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Use of 6-*O-tert.*-butyldimethylsilylated ß-cyclodextrins for the enantioseparation of chiral organochlorine compounds

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Abstract

Enantioseparations of 26 chiral organochlorine compounds (α-hexachlorocyclohexane, toxaphene, chlordane, atropisomeric polychlorinated biphenyls) were studied with GC–electron-capture detection. The chiral stationary phases (CSPs) consisted of 35% heptakis (6-*O-tert.*-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin (β-TBDM) diluted in OV1701. One CSP was made of pure β-TBDM (purity>99%) while the other was randomly silylated β-TBDM. High-temperature gas chromatography showed that this CSP contained five products in almost equal amounts. Both β-TBDM columns were installed in parallel in the same GC system. On the randomly silylated β-TBDM, aromatic compounds had shorter and aliphatic compounds had longer retention times than on the purified β-TBDM. The randomly silylated β-TBDM resolved the enantiomers of 24 compounds (16 of them were baseline separated) and the purified β-TBDM only six (four of them were baseline separated). Thus, the purification of β-TBDM decreased the efficiency of the chiral resolution of organochlorine compounds on β-TBDM. The results clearly demonstrate that the randomly silylated β-TBDM contains one or more side products more suitable for the enantioseparation of organochlorine compounds than the purified β-TBDM.

Keywords: Enantiomer separation; Chiral stationary phases, GC; Heptakis (6-*O-tert.*-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin stationary phases; Organochlorine compounds; Pesticides; Polychlorinated biphenyls; Toxaphene

1. Introduction

The enantioseparation of chiral organochlorines and particularly compounds of technical toxaphene (CTTs) is still a challenge for analytical chemists and producers of chiral stationary phases (CSPs). The first successful enantioseparations of compounds of technical toxaphene (CTTs) were published in 1994 [1,2]. In these and follow-up studies, *tert.*-butyldimethylsilylated β-cyclodextrin (β-BSCD) was applied as the CSP [1–6]. β-BSCD was introduced by Blum and Aichholz in 1990 [7]. The authors already described that the product of the synthesis was composed of several silylation products, and randomly *tert.*-butyldimethylsilylated β-cyclodextrin seems to be an appropriate description of the β-BSCD phase. Unfortunately, the composition and quality of β-BSCD phases varied from batch to batch. At least in the case of the enantiomers of the α-hexachlorocyclohexane (α-HCH), the elution order was reversed on β-BSCD columns prepared from different batches [6,8]. This is dissatisfying for analytical chemists. On the other hand, for a long time it was thought that enantioseparations of CTTs are restricted to β-BSCD which made the β-BSCD indispensable for environmental analysis [3]. Recent-

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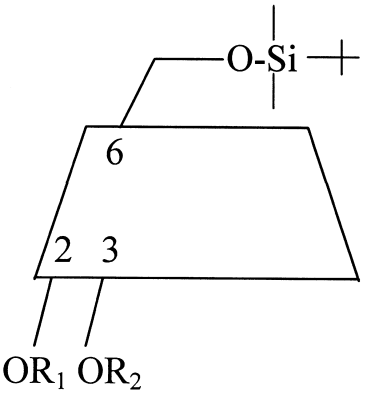
ly, it was shown that heptakis (6-*O*-*tert.*-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin (β-TBDM) also enantioseparated several CTTs [9]. As in the case of β-BSCD, β-TBDM benefits from the huge substituent on the primary carbon C-6 of the cyclodextrin's glucose units (see Fig. 1). This bulky substituent blocks the narrow entrance of the torsus-shaped cyclodextrins, having an improving effect on the enantioseparation of non-polar compounds [10]. The first step of the β-TBDM synthesis is silylation of the primary carbons [11–13], followed by methylation of the secondary carbons C-2 and C-3 of the glucose units of cyclodextrins. The purification step prior to methylation is necessary in order to obtain well-defined β-TBDM phases [12]. Unfortunately, such purification steps were obviously not performed with the chiral stationary β-TBDM phases used in our earlier presentations [9,14]. Since these β-TBDM phase were only randomly silylated we expected differences from batch to batch and also in comparison with the purified β-TBDM phase [15]. To investigate this, chiral resolution characteristics were compared on two β-TBDM phases: randomly silylated β-TBDM and purified β-TBDM which contained >99% of heptakis (6-*O*-*tert.*-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin.

Enantioseparations of 26 chiral organochlorine compounds [CTTs, polychlorinated biphenyl (PCB) atropisomers, chlordane, α-HCH, *o,p′*-DDT] were studied on the two β-TBDM phases.



ß-TBDM: $R_1 = R_2 =$ methyl,

ß-BSCD: $R_1 = R_2 =$ *tert.*-butyldimethylsilyl

Si = *tert.*-butyldimethylsilyl

Fig. 1. Schematic representation of *tert.*-butyldimethylsilylated β-cyclodextrins.

2. Material and methods

2.1. Standard compounds and chemicals

Enantioenriched e-aeee-pentachlorocyclohexene-1 (β-PCCH) and aaeeee-1,2,3,4,5,6-hexachlorocyclohexane (α-HCH) were obtained by treatment with brucine followed by isolation on silica [8]. Enantioenriched oxychlordane was from Dr. Ehrenstorfer, Augsburg, Germany. Enantiopure PCB 84 and PCB 132 were gifts from Dr. Haglund, Umeå University, Sweden, produced according to the method recently described [16]. All further solutions were only available as racemates, which were purchased from Promochem, Wesel, Germany or from Dr. Ehrenstorfer, Augsburg, Germany. The following compounds of technical toxaphene were studied: 2,2,5-endo,6-exo,8,9,10-heptachlorobornane (systematic AV-code: B7-515 [17]; Parlar-number: No. 32 [18]), 2-endo,3-exo,5-endo,6-exo,8,8,9,10-octachlorobornane (B8-1412; –), 2-endo,3-exo,5-endo,6-exo,8,8,10,10-octachlorobornane (B8-1413; No. 26), 2-endo,3-exo,5-endo,6-exo,8,9,10,10-octachlorobornane (B8-1414; No. 40), 2-exo,3-endo,5-exo,8,9,9,10,10-octachlorobornane (B8-1945; No. 41), 2-exo,5,5,8,9,9,10,10-octachlorobornane (B8-2229; No. 44), 2,2,5,5,8,9,9,10,10-nonachlorobornane (B9-1025; No. 62), and 2-endo,3-exo,5-endo,6-exo,8,8,9,10,10-nonachlorobornane (B9-1679; No. 50).

2.2. Chiral stationary phases

The two β-TBDM columns consisted of 35% heptakis (6-*O*-*tert.*-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin diluted in OV1701, respectively. Two different qualities of the CSP were available to us.

The first one contained randomly silylated β-TBDM. The CSP was a gift from M.D. Müller (Swiss Federal Research Station, Wädenswil, Switzerland) and the column was prepared by G. Hottinger. This phase was used in our earlier presentations [9,19].

The second β-TBDM phase consisted of 6-*O*-persilylated β-TBDM (saturation degree approx. 99%). This column is commercially available from BGB Analytik (Switzerland).

Both columns had a length of 20 m length, an internal diameter of 0.25 mm, and a film thickness of 0.15 μm. The columns were made with the same method and the same chemicals except the modified cyclodextrins.

2.3. Enantioselective gas chromatography with electron-capture detector (GC–ECD)

Enantioseparations were performed on an HP 5890 (Hewlett-Packard) gas chromatograph. The splitless injector was connected to a hose which divided the sample onto the two β-TBDM columns installed in the GC oven, each of them ending in ^{63}Ni electron-capture detectors. The injector and detector temperatures were set at 230°C and 270°C, respectively. Helium was used as carrier gas (column head pressure 1.0 bar) and nitrogen as the make-up gas.

The GC oven was programmed in the following manner: 120°C, 2 min, with 15°C/min to 150°C (50 min) in the case of α-HCH or to 180°C (100 min), then 20°C/min to 220°C (10 min) in the case of the CTTs.

All other compounds were eluted with the following temperature program: 60°C, 2 min, then at 25°C/min to 160°C, 10 min, 1°C/min to 200°C, 10°C/min to 220°C, 20 min. The compounds eluted during the slow heating rate of 1°C/min.

2.4. High-temperature gas chromatography with flame ionisation detection (HTGC–FID)

High-temperature GC analyses of the chiral stationary phases were performed according to Blum and Aichholz [20] and Deege et al. [21]. A Carlo Erba HR 5300 Mega gas chromatograph was used which was equipped with a flame ionisation detector. Hydrogen was the carrier gas at a column head pressure of 0.2 bar. The samples were introduced via a high oven temperature (h.o.t.) cold on column injector. The detector temperature was set at 480°C. A high-temperature fused-silica (10 m×0.32 mm I.D.) coated with 0.2 μm d_f PS089 was used as the stationary phase. The GC oven program started at 300°C, after 1 min the temperature was raised at 5°C/min to 420°C which was held for 5 min. The total run time was 30 min.

3. Results

Characterization of the β-TBDM phases tested in this study was performed with thin layer chromatography according to method described by Dietrich et al. [12]. Only one compound was detected for the purified product which corresponded to the pure β-TBDM. This observation is in agreement with findings of Mosandl et al. which described synthesis and isolation of the purified β-TBDM [12] and similar CSPs [13,22,23]. On the other hand, several dots were detected in the case of the randomly silylated β-TBDM. For a closer study, high-temperature gas chromatography [20,21] was applied to the randomly silylated β-TBDM. The gas chromatogram showed five significant peaks and based on the FID peak height, the pure 6-*O*-*tert.*-butyldimethylsilyl-2,3-di-*O*-methyl-β-cyclodextrin only arose for approx. 20% of the CSP. Due to the shorter retention times of the side products, it is plausible that the side products have more methyl groups than β-TBDM. This was recently confirmed by LC–MS studies of the randomly silylated β-TBDM [24]. With this technique, the Oehme group identified peaks corresponding to one to three additional methyl groups instead of *tert.*-butyldimethylsilyl groups on the β-cyclodextrin [24].

Installation of the two β-TBDM columns in the same GC oven allowed a direct comparison of the separation characteristics. Table 1 lists elution temperatures, retention times and chiral resolution characteristics of 26 chiral organochlorine compounds on the two columns. A remarkable phenomenon was observed with respect to the elution temperatures. The PCB atropisomers, *o,p′*-DDE and β-PCCH eluted faster from the randomly silylated β-TBDM, while all other compounds (CTTs, chlor-

Table 1
Elution characteristics of chiral organochlorines on two β-TBDM phases

Compound	Randomly silylated β-TBDM		Purified β-TBDM	
	Chiral resolution (%)	Elution temp.; t_R1/t_R2	Chiral resolution (%)	Elution temp.; t_R1/t_R2
α-HCH	100	150°C; 20.8C/21.7	>100	150°C; 19.9/21.8
β-PCCH	25	160°C; 13.8/13.9	>100	161/164°C;17.5/20.0
Oxychlordane	30	189°C; 45.5/45.7	>20	178°C; 33.7/33.9
cis-Chlordane	100	194°C; 49.9/50.5	100	185/186°C;41.2/41.8
trans-Chlordane	>100	194/196°C; 50.5/51.8	0	185°C; 41.2/41.2
Heptachlor	10	179°C; 35.2/35.4	0	167°C; 23.5/23.5
cis-Heptachlorepoxide	50	192°C; 47.7/48.0	0	170°C; 35.5/35.5
trans-Heptachlorepoxide	>100	191/192°C; 47.0/48.4	0	181°C; 36.9/36.9
o,p-DDT	100	196°C; 51.9/52.4	0	198°C; 53.9/53.9
PCB 84	100	180°C; 35.8/36.4	0	187°C; 42.9/42.9
PCB 95	25	175°C; 30.8/31.0	100	183°C; 38.9/39.4
PCB 132	100	193°C; 48.5/49.3	0	210°C; 57.4/57.4
PCB 144	10	185°C; 40.5/40.6	0	194°C; 49.8/49.8
PCB 149	70	186°C; 41.7/42.1	30	196°C; 51.4/51.7
PCB 171	100	220°C; 58.4/58.9	0	220°C; 63.4/63.4
PCB 174	0	200°C; 55.9/55.9	0	220°C; 62.7/62.7
PCB 183	75	197°C; 52.5/52.9	0	220°C; 59.9/59.9
B7-515 (Parlar No. 32)	>100	180°C; 76.9/80.1	0	180°C; 49.6/49.6
B7-1453 (−)	>100	180°C; 30.1/32.2	0	180°C; 24.9/24.9
B8-1412 (−)	100	180°C; 40.6/41.6	0	180°C; 34.9/34.9
B8-1413 (Parlar No. 26)	0	180°C; 41.1/41.1	0	180°C; 31.4/31.4
B8-1414 (Parlar No. 40)	>100	180°C; 72.4/77.1	80	180°C; 60.9/61.9
B8-1945 (Parlar No. 41)	>100	180°C; 66.5/72.4	90	180°C; 54.8/55.4
B8-2229 (Parlar No. 44)	>100	180°C; 84.5/96.4	0	180°C; 54.8/54.8
B9-1025 (Parlar No. 62)	>100	220°C; 113.3 /115.5	0	180°C; 89.5/89.5
B9-1679 (Parlar No. 50)	>100	180°C; 72.4/75.7	0	180°C; 59.8/59.8

dane related compounds, and α-HCH) had shorter retention times on the purified β-TBDM column: except for β-PCCH, the *aromatic* compounds eluted *faster* from the *randomly silylated* β-TBDM and the *aliphatic* compounds eluted *faster* from the *purified* β-TBDM. Another example to support this observation is (the non-chiral) hexachlorobenzene (HCB). On the randomly silylated β-TBDM, HCB (t_R 12.5 min) eluted much earlier than α-HCH (t_R 17.4, 18.0 min) while on purified β-TBDM, HCB (t_R 18.0 min) eluted between the α-HCH enantiomers (t_R 17.3, 18.3 min) using the same temperature program as for PCBs.

Enantioseparations of chiral organochlorines are usually governed by enthalpy control [3] and, therefore, the best chiral resolution is obtained with isothermal elution. In this presentation enantioseparation characteristics of most of the compounds (except the CTTs and α-HCH) were studied using a slow GC heating rate of 1°C/min. Although less suitable than isothermal elution, this technique provides many results within a few injections. In fact, all compounds except CTTs and α-HCH were chromatographed using the same temperature program (see Section 2). On the other hand, the enantioseparations were not optimized and, consequently, presentation of distinct chiral resolution values (R_s) or separation factors α was avoided. Instead of R_s or α we chose a value representing the percentage of the valley between the two enantiomers. A value of 0 means no chiral resolution ($R_s=0$), a value of 50 corresponds with a valley of 50%, a value of 100 with baseline separation of the enantiomers ($R_s\approx1$), and so on. Together with the retention times, this allowed a simple comparison of the values obtained on the two β-TBDM columns.

On the randomly silylated β-TBDM all components except PCB 174 and B8-1413 (Parlar No. 26) were at least partly enantioresolved. Sixteen of the 26 compounds were baseline resolved. In contrast to

that, only four compounds were baseline separated on the purified β-TBDM while the enantiomers of 18 compounds remained unresolved. In all cases except β-PCCH and PCB 95 the enantioseparation on the randomly silylated β-TBDM was more efficient.

The elution order of the PCB atropisomers was the same on both CSPs, but in the CTT group some deviations were observed. For example, B8-1413 (Parlar No. 26) eluted in front of B8-1412 from the purified β-TBDM but the unresolved peak of B8-1413 eluted between the B8-1412 enantiomer on the randomly silylated β-TBDM column. B7-515 (Parlar No. 32) and B8-2229 (Parlar No. 44) eluted comparably later, B8-1414 (Parlar No. 40) earlier from the randomly silylated β-TBDM phase. Fig. 2 shows the ECD chromatograms of a seal blubber extract of B7-1453, B8-1412 and B8-2229 (Parlar No. 44) on the two β-TBDM columns. None of the compounds was enantioseparated on the purified β-TBDM while the randomly silylated β-TBDM exhibited fantastic enantioseparation characteristics [9].

Only a few enantiopure or enantioenriched compounds were available. Elution sequences were determined for the enantiomers of α-HCH, oxychlordane, PCB 84, and PCB 132. On the randomly silylated β-TBDM column the (−)-enantiomer eluted prior to the (+)-enantiomer in each of the four cases. On the purified β-TBDM phase, only oxychlordane and α-HCH were resolved. Interestingly, (+)-α-HCH eluted before the levorotary enantiomer (see Fig. 3). In this case peak reversal was observed in dependence of the quality of β-TBDM.

4. Discussion

The peak reversal of the α-HCH enantiomers which was observed on the two β-TBDM columns of different quality adds another example to the case recently discussed for *tert.*-butyldimethylsilylated β-cyclodextrin (β-BSCD) [6]. On β-BSCD, this effect was interpreted in the following manner: some components of the chiral stationary phase eluted the (+)-enantiomer prior to the (−)-enantiomer and others vice versa [6]. Due to the experience with (randomly silylated) β-BSCD it was expected that randomly silylated β-TBDM may also be obtained with different composition. Consequently, chiral resolution obtained on the present randomly silylated β-TBDM phase may not be reproducible on another

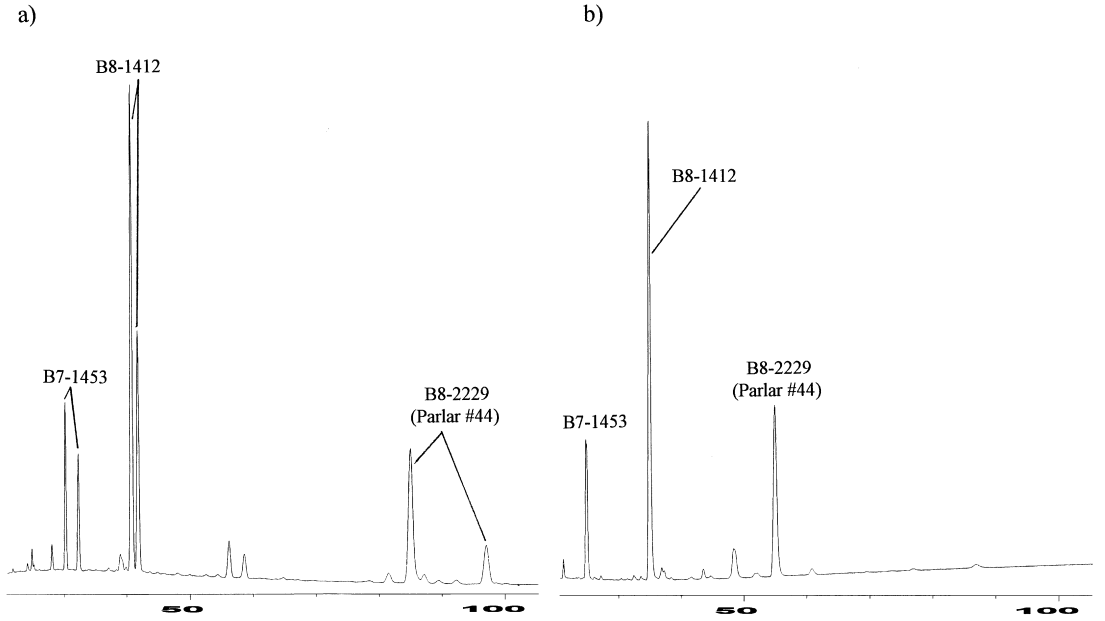


Fig. 2. Enantioseparation of B7-1453, B8-1412, and B8-2229 (Parlar No. 44) in an isolate from seal blubber. (a) randomly silylated β-TBDM; (b) purified β-TBDM.

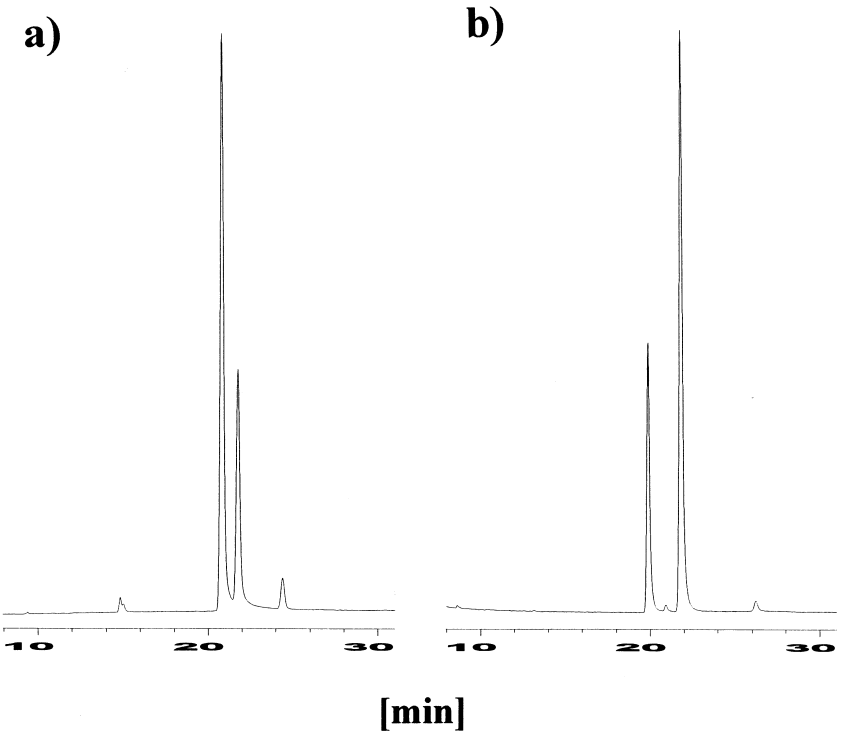


Fig. 3. Elution order of the enantiomers of α-HCH in dependence on the quality of β-TBDM. Injection of a solution with enantioenriched (−)-α-HCH. (a) randomly silylated β-TBDM: (−)-α-HCH eluted prior to (+)-α-HCH; (b) purified β-TBDM: (+)-α-HCH eluted prior to (−)-α-HCH.

randomly silylated β-TBDM phase made from another synthesis batch. This explains some deviating results on the present randomly silylated β-TBDM and another (randomly silylated) β-TBDM phase earlier described by some of us [14]. For example, (+)-α-HCH was the first eluted enantiomer on the earlier tested β-BSCD [14] while it eluted later from the present randomly silylated β-TBDM (see Table 1). This is a further argument for the checking of elution orders with enantioenriched or enantiopure standards [8].

Furthermore, these uncertainties in the composition and properties point against the use of randomly silylated chiral stationary phases such as β-TBDM and β-BSCD. On the other hand, the purified β-TBDM was only suitable for the enantioseparation of a few organochlorines (see Table 1). Of the four baseline separated compounds, the first eluted α-HCH and the second eluted β-PCCH enantiomer co-eluted with the temperature program applied. The first eluted *cis*-chlordane enantiomer co-eluted with the unresolved *trans*-chlordane; both compounds are usually present in environmental samples. PCB 95 was the only compound which allows for an application of purified β-TBDM in environmental studies. Enantioseparation of PCB 95 is, however, also possible on 2,3,6-tri-*O*-methyl-β-cyclodextrin [25], 2, 3-di- *O* -methyl-6- *O* -thexyldimethylsilyl- β -cyclodextrin [26], and 2,6-di-*O*-methyl-3-*O*-*n*-pentyl-β-cyclodextrin [27] which also enantioseparated further chiral organochlorines. Therefore, the purified β-TBDM which successfully enantioseparated a high number of chiral compounds [12], is barely suitable for the enantioseparation of chiral organochlorines.

On the other hand, the present randomly silylated β-TBDM enantioseparated a high number of chiral organochlorines. Most of the compounds were baseline separated and sometimes the chiral resolution values were exceptionally high [9]. This fact, however, deserves a closer study of the randomly

silylated β-TBDM phase. Obviously, the intermediate purification step reduced the chiral resolution of almost all of the compounds studied. There must be side products of the synthesis which enable a better enantioseparation than the purified well-defined β-TBDM. Therefore, we suggest isolation of well-defined side products of β-TBDM synthesis which may allow for a better enantioseparation of chiral organochlorines than the purified β-TBDM and maybe also the randomly silylated β-TBDM. Unfortunately, we have to annotate that the results obtained by some of us during recent years on randomly silylated β-TBDM [9,14] may not be reproducible on other randomly silylated β-TBDM phases. This also points to the production of well-defined β-TBDM phases, which should, however, not be restricted to the isolation of per-modified cyclodextrins.

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