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Synthesis of [$^2H_5$]-ebastine fumarate and [$^2H_5$]-hydroxyebastine

Zhoujie Yu,[a] Wei Wang,[b] and Liqin Chen[c]*

This study describes the synthesis of deuterium-labelled ebastine fumarate and its deuterium-labelled metabolite hydroxyebastine. The synthesis of the two desired compounds both used [$^2H_5$]-bromodiphenylmethane as deuterium-labelled reagent, which was synthesized beforehand in three steps. [$^2H_5$]-ebastine was synthesized in further three steps with a 27% overall yield and [$^2H_5$]-hydroxyebastine was synthesized in further seven steps with a 13% overall yield.

Keywords: deuterium-labelled; ebastine; hydroxyebastine; metabolite

Introduction

Histamine plays a very important role in the development of allergic symptoms and the most widely used treatments for disease relief are H_1-antihistamines.[1] Ebastine is a selective histamine H_1-receptor antagonist which belongs to second generation of nonsedating H_1-antihistamine.[2] It is an effective and well-tolerated treatment for allergic rhinitis and chronic idiopathic urticaria and has no clinically relevant adverse effects on cognitive function, psychomotor performance or on cardiovascular function.[3] Ebastine undergoes extensively sequential metabolism in the liver and one of the major primary metabolites identified in the human body is hydroxyebastine.[4–6] Ebastine labelled with stable isotopes of hydrogen were required for drug metabolism (excretion, distribution and absorption) studies and to develop bioanalytical methods to support clinical studies, while deuterium-labelled hydroxyebastine could be used as an important internal standard for bioavailability studies of ebastine.

Experimental

General

[$^2H_6$]-benzene was purchased from Cambridge Isotope Laboratories. All other reagents were obtained from Sigma-Aldrich. All solvents were of analytical grade and used without further purification. Mass spectra were obtained with a Quattro micro API mass spectrometer. ^{1}H NMR spectra was acquired on a Bruker 300 MHz spectrometer with TMS as internal standard. Chemical purities were determined by an agilent 1200 series HPLC with an Agilent XDB-C18 column, 5 μm, 4.6 × 150 mm.

[$^2H_5$]-benzophenone (2)

To [$^2H_6$]-benzene (1.85 g, 22.0 mmol) was added aluminium chloride (36.2 g, 27.1 mmol) and the slurry was stirred under N_2 atmosphere for 0.5 h at 0°C. Benzoyl chloride (3.04 g, 21.6 mmol) was added slowly at 0–10°C. The mixture was warmed to ambient temperature and monitored by TLC analysis for completion (2 h). After completion, the reaction was quenched slowly with 10% HCl at 0–10°C and the mixture was extracted with dichloromethane (3 × 50 mL). The combined dichloromethane extracts were washed with brine (50 mL), dried over $MgSO_4$ and concentrated under reduced pressure to afford (**2**) as a white solid (3.77 g, 89%).

[$^2H_5$]-diphenylmethane (3)

[$^2H_5$]-benzophenone (**2**) (3.77 g, 20.1 mmol) was hydrogenated in methanol (50 mL) in the presence of 10% Pd/C (0.35 g) at ambient temperature for 12 h. The Pd/C catalyst was then filtered and washed with methanol (3 × 5 mL). The solvent was removed under reduced pressure to give the title compound as a colorless liquid with pleasant aroma (3.37 g, 96%).

^{1}HNMR ($CDCl_3$): δ 3.99 (s, 2H), δ 7.18–7.22 (m, 3H), δ 7.26–7.31 (m, 2H).

[$^2H_5$]-bromodiphenylmethane (4)

To a solution of compound (**3**) (3.37 g, 19.4 mmol) in carbon tetrachloride (30 mL) was added benzoyl peroxide (0.24 g, 1.0 mmol) and 0.5 Eq of *N*-bromosuccinimide (1.73 g, 9.7 mmol). The reaction mixture was refluxed at 80°C for 0.5 h and another 0.5 Eq of *N*-bromosuccinimide (1.75 g, 9.8 mmol) was added.

[a]*College of Material Science and Technology, Nanjing University of Aeronautics and Astronautics, 29 Yudao Street, Nanjing, Jiangsu 210016, People's Republic of China*

[b]*College of Pharmaceutical Sciences, Nanjing University of Technology, 5 Xinmofan Road, Nanjing, Jiangsu 210009, People's Republic of China*

[c]*Hi-Tech Research Institute and State Key Laboratory of Materials-Oriented Chemical Engineering, Nanjing University of Technology, 5 Xinmofan Road, Nanjing, Jiangsu 210009, People's Republic of China*

**Correspondence to: Liqin Chen, Hi-Tech Research Institute and State Key Laboratory of Materials-Oriented Chemical Engineering, Nanjing University of Technology, 5 Xinmofan Road, Nanjing, Jiangsu 210009, Peoples' Republic of China.*
E-mail: liqin_chen@hotmail.com

After stirring for further 0.5 h, the reaction mixture was cooled to ambient temperature, filtered and filtercake was washed with carbon tetrachloride (3 × 5 mL). The filtrate was washed with saturated sodium bicarbonate solution, dried over $MgSO_4$ and evaporated to afford compound (**4**) as a brown solid (4.35 g, 90%). The compound (**4**) was used directly without further purification.

1-[4-(1,1-dimethylethyl)phenyl]-4-[4-hydroxy-1-piperidineyl]-butanone (5)

To a suspension of anhydrous Na_2CO_3 (2.68 g, 25.2 mmol) and KI (4.25 g, 25.6 mmol) in acetone (50 mL) was added 4-chloro-1-[4-(1,1-dimethylethyl)phenyl]-1-butanone (5.97 g, 25.0 mmol) and 4-hydropiperidine (5.06 g, 50.1 mmol). The reaction mixture was refluxed at 70°C overnight. After completion, the mixture was cooled to ambient temperature and filtered. The filter cake was washed with acetone (3 × 5 mL) and the filtrate was evaporated to afford a brown sticky liquid. The crude product was purified by chromatography on silica gel (120 g) column, eluted with 0–5% MeOH in dichloromethane. The title compound was obtained as a pale yellow solid (4.57 g, 60%).

^{1}HNMR ($CDCl_3$): δ 1.34 (s, 9H), δ 1.55–1.65 (m, 2H), δ 1.93–2.02 (m, 4H), δ 2.27 (t, J = 12.0 Hz, 2H), δ 2.49 (t, J = 9.0 Hz, 2H), δ 2.81–2.88 (m, 2H), δ 3.00 (t, J = 6.0 Hz, 2H), δ 3.71-3.76 (m, 1H), δ 7.46 (d, J = 9.0 Hz, 2H), δ 7. 89 (d, J = 9.0 Hz, 2H).

[$^2H_5$]-ebastine (6)

To a suspension of anhydrous Na_2CO_3 (1.23 g, 11.6 mmol) in toluene (10 mL) was added [$^2H_5$]-bromodiphenylmethane (**4**) (0.91 g, 3.6 mmol) and compound (**5**) (1.12 g, 3.7 mmol). The reaction mixture was refluxed at 125°C for 6 h and then cooled to amibent temperature. The precipitate were filtered and filtercake was washed with toluene (3 × 2 mL). The solvent was removed under reduced pressure to give the product as a dark brown sticky liquid. The crude product was purified by chromatography on silica gel (60 g) column, eluted with 0–40% EtOAc in hexane to afford (**6**) as a white solid (1.10 g, 65%).

^{1}HNMR ($CDCl_3$): δ 1.34 (s, 9H), δ 1.67–1.73 (m, 2H), δ 1.87–1.97 (m, 4H),δ 2.12 (s, 2H), δ 2.38 (t, J = 9.0, 2H), δ 2.76 (t, J = 6.0, 2H), δ 2.97 (t, J = 9.0, 2H), δ 3.41–3.43 (m, 2H), δ 5.51 (s, 1H), δ 7.21–7.35 (m, 5H), δ 7.45 (d, J = 9.0 Hz, 2H), δ 7. 89 (d, J = 9.0 Hz, 2H).

[$^2H_5$]-ebastine fumarate(7)

[$^2H_5$]-ebastine (**6**) (1.10 g, 2.3 mmol) was dissolved in ethanol (2 mL) and stirred at amibent temperature. Fumaric acid (0.26 g, 2.3 mmol) was dissolved in ethanol (4 mL) and added to the reaction flask drop wise. The reaction mixture was stirred for 20 min. Some white precipitate started to form. The precipitate was filtered and washed with ethanol (2 × 1 mL). The solid was dried under vacuum to afford (**7**) as a white solid (1.26 g, 92%).

^{1}HNMR (DMSO-d_6): δ 1.29 (s, 9H), δ 1.54–1.62 (m, 2H), δ 1.80–1.84 (m, 4H), δ 2.34 (s, 2 H), δ 2.50 (t, J = 3.0 Hz, 2H), δ 2.84 (t, J = 9.0 Hz, 2H), δ 3.00 (t, J = 6.0 Hz, 2H), δ 3.39 (s, 1H), δ 5.51 (s, 1H), δ 6.57 (s, 2H), δ 7.29–7.38 (m, 5H), δ 7.51 (d, J = 9.0 Hz, 2H), δ 7.87 (d, J = 9.0 Hz, 2H). MS-EI (*m/z*): 474.2 (5), 475.2 (M^+, 100), 476.2 (40), 477.2 (10), 487.2 (2). HPLC (XDB-C18, CH_3OH/10mmol/L CH_3COONH_4 +0.05% TEA = 90/10, 1.0 mL/min): t_R 6.1 min (>99.5%).

Methyl-2,2-dimethyl-2-phenyl acetate (8)

Methanol (25 mL), 2,2-dimethyl-2-phenyl-aceic acid (5.03 g, 30.6 mmol) and concentrated sulphuric acid (98%, 1 mL) were charged to a flask at ambient temperature. The temperature was raised to reflux at 70°C and maintained for 12 h. After completion, the mixture was cooled to ambient temperature, neutralized with aqueous ammonia and extracted with dichloromethane (3 × 50 mL). The combined dichloromethane layers were dried over $MgSO_4$ and concentrated to obtain compound (**8**) as a colorless liquid (5.12 g, 94%).

^{1}H-NMR ($CDCl_3$): δ 1.58 (s, 6H), δ 3.65 (s, 3H), δ 7.21–7.26 (m, 2H), δ 7.33 (d, J = 3.0 Hz, 3H).

2-methyl-2-phenyl propanol (9)

To a chilled solution of compound (**8**) (5.12 g, 28.7 mmol) in dry THF (20 mL) was added sodium borohydride (1.06 g, 28.0 mmol) in portions within 0.5 h. The mixture was stirred at ambient temperature for 3 h. After completion, the PH was adjusted to 3–4 with 10% HCl and the mixture was filtered. The filtrate was extracted with dichloromethane (3 × 50 mL) and the combined extracts were dried over $MgSO_4$, concentrated under reduced pressure to obtain compound (**9**) as a colorless liquid (3.95 g, 92%).

2-methyl-2-phenylpropyl acetate (10)

To a chilled solution of compound (**9**) (3.95 g, 26.3 mmol) in pyridine (20 mL) was added acetic anhydride (5.31 g, 52.1 mmol) slowly. The mixture was stirred for 2 h at 10–20°C, then ethyl acetate (50 mL) and chilled water (25 mL) were added to quench the reaction. After stirring for further 0.5 h, 10% chilled HCl (50 mL) was added to the mixture. The organic layer was separated and washed with saturated sodium bicarbonate solution until PH of 7–8 was obtained. The separated organic layer was dried over $MgSO_4$ and concentrated under reduced pressure to obtain 2-methyl-2-phenylpropyl acetate (**10**) as a pale green liquid (4.45 g, 88%).

Ethyl-2-[4-(4-chloro-butyryl)phenyl]-2-methyl propanoate (11)

Dry Dichloromethane (30 mL) and aluminium chloride (4.61 g, 34.6 mmol) were charged to a flask and chilled to −10°C in a ice–salt bath. Compound (**10**) (4.45 g, 23.1 mmol) was stripped with toluene (2 × 10 mL), dissolved in dry dichloromethane (10 mL) and added slowly. The mixture was stirred for 0.5 h at −10°C under N_2 atmosphere. 4-chloro butyryl chloride (3.90 g, 27.7 mmol) was dissolved in dry dichloromethane (10 mL) and added slowly to the reaction mixture. The mixture was stirred for additional 2 h at 0°C. After completion, the mixture was quenched slowly with 10% HCl at 10–20°C. The dichloromethane layer was separated and the aqueous layer was extracted with dichloromethane (2 × 80 mL). The combined dichloromethane layers were dried over $MgSO_4$ and concentrated under vacuum to obtain crude product as a brown sticky liquid. The crude product was purified by chromatography on silica gel (120 g) column, eluted with EtOAc/Hexanes (1:10) to obtain (**11**) as a pale brown liquid (4.78 g, 70%).

^{1}H-NMR ($CDCl_3$): δ 1.39 (s, 6H), δ 2.00 (s, 3H), δ 2.19–2.28 (m, 2H), δ 3.18 (t, J = 9.0 Hz, 2H), δ 3.70 (t, J = 3.0 Hz, 2H), δ 4.16 (s, 2H), δ 7.46 (d, J = 6.0 Hz, 2H), δ 7.93 (d, J = 9.0 Hz, 2H).

Ethyl-2-[4-(4-(4-hydroxy-1-piperidineyl)butyryl)phenyl]-2-methyl propanoate (12)

To a suspension of anhydrous Na_2CO_3 (1.75 g, 16.5 mmol) and KI (2.70 g, 16.3 mmol) in acetone (30 mL) were added compound (**11**) (4.78 g, 16.2 mmol) and 4-hydropiperidine (3.28 g, 32.5 mmol). The reaction mixture was refluxed at 70°C overnight, and then cooled to ambient temperature. The precipitate was filtered and filter cake was washed with acetone (3 × 5 mL). The filtrate was evaporated to afford a sticky liquid. The crude product was purified by chromatography on silica gel (100 g) column, eluted with 0–5% MeOH in dichloromethane to obtain the title compound as pale yellow solid (3.75 g, 64%).

^{1}H-NMR ($CDCl_3$): δ 1.24 (t, J = 9.0 Hz, 1H), δ 1.40 (s, 6H), δ 1.60–1.63 (m, 2H), δ 1.99–2.05 (m, 6H), δ 2.36 (s, 2H), δ 2.54 (s, 2H), δ 2.88 (s, 2H), δ 3.03 (t, J = 6.0 Hz, 2H), δ 3.79 (s, 1H), δ 4.15 (s, 3H), δ 7.44 (d, J = 9.0 Hz, 2H), δ 7.92 (d, J = 9.0 Hz, 2H).

Ethyl-2-[4-(4-(4-[$^2H_5$]-diphenylmethoxy-1-piperidineyl)butyryl)phenyl]-2-methylpropanoate (13)

To a suspension of anhydrous Na_2CO_3 (1.65 g, 15.6 mmol) in toluene (30 mL) was added compound (**4**) (2.60 g, 10.3 mmol) and compound (**12**) (3.75 g, 10.4 mmol). The reaction mixture was refluxed at 125°C for 6 h, then cooled to ambient temperature and filtered. The filter cake was washed with toluene (3 × 5 mL) and filtrate was evaporated to afford a dark brown sticky liquid. The crude product was purified by chromatography on silica gel (100 g) column, eluted with 0–40% EtOAc in hexane to obtain pure product as a white solid (3.22 g, 57%).

[$^2H_5$]-hydroxyebastine (14)

To a solution of Compound (**13**) (3.22 g, 6.0 mmol) in methanol (20 mL) was added 10% aqueous sodium hydroxide (8 mL). The mixture was stirred at ambient temperature overnight. Methanol was concentrated, then pure water (20 mL) and dichloromethane (40 mL) was added to the remaining. The dichloromethane layer was separated and the aqueous layer was extracted with dichloromethane (2 × 40 mL). The combined dichloromethane extracts were washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure to afford an off-white solid. After purification by recrystallization in isopropanol, the desired product was obtained as a white solid (2.45 g, 85%).

^{1}HNMR ($CDCl_3$): δ 1.30 (t, J = 9.0 Hz, 1H), δ 1.36 (s, 6H), δ 1.59–1.68 (m, 2H), δ 1.83–1.96 (m, 4H), δ 2.08 (t, J = 6.0 Hz, 2H), δ 2.36 (t, J = 9.0 Hz, 2H), δ 2.72 (t, J = 6.0 Hz, 2H), δ 2.99 (t, J = 6.0 Hz, 2H), δ 3.35–3.45 (m, 1H), δ 3.63 (d, J = 6.0 Hz, 2H), δ 5.52 (s, 1H), δ7.26 (d, J = 6.0 Hz, 1H), δ 7.31–7.33 (m, 4H), δ 7.46 (d, J = 6.0 Hz, 2H), δ 7.93 (d, J = 6.0 Hz, 2H). MS-EI (*m/z*): 490.2 (9), 491.2 (M^+, 100), 492.3 (35), 493.3 (7). HPLC (XDB-C18, CH_3OH/10 mmol/L CH_3COONH_4 + 0.03% TEA = 83/17, 1.0 mL/min): t_R 4.7 min (> 99.1%).

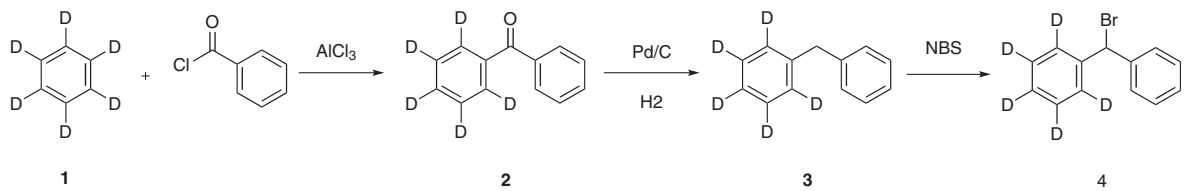
Results and discussion

The synthesis of ebastine has been readily reported,[7–9] while the synthesis of [$^2H_5$]-ebastine fumarate has not been described previously. Although, several reports have covered the likelihood of transformation of ebastine into hydroxyebastine by cytochrome P450 enzymes in human liver and Buisson *et al.* described biooxidation of ebastine to hydroxyebastine on 100 mg scale using streptomyces platensis,[4–6,10,11] the synthesis of [$^2H_5$]-hydroxyebastine has also not been covered before.

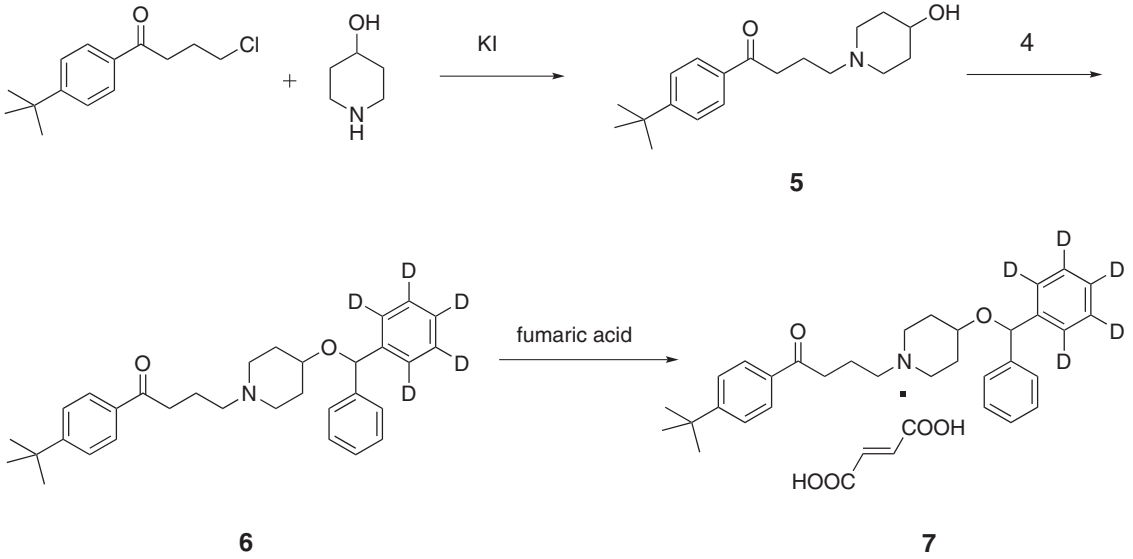
Scheme 1 presents the synthetic route of preparing [$^2H_5$]-bromodiphenylmethane (**4**). Compound (**4**) was synthesized in three steps and the overall yield was a satisfactory 76%.[12–14] It began from Friedel–Crafts acylation of [$^2H_6$]-benzene and benzyl chloride to obtain [$^2H_5$]-benzophenone (**2**) as a white solid. Catalytic hydrogenation of compound (**2**) with 10% Pd/C gave [$^2H_5$]-diphenylmethane (**3**) as a colorless liquid with pleasant aroma. Compound (**3**) was then brominated by NBS in the presence of benzoyl peroxide to afford Compound (**4**), which was used directly without further purification in the following reactions.

Scheme 2 describes the synthetic route of preparing [$^2H_5$]-ebastine fumarate (**7**).[7–9] We chose the most concise synthesis route began with condensation of commercially available 4-hydropiperidine and 4-chloro-1-[4-(1,1-dimethylethyl)phenyl]-1-butanone to form compound (**5**), followed by reaction with [$^2H_5$]-bromodiphenylmethane (**4**) to give [$^2H_5$]-ebastine (**6**) as a white solid. Compound (**6**) was treated with fumaric acid to form [$^2H_5$]-ebastine fumarate (**7**) as a white solid. The overall yield was a reasonable 27%.

Scheme 3 shows the synthetic route of preparing [$^2H_5$]-hydroxyebastine (**14**).[15] Methyl-2,2-dimethyl-2-phenyl acetate (**8**) was prepared by esterification of 2,2-dimethyl-2-phenyl-aceic acid and methanol. Reduction of compound (**8**) by sodium borohydride gave 2-methyl-2-phenyl propanol (**9**) as a colorless oil. Treatment of compound (**9**) with acetic anhydride at 10–20°C obtained ethyl-2-methyl-2-phenyl propanoate (**10**). Friedel–Crafts acylation of compound (**10**) and 4-chloro butyryl chloride was the key step, in which ortho-substituted by-product was inavoidable and the crude product was purified by chromatography on silica gel column to yield compound (**11**) as a pale brown liquid. Reaction of compound (**11**) with 4-hydroxypiperidine produced compound (**12**), followed by condensation with [$^2H_5$]-bromodiphenylmethane (**4**) at reflux in toluene to give compound (**13**) as a white solid. Finally, compound (**13**) was hydrolyzed to generate an off-white solid product. After purification by recrystallization from isopropanol, [$^2H_5$]-hydroxyebastine (**14**) was obtained as a white solid in 13% overall yield from [$^2H_6$]-benzene.

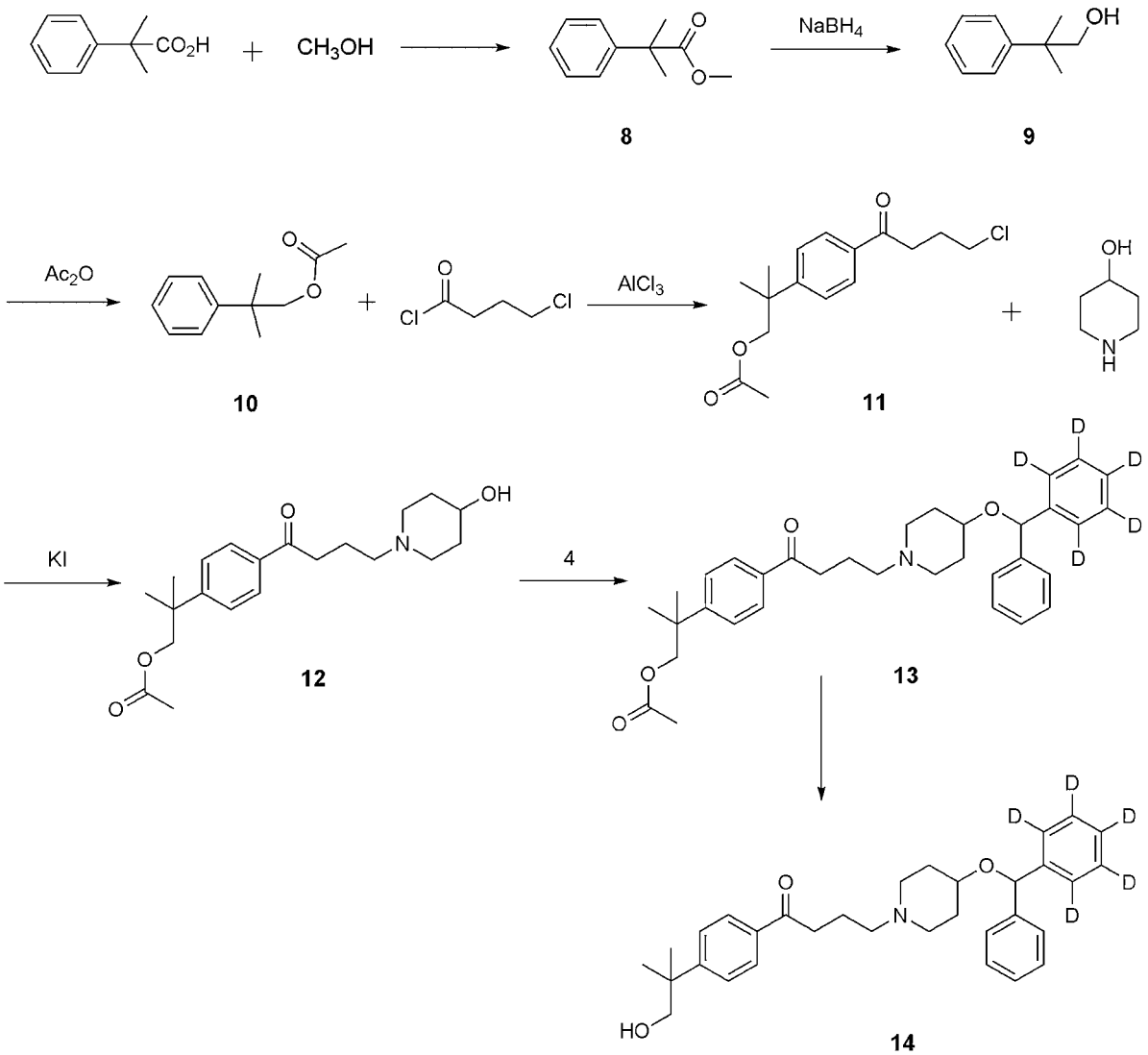


Scheme 1.



5 **6** **7**

Scheme 2.



8 **9** **10** **11** **12** **13** **14**

Scheme 3.

HPLC results showed that both [$^2H_5$]-ebastine fumarate (**7**) and [$^2H_5$]-hydroxyebastine (**14**) were obtained with over 99% chemical purity. Mass spectrometry analysis of compound (**7**) and compound (**14**) revealed over 98% deuterium enrichment.

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