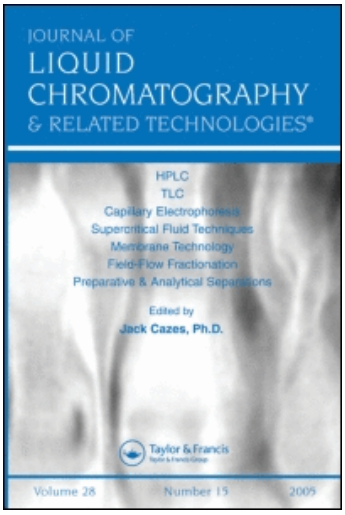

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CHIRAL RECOGNITION OF *N*-ACYL-1-(2-FLUORENYL)-1-AMINOALKANES BY π-ACIDIC CHIRAL STATIONARY PHASES: A MECHANISTIC VIEW

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ABSTRACT

The enantiomers of *N*-acyl-1-(2-fluorenyl)-1-aminoalkanes are separable on a number of chiral stationary phases derived from *N*-(3,5-dinitrobenzoyl)amino acid esters and amides. Previously, these separations and some structure-dependent inversions in the elution order of the enantiomers of these analytes were rationalized on the basis of two competing chiral recognition mechanisms, one involving stacking of amide dipoles, the other hydrogen bonding. To rationalize data obtained using several new chiral stationary phases not available at the time of the original study, the original mechanistic proposal accounting for the origins of chiral recognition for these analytes is now modified. Two competing hydrogen bonding mechanisms, each modified by attendant intercalation effects, are thought to better account for the experimental observations.

INTRODUCTION

"The matter is a perfectly trivial one but there are points in connection with it which are not entirely devoid of interest and even of instruction." [1]

The liquid chromatographic separation of enantiomers using chiral stationary phases (CSPs) is perhaps one of the most promising yet most

underappreciated tools currently available for the study of molecular recognition processes. In this laboratory, chromatography of homologous series of racemates on CSPs has afforded data from which structure-enantioselectivity relationships may be extracted. These relationships aid in both the understanding of chiral recognition processes and in the design of new chiral stationary phases. In several instances, an inversion in the elution order of enantiomers has been noted when one chromatographs a series of analytes in which the length of a given alkyl substituent is progressively increased [2, 3]. For example, such inversions occur when homologous series of racemic *N*-acyl-1-(2-fluorenyl)-1-aminoalkanes, shown in Figure 1, are chromatographed on a several different *N*-(3,5-dinitrobenzoyl)amino acid amide-linked CSPs. The point in the series at which the inversion occurs depends upon the length and size of the alkyl substituent on the stereogenic center (R_1) and the length of the *N*-acyl group (R_2).

Data from the original study were rationalized by suggesting that two competing opposite sense mechanisms might be operative, the contributions of each process to the overall retention of each enantiomer being influenced by the analyte's alkyl and *N*-acyl substituents. Both processes were thought to involve face-to-face π-π interaction between the selector's 3,5-dinitrobenzoyl group and the analyte's 2-fluorenyl system. Additionally, hydrogen bonding and "dipole stacking" of carboxamide groups were proposed to augment the π-π interactions [3]. Dipole-dipole interaction is well documented, and the antiparallel "stacking" of amide dipoles was suggested as possibly aiding the face-to-face approach of analyte and selector. No evidence contrary to the stacking of amide dipoles in this system has ever been presented, and the concept has been taken up (in other systems) by others [4-7]. However, additional observations and experience suggest that refined versions of the original rationale better account for the experimental data. These data, acquired using CSPs not available at the time of the original publication, along with an increased awareness of the importance of intercalative effects, lead us to modify the original rationale. The principle modification is to forego the suggestion that dipole stacking plays an essential role in the chiral recognition of these analytes and to incorporate the view that both faces of the selector's 3,5-dinitrobenzoyl system may be approached by the analyte enantiomers, although not necessarily with equal ease. Differential ease of approach to the two faces of a dinitrobenzoyl group has always been an essential feature of our chiral recognition models. However, the consequences of analyte approach to the more hindered face of the

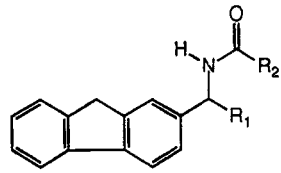


Figure 1. Structure of the analytes used in the investigation

selector have been considered explicitly only in a few instances, and then only to maintain that such approach is less likely and not apt to be the dominant contributor to chiral recognition [7, 8]. In the modified rationale, both analyte enantiomers are presumed capable of face-to-face π-π interactions with either face of the dinitrobenzoyl system. Moreover, both analyte enantiomers are thought to participate in hydrogen bonding interactions as well, although different modes of hydrogen bonding may be employed by each enantiomer. Intercalative interactions between the analyte's *N*-acyl or alkyl substituent are thought, as in the earlier rationale, to influence the relative extents to which each enantiomer binds to either face of the selector.

EXPERIMENTAL

Chromatography

Chromatography was performed with an Aspec-Bischoff model 2200 isocratic HPLC pump, a Rheodyne Model 7125 injector equipped with a 20 µl sample loop, a Milton-Roy LDC UV Monitor D™ fixed wavelength detector operating at 254 nm, and a Hewlett-Packard 3394A recording integrator. Signs of rotation were measured using an Aspec-Bischoff model 2200 isocratic HPLC pump, a Rheodyne Model 7125 injector equipped with a 20 µl sample loop, a Milton-Roy LDC UV Monitor D™ fixed wavelength detector operating at 254 nm in series with a Rudolph Autopol III digital polarimeter equipped with a 20 cm flow cell, and a Kipp and Zonen BD 41 dual channel recorder. Void volumes were determined using dodecane.

The Chiral Stationary Phases

A commercially available column (Regis Chemical Co., Morton Grove, IL) containing CSP 1 was employed. CSP 3 was prepared using a modification of the procedure used to prepare CSP 1 [9]. The preparation of CSPs 2 and 4 is reported elsewhere [10, 11].

The Analytes

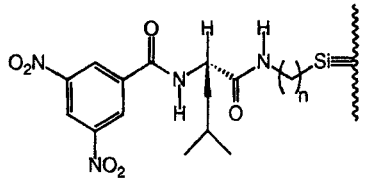
All *N*-acyl-1-(2-fluorenyl)-1-aminoalkanes were available from prior studies [3].

RESULTS AND DISCUSSION

The structures of the CSPs used in the present investigation are shown in Figure 2.

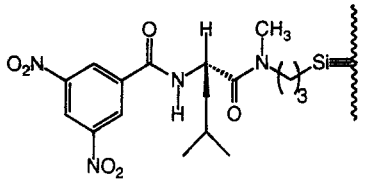
CSP 2 differs from CSP 1 in that the length of the tether which anchors the phase to the silica support contains eleven methylene groups instead of three. CSP 3 differs from CSP 1 only in that the C-terminal carboxamide bears a methyl group instead of a hydrogen on nitrogen. This amide N-H is thought to be unnecessary for chiral recognition of a number of analytes. Since non-essential interactions sites are detrimental to the enantioselectivity of a CSP, this amide N-H was replaced by a less interactive methyl group. Indeed, CSP 3 typically provides decreased retention and increased enantioselectivity relative to CSP 1. CSP 4 was designed to mimic CSP 3, except that it is oriented differently with respect to the underlying silica support.

Data obtained when several series of (*R*,*S*)-*N*-acyl-1-(2-fluorenyl)-1-aminoisobutanes are chromatographed on CSPs 1, 2, 3 and 4 are shown in Table 1. As indicated by the signs of rotation, an inversion in the elution order of the enantiomers occurs on CSP 1 when the acyl group reaches three carbons in length. The (*R*)-(+)-enantiomers are more retained when the acyl group is short, and the (*S*)-(-)-enantiomers are more retained throughout the remainder of the homologous series. On CSP 2, no such inversion takes place, the (*S*)-enantiomers being the more retained throughout the series. Similar trends are observed when these analytes are chromatographed on CSPs 3 and 4, although a few early members of the series are not resolved on CSPs 3 and 4.

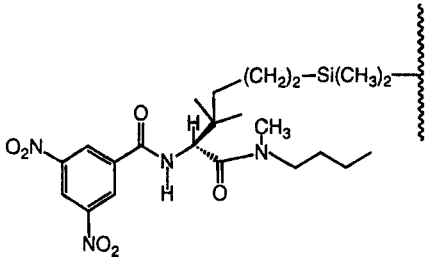


CSP 1: "n" = 3

CSP 2: "n" = 11



CSP 3



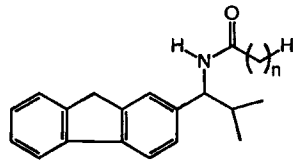
CSP 4

Figure 2. Structures of the chiral stationary phases (CSPs) used in the investigation

Data obtained when the enantiomers of various *N*-butanoyl-1-(2-fluorenyl)aminoalkanes are chromatographed on CSPs 1-4 are presented in Table 2. To a great extent, mechanistic studies have focused on the primary modes of retention of the more retained enantiomer, since the less retained enantiomer forms less stable and, in all likelihood, a greater variety of adsorbates. Situations in which some anomalous event (such as an inversion in the elution order of enantiomers) manifests itself are unusually rich in information and any mechanistic

TABLE 1

Separation of the Enantiomers of *N*-Acyl-1-(2-fluorenyl)-1-aminoisobutane on CSPs 1, 2, 3 and 4

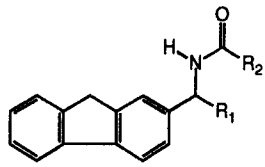


	CSP 1			CSP 2			CSP 3			CSP 4		
"n"	k'_1	α	$[\alpha]_D$ (*)	k'_1	α	$[\alpha]_D$ (*)	k'_1	α	$[\alpha]_D$ (*)	k'_1	α	$[\alpha]_D$ (*)
0	4.25	1.34	(+) (*R*)	2.35	1.17	(-) (*S*)	3.61	1.00		2.49	1.00	
1	8.82	1.30	(+) (*R*)	3.56	1.12	(-) (*S*)	5.70	1.00		2.53	1.18	(-) (*S*)
2	8.91	1.00		3.04	1.34	(-) (*S*)	3.79	1.18	(-) (*S*)	1.67	1.45	(-) (*S*)
3	7.00	1.03	(-) (*S*)	2.71	1.35	(-) (*S*)	2.95	1.20	(-) (*S*)	1.41	1.47	(-) (*S*)
4	6.05	1.15	(-) (*S*)	2.63	1.37	(-) (*S*)	2.48	1.27	(-) (*S*)	1.21	1.47	(-) (*S*)
6	4.36	1.27	(-) (*S*)	2.19	1.41	(-) (*S*)	1.86	1.33	(-) (*S*)	0.97	1.51	(-) (*S*)
7	3.86	1.37	(-) (*S*)	2.19	1.40	(-) (*S*)	1.71	1.36	(-) (*S*)	0.73	1.52	(-) (*S*)
9	3.18	1.47	(-) (*S*)	1.84	1.43	(-) (*S*)	1.51	1.38	(-) (*S*)	0.57	1.56	(-) (*S*)
13	2.41	1.58	(-) (*S*)	1.54	1.48	(-) (*S*)	1.21	1.44	(-) (*S*)	0.43	1.53	(-) (*S*)
17	1.95	1.68	(-) (*S*)	1.35	1.51	(-) (*S*)	1.03	1.47	(-) (*S*)	0.32	1.53	(-) (*S*)

On CSPs 1, 2 and 3, the mobile phase consists of 20% 2-propanol in hexane; on CSP 4 the mobile phase consists of 10% 2-propanol in hexane; "n" = number of methylene units in analyte's acyl substituent; k'_1 = capacity factor for the first eluted enantiomer; α = chromatographic separation factor; $[\alpha]_D$ = sign of rotation for the more retained enantiomer, (*) = absolute configuration of the more retained enantiomer

TABLE 2

Separation of the Enantiomers of *N*-Butanoyl-1-(2-fluorenyl)-1-aminoalkanes on CSPs 1, 2, 3 and 4



		CSP 1			CSP 2			CSP 3			CSP 4		
R_1	R_2	k'_1	α	$[\alpha]_D$ (*)	k'_1	α	$[\alpha]_D$ (*)	k'_1	α	$[\alpha]_D$ (*)	k'_1	α	$[\alpha]_D$ (*)
CH_3-	C_3H_7	9.17	1.42	(-) (*S*)	3.44	1.46	(-) (*S*)	4.32	1.33	(-) (*S*)	2.91	1.43	(-) (*S*)
$(CH_3)_2CH_2$-	C_3H_7	7.00	1.03	(-) (*S*)	2.71	1.35	(-) (*S*)	2.95	1.20	(-) (*S*)	1.41	1.47	(-) (*S*)
C_6H_{13}-	C_3H_7	5.18	1.11	(+) (*R*)	2.43	1.35	(-) (*S*)	2.32	1.17	(-) (*S*)	0.95	1.54	(-) (*S*)

On CSPs 1, 2 and 3, the mobile phase consists of 20% 2-propanol in hexane; on CSP 4 the mobile phase consists of 10% 2-propanol in hexane; k'_1 = capacity factor for the first eluted enantiomer; α = chromatographic separation factor; $[\alpha]_D$ = sign of rotation for the more retained enantiomer, (*) = absolute configuration of the more retained enantiomer

hypothesis must account for the anomaly to be viable. Moreover, a rationale consistent with a large body of data is more compelling than one which accounts for but a few observations. In the present study, the length of the analyte's acyl substituent differentially influences the stabilities of the diastereomeric adsorbates formed with CSP 1. When the length of the acyl group is held constant and the size of the alkyl group on the stereogenic center is varied, enantioselectivity and elution order can be altered. However, on CSPs 2, 3 and 4, all of which are but slight structural variations of CSP 1, this behavior is not observed. The retention of each enantiomer is affected by contributions from the numerous processes by which the enantiomers interact with the CSP. Not all such processes are equally probable,

and it is instructive to limit discussion to the one or two modes of interaction between each enantiomer and the CSP which are thought to be the primary contributors to retention. Upon these basic models are then superimposed those subtle factors which influence the contribution of each of the competing processes.

How might the (*S*)-enantiomers of the present class of analytes interact with CSP 1? Examination of space-filling models augmented by conformational analysis suggests that these enantiomers may appoach the less hindered faces of CSPs 1, 2 and 3 (*i.e.* that face not presenting the isobutyl group) and in so doing participate in 1) a face-to-face π-π interaction between the 3,5-dinitrobenzoyl group and the π-basic fluorenyl moiety, and 2) a hydrogen bond between the 3,5-dinitrobenzamide N-H and the amide carbonyl oxygen of the analyte. This orients the methine hydrogen of the analyte toward the chiral selector and the bulky isopropyl substituent away from the selector, more or less toward the underlying silica support. The (*S*)-enantiomer's acyl group (R_2) is directed away from the tether and silica support and into the bulk mobile phase. A cartoon-like representation of the CSPs and the analytes is introduced in Figure 3, and the situation described above is represented using this convention in Figure 4.

The (*R*)-enantiomer, if it approaches the CSP from its less sterically congested face, cannot enjoy the same bonding interactions as does its antipode unless it adopts a higher energy conformation where the bulky isopropyl group on the stereogenic center must eclipse (approximately) the amide carbonyl oxygen. However, by approaching the CSPs dinitrobenzoyl group from its more hindered face (the one *syn* to the isobutyl group), this enantiomer can be oriented so as to place its (small) methine hydrogen toward the CSP's isobutyl group, undergo a face-to-face π-π interaction between the 3,5-dinitrobenzoyl group and the π-basic fluorenyl moiety, and maintain a hydrogen bond between the 3,5-dinitrobenzamide N-H and the analyte's amide carbonyl oxygen. In so doing, the (*R*)-enantiomer now enjoys the same interactions with the CSP as does the (*S*)-enantiomer, while placing the isopropyl group on the analyte's stereogenic center in a position where it undergoes little steric encumbrance. However, this also requires that the (*R*)-enantiomer direct its acyl group alongside the tether and toward the silica support. In normal phase solvents, this "intercalation" process is energetically costly and reduces the stability of that diastereomeric complex. Moreover, the extent of this reduction in stability increases with the length of the acyl group. Approach of the analyte from this face of the CSP is illustrated in Figure 5.

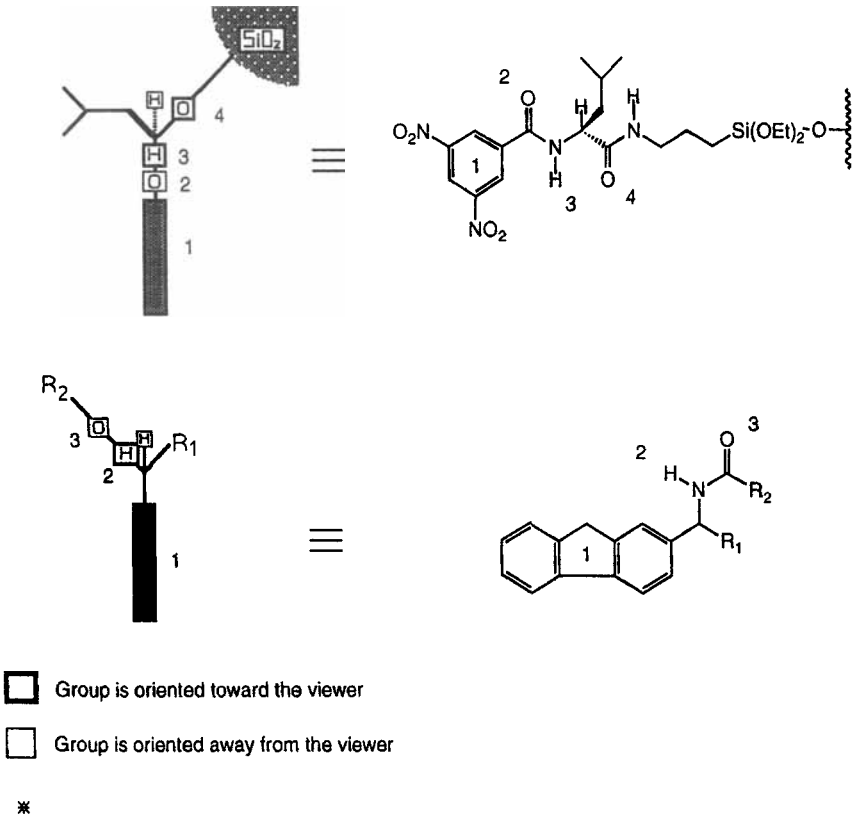


Figure 3. Legend to accompany Figures 4 and 5.

If one accepts the foregoing, it follows that Hydrogen Bonding Scheme B (Figure 5) is more exergonic than Hydrogen Bonding Scheme A (Figure 4) when both the alkyl group and the acyl group of the analyte are short, since the (*R*)-enantiomer is the more retained on CSP 1. As the acyl group (R_2) of the (*R*)-enantiomer becomes longer, the complex is rendered less stable by intercalative effects, the retention of the (*R*)-enantiomers is reduced relative to their antipodes, and, eventually, an inversion of the elution order occurs.

The data in Table 2 are consistent with intercalation of the alkyl group (R_1) on the stereogenic center of the (*S*)-enantiomers of these analytes. Progressive

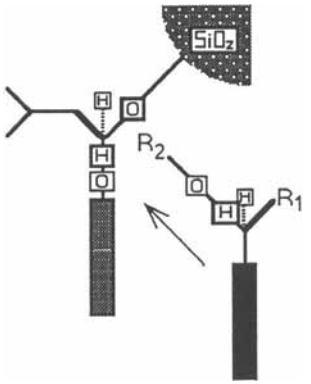


Figure 4. Hydrogen Bonding Scheme "A": The (*S*)-enantiomers of the analytes may approach the CSP from the less hindered face. The essential bonding interactions are described in the text.

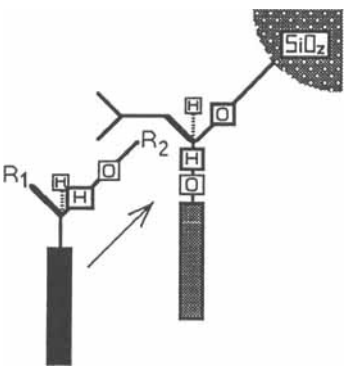


Figure 5. Hydrogen Bonding Scheme "B": The (*R*)-enantiomers of the analytes may approach the CSP from the more hindered face, causing intercalation of R_2. The essential bonding interactions are described in the text.

lengthening of this group likewise produces an inversion in the elution order of the enantiomers of (*R*,*S*)-*N*-butanoyl-1-(2-fluorenyl)-1-aminoalkanes. This, in effect, restores the original elution order as the effects of intercalation of the longer alkyl substituents of the (*S*)-enantiomers more than compensate for the intercalation of the butanoyl group of the (*R*)-enantiomers. The (*S*)-enantiomers, however, are the more retained throughout the series on CSPs 2, 3 and 4.

Thus, the data in Tables 1 and 2, when taken collectively, imply that CSPs 2, 3 and 4, by virtue of their structural dissimilarities to CSP 1, relieve the energetic consequences of intercalation of R_1 during Hydrogen Bonding Scheme A and the energetic consequences of intercalation of R_2 during Hydrogen Bonding Scheme B. How might this occur?

Owing to the long tether utilized to anchor CSP 2 to the silica support, the effect of intercalation of either R_1 or R_2 is never severe enough to produce an inversion in elution order. The complexes formed between the (*S*)-enantiomers of the analytes and CSP 2 are more stable than those involving the (*R*)-enantiomers, regardless of the length of R_1 or R_2. On CSPs 1 and 3, the short three-carbon tether causes the energetic consequences of intercalation to be greater, thereby more profoundly influencing retentions. However, the effects of intercalation are not so evident on CSP 3 as they are on CSP 1. This difference is suspected to stem from the fact that the C-terminal amide of CSP 1 has a very strong preference for the "Z"-amide rotamer whereas CSP 3 presumably has no great preference for either rotamer. Examination of the ^1H NMR spectrum of the silane precursor to CSP 3 in $CDCl_3$ indicates that the Z : E ratio for this amide is approximately 1:1. Furthermore, this ratio depends upon solvent and temperature and may be altered by complexation with an analyte. It appears likely, based on the inspection of space-filling models, that the intercalation problem introduced by Hydrogen Bonding Scheme A is less severe in the E-rotamer than in the Z-rotamer. For this reason, separation of the analyte enantiomers on CSP 3 occurs only when the R_2 groups on the (*R*)-enantiomers are long enough so that their intercalation has significant energetic consequences. As a consequence of this, no elution order inversions are noted on CSP 3 and enantioselectivity increases steadily throughout the homologous series. On CSP 4, contributions to retention from Hydrogen Bonding Scheme B should be minor, since access to the more hindered face of the CSP should be effectively blocked by the surface of the silica itself. Again, no inversion in the elution order of the enantiomers is detected as the lengths of the R

substituents are increased. Note that the retention of both enantiomers drops rather rapidly as the lengths of the acyl groups (R_2) are increased. No dependence of elution order of the enantiomers on the length of R_1 is noted with CSP 4, since the orientation of this CSP relative to the silica support precludes any intercalative effects by this group.

CONCLUSION

It is important to understand those factors which are capable of altering the elution order of enantiomers from chiral stationary phases if absolute configurations are to be assigned from observed elution orders. Moreover, such understanding is crucial to the rational design of new chiral stationary phases. It is herein demonstrated that relatively small structural changes in a CSP can sometimes influence elution order of enantiomers from that CSP and that the elution orders of analytes in a homologous series may depend upon the influence of subtle factors possibly in delicate balance. In such instances, enantioselectivities are apt to be modest. While one would perhaps wish to avoid such circumstances, this can only be done if one recognizes that the CSP being used is a poor choice for the analytes of interest. A clear understanding of the origins of chiral recognition is a great aid in selecting an appropriate CSP. As our studies progress, ideas on the origins of chiral recognition evolve and become more sophisticated. The present modification of the early rationale is felt to be a closer approximation of reality and to better accommodate a body of data.

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