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SYNTHESIS OF OPTICALLY PURE (D)-PHENYL[3-^{14}C]ALANINE

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SUMMARY

Lithium aluminium hydride reduction of methyl [7-^{14}C]benzoate gave [7-^{14}C]benzyl alcohol which was transformed (HBr, H_2SO_4) to [7-^{14}C]benzyl bromide. The latter was reacted with lithium N-(bis-methylthiomethylenimino)-acetyl-(2R)-bornane-10,2-sultam (Oppolzer chiral synthon) followed by hydrochloric acid then lithium hydroxide hydrolysis and chromatography on a Dowex 50 column to give (D)-phenyl[3-^{14}C]alanine with 40% overall yield. The molar activity was higher than 50 mCi/mmol and e.e. > 99% as measured by the Marfey method and a modified Marfey method with TLC scanning. The latter method is suitable for the measurement of the optical purity of any amino acid.

Key words: (D)-Phenyl[3-^{14}C]alanine, amino acid, optical purity, chromatography, Oppolzer method

INTRODUCTION

In the last few years, stereoselective syntheses have became common preparative methods in organic chemistry, but so far they are relatively rare in the field of labelled compounds[1]. Stereoselective syntheses have great importance in the synthesis of optically active, labelled amino acids, especially with regard to the unnatural ones (including D-isomers), because the natural amino acids can be manufactured by biosynthesis. The peptide pharmaceuticals often contain unnatural amino acids, so there is an increasing need for them. The most convenient route to obtain them is by asymmetric synthesis.

There are several possibilities for labelling optically active amino acids[2] (e.g. Shoellkopf approach and the use of chiral auxiliaries), but it seems that the best choice is the Oppolzer method[3] (including both alkylation or amination on the α-carbon atom), which provided better yields and optical purity[4]. We chose the Oppolzer alkylation method because it needs fewer active steps.

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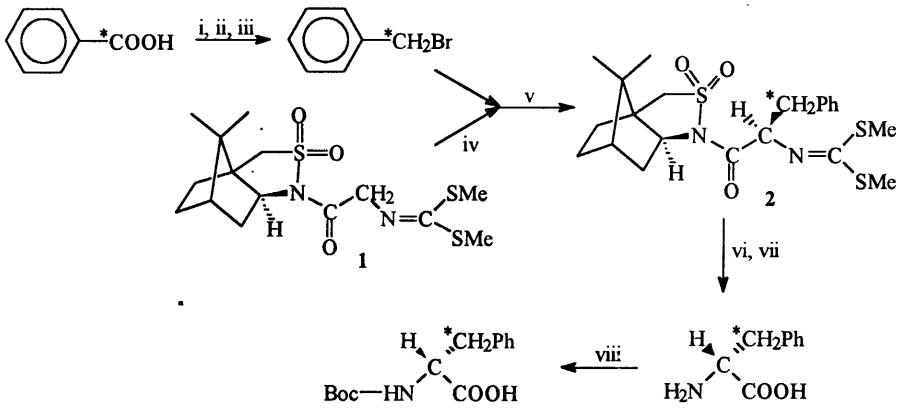
All the methods mentioned were applied to the synthesis of amino acids labelled with ^{13}C and ^{15}N,[5,6] but the preparation of ^{14}C-labelled amino acids in this way have not been published so far. Therefore, below we describe the synthesis of (D)-phenyl[3-^{14}C]alanine.

SYNTHESIS

Our synthesis started from [7-^{14}C]benzoic acid. It was esterified with diazomethane, reduced with lithium aluminium hydride and treated with conc. HBr to give [7-^{14}C]benzyl bromide. Then it was added to the cooled solution of lithiated **1** (Oppolzer synthon) to give **2**. It was purified by chromatography and hydrolyzed in two steps to give (D)-phenyl[3-^{14}C]alanine, with 40% overall radiochemical yield (calculated from benzoic acid). The product contained about 5% of inorganics (this was shown by the decrease in the molar activity), but it did not disturb its transformation to tert-butyloxycarbonyl (D)-phenyl[3-^{14}C]alanine.

Scheme 1.



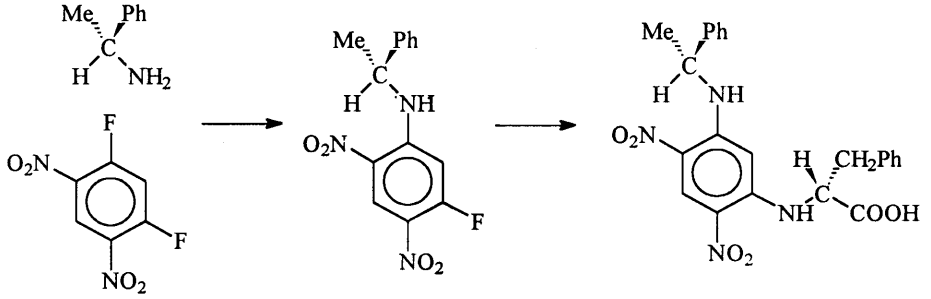
i = diazomethane, ether; ii = $LiAlH_4$, ether, then methanol; iii = conc. HBr, conc. H_2SO_4; iv = THF, BuLi, -78°C; HMPA, -78°C → r.t.; vi = 1 n HCl; vii = 1 n LiOH, 0°C; viii = Boc_2O, NEt_3, dioxane, water;

The optical purity of the product was checked by the Marfey method[7], showing that ee >99%. The Marfey method needs HPLC evaluation with a relatively long elution time, so that by modifying the Marfey reagent (using α-phenylethylamine instead of alaninamide, see Scheme 2.) a new method was developed, which uses TLC instead of HPLC. In the case of ^{14}C-labelled amino acids radioscanning of the TLC plates makes the quantitative evaluation of the samples possible.

This method is suitable for the determination of the optical purity of almost every amino acid. The clearly visible yellow spots facilitate a simple, semi-quantitative determination of optical purity. even in the case of inactive amino acids. Phenylalanine, leucine, isoleucine, nor-leucine, valine, tryptophane, tyrosine,

lysine, ornithine and methionine separated well in benzene - acetonitrile - acetic acid 90:5:5; threonine, serine, histidine, aspartic acid and glutamic acid in chloroform - acetonitrile - acetic acid 50:50:4; alanine and cysteine in chloroform - acetic acid 97:3 and glutamine in acetonitrile - acetic acid 10:1 solvent systems. In the case of proline and arginine the differences in R_f values were too small for determination. In all cases investigated, the L isomer eluted faster, than the D form.

Scheme 2.



EXPERIMENTAL

Melting points are uncorrected and were determined with a PHMK microscope. Chromatography was performed on Silica gel 60 HF_{254} plates (MERCK) and Silica gel 60 (0.063-1.00 mm), respectively. The spots were visualized by UV light, or developped with ninhydrine and chloro - tolidine reagents. The plates were evaluated by a Berthold tracemaster 20 scanner. Radioactivity was measured on an LKB 1217 rack beta liquid scintillation counter. [7-^{14}C]Benzoic acid was prepared in our laboratory according to known procedures[8]. Oppolzer synthon (**1**) was prepared according to the known procedures[9,10,11] from (1R)-(-)-10-camphorsulfonic acid. Evaporations were performed on a rotary evaporator.

[7-^{14}C]Benzyl bromide

This compound was prepared as described earlier[12] from 258 mg (2.08 moles, 114.6 mCi) of [7-^{14}C]benzoic acid. 322 mg of brownish oil was obtained, which was distilled in a "Kugelrohr" apparatus at 20 Hg mm pressure to give 284 mg (1.64 mmoles, 90.5 mCi, 79%) of [7-^{14}C]benzyl bromide as a colourless oil.

N-{(2'R)-2'-(Bis-methylthiomethylenimino)-3'-phenyl-[3'-^{14}C]propanoyl}-(2R)-bornane-10,2-sultam (2)

N-(Bis-methylthiomethylenimino)-acetyl-(2R)-bornane-10,2-sultam (Oppolzer synthon, **1**) (582 mg, 1.55 mmoles) was dissolved in dry THF (7 ml) under

an inert atmosphere, cooled to -70°C and BuLi (640 µl, 1.6 mmoles, 2.5 n solution in hexane) was added. The solution was stirred for 4 hours at (-70) - (-60)°C, then a solution of [7-^{14}C]benzyl bromide (284 mg, 1.64 mmoles, 90.5 mCi) in THF (1 ml) and freshly distilled HMPA (270 µl, 277 mg, 1.55 mmoles) were added successively. The mixture was stirred at -70°C for 30 minutes, then allowed to warm to room temperature. The next day, water (10 ml) was added (at the beginning slowly) and the mixture was extracted with ether (3 x 25 ml), washed with brine, dried over $MgSO_4$ and evaporated. 723 mg of yellow oil was obtained. According to radio TLC (benzene - ethyl acetate 9:1) it was 95% pure, apart from some inactive components. It was purified by chromatography (22 g of Silica gel, benzene), then treated with pentane. **2** was obtained as white crystals (397 mg). M.p.: 129.5-131.5°C (Lit.: 132-133°C)[13]. Radio TLC showed 2% of impurties. Radiochemical yield 52%. A_{sp} = 121.7 mCi/g; A_m = 56.8 mCi/mmol; A_t = 48.15 mCi.

(D)-Phenyl[3-^{14}C] alanine

2 (397 mg, 0.847 mmoles, 48.15 mCi) was dissolved in THF (7 ml), water (4 ml) and 2 n HCl (2 ml) were added and it was stirred overnight at room temperature. Next day TLC (butanol - acetic acid - water 4:1:1) showed, that **2** disappeared and a ninhydrine-active material was obtained. It was hydrolysed further without isolation. The solution was basified by adding LiOH (10 ml) and stirred for 2.5 hours at 0°C, acidified to pH 3 with 2 N HCl, THF was evaporated at reduced pressure, and then the mixture was extracted with ether (2 x 15 ml) to separate camphor sultame. The aqueous phase was fed into a Dowex 50 (W x 4) column (20 ml resin in H^+ cycle), washed with water (100 ml), then eluted with 1 N NH_4OH. The radioactive fractions were pooled and evaporated. (D)- Phenyl[3-^{14}C]alanine was obtained as a white powder (131.5 mg, 93%). It showed only one spot by TLC (butanol - acetic acid - water 4:1:1). A_{sp} = 314.7 mCi/g; A_m = 52.56 mCi/mmol; A_t = 41.23 mCi. The lower molar activity showed that the material contained about 5% inorganics, but this did not affect the next step.

***tert*-Butyloxycarbonyl-(D)-phenyl[3-^{14}C]alanine**

(D)-Phenyl[3-^{14}C] alanine (91 mg, 0.54 mmol, 22,57 mCi) was suspended in a mixture of water (2 ml), dioxane (2 ml) and triethylamine (0.5 ml) and stirred until a clear solution was obtained. Then di-*tert*-butyl dicarbonate (150 mg, mmol) was added and the mixture was stirred for 2 hours at 40-50°C. Then about half of the solvents were evaporated, the residue was diluted with water (10 ml), CH_2Cl_2 was added, the mixture was cooled to 0-5°C and cautiously acidified with 1 N HCl to pH 1. It was extracted with CH_2Cl_2 (3 x 15 ml), washed with brine (2 x 15 ml), dried over $MgSO_4$ and evaporated. The residue was treated with hexane to give 98 mg of Boc-(D)-phenyl[3-^{14}C]alanine as a white powder. TLC and radio TLC showed only one integrable peak (benzene - acetic acid 95:5). A_{sp} = 214.4 mCi/g; A_m = 56.70 mCi/mmol; A_t = 20.77 mCi. Radiochemical yield: 92%.

Determination of optical purity

A) - According to Marfey

0.3 mg of phenyl[3-^{14}C]alanine was dissolved in water (50 µl), and Marfey reagent[7] (100 µl) and 5% $NaHCO_3$ solution (50 µl) were added and the mixture was kept at 40-50°C for 15 minutes. Then dimethyl sulfoxide (500 µl) was added and this mixture was injected onto the HPLC column.

The HPLC system was made by Gilson. An ABI 783A UV detector at 340 nm and a Berthold LB 503 radiodetector were used. A Hypersil ODS 10 mm reverse-phase column was used and elution was on an acetonitrile - water gradient from 10% acetonitrile to 50 % during 20 minutes, then this ratio kept for 3 minutes.

The peaks were the following:

Marfey reagent (unreacted)	at 19.8 min
the derivate of (L)-phenylalanine	at 21.2 min
the derivate of (D)-phenylalanine	at 23.0 min

The product was analysed by this method, and the only peak detected by the radiomonitor was the derivative of (D)-phenyl[3-^{14}C]alanine. It means that ee > 99%, so the optical purity of the product was sufficient for peptide synthesis.

B) - Modification of the Marfey method

Preparation of modified Marfey reagent:

2,4-Dinitro-1,5-difluoro-benzene (254 mg, 1.24 mmoles) was dissolved in ether (15 ml), then a mixture of R(+)-α-phenyl-ethylamine (141 mg, 1.16 mmoles) and ether (5 ml) was added over 20 minutes. The mixture was stirred for an additional 30 minutes, then aq. 5% $NaHCO_3$ solution (10 ml) was added to the yellow solution and stirred for 10 minutes. The phases were separated, extracted with ether, dried over $MgSO_4$ and evaporated. A yellow syrup was obtained (337 mg). It was purified by chromatography (hexane - benzene 1:1 → benzene). 310 mg of yellow oil was obtained, which was dissolved in acetone (20 ml), and this solution was used as reagent.

Derivatisation process:

About 1 mg of (D)-Phe-OH was dissolved in a mixture of water (100 µl), acetone (100 µl) and triethylamine (50 µl), then 100 µl of reagent was added. The mixture was allowed to stand for about 30 minutes at room temperature, then investigated by TLC (benzene - acetonitrile - acetic acid 90:5:5). The R_f values of the L and D isomers were 0.7 and 0.6, respectively. The quantitative evaluation of the plate was performed by radio scanning. Our sample did not show detectable amounts of the L isomer.

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