Journal of Chromatography A, 1218 (2011) 3496–3501

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Novel β-cyclodextrin chiral stationary phases with different length spacers for normal-phase high performance liquid chromatography enantioseparation

Xianghua Lai [a], Weihua Tang [b,*], Siu-Choon Ng [a,**]

[a] Division of Chemical and Biomolecular Engineering, College of Engineering, Nanyang Technological University, 16 Nanyang Drive, Singapore 637722, Singapore
[b] Key Laboratory of Soft Chemistry and Functional Materials, Ministry of Education, Nanjing University of Science and Technology, 200 Xiaolingwei Street, Nanjing 210094, China

ARTICLE INFO

Article history:
Received 15 February 2011
Received in revised form 26 March 2011
Accepted 28 March 2011
Available online 4 April 2011

Keywords:
Chiral stationary phase
Cyclodextrin
Spacer
Enantioseparation

ABSTRACT

Cyclodextrin and its derivatives are widely used as selectors of chiral stationary phases (CSPs) for high performance liquid chromatography (HPLC) due to their unique molecular structure and resolution capability. Three mono(6^A-*N*-(ω-alkenylamino)-6^A-deoxy)perphenylcarbamoylated β-cyclodextrin (**PICD**) based CSPs with different length spacers have been prepared, with their enantioseparation abilities evaluated with 10 model racemates including aromatic alcohols, flavanone compounds, amine and non-protolytic compounds under normal-phase conditions. The effect of spacer length and surface loading on the enantioseparation performance of CSPs is investigated herewith. The results indicate that higher surface loading **6C-PICD** displays the best enantioselectivities towards selected racemates under normal-phase conditions.

1. Introduction

Liquid chromatographic separation of racemates using chiral stationary phases (CSPs) has become an indispensable tool in many areas such as pharmaceutical industry, food and life sciences. Currently, there are two main approaches to prepare CSPs. The first is to prepare CSPs by physically coating functionalized chiral selectors, such as cellulose and amylose derivatives, onto silica gel; the second approach is to prepare CSPs by chemical bonding of chiral selectors onto siliceous support through an appropriate spacer arm. CSPs prepared by these two approaches exhibit excellent enantioseparation abilities towards a wide range of structurally diverse racemic compounds. To date, the chemically bonded CSPs have received prominent attention based upon their stability and utility with many solvents [1,2]. Chemically bonded CSPs have been developed into several categories: Pirkle-type, protein-based, polysaccharide-based, macrocyclic antibiotic, crown ethers, imprinted polymers, chiral ligand exchange and cyclodextrin (CD)-based CSPs [2–11].

CDs have an internal toroidal and relatively non-polar cavity. It may easily accommodate molecules with suitable size. Based on the unique molecular structure and natural chirality, CD-based CSPs have been used extensively in HPLC enantioseparation *via* chemically bonding onto silica gel through various spacer arms [1,3,12–15]. It was reported that the enantioseparations of CD-based CSPs are attributed, at least partially, to the spacer which kept the cavity of CD chiral selectors away from the support surface [16]. Berthod et al. reported that the length of spacer tethering the chiral selector to the chromatographic support has a dramatic influence on the chiral separation because the spacer makes the solutes' access easier to facilitate the occurrence of chiral discrimination [17]. On the contrary, no significant difference was also observed in the enantioseparation abilities of CD-bonded CSPs with different length spacers by Lai and Ng [18]. Based upon conflicting information, we think that the length of spacer might influence the enantioseparation abilities under some conditions because the chiral recognition is assumed to occur based upon differential energies as well as access between the analytes and chiral selector.

Previously, we reported a facile synthetic protocol for a series of CD-based CSPs by immobilizing mono(6^A-*N*-allylamino-6^A-deoxyl)perfunctionalized CDs onto the surface of pristine silica *via* hydrosilylation [19]. As an extension of our study, this work aims to provide an insight view on the effect of spacer length on the enantioseparation abilities of this series of CD-based CSPs. Three CSPs based on mono(6^A-*N*-(ω-alkenylamino)-6^A-deoxy)perphenylcarbamoylated CD (PICD) with different length spacer arms of different length were prepared by immobilizing onto silica surface *via* hydrosilylation. Their chromatographic performance under normal-phases was evaluated with 10 model racemates including aromatic alcohols, flavanone compounds, amine and non-protolytic compounds. The effect of spacer length and surface loading on the enantioseparation of the resulted CSPs is discussed in detail.

* Corresponding author. Tel.: +65 67904067; fax: +65 67947553.
** Corresponding author.
E-mail addresses: whtang@mail.njust.edu.cn (W. Tang), ngsc@ntu.edu.sg (S.-C. Ng).

0021-9673/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2011.03.071

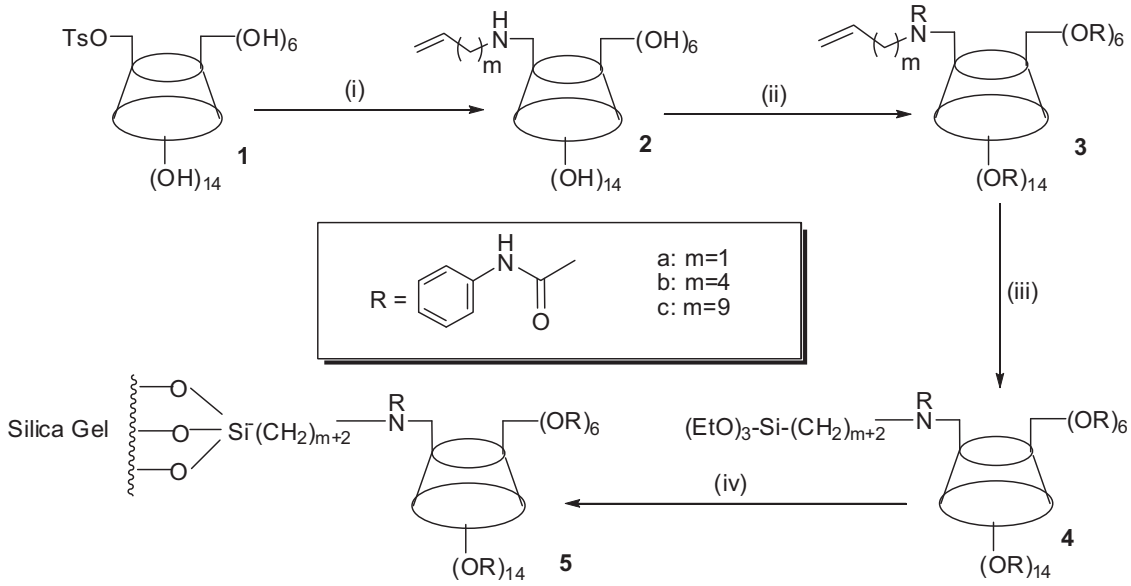


Fig. 1. Synthetic route to per functionalized cyclodextrin immobilized silica gel *Reagents and conditions:* (i) ω-alkenyl amine/Δ; (ii) phenylisocyanate/pyridine (dry)/Δ; (iii) triethoxysilane/Pt cat./THF (dry)/reflux; (iv) silica gel/toluene/Δ.

2. Experimental

2.1. Chemicals and materials

β-CD and triethylamine were obtained from Fluka (Buchs, Switzerland). Allylamine was purchased from TCI, Tokyo. Phenylisocyanate was purchased from Aldrich. Pyridine was distilled over calcium hydride before use; tetrahydrofuran (THF) and toluene were distilled over sodium before use. The silica gel used for HPLC was supplied by Kromasil (mean pore size 100 Å, particle size 5 μm and surface area of 300 m^2/g). All chiral samples used were obtained from Aldrich.

2.2. Instrumentations

NMR spectra were collected on a Brucker ACF300 FT-NMR spectrometer; FT-IR spectra were performed on a Bio-Rad TFS156 instrument using KBr pellets. Elemental analysis was carried out on a Perkin-Elmer 2400 CHN analyzer and the optical rotations were measured on a Perkin Elmer 241 polarimeter. The HPLC system including a Perkin Elmer series 200 LC pump and Perkin Elmer 785A UV/vis detector, is connected to a computer *via* Perkin Elmer Nelson 900 series interface and 600 series link. Stainless steel HPLC empty columns (⌀4.6 mm × 250 mm) were purchased from Phenomenex (USA).The CSP was packed into the empty column by a high-pressure slurry packing procedure using an Alltech® air compression pump (Alltech, USA). All the chromatograms were obtained at ambient temperature.

2.3. Preparation of silica gel with chemically bonded cyclodextrin

Mono(6^A-*N*-(ω-alkenylamino)-6^A-deoxy)perphenylcarbamoylated-β-cyclodextrin based CSPs with different spacer lengths were prepared according to the synthetic route depicted in Fig. 1.

Mono(6^A-(*p*-tolysulfonyl)-6^A-deoxy)-β-CD **1** [20–25] was readily converted to the key intermediate **2** in high purity and good yield by refluxing with ω-alkenylamine in DMF for 5 h and then precipitating the product in acetonitrile [10,17]. 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 resultant CSP **5**.

2.3.1. Preparations of ω-alkenylamines

The spacers with different arm-length were synthesized with a three-step Gabriel reaction [26–28], as shown in Fig. 2. ω-alkenyl alcohols with different alkyl chains first underwent an iodinization to obtain 1-iodo-olefines, which reacted with potassium phthalimide to give the intermediate 1-phthalimide-olefine. The intermediate was further treated with hydrazine to afford the desired products: 10-undecenylamine and 5-hexenylamine.

The synthesis protocol for 10-undecenylamine is described as follows. Triphenylphosphine (25.2 g, 96 mmol), imidazole (96.5 g, 96 mmol) and iodine (24.4 g, 96 mmol) were added subsequently to 300 mL dichloromethane (DCM) under cooling. A solution of 10-undecen-1-ol (15.3 g, 90 mmol) in DCM (70 mL) was then added dropwise to the previously obtained solution and the resulting mixture was stirred at room temperature for 3 h. After filtering off the insoluble, the filtrate was concentrated to about 50 mL and then poured into vigorously stirred hexane (500 mL). After filtration, the filtrate was concentrated to afford10-undecen-1-iodine. 10-undecen-1-iodine was further reacted with potassium phthalimide (8.3 g, 45.0 mmol) in DMF (ca. 100 mL) at 80 °C for 5 h. The reaction mixture was then poured into iced water (500 mL), the white precipitate was collected and vacuum-dried over P_2O_5. The dried precipitate was then dissolved in methanol and refluxed with hydrazine hydrate for 2 h. Afterwards, concentrated HCl (50 mL, 10 M) was added and the solution was refluxed for another 1.5 h.

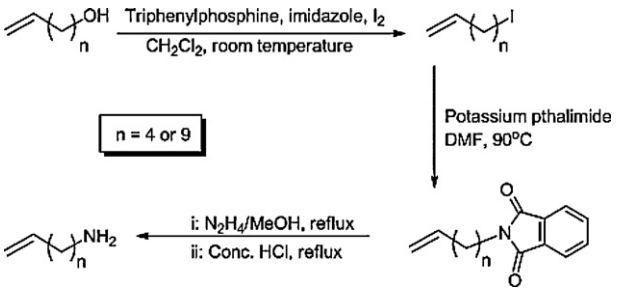
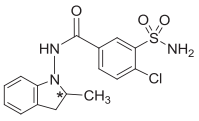
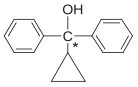
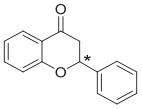
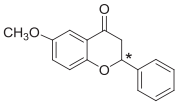
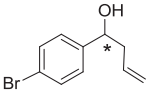
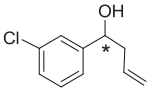
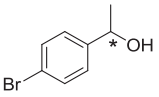
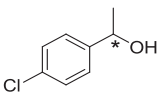
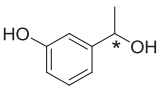


Fig. 2. Preparation of the spacer arms.

Table 1
Characteristics of the resultant CSPs.

CSP	IR peak (cm^{-1})	Elemental analysis (%)			Surface loading ($\times 10^{-8}$ mol m^{-2})	Column efficiency (plates/m)
		C	H	N		
3C-PICD	1733	4.98	0.73	0.45	7.8	36,000
6C-PICD	1740	8.79	1.07	0.88	14.5	38,000
11C-PICD	1738	4.43	0.64	0.43	6.6	30,000

Table 2
Enantioseparation of racemic compounds on the three columns.

Entry	Compounds	Columns	Chromatographic data*				
			k_1	k_2	α	Rs	Cond.
#1. Indapamide		**3C-PICD**	4.62	4.62	1.00	0	I
		6C-PICD	5.57	7.09	1.27	1.36	I
		11C-PICD	5.38	5.38	1.00	0	I
#2. Ancymidol		**3C-PICD**	2.20	2.20	1.00	0	I
		6C-PICD	2.66	3.06	1.15	1.24	I
		11C-PICD	2.54	2.54	1.00	0	I
#3. 4-Chromanol		**3C-PICD**	5.13	5.13	1.00	0	II
		6C-PICD	5.54	6.21	1.12	1.92	II
		11C-PICD	3.75	4.20	1.12	1.75	II
#4. Flavanone		**3C-PICD**	0.85	1.17	1.38	1.25	III
		6C-PICD	1.13	1.58	1.40	3.00	III
		11C-PICD	0.87	1.16	1.33	2.35	III
#5. 6-Methoxyflavanone		**3C-PICD**	1.25	1.45	1.16	0.50	IV
		6C-PICD	2.00	2.26	1.13	0.88	IV
		11C-PICD	2.32	2.32	1.00	0	IV
#6. 1-4-Bromophenyl-3-buten-1-ol		**3C-PICD**	0.55	1.2	2.18	2.05	IV
		6C-PICD	0.84	1.74	2.07	3.20	IV
		11C-PICD	0.44	0.49	1.11	0.92	IV
#7. 1-3-Chlorophenyl-3-buten-1-ol		**3C-PICD**	1.18	1.45	1.23	0.8	II
		6C-PICD	1.41	1.82	1.29	1.16	II
		11C-PICD	1.07	1.07	1.00	0	II
#8. 1-(4-Bromophenyl)-ethanol		**3C-PICD**	0.82	1.35	1.65	2.45	IV
		6C-PICD	1.0	1.79	1.79	3.92	IV
		11C-PICD	0.47	0.51	1.09	0.71	IV
#9. 1-(4-Chlorophenyl)-ethanol		**3C-PICD**	2.16	2.16	1.00	0	II
		6C-PICD	2.82	3.72	1.32	1.50	II
		11C-PICD	2.26	2.71	1.20	1.38	II
#10. 1-(3-Hydroxylphenyl)-ethanol		**3C-PICD**	2.22	2.9	1.31	1.69	IV
		6C-PICD	3.11	4.26	1.37	2.25	IV
		11C-PICD	2.24	2.24	1.00	0	IV

HPLC conditions: flowrate, 0.80 mL/min; I, hexane/IPA (75/25); II, hexane/IPA (97/3); III, hexane/IPA (95/5); IV, hexane/IPA (90/10).

After filtration, the filtrate was adjusted to alkaline by cautiously adding with KOH solution. The filtrate was extracted twice with ether and dried over anhydrous $CaCl_2$. The residue was vacuum distilled to afford pure product 10-undecenylamine as colorless oil (yield 65%). Characterization data for 10-undecenylamine: IR (cm^{-1}) 3374, 3293 (N–H str), 2926, 2854 (C–H str), 1641 (–NH_2 def), 1066 (C–NH_2 str); ^{1}H NMR ($CDCl_3$) δ (ppm) 1.28 (16H, ($C\underline{H}_2$)$_8$–CH_2–NH_2), 2.04 (2H, CH_2–$N\underline{H}_2$), 2.68 (2H, $C\underline{H}_2$–NH_2), 4.96 (2H, $C\underline{H}_2$=CH–), 5.81 (1H, CH_2=C$\underline{H}$–); ESI-MS *m*/*z* calcd for $C_{11}H_{23}N$ 169, found 170 for $[M]^+$.

The characterization data for 5-hexenylamine: IR (cm^{-1}) 3226 (N–H str), 2948, 2836 (C–H str), 1656 (–NH_2 def), 1112 (C–NH_2 str); ^{1}H NMR ($CDCl_3$) δ (ppm) 1.29 (6H, ($C\underline{H}_2$)$_3$–CH_2–NH_2), 2.05 (2H, CH_2–$N\underline{H}_2$), 2.72 (2H, $C\underline{H}_2$–NH_2), 4.97 (2H, $C\underline{H}_2$=CH– 5.78 (1H, CH_2=C$\underline{H}$–); ESI-MS *m*/*z* calcd for C6H13N 99, found 100 for $[M]^+$.

2.3.2. Synthesis of mono(6^A-N-(5-hexenylamino)-6^A-deoxy) β-cyclodextrin **2b**

We have previously reported the synthesis of mono(6^A-N-allylamino-6^A-deoxy)-β-cyclodextrin **3b** [19,29]. By following a similar synthetic procedure as for **2a**, 5-hexenylamine (20.0 g, 202.0 mmol) and **1** (6.5 g, 5.0 mmol) were employed to afford **2b** (3.0 g, 48%). Characterization data for **2b**: m.p. >229 °C; $[\alpha]_D^{25}$ + 109.8° (*c* = 1.4, H_2O); IR (cm^{-1}, KBr): 3380 (O–H, s), 2924 (sp^3C–H, m), 1640 (C=C, m), 1142 (C–N, m), 1035 (C–O–C, s); ^{13}C NMR(75 MHz, DMSO-d_6) δ: 29.51–34.40 (($C\underline{H}_2$)$_3$CH=CH_2), 48.50 ($C\underline{H}_2$NH), 59.83 (C-6), 71.95 (C-2), 72.18–72.35 (C-5), 72.80 (C-3), 81.09–81.46 (C-4), 101.44–101.87 (C-1), 114.83 (CH=$C\underline{H}_2$), 138.46 ($C\underline{H}$=CH_2); Anal. Calcd. (%) for $C_{48}H_{81}O_{34}N{\cdot}3H_2O$: C, 45.39; H, 6.90; N, 1.10, Found (%): C, 43.55, H, 6.79, N, 1.13, ESI-MS for $C_{48}H_{81}O_{34}N$ (1216), *m*/*z*: 1217 (M^+, 100%).

2.3.3. Synthesis of mono(6^A-N-(10-undecenylamino)-6^A-deoxy) β-cyclodextrin **2c**

By following a similar synthetic procedure as for **2a**, 10-undecenylamine (25.0 g, 142.0 mmol) and **1** (6.5 g, 5.0 mmol) were employed to afford **2c** (3.4 g, 52%). Characterization data for **2c**: m.p. >220 °C, decomposed; $[\alpha]_D^{25}$ + 103.0° (*c* = 0.9, H_2O); IR (cm^{-1}, KBr): 3377 (O–H, s), 2925 (sp^3C–H, m), 1643 (C=C, m), 1142 (C–N, m), 1034 (C–O–C, s); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 26.52–35.39 (($C\underline{H}_2$)$_8$CH=CH_2), 49.11 ($C\underline{H}_2$NH), 59.80 (C-6), 71.95 (C-2), 72.35 (C-5), 72.97 (C-3), 81.46 (C-4), 101.87 (C-1), 114.63 (CH=$C\underline{H}_2$), 138.69 ($C\underline{H}$=CH_2); Anal. Calcd. (%) for $C_{53}H_{91}O_{34}N{\cdot}6H_2O$: C, 45.64; H, 7.45; N, 1.0, Found (%): C, 45.28, H, 7.25, N, 0.89, ESI MS for $C_{53}H_{91}O_{34}N$ (1286), *m*/*z*: 1287 (M^+, 58%), 1286 (100%).

2.3.4. Synthesis of (6^A-N-(5-hexenyl-1-amino)-6^A-deoxy)-heptakis (2,3-di-O-phenylcarbamate)-6^B,6^C,6^D,6^E,6^F,6^G-hexakis-O-phenylcarbamoylated β-cyclodextrin, **3b**

By following a similar synthetic procedure as for (6^A-N-allylamino-6^A-deoxy)heptakis (2,3-di-O-phenylcarbamate)-6^B,6^C,6^D,6^E,6^F,6^G-hexakis-O-phenylcarbamoylated β-cyclodextrin **3a** [19,29], phenylisocyanate (6.0 g, 50.0 mmol) and **2b** (2.4 g, 2.0 mmol) were employed to afford **3b** (4.2 g, 56%). Characterization data for **3b**: m.p. 193–200 °C; $[\alpha]_D^{25}$ + 6.0° (*c* = 1.7, $CHCl_3$); IR (cm^{-1}, KBr): 3379, 3310 (N–C=O, m); 3058 (sp^2C–H, m); 2929 (sp^3C–H, m); 1728 (C=O, s); 1604, 1537, 1435 (arom C=C ring, s); 1224, 1049 (C–O–C, s); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 25.04–30.58 (($C\underline{H}_2$)$_3$CH=CH_2), 32.91 ($C\underline{H}_2$NH), 58.30 (C-6), 62.91 (C-6^A), 68.76–69.78 (C-2), 70.43–71.01 (C-5), 72.01 (C-3), 98.31 (C-1), 114.54 (CH=$C\underline{H}_2$), 117.18–128.60 (aromatic C), 138.36 ($C\underline{H}$=CH_2), 152.52–153.51 ($\underline{C}$=O); Anal. Calcd. (%) for $C_{195}H_{186}O_{55}N_{22}{\cdot}2H_2O$: C, 62.39; H, 5.10; N, 8.21, Found (%): C, 63.07, H, 5.07, N, 8.11.

2.3.5. Synthesis of (6^A-N-(10-undecenyl-1-amino)-6^A-deoxy)-heptakis (2,3-di-O-phenylcarbamate)-6^B,6^C,6^D,6^E,6^F,6^G-hexakis-O-phenylcarbamoylated β-cyclodextrin, **3c**

By following a similar procedure as for **3a**, phenylisocyanate (6.0 g, 50.0 mmol) and **2c** (2.6 g, 2.0 mmol) were employed to afford **3c** (3.8 g, 50%). Characterization data for **3c**: m.p. 185–191 °C; $[\alpha]_D^{25}$ + 6.8° (*c* = 1.7, $CHCl_3$); IR (cm^{-1}, KBr): 3379, 3306 (N–C=O, m); 3058 (sp^2C–H, m); 2958 (sp^3C–H, m); 1728 (C=O, s); 1604, 1528, 1435 (arom C=C ring, s); 1223, 1049 (C–O–C, s); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 19.80–29.02 (($C\underline{H}_2$)$_8$CH=CH_2), 33.10 ($C\underline{H}_2$NH), 59.69 (C-6), 62.92 (C-6^A), 69.68 (C-2), 70.64–71.06 (C-5), 71.56–71.99 (C-3), 98.25 (C-1), 114.38 (CH=$C\underline{H}_2$), 117.19–129.65 (aromatic C), 138.10–138.80 ($C\underline{H}$=CH_2), 152.53–152.93 ($\underline{C}$=O); Anal. Calcd. (%) for $C_{200}H_{196}O_{55}N_{22}{\cdot}H_2O$: C, 63.38; H, 5.27; N, 8.13. Found (%): C, 62.62, H, 5.18, N, 8.48.

2.3.6. Hydrosilylation and immobilization of compound **3**

Triethoxysilane (7 g, 43.8 mmol) was stirred with a solution of **3** (0.5 mmol) and tetra(triphenylphosphine)platinum (0) (10 mg) in dried THF (8 mL) at 85 °C for 3 days. The mixture was subjected to fast filtration through silica gel column with anhydrous diethyl ether (150 mL). After solvent removal, the as-prepared **4** was dissolved in anhydrous toluene (100 mL), followed by the addition of silica gel (4.5 g, dried at 130 °C/0.5 mmHg for 10 h). 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 soxhlet extracted with acetone for 24 h and then vacuum-dried overnight at 90 °C to give the target CSPs 5. The elemental analysis data of the three target CSPs are listed in Table 1. The carbon content in the elemental analysis as well as the appearance of FT-IR peak at about 1740 cm^{-1}, attributable to carbonyl stretching in the CSPs, provides the corroborative evidence that CD moieties have been successfully immobilized onto the surface of silica gel. According to the microanalysis data, surface concentration of CD derivatives on silica gel is calculated as shown in Table 1.

2.4. Packing of the column

The slurry method was applied to pack the derived CSP into HPLC columns using *n*-hexane as the packing solvent. After suspending the CSP (3.5 g) in *n*-hexane (30 mL) and sonicating for 20 min, the CSP slurry was packed into the stainless steel column at a pressure of 7800 psi maintained for 20 min before gradual release of the pressure. The column was conditioned with mobile phase before use.

3. Results and discussions

Table 1 depicts the elemental analysis data for the three CSPs; and accordingly, surface loading of the CD derivative on silica gel is calculated [18]. It is evident that the surface loading for **6C-PICD** is the best whilst **11C-PICD** has the lowest coverage, which may result from the higher steric hindrance with longer spacer arms. The efficiencies of each column have been measured under normal phase (hexane/IPA, 90/10 (v/v), 0.80 mL/min) using biphenyl as the testing compound. As also shown in Table 1, **6C-PICD** presents the highest number of theoretical plates (38,000 plates/m), in comparison to 36,000 plates/m for **3C-PICD** and 30,000 plates/m for **11C-PICD**.

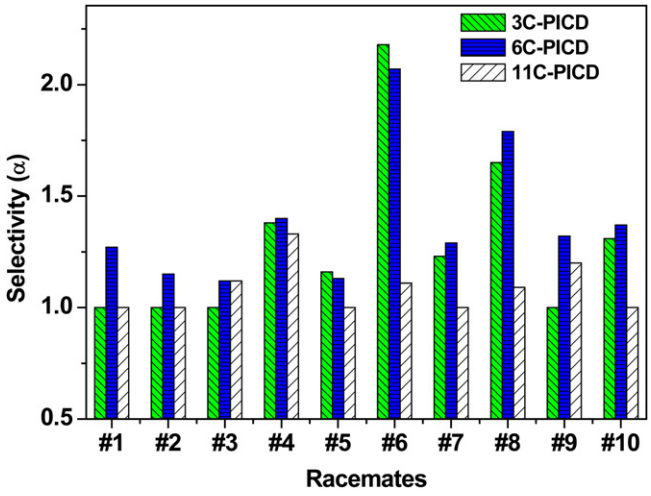


Fig. 3. Selectivity factor (α) versus spacer arm length for selected racemic samples.

To investigate the effect of the spacer length on enantioseparation abilities, 10 racemic samples were selected for the evaluation of these three columns. The chromatographic data obtained under normal-phase conditions using hexane/IPA mixtures as eluents are summarized in Table 2. For comparison, each model analyte was separated under the same chromatographic conditions on CSPs with three different spacer lengths.

From the separation data listed in Table 2, one can find that **6C-PICD** with higher surface loading displays the best enantioseparation performance for all selected analytes. A close look at the resolution data for compounds #3 and #4 with **3C-PICD** and **11C-PICD**, we can find though they have similar surface loading on silica gel **11C-PICD** presents better resolution ability towards flavanone compounds; whilst **3C-PICD** displays better resolutions towards single aromatic ring containing alcohols (compounds #6–#8, #10). The data suggest that the spacer length does affect the enantioselectivity abilities of the CSPs towards selected racemates except compounds #1 and #2. The same analytes have quite different enantioseparations on different CSPs even when they were separated under same chromatographic conditions.

Fig. 3 shows the histogram of selectivity factor (α) related to the spacer arm length for the racemic samples. In many cases, CSP **6C-PICD** displayed the best enantioseparation ability. The data suggest that there may exist an optimum spacer length (approximately 6 carbons) to achieve better enantioseparation when using this series of CSPs.

The existence of optimum length might be explained by the fact that the longer spacer arm pushes the CD-selectors further away from the surface of silica gel, which has the tendency of reducing the achiral hydrogen bonding interaction between the analytes and the hydroxyl groups on silica surface. The diminished participation of achiral molecular structures is beneficial for enantioresolution. On the other hand, if the spacer arm is long enough that the chiral selectors are able to contact with each other freely, then the mutual interactions between the chiral selectors (CD) in the stationary phase could increase. These interactions between CDs may lead to a possible decrease of the chiral-selective interaction between the analytes and the chiral selectors [20]. In other words, there may be an optimal spacer length for CD CSPs in HPLC enantioseparation.

The effect of optical spacer length was compared at the same separation conditions for **3C-PICD**, **6C-PICD** and **11C-PICD**. Taking compound #4 for example, even when eluent with stronger eluting ability was utilized on **6C-PICD**, longer retention was afforded on this CSP than that on the other two CSPs. From the chromatograms shown in Fig. 4, one can find

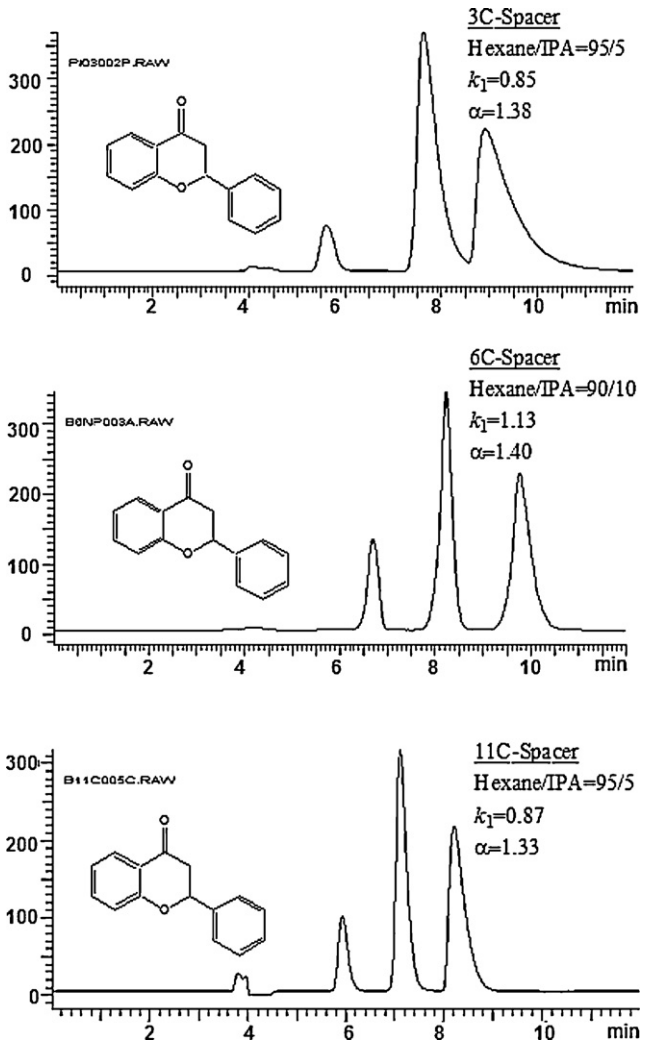


Fig. 4. Chromatograms for flavanone on the three columns. (Flowrate: 0.80 mL/min, first peak is the internal reference peak for biphenyl).

that better resolution peaks with reduced tailing are observed with **6C-PICD**, which partially accounts for the best resolution achieved with **6C-PICD**. Similar improvement in chromatographic resolution is also observed with **6C-PICD** for the rest model analytes.

As shown in Table 2, it is notable that the higher the surface coverage, the stronger retention for all the selected compounds. **6C-PICD** has the highest surface loading among the three CSPs (see Table 1). As a result, **6C-PICD** exhibited strongest retention to all the racemic samples (see Table 2).

It is reasonable to assume that higher surface loading of CD moieties on silica gel increases the enantioseparation abilities of CSPs. It is well known that the hydrophobic effect may drive the analytes to interact with the CD selector under reverse phase for the CD-CSPs, as demonstrated by permethylated-β-CD CSPs on silica gel [16]. However, no such drive is anticipated under normal-phase condition since no inclusion phenomena occur. There is a common assumption that surface loading principally affects the mass transfer kinetics of the selector-select and association. In addition to enantioselective binding sites, CSP surfaces usually harbor a considerable amount of excess functionality, emanating from the supporting matrix, linker groups and spacer units, and this may undergo nonspecific interactions with the analytes [30]. In this case, a higher surface coverage may lead to higher possibilities for interactions (ca. hydrogen bonding, π–π

interaction or probably dipole interaction) between analytes and chiral selectors. In our CSPs, CD is tethered to the silica support *via* a single amino (–NH–) linkage. The racemate bearing polar functional group, such as –OH (entry 2,3, 6–10) –NH or $–SO_2NH_2$ (entry 1), and $–OCH_3$ (entry 5) group may form a proximal hydrogen bond (possibly also dipole interaction) between its polar groups and the amino linkage on the spacer. Consequently, the CSP (ca **6C-PICD**) with higher surface loading might exhibit stronger retention than **3C-PICD**. At the same time, the strengthened retention contributes, more or less, to the better enantioselectivities.

Besides normal-phase separations, our **PICDs** also perform in reversed-phase condition. Taking **3C-PICD** for instance, a chiral resolution of 4.46 was obtained for alprenolol and 1.73 for pindol with MeOH-1% tetraethylammonium acetate buffer (pH = 5.5, 35/65, v/v) and 0.5 mL/min flow-rate. By using Shinwa phenylcarbamoylated CD CSPs, the resolution for alprenolol and pindol was 2.70 and 1.73, respectively, with 20 mM phosphate buffer (pH 4.6)-acetonitrile (65:35) and1.0 mL/minflow-rate [31]. For the resolution of flavanone compounds, under both normal-phase (Table 2) and the above-mentioned reversed-phase conditions, compounds #4 and #5 achieved comparable resolution as Cyclobond CSPs, where resolutions of 1.3 and 1.1 were obtained for #4 and #5, respectively, with methanol-acetonitrile (65:35) and1.0 mL/min flowrate [32].

4. Conclusions

Three amino-linked chiral stationary phases by immobilizing mono(6^A-*N*-(ω-alkenylamino)-6^A-deoxyl)perphenylcarbamoylated β-CD onto silica gel *via* hydrosilylation have been conveniently prepared. The present study has demonstrated that the performance of these CDs-based CSPs is strongly dependent upon the spacer length and their surface loading on silica surface. Under normal-phase mode, higher surface-loaded **6C-PICD** presents the best resolution towards all model analytes. With similar surface loading, **11C-PICD** displays different chiral resolution ability from **3C-PICD**, which indicates there maybe exits an optimal spacer length in this series of CSPs. At the same time, surface loading plays a role in chiral discrimination under normal-phase conditions by increasing the possibilities of interaction between analytes and the chiral selectors.

Acknowledgements

Funding from the National University of Singapore (NUS) and Natural Science Foundation of Jiangsu Province (grant no. BK2010486) in support of this work is gratefully acknowledged. Xiang-Hua Lai thanks NUS for the award of a research scholarship.

References

[1] T.J. Ward, K.D. Ward, Anal. Chem. 82 (2010) 4712.
[2] I.W. Muderawan, T.T. Ong, S.C. Ng, J. Sep. Sci. 29 (2006) 1849.
[3] D.W. Armstrong, B. Zhang, Anal. Chem. 73 (2001) 557A.
[4] S. Li, D.K. Lloyd, Anal. Chem. 65 (1993) 3684.
[5] W.P. Karen, Anal. Bioanal. Chem. 372 (2002) 22.
[6] A.S. Carter-Finch, N.W. Smith, J. Chromatogr. A 848 (1999) 375.
[7] M. Laëmmerhofer, W. Lindner, J. Chromatogr. A 829 (1998) 115.
[8] A. Zhou, X. Lv, Y. Xie, C. Yan, R. Gao, Anal. Chim. Acta 547 (2005) 158.
[9] V.A. Davankov, J. Chromatogr. A 1000 (2003) 891.
[10] B. Sellergren, J. Chromatogr. A 906 (2001) 227.
[11] M. Yoshio, N. Hiroyuki, N. Kouji, N. Hideo, S. Tadashi, J. Chromatogr. A 805 (1998) 85.
[12] V. Schurig, Trac-Trend Anal. Chem. 21 (2002) 647.
[13] L. Chen, L.F. Zhang, C.B. Ching, S.C. Ng, J. Chromatogr. A 950 (2002) 65.
[14] I. Ciucanu, W.A. Konig, J. Chromatogr. A 685 (1994) 166.
[15] I. Ciucanu, J. Chromatogr. A 727 (1996) 195.
[16] G. Felix, C. Cachau, A. Thienpont, M.H. Soulard, Chromatographia 42 (1996) 583.
[17] A. Berthod, C.D. Chang, D.W. Armstrong, Talanta 40 (1993) 1367.
[18] X.H. Lai, S.C. Ng, Tetrahedron Lett. 44 (2003) 2657.
[19] T. Ihara, Y. Sugimoto, M. Asada, T. Nakagama, T. Hobo, J. Chromatogr. A 694 (1995) 49.
[20] W.H. Tang, S.C. Ng, Nat. Protoc. 3 (2008) 691.
[21] R.C. Petter, J.S. Salek, C.T. Sikorski, G. Kumaravel, F.T. Lin, J. Am. Chem. Soc. 112 (1990) 3860.
[22] W.H. Tang, I.W. Muderawan, T.T. Ong, S.C. Ng, H.S.O. Chan, J. Chromatogr. A 1094 (2005) 187.
[23] W.H. Tang, S.C. Ng, Nat. Protoc. 2 (2007) 3195.
[24] W.H. Tang, I.W. Muderawan, T.T. Ong, S.C. Ng, Tetrahedron Asymmetry 18 (2007) 1548.
[25] W.H. Tang, I.W. Muderawan, T.T. Ong, S.C. Ng, Anal. Chim. Acta 546 (2006) 119.
[26] G.L. Lange, C. Gottardo, Synth. Commun. 20 (1990) 1473.
[27] B. Boyer, S. Durand, G. Lamaty, J.M. Moussanou-Missima, A.A. Pavia, J. Chem. Soc. Perkin Trans. 8 (1991) 1311.
[28] M.N. Khan, J. Org. Chem. 61 (1996) 8063.
[29] X.H. Lai, S.C. Ng, J. Chromatogr. A 1031 (2004) 135.
[30] G. Subramanian, Chiral Separation Techniques, third ed., Wiley-VCH Verlag/GmbH & Co. KGaA, Weinheim, 2007.
[31] K. Nakamura, H. Fujima, H. Kitagawa, H. Wada, K. Makino, J. Chromatogr. A 694 (1995) 111.
[32] M. Krause, R. Galensa, J. Chromatogr. A 514 (1990) 147.