HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC STUDY ON THE ENANTIOSEPARATION OF FLUORINE CONTAINING CYCLIC AMINO ACID DERIVATIVES

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Abstract
The stereoisomers of several fluorinated cyclic β^3-amino acid derivatives and their nonfluorinated counterparts were separated on chiral stationary phases containing as chiral selectors cellulose tris-(3,5-dimethylphenyl carbamate), cellulose tris-(3-chloro-4-methylphenyl carbamate), cellulose tris-(4-methylbenzoate), cellulose tris-(4-chloro-3-methylphenyl carbamate), amylose tris-(3,5-dimethylphenyl carbamate) or amylose tris-(5-chloro-2-methylphenyl carbamate). The enantioseparations were carried out in normal-phase mode with n-hexane/alcohol/alkylamine mobile phases in the temperature range 5–40 °C. The effects of the mobile phase composition, the nature and concentration of the alcohol and alkylamine additives, the structures of the analytes and temperature on the separations were investigated.

Introduction
Chirality is eminently important for the modern pharmaceutical industry since many drug compounds are chiral molecules whose stereoisomers usually possess variant toxicological and pharmacological properties. In recent years, fluorine chemistry is an increasing interest in synthetic and medicinal chemistry in recent years because of its considerable impact in drug design. The property changes resulting from the replacement of one or more atoms in a molecule by fluorine may be especially significant from the aspect of biological activity. Fluorinated amino acid derivatives, for example, are of significance in medicinal chemistry in view of their more effective biological properties in comparison with those of their nonfluorinated derivatives: the special characteristics of the C–F bond and the fluorine atom can lead to modified properties as concerns the interactions with active sites of bioreceptors or enzymes [1, 2].

Experimental
The chromatographic system was a 1100 Series HPLC system which was equipped with a solvent degasser, a pump, an autosampler, a column thermostat, a multiwavelength UV–Vis detector and ChemStation chromatographic data software (Agilent Technologies, Waldbronn, Germany), and a corona-charged aerosol detector from ESA Biosciences Inc. (Chelmsford, MA, USA). Chromatography was performed in isocratic mode at a flow rate of 1.0 mL/min and a column temperature of 25 °C. Detection was accomplished with a corona detector. The HPLC columns used were Lux Cellulose-1, Lux Cellulose-2, Lux Cellulose-3, Lux Cellulose-4, Lux Amylose-1 and Lux Amylose-2 (250 × 4.6mm i.d., 5 μm particle size for all columns; Phenomenex, Torrance, CA, USA). The dead-time (t_0) of the column was determined via the injection of tri-t-butylbenzene.

Results and discussion

Effects of the chromatographic conditions on the chiral separation of fluorinated cyclic amino acid derivatives

Compounds investigated in this study were chromatographed on six different chiral stationary phase (CSPs) with mobile phases of n-hexane containing different alcohols (EtOH, PrOH, 2-PrOH, BuOH or t-BuOH) and alkylamine additives. The nature and concentration of the alcohol added were expected to exert considerable effects on the retention, selectivity and resolution, that is, the ratio of the nonchiral and chiral interactions between the CSP and the analytes might depend on these factors. The nonfluorinated derivatives exhibited lower k_1 values than those of the fluorinated ones. The highest α values were observed for the fluorinated derivatives when 2-PrOH was added. The changes in selectivity might be explained by specific changes in the supramolecular structure of the selector. The change caused in the structure of the CSP by the change of the nature and concentration of the alcohol may affect the chiral selectivity of the CSP, depending on the size and structure of the analyte [3].

The six polysaccharide-based CSPs display complementary features, with either some degree of separation efficiency or alternatively probable baseline separation. Separations were carried out with the same mobile phase composition, *n*-hexane–2-PrOH–DEA (90:10:0.1 v/v/v). Of the cellulose-based columns, CSP-3 appeared to be the least effective, since the tris-4-methylbenzoate substitution on the cellulose backbone was disadvantageous in these cases. Comparison of CSP-2 and CSP-4 revealed that k_1 was generally higher on CSP-4 than on CSP-2, while the α and R_S values obtained on CSP-2 were higher. It seems that the nonfluorinated and fluorinated cyclic amino acid derivatives may have stronger nonselective interactions with CSP-4 than with CSP-2. Comparison of the cellulose- and amylose-based selectors substituted with the same tris-(3,5-dimethylphenyl carbamate) (CSP-1 and CSP-5) or similar tris-(3- chloro-4-methylphenyl) carbamate (CSP-2) and amylose tris-(5- chloro-2-methylphenyl) carbamate (CSP-6) moieties demonstrated that k_1 was generally higher on the amylose-based CSPs. Higher α and R_S values were usually obtained on CSP-5 than on CSP-1 indicating more favorable interactions with the selector possessing a helical structure. The selectors' distinct structures and the different positions of the methyl and chlorosubstituents on the aromatic ring in the two selectors affectedthe selector–selectand interactions in different ways, resulting in different chromatographic behavior.

Effect of fluoro substitution

Fluoro substitution on the ring affects the polarity and steric arrangement and therefore the interactions between the selector and the analyte. Nonfluorinated derivatives was observed to have generally smaller k_1 values on all of the columns with the exception of CSP-6, on which the nonfluorinated derivatives were retained more strongly. The higher retention of the fluorinated derivatives was generally accompanied by higher α and R_S values. The fluorinated derivatives are probably capable of H-bonding interactions with the carbamate moiety of the selector. On the cellulose-based CSPs, higher k_1 values were generally obtained for analytes containing a double bond. Enhanced polarity of the molecules may improve the aromatic π–π interactions between the selectand and the selector, resulting in higher k_1 values, but this was not always accompanied by increased selectivity.

Effects of temperature and thermodynamic parameters

In order to investigate the effects of temperature on the chromatographic parameters, a variable-temperature study was carried out on CSP-2 and CSP-6 over the temperature range 5–40 °C. The retention factors and selectivities generally decreased with increasing temperature. All of the van't Hoff plots (ln α vs. 1/T) were linear, with good correlation coefficients. The Δ(ΔH)° and Δ(ΔS)° data exhibit both negative and positive values. When Δ(ΔH)° and Δ(ΔS)° were both negative, the secondeluted enantiomer fitted more strongly into the cavity of the selector, forming a more stable complex than in the case of the first-eluted enantiomer, and the negative entropy was less favorable for enantioseparation. The enantioseparation was enthalpically driven, as in the common case, and the selectivity decreased with increasing temperature. When Δ(ΔH)° and Δ(ΔS)° were both positive, the separation factor increased with increasing temperature, in parallel with decreasing retention. In this case, the change in the adsorption enthalpy with increasing temperature exerted a positive effect on the enantioselectivity. On the other hand, the positive Δ(ΔS°) compensated for the positive Δ(ΔH°) and resulted in a negative Δ(ΔG°). The enantioseparation was entropically driven.

Conclusion

The chromatographic retention behavior and resolution proved to depend on the nature and concentration of the alcohol additive, the temperature and the positions of the substituents. The nature of the alkylamine additives exhibited only slight effects on the chromatographic parameters. Of the studied CSPs, cellulose tris-(3-chloro-4-methylphenyl carbamate) and amylose tris-(3,5-dimethylphenyl carbamate) appeared the best suitable for the direct enantioseparation of the studied derivatives. The values of thermodynamic parameters such as the changes in Δ(ΔH°), Δ(ΔS°) and Δ(ΔG°) proved to depend on the structures of the analytes and on the chiral selector used. Most of the separations were enthalpically driven, but entropically driven separations were also observed.

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