Published in final edited form as:

J Liq Chromatogr Relat Technol. 2015 March ; 38(5): 550–560. doi:10.1080/10826076.2014.917668.

PREPARATION AND EVALUATION OF HPLC CHIRAL STATIONARY PHASES BASED ON CATIONIC/BASIC DERIVATIVES OF CYCLOFRUCTAN 6

Nilusha L. Padivitage¹, Jonathan P. Smuts¹, Zachary S. Breitbach^{1,2}, Daniel W. Armstrong^{1,2}, and Alain Berthod^{3,*}

¹Department of Chemistry, University of Texas at Arlington, Planetarium Place, Arlington, TX 76019, USA

²AZYP LLC, 700 Planetarium Place, Arlington, TX 76019, USA

³Institut des Sciences Analytiques, Université de Lyon 1, CNRS, 5 rue de la Doua, 69100 Villeurbanne, France

Abstract

The cyclofructan 6 (CF6) macrocyclic-oligosaccharide was derivatized with five different substituents able to bear positive charges: propyl imidazole (IM) methyl benzimidazole (BIM), dimethyl aminopropyl (AP), pyridine (PY) and dimethyl aminophenyl (DMAP). The derivatized cyclofructans were reacted with triethoxysilyl-propylisocyanate as a linker to bond them to 5 μ m spherical silica gel particles and then used to prepare HPLC columns. The bonded silica particles were analyzed to establish the bonding densities. A set of 34 chiral compounds including acids, neutral compounds and bases was tested with nine different mobile phase compositions including two reverse phase (RP) acetonitrile/pH 4 buffer, three polar organic (PO) acetonitrile/methanol and four normal phase (NP) heptane/ethanol mobile phases. No compounds could be separated in the RP mode. Eight compounds only could be enantioseparated in the PO mode and 21 compounds in the NP mode. The most effective chiral stationary phase was the propyl imidazole derivatized CF6 phase, provided that no more than six imidazole substituents and two linkers are attached per CF6 unit.

Keywords

Liquid chromatography; chiral separation; cyclofructan; cationic derivatives; enantiomer separation

INTRODUCTION

Cyclofructans (CF) are cyclic oligosaccharides consisting of β -2,1 linked D-fructofuranose units (Figure 1). The oligosaccharide with six fructofuranose units is called cyclofructan 6 or CF6. CF7 and CF8, with respectively 7 and 8 units ($n = 2$ and 3 in Figure 1) also are jointly

*Corresponding author, berthod@univ-lyon1.fr.

produced by fermentation of inulin, an edible carbohydrate fiber coming from chicory, by specific strains of *Bacillus circulans*.^[1] Recently we employed native and derivatized cyclofructans as chiral selectors for capillary electrophoresis,^[2, 3] gas chromatography^[4] and HPLC.^[5–9] Native and sulfonated CF6 gave remarkable results in hydrophilic interaction chromatography, especially with amine containing solutes.^[10, 11]

All previously tested chiral selectors, based on derivatized CFs, were neutral or negatively charged. Thus it is logical to investigate if positively charged CF derivatives could produce enhanced or different enantioseparations. This is the purpose of the present work. Five different CF6 derivatives were synthesized and bonded on silica to produce seven different chiral stationary phases (CSPs) for HPLC. The five derivatives were based on amine, imidazole or pyridine moieties, all groups being basic and with a positive charge in acid containing mobile phases. The derivative syntheses as well as the silica bonding procedures are described. A series of chiral solutes, including acids, neutral compounds and bases, was tested to evaluate the enantiomer separation capability of the seven newly obtained CSPs.

EXPERIMENTAL

Chemical and reagents

CF6, C₃₆H₆₀O₃₀, m.w. 972, was obtained from AZYP (Arlington, TX). Daiso silica gel particles SP-100-5-P with 5 µm spherical diameter (10 nm pore diameter, 1.1 mL/g pore volume, 440 m²/g surface area and 1.25 particle size distribution as d₄₀/d₉₀) was obtained from Daiso Fine Chemical USA, Inc. (Torrance, CA). The derivatized silica particles were slurry packed into 25 cm × 0.46 cm (i.d) stainless steel columns at 84 MPa (12,000 psi). Anhydrous toluene, anhydrous pyridine, anhydrous dimethylformamide (DMF), ethyl acetate, acetic acid (AA), triethylamine (TEA), trifluoroacetic acid (TFA), ammonium acetate, 3-(triethoxysilyl) propyl isocyanate, 1,1'-carbonyl diimidazole (CDI), 1-(3-aminopropyl) imidazole, 2-(aminomethyl) benzimidazole dihydrochloride, 3-(dimethylamino)-1-propylamine, 4-(dimethylamino) phenyl isocyanate and 3-pyridyl isothiocyanate were purchased from Sigma-Aldrich (St. Louis, MO). All of 34 racemic analytes tested in this study were purchased from Sigma-Aldrich and Anichem (North Brunswick, NJ, USA). Acetonitrile (ACN), methanol (MeOH), n-heptane (Hep), isopropyl alcohol (IPA) of HPLC grade were obtained from VWR (Sugarland, TX). Water was purified by a Milli-Q-water purification system (Millipore, Billerica, MA).

Preparation of derivatized CF6

The synthesis of imidazole (IM), benzimidazole (BIM) and dimethylaminopropyl (AP) CF6 derivatives was done in two steps, but one pot, using the 1,1'-carbonyldiimidazole reagent (CDI) in DMF solution as described by Rannard and Davis.^[12] The two imidazole groups of this reagent are excellent leaving groups forming an acyl imidazole bond with CF hydroxyl groups in a first step and a carbamate bond with amines in the second step as illustrated by Figure 2. Note that the acyl imidazole intermediate is highly reactive and cannot be isolated. A total of 18 hydroxyl groups of CF6 could be derivatized depending on the reagent concentration. With the conditions: CDI/CF6 molar ratio of 6, temperature 55°C for 5 hours, an average of six CF6 hydroxyl groups were derivatized with acyl imidazoles (Figure 2).

With the conditions: CDI/CF6 molar ratio of 12, temperature of 55°C for 6 hours, an average of ten CF6 hydroxyl groups were derivatized. In the second step, the reagent 1-(3-aminopropyl) imidazole or 2-(aminomethyl) benzimidazole or 3-(dimethylamino)-1-propylamine were added with a 20% molar excess to the CDI reagent. The reaction was done at 70°C for 12 hours giving respectively the CF6-IM, CF6-BIM or CF6-AP derivatives with a yield of 90% (Figure 2).

Three different CF6-IM derivatives were prepared varying the experimental conditions as follows: CF6-IM synthesis - 1.00 g (1.03 mmol) of CF6 was dissolved in anhydrous DMF (40 mL) in a 100 mL round bottom flask. Next, 1.017 g of CDI (6.27 mmol) was added to this solution and then placed in an oil bath. The temperature of the oil bath was slowly raised to 55°C and held there for five hours. By this time the CDI was entirely consumed and the acyl imidazole intermediate was confirmed by electrospray ionization mass spectrometry (ESI-MS). Then, 1 mL of 1-(3-aminopropyl) imidazole (8.23 mmol) was added to the same pot and the temperature was increased to 70°C and the reaction continued overnight. The following day the mixture was transferred into a large volume of toluene. A white precipitate formed and the product (CF6-IM1 and 2) was recovered by suction filtration and dried in a vacuum oven (P₂O₅) overnight. The molecular mass distribution of the product was determined by ESI-MS (3–8 imidazole groups with the average degree of substitution of six). The final yield was 90 %. The preparation of CF6-IM3 was achieved by doubling the amounts of CDI (2 g or 12.5 mmoles) and aminopropyl imidazole (2 mL or 16.5 mmoles) reagents to afford a more highly substituted derivative which contained 8–14 imidazole groups with the average degree of substitution of ten per CF6 unit (same 90 % yield).

CF6-DMAP and CF6-PY were prepared in a single step procedure. First, 1.00 g (1.03 mmol) of CF6 was dissolved in 30 mL anhydrous pyridine in a 100 mL round bottom flask and heated to 60°C in an oil bath under nitrogen gas for two hours. Next, 1.335 g of 4-(dimethylamino) phenyl isocyanate (8.23 mmol) was dissolved in 10 mL of dry pyridine (or 0.92 mL of 3-pyridyl isothiocyanate (also 8.23 mmol)) and added drop wise for 30 min to the CF6 solution stirring under dry nitrogen. The reaction was carried out for five hours at 60°C. The reaction mixture turned slightly yellow. After cooling, the solution was rotary evaporated under reduced pressure. The crude product was washed twice with ethyl acetate. The purified, final product was recovered by suction filtration and dried in a vacuum oven (P₂O₅) overnight. The mass spectrum showed that CF6-DMAP is a mixture containing four to eight 4-(dimethylamino) phenyl groups per CF6 unit with the average degree of substitution of six. The yield was 88 %. CF6-PY contained three to seven pyridyl groups per CF6 unit with an average degree of substitution of five and an 85% yield.

Binding CF6 to silica

To bond the derivatized CF6 to silica particles, the derivatized cyclofructans were further modified with a “linker”: (triethoxysilyl) propylisocyanate. The procedure was the same for all derivatized CF6 (except CF6-IM1): 2 g of derivatized CF6 (about 1 mmol) was dissolved in 40 mL anhydrous DMF in a 100 mL flask. The flask was mounted in an oil bath and heated at 90°C. 0.54 mL of 3-(triethoxysilyl) propylisocyanate (2 mmol) was dissolved in 10

mL of dry pyridine and added drop wise to the flask maintained for 5 h at 90°C. Two triethoxysilyl-propyl linkers are attached to each derivatized CF6. To obtain CF6-IM1, four triethoxysilyl-propyl linkers were attached to each derivatized CF6 doubling the isocyanate volume (1.08 mL or 4 mmol) added to the IM derivatized CF6. Next for all prepared CF6, 3 g of dried silica particles were mixed to 100 mL dry toluene in a 250 mL flask and the derivatized CF6 DMF solution was poured in it under dry nitrogen. The stirred mixture was heated at 110°C and allowed to react overnight. The bonded silica particles were washed in toluene, pyridine, water/methanol, acetone and dichloromethane, dried in vacuum and a sample was sent for elemental analysis.

Table 1 lists the carbon and nitrogen loading of the seven CSPs prepared. These values allowed for the computation of the bonding densities using the listed moiety masses and carbon and nitrogen atom numbers present in the complete bonded CF6 unit (Figure 3). The bonding densities obtained using the N percentage versus the C percentage differed by less than 30%, the N value being smaller, except for the SP-CF6-DMAP that returned a N-based bonding density 33% higher ($0.48 \mu\text{mol}/\text{m}^2$) than the C-based bonding density ($0.33 \mu\text{mol}/\text{m}^2$). The listed bonding densities are the average of the N and C obtained values. Figure 3 shows the CSP structures.

Chromatographic protocol

An Agilent 1200 HPLC chromatograph in isocratic mode was used for the enantiomer separation study (Agilent, Palo Alto, CA, USA). The Chemstation software version B.01.03 was used for data handling and chromatogram edition. All separations were carried out at room temperature (21–23°C). The injection loop was 5 μL and the flow rate was 1 mL/min. The diode array detector allowed monitoring 195, 210, 254 and 280 nm simultaneously.

The separations were initially done in the three chromatographic modes: normal phase (NP) with heptane/ethanol mobile phases, polar organic (PO) with acetonitrile/methanol mobile phases, all waterless mobile phases containing 0.1% v/v trifluoroacetic acid, and aqueous based reversed phase (RP) with acetonitrile/buffer mobile phases adjusted at pH around 4 with acetic acid. The mobile phases were degassed and filtered on 0.5 μm .

RESULTS AND DISCUSSION

Previous studies showed that aliphatic and aromatic functionalization of cyclofructans significantly broadened the application range of these CSPs.^[5–7] Therefore, basic/cationic substitution groups on cyclofructan 6 (CF6), including amine and aromatic groups, such as imidazole, benzimidazole, pyridine, aliphatic and aromatic amines, were tested. Chiral recognition of these stationary phases may be attributed to the presence of hydrogen bonding sites, π - π interactions, electrostatic interactions (for ionizable analytes) and/or steric hindrance which are mainly provided by the substitutions on the CF6. Table 2 lists the chromatographic data for all compounds examined by the newly synthesized CSPs. The set of chiral compounds listed in Table 2 shows the variety of functionalities with compounds containing different acidic groups, neutral groups, or amines. The positive character of the imidazole (IM), benzimidazole (BIM), propyl amine (AM), pyridine (PY) and dimethyl phenylamine (DMAP) substituting groups is certain in aqueous RP mobile phase with

measured pH 4. It is only speculated in waterless NP and PO mobile phases with 0.1% TFA in which pH is more difficult to define and ion formation may or may not occur.

Mode of operation

Figure 4 is a figure showing Table 2 information to make it quickly seen. The striking observation is that only normal phase and polar organic modes are presented with much more results for the first mode. Indeed, no pairs of enantiomers could be separated in the reversed phase mode with acetonitrile/pH4 buffer mobile phases. The cationic CF6 stationary phases exhibited better enantioselectivities in the normal phase mode with 22 compounds separated out of 34 (8 enantiomers were baseline separated, Figure 4, top). The polar organic mode was able to separate only 8 of the 34 compounds with a single baseline resolution (Figure 4, bottom, and Table 2). It seems that hydrogen-bonding and dipole-dipole stacking, two interactions greatly quenched by water molecules, are predominant for enantiorecognition of these analytes in the normal phase and polar organic waterless modes. This observation is in agreement with recent results obtained with cationic derivatized cyclodextrins.^[13]

Figure 5 shows the separation of the warfarin enantiomers (compound 14) on three different basic CSPs with the same NP mobile phase. The chromatographic parameters are listed in the figure. A CSP that is unable to separate warfarin enantiomers has little value.^[14] All newly synthesized cationic CSPs were able to separate the warfarin enantiomers in the normal phase mode with significant enantiomeric selectivity factors (Table 2). The enantiomeric resolution factors depend on column efficiency. As shown in Figure 5, the experimental column efficiencies can be very different. This is due to the packing procedure not being optimized because of the limited amount of the newly synthesized CSPs.

It must be pointed out here that the focus of this work is to scout for effective new chiral selectors. Thus no optimization of the column packing procedure was done at this stage. Such packing optimization will be done when a new CSP has established its capability to provide chiral recognition for many enantiomeric pairs. Only then would significant amounts of such a CSP be prepared to allow for packing optimization. Thus, a focus on comparing selectivity rather than on resolution between chiral selectors is the most accurate way to evaluate the ability for a selector to differentiate the test set of enantiomers. However, we chose to use the resolution factor because it goes from 0 (no separation) to higher values as the separation improves clearly pointing out successful separations. The corresponding enantio-selectivity factors are one for no separation and increasing higher than one for better separations. It is more difficult to see that e.g. the enantio-selectivity factor of 1.01 of coumachlor (compound 15) on CF6-BIM is good enough giving a resolution factor of 1.0 with two clearly visible peaks (Table 2). Of course, a better selectivity does induce an enhanced resolution factor and *vice versa*.

Figure 5 shows that the CF6-IM1 CSP column was acceptably well packed giving 11,000 plates and symmetric peaks. The 11,000 plate number corresponds to a HETP of 23 μm or almost five times the particle diameter (5 d_p). Columns made with the two other CSPs were less well packed giving lower efficiencies and clearly distorted peaks at base. Since the peak width at base is used to calculate the resolution factor, reduced R_s values are obtained with

such distorted peaks. However, with the highest enantioselectivity factor of 1.43 obtained in this study, the CF6-PY CSP is the most effective of the three tested CSPs in separating the warfarin enantiomers. The corresponding resolution factor is 1.8 showing a very good baseline separation of the two enantiomers (Figure 5).

Comparing cationic derivatives

Figure 6 is a compilation of the number of enantiomeric separations obtained for each newly synthesized CSP. It also shows the number of baseline separations obtained as well as the average enantioresolution factor value ($\Sigma R_s/(\text{number of separations})$). As indicated above, selectivity is the critical parameter; however it is easier to compare resolution factors knowing that they would be even better with enhanced efficiency obtained after packing optimization. Clearly, in both normal phase and polar organic modes, the propyl imidazolium derivatized CSP (SP-CF6-IM) stands out with more enantiomeric separations (16 for SP-CF6-IM2 in the NP mode + 4 in the PO mode, Figure 6) than the four other derivatized CSPs. The second most efficient CSP is the dimethyl benzyl CSP (SP-CF6-DMAP) but with only nine separations in the NP mode plus one in the PO mode, that is half as many as the SP-CF6-IM2. The average enantioresolution factor is highest for the SP-CF6-BIM. This is because the benzyimidazolium derivatized CSP rarely worked (only 5 compounds resolved in the NP mode and one in the PO mode, Figure 6), but when it worked, it worked well (Table 2).

Three different propyl imidazolium CSPs were prepared varying the linker number and the substitution number (Table 1). As mentioned, the SP-CF6-IM2 CSP showed the best overall effectiveness. Grafting four linkers per CF6 units with SP-CF6-IM1 produced a greatly enhanced bonding density ($0.66 \mu\text{mol}/\text{m}^2$). Unfortunately, it appears that the solutes have difficulty reaching enantioselective areas of the chiral selector in the densely packed CSP CF6-IM1: the number of enantioseparations is only four in the NP mode and none in the PO mode (Figure 6). The SP-CF6-IM3 CSP was prepared with CF6 containing 10 imidazole substituents instead of 6 in CF6-IM1 and IM2. Only 9 enantiomeric pairs could be separated in the NP mode and none in the PO mode (Figure 6). All compounds that were separated by the CF6-IM1 or CF6-IM3 CSPs were also separated by the CF6-IM2 showing the expected similar enantio-recognition capability for the same selector. The deleterious effect of the increased degree of CF6 substitution is also likely due to steric crowding with less available hydroxyl groups to interact with the analytes. Similar results were reported with highly aromatic derivatized CF6 selectors.^[5] It must be noted that the low efficacy of the CF6-BIM and CF6-PY CSPs also could be due to steric crowding of the CF6 rings since both these CSPs have a degree of substitution of 9 and 11, respectively (Table 1).

Compound structure and enantio-recognition

Figure 4 shows neutral compounds with a circled number, basic compounds with a triangle and no symbol for acidic compounds. Basic compounds contain amine groups that can bear a positive charge at a pH around 4. Acidic compounds can be ionized bearing a negative charge or can be neutral at pH 4. The neutral uncharged racemates are best separated by the cationic derivatized CF6 CSPs (Figure 4 and Table 2). The basic compounds, likely positively charged, are less often separated than neutrals, however all of them were

separated by at least one CSP in one mode. The acidic compounds were the least likely to be separated by the cationic CF6 CSPs: out of 14 acidic compounds, 7 could not be separated by any CSPs or conditions. The other 7 acidic compounds could be very partially separated by a single CSP or 2 CSPs for compounds 1, 2 and 5. Since most separations were obtained in the NP mode, it is not surprising that charge-charge interaction play little or no role in this apolar mode. The partial separation of the enantiomers of phenylethyl sulfamic acid (compound 1) and acetyl mandelic acid (compound 2) by several cationic CF6 CSPs should be noted since these racemic solutes were not separated with previously studied CF6 CSPs.^[5, 6]

Figure 7 (top) shows the separation of two structurally similar chiral compounds: compound 24: benzyl-6-oxo- *cis*-2,3-diphenyl-4-morpholine carboxylate and compound 21 that is the *t*-butyl-6-oxo equivalent. The chromatographic data are very similar showing that it is not the terminal group, (benzyl or *t*-butyl) that has a critical part in the enantio-recognition of these isomers by the CF6-IM2 CSP. However, for the CF6-BIM CSP, Table 2 lists a baseline separation of the benzyl (compound 24) and no separation of the *t*-Bu (compound 21). In this case, the increased volume of the *t*-Bu terminal group may impede chiral recognition by the CF6-BIM CSP. Figure 7 (bottom) shows highly retained compounds 16 (binaphthol) and 22 (diperodon). Binaphthol (atropoisomery) was separated by most of the cationic derivatized CF6 CSPs (Table 2) and it was always highly retained including by the SP-CF6-AP CSP that does not contain any aromatic ring. Apparently π - π interactions are not necessary to separate biaryl atropoisomers with cationic derivatized cyclofructan based chiral selectors. Diperodon (compound 22) could only be separated by the CF6-BIM CSP and only with the NP4 mobile phase (lowest ethanol content).

CONCLUSION

The capabilities of CSPs containing basic derivatives of cyclofructan 6 did not show the extreme differences in enantiomeric separation capabilities (compared to commercially available cyclofructan based (LARIHCTM) columns) that was expected. Surprisingly, none of the 34 racemic compounds, containing acids, neutral analytes and bases, could be enantioresolved in the reverse phase mode with acetonitrile/pH 4 buffer mobile phases. The CSPs were designed to be able to carry positive charges with protonated imidazolium, pyridinium or ammonium groups. Seven acidic compounds could not be separated at all. The 27 remaining compounds could be separated mainly in the normal phase mode (21 compounds) or in the polar organic mode (8 compounds). Ultimately, the imidazole based CSP showed better enantioselectivity than the other basic derivatives. It was confirmed that bulkiness of the substituent group can be a major hindrance in chiral recognition by CF6 based CSPs. Also, a degree of substitution of the cyclofructan 6 greater than six is detrimental to the enantio-recognition ability of the substituted selector.

Acknowledgments

Research reported in this publication was supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Number R44GM103359. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. AB thanks the French National Center for Scientific Research (CNRS, ISA, UMR5280) for continuous support.

References

1. Wang, C.; Armstrong, DW. Cyclofructans, a new class of chiral stationary phases. In: Berthod, A., editor. Chiral recognition in separation methods: mechanisms and applications. Vol. Ch 4. Springer; Heidelberg: 2010. p. 77-96.
2. Jiang C, Tong MY, Breitbach ZS, Armstrong DW. Synthesis and examination of sulfated cyclofructans as a novel class of chiral selectors for CE. Electrophoresis. 2009; 30:3897–3909. [PubMed: 19938182]
3. Zhang YJ, Huang MX, Zhang YP, Armstrong DW, Breitbach ZS, Ryoo JJ. Use of sulfated cyclofructan 6 and sulfated cyclodextrins for the chiral separation of four basic pharmaceuticals by CE. Chirality. 2013; 25:735–742. [PubMed: 23939835]
4. Zhang Y, Breitbach ZS, Wang C, Armstrong DW. The Use of Cyclofructans as Novel Chiral Selectors for Gas Chromatography. Analyst. 2010; 135:1076–1083. [PubMed: 20419259]
5. Sun P, Wang C, Breitbach ZS, Zhang Y, Armstrong DW. Development of New HPLC Chiral Stationary Phases Based on Native and Derivatized Cyclofructans. Anal Chem. 2009; 81:10215–10226. [PubMed: 20000643]
6. Sun P, Armstrong DW. Effective enantiomeric separations of racemic primary amines by the isopropyl carbamate-cyclofructan 6 chiral stationary phase. J Chromatogr A. 2010; 1217:4904–4918. [PubMed: 20561631]
7. Sun P, Wang C, Padivitage N, Lasanthi T, Nanayakkara YS, Perera S, Qiu H, Zhang Y, Armstrong DW. Evaluation of aromatic-derivatized cyclofructans 6 and 7 as HPLC chiral selectors. Analyst. 2011; 136:787–800. [PubMed: 21140011]
8. Breitbach ZS, Feng Q, Doddiba E, Koswatta PB, Lovely CJ, Armstrong DW. Enantiomeric Separation of 4,5-disubstituted Imidazoles by HPLC and CE using Cyclodextrin-Based Chiral Selectors. Supramolecular Chemistry. 2010; 22:758–767.
9. Padivitage NL, Doddiba E, Breitbach ZS, Armstrong DW. Enantiomeric separations of illicit drugs and controlled substances using cyclofructan-based (LARIHC) and cyclobond I 2000 RSP HPLC chiral stationary phases. Drug Test Anal. 2014; 6 in press. 10.1002/dta.1534
10. Qiu H, Loukotkova L, Sun P, Tesarova E, Bosakova Z, Armstrong DW. Cyclofructan 6 based stationary phases for hydrophilic interaction liquid chromatography. J Chromatogr A. 2011; 1218:270–279. [PubMed: 21167492]
11. Padivitage NL, Armstrong DW. Sulfonated cyclofructan 6 based stationary phase for hydrophilic interaction chromatography. J Sep Sci. 2011; 34:1636–1647. [PubMed: 21710525]
12. Rannard SP, Davis NJ. The Selective Reaction of Primary Amines with Carbonyl Imidazole Containing Compounds: Selective Amide and Carbamate Synthesis. Org Lett. 2000; 2:2117–2120. [PubMed: 10891244]
13. Wang R, Ong T, Tang W, Ng S. Cationic cyclodextrins chemically-bonded chiral stationary phases for high-performance liquid chromatography. Anal Chim Acta. 2012; 718:121–129. [PubMed: 22305907]
14. Berthod A. Chiral recognition mechanisms. Anal Chem. 2006; 78:2093–2099. [PubMed: 16791981]

FIGURE 1.

Cyclofructan structure. **A-** Space filling model of CF6 showing the covering of the crown ether cavity by the fructofuranose units. **B-** Molecular structure of CF6 ($n=1$); CF7 ($n=2$) and CF8 ($n=3$).

FIGURE 2.

Synthesis of derivatized CF6 in two steps in one pot using the 1,1'-carbonyl diimidazole reagent. (1) 1-(3-aminopropyl) imidazole forming CF6-IM; (2) 2-(aminomethyl) benzimidazole forming CF6-BIM; (3) 3-(dimethylamino)-1-propylamine forming CF6-AP. The reaction of a single functional hydroxyl group is indicated. There are 18 –OH groups on each CF6 ring.

FIGURE 3.

The chiral CF6 based stationary phases prepared with basic amine containing moieties. IM: propyl imidazole; BIM: benzimidazole; AP: dimethyl aminopropyl; PY pyridinyl; DMAP: dimethyl aminophenyl. The reaction of a single functional hydroxyl group is indicated. There are 18 -OH groups on each CF6 ring.

FIGURE 4.

Enantioresolution factors obtained with the seven cationic CF6 CSPs with the 34 solutes of Table 2. Baseline resolution corresponds to $R_s > 1.5$. Circled compounds are neutral, bases have a triangle and unlabeled numbers correspond to acids. The best enantioresolution factor (obtained with different mobile phase compositions) is shown; it is most often also the best selectivity factor.

FIGURE 5.

Separation of the warfarin enantiomers (compound 14) with the NP2 mobile phase (heptane/ethanol 80/20 v/v with 0.1% trifluoroacetic; 1 mL/min) on three different basic CF6 chiral stationary phases. Column 25×0.46 cm.

FIGURE 6.

Cationic derivatized CF6 chiral stationary phase efficacy. Top: The most efficient normal phase mode; bottom: polar organic mode. Not a single enantioseparation was obtained in reversed phase mode. See Table 2 and Figure 4 for full details on individual compound results.

FIGURE 7.

Separations of the enantiomers of compounds 24, 21, 16 and 22 on cationic derivatized CF6 chiral stationary phases. Top chromatograms: structurally similar compounds 24 and 21 separated with the same NP2 mobile phase (heptane/ethanol 80/20 v/v with 0.1% trifluoroacetic acid). Bottom chromatograms: highly retained compounds, atropisomers of binaphthol (compound 16) with NP4 mobile phase (heptane/ethanol 80/20 v/v with 0.1% trifluoroacetic acid) and the only separation of dipiperodon (compound 22) also with the NP4 mobile phase.

TABLE 1

Elemental analysis data and bonding density for the 7 synthesized chiral stationary phases.

Name	C %	N %	Bonded moiety			Ratio CF6: Linker	CF6 substitution	Bonding density $\mu\text{mol}/\text{m}^2$
			C	N	m.w.			
SP-CF6-IMI	19.4	5.6	94	22	2214	1:4	6	0.66
SP-CF6-IM2	14.8	3.5	86	20	2046	1:2	6	0.42
SP-CF6-IM3	12.2	3.8	114	32	3650	1:2	10	0.26
SP-CF6-BIM	16.6	4.2	125	29	2697	1:2	9	0.34
SP-CF6-AP	11.3	2.7	109	24	2548	1:2	11	0.24
SP-CF6-PY	12.6	1.8	80	14	1956	1:2	6	0.33
SP-CF6-DMAP	12.1	2.5	89	12	1950	1:2	5	0.40

C% and N% are the experimental values obtained by elemental analysis. The number of carbon atoms, C, and nitrogen atoms, N, and the mass of the bonded moiety, m.w., are listed. The ratio CF6:linker is the number of ethoxysilyl propyl carbamate units per Cyclofructan 6 ring. The CF6 substitution number indicates the number of cationic derivatives added per CF6 ring. 5 μm Daiso SP-100-5P particle bonded with the Figure 3 derivatized cyclofructan 6. The listed bonding density is the average value of the bonding densities obtained using the C% and the N%.

TABLE 2

Chromatographic data for all analytes examined on the basic CF6 CSPs.

	Compound	Structure	CSP SP-CF6-	Mobile Phase	k _i	α	R _s
1	Phenethylsulfamic acid		IM2	NP2	6.64	1.05	1.0
			IM3	NP2	6.58	1.02	0.8
2	Acetylmandelic acid		PY	PO2	0.62	1.10	1.0
			IM2	NP3	5.71	1.00	0.4
			DMAP	NP2	0.53	1.01	0.5
3	2-(2-Chlorophenoxy)-propionic acid		DMAP	PO1	3.06	1.03	0.6
4	2-Phenylpropionic acid		PY	PO1	0.78	1.01	0.5
5	3-Oxo-1-indancarboxylic acid		IM2	NP2	3.74	1.02	0.5
			IM3	NP2	14.3	1.05	0.5
6	5-Methoxy-1-indanone-3-acetic acid		IM2	PO3	6.19	1.06	0.5

Compound	Structure	CSP SP-CF6-	Mobile Phase	k ₁	α	R _s
7		IM1	NP2	2.76	1.00	-
		IM2	NP3	1.61	1.00	-
		IM3	NP3	5.79	1.00	-
8		IM1	NP2	1.94	1.00	-
		IM2	NP3	0.68	1.00	-
		IM3	NP3	4.53	1.00	-
9		IM1	NP2	1.24	1.00	-
		IM2	NP3	4.60	1.00	-
		IM3	NP3	2.82	1.00	-
10		IM1	NP2	7.75	1.00	-
		IM2	NP3	3.33	1.00	-
		IM3	NP3	5.20	1.00	-
		BIM	NP2	7.10	1.00	-
11		IM1	NP4	11.58	1.00	-
		IM2	NP2	4.51	1.00	-
		IM3	NP3	4.53	1.00	-
12		IM1	NP2	11.59	1.00	-
		IM2	NP2	6.97	1.00	-
		IM3	NP2	8.96	1.00	-
13		IM1	NP2	8.96	1.00	-
		IM2	NP2	8.96	1.00	-
		IM3	NP2	8.96	1.00	-

Compound	Structure	CSP SP-CF6-	Mobile Phase	k _i	α	R _s
14 Warfarin (3-(alpha-Acetylbenzyl)-4-hydroxycoumarin)		IM1	NP2	1.36	1.32	3.1
		IM2	NP2	1.12	1.17	1.5
		IM3	NP1	1.30	1.17	1.6
		BIM	NP2	0.88	1.28	2.0
		AP	NP2	0.67	1.40	1.4
		PY	NP2	0.93	1.43	1.8
		DMAP	NP3	8.26	1.05	1.3
15 Coumachlor		IM1	NP1	2.91	1.17	1.5
		IM2	NP2	2.60	1.17	1.5
		IM3	NP1	2.82	1.16	1.5
		AP	NP2	2.60	1.17	1.5
		PY	NP2	2.51	1.03	1.0
		BIM	NP2	2.42	1.01	1.0
		DMAP	NP3	2.75	1.11	2.0
16 1,1'-Bi-2-naphthol		DMAP	NP4	6.88	1.08	1.5
		IM1	NP2	7.05	1.07	1.3
		IM2	NP3	6.67	1.04	1.0
		IM3	NP2	5.36	1.03	0.8
		AP	NP1	5.21	1.05	1.0
		DMAP	NP3	5.70	1.04	1.0
		IM2	NP3	1.94	1.17	0.8
17 6,6'-Dibromo-1,1'-bi-2-naphthol		IM3	NP2	1.65	1.13	0.8

Compound	Structure	CSP SP-CF6-	Mobile Phase	k ₁	α	R _s
18		DMAF	NP3	7.98	1.03	0.8
19		IM2	PO2	0.67	1.02	0.6
20		IM2	NP3	0.39	1.02	0.5
21		IM2	NP2	1.48	1.18	2.0
		IM3	NP2	0.89	1.08	1.5
22		BIM	NP4	18.6	1.16	2.0

Compound	Structure	CSP SP-CF6-	Mobile Phase	k ₁	α	R _s
23		IM3 BIM	NP2 PO3	1.25 1.25	1.18 1.18	1.7 1.7
24		IM2 IM3 BIM1	PO3 NP2 NP2 NP2	2.34 1.25 1.21 1.29	1.17 1.14 1.14 1.14	1.0 1.5 1.5 1.5
25		IM2	NP2	0.96	1.18	1.6
26		BIM IM2	NP2 NP2	7.82 7.21	1.08 1.03	1.4 0.8
27		PY DMAP	NP2 NP2	6.92 7.02	1.05 1.01	0.8 0.5
28		IM2	NP3	3.01	1.02	0.7

Compound	Structure	CSP SP-CF6-	Mobile Phase	k ₁	α	R _s
29		IM2	NP2	3.63	1.03	0.8
30		IM1	NP2	1.32	1.02	0.6
31		DMAp	NP2	6.72	1.01	0.5
32		DMAp	NP2	5.63	1.01	0.5
33		PY	PO1	6.12	1.01	0.5

Compound	Structure	CSP SP-CF6-	Mobile Phase	k ₁	α	R _s
34 Chlrophedianol		IM2	PO3	2.46	1.04	0.5

Mobile phase composition: Normal phase mode, NP1 = heptane/ethanol 70/30 v/v; NP2 = 80/20 v/v; NP3 = 90/10 v/v; NP4 = 95/5 v/v, all NP phases contain 0.1% trifluoroacetic acid.

Polar organic mode, PO1 = acetonitrile/methanol 60/40 v/v; PO2 = 80/20 v/v; PO3 = 98/2 v/v, all PO phases contain 0.3% acetic acid and 0.2% triethylamine.

k₁ is the retention factor of the first eluting enantiomer, α and R_s are respectively the enantioselectivity and resolution factors.