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# Development of New HPLC Chiral Stationary Phases Based on Native and Derivatized Cyclofructans

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**An unusual class of chiral selectors, cyclofructans, is introduced for the first time as bonded chiral stationary phases. Compared to native cyclofructans (CFs), which have rather limited capabilities as chiral selectors, aliphatic- and aromatic-functionalized CF6s possess unique and very different enantiomeric selectivities. Indeed, they are shown to separate a very broad range of racemic compounds. In particular, aliphatic-derivatized CF6s with a low substitution degree baseline separate all tested chiral primary amines. It appears that partial derivatization on the CF6 molecule disrupts the molecular internal hydrogen bonding, thereby making the core of the molecule more accessible. In contrast, highly aromatic-functionalized CF6 stationary phases lose most of the enantioselective capabilities toward primary amines, however they gain broad selectivity for most other types of analytes. This class of stationary phases also demonstrates high "loadability" and therefore has great potential for preparative separations. The variations in enantiomeric selectivity often can be correlated with distinct structural features of the selector. The separations occur predominantly in the presence of organic solvents.**

Enantiomeric separations have attracted great attention in the past few decades. Early enantioselective LC work in the 1980s provided the impetus for the 1992 FDA policy statement on the development of stereoisomeric drugs.[1] This was because the facile analysis and preparation of many pharmaceutically active enantiomers became possible for the first time. Such broadly applicable techniques were essential for pharmacokinetic and pharmacodynamic studies, development, quality control, and sometimes production of enantiomeric drugs. HPLC with chiral stationary phases (CSPs) is far and away the most powerful and widely used technique for solvent-based enantiomeric separations at both analytical and preparative scales. Supercritical fluid separations are increasing in importance, particularly for preparative separations.

Currently, over a hundred CSPs have been reported, and these CSPs are made by coating or bonding the chiral selectors, usually to silica gel supports. Interestingly, only a few types/classes of CSPs dominate the field of enantiomeric separations, for example, polysaccharide-based CSPs,[2−11] macrocyclic antibiotic CSPs,[12−22] and $\pi$ complex CSPs.[23−26] Researchers continue to make great efforts to develop new HPLC CSPs, which could make a substantial impact on enantiomeric separations. It has been stated that today in order for any new CSPs to have an impact, they must fulfill one or more of the following requirements:[27] (a) broader applicability than existing CSPs, (b) superior separations for specific groups of compounds, or (c) fill an important unfulfilled separation niche. In the present article, a unique class of CSPs based on cyclofructan (CF) is introduced and is shown to have the potential to satisfy all of the above-mentioned requirements.

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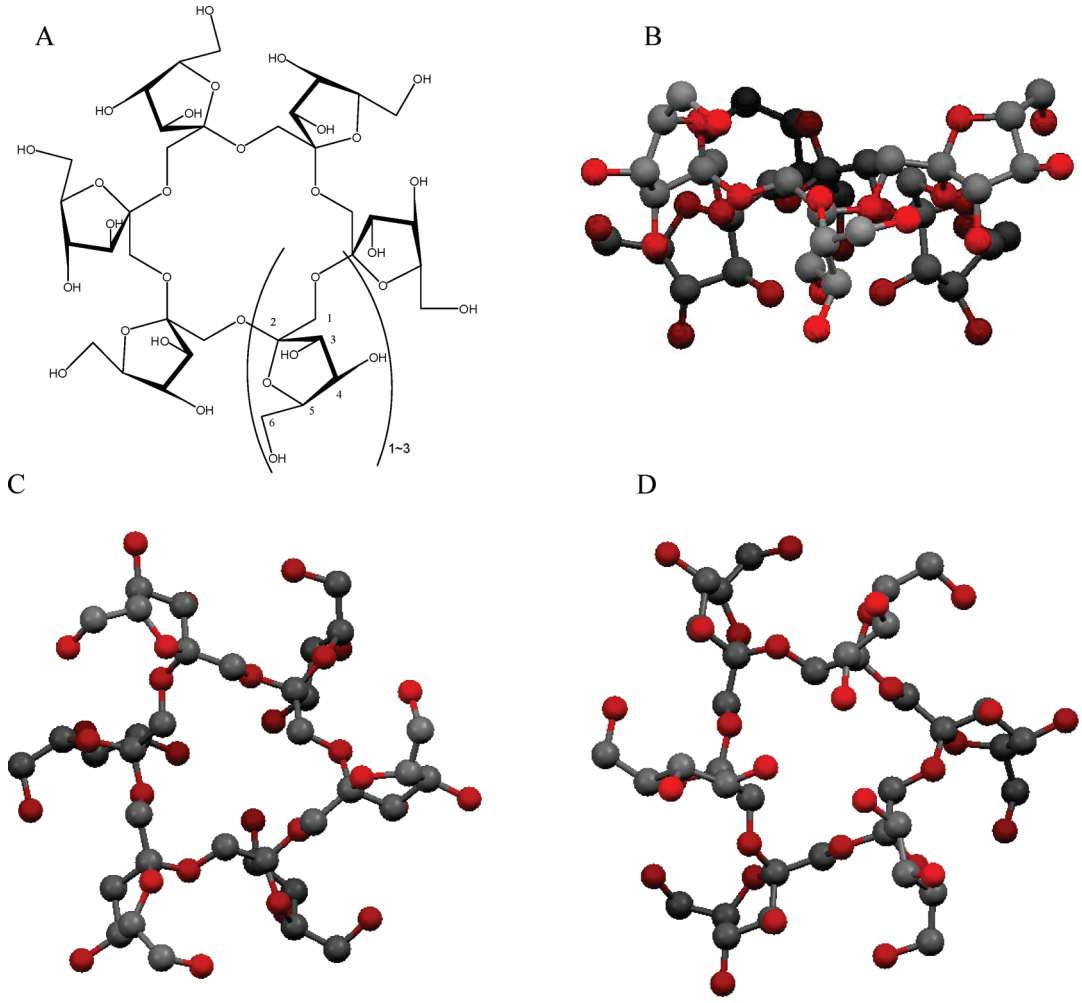


**Figure 1.** Structure of cyclofructan: (A) molecular structure of CF6, CF7, and CF8; (B−D) crystal structure of CF6 (B) side view; (C) hydrophobic side up; (D) hydrophilic side up. Color scheme: oxygen atoms are red and carbons are black. Hydrogens are not shown.

Cyclofructans (CFs) are one of a relatively small group of macrocyclic oligosaccharides.[28,29] Cyclodextrins are perhaps the best known member of this class of molecules.[30,31] However, as will be shown, cyclofructans are quite different in both their structure and behavior. Cyclofructans consist of six or more $\beta$-(2 → 1) linked D-fructofuranose units (see Figure 1). Common abbreviations for these compounds are CF6, CF7, CF8, etc., which indicate the number of fructose units in the macrocyclic ring. Cyclofructans were first reported by Kawamura and Uchiyama in 1989.[28] Since then, cyclofructans have been used in a variety of applications mostly as additives to consumer products, such as moderators of food and drink bitterness and astringency,[32−34] browning prevention agent,[35] ink formulation agents,[36] lubricants,[37] and so on. In addition, cyclofructans have been shown to have cryoprotective effects[38] and complexing abilities toward metal ions.[39] However, to date and to our knowledge, there have been no published reports on HPLC enantiomeric separations using either native cyclofructan or derivatized cyclofructans as chiral selectors.

In the present work, we described the unique structure of CF6, synthesis of CF6-based CSPs, chiral separation mechanistic considerations, their chromatographic performance in terms of enantiomeric separations, and the pronounced effect of certain derivatization approaches on CF6 structure and selectivity.

## EXPERIMENTAL SECTION

**Materials.** Cyclofructans (CFs) can be produced either by fermentation of inulin via any of five microorganisms (for example,

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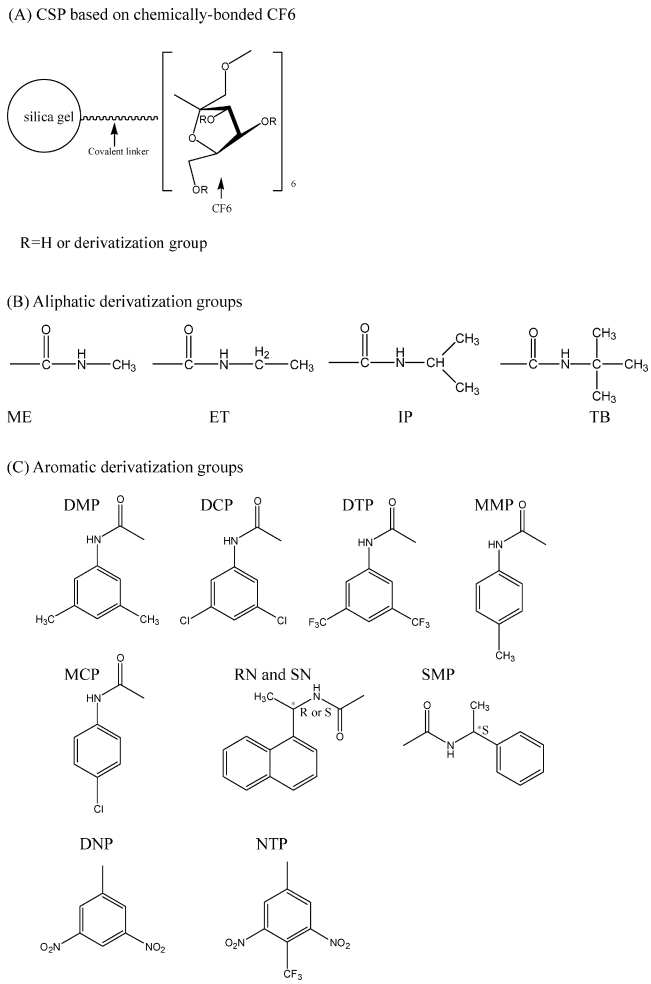


**Figure 2.** Scheme of chemically bonded CF6 stationary phase and derivatized-CF6 CSPs and chemical structure of all derivatizing groups.

*Bacillus circulans* OKUMZ 31B and *B. circulans* MCI-2554),[28,40−46] or incubation of inulin with the active enzyme cycloinulooligosaccharide fructanotransferase (CFTase).[29] Also, the CFTase gene has been isolated, and its sequence has been determined and incorporated into common baker's yeast.[29,43] Therefore, mass-produced CFs could be available at low cost. CF6 and a mixture of CF7 (80%) + CF6 (20%) were generously donated by Mitsubishi Chemical Group (Tokyo, Japan). Different types of silica (all of 5 $\mu$m spherical diameters) were utilized. They include Daiso silica of 5 $\mu$m spherical diameter (300 Å pore size, 107 $m^2/g$ surface area; 200 Å, 170 $m^2/g$; 120 Å, 324 $m^2/g$; 100 Å, 440 $m^2/g$), and Kromasil silica (300 Å, 116 $m^2/g$; 200 Å, 213 $m^2/g$; 100 Å, 305 $m^2/g$). Anhydrous *N*,*N*-dimethylformamide (DMF), anhydrous toluene, anhydrous pyridine, acetic acid (AA), triethylamine (TEA), trifluoroacetic acid (TFA), butylamine, sodium hydride, 3-(triethoxysilyl)propyl isocyanate, 1,6-diisocyanatohexane, (3-aminopropyl)triethoxysilane, 3-glycidoxypropyltrimethoxy silane, and all 81 racemic analytes tested in this study were purchased from Sigma-Aldrich (Milwaukee, WI). All isocyanate and isothiocyanate derivatization reagents were also obtained from Sigma-Aldrich. They include methyl isocyanate, ethyl isocyanate, isopropyl isocyanate, *tert*-butyl isocyanate, methyl isothiocyanate, 3,5-dimethylphenyl isocyanate, 3,5-dichlorophenyl isocyanate, *p*-tolyl isocyanate, 4-chlorophenyl isocyanate, 3,5-bis(trifluoromethyl)phenyl isocyanate, *R*-1-(1-naphthyl)ethyl isocyanate, *S*-1-(1-naphthyl)ethyl isocyanate, and *S*-α-methylbenzyl isocyanate. 1-Chloro-3,5-dinitrobenzene and 4-chloro-2,6-dinitrobenzotrifluoride were obtained from Alfa Aesar (Ward Hill, MA). Acetonitrile (ACN), 2-propanol (IPA), *n*-heptane, ethanol (ETOH), and methanol (MEOH) of HPLC grade were obtained from EMD (Gibbstown, NJ). Water was obtained form Millipore (Billerica, MA).

**Table 1. Elemental Analysis Results of Three Representative CF6-Based Stationary Phases**

| chiral selector | degree of substitution | C % | H % | N % |
|---|---|---|---|---|
| native CF6 | 0 | 10.19 | 1.78 | 0.41 |
| methyl carbamate CF6 | low[a] | 12.02 | 1.90 | 1.01 |
| *R*-naphthylethyl carbamate CF6 | very high[b] | 19.11 | 2.52 | 1.57 |

[a] DS ~ 6. [b] Complete derivatization (DS = 18).

**Synthesis of CF-Based CSPs.** Native or derivatized cyclofructans were bonded to silica support by a variety of different methods. Native CF6 is used as an example to describe the procedures for the three binding chemistries tested. In the first method, silica (3 g) was dried at 110 °C for 3 h. Anhydrous toluene was added, and any residual water was removed using a Dean−Stark trap for 3 h. The mixture was cooled down <40 °C, and 1 mL of (3-aminopropyl)triethoxysilane was added dropwise to the 3 g of the silica−toluene slurry. Next, the mixture was refluxed for 4 h and then cooled, filtered, washed, and dried to obtain amino-functionalized silica (3.3 g). Then, 2 mL of 1,6-diisocyanatohexane was added to the dry amino−silica toluene slurry, which was kept in an ice bath. Next, the slurry mixture was heated to 70 °C for 4 h. The excess reactant was removed by vacuum filtration, and the solid product was washed with anhydrous toluene twice. Lastly, 1 g of dried cyclofructan dissolved in 20 mL of pyridine was added, and the mixture was heated to 70 °C and allowed to react for 15 h. Finally, 3.7 g of product was obtained.

The second binding chemistry also forms the carbamate linker. Cyclofructan (1 g) was dissolved in 40 mL of anhydrous pyridine. To this solution, 0.7 mL of 3-(triethoxysilyl)propyl isocyanate was added dropwise under dry argon atmosphere protection. And the mixture was heated at 90 °C for 5 h. Next, residual water was removed from silica gel (3 g) using a Dean−Stark trap and 150 mL of anhydrous toluene. After the two mixtures were cooled to room temperature, the cyclofructan reaction mixture was added to the silica−toluene slurry and heated at 105 °C overnight. The final mixture was cooled and washed. After the mixture was dried in vacuum overnight, 3.4 g of product was obtained.

The third binding chemistry forms an ether linkage. Epoxy-functionalized silica was synthesized as previously described.[12] First, CF6 (1 g) was dissolved in 30 mL of anhydrous DMF. Then, 0.2 g of NaH was added to the solution under dry argon protection and stirred for 10 min. Unreacted NaH was removed by vacuum

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**Table 2. Physical Properties of Cyclofructans 6–8**

| macrocycle | molecular weight | melting point (°C) | $[\alpha]_D^{20}$ (deg) in $H_2O$ | cavity i.d. (Å)[c] | macrocycle o.d. (Å)[c] | macrocycle height (Å)[c] |
|---|---|---|---|---|---|---|
| CF6 | 972.84 | 210–219[a]<br>231–233[b] | −64.6[b]<br>−63.5[d] | 2.3 | 14.6 | 8.7–9.4 |
| CF7 | 1134.98 | 215–222[a] | −59.1[d] | 4.1 | 15.9 | 8.5–8.9 |
| CF8 | 1297.12 | N/A | N/A | 4.7 | 16.1 | 8.5–9.2 |

[a] Melts and decomposes in this range. [b] Values taken from ref 28. [c] Values estimated from ref 46. Estimates accounting for van der Waals radii. [d] Measured in our lab.

filtration. Lastly, dry epoxy-functionalized silica (3.3 g) was added to the filtrate, and the mixture was heated at 140 °C for 3 h and subsequently cooled down to room temperature. After filtration and drying, 3.5 g of product was obtained.

Syntheses of derivatized cyclofructan chiral stationary phases (CSPs) have been conducted in a variety of ways. For example, native cyclofructan can be chemically bonded to silica, then derivatized, or cyclofructan can be first partially derivatized, then bonded to silica. Figure 2 shows the diagram of covalently bonded CF6 and the structures of the aliphatic and aromatic derivatization groups used in this study. All derivatization groups were bonded to CF6 via a carbamate or a thiocarbamate linkage, with the exception of dinitrophenyl and dinitrophenyl-trifluoromethyl groups, which are attached to CF6 via an ether linkage.[47] Table 1 shows elemental analysis data for the native CF6-CSP, partially derivatized methyl carbamate-CF6 CSP, and completely derivatized *R*-naphthylethyl carbamate-CF6 CSP. After the cyclofructan derivative is bonded to the support, it can be further derivatized to achieve more complete coverage or to have two different derivative groups on the cyclofructan ($\pi$-acid and $\pi$-basic groups).

**HPLC Method.** The HPLC column packing system is composed of an air driven fluid pump (HASKEL, DSTV-122), an air compressor, a pressure regulator, a low pressure gauge, two high-pressure gauges (10 000 and 6 000 psi), a slurry chamber, check valves, and tubings. The CSP was slurry packed into a 25 cm × 0.46 cm (i.d.) stainless steel column.

The HPLC system was an Agilent 1100 system (Agilent Technologies, Palo Alto, CA), which consists of a diode array detector, an autosampler, a binary pump, and Chemstation software. All chiral analytes were dissolved in ethanol, methanol, or appropriate mobile phases. For the LC analysis, the injection volume and the flow rate were 5 $\mu$L and 1 mL/min, respectively. Separations were carried out at room temperature (~20 °C) if not specified. The mobile phase was degassed by ultrasonication under vacuum for 5 min. Each sample was analyzed in duplicate. Three operation modes (the normal phase mode, polar organic mode, and reversed phase mode) were tested. In the normal phase mode, heptane with ethanol or isopropanol was used as the mobile phase. In some cases, TFA was used as an additive. The mobile phase of the polar organic mode was composed of acetonitrile/methanol and small amounts of acetic acid and triethylamine. Water/acetonitrile or acetonitrile/acetate buffer (20 mM, pH = 4.1) was used as the mobile phase in the reversed-phase mode. The supercritical fluid chromatographic instrument is a Berger supercritical fluid chromatography (SFC) unit with an FCM1200 flow control module, a TCM 2100 thermal column module, a dual pump control module, and a column selection valve. The flow rate was 4 mL/min. The cosolvent was composed of methanol/ethanol/isopropanol = 1:1:1 and 0.2% DEA (diethylamine). The gradient mobile phase composition was 5% cosolvent hold during 0–0.6 min, 5–60% during 0.6–4.3 min, 60% hold during 4.3–6.3 min, 60%–5% during 6.3–6.9 min, and 5% hold during 6.9–8.0 min.

For the calculations of chromatographic data, $t_0$ was determined by the peak of the refractive index change due to the sample solvent or determined by injecting 1,3,5-tri*tert*-butylbezene in the normal phase mode. The molecular structure modeling program was ACD/3D viewer freeware.

## RESULTS AND DISCUSSION

**Structure and Properties of Cyclofructans.** Cyclofructans consist of six or more D-fructofuranose units (Figure 1), and each fructofuranose unit contains four stereogenic centers and three hydroxyl groups. Their central core has the same structure as the respective crown ethers. Table 2 gives relevant physicochemical data for CF6, CF7, and CF8. It indicates that the "cavity" inner diameter (i.e., distance between opposing oxygen atoms in the molecular core) increases significantly from 2.3 Å for CF6 to 4.1 Å for CF8.[46] Also, the macrocycle outer diameter of CF6 to CF8 demonstrates the same trend. However, the macrocycle heights are all quite similar.

Among cyclofructans, CF6 has attracted the most attention due to its availability in pure form and its highly defined geometry. The crystal structure of CF6 (shown in Figure 1B–D) reveals that six fructofuranose rings are arranged in spiral fashion around the crown ether skeleton, either up or down toward the mean plane of the crown ether.[44,45] Six three-position hydroxyl groups alternate to point toward or away from the molecular center, and three oxygen atoms pointing inside (oxygens in the molecular center of Figure 1D) are very close to each other (~3 Å). As a result, access to the 18-crown-6 core on that side of the macrocycle is blocked by the hydrogen bonded hydroxyl groups[45] (Figure 1D). This side of the macrocycle is relatively hydrophilic as the result of the directionality of all its hydroxyl groups (Figure 1D). The other side of CF6 appears to be more hydrophobic, resulting from the methylene groups of $-O-C-CH_2-O-$ around the central indentation (Figure 1C). A computational lipophilicity pattern of CF6 also confirms that CF6 shows a clear "front/back" regionalization of hydrophilic and hydrophobic surfaces.[46] Both the crystal structure and computational modeling studies demonstrate that CF6 appears to have considerable additional internal hydrogen bonding. The fact that three 3-OH groups completely cover one side of the 18-crown-6 ring and the core crown oxygens are almost folded inside the molecule makes CF6 very different from other 18-crown-6 ether based chiral selectors. It is worth emphasizing that CF6–CF8 do not possess central hydrophobic

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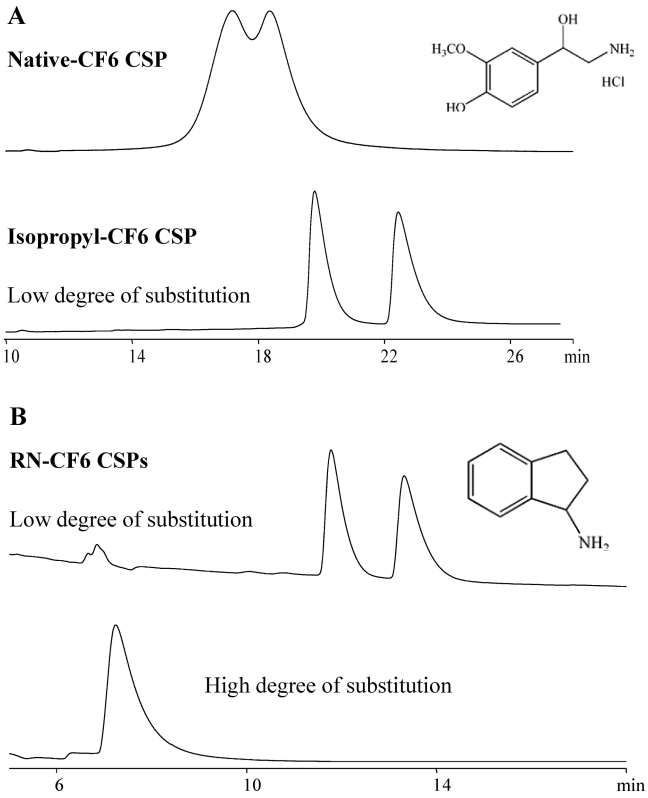


**Figure 3.** Separation of primary amines on derivatized-CF6 stationary phases with different substitution degrees. Analytes and mobile phases are (A) normetanephrine hydrochloride, 75ACN/25MEOH/0.3AA/0.2TEA (top), 60ACN/40MEOH/0.3AA/0.2TEA (bottom) and (B) 1-aminoindan, 60ACN/40MEOH/0.3AA/0.2TEA (top and bottom).

cavities, as do cyclodextrins.[30,31] Consequently, hydrophobic inclusion complexation, which plays an important role in the association of organic molecules with cyclodextrins, does not seem to be relevant for cyclofructans.

For the above-mentioned structural reasons, native CF6 appears to have limited capabilities to form either hydrophobic inclusion complexes or ion/crown ether inclusion complexes. In order to investigate whether or not CF6 provides any chiral recognition capabilities, we synthesized the covalently bonded native-CF6 stationary phase. We also conducted extensive capillary electrophoresis (CE) experiments investigating CF6 as a chiral run buffer additive.[48] The native CF6-based column was evaluated by injecting a set of 81 analytes with a wide variety of functionalities, including amines, carboxylic acids, alcohols, and so forth. This CSP based on native CF6 only partially separated enantiomers of a few primary amines (Figure 3A, top) and two binaphthyl compounds [i.e., 1,1′-bi(2-naphthyl diacetate) and 1,1′-bi-2-naphthol bis(trifluoromethanesulfonate)]. It appeared that few of the separations were baseline due to a combination of marginal selectivity, inefficient separations often coupled with poor peak shapes and often long retention. For all other analytes, the CF6-CSP exhibited negligible enantioselectivity. Furthermore no enantiomeric separations were obtained with native CF6 in CE.

**Initial Observations on the Effects of Derivatization of Cyclofructan 6.** The hypothesis that extensive intramolecular hydrogen bonding in CF6 (see Figure 1D) and its compact configuration has detrimental effects on its enantioselective separation ability could be tested if one could disrupt its internal hydrogen bonding and allow the structure to "relax" or open somewhat. One way to do this is to block a few of the crucial hydrogen bonding groups within the CF6 structure. Interestingly, when CF6 CSPs were made with either aliphatic or aromatic substituted-CF6s (with a low degree of hydroxyl group substitution), they exhibited tremendous enantioselectivity toward chiral primary amines (see Figure 3A). Molecular modeling was performed for the derivatized CF6 (Figure 4A) and helps to explain the improved enantiomeric resolution toward primary amines. In this simplest case, it is supposed that initial derivatization with the methyl substituent (ME) occurs with the 6-OH groups. Figure 4A shows a side view of the molecular model of ME-carbamate CF6 obtained with the ACD/3D viewer freeware program. It suggests that the CF6 intramolecular hydrogen bonding is disrupted after partial derivatization, causing a "relaxation" of the molecular structure. This may expose the crown ether core and/or other previously inaccessible hydrogen bonding sites of the CF6. Substitution of a few of the hydroxyl groups, especially with larger derivatization groups can increase the steric bulk, which can be beneficial for improving peak efficiency (as will be discussed). However, further increases in the substitution degree on the CF6 molecule (Figure 3B) results in significantly worse capabilities for separating primary amines. Enantioselectivity for all primary amines was completely lost on the RN-CSP with complete substitution. The reason for this becomes apparent from the structure of CF6 with 18 RN groups (Figure 4B), which shows the RN groups are positioned up or down, thus enlarging the depth of the molecule and again sterically blocking the molecular core. The conversion of all hydroxyl groups to carbamate groups also removes all hydrogen bonding donor groups, and most oxygens are buried deep inside the molecule. Thus, having many bulky functional groups can sterically hinder the chiral recognition of primary amines. Also, it is found that aliphatic- and aromatic-functionalized CF6 CSPs provide different capabilities for separating primary amines. The IP- and ME-derivatized columns gave higher enantioselectivity and resolution for tryptophanol (Figure S1 in the Supporting Information). A noticeable improvement in peak efficiency was observed on these aliphatic-functionalized columns. The aliphatic-functionalized CF6 stationary phases separate primary amines more effectively, providing higher selectivity and/or higher efficiency. These results demonstrate that the size of the derivatizing group also plays an important role in the separation of primary amines. Although aliphatic-functionalized CF6 stationary phases with a low substitution degree were highly successful for the separations of primary amines, they show poor capabilities for separating most other analytes.

Aromatic-functionalization of a native chiral selector is a common strategy used to develop new chiral stationary phases, such as dimethylphenyl and dichlorophenyl substitution of amylose and cellulose,[2−11] and dimethylphenyl and naphthylethyl substitution of $\beta$-cyclodextrin.[49−52] Compared to native chiral

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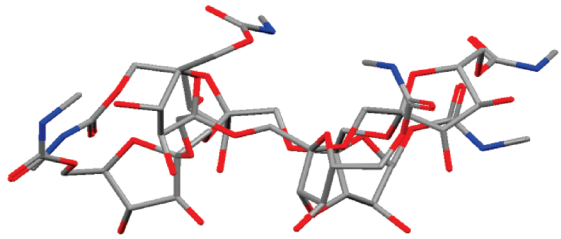
(A) Low degree of hydroxyl substitution with small aliphatic groups



(B) High degree of hydroxyl substitution with bulky aromatic groups

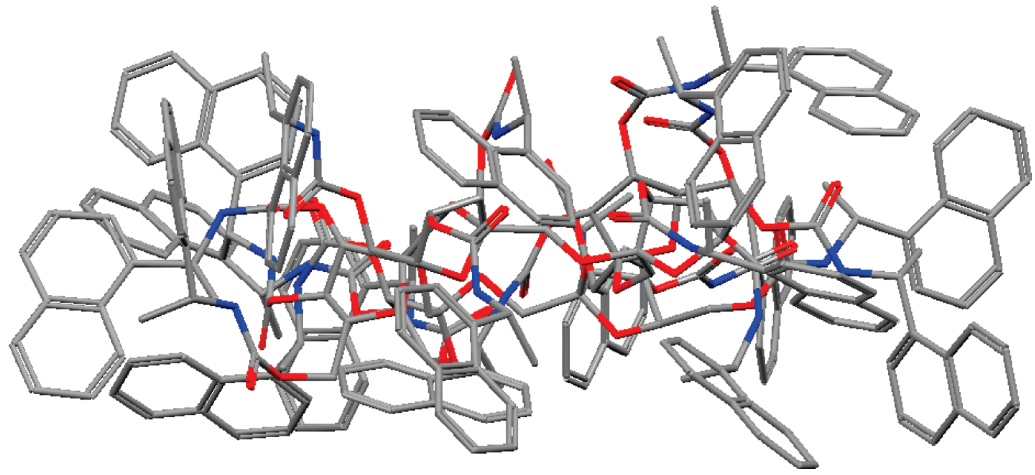


**Figure 4.** Edge view of CF6 derivatized with (A) six methyl carbamate groups and (B) 18 *R*-naphthylethyl carbamate groups. The color coding is oxygen atoms are red, carbon atoms are black, and nitrogen atoms are blue. Hydrogen atoms are not shown. Compare these edge views with that of native cyclofructans in Figure 1, part B.

selectors, the aromatic derivatized-types provide improved enantioseparation capabilities and separate a wider range of analytes. Therefore it was logical to synthesize aromatic-functionalized CF6 stationary phases with a higher substitution degree. Evaluations of these stationary phases show that highly aromatic-functionalized CSPs negate the previously found almost universal selectivity for chiral primary amines. However, there was a dramatic enhancement in enantiomeric selectivity for most other types of compounds. For example, *N*-(3,5-dinitrobenzoyl)-DL-leucine was successfully separated ($\alpha = 1.91$, $R_s = 4.4$) by the RN-CF6 column, while no separation was observed on the CF6-CSP (Figure S2 in the Supporting Information). The aromatic-functionalized CF6 CSPs provide ample opportunities for multiple hydrogen bonding interactions, $\pi-\pi$ interaction, and dipole−dipole interaction, aided by steric interactions to obtain effective chiral recognition.

It appears from these initial results that cyclofructan can be functionalized in such ways as to provide two completely different types of chiral selectors which separate enantiomers via two different mechanisms. The minimally functionalized CF6 (with smaller aliphatic moieties) has a relaxed structure that exposes its crown ether core and additional hydroxyl groups. This allows for interactions with and separation of chiral primary amines in organic solvents for the first time. More highly aromatic derivatized CF6 has a sterically crowded structure (Figure 4B) that hinders access to its molecular core but provides other ample interaction sites about its periphery. It is these sites that provide chiral recognition for a broad range of compounds. In the following sections, more detailed examination and optimization of these two functionalized cyclofructan formats are discussed.

**Cyclofructan CSPs Based on Aliphatic-Functionalized CF6.** Initial studies (supra vide) showed that aliphatic-CF6 CSPs provide excellent enantiomeric separation abilities for primary amines, so it was necessary to conduct more comprehensive studies on these aliphatic-functionalized stationary phases. Aliquots of CF6 were, respectively, derivatized with four different aliphatic moieties: methyl (ME), ethyl (ET), isopropyl (IP), and *tert*-butyl (TB) groups. Among them, IP- and ME-functionalized CF6 appeared to give the best enantiomeric separations of primary amines. Representative chromatographic data are shown in Table 3. For comparison, data generated on the aromatic RN-CF6 CSP with a low substitution degree also are included. The IP CSP gave the highest selectivity toward nine primary amines, while slightly higher selectivity was obtained on the methyl-derivatized CF6 column for the other two compounds. However, in those two cases, the IP-CSP gave better or the same resolution, due to its higher efficiency. In most cases the aromatic derivatized RN-CSP gave the poorest enantioselectivities and resolutions. Compared to all other columns (Figure 2), the isopropyl-derivatized CF6 CSP provided higher selectivity and/or higher efficiency and it produced baseline separations of all racemic primary amines tested.

Furthermore, thiocarbamate linkages between CF6 and various functional groups were also tested using the analogous isothiocyanate derivatization reagent. A comparison of the separations obtained from CF6 after derivatization with methyl isocyanate versus methyl isothiocyanate shows that the methyl-functionalized CSP based on the carbamate linkage produced significantly higher selectivity and resolution.

**Table 3. Chromatographic Data of Primary Amines Separated on Derivatized-CF6 Stationary Phases in Optimized Conditions**

| no. | compound name | CSP[a] | $k_1$ | α | $R_s$ | mobile phase[b] |
|---|---|---|---|---|---|---|
| 1 | *trans*-1-amino-2-indanol | IP | 2.85 | 1.31 | 3.9 | 60A40M0.3AA0.2T |
| | | ME | 2.44 | 1.28 | 3.5 | 60A40M0.3AA0.2T |
| | | RN-L | 1.43 | 1.23 | 1.6 | 60A40M0.3AA0.2T |
| 2 | *cis*-1-amino-2-indanol | IP | 2.69 | 1.12 | 1.6 | 60A40M0.3AA0.2T |
| | | ME | 2.47 | 1.10 | 1.5 | 60A40M0.3AA0.2T |
| | | RN-L | 3.00 | 1.07 | 0.8 | 75A25M0.3AA0.2T |
| 3 | normetanephrine hydrochloride | IP | 5.83 | 1.16 | 2.6 | 60A40M0.3AA0.2T |
| | | RN-L | 2.84 | 1.15 | 1.6 | 60A40M0.3AA0.2T |
| | | ME | 5.24 | 1.14 | 2.0 | 60A40M0.3AA0.2T |
| 4 | DL-octopamine hydrochloride | IP | 6.09 | 1.14 | 2.1 | 60A40M0.3AA0.2T |
| | | ME | 5.46 | 1.12 | 1.8 | 60A40M0.3AA0.2T |
| | | RN-L | 2.74 | 1.10 | 1.5 | 60A40M0.3AA0.2T |
| 5 | phenylpropanolamine hydrochloride | IP | 3.64 | 1.13 | 2.2 | 60A40M0.3AA0.2T |
| | | RN-L | 1.81 | 1.13 | 1.6 | 60A40M0.3AA0.2T |
| | | ME | 3.17 | 1.11 | 1.9 | 60A40M0.3AA0.2T |
| 6 | 1-aminoindan | IP | 3.90 | 1.17 | 3.1 | 60A40M0.3AA0.3T |
| | | RN-L | 3.21 | 1.17 | 2.1 | 60A40M0.3AA0.2T |
| | | ME | 3.37 | 1.15 | 2.7 | 60A40M0.3AA0.2T |
| 7 | 1,1-diphenyl-2-aminopropane | IP | 1.12 | 1.09 | 1.5 | 60A40M0.3AA0.2T |
| | | RN-L | 3.31 | 1.07 | 1.5 | 85A15M0.3AA0.2T |
| | | ME | 1.94 | 1.07 | 1.3 | 75A25M0.3AA0.2T |
| 8 | 2-amino-1-(4-nitrophenyl)-1,3- propanediol | ME | 2.40 | 1.18 | 1.9 | 60A40M0.3AA0.2T |
| | | IP | 2.16 | 1.15 | 2.3 | 60A40M0.3AA0.2T |
| | | RN-L | 6.74 | 1.14 | 1.7 | 85A15M0.3AA0.2T |
| 9 | α-methylbenzylamine | ME | 3.07 | 1.17 | 1.5 | 60A40M0.3AA0.2T |
| | | IP | 2.77 | 1.15 | 1.5 | 60A40M0.3AA0.2T |
| | | RN-L | 8.12 | 1.09 | 0.8 | 85A15M0.3AA0.2T |
| 10 | DL-tryptophanol | IP | 3.39 | 1.15 | 2.7 | 60A40M0.3AA0.2T |
| | | ME | 3.03 | 1.12 | 1.7 | 60A40M0.3AA0.2T |
| | | RN-L | 4.66 | 1.12 | 1.4 | 75A25M0.3AA0.2T |
| 11 | 1,2,2-triphenylethylamine | IP | 1.14 | 1.16 | 1.6 | 75A25M0.3AA0.2T |
| | | ME | 0.48 | 1.07 | 0.6 | 75A25M0.3AA0.2T |

[a] Abbreviations of CSPs: ME, methyl carbamate-CF6 CSP; IP, isopropyl carbamate-CF6 CSP; RN-L, *R*-naphthylethyl carbamate-CF6 CSP with a low substitution degree. [b] Abbreviations of mobile phases: A, acetonitrile; M, methanol; AA, acetic acid; T, triethylamine.

For primary amines, enantioresolution was observed in both the polar organic mode and normal phase mode. While operating under normal phase conditions, both acidic additives (trifluoroacetic acid) and basic additives (butylamine) were tested. In this instance, the acidic modifier is thought to act to maintain an ion-pair. Fronting asymmetric wide peaks were always observed in the normal phase mode with an acidic additive, due to strong interactions between basic analyte and the weakly acidic silica-based stationary phase. The selectivity of primary amines on the CF6 column was lost in most cases when using a butylamine additive, although symmetric peaks were observed (data not shown). The effect of butylamine in decreasing the retention and selectivity can be attributed to the fact that this basic additive simply competes with basic analytes for the primary interaction sites on the chiral stationary phase. In the polar organic mode, better resolution ($R_s$ = 1.6) of 1,2,2-triphenylethylamine was observed due to good selectivity (α = 1.16) and highest efficiency (symmetric sharp peaks) (Figure S3 in the Supporting Information). However, higher enantioselectivity (α = 1.25) often was obtained in the normal phase mode. This trend is true for all tested primary amines: better resolution was obtained in the polar organic mode. All primary amines were baseline separated using the same or similar mobile phase compositions, which streamlines the method development process. In addition, the polar organic mode offers other advantages, for example, short analysis times, low back-pressure, and better analyte solubility in the mobile phase. In order to evaluate the effects of acidic and basic additives in the polar organic mode on the separation of primary amines, different types and amounts of basic additives were investigated and the results are shown in Table 4. The highest enantioselectivity was obtained using the combination of triethylamine and acetic acid as additives. Also, the ratio of acidic/basic additives has been optimized, and it was determined that addition of 0.3% acetic acid/0.2% triethylamine commonly results in the highest selectivity.

**Table 4. Additive Effect on the Separation of (±) *trans*-1-Amino-2-Indanol (Primary Amine Type) in the Polar Organic Mode on the IP-CF6 Column**

| | | $k_1$ | α | $R_s$ |
|---|---|---|---|---|
| change basic additive type[a] | triethylamine | 2.85 | 1.31 | 4.0 |
| | trimethylamine | 3.36 | 1.29 | 5.3 |
| | ethanolamine | 1.97 | 1.14 | 2.6 |
| | butylamine | 2.36 | 1.16 | 2.3 |
| | diethylamine | 3.67 | 1.29 | 1.6 |
| change additive amount ratio[b] | 0.30AA/0.20TEA | 2.85 | 1.31 | 4.0 |
| | 0.20AA/0.30TEA | 2.69 | 1.24 | 3.9 |
| | 0.25AA/0.25TEA | 3.24 | 1.27 | 4.4 |

[a] The mobile phase is composed of 60%acetonitrile/40%methanol/0.3%acetic acid (equals 52 mM)/14 mM basic additive. [b] Volume percentage.

The most important feature of the derivatized-CF6 CSPs is their extremely high success rate for separating primary amines. Indeed, 100% of the tested primary amines were baseline separated. Currently, the most effective CSPs available for separating

**Table 5. Chromatographic Data of Other Compounds Separated on Six Derivatized-CF6 Stationary Phases in Optimized Conditions**

| no. | compound name | CSP[a] | $k_1$ | α | $R_s$ | mobile phase[b] |
|---|---|---|---|---|---|---|
| | Acids | | | | | |
| 1 | *O*-acetylmandelic acid | IP | 11.00 | 1.04 | 1.2 | 98H2E0.1TFA |
| | | SMP | 9.86 | 1.04 | 0.9 | 98H2E0.1TFA |
| 2 | 2,3-dibenzoyl-DL-tartaric acid | IP | 5.93 | 1.08 | 1.0 | 99A1M0.3AA0.2T |
| | | RN | 7.74 | 1.04 | 1.2 | 99.8A0.2M0.3AA0.2T |
| 3 | ketorolac | IP | 8.30 | 1.03 | 0.9 | 95H5E0.1TFA |
| | | SMP | 4.77 | 1.03 | 0.6 | 90H10E0.1TFA |
| | | RN | 4.65 | 1.03 | 0.6 | 90H10E0.1TFA |
| 4 | phenethylsulfamic acid | IP | 3.24 | 1.14 | 2.6 | 60A40M0.3AA0.2T |
| | | SMP | 3.18 | 1.13 | 0.8 | 70H30E0.1TFA |
| | | RN | 5.90 | 1.10 | 1.3 | 80A20M0.3AA0.2T |
| | | DMP | 2.03 | 1.10 | 0.6 | 70H30E/0.1TFA |
| 5 | 1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline hydrobromide | RN | 8.87 | 1.16 | 1.6 | 70H30E0.1TFA |
| | | SMP | 7.19 | 1.14 | 2.2 | 70H30E0.1TFA |
| | | IP | 2.74 | 1.11 | 1.8 | 60A40M0.3AA0.2T |
| | | DMP | 4.12 | 1.07 | 1.2 | 70H30E0.1TFA |
| | Secondary and Tertiary Amines | | | | | |
| 1 | bis-[(*R*/*S*)-1-phenylethyl]amine HCl | RN | 4.04 | 1.16 | 2.5 | 90H10E0.1TFA |
| | | SMP | 7.07 | 1.13 | 1.5 | 98H2E0.1TFA |
| 2 | bendroflumethiazide | RN | 5.50 | 1.16 | 2.3 | 70H30E0.1TFA |
| | | SMP | 4.27 | 1.03 | 0.5 | 70H30E0.1TFA |
| 3 | Tröger's base | DMP | 0.95 | 1.59 | 5.7 | 70H30E |
| | | RN | 0.96 | 1.53 | 4.2 | 70H30E |
| | | DCP | 1.25 | 1.28 | 2.0 | 80H20E |
| | | SMP | 0.94 | 1.19 | 1.5 | 80H20E |
| | | IP | 0.62 | 1.11 | 0.9 | 80H20E |
| 4 | orphenadrine citrate salt | DCP | 8.28 | 1.51 | 3.0 | 80H20E |
| 5 | diperodon hydrochloride | DCP | 2.89 | 1.21 | 1.2 | 80H20E |
| | | SMP | 10.36 | 1.06 | 0.7 | 70H30E0.1TFA |
| | Amino Acid Derivatives | | | | | |
| 1 | *N*-(3,5-dinitrobenzoyl)-DL-leucine | RN | 0.80 | 1.91 | 4.4 | 50H50E0.1TFA |
| | | DMP | 5.54 | 1.12 | 2.3 | 90H10E0.1TFA |
| | | SMP | 1.85 | 1.11 | 1.5 | 80H20E0.1TFA |
| | | DCP | 7.45 | 1.06 | 0.6 | 80H20E0.1TFA |
| 2 | *N*-(3,5-dinitrobenzoyl)-DL- phenylglycine | RN | 7.22 | 1.12 | 1.8 | 70H30E0.1TFA |
| | | DMP | 11.01 | 1.08 | 1.5 | 90H10E0.1TFA |
| | | SMP | 10.80 | 1.05 | 1.1 | 90H10E0.1TFA |
| 3 | carbobenzyloxy-alanine | SMP | 3.83 | 1.10 | 1.6 | 90H10I0.1TFA |
| | | DCP | 6.49 | 1.06 | 1.3 | 95H5E0.1TFA |
| 4 | *N*-benzoyl-DL-phenylalanine $\beta$-naphthyl ester | SMP | 4.49 | 1.19 | 3.2 | 90H10E0.1TFA |
| | | DMP | 4.00 | 1.10 | 1.7 | 90H10I0.1TFA |
| | | IP | 6.19 | 1.08 | 1.5 | 95H5E0.1TFA |
| | | RN | 10.25 | 1.06 | 0.6 | 95H5E0.1TFA |
| | | DCP | 3.63 | 1.05 | 0.7 | 90H10I0.1TFA |
| 5 | 3,5-dinitrobenzoyl-tryptophan methyl ester | RN | 3.99 | 1.17 | 2.2 | 50H50E0.1TFA |
| | | SMP | 2.76 | 1.10 | 1.5 | 70H30E0.1TFA |
| | | DMP | 5.19 | 1.09 | 1.5 | 80H20E0.1TFA |
| 6 | *N*-2,4-dinitrophenyl-DL-norleucine | DMP | 5.54 | 1.10 | 1.7 | 95H5I0.1TFA |
| | | RN | 10.91 | 1.07 | 1.3 | 95H5E0.1TFA |
| 7 | dansyl-norleucine cyclohexylammonium salt | SMP | 6.95 | 1.07 | 1.4 | 90H10E0.1TFA |
| | | DMP | 15.05 | 1.05 | 1.2 | 95H5E0.1TFA |
| | | IP | 6.93 | 1.04 | 0.8 | 90H10E0.1TFA |
| | Alcohols | | | | | |
| 1 | α-methyl-9-anthracenemethanol | RN | 5.05 | 1.08 | 1.7 | 98H2I0.1TFA |
| | | DMP | 7.61 | 1.03 | 0.7 | 99H1I0.1TFA |
| | | SMP | 5.18 | 1.02 | 0.5 | 98H2E0.1TFA |
| 2 | benzoin | DMP | 5.91 | 1.07 | 1.5 | 99H1I0.1TFA |
| | | SMP | 4.89 | 1.05 | 1.3 | 98H2E0.1TFA |
| | | RN | 6.22 | 1.04 | 0.8 | 98H2E0.1TFA |
| 3 | *N*,*N*′-dibenzyl-tartramide | DMP | 18.03 | 1.10 | 1.5 | 95H5I0.1TFA |
| | | DCP | 3.34 | 1.10 | 1.2 | 80H20E0.1TFA |
| | | RN | 11.30 | 1.06 | 1.3 | 90H10E0.1TFA |
| | | SMP | 9.26 | 1.05 | 0.8 | 90H10E0.1TFA |
| 4 | furoin | IP | 9.91 | 1.03 | 1.3 | 95H5E0.1TFA |
| | | DMP | 9.98 | 1.03 | 0.6 | 98H2E0.1TFA |
| | | SMP | 5.64 | 1.02 | 0.5 | 90H10E/0.1TFA |
| 5 | cromakalim | DMP | 16.00 | 1.05 | 1.5 | 95H5E0.1TFA |
| | | DCP | 8.69 | 1.05 | 1.1 | 90H10E0.1TFA |
| | | RN | 8.84 | 1.02 | 0.4 | 90H10E0.1TFA |
| | Others | | | | | |
| 1 | 1,1′-bi(2-naphthyl diacetate) | IP | 0.40 | 1.35 | 2.9 | 70H30E |

**Table 5. Continued**

| no. | compound name | CSP[a] | $k_1$ | α | $R_s$ | mobile phase[b] |
|---|---|---|---|---|---|---|
| | | DMP | 0.74 | 1.21 | 1.7 | 70H30E |
| | | SMP | 0.77 | 1.17 | 1.9 | 70H30E |
| | | RN | 1.79 | 1.11 | 1.6 | 90H10E0.1TFA |
| | | DCP | 1.15 | 1.08 | 1.1 | 90H10E0.1TFA |
| 2 | 5,5′,6,6′,7,7′,8,8′-octahydro(1,1′-binaphthalene)-2,2′diol | DMP | 5.47 | 1.18 | 2.8 | 99H1I0.1TFA |
| | | RN | 5.34 | 1.11 | 1.9 | 98H2E0.1TFA |
| | | SMP | 4.47 | 1.09 | 1.5 | 98H2E0.1TFA |
| 3 | 2,2′-diamino-1,1′-binaphthalene | DMP | 1.43 | 1.45 | 5.3 | 70H30E |
| | | DCP | 1.77 | 1.22 | 2.0 | 80H20E |
| | | SMP | 1.91 | 1.20 | 3.1 | 70H30E |
| | | RN | 2.31 | 1.18 | 2.8 | 70H30E |
| | | IP | 2.15 | 1.16 | 3.0 | 80H20E |
| 4 | 6,6′-dibromo-1,1′-bi-2-naphthol | DMP | 0.74 | 1.58 | 4.7 | 70H30E |
| | | DCP | 2.13 | 1.24 | 5.0 | 90H10E |
| | | SMP | 0.93 | 1.19 | 2.1 | 70H30E |
| | | RN | 1.17 | 1.15 | 1.5 | 70H30E |
| | | IP | 3.63 | 1.07 | 1.5 | 90H10E |
| 5 | althiazide | RN | 2.83 | 1.16 | 1.9 | 50H50E0.1TFA |
| | | DMP | 8.80 | 1.04 | 0.7 | 80H20E0.1TFA |
| | | IP | 8.29 | 1.02 | 0.8 | 70H30E0.1TFA |
| | | SMP | 7.10 | 1.02 | 0.5 | 70H30E/0.1TFA |
| 6 | 1,1′-bi-2-naphthol bis(trifluoromethanesulfonate) | SMP | 3.84 | 1.17 | 2.0 | 100H |
| | | IP | 1.17 | 1.08 | 1.5 | 100H |
| | | RN | 6.13 | 1.08 | 1.3 | 100H |
| 7 | *cis*-4,5-diphenyl-2-oxazolidinone | DMP | 5.41 | 1.09 | 1.6 | 90H10I0.1TFA |
| | | RN | 6.82 | 1.04 | 0.8 | 90H10I0.1TFA |
| 8 | 2,3-dihydro-7a-methyl-3-phenylpyrrolo[2,1-b]oxazol-5(7aH)-one | RN | 3.20 | 1.12 | 1.9 | 85H15I0.1TFA |
| | | DMP | 5.13 | 1.05 | 1.1 | 98H2E0.1TFA |
| | | SMP | 2.46 | 1.03 | 0.7 | 98H2E0.1TFA |
| | | DCP | 2.67 | 1.02 | 0.6 | 90H10E0.1TFA |
| 9 | ethyl 11-cyano-9,10-dihydro-endo-9,10-ethanoanthracene-11-carboxylate | SMP | 1.44 | 1.13 | 2.0 | 90H10I0.1TFA |
| | | RN | 3.21 | 1.08 | 1.5 | 98H2E0.1TFA |
| | | IP | 1.42 | 1.08 | 1.5 | 95H5E0.1TFA |
| | | DMP | 2.07 | 1.03 | 0.5 | 98H2E0.1TFA |
| 10 | lormetazepam | SMP | 3.87 | 1.08 | 1.5 | 80H20E0.1TFA |
| | | IP | 8.46 | 1.06 | 1.5 | 90H10E0.1TFA |
| | | RN | 5.02 | 1.04 | 0.7 | 80H20E0.1TFA |
| 11 | 3a,4,5,6-tetrahydro-succininido[3,4-b]acenaphthen-10-one | SMP | 2.36 | 1.13 | 2.2 | 70H30E0.1TFA |
| | | IP | 2.14 | 1.12 | 1.9 | 70H30E0.1TFA |
| | | RN | 3.25 | 1.10 | 1.5 | 70H30E0.1TFA |
| | | DMP | 7.35 | 1.08 | 1.5 | 90H10E0.1TFA |
| 12 | 3-(α-acetonyl-4-chlorobenzyl)-4-hydroxycoumarin | DMP | 5.35 | 1.17 | 2.0 | 90H10I0.1TFA |
| | | SMP | 5.16 | 1.14 | 2.2 | 90H10E0.1TFA |
| | | RN | 15.94 | 1.10 | 1.6 | 95H5E0.1TFA |
| | | DCP | 4.65 | 1.10 | 1.4 | 90H10E0.1TFA |
| | | IP | 4.61 | 1.09 | 1.5 | 90H10E0.1TFA |
| 13 | warfarin | DMP | 9.87 | 1.10 | 1.6 | 95H5I0.1TFA |
| | | SMP | 7.19 | 1.08 | 0.9 | 90H10I0.1TFA |
| | | DCP | 4.98 | 1.07 | 0.8 | 90H10E0.1TFA |
| | | RN | 11.94 | 1.05 | 1.2 | 95H5E0.1TFA |
| | | IP | 4.60 | 1.02 | 0.5 | 90H10E0.1TFA |
| 14 | fipronil | SMP | 16.03 | 1.09 | 2.0 | 98H2E0.1TFA |
| | | IP | 2.83 | 1.08 | 1.5 | 90H10E0.1TFA |
| | | RN | 12.02 | 1.07 | 1.5 | 97H3E0.1TFA |
| | | DCP | 0.55 | 1.03 | 0.4 | 80H20E |
| 15 | *trans*-stilbene oxide | IP | 0.62 | 1.10 | 1.3 | 100H |
| | | SMP | 2.02 | 1.09 | 1.5 | 100HEP |
| | | RN | 2.75 | 1.07 | 1.0 | 100HEP |
| 16 | thalidomide | SMP | 5.89 | 1.10 | 1.9 | 70H30E0.1TFA |
| | | DMP | 8.03 | 1.08 | 1.5 | 80H20E0.1TFA |
| | | DCP | 10.69 | 1.05 | 1.0 | 80H20E |
| | | RN | 7.85 | 1.04 | 0.7 | 70H30E0.1TFA |
| 17 | 3,5-dinitrobenzoyl-2-aminoheptane | RN | 1.68 | 1.15 | 2.2 | 80H20E0.1TFA |
| 18 | 3,5-dinitro-*N*-(1-phenylethyl)-benzamide | RN | 1.24 | 1.92 | 8.2 | 50H50E0.1TFA |
| | | SMP | 0.93 | 1.14 | 1.5 | 70H30E0.1TFA |

[a] Abbreviations of CSPs: DMP, dimethylphenyl carbamate-CF6 CSP; DCP, dichlorophenyl carbamate-CF6 CSP; RN, *R*-naphthylethyl carbamate-CF6 CSP; SMP, *S*-methylbenzyl carbamate-CF6 CSP; IP, isopropyl carbamate-CF6 CSP; ME, methyl carbamate-CF6 CSP. [b] Abbreviations of mobile phases: H, heptane; I, isopropanol; E, ethanol; A, acetonitrile; M, methanol; AA, acetic acid; T, triethylamine; TFA, trifluoroacetic acid.

racemic primary amines are synthetic chiral crown ether-based stationary phases.[53−57] However, their applications are intrinsically restricted to primary amines, with the partial exception of 18-crown-6 tetracarboxylic acid. Furthermore, strong acidic, aqueous mobile phases are always necessary to protonate and separate these primary amines. The derivatized CF6-based CSPs differ

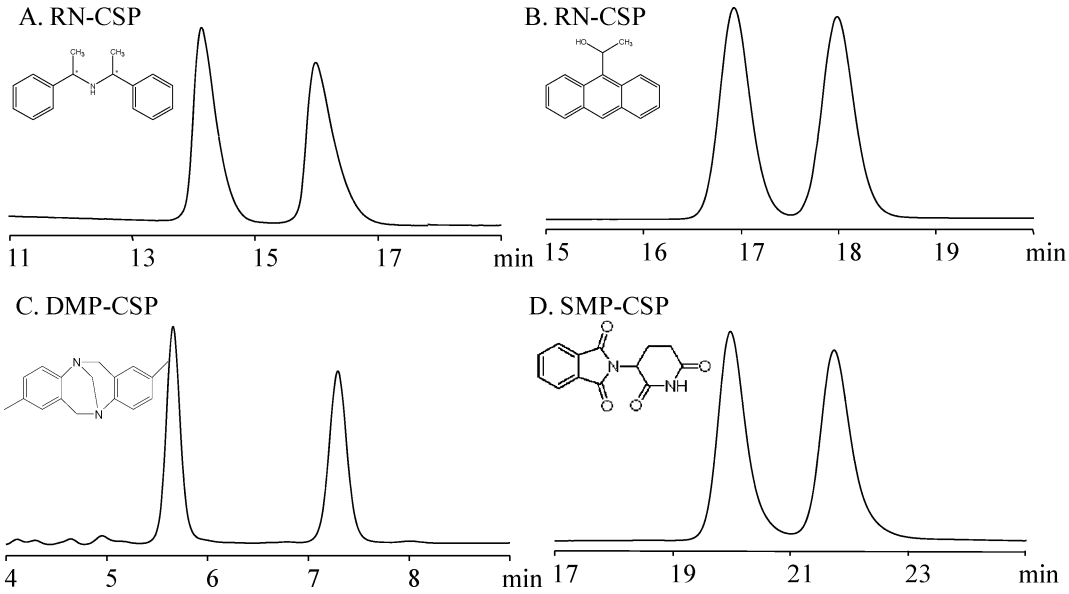



**Figure 5.** Selected chromatograms showing enantioseparations of various analytes on different derivatized-CF6 columns. The analytes and mobile phases are (A) bis-[(*R*/*S*)-1-phenylethyl]amine, 90H10E0.1TFA; (B) α-methyl-9-anthracenemethanol, 98heptane/2IPA/0.1TFA; (C) Tröger's base, 70heptane/30ETOH; (D) thalidomide, 70heptane/30ETOH/0.1TFA.

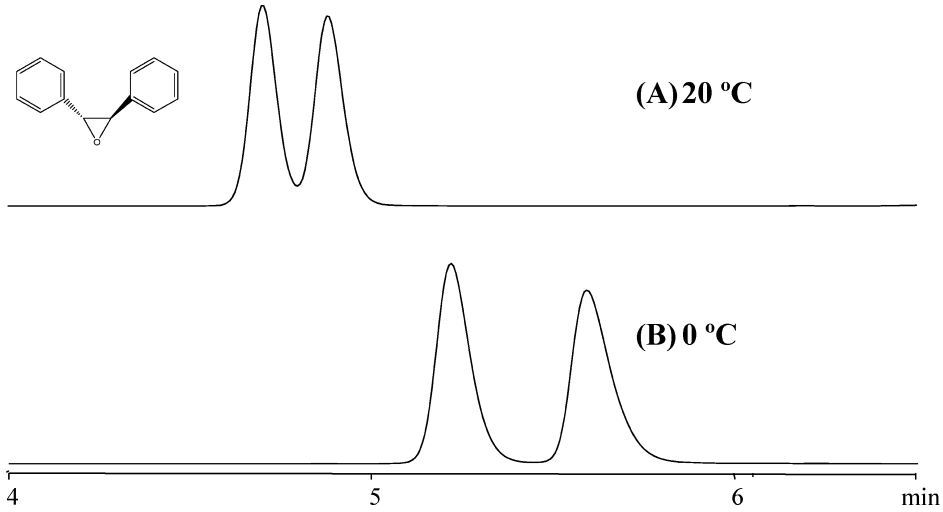



**Figure 6.** Temperature effect on separation of *trans*-stilbene oxide on the IP-CF6 column. The mobile phase is 100% heptane. The chromatographic data: (A) $k_1 = 0.62$, $\alpha = 1.10$, $R_s = 1.3$; (B) $k_1 = 0.80$, $\alpha = 1.16$, $R_s = 2.0$.

significantly from all other crown ether-based ones, in that they work best in polar organic and normal phase solvents for separating primary amines, and their applications are not always limited to primary amines.

**Cyclofructan CSPs Based on Aromatic-Functionalized CF6.** A total of 10 different CSPs were made which consisted of silica bonded cyclofructan, highly functionalized with 10 different aromatic moieties (See Figure 2). Their respective chromatographic performances were evaluated by injecting all 70 probe molecules (nonprimary amine types). Among these 10 aromatic derivatized-CF6 CSPs, four CSPs appeared to produce superior results. They are 3,5-dimethylphenyl (DMP), 3,5-dichlorophenyl (DCP), *R*-1-(1-naphthyl)ethyl (RN), and *S*-α-methylbenzyl (SMP). The chromatographic data of the 70 probe analytes on these four columns are listed in Table 5. If one analyte is separated on more than one column, the results are listed in descending order of selectivity. If the IP-functionalized CSP showed an enantioseparation, the data also was included for comparison purposes. Chromatograms of four representative separations under optimized conditions are shown in Figure 5. In summary, 40 analytes out of 70 were separated, including 35 baseline ($R_s \geq 1.5$) and 5 partial resolution ($0.3 < R_s < 1.5$). Table 5 clearly demonstrates that the aromatic derivatized-CF6 columns show excellent enantioselectivity toward various types of analytes, including acids, secondary amines, tertiary amines, alcohols, and others. Although they are not universally effective for all of the tested enantiomers,

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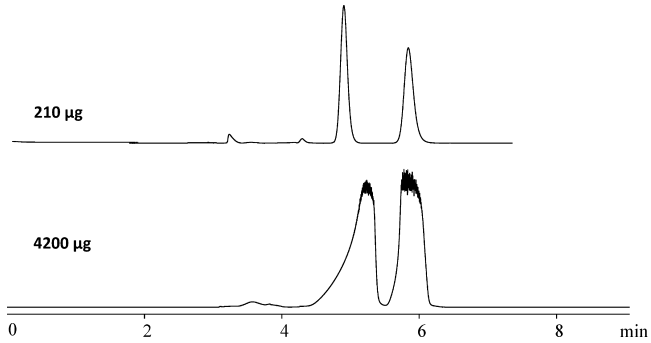


**Figure 7.** Loading test on the RN-CF6 column. The analyte is *N*-(3,5-dinitrobenzoyl)-DL-phenylglycine. The mobile phase is 85ACN/15MEOH/0.3AA/0.2TEA. Injection volumes are 5 (top) and 100 $\mu$L (bottom). UV detection: 350 nm.

the variety of compounds separated by derivatized-CF6 was encouraging.

Table 5 clearly demonstrates that the nature of the aromatic group plays a major role in chiral recognition. The aromatic-functionalized CF6 CSPs demonstrate complementary capability of enantiomeric selectivities, in that some analytes were baseline separated on one column, while only a partial separation or no separation was observed on other aromatic functionalized CF6 columns. For example, 3,5-dinitro-*N*-(1-phenylethyl)benzamide which was well separated ($R_s = 8.2$) on the RN-CF6 column with an extremely high selectivity ($\alpha = 1.92$) and also baseline separated ($\alpha = 1.14$, $R_s = 1.5$) on the SMP column (Figure S4 in the Supporting Information). However, a single peak was observed using the DMP-CF6 and DCP-CF6 columns. The stereogenic center of 3,5-dinitro-*N*-(1-phenylethyl)benzamide is directly attached to a phenyl ring, which participates in $\pi-\pi$ interactions with the aromatic moieties of the derivatized CF6. Compared to DMP, both RN and SMP contain a stereogenic center, directly connected to the aromatic ring (phenyl or naphthyl), which may be beneficial for chiral recognition of this analyte.

One advantage for all CF6-based CSPs is their excellent stability. These columns are stable in all common organic solvents, and no detrimental changes in column performance was observed after more than 1000 injections. The selection of the mobile phase is an important parameter in enantioselective chromatography. Therefore, it was necessary to compare all three operation modes (i.e., the normal phase mode, polar organic mode, and the reversed phase mode) when evaluating these CF6-based columns. Further, the effects of other experimental factors, such as alcohol modifier type in the normal phase mode and the column temperature on the separations were studied. Such fundamental information offers guidance for methods development.

For neutral and acidic compounds, better resolution was typically obtained in the normal phase mode. 6,6′-Dibromo-1,1′-bi-2-naphthol is baseline separated ($\alpha = 1.58$, $R_s = 4.7$) using 70% heptane/30% ethanol, while only a tiny peak split ($\alpha = 1.03$, $R_s = 0.6$) was observed using the reversed phase mode (Figure S5 in the Supporting Information). With the polar organic mobile phase, no retention of this analyte was obtained. Water in the reversed phase system may compete too effectively for hydrogen bonding sites on the chiral stationary phase, and thus it has a negative effect on the separation of enantiomeric compounds. It is well-known that certain interactions are enhanced in less polar solvents.[23,49,58,59] Specifically these are $\pi-\pi$, $n-\pi$, dipolar, and hydrogen bonding interactions. Since these CSPs are effective mainly in the normal phase mode and polar organic mode, these must be the dominant associative interactions, while steric repulsion is important in all solvent systems.

In the normal phase mode, ethanol and isopropanol are commonly used as alcohol modifiers. Ordinarily, the ethanol modifier yields good enantioselectivity, better peak efficiency, and faster elution; therefore, it was chosen as the primary polar organic modifier. Isopropanol also was tested for further optimization because in some cases higher enantioselectivity is observed using isopropanol rather than ethanol. The value of $\alpha$ for the enantioseparation of *N*-benzoyl-DL-phenylalanine $\beta$-naphthyl ester is improved from 1.05 to 1.10, when replacing ethanol with isopropanol.

At lower temperatures, enantioselectivity usually increases, at the expense of efficiency. Figure 6 illustrates that reducing the column temperature from 20 to 0 °C significantly improves enantioselectivity and resolution. Therefore, lowering the column temperature is another strategy to optimize enantioseparations with these CSPs, as it is with most other CSPs.

In order to assess the potential for preparative HPLC, it is necessary to perform loading tests on any new stationary phases. Sample loading was examined by injecting *N*-(3,5-dinitrobenzoyl)-phenylglycine on the RN-CF6 column in the polar organic mode. A total of 4200 $\mu$g of this racemate has been baseline separated on an analytical column (shown in Figure 7). It should be noted that the injection amount was limited by the solubility of the analyte in the mobile phase. It is clear that more sample could be loaded while maintaining baseline resolution. These brush-type CSPs based on derivatized-CF6 demonstrate great potential for

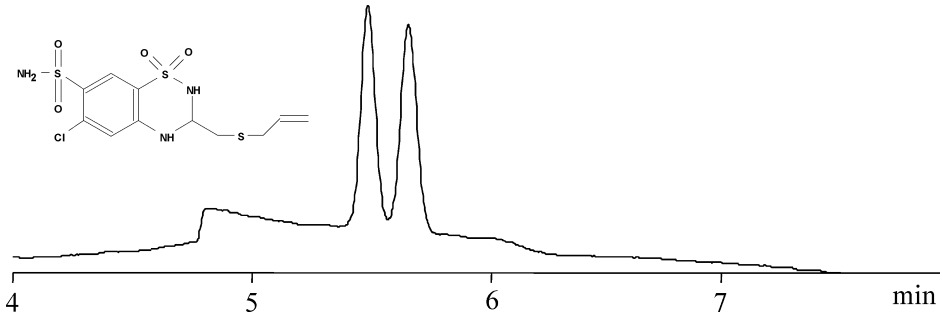


**Figure 8.** SFC chromatogram of althiazide on the RN-CF6 column. The gradient mobile phase is as described in the Experimental Section.

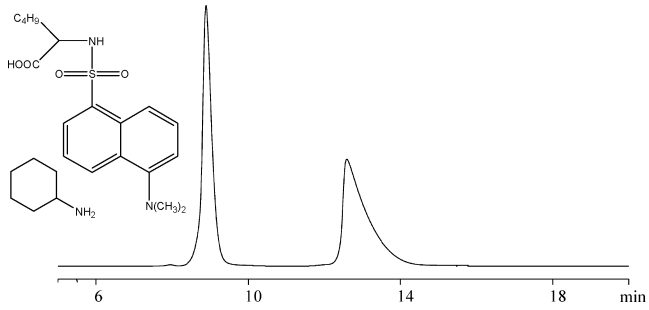


**Figure 9.** Separation of dansyl-norleucine cyclohexylammonium salt on the dimethylphenyl carbamate-CF7 CSP. The mobile phase is 80heptane/20ETOH/0.1TFA.

preparative HPLC. In addition, comprehensive SFC investigations of these cyclofructan-based CSPs are under way, and a representative chromatogram is shown in Figure 8.

Most recently, we have begun to study the cyclofructan containing seven units (CF7) as a chiral selector. Preliminary studies show that the derivatized-CF7 CSP also successfully separates various types of analytes and an initial result is shown in Figure 9. It is worth mentioning that this analyte was only partially separated by all derivatized-CF6 columns. This demonstrates that the dimethylphenyl carbamate-CF7 likely has somewhat different enantioselectivities. This work is ongoing and will be presented in a subsequent publication.

## CONCLUSIONS

Cyclofructans are a completely new class of chiral selectors. While native cyclofructan 6 has limited capabilities as a chiral selector, specific, derivatized cyclofructans appear to be exceptional chiral selectors which can be "tuned" to separate enantiomers of different types of molecules. Partial derivatization of the cyclofructan hydroxyl groups appears to disrupt internal hydrogen bonding and "relax" the structure. When "lightly" derivatized with aliphatic functionalities, CF6 becomes exceptionally adept at separating enantiomers of primary amines and does so in organic solvents and supercritical $CO_2$. This is in contrast to all known chiral crown ether CSPs that work exclusively with aqueous acidic solvents. When CF6 is extensively functionalized with aromatic moieties, it no longer effectively separates primary amine racemates. However, it does separate a broad variety of other enantiomers. Furthermore, its preparative separation capabilities appear to be exceptional, and this indicates that it is likely that multiple analytes can associate simultaneously with a single chiral selector. While this class of chiral selectors is in its infancy, it is clear that they will be further developed and play an important role in future enantiomeric separations.

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## SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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