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RESEARCH ARTICLE

Synthesis of stable isotope labeled D_9-Mabuterol, D_9-Bambuterol, and D_9-Cimbuterol

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Three stable and simple synthetic routes of labeled D_9-Mabuterol, D_9-Bambuterol, and D_9-Cimbuterol were described with 98.5%, 99.7%, and 98.4% isotopic abundance and good purity. These structures and isotope-abundance were confirmed according to ^{1}H NMR and liquid chromatography-tandem mass spectrometry.

KEYWORDS

β-agonist, internal standards, isotope labeling, synthesis

1 | INTRODUCTION

As β-agonists, mabuterol, bambuterol, and cimbuterol were bronchodilators and anti-allergic effects, which were stronger than clenbuterol on relaxation of tracheal smooth muscle, addition of histamine-induced airway resistance, and inhibition of experimental asthma.[1–3] They were also used as feed additives to improve the ratio of lean tissue. However, residue would accumulate in the tissue of animals after using, which directly endangered human health. Thus, these drugs have been prohibited as growth promoters of farm animals in several countries, such as the European Union, Japan, and China. The detection of the β-agonists' residue in food has attracted wide spread attention in recent times.[4,5]

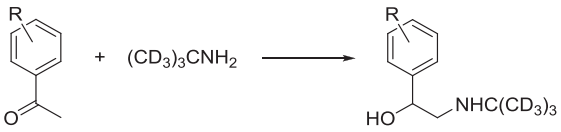
In recent years, there have been many analysis methods for detecting these β-agonists, such as liquid chromatography (LC), capillary electrophoresis, gas chromatography-tandem mass spectrometry, liquid chromatography-tandem mass spectrometry (LC-MS), and etc.[6–8] But there were still some defects with these methods, such as pretreatment of samples and the interferences of impurities. Thus more accurate and effective analysis methods had brought to the forefront of researchers. Isotope Dilution Mass Spectrometry was widely used in detecting the content of β-agonists in food, which using isotopically labeled compounds as internal standards could avoid these defects above and make the results more accurate.[9]

The deuterium labeled β-agonists could be used as internal standards to detect the residue in food and explore other mechanism studies. To our knowledge, there were few reports about the synthesis of D_9-mabuterol, D_9-bambuterol, and D_9-cimbuterol. Herein, we developed a convenient method to synthesize three kinds of stable isotope labeled D-ring *β*-agonistic drugs (D_9-mabuterol, D_9-bambuterol, and D_9-cimbuterol) using the D_9-tert-Butylamine as the labeled precursor (Scheme 1). And the deuterium atoms were labeled on the saturated chain rather than the benzene ring, which was stable enough without the dilution of the isotopic abundance.

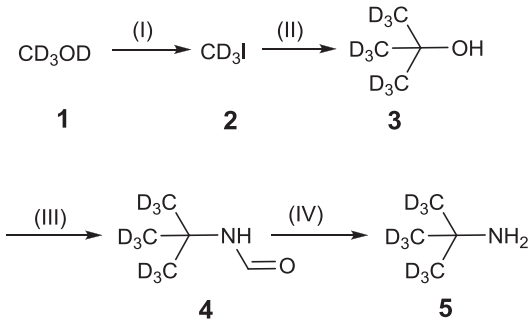
2 | RESULTS AND DISCUSSION

Based on the previous works,[10] we summarized the route of synthesis of D_9-tert-Butylamine depicted in Scheme 2. The 4-step synthesis of D_9-tert-Butylamine **5** started with **1** as starting material. First of all, **2** was yielded by the iodination of D_4-methanol with 85.0% yield. Through the Grignard reaction of **2** and nucleophilic addition with D_6-acetone, the D_9-tert-Butanol **3** was obtained with 45.0% yield. The **4** was obtained through amidation of **3** with 51.0% yield. At last, hydrolysis of **4** with KOH aq gave **5** (98.5% atom D according to its Mass Spectra (MS) spectrum of Figure 1) in 76.0% yield at 100°C for 6 hours.

The 4-step synthesis of stable isotope labeled Mabuterol **9** started with 3-chloro-4-amino-5-trifluoromethyl-acetophenone **6** as starting material (Scheme 3). The 3-chloro-4-amino-5-trifluoromethyl-α-bromoacetophenone **7** was obtained with 70.2% yield by the bromination of **6** with $CuBr_2$ at 70°C. Through the subsequent nucleophilic substitution of **7** with D_9-tert-Butylamine, the product **8** was obtained with 27.5% yield. At last, the target product **9** with



SCHEME 1 Synthesis of β-agonists



SCHEME 2 Synthesis of D_9-tert-Butylamine. Reagents and conditions: (I) I_2, H_2O, P, P_2O_5, 70°C, 1.5 hours; (II) diethyl ether, Mg, acetone-D_6, r.t, overnight; (III) NaCN, D_2SO_4, diethyl ether, acetic acid-D_3, overnight; and (IV) KOH aq, 6 hours, 100°C, 98.5% atom D.[a] [a] Calculated by Mass Spectra

80.0% yield was got by the reduction of **8** with sodium borohydride. The isotopic abundance was 98.5% atom D according to its MS spectrum of Figure 2.

For the synthesis of D_9-Bambuterol **13** as depicted in Scheme 4, 5-acetyl-1,3-phenylenebis(dimethylcarbamate) **10** was reacted with cupric bromide at 70°C to get 1-[bis-3′,5′-(*N,N*-dimethylcarbamoyloxy)-phenyl]-2-bromoethanone **11** with 75.4 % yield via further crystallization from chloroform. Through the subsequent amination of the D_9-tert-Butylamine, corresponding labeled intermediate 1-[bis-3′,5′-(*N,N*-dimethylcarbamoyloxy)-phenyl]-2-(*t*-butylamino) ethanone **12** was obtained with 31.5% yield. Then reduction of **12** with sodium borohydride gave **13** with 99.7 % atom D according to its MS spectrum of Figure 3.

D_9-Cimbuterol **19** was synthesized using a known method,[11] which using 1-(4-aminophenyl)ethan-1-one **14** as starting material (Scheme 5). The compound **15** was obtained through the bromination of **14** with an equal amount of *N*-bromosuccinimide. Then **15** was converted to the corresponding 5-acetyl-2-aminobenzonitrile **16** with 66.6% yield. Subsequently, crude **16** was brominated with cupric bromide at 70°C, which giving 2-amino-5-(2-bromoacetyl) benzonitrile **17** in 71.5% yield. **19** was obtained through the amination of **17** with the D_9-tert-Butylamine and reduction with sodium borohydride with 26.7% yield, (98.4% atom D according to its MS spectrum of Figure 4).

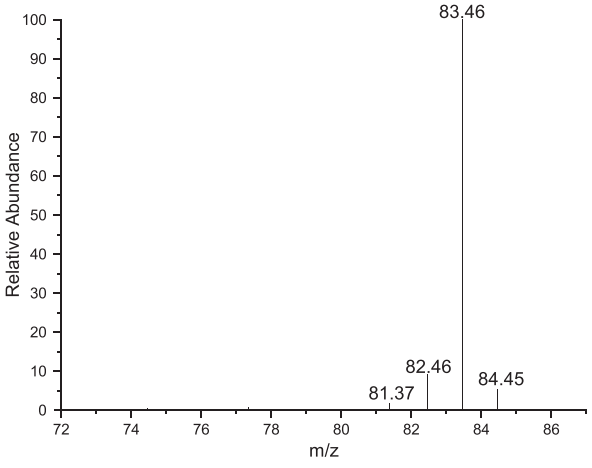
3 | EXPERIMENT

3.1 | Materials and instruments

All commercial reagents and solvents were used without further purification, unless otherwise specified. The labeled raw materials were purchased from J&K Chemical. Reactions were monitored by thin-layer chromatography using silica gel plate. ^{1}HNMR spectra were recorded on Bruker Avance III 600 MHz spectrometers using $CDCl_3$ unless otherwise stated with tetramethylsilane as an internal standard. The chemical shifts are reported in ppm relative to residual $CHCl_3$ ($\delta = 7.26$). Mass Spectra were obtained from TSQ Quantum Access spectrometer equipped with electrospray ionization source and reported as m/z. Purity was detected by HPLC (Essentia LC-15C) that used WondaSil C18-WR 4.6 × 150 mm, 5 um column, and the solvent which contained water and acetonitrile.

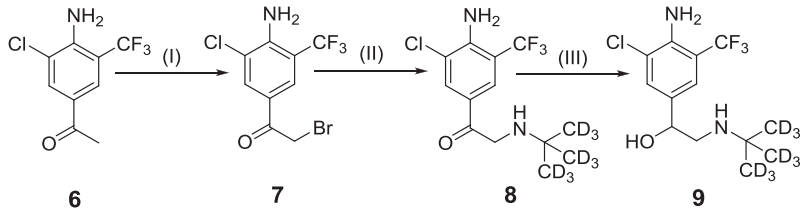
3.2 | D_3-Iodomethane (2)

A dry 250 mL flask, equipped with a reflux condenser, was charged with P (50.0 g, 1.6 mol), H_2O (100 mL), then I_2 (250.0 g, 1.0 mol) was added slowly into the flask at −15°C over 0.5 hour. After completion of the addition, D_4-methanol **1** (30.0 g, 0.8 mol) was added gradually. The reaction mixture was heated to 65°C and remained for 2 hours. After cooling, the mixture was distilled to give D_3-iodomethane **2** at 45°C in 85.0% yield (based on D_4-methanol).

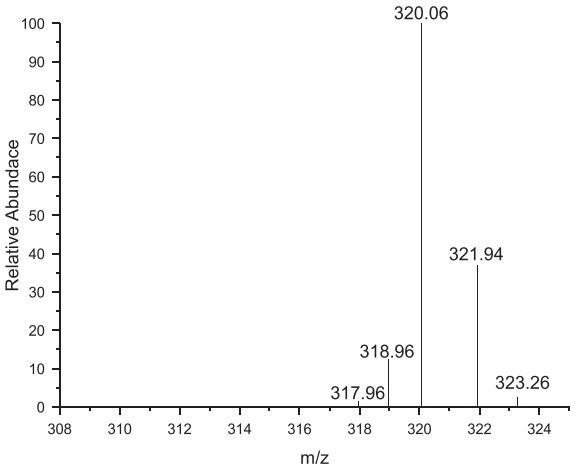


m/z	Intensity	Relative
73.44	4660.9	0.07
74.47	33079.5	0.50
75.43	2817.8	0.04
76.43	1028.8	0.02
77.35	46031.8	0.70
78.40	2693.1	0.04
79.42	2793.2	0.04
80.47	7895.1	0.12
81.37	111619.5	1.69
82.46	611133.7	9.23
83.46	6618931.1	100.00
84.45	348140.7	5.26
85.40	10659.4	0.16

FIGURE 1 Mass Spectra spectrum of **5**

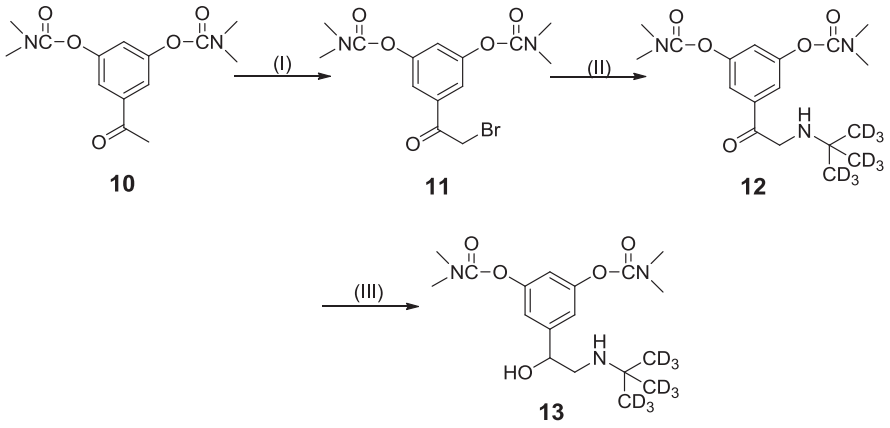


SCHEME 3 Synthesis of stable isotope labeled D_9-Mabuterol. Reagents and conditions: (I) $CuBr_2$, $CHCl_3$, ethyl acetate, ethanol, 70°C, 2.5 hours; (II) $(CD_3)_3CNH_2$, $CHCl_3$, 65°C, 2.5 hours; and (III) sodium borohydride, H_2O, CH_3OH, 6 N HCl, NH_3, 97.3%,[a] 98.5% atom D.[b] [a] Detected by HPLC. [b] Calculated by Mass Spectra

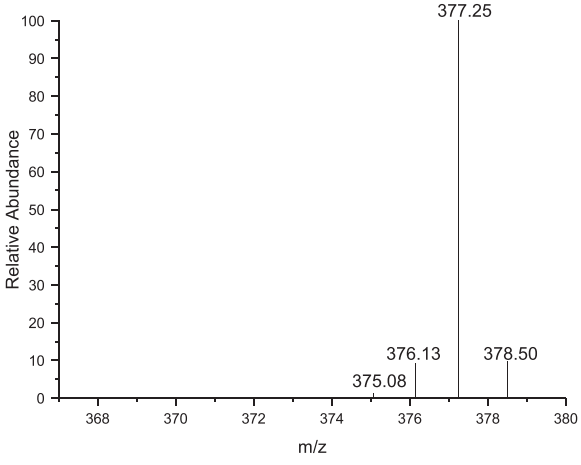


m/z	Intensity	Relative
309.22	5056.4	0.05
310.70	4605.8	0.05
312.03	3505.8	0.04
313.16	3749.1	0.04
314.25	6198.7	0.06
315.90	2712.6	0.03
316.84	20682.4	0.21
317.96	143581.4	1.48
318.96	1022405.8	12.42
320.06	9677398.7	100.00
321.94	3571649.3	36.91
323.26	230928.9	2.39

FIGURE 2 Mass Spectra spectrum of **9**

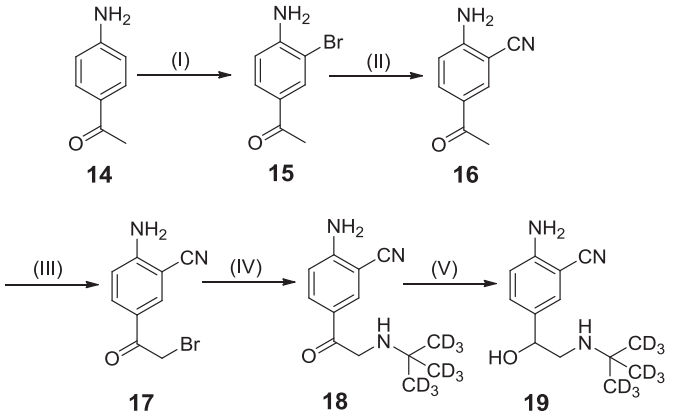


SCHEME 4 Synthesis of stable isotope labeled D_9-Bambuterol. Reagents and conditions: (I) $CuBr_2$, $CHCl_3$, ethyl acetate, ethanol, 70°C, 2.5 hours; (II) $(CD_3)_3CNH_2$, $CHCl_3$, 65°C, 2.5 hours; and (III) sodium borohydride, H_2O, CH_3OH, 6 N HCl, NH_3, 98.3%,[a] 99.7% atom D.[b] [a] Detected by HPLC. [b] Calculated by Mass Spectra

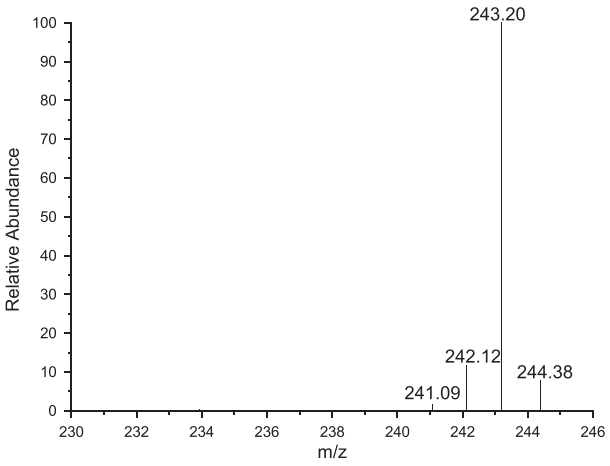


m/z	Intensity	Relative
368.01	4674.9	0.11
368.82	2687.2	0.06
370.08	2054.1	0.05
370.75	1104.5	0.03
372.40	50.2	0.00
373.03	1108.3	0.03
374.04	3637.7	0.09
375.08	50093.6	1.17
376.13	395764.4	9.26
377.25	4272871.1	100.00
378.50	416546.1	9.75

FIGURE 3 Mass Spectra spectrum of **13**



SCHEME 5 Synthesis of stable isotope labeled D_9-Cimbuterol. Reagents and conditions: (I) *N*-bromosuccinimide, 5% $NaHCO_3$, H_2O, ethanol, 35°C, 10 minutes; (II) *N,N*-dimethylformamide, cuprous cyanide, $FeCl_3$, HCl, H_2O, CH_2Cl_2; (III) $CuBr_2$, THF, H_2O, $MgSO_4$; (IV) $(CD_3)_3CNH_2$, ethanol, ethyl acetate, H_2O; and (V) ethanol, ethyl acetate, H_2O, sodium borohydride, 98.1%[a], 98.4% atom D.[b] [a] Detected by HPLC.[b] Calculated by Mass Spectra



m/z	Intensity	Relative
231.89	592.3	0.02
232.91	1612.2	0.07
233.94	5711.5	0.24
234.96	1932.0	0.08
235.98	1648.9	0.07
237.08	841.8	0.04
237.90	628.7	0.03
239.04	1167.7	0.05
240.22	4583.1	0.19
241.09	36770.9	1.54
242.12	277085.5	11.64
243.20	2380461.4	100.00
244.38	187489.0	7.88

FIGURE 4 Mass Spectra spectrum of **19**

3.3 | D_9-tert-Butanol (3)

D_3-iodomethane **2** (90.0 g, 0.6 mol) in dried ether (100 mL) was slowly added to magnesium (12.8 g, 0.8 mol) under nitrogen, to produce the Grignard reagent. After heating at reflux for 45 minutes, a solution of D_6-acetone (31.5 g, 0.5 mol) in dry ether (250 mL) was added dropwise in an ice bath. The mixture was stirred overnight at room temperature (r.t), and the magnesium complex was then decomposed by slow addition of 10% H_2SO_4 aq. The ether layer was separated, and the aqueous layer was subjected to extraction with ether. The combined extracts were dried over anhydrous magnesium sulfate. The solution was then reduced by distillation through a short fractionating column to give **3** at 85°C in 45% yield (based on D_6-acetone).

3.4 | D_9-*t*-butylformamide (4)

A dry 250 mL 3-necked flask equipped with a dropping funnel was charged with sodium cyanide (5.5 g, 0.1 mol). The mixture of D_2SO_4 (25.0 g, 0.3 mol) and D_3-acetic acid (10 mL) was slowly added. The stirred solution was heated to 55°C, and a solution of the compound **3** (5.0 g, 60.0 mmol) in D_3-acetic acid (15 mL) was added dropwise. Stirring was continued for 12 hours at r.t. Then the mixture was poured onto ice and neutralized with 15% NaOH aq (100 mL). The mixture was extracted with ether and reduced distilled to give **4** at 100°C at 30 mmHg (98.5% atom D) in 51.0% yield (based on D_9-tert-Butanol).

3.5 | D_9-tert-Butylamine (5)

The D_9-*t*-butylformamide **4** (4.0 g, 37.7 mmol) was boiled under reflux conditions with 30% KOH aq (50 mL) for 6 hours. After cooling, the solution was distilled to give **5** at 45°C in 76.0% yield (based on D_9-tert-Butanol). LC-MS: $[M - H]^-$ m/z 83.

3.6 | 3-chloro-4-amino-5-trifluoromethyl-α-bromoacetophenone (7)

A dry 250 mL 3-necked flask, equipped with a dropping funnel, was charged with **6** (7.0 g, 29.0 mmol), $CuBr_2$ (14.0 g, 63.0 mmol), $CHCl_3$ (60 mL), and ethyl acetate (60 mL). The reaction mixture was heated to 70°C and remained for 30 minutes; then ethanol (32 mL) was added slowly. After completion of the addition, the mixture was stirred at 70°C for 2 hours and was filtered. The filtrate was washed with water until no white precipitate. The organic phase was dried over anhydrous magnesium sulfate and evaporated under

vacuum to give the crude product. The pale yellow crystal **7** (6.5 g, 70.2%) was obtained through recrystallization by isopropyl ether. ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 4.33 (s, 2H), 5.24 (s, 2H), 8.03 (d, J = 1.5 Hz, 1H), 8.09 (d, J = 1.8 Hz, 1H). LC-MS: $[M - H]^-$ m/z 318.

3.7 | D_9-1-(3-amino-5-chloro-4-trifluoromethylphenyl)-2-(t-butylamino)ethanone (8)

A dry 100 mL 3-necked flask with a reflux condenser was charged with **7** (2.0 g, 6.2 mmol) and chloroform (25 mL). The solution was heated to 65°C and remained 30 minutes; then the D_9-tert-Butylamine (1.0 g, 12.0 mmol) was injected slowly over 30 minutes. The reaction mixture was stirred for 2 hours while some white solid was generated. After cooling, the mixture was filtered and to filtrate was added an isopropanol solution of hydrochloric acid giving a turbid solution. The pale yellow solid powder **8** (1.1 g, 27.5% based on D_9-tert-Butylamine) was obtained by filtration. ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 4.62 (s, 2H), 8.12 (d, J = 1.2 Hz, 1H), 8.22 (d, J = 1.2 Hz, 1H). LC-MS: $[M + H]^+$ m/z 318.

3.8 | D_9-Mabuterol (9)

To a stirred suspension of **8** (1.1 g, 3.5 mmol) and water (5 mL) in methanol (10 mL), sodium borohydride (0.6 g, 15.9 mmol) in water (1 mL) was added gradually at 0°C. The system was stirred for 2 hours at r.t, adjusted to acidity by 6 N hydrochloric acid. After stirring overnight, sodium borohydride (0.6 g, 15.9 mmol) in water (1 mL) was added slowly into the solution. Adjusted the pH to 2 ~ 5 by 6 N hydrochloric acid, the system was filtered and to the filtrate was added ammonia until no white precipitate appeared. The white solid **9** (0.9 g, 80.0%) was obtained by filtration. ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 2.53 (d, J = 0.6 Hz, 1H), 2.87 (d, J = 1.2 Hz, 1H), 4.53 (m, 1H), 4.60 (s, 1H), 7.36 (s, 1H), 7.47 (s, 1H). LC-MS: $[M + H]^+$ m/z 320.

3.9 | 1-[bis-3′,5′-(*N*,*N*-dimethylcarbamoyloxy)-phenyl]-2-bromoethanone (11)

To a suspension of **10** (8.8 g, 30.0 mmol) and $CuBr_2$ (13.4 g, 60.0 mmol) in $CHCl_3$ (80 mL) and ethyl acetate (80 mL) at 70°C was added ethanol (40 mL) over 30 minutes. The reaction mixture was stirred for 2 hours, then filtered. The cake was washed with ethyl acetate (40 mL), and the filtrate was washed with water until no white precipitate. The organic layer was dried over anhydrous magnesium sulfate and removed in vacuum and crystallized in chloroform to give a yellow solid **11** (8.4 g, 75.4%). ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 3.00 ~ 3.08 (s, 12H), 4.41 (s, 2H), 7.25 (t, J = 1.2 Hz, 1H), 7.57 (d, J = 1.8 Hz, 2H). LC-MS: $[M + H]^+$ m/z 374.

3.10 | D_9-1-[bis-3′,5′-(*N*,*N*-dimethylcarbamoyloxy)-phenyl]-2-(*t*-butylamino)ethanone (12)

To a stirred solution of **11** (3.7 g, 10.0 mmol) in chloroform (40 mL) at reflux state was added the D_9-tert-Butylamine (1.6 g, 20.0 mmol) over 30 minutes. The reaction mixture was stirred for 6 hours, while white solid generating. Then, the mixture was filtered, and the filtrate was washed with water, which was dried over anhydrous magnesium sulfate and concentrated in vacuum to give solid. The solid was washed with isopropyl ether to give **12** (2.4 g, 31.5 % based on D_9-tert-Butylamine). ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 2.98 ~ 3.07 (s, 12H), 3.77 (s, 2H), 7.16 (d, J = 1.8 Hz, 1H), 7.40 (d, J = 1.8 Hz, 2H). LC-MS: $[M + H]^+$ m/z 375.

3.11 | D_9-Bambuterol (13)

To a solution of **12** (2.4 g, 6.3 mmol) and water (20 mL) in ethanol (20 mL) at 0°C was added sodium borohydride (1.0 g, 25.2 mmol) gradually. The reaction mixture was stirred for 4 hours at r.t and then added water (30 mL) and ethyl acetate (40 mL). The layers were separated, and the organic layers was washed with water (20 mL × 2), dried over anhydrous magnesium sulfate, and concentrated in vacuum to yield white solid **13** (1.9 g, 81.3%). ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 2.94 ~ 3.02 (m, 12H), 2.85 ~ 3.20 (m, 2H), 5.35 (s, 1H), 6.90 (t, J = 2.4 Hz, 1H), 7.10 (d, J = 2.4 Hz, 2H), 8.09 (br, 1H), 9.80 (br, 1H). LC-MS: $[M + H]^+$ m/z 377.

3.12 | 1-(4-amino-3-bromophenyl)ethan-1-one (15)

The 1-(4-aminophenyl)ethan-1-one **14** (4.1 g, 30.0 mmol) and toluene (50 mL) were added to a round-bottom flask equipped with a reflux condenser. *N*-bromosuccinimide (5.3 g, 30.0 mmol) was added to the solution over 10 minutes. Stirring was continued for 10 minutes at 35°C. After completion of reaction, the organic layers were successively washed with a solution of 5% $NaHCO_3$ (50 mL) and water (50 mL × 2). Removal of the solvent under vacuum yielded a dark brown oil. The water was added into the crude product, which was dissolved in ethanol, as the white crystal appearing. Then **15** was collected by filtration (5.9 g, 91.2%). ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 2.51 (s, 3H), 4.57 (brs, 2H), 6.74 (d, J = 12.6 Hz, 1H), 7.74 (dd, J = 3.0, 3.0 Hz, 1H), 8.07 (d, J = 3.0 Hz, 1H). LC-MS: $[M - H]^-$ m/z 212.

3.13 | 5-acetyl-2-aminobenzonitrile (16)

Cuprous cyanide (1.8 g, 20.0 mmol) was added to a mixture of **15** (3.6 g, 16.6 mmol) dissolved in *N*,*N*-dimethylformamide (20 mL). The mixture was stirred at 160°C under nitrogen for 6 hours. The progress of the reaction was monitored using thin-layer chromatography (hexane : ethyl acetate = 2:1). The resulting mixture was

cooled to r.t, and the mixture was treated with a solution of ferric chloride (prepared from ferric chloride (6.6 g), hydrochloric acid (6.6 mL), and water (20 mL)) and stirred for 30 minutes at 25°C. The reaction mixture was poured into H_2O (50 mL) followed by extraction with dichloromethane (30 mL × 3). The combined organic layers were dried over anhydrous magnesium sulfate and filtered, and the solvent was removed in vacuum to give 5-acetyl-2-aminobenzonitrile **16** (1.8 g, 66.6%) as yellow solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 2.51 (s, 3H), 4.89 (brs, 2H), 6.77 (d, J = 9.0 Hz, 1H), 7.96 (dd, J = 2.4, 1.8 Hz, 1H), 8.03 (d, J = 1.8 Hz, 1H). LC-MS: $[M - H]^-$ m/z 159.

3.14 | 2-amino-5-(2-bromoacetyl)benzonitrile (17)

The 5-acetyl-2-aminobenzonitrile **16** (3.0 g, 18.7 mmol), $CuBr_2$ (8.4 g, 37.6 mmol) and tetrahydrofuran (30 mL) were added to a flask equipped with a reflux condenser. The mixture was stirred at 70°C for 2 hours. After completion of reaction, the mixture was poured into ice water (300 mL) and extracted with ethyl acetate (50 mL × 3). The combined organic layer was dried over anhydrous magnesium sulfate and filtered, and the solvent was removed in vacuum to give **17** (3.2 g, 71.5%) as pale yellow solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 4.31 (s, 2H), 4.97 (brs, 2H), 6.79 (d, J = 8.4 Hz, 1H), 7.98 (dd, J = 1.8, 2.4 Hz, 1H), 8.09 (d, J = 1.8 Hz, 1H). LC-MS: $[M - H]^-$ m/z 237.

3.15 | D_9-Cimbuterol (19)

2-Amino-5-(2-bromoacetyl)benzonitrile **17** (2.0 g, 8.5 mmol) and ethanol (25 mL) were added to a round-bottom glass flask equipped with a magnetic stirring bar using an ice bath. D_9-tert-Butylamine (1.1 g, 16.9 mmol) was added to the reaction mixture maintaining temperature at 0 ~ 10°C, followed by stirring in the ice bath for 0.5 hour. After the completion of addition, the reaction mixture was stirred at 60°C under nitrogen for 2 hours. The resulting mixture was cooled to r.t, while the sodium borohydride (0.5 g, 13.5 mmol) was added slowly. After stirring for 4 hours, the reaction mixture was diluted with ethyl acetate (200 mL) and dried over anhydrous magnesium sulfate. The crude product was purified by column chromatography on a silica gel column using chloroform/methanol (15:1) as eluent to give white solid **19** (1.4 g, 36.7% based on D_9-tert-Butylamine, 98.4% atom D). ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 4.34 (s, 1H), 4.37 (s, 1H), 5.90 (s, 2H), 6.74 (d, J = 8.4 Hz, 1H), 7.27 (d, J = 1.8 Hz, 1H), 7.29 (s, 1H). LC-MS: $[M + H]^+$ m/z 243.

4 | CONCLUSIONS

In summary, 3 synthetic methods of stable isotope labeled D_9-Mabuterol, D_9-Bambuterol, and D_9-Cimbuterol were described with 98.5%, 99.7%, and 98.4% isotopic abundance, respectively, and their structure and isotope-abundance were confirmed by ^{1}H NMR and LC-MS.

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REFERENCES

1. Braman SS. The global burden of asthma. *Chest*. 2006;130:4–12.
2. Kikkawa H, Kanno K, Ikezawa K. TA-2005, a Novel, Long-Acting, and Selective β_2-Adrenoceptor Agonist: Characterization of Its in Vivo Bronchodilating Action in Guinea Pigs and Cats in Comparison with Other β_2-Agonists. *Biol Pharm Bull*. 1994;17(8):1047–1052.
3. Vilsvik JS, Langaker O, Persson G, et al. Bambuterol: a new long acting bronchodilating prodrug. *Ann Allergy*. 1991;66(4):315–319.
4. Kruger G, Keck K. *Drug Res*. 1984;34:1612
5. Kruger G, Noll K, Pieper H, et al. Aminophenylethanolamines: DE, 2354961 [P].1975.
6. Shao B, Jia X, Zhang J, et al. Multi-residual analysis of 16 β-agonists in pig liver, kidney and muscle by ultra performance liquid chromatography tandem mass spectrometry. *Food Chem*. 2009;114(3):1115–1121.
7. He P, Shen L, Liu R, Lou Z, Li Z. Direct Detection of β-Agonists by Use of Gold Nanoparticle-Based Colorimetric Assays. *Anal Chem*. 2011;83 (18):6988–6995.
8. Wang W, Zhang Y, Wang J, Shi X, Ye J. Determination of β-agonists in pig feed, pig urine and pig liver using capillary electrophoresis with electrochemical detection. *Meat Sci*. 2010;85(2):302–305.
9. Blanca J, Muñoz P, Morgado M, et al. Determination of clenbuterol, ractopamine and zilpaterol in liver and urine by liquid chromatography tandem mass spectrometry. *Anal Chim Acta*. 2005;529(1-2):199–205.
10. Holman RJ, Perkins MJ. A probe for homolytic reactions in solution. Part V. Perdeuterionitrosobutane: an improved spin trap. *J Chem Soc. C* 1971;2324–2326.
11. Asato G, Baker PK, Bass RT, et al. Repartitioning Agents: 5-[11-Hydroxy- 2-(isopropylamino)ethyl]- anthranilonitrile and Related Phenethanolamines; Agents for Promoting Growth, Increasing Muscle Accretion and Reducing Fat Deposition in Meatproducing Animals. *Agric Biol Chem*. 1984;48 (11):2883–2888.

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