THE SYNTHESIS OF 4-(4-[^{11}C]METHOXYPHENYL)-(5-FLUORO-2-HYDROXYPHENYL)-METHYLENE-AMINOBUTYRIC ACID, AS A POTENTIAL RADIOLIGAND FOR THE GABA RECEPTOR IN THE BRAIN.

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SUMMARY

A procedure for the synthesis of 4-(4-[^{11}C]methoxyphenyl)-(5-fluoro-2-hydroxyphenyl)-methylene-aminobutyric acid has been developed. The production entailed a O-methylation of 5-fluoro-2-hydroxy-4'-hydroxybenzophenone with cyclotron produced [^{11}C]iodomethane in the presence of alkali and a subsequent Schiff reaction of 5-fluoro-2-hydroxy-4'-[^{11}C]methoxybenzophenone with γ-aminobutyric acid. 5-Fluoro-2-hydroxy-4'-hydroxybenzophenone was obtained by a demethylation of the 4'-methoxyderivative with boron tribromide. Subsequent purification by HPLC and sterilisation by filtration gave 740 MBq(20 mCi) of an injectable solution. The radiochemical yield (decay-corrected) from [^{11}C]iodomethane achieved 27%. The specific activity was 3.7 GBq/μmol(100 mCi/μmol) at the end of the radiosynthesis (45 min from EOB). The preparations have been demonstrated to be chemically and radiochemically pure by HPLC and TLC.

Key-words: GABA-receptor, O-methylation, Schiff reaction, ^{11}C, progabidic acid derivative.

INTRODUCTION

Positron Emission Tomography (PET) is widely used as a technique for studying neurotransmitters in the brain. Different neurotransmitter systems such as the dopamine, serotonine, benzodiazepine and muscarine receptors have already been investigated with PET (1). Up till now no PET studies of the γ-aminobutyric acid receptor have been reported. Nevertheless

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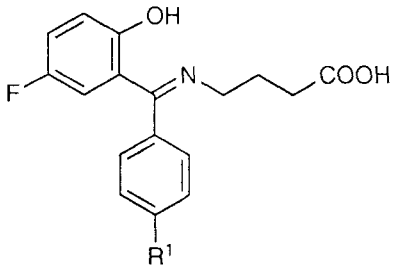
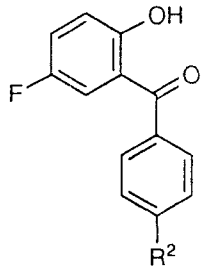
GABA seems to be involved in many neurological and psychiatric diseases such as Huntington's chorea, epilepsia and schizophrenia (2,3).

GABA has already been labelled with ^{11}C (4) and ^{13}N (5). The poor passage of GABA through the blood brain barrier makes this radioligand unattractive for PET imaging of the GABA receptor in the brain. A ligand that shows enough lipophilicity to penetrate into the blood brain barrier and that binds with great affinity to the GABA-receptor is necessary. Kaplan et al. (6) described the formation of GABA derivatives with an imine link (Schiff base) to a benzophenone molecule. These molecules show low toxicity. In vitro experiments show displacement of [^{3}H]GABA from the GABA-receptor. Moreover they have a satisfactory lipophilicity to pass the blood brain barrier (7,8). SL 75 102 or progabidic acid (4-(4-chlorophenyl)-(5-fluoro-2-hydroxyphenyl)-methylene-aminobutyric acid(**1**) (9) is an example of such a molecule.
This paper describes the radiosynthesis of a derivative namely 4-(4-[^{11}C]methoxyphenyl)-(5-fluoro-2-hydroxyphenyl)-methylene-aminobutyric acid ([^{11}C]methoxyprogabidic acid) (**2**).





1; R^1 = Cl	progabidic acid
2; R^1 = $O^{11}CH_3$	[^{11}C]methoxyprogabidic acid
3; R^1 = OCH_3	methoxyprogabidic acid
4; R^1 = OH	hydroxyprogabidic acid
5; R^2 = OCH_3	5-fluoro-2-hydroxy-4'-methoxybenzophenone
6; R^2 = $O^{11}CH_3$	5-fluoro-2-hydroxy-4'-[^{11}C]methoxybenzophenone
7; R^2 = OH	5-fluoro-2-hydroxy-4'-hydroxybenzophenone

In a first reaction 5-fluoro-2-hydroxy-4'-hydroxybenzophenone (**7**) was converted to 5-fluoro-2-hydroxy-4'-[^{11}C]methoxybenzophenone(**5**) by a O-methylation with [^{11}C]CH_3I. A subsequent

Schiff reaction with an excess of γ-aminobutyric acid in the presence of sodium methoxide as catalyst resulted in [^{11}C]methoxyprogabidic acid (**2**).

EXPERIMENTAL AND RESULTS

Materials

p-Methoxybenzoylchloride, p-fluorophenol, triethylamine, boron tribromide, titanium chloride, sodium methoxide and γ-aminobutyric acid were purchased from Janssen Chimica. All other chemicals were of either 'HPLC' or 'pro analyse' grade and obtained from Janssen Chimica, UCB and Analar.

Preparation of 4-methoxybenzoic acid-4-fluorophenyl ester

70 mmol (7.85g) p-methoxybenzoylchloride, dissolved in dry dichloromethane, was added dropwise to a stirred solution of p-fluorophenol (70 mmol, 11.94g) and triethylamine (86 mmol, 12 ml) in dry dichloromethane. The mixture was refluxed for 1 h. After cooling the reaction mixture was extracted with 1 M NaOH, 1 M HCl and water, dried over $MgSO_4$ and evaporated under reduced pressure. The obtained residue was recrystallized from petroleum ether (bp 40°-60°) to give 14.1 g (η = 81%) of white crystals. The purity is confirmed by TLC (Silicagel, Polygram SILG/UV 254; elution: carbon tetrachloride: piperidine: ethylacetate (4:1:1 v/v). Only one spot could be observed under UV irradiation (Rf 0.8).
mp: 65-66°C, ^{1}**H NMR**: (CD_3OD) δ=8.1 (m,2H,aryl); 7.1-7.0 (m,6H, aryl); 3.9 (s,3H,OCH_3) **MS**: m/z 246 ($[M]^+$,5%); 135 ($[CH_3OPhC{=}O]^+$,100%); 108 ($[CH_3OPh]^+$,10%).

Preparation of 5-fluoro-2-hydroxy-4'-methoxybenzophenone (5)

20 mmol (4.92g) 4-methoxybenzoic acid-4-fluorophenyl ester was melted in a round bottom flask on an oil bath at 120°C. 34 mmol (3800μl) $TiCl_4$ was added dropwise. The temperature was increased to 160°C and the reaction mixture was heated for 20 min. After cooling the mixture was extracted with diethyl ether/water. The organic layer was separated, dried over $MgSO_4$ and evaporated under reduced pressure. TLC of the resulting brown oil (eluent carbon tetrachloride :piperidine: ethylacetate (4:1:1 v/v)) showed two spots (Rf 0.8: starting product and Rf 0.42: reaction product). A pure sample was obtained by column chromatography (3cm i.d., 30 cm height) with silicagel (0.05 - 0.2 mm) using carbon tetrachloride:

piperidine: ethylacetate (4:1:1 v/v) as eluent. The collected fraction was evaporated under reduced pressure and recrystallized from methanol/water to give 2.41 g (η=49%) of a yellow powder.

mp: 79.5 °C, **^{1}H NMR:** (CD_3OD) δ=7.7 (m,2H,aryl); 7.2-7.1 (m,4H, aryl); 7.0 (m,1H,aryl); 3.9 (s,3H,O-CH_3) **MS:** m/z 246 ($[M]^+$,30%); 139 ($[M-PhOCH_3]^+$,16%); 135 ($[CH_3OPhC{=}O]^+$,28%); 108 ($[PhOCH_3]^+$,100%).

Preparation of 5-fluoro-2-hydroxy-4'-hydroxybenzophenone (7)

0.32 mmol (79 mg) 5-fluoro-2-hydroxy-4'-methoxybenzophenone (5) was stirred under nitrogen in a small conical reaction vial with an excess of boron tribromide (1.6 mmol, 250 μl) at -70°C for 2 min. The reaction mixture was brought to 0°C and allowed to react for 1 h. 600 μl 1 M HCl was added dropwise with a syringe. The solution is extracted twice with dichloromethane. The organic phase is washed with water and extracted with 1 M NaOH. The aqueous layer is acidified with HCl and extracted with dichloromethane. The organic layer is dried over $MgSO_4$ and evaporated under reduced pressure. 35 mg (η= 47%) of a yellow powder were obtained.

The purity of the product is determined by HPLC (column: Spherisorb 5 μm, 125 mm x 4mm, Merck; mobile phase: McIlvaine phosphatebuffer pH 6.0:methanol (35:65 v/v); flow 1 ml/min; detection: UV 255 nm). Only one peak with a retention time of 3.5 min was observed. No peak for 5-fluoro-2-hydroxy-4'-methoxybenzophenone (5) was detected.

mp: 104-105°C **^{1}H NMR:** (CD_3OD) δ=7.7 (m,2H,aryl); 7.2 (m,2H,aryl); 7.0 (m,1H,aryl); 6.9 (m,2H,aryl) **MS:** m/z 232 ($[M]^+$,49%); 139 ($[M-PhOH]^+$,100%); 121 ($[HOPhC{=}O]^+$,61%).

Preparation of methoxyprogabidic acid (3)

2 mmol (498 mg) of 5-fluoro-2-hydroxy-4'-methoxybenzophenone (5), 6 mmol of γ-aminobutyric acid (619 mg) and 12 mmol (648 mg) of sodium methoxide were dissolved in methanol. The resulting solution was evaporated to dryness at 120°C under a slow stream of nitrogen. The yellow residue was taken up in 0.1 M citric acid and extracted with dichloromethane. The organic layer was separated, dried over $MgSO_4$ and evaporated under reduced pressure. Subsequently the residue was dissolved in a minimal amount of chloroform and transferred on a silica column (silicagel 0.05-0.2 mm, 3 cm ID, 30 cm height).

The impurities were washed off with 300 ml chloroform. Finally the product was eluted with 200 ml methanol. Evaporation under reduced pressure gave 360 mg (η= 54%). Purity was confirmed by TLC (Silicagel, Polygram SILG/UV 254; elution dichloromethane:methanol:water:acetic acid (97:2.5:0.15:0.3 v/v). Only one spot could be observed under UV irradiation (Rf: 0.28).

mp: 113°C **^{1}H NMR:** (CD_3OD) δ=7.2 (m,2H,aryl); 7.1 (m,2H,aryl); 7.1-7.0(m,1H,aryl); 6.8 (m,1H,aryl); 6.5 (m,2H,aryl); 4.9 (s,OH); 3.9(s,3H,OCH_3); 3.4(t,2H,-NCH_2-); 2.5 (t,2H,$\underline{CH_2}COOH$); 1.9 (m,2H,-$CH_2\underline{CH_2}CH_2$-) **MS:** m/z 331 ([M]$^+$,5%); 272 ([M -CH_2-COOH]$^+$,4%); 246([OHFPhC=OPhOCH_3]$^+$,26%); 139([OHFPhC=O]$^+$, 50%); 135 ([CH_3OPhC=O]$^+$,37%); 108 ([PhOCH_3]$^+$,100%).

Preparation of [^{11}C]CH_3I

The set up is given in Figure 1 . Protons of 18 MeV with a beam intensity of 15 μA are used in a ^{14}N (p,α)^{11}C reaction. The target was irradiated for 20 min. The produced [^{11}C]CO_2 was trapped in a copper coil cooled with liquid argon. After complete trapping the copper coil was heated by a stream of hot air. The [^{11}C]CO_2 was swept through 200 μl 0.5 M $LiAlH_4$ in tetrahydrofuran (THF) by a flow of nitrogen at room temperature. After evaporation of THF (160°C) the [^{11}C]methanolate

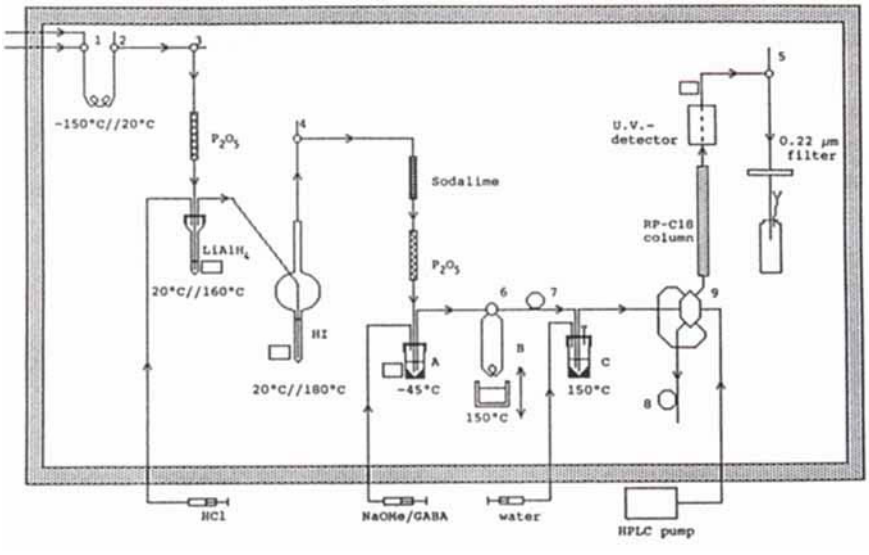


Figure 1: Set up of the apparatus: GM-tubes; 1-5: electromagnetic 3-way valves; 6: electromagnetic 4-way valve; 7-8: peristaltic pumps 9: 6-way injection valve.

was hydrolysed with 100 µl 12 M HCl. The [^{11}C]CH_3OH was distilled into 1 ml of HI. All steps were remote and microprocessor controlled.

Preparation of [^{11}C]methoxyprogabidic acid (2)

[^{11}C]CH_3I was transferred by nitrogen gas (flow rate 20 ml/min) from the HI vial by increasing the temperature to 180°C, into a 2 ml conical reaction vial (vial A) cooled at -45°C and containing a mixture of 3 µmol 5-fluoro-2-hydroxy-4'-hydroxybenzophenone (7) in 150 µl dimethylformamide and 2.5 µl 0.8M NaOH in water. After complete transfer of [^{11}C]CH_3I (monitored by a GM-tube) the mixture was transferred with the aid of a peristaltic pump to a reaction loop B. The loop was closed by means of valve 6 and heated at 150°C for 5 min. After cooling the content of the reaction loop was pumped into reaction vessel C. 9 µMol γ-aminobutyric acid and 9 µmol sodium methoxide were added. The mixture was allowed to react at 150°C for 8 min. The residue was diluted to 250 µl with water.

The resulting solution was injected on the HPLC system. The column (Spherisorb 10 µm, 250 mm x 9.2 mm) was eluted with a mixture of phosphate buffer 33 mM: sodium chloride 0.1 M: ethanol (45:15:40 v/v) at a flow rate of 4 ml/min. The eluate was simultaneously monitored with a UV-detector (Pye Unicam LK 3) set at 254 nm and a GM-tube. A typical chromatogram obtained from a production is shown in Figure 2. By means of the electromagnetic valve 5 the fraction containing the [^{11}C]methoxyprogabidic acid (2) was isolated from the reaction mixture and filtered through a 0.22 µm sterile Acrodisc filter into a sterile and pyrogen free vial. 5ml of sterile bidistilled water was added so that a final concentration of 20 % ethanol was obtained.
740 MBq(20 mCi) of radioactive product was obtained. The radiochemical yield (decay-corrected) averaged 17% (from cyclotron produced [^{11}C]CO_2) and 27% (from [^{11}C]CH_3I). The total preparation time was about 45 min. All steps were remote controlled.

Analysis and quality control of [^{11}C]methoxyprogabidic acid (2)

The radioachemical purity of the synthesized ^{11}C-product was controlled by HPLC and TLC. The HPLC system consisted of a Spherisorb RP-C18 column (Merck, 125mm x 4 mm) eluted with a

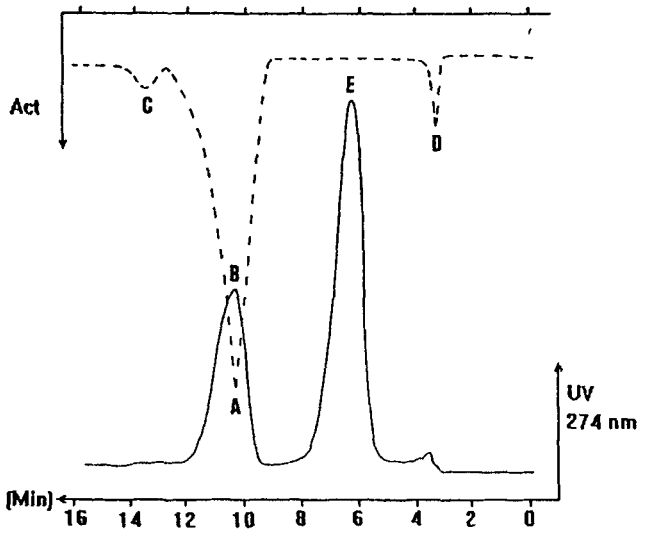


Figure 2: Typical chromatogram of on-line product purification in the synthesis of the labelled compound. **A** is [^{11}C]methoxyprogabidic acid (**2**), **B** methoxyprogabid acid (**3**), **C** is a unknown sideproduct, **D** is unreacted [^{11}C]CH_3I and **E** is hydroxyprogabidic acid (**4**).

McIlvaine phosphate buffer pH 6.5: methanol (48:52 v/v) mixture. Detection was achieved with a UV-detector set at 255 and 220 nm (chemical purity and determination of the specific activity) and a NaI(Tl) detector (radiochemical purity). The system for the TLC is described under the preparation of methoxyprogabidic acid (**3**).

The HPLC system shows one single peak for both the UV- and radiochromatogram with the same retention time as for reference methoxyprogabidic acid (**3**). The radioactive product and reference have the same Rf-value on TLC. No chemical impurities were detected. The specific activity was found to be 3.7 GBq/μmol(100 mCi/μmol).

DISCUSSION

[^{11}C]Methoxyprogabidic acid (**2**) was synthesized in a two step reaction, as shown in figure 3. The label in the benzophenone moiety has the advantage that no radioactivity will be excreted as $^{11}CO_2$, as observed with 4-(4-methoxy-phenyl)-(5-fluoro-2-hydroxyphenyl)-methylene-[^{14}C]butamide by Allen et al. (10).

As 5-fluoro-2-hydroxy-4'-hydroxybenzophenone (**7**) is not commercially available, it had to be synthesised from 5-fluoro-2-hydroxy-4'-methoxybenzophenone (**5**) by a demethylation

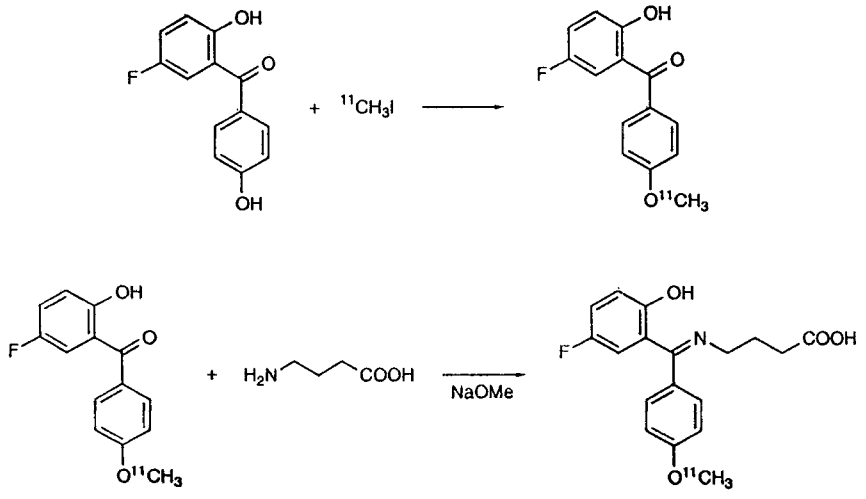


Figure 3: Synthesis of [^{11}C]methoxyprogabidic acid (**2**)

reaction. Different reagents such as piperidine, trifluoromethylsilane and boron tribromide could be used to demethylate phenolic ethers (11-14). In our case the best results were obtained with boron tribromide. A 5-fold excess of reagent to the amount of 5-fluoro-2-hydroxy-4'-methoxybenzophenone (**5**) is used to obtain a sufficient yield (η= 47%).

5-Fluoro-2-hydroxy-4'-methoxybenzophone (**5**) was produced by an esterification between p-fluorophenol and p-methoxybenzoylchloride, followed by a Fries rearrangement with titanium chloride (15).

[^{11}C]CH_3I is a widely used reagent for the methylation of primary and secondary amino groups. On the other hand, O-methylations with [^{11}C]CH_3I are limited to a small number of examples (16-19). These reactions are examples of the Williamson synthesis. Phenoxide ions, produced from phenols in the presence of alkali, react with alkylhalides to form unsymmetric ethers. This nucleophilic reaction is promoted by the use of a dipolar aprotic solvent such as dimethylformamide (DMF) or dimethylsulphoxide (DMSO) (20). Under these conditions the solvation of the phenoxide ion is discourraged so that carbon alkylation in ortho or para positions of the phenol does not occur. We preferred DMF because of its lower freezing point, in order to trap the [^{11}C]CH_3I at low temperature. The [^{11}C]CH_3I trapping was almost complete at -45°C.

For the methylation step, the reaction mixture was transferred to a reaction coil B which could be closed hermetically. These conditions were necessary because otherwise a large amount of the $[^{11}C]CH_3I$ is lost by evaporation during the reaction due to the high temperatures necessary for the methylation (19).

$[^{11}C]$Methoxyprogabidic acid is purified by reverse phase HPLC. The method of Decourt et al.(21) is modified and extended to a preparative scale. The deliverence under an appropriate pharmaceutical preparation is limited to a simple dilution with bidistilled water.

Quality control was done by HPLC (combined UV and NaI(Tl) detection) and TLC (UV irradiation). For checking the chemical purity by HPLC, two different wavelengths were used. At 220 nm the absence of DMF was controlled. The amount was found to be lower than the detection limit (1μg/ml). 255 nm was the wavelength for the detection of benzophenone containing molecules. No peak other than the desired product was observed. A linear correlation between the peak area and the concentration of methoxyprogabidic acid (**3**) was found at 255 nm for concentrations from 3 to 100 μg/ml.

The obtained specific activity of 3.7 GBq/μmol(100 mCi/μmol) (66 μg of carrier) is high enough for receptor studies in the brain. The radiochromatogram showed only one single peak with the same retention time as reference methoxyprogabidic acid (**3**).

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