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Synthesis of $^{13}C,^{2}H_3$-Salmeterol: An Analytical Internal Standard for Pharmacokinetic Studies

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SUMMARY

A convenient synthesis of an isotopically-labelled version ($^{13}C,^{2}H_3$) of the long-acting β_2-adrenergic agonist, salmeterol, is described, starting from methyl salicylate, 1,6-dibromohexane and 4-phenyl-1-butanol. This compound was designed as an analytical internal standard for mass spectrometric determination of salmeterol in pharmacokinetic studies.

Key Words: $^{13}C,^{2}H_3$-salmeterol, β_2-adrenergic agonist, pharmacokinetics, mass spectrometry.

INTRODUCTION

Salmeterol (**1**), originally described by Skidmore *et al.* (1,2) is a potent, long-acting β_2-adrenergic agonist that is widely used as an inhaled medication for the treatment of asthma (3). Salmeterol, administered as its xinafoate (1-hydroxy-2-naphthoate), is reported (4) to be over 10,000 times more hydrophobic than its predecessor, albuterol (also known as salbutamol; structure **1** where a *t*-butyl

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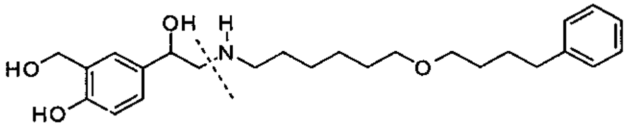
group replaces the lengthy *N*-substituent). This increased hydrophobicity accounts for the long-acting bronchodilating effect from inhalation of salmeterol (5).



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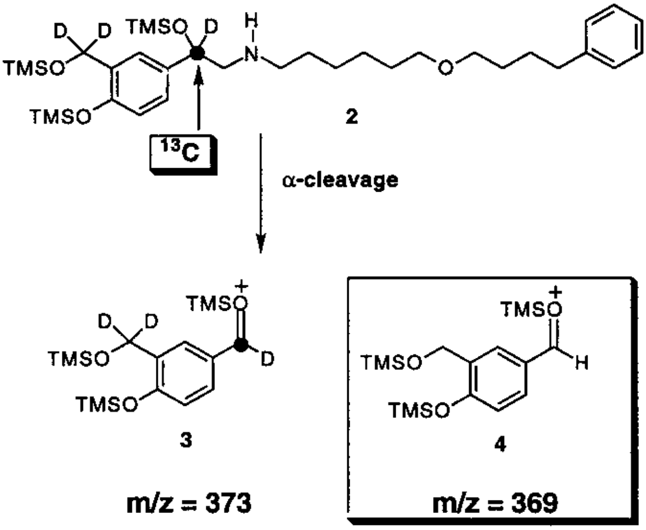
A number of racemic salmeterol syntheses have been carried out, virtually all of which have involved the strategic bond connection illustrated (dashed line) on structure **1** (6). Most syntheses have involved an α-bromoacetophenone, leading to the left half of structure **1**, as the electrophilic partner in an Sn2 reaction with a long-chain amine, the right half of structure **1** (1,2,7). Two enantioselective syntheses of R- and S-salmeterol have been reported, both of which proceed from an achiral α-bromoacetophenone. One approach relied upon a chiral auxiliary derived from optically active phenylglycinol (8), while in the second synthesis, chirality was conferred by a catalytic asymmetric borane reduction (9).

The pharmacokinetics of inhaled salmeterol have not been well described due to the difficulty of measuring low plasma levels of the drug following inhalation. Recent reports from our laboratories described a GC-MS assay to quantitate plasma levels of albuterol as low as 50 pg/mL (10), and the use of this technique to study the pharmacokinetics of inhaled albuterol in humans (11). This assay relied upon utilization of an internal standard which was an isotopically-labelled version of albuterol. Ideally, an internal standard (or its derivative) should exhibit an easily-measured major fragment in the mass spectrum at a m/z where the spectrum of the parent compound is blank.

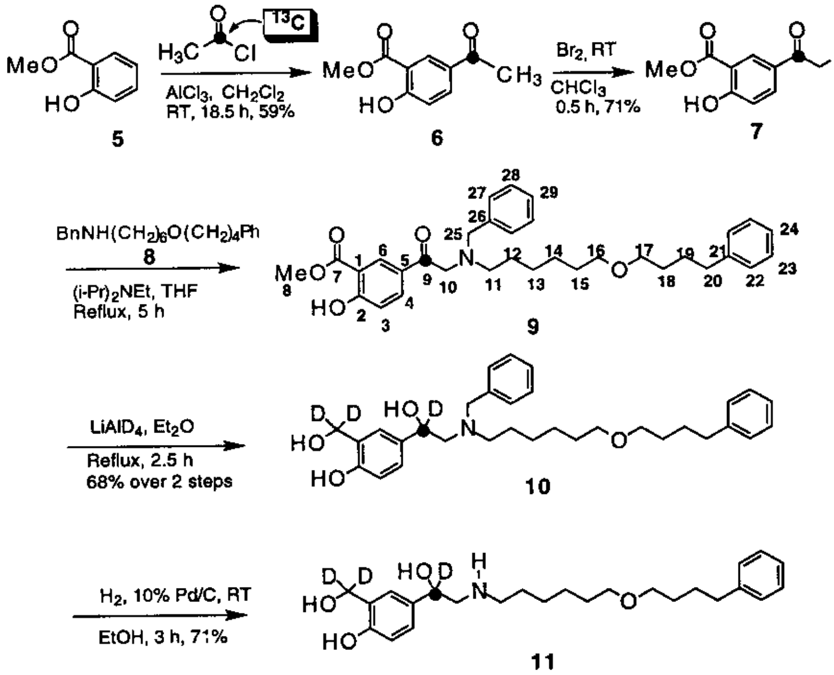
A $^{13}C,^{2}H_3$-salmeterol could serve as an excellent internal standard for the quantitation of salmeterol in plasma. For example, the tris-trimethylsilyl (TMS) ether derivative **2** should produce the α-cleavage cation **3** as a major fragment in its electron impact mass spectrum (10). The analogous tris-TMS derivative of salmeterol itself displays fragment **4** at m/z 369 and nothing is seen at m/z 373 (12,13). This paper details a straightforward and efficient synthesis of the requisite $^{13}C,^{2}H_3$-salmeterol, *i.e.* the precursor to TMS derivative **2** (14).



RESULTS AND DISCUSSION

Friedel-Crafts acylation of methyl salicylate (**5**) with acetyl-1-^{13}C chloride provided ketone **6** (15), which was brominated to give α-bromoacetophenone **7** (16). The next step was envisaged to be a nucleophilic substitution by benzylamine **8**, which was prepared from 1,6-dibromohexane and 4-phenyl-1-butanol using minor modifications of published procedures (9,17). This product was conveniently purified and stored as the hydrochloride (7). Reaction of bromide **7** with benzylamine **8** in the presence of diisopropylethylamine afforded the keto-ester **9** as a pale yellow oil which decomposed upon attempted silica gel chromatography, as well as upon prolonged cold storage (18).

Three deuterium atoms were introduced simultaneously by reduction of compound **9** with excess $LiAlD_4$ to give the racemic alcohol **10**. In previous salmeterol syntheses, $LiAlH_4$ reduction of unprotected phenolic compounds such as **9** were plagued by formation of emulsions during workup, thus leading to low yields of the desired product (1,2,19). As described in the experimental section, we have circumvented this problem by careful control of the pH during workup. Next, debenzylation was effected with H_2/Pd at atmospheric pressure (8) to give the desired ^{13}C,^{2}H$_3$-salmeterol (**11**), which could be converted to its xinafoate salt by treatment with 1-hydroxy-2-naphthoic acid (1,2,7,8).



The carbon-13 and deuterium substitutions on the compounds described above produce some typical coupling and chemical shift perturbations of the ^{13}C and ^{1}H NMR spectra as reflected in the assignments detailed in the experimental section. Since $^{1}H,^{2}H$ coupling constants are only about one-sixth of the corresponding $^{1}H,^{1}H$ coupling constants, they are often detectable only as a slight broadening of the resonance lines (20). Interestingly, in the ^{13}C NMR spectra for compounds **10** and **11**, the quintet for C-7 (see numbering (18) on structure **9**) has "disappeared" into the baseline noise. It is smaller not only by virtue of being split into five peaks, but also because NOE enhancement is lost that results from proton decoupling in the unlabelled compound (21). In addition, there is an expected deuterium-induced ^{13}C NMR chemical shift shielding for C-9 as exemplified by compound **10**, for which the C-9 resonance appears at 68.9 ppm, versus 69.3 ppm for the unlabelled analogue.

CONCLUSION

In summary, a convenient synthesis of a $^{13}C,^{2}H_3$-salmeterol (**11**) from methyl salicylate, 1,6-dibromohexane and 4-phenyl-1-butanol has been accomplished. Indeed, the tris-TMS ether derivative **2** produces the expected α-cleavage cation **3** upon GC-MS analysis in the EI mode. The use of this compound as an analytical internal standard for pharmacokinetic studies to quantitate inhaled, unlabelled salmeterol in human blood plasma will be described in due course.

EXPERIMENTAL

Melting points are uncorrected. IR spectra: Nicolet Magna-IR 550 Series II FT-IR spectrometer. ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectra: Varian Gemini 300, solvent $CDCl_3$, reference TMS. FAB LRMS: Micromass ZAB-SE. FAB and EI HRMS: Kratos MS-50. GC-MS (EI): Hewlett Packard 5988. Preparative TLC: Merck silica gel, TLC grade 7749. Solutions of unpurified reaction products were dried over anhydrous Na_2SO_4.

Methyl 4-Acetyl-2-hydroxybenzoate-^{13}C (6). Solid $AlCl_3$ (3.340 g, 0.0251 mmol) was added in portions (effervescence) to a stirred solution of methyl salicylate (**5**, 1.866 g, 0.0123 mmol) and acetyl-1-^{13}C chloride (99 atom % ^{13}C, Aldrich; 0.973 g, 0.124 mmol) in CH_2Cl_2 (25 mL) which was cooled to 0 °C in an ice bath. The ice bath was removed and stirring at room temperature was continued for 18.5 h at which time the dingy, but mostly clear liquid was poured on ice (50 g). The layers were separated and the aqueous phase was extracted with CH_2Cl_2 (2 x 15 mL). The combined organic solutions were washed successively with H_2O (2 x 20 mL) and saturated, aqueous $NaHCO_3$ (20 mL), then dried and concentrated to provide the desired product **6** (2.147 g, 90%) as a tan solid. Purification was effected by recrystallization from hexane to provide white crystals (1.421 g, 59%); mp 60-62 °C; ref. 15 (unlabelled compound): mp 60-62 °C; IR (KBr) 3116 (m), 1684 (s), 1653 (s) cm^{-1}; ^{1}H NMR δ 2.58 (d, 3H, J = 6.0 Hz, H-10), 4.00 (s, 3H, H-8), 7.04 (d, 1H, J = 8.7 Hz, H-3); 8.09 (ddd, 1H, J = 8.7, 3.2, 2.4 Hz, H-4), 8.48 (dd, 1H, J = 4.1, 2.4 Hz, H-6), 11.3 (s, 1H, OH); ^{13}C NMR δ 26.0 (d, J = 43.2 Hz, C-10), 52.5 (C-8), 112.2 (d, J = 4.5 Hz, C-1), 118.1 (d, J = 4.0 Hz, C-3), 129.3 (d, J = 54.7 Hz, C-5), 131.5 (d, J = 3.5 Hz, C-6), 135.5 (d, J = 3.4 Hz, C-4), 165.4 (C-2), 170.3 (C-7), 195.8 (C-9); LRMS (EI) m/z (%) = M$^+$ 195 (56), 180 (72), 163 (34), 148 (100); HRMS (EI) *m/z* = Found 195.0603, $C_9{}^{13}CH_{10}O_4$ (M$^+$) requires 195.0613.

Methyl 4-Bromoacetyl-2-hydroxybenzoate-^{13}C (7). A solution of bromine (629 mg, 3.936 mmol) in $CHCl_3$ (10 mL) was added dropwise over approx. 0.5 h to a stirred solution of ketone **6** (730 mg, 3.740 mmol) in $CHCl_3$ (6 mL). Initially the bromine color dissipated slowly, but once some HBr was formed the color loss was rapid. Solid $NaHCO_3$ (2.20 g) was added cautiously (effervescence) with stirring. After drying and concentrating, the desired product remained as a white solid (1.010 g, 99%). The solid was recrystallized from ligroin to provide bromoketone **7** (730 mg, 71%) as white crystals; mp 91-92 °C; ref. 16 (unlabelled compound): mp 90-92 °C; IR (KBr) 3063 (m), 1684 (s), 1647 (s) cm^{-1}; ^{1}H NMR δ 4.02 (s, 3H, H-8), 4.41 (d, 2H, J = 3.6 Hz, H-10), 7.07 (d, 1H, J = 8.9 Hz, H-3); 8.10 (ddd, 1H, J = 8.9, 3.6, 2.3 Hz, H-4), 8.53 (dd, 1H, J = 4.1, 2.3 Hz, H-6), 11.4 (s, 1H, OH); ^{13}C NMR δ 30.0 (d, J = 44.4 Hz, C-10), 52.7 (C-8), 112.5 (d, J = 4.5 Hz, C-1), 118.5 (d, J = 4.0 Hz, C-3), 125.8 (d, J = 58.6 Hz, C-5), 132.3 (d, J = 3.5 Hz, C-6), 136.1 (d, J = 2.9 Hz, C-4), 166.0 (C-2), 170.0 (C-7), 189.4 (C-9); LRMS (EI) m/z (%) = M$^+$ (^{81}Br) 275 (3), M$^+$ (^{79}Br) 273 (3), 180 (100), 148 (78); HRMS (EI) *m/z* = Found 272.9719, $C_9{}^{13}CH_9{}^{79}BrO_4$ (M$^+$) requires 272.9718.

Methyl 2-hydroxy-5-{[(phenylmethyl)[6-phenylbutoxy)hexyl]amino]acetyl]-benzoate-^{13}C (9). A mixture of bromoketone **7** (219 mg; 0.800 mmol), benzylamine **8** (269 mg; 0.794 mmol; prepared by thoroughly washing a CH_2Cl_2 solution of its hydrochloride salt with 10% aqueous NaOH, then drying and concentrating), and diisopropylethylamine (207 µL; 1.191 mmol) in THF (10 mL) was stirred and refluxed for 5 h. After cooling and filtration, the mixture was concentrated to dryness. The semisolid residue was rinsed with several portions of Et_2O which were combined, dried and concentrated to provide the desired keto-ester **9** as a golden, non-viscous liquid (440 mg, 103%). This product was not purified owing to instability, was stored cold, and was used as quickly as possible in the next reaction; IR (neat) 3100 (m), 2929 (s), 2862 (s), 1683 (s), 1647 (s), 1212 (s), 1114 (s) cm^{-1}; ^{1}H NMR δ 1.22-1.73 (m, 12H, H-12,13,14,15,18,19), 2.54 (t, 2H, J = 7.4 Hz, H-20), 2.62 (t, 2H, J = 7.4 Hz, H-11), 3.33 (t, 2H, J = 6.6 Hz, H-17), 3.39 (t, 2H, J = 6.3 Hz, H-16), 3.68 (s, 2H, H-25), 3.73 (d, 2H, J = 4.2 Hz, H-10), 3.98 (s, 3H, H-8), 6.98 (d, 1H, J = 8.8 Hz, H-3), 7.12-7.34 (m, 10H, H-22,23,24,27,28,29), 8.07 (ddd, 1H, J = 8.8, 2.8, 2.6, H-4), 8.63 (dd, 1H, J = 3.5, 2.6, H-6); ^{13}C NMR δ 26.0, 27.0, 27.9, 29.3, 29.7 (the previous 5 peaks represent C-12,13,14,15,18,19; two signals apparently overlap), 35.6 (C-20), 52.4 (C-8), 54.5 (C-11), 58.9 (C-25), 61.0 (d, J = 44.3 Hz, C-10), 70.7 (C-17), 70.9 (C-16), 112.0 (d, J = 4.6, C-1), 117.7 (d, J = 3.4, C-3), 125.7 (C-24), 127.2 (C-29), 127.8 (C-5), 128.3, 128.5, 129.2 (the previous 3 peaks represent C-22,23,27,28; two signals apparently overlap), 131.9 (d, J = 2.9 Hz, C-6), 136.0 (C-4), 139.0 (C-26), 142.6 (C-21), 165.4 (C-2), 170.4 (C-7), 197.0 (C-9); LRMS (FAB) m/z (%) = $(M + H)^+$ 533 (13), 368 (90), 340 (100), 180 (42); HRMS (FAB) *m/z* = Found 533.3090, $C_{32}{}^{13}CH_{42}NO_5$ $(M + H)^+$ requires 533.3096.

(R/S)-4-Hydroxy-α^1-[[(phenylmethyl)[6-(4-phenylbutoxy)hexyl]amino]methyl]-1,3-benzenedimethanol-$^{13}C,^{2}H_3$ (10). Unpurified keto-ester **9** (224 mg 0.421 mmol) was stirred in diethyl ether (10 mL) at the ambient temperature while $LiAlD_4$ (258 mg, 6.143 mmol) was added cautiously in small portions. The mixture was stirred and gently refluxed for 2.5 h under a drying tube, then cooled in ice as H_2O (approx. 2 mL) was cautiously added dropwise (vigorous effervescence). During the H_2O addition, the mixture became thick and chalky and was manually swirled to mix before eventual ease of stirring resumed. After concentration *in vacuo*, the residue was dissolved in aqueous HCl (2.5 mL con. HCl, 22.5 mL H_2O), then adjusted to pH 7 by cautious addition of solid $NaHCO_3$ with mixing (gas evolution is vigorous initially). (Note: if the pH was adjusted to 8 or above, an intractable, white, gelatinous mass formed, thus necessitating addition of more acid to readjust to pH 7.) The mixture was extracted with CH_2Cl_2 (3 x 30 mL). The combined extracts were washed with 10% (w/v) aqueous $NaHCO_3$ (30 mL), dried and concentrated to provide a dull yellow oil (177 mg). Purification was effected by preparative TLC (1:1:0.013 hexanes:EtOAc:conc. NH_4OH; R_f = 0.12-0.39) to provide the desired product **10** as a colorless oil (146 mg; 68% over two steps); IR (neat) 3311 (s), 2937 (s), 2853 (s), 1263 (s), 1116 (s) cm^{-1}; ^1H NMR δ 1.18-1.73 (m, 12H, H-12,13,14,15,18,19), 2.42 (m, 1H, one H-20), 2.53 (br s, 2H, H-10), 2.56 (m, 1H, one H-20), 2.62 (t, 2H, J = 7.1 Hz, H-11), 3.37 (t, 2H, J = 6.5 Hz, H-16), 3.41 (t, 2H, J = 6.3 Hz, H-17), 3.46 (d, 1H, J = 13.5 Hz, one H-25), 3.86 (d, 1H, J = 13.5 Hz, one H-25), 4.90 (br, 2H, two OH), 6.73 (d, 1H, J = 8.1 Hz, H-3), 6.88 (dd, 1H, J = 3.9, 2.2 Hz, H-6), 7.04 (ddd, 1H, J = 8.1, 3.9, 2.2 Hz, H-4), 7.12-7.37 (m, 10H, H-22,23,24,27,28,29); ^{13}C NMR δ 26.0, 26.8, 27.0, 27.9, 29.3, 29.6 (the previous 6 peaks represent C-12,13,14,15,18,19), 35.6 (C-20), 54.0 (C-11), 58.7 (C-25), 62.3 (d, J = 38.2 Hz, C-10), 68.9 (t, J = 21.1 Hz, C-9), 70.7 (C-17), 70.8 (C-16), 116.3 (d, J = 4.0 Hz, C-3), 125.1 (d, J = 4.0 Hz, C-6), 125.5 (d, J = 2.9 Hz, C-4), 125.7 (C-24), 126.9 (d, J = 3.5 Hz, C-1), 127.3 (C-29), 128.3, 128.46, 128.49, 129.1 (the previous 4 peaks represent C-22,23,27,28), 133.3 (d, J = 50.6, C-5), 138.8 (C-26), 142.6 (C-21), 155.7 (C-2); LRMS (FAB) m/z (%) = $(M + H)^+$ 510 (100), 352 (99), 340 (50), 307 (46); HRMS (FAB) *m/z* = Found 510.3496, $C_{31}{}^{13}CH_{41}{}^{2}H_3NO_4$ $(M + H)^+$ requires 510.3492.

(R,S)-4-Hydroxy-α^1-[[[6-(4-phenylbutoxy)hexyl]amino]methyl]-1,3-benzenedimethanol-$^{13}C,^{2}H_3$ (11; $^{13}C,^{2}H_3$-salmeterol). A mixture of N-benzyltriol **10** (105 mg, 0.206 mmol) and 30 mg of 10% Pd on activated carbon was stirred in abs. EtOH (13 mL) under H_2 at atmospheric pressure and room temperature for 3 h, filtered over Celite, then concentrated to provide 96 mg of a pale yellow, viscous oil. This was purified by preparative TLC (60:8:1 CH_2Cl_2:MeOH:conc. NH_4OH; R_f = 0.19-0.38) to provide $^{13}C,^{2}H_3$-salmeterol **11** as a pale yellow liquid (61 mg, 71%); IR (neat) 3305 (s), 2932 (s), 2853 (s), 1253 (s), 1105 (s) cm^{-1};

^{1}H NMR δ 1.18-1.73 (m, 12H, H-12,13,14,15,18,19), 2.57 (m, 2H, H-20), 2.62 (t, 2H, J = 7.5 Hz, H-11), 2.64 (br s, 2H, H-10), 3.36 (t, 2H, J = 6.3 Hz, H-16), 3.40 (t, 2H, J = 6.3 Hz, H-17), 5.00 (br, 4H, 3 OH, NH), 6.69 (d, 1H, J = 8.4 Hz, H-3), 6.89 (br s, 1H, H-6), 6.96 (m, 1H, H-4), 7.12-7.30 (m, 5H, H-22,23,24); ^{13}C NMR δ 25.9, 26.9, 27.9, 29.2, 29.3, 29.6 (the previous 6 peaks represent C-12,13,14,15,18,19), 35.7 (C-20), 49.2 (d, J = 2.9 Hz, C-11), 56.3 (d, J = 38.7 Hz, C-10), 70.75 (br s, C-9), 70.82 (C-17), 71.2 (C-16), 116.5 (C-3), 125.77 (C-4, C-6 overlapped), 125.84 (C-24), 126.7 (C-1), 128.4, 128.5 (the previous 2 peaks represent C-22,23), 133.5 (d, J = 49.5, C-5), 142.6 (C-21), 155.9 (C-2); LRMS (FAB) m/z (%) = $(M + H)^+$ 420 (100), 402 (19), 307 (22), 262 (70), 250 (30); HRMS (FAB): *m/z* = Found 420.3023, $C_{24}{}^{13}CH_{35}{}^{2}H_{3}NO_{4}$ $(M + H)^+$ requires 420.3023.

The xinafoate salt was prepared as described in the literature (8) to give a white solid; mp 133-134 °C; ref. 1,2 (racemic, unlabelled compound): mp 137-138 °C.

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