Journal of Labelled Compounds and Radiopharmaceuticals
J. Labelled Cpd. Radiopharm. **42**, 109-119 (1999)

SYNTHESIS OF [TRIFLUOROMETHYL-[$^{14}C_6$]-PHENYL]- SR 57746A

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SUMMARY

[Trifluoromethyl-[$^{14}C_6$]-phenyl]- SR 57746A **4**, a neurotrophic and neuroprotective compound, has been synthesized for metabolic and pharmacokinetic studies. The key step for its synthesis was the preparation of 3-trifluoromethyl-[$^{14}C_6$]-bromobenzene **1**. This compound was obtained in 4 steps from [$^{14}C_6$]-nitrobenzene **10** via [$^{14}C_6$]-3-iodo-nitrobenzene **11**, 3-trifluoromethyl-[$^{14}C_6$]-nitrobenzene **8**, 3-trifluoromethyl-[$^{14}C_6$]-aniline **9** . The overall yield for the 4 steps was 35%, the specific activity was 29 mCi/mmol and the radioactive purity was better than 98%. The Grignard reagent of **1** gave the title compound after condensation with N-(2-naphthylethyl)-4-piperidone **12** and dehydration.

Key words: trifluoromethyl-[$^{14}C_6$]-benzene, [$^{14}C_6$]-3-iodo-nitrobenzene, 3-bromo-trifluoromethyl-[$^{14}C_6$]-benzene.

INTRODUCTION

SR 57746A [C.A. nomenclature: pyridine, 1,2,3,6-tetrahydro-1-[2-(2-naphthalenyl)ethyl]-4-[3-(trifluoromethyl)-phenyl]-, hydrochloride [CAS Registry number: 90494-79-4] is a potent, orally active neurotrophic and neuroprotective compound under development by Sanofi Recherche. It is active *in vitro* and *in vivo* in a variety of animal models of central and peripheral neurodegenerative diseases (**1, 2**) making it of potential use for conditions such as amyotrophic lateral sclerosis (ALS), Alzheimer's disease (DAT), and peripheral neuropathies.

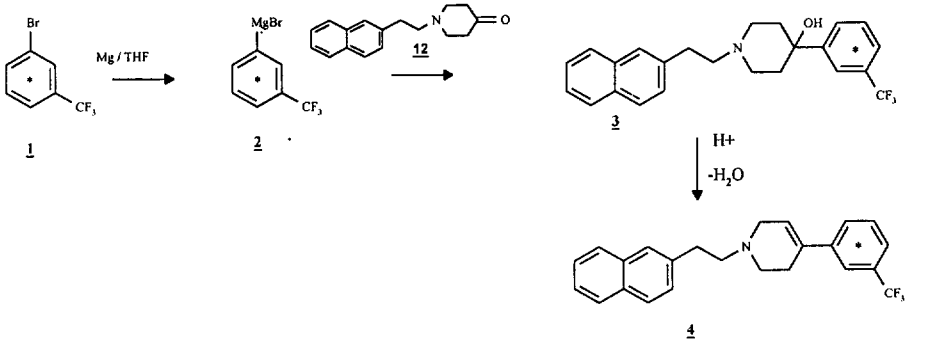
CCC 0362–4803/99/020109–11$17.50

Received 27 March 1998
Revised 3 August 1998
Accepted 4 August 1998

^{14}C-labelled SR 57746A was required for metabolism and pharmacokinetic studies. The synthesis of this compound is described within a program designed to provide a general method for preparing trifluoromethyl-[$^{14}C_6$]-aryl derivatives.

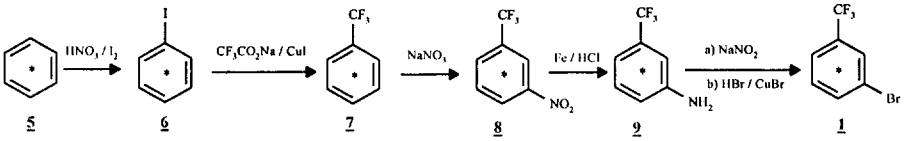
RESULTS AND DISCUSSION

Metabolic considerations led us to label SR 57746A with ^{14}C in its trifluoromethyl-[$^{14}C_6$]-phenyl moiety. Thus 3-trifluoromethyl-[$^{14}C_6$]-bromobenzene 1 became the key intermediate for this radioactive synthesis. (*Scheme 1*)



Scheme 1

Some 16 years ago one of us **(3)** described the synthesis of trifluoromethyl-[$^{14}C_6$]-benzene 7 by the trifluoromethylation of [$^{14}C_6$]-iodobenzene with CF_3I in DMF in a steel bomb under pressure. A good yield has been reported for the trifluoromethylation of iodoaryl compounds with iodotrifluoromethane **(4)**. However, an isotope effect was reported by Parker **(5)** in the radioactive synthesis of 4-chloro-trifluoromethyl-[$^{14}C_6$]-benzene. In order to circumvent the use of pressure with radioactive and volatile products and to avoid an isotope effect, we used (*Scheme 2*) the trifluoromethylation of aryl halides with a mixture of CF_3COONa / CuI in N-methylpyrrolidone at 160°C at atmospheric pressure **(6)**.

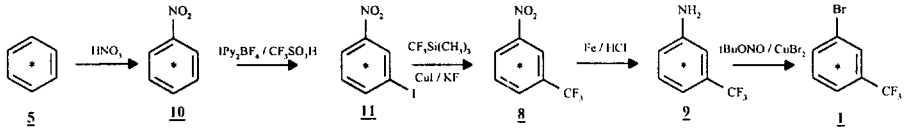


Scheme 2

[$^{14}C_6$]-Iodobenzene **6** was made according to (7) by iodination of [$^{14}C_6$]-benzene with a mixture of HNO_3 / I_2 (60% yield) and extracted from the reaction mixture using methylene chloride. Trifluoromethylation of **6** gave a mixture of three products analyzed by GC with an FID detector and a radioactive detector. This mixture consisted of 50% of unreacted [$^{14}C_6$]-iodobenzene, 30% of the required product and 20% of a radioactive impurity. The impurity, as analyzed by GC/MS, was found to be [$^{14}C_6$]-chlorobenzene. Coinjection with cold chlorobenzene gave only one peak by GC. This by-product probably arose from the traces of methylene chloride which were not completely removed from [$^{14}C_6$]-iodobenzene (see experimental). This is supported by the following experimental evidence.

Cold iodobenzene obtained from benzene under the same experimental conditions did not give chlorobenzene. In this cold synthesis, methylene chloride was probably completely removed. In the radioactive synthesis the distillation was stopped as soon as radioactive iodobenzene distilled. In fact, chlorobenzene was obtained in a cold synthesis when iodobenzene was heated in a mixture of N-methylpyrrolidone / methylene chloride (2:1) for 72 hours instead of 15 hours for [$^{14}C_6$]-iodobenzene. The formation of chlorobenzene seems to be slower in the cold synthesis than in ^{14}C synthesis in spite of the higher proportion of methylene chloride.

To avoid radioactive contamination during the purification of the mixture of these 3 products by liquid chromatography, we looked for a safer route using less volatile compounds.(*Scheme 3*)



Scheme 3

We used the meta orientation of a nitro group in an aromatic compound for electrophilic substitution to obtain [$^{14}C_6$]-3-nitro-iodobenzene **11** . The iodination of cold nitrobenzene was achieved with a mixture of bis-(pyridine)iodonium(I)tetrafluoroborate (IPy_2BF_4) and trifluorosulfonic acid in dry methylene chloride with the molar ratio nitrobenzene / IPy_2BF_4 / CF_3SO_3H = 1:3:6 (**8**). This reaction gave only 3-nitro-iodobenzene in 82% yield. In our hands **11** was obtained in 81% yield after liquid chromatography purification.

The trifluoromethylation of 4-iodo-nitrobenzene was reported (**9**) using trifluoromethyl-trimethylsilane ($CF_3Si(CH_3)_3$) / KF / CuI) in a sealed pyrex tube at 80°C. We modified this method and found that at atmospheric pressure and at 80°C 3-nitro-trifluoromethyl-[$^{14}C_6$]-benzene **8** was obtained in 71% yield after liquid chromatography.

Reduction of the nitro derivative with Fe / HCl gave 3-trifluoromethyl-[$^{14}C_6$]-aniline **9** in 78% yield. In order to circumvent the difficulties associated with the classical Sandmeyer reaction (**10**) to give 3-trifluoromethyl-[$^{14}C_6$]-bromobenzene **1** (diazotation of **9** followed by the addition of this diazonium salt to HBr / CuBr at 120°C, formation of by-products), we used deamination with *tert*-butyl nitrite and copper(II) bromide of 3-trifluoromethyl-[$^{14}C_6$]-aniline (**11**). **1** was obtained in one step at room temperature without by-products in 78% yield with a purity better than 98% by GC.

The Grignard reaction of **1** with magnesium followed by condensation with N-(β-naphthylethyl)piperidone **12** gave [trifluoromethyl-[$^{14}C_6$]-benzene]-SR 59322A **3** in 6.8% yield after HPLC purification. This poor yield based on **1** can be explained by the difficulty of synthesizing a Grignard reagent on a radioactive product and on a small scale. On the other hand the condensation of this ketone seems to be difficult: in cold synthesis the yield based on pure 3-trifluoromethyl-bromobenzene was 53% with 1.4 equivalents of Grignard product.

The dehydration of **1** under acidic conditions gave [trifluoromethyl-[$^{14}C_6$]-benzene] SR 59322A **4** in 82% yield after purification.

The condensation of the lithio derivative, obtained from **1** - by lithium / bromine exchange with an alkyl lithium- with the piperidone **12**, was not investigated.

EXPERIMENTAL

General Methods:

GC analyses were performed on a Varian 3300 instrument through a Carbowax 10 capillary column (30 m). A Raytest detector was used to detect radioactivity. MS/GC analyses were performed through a CP Sil 5 CB column (25 m). Radioactive TLCs were recorded on a Berthold model LB 511. The HPLC radioactive monitor was a Berthold model LB 503. ^1H NMR spectra were recorded at 300 MHz on a Brüker AM 400. A Wallac 1409 was used for liquid scintillation counting. Specific activities were determined by MS on a Finnigan MAT 4600 spectrometer.

[$^{14}C_6$]-Iodobenzene **6**:

990 MCi of [$^{14}C_6$]-benzene (SA = 40 mCi / mmol) were transferred under vacuum into a round bottom flask fitted with a condenser, containing 6 mL of HNO_3 (d = 1.43) and 3.4 g of twice sublimed iodine.

The reaction mixture was stirred under nitrogen at 90°C for 2 hours. The solution was allowed to reach 0°C and 15 mL of an NaOH solution (240 g/L) were added. After extraction with methylene chloride, 785 mCi of a mixture of [$^{14}C_6$]-benzene and [$^{14}C_6$]-iodobenzene (20:80) were obtained. [$^{14}C_6$]-Benzene was kept and eliminated subsequently. Methylene chloride was distilled at atmospheric pressure and the traces of water were eliminated by addition and distillation of 100 mL of dry methylene chloride.

GC: capillary column Supelcowax 10: temperature programming: 50°C for 2 min then 50°C to 200°C at 35°C/min, injector temperature = 150°C, detector temperature = 200°C. t_R (benzene) = 2.2 min; t_R (iodobenzene) = 5.8 min

Trifluoromethyl-[$^{14}C_6$]-benzene 7:

The mixture of [$^{14}C_6$]-iodobenzene and [$^{14}C_6$]-benzene (80:20) (785 mCi , 40 mCi/mmol), sodium trifluoroacetate (7.9 g) and copper iodide (5.6 g) in N-methylpyrrolidone (110 mL) were heated at 160°C under an argon atmosphere for 5 hours. The solution was extracted with a mixture of diethyl ether / pentane (1:1). The organic layer was washed twice with a saturated NaCl solution, twice with water, and was then dried over anhydrous sodium sulfate.

GC: capillary column Supelcowax 10: temperature programming: 50°C for 2 min then 50°C to 200°C at 35°C/min, injector temperature = 150°C, detector temperature = 200°C. t_R (benzene) = 2.2 min; t_R (trifluoromethylbenzene) = 2.5 min; t_R (chlorobenzene) = 4.3 min; t_R (iodobenzene) = 5.8 min .

[$^{14}C_6$]-3-Iodo-nitrobenzene 4:

IPy_2BF_4 was prepared according to **(12)**. [$^{14}C_6$]-nitrobenzene was made from [$^{14}C_6$]-benzene by nitration with HNO_3 **(13)**.

A: preparation from 214 mCi of [$^{14}C_6$]-nitrobenzene:

6.2 G of IPy_2BF_4 dissolved in 50 mL of dry methylene chloride were added slowly, and 5 g of CF_3SO_3H rapidly to [$^{14}C_6$]-nitrobenzene (214 mCi, 50 mCi/mmol) dissolved in 20 mL of dry methylene chloride in a two-necked flask, under nitrogen. The mixture was stirred at room temperature under nitrogen. The yield measured by GC after 15 hours of stirring was 20%.

For the unlabeled compound, the yield was 90% under the same experimental conditions.

890 mg of IPy_2BF_4 and 5 g of CF_3SO_3H were added to the solution and the mixture was stirred for 64 hours. The yield was 96% measured by GC.

The reaction mixture was treated with aqueous sodium thiosulfate, extracted with methylene chloride, treated with a NaOH solution (14 g/L) and washed with water. 190 MCi of crude [$^{14}C_6$]-3-iodo-nitrobenzene were obtained.

B: preparation from 484 mCi of [$^{14}C_6$]-nitrobenzene:

A second synthesis from 484 mCi of [$^{14}C_6$]-nitrobenzene under the same experimental conditions gave 450 mCi of crude [$^{14}C_6$]-3-iodo-nitrobenzene.

The two batches of [$^{14}C_6$]-3-iodo-nitrobenzene were both purified by liquid chromatography on a silicagel column (Lichroprep Si60 Merck 40-63 μm) with cyclohexane / methylene chloride (7:3). 565 MCi of pure [$^{14}C_6$]-3-iodo-nitrobenzene were obtained. Yield: 81%.

Radiochemical purity >99%

TLC: Silicagel 60 F_{254} Merck, cyclohexane / methylene chloride (7:3). Rf = 0.43

GC: capillary column Supelcowax 10, temperature programming: 80°C for 2 min and 80°C to 240°C at 35°C/min, temperature of injector: 200°C. temperature of detector: 250°C. t_R = 7.5 min

3-Trifluoromethyl-[$^{14}C_6$]-nitrobenzene 8:

20 ML of dry tetrahydrofuran (distilled over sodium/benzophenone) and 20 mL of dry 1-methylpyrrolidone were added to 565 mCi of [$^{14}C_6$]-3-iodo-nitrobenzene. The reaction mixture was stirred slowly at room temperature. 3.7 G of CuI, 850 mg of KF and 2.3 mL of trifluoromethyltrimethylsilane were added to this solution. The mixture was stirred at 80°C for 20 hours. The reaction mixture was allowed to reach room temperature, 100 mL of water were added and the solution was extracted with a mixture of ether/pentane (1:1). The organic layer was washed with water, N/1000 hydrochloric acid and saturated aqueous NaCl solution and twice with water. The crude product (505 mCi) containing 85% of 3-trifluoromethyl-[$^{14}C_6$]-nitrobenzene (determined by GC) was purified on a silicagel column (Lichroprep Si60 Merck 40-63 μm) with cyclohexane / methylene chloride (8:2).

400 MCi of 3-trifluoromethyl-[$^{14}C_6$]-nitrobenzene were obtained. Yield: 71%. Radiochemical purity: >99%

TLC: Silicagel 60 F_{254} Merck, cyclohexane / methylene chloride (8:2). Rf = 0.35

GC: capillary column Supelcowax 10, temperature programming: 80°C for 2 min and 80°C to 240°C at 35°C/min, temperature of injector: 200°C. temperature of detector: 250°C. t_R = 4.7 min.

3-Trifluoromethyl-[$^{14}C_6$]-aniline 9:

360 MCi of 3-trifluoromethyl-[$^{14}C_6$]-nitrobenzene, 2 g of iron powder and 6 mL of ethanol were stirred at room temperature and 30 mL of 1 N aqueous hydrochloric acid were added dropwise. The reaction mixture was stirred at room temperature for 4 hours and extracted twice with ether to remove the unreacted 3-trifluoromethyl-[$^{14}C_6$]-nitrobenzene. The aqueous layer containing 3-trifluoromethyl-[$^{14}C_6$]-aniline hydrochloride was made basic with 2 N sodium hydroxide solution and extracted twice with ether. The organic layer was washed with water. 280 MCi of 3-trifluoromethyl-[$^{14}C_6$]-aniline were obtained. Yield = 78%. Radiochemical purity >99%.

TLC: Silicagel 60 F_{254} Merck, cyclohexane / methylene chloride (8:2). Rf = 0.14

GC: capillary column Supelcowax 10, temperature programming: 80°C for 2 min and 80°C to 240°C at 35°C/min, temperature of injector: 200°C. temperature of detector: 250°C. t_R = 5.75 min.

3-Trifluoromethyl-[$^{14}C_6$]-bromobenzene 1:

1.8 G of anhydrous copper(II) bromide, 1.1 g of *tert*-butylnitrite and 15 mL of anhydrous acetonitrile were added to a two-necked flask fitted with an addition funnel and a gas outlet tube. The resulting mixture was cooled to 0°C. 280 MCi of 3-trifluoromethyl-[$^{14}C_6$]-aniline in 15 mL of dry acetonitrile were added over a period of 10 min to the reaction solution. The mixture was stirred at room temperature for 3 hours. The reaction solution was poured into 200 mL of 20% aqueous hydrochloric acid and extracted with 150 mL of ether. The organic layer was washed with 200 mL of 20% aqueous hydrochloric acid and three times with 100 mL of water. The resulting ether solution was dried over anhydrous sodium sulfate.

275 MCi of crude product were obtained: 93% purity. 900 Mg of cold 3-trifluoromethyl-bromobenzene were added to minimize distillation loses. The specific activity was 29 mCi / mmol. Ether was distilled under atmospheric pressure and the residua were transferred under vacuum at 60°C.

220 MCi of 3-trifluoromethyl-[$^{14}C_6$]-bromobenzene were obtained with a purity better than 98% by GC. Yield = 78%. Radiochemical purity >98%

GC: capillary column Supelcowax 10, temperature programming: 80°C for 2 min and then to 240°C at 35°C/min, temperature of injector: 150°C. temperature of detector: 250°C. t_R = 4.3 min.

[Trifluoromethyl-[$^{14}C_6$]-benzene]-SR 59322A 3:

Tetrahydrofuran was distilled over sodium and benzophenone.

To a stirred suspension of 440 mg (19.1 mmol) of magnesium in 3 mL of dry tetrahydrofuran, heated to reflux, were added slowly, under nitrogen, 220 mCi of 3-trifluoromethyl-[$^{14}C_6$]-bromobenzene, 0.5 mL of cold 3-trifluoromethyl-bromobenzene and 0.1 mL of 1,2-dibromoethane in 15 mL of dry tetrahydrofuran.

The mixture was stirred, under nitrogen, at 70 °C for 30 min. The temperature was then lowered to 0 °C and 2.6 g of N-(β-naphthylethyl)-4-piperidone (SR 59609: **12**) in 10 mL of dry tetrahydrofuran were added slowly. This solution was allowed to reach room temperature and was stirred for 2 hours. The reaction mixture was treated with an aqueous solution of ammonium chloride and extracted with ether.

After purification on a silicagel column (Merck Lichroprep Si60 40-63 μm) with chloroform / hexane / methanol (75 / 20 / 5), 30 mCi of [trifluoromethyl-[$^{14}C_6$]-benzene]-SR 59322A (76% purity) were obtained.

HPLC purification [column: Zorbax SB-C18; mobile phase: acetonitrile/water/triethylamine (70/30/0.1)] gave 15 mCi of **3**, 99% radiochemical purity, overall yield 6.8% based on **1**, specific radioactivity 19.6 mCi/mmol.

TLC: Silicagel Si60 Merck F_{254}, chloroform / hexane / methanol (75:20:5) Rf = 0.24

Silicagel Si60 Merck F_{254}, methylene chloride / acetone (30:70), Rf = 0.38

HPLC: column SB C18, acetonitrile / water / triethylamine (70:30:0.1), t_R = 6.24 min

[Trifluoromethyl-[$^{14}C_6$]-benzene]-SR 57746A 4:

6 ML of glacial acetic acid and 3 mL of 37% hydrochloric acid were added to a 100 mL flask containing [trifluoromethyl-[$^{14}C_6$]-benzene]-SR 59322A. This mixture reaction was stirred at 130 °C for 30 hours then cooled to 0 °C, neutralized with a 2 N sodium hydroxide and extracted with ether. The organic layer was washed with water.

After purification by HPLC [column: Zorbax SB-C18; mobile phase: acetone / water / triethylamine (70/ 30/ 0.1)], 13 mCi of 4 base form were obtained and precipitated with 60 µL of 37% hydrochloric acid. After filtration 12.3 mCi were obtained. Yield: 82%.

TLC: Silicagel Si60 Merck F_{254} , hexane / ethylacetate / triethylamine (65:35:1). Rf = 0.3

radiochemical purity: 98.9%

Silicagel KC18 Merck, methanol / water / triethylamine (95:5:1). Rf = 0.44

radiochemical purity: 99.6%

HPLC: Column Zorbax SB-C18 (250*4.6), acetonitrile / water / triethylamine (80:20:0.1)

t_R = 7.25 min. Radiochemical purity >99%

^{1}H NMR: 300 MHz in methanol d_4 (in agreement with the spectrum of an authentic sample)

Acknowledgments:

We thank H. Virelizier for the GC/MS analyses. We also thank Sanofi Recherche for supporting the work.

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