Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXXIV, No. 2

A Practical Approach to the Synthesis of ^{14}C-labeled Amino Acid and Simple Peptide

I. Victor Ekhato* and Che C. Huang

Radiochemistry, Dept. of Chemical Development
Parke-Davis Pharmaceutical Research
Division of Warner-Lambert Company
Ann Arbor, Michigan 48105

SUMMARY

A convenient method for the synthesis of phenylmethyl N-methyl[carboxy-^{14}C]glycine hydrochloride (7) is described. It involves the carboxylation of transmetalated organostannane compound 4 which itself is accessible by established procedure. Success in preparing the labelled amino acid 7 enabled us to synthesize target N-glycyl-N-methyl[carboxy-^{14}C]glycine (10) by conventional coupling protocol.

KEY WORDS: *Intestinal peptide transporter, Caco-2 cell line, N-glycyl-N-methyl[carboxy-^{14}C]glycine, organostannane compound, and transmetalation.*

Introduction

In order to characterize the expression of intestinal peptide transporter in a Caco-2 cell line, we had a need to develop an efficient and rapid access to fairly high specific activity ^{14}C-labelled amino acid to make a peptide substrate of the transporter (1). It was highly desirable to develop a method which could be applied to the preparation of amino acids as may be required by this study, and needed in other peptide programs. We approached the synthetic task by employing organostannane reactions. A labelled pro-chiral amino acid was made, but the approach is potentially applicable to achiral or chiral amino acids and peptides thereof since the crucial α-substituted organostannane precursors are accessible (2). The results of such application to several other amino acids and peptides are being prepared for publication in appropriate journal.

Herein, we report the synthesis of phenylmethyl N-methyl[carboxy-^{14}C]glycine hydrochloride (7) which was facilitated by the transmetalation of N′-[(1,1-dimethylethoxy)carbonyl]-protected α-aminoorganostannane precursor, carboxylation of

0362–4803/94/020107–09$09.50

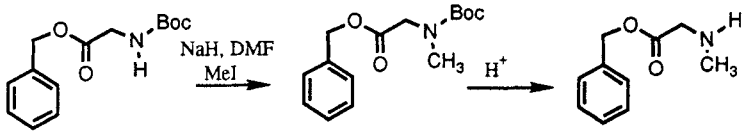
Received 21 May, 1993
Revised 12 October, 1993

the product, followed by coupling with glycine to make the ^{14}C-labelled dipeptide substrate 10 of target transporter. We found this method to be facile and more practical than other alternative syntheses including the Strecker approach, or a modification (3) thereof by which we could have prepared our target compound 7 from $K^{14}CN$.

Results and Discussion

Conventionally, N-methylglycine (sarcosine) may be prepared by the N-alkylation of glycine with methyl iodide. The potentially troublesome problem of dialkylation may be avoided by protecting the amino group as the carbamate prior to deprotonation and alkylation. Removal of the N′-[(1,1-dimethylethoxy)carbonyl] (boc) group at the end of the transformation provides N-methylglycine. We obtained a poor yield of N-methylglycine by such a sequence as in **Scheme I**, and that prompted us to investigate the organostannane route.

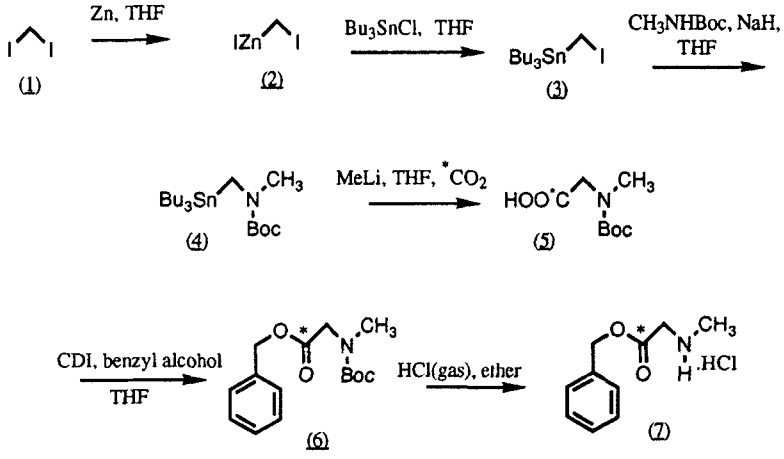
Scheme I



Furthermore, we would utilize in place of methyl iodide the comparatively inexpensive $Ba^{14}CO_3$ as a source of ^{14}C-label in this alternative route. The synthesis was accomplished as outline in **Scheme II**, and the sequence required us to make the crucial compound tributyl(iodomethyl)stannane (3), which is accessible by published procedure (4). We hoped to be able to use compound 3 in N′-alkylation of 1,1-dimethylethyl methylcarbamate and obtain 1,1-dimethylethyl methyl[tributylstannyl)methyl]carbamate (4), a desired precursor to labelled N-methylglycine. First, diiodomethane was converted in a one pot operation to iodomethylzinc iodide (2), which then reacted with tributylstannyl chloride to furnish tributyl(iodomethyl)stannane (3). The compound 3 was isolated by distillation in 77.6 % yield. The requisite 1,1-dimethylethyl methylcarbamate in the subsequent step was prepared by the derivatization of methylamine as the carbamate. Following the deprotonation of 1,1-dimethylethyl methylcarbamate with NaH, it reacted with compound 3 to give the monoalkylated N-methylglycine precursor , 1,1-dimethylethyl methyl[(tributylstannyl)methyl]carbamate

(4). We were now ready to make labelled N-methylglycine. The compound 4 was transmetalated at -95°C by reaction with methyllithium, and carboxylated with $^{14}CO_2$ generated by the action of conc. H_2SO_4 on $Ba^{14}CO_3$. Acidification of the completed carboxylation reaction was preferably carried out with an organic acid so as to minimize cleavage of protective group and maximize recovery of product as the N′-[(1,1-dimethylethoxy)carbonyl]-protected N-methyl[carboxy-^{14}C]glycine 5 .

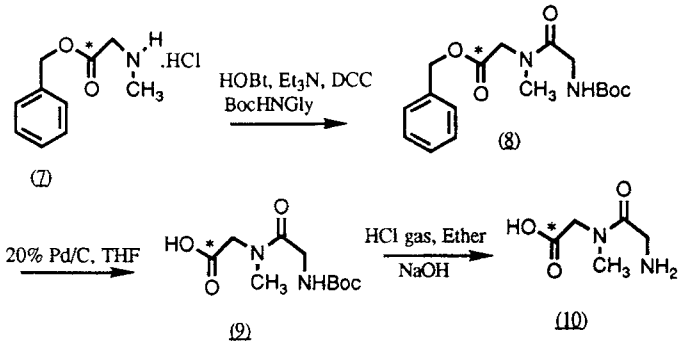
Scheme II



Without further purification, the dried N-[(1,1-dimethylethoxy)carbonyl]-N-methyl[carboxy-^{14}C]glycine (5) was activated with 1,1′-carbonyldiimidazole (CDI) for reaction with benzyl alcohol to the benzyl ester . Selective removal of the boc group with HCl (gas) furnished crystalline phenylmethyl N-methyl[carboxy-^{14}C]glycine hydrochloride (7) in 52 % yield from 4. We applied the conventional method (5) for coupling amino acids to the labelled N-methylglycine 7 and N′-[(1,1-dimethylethoxy)carbonyl]glycine to make phenylmethyl N-[N[(1,1,-dimethylethoxy)carbonyl]glycyl]-N-methyl[carboxy-^{14}C]glycine (8). About 5 % of starting labelled phenylmethyl N-methyl[carboxy-^{14}C]glycine hydrochloride (7) remained after coupling, and had to be removed by chromatography prior to Pd/C catalyzed hydrogenolysis of the benzyl ester group from compound (8). Cleavage of the N′-[(1,1-dimethylethoxy)carbonyl] group was accomplished with HCl (gas). At this point, good radiochemical purity grade (96 %) of the hydrochloride salt of the dipeptide

was obtained, but it proved to be problematic, being very hygroscopic upon isolation. On the other hand, the free base was a readily isolable, stable crystalline solid.

Scheme III



(7) (8) (9) (10)

A solution of this hydrochloride salt of N-glycyl-N-methyl[carboxy-^{14}C]glycine was brought to pH 7 with a solution of NaOH and re-isolated by filtration through a reverse phase (C_{18}) column, and then characterized. A substantial quantity of the peptide was lost by cyclization to a putative diketopiperazine during the process. All intermediate compounds were characterized by proton nmr analysis and comparison with authentic samples. The final product N-glycyl-N-methyl[carboxy-^{14}C]glycine (10), matched commercially obtained 'cold' reference in all its physical measurements.

In conclusion, this work demonstrates that α-aminoorganostannane may offer an efficient, direct route to ^{14}C -labelled amino acids. We believe that as a consequence of this approach, inexpensive preparation of labelled analogs of the various physiologically important peptides may now be readily accomplished.

Experimental Section

General Methods

All reactions involving organometallic compounds were carried out under inert atmosphere and reaction solvents were freshly distilled from appropriate drying agent. ^{1}H-NMR spectra were recorded on a Gemini 200 MHz or a Varian XL 300 MHz spectrometer. Radiochemical purity (RCP) of every labeled compound was determined by tlc using a Bioscan 200 imaging scanner. Radiochemical counting was performed on a Packard 574 liquid scinitillation counter using Beckman Read-Solv MP cocktail.

HPLC analysis of final products were performed on a Waters Associates 600E system with on-line Applied BioSystems 1000S diode array detector and either a β-RAM radioactivity detector or Radiomatic series A-200 radioactivity flow detector. Column chromatography was carried out on a J.T. Baker Bakerbond octadecyl (C_{18}) prep LC silica gel packing (40μm).

Tributyl (iodomethyl) stannane (3)

Cupric acetate monohydrate (250 mg, 1.25 mmoL) was dissolved in glacial acetic acid (25 mL) by heating and shaking on a steam water bath. Zinc granules (9.8 g, 150 mmol) were added to the hot solution and shaken for 2 min while heating. The acetic acid was decanted and fresh acetic acid (25 mL) was added. After shaking for additional 2 min, it was decanted, cooled and washed with anhydrous ether (3X50 mL), and then dried under a stream of nitrogen.

The three-necked flask (500 mL) was then equipped with a dropping funnel, reflux condenser, a gas inlet tube and a magnetic stirrer. After it was flame dried under nitrogen atmosphere, dry THF (42 mL) and a few drops of CH_2I_2 were added and stirred. A purple coloration appeared in a few minutes of stirring, and additional THF (81 mL) was then added. From the dropping funnel CH_2I_2 (940.11 g, 150 mmol) in 42 mL of dry THF was added dropwise such that the reaction temperature was maintained at 40°C. One hr into the reaction, it was placed in a water bath maintained at 40°C and stirred such for 3 hr, during which the Zn-Cu couple was used up. It was cooled in an ice bath and then filtered into another 500 mL flask that was similarly equipped.
The dropping funnel was charged with Bu_3SnCl (27.12 mL, 100 mmol) in 60 mL of dry THF. It was added dropwise to the prepared ICH_2ZnI solution over a period of 30 min while maintaining 40°C temperature. After stirring for 18 hr (overnight) at this temperature, it was poured onto petroleum ether (300 mL) , washed with water (2 X 200 mL), and then dried on anhydrous $MgSO_4$. The crude product was distilled under reduced pressure to give 38.86 g (90 %). Proton nmr (300 MHz) ($CDCl_3$) 1.94, (-C<u>H</u> 2I) and satellites at 1.97 and 1.91; 1.55, (m, -C<u>H</u> 2) 1.39, (-C<u>H</u> 2); 0.93, (C<u>H</u> 3); and 0.91 (C<u>H</u> 3).

1,1-Dimethylethyl methylcarbamate

A solution of methylamine (60 mL, 481 mmol; 8.03 M in absolute ethanol) was diluted with dry THF (100 mL) and cooled in an ice-water bath. Di-*tert*-butyl

dicarbonate (98.8 g, 458 mmol) in dry THF (100 mL) was added dropwise to the cooled solution of amine. The ice-water bath was removed, and the reaction was stirred at room temperature for 2.5 hr. It was concentrated to an oil which was taken up in ether, washed successively with water, sat'd $NaHCO_3$, and brine, and finally dried over $MgSO_4$. The product was distilled under reduced pressure to give 48.9 g (78 %). Proton nmr (300 MHz) ($CDCl_3$) 4.60, (-NH Boc), 2.72 and 2.70, 1.50 and 1.42.

1,1-Dimethylethyl methyl[(tributylstannyl)methyl]carbamate (4)

1,1-Dimethylethyl methylcarbamate (6.5 g, 49.6 mmol) was stirred under an atmospherre of argon at 65°C in dry DMF (20 mL), containing NaH (2.0 g, 50 mmol; as a 60% dispersion in oil). After 2 hr, iodomethyltributyltin (19.37 g, 45.0 mmol) in dry DMF (20 mL) was added in one portion. A clear solution was attained in less than 1 hr, and at which time tlc(5% EtOH in CH_2Cl_2) showed that Bu_3SnCH_2I had been consumed. The solvent was stripped and the residue was taken up in CH_2Cl_2 (200 mL), and washed with water and brine. After drying on anhydrous $MgSO_4$, the solvent was stripped and product was distilled under reduced pressure to give 12.8 g (66 %). Proton nmr (300MHz) ($CDCl_3$) 3.08 and 2.92, (- Bu_3SnCH_2); 2.85 and 2.83, (-NCH_3); 1.54-1.43 (m, -CH_2); 1.36-1.26 (m, -CH_2); and 0.89, (tr, -CH_3) .

Phenylmethyl N-methyl[carboxy-^{14}C]glycine hydrochloride (7).

Barium carbonate (1.097 g, 5.55 mmol, 59.5 mCi/mmol) was treated with excess H_2SO_4, and the carbon dioxide evolved was trapped in a cold finger that was immersed in liq. nitrogen. 1,1-Dimethylethyl methy[(tributylstannyl)methy]carbamate (4) (2.5 g, 5.76 mmol) in 50 mL of dry THF was cooled to -95°C and following equilibration at this temperature for 45 min, methyllithium (4.11 mL, 5.76 mmol; 1.4 M solution in diethyl ether) was added dropwise over 10 min with stirring under argon. After a further 15 min reaction, it was frozen in liquid N_2 and transferred to a vaccum line connected to the cold finger containing the $^{14}CO_2$. The labelled carbon dioxide was vaccum transferred into the reaction flask, and the reaction was allowed to proceed for 20 min, after which it was quenched with 20% AcOH in THF. It was poured onto a cold solution of brine and extracted with ethyl acetate, washed with water, and dried. After the solvent was removed, the product was dried on high vaccum pump overnight. The crude reaction product 5 (836 mg) was taken up in dry THF (20 mL), and 1,1′-carbonyldiimidazole (CDI) (2.512 g, 15.5 mmol) was added and stirred at room

temperature for 2 hr. Benzyl alcohol (1.672 g, 15.49 mmol) was added and stirring was continued under argon overnight, at which time tlc showed 88 % completion of reaction. It was concentrated and the residue was chromatographed on a silica gel column, eluting first with hexane and then with 20 % ethyl acetate in hexane to give product 6 (918.3 mg). Proton nmr (300MHz) ($CDCl_3$), 7.36, s, C_6H_5; 5.19 and 5.18, (-CH_2O); 4.04 and 3.94, (-NCH_2COO-); 2.95 and 2.92, (-NCH_3); 1.47 and 1.38, (Boc methyls). A solution of the crude product was treated with hydrogen chloride gas, bubbled through for 15 min after which starting ester was absent. After the solvent was evaporated, the white solid was re-dissolved in methanol and reprecipitated with ether to give 7 (612 mg 52 %, 171.6 mCi, 60 mCi/mmol). Proton nmr (300 MHz) (D_2O), 7.48, (C_6H_5); 5.33, (-CH_2O-); 4.05, (-NCH_2COO-); 2.80, (-NCH_3).

N-Glycyl-N-methyl[carboxy-^{14}C]glycine (10)

To 1-hydroxybenzotriazole hydrate (403.03 mg, 2.98 mmol) and phenylmethyl N-methyl[carboxy-^{14}C]glycine (612 mg, 2.84 mmo) in DMF (8.0 mL) was added N′-[(1,1-dimethylethoxy)carbonyl]glycine (507.5 mg, 2.89 mmol) and then cooled in an ice bath. While stirring triethylamine (807 μL) was added, followed by dropwise addition of 1,3-dicyclohexylcarbodiimide (DCC) (637 mg, 3.08 mmol) in ethyl acetate (20 mL) over 30 min. After a further 10 min stirring at this temperature, the bath was removed and the reaction was stirred overnight at room temperature. It was diluted with ethyl acetate (80 mL) and filtered, and the solid was washed with ethyl acetate. The combined ethyl acetate solution was washed with water (20 mL), sat'd $NaHCO_3$ solution, and brine (40 mL). The product phenylmethyl N-[N-[(1,1-dimethylethoxy)carbonyl]glycyl]-N-methoxy[carboxy-^{14}C]glycine (8) was shown by tlc to be 94 % radiochemically pure, and about 5 % phenylmethyl N-methyl[carboxy-^{14}C]glycine. It was purified by column chromatography on silica gel eluted with ethyl acetate:hexane (60:40) to give phenylmethyl N-[N-(1,1-dimethylethoxy)carbonyl]glycyl]-N-methyl[carboxy^{14}C]glycine (8) (906 mg), shown by tlc to be of a radiochemical purity greater than 98%. The compound phenylmethyl N-[N-[(1,1-dimethylethoxy)carbonyl]glycyl]-N-methyl[carboxy-^{14}C]glycine 8) (906 mg) in dry THF (100 mL) containing 20 % Pd/C (136 mg) was hydrogenated at 60 psi overnight and filtered through a bed of Celite, which was subsequently washed with dry THF. The combine solution was evaporated to dryness and the residue was redissolved in ether (50 mL), and HCl gas was bubbled

through the solution for 25 min during which the solid HCl salt of the peptide separated. The solvent was decanted and the solid was dried to give 409.15 mg (132mCi, 59.0 mCi/mmol) of 10, with radiochemical purity of 96 %. Proton nmr 300mHz, (D_2O) 4.23, 4.21, 4.09, 3.96, 3.07 and 2.98. Re-isolation with methanol-ether yielded very hygroscopic hydrochloride salt. A solution of peptide in water (6.0 mL) was brought to pH 7.0 by the dropwise addition of 0.5N NaOH with stirring. The solution was then applied to C_{18} column and eluted with methanol. The desired earlier fractions were combined, evaporated to dryness and redissolved in water. The solution was lyophilyzed, and solid residue was washed with methanol to give N-glycyl-N-methyl[carboxy-^{14}C]glycine (147 mg, 54 mCi/mmol) . Tlc reverse phase (C_{18}), (MeOH:H_2O 75:25), Rf 0.84, 98.24 %; HPLC T_r 2.8 min, radiochemical purity 100 %, chemical purity 99.3 % (by area normalization), on Vydac Protein & Peptide C_{18}, 5μ, 4.6 mm ID X 25 mm, 0.05M $NH_4H_2PO_4$ for chemical purity assay and H_2O: CH_3CN (65:35) for radiochemical purity assay, flow rate 1.0 mL/min, UV detection at $\lambda = 214$ nm. Proton nmr (D_2O) 4.07, 3.98, 3.92 3.04 and 2.99.

Acknowledgement

We are grateful to Mr. Yun Huang for the radioactivity measurements on title peptide.

Reference:

(1) Taylor E., Burston D., and Mathews D.M. -Clinical Science 58: 221 (1980). Burston D., and Mathews D.M. -Clinical Science 79: 267 (1990)

(2) Still C. W. -J. Am. Chem. Soc. 100: 1481 (1978). Park S.B. and Chong J. M. - J. Org. Chem. 57: 2220 (1992). Ye J. , Shin D., Bhatt K.R. Swain A.P., and Falck J.R.- Synlett. 205 (1993). Burchat A. F. , Chong J. M., Park S. B. - Tetrahedron Lett. 34: 51 1993.

(3) Steiger R.E. - Org. Syn. Coll. Vol. III, 84, (1955); Wheeler W.J., O'Bannon D.D.- "Synthesis and Application of Isotopically Labelled Compounds," Elsevier Science Publishers B.V, 181, (1992); Maldelmont J.C., Guerin P., Domurado D.- J. Labelled Compds Radiopharm. 31(9), 694, (1992).

(4) Seyferth D. and Andrews S.B. -J. Organomet. Chem. 30: 151 (1971).

(5) Bodansky M. - ' Priciples of Peptide Synthesis', Springer-Verlag, N. Y. 36 (1984). Patt W.C., Hamilton H.W., Taylor M.D, Ryan M.J., Taylor D.G., Connolly C.J.C., Doherty A.M, Klutchko S.R., Sircar I, Steinbaugh B.A., Batley B.l., Painchaud C.A., Rapundalo S.T., Michniewicz B.M, and Olson S.C. - J. Med. Chem. 35(14): 2562 1992. Hoffman R.V and Kim H.O - Tetrahedron 48(15): 3007 1992.