SYNTHESIS OF (*S*)-*N*-(*endo*-8-METHYL-8-AZABICYCLO[3.2.1] OCT-3α-YL)-4-AMINO-5-CHLORO-2-(1-METHYL-2-BUTYNYLOXY) [*CARBONYL*-^{14}C]BENZAMIDE MONOHYDROCHLORIDE (^{14}C-E3620)

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Summary

(*S*)-*N*-(*endo*-8-Methyl-8-azabicyclo[3.2.1]oct-3α-yl)-4-amino-5-chloro-2-(1-methyl-2-butynyloxy)[*carbonyl*-^{14}C]benzamide monohydrochloride (^{14}C-E3620, **1 1**), which was required for a study of the pharmacokinetic profile of E3620, is not only a potent antagonist of 5-HT$_3$ receptors but also a potent agonist of 5-HT$_4$ receptors. It was synthesized using (*S*)-5-chloro-4-(2,5-dimethylpyrrol-1-yl)-2-(1-methyl-2-butynyloxy)[*carboxy*-^{14}C]benzoic acid (**8**) as the labelled starting material.
The product was produced at high radiochemical purity with a specific activity of 2.02GBq / mmol.

Key words: 5-HT$_3$ receptor antagonist, 5-HT$_4$ receptor agonist, ^{14}C-E3620

Introduction

(-)-(*S*)-*N*-(*endo*-8-Methyl-8-azabicyclo[3.2.1]oct-3α-yl)-4-amino-5-chloro-2-(1-methyl-2-butynyloxy)benzamide monohydrochloride dihydrate (E3620, **1**) has been developed in our laboratories as not only a 5-HT$_3$ receptor antagonist but also a 5-HT$_4$ receptor agonist[1]. Clinical evaluation of **1** are ongoing, it shows a very potent gastrointestinal motor activity, and antiemesis. In this paper, we describe the synthesis of ^{14}C-labelled E3620. This synthesis was carried out to study the pharmacokinetic profile of E3620.

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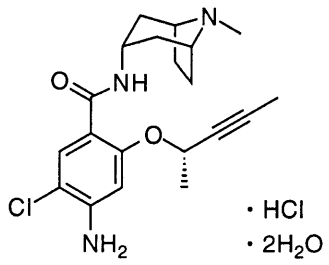

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Results and discussion

^{14}C-E3620 (**1** 1) was prepared from (*S*)-5-chloro-4-(2,5-dimethylpyrrol-1-yl)-2-(1-methyl-2-butynyloxy)[*carboxy*-^{14}C]benzoic acid (**8**) in three steps. The required unlabelled bromide **7** was prepared from 2-chloro-5-methoxyacetanilide (**2**) in five steps.The synthetic pathway is shown in Figure 1.

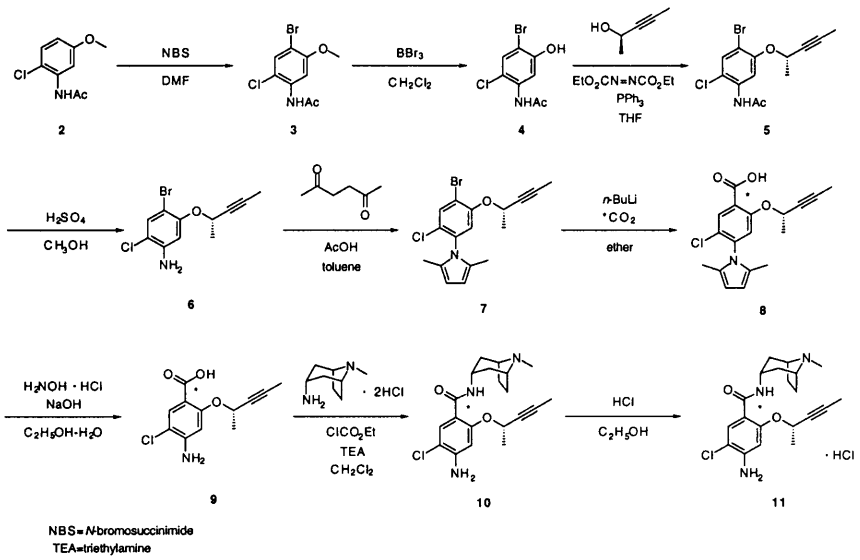

Figure 1. Synthesis of (*S*)-*N*-(*endo*-8-Methyl-8-azabicyclo[3.2.1]oct-3α-yl)-4-amino-5-chloro-2-(1-methyl-2-butynyloxy)[*carbonyl*-^{14}C]benzamide monohydrochloride (^{14}C-E3620)

The treatment of **2** with *N*-bromosuccinimide afforded the corresponding bromide **3**. The demethylation of **3** with boron tribromide furnished **4** in good yield. Compound **4** was transformed into the alkynyl ether **5** using the Mitsunobu reaction[2]. The hydrolysis of **5** with conc.H_2SO_4 gave **6**, which was then protected with acetonylacetone[3] and transformed into **7**. The

carboxylation of **7** with ^{14}C-labelled CO_2 and *n*-butyllithium[4] afforded the key intermediate **8**, which was then converted to **9** by deprotection with hydroxylamine hydrochloride[3]. The reaction of **9** with *endo*-3-aminotropane[5] in the presence of ethyl chloroformate as a condensing agent afforded **10**. Finally, the treatment of **10** with 1N aqueous HCl afforded the desired ^{14}C-E3620 (**11**). The structure of **11** was confirmed by comparison (TLC and HPLC) with an authentic unlabelled sample of E3620. Purified **11** had 100% radiochemical purity by HPLC and a specific activity of 2.02GBq / mmol.

Experimental

General Method Reagents and solvents were purchased from usual commercial sources. Silicagel (Kieselgel 60, Merck) was used for column chromatography. Thin layer chromatography was carried out using Kieselgel 60 F_{254} plate (Merck). All organic extracts were dried over anhydrous $MgSO_4$, and the solvent was removed with a rotary evaporator under reduced pressure. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a Varian Unity 400 (400 MHz) spectorometer, and chemical shifts are expressed in ppm downfield from tetramethylsilane (TMS) as an internal reference. Abbreviations are as follows; s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br., broad peak. Measurements of radioactivity were carried out using an Aloka LSC-9000 type Liquid Scintillation Spectrometer.

4-Bromo-2-chloro-5-methoxyacetanilide (3)

To a cooled (ice-bath) solution of 2-chloro-5-methoxyacetanilide (**2**, 2.0g, 10mmol) in DMF (25mL) was added *N*-bromosuccinimide (1.8g, 10mmol). The resulting solution was stirred at 0℃ for 0.5h. The mixture was stirred at room temperature overnight, poured into saturated aqueous $NaHCO_3$, and extracted with ethyl acetate. The extract was washed twice with brine. The solvent was evaporated to give **3** (2.22g, 80%) as white crystals.
^{1}H-NMR (400MHz, $CDCl_3$) δ; 2.25 (s, 3H), 3.90 (s, 3H), 7.51 (s, 1H), 7.59 (br.s, 1H), 8.20 (s, 1H) ppm.

4-Bromo-2-chloro-5-hydroxyacetanilide (4)

To a cooled (ice-bath) solution of **3** (2.22g, 8mmol) in CH_2Cl_2 (50mL) was added boron tribromide (1.0M in CH_2Cl_2, 42mL, 42mmol). The mixture was stirred at room temperature for 1.5h and poured into water. The precipitate was collected by filtration. The filtrate was extracted with CH_2Cl_2. The precipitate was dissolved in 1N aqueous NaOH, acidified with 1N aqueous HCl, neutralized with saturated aqueous $NaHCO_3$, and then extracted with ethyl acetate. The ethyl acetate layer and the CH_2Cl_2 layer were combined and concentrated to give **4** (1.82g, 86%) as colorless crystals.
^{1}H-NMR (400MHz, $CDCl_3$+DMSO-d_6) δ; 2.15 (s, 3H), 7.38 (s, 1H), 7.69 (br.s, 1H), 8.05 (s, 1H), 9.56 (s, 1H) ppm.

(*S*)-4-Bromo-2-chloro-5-(1-methyl-2-butynyloxy)acetanilide (5)

To a solution of triphenyphosphine (10g, 41mmol) in THF (200mL) was added diethyl azodicarboxylate (7.0mL, 41mmol), *(R)*-3-butyn-2-ol[6] (3.5g, 41mmol), and a solution of **4** (11.9g, 45mmol) in a small amount of THF at -70℃ under nitrogen. The mixture was stirred at room temperature overnight and evaporated. The residue was purified by silicagel column chromatography (*n*-hexane / ethyl acetate 95:5~85:15) to give **5** (7.7g, 57%) as white crystals.
^{1}H-NMR (400MHz, $CDCl_3$) δ; 1.66 (d, J = 8Hz, 3H), 1.84 (d, J = 2Hz, 3H), 2.26 (s, 3 H), 4.87 (m, 1H), 7.51 (s, 1H), 7.54 (br.s, 1H), 8.36 (s, 1H) ppm.

(*S*)-4-Bromo-2-chloro-5-(1-methyl-2-butynyloxy)aniline (6)

To a suspension of **5** (7.7g, 23mmol) in CH_3OH (200mL) was added conc.H_2SO_4 (15mL) at 0℃. The resulting solution was stirred at room temperature overnight. The reaction mixture was neutralized with saturated aqueous $NaHCO_3$, and then extracted twice with ethyl acetate. The solvent was evaporated to give **6** (6.8g, 100%) as white crystals.
^{1}H-NMR (400MHz, $CDCl_3$) δ; 1.64 (d, J = 7Hz, 3H), 1.83 (d, J = 2Hz, 3H), 4.05 (br.s, 2H), 4.72 (m, 1H), 6.56 (s, 1H), 7.37(s, 1H) ppm.

(*S*)-1-[4-Bromo-2-chloro-5-(1-methyl-2-butynyloxy)phenyl]-2,5-dimethylpyrrole (7)

The mixture of **6** (39g, 135mmol), acetonylacetone (77g, 675mmol), acetic acid (50mL), and toluene (500mL) was heated to reflux with a Dean-Stark trap for 5h and then cooled to room temperature. The reaction mixture was poured into saturated aqueous $NaHCO_3$ and extracted with ethyl acetate. The extract was washed with brine. The solvent was evaporated off. The residue was purified by silicagel column chromatography (*n*-hexane / ethyl acetate 95:5~90:10). After removal of the solvent, the residue was treated with *n*-hexane to give **7** (44.7g, 90%) as colorless crystals.
^{1}H-NMR (400MHz, $CDCl_3$) δ; 1.64 (d, J = 8Hz, 3H), 1.76 (d, J = 2Hz, 3H), 1.98 (d, J = 5Hz, 6H), 4.79 (m, 1H), 5.93 (s, 2H), 7.05 (s, 1H), 7.72 (s, 1H) ppm.

(*S*)-5-Chloro-4-(2,5-dimethylpyrrol-1-yl)-2-(1-methyl-2-butynyloxy) [*carboxy*-^{14}C]benzoic acid (8)

The key intermediate **8**, which was prepared from **7** and ^{14}C-CO_2, was purchased from Amersham International Ltd. : Specific activity; 2.1GBq / mmol: Radiochemical purity by HPLC 98.3%. HPLC conditions: Spherisorb ODS2 150mm x 4.6mm, mobile phase H_2O / CH_3CN 50:50~0:100 gradient over 10 min, flow rate 1mL / min, detector UV240nm.

(*S*)- 4-Amino-5-chloro-2-(1-methyl-2-butynyloxy)[*carboxy*-^{14}C] benzoic acid (9)

A solution of **8** (0.9g, 2.68mmol, 5.62GBq), H_2NOH•HCl (12.85g, 185mmol),

and KOH (7.4g, 112mmol) in C_2H_5OH-H_2O (8:3, 142mL) was heated to reflux for 23h under nitrogen and then cooled to room temperature. The reaction mixture was poured into 1N aqueous HCl and extracted three times with $CHCl_3$. The combined organic layer was evaporated. The residue was purified by silicagel column chromatography (*n*-hexane / ethyl acetate 90:10~70:30) to give **9** (0.35g, 2.84GBq) as white crystals.

(*S*)-*N*-(*endo*-8-Methyl-8-azabicyclo[3.2.1]oct-3α-yl)-4-amino-5-chloro-2-(1-methyl-2-butynyloxy)[*carbonyl*-^{14}C]benzamide (10)

To a solution of **9** (0.35g, 1.37mmol, 2.84GBq) and triethylamine (0.35g, 3.5mmol) in CH_2Cl_2 (40mL) was added ethyl chloroformate (0.22g, 2mmol) at 0℃ under nitrogen. After the solution was stirred at room temperature for 15min, *endo*-3-aminotropane dihydrochloride (0.32g, 1.5mmol) was added. The reaction mixture was stirred at room temperature for 3h, poured into saturated aqueous $NaHCO_3$, and extracted four times with CH_2Cl_2. The combined organic layer was evaporated. The residue was purified by silicagel column chromatography (CH_2Cl_2 / CH_3OH 100:0~75:25) and two kinds of preparative HPLCs. Preparative HPLC conditions: DAISEL CHIRALCEL OD 250mm x 20mm, mobile phase *n*-hexane / C_2H_5OH / triethylamine 80:20:0.1, flow rate 10mL / min, detector UV275nm: Merck LiChrosphar RP-select B 250mm x 10mm, mobile phase CH_3CN / H_2O / $HClO_4$ 25:75:0.1, flow rate 6mL / min, detector UV275 nm. The eluent was evaporated to remove CH_3CN, the pH adjusted to 14 with 5N aqueous NaOH, and extracted four times with $CHCl_3$. The solvent was evaporated to give **10** (0.315g, 1.72GBq) as a colorless viscous oil.

(*S*)-*N*-(*endo*-8-Methyl-8-azabicyclo[3.2.1]oct-3α-yl)-4-amino-5-chloro-2-(1-methyl-2-butynyloxy)[*carbonyl*-^{14}C]benzamide monohydrochloride (^{14}C-E3620 , 11)

To a solution of **10** (0.315g, 0.83mmol, 1.72GBq) in C_2H_5OH (100mL) was added 1N aqueous HCl (0.83mL) to give a solution of **11** in C_2H_5OH: Specific activity; 2.02GBq / mmol: Radiochemical purity by HPLC 100%. HPLC conditions: Merck LiChrosphar RP-select B 250mm x 4.0mm, mobile phase CH_3CN / H_2O / $HClO_4$ 300:700:2, flow rate 1mL / min, RI detector: Optical purity by HPLC 100%. HPLC conditions: DAISEL CHIRALCEL ODR 250mm x 4.6mm, mobile phase CH_3CN / H_2O / $HClO_4$ 300:700:2, flow rate 0.5mL / min, RI detector.

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