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1 **Polysaccharide-based chiral stationary phases as halogen bond acceptors: a novel**
2 **strategy for detection of stereoselective σ -hole bonds in solution**

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9

10 *RUNNING TITLE:* Polysaccharide-based polymers as halogen bond acceptors

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14

15 **Abbreviations:** **ACMPC**, amylose *tris*(5-chloro-2-methylphenylcarbamate); **ADMPC**, amylose *tris*(3,5-
16 dimethylphenylcarbamate); **CCMPC**, cellulose *tris*(3-chloro-4-methylphenylcarbamate); **CDMPC**, cellulose *tris*(3,5-
17 dimethylphenylcarbamate); **CSP**, chiral stationary phase; **ECD**, electronic circular dichroism; **EEC**, entropy-enthalpy
18 compensation; **EEO**, enantiomer elution order; **EP**, electrostatic potential; **EPS**, electrostatic potential surface; **ESH**, explicit
19 σ -hole; **HB**, hydrogen bond; **Hex**, *n*-hexane; **IPA**, isopropyl alcohol; **MD**, molecular dynamic; **MeOH**, methanol; **PO**, polar
20 organic; **XB**, halogen bond; **XBA**, halogen bond acceptor; **XBD**, halogen bond donor; **XRD**, X-ray diffraction

21

22 **Keywords:** Atropisomers / Enantioseparation / Halogen bond / Molecular dynamic / Polysaccharide-
23 based chiral stationary phases

24

25 **Abstract**

26 In the last years, halogen bonds (XBs) have been exploited in a variety of research areas both in the solid
27 state and in solution. Nevertheless, several factors make formation and detection of XBs in solution
28 challenging. In addition, to date few chiral molecules containing electrophilic halogens as recognition
29 sites have been reported. Recently, we described the first series of XB-driven enantioseparations
30 performed on cellulose *tris*(3,5-dimethylphenylcarbamate) (CDMPC) by HPLC. On this basis, herein
31 the performances of amylose *tris*(3,5-dimethylphenylcarbamate) (ADMPC) as XB acceptor were also
32 investigated and compared with respect to CDMPC. With the aim to explore the effect of polysaccharide
33 backbone on the enantioseparations, the thermodynamic parameters governing the halogen-dependent
34 enantioseparations on both CDMPC and ADMPC were determined by a study at variable temperature
35 and compared. Molecular dynamic simulations were performed in parallel in order to model the halogen
36 bond in polysaccharide-analyte complexes. Chiral halogenated 4,4'-bipyridines were used as test
37 compounds (XB donors). On this basis, a practical method for detection of stereoselective XBs in
38 solution was developed, which is based on the unprecedented use of HPLC as technical tool with
39 polysaccharide-based polymers as molecular probes (XB acceptors). The analytical strategy showed
40 higher sensitivity for the detection of weak XBs compared to some spectroscopic techniques currently
41 used in this field.

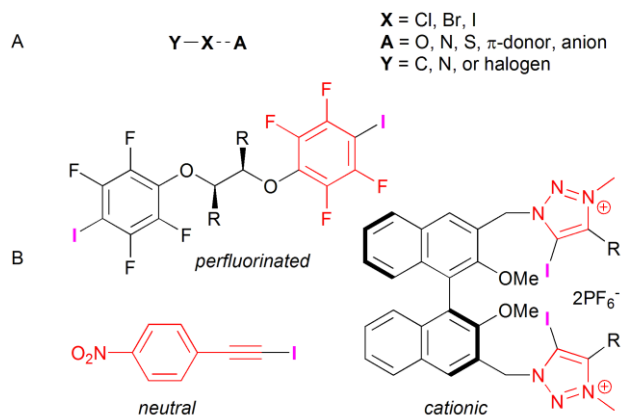
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45 **1 Introduction**

46 A halogen bond (XB) is described as a noncovalent interaction between a covalently-bonded halogen
47 (donor), bearing a region with a positive electrostatic potential (EP) (σ -hole) on unpopulated σ^* orbitals,
48 and a negative site (acceptor) (Fig. 1A) [1].



49

50 **FIGURE 1** A: general description of XB; B: examples of perfluorinated, cationic, and neutral XBDs

51 This highly-directional attractive interaction, where halogens are involved not exclusively for their
52 hydrophobic, inductive or steric effects [2], is characterized by R-X \cdots acceptor angles close to 180°, and
53 a strength increase for heavier halogens (Br, I). Indeed, the size of the σ -hole increases with the
54 polarizability of the halogen following the order Cl < Br < I. Therefore, directionality and tunability are
55 the key features which make XB a useful tool in crystal engineering and for the control of molecular
56 assembly [3]. Although similar to the hydrophilic hydrogen bond (HB), the XB is hydrophobic, and this
57 complementarity provides new opportunities for molecular recognition, particularly in solvated
58 environment. Nevertheless, the exploration of solvent effects on XB has begun later compared to solid-
59 state studies, and to date the applications in solution are still challenging [4]. Indeed, formation and
60 detection of XBs in solution are more complicated compared to the solid state because the interactions
61 can be influenced by conformational freedom of the molecules, a less ordered medium, and solvent
62 effects on both donor and acceptor [5]. Another open issue concerns the application of XB in chiral
63 systems, and few chiral molecules having σ -holes on halogens as recognition sites were described

64 recently [5-11]. Perfluorinated and cationic *N*-heterocyclic substructures (Fig. 1B) are able to activate
65 potential σ -hole sites and generate strong XBs, which are detectable by means of a variety of
66 spectroscopic techniques, NMR being the most widely used [12, see new ref]. On the other hand,
67 iodinated and brominated neutral XB donors (XBDs) lacking perfluorination are needed for real-life
68 applications in drug design [13] and supramolecular chemistry [14]. In general, in these cases,
69 spectroscopy weakly confirms the XB activity in solution. Therefore, in the last decade, the quest for
70 analytical methods characterized by higher sensitivity has been tackled [15].

71 Our groups have recently developed new procedures for the syntheses [16,17] and enantioseparations
72 [18] of halogenated chiral 4,4'-bipyridines which behaved as XBDs both in solution [19,20] and in the
73 solid state [17,21]. We discovered that XB-driven HPLC enantioseparations can be performed on
74 cellulose *tris*(3,5-dimethylphenylcarbamate) (CDMPC) as chiral stationary phase (CSP) [20].

75 Envisaging for polysaccharide derivatives a novel function other than resolution of racemic mixtures,
76 in this paper we show that HPLC coupled with polysaccharide-based CSPs as XB acceptors (XBAs)
77 can serve as analytical means for detection of weak stereoselective XBs in solution. Chiral halogenated
78 4,4'-bipyridines lacking perfluorination were used as XBD test compounds. The mechanistic bases of
79 this strategy were confirmed through a thermodynamic study performed at variable temperature as well
80 as enantiomer elution order (EEO) evaluation. Moreover, molecular dynamic (MD) simulations were
81 performed in order to model XB-based polysaccharide-analyte complexes.

82 **2 Materials and methods**

83 **2.1 Instrumentation**

84 An Agilent Technologies (Waldbronn, Germany) 1100 Series HPLC system [high-pressure binary
85 gradient system equipped with a diode-array detector operating at multiple wavelengths, a
86 programmable autosampler with a 20 μ l loop and a thermostatted column compartment] was employed.
87 Data acquisition and analysis were carried out with Agilent Technologies ChemStation Version B.04.03

88 chromatographic data software. The UV absorbance is reported as milliabsorbance units (mAU). Lux
89 Cellulose-1 (cellulose *tris*(3,5-dimethylphenylcarbamate) (CDMPC)), Lux Cellulose-2 (cellulose *tris*(3-
90 chloro-4-methylphenylcarbamate) (CCMPC)), Lux Amylose-1 (amylose *tris*(3,5-
91 dimethylphenylcarbamate) (ADMPC)), Lux Amylose-2 (amylose *tris*(5-chloro-2-
92 methylphenylcarbamate) (ACMPC) (Phenomenex, USA), Chiralcel OD-H (CDMPC), and Chiralpak IA
93 (ADMPC) (Chiral Technologies Europe, France) were used as analytical (5 μ m, 4.6 \times 250 mm) chiral
94 columns. Dead time (t_0) was measured by injection of tri-*tert*-butylbenzene (Sigma-Aldrich, Germany)
95 as a non-retained compound [22]. Analyses were performed in isocratic mode. Chromatographic
96 separations were performed at 25°C. The flow rate (*FR*) was set at 0.8 ml/min for analytical separations.
97 The EEO was determined by injecting enantiomers of known absolute configuration (SI). The van't Hoff
98 experiments were conducted at 10, 15, 20, 25, 30 and 35 °C in a thermostatted column chamber
99 equipped with a cooling system. When the temperature was changed, the column was allowed to
100 equilibrate for 1 h before injecting the samples. Additional details on determination of thermodynamic
101 parameters are reported in SI.

102 2.2 Chemicals and reagents

103 *Rac*-**1-10** were synthesized as previously reported [16,17]. HPLC grade *n*-hexane (Hex), *n*-heptane,
104 methanol (MeOH) and 2-propanol (IPA) were purchased from Sigma-Aldrich (Taufkirchen, Germany).

105 2.3 Computational

106 For 4,4'-bipyridines geometry optimization and computation of electrostatic potential surfaces (EPSs)
107 and related parameters (EP values are given in kJ/mol) were performed and graphically generated using
108 the Spartan'10 Version 1.1.0 [23] (Wavefunction Inc., Irvine, CA) program and employing the ab initio
109 DFT method with the B3LYP functional and the 6-311G* basis set (available for elements H-Ca, Ga-Kr
110 and I). The EP describes the value of the electrostatic potential onto an electron density surface and it
111 was used as an indicator of the charge distribution on the molecules. Min EP and max EP are the
112 minimum and the maximum values of the mapped property. EPS and molecular property calculations of

113 polysaccharide side chains and solvents were performed with the B3LYP functional and the 6-31G**
114 basis set. The carbamate frameworks were designed by substituting the glucosyl moiety with a methyl
115 group. All calculated EPS and experimental details for MD simulations are available in the SI.

116 **3 Results and discussion**

117 **3.1 HPLC as technical tool**

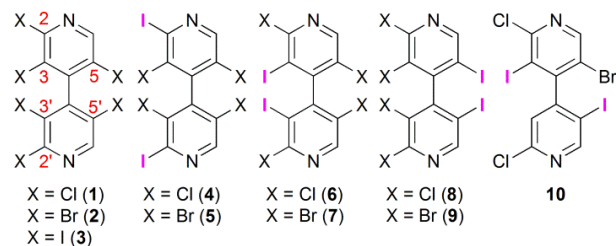
118 The strength of the XB is regulated by the depth of the σ -hole, the Lewis basicity of the XBA and the
119 stereoelectronic properties of the medium. Thus, with the aim to develop a sensitive analytical procedure
120 for detection and investigation of stereoselective XBs, HPLC on a chiral support acting as XBA was
121 envisaged as a versatile technical tool on the basis of the following remarks: *i*) detailed information on
122 primary and secondary stereoselective interactions between a chiral analyte, acting as potential XBD,
123 and a selector able to function as XBA could be derived from the chromatographic parameters [24,25];
124 *ii*) this technique works in a solvated medium, namely the mobile phase (MP), which can be easily
125 tuned, allowing solvent effects on both donor and acceptor to be studied; *iii*) the interaction is studied
126 under pressure (30-40 bar) inducing close contacts with an amplification of the contact extent compared
127 to techniques working at ambient pressure. In addition, several adsorption-desorption steps contribute to
128 the separation, increasing the sensitivity of the technique; *iv*) retention and separation are influenced by
129 temperature, so that thermodynamic parameters associated with halogen-dependent enantioseparation
130 could be determined by van't Hoff plots [26]; *v*) finally, the availability of the EEO is of high
131 importance in order to gain information on the topological approach toward the selector.

132 **3.1.1 Polyhalogenated 4,4'-bipyridines as XBDs**

133 4,4'-bipyridines **1-10** (Table 1) were used as neutral chiral non-perfluorinated XBD analytes. For all
134 halogenated substituents, the EPs were calculated in order to evaluate the σ -hole depth [20,27]. The
135 design was performed by means of halogen-driven structural and electronic engineering on the electron-

136 poor 4,4'-bipyridine core with the aim to obtain analytes whose chromatographic response was strictly
 137 dependent on the σ -hole depth.

138 **TABLE 1** Calculated max EPs on the halogens (I, Br, Cl)



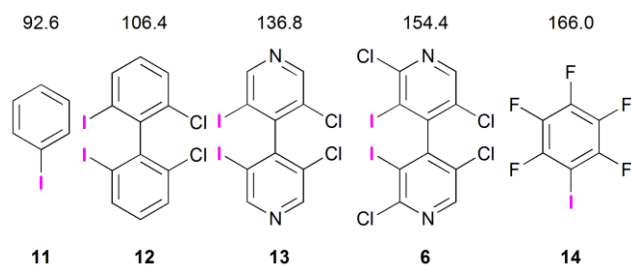
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XBDs	max EP (kJ/mol)					
	X _{C2}	X _{C2'}	X _{C3}	X _{C3'}	X _{C5}	X _{C5'}
1	64.5	64.5	89.9	89.9	90.9	90.9
2	95.2	95.2	119.8	119.8	120.9	120.9
3	120.5	120.5	144.2	144.2	146.8	146.8
4	127.4	127.4	86.2	86.2	88.0	88.0
5	123.1	123.1	118.9	118.9	120.5	120.5
6	60.2	60.2	154.3	154.3	85.4	85.4
7	93.1	93.1	148.0	148.0	118.8	118.8
8	62.4	62.4	85.5	85.5	154.4	154.4
9	94.8	94.8	118.3	118.3	149.3	149.3
10	60.9	52.3	151.3	--	121.3	144.1

140

141 The comparison of the max EPs on iodine in compounds **6** and **11-14** (Fig. 2) evidenced the
 142 combined electronic activation which is exerted on iodine σ -hole by the heterocyclic core and the
 143 chlorine introduction at positions 2,2', **14** being a known perfluorinated XBD used as reference [28]. In
 144 particular, compounds **1-3** were used in order to assess the behaviour of iodine, bromine and chlorine as
 145 XBD sites. As in the solid state XBs can be assessed by the reduction of the sum of van der Waals radii
 146 [29] between the interacting atoms *via* single crystal XRD, for **1-3** the extent of the penetration of the
 147 van der Waals atomic spheres was shown to follow the reported XB strength (I > Br > Cl) (SI). On the
 148 other hand, compounds **4-9** were selected as models focusing on the stereoelectronic environment of

149 iodine as privileged XBD site. In addition, **10** was specifically used in order to have two types of
150 iodinated XBD sites in a single molecule, I_{C3} being (151.3 kJ/mol) more electronically activated and less
151 sterically available than I_{C5} (144.1 kJ/mol). Indeed, as halogens are much larger and polarizable than
152 hydrogen, XB is more sensitive to steric hindrance than HB [1].



153 **FIGURE 2** Comparison of calculated max EPs (kJ/mol) on iodine for **6** and **11-14** (Spartan'10 Version 1.1.0, DFT,
154 B3LYP/6-311G*)

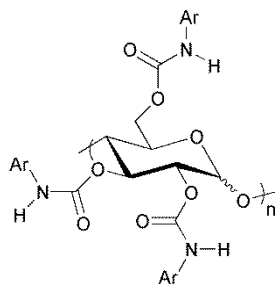
156 Therefore, in this context, halogen atoms serve as σ -hole donors, stereoelectronic modulators, and
157 atropisomerism inductors by blocking 4,4'-axis rotation.

158 **3.1.2 Polysaccharide dimethylphenylcarbamates as XBAs**

159 We have recently demonstrated that XB-driven enantioseparations of halogenated 4,4'-bipyridines
160 can be performed by HPLC on CDMPC [20], where XBs are formed between halogen substituents on
161 the analyte and the carbonyl groups of the CSP. On this basis, we envisaged the possibility to use both
162 cellulose- and amylose-based polymers as chiral probes for detection and systematic study of XBs
163 (Table 2). In these CSPs, conformational chirality depends on the helical twist generated by the specific
164 glycosidic 1,4 linkages in the cellulose (β) and amylose (α) chains [30]. Consequently, a chiral
165 supramolecular environment surrounds the carbonyl XBA sites located in the inner polar layer, whereas
166 an outer layer containing substituted aromatic rings is able to exert π - π , π -X and hydrophobic
167 interactions [24,25]. On this basis, CDMPC and ADMPC were chosen as privileged probes which
168 contain carbonyl oxygens with good properties as XBAs (min $EP_{CO} = -170$ kJ/mol). On the other hand,
169 CCMPC and ACMPC were considered as terms of comparison. Indeed, for analytes able to exert XBs
170 on CDMPC or ADMPC, lower separation factors were expected on CCMPC and ACMPC as results of

171 the reduced XBA capability of the carbonyls induced by the electron-withdrawing chloro-substituent
172 [31].

173 **TABLE 2** Structures of polysaccharides used as XBAs



174

CSP	Ar- = (R,R')C ₆ H ₃ -	linkage	min EP _{CO} ^a
CDMPC	(3,5-dimethyl)	β-D	-169.98
ADMPC	(3,5-dimethyl)	α-D	-169.98
CCMPC	(3-chloro-4-methyl)	β-D	-155.82
ACMPC	(5-chloro-2-methyl)	α-D	-158.77

175 ^a[kJ/mol]

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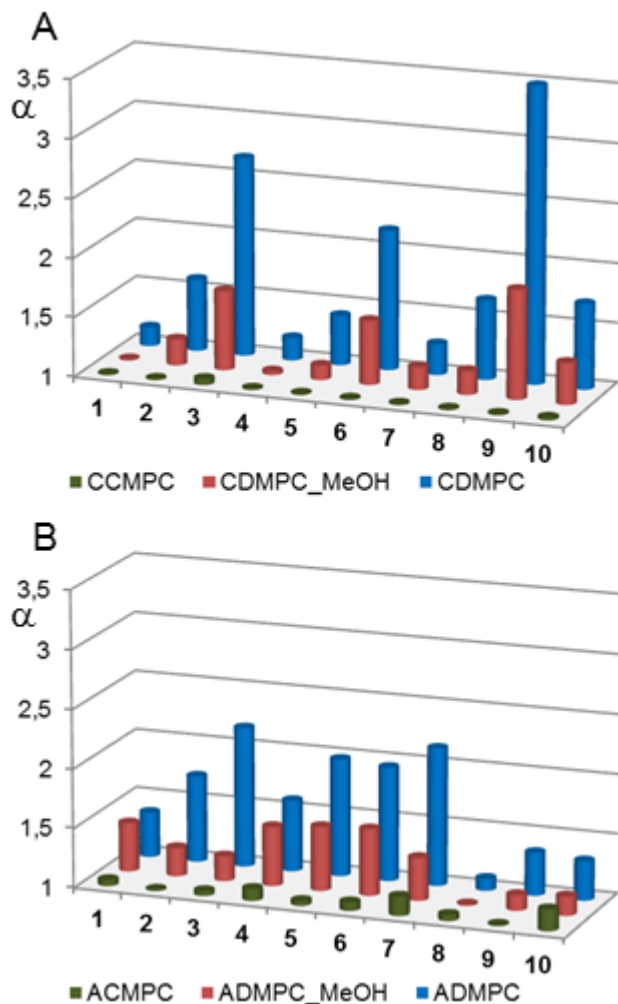
177 **3.1.3 The mobile phase as XB medium: solvent effect**

178 Solvent effect could be evaluated by means of HPLC. On CDMPC the mixture Hex/IPA 90:10 (*mix*
179 *A*) had proved to assist XBs and, consequently, to produce high/moderate α values for compound with
180 good properties as XBDs. On the contrary, by enhancing MP polarity through addition of methanol
181 (MeOH) (Hex/IPA/MeOH 90:5:5) (*mix B*), selectivity decreased. Indeed, MeOH is able to destabilize
182 XBs [4] by penetrating inside the chiral cavity and forming HBs with the carbonyl oxygens better than
183 IPA due to its stereoelectronic properties (SI). Meanwhile, MeOH favours hydrophobic contacts [20].

184 **3.2 HPLC XB-driven enantioseparations: CDMPC vs ADMPC**

185 Six chromatographic systems generated by the combination of CDMPC, ADMPC, ACMPC with *mix*
186 *A*; and CDMPC and ADMPC with *mix B* as MPs were evaluated toward compounds **1-10** (SI). The
187 obtained α values are comparatively summarized in Fig. 3. Moreover, as both retention and separation
188 are influenced by temperature, a variable temperature study was carried out between 10 and 35 °C for all

189 bipyridines on the five chromatographic systems. Because the van't Hoff plots were linear in the
190 considered temperature range ($r^2 \geq 0.9900$), enthalpy and entropy values derived from the plots allowed
191 to evaluate the enthalpic and entropic contribution to halogen-dependent enantioseparations.



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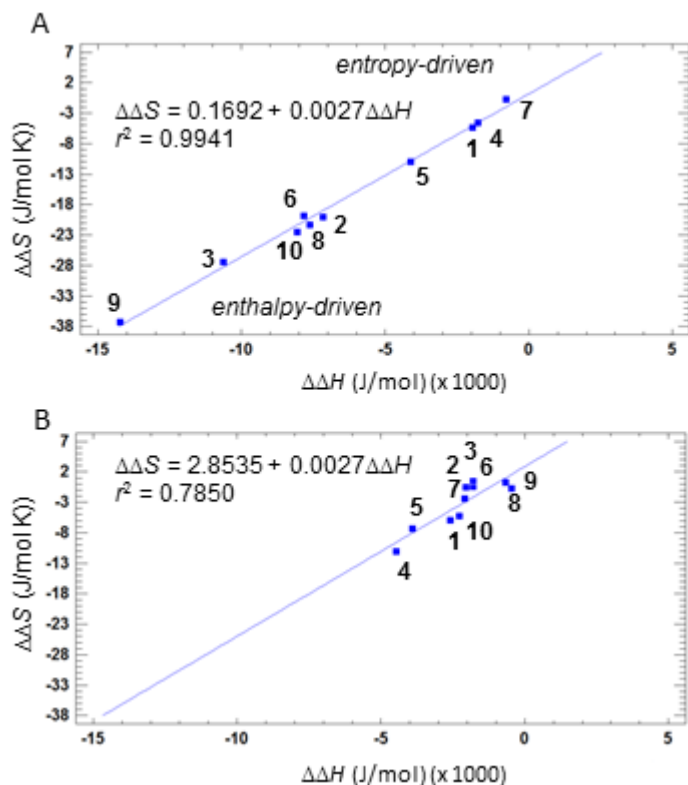
193 **FIGURE 3** Comparative outcomes of the XBD screening on cellulose- (A) and amylose-based (B) CSPs

194 On both CDMPC and ADMPC with *mix A*, retention and selectivity for compounds **1-3** increased
195 following the order Cl < Br < I ($0.44 \leq k_1 \leq 8.00$; $1.16 \leq \alpha \leq 2.68$), with *M-P* EEO in each case. In
196 accord with our initial hypotheses, evaluation of the chromatographic parameters obtained on both
197 CCMPC and ACMPC showed a dramatic decrease of selectivity values ($1.00 \leq \alpha \leq 1.05$).

198 Interestingly, on ADMPC selectivity increased compared to CDMPC for **1** (+19%) and **2** (+7.4%),
199 whereas it decreased for **3** (-18.6%). This behaviour could be explained taking into account that for

200 CDMPC the cavities are slightly bigger than for ADMPC which presents stronger intramolecular HBs
201 [33]. Consequently, ADMPC cavity was more accessible for **1** (ESP volume 277.08 Å³) and **2** (310.97
202 Å³) and less available for **3** (373.43 Å³). Therefore, a mixed enantioseparation mechanism controlled by
203 halogen-dependent interactions as well as analyte steric fit seemed to be active on the amylose-based
204 CSP, with chromatographic outcomes depending on both volume and XBD ability of the analyte.
205 Differently, on CDMPC the σ -hole on halogens were shown to control enantioseparation exclusively
206 [20]. The occurrence of two different mechanisms as the polysaccharide backbone changes was
207 supported by the following remarks: *i*) $\Delta\Delta S$ values determined for **1-3** on CDMPC (-5.36 (**1**) > -19.78
208 (**2**) > -27.47 (**3**)) ranged following an opposite order compared to ADMPC (-5.95 (**1**) < -2.38 (**2**) < -0.55
209 (**3**)); *ii*) by enhancing MP polarity with *mix B* on CDMPC retention increased ($0.51 \leq k_1 \leq 8.11$),
210 whereas selectivity decreased ($1.00 \leq \alpha \leq 1.67$), keeping again the order Cl < Br < I. Indeed, a percent
211 increase of k_1 following the order Cl (15.9%) > Br (10.6%) > I (1.4%) could be observed, reasonably
212 due to more favorable conditions for hydrophobic or steric fit driven retention mechanisms. On the
213 contrary, retention of the second eluted enantiomer (*P*) (k_2) showed a percent decrease following the
214 order Cl (0%) < Br (-14.50%) < I (-36.7%), corresponding to the strength trend of XB interactions [1].
215 Differently, on ADMPC with *mix B*, retention of both enantiomers decreased with a percent decrease of
216 k_1 following the order Cl (-31.9%) > Br (-12.6%) > I (-11.0%) and, conversely, following the order I (-
217 50.3%) > Br (-37.5%) > Cl (-30.8%) for k_2 . As a result, on ADMPC selectivity increased with *mix B*
218 compared to *mix A* for **1** (+2.2%), whereas it decreased for **2** (-28.3%) and **3** (-44.5%); *iii*) for CDMPC
219 the enthalpy-entropy compensation (EEC) straight line ($r^2 = 0.9941$) showed that the
220 enantiodiscrimination mechanism (XB-driven) does not change over the analyte series (Fig. 4A). On the
221 contrary, on ADMPC a deviation from linearity ($r^2 = 0.7850$) was observed in the EEC line, which
222 confirmed the presence of other entropy-driven forces along with the XB (Fig. 4B); *iv*) on CDMPC,
223 $\Delta\Delta H$ and $\Delta\Delta S$ values range from -0.79 to -14.23 kJ/mol and from -0.71 to -27.47 J/(mol K), respectively,

224 whereas on the ADMPC, higher $\Delta\Delta H$ (from -0.46 to -4.47 kJ/mol) and $\Delta\Delta S$ (from 0.31 to -11.07 J/(mol
225 K)) values were in a shorter range^[VM1];



226
227 **FIGURE 4** EEC lines for 4,4'-bipyridines **1-10** on CDMPC (A) and ADMPC (B)

228 v) for compounds **4-10** selectivity decreased by using *mix B* on both CDMPC and ADMPC.
229 Nevertheless, only on CDMPC, both $\Delta\Delta H$ and $\Delta\Delta S$ values increased ranging from 0.06 to -7.59 kJ/mol
230 and from 0.48 to -20.08 J/(mol K), respectively. On the contrary, on ADMPC, $\Delta\Delta H$ and $\Delta\Delta S$ tended to
231 decrease, with values ranging from -0.45 to -8.74 kJ/mol and from 1.31 to -17.55 J/(mol K),
232 respectively; vi) on CDMPC, the topological approach to the polymeric XBA was iodine-dependent.
233 Indeed, iodine at positions 5,5' (**8** and **9**) induces a preference for *M-P* as EEO, whereas iodine at
234 positions 3,3' (**6** and **7**) induces the reverse EEO, *P-M*. As **10** also showed *P-M* as EEO, we assumed the
235 pivotal role of the 3-iodine in this case, although the medium extent of separation^{[VM2][PP3][VM4]} (α : 1.67 (**8**) < 1.72
236 (**10**) < 2.18 (**6**)) suggested a cooperative effect of the two kind of iodines. On ADMPC the EEO was *M-*
237 *P* in almost all cases, confirming that the approach of the analytes tended to depend on their shape (steric

238 fit). In this regard, it is interesting to note that on ADMPC, MeOH-induced inversion of the elution order
239 (*P-M*) could be observed for **3** and **9** compared to *mix A* (*M-P*). Differently, by using *mix A*, backbone-
240 induced inversion of EEO could be observed for **6** and **7** on ADMPC (*M-P*) with respect to CDMPC (*P-*
241 *M*).

242 The comparative evaluation of the chromatographic parameters of tetrachlorinated **4**, **6**, and **8**
243 obtained with the system CDMPC-*mix A* highlighted the pivotal role of iodine as XBD site able to exert
244 stereoselective interactions. Indeed, α of **6** and **8**, bearing iodines close to the chiral axis, are higher
245 (+83.6% and +40.3%, respectively) than the corresponding value of **4**, whose 2,2'-iodines are located far
246 from the chiral axis. Analogously to that observed for **4** and **8**, α value derived from α obtained for the
247 2,2',3,3'-tetrabromo-5,5'-diiodo **9** is higher (+146%) than the corresponding value of the 2,2'-diiodo **5**.
248 Differently, for the 3,3'-diiodo **7** α decreased compared to **5**. In this case, the comparison of the $\Delta\Delta S$
249 values derived from the van't Hoff plots and associated with the enantiodiscriminations ($[J/(\text{mol}\cdot\text{K})]$, **5**: -
250 10.92; **7**: -0.71) proved that entropic factors, caused by the larger 2,2'-bromines close to the 3,3'-iodines,
251 exert a detrimental effect on selectivity [20]. Differently, on ADMPC-*mix A*, iodine at position 5,5'
252 seemed to be detrimental for enantioseparation. Indeed, **8**, **9** and **10** showed lower values of selectivity
253 ($1.10 \leq \alpha \leq 1.36$) compared to compounds bearing iodine in positions 3,3' and 2,2' ($1.60 \leq \alpha \leq 2.16$).

254 **3.3 Molecular dynamics of 6 and 8 on CDMPC and ADMPC**

255 MD calculations (see SI for details) were performed to simulate the interaction modes of the 4,4'-
256 bipyridines **6** and **8** with CDMPC and ADMPC. In this study, molecular models of 9-mer CDMPC and
257 ADMPC were constructed in order to confirm the halogenated binding sites for the enantiomers of **6** and
258 **8**. On the basis of our previous work in the field [20], the extra point [34], or explicit σ -hole (ESH) [35]
259 concept was used to model the XB in polysaccharide-haloanalytes complexes. Moreover, with the aim to
260 compare qualitatively the MD outcomes associated with different molecular situations, the calculations
261 were performed with and without the ESH, and Hex and MeOH solvent effects were taken into account

262 in accord with the methods used in the HPLC screening. Table 3 shows the values associated with I-
 263 driven contacts found in the MD runs over 10 ns (ESH, solvent box, *n*-hexane).

264 **TABLE 3** Calculated geometrical parameters of (I···O) polysaccharide-analyte interactions^a

polysaccharide	XBD	HPLC ^b		MD			
		<i>t_R</i>	obs.	CDMPC-enantiomer complex	d _{I···O} (Å) ^{c,d}	C-I···O angle (°)	I···O=C angle (°)
CDMPC	6	8.27	(<i>P</i>)	(<i>P</i>)	3.41 (-2.6)	146.86	121.22
		13.83	(<i>M</i>)	(<i>M</i>)	3.08 (-12.0)	166.70	125.15
	8				3.14 (-10.3)	170.38	125.56
		7.98	(<i>M</i>)	(<i>M</i>)	3.00 (-14.3)	171.25	162.10
		10.95	(<i>P</i>)	(<i>P</i>)	3.26 (-6.9)	174.25	127.19
					3.60 (π···I)		
ADMPC	6	9.10	(<i>M</i>)	(<i>M</i>)	3.70 (π···I)		
		14.46	(<i>P</i>)	(<i>P</i>)	3.17 (-9.4)	169.42	135.03
					3.40 (-2.8)	160.39	122.95
	8	7.06	(<i>M</i>)	(<i>M</i>)	3.30 (π···I)		
		7.43	(<i>P</i>)	(<i>P</i>)	3.14 (-10.3)	169.15	159.57
					3.30 (-5.7)	164.79	123.50

265 ^aMD conditions: ESH; solvent box, *n*-hexane.

266 ^bLux Cellulose-1 (CDMPC) or Lux Amylose-1 (ADMPC), Hex/IPA 90:10 v/v, *FR* 0.8 ml/min, *t_R* [min].

267 ^cΣrvdW (I,O) = 3.5 Å [28].

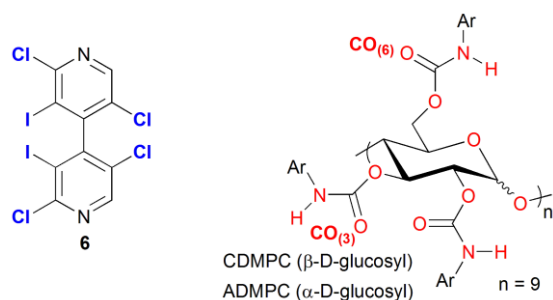
268 ^dPenetration parameters I···O% = 100 × {(d_{I···O})/(r_{vdWI} + r_{vdWO}) - 1}, where d_{I···O} is the interatomic distance and r_{vdW} the corresponding
 269 van der Waals radii, are reported in brackets.

270

271 The iodinated analogues **6** and **8** showed distribution of I···O distances clustering around 3.00-3.41 Å
 272 with penetration parameters ranging from -2.6% to -14.3% (sum of the van der Waals radii for I,O = 3.5
 273 Å) [24]. Moreover, the C—I···O angles ranged from 160.39° to 174.25° in almost all cases and only for
 274 the complex CDMPC-(*P*)-**6** a lower value of 146.86° was observed. In general, angles ranging from
 275 160° to 180° are considered acceptable to decide if the interaction corresponds to a halogen bond [20].
 276 Analogously, the X···O—C angles showed a distribution ranging from 121.22° to 135.03° and only for
 277 the complexes of (*M*)- and (*P*)-**8** on CDMPC (162.10°) and ADMPC (159.57°), respectively, a deviation
 278 from the reference value of 120° was observed. [All_[PP6][VM7] these observations (penetration of van der
 279 Waals spheres and interaction angles) can be considered as an evidence of the implication of XB. [VM8]It
 280 is worth mentioning that in all cases two I-driven interactions are observed for the second eluted
 281 enantiomers compared to the single I-driven interaction observed for each first eluted enantiomers. As
 282 consequence, the MD outcomes was showed to be in accord with the experimental EEO. As expected,

283 no I...O interactions were observed when ESH correction was not applied or when MeOH was
284 introduced in the solvent box instead of Hex.

285 In addition, with the aim to explore the recognition sites on both donor-analyte and acceptor-
286 polysaccharide, the potential contacts occurring in the course of the molecular dynamics were examined.
287 Indeed, taking into account the dynamic feature of the enantioseparation event, we analysed statistically
288 the distances between each of the six halogen (2 iodines, 4 chlorines) on 4,4'-bipyridine **6** (as donor
289 recognition sites) and fourteen points (N, O, H) located on each monomers of the CDMPC and ADMPC
290 nonamers (Fig. 5).

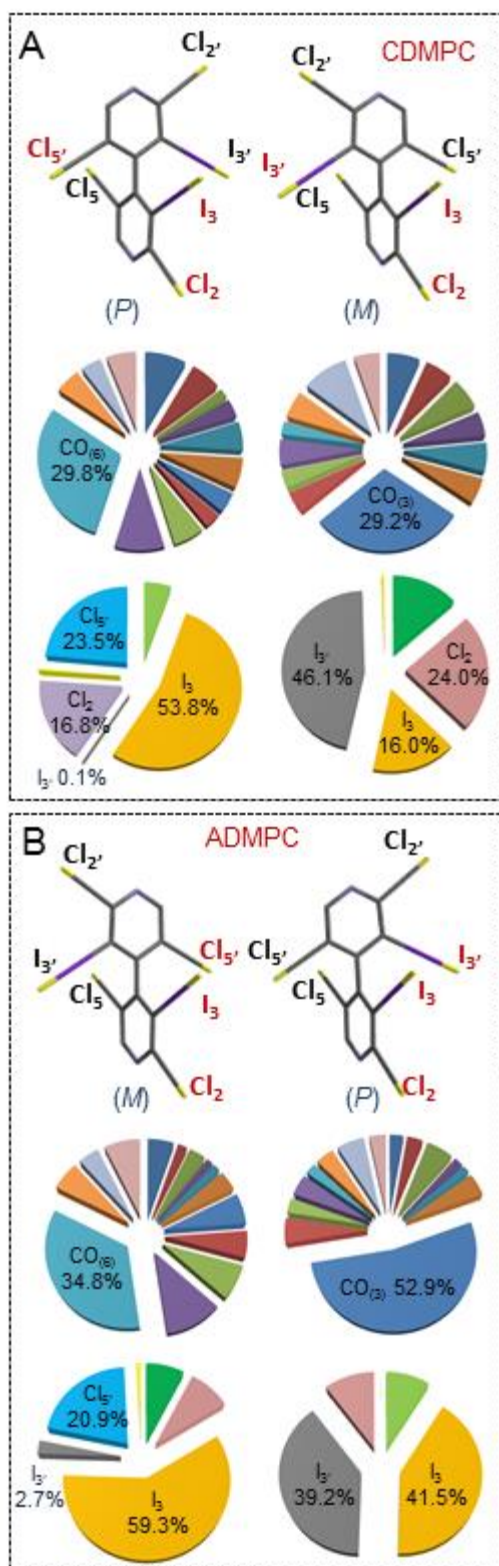


291

292 **FIGURE 5** Schematic representations of the contact sites analysed on **6**, CDMPC, and ADMPC in the course of MD
293 simulations

294 The contact distances were acquired (5000 steps) during the molecular dynamic time (10 ns). All
295 collected values within 6 Å were extracted (see SI for details) in order to evaluate the sites involved in
296 close contacts, without considering the type of interactions. From the calculated distribution values
297 associated with the selected recognition sites (Fig. 6), we observed that the (*P*) and (*M*) atropisomers
298 which are the first eluted enantiomers on CDMPC and ADMPC, respectively, presented the 3-iodine as
299 privileged recognition site (53.8% and 59.3%) and the carbonyl of the carbamate framework at C₆ as the
300 most frequent recognition sites (29.8% and 34.8%). Differently, on CDMPC the atropisomer (*M*) as
301 second eluted enantiomers showed two iodines as privileged sites with a distribution of 62.1%.
302 Analogously, for the second eluted atropisomer (*P*) on ADMPC the two iodine at 3,3'-positions
303 interacted with the polysaccharide with a distribution value of 80.7%. For both second eluted

304 enantiomers, the privileged recognition site is the carbonyl framework at C₃ (29.2% and 52.9% on
 305 CDMPC and ADMPC, respectively).



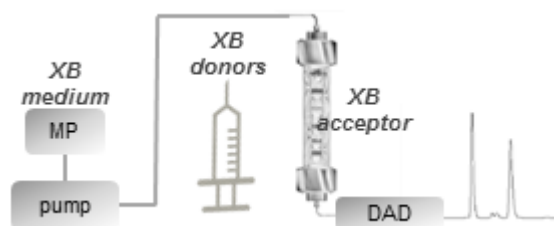
306
 307 **FIGURE 6** Distribution of the interaction sites in the course of MD simulations (10 ns) of **6** enantiomers on 9-mers of both
 308 CDMPC and ADMPC

309 Again, the results of the statistical evaluation of the observed contacts in the course of the four MDs
 310 appeared consistent with the experimental EEOs.

311 3.4 A novel strategy for detection of stereoselective σ -hole bonds in solution

312 By the chromatographic, thermodynamic and computational evaluation, a protocol based on a three-
 313 step orthogonal on-column screening has been set up to probe and detect XBs. Cellulose-based CSPs
 314 appeared suitable for the purpose furnishing for **1-10**, used as XBD probes, chromatographic responses
 315 strictly dependent on the occurrence of XBs, σ -hole depth and iodine regiochemistry. In Table 4, the
 316 expected behaviours, as XBA, XBD and MP change, are summarized. Nevertheless, also amylose-based
 317 CSPs proved to be useful in this field allowing to assess steric effects on XBs.

318 **TABLE 4** HPLC protocol to detect XB interactions



319

XBD	mechanism	CSP ^a	MP ^b	α^c range
good	XB-driven	CDMPC	A	1.26-3.50
good	hydrophobic	CCMPC	A	1.00-1.05
good	XB-driven	CDMPC	B	1.00-1.92
poor	hydrophobic	CDMPC	A	< 1.2

320 ^aCDMPC cellulose *tris*(3,5-dimethylphenylcarbamate), CCMPC
 321 cellulose *tris*(3-chloro-4-methylphenylcarbamate), ADMPC amylose
 322 *tris*(3,5-dimethylphenylcarbamate), ACMPC amylose *tris*(5-chloro-2-
 323 methylphenylcarbamate).

324 ^bHex/IPA 90:10, *FR* = 0.8 ml/min (A), Hex/IPA/MeOH 90:5:5, *FR* =
 325 0.8 ml/min (B); hex = *n*-hexane, IPA = 2-propanol, MeOH =
 326 methanol.

327 ^c α separation factor.

328

329 Finally, as spectroscopic techniques have been widely used in this field [12], ESR and NMR
 330 experiments were carried out (SI) to gain an insight into the sensitivity of the techniques toward some
 331 4,4'-bipyridyl XBD probes. These experiments poorly confirmed the XBD activity proving the higher
 332 sensitivity of HPLC for detection of weak stereoselective XBs in solution.

333 3.5 Halogen-dependent enantioseparations under aqueous-organic elution mode: a perspective

334 With the aim to explore the effect of water on halogen-dependent enantioseparations, the
335 chromatographic behaviour of compounds **1-3** and **6** were investigated on ADMPC by using MeOH
336 100%, ACN 100%, MeOH/water 90:10, ACN/water 90:10 and ACN/water 80:20. The results of the
337 screening are reported in Table 5.

338 **TABLE 5** HPLC parameters (α , R_s) with PO and aqueous MPs^a

XBD		MP				
		MeOH/water		ACN/water		
		10:0	9:1	10:0	9:1	8:2
1	α	1.37	1.00	1.06	1.05	1.04
	R_s	2.4	0.0	0.2	0.4	0.6
2	α	1.52	1.00	1.00	1.00	1.03
	R_s	3.0	0.0	0.0	0.0	0.4
3	α	1.00	1.00	1.46	1.50	1.53
	R_s	0.0	0.0	3.7	6.3	6.7
6	α	2.27	1.00	1.00	1.12	1.13
	R_s	3.6	0.0	0.0	2.1	2.2

339 ^aColumn: Lux Amylose-1 (ADMPC), $FR = 0.8$ ml/min.

340
341 By using pure MeOH as MP, only compound **3** was completely unresolved indicating that ~~the~~ the
342 steric fit ~~seemed to have~~ a pivotal effect on enantioseparation extent. ~~and~~ The Addition of water in the
343 MP ~~proved to be~~ was detrimental for the enantioseparation of **1**, **2** and **6**_[VM9]. ~~Also with the aqueous MP,~~
344 ~~the steric fit seemed to have a pivotal effect on enantioseparation extent~~_[VM10]. On the contrary, by using
345 pure ACN which favours polar interactions more than MeOH, ~~only~~ compound **3** was well
346 enantioseparated and the gradual increase of water content in the MP had a beneficial effect only on ~~the~~
347 iodine-substituted compounds **3** and **6**_[VM11]. These results evidenced the complementary behaviour of
348 ACN compared to MeOH as MP in halogen-dependent enantioseparations, and an interesting effect of
349 water in ~~the~~ iodine-dependent enantioseparations performed in aqueous ACN.

350 4 Concluding remarks

351 The on-column screening of halogenated 4,4'-bipyridines, used as test compounds, proved the
352 efficacy and the potential of HPLC and cellulose-based polymers as technical and molecular tools,
353 respectively, for the detection of stereoselective σ -hole bonds. On the other hand, on amylose-based

354 CSPs both chromatographic and thermodynamic results evidenced that other entropy-driven forces act
355 along with halogen-dependent interactions allowing to study steric effects acting on halogen bonds in
356 solution. Significantly, halogen-dependent enantioseparations have been observed on ADMPC by using
357 water-containing MPs. This result allows to envisage the possibility to study water effects on σ -hole-
358 driven interactions by HPLC. In this regard, it is worth mentioning that for medicinal chemistry
359 applications, water is the exclusive solvent, and the behaviour of XB in water is still poorly understood.

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