THE SYNTHESIS OF [^{14}C]-L-LYSYL-N-(2-BENZOYL-4-CHLOROPHENYL)-N-METHYL GLYCINAMIDE

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SUMMARY

A procedure has been developed for the synthesis of L-lysyl-N-(2-benzoyl-4-chlorophenyl)-N-methyl glycinamide labelled with carbon-14. The label was introduced using [^{14}C]carbon dioxide to prepare the intermediate [carboxyl-^{14}C]benzoic acid.

KEY WORDS:- CARBON-14, PRO-DRUG, DIAZEPAM

Incubation of the title compound (1) with the whole blood of various mammalian species including man results in the cleavage of the terminal amino acid residue to produce an intermediate which at physiological pH cyclizes to give the minor tranquillizer diazepam (2). The level of conversion of the pro-drug to diazepam is high ($\geq$ 95%). In salt form (dihydrobromide, citrate etc), the pro-drug has the advantage of water solubility over diazepam providing an alternative convenient method of presentation of the drug *in vivo*. To facilitate metabolic studies and the comparison with diazepam, a carbon-14 labelled form was required.

The synthetic route adopted is shown in the Scheme.

[^{14}C]Carbon dioxide was generated from barium [^{14}C]carbonate with conc. sulphuric acid and absorbed into phenylmagnesium bromide at -78°. To obtain acceptable yields (80%) of [carboxyl-^{14}C]benzoic acid, it was necessary to use a mole excess of the Grignard reagent. Benzonitrile was prepared directly from the acid using the method of Oxley et al (3). The crude product was of high purity and was converted directly into the appropriately substituted benzophenone

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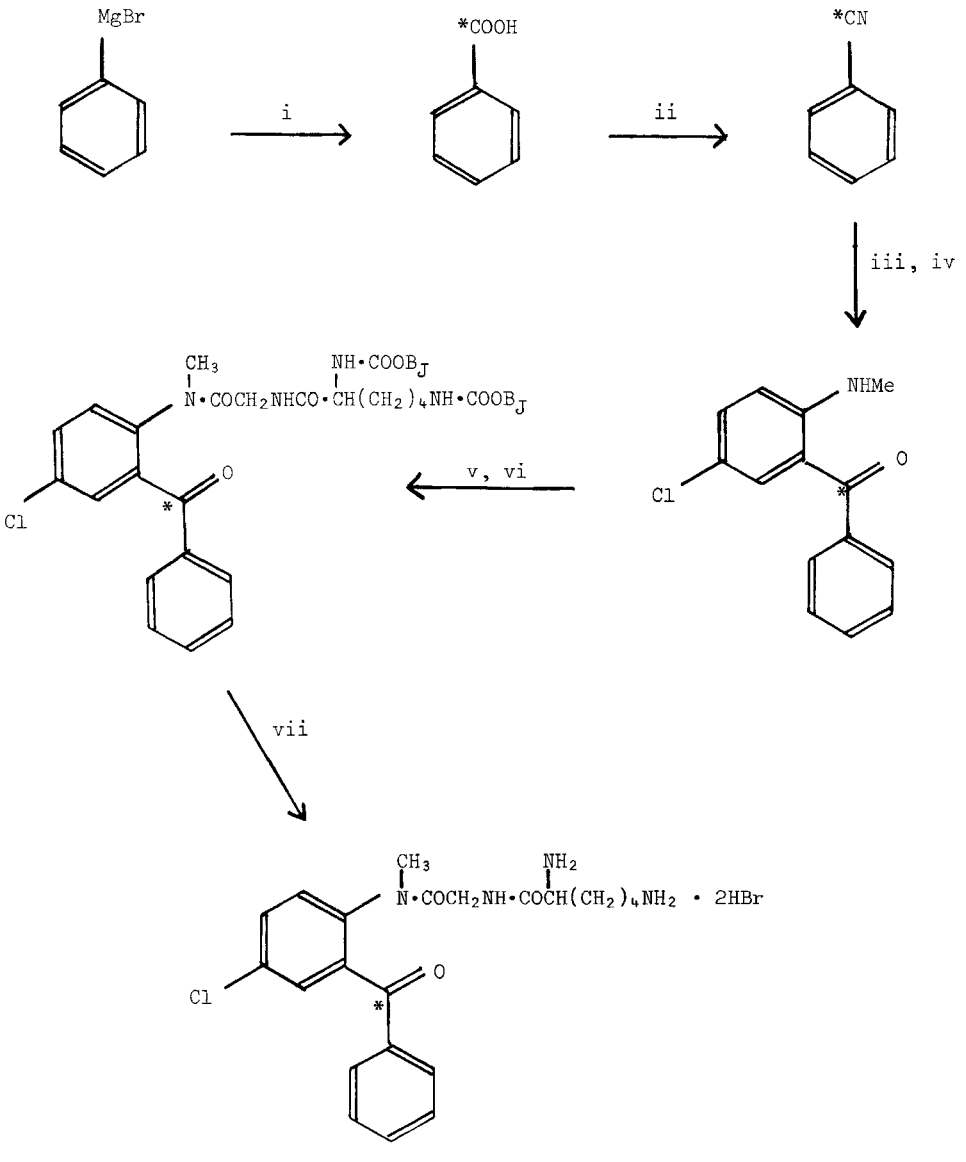

Reagents:- i $*CO_2$; ii $PhSO_2NH_2$-H^+; iii N-methyl-4-chloroaniline-BCl_3-$AlCl_3$; iv H_2O/H^+; v $(CF_3CO)_2O$; vi Dicarbobenzyloxylysylglycine-NEt_3 vii HBr-AcOH.

SCHEME

by reaction with N-methyl-4-chloroaniline using the procedure of Sugasawa and co-workers (4). Coupling of the aminobenzophenone with the protected dipeptide, N^{α}, N^{ε}-dicarbobenzyloxylysyl glycine was achieved by the mixed anhydride method using an excess of dipeptide to obtain good yields. De-protection was readily achieved using hydrogen bromide in acetic acid. The crude product is of high purity and essentially homogeneous by HPLC analysis. It is hygroscopic and light sensitive and any attempts to recrystallise or otherwise manipulate result in a decrease in purity rather than an improvement.

EXPERIMENTAL

Tlc was performed by upward irrigation on microscope slides coated with Merck silica gel G and column chromatography with Merck silica gel of particle size 0.05-0.2 mm in the same solvent as used for tlc. Autoradiograms were obtained from thin layer chromatograms using Kodirex (Kodak) X-ray film. The identity and homogeneity of labelled compounds was established by full spectroscopic analysis (ir, nmr, ms and uv) of products obtained under comparable experimental conditions using unlabelled reactants and were homogeneous by tlc in several solvent systems. Radiochemical homogeneity was established by autoradiography.

Scintillation counting was carried out on a Packard Model 3375 Scintillation Counter fitted with automatic external standardisation. ^{1}H nmr spectra were determined on a Jeol MH 100 spectrometer whilst ^{13}C nmr spectra were obtained on a Jeol FX 60Q instrument. Spectra were run in deuteriochloroform.

[Carboxyl-^{14}C]Benzoic acid - Dry bromobenzene (2.2 ml) in dry ether (15 ml) was added to magnesium (0.5 g) stirred in ether (5 ml) to prepare a solution of phenylmagnesium bromide. An aliquot (10 ml) of this solution was diluted with dry ether (10 ml) and allowed to react at -78° with [^{14}C]carbon dioxide

generated from an intimate mixture of barium [^{14}C]carbonate (430 mg, 12.5 mCi) and unlabelled barium carbonate (570 mg) and conc. sulphuric acid (5 ml) using a simplified form of the procedure described by Dauben et al (5). The reaction mixture was allowed to warm to room temperature and processed conventionally to give [carboxyl-^{14}C]benzoic acid (500 mg, 82%).

Benzo[^{14}C]nitrile - A mixture of [carboxyl-^{14}C]benzoic acid (0.5 g), benzene sulphonamide (1.7 g) and toluene sulphonic acid (0.1 g) was heated at 220-230° for 2 h. The product was flash distilled out of the mixture in vacuo (0.1 mm) and was collected directly into a receiver cooled in a dry ice-carbon tetrachloride bath (-20°). The yield of crude benzo[^{14}C]nitrile was ca 400 mg (80%). The method is that of Oxley et al (3).

2-Methylamino-4-chloro[carbonyl-^{14}C]benzophenone - N-methyl-4-chloroaniline (0.8 g, 0.005 mole) was added to a solution of boron trichloride (600 mg, 0.005 ml) in dry tetrachloroethane (3 ml) stirred in an ice-water bath and the solution then heated at 80-100° for 20 min under dry nitrogen. The mixture was cooled and benzo[^{14}C]nitrile (400 mg) in dry tetrachloroethane (2 ml) added followed by anhydrous aluminium trichloride (0.7 g). The mixture was stirred and heated under reflux for 5.5 h when after cooling 2N hydrochloric acid (10 ml) was added and then boiled for an additional 0.5 h. Conventional processing gave a dark oil which was chromatographed over silica eluting with petrol-ether, 19:1 to afford the benzophenone as a yellow solid (500 mg).

[N^{α},N^{ε}-Dicarbobenzyloxy-L-lysyl]-N-(2-[carbonyl-^{14}C]benzoyl-4-chlorophenyl)-N-methylglycinamide - Trifluoroacetic anhydride (0.45 ml, 0.003 moles) was added to a mixture of N^{α},N^{ε}-dicarbobenzyloxylysylglycine (1.5 g, 0.003 moles) and triethylamine (0.45 ml, 0.003 moles) in dry acetonitrile (5 ml) stirred in an ice-water bath. After 10 min 2-methylamino-4-chloro[carbonyl-^{14}C]benzophenone

(500 mg, 0.002 mole) was added and the mixture stirred at 0-5° for 10 min and then at room temp for 1 h. The reaction mixture was treated with excess sodium bicarbonate solution and worked up in the usual way to afford a brown gum that was purified by column chromatography over silica with chloroform-methanol, 38:1 (yield is 800 mg).

L-Lysyl-N(2-(Carbonyl-^{14}C)benzoyl-4-chlorophenyl)-N-methyl glycinamide dihydrobromide - The [^{14}C]dicarbobenzyloxy derivative (400 mg) was dissolved in a saturated solution of hydrogen bromide in acetic acid (4 ml) and stored at room temperature for 2.5 h. Dry ether (40 ml) was added to the mixture and decanted from the precipitated solid which was washed with additional portions of dry ether (2 x 40 ml). The final traces of ether were removed in vacuo to afford a white solid. The hydrobromide was homogeneous by reverse phase HPLC analysis using an RP18 column (25 x 0.46 cm) and eluting with acetonitrile-water, 1:1, 0.1 M $HClO_4$/0.2 M $NaClO_4$. The retention time was 3 min 13 sec at a flow rate of 2 ml/min. The UV detector wavelength was 254 nm. The use of acetonitrile-water, 4:6 (ret time 5.35) and acetonitrile-water, 3:7 (ret time 17.35) as eluting solvents confirmed homogeneity. The yield was 210 mg. The specific activity was 2.66 mCi/mmol.

References

1. British Patent No 1517164 (1978).

2. Hassall C.H., Holmes S.W., Johnson W.H., Kröhn A., Smithen C.E. and Thomas W.A. - Experientia 33 1492 (1977).

3. Oxley P., Partridge M.W., Robson T.D. and Short W.F. - J.Chem.Soc - 763 (1946).

4. Sugasawa T., Toyoda T., Adachi M. and Sasakura K. - J.Amer.Chem.Soc 100 4842 (1978).

5. Dauben W.G., Reid J.C. and Yankwich P.E. - Anal.Chem. - 19 828 (1947).