SYNTHESIS OF 2-^{14}C-3-AMINO-1-CHLORO-5-METHYLHEXAN-2-ONE HYDROCHLORIDE

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Received August 13, 1976
Revised September 3, 1976

Summary

A convenient synthesis of the ^{14}C-labelled chloromethyl ketone analog of leucine is reported. Modification of previously published conditions allowed for the facile synthesis of: 2-^{14}C-3-amino-1-chloro-5-methylhexan-2-one hydrochloride in four steps from 1-^{14}C-DL-leucine in 85.7% yield. This represented a four-fold improvement over yields reported previously for compounds of this class.

Key Words: ^{14}C-chloromethyl ketones, 2-^{14}C-3-amino-1-chloro-5-methylhexan-2-one

Chloromethyl ketone analogs of amino acids have been reported to act as specific inhibitors of enzyme action. Compounds of this class have been reported to inhibit the action of trypsin, and chymotrypsin[1,2] and it was suggested recently that the α-chloromethyl ketone analog of leucine, DL-3-amino-1-chloro-5-methylhexan-2-one hydrochloride (1) had selectivity as a reversible inhibitor of leucine aminopeptidase and as an irreversible inhibitor of aminoacyl-t-RNA.[3] Recent findings in our laboratories demonstrated a marked selectivity for the inhibition of neutral amino acid transport systems in ascites S37 tumor cells and it was desirable to synthesize the ^{14}C-labelled compound for the isolation and identification of membrane bound amino acid transport system components.[4] Preliminary studies in our laboratories confirmed the earlier findings of others[3] that DL-amino acid precursors were more readily converted to their crystalline α-chloromethyl ketone analogs than were the corresponding L-amino acids.

The title compound was prepared in 85.7% overall yield via a four step reaction sequence which optimized our initial yields of 20% from the unlabelled DL-leucine. The previously reported unlabelled amino acid derivatives of this class[3] were never reported in greater than 22% yield but modification of the procedures of Birch *et al.* gave high yields with excellent incorporation of

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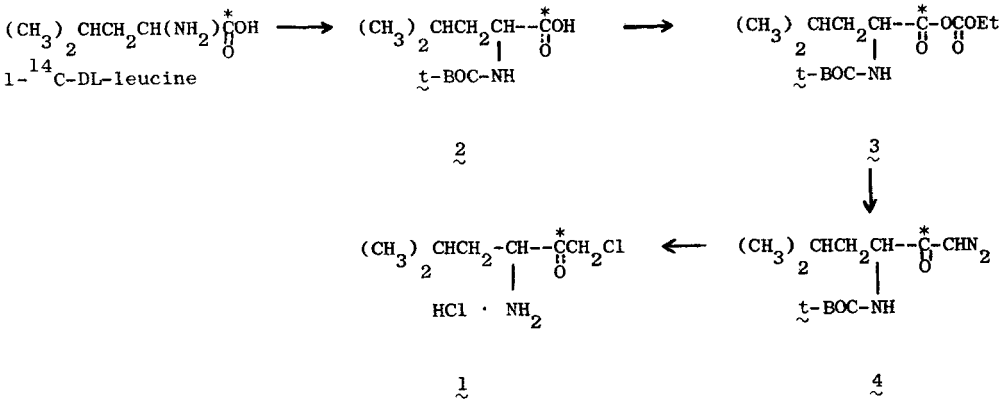
radiolabel into the carbonyl group with 1-^{14}C-DL-leucine* as the starting material.



The DL-amino acid was chosen because of 1) ready availability 2) higher overall yields with our synthetic routes than for the L-leucine. In addition to these factors, the 1-^{14}C-DL-leucine was significantly less expensive (less than half the cost) than the 1-^{14}C-L-leucine.

The 2-^{14}C-DL-chloromethyl ketone was prepared by the conversion of the 1-^{14}C-DL-amino acid to the respective N-t-butyloxycarbonyl (N-t-BOC) derivative (2) in nearly quantitative yield by modification of the procedure of Grzonka and Lammek.[5] The isolated N-t-BOC derivative contained virtually all radioactivity of the starting amino acid and was then converted to the reactive DL-N-t-BOC-alanyl ethoxyformyl anhydride (3) by treatment with ethylchloroformate. The compound 3 was reacted with excess diazomethane to produce DL-3-N-t-BOC-amido-1-diazo-butane-2-one (4), a diazo ketone. The diazo ketone 4 was treated with anhydrous hydrogen chloride to yield the α-chloromethyl ketone 1 as the hydrochloride salt.

The ^{14}C-radiolabel at the 1-position of the DL-amino acid would be expected to be fully incorporated into the final product since no decarboxylation reactions were observed to occur in N-t-BOC formations, mixed anhydride, diazo ketone formation or in the HCl treatment of the diazo ketone. The final product was isolated as white crystals which precipitated from anhydrous ether after treatment of 4 with dry HCl. Through this method 1.0 mCi of 1-^{14}C-DL-leucine was converted to the 2-^{14}C-DL-leucine chloromethyl ketone (1) with specific activity of 20.7μCi/mM.

Experimental

All inert solvents were distilled from $LiAlH_4$ and stored over sodium. Melting points were determined on a Hoover-Thomas Unimelt apparatus in sealed capillary tubes and are uncorrected. Specific activity was determined after sample combustion in a Packard-Tri-Carb Sample Oxidizer 306 and liquid scintillation counting in Perma-Fluor V cocktail with a Beckman Counter Model LS-355 using an internal standard. Intermediate compound purity was determined by tlc and total cpm analysis.

Preparation of 2-^{14}C-1-chloro-3-amino-5-methylhexan-2-one (leucine chloromethyl ketone 1)

1-^{14}C-N-t-BOC-DL-Leucine (2)

Compound 2 was prepared by modification of the procedure described by Grzonka et al. (5).

A suspension of 5.24 (0.04 mol) of DL-leucine combined with 1.0 mCi of 1-^{14}C-DL-leucine (sp. act. 59 mCi/mM) in 60 ml H_2O with 16.8 ml of Et_3N was cooled in an ice bath. t-BOC-azide (6.8 ml 0.04 mol) in 60 ml dioxane was added dropwise with stirring. The reaction mixture was stirred 16 hrs at room temperature until a clear solution resulted. Organic solvents were removed in vacuo, the residual solution was extracted (2 x 200 ml) with Et_2O, cooled to 0°C and carefully acidified to pH 2.0 with 1N HCl. The product was extracted (3 x 120 ml) with EtOAc, the organic layer dried (Na_2SO_4) and solvent evaporated in vacuo. The resulting oil (9.05 g, 98%) was of sufficient purity as judged from tlc (silica gel, EtOAc) to be used directly in the preparation of 3. The product 2 contained 0.989 mCi.

1-^{14}C-N-t-BOC-DL-leucyl ethoxyformyl anhydride (3).

A 300 ml etheriel solution of 9.05 g (0.039 mol) of 2 with 3.84 g Et_3N was cooled in an ice bath and 4.1 g of ethylchloroformate in 50 ml of dry Et_2O was added dropwise. The reaction mixture was stirred 2 hrs at 0°C, the precipitated $Et_3N \cdot HCl$ was filtered off and solvent removed under reduced pressure. This afforded 11.6 g (98%) of 3 as a reactive oil which was utilized without further puricication.

2-^{14}C-1-diazo-3-N-t-BOC-amino-5-methylhexan-2-one (4).

A 300 ml etheriel solution of 11.6 g (0.038 mol) of the anhydride 3 was treated with a large excess of CH_2N_2 at 0°C for 2 hrs. The yellow Et_2O solution was extracted with 200 ml H_2O, dried (Na_2SO_4), filtered and the solvent evaporated in vacuo. The residual oil (8.74 g, 90%) was not isolated and was used directly for the synthesis of 1.

2-^{14}C-1-chloro-3-amino-5-methylhexan-2-one hydrochloride (1).

The solution of 8.74 g (0.034 mol) of diazo ketone 4 in 300 ml of anhydrous Et_2O was saturated with dry HCl gas and kept at -10°C for three days. Additional HCl gas was introduced on the third day and the mixture kept at -10°C another three days. The white crystalline product was filtered off in a dry box under an argon atmosphere. This afforded 6.84 g (99%) of crystalline chloromethyl ketone 1, m.p. 146-147°C (lit:[3,4] 134-140°C; 147-148°C) specific activity 20.7 μCi/mM

The overall yield was 85.7% based on the starting DL-leucine.

Acknowledgement

We are grateful to the National Cancer Institute, National Institutes of Health Grant Numbers CA-17925-01 and CA-16058 for support of this work.

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