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**SEPARATION MATERIALS FOR CHROMATOGRAPHY AND
ELECTROPHORESIS APPLICATIONS COMPRISING CYCLODEXTRINS
CROSS-LINKED AND CHEMICALLY BONDED TO A SUPPORT VIA
URETHANE LINKAGES**

FIELD OF THE INVENTION

The present invention relates to the development of novel separating materials for use in processes such as high performance liquid chromatography (HPLC), liquid chromatography (LC), thin layer chromatography (TLC), capillary electrophoretic chromatography (CE) and counter-current chromatograph. The materials comprise amine-bearing support materials and cyclodextrins mutually cross-linked and chemically bonded via urea linkages. The invention further relates to processes for the production of these separating materials and their use in separating compounds and especially resolving enantiomeric mixtures.

10 BACKGROUND OF THE INVENTION

The generic applicability of cyclodextrins in chromatographic separation and purification processes is described at length in reviews by W. L. Hinze, *Separation and Purification Methods*, 1981, **10(2)**, 159-237; Y. Kawaguchi, et al., *Anal. Chem.*, 1983, **55**, 1852; D. M. Armstrong, et al., *Anal. Chem.*, 1985, **57**, 234 and S. Li, et al., *Chem. Rev.*, 1992, **92**, 1457. Chromatographic separation on chiral stationary phases (CSP) is also the most convenient analytical method for the determination of enantiomeric purity (see for example S. G. Allenmark, *Chromatographic Enantioseparations: Methods and Applications*, 2nd ed., Prentice Hall, NJ, 1991). In recent years, tremendous research efforts were made in bonding cyclodextrins to solid matrices, such as silica gel, via amino or amido linkages. However, these bonds are inherently unstable to hydrolysis, thus placing severe limitations on use of these materials in aqueous media. Alternative approaches for immobilizing cyclodextrin using hydrolytically more stable ether linkages (US. Pat. No. 4,539, 399) or carbamic acid moieties (US. Pat. No. 503,898) were also investigated. However, in all these approaches, regioselective derivatization of cyclodextrin cannot be readily effected due to the presence of multiple hydroxy

moieties in the cyclodextrin starting materials. Thus, reaction may take place on the 2, 3 or 6- position of cyclodextrin which is hard to resolve.

It was often reported that derivatized cyclodextrin stationary phases for liquid chromatography show definite enantioselectivity for a variety of compounds while
5 pristine cyclodextrin bonded stationary phases depict low enantioselectivity. Thus, as an example, enantioselectivity of the materials was generally improved with increasing degree of derivatization of the -OH groups on cyclodextrin with carbamate groups, and by increasing the surface concentration of cyclodextrin immobilized on the support materials (D. W. Armstrong et al., *Anal. Chem.*, 1990, **62**, 1610; T. Hargitai et al., *J.*
10 *Chromatogr.*, 1993, **628**, 11; T. Hargitai, et al., *J. Liq. Chromatogr.*, 1993, **16**(4), 843). In order to maximize the extent of cyclodextrin derivatization, large molar excesses of derivatizing reagents under vigorous conditions were often used. However, the derivatization processes invariably involved the prior immobilization of underivatized cyclodextrin on the support material followed by functionalization procedures
15 involving solid-liquid phases. This usually results in partial derivatization of the hydroxyl groups of the cyclodextrin with large, sterically encumbered substituents consistently having a lower extent of derivatization. In addition, these methods did not give good reproducibility or uniformity of product with the consequence that separation of enantiomers may vary from batch to batch of the cyclodextrin-based CSP obtained.

20 Recently a procedure of immobilizing perfunctionalized cyclodextrin onto the surface of a support via a urethane linkage has been patented which represents an efficient method with well defined chemical structure and very good reproducibility (Ng *et. al.*, Singapore Pat. No. 9703059-7; U.S. Patent Application No. 09/140,073). However, in these examples, and in the other instances listed above, the cyclodextrins
25 were immobilized onto the support as small molecules, which potentially limits their stability in mobile phases with high aqueous content.

Polysiloxane with cyclodextrin anchored to its side chain has been prepared and coated onto the surface of silica gel. This material exhibits interesting properties in reverse phase HPLC (V. Schurig, et al., *J. Chromatogr. A*, 1996, **755**, 299; V. Schurig,
30 et al., *Ger offen DE 43 24 636 A1*(1994); V. Schurig, et al., *Angew. Chem., Int. Ed. Engl.*, 1994, **33**, 2222.]. However, there is no report of the cyclodextrin polymer immobilized onto a support and applied in chiral separation.

SUMMARY OF THE INVENTION

One objective of this invention is to provide novel and improved CSP materials comprising a support material and cyclodextrin or perfunctionalized cyclodextrins mutually cross-linked and chemically bonded to the support via multiple urea moieties.

5 Another objective of the invention is to provide methods for the preparation of such materials.

Yet another objective of the invention is to provide the use of the separating material in chromatography such as in HPLC, LC, TLC and CE, for separating or purifying compounds.

10 A method for enantioseparation of racemic mixtures of chiral compounds is a further object of the invention.

A further objective of the invention is to provide a process for the production of the separating material in which 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G-heptakisazido- 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G-heptakisdeoxy cyclodextrin and 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G-heptakisazido- 6^A,
15 6^B, 6^C, 6^D, 6^E, 6^F, 6^G-heptakisdeoxy- perfunctionalized cyclodextrin is:

- a. coupled directly to any support which is carrying free -NH₂ groups on the surface of the support;
- b. reacted with any alkenyl amines to give the corresponding perfunctionalized cyclodextrin with 6-position alkenyl substituted side chains via urea linkages, then this derivative is hydrosilylized with
20 HSiR_nX_{3-n} (where R and X are alkyl, alkyloxy, aryl or halide) and the derivatized cyclodextrin is immobilized onto the surface of a support material; or
- c. reacted with any aminosilanes containing at least one further reactive
25 group to afford the corresponding cyclodextrin-silane derivative and the resulting cyclodextrin-silane derivative is reacted with silica gel to obtain a perfunctionalized cyclodextrin cross-linked and chemically bonded silica gel.

The cyclodextrin-based CSP of the invention can be used in counter-current
30 chromatographic processes to provide an efficient means of industrial scale enantioseparation.

These and other objectives will become apparent or will be highlighted in the ensuing description.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig.1 shows the synthesis procedure (a) of the invention. The product is (a
5 representative) cyclodextrin or completely substituted cyclodextrin cross-linked mutually and immobilized onto the surface of a support via multiple urea bonds. R = H, alkyl, aryl, ester or carbamate.

Figs. 2A and 2B are chromatograms showing chiral separations using the material of Example 4. In Figure 2A, enantiomers of propanolol are separated using
10 H₂O/acetonitrile 80/20 as the mobile phase. $k_1 = 3.91$, $k_2 = 4.93$, $\alpha = 1.27$, $R_s = 2.61$. Figure 2B shows the separation of enantiomers of 1-p-Br-phenyl ethanol using Hexane/IPA 90/10 as the mobile phase. $k_1 = 1.38$, $k_2 = 3.40$, $\alpha = 2.00$, $R_s = 5.55$.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is based on a quantitative reaction of (6^A-azido-6^A-
15 deoxy)-perfunctionalized cyclodextrin with primary amines. When aminized silica gel is used in place of the primary amine under similar conditions, the 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G-heptakisazido- 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G-heptakisdeoxy cyclodextrin can be mutually cross-linked and at the same time immobilized onto the surface of silica gel via multiple stable urethane linkages.

20 Supports that can be employed include silica gel or any alternative inorganic materials such as Al₂O₃, TiO₂ or ZrO₂ or synthetic polymer supports. The support preferably has free NH₂ groups on the surface. A preferred support is silica gel, which is commercially available in a wide range of different shapes and sizes. Spherical particles are preferred. The particle size is typically from 1 to 20 μ M, more typically
25 from 2 to 10 μ M. For use in HPLC analytical separation media, a particle size of about 5 μ M is preferred. The CSP according to the invention can be produced by three different approaches as in methods a to c below. However, the key step in all the methods is the same, involving reacting 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G-heptakisazido- 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G-heptakisdeoxy cyclodextrin and 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G-
30 heptakisazido- 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G-heptakisdeoxy- perfunctionalized cyclodextrin

with free amines via a facile one-pot procedure in high yield or providing good immobilization contents, or both. The cyclodextrin used may be of any of the α , β or γ form or combination thereof; the derivatization substituent groups may be alkyl, aryl, ester or carbamate.

5 In method a described above, the 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G -heptakisazido- 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G -heptakisdeoxy cyclodextrin and 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G -heptakisazido- 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G -heptakisdeoxy- perfunctionalized cyclodextrin is cross-linked and coupled to a support that has already been modified with amino groups. This NH₂-bearing material can either be obtained commercially in finished
10 form or prepared by a silanization reaction of the support with an appropriate aminosilane. For a silanization, an aminosilane such as, for example, 3-aminopropyltrimethoxysilane, 3-aminopropyltriethoxysilane, 3-(2-aminoethyl)amino propyltriethoxysilane and 3-(2-aminoethyl)amino propyltrimethoxysilane, is preferred.

In the method b described above, a suitable alkenyl amine can be the
15 $\text{CH}_2=\text{CH}(\text{R}_1)_l(\text{R}_2)_m\text{NH}_2$, wherein l and m are integers from 0 to 20. L and m are each typically from 1 to 10, more typically from 1 to 5. R₁ is typically any alkyl moiety but preferably -CH₂- and m is typically 0 (R₂ is absent). If m>0, R₂ is typically an aromatic moiety. For hydrosilylating the terminal C=C group, the active HSiR_nX_{3-n} compound is one in which R and X are independently alkyl, alkyloxy, aryl or halide and n is an
20 integer from 1 to 3. In instances where R and/or X are alkyl or alkoxy, each preferably contains 1 to 3 carbon atoms. In instances where R and/or X are aryl, each is preferably phenyl.

In the method c described above, the aminosilanes described in method a are suitable for this purpose. Also useful are aminosilanes of the formula H₂N-(Y₁)_l(Y₂)_m-
25 SiR_nX_{3-n}, wherein R and X are independent alkyl, alkyloxy, aryl or halide and n is an integer from 1 to 3. L and m are integers from 0 to 20, L and m are each typically from 1 to 10, but more typically from 1 to 5. Y is typically -CH₂- and m is typically 0 (Y₂ is absent) or if m>0, Y₂ is then typically an aromatic moiety. In all of these instances, urea linkages are formed between the NH₂ groups of the support and the cyclodextrin.

30 The connecting spacer between the support and the cyclodextrin may comprise a relatively short or relatively long chain. This chain preferably has 3-20 atoms and may contain nitrogen, oxygen or silicon atoms in addition to carbon atoms.

The material obtained according to the invention can, if desired, be further subjected to an "end-capping" reaction. In this, the remaining hydroxyl groups on the silica gel surface are reacted in a known manner with a reactive silane, such as, for example, trimethylchlorosilane or hexamethyldisilazane, to complete the blocking of the surface hydroxyl groups.

Above all, by present invention, the cyclodextrin can be cross-linked mutually and immobilized onto the surface of the support via multiple urea linkage(s). In the CSP material obtained by the present invention, the cyclodextrin is cross-linked mutually and the immobilization can be effected with good reproducibility.

In the products produced by present invention, the cyclodextrin or perfunctionalized cyclodextrin is cross-linked mutually and connected to the support material via multiple urea linkages providing a material that is more stable than when the cyclodextrins are immobilized as a single molecule. Very good reproducibility of separation is desired, even after long run times in reverse phase separations using mobile phases having a high aqueous concentration.

Cyclodextrin with the 6-positions substituted by iodine or bromo moieties can be prepared using the procedure of B. I. Gorin, *Tetrahedron Lett.*, 1996, **37**(27), 4647; D. Alker, *Tetrahedron Lett.*, 1994, **35**(48), 9091; C. Roehri-Stoeckel, *Tetrahedron Lett.*, 1997, **38**(9), 1551; or Yoshinaga, United States Patent No. 5,241,059. This step is followed by reaction with NaN_3 in DMF to give 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G - heptakisazido- 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G -heptakisdeoxy cyclodextrin. The other hydroxyl groups on the 2,3 positions of the 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G -heptakisazido- 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G -heptakisdeoxy cyclodextrin can subsequently be completely derivatized with different substituents groups such as alkyl, aryl, ester and carbamate.

6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G -heptakisazido- 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G -heptakisdeoxy-2^A, 2^B, 2^C, 2^D, 2^E, 2^F, 2^G -heptakis-O-acetyl-3^A, 3^B, 3^C, 3^D, 3^E, 3^F, 3^G -heptakis-O-acetyl-β-cyclodextrin can be prepared by stirring 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G -heptakisazido- 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G -heptakisdeoxy-β-cyclodextrin with (AcO)₂O/Pyridine at 40 °C in 90% yield.

6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G -heptakisazido- 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G -heptakisdeoxy-2^A, 2^B, 2^C, 2^D, 2^E, 2^F, 2^G -heptakis-O-methylated- 3^A, 3^B, 3^C, 3^D, 3^E, 3^F, 3^G -heptakis-O-methylated-β-cyclodextrin can be prepared by reaction of 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G -

heptakisazido- 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G -heptakisdeoxy-β- cyclodextrin in CH₃I/DMF/NaH at 40 °C in 65% yield.

6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G -heptakisazido- 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G -heptakisdeoxy- 2^A, 2^B, 2^C, 2^D, 2^E, 2^F, 2^G -heptakis-O-phenylcarbamoylated- 3^A, 3^B, 3^C, 3^D, 3^E, 3^F, 3^G -
5 heptakis-O-phenylcarbamoylated-β- cyclodextrin can be prepared by reaction of 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G -heptakisazido- 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G -heptakisdeoxy-β- cyclodextrin in PhNCO/ Pyridine at 80 °C in 75% yield.

Aminized silica-gel can be prepared according to the method of T. Hargital et al., *J. Chromatogr.*, 1993, **628**, 11, giving the following composition as determined
10 from elemental analysis: C% 3.25, H% 0.96, N% 0.98.

The following examples illustrate the practice of the present invention.

EXAMPLE 1

4 g of aminized silica gel was stirred in 30 ml of anhydrous THF through which a continuous stream of CO₂ gas was passed. After 20 mins, 1.2g of 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G -heptakisazido- 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G -heptakisdeoxy- 2^A, 2^B, 2^C, 2^D, 2^E, 2^F, 2^G -
15 heptakis-O-acetyl- 3^A, 3^B, 3^C, 3^D, 3^E, 3^F, 3^G -heptakis-O-acetyl-β- cyclodextrin in 10 ml anhydrous THF was added. Stirring was continued another 5 mins, whence 2.0 g of triphenylphosphine in 20 ml of anhydrous THF was added. The mixture was stirred for 10 hrs with constant bubbling of CO₂ at room temperature. The reaction mixture was
20 then transferred to a soxhlet extraction apparatus and extracted with acetone for 24 hrs. After removal of the acetone in vacuo, the peracetylated cyclodextrin cross-linked and immobilized silica gel was obtained having the following composition as determined from elemental analysis: C 12.46%, H 1.97%, N 1.03%.

EXAMPLE 2

25 4 g of aminized silica gel was stirred in 30 ml of anhydrous DMF through which a continuous stream of CO₂ gas was passed. After 20 mins, 1.2 g of 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G -heptakisazido- 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G -heptakisdeoxy-β- cyclodextrin in 30 ml of anhydrous DMF was added. Stirring was continued another 5 mins, whence 2.0 g of triphenylphosphine in 20 ml of anhydrous DMF was added. The mixture was stirred
30 for 10 hrs with constant bubbling of CO₂ at room temperature. After filtering through a

glass frit and rinsing with pyridine, water, ethanol and petroleum ether (in this sequence), a cyclodextrin cross-linked and immobilized silica gel was obtained having the following composition as determined from elemental analysis: C 7.93%, H 1.65%, N 1.82%.

5 EXAMPLES 3 AND 4

Example 1 was repeated using 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G -heptakisazido- 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G -heptakisdeoxy- 2^A, 2^B, 2^C, 2^D, 2^E, 2^F, 2^G -heptakis-O-methylated- 3^A, 3^B, 3^C, 3^D, 3^E, 3^F, 3^G -heptakis-O-methylated-β-cyclodextrin (Example 3) or 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G -heptakisazido- 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G -heptakisdeoxy- 2^A, 2^B, 2^C, 2^D, 2^E, 2^F, 2^G -heptakis-O-phenylcarbamoylated- 3^A, 3^B, 3^C, 3^D, 3^E, 3^F, 3^G -heptakis-O-phenylcarbamoylated-β-cyclodextrin (Example 4) in place of the 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G -heptakisazido- 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G -heptakisdeoxy- 2^A, 2^B, 2^C, 2^D, 2^E, 2^F, 2^G -heptakis-O-acetyl- 3^A, 3^B, 3^C, 3^D, 3^E, 3^F, 3^G -heptakis-O-acetyl-β-cyclodextrin of Example 1. The elemental analyses were as follow:

Example 3. Bonded with cross-linked permethylated-β-cyclodextrin: C 11.45%, H 1.85%, N 1.14%.

Example 4. Bonded with cross-linked β-cyclodextrin perphenylcarbamate: C 13.78%, H 2.02%, N 2.38%.

EXAMPLE 5

A solution of the allylamine (14 mmol) in 20 ml of anhydrous THF was saturated with dry CO₂ at room temperature with stirring. A precipitate of ammonium carbanate derivative was immediately observed. With continuous bubbling of CO₂ into the suspension, 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G -heptakisazido- 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G -heptakisdeoxy- 2^A, 2^B, 2^C, 2^D, 2^E, 2^F, 2^G -heptakis-O-acetyl- 3^A, 3^B, 3^C, 3^D, 3^E, 3^F, 3^G -heptakis-O-acetyl-β-cyclodextrin (1.80 mmol) in 10 ml of anhydrous THF was added in a single portion, followed by addition of PPh₃ (12.6 mmol) in 20 ml of anhydrous THF. This mixture was allowed to react for about 5 hrs after TLC revealed that no starting materials remained. After evaporation to dryness, the product was purified by column chromatography with ethyl acetate-acetone (1:1) as eluent in 90 % yield.

EXAMPLES 6 AND 7

Example 5 was repeated by using 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G -heptakisazido- 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G -heptakisdeoxy- 2^A, 2^B, 2^C, 2^D, 2^E, 2^F, 2^G -heptakis-O-methylated- 3^A, 3^B, 3^C, 3^D, 3^E, 3^F, 3^G -heptakis-O-methylated-β- cyclodextrin (Example 6) or 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G -heptakisazido- 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G -heptakisdeoxy- 2^A, 2^B, 2^C, 2^D, 2^E, 2^F, 2^G -heptakis-O-phenylcarbamoylated- 3^A, 3^B, 3^C, 3^D, 3^E, 3^F, 3^G -heptakis-O-phenylcarbamoylated-β- cyclodextrin (Example 7) in place of the 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G -heptakisazido- 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G -heptakisdeoxy- 2^A, 2^B, 2^C, 2^D, 2^E, 2^F, 2^G -heptakis-O-acetyl- 3^A, 3^B, 3^C, 3^D, 3^E, 3^F, 3^G -heptakis-O-acetyl-β- cyclodextrin of Example 5.

EXAMPLE 8

1.5 g of product obtained in Example 5 was stirred with 5 ml of triethoxysilane and 10 mg of tetrakis(triphenylphosphine)platinum at 60°C. After 24 hrs the mixture was adsorbed with 2 cm³ high molecular weight silica gel in a Buchner funnel and washed with 100 ml ether. After removal of the ether and volatiles by vacuum, the residue was dissolved in 50 ml anhydrous toluene, 4.0 g of silica gel (which had already been dried over vacuum at 120°C overnight) was added, the mixture was stirred at 80°C for 8 hrs. 1 ml of water was added and the reaction was continued for another 3 hrs. After removing the volatiles, the residue was heated to 200°C for 5 hrs. After filtering and extracting in a soxhlet apparatus with acetone for 24 hrs, a product with the following elemental analysis was obtained: C 8.45%, H 2.00%, N 0.10%.

EXAMPLES 9 AND 10

Example 8 was repeated by using the product of example 6 (Example 9) or the product of example 7 (Example 10) in place of the product of example 5 with elemental analyses shown as following:

Example 9. Bonded with cross-linked permethylated-β-cyclodextrin: C 7.45%, H 1.76%, N 0.09%.

Example 10. Bonded with cross-linked β-cyclodextrin perphenylcarbamate: C 9.03%, H 2.03%, N 0.25%.

EXAMPLE 11

The material produced as in Example 4 was introduced into a column (250× 4.6 mm). Quite good chiral separations could be achieved both in normal phase and reverse phase. Peaks were deviated by UV absorbance at 254 nm. A wide variety of chiral compounds and pharmaceutical active ingredients could be separated easily. A separation factor of more than 5 could be easily achieved for some drugs.

Table 1: Enantiomeric separation of various chiral compounds

Substance	HPLC Condition	Flow rate (ml/min)	Retention Time (in minutes)	α	Rs
Atropine	Condition2	0.5	7.02 / 11.14	5.62	4.46
Acebutalol	Condition1	0.5	14.56 / 17.20	1.30	2.55
Alprenolol	Condition1	0.5	18.55 / 24.72	1.50	1.85
Pindolol	Condition1	0.5	11.72 / 13.60	1.34	1.21
Isopreteralol	Condition1	0.5	7.50 / 13.04	5.06	3.29
Propranolol	Condition5	1.0	22.50 / 38.83	1.43	1.52
Bendroflu-methiazide	Condition3	0.5	25.50 / 36.10	1.49	3.19
H2	Condition3	0.5	15.62 / 17.32	1.18	1.16
p-bromo-phenyl ethanol	Condition4	1.0	7.70 / 12.50	2.14	4.05

*Condition 1: MeOH/Buffer(98mM TEAA, pH=5.30) = 30/70 (v/v)

Condition 2: MeOH/Buffer(98mM TEAA, pH=4.67) = 50/50 (v/v)

Condition 3: MeOH/Water = 70 / 30 (v/v)

10 Condition 4: Hexane/IPA= 90/10 (v/v)

Condition 5: Hexane/IPA= 80/20 (v/v)

Having now described the invention, it is not intended that it be limited except as may be required by the appended claims.

CLAIMS

What is claimed is:

1. A process for producing a chromatographic separation material comprising any of the following steps:
 - (a) reacting 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G -heptakisazido- 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G -
5 heptakisdeoxy cyclodextrin or 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G -heptakisazido- 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G -heptakisdeoxy-perfunctionalized cyclodextrin with a support bearing free NH₂ groups;
 - (b) producing a product with side chains of terminal C=C group via urea linkages by reacting 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G -heptakisazido- 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G -
10 heptakisdeoxy cyclodextrin or 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G -heptakisazido- 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G -heptakisdeoxy-perfunctionalized cyclodextrin with an alkenyl amine, and then chemically immobilizing and cross-linking the product to the surface of a support by hydrosilylating the terminal C=C group of said product by reacting the product with an active HSiR_nX_{3-n}, wherein R and X are independently alkyl, alkyloxy, aryl or halide and n is from 1 to 3; or
 - 15 (c) chemically immobilizing and cross-linking cyclodextrin to a support by reacting 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G -heptakisazido- 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G -heptakisdeoxy cyclodextrin or 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G -heptakisazido- 6^A, 6^B, 6^C, 6^D, 6^E, 6^F, 6^G -heptakisdeoxy-perfunctionalized cyclodextrin with an aminosilane containing at least one further reactive group.
- 20 2. The process of claim 1 wherein the substitution groups of the perfunctionalized 6-azido cyclodextrin are alkyl, aryl, ester or carbamate.
3. The process of claim 1 wherein the support is silica gel, Al₂O₃, TiO₂, ZrO₂ or a synthetic polymer.
4. The process of claim 1 wherein steps (a), (b) or (c) are effected in the
25 presence of PPh₃/CO₂ and an anhydrous organic solvent.
5. The process of claim 1, wherein in step (b) the alkenyl amine has three to twenty carbon atoms in the chain.



6. The process of claim 1, wherein in step (c) the aminosilane is of the formula $H_2N-(Y)-SiR_nX_{3-n}$, wherein Y is any aromatic or alkyl spacer group having up to 20 carbon atoms, R and X are alkyl, alkyloxy, aryl or halide and n is from 1 to 3.

7. The process of claim 1 wherein the cyclodextrin is an alpha-, beta- or gamma- cyclodextrin.

8. A product of the process of any of claims 1 through 7.

9. A process for the separation of substance mixtures, comprising contacting said substance mixture with a separation material that comprises cyclodextrin cross-linked via multiple urea groups and chemically bonded to an insoluble support via multiple urea groups and chromatographically or electrophoretically separating the substances in said substance mixture.

10. The process of claim 9, wherein said substance mixture comprises a mixture of enantiomers of a compound.

