Mosher ester analysis for the determination of absolute configuration of stereogenic (chiral) carbinol carbons

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Published online 4 October 2007; doi:10.1038/nprot.2007.354

This protocol details the most commonly used nuclear magnetic resonance (NMR)-based method for deducing the configuration of otherwise unknown stereogenic, secondary carbinol (alcohol) centers (R^1R^2CHOH (or the analogous amines where OH is replaced by NH_2)). This 'Mosher ester analysis' relies on the fact that the protons in diastereomeric α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) esters (i.e., those derived from conjugation of the carbinol under interrogation with MTPA) display different arrays of chemical shifts (δs) in their ^{1}H NMR spectra. The protocol consists of the following: (i) preparation of each of the diastereomeric *S*- and *R*-MTPA esters and (ii) comparative ($\Delta\delta^{SR}$) analysis of the ^{1}H NMR spectral data of these two esters. By analyzing the sign of the difference in chemical shifts for a number of analogous pairs of protons (the set of $\Delta\delta^{SR}$ values) in the diastereomeric esters (or amides), the absolute configuration of the original carbinol (or amino) stereocenter can be reliably deduced. A typical Mosher ester analysis requires approximately 4–6 h of active effort over a 1- to 2-d period.

INTRODUCTION

The absolute configuration of a non-racemic sample of a chiral molecule is a fundamental and essential property of that compound. This is especially so when the compound is of biological relevance (e.g., a natural product or a synthetic compound under development as a possible therapeutic agent). For evidence, one needs to look no further than the infamous history of the differences in biological response to each of the two enantiomers (mirror image antipodes) of thalidomide[1]. Assignment of absolute configuration to compounds where that important structural detail is not yet known is accomplished by a number of different methods. These include (i) correlation with compounds of known configuration by synthetic interconversions and comparison of optical rotation by polarimetric methods, (ii) approaches based on X-ray crystallographic, optical rotary dispersion, circular dichroism or exciton chirality methods and (iii) various empirical methods based on NMR spectroscopy[2]. Among the NMR-based methods, what is now commonly referred to as Mosher ester (or amide) analysis is the most frequently used.

In 1973, Dale and Mosher reported[3] an NMR-based method, which has come to be known as Mosher ester analysis, for deducing the absolute configuration of the stereogenic carbon center in secondary alcohols (i.e., R^1R^2CHOH, **1**, wherein $R^1 \neq R^2$). They used a chiral, enantiomerically pure carboxylic acid to derivatize **1**, the latter having an unknown configuration. Although various acids have been studied in this role[2], the discussion here will be focused on the most widely used α-methoxy-α-trifluoromethylphenylacetic acid (MTPA-OH, **2**), commonly known as Mosher's acid. By direct analogy, this method can also be applied to the analysis of α-chiral amines (e.g., $R^1R^2CHNH_2$ or $R^1R^2CHNHR^3$; Mosher amide analysis[3,4]), but this analysis is required less commonly, so the rest of the discussion in this protocol is confined to ester derivatives of alcohol (carbinol) substrates.

The first step in the protocol for Mosher ester analysis for the determination of absolute configuration is shown in **Figure 1**. It involves coupling of the hydroxyl group of the alcohol with, in two separate but analogous experiments, each enantiomer of Mosher's acid (i.e., **2R** and **2S**). This acylation reaction is commonly performed using a carboxylic acid-activating agent such as *N,N′*-dicyclohexylcarbodiimide (DCC). Alternatively, the acid chlorides **3S** and **3R** (MTPA-Cl), derived from **2R** and **2S**, respectively, can be used directly as active acylating agents[5,6]. This results in the formation of each of the two diastereomeric Mosher esters **4R** and **4S**, respectively. As diastereomers have different physical and spectroscopic properties, Dale and Mosher recognized that their ^{1}H (proton) NMR spectra would differ. They proceeded to show that by comparison of the different chemical shifts of resonances obviously corresponding to protons residing within the substituents R^1 and R^2 in **4** (or the ^{19}F atoms of the CF_3 group in the MTPA moiety[7]), they could deduce the absolute configuration of the carbinol center in the original alcohol **1**.

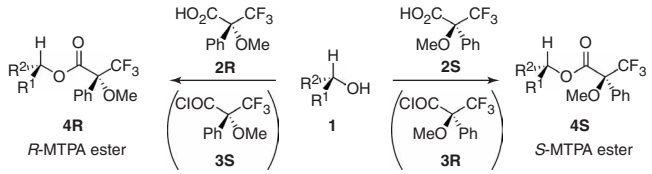


Figure 1 | Scheme for the synthesis of *R*- and *S*-Mosher esters **4** from the generic carbinol **1**.

In the late 1980s, the Kakisawa group[8–10] at Tsukuba described a major development in the method, which has come to be known as the modified (or advanced) Mosher ester analysis. Technological developments had made access to more powerful (higher field), superconducting magnetic resonance spectrometers more commonplace. Among other things, this meant that a greater portion of the individual protons could be routinely assigned in 'small-molecule' compounds (e.g., those having <2,000 amu) having moderate-to-high structural complexity. Today, it is not uncommon

to be able to confidently assign nearly all proton resonances in such molecules. The thrust of the Kakisawa contribution was that many analogous pairs of protons residing within the R^1 and R^2 moieties in **4R** versus **4S** (i.e., many data points for a single carbinol analyte) could be analyzed, resulting in a much higher level of confidence in the deduced absolute configurations for a much broader array of substrate carbinols **1**. The self-reinforcing nature of this analysis is an integral feature of this modified Mosher method.

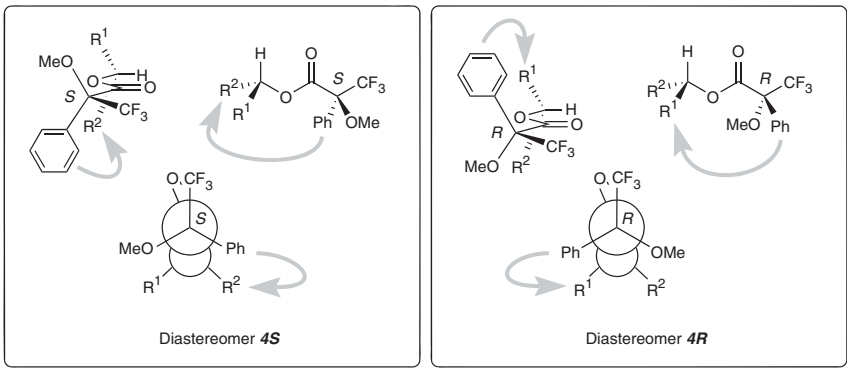


Figure 2 | Commonly encountered representations of the conformations (rotational isomers) used for the analysis of each of the diastereomeric MTPA esters **4S** and **4R**. The phenyl group shielding effect is indicated by the gray arrows in each representation.

In addition to absolute configuration, Mosher ester derivatives can be used to gain other types of stereochemical information. Mosher showed that one can deduce the ratio of two enantiomers in a given sample (i.e., enantiomeric ratio (er = [major]:[minor]), sometimes expressed as the enantiomeric excess (%ee = %major − %minor)) by measuring the relative intensities of analogous resonances (^{1}H and/or ^{19}F) in each of the diastereomers **4R** and **4S**[5]. (Complementary measurement of this ratio is sometimes performed by gas or liquid chromatographic analysis.) While valuable[11], this variant is not further discussed in this protocol, but it is mentioned here since one encounters language like 'Mosher ester analysis was used to determine the enantiomeric purity of this mixture' in the literature.

The success of the Mosher ester method for deducing the absolute configuration of a secondary alcohol relies upon the empirically based (and validated) conformational picture that is shown in **Figure 2**. Briefly, the important conformation for each diastereomer is taken to be the one in which the ester adopts the usual *s-trans* arrangement about its O–CO bond (analogous to the major conformation for acyclic, secondary amide C–N bonds), and both the trifluoromethyl (CF_3) substituent of the MTPA moiety and the methine proton of the secondary alcohol moiety are syn-coplanar (0° dihedral angle) with the carbonyl group. Thus, all of the underlined atoms in the $R^1R^2\underline{C}(\underline{H})–\underline{O}–\underline{C}(=\underline{O})–\underline{C}–\underline{C}F_3$ substructure of each Mosher ester are coplanar. Although this is not the only conformation populated in the ensemble of (rapidly interconverting) rotamers available to **4**, it is the one that dominates the spectroscopic features that distinguish the diastereomeric MTPA esters. Or, as Mosher and coworkers stated so clearly, these conformations "are intended to represent a model which successfully correlates the known results. These are not intended to represent the preferred ground state conformation of the molecules under consideration. They may in fact measure an effective average of many conformations or may represent a minor conformation which, however, exerts a proportionately large differential shielding of the [R^1] and [R^2] groups. Admittedly the success of the correlation tends to reinforce the belief that these do indeed represent major conformations of the molecules in question, but it must still be borne in mind that the possibility exists that this is a fortuitous array which happens to serve as an empirical correlation of the results"[7].

An aryl group (e.g., the phenyl substituent in each of the MTPA esters **4**) is known to impose an anisotropic, magnetic shielding effect on protons residing above (or below) the plane of the aryl ring. This shielding results in a more upfield chemical shift for the affected (spatially proximal) proton(s) in the NMR spectrum. Inspection of the dominant conformer for **4S** versus that for **4R** reveals a key distinguishing feature. Protons residing within the R^2 substructure of **4S** are relatively more shielded (and upfield in its spectrum) and, conversely, protons within the R^1 substructure of **4R** are relatively more shielded; this is the crux of the Mosher method[3,7]. Moreover, the 'reach' of this anisotropic shielding effect extends a considerable distance within the molecule so that a fairly large number of (even remote) protons within each R group are differentially shielded in the two diastereomers; this is the crux of the modified (or advanced) Mosher method[10]. A consequence of all of the above is that the *signs* of the $\Delta\delta^{SR}$ (defined, by convention, to be $\delta_S - \delta_R$[8,10]) values for protons residing in R^1 will be positive and those in R^2 will be negative. Thus, by knowing which protons have a positive versus a negative $\Delta\delta^{SR}$ value, one can deduce the (sub)structures of R^1 versus R^2, which translates directly to the absolute configuration of the secondary carbinol center in **1**—the original objective of the exercise.

Mosher ester (and amide) analysis, especially the modified version, is remarkably powerful and general. The most complementary alternative, predicated on the same concepts, is the use of *O*-methylmandelate esters (or methoxyphenyl acetates), which was introduced by Trost *et al.*[12]. Although there are instances where the Mosher method must be applied with caution (e.g., for substrates with more than one carbinol and/or amine functional groups), discussion of such subtleties is beyond the scope of this protocol. Interested readers are encouraged to consult the comprehensive review by the Riguera group[2]. Finally, note that some of the developers of more recent NMR-based methods similar to the Mosher analysis have elected to use the convention $\Delta\delta^{RS} = \delta_R - \delta_S$. This is opposite to the $\Delta\delta^{SR}$ convention used universally in Mosher analyses. One will get to the correct answer either way, but it is essential that users unambiguously state which way the data are being interpreted and reported.

The protocol detailed below consists of two main components: (i) individually prepare (synthesize) each of the two diastereomeric MTPA esters and then (ii) comparatively analyze the ^{1}H NMR spectral data of each of the two diastereomeric MTPA esters. We have chosen (−)-menthol (**5**; **Fig. 3**) as a representative example of the universe of secondary carbinols **1**. Of course, the absolute configuration of (−)-menthol is known, but the synthesis and analysis methodologies are unchanged by that fact. Given the sensitivity of ^{1}H NMR spectroscopy, it is virtually never necessary to carry out the preparation of the MTPA esters for a Mosher analysis on a very large scale, even when the supply of carbinol **1** is

large. The more common situation is that **1** is precious and in short supply (e.g., in the case of initial structural studies of a newly isolated natural product); if necessary, the method can be carried out routinely on scales even below 100 μg (<1 μmol). Accordingly, we have provided (in Step 1) three complementary, representative procedures for synthesis of MTPA-menthyl esters **6** (**Fig. 3**) on different scales and using different acylating agents. Namely, in option A we describe the preparation of **6S** from *R*-(−)-MTPA-Cl (**3R**) on a 10 mg scale; in option B, **6S** from *R*-(−)-MTPA-Cl (**3R**) on a 50 μg scale and in option C, **6R** from *R*-(+)-MTPA-OH (**2R**) on a 10 mg scale. The interpretation of the data (described in Steps 2–7) is performed in an identical manner, regardless of how the pair of diastereomeric esters was prepared.

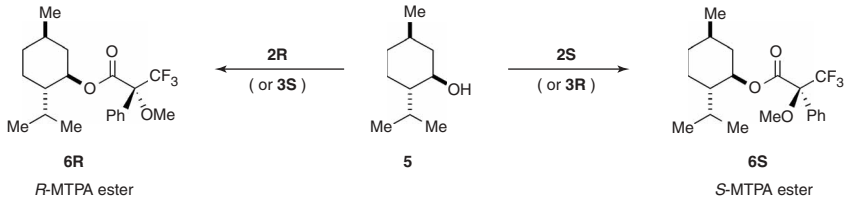


Figure 3 | Scheme for the synthesis of *R*- and *S*-MTPA menthyl esters **6** from (−)-menthol (**5**).

Throughout the protocol, it is essential that the individual samples, spectra and data for each of the two diastereomers never be confused/interchanged. For this reason, we recommend that the sequence of synthesis and NMR data collection and tabulation be performed for each of the diastereomeric MTPA esters serially rather than in parallel. Although this may seem like an obvious point (as an interchange of the two samples will lead to the assignment of precisely the wrong absolute configuration!), experience teaches that it is an easy issue over which uncertainty can arise in the mind of the experimenter.

MATERIALS

REAGENTS

- (*R*)-(+)-α-Methoxy-α-trifluoromethylphenylacetic acid (**2R**, *R*-(+)-MTPA-OH; e.g., Acros)
- (*S*)-(−)-α-Methoxy-α-trifluoromethylphenylacetic acid (**2S**, *S*-(−)-MTPA-OH; e.g., Aldrich)
- (*S*)-(+)-α-Methoxy-α-trifluoromethylphenylacetyl chloride (**3S**, *S*-(+)-MTPA-Cl; prepared from *R*-(+)-MTPA-OH[5,6,13], but this reagent is also commercially available; e.g., Fluka)
- (*R*)-(−)-α-Methoxy-α-trifluoromethylphenylacetyl chloride (**3R**, *R*-(−)-MTPA-Cl; prepared from *S*-(−)-MTPA-OH[5,6,13], but this reagent is also commercially available; e.g., Lancaster)
- (−)-Menthol (**5**, (1*R*,2*S*,5*R*)-(−)-2-isopropyl-5-methylcyclohexanol; e.g., Aldrich)
- *N*,*N*′-DCC (e.g., Acros)
- Pyridine (e.g., Aldrich)
- 4-Dimethylaminopyridine (e.g., Aldrich)
- Chloroform-*d* (e.g., Cambridge Isotope Lab)
- Hexanes, ethyl acetate, diethyl ether, dichloromethane (CH_2Cl_2; standard reagent-grade laboratory solvents)
- Silica gel, bulk (typically 40- to 63-μm-diameter particle size, with nominal 60 Å pore size)
- Silica gel thin-layer chromatography (TLC) plates (SIL G/UV254, 0.25 mm layer thickness; Machery Nagel, M805024)
- Ceric ammonium molybdate, phosphomolybdic acid, anisaldehyde or potassium permanganate (e.g., TCI America, Fisher Scientific or Aldrich)

EQUIPMENT

- Screw-capped glass vial or other similar reaction vessel with a relatively small headspace with respect to the volume of reaction solvent
- Glass microliter syringes (Hamilton) or Wiretrols (Drummond) for measuring small volumes of liquid
- Common glassware (round-bottomed flask, Erlenmeyer flask, disposable pipets, etc.)
- Magnetic stirrer and stir bar small enough to fit the reaction vial
- Rotary evaporator (Büchi)
- Balance (Mettler)
- Handheld UV lamp (UVP)
- Small chromatography column
- NMR sample tube (5 mm diameter; New Era)
- ^{1}H NMR spectrometer (≥200 MHz; Varian, Bruker)

PROCEDURE

Individually prepare (synthesize) and collect the ^{1}H NMR spectrum for each of the two diastereomeric MTPA esters.

1| Examples of three methods are provided as follows: (A) preparation of *S*-MTPA-menthyl ester **6S** from (−)-menthol (**5**) and the *R*-acid chloride (**3R**, *R*-(−)-MTPA-Cl) on a 10 mg scale; (B) preparation of *S*-MTPA-menthyl ester **6S** from (−)-menthol (**5**) and the *R*-acid chloride (**3R**, *R*-(−)-MTPA-Cl) on a 50 μg scale; and (C) preparation of *R*-MTPA-menthyl ester **6R** from (−)-menthol (**5**), the *R*-Mosher acid (**2R**, *R*-(+)-MTPA-OH) and DCC on a 10 mg scale.

(A) *S*-MTPA-menthyl ester 6S from (−)-menthol (5) and the *R*-acid chloride (3R, *R*-(−)-MTPA-Cl) on a 10 mg scale

(i) Fit a screw-capped 4 ml glass vial with a Teflon-coated magnetic stir bar.

(ii) Transfer (−)-menthol (**5**, 10.0 mg, 64 μmol) and dry pyridine (16 μl, 0.20 mmol, 3.1 equiv.) to the vial.

(iii) Dissolve the contents of the vial in anhydrous CH_2Cl_2 or chloroform ($CHCl_3$) (1 ml, [**5**] =0.064 M). If a source of one of these anhydrous solvents is not readily available, either can be dried using the same procedure described in the following critical step.

▲ **CRITICAL STEP** If $CHCl_3$ is selected as the solvent, it should first be passed through a column of fresh silica gel or alumina immediately before use to remove the ethanol (~0.75% (vol/vol)) that is present as a stabilizer in most commercial sources. For example, insert a small cotton plug into the neck of a disposable pipet and load the pipet with fresh silica gel (or alumina) to a height of approximately 5 cm. Pass several milliliters of $CHCl_3$ through this column using pressure applied from a pipet bulb.

(iv) Add the *R*-(−)-MTPA-Cl (**3R**, 23 μl, 0.12 mmol, 1.9 equiv.) to the mixture. This transfer can be performed using a microliter syringe or Wiretrol.
(v) Cap the vial and stir at ambient temperature.
(vi) Monitor the progress of the reaction by TLC analysis, by eluting with a solvent composed of hexanes and ethyl acetate in a 4:1 ratio, and visualizing the plate by UV monitoring and/or staining with an appropriate TLC stain (e.g., ceric ammonium molybdate, phosphomolybdic acid, anisaldehyde or potassium permanganate). Because they are less polar, the MTPA esters will elute on silica gel more rapidly than the precursor carbinol (i.e., with a higher retention factor (R_f) on the TLC plate). For the case of menthol (**5**) to **6R**, the former has an R_f of 0.46 and the latter 0.82. The choice of TLC elution solvent will vary depending on the polarity of the starting substrate under analysis.
(vii) After the reaction is complete (typically less than 2 h), partition the reaction mixture between diethyl ether (3 ml) and water (1 ml).
(viii) Mix the layers thoroughly.
(ix) Separate the layers and place the ether layer into a small Erlenmeyer flask.
(x) Add 3 ml of ether to the aqueous layer that remains in the reaction vial and repeat Steps (viii) and (ix), adding this ether layer to the original one in the Erlenmeyer flask.
(xi) Repeat Step (x).
(xii) Dry the combined ether extracts with a suitable solid drying agent (anhydrous solid Na_2SO_4 or $MgSO_4$) in a 25- to 50-ml Erlenmeyer flask. Typically, approximately 1 g of drying agent is used for the approximately 10 ml of combined organic extracts. Swirl the suspension occasionally over a period of approximately 5 (for $MgSO_4$) or 30 (for Na_2SO_4) min. In instances where the substrate might contain acid-sensitive functionality, the use of Na_2SO_4 is recommended.
(xiii) Filter the ether, containing the Mosher ester, from this suspension into a round-bottomed flask (~25 ml).
(xiv) Evaporate the volatiles using a rotary evaporator under reduced pressure using a 20–25 °C water bath.
(xv) Purify the product by chromatography on silica gel (any of flash[14], medium pressure liquid chromatography (MPLC), HPLC, gravity column or prep-TLC plate methods can be used), eluting with hexanes/ethyl acetate (40:1) and monitoring the fractions using TLC (4:1, hexanes/ethyl acetate) (or another commonly available detection method like UV absorbance or differential refractive index).
(xvi) Combine the fractions containing the purified ester product **6S** and remove the solvents under reduced pressure using a 20–35 °C water bath.
(xvii) If necessary or desired, remove the remaining traces of ethyl acetate and hexane elution solvents from the purified product by attaching the sample flask to a high-vacuum source (e.g., 0.1–1 torr) at ambient temperature for approximately 1 h.
(xviii) Record the ^{1}H NMR spectrum of **6S** in deuterochloroform ($CDCl_3$).
(xix) Prepare the *R*-MTPA-menthyl ester **6R** by repeating Steps (i–xvii) using *S*-(+)-MTPA-Cl in place of *R*-(−)-MTPA-Cl.
(xx) Record the ^{1}H NMR spectrum of **6R** in $CDCl_3$.

(B) *S*-MTPA-menthyl ester 6S from (–)-menthol (5) and the *R*-acid chloride (3R, *R*-(–)-MTPA-Cl) on a 50 μg scale

(i) Fit a screw-capped 2 ml glass vial with a Teflon-coated magnetic stir bar.
(ii) Transfer (−)-menthol (**5**, 50 μg, 0.32 μmol) and dry pyridine (1 μl, 12.5 μmol, 39 equiv.) to the vial.
(iii) Dissolve the contents of the vial in anhydrous $CDCl_3$ (100 μl, [**5**] = 3.2 mM). Use of $CDCl_3$ as the solvent allows one to assay the reaction mixture directly by ^{1}H NMR spectroscopy.
(iv) Add the *R*-(−)-MTPA-Cl (**3R**, 1 μl, 5.2 μmol, 16 equiv.) to the vial, cap the vial and stir the contents at room temperature.
(v) Perform Step 1A(vi).
(vi) After the reaction is complete (typically less than 1 h), dilute the reaction mixture with dry $CDCl_3$ (0.6 ml).
(vii) Transfer the entire $CDCl_3$ solution to a standard (5 mm diameter) NMR sample tube.
(viii) Record a ^{1}H NMR spectrum.
(ix) Prepare the *R*-MTPA-menthyl ester **6R** by repeating Steps 1B(i–vii), using *S*-(+)-MTPA-Cl in place of *R*-(−)-MTPA-Cl.
(x) Record the ^{1}H NMR spectrum of **6R** in $CDCl_3$.

(C) *R*-MTPA-menthyl ester 6R from (–)-menthol (5), the *R*-Mosher acid (2R, *R*-(+)-MTPA-OH) and DCC on a 10 mg scale

(i) Fit a screw-capped 4 ml glass vial with a Teflon-coated magnetic stir bar.
(ii) Transfer (−)-menthol (**5**, 10.0 mg, 64 μmol) and *R*-(+)-MTPA-OH acid (**2R**, 32 μl, 0.2 mmol, 3.1 equiv.) to the vial.
(iii) Dissolve the contents of the vial in anhydrous CH_2Cl_2 or $CHCl_3$ (1 ml, [**5**] = 0.064 M). If a source of one of these anhydrous solvents is not readily available, either can be dried using the same procedure described in the critical step above, following Step 1A(iii).
(iv) Add DCC (42 mg, 0.2 mmol, 3.1 equiv.) to the vial.
! CAUTION DCC is an irritant and can lead to sensitization. Avoid any direct contact with the skin or inhalation of particulates.
(v) Add 4-dimethylaminopyridine (25 mg, 0.20 mmol, 3.1 equiv.), tightly cap the vial and stir the contents at room temperature.
(vi) Perform Step 1A(vi).

(vii) After the reaction is complete (typically 12–24 h), filter the white precipitate through a cotton plug. This solid is largely the unwanted by-product, *N,N'*-dicyclohexylurea.
(viii) Purify the product as described in Steps 1A(xiv–xvii).
(ix) Record the ^{1}H NMR spectrum of **6R** in $CDCl_3$.
(x) Prepare the *S*-MTPA-menthyl ester **6S** by repeating Steps (i–viii), using *S*-(−)-MTPA-OH (**2S**) in place of *R*-(+)-MTPA-OH (**2R**).
(xi) Record the ^{1}H NMR spectrum of **6S** in $CDCl_3$.

Comparatively analyze the ^{1}H NMR spectral data of each of the two diastereomeric MTPA esters

2| Unambiguously assign as many proton resonances as possible in the ^{1}H NMR spectrum for each of the diastereomeric esters **4S** and **4R**.

3| Determine the difference in chemical shifts ($\Delta\delta^{SR}$) for each of the assignable analogous pairs of protons for the *S*- and *R*-MTPA esters according to the following convention: $\Delta\delta^{SR} = \delta$(*S*-MTPA ester)$-\delta$(*R*-MTPA ester) (or $\Delta\delta^{SR} = \delta_S - \delta_R$). Gather all of the resonances of positive and, then, negative $\Delta\delta^{SR}$ values together. These $\Delta\delta^{SR}$ data for the menthyl MTPA esters **6S** versus **6R** are presented in **Table 1**, listed in the order from most positive to most negative.

TABLE 1 | $\Delta\delta$ $(=\delta_S - \delta_R)$ data for the *S*- and *R*-MTPA-menthyl Mosher esters 6S and 6R.

	δ *S*-ester (6S) (ppm)	δ *R*-ester (6R) (ppm)	$\Delta\delta^{SR}$ $(=\delta_S - \delta_R)$	
			ppm	Hz (500 MHz)
6ax	1.12	0.98	+0.14	+70
6eq	2.13	2.08	+0.05	+25
5Me	0.94	0.91	+0.03	+15
5ax	1.54	1.52	+0.02	+10
1ax	4.90	4.88	+0.02	+10
4ax	0.87	0.86	+0.01	+5
4eq	1.70	1.70	0.00	0
3ax	1.04	1.06	−0.02	−10
3eq	1.67	1.69	−0.02	−10
2ax	1.42	1.45	−0.03	−15
2′Me_a	0.67	0.77	−0.10	−50
2′Me_b	0.74	0.87	−0.13	−65
2′	1.56	1.88	−0.32	−160

For the menthyl MTPA esters 6S and 6R, all proton resonances are uniquely identifiable. However, this need not be the case, and, for many (most?) complex structures, not every proton in the compound is uniquely identifiable in its ^{1}H NMR spectrum. In such cases, it is prudent to use only the subset of analogous proton pairs for which unambiguous assignments are known.

4| Using the conformations shown in **Figure 2** for the MTPA esters of generic secondary alcohols, decide which protons of the esters under study are part of the R^1 substituent and which part of R^2. More specifically, those protons that have positive $\Delta\delta^{SR}$ values reside within R^1, whereas those with negative values 'belong to' R^2. The structures shown in **Figure 4** for the menthyl esters **6S** and **6R** clearly show that the protons with positive $\Delta\delta^{SR}$ values (i.e., those in R^1) are all on one side (front) of the plane of the MTPA moiety, whereas those with negative values are all on the opposite (back) side of that plane.

! CAUTION Occasionally, one of the proton resonances proves to be an exception to this trend. If so, it is usually for a proton with a $\Delta\delta^{SR}$ value that is relatively small in magnitude because this exceptional proton resides either near the plane of the MTPA ester moiety or quite remotely. For this reason, the secondary carbinol proton itself (i.e., H1 in **6**) is typically ignored in the analysis; an important corollary is that protons having the largest $\Delta\delta^{SR}$ values are, qualitatively, weighted very heavily in the analysis.

5| Use the Cahn Ingold Prelog convention[15] to assign the configuration of the original carbinol center as *R* or *S*. C1 of (−)-menthol has the *R* configuration, as the substituent priority sequence is 1, OH; 2, C2; 3, C6; and 4, H.

6| Report the data. A typical 'chemist-friendly' format for reporting the synthesis and spectral properties of a Mosher ester is presented in **Box 1**. Specifically, it describes the preparation of both the *S*- and *R*-MTPA esters of (−)-menthol (**6S** and **6R**, respectively).

7| Finally, one particularly insidious aspect of the Mosher method relates to the Cahn Ingold Prelog convention nomenclature mentioned above and represents a potential pitfall. This problem is presented as a caveat in **Box 2**.

? TROUBLESHOOTING

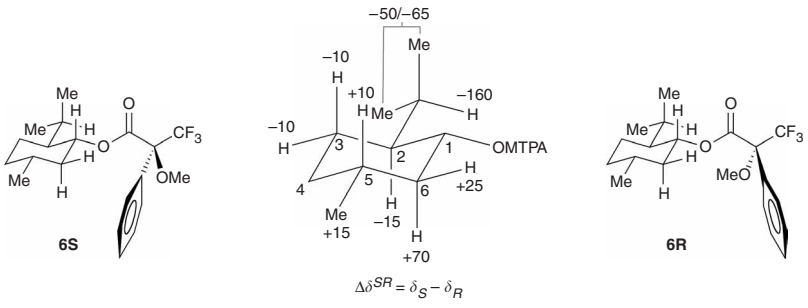


Figure 4 | Conformations used for the analysis of the menthyl esters **6S** and **6R** and the resulting $\Delta\delta^{SR}$ $(=\delta_S - \delta_R)$ values (in Hz) for each of the protons on the 'front' and 'back' sides of the menthyl moiety.

● TIMING

Steps 1A(i–iv): 15–20 min
Steps 1A(v) and (vi): 1–3 h
Steps 1A(vii–xiv): 20–40 min
Steps 1A(xv) and (xvi): 20–60 min
Step 1A(xvii): 5–30 min
Step 1A(xviii): ~3.5 h
Step 1A(xix): 5–30 min
Steps 1B(i–iv): 15–20 min
Step 1B(v): 1–3 h
Steps 1B(vi) and (vii): 2–5 min
Step 1B(viii): 5–40 min

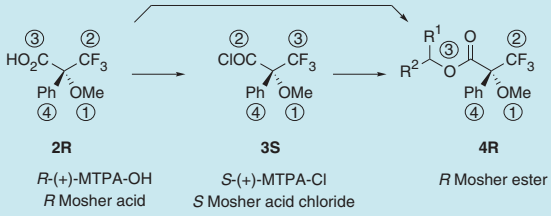
BOX 1 | EXAMPLE FORMAT FOR REPORTING RESULTS OF A MOSHER ESTER ANALYSIS

Preparation of the *S*- and *R*-MTPA-menthyl esters 6S and 6R

To a stirred solution of (−)-menthol (**5**, 10.0 mg, 64 μmol) and dry pyridine (16 μl, 0.20 mmol, 3.1 equiv.) in dry deuterochloroform (1 ml, [**5**] = 0.064 M) at room temperature, *R*-(−)-MTPA-Cl (**3R**, 23 μl, 0.12 mmol, 1.9 equiv.) was added. The reaction progress was monitored by thin-layer chromatography (TLC) on silica gel (4:1::Hex:EtOAc). After complete consumption of the menthol (~2 h), the reaction mixture was quenched by the addition of water (~1 ml) and ether (~3 ml). The aqueous layer was extracted with two additional portions of ether (~3 ml), and the combined organic layers were dried (Na_2SO_4), filtered and concentrated *in vacuo*. The crude product mixture was purified by silica-gel chromatography (MPLC, eluting with hexanes/ethyl acetate (40:1, $R_f = 0.18$)) to give the *S*-MTPA-menthyl ester **6S** (21.5 mg, 90%) as a white solid. **6S**: ^{1}H NMR ($CDCl_3$) δ 7.54 (m, 2H), 7.39 (m, 3H), 4.90 (ddd, 1H, $J = 4.5, 11.0, 11.0$ Hz), 3.59 (q, 3H, $J = 1.0$ Hz), 2.13 (dddd, 1H, $J = 2.0, 3.5, 4.0, 11.5$ Hz), 1.70 (dddd, 1H, $J = 3.5, 3.5, 3.5, 12.5$ Hz), 1.67 (dddd, 1H, $J = 3.5, 3.5, 3.5, 13$ Hz), 1.56 (dsept, 1H, $J = 3.0, 7.0$), 1.54 (ddddq, 1H, $J = 3.5, 3.5, 12, 12, 7.0$ Hz), 1.42 (dddd, 1H, $J = 3.0, 3.0, 11.0, 12.5$ Hz), 1.12 (ddd, 1H, $J = 12, 12, 12$ Hz), 1.04 (dddd, 1H, $J = 3.5, 12.5, 12.5, 12.5$ Hz), 0.94 (**d**, 3H, $J = 7.0$ Hz), 0.87 (dddd, 1H, $J = 4.0, 12.5, 13, 13$ Hz), 0.74 (d, 3H, $J = 7.0$ Hz), and 0.67 (d, 3H, $J = 7.0$ Hz). In an entirely analogous fashion, the *R*-MTPA-menthyl ester **6R** was prepared using *S*-(+)-MTPA-Cl (**3S**). **6R**: ^{1}H NMR ($CDCl_3$) δ 7.52 (m, 2H), 7.40 (m, 3H), 4.88 (ddd, 1H, $J = 4.5, 11.0, 11.0$ Hz), 3.53 (q, 3H, $J = 1.0$ Hz), 2.08 (dddd, 1H, $J = 2.0, 3.5, 4.0, 12$ Hz), 1.88 (dsept, 1H, $J = 3.0, 7.0$), 1.70 (dddd, 1H, $J = 3.5, 3.5, 3.5, 13$ Hz), 1.69 (dddd, 1H, $J = 3, 3, 3, 13$ Hz), 1.52 (ddddq, 1H, $J = 3.5, 3.5, 12, 12, 6.5$ Hz), 1.45 (dddd, 1H, $J = 3.0, 3.0, 11.0, 12.5$ Hz), 1.06 (dddd, 1H, $J = 3.5, 13, 13, 13$ Hz), 0.98 (ddd, 1H, $J = 12, 12, 12$ Hz), 0.91 (d, 3H, $J = 6.5$ Hz), 0.87 (d, 3H, $J = 7.0$ Hz), 0.86 (dddd, 1H, $J = 3.5, 12.5, 12.5, 12.5$ Hz), and 0.77 (d, 3H, $J = 7.0$ Hz).

BOX 2 | THE DIRTY LITTLE SECRET ABOUT THE ABSOLUTE CONFIGURATION OF THE MOSHER ACID CHLORIDE

Upon conversion of the MTPA acid (MTPA-OH) to the MTPA acid chloride (MTPA-Cl), there is a switch in the relative priority of two of the groups. Specifically, the trifluoromethyl group (CF_3) is higher in priority than the carboxyl group (COOH) in the acid, but lower than the chlorocarbonyl group (COCl) in the acid chloride. Thus, the *R* enantiomer of the Mosher acid (*R*-(+)-MTPA-OH, **2R**) gives rise to the *S* enantiomer of the Mosher acid chloride (*S*-(+)-MTPA-Cl, **3S**) (and vice versa for the *S* acid **2S** to the *R* acid chloride **3R**). This represents a somewhat rare instance in which the absolute configuration of a stereocenter changes as a result of a chemical reaction, even though none of the four bonds to the stereogenic carbon was involved in the reaction.



As was shown but not otherwise emphasized earlier in **Figure 1**, it follows that the *R* Mosher acid gives rise to the *R* Mosher ester, but that it is the *S* Mosher acid chloride that gives rise to the *R* Mosher ester (since there is another change in relative priority: CF_3 is lower than COCl but higher than COOR). It is, of course, essential that every user of Mosher ester analysis considers this fact when performing the analysis. Otherwise, again, precisely the opposite (and wrong) absolute configuration will be assigned[16]. Somewhat ironically, this potential problem has been exacerbated by the fact that the acid chloride became commercially available in the early 1990s. That is, earlier workers would often prepare the acid chloride from purchased, say, *R* acid, convert the derived acid chloride to the *R* Mosher ester and properly deduce the correct absolute configuration, without even realizing that they were passing by way of the *S* acid chloride.

Finally, it is also important that users report the method by which they made their Mosher esters with absolute clarity, so that no doubt is left in readers' minds about the configuration of each MTPA ester. The experimental description in **Box 1** represents one such unambiguous presentation. If the publication venue does not lend itself to full experimental description, then an unambiguous statement like "esterification of # with (*S*)- and (*R*) MTPA-Cl occurred only at the C-8 hydroxy group to give the (*R*)- and (*S*)-MTPA esters **#a** and **#b**, respectively"[17] is to be highly commended and recommended. All too often and in fact typically, authors are ambiguous on the issue of the precise origin of their *R*- and *S*-esters. For example, simple statements like 'Mosher ester analysis led to the assignment of the *R* configuration…' and '*R*- and *S*-MTPA esters were prepared from reaction of carbinol X with the MTPA acid chlorides' abound and leave doubt. This does not mean that the method was misused (i.e., that the user was unaware of the CIP switch), but it does (and should) erode the confidence that readers can have in the conclusions reached. The only instances in which it is clear that an error was made—and we have found a number of published examples of this—is where it is unambiguously stated that 'the R-Mosher ester was prepared using *R*-MTPA-Cl'. Ironically, these cases are at least somewhat self-correcting, since the informed reader will know to reverse whatever conclusions were drawn from these analyses.

The bottom line is that Mosher ester analysis is an extremely reliable method for determining absolute configuration, provided that users be aware of the above pitfall and that they demonstrate that awareness by reporting the method of synthesis (origin) of each Mosher ester in an unambiguously stated manner so that others will have the full measure of confidence in the conclusions.

Step 1B(ix): ~3.5 h
Step 1B(x): 5–40 min
Steps 1C(i–iv): 15–20 min
Steps 1C(v) and (vi): 12–24 h
Step 1C(vii): 5 min
Step 1C(viii): 30–70 min
Step 1C(ix): 5–30 min
Step 1C(x): ~24 h
Step 1C(xi): 5–30 min
Step 2: 15–90 min
Step 3: 30–60 min
Step 4: 20 min
Step 5: 5 min
Step 6: 1–2 h

? TROUBLESHOOTING

Troubleshooting advice regarding potential issues with the preparation, purification, and/or NMR data collection for esters **4R** and **4S** can be found in **Table 2**.

TABLE 2 | Troubleshooting table.

Problem	Possible reason	Solution
Poor conversion to product	Moisture in reaction mixture that consumes MTPA-Cl or DCC-activated MTPA-OH	Use anhydrous solvents and oven-dried glassware
Slow reaction	Reaction solution too dilute	Make sure substrate alcohol and MTPA-OH or MTPA-Cl are present at $\geq$0.05 M, and monitor reaction conversion carefully by TLC analysis
Product contamination, especially for microscale	Leaching of impurities (e.g., plasticizers) from plastic labware by organic solvents	Use only glass reaction vessels, pipets, etc., and Teflon rather than plastic tubing
Product contamination by excess MTPA-Cl, again, especially for microscale	Need to use $\geq$10 equiv. of MTPA-Cl because of limited availability of carbinol	Quench the reaction mixture $Me_2N(CH_2)_3NH_2$ before adding dilute HCl during workup[3] Use stock solutions to accurately measure reagent quantities[18]
Overlapping resonances complicating NMR interpretation	Overall molecular complexity and/or coincidental chemical shift similarity	Carry out 2D-NMR analysis (e.g., COSY or HMQC) to unambiguously assign overlapping resonances

ANTICIPATED RESULTS

Each purified diastereomeric Mosher ester (**4R** and **4S**) will be prepared and isolated in 50–80% yield from the starting carbinol **1**. ^{1}H NMR spectroscopic data of sufficient quality for subsequent $\Delta\delta$ analysis will be obtained for each ester. After the prescribed $\Delta\delta^{SR}$ analysis is performed, the absolute configuration of the carbinol **1** will have been deduced.

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