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Chemical Synthesis of [^{2}H]-Ethyl Tosylate and Exploration of Its Crypto-optically Active Character Combining Complementary Spectroscopic Tools

Timothée Naret, Philippe Lesot, Andrew R. Puente, Prasad L. Polavarapu, David-Alexandre Buisson, Jeanne Crassous, Grégory Pieters, and Sophie Feuillastre*

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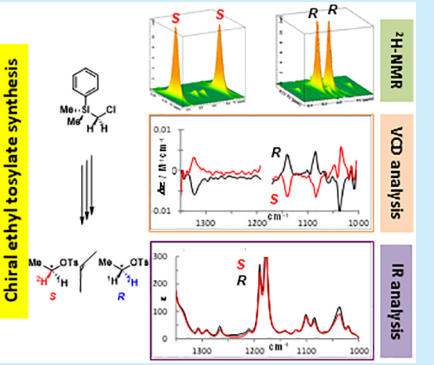
ABSTRACT: Small chiral molecules are excellent candidates to push the boundaries of enantiodiscrimination analytical techniques. Here is reported the synthesis of two new deuterated chiral probes, (*R*)- and (*S*)-[^{2}H]-ethyl tosylate, obtained with high enantiomeric excesses. Due to their crypto-optically active properties, the discrimination of each enantiomer is challenging. Whereas their enantiopurity is determined by ^{2}H NMR in chiral anisotropic media, their identification was performed by combining quantum chemical calculations and vibrational circular dichroism analysis.



Chirality is a key molecular property that confers specific characteristics to matter. Due to the single spatial orientation of chiral compounds, numerous applications are found in Biology, Chemistry, and Physics and also in Materials Science.[1] Enantiodiscrimination based on planar light polarization is a routine tool to distinguish optically active isomers. However, this approach fails for analyzing crypto-optically active compounds,[2] which are ideal candidates to explore the limits of detection and identification of modern analytical techniques.

From an application point of view, crypto-optically active entities show a strong potential as probes for enzymatic or chemical mechanism studies.[3] The smallest of them, the chiral methyl group, C^{1}H^{2}H^{3}H, has been widely described and used to advance knowledge of living processes. Notably, it has been very helpful for the understanding of methyl transferase enzyme mechanisms.[4] Nevertheless, the use of the chiral methyl group suffers from the following drawbacks: (i) the presence of a radioactive tritium atom causing difficulties in terms of synthesis, handling, storage, analysis, and waste management; (ii) the challenging measurement of enantiomeric excess (ee) in the case of isotopic chirality. So far, only fastidious syntheses of a well-controlled diastereoisomer (without epimerization of the chiral center) or enzymatic resolution, both associated with ^{3}H NMR analysis, have been disclosed to determine ee.[4a,5] To circumvent these limitations, the design of specific deuterium-labeled probes may simplify the generation of stereogenic centers thanks to the nonradioactive nature of these analytes and their analysis with the possibility to use advanced techniques such as ^{2}H NMR in chiral liquid crystals (CLCs) to evaluate their enantiopurity ratios.[6]

In this work, we propose to replace the tritium atom of the chiral methyl group with a methyl moiety to obtain the nonradioactive ethyl chiral group. After the achievement of the first chemical synthesis of such crypto-optically active probes with a high deuterium atom incorporation,[7] to completely describe each enantiomer, modern analytical techniques were used (Figure 1). It is important to note that some alkyl

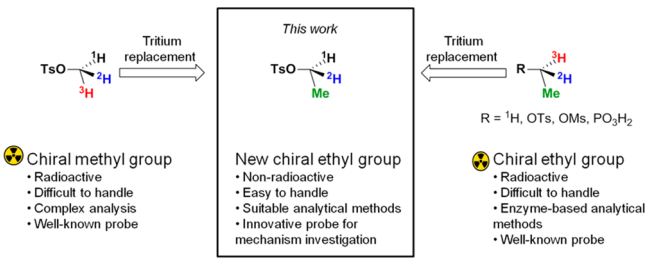


Figure 1. Goals of this work.

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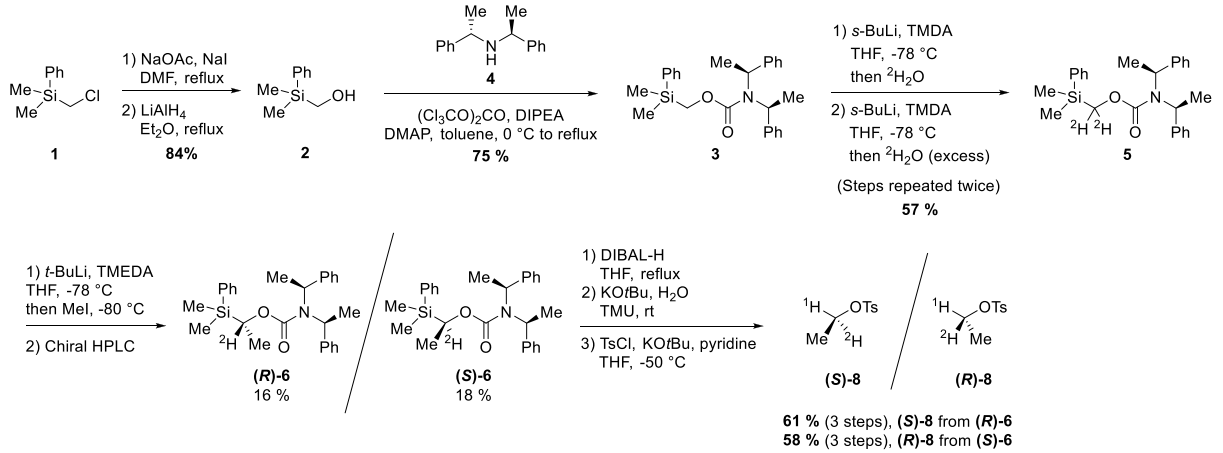
Scheme 1. Synthesis of Deuterated Crypto-optically Active Probes (*R*)-8 and (*S*)-8



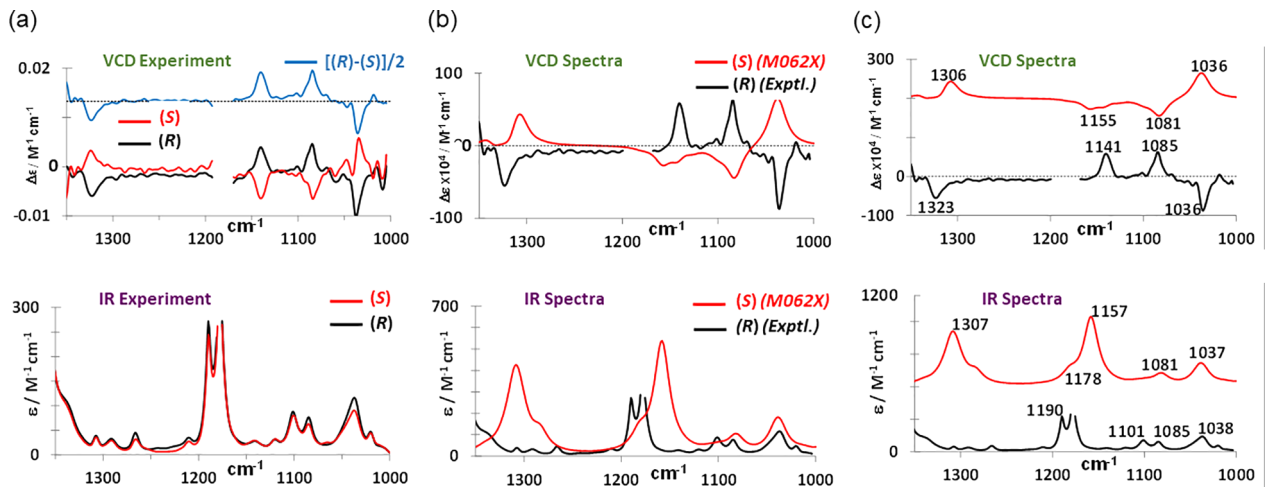


Figure 2. (a) Solvent-subtracted experimental VCD (top panel) and (bottom panel) and IR spectra of (*S*)-**8** (red) and (*R*)-**8** (black) in the 1350−1000 cm^{-1} region measured in CD_2Cl_2 solvent. The topmost trace (blue), obtained as one-half of the difference between VCD spectra of *R*- and *S*-enantiomers, gives the VCD spectrum of (*R*)-**8** with improved S/N ratio (slighlty shifted upward for clarity). VCD spectra in the 1168−1198 cm^{-1} region and IR peak at ∼1178 cm^{-1} are not displayed due to noise associated with excess absorbance. (b) Comparison of the experimental VCD and IR spectra for (*R*)-**8** (black traces) and the predicted spectra for the *S*-enantiomer at the M06-2X/6-311++G(2d,2p) level (red traces). (c) Spectra in panel (b) are shifted upward and stacked above each other to display the peak positions. All calculated frequencies are scaled by a factor of 0.9725. Note that the strongest IR band around 1180 cm^{-1} has been truncated as strong absorption may induce artifacts.

transferase enzymes tolerate various alkyl groups as ethyl, allyl, or propargyl during the transfer process,[8] and so chiral ethyl probes could be helpful for studies of this class of enzymes. As a matter of fact, the radioactive chiral ethyl group has already been used for the understanding enzymatic mechanisms.[9]

Our synthetic approach to the chiral ethyl group was inspired by the one described by Hammerschmidt et al. for the synthesis of chiral methyl tosylate[10] (Scheme 1). First, the chloromethyldimethylphenylsilane **1** was converted to the corresponding alcohol **2** in good yield. Then, carbamate **3** was obtained by the condensation of bis((*S*)-1-phenylethyl)amine **4** with triphosgene followed by the addition of alcohol **2**. Carbamate **3** was then deuterated in the α-position of the silicon atom via two consecutive lithiation/deuteration sequences, allowing a ^2H incorporation over 99%. Then, the dideuterated carbamate **5** was alkylated using iodomethane through an optimized lithiation process (see Table S1 for optimization). The use of *t*-BuLi at −50 °C (Table S1, entry 4) led to a mixture of diastereoisomers **6** (Scheme 1) with a high conversion. Then the two diastereoisomers were separated using chiral HPLC. Treatment of monodeuterated **6** with DIBAL-H cleaved the carbamate moiety to give the corresponding chiral alcohol (not isolated due to its high volatility). A consecutive Brook rearrangement followed by the tosylation of the ethanol intermediate gave chiral ethyl tosylates **8**.

As the determination of the absolute configuration (AC) is essential for the use of such chiral molecules as probes,[11,12] a combination of several modern analytical methods for the characterization of isotopic chirality has been described. First, in 2007, X-ray crystallography and vibrational circular dichroism (VCD) were used to determine the AC of (*R*)-4-ethyl-4-methyloctane.[13] Then, quantum chemical (QC) computations were coupled with Raman optical activity[14] or VCD[15−17] experiments to provide essential information on various crypto-optically active compounds.

During our whole synthetic pathway, no information regarding the AC could be obtained by optical rotation, so VCD analysis was performed.[18] Figure 2a (top) reveals mirror image signals for enantiomers (for complete VCD spectra, see Figure S17). The most relevant signatures were observed

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between 1000 and 1050 cm^{-1} and between 1250 and 1350 cm^{-1}. To assign signals to products, QC calculations were performed (Figure 2b (top)), and then calculated and experimental IR and VCD spectra were compared (Figure 2b,c). The M06-2X-based frequencies were scaled down by a factor of 0.9725, giving the maximum similarity with the experimental VCD and IR spectra. Interestingly, although [^{2}H]-ethyl tosylate **8** is called crypto-optically active, it actually displays a VCD activity.

A comparison using the B3LYP functional (Figures S19 and S20) shows that M06-2X gives better agreement to the experiment overall. The VCD band signs predicted that the *S*-stereoisomer appears clearly opposite to those seen for (*R*)-**8**. Therefore, both levels of calculation enabled confirmation of the AC of (*R*)-**8** and (*S*)-**8** enantiomers in relation to the signs of the VCD signals (Figure 2). It is interesting to note that the corresponding *S*-(−) and *R*-(+) AC of [1-$^2H_1$]ethanol were determined by three methods (enzymatic,[19] chemical correlation from sugars,[20] and VCD[21]).

Finally, the isotopic enantiomers of **8** were studied with the help of ^{2}H-{^{1}H} 2D NMR recorded at 92.1 MHz and 297 K in the poly(γ-benzyl-L-glutamate) (PBLG)-based chiral mesophase (20.7% m_{PBLG}/m_{tot}). In such lyotropic CLCs, each monodeuterated enantiomer generates a single ^{2}H quadrupolar doublet (^{2}H-QD) when the spectral enantiodiscrimination occurs;[6] two ^{2}H-QD are therefore theoretically expected if the *R*/*S* ^{2}H signals are spectrally resolved.

This occurrence has been observed on the ^{2}H-{^{1}H} 2D NMR spectrum recorded at the natural abundance deuterium (NAD) level of commercial ethyl tosylate when DMF is used as organic cosolvent (Figures 3a and S22 -see details in SI-). In this case, each monodeuterated enantioisotopomer ((*S*)-C*DH- and (*R*)-C*HD-) associated with the pro-(*S*) and pro-(*R*) hydrogenated enantiotopic position are detected. Note that using chlorofom, a weakly polar cosolvent, no spectral enantiodiscrimination occurs.[6b,c]

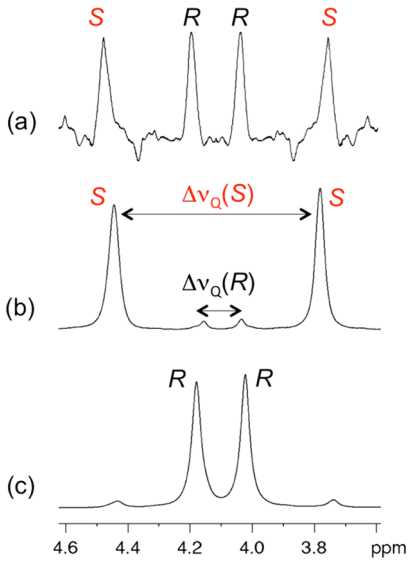

Figure 3. (a) NAD ^{2}H-{^{1}H} 1D NMR projection (sum of columns) extracted from the tilted and symmetrized *Q*-resolved Fz 2D experiment (details are in Supporting Information) of racemic ethyl tosylate (42 mg), PBLG (130 mg), DMF (450 mg). (b,c) Same as (a) but obtained with (*S*)-**8** and (*R*)-**8**, respectively (5 mg of labeled plus 35 mg of unlabeled *R*/*S*-solute), PBLG (130 mg), and DMF (452 mg). The degree of polymerization of PBLG is equal to 710.

The enantiopurity of the two isotopically labeled compounds **8** (Figure 3b,c) has been determined at the same *T* (297 K) and using identical PBLG mass ratio (20.9%), thus leading to a magnitude of ^{2}H splittings identical to those observed on the anisotropic NAD NMR spectra. The large difference in intensity between each ^{2}H-QD of (*S*)-**8** and (*R*)-**8** reveals the presence of a significant enantioenrichment. Deconvolution of the signals indicates that the chiral ethyl tosylate was prepared with an ee greater than 90% for both isomers.

In conclusion, the first chemical synthesis of a nonradioactive chiral ethyl group was developed. Although the anisotropic ^{2}H NMR allowed enantiopurity evaluation, the VCD analysis/QC calculation combination permitted the AC of each synthesized enantiomer of the chiral ethyl tosylate to be assigned. Due to their easier synthetic access and handling compared to that with their radioactive counterparts, such small crypto-optically active probes possess a great potential of applications to decipher alkyl transferase enzyme reaction mechanisms.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.0c03219.

Experimental procedure details, NMR data for compounds **2**−**8**, QC calculation, anisotropic ^{2}H NMR analysis (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Sophie Feuillastre − *Université Paris-Saclay, CEA, INRAE, Département Médicaments et Technologies pour la Santé (DMTS), F-91191 Gif-sur-Yvette, France;* orcid.org/0000-0002-9327-2050; Email: sophie.feuillastre@cea.fr

Authors

Timothée Naret − *Université Paris-Saclay, CEA, INRAE, Département Médicaments et Technologies pour la Santé (DMTS), F-91191 Gif-sur-Yvette, France*

Philippe Lesot − *Université Paris-Saclay, ICMMO, UMR CNRS 8182, RMN en Milieu Orienté, F-91405 Orsay cedex, France;* orcid.org/0000-0002-5811-7530

Andrew R. Puente − *Department of Chemistry, Vanderbilt University, Nashville, Tennessee 37235, United States*

Prasad L. Polavarapu − *Department of Chemistry, Vanderbilt University, Nashville, Tennessee 37235, United States;* orcid.org/0000-0001-6458-0508

David-Alexandre Buisson − *Université Paris-Saclay, CEA, INRAE, Département Médicaments et Technologies pour la Santé (DMTS), F-91191 Gif-sur-Yvette, France*

Jeanne Crassous − *Univ. Rennes, Institut des Sciences Chimiques de Rennes, UMR CNRS 6226, F-35042 Rennes cedex, France;* orcid.org/0000-0002-4037-6067

Grégory Pieters − *Université Paris-Saclay, CEA, INRAE, Département Médicaments et Technologies pour la Santé (DMTS), F-91191 Gif-sur-Yvette, France;* orcid.org/0000-0002-3924-8287

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.orglett.0c03219

Notes

The authors declare no competing financial interest.

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