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Racemic Synthesis and Enantiomeric Conversion of [1-^{13}C] Valine

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SUMMARY

A Strecker synthesis of isobutyraldehyde, K^{13}CN and ammonia produced D,L-[1-^{13}C] valine in 83% yield. This racemic mixture was then converted to the L-form via a D-amino acid oxidase - branched amino acid aminotransferase system in an overall yield of 75% based on K^{13}CN. Given the generality of the Strecker synthesis as well as the broad substrate specificity of the oxidase and aminotransferase enzymes, it is anticipated that this procedure will be comparably efficient for most standard aliphatic and aromatic amino acids.

Key Words: [1-^{13}C] valine, enantiomeric conversion, D-amino acid oxidase, branched amino acid aminotransferase.

INTRODUCTION

The desire to produce enantiomerically pure amino acids has been one of the most important stimuli for the active development of chiral synthesis techniques during the last two decades. Indeed the use of rhodium - chiral phosphine catalysts (1) for the asymmetric production of aromatic amino acids represents one of the earliest major successes in this field. Unfortunately, chiral synthesis techniques have generally been of more limited utility in the area of isotopically labeled amino acid chemistry. This is in large part because the significantly elaborated precursors often demanded for chiral synthesis are not readily compatible with the desire to introduce the labeled atom(s) late in the synthetic scheme using a simple precursor form. In the specific example of present interest, stereoselective

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Strecker reactions have been reported by addition of cyanide to Schiff bases formed from aldehydes and chiral amines. In initial work in this field Harada and Okawara (2) used the stereoisomers of α-methylbenzylamine in order to obtain synthetic yield of various amino acids in a range of 9-58% having optical purities of 22-58%. More recent improvements have led to increased synthetic yields for amino acids having enantiomeric excesses of 50-90% (3). However, these later procedures have used trimethylsilylcyanide as the addition agent which reduce their utility for the synthesis of carboxyl labeled amino acids.

As a result, protocols for synthesis of labeled amino acids have continued to rely heavily on more classical reaction schemes which produce racemic products. If a chiral amino acid sample is ultimately desired, resolution by enzymatic means or by fractional crystallization are generally used. In addition to the added steps required by these approaches, resolution procedures generally suffer from the wasting of the portion of the label initially incorporated into the undesired enantiomer. A more appealing approach involves the use of the simpler classical racemic syntheses combined with an enzyme catalyzed reaction scheme which converts the initial product into the enantiomerically pure L-form. We have recently developed such an enantiomeric conversion system (Shah, S. A., Schafer, P. H., Recchia, P. A., Polach, K. J. and LeMaster, D. M., manuscript submitted for publication). Herein we report application of this approach to a 30 mmole scale synthesis of L-[1-^{13}C] valine.

RESULTS AND DISCUSSION

In recent years considerable effort has been devoted toward optimization of the cyanohydrin reaction, particularly as it applies to carbohydrate chemistry (4). This research has served to demonstrate the strong dependence of the overall yield on pH of the reaction medium. We have adapted an earlier published lactonitrile synthesis (5) in which a weakly acidified solution containing acetaldehyde and potassium cyanide was neutralized with an equivalent of NaOH, stirred at 4^{0}C and then made basic with excess sodium hydroxide.

We modified this procedure by substituting ammonium hydroxide for the two aliquots of sodium hydroxide. After acid hydrolysis and cation exchange chromatography, D,L-alanine was isolated in 84 % yield. In contrast, using isobutyraldehyde in this protocol gave a much lower yield. However, increasing the incubation period in concentrated ammonium hydroxide from 20 minutes to 24 hours at room temperature resulted in a yield of D,L-valine of 83 % (25 mmol), presumably reflecting the increased steric hindrance of the isobutyryl group.

The enantiomeric conversion system utilizes D-amino acid oxidase to convert the D-form of the amino acid to the corresponding α-keto acid. The branched-chain aminotransferase, which we have recently overexpressed from E. coli, (Chanatry, J. A., Schafer, P. H., Kim, M. S. and LeMaster, D. M., manuscript submitted for publication), then serves to convert the α-keto acid to the L-form amino acid. In order to drive the aminotransferase reaction toward completion an additional amine donor is required. L-glutamate is not only the normal physiological donor for the aminotransferase reaction, it is useful choice for several other reasons as well. Due primarily to its use in the flavoring industry, L-glutamate is manufactured in considerably larger quantities than any other amino acid and has a correspondingly reduced price (6). Since the equilibrium constants for the aminotransferase reactions are near unity (7), a substantial molar excess of L-glutamate is required in order to convert most of the α-keto acid into the L-form. In these experiments we have used a 10-fold excess of L-glutamate. Another benefit of L-glutamate is its high solubility at neutral pH as the sodium salt. We detected no significant inhibition of either the oxidase or aminotransferase activities at 0.5 M L-glutamate. L-glutamate is noteworthy for the ease with which it can be removed from the other neutral amino acids by simple ion exchange displacement chromatography techniques.

The amount of oxidase and aminotransferase used was determined by qualitative estimation of the apparent half time of the two separate partial reactions as analyzed by thin layer chromatography. Concentrations were

used sufficient to generate a half time of approximately 6 hours. The degree of enantiomeric purity was determined by HPLC analysis following derivatization with Nα-(2,4-dinitro-5-fluoro-phenyl)-L-alaninamide (8).

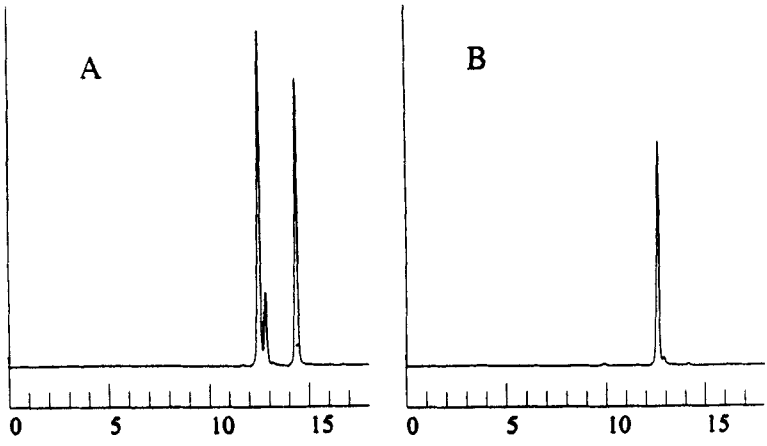

Figure 1. Reversed phase HPLC analysis of valine derivatized with Marfey's reagent (8). In panel A is separated a nearly equal mixture of L-valine (12.6 min) and D-valine (14.3 min). The peak at 12.9 minutes is due to the hydrolyzed reagent. The L-[1-^{13}C] valine is analyzed in panel B.

As illustrated in Figure 1, only 0.8 % of the D-form was present in the final valine product. Since the degree of enantiomeric purity is in practice determined by the D-amino acid oxidase concentration, a correspondingly higher amount can be used in cases where a higher enantiomeric purity is required. Both genetic (9) and biochemical (10, personal observations) studies have demonstrated the fairly broad substrate specificity of the branched-chain amino acid aminotransferase. The very broad substrate range of the D-amino acid oxidase is well-known (11). As a result it is anticipated that an analogous procedure will prove equally useful for most of the standard aliphatic and aromatic amino acids.

EXPERIMENTAL

K^{13}CN was obtained via a grant from the Stable Isotopes Division of Los Alamos National Laboratories. All other chemicals were reagent grade. Porcine renal D-amino acid oxidase and bovine liver catalase were obtained from Sigma Chemical Co. Branched-amino acid aminotransferase was

produced as described elsewhere (Chanatry, J. A., Schafer, P. H., Kim, M. S. and LeMaster, D. M., manuscript submitted for publication).

[1-^{13}C] D,L-Valine Thirty mmol (1.98 g) of $K^{13}CN$ was dissolved in 6 ml of water at 4^{0}C. In a sealed system 15.25 ml of 2.15 N H_2SO_4 was added slowly via an addition funnel followed by 33 mmol (3 ml) of isobutyraldehyde. The mixture was stirred for 2 hours at 4^{0}C. Three ml of 1 M NH_4OH was added and the solution was stirred an additional hour. Four ml of concentrated NH_4OH was added and the solution continued to be stirred for 24 hours at room temperature. After addition of sixty ml of concentrated HCl the solution was stirred for another 24 hours followed by reflux for 8 hours. The solvent was removed by rotary evaporation followed by two rounds of addition of water and reconcentration to remove residual acid. The final crude product was taken up in water and passed onto a Dowex 50 X-8 column (2.5x20 cm) in H^+ form. After washing with water, the D,L-valine was displaced using 150 mM pyridine. Rotary evaporation yielded a crystalline powder of 2.91 g (83% yield) of D,L-valine.

[1-^{13}C] L-Valine 2.91 g (25 mmol) of D,L-valine was taken up in 500 ml of 100 mM Tris HCl, pH 8.5 containing 1mM EDTA and 42.3 g (250 mmol) of monosodium glutamate. To this solution was added in order: 500 mg bovine serum albumin (final 1 mg/ml), 24.71 mg pyridoxal 5'-phosphate (final 0.2 mM), 7.5 mg of catalase (stock solution of 75 mg/ml), 80 mg of D-amino acid oxidase (0.8U/mg) and 0.5 mg (25U/mg) of branched-amino acid aminotransferase (from frozen stock solution). The 2 L flask was covered with perforated aluminum foil and incubated at 37^{0}C with agitation for 3 days.

The solution was diluted 3-fold and loaded onto a Dowex 50 X-8 column (300 ml bed volume) in H^+ form. After washing with water, the valine and glutamate were displaced with 150 mM pyridine. The amino acid containing fractions up to the pyridine containing front can be directly loaded onto a Dowex 1 X-8 (350 ml bed volume) in acetate form. The portion

of sample from the Dowex 50 column contaminated by pyridine is concentrated by rotary evaporation to remove the pyridine and then resuspended in water and loaded onto the Dowex 1 column. After washing with approximately two column volumes of water, the effluent from the Dowex 1 column is concentrated to yield 2.66 g (22.5 mmol) of l-[1-^{13}C] valine for an overall yield of 75 % based on ^{13}CN.

Determination of Enzyme Kinetics and Enantiomeric Purity The kinetics of the oxidase and transaminase reactions were monitored using D-valine and α-ketoisovaleric acid + L-glutamate, respectively, for the partial reactions. The samples were analyzed by silica TLC (1-butanol, acetic acid, water - 4:1:1) developed with ninhydrin. Qualitative determination of enantiomeric purity was carried by derivatization with Nα-(2,4-dinitro-5-fluoro-phenyl)-L-alaninamide and analysis via C_{18} reverse phase TLC (12). For quantitative determination of enantiomeric purity the derivatized samples were separated by HPLC on a C_{18} reverse phase column using a 50mM ammonium phosphate pH 3.0 - acetonitrile gradient (25 to 60%) (8).

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REFERENCES

1. Knowles, W. S., Sabacky, M.J., Vineyard, B.D. and Weinkauff, D. J. - J. Amer. Chem. Soc. 97: 2567 (1975)
2. Harada, K. and Okawara, T. - J. Org. Chem. 38: 707 (1973)
3. Kunz, K., Sager, W., Pfrengle, W. and Schanzenbach, D. - Tet. Lett. 29: 4397 (1988)

4. Serianni, A. S. and Barker, R. in Isotopes in the Physical and Medical Sciences, Ed. Buncel, J., Elsevier, Amsterdam, 1987 pp. 212
5. Sakami, W., Evans, W. E. and Gurin, S. - J. Amer. Chem. Soc. 69: 1110 (1947)
6. Martens, J. - Top. Curr. Chem. 125: 165 (1984)
7. Jenkins, W. T. in Vitamin B_6 - Pyridoxal Phosphate - Chemical, Biochemical and Medical Aspects, Ed. Dolphin, D., Poulson, R. and Avramovic, O., John Wiley & Sons, New York, 1986 pp. 117
8. Marfey, P. - Carlsberg Res. Commun. 49: 591 (1984)
9. Gelfand, D. H. and Steinberg, R. A. - J. Bacteriol. 130: 429 (1977)
10. Inoue, K., Kuramitsu, S., Aki, K., Watanabe, Y., Takagi, T., Nishigai, M., Ikai, A. and Kagamiyama, H. - J. Biochem. 104: 777 (1988)
11. Greenstein, J. P., Birnbaum, S. M. and Otey, M. C. - J. Biol. Chem. 204: 307 (1953)
12. Ruterbories, K. J. and Nurok, D. - Analyt. Chem. 59: 2735 (1987)