

**SYNTHESIS OF (S)-N-(endo-8-METHYL-8-AZABICYCLO[3.2.1]OCT-3 $\alpha$ -YL)-4-AMINO-5-CHLORO-2-(1-METHYL-2-BUTYNYLOXY)[CARBONYL-<sup>14</sup>C]BENZAMIDE MONOHYDROCHLORIDE (<sup>14</sup>C-E3620)**

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**Summary**

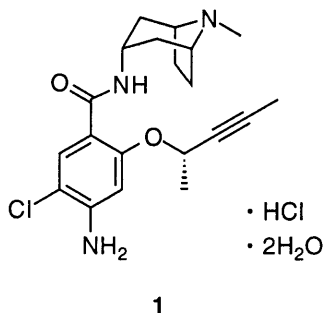
(S)-N-(endo-8-Methyl-8-azabicyclo[3.2.1]oct-3 $\alpha$ -yl)-4-amino-5-chloro-2-(1-methyl-2-butyntyloxy)[carbonyl-<sup>14</sup>C]benzamide monohydrochloride (<sup>14</sup>C-E3620, **1**), which was required for a study of the pharmacokinetic profile of E3620, is not only a potent antagonist of 5-HT<sub>3</sub> receptors but also a potent agonist of 5-HT<sub>4</sub> receptors. It was synthesized using (S)-5-chloro-4-(2,5-dimethylpyrrol-1-yl)-2-(1-methyl-2-butyntyloxy)[carboxy-<sup>14</sup>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<sub>3</sub> receptor antagonist, 5-HT<sub>4</sub> receptor agonist, <sup>14</sup>C-E3620

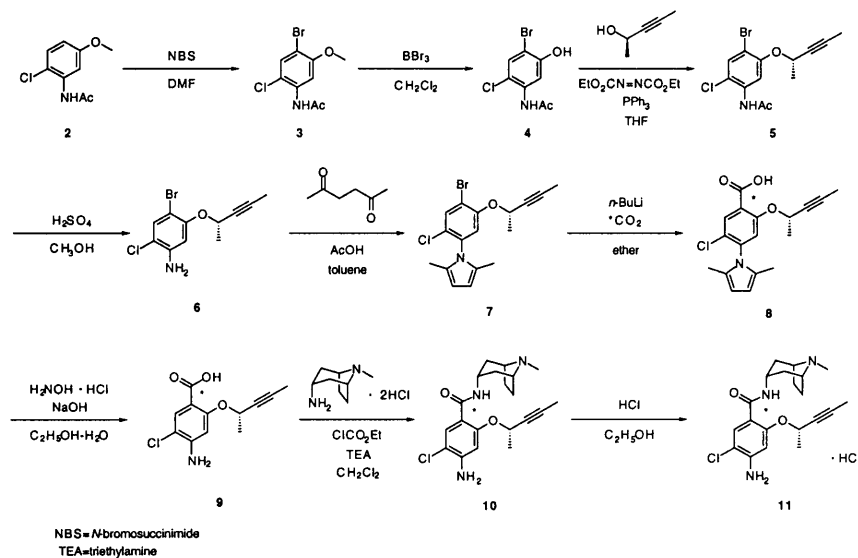
**Introduction**

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



## Results and discussion

$^{14}\text{C}$ -E3620 (**1**) was prepared from (*S*)-5-chloro-4-(2,5-dimethylpyrrol-1-yl)-2-(1-methyl-2-butynyloxy)[*carboxy*- $^{14}\text{C}$ ]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-3-yl)-4-amino-5-chloro-2-(1-methyl-2-butynyloxy)[*carbonyl*- $^{14}\text{C}$ ]benzamide monohydrochloride ( $^{14}\text{C}$ -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<sup>21</sup>. The hydrolysis of **5** with conc.  $\text{H}_2\text{SO}_4$  gave **6**, which was then protected with acetonylacetone<sup>31</sup> and transformed into **7**. The

carboxylation of **7** with  $^{14}\text{C}$ -labelled  $\text{CO}_2$  and *n*-butyllithium<sup>31</sup>) afforded the key intermediate **8**, which was then converted to **9** by deprotection with hydroxylamine hydrochloride<sup>31</sup>. The reaction of **9** with *endo*-3-aminotropane<sup>31</sup> in the presence of ethyl chloroformate as a condensing agent afforded **10**. Finally, the treatment of **10** with 1N aqueous HCl afforded the desired  $^{14}\text{C}$ -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 F254 plate (Merck). All organic extracts were dried over anhydrous  $\text{MgSO}_4$ , and the solvent was removed with a rotary evaporator under reduced pressure. Proton nuclear magnetic resonance ( $^1\text{H}$ -NMR) spectra were recorded on a Varian Unity 400 (400 MHz) spectrometer, 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  $0^\circ\text{C}$  for 0.5h. The mixture was stirred at room temperature overnight, poured into saturated aqueous  $\text{NaHCO}_3$ , 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.

$^1\text{H}$ -NMR (400MHz,  $\text{CDCl}_3$ )  $\delta$ : 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  $\text{CH}_2\text{Cl}_2$  (50mL) was added boron tribromide (1.0M in  $\text{CH}_2\text{Cl}_2$ , 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  $\text{CH}_2\text{Cl}_2$ . The precipitate was dissolved in 1N aqueous NaOH, acidified with 1N aqueous HCl, neutralized with saturated aqueous  $\text{NaHCO}_3$ , and then extracted with ethyl acetate. The ethyl acetate layer and the  $\text{CH}_2\text{Cl}_2$  layer were combined and concentrated to give **4** (1.82g, 86%) as colorless crystals.

$^1\text{H}$ -NMR (400MHz,  $\text{CDCl}_3+\text{DMSO}-d_6$ )  $\delta$ : 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 triphenylphosphine (10g, 41mmol) in THF (200mL) was added diethyl azodicarboxylate (7.0mL, 41mmol), (*R*)-3-butyn-2-ol<sup>®</sup> (3.5g, 41mmol), and a solution of **4** (11.9g, 45mmol) in a small amount of THF at -70°C 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. <sup>1</sup>H-NMR (400MHz, CDCl<sub>3</sub>) δ; 1.66 (d, *J* = 8Hz, 3H), 1.84 (d, *J* = 2Hz, 3H), 2.26 (s, 3H), 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<sub>3</sub>OH (200mL) was added conc.H<sub>2</sub>SO<sub>4</sub> (15mL) at 0°C. The resulting solution was stirred at room temperature overnight. The reaction mixture was neutralized with saturated aqueous NaHCO<sub>3</sub>, and then extracted twice with ethyl acetate. The solvent was evaporated to give **6** (6.8g, 100%) as white crystals. <sup>1</sup>H-NMR (400MHz, CDCl<sub>3</sub>) 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<sub>3</sub> 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. <sup>1</sup>H-NMR (400MHz, CDCl<sub>3</sub>) 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-<sup>14</sup>C]benzoic acid (8)**

The key intermediate **8**, which was prepared from **7** and <sup>14</sup>C-CO<sub>2</sub>, 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<sub>2</sub>O / CH<sub>3</sub>CN 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-<sup>14</sup>C]benzoic acid (9)**

A solution of **8** (0.9g, 2.68mmol, 5.62GBq), H<sub>2</sub>NOH•HCl (12.85g, 185mmol),

and KOH (7.4g, 112mmol) in C<sub>2</sub>H<sub>5</sub>OH-H<sub>2</sub>O (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<sub>3</sub>. 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-3 $\alpha$ -yl)-4-amino-5-chloro-2-(1-methyl-2-butynyloxy)[carbonyl-<sup>14</sup>C]benzamide (**10**)**

To a solution of **9** (0.35g, 1.37mmol, 2.84GBq) and triethylamine (0.35g, 3.5mmol) in CH<sub>2</sub>Cl<sub>2</sub> (40mL) was added ethyl chloroformate (0.22g, 2mmol) at 0°C 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<sub>3</sub>, and extracted four times with CH<sub>2</sub>Cl<sub>2</sub>. The combined organic layer was evaporated. The residue was purified by silicagel column chromatography (CH<sub>2</sub>Cl<sub>2</sub> / CH<sub>3</sub>OH 100:0~75:25) and two kinds of preparative HPLCs. Preparative HPLC conditions: DAISEL CHIRALCEL OD 250mm x 20mm, mobile phase *n*-hexane / C<sub>2</sub>H<sub>5</sub>OH / triethylamine 80:20:0.1, flow rate 10mL / min, detector UV275nm: Merck LiChrosphar RP-select B 250mm x 10mm, mobile phase CH<sub>3</sub>CN / H<sub>2</sub>O / HClO<sub>4</sub> 25:75:0.1, flow rate 6mL / min, detector UV275 nm. The eluent was evaporated to remove CH<sub>3</sub>CN, the pH adjusted to 14 with 5N aqueous NaOH, and extracted four times with CHCl<sub>3</sub>. 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-3 $\alpha$ -yl)-4-amino-5-chloro-2-(1-methyl-2-butynyloxy)[carbonyl-<sup>14</sup>C]benzamide monohydrochloride (<sup>14</sup>C-E3620 , **11**)**

To a solution of **10** (0.315g, 0.83mmol, 1.72GBq) in C<sub>2</sub>H<sub>5</sub>OH (100mL) was added 1N aqueous HCl (0.83mL) to give a solution of **11** in C<sub>2</sub>H<sub>5</sub>OH: Specific activity; 2.02GBq / mmol: Radiochemical purity by HPLC 100%. HPLC conditions: Merck LiChrosphar RP-select B 250mm x 4.0mm, mobile phase CH<sub>3</sub>CN / H<sub>2</sub>O / HClO<sub>4</sub> 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<sub>3</sub>CN / H<sub>2</sub>O / HClO<sub>4</sub> 300:700:2, flow rate 0.5mL / min, RI detector.

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