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BROAD SPECTRUM RESOLUTION OF OPTICAL ISOMERS USING CHIRAL HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC BONDED PHASES*

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SUMMARY

Using a chiral recognition rationale, a chiral fluoroalcoholic bonded stationary phase has been devised which proves capable of separating the enantiomers of a large number of solutes including sulfoxides, lactones, and derivatives of alcohols, amines, amino acids, hydroxy acids, and mercaptans.

Chiral recognition being a reciprocal event, chiral stationary phases modeled after solutes resolvable upon the fluoroalcoholic columns successfully separate the enantiomers of a number of fluoroalcohols.

INTRODUCTION

The development of chiral stationary phases (CSP) for the direct chromatographic separation of optical isomers is a problem that has been addressed by many researchers. Despite impressive but scattered successes, no CSP has been devised to suffice for the separation of more than a relatively narrow range of structurally related compounds. To date, most liquid chromatographers have confined their efforts to amino acids[1–4], helicenes[5–7], and di- and triphenic acids[8].

In this paper, we set forth a chiral recognition rationale that can be used to design CSP for the chromatographic separation of an assortment of optical isomers. Several successful applications of this rationale are presented.

RATIONALE

It should be generally appreciated that a minimum of three reference points are needed to distinguish the handedness of a chiral object. In molecular terms, this means that a CSP, in order to interact preferentially with one solute enantiomer, must undergo a minimum of three simultaneous interactions with that enantiomer.

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Nuclear magnetic resonance (NMR) studies of the interactions of chiral fluoroalcohols such as I with a wide variety of solutes have shown that, whenever possible, the fluoroalcohol employs a two-point chelate-like mode of solvation[9–20]. The principal interaction is hydrogen bonding; the secondary interaction is carbinyl hydrogen bonding[20]. Many commonly occurring structural subunits suffice as interaction sites as indicated in Fig. 1.

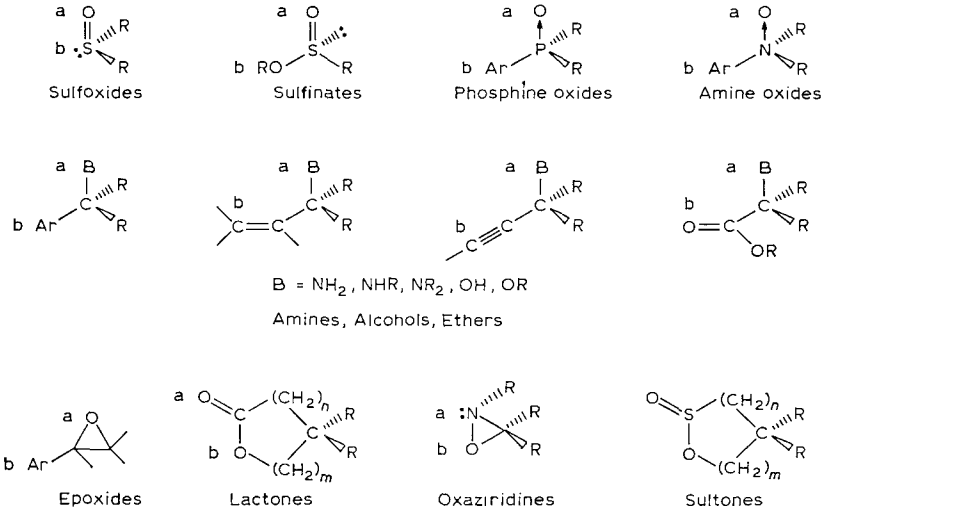
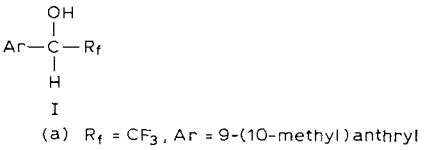


Fig. 1. A representative sampling of solutes known to give chelate-like solvates upon solvation by type I fluoroalcohols. The site of primary hydrogen bonding is designated as a; the site of secondary interaction is designated as b.

Interaction of chiral type I fluoroalcohols with enantiomeric solutes gives rise to diastereomeric solvates, the chelate-like diasteromeric solvates of present interest being represented in a general way as IIa and b. Although diastereomeric, these solvates differ significantly in stability only if the alcohol substituents, R_f and W, interact, either directly or indirectly, with the remaining solute substituents, Y and Z.



I

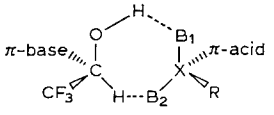
(a) $R_f = CF_3$, Ar = 9-(10-methyl)anthryl

(IIa) (IIb)

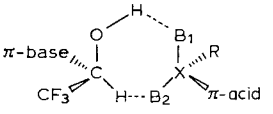
X = C, N, P, S
B_1 = hydrogen bond receptor
B_2 = less basic (than B_1) site

In order to incorporate the widespread chelating ability of type I fluoroalcohols into a chiral stationary phase that would truly have a greater affinity for one solute enantiomer than the other, it was evident that the alcohol substituents, R_f and W, would have to interact with and distinguish between Z and Y. Since the principal function of the simple perfluoroalkyl substituent, R_f, is to strengthen the primary and secondary interactions by conferring acidity upon the hydroxyl and carbinyl hydrogens, the major role in differentiating between Y and Z was expected to fall to the remaining alcohol substituent W. To differentiate between Y and Z, W must take advantage of some structural difference such as size, charge distribution, additional hydrogen bonding sites, or hydrophobic interactions. Since the ease of enantiomer separation stems from the magnitude of the stability difference of the diastereomeric solvates, the strength of the stereochemically dependent third interaction should be appreciable. (Future chiral stationary phases might contain modified "R_f" substituents so as to lead to a "push-pull" situation involving a fourth stereochemically dependent interaction to further augment chiral recognition.)

In our first chiral stationary phase, we have chosen to use π–π donor–acceptor interactions as the stereochemically dependent third interaction. Prior work[21,22] had shown that the diastereomeric solvates derived from 2,2,2-trifluoro-1-[(10-methyl)-9-anthryl]ethanol, Ia, and the enantiomers of methyl 2,4-dinitrophenyl sulfoxide were of different stability owing to the stereochemical dependence of the interaction between the electron-rich anthryl group and the electron-deficient dinitrophenyl substituent. A general representation of these diastereomers is shown in IIIa and b and provides a basis for anticipating the selective interaction of a CSP derived from Ia with a number of π-acid substituted chelate-forming solutes.



III a



III b

X = C, N, S, P
B_1 = hydrogen bond receptor
B_2 = carbinyl hydrogen bond receptor

EXPERIMENTAL

Chromatography was performed using an Altex 100A pump, a Valco 7000 p.s.i. injector with a 10-μl loop and an Altex Model 152 dual wavelength (254 and 280 nm) detector. Columns were slurry packed using conventional methods. Solutes were available from prior studies, prepared from commercially available materials, or generously provided by colleagues throughout the world.

Fluoroalcoholic stationary phase IV

The preparation of this CSP has been described[23].

Second-generation chiral stationary phases XIII and XIV

Owing to the preliminary nature of this work and the number of bonded phases prepared, we have initially employed an inexpensive silica gel (Ventron 58 μm,

large pore, 600 m^2/g) ball-milled to a powder. While the theoretical plate count of columns so derived is far from "state of the art", these columns adequately serve the intended purpose. More efficient columns based upon 5-μm spherical particles of silica gel will be prepared ultimately.

Aminopropyl functionalized silica. A slurry of 50 g of ball-milled silica in 500 ml of toluene was heated to reflux until water was no longer removed azeotropically (Dean-Stark trap). Thereupon, 100 g of γ-aminopropyltriethoxysilane was added and solvent was slowly and intermittently distilled until the infrared spectrum of the distillate no longer (18–36 h) showed hydroxy adsorption. The silica was isolated by filtration and washed repeatedly with toluene, methanol, ether and finally pentane. After drying, appreciable weight gain (*ca.* 40%) is noted. Anal. found: C, 10.91; H, 2.53; N, 2.78; Si, 39.28%.

3,5-Dinitrobenzoylphenylglycine. A mixture of 50 g of D-(—)-phenylglycine and 72 g of 3,5-dinitrobenzoyl chloride in 600 ml of dry tetrahydrofuran (THF) was stirred for one week at room temperature. The THF was removed under vacuum, the residue was dissolved in 5% aqueous sodium bicarbonate, and this solution was washed with two (100-ml) portions of ether. The aqueous extract was acidified to a pH of 5.3 and continuously extracted with ether until material was no longer being extracted into the ether. The ether extracts were dried over anhydrous $MgSO_4$ and the ether was removed under vacuum to afford 57.72 g of colorless crystalline 3,5-dinitrobenzoylphenylglycine (54%). The enantiomeric purity of this material was determined by chromatography upon CSP IV (Table IV) to be 96%. Recrystallization (methanol)* can be used to increase enantiomeric purity. The enantiomerically pure material has a m.p. of 211–213°.

NMR: (d_6 Acetone) δ 5.81 (d, 1 H), δ 7.30–7.65 (m, 5 H), δ 8.90–9.20 (m, 4 H). Infrared (IR): (KBr disc) 3400–3085, 1733, 1652, 1580, 1345, 1218, 1190, 1080, 920, 732, 722 cm^{-1}. Anal. Calculated for $C_{15}H_{11}N_3O_7$: C, 52.18; H, 3.21; N, 12.17. Found: C, 52.12; H, 3.24; N, 12.20.

Chiral stationary phase XIII. A solution of 10 g of D-3,5-dinitrobenzoylphenylglycine in 200 ml of dry THF was poured over 10 g of dry aminopropyl silica gel and 7.9 g of N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) was added with swirling. After 8 h at room temperature, the silica was isolated by filtration and washed repeatedly with methanol, acetone and ether. These last washings employed centrifugation–decantation and some fines were thus removed. After drying, *ca.* 12 g of the silica bonded phase was obtained.

Anal. Calculated: 0.51 mmoles/g (based on C). Found: C, 14.64; H, 2.28; N, 3.09; Si, 31.39%.

It is at this last stage that partial racemization might occur. Use of *n*-butylamine as a trapping nucleophile leads to the nonracemized amide. However, racemization and trapping would be competitive reactions and the aminopropyl silica could be less reactive than *n*-butylamine.

After being packed, the column was washed with 10 g of trifluoroacetic acid in 150 ml of methylene chloride to protonate remaining free aminopropyl groups.

N-(3,5-Dinitrobenzoyl)phenylglycinol. To a solution of 16.72 g of D-phenylglycinol (122 mmoles) and 14.50 g of pyridine (183 mmoles) in 250 ml of methylene

* This solvent can lead to esterification upon heating or long standing.

chloride was slowly added 28.10 g of 3,5-dinitrobenzoyl chloride (122 mmoles). The mixture was heated at reflux for 4 h. Acetonitrile ($\approx$ 250 ml) was added to dissolve the solid product and the organic phase was washed with 5% hydrochloric acid (2 $\times$ 100 ml) and 3 *N* sodium hydroxide solution (4 $\times$ 200 ml). After the organic phase was dried over anhydrous $MgSO_4$, the solvents were removed under vacuum to afford 31.67 g (78%) of a pale yellow solid (m.p. 231–234°). The enantiomeric purity of this material can be ascertained by chromatography upon CSP IV.

NMR: (d_6 Acetone) δ 2.8 (s, 1 H), δ 3.8 (d, 2 H), δ 5.2 (t, 1 H), δ 7.1–7.5 (m, 5 H), δ 8.7 (s, 1 H), δ 9.0–9.1 (m, 3 H). IR: (Nujol) 3500, 1660, 1540, 1470, 1350, 1260, 1080, 1020, 920 cm^{-1}.

N-(3,5-Dinitrobenzoyl)phenylglycinol chloroformate. To a solution of phosgene (9.5 g, 96 mmoles) in 150 ml of methylene chloride cooled to $-5°$ was added dropwise a solution of 14.23 g of N-(3,5-dinitrobenzoyl)phenylglycinol (43 mmoles) and 3.4 g of pyridine (43 mmoles) in 300 ml of methylene chloride. After the addition was completed, stirring was continued for 30 min at 0°. The solution was filtered under nitrogen and the solvent was removed under vacuum to give the crude chloroformate as an orange syrup.

NMR: ($CDCl_3$) δ 5.0 (d, 2 H), δ 5.4 (t, 1 H), δ 7.1–7.5 (m, 5 H), δ 8.7 (t, 1 H), δ 9.0–9.1 (m, 3 H).

The crude chloroformate was not otherwise characterized but was used immediately.

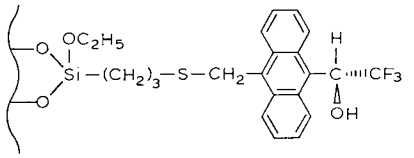
Chiral stationary phase XIV. To a stirred mixture of aminopropyl silica (12.45 g) and triethylamine (2.52 g, 25 mmoles) in 50 ml of methylene chloride was added the crude chloroformate from the above reaction. The mixture was stirred at room temperature for two days. The solution was filtered and the silica washed with methylene chloride, methanol, acetone, ether, and pentane.

Anal. Found: C, 14.82%, H, 2.16%, N, 3.73%; Si, 32.67%. Calcd: 0.37 mmoles per g (based on C).

After being packed, the column was treated with 10 g of trifluoroacetic acid in 100 ml of methylene chloride to protonate any residual aminopropyl groups.

RESULTS

Chiral stationary phase IV, derived from R-Ia, has shown an impressive ability to separate the enantiomers of solutes falling into the purview of its chiral recognition scheme*. First, it separates the enantiomers of a wide range of π-acid



IV

substituted sulfoxides (Fig. 2, Table I). In general, the ability of CSP IV to separate sulfoxide enantiomers correlates with the π-acidity of the sulfoxide substituent and is

* Experimental details for the preparation of IV and a sampling of the enantiomer separations achieved thereon are being reported elsewhere[23].

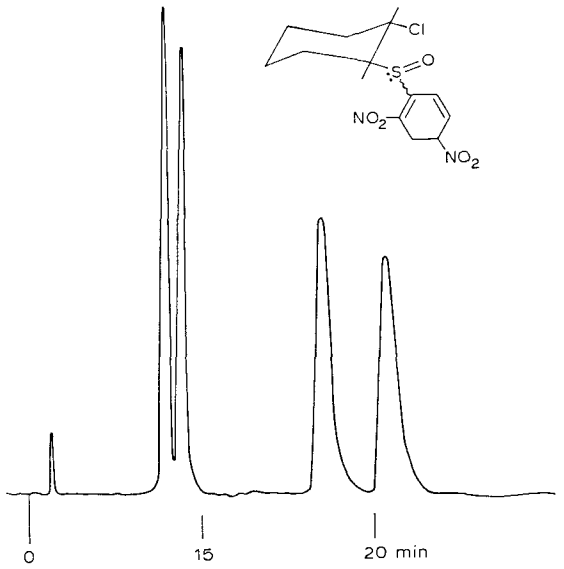
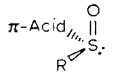


Fig. 2. Separation of the four stereoisomers (two racemic diastereomers) of *trans*-1-chloro-2-(2,4-dinitrophenylsulfinyl)cyclohexane upon chiral stationary phase IV using 5% isopropyl alcohol in hexane.

TABLE I

SEPARATION OF SULFOXIDE ENANTIOMERS UPON CHIRAL STATIONARY PHASE IV

Eluent was 20% isopropyl alcohol in hexane. Stationary phase IV was bonded onto 10 μm Porasil and slurry packed into a 9 × 250 mm column. The absolute configuration of the initially eluted sulfoxide enantiomer is presumed in all cases to be that shown above. The elution orders of the first and twelfth entries in this table are as indicated and C.D. spectroscopy shows the elution order of entries two to six to be the same as that of one. The absolute configurations of the remaining sulfoxides have not been assigned previously.



R	*π-Acid*	α	k_1'
CH_3	2,4-$(NO_2)_2C_6H_3$	1.12	13.05
n-Butyl	2,4-$(NO_2)_2C_6H_3$	1.19	6.32
n-Octyl	2,4-$(NO_2)_2C_6H_3$	1.24	4.44
n-Dodecyl	2,4-$(NO_2)_2C_6H_3$	1.26	3.33
n-Octadecyl	2,4-$(NO_2)_2C_6H_3$	1.28	2.44
Phenyl	2,4-$(NO_2)_2C_6H_3$	1.23	6.82
Benzyl	2,4-$(NO_2)_2C_6H_3$	1.22	7.70
n-Dodecyl	4-NO_2-C_6H_4	1.09	2.78
Phenyl	4-NO_2-C_6H_4	1.05	6.95
n-Dodecyl	2,4-$(NO_2)_2$-C_6H_3-CH_2	1.38	6.39
n-Dodecyl	C_6F_5	1.09	0.51
n-Dodecyl	4-ClC_6H_4	1.02	1.26
CH_3	C_6Cl_5	1.04	2.51

but slightly dependent upon the remaining "inert" (which means that additional functionality which would interfere with the primary or secondary interactions is lacking) sulfoxide substituent. [It is not yet known whether the observed modest variation in α values stems from variations in chiral recognition (higher order interactions) or contributions from additional retention mechanisms that do not involve chiral recognition. The presence of free silanol groups could well give rise to such additional retention.] Elution orders are those expected from the rationale[23].

Chiral stationary phase IV also separates the enantiomers of the π-acid bearing lactone V (Fig. 3).

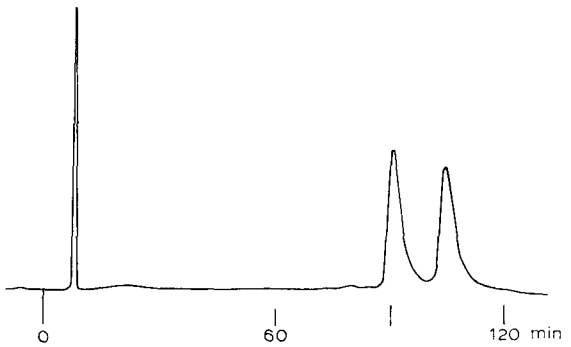
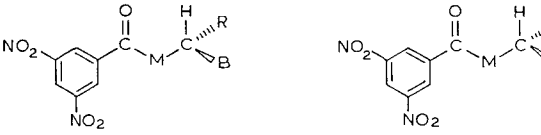


Fig. 3. Separation of the enantiomers of γ-2,4-dinitrophenyl-γ-butyrolactone upon chiral stationary phase IV using 20% isopropyl alcohol in hexane.

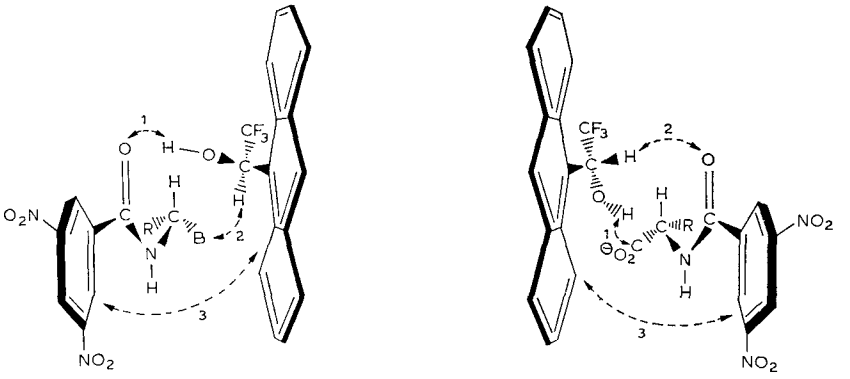
Solutes lacking π-acid substituents can often be derivatized to incorporate such functionality so as to markedly increase the scope of CSP IV. Amines, alcohols, and thiols react readily with 2,4-dinitrofluorobenzene or 3,5-dinitrobenzoyl chloride. The latter has proven to be generally useful in leading to derivatives resolvable upon CSP IV. (The 3,5-dinitrobenzoyl moiety is both a π-acid and a basic site suitable for either the primary or secondary interactions. It is also a strongly absorbing ultraviolet chromaphore that facilitates detection. Moreover, this group can generally be removed hydrolytically if the original solute enantiomer is to be recovered after resolution. An additional advantage, shared by all achiral derivatizing agents, is that no optical fractionation occurs during reaction with a solute, the enantiomeric purity of which is to be determined chromatographically after derivatization.)

The 3,5-dinitrobenzoyl (DNB) derivatives of a number of primary amines, alcohols and mercaptans are represented by generalized structures VI–VIII. These derivatives preferentially populate conformations that place the DNB carbonyl oxygen near the methine hydrogen (presumably, for reasons of carbinyl hydrogen bonding)[20]. This conformational preference, greater in amides than in esters, appears to influence the chromatographic behavior of DNB derivatives upon CSP IV. Both the DNB carbonyl oxygen and group B can serve as basic sites to afford transient chelate-like solvates with CSP IV. If the DNB carbonyl oxygen is more basic than group B, the primary and secondary interactions occur as shown in solvate IX. If group B is more basic than the DNB carbonyl oxygen, a solvate such as X is preferentially afforded. The consequence of chelate-like solvation of derivatives VI–VIII by CSP IV is that, for the solvate diastereomers shown in IX–X, the π-acid moiety is,

using the chelate ring for reference, *cis* to the π-acceptor moiety and a π–π tertiary interaction can occur. In the other diastereomers, the π-acid and π-base groups are *trans* to one another, rendering impossible the tertiary interaction. Once again, a stereochemically dependent π–π donor–acceptor interaction leads to enantiomer separation and predictable elution orders so long as one correctly assesses the relative basicities of the DNB carbonyl oxygen and B. Note that a basicity inversion inverts the elution order expected for a given absolute configuration*.



VI M = NH
VII M = O B = basic site
VIII M = S



IX X

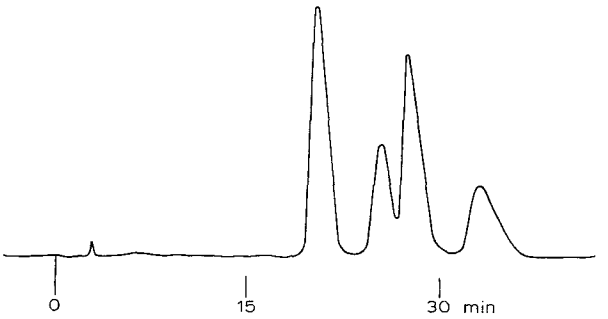
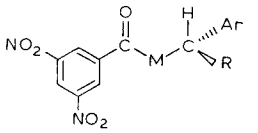


Fig. 4. Separation of the enantiomers of the racemic *trans* and *cis* diastereomers of 2-phenyl-1-aminocyclohexane upon chiral stationary phase IV using 5% isopropyl alcohol in hexane.

* The astute reader will realize that even in situations where there is a clear basicity difference between B_1 and B_2, the least stable solvate (*i.e.*, the one expected to have but two simultaneous interactions) can in principle have three simultaneous interactions by adopting the less favored chelation mode. What is experimentally observed, of course, is a weighted time average of all possible interaction modes.

TABLE II

SEPARATION OF THE ENANTIOMERS OF 3,5-DINITROBENZOYL DERIVATIVES OF AMINES, ALCOHOLS AND THIOLS UPON CHIRAL STATIONARY PHASE IV



Solvent was 20% isopropyl alcohol in hexane. The elution order of each starred* solute was determined by chromatography of a partially resolved configurationally established sample. In every instance, the configuration of the initially eluted enantiomer proved to be that shown above. The presumption is that the elution orders of the remaining solutes also follow this pattern.

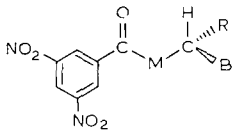
R	*Ar*	*M*	α	k_1'
CH_3	Phenyl	NH	1.19*	3.01
CH_3	*p*-Anisyl	NH	1.18*	5.19
CH_3	*p*-$CF_3C_6H_4$	NH	1.11	2.03
CH_3	α-Thienyl	NH	1.14*	3.04
CH_3	α-Naphthyl	NH	1.10*	3.53
CH_3	*p*-Biphenyl	NH	1.17	3.64
C_2H_5	*p*-Biphenyl	NH	1.21	3.09
C_2H_5	Phenyl	NH	1.29*	2.63
iso-C_3H_7	Phenyl	NH	1.26*	2.29
n-C_3H_7	4-$CH_3OC_6H_4$	NH	1.30	3.49
n-C_3H_7	4-$C_3H_7OC_6H_4$	NH	1.34	2.35
$CH_3O_2C(CH_2)_2$	Phenyl	NH	1.18	6.33
CH_3	Phenyl	O	1.08*	1.56
C_2H_5	Phenyl	O	1.10	1.23
n-C_3H_7	Phenyl	O	1.12	1.09
iso-C_3H_7	Phenyl	O	1.14	0.81
Cyclopropyl	Phenyl	O	1.09	1.32
tert.-C_4H_9	Phenyl	O	1.13	0.81
Cyclobutyl	Phenyl	O	1.12	1.09
Cyclopentyl	Phenyl	O	1.16	0.97
Cyclohexyl	Phenyl	O	1.13	0.93
Benzyl	Phenyl	O	1.03	1.69
CH_3	*p*-ClC_6H_4	O	1.04	1.28
CH_3	α-Naphthyl	O	1.04	2.21
n-C_3H_7	2,5-$(CH_3O)_2C_6H_3$	O	1.09	2.01
CH_3	9-Anthryl	O	1.06	3.49
CF_3	*m*-$NO_2C_6H_4$	O	1.06	3.93
CF_3	α-Naphthyl	O	1.10*	1.76
CF_3	3-Pyrenyl	O	1.10*	3.07
CCl_3	Phenyl	O	1.11	1.21
CBr_3	Phenyl	O	1.10	1.67
iso-C_3H_7	Phenyl	O	1.14	0.81
iso-C_3H_7	Phenyl	S	1.16	1.05
CH_3	Phenyl	S	1.07	1.47

Table II contains data pertinent to the separation of 3,5-DNB derivatized amines, alcohols and thiols in which the B moiety is an aryl system. In all such instances, chromatography of partially resolved configurationally known samples leads to elution orders indicating the DNB carbonyl oxygen to be the site of primary interaction (see IX), a chemically reasonable result. Fig. 4 illustrates such separations.

TABLE III

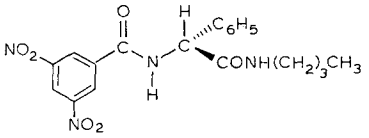
SEPARATION OF THE ENANTIOMERS OF 3,5-DINITROBENZOYL DERIVATIVES OF AMINO ACIDS, AMINO ALCOHOLS AND HYDROXY ACIDS

Separations were performed using 20% isopropyl alcohol in hexane unless otherwise specified. The elution order of each starred solute was determined by chromatography of a partially resolved configurationally established sample. In every instance, the configuration of the initially eluted enantiomer proved to be that shown above. The presumption is that the elution orders of the remaining solutes also follow this pattern.



R	*B*	*M*	α	k_1'
CH_3	CO_2CH_3	NH	1.08*	4.16
CH_3	$CO_2C_2H_5$	NH	1.10	3.32
CH_3	CO_2-*i*-C_3H_7	NH	1.14	2.76
iso-C_3H_7	CO_2CH_3	NH	1.05*	2.75
iso-C_4H_9	CO_2CH_3	NH	1.07*	2.00
$CH_3S(CH_2)_2$	CO_2CH_3	NH	1.04*	4.81
Phenyl	CO_2CH_3	NH	1.19*	4.17
Benzyl	CO_2CH_3	NH	1.10*	4.00
CH_3	$CONHC_4H_9$	NH	1.34*	12.24§
iso-C_3H_7	$CONHC_4H_9$	NH	1.33*	0.82
Isobutyl	$CONHC_4H_9$	NH	1.65*	3.76§
$CH_3S(CH_2)_2$–	$CONHC_4H_9$	NH	1.38*	9.93§
Phenyl	$CONHC_4H_9$	NH	1.78*	0.75
Benzyl	$CONHC_4H_9$	NH	1.52*	6.78§
α-Naphthyl	$CONHC_4H_9$	NH	1.65	1.15
Phenyl	CH_2OH	NH	1.38*	2.36
Benzyl	$CH_2O(CH_2)_2OCH_3$	NH	1.18	5.00
Phenyl	$CH_2O_2CCH_3$	NH	1.18	5.30
Phenyl	$C(CH_3)_2OH$	NH	1.35	5.94§§
CH_3	CH_2OH	NH	1.06*	8.40§§
Isopropyl	CH_2OH	NH	1.19*	3.68§§
CH_3	$(CH_2)_2OH$	NH	1.15	4.30§§
CH_3	CO_2CH_3	O	1.05*	3.39
Phenyl	$CONHCH_3$	O	1.12	9.88
CH_3	$CONH_2$	O	1.10	8.90
2,5-$(CH_3)_2C_6H_3$	CO_2CH_3	O	1.05	2.51
CCl_3	$CH_2CO_2CH_3$	O	1.06	1.80
$CH_3O_2CCH_2$	CO_2CH_3	O	1.12	6.95
Phenyl	$PO(OC_2H_5)_2$	NH	1.38	4.75§§
p-Tolyl	$PO(OC_2H_5)_2$	NH	1.40	4.38§§
p-Anisyl	$PO(OC_2H_2)_5$	NH	1.37	7.50§§
p-ClC_6H_4	$PO(OC_2H_5)_2$	NH	1.26	4.38§§
p-BrC_6H_4	$PO(OC_2H_5)_2$	NH	1.25	4.50§§

§ 5% Isopropyl alcohol in hexane.
§§ 10% Isopropyl alcohol in hexane.



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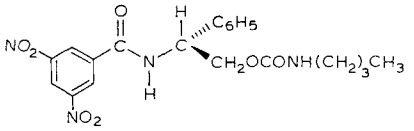

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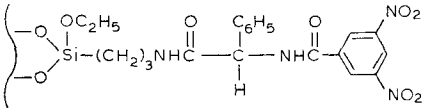

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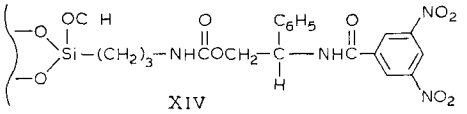


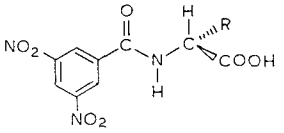
XIV

TABLE IV

DIRECT AND REVERSED-PHASE SEPARATION OF ENANTIOMERS OF 3,5-DINITROBENZOYL DERIVATIVES OF α-AMINO ACIDS UPON CHIRAL STATIONARY PHASE IV

The elution order of each starred solute was determined by chromatography of a partially resolved configurationally established sample. In every instance, the configuration of the initially eluted enantiomer proved to be that shown above. The presumption is that the elution orders of the remaining solutes also follow this pattern.

a = 10% isopropyl alcohol in hexane. b = 80% water, 20% methanol, 0.25% $NaHCO_3$. In this solvent, the carboxyl group is ionized.



	α a/b	k_1 *a/b*
Alanine	1.17/1.40*	6.05/0.75
Valine	1.14/1.36*	3.76/1.4
Leucine	1.12/1.42*	2.82/2.8
Phenylalanine	1.17/1.40*	4.88/6.0
Methionine	1.12/1.31*	6.10/3.2
Phenylglycine	1.27/1.73*	7.48/3.0
α-Naphthylglycine	1.21/1.74	10.0/15.5
Tyrosine	1.16/1.41*	11.9/2.2
Glutamic acid	1.09/—	10.6/*ca.* 0.0
Isoleucine	1.16/1.50	3.26/2.1

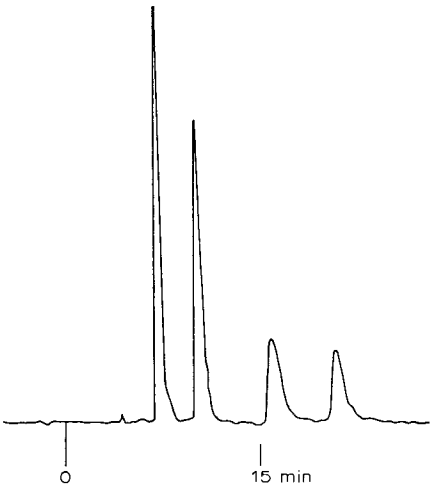


Fig. 5. Separation of the enantiomers of the racemic N-3,5-dinitrobenzoyl derivatives of leucine and alanine *n*-butyl amides upon chiral stationary phase IV using 5% isopropyl alcohol in hexane. The order of elution is D-leu, L-leu, D-ala and L-ala.

Tables III and IV contain data relevant to the separation of enantiomers of 3,5-DNB derivatives of amines and alcohols in which the basic site B is more basic than the DNB carbonyl oxygen. The primary hydrogen bonding interaction now occurs at B and the DNB carbonyl oxygen is the site of the secondary interaction (see X). This role change must be borne in mind when elution orders are correlated with absolute configurations. Fig. 5 illustrates the separation of the enantiomers of two racemic amino acids. Note that, as one increases the basicity difference between B and the DNB carbonyl oxygen, the quality of the chiral recognition (*i.e.*, the magnitude of α) increases. This trend is easily noted for the α-amino acids as one progresses from the acids to esters to the carboxylate anions to the amides. Derivatized amino phosphonic acids have been similarly resolved (Table III) as have (without elaboration at this time) 3,5-DNB derivatized di- and tripeptide enantiomers and diastereomers.

The degree of chiral recognition, as judged by the magnitude of α, is not especially sensitive to the eluent employed; indeed, aqueous solvents have been employed for neutral solutes without substantial alteration of the observed α values. Table IV and Fig. 6 document the separation of a number of α-amino acid enantiomers (as the DNB derivatives) upon CSP phase IV using a water–methanol–sodium bicarbonate eluent. In the latter solvent, the solutes are present as carboxylate anions rather than free acids. Hence, α values do change. To date, solvent changes have never altered elution order of the enantiomers from that expected on the basis of the chiral recognition rationale. Reduction in temperature increases the magnitudes of the α values but concomitant loss in column efficiency (owing to slowed mass transport) tends to offset any gain in resolution ability. Each case should be judged individually, however.

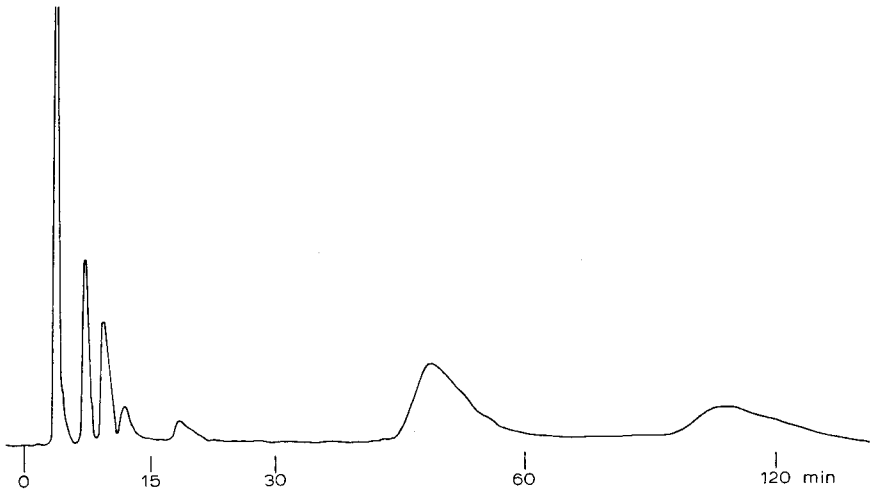


Fig. 6. Reversed-phase separation of the enantiomers of the N-3,5-dinitrobenzoyl derivatives of racemic isoleucine, phenylglycine, and α-naphthyl glycine. In order of elution: 3,5-dinitrobenzoic acid, D-isoleu, L-isoleu, D-phenyl gly, L-phenyl gly, D-α-naphthyl gly and L-α-naphthyl gly. Solvent was 80% water, 20% methanol and 0.25% sodium bicarbonate.

Second-generation chiral stationary phases

If is useful to remember that chiral recognition works in two directions. That is, a "second-generation" stationary phase appropriately* prepared from a single enantiomer of a solute resolvable upon chiral fluoroalcoholic stationary phase IV should, in turn, separate the enantiomers of fluoroalcohol Ia and its analogs. By comparing the quality of the enantiomer separation (*i.e.*, the α value) of Ia with those of its analogs, one can, with rather small racemic samples, quickly ascertain the relative suitabilities of the various fluoroalcohols as chiral stationary phase precursors. Such "iterative" procedures will allow facile optimization of CSP design.

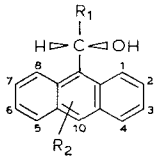
Using CSP IV, the enantiomers of phenylglycine derivative XI and phenylglycinol derivative XII were found to have α values of 1.78 and 1.26, respectively. Using D-phenylglycine, second-generation chiral stationary phases XIII and XIV were prepared and found to separate the enantiomers of a series of fluoroalcohols analogous to Ia. Table V lists representative chromatographic parameters for resolutions of these alcohols upon XIII and XIV. Fig. 7 shows several such resolutions upon XIII. Chromatography of partially resolved configurationally known fluoroalcohols shows that the R-enantiomers are first to be eluted, as expected. (The D-enantiomers of phenylglycine derivatives XI and XII are first to be eluted from R-IV.) As shown in Table V, increasing the size of the R_f group increases the magnitude of the α value while introduction of substituents into the anthryl system can either increase or diminish α, depending upon the electron donating or withdrawing properties of the substituent. The α value can also be diminished if the substituent

* It should be evident that bonding to the solid support must not interfere with the essential chiral recognition interactions.

TABLE V

SEPARATION OF FLUOROALCOHOL ENANTIOMERS UPON SECOND GENERATION CHIRAL STATIONARY PHASES XI AND XII

Unless otherwise specified, solvent was 5% isopropyl alcohol in hexane. The elution order of each starred solute was determined by chromatography of a partially resolved configurationally established sample. In every instance, the configuration of the initially eluted enantiomer proved to be that shown above. The presumption is that the elution orders of the remaining solutes also follow this pattern.



R_1	R_2	α upon		k_1' [§] upon	
		XI	*XII*	*XI*	*XII*
CF_3	H	1.33*	1.13*	4.74	13.0
CF_3	10-CH_3	1.44*	1.22*	6.0	13.3
CF_3	3-CH_3	1.32	1.12	4.36	12.2
CF_3	10-Br	1.34*	1.24*	5.07	9.70
CF_3	4-Cl	1.32	1.15	3.7	8.52
CF_3	10-CN		1.00		4.14
CF_3	10-n-C_4H_9	1.42	1.17	3.13	8.00
CF_3	10-Benzyl	1.30	1.12	2.10[§]	10.4
CF_3	10-CH_3O	1.39	1.52	8.50	9.75
CF_3	10-CH_3S	1.29	1.13*	3.5	9.82
CF_3	10-Phenyl	1.19*		2.4	
CH_3	H	1.28	1.20	7.07	8.21
C_2F_5	H	1.37	1.20	2.43	8.76
C_3F_7	H	1.40*	1.26*	2.14	6.66

[§] 10% Isopropyl alcohol in hexane.

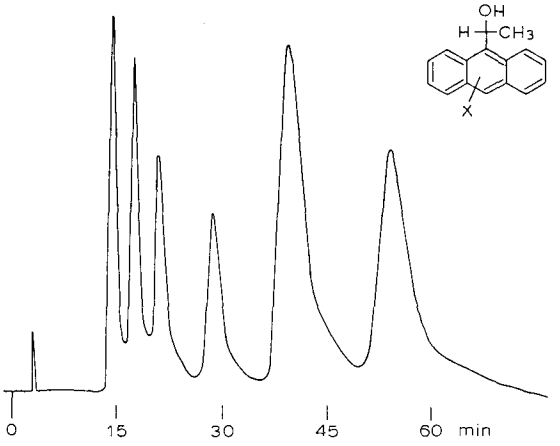


Fig. 7. Separation of the enantiomers of three type I fluoroalcohols upon chiral stationary phase XIII. In order of elution, the chromatographic bands arise from the *R*- and *S*-enantiomers respectively of 2,2,2-trifluoro-1-[(4-chloro)-9-anthryl]ethanol, 2,2,2-trifluoro-1-[(10-methyl)-9-anthryl]-ethanol and 2,2,2-trifluoro-1-[(10-methoxy)-9-anthryl]ethanol. Eluent used was 5% isopropyl alcohol in hexane.

sterically interferes with the close approach (third interaction) of the π-acid moiety. A clear inference is that CSPs derived from carbinols bearing large R_f groups and anthryl (or other polynuclear aromatic) systems bearing small electron donating substituents would outperform our initial CSP IV in terms of scope and quality of chiral recognition. Such CSPs are being prepared. It is interesting to note that replacing the R_f group with a simple alkyl group does not drastically interfere with chiral recognition. Possibly, the diminished acidity of the nonfluorinated carbinols is offset somewhat by unfavorable hydrophobic-like interactions between perfluoroalkyl groups and polar solutes.

It will be noted that the α values realized upon chiral stationary phase XIII, although appreciable, are not as large as the α value shown by phenylglycine derivative XI upon chiral stationary phase IV. These low α values may stem from partial racemization of the phenylglycine during preparation of XIII, insufficient length of the connecting "arm", or residual polar sites on the silica gel that give rise to retention mechanisms other than those responsible for the chiral recognition*. These questions are being studied and will be commented upon at a later date. Similarly, it can be noted that essentially the same α value is noted whether phenylglycinol derivative XII is chromatographed upon IV or whether 2,2,2-trifluoro-1-[9-(10-alkyl)anthryl]-ethanols are chromatographed upon chiral stationary phase XIV. Racemization is known not to have occurred during preparation of XIV; moreover, a longer connecting arm is used.

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* Additional retention mechanisms attenuate the α value. Hence, stability differences between diastereomeric solvates calculated from observed α values will represent minimum values.

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