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Diastereo- and enantioseparation of a N^{α}-Boc amino acid with a zwitterionic quinine-based stationary phase: Focus on the stereorecognition mechanism

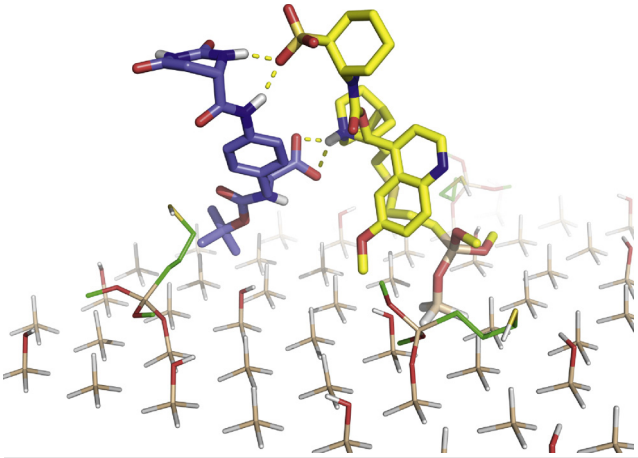
Federica Ianni[a], Andrea Carotti[a], Maura Marinozzi[a], Gloria Marcelli[a], Alessandro Di Michele[b], Roccaldo Sardella[a,*], Wolfgang Lindner[c], Benedetto Natalini[a]

[a] Department of Pharmaceutical Sciences, University of Perugia, Via Fabretti 48, 06123 Perugia, Italy
[b] Department of Physics and Geology, University of Perugia, Via Pascoli 1, 06123 Perugia, Italy
[c] Department of Analytical Chemistry, University of Vienna, Währinger Strasse 38, 1090 Vienna, Austria

HIGHLIGHTS

- The ZWIX(+) column allowed getting the Boc-Aph(Hor)-OH (**1**) isomeric peaks resolved.
- ECD studies and molecular dynamic simulations allowed to assign the elution order.
- Molecular descriptors revealed the active role of achiral elements of the CSP.

GRAPHICAL ABSTRACT



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ABSTRACT

A chiral chromatography method enabling the simultaneous diastereo- and enantioseparation of N^{α}-Boc-N^{4}-(hydroorotyl)-4-aminophenylalanine [Boc-Aph(Hor)-OH, **1**] was optimized with a quinine-based zwitterionic stationary phase. The polar-ionic eluent system consisting of ACN:MeOH:water—49.7:49.7:0.6 (v/v/v) with formic acid (4.0 mM) and diethylamine (2.5 mM), allowed the successful separation of the four acid stereoisomers: $\alpha_{\text{D,D-/D,L-}\mathbf{1}} = 1.08$; $\alpha_{\text{D,L-/L,D-}\mathbf{1}} = 1.08$; $\alpha_{\text{L,D-/L,L-}\mathbf{1}} = 1.40$.

According to the in-house developed synthetic procedure and the recorded electronic circular dichroism spectra, the following stereoisomeric elution order was readily established in the optimal chromatographic conditions: D,D-**1** < D,L-**1** < L,D-**1** < L,L-**1**.

With the aim of better understanding the molecular basis of the retention behaviour of the four stereoisomers in the employed chromatographic system and conditions, a computational protocol consisting in molecular dynamics simulations was applied. The use of the three descriptors INTER (in kcal mol^{-1}, encoding for the interaction energy between the selector SO unit and the whole system), INTER_SA (in kcal mol^{-1}, encoding for the interaction energy between SO and the sole selectand SA), and

* Corresponding author. Fax: +39 75 5855161.
E-mail address: roccaldo.sardella@unipg.it (R. Sardella).

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chiral stationary phases
Electronic circular dichroism

SELF (in kcal mol^{-1}, encoding for the conformational energy of SA relative to its minimum energy registered by the collected snapshots) revealed the active role of achiral sub-structural elements of the chiral stationary phase and eluent components in the overall stereorecognition mechanism.

1. Introduction

Despite the initial skepticism from the pharmaceutical industry, peptide therapeutics are quickly increasing in the global drug market while advances in fields such as chemical synthesis and peptide formulation have been made in recent decades [1]. At present, around 60–70 approved peptide drugs are on the market with a considerable commercial success. The main therapeutic indications include cancer and metabolic diseases, even if some progress is being made in other clinical areas [2,3]. Evidently, chemical synthesis of peptide drugs requires starting materials that often are unnatural amino acids, endowed with high stereochemical purity. For this reason, the rapid and accurate determination of the optical purity of free or *N*-protected constitutive amino acids and the deriving peptides still remains a field of great interest.

In the frame of a research collaboration with a company committed to manufacturing active pharmaceutical ingredients, we became interested in the multigram scale synthesis of N^{α}-Boc-N^{4}-(L-hydroorotyl)-L-4-aminophenylalanine [Boc-Aph (Hor)-OH, L,L-**1**] (Scheme 1), the *N*-protected form of the fifth amino amino acid of a synthetic peptide, used for the treatment of prostate cancer. Although the synthesis of **1** was already known [4] a method for the determination of its optical purity has not been published so far. More to the point, in consideration of the fact that **1** is endowed with two asymmetric carbons, a method providing simultaneous information about the diastereo- as well as the enantiopurity of the compound would be highly desirable. In this connection, the large scale preparation of L,L-**1** by a one-pot, telescoped procedure, as well as of the other three corresponding stereoisomers (Scheme 2) has been carried out.

So far, various CSPs operating with different stereorecognition mechanisms have been successfully employed for the enantioseparation of uncommon *N*-protected amino acids carrying multiple stereocentres [5–9]. However, in most cases, the selected "chiral column" allowed high enantioselectivity but suffered from low chemoselectivity, or *vice-versa*. One potential solution to overcome this limitation is to use multidimensional liquid chromatography (2D-LC) approaches, where the two dimensions are often based on different separation mechanisms [10–12]. Much more rarely, tandem column systems consisting of columns connected in series showed promising results with a significantly improved overall separation performance [13–16].

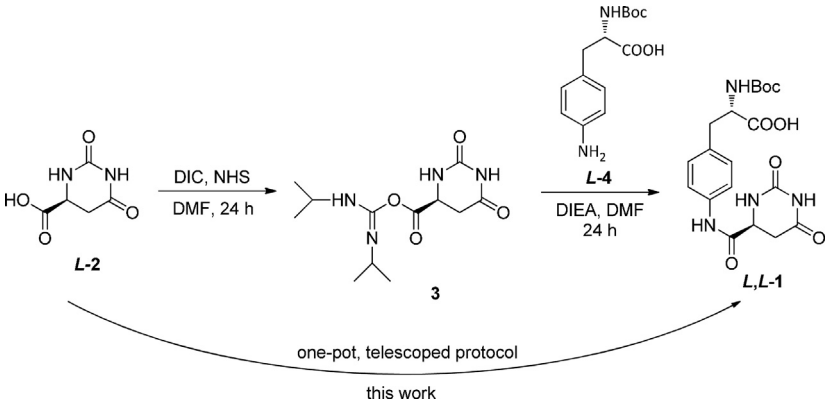
In a recent study [17], we described the successful simultaneous diastereo- and enantioseparation of two farnesoid X receptor (FXR) agonists carrying a carboxylic group with a weak anion-exchanger-based CSP (WAX-CSP), incorporating a quinine (QN) carbamate derivative as the chiral selector (SO) unit. A considerable increase in terms of overall stereoselectivity towards acidic compounds is exhibited by the second generation of this class of CSPs based on *Cinchona* alkaloids: the zwitterionic (ZWIX) CSPs, prepared by the rational fusion of the QN- or quinidine (QD)-based scaffold (that is, the WAX site) with aminocyclohexanesulfonic acid residues (acting as strong cation-exchange sites, SCX) (Fig. 1, CSP 1 and CSP 2, respectively) [18–22].

The appreciable chemo- and stereoselectivity demonstrated by CSP 1 and CSP 2 for acids, bases and zwitterion compounds with one or more chiral centres [19,23] along with the chemical nature of **1** (Scheme 1) directed us to select this class of material for the overall stereoseparation of its isomers. Besides the efforts made to improve the macroscopic manifestations of stereoselectivity, we also tried to understand the details of the molecular recognition mechanism underlying the selective binding phenomena. To fulfil this task, molecular dynamic simulations and electronic circular dichroism (ECD) investigations were independently carried out, taking advantage from the virtues of both techniques. Importantly, we demonstrated that to combine information from spectroscopic and computational investigations can be of practical aid to determine the stereoisomeric elution order when single reference stereoisomers are not available.

2. Material and methods

2.1. Chemicals

All the reagents used were of analytical grade. Acetonitrile (ACN), methanol (MeOH), and formic acid (FA), anhydrous *N*,*N*-dimethylformamide (DMF), L- and D,L-dihydroorotic acids were purchased from Sigma–Aldrich (Milano, Italy). Diethylamine (DEA) was purchased from Fluka (Buchs, Switzerland). 1,3-Diisopropylcarbodiimide (DIC) and *N*-hydroxy succinimide (NHS) were purchased from Alfa Aesar (Karlsruhe, Germany).



Scheme 1. Two-step- and one-pot, telescoped procedure for the preparation of Boc-Aph(Hor)-OH (L,L-**1**).