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Chiral properties of tetrathiatriarylmethyl spin probes†

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We report that tetrathiatriarylmethyl (trityl) EPR probes are chiral molecules at room temperature, the two stereoisomers that differ in their helicity being configurationally stable enough to be separated and stored independently.

Stable tetrathiatriarylmethyl radicals, **1a** (Fig. 1), belong to a family of trityl radicals extensively used for electron paramagnetic resonance imaging (EPRI),¹ oximetry,² dynamic nuclear polarization (DNP)³ and the detection of superoxide radical anions.⁴ These water soluble, paramagnetic species have unique properties, namely an exceptional biostability, a narrow EPR linewidth (the anoxic linewidth is <100 mG), and they display a low concentration-dependent broadening of the EPR line.⁵

Accordingly, this class of carbon-centered radicals has been selected by several laboratories for chemical modifications with the aim of diversifying their applications and improving their performances. In this context, a series of new tetrathiatriarylmethyl radicals have recently been synthesized for various applications: dendritic trityl radicals that possess a higher stability,⁶ ester-derivatized forms used for intracellular oxygen measurement,⁷ amino-derivatized forms allowing dual pH/oxygen assessment,⁸ fluorinated variants used to quantify tissue oxygenation with a high sensitivity in perfluorocarbon formulations,⁹ aldehyde derivatives with enhanced sensitivity to oxygen¹⁰ and trityl-nitroxide biradicals dedicated to the simultaneous measurement of redox status and oxygenation.¹¹

Surprisingly, during these numerous developments of trityl radicals for biomedical uses, the fundamental question of the potential chirality associated with their helicoidal conformation has never been addressed. However, it has been shown that tetrathiatriarylmethyl radicals adopt a propeller shape in which all of the rings twist in the same direction.¹² This geometrical arrangement allows two conformations to occur, namely the right-handed (*P*) and left-handed (*M*) helices,

which feature an enantiomeric relationship (Fig. 2). If the conformational equilibration between these two helices is rapid, then the molecules will not display chiral properties. However, due to the large steric bulk of the three aryl moieties, one can expect the rotation around the three carbon–carbon bonds connected to the central carbon to be restricted, thus limiting the stereoisomerisation of the two helices. If this is the case and the interconversion between the two enantiomeric helices is slow, then the two enantiomeric helices could potentially be separately isolated. It is therefore crucial to study the dynamics of this conformational equilibrium, since the chirality could have important consequences for the applications, especially as different enantiomers can display different biological activities. In addition, the connection of chiral substituents to the trityl core would result in the formation of diastereoisomeric mixtures¹¹ possibly with different chemical, biological and physical properties (*e.g.* different EPR spectra). Finally, the chirality associated with an open-shell system would confer interesting chiro-optical properties to the tetrathiatriarylmethyl radicals, such as nonlinear optical (NLO) responses.¹³

As trityl alcohol is also a propeller-shaped molecule,¹⁴ tetrathiatriarylmethyl alcohols, the direct precursors of the radicals (see Fig. 1), might similarly be chiral if the rotations around the three carbon–carbon bonds connected to the central carbon are restricted. Indeed, when we added Pirkle's alcohol, a known chiral solvating agent (CSA), to a solution of **2d**¹⁵ in CDCl₃, we observed splitting of the ¹H and ¹³C NMR signals on the spectra measured at room temperature (see ESI†), indicating the formation of two diastereoisomeric solvation complexes. This observation shows that the interconversion of the helicity does not occur rapidly on the NMR

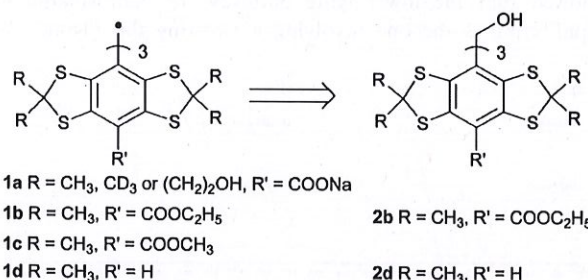


Fig. 1 Representative tetrathiatriarylmethyl radicals with biomedical uses (**1a**) and their precursors.

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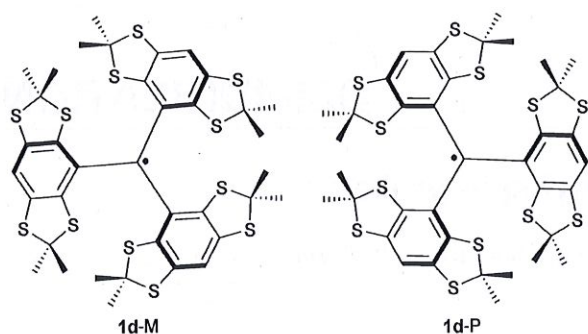


Fig. 2 The two conformational enantiomers of tetrathiatriarylmethyl radical **1d**.

time scale at 25 °C. Further experimental evidence of the chirality of tetrathiatriarylmethyl alcohols was obtained for alcohol **2b**¹⁵ by analytical HPLC carried out on a chiral stationary phase (CSP HPLC) at room temperature. Indeed, the resolution of the peaks corresponding to the two enantiomers of **2b**† shows that there is no rapid isomerisation process on the HPLC time scale either (Fig. 3a). Under the same conditions, resolution of the tetrathiatriarylmethyl radical **1b** was also very visible (Fig. 3b). As expected for enantiomers, the CD spectra recorded by stopped-flow spectrometry at the maximum of CD intensity for the two peaks of **1b** are mirror images (see ESI†). This is the first direct experimental proof of the chirality of a tetrathiatriarylmethyl radical at room temperature.

In order to study the kinetic of isomerization, we first investigated the conformational pathway using computational (DFT) methods.§ There are four potential pathways for interconversion: the *n*-ring flip mechanisms (*n* = 0, 1, 2, 3) in which zero, one, two or three aromatic ring(s) rotate(s) in a conrotatory fashion across the plane perpendicular to the one formed by the three aromatic carbons linked to the central carbon, while the other ring(s) rotate(s) in the opposite direction through the reference plane (Fig. 4, for the complete pathways see ESI†).¹⁶ We have investigated all these four possible mechanisms for radical **1d** (the model compound). Transition state structures could be found for the two- and the three-ring flip mechanisms, **TS-two** and **TS-three**, respectively. For the other mechanisms, our calculations revealed that bringing two of the rings into the same plane would imply such a spatial proximity between the thio substituents of these aryl groups (<0.5 Å between two S atoms) that these structures are simply not realistic. Calculating the transition state energies showed that the lower lying pathway for conformational equilibrium is the one involving a two-ring flip (Table 1).

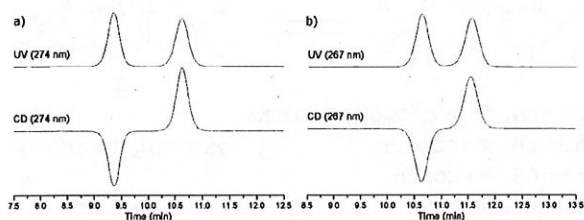


Fig. 3 CSP HPLC chromatograms with UV and CD detection of trityl alcohol **2b** at 274 nm (a) and radical **1b** at 267 nm (b).

It is interesting to note that our calculation suggests that solvation should not have an important effect on the activation free energy.

According to the calculated activation free energy in solution (29.5 kcal mol⁻¹), the two enantiomers of **1d** should be configurationally stable enough to be isolated and stored separately. We thus undertook the resolution of a tetrathiatriarylmethyl radical on a preparative scale.

We first aimed to synthesize the diastereoisomers by derivatization of the trityl core with an enantiomerically pure compound. Our strategy consisted of derivatizing the corresponding trityl alcohol instead of the radical because of its higher chemical stability; the radical could easily be generated from the alcohol¹⁷ after separation of the diastereoisomers. Accordingly, trityl alcohol **3a** has been synthesized by aminolysis of **2b** with (*S*)- α -methylbenzylamine as the chiral derivatizing agent (Scheme 1). The ¹H and ¹³C NMR spectra of **3a** clearly show the presence of two diastereoisomers contrasting with the benzylamine analogue **3b** that does not possess an additional chiral center (see ESI†). Unfortunately, all of our attempts to separate diastereoisomers **3a** by crystallization or chromatography failed. Notably, no induction of asymmetric carbons on the helicity has been observed as the ratio of diastereoisomers of **3a** was 50:50 (determined by NMR).

We then investigated the possibility of direct resolution of the enantiomers using semi-preparative CSP HPLC. Again, the resolution of trityl alcohol **2b** instead of radical **1b** has been considered, due to the better resolution obtained for alcohol **2b** by analytical CSP HPLC (see Fig. 3). Transposition of the analytical CSP HPLC method to a semi-preparative scale allowed us to obtain the two enantiomers of **2b** in milligram amounts with an enantiomeric excess of 100% (see ESI†). Further scaling-up is limited due to the low solubility of **2b** in the eluent. The two optically pure enantiomers of **2b** were then separately converted into the corresponding enantiomers of radical **1b** by treatment with neat trifluoroacetic acid, a known procedure for the quantitative conversion of tetrathiatriarylmethyl alcohols into radicals.¹⁷ Analytical CSP HPLC of the two enantiomers confirmed their enantiomeric purity (see ESI†).

Having these two enantiomers **1b** in hand, we investigated their conformational stability in solution by following the kinetics of their racemization (see ESI†). In the event, we found that, at room temperature, **1b** isomerizes slowly in EtOAc (*t*_{1/2} enantio ≈ 1 month), thus confirming that the two enantiomers of **1b** can be separately stored for months in the freezer. The free activation energy calculated from the obtained kinetic constant, using the Eyring equation, is 25.98 ± 0.02 kcal mol⁻¹ at 25 °C. This experimental value is in good agreement with our calculations and supports the two-ring flip as the enantiomerization mechanism. A complete study of the enantiomerization mechanism and the determination of the activation parameters for other substituted tetrathiatriarylmethyl radicals, tetrathiatriarylmethanols and tetrathiatriarylmethanes are currently under study and will be reported elsewhere.

In conclusion, we have shown that tetrathiatriarylmethyl radicals, which are very attractive spin probes, are chiral molecules at room temperature. The two enantiomers that

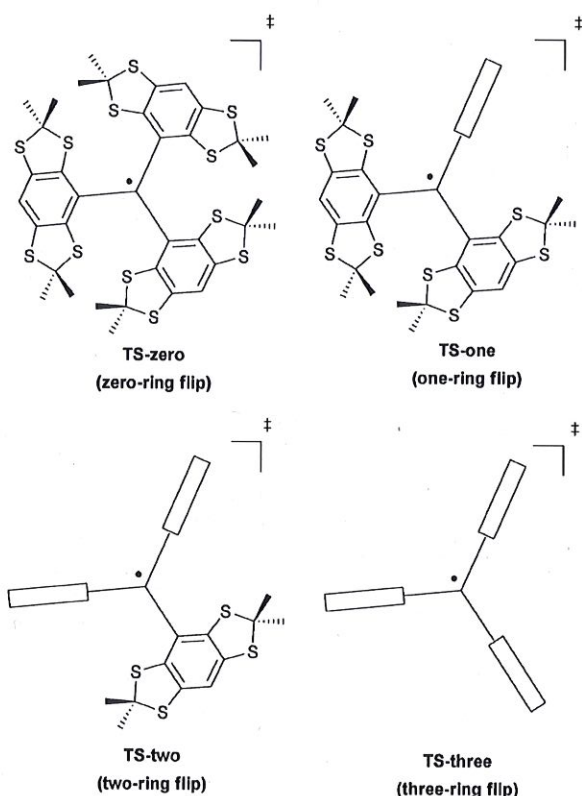
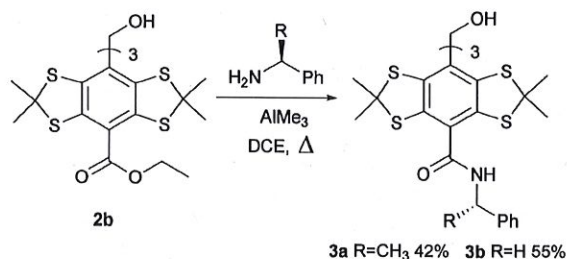


Fig. 4 The four potential transition states for the isomerization of radical 1d.

Table 1 The computed energy barrier ($\Delta E^\ddagger$) and activation free energy ($\Delta G^\ddagger$) in the gas phase and in solution for radicals 1d and, in brackets, 1c (energies are given in kcal mol⁻¹ relative to reactant)

	$\Delta E^\ddagger$	$\Delta G^\ddagger$	$\Delta G^\ddagger$ (EtOAc)
TS-zero	— ^a	— ^a	— ^a
TS-one	— ^a	— ^a	— ^a
TS-two	25.6(27.8)	28.3	29.5
TS-three	74.8	77.9	79.6

^a All of our attempts to obtain such a transition state structure showed the unfeasibility of having two (or three) of the aromatic rings on the same plane without breaking a bond. This enantiomerization pathway is thus not accessible.



Scheme 1

differ in their helicity are configurationally stable enough to be separated and independently stored for months in the freezer but slow racemization occurs in solution at room temperature.

These observations are of primary importance and should be taken into account in the further development and derivatization of these species for biomedical uses.

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Notes and references

‡ The right- and left-handed helices showing opposing helicity in the circular dichroism (CD) chromatogram and identical UV spectra (see ESI†).

§ Geometry optimization was carried out using the UB3LYP hybrid density functional, as implemented in Jaguar 7.5, with the standard split valence polarized 6-31G* basis. Single point energy calculations were carried out at the UB3LYP-D level of theory (including an approximation correction for dispersion) with the larger 6-311+G** basis, using the ORCA package. Solvation effects were estimated at the UB3LYP-D/6-31G* level using the conductor-like screening model (COSMO) as implemented in ORCA, using the parameters appropriate for ethyl acetate. See ESI† for full computational details and related references.

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