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Note

Generation of extreme selectivity in chiral recognition

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In previous papers, we have described rational approaches to the design of chiral stationary phases (CSPs) for the liquid chromatographic separation of the enantiomers of a large and diverse array of chiral molecules[1–3]. Efforts have been focused on attaining an understanding of the mechanistic origins of chiral recognition, for such understanding can be used to design CSPs of enhanced selectivity and expanded scope. In this paper, we describe an approach to analyte modification which can sometimes be used to confer an extreme degree of selectivity. Although illustrated by the separation of enantiomers on a chiral column, the approach is more general and is not restricted just to the separation of enantiomers.

Separation of enantiomers on a CSP requires that the diastereomeric adsorbates formed have non-identical (*i.e.* $\Delta\Delta G \neq 0$) free energies, ΔG, of association with the CSP. The extent of selectivity, α, is related to $\Delta\Delta G$ by the expression: $\Delta\Delta G = -RT \ln \alpha$. If one could double $\Delta\Delta G$, one would increase α to the square of its original value. Many compounds are amenable to selectivity-enhancing modification. For example, when analytes such as methyl or ethyl esters, acetates or propionates, or amides of simple amines are encountered, one may have the option of making bis-esters or bis-amides from the chiral constituent of interest and achiral diols, diacids or diamines. If the spacing between the ends of the bis-derivative permits the chiral moieties to interact independently and simultaneously with the CSP, one expects, on *simplistic* grounds, that the $\Delta\Delta G$ observed for the enantiomers of the bis-analyte will be roughly twice that observed for the enantiomers of the corresponding mono-analyte. Hence, the α noted for the enantiomers of the bis-analyte should be *roughly* the square of the α observed for the enantiomers of the mono-analyte. We herein report the use of CSP 1 to compare the chromatographic behavior of the enantiomers of mono-analyte 2 with those of the corresponding bis-analyte 3.

EXPERIMENTAL

Chromatography was performed isocratically using a Rainin Rabbit HPX pump, an LDC/Milton Ray UV Monitor D fixed-wavelength (254 nm) detector, a Kipp-Zonen BD-41 recorder, a Reodyne injector and a (*S*)-N-(2-naphthyl)alanine column (Regis, Morton Grove, IL, U.S.A.).

The mono- and bis-amides of N-(3,5-dinitrobenzoyl)leucine[4] were prepared by activation of the carboxyl group with 1-ethoxycarbonyl-2-ethoxy-1,2-dihydroqui-

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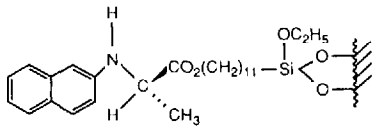

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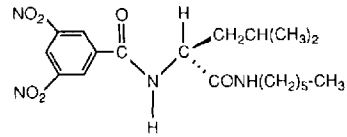

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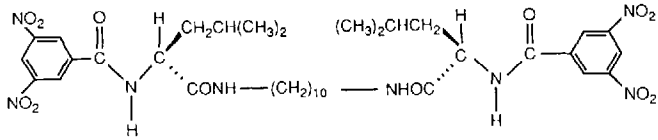

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noline (EEDQ) (Aldrich) in methylene chloride and subsequent slow addition of dilute methylene chloride solutions of either *n*-hexylamine or 1,10-diaminodecane. These solutions were washed sequentially with 1 *M* sodium hydroxide, 1 *M* hydrochloric acid, water and brine, then dried over anhydrous sodium sulfate. Filtration afforded analyte solutions which can be used directly. Crystallization of bis-amide 3 was avoided so as to not alter the ratio of diastereomers. Derivatives of both racemic and (*S*)-N-(3,5-dinitrobenzoyl)leucine were made and chromatographed to establish rigorously elution orders. On a racemic Regis N-(2-naphthyl)alanine column, the enantiomers are coeluted and, in the case of the bis-amide, 3, they are coeluted with the meso diastereomer.

RESULTS AND DISCUSSION

The recently described and now from Regis available CSP 1, derived from N-(2-naphthyl)alanine, shows a high degree of chiral recognition toward the enantiomers of the N-3,5-dinitrobenzoyl derivatives of amino acids and achiral amines[3]. For example, the enantiomers of N-(3,5-dinitrobenzoyl)leucine *n*-hexylamide, 2, show separation factors of 2.35 and 10.5, respectively at 25°C in mobile phases of methanol or 30% 2-propanol in hexane, the (*R*)-enantiomer being eluted before the (*S*)-enantiomer. Activation of the carboxyl of racemic N-(3,5-dinitrobenzoyl)leucine with EEDQ, followed by reaction with 1,10-diaminodecane, affords a 1:2:1 ratio of the (*R,R*)-, (*R,S*)- and (*S,S*)-bis-derivatives, 3. Chromatography on CSP (*S*)-1 cleanly separates the three stereoisomers, the *meso* (*R,S*)-isomer eluting after the (*R,R*)- but before the (*S,S*)-enantiomer, *as expected*. The separation factors observed for the enantiomers in methanol and 30% 2-propanol in hexane are 10.0 and 121.0, respectively! In the second instance, the (*R,R*)-enantiomer eluted after 8 min, the *meso* after 96 min, and the (*S,S*) after 590 min, almost 10 h after its enantiomer. Even so, the resolution factor for the enantiomers exceeds eighty since good peak shapes are retained despite the strong retention.

It should be evident that, by making tris, tetrakis, etc. derivatives, selectivity can be elevated to rather large values. For example, the enantiomers of a tetrakis-analogue of compound 3 would be expected to show a separation factor in excess of 10^4 on CSP 1. While such selectivity is unnecessary (even highly undesirable) for analytical work, it would trivialize the preparative separation of enantiomers, reduc-

ing the process to filtration (à la affinity chromatography) rather than chromatography. However, the reduced solubility to be anticipated for such analytes and the number of stereoisomers expected to arise from *non-selective* derivatization are disadvantages that would be encountered in practice.

The simplistic doubling of $\Delta\Delta G$ seemingly underestimates the consequences of progressing from a mono-analyte to a bis-analyte. Our initial expectations were that, for entropic reasons, the mono-analytes would not be an accurate "half-model" for the bis-analytes. We expected that the $\Delta\Delta H$ contribution from the bis-analyte would be somewhat less than twice that of the mono-analyte since simultaneous dual interaction might not always be achieved. The bis-analyte was expected to show a significantly larger $\Delta\Delta S$, owing to its greater restriction of freedom while adsorbed. The entropic considerations here are similar to the well-known "chelation effect" in coordination chemistry[5]. To obtain values of $\Delta\Delta H$ and $\Delta\Delta S$, the temperature dependence of α was determined for analytes 2 and 3 on CSP 1. Methanol was used as a mobile phase to avoid runs of multi-hour duration. The α values observed for compounds 2 and 3 ranged from 3.31 and 29.5 at 0°C to 1.93 and 4.15 at 50°C. Plots of ln α *vs.* $1/T$ are linear and afford values for $\Delta\Delta H$ and $\Delta\Delta S$ of -0.52 ± 0.01 kcal mol^{-1} and -1.32 eu for compound 2 and -1.73 ± 0.1 kcal mol^{-1} and -4.65 eu for compound 3. While we believe that compounds 2 and 3 have basically the same chiral recognition processes available to them, the question as to why the $\Delta\Delta H$ value observed for compound 3 is so much larger than anticipated is fascinating and presently lacks a detailed answer. Analytes are retained by a blend of interactions. Clearly, the blend is different for analytes 2 and 3, but different in what way? If the answer were known, one might employ this insight in designing improved CSPs.

We are engaged in a general study of bis-analytes so as to refine our understanding of how to best employ this approach to the amplification of chiral recognition. Such studies may also provide a probe for estimation of average interstrand spacing and give insight into types of retention processes which might otherwise be difficult to study.

CONCLUSION

The use of multidentate analytes has been shown to amplify vastly chromatographic selectivity owing to the additivity of the binding effects. The ramifications of the approach are apt to be of greater preparative than analytical significance.

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