Journal of Pharmaceutical and Biomedical Analysis 76 (2013) 183–191

Contents lists available at SciVerse ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Development of the high-performance liquid chromatographic method for the enantioseparation of unusual glycine ester analogs on polysaccharide-based chiral stationary phases

Anita Aranyi[a], István Ilisz[a], Nóra Grecsó[a], Renáta Csütörtöki[b], István Szatmári[b], Ferenc Fülöp[b], Antal Péter[a,*]

[a] Department of Inorganic and Analytical Chemistry, University of Szeged, Dóm tér 7, H-6720 Szeged, Hungary
[b] Institute of Pharmaceutical Chemistry, University of Szeged, Eötvös u. 6, H-6720 Szeged, Hungary

ARTICLE INFO

Article history:
Received 20 November 2012
Received in revised form 5 December 2012
Accepted 27 December 2012
Available online 3 January 2013

Keywords:
Column liquid chromatography
Enantiomer separation
Gly analogs
Lux Cellulose columns
Lux Amylose-2 column

ABSTRACT

The stereoisomers of ten unusual amino acid analogs, 1- and 2-naphthol-substituted glycine and its ester derivatives, were separated on chiral stationary phases containing the chiral selectors cellulose *tris*-(3,5-dimethylphenylcarbamate) (Cellulose-1), cellulose *tris*-(3-chloro-4-methylphenylcarbamate) (Cellulose-2), cellulose *tris*-(4-methylbenzoate) (Cellulose-3), cellulose *tris*-(4-chloro-3-methylphenylcarbamate) (Cellulose-4) and amylose *tris*-(5-chloro-2-methylphenylcarbamate) (Amylose-2). Experiments were performed in normal-phase mode with *n*-heptane/alcohol/diethylamine mobile phases in a wide temperature range: 5–50 °C. Thermodynamic parameters were calculated from plots of $\ln k$ or $\ln \alpha$ *vs.* $1/T$. $\Delta(\Delta H^\circ)$ ranged from −10.1 to 6.2 kJ mol^{-1}, $\Delta(\Delta S^\circ)$ from −31.5 to 22.5 J mol^{-1} K^{-1} and $-\Delta(\Delta G^\circ)$ from 0.4 to 1.4 kJ mol^{-1}, and both enthalpy and entropy-controlled enantioseparations were observed. The latter was advantageous with regard to the shorter retention and greater selectivity at high temperature. The sequence of elution of the stereoisomers was determined in some cases.

1. Introduction

The Mannich reaction [1] is an important C—C bond formation reaction that is widely used in the syntheses of secondary and tertiary amine derivatives and as a key step in the syntheses of many bioactive molecules and complex natural products [2]. One of its special variations is the modified Mannich reaction (mMR) in which electron-rich aromatic compounds such as 1- or 2-naphthol are applied. The reaction was later extended to use quinolinol or isoquinolinol as starting compounds in the mMR [3].

Many important biological and pharmacological activities have been found for aminonaphthols, aminoquinolinols and aminoisoquinolinols prepared *via* mMR. Functionalized quinolines and isoquinolines are widely employed as antimalarial, anti-asthmatic or anti-inflammatory agents or as antibacterial, antihypertensive and tyrosine kinase PDGF-RTK-inhibiting agents. In an integrated, virtual database screening, 7-[anilino(phenyl)methyl]-2-methyl-8-quinolinol, and its derivatives, were found to be a promising new class of nonpeptide inhibitors of the MDM2-p53 interaction [4]. Recent, biochemical and X-ray crystallographic studies confirmed that anilinoquinolinol derivatives were actually covalent inhibitors of MIF tautomerase [5], while another virtual screening demonstrated that 7-[(3-fluorophenylamino)(pyridin-2-yl)methyl]-8-quinolinol was an MIF-CD74 inhibitor at low micromolar concentrations, acting as a weak tautomerase inhibitor [6]. The *in vitro* efficacy of 7-substituted-8-hydroxyquinolines containing sulphonamide moiety, against *Escherichia coli*, *Vibria cholerae*, *Achromobacter hydrophilis*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Salmonella typhii*, *Plesiomonas schigellaides*, *Proteus vulgaris*, *Citrobacter* and *C. ovis* revealed that derivatives containing a 2-pyrimidinyl group on the sulphonamide side were the most effective [7]. de Witte et al. evaluated the *in vitro* and *in vivo* P-glycoprotein (P-gp)-modulating activities of the products of α-aminobenzylnaphthols and tylosine by using human MDR1 gene-transfected and parental L5178 mouse lymphoma cell lines. The most promising compound was *N*-tylosyl-1-α-amino-(3-bromophenyl)methyl-2-naphthol [8]. On the other hand, a series of racemic 1-((4-(2-(dialkylamino)ethoxy)phenyl)-(2-hydroxynaphthalen-1-yl)methyl)piperidin-4-ols were evaluated against estrogen-responsive human MCF-7 breast cancer cells [9]. On the other hand, aminonaphthols, aminoquinolinols and aminoisoquinolinols following the ring closure can serve new compounds with potential pharmacological activities.

* Corresponding author. Tel.: +36 62 544000x3656; fax: +36 62 544340.
E-mail address: apeter@chem.u-szeged.hu (A. Péter).

0731-7085/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jpba.2012.12.030

Naphthoxazinone derivatives have received considerable attention in view of the interesting pharmacological properties associated with this heterocyclic scaffold [10–12]. It has been reported that they act as antibacterial agents [13], while a benzoxazinone derivative was approved by the FDA as a non-nucleoside reverse transcriptase inhibitor in 1998, and is currently in clinical use for the treatment of AIDS [14].

Non-proteinogenic amino acids have often been used as important building blocks for the construction of compound libraries in the field of combinatorial chemistry for drug discovery [15]. In our previous experiments, hydroxynaphthyl-substituted glycine derivatives **2** and **7** were successfully prepared from 2- or 1-naphthol, glyoxylic acid and benzyl carbamate in methanol (MeOH) *via* a mMR in the presence of *p*-toluenesulfonic acid (*p*-TSA), followed by removal of the protecting group. Acidic hydrolysis of **2** and **7** resulted in the expected α-amino acids **1** and **6**. The optimized reaction conditions were extended by starting from ethanol (EtOH) leading to **3** and **8**, respectively (Fig. 1) [16].

Since the behavior of glycine analogs in biological systems depends strongly on their stereochemistry, there is a clear need for the elaboration of precise separation and identification methods by which their configurations can be assigned. One of the most frequently applied techniques is chiral high-performance liquid chromatography (HPLC). HPLC enantioseparations of analytes containing 1- or 2-naphthol-groups have been performed on cellulose- or amylose-based chiral stationary phases (CSPs) by Sztojkov-Ivanov et al. [17,18] and by Ilisz et al. [19,20], on β-cyclodextrin based CSP by Berkecz et al. [21] and by capillary electrophoresis with the application of substituted β-cyclodextrins and chiral crown ethers by Ilisz et al. [22]. Chankvetadze et al. [23–27] proposed chloromethylphenylcarbamate derivatives of cellulose and amylose as useful CSPs for HPLC enantioseparations. These CSPs have recently been commercialized and applied for the enantioseparation of various chiral compounds [28–30].

Enantioselective retention and separation are influenced by the concentration and nature of the mobile phase components, together with other variables, such as the pH and temperature. In chromatographic enantioseparations, the relationship between the chromatographic data and the column temperature is as follows:

$$\ln k = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} + \ln \phi \quad (1)$$

in which k is the retention factor, α is the separation factor, ΔH° is the enthalpy of transfer of the solute from the mobile phase to the stationary phase, ΔS° is the entropy of transfer of the solute from the mobile phase to the stationary phase, R is the gas constant, T is temperature and ϕ is the reversal of the phase ratio (V_M/V_S) of the column.

Eq. (1) reveals that a plot of $\ln k$ *vs.* $1/T$ is linear, with slope $-\Delta H^\circ/R$ and intercept $\Delta S^\circ/R + \ln \phi$, if ΔH° is invariant with temperature. Since the value of ϕ is often not known, the $\Delta S^{\circ *}$ values [$\Delta S^{\circ *} = \Delta S^\circ + R \ln \phi$] calculated from the intercepts of the plots *via* Eq. (1) are generally used. Any uncertainty in the phase ratio affects all $\Delta S^{\circ *}$ values in the same manner.

$\Delta(\Delta H)^\circ$ and $\Delta(\Delta S)^\circ$ are the differences in ΔH° and ΔS°, respectively, for a given pair of enantiomers:

$$\Delta(\Delta H)^\circ = \Delta H_2{}^\circ - \Delta H_1{}^\circ \quad \text{and} \quad \Delta(\Delta S)^\circ = (\Delta S_2^{\circ *} - R \ln \phi) - (\Delta S_1^{\circ *} - R \ln \phi) = \Delta S_2^{\circ *} - \Delta S_1^{\circ *} \quad (2)$$

In chiral chromatography, however, the van't Hoff plots often deviate from linearity, possibly as a result of the inhomogeneity of the CSP surface, leading to a mixed retention mechanism [31]. Additionally, there are both achiral and chiral contributions to retention that can vary with a wide variety of experimental parameters [32–37].

If $\Delta(\Delta H)^\circ$ and $\Delta(\Delta S)^\circ$ are both negative, the enantioseparation is enthalpically driven, as in the common case. The second-eluted enantiomer forms a more stable complex with the selector than does the first-eluted enantiomer, with more unfavorable entropy for enantioseparation. The separation factor decreases with increasing temperature. If the enantioseparation is entropically driven, the separation factor increases with increasing temperature, and the column efficiency increases concurrently. These two features make entropically driven enantioseparation especially attractive.

The present paper describes normal-phase HPLC methods for the enantioseparation of new racemic Gly ester analogs possessing 1-naphthol and 2-naphthol side-chains (Fig. 1). The synthetically applicable series of Gly esters was designed and synthesized to contain methyl, ethyl, propyl and 2-propyl groups, thereby allowing a systematic study of the steric effects of ester groups on the HPLC parameters. The HPLC methods rely on the use of commercial polysaccharide-based chiral CSPs: cellulose *tris*-(3,5-dimethylphenylcarbamate) (Lux Cellulose-1), cellulose *tris*-(3-chloro-4-methylphenylcarbamate) (Lux Cellulose-2), cellulose *tris*-(4-methylbenzoate) (Lux Cellulose-3), cellulose *tris*-(4-chloro-3-methylphenylcarbamate) (Lux Cellulose-4) and amylose *tris*-(5-chloro-2-methylphenylcarbamate) (Lux Amylose-2). The effects of the mobile phase composition, the nature and concentration of the alcoholic modifier, the specific structural features of the analytes and selectors and temperature on the retention are discussed on the basis of the experimental data. The elution sequence was determined in some cases.

2. Experimental

2.1. Materials and methods

In our initial experiments, the syntheses of propyl (**4**, **9**) and 2-propyl (**5**, **10**) glycine ester derivatives were planned in a manner similar to that for their methyl and ethyl analogs, starting from 2- or 1-naphthol, glyoxylic acid and benzyl carbamate in the corresponding alcohol by using *p*-TSA as catalyst [16]. Since the reaction did not result in the desired products even after a long reaction time, **4**, **5**, **9** and **10** were synthesized by the thionyl chloride-mediated esterification of the correspondent α-amino acids **1** and **6** (Fig. 1).

2.2. General procedure for the synthesis of glycine ester analogs *4*, *5*, *9* and *10*

To cooled propanol (*n*-PrOH) or 2-propanol (2-PrOH) (20 ml) at −10 °C, 0.2 ml (2.76 mmol) of thionyl chloride was added dropwise with the temperature kept between −5 and +5 °C, the amino acid **1** or **6** (0.3 g, 1.38 mmol) was added in small portions. The mixture was allowed to reach room temperature and then heated under reflux until the thin-layer chromatography (TLC) showed no presence of the starting material (5–6 h). The solution was then concentrated under reduced pressure. The residue was made alkaline with 10% Na_2CO_3 solution and extracted with EtOAc (3× 10 ml). The organic extracts were combined, dried (Na_2SO_4) and concentrated under reduced pressure. The residue was crystallized with Et_2O (10 ml), filtered and recrystallized from 2-Pr_2O:EtOAc (3:1).

2.2.1. Propyl 2-amino-2-(2-hydroxynaphthalen-1-yl)acetate (*4*)

Beige crystals, mp: >350 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.63 (t, 3H, J = 7.0 Hz), 1.37–1.50 (m, 2H), 4.02 (t, 2H, J = 7.1 Hz), 5.74 (s, 1H), 7.09 (d, 1H, J = 8.1 Hz), 7.30 (t, 1H, J = 7.5 Hz), 7.46 (t, 1H, J = 7.2 Hz), 7.66–7.81 (m, 2H), 7.90 (d, 1H, J = 7.9 Hz). Anal. calcd. for