THE SYNTHESIS OF ^{14}C-LABELED N-CARBONYLBENZYLOXYPHENYLALANINE METHYL-ORTHOESTER

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## SUMMARY

Phenylpropionitrile-1-^{14}C was prepared by the reaction of potassium cyanide-^{14}C with phenethyl chloride. Conversion of the nitrile to methylphenyl-propioimidate-1-^{14}C hydrochloride, chlorination with Cl_2 in NaOCl to produce methyl N-chlorophenylpropioimidate-1-^{14}C, followed by treatment with sodium methoxide produced phenylalanine methylorthoester-1-^{14}C. Treatment of the amine with n-butyllithium followed by addition of benzylchloroformate produced N-carbonylbenzyloxy-phenylalanine methylorthoester-1-^{14}C.

INTRODUCTION

Orthoesters of N-protected α-amino acids have been shown to be useful reagents for the selective aminoacylation of cis-glycol residues (1,2,3,4). Our interest in selective aminoacylation reactions and our desire to investigate the biological activity of the aminoacyl products has led us to prepare N-carbonylbenzyloxyphenylalanine methylortho-ester-1-^{14}C. Among the several methods for the synthesis of ortho-esters (5,6), the procedure of Graham (7) proved to be the most suitable for α-amino orthoesters. The synthesis of the title compound recently has been reported (8).
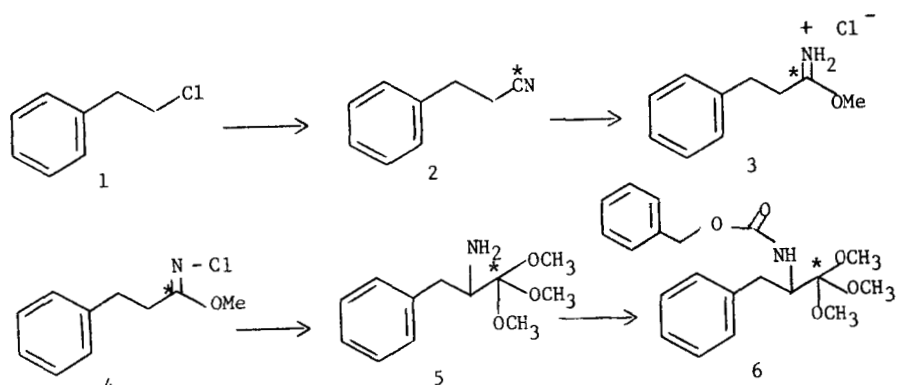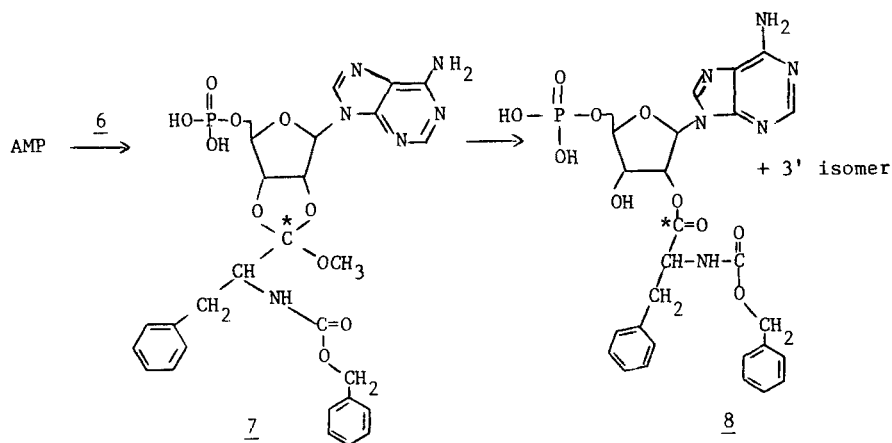
Scheme 1

RESULTS AND DISCUSSION

Initial approaches to the synthesis of the nitrile, 2, involved the use of potassium cyanide-^{14}C and excess phenethylchloride (1), but the presence of 1 was found to inhibit the next step in the sequence, and purification at this point was neither ready nor desirable (Scheme 1). The use of the potassium cyanide-^{14}C 18-Crown-6 complex for the nucleo-philic substitution of cyanide proved to be entirely satisfactory in that it allowed the use of stoichiometric amounts of cyanide and 1, and produced 2 which was relatively free from contamination with large amounts of 1. Conversion of 2 to the imidate 3 through treatment with one equivalent of methanol and excess hydrogen chloride proceeded in good yield provided the conditions were kept strictly anhydrous, but it was found that the conversion of 3 to the N-chloroimidate 4 could be accomplished in good yield only if base washed Pyrex glassware was used. The N-chloroimidate 4 was converted to the orthoester of phenylalanine-1-^{14}C (5) in good yield by treatment with 0.5N sodium methoxide at 60°. The use of classical methods for the attachment of the carbonylbenzyloxy group to the amine of 5 produced very poor yields of the title compound, 6, and relatively large yields of N-carbonylbenzyloxyphenylalanine methylester, possibly through an intramolecular proton transfer followed by hydrolysis of the orthoester. Treatment of 5 with nBuLi to remove

one of the amine protons followed by treatment with benzylchloroformate
avoided this hydrolysis and produced 6 in excellent yield.

Orthoesters such as 6 are extremely sensitive to acid catalyzed
hydrolysis, and were found to decompose extensively on silica gel unless
great care was taken to neutralize acidic residues of the silica. The
most satisfactory method for accomplishing this was to extensively wash
the silica plate with triethylamine, followed by reactivation of the
plate at elevated temperatures and chromatography in solvents containing
2% triethylamine. Using EtOAc as the eluent, it was possible to readily
separate 6 from the impurities introduced in the 5 steps of the synthetic
scheme. Through the use of this technique, intermediates 2-5 were not
purified, but carried directly on to the final step, where the impurities
were removed chromatographically. The title compound (6) was obtained
in chromatographically pure form in an overall radiochemical yield of
73%.

Scheme 2

The structure of 6 was confirmed by its chromatographic behavior
(Table I), and by its reactivity towards AMP (Scheme 2). The results of
the exchange of 6 with AMP, listed in Table II, show that 11.8% of the
radioactivity from 6 was found in the exchange product 8.[9] The uv data
indicate that 7.7% of the AMP present was converted to 8, but we believe

this value to be artifically low due to incomplete removal of 8 from
the paper.

Compound	Rf
5	0.16
N-CBZ-phenylalanine methylester	0.37
$PhCH_2CH_2COOCH_3$	0.47
6	0.67

Table 1: Rf values on Et_3N washed silica gel developed with EtOAc
          containing 2% Et_3N.

Compound	Rf	cpm Reaction	cpm Rxn mixture without AMP
AMP	0.20	266.6 ± 5%	252.6 ± 5%
8	0.80	31,025 ± 0.5%	267.2 ± 5%
6	0.89	232,031 ± 0.1%	251,072 ± 0.1%

	A_{260}/ml buffer Reaction	A_{260}/ml buffer Rxn mixture without 6
AMP	1.80	1.93
8	0.150	0.00
6	0.010	0.00

Table II: Results of exchange reaction (Scheme II) compounds separated
           by descending paper chromatography on Whatman No. 1 using
           $nBuOH:HOAc:H_2O$ (5:2:3). Values reported are the average of
           3 spots.

EXPERIMENTAL

Radioactivity was assayed in a Beckmann liquid scintillation counter,
using a 0.5%solution of PPO in toluene as the fluorescent cocktail. Organic
solution of the compounds were dissolved directly in the cocktail; the
paper circles from paper chromatography were suspended in the cocktail
for counting. The counting efficiency of the system was greater than 90%.

Phenylpropionitrile-1-^{14}C (2). Potassium cyanide-^{14}C (3.57 mg;

0.055 mmole; 3.0 mCi) and 18-Crown-6 ether (14.5 mg; 0.055 mmole) were

dissolved in 2.0 ml anhydrous methanol, the solution evaporated to

dryness under reduced pressure, and the resulting solid dried at 50°

(20 μHg) for 15 min. This slightly yellow solid was taken up in 0.5 ml

dry dimethylsulfoxide, phenethyl chloride (7.70 mg; 0.055 mmole) added,

and the solution stirred at 27° under nitrogen for 48 hours. Saturated

sodium chloride (1.0 ml) was added, the solution extracted with ether

(4 x 1.0 ml), and the combined ether layers dried (MgSO$_4$) and concentrated

in a small Craig tube under a stream of N$_2$ to produce 5.82 mg (81%) of

2 with a total activity of 2.43 mCi. This material was not purified

further, but carried directly on to 3.

Methyl 1-^{14}C-phenylpropioimidate hydrochloride (3). Methanol (1.43

mg; 0.44 mmol) was added to the nitrile 2, the solution immediately

cooled to 0° under a stream of nitrogen, and dry hydrogen chloride

bubbled through the solution via a very fine capillary for 45 min. The

reaction vessel was tightly stoppered and allowed to stand at 2° for

12 hours. The now viscous solution was washed with 0.5 ml ether and

the white crystalline product collected by centrifugation to yield

8.88 mg (99.5%) 3 having an activity of 2.42 mCi.

Methyl N-chloro-1-^{14}C-phenylpropioimidate (4). The imidate hydro-

chloride 3 still in the Craig tube was cooled to 0° under a stream of

N$_2$, 0.5 ml 1.2N sodium hypochlorite solution [made by dissolving 0.226 g

(5.65 mmole) sodium hydroxide in 1.8 ml of water and bubbling 0.294 g

(4.14 mmole) chlorine into the solution while the temperature was

maintained at 0°] was added, and the solution allowed to stand at 0°

for 30 min. The now yellow solution was extracted with pentane (4 x

0.5 ml), and the combined pentane extracts dried (MgSO$_4$) and concentrated

under a stream of nitrogen to produce 8.77 mg (99%) of 4 as a yellow

oil with an activity of 2.40 mCi.

1-^{14}C-Phenylalanine methylorthoester (5). The N-chloroimidate 4

(8.77 mg; 0.044 mmole) was dissolved in 0.5 ml methanol, placed in a

thick walled (3 mm) Pyrex tube, 0.5 ml 1.0N sodium methoxide added, the

solution cooled to -78°, and the tube sealed under the vacuum of 30 mm

Hg. The reaction vessel was then heated to 60° for 3 hours, cooled to

room temperature, opened, and the contents poured into 1.0 ml saturated

sodium chloride solution containing a small amount of sodium carbonate.

This cloudy solution was extracted with methylene chloride (4 x 0.5 ml),

and the combined organic layers dried (Na_2CO_3) and concentrated under

a stream of nitrogen to produce 9.80 mg (98.9%) of 5 as a slightly yellow

oil having an activity of 2.36 mCi.

   N-Carbonylbenzyloxy-1-^{14}C-phenylalanine Methylorthoester (6). The

orthoester 5 (9.80 mg; 0.043 mmole) was taken up in 0.5 ml of anhydrous

ether, the solution stirred, cooled to -78°, and n-butyllithium added

(0.05 ml of a 2.0 N solution). The now yellow solution was allowed to

stir at -78° for 15 min, benzylchloroformate (17.0 mg; 0.10 mmole) added,

and the solution stirred at -78° for 30 min, and then at room temperature

for 30 min. The resulting pale yellow solution was poured into 1.0 ml

of saturated sodium chloride solution containing a small amount of

sodium carbonate, extracted with ether (4 x 0.5 ml), and the combined

ether layers dried (Na_2CO_3) and concentrated to a volume of ca. 1.0 ml

under a stream of N_2. This material was purified by preparative thin

layer chromatography on a 20 x 20 cm x 0.5 mm silica gel plate which

had been neutralized by exhaustively washing with triethylamine followed

by activation at 100° C for 2 hours. The plate was developed with

ethyl acetate containing 2% triethylamine, the band corresponding to

the N-protected orthoester (R_f = 0.67) scraped off, and the product

washed from the silica gel with ethyl acetate. Removal of the solvent

under a stream of nitrogen afforded 12.3 mg (86%) of 6 was a slightly

yellow solid having a total activity of 2.20 mCi.

Exchange of 6 with Adenosine 5'-phosphate. (Scheme II) Adenosine 5'-phosphate (0.5 mg; 1.4 μmole) was placed in a capillary tube, 10 μl of a solution containing 12.3 mg 6 (37 μmole) and 30 μl methane sulfonic acid in 0.100 ml dimethylformamide was added, the capillary sealed, and the reaction mixture allowed to stand at 27° for 48 hours. The reaction mixture was then spotted in six spots on Whatman No. 1 paper, and the chromatogram developed with butanol:acetic acid:water (5:2:3). The spots corresponding to the reaction product and starting materials from half of these were cut out and assayed directly by scintillation counting, while the remainder were cut out, washed with 2.0 ml 1.0 M phosphate buffer (pH 7.5), and the adenosine chromophore assayed by UV (260 nm). The results are summarized in Table II.

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9. The structure of 8 was verified by comparison with a non-radioactive sample of 8 prepared via scheme 2 and shown to be identical to that reported in Harpold, M. A. and Calvin, M. Biochim. Biophys. Acta 308: 117 (1973).