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Convenient method for synthesis of a carbon-14 analogue of DL-Phenyl alanine

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Abstract

DL-Phenyl alanine labeled with carbon-14 in the α-position has been synthesized from ethyl [2-^{14}C] acetate as part of a 6-step sequence.

Keywords: Phenyl alanine; Carbon-14; Diethyl[2-^{14}C] malonate; Labeling**.**

1. Introduction

Phenylalanine, as an essential amino acid has been known as a rich source of biologically active molecular entity [1, 2]. The intricate coordination of systems in the human body is seen in the enzyme-catalyzed process by which phenylalanine is converted into tyrosine, which in turn is converted into DOPA, the metabolic precursor of such other vitally important molecules as dopamine, norepinephrine (noradrenaline), and epinephrine (adrenaline) as neurotransmitters [3, 4]. The serious disorder phenylketonuria causes mental retardation, is a disruption of such harmony due to a genetic defect that inhibits the body's production of the key enzyme required for converting phenylalanine to tyrosine [5]. People suffering from Parkinson's disease used DL-phenyl alanine to treat depression and the D form may also be helpful in the treatment of Parkinson's disease and chronic pain in both osteo-arthritis and rheumatoid arthritis with mixed results Increases blood levels of norepinephrine, epinephrine and dopamine - all three required for neurotransmission [6, 7]. To further elucidate the mechanism of action and to support the on-going metabolism studies the need arose to synthesize the corresponding carbon-14 compound, with the label situated in a biologically stable site [8]. In pervious report we reported the synthesis of [4,5-^{3}H]-leucine and in this report, the synthesis of DL-phenylalanine labeled with carbon-14 in α-position 1 is described [9].

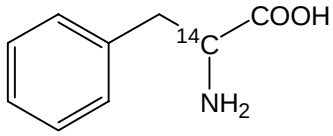

Fig. 1. Chemical structure of phenyl alanine.

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2. Experimental

Ethyl [2-^{14}C] acetate was prepared according to the standard procedure [10]. IR spectra were recorded on a Bruker FT-IR, Vector 22 instrument and the ^{1}H- NMR spectra were recorded on a Varian unity plus 400 spectrometer (400MHz). Radioactivity was determined using a Beckman LS6500 liquid scintillation spectrometer. Mass spectra were obtained on a Finnigan TSQ-70 instrument.

2.1. Diethyl [2-^{14}C]malonate 3

A flame-dried, 100-mL, round-bottomed Schlenk flask equipped with a rubber septum and a magnetic stirring bar was purged with argon. The flask was charged with 15 mL of anhydrous tetrahydrofuran (THF) and 5.8 mL (28 mmol) of hexamethyldisilazane (HMDS). After the solution was cooled to 0°C in an ice-water bath, 9.4 mL (23.5 mmol) of a solution of butyllithium (2.5 mol L^{-1} in hexanes) was added slowly via a syringe to the stirred solution. The ice bath was removed and the mixture was allowed to warm to room temperature.After the solution was stirred for 30 min, it was cooled to −78 °C using an acetone-dry ice bath and equilibrated for 5 min at the same temperature. Then 1.00 g (11.2 mmol, 414MBq) of ethyl [2-^{14}C]acetate was added within 5 min via a syringe, and the acetate-containing flask was rinsed with 0.5 mL of anhydrous THF. Stirring was continued at −78°C for 20 min, and 1.07 mL (11.2 mmol, 1 equiv) of ethyl chloroformate was added within 5 min via a syringe. The mixture was stirred for 1 hr at −78°C, and 5 mL of 6 mol L^{-1} hydrochloric acid (HCl) was added in one portion.

The mixture was allowed to warm to room temperature, and after the addition of 20 mL of water, the pH of the solution was adjusted to 1-2 with 2 mol L^{-1} HCl. The mixture was extracted with diethyl ether (3×50 mL), and the combined organic phases were washed successively with 2 mol L^{-1} HCl, water, and brine (30 mL each). The HCl and water phases were combined and reextracted with ether (50 mL). The organic layer was washed with brine (20mL) and added to the combined organic phases. The combined extracts were dried over anhydrous sodium sulfate (Na_2SO_4), filtered and concentrated under reduced pressure with a rotary evaporator. The crude product was distilled in a microdistillation apparatus at 90 mbar (67.5 mm) to give (1.66 g,10.3 mmol, 380MBq) of diethyl [2-^{14}C]malonate as a colorless liquid. ^{1}H-NMR ($CDCl_3$): δ4.19 (q,4H, J=7.18), δ3.34 (s,2H) δ1.27 (t,6H, J=7.18).

2.2. Diethyl isonitrosomalonate 4

In a 25-mL. three-necked, round-bottomed flask, equipped with a mechanical stirrer and thermometer, was placed (1.66g, 380MBq) of diethyl malonate 3. The flask was cooled in an ice bath, and a mixture of 1.9 mL. of glacial acetic acid and 2.7 mL. of water was added with stirring. With the temperature at about 5 °C, a total of 2.185 g. of sodium nitrite was added in portions over a period of 1.5 hours, the temperature being maintained around 5 °C during the addition. After all the sodium nitrite was added, the ice bath was removed, and the stirring was continued for 4 hours. The reaction mixture was transferred to a 10-mL. separatory funnel and was extracted with two 2-mL. portions of ether. The combined ethereal solution of diethyl isonitrosomalonate was used in the next step immediately.

2.3. Diethyl acetamidomalonate 5

The solution of diethyl isonitrosomalonate 4 described above, 2.85 g. of acetic anhydride, and 7.5 mL. of glacial acetic acid are placed in a 50-mL., three-necked, round-bottomed flask fitted with a mechanical stirrer, a thermometer, and a dropping funnel. With vigorous stirring 2.6 g. of zinc dust was added in small portions over a period of 1.5 hours in such a manner that the

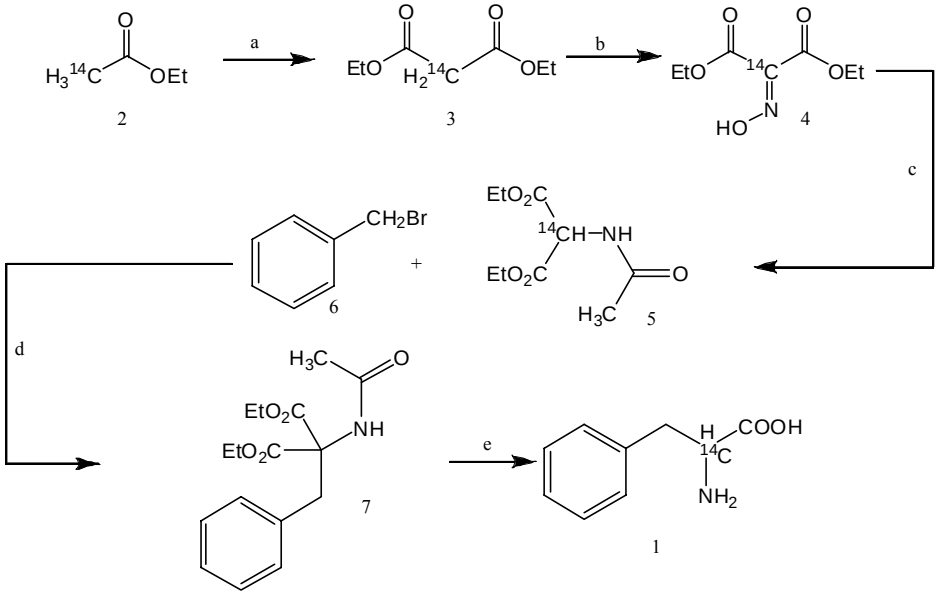
temperature of the reaction was maintained at 40–50 °C. The reaction was markedly exothermic during most of the zinc addition, and intermittent cooling (water bath) was required. After all the metal has been added, the mixture was stirred for an additional 30 minutes. The reaction mixture was filtered with suction and the cake was washed thoroughly with two 7-mL. portions of glacial acetic acid. The combined filtrate and washings are evaporated under reduced pressure on the steam bath until a thick oil, which generally partially crystallizes, remains. To purify the crude product, 3.5 mL. of water was added, and the flask was warmed on a steam bath until the solid melts. The mixture of water and oil was stirred rapidly in an ice bath, and diethyl acetamido[2-^{14}C]malonate crystallized as a fine white product. After cooling in an ice bath for an additional hour, the product was collected by filtration, washed once with cold water, and dried in air at 50°. A second crop was obtained by concentrating the mother liquor under reduced pressure. The yield of diethyl Diethyl acetamidomalonate 5, (1734mg, 292MBq). (77%). ^{1}H-NMR ($CDCl_3$): δ6.54 (bs, 1H), δ5.16 (s,1H), δ4.27 (q,4H, J=7.21), δ2.1 (s,3H), δ1.3 (t,6H, J=7.21).

2.4. 2-Acetylamino-2-benzyl-malonic acid [2-^{14}C]diethyl ester 7

A solution of 119 mg of sodium in 5 mL of absolute ethanole was added to solution of (1085 mg, 182.7 MBq) ethylacetamido cyano acetate and 5mL of absolute alcohole in a 100 mL 3 necked flask equipped with a mercury- sealed stirrer, reflux condenser and dropping funnel. Benzyl bromide (948 mg) was added dropwise with cooling, the mixture was stirred for 2 hours at r.t, then diluted with 10 mL of water and the product was collected, yield (1474 mg, 175.4 MBq) (yield: 96%). ^{1}H-NMR ($CDCl_3$): δ7.1-7.2 (5H-aromatic), δ6.54 (bs, 1H), δ4.27 (q, 4H, J=7.19), δ3.51 (s, 2H) δ2.1 (s,3H, J=7.19), δ1.3 (t, 6H).

2.5. DL-Phenyl alanine[α-^{14}C] 1

A mixture of (1474 mg, 175.4 MBq) of 2-Acetylamino-2-benzyl-malonic acid [2-^{14}C]diethyl ester 7 and 15 mL of 48% HBr was refluxed for 4 hours. The excess acid was vacuum- distilled, and the residue was dissolved in 5mL of water and decolorized with charcoal. A slight excess of ammonium hydroxide and then a slight excess of acetic acid were added and the product was collected, washed with water and alcohol and dry and gave (753 mg, 166.6 MBq) of 1 (yield 95%).^{1}H-NMR (D_2O): δ7.1-7.2 (5H-aromatic), δ4.18 (t, 1H), δ3.2-3.4 (m, 2H). MS (70eV): m/z = 167 (M^+).



Scheme 1 a) LiHMDS, EtOCOCl -78 °C, b)$NaNO_2$/HOAc, c) Zn, HOAc, Ac_2O, d) t-BuOK, t-Bu-OH, e) HBr (48%).

3. Results and discussion

In this approach, according to the synthetic pathway shown in scheme **1**, Ethyl-[2-^{14}C] acetate **2** was converted to diethyl [2-^{14}C] malonate ester **3** by reaction with hexamethyl disilazane, buthyl lithium and ethylchloro formate in THF with good yield [11]. In the next step, diethyl isonitroso malonate **4** was derived from the reaction of diethyl [2-^{14}C] malonate ester **3** with sodium nitrite in acetic acid. The latter product without anymore purification, was converted to diethylacetamido malonate **5** in one pot operation by reduction with Zinc powder, acetic acid in the presence of anhydride acetic acid [12]. The diethylacetamido malonate **5** was coupled with benzyl bromide in the presence of base (Santiago et al., 1996). In the final step, the resulted product 7 was hydrolysed to DL- phenyl-alanine-[α-^{14}C] with hydrogen bromide (48%) under reflux condition [13].

Conclusion

In this paper, a convenient synthetic pathway for labeling of DL-Phenyl alanine with carbon-14 in the α-position has been presented as part of a 6-step sequence from ethyl [2-^{14}C] acetate.

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