 (-OCH$_2$O**C**H$_3$), 64.3 (C6), 74.8, 77.4, 79.5, 83.7 (C2, C3, C4, C5), 99.3 (C1), 101.1 + 103.7 (-O**C**H$_2$OCH$_3$).

4.3.2 Separation characteristics

The spectrum of compounds for which enantioseparations could be achieved using 2,3-MOM-TBDMS-α-CD as chiral stationary phase was rather limited compared to the β- and γ-CD analogs. Separation data for compounds which could be resolved are listed in Table 4.3.2.

Methyl branched compounds

As shown in chapter 4.1, 2,3-MOM-TBDMS-γ-CD is a chiral stationary phase suitable for enantiodifferentiation of methyl branched volatiles containing various functional groups. In contrast, on 2,3-MOM-TBDMS-α-CD the enantiomers of 2-methyl branched aldehydes (2-methylbutanal), esters (methyl 2-methylbutanoate, ethyl 2-methylbutanoate) and ketones (3-methyl-2-pentanone, 2-methylcyclopentanone) could not be separated. A significant difference is the separation behavior of 3,3,5-trimethylcyclohexanone, which exhibits a separation factor of 1.60 on 2,3-MOM-TBDMS-γ-CD but is not separated on the α-analog.

Table 4.3.2 Separation characteristics of 2,3-MOM-6-TBDMS-α-CD

Compound	*T* (°C)	k_1	α	*R*s
Methyl branched compounds				
2-Methylbutanol	65	16.88	1.06	2.78
2-Methylbutanoic acid	95	10.12	1.06	3.37
2-Methylpentanoic acid	105	8.81	1.05	3.04
2-Methylhexanoic acid	105	15.48	1.02	1.57
2-Methylheptanoic acid	110	20.84	1.02	1.47
5-Methyl-2-hepten-4-one	65	16.66	1.03	1.79
Lactones				
gamma-Pentalactone	105	12.27	1.09	5.71
gamma-Hexalactone	115	10.17	1.04	3.01
gamma-Heptalactone	120	13.29	1.03	2.49
gamma-Octalactone	125	17.57	1.02	1.88
gamma-Nonalactone	135	17.15	1.02	1.64
delta-Hexalactone	105	15.07	1.04	2.87
delta-Heptalactone	115	14.24	1.03	1.91
delta-Octalactone	125	14.53	1.03	1.99
delta-Nonalactone	135	14.88	1.02	1.31
Aromatic compounds				
1-Phenylethanol	100	14.98	1.03	1.98
1-Phenylethyl acetate	90	19.94	1.02	1.52
Miscellaneous compounds				
3,5-Dimethyl-1,2-cyclopentandione	65	13.43	1.09	6.30

Acetoin	65	15.10	1.10	4.95
Limonene	70	14.88	1.06	3.37

Among the methyl branched alcohols 3-methylpentanol which showed a very good separation on 2,3-MOM-TBDMS-γ-CD (α = 1.19) and a slight resolution on 2,3-MOM-TBDMS-β-CD could not be separated on 2,3-MOM-TBDMS-α-CD. On the other hand, 2-methylbutanol is well separated on 2,3-MOM-TBDMS-α-CD (α = 1.06) and the separation efficiency decreases with increasing torus size (α = 1.04 for 2,3-MOM-TBDMS-β-CD; α = 1.02 for 2,3-MOM-TBDMS-γ-CD) for the other CD analogs.

Another class of compounds which showed good separation characteristics on 2,3-MOM-TBDMS-α-CD are 2-methylalkanoic acids. As shown in Figure 4.3.2.1 the enantiomers of a homologous series (from 2-methylbutanoic acid to 2-methylheptanoic acid) were all baseline-separated. Considering the carboxylic function and the strong tailing normally encountered with this moiety, the peak shapes are very good.

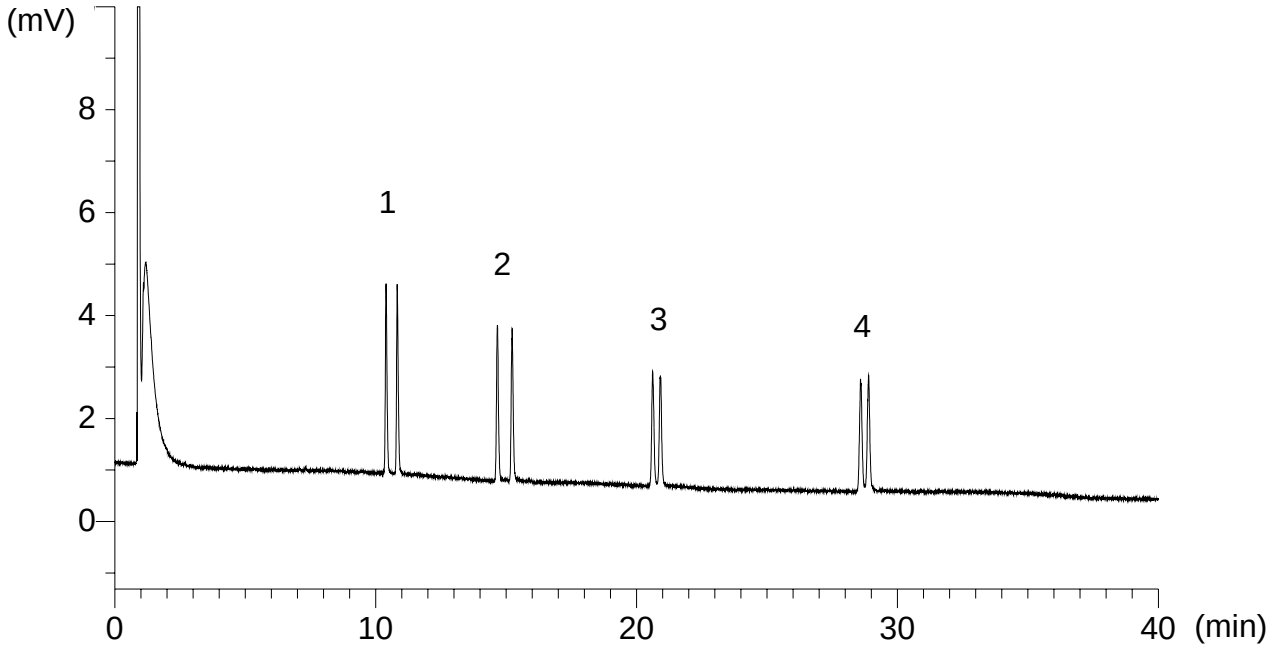


Figure 4.3.2.1 Separation of the enantiomers of 2-methylalkanoic acids on 2,3-MOM-6-TBDMS-α-CD: 2-methylbutanoic acid (1), 2-methylpentanoic acid (2), 2-methylhexanoic acid (3) and 2-methylheptanoic acid (4); Temperature programmed run: 90 °C (initial, 2 min hold) then ramp at 1.0 °C/min rate.

Lactones

Another class of compounds which could be separated into their enantiomers on 2,3-MOM-TBDMS-α-CD are lactones. As shown in Figure 4.3.2.2, temperature-programmed runs allow the enantioseparation of γ- and δ-lactones. Especially the lower homologues are well separated. 2,3-MOM-TBDMS-α-CD is superior to the β-CD analog as regards the separation of δ-lactones (except for δ-heptalactone). The γ-lactone homologs are generally better resolved on 2,3-MOM-TBDMS-β-CD and 2,3-MOM-TBDMS-γ-CD.

2-Alkyl esters

The data for the separation of enantiomers of 2-alkyl esters which showed outstanding α-values especially on 2,3-MOM-TBDMS-β-CD (see 4.2) are listed in Table 4.3.2.1. Out of the series of homologues tested only the smallest representative 2-pentyl acetate could be resolved.

Table 4.3.2.1 2-Alkyl ester separation on 2,3-MOM-6-TBDMS-α-CD

	α	k_1	*T* (°C)
2-Pentyl acetate	1.08	20.94	40
2-Pentyl butanoate	1	14.65	70
2-Pentyl hexanoate	1	15.25	95
2-Heptyl acetate	1	14.15	80
2-Heptyl butanoate	1	17.24	95
2-Heptyl hexanoate	1	13.70	120
2-Nonyl acetate	1	14.40	100
2-Nonyl butanoate	1	13.11	120
2-Nonyl hexanoate	1	13.34	140

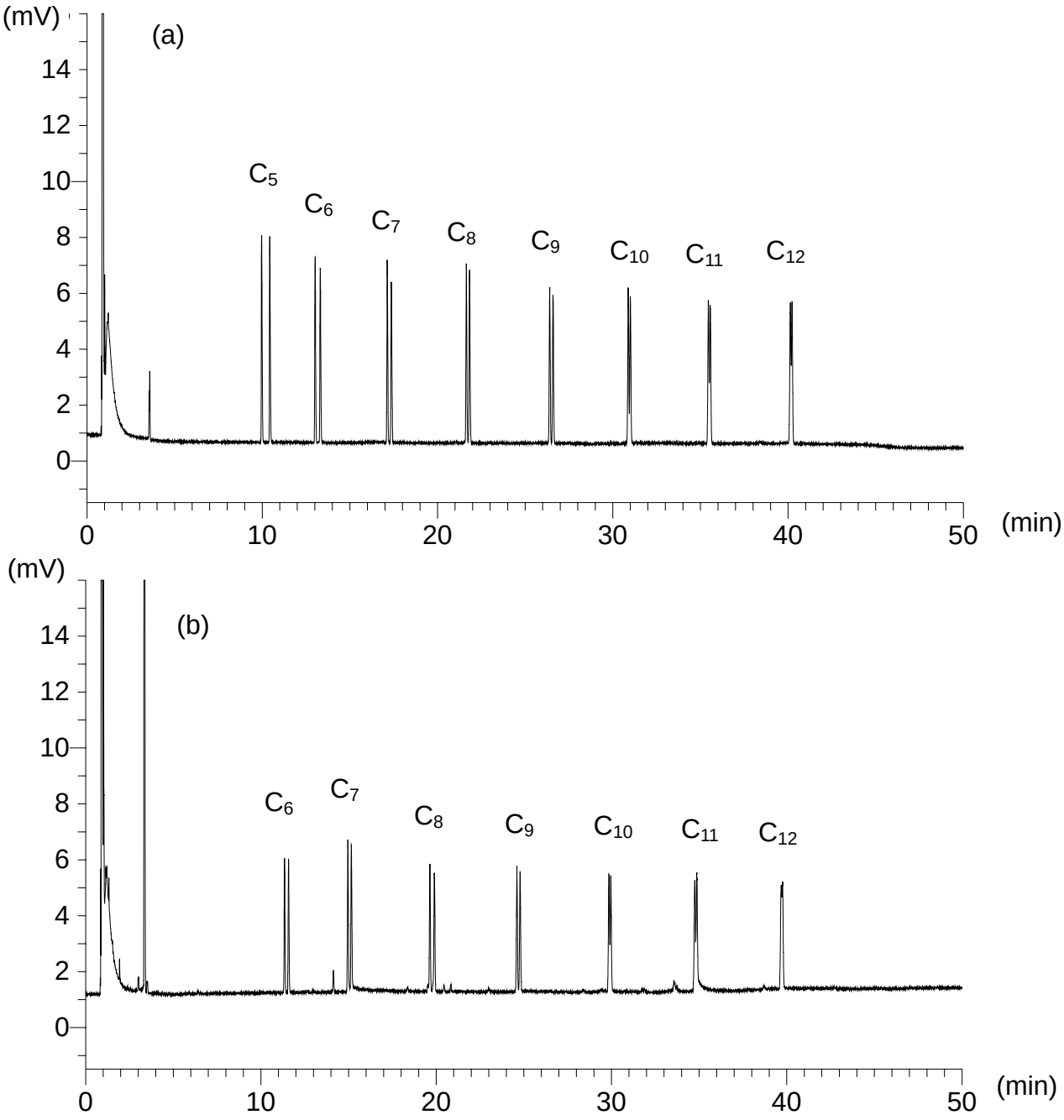


Figure 4.3.2.2 Separation of series of: (a) γ-lactones (C_5 - C_{12}) and (b) δ-lactones (C_6 - C_{12}) on 2,3-MOM-6-TBDMS-α-CD. Temperature programming: 90 °C (initial, 2 min hold) then ramp at 2 °C/min rate.

Aromatic compounds

Both, 1-phenylethanol and its acetate are only moderately resolved into the enantiomers on 2,3-MOM-6-TBDMS-α-CD. This is in contrast to the phenomena observed on the 2,3-MOM-6-TBDMS-β-CD and 2,3-MOM-6-TBDMS-γ-CD analogues, on which either the free alcohol (γ: α = 1.14) or the acetate (β: α = 1.22) exhibit pronounced enantioseparation.

Miscellaneous compounds

The α-value determined for the monoterpene hydrocarbon limonene is in the same order of magnitude as those observed on 2,3-MOM-6-TBDMS-β-CD and 2,3-MOM-6-TBDMS-γ-CD. On the other hand, camphene and α-pinene and the monoterpene alcohols linalool and α-terpineol could not be separated on 2,3-MOM-6-TBDMS-α-CD. No enantioseparation was possible for the secondary alcohols 2-butanol, 3-octanol, 3-buten-2-ol and 1-penten-3-ol, for the sulfur-containing 2-methylbutanthiol and for the halo-compound 2-iodobutane.

Only moderate separation was possible for 5-methyl-2-hepten-4-one (Filbertone) and although well separated on 2,3-MOM-6-TBDMS-α-CD, this was also true for 3,5-dimethyl-1,2-cyclopentandione (Coronol) and acetoin. For 3-methyl-2-pentanone no resolution was observed.

The hydroxy ketone acetoin, some methyl branched ketones and some cyclic pentenolone and furanone derivatives exhibited pronounced enantioseparation on 2,3-MOM-6-TBDMS-γ-CD. A comparison of the separations of representatives of these compound classes on the three different MOM-type CD homologues are shown in Figure 4.3.2.3. With decreasing torus size the α-values drastically decrease.

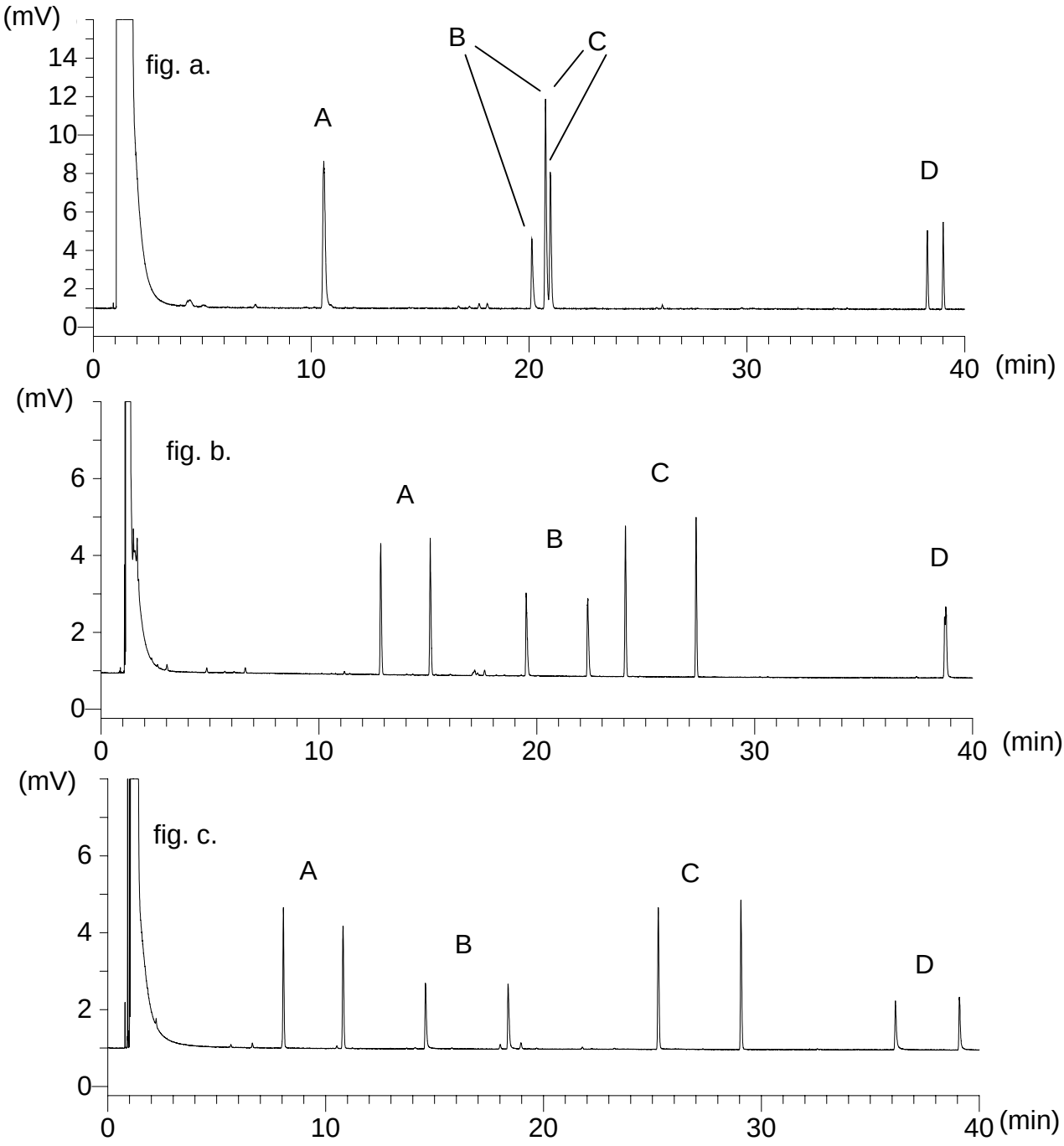


Figure 4.3.2.3 Separation of α-hydroxyketones and methyl branched ketones on 2,3-MOM-6-TBDMS-α-CD (a), 2,3-MOM-6-TBDMS-β-CD (b) and 2,3-MOM-6-TBDMS-γ-CD (c) column. Temperature programming: 40 °C (initial, 2 min. hold), then ramp at 2°C/min rate. A: 3-Methyl-2-pentanone, B: Acetoin, C: 5-methyl-2-hepten-4-one (Filbertone®), D: 2-hydroxy-3,5-di-methyl-2-cyclopentanone (Coronol®).

4.4 Variation of alkoxymethyl side-chains

4.4.1 Synthesis

Modifications of the alkoxymethyl side chains were achieved by introducing (i) the elongated ethoxymethyl moiety, (ii) the polar (2-methoxyethoxy)methyl group and (iii) the apolar and bulky (2-trimethylsilylethoxy)methyl group.

Octakis(2,3-di-*O*-ethoxymethyl-6-*O*-*tert*-butyldimethylsilyl)-γ-cyclodextrin (2,3-di-EOM-6-TBDMS-γ-CD), octakis(2,3-di-*O*-(2-methoxyethoxy)methyl-6-*O*-*tert*-butyldimethylsilyl)-γ-cyclodextrin (2,3-MEM-6-TBDMS-γ-CD) and octakis-(2,3-di-*O*-(2-trimethylsilylethoxy)methyl-6-*O*-*tert*-butyldimethylsilyl)-γ-cyclo-dextrin (2,3-SEM-6-TBDMS-γ-CD) were synthesized according to the same procedure as described for 2,3-MOM-6-TBDMS-γ-CD starting from 6-O-TBDMS-γ-CD using ethoxymethylchloride, (2-methoxyethoxy)methyl-chloride and (2-trimethylsilylethoxy)methylchloride as derivatization reagents (Greene and Wuts, 1999). The structures are shown in Figure 4.4.

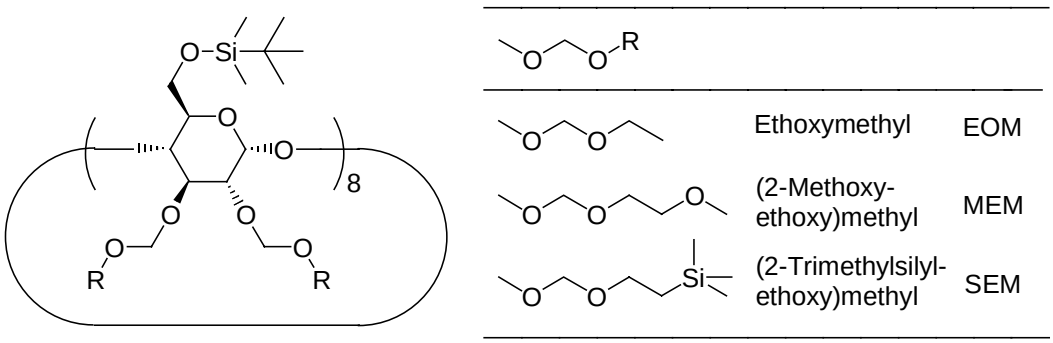


Figure 4.4 Structures of the synthesized TBDMS-γ-CD derivatives.

4.4.2 Separation characteristics of 2,3-EOM-6-TBDMS-γ-cyclodextrin

The compounds separated into their enantiomers using 2,3-EOM-6-TBDMS-γ-CD as chiral stationary phase are listed in Table 4.4.2.
The data obtained for the enantioseparation of methyl branched compounds demonstrate a general trend observed for 2,3-EOM-6-TBDMS-γ-CD: The α-values are almost identical or at least in the same order of magnitude as those determined on 2,3-MOM-6-TBDMS-γ-CD. However, due to peak broadening the resolutions are decreased up to 50%. The enantioseparation of ethyl 2-methylbutanoate shown in Figure 4.4.2 is a typical example for this phenomenon.

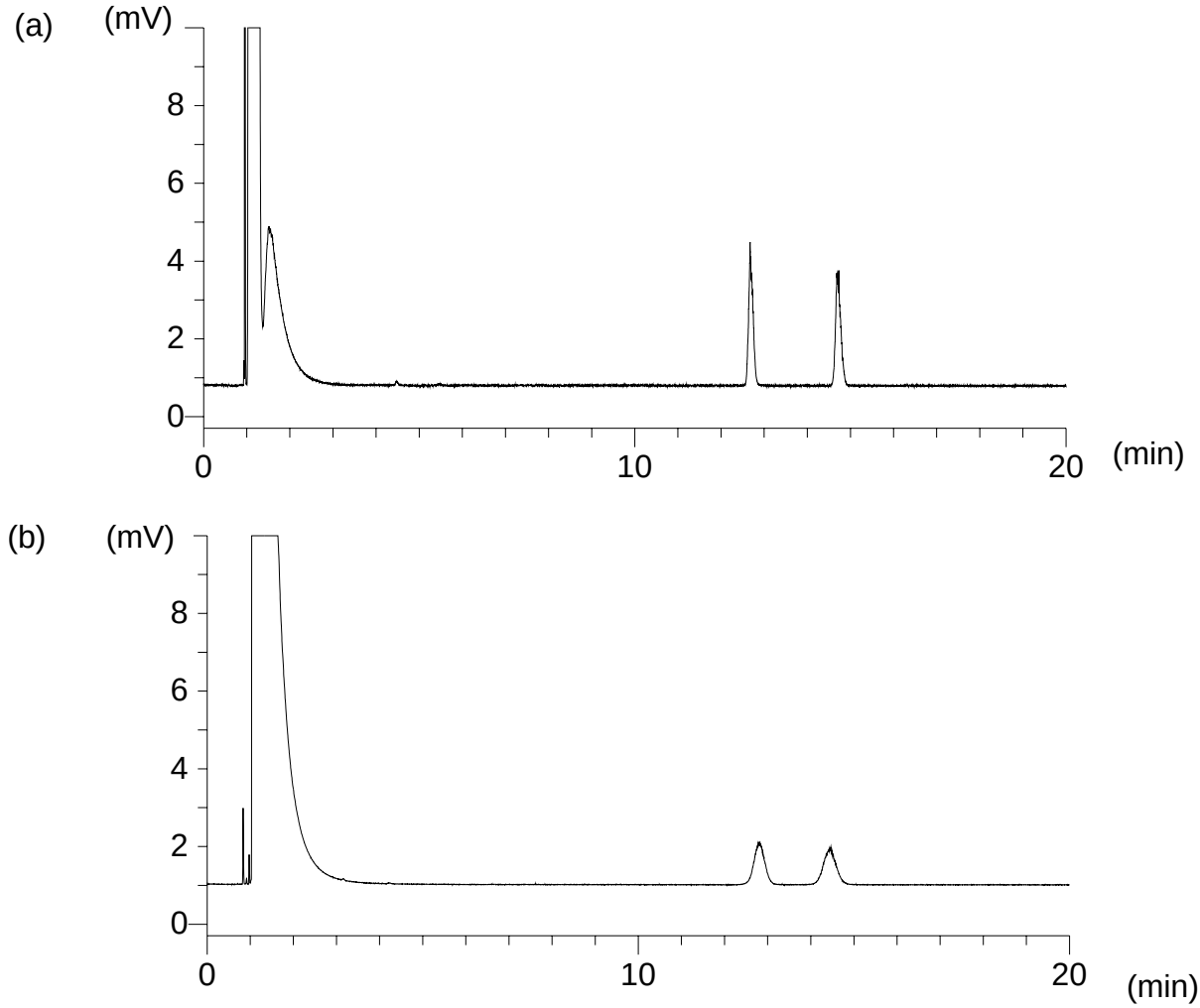


Figure 4.4.2 Enantioseparation of ethyl 2-methylbutanoate on: (a) TBDMS-MOM-γ-CD and (b) TBDMS-EOM-γ-CD, 40 °C isothermal.

For the γ- and δ-lactones, the trend is similar, although the discrepancies between the resolutions become less with increasing chain lengths of the substances.

The fact that the differences between 2,3-EOM-6-TBDMS-γ-CD and 2,3-MOM-6-TBDMS-γ-CD become less pronounced within a homologous series with increasing size of the compounds is also reflected in the data obtained for the 2-alkyl esters.

Table 4.4.2 Separation characteristics of 2,3-EOM-6-TBDMS-γ-CD

Compound	*T* (°C)	*k*	α	R_S
Methyl branched compounds				
Esters				
Methyl 2-methylbutanoate	40	8.00	1.14	3.18
Ethyl 2-methylbutanoate	40	16.46	1.14	3.48
Propyl 2-methylbutanoate	50	19.23	1.08	3.24
iso-Propyl 2-methylbutanoate	40	18.76	1.14	2.73
Butyl 2-methylbutanoate	60	23.30	1.04	2.29
Aldehyde				
2-Methylbutanal (50 kPa)	30	8.65	1.05	1.12
Ketones				
3-Methyl-2-pentanone	40	10.97	1.50	6.45
3,3,5-Trimethylcyclohexanone	75	16.67	1.56	26.11
Acids				
2-Methylpentanoic acid	90	16.11	1.06	4.05
2-Methylhexanoic acid	100	16.56	1.05	3.72

Secondary alcohols				
2-Pentanol	40	13.96	1.10	2.73
2-Hexanol	50	17.30	1.08	3.36
2-Heptanol	60	20.12	1.05	2.57
2-Octanol	70	23.21	1.02	1.37
3-Heptanol	60	17.56	1.07	2.99
3-Octanol	75	14.75	1.03	2.07
4-Octanol	70	19.00	1.04	1.98
1-Penten-3-ol	40	13.45	1.14	3.21
1-Octen-3-ol	70	20.38	1.07	4.55
3-Butyn-2-ol	30	16.84	1.03	1.10
2-Alkyl esters				
2-Pentyl acetate	40	11.05	1.87	14.56
2-Pentyl butanoate	65	11.63	1.394	17.79
2-Pentyl hexanoate	85	17.17	1.11	5.73
2-Heptyl acetate	65	13.78	1.22	8.64
2-Heptyl butanoate	85	16.89	1.12	5.87
2-Heptyl hexanoate	105	21.71	1.03	1.87
2-Nonyl acetate	85	20.99	1.10	5.00
2-Nonyl butanoate	105	22.44	1.05	2.95
2-Nonyl hexanoate	130	19.98	1.01	0.82
Lactones				
gamma-Hexalactone	115	15.21	1.07	5.30

gamma-Heptalactone	125	14.15	1.10	7.81
gamma-Octalactone	135	10.84	1.15	10.67
gamma-Nonalactone	145	9.89	1.04	2.75
gamma-Decalactone	155	10.32	1.02	1.35
gamma-Undecalactone	160	13.40	1.01	1.25
gamma-Dodecalactone	160	21.08	1.01	1.11
Whiskey lactone (1)	120	16.30	1.35	27.23
Whiskey lactone (2)	120	21.48	1.02	1.57
Sotolone	120	13.87	1.10	7.40
delta-Hexalactone	110	13.55	1.12	8.49
delta-Heptalactone	120	12.28	1.14	10.16
delta-Octalactone	130	10.23	1.09	6.88
delta-Nonalactone	140	10.85	1.04	3.12
delta-Decalactone	150	11.68	1.02	1.59
delta-Undecalactone	160	12.66	1.02	1.22
delta-Dodecalactone	170	13.41	1.01	1.30
epsilon-Decalactone	140	14.57	1.21	15.44
Aromatics				
1-Phenylethyl acetate	90	21.29	1.07	5.24
1-Phenylethyl propanoate	100	21.13	1.07	5.13
1-Phenylethyl butanoate	110	21.40	1.02	1.85
Ethyl methylphenylglycidate (I)	120	24.17	1.02	1.78
Ethyl methylphenylglycidate	130	28.26	1.06	5.03

Sulfur-containing compounds				
2-Pentanthiol	40	11.04	1.18	3.75
2-Methyl-4-propyl-1,3-oxathiane (1)	85	17.97	1.27	17.23
2-Methyl-4-propyl-1,3-oxathiane (2)	85	22.47	1.27	20.65
Miscellaneous				
Menthol	100	12.68	1.03	2.49
alpha-Damascone	110	22.84	1.01	1.07
Acetoin	55	9.60	1.22	9.73
Acetoin *n*-butyryl ester	85	14.12	1.07	5.10
Methyl corylone	100	12.64	1.20	12.64
Homofuraneol	60	15.19	1.05	1.67
Ethyl 3-hydroxybutanoate	80	16.41	1.03	1.76
Ethyl 3-hydroxyhexanoate	90	23.98	1.03	2.56
Propylene glycol	65	14.97	1.05	3.65
2,3-Butanediol	65	18.03	1.11	5.97
iso-Valeraldehyde PGA	50	16.22	1.09	3.54
iso-Valeraldehyde PGA	50	19.66	1.06	2.00
2-Iodobutane	40	11.95	1.11	2.23
2-Methylpiperazine	55	22.63	1.08	2.86

4.4.3 Influence of alkoxymethyl side chain

The methyl branched ketones 3-methyl-2-pentanone and 5-methyl-2-hepten-4-one, the hydroxy ketone acetoin and the 3,5-dimethyl-2-cyclopentenone were used for comparison of the separation characteristics of 2,3-MOM-6-TBDMS-γ-CD, 2,3-EOM-6-TBDMS-γ-CD, 2,3-MEM-6-TBDMS-γ-CD and 2,3-SEM-6-TBDMS-γ-CD.

Using 2,3-EOM-6-TBDMS-γ-CD as stationary phase, the separation performance for 3-methyl-2-pentanone, acetoin, filbertone and coronol decreased slightly (Figure 4.4.3b). On 2,3-MEM-6-TBDMS-γ-CD a complete loss of enantioselectivity for acetoin was observed and the separation factors for the other compounds were also substantially reduced (Figure 4.4.3c). On 2,3-SEM-6-TBDMS-γ-CD a severe reduction in separation efficiency as well as considerable peak broadening were observed (Figure 4.4.3d).

Reasons for the broadening peaks may be slow mass transfer of the analyte when interacting with the CD cavity or decreased solubility of the cyclodextrin phases with longer side-chains

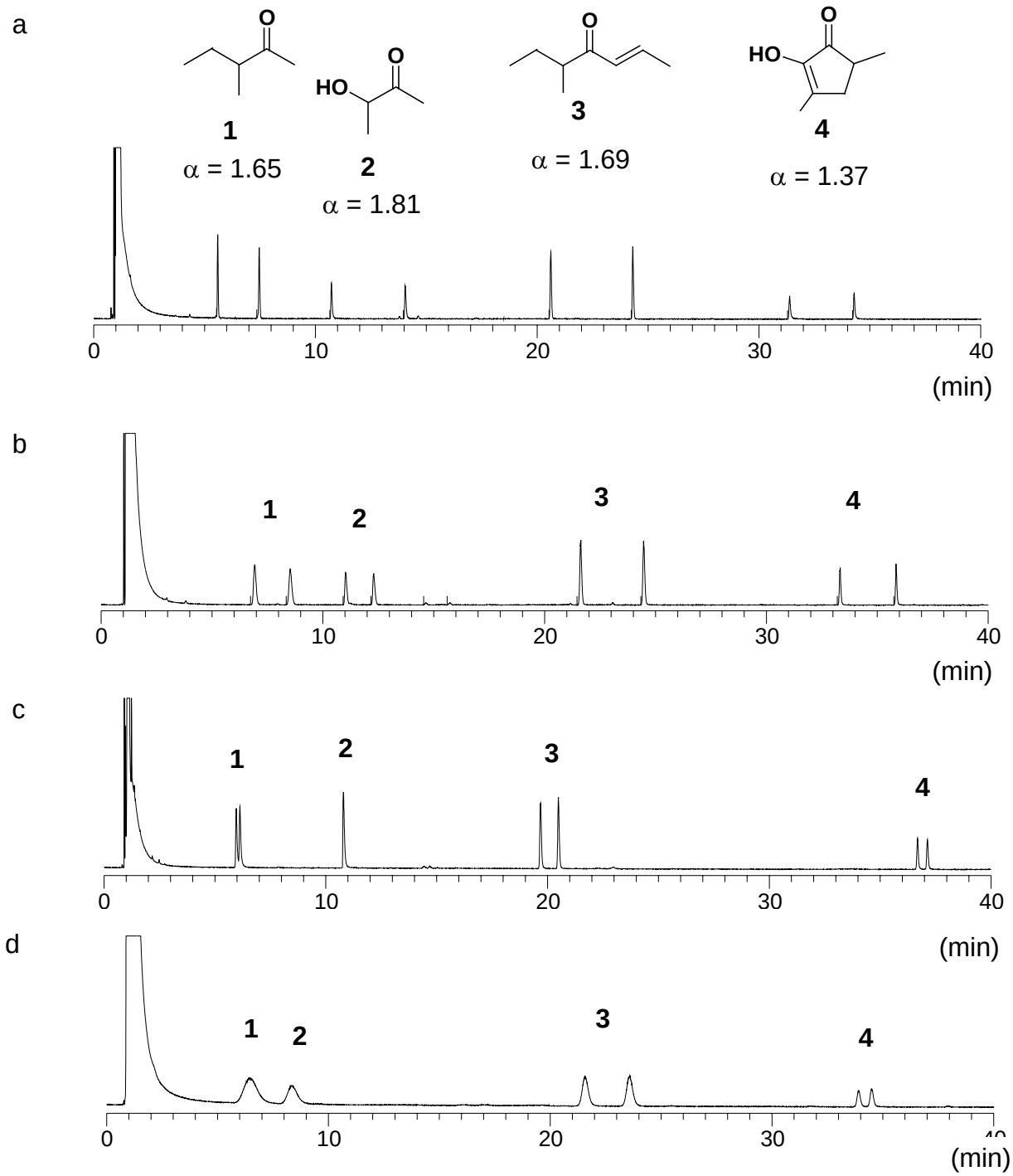


Figure 4.4.3 Separation of enantiomers of 3-methyl-2-pentanone (1), acetoin (2), 5-methyl-2-hepten-4-one (3) and 3,5-dimethyl-2-cyclopentenone (4). Columns: (a) 2,3-MOM-6-TBDMS-γ-CD; (b) 2,3-EOM-6-TBDMS-γ-CD; (c) 2,3-MEM-6-TBDMS-γ-CD; (d) 2,3-SEM-6-TBDMS-γ-CD. Temperature programming: 40 °C (2 min hold), ramp at 2.0 °C/min rate.

4.5 2,3-*O*-(2-methylbutyryl)-6-*O*-TBDMS-γ-cyclodextrin

4.5.1 Synthesis

Octakis-2,3-*O*-(2-methylbutyryl)-6-*O*-TBDMS-γ-cyclodextrin (2MB-6-TBDMS-γ-CD) was synthesized according to the reaction scheme shown in Figure 4.5.1.1 for the (*S*)-2-methylbutyryl derivative.

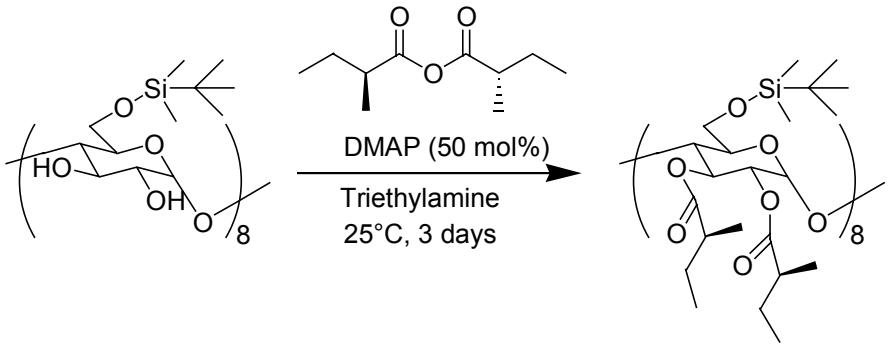


Figure 4.5.1.1 Synthesis of octakis-2,3-*O*-(2-methylbutyryl)-6-*O*-TBDMS-γ-cyclodextrin (as example: preparation of a CD derivative with (*S*)-configured 2-methylbutyryl side chain).

A 2-methyl branched compound, i.e. one of the simplest chiral molecules, was selected to introduce an additional chiral center into the CD via the side chains at positions 2 and 3 of the glucose units. Considering the relatively low reactivity achieved when alkylating cyclodextrins with alkyl halides (Miranda et al., 1998; Bicchi et al., 1999b) and the ready availability of the chiral starting material, it was decided to select 2-methylbutyric acid rather than 2-methylbutanol as the moiety to be introduced.

Acylations of cyclodextrins are conventionally carried out using the corresponding acid anhydride as derivatization reagent. This approach has the inherent disadvantage that 50 % of the chiral material is lost in the course of the substitution reaction. Therefore, several attempts were made to synthesize 2MB-6-TBDMS-γ-CD by using the corresponding acid chloride as reagent. However, this approach turned out to be unsuccessful due to incomplete

substitution patterns (room temperature) or decomposition of the cyclodextrin ring (elevated temperature). It is rather unusual that an acid chloride is inferior in terms of reactivity towards hydroxy groups compared to the corresponding acid anhydride. However, it has been reported that acetylation of β-cyclodextrins using acetyl chloride, at room temperature, resulted in selective acetylation of the 2-OH groups but did not lead to complete derivatization of the glucose units (Sutyagin et al., 2002). The authors explained this phenomenon by the characteristic inclusion properties of the cyclodextrin molecule. The decomposition of the cyclodextrin ring system at elevated temperature may be explained by the cleavage of the glucosidic bond (a mixed acetal) under the influence of acetyl chloride. It is known that a tetrahydropyranyl ether (THP), also a mixed acetal, can be transformed with excellent yield (91%) into an acetate under heating in the presence of acetyl chloride and acetic acid (Jacobson et al., 1970). THP ether could be considered as a model structure showing similarity to the glucosidic bonds of cyclodextrins. There are also reports of cleavage of an acetal function with acetyl chloride under influence of guanidinium chloride (Gros et al., 1995). Therefore, it is plausible that the type of reaction as shown in Figure 4.5.1.2 has contributed to the decomposition of the cyclodextrin ring.

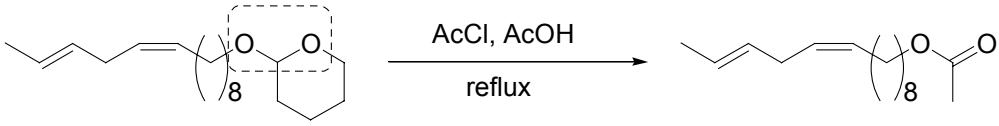


Figure 4.5.1.2 Cleavage of an acetal function with acetyl chloride.

Since the employed acid anhydride is relatively precious, the acylation reaction needs to be accomplished with minimum excess of the acylating reagent possible. In this respect, the dehydration of the intermediate TBDMS-CD plays an essential role in yielding satisfactory amount of the desired material. The utilization of the bulb-to-bulb distillation apparatus along with a high vacuum source up to 10^{-4} mmHg allowed strict drying and was found to be a convenient and efficient procedure to accomplish this criterion.

4.5.2 Structural characterization

The structures of (*S*)- and (*R*)-2MB-6-TBDMS-γ-CD were elucidated using ^{1}H and ^{13}C NMR. The unbranched analog 2,3-*n*-butyryl-6-TBDMS-γ-cyclodextrin was used as reference material; its ^{1}H NMR spectrum is shown in Figure 4.5.2.1 Although this chiral stationary phase has been widely applied (Maas et al., 1996; Mosandl et al., 1998), NMR data had not been published. Therefore, 2,3-*n*-propionyl-6-TBDMS-γ-cyclodextrin, for which NMR data had been published (Beck et al., 2000b) was used as comparator. As it can be seen in Table 4.5.2.1, the ^{1}H NMR spectra of the two cyclodextrin derivatives are very similar, except for the hextet resonance at 1.64 ppm which could be assigned to the proton attached to the β-carbon of the butyryl side chain. The ^{13}C NMR data sets were also quite similar except for the new signal at 18.3 ppm arising from the β-carbon of the butyryl side chain (Table 4.5.2.2).

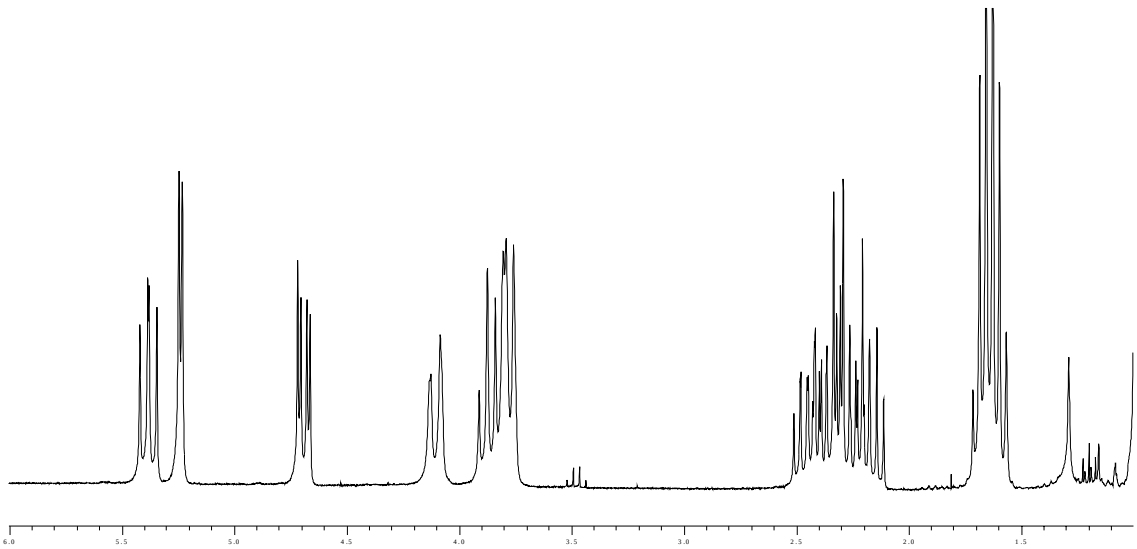


Figure 4.5.2.1 NMR spectrum of 2,3-*n*-butyryl-6-TBDMS-γ-CD between 1.0 - 6.0 ppm at 295 K (22 °C).

The ^{1}H NMR data of (*S*)- and (*R*)-2-MB-6-TBDMS-γ-CD are summarized in Table 4.5.2.3. For the structural elements common to the homolog 2,3-*n*-butyryl-γ-CD the expected signals were observed. The additionally present protons at the α-methyl moieties resulted in two doublets (due to the coupling with the methyne proton) for the (S)-configured (1.14 and 1.18 ppm) and for the (*R*)-configured (1.09 and 1.13 ppm) cyclodextrin. Due to the chirality introduced, the protons

attached to the methylene carbons of the 2-methylbutyryl side chains experience different magnetic environments leading to two independent resonance patterns (1.24-1.50 and 1.63-1.79 ppm for the (*S*)-type; 1.35-1.57 and 1.68-1.95 ppm for the (*R*)-type). This split of the hextet signal at 1.64 ppm of the 2,3-*n*-butyryl-γ-CD is a characteristic difference arising from the introduction of the methyl group in α-position of the butyryl side chain. Similar phenomena are observed for (*S*)- and (*R*)-2-methylbutanoic acid.

The ^{13}C NMR data for (*S*)- and (*R*)-2MB-6-TBDMS-γ-CD are summarized in Table 4.5.2.4. For the structural elements common to the 2,3-*n*-butyryl-γ-CD the expected NMR signals were observed. Characteristic, heavily shifted resonance pairs around 175 ppm indicate the full derivatization of the 2,3-hydroxyl group. Additionally, the ^{13}C NMR signal attributed to the newly attached 2-methylbutyryl side-chain could be observed for the terminal methyl moiety (15.4, 16.4 for (*S*); 15.7, 16.0 for (*R*)) and also for the α-methyl carbon (26.23, 26.3 for (*S*); 25.6, 26.2 for (*R*)).

Although the NMR signal patterns for 2-methylbutyryl-6-TBDMS-γ-CD were in agreement with the homologous *n*-butyryl-6-TBDMS-γ-CD, a unique phenomenon was observed for 2-methylbutyryl-6-TBDMS-γ-CD. The NMR signals were extremely broad when the NMR experiments were performed at room temperature (Figure 4.5.2.2a and c). Assuming that this phenomenon was the result of a slow conformational exchange rate of the 2-methylbutyryl-6-TBDMS-γ-CD, due to the presence of the methyl group in α position of the side chain, an additional experiment was carried out at elevated temperature of 343 K (70 °C). For the protons belonging to the *tert*-butyldimethylsilyl group this resulted in an only minor enhancement of the signals (Figure 4.5.2.2c and d). On the other hand, the resolution of the signals related to the 2-methylbutyryl side chains were drastically improved (Figure 4.5.2.2a and b). For the α-proton attached directly to the chiral center the improvement of the signal (2.36 ppm) upon temperature increase was considerably lower compared to its neighboring protons.

This type of peak broadening is not regularly observed for CD derivatives. For 2,6-dipentyl-3-acetyl-γ-CD, a similar broadening of NMR peaks has been reported, however only for a certain type of protons (H_3 on the glucose ring) (Schmarr et al., 1991a).

Table 4.5.2.1 Comparison of the ^{1}H NMR data of 2,3-*n*-butyryl-6-TBDMS-γ-CD and its homolog 2,3-propionyl-6-TBDMS-γ-CD.

2,3-Propionyl-6-TBDMS-γ-CD [a]					2,3-*n*-Butyryl-6-TBDMS-γ-CD [b]				
Shift (ppm)	Multi.	*J* (Hz)	Int.	Assignment	Shift (ppm)	Multi.	*J* (Hz)	Int.	Assignment
0.03, 0.04	*s*		48	-Si(C**H**$_3$)$_3$-^{t}Bu	0.06, 0.07	*s*		48	-Si(C**H**$_3$)$_3$-^{t}Bu
0.88	*s*		72	-SiMe$_2$C(C**H**$_3$)$_3$	0.91	*s*		72	-SiMe$_2$C(C**H**$_3$)$_3$
1.06-1.13	2*t*	7.4, 7.5	48	-COCH$_2$C**H**$_3$	0.95	*t*	7.3	48	-COCH$_2$CH$_2$C**H**$_3$
					1.64	*hex*	7.3	32	-COCH$_2$C**H**$_2$CH$_3$
2.16-.248	*m*		32	-COC**H**$_2$CH$_3$	2.11-2.51	*m*		32	-COC**H**$_2$CH$_2$CH$_3$
3.73-3.88	*m*		24	H$_4$ + H$_5$ + H$_{6b}$	3.78-3.80	*m*		16	H$_5$ + H$_{6b}$
					3.87	*t*	9.0	8	H$_4$
4.05	*d*	10.9	8	H$_{6a}$	4.11	*d*	11.0	8	H$_{6a}$
4.65	*dd*	3.5, 10.2	8	H$_2$	4.69	*dd*	3.5, 10.3	8	H$_2$
5.22	*d*	3.4	8	H$_1$	5.24	*d*	3.5	8	H$_1$
5.36	*t*	9.6	8	H$_3$	5.38	*dd*	8.8. 10.3	8	H$_3$

(a) 250 MHz, $CDCl_3$ (b) 300 MHz, $CDCl_3$ (Beck et al., 2000).

Table 4.5.2.2 Comparison of the ^{13}C NMR data of 2,3-*n*-butyryl-6-TBDMS-γ-CD and its homolog 2,3-propionyl-6-TBDMS-γ-CD.

2,3-Propionyl-6-TBDMS-γ-CD [a]		2,3-Butyryl-6-TBDMS-γ-CD [b]	
Shift (ppm)	Assignment	Shift (ppm)	Assignment
-5.3	Si(**C**H_3)$_3$-^{t}Bu	-5.0	Si(**C**H_3)$_3$-^{t}Bu
-5.1	Si(**C**H_3)$_3$-^{t}Bu	-4.8	Si(**C**H_3)$_3$-^{t}Bu
8.90 / 8.96	-COCH_2**C**H_3	13.7 × 2	-COCH_2CH_2**C**H_3
		18.3	-COCH_2**C**H_2CH_3
18.0	-SiMe$_2$**C**(CH_3)$_3$	18.5	-SiMe$_2$**C**(CH_3)$_3$
25.9	-SiMe$_2$C(**C**H_3)$_3$	26.1	-SiMe$_2$C(**C**H_3)$_3$
27.2 / 27.4	-CO**C**H_2CH_3	36.0 / 36.3	-CO**C**H_2CH_2CH_3
61.8	C6	62.3	C6
70.5	C3	70.8	C3
71.4	C2	71.6	C2
72.0	C5	72.4	C5
73.7	C4	74.4	C4
95.5	C1	96.0	C1
172.7 / 174.0	-**C**OCH_2CH_3	171.7 / 173.2	-**C**OCH_2CH_2CH_3

(a) 62.5 MHz, $CDCl_3$(b) 50 MHz, $CDCl_3$ (Beck et al., 2000).

Table 4.5.2.3 ^{1}H NMR (250 MHz, $CDCl_3$) data of 2,3-(*S*)- and (*R*)-2-methylbutyryl-6-TBDMS-γ-cyclodextrin

2,3-[(*S*)-2-Methylbutyryl]-6-TBDMS-γ-CD					2,3-[(*R*)-2-Methylbutyryl]-6-TBDMS-γ-CD				
Shift (ppm)	Multi.	*J* (Hz)	Int.	Assignment	Shift (ppm)	Multi.	*J* (Hz)	Int.	Assignment
0.06	*s*		48	-Si(C**H**$_3$)$_3$-^{t}Bu	0.08	*s*		48	-Si(C**H**$_3$)$_3$-^{t}Bu
0.89	*t*	7.3	48	-COCH(CH$_3$)CH$_2$C**H**$_3$					
0.90	*s*		72	-SiMe$_2$(C**H**$_3$)$_3$	0.91	*s*		72	-SiMe$_2$C(C**H**$_3$)$_3$
					0.94	*t*	7.0	48	-COCH(CH$_3$)CH$_2$C**H**$_3$
1.14 / 1.18	*d*	7.0	48	-COCH(C**H**$_3$)CH$_2$CH$_3$	1.09 / 1.13	*d*	7.0	48	-COCH(C**H**$_3$)CH$_2$CH$_3$
1.24-1.50	*m*		16	-COCH(CH$_3$)C**H**$_2$CH$_3$	1.35-1.57	*m*		16	-COCH(CH$_3$)C**H**$_2$CH$_3$
1.63-1.79	*m*		16	-COCH(CH$_3$)C**H**$_2$CH$_3$	1.68-1.95	*m*		16	-COCH(CH$_3$)C**H**$_2$CH$_3$
2.36	*2×q*	7.0	16	-COC**H**(CH$_3$)CH$_2$CH$_3$	2.32-2.48	*m*		16	-COC**H**(CH$_3$)CH$_2$CH$_3$
3.76-3.82	*m*		16	H$_5$+H$_{6b}$	3.77-3.83	*m*		16	H$_5$+H$_{6b}$
3.95	*t*	8.5	8	H$_4$	3.95	*t*	8.5	8	H$_4$
4.17	*d*	11.5	8	H$_{6a}$	4.19	*d*	11.5	8	H$_{6a}$
4.75	*dd*	3.5, 10.0	8	H$_2$	4.78	*dd*	3.5, 11.5	8	H$_2$
5.21	*d*	3.5	8	H$_1$	5.19	*d*	3.5	8	H$_1$
5.35	*d*	9.0	8	H$_3$	5.37	*d*	9.0	8	H$_3$

Table 4.5.2.4 ^{13}C NMR (250 MHz, $CDCl_3$) data of 2,3-(*S*)- and (*R*)-2-methylbutyryl-6-TBDMS-γ-cyclodextrin

2,3-[(*S*)-2-Methylbutyryl]-6-TBDMS-γ-CD		2,3-[(*R*)-2-Methylbutyryl]-6-TBDMS-γ-CD	
Shift (ppm)	Assignment	Shift (ppm)	Assignment
-5.0	-Si(**C**H_3)$_3$-^{t}Bu	-4.9	-Si(**C**H_3)$_3$-^{t}Bu
-4.8	-Si(**C**H_3)$_3$-^{t}Bu	-4.7	-Si(**C**H_3)$_3$-^{t}Bu
11.6	-COCH(CH_3)CH_2**C**H_3	11.4	-COCH(CH_3)CH_2**C**H_3
15.4 / 16.4	-COCH(CH_3)**C**H_2CH_3	15.7 / 16.0	-COCH(CH_3)**C**H_2CH_3
18.5	-SiMe_2**C**(CH_3)$_3$	18.5	-SiMe_2**C**(CH_3)$_3$
26.16	-SiMe_2C(**C**H_3)$_3$		
26.23	-COCH(**C**H_3)CH_2CH_3	25.6	-COCH(**C**H_3)CH_2CH_3
		26.2	-SiMe_2C(**C**H_3)$_3$
26.3	-COCH(C**H**$_3$)CH_2CH_3		
40.6 / 40.7	-CO**C**H(CH_3)CH_2CH_3	40.3 / 40.5	-CO**C**H(CH_3)CH_2CH_3
62.5	C6	62.6	C6
70.8	C3	70.7	C3
71.2	C2	71.1	C2
72.4	C5	72.6	C5
74.5	C4	74.8	C4
95.9	C1	96.4	C1
174.3 / 176.3	-**C**OCH(CH_3)CH_2CH_3	174.2 / 176.0	-**C**OCH(CH_3)CH_2CH_3

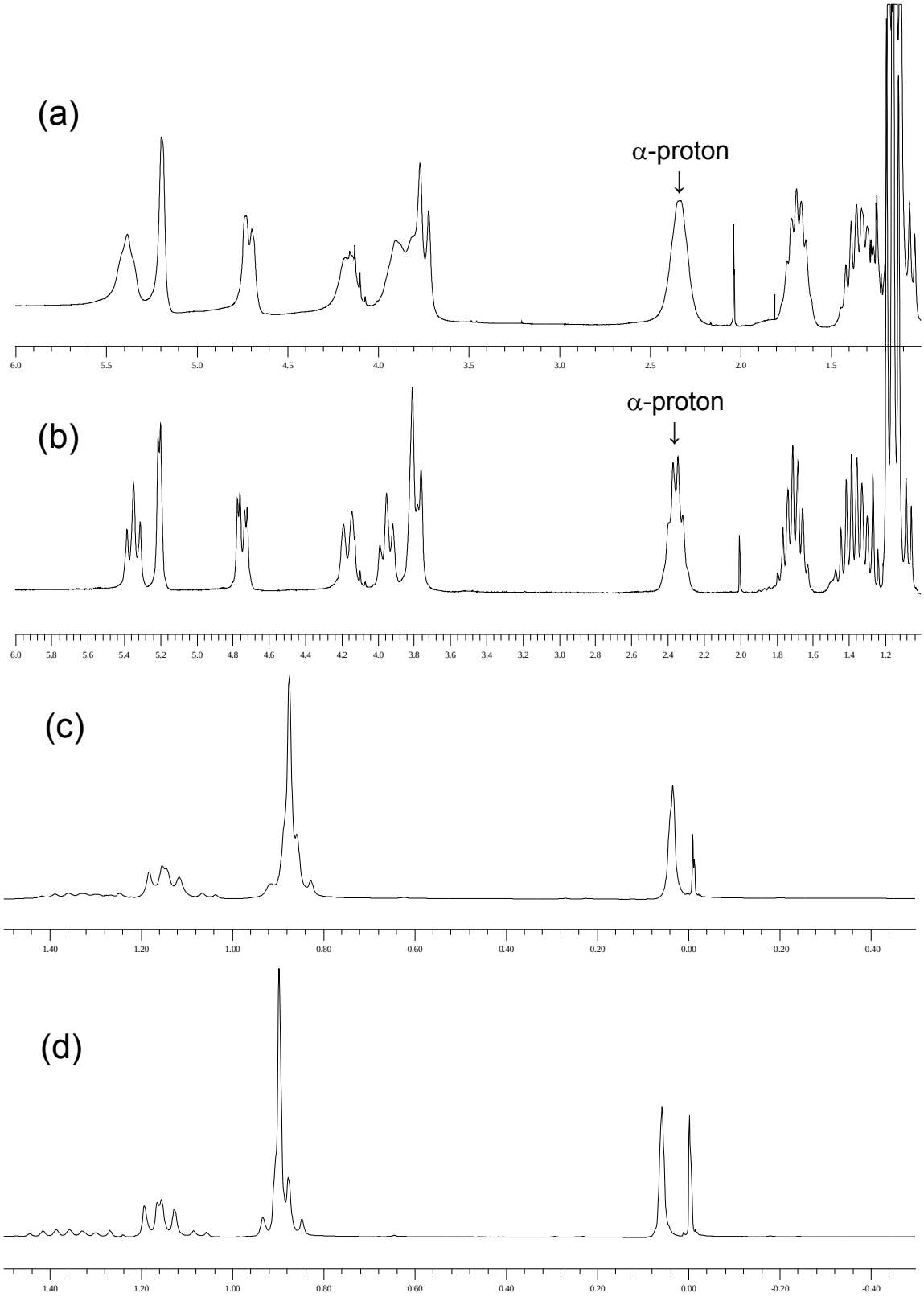


Figure 4.5.2.2 NMR spectra of (*S*)-2-methylbutyryl-6-TBDMS-γ-CD. (a): 1.0 - 6.0 ppm; 295 K (22 °C); (b): 1.0 - 6.0 ppm, 343K (70 °C); (c): -0.5 - 1.5 ppm, 295 K (22 °C); (d) -0.5 -1.5 ppm, 343K (70 °C).

Considering the objective to introduce a chiral 2-methylbutyryl moiety onto the cyclodextrin rim it was necessary to avoid harsh conditions that might result in racemization of the acyl side chain. If moderate bases such as DMAP, triethylamine or pyridine are used, normally no racemizations of 2-methylalkanoic acid moieties are observed. Retention of the configuration has been demonstrated for numerous examples using either (*S*)- or (*R*)-2-methylbutyric anhydride as acylating agent (Moher et al., 1992; Wess et al., 1994; Araki and Konoike, 1997; Lu et al., 1997; Oliver et al., 2003; Cortes-Selva et al., 2004; Ley et al., 2004). In the present study, the absence of such a racemization could be proven by the small but distinct chemical shift differences between the (*S*)- and the (*R*)-2-methylbutyryl-6-TBDMS-γ-cyclodextrins reflecting the diastereomeric differences which alter the magnetic environment around the observed protons. The terminal methyl group signal of the 2-methylbutyryl side chain of the (*R*)-derivative appears at 0.94 ppm slightly lower than that of the (*S*)-derivative (0.89 ppm), whereas the α-methyl group on the 2-methylbutyryl side chain on the (*S*)-derivative is shifted further compared to the (*R*)-derivative (1.14, 1.18 vs. 1.09, 1.13 ppm). In addition, the signals for the protons at the β-carbon of the 2-methylbutyryl side chain of the (*R*)-derivative are shifted downfield compared to the (*S*)-derivative (1.35-1.57, 1.68-1.95 vs. 1.24-1.50, 1.63-1.79 ppm). The signal for the proton at C1 of the (*S*)-derivative is shifted downfield compared to the (*R*)-derivative (5.21 vs. 5.19 ppm). Such differences in chemical shifts are known from other diastereoisomers of 2-methylbutyrated saccharides (York et al., 1997). They confirm that the preparation of the 2-methylbutyryl-6-TBDMS-γ-cyclodextrin proceeded without racemization at the α-methyl position of the side chain.

To further confirm the expected structure, particularly the complete derivatization pattern, additional MS analysis was performed. As shown in Figure 4.5.2.3, the molecular ion was detectable as sodium ion adduct (M+Na: m/z = 3578), accompanied by a small peak of double charged molecular ion. Further MS-MS analysis revealed fragments in 101 m/z intervals representing the consecutive cleavage of 2-methylbutyryl moieties.

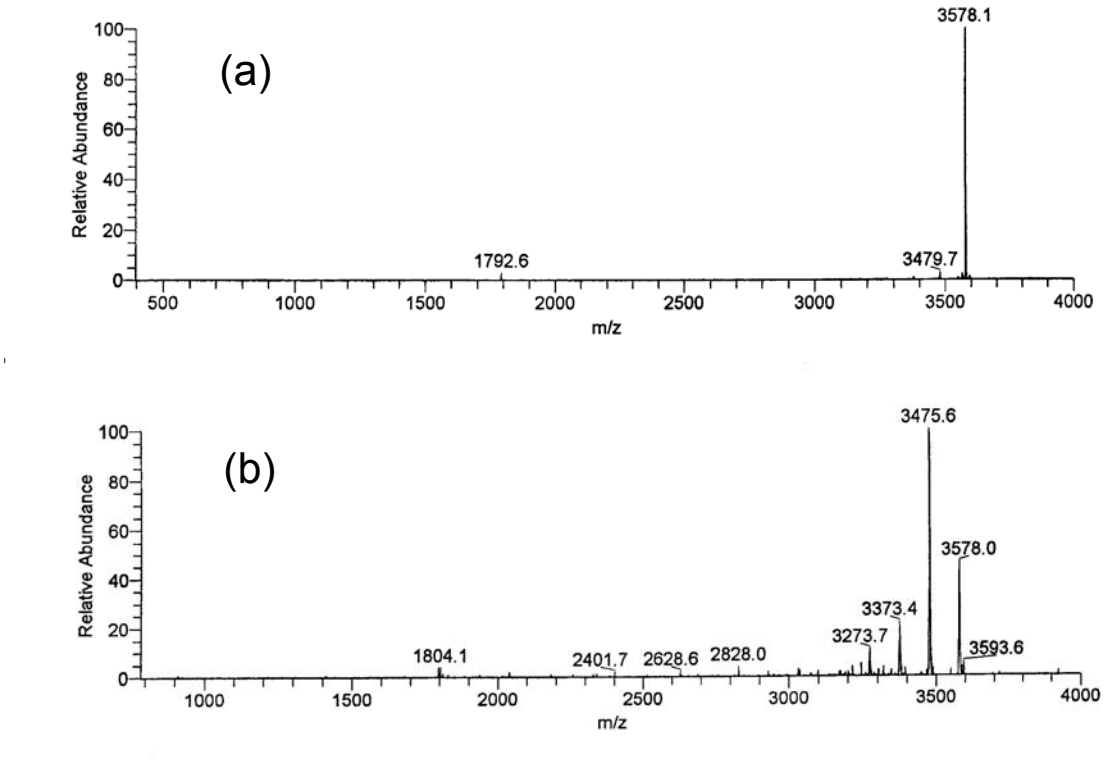


Figure 4.5.2.3 Mass spectral data of (*S*)-2-methylbutyryl-6-TBDMS-γ-CD: (a) MS, (b) MS/MS of m/z = 3578 (for conditions see Material and Methods).

4.5.3. Separation characteristics

A preliminary screening demonstrated that the suitability of (*S*)-2-methylbutyryl-6-TBDMS-γ-CD as chiral stationary phase was rather limited. No enantioseparation could be achieved for representatives of monoterpene hydrocarbons (e.g., limonene, α-pinene, camphene), monoterpene alcohols (e.g. perilla alcohol, linalool), monoterpene ketones (e.g., carvone, pulegone), aliphatic esters (e.g., 2-methylbutyl acetate), γ-lactones (e.g., trans-whiskey lactone) and acids (e.g. mandelic acid).

Two types of compounds which turned out to be resolved into their enantiomers were the aromatic alcohol 1-phenylethanol and δ-lactones. Therefore, these were used for studies on the separation characteristics of (*S*)-2-methylbutyryl-6-TBDMS-γ-CD. The data were compared to those obtained on the corresponding (*R*)-2-methylbutyryl-6-TBDMS-γ-CD and on the CD analog with un-branched side chain, i.e. *n*-butyryl-6-TBDMS-γ-CD.

δ-Lactones

The separations of a homologous series of aliphatic δ-lactones (C_6-C_{12}) on 2,3-(*S*)-2-methylbutyryl-6-TBDMS-γ-CD, 2,3-(*R*)-2-methylbutyryl-6-TBDMS-γ-CD and *n*-butyryl-6-TBDMS-γ-CD are shown in Figure 4.5.3.1. A comparison of the α-values determined under isothermal conditions is given in Table 4.5.3.

Table 4.5.3 α-Values for δ-lactones separated on *n*-butyryl-6-TBDMS-γ-CD (column A), 2,3-(*S*)-2-methylbutyryl-6-TBDMS-γ-CD (column B) and 2,3-(*R*)-2-methylbutyryl-6-TBDMS-γ-CD (column C)

	Column A	Column B	Column C
δ-Hexalactone	1.017 (120 °C)	1.074 (100 °C)	1.000 (100 °C)
δ-Heptalactone	1.058 (120 °C)	1.294 (110 °C)	1.144 (110 °C)
δ-Octalactone	1.118 (120 °C)	1.354 (110 °C)	1.258 (110 °C)
δ-Nonalactone	1.014 (120 °C)	1.049 (110 °C)	1.037 (110 °C)
δ-Decalactone	1.028 (130 °C)	1.013 (120 °C)	1.013 (120 °C)
δ-Undecalactone	1.017 (140 °C)	1.000 (130 °C)	1.000 (130 °C)
δ-Dodecalactone	1.011 (150 °C)	1.000 (140 °C)	1.000 (140 °C)

The separations obtained on the reference column *n*-butyryl-6-TBDMS-γ-CD in this study are better than those previously reported (Maas et al., 1996). This may be explained by the different achiral stationary phases used to dissolve the CD derivative (SE54 vs. OV-1701). The fact that enantioseparations improve with decreasing polarity of the dissolving achiral stationary phase has been reported (Bicchi et al., 1993; Jung and Schurig, 1993; Dietrich et al., 1995).

The δ-lactones show similar separation profiles on the three chiral stationary phases depending on the chain lengths. On all columns δ-octalactone exhibits the best enantioseparation. The resolution drops drastically when the length of the alkyl chain is further increased. For the lactones up to C_9 the α-values on (*R*)-2-methylbutyryl-6-TBDMS-γ-CD are significantly higher than on the un-branched *n*-butyryl-6-TBDMS-γ-CD. On the diastereoisomeric (*S*)-2-methylbutyryl-6-TBDMS-γ-CD the α-values are slightly decreased. However, it is important to note that the order of elution of the enantiomers remained the same as shown for δ-octalactone in Figure 4.5.3.2.

1-Phenylethanol

For 1-phenylethanol separation characteristics comparable to those of the δ-lactones were observed as shown in Figure 4.5.3.3. The enantioseparations on the 2-methylbutyryl-6-TBDMS-γ-CD phases were higher than those on the reference column *n*-butyryl-6-TBMDMS-γ-CD. Again, the α-value on the (*R*)-2-methylbutyryl-6-TBDMS-γ-CD is higher than that on (*S*)-2-methylbutyryl-6-TBDMS-γ-CD. However, the order of elution of the enantiomers remains unchanged.

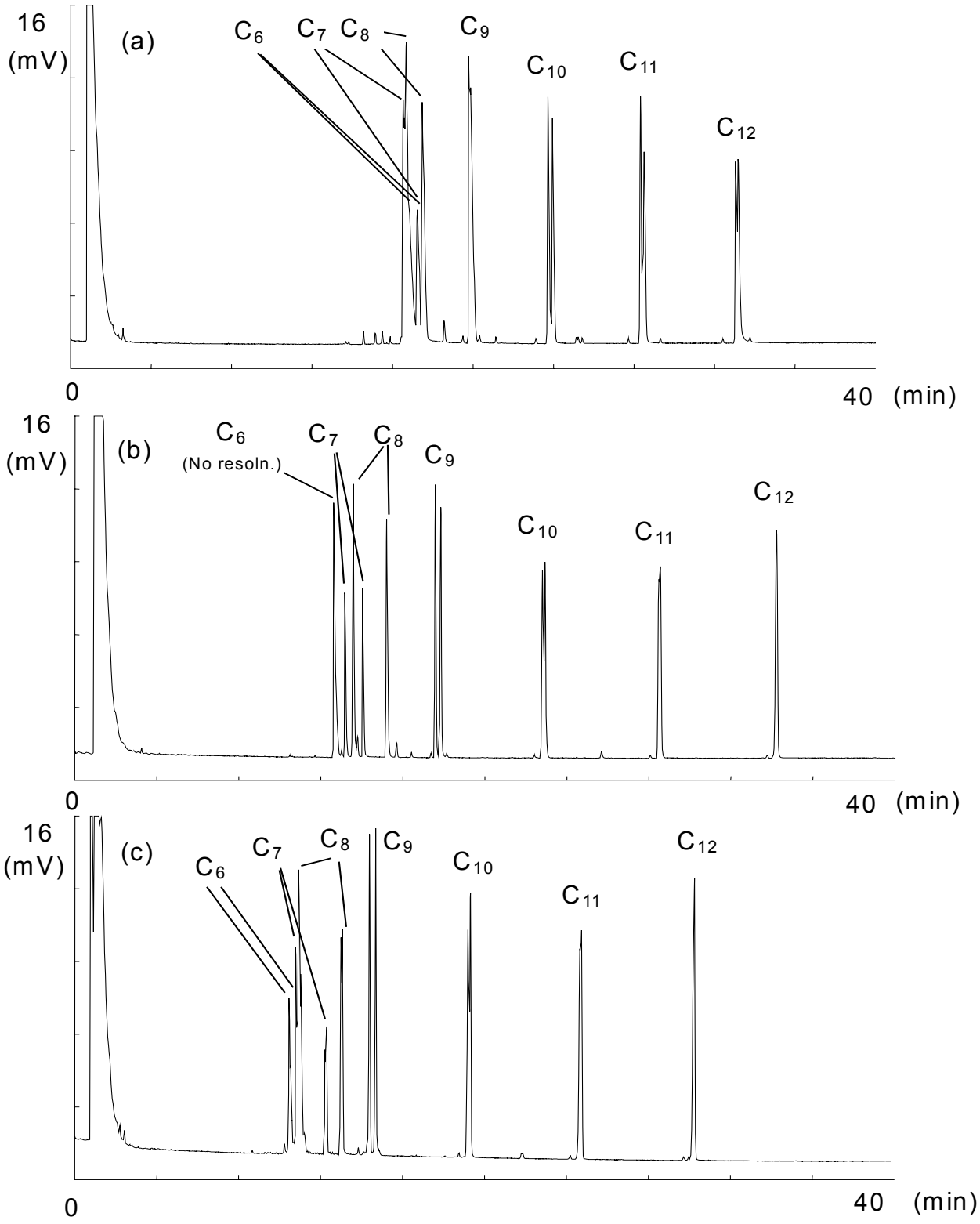


Figure 4.5.3.1 Separation of the enantiomers of δ-lactones on: (a) *n*-butyryl-6-TBDMS-γ-CD, (b) 2,3-(*S*)-2-methylbutyryl-6-TBDMS-γ-CD, (c) 2,3-(*R*)-2-methylbutyryl-6-TBDMS-γ-CD. Temperature program: 100 °C (initial, 2 min hold) then ramp at 2.0 °C/min rate.

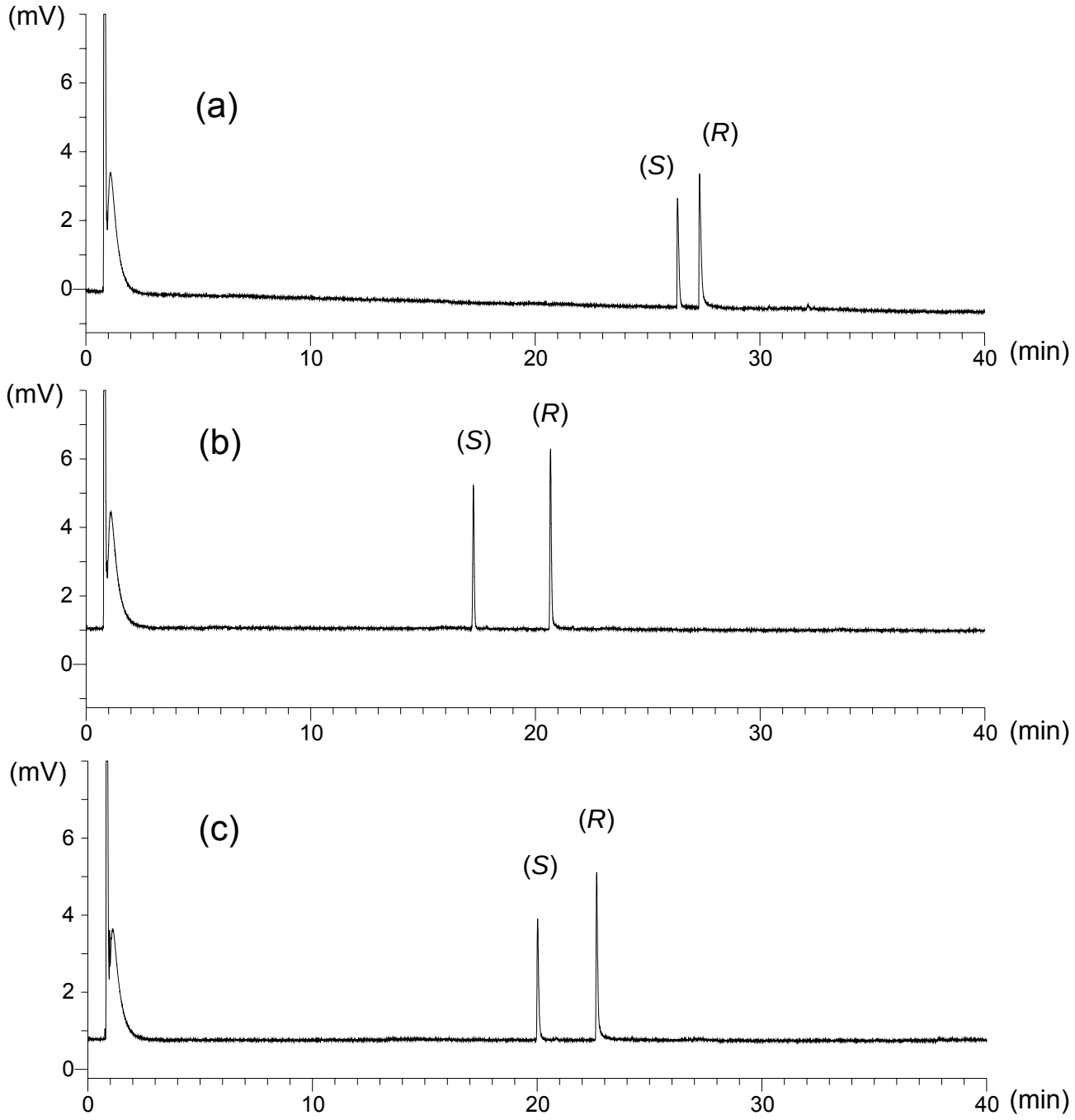


Figure 4.5.3.2 Separation of delta-octalactone [enantiomeric excess (*R*): 12 %] on: (a) 2,3-*n*-butryryl-6-TBDMS-γ-CD; (b) 2,3-(*S*)-2-methyl-butyryl-6- TBDMS-γ-CD; (c) 2,3-(*R*)-2-methyl-butyryl-6-TBDMS-γ-CD. Temperature program: 80 °C (initial, 2 min hold) then ramp at 2.0 °C/min rate.

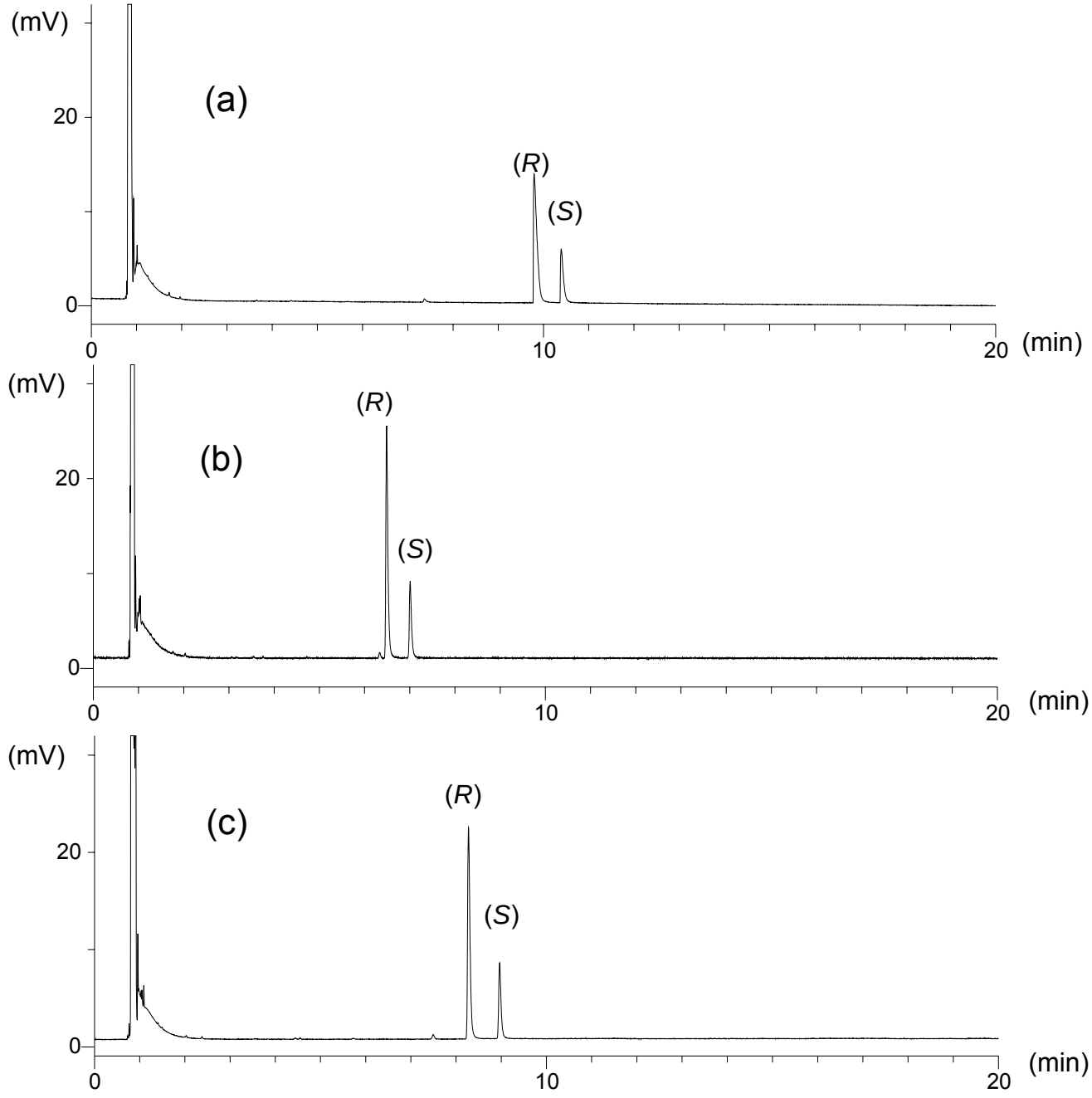


Figure 4.5.3.3 Separation of the enantiomers of 1-phenylethanol [enantiomeric excess (*R*): 47 %] on:
(a) 2,3-*n*-butryryl-6-TBDMS-γ-CD
(b) 2,3-(*S*)-2-methylbutyryl-6- TBDMS-γ-CD
(c) 2,3-(*R*)-2-methylbutyryl-6-TBDMS-γ-CD.
Temperature program: 80 °C (initial, 2 min hold) then ramp at 2.0 °C/min rate.

A wide spectrum of derivatizations of TBDMS-cyclodextrins via alkylations or acylations has been described (see 2.5). However, only one CD phase has been reported which bears a chiral center in its side chain. Permethylated 2-Hydroxypropyl (PMHP) cyclodextrin has been prepared by reaction of cyclodextrin with propylene oxide under alkaline aqueous conditions and subsequent methylation (Armstrong et al., 1990). This reaction sequence does not proceed selectively and hydroxy groups of the cyclodextrin are randomly substituted. Despite the usefulness of this chiral stationary phase for enantioseparations, its structure is not clearly defined. The actual distribution of substituents can be demonstrated by MS analysis (Armstrong et al., 1990).
In contrast, the procedure applied in this study, i.e. derivatization using an acid anhydride, resulted for the first time in a CD with complete derivatization of the 2,3 hydroxy groups with chiral acyl moieties. Therefore, the results obtained as regards the influence of the configuration of the side chain on the order of elution of enantiomers should be more meaningful than those obtained on the above-described CD derivative in which the substitution with chiral moieties proceeded incompletely and randomly. The fact that the configuration of the 2-methylbutyryl side chains had no influence on the order of elution of the enantiomers indicates that the inherent chirality of the CD torus is more important for chiral recognition than the diastereomeric differences induced by the chiral side chains. However, this conclusion should be considered preliminary because the limited versatility of the synthesized 2,3-(*S*)- and (*R*)-2MB-6-TBDMS-γ-CD, respectively, only allowed studies on representatives from two chemical classes (1-phenylethanol and δ-octalactone). In addition, cyclodextrins derivatized with other chiral moieties should be considered.

The spectrum of compounds which could be separated into enantiomers on both (*S*)-2-methylbutyryl-TBDMS-γ-CD and (*R*)-2-methylbutyryl-TBDMS-γ-CD is very limited compared to the unbranched *n*-butyryl-TBDMS-γ-CD, which has been reported as rather effective for enantioseparations of many functional classes (Maas et al., 1996). On the other hand, as shown for the δ-lactones the separation factors are significantly improved on the CD derivatives with the 2-methylbutyuryl side chains compared to the *n*-butyryl-TBDMS-γ-CD for the homologs C_6-C_9. This indicates considerable chiral recognition at the edge of the

cavity or the rim part of the cyclodextrin torus, because the methyl group is only a rather small moiety which would not cover up the whole cavity opening of the γ-CD. If the chiral recognition were solely based on induced-fit mechanisms, this significant impact of a methyl group on the versatility of the chiral stationary phase would not be expected. Taking into account the NMR data, probably the sterically rigid environments adjacent to the 2,3-OH moieties make possible this loss of versatility on one hand and the enhancement of separation performance for a very narrow range of chiral molecules by close-fit interactions on the other hand.

4.6 Outlook

The introduction of acetal moieties as side chains proved to be a useful strategy to improve the properties of cyclodextrins as chiral stationary phases in GC. The outstandingly high α-values observed for important classes of flavor compounds make this type of CD derivatives attractive in terms of preparative applications. It should eventually be possible to isolate the separated enantiomers at large scale, as so far only described for the fluorinated chiral compound 2-chloro-1-(difluoromethoxy)-1,1,2-trifluoroethane (enflurane) using Lipodex E (Schurig et al., 1993).

The phases should also be valuable for sensory assessments of enantiomers via capillary gas chromatography / olfactometry. The high separation factors obtained for many flavor compounds would make it possible to assess the odor properties of enantiomers without the difficulties arising from peak overlapping.

In addition, the new stationary phases should be useful for mechanistic studies. In accordance with former considerations (Schurig and Juza, 1997), the fact that for many compound classes chiral separation factors α significantly higher than 1.3 were observed, should qualify these CD derivatives as useful candidates to determine thermodynamic data and to broaden the knowledge on the mechanisms underlying enantioseparations.

The conclusions drawn for (*S*)- and (*R*)-2MB-6-TBDMS-γ-CD as regards the influence of chiral moieties as side chains must be considered as preliminary, because the limited versatility of the two stationary phases only allowed studies on a few compounds. CD derivatives with other chiral substituents should be investigated to reach more general conclusions.

5. Summary

Cyclodextrin (CD) derivatives are widely used as chiral stationary phases in capillary gas chromatography. Their performance depends substantially on the side chains attached to the hydroxyl groups of the glucose moieties. In this study new classes of CD-derivatives suitable for gas chromatographic (GC) separation of enantiomers were synthesized by introducing (i) acetal functions and (ii) chiral acyl moieties at positions 2 and 3 of the glucose units.

Octakis(2,3-di-*O*-methoxymethyl-6-*O*-tert-butyldimethylsilyl)-γ-cyclodextrin (2,3-MOM-6-TBDMS-γ-CD) obtained by reaction of 6-O-TBDMS-γ-cyclodextrin with methoxymethylchloride (MOM-Cl) was synthesized as first representative of cyclodextrin GC stationary phases containing alkoxymethyl side chains. The structure was confirmed by NMR and mass spectrometry. The suitability of the material diluted in polysiloxane as GC stationary phase was shown. Enantioseparations could be achieved for a broad spectrum of chiral volatiles from various chemical classes. Structural influences of the analytes on the enantiodifferentiations were demonstrated. High separation factors were observed for the hydroxyketone acetoin (α = 1.8) and some methyl branched ketones. Pronounced enantioseparations were also determined for cyclic pentenolone and furanone derivatives.

To investigate the impact of the size of the CD torus, the 2,3-MOM-derivatives of the β- and α-CD analogs were synthesized. The spectrum of compounds for which enantiomers could be separated on 2,3-MOM-6-TBDMS-β-CD was more limited and the enantioseparations achieved were generally less pronounced compared to the γ-CD derivative. However, for 2-alkyl esters unusually high separation factors (α up to 4.31) were observed. Using 2-pentyl acetate as example, phenomena underlying the enantioseparation were investigated by determining thermodynamic parameters. The data showed that only one enantiomer is retained significantly on the chiral stationary phase whereas the other one behaves like the hydrocarbons used as references. The limited spectrum of compounds which could be separated on 2,3-MOM-6-TBDMS-α-CD demonstrated the critical impact of the size of the cyclodextrin on enantioseparations achievable with MOM-type chiral selectors.

The influence of the alkoxymethyl side chains on enantioseparations was

assessed by introducing the elongated ethoxymethyl moiety, the polar (2-methoxyethoxy)methyl group, and the apolar and bulky (2-trimethylsilylethoxy)methyl group, respectively. These modifications reduced the column performance as regards separation factors and/or peak shape.

The second approach was based on the introduction of additional asymmetric centers in the side chains by attaching 2-methylbutyryl groups in positions 2 and 3 of the glucose units. By using the corresponding acid anhydrides, 2,3-di-*O*-[(*S*)-2-methylbutyryl-6-TBDMS]-γ-cyclodextrin and the (*R*)-configured analog could be synthesized. The structures of these first examples of cyclodextrin stationary phases exhibiting defined substitution patterns with chiral moieties were confirmed by NMR and mass spectrometry. Compared to the unbranched *n*-butyryl-TBDMS-γ-CD, an established chiral stationary phase, the presence of the additional α-methyl groups in the side chains reduced the number of compounds for which enantiomers could be resolved. Using 1-phenylethanol and δ-lactones as examples, it could be demonstrated that the configurations of the chiral side chains influenced the separation factors but had no effect on the order of elution of the enantiomers.

The introduction of acetal moieties as side chains proved to be a useful strategy to improve the properties of cyclodextrins as chiral stationary phases in GC. The outstandingly high α-values observed for important classes of flavor compounds make this type of CD-derivatives attractive in terms of preparative applications and sensory assessments of enantiomers by gas chromatography/olfactometry. In addition, these phases seem ideal for further studies on mechanisms underlying the separation of enantiomers via capillary GC.

6. Zusammenfassung

Cyclodextrin (CD) Derivate werden als chirale stationäre Phasen in der Kapillargaschromatographie (GC) eingesetzt. Ihre Eigenschaften werden wesentlich durch die an die Hydroxygruppen der Glucosebausteine gebundenen Seitenreste bestimmt. In dieser Arbeit wurden neue Klassen von Cyclodextrin Derivaten synthetisiert, indem (a) Acetalfunktionen und (b) chirale Gruppen in den Positionen 2 und 3 der Glucoseeinheiten eingeführt wurden.

Oktakis(2,3-di-*O*-methoxymethyl-6-*O*-tert-butyldimethylsilyl-cyclodextrin (2,3-MOM-6-TBDMS-γ-CD) wurde durch Umsetzung von 6-*O*-TBDMS-γ-Cyclodextrin mit Methoxymethylchlorid (MOM-Cl) als erster Vetreter von Cyclodextrin-GC-Phasen, die Alkoxymethyl-Seitenreste aufweisen, synthetisiert. Die Struktur wurde mittels NMR and massenspektrometrischer Untersuchungen bestätigt. Die Eignung des in Polysiloxan verdünnten Materials als stationäre Phase für die GC wurde gezeigt. Enantiomerentrennungen gelangen für ein breites Spektrum chiraler flüchtiger Verbindungen aus unterschiedlichen Stoffklassen. Strukturelle Einflüsse der Analyten auf die Enantiodifferenzierungen wurden aufgezeigt. Hohe Trennfaktoren (α = 1.8) wurden für das Hydroxyketon Acetoin und einige methylverzweigte Ketone ermittelt. Ausgeprägte Enantiomerentrennungen wurden auch für zyklische Pentenolone und Furanon-Derivate beobachtet.

Um den Einfluss der Größe des Cyclodextrinrings zu verfolgen, wurden die 2,3-MOM-Derivate von β- and α-Cyclodextrin synthetisiert. Das Spektrum an Verbindungen, für die auf 2,3-MOM-6-TBDMS-β-CD eine Trennung der Enantiomere möglich war, war kleiner und die erzielten Trennungen grundsätzlich schlechter als auf der γ-CD Phase. Aussergewöhnlich hohe Trennfaktoren (α bis zu 4.31) wurden jedoch für 2-Alkylester beobachtet. Am Beispiel von 2-Pentylacetat wurden die der Enantiomerentrennung zugrunde liegenden Phänomene durch Bestimmung thermodynamischer Parameter untersucht. Die Daten zeigten, dass nur eines der Enantiomere durch die chirale stationäre Phase deutlich zurückgehalten wurde, während das andere sich wie die als Referenz benutzten Kohlenwasserstoffe verhielt. Das begrenzte Spektrum von Verbindungen, die auf 2,3-MOM-6-TBDMS-α-CD getrennt werden konnten, verdeutlichte den Einfluss der Größe des Cyclodextrinrings

auf Enantiomerentrennungen mittels chiraler Pasen vom MOM-Typ.

Zur Untersuchung des Einflusses der Alkoxymethyl-Seitenketten auf die Enantiomerentrennungen wurden die verlängerte Ethoxymethyl Gruppe, die polare (2-Methoxyethoxy)methyl Guppe bzw. der unpolare und sperrige (2-Trimethylsilylethoxy)methyl Rest eingeführt. Diese Modifizierungen verschlechterten die Trennleistungen hinsichtlich Trennfaktoren und/oder Form der Peaks.

Der zweite Ansatz beruhte auf der Einführung zusätzlicher asymmetrischer Zentren in den Seitenketten durch Verknüpfung mit 2-Methylbutyrylresten an den Positionen 2 and 3 der Glucoseeinheiten. Mit Hilfe der entsprechenden Säureanhydride konnten 2,3-Di-*O*-[(*S*)-2-Methylbutyryl-6-TBDMS]-γ-cyclodextrin und das analoge (*R*)-konfigurierte Derivat synthetisiert werden. Die Strukturen dieser ersten Beispiele von Cyclodextrinphasen, die definierte Substitutionsmuster mit chiralen Gruppen aufweisen, wurden mittels NMR und Massenspektrometrie bestätigt. Im Vergleich zum unverzweigten *n*-Butyryl-TBDMS-γ-CD, einer etablierten stationären Phase, wurde durch die Anwesenheit der zusätzlichen α-Methylgruppen in den Seitenketten die Zahl der Verbindungen, deren Enantiomere getrennt werden konnten, reduziert. Am Beispiel von 1-Phenylethanol und von δ-Lactonen konnte gezeigt werden, dass die Konfigurationen der chiralen Seitenketten die Trennfaktoren beeinflussen, jedoch keinen Effekt auf die Elutionsreihenfolge der Enantiomere haben.

Die Einführung von Acetalen als Seitenketten erwies sich als nützliche Strategie, um die Eigenschaften von Cyclodextrinen als chirale stationäre Phasen für die GC zu verbessern. Die aussergewöhnlich hohen α-Werte, die für Vertreter wichtiger Klassen von Aromastoffen beobachtet wurden, machen diesen Typ von Cyclodextrin Derivaten attraktiv für präparative Anwendungen und sensorische Bewertungen von Enantiomeren mittels Gas-chromatographie/Olfaktometrie. Darüber hinaus erscheinen diese Phasen ideal für weiterführende Studien zu Mechanismen von Enantiomerentrennungen mittels Kapillar GC.

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1. Takahisa, E. and Engel, K.-H. 2,3-Di-*O*-methoxymethyl-6-*O*-*tert*-butyldimethylsilyl-γ-cyclodextrin: a new class of cyclodextrin derivatives for gas chromatographic separation of enantiomers. *J. Chromatogr., A*. **2005**, 1063, 181-192.

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Curriculum vitae

Name:	Eisuke TAKAHISA
Date and Place of Birth:	October 05, 1974 in Tokyo (Japan)
Nationality:	Japanese

Educational background:

01, September, 1981 – 31, March, 1987
Elementary School
(Sir Wilfrid Laurier elementary school in Vancouver, Canada)

01, April, 1987 – 90, March, 1975
Junior High School (Hachioji-dainana junior high school in Tokyo)

01, April, 1990 – 31, March, 1993
Senior High School (Hachioji-higashi senior high school in Tokyo)

01, April, 1993 – 31, March, 1997
Tokyo Institute of Technology (Department of Biotechnology, School of Bioscience and Biotechnology)

31, March, 1997
Bachelor degree in Biotechnology

01, April, 1997 – 31, March, 1999
Graduate School of Science and Engineering (Department of Environmental Chemistry and Engineering)

31, March, 1999
Master degree in Engineering

01, April, 1999 －
Researcher (Synthetic chemist) at T. Hasegawa Co., Ltd. (Tokyo, Japan)

01, April, 2001 –
Visiting Scholar at TU München