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Enantioselective recognition of 2,3-benzodiazepin-4-one derivatives with anticonvulsant activity on several polysaccharide chiral stationary phases

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Abstract

The retention behaviour of racemic 1-(4-aminophenyl)-1,2,3,5-tetrahydro-7,8-methylendioxy-4*H*-2,3-benzodiazepin-4-one derivatives with anticonvulsant activity on several chiral stationary phases was investigated. The selective performances of six polysaccharide phases, namely, Chiralcel OA, OD, OF, OG, OJ and Chiralpak AD were studied and normal phase HPLC methods were optimized to separate the enantiomeric forms of this class of compounds. The chiral recognition mechanism between the analytes and the chiral selectors was discussed. A molecular modeling study was carried out with the aim to explore the enantioselective molecular recognition process with the Chiralcel OG stationary phase.

Keywords: Chiral separation; Benzodiazepines; Polysaccharide chiral stationary phases; Enantiomer separation; Docking

1. Introduction

In recent years, the impact of chirality in the design, development and utilization of drugs has gained widespread interest.

Chiral drugs can differ substantially in their pharmacological activity depending on their absolute configuration. Often only one of the antipodes is pharmacologically active, while the other may be inactive, or even toxic.

Therefore, there is a dramatically increasing demand for enantioselective techniques useful to separate and isolate racemates, as well as to control the optical purity of the separated enantiomers along the development process of new targets.

Polysaccharides such as cellulose and amylose are the most accessible optical active polymers and their derivatives were widely used in HPLC for chiral resolution of many different racemic compounds [1]. In this work polysaccharide phases were used for the separation of a homologous series of congeneric 2,3-benzodiazepines.

In a search for anticonvulsant agents, racemic 1-(4-aminophenyl)-7,8-methylenedioxy-1,2,3,5-tetrahydro-4*H*-2,3-benzodiazepin-4-one and its 3-N-alkylcarbamoyl derivatives (Fig. 1) were synthesized [2] and proved to possess marked anticonvulsant properties. Normal phase HPLC methods were optimized to separate the enantiomeric forms of this class of compounds. Cellulose tris acetate and tris 4-methylbenzoate (Chiralcel OA and OJ) and cellulose or amylose tris phenyl carbamate (Chiralcel OD, OG, OF and Chiralpack AD) derivatives as chiral stationary phases were used and interactions between substances and the chiral phases were studied.

The investigation of the influence of small structural changes in this series of related analytes on the enantioseparation would provide some information about the recognition mechanism on polysaccharide stationary phases. Therefore, the selectivity (α), the enantiomeric resolution (Rs) of the racemates, and the capacity factors of the least and of the most retained enantiomers (k_1 and k_2, respectively) were calculated and compared.

Recently Okamoto et al. have reported detailed computational studies about enantioselective recognition processes between CSP based on phenylcarbamate derivatives of cellulose using different theoretical approaches [3]. Extensive reviews published by the same Japanese group are useful to understand

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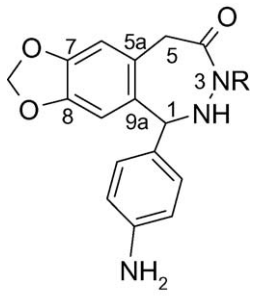


1, R=H
2, R=CONHCH$_3$
3, R=CONHC$_2$H$_5$
4, R=CONHC$_3$H$_7$

Fig. 1. Structures of the 1-(4-aminophenyl)-7,8-methylendioxy-1,2,3,5-tetrahydro-4*H*-2,3-benzodiazepin-4-one derivatives **1**–**4**.

details about the separation using polysaccharide derivatives as CSP [4–6].

In order to achieve more information about recognition mechanism, a docking study concerning the interactions of the lead compound (**1**) with the Chiralcel OG stationary phase was carried out.

2. Experimental

2.1. Chemicals and reagents

1-(4-Aminophenyl)-7,8-methylenedioxy-1,2,3,5-tetrahydro-4*H*-2,3-benzodiazepin-4-ones (**1**–**4**) were synthesized as previously reported [2]. The solvents used were HPLC grade *n*-hexane, 2-propanol and ethanol (Merck, Darmstadt, Germany).

2.2. Chromatographic conditions

HPLC apparatus was a Merck Hitachi chromatographic system pump (LaChrom L-7100) equipped with a septumless injector (Rheodyne 7125-075) and a column oven (LaChrom L-7300). An UV detector (LaChrom L-7400) was used. Peak area integrations were performed using a D-7000 HPLC system manager program. The columns used were: Chiralcel OA (cellulose tris acetate), Chiralcel OD (cellulose tris 3,5-dimethylphenylcarbamate), Chiralcel OF (cellulose tris 4-chlorophenylcarbamate), Chiralcel OG (cellulose tris 4-methylphenylcarbamate), Chiralcel OJ (cellulose tris 4-methylbenzoate) and Chiralpak AD (amylose tris 3,5-dimethylphenylcarbamate). All the columns were 25 cm × 0.46 cm size packed with a chiral stationary phase of 10 μm size and were purchased from Daicel Chemical Industries (Tokyo, Japan).

HPLC conditions for mobile phases were as follows: *n*-hexane/ethanol, 20/80 (v/v), flow rate 0.8 mL min^{-1} for Chiralcel OD, OG, OJ and Chiralpak AD; *n*-hexane/2-propanol, 20/80 (v/v), flow rate 1.0 mL min^{-1} for Chiralcel OA; *n*-hexane/2-propanol, 50/50 (v/v), flow rate 0.65 mL min^{-1} for Chiralcel OF. Strong retention of derivatives under study involves chromatographic conditions next to limits recommended by manufacturers for each column, such as the choice of ethanol than 2-propanol. This caused high backpressure that limited the flow rate. For Chiralcel OA the alcoholic modifier was changed because with ethanol no resolution was obtained and the flow rate was increased in order to obtain shorter retention times; for Chiralcel OF the alcoholic modifier was changed because the ethanol is not allowed by instruction manual of the column. All the experiments were carried out at 20 ± 1 °C. The detection was recorded at 247 nm. The solutes (0.1 mg mL^{-1}) were dissolved in ethanol and filtered through a 0.45 μm Sartorius filter membrane. The stability of the solutions at 20 °C was checked by HPLC analysis every third day for two months and no racemization was observed.

2.3. Molecular modeling study

2.3.1. First step: conformational search of analyte *1*

The (*S*)-**1** enantiomer was built by the Maestro Graphical User Interface (GUI) of the MacroModel package [7]. The conformational search was performed by Monte Carlo 2000 randomizing the rotatable bonds of (*S*)-**1** and energy minimizing all accepted conformations according to the AMBER* united atoms force field. The conformational deduplication was carried out applying the standard criterion proposed in the BatchMin program (internal energy difference within 1 kcal mol^{-1} and root mean square deviation in the atomic coordinates lower than 0.25 Å). The (*R*)-**1** enantiomer was obtained by sign inversion of the *Z* atomic coordinates.

2.3.2. Second step: model of Chiralcel OG polymer

According to a previously reported procedure [8] the Maestro GUI was used to build glucose units and to assembly in a helical conformation. The energy calculation was carried out with the same force field used in the previous step.

2.3.3. Third step: docking experiments

According to the MOLINE method [9] run with a resolution GRID equal to 6, for each couple of analyte conformer and the CSP model, 65712 configurations were systematically generated. Globally, 788544 configurations were computed and submitted to the SEL-5FD module of the MOLINE procedure obtaining 15185 and 15348 bimolecular complexes for the [(*R*)-**1**:CSP] and [(*S*)-**1**:CSP], respectively. The OGRID module performed a further optimization relaxing these configurations and deduplicating identical ones up to 44 and 69, respectively within the first 3 kcal mol^{-1} above their energy global minimum. Also in this step the interaction energies were computed according to the AMBER* united atoms force field.

3. Results

The series of compounds examined shows a different pattern of substitution at 3-N position of 2,3-benzodiazepine nucleus,

which can influence different chromatographic parameters, useful in obtaining information about the possible chiral recognition mechanism.

Although the chiral recognition process is the sum of all the possible enantioselective and non enantioselective interactions between the solute and the CSP, a sequence of steps can be evaluated. This procedure provides an assessment of the different contributions and on overall view of the mechanism operating on the CSP.

It is generally recognized [10–15] that the main reason for optical resolution on CSPs is a stability difference in transient diastereomeric complexes between the enantiomers and the CSP. Moreover, the chiral recognition is also a function of the fit of the asymmetric analyte into a chiral groove of the CSP. Other potential interactions include π–π interactions between the aromatic ring of the CSP and the phenyl moiety of analyzed compounds, dipole-dipole interactions between the chiral solutes and the polar functionalities of the CSP (–OCOR or –OCONHR), Van der Waals interactions between the solute and the CSP and H-bonding interactions, which have significant impact on retention and enantioselectivity.

Thus, not only the steric, but also the electronic properties of a certain chiral compound, and of the CSP have to be taken into consideration [16–20].

With the aim of separating and studying the chromatographic behaviour of racemic 2,3-benzodiazepines **1**–**4**, an initial analysis was performed, for all CSPs at 20 °C, with *n*-hexane/ethanol, 20/80 (v/v). As baseline separation was not achieved for all the compounds under these conditions, the analysis was optimized by varying the alcoholic modifier. The selectivity with polysaccharide CSPs can be changed by using different alcohols in the mobile phase, as hydrogen bonding is possible both for the solute and for protic (proton-donating) modifiers. This results in competition between solute and solvent for the stationary phase, and this affects enantioselectivity and resolution [21]. The effect of structurally different alcohols on retention and stereoselectivity on polysaccharide-based CSPs was investigated by Wainer and co-workers [16,22–25]. It was suggested that alcohol in the mobile phase not only competes for chiral bonding sites with chiral solutes, but can also alter the steric environment of the chiral grooves on the CSP by binding to achiral sites at the groove or close to.

All the compounds under study showed strong retention, thus a relatively polar mobile phase, though still in normal phase chromatography, has to be used.

The benzodiazepine derivatives differ structurally in the substitution at N-3 position, which is not located on the chiral carbon atom. Yet, the length of the alkyl chain affects the selectivity though the parent compound **1** does not follow the same trend. This suggests interactions involving the lipophylic moiety of these substances, together with polar interactions of the amino group of the aniline moiety, that causes long retention and the necessity of high percentage of alcohol.

The chromatographic parameters, k, α and Rs, for all the compounds tested are given in Tables 1–3. The resolution factor (Rs) was calculated by using the equation: $\mathrm{Rs} = 1.18(\mathrm{tr}_2 - \mathrm{tr}_1)/(w_1 + w_2)$, where tr_2 and tr_1 are the retention times, and w_1 and w_2 are the peak widths at half height for the first and second eluted enantiomers, respectively. Figs. 2 and 3 report, respectively, enantioselectivity and resolution of compounds **1**–**4** on six polysaccharide phases. Representative chromatograms obtained

Table 1
The chromatographic parameters, k, α and Rs for enantiomeric resolution of the compounds tested (**1**–**4**) on polysaccharide chiral stationary phases using *n*-hexane–ethanol, 20:80, v/v as the mobile phase with a flow rate of 0.8 mL min^{-1}

	k_1	k_2	α	Rs
Chiralcel OJ				
1	5.11	14.69	2.88	3.21
2	2.46	6.83	2.78	2.99
3	4.83	13.11	2.71	2.69
4	4.01	9.48	2.36	2.36
Chiralcel OG				
1	7.64	38.71	5.07	7.59
2	6.15	6.59	1.18[a]	1.05
3	5.46	–	1	–
4	5.32	–	1	–
Chiralcel OD				
1	4.06	7.63	1.88	4.24
2	4.90	6.59	1.34	2.27
3	4.20	6.63	1.58	3.44
4	3.61	7.37	2.04	4.95
Chiralpak AD				
1	1.56	2.04	1.30	2.36
2	3.17	4.39	1.39	5.48
3	3.16	3.69	1.17	4.18
4	3.40	3.89	1.15	1.69

For details see Section 2.
[a] Mobile phase: *n*-hexane-ethanol, 20:80, v/v, containing 0.1% DEA.

Table 2
The chromatographic parameters, k, α and Rs for enantiomeric resolution of the compounds tested (**1**–**4**) on Chiralcel OA as chiral stationary phase using *n*-hexane-2-propanol, 20:80, v/v as the mobile phase with a flow rate of 1.0 mL min^{-1}

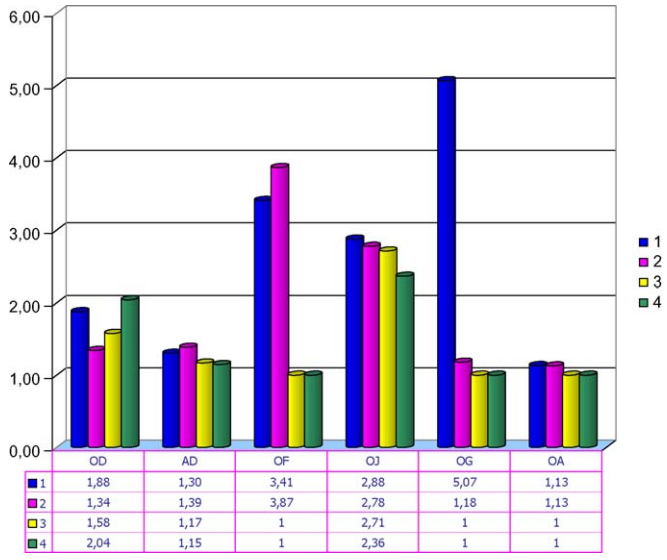
	k_1	k_2	α	Rs
Chiralcel OA				
1	39.78	45.09	1.13	0.29
2	32.91	–	1	–
3	14.54	–	1	–
4	14.27	–	1	–

For details see Section 2.

Table 3
The chromatographic parameters, k, α and Rs for enantiomeric resolution of the compounds tested (**1**–**4**) on Chiralcel OF as chiral stationary phase using *n*-hexane-2-propanol, 50:50, v/v as the mobile phase with a flow rate of 0.65 mL min^{-1}

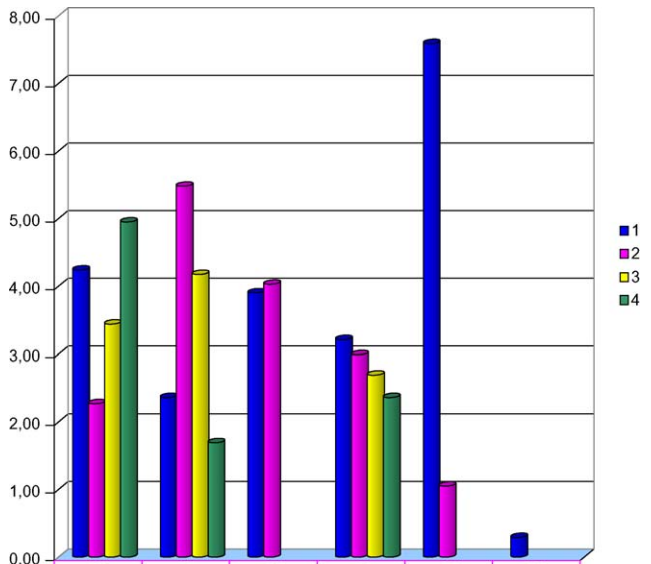
	k_1	k_2	α	Rs
Chiralcel OF				
1	17.95	61.22	3.41	3.90
2	7.66	29.61	3.87	4.03
3	4.71	–	1	–
4	4.37	–	1	–

For details see Section 2.



	OD	AD	OF	OJ	OG	OA
1	1,88	1,30	3,41	2,88	5,07	1,13
2	1,34	1,39	3,87	2,78	1,18	1,13
3	1,58	1,17	1	2,71	1	1
4	2,04	1,15	1	2,36	1	1

Fig. 2. Enantioselectivity of compounds **1–4** on six polysaccharide phases.



	OD	AD	OF	OJ	OG	OA
1	4,24	2,36	3,90	3,21	7,59	0,29
2	2,27	5,48	4,03	2,99	1,05	
3	3,44	4,18		2,69		
4	4,95	1,69		2,36		

Fig. 3. Resolution of compounds **1–4** on six polysaccharide phases.

for compounds in study when using Chiralcel OD and Chiralpak AD are reported, respectively, in Figs. 4 and 5.

Chiralcel OA and OJ are cellulose ester derivatives. Cellulose is a crystalline polymer composed of a linear poly-β-D-1,4-glucoside, with a helical structure in which each glucose residue lies near the helical axis but is twisted relative to the previous one [26,27]. Such chains are held together by intramolecular and intermolecular hydrogen bonds, thus forming a sort of sheets, that stack vertically and are staggered half a glucose unit. Thus, chiral grooves are created between sheets.

The absence of aromatic moiety in the Chiralcel OA is responsible of a low, or none resolution for all derivatives studied (Table 2). The phenyl moiety in the Chiralcel OJ, which represents another interaction site, allows to achieve good selectivity and resolution for all the compounds (Table 1). Such moiety is the most important in enantioselective recognition of these analytes, as it provides strong π–π bonding with aromatic groups of the compounds and increases the electron density on carbonyl, probably responsible of the hydrogen bonding with 4′-NH_2 and NH-groups of the benzodiazepine ring in compound **1**.

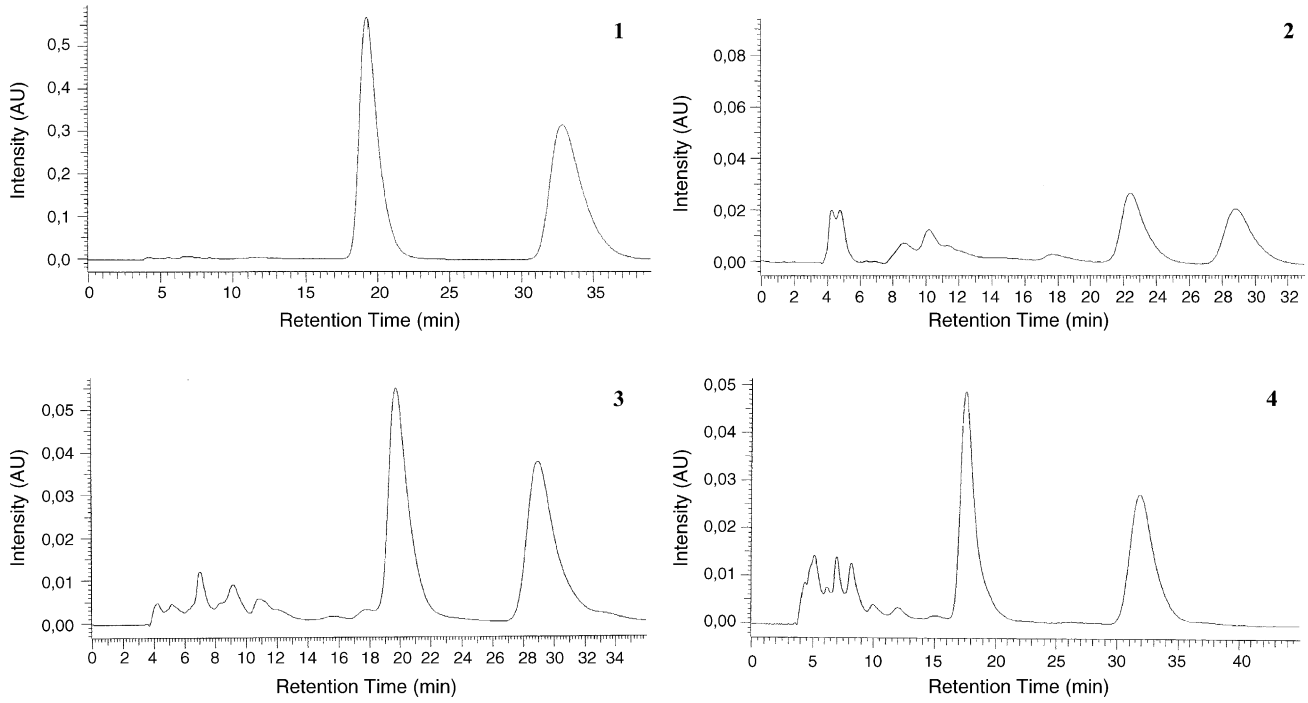


Fig. 4. Enantiomeric resolution of compounds **1–4** on Chiralcel OD column. Chromatographic conditions are described in Section 2.

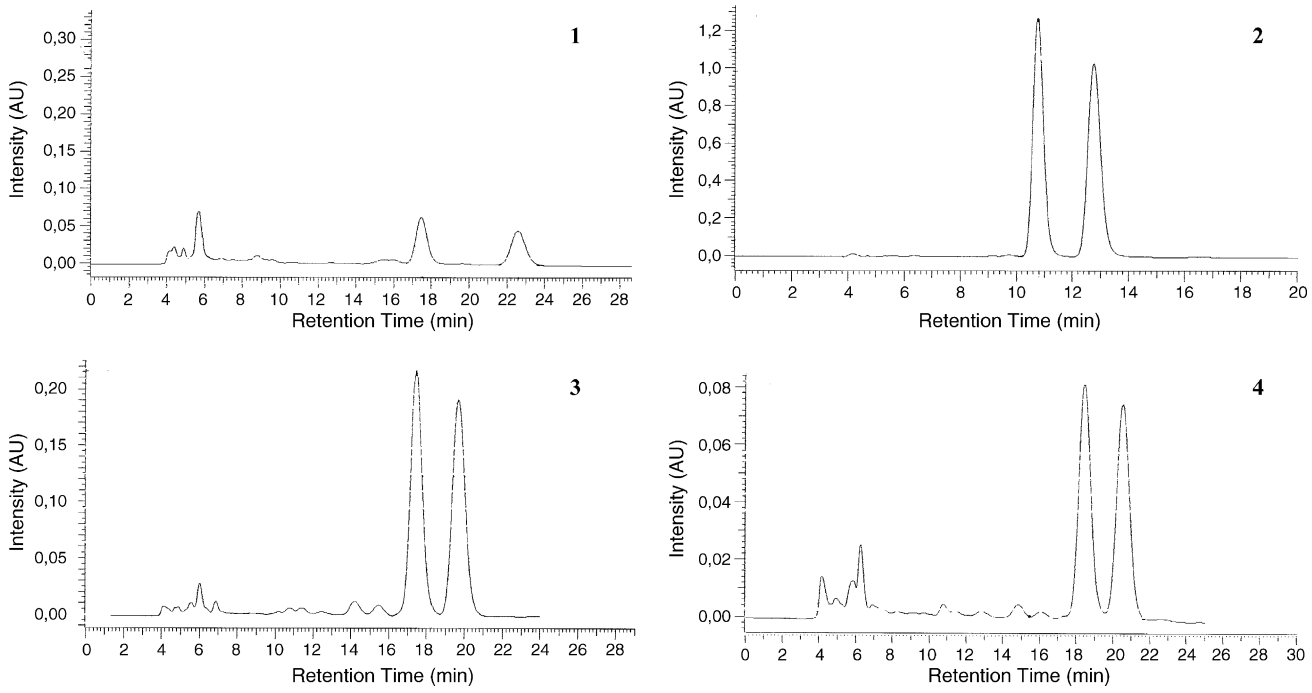


Fig. 5. Enantiomeric resolution of compounds **1–4** on Chiralpak AD column. Chromatographic conditions are described in Section 2.

The presence of different substituents at N-3 in derivatives **2–4** does not allow this kind of interaction with CSP, though the 3-*N*-alkylcarbamoyl moiety provides another hydrogen bonding site. The length of the chain decreases selectivity and resolution for derivatives with the highest degree of steric hindrance, but the retention has a different trend for the derivative **2**, where probably contribution of steric hindrance and hydrogen bonding interaction is less incisive.

Different interactions are involved by using OG, OF and OD columns, based on cellulose phenylcarbamate, that increase, in all cases, the retention. Moreover, the electron-withdrawing or electron-donating power of the substituent present on the phenyl ring influences differently the stereoselectivity.

The OG column presents the same substituent on the phenyl group with respect to the column OJ and shows improved α and Rs values only for compound **1** (Table 1). Under the same chromatographic conditions derivatives **2–4** are not separated and with the addition of diethylamine (DEA) only component **2** is resolved, while the other derivatives show low retention. Strongly basic compounds tend to adsorb on Daicel polysaccharide columns, resulting in broad/tailing peaks or in a decrease of enantioselectivity. The adsorption occurs at the most active sites on the silica support in the column. Amine additives, such as DEA, are commonly added to the mobile phase to improve peak shapes of basic compounds on polysaccharide-based CSPs [28–32]. These additives, adsorbed on the active sites, displace solute molecules and make these sites unavailable for solute adsorption.

Addition of amines rarely gives dramatic improvements in enantioselectivity, as nonideal separations in chiral chromatography are more due to non-covalent interactions different than silanol ones [33].

The presence of the electron-withdrawing substituent in column OF reduces the electronic density on the carbamate group and affects stereoselectivity (Table 3). Good selectivity and resolution are obtained only for compounds **1** and **2**, which present the lowest degree of steric hindrance; derivatives **3** and **4** are not resolved and show low retention. This is probably due to the prevalence of inclusion mechanism over electrostatic interactions during the process of chiral separation.

The Chiralcel OD and Chiralpack AD present the same kind of derivatization and differ only for the polysaccharide chiral part. The tris(3,5-dimethylphenylcarbamate) derivative of amylose (AD) is reported to possess a complementary character to its cellulose analogue (OD) for the enantioseparation of certain analytes [34]. On polysaccharide stationary phases, hydrogen bonding and π–π interactions are supposed to be the major analyte-chiral stationary phase interactions. However, the spatial arrangement of the enantiomers in the created chiral environment may play an important role too.

Good separations are attained with both, employing a mobile phase based on *n*-hexane and ethanol 20/80 (v/v), with α values higher for the OD column (Table 1). The effect of DEA was also investigated, but no improvement in separation was observed in all cases.

A linear increase of α and Rs values in function of the length of the alkyl chain is observed on OD column for the **2–4** 3-N-alkylcarbamoyl derivatives. An opposite behaviour is observed with the amylose-based AD column. This is due to the conformation of the chiral portion; the more planar surface of cellulose enables a better fitting of the components inside the chiral grooves; moreover, selectivity is higher for the longer-chained compounds; this is probably due to additional lipophylic interactions which stabilize the diastereomeric complex. This is not

observed for the AD column, as the helical structure of amylose enables a better fit for compounds with lower steric hindrance.

The computational study was carried out focusing our attention onto the separation of derivative **1** enantiomers by means of the OG column. This choice was justified mainly because the highest enantioselectivity value, that corresponds to a 0.964 kcal mol^{-1} of free energy difference ($\Delta\Delta G$) of complexation. Since the error of molecular mechanics energy calculation can be considered within the order of 0.5 kcal mol^{-1} value we modeled only this enantioselective interaction. Moreover, the stereochemical assignment was performed only for derivative **1**. The absolute configuration of (*R*)-**1** was assigned by X-ray crystallographic analysis on the second-eluted fraction [35].

In the first step the conformational search of the analyte **1** was carried out with standard molecular mechanics methods (see Section 2.3). In this search six conformers were obtained for each enantiomer: the first two conformers represent almost 100% of the Boltzmann population at room temperature. They differ mainly in the seven-membered ring conformation that induces a first extended (Conf 1) and a second folded (Conf 2) energy minimum structures (Fig. 6).

The second step was dedicated to build a model of Chiralcel OG stationary phase according to the procedure described by Yashima et al. for a similar CSP [8]. The glucose unit built with methoxy groups in positions 1 and 4 was energy minimized. A tetramer (4-mer) with a left-handed threefold (3/2) helix was assembled according to the X-ray analysis carried out by Vogt and Zugenmaier [36]. According to the IUPAC recommendations [37], linkage torsional angles between monomers were

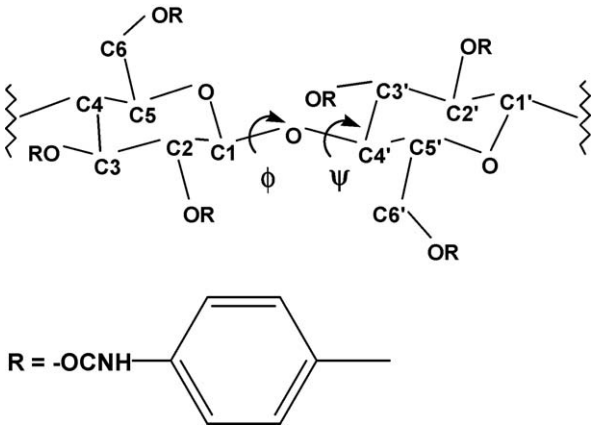


Fig. 7. Linkage between glucose monomers considered in the Chiralcel OG CSP.

defined by C5-O-C1-O-C4′ (ϕ) and C1-O-C4′-C5′ (ψ) and fixed to −85.6 and 82.0°, respectively, as shown in Fig. 7.

The third step was dedicated to the docking experiments performed according to the methodology developed and published, corresponding to the MOLINE approach [9]. The interaction energies of the most stable complexes were compared to the experimental elution data obtaining a good agreement. Basing on the most stable configurations (relative orientations), the (*R*)-**1** complex with the CSP resulted more favoured than the (*S*)-**1** one, with an interaction energy equal to −32.21 kcal mol^{-1} versus −31.05 kcal mol^{-1}.

In Fig. 8, the structural comparison of the two global minimum configurations is reported. Both of them highlight a

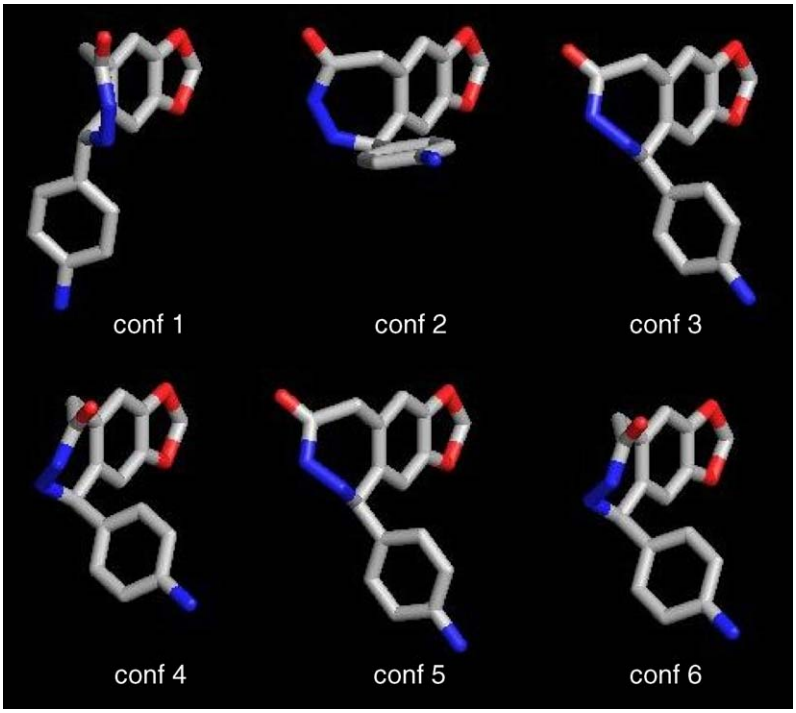


Fig. 6. Conformational Monte Carlo analysis results of compound **1** displayed in polytube rendering. For clarity no hydrogen atoms were reported. For each conformer the relative energy in kcal mol^{-1} and the Boltzmann population % at 300 K are reported in brackets: Conf 1 (0.00, 68.28); Conf 2 (0.47, 31.37); Conf 3 (3.56, 0.18); Conf 4 (3.56, 0.18); Conf 5 (6.10, 0.00); Conf 6 (6.57, 0.00).

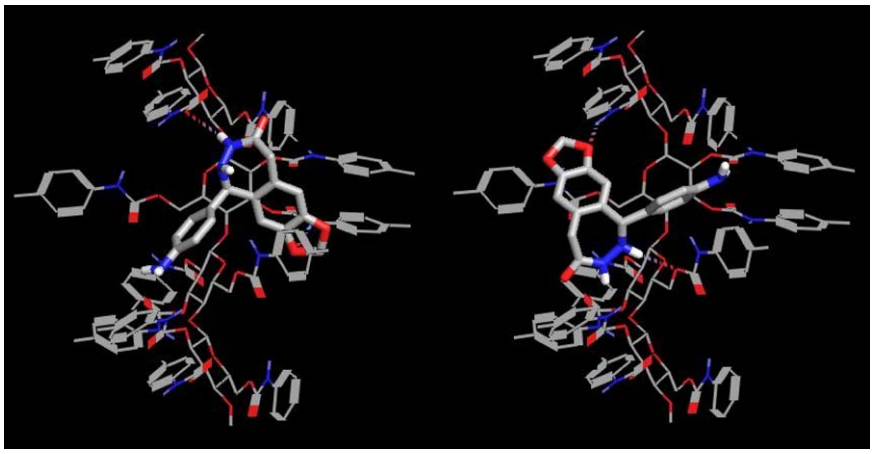


Fig. 8. Structural comparison between (*R*)-**1** (left polytube model) and (*S*)-**1** (right polytube model) most stable complexes with the Chiralcel OG (wireframe model). Intermolecular hydrogen bonds are reported as red dash lines.

common preference to accommodate the aromatic moiety in a binding pocket delimited by the 2-mer and 3-mer sugar rings and the 4-methylphenyl carbamate sidechain in 3′ and 2′ of the 1-mer and 4-mer residues, respectively. Derivative **1** enantiomers form intermolecular hydrogen bonds through the H on N-2 or N-3 and the O in 7 as donor and acceptor atoms, respectively. The counterparts onto the CSP are represented by both oxygen atoms and the amidic hydrogen of the carbamate sidechain (Fig. 8).

This study indicates that the capacities of the enantiomeric resolution of all 2,3-benzodiazepines studied on several polysaccharide based CSPs were in the order of Chiralcel OD > Chiralpak AD > Chiralcel OJ. The latter shows higher α values, but the capacity factors are increased. Onto the other CSPs, the enantiomeric resolution was observed only for derivatives **1–2** in the order of Chiralcel OG > OF > OA. Taking into the consideration the results obtained, one can conclude that the enantiomeric resolution of these anticonvulsant agents on the used CSPs is governed mainly by hydrogen bonds together with π–π and dipole–dipole interactions; moreover the chiral recognition is also a function of the fit of the lipophylic moiety of the analyte into a chiral cavity of the CSP.

The reported HPLC system can be used for the resolution of the racemic 1-(4-aminophenyl)-1,2,3,5-tetrahydro-7,8-methylendioxy-4*H*-2,3-benzodiazepin-4-one derivatives on a semi-preparative scale for further pharmacological investigations of the individual enantiomer of these anticonvulsant agents.

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