Comparative analysis of enantioselective separation of novel PPAR agonists by HPLC on cellulose- and amylose-based chiral stationary phases

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Abstract
Chiral chromatographic resolution of a series of dual PPARα/γ agonists was performed on the commercially available Chiralcel OD and Chiralpak AD columns in normal phase mode using a mobile phase consisting of a mixture of *n*-hexane, 2-propanol and trifluoroacetic acid at constant volume ratio. The selected CSPs resolved most compounds as underivatized acids without requiring time consuming analysis. Both electronic features and steric hindrance affected chiral interactions between the analytes and the CSPs. Additional information on the chiral recognition mechanism was deduced from the chromatographic behaviour of some selected methyl esters.

Keywords: Chiral recognition mechanism, enantiomeric resolution, α-substituted acetic acids, polysaccharide-based chiral stationary phases, PPAR agonists

Introduction

Chiral chromatographic techniques have been used for the analytical and preparative separation of enantiomers for many years. Enantioselective HPLC, based on the formation of transient diastereomeric complexes between a chiral selector and a chiral selectand, is today the most widely used methodology for the separation of optical isomers both in academia and industry.[1] In particular, this technique is largely employed for chiral drugs whose resolution is an issue of the greatest importance due to the strict relationship often existing between absolute configuration and pharmacological and toxicological effects.

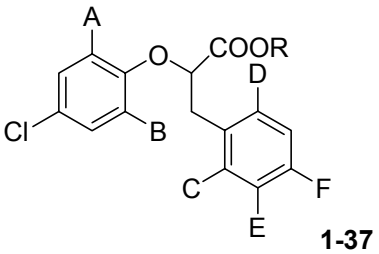
Our interest for stereochemistry and biological activity started more than two decades ago and recently focused on the synthesis and pharmacological evaluation of new PPAR agonists. Peroxisome Proliferator-Activated Receptors (PPARs) belong to the nuclear receptors superfamily,[2,3] and they are transcription factors activated by specific ligands, which are usually

lipophilic small molecules.[4] Binding of these ligands results in conformational changes of the receptors that facilitate their interaction with coactivator proteins in the nucleus.[3,5,6] The resulting protein complexes activate the transcription of specific target genes, resulting in the induction of intracellular signalling cascades that mediate the physiological effects of the ligands.[7,8] So far, three PPAR subtypes have been described in mammals: PPARα, PPARγ, and PPARδ. All of these are targets for treatment of the metabolic syndrome, a cluster of risk factors for cardiovascular disease and diabetes including obesity, atherogenic dyslipidemia, hypertension, insulin resistance, and elevated fasting blood glucose.[3] Because of the well-documented therapeutic actions of their synthetic agonists, PPARs have been the focus of intense academic and pharmaceutical research since their discovery in the early 1990s.

Fibrates are a class of drugs that reduce serum triglycerides and increase HDL cholesterol through activation of PPARα which is expressed predominantly in the liver.[9-11]

Very recently, we reported the effects on PPARs of a series of 2-aryloxy-3-aryl-propanoic acids which can be considered chiral analogs of clofibric acid, the active metabolite of the hypolipidemic drug clofibrate. Some of these derivatives showed an agonist activity strictly related to the absolute configuration,[12] which prompted us to carefully investigate their optical purity. The enantiomeric excesses of these stereoisomers, therefore, were determined by HPLC on chiral stationary phase (CSP) and, for this purpose, we chose the tris(3,5-dimethylphenylcarbamate) of cellulose and amylose (Chiralcel OD and Chiralpak AD, respectively) because of their peculiar enantioselective properties and their capability to separate a wide spectrum of stereochemically interesting organic molecules and chiral drugs.[13-16] The study of the chromatographic behavior, however, was extended to all compounds of the series; due to our continuous research on the synthesis of new chiral analogs of clofibric acid as PPAR agonists,[17-18] we were interested, in fact, in a systematic investigation of the chromatographic enantioseparation of this class of drugs in relation with their structure and substitution pattern with the aim to gain useful information for method development and optimization.

In this study, therefore, the direct HPLC enantiomeric separation of a series of PPAR ligands (Figure 1) has been comparatively evaluated using the above-mentioned cellulose- and amylose-based chiral stationary phases and a mobile phase consisting of a mixture of *n*-hexane, 2-propanol and trifluoroacetic acid at constant volume ratio. The use of trifluoroacetic acid as an additive into the mobile phase allows to perform the chiral resolution of numerous acid analytes[19] without any derivatization which represents, on the contrary, a prerequisite for other CSPs.



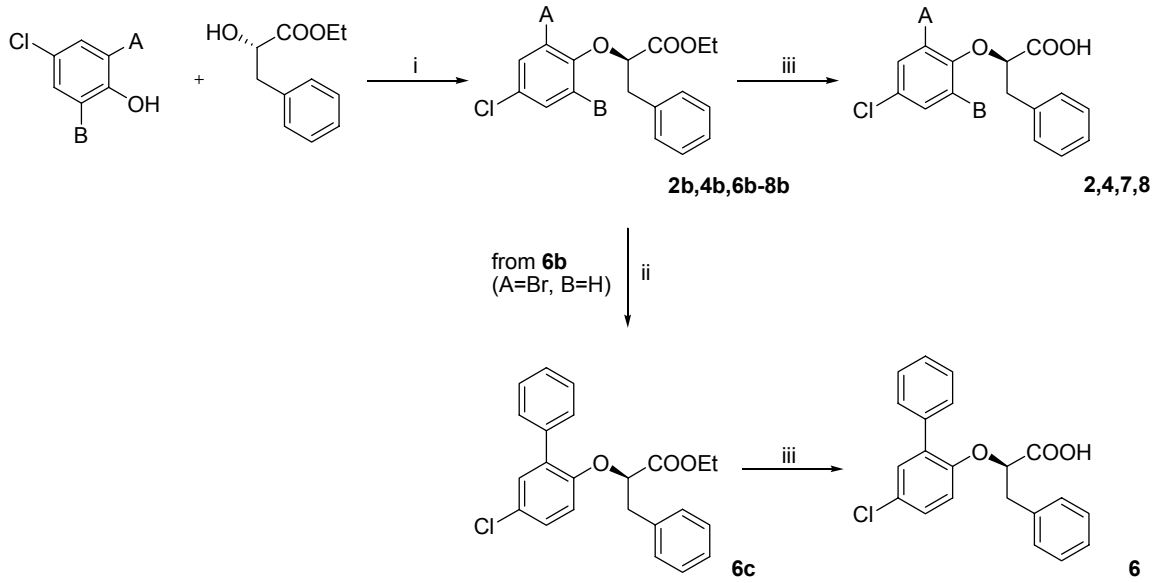
A,B,C,D,E,F= alkyl,aryl,halogen
R= H, CH_3

Figure 1. General structure of the investigated PPAR agonists.

Results and Discussion

Racemates **1-21**, **23-25** and **27** as well as the enantiomers of compounds **1**, **21**, **22**, **26** and **28** were synthesized according to previously reported procedures.[12,17] For the analytes **22**, **26** and **28**, non-equimolecular mixtures of the corresponding optical isomers were injected in place of racemates. The *R*-enantiomers of **2**, **4**, **6**, **7** and **8** were prepared as reported for racemates[12] starting, in this case, from commercially available (*S*)-phenyllactic acid (scheme 1). The Mitsunobu condensation of its ethyl ester with the suitable 4-chlorophenols afforded the *R*-phenoxyesters **2b**, **4b** and **6b–8b**. The hydrolysis of these intermediates, except for **6b**, afforded the desired *R*-acids **2**, **4**, **7** and **8**. Alternatively, the bromo-derivative **6b** was condensed with phenylboronic acid in Suzuki conditions to give the corresponding intermediate **6c** whose hydrolysis afforded acid ***R*-6**. ***R*-19** was obtained by fractional crystallization from ethyl acetate of the diastereomeric salts prepared from the corresponding racemate with (*S*)-1-phenylethylamine (see Experimental section).

The absolute configuration of the stereoisomers of **1**, **22**, **26** and **28** was previously assigned on the basis of the known stereochemical course of their synthetic pathways.[12,17] Similarly, the absolute configuration of the *R*-enantiomers of **2**, **4**, **6**, **7** and **8** was unequivocally established by Mitsunobu reaction which is known to occur with inversion of configuration.[20] Only for the stereoisomers of the analytes **19** and **21** the absolute configuration was attributed by circular dichroism analysis. The CD curves of **(–)-19** and **(–)-21**, in fact, show a negative Cotton effect around 220-250 nm as well as the *R*-isomers of **1** and **28** with known absolute configuration. The high structural similarity of these compounds, differing only for a moiety of the molecule which is located far away from the stereogenic center, allows to argue that, with reasonable confidence, the levo-rotatory isomers of **19** and **21** have the *R*-configuration too.

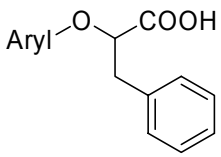


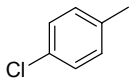
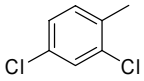
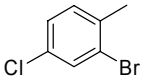
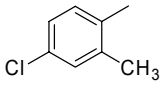
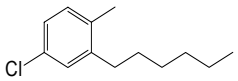
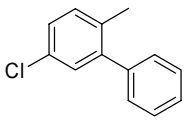
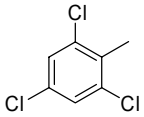
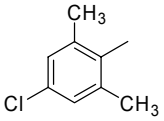
Scheme 1. Preparation of the *R*-enantiomers of **2**, **4**, **6**, **7**, **8**. i) DVB-$(Ph)_3P$, DIAD, dry toluene; ii) phenylboronic acid, Cs_2CO_3, $Pd[(Ph)_3P]_4$, dry toluene, 95°C; iii) 2N NaOH/THF 1:1.

Table 1 provides chromatographic data for the normal-phase separation of the enantiomers of compounds **1-8** on cellulose- and amylose-derived CSPs. In this series, we used derivative **1** as a reference compound and analyzed the chromatographic behavior resulting from the introduction of additional substituents in position 2 (ortho) or 2,6 (di-ortho) of the phenoxylic nucleus.

Five out of eight compounds were baseline resolved on OD column, whereas the poly-halogenated derivatives **2**, **3** and **7** were poorly or not resolved. The 2-phenyl-substituted derivative **6** displayed the highest separation factor (see Figure 2); the selectivity decreased for the alkyl-substituted compounds **4**, **5** and **8** but still remained higher than reference **1**.

Table 1. Chromatographic parameters for the enantioseparation of acids **1–8**



		OD column				AD column			
Comp	Aryl	k_1	α	Rs	First eluted enantiomer	k_1	α	Rs	First eluted enantiomer
1		2.59	1.20	3.48	*R*	4.72	1.05	n.d.	*R*
2		2.37	1.00	-		3.18	1.11	1.30	*S*
3		2.40	1.00	-		3.34	1.00	-	
4		2.28	1.40	6.11	*R*	3.14	1.12	1.44	*R*
5		1.80	1.35	5.30		1.96	1.20	2.26	
6		2.16	1.53	7.57	*S*	2.86	1.07	0.72	*R*
7		1.76	1.04	0.69	n.d.	2.41	1.35	3.71	*S*
8		1.68	1.31	5.10	*S*	2.57	1.07	0.73	*S*

Mobile phase: *n*-hexane/2-propanol/trifluoroacetic acid 95/5/0.02 (% v/v); n.d.: not determined**.**

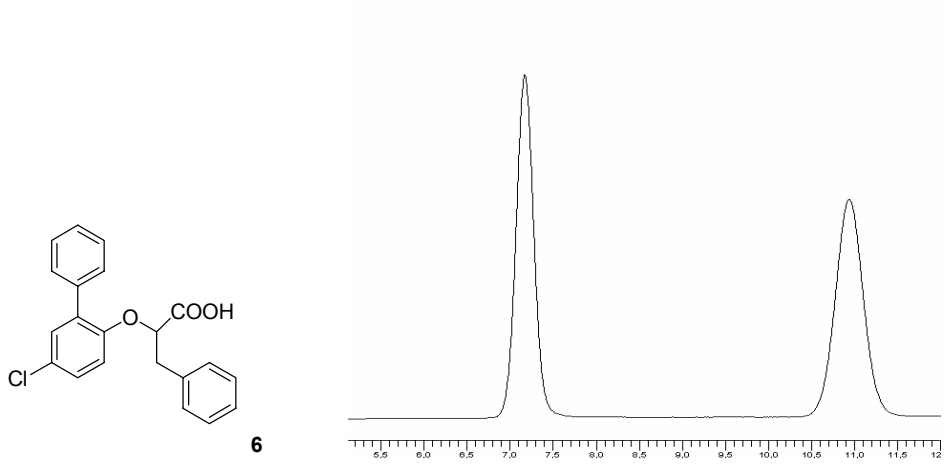


Figure 2. Chromatographic trace on Chiralcel OD of racemate **6**. Experimental conditions are reported in the text.

Using the amylose-derived AD CSP, all compounds displayed a marked increment of the retention time with concomitant substantial improvement in the separation only for the poly-halogenated derivatives **2** and **7** (see Figure 3).

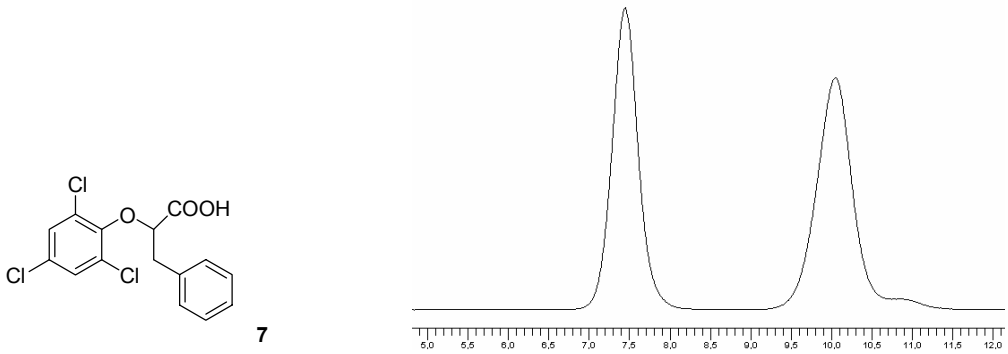


Figure 3. Chromatographic trace on Chiralpak AD of racemate **7**. Experimental conditions are reported in the text.

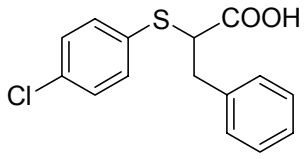
A significant decrease of selectivity was observed for all of the other acids, whereas the 2-bromo-derivative **3** still remained unresolved. Interestingly, the enantiomer elution order inverted only for the analyte **6**; for all of the other analytes whose enantiomers were available, it remained unchanged differently from what often observed switching from OD to AD CSP.[14]

From a general overview of the data here reported, it appears evident the high recognition ability of both the cellulose- and amylose-based CSPs towards the investigated analytes; except for the 2-bromo-derivative **3**, in fact, all of them were well enantiodiscriminated at least on one of the two examined columns. These polysaccharide CSPs are semisynthetic polymers

containing chains of derivatized D-(+)-glucose residues in β-1,4-linkage in cellulose and in α-1,4-linkage in amylose. These chains lie side-by-side in a helical fashion. It is well known that the binding of the analytes to these CSPs is steered by the difference in volume between the helical groove of the cellulose derivative (OD) and that of the amylose derivative (AD). The former appears to be more linear and rigid in nature, whereas the latter gives a more compact and wider helix with well-defined grooves.[21,22] In both cases the polar carbamate groups are oriented inward the groove and the hydrophobic aromatic groups outward it. The former allow H-bonding and the latter π–π interactions with the analytes. These bonding types, together with dipole-dipole interactions and steric effects, determine the stereogenically different fit of enantiomers into the chiral cavities.[14,22]

The experimental data obtained with the AD column shows longer retention times for all the compounds of this set compared to OD. This could be explained suggesting an easier accessibility of the helical cavity of the amylose-derived CSP. However, only for compounds **2** and **7** the higher capacity factors result in better selectivity and resolution probably due to a supplementary π–π interaction of their electron-poor aryloxylic rings with the phenyl rings of the tris(3,5-dimethylphenylcarbamate) of amylose. The lower electron-withdrawing effect as well as the larger size of bromine compared to chlorine could explain, instead, the lack of stereoselectivity towards the bromo-derivative **3**.

The influence of a possible H-bond interaction between the phenoxylic oxygen atom of these compounds and the carbamoyl NH group of both OD and AD CSPs was also evaluated. For this purpose, the sulfur analog **1a**[18] (Figure 4) was prepared and analyzed; the introduction of a sulfur in place of the ether oxygen atom produced a concomitant decrease of capacity, selectivity and resolution factors on OD column (k_1 = 2.24, α = 1.11, Rs = 2.15) compared to the isosteric analyte **1**. The influence of this substitution appears less important on AD CSP; the selectivity factor towards the sulfur analog **1a**, in fact, remained unchanged compared to OD in spite of the higher capacity factor (k_1 = 4.59, α = 1.11, Rs = 1.15).



1a

Figure 4

Table 2 provides chromatographic data for the normal-phase separation of the enantiomers of compounds **9-28** on cellulose- and amylose-derived CSPs.

In this series, we analyzed the chromatographic behavior resulting from the introduction of additional substituents in different positions of the benzylic aromatic ring of **1**. All ortho-substituted compounds, except for the trifluoromethyl derivative **25**, were baseline resolved on

OD column (as an example, see Figure 5) as well as the meta-phenoxyl compound **20**. All para-substituted analytes were poorly or not resolved with the exception of the para-phenoxyl-derivative **21**. In any case, the alkyl-substituted analytes mostly displayed the highest separation factors.

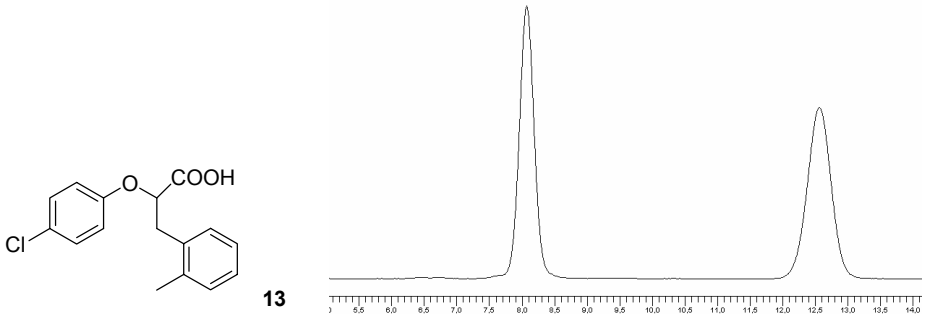


Figure 5. Chromatographic trace on Chiralcel OD of racemate **13**. Experimental conditions are reported in the text.

Using the amylose-derived AD CSP, all compounds displayed a marked increment of the retention time; 13 out of 20 analytes showed a concomitant substantial improvement in the enantioseparation. A significant decrease of selectivity was observed, instead, for the best resolved acids on OD column **13**, **15**, **17**, **24** and **27** as well as for the 3-phenoxyl-derivative **20**, whereas the separation factor of the 2-phenoxyl-derivative **19** remained unchanged. Only the 3-phenoxyl- and 2-phenyl-substituted analytes **20** and **27** were not resolved. Interestingly, the AD CSP showed a reverse chromatographic behavior compared to OD. The para-substituted acids, in fact, turned out to be the best resolved analytes with the 4-thienyl-derivative **22** showing, by far, the highest selectivity factor.

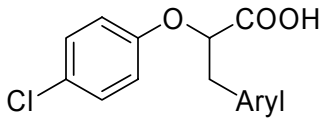
No reverse elution order was observed for the only three analytes **19**, **21** and **22** whose enantiomers were available and resolved on both cellulose- and amylose-derived CSPs.

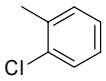
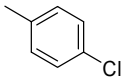
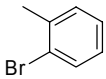
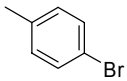
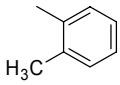
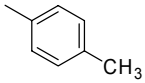
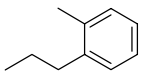
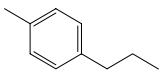
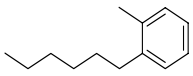
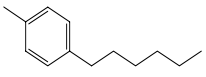
The analysis of the chromatographic data of **9-28** shows, once again, the highly versatile and complementary properties of both the cellulose- and amylose-based CSPs towards the investigated analytes; except for the 2-trifluoromethyl-derivative **25**, in fact, all of them were baseline resolved at least on one of the two examined columns.

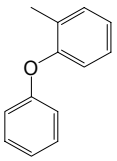
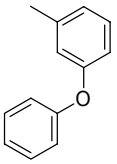
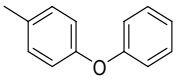
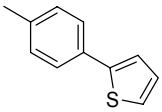
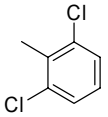
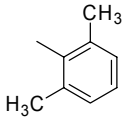
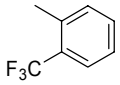
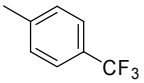
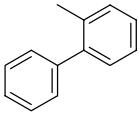
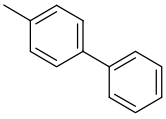
These analytes, again, showed higher capacity factors on AD compared to OD column suggesting, also in this case, an easier accessibility of the helical cavity of the amylose-derived CSP. The benzylic moiety as well as its substituents, therefore, would be able to realize supplementary interactions with AD CSP accounting for the better enantioresolution of 13 out of 20 analytes compared to OD (as an example, see Figure 6). Interestingly, the AD column exhibited a very efficient enantiodiscrimination process towards the para-thienyl derivative **22** which might be ascribed to the strong electrostatic interaction realized by the thiophene ring with

the π-cloud of the aromatic ring of the tris(3,5-dimethylphenylcarbamate) of amylose. In particular, sulfur-arene interactions are known to be strongly attractive.[23]

Table 2. Chromatographic parameters for the enantioseparation of acids **9–28**



		OD column				AD column			
Comp	Aryl	k_1	α	Rs	First eluted enantiomer	k_1	α	Rs	First eluted enantiomer
9		2.57	1.12	2.16		4.22	1.17	2.04	
10		2.20	1.00	-		5.01	1.33	3.43	
11		2.77	1.11	1.95		4.30	1.17	1.60	
12		2.28	1.00	-		5.15	1.37	3.17	
13		2.42	1.56	8.09		3.78	1.15	1.40	
14		1.93	1.05	0.78		4.39	1.19	1.86	
15		1.71	1.26	4.36		3.07	1.09	0.85	
16		1.75	1.05	0.86		3.50	1.30	2.66	
17		1.66	1.52	7.20		2.61	1.09	0.83	
18		1.63	1.04	0.65		2.81	1.42	2.95	

19		2.96	1.11	1.95	*R*	4.37	1.10	0.77	*R*
20		3.72	1.09	1.51		5.30	1.00	-	
21		3.48	1.09	1.25	*R*	6.03	1.23	1.84	*R*
22		2.98	1.09	1.09	*R*	3.17	2.94	10.53	*R*
23		1.96	1.10	1.94		4.70	1.24	1.87	
24		1.76	1.21	3.55		2.65	1.08	0.61	
25		1.82	1.00	-		3.43	1.08	0.68	
26		2.14	1.00	-		3.94	1.32	2.97	*R*
27		2.05	1.14	2.46		4.98	1.00	-	
28		3.53	1.00	-		5.65	1.21	2.03	*R*

Mobile phase: *n*-hexane/2-propanol/trifluoroacetic acid 95/5/0.02 (% v/v).

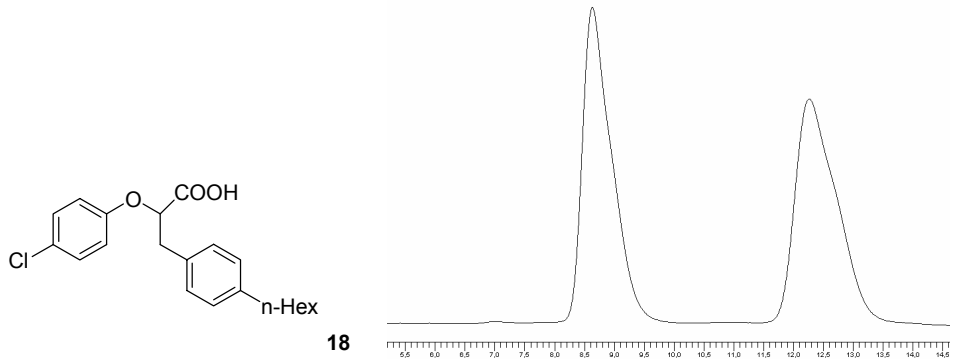
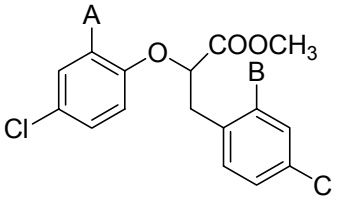


Figure 6. Chromatographic trace on Chiralpak AD of racemate **18**. Experimental conditions are reported in the text.

The only analytes displaying lower enantioselectivity factors on AD column compared to OD were the ortho-alkyl-substituted derivatives **13**, **15**, **17** and **24**, and compounds **20** and **27** bearing the meta-phenoxyl and the ortho-phenyl substituents, respectively. These substituents could force the benzylic aromatic ring to assume a conformation not particularly favorable for interactions with the amylose-CSP.

With the aim to gain insight into the factors influencing the chiral recognition of the acids reported in this work, we analyzed the chromatographic behavior of the methyl esters **29-37**, obtained by reaction of the corresponding acids with a solution of diazomethane in Et_2O, on both the polysaccharide CSPs. These derivatives were chosen because of the different steric and electronic properties of the substituents present on both aromatic rings. Moreover, esters **32**, **33** and **35-37** derived from acids which were not resolved on OD column (**10**, **12**, **25**, **26** and **28**), whereas ester **29** derived from the acid **3** which was unresolved on both OD- and AD-CSPs.

Table 3. Chromatographic parameters for the enantioseparation of methyl esters **29–37**



29-37

				OD column				AD column			
Comp	A	B	C	k_1	α	Rs	First eluted enantiomer	k_1	α	Rs	First eluted enantiomer
29	Br	H	H	2.21	1.06	1.27		1.90	1.00	-	
30	CH_3	H	H	2.00	1.48	7.98	*R*	1.61	1.16	1.33	*R*
31	Ph	H	H	2.33	1.06	1.11	*S*	1.88	1.00	-	
32	H	H	Cl	2.09	1.00	-		2.24	1.12	1.17	
33	H	H	Br	2.14	1.00	-		2.33	1.12	1.52	
34	H	CH_3	H	2.19	1.57	9.47		1.80	1.02	0.18	
35	H	CF_3	H	1.59	1.00	-		1.78	1.00	-	
36	H	H	CF_3	2.06	1.00	-		2.99	1.11	1.00	
37	H	H	Ph	4.51	1.26	3.28		1.99	1.07	n.d.	

Mobile phase: *n*-hexane/2-propanol 95/5 (% v/v); n.d.: not determined.

The experimental data reported in Table 3 shows that it is possible to perform the resolution of all methyl esters, with the exception of **35**, at least on one of the two investigated columns. All the analytes were less retained than the corresponding acids with the exception of **31** and **37** on OD. This implicates that the formation of a H-bond between the carboxylic group of acid analytes and CSPs is an important feature; other interactions, however, play a crucial role in the recognition mechanism as confirmed from the better separation of some esters compared to their corresponding acids (see esters **29**, **30** and **37** on OD, ester **30** on AD). One of these interactions could be realized from the methyl group of these esters with the π-cloud of the CSP aromatic rings.

To summarize, the investigated CSPs were very efficient in accomplishing chiral resolution of both acids and esters without requiring time consuming analysis. This holds true especially if

one considers that no attempts were made to optimize the enantioseparation conditions. They could, therefore, be used to provide the enantiomers of many of the examined compounds even on a semi-preparative scale. As an example, the chromatographic traces on Chiralpak AD relating to the racemates and *S*-isomers of the potent PPAR agonists **21** and **26** are reported in Figure 7. In both cases, the high resolution allows to distinguish the presence of the *R*-isomer (distomer) as an impurity (lower than 1-2 %) of the biologically active *S*-isomer (eutomer).

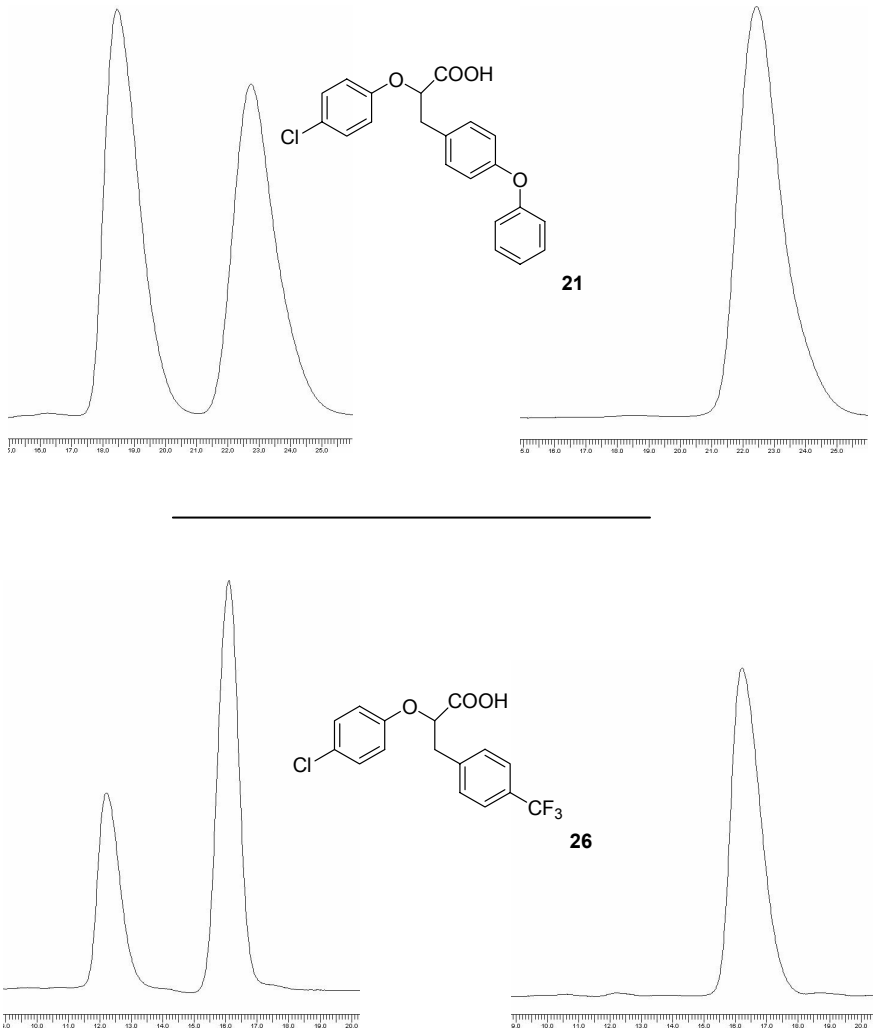


Figure 7. Chromatographic traces on Chiralpak AD of the racemates **21** and **26** and their *S*-isomers. Experimental conditions are reported in the text.

As regards the chiral discrimination abilities of the two CSPs, besides the hydrogen bonding pattern claimed to form between the carboxylic function of acid analytes and both carbamate group and pyranyl oxygen of CSP, also steric effects and π–π interactions of both the aromatic parts of the analytes with the tris(3,5-dimethylphenylcarbamate) substituent of cellulose or amylose play a crucial role in the chiral recognition process. However, other strong interactions

occur in methyl esters accounting for the better enantioresolution of some of these derivatives compared to the corresponding acids.

Experimental Section

General. Chromatography was performed on a Perkin-Elmer chromatograph, equipped with a Rheodyne 7725i model injector, a 785A model UV/Vis detector, a series 200 model pump, and NCI 900 model interface. The analyses were carried out at room temperature, with detection at 280 nm. The chromatographic data were collected on a computer running Perkin-Elmer TotalChrom version 6.3.1.0504 software. Chiralcel OD and Chiralpak AD columns (4.6 mm i.d. x 250 mm) were supplied by Daicel Chemical Industries, Ltd, Tokyo, Japan; HPLC grade *n*-hexane and 2-propanol were purchased from Carlo Erba (Milan, Italy); trifluoroacetic acid (TFA, 99+%, spectrophotometric grade) was obtained from Sigma Aldrich (Milan, Italy).

Mobile phase elution was made isocratically at flow 1.0 mL/min using *n*-hexane/2-propanol 95/5 (% v/v) or *n*-hexane/2-propanol/TFA 95/5/0.02 for methyl esters and acids, respectively. A conditioning time of 120 minutes was applied whenever the eluent composition was changed to obtain a good reproducibility. Injected solutions (filtered through a 0.45 μm membrane filter) were obtained dissolving 2-3 mg of analytes in 2 mL of 2-propanol. The retention factor (*k*) was calculated using the equation $k = (t_r/t_o)-1$, where t_r is the retention time of the analyte and t_o is the retention time of an unretained compound. In this study t_o was determined as the first disturbance of the baseline after injection. The separation factor (α) was calculated using the equation, $\alpha = k_2/k_1$ where k_1 and k_2 are the retention factors for the first and last eluted enantiomers, respectively. Resolution is calculated from the equation $Rs = 2(t_2 - t_1)/(w_1 + w_2)$, where t_1 and t_2 are the retention times of the first- and second-eluted peaks, respectively, and w_1 and w_2 are the peak widths. The determination of the enantiomeric elution order was carried out by injecting solutions of pure enantiomers under the same experimental conditions of the racemates.

Racemates **1-21**, **23-25** and **27** as well as the enantiomers of compounds **1**, **21**, **22**, **26** and **28** were synthesized according to previously reported procedures.[12,17] The enantiomeric excesses of the optically active acids were >90% as determined by HPLC analysis of their methyl esters, obtained by reaction with a solution of diazomethane in Et_2O, on Chiralcel OD (**1**, **21**, **22**, **28**) or Chiralpak AD (**26**) columns using *n*-hexane/*i*-PrOH 98:2 (**1** and **26**) or 95:5 (**21**, **22** and **28**) as the mobile phase, flow rate 0.5 mL/min, detection 230 nm.

The *R*-enantiomers of **2**, **4**, **6**, **7** and **8** were prepared as reported for racemates[12] starting, in this case, from commercially available (*S*)-phenyllactic acid. The physico-chemical properties of these isomers were identical to those of the corresponding racemates. Chemical yields were 20-75%. The enantiomeric excesses were determined by HPLC analysis on Chiralpak AD column using the experimental conditions employed in this study (*n*-hexane/2-propanol/TFA 95:5:0.02 as the mobile phase, flow rate 1.0 mL/min, detection 280 nm). All of the optically active acids

had enantiomeric excesses > 95% except for **6** (e.e. 65%).

***R*-19** was obtained by resolution of the corresponding racemate as follows. 1.10 g of ***R,S*-19** (2.9 mmol) and 0.39 g of (*S*)-1-phenylethylamine (3.2 mmol) were dissolved in 20 mL of absolute ethanol and the mixture was stirred overnight at room temperature. The solvent was evaporated under vacuo and the solid residue was crystallized from ethyl acetate. After three crystallizations, the diastereomeric salt was treated with 2N HCl and then extracted with Et_2O. The organic layer was washed with brine, dried over Na_2SO_4 and evaporated to dryness affording the final acid which was crystallized from $CHCl_3$/*n*-hexane. Yield: 15%. The physico-chemical properties of this isomer were identical to those of the corresponding racemate.[12] $[\alpha]_D$ = - 29 (c 0.1, MeOH); e.e. 96% (HPLC on Chiralcel OD; *n*-hexane/2-propanol/TFA 95:5:0.02 as the mobile phase, flow rate 1.0 mL/min, detection 280 nm).

References

1. Lämmerhofer, M.; Lindner, W. Recent developments in liquid chromatographic enantioseparation, In: Valko, K. Ed., *Handbook of Analytical Separations*, Vol. 1, *Separation methods in drug synthesis and purification*, Elsevier: Amsterdam 2000, p 337.
2. Berger, J.; Moller, D. E. *Annu. Rev. Med.* **2002**, *53*, 409.
3. Berger, J. P.; Akiyama, T. E.; Meinke, P. T. *Trends Pharmacol. Sci.* **2005**, *26*, 244.
4. Kliewer, S. A.; Sundseth, S. S.; Jones, S. A.; Brown, P. J.; Wisely, G. B.; Koble, C. S.; Devchand, P.; Wahli, W.; Willson, T. M.; Lenhard, J. M.; Lehmann, J. M. *Proc. Natl. Acad. Sci. U.S.A*. **1997**, *94*, 4318.
5. Nagy, L.; Schwabe, J. W. *Trends Biochem. Sci.* **2004**, *29*, 317.
6. Nettles, K. W.; Greene, G. L. *Annu. Rev. Physiol.* **2005**, *67*, 309.
7. Renaud, J. P.; Moras, D. *Cell. Mol. Life Sci.* **2000**, *57*, 1748.
8. Desvergne, B.; Wahli, W. *Endocr. Rev.* **1999**, *20*, 649.
9. Issemann, I.; Prince, R. A.; Tugwood, J. D.; Green, S. *J. Mol. Endocrinol.* **1993**, *11*, 37.
10. Staels, B.; Dallongeville, J.; Auwerx, J.; Schoonjans, K.; Leitersdorf, E.; Fruchart, J.-C. *Circulation* **1998**, *98*, 2088.
11. Rubins, H. B.; Robins, S. J.; Collins, D.; Fye, C. L.; Anderson, J. W.; Elam, M. B.; Faas, F. H., Linares, E.; Schaefer, E. J.; Schectman, G.; Wilt, T. J.; Wittes, J. *Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial Study Group*. *N. Engl. J. Med.* **1999**, *341*, 410.
12. Fracchiolla, G.; Lavecchia, A.; Laghezza, A.; Piemontese, L.; Trisolini, R.; Carbonara, G.; Tortorella, P.; Novellino, E.; Loiodice, F. *Bioorg. Med. Chem.* **2008**, *16*, 9498.
13. Yashima, E.; Okamoto, Y. Chiral recognition mechanisms of polysaccharide stationary phases. In Aboul-Enein, H. Y.; Wainer, I. W., Eds., *The impact of stereochemistry on drug development and use, chemical analysis series*. Wiley: New York, 1997, p 403.
14. Okamoto, Y.; Yashima, E. *Angew. Chem. Int. Ed.* **1998**, *37*, 1020.

15. Aboul-Enein, H. Y.; Ali, I. Polysaccharide-based chiral stationary phases. In Cazes Ed., *Chiral separations by liquid chromatography and related technologies*. Marcel Dekker: New York, 2003, p 21.
16. Aboul-Enein, H. Y.; Ali, I. Applications of polysaccharide-based chiral stationary phases for resolution of different compound classes. In *Methods Mol. Biol.,* Gübitz, G., Schmid, M. G. Eds., Humana Press: Totowa, NJ, **2004**, 186.
17. Pinelli, A.; Godio, C.; Laghezza, A.; Mitro, N.; Fracchiolla, G.; Tortorella, V.; Lavecchia, A.; Novellino, E.; Fruchart, J.-C.; Staels, B.; Crestani, M.; Loiodice, F. *J. Med. Chem.* **2005**, *48*, 5509.
18. Fracchiolla, G.; Laghezza, A.; Piemontese, L.; Carbonara, G.; Lavecchia, A.; Tortorella, P.; Crestani, M.; Novellino, E.; Loiodice, F. *ChemMedChem.* **2007**, *2*, 641.
19. Piras, P.; Roussel, C.; Pierrot-Sanders, J. *J. Chromatog.* **2001**, *906*, 443.
20. Mitsunobu, O. *Synthesis* **1981**, 1.
21. Vogt, U.; Zugenmaier, P. *Ber. Bunsenges. Phys. Chem.* **1985**, *89*, 1217.
22. Yamamoto, C.; Okamoto, Y. *Bull. Chem. Soc. Jpn.* **2004**, *77*, 227.
23. Meyer, E. A.; Castellano, R. K., Diederich, F. *Angew. Chem. Int. Ed.* **2003**, *42*, 1210.