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ENANTIOSELECTIVE GAS CHROMATOGRAPHY WITH MODIFIED CYCLOMALTO-OLIGOSACCHARIDES AS CHIRAL STATIONARY PHASES

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ABSTRACT

2,3,6-Tri-*O*-pentylated as well as 3-*O*-acetylated/2,6-di-*O*-pentylated derivatives of cyclomalto-hexaose and -heptaose are useful chiral stationary phases for enantioselective capillary g.l.c.

INTRODUCTION

The separation of enantiomers by capillary g.l.c. has become an efficient and sensitive technique for the stereochemical analysis of such natural compounds as pheromones, flavour constituents, amino acids, carbohydrates, *etc.*[1,2]. The technique has been applied also to analyse the enantiomeric composition of the products of asymmetric syntheses[3] and biotransformations[4], to prove the enantiomeric purity of chiral building blocks for the synthesis of natural compounds and pharmaceuticals, and to study the stereochemistry of chemical reactions[5].

Polysiloxanes[6,7] that carry amino acid amides as the chiral selectors have been used almost exclusively for these purposes. The separation of enantiomers is brought about by the formation of diastereoisomeric association complexes formed mainly by hydrogen bonding[8]. Alternatively, chiral transition-metal compounds can be added to the stationary phase[9]. This technique can be used to separate substrates that will co-ordinate with the chiral metal complex.

Although cyclomalto-oligosaccharides (cyclodextrins, CDs) covalently bound to silica gel have been used for the separation of enantiomers by liquid chromatography[10], there have been few reports on the use of CDs[11,12] or methylated CDs[13,14] in g.l.c. Armstrong and his associates have listed[10] the requirements for chiral recognition by cyclomaltoheptaose (βCD): "*The formation of an inclusion complex requires the presence of an aromatic ring system and at least one strong interaction of the included guest molecule with the hydroxyl groups at the entrance of the macrocyclic cavity.*"

Chiral recognition of βCD has been attributed[15,16] to different hydrogen-

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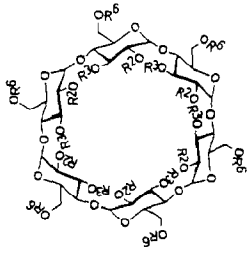
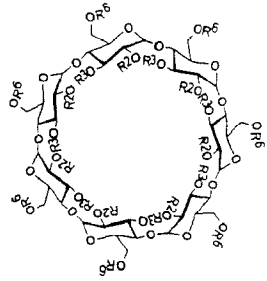
bonding interactions of the enantiomers of the guest molecule and the CD host molecules.

Enantioselective host–guest interactions of rather unpolar substrates and highly hydrophobic derivatives of CD are now reported.

RESULTS AND DISCUSSION

Pentylation of HO-2,6 of cyclomaltohexaose (αCD) and βCD was achieved with pentyl bromide and sodium hydroxide in dimethyl sulfoxide[17]. Further pentylation of the less reactive HO-3 was effected with pentyl bromide and sodium hydride in boiling tetrahydrofuran (5 days under reflux). The 3-*O*-acetyl derivatives were prepared with acetic anhydride–triethylamine in boiling dichloromethane after addition of 4-dimethylaminopyridine[18]. The alkylation products were analyzed for over- or under-alkylation by g.l.c. and mass spectrometry after reductive cleavage. By this method, even 0.1 mol% under- or over-substituted products can be detected[19]. Glass capillary columns were coated with the CD derivatives, and hydrogen was used as the carrier gas.





1 $R^2 = R^3 = R^6 =$ nPn (Lipodex A) **2** $R^2 = R^3 = R^6 =$ nPn (Lipodex C)

3 $R^2 = R^6 =$ nPn, $R^3 =$ Ac (Lipodex B) **4** $R^2 = R^6 =$ nPn, $R^3 =$ Ac (Lipodex D)

Hexakis(2,3,6-tri-O-pentyl)cyclomaltohexaose (**1**, *Lipodex A*)*. — This hydrophobic derivative is soluble in dichloromethane and displays enantioselective interaction with many different types of trifluoroacetylated hydroxy compounds[18], such as 1-phenylethanol, 1,2-*O*-isopropylideneglycerol, epoxyalcohols[20], methyl lactate, 2- and 3-hydroxybutanoate, glycerate, tartrate (Fig. 1), some aldonic acids and mandelic acid, acyclic and cyclic 1,2-diols (Fig. 2), chiral triols, polyols[21], amino alcohols (*e.g.*, sphingosine), tetroses, pentoses, and hexoses (also as methyl glycosides and partially *O*-methylated derivatives)[21], 1,4- and 1,5-anhydroalditols[21], α-halogenated carboxylic acid esters, chiral α-carbonyl compounds (Fig. 3), alkyl halides, spiroacetals[22], and barbiturates[22].

*Capillary columns with the chiral stationary phases Lipodex A–D (protected trade names) are available from Macherey-Nagel, D-5160 Düren, F.R.G.

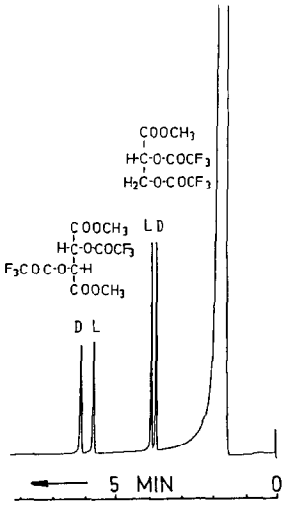


Fig. 1. Separation of the enantiomers of glyceric acid and tartaric acid (40-m glass capillary column with Lipodex A at 80°).

*Heptakis(2,3,6-tri-*O*-pentyl)cyclomaltoheptaose* (**2**, *Lipodex C*). — Trifluoroacetylated alcohols, carbohydrates (Fig. 4), hydroxyacid esters, and cyanohydrins[23] can be separated on this phase. However, the ability to separate the enantiomers of acyclic, monocyclic, and bicyclic olefins is of particular interest[24]. Chiral recognition is observed as long as the chiral centre is adjacent to the double bond (Fig. 5). Enantioselective interaction is also obtained for acyclic and cyclic

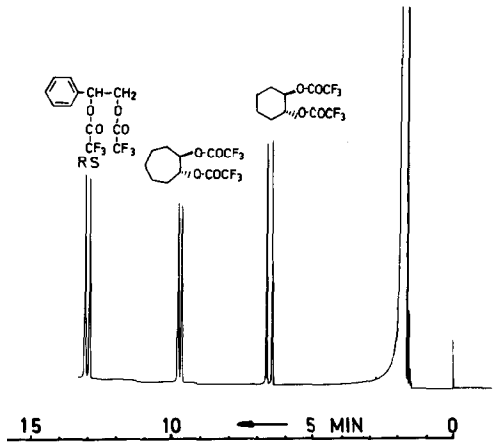


Fig. 2. Separation of the enantiomers of cyclohexane-*trans*-1,2-diol, cycloheptane-*trans*-1,2-diol, and 1-phenylethane-1,2-diol (40-m glass capillary column with Lipodex A at 75° and 3°/min).

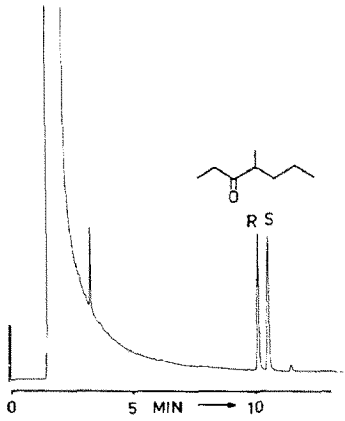


Fig. 3. Separation of the enantiomers of 4-methyl-3-heptanone (40-m glass capillary column with Lipodex A at 85°).

alkyl halides. Baseline separation is limited to compounds with eight or less carbon atoms.

Hexakis(3-O-acetyl-2,6-di-O-pentyl)cyclomaltohexaose (**3**, *Lipodex B*). — This derivative can form inclusion complexes with 5-membered heterocycles. Thus, high enantioselectivity was observed toward γ-lactones (Fig. 6), cyclic carbonates

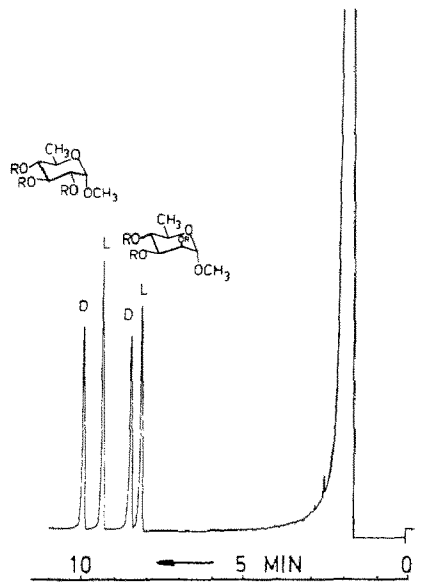


Fig. 4. Separation of the enantiomers of methyl α-rhamnopyranoside and methyl α-quinovopyranoside; R = CF_3CO (42-m glass capillary column with Lipodex C at 90°).

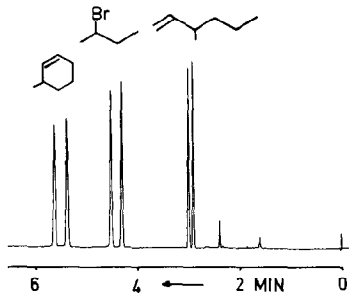


Fig. 5. Separation of the enantiomers of 3-methyl-1-hexene, 2-bromobutane, and 3-methylcyclohexene (42-m glass capillary column with Lipodex C at 20°; head-space injection).

of 1,2-diols, 1-*O*-alkylglycerols, and succinimides[25]. Chiral γ-lactones, which have not been separated hitherto, are common flavour constituents in many fruits[26] and have been identified as pheromones in ants. Large separation factors were observed also for trifluoroacetylated carbohydrate derivatives and other hydroxyl compounds, such as aldols, cyanohydrins (Fig. 7), and amino alcohols.

Hepakis(3-O-acetyl-2,6-di-O-pentyl)cyclomaltoheptaose (**4**, *Lipodex D*). — In addition to the inclusion effect, the Ac group serves as a receptor for hydrogen bonding in **3** and **4**, which may increase the selectivity and allow the separation of polar compounds. High separation factors were obtained for trifluoroacetylated α- and β-chiral amines, amino alcohols (Fig. 8), β-amino acid esters, cyclic *E*-1,2- and 1,3-diols[27], and some lactones and bicyclic ethers (frontalin, brevicomin), which are important pheromone constituents of bark beetles (Fig. 9).

Mechanism of separation. — Whereas hydrogen bonding is the major attractive force for chiral diamide phases (chiral polysiloxanes), molecular inclusion

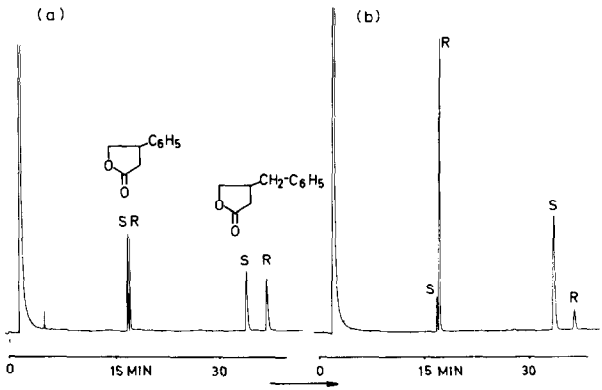


Fig. 6. (a) Separation of the enantiomers of 3-phenyl- and 3-benzyl-butyrolactone and (b) determination of enantiomeric composition of samples prepared by enantioselective synthesis (courtesy of Professor D. Enders, Universität Aachen) (40-m glass capillary column with Lipodex B at 175°).

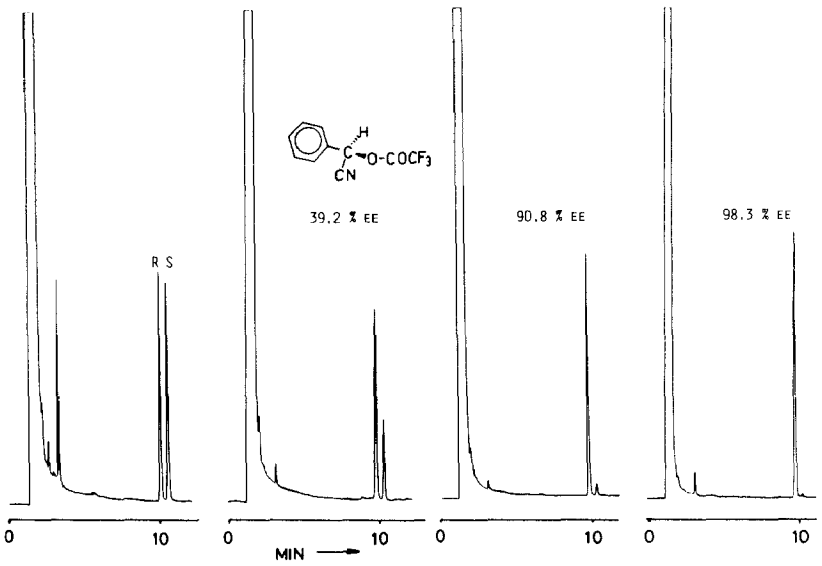


Fig. 7. Separation of the enantiomers of trifluoroacetylated mandelonitrile and determination of enantiomeric excess of samples prepared by enzymic synthesis (courtesy of Dr. T. Ziegler, Universität Stuttgart) (40-m glass capillary column with Lipodex B at 115°).

is essential for the enantioselectivity of CDs. Pentylated maltohexaose, in contrast, exhibited weak enantioselectivity toward chiral substrates. From a large number of racemates which could be resolved on a column with pentylated αCD, only cyclohexane-*trans*-1,2-diol and 1-phenylethylamine could be resolved on a column with pentylated maltohexaose. Since the CD derivatives are amorphous liquids, the structures of inclusion complexes cannot be studied as for the crystalline

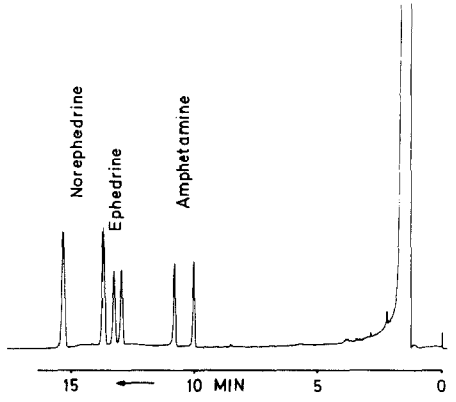


Fig. 8. Separation of the enantiomers of amphetamine, ephedrine, and norephedrine (as *N,O*-trifluoroacetyl derivatives) (40-m glass capillary column with Lipodex D at 150°).

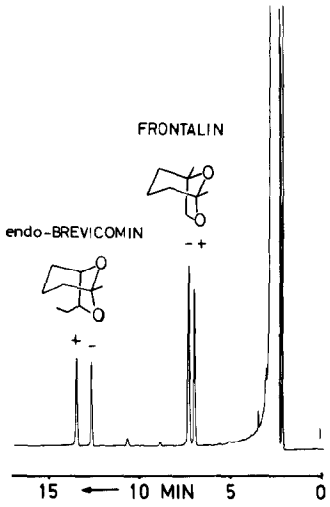


Fig. 9. Separation of the enantiomers of the pheromones frontalin and endo-brevicomin from bark beetles (samples by courtesy of Professor W. Francke, Universität Hamburg) (50-m glass capillary column with Lipodex D at 78°, 5 min isothermal, then 3°/min to 120°).

unmodified CDs. Calculations of lowest energy conformations are also difficult and ambiguous due to the high flexibility of the alkyl chains. The bottom sides of CDs are compressed by polyalkylation[28,29]. There are indications that the diameter of the cavity has a strong influence on the difference in energies of diastereomeric inclusion complexes, as demonstrated in Fig. 10 where the separation of a homologous series of 2-hydroxyacid methyl esters on a column with **1** and **2** is compared. The separation of racemic *o*-, *m*-, and *p*-methyl-substituted 1-phenylethanols on a column of **1** is shown in Fig. 11. All isomers and the unsubstituted derivative are separated except for the *o*-methyl derivative, the shape of which is probably unfavourable for inclusion in the cavity. On a corresponding column with **2**, none of the racemates was separated. The separation factors for trifluoroacetylated 1,2-diols, α-chiral amines, γ-lactones, and some other compounds do not decrease with increasing length of the alkyl chain, at least up to C_{14}. In other compounds, enantioselectivity depends very significantly on the chain length, as observed for 2-hydroxyacid esters and alkyl halides. Thus, not only the functional site but also the size and shape of a molecule may effect enantioselective inclusion. Further investigations are needed in order to obtain a better insight into the mechanisms of the enantioselective host–guest interactions.

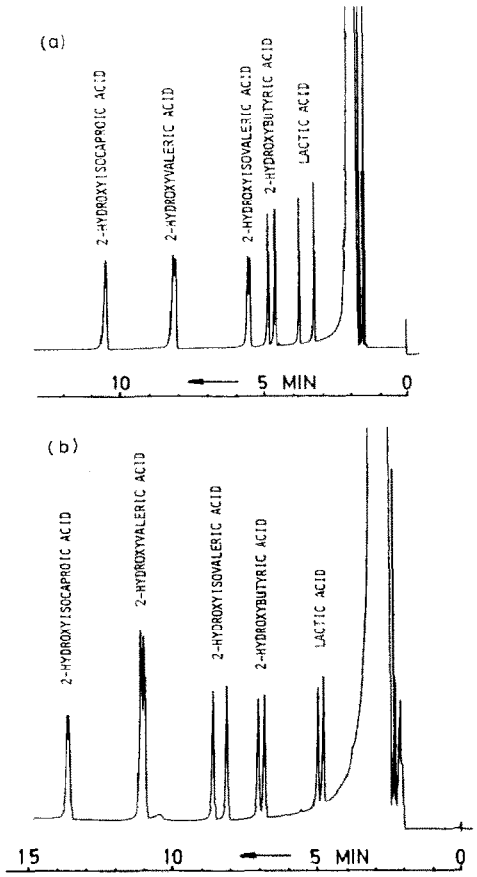


Fig. 10. Separation of the enantiomers of 2-hydroxyacid methyl esters (as the *O*-trifluoroacetyl derivatives) (40-m glass capillary column with (a) Lipodex A and (b) Lipodex C at 60°).

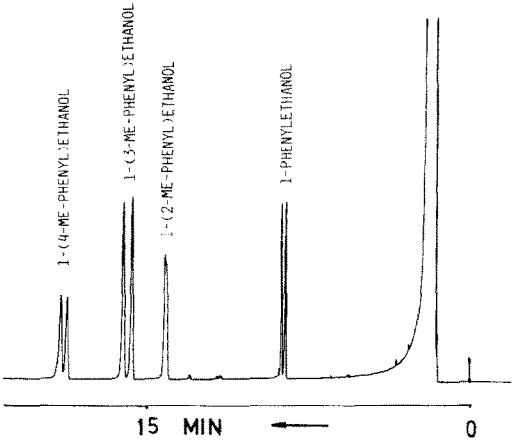


Fig. 11. Separation of the enantiomers of 1-phenylethanol and its *o*-, *m*-, and *p*-methyl derivatives (as the *O*-trifluoroacetyl derivatives) (40-m glass capillary column with Lipodex A at 75°).

EXPERIMENTAL

The preparation of **1** was described previously[21]; **2** was prepared in the same manner by using βCD. The completion of alkylation was proved by g.l.c. and mass spectrometry after reductive depolymerization[19]. The 3-*O*-acetylated products **3** and **4** were obtained by acetylation of hexakis(2,6-di-*O*-pentyl)-α- or βCD[21] with 7 mmol of acetic anhydride, 8 mmol of triethylamine and 0.175 mmol of 4-dimethylaminopyridine. This mixture was boiled under reflux for 24 h in dichloromethane. Another portion of 8 mmol of triethylamine and 7 mmol of acetic anhydride was added and refluxing was continued for another 72 h. The solvent was removed *in vacuo* and the product was taken up in *tert*-butyl methyl ether. After washing the organic phase with water, dil. aq. $NaHCO_3$, water, dil. aq. NaH_2PO_4, and water, the organic solution was concentrated and dried at 0.05 Torr.

The static procedure[30] was used for coating Pyrex-glass capillaries as described previously[21].

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REFERENCES

1 W. A. KÖNIG, *The Practice of Enantiomer Separation by Capillary Gas Chromatography*, Huethig, Heidelberg, 1987.
2 V. SCHURIG, *Angew. Chem.*, 96 (1984) 733–822; *Angew. Chem. Int. Ed. Engl.*, 23 (1984) 747–836.
3 W. A. KÖNIG, E. SCHMIDT, AND R. KREBBER, *Chromatographia*, 18 (1984) 698–700.
4 K. LAUMEN AND M. P. SCHNEIDER, *J. Chem. Soc., Chem. Commun.*, (1988) 598–600.
5 T. BOLTE, D. YU, H.-T. STÜWE, AND W. A. KÖNIG, *Angew. Chem.*, 99 (1987) 362–363; *Angew. Chem. Int. Ed. Engl.*, 26 (1987) 331–332.
6 H. FRANK, G. J. NICHOLSON, AND E. BAYER, *Angew. Chem.*, 90 (1978) 396–397; *Angew. Chem. Int. Ed. Engl.*, 17 (1978) 363–364.
7 W. A. KÖNIG, I. BENECKE, AND S. SIEVERS, *J. Chromatogr.*, 217 (1981) 71–79.
8 B. FEIBUSH, A. BALAN, B. ALTMAN, AND E. GIL-AV, *J. Chem. Soc., Perkin Trans. 2*, (1979) 1230–1236.
9 V. SCHURIG, *Kontakte (Darmstadt)*, (1986) 3–22.
10 D. W. ARMSTRONG, T. J. WARD, R. D. ARMSTRONG, AND T. E. BEESLEY, *Science*, 232 (1986) 1132–1135.
11 E. SMOLKOVA-KEULEMANSOVA, *J. Chromatogr.*, 251 (1982) 17–34.
12 T. KOSCIELSKI, D. SYBILSKA, S. BELNIAK, AND J. JURCZAK, *Chromatographia*, 21 (1986) 413–416.
13 J. SZEJTLI, *Staerke*, 39 (1987) 357–362.
14 V. SCHURIG AND H.-P. NOWOTNY, *J. Chromatogr.*, 441 (1988) 155–163.
15 J. A. HAMILTON AND L. CHEN, *J. Am. Chem. Soc.*, 110 (1988) 5833–5841.
16 K. HARATA, K. UEKAMA, M. OTAGIRI, AND F. HIRAYAMA, *Bull. Chem. Soc. Jpn.*, 60 (1987) 497–502.
17 I. CIUCANU AND F. KEREK, *Carbohydr. Res.*, 131 (1984) 209–217.
18 W. A. KÖNIG, S. LUTZ, AND G. WENZ, *Angew. Chem.*, 100 (1988) 989–990; *Angew. Chem. Int. Ed. Engl.*, 27 (1988) 979–980.

19 P. MISCHNICK-LÜBBECKE AND R. KREBBER, *Carbohydr. Res.*, 187 (1989) 197–202.
20 W. A. KÖNIG, S. LUTZ, G. WENZ, G. GÖRGEN, C. NEUMANN, A. GÄBLER, AND W. BOLAND, *Angew. Chem.*, 101 (1989) 180–181; *Angew. Chem. Int. Ed. Engl.*, 28 (1989) 178–179.
21 W. A. KÖNIG, P. MISCHNICK-LÜBBECKE, B. BRASSAT, AND S. LUTZ, *Carbohydr. Res.*, 183 (1988) 11–17.
22 W. A. KÖNIG, S. LUTZ, AND G. WENZ, *Proc. Int. Symp. Cyclodextrins, 4th, Munich, 1988*, Kluwer Academic Publishers, Dordrecht, 1989 p. 456.
23 W. A. KÖNIG, S. LUTZ, R. KREBER, G. WENZ, K. BALDENIUS, J. EHLERS, AND H. TOM DIECK, *J. High Res. Chromatogr., Chromatogr. Commun.*, 12 (1989) 35–39.
24 J. EHLERS, W. A. KÖNIG, S. LUTZ, G. WENZ, AND H. TOM DIECK, *Angew. Chem.*, 100 (1988) 1614–1615; *Angew. Chem. Int. Ed. Engl.*, 27 (1988) 1556–1557.
25 W. A. KÖNIG, S. LUTZ, C. COLBERG, N. SCHMIDT, G. WENZ, E. VON DER BEY, A. MOSANDL, C. GÜNTHER, AND A. KUSTERMANN, *J. High Res. Chromatogr., Chromatogr. Commun.*, 11 (1988) 621–625.
26 A. MOSANDL, A. KUSTERMANN, U. PALM, H.-P. DORAU, AND W. A. KÖNIG, *Z. Lebensm.-Unters. Forsch.*, 1989, in press.
27 W. A. KÖNIG, S. LUTZ, E. VON DER BEY, AND G. WENZ, *J. High Res. Chromatogr., Chromatogr. Commun.*, 11 (1988) 506–509.
28 K. HARATA, K. UEKAMA, M. OTAGIRI, AND F. HIRAYAMA, *Bull. Chem. Soc. Jpn.*, 55 (1982) 3904–3910.
29 R. BRESLOW, M. F. CZARNIECKI, J. EMERT, AND H. HAMAGUCHI, *J. Am. Chem. Soc.*, 102 (1980) 762–769.
30 J. BOUCHE AND M. VERZELE, *J. Gas Chromatogr.*, 6 (1968) 501–505.