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Stereoselective Synthesis and Application of L-[^{15}N]Amino Acids

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Abstract

We have developed two general approaches to the stereoselective synthesis of ^{15}N- and ^{13}C-labeled amino acids. First, labeled serine, biosynthesized using the methylotrophic bacterium *M. extorquens* AM1, serves as a chiral precursor for the synthesis of other amino acids. For example, pyridoxal phosphate enzymes can be used for the conversion of L-[α-^{15}N]serine to L-[α-^{15}N]tyrosine, L-[α-^{15}N]tryptophan, and L-[α-^{15}N]cysteine. In the second approach, developed by Oppolzer and Tamura, an "electrophilic amination" reagent , 1-chloro-1-nitrosocyclohexane, was used to convert chiral enolates into L-α-amino acids. We prepared 1-chloro-1-[^{15}N]nitrosocyclohexane and used it to aminate chiral enolates to produce L-[α-^{15}N]amino acids. The stereoselectivity of this scheme using the Oppolzer sultam chiral auxiliary (**6**) is remarkable, producing enantiomer ratios of 200 to 1.

Introduction

Stable isotope-labeled amino acids are used by biomedical researchers to probe important biochemical problems. For example, [α-^{15}N] and [1-^{13}C]amino acids are used to determine their nutritional requirements in humans and to examine inborn errors in amino acid metabolism[1]. In addition, stable isotope-labeled compounds are used to unravel the intricate chemistry involved in the biosynthesis of natural products[2]. Because many natural products including enzyme cofactors[3] and antibiotics[2] are derived from amino acids, labeled amino acids are useful for biosynthetic studies. Finally, stable isotope-labeled amino acids are used in combination with NMR spectroscopy to study enzymes and proteins. Historically, proteins were labeled with amino acids enriched with ^{13}C or ^{15}N and were examined by direct detection NMR methods[4]. Emphasis was on labeling only those amino acyl residues involved in binding or catalysis. More recently, NMR has been used to probe the dynamics and solution structure of proteins; therefore, there is interest in labeling essentially all of the amino acyl residues[5]. Development of multiple quantum NMR methods which allow indirect detection of a ^{13}C or ^{15}N nucleus based on its coupling interactionwith the directly bonded protons[6] and of three-[7]

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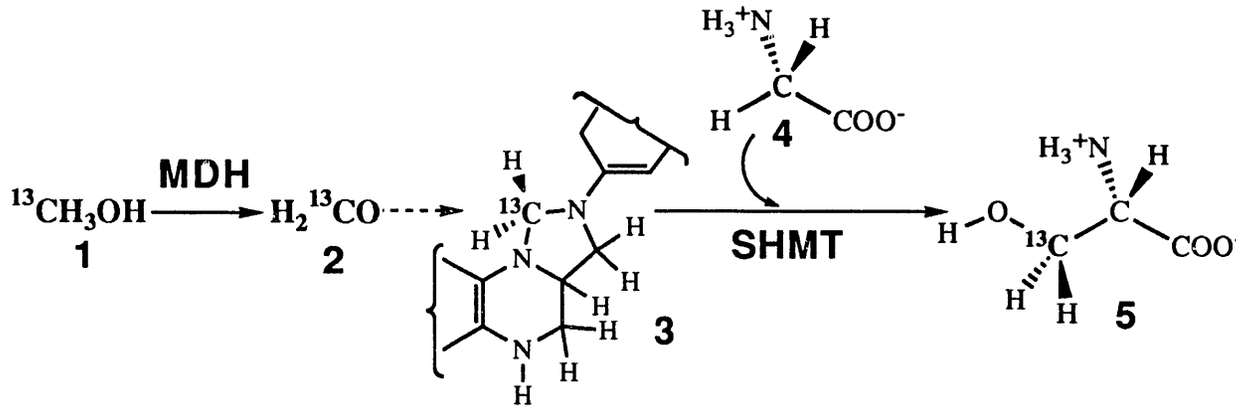
and four-dimensional[8] NMR methods which allow correlation of proton, carbon, and nitrogen resonances has increased interest in labeled amino acids.

For many of the applications described above, the naturally occurring L-configuration of the labeled amino acid is required. In general, specific labels have been introduced into racemic mixtures of α-amino acids which were resolved using hog kidney acylase. We are developing strategies for the stereoselective synthesis of specifically labeled L-α-amino acids. Described briefly here are two general schemes for the stereoselective synthesis of ^{13}C- and ^{15}N-labeled amino acids. The first involves the stereospecific biosynthesis of L-serine and its conversion to other amino acids. The second, a more general scheme, involves the use of the Oppolzer chiral auxiliary.

Biosynthesis of Labeled Serine

As diagrammed below, we developed a method for the biosynthesis of L-serine which uses the serine-type methylotrophic bacterium, *Methylobacterium extorquens* AM1[9]. This organism contains large amounts of the enzymes methanol dehydrogenase (MDH) and serine hydroxymethyl-transferase (SHMT) which can be used for the net synthesis of methanol[10]. MDH catalyzes the oxidation of methanol (**1**) to formaldehyde (**2**); SHMT catalyzes the stereospecific aldol condensation of formaldehyde (**2**), as methylenetetrahydrofolate (**3**), with glycine (**4**) to produce L-serine (**5**). In this process, C-3 of serine is derived from methanol; C-1, C-2 and the α-amino group of serine are derived from glycine. Therefore, by starting with the appropriately labeled precursors, we can synthesize most of the ^{2}H, ^{13}C, and/or ^{15}N isotopomers of L-serine.



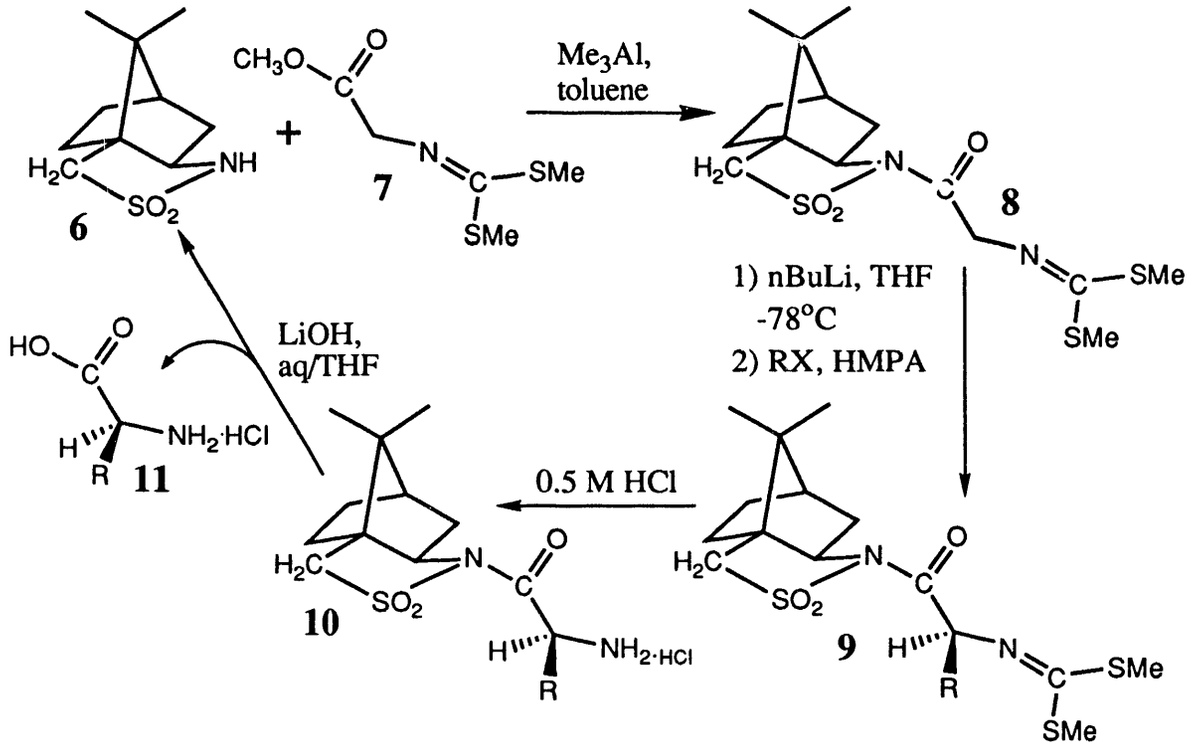
Stereoselective Conversion of Serine to Other L-α-Amino Acids

L-serine is an important amino acid because it can serve as a precursor for the synthesis of more complex amino acids; the stereochemistry at the α-carbon produced during the biosynthesis of serine can be retained in the product amino acid. For example, Vederas and coworkers[11,12] have described the synthesis of the N-urethane-protected β-lactone of L-serine;

the β-lactone can serve as a template for homologation reactions at the β-carbon. Treatment of the L-(N-*t*-boc)-serine-β-lactone with a series of nucleophiles yields α-amino acids with retention of configuration at the α-carbon. In addition, L-serine can serve as substrate for a number of pyridoxal phosphate enzymes which can be used for the net synthesis of aromatic amino acids[13-15]. As has been reported elsewhere, we have converted ^{13}C- and ^{15}N-labeled serine into labeled L-tyrosine[15], L-aspartate[16], L-tryptophan[17], and L-cysteine[18].

Labeled Amino Acid Synthesis Using the Camphor Sultam Chiral Auxiliary

Recently, Oppolzer and coworkers[19,20] developed strategies for the synthesis of enantiomerically pure amino acids which involve the camphor sultam chiral auxiliary (**6**). First they described a camphor-based chiral glycine equivalent (**8**) which, as diagrammed below, is useful for the stereoselective synthesis of the common α-amino acids. Oppolzer's chiral auxiliary (**6**) contains a sultam ring fused to the camphor nucleus; N-protected glycine (N-[bis(methylthio)-methylene]-glycine) (**7**) is linked as an amide to the nitrogen in the sultam ring. This chiral glycine equivalent (**8**) is metallated by treatment with n-butyl lithium in THF at -78°C. Decomposition of the enolate with electrophiles is carried out in the presence of hexamethylphosphoramide. The N-blocking group is removed by acid hydrolysis. After treatment with dilute lithium hydroxide, the product amino acid (**11**) and the camphor sultam auxiliary (**6**) are separated and recovered.



Scheme 1

This process occurs with remarkable stereoselectivity. Starting with the (2*S*)-camphor sultam glycinate and using a series of alkyl iodides as electrophiles, L-alanine, L-valine, L-leucine, L-phenylalanine, and L-aspartic acid were produced in enantiomeric excess, after crystallization, of greater than 99.5%. Using a three-fold excess of primary alkyl iodides, the alkylation reaction was uniformly efficient (yield 85 to 93% based on **8**) and had remarkable enantioselectivity (d.e. 95.6 to 96.8%). Efficient π-face-selective alkylations were also achieved using secondary alkyl iodides or alkyl bromides in the presence of tetrabutylammonium iodide. Efficient procedures for deblocking the product amino acid were carried out without racemization of the product.

Synthesis of L-[β-^{13}C]Phenylalanine--We prepared L-[β-^{13}C]phenylalanine by alkylation of the sultam glycinate with [α-^{13}C]benzyl iodide which was prepared as follows. Phenylmagnesium bromide was treated with $^{13}CO_2$ to yield [*carboxyl*-^{13}C]benzoic acid which was reduced directly to [α-^{13}C]benzyl alcohol by treatment with lithium aluminum hydride. Benzyl alcohol was converted to the corresponding chloro-compound by treatment with HCl. Displacement of chloride by iodide was effected by treatment with NaI. Although preparation of [α-^{13}C]benzyl iodide required four manipulations of the label, these reactions were all very efficient, with >94% of the $^{13}CO_2$ being recovered as the product [α-^{13}C]benzyl iodide.

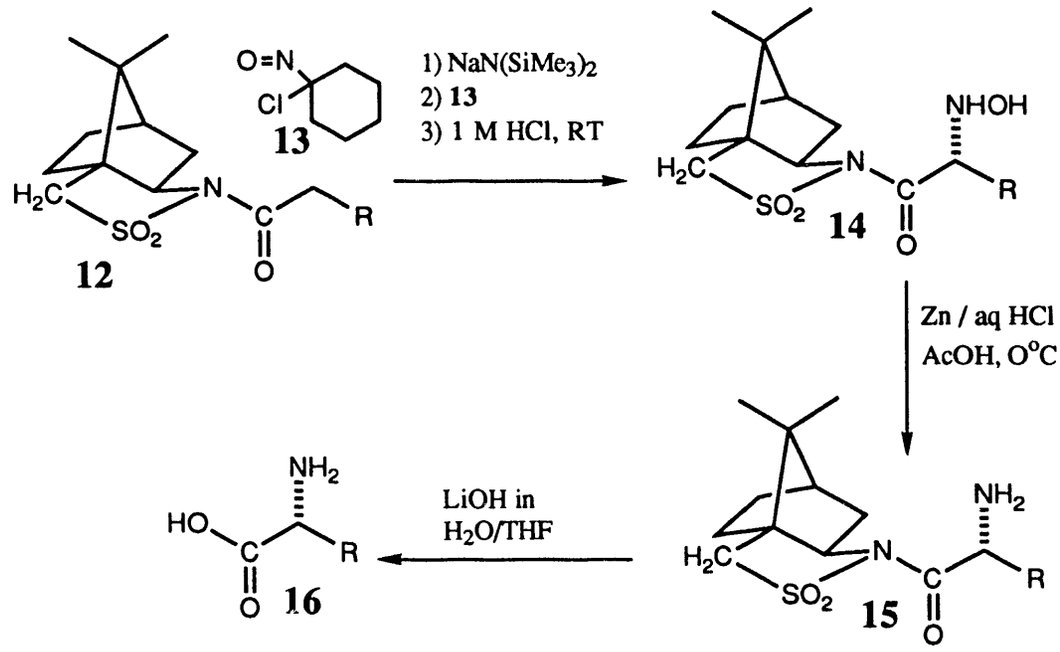
The sultam glycine enolate was treated with 3 equivalents of [α-^{13}C]benzyl iodide in THF at -78°C. ^{13}C NMR analysis of the crude reaction products showed a mixture of ^{13}C-labeled alkylated sultam and [α-^{13}C]benzyl iodide; these compounds were separated and recovered. The recrystallized yield of the phenylalanine sultam derivative was 67% based on the sultam glycine enolate. The overall recovery of label in benzyl iodide (60%) and phenylalanine (22%) was 82%. ^{13}C NMR analysis of the recrystallized phenylalanine sultam derivative showed that the (2*S*) enantiomer was produced in diastereomeric excess of >99%.

Preparation of ^{15}N-Labeled Amino Acids Using the Camphor Sultam Chiral Auxiliary

Oppolzer and Tamura[20] developed an "electrophilic amination" approach which incorporates the camphor sultam chiral auxiliary (Scheme 2). Deprotonation of the N-acyl camphor sultam (**12**) is effected by treatment with sodium hexamethyldisilazide. The enolate is treated with 1.1 equivalents of 1-chloro-1-nitrosocyclohexane (**13**) followed by HCl to quench the reaction. The isolated sultam hydroxylamino acid (**14**) is treated with Zn to yield the sultam-linked amino acid (**15**). The sultam is removed by saponification with dilute LiOH to yield the free amino acid (**16**). Again, the stereoselectivity of this scheme produces enantiomer ratios of 200 to 1. As diagrammed below, the acyl derivatives of the (2*S*)-camphor sultam lead to D-amino acids; acyl derivatives of the antipode, (2*R*)-camphor sultam yield L-amino acids.

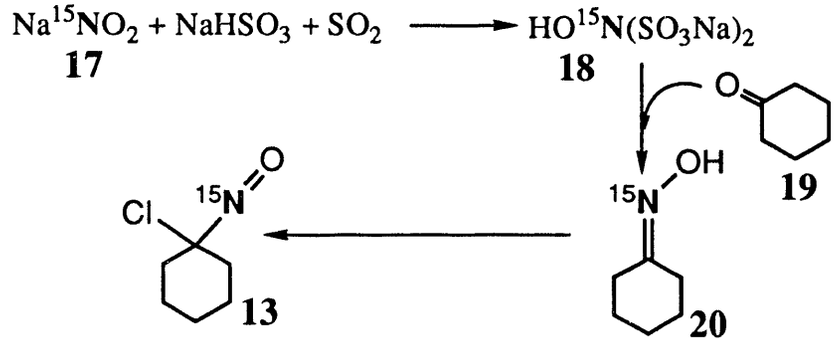
We developed an efficient synthesis of ^{15}N-labeled 1-chloro-1-nitrosocyclohexane to make this a convenient method for the synthesis of L-[α-^{15}N]amino acids.

Preparation of 1-chloro-1-[^{15}N]nitrosocyclohexane--Scheme 3 shows the preparation of 1-chloro-1-[^{15}N]nitrosocyclohexane. First we prepared cyclohexanone [^{15}N]oxime using a



Scheme 2

modification of a procedure described by Eck and Marvell[21]. Potassium [^{15}N]nitrite (**17**) was converted into the intermediate hydroxylamine disulfonate (**18**) by treatment with sodium bisulfide and sulfur dioxide at 0°C. Addition of an excess of cylohexanone (**19**) followed by heating at 50°C for one hour gave a mixture of the cyclohexanone [^{15}N]oxime (**20**) and unreacted cyclohexanone (**19**). Cyclohexanone (**19**) was removed *in vacuo* to yield pure [^{15}N]oxime (**20**) in 60% yield based on potassium [^{15}N]nitrite (**17**). The [^{15}N]oxime was then converted to 1-chloro-1-[^{15}N]nitrosocyclohexane (**13**) by treatment with chlorine gas[22] in quantitative yield.



Scheme 3

Treatment of acyl sultams corresponding to L-alanine, L-valine, L-[1-^{13}C]valine, L-leucine, and L-phenylalanine with 1-chloro-1-[^{15}N]nitrosocyclohexane gave the related L-hydroxylamino acyl sultams (**14**) in good to excellent yields. NMR analysis of the crude compounds (**14**) showed no evidence of the D-isomers.

References

1. Matthews, D.E. and Bier, D.M. (1983) *Ann. Rev. Nutr.* 3: 309.
2. Floss, H.G. and Beale, J.M. (1989) *Angew. Chem. Int. Ed. Engl.* 28:146-177.
3. Houck, D.R., Hanners. J.L., and Unkefer, C.J. (1991) *J. Am. Chem. Soc.* 113:3162-3166.
4. London, R.E. (1984) in *Topics in Carbon-13 NMR Spectroscopy*, Volume 4 (G.C. Levy, ed.) John Wiley and Sons, New York, pp. 54-90.
5. London, R.E. (1980) in *Magnetic Resonance in Biology, Volume 1* (J.S. Cohen, Ed.) John Wiley and Sons, New York, pp. 1-69.
6. Griffey, R.H. and Redfield, A.G. (1987) *Quart. Rev. Biophysics* 19:51-82.
7. Oschkinat, H., Griesinger, C., Kraulis, P.J., Sorensen, O.W., Ernst, R.R., Gronenborn, A.M., and Clore, G.M. (1988) *Nature* 332:374-376.
8. Kay, L., Clore, M., Bax, A., and Gronenborn, A. (1990) *Science* 249:411-413.
9. Hanners, J. L., Gibson, R., Velarde, K., Hammer, J., Alvarez, M., Griego, J., and Unkefer, C.J. (1991) *J. Labelled Comp. Radiopharm.* 29: 781-790.
10. Anthony, C. (1982) in *The Biochemistry of Methylotrophs*, Academic Press, New York pp.19-132.
11. Arnold, L. D., Kalantar, T. H., and Vederas, J. C. (1985) *J. Am. Chem. Soc.* 107: 7105-7109.
12. Arnold, L. D., May, R. G., and Vederas, J. C. (1988) *J. Am. Chem. Soc.* 110: 2237-2241.
13. Houck, D. R., Chen, L. C., Keller, P. J., Beale, J. M., and Floss, H. G. (1988) *J. Am. Chem. Soc.* 110: 5800-5806.
14. Soda, K. and Esaki, N. (1987) *Methods in Enzymology* 143: 291-297.
15. Walker, T. E., Matheny, C., Storm, C.N., and Hayden, H. (1986) *J. Org. Chem.* 51:1175-1179.
16. Lodwig, S.N. and Unkefer, C.J. (1991) *J. Labelled Comp. Radiopharm.* In press.
17. Unkefer, C.J., Lodwig, S.N., Hanners, J.L., Ehler, D.S., and Silks, L.A. III (1991) *J. Labelled Comp. Radiopharm.* In press.
18. Unkefer, C.J., Hanners, J.L., and Ehler, D.S. (1991) *J. Labelled Comp. Radiopharm.* In press.
19. Oppolzer, W., Moretti, R., and Thomi, S. (1989) *Tetrahedron Lett.* 30:6009-6010.
20. Oppolzer, W. and Tamura O. (1990) *Tetrahedron Lett.* 31:991-994.
21. Eck, J.C. and Marvel, C.S. (1943) *Org. Syn. Coll. Vol.* 2:76-78.
22. Muller, E., Metzger, H., and Fries, D. (1954) *Chem. Ber.* 87:1449-53.

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