

Synthesis of deuterium-labelled trantinterol

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Trantinterol, a selective β_2 -adrenoceptor agonist, is currently in Phase II clinical trials for the treatment of asthma and bronchitis. The synthesis of deuterium-labelled trantinterol is described. The labelled trantinterol is used as an internal standard for the analysis and metabolic studies.

Keywords: deuterium; labelled; synthesis; trantinterol

Introduction

β_2 -Adrenoceptor is a surface-membrane bound, G-protein coupled 7TM-receptor and distributes widely in human lung, occurring not only in airway smooth muscle but also on other cells such as epithelial and endothelial cells, type II cells, and mast cells.^{1,2} When binding to β_2 -agonists, it carries out the signal transduction to the interior of the cell by coupling to heterotrimeric G proteins. The β_2 -adrenoceptor couples to the stimulatory G protein termed G_s , which acts to stimulate the effector adenylyl cyclase catalyzing the conversion of adenosine triphosphate to cyclic AMP (cAMP). cAMP acts as a classic second messenger in the cell through its activation of protein kinase A, which phosphorylates multiple targets.³ In the lung, β_2 -adrenoceptor's stimulation acts to relax airway smooth muscle. β_2 -Adrenoceptor is an important target of drug discovery efforts. Salbutamol, terbutaline, salmeterol, and formoterol selectively activate β_2 -adrenoceptors in bronchioles and result in the relaxation of airway smooth muscle.⁴⁻⁷ β_2 -Adrenoceptor agonists are the most effective and safe bronchodilators currently available. Studies in this field led to the discovery of trantinterol (2-(4-amino-3-chloro-5-trifluoromethyl-phenyl)-2-*t*-butylaminoethanol), which exhibited selective β_2 -adrenoceptor agonist activity and potent bronchodilatation.⁸ Trantinterol is being evaluated in Phase II human clinical trials for the treatment of asthma and bronchitis. To support the pharmacokinetic and metabolism studies of trantinterol, a stable isotopically labelled standard was needed. This report describes the experimental details for the preparation of deuterium-labelled trantinterol.

Results and discussion

The procedures used for the preparation of [²H₉]trantinterol were modifications of the synthetic route developed by Cheng *et al.*⁹ As shown in Scheme 1, 4-amino-3-chloro-5-trifluoromethylbenzoic acid (**1**) reacted with thionyl chloride to give 4-amino-3-chloro-5-trifluoromethylbenzoyl chloride (**2**), with no further purification (**2**) reacted with diethyl malonate in the presence of magnesium ethoxide in THF to afford 4-amino-3-chloro-5-trifluoromethylacetophenone (**3**). The 4-amino-3-chloro-5-trifluoromethylacetophenone

(**3**) was brominated in acetic acid to give 4-amino-3-chloro-5-trifluoromethyl- α -bromoacetophenone (**4**). The keto group of (**4**) was reduced with NaBH₄ in MeOH/H₂O to give 1-(4-amino-3-chloro-5-trifluoromethylphenyl)-2-bromoethanol (**5**), which was converted to 2-chloro-4-oxiran-2-yl-6-(trifluoromethyl)aniline (**6**) under basic conditions. Aminolysis of 2-chloro-4-oxiran-2-yl-6-(trifluoromethyl)aniline (**6**) with [²H₉]t-butylamine gave [²H₉]trantinterol as a minor isomer because the attack on the less hindered side of the epoxide is preponderant. After purification of the free base by flash chromatography, the hydrochloride of [²H₉]trantinterol was prepared.

Experimental

General

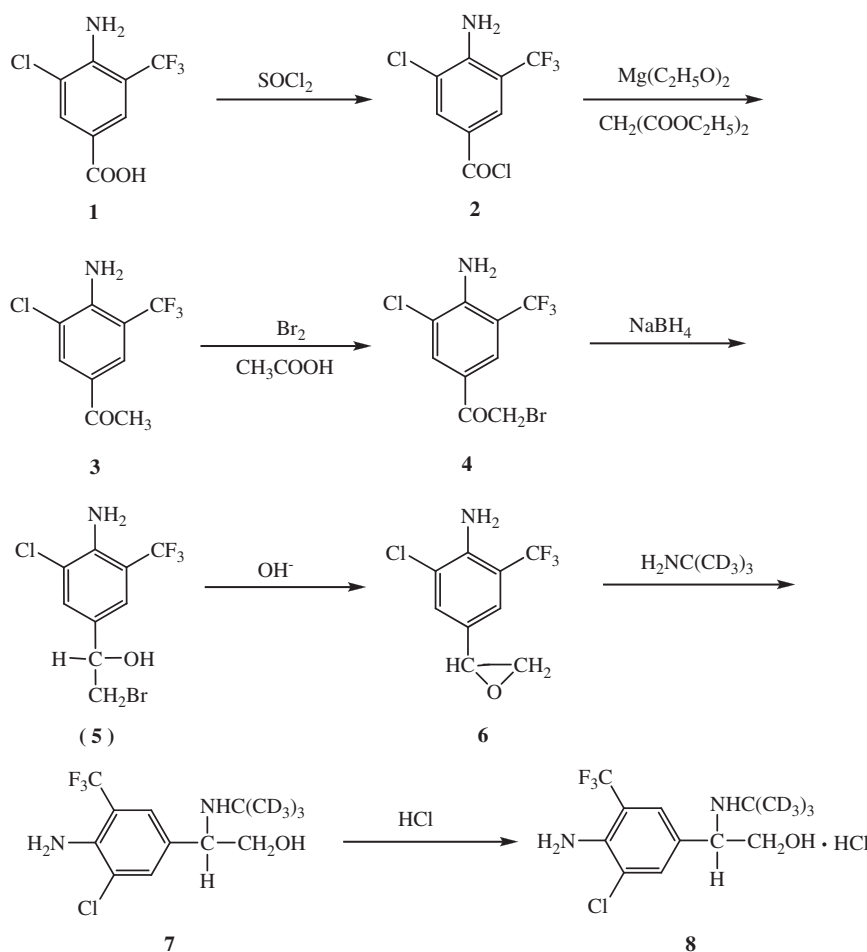
Melting points were determined on a XT4A microscopic digital melting-point apparatus and are uncorrected. ¹H-NMR (400 MHz) and ¹³C-NMR (100 MHz) spectra were recorded on a Bruker NMR spectrometer in CDCl₃. Chemical shifts (δ) are reported in ppm and TMS as an internal standard. LC-MS Spectra were obtained with a Thermo Scientific LTQ Orbitrap XLM mass spectrometer. 4-Amino-3-chloro-5-trifluoromethylbenzoic acid was obtained from Betapharma Co., Ltd (No. 17#-604, 830 Lane, Maotai Road, Shanghai, China), thionyl chloride, diethyl ether, and tetrahydrofuran were distilled before use. All other chemicals were of analytical grade. [²H₉]t-Butylamine was purchased from CDN Isotopes (88 Leacock St., Pointe-Claire-dorval; Quebec, Canada H9R 1H1) with isotopic purity of 99.4%.

All reactions were initially optimized using unlabelled compounds.

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Scheme 1. This paper describes the preparation of deuterium-labelled trantinterol, a selective β_2 -adrenoceptor agonist. 4-Amino-3-chloro-5-trifluoromethylbenzoic acid reacted with thionyl chloride to give 4-amino-3-chloro-5-trifluoromethylbenzoyl chloride. With no further purification it reacted with diethyl malonate in THF to afford 4-amino-3-chloro-5-trifluoromethylacetophenone. After bromination and reduction, 4-amino-3-chloro-5-trifluoromethylacetophenone was transferred to (4-amino-3-chloro-5-trifluoromethylphenyl)-ethylene oxide. The reaction between [$^2\text{H}_9$]t-butylamine and (4-amino-3-chloro-5-trifluoromethylphenyl)-ethylene oxide gave [$^2\text{H}_9$]trantinterol. After purification of the free base by flash chromatography, the hydrochloride of [$^2\text{H}_9$]trantinterol was prepared. The produced [$^2\text{H}_9$]trantinterol hydrochloride could be used as a mass spectroscopic standard that was nine mass units separated from the original drug substance.

4-Amino-3-chloro-5-trifluoromethylbenzoyl chloride (2)

4-Amino-3-chloro-5-trifluoromethylbenzoic acid (2.6 g, 10.8 mmol) was added to thionyl chloride (10 mL, 140 mmol). The suspension was heated until the solid dissolved and then refluxed for 2 h. After cooling to room temperature, the reaction mixture was evaporated under vacuum and gave crude product **2** as a yellow solid (2.46 g, 87.9%), m.p. 110–113°C (Lit. 110–115°C).⁹

4-Amino-3-chloro-5-trifluoromethylacetophenone (3)

To a solution of $\text{Mg}(\text{C}_2\text{H}_5\text{O})_2$ (6.21 g, 54.2 mmol) in anhydrous THF (15 mL) a mixture of absolute ethanol (4.8 mL), diethyl malonate (8.1 mL, 53.2 mmol), and THF (5.6 mL) was added dropwise. The reaction mixture was refluxed for 2 h. A solution of **2** (13.1 g, 50.8 mmol) in THF (40 mL) was added over 0.5 h and the mixture was refluxed for another 2 h. After cooling to room temperature, 2 M sulfuric acid was added dropwise to adjust pH to 2. The organic phase was separated, evaporated under reduced pressure to give a yellow oil. A mixture of glacial acetic acid (45 mL), water (30 mL), and concentrated sulfuric acid (5.7 mL) was added and refluxed for 5 h. After removal of the solvent under reduced pressure, the residue was taken up in

cold water, basified with 1 M NaOH, and washed with CHCl_3 (30 mL). The combined organic fraction was dried over Na_2SO_4 and evaporated to give a white solid. Purified by flash chromatography (petroleum ether:ethyl acetate = 8:1) afforded **3** as a white solid (9.94 g, 82.4% yield), m.p. 123–125°C (Lit. 120–130°C).⁹ $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ : 2.54 (s, 3H), 5.13 (s, 2H), 8.01 (d, 1H, $^4J = 1.76$ Hz), 8.06 (d, 1H, $^4J = 1.76$ Hz); $^{13}\text{C-NMR}$ (100 MHz, CDCl_3) δ : 26.01, 120.35, 125.27, 126.35, 126.41, 126.62, 132.86, 144.60, 194.51. MS (ESI) m/z : 239 [M+1].

4-Amino-3-chloro-5-trifluoromethyl- α -bromoacetophenone (4)

Compound **3** (2.125 g, 8.9 mmol) was dissolved in glacial acetic acid (20 mL). A solution of bromine (0.5 mL, 9.7 mmol) in glacial acetic acid (4 mL) was added dropwise at 45–50°C and kept at this temperature for 0.5 h. After concentration under reduced pressure, the residue was taken up in EtOAc (20 mL), washed with 0.5 M NaHCO_3 (20 mL) and water (10 mL). The organic layer was dried over Na_2SO_4 and evaporated to give a yellow solid. Purification by flash chromatography (petroleum ether:ethyl acetate = 10:1) produced **4** as a white solid (1.47 g, 51.9% yield), m.p. 119–120°C (Lit. 113–115°C).⁹ $^1\text{H-NMR}$ (400 MHz,

CDCl₃δ: 3.41 (d, 1H, ²J = 10.4 Hz), 3.50 (d, 1H, ²J = 10.4 Hz), 4.63 (s, 2H), 7.32 (d, 1H, ⁴J = 1.2 Hz), 7.42 (d, 1H, ⁴J = 1.2 Hz); ¹³C-NMR (100 MHz, CDCl₃)δ: 29.79, 120.61, 123.21, 127.26, 128.16, 128.62, 133.60, 145.35, 188.20. MS (ESI) *m/z*: 318 [M+1].

2-Chloro-4-oxiran-2-yl-6-(trifluoromethyl)aniline (6)

Compound **4** (1.5 g, 4.7 mmol) was dissolved in MeOH (10 mL) and water (1 mL) was added. Sodium borohydride (0.258 g, 6.8 mmol) was added in small portions and the reaction mixture was stirred at room temperature for 3 h, then acidified to pH = 2 with 2 M HCl at 0°C. The reaction mixture was concentrated and the residue was taken up in chloroform (15 mL), washed with water to neutrality. The organic layer was dried over Na₂SO₄, concentrated, and purified by flash chromatography (petroleum ether:ethyl acetate = 10:1) to give **6** as yellow oil (0.324 g, 28.8% yield). ¹H-NMR (400 MHz, CDCl₃)δ: 3.48 (m, 1H), 3.59 (m, 1H), 4.70 (s, 2H), 4.83 (m, 1H), 7.39 (d, 1H, ⁴J = 1.6 Hz), 7.49 (d, 1H, ⁴J = 1.6 Hz); ¹³C-NMR (100 MHz, CDCl₃)δ: 64.51, 72.62, 120.98, 123.07, 123.11, 125.61, 129.52, 130.57, 141.06. MS (ESI) *m/z*: 475 [2M+1].

2-(4-Amino-3-chloro-5-trifluoromethylphenyl)-2-[²H₉]-*t*-butylaminoethanol (7)

Compound **5** (0.342 g, 1.4 mmol) was dissolved in anhydrous ethanol (8 mL) and [²H₉]*t*-butylamine (0.164 g, 2 mmol) was added. The mixture was refluxed for 13 h and concentrated. The residue was purified by flash chromatography (chloroform:methanol = 25:1) to give **7** as a white solid (0.202 g, 44.1% yield), m.p. 86–88°C (Lit. 85–90°C for the unlabelled analogue).⁹ ¹H-NMR (400 MHz, CDCl₃)δ: 1.98 (brs, 2H, –OH, –NH) 3.26 (m, 1H), 3.53 (m, 1H), 3.78 (m, 1H), 4.60 (s, 2H), 7.29 (d, 1H, ⁴J = 1.4 Hz), 7.40 (d, 1H, ⁴J = 1.4 Hz); ¹³C-NMR (100 MHz, CDCl₃)δ: 57.45, 66.78, 120.82, 122.92, 123.41, 125.63, 131.18, 133.48, 139.89. MS (ESI) *m/z*: 320 [M+1].

2-(4-Amino-3-chloro-5-trifluoromethylphenyl)-2-[²H₉]-*t*-butylaminoethanol hydrochloride (8)

To a solution of compound **6** (0.134 g, 0.4 mmol) in diethyl ether (10 mL) was added saturated HCl-isopropanol solution dropwise at 0°C until pH 2. The solution was stirred at this temperature for 0.5 h. The precipitate was collected by filtration, washed with anhydrous ether, and dried under vacuum to afford **8** as a white solid (0.133 g, 89.1% yield), m.p. 215–217°C (dec) (Lit. 205–206°C for the unlabelled analogue).⁹

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