A Convenient Synthesis of a Permethyl-Substituted β-Cyclodextrin-Containing Polysiloxane Stationary Phase Using an Amide Linking Group

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Received September 1, 1995

A novel amide-linked permethyl-substituted β-cyclodextrin-bound polysiloxane stationary phase was prepared in only four steps. First, mono(6-*O*-toluenesulfonyl)-β-cyclodextrin was treated with sodium azide. Second, the resulting azide derivative was treated with methyl iodide and base followed by reduction with hydrogen to give amine-substituted permethylcyclodextrin **3**. Third, cyclodextrin **3** was treated with *p*-(allyloxy)benzoyl chloride to form 6^A-(*p*-allyloxybenzamido)-substituted permethyl-β-cyclodextrin **4**. Lastly, β-cyclodextrin **4** was hydrosilylated onto a polysiloxane backbone containing hydrogen, methyl, and *p*-tolyl substituents. This new phase separated the enantiomers of certain chiral lactones and alcohols in capillary supercritical fluid chromatography.

J. Heterocyclic Chem., **32**, 1715 (1995).

Introduction.

The analytical separation of enantiomers has immediate significance in the pharmaceutical industry. Chiral separations can be realized with either chiral stationary phases or chiral mobile phases. Chiral stationary phases, because of their effectiveness and convenience, play a dominant role in modern chromatographic separations using gas chromatography (GC) [1-5], liquid chromatography (LC) [6,7], and supercritical fluid chromatography (SFC) [8-12]. There continues to be much research interest and effort to find stable and universal enantioselective stationary phases.

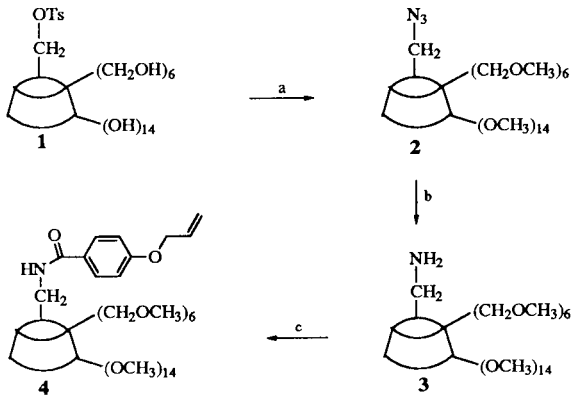
Because of superior efficiency, thermal and chemical stability, and chiral selectivity, different chiral stationary phases based on the polysiloxane backbone have been prepared. The phases can be categorized according to their chiral functional groups, namely amino acid derivatives [13], metal complexes [14], and cyclodextrin-containing [1] phases. The phases containing cyclodextrin are the most widely applicable and available stationary phases today. The relationships between cyclodextrin phases with different substituents [2], different weight percentages, different methods and positions of cyclodextrin attachment, different lengths and structures of the attaching units, and the possibility to immobilize the phase have been systematically investigated [1-12,15-17]. To our knowledge, a cyclodextrin phase with an amide connecting group has not been reported.

From our recent and systematic studies on cyclodextrin-containing polysiloxanes as chiral stationary phases, we found that the phase which has a pendant permethyl-substituted β-cyclodextrin attached to a polysiloxane backbone by one *p*-(allyloxy)phenyl spacer provides superior chiral resolution in both SFC and GC [10,15-18]. In the course of developing new cyclodextrin based chiral stationary phases, a more efficient and straight-forward method to attach the cyclodextrin was desired. Most of the usual methods to prepare the cyclodextrin-containing polysiloxane stationary phases involve a multi-step process which includes either comparatively expensive reagents or difficulties in product purification [15]. In order to simplify the preparation procedure, the amide group combined with the *p*-(allyloxy)phenyl group was used as a new spacer to connect the cyclodextrin with the polysiloxane backbone. The resulting amide-linked stationary phase not only takes just four steps to prepare, but the intermediate alkenylamidocyclodextrin is easier to purify. In this paper, the synthesis of the β-cyclodextrin-containing polysiloxane stationary phase containing the amide-linkage is reported. The utility of the new phase is demonstrated by the separation of three chiral solutes in SFC.

Results and Discussion.

The chiral stationary phase composed of a permethylated β-cyclodextrin chemically bonded to a polysiloxane backbone has proven to be one of the most universally applicable stationary phases [4]. The nature of the connecting spacer between the cyclodextrin and polysiloxane backbone [5] and the connection point at specific cyclodextrin positions [17] have been major research areas for cyclodextrin stationary phases. Usually, the preparation of β-cyclodextrin intermediates involves protecting the primary hydroxy groups with (*tert*-butyl)dimethylsilyl chloride which is relatively expensive. Another route to these cyclodextrin-containing phases is to make the permethylated 3-*O*-(*p*-allyloxy)benzoyl-β-cyclodextrin from native β-cyclodextrin. However, purification of mono[3-*O*-(*p*-allyloxybenzoyl)]heptakis (2,6-di-*O*-methyl)-β-cyclodextrin is not easy [17]. The new synthesis of a chiral phase containing an amide linkage reported herein not only avoids these problems, but the phase can be made on a large scale. The procedures to prepare this new stationary phase are shown in Schemes I and II. The amide moiety

Scheme I. Preparation of Permethyl-Substituted 6-*O*-(*p*-Allyloxybenzamido)-β-Cyclodextrin **4** [a]

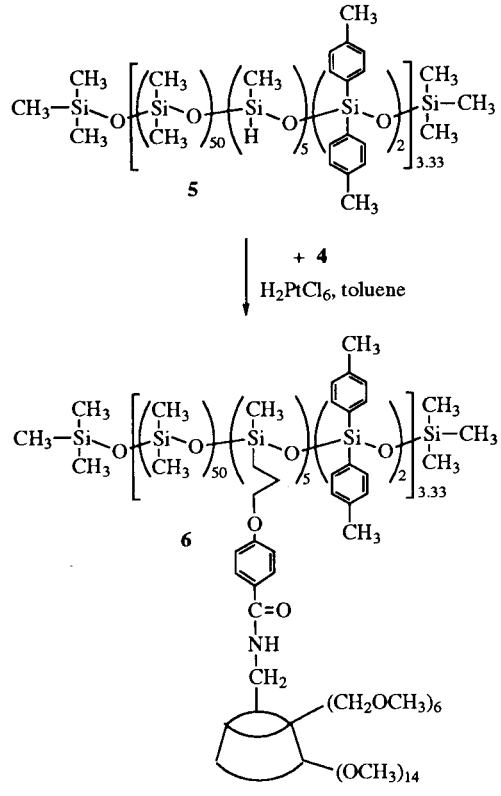


[a] Key: (a) 1. NaN_3, DMF; 2. NaH, CH_3I, DMF; (b) H_2, PtO_2, C_2H_5OH; (c) *p*-(allyloxy)benzoyl chloride, NEt_3, toluene.

does not interfere in the subsequent hydrosilylation reaction shown in Scheme II [19].

Mono (6-*O*-toluenesulfonyl)-β-cyclodextrin **1** was prepared in a 31% yield following the reported procedure [20]. Nucleophilic substitution by sodium azide and permethylation of **1** were accomplished to produce permethylated 6-azido-6-deoxy-β-cyclodextrin **2** (Scheme I). TLC analysis (silica gel, using 20/1: chloroform/methanol) of the crude product indicated the presence of two minor by-products. Their R_f values were very close [0.57, 0.53 (**2**), 0.38].

Scheme II. Preparation of Permethylated β-Cyclodextrin-Bound Polysiloxane **6**



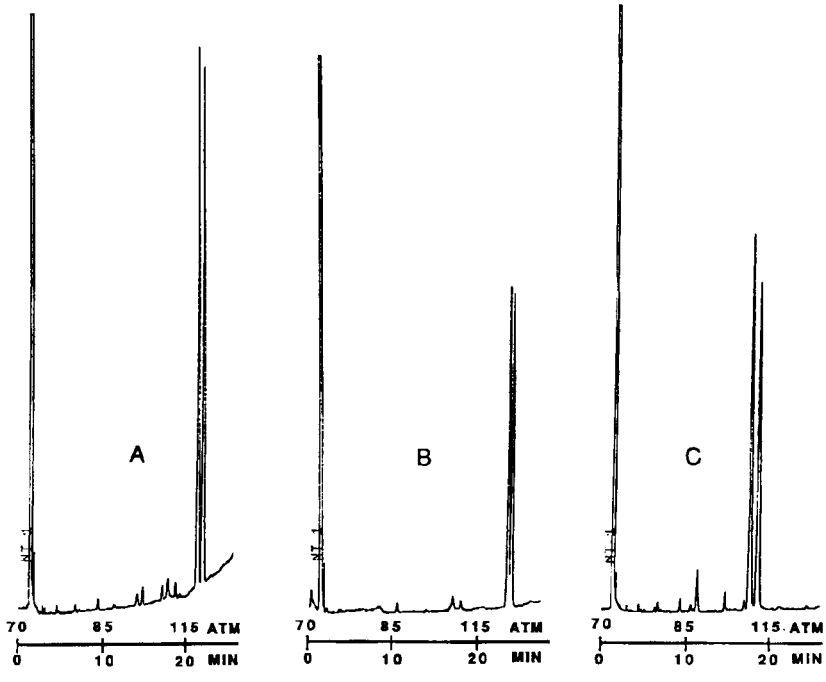


Figure 1. SFC chromatogram of (±)-*t*-2-phenyl-1-cyclohexanol (A), (±)-γ-phenyl-γ-butyrolactone (B), and (±)-pentolactone (C). Conditions: 10 m x 50 μm i.d. capillary column coated with the newly synthesized stationary phase (d_f = 0.2 μm), integral restrictor, 80°, FID, CO_2, pressure programmed from 70 to 180 atm at a rate of 3 atm per minute after a 5-minute initial isobaric period.

Fortunately, product **2** was obtained by column chromatography, and was directly hydrogenated [21] in the presence of platinum dioxide in ethanol to give crude product **3**, which also contained two minor by-products. In this case, their R_f values were quite different. Pure **3** (R_f value of 0.45) was obtained by column chromatography. The overall yield of **3** was 74% (from monotosylate ester **1**). Treatment of **3** with a little less than one equivalent of *p*-(allyloxy)benzoyl chloride in the presence of triethylamine in toluene gave permethylated *p*-(allyloxy)benzamido-β-cyclodextrin **4** in a yield of 86%.

Preparation of permethylated β-cyclodextrin-bound polysiloxane **6** is shown in Scheme II. Copolymer **5** was prepared as reported [17]. Alkene-substituted **4** and the appropriate amount of **5** to provide an equimolar concentration of Si-H functions were used in the hydrosilylation reaction. Assuming that all of the alkene-substituted cyclodextrin reacted with **5**, the resulting polymer would have a ratio of 50 dimethylsiloxanes to 5 permethylcyclodextrin-substituted siloxanes to 2 ditolyl-substituted siloxanes. The tolyl groups are present for cross-linking purposes.

Open-tubular column SFC is will-suited to the separation of enantiomers [5,8,10,11]. SFC offers faster analysis than LC and lower analysis temperatures than GC. This not only enhances enantioselectivity, but also prevents thermal decomposition and racemization. Figure 1 shows SFC separations of the enantiomers of (±)-*t*-2-phenylcyclohexanol, (±)-γ-phenyl-γ-butyrolactone and (±)-pentolactone. It can be seen that under the experimental conditions, the new phase did exhibit excellent enantioselectivity towards these chiral solutes. The phase separated other chiral solutes such as (±)-α-pinane (α = 1.03) and (±)-3,3-dimethyl-2-butanol (α = 1.03). A separation factor (α) of 1.03 indicates baseline separation. This column was stable for over six months of normal operation.

EXPERIMENTAL

Proton and carbon nmr spectra were recorded in deuteriochloroform on a Varian Gemni 200 MHz spectrometer. β-Cyclodextrin (Aldrich) was dried over phosphorus pentoxide under vacuum at 100° for 24 hours before use. Organic extracts were dried over anhydrous magnesium sulfate. Mono (6-*O*-toluenesulfonyl)-β-cyclodextrin (**1**) was prepared as reported [20].

6^A-Azido-6^A-deoxyheptakis(2,3-di-*O*-methyl)-6^B,6^C,6^D,6^E,6^F,6^G-hexa-*O*-methyl-β-cyclodextrin (**2**) (Scheme I).

A mixture of **1** (16.5 g, 12.8 mmoles) and sodium azide (5.00 g, 76.9 mmoles) in 300 ml of dimethylformamide was stirred at 120° for 100 minutes. After cooling to room temperature and filtering, the solution was added dropwise to a suspension of sodium hydride (18.5 g, 0.77 mole) in 700 ml of dimethylformamide, and then iodomethane (120.1 g, 0.85 mole) was dropped slowly into the mixture at 0°. The resulting mixture was stirred at room temperature for 24 hours. Methanol (20 ml) was slowly added to the reaction mixture at 0° to decompose the excess sodium hydride. Most of the dimethylformamide was distilled under reduced pressure and the residue was partitioned between chloroform and water. The organic layer was separated, washed successively with water, aqueous sodium thiosulfate and water, and then dried and concentrated. The residual dimethylformamide was removed under vacuum. One tenth of the crude product was chromatographed on silica gel (80/1: chloroform/methanol) to give pure compound **2**, mp 97-99°; $[\alpha]_D^{25}$ + 155.1° (c = 1.37, chloroform); ir (potassium bromide): 2924, 2100, 1458, 1400, 1158, 1107, 1041, 971 cm^{-1}; ^1H nmr δ 5.16-5.00 (m, 7H), 4.00-3.26 (m, 95H), 3.17 (dd, J_1 = 3.0 Hz, J_2 = 9.24 Hz, 7 H); ^{13}C nmr: δ 99.6, 99.5, 99.4, 82.6, 82.4, 82.3, 81.9, 80.8, 80.7, 80.6, 71.9, 71.6, 71.4, 71.3, 62.0, 61.8, 59.4, 59.0, 58.9, 52.8.

Anal. Calcd. for $C_{62}H_{109}N_3O_{34}$: C, 51.69; H, 7.63; N, 2.92. Found: C, 51.49, H, 7.49; N, 2.88.

6^A-Amino-6^A-deoxyheptakis(2,3-di-*O*-Methyl)-6^B,6^C,6^D,6^E,6^F,6^G-hexa-*O*-methyl-β-cyclodextrin (**3**) (Scheme I).

A solution of **2** (16.1 g, 11.2 mmoles) in 200 ml of ethanol was shaken with hydrogen (50 psi) in the presence of platinum oxide (0.65 g) at room temperature for 4 days. The mixture was filtered through a Celite 545 pile and the filtrate was evaporated to dryness under reduced pressure. The residue was purified by column chromatography (20/1: chloroform/methanol) to give 11.67 g (74%) of **3**, mp 91-93°; $[\alpha]_D^{25}$ + 143.4° (c = 0.93, chloroform); ir (potassium bromide): 3434, 2927, 1508, 1458, 1400, 1143, 1108, 1040, 971 cm^{-1}; ^1H nmr: δ 5.20-4.96 (m, 7 H), 3.94-3.24 (m, 95 H), 3.16 (dd, J_1 = 3.30 Hz, J_2 = 9.44 Hz, 7 H), 3.00 (s, NH, 2 H); ^{13}C nmr: δ 99.5, 99.4, 99.3, 83.1, 82.5, 82.4, 82.2, 81.0, 80.9, 80.6, 80.4, 77.7, 72.1, 71.7, 71.6, 71.4, 71.3, 69.6, 62.0, 61.8, 61.6, 59.6, 59.4, 59.1, 58.9, 58.7, 43.2.

Anal. Calcd. for $C_{62}H_{111}NO_{34}\cdot 2H_2O$: C, 51.34; H, 7.99; N, 0.97. Found: C, 51.44; H, 7.80; N, 0.99.

6^A-[*p*-(Allyloxy)benzamido]-6^A-deoxyheptakis(2,3-di-*O*-methyl)-6^B,6^C,6^D,6^E,6^F,6^G-hexa-*O*-methyl-β-cyclodextrin (**4**) (Scheme I).

A solution of *p*-(allyloxy)benzoyl chloride (1.70 g, 8.7 mmoles) in 100 ml of toluene was added dropwise to a stirred solution of **3** (11.7 g, 8.3 mmoles) and triethylamine (2.0 g, 19.8 mmoles) in 150 ml of toluene at room temperature over 1 hour. The mixture was stirred at room temperature for 24 hours. The mixture was filtered and the filtrate was diluted with 150 ml of toluene and washed twice with water. The organic layer was dried and concentrated. The residue was crystallized from ethyl ether/hexane to give white crystalline **4** (11.1 g, 86%), mp 110-112°; $[\alpha]_D^{25}$ + 141.0° (c = 1.40, chloroform); ir (potassium bromide) 3446, 2930, 2832, 1654, 1606, 1499, 1458, 1384, 1249, 1161, 1109, 1038, 973, 853, 755, 706; ^1H nmr: δ 7.73 (d, J = 8.73 Hz, 2 H), 6.90 (d, J = 8.73 Hz, 2 H), 6.72 (broad s, 1 H), 6.03 (m, 1 H), 5.46-4.99 (m, 9 H), 4.53 (d, J = 3.22 Hz, 2 H), 4.20-3.05 (m, 102 H); ^{13}C nmr: δ 167.3, 161.6, 133.1, 129.3, 127.4, 118.5, 100.1, 99.5, 99.4, 99.3, 82.9, 82.6, 82.5, 82.3, 82.2, 82.0, 81.2, 81.1, 81.0, 80.5, 80.3, 77.8, 72.1, 71.9, 71.6, 71.5, 71.3, 70.8, 69.3, 62.1, 62.0, 61.8, 61.7, 61.6, 59.8, 59.4, 59.2, 58.9, 58.8, 58.6, 41.0.

Anal. Calcd. for $C_{72}H_{119}NO_{36}$: C, 54.40; H, 7.70; N, 0.90.

Found: C, 54.20; H, 7.55; N, 0.84.

Preparation of β-Cyclodextrin-containing Methylpolysiloxane (**6**) (Scheme II).

Alkene **4** (1.8 g, 1.2 mmoles), copolymer **5** [17] (1.03 g, 1.2 mmoles), and 12 g of toluene were placed in a 50 ml Teflon centrifuge tube. Parafilm was placed around the cap to keep out moisture. The mixture was heated in an oil bath at 85-90° for 72 hours, and the solvent was evaporated. A solution of the residue in 10 ml of methylene chloride was washed with 10 ml of methanol and 10 ml of water. The mixture was centrifuged and the water-methanol layer was removed. This process was repeated five more times. The solvent was evaporated and the residue was dried under vacuum at 60° for 20 hours to give 2.51 g (89%) of **6**. The proton nmr spectrum of **6** was consistent with this structure.

Column Preparation.

Fused silica capillary tubing was first deactivated using a cyano deactivation reagent [22]. The deactivated tubing was then statically coated with the synthesized chiral stationary phase at 40° using a 1:1 methylene chloride/*n*-pentane mixture as solvent. The concentration of the coating solution was controlled to give an approximate film thickness of 0.2 μm. After coating, the column was first purged with pure nitrogen for about 30 minutes, then purged with nitrogen bubbled through azo-*tert*-butane (ATB) for *ca.* 60 minutes. The column was sealed at both ends and the stationary phase was cross-linked under temperature programming from 40° to 220° at a rate of 4° per minute and held at the final temperature for 30 minutes. After cross-linking, the column was rinsed with 0.5 ml of methylene chloride, and dried with a nitrogen purge. Finally, the column was conditioned in a GC system with helium as carrier gas by temperature programming from 40° to 230° at a rate of 1° per minute, and holding for 2 hours at 230°.

Column Evaluation.

The finished capillary column with integral restrictor at the end was directly connected to a Lee Scientific SFC system and conditioned under SFC conditions at 100° with pressure programming from 80 to 280 atm at a rate of 3 atm per minute, and holding overnight before use. The injector was kept at ambient temperature, and the injection time was 1.2 seconds. The flow split ratio was approximately 1:10. The detector was kept at 375°. The expand carbon dioxide flow at 100 atm inlet pressure was approximately 0.8 ml per minute. The separation conditions are listed in Figure 1.

Acknowledgment.

We thank Merck Research Laboratories (Department of Pharmaceutical Research and Development, West Point, PA, USA) for supporting this work.

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