Synthesis of the ^{3}H-labelled 5-HT$_3$ Antagonist (RS-25259-197) at High Specific Activity

Leyi Gong and Howard Parnes
Chemical Research and Development
Roche Bioscience
3401 Hillview Ave.
Palo Alto, CA 94304 USA

Summary

The preparation of the title compound, a selective 5-HT$_3$ antagonist with anti-emetic properties, is described. The key intermediate involved is 6-bromo-1,2-dihydronaphthoic acid (**5**), which was synthesized from 4-bromophenylacetic acid by Micheal addition, acid-induced ring cyclization, reduction and dehydration. Compound (**5**) was selected because it has two labelling sites to ensure high specific activity of the final product. Reduction of amide **6** with carrier-free tritium gas, followed by reduction of the amide functional group with BF_3-OEt_2 and intramolecular cyclization furnished the title compound having a specific activity of 70.4 Ci/mmol and >99% purity.

Key Words: 5-HT$_3$ antagonist, RS-25259-^{3}H, anti-emetic agent.

Introduction

It has been found that activation of 5-HT$_3$ receptors at a peripheral and/or central locus is responsible for the side effects of nausea and emesis associated with anti-cancer therapy[1,2,3,4]. In fact, several selective 5-HT$_3$ receptor antagonists including ondansetron, granisetron, and tropisetron have shown their clinical effect in reducing or even preventing emesis induced by chemotherapy[3]. However, their clinical usefulness is impacted by their varying potency, efficacy, and duration of action[3]. RS-25259-197 (**9**)[5], (3aS)-2-[(S)-1-azabicyclo[2.2.2]oct-3-yl]2,3,3a,4,5,6-hexahydro-1-oxo-1-1H-benz[de] isoquinoline hydrochloride, emerged as a selective 5-HT$_3$ antagonist by receptor binding study in vitro[6] and showed a significant improvement over ondansetron as an anti-emetic agent with respect to potency and duration of action in animal models[4]. It is also an effective anti-emetic agent in human. In the

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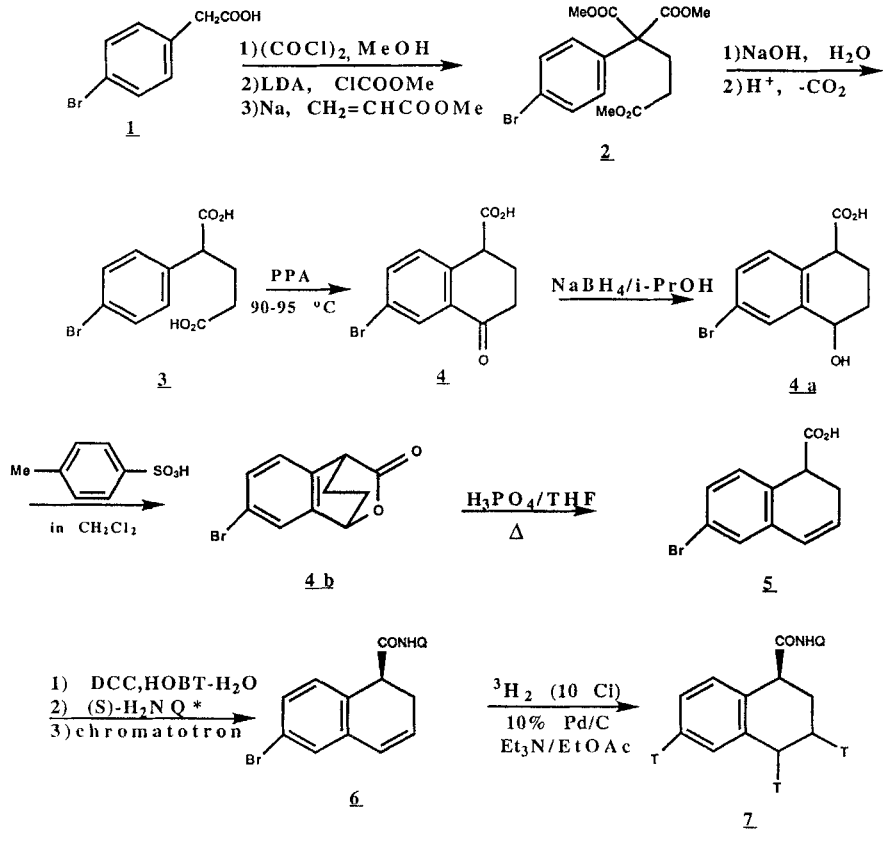
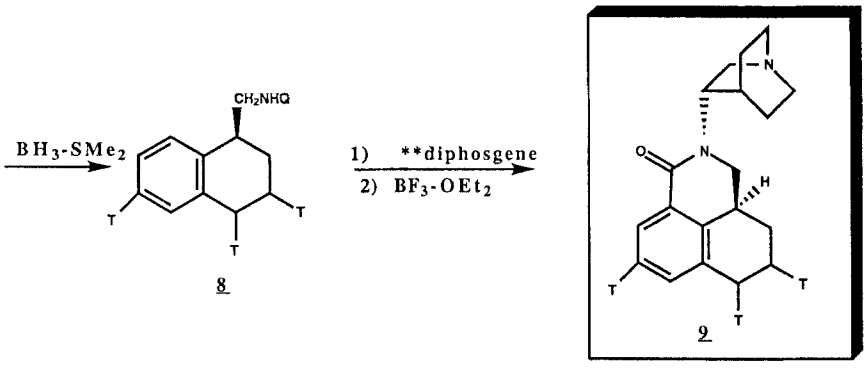
process of its evaluation and development, the ^{3}H-labelled title compound with high specific activity was required for both metabolism and receptor mapping/binding studies. Described herein is the synthesis of ^{3}H-RS-25259-197 at high specific activity.

Results and Discussion

RS-25259-197 (**9**) having two chiral centers constitutes one of four possible stereoisomers. Use of enantiomerical pure (S)-3-aminoquinuclidine reduces the possible isomers to two. However, work done previously in this laboratory has shown that it is very difficult to separate the two tritiated diastereomers (S,S and R,S) by preparative HPLC or by radial chromatography. Thus, a synthetic strategy was devised so that a resolution step was incorporated at an early stage of the synthesis to afford the product as a single diastereomer with the desired (S,S) configuration. In addition, two labelling sites were introduced to ensure high specific activity as shown in **Scheme I**.

Scheme I





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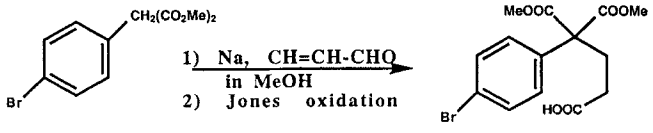
* Q=quinuclidine

**Diphosgene= $Cl_3COCOCl$

4-Bromophenylacetic acid (1) was first esterified as the methyl ester, then deprotonated with lithium diisopropylamide, and reacted with methyl chloroformate to give the substituted malonic diester (10). This then underwent Michael addition with methyl acrylate in the presence of sodium methoxide in methanol to give **2**. Ester hydrolysis under basic conditions, followed by decarboxylation with acid gave diacid **3** in quantitative yield. To convert **3** to **4** through cyclization, several methods were investigated. Friedel-Crafts acylation was attempted first by transformation of acid **3** to the acid chloride, followed by complexion with $AlCl_3$. The reaction was carried out at ambient temperature and gave a complex mixture. The complication might have been due to the presence of two acid chloride functional groups, resulting in possible inter and intra-molecular acylation. To circumvent this problem, malonic diester (10) was reacted with acrolein, followed by oxidation with Jones reagent to give mono-acid (11). However, when 11 was subjected to Friedel-Crafts conditions, only starting material was recovered.



10 11

Treatment of **3** with conc. H_2SO_4, also gave only starting material. The cyclization, however, was best accomplished with polyphosphoric acid (PPA). When **3** was heated in PPA at 90°C for 4h, 46% of desired dihydroketone **4** was obtained. All the by-products were neutral polymeric materials that were easily separated from the product

by acid-base extraction. Sodium borohydride reduction of **4** gave the desired alcohol (**4a**). Surprisingly, attempted dehydration with p-toluenesulfonic acid in CH_2Cl_2 afforded lactone **4b**, instead of the expected olefin **5**. However, treatment of **4b** with H_3PO_4 in THF at reflux temperature transformed **4b** to the desired key intermediate **5**. Coupling of the acid **5** with enantiomerically pure (S)-3-aminoquinuclidine using DCC, HOBT-H_2O afforded two diastereoisomeric amides, which were readily separated by column chromatography and subsequently, by chromatotron. The pure (S,S) isomer (**6**) was isolated in 12% yield.

Tritiation of **6** with carrier-free tritium gas in the presence of 10% Pd/C in EtOAc/Et_3N gave tritium labelled amide **7**. Micro-scale reduction of **7** with BH_3-SMe_2 in THF under reflux provided amine **8**. Treatment of **8** with diphosgene (trichloromethylchloroformate), followed by BF_3–OEt_2 resulted in amidation and intramolecular cyclization to afford enantiomerically pure RS-25259-197-[^{3}H] (**9**). This product was readily purified by radial chromatography. However, upon evaporation of the solvent and exposure to air, a more polar material was formed. We suspected that since the product had a tertiary nitrogen, it was susceptible to air oxidation to the N-oxide, especially in this case, where the quantity was extremely small (0.14 μmol). This assumption was verified by quantitative reduction of the polar material with $TiCl_3$ back to the desired product. To avoid this problem, the Chromatotron fractions were combined and acidified with HCl/ethanol solution to trap the product as the HCl salt prior to concentration. The final product was obtained in >99% purity both chemically and radiochemically and had specific activity of 70.4 Ci/mmol by HPLC using external standard.

Conclusion

An effective synthetic route to ^{3}H-labeled 5-HT_3 antagonist, RS-25259-197 was designed and implemented, resulting in high incorporation of tritium (70 Ci/mmol) and high diastereomeric purity (>99%).

Experimental

Carrier free tritium gas was purchased from New England Nuclear. Unlabelled reagents were purchased from Aldrich Chemical Co. and were used without further purification. Radiochromatography was performed on a Bioscan 200 scanner. Radioassays were performed on a Packard 4000 liquid scintillation counter. NMR spectra were recorded using a Varian EM 390 spectrometer. Chemical shifts (δ) were

reported downfield from tetramethylsilane. Mass spectra were obtained on a Finning-MAT 8230 spectrometer and for multibromination compounds, M/Z values were reported as that of the highest isotope distribution peak. HPLC analyses of final products were performed on a Beckman System Gold chromatograph. Specific Activity of tritium labelled products was determined by injecting known radioactivity concentration of labelled compound followed by known concentration of unlabelled compound, and comparing both uv absorption peak areas.

Methyl 4-(4'-Bromophenyl)-4,4-dimethoxycarbonylbutanoate (2)

$SOCl_2$ (44 mL, 0.6 mol) was added dropwise to a solution of 4-bromophenyacetic acid (1) (43.0 g, 0.2 mol) in 200 mL of CH_2Cl_2. Two drops of DMF were added, and the solution was stirred overnight at ambient temperature. Volatile material was removed by rotary evaporation, and the residue was dissolved in 20 mL of CH_2Cl_2. MeOH (24 mL, 0.6 mol) and Et_3N (80 mL, 0.6 mol) in 100 mL of CH_2Cl_2 were added to the above solution at 0°C. The mixture was stirred at ambient temperature for 2h. The white solid which formed was filtered and the filtrate was concentrated. The residue was then dissolved in 50 mL of CH_2Cl_2, washed with 5% HCl, NaCl (sat.), dried over Na_2SO_4. Column chromatography (silica gel) with 15% EtOAc in hexane gave 45.5 g of methyl ester as an oil (yield: 99%). (^{1}H NMR ($CDCl_3$): 7.46 (d, 2H, J=8), 7.16 (d, 2H, J=8), 3.70 (s, 3H), 3.58 (s, 2H); MS (EI): M/Z 288, 230).

To a solution of i-Pr_2NH (9.8 mL, 70.2 mmol) in 100 mL of THF at 0°C was added n-BuLi (52.7 mL, 84.2 mmol, 1.6 M in hexane) dropwise. After addition, the solution was stirred at 0°C for 15 min and cooled to -78°C. The above methyl ester (13.4 g, 58.5 mmol) was added slowly to this solution. After the mixture was stirred at -78°C for 15 min, methyl chloroformate was added (6.7 mL, 86.1 mmol). The reaction was then stirred at -78°C for 40 min during which time a white precipitate formed. The reaction was quenched with NH_4Cl (sat.) and partitioned between EtOAc and H_2O. The EtOAc layer was washed with H_2O, NaCl (sat.) and dried over Na_2SO_4. Purification by column chromatography (silica gel) with 12% EtOAc in hexane provided 3.8 g of starting material, methyl ester and 9.8 g of malonate (yield: 57%). (TLC: EtOAc-Hexane (2:8); Rf 0.61; ^{1}H NMR ($CDCl_3$): 7.50 (d, 2H, J=8.6), 7.28 (d, 2H, J=8.6), 4.61 (s, 1H), 3.76 (s, 6H); MS (EI): M/Z 286, 288).

Sodium (0.21 g, 9 mmol) was added to 50 mL of methanol at ambient temperature. After H_2 evolution ceased, the above malonate (9.0 g, 31.4 mmol) was added, followed by addition of methyl acrylate (4.5 mL, 50.0 mmol). The resulting mixture was stirred at

ambient temperature for 6h and then quenched with 10% HCl until acidic. MeOH was removed *in vacuo* and the residue was partitioned between EtOAc and H_2O. The EtOAc layer was washed with NaCl (sat.), and dried over Na_2SO_4. Concentration and column purification (silica gel) with 20% EtOAc in hexane afforded 2.6 g of starting material and 7.5 g of **2** (yield: 64%).

TLC: EtOAc-Hexane (2:8); Rf 0.43.

^1H NMR ($CDCl_3$): 7.48 (d, 2H, J=8.7), 7.25 (d, 2H, J=8.9), 3.76 (s, 6H), 3.65 (s, 3H), 2.62 (t, 2H, J=7.8), 2.29 (t, 2H, J=7.8); MS (EI): M/Z 372, 374.

4-(4'-Bromophenyl)-4-hydroxycarbonylbutanoic Acid (3)

Compound **2** (4.8 g, 12.9 mmol) was heated at reflux with KOH (2.88 g, 51.4 mmol) in 48 mL of H_2O overnight. The reaction was cooled to ambient temperature and H_2SO_4 (2.49 mL conc.) in 6.5 mL of H_2O was added. The resulting mixture was heated at reflux for 2h. TLC showed the reaction to be complete. The mixture was then partitioned between EtOAc and H_2O. The EtOAc layer was washed with NaCl (sat.) and dried over Na_2SO_4. Concentration gave 3.69 g of **3** (yield: >99%).

^1H NMR ($CDCl_3$): 7.48 (d, 2H, J=8.5), 7.21 (d, 2H, J=8.5), 3.65 (dd, 1H, J=5.9, 8.2), 2.43 (m, 4H).
MS(EI): M/Z 286, 288.

7-Bromo-4-Hydroxycarbonyltetralone (4)

Polyphosphoric acid (PPA) (220 g) was heated at 90°C and **3** (1.80 g, 6.3 mmol) in 15 mL of Et_2O was added. Et_2O was evaporated and the mixture was stirred at 90°C for 4h. The reaction was cooled and poured into ice-cold water slowly. Organic material was extracted with EtOAc three times. The EtOAc layer was extracted with K_2CO_3 (sat.). The basic aqueous layer was then acidified with 10% HCl and extracted with EtOAc. The EtOAc layer was washed with H_2O, NaCl (sat.), and dried over Na_2SO_4. Concentration and column chromatography (silica gel) with 35% EtOAc, 0.5% HOAc in hexane gave 0.78 g of white solid, **4** (yield: 46%).

TLC: EtOAc-HOAc-Hexane (35:65:0.5); Rf 0.23.

^1H NMR ($CDCl_3$): 8.20 (d, 1H, J=2.1), 7.66 (dd, 1H, J=2.2, 8.3), 7.29 (d, 1H, J=8.3), 3.97 (t, 1H, J=4.7), 2.92 (m, 1H), 2.71 (m, 1H), 2.54 (m, 1H), 2.43 (m, 1H).
MS (EI): M/Z 268, 270.

6-Bromo-1,2-dihydronaphthoic Acid (5)

$NaBH_4$ (0.81 g, 2.1 mmol) was added to 10 mL of i-PrOH at 0°C. To this suspension, 0.60 g of **4** (0.21 mmol) in 5 mL of i-PrOH was added, and the mixture was stirred overnight. It was then acidified with 10% HCl, and extracted with EtOAc. The EtOAc layer was washed with H_2O, NaCl (sat.) and dried over Na_2SO_4. Concentration gave 0.62 g of alcohol (**4 a**) (TLC: EtOAc-HOAc-Hexane (35:0.5:65); Rf 0.20).

To a solution of the above alcohol (**4 a**) (0.62g, 0.21 mmol) in 50 mL of CH_2Cl_2 was added 3.0 g of p-toluenesufonic acid (p-TSA). After the mixture was stirred at rt for 3h, H_2O was added and CH_2Cl_2 layer was separated, washed with NaCl (sat.) and dried over Na_2SO_4. Purification by column (silica gel) with 35/65/0.5 of EtOAc/Hexane/HOAc gave 0.39 g of lactone **4 b** (yield: 74%) (TLC: EtOAc-Hexane-HOAc (35:65:0.5); Rf 0.82; ^1H NMR ($CDCl_3$): 7.50 (dd, 2H, J=2, 7.5), 7.20 (d, 1H, J=7.6), 5.59 (d, 1H, J=3.7), 3.94 (t, 1H, J=2.7), 2.41 (m, 1H), 2.21 (m, 1H), 1.74 (m, 1H), 1.66 (m, 1H); MS (EI): M/Z 252, 254; IR (KBr): 1748 cm^{-1}; m.p. 103.0-104.5 °C; Elem. Anal. calcd for $C_{11}H_9O_2Br$: C, 52.38; H, 3.57. Found: C, 52.39; H, 3.50).

Lactone **4 b** (0.39 g, 1.5 mmol) was dissolved in 10 mL of THF and 10 mL of H_3PO_4 and heated at 100°C for 4h. H_2O was added and the mixture was partitioned between H_2O and EtOAc. The EtOAc layer was washed with H_2O, NaCl (sat.) and dried over Na_2SO_4. Concentration and column purification with EtOAc-Hexane-HOAc (35:65:0.5) gave 0.30 g of white solid (77%).

TLC: EtOAc-Hexane-HOAc (35:65:0.5); Rf 0.38.

^1H NMR ($CDCl_3$): 7.32 (dd, 1H, J=2.2, 8.0), 7.22 (d, 1H, J=2.0), 7.09 (d, 1H, J=8.0), 6.40 (dd, 1H, J=2.4, 9.7), 6.06 (m, 1H), 3.75 (dd, 1H, J=3.5, 7.6), 2.88 (m, 1H), 2.82 (m, 1H), 2.59 (m, 1H), 2.54 (m, 1H).
MS (EI): M/Z 252, 254.

6-Bromo-1,2-dihydronaphthamide (6)

To a solution of compound **5** (0.30 g, 1.19 mmol) in 2 mL of CH_3CN were added DCC (0.25 g, 1.19 mmol) and HOBT·H_2O (0.16 g, 1.19 mmol). After the mixture was stirred at ambient temperature for 0.5h, (S)-3-aminoquinuclidine (0.15 g, 1.19 mmol) in 1 mL of CH_3CN was added. The reaction was stirred for 48h and filtered. The filtrate was concentrated and the residue was partitioned between 10% Na_2CO_3 and CH_2Cl_2. The organic layer was washed with NaCl (sat.) and dried over Na_2SO_4. Column purification

(silica gel) with 1% NH_4OH, 9% MeOH in CH_2Cl_2 gave 80 mg of (S,S)-isomer, 0.21 g of mixture (both S,S and R,S isomers) and 36 mg of (R,S) isomer (yield: 76%).
HPLC showed that the (S,S) isomer obtained above was 99.5% pure. It was therefore further purified by chromatotron (0.5% NH_4OH, 4.5% MeOH in CH_2Cl_2) to give 100% pure (S,S) isomer (53 mg).

TLC: NH_4OH-MeOH-CH_2Cl_2 (1.5:13.5:85); Rf 0.43.
HPLC: Biotage, 24% ACN in (0.2% NH_4OH, 0.1% Et_2NH in H_2O), 220 nm, 1mL/min, Rt of SS-isomer, 11.9 min; Rt of RS-isomer, 15.7 min.
^1H NMR ($CDCl_3$): 7.36(dd, 1H, J=2.1, 8.0), 7.30 (d, 1H, J=2.1), 7.07 (d, 1H, J=8.0), 6.44 (dd,, J=2.9, 9.8), 6.11 (m, 1H), 5.5 (br.d. NH), 3.8 (m, 1H), 3.55 (dd, 1H, J=3.2, 7.8), 3.20 (m, 1H), 3.0 (m, 1H), 2.57 (m, 3H), 2.6 (m, 2H), 2.2 (m, 1H), 1.75 (dd, 1H, j=3.1, 6.3), 1.55 (m, 2H), 1.3 (m, 2H); MS (EI): M/Z 360, 362.

Tritiated Tetrahydronaphthamide (7)

A side arm septum flask was charged with compound **6** (11 mg, 0.03 mmol) and 10% Pd/C (20 mg). The flask was connected to a high vacuum line and the system was evacuated for 30 min. EtOAc (1 mL) and Et_3N (0.3 mL) were then added by a syringe. After the system was degassed, $^3H_2$ (10 Ci, 0.17 mmol) was introduced using a Toepler pump. The reaction was stirred at ambient temperature for 24h. Volatile materials were removed and the reaction was filtered. Exchangable material was dissolved in methanol and removed by rotary evaporation. Product **7** (2.57 Ci) was obtained at >95% purity. Purification of crude **7** (771 mCi) by column chromatography (silica gel) with 1.0% NH_4OH, 9% MeOH in CH_2Cl_2 afforded 705 mCi of pure **7**.

Radio-TLC: NH_4OH-MeOH-CH_2Cl_2 (1:9:90); Rf 0.35.

Tritiated Tetrahydronaphthamine (8)

Compound **7** (141 mCi) was dissolved in 0.4 mL of anhydrous THF and 0.13 mL of BH_3-SMe_2 (0.26 mmol, 2.0 M in THF) was added. The mixture was heated at reflux for 8h. HCl (10%) was added and the mixture was heated at reflux for another hour. It was then made basic with 15% NaOH and partitioned between H_2O and EtOAc. The EtOAc layer was washed with NaCl (sat.) and dried over Na_2SO_4. Column purification (silica gel) with 1% NH_4OH, 19% MeOH in CH_2Cl_2 gave 93 mCi of 94% pure **8**.

Radio-tlc: NH_4OH-MeOH-CH_2Cl_2 (1:19:80); Rf 0.24.

(3S)-2-[(S)-1-Azabicyclo[2,2,0]oct-3-yl]-2,3,3a,4-hexahydro-1-oxo1-H-benz[de]isoquinoline-^{3}H (RS-25259-197-^{3}H) (9)

To a solution of 8 (31 mCi) in 0.5 mL of toluene was added 0.022 mL of diphosgene in toluene ($3.6x10^{-3}$ mmol, prepared from 0.02 mL neat in 1mL of toluene). The mixture was stirred at ambient temperature for 15 min then heated at reflux for 3h. BF_3-OEt_2 (0.05 mL) was then added and reflux was continued for another hour. 10% HCl (1 mL) was added and the mixture was heated at 80°C for 0.5h. It was then made basic with 15% NaOH and partitioned between H_2O and EtOAc. The EtOAc layer was separated, washed with NaCl (sat.) and dried over Na_2SO_4. TLC showed 79% of product with 20% of 8 remaining. Purification by radial chromatography two times gave 8.27 mCi of >99% pure 9 having a specific activity of 70.4 Ci/mmol.

Radio-tlc: NH_4OH-MeOH-CH_2Cl_2 (1:9:90); Rf 0.50.
Radio-HPLC: Biotage, 20% ACN in (0.2% NH_4OH, 0.1% Et_2NH in H_2O), 220 nm, 1 mL/min, Rt 16.3 min.

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