Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXXIX, No. 7

SYNTHESIS OF 4-TERT-BUTYL-3-(2-CHLORO-[-2-^{14}C]ETHYL)UREIDO BENZENE

El-mostafa Azim[1], Jean-Michel Dupuy[1], Jean-Claude Maurizis[1], René C.-Gaudreault[2], Annie Veyre[3], Jean-Claude Madelmont[1]

[1]INSERM U 71, rue Montalembert, B.P. 184, 63005 CLERMONT-FERRAND CEDEX

[2]Centre de Recherche, Pavillon Saint-François d'Assise, CHUQ, 10, rue de l'Espinay, Québec, G1L 3L5, Canada

[3]Laboratoire de Biophysique Médicale, Faculté de Médecine, B.P. 38,28, Place Henri-Dunant, 63001 CLERMONT-FERRAND CEDEX

SUMMARY

Carbonation of CH_3MgI with $^{14}CO_2$ led to sodium [1-^{14}C] acetate: 1 which was successively brominated and esterifed with diazomethane to give rise to methyl 2-bromo-[-1-^{14}C] acetate: 3, the reduction of which with H_3Al gave 2-bromo-[-1-^{14}C] ethanol 4. 4 was then reacted with NaCN; the intermediate 3-hydroxy-[-3-^{14}C] propionitrile 5 was hydrolyzed with HCl at 130°C into the corresponding acid: 6 which was transformed into 3-chloro-[-3-^{14}C] propionyl chloride: 7 ; 7 reacted with NaN_3 gave 3-chloro-[-3-^{14}C] propyl azide: 8 which by Curtius rearrangment led to 2-chloro-[-2-^{14}C] ethyl isocyanate: 9 which finally was condensed with 4-t-butylaniline leading to the title compound (radiochemical purity 99%) in an overall yield of about 10% based on [^{14}C] barium carbonate.

Key words: Methy bromo-[-1-^{14}C] acetate; 2-Bromo-[-1-^{14}C] ethanol; 3-chloro-[3-^{14}C] propionic acid; 3-chloro-[-3-^{14}C] propylazide; 2-chloro-[-2-^{14}C] isocyanate.

INTRODUCTION

4-tert-butyl-3-(2-chloroéthyl)ureido benzene is a member of a new class of potent antineoplastic agents; 1-aryl 3-(2-chloroethyl)ureas (CEUs) (1). These molecules were designed from the combination of the aromatic moiety of aromatic nitrogen mustards with the unnitrosated pharmacophore of nitrosoureas. CEUs are active against a large number of tumor cell lines even those having developed resistance

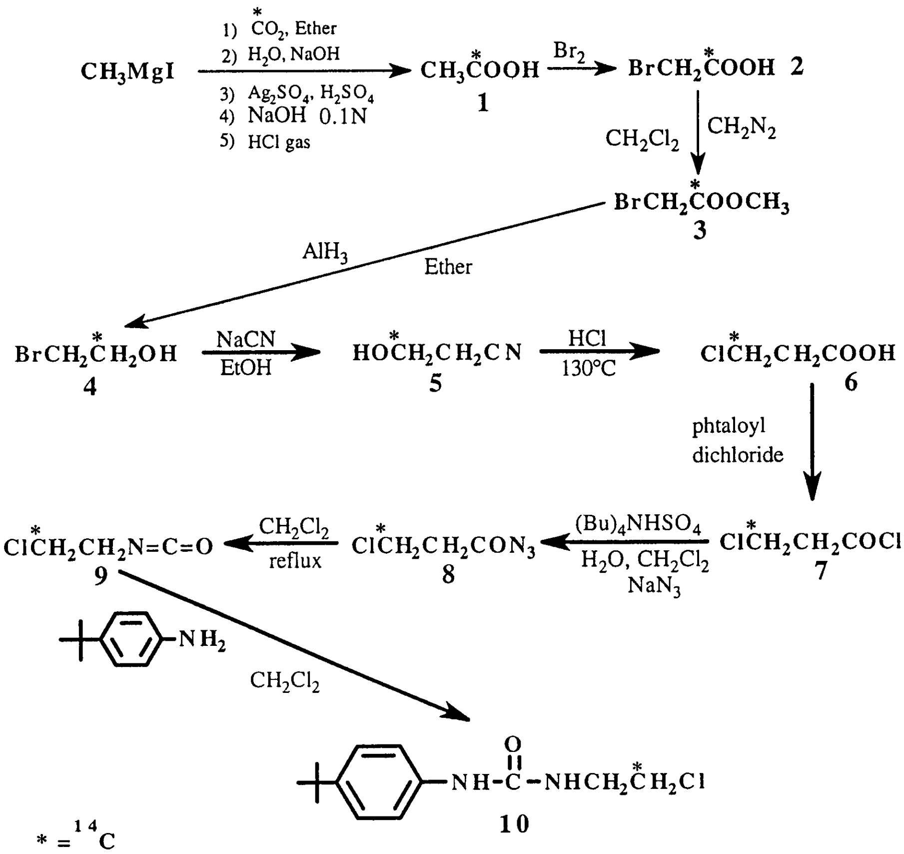
CCC 0362–4803/97/070559–08$17.50

Received 17 February 1997
Revised 10 March 1997

mechanisms toward antineoplastic agents (2). These compounds exert their cytotoxicity through the extensive depolymerization of cellular microtubules (3). In order to characterize the major urinary metabolite deriving from the 2-chloroethyl moiety, we have labelled 4-tert-butyl-3-(2-chloroéthyl)ureido benzene with ^{14}C on the carbon adjacent to the chlorine atom.

SYNTHESIS

The preparation of 4-tert-butyl-3-(2-chloro-[-2-^{14}C]éthyl)ureido benzene, was performed according to Scheme 1.



Scheme 1

The carbonation of methyl magnesium iodide (4), followed by the phosphorus catalysed bromination of [1-^{14}C] acetic acid (5) led to bromoacetic acid 2 in 85% yield. Esterification of bromoacetic acid 2 with diazomethane in methylene dichloride at 0°C followed by reduction of the ester function by aluminium hydride (6), led to the ^{14}C-labelled bromoethanol $\underline{4}$ in 50% yield from methyl-2-bromoacetate $\underline{3}$.3-Hydroxypropionitrile 3-^{14}C $\underline{5}$ was prepared in 85% yield by reaction of bromoethanol $\underline{4}$ with sodium cyanide (7). Subsequent hydrolysis of $\underline{5}$ using concentrated hydrochloric acid gave 3-chloropropionic acid-^{14}C $\underline{6}$ in 90% yield. Treatment of the latter compound with phthaloyl dichloride afford the corresponding acid chloride $\underline{7}$ in 85%. Azide $\underline{8}$ was prepared in 79% yield by treatment of $\underline{7}$ with sodium azide in presence of tetrabutylammonium hydrogen sulphate. Curtius rearrangement of the azido derivative $\underline{8}$ to 2-chloroethylisocyanate $\underline{9}$ in methylene dichloride followed by its nucleophilic addition to 4-tert-butylaniline gave 4-tert-butyl-3-(2-chloroéthyl)ureido benzene-^{14}C $\underline{10}$ in 50% yield.

EXPERIMENTAL

General Comments. All organic chemicals were obtained from Aldrich Chemical Co. The evaporation of organic solvents was performed under vacuum using a rotary evaporator. ^{1}H-NMR spectra were recorded on a Bruker WB 200AM spectrometer. All ^{1}H-chemical shifts are reported in part-per million down field relative to tetramethylsilane (TMS) used as internal standard. Analytical thin layer chromatography (TLC) were conducted on precoated silica gel plates (Merck 60 F_{254}; 0.2 mm thick). Visualization of TLC spots was achieved using ultra violet light at 254 nm and iodine as well. Column chromatography was performed using Silica gel 60 (chromagel, 230-400 mesh, SDS) as adsorbent. Radioactivity of samples was assessed using a Packard 4550 scintillation counter. Radiochemical purities were measured using the AMBIS 4000 detector (computer-controlled multi-vire proportional counter (Braun Sciencetec France)).

1) Sodium [1-^{14}C]acetate, $\underline{1}$

On a vacuum manifold, at 4×10^{-3} mbar, 2.368g (12.00 mmol, 3128 MBq, 85 mCi) of barium carbonate-^{14}C were cautiously treated with 50 ml of concentrated sulphuric acid to obtain a slow evolution of carbon dioxide-^{14}C. The carbon dioxide-^{14}C was vacuum transferred into a liquid nitrogen cooled flask containing a degassed solution of methyl magnesium iodide (40 mmoles, 40 ml) in 50 ml of dry ether. The carboxylation of the stirred Grignard reagent was carried-out at -20°C for 40 min. The reaction vessel was flushed with dry nitrogen. The reaction mixture was treated at -10°C by slow addition of 40 ml of water followed by 30 ml of 20% sodium hydroxide solution and transferred into a separatory funnel. The aqueous layer was collected and the organic phase was extracted with water (5 x 10ml). The aqueous layers were pooled together and 10 g of silver sulphate were added. The resulting slurry was stirred at room temperature for 90 min., concentrated under vacuum to half of its original volume and acidified with 30 ml of 50% sulphuric acid. The free acetic acid-^{14}C was isolated by steam distillation and the distillate was titrated potentiometrically with 114 ml of 0.1N aqueous sodium hydroxide. The resulting sodium (carboxyl-^{14}C) acetate solution was evaporated in vacuo to a small volume, transferred to a 100 ml round bottom long-necked flask and freeze dried. Finally, the crystalline residue was dried under vacuum at 120°C for 2 hours to yield 935 mg of 1 (11.4 mmol, 95%).

2) Bromo[1-^{14}C]acetic acid, 2

Nine hundred thirty five milligrams of sodium (carboxyl-^{14}C) acetate (11.4 mmol) were placed in 100 ml round bottom long-necked flask equipped with a magnetic stirring bar and connected to a vacuum line. Anhydrous hydrogen chloride, obtained from the reaction of 702 mg (12.00 mmol) of sodium chloride and 20 ml of concentrated sulphuric acid, was vacuum transferred into the flask cooled with liquid nitrogen. The resulting reaction mixture was stirred at room temperature for 1 hour, cooled to -180°C and subsequently slowly warmed to -80°C (dry ice-acetone mixture) to pump-off the excess gaseous HCl. The residual (carboxyl-^{14}C) acetic acid was vacuum transferred into a liquid nitrogen cooled flask and treated with 10 ml of acid anhydride at 100°C for 1 hour under anhydrous conditions. After addition of phosphorus (5 mg, 0.16 mmol) and dry bromine (2.100 g, 15.00 mmol) at room temperature the reaction mixture was heated for 2 hours at 125°C; then hydrogen bromide and excess of bromine were removed by bubbling of dry nitrogen at 50°C. Bromoacetic-^{14}C was isolated in 90% yield and used without further purification.

S.A. = 257.6 MBq/mmol, 7 mCi/mmol.

Acetic acid-^{14}C, 1

^{1}H-NMR (200 MHz, $CDCl_3$): δ 10.20 ppm (s, 1H, COO $\underline{H}$); 2.09 ppm (s, 3H, C $\underline{H}_3$).

Bromoacetic acid-^{14}C, 2

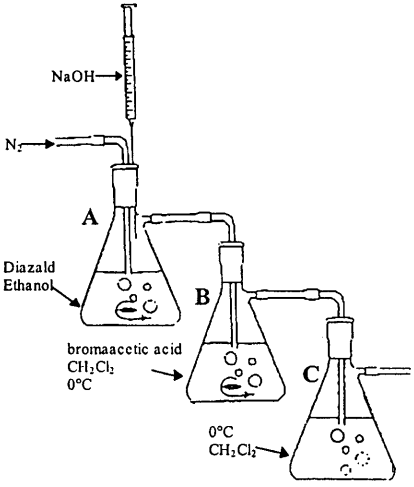
^{1}H-NMR (200 MHz, $CDCl_3$): δ 10.20 ppm (s, 1H, COO $\underline{H}$); 3.90 ppm (s, 2H, Br $\underline{CH_2}$).

3) Methyl bromo[-1-^{14}C]acetate, $\underline{3}$

Flask (A) was charged with p-toluenesulphonylmethylnitrosamide (Diazald, 3.640 g, 17.00 mmol) suspended in ethanol (60 ml). Flask (B) was charged with bromo-2-(carboxyl-^{14}C) acetic acid $\underline{3}$ (1.420 g, 10.20 mmol) dissolved in methylene dichloride (80 ml) and cooled with an ice- bath. A slow stream of nitrogen was then allowed to pass through the whole system (scheme 2). Then a concentrated aqueous solution of sodium hydroxide was slowly injected using a hypodermic syringe into flask (A) through a rubber septum. The yellow diazomethane generated in flask (A) was then brought by the stream of nitrogen into flask (B) to react with $\underline{3}$. Sodium hydroxide solution was added in flask (A) until the yellow colour in flask (A) was completely discharged. Solution in flask (B) showed on TLC complete methylation of acid $\underline{3}$ after 15 min (96% yield).

S.A. = 257.6 MBq/mmol, 7mCi/ mmol.

^{1}H NMR (200 MHz, $CDCl_3$): δ 3.85 ppm (s, 2H, $BrC\underline{H}_2$); 3.79 ppm (s, 2H, $C\underline{H}_3$)



Scheme 2

4) 2-Bromo[1-^{14}C]ethanol $\underline{4}$

Into a three necked flask (100 ml) equipped with a condenser, a thermometer and a rubber septum were place an ether suspension (20 ml) of lithium aluminium hybride (424 mg, 11.17 mmol,). To the mixture, aluminium chloride (374 mg, 2.80 mmol) in ether (4 ml) was added dropwise over a period of 5 min. To the aluminium hydride slurry, methylbromoacetate-^{14}C (1.50 g, 9.80 mmol) in ether (20 ml) was slowly added at 0°C over 15 min. During this addition, hydrogen was evolved. The temperature of the solution was maintained at 0°C. After 15 min, the excess hydride was cautiously destroyed by the addition of 0.7 ml of water. The precipitate was filtered-off and washed twice with 20 ml of ether. Distillation of ether afforded bromoethanol-^{14}C in 50% yield.

S.A. = 257.6 MBq/mmol, 7 mCi/mmol.

^{1}H-NMR (200 MHz, $CDCl_3$): δ 3.92 ppm (t, 2H, BrC $\underline{H}_2$); 3.54 ppm (t, 2H, C $\underline{H}_2$OH).

5) 3-hydroxy-[-3-^{14}C] propionitrile 5

To a solution of bromoethanol-^{14}C (612 mg, 4.90 mmol) in ethanol (5 ml) was added sodium cyanide (294 mg, 6.00 mmoles). The mixture was stirred and refluxed for 20 hrs. Then, the solution was cooled, diluted by addition of 100 ml of acetone and filtered. Removal of acetone under reduced pressure yielded 85% of 3-hydroxy-[-3-^{14}C]propionitrile (295 mg 4.16 mmol) which was used without further purification.

S.A. = 257.6 MBq/mmol, 7 mCi/mmol.

^{1}H-NMR (200 MHz, $CDCl_3$): δ 3.87 ppm (t, 2H, C$\underline{H}_2$OH); 2,77 ppm (s, 1H, O$\underline{H}$); 2.60 ppm (t, 2H, C$\underline{H}_2$CN).

6) 3-chloro-[-3-^{14}C]propionic acid 6

A mixture of 5 (4.10 mmol) and 6 ml of concentrated hydrochloric acid were heated at 130°C for 4 hours in a pressure-sealed flask. After cooling, the solution was extracted with ether (3 x 50 ml). The organic layer was dried over anhydrous magnesium sulphate, filtered, and. Ether was evaporated under reduced pressure to afford 400 mg (3.69 mmol, 90% yield) of 3-chloropropionic acid 3-^{14}C.

S.A. = 257.6 MBq/mmol, 7mCi/mmol.

^{1}H-NMR (200 MHz, $CDCl_3$): δ 9.50 ppm (s, 1H, COO$\underline{H}$); 3.76 ppm (t, 2H, C$\underline{H}_2$COOH); 2.86 ppm (t, 2H, C$\underline{H}_2$Cl).

7) 3-chloro-[-3-^{14}C]propionic acid chloride 7

6 (390 mg, 3.60 mmol) and phthaloyl dichloride (5 ml) were heated at 140°C for 3 hours in a

pressure sealed flask. Further 388 mg (3.06 mmol, 85% yield) of chemically and radiochemically pure 3-chloro-[-3-^{14}C]propionic acid chloride were obtained by vacuum transfer into a flask cooled with liquid nitrogen.

S.A. = 257.6 MBq/mmol, 7mCi/mmol.

^{1}H-NMR (200 MHz, $CDCl_3$): δ 3.76 ppm (t, 2H, C $\underline{H}_2$COCl); 3.35 ppm (t, 2H, ClC $\underline{H}_2$).

8) 3-chloro-[3-^{14}C]propylazide $\underline{8}$

Sodium azide (390 mg, 6.00 mmol) and tetrabutylammonium hydrogen sulphate (7 mg) in water (2 ml) were added to a solution of the acid chloride $\underline{7}$ (381 mg, 3.00 mmol) in methylene dichloride (20 ml). The mixture was stirred at 0°C for 2 hours, and then the organic layer was washed with water (3 x 6 ml), dried over anhydrous magnesium sulphate and filtered. The solvent was removed under reduced pressure to yield 411 mg (2.370 mmol, 79%) of azide $\underline{8}$.

S.A. = 257.6 MBq/mmol, 7mCi/mmol.

^{1}H-NMR (200 MHz, $CDCl_3$): δ 3.76 ppm (t, 2H, C $\underline{H}_2CON_3$); 2.83 ppm (t, 2H, C $\underline{H}_2$Cl).

9) 2-chloro-[2-^{14}C]ethylisocyanate $\underline{9}$

A solution of $\underline{8}$ (400 mg, 2.30 mmol) in 20 ml of methylene dichloride was stirred for 20 hours under reflux. Removal of the solvent under reduced pressure afforded 218 mg (2.07 mmol, 90% yield) of 2-chloro-[2-^{14}C]ethylisocyanate $\underline{9}$.

S.A. = 257.6 MBq/mmol, 7mCi/mmol.

^{1}H-NMR (200 MHz, $CDCl_3$): δ 3.74-003.55 ppm (m, 4H, C $\underline{H}_2C\underline{H}_2$).

10) 4-tert-butyl-3-(2-chloro-[2-^{14}C]éthylureido benzene $\underline{10}$

To a solution of 2-chloroethylisocyanate 2-^{14}C $\underline{9}$ (211 mg, 2.00 mmol) in methylene dichloride (50 ml) were added 596 mg of 4-tert-butylaniline (4.00 mmol). The mixture was heated at 40°C for 20 hours. The solution was evaporated under vacuum and the residue was purified by column chromatography (20/80; pentane/ethyl acetate) to yield 255 mg (1.00 mmol, 50%) of pure 4-tert-butyl-3-(2-chloroéthyl)ureido benzene-^{14}C.

S.A. = 257.6 MBq/mmol, 7mCi/mmol.

^{1}H-NMR (200 MHz, $CDCl_3$): d 7.33 ppm (dd, 2H, Ar); 7.19 ppm (d, 2H, Ar) ; 6.95 ppm (s, 1H, NHCO); 5.65 ppm (s, 1H, N H); 3.70-3.50 ppm (m, 4H, C H_2CH_2); 1.28 ppm (s, 9H, C H_3).

REFERENCES

1) R. C.-Gaudreault, J. Lacroix, M. Pagé and L.P. Joly, J. Pharm. Sci., 185-188, 77, (1988).
2) Poyet, P., Ritchot, N., Béchard, P. and C.-Gaudreault, R. Anticancer Res., 13(5), 1447-1452, (1993).
3) C.-Gaudreault, R., Alaoui-Jamali, M.A., Batist G., Béchard, P., Lacroix, J. and Poyet, P. Cancer Chemother. Pharmacol., 33(6), 489-492, (1994).
4) B.M. Tolbert, J. Biol. Chem., 173-205, (1948).
5) B. Chepartz and S. Gurin, J. Biol. Chem., 180-663, (1949).
6) J. Lintermans, A. Benakis and J. Williams, J. Lab. Comp., Vol VII, no 4, 533-540 (1971).
7) M. Azim, J.-C. Madelmont, C. Cussac, M. Rapp, J.-C. Maurizis, R. C.-Gaudreault, D. Godeneche, A. Veyre, J. Lab. Comp. and Radiopharm., Vol XXXIII, no 11, 1079-1082, (1993).