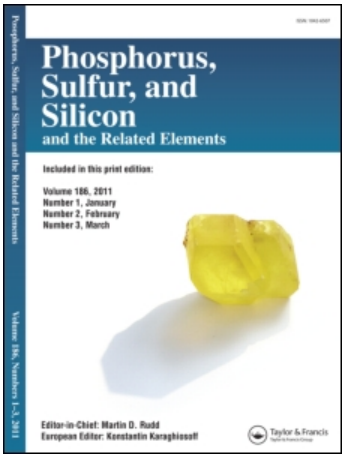

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SYNTHESIS OF SE- AND TE-CONTAINING AMINO ACIDS: USEFUL PROBES FOR STRUCTURAL STUDIES OF PROTEINS.

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ABSTRACT The synthesis of (l)-Se-cystine, (l)-[^{77}Se]-cystine and (l)-Te-cystine in 4 steps from commercially available 2(S)-[(*tert*-butoxycarbonyl)amino]-3-hydroxy-propionate has been accomplished. [4-^{13}C]Te-Met has been synthesized in a one pot reaction.

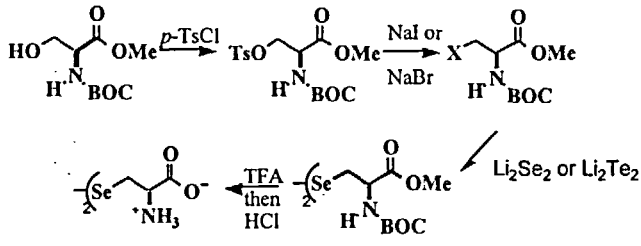
Keywords: Selenium and tellurium-containing amino acids, [^{77}Se]-cystine and Te-cystine, telluromethionine, and [4-^{13}C]telluro-methionine.

Techniques for the biosynthetic incorporation of selenium- and tellurium- containing amino acids into biomacromolecules have been exploited to produce both heavy-atom derivatives and nuclear magnetic resonance probes.[1] These chalcogen-based derivatives have played a significant role in the elucidation of both the local and global structures of a variety of biomacromolecules. Although the replacement of methionine with selenomethionine (Se-Met) in a protein was reported as early as 1957, it was not until recently that a selenium containing amino acid was successfully used as a "heavy-atom" protein derivative by Hendrickson.[2] More recently, Odom, Dunlap and coworkers reported the

incorporation of telluromethionine (Te-Met) into a protein.[3] Two of the more interesting features of this body of work are that the Te-Met was not only incorporated but also was selectively introduced into only the buried methionyl residues. Odom and Dunlaps method has recently been shown to be an effective and rational approach for heavy-atom derivatization of a number of proteins.[4]

Prompted by the increased demand for these compounds, we have initiated a program to synthesize optically active and isotopically enriched

Scheme 1

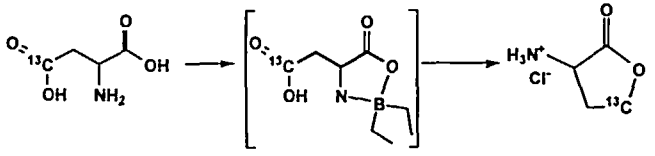


selenium and tellurium containing amino acids. We have been exploring synthetic routes for the synthesis of stable isotope labeled amino acids which contain either a selenium or tellurium atom. (l)-Se-cystine, (l)-[^{77}Se]-cystine and (l)-Te-cystine have been constructed in 4 steps from commercially available 2(S)-[(*tert*-butoxycarbonyl)amino]-3-hydroxy-propionate according to Scheme 1.[5] The ^{77}Se chemical shift of the (l)-[^{77}Se]-cystine was observed at 294 ppm.[6]

In order to gain solution structural information by NMR spectroscopy of the Te-Met derivatized proteins it is essential to construct the various isotopomers of this amino acid. A one-pot synthesis of both racemic and optically active (l)-Te-Met from homoserine lactone

(HSL) has been reported (7). Using a synthetic route identical to one which we recently developed for the synthesis of (l)-Te-Met, it was envisioned that

Scheme 2



a suitably labeled HSL would serve quite well as the substrate for the reaction. Synthesis of the (l)-[4-^{13}C]HSL is accomplished using the procedure developed by Bong and Lynn (Scheme 2).[8] Aspartic acid is regioselectively reduced using an *in situ* protection of the 1-carboxylic acid with triethylborane. Reduction of the remaining acid is then accomplished using diborane. Annulation is effected with HCl, giving the (l)-[4-^{13}C]HSL. Using MPTA, the HSL enantiomeric excess was determined to be greater than 99%, as reported. In addition, the yields were similar to those that were reported. Addition of 1.8 equivalents of lithium methyl tellurolate in THF to a -78°C methanolic solution of (l)-[4-^{13}C]HSL gave rise to labeled Te-Met. While it has been suggested by Karnbrock *et al.*[9] that using methanol as a solvent for the reaction may be problematic, it is well known that the tellurolate should remain completely ionized in methanolic solutions. Methanol in fact, serves as an ideal solvent by both solubilizng the lactone and allowing the tellurolate to remain ionized and therefore more nucleophilic.

Interestingly, when the reaction was carefully monitored by TLC, it is apparent that the reaction does not take place until sometime during

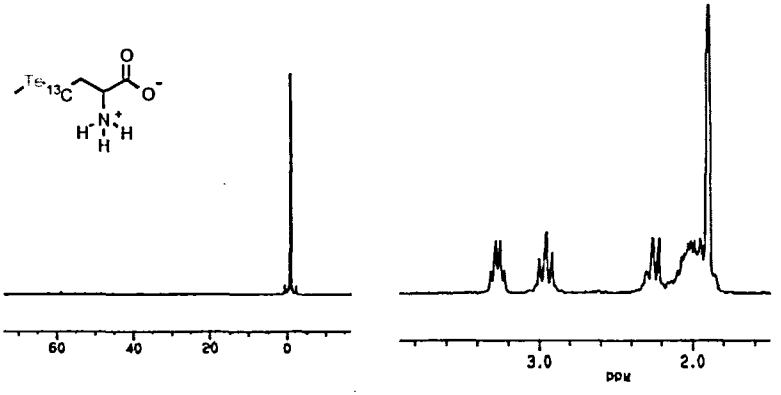


Figure 1. Left panel is a trace of the ^{13}C NMR spectrum of (l)-[4-^{13}C]Te-Met. Right panel is a trace of the ^{1}H NMR spectrum of (l)-[4-^{13}C]Te-Met.

the concentration process. Evidently, during the removal of the solvents over a 2-4 hour period the ring opening process occurs.[10] TLC analysis of this mixture indicates the presence of a product which possesses an R_f similar to the authentic natural abundance Te-Met (bright navy blue as indicated with ethanolic phosphomolybdic acid). ^{13}C NMR analysis of the crude reaction mixture clearly indicates only two major ^{13}C resonances. The largest resonance (~80%) occurs at ~-3 ppm and is indicative of [4-^{13}C]Te-Met, which is shielded by the heavy tellurium atom. A smaller resonance occurs at 59 ppm and is indicative of homoserine. Careful analysis of the -3 ppm resonance showed presence of satellite peaks which were ascribed to ^{13}C-^{125}Te couplings, thereby providing additional evidence of C-O ring opening. This process afforded the labeled Te-Met in 58% yield. Figure 1 clearly shows the ^{13}C enriched Te-Met.

Using this method we are currently exploring additional synthetic targets, such as the remaining single and multiple ^{13}C isotopomers of Te-Met, and these will be reported in due course. In addition, we are actively pursuing the synthesis of both selenium and tellurium-containing trytophan analogs. Considering both the increase in atomic radius progressing from S to Te as well as the significant decrease in electronegitivity, labeled Te-Met may provide critical NMR spectroscopic information regarding potential solution conformational changes that occur upon substitution of Te-Met for Met in proteins.

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10. Typical procedure. Caution: methyl tellurol, methyl telluride, and dimethyl ditelluride are noxious, volatile, and probably toxic materials. All manipulations must be carried out in a high velocity hood and protective clothing and gloves should be worn. The HSL was added to a 250 mL three-necked round bottom flask which was fitted with two rubber septa and a magnetic stir bar. The flask was directly attached to a glass vacuum manifold. Methanol was added (100 mL) and the system was then pumped down to a constant pressure of 0.030 Torr. To this was added a THF solution of methyltellurolate (1.80 equivalents). The resulting yellow solution was warmed to room temperature. The solvents were then slowly removed over a 2-4 hour period. During this time ice formed on the outside surface of the round bottom flask. The resulting brown foam was taken up in 20 mL of water. The mixture was stirred for 1h under a light vacuum (enough to chill the flask). The cooled aqueous mixture was then filtered through prewashed and hydrated Celite (more than one filtration may be required). The Celite was washed one time with a minimal amount of cold water. The solution was then acidified with solid citric acid monohydrate. The solids which formed were collected and then dried. All compounds have been characterized spectrally (^{1}H, ^{13}C, and ^{125}Te NMR, MS and/or elemental analysis). We have supplied (l)-Te-Met to accredited researchers since 1990.