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Direct determination of absolute stereochemistry of α-methylselenocysteine using the Mosher method

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ABSTRACT

Mosher amides of α-methylselenocysteine were synthesized to determine the absolute stereochemistry of the sterically hindered α-carbon utilizing ^{1}H, ^{13}C, ^{19}F, and ^{77}Se NMR spectroscopies. After analysis of these spectra using the established Mosher method, the stereochemistry of the α-carbon was determined to be (*R*), which was subsequently confirmed using x-ray crystallography.

1. Introduction

α-Methyl amino acids have seen a resurgence of interest in recent years. Incorporating α-methyl amino acids into proteins and peptides results in increased stability [1,2] and distinctive secondary structures [3–6]. We have previously synthesized several α-methyl amino acids from a common starting material, which most recently includes α-methylselenocysteine [7–10]. Scheme 1 illustrates our method of preparing α-methylselenocysteine utilizing Pig Liver Esterase (PLE) to provide the needed chirality with a high level of optical purity. In general, PLE hydrolysis tends to produce the (*R*)-enantiomer. However, there are cases where PLE hydrolysis favors the formation of the (*S*)-enantiomer which can lead to uncertainty in the assignment of the resulting stereocenter for unexplored substrates [11,12]. Therefore, it is necessary to have a convenient method that can reliably assign the absolute stereochemistry of the α-carbon.

The Mosher method has seen numerous uses as a reliable method to analyze chiral molecules by NMR [13]. The Mosher method is successful in identifying enantiomeric excess as well as establishing absolute stereochemistry to an unassigned stereocenter. This method typically employs a secondary stereogenic carbon bearing an alcohol or amine that is converted to an ester or amide, respectively, by coupling α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) [14]. While alternative chiral derivatizing reagents and techniques have been developed by other labs [15–17], the Mosher method using MTPA is more commonly used. However, if the stereogenic carbon is tertiary, the Mosher method becomes problematic since the steric bulk can prevent access to the expected conformation as there is no longer an α-hydrogen to sit in the MTPA plane on the original substrate [13,18]. With this added complication, many opt to use the method for enantio-purity determination for these congested systems [19–21]. To the best of our knowledge, there is only a single report employing the Mosher method on tertiary alcohols [18]. We chose to explore the possibility to achieve direct stereocenter assignment in the congested systems of α-methyl amino acids utilizing the Mosher method.

2. Results and discussion

We utilized the Mosher method in an attempt to determine the absolute stereochemistry of α-methylselenocysteine **2**, which we hypothesized to be the (*R*)-enantiomer (ca. 88% ee) based on comparisons of the optical rotation data with that of an α-methylcysteine homologue of known absolute configuration [22]. Amino acid **2** was converted to two diasteromeric MTPA-amides, (*X*,*R*)-**5** and (*X*,*S*)-**5** (where X is the unassigned stereochemistry of the α-carbon of the amino acid), through a series of reactions (Scheme 2), with retainment of the original stereochemistry at the stereocenter in question. Several spectra were obtained comparing the chemical shift differences due to anisotropic effects of the two diastereomers.

The α-methyl hydrogen atoms show a strong shielding effect from the MTPA-phenyl (+13.4 Hz with a 400 MHz spectrometer using the δ(*X*,*S*)-δ(*X*,*R*) convention) in ^{1}H NMR for (*X*,*R*)-**5** which strongly correlates to an ipsilateral position to the phenyl, whereas the opposite is observed when considering (*X*,*S*)-**5** (Fig. 1). Due to their diastereotopic nature, the methylene protons (+1.7 and + 11.6 Hz) adjacent to the selenium did not shift, and is presumed to be caused by the

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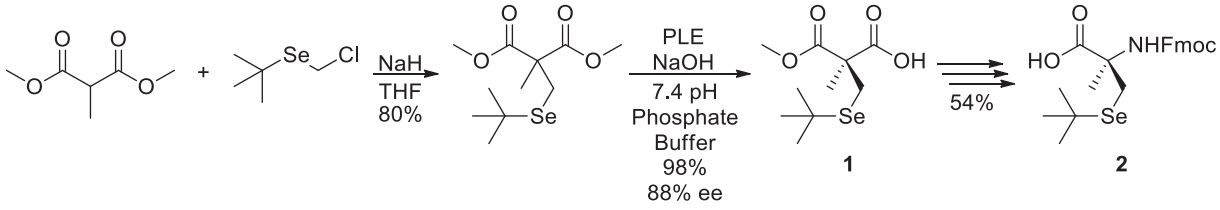
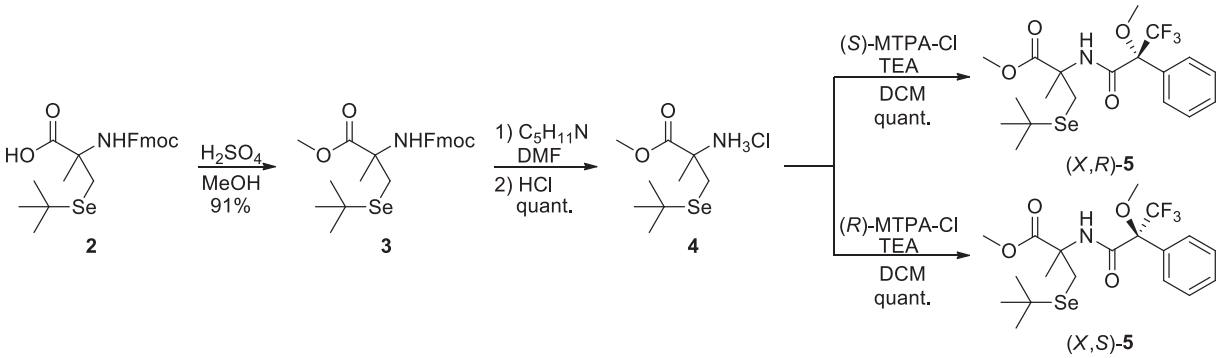


Scheme 1. Synthesis of **2** showing the formation of the stereocenter from PLE hydrolysis [7].



Scheme 2. Synthesis of the MTPA-amides from **2**.

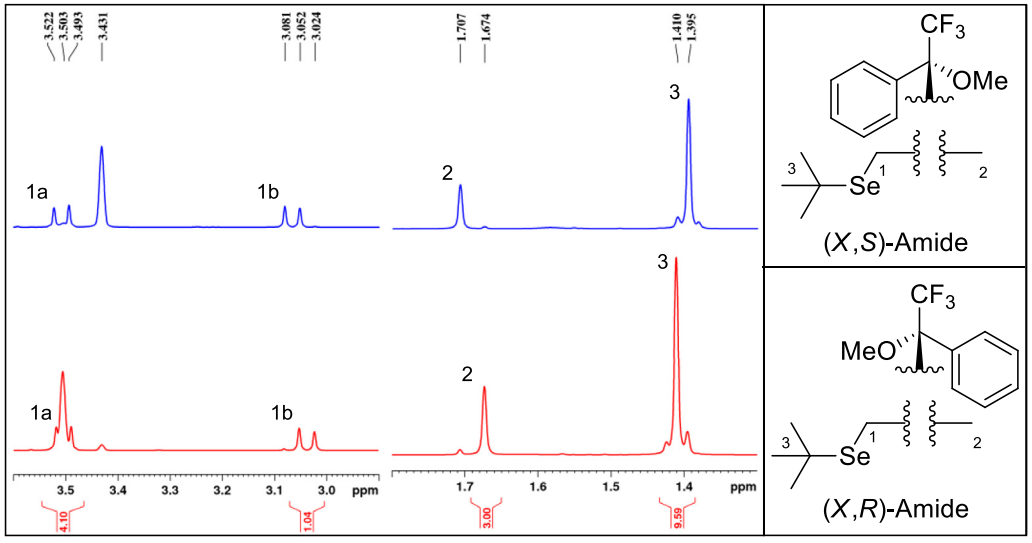


Fig. 1. ^{1}H spectra of (*X,S*)-**5** (blue) and (*X,R*)-**5** (red) showing the sp^{3} hybridized region as well as structures showing the predicted placements of groups off of the α-carbon. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

geminal ^{2}J-coupling or shielding from the adjacent selenium atom interfering with the anisotropic effects. The *tert*-butyl protons experience only a small anisotropic shift (−6.2 Hz), believed to result from the larger distance from the MTPA-phenyl. Considering the more reliable anisotropic shifts presented in the ^{1}H spectra, the side groups take the configurations that are presented in Fig. 1 for each diastereomer.

The ^{13}C spectra were more conclusive with stereocenter assignment than the ^{1}H spectra. In agreement with the ^{1}H spectra, the α-methyl carbon (+28.6 Hz) experienced a large anisotropic shift upfield with (*X,R*)-**5** (Fig. 2). Inversely, the methylene carbon atom (−43.5 Hz) as well as the quaternary *tert*-butyl carbon atom (−14.8 Hz) are shifted upfield on (*X,S*)-**5**. The *tert*-butyl methyl carbon atoms on both diastereomers did not experience any noticeable anisotropic shifts, which could be attributed to the increasing distance from the MTPA-phenyl tapering its influence on the methyl carbon atoms. A diminished anisotropic effect was also noticed in the ^{1}H spectra as the *tert*-butyl protons also experienced very little anisotropic shifting. Considering the observed anisotropic shifts in the ^{13}C spectra, the side groups match with the configurations proposed for the ^{1}H spectra as suggested in Fig. 1, with even more confidence.

The resonances of the trifluoromethyl groups of each diastereomer are markedly magnetically inequivalent in the ^{19}F NMR (Fig. 3) spectra. The ^{19}F signal of (*X,R*)-**5** is downfield compared to (*X,S*)-**5**. Based on literature precedence [13,23,24], this is caused by the steric effects of a large side group interacting with the MTPA-phenyl, destabilizing the rotomer.

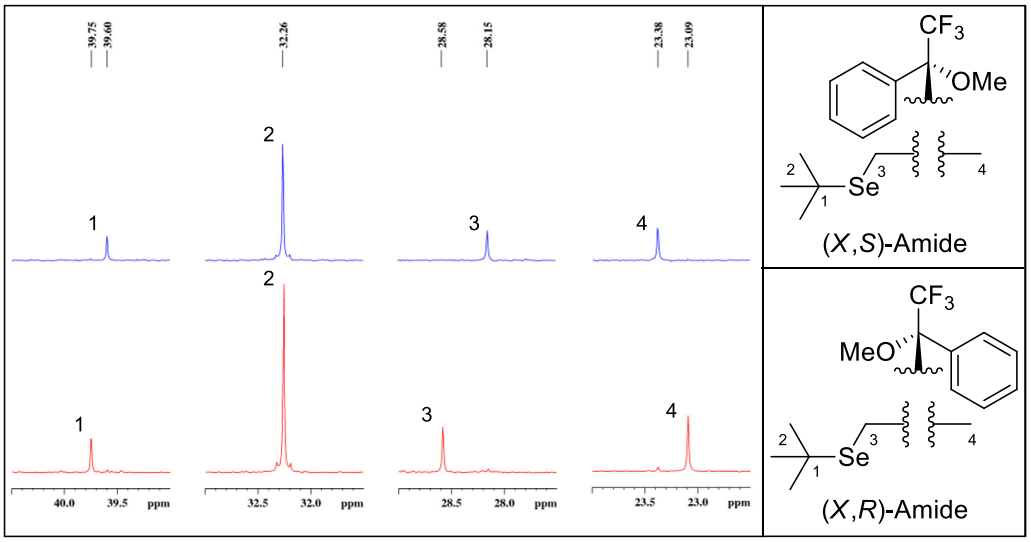


Fig. 2. Sp^3 regions of the ^{13}C spectra of (*X,S*)-**5** (blue) and (*X,R*)-**5** (red). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

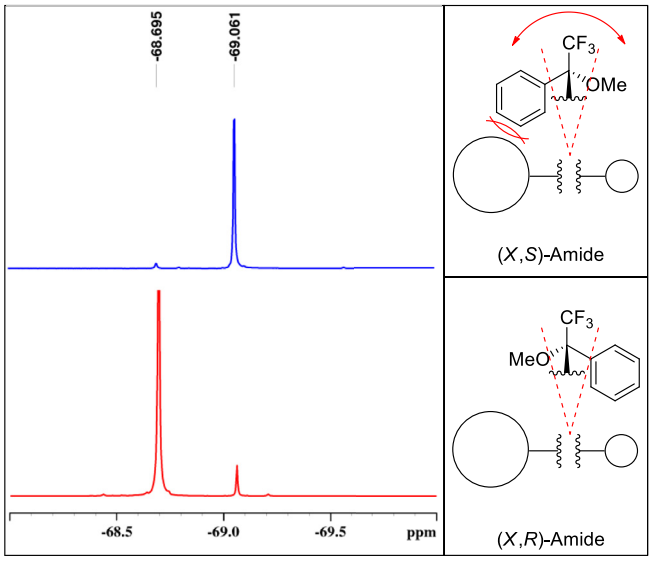


Fig. 3. The ^{19}F spectra of the diastereomers ((*X,S*)-**5**, blue; (*X,R*)-**5**, red) comparing the trifluoromethyl fluorine shifts. In (*X,S*)-**5**, the trifluoromethyl group is shielded, so the larger selenium containing group must be on the same side as the phenyl. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

This destabilization causes the trifluoromethyl to spend less time in the preferred deshielding cone of the MTPA-carbonyl resulting in an overall upfield anisotropic shift when the MTPA-phenyl and the large side group are ipsilateral. If the MTPA-phenyl is on the same side as the smaller group, there is less destabilization, and therefore the trifluoromethyl remains longer in the carbonyl deshielding cone. With the selenium containing side group being much larger than the methyl group, and the trifluoromethyl group in (*X,S*)-**5** experiencing the upfield shift, the ^{19}F NMR corroborates the previously proposed configurations presented in Figs. 1 and 2.

We hypothesized that ^{77}Se NMR would give a large anisotropic shift difference between the two disateromers since selenium is sensitive to its electronic environment [25–27]. However, the ^{77}Se shift difference was small (ca. 1 ppm) (Fig. 4). The ^{77}Se spectra gave results that appear contradictory to the hypothesized configuration of the α-carbon. Other effects, such as steric hindrance, atom distance, or the large electron cloud shielding the nucleus may prevent the observance of significant chemical shift differences.

Based on all the spectra collected, we have determined the configuration of the side groups on the α-carbon, leaving the ester to sit in the MTPA-plane. We propose that the ester will sit on the same side of the molecule as the amide hydrogen, allowing for hydrogen bond formation. With the ester sitting *syn*-periplanar to the amide hydrogen, the absolute configuration of the α-carbon would be (*R*) as illustrated in Fig. 5. This configuration coincides with what is suspected when compared to the α-methylcysteine analogues synthesized using our PLE method. Both α-methylcysteine and α-methylselenocysteine products have the same direction of rotation, -1° (c = 1.00 in $CHCl_3$ at 91% ee) [28] and -2.1° (c = 1.07 in $CHCl_3$ at 88% ee) [7] respectively, supporting the hypothesis that the stereocenters are the same configuration.

After completing the Mosher analysis, it was fortuitous that x-ray quality crystals of **1** could be obtained. The crystals of **1** were grown from octane by slow evaporation. The enzymatic product **1** shares the same configuration as **2** as it is well established that the Curtius rearrangement employed in the synthesis is known to preserve the stereochemistry at the α-carbon [28]. X-ray diffraction on this crystal confirmed the assignment of an (*R*)-stereocenter of the parent amino acid, ultimately validating the assignment of the stereocenter utilizing the Mosher method (Fig. 6).

Based on our findings, a sterically hindered, tertiary, stereocenter bearing an amine can be assigned utilizing the classic Mosher method. With the added steric effects provided from the α-methyl group, these structures do not sit in the MTPA-plane as normally observed when an α-hydrogen is present. This procedure provides a convenient method of determining the absolute stereochemistry of novel α-methyl amino acids.

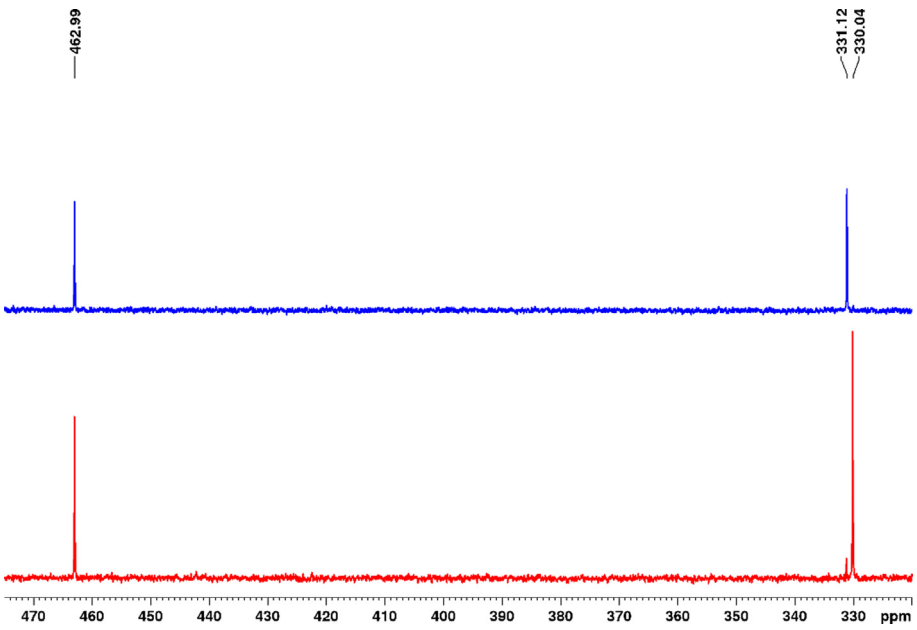


Fig. 4. ^{77}Se NMR spectra of (*X,S*)-**5** (blue) and (*X,R*)-**5** (red) spiked with diphenyl diselenide (463 ppm) as an internal standard. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

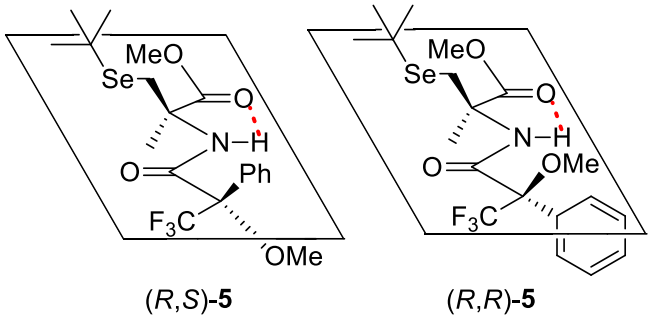


Fig. 5. Both diastereomeric amides laid in a plane. Based on the data the amides appear to sit in these conformations with the parent amino acid having the (*R*)-configuration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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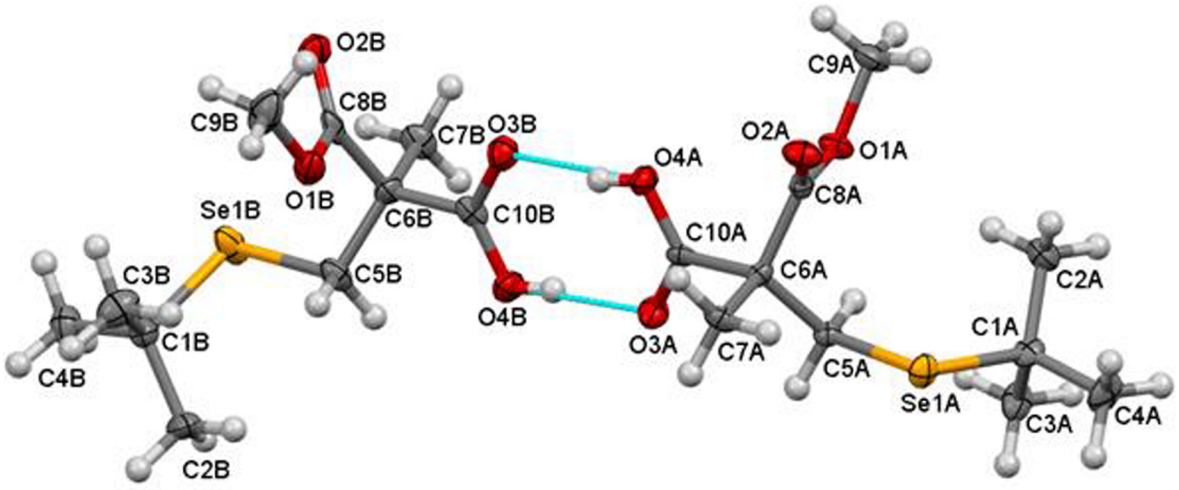

Fig. 6. X-ray crystal structure of the enzymatic hydrolysis product at an elipsoid displacement probability of 50%. The α-carbons (C6A and C6B) have (*R*)-absolute stereochemistry.

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