

SYNTHESIS OF 6-CHLORO 2-(ETHYLAMINO)-4-METHYL-4-PHENYL- [-4-¹⁴C]-4H,-3,1-BENZOXAZINE (ETIFOXINE)

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Summary

Carbonylation of phenyl lithium with ¹⁴CO₂, followed by reduction of [¹⁴CO₂] benzoic acid, led to [α-¹⁴C] benzyl alcohol **3**, the oxidation of which afforded the [α-¹⁴C] benzaldehyde **4**. The latter, then condensed with pivoylamide **6** (trimethyl acetamide-N-(2-lithio-4chlorophenyl) gave 5-chloro-2 (t-Butylamide)-α-phenyl-[α-¹⁴C] benzyl alcohol **7** which was then oxidized to ketone **8** which it self were subsequently hydrolysed to 5-chloro-2-amino-[α-¹⁴C]benzophenone **9**. Grignard reaction of **9** with methylmagnesium iodide gave 5-chloro-2-amino-α-methyl-α-phenyl-[α-¹⁴C] benzyl alcohol **10**. Treatment of the alcohol **10** with ethylthioisocyanate, followed by cyclisation led to 6-chloro-2-(ethylamino)-4-methyl-4-phenyl-[-4-¹⁴C]-4H-3,1 benzoxazine **12**. The latter was acidified to give rise to Etifoxine hydrochloride **13** in an overall yield of about 31%based on [¹⁴C] barium carbonate.

Key words: benzyl alcohol-α-¹⁴C; benzaldehyde-α-¹⁴C; 5-chloro-2 (t-Butylamide)-α-phenyl-[α-¹⁴C] benzyl alcohol; 5-chloro-2-amino-[α-¹⁴C]benzophenone; 5-chloro-2-amino-α-methyl-α-phenyl-[α-¹⁴C] benzyl alcohol; 6-chloro-2-ethylamino-4-methyl-4-phenyl-[-4-¹⁴C]-4H-3,1 benzoxazine; Etifoxine hydrochloride

Introduction

In order to study the distribution and metabolic fate of Etifoxine hydrochloride in pigmented rat and in humans a radio-labelled labelled form of the compound was needed. Heidelberger and Ricke (1) have already described a procedure for preparing 5-chloro-2-amino-benzophenone. The disadvantages of this procedure are that the product of the reaction was

difficult to isolate and the yield is less than 10 %. We therefore embarked on an improved procedure.

The preparation of Etifoxine hydrochloride labelled with ^{14}C , was performed according to the reaction sequence depicted in scheme 1.

Synthesis

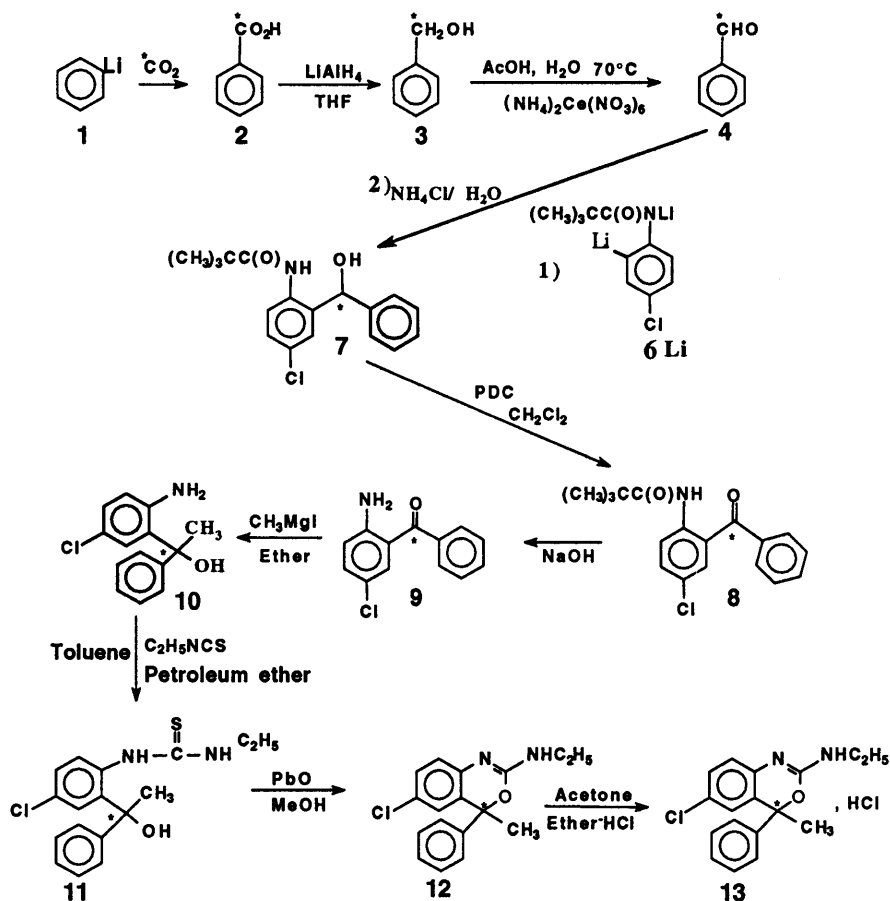
Carbonation of phenyl lithium with $^{14}\text{CO}_2$ (2), followed by reduction of the intermediate [$^{14}\text{CO}_2\text{H}$] benzoic acid with lithium aluminium hydride, led to benzyl alcohol **3** in 85% yield from $\text{Ba}^{14}\text{CO}_3$ (3). Subsequent oxidation of the alcohol function with ceric ammonium nitrate (4) afforded the benzaldehyde **4** in 70 % overall yield from $^{14}\text{CO}_2$. The trimethyl acetamide-N-(2 lithio 4-chlorophenyl) was prepared by ortholithiation (5) of the corresponding amide prepared according to scheme 2: condensation of trimethylacetyl chloride with 4-chloroaniline in acetonitrile (6). This amide was treated with 2,1 equivalent of BuLi. The resulting mixture was reacted with [^{14}CHO] benzaldehyde **4** to produce the desired alcohol **7** in 90 % yield. The alcohol **7** was oxidized to ketone **8** by PDC and subsequent hydrolysis provided the aminoketone **9** in 58 % yield. Grignard reaction of **9** with methylmagnesium gave 5-chloro-2-amino- α -methyl- α -phenyl-[- α - ^{14}C] benzyl alcohol **10**.

Treatment of the alcohol **10** with ethylthioisocyanate, followed by cyclisation with PbO led to 6-chloro-(2-ethylamino)-4-methyl-4-phenyl-[- 4 - ^{14}C]-4H-3,1 benzoxazine **12**, which was acidified with ether-hydrochloric acid to give Etifoxine hydrochloride **13** in 80 % yield.

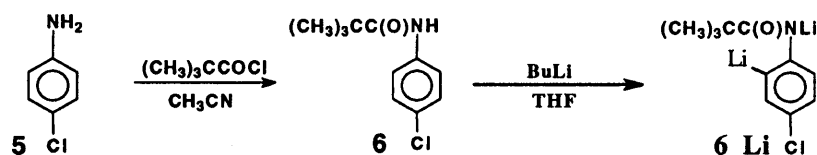
Experimental

General comments. Proton nuclear magnetic resonance (^1H -NMR) spectra were performed on a Brücker AM 200 (4.5 T) spectrometer. Chemical shifts are reported in parts per million relative to internal tetramethyl silane (TMS) standard. Melting points (mp) were determined on an Electrothermal digital apparatus. Analytical thin layer chromatography (TLC) was carried out on precoated silica gel plates (Merck 60 F 254, 0,2 mm thick) with both

detection by ultra violet light at 254 nm and visualisation by iodine. Silica gel 60 (chromagel, 230-400 mesh, SDS) was used. Radioactive samples were measured using a Packard 4550 scintillation counter



Scheme 1



Scheme 2

1) [$^{14}\text{CO}_2\text{H}$] Benzoic acid, 2

The carbonation of phenyllithium with $^{14}\text{CO}_2$ has been reported in the literature (7) to give [$^{14}\text{CO}_2\text{H}$] benzoic acid in 95 % yield

S.A. 10 mCi/mmole, 370 MBq/mmole.

^1H NMR (200 MHz, CDCl_3) : δ 8.14 ppm (d, 1H); 7.85-7.40 ppm (m, 3H).

2) Benzyl alcohol, α - ^{14}C 3

To a solution of benzoic acid 2 (1.16 g, 9.5 mmol) in THF (30 ml) were added at 0°C 12 ml of LiAlH_4 (1N) in THF. This mixture was stirred at room temperature for 18 h. Work up was accomplished by adding H_2SO_4 (6 N) (10 ml) and the residue was extracted with ether (5 x 60 ml). The combined organic layers were washed with NaOH (1N) (10 ml), water (20 ml), and dried over anhydrous magnesium sulphate. Removal of the ether by evaporation under reduced pressure afforded benzyl alcohol α - ^{14}C in 90 % yield from $^{14}\text{CO}_2$.

S.A. = 10 mCi/mmole, 370 MBq/mmole

^1H NMR (200 MHz, CDCl_3) : δ 7.50-7.35 ppm (m, 5 H); 4.69 ppm (s, 2H); 2.67 ppm (s, 1H)

3) [^{14}CHO] Benzaldehyde 4

To ammonium cerium nitrate (9.870 g, 18.00 mmol) in acetic acid (25 ml) and water (25 ml) was added benzyl alcohol- α - ^{14}C (972 mg, 9.00 mmol). The resulting mixture was stirred at 70°C for 90 min, then extracted with ether (4 x 60 ml). The organic layer was washed in successive order with NaOH (50 ml) (1,5 N) and water (50 ml), dried over anhydrous magnesium sulphate and subsequently filtered. Removal of the ether by evaporation under reduced pressure afforded [^{14}CHO] benzaldehyde in 80 % yield.

S.A. = 10 mCi/mmole, 370 MBq/mmole

^1H NMR (200 MHz, CDCl_3): δ 9.97 ppm (s, 1H); 7.90-7.75 ppm (m, 2 H); 7.65-7.40 ppm (m, 3H).

4) N-(4-chlorophenyl) trimethyl acetamide 6

A solution of 4-chloroaniline (30 mmol) in acetonitrile (50ml) was treated with anhydrous potassium carbonate (30mmol) and trimethylacetyl chloride (32mmol) with vigorous stirring and cooling. After stirring for 2h at room temperature the mixture was poured over crushed ice (200g). After further extraction with ether (2 x 100ml) the combined organic layer was dried with magnesium sulphate. Evaporation of the organic layer gives the crude 6 in 90% yield.

^1H NMR (200MHz, CDCl_3): δ 7.49 ppm (d, 2H); 7.38 ppm (s, 1H); 7.24 ppm (d, 2H); 1.38 ppm (s, 9H).

5) 5-chloro-2 (t-Butylamide)- α -phenyl-[α - ^{14}C] benzyl alcohol 7

The pivalamide 6 (1.521 g, 7.20 mmole) was dissolved in dry THF (40 ml) and the solution was cooled to -78°C . BuLi (15.80 mmol, 2,2 eq) was added to the solution at -78°C and the mixture was stirred at 0°C for 3 h. The [α - ^{14}C] benzaldehyde (763 mg, 7.2 mmol) in THF (10 ml) was then added dropwise to the mixture over 3 min and the mixture was stirred at 0°C for another 2 hours. The reaction was stopped by addition of a saturated aqueous NH_4Cl solution (20 ml). Then the aqueous fraction was extracted with ether (50 ml); the organic layer was separated, washed with water brine solution, dried over anhydrous magnesium sulphate and subsequently filtered. The solvent was evaporated under reduced pressure and the residue was purified using a silica gel column (hexane/AcoEt) (9/1) to provide 7 in 80 % yield.

S A = 10 mCi/mmole, 370 MBq/mmole

m.p. = 154°C

^1H NMR (200 MHz, CDCl_3): δ 8.78 (s, 1H); 8.12 ppm (d, 1H); 7.45-7.10 ppm (m, 6H) ; 7.08 ppm (d, 1H); 5.82 ppm (s, 1H); 3.51 ppm (s, 1H); 1.05 ppm (s, 9H).

6) 5-chloro-2-amino-[-¹⁴C] benzophenone 9

The alcohol 7 (1.736 g, 5.76 mmol) was dissolved in methylene dichloride (20 ml) and the solution was added into a suspension of pyridinium dichromate (PDC) (8.64 mmol, 1.5 eq) in methylene dichloride (50 ml). The resulting mixture was refluxed for 1 h. After ether (50 ml) had been added, the dark solution was filtered through a column of silica gel (AcoEt/pentane) (1/9) to provide 8.

m.p. = 98°C

¹H NMR (200 MHz, CDCl₃): δ 11.07 ppm (s, 1H); 8.69 ppm (d, 1H); 7.75-7.30 ppm (m, 7H); 1.34 ppm (s, 9H)

To a solution of ketone 8 in ethanol (20 ml) was added NaOH (10 %) (4 ml). The reaction mixture was stirred for 7 hours at room temperature then extracted with ether (3x40 ml). The organic layer was separated, dried over anhydrous magnesium sulphate and filtered. Removal of the organic solvent by evaporation under reduced pressure afforded 9 in 72 % yield.

S.A. 10 mCi/mmol, 370 MBq/mmol

¹H NMR (200 MHz, CDCl₃): δ 8.69-8.46 ppm (m, 5H); 8.41 ppm (d, 1H); 8.24 ppm (dd, 1H); 7.69 ppm (d, 1H); 7.06 ppm (s, 2H)

7) 5-chloro-2-amino-α-methyl-α-phenyl-[-α-¹⁴C] benzyl alcohol 10

To a solution of methyl magnesium bromide (12.42 mmol) in n-butyl ether (20 ml) at 45°C was added dropwise ketone 9 (959 mg, 4.14 mmol) in n-butyl ether (25 ml). The mixture was stirred for 2 h. After cooling this mixture to 10°C, an aqueous solution of NH₄Cl (36 %)(15 ml) was added. The aqueous layer was extracted with ether (3 x 60 ml) and the organic layer was separated, dried over anhydrous magnesium sulphate and filtered. Removal of the solvent by evaporation under reduced pressure afforded pure alcohol 10 in 100 % yield.

S.A. = 10 mCi/mmol, 370 Mbq/mmol.

^1H NMR (200 MHz, CDCl_3): δ 7.30-7.05 ppm (m, 6H); 6.94 ppm (dd, 1H); 6.40 ppm (d, 1H); 3.55 ppm (s, 3H); 1.70 ppm (s, 3H)

8) 5-chloro-2-(β ethylthiourido)- α -methyl- α -phenyl-[- α - ^{14}C] benzyl alcohol 11

Ethylthioisocyanate (440 mg, 5.05 mmol) was added to a flask containing alcohol 10 (975 mg, 4.14 mmol) in toluene (4 ml) and petroleum ether (4 ml) maintained at 60°C. The mixture was stirred for 5 h. Then the volatile organic solvents were removed under reduced pressure. The residue was crystallised in CH_2Cl_2 /pentane (1/50) (V/V) (90 % overall yield).

S.A. 10 mCi/mmol, 370 MBq/mmol

m. p. 125°C

^1H NMR (200 MHz, CDCl_3): δ 8.08 ppm (s, 1H); 7.64 ppm (s, 1H); 7.40-7.10 ppm (m, 6H); 5.34 (s, 1H); 3.64 ppm (s, 1H); 3.35-3.10 (m, 2H); 1.94 ppm (s, 3H); 1.61 (s, 1H); 0.93 ppm (t, 3H)

9) 6-chloro-2-ethylamino-4-methyl-4-phenyl-[-4- ^{14}C]-4H-3,1 benzoxazine 12

To a solution of the thiourea 11 (1.147 g, 3.72 mmol) in methanol (12 ml) maintained at 45°C was added PbO (2.41 g). After 1 hour the reaction mixture was filtered and the residue washed with methanol (100 ml). The filtrate was evaporated under reduced pressure and the residue was purified by liquid chromatography on a silicagel column (CH_2Cl_2 /EtOH) (9/1) (95 % yield).

S.A. 10 mCi/mmol, 370 MBq/mmol

^1H NMR (200 MHz, CDCl_3): δ 7.40-7.22 ppm (m, 6H); 7.18 ppm (dd, 1H); 7.00-6.93 ppm (m, 1H); 4.40 ppm (s, 1H); 3.35 ppm (dq, 2H); 1.94 ppm (s, 3H); 1.16 ppm (t, 3H)

10) 6-chloro-2-éthylamino-4-méthyl-4-phenyl-[-4- ^{14}C]-4H-3,1benzoxazine hydrochloride 13.

A solution of Etifoxine-base 12 (1.060 g, 3.53 mmol) in acetone (40 ml) was acidified with a solution of ether-HCl (1N). The mixture was stirred for 30 min after which the solvents

were removed at reduced pressure and the residue was crystallised in methyl acetate at -5°C (93 % yield).

S.A. = 10 mCi/mmol, 370 MBq/mmol

m.p. $138-140^{\circ}\text{C}$

^1H NMR (200 MHz, CDCl_3): δ 12.85 ppm (s, 1H); 9.67 ppm (s, 1H); 7.50-7.00 ppm (m, 8H); 3.47 ppm (qt, 2H); 2.15 ppm (s, 3H); 1.27 ppm (t, 3H)

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