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# Efficient and facile synthesis of novel stable monodeuterium labeled ractopamine

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**A novel synthetic route to stable deuterium labeled ractopamine was disclosed with 6.49% total yield and 97.7% isotopic abundance. Its structure and the isotope-abundance were confirmed according to $^1$H-NMR and high-resolution mass spectrometry.**

**Keywords:** deuterium labeling; drug standards; ractopamine; $\beta$-adrenergic agonists

## Introduction

Ractopamine, a $\beta$-adrenergic agonist, can improve the efficiency of feed utilization and enhance the meat leanness in livestock.[1,2] However, because of the residue of the ractopamine, the meat products obtained from treated animals may pose a potential risk for consumer health, such as asthma or angiocardiopathy.[3] Thus, it had been prohibited as a growth promoter of farm animals in several countries, such as the European Union, Japan, and China.[4,5]

Recently, many methods had been developed for the analysis of ractopamine, including high-performance liquid chromatography, gas chromatography-mass spectrometry, and liquid chromatography-mass spectrometry. But these methods had some defects for determining the residue of ractopamine in animal tissues because of interference from other impurities and losses in the sample purification process. More prominent and reliable analytical methods had aroused the researchers' interest,[6–8] in which employing isotopically labeled compounds as internal standards could adjust for the losses in sample pretreatment and purification, offset the error caused by ion suppression in the mass spectrometric detection process, and make the results closer to the true value efficiently.

This deuterium labeled ractopamine could be used for the preliminary and qualitative screening of veterinary drug residues and metabolic mechanism study of ractopamine as its metabolic stability.[9] To date, there were very few procedures described in the literature for the preparation of deuterium labeled ractopamine. Li's group applied a patent for the preparation of $d_3$- and $d_4$-ractopamine.[10] Therefore, a promising and practical route is reported in this article.

## Results and discussion

The nine-step synthesis of the labeled ractopamine **10** started with commercially available *p*-methoxybenzaldehyde as depicted in Scheme 1. First of all, in the presence of NaOH as the catalyst and at 25 °C, *p*-methoxybenzaldehyde was reacted with the excess amount of acetone to furnish $\alpha$, $\beta$-unsaturated ketone **2** with 67% yield via aldol condensation and subsequent dehydration. Through the subsequent hydrogenation of the carbon-carbon double bond, corresponding saturated ketone **3** was obtained with 61% yield.

Reduction of **3** using 99% sodium borodeuteride gave **4** in 93.1% yield. Efficient tosylation was achieved in the presence of 4-dimethylaminopyridine and triethylamine in 30 min at room temperature. Substitution of the tosyloxy group of **5** with sodium azide furnished azide **6**, which was subsequently reduced to the corresponding amine **7** under reduction conditions with 95% yield over two steps.

Compound **7** coupled with 2-bromo-4′-methoxyacetophenone in the presence of N, N-diisopropylethylamine in tetrahydrofuran to furnish compound **8**, which existed in its enol form as confirmed by $^1$H-NMR of non-deuterium labeled compound **8′**. In Figure 1, the chemical shift of $H_A$, attached to the enol, was found at 8.02 ppm as a single peak, while no characteristic signals of its corresponding keto-tautomer (the CO-C$\underline{H_2}$-NH signal might occur at 3.00–4.00 ppm with two hydrogen atoms) was found in this spectrum.

Subsequently, quick reduction of crude **8** in the presence of catalyst 10% Pd/C under an atmosphere of hydrogen gave the desired product **9**. After the demethylation of **9** by treatment with borontribromide at −60 °C, the desired compound **10** was obtained with 70% yield.

From the $^1$H-NMR spectrum Figure 2, the $H_B$ of **10′**, non-deuterium labeled ractopamine, was observed at 3.27–3.30 ppm, while no corresponding signal was observed in the spectrum of **10**. So the deuterium atom was successfully introduced to the desired position, and the isotopic abundance was 97.7% according to its high-resolution mass spectrometry (HRMS) of Figure 3.

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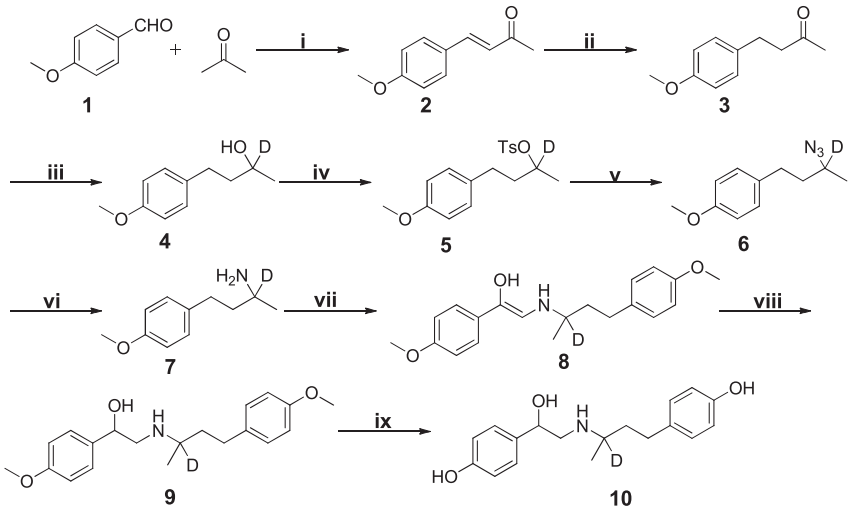



**Scheme 1.** Synthesis of monodeuterium labeled ractopamine. Reagents and conditions: (i) 10% NaOH(aq), rt, 1.5 h, 67%; (ii) H2, Raney Ni, EA, rt, 4 h, 61%; (iii) NaBD4, THF, rt, 5 h, 93%; (iv) TsCl, NEt3, DMAP, DCM, rt, 30 min, 64.0%; (v) NaN3, DMF, rt, 10 h, 96.8%; (vi) H2, 10% Pd/C, MeOH, rt, 10 h, 98%; (vii) 2-bromo-4′-methoxyacetophenone, iPr2NEt, THF, rt, 3 h; (viii) H2, 10% Pd/C, MeOH, rt, 4 h; and (ix) BBr3, DCM, rt, 6 h, 70%.

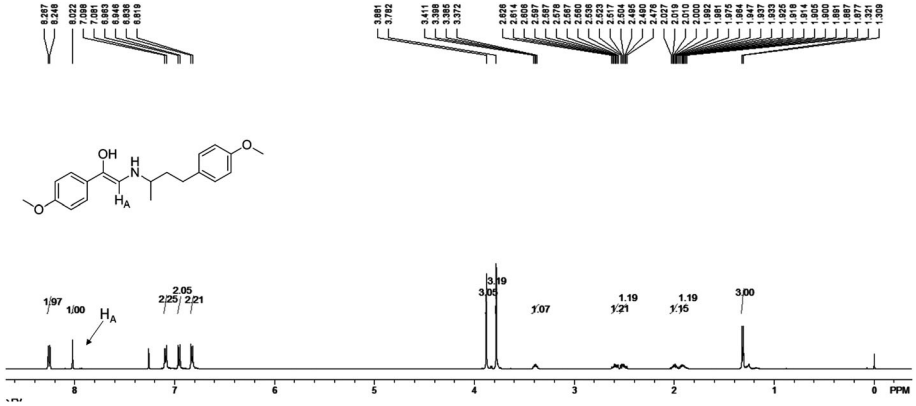



**Figure 1.** $^{1}$H-NMR spectrum of **8′**.

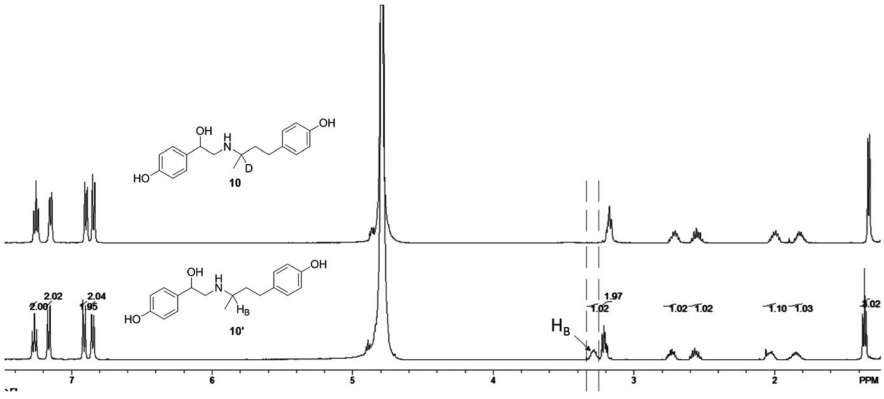
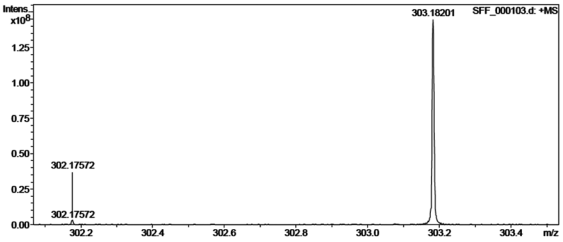



**Figure 2.** $^{1}$H-NMR spectrum of **10** and **10′**.




| m/z | z | I | Res. |
|---|---|---|---|
| 302.17572 | | 3455844 | 54482 |
| 303.07579 | | 703093 | 61287 |
| 303.15884 | | 985231 | 104219 |
| 303.16274 | | 1420560 | 92352 |
| 303.16859 | | 2577554 | 69036 |
| 303.18201 | | 145719824 | 47362 |
| 303.20430 | | 1266844 | 91011 |
| 303.21081 | | 973470 | 91239 |

**Figure 3.** HRMS spectrum of **10**.

## Conclusion

In summary, the stable isotope labeled ractopamine had been prepared with 6.49% total yield and 97.7% isotopic abundance. Its structure was confirmed by $^1$H-NMR and HRMS.

## Experimental

### General

All reactions were carried out under an atmosphere of nitrogen. All commercially available reagents and solvents were used without purification. The stable labeled raw material, sodium borodeuteride, was purchased from CIL. $^1$H-NMR spectra were recorded on Bruker Avance III 500 MHz spectrometers using $CDCl_3$, $D_2O$, and DMSO-$d_6$ unless otherwise stated. HRMS data were obtained from Bruker solanX 70 equipped with Electrospray ionization source. Column chromatography and preparative thin-layer chromatography were carried out by using Merck silica gel 60 (230–400 mesh), respectively. Purity was determinated by high-performance liquid chromatography (Essentia LC-15C) that used WondaSil C18-WR 4.6 * 150 mm, 5 μm column, and the solvent which contained water and acetonitrile.

*(E)-4-(4-methoxyphenyl)but-3-en-2-one (**2**)*

A 5% aqueous sodium hydroxide (2 mL) was added gradually to a solution of *p*-methoxybenzaldehyde (64.58 g, 0.47 mol) and acetone (177 mL, 2.38 mol) in water at 0 °C. The reaction mixture was stirred at room temperature for 1.5 h and then neutralized with 1 N hydrochloric acid to form a yellow solid. The crude product was filtered, washed with cold water, and dried then purified by column chromatography on a silica gel column using petroleum ether/ethyl acetate (7:1) as eluent to yield **2** (55.79 g, 67%). $^1$H NMR (500 MHz, $CDCl_3$) δ ppm 7.50 (d, $J$ = 8.7 Hz, 2H), 7.47 (d, $J$ = 16.8 Hz, 1H), 6.92 (d, $J$ = 8.8 Hz, 2H), 6.61 (d, $J$ = 16.2 Hz, 1H), 3.84 (s, 3H), and 2.36 (s, 3H).

*4-(4-methoxyphenyl)butan-2-one (**3**)*

To a stirred solution of **2** (7.20 g, 0.04 mol) in ethyl acetate, (35 mL) was added Raney nickel (0.8 mL), the solution was stirred for 4 h under hydrogen atmosphere at room temperature. The reaction mixture was filtered, washed with ethyl acetate, and the crude product obtained by the evaporation of the solvent was chromatographed over a silica gel column using petroleum ether/ethyl acetate (15:2) as eluent to furnish **3** (4.47 g, 61%). $^1$H NMR (500 MHz, $CDCl_3$) δ ppm 7.09 (d, $J$ = 8.6 Hz, 2H), 6.82 (d, $J$ = 8.6 Hz, 2H), 3.77 (s, 3H), 2.83 (t, $J$ = 7.3 Hz, 2H), 2.71 (t, $J$ = 7.2 Hz, 2H), and 2.12 (s, 3H).

*4-(4-methoxyphenyl)butan-2-ol (**4**)*

Reduction of **3** (2.25 g, 12.6 mmol) was carried out with sodium borodeuteride (0.82 g, 19.6 mmol) in tetrahydrofuran (100 mL) for 5 h at 25 °C. After completion of the reaction, the reaction mass was neutralized with dilute HCl (5%) and extracted with dichloromethane. The organic layer was dried over $Na_2SO_4$ and evaporated under vacuum. Compound **4** was obtained as colorless oil in 93% yield. $^1$H NMR (500 MHz, $CDCl_3$) δ ppm 1.21 (s, 3H), 1.44 (s, 1H), 1.68–1.77 (m, 2H), 2.59–2.72 (m, 2H), 3.79 (s, 3H), 6.83 (d, $J$ = 8.5 Hz, 2H), 7.12 (d, $J$ = 8.4 Hz, 2H). HRESIMS: $[M + Na]^+$ $m/z$ 204.1111 (Calcd for $[C_{11}H_{15}DO_2+Na]^+$ 204.1111)

*4-(4-methoxyphenyl)butan-2-yl 4-methylbenzenesulfonate (**5**)*

To a stirred solution of **4** (2.13 g, 11.7 mmol) in dichloromethane (21 mL), containing a catalytic amount of 4-dimethylaminopyridine (3.12 g, 25.5 mmol) and triethylamine (21 mL, 151 mmol), was added dropwise the *p*-toluensulfonyl chloride (9.05 g, 47.47 mmol) in dichloromethane (30 mL) at 0 °C under nitrogen. The reaction was monitored by thin-layer chromatography. At the end of the reaction, the reaction mixture was concentrated to give the crude product, which was purified by flash column chromatograph on silica gel using petroleum ether/ethyl acetate (7:1) as eluent to yield **5** (2.53 g, 64.0%) as a pale yellow liquid. $^1$H NMR (500 MHz, $CDCl_3$) δ ppm 1.28 (s, 3H), 1.73–1.93 (m, 2H), 2.43 (s, 3H), 2.42–2.58 (m, 2H), 3.78 (s, 3H), 6.79 (d, $J$ = 8.5, 2H), 6.98 (d, $J$ = 8.5, 2H), 7.33 (d, $J$ = 8.1, 2H), 7.79 (d, $J$ = 8.2, 2H). HRESIMS: $[M + Na]^+$ $m/z$ 358.1190 (Calcd for $[C_{18}H_{21}DO_4S+Na]^+$ 358.1199)

*1-(3-azidobutyl)-4-methoxybenzene (**6**)*

To a stirred solution of **5** (2.52 g, 7.51 mmol) in DMF (100 mL) under nitrogen atmosphere was added sodium azide (1.52 g, 23.4 mmol). After stirring for 10 h at room temperature, the reaction mixture was quenched with water and extracted with ethyl acetate. The combined organic extracts were dried over anhydrous $Na_2SO_4$ and concentrated to give the crude product, which was purified by flash column chromatograph on silica gel using petroleum ether/ethyl acetate (19:1) as eluent to yield azide **6** (1.50 g, 96.8%). $^1$H NMR (500 MHz, $CDCl_3$) δ ppm 1.28 (s, 3H), 1.69–1.82 (m, 2H), 2.58–2.73 (m, 2H), 3.79 (s, 3H), 6.84 (d, $J$ = 8.6 Hz, 2H), 7.11 (d, 2H, $J$ = 8.5 Hz). HRESIMS: $[M + H]^+$ $m/z$ 207.1288 (Calcd for $[C_{11}H_{14}DN_3O+H]^+$ 207.1356 )

*4-(4-methoxyphenyl)butan-2-amine (**7**)*

To a solution of **6** (1.50 g, 7.27 mmol) in methanol (50 mL) was added 10% palladium on carbon, and hydrogenated under hydrogen atmosphere at room temperature for 10 h. The reaction mixture was filtered, and the filtrate was concentrated to yield **7** (1.29 g, 98%). $^1$H NMR (500 MHz, $CDCl_3$) δ ppm 1.09 (s, 3H), 1.61 (m, 2H), 2.57–2.62 (m, 2H), 3.78 (s, 3H), 6.82 (d, $J$ = 8.5 Hz, 2H), 7.10 (d, $J$ = 8.5 Hz, 2H). HRESIMS: $[M+H]^+$ $m/z$ 181.1434 (Calcd for $[C_{11}H_{16}DNO+H]^+$ 181.1451 )

*(Z)-1-(4-methoxyphenyl)-2-((4-(4-methoxyphenyl)butan-2-yl) amino)ethanol (**8**)*

N, N-diisopropylethylamine (1.70 mL, 9.73 mmol) was added dropwise to a mixture of 2-bromo-4′-methoxyacetophenone (72.6 mg, 0.317 mmol), powdered molecular sieves (4 Å, 0.5 g) and **7** (114 mg, 0.632 mmol) in tetrahydrofuran (10 mL) and stirred for 3 h under nitrogen. The reaction mixture was filtered, and the solvent removed under reduced pressure to afford crude **8** which was used in the next step without further purification.

*1-(4-methoxyphenyl)-2-((4-(4-methoxyphenyl)butan-2-yl)amino) ethanol (**9**)*

To a stirred solution of **8** in methanol (10 mL) was added palladium (10% on carbon, 20 mg), the solution was stirred for 4 h under hydrogen atmosphere at room temperature. The reaction mixture was filtered, washed with methanol, and the crude product obtained by the evaporation of the solvent was chromatographed over a silica gel column using dichloromethane/methanol (7:3) as eluent to furnish **9** (84 mg, 40.2%). $^1$H NMR (500 MHz, $CDCl_3$,) δ ppm 1.25 (d, $J$ = 6.7 Hz, 3H), 1.73–1.99 (m, 2H), 2.55–2.69 (m, 2H), 2.72–3.05 (m, 2H), 3.77 (d, $J$ = 0.9 Hz, 3H), 3.79 (s, 3H), 4.90 (d, $J$ = 7.8 Hz, 1H), 6.80 (d, $J$ = 8.5 Hz, 2H), 6.85 (d, $J$ = 8.2 Hz, 2H), 7.08 (dd, $J$ = 8.5, 4.0 Hz, 2H), 7.28 (dd, $J$ = 8.7, 2.0 Hz, 2H). HRESIMS: $[M+H]^+$ $m/z$ 331.2122 (Calcd for $[C_{20}H_{26}DNO_3+H]^+$ 331.2132)

*4-(1-hydroxy-2-((4-(4-hydroxyphenyl) butan-2-yl) amino)ethyl)phenol (**10**)*

To a solution of **9** (62 mg, 0.19 mmol) in dichloromethane (5 mL) cooled at −60 °C under nitrogen was slowly added boron tribromide (0.5 mL, 5.3 mmol). The reaction mixture was stirred for 1 h at −60 °C and then allowed to warm to room temperature. After quenching with 10% $NaHCO_3$ at 0 °C, the mixture was extracted with ethyl acetate. The organic phases were dried over $MgSO_4$ and concentrated under reduced pressure to give **10** (40 mg, 70%). $^1$H NMR (500 MHz, $D_2O$) δ ppm 1.32 (d, $J$ = 6.4 Hz, 3H), 1.77–2.03 (m, 2H), 2.50–2.74 (m, 2H), 3.17 (m, 2H), 4.86 (m, 1H), 6.83 (d, $J$ = 8.4 Hz, 2H), 6.89 (d, $J$ = 8.3 Hz, 2H), 7.14 (d, $J$ = 6.9 Hz, 2H), 7.24 (t, $J$ = 8.2 Hz, 2H). HRESIMS: $[M+H]^+$ $m/z$ 303.1820 (Calcd for $[C_{18}H_{22}DNO_3+H]^+$ 303.1819)

## Conflict of interest

The authors did not report any conflict of interest.

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