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Novel cyclodextrin chiral stationary phases for high performance liquid chromatography enantioseparation: Effect of cyclodextrin type

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ABSTRACT

Three novel chiral stationary phases (CSPs) were prepared by regioselective chemical immobilization of mono(6^A-*N*-allylamino-6^A-deoxy)perphenylcarbamoylated (PICD) α-, β-, and γ-cyclodextrins (CDs) onto silica support via hydrosilylation. Their enantioseparation properties in high performance liquid chromatography (HPLC) were evaluated with a large spectrum of racemates including flavanone compounds, β-adrenergic blockers, amines and non-protolytic compounds. The effect of CD's cavity size on enantioseparation abilities was studied and discussed. The results indicated that CD's surface loading at silica support played an important role in the enantioseparation on these CSPs under normal-phase conditions while inclusion phenomena contributed the major driving force under reverse-phase conditions. As expected, α-PICD demonstrated the best resolutions towards flavonone and most aromatic alcohols under normal-phase conditions with the highest surface loading; while Fujimura's competitive inclusion model can be applied to explain the better enantioseparations towards β-adrenergic blockers, amines and non-protolytic compounds with α- and β-PICD CSPs. γ-PICD CSP showed superior enantioseparation ability for sterically encumbered analytes like flavanone compounds under both normal-phase and reversed phase conditions.

1. Introduction

With a growing demand for the exploration of different properties like toxicities and metabolic pathways of enantiomers, increasing efforts have been made on the resolution optically active compounds with respect to their optical purity. Direct enantioseparation by chiral stationary phases (CSPs) in high-performance liquid chromatography (HPLC) remains one of the most important techniques for both analysis of enantiomeric purity and quick obtainment of optically pure materials [1–3]. Several categories of chemically bonded CSPs such as Pirkle-type, protein-based, polysaccharide-based, macrocyclic antibiotic, crown ethers, imprinted polymers, chiral ligand exchange and cyclodextrin (CD)-based CSPs have been designed and applied for enantioseparation [4–13]. Among them, CD-base CSPs are especially attractive for their versatility and durability under all kinds of conditions [8–13].

Cyclodextrins (CDs) are chiral, toroid-shaped cyclic oligosaccharides comprising six, seven or eight glucopyranose units bonded via (1,4)-linkage, assigned as α-, β-, or γ-CD, respectively [2,14]. CDs and their derivatives are used extensively as chiral selectors for CSPs for HPLC chiral separation due to their natural chirality and ability to form inclusion complex with molecules via hydrophobic cavity [15–18]. It was reported that the combination of hydrophobic interactions and steric effects from the substituents present on the cavity entrance are believed to be responsible for the observed enantioselectivity in reversed-phases HPLC [15,18].

The chiral recognition of CD CSPs under reverse-phase conditions is thought to be driven by the inclusion complexation between the hydrophobic moiety of analyte and the relatively non-polar interior of the CD cavity [19]. Therefore, the dimension of CD-cavity is likely to have substantial effects on the enantioseparation ability of CD-bonded CSPs under reversed-phase conditions. Under normal-phase conditions, however, the CD-cavity is more likely to be occupied by the non-polar molecules of the mobile phase [20]; and the chiral recognition was mainly attributed to the π–π interaction and hydrogen bonding between sites provided by the aromatic and carbonyl substituents on the derivatized CD [21].

We previously reported a novel approach for preparing a CSP based on mono(6^A-*N*-allylamino-6^A-deoxy)perphenylcarbamoylated β-CD (β-PICD), which was immobilized onto porous silica via hydrosilylation [22,23]. This CSP exhibited outstanding enantioseparation abilities towards a wide range of chiral compounds. The effect of spacer length of mono(6^A-*N*-(ω-alkenylamino)-6^A-deoxy)perphenylcarbamoylated β-CD based

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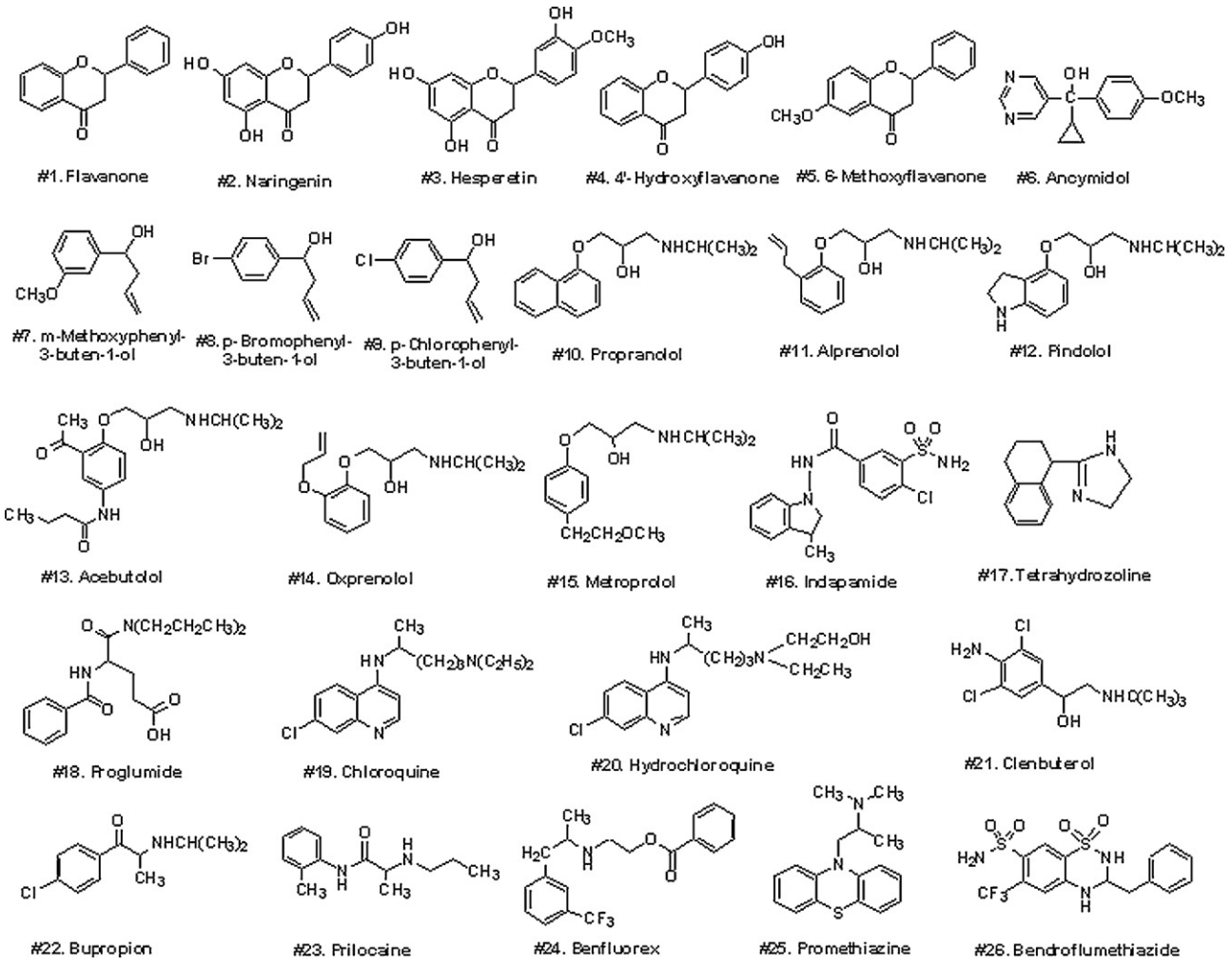

Fig. 1. Structure of flavanone, β-adrenergic blockers, amines and non-protolytic racemates studied.

CSPs was recently studied [24], which indicated an optimal spacer length (6–11 C atoms) existed. To further investigate the influence of CD cavity size on the enantioseparation performance, two analogue CSPs based on mono(6^A-*N*-allylamino-6^A-deoxy)perphenylcarbamoylated α-, and γ-CD (α-PICD and γ-PICD) were prepared in this paper. Enantioseparation of these three CSPs were presented herewith, and the effect of the CD-cavity dimension on the enantioseparation was discussed.

2. Experimental

2.1. Materials and instrumentation

α-, γ-cyclodextrins were purchased from TCI (Tokyo, Japan). All racemic samples (Fig. 1) and other reagents were procured from Sigma–Aldrich (Saint Louis, MO, USA) and used without further purification. Other chemicals and all instrumentations are the same as in Ref. [24].

2.2. Preparation of CSPs

Mono(6^A-*N*-allylamino-6^A-deoxy)perphenylcarbamoylated α-, β-, and γ-CD based CSPs were prepared according to the reported synthetic route by using different CD [22–24]. By refluxing mono[6^A-(*p*-tolysulfonyl)-6^A-deoxy]-α-, β-, or γ-CD **1** with allylamine [25–27], the key intermediate mono-(6^A-*N*-allylamino-6^A-deoxy)-α-, β-, or γ-CD **2** was obtained in high purity and good yield. Further reaction of **2** with phenyl isocyanate, (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 cyclodextrin **3** can be prepared. A further hydrosilylation of **3** with triethoxylsilane with 0.5% eq. tetrakis(triphenylphosphine)-platinum(0) gave the reactive siloxane, which was directly immobilized onto silica gel to afford the resultant CSPs.

Characterization data for mono-(6^A-*N*-allylamino-6^A-deoxy)-α-CD **2a**: IR (cm^{-1}, KBr): 3392 (O-H, str), 2935 (C–H, str), 1662 (C=C, m), 1024 (C–O str); ^{13}C NMR (75 Hz, DMSO-d_6) δ: 104.53 (C-1), 83.79 (C-4), 75.77 (C-2), 74.73 (C-3), 74.52 (C-5), 62.93 (C-6), 114.37(CH=$\underline{C}H_2$), 137.45 ($\underline{C}H$=CH_2); Anal. Calcd. (%) for $C_{39}H_{65}NO_{29}$: C 46.29, H 6.47, N 1.38, Found: C 45.01, H 6.59, N 1.19; ESI-MS for $C_{39}H_{65}NO_{29}$ (1012), *m*/*z*: 1013 for $[M]^+$.

Characterization data for mono-(6^A-*N*-allylamino-6^A-deoxy)-γ-CD **2c**: IR (cm^{-1}, KBr): 3401, 3308 (O-H, str); 2922 (C–H, str), 1640 (C=C, m), 1038 (C–O str); ^{13}C NMR (75 Hz, DMSO-d_6) δ: 101.63 (C-1), 80.86 (C-4), 73.81 (C-2), 73.48 (C-3), 72.84 (C-5), 61.77 (C-6), 114.99 (CH=$\underline{C}H_2$), 137.54 ($\underline{C}H$=CH_2); Anal. Calcd. (%) for $C_{51}H_{85}NO_{39}$: C 45.84, H 6.41, N 1.05; Found: C 44.01, H 6.63, N 1.09; ESI-MS for $C_{51}H_{85}NO_{39}$ (1336), *m*/*z*: 1337 for $[M]^+$.

Characterization data 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 α-CD **3a**: IR (cm^{-1}, KBr): 2920 (C–H, str), 1658 (C=C, m), 1045 (C–O str); ^{13}C NMR (75 Hz, DMSO-d_6) δ:

Table 1
Enantioseparation data under normal-phase conditions.

Entry	Column	Chromatographic data and conditions				
		k_1	k_2	α	R_s	Cond.
#1. Flavanone	α-PICD	0.92	1.40	1.52	2.79	II
	β-PICD	0.85	1.17	1.38	1.25	II
	γ-PICD	0.49	0.79	1.61	2.38	II
#2. Naringenin	α-PICD	2.76	2.76	1.00	0	I
	β-PICD	4.54	4.54	1.00	0	I
	γ-PICD	4.38	4.75	1.08	0.67	I
#3. Hesperetin	α-PICD	4.33	4.33	1.00	0	I
	β-PICD	6.80	6.80	1.00	0	I
	γ-PICD	7.62	9.68	1.27	1.95	I
#4. 4′-Hydroxyflavanone	α-PICD	7.94	7.94	1.00	0	III
	β-PICD	11.20	11.20	1.00	0	III
	γ-PICD	9.44	13.71	1.45	3.06	III
#5. 6-Methoxyflavanone	α-PICD	0.87	1.23	1.41	2.67	I
	β-PICD	1.25	1.45	1.16	0.50	I
	γ-PICD	0.62	1.05	1.69	2.69	I
#6. Ancymidol	α-PICD	8.54	10.85	1.27	1.44	I
	β-PICD	5.13	5.13	1.00	0	I
	γ-PICD	4.02	4.55	1.13	0.94	I
#7. m-Methoxyphenyl-3-buten-1-ol	α-PICD	0.80	1.40	1.75	4.57	II
	β-PICD	0.61	1.32	2.16	3.20	II
	γ-PICD	0.51	0.91	1.78	2.22	II
#8. p-Bromophenyl-3-buten-1-ol	α-PICD	1.40	1.80	1.29	1.63	II
	β-PICD	1.07	1.85	1.73	2.16	II
	γ-PICD	0.85	0.85	1.00	0	II
#9. p-Chlorophenyl-3-buten-1-ol	α-PICD	1.22	1.45	1.21	1.09	II
	β-PICD	1.02	1.59	1.56	1.54	II
	γ-PICD	0.77	0.77	1.00	0	II

HPLC conditions: I. hexane/IPA = 90/10, 0.80 mL/min; II. hexane/IPA = 95/5, 0.80 mL/min; III. hexane/IPA = 97/3, 1.00 mL/min.

29.43 (CH_2NH), 61.57 (C-6), 69.76 (C-2), 70.02–70.49 (C-5), 71.29 (C-3), 101.32 (C-1), 114.65 (CH=CH_2), 119.33–126.50 (aromatic C), 137.86 (CH=CH_2), 160.42–165.49 (C=O); Anal. Calcd. (%) for $C_{158}H_{150}N_{18}O_{46}$: C 62.49, H 4.98, N 8.30; Found (%): C 61.73, H 5.04, N 8.33; ESI-MS for $C_{158}H_{150}N_{18}O_{46}$ (3037), *m*/*z*: 3038 for $[M]^+$.

Characterization data 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 γ-CD **3c**: IR (cm^{-1}, KBr): 2943 (C–H, str), 1645 (C=C, m), 1029 (C–O str); ^{13}C NMR (75 Hz, DMSO-d_6) δ: 26.54 (CH_2NH), 62.18 (C-6), 70.21 (C-2), 70.55 (C-5), 71.48 (C-3), 99.76 (C-1), 115.64 (CH=CH_2), 122.26–127.43 (aromatic C), 137.75 (CH=CH_2), 161.43–167.59 (C=O); Anal. Calcd. (%) for $C_{212}H_{200}N_{24}O_{62}$: C 62.47, H 4.95, N 8.25; Found: C 63.14, H 5.11, N 7.96; ESI-MS for $C_{212}H_{200}O_{62}N_{24}$ (4076), *m*/*z*: 4077 for $[M]^+$.

The slurry method was applied to pack the different CD derived CSPs into HPLC columns (250 mm × 4.6 mm I.D.) using *n*-hexane as the packing solvent according to our reported procedure [27].

3. Results and discussion

3.1. Characterization of CSP columns

According to the microanalysis data, the surface concentration of CD derivatives on the silica gel can be calculated [24,25]. Carbon content for CSP 5 further corroborated the success of the immobilization procedure. The surface loading data indicated that α-PICD possessed the highest (13.3×10^{-8} mol/m^2) coverage while β-PICD had the lowest. These three columns gave efficiencies of 33,000 plates per meter for α-PICD, while 36,000 for β-PICD and 34,000 for γ-PICD, respectively, by using biphenyl as the testing-sample under normal phase (hexane and IPA in 90/10, v/v ratio, 0.8 mL/min). It is still unclear why the lowest CD surface-loaded β-PICD CSP presented the highest column efficiency, probably due to the best mach of biphenyl ring from test compound with the cavity size of β-CD.

3.2. Enantioseparation under normal-phase conditions

The chiral resolution capability of three CD based CSPs was first evaluated under normal-phase conditions. Separation data of 5 flavanone compounds and 4 aromatic alcohols are tabulated and compared in Table 1. For comparison, each model analyte was separated under the same chromatographic conditions on CSPs with different CDs. It is generally believed that inclusion complexation is no longer the major driving forces for CD-based CSPs under normal phases [19,20]. Instead, the chiral recognition mechanisms capitalizing on specific interactions between the exterior of the CD-cavity and analyte prevail. By evaluating the chiral recognition of CSPs containing same functionalities but different CD cavity size bonded onto silica gel surface via the primary hydroxyl side of CD, the validity of the statement can be examined.

From the separation data listed in Table 1, one can find that α-PICD with highest surface loading displayed excellent enantioseparation for model analytes **#1** and **#6**–**#9**; while γ-PICD with higher surface loading displays good satisfactory chiral resolution for analytes **#1**–**#5** and **#7**, with the biggest separation factors (α) with the shortest retention time (k) were achieved on γ-PICD. It is obvious that the flavor molecules (**#1**–**#5**) possess a tri-cycle structure, which is suitable to be included into the cavity of γ-CD but almost impossible to be included by the cavity of α- or β-CD. Thus, we may assume that the size of CD ring contributed greatly to the enantioseparation, which might be applied to explain

that γ-PICD exhibited the best enantioselectivities towards the flavanones under normal phases. Among the selected analytes, flavanone (entry **#1**) and aromatic alcohol (entry **#7**) were well separated on all three CSPs, with the highest separation factors (α) and the longest retention time (k) achieved on α-PICD, attributed to its high surface loading. As known, the excess functionality harbored around CSP surfaces may undergo non-specific interactions with the analytes [28]. 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. Under this condition, α- and γ-PICD presented better resolutions and stronger retention towards selected analytes due to the intensified interactions.

A close look at the separation data for flavonone compounds (entries **#1**–**#5**), one would find when flavonone's its bicyclic ring is substituted with hydrophilic group like –OH as in naringenin and hesperedin (entries **#2** and **#3**), much longer retention time and lower separation factor were observed compared with flavanone. This behavior can be explained by the fact that this hydroxyl group can form non-chiral-selective hydrogen-bonding (H-bonding) with –NH groups from the pendant functionalities in secondary face of CD cavity or hydroxyl groups on the surface of silica gel. Therefore, these hydroxyl-containing analytes displayed stronger retention with CSPs but decreased chiral resolution due to this achiral hydrogen-bonding: i.e., γ-PICD presents better enantioseparation but shorter retention time for compound **#1** in comparison with compounds **#2** and **#3**. The non-chiral selective H-bonding between the solutes of compounds **#2**–**#4** and silica gel submerged the chiral selective interaction between solutes and chiral selectors, resulting in reduced enantioseparation but enhanced retention.

The absence of inclusion phenomena under normal-phase separation was further observed in the resolution of aromatic alcohols **#7**–**#9**, where only one benzyl ring is contained. These analytes were all well separated on α- and β-PICD CSPs. As observed, the capacity factors were observed to be almost the same for both columns and the difference in selectivity and resolution was not too drastic, probably implying that no inclusion complexation occurs in normal-phase conditions. In a word, enantioseparation capability of PICD series CSPs is mainly mediated by surface loading of functionalized CD on CD cavity-dependent interactions and CD surface loading on the silica support.

3.3. Enantioseparation under reversed-phase conditions

Table 2 summarizes the enantioseparation results of 18 racemates including β-adrenergic blockers, amines and non-protolytic compounds on these three CSPs under reversed-phase condition. It is interesting to note that the chiral center of all the analytes (entries **#12**, **#14**, **#15** and **#18**–**25**) that can be separated on α-PICD is located in a side-chain. Fujimura et al. proposed a chiral recognition mechanism based on competitive inclusion mode [29], which involved the formation of an inclusion complex of CD with an aromatic group as well as with a side chain on an asymmetric carbon. For CD-CSPs, it is reasonable to assume that sufficient differentiation of chiral discriminated interaction between the R-/S-form of the analyte and the CSPs may not be provided if the stereogenic center is far away from the chiral environment (CD cavity). Consequently, enhanced enantioselectivity might be afforded if the analyte's stereogenic center can get close to CD's chiral cavity easily. Based on Fujimura's competitive inclusion model [29], the aliphatic pendant chain containing the chiral center could be included into the cavity of α-CD while the steric-bulky cyclic moieties cannot. Consequently, the stereogenic center in the aliphatic pendant chain was exposed to the chiral environment and enhanced chiral discrimination likely occurred. Similar recognition mechanism may be applied to explain the enantioseparation of indapamide and tetrahydrozoline (entries **#16** and **#17**) on β-PICD. Their bicyclic moiety containing the chiral center was apt to be "tight-included" into the cavity of β-CD, which resulted in their separation on β-PICD.

Table 2
Enantioseparation data under reversed-phase condition.

Entry	Column	Chromatographic data			
		k_1	k_2	α	R_s
#10. Propranolol	α-PICD	1.67	1.67	1.00	0
	β-PICD	2.23	3.56	1.51	4.70
	γ-PICD	1.52	1.52	1.00	0
#11. Alprenolol	α-PICD	2.14	2.14	1.00	0
	β-PICD	1.54	2.13	1.38	4.46
	γ-PICD	1.37	1.37	1.0	0
#12. Pindolol	α-PICD	1.42	1.71	1.20	1.53
	β-PICD	0.71	0.87	1.23	1.73
	γ-PICD	0.57	0.57	1.00	0
#13. Acebutolol	α-PICD	2.94	2.94	1.00	0
	β-PICD	1.63	1.79	1.10	0.95
	γ-PICD	1.31	1.31	1.00	0
#14. Oxprenolol	α-PICD	1.56	1.74	1.12	0.67
	β-PICD	1.75	1.84	1.05	0.53
	γ-PICD	1.44	1.44	1.00	0
#15. Metroprolol	α-PICD	1.63	1.82	1.12	0.89
	β-PICD	1.20	1.20	1.00	0
	γ-PICD	1.79	1.79	1.00	0
#16. Indapamide	α-PICD	2.76	2.76	1.00	0
	β-PICD	4.25	4.76	1.12	1.33
	γ-PICD	3.42	3.42	1.00	0
#17. Tetrahydrozoline	α-PICD	1.46	1.46	1.00	0
	β-PICD	1.26	1.35	1.07	0.86
	γ-PICD	1.52	1.52	1.00	0
#6. Ancymidol	α-PICD	4.39	4.39	1.00	0
	β-PICD	6.66	7.05	1.06	0.71
	γ-PICD	5.12	5.12	1.00	0
#18. Proglumide	α-PICD	3.84	4.32	1.12	0.93
	β-PICD	3.76	4.29	1.14	1.66
	γ-PICD	2.79	2.79	1.00	0
#19. Chloroquine	α-PICD	3.44	4.29	1.25	2.31
	β-PICD	3.51	3.51	1.00	0
	γ-PICD	3.02	3.02	1.00	0
#20. Hydrochloroquine	α-PICD	2.31	2.89	1.25	1.69
	β-PICD	3.32	3.32	1.00	0
	γ-PICD	3.40	3.40	1.00	0
#21. Clenbuterol	α-PICD	0.79	0.94	1.19	0.86
	β-PICD	0.83	0.83	1.00	0
	γ-PICD	0.51	0.51	1.00	0
#22. Bupropion	α-PICD	1.66	3.34	2.01	3.84
	β-PICD	1.46	1.46	1.00	0
	γ-PICD	1.20	1.20	1.00	0
#23. Prilocaine	α-PICD	0.60	0.92	1.53	2.0
	β-PICD	1.00	1.00	1.00	0
	γ-PICD	0.57	0.57	1.00	0
#24. Benfluorex	α-PICD	5.16	5.77	1.12	1.06
	β-PICD	3.25	3.25	1.00	0
	γ-PICD	2.39	2.39	1.00	0
#25. Promethiazine	α-PICD	4.0	7.1	1.78	5.70
	β-PICD	3.63	6.71	1.85	5.37
	γ-PICD	2.42	2.98	1.23	2.07
#26. Bendroflumethiazide	α-PICD	0.94	0.94	1.00	0
	β-PICD	1.33	1.33	1.00	0
	γ-PICD	2.06	2.97	1.44	2.27

HPLC condition: buffer (1% TEAA, pH 5.5)/MeOH = 65/35, 0.5 mL/min, preparation as in Ref. [24].

Table 3
Enantioseparation of flavonones with γ-PICD and acetylated Cyclobond I CSP [32].

Entry	Column	Chromatographic data and conditions			
		k_1	α	R_s	Cond.
Flavanone	γ-PICD	5.81	1.33	1.45	I
	Cyclobond I	4.40	1.07	0.7	II
4′-Hydroxyflavanone	γ-PICD	2.81	1.20	1.36	I
	Cyclobond I	3.22	1.00	0	II
5-Methoxyflavanone	γ-PICD	5.02	1.12	0.67	I
	Cyclobond I	3.05	1.07	0.6	II
6-Methoxyflavanone	γ-PICD	2.87	1.31	1.92	I
	Cyclobond I	5.00	1.06	0.70	II

HPLC condition: I. buffer (1% TEAA, pH 5.5)/MeOH = 65/35, 0.50 mL/min; II. water/methanol = 50/50, 1 mL/min.

β-Adrenergic blockers are a series of hydroxyl-amines containing aromatic rings with different substituents. It is well known that their enantiomers have different potencies and pharmacological effects. Some β-blockers were separated on our CSPs (entries **#10**–**15**). It appears that β-PICD showed better chiral selectivity towards β-blockers racemates, especially those containing bicyclic ring structure (e.g. entries **#10** and **#12**). In comparison with our previous reported urea-bonded CSPs [15], β-PICD presented superior resolution capability towards β-adrenergic blockers under the similar chromatographic condition. Our β-PICD CSP also demonstrated much better resolutions towards propranolol, alprenolol and proglumide than commercially available Shinwa phenylcarbomoylated CD CSPs [30].

Among the 18 racemates listed in Table 2, only promethiazine (entry **#25**) and bendroflumethiazide (entry **#26**) were both well separated on γ-PICD while the later cannot be discriminated on α- and β-PICD. As mentioned before, promethiazine and bendroflumethiazide have bulky molecular structure whose size is compatible with the cavity of γ-CD. Promethiazine, which has an aliphatic side-chain containing the chiral carbon, was also separated on α- and β-PICD CSPs; the possible chiral recognition mechanism may result from the above mentioned competitive inclusion model. For bendroflumethiazide, because its chiral center is a member of a ring, it could be expected that the molecular structure was too bulky to be included into the hydrophobic cavity of α- and β-CD while it could be easily included by the larger cavity of γ-CD. Consequently, the γ-CD bonded CSP is superior to that of α- and β-CD bonded CSP in recognizing chiral molecules with larger steric configuration. This CD-cavity size dependent resolution was also observed in our earlier study [31]. This point can be further confirmed by the enantioseparation of the flavanone compounds under reversed phase (see Table 3). As expected, all seven flavanones were well separated on CSP γ-PICD but the separation cannot be achieved on both α- and β-PICD CSPs under the same separation conditions. It should note that our CSPs present better resolutions for flavonone compounds than the commercially available Acetylated Cyclobond I CSP [32] (Table 3).

4. Conclusions

Based on mono(6^A-*N*-allylamino-6^A-deoxy)perphenylcarbamoylated α-, β-, and γ-CDs, three novel chiral stationary phases were successfully prepared. Their enantioseparation properties were evaluated under both normal and reversed phase conditions. Obviously, γ-PICD exhibits the best chiral selectivity towards the flavanone compounds under both normal and reversed phases; as expected, γ-PICD also seemed to be useful for discriminating sterically encumbered analytes while CSP α- and β-PICD are good for enantioseparation of analytes with relatively small molecular size under reversed-phase conditions.

It appears that the surface loading of CD on silica gel plays an important role in the enantioseparation on our CSPs under normal phases; while inclusion is assumed to be the major driving force for chiral separation on the columns under reversed phase, while the size, shape, and side chain of the analyte molecule may also affect the enantioselectivity.

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