Received: 6 January 2017 | Revised: 27 February 2017 | Accepted: 19 March 2017

DOI: 10.1002/chir.22706

REGULAR ARTICLE

WILEY

New chiral stationary phases based on xanthone derivatives for liquid chromatography

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Funding information
Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES/FCT), Grant/Award Number: Project 214/08; Structured Program of R&D&I INNOVMAR - funded by the Programme NORTE2020 under the PORTUGAL 2020 Partnership Agreement, through ERDF and FCT, Grant/Award Number: NORTE-01-0145-FEDER-000035; Research Line NOVELMAR; Research project PTDC/MAR-BIO/4694 /2014 funded by FCT/MCTES, PIDDAC and ERDF through the COMPETE (POFC) programme in the framework of the programme PT2020 reference POCI-01-0145-FEDER-016790 3599-PPCDT; Cooperativa de Ensino

Abstract

Six chiral derivatives of xanthones (CDXs) were covalently bonded to silica, yielding the corresponding xanthonic chiral stationary phases (XCSPs). The new XCSPs were packed into stainless-steel columns with 150 x 4.6 mm i.d. Moreover, the greening of the chromatographic analysis by reducing the internal diameter (150 x 2.1 mm i.d.) of the liquid chromatography (LC) columns was also investigated. The enantioselective capability of these phases was evaluated by LC using different chemical classes of chiral compounds, including several types of drugs. A library of CDXs was evaluated in order to explore the principle of reciprocity as well as the chiral self-recognition phenomenon. The separation of enantiomeric mixtures of CDXs was investigated under multimodal elution conditions. The XCSPs provided high specificity for the enantiomeric mixtures of CDXs evaluated mainly under normal-phase elution conditions. Furthermore, two XCSPs were prepared with both enantiomers of the same xanthonic selector in order to confirm the inversion order elution.

KEYWORDS
chiral derivatives of xanthones, chiral stationary phase, enantioselectivity, liquid chromatography

Superior Politécnico e Universitário COXANT-CESPU-2016; Erasmus Mundus Action 2, Grant/Award Number: PhD's scholarship; QOPNA research project and Portuguese NMR network, Grant/Award Number: FCT UID/QUI/00062/2013

1 | INTRODUCTION

Analytical and preparative enantiomer separations by chromatography and related techniques play more than ever a crucial role in the chemical industry and academic research.[1] Over the years, liquid chromatography (LC) using chiral stationary phases (CSPs) has become a very helpful and highly applicable method for preparative resolution of racemates,[2] determination of the enantiomeric purity in quality control,[3] analysis of the stereochemistry of natural compounds,[4,5] monitoring enantiomeric reactions,[6,7] and for pharmacokinetic and environmental studies.[8,9] LC can be considered the method of choice in analytical laboratories due to its high speed, sensitivity, and reproducibility. Moreover, a number of CSPs based on polysaccharide derivatives, macrocyclic antibiotics, small chiral molecules, cyclodextrins, crown ethers, proteins, synthetic polymers, molecularly imprinted polymers, among others, have been developed for successful enantioseparations.[10-18] However, in spite of a large number of different types of CSPs described for enantiomeric separation by LC, there is no universal CSP, i.e., one CSP can only separate a limited number of chiral compounds and, in many cases, the choice of CSP may became a difficult task. Furthermore, even though there are many CSPs commercially available, many chiral compounds remain to be resolved. For this reason, the development of new CSPs continues to be a field of great importance.

Xanthone derivatives comprise an important class of oxygenated heterocycles, many of which possess a broad spectrum of biological/pharmacological activities.[19-23] In recent years, the synthesis of new bioactive xanthone derivatives using different synthetic methodologies, including chiral derivatives of xanthones (CDXs),[24,25] has been an area of great interest in our group.[26-29]

The CDXs, when anchored to a chromatographic support by covalent linkage through a spacer, possess all the necessary attributes to constitute chromatographic selectors.[30] The 3D *quasi*-planar structure and peculiar electronic properties of the xanthone scaffold,[31] associated with a diversity of functional groups and chiral moieties, constitute structural characteristics affording the establishment of different types of interactions, as well as contributing three-dimensionality factors that influence the enantioselectivity. In fact, the xanthone-based chiral selectors may contain aromatic, polar, and bulky nonpolar groups, forming interactions through hydrogen bonding, dipole–dipole, π–π interactions, and steric hindrance effects,[32] similar to what occurs with Pirkle-type CSPs.[33,34]

Herein we report the synthesis and evaluation of six new CSPs, based on xanthone derivatives (Figure 1). These xanthonic chiral stationary phases (XCSPs) are based on a completely different type of small molecule from those commercially available. The enantioresolution capability of the XCSPs was evaluated by LC using different chemical classes of chiral compounds, including drugs. A library of enantiomeric mixtures of CDXs was also evaluated in order to explore the principle of reciprocity,[34-36] as well as the chiral self-recognition phenomenon.[37,38] Two XCSPs possessing enantiomers of the same CDX were prepared to evaluate the resolving power of both enantiomers as selector and to confirm the inversion of the elution order. The greening choice of the chromatographic analysis, by reducing the internal diameter of the LC columns, was also investigated. To the best of our knowledge, this is the first report of the use of CDXs as LC chiral selectors.

2 | MATERIALS AND METHODS

2.1 | Chemicals

Enantiomerically pure reagents, (*S*)-(+)-valinol, (*R*)-(–)-α-phenylglycinol, (*S*)-(+)-α-phenylglycinol, (1*R*,2*S*)-(–)-2-amino-1,2-diphenylethanol, and (*R*,*R*)-(+)-2-amino-1,2-diphenylethanol, were obtained from Sigma-Aldrich (St. Louis, MO). *O*-(Benzotriazol-1-yl)-*N*-*N*-*N*'-*N*'-

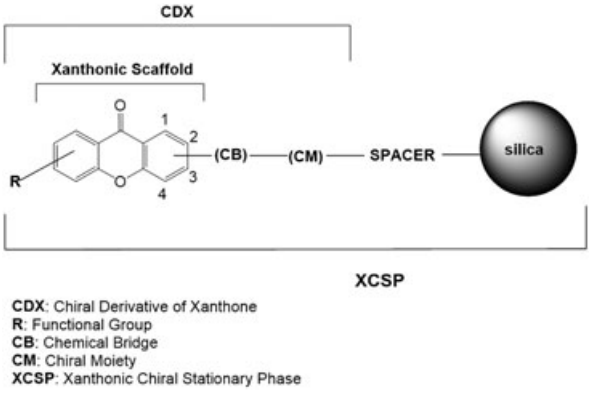


FIGURE 1 Schematic representation of a stationary phase based on chiral derivatives of xanthones

tetramethyluronium tetrafluoroborate (TBTU), 3-(triethoxysilyl)propylisocyanate, and other reagents and solvents were commercially available materials as *pro analysis*, from Sigma-Aldrich or Merck (Darmstadt, Germany), and were used without further purification. Porous spherical silica gel Nucleosil-100Å-5μm from Macherey-Nagel (Dören, Germany) was used as the support material of the six XCSPs. Ethanol (EtOH), 2-propanol (2-PrOH), *n*-hexane (Hex), methanol (MeOH), and acetonitrile (ACN) for high-performance liquid chromatography (HPLC) were purchased from Sigma-Aldrich. Triethylamine (TEA), diethylamine (DEA), and trifluoroacetic acid (TFA) (all p.a. grade) were also obtained from Sigma-Aldrich. Ultrapure water was produced by a Millipore Milli-Q system (Millipore, Bedford, MA). Commercial chiral test compounds used to evaluate the XCSPs were obtained from Sigma-Aldrich or from Merck. Kielcorins and CDXs were synthesized previously in our laboratory according to procedures described elsewhere.[24,25,39]

2.2 | Instrumentation and chromatography

Data melting points are uncorrected and were obtained with a Köfler microscope. Optical rotation measurements were carried out on a Polartronic Universal polarimeter. Infrared spectra were recorded in a KBr microplate (cm^{-1}) in an FTIR spectrometer Nicolet iS10 from Thermo Scientific (Waltham, MA) with Smart OMNI-Transmission accessory (Software OMNIC 8.3). ^{1}H and ^{13}C NMR spectra were taken in DMSO-d_6 at room temperature, on Bruker Avance 300 and 500 instruments (Billerica, MA; 300.13 and 500.13 MHz for ^{1}H and 75.47 and 125.77 MHz for ^{13}C). Chemical shifts are expressed in δ (ppm) values relative to tetramethylsilane (TMS) as an internal reference. Coupling constants are reported in Hz. ^{13}C nuclear magnetic resonance (NMR) assignments were made by 2D HSQC and HMBC experiments (long-range C, H coupling constants were optimized to 7 Hz). HRMS mass spectra were measured on a Bruker Daltonics micrOTOF Mass Spectrometer, recorded as ESI (electrospray) mode. Elemental analyses were conducted on an Elemental Carlo Erba 1108 apparatus. Flash chromatography was carried out using Grace Resolv, silica gel 5 g/25 mL cartridges. Thin-layer chromatography (TLC) was performed using Merck silica gel 60 (GF_{254}) plates, with appropriate mobile phases and detection at 254 and/or 365 nm. The column packing system was an SSI Lab Alliance – Pack in a Box, portable HPLC column packing system model CP constant pressure pump, nonflush, 0.1–24 mL/min, 10.000 psig, with transducer and RS-232 control, SS, 110/220V column packer assembly (50 mL reservoir, column adapter 5/16" 4.6 mm) with Quick-Set pump control software. A Shandon column packing pump was also used. The HPLC system used consisted of a JASCO (Tokyo, Japan) model 880-PU pump, equipped with a Rheodyne 7125 injector fitted with a 20 μL loop, a JASCO model 880-30 solvent mixer, a 875-UV Intelligent U*V*/Vis Detector, and a QR-2090 Plus Chiral Polarimeter Detector. Data acquisition was performed using Chromatography Station for Windows, v. 1.7 DLL. Two more HPLC systems were employed: a system consisted of one Shimadzu (Kyoto, Japan) LC-10ATVP pump, equipped with a Rheodyne 7725i injector fitted with a 100 μL loop, an SPD-10AV U*V*/Vis detector with an SCL-10Avp interface, controlled using Shimadzu CLASS-VP software; and a system consisted of two Shimadzu LC 10-ADvp pumps, an FCV-10AL solvent selector valve, an automatic injector SIL10-Advp, an SPD-10AV U*V*/Vis detector with an SCL-10Avp interface. A CD detector model JASCO CD 2095 Plus was also used. Data acquisition was performed using Shimadzu LC SOLUTIONS software. Analyses were performed at room temperature in an isocratic mode with a multimodal operation. The stock solutions of chiral test compounds were prepared by dissolution in EtOH at a concentration of 1 mg/mL and further diluted in the same solvent. Working solutions of mixtures of enantiomeric pure compounds were prepared mixing equal aliquots of each enantiomer. The mobile phase compositions were Hex and EtOH or 2-PrOH as a modifier, with or without TFA, TEA, or DEA, in normal-phase conditions. The evaluations in polar organic mode were carried out using MeOH, EtOH, ACN, or mixtures of these solvents. MeOH and water or ACN and water, with or without TFA or TEA, were used in reversed-phase conditions. The mobile phases were prepared in a volume/volume relation and degassed in an ultrasonic bath for 15 min before use. The flow rate used was 0.5 mL/min (columns with 150 x 4.6 mm i.d.) or 0.2 mL/min (columns with 150 x 2.1 mm i.d.) and the chromatograms were monitored by UV detection at a wavelength of 254 nm and/or polarimeter detection and/or CD detection. Sample injections (20 μL) were carried out in triplicate. The dead time (t_0) was considered to be equal to the peak of the solvent front and was taken from each particular run. The chromatographic parameters were established as follows: the retention factor (k) was determined as $[k = (t_R – t_0) / t_0]$ where t_R is the retention time for each enantiomer; the enantioselectivity factor (α) was calculated as $[\alpha = k_2 / k_1]$; the resolution (R_s) was determined as $[R_s = 1.18 \times (t_{R2} - t_{R1}) / (W_{1\ 0.5} + W_{2\ 0.5})]$ where t_{R1}, $W_{1\ 0.5}$ and t_{R2}, $W_{2\ 0.5}$ are the retention times and the peak width at half height of the first and second eluted enantiomer, respectively. The chiral selector loadings ($\mu mol/m^2$) were determined from the elemental analyses of carbon as follows: selector loading $= (10^6 \times \%C) / S\ [(100 \times n \times 12) – (\%C \times Mw)]$ where %C is the elemental analyses of carbon, S is the surface area, n is the number of carbon atoms in the modified chain, and Mw is the relative molecular mass.

2.3 | Development of XCSPs 1-6

XCSPs 1–6 were prepared following similar synthetic procedures according to Schemes 1 and 2.

2.4 | General procedure for the synthesis of CDXs 7–11 and 18

CDXs **7–11** and **18** were synthesized and characterized according to the described procedure.[24,25]

2.5 | General procedure for the synthesis of silylated derivative of CDXs 12–16 and 19

The appropriate CDX (2.12 mmol) was dissolved in anhydrous toluene (60 mL), and triethylamine (303 μL, 2.18 mmol) and 3-(triethoxysilyl)propylisocyanate (550 μL, 2.22 mmol) were added. The mixture was refluxed for 76 h. The solvent was evaporated under reduced pressure and the crude product was purified by flash chromatography (Grace Resolv, silica gel 5 g / 25 mL, *n*-hexane/ethyl acetate in gradient) and by crystallization from ethyl acetate / *n*-hexane affording the silylated derivative.

2.5.1 | (*S*)-2-(6-methoxyxanthone-2-carboxamido)-3-methylbutyl [3-(triethoxysilyl) propyl] carbamate (12)

Compound **12** was obtained as white crystals (yield: 91%); m.p.: 144–146°C (ethyl acetate / *n*-hexane); $[\alpha]_D^{20}$ = +15.8° (7.58 mg/mL, dichloromethane); ^{1}H NMR, ^{13}C NMR, IR, and HRMS data are shown in the Supplementary Material.

2.5.2 | (*R*)-2-(6-methoxyxanthone-2-carboxamido)-2-phenylethyl [3-(triethoxysilyl) propyl] carbamate (13)

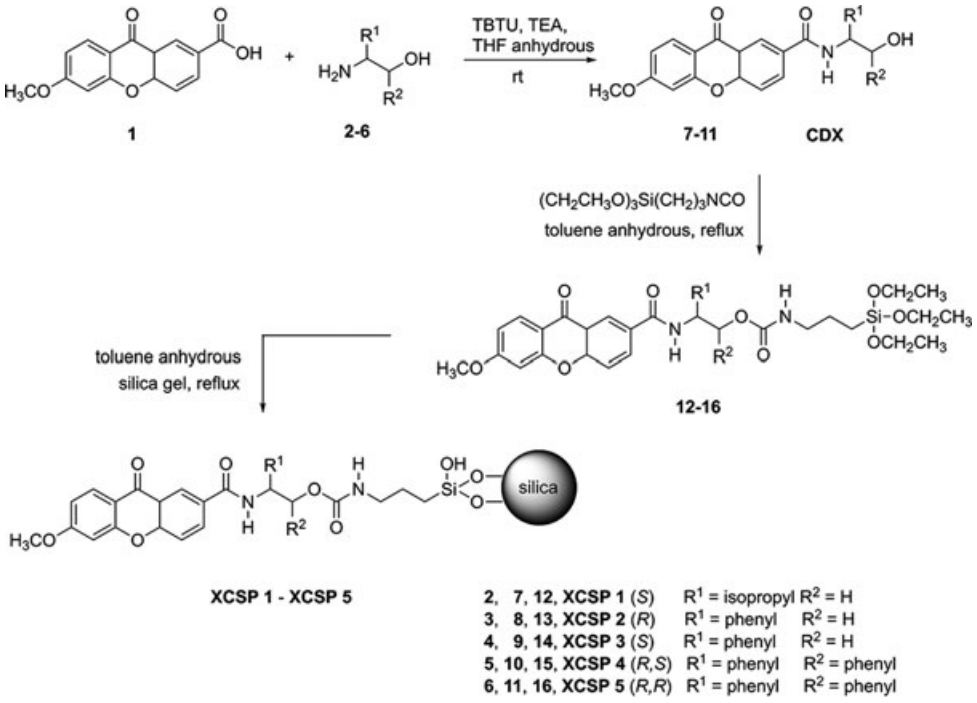
Compound **13** was obtained as white crystals (yield: 87%); m.p.: 96–98°C (ethyl acetate); $[\alpha]_D^{20}$ = +44.3° (7.5 mg/mL, ethyl acetate); ^{1}H NMR, ^{13}C NMR, IR, and HRMS data are shown in the Supplementary Material.

2.5.3 | (*S*)-2-(6-methoxy-9-oxo-9*H*-xanthene-2-carboxamido)-2-phenylethyl [3-(triethoxy-silyl) propyl] carbamate (14)

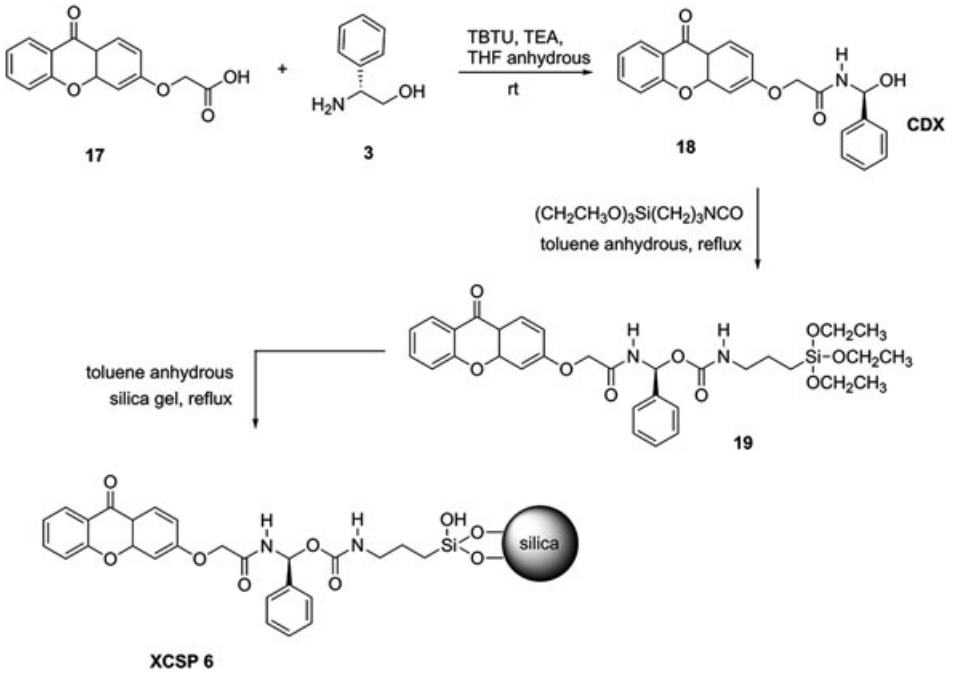
Compound **14** was obtained as white crystals (yield: 74%); m.p.: 96–98°C (ethyl acetate / *n*-hexane); $[\alpha]_D^{20}$ = –44.3° (7.5 mg/mL, ethyl acetate); ^{1}H NMR, ^{13}C NMR, IR, and HRMS data are shown in the Supplementary Material.

2.5.4 | (1*S*,2*R*)-2-(6-methoxy-9-oxo-9*H*-xanthene-2-carboxamido)-1,2-diphenylethyl [3-(triethoxysilyl)propyl] carbamate (15)

Compound **15** was obtained as white crystals (yield: 63%); m.p.: 219–220°C (ethyl acetate / *n*-hexane); $[\alpha]_D^{20}$ = –150.0° (0.20 mg/mL, dichloromethane); ^{1}H NMR, ^{13}C NMR, IR, and HRMS data are shown in the Supplementary Material.



SCHEME 1 Synthesis of XCSPs 1–5



SCHEME 2 Synthesis of XCSP 6

2.5.5 | (1*R*,2*R*)-2-(6-methoxy-9-oxo-9*H*-xanthene-2-carboxamido)-1,2-diphenylethyl [3-(triethoxysilyl)propyl] carbamate (16)

Compound **16** was obtained as white crystals (yield: 51%); m.p.: 155–158°C (ethyl acetate / *n*-hexane); $[\alpha]_D^{20}$ = +22.0° (0.5 mg/mL, chloroform); ^{1}H NMR, ^{13}C NMR, IR, and HRMS data are shown in the Supplementary Material.

2.5.6 | (*R*)-2-[2-(3-oxyxanthone)acetamide]-2-phenylethyl [3-(triethoxysilyl)propyl] carbamate (19)

Compound **19** was obtained as light pink crystals (yield: 76%); m.p.: 108–110°C (ethyl acetate / *n*-hexane); $[\alpha]_D^{20}$ = –19.2° (3.1 mg/mL, ethyl acetate); ^{1}H NMR, ^{13}C NMR, IR, and HRMS data are shown in the Supplementary Material.

2.6 | General procedure of covalent linkage of silylated derivative of CDXs onto the silica gel and HPLC column packing

Anhydrous toluene (30 mL) was added to silica gel Nucleosil (2890 mg, 100 Å-5µm, Macherey-Nagel), previously dried in a desiccator under vacuum and with phosphorus pentoxide for 24 h. Following this, the appropriate silylated derivative of CDX (1.42 mmol) dissolved in the same solvent (30 mL) was added. The mixture was gently stirred at reflux for 76 h. The solid was then filtered and washed successively with (100 mL) toluene, methanol, acetone, ethyl acetate, dichloromethane, and *n*-hexane. The bonded phase was dried in a desiccator under vacuum for 24 h, yielding the **XCSP**. Each new CSP (2.30 g) was mixed with Hex:2-PrOH (50:50 *v/v*) (50 mL), and then sonicated for 3 min. The suspension was poured into the chamber of a column packer and was packed into an empty HPLC column (150 x 4.6 mm i.d.) with Hex:2-PrOH (90:10 *v/v*) as packing solvent, under a pressure no more than 7500 psi.

3 | RESULTS AND DISCUSSION

Six new CSPs, based on xanthone derivatives (XCSP **1**–**6**), were developed according to Schemes 1 and 2. XCSPs **1**–**5** were prepared using the xanthone derivative 6-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid (**1**) (Scheme 1), synthesized via Ullmann reaction with the formation of diaryl ether intermediate according to the previously described procedure,[25] as a chemical substrate. For **XCSP 6**, the xanthone derivative 2-[(9-oxo-9*H*-xanthen-3-yl)oxy]acetic acid (**17**), synthesized via benzophenone intermediate,[24] was used as a substrate (Scheme 2). The CDXs were synthesized by coupling these two suitable functionalized xanthone derivatives with commercially available enantiomerically pure building blocks.[24,25] The activation of the carboxylic acid group of the xanthone scaffold was carried out with TBTU. Accordingly, xanthone **1** was coupled with the enantiomerically pure amino alcohols (*S*)-(+)-valinol (**2**), (*R*)-(–)-α-phenylglycinol (**3**), (*S*)-(+)-α-

phenylglycinol (**4**), (1*R*,2*S*)-(–)-2-amino-1,2-diphenylethanol (**5**), and (1*R*,2*R*)-(+)-2-amino-1,2-diphenylethanol (**6**), to afford CDXs (**7**–**11**), respectively. CDX **18** was synthesized by coupling xanthone **17** with the amino alcohol (*R*)-(–)-α-phenylglycinol (**3**). Our selection included enantiomerically pure building blocks with no tendency towards racemization or enantiomeric interconversion, having a primary amine as a reactive group for the coupling reaction with the carboxyxanthone derivative and a hydroxyl group to further linkage to the spacer.

The amino alcohols valinol and phenylglycinol were chosen to obtain XCSPs, similar to valine-derived[40] and phenylglycine-derived[41] CSPs developed by Pirkle et al. In fact, these amino acid-derived CSPs were shown to be useful to separate a broad spectrum of racemates on both analytical and preparative scales.[33,34] Recognizing the crucial role of π–π interactions between each enantiomer and this type of CSPs for chiral recognition, the amino alcohol 2-amino-1,2-diphenylethanol was also selected in order to increase these interaction types, as well as to obtain a more rigid structure (cleft type selector), similar to the most employed and successful Pirkle-type CSP, i.e., Whelk-O1 CSP.[42]

The strategy used for binding the CDXs (chiral selectors) to the chromatographic support was through the synthesis of derivatives that allowed the covalent linkage to the silica, specifically silylated derivatives.[43,44] Consequently, all CDXs reacted with 3-(triethoxysilyl) propylisocyanate giving the corresponding silylated derivatives. Then, the silylated derivatives of all the CDXs were anchored to silica gel (Nucleosil-100-5) giving the correspondent XCSPs **1**–**6** (Schemes 1 and 2). This synthetic methodology involves only a few steps and the reactions provided good yields.

The chiral selector loadings for all the XCSPs were calculated from the elemental analyses of carbon (Table 1).

Finally, all the XCSPs were packed in a column with 150 x 4.6 mm i.d. using Hex:2-PrOH (90:10 *v/v*). XCSP **2** and XCSP **5** were also packed in a column with 150 x 2.1 mm i.d.

The LC enantioselective resolution capabilities of **XCSPs 1-6** were evaluated with several enantiomeric mixtures of structurally different chiral compounds, including some types of drugs. Furthermore, a library of CDXs was also chosen that incorporates the enantiomeric precursors to XCSPs **1**–**3** and XCSP **5** and analogs.

The enantioseparation by XCSPs **1**–**6** was evaluated under normal, polar organic, and reversed-phase elution conditions. Table 2 shows the overall best results.

The results in Table 2 show that the best enantioselectivity was achieved mainly under normal-phase elution conditions using EtOH as a modifier.

It was found that **XCSP 1** showed enantioselective capabilities only for enantiomeric mixtures of CDXs. Concerning, for example, the enantiomeric mixtures of CDXs **23**, **24,** and **25**, good enantioselectivity (α = 1.14, 1.15, and 1.15) and resolution (R_S = 2.16, 2.24, and 2.18) were respectively obtained, with Hex:EtOH (90:10 *v/v*) as mobile phase.

XCSP 2 was found to have more versatility, showing enantioselectivity not only for the enantiomeric mixtures of CDXs (α ranging from 1.04 to 1.32) but also for the chiral compounds 1-(9-anthryl)-2,2,2-trifluoroethanol (**19**) (α = 1.07), *N*-[1-(1-naphthyl)ethyl]-3,5-dinitrobenzamide (**20**) (α = 1.31), and bi-naphthol (**22**) (α = 1.08). Unsurprisingly, in general, **XCSP 2** showed the best performance and versatility, when taking into account that: 1) **XCSP 2** is similar to the successfully commercially available Phenylglycine CSP[41]; 2) the selector of this CSP has another aromatic group in addition to the xanthone scaffold, both of which were essential for chiral recognition by this type of CSPs through π–π interactions between enantiomers and the CSP; and 3) an excellent enantioselectivity and resolution were achieved for the enantiomeric mixture of chiral selector of **XCSP 2** using macrocyclic antibiotics,[7] (*S*,*S*)-Whelk-O1,[32] and polysaccharide-based CSPs.[6]

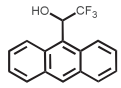
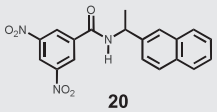
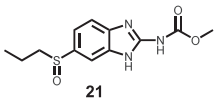
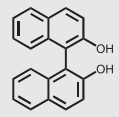
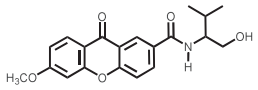
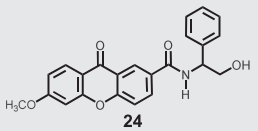
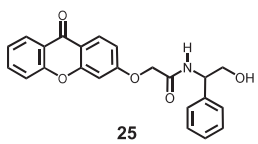
As expected, **XCSP 3** proved to have similar enantioselectivity capabilities to **XCSP 2**. Actually, these two XCSPs have the same chiral selector but with opposite configurations. However, the **XCSP 3** generally presented higher retention for the enantiomers, which can be explained by its slightly higher selector loading (1.31 μmol/m^2) when compared with **XCSP 2** (0.90 μmol/m^2).

Regarding **XCSP 4**, the additional aromatic groups of the chiral selector of this CSP did not contribute to its potential enantiorecognition. In fact, the steric hindrance of the bulky and rigid structural elements may be responsible for non-

TABLE 1 Elemental analysis and chiral selector loadings for XCSPs **1–6**

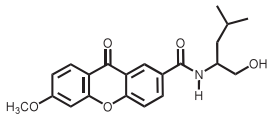
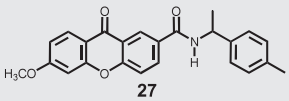
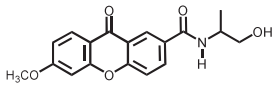
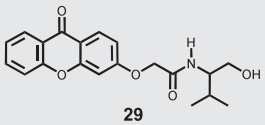
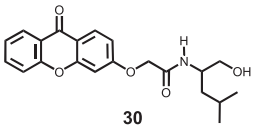
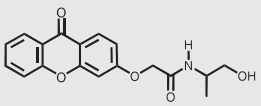
Elemental analysis	XCSP 1	XCSP 2	XCSP 3	XCSP 4	XCSP 5	XCSP 6
% C	7.09	8.66	11.87	8.36	10.11	9.87
% N	1.01	0.82	1.14	1.10	0.85	0.89
% H	2.60	1.84	1.65	1.59	0.97	1.76
Selector loading (based on C) μmol/m^2	0.80	0.90	1.31	0.70	0.85	1.04

TABLE 2 Chromatographic resolution of enantiomeric mixtures on XCSPs **1-6**

Enantiomeric mixture	XCSP	Mobile phase (*v/v*)	k_1	α	R_s
 **19** 1-(9-anthryl)-2,2,2-trifluoroethanol	XCSP 2	Hex/2-PrOH: 90/10	1.81	1.07	< 1.00
 **20** *N*-[1-(1-naphtyl)ethyl]-3,5-dinitrobenzamide	XCSP 2	Hex/EtOH: 80/20	2.90	1.17	1.95
		Hex/2-PrOH: 70/30	2.33	1.31	1.93
	XCSP 3	Hex/EtOH: 80/20	3.07	1.17	1.95
	XCSP 6	Hex/EtOH: 80/20	3.35	1.22	2.96
 **21** albendazole sulfoxide	XCSP 4	ACN/H_2O: 10/90	10.29	1.07	< 1.00
		MeOH/H_2O: 15/85	8.97	1.06	< 1.00
		EtOH/H_2O: 15/85	4.61	1.07	< 1.00
 **22** bi-naphthol	XCSP 2	Hex/EtOH: 90/10 with 0.01% TFA	3.96	1.08	< 1.00
 **23** *N*-(1-hydroxy-3-methylbutan-2-yl)-6-methoxy-9-oxo-9*H*-xanthene-2-carboxamide	XCSP 1	Hex/EtOH: 90/10	9.76	1.14	2.16
		Hex/EtOH: 80/20	5.27	1.13	1.49
		Hex/2-PrOH: 70/30	2.36	1.18	< 1.00
	XCSP 2	Hex/EtOH: 80/20	6.12	1.12	1.33
		Hex/EtOH: 85/15	9.59	1.13	1.57
		Hex/2-PrOH: 70/30	3.48	1.15	< 1.00
	XCSP 3	Hex/EtOH: 80/20	6.72	1.11	1.30
	XCSP 6	Hex/EtOH: 80/20	6.01	1.06	< 1.00
 **24** *N*-(2-hydroxy-1-phenylethyl)-6-methoxy-9-oxo-9*H*-xanthene-2-carboxamide	XCSP 1	Hex/EtOH: 90/10	18.79	1.15	2.24
		Hex/EtOH: 80/20	7.53	1.13	1.68
		Hex/2-PrOH: 70/30	4.35	1.16	< 1.00
	XCSP 2	Hex/EtOH: 80/20	10.99	1.15	1.98
		Hex/EtOH: 85/15	17.89	1.15	1.60
		Hex/2-PrOH: 70/30	6.82	1.32	< 1.00
		EtOH	0.96	1.10	< 1.00
		ACN/MeOH: 50/50	0.57	1.10	< 1.00
		ACN/MeOH: 5:95	0.96	1.08	< 1.00
	XCSP 3	Hex/EtOH: 80/20	10.67	1.12	1.95
	XCSP 6	Hex/EtOH: 80/20	12.35	1.05	< 1.00
	XCSP 5	Hex/EtOH: 80/20	4.45	1.10	< 1.00
 **25** *N*-(2-hydroxy-1-phenylethyl)-2-((9-oxo-9*H*-xanthen-3-yl)oxy) acetamide	XCSP 1	Hex/EtOH: 90/10	16.63	1.05	< 1.00
		Hex/EtOH: 80/20	6.94	1.04	< 1.00

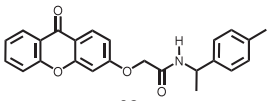
(*Continues*)

TABLE 2 (Continued)

Enantiomeric mixture	XCSP	Mobile phase (*v/v*)	k_1	α	R_s
 **26** *N*-(1-hydroxy-4-methylpentan-2-yl)-6-methoxy-9-oxo-9*H*-xanthene-2-carboxamide	XCSP 1	Hex/EtOH: 90/10	7.59	1.15	2.18
		Hex/EtOH: 80/20	3.55	1.13	1.38
		Hex/2-PrOH: 70/30	1.86	1.18	< 1.00
	XCSP 2	Hex/EtOH: 80/20	4.85	1.11	1.21
		Hex/EtOH: 85/15	6.69	1.11	1.40
		Hex/2-PrOH: 70/30	2.63	1.16	< 1.00
	XCSP 3	Hex/EtOH: 80/20	6.42	1.11	1.10
	XCSP 6	Hex/EtOH: 80/20	4.79	1.08	1.06
 **27** 6-methoxy-9-oxo-*N*-(1-(*p*-tolyl)ethyl)-9*H*-xanthene-2-carboxamide	XCSP 1	Hex/EtOH: 90/10	5.88	1.07	< 1.00
		Hex/EtOH: 80/20	3.66	1.06	< 1.00
		Hex/2-PrOH: 70/30	1.82	1.08	< 1.00
	XCSP 2	Hex/EtOH: 80/20	4.92	1.09	1.14
		Hex/EtOH: 85/15	7.07	1.10	1.38
		Hex/2-PrOH: 70/30	2.92	1.12	< 1.00
		EtOH	0.78	1.08	< 1.00
	XCSP 3	Hex/EtOH: 80/20	6.72	1.10	1.15
	XCSP 6	Hex/EtOH: 80/20	4.99	1.08	1.11
 **28** *N*-(1-hydroxypropan-2-yl)-6-methoxy-9-oxo-9*H*-xanthene-2-carboxamide	XCSP 1	Hex/EtOH: 90/10	18.08	1.06	< 1.00
		Hex/EtOH: 80/20	7.06	1.05	< 1.00
	XCSP 2	Hex/EtOH: 80/20	9.59	1.04	< 1.00
		Hex/EtOH: 85/15	13.52	1.04	< 1.00
	XCSP 3	Hex/EtOH: 80/20	12.54	1.03	< 1.00
 **29** *N*-(1-hydroxy-3-methylbutan-2-yl)-2-((9-oxo-9*H*-xanthen-3-yl)oxy)acetamide	XCSP 1	Hex/EtOH: 90/10	10.07	1.05	< 1.00
	XCSP 3	Hex/EtOH: 80/20	7.53	1.05	< 1.00
 **30** *N*-(1-hydroxy-4-methylpentan-2-yl)-2-((9-oxo-9*H*-xanthen-3-yl)oxy)acetamide	XCSP 1	Hex/EtOH: 90/10	8.40	1.08	< 1.00
		Hex/EtOH: 80/20	3.43	1.06	< 1.00
	XCSP 2	Hex/EtOH: 80/20	4.82	1.07	< 1.00
	XCSP 3	Hex/EtOH: 80/20	6.24	1.07	< 1.00
 **31** *N*-(1-hydroxypropan-2-yl)-2-((9-oxo-9*H*-xanthen-3-yl)oxy) acetamide	XCSP 1	Hex/EtOH: 90/10	18.44	1.04	< 1.00
	XCSP 3	Hex/EtOH: 80/20	12.35	1.02	< 1.00

(*Continues*)

TABLE 2 (Continued)

Enantiomeric mixture	XCSP	Mobile phase (*v/v*)	k_1	α	R_s
 **32** 2-((9-oxo-9*H*-xanthen-3-yl)oxy)-*N*-(1-(*p*-tolyl)ethyl)acetamide	XCSP 1	Hex/EtOH: 90/10	5.89	1.04	< 1.00
	XCSP 2	Hex/EtOH: 80/20	4.66	1.06	< 1.00
	XCSP 3	Hex/EtOH: 80/20	6.33	1.07	< 1.00
	XCSP 6	Hex/EtOH: 80/20	4.79	1.04	< 1.00

enantioselective interactions with the chiral compounds. Interestingly, it was found that **XCSP 4** presented some enantioselectivity (α ~1.07) only for the chiral compound albendazole sulfoxide (**21**) under reversed-phase mode. This result indicates that the chiral recognition mechanism of **XCSP 4** is different from other XCSPs.

Interestingly, although the chiral selector of **XCSP 5** differs only in the configuration of one stereogenic center compared with that of **XCSP 4**, the chromatographic behavior of **XCSP 5** was similar to that of **XCSPs 1**–**3**. However, **XCSP 5** revealed poor enantioresolution capabilities for the tested compounds.

On the other hand, the best chromatographic value was achieved with **XCSP 6**, with α = 1.22 and R_S = 2.96 for *N*-[1-(1-naphthyl)ethyl]-3,5-dinitrobenzamide (**7**) with Hex:EtOH (80:20 *v/v*) as a mobile phase. The selector of **XCSP 6** comprises the same chiral moiety as that of **XCSP 2,** but with different substituents on the xanthone scaffold. Nevertheless, in general, less discrimination ability was observed compared to **XCSP 1** and **XCSP 2** (Table 2).

It is important to highlight that the enantioselectivity for the enantiomeric mixtures of CDXs, tested on XCSPs, was mainly achieved using a low percentage of the modifier (5–30%). However, with those elution conditions high retention factors were observed (k_1 ranging from 1.82 to 18.79). To overcome this situation, the polarity of the mobile phase was increased to consequently decrease the retention time, but concomitantly the enantioselectivity also decreased. Considering evaluation on the polar organic and reversed-phase elution conditions, the chromatographic results were not satisfactory. In general, short retention times were observed with absence or very poor resolutions.

Characteristic chromatograms obtained during the evaluation of the enantioselective resolution capabilities of the XCSPs are shown in Figure 2.

Considering the overall chromatographic results, it was also found that all the XCSPs (except for **XCSP 4**) provided enantioselectivity for the enantiomeric mixtures of CDXs. Accordingly, for example, from the 12 enantiomeric mixtures of CDXs tested, 10 of them (83%) showed enantioselectivity on **XCSP 1** and 9 of them (75%) on **XCSP-2**. This specificity for the CDXs is very important, since previous studies, using commercial CSPs, namely, (*S*,*S*)-Whelk-O1 and *L*-Phenylglycine CSPs, demonstrated less versatility for this important class of chiral compounds.[32] In fact, the enantiomeric mixture of CDX **27** was the only pair resolved on *L*-Phenylglycine CSP, while (*S*,*S*)-Whelk-O1 CSP showed enantioselectivity only for CDXs possessing an aromatic moiety bonded to the stereogenic center.

The chromatographic results also suggested that XCSPs can be developed by applying the concept of reciprocity.[35,36] As an example, the best resolution achieved on **XCSP 1** was with the enantiomeric mixture of CDX **24**, with R_S = 2.24 using Hex:EtOH (90:10 *v/v*) as a mobile phase. Therefore, the immobilized enantiomers of CDX **24** (**XCSP 2** and **XCSP 3**), using similar interactions, were able to distinguish between the enantiomeric precursors of **XCSP 1**, i.e., CDX **23** (Table 2). For example, good enantioselectivity (α = 1.13) and resolution (R_S = 1.57) were obtained on **XCSP-2** with Hex:EtOH (85:15 *v/v*).

It was also found that the chiral self-recognition phenomenon occurs with the XCSPs. As, for example, **XCSP 1** and **XCSP 2** can effectively separate the enantiomeric mixtures of their precursors, namely, CDX **23** and CDX **24**, with R_S = 2.16 and 1.98, respectively. In both cases, the enantiomer that forms the homochiral diastereisomeric complex with the CSP was more retained. Similar results were described in the literature using Pirkle-type CSPs.[33,37,38]

In order to confirm the inversion of elution order of XCSPs, the two enantiomers of CDX **24** (compounds **8** and **9**) were also eluted separately on **XCSP 2**, and on the opposite enantiomeric XCSP (**XCSP 3**). The chromatograms are shown in Figure 3.

As shown in Figure 3, with these new CSPs, the elution order of the enantiomers of CDX **24** is inverted by using the same type of CSP. This is an important advantage for enantiomeric purity determinations or preparative separations, giving the possibility to elute before the trace enantiomer or the desired enantiomer, respectively.

Additionally, concerning ecological, practical and economic reasons, the greening of the chromatographic

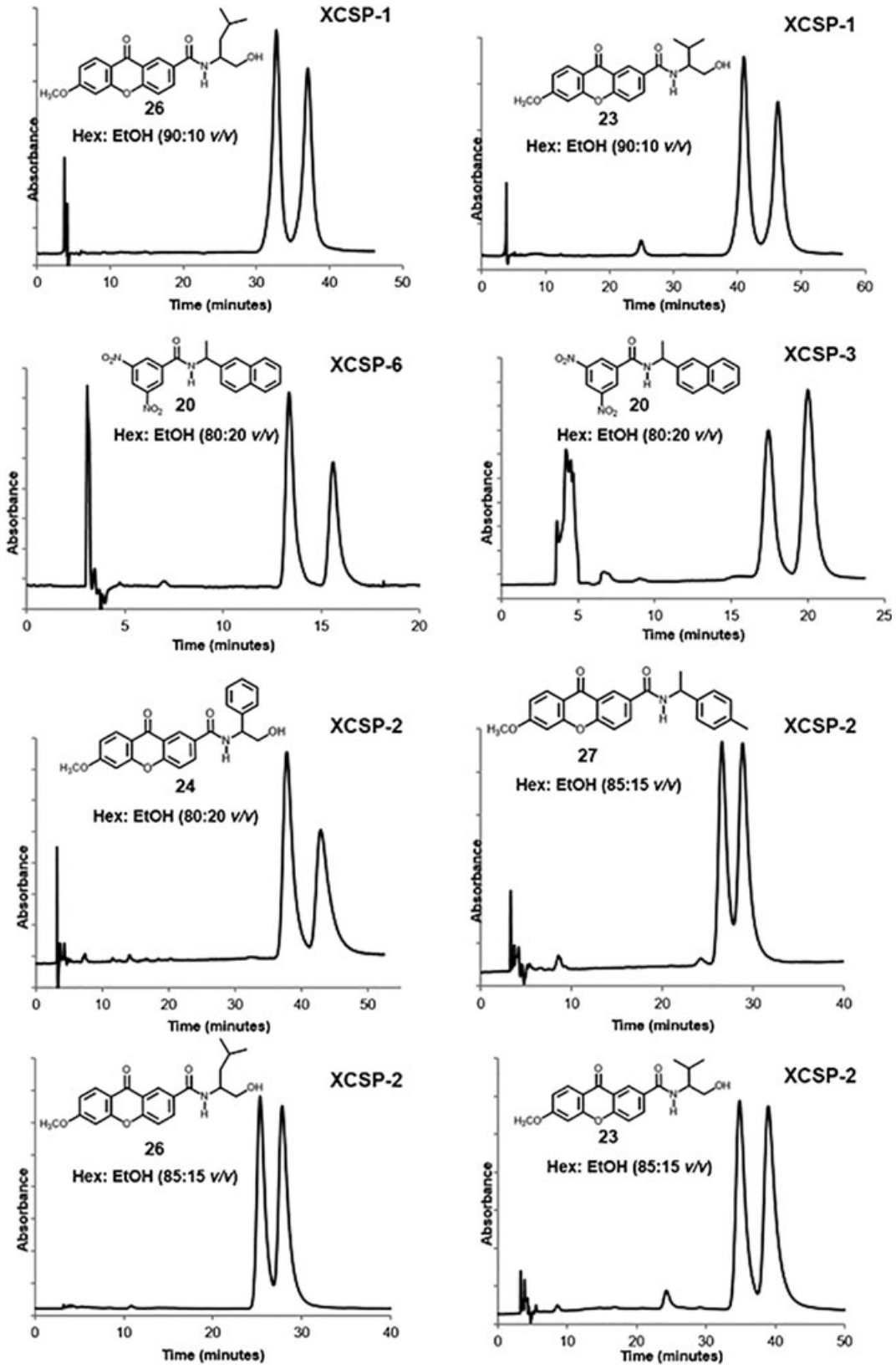


FIGURE 2 Chromatograms of enantiomeric mixtures of compounds 20, 23, 24, 26, and 27 on XCSPs. Flow rate, 0.5 mL/min; detection, 254 nm

analysis[45,46] was investigated by reducing the internal diameter of the LC columns from 4.6 to 2.1 mm i.d. Accordingly, the best obtained XCSPs, **XCSP 2,** and **XCSP 5** were packed in a column with 150 x 2.1 mm i.d. and their enantiomeric performance was evaluated using a flow rate of 0.2 mL/min. The major advantage

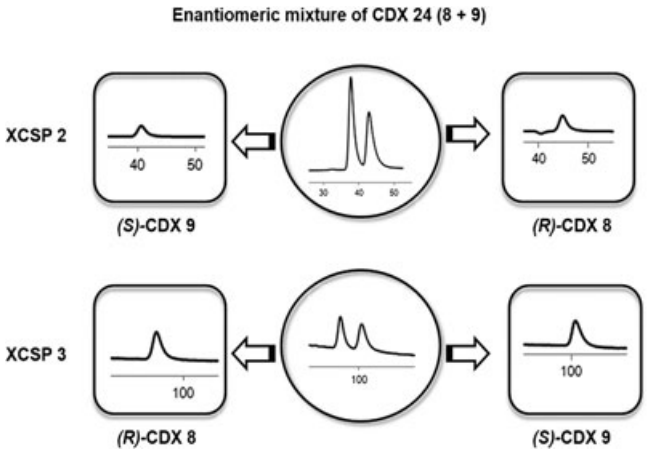


FIGURE 3 Schematic chromatograms of enantiomeric mixture of CDX 24, (S)-CDX 9, and (R)-CDX 8 on XCSP 2 and XCSP 3. Mobile phase, Hex:EtOH (80:20 *v/v*); flow rate, 0.5 mL/min; detection, 254 nm

was the decrease of the solvent consumption without compromising the enantioseparation.

4 | CONCLUSION

Six new xanthonic chiral stationary phases (XCSPs) were developed using a well-established synthetic methodology that involves only a few steps of reactions with good yields. The XCSPs were covalently bonded to fully porous silica, which guarantee long-lasting columns, and show high stability, versatility in the selection of the mobile phase composition, and reproducibility. It was found that, in general, they show enantioselectivity for CDXs and other chiral compounds, such as 1-(9-anthryl)-2,2,2-trifluoroethanol, *N*-[1-(1-naphthyl)ethyl]-3,5-dinitrobenzamide, binaphthol, and albendazole sulfoxide. Moreover, this type of chiral stationary phase gives the possibility to invert the elution order (by using the opposite enantiomer as chiral selector). Therefore, this work represents a breakthrough that is extremely interesting not only in the field of chiral LC but also as new chemical tools for further studies of chiral molecular recognition.

ACKNOWLEDGMENTS

This research was partially supported by the Structured Program of R&D&I INNOVMAR – Innovation and Sustainability in the Management and Exploitation of Marine Resources (reference NORTE-01-0145-FEDER-000035, Research Line NOVELMAR), funded by the Northern Regional Operational Programme (NORTE2020) through the European Regional Development Fund (ERDF) and by Foundation for Science and Technology (FCT), under the project PTDC/MAR-BIO/4694/2014 (reference POCI-01-0145-FEDER-016790) supported through national funds provided by FCT – Foundation for Science and Technology and European Regional Development Fund (ERDF) through the COMPETE – Programa Operacional Factores de Competitividade (POFC) programme and the Project 3599 – Promover a Produção Científica e Desenvolvimento Tecnológico e a Constituição de Redes Temáticas (3599-PPCDT), COXANT–CESPU-2016, QOPNA research project (FCT UID/QUI/00062/2013) and Portuguese NMR network. Y.Z. Phyo thanks the Erasmus Mundus Action 2 (Lotus Plus project) for a PhD's scholarship. We thank also Coordenação de Aperfeiçoamento Pessoal de Nível Superior (CAPES) and FCT for the joint international Project 214/08.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Fernandes C, Tiritan ME, Cravo S, et al. New chiral stationary phases based on xanthone derivatives for liquid chromatography. *Chirality.* 2017;1-13. https://doi.org/10.1002/chir.22706