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Liquid chromatographic separation of the enantiomers of cyclic β-amino esters as their N-3,5-dinitrobenzoyl derivatives

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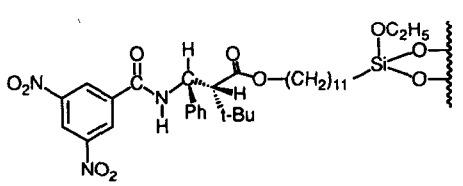
Abstract

A variety of cyclic N-3,5-dinitrobenzoyl β-amino esters has been synthesized and resolved by chiral HPLC. The β-amino esters were derived from β-lactams formed by the [2 + 2] cycloaddition of N-chlorosulfonyl isocyanate with simple olefins. Chromatographic separation of the enantiomers of these N-(3,5-dinitrobenzoyl)-β-amino esters on three π-basic chiral stationary phases is described and the origin of the observed chiral recognition considered.

1. Introduction

A chiral stationary phase (CSP) derived from a conformationally restricted β-amino acid, CSP 1, was developed in these laboratories several years ago [1]. Despite the greater distance between two of the potential recognition sites, CSP 1 has broader analyte scope and generally affords larger separation factors than its α-amino acid analogue, N-(3,5-dinitrobenzoyl)phenylglycine [2]. It is presumed that greater degrees of recognition site preorganization and conformational rigidity are present and are responsible for CSP 1's improved performance. To explore this hypothesis, a series of cyclic (and thereby conformationally restricted) β-amino acids were prepared and the enantiomers of their 3,5-dinitrobenzamide derivatives were examined chromatographically on three CSPs, each of which has a somewhat different conformational preference. Should large separation factors be encountered for the enantiomers of one or more of the analytes in this series, one might, in view of the reciprocity often noted for chiral recognition [3], use this information to aid in the development of a still more efficacious β-amino acid-derived CSP. The understanding one might gain concerning the relationship between structure and chiral recognition using this series of analytes would be an added benefit.



CSP 1

The synthetic route used to prepare the β-lactam precursor of CSP 1 is not suitable for the preparation of cyclic β-amino acids [1,4]. A convenient method for the rapid synthesis of small quantities of β-lactams (and the derivative β-amino acids) is the [2 + 2] cycloaddition of

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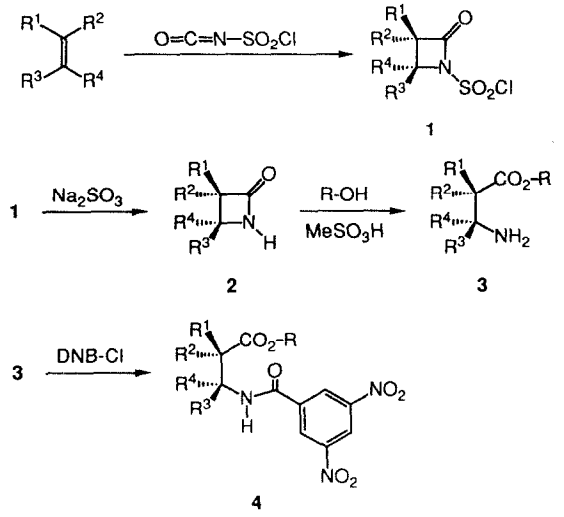


Fig. 1. The synthetic sequence used to prepare racemic analytes **5–14**.

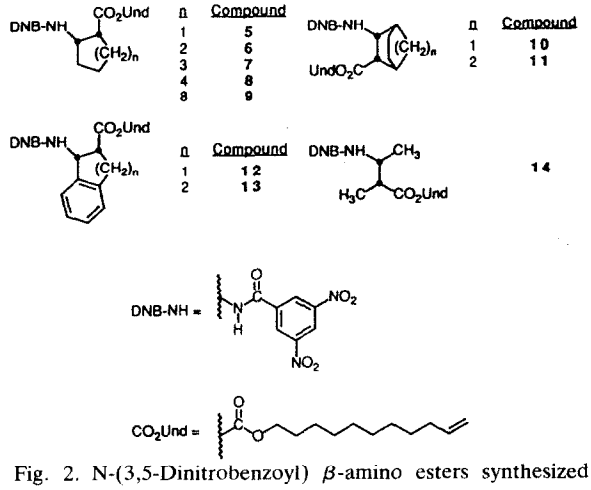
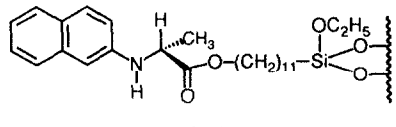


Fig. 2. N-(3,5-Dinitrobenzoyl) β-amino esters synthesized for use in this study.

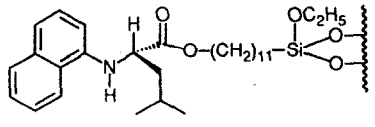
N-chlorosulfonyl isocyanate (CSI) to simple olefins, either cyclic or acyclic (see Fig. 1) [5]. The [2 + 2] cycloaddition reaction proceeds with net retention of the stereochemistry of the starting olefin. Hence, either *cis* or *trans* disubstituted β-lactams can be obtained.

The N-chlorosulfonyl β-lactams, **1**, obtained by [2 + 2] cycloaddition of the isocyanate to an alkene, were hydrolyzed to give the corresponding β-lactams, **2**. These were then ring opened, esterified and acylated as previously described to give the N-3,5-dinitrobenzoyl (DNB) amino esters, **4** [1]. The compounds synthesized by this route are shown in Fig. 2.

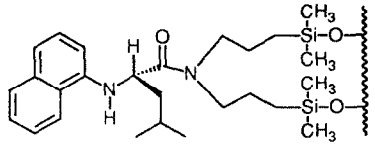
The liquid chromatographic separation of the enantiomers of the DNB derivatives of a series of acyclic β-amino acids on several π-basic CSPs was reported earlier [6,7]. The chromatographic separation of the enantiomers of derivatized β-amino acids by liquid chromatography [8,9], ligand-exchange chromatography [10–13], gas chromatography [11], diastereomeric derivatization [14–16] and mobile phase additives [17] have been reported elsewhere. In this study, DNB β-amino esters were evaluated on π-basic CSPs 2–4. CSPs 2 and 3 are commercially available (see Experimental). The synthesis and evaluation of CSP 4 are being reported elsewhere.



CSP 2



CSP 3



CSP 4

2. Experimental

2.1. Equipment

Chromatography was performed with an Alcott 760 HPLC pump, a Rheodyne Model 7125 injector with a 20-μl sample loop, a 250 × 4.6 mm stainless-steel column packed with either CSP 2, 3 or 4, a LDC Analytical UV Monitor D

fixed-wavelength detector (254 nm), a Rudolph Research Autopol III automatic polarimeter using a 2-dm flow-cell and a Hewlett-Packard HP 3394A integrator.

CSPs 2 and 3 are available from Regis (Morton Grove, IL, USA).

2.2. Chemicals

The CSI, undecenyl alcohol and olefins were purchased from Aldrich. Chromatography solvents were generously provided by EM Science. The normal-phase void volume was determined using tri-*tert.*-butylbenzene [18].

2.3. General synthetic approach

The β-lactams were synthesized according to published procedures or minor modifications thereof [19–22]. The ring opening of the β-lactams and esterification of the resulting β-amino acids was accomplished by heating a 1:1.2:1.2 molar ratio of β-lactam, undecenyl alcohol and methanesulfonic acid, respectively, in benzene at reflux overnight with azeotropic removal of the water formed. The resulting reaction mixture was washed successively with saturated $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and isolated in vacuo. The crude amino ester was then acylated with 3,5-dinitrobenzoyl chloride according to standard procedure [1]. Compounds were purified by silica gel flash chromatography or preparative TLC with an ethyl acetate–hexane solvent system.

3. Results and discussion

The enantiomers of each of the DNB β-amino esters studied were successfully resolved by each of the three CSPs examined, see Table 1. In most cases, baseline (or better) resolution of the enantiomers was obtained. The enantiomers of the five-, six- and seven-membered rings, **5–7**, are the most readily separated by these CSPs. Analytes having still larger rings (*e.g.* **8** and **9**) are less well resolved. The decrease in the separation factors of the enantiomers of a larger-ringed analyte is presumably due to the increased degree of conformational flexibility. This is believed to result in a loss of preorganization and to lead to a blend of retention processes which causes increased retention of the least-retained enantiomer and reduced retention of the more retained enantiomer.

The relative placement of interaction sites and the rigidity of two of the smaller ringed analytes was altered either by incorporation of a bridge, **10** and **11**, or by fusing a benzo substituent, **12** and **13**, onto the cyclic analytes. Increased ana-

Table 1
Separation of the enantiomers of N-3,5-dinitrobenzoyl β-amino esters

Compound	CSP 2			CSP 3			CSP 4		
	k'_1	α	Sign[a]	k'_1	α	Sign[a]	k'_1	α	Sign[a]
5	1.88	1.63	(+)	1.51	3.76	(−)	1.13	6.72	(−)
6	2.45	1.87		2.27	3.67		1.19	9.40	
7	2.01	2.07	(+)	1.89	4.57	(−)	1.08	10.3	(−)
8	1.89	1.57	(+)	1.79	2.77	(−)	1.13	5.36	(−)
9	1.76	1.56		2.19	2.28		1.35	2.94	
10	2.11	1.52	(+)	1.76	2.55	(−)	1.02	4.67	(−)
11	3.00	1.17		2.17	1.99		0.91	5.08	
12	3.28	1.16	(−)	1.99	2.78	(+)	2.53	1.66	(+)
13	2.93	1.60	(+)	2.60	1.50	(+)	3.76	1.06	(−)
14	1.89	1.31	(−)	1.65	2.30	(+)	1.38	3.96	(+)

Chromatographic conditions: mobile phase, 2-propanol–hexane (20:80); flow-rate, 2.0 ml/min.
[a] The sign of rotation at 589 nm of the most retained enantiomer is given.

lyte rigidity may be either beneficial or detrimental to chiral recognition by a given selector. Should a structural change in an analyte more heavily populate a conformation having good spatial complementarity of its interaction sites with those of the selector, one can increase the retention of the more retained enantiomer while diminishing the retention of the least-retained enantiomer. By locking the analyte enantiomers into an unfavorable (for chiral recognition) conformation, rigidity can reduce or destroy the selectors' ability to distinguish between the enantiomers.

The enantiomers of the bridged **10** and **11** exhibit separation factors adequate for resolution but smaller than those of their monocyclic counterpart, **6**. In retrospect, it seems likely that, to some extent, the rigid bridge interferes sterically with the selector–analyte interactions principally responsible for chiral recognition. Benzo analogues **12** or **13** show less enantioselectivity on CSPs 2–4 than do the monocyclic analogues **5** and **6**. The added benzo substituent may function as an additional interaction site, either leading to non-chiral retention or to an alternative and opposite-sense chiral recognition process. In fact, **12** and **13** exhibit larger separation factors on CSP 3 than on CSP 4, **13** being best resolved on CSP 2. The order of elution of the enantiomers of **13** from CSP 4 differs from that noted on CSPs 2 and 3. This aspect of chromatographic behavior is not consistent with the general trends observed in Table 1 and seems to support the contention that the benzo substituent can alter the (otherwise) dominant recognition process.

The structurally simple non-cyclic analyte, **14**, prepared for comparative purposes, is well resolved by all three CSPs, demonstrating that the enantiomers of simple DNB β-amino esters bearing sterically small substituents can be separated by CSPs 2–4.

The separation factors for the enantiomers of most DNB β-amino esters on CSPs 2 and 3 are much smaller those found for simple DNB α-amino esters under similar chromatographic conditions [7,23]. This is more or less to be expected, since a greater distance and a larger number of bonds between interaction sites typically leads to greater conformational flexibility and less preorganization. Similar considerations apply to chiral selectors. While CSPs 2 and 3 are essentially the same mechanistically, a claim consistent with a body of experimental data [24–26], the latter often show significantly larger separation factors for the enantiomers of DNB α-amino acid derivatives than do the former. CSP 3 is conformationally more rigid and apparently better preorganized to accommodate the more retained enantiomer of the DNB derivative of either an α- or β-amino acid (see Table 1).

Although in a slightly different spatial arrangement, the necessary (for chiral recognition) interaction sites present in DNB α-amino acid derivatives are also present in DNB β-amino acid derivatives. There is no reason to suspect that the chiral recognition processes of the two are conceptually much different even though the enantioselectivity shown by the latter is reduced for the aforementioned reasons. The chiral recognition process proposed to account for the ability of CSPs 2–4 to "recognize" the enantiomers of DNB β-amino esters involves face to face $\pi-\pi$ interaction of the electron-rich naphthyl group and the electron-poor DNB group, a hydrogen bond between the aryl NH and the terminal carbonyl of the β-amino ester and a second hydrogen bond between the DNB amido hydrogen and the terminal carbonyl of the α-amino acid derivative of the CSP. These processes must occur simultaneously (Fig. 3) for the more retained enantiomers of these analytes.

The preceding chiral recognition mechanism readily accounts for the greater enantioselectivity of CSP 4 relative to CSP 3. The amide carbonyl oxygen of CSP 4 bears a higher electron density than that of the ester carbonyl oxygen of CSP 3 and is hence a better hydrogen bond acceptor. By moderately increasing the strength of an interaction essential to the chiral recognition process, one expects to increase enantiodiscrimination. The data in Table 1 are consistent with this argument. The separation factors for the enantiomers of every analyte (with the exception of **12** and **13**) are greater on CSP 4 than on CSP 3.

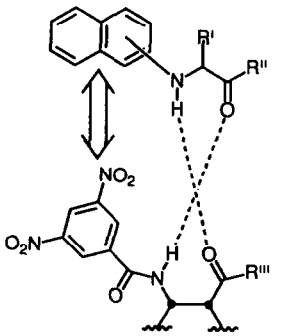


Fig. 3. A generic chiral recognition mechanism proposed to account for the separation of the enantiomers of DNB β-amino esters on CSPs 2-4.

As is the case with most brush-type CSPs, CSPs 2-4 generally exhibit good chromatographic efficiency and resolution factors (R_s) sufficient for complete separation of the enantiomers of these analytes. For example, the resolutions of one of the less-well separated analytes, compound **11**, on these phases (CSP 2, $R_s = 1.2$; CSP 3, $R_s = 3.5$; CSP 4, $R_s = 8.6$) are of a magnitude adequate for most analytical and preparative applications. Compound **13** does show a decrease in resolution from CSPs 2 to 4 (CSP 2, $R_s = 4.0$; CSP 3, $R_s = 3.3$; CSP 4, $R_s = 0.61$) consistent with the previously noted unusual chromatographic behavior of this analyte.

The depiction of CSP 4 shows (*S*)-N-(1-naphthyl)leucine di-*n*-propyl amide doubly linked to the silica support. This is an idealized picture, a mixture of linkage modes doubtless occurs. A selector tethered by both trimethylene legs, as is CSP 4, is expected to manifest a degree of rigidity not present in CSP 3. Plausibly, "two-legged" attachment may lead to a greater spacing between the strands of bonded phase than occurs with "one-legged" attachments, a point under study. Strand spacing can influence the level of enantioselectivity afforded by a given immobilized selector.

Conclusions

The enantiomers of a variety of DNB β-amino esters have been chromatographically separated on three π-basic CSPs. From the ease of separation observed, it is likely that the enantiomers of a wide variety of substituted β-amino acids derivatives can be resolved by CSPs 2–4. The chiral recognition mechanism earlier advocated as responsible for the separation of the enantiomers of α-amino acid derivatives on CSPs 2 and 3 also accounts for the differential affinities shown toward the enantiomers of β-amino acid derivatives by these CSPs. From the magnitudes of the separation factors observed, one presumes that separation of the enantiomers of C-terminal amides of DNB β-amino acids will be separated easily using CSPs 2–4.

CSPs corresponding to analytes **12** and **13** have been synthesized and their performance will be reported in due course.

Acknowledgements

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