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Synthesis and chromatographic properties of a novel chiral stationary phase derived from heptakis(6-azido-6-deoxy-2,3-di-*O*-phenylcarbamoylated)-β-cyclodextrin immobilized onto amino-functionalized silica gel via multiple urea linkages

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Abstract

A novel chiral stationary phase (PPHCDN7) was prepared by immobilization of heptakis(6-azido-6-deoxy-2,3-di-*O*-phenylcarbamoylated)-β-cyclodextrin (PPHCD) onto the surface of amino-functionalized silica gel via multiple urea linkages derived from an extended application of the Staudinger reaction. A wide range of structurally divergent racemic drugs and other compounds were successfully separated into their enantiomers under both normal and reversed-phase conditions. β-Adrenergic blockers and racemic tertiary, secondary and primary amines were readily separated using a mixture of methanol and aqueous triethylammonium acetate buffer. The optimal pH value for the separation falls in the range of 4.65 to 6.30. With atropine and isoproterenol, good enantioseparations with separation factors of $\alpha > 5$ were easily attainable. © 2002 Published by Elsevier Science B.V.

Keywords: Chiral stationary phases, LC; Enantiomer separation; Cyclodextrins

1. Introduction

Chromatographic enantioseparation on chiral stationary phases (CSPs) represents one of the most direct and facile approaches for the determination of enantiomeric purity [1–4] with strong potentials for development into convenient bulk-phase enantioseparation processes as exemplified in their application in simulated counter-current production of pure enantiomeric drugs [5]. It is not surprising therefore that there have been tremendous research impetus focused on the development of efficacious CSPs over the past decades [6]. Commercially available CSPs are largely based on functionalized cellulose [3] and cyclodextrins (CD) [7], which exhibit good enantiomeric separation abilities toward a broad range of structurally diverse racemic drugs and other compounds.

Most of CD-CSPs have been prepared by bonding native CDs to supports such as silica gel before the

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modification of the CDs [8–10]. Generally, these CD-CSPs demonstrated unusual selectivity under either reversed- or normal-phase modes mainly attributable to inclusion complex properties and hydrogen bonding interactions, respectively. In addition, Armstrong et al. reported a unique CD-based CSP with functionalization of the hydroxyl moieties with chiral pendant 1-(1-naphthyl) ethylcarbamate, which exhibited good enantioseparation ability under both reversed-phase (use of polar miscible organic-aqueous eluents) and normal-phase (use of non-polar organic eluents) towards a variety of racemic compounds. These results enlarged the applications of CD-CSPs [11–13].

Although the above-mentioned CD-based CSPs are chemically anchored to the support material via amino/amido linkages [14,15] or the solvolytically more stable ether [12,13,16,17]/carbamate [8,9,18, 19] moieties and are therefore more solvent tolerant, most of the synthetic generations of these CD-CSPs involved post-immobilization derivatization of the CD. As these processes involved heterogeneous solid–liquid reactions, complete functionalization of the CD-hydroxyl moieties were not readily accomplished [9,11,16] which may result in poor batch-to-batch reproducibility.

To overcome these shortcomings, there are intense research efforts in the synthetic generation of CSPs with well-defined chemical structures as well as good stability, particularly for use in mobile phases with high aqueous contents. Accordingly, we have focused our attention on immobilized β-cyclodextrin CSPs and reported a novel and facile methodology for the preparation of a series of patented CD-based CSPs [20,21] by immobilization of mono(6-azido-6-deoxy)-perfunctionalized β-cyclodextrin on amino-functionalized silica gel via a single stable urea linkage using the Staudinger reaction approach [22–25]. These CSPs demonstrated excellent enantioseparation abilities and stability and the enantioseparation results will be reported later.

In this paper, we report on the extension of the above research work to a more durable CSP having multiple covalent linkages onto the inert support. The key step relies on the immobilization of heptakis(6-azido-6-deoxy-2,3-di-*O*-phenylcarbamoylated)-β-cyclodextrin (PPHCD) onto the surface of amino-functionalized silica gel using the Staudinger reaction. As the derived CSP possesses high hydrophobicity, together with multiple urea bonds which are more stable particularly in acidic media, this novel CSP is expected to have good stability particularly when used in mobile phases with high aqueous content. Its chromatographic performance was evaluated using a wide range of chiral drugs/compounds under various conditions. Studies on optimization of separation conditions, particularly the pH values for enantioseparations were also investigated.

2. Experimental

2.1. Chemicals and materials

β-Cyclodextrin and triethylamine were obtained from Fluka (Buchs, Switzerland). Phenyl isocyanate was purchased from Aldrich. The silica gel used was Hypersil 5 μm silica gel (pore size 120 Å, surface area 170 m^2/g). Other solvents used in the preparation of the stationary phase were of at least analytical reagent grade and were carefully purified and dried before use. Heptakis(6-azido-6-deoxy)-β-cyclodextrin was prepared according to literature methods [26,27]. Amino-functionalized silica gel prepared according to the literature [9] has an elemental content of C 3.42%, H 0.95%, N 1.11%. 1-Phenylethanol and its analogs were prepared by reducing the corresponding ketones with $LiAlH_4$ in anhydrous tetrahydrofuran (THF). All other drugs and chiral compounds used were obtained from Sigma.

2.2. Equipment and measurements

FT-IR spectra of all samples were obtained with a Bio-Rad TFS156 instrument using the KBr technique. Microanalyses were effected on a Perkin-Elmer 2400 CHN analyzer. HPLC was performed with a Perkin-Elmer Binary LC pump model 250, a Perkin-Elmer Bip UV–Vis Spectrophotometric detector model LC 290 (Foster City, CA, USA), and a CR-6A integrator (Shimadzu, Kyoto, Japan). The injection loop has a 20 μl capacity. Detection was

carried out at 254 nm. The CSP was packed into the stainless steel HPLC column (250×4.6 mm I. D., from Phenomenex) by a conventional high-pressure slurry packing procedure using an Alltech® air compression pump (Alltech, USA). All the chromatograms were obtained at room temperature with a constant flow-rate of 0.5 ml/min for reversed-phase and 1.0 ml/min in normal-phase. The column gave efficiency of 38 000 plates per meter using biphenyl as the test probe under normal-phase conditions (*n*-hexane-2-propanol, 90:10, v/v).

2.3. Preparation of chiral stationary phase

The synthetic scheme to the CSP PPHCDN7 (**4**) is depicted in Fig. 1. In the first step, β-CD was converted to heptakis(6-iodo-6-deoxy)-β-CD (**1**) [28,29]. Thereafter, it was reacted with sodium azide in DMF to afford heptakis(6-azido-6-deoxy)-β-CD (**2**), which was then perphenylcarbamoylated with phenyl isocyanate to afford (**3**).

2.3.1. Preparation of heptakis(6-iodo-6-deoxy)-β-cyclodextrin (*1*)

Iodine (48.1 g, 0.19 mol) and triphenylphosphine (50.0 g, 0.19 mol) were mixed in dried DMF (ca. 300 ml) and stirred for 5 min, followed by adding the solution of vacuum-dried β-CD (8.84 g, 7.79 mmol) in dried DMF (ca. 80 ml). The reaction mixture was stirred for 20 h at 90–95 °C under nitrogen atmosphere and was concentrated under reduced pressure later. Then the reaction flask was cooled with an ice-bath and 3.5 *M* sodium methanoate (100 ml) was added to the mixture to adjust the pH of the reaction mixture at 9–10. After filtration, the brown orange precipitate was washed with a large amount of acetone, yielding light brown solid, (12.6 g, 84.0%) Mp: >205.79 °C, decomposed; $[\alpha]_D^{25}$ +180 (*c* 0.05, DMSO); IR (cm^{-1}): 3366 (O–H str), 2914, (C–H str), 1038 (C–O–C str); ^{1}H NMR (DMSO-d_6, TMS) δ (ppm): 6.2–5.6 (m, 14H), 5.1–4.9 (m, 7H), 4.0–3.5 (m, 42H); Microanalysis for $C_{42}H_{63}O_{28}I_7$ (1904.98): Calculated C 26.27%, H 3.33%; Found C 27.01%, H 3.36%.

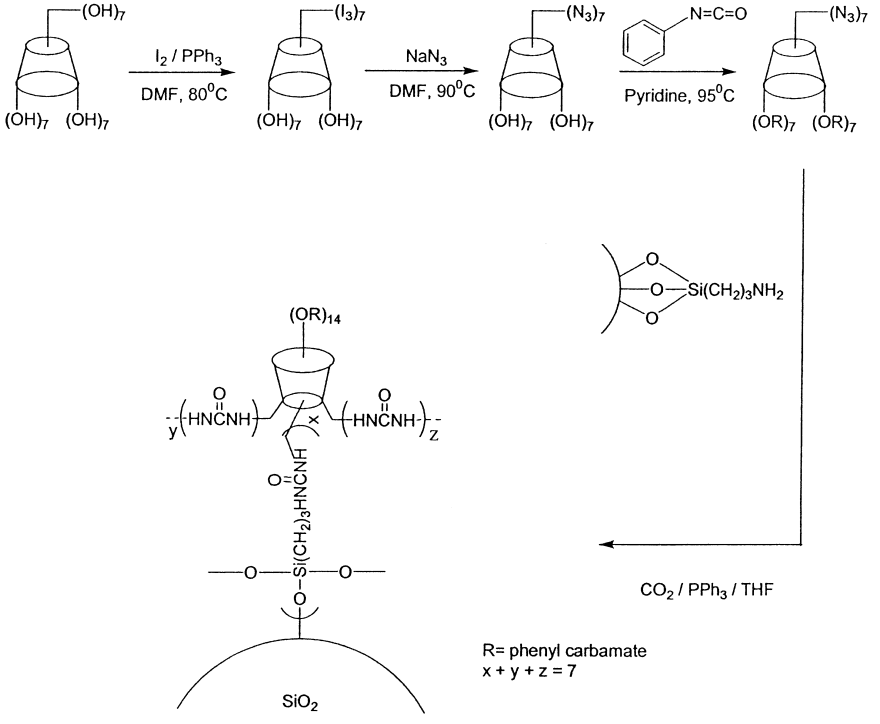


Fig. 1. Synthesis of CSP.

2.3.2. Preparation of heptakis(6-azido-6-deoxy)-β-cyclodextrin (2)

(**1**) (13.0 g, 6.82 mmol) was dissolved in dried DMF (200 ml) followed by adding sodium azide (15.52 g, 0.28 mol, 5 equiv. per iodide atom) and stirred at 95 °C overnight under nitrogen atmosphere. The reaction mixture was concentrated under reduced pressure and washed with ultra-pure water. After filtration, the residue was washed with water, yielding light brown solid (**2**) (7.35 g, 82%) Mp: >220.49 °C, decomposed; $[\alpha]_D^{25}$ +240 (*c* 0.05, DMSO); IR (cm^{-1}): 3366 (O–H str), 2925, (C–H str), 2106 (N_3 str), 1053 (C–O–C str); ^{1}H NMR (DMSO-d_6, TMS) δ (ppm): 5.9–5.7 (m, 14H), 4.0–3.5 (m, 42H); Microanalysis for $C_{42}H_{63}O_{28}N_{21}$ (1310.08): Calculated C 38.51%, H 4.85%, N 22.45%; Found C 38.90%, H 4.85%, N 22.11%.

2.3.3. Preparation of heptakis(6-azido-6-deoxy-2,3-di-O-perphenyl carbamoylated)-β-cyclodextrin (3)

Vacuum-dried (**2**) (2.0 g, 1.50 mol) dissolved in dried pyridine (30 ml) and phenyl isocyanate (10 ml) was added. The reaction mixture was stirred for 15 h at 90 °C under a nitrogen atmosphere. After removal of pyridine and unreacted phenyl isocyanate under reduced pressure, the residue was dissolved in dried ethyl acetate (100 ml). The solution was washed with water (3×50 ml). The organic layer was combined and dried with anhydrous magnesium sulphate. The solution was filtered, concentrated and the resulting yellowish residue was purified by column chromatography over silica gel using *n*-hexane–chloroform (1:4) as eluent, yielding light yellow powder, (**3**) (3.06 g) Mp: 202–204.8 9 °C, $[\alpha]_D^{25}$ +150 (*c* 0.05, $CHCl_3$); IR (cm^{-1}): 3393, 3309 (N–H str), 3053 (arom C–H str), 2106 (N_3 str), 1738(C=O str), 1542, 1497 (arom C=C ring str), 1041 (sym C–O–C str), 767 (C–H arom op bend); ^{1}H NMR ($CDCl_3$, TMS) δ (ppm): 7.60–6.70 (m, 84H), 4.5–3.5 (m, 49H); Microanalysis for $C_{140}H_{133}O_{42}N_{35}$ (2977.80): Calculated C 56.45%, H 4.50%, N 16.46%; Found C 56.65%, H 4.47%, N 16.84%.

2.3.4. Preparation of heptakis(6-azido-6-deoxy-2,3-di-O-perphenyl carbamoylated)-β-cyclodextrin (PPHCD) cross-linkly immobilized silica gel PPHCDN7 (4)

Amino-functionalized silica gel (4.0 g) was stirred in anhydrous THF (30 ml) through which a continuous stream of CO_2 gas was passed. After 20 min, compound (**3**) (1.2 g) in anhydrous THF (10 ml) was added. Stirring was continued for another 5 min, when triphenylphosphine (2.0 g) in anhydrous THF (20 ml) was added. The mixture was stirred for 3 h with constant passage of CO_2 at ambient temperatures (ca. 25 °C). The reaction mixture was then transferred to a Soxhlet extraction apparatus and extracted with acetone for 24 h. After the removal of the acetone, the derived CSP (**4**) has an elemental composition of: C 13.78%, H 2.02% and N 2.38%.

2.4. Preparation of mobile phase and samples

Triethylammonium acetate buffers were prepared using 1% aqueous triethylamine, which were adjusted by addition of glacial acetic acid to the desired pH. The mobile phase, comprising of triethylammonium acetate buffer and the appropriate amount of the organic modifier, were freshly prepared, filtered, and degassed under vacuum using a DEGASYS DG-2410 degasser. A period of 1–2 h of equilibration after a pH change of the mobile phase was allowed in order to obtain reproducible results.

The concentration of sample solutions was 1.0 mg ml^{-1} using the mobile phase as solvent. All the experiments were carried out at room temperature (ca. 25 °C).

3. Results and discussions

3.1. Structural characterization of modified CD

As described earlier, the proposed structure of the immobilized perphenyl carbamoylated β-cyclodextrin CSP was corroborated by the elemental analysis and FT-IR spectra.

On account of the higher reactivity of the seven primary 6-hydroxyl groups located at the narrow end of the β-CD cone, they undergo facile transformation to azido groups. These were then used to link perfunctionalized β-CD onto the silica gel with likelihood of some urea linkages between the CD molecules. Meanwhile, the 14 less reactive secondary 2- and 3-hydroxyl groups on the wider end of

the β-CD were perphenyl carbamoylated, which has the effect of extending the cavity in addition to enhancing its hydrophobicity, which may play an important role in the enantioseparation process. Full functionalization of the hydroxyl groups with phenyl carbamate groups at 2- and 3-position of β-CD residues corroborated by matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) using 2,4,6-trihydroxy acetophenone (THAP) as matrix. The intense and exclusive molecular ion $[M+Na]^+$ in Fig. 2 further confirmed the proposed structure of heptakis(6-azido-6-deoxy-2,3-di-*O*-phenylcarbamoylated)-β-cyclodextrin.

The carbon content in the elemental analysis as well as the appearance of FT-IR vibrational bands in the 1800–1600 cm^{-1} range attributable to carbonyl stretching in PPHCD provides for corroborative evidence that the cyclodextrin moieties have been successfully immobilized onto the surface of the silica gel.

3.2. Influence of structure of the CSP

By forming the crosslinked network-like polymer structure, this CSP possesses three functions: chiral recognition ability by the β-CD cone that has chiral centers in its cavity, a hydrophobic interaction by the phenyl cluster, and a significant improvement of stability of this cross-linked CSP, particularly under the aqueous phase.

However, it should be noted that, since the present functionalized CD was attached to the surface of amino-functionalized silica gel at a relatively short distance (just a 3 carbon spacer), the chiral resolution may be affected by a surface effect of the silica gel. Further studies on these aspects will be reported in due course.

3.3. Enantioseparation results

Enantioseparations of (1-aryl) ethanol and racemic

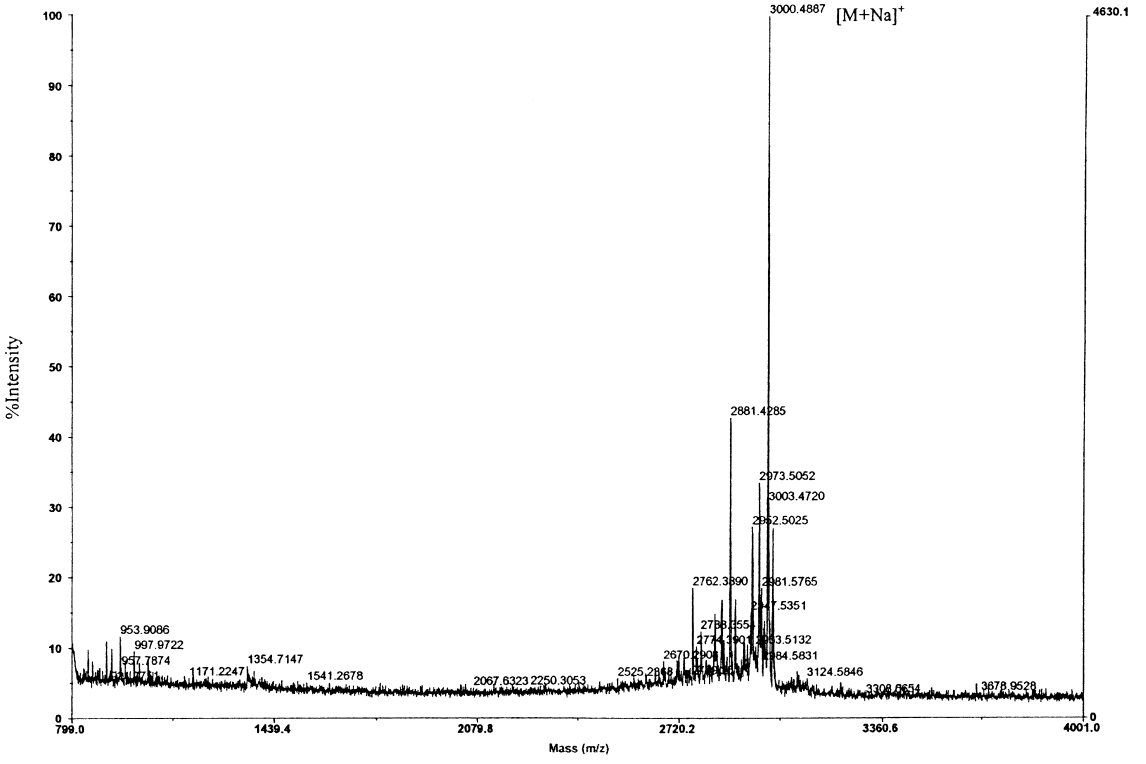


Fig. 2. MALDI-MS spectra of heptakis(6-azido-6-deoxy-2,3-di-*O*-phenylcarbamoylated)-β-cyclodextrin. ($C_{140}H_{133}O_{42}N_{35}$, M=2977.80 daltons).

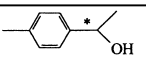
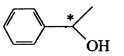
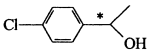
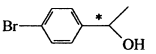
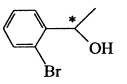
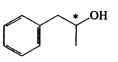
drugs were summarized in Tables 1–4 and representative chromatograms of atropine, alprenolol, bendroflumethiazide 1-(4-bromophenyl)-ethanol were demonstrated in Fig. 3.

3.3.1. Enantioseparation of some aromatic alcohols

From Table 1, it is evident that five (1-aryl) ethanol analogs exhibited excellent enantioseparations while no enantioseparation was observed for 1-phenyl-2-propanol where the chiral center is the carbon removed from the aromatic ring.

With chiral aromatic alcohols (S/N) 1 to 4, it is apparent that the retention time, separation factors (α) and resolution (R_s) increased when the *para* substituent on the aromatic ring is changed from methyl to H to chloro/bromo. This trend is similar to

Table 1
Enantioseparations of aromatic substituted alcohols

S/N	Compounds	k_1	α	Rs
1.		0.62	1.12	1.06
2.		0.70	1.20	1.53
3.		1.02	1.65	4.03
4.		1.10	1.98	5.62
5.		0.77	1.18	1.83
6.		N. A.	N. A.	N. A.

HPLC conditions: flow-rate, 1.0 ml min^{-1}; detection, 254 nm; mobile phase: *n*-hexane/IPA=90/10 (v/v).

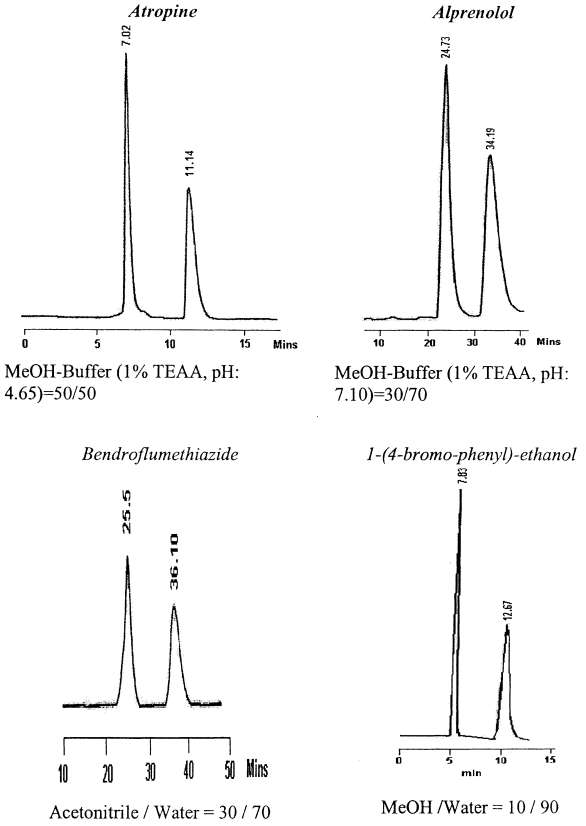


Fig. 3. Representative chromatograms for atropine, bendroflumethiazide, alprenolol and 1-(4-bromo-phenyl)-ethanol on CSP.

that of the perfunctionalized β-cyclodextrin stationary phases immobilized via a single urea linkage reported earlier by us [20,21]. It is obvious that the substituents are changed from being electron donation ($-CH_3$) to electron withdrawing (–Cl) for compounds 1–3. This result suggests that for our novel CSP, chiral discrimination is largely the result of π–π interaction or dipole–dipole interaction. When the *para* substituent is electron withdrawing, the aromatic ring will exhibit a π-acidic property. Meanwhile, the aromatic phenylcarbamate group in the CSP is π-basic in nature. Consequently, the interaction of the CSP with compounds will be stronger with increasing electron withdrawing effects giving rise to a longer retention time. The stronger interaction also helps in providing stronger interaction of the aromatic ring of the racemic solutes and consequently, contributes more to the chiral discrimination effect.

Steric effects also appear to play an important role. Thus, *o*-bromo compound 5 was less efficiently resolved than *para* isomer 4. This might be due to its larger steric hindrance in its interaction with the CSP.

No enantioseparation was observed for compound 6 indicating that the chiral carbon adjacent to the aromatic ring is essential to the enantioseparation on this CSP.

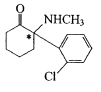
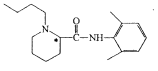
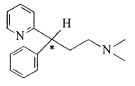
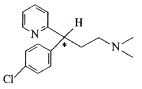
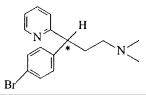
3.3.2. Enantioseparation of primary, secondary and tertiary amines

We investigated the chiral recognition ability of PPHCDN7 using six different racemic amines including one primary amine, one secondary amine and four tertiary amine derivatives. Table 2 summarized their retention factors (k_1) for the first eluted enantiomers, separation factors (α) and resolution factors (R_s).

The results show that separation ability increases from primary to secondary and then to tertiary amine. With 1-phenylethylamine, no enantioseparation could be achieved using the current CSP. The R_s and α values of ketamine (a secondary amine) fell in between those of primary amine and tertiary amine. Amongst these amines, tertiary amine afforded the best separation results. Thus, the resolution order of this column towards these samples is tertiary amine>secondary amine>primary amine.

For the tertiary amines of compounds 3–6, *p*-substituent groups at the aromatic rings may affect enantioseparation effects. It is interesting to see that enantioseparation is improved from chloro to bromo substituents. When the 4-position of the aromatic ring was substituted by a bromine, the analyte separated the best with an α of 1.25 while the R_s was 1.77. When the substituents were Cl- and H-, enantioseparation decreased. Therefore, the resolution is in the order of Br–pheniramine>Cl–pheniramine>pheniramine, which is consistent with the separation results of the (1-aryl) ethanol analogs discussed above. These results also confirm that, although the enantiomeric mechanism under normal-phase and reversed-phase is different, the enantioseparation mode for the same analyte is similar.

Table 2
Enantioseparations of primary, secondary and tertiary amines

S/N	Drugs	Structure	k_1	α	Rs
1.	1-phenylethylamine		0.161	1.00	--
2.	Ketamine		0.48	1.10	0.71
3.	Bupivacaine		0.90	1.27	1.22
4.	Pheniramine		0.96	1.12	0.73
5.	Chlorpheniramine		2.16	1.20	1.18
6.	Brompheniramine		2.27	1.25	1.77

HPLC conditions: flow-rate, 0.5 ml min^{-1}; detection, 254 nm; mobile phase, MeOH/1% TEAA buffer (pH=5.33)=30/70 (v/v).

3.3.3. Enantioseparations of β-adrenergic blockers (amino alcohol)

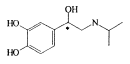
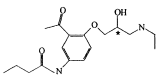
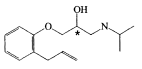
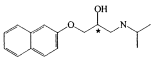
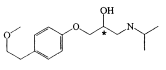
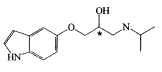
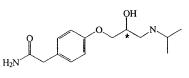
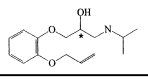
β-Adrenergic blockers are hydroxyl amines with the functional groups bearing secondary amines or N-isopropyl amines. These drugs also contain aromatic rings with different substituent moieties. The chromatographic separation of these compounds has been dominated by protein-based chiral stationary phases which are limited by low capacity and poor stability in spite of their high enantioseparation abilities.

Eight β-blockers were separated on this column under both normal and reversed-phase processes (Table 3). For most of these drugs, good resolutions were achieved under reversed-phase conditions using mixtures of aqueous triethylammonium acetate buffer and methanol. Since the pK_a values of these drugs are around 6.0, it is expected that they are separated in media with the pH value below 6.0, which will suppress ionization of these drugs, which may otherwise weaken the interactions with the CSP and allow for a more facile elution from the CSP.

Isoproterenol gave the best separation factor of 5.06 and resolution of 3.88. This was anticipated since it has a chiral center at the α-position of the aromatic ring while other drugs have the chiral carbons more remote from the aromatic ring. This tendency coincided with the separation of aromatic alcohols discussed above where the best resolution was achieved when the chiral carbon is at the α-position to the aromatic ring. The presence of two hydroxyl groups on the aromatic ring makes the molecule possess a relatively higher hydrophilicity. This, then, makes retention weaker indicated by the small k_1 value.

Alprenolol and oxprenolol gave very different results under the same conditions although their

Table 3
Enantioseparations of β-adrenergic blockers (amino alcohols)

S/N	Drugs	Structures	k_1	α	Rs	Conditions
1.	Isoproterenol		0.22	5.06	3.88	1
2.	Acebutolol hydrochloride		1.39	1.30	3.00	1
3.	Alprenolol		2.02	1.50	2.18	1
4.	Propranolol		4.29	1.43	1.79	4
5.	Metoprolol		4.04	1.26	1.18	3
6.	Pindolol		0.91	1.34	1.43	2
7.	Atenolol		0.34	1.31	0.98	2
8.	Oxprenolol		1.10	1.09	<0.5	2

HPLC conditions: flow-rate, 0.5 ml min^{-1} for mobile phase 1–2 and 1.0 ml min^{-1} for 3–4; detection, 254 nm; mobile phase, 1: MeOH/1% TEAA buffer (pH=4.33)=20/80 (v/v), 2: MeOH/1% TEAA buffer (pH=5.33)=30/70 (v/v), 3: *n*-hexane/IPA=90/10 (v/v), 4: *n*-hexane/IPA=80/20 (v/v).

chemical structures are similar except for the substituted groups which are $-CH_2CH=CH_2$ and $-OCH_2CH=CH_2$, respectively. It's obvious that the stronger electron donating ability of the latter leads to a more π-excessive and consequently more π-basic aromatic ring, which results in its poor π−π interaction with a CSP whose aromatic rings are also π-basic. The larger separation factors (α) and the resolutions (R_s) of alprenolol are attributable to the increase in π−π interaction between the solute and CSP.

It was previously reported [14,15] that the presence of two aromatic rings in chiral solutes may contribute to accentuated resolution because the two aromatic rings can fit into the cavity of native β-CD. However, we didn't observe this result with our CSP in that neither propranolol nor pindolol showed enhanced enantioseparation over other analogs. With our CSP, this may be attributable to the introduction of a large amount of the phenyl carbamate groups which has greatly expanded the cavity of cyclodextrin; which makes the complexing interaction mode less crucial to other decisive interactions such as hydrophobic, hydrogen bonding and π−π interactions.

3.3.4. Enantioseparation of non-protolytic drugs and weak acids

Whilst benzoin and dihydrobenzoin can be separated by adding TEAA as the additive under reversed-phase with buffer/MeOH, bendroflumethiazide gave good enantioseparation with $R_s>3$ in a mobile phase comprising just acetonitrile and water without any buffer.

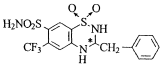
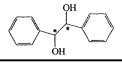
Bendroflumethiazide has a chiral carbon next to a –NH group and a SO_2NH group, and consequently has a strong tendency to form a hydrogen bond for enantioseparation; therefore bendroflumethiazide should be separated under reversed-phase with a simple composition of mobile phase as was observed here (Table 4).

3.4. Stability of the column under the reversed-phase mode

The cross-linked polymer structure enables the CSP to have a high stability in a mobile phase with high aqueous phase content. Some drugs, such as isoproterenol, can be separated using a mobile phase with very high aqueous contents such as having the buffer up to 99%. The baseline is very stable and the resolution results were readily reproducible.

The column also shows excellent properties under

Table 4
Non-protolytic drugs and weak acids

S/N	Drugs	Structures	k_1	α	Rs	Conditions
1.	Bendroflumethiazide		5.80	1.49	3.76	5
2.	Benzoin		1.18	1.09	1.00	6
3.	Dihydrobenzoin		4.9	1.05	0.77	7

HPLC conditions: flow-rate, 0.5 ml min^{-1}; detection, 254 nm; mobile phase, 5: acetonitrile/water=30/70 (v/v), 6: MeOH/1% TEAA buffer (pH=5.60)=50/50 (v/v), 7: MeOH/1% TEAA buffer (pH=5.60)=30/70 (v/v).

media with a wide range of pH values from 2.5 to 7.1. To those drugs, which should be applied under very severe condition such as high acetic media, this column is still expected to possess a very significant stability.

3.5. Effect of the pH value and salt under reversed-phase mode

Results showed that pH plays an important role in enantioseparation under the reversed-phase mode. This is not surprising since ionization of the solute molecule (which is pH dependent) is an important factor in enantioseparation. Addition of buffer can improve the chiral resolution in three ways. First, it controls the ionization of solute molecules which have weakly acidic or basic functional groups. Whether the chiral solute is neutral or in an ionized state has much influence on its retention and selectivity on the column which was demonstrated via the separation of β-blockers. Without a buffer media, the drugs exhibited poor separations with the exception of propranolol. After introducing the buffer, most of the drugs were baseline-resolved. Secondly, changes of pH may vary the interaction of a functional group with the cyclodextrin or its hydrogen-bonding ability. Finally, the buffer can improve the efficiency because it smooths the surface of the CSP and modifies it by masking strong adsorption sites on the stationary phase. Thus the phase will be more uniform and gives better enantioseparations.

4. Conclusion

A novel chiral stationary phase derived from heptakis(6-azido-6-deoxy-2,3-di-*O*-phenylcarbamoylated)-β-cyclodextrin immobilized on the surface of aminized silica gel via multiple urea linkages has been developed successfully. Various kinds of chiral drugs and compounds were successfully enantioseparated on this column under both normal and reversed-phase conditions not only by the mechanism of the formation of an inclusion complex, but also by the interaction of hydrogen bonding, π–π interaction and dipole–dipole interaction. The stability of the column is excellent and it proved to have good enantioseparation ability under a wide range of conditions.

The mobile phase constitution, pH value of the system and addition of salts play very important roles in the enantioseparation under the reversed-phase.

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