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High-performance liquid chromatographic enantioseparation of unusual amino acid derivatives with axial chirality on polysaccharide-based chiral stationary phases

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ABSTRACT

The successful enantioseparation of axially chiral amino acid derivatives containing a cyclohexylidene moiety on an analytical and semipreparative scale was achieved for the first time by HPLC using polysaccharide-based chiral stationary phases. Racemic methyl *N*-benzoylamino esters, easily obtained by methanolysis of the corresponding 5(4*H*)-oxazolones, were subjected to chiral HPLC resolution using chiral stationary phases based on immobilized 3,5-dimethylphenylcarbamate derivatives of amylose (Chiralpak® IA column) or cellulose (Chiralpak® IB column). The behaviour of both selectors under different elution conditions was evaluated and compared. The amylose column showed better performance than the cellulose column for all enantiomers tested. The semipreparative resolution of axially chiral amino acid derivatives with different side chains has been achieved on a 250 mm × 20 mm ID Chiralpak® IA column using the appropriate mixture of *n*-hexane/chlorofom/ethanol as eluent by successive injections of a solution of the sample in chloroform. Using this protocol up to 120 mg of each enantiomer of the corresponding axially chiral amino acid derivative were obtained from 300 mg of racemate. [(*Sa*)-**2a**, 105 mg; (*Ra*)-**2a**, 60 mg, [(*Sa*)-**2b**, 105 mg; (*Ra*)-**2b**, 90 mg, [(*Sa*)-**2c**, 120 mg; (*Ra*)-**2c**, 100 mg].

1. Introduction

α-Amino acids are considered to be amongst the most important building blocks in chemistry. Apart from being the structural subunits of proteins, peptides and many secondary metabolites they are versatile chiral starting materials for the synthesis of peptides, alkaloids, antibiotics and more complex molecules with biological activities [1–3]. Amino acids have also been used as chiral auxiliaries, ligands and catalysts in asymmetric synthesis [4–8].

The design and synthesis of new α-amino acids with unusual structural features that can provide peptides with improved biological properties, more versatile chiral synthons or catalysts capable of inducing higher asymmetry is a subject of continued interest [9–14].

In most of the newly designed chiral amino acids, chirality relies on the presence of one or more a stereogenic atoms. Chirality may arise from another type of molecular asymmetry, namely the presence of a chiral axis. In this context, atropoisomeric α-amino acids with a biaryl axis in their structure have been synthesised [15–19] and resolved [20,21], and the behaviour of model peptides that incorporate these unusual amino acids has been studied in detail [22–27].

In the course of our research we prepared racemic (4-substituted cyclohexylidene)glycines (Fig. 1), another family of axially chiral amino acids, which can be considered as elongated structural analogues of parent amino acids, and small peptides derived from them [28–30]. We became interested in the development of a practical procedure for the isolation of these axially chiral amino acids in enantiomerically pure form.

High-performance liquid chromatography using chiral stationary phases is a powerful tool for the direct analysis of enantiomers. High-performance liquid chromatography on a semipreparative scale is considered to be one of the most efficient approaches to obtain small amounts of enantiomerically pure compounds in a reasonable time [31–33], which is of paramount importance in pharmaceutical research and drug development. Different protocols to perform the enantiomeric separation of chiral nonproteinogenic amino acids with stereogenic atoms by high-performance liquid chromatography have been described [34]. As far as axially chiral amino acids are concerned, the analytical resolution of atropoisomeric α-amino acid Bin has been performed on a β-cyclodextrin-based chiral stationary phase, ChiralDex [35].

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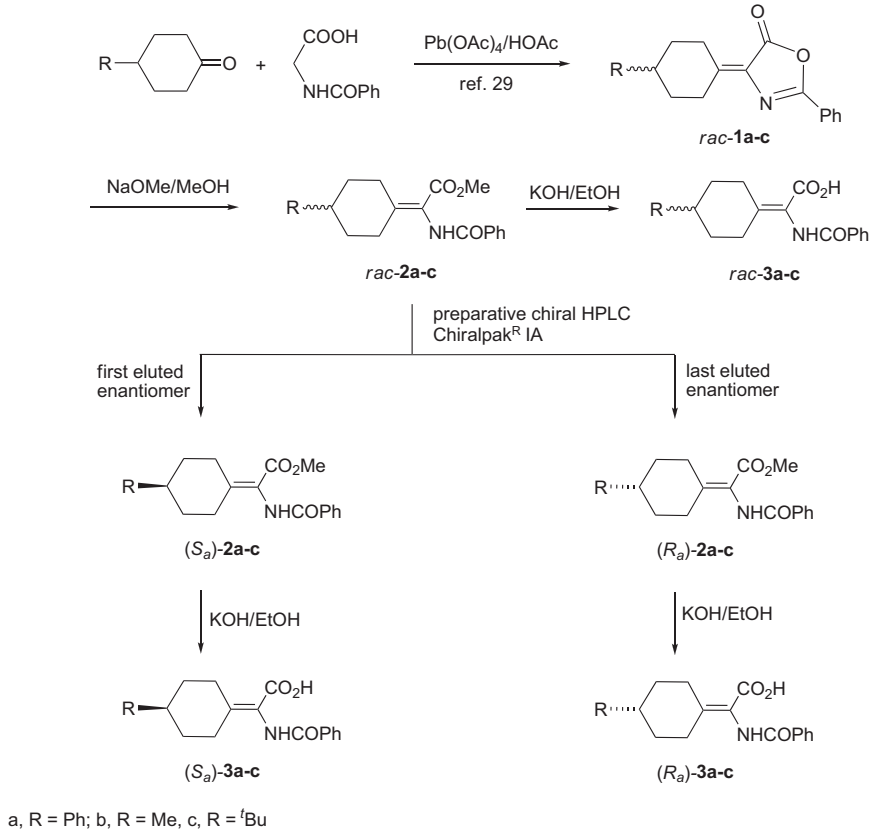

Fig. 1. Synthesis and structures of compounds **2a**–**c**.

Nevertheless, to the best of our knowledge work has not been published on the development of enantioselective chromatographic protocols for the quantitative determination and preparative resolution of axially chiral amino acids containing a cyclohexylidene moiety [36].

Our efforts were focused on developing chromatographic protocols to perform the enantioseparation of axially chiral (4-substituted cyclohexylidene)glycine derivatives on an analytical and semipreparative scale by high-performance liquid chromatography using chiral stationary phases. Among the different chiral stationary phases available, those based on polysaccharides are exceptionally versatile for the analytical separation of many different chiral compounds [37]. In the work described here, chiral stationary phases (CSPs) based on immobilized amylose and cellulose were chosen since they are particularly useful for preparative-scale enantioseparation due to the combination of excellent chiral recognition properties, compatibility with organic solvents and high loading capacity [38,39]. Moreover, these stationary phases are commercially available.

2. Experimental

2.1. Materials and methods (chemicals and reagents)

All the reagents and solvents were reagent grade and were used without further purification unless otherwise specified. *n*-Hexane, isopropanol, ethanol, acetone and chloroform used for HLPC separations were chromoscan grade from LabScan (Avantor Performance Materials Poland S.A., Gliwice, Poland). Reactions were magnetically stirred and monitored by thin-layer chromatography (TLC) on 0.25-mm silica gel plates. UV light, *p*-anisaldehyde, ninhydrin and phosphomolybdic acid sprays were applied for visualization. 5(4*H*)-Oxazolones **1a**, **1b** and **1c** were prepared according to our previously described procedure [29].

2.2. Instrumentation

HPLC separations were carried out on a Waters HPLC system (Waters Corporation, Milford, USA) consisting of an M-600 low-pressure gradient pump, an M-2996 photodiode array detector and an M-2487 dual wavelength absorbance detector, to monitor analytical and preparative separations, respectively. The chromatographic data were acquired and processed with Millennium® chromatography manager software (Waters). A rheodine 7125 syringe-loading sample injector was equipped with 20- and 500-μL loops for analytical or semipreparative chromatography. Commercially available polysaccharide chiral stationary phases based on amylose tris(3,5-dimethylphenylcarbamate), Chiralpak® IA column, and cellulose tris(3,5-dimethylphenylcarbamate), Chiralpak® IB column (Chiral Technologies Europe, Illkirch Cedex, France), were used.

The HPLC analytical assays were carried out operating under isocratic conditions at room temperature on Chiralpak® IA and Chiralpak® IB 250 × 4.6 mm ID columns. Different binary and ternary mixtures of solvents were used as eluents. Samples were manually injected. The flow rate was 1 mL/min. The analyte concentration in injected solutions was 5 mg/mL and the injection volume was 5 μL. Detection was performed at multiple wavelengths for each compound. The capacity (k'), selectivity (α) and resolution (R_s) factors were calculated according to the equations $k' = (t_r - t_0)/t_0$, $\alpha = k'_2/k'_1$, $R_s = 2(t_2 - t_1)/(w_2 + w_1)$. Subscripts 1 and 2 refer to the first and second eluted enantiomer, respectively, t_r ($r = 1, 2$) are their retention times, and w_1 and w_2 denote their baseline peak widths; t_0 is the dead time.