Comparative enantioseparation of planar chiral ferrocenes on polysaccharide-based chiral stationary phases

Alessandro Dessi, Barbara Sechi, Roberto Dallocchio, Bezhan Chankvetadze, Mireia Pérez- Baeza, Sergio Cossu, Victor Mamane, Patrick Pale, Paola Peluso

To cite this version:
Alessandro Dessi, Barbara Sechi, Roberto Dallocchio, Bezhan Chankvetadze, Mireia Pérez- Baeza, et al.. Comparative enantioseparation of planar chiral ferrocenes on polysaccharide-based chiral stationary phases. Chirality, Wiley, In press, 10.1002/chir.23417. hal-03550399

HAL Id: hal-03550399
https://hal.archives-ouvertes.fr/hal-03550399
Submitted on 1 Feb 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
Comparative enantioseparation of planar chiral ferrocenes on polysaccharide-based chiral stationary phases

Alessandro Dessì,1,‡ Barbara Sechi,1,‡ Roberto Dallocchio,1 Bezhan Chankvetadze,2 Mireia Pérez-Baeza,3 Sergio Cossu,4 Victor Mamane,5,* Patrick Pale,5 and Paola Peluso1,*

1 Istituto di Chimica Biomolecolare ICB, CNR, Sede secondaria di Sassari, Traversa La Crucca 3, Regione Baldinca, I-07100 Li Punti - Sassari, Italy
2 Institute of Physical and Analytical Chemistry, School of Exact and Natural Sciences, Tbilisi State University, Chavchavadze Ave 3, 0179 Tbilisi, Georgia
3 Departamento de Química Analítica, Universitat de València, Burjassot, València, Spain
4 Dipartimento di Scienze Molecolari e Nanosistemi DSMN, Università Ca’ Foscari Venezia, Via Torino 155, I-30172 Mestre Venezia, Italy
5 Institut de Chimie de Strasbourg, UMR CNRS 7177, Equipe LASYROC, 1 rue Blaise Pascal, 67008 Strasbourg Cedex, France

SHORTENED TITLE: ENANTIOSEPARATIONS OF SUBSTITUTED FERROCENES

KEY WORDS: Electrostatic potential / Enantioseparation / Ferrocenes / Planar chirality / Polysaccharide-based chiral stationary phases

CORRESPONDENCE TO:
Dr. Paola Peluso
Istituto di Chimica Biomolecolare ICB CNR – UOS di Sassari, Traversa La Crucca, 3, Regione Baldinca, I-07100 Li Punti - Sassari, Italy
E-mail address: paola.peluso@cnr.it

Dr. Victor Mamane
Institut de Chimie de Strasbourg, UMR CNRS 7177, Equipe LASYROC, 1 rue Blaise Pascal, 67008 Strasbourg Cedex, France
E-mail address: vmamane@unistra.fr

‡ These authors contributed equally to this work
**Graphical abstract** - High-performance liquid chromatography (HPLC) enantioseparations of ten 1,2- and 1,3-disubstituted planar chiral ferrocenes on five polysaccharide-based chiral stationary phases (CSPs) were comparatively explored under multimodal elution conditions. The presence of π-extended systems in the analyte structure was shown to impact affinity of the most retained enantiomer toward amylose-based selectors, observing retention times higher than 80 min with methanol-containing mobile phases (MPs).
**ABSTRACT** - Planar chiral ferrocenes are well-known compounds which have attracted interest for application in synthesis, catalysis, material science, and medicinal chemistry for several decades. In spite of the fact that asymmetric synthesis procedures for obtaining enantiomerically enriched ferrocenes are available, sometimes the accessible enantiomeric excess of the chiral products is unsatisfactory. In such cases and for resolution of racemic chiral ferrocenes, enantioselective high-performance liquid chromatography (HPLC) on polysaccharide-based chiral stationary phases (CSPs) has been used in few literature reports. However, while moderate/high enantioselectivities have been obtained for planar chiral ferrocenes bearing polar substituents, the enantioseparation of derivatives containing halogens or exclusively alkyl groups remains rather challenging. In this study, the enantioseparation of ten planar chiral 1,2- and 1,3-disubstituted ferrocenes was explored by using five polysaccharide-based CSPs under multimodal elution conditions. Baseline enantioseparations were achieved for nine analytes with separation factors (α) ranging from 1.20 to 2.92. The presence of π-extended systems in the analyte structure was shown to impact affinity of the most retained enantiomer toward amylose-based selectors, observing retention times higher than 80 min with methanol-containing mobile phases (MPs). Electrostatic potential (V) analysis and molecular dynamics (MD) simulations were used in order to inspect interaction modes at the molecular level.
1. INTRODUCTION

Ferrocene ([Fe(η⁵-C₅H₅)₂]) is an iconic framework in organometallic chemistry,¹ which, in 1952, was characterized as the first sandwich compound by Woodward and co-authors.² Structurally, ferrocene is a rigid three-dimensional unit wherein iron is located between two cyclopentadienyl (Cp) rings. Owing to its stability, and the possibility of functionalization at the cyclopentadienyl rings,³⁻⁷ ferrocene derivatives have over time found application in several fields such as catalysis,⁸⁻¹⁰ medicinal chemistry,¹¹ and material chemistry.¹²⁻¹⁴ Moreover, the ferrocenium/ferrocene system (Fc/Fc⁺) is a versatile redox couple which have proven to be suitable for the preparation of switchable functional systems.¹¹,¹⁵,¹⁶

Due to their versatility to access molecular wires, and responsive switchable systems and materials,¹⁷⁻¹⁹ particular attention has been devoted to the so-called “π-extended ferrocenyl frameworks”, which are featured by delocalized orbital networks based on the combination of a ferrocene unit and a π-conjugated extended cloud, such as the ethynyl group.¹³,²⁰ Planar chiral aryl ethynyl ferrocenes have been used as intermediates for the preparation of helical ferrocenes showing peculiar chiroptical properties with very high optical value and huge intensity of the CD signals.²¹

Recently, our groups developed the design, synthesis, and characterization of compounds 1-8 as new chiral iodoethynyl ferrocenes showing three key features (Figure 1):⁶ a) planar chirality due to the presence of two different substituents on the same Cp ring, b) a π-extended ethynyl ferrocenyl moiety, with the possibility to further extend the π-conjugation to the second Cp-substituent depending on its electronic features (Figure 2a,b), and c) an iodine substituent as an electrophilic σ-hole donor activated by C≡C group (Figure 2d).²²,²³ In this regard, it is worth mentioning that halogen bonds (XBs) were identified in the crystal packing of compounds 1, 3-5, and 7 with both lengths and angles falling within the range of values typically observed for this type of σ-hole bonds.⁶

These features also make compounds 1-8 suitable as test probes to explore the impact of both π-extended clouds and electrophilic σ-holes in enantioselective processes occurring in HPLC environment, with the aim to profile noncovalent interactions involved in the enantioselective recognition. In previous
studies, we demonstrated that σ-hole interactions, such as XB s and chalcogen bonds (ChBs), which involve electrophilic regions of electron charge density depletion (σ-holes), may participate in the enantioselective interaction between polysaccharide-based selectors and selectands containing electrophilic σ-holes.  

On this basis, we explored the enantioseparation of chiral ferrocenes 1-8 along with 9 and 10, as reference compounds for comparison, by using five polysaccharide carbamate-based chiral stationary phases (CSPs) (Supporting Information, Table S1) under multimodal elution conditions. The experiments were integrated with computational analysis by using electrostatic potential (V) calculations and molecular dynamics (MD) simulations in order to disclose the mechanistic features of these enantioseparations.

The second aim of this study was to identify suitable methods for baseline enantioseparation of chiral ferrocenes containing small or nonpolar groups. Indeed, while moderate/high enantioselectivities have been obtained under normal-phase (NP) elution conditions for planar chiral ferrocenes bearing hydroxyl, carbonyl, and carboxyl groups as substituents, 21,28-30 the enantioseparations reported for derivatives containing small groups, halogens or exclusively alkyl groups are rather unsatisfying. 31,32 It is worth mentioning that few papers have reported systematic studies on enantioseparation of organometallic compounds on polysaccharide-based CSPs so far. 28,31-39

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

Compounds 1-10 were prepared and characterized as previously reported. 6,40 HPLC grade n-hexane (Hex), methanol (MeOH), isopropyl alcohol (IPA), acetonitrile (ACN) and water were purchased from Sigma-Aldrich (Taufkirchen, Germany).

2.2. Chromatography

An Agilent Technologies (Waldbonn, Germany) 1100 Series HPLC system (high-pressure binary gradient system equipped with a diode-array detector operating at multiple wavelengths (220, 254, 280,
360 nm), a programmable autosampler with a 20 μl loop, and a thermostated column compartment) was employed. Data acquisition and analyses were carried out with Agilent Technologies ChemStation Version B.04.03 chromatographic data software. The UV absorbance is reported as milliabsorbance units (mAU). Lux Cellulose-1 (C-1) (cellulose tris(3,5-dimethylphenylcarbamate), CDMPC), Lux i-Cellulose-5 (iC-5) (cellulose tris(3,5-dichlorophenylcarbamate), CDCPC), Lux Amylose-1 (A-1) and Lux i-Amylose-1 (iA-1) (amylose tris(3,5-dimethylphenylcarbamate), ADMPC), and Lux i-Amylose-3 (iA-3) (amylose tris(3-chloro-5-methylphenylcarbamate), ACMPC) (5 μm) (Phenomenex Inc., Torrance, CA, USA) were used as chiral columns (250 × 4.6 mm) (Table S1). Analyses were performed in isocratic mode at 25°C. The flow rate (FR) was set at 0.8 ml/min. For compounds 1, 3-6, 7, and 8, the enantiomer elution order (EEO) was determined by injecting enantiomers of known absolute configuration prepared by asymmetric syntheses. For compounds 9 and 10, the relative EEO was assigned by injecting pure enantiomers of unknown absolute configuration which are denoted as X9, Y9 and X10, Y10.

2.3. Computationals

V extrema calculated on the molecular electron density isosurfaces (maxima and minima) (VS,max and VS,min) (au, electrons/bohr) were computed by using Gaussian 09 (Wallingford, CT 06492, USA), at the density functional theory (DFT) level of theory, using the B3LYP functional and the def2TZVPP basis set. Search for the exact location of VS,max and VS,min was made through the Multiwfn code and through its module enabling quantitative analyses of molecular surfaces (isovalue 0.002 au). In our computations, .wfn files were obtained through the Gaussian 09 package. Details for molecular dynamics (MD) are reported in the Supporting Information file.

3. RESULTS AND DISCUSSION

3.1. Chromatographic screening

In the frame of this study, analytes, CSP and mobile phase (MP) were considered as experimental variables, examined individually, and their influence on retention and selectivity was examined through
the evaluation of retention and separation factors \((k\) and \(\alpha\)), on the basis of the following preliminary remarks:

\(\text{a) within the 1,2-disubstituted 1-5 and 1,3-disubstituted 6-8 series, the influence of the distinctive functional group on enantioseparation was evaluated. As shown in Figure 2, different substituents on the Cp ring may determine different degrees of delocalization across the entire system. For instance, for compound 4 (Ph) and 5 (2-Naphth) (Figure 2b,c) the delocalization is across the entire molecule, whereas for compound 3, the calculated HOMO does not extend on the second substituent (Me) (Figure 2a). The impact of the triple bond on the enantioseparation was examined, and for this purpose compounds 9 and 10 served as reference systems for comparison. The effect of 1,2- and 1,3-disubstitution on the enantioseparation was also considered. The local electron charge density of specific molecular regions of the analytes was inspected in terms of calculated \(V_S\) (Supporting Information, Table S2), positive and negative \(V_S\) values being associated with electrophilic and nucleophilic regions;}

\(\text{b) the performances of different polysaccharide selectors were evaluated under multimodal elution conditions, and compared in terms of polysaccharide backbone (cellulose-based C-1} \text{ versus amylose-based A-1}, \text{ and type of carbamate pendant groups (methylated A-1, iA-1, C-1, chlorinated iC-5, methylated and chlorinated iA-3). In this regard, the electronic properties of the carbamate moiety, which are tuned by methyl and chlorine substituents located on the phenyl ring of the carbamate pendant groups, were determined by DFT calculations (Table 1). The impact of the anchoring technique (immobilization} \text{ versus coating})^{44,45} \text{ was also considered by comparing the performances of amylose tris(3,5-dimethylphenylcarbamate) (ADMPC)-based columns (A-1} \text{ versus iA-1);}

\(\text{c) the effect of MP on the enantioseparations was evaluated comparatively by using } n\text{-hexanic mixtures (Hex/IPA 95:5 (A) and Hex/IPA/MeOH 95:2.5:2.5 (B) v/v), polar organic (PO) conditions (MeOH 100\% (C)), and aqueous-organic mixtures (MeOH/water 95:5 (D) and 90:10 \€ v/v). In particular, the comparative use of A-E, as MPs, allowed for evaluating the effect of increasing hydrophobicity of the medium. The introduction in the MP of 2.5\% MeOH (B) allows modulation of the}
binding between analyte and polysaccharide-based selector by favouring a better penetration of the analyte into the groove, and tuning hydrophobic versus hydrogen bond (HB) interactions, while keeping intact the high-ordered three-dimensional structure of the polysaccharide. Otherwise, the use of pure MeOH (C), as a MP, impacts intramolecular HBs determining the high-ordered structure of the polysaccharide, thus producing a huge effect within the polysaccharide structure. In particular, the hydroxyl groups of MeOH molecules strongly interact, as HB donors/acceptors, with C=O and N-H groups of the polymer, competing with intramolecular HBs within the selector, and with selector-selectand intermolecular HBs. Indeed, with MeOH, hydrophobic interactions tend to be more favoured compared to HBs, and the addition of water was expected to enhance hydrophobic interactions and increase capacity factors in accordance with a typical reversed-phase (RP) system.

On this basis, twenty-five chromatographic systems generated by the combination of C-1, iC-5, A-1, iA-1, and iA-3 with the mixtures A-E, as MPs, were evaluated and characterized by $k$ (Supporting Information, Figure S1) and $\alpha$ (Figure S2) values toward ferrocenes 1-10 (Tables S3-S12). Baseline enantioseparations were obtained for compounds 1-8 and 10 with $\alpha$ values ranging from 1.10 to 11.41, whereas partial enantioseparation ($\alpha = 1.07$) was obtained only for 9, with the system C-1/A. For compounds 1, 3, and 10, baseline enantioseparations were achieved under NP elution conditions and with aqueous-MeOH mixtures as MPs, but MeOH showed to exert a detrimental effect on the enantioseparation in these cases. Compounds 2, and 4-8 were baseline enantioseparated under all three elution modes, namely $n$-hexanic mixtures, PO and aqueous-organic elution conditions.

Chromatographic conditions and parameters of the best enantioseparations obtained for compounds 1-8 and 10, as a suitable compromise between a sufficiently high enantioselectivity value, a baseline resolution, and a run time as short as possible, are reported in Table 2 (see Supporting Information, Figure S3 for chromatographic traces). For compounds 3-5, 7, and 8 optimized enantioseparations were obtained by using chlorinated chiral columns (iA-3 or iC-5) with selectivity factors ranging from 1.46 to 2.92. Otherwise, for compounds 1, 2, 6, and 10, optimized enantioseparation methods were developed...
by using coated methylated chiral columns (C-1 or A-1) \((1.19 \leq \alpha \leq 2.13)\). In the series of optimized enantioseparations, methanol-containing MPs were successfully used for compounds 1-4, 6, and 10, and NP elution conditions for 5, 7, and 8.

3.2. Impact of analyte structure on enantioseparation

With the aim to evaluate the impact of the structural features of each analyte on its enantioseparability, the rate of baseline enantioseparations \((\text{rbs})\) was determined from the wealth of chromatographic results (Figure 3). In the frame of the twenty-five chromatographic systems explored in this study, \(\text{rbs}\) increased following the order \(7, 8 (64\%, 62\%) > 2 (32\%) > 4, 5 (28\%) > 6 (16\%) > 3, 10 (12\%) > 1 (8\%) > 9 (0\%).\) From this trend, some remarks emerged:

- \(\text{a)}\) 1,3-disubstituted ferrocenes showed high enantioseparability compared to the 1,2-disubstituted analogues \((7 > 4, 8 > 5 \text{ and } 6 > 1)\). Indeed, average retention factors (Figure S4a) and selectivity factors (Figure S4b) of 6, 7, and 8 \((1.56 \leq k \leq 11.88; 1.05 \leq \alpha \leq 3.45)\) were, in general, higher than those of 1, 4, and 5 \((0.65 \leq k \leq 1.62; 1.05 \leq \alpha \leq 1.32)\), respectively. This behaviour could be caused by the fact that in the 1,3-pattern both substituents are accessible to the selector, whereas the substituents are sterically constrained in 1,2-substituted derivatives. On the other hand, no relevant difference in terms of \(V_S\) values (Table S2), justifying the different \(\text{rbs}\), was observed between 1,2- and 1,3-substituted derivatives;

- \(\text{b)}\) the ethynyl framework represents a key structural element given that the enantioseparability decreased moving from 1 and 2 \((\text{rbs}: 8\% \text{ and } 32\%)\) to 9 and 10 \((\text{rbs}: 0\% \text{ and } 12\%)\), respectively. In terms of \(V_S\) values associated to nucleophilic and electrophilic region, it is worth mentioning that in 1 and 2 the ethynyl group contributes to increase the electrophilic character of the iodine \((V_{S,\text{max}} (I) = 0.0721 \text{ au (1) and } 0.0755 \text{ au (2))})\), compared to the analogues 9 and 10 \((V_{S,\text{max}} (I) = 0.0465 \text{ au (9) and } 0.0529 \text{ au (10))})\), respectively. Interestingly, both \(\text{rbs}\) and positive \(V_{S,\text{max}}\) on iodine decreases along the bromoiodo-substituted series 6 \((\text{rbs } 16\%, \ V_{S,\text{max}} (I) = 0.0737 \text{ au}), 1 \ (\text{rbs } 8\%, \ V_{S,\text{max}} (I) = 0.0721 \text{ au}), 9 \ (\text{rbs } 0\%, \ V_{S,\text{max}} (I) = 0.0465 \text{ au})\). In addition, compound 2 presents higher electron density on the C≡N
group \((V_{S,\text{min}}(N) = -0.0721 \text{ au})\) and a more extended \(\pi\)-system compared to the analogue \(10\) \((V_{S,\text{min}}(N) = -0.0714 \text{ au})\);

c) the presence of a \(\pi\)-extended system involving the second substituent of the iodoethynyl ferrocene impacts both retention and selectivity. Indeed, compounds 2, 4, 5, 7, and 8 showed average retention for both first and second eluted enantiomer, average selectivity and \(rbs\) \((0.76 \leq k \leq 11.88; \ 1.20 \leq \alpha \leq 3.45;\)
\(28\% \leq rbs \leq 64\%\) higher compared to compounds 1, 3, 6, 9, and 10 \((0.54 \leq k \leq 1.64; \ 1.01 \leq \alpha \leq 1.11;\)
\(0\% \leq rbs \leq 16\%\); d) the EEO was determined for each enantioseparation, and the assignments are summarized in Table S5 (Supporting Information). S-R was the most frequent EEO, and only compound 2 retained its EEO in all chromatographic systems. Several cases of reversal of EEO dependent on analyte structure, selector structure, and MP type could be observed, but in general they are not easy to explain. Compounds containing \(\pi\)-extended systems provided interesting cases of EEO. For instance, on C-1/A and C-1/B all compounds showed S-R as EEO with the exception of 7 and 8 \((R-S)\). Interestingly, by changing the \(n\)-hexanic mixtures to pure MeOH with the same chiral column (C-1/C), only compound 8 retained the \(R-S\) sequence as EEO, whereas compound 7 showed MP-dependent EEO reversal to S-R, despite the structural similarity of the two analytes. Compound 5 showed the S-R elution order on all amylose-based selectors with mixture A, but adding 2.5% MeOH provided EEO reversal to R-S on the same selectors. Again, only for compound 5, a reversal of elution order was observed on immobilized iA-1 \((R-S)\) compared to the coated A-1 \((S-R)\) by using MeOH or the aqueous mixture D as MPs. The same phenomenon was not observed with \(n\)-hexanic mixtures (mixtures A and B).

### 3.3 Impact of selector structure on enantioseparation

Among the chiral columns used in this study, better results were obtained on the A-1 and iA-3, each column providing baseline enantioseparations for seven compounds. C-1 was also able to enantioseparate six compounds, whereas iC-5 and iA-1 provided baseline enantioseparations only for compounds 2, 4, 5, 10 and 7, 8, respectively (Supporting Information, Figure S6). Compounds 2 and 10
containing the C≡N group as a strong HB acceptor showed very high retention on the iC-5 (5.39 ≤ k ≤ 6.29) under NP conditions, but moderate selectivity on this chiral column (α = 1.11 (2), 1.15 (10)). Thus, the acidic amidic hydrogen of the CDCPC strongly contributes to determine the affinity of the analyte toward the selector participating in the C≡N···H-N HB, but less to enantioselectivity.

Among all compounds, 1,3-disubstituted 7 and 8 showed an interesting behaviour with very high affinity for the amylose-based selectors by using methanol-containing MPs (Tables S9 and S10). The same behaviour was not observed for the 1,2-analogues 4 and 5.

3.4 Enantioseparation of compounds 7 and 8: CDMPC vs ADMPC

As shown in Figure 4, 1,3-disubstituted ferrocenes 7 and 8 showed a complementary behaviour on C-1 as the MP changes, and in both cases two mechanisms seem to control enantioseparation on this column depending on MP polarity:

i) a mechanism based on polar interactions occurring with mixture A, which proved to be more effective, in terms of selectivity, for compound 8 (R = naphthyl) (α = 1.25) compared to derivative 7 (R = Ph) (α = 1.08). The addition of methanol to mixture A, or the use of pure MeOH as a MP, appeared to be detrimental for the enantioseparation in both cases;

ii) a mechanism occurring under hydrophobic conditions when increasing amounts of water (5% and 10%) were added to the MP consisting of pure methanol. In these cases, the separation system behaves as a typical reversed-phase system, and selectivity factors increased with increasing amount of water additive [52]. Under these conditions, compound 7 (α = 1.19) was enantioseparated better than compound 8 (α = 1.09).

Interestingly, for compound 7 the transition between the two mechanisms is associated with a MP-dependent reversal of EEO, which is R-S with A and B as MPs, and S-R with pure methanol and aqueous mixtures D and E. Otherwise, the naphthyl derivative 8 retained the same R-S elution sequence with all MPs (A-E).
The features of the enantioseparations of compounds 7 and 8 on A-1 were shown to be completely different (Figure 4). In these cases, with mixture A the phenyl derivative 7 was enantioseparated better \((\alpha = 2.27)\) than compound 8 \((\alpha = 1.29)\), showing an opposite trend compared to the enantioseparations on CDMPC, also in terms of EEO (CDMPC: R-S; ADMPC: S-R). On the amylose-based selector, the use of methanol-containing MPs such as B and C increased retention and selectivity producing large enantioseparation for both compounds 7 \((\alpha_B = 8.87; \alpha_C = 11.41)\) and 8 \((\alpha_B = 4.50; \alpha_C = 8.92)\). In particular, for compound 7, the addition of only 2.5% MeOH to the binary mixture A caused the increase of the retention time of the second eluted \((R)\)-enantiomer from 11.43 min to 83.46 min, and a further increase to 96.25 min by using pure MeOH as MP. The effect occurred in less degree for compound 8, the retention time of the second eluted enantiomer changing from 14.08 min to 24.38 and 46.6 min with A, B, and C as MPs, respectively.

The application of van’t Hoff analysis allowed for identifying different thermodynamic profiles for compound 7 and 8 as the cause of the different selectivity observed by changing the ADMPC to CDMPC. Thus, retention and selectivity of compounds 7 and 8 on C-1 and A-1 with hex/IPA 95:5 v/v as a MP, were determined at different temperatures from 5 to 45 °C in 5 °C increments (Supplementary material, Table S13). The thermodynamic quantities derived from van’t Hoff plots (Figure S7) are reported in Table S14 (Supplementary material). The enantioseparations of both analytes on C-1 were shown to be enthalpy-driven with \(T_{iso} = 147°C\) and 85°C for 7 and 8, respectively. Otherwise, the enantioseparation of 8 on A-1 is entropy-driven \((|T\Delta S^0| > |\Delta H^0|)\) and a \(T_{iso} = -37°C\) was calculated in this case. An enthalpy-driven process also occurred \((|T\Delta S^0| < |\Delta H^0|)\) for the enantioseparation of 7 on A-1, where the difference between the free energies associated to the transfer of the enantiomers from the MP to the selector surfaces is essentially due to a negative enthalpy contribution \((-281.3 \text{ cal/mol})\), whereas the entropy term is positive and close to zero \((0.78 \text{ cal-K-1\cdotmol-1})\). Indeed, the enantioselectivity is almost independent of the temperature variation. Moving from C-1 to A-1, an increase of the entropy contribution to enantioselection could be determined by comparing the values of
the thermodynamic ratio $Q = \Delta \Delta H^\circ/(298 \times \Delta \Delta S^\circ)$: 7, 1.41 (C-1) → 1.21 (A-1); 8, 1.20 (C-1) → 0.79 (A-1).

Large separations for compounds 7 and 8 were also observed on iA-1 (Supporting Information, Figures S8 and S9) and iA-3 (Figures S10 and S11), but not with cellulose-based selectors, showing that the high affinity of the second eluted enantiomers of these compounds toward the selectors is mainly determined by the features of backbone with methanol-containing MP, thus occurring under hydrophobic conditions. With the aim to confirm this feature, we explored the impact of adding polar and nonpolar components to the MP on the retention of the second (R)-enantiomer of compounds 7 and 8 by using iA-1 (Figures S8 and S9). Considering that solvents favour and disfavour hydrophobic and polar interactions, respectively, following the order acetonitrile (ACN) < IPA < MeOH < water, changing the aqueous mixture D to MeOH/IPA and MeOH/acetonitrile (ACN) as MPs caused a decrease in the retention of the (R)-enantiomer of 7 decreased from 113.10 min to 23.28 and 18.38 min, respectively. Otherwise, by adding 5% water to pure ACN, retention time of the (R)-enantiomer increased from 15.12 min to 26.89 min. On this basis, by changing MeOH to ACN in mixture D a drop of retention time of the (R)-enantiomer from 113.10 min to 26.89 was observed. On the other hand, the retention factors of the first eluted (S)-enantiomer ($0.56 \leq k_2 \leq 3.50$) showed to be less influenced by the polarity of the MP compared to the (R)-enantiomer ($2.56 \leq k_2 \leq 27.79$). A similar trend was obtained for compound 8. The evaluation of the ‘dance’ of the second peak on the time scale under multimodal conditions, allowed to confirm that the high affinity of the analyte toward amylose-based selectors actually originates from hydrophobic conditions.

With the aim to explore the mechanism at the molecular level, a MD simulation was performed by using the (R)-enantiomer of compound 7 as selectand, an ADMPC nonamer as selector, and MeOH as a virtual solvent (Fig. 5). The modelling of the experimental enantioseparation confirmed that the (R)-enantiomer of compound 7 penetrates deeply in the groove of the selector (Fig. 5a). The selectand remained blocked during 100 ns of MD and confined in a hydrophobic cavity which appeared to be
profiled by six aromatic rings of the selector (Fig. 5b). Interestingly, a HB between the \( \pi \)-ethynyl cloud and the amidic hydrogen of the selector \( (d = 2.347 \, \text{Å}) \) was observed to contribute to the binding of the \( (R) \)-enantiomer into the polymer groove, confirming the pivotal role of this structural element on enantioseparation.

4. CONCLUSION

In this study, the enantioseparation of ferrocenes 1-10 has been explored systematically and, as a result, methods for baseline enantioseparations were successfully developed for nine compounds with selectivity factors ranging from 1.20 to 2.92. Otherwise, 1-bromo-2-ido-ferrocene (9) could be only partially enantioseparated, confirming that the enantioseparation of small nonpolar planar chiral ferrocenes remains rather challenging. In most cases, amylose-based CSPs provided better enantioseparation performances compared to cellulose-based ones. Due to the hydrophobic feature of the ferrocenes used in this study as analytes, aqueous methanol-containing MPs allowed for improving enantioseparation performances in several cases.

The impact of \( \pi \)-extended clouds on the enantioseparations was clearly demonstrated, compounds 7 and 8 providing interesting trends and behaviours as both selector and MP features changes. Interestingly, the addition of small (2.5%) percentages of methanol to the Hex/IPA 95:5 increased significantly the affinity of the second eluted enantiomers of compounds 7 and 8, producing large enantioseparations on amylose-based selectors ranging from 4.69 to 11.41, and from 4.50 to 8.92, respectively.

MD simulations disclosed a) the confinement of the analyte in a hydrophobic cavity deeply inside the ADMPC groove and b) the stabilization of the selector-selectand complex exerted by a HB between the ethynyl \( \pi \)-cloud and the amidic hydrogen of the selector, as the molecular basis underlying the high affinity of analytes 7 and 8 toward amylose-based selectors.
REFERENCES


FIGURE CAPTIONS

FIGURE 1 Structures and numbering of chiral ferrocenes 1-10

FIGURE 2 The highest occupied molecular orbitals (HOMO) (contour value = 0.02 au) for compounds 3-5 (a-c), and electrostatic potential isosurface ($V_S$) (isovalue 0.002 au) of compound 6 (d) (DFT/B3LYP/def2TZVP; color legend: minimum and maximum $V_S$ values are depicted in red and blue, respectively, and colors in between (orange, yellow, green) depict intermediate values)

FIGURE 3 Rate of baseline enantioseparations of compounds 1-8 and 10 under multimodal elution conditions

FIGURE 4 Comparison of the enantioseparation traces of compounds 7 and 8 on C-1 and A-1 under multimodal elution conditions

FIGURE 5 Representative snapshot from the simulated MD trajectories (100 ns) of (R)-7 complex with ADMPC (solvent box, MeOH): a) electron density surface (legend colours: green, aromatic ring; red, C=O; blue, N-H; grey, Ph + Cp + C≡C of (R)-7; magenta, iodine), b) tube model of the (R)-7/ADMPC complex (legend colours: orange, Ph + Cp + Fe + C≡C of (R)-7; magenta, iodine; green, aromatic rings featuring and delimiting the hydrophobic binding cavity of ADMPC)
FIGURE 1 Structures and numbering of chiral ferrocenes 1-10

<table>
<thead>
<tr>
<th>R</th>
<th>1</th>
<th>6</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Br</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CN</td>
<td></td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Me</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ph</td>
<td>4</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>2-Naph</td>
<td>5</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

FIGURE 2 The highest occupied molecular orbitals (HOMO) (contour value = 0.02 au) for compounds 3-5 (a-c), and electrostatic potential isosurface ($V_S$) (isovalue 0.002 au) of compound 6 (d) (DFT/B3LYP/def2TZVPP; color legend: minimum and maximum $V_S$ values are depicted in red and blue, respectively, and colors in between (orange, yellow, green) depict intermediate values).
FIGURE 3 Rate of baseline enantioseparations of compounds 1-8 and 10 under multimodal elution conditions
FIGURE 4 Comparison of the enantioseparation traces of compounds 7 and 8 on C-1 and A-1 under multimodal elution conditions.
FIGURE 5 Representative snapshot from the simulated MD trajectories (100 ns) of (R)-7 complex with ADMPC (solvent box, MeOH): a) electron density surface (legend colours: green, aromatic ring; red, C=O; blue, N-H; grey, Ph + Cp + C≡C of (R)-7; magenta, iodine), b) tube model of the (R)-7/ADMPC complex (legend colours: orange, Ph + Cp + Fe + C≡C of (R)-7; magenta, iodine; green, aromatic rings featuring and delimiting the hydrophobic binding cavity of ADMPC)
TABLE 1  $V_{S,\text{max}}$ and $V_{S,\text{min}}$ values (a.u.) associated with the main recognition sites (carbamate N–H, C=O, and Ar) of cellulose- and amylose-based selectors used in the study (DFT/B3LYP/def2TZVPP)

<table>
<thead>
<tr>
<th>Chiral selector</th>
<th>Side chain</th>
<th>$V_{S,\text{max}}$ (N–H)</th>
<th>$V_{S,\text{min}}$ (C=O)</th>
<th>$V_{S,\text{min}}$ (Ar)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDMPC/ADMPC</td>
<td>3,5-dimethylphenylicarbamate</td>
<td>0.0843</td>
<td>-0.0625</td>
<td>-0.0271</td>
</tr>
<tr>
<td>CDCPC</td>
<td>3,5-dichlorophenylicarbamate</td>
<td>0.0990</td>
<td>-0.0536</td>
<td>-0.0066</td>
</tr>
<tr>
<td>ACMPC</td>
<td>3-chloro-5-methylphenylcarbamate</td>
<td>0.0914</td>
<td>-0.0595</td>
<td>-0.0163</td>
</tr>
</tbody>
</table>

TABLE 2 Chromatographic parameters ($t$, $k$, $\alpha$) for optimized baseline enantioseparations of rac-1-8 and 10 on polysaccharide carbamate-based chiral columns under multimodal elution conditions

<table>
<thead>
<tr>
<th>Compound</th>
<th>Column</th>
<th>MP</th>
<th>$t_1$ [min]</th>
<th>$t_2$ [min]</th>
<th>$k_1$</th>
<th>$k_2$</th>
<th>$\alpha$</th>
<th>EEO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A-1</td>
<td>E</td>
<td>7.42</td>
<td>8.11</td>
<td>0.88</td>
<td>1.05</td>
<td>1.20</td>
<td>S-R</td>
</tr>
<tr>
<td>2</td>
<td>A-1</td>
<td>D</td>
<td>4.80</td>
<td>5.80</td>
<td>0.23</td>
<td>0.49</td>
<td>2.13</td>
<td>S-R</td>
</tr>
<tr>
<td>3</td>
<td>iA-3</td>
<td>E</td>
<td>6.79</td>
<td>8.09</td>
<td>0.71</td>
<td>1.03</td>
<td>1.46</td>
<td>S-R</td>
</tr>
<tr>
<td>4</td>
<td>iA-3</td>
<td>C</td>
<td>4.79</td>
<td>5.63</td>
<td>0.22</td>
<td>0.44</td>
<td>1.97</td>
<td>R-S</td>
</tr>
<tr>
<td>5</td>
<td>iC-5</td>
<td>A</td>
<td>7.22</td>
<td>9.29</td>
<td>0.95</td>
<td>1.51</td>
<td>1.59</td>
<td>S-R</td>
</tr>
<tr>
<td>6</td>
<td>A-1</td>
<td>C</td>
<td>7.91</td>
<td>8.68</td>
<td>1.05</td>
<td>1.25</td>
<td>1.19</td>
<td>R-S</td>
</tr>
<tr>
<td>7</td>
<td>iA-3</td>
<td>A</td>
<td>6.16</td>
<td>11.03</td>
<td>0.70</td>
<td>2.05</td>
<td>2.92</td>
<td>S-R</td>
</tr>
<tr>
<td>8</td>
<td>iA-3</td>
<td>A</td>
<td>7.34</td>
<td>10.12</td>
<td>1.03</td>
<td>1.80</td>
<td>1.75</td>
<td>S-R</td>
</tr>
<tr>
<td>10</td>
<td>C-1</td>
<td>D</td>
<td>6.18</td>
<td>7.60</td>
<td>0.55</td>
<td>0.90</td>
<td>1.65</td>
<td>Y-X</td>
</tr>
</tbody>
</table>

Abbreviation: EEO, enantiomer elution order; the notation X or Y is reported for unknown absolute configuration; C-1, Lux Cellulose 1; iC-5, Lux i-Cellulose-5; A-1, Lux Amylose-1; iA-1, Lux i-Amylose-1; iA-3, Lux i-Amylose-3; A, Hex/IPA 95:5 v/v; B, Hex/IPA/MeOH 95:2.5:2.5 v/v/v; C, MeOH 100%; D, MeOH/water 95:5 v/v; E, MeOH:water 90:10 v/v