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Enantioseparation of fluorinated 3-(aryl)thio-4,4'-bipyridines: insights into chalcogen and π -hole bonds in high-performance liquid chromatography

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ABSTRACT

A chalcogen bond (ChB) is a σ -hole-based noncovalent interaction between a Lewis base and an electrophilic element of Group VI (O, S, Se, Te), which behaves as a Lewis acid. Recently, we demonstrated that halogen bond, the more familiar σ -hole-based interaction, is able to promote the enantioseparation of chiral compounds in HPLC environment. On this basis, an investigation to detect ChBs, functioning as stereoselective secondary interactions for HPLC enantioseparations, was started off and the results of this study are described herein. Our investigation also focused on the impact of the perfluorinated aromatic ring as a π -hole donor recognition site. For these purposes, seven atropisomeric fluorinated 3-(aryl)thio-4,4'-bipyridines were designed, synthesized and used as potential ChB donors (ChBDs) with two cellulose-based chiral stationary phases (CSPs) containing carbonyl groups as ChB

acceptors (ChBAs). In addition, one and two analogues lacking fluorine and sulphur, respectively, were prepared as terms of comparison. The design of the test analytes was computationally guided. In this regard, electrostatic potentials (EPs) associated with σ - and π -holes were computed and the atomic contributions to the sulphur EP maxima were derived using a molecular space partitioning in terms of Bader's atomic basins. This procedure is akin to the Bader-Gatti electron density source function (SF) decomposition, yet suitably extended to the EP field. For five 3-substituted-4,4'-bipyridines, thermodynamic parameters were derived from van't Hoff plots. Finally, the use of molecular dynamic (MD) simulation to model ChB in cellulose-analyte complexes was explored. Evidences that σ -hole and π -hole interactions can jointly drive HPLC enantioseparations through recognition sites generated by local electron depletion emerged from both experimental results and theoretical data.

Keywords: Bipyridines; Chalcogen bond; Electrostatic potential surfaces; Molecular dynamic; Polysaccharide-based chiral stationary phases; Source function

1. Introduction

The enantioseparation of chiral compounds through the so-called direct approach is an important matter in several fields of chemistry, biochemistry and life science [1,2]. Easy in its essence, the direct approach is based on the transformation of enantiomers into transient diastereomeric complexes in a chiral environment generated by a chiral selector. Nevertheless, understanding the recognition pattern occurring in these processes keeps on being a demanding issue in separation science [3]. Indeed, along with strong long-range interactions involved in the non-stereoselective binding, diverse short-range directional interactions based on the complementarity of selector and analyte (electrostatic fit), including hydrogen bonds, π - π , dipole-dipole, and van der Waals interactions [4], are able to promote the enantioseparation. Moreover, steric and hydrophobic factors can also play a role [1,5].

Currently, σ -hole bonds involving atoms of Groups IV–VII are also considered as important directional noncovalent interactions and applied in several fields [6,7]. A σ -hole indicates the region of positive electrostatic potential (EP) found on an anti-bonding σ^* orbital, located along the elongation of a covalent bond. Typically, a σ -hole bond can be written as EWG–D σ ...A (B_L), where D σ is the donor atom, which serves as a Lewis acid, and A is the acceptor moiety with properties as a Lewis base (B_L). The electron-withdrawing group (EWG) covalently attached to the D σ redistributes the electron density on the D σ itself (anisotropy), forming electron-deficient σ -holes which, consequently, act as a Lewis acid. In general, the depth of a σ -hole increases as the polarizability of D σ [8].

Recently, our groups discovered that halogen bonds (XBs), belonging to the family of the σ -hole bonds, can drive HPLC enantioseparations of halogenated analytes (XB donors) [9] by using cellulose tris(3,5-dimethylphenylcarbamate) (CDMPC) as a chiral selector (XB acceptor) [10,11], enlarging the range of interactions which are active in HPLC environment. Later, envisaging for the polysaccharide derivatives a novel function other than resolution of racemic mixture [12], we showed that HPLC and

polysaccharide-based chiral stationary phases (CSPs) can be used as technical and molecular tools, respectively, for detection of stereoselective XBs [13] (Fig. 1).

A halogen bond

Hal = Cl, Br, I

EWG = electron-withdrawing group

B polysaccharide-based polymers as halogen bond acceptors

70

Fig. 1. Description of halogen bond (XB) (A) and polysaccharide-based polymers as XB acceptors (B).

Less studied than XBs as σ -hole interactions, in the last years chalcogen bonds (ChBs) have found application in materials and crystal engineering [14], self-assembly processes [15], anion binding and transportation [16,17], and catalysis [18,19].

75

Fig. 2. General description of chalcogen (ChB) (A) and π -hole bond (B).

The general scheme of the ChB is depicted in Figure 2A, in which Ch is an electrophilic chalcogen atom (Group VI: O, S, Se, Te) and B_L is a donor site. Analogously to the XB, the main characteristics of

ChB are directionality and tunability. Indeed, two regions of positive EP are localized on the outer part of the Ch atom, opposite to each EWG (typical EWG–Ch··· B_L bond angles are close to 180°), and the strength of the ChB can be tuned by varying Ch atom, EWG and B_L [14].

Conceptually analogous to the σ -hole bond, a π -hole bond is a noncovalent interaction which involves an unpopulated π^* orbital (π -hole), as a donor, and a B_L as acceptor (anion or lone-pair). A typical π -hole is located perpendicular to the molecular framework of perfluorinated aromatic rings (Fig. 2B) [6,20].

Further to our recent results on the XB interactions, in this paper we describe the results of a study aiming to detect, in HPLC environment, other interactions involving regions of electron depletion as recognition sites at both chiral and achiral level. For this purpose, atropisomeric 4,4'-bipyridines **1**, **2** and **5-10** (Fig. 3) were designed as potential ChB donors (ChBDs) and synthesized in their racemic form through focused procedures. In addition, perfluorinated compounds **3** and **4**, lacking sulphur at position 3, were designed and prepared as terms of comparison with the aim to evaluate the impact of the pentafluorophenyl-centred π -hole on the enantioseparation.

Fig. 3. Structures of the 5,5'-dibromo-2,2-dichloro-3-substituted-4,4'-bipyridines **1-10**.

For all compounds, EPs were computed [21], and atomic contributions to the EPs maxima of sulphur in **1-5**, associated to ChBs, were derived using a molecular space partitioning in terms of Bader's atomic

basins [22]. This procedure is akin to the Bader-Gatti electron density source function (SF) approach [23,24], suitably extended to the EP field [25,26].

The chromatographic behaviour of compounds **1-10** was evaluated on cellulose-based CSPs upon structural variations of selectors, analytes and mobile phase (MP) polarity. Our previous studies proved the suitability of polysaccharide polymers as probes to detect stereoselective σ -hole interactions (Fig. 1B). Then, a study at variable temperature was performed to determine the thermodynamic parameters associated with the enantioseparation processes. Moreover, an exploration of the potential role of sulphur as a ChBD site was performed by molecular dynamic (MD) [11].

It is worth mentioning that studies on both stereoselective ChB and π -hole bonds are unprecedented. In addition, currently, $S\cdots O=C$ contacts have attracted interest because they have proved to control protein folding and likely regulate enzymatic functions [6].

2. Experimental

2.1. Chemicals

The names and the syntheses of compounds **1-10** together with the ^1H and ^{13}C NMR, and HRMS spectra are available in the Supplementary data.

2.2. Chromatography

An Agilent Technologies (Waldbronn, 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 thermostatted column compartment) was employed for both analytical and multimilligram separations. 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 (Phenomenex, USA), Chiralcel OD (Daicel, Tokyo, Japan) (cellulose tris(3,5-dimethylphenylcarbamate); 10 μm), Lux Cellulose-2 (cellulose tris(3-chloro-4-methylphenylcarbamate); 5 μm , Phenomenex, USA), Chiralpak IC (cellulose

121 tris(3,5-dichlorophenylcarbamate); 5 μ m) and Chiralpak IA (amylose tris(3,5-
122 dimethylphenylcarbamate); 5 μ m) (Chiral Technologies Europe, Illkirch, France) were used as chiral
123 columns (250 $\times$ 4.6 mm). HPLC grade ethanol (EtOH), *n*-hexane (Hex), *n*-heptane, methanol (MeOH),
124 and 2-propanol (IPA), were purchased from Sigma-Aldrich (Taufkirchen, Germany). The retention
125 factor (*k*) was determined as $k = (t_R - t_0)/t_0$, where t_R is the retention time for the eluted enantiomer. Dead
126 time (t_0) was measured by injection of tris-*tert*-butylbenzene (Sigma-Aldrich, Taufkirchen, Germany) as
127 a non-retained compound [27]. The separation factor (α) was calculated as $\alpha = k_2/k_1$, where k_1 and k_2 are
128 the retention factors of the first- and the second-eluted enantiomer, respectively. The resolution (R_s) was
129 determined as $2(t_{R2} - t_{R1})/(W_1 + W_2)$ where *W* is the basewidth of peak. Analyses were performed in
130 isocratic mode at 25°C. The flow rate (*FR*) was set at 0.8 ml/min for analytical separations. For
131 compounds **1**, **2** and **4**, the enantiomer elution order (EEO) was determined by injecting enantiomers of
132 known absolute configuration. The absolute configuration was assigned as reported in the
133 Supplementary data. The van't Hoff experiments were conducted at 10, 15, 20, 25, 30 and 35°C in a
134 thermostatted column chamber equipped with a cooling system. When the temperature was changed, the
135 column was allowed to equilibrate for 1 h before injecting the samples. Additional details on
136 determination of thermodynamic parameters are reported in Supplementary data.

137 2.3. Computational

138 Conformational search was performed through molecular mechanics, using the MMFF94 force field
139 and the Spartan '10 Version 1.1.0 (Wavefunction Inc., Irvine, CA) program [28]. Geometry optimization
140 and computation of electrostatic potentials surfaces (EPSs) and related parameters (EP extrema, maxima
141 and minima values, given in kJ/mol) were performed and graphically generated (Spartan '10 Version
142 1.1.0) employing the density functional theory (DFT) method with the B3LYP functional and the 6-
143 311G* basis set (available for elements H-Ca and Ga-Kr). Calculated EPs for 4,4'-bipyridines **1-10** are
144 available in the Supplementary data. Values of the electrostatic potential onto an isovalue electron

density surface were used as an indicator of the anisotropy of the molecular charge distribution. The surface mapped values of the EP as derived from Spartan '10, used the default values of the program (electron density isovalue 0.002 au, high resolution). EPS colours towards red depict negative mapped potentials, while colours towards blue depict positive potentials and colours in between (orange, yellow, green) depict intermediate values of the mapped potential. Min EP and max EP are the local minimum(a) and the maximum(a) values of the mapped property. Search for the exact location of such EP extrema was made through the Multiwfn code [29] and through its module enabling quantitative analyses of molecular surfaces [30] for several isovalue surface fields and mapped properties thereon.

Experimental details for SF calculations and MD are available in the Supplementary data.

3. Results and discussion

3.1. Design and syntheses of analytes **1** and **2** with properties as chalcogen bond donors

The design of new 4,4'-bipyridines serving as ChBDs was performed by means of halogen-driven structural and electronic engineering on the electron-poor 4,4'-bipyridine core with the aim to obtain analytes whose chromatographic response was dependent on the σ -hole depth of sulphur. The theoretical approach started from the chiral 4,4'-bipyridine core **11** (Fig. 4), where two bromines and one sulphur close to the stereogenic axis serve as atropisomerism inductors and potential Ch site, respectively. Thus, optimization of the system was tackled in principle by means of a two-step stereoelectronic activation of the sulphur site, which consists of i) regiospecific activation of **11** with chlorines, and ii) perfluorination of the phenyl group of compound **1** in order to obtain **2**.

Fig. 4. Design of potential 4,4'-bipyridine-based ChBDs.

3.1.1 Electrostatic potentials and their source contribution at the sulphur electrostatic potential maxima

To prove the efficacy of the double activation engineered on **11** to obtain **2**, EPSs of the compounds were generated by mapping the EP onto electron density isosurfaces (Supplementary data). The EP analysis is widely used for exploring and studying noncovalent interactions [31,32].

The distribution of the mapped EPs showed two sulphur-centred σ -holes, σ_I and σ_2 , as two narrow separated positive regions which surround the most electron-poor sites corresponding to the max EP values on sulphur. These regions are located along the elongation of each C-S bond (Fig. 4), σ_I (at the top of Het-S bond, Het = C₁₀H₅Br₂N₂ for **11**, and C₁₀H₃Br₂Cl₂N₂ for **1** and **2**) being more electronically activated and sterically accessible than σ_2 (at the top of S-Ar bond, Ar = C₆H₅ for **11** and **1**, and C₆F₅ for **2**) (Table 1). Indeed, in compound **2** the max EP associated to σ_I (max EP 114.4 kJ/mol) increases (+115%) more than the max EP of σ_2 (max EP 52.0 kJ/mol) (+38.3%), relative to the corresponding EP values in **11** (σ_I , σ_2 : 53.2, 37.6 kJ/mol).

Table 1
Calculated EP^a values [kJ/mol] for compounds **1**, **2** and **11** (Spartan '10 V1.1.0, DFT, B3LYP/6-311G*).

4,4'-bipy	max EP (S) (σ_I , σ_2)	max EP (Ar _{pyr})	min EP (N _{pyr})	max EP (Ar _F)	max EP (3-Br)	max EP (3'-Br)	max EP (2-Cl)	max EP (2'-Cl)
11	53.2, 37.6	46.3	-164.8	--	94.5	84.3	--	--
1	59.9, 52.5	73.0	-148.6	--	114.3	106.2	49.7	48.0
2	114.4, 52.0	86.1	-144.0	105.7	124.5	116.3	69.5	50.8

^a EP electrostatic potential.

From the EP analysis, different effects on σ_I and σ_2 can be assigned to the two types of activation: i) the introduction of two chlorines, at positions 2 and 2', increases the max EP value of σ_2 (**1**: max EP 52.5 kJ/mol, + 39.6%) more than for σ_I (**1**: max EP 59.9 kJ/mol, + 12.6%); ii) on the contrary, with the subsequent perfluorination, the max EP value of σ_I increases dramatically (**2**: max EP 114.4, + 91%), whereas the max EP in σ_2 remains almost unchanged.

In addition, in compound **2** a positive EP (**2**: max EP 105.7 kJ/mol) on the perfluorinated aromatic ring revealed the presence of a π -hole as a potential recognition site. Moreover, in **1** and **2** the common 5,5'-dibromo-2,2'-dichloro-4,4'-bipyridyl scaffold showed four halogen-centred σ -holes.

With the aim to examine in depth the origin of the EP on sulphur, we applied the SF [33] EP decomposition [25,26] to the sulphur-centred σ -holes in both **1** and **2**. Within this approach, each EP value may be envisaged as due to source contributions from the atoms or groups of atoms of a system. On this basis, it is possible to evaluate how the EP at the σ -hole is influenced by the remaining parts of the system and the small or large extent of such influence is just quantified by the SF. In Table 2, the EP SF decomposition in **1** and **2** at the EP maxima of sulphur σ_1 and σ_2 is reported. The values of V represent the SF contributions of the listed atomic groups, while those of ΔV express the change of such contributions on passing from **1** to **2** [$\Delta V = V(2) - V(1)$]. The sign of source is positive or negative whether the atomic (or group) source concurs or opposes to the positive potential of σ_1 and σ_2 .

Table 2

EP (au) SF^a decomposition in **1** and **2** at the electrostatic potential maxima of sulphur σ_1 and σ_2 on the 0.002 au electron density surface.^b

Atomic groups	1	2	1 → 2
	V ^c [au]	V ^c [au]	ΔV^d [au]
	σ_1 (1.90 Å ^e ; 0.0285 au) ^f	σ_1 (1.94 Å ^e ; 0.0495 au) ^f	(0.0210) ^f
Het	-0.0041	0.0019	0.0060
All Halogen atoms	-0.0329	-0.0325	0.0004
All [C _{Ar} + H _{Ar} (F) _{Ar}]	-0.0022	-0.0184	-0.0162
S	0.0359	0.0665	0.0306
	σ_2 (1.94 Å ^e ; 0.0268 au) ^f	σ_2 (2.02 Å ^e ; 0.0272 au) ^f	(0.0004) ^f
Het	-0.0175	-0.0118	0.0057
All Halogen atoms	-0.0433	-0.0451	-0.0018
All [C _{Ar} + H _{Ar} (F) _{Ar}]	0.0038	-0.0119	-0.0157
S	0.0416	0.0515	0.0099

^a EP electrostatic potential; SF source function.

^b Full source function calculation data are reported in the Supplementary data

^c V, SF atom/group contribution.

^d $\Delta V = V(2) - V(1)$, EP atom/group contribution change on passing from **1** to **2**

^e σ distance from sulphur nucleus.

^f Reference values of EP at σ_1 and σ_2 were calculated at DFT level of theory with the B3LYP functional and the 6-311G* basis set.

In Table 2, the sum of V(Het)+V{All [C_{Ar} + H_{Ar} (or F)_{Ar}]}+V(S) reproduces, as expected, the observed EP maxima in **1** and **2**. The V(Het) contributions include those of the halogen atoms, which

are also separately listed in Table 2. These latter remain almost constant, on passing from **1** to **2**, for both σ_I and σ_2 maxima ($\Delta V(\text{Het}) = 0.0004$ and -0.0018 au, respectively), while $V(\text{Het})$ similarly increase for both maxima in **2** ($\Delta V(\text{Het}) = 0.0060$ and 0.0057 au, respectively). What leads to a much larger σ_I maximum upon fluorination is the almost doubling of the S atom contribution, from 0.0359 (**1**) to 0.0665 au (**2**). The $\Delta V_{\text{s atom contribution}} = 0.0306$ au largely overwhelms the negative contribution of the $[\text{C}_{\text{Ar}} + \text{F}_{\text{Ar}}]$ group ($\Delta V = -0.0162$ au), while for the σ_2 maximum the opposite occurs ($\Delta V_{\text{s atom contribution}} = 0.0099$ au; ($\Delta V_{\text{All } [\text{C}_{\text{Ar}} + \text{F}_{\text{Ar}}]} = -0.0157$ au), due to the different geometrical location of the two maxima. Due to the large electron-withdrawing power of fluorine, the sulphur atom becomes more positively charged upon fluorination ($q(\text{S}) = 0.122$ and $0.208 e^-$, where $q(\text{S})$ is the Bader's net charge in **1** and **2**, respectively), hence more able to produce positive EP regions in **2**, relative to **1**. However, such an effect is three times as big at the top of Het-S bond (σ_I) than at the top of S-Ar bond (σ_2), [$\Delta V(\text{S}) = 0.0306$ and 0.0099 au], owing to the much closer proximity of the former to the strong electron-withdrawing F atoms. The presence of F atoms makes the C_{Ar} atoms highly electropositive ($\Delta q(\text{C})$ about $0.5 e^-$), the sulphur more positively charged and polarized in a direction counter to C-F and S-F charge transfer. Fluorination of the aryl ring has therefore a large enhancement effect on the σ_I hole of sulphur due to the induced large EP contribution increase from sulphur, despite the presence of F renders $V(\text{C}_{\text{Ar}} + \text{F})$ highly negative, relative to $V(\text{C}_{\text{Ar}} + \text{H})$ and even more so for σ_I . In addition, geometrical parameters for the two maxima on sulphur were derived from calculations (**1**, σ_I : $1.90 \text{ \AA}$, Het-S- σ angle xx° ; σ_2 : $1.94 \text{ \AA}$, Ar-S- σ angle xx°) (**2**, σ_I : $1.94 \text{ \AA}$, Het-S- σ angle xx° ; σ_2 : $2.02 \text{ \AA}$, Ar-S- σ angle xx°).

3.1.2 Syntheses of 3-(aryl)thio-4,4'-bipyridines **1** and **2**

In practice, racemic **1** and **2** were prepared by reaction of the *in situ* generated lithio intermediate **12** with 1,2-diphenyldisulfide and 1,2-bis(pentafluorophenyl)disulfide, respectively. The intermediate **12** was prepared by iodine – lithium exchange performed starting from 4,4'-bipyridine **13** (Scheme 1) [34].

Scheme 1. Synthesis of 4,4'-bipyridines **1** and **2**.

Under these conditions, after addition of electrophile, **1** and **2** were obtained with 65% and 21% yield, respectively (see Supplementary data for details), and enantioseparated by HPLC. Assignment of absolute configuration for **1** was achieved by single crystal XRD analysis, whereas for **2**, ECD spectroscopy coupled with TD-DFT calculations was used because it could not be crystallized (Supplementary data).

Having at disposal compounds **1** and **2**, chromatographic responses strictly dependent on the electronic features of the distinctive substituent at position 3 were expected.

3.2. Comparative enantioseparation of 3-(phenyl)thio- and 3-(pentafluorophenyl)thio-4,4'-bipyridine

Four chromatographic systems were generated by the combination of CDMPC with either Hex/IPA 90:10 (*mix A*), hex/IPA/MeOH 90:5:5 (*mix B*) or MeOH 100% (*mix C*) and cellulose tris(3-chloro-4-methylphenylcarbamate) (CCMPC) with *mix A*: CDMPC/*mix A*, CCMPC/*mix A*, CDMPC/*mix B* and CDMPC/*mix C*. These four systems were used in parallel, as an orthogonal HPLC protocol [13].

Recently, cellulose-based CSPs proved to be suitable molecular tools to study XBs in HPLC environment [10]. On this basis, we envisaged that these polysaccharide-based CSPs could be used as tools also for exploring other stereoselective interactions involving recognition sites generated by local electron depletion, such as ChBs. Indeed, in these CSPs the glucosyl backbone determines conformational chirality [35], whereas the derivatization of the native polymer allows a perpendicular molecular expansion (with respect to the backbone axis) and the transfer of the chiral information to generate a chiral polymeric groove which is able to host and recognize enantiomers. In this context, a

polar layer containing carbonyl oxygens is located inside the polymer groove, whereas a hydrophobic layer containing substituted aromatic rings is located outside the polymer groove [36]. The presence of methyl, as electron donating group, or chlorine, as EWG, on the aromatic ring influences the capability of the carbonyl oxygens as Lewis bases [12] and, consequently, their properties as σ - / π -hole acceptors. On this basis, in CDMPC the carbonyl oxygens show enhanced properties as acceptors (min EP_{C_O} = -170 kJ/mol). On the other hand, in CCMPC the carbonyls present reduced acceptor capability induced by EW chlorine substituents (min EP_{C_O} = -158 kJ/mol) [37]. Moreover, the properties of the carbonyl oxygens can be triggered by tuning the MP. Indeed, hex/IPA-containing normal phase (NP) elution conditions (such as *mix A*) has proved to assist interactions involving the polar layer. On the contrary, MeOH-containing MPs (*mix B* and *C*) was found to destabilize σ -hole bonds forming hydrogen bonds with the polar layer and promoting hydrophobic contacts [11,12].

On this basis, the orthogonal HPLC protocol was applied to **1** and **2** (Table 3). Retention was shown to be slightly affected by the orthogonal conditions and, indeed, k_1 values for the first eluted (*M*)-enantiomers range from 1.01 to 1.14 and from 0.87 to 1.26 for **1** and **2**, respectively. Analogously, both k_2 and α values of **1** were scarcely influenced by any change of the chromatographic system (CSP and/or MP) (entries 1-4). In these cases, hydrophobic forces seemed to control retention and selectivity. On the contrary, a different behaviour was observed for compound **2** because k_2 for the second eluted (*P*)-enantiomer and α values seemed to be deeply influenced by changes of analyte and CSP structures as well as MP polarity (entries 5-8). On this basis, a thorough comparative analysis of chromatographic data, computed electronic properties and thermodynamic parameters, derived from van't Hoff plots, suggested that a stereoselective secondary interaction, strictly dependent on the (pentafluorophenyl)thio substituent at the position 3 of the 4,4'-bipyridine scaffold and involving the carbamate carbonyl oxygen of the CDMPC, could explain the diverse separation outcomes of **2** and **1**. In fact, taking into account the effect of focused changes of donor/acceptor structures and MP, the following observations emerged:

Table 3
 Substitution pattern, retention and selectivity of 5,5'-dibromo-2,2'-dichloro-4,4'-bipyridines **1** and **2** on CDMPC and CCMPC under normal phase (NP) and polar organic (PO) elution modes (*FR* 0.8 ml/min, *T* = 25°C).

entry	bipy	CSP ^a	MP ^b	<i>k</i> ₁ ^c	<i>k</i> ₂ ^c	α
1	1	CDMPC	A	1.08 (<i>M</i>)	1.25 (<i>P</i>)	1.16
2	1	CCMPC	A	1.07 (<i>M</i>)	1.19 (<i>P</i>)	1.11
3	1	CDMPC	B	1.14 (<i>M</i>)	1.29 (<i>P</i>)	1.13
4	1	CDMPC	C	1.01	1.01	1.00
5	2	CDMPC	A	1.14 (<i>M</i>)	6.12 (<i>P</i>)	5.34
6	2	CCMPC	A	0.90 (<i>M</i>)	1.18 (<i>P</i>)	1.31
7	2	CDMPC	B	1.26 (<i>M</i>)	4.61 (<i>P</i>)	3.67
8	2	CDMPC	C	0.87 (<i>M</i>)	1.80 (<i>P</i>)	2.06

^a CDMPC cellulose tris(3,5-dimethylphenylcarbamate); CCMPC cellulose tris(3-chloro-4-methylphenylcarbamate).

^b MP, Hex/IPA 90:10 (A); Hex/IPA/MeOH 90:5:5 (B); MeOH 100% (C).

^c Absolute configuration of the eluted peaks is reported.

i) *effect of changing analyte structure (entries 1 and 5)*. The substitution pattern of the 5,5'-dibromo-2,2'-dichloro-3-(aryl)thio-4,4'-bipyridines **1** and **2** could be correlated by changing the group at the 3-position, thus the pivotal role of σ_I depth on selectivity could be hypothesized. Indeed, the distinctive (pentafluorophenyl)thio group (max $EP_S = 114.4$ kJ/mol) in **2** generates a more electrophilic σ_I than the (phenyl)thio group (max $EP_S = 59.9$ kJ/mol) in **1**, giving, therefore, a possible explanation for the different behaviour of the two (*P*)-enantiomers (**2**: $k_2 = 6.12$, $\alpha = 5.34$; **1**: $k_2 = 1.25$, $\alpha = 1.16$), with a

79.5% decrease in the k_2 value of **1** compared to **2**, under identical elution conditions (CDMPC/*mix A*). In addition, in **2** the distinctive π -hole could have an impact, as a recognition site, on the enantioseparation extent, hypothesizing a π -hole interaction involving CSP carbonyl oxygen as B_L (Fig. 2B). On the other hand, in this compound the two chlorine-centred σ -holes at positions 2,2' showed low depth and scarce capability to control selectivity [11,37]. Analogously, our previous studies on XB-driven enantioseparations had showed that 5,5'-bromine-centred σ -holes, with max EP = 123.3 kJ/mol, are only able to produce moderate selectivity ($\alpha = 1.47$) [11];

ii) *effect of changing polysaccharide structure (entries 1 vs 2 and 5 vs 6)*. By changing CDMPC to CCMPC, the separation factor of **2** dramatically dropped with a 75.2% decrease, whereas the separation factor of **1** showed a decrease of just 3.4%. This behaviour could be related to the reduced Lewis basicity of the carbonyl groups of the CCMPC;

iii) *effect of changing medium polarity (entries 1 vs 3-4 and 5 vs 7-8)*. On CDMPC, the addition of MeOH (*mix B* and *mix C*) enhanced MP polarity and introduced in the chromatographic system a competitive electrophile. Consequently, selectivity decreased for **1** and **2** of 2.6% and 31.3% with *mix B* and of 13.8% and 61.4% with *mix C*, respectively;

iv) *analysis of thermodynamic parameters (Table 4)*. As both retention and separation are influenced by temperature, a variable temperature study was carried out between 10 and 35°C for 4,4'-bipyridines **1** and **2** on the four chromatographic systems. Because the van't Hoff plots were linear in the considered temperature range ($r^2 \geq 0.9958$) (Supplementary data), enthalpy and entropy values derived from the plots allowed to evaluate the enthalpic and entropic contribution to enantioseparations. The derived thermodynamic parameters showed that the enantiomeric separation of **1** and **2** are enthalpically driven ($\Delta\Delta H < 0$ and $\Delta\Delta H < T\Delta\Delta S$) under all elution conditions. Negative ΔS indicated an increase in the order of the chromatographic system as the solute moved from mobile phase to stationary phase. In particular, with the system CDMPC/*mix A*, the second eluted enantiomer (*P*)-**2** showed values of enthalpy, entropy

and free energy ($\Delta H = -34.01 \text{ kJ}\cdot\text{mol}^{-1}$, $\Delta S^* = -98.86 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$, $\Delta G = -4.53 \text{ kJ}\cdot\text{mol}^{-1}$) much lower than those of (*P*)-**1** ($\Delta H = -12.27 \text{ kJ}\cdot\text{mol}^{-1}$, $\Delta S^* = -39.21 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$, $\Delta G = -0.58 \text{ kJ}\cdot\text{mol}^{-1}$), confirming the pivotal role of the $-\text{SC}_6\text{F}_5$ group to exert polar interactions with the CSP. It is worth mentioning that, under the same elution conditions (CDMPC/*mix A*), the thermodynamic behaviour of the couple **1/2** seemed to follow the same trend of the couple 2,2',3,3',5,5'-hexachloro- (**14**) / 2,2',3,3',5,5'-hexaiodio-4,4'-bipyridine (**15**) [13] which differ in the capability to exert σ -hole interactions in HPLC environment, iodine being privileged σ -hole donor. Indeed, $\Delta\Delta H$, $\Delta\Delta S$ and $\Delta\Delta G$ values associated to **1** ($\Delta\Delta H = -1.96 \text{ kJ}\cdot\text{mol}^{-1}$, $\Delta\Delta S = -5.32 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$, $\Delta\Delta G = -0.37 \text{ kJ}\cdot\text{mol}^{-1}$) are very similar to the values of the poor σ -hole bond donor **14** ($\Delta\Delta H = -1.95 \text{ kJ}\cdot\text{mol}^{-1}$, $\Delta\Delta S = -5.36 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$ and $\Delta\Delta G = -0.35 \text{ kJ}\cdot\text{mol}^{-1}$), whereas **2** was characterized by higher entropic stabilization ($\Delta\Delta S = -50.73 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$) as it occurs with the good σ -hole donor **15** [$\Delta\Delta S = -27.47 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$] [13].

Table 4
Thermodynamic parameters calculated from the van't Hoff plots (see Supplementary data) for 2,2'-dichloro-5,5-dibromo-4,4'-bipyridines **1** and **2** on CDMPC and CCMPC under normal phase (NP) and polar organic (PO) elution modes (*FR* 0.8 ml/min).

bipy ^a	CSP ^b	MP ^c	ln <i>k</i> vs 1/ <i>T</i>			ln α vs 1/ <i>T</i>		
			ΔH (kJ·mol ⁻¹)	ΔS^* (J·K ⁻¹ ·mol ⁻¹)	$\Delta G_{298 \text{ K}}$ (kJ·mol ⁻¹)	$\Delta\Delta H$ (kJ·mol ⁻¹)	$\Delta\Delta S$ (J·K ⁻¹ ·mol ⁻¹)	$\Delta\Delta G_{298 \text{ K}}$ (kJ·mol ⁻¹)
1	(<i>M</i>)	CDMPC	A	-10.31	-33.89	-0.21	-1.96	-5.32
	(<i>P</i>)			-12.27	-39.21	-0.58		
1	(<i>M</i>)	CCMPC	A	-10.04	-33.07	-0.18	-1.59	-4.46
	(<i>P</i>)			-11.63	-37.53	-0.44		-0.26
1	(<i>M</i>)	CDMPC	B	-9.80	-31.76	-0.33	-0.78	-1.59
	(<i>P</i>)			-10.58	-33.35	-0.64		-0.31
1	(<i>M, P</i>)	CDMPC	C	-8.10	-27.10	-0.02	0	0
2	(<i>M</i>)	CDMPC	A	-14.69	-48.13	-0.34	-19.32	-50.73
	(<i>P</i>)			-34.01	-98.86	-4.53		-4.19
2	(<i>M</i>)	CCMPC	A	-12.21	-41.88	0.28	-3.56	-9.64
	(<i>P</i>)			-15.77	-51.52	-0.41		-0.69
2	(<i>M</i>)	CDMPC	B	-13.75	-44.22	-0.57	-14.59	-38.09
	(<i>P</i>)			-28.34	-82.31	-3.80		-3.23
2	(<i>M</i>)	CDMPC	C	-9.99	-34.61	0.33	-8.46	-22.32
	(<i>P</i>)			-18.45	-56.93	-1.47		-1.80

^a Absolute configuration of the eluted peaks is reported.

^b CDMPC cellulose tris(3,5-dimethylphenylcarbamate); CCMPC cellulose tris(3-chloro-4-methylphenylcarbamate).

^c MP, Hex/IPA 90:10 (A); Hex/IPA/MeOH 90:5:5 (B); MeOH 100% (C).

On the other hand, the values of the thermodynamic parameters, obtained for **1** and **2** with the chromatographic system CDMPC/*mix C*, proved the higher recognition ability of the selector toward the enantiomers of **2** ($\Delta\Delta H = -8.46 \text{ kJ}\cdot\text{mol}^{-1}$, $\Delta\Delta S = -22.32 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$, $\Delta\Delta G = -1.80 \text{ kJ}\cdot\text{mol}^{-1}$) compared to **1**

(not separated). This behaviour under polar organic (PO) elution conditions could be due to higher hydrophobicity and more efficient π - π stacking of **2** with respect to **1** ($\text{Log } P_{\text{partition coefficient}}$: 6.09 (**1**) < 6.88 (**2**)).

Having identified the main features of the chromatographic behaviour of **1** and **2** with the four orthogonal chromatographic systems, the enantioseparation of compounds **5-10** was explored with the aim to evaluate the effect of diverse fluorine substitution patterns on the enantioseparation.

3.3. Enantioseparation behaviour of compounds **5-10**: effect of fluorine regiochemistry and number

Compounds **5-10** were designed with the aim to derive focused information on the effect of fluorine regiochemistry (**5-7**) and number (**8**, **9** and **10**) from the chromatographic analysis. All this series of compounds was prepared by iodine – lithium exchange performed on 4,4'-bipyridine **13** and subsequent electrophilic trapping of the lithio intermediate **12** with the proper disulphide (30-71% yields), as reported in Scheme 1 for **1** and **2** [34].

In Table 5, retention and selectivity factors observed for compounds **5-10** with the chromatographic system CDMPC/mix A are reported (entries 2-7) along with the corresponding parameters of **1**, as a term of comparison (entry 1).

Table 5
Substitution pattern, retention, selectivity, max EP at σ_I and Ar_F of 5,5-dibromo-2,2'-dichloro-4,4'-bipyridines **1** and **5-10** on CDMPC under normal phase (NP) elution mode (Hex/IPA 90:10, FR 0.8 ml/min, $T = 25^\circ\text{C}$).

entry	bipy	fluorine pattern	max EP ^a σ_I	max EP (Ar_F)	k_1^a	k_2^a	α
1	1	--	59.9	--	1.08	1.25	1.16
2	5	<i>o</i> -F	69.8	--	1.21	1.42	1.17
3	6	<i>m</i> -F	81.4	--	1.22	1.42	1.16
4	7	<i>p</i> -F	68.6	12.0	1.20	1.30	1.08
5	8	<i>o,o</i> -F ₂	80.2	21.6	1.43	1.68	1.18
6	9	<i>o,p</i> -F ₂	79.9	25.1	1.16	1.29	1.11
7	10	<i>o,o,m</i> -F ₃	92.0	48.1	1.83	2.64	1.45

^a EP electrostatic potential [kJ/mol].

For the mono substituted series **5-7** (entries 2-4), containing one fluorine on the phenyl ring directly bonded to sulphur, retention was slightly affected by the substitution regiochemistry and, indeed, k_1

values for the first eluted enantiomers range from 1.20 to 1.22. Analogously, both k_2 and α values of **5-7** were scarcely influenced by any change of fluorine position, nevertheless selectivity was shown to decrease following the order *o*-F (**5**, $\alpha = 1.17$) > *m*-F (**6**, $\alpha = 1.16$) > *p*-F (**7**, $\alpha = 1.08$) (Fig. S7). For these compounds, a clear correlation between max EP on σ_I , max EP on Ar_F and k_2 or α was not observed. In particular, no positive value of EP related to π -hole was found for **5** and **6**, whereas a slight depletion of the electron density (max EP (Ar_F) = 12 kJ/mol) characterizes **7**.

Table 6
EP(au) SF^a decomposition in **5-7** at the electrostatic potential maximum of sulphur σ_I on the 0.002 au electron density surface.^b

	5 σ_I (1.91 Å ^c ; 0.0323 au) ^d	6 σ_I (1.98 Å ^c ; 0.0367 au) ^d	7 σ_I (1.91 Å ^c ; 0.0322 au) ^d
Atom	V ^e	V ^e	V ^e
C ₂₁	-0.0394	-0.0308	-0.0393
C ₂₃	0.0733	0.0030	0.0023
C ₂₄	-0.0089	-0.0095	-0.0112
C ₂₆	0.0031	0.0504	0.0010
C ₂₅	-0.0006	0.0007	0.0006
C ₂₂	-0.0008	0.0008	0.0526
(C+H) _{Ar}	0.0761	0.0641	0.0621
Het	-0.0015	-0.0028	-0.0039
S	0.0442	0.0353	0.0369
F	-0.0858	-0.0589	-0.0618

^a EP electrostatic potential; SF source function.

^b Full source function calculation data are reported in the SI. Atoms are numbered in accordance with the source function calculation output (Supplementary data).

^c σ_I distance from sulphur nucleus.

^d Reference values of EP at σ_I were calculated at DFT level of theory with the B3LYP functional and the 6-311G* basis set.

^e V, SF atom/group contribution. Those for the C linked to F atoms are denoted in bold.

Application of the SF descriptor to the sulphur-centred σ -holes in **5-7** is illustrated in Table 6. Each σ_I EP value may be expressed as a sum of contributions from the S and F atoms and from the Het and (C+H)_{Ar} groups. Data in Table 6 reveal that the single F substitution largely decreases the SF contribution of the S atom to σ_I , from 0.0665 au in **2** (Table 2) to an average value of only 0.0388 au in **5-7**, slightly larger than for the unsubstituted compound **1** (0.0359 au). Indeed the average (**5-7**) net positive charge of sulphur is 0.139 e⁻, much closer to that found in **1** (0.122) than in **2** (0.208). Increase

of the sulphur positive charge upon fluorination follows the substitution pattern, being larger when the fluorine is closer and smaller when it is further from S (0.152, 0.140 and 0.125 for *ortho*, *meta* and *para* F-substitution). Thus, the highest V(S) contribution turns out to be that of the *ortho*-F compound **5**. The V(S) contribution for the *para*-F compound is, instead, surprisingly similar and even slightly larger than that of the *meta*-F compound. This becomes no longer a surprise when it is realized that the σ_I -hole is indeed slightly closer to the F nucleus in **7** than in **6** (5.44 and 5.70 Å, respectively), because *meta*-F substitution leads to a *trans* rather than to a *cis* σ_I -S-C_{Ar}-F minimum geometrical conformation. As a result, the negative V(F) contribution, counteracting the positive V(S) contribution, is lowest in magnitude for the *meta*-F compound. The sum of V(F) and V(S) contributions already accounts for the σ_I trend in **6-7** compounds (-0.0236 and -0.0249 au, respectively). Despite the more negative V(S+F) contribution in **5** relative to **7** (-0.0416 and -0.0249 au, respectively), almost equal σ_I values occur for *ortho* (**5**) and *para* (**7**) F-substitution. This arises because of a larger positive contribution of the C atom linked to the fluorine (these contributions are denoted in bold in Table 6) which compensates the more negative V(S+F) contribution (-0.0416 and -0.0249 au, respectively). The small changes in the V(Het) values play only a minor role in defining the σ_I trend in **5-7** compounds. The SF analysis of the σ -holes in **5-7** allows for an unprecedented insight on the effect of the F-substitution pattern. Minimum energy geometrical conformation also plays a significant role and it cannot be ignored. If not taken into account, the largest σ_I value for **6** could not find an easy explanation. The largest F distance from σ_I in the *meta* substituted compound realizes the best compromise between the activation of S induced by the presence of the F atom and the counteracting effect on the EP positive σ -hole due to the negative EP contribution from this same atom.

This kind of analysis confirmed the pivotal role of sulphur in the enantioseparation and, in this perspective, the decrease of selectivity changing the 2,6-difluoro pattern of **8** (Table 5, entry 5, $\alpha = 1.18$) to the 2,4-difluoro of **9** (Table 5, entry 6, $\alpha = 1.11$) could be explained on the basis of the minor

contribution of the carbon skeleton to σ_I induced by fluorine located at the *para* position compared to *ortho* position.

Concerning the effect induced by the number of fluorine atoms on the aromatic ring, in the series **5**, **8** and **10** (Table 5, entries 2, 5 and 7) containing 1, 2, and 3 fluorine atoms, respectively, k_1 , k_2 and α increased as the number of fluorine atoms (Fig. S8) and, in particular, selectivity tended to increase moderately as the max EP on σ_I and ArF (*o*-F (**5**, $\alpha = 1.17$; max EP $_{\sigma_I}$ = 69.8 kJ/mol) < *o,o*-F₂ (**8**, $\alpha = 1.18$; max EP $_{\sigma_I}$ = 80.2 kJ/mol; max EP $_{ArF}$ = 21.6 kJ/mol) < *o,o,m*-F₃ (**10**, $\alpha = 1.45$; max EP $_{\sigma_I}$ = 92.0 kJ/mol; max EP $_{ArF}$ = 48.1 kJ/mol)). Accordingly, thermodynamic parameters derived for compound **8** (Table S7) are lower ($\Delta\Delta H = -2.49$ kJ·mol⁻¹, $\Delta\Delta S = -6.96$ J·K⁻¹·mol⁻¹, $\Delta\Delta G = -0.41$ kJ·mol⁻¹) than the corresponding values associated to the enantioseparation of **1** ($\Delta\Delta H = -1.96$ kJ·mol⁻¹, $\Delta\Delta S = -5.32$ J·K⁻¹·mol⁻¹, $\Delta\Delta G = -0.37$ kJ·mol⁻¹), under the same elution conditions (CDMPC/*mix A*).

3.4. Impact of recognition sites generated by local electron depletion: σ -hole vs π -hole.

Finally, with the aim to distinguish the contribution of the chalcogen site (sulphur) from that of the fluorinated π -hole, compounds **3** and **4** were designed and prepared by deprotolithiation of 2,2'-dichloro-5,5'-dibromo-4,4'-bipyridine **16** [34]. Then, lithio intermediate **12** was trapped by the proper electrophile (pentafluorobenzyl bromide or hexafluorobenzene), furnishing **3** and **4** (18% and 5% yields, respectively) (Scheme 2). Assignment of absolute configuration for **4** was achieved by single crystal XRD analysis (Supplementary data).

Scheme 2. Synthesis of 4,4'-bipyridines **3** and **4**.

429 The orthogonal HPLC protocol was applied to compounds **3** and **4** and the obtained chromatographic
430 results were compared to the corresponding outcomes of **2** (Table 7).

431 The comparison between the chromatographic parameters of compound **2**, **3** and **4** (Fig. S9) proved
432 the pivotal role of the π -hole on the perfluorinated phenyl ring as recognition site. Indeed, the following
433 observations emerged:

434 **Table 7**
435 Substitution pattern, retention, selectivity and max EP at the π -hole (Ar_F) of 5,5-dibromo-2,2'-dichloro-4,4'-bipyridines **2**, **3** and **4** on
436 CDMPC and CCMPC under normal phase (NP) and polar organic (PO) elution modes (FR 0.8 ml/min, $T = 25^\circ\text{C}$).

2

3

4

CSP ^a	MP ^b	Parameter ^c	2 (-SC ₆ F ₅) 105.7 kJ/mol ^d	3 (-CH ₂ C ₆ F ₅) 89.8 kJ/mol ^d	4 (-C ₆ F ₅) 101.1 kJ/mol ^d
CDMPC	A	k_1	1.14 (<i>M</i>)	1.21	0.65 (<i>M</i>)
		k_2	6.12 (<i>P</i>)	4.39	0.97 (<i>P</i>)
		α	5.34	3.62	1.48
CCMPC	A	k_1	0.90 (<i>M</i>)	1.22	0.48 (<i>M</i>)
		k_2	1.18 (<i>P</i>)	1.80	0.51 (<i>P</i>)
		α	1.31	1.48	1.07
CDMPC	B	k_1	1.26 (<i>M</i>)	1.32	0.71 (<i>M</i>)
		k_2	4.61 (<i>P</i>)	3.68	1.10 (<i>P</i>)
		α	3.67	2.78	1.56
CDMPC	C	k_1	0.87 (<i>M</i>)	0.67	0.51 (<i>M</i>)
		k_2	1.80 (<i>P</i>)	1.36	0.67 (<i>P</i>)
		α	2.06	2.02	1.31

438 ^a CDMPC cellulose *tris*(3,5-dimethylphenylcarbamate); CCMPC cellulose *tris*(3-chloro-4-methylphenylcarbamate).

439 ^b MP, Hex/IPA 90:10 (A); Hex/IPA/MeOH 90:5:5 (B); MeOH 100% (C).

440 ^c For compounds **2** and **4**, absolute configuration of the eluted peaks is reported.

441 ^d max EP (electrostatic potential) π -hole (Ar_F).

442 i) in general, compound **4** showed lower retention and selectivity compared to **2** and **3** and a different
443 recognition mechanism seemed to occur. From the EPS generated for **4** by calculation, it could be
444 observed that the aromatic ring at the position 3 of the 4,4'-bipyridine scaffold is sterically hindered by
445 the adjacent chlorine at the 2 position. This condition prevents the penetration of the analyte into the
446 groove as proved by the low values of retention of both first and second eluted peaks ($0.48 \leq k_1 \leq 0.71$;
447 $0.51 \leq k_2 \leq 1.10$) obtained for **4** with all four chromatographic systems. This hypothesis appeared in
448 agreement with the positive values of $\Delta G_{298\text{ K}}$ derived for **4** in almost all cases ($0.08 \leq \Delta G_{298\text{ K}} [\text{kJ}\cdot\text{mol}^{-1}]$)

449 ≤ 1.81) (Table S8), depending on the lower value of the entropic term $T\Delta S$ ($-15.15 \leq T\Delta S$ [$\text{kJ}\cdot\text{mol}^{-1}$] $\leq -$
 450 9.05) compared to the enthalpic term ΔH ($-15.07 \leq \Delta H$ [$\text{kJ}\cdot\text{mol}^{-1}$] ≤ -7.38) in the Gibbs equation ($\Delta G =$
 451 $\Delta H - T\Delta S$, $\Delta H < 0$ and $T\Delta S < \Delta H$). On this basis, weak hydrophobic π - π interactions between the
 452 analyte and the aromatic moieties in the outer regions of the CSP are likely to govern selectivity in this
 453 case. Indeed, by introducing MeOH in the MP (*mix B*), which favours hydrophobic behaviours,
 454 selectivity of **4** on CDMPC increases ($\alpha = 1.56$) compared to the selectivity obtained with *mix A* on the
 455 same CSP ($\alpha = 1.48$).

456 ii) it is worth comparing the chromatographic behaviours of the couple 5,5'-dibromo-2,2'-dichloro-3-
 457 phenyl-4,4'-bipyridine (**17**) / **1** (Fig. S10) and the couple **4** / **2** (Fig. S9). In both cases, the substitution
 458 pattern of **17**, **4** and **1**, **2** can be correlated by introducing a sulphur atom as spacer between the
 459 heteroaromatic scaffold and the phenyl ring. Interestingly, this structural variation has a dramatic effect
 460 on selectivity only for **2**, which bears both σ - and π -hole ($\text{max EP}_{\sigma I} = 114.4$ kJ/mol; $\text{max EP}_{\text{ArF}} = 105.7$
 461 kJ/mol), whereas it has no effect for **1** ($\text{max EP}_{\sigma I} = 59.9$ kJ/mol). Indeed, the enantioseparation traces of
 462 **1** and **17** are superimposable (Fig. S10);

463 iii) the EPS generated for compound **3** showed a more accessible π -hole due to the presence of a
 464 methylene ($-\text{CH}_2-$) spacer between the 4,4'-bipyridine and the perfluorinated ring, even with a lower
 465 depth (89.9 kJ/mol) compared to **4** (101.1 kJ/mol). For **3**, higher retention and selectivity were observed
 466 compared to **4** (**3**: $k_1 = 1.21$, $k_2 = 4.39$, $\alpha = 3.62$; **4**: $k_1 = 0.65$, $k_2 = 0.97$, $\alpha = 1.48$), which are likely due
 467 to the conformational flexibility induced by the methylene spacer. In particular, on CDMPC, **3** showed
 468 lower values of $\Delta\Delta H$ ($-12.99 \leq \Delta\Delta H$ [$\text{kJ}\cdot\text{mol}^{-1}$] ≤ -7.11) and $\Delta\Delta S$ ($-32.81 \leq \Delta\Delta S$ [$\text{J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$] ≤ -17.98)
 469 compared to **4** ($-2.29 \leq \Delta\Delta H$ [$\text{kJ}\cdot\text{mol}^{-1}$] ≤ -1.87 ; $-4.03 \leq \Delta\Delta S$ [$\text{J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$] ≤ -3.15). On the contrary, on
 470 CCMPC higher values of $\Delta\Delta H$ and $\Delta\Delta S$ were derived for **3** (**3**: $\Delta\Delta H = -3.64$ $\text{kJ}\cdot\text{mol}^{-1}$; $\Delta\Delta S = -8.84$ $\text{J}\cdot\text{K}^{-1}$
 471 $\cdot\text{mol}^{-1}$; **4**: $\Delta\Delta H = -3.94$ $\text{kJ}\cdot\text{mol}^{-1}$; $\Delta\Delta S \leq -12.68$ $\text{J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$). These variations dependent on the CSP
 472 structures appeared consistent with a mechanism controlled by a lone pair- π -hole bond, involving **3** and

the carbonyl lone pair in the inner part of the CSP. In this perspective, as the Lewis basicity of the carbonyl decreases in the CCMPC, consequently, enantiomer thermodynamic stabilization also decreases. On the contrary, for **4** the thermodynamic stabilization by means of π - π interactions is higher with the distinctive 3-chloro-4-methylphenyl (CCMPC) compared to the 3,5-dimethylphenyl (CDMPC), likely because of different stereoelectronic properties;

iv) compound **2** showed higher k_2 and selectivity than **3** on CDMPC (**2**: $k_2 = 6.12$, $\alpha = 5.34$; **3**: $k_2 = 4.39$, $\alpha = 3.62$), whereas lower retention and selectivity on CCMPC (**2**: $k_1 = 0.90$, $\alpha = 1.18$; **3**: $k_1 = 1.22$, $\alpha = 1.48$) was observed. On the contrary, the two compounds showed very similar selectivity on CDMPC with *mix C* (**2**: $\alpha = 2.06$; **3**: $\alpha = 2.02$), where pure MeOH, as MP, prevent formation of polar interactions. Taking into account that the substitution pattern of **3** and **2** could be correlated by changing the methylene spacer to sulphur at the 3-position, the critical function of sulphur on selectivity emerged.

Fig. 5. A. Enthalpy-entropy ($\Delta\Delta S$ vs $\Delta\Delta H$ values x 10000) compensation for compounds **1**, **2**, **3**, **4** and **8** on CDMPC and CCMPC under normal phase (NP) and polar organic (PO) elution modes (*FR* 0.8 ml/min); B. Residual plot ($\Delta\Delta S$ values).

Analysis of the collected thermodynamic parameters for **1-4** and **8** (Tables 4, S7 and S8) revealed that $\Delta\Delta H$ and $\Delta\Delta S$ change proportionally. The changes in the two quantities essentially cancel out each

other, resulting in only small changes in $\Delta\Delta G$. This phenomenon is known as entropy-enthalpy compensation (EEC) and is illustrated graphically by the linear relationship between $\Delta\Delta H$ and $\Delta\Delta S$ (Fig. 5). Enthalpy-entropy compensation gave a straight line with a good correlation coefficient ($r^2 = 0.9954$) and no deviation from linearity was observed. This could confirm that the same type of discrimination mechanism governs the examined enantioseparations with a level of efficacy dependent on the substitution pattern.

With the aim to examine in depth the results of the EEC, the residual ($\Delta\Delta S_{\text{predicted}} - \Delta\Delta S_{\text{experimental}}$) plot values (Table S9) derived from the linear regression analysis were compared. Taking the sum of the residual values of **2** as reference absolute value (1.46), interestingly, in accord with the experimental outcomes, the corresponding absolute values calculated for **1**, **3** and **4** showed a deviation from **2** following the order **2** (1.46) < **1** (1.48) < **3** (2.0) < **4** (6.58_[A1]).

502

503 **Fig. 6.** Linear regression analysis describing the relationships between $\ln k_2$ and the max EP Ar_F (π -hole)
 504 (A) and the max EP on σ_1 (B) for compound **1-3** and **5-10**.

505 Finally, the influence of the two regions of electron depletion on retention of the second eluted
506 enantiomers was verified by simple linear regression using $\ln k_2$ and max EPs on Ar_F (π -hole) and σ_1 as
507 dependent and independent variables, respectively. On the basis of the described chromatographic
508 behaviour, compound **4** was excluded from the analysis. For compounds **2**, **3** and **7-10**, which are
509 characterized by positive values of EPs on Ar_F, the result of fitting a simple linear regression model to
510 describe the relationship between $\ln k_2$ and max EPs on Ar_F is reported in Fig. 6A ($r^2 = 0.9645$). In this
511 case, the P-value of the considered independent variable, max EPs on Ar_F, is 0.0005 indicating a
512 statistically significant relationship (P-value > 0.05).

513 Then, for compounds **1**, **2** and **5-10**, the correlation between $\ln k_2$ and the max EPs on σ_1 is reported
514 in Fig. 7B ($r^2 = 0.8633$). The P-value of the considered independent variable, the max EPs on σ_1 , is low
515 (0.0008), showing again a statistical significant relationship (P-value < 0.05) between selectivity and the
516 electron depletion on sulphur.

517 On the basis of experimental results, theoretical data and statistical evaluations, it is reasonable to
518 conclude that both σ - and π -hole bond concur to the enantioseparation outcomes of **2**. The study of
519 structurally similar compounds showed that the strength of each interaction strictly depends on analyte
520 and CSP structures and MP polarity. In this regard, it is interesting to mention that several recent articles
521 reported on the interplay between σ -hole and π -hole bonds [38].

522 3.5. Molecular dynamics.

523 MD calculations were performed to simulate the interaction modes of compound **2** with the CDMPC.
524 A massless dummy atom (MA_D) connected to sulphur was introduced manually in **2**, by using distance
525 (1.6 Å) and charge (0.2 units of positive charge) fixed arbitrarily (see Supplementary data for details).
526 On this basis, with the aim to compare qualitatively the MD outcomes under different molecular
527 situations, the calculations were performed with and without the MA_D, and Hex solvent effects were
528 taken into account in accord with the method used in the HPLC screening.

529 The MD protocol proposed herein may not lead to the selector-selectand complexes with the global
530 minimum energy, however some interesting behaviour could be observed, which confirmed the pivotal
531 role of sulphur as a chalcogen recognition site in **2**:

532 i) by carrying out 10 ns MD, the trajectory plots for CDMPC and **2** showed stable interactions when
533 the MA_D correction was applied, with narrow fluctuations over 10 ns compared to the larger fluctuations
534 observed when MA_D correction was not applied (Fig. S30);

535 ii) in all simulations performed by using the MA_D correction, contacts between sulphur and the
536 carbonyl groups of the carbamate moieties of the CSP were observed. Taking into account that the MA_D
537 correction was introduced arbitrarily, a measure of the geometrical parameters of the contact was not
538 performed;

539 iii) finally, the occupancy graphs generated from the MD trajectories for the complexes CDMPC /
540 (*M*)-**2** (with MA_D), CDMPC / (*P*)-**2** (with MA_D), and CDMPC / (*P*)-**2** (without MA_D) were compared
541 (Fig. S31). The occupancy analysis allowed to evaluate which regions of space were highly populated by
542 the analysis over 10 ns MD. Interestingly, by using the MA_D, the first eluted enantiomer (*M*)-**2** showed
543 occupancy volumes in the outer region of the CSP, whereas for the second eluted enantiomer (*P*)-**2**
544 occupancy volumes were also generated in the inner regions of the polymer. On the contrary, not
545 applying the MA_D correction, for the enantiomer (*P*)-**2** the occupancy volumes were shown to move
546 toward the outer regions of the polymer.

547 **4. Conclusions**

548 The enantioseparability of fluorinated 3-(aryl)thio-4,4'-bipyridines on CDMPC was shown to
549 increase as the EP related to regions of local electron depletion on the analyte becomes increasingly
550 positive. This observation represents the core of our study which, on the basis of theoretical data and
551 experimental results, furnishes evidences that stereoselective chalcogen and π -hole bonds, so far
552 unexplored in HPLC environment, can drive enantioseparations. A new approach, based on the

553 extension of the Source Function tool to the EP and enabling to get insights on the chemical origin of
554 these enantioseparation “drivers”, has for the first time been proposed.

555 Computational evaluation of molecular properties allowed to design analytes suitable as probes and,
556 in parallel, to clarify some aspects of the observed chromatographic behaviours. Finally, the introduction
557 of a massless dummy ligand, positively charged to mimic the electron depletion on sulphur, allowed to
558 explore the dynamic behaviour of the enantiomers of **2** on CDMPC, obtaining theoretical results
559 qualitatively in accord with the experimental outcomes.

560 On this basis, enantioseparations of atropisomeric test probes containing heavier chalcogen atoms are
561 currently under investigations.

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568 **Appendix A. Supplementary data**

569 Supplementary data associated with this article can be found, in the online version, at doi:

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FIGURE CAPTIONS

Fig. 1. Description of halogen bond (XB) (A) and polysaccharide-based polymers as XB acceptors (B).

Fig. 2. General description of chalcogen (ChB) (A) and π -hole bond (B).

Fig. 3. Structures of the 5,5'-dibromo-2,2-dichloro-3-substituted-4,4'-bipyridines **1-10**.

673 **Fig. 4.** Design of potential 4,4'-bipyridine-based ChBDs.

674 **Fig. 5.** A. Enthalpy-entropy ($\Delta\Delta S$ vs $\Delta\Delta H$ values x 10000) compensation for compounds **1**, **2**, **3**, **4** and **8**
675 on CDMPC and CCMPC under normal phase (NP) and polar organic (PO) elution modes (FR 0.8
676 ml/min); B. Residual plot ($\Delta\Delta S$ values).

677 **Fig. 6.** Linear regression analysis describing the relationships between $\ln k_2$ and the max EP Ar_F (π -hole)
678 (A) and the max EP on σ_1 (B) for compound **1-3** and **5-10**.

679 **Scheme 1.** Synthesis of 4,4'-bipyridines **1** and **2**.

680 **Scheme 2.** Synthesis of 4,4'-bipyridines **3** and **4**.