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Contribution of Chiral HPLC in Tandem with Polarimetric Detection in the Determination of Absolute Configuration by Chemical Interconversion Method: Example in 1-(thi)oxothiazolinyl-3-(thi)oxothiazolinyl Toluene Atropisomer Series

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ABSTRACT We report the determination of the absolute configuration of eight stereoisomers in the series of chiral 1-(thi)oxothiazolinyl-3-(thi)oxothiazolinyl toluene atropisomers **1–3,** from the known absolute configuration of one stereoisomer, determined by X-ray crystallography. The method uses the affiliation between signs of rotation of polarised light during chemical transformations which preserve the absolute configuration and also during rotation around a single pivot bond producing a compound of known configuration. The use of chiral HPLC in tandem with a chirality detector gives a decisive advantage since such correlation can be performed on a mixture of a very limited quantity of compounds, without tedious purification steps. The method shown as an example in this article, which uses chiral HPLC with chirality detection, may prove useful in many other cases where the determination of the absolute configuration is necessary and where a chemical interconversion method can be used on a microscale. *Chirality 14:665–673, 2002.*

KEY WORDS: chemical correlation method; chiroptical detection; heterocycles; heteroaryl atropisomers; micro-scale.

Chemical interconversion is a classical method used to determine the absolute configuration of optically active compounds.[1] Basically, it involves the application of a series of reactions linking the compounds of unknown configuration to an optically pure compound of known configuration. A well-established relationship must exist for the reference compound between the absolute configuration and an available chirality assessment (for instance, the sign of the rotatory power). The reference compound may be a starting material or a final product. The chemical interconversion may or may not involve the chiral centre. In the former case, particular attention must be paid to the stereospecificity of the applied transformation. Since all the materials engaged in the chemical interconversion method are optically active, particular care must be taken in the different purification steps. It generally results in an increase of the consumption of products.

In summary, purification steps and chirality assessment are the two main issues in the chemical interconversion method. In principle, these two issues may be solved using chiral HPLC (separation method) in tandem with a chirality detection (chirality assessment).

In this report, we describe a micromethod of chemical interconversion aimed at the determination of the absolute configuration of a series of atropisomers of interest to our laboratory. The micromethod associates chiral HPLC and polarimetric detection.

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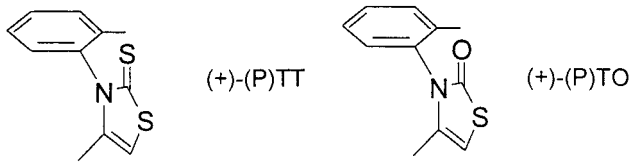


Scheme 1. (+)-(P)-N-(2-methylphenyl)-4-methyl-thiazoline-2-thione (TT) and (+)-(P)-N-(2-methylphenyl)-4-methyl-thiazolin-2-one (TO).

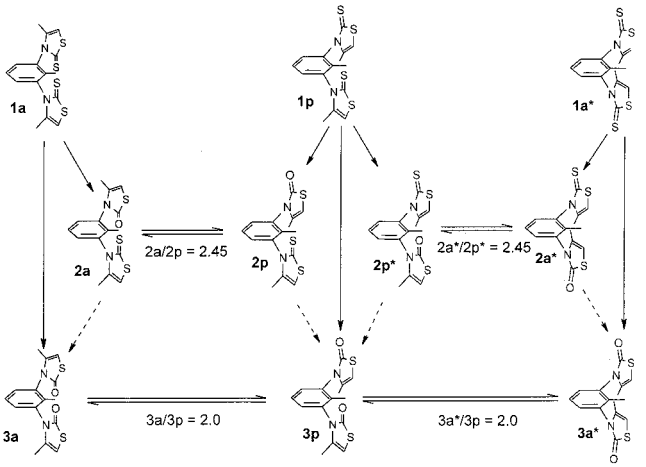
METHODOLOGY AND COMPOUNDS PRESENTATION

N-arylthiazoline-(thi)one derivatives have long been used as model compounds for the investigation of chiral recognition mechanisms during the chromatographic separation of enantiomers on chiral stationary phases (CSP).[2–6] The high barrier to rotation around the N–C bond gives rise to stable atropisomers at room temperature in which the planes of the lipophilic aryl group and the highly dipolar heterocycle are perpendicular.[7] In order to establish these compounds as model compounds several steps were accomplished: determination of the barriers to rotation, X-ray data, determination of the absolute configuration of the enantiomers of thiazoline-thione and thiazolin-one derivatives. The absolute configuration of one enantiomer of the thiazoline-thione analogue was determined by X-ray analysis of a menthol derivative, whereas the absolute configuration of the thiazoline-one analogue was determined by a nonracemizing chemical interconversion converting the thiocarbonyl into a carbonyl group.[8] Thus, starting from the (+)-(P) N-(2-methylphenyl)-4-methyl-thiazoline-2-thione, the (+) thiazolin-one was obtained indicating that the absolute configuration of the (+) thiazolin-one is (P) (Scheme 1).

We recently described the first synthesis of various atropisomeric derivatives **1–3** in which the two heterocyclic rings are perpendicular to the plane of the toluene giving rise to what is called hereafter a parallel or antiparallel arrangement, depending on the relative orientation of the exocyclic C=X groups of the two heterocycles.[9] In compounds **1** and **3,** the parallel arrangement corresponds to a meso form, whereas two enantiomers are found in the antiparallel arrangement; in compound **2** both parallel and anti-parallel forms are chiral (Scheme 2).

In comparison with the Parent N-arylthiazoline-(thi)one derivatives compounds **1–3** present some interesting topographic characteristics which might prove useful for a better understanding of the chiral recognition or nonchiral interaction with various CSPs. Each of them mimics twice what is topographically found in the monoderivative analogue during interaction with the chiral support. Chiral HPLC on cellulose triacetate with on-line polarimetric detection was used in our previous study primarily in order to clearly identify the meso from the racemic forms and these diastereomers were then obtained by achiral chromatography on silica.

If compounds **1–3** present in their design some promising structural features for chiral recognition studies, a serious drawback is that nothing is known about the absolute configuration of the different enantiomers. We have



Scheme 2. Description interconversions through stereospecific reactions of compounds **1–3,** (arrows stereospecific reactions, dotted arrows possible stereospecific reactions); interconversions through rotation equilibrium around C-N pivot bond (double arrows).

already stressed on several occasions that knowledge of the absolute configuration is a prerequisite to any attempts for molecular modelling of chiral recognition. The availability of on-line specific chirality detectors such as a polarimeter or a CD instrument provides an easy way to determine the order of elution on different CSPs after checking the effect of the eluent composition on the sign of the rotatory power.[10–13] It is thus quite easy to trace a possible reversal of elution order going from one CSP to another when using specific chirality detectors. More significant information in terms of molecular recognition is obtained when it is possible to link the elution order to the absolute configuration. It is important to stress again that the determination of elution order based on chiroptical detection requires knowledge of the mobile phase composition effect on the sign of the optical rotation or CD signal. For instance, the signal of optical rotation is inverted for warfarin enantiomers when chiral separation is performed in hexane/propan-2-ol 90:10 or heptane/ethyl acetate 80:20.[14]

In this article, we report the determination of the absolute configuration of each molecule of a series of compounds **1–3,** from the known absolute configuration of an enantiomer of one compound, determined by X-ray crystallography. This method uses the affiliation between signs of rotation of polarised light during chemical interconversions occuring with conservation of the absolute configuration and also during rotation around a single pivot bond producing a compound of known configuration. Scheme 2 displays the possible relationships between the various stereoisomers of compounds **1–3,** using the transformation from *-thione* to *-one* whilst retaining the absolute configuration and equilibrium accessible through rotation of the *-one* heterocycle around the C-N pivot bond.

The stereoisomers of compound **1** were obtained by direct synthesis in a mixture close to the statistical repartition. **1a** and **1a*** (**a** stands for antiparallel) are enantiomers, **1p** (**p** stands for parallel) is a meso form. It is worth noting that rotation around the pivot bond is impossible and decomposition occurs when boiling in toluene without

any rotation. **1a, 1a*,** and **1p** are thus noninterconverting stereoisomers. Compounds **2** are obtained when the mixture **1a, 1a*,** and **1p** is treated at room temperature successively with methyl iodide/acetone and MeONa/MeOH. More precisely, the meso derivative **1p** gives the pair of enantiomers **2p** and **2p*,** whereas **1a** gives **2a** and **1a*** gives **2a*,** respectively. **2a, 2p, 2p*,** and **2a*** are stable atropisomers at room temperature and at elevated temperature parallel and antiparallel forms slowly equilibrate. The position of the equilibrium and the associated barrier to rotation have been determined using a racemic mixture ($\Delta G^{\#}$ = 125.04 KJ/mole at 110°C in tetrachloroethylene for 2p→2a).[15] The parallel forms in which the two dipoles are in direct interaction are disfavoured; the parallel–antiparallel transformation occurs solely by the rotation around the N-C bond of the *-one* heterocycle and not by rotation around the N-C bond of the *-thione* heterocycle. The fact that the *-one* heterocycle can rotate on heating, whereas the *-thione* form does not, is a cornerstone in our study. It means that starting from **2p, 2a** will be exclusively obtained and not a mixture composed of **2a** and **2a*.** Compound **3** exists in three forms, **3a, 3a*,** and **3p,** which can be obtained by direct synthesis from the **1** or **2** analogues. The mixture **3a, 3a*, 3p** equilibrates very slowly to reach an equilibrium position close to the statistical repartition through rotation around the N-C bond of the *-one* heterocycle ($\Delta G^{\#}$ = 129.03 KJ/mole at 110°C in tetrachloroethylene for 3p→3a).[15]

Analysis of the possible interconversion pathways using either chemical transformation or rotation processes which have been established on racemic mixtures results in a strategy to determine the absolute configuration of the four pairs of enantiomers (**1a:1a*, 2a:2a*, 2p:2p*, 3a:3a***) as soon as the absolute configuration and the associated sign of the rotatory power of one of the enantiomers in the pair **1a:1a*** is available. Analytical or semipreparative chiral chromatography with chirality detection (polarimeter) was used in all the experimental steps of this study. Thus, in the first step the three forms of compound **1** have to be separated by semipreparative chiral chromatography affording each of the pure (+) and (–) enantiomers of the antiparallel forms (**1a** or **1a***) and achiral form **1p.** In the second step, the absolute configuration of the (+) or the (–) form must be determined by X-ray analysis. This step linking the sign of the rotatory power to a given absolute configuration thus selects the left or the right domains of Scheme 2 starting either from **1a** or **1a*.** In the third step, optically pure **1a** will be transformed into a mixture of **1a, 2a,** and **3a,** which will be submitted to chiral HPLC analysis with a chirality detector. This step, which maintains the absolute configuration during the transformations, is a straightforward method to link the absolute configuration with one macroscopic property the sign of the rotatory power. In the fourth step, the meso form **1p** will be transformed into the mixture of enantiomers (+) and (–) corresponding to either **2p** or **2p*** (together with some meso **3p**) which will next be resolved by semipreparative chiral HPLC. In the fifth step, one of the (+) or (–) forms corresponding to either **2p** or **2p*** has to be equilibrated in order to determine by chiral HPLC which form (+) or (–) of the related antiparallel **2** will be obtained during that interconversion. Since the absolute configuration of the (+) and (–) forms of the antiparallel **2** will be known after step 3, the absolute configuration of the (+) and (–) forms of the parallel arrangement **2** will be determined. A variation for determining the absolute configuration of (+) or (–) parallel arrangements in **2** will involve 1) isolation of **2a,** whose associated sign is known after step 3, out from the mixture **1a, 2a,** and **3a** obtained in step 3; 2) equilibration by rotation and further chiral HPLC to see if **2p** is (+) or (–). This variation will not be easy, due to the unfavourable equilibrium of **2a** vs. **2p.**

MATERIALS AND METHODS

Chiral Chromatography

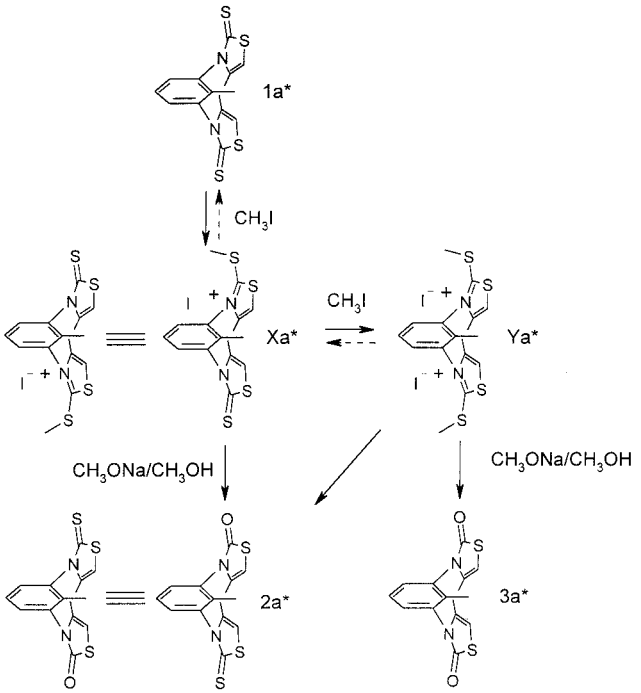
The chiral HPLC experiments were performed on a screening unit composed of a Merck D-7000 system manager, Merck-Lachrom L-7100 pump, Merck-Lachrom L-7360 oven which accommodates 12 columns alimented by a Valco 12 positions valve, Merck-Lachrom L-7400 UV-detector, and Jasco OR-1590 polarimeter detector. Hexane, propan-2-ol and EtOH are HPLC grade from SDS (Peypin, France), the solvents for chromatography experiments were degassed and filtered on Millipore (Bedford, MA) membrane 0.45 µm before use. Cellulose-based chiral stationary phases CHIRALCEL OD-H, CHIRALCEL OJ, or amylose based CSP CHIRALPAK AD DAICEL columns are available from Merck-Eurolab, all of them being (250*4.6 mm) in size. For semipreparative separation a (250*10 mm) CHIRALCEL OJ was used. (S,S) Whelk –O1 (250*4.6 mm) was obtained from Regis (Morton Grove, IL, USA). TLC were performed on silica, eluent $CHCl_3$/AcOEt, 9:1, UV λ = 254 nm.

Experiments

The parallel and antiparallel 1,3-bis-(4-methyl-3-thiazolinyl-2-thione)-2-methylbenzene products used for the following experiments were separated by LC (silica, 150 × 30 mm, eluent $CHCl_3$/AcOEt, 9:1) as reported previously.[9]

Synthesis of thione-one 2a* and bis-one 3a* from 1a* (Scheme 3) and thione-one 2a and bis-one 3a from 1a. This two-step method is adapted from the method described for the racemate and for other heterocycles.[9,16]

To 1.4 mg (4×10^{-6} mol) of (–) **1a*** (**1a*** : $[\alpha]_D^{298}$ = –370° (c = 0.02, $CHCl_3$) obtained by semipreparative chiral HPLC) dissolved in 2.0 ml anhydrous acetone was added 2.4 µl (4×10^{-5} mol) methyl iodide and the reaction was left under agitation at room temperature, in a closed vessel; until all of the bis-thione was consumed. The reaction was monitored by TLC; the thiazolium salts formed were not eluted. After 22 h, the transformation of the bis-thione into the salts **Xa*** and **Ya*** was complete and the solvent was evaporated to obtain a mixture of the salts, which was then used for the second step without further analysis. The mixture of the thiazolium salts **Xa*** and **Ya*** with 1.7 mg (3.2×10^{-5} mol) of sodium methoxide dissolved in 0.4 ml of methanol was stirred at room temperature in a closed vessel until all of the thiazolium salts **Xa*** and **Ya*** had been



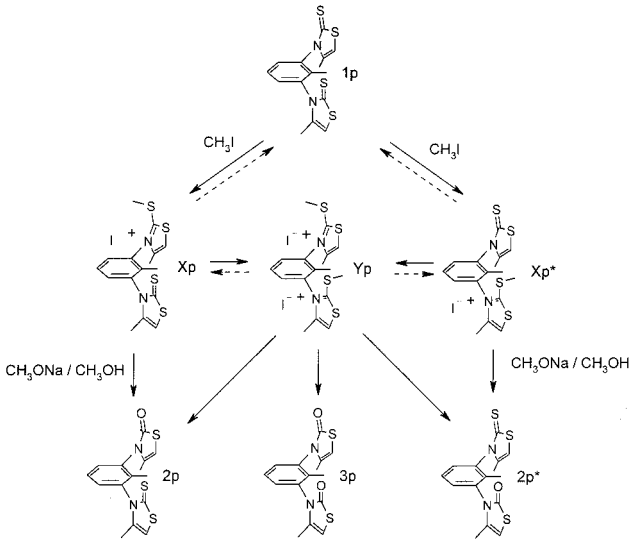
Scheme 3. Procedure for the synthesis of the oxygenated compounds (thione-one **2a*** and bis-one **3a***) from bis-thione **1a*.**

consumed. On completion of the reaction (the complete transformation of the salts into the oxygenated products), monitored by TLC, after 28 h, 5 ml of water was added, on which some of the oxygenated products precipitated. The products (precipitate and in solvent) were extracted with 3 × 5 ml of dichloromethane, which was then dried over $MgSO_4$, filtered, and evaporated. It was noted by TLC that there were four products, the bis-thione **1a*** (Rf = 0.58), thione-one **2a*** (Rf = 0.37), bis-one **3a*** (Rf = 0.35) as expected and also a product with a Rf of 0.50. The mixture was analysed by chiral HPLC on CHIRALCEL OJ.

2a and **3a** were obtained starting from **1a** (10.5 mg) using the same procedure in a quantity sufficient for the measurement of the rotatory powers. The oxygenated products were then separated by preparative plate and semipreparative HPLC. Chiral HPLC analysis showed on CHIRALCEL OJ, as expected, that the oxygenated products obtained rotated polarised light in a positive direction. **2a:** $[\alpha]_D^{298}$ = 140° (c = 0.025, $CHCl_3$); **3a:** $[\alpha]_D^{298}$ = 215° (c = 0.026, $CHCl_3$).

Synthesis of thione-one 2p, 2p*, and bis-one 3p from 1p (Scheme 4). The reaction was carried out in order to obtain the two enantiomers of the parallel thione-one in a quantity sufficient for the measurement of the rotatory power and the parallel bis-one.

To 11.1mg (3.2×10^{-5} mol) of parallel bis-thione **1p,** dissolved in 5 ml acetonitrile, was added 0.020 ml, (3.2×10^{-4} mol) methyl iodide and the reaction was left under agitation at room temperature, in a closed vessel, until all of the bis-thione was consumed. The reaction was monitored by TLC (silica, eluent $CHCl_3$/AcOEt, 9:1, UV λ = 254 nm);



Scheme 4. Procedure for the synthesis of the oxygenated compounds (thione-one **2p, 2p*,** and bis-one **3p**) from bis-thione **1p.**

the thiazolium salts formed are not eluted. After 48 h, the transformation of the bis-thione into the salts **Xp, Xp*,** and **Yp** was complete and the solvent was evaporated to obtain a mixture of the salts which was then used for the second step without further purification. The mixture of the thiazolium salts **Xp, Xp*,** and **Yp** with 15.7 mg (2.9×10^{-4} mol) of sodium methoxide (rmm = 54.024), dissolved in 5 ml of methanol, was stirred at room temperature in a closed vessel until all of the thiazolium salts **Xp, Xp*,** and **Yp** had been consumed. On completion of the reaction (the complete transformation of the salts into the oxygenated products), monitored by TLC, after 96 h, 20 ml of water was added, on which some of the oxygenated products precipitated. The products (precipitate and in solvent) were extracted with 3 × 20 ml of dichloromethane, which was then dried over 10 g $MgSO_4$, filtered, and evaporated. It was noted by TLC that there were four products, the bis-thione **1p** (Rf = 0.24), thione-ones **2p** and **2p*** (Rf = 0.17), bis-one **3p** (Rf = 0.08), as expected, and a product with a Rf of 0.36.

The oxygenated products were then separated by preparative plate and the resulting sample of the thione-one was separated into enantiomers using semipreparative chiral HPLC on CHIRALCEL OJ (eluent 100% EtOH). The enantiomer giving a positive rotatory power was used to measure the rotatory power. **2p:** $[\alpha]_D^{298}$ = 115° (c = 0.022, $CHCl_3$)

Procedure for the Rotation of the Parallel Thione-one 2p to the Antiparallel Thione-one 2a

The equilibria between the parallel and antiparallel conformers of **2** has been studied by NMR and it is known that from an initial sample of the parallel thione-one 100% pure, after 24 h at 110°C, the equilibrium lies in favour of the antiparallel conformer, at 0.71/0.29.[9]

The following rotation was carried out on the enantiomer of the parallel thione-one **(+) 2p.** The rotation was carried

TABLE 1. Data for the chiral chromatographic separation of compounds 1

CSP[a]	Cpd	Eluent[b]	Flow[c]	T	Rt_1	Rt_2	k_1	α	Sign[d]
OD-H	1a/1à*	A	1	18	22.56	29.12	6.65	1.33	(+)
OD-H	1p	A	1	18	76.59	—	24.96	—	meso
AD	1a/1à*	A	1	18	19.71	31.08	5.68	1.68	(+)
AD	1p	A	1	18	116.59	—	37.86	—	meso
AD	1a/1a*	B	0.5	18	8.06	11.23	0.31	2.74	(+)
AD	1p	B	0.5	18	12.68	—	1.05	—	meso
OJ	1a/1a*	B	0.5	25	14.62	19.39	1.42	1.56	(+)
OJ	1p	B	0.5	25	15.52	—	1.57	—	meso

[a]OD-H is CHIRALCEL OD-H, AD is CHIRALPAK AD, OJ is CHIRALCEL OJ.
[b]Eluent A = 90:10 Hexane/propan-2-ol, Eluent B = 100% EtOH.
[c]In mL/min.
[d]Sign of the first eluted enantiomer (polarimetric detection).
UV detection at 254 nm.

out in order to study the affiliation between the signs of rotation of polarised light given by the parallel and antiparallel conformers of the thione-one. The (+) enantiomer was taken as two peaks are found at the same retention time on CHIRALCEL OJ, these being the parallel **(–) 2p*** and antiparallel **(+) 2a.** This avoids the possible complication of the minus giving the plus.

Thus, 0.7 mg of the enantiomer (+) **2p** (99.5% enantiomerically pure) was dissolved in 1.5 ml of tetrachloroethene, placed in a Schlenk apparatus, and put under argon. The solution was heated to 105°C and left under agitation for 3 h. The tetrachloroethene was evaporated, washed with dichloromethane, and the solvent evaporated. Inspection by TLC showed three products, the expected parallel and antiparallel conformers of the thione-one **2p** (Rf = 0.17) and **2a** (Rf = 0.37) and a third unidentified product with a Rf of 0.71.

HPLC analysis of the mixture (CHIRALCEL OJ) showed that the antiparallel enantiomer obtained upon rotation is that giving a positive rotation of polarised light.

RESULTS AND DISCUSSION

Step One: Separation of Compound 1

As stated earlier, compound **1** was obtained as a mixture of the noninterconverting forms **1a, 1a*,** and **1p.** The separation of these three forms was screened on three chiral columns (CHIRALCEL OD-H, CHIRALPAK AD, and CHIRALCEL OJ) with UV and polarimetric detection. The results are given in Table 1.

All three columns gave good separation of the **1a/1a*** pair with α values ranging from 1.32–1.68 in the chosen elution solvent. It is worth noting that in each case the (+) form is eluted first. It is critical to check the possible inversion of the sign due to solvent change when data using different eluents are compared: for compounds **1–3,** we checked that solvent change (hexane/propan-2-ol, EtOH, $CHCl_3$) did not affect the sign. On CHIRALCEL OD-H and CHIRALPAK AD, the meso form, which has a very high dipole moment, was too strongly retained to give a convenient preparative separation of the pair **1a/1a*** using hexane/propan-2-ol mixture. Elution with pure EtOH on CHIRALPAK AD and CHIRALCEL OJ gave a much shorter retention but **1p** was found too close to the second eluted enantiomer of the **1a/1a*** pair in the former case or intermeshed in the latter. EtOH is good option for semipreparative separation since the solvent is easily recycled, the solubility of the analyte is poor in EtOH, but it is far better than in hexane/propan-2-ol. The use of ethanol required an initial semipreparative separation of the parallel and antiparallel forms by achiral chromatography on silica. The chiral separation of the **1a/1a*** pair was thus performed on CHIRALCEL OJ with ethanol affording 20 mg of each enantiomer after collection of the samples resulting from 110 injections (1 mg/mL, 500 μL loop).

*Step Two: Determination of the Absolute Configuration of the (+) Form of the Pair 1a/1a**

The absolute configuration of the (+) form of the **1a/1a*** pair was determined by X-ray crystallography.†

The crystals are clear, light yellow, and well-defined and a sample, size 0.15 × 0.15 × 0.20 mm was used for investigation. The X-ray diffraction data were measured at room temperature using a KUMA diffractometer equipped with graphite-monochromated CuK_α-radiation. Lattice constants were refined by least-square fits of 27 reflections in θ-range 14.0–30.8°. Intensity data were collected using a ω-2θ scan mode up to θ = 78.2° in the limiting indices region $0 \leq h \leq 17$, $0 \leq k \leq 17$, $-20 \leq 1 \leq 20$, which included the *Bijvoet pairs.* Three standard reflections were measured after every 200 reflections, showing no decay of the crystal during the data collection. In all, 7,353 reflections were collected yielding 3,494 unique (R_{int} = 0.0467) and of these 2,918 with $I > 2\sigma(I)$.

Crystals are tetragonal, space group $P4_12_12$, a = 14.126(2), b = 14.126(1), c = 16.308(2)Å. An initial structure model was obtained by direct methods and all the nonhydrogen atoms were refined with anisotropic thermal parameters by a full-matrix least-squares procedure based on F^2. The hydrogen atoms were placed in calculated positions and were refined as constrained to bonding atoms. All

†Crystallographic data for the structure reported in this article have been deposited with the Cambridge Crystallographic Data Centre (CCDC 177991). Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB21EZ, United Kingdom (fax:44-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

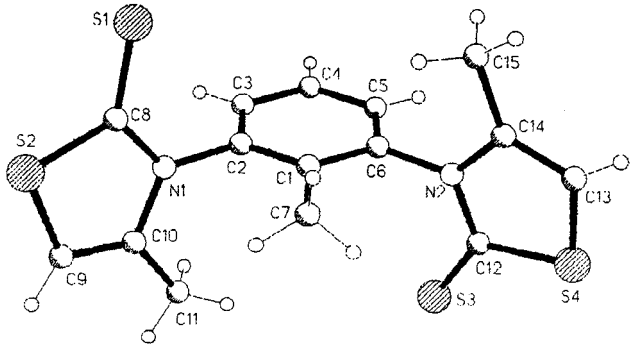


Fig. 1. Absolute configuration by X-ray crystallography for (+)1a: **(P;P).**

H atoms were included in the model of the structure with isotropic *U*-values fixed at 1.5 times U_{eq} of C atoms for CH_3 groups and 1.2 U_{eq} for others. Final *R1* = 0.037 for observed (with $I>2\sigma(I)$) and *wR2* = 0.0917 for all reflections. The Flack *x* parameter was 0.02(2), confirming the choice of correct enantiomer. The largest difference map residual peak and hole were 0.266 and –0.257 e/Å^3, respectively. The refinement of the inverted structure in the counterpart space group $P4_32_12$, which is the other member of the enantiomorphous pair, was also carried out. The corresponding convergence at *R1* = 0.047 and *wR2* = 0.1314 and the Flack *x* parameter 0.99(3) additionally justify the correct choice of the enantiomer. Calculations were carried out using the SHELX-97 system of computer programs (G.M. Sheldrick, SHELX-97: programs for the solution and refinement of the crystal structures. University of Göttingen, Germany, 1997).

The (P;P) absolute configuration was found for the (+) enantiomer and thus **1a** in Scheme 2 is the dextrorotatory form. The ORTEP drawing is given in Figure 1. Crystal packing results in different torsion angles between the mean plane of the tolyl framework and the heterocycles: 98.09(4)° and 88.134(4)°, respectively. Dextrorotatory enantiomers have the same absolute configuration (P) and (P;P) in the N-(2-methylphenyl)-4-methyl-thiazoline-2-thione and 1,3-bis-(4-methyl-3-thiazolinyl-2-thione)-2-methylbenzene.

Thus, in Scheme 2 **1a** = (+)-(P;P)-[2-Methyl-1,3-phenylene]bis[4-methyl-2(3H)-thiazole-thione], **1a*** = (–)-(M;M)-[2-Methyl-1,3-phenylene]bis[4-methyl-2(3H)-thiazole-thione].

Step Three: Determination of the Sign of Rotation Associated with (M;M)-2a and (M;M)-3a**

Starting from optically pure (–)-(M;M) **1a*,** optically pure (M;M) **2a*,** and (M;M) **3a*** are formed according to Scheme 3.

The chemical interconversion goes through the formation of the intermediates **Xa*** and **Ya*,** which correspond to mono- or di-methylation by methyl iodide, respectively, of **1a*. 2a*** is obtained directly from **Xa*** or from **Ya*,** in the latter case demethylation of the thiomethyl group competes with the addition–elimination mechanism. Such a demethylation is also possible from **Xa*** under treatment with MeONa in MeOH accounting for the formation of **1a*** even if the total transformation of initially engaged **1a*** was checked. **3a*** is obtained from **Ya*.** It follows that the reaction produces a mixture of three optically pure compounds. A classical way to address the determination of the sign of rotation associated with (M;M)-**2a*** and (M;M)-**3a*** could be to recover **2a*** and **3a*** in a pure state from the mixture and to determine the sign of the rotation. We explored another way that minimises the amount of optically pure **1a*** consumed in such experiments: Step 3 was successfully achieved using chiral chromatography without isolation of the products. Using racemic antiparallel **1, 2,** and **3,** several CSPs and eluting conditions were screened in order to find a column and conditions allowing the complete separation of the six resulting peaks and the assignment of their order of elution. This goal was achieved on CHIRALCEL OJ using EtOH as the eluent as shown in Figure 2.

The peaks at 7.76 and 8.52 min correspond to the antiparallel pair **3,** (+) and (–) forms, respectively. The peaks at 9.55 and 11.03 min correspond to the antiparallel pair **2,** (+) and (–) forms, respectively. The peaks at 13.50 and 17.05 min correspond to the antiparallel pair **1,** (+)-(P;P) and (–)-(M;M) forms, respectively (see above). Starting from optically pure (–)-(M;M) **1a*,** the last eluted peak, analysis of the crude reaction mixture showed that **3a*** (retention 8.63 min) and **2a*** (retention 11.73 min) are both (–) forms. The peak at 19.43 min corresponds to recovered (–)-(M;M) **1a*** and the peak at 16.23 min (–) form corresponds to an unknown impurity (Fig. 3).

The chemical interconversion was checked again starting from (+)-(P;P)-**1a.**

Thus, in Scheme 2 **2a** = (+)-(P;P)-4-Methyl-3-[2-methyl-3-(4-methyl-2-thioxo-3(2H)-thiazolyl)phenyl]-2(3H)-thiazolone; **2a*** = (–)-(M;M)-4-Methyl-3-[2-methyl-3-(4-methyl-2-thioxo-3(2H)-thiazolyl)phenyl]-2(3H)-thiazolone; **3a** = (+)-(P;P)-)-[2-Methyl-1,3-phenylene]bis[4-methyl-2(3H)- thiazolone]; **3a*** = (–)-(M;M)-[2-Methyl-1,3-phenylene]bis[4-methyl-2(3H)-thiazolone].

*Step Four: Synthesis and Separation of 2p and 2p**

Starting from meso **1p,** treatment with methyl iodide and then with MeONa/MeOH produced **2p** and **2p*** together with meso **3p.** Analysis on CHIRALCEL OJ (eluent 100% EtOH) gave three well-resolved peaks. The first, eluting at 7.23 min, corresponds to the meso form **3p,** as confirmed by the absence of signal under polarimetric detection and independent injection of pure **3p.** The peaks eluting at 8.29 and 9.19 min are the (+) and (–) forms, respectively, of the parallel thione-one pair (**2p:2p***) as shown by polarimetric detection. The optically pure (+) form was obtained by semipreparative separation (Fig. 4).

Step Five: Equilibration

The optically pure (+) form corresponding to either **2p** or **2p*** was heated in tetrachloroethene for 3 hrs at 105°C in a Schlenk tube under argon. Evaporation of the

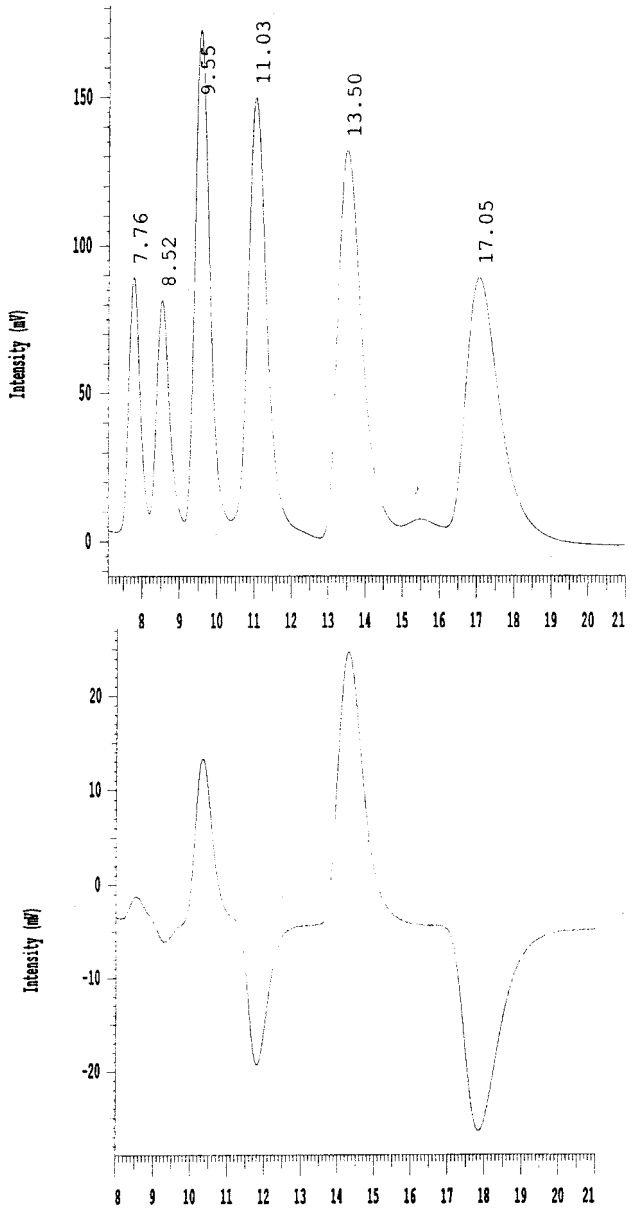


Fig. 2. Chromatography on CHIRALCEL OJ (100 % EtOH) on the racemic mixture **3a/3a*, 2a/2a*,** and **1a/1a*** (in the increasing order of elution times) and corresponding polarimetric trace.

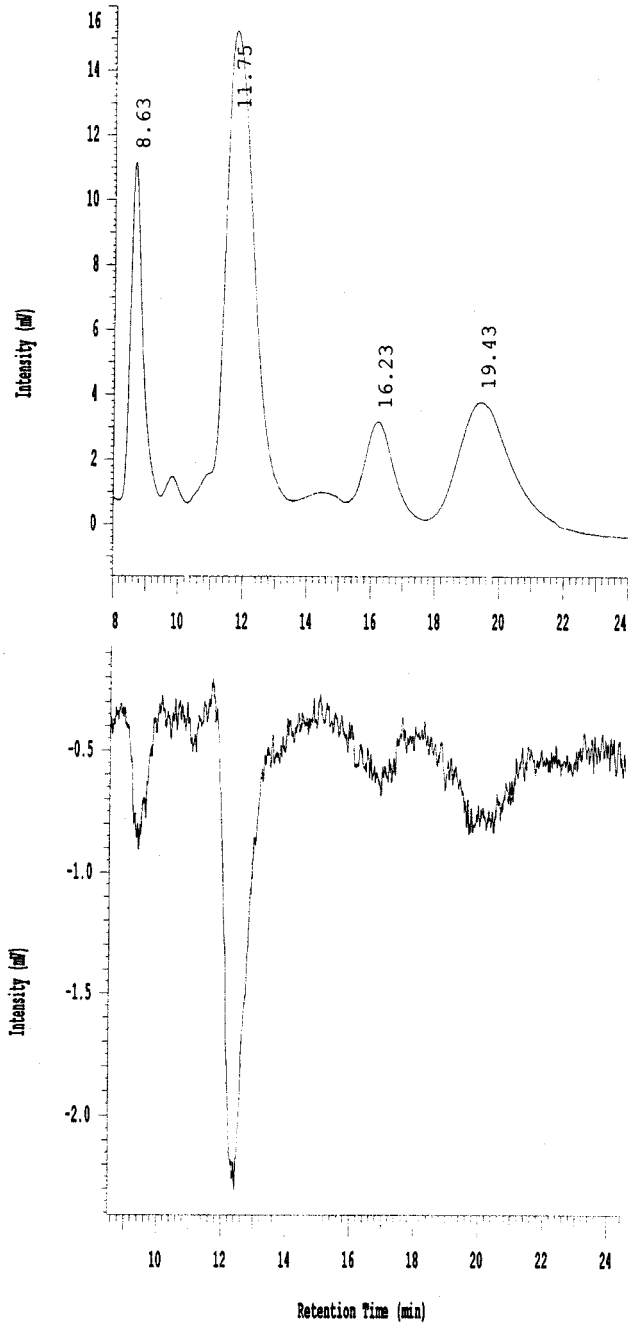


Fig. 3. Chiral HPLC trace of the products resulting from the transformation of **(–)-(M;M)1a*** (8.63 min) into **2a*** (11.75 min) and **3a*** (19.46 min). Polarimetric analyse (CHIRALCEL OJ, 100% EtOH) shows that the three compounds are levorotatory.

solvent on a vacuum line and analysis of the residue on CHIRALCEL OJ showed that the (+) form of the parallel thione one derivative **2** is in equilibrium with the (+) form of the antiparallel **2.** The achiral and highly absorbing compound which elutes at 7.21 min is an impurity formed when heating. Since the absolute configuration of the (+) form of the antiparallel **2** is (+)-(P;P)-**2a** (Step 3), the (+) form of the parallel **2** is **2p** (Fig. 5).

Thus, in Scheme 2 **2p** = (+)-(M)-4-Methyl-3-(P)-[2-methyl-3-(4-methyl-2-thioxo-3(2H)-thiazolyl)phenyl]-2(3H)-thiazolone; **2p*** = (–)-(P)-4-Methyl-3-(M)-[2-methyl-3-(4-methyl-2-thioxo-3(2H)-thiazolyl)phenyl]-2(3H)-thiazolone.

Attempts to Determine the Absolute Configuration by Application of a Chiral Recognition Model on Whelk-O1 Chiral Column

During the time this work was in progress, we received a new so-called (S,S) Whelk-O1 column from Regis. We found that this was able to separate the N-aryl thiazoline-thione atropisomers whereas we found older versions of this column gave very distorted peaks or no elution for thione heterocycles under the same conditions. Both ver-

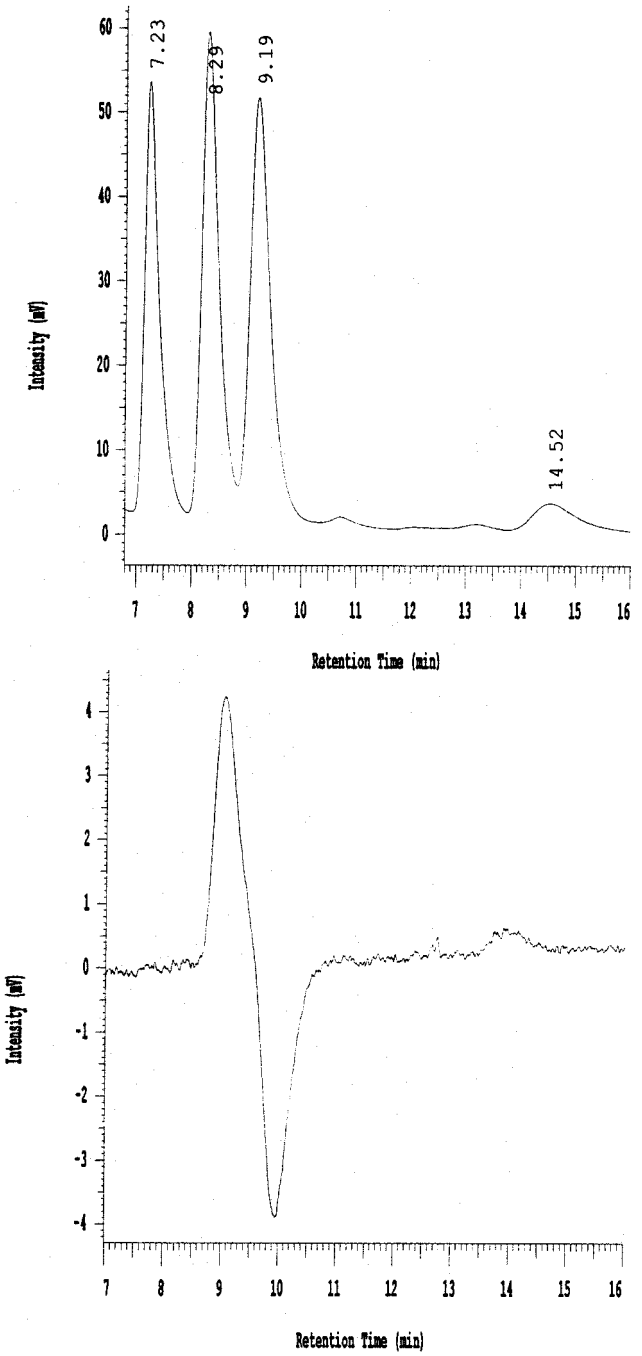


Fig. 4. Chiral HPLC trace showing the retention of the meso **3p** and racemic pair **2p/2p*:** UV and polarimetric analyses (CHIRALCEL OJ, 100% EtOH).

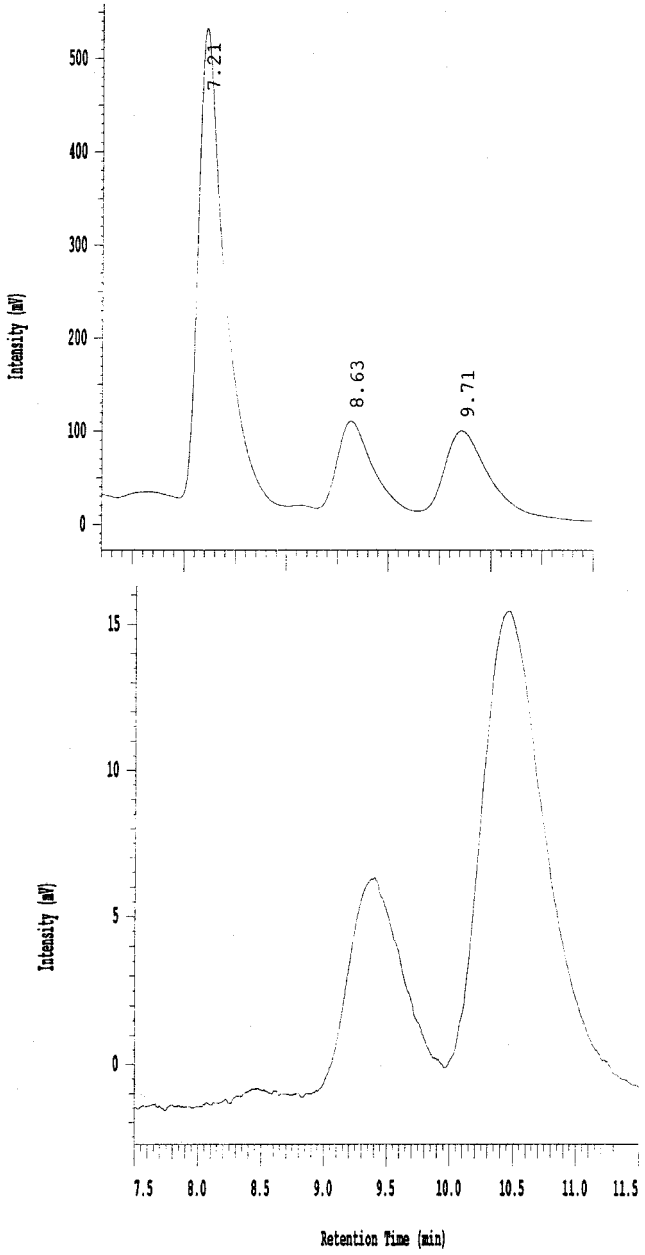


Fig. 5. Chiral HPLC trace of the products of the rotation from the (+) form of the pair **2p:2p*** resulting in **(+)-(P;P) 2a:** UV and polarimetric analyses (CHIRALCEL OJ, 100% EtOH).

sions gave a good separation of the thiazolinone derivatives. This prompted us to attempt the separation of **1a/1a*, 2a/2a*, 2p/2p*,** and **3a/3a*** pairs of racemates on the Whelk-O1 CSP, for which a model of chiral recognition has been proposed.[17] This model claims the determination of the absolute configuration of atropisomeric racemates, including N-aryl-thiazoline-(thi)ones from the order of elution.[17] In other words, our purpose was to check if we could have replaced all the lengthy experimental work we have described above by chiral chromatographic analysis on the new version of Whelk-O1. The results are reported in Table 2.

Satisfactory resolutions were obtained except for the **2p/2p*** pair, which was poorly separated and highly retained. Due to very poor solubility in the solvent it was not possible to determine the order of elution for this compound. The very high retention is probably due to the high dipole found in **2p/2p*** forms. The first eluted enantiomer for N-(2-methylphenyl)-4-methyl-thiazoline-thione and for N-(2-methylphenyl)-4-methyl-thiazolin-one analogue is (+)-(P), the same order is found for the pair **1a/1a*** in which

TABLE 2. Chromatographic data on (S,S)-Whelk-O CSP (Eluent 80:20 Hexane/propan-2-ol)

Cpds	T	Flow	First	Rt_1	Rt_2	k_1	α
TT	25	1	(+)-(P)	10.27	14.44	2.66	1.56
TO	25	1	(+)-(P)	14.15	26.53	3.71	2.11
1a/1a*	25	1	(+)-(P;P)	30.03	33.92	9.1	1.13
2a/2a*	25	1	(–)-(M;M)	43.45	49.04	13.48	1.14
2p/2p*	35	1.5	?[a]	62	68	19.6	1.10
3a/3a*	25	1	(–)-(M;M)	47.57	62.33	14.85	1.33
1p	35	2	meso	118.83	—	77.6	—
3p	35	2	meso	81.95	—	53.6	—

[a]Very broad peaks and poor solubility prevented polarimetric determination of the order of elution.

the (+)-(P;P) form is eluted first. This order of elution fits the chiral recognition model. To our surprise the (–)-(M;M) forms are eluted first in both **2a/2a*** and **3a/3a*** pairs. The model of chiral recognition on Whelk-O1 has to be revisited in the case of rather large structures such as those depicted in this article.

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REFERENCES

1. Eliel EL, Wilen SH. Stereochemistry of organic compounds. New York: John Wiley & Sons; 1994. p 126–147.
2. Roussel C, Djafri A. Separation of enantiomers on triacetylcellulose and barriers to rotation in m-substituted N-phenyl-delta-4 thiazoline-2-thiones. Nouv J Chim 1986;10:399–404.
3. Roussel C, Popescu C. Effects of alkyl substituents on chiral separation of N-arylthiazolin-2-(thi)-one atropisomers on tris(p-methylbenzoylcellulose) beads and cellulose triacetate: lipophilicity aspects. Chirality 1994;6:251–260.
4. Roussel C, Bonnet B, Piederriere B, Suteu C. Enantioselective correlation between retention factor and lipophilicity index in chiral separation on cellulose and amylose tris(3,5-dimethylphenylcarbamate) CSPs in reversed mode: a case study. Chirality 2001;13:56–61.
5. Roussel C, Suteu C. An investigation into chiral recognition mechanism of N-arylthiazolin-2(thi)one atropisomers on CHIRALCEL OJ by factorial design and lipophilicity approaches. J Chromatogr A 1997;761:129–138.
6. Pirkle WH, Welch CJ. Use of simultaneous face to face and face to edge π-π interactions to facilitate chiral recognition. Tetrahedron: Asymmetry 1994;5:777–780.
7. Roussel C, Stein JL, Beauvais F. Separation of atropisomers on CSP microcrystalline cellulosetriacetate and barriers to rotation in some polymethyl-3-phenyl-Δ-4-thiazoline-2-thiones and their oxygen analogues. New J Chem 1990;14:169–173.
8. Roussel C, Chemlal A. Resolution of N-aryl-4-thiazoline-2-thione atropisomers on microcrystalline cellulose triacetate: determination of the absolute configuration of 3-(2-tolyl)-4-t-butyl-4-thiazoline-2-thione atropisomers and their oxygen analogues. New J Chem 1988;12:947–952.
9. Hirtopeanu A, Suteu C, Uncuta C, Mihai G, Roussel C. Bis[oxo/thioxothiazolinyl]aromatic compounds—synthesis and conformational assigment. Eur J Org Chem 2000;1081–1090.
10. Mannschreck A. Chiroptical detection during liquid chromatography. Chirality 1992;4:163–169.
11. Brandl F, Pustet N, Mannschreck A. Chiroptical detection during liquid chromatography 7. The rotation angle/absorbance ratio of chiral molecules. Its possible use for on-line analysis during preparative separations of enantiomers. J Chromatogr 2001;909:147–154.
12. Goodall DM. Chiral analysis by polarimetric detection. Trends Anal Chem 1993;12:177–184.
13. Hanai T, Suzuki I, Nakanishi K. Polarimeter as a liquid chromatography detector for optical isomers. Anal Sci 1985;1:483–484.
14. Francotte ER. Enantioselective chromatography as a powerful alternative for the preparation of drug enantiomers. J Chromatogr A 2001;906:379–397.
15. Hirtopeanu A, Mihai M, Mihai G, Roussel C. Bis[oxo/thioxothiazolinyl]aromatic compounds-Stereochemical aspects. Heterocycles 2000;53:1669–1675.
16. Roussel C, Adjimi A, Chemlal A, Djafri A. Comparison of racemization processes in 1-aryl-pyrimidine-2-thione and 3-aryl-thiazoline-2-thione atropisomers and their oxygen analogues. J Org Chem 1988;53:5076–5080.
17. Pirkle WH, Koscho ME, Wu Z. High-performance liquid chromatographic separation of the enantiomers of N-aryl thiazolinones and their sulfur derivatives on a synthetic chiral stationary phase. J Chromatogr A 1996;726:91–97.