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Synthesis of chiral fluorine-tagged reference standards for the ^{19}F NMR-based stereochemical analysis of sulfoxides at trace analytical levels

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ABSTRACT

Chiral fluorine-tagged sulfoxides of known absolute configuration have been synthesized. These compounds are required as reference standards to validate a ^{19}F NMR-based micromethod for the stereochemical analysis of biosynthetic fatty acyl sulfoxides.

^{19}F NMR is a useful method for tracking fluorinated substrates in enzymatic reactions in vitro or in vivo. The lack of naturally occurring interferences, inherently high sensitivity and the wide chemical shift range featured in ^{19}F NMR-based methodologies has a led to numerous applications in the study of biological systems.[1] Our group has shown that ω-fluorine-tagged substrate analogues[2a] are useful mechanistic probes for a large family of medicinally important enzymes known as fatty acid desaturases.[3] Recently, we demonstrated that ^{19}F NMR could be used in combination with a well-characterized, chiral solvating agent, (*S*)-(+)-α-methoxyphenylacetic acid ((*S*)-(+)-MPAA), to distinguish between fluorine-tagged sulfoxide enantiomers at the trace (nanomole) level.[4] Further exploration of the scope and limitations of this methodology was prompted by a recent theoretical study[5] that predicted possible anomalies in the behaviour of ^{19}F NMR chemical shifts of fluorinated sulfoxides complexed to MPAA. In this Letter, we show that the direction of induced non-equivalence for ^{19}F and ^{1}H chemical shifts of a remote CH_2F reporter group may differ depending on the nature of the solvating agent.

In a previously reported, 'proof of principle' experiment,[4] methyl 15-fluoro-11-thiapentadecanoate **1** was converted (in part) to the corresponding sulfoxide **2** by actively growing *Saccharomyces cerevisiae* cultures.[6] Stereochemical analysis of biosynthetic methyl 15-fluoro-11-thiapentadecanoate S-oxide **2** at the trace analytical level (40 nmol) was performed using ^{19}F NMR in combination with (*S*)-(+)-MPAA.[7] Application of a Pirkle-type binding model (Fig. 1a), that had been previously validated for ^{1}H and ^{13}C NMR applications,[7] led to the conclusion that the predominant enantiomer of **2** (32% ee) was (*S*)-configured. We now describe how this inference was put to a rigorous test using authentic samples of structurally related reference standards, namely, (*R*)- and (*S*)-15-fluoro-11-thiapentadecane S-oxide **3**.

(*R*)- and (*S*)-**3** were synthesized in four steps from commercially available 1-bromo-4-fluorobutane (Scheme 1) in 5% and 20% yield, respectively. The key step in the synthetic sequence is the preparation of diastereomerically enriched DAG-(*S*)- or (*R*)-ω-fluorobutanesulfinates (de 95% and >99%, respectively) (DAG = diacetone-D-glucose) from the corresponding ω-fluorobutanesulfinyl chlorides using the method of Alcudia and co-workers[8,11] The absolute configuration of the sulfinates was confirmed by a comparison of the chiroptical and NMR data (characteristic ^{1}H resonances (H2) and ^{13}C resonances (C1–C4) for these compounds with that of literature values reported for the two diastereomeric DAG *n*-propylsulfinates[11] and DAG *n*-butylsulfinates.[12] Treatment of DAG-(*S*)- or (*R*)-ω-fluorobutanesulfinates with 3 equiv[13]of decylmagnesium bromide yielded enantiomerically enriched samples of (*R*)- and (*S*)-**3**, respectively (ee 90% and >98%, respectively).[14] This substitution reaction is known to proceed with inversion of configuration at the sulfinyl centre of the DAG-ω-fluorobutanesulfinates.[11]

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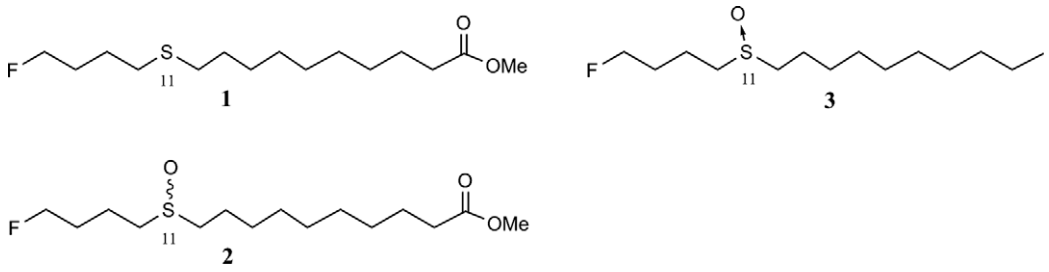


With the provision of fluorine-tagged sulfoxy reference standards, we were in a position to evaluate the ability of ^{19}F NMR versus ^{1}H NMR to report stereochemical information. The ^{1}H-decoupled ^{19}F NMR spectrum of the mixture of (*R*)-**3** and (*S*)-**3** (1:2 ratio, ca 20 μmol total) to which (*S*)-(+)-MPAA (4 equiv) was added was obtained and is shown in Figure 2A. The corresponding ^{1}H NMR signals (doublet of triplets ($^{2}J_{HF}$ = 47.4, $^{3}J_{HH}$ = 5.6, 2H)) due to the CH_2F reporter resonance are shown in Figure 2B. As expected, the ^{1}H resonances of the (*R*)-sulfoxide were shifted significantly upfield relative those of the (*S*)-sulfoxide ($\Delta\delta$ = 0.018 ppm). This result is in agreement with the shielding effects portrayed in the Pirkle-type[15] interaction of **2** with this solvating agent (Fig. 1a) and previous validation experiments.[7,12] However, the corresponding ^{19}F resonance of the (*R*)-sulfoxide was shifted *downfield* relative to the peak for the (*S*)-sulfoxide ($\Delta\delta$ = 0.01 ppm). This phenomenon was also observed in the NMR analysis of the (*R*)-**3**:(*S*)-**3** mixture at lower amounts of analytes (40 nm) (data not shown).

The generality of the results documented above was probed by conducting NMR experiments with two other solvating agents: (1) the anthryl analogue of (*S*)-MPAA, that is, (*S*)-(+)-(9-anthryl)methoxyacetic acid ((*S*)-(+)-AMA)[16–18] and (2) the corresponding 'Pirkle alcohol'—(*R*)-(−)-2,2,2-trifluoro-1-(9-anthryl)-ethanol ((*R*)-(−)TFAE).[19a,b] In both cases, the expected shielding effects (Fig. 1b and c) were observed for the ^{1}H NMR resonances assigned to the reporter group as shown in Figure 2D and F. The larger $\Delta\delta$ observed for the anthryl- versus phenyl-based solvating agents was also anticipated.[16] However, the corresponding ^{1}H-decoupled ^{19}F NMR spectra for these complexation experiments (Fig. 2C and E) could not have been predicted: a slight deshielding ($\Delta\delta^{R-S}$ = +0.002 ppm) effect of the signal for the (*R*)-enantiomer relative to that of (*S*)-enantiomer was observed using the (*S*)-(+)-AMA solvating agent but a shielding effect ($\Delta\delta^{R-S}$ = −0.01 ppm) when ((*R*)-(−)-TFAE) was used. The results of these NMR experiments are summarized in Table 1.

In conclusion, we would like to make the following comments:

(1) ^{1}H-decoupled ^{19}F NMR remains an attractive means to probe the stereochemistry of enzymatic sulfoxidation of fluorine-tagged substrates at trace analytical levels. However, the use of authentic chiral reference standards is required in order to properly assign the absolute configuration of analytes. This is because the behaviour of ^{19}F NMR chemical shifts of a remote reporter group is highly dependent on the nature of the chiral NMR solvating agent. This phenomenon points to the exquisite sensitivity of ^{19}F NMR to subtle differences in the structures of the sulfoxide-solvating agent complex. Further in silico modelling studies may clarify the nature of these differences.

(2) Based on these considerations, the previous assignment[4] of absolute configuration to the predominant enantiomer of fatty acyl 11-sulfoxide **2** should be reversed. It should be noted that **2** was produced from **1** in very low yield and in low ee (32%) by *S. cerevisiae*—this is in contrast to the much more efficient,

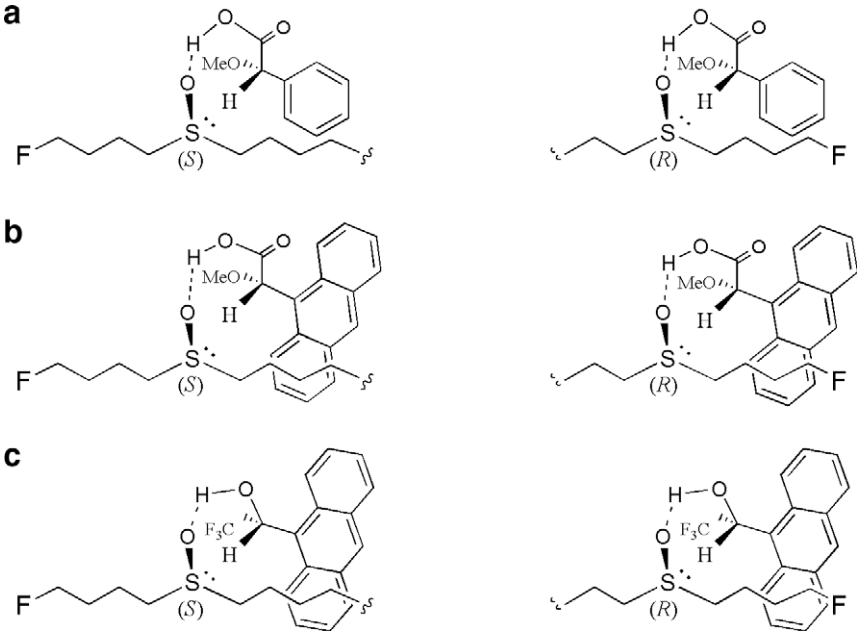


Figure 1. Pirkle-type binding model for the interaction of (a) (*S*)-MPAA, (b) (*S*)-AMA, (c) (*R*)-TFAE with each enantiomer of methyl 15-fluoro-11-thiapentadecane S-oxide **3**.

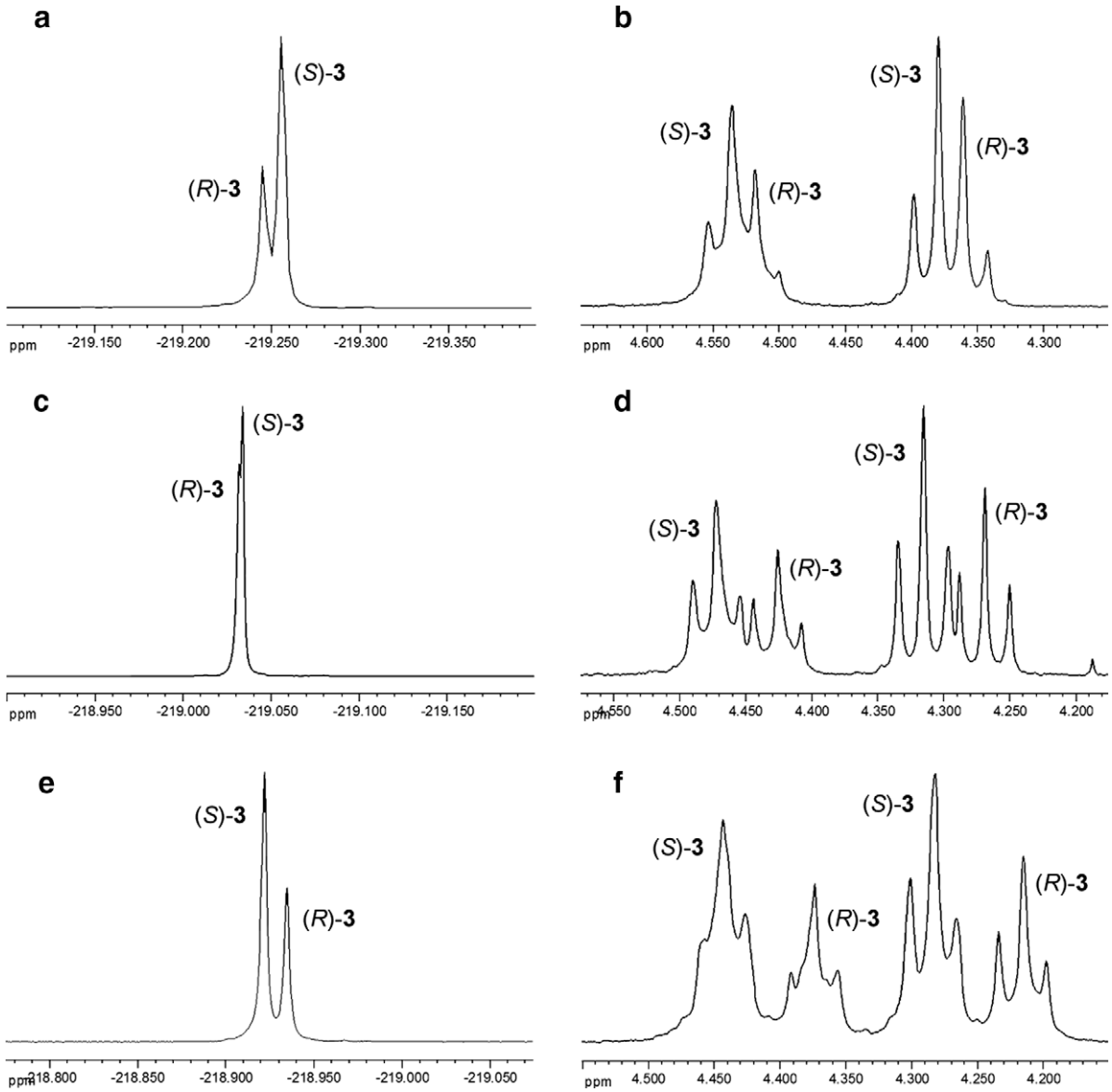


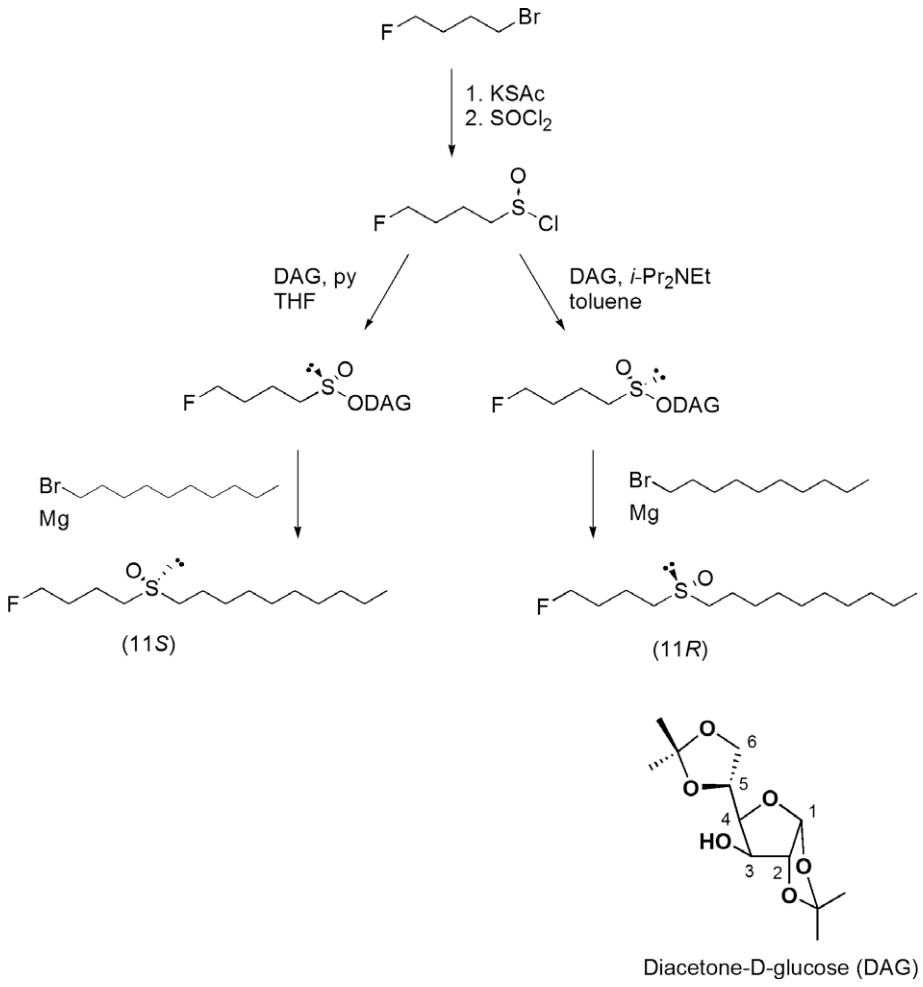
Figure 2. NMR signals for a CH_2F reporter group of a mixture of (*R*)-**2** and (*S*)-**2** (1:2 ratio, ca. 20 μmol total) in the presence of 4 equiv of various chiral solvating agents. (a) (*S*)-(+)-MPAA, ^{1}H-decoupled ^{19}F NMR; (b) (*S*)-(+)-MPAA, ^{1}H NMR; (c) (*S*)-AMA, ^{1}H-decoupled ^{19}F NMR; (d) (*S*)-AMA, ^{1}H NMR; (e) (*R*)-TFAE, ^{1}H-decoupled ^{19}F NMR; (f) (*R*)-TFAE, ^{1}H NMR.

Δ9 desaturase-mediated sulfoxidations of 9-thia substrate analogues that are typically highly enantioselective and match the stereochemistry of H-removal of the parent reaction.[7] Thus, in retrospect, the slightly *pro R*-selective 11-sulfoxidation of **1** is not an entirely surprising result.

(3) The Pirkle-based binding model (Fig. 1a–c) can be used with a high degree of confidence for the stereochemical analysis of chiral sulfoxides when ^{1}H NMR is employed as the probe. The ^{1}H NMR-based methodology has been validated using a number of authentic reference standards[7] including the compounds described in this work.

Table 1
Summary of the predicted and observed $\Delta\delta^{R-S}$ for a 1:2 mixture of (*R*)-**3**:(*S*)-**3** in the presence of various chiral solvating agents (3–4 equiv)

$\Delta\delta^{R-S}$ (ppm)	Predicted	Observed		
		(*S*)-(+)-MPAA	(*S*)-(+)-AMA	(*R*)-TFAE
^{1}H NMR	*Shielded*	*Shielded* −0.02	*Shielded* −0.05	*Shielded* −0.07
^{19}F NMR	*Shielded*	*Deshielded* 0.01	*Deshielded* +0.00(2)	*Shielded* −0.01



Scheme 1. Synthesis of chiral fluorine-tagged sulfoxides from diastereomeric DAG sulfinates.

Acknowledgments

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8. (a) *1,2:5,6-Di-O-isopropylidine-a-D-glucofuranosyl-(−)-(S)-ω-fluorobutanesulfinate.* The title compound was obtained by reaction of DAG and $FCH_2CH_2CH_2CH_2SOCl$ (derived from 1-bromo-4-fluorobutane[9,10]) in the presence of *i*-Pr_2NEt as previously described.[11] After purification of the crude product by flash chromatography (SiO_2, hexane/EtOAc 1:1), the (*S*)-sulfinate was obtained as a colourless oil at rt: $[\alpha]_D^{25}$ − 36.5 (*c* 1.1, acetone); de = 95%; R_f (SiO_2, hexane/EtOAc 1:1) = 0.44; IR (film): 2987, 1714, 1456, 1374, 1255, 1217, 1165, 1135, 1075, 1021, 837, 754 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz, δ (ppm) referenced to TMS) δ 5.91 (d, *J* = 3.7, 1H), 4.75 (d, *J* = 2.5, 1H), 4.61 (d, *J* = 3.7, 1H), 4.49 (dt, $^2J_{HF}$ = 47.4, $^3J_{HH}$ = 5.6, 2H); 3.95–4.35 (m, 4H); 2.86 (m, 2H); 1.74–1.94 (m, 4H); 1.51 (s, 3H), 1.43 (s, 3H), 1.34 (s, 3H), 1.32 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz, δ (ppm) referenced to TMS) δ 112.47, 109.29, 104.96, 83.57, 83.19 (d, J_{CF} = 164.9), 80.35, 79.28, 72.30, 67.78, 56.67, 29.42 (d, J_{CF} = 19.9), 26.76, 26.69, 26.25, 25.16, 17.66 (d, J_{CF} = 4.6); ^{19}F NMR (282.4 MHz, $CDCl_3$, δ (ppm) referenced to $CFCl_3$ using default instrumental setting) δ −219.51; IR (film): ν_{max} 2987, 1714, 1456, 1374, 1255, 1217, 1165, 1135, 1075, 1021, 837, 754 cm^{-1}; EI-MS: *m/z* 367 (17 $[M-15]^+$), 309 (5), 249 (8), 245, (8), 190 (9), 185 (9), 127 (29, $[C_6H_7O_3]^+$), 123 (20, $[FC_4H_8SO]^+$), 113 (11, $[C_4H_7SO]^+$), 101 (100, $[C_5H_9O_2]^+$), 85 (14), 59 (25), 43 (47, $[C_2H_3O]^+$); HR-EI-MS *m/z* 367.1218 ($[M-CH_3]^+$, $C_{15}H_{24}O_7FS$ requires 367.1227).; (b) *1,2:5,6-Di-O-isopropylidine-a-D-glucofuranosyl-(−)-(R)-ω-fluorobutanesulfinate.* The title compound was prepared as above with the exception of using pyridine as base instead of *i*-Pr_2NEt a colourless oil at rt: $[\alpha]_D^{25}$ + 3.91 (*c* 1.1, acetone); de >98%; R_f (SiO_2, hexane/EtOAc 1:1) = 0.49; IR (film): 2988, 1714, 1456, 1374, 1256, 1219, 1166, 1075, 1022, 956, 887, 838, 754, 687 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) 300 MHz) δ 5.89 (d, *J* = 3.5, 1H), 4.76 (d, *J* = 3.5, 1H), 4.71 (s, 1H), 4.46 (dt, $^2J_{HF}$ = 47.2, $^3J_{HH}$ = 5.6, 2H); 3.95–4.17 (m, 4H); 2.86 (m, 2H); 1.74–1.94 (m, 4H); 1.48 (s, 3H), 1.40 (s, 3H), 1.30 (s, 3H), 1.28 (s, 3H); ^{13}C

NMR ($CDCl_3$, 75 MHz) δ 112.37, 109.43, 105.31, 83.79, 83.14, 83.12 (d, J_{CF} = 165.0), 80.88, 72.10, 67.66, 57.15, 29.39 (d, J_{CF} = 19.8), 26.83, 26.70, 26.15, 25.15, 17.38 (d, J_{CF} = 4.8); ^{19}F NMR (282.4 MHz, $CDCl_3$) δ -219.53; EI-MS: *m/z* 367 (10 $[M-15]^+$), 309 (2), 249 (4), 245, (30), 187 (11), 185 (6), 159 (3), 127 (24, $[C_6H_7O_3]^+$), 123 (12, $[FC_4H_8SO]^+$), 113 (8, $[C_4H_7SO]^+$), 101 (100, $[C_5H_9O_2]^+$), 85 (17), 59 (40), 43 (16, $[C_2H_3O]^+$); HR-EI-MS *m/z* 367.1240 ($[M-CH_3]^+$, $C_{15}H_{24}O_7FS$ requires 367.1227).

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13. It is important to note that synthesis of ω-fluoro-tagged sulfoxides by Anderson-type methodology fails when less than 3 equiv of Grignard reagent is used. We attribute this phenomenon to possible coordination of fluorine to the magnesium ion of the Grignard reagent.
14. (a) *(R)-15-Fluoro-11-thiapentadecanoate S-oxide* (*R*-**3**): The title compound was prepared by addition of decyl magnesium bromide to *1,2:5,6-Di-O-isopropylidine-α-D-glucofuranosyl-(−)-(S)-ω-fluorobutanesulfinate*, according to a literature procedure.[12] Purification of the crude product was accomplished by flash chromatography (80% EtOAc/hexane) and a white solid was obtained (34% yield): $[\alpha]_D^{25}$ − 1.4 (*c* 1.1, $CHCl_3$); ee = 90% (^{19}F NMR, (*R*)-TFAE); mp 56–57 °C; R_f (SiO_2, hexane/EtOAc 25:75) = 0.10; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.49 (dt, $^2J_{HF}$ = 47.1, $^3J_{HH}$ = 5.6, 2 H), 2.57–2.77 (m, 4H), 1.93 (m, 2H), 1.70–2.02 (m, 2H), 1.70–1.88 (m, 2H), 1.44 (m, 2H), 1.20–1.52 (m, 12H), 0.87 (*t*, *J* = 6.7, 3H); ^{13}C NMR (75 MHz , $CDCl_3$): 83.37 (d, J_{CF} = 164.7), 52.57, 51.81, 31.85, 29.51 (d, J_{CF} = 19.9), 29.47, 29.35, 29.25, 29.19, 28.86, 22.65, 22.59, 19.06 (d, J_{CF} = 4.7), 14.08. ^{19}F NMR (282.4 MHz, $CDCl_3$): -219.23; IR (KBr): ν_{max} 2919, 2849, 1470, 1412, 1047, 1012, 923, 725 cm^{-1}; EI-MS: *m/z* 247 (79, $[M-17]^+$), 189 (9), 173 (16), 161 (6), 124 (15), 85 (19), 71 (24), 55 (96), 43 (100); HR-EI-MS *m/z* 247.1895 ($[M-OH]^+$, $C_{14}H_{28}FS$ requires 247.1897).; (b) *(S)-15-Fluoro-11-thiapentadecanoate S-oxide* (*S*-**3**): The title compound was prepared as above from *1,2:5,6-Di-O-isopropylidine-α-D-glucofuranosyl-(−)-(R)-ω-fluorobutanesulfinate*. Purification of the crude product by flash chromatography (80% EtOAc/hexane) provided a white solid (35% yield). The analytical data of this compound was identical to its enantiomer except for $[\alpha]_D^{25}$ + 1.7 (*c* 1.1, $CHCl_3$); ee >98% (^{19}F NMR, (*R*)-TFAE).
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19. (a) Pirkle, W. H.; Beare, S. D. *J. Am. Chem. Soc.* **1969**, *91*, 5150; (b) Similar trends in shielding effects were obtained for ((*R*)-(−)TFAE) and its phenyl analogue ((*R*)-(−)TFPE) (data not shown).