Chiral separation of helical chromenes with chloromethyl phenylcarbamate polysaccharide-based stationary phases

Journal:	<i>Journal of Separation Science</i>
Manuscript ID	Draft
Wiley - Manuscript type:	Original Paper
Date Submitted by the Author:	n/a
Complete List of Authors:	Ianni, Federica; Universita degli Studi di Perugia Scorzoni, Stefania; Universita degli Studi di Perugia Gentili, Pier Luigi; Universita degli Studi di Perugia Di Michele, Alessandro; Universita degli Studi di Perugia Frigoli, Michel; Institut Lavoisier de Versailles Camaioni, Emidio; Universita degli Studi di Perugia Ortica, Fausto; Universita degli Studi di Perugia Sardella, Roccaldo; University of Perugia, Chimica e Tecnologia del Farmaco
Keywords:	Chloromethyl phenylcarbamate-based chiral stationary phases, Electronic circular dichroism, Helical chromenes, Helicene-like structure, Theoretical calculations

SCHOLARONE™
Manuscripts

**Chiral separation of helical chromenes with chloromethyl phenylcarbamate
polysaccharide-based stationary phases**

*Federica Ianni¹, Stefania Scorzoni¹, Pier Luigi Gentili², Alessandro Di Michele³, Michel Frigoli⁴,
Emidio Camaioni¹, Fausto Ortica^{2,5}, Roccaldo Sardella^{1*}*

¹ *Department of Pharmaceutical Sciences, University of Perugia, Via Fabretti 48,
06123 Perugia, Italy*

² *Department of Chemistry, Biology and Biotechnology, University of Perugia, Via Elce di Sotto 8,
06123 Perugia, Italy*

³ *Department of Physics and Geology, University of Perugia, Via Pascoli, 06123 Perugia, Italy*

⁴ *Institut Lavoisier de Versailles, UMR CNRS 8080 Université de Versailles Saint-Quentin-en-
Yvelines, 45 av des États-Unis Bâtiment Lavoisier, 78035 Versailles, France*

⁵ *Istituto Nazionale di Fisica Nucleare (INFN) Sezione di Perugia, Via Pascoli,
06123 Perugia, Italy*

RUNNING TITLE:

Chiral separation of helical chromenes with polysaccharide-based CSPs

Correspondence:

Dr. Roccaldo Sardella

Department of Pharmaceutical Sciences, University of Perugia, Via Fabretti 48, 06123 Perugia,
Italy

Email: roccaldo.sardella@unipg.it

26 Fax number: +49-075 585 5161

27 **LIST OF NON-STANDARD ABBREVIATIONS:**

28 CSP - chiral stationary phase; ECD - electronic circular dichroism; EEO - enantiomeric elution
29 order; IPA -2-propanol; T_{iso} - Isoenantioselective temperature

30

31

32 **KEYWORDS**

33 Chloromethyl phenylcarbamate-based chiral stationary phases; Electronic circular dichroism;
34 Helical chromenes; Helicene-like structure; Theoretical calculations

35

36

37

38

39

40

41

42

43

44

45

46

47

48

49

50

ABSTRACT

Two chloromethyl phenylcarbamate-based chiral stationary phases, one containing an amylose type chiral selector (Lux Amylose 2, from Phenomenex) and the other a cellulose type one (Lux Cellulose-4, from Phenomenex), were successfully used for the chiral resolution of three helical chromenes featuring a helicene-like structure.

The compound bearing a phenyl substituent on the helicene-like structure was enantioresolved at 25 °C with Lux Cellulose-4 and a n-hexane/PrOH - 99/1 (v/v) eluent. With a n-hexane/IPA - 99.8/0.2 (v/v) mobile phase, the same column (operated at 35 °C) provided the separation of the four isomers of the compound having a hexyl residue on the helicene-like motif and an additional asymmetric carbon. Lux Amylose-2 was necessary for the enantioseparation of compound having the sole hexyl residue on the helical scaffold. For the last compound a n-hexane/PrOH - 99.8/0.2 (v/v) eluent was used and the column temperature fixed at 5 °C.

The enantiomer elution order was appraised *via* electronic circular dichroism coupled to theoretical calculations.

Noteworthy, different thermodynamics of retention and enantioseparation were observed for molecules with pronounced structural similarity, that is, the enantiomer pairs of the compound containing the additional asymmetric carbon atom. Indeed, both entropically and enthalpically controlled adsorption and separation processes were observed.

1 INTRODUCTION

Even though photochromic phenomena had already been serendipitously observed since 1867 [1-3], an aware definition of photochromism as the reversible change of colour of a chemical species induced by light only dates back to 1950 [4]. A decisive growth of this field of research took place since the 60's, due to continuous progresses in synthetic, spectroscopic and analytical techniques on the one hand, and the development of several applications, such as photochromic glasses and micro-image process, on the other [5, 6].

The interest in photochromic compounds has then significantly increased over the last decades, particularly as a consequence of the development of fatigue-resistant molecules belonging to the classes of spirooxazines and chromenes [7-12], which have found various important applications, the most well-known of them in the production of photochromic ophthalmic lenses.

These compounds usually display either a thermo- or both a thermo- and photo-reversible behaviour, while other deeply investigated photochromic systems such as diarylethenes [13, 14] and azobenzenes [15, 16] only exhibit pure photoreversible features and are object of interest in light of their potential utility in applications which range from molecular electronics to bioimaging. Undoubtedly, the mechanism which drives the reversible reaction of a photochromic system is important from the twofold aspect of their physical-chemical characterization and practical applications and indeed one of the most widespread way to classify these molecules refers to their either thermoreversible (type T photochromism) or photoreversible (type P photochromism) back reaction [7].

In chromene molecules, the electrocyclic ring-opening at the pyran C-O bond induced by UV irradiation, investigated for the first time by Lenoble and co-workers [17], yields open metastable photoproduct(s) having o-quinone-allides structures, which are generally coloured due to an enhanced electronic delocalization compared to the initial chromene molecule in its closed form [18, 19]. Chromenes usually exhibit a rather complicate photochromic behaviour, due to the

concomitant presence of thermal and photochemical processes in the mechanism which restores the starting material. The classical behaviour of these molecules, where the UV irradiation of the closed form leads to photoproduction of the *transoid-cis* (TC) and *transoid-trans* (TT) structures, can be exemplified by the 3,3-diphenyl-[3H]-naphtho[2-1b]pyran [20, 21]. The TC is generally more thermally labile compared to TT while its thermodynamic stability is usually higher than that of TT [22, 23].

Some of the authors of the present work have recently shown that the thermal persistence of the colour generated upon UV irradiation can be strongly enhanced upon shifting the photoequilibrium to the TT isomer, thus increasing the P-type character of the chromene molecule [24] and the subsequent introduction of a helical scaffold into the molecular structure represents a further step in this direction [25]. Moreover, the presence of the axial chirality may give rise to interesting potential applications for these helical chromenes, such as the possibility of obtaining photochromic chiroptical materials [26, 27] to be used as chiral photo-switches for developing smart materials distinguishing right- from left-circularly polarized UV-visible radiation [28-30].

It is worth mentioning that this specific axial chirality, also called helicity, has a peculiar nomenclature with P (plus) for the right-handed helix and M (minus) for the left-handed one.

In this scenario, the three compounds (**1-3**) shown in Fig. 1 were considered of particular importance. All of three are characterized by a helicene-type fashion with either a phenyl (in compound **1**) or a hexyl (in compounds **2**, and **3**) substituent. An additional asymmetry contribution is given in **2** by the presence of a “chiral” carbon atom.

To fully exploit the sensory functions of helical chromenes **1-3** (Fig. 1), it is important to separate their enantiomeric isomers. Therefore, interested in evaluating the photochromic behavior of the single isomers of compounds **1-3** upon UV-Vis irradiation, we decided to develop suitable enantioselective chromatography methods enabling to isolate sufficient amounts of each isomer.

Various CSPs incorporating both low- and high-molecular weight chiral selectors were profitably used over the years for the chiral resolution of carbohelicenes and their congeners [31].

Furthermore, relevant chromatographic performances were achieved with the more relevant modes of elution, that is, under normal-, polar organic/polar ionic- as well as reversed-phase conditions [31]. However, to the best of our knowledge, this is the first study in which the chiral separation of helicene-like compounds (**1-3**, Fig. 1) was successfully performed with chloromethyl phenylcarbamate cellulose- and amylose-based stationary phases. This sub-family of polysaccharide derivatives used as chiral selector, proved extraordinary enantioresolution ability towards different classes of compounds. Their excellent enantioselective power is explained by the elevated number of –NH and –C=O groups available for stereoselective contacts with the analyte, at expenses of those occurring intramolecularly between adjacent carbamate motifs along the polymer chain [32, 33].

The chromatographic study (including interesting thermodynamics considerations) as well as the electronic circular dichroism (ECD) analyses coupled with *in-silico* ECD spectra predictions to appraise the enantiomeric elution order (EEO), are described in the following sections.

2 MATERIALS AND METHOD

2.1 Chemicals and Reagents

Compounds **1-3** were synthesized according to the procedure described in [25]. Analytical grade n-hexane, 1-propanol (PrOH), 2-propanol (IPA), were purchased from Sigma-Aldrich (Milano, Italy). The compound 1,3,5-tri-*tert*-butylbenzene was used as the unretained marker and purchased from Sigma-Aldrich (Milano, Italy). All the employed mobile phases were degassed with 10 min sonication before use. Compounds to be injected were solubilized in the selected mobile phase and analyzed at the approximate concentration of 0.5-1.0 mg/mL.

2.2 Instrumentations

HPLC analyses were performed with the following polysaccharide-based chiral stationary phases: Lux Cellulose-2 (CSP 1) (250 mm x 4.6 mm I.D., containing cellulose tris(3-chloro-4-

155 methylphenylcarbamate) adsorbed onto a 5 μm silica gel), Lux Cellulose-4 (CSP 2) (250 mm x 4.6
156 mm I.D., containing cellulose tris(4-chloro-3-methylphenylcarbamate) adsorbed onto a 5 μm silica
157 gel), and Lux Amylose-2 (CSP 3) (250 mm x 4.6 mm I.D., containing amylose tris(5-chloro-2-
158 methylphenylcarbamate) adsorbed onto a 5 μm silica gel) were purchased from Phenomenex
159 (Torrance, CA, USA).

160 Before the use, the selected column was conditioned with the selected mobile phase at a 1.0 mL/
161 min flow rate for not less than least 40 min. All the analyses were run at 1.0 mL/min flow. HPLC
162 analyses were made on a Shimadzu (Kyoto, Japan) LC-20A Prominence, equipped with a CBM-
163 20A communication bus module, two LC-20AD dual piston pumps, a SPD-M20A photodiode array
164 detector, and a Rheodyne 7725i injector (Rheodyne Inc., Cotati, CA, USA) with a 20 μL stainless
165 steel loop. A Grace (Sedriano, Italy) heater/chiller (Model 7956R) was used as column thermostat.
166 ECD spectra were recorded by means of a Jasco J-810 Spectropolarimeter (Jasco Corporation,
167 Tokyo, Japan) at 25 $^{\circ}\text{C}$, using 1 mm quartz cell. Solution of stereoisomers of compounds **1-3** were
168 prepared in n-hexane and analyzed in the global spectral range of 225-400 nm.

170 2.3 Thermodynamics of retention and selectivity

171 Van't Hoff plots represent a convenient way for calculating the thermodynamics parameters of a
172 chromatographic process [34]. The plot of the natural logarithm of retention factor values ($\ln k$)
173 against the reciprocal of column temperature ($1/T$) values allows to derive the standard molar
174 enthalpy of adsorption ΔH° , from the slope of the line formalized by equation (1), while the
175 intercept is related to the adsorption entropy ΔS° ,

$$177 \ln k = -\frac{\Delta H^{\circ}}{R} \times \frac{1}{T} + \frac{\Delta S^{\circ}}{R} + \ln \phi \quad (1)$$

with R being the gas constant (equal to 8.341 J/mol K) and ϕ the phase ratio of the column. It should be pointed out that the intercept often represents an apparent entropy change $\Delta S^\# (= \Delta S^\circ + \ln \phi)$, when the phase ratio is unknown.

The two differences $\Delta H^\circ_2 - \Delta H^\circ_1 (\Delta\Delta H^\circ)$ and $\Delta S^\circ_2 - \Delta S^\circ_1 (\Delta\Delta S^\circ)$ are instead derived from the plot of $\ln \alpha$ vs the $1/T$ values [Eq. (2)], with α representing the enantioselectivity factor.

$$\ln \alpha = -\frac{\Delta_{S,R} \Delta H^\circ}{R} \times \frac{1}{T} + \frac{\Delta_{S,R} \Delta S^\circ}{R} \quad (2)$$

Isoenantioselective temperature (T_{iso}) represents the temperature value where the enthalpy-entropy compensation occurs and the two enantiomers are no longer separated. T_{iso} can be calculated as follow (Eq. 3).

$$T_{iso} = \frac{\Delta_{S,R} \Delta H^\circ}{\Delta_{S,R} \Delta S^\circ} \quad (3)$$

2.4 Theoretical calculations

All the ground-state conformations of the isomers of compounds **1** and **2** were optimized at the DFT/B3-LYP level by employing 3-21g basis set. All the excited-state calculations of the isomers of compounds **1** and **2** were performed at the ground-state geometries by using both DFT/B3-LYP, with 6-31G as basis set, and semi-empirical ZINDO method. The results correspond to vertical transitions, and the excitation energies can be approximately compared with the band maxima of the experimental spectra. The ECD spectra were simulated by using the TD-DFT method. The output is the rotatory strength for every electronic transition that is given by the following equation, expressed in cgs units:

$$R = 2.297 \times 10^{-39} \int_{\lambda_1}^{\lambda_2} \frac{\Delta \epsilon(\lambda)}{\lambda} d\lambda \quad (4)$$

204

205 In Eq. 4, λ_1 and λ_2 are the wavelengths delimiting the transition and $\Delta\epsilon$ is the difference of the
206 absorption coefficients for left and right circularly polarized light [35].

207 All the calculations have been carried out by using Gaussian 03 software [Gaussian 03, Revision
208 E.01, Gaussian, Inc., Wallingford CT, 2004].

209

210 3 RESULTS AND DISCUSSION

211 With the aim of selecting the best CSP for the enantioselective analysis of compounds **1** and **3** and
212 the chemo- and enantioselective analysis of compound **2** (Fig. 1), three polysaccharide-based CSPs
213 (CSPs 1-3, Fig. 2) were scrutinized. CSPs 1-3 (see section 2.2 for details) were chosen with the aim
214 of evaluating the impact of the polymer winding and the position of the phenyl substituents, on the
215 overall chromatographic performance.

216 For all compounds, the good chromatographic results achieved in the identified best eluent
217 conditions, facilitated the collection of all stereoisomeric species for the following electronic
218 circular dichroism (ECD) study. For the assignment of the enantiomer elution order (EEO), please
219 see section 3.1.

220

221 **Please, insert Figure 1 near here**

222 **Please, insert Figure 2 near here**

223

224 CSP **2** was the sole polymeric material among those screened in the study, enabling the
225 enantioresolution of **1**. Only few modifications of the eluent composition allowed us to identify the
226 n-hexane/PrOH - 99/1 (v,v) combination as suitable to get the two peaks fully resolved (Table 1)
227 with the following EEO: M-(-)-**1** < P-(+)-**1**. Different proportions of n-hexane and PrOH, as well as
228 the replacement of the latter with other alcoholic modifiers were tested and produced a sensitive

deterioration of the overall chromatographic performance. The chromatogram in the optimal eluent system is depicted in Fig. 3(A), while details on the other experimental conditions are included in the figure caption.

The comparative evaluation performed with standard normal-phase eluent systems for polysaccharide-based CSPs, revealed CSP 2 as the most promising chiral medium for the simultaneous diastereo- and enantioseparation of **2**. Only marginal levels of enantioselectivity were produced by CSP 3, while all the remaining CSPs resulted definitely ineffective.

Please, insert Table 1 near here

Please, insert Figure 3 near here

Further optimization of the mobile phase composition with CSP 2 allowed us to record the complete stereoselectivity, when 0.2 % (in volume) IPA was added to n-hexane. However, running the analysis at a 1.0 mL/min flow rate and with a column temperature fixed at 25 °C (298.15 K) did not produced the base-line separation for both enantiomer couples ($R_s < 1.5$). Increasing amount of IPA progressively attenuated the enantioselectivity of the first eluted enantiomer pair, and concurrently increased that of the second eluted one (data not shown). Instead, with the replacement of IPA with other alcohols (namely, EtOH or PrOH), the overall chromatographic performance sensitively got worse, even when tested in the presence of basic and/or acidic additives.

A series of results not reported in the manuscript clearly indicated a contribution of H-bonding interactions to analyte retention: increasing amounts of alcohol in the eluent markedly shortened analyte residence in the column. However, one could speculate that increased retention with low alcohol containing mobile phases systems might principally derive from stronger π - π stacking interactions between the aromatic portion of the chiral selector and that of analyte [36]. More realistically, both forces are active in governing analyte retention.

The identification of the enantiomeric pairs was facilitated by the previous diastereomeric separation *via* preparative-scale reversed-phase achiral chromatography. Whilst, the elution order $M(-)-(S)-2 < P(+)-(R)-2 < P(+)-(S)-2 < M(-)-(R)-2$ was elucidated with the aid of ECD studies coupled with theoretical calculations (see section 3.1 for details). The latter giving also information on the stereochemistry of the asymmetric carbon atom.

Being aware that enantioselective retention mechanisms are often influenced by temperature to a greater extent than are ordinary separations, a series of analyses was performed at 10, 20, 25, 30, and 35 °C (respectively, 283.15, 293.15, 298.15, 303.15 and 308.15 K) with the n-hexane/IPA – 99.8/0.2% (v,v) eluent. From data listed in Table 1 it is readily evident that enantioselectivity improved at high temperatures for the first eluted enantiomer pair $M(-)-(S)-2/P(+)-(R)-2$, while an opposite trend emerged for second eluted one $P(+)-(S)-2/M(-)-(R)-2$. Good levels of chemoselectivity (diastereoselectivity between $P(+)-(R)-2$ and $P(+)-(S)-2$) were instead maintained over the entire temperature range.

As far as resolution is concerned, a trend coherent with enantioselectivity data was obtained for $M(-)-(S)-2/P(+)-(R)-2$ while a maximum (with base-line separation) was reached for $P(+)-(S)-2/M(-)-(R)-2$ at 30 °C. In Fig. 3(B), the chromatogram in the optimal compromise analysis condition is shown.

The singular and opposite behavior displayed by the two enantiomer pairs stimulated us to gain further insights into the thermodynamics of the stereorecognition processes with the above eluent system and CSP 2. The influence of temperature on retention and selectivity was studied through the inspection of the van't Hoff plots (Fig. 4) [34].

Please, insert Figure 4 near here

The thermodynamic quantities derived from the van't Hoff plots (Table 2) reveal endothermic adsorption processes (that is, $\Delta H > 0$) for all the four isomers of **2**. Moreover, entropically controlled adsorptions are active in the chromatographic process: $|\Delta H| < |T\Delta S|$ [37, 38].

The van't Hoff plots are all linear, thereby indicating that the conformation of the chiral selector remains essentially invariant between 10 °C and 35 °C. Therefore, multiple retention and chiral recognition mechanisms do not take place within the explored temperature range [34, 39]. Additionally, the persistence of the same enantiomer elution order (that is, M-(-)-(S)-**2** < P-(+)-(R)-**2** < P-(+)-(S)-**2** < M-(-)-(R)-**2**) over the above range was demonstrated by ECD analysis on peak fractions collected at different temperatures (Figs. S1 and S2, Supporting Information).

Please, insert Table 2 near here

The apparent entropy change, $\Delta S^\ddagger$, is different between the enantiomers of each pair. The dissimilarity may hypothetically depend on a different number of solvent molecules released by the two enantiomers as they associate with the complementary sites on the CSP [39].

For the two enantiomers M-(-)-(S)-**2**/P-(+)-(R)-**2**, $|\Delta(\Delta H)|$ was always found higher than $|T\Delta(\Delta S)|$, thus indicating the occurrence of an entropically controlled separation process: enantioselectivity improves on increasing column temperature [34]. The only exception is represented by the elution at 10 °C (283.15 K), where the two quantities are quite similar as a result of the closeness to the isoenantioselective temperature (T_{iso} about 282.9 K). Below 20 °C, chiral recognition is progressively abolished.

For the pair P-(+)-(S)-**2**/M-(-)-(R)-**2**, $|\Delta(\Delta H)| > |T\Delta(\Delta S)|$ over the entire temperature range investigated, which means an enthalpically controlled enantioseparation process: enantioselectivity improves on decreasing temperature. For the more retained enantiomer pair, a T_{iso} of about 332.3 K was estimated and, therefore, all the analyses were run rather below the so called “temperature-

induced blind zone” for chiral recognition [38]. This temperature is well above the highest allowed with this enantiorecognition material. Indeed, temperatures higher than 50 °C could irreversibly damage the chiral selector.

The results described in the present study are in line with those reported by other authors [37]: even subtle differences in the chemical structure of analytes can produce different sorption and enantiorecognition behaviours.

Differently from the previous compounds, the peculiar winding of the amylose-based chiral selector in CSP 3 was necessary to obtain favorable enantioseparation values for **3**. For this compound, some level of stereoselectivity was also obtained with CSP 2, along with unprofitable elution efficiencies.

The n-hexane/ PrOH - 99.8/0.2% (v,v) eluent system was used as starting point for following analyses pursued with the aim to achieve enantioresolution of the peaks. Also for this compound, modifying the eluent composition with alternative alcohols and relative concentrations as well as with the addition of common “efficiency enhancers” [40] did not produce noticeable ameliorations. Therefore, also for **3**, the effect of column temperature was in the range 278.15-308.15 K.

Examination of van’t Hoff plots for **3** (Fig. 4C and 4D) clearly suggests similar thermodynamic considerations as for P-(+)-(S)-**2**/M-(-)-(R)-**2** (Table 2): endothermic adsorption processes are active for both enantiomers along with an enthalpically controlled enantioseparation mechanism. For compound **3**, all the analyses were run rather below T_{iso} , which was estimated to be about 339.1 K.

Also for **3**, the perdurability of the EEO (that is, P-(+)-**3** < M-(-)-**3**) over the investigated temperature range was verified through ECD analyses (data not shown).

Acceptable extent of α and R_s values were reached running the analysis at 5 °C (Fig. 3(C)).

3.1 Stereochemical characterization of isomers of compounds 1-3 and evaluation of their elution order

To determine the helicity M or P of the isolated isomers and, consequently, the stereoisomeric elution order for compounds **1-3** in the optimized experimental conditions, ECD spectra were recorded in n-hexane for all the isolated isomers. Then, the experimental spectra were interpreted by carrying out theoretical calculations and by comparing our results with those of other helicenes that are retrievable in literature [41-43].

The experimental ECD spectrum of the first eluted enantiomer of compound **1** is shown in Fig. 5 wherein the results of the simulations are also reported. The compound exhibits a strong negative Cotton effect. The electronic transitions at lower energies involve principally the helicene backbone fused with the benzopyran ring (as usually occurs with these compounds [44]). The simulations, which reproduce quite well the experimental data (see section 2.4 for the details of the simulations), allow to assign the ECD spectrum of Fig. 5 to the M isomer, whose structure is shown in the same figure. The second eluted enantiomer of **1** has an ECD that is the mirror image of that shown in Fig. 5 (see Supporting Information), with a strong positive Cotton effect, because it is due to the P isomer of **1**. Therefore, the EEO is M-(-)-**1** < P-(+)-**1**. The assignment made by using the output of the calculations is in agreement with what expected from previous results that can be found in literature [3].

Please, insert Figure 5 near here

An opposite EEO was instead deduced from the ECD spectra of the enantiomers of compound **3**: P-(+)-**3** < M-(-)-**3**. More complicated is the situation for compound **2**, because it exists as four isomers: P-(R), P-(S), M-(R), and M-(S), because besides the helical structure, it has a chiral centre in the spiro-carbon. The ECD spectra of all the four eluted isomers were recorded. Moreover, the ECD spectra were simulated by calculating the rotatory strengths, R, of the isomers (see section 2.4 for the equation defining R). The results for two isomers are shown in Fig. 6; the other results are reported in the Supporting Information. Fig. 6A shows the experimental and calculated ECD of the first isomer that is eluted, which is M-(-)-(S)-**2**. The second isomer to be eluted is the mirror image of the first: P-(+)-(R)-**2** (see Supporting Information). The experimental and calculated ECD of the

third isomer is shown in Fig. 6B: it is P-(+)-(S)-**2**. Finally, the last is, as expected, M-(-)-(R)-**2**, which is the mirror image of the third one (see tary Material). In summary, the order of isomer elution is: M-(-)-(S)-**2** < P-(+)-(R)-**2** < P-(+)-(S)-**2** < M-(-)-(R)-**2**.

Please, insert Figure 6 near here

4 CONCLUDING REMARKS

Two chloromethyl phenylcarbamate-based CSPs, one containing an amylose type chiral selector and the other a cellulose type one, were successfully used for the chiral resolution of the three helical chromenes **1-3**.

Noteworthy, even small differences in the analyte structure dictated preferential stereoselective bindings for a specific polymer type. Indeed, enantiomers of compound **1** and all isomers of compound **2** were well separated with a cellulose-based chiral stationary phase (in Lux Cellulose-4, CSP 2), the amylose winding (in Lux Amylose-2, CSP 3) was instead necessary to succeed in the enantioseparation of **3**.

Even more intriguingly, different thermodynamics of retention and stereoseparation were observed for molecules with pronounced structural similarity, that is the diastereomers of compound **2**. The optimized normal-phase eluent conditions allowed to get the isomers of each compound sufficiently separated to be isolated and then submitted to following photochemistry investigations. The results of the photochromic behavior of the single isomers of compounds **1-3** upon UV-Vis irradiation falls outside the scope of the present study and is therefore the object of a manuscript under preparation.

Acknowledgement

P. L. Gentili and F. Ortica acknowledge the financial support by the University of Perugia (Fondo Ricerca di Base 2014, D. D. n. 170, 23/12/2014). F. Ortica gratefully acknowledges I.N.F.N. for financial support.

Conflict of interest statement

The authors declare no conflict of interest.

REFERENCES

- [1] Fritzsche, J., Photochromism of tetracene. *Comptes Rendus Acad. Sci. Paris* 1867, 69, 1035.
- [2] Ter Meer, E., Über Dinitroverbindungen der Fettreihe. *Justus Liebigs Ann. Chem.* 1876, 181, 1-22.
- [3] Markwald, W. Z., Über Phototropie. *Z. Phys. Chem.* 1899, 30, 140- 145.
- [4] Hirshberg, Y., Photochromy in the bianthrone series. *Comptes Rendus Acad. Sci. Paris* 1950, 231, 903-904.
- [5] Brown, G.H. (Ed.), Techniques of Chemistry, Vol. III, Photochromism. Wiley-Interscience, New York, 1971.
- [6] Bertelson, R. C., Reminiscences About Organic Photochromics. *Mol. Cryst. Liq. Cryst.* 1994, 246, 1-8.
- [7] Bouas-Laurent, H., Dürr, H., Organic photochromism (IUPAC Technical Report). *Pure Appl. Chem.* 2001, 73, 639-665.
- [8] Guglielmetti, R., in: Dürr, H., Bouas-Laurent, H. (Eds.), Photochromism: Molecules and Systems. Elsevier, Amsterdam 1990, pp. 314-466.
- [9] Chu, N. Y. C., in: Dürr, H., Bouas-Laurent, H. (Eds.), Photochromism: Molecules and Systems. Elsevier, Amsterdam 1990, pp. 493-513.
- [10] Maeda, S., in: Crano, J. C., Guglielmetti, R. J. (Eds.), Organic Photochromic and Thermochromic Compounds. Kluwer Academic/Plenum Publishers, New York 1999, pp. 85-109.

- [11] Van Gemert, B., in: Crano, J. C., Guglielmetti, R. J. (Eds.), *Organic Photochromic and Thermochromic Compounds*. Kluwer Academic/Plenum Publishers, New York 1999, pp. 111-140.
- [12] Hepworth, J. D., Heron, B. M., in: Kim, S. H. (Ed.), *Functional dyes*. Elsevier, Amsterdam 2006, pp. 85-135.
- [13] Irie, M., *Diarylethenes for memories and switches*. *Chem Rev.* 2000, *100*, 1685-1716.
- [14] Matsuda, K., Irie, M., *Diarylethene as a photoswitching unit*. *J. Photochem. Photobiol. C: Photochem. Rev.* 2004, *5*, 169-182.
- [15] Russew, M. M., Hecht, S., *Photoswitches: from molecules to materials*. *Adv. Mater.* 2010, *22*, 3348-3360.
- [16] Beharry, A. A., Woolley, G. A., *Azobenzene photoswitches for biomolecules*. *Chem. Soc. Rev.* 2011, *40*, 4422-4437.
- [17] Lenoble, C., Becker, R. S., *Photophysics, photochemistry and kinetics of photochromic 2H-pyrans and chromenes*. *J. Photochem.* 1986, *33*, 187-197.
- [18] Kolc, J., Becker, R. S., *Proof of structure of the colored photoproducts of chromenes and spiropyrans*. *J. Phys. Chem.* 1967, *71*, 4045-4048.
- [19] Gentili, P. L., Danilov, E., Ortica, F., Rodgers, M. A. J., Favaro, G., *Dynamics of the excited states of chromenes studied by fast and ultrafast spectroscopies*. *Photochem. Photobiol. Sci.* 2004, *3*, 886-891.
- [20] Delbaere, S., Luccioni-Houze, B., Bochu, C., Teral, Y., Campredon, M., Vermeersch, G., *Kinetic and structural studies of the photochromic process of 3H-naphthopyrans by UV and NMR spectroscopy*. *J. Chem. Soc.-Perkin Trans. 2* 1998, 1153-1158.
- [21] Ottavi, G., Favaro, G., Malatesta, C., *Spectrokinetic study of 2,2-diphenyl-5,6-benzo(2H)chromene: a thermoreversible and photoreversible photochromic system*. *J. Photochem. Photobiol. A Chem.* 1998, *115*, 123-128.

- 429 [22] Demadrille, R., Rabourdin, A., Campredon, M., Giusti, G., Spectroscopic characterisation and
430 photodegradation studies of photochromic spiro[fluorene-9,3'-[3'H]-naphtho[2,1-b]pyrans]. *J.*
431 *Photochem. Photobiol. A: Chem.* 2004, 168, 143.
- 432 [23] Gabbutt, C. D., Heron, M., Instone, A. C., Kolla, S. B., Mahajan, K., Coelho, P. J., Carvalho, L.
433 M., Synthesis and photochromic properties of symmetrical aryl ether linked bi- and tri-
434 naphthopyrans. *Dyes Pigm.* 2008, 76, 24-34.
- 435 [24] Frigoli, M., Maurel, F., Berthet, J., Delbaere, S., Marrot, J., Oliveira, M. M., The Control of
436 photochromism of [3H]-naphthopyran derivatives with intramolecular CH- π bonds. *Org. Lett.*
437 2012, 14, 4150-4153.
- 438 [25] Frigoli, M., Marrot, J., Gentili, P. L., Jacquemin, D., Vagnini, M., Pannacci, D., Ortica, F., P-
439 type photochromism of new helical naphthopyrans synthesis and photochemical, photophysical
440 and theoretical study. *Chem. Phys. Chem.*, 2015, 16, 2447-2458.
- 441 [26] Isla, H., Crassous, J., Helicene-based chiroptical switches. *C. R. Chim.* 2016, 19, 39-49.
- 442 [27] Shen, C., He, X., Toupet, L., Norel, L., Rigaut, S., Crassous, J., Dual redox and optical control
443 of chiroptical activity in photochromic dithienylethenes decorated with hexahelicene and bis-
444 ethynyl-ruthenium units. *Organometallics* 2017, DOI: 10.1021/acs.organomet.7b00534.
- 445 [28] Chien, C., Liu, J., Optical behaviors of cholesteric liquid crystalline polyester composites with
446 various chiral photochromic dopants. *Langmuir* 2015, 31, 13410-13419.
- 447 [29] Kim, Y., Frigoli, M., Vanthuyne, N., Tamaoki N., A helical naphthopyran dopant for
448 photoresponsive cholesteric liquid crystals. *Chem. Commun.* 2017, 53, 200-203.
- 449 [30] Gentili, P. L., Small steps towards the development of chemical artificial intelligent systems.
450 *RSC Adv.* 2013, 3, 25523-25549.
- 451 [31] Gingras, M., Félix G., Peresutti R., One hundred years of helicene chemistry. Part 2:
452 stereoselective syntheses and chiral separations of carbohelicenes. *Chem. Soc. Rev.* 2013, 42,
453 1007-1050.

- 454 [32] Chankvetadze, B., Yashima, E., Okamoto, Y., Chloromethylphenylcarbamate derivatives of
455 cellulose as chiral stationary phases for high-performance liquid chromatography. *J.*
456 *Chromatogr. A* 1994, 670, 39-49.
- 457 [33] Chankvetadze, B., Yashima, E., Okamoto, Y., Dimethyl-, dichloro- and
458 chloromethylphenylcarbamates of amylose as chiral stationary phases for high-performance
459 liquid chromatography. *J. Chromatogr. A* 1995, 694, 101-109.
- 460 [34] Lämmerhofer, M., Chiral recognition by enantioselective liquid chromatography: Mechanisms
461 and modern chiral stationary phases. *J. Chromatogr. A* 2010, 1217, 814-856.
- 462 [35] Diedrich, C., Grimme, S., Systematic Investigation of Modern Quantum Chemical Methods to
463 Predict Electronic Circular Dichroism Spectra. *J. Phys. Chem. A* 2003, 107, 2524-2539.
- 464 [36] Yashima, E., Yamada, M., Kaida, Y., Okamoto, Y., Computational studies on chiral
465 discrimination mechanism of cellulose trisphenylcarbamate. *J. Chromatogr. A* 1995, 694, 347-
466 354.
- 467 [37] Yaku, K., Aoe, K., Nishimura, N., Morishita, F., Thermodynamic study and separation
468 mechanism of diltiazem optical isomers in packed-column supercritical fluid chromatography.
469 *J. Chromatogr. A* 1999, 848, 337-345.
- 470 [38] Yao, B., Zhan, F., Yu, G., Chen, Z., Fan, W., Zeng, X., Zeng, Q., Weng, W., Temperature-
471 induced inversion of elution order in the chromatographic enantioseparation of 1,1'-bi-2-
472 naphthol on an immobilized polysaccharide-based chiral stationary phase. *J. Chromatogr. A*
473 2009, 1216, 5429-5435.
- 474 [39] Péter, A., Vékes, E., Armstrong, D. W., Effects of temperature on retention of chiral
475 compounds on a ristocetin A chiral stationary phase. *J. Chromatogr. A* 2002, 958, 89-107.
- 476 [40] Sardella, R., Ianni, F., Lisanti, A., Marinozzi, M., Scorzoni, S., Natalini, B., The effect of
477 mobile phase composition in the enantioseparation of pharmaceutically relevant compounds
478 with polysaccharide-based stationary phases. *Biomed. Chromatogr.* 2014, 28, 159-167.

- [41] Newman, M. S., Darlak, R. S., Tsai, L., Optical properties of hexahelicene. *J. Am. Chem. Soc.* 1967, *89*, 6191-6193.
- [42] Samal, M., Chercheja, S., Rybacek, J., Vacek-Chocholousova, J., Vacek, J., Bednarova, L., Saman, D., Stara, I. G., Stry, I., An ultimate stereocontrol in asymmetric synthesis of optically pure fully aromatic helicenes. *J. Am. Chem. Soc.* 2015, *137*, 8469-8474.
- [43] Laarhoven, W. H., Prinsen, W. J. C., Carbohelicenes and Heterohelicenes. *Top. Curr. Chem.* 1984, *125*, 63-130.
- [44] Gentili, P. L., Rightler, A. L., Heron, B. M., Gabbutt, C. D., Extending human perception of electromagnetic radiation to the UV region through biologically inspired photochromic fuzzy logic (BIPFUL) systems. *Chem. Commun.* 2016, *52*, 1474-1477.

FIGURE CAPTIONS

Figure 1. Structure of the compounds investigated in the present study.

Figure 2. Structure of the CSPs utilized in the present study.

Figure 3. Chromatograms of (A) **1**, (B) **2** and (C) **3** in the optimized analysis conditions.

Experimental conditions: eluent, (A) n-hexane/PrOH - 99/1 (v/v), (B) n-hexane/IPA - 99.8/0.2 (v/v), (C) n-hexane/PrOH - 99.8/0.2 (v/v); column temperature, (A) 25 °C, (B) 35 °C, (C) 5 °C; flow-rate, 1.0 mL/min; detection at 254 nm; EEO, (A) M-(-)-**1** < P-(+)-**1**, (B) M-(-)-(*S*)-**2** < P-(+)-(*R*)-**2** < P-(+)-(*S*)-**2** < M-(-)-(*R*)-**2**, (C) P-(+)-**3** < M-(-)-**3**.

Figure 4. Plots of van't Hoff. Temperature dependence of (A) retention for compound **2**, (B) enantioselectivity for compound **2**, (C) retention for compound **3**, (D) enantioselectivity for compound **3**. Experimental conditions: eluent, (A,B) n-hexane/IPA - 99.8/0.2 (v/v), (C,D) n-hexane/PrOH - 99.8/0.2 (v/v); flow-rate, 1.0 mL/min; detection at 254 nm; EEO, (A) M-(-)-(*S*)-**2** < P-(+)-(*R*)-**2** < P-(+)-(*S*)-**2** < M-(-)-(*R*)-**2**, (C) P-(+)-**3** < M-(-)-**3**.

Figure 5. Experimental ECD and calculated rotatory strengths *R*, depicted as vertical bars, for the first peak of compound **1**. The structure of the isomer is also shown.

Figure 6. Experimental ECD and calculated rotatory strengths R , depicted as vertical bars, for the first (A) and the third (B) peak of compound **2**. The structures of the isomers giving the two ECD spectra are also reported.

For Review Only

Figure 1

Figure 1. Structure of the compounds investigated in the present study.

297x209mm (300 x 300 DPI)

Polymer Type	R	CSP #	Tradename
1	Lux Cellulose-2
	Tris(3-chloro-4-methylphenylcarbamate)		
Cellulose		2	Lux Cellulose-4
	Tris(4-chloro-3-methylphenylcarbamate)
3	Lux Amylose-2
	Tris(5-chloro-2-methylphenylcarbamate)		
Amylose			

Figure 2

Figure 2. Structure of the CSPs utilized in the present study.

297x209mm (300 x 300 DPI)

Figure 3

Figure 3. Chromatograms of (A) 1, (B) 2 and (C) 3 in the optimized analysis conditions. Experimental conditions: eluent, (A) n-hexane/PrOH - 99/1 (v/v), (B) n-hexane/IPA - 99.8/0.2 (v/v), (C) n-hexane/PrOH - 99.8/0.2 (v/v); column temperature, (A) 25 °C, (B) 35 °C, (C) 5 °C; flow-rate, 1.0 mL/min; detection at 254 nm; EEO, (A) M-(-)-1 < P-(+)-1, (B) M-(-)-(S)-2 < P-(+)-(R)-2 < P-(+)-(S)-2 < M-(-)-(R)-2, (C) P-(+)-3 < M-(-)-3.

209x297mm (300 x 300 DPI)

Figure 4

Figure 4. Plots of van't Hoff. Temperature dependence of (A) retention for compound 2, (B) enantioselectivity for compound 2, (C) retention for compound 3, (D) enantioselectivity for compound 3. Experimental conditions: eluent, (A,B) n-hexane/IPA - 99.8/0.2 (v/v), (C,D) n-hexane/PrOH - 99.8/0.2 (v/v); flow-rate, 1.0 mL/min; detection at 254 nm; EEO, (A) M-(-)-(S)-2 < P-(+)-(R)-2 < P-(+)-(S)-2 < M-(-)-(R)-2, (C) P-(+)-3 < M-(-)-3.

297x209mm (300 x 300 DPI)

Figure 5

Figure 5. Experimental ECD and calculated rotatory strengths R, depicted as vertical bars, for the first peak of compound 1. The structure of the isomer is also shown.

297x209mm (300 x 300 DPI)

Figure 6

Figure 6. Experimental ECD and calculated rotatory strengths R , depicted as vertical bars, for the first (A) and the third (B) peak of compound 2. The structures of the isomers giving the two ECD spectra are also reported.

297x209mm (300 x 300 DPI)

Table 1. Selected chromatographic parameters evaluated in the present study.

Compound	CSP #	Mobile Phase	T (°C) [T (K)]	Chromatographic Parameters ^a									
				k ₁	k ₂	k ₃	k ₄	α (1-2)	α (2-3)	α (3-4)	R _s (1-2)	R _s (2-3)	R _s (3-4)
1	2	A	25 [298.15]	0.98	1.38			1.41			2.62		
			10 [283.15]	3.43	3.43	5.37	7.28	1.00	1.57	1.36	nc	1.96	1.37
			20 [293.15]	5.02	5.53	7.68	9.62	1.10	1.39	1.25	nc	1.48	1.30
2	2	B	25 [298.15]	5.81	6.72	9.04	10.69	1.16	1.35	1.18	0.93	1.85	1.04
			30 [303.15]	6.50	7.80	10.19	12.16	1.20	1.31	1.19	1.51	2.22	1.47
			35 [308.15]	7.53	9.42	11.91	13.71	1.25	1.26	1.15	2.10	2.18	1.29
3	3	C	5 [278.15]	4.21	6.84			1.62			1.60		
			10 [283.15]	4.73	7.38			1.56			1.34		
			25 [298.15]	5.29	7.53			1.42			1.07		
			35 [308.15]	6.45	7.87			1.22			0.66		

A, n-hexane/PrOH - 99/1 (v/v)
B, n-hexane/IPA - 99.8/0.2 (v/v)
C, n-hexane/PrOH - 99.8/0.2 (v/v)
Flow rate, 1.0 mL/min
^a number refers to the elution order
nc: not calculated by the software

Table 2. Thermodynamic parameters obtained by the van't Hoff plots and relative correlation coefficients.

Compound ^a	ΔH° (kJ/mol)	$\Delta S^\#$ (J/mol K)	R^2	$\Delta(\Delta H^\circ)$ (kJ/mol)	$\Delta(\Delta S^\#)$ (J/mol K)	R^2
2						
M-(-)-(S)	22.58	90.19	0.993			
P-(+)-(R)	29.08	113.14	0.995	6.49	22.95	0.999
P-(+)-(S)	22.93	95.05	0.998	-4.70	-14.14	
M-(-)-(R)	18.23	80.92	0.999			0.937
3						
P-(+)	9.17	45.02	0.953			
M-(-)	2.78	26.19	0.953	-6.39	-18.83	0.944

^aStereoisomers are listed according to their elution order