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# Preparation and chiral recognition of a novel chiral stationary phase for high-performance liquid chromatography, based on mono(6$^A$-*N*-allylamino-6$^A$-deoxy)-perfunctionalized β-cyclodextrin and covalently bonded silica gel

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**Abstract**

A novel chiral stationary phase (CSP) was prepared by immobilizing mono(6$^A$-*N*-allylamino-6$^A$-deoxy)-perphenylcarbamoylated β-cyclodextrin onto the surface of silica gel via hydrosilylation. The chromatographic properties of this column were tested with a wide range of structurally diverse racemic compounds and drugs under reverse phases. Separation mechanisms involved are also discussed.


*Keywords:* Chiral stationary phases, LC; Cyclodextrins; Beta-blockers; Pyrimidines; Drugs; Alcohols

## 1. Introduction

The analytical determination of stereoisomeric compounds, especially those of pharmaceutical importance is gaining significance on account of the role of chirality in living systems. 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 preparative enantioseparation processes. Amongst the many types of CSP reported, those based on cyclodextrins (CDs) are attracting burgeoning research interests. CDs are cyclic oligosaccharides containing six or more D-(+)-glucopyranose units, which are bonded through α-(1,4) linkages. The structures of CDs give rise to their remarkable ability in forming inclusion complexes with a variety of molecules [5–7] as well as ions [8,9]. Moreover, CDs are themselves chiral, making them amenable for enantioisomeric resolution. Accordingly, it is not surprising that CDs and their derivatives are widely used as chiral separation selectors in enantioselective chromatography [10–12].

We have previously reported on a novel methodology for the preparation of a series of CD-based CSPs by immobilization of monoazidoperfunctionalised CDs onto the surface of aminized silica gel via stable urea linkages using a Staudinger reaction [13–15]. However, the use of aminised silica would invariably result in remnant unreacted amine moieties on the surface of the CSPs. The presence of free amine groups on the surface may be undesirable under some conditions, since they may interact with analytes via H-bonding affording a longer retention time and/or have an adverse influence on the selectivity of the CSP.

We present herein an alternative facile approach that can effectively avoid the use of aminised silica gel. Thus, mono(6$^A$-*N*-allylamino-6$^A$-deoxy)-perphenylcarbamoylated β-cyclodextrin was first synthesized from the readily available mono(6$^A$-(*p*-toluenesulfonyl)-6$^A$-deoxy)-β-cyclodextrin, compound **1** [16–20]. Hydrosilylation with triethoxylsilane and then reaction of the reactive siloxane intermediate with pristine silica gel afforded a durable CSP **5** without free amine groups on the surface.

The chromatographic properties of the derived column were evaluated with a range of structurally diverse racemic compounds and drugs. Separation mechanisms involved are also discussed. With a view of comparing the enantioseparation abilities of the CSPs derived from different methodologies, we compared CSP **5** with the column SINU-PC[1]

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[1] Columns are now commercially available. For further information, pleases refer to website at: http://www.chiralpure.com.

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[14,21] from aminised silica and the Staudinger immobilization approach.

## 2. Experimental

### 2.1. Chemicals and materials

β-Cyclodextrin and triethylamine were obtained from Fluka (Buchs, Switzerland). Allylamine was purchased from TCI, Tokyo. Phenyl isocyanate was purchased from Aldrich. The silica gel used for HPLC was supplied by Hypersil (UK) with a mean pore size of 100 Å, particle size of 5 μm and surface area of 300 $m^2$/g. Solvents used in synthesis were analytical reagent grade and were carefully purified and dried if necessary before use. Aromatic substituted alcohols were prepared by reducing the corresponding ketones with $LiAlH_4$ in anhydrous tetrahydrofuran (THF); chiral pyrimidine compounds were provided by Dr. W.K. Chui (Department of Pharmacy, National University of Singapore); all other chiral samples used were obtained from Sigma–Aldrich.

### 2.2. Instrumentation

$^{13}$C NMR spectra were performed on a Brucker ACF300 FT-NMR spectrometer; FT-IR spectra were performed on a Bio-Rad TFS156 instrument using KBr pellets. Elemental analysis was measured on a Perkin-Elmer 2400 CHN analyzer and the optical rotations were tested on a Perkin-Elmer 241 polarimeter. The employed HPLC system comprised of a Perkin-Elmer series 200 LC pump, Perkin-Elmer 785A UV-Vis detector, connected to a computer via Perkin-Elmer Nelson 900 series interface and 600 series link. The stainless steel HPLC empty columns (250 mm × 4.6 mm) were purchased from Phenomenex (USA). The CSP was packed into the empty column by following a conventional high-pressure slurry packing procedure using an Alltech (USA) air compression pump. All the chromatograms were obtained at ambient temperatures.

### 2.3. Preparation of silica gel with chemically bonded cyclodextrin

The synthesis of amido-bonded perphenylcarbomoylated β-cyclodextrin (CSP **5**) was effected in a relatively straightforward manner according to the synthetic route depicted in Fig. 1.

Mono[$6^A$-(*p*-toluenesulfonyl)-$6^A$-deoxy]-β-cyclodextrin, compound **1** [16–20] was readily converted to the key intermediate **2** in high purity and good yield by refluxing in allylamine for 5 h and then precipitating the product in acetonitrie [22]. Reaction of **2** with phenyl isocyanate afforded **3**. Thereafter, hydrosilylation of **3** with $(EtO)_3SiH$ in the presence of catalytic amount of tetrakis(triphenylphosphine)platinum(0) gave the reactive siloxane **4**, which was directly immobilized onto the surface of silica gel to afford the CSP **5**.

#### 2.3.1. Synthesis of mono($6^A$-N-allylamino-$6^A$-deoxy)-β-cyclodextrin, compound **2**

A solution of compound **1** (2.23 g, 1.73 mmol) in allylamine (23 g, 400 mmol) was refluxed for 5 h; the resultant solution was cooled to room temperature and diluted with methanol (30 ml). After addition of acetonitrile (200 ml) with stirring, the white precipitate was then filtered and dried under vacuum to afford the product (1.65 g, 82%): mp ≥ 195 °C (dec.); $[\alpha]_D$ + 122° ($c$ = 0.93, water); IR ($cm^{-1}$, KBr): 3395 (O–H, s), 2927 ($sp^3$C–H, m), 1657 (C=C, m), 1158 (C–N, m), 1033 (C–O–C, s); $^{13}$C NMR {75 MHz, [$^2H_6$]dimethyl sulfoxide (DMSO-$d_6$)} $\delta$: 51.47 ($CH_2$NH), 59.84 (C-6), 71.93 (C-2), 72.31 (C-5), 72.95 (C-3), 81.39–81.46 (C-4), 101.73–101.87 (C-1), 115.23 (CH=$CH_2$), 137.23 ($CH$=$CH_2$); anal. calcd. (%) for $C_{45}H_{75}O_{34}N$: C, 46.03; H, 6.44; N, 1.19. Found (%): C, 45.62; H, 6.83; N,

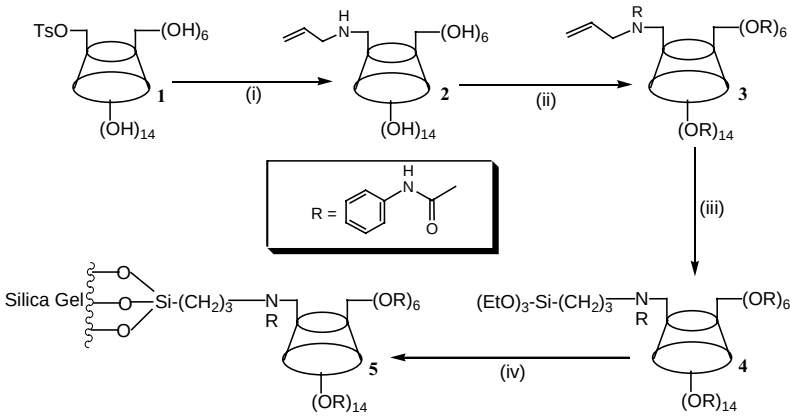



Fig. 1. Synthetic route to perfunctionalized cyclodextrin immobilized silica gel. Reagents and conditions: (i) allylamine/reflux; (ii) phenylisocyanate/pyridine (dry)/Δ; (iii) triethoxysilane/Pt cat./THF (dry)/reflux; (iv) silica gel/tolune/Δ.

1.67; ESI MS for $C_{45}H_{75}O_{34}N$ (1174), *m/z*: 1175 ($M^+$, 100%).

### 2.3.2. Synthesis of ($6^A$-N-allylamino-$6^A$-deoxy)heptakis (2,3-di-O-phenylcarbamoylated)-$6^B$,$6^C$,$6^D$,$6^E$,$6^F$,$6^G$-hexa-O-phenylcarbamoylated β-cyclodextrin, compound **3**

Phenyl isocyanate (14 g, 115.0 mmol) was added into a solution of compound **2** (1.60 g, 1.4 mmol) in dried pyridine (30 ml) and the mixture was stirred overnight at 85 °C. The solvent was removed by distillation under pressure to give a yellow–brown gel. The gel was dissolved in ethyl acetate (150 ml) and stirred for about 2 h after water (60 ml) was added. Filtration was applied and the organic layer of the filtrate was dried on anhydrous magnesium sulfate. Crude product was obtained after removing the solvent. The crude product was then subjected to flash chromatography over silica gel using *n*-hexane–ethyl acetate (2:1) as eluent to afford compound **3** as a light yellow solid (2.48 g, 52%): $R_f = 0.14$ (*n*-hexane–ethyl acetate, 2:1); mp 204–207 °C; $[\alpha]_D + 14.6°$ ($c = 0.005$, $CHCl_3$); IR ($cm^{-1}$, KBr): 3395, 3304 (N–C=O, m); 3056 ($sp^2$C–H, m); 2954 ($sp^3$C–H, m); 1737 (C=O, s); 1602, 1535, 1445 (arom C=C ring, s); 1228, 1083 (C–O–C, s); $^{13}$C NMR (75 MHz, DMSO-$d_6$) $\delta$: 31.23 ($CH_2$NH), 59.66 (C-6), 62.92 (C-$6^A$), 69.45–69.92 (C-2), 70.59–70.75 (C-5), 72.28 (C-3), 98.32 (C-1), 115.54 (CH = $CH_2$), 118.29–128.11 (aromatic C), 138.35 ($CH$ = $CH_2$), 148.52–152.49 (C=O); anal. calcd. (%) for $C_{192}H_{180}O_{55}N_{22}$: C, 62.74; H, 4.94; N, 8.38. Found (%): C, 62.55, H, 4.91, N, 8.29. Full functionalization of the hydroxyl groups with phenyl carbamate groups at 2- and 3- as well as 6-position of β-CD residues was corroborated by matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) using 2,3-dihydroxybenzoic acid (DHBA) as matrix. The sample was dissolved in acetone; the intense and exclusive molecular ion $[M + CH_3COCH_3]^+$ in Fig. 2 further confirmed the proposed structure of ($6^A$-*N*-allylamino-$6^A$-deoxy)heptakis(2,3-di-*O*-phenylcarbamoylated) -$6^B$,$6^C$,$6^D$,$6^E$,$6^F$,$6^G$-hexa-*O*-phenylcarbamoylated β-cyclodextrin.

### 2.3.3. Hydrosilylation and immobilization of compound **3**

Triethoxysilane (7 g, 43.8 mmol) was stirred with a solution of compound **3** (1.80 g, 0.5 mmol) and tetra(triphenylphosphine)platinum(0) (10 mg) in dried THF (8 ml) at 85 °C for 3 days, then the mixture was subjected onto silica gel in a Buchner funnel and eluted out quickly by addition of anhydrous diethyl ether (150 ml) under vacuum. After removal of solvent, the residue (**4**) was dissolved in anhydrous toluene (100 ml), followed by addition of silica gel (4.0 g, dried at 180 °C/0.5 mmHg for 10 h; 1 mmHg = 133.322 Pa). The mixture was then refluxed overnight, thereafter, 2 ml of water was added, and the resultant mixture was stirred at 90 °C for another 3 h. After filtration, the residue was washed with acetone in a Soxhlet apparatus for 24 h and then dried under vacuum at 90 °C overnight to give the tar-

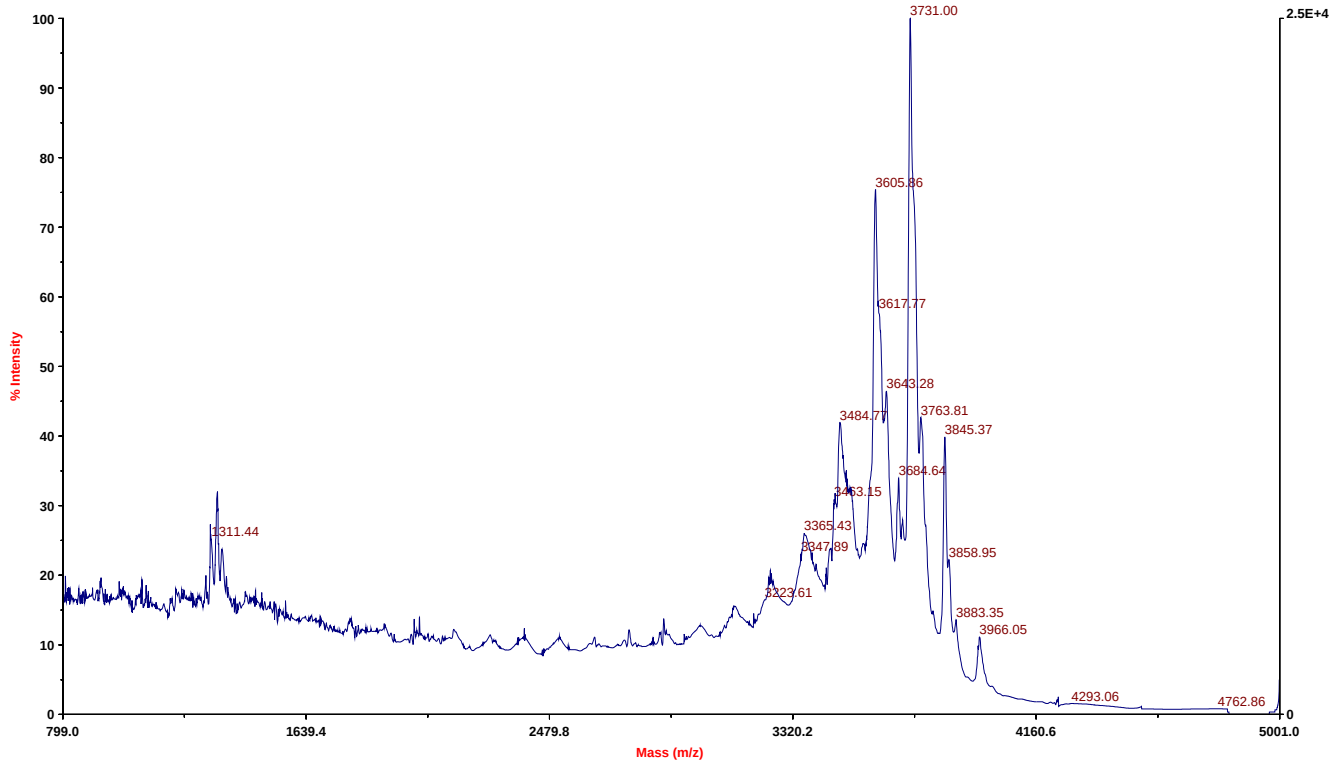



Fig. 2. MALDI-MS spectra of ($6^A$-*N*-allylamino-$6^A$-deoxy)heptakis(2,3-di-*O*-phenylcarbamoylated)-$6^B$,$6^C$,$6^D$,$6^E$,$6^F$,$6^G$-hexa-*O*-phenylcarbamoylated β-cyclodextrin. ($C_{192}H_{180}O_{55}N_{22}$: *m/z* anal. calcd. 3673. Found: 3731 for $[M + CH_3COCH_3]^+$).

get CSP **5** (elementary analysis: C, 4.98%; H, 0.73%; N, 0.45%).

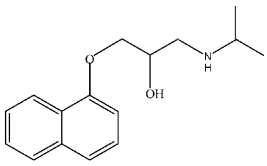
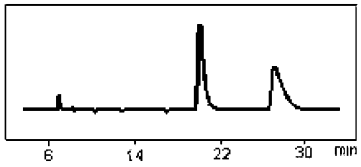
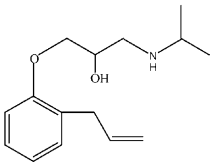
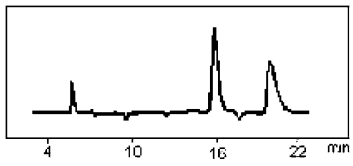
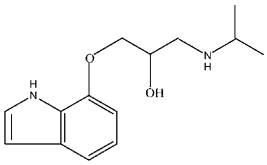
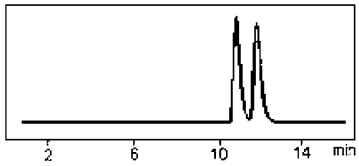
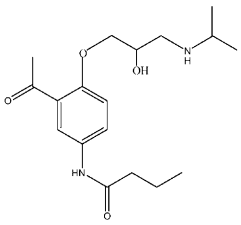
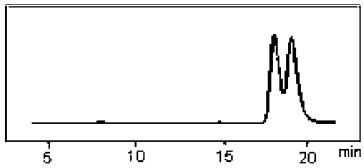
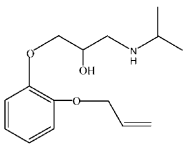
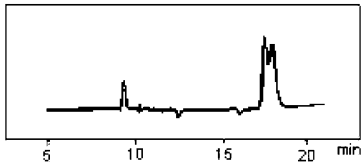
The carbon content in the elemental analysis as well as the appearance of FT-IR peak at 1733 $cm^{-1}$ attributable to carbonyl stretching in CSP **5** provides corroborative evidence that the cyclodextrin moieties have been successfully immobilized onto the surface of the silica gel. According to the microanalysis data, surface concentration [23] of the cyclodextrin derivative on the silica gel is calculated to be $7.8 \times 10^{-8}$ mol/$m^2$.

### 2.4. Preparation of column and mobile phase

The slurry method (using $CCl_4$–dioxane) was applied to prepare the HPLC column with methanol as the packing solvent. After suspended in $CCl_4$–dioxane (20 ml/10 ml) and sonicated for 20 min, slurry of CSP **5** (3.5 g) was packed into a stainless steel column (250 mm × 4.6 mm) with standing at the maximum pressure of 7800 psi for 20 min before a gradual release of pressure (1 psi = 6894.76 Pa). The column was conditioned with mobile phase before use.

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, was freshly prepared, filtered, and degassed under vacuum using a Degasys DG-2410 degasser. Duration of 1–2 h of equilibration after a pH change of the mobile phase was allowed in order to ob-

Table 1
Chromatographic results of five β-blockers

| S.No. | Compound | Separation data | Chromatogram |
|---|---|---|---|
| 1 | Propranolol  | $k_1 = 2.35$<br>$k_2 = 3.56$<br>$\alpha = 1.51$<br>$R_s = 4.70$<br>Condition: **II** |   |
| 2 | Alprenolol  | $k_1 = 1.54$<br>$k_2 = 2.13$<br>$\alpha = 1.38$<br>$R_s = 4.46$<br>Condition: **II** |   |
| 3 | Pindolol  | $k_1 = 0.71$<br>$k_2 = 0.87$<br>$\alpha = 1.23$<br>$R_s = 1.73$<br>Condition: **II** |   |
| 4 | Acebutolol (HCl)  | $k_1 = 1.63$<br>$k_2 = 1.79$<br>$\alpha = 1.10$<br>$R_s = 0.95$<br>Condition: **III** |   |
| 5 | Oxprenolol  | $k_1 = 1.75$<br>$k_2 = 1.84$<br>$\alpha = 1.05$<br>$R_s = 0.53$<br>Condition: **III** |   |

Conditions for all separations listed in Tables 1–4 are given as follows: (**I**) buffer (1% TEA, pH 4.75)-methanol (70:30), flow rate: 0.5 ml/min. (**II**) Buffer (1% TEA, pH 5.50)-methanol (65:35), flow rate: 0.5 ml/min. (**III**) Buffer (1% TEA, pH 5.50)-methanol (75:25), flow rate: 0.5 ml/min.

tain reproducible results. All the experiments were carried out at ambient temperature (ca. 25 °C).

## 3. Results and discussion

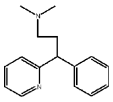
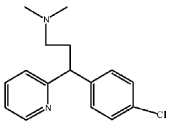
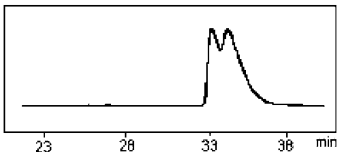
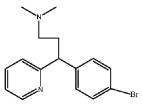
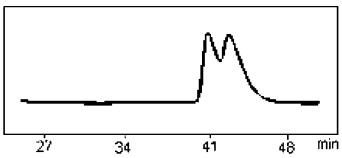
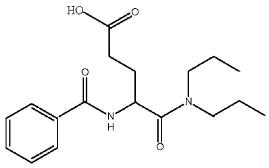
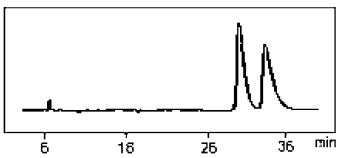
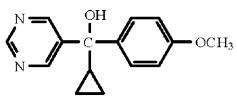
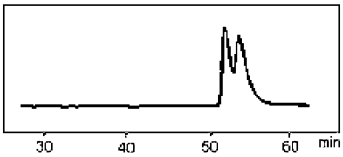
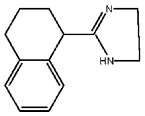
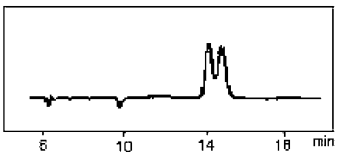
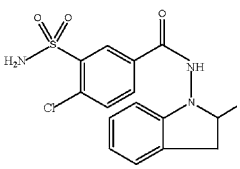
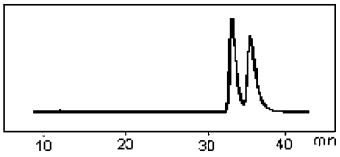
The column gives the efficiency of 37 250 plates per meter using biphenyl as a test probe under normal phase (isopropanol–hexane in 5:95 (v/v)). Chromatographic properties of this column were tested with a wide range of structurally diverse racemic compounds and drugs. The enantioseparation results are summarized in Tables 1–4.

### 3.1. Retention behavior of β-adrenergic blockers

β-Adrenergic blockers are a series of hydroxylamine containing aromatic rings with different substituent moieties. It is well known that their enantiomers have different potencies and pharmacological effects but most of them are marketed as racemic compounds. The chromatographic separation of these compounds has been dominated by protein-based CSPs which are limited by poor stability under certain HPLC conditions in spite of their high enantioseparation abilities [24].

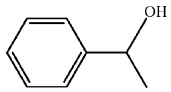
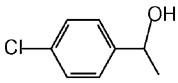
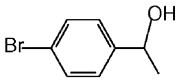
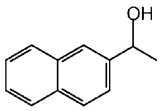
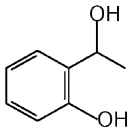
Some β-blocker drugs have been separated using this column under reverse phase condition (see Table 1). As ex-

Table 2
Chromatographic results of some other chiral drugs

| S.No. | Compound | Separation data | Chromatogram |
|---|---|---|---|
| 1 | Pheniramine  | $k_1 = k_2 = 4.0$<br>$\alpha = 1.0$, $R_s = 0$<br>Condition: **I** | No separation |
| 2 | Chlorpheniramine  | $k_1 = 4.24$<br>$k_2 = 4.41$<br>$\alpha = 1.04$<br>$R_s = 0.35$<br>Condition: **I** |   |
| 3 | Bromopheniramine  | $k_1 = 5.57$<br>$k_2 = 5.81$<br>$\alpha = 1.04$<br>$R_s = 0.65$<br>Condition: **I** |   |
| 4 | Proglumide  | $k_1 = 3.76$<br>$k_2 = 4.29$<br>$\alpha = 1.14$<br>$R_s = 1.66$<br>Condition: **II** |   |
| 5 | Ancymidol  | $k_1 = 6.66$<br>$k_2 = 7.05$<br>$\alpha = 1.06$<br>$R_s = 0.71$<br>Condition: **III** |   |
| 6 | Tetrahydrozoline  | $k_1 = 1.26$<br>$k_2 = 1.35$<br>$\alpha = 1.07$<br>$R_s = 0.86$<br>Condition: **III** |   |
| 7 | Indapamide  | $k_1 = 4.25$<br>$k_2 = 4.76$<br>$\alpha = 1.12$<br>$R_s = 1.33$<br>Condition: **II** |   |

Conditions for separations: see Table 1.

Table 3
Chromatographic results of some aromatic alcohol

| S.No. | Chiral compounds | Separation data | |
|---|---|---|---|
| 1 |  | No separation | |
| 2 |  | $k_1 = 2.28$<br>$k_2 = 3.50$ | $\alpha = 1.54$<br>$R_s = 5.00$ |
| 3 |  | $k_1 = 0.84$<br>$k_2 = 3.17$ | $\alpha = 3.77$<br>$R_s = 12.22$ |
| 4 |  | $k_1 = 4.77$<br>$k_2 = 5.14$ | $\alpha = 1.08$<br>$R_s = 1.09$ |
| 5 |  | $k_1 = 1.37$<br>$k_2 = 1.49$ | $\alpha = 1.09$<br>$R_s = 0.74$ |

Conditions: methanol–buffer (pH 5.5) (75:25), 0.5 ml/min.

pected, propranonol and pindolol showed enhanced enantioseparation over other analogs, this result is consistent with the experiment data reported by Fujimura et al. [25] and Kawaguchi et al. [26], they highlighted that the presence of two aromatic rings in chiral solutes may contribute to accentuated resolution because the two aromatic rings can fit better into the cavity of β-CD. It was also reported [27,28] that the more rigid the solute, the bigger is the energy difference during formation of inclusion complexes. Consequently, it is likely that the more rigid molecular structure afford better enantioseparation. The bicyclic aromatic moieties make propranolol and pindolol relatively more rigid than others, which may result in a larger energy difference between the diastereomeric inclusion complexes as mentioned above, thus contributing to enhanced chiral discrimination.

Alprenolol and oxprenolol exhibited different separation results even though their chemical structures are quite similar except that the substituted groups are $CH_2$=$CHCH_2$– and $CH_2$=$CHCH_2O$–, respectively. The same result was observed with another CSP reported by us previously [29]. It might be due, in part, to the electron donating ability of the $CH_2$=$CHCH_2O$– group. This conjugative ether moiety in the pendant would make the aromatic ring attached to $CH_2$=$CHCH_2O$– electron rich, thereby reducing the π–π interaction between the analyte and the aromatic rings on the CSP which are also electron rich. The bigger separation factors ($\alpha = 1.38$) and the resolutions ($R_s = 4.46$) of alprenolol may be attributable to the stronger π–π interaction between the solute and CSP.

### 3.2. Retention behavior of some other chiral drugs

Table 2 depicts the chromatographic data of some other chiral drugs on CSP **5**. The data listed in the table indicates that, from bromopheniramine ($R_s = 0.65$), chloropheniramine ($R_s = 0.35$) to pheniramine ($R_s = 0$), the resolutions decrease under the same condition. Considering the structure of the three molecules, the difference is the substituents attached to the chiral center, which are *p*-bromophenyl, *p*-chlorophenyl and phenyl, respectively. To explain the separation result, we assume that the existence of halogen atom (Br–, Cl–) reduces the electron density of the benzene ring making the ring π-deficient. Meanwhile, the aromatic phenylcarbamate group in the CSP is π-excessive. Consequently, the π-acceptor/π-donor interaction between the benzene ring of the analytes and the aromatic ring of the CSP contributes to improved enantioseparation. Analogous results are observed in the enantioseparation of the aromatic alcohols (see Table 3).

### 3.3. Retention behavior of some chiral aromatic alcohols

As shown in Table 3, 1-(*p*-bromophenyl)ethanol and 1-(*p*-chlrorophenyl)ethanol were separated better than the other aromatic alcohols. In comparing 1-(*p*-bromophenyl)ethanol and 1-(*p*-chlorophenyl)ethanol, the former afforded accentuated $\alpha$ and $R_s$ values. The proposed interaction mechanism mentioned above is assumed to be the reason for this result.

### 3.4. Retention behavior of a series of pyrimidine compounds

Pyrimidine compounds tested here are a series of potential antimalaria drugs with the molecular structure listed in Fig. 3. Table 4 shows that almost all of the eighteen pyrimidine compounds selected can be well separated on the column. Some representative chromatograms are depicted in Fig. 4. Perusing the generic structure of pyrimidine compounds, we find that there is a $sp^2$ hybridized carbon from the phenyl group attached to the chiral carbon. It is reported that the $sp^2$ hybridization of groups attached to the chiral center affects the chiral recognition greatly. The more $sp^2$ hybridized atoms attached to the chiral center, the better the enantioseparation achievable on CD-CSPs [30,31]. Armstrong and co-workers also pointed out that if the chiral center is part of a ring structure, the chiral recognition by CD-CSPs is enhanced [32]. Consequently, we would expect a good resolution of this series of chiral compounds on our column. As seen, the separation results listed in Table 4

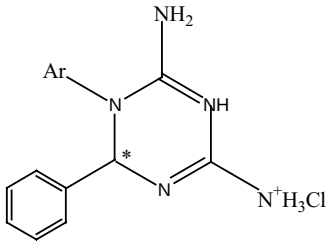



Fig. 3. General structure of pyrimidine compounds.

Table 4
Separation results for some pyrimidine compounds

| S.No. | Ar group | Chromatographic data | | | | Separation condition |
|---|---|---|---|---|---|---|
| | | $k_1$ | $k_2$ | $\alpha$ | $R_s$ | |
| 1 | *p*-Chlorophenyl | 1.69 | 1.94 | 1.15 | 2.19 | **II** |
| 2 | *p*-Bromophenyl | 2.52 | 3.06 | 1.21 | 2.72 | **I** |
| 3 | *p*-Toluyl | 2.05 | 2.37 | 1.16 | 1.62 | **I** |
| 4 | *p*-Methoxyphenyl | 2.78 | 3.19 | 1.15 | 2.00 | **I** |
| 5 | *m*-Chlorophenyl | 2.63 | 3.05 | 1.16 | 2.23 | **I** |
| 6 | *m*-Bromophenyl | 2.95 | 3.19 | 1.08 | 1.18 | **III** |
| 7 | *m*-Toluyl | 2.14 | 2.43 | 1.14 | 1.67 | **I** |
| 8 | *m*-Nitrophenyl | 1.68 | 2.03 | 1.21 | 2.66 | **I** |
| 9 | *m*-Cyanophenyl | 3.63 | 4.00 | 1.10 | 1.54 | **I** |
| 10 | *o*-Chlorophenyl | 2.22 | 2.42 | 1.09 | 1.38 | **II** |
| 11 | *o*-Bromophenyl | 1.77 | 2.06 | 1.16 | 2.27 | **II** |
| 12 | *o*-Toluyl | 2.59 | 2.95 | 1.14 | 2.12 | **I** |
| 13 | *o*-Methoxyphenyl | 1.17 | 2.17 | 1.85 | 9.79 | **II** |
| 14 | 3′-Chloro-4′-methylphenyl | 1.29 | 1.52 | 1.18 | 1.89 | **I** |
| 15 | 3′-Chloro-4′-methoxyphenyl | | No separation | | | **I–III** |
| 16 | 3′-Nitro-4′-chlorophenyl | 4.70 | 5.03 | 1.07 | 1.20 | **III** |
| 17 | 3′-Nitro-4′-methylphenyl | 2.17 | 2.28 | 1.05 | 0.57 | **III** |
| 18 | 3′,4′-Dimethylphenyl | 2.03 | 2.25 | 1.11 | 0.98 | **III** |

Conditions for separations: see Table 1.

are in accordance with the general expectation and all compounds except 15 of this series were well separated.

### 3.5. Comparison of CSP **5** with single urea-covalent bonded column SINU-PC

For the purpose of structure-property correlation of our CSPs, we compared the enantioseparation ability of CSP **5** with that of the SINU-PC column [21]. These two CSPs were prepared based on the same silica gel and CD derivatives involved were perfunctionalised by the same substituent; the difference lies in the methodology of immobilization: hydrosilylation of mono(allylamino)perfunctionalised CD **3** with triethoxylsilane and then reaction of the reactive siloxane **4** with pristine silica gel afforded a durable CSP **5** without free amine groups on the surfaces while SINU-PC was prepared by immobilizing mono($6^A$-azido-$6^A$-deoxy)-perphenylcarbamoylated β-CD, onto the surface of aminized silica gel via Staudinger reaction. Enantioseparation data of β-adrenergic blockers and some drugs on SINU-PC are summarized in Table 5.

From Tables 1, 2 and 5, it is evident that the derived CSP **5** depicted complementary enantioresolution abilities from the SINU-PC. It is also suggestive that for those racemates which can be separated on both of the two columns, better $\alpha$ as well as $R_s$ values and shorter retention ($k$) were achieved on CSP **5**. All these indicate that the current synthetic procedure can afford improved CSPs.

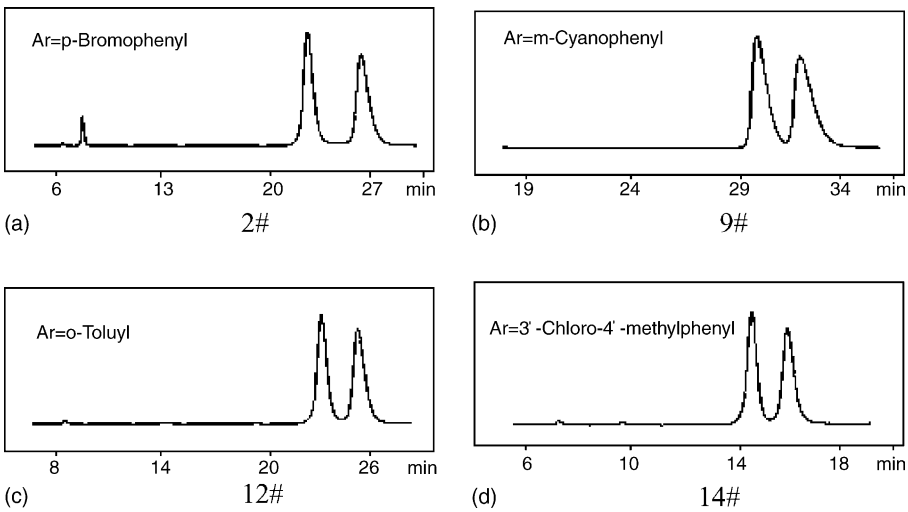



Fig. 4. Some representative chromatograms of chiral pyrimidine compounds on the column packed with CSP **5**.

Table 5
Summary of the enantioseparation data on SINU-PC[a]

| Compound | Separation result | | | | Separation condition |
|---|---|---|---|---|---|
| | $k_1$ | $k_2$ | $\alpha$ | $R_s$ | |
| Propranolol | 3.91 | 4.97 | 1.27 | 3.08 | Water–$CH_3CN$ (70:30) |
| | 1.83 | 2.53 | 1.38 | 1.63 | Hexane–isopropanol (80:20) |
| Alprenolol | 12.6 | 14.49 | 1.15 | 1.25 | Water–$CH_3CN$ (85:15) |
| Oxprenolol | 3.67 | 4.40 | 1.20 | 1.00 | Hexane–isopropanol (95:5) |
| Acebutolol (HCl) | | | | | No separation |
| Pindolol | | | | | No separation |
| Chlorpheniramine | | | | | No separation |
| Brompheniramine | | | | | No separation |
| Proglumide | | | | | No separation |
| Ancymidol | | | | | No separation |
| Tetrahydrozoline | | | | | No separation |
| Indapamide | | | | | No separation |

[a] The chromatographic data listed in this table come from ref. [21].

## 4. Conclusions

A novel chiral stationary phase with amino-linked and immobilized mono($6^A$-*N*-allylamino-$6^A$-deoxyl)-perphenyl carbamoylated β-cyclodextrin onto silica gel via hydrosilylation has been conveniently prepared. A wide range of racemic drugs and compounds can be successfully separated on this column. The enantioseparation data indicate that the current synthetic procedure can afford improved CSPs.

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