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Letter

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Computer-Aided Molecular Design of Asymmetric Pyrazole Derivatives with Exceptional Enantioselective Recognition toward the Chiralcel OJ-H Stationary Phase

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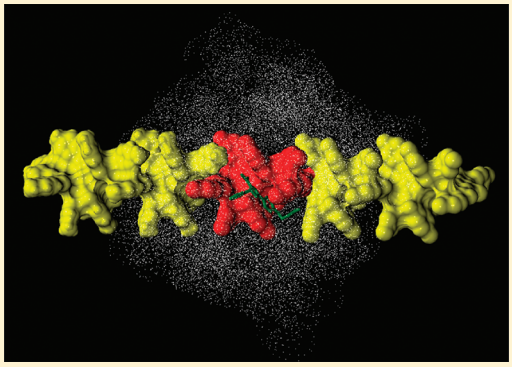
Supporting Information



ABSTRACT: A computer-aided design of novel asymmetric pyrazoles with improved enantioselective properties was performed by docking experiments starting from a model of Chiralcel OJ chiral in the stationary phase. Synthesis and HPLC experiments confirmed the theoretical prediction and led to a detailed investigation of the enantioselective recognition process. For the first time, looking at the time spent by each enantiomer in contact with the CSP during long molecular dynamic simulations, the experimental analytical trend has been reproduced.

The enantioselective high-performance liquid chromatography (HPLC) is a largely used technique to determine the enantiomeric excess of natural and synthetic chiral compounds and to obtain single enantiomers with high optical purity on a preparative scale. The use of chiral stationary phases (CSPs) in HPLC permits to resolve a chiral compound in a direct way, often without prior derivatization with optically active agents. The general mechanism of resolution is based on the formation of temporary labile diastereomeric associations between the enantiomers to be separated (selectands) and the component of the chiral chromatographic system (selector), fixed appropriately to an inert support, usually silica gel. Stereodependent interactions between the enantiomers and the selector lead to a thermodynamic stability difference in the labile diastereomeric complexes and consequently to different retention times within the column. The enantiomer establishing the least stable complex is the first eluting one. The recognition ability of a CSP can be quantitatively evaluated using the enantioseparation factor (α), defined as the ratio between the retention factors (k′) of the two enantiomers. The chromatographic parameter α is correlated to the difference in free standard energy of two transient diastereomeric enantiomer−CSP complexes ($\Delta\Delta G$) by the following equation: $\Delta\Delta G = -RT \ln \alpha$, where R is the gas constant and T the absolute temperature.

Usually the area of interest for HPLC applications is bounded by α values between 1.1 and about 5. Values out of this range determine an incomplete enantioseparation or involve a long time of analysis and large utilize of solvents. However, higher enantioselectivities may be useful to clarify some aspects of the enantiorecognition mechanism, especially for those chiral stationary phases for which the three-dimensional structure is lacking and the spectroscopic and computational studies are not exhaustive yet. This is the case of the benzoates and arylcarbamates of cellulose and amylose, which are the most used selectors to resolve chiral compounds by HPLC on analytical and preparative scale.

Particularly, the cellulose tris(4-methylbenzoate) has been largely employed to resolve a broad range of racemates in normal phase, polar organic, and reversed-phase conditions. In the commercially available 4-methylbenzoate, cellulose-based CSPs are physically adsorbed on 3 (Chiralcel OJ-3), 5 (Chiralcel OJ-H), or 10 μm (Chiralcel OJ) particles of macroporous silica gel.

We have recently reported a very high enantioselectivity with enantiomers of **1** (Figure 1) resolved onto a Chiralcel OJ-H

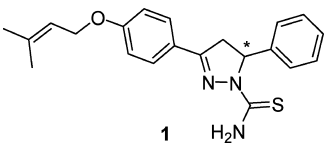


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Figure 1. Chemical structure of the chiral analyte **1**.

with a remarkable α = 73.2, using at room temperature pure ethanol as the mobile phase.[1] This compound belongs to a

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family of asymmetric pyrazolines endowed with nanomolar biological activities against monoamminooxydases.[2]

It has been suggested that the mechanism of chiral recognition of the Chiralcel OJ-H CSP is based on the formation of transient complexes CSP-enantiomer through the different inclusion of the enantiomers in the cavities created by the arrangement of the selector. At the molecular level, the OJ-H CSP, as well as other polysaccharides and unlike monomeric selectors consisting of small molecules, contains multiple sites of interaction that do not act independently as if they were isolated units. Presently, it is not possible to have an adequate crystallographic model to investigate the nature of the selective interactions. So, starting from our previously reported experiences,[3,4] we have developed a theoretical molecular model with the aim to clarify the reasons of the high experimental enantioselectivity of **1** toward the OJ-H CSP. During analysis of the most stable binding modes, we have noted a possible improvement in the enantioselectivity modifying the prenyloxy chemical moiety with an aromatic substituent.

In this communication, we report, for the first time, the successful design of a novel analyte derivative guided by a computational molecular model of the OJ-H CSP, leading to a consistent improvement to the enantioselective α value. Moreover, intensive molecular dynamics experiments carried out to explain the enantioselective recognition were rationalized, measuring the time spent by each enantiomer in contact with the CSP model.

The work has been carried out in eight consecutive steps briefly reported in the following list.

Step 1: OJ-H CSP model construction
Step 2: Conformational search of **1** and docking with OJ-H CSP
Step 3: Analysis of thermodynamic and structural models
Step 4: Design of derivative **2** and molecular modeling
Step 5: Synthesis of **2**
Step 6: HPLC enantioseparation and absolute configuration assignment of the enantiomers of **2**
Step 7: Molecular dynamics of the complexes [**1**/**2**·OJ-H CSP]
Step 8: Molecular dynamics analysis

OJ-H CSP MODEL CONSTRUCTION

The first step of the work was dedicated to the construction of the OJ-H CSP polymer structure starting from our four-glucose backbone OD and OG models.[3,4] This last CSP was structurally closer to the OJ-H side chain: A simple replacement of the carbamate with the benzoate moieties was sufficient to generate a preliminary model in helical conformation according to the X-ray data obtained by Vogt and Zugenmaier.[5] The building procedure (Supporting Information) corresponds to that described by Yashima and co-workers for similar CSPs.[6]

CONFORMATIONAL SEARCH OF 1 AND DOCKING WITH OJ-H CSP

The conformational search was carried out by Monte Carlo simulations starting with an initial model of the *(S)*-**1** enantiomer (Supporting Information). Twenty-four unique low energy conformers were identified and converted into the *(R)*-**1** enantiomer structures. The docking experiments were carried out submitting all 24 conformers of both **1** enantiomers against the OJ-H CSP model, accordingly to the MOLINE method.[7,8] In order to take into account the best nonpolar mobile phase environment obtained with an unique solvent, we set the dielectric constant at 37.5, mimicking the ethanol properties (Supporting Information).

ANALYSIS OF THERMODYNAMIC AND STRUCTURAL MODELS

The analysis of the docking results were carried out considering both thermodynamics and structural aspects. The free energies of complexation were computed at room temperature and compared to experimental data.

In agreement with the chromatographic values, complexation free energies of *(S)*-**1** and *(R)*-**1** analytes with the OJ-H model resulted, respectively, equal to −43.90 and −41.70 kcal mol^{-1}. The enantioselective difference $\Delta\Delta G_{S\text{-}R}$ was therefore equal to −2.20 kcal mol^{-1}. The inspection of the lowest energy poses of the analytes with the OJ-H CSP revealed the reasons of the enantioselectivity. The *(S)*-**1** enantiomer's best pose was stabilized by two $\pi-\pi$ stacking interactions established by both phenyl rings with the 4-methyl benzoate side chains of I and IV glucose residues (Figure 2). In this configuration, the thioamide moiety was positioned toward the CSP, establishing intermolecular hydrogen bonds with two oxygen atoms of the III glucose residue. This finding was apparently in contrast to the hypothesis usually advanced in the literature regarding the sites of selector capable to establish productive interactions with selectands. In fact, the oxygen atom of carbonyl groups is generally recognized as the most important hydrogen bond acceptor.[9−11]

Conversely, the *(R)*-**1** enantiomer showed an inverted head−tail best pose with a reduced stabilization pattern: one $\pi-\pi$ stacking (phenyl in position 3 and the 4-methyl benzoate side chain of the IV glucose residue) and one intermolecular hydrogen bond (thioamide and III glucose residue). This difference in the two consistent nonbonding contributions can explain the reason of the experimental enantioselectivity also using ethanol as a unique mobile phase.

DESIGN OF DERIVATIVE 2 AND MOLECULAR MODELING

Interestingly, a deeper analysis of both best poses did not reveal a leading role of the prenyl moiety in the enantioselective recognition of **1** enantiomers with the OJ-H CSP model. In fact, this moiety was partially exposed to the solvent, especially in the lowest energy configuration of the [*(S)*-**1**·OJ-H] complex. Starting from this observation, the design work has been carried out focusing the attention onto alternative chemical derivatives of the prenyl moiety. With the aim to improve the enantioselective performances of new derivatives, easily derivable from the pyrazole chiral scaffold, we have focused the attention onto the substitution of the prenyl moiety with a chemical group able recognize the OJ-H CSP. Taking into account that stationary phase contains an aromatic side chain and that the mobile phase is polar, we have designed the derivative **2** on the basis of the replacement of the prenyl with a phenyl moiety (Figure 3).

This substitution should exert a general improvement of the retention times in both enatiomers, and also on the basis of the position of the prenyl moiety in the best pose of the *(S)*-**1** enantiomer, add a new stacking interaction with a side chain of the III glucose residue. Before going on with the synthesis of **2** and the analytical separation with the OJ-H, we have performed

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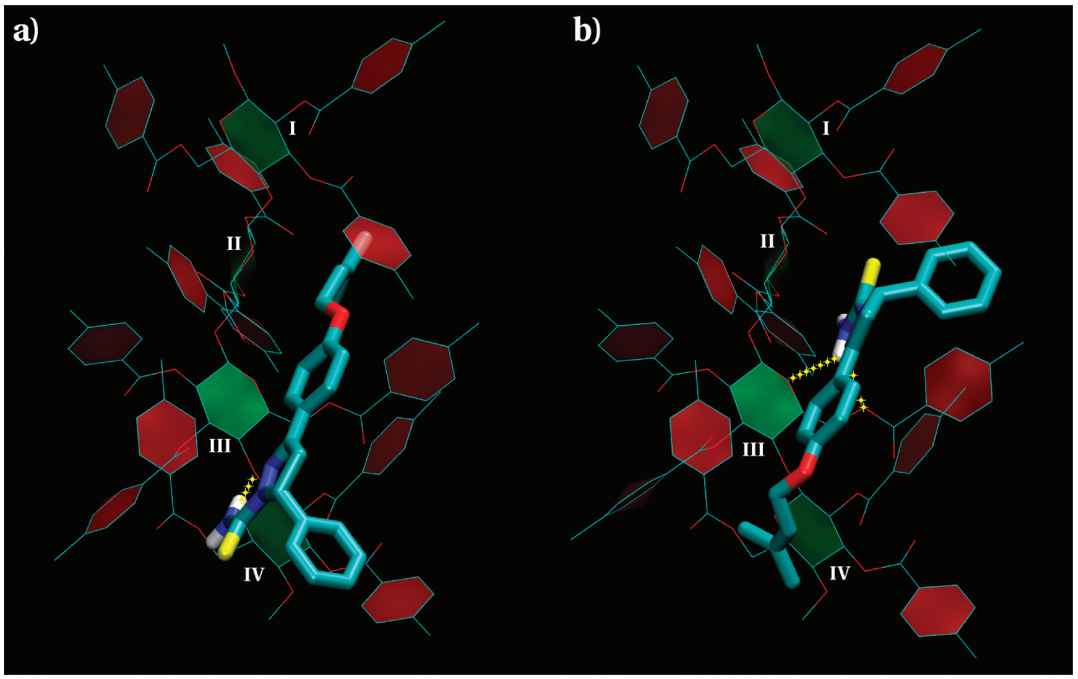

Figure 2. Best poses of a) *(R)*-**1** and b) *(S)*-**1** in polytube models complexed with the OJ-H CSP, represented in paper chain rendering. Yellow dots display intermolecular hydrogen bonds. Four glucose residues of the OJ-H model are labeled with roman numbers I–IV.

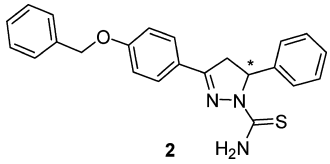

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Figure 3. Chemical structure of the designed chiral analyte **2**.

the conformational analysis and the docking experiments as already done for **1**. With the novel compound, the number of unique conformers identified in the Monte Carlo search was only 14 because of the symmetrical effect of the new phenyl moiety (Supporting Information). As previously carried out with the original compound, all these conformers were submitted to the docking procedure exactly in the same environment conditions (Figure 4).

The thermodynamic results predicted better performances in terms of free energy of complexation and difference between them.

[*(S)*-**1**·OJ-H] and [*(R)*-**1**·OJ-H] resulted, respectively, as the most and less stable complexes, with values equal to −46.60 and −44.00 kcal mol^{-1}. As expected in the design process, these values were both lower than those computed with the corresponding **1** enantiomers. Moreover, the predicted difference in the free energy of complexation $\Delta\Delta G_{S\text{-}R}$ was equal to −2.60, with a theoretical gain of 0.4 kcal ·mol^{-1} with respect to the previous case.

The visual inspection of the best poses revealed the most relevant contributions in the enantioselective recognition. Surprisingly, the derivative *(S)*-**2** preferred to bind the OJ-H CSP with the inverted head–tail configuration, similarly to the *(R)*-**1** mode. Two π–π stacking interaction were detected (a) between the analyte phenyl in position 3 and the side chain of the IV glucose residue (similar to the original found with both **1** enantiomers) and (b) between the new phenyl moiety and the aromatic side chain of the III glucose unit. In this configuration, the thioamide was still oriented toward the cellulose backbone, keeping one intermolecular hydrogen bond with the OJ-H CSP. Conversely the *(R)*-**2** enantiomer recognized the CSP only by one π–π stacking interaction established between the new phenyl moiety and the aromatic side chain of the II glucose unit. Notably in this configuration, the *(R)*-**2** thioamide moiety was exposed to the solvent, missing one relevant anchoring option with the CSP and gaining the opportunity to establish hydrogen bonds the mobile phase.

SYNTHESIS OF COMPOUND 2

On the basis of the docking experiments, we judged it appropriate to synthesize compound **2**. It has been synthesized as reported in Scheme 1 (Supporting Information). The intermediate chalcone was obtained by direct Claisen–Schmidt condensation between benzaldehyde and 4-benzyloxy acetophenone in a basic medium in ethanol 96%. A solution of chalcone in 50 mL of ethanol and sodium hydroxide was refluxed for 8 h in the presence of thiosemicarbazide to obtain the new derivative **2**.

HPLC ENANTIOSEPARATION AND ABSOLUTE CONFIGURATION ASSIGNMENT OF THE ENANTIOMERS OF 2

The direct HPLC enantoseparation of **2** was accomplished on the polysaccharide-based Chiralcel OJ-H CSP using pure ethanol as the mobile phase. Under the polar organic mode, an enantioseparation factor value of α = 79.8 was obtained. The retention factors of the **2** enantiomers were, respectively, 9.1 and 723.7, values about twice that found for the enantiomers of **1** (k1 = 4.6 and k2 = 336.0).[1] The enantiomer elution order of **2** was established by circular dicroism (CD) correlation, using the enantiomers of **1** as references of known stereochemistry (Supporting Information).[1] The CD analysis results confirmed *(R)* configuration as the first eluted enantiomer and *(S)* as the most retained one also for the analyte **2**.

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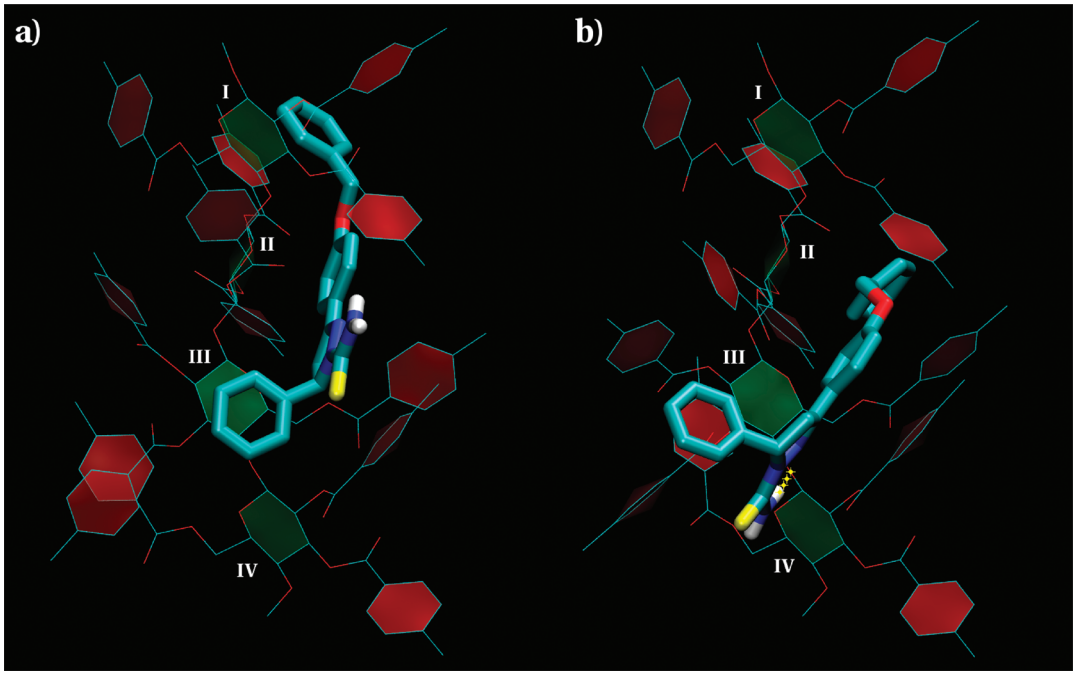

Figure 4. Best poses of (a) *(R)*-**2** and (b) *(S)*-**2** in polytube models complexed with the OJ-H CSP, represented in paper chain rendering. Yellow dots display intermolecular hydrogen bonds. Four glucose residues of the OJ-H model are labeled with roman numbers I–IV.

MOLECULAR DYNAMICS OF THE COMPLEXES [1/2·OJ-H CSP]

In order to simulate the polymeric environment of the chromatographic matrix in a more realistic model, the enantioselective recognition of compounds **1** and **2**, we have set up and run molecular dynamics (MD) experiments starting with the best docking poses. The MD models were built extending the number of the CSP OJ-H glucose residues up to 16 units, inserting the complexes into a boundary condition box of about 76,200 Å^3 and explicitly adding 407 molecules of ethanol solvent.

During these calculations, carried out by the Desmond program,[12] 6000 structures of each complex were collected at regular time intervals of 10 ps. Graphical inspection of the sampled trajectories revealed an exhaustive exploration of the stationary phase. In fact, all enantiomers, because of the applied boundary conditions, were able to move from their starting position visiting several other sites available onto the OJ-H CSP. It is worth noting that the model of ethanol was not available into the Desmond software, so it has been obtained by modifying the original cubic methanol box that contained 512 molecules into a volume of about 35,000 Å^3. Using the Maestro graphic user interface,[13] each methyl group was replaced by the ethyl moiety. The resulting ethanol box was submitted to energy minimization using the OPLS-2005[14] force field with a convergence threshold equal to 0.05 kcal/mol/Å. The optimized box has not reported van der Waals clashes, and its dimensions were about 50,000 Å^3. In order to obtain an equilibrated explicit solvent model, the relaxed box was submitted to 12 ns of molecular dynamics simulation carried out at 300 K, with time step equal to 2.0 fs and constant pressure fixed to 1.01 bar. Final molecular dynamics structure, after energy minimization, was considered as our explicit ethanol solvent model.

MOLECULAR DYNAMICS ANALYSIS

With the aim to quantitatively report the differences of **1** and **2** enantiomers with respect to the stationary phase recognition, the number of selector/selectand van der Waals contacts (HGC) was computed for each sampled complex. Such information allowed us to distinguish among strongly interacting complexes (SIC), characterized by HGC values larger than 10, weak interacting complexes (WIC) with HGC values between 1 and 10, and totally guest solvent exposed (GSE) with no contact to the OJ-H CSP. Taking into account the HGC data and the trajectories sampling interval, we have computed the time of selector/selectand recognition (RT) as a descriptor directly related to the affinity of our compounds with respect to the stationary phase (Figure S8, Supporting Information). In fact, the simulation times spent by our ligands in SIC or in WIC configuration, reported for both *(S)*-**1** and *(S)*-**2** longer values than their *(R)*- enantiomers, were 1 and 3.8 ns, respectively (Figure S8, Supporting Information). The differential analysis focused onto the SIC RT data indicated a consistent gain of the enantiorecognition time passing form the **1** (4.18 ns) to **2** enantiomers (5.23 ns).

These theoretical measurements were also in qualitative agreement with the larger α value experimentally reported for compound **2** with respect to **1**. The good agreement among RT and experimentally determined separation factors prompted us to progress the analysis of the **1** and **2** OJ-H CSP recognition patterns by means of single linkage cluster analysis carried out for all SIC complexes (Supporting Information). Such a study revealed in all cases a large population of the cluster containing the docking generated starting configuration and, for the lower retained *(R)*- enantiomers, the presence of at least other 2 populated clusters (Figure 5).

According to the docking interaction energies, the SIC times of *(S)*- enantiomers longer than *(R)*- one and the three populated clusters observed for complexes of these last isomers, could contribute in rationalizing the experimental results.

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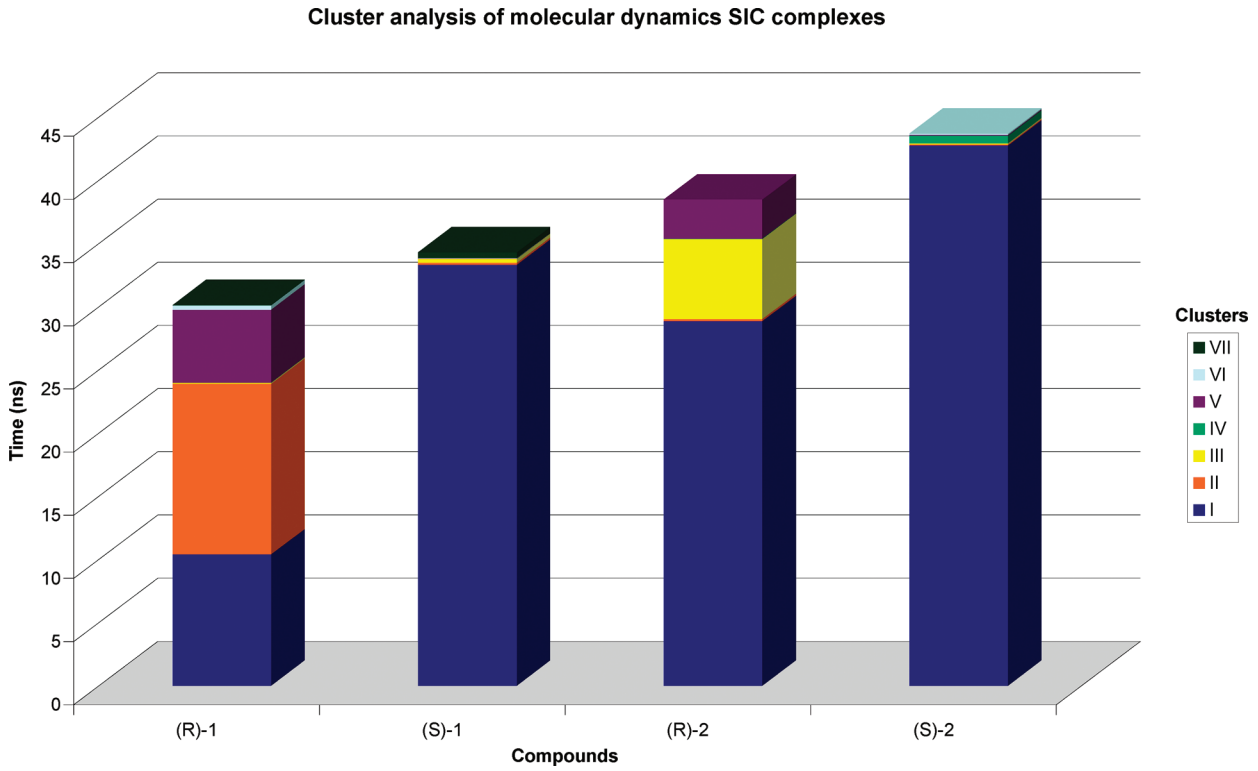

Figure 5. Clustering of **1** and **2** SIC complexes. Cluster I is referred to the starting docking poses.

Then we focused our attention onto the HB intermolecular network established by the analytes with respect the CSP and the mobile phase during the MD simulations. In this analysis, we have considered two selectand HB donors (the hydrogen atoms of the thioamide) and two HB acceptors (the pyrazole nitrogen at position 1 and the ether oxygen). The donor atoms established more than 90% of the HB interactions prevalently with the solvent. The interactions with the CSP resulted less frequent but uniquely with the carbonyl oxygen atoms of the OJ-H model, in agreement with the literature data.[9−11] Conversely, the acceptor atoms of the analytes established few solvent HB contacts in all cases for less than 10% of the monitored interactions (Figures S10−S15, Supporting Information).

From this observation, we can argue that the recognition driving force of the selector/selectand interaction is based for our analytes mainly on the hydrophobic and π-stacking interactions rather than on HB contributions. Actually, the introduction guided by the modeling work of an additional phenyl moiety increased both retention and enantioselective factors in the designed analyte.

In conclusion, in this communication, we report a mixed theoretical and experimental study in the field of the HPLC enantioseparation. For the first time, we have performed a successful computer-aided design of a chiral derivative with improved enantioselective properties. The synthesis and separation of designed enantiomers confirmed the good theoretical prediction and prompted an investigation of a more sophisticated model of enantioselective recognition. This issue was carried out, for the first time, looking at the time spent by each enantiomer in contact with the CSP during long MD simulations. The data obtained with this innovative analysis were consistent with both docking and analytical results.

■ ASSOCIATED CONTENT

Supporting Information

All experimental details regarding the computational, synthetic, and analytical steps are reported for each step. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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