

SYNTHESIS OF  $^{14}\text{C}$ -LABELLED 1-(3,5-BIS-TRIFLUOROMETHYL)-  
PHENYL-4-METHYL THIOSEMICARBAZIDE, C 2696-GO<sup>†</sup>

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SUMMARY

For pharmacokinetic and metabolism studies in experimental animals C 2696-Go was labelled initially with  $^{14}\text{C}$  on the thiocarbonyl carbon of the thiosemicarbazide moiety of the molecule, having a specific activity of 3.14  $\mu\text{Ci}/\text{mg}$ , in a radiochemical yield of 43%, based on the starting potassium [ $^{14}\text{C}$ ]thiocyanate.

C 2696-Go alternatively labelled with  $^{14}\text{C}$  on one of the trifluoromethyl groups, having a specific activity of 3.6  $\mu\text{Ci}/\text{mg}$  was also synthesized in an overall radiochemical yield of 5.1%, starting from  $\text{Ba}[^{14}\text{C}]\text{O}_3$ .

Key words : C 2696-Go Carbon-14, antifertility agent,  
antiimplantation activity, synthesis

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INTRODUCTION

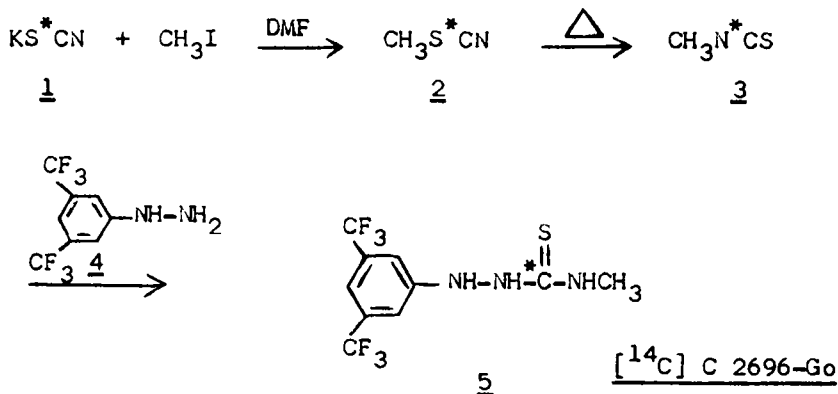
The compound C 2696-Go, 1-(3,5-bistrifluoromethyl)phenyl-4-methylthiosemicarbazide is an antifertility agent exhibiting potent antiimplantation activity in the rat.<sup>1</sup>

For pharmacokinetic and metabolism studies in laboratory animals C 2696-Go was labelled initially with carbon-14 on the thiocarbonyl carbon of the thiosemicarbazide moiety of the molecule (5, Scheme A) having a specific activity of 3.14  $\mu\text{Ci}/\text{mg}$ .

A second <sup>14</sup>C labelled preparation of C 2696-Go (16, Scheme B) having a specific activity of 3.6  $\mu\text{Ci}/\text{mg}$  was also synthesized, the label now being located on one of the trifluoromethyl substituent groups on the phenyl ring. This position for the <sup>14</sup>C label is considered to be more secure than that in the earlier labelled preparation.

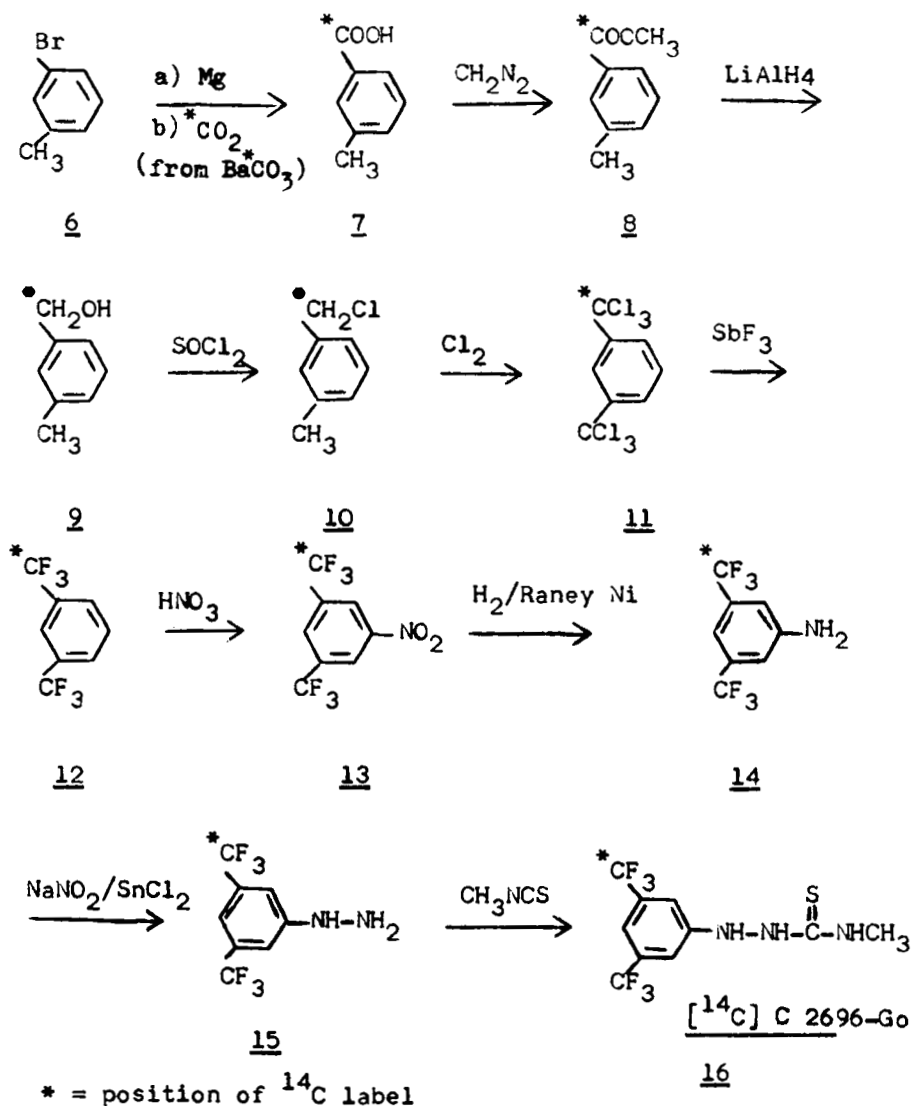
DESIGN OF THE SYNTHESSES AND DISCUSSION

The syntheses of the two labelled preparations of C 2696-Go are outlined in the following schemes A and B and the positions of the label are indicated by the asterisk (\*) in the respective structures.

Scheme A

\* = position of <sup>14</sup>C label

## Scheme B



The synthesis was carried out on a 2 mmol scale starting with potassium[<sup>14</sup>C]thiocyanate (2 mCi). Reaction with methyl iodide at RT led to methyl[<sup>14</sup>C]thiocyanate (2) which was pyrolyzed to methyl[<sup>14</sup>C]isothiocyanate(3)<sup>2</sup> by refluxing in DMF solution at 165 °C for two hours. The DMF distillate

containing (3) on reaction with 3,5-bistrifluoromethylphenylhydrazine (4) afforded  $^{14}\text{C}$ -C 2696-Go (5) having a specific activity of 3.14  $\mu\text{Ci}/\text{mg}$  in a radiochemical yield of 43% based on potassium[ $^{14}\text{C}$ ]thiocyanate.

Carbonation of the Grignard reagent from 3-bromotoluene (6), with  $^{14}\text{CO}_2$  obtained from  $\text{Ba}^{14}\text{CO}_3$  yielded 1-[ $^{14}\text{C}$ ]carboxy-3-methyl benzene (7) which was esterified with ethereal diazomethane to the methyl ester (8), followed by reduction with lithium aluminium hydride to the corresponding benzyl alcohol (9) which was then converted to the chloromethyl compound (10) by reaction with thionyl chloride. Subsequent chlorination of (10) with chlorine photochemically afforded the bistrichloromethyl benzene (11) which on heating with antimony trifluoride gave the bistrifluoromethyl benzene (12) bearing the  $^{14}\text{C}$ -label on one of the trifluoromethyl groups. Nitration of the latter by heating with fuming nitric acid in 25% oleum gave 1-[ $^{14}\text{C}$ ]trifluoromethyl-3-trifluoromethyl-5-nitrobenzene (13) which on catalytic hydrogenation in the presence of Raney Nickel yielded the corresponding amino compound (14). Diazotisation of (14) followed by reduction of the diazonium compound *in situ* with stannous chloride led to 1-[ $^{14}\text{C}$ ]trifluoromethyl-3-trifluoromethyl-5-hydrazinobenzene (15). Finally reaction of (15) with methyl isothiocyanate gave [ $^{14}\text{C}$ ] C 2696-Go (16) bearing the  $^{14}\text{C}$  label on one of the trifluoromethyl groups. This labelled preparation having a specific activity of 3.6  $\mu\text{Ci}/\text{mg}$  was obtained in a overall radiochemical yield of 5.1% based on the starting  $\text{Ba}^{14}\text{CO}_3$ .

## EXPERIMENTAL

### 1. Materials and methods

Relevant data for the final synthetic sequence of Scheme B using unlabelled intermediates is given below :

Compound No.	Mol. wt.	m.p./b.p. °C
<u>7</u>	136	111-3
<u>9</u>	122	120-3/26 mm
<u>10</u>	141	95-100/8 mm
<u>11</u>	313	109-110
<u>12</u>	214	115-117/760 mm
<u>13</u>	259	70-2/7 mm
<u>14</u>	229	80-5/15 mm
<u>15</u>	244	75-7
<u>16</u>	317	203-4

Melting and boiling points are uncorrected. Potassium [<sup>14</sup>C] thiocyanate (101.4 mg; specific activity 1.9 mCi/mmol; 2 mCi) was procured from the Isotope Division, Bhabha Atomic Research Centre, Trombay, Bombay 400 085, INDIA.

Non-radioactive potassium thiocyanate was of AnalaR grade. Non-radioactive 3,5-bistrifluoromethylphenylhydrazine m.p. 75-77°C was prepared internally<sup>1</sup> from 3,5-bistrifluoromethylaniline which was purchased from Fluka Chemicals AG, Buchs, Switzerland. All other reagents and chemicals were of reagent grade. Dimethylformamide (bp 153°C) was distilled before use. Anhydrous ether was prepared by allowing the commercially available solvent ether to stand over anhydrous calcium chloride, at first, for three to four days, then filtered and stored over sodium wire in an amber coloured bottle. Methyl iodide was refluxed, distilled and stored over silver powder.

Measurements of radioactivity and radiometric TLC were carried out as described earlier<sup>2</sup>.

The identity and chemical and radiochemical purity of the labelled preparations were established by comparison of m.p., mmp, UV and TLC ( $R_f$ ) with the analytically pure unlabelled C 2696-Go synthesized internally and radio-scanning on a Berthold scanner. The  $R_f$ s of C 2696-Go in the solvent systems used were as given below :

<u>Solvent systems</u>	<u><math>R_f</math></u>
a) Chloroform - ethyl acetate (3:1 v/v)	0.68
b) Chloroform - methanol (93:7 v/v)	0.63
c) Chloroform	0.12
d) Benzene-methanol (10:1 v/v)	0.28

C 2696-Go is a colourless crystalline compound having a molecular formula  $C_{10}H_9F_6N_3S$ ; a molecular weight of 317 and a mp of 203-204°C. It exhibited in ethanol solution a UV absorption maximum at 248 nm.

2. 1-(3,5-Bistrifluoromethyl)phenyl-4-methyl-3-[ $^{14}C$ ]thio-  
semicarbazide

Methyl[ $^{14}C$ ]isothiocyanate (3)

This compound was prepared as described<sup>2</sup> using potassium[ $^{14}C$ ]thiocyanate (1) (101.4 mg; specific activity 1.9 mCi/mmol, 2 mCi), nonradioactive potassium thiocyanate (93 mg), DMF (1.2 ml) and methyl iodide (0.18 ml; 426 mg; 3 mmoles). After the pyrolysis of methyl[ $^{14}C$ ]thiocyanate(2) to methyl[ $^{14}C$ ]isothiocyanate (3) and cautious distillation (Kugelrohr), the condensing tube was washed with anhydrous ether (5 ml); the washings and the distillate were combined and used in the next step.

1-(3,5-Bistrifluoromethyl)phenyl-4-methyl-3-[<sup>14</sup>C]thio-  
semicarbazide (5)

To the DMF-ether solution containing (3) was added a solution of 3,5-(bistrifluoromethyl)phenylhydrazine (4) (490 mg; 2 mmoles) in anhydrous ether (5 ml) and the mixture swirled for a few minutes. Then all the ether was evaporated off on a water-bath and the residue was allowed to stand at 25°C overnight. Water (4 ml) was added and the resulting suspension stirred with a mixture of benzene (1 ml) and hexane (5 ml) for 30 min and allowed to stand in the refrigerator for 1 hr. The solid was collected on a Büchner funnel, washed with hexane and dried to get the crude C 2696-Go (5) (310 mg). Recrystallisation, twice, from hexane, yielded pure (5) 274 mg; specific activity 3.14 µCi/mg; radiochemical yield 43%; radiochemical purity >98%.

3. 1-{3-[<sup>14</sup>C]-Trifluoromethyl-5-trifluoromethyl}phenyl-4-methyl-  
thiosemicarbazide

1-[<sup>14</sup>C]Carboxy-3-methylbenzene (7)

To the reaction flask containing Mg-turnings (0.27 g, 11 mmoles) in anhydrous diethyl ether (10 ml), under magnetic stirring, 3-bromotoluene (1.7 g, 10 mmoles) in ether (5 ml) was added within 15 min, keeping the reaction mixture under slight reflux. The mixture was heated for an additional hr under reflux. The GRIGNARD solution was cleared by filtration, transferred to another round bottom flask and chilled with liquid nitrogen. Under vacuum, the CO<sub>2</sub> generated from Ba<sup>14</sup>CO<sub>3</sub> (0.59 g, 5 mmoles, 39.8 mCi) and conc. H<sub>2</sub>SO<sub>4</sub> (20 ml), was frozen on top of the Grignard reagent. The mixture was

stirred for 20 min at  $-20^{\circ}$  and again for 20 min at  $0^{\circ}$ , subsequently acidified with 2N HCl and extracted with ether. In order to remove neutral by-products, the organic layer was extracted with two portions of saturated aqueous  $\text{Na}_2\text{CO}_3$ , 10 ml each. The aqueous phase was again acidified with conc. HCl and extracted with ether. After drying of the ethereal solution with  $\text{Na}_2\text{SO}_4$  and removal of the solvent, crude acid (7) (0.54 g, 3.96 mmoles) was obtained.

1-[ $^{14}\text{C}$ ]Hydroxymethyl-3-methylbenzene (9)

The above acid (7) (0.54 g, 3.96 mmoles, 32 mCi) was dissolved in ether (20 ml) and methylated by addition of a slight excess of an ethereal diazomethane solution. After standing of the reaction mixture at room temperature for 30 min, the volume was reduced to 10 ml by evaporation. This solution, containing the methylester (8) was added dropwise within 15 min to the suspension of  $\text{LiAlH}_4$  (0.38 g, 10 mmoles) in anhydrous ether (10 ml), followed by heating under reflux for 4 hrs. Ice-water (10 ml) and 2N HCl (10 ml) were added cautiously. The mixture was stirred again for one hr at room temperature, and finally, non-radioactive 3-methyl-benzyl alcohol (2.56 g, 21 mmoles) was added. Extraction with ether, removal of the solvent and distillation at  $120-140^{\circ}/12$  mm Hg in a bulb-tube (Kugelrohr) yielded (9) (2.8 g, 23 mmoles, approximately 29 mCi).

1-[ $^{14}\text{C}$ ]Chloromethyl-3-methylbenzene (10)

The product (9) (2.8 g, 23 mmoles) was placed in a round bottom flask with an ice-bath and thionyl chloride (5.95 g, 50 mmoles) and one drop of pyridine were added. Then the mixture was stirred at  $100^{\circ}$  for 4 hrs. Ice was added and the product



extracted with ether. After evaporation of the solvent, the residue was distilled in a bulb-tube at 130-150°/12 mm Hg. to give the chloromethyl compound (10) (1.93 g, 13.7 mmoles).

1-[<sup>14</sup>C]Trichloromethyl-3-trichloromethylbenzene (11)

The above product (10)(1.93 g, 13.7 mmoles) was dissolved in CCl<sub>4</sub> (3 ml) in a reaction flask, which was heated in an oil bath at 130° and irradiated with a lamp of 250 Watt under a continuous stream of dry chlorine. After 16 hrs, the mixture turned yellow, indicating completion of the chlorination reaction. The solvent was evaporated and the product distilled in a bulb-tube at 120-135°/0.1 mm Hg, affording crystalline (11)(4.07 g, 13 mmoles).

1-[<sup>14</sup>C]Trifluoromethyl-3-trifluoromethylbenzene (12)

The mixture of (11)(3.82 g, 12.2 mmoles) and SbF<sub>3</sub> (4.48 g, 25 mmoles) was heated quickly under an atmosphere of N<sub>2</sub> with a burner until the reaction started. After 15 sec.

the reaction mixture was cooled down. Using a vacuum manifold (10<sup>-2</sup> mm Hg), the product was lyophilized through a tube containing KOH into a trap chilled with liquid N<sub>2</sub>, yielding (12) (1.92 g, 8.95 mmoles).

1-[<sup>14</sup>C]Trifluoromethyl-3-trifluoromethyl-5-nitrobenzene (13)

To the reaction flask containing (12)(1.92 g, 8.95 mmoles) oleum (2.9 ml) containing 25% free SO<sub>3</sub> was added followed by 100% fuming HNO<sub>3</sub> (2.25 ml). Nitration was achieved by refluxing in an oil bath at 120° for 5 hrs. Into the cooled mixture ice was added and the product was extracted with CH<sub>2</sub>Cl<sub>2</sub>. After removal of the solvent, distillation in a bulb-tube at 120-135°/40 mm Hg provided (13)(1.32 g, 5.1 mmoles).

1-[<sup>14</sup>C]Trifluoromethyl-3-trifluoromethyl-5-aminobenzene (14)

The above nitro derivative (13)(1.32 g, 5.1 mmoles) was hydrogenated catalytically with Raney-Ni (100 mg) in ethanol (15 ml) at a temperature of 30-40°. After 20 hrs the uptake of H<sub>2</sub> ceased; the catalyst was removed by filtration, the ethanol evaporated and the remaining oil distilled in a bulb-tube at 110-125°/30 mm Hg. to yield the aniline (14) (1.09 g, 4.75 mmoles).

1-[<sup>14</sup>C]Trifluoromethyl-3-trifluoromethyl-5-hydrazinobenzene (15)

To the solution of (14)(1.09 g, 4.75 mmoles) in water (3 ml), chilled in an ice-bath, was added dropwise under vigorous stirring conc. HCl (4 ml) yielding a fine suspension. Within 20 min sodium nitrite (0.38 g, 5.5 mmoles) in water (0.5 ml) was added and the resulting yellow solution was stirred for 10 more min. At the temperature of 0° SnCl<sub>2</sub>.2H<sub>2</sub>O (3.21 g, 14.2 mmoles) in conc. HCl (4.8 ml) was added within 40 min and stirring was continued for 30 min. The precipitated slightly yellow crystals were separated by filtration and washed with conc. HCl (1 ml). The solid product was dissolved in water (5 ml) and filtered with charcoal. To the clear aqueous phase NaOH was added to make the solution slightly alkaline. The precipitated crystalline product was removed and dried under vacuum affording (15) (0.74 g, 3.03 mmoles).

1-{3-[<sup>14</sup>C]Trifluoromethyl-5-trifluoromethyl}phenyl-4-methyl-thiosemicarbazide (16)

The solution of (15) (0.54 g, 2.2 mmoles) in benzene (5 ml) was filtered with charcoal. To the clear filtrate, methyl-isothiocyanate (0.17 g, 2.3 mmoles) was added and the mixture stirred at 80° for 4 hrs. From the resulting solution,

upon addition of n-hexane (4 ml) and chilling in an ice-bath, the crystalline product precipitated. Removal by filtration and drying yielded pure (16)(0.564 g, 1.78 mmoles); specific activity 3.6  $\mu$ Ci/mg; radiochemical yield 5.1%; radiochemical purity >98%.

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