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TETRAHEDRON: *ASYMMETRY*

Enantioselective synthesis of (*S*)-salmeterol via asymmetric reduction of azidoketone by *Pichia angusta*

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Abstract—An efficient enantioselective route to (*S*)-salmeterol involving asymmetric reduction of an azidoketone intermediate to an azido alcohol by *Pichia angusta* is described.

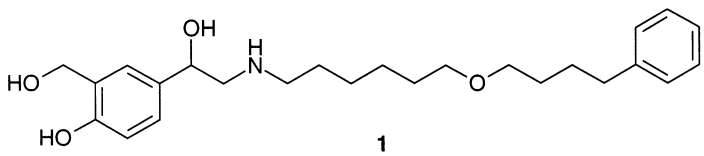
1. Introduction

Salmeterol (Serevent®) **1** is a potent, long acting β2-adrenoreceptor agonist used as a bronchodilator for the prevention of bronchospasm in patients with asthma and chronic obstructive pulmonary disease.[1] Interest in the (*S*)-enantiomer of salmeterol had recently been stimulated by the publication of a patent by Sepracor which claimed that the (*S*)-enantiomer of salmeterol had a higher selectivity for β2 receptors and that it did not precipitate certain adverse effects associated with the administration of (±)- or (*R*)-salmeterol.[2]

We were interested in a practical and efficient synthesis of (*S*)-salmeterol having >95% enantiomeric excess (e.e.) for further pharmacological screening. Surprisingly, the synthesis of non-racemic salmeterol has been described in only two Glaxo patents and also in a communication by Helquist. The first patent described the resolution of the ethanolamine **2** using (−)-di-*p*-toluolyl tartaric acid[3] and the second patent described the use of phenylglycinol as a chiral auxiliary, followed by chromatographic separation.[4] The Helquist synthesis involved the enantioselective reduction of the phenacyl bromide **3** to the corresponding bromohydrin using Corey's CBS-oxazaborolidine catalyst.[5]

2. Results and discussion

We envisaged an enantioselective reduction of an aromatic α-azidoketone to provide an azido alcohol, using a procedure similar to that described by Yadav et al.[6] Thus, the salmeterol intermediate **4** was first protected as the acetonide **5** and then converted to the azidoketone **6** (95% yield for the two steps) (Scheme 1). Reduction of **6** with borane in the presence of 7.5% (*S*)-2-methyl-CBS-oxazaborolidine provided the (*S*)-azido alcohol **7** but with an unexpectedly low enantio-



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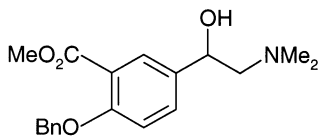

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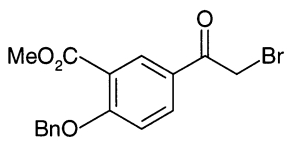

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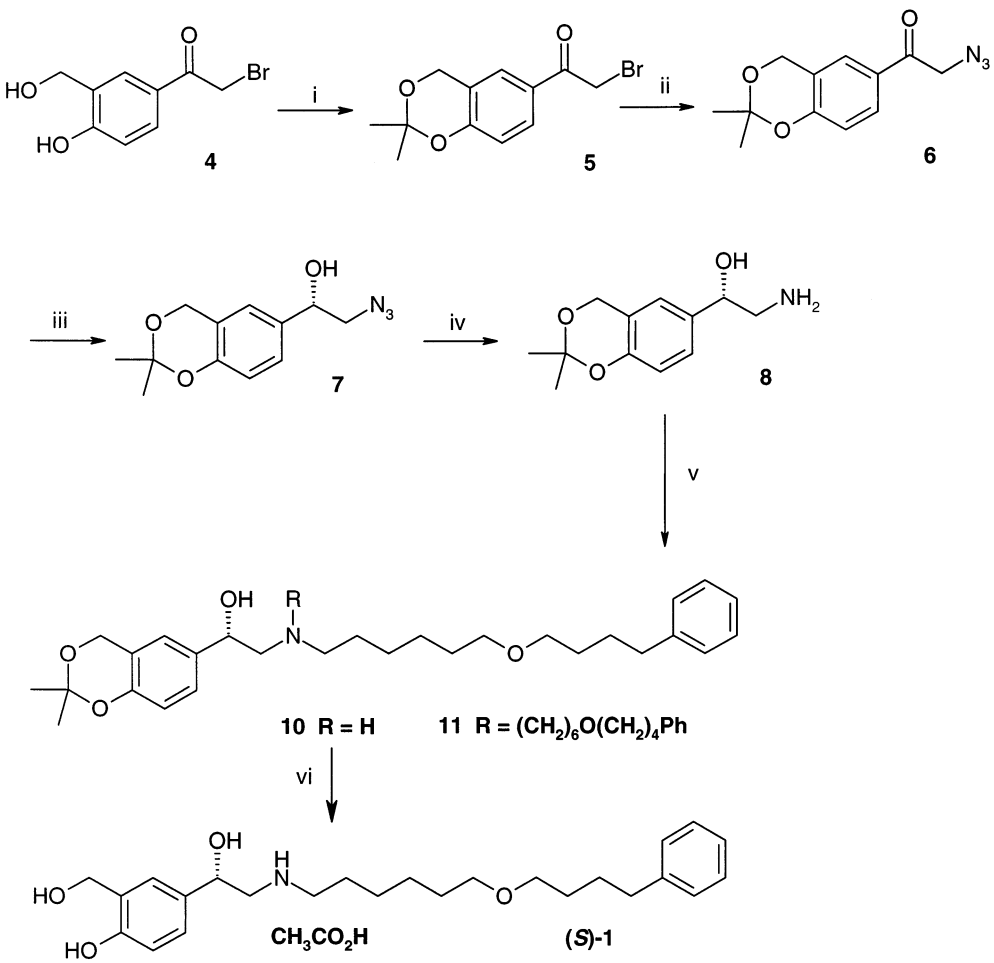

Scheme 1. *Reagents and conditions*: (i) 2-methoxypropene, dichloromethane, TsOH; (ii) NaN_3, DMF; (iii) *Pichia angusta*; (iv) H_2, 10% Pd/C, EtOH; (v) $Br(CH_2)_6O(CH_2)_4Ph$ **9**, DMF; (iv) AcOH, water.

meric ratio (5:1). Since (*R*)-salmeterol is more potent than the (*S*)-enantiomer even a small amount of the (*R*)-isomer in the product could produce misleading pharmacological results. Rather than attempting lengthy and expensive purification, we investigated an alternative procedure for the reduction of **6**. The biotransformation of ketones to alcohols can be performed with a variety of enzymes or whole cell preparations. The latter are usually preferred on an industrial scale in order to avoid the problem of cofactor regeneration.[7] Moreover, although the prevalent enantiopreference in the reduction is for alcohols with the (*R*)-configuration a number of micro-organisms provide alcohols with the (*S*)-configuration.[8,9] Forty two micro-organisms were screened for their ability to reduce the ketone group of **6** and five were identified that gave selective reduction. *Mucor racemosus* C142, *Curvularia lunata* NRRL2434 and *Aureobasidium pullulans* ATCC16623 preferentially converted the azidoketone **6** to the (*R*)-enantiomer of **7**, whereas *Zygosaccharomyces rouxii* NCYC381 and *Pichia angusta* ATCC16623 preferentially converted **6** to the (*S*)-enantiomer of **7** (Table 1). On scale-up it was found that the yeast *Pichia angusta* produced **7** with excellent enantioselectivity (e.e. >98%) and 94% yield. Chiral HPLC analysis indicated no detectable (*R*)-isomer or residual ketone. The azido alcohol **7** was then hydrogenated over palladium on charcoal in ethanol to provide the amino alcohol **8** in 86% yield. The enantiomeric purity of **8** was assessed by 750 MHz ^{1}H NMR using the chiral complexing agent (*R*)-(−)-2,2,2-trifluoro-1-(9-anthryl)ethanol. The benzylic C*H*OH of the (*R*)-isomer appears as a dd at 4.29, whereas the analogous proton in the (*S*)-isomer appears as a dd at

Table 1. Reduction of ketone **6** by a selection of micro-organisms

Organism	Hours post-ketone addition	E.e. % (config.)
Mucor racemosus C142	18	85 (*R*)
	42	88 (*R*)
Curvularia lunata	18	80 (*R*)
NRRL2434	42	83 (*R*)
Aureobasidium pullulans	18	85 (*R*)
ATCC16623	42	83 (*R*)
Zygosaccharomyces rouxii	6	94 (*S*)
NCYC381	24	94 (*S*)
Pichia angusta ATCC16623	18	94 (*S*)
	42	93 (*S*)

4.33. The higher field dd was not detected indicating that compound **8** was >95% pure. Reaction of **8** with the bromide **9** (prepared from 4-phenyl-1-butanol and 1,6-dibromohexane under phase transfer conditions)[10] gave the secondary amine **10** together with a small amount of dialkylated product **11** which was removed by filtration on silica gel. Deprotection of **10** with aqueous acetic acid gave (*S*)-salmeterol acetate salt in quantitative yield and 98.6% e.e. (chiral HPLC).

3. Conclusion

In conclusion, we have described an efficient and practical synthesis of (*S*)-salmeterol in very high e.e. and an overall yield of 54%.

4. Experimental

LCMS analysis was conducted on a Supelcosil LCABZ+PLUS column (3.3 cm×4.6 mm) eluting with 0.1% HCO_2H and 0.01 M NH_4OAc in water (solvent A), and 0.05% HCO_2H and 5% water in MeCN (solvent B), using the following elution gradient 0–0.7 min 0% B, 0.7–4.2 min 100% B, 4.2–5.3 min 0% B, 5.3–5.5 min 0% B at a flow rate of 3 mL/min. The mass spectra were recorded on a Fisons VG Platform spectrometer using electrospray positive and negative mode (ES+ve and ES-ve).

4.1. 2-Bromo-1-(2,2-dimethyl-4*H*-1,3-benzodioxin-6-yl)ethanone 5

2-Methoxypropene (24 mL, 250 mL) was added to an ice-cooled mixture of bromide **4** (50 g, 204 mmol) (**Caution**: potent alkylating agent) and *p*-toluenesulfonic acid monohydrate (230 mg, 1.2 mmol) in dichloromethane (500 mL). After the addition was complete, the mixture was stirred for 30 min and then more 2-methoxypropene (7.5 mL, 78 mmol) was added and the mixture was stirred for an additional 10 min. Aqueous sodium bicarbonate solution was added and the two phases were separated. The organic phase was washed with aqueous sodium bicarbonate, dried ($MgSO_4$) and evaporated to dryness to give **5** as a beige solid (58.3 g, 100%). ^{1}H NMR ($CDCl_3$; 400 MHz): δ 7.82 (1H, dd, *J* 8, 2 Hz), 7.70 (1H, d, *J* 2 Hz), 6.88 (1H, d, *J* 8 Hz), 4.90 (2H, s), 4.39 (2H, s), 1.58 (6H, s); ^{13}C NMR ($CDCl_3$; 100 MHz): δ 188.9, 155.3, 128.6, 125.7, 125.5, 118.5, 116.3, 99.8, 59.6, 29.7, 23.8.

4.2. 2-Azido-1-(2,2-dimethyl-4*H*-1,3-benzodioxin-6-yl)ethanone 6

A suspension of bromoketone **5** (52 g, 181 mmol) (**Caution**: potent alkylating agent) in DMF (300 mL) was treated with sodium azide (12.24 g, 188 mmol) and the mixture was stirred at 20°C for 2 h, by which time the mixture became homogeneous and red colored. The mixture was diluted with ethyl acetate and washed with water, brine and dried ($MgSO_4$). The filtrate was evaporated to dryness to give **6** as a pale yellow solid (42.5 g, 95%): ^{1}H NMR ($CDCl_3$; 400 MHz): δ 7.72 (1H, dd, *J* 8, 2 Hz), 7.62 (1H, br s), 6.88 (2H, d, *J* 8 Hz), 4.89 (2H, s), 4.49 (2H, s), 1.57 (6H, s); ^{13}C NMR ($CDCl_3$; 100 MHz): δ 191.7, 156.5, 128.5, 126.8, 125.5, 119.6, 117.5, 100.8, 60.6, 54.5, 24.8; MS (TSP+ve) *m*/*z* 248 $(M+H)^+$. Found: C, 58.40; H, 5.22; N, 16.78. $C_{12}H_{13}N_3O_3$ requires C, 58.29; H, 5.30; N, 17.00%.

4.3. Biotransformations

Organisms were grown in 10 mL YEPD medium (yeast extract 2%, peptone 2% and dextrose 2%) at 28°C with shaking at 250 rpm. azidoketone **6** (2 mg) in acetonitrile (200 μL) was added after 2 days growth, and incubation continued. The results are shown in the Table 1.

4.4. (1*S*)-2 Azido-1-(2,2-dimethyl-4*H*-1,3-benzodioxin-6-yl)ethanol 7

4.4.1. Biotransformation method. The yeast *Pichia angusta* ATCC16623 was grown in 5.2 L of YEPD medium in 13 Florence flasks. The flasks were incubated at 28°C with shaking until the organism had reached late growth stage. After 16 h growth, a solution of ketone **6** (5.2 g, 21 mmol) in acetonitrile (52 mL) was added (4 mL to each flask) and incubation continued at 28°C for 48 h. At this stage all the ketone was consumed and the contents of the flasks were combined. The broth was centrifuged (4200 rpm for 30 min) and the supernatant was decanted and filtered. The filtrate (4.9 L) was extracted with *tert*-butyl methyl ether (4.9 L), the organic phase was separated, dried (Na_2SO_4), filtered and concentrated to a pale yellow oil (5.35 g). HPLC (Kromasil C8, 15 cm×4.6 mm, eluting with 49% MeCN in 25 mM aq. NH_4OAc isocratically at a flow rate of 1 mL/min) showed no detectable residual ketone **6**. HPLC (ChromTech Chiral AGP, 15 cm×4.6 mm, eluting with 17.5% MeCN in 10 mM aq. KH_2PO_4 pH 7 isocratically with a flow rate of 0.5 mL/min) rt = 4.39 min; rt [(*R*)-isomer] = 3.71 min; rt [(*S*)-isomer] = 4.49 min. The crude product was purified by chromatography on silica gel (90 g Biotage cartridge) eluting with 15% diethyl ether–light petroleum (40–60°C) (1 L), followed by 20% diethyl ether–light petroleum (1.5 L) to give **7** as a colorless oil (4.92 g, 94%): NMR ($CDCl_3$; 400 MHz): δ 7.12 (1H, dd, *J* 8, 2 Hz), 6.99 (1H, d, *J* 2 Hz), 6.80 (1H, d, *J* 8 Hz), 4.81 (2H, s), 4.78 (1H, dd, *J* 8, 4 Hz), 3.43 (1H, dd, *J* 12, 8 Hz), 3.37 (1H, dd, *J* 12, 4 Hz), 2.64 (1H, br s), 1.53 (6H, s), ^{13}C NMR ($CDCl_3$; 100 MHz): δ 151.2, 132.5, 125.8, 122.3, 119.6, 117.3, 99.7, 73.0, 60.8, 58.0, 24.7, 24.6; LCMS rt = 2.82 min, ES+ve *m*/*z* 250 $[(M+H)^+$, 14%], ES-ve *m*/*z* 294 $[(M+HCO_2)^-$, 24%]. Found: C, 57.89; H, 6.01; N, 16.46. $C_{12}H_{15}N_3O_3$ requires C, 57.83; H, 6.07; N, 16.86%.

4.4.2. Chemical method. (*S*)-2-Methyl-CBS-oxazaborolidinine (1 M solution in toluene, 4.4 mL) was diluted with THF (43.6 mL), cooled to 0°C and then treated with a solution of BH_3·THF (1 M, 72.6 mL) under nitrogen. After 15 min at 0°C, a solution of **6** (14.37 g, 58.1 mmol) in THF (145 mL) was added dropwise over 1.5 h at 5°C. The reaction mixture was stirred for a further hour and then quenched by the addition of

aqueous HCl (2 M, 58.1 mL) and extracted with ethyl acetate. The organic layer was washed with aqueous HCl (2 M), aq. $NaHCO_3$, brine and dried ($MgSO_4$). The residue was purified by chromatography on silica gel (90 g Biotage cartridge) eluting with light petroleum (40–60°C) dichloromethane (2:3) to give **7** as a yellow liquid (3.0 g, 21%). The enantiomeric purity of this material was not determined at this stage, but was examined after conversion to the amine **8**.

4.5. (1*S*)-2-Amino-1-(2,2-dimethyl-4*H*-1,3-benzodioxin-6-yl)ethanol 8

The azide **7** (4.92 g, 19.7 mmol) was hydrogenated over 10% Pd/C (0.5 g) in ethanol (150 mL) over 3 h. The catalyst was removed by filtration and washed with ethanol. The combined filtrate and washings were evaporated to dryness. The residue was filtered on silica gel (40 g Biotage cartridge) eluting with toluene:ethanol:aq. 880 ammonia (85:14:1) to give **8** as a white solid (3.77 g, 86%): $[\alpha]_D^{20}$ +17 (*c* 0.83 in MeOH); ^{1}H NMR ($CDCl_3$; 400 MHz): δ 7.12 (1H, dd, *J* 8, 2 Hz), 7.00 (1H, d, *J* 2 Hz), 6.80 (1H, d, *J* 8 Hz), 4.84 (2H, s), 4.54 (1H, dd, *J* 8, 4 Hz), 2.98 (1H, dd, *J* 13, 4 Hz), 2.77 (1H, dd, *J* 13, 8 Hz), 2.05–1.70 (3H, br), 1.54 (6H, s); ^{13}C NMR ($CDCl_3$; 100 MHz): δ 150.6, 134.4, 125.8, 122.2, 119.3, 117.0, 99.5, 74.0, 61.0, 49.2, 24.8, 24.6; LCMS rt = 1.75 min, ES+ve *m*/*z* 206 [$(MH-H_2O)^+$, 100%]; 224 [$(M+H)^+$, 10%]. Found: C, 64.58; H, 7.58; N, 6.20. $C_{12}H_{17}NO_3$ requires C, 64.54; H, 7.67; N, 6.27%. The enantiomeric excess of **8** was determined by 750 MHz ^{1}H NMR using **8** (1 mg) in $CDCl_3$ (0.8 mL) and (*R*)-(−)-2,2,2-trifluoro-1-(9-anthryl)ethanol (18 mg).

4.6. (1*S*)-1-(2,2-Dimethyl-4*H*-1,3-benzodioxin-6-yl)-2-{[6-(4-phenylbutoxy)hexyl]amino}ethanol 10

A mixture of the amino alcohol **8** (223 mg, 1 mmol) and bromide **9** (178 mg, 0.57 mmol) in DMF (2 mL) was stirred at 20°C for 67 h. The reaction mixture was concentrated under reduced pressure, the residue was dissolved in ethyl acetate and washed with water and brine. The organic solution was dried ($MgSO_4$) concentrated to a colorless oil, and purified by chromatography (10 g silica Bond Elut cartridge) eluting with 1, 2 and 5% 2 M ammonia in methanol–dichloromethane to give **10** as a colorless oil (181 mg, 70%). ^{1}H NMR ($CDCl_3$; 400 MHz): δ 7.3–7.25 (2H, m), 7.2–7.1 (4H, m), 7.00 (1H, d, *J* 2 Hz), 6.78 (1H, d, *J* 8 Hz), 4.83 (2H, s), 4.60 (1H, dd, *J* 9, 4 Hz), 3.41 (2H, t, *J* 7 Hz), 3.38 (2H, t, *J* 7 Hz), 2.83 (1H, dd, *J* 12, 4 Hz), 2.70–2.55 (5H, m), 2.55–2.30 (2H, br), 1.7–1.4 (8H, m), 1.54 (6H, s), 1.4–1.28 (4H, m); LCMS rt = 3.21 min, ES+ve *m*/*z* 456 $(M+H)^+$, ES-ve *m*/*z* 500 $(M+HCO_2)^-$

4.7. (*S*)-Salmeterol acetate 1

The acetonide **10** (59 mg, 0.13 mmol) was heated in a mixture of acetic acid (5 mL) and water (2 mL) at 71°C for 0.5 h. The mixture was concentrated under reduced pressure, the residue was dissolved in methanol and evaporated to dryness to give the acetate salt of **1** as a colorless gum (62 mg, 100%). ^{1}H NMR (CD_3OD; 400 MHz): δ 7.34 (1H, d, *J* 2 Hz), 7.23 (1H, t, *J* 8 Hz), 7.21 (1H, s), 7.20–7.10 (4H, m), 6.79 (1H, d, *J* 8 Hz), 4.86 (1H, dd, *J* 9, 4 Hz), 4.65 (2H, s), 3.43 (2H, t, *J* 7 Hz), 3.41 (2H, t, *J* 7 Hz), 3.15–2.95 (4H, m), 2.61 (2H, t, *J* 7 Hz), 1.92 (3H, s), 1.75–1.53 (8H, m), 1.45–1.35 (4H, m). Chiral HPLC on a Sumichiral OA-4100 column (25 cm×4.6 mm) eluting with hexane:ethanol:dichloromethane:TFA (240:30:130:1), at a flow rate of 1 mL/min, detecting at 276 nm: rt = 17.45 min, 99.3% [(*S*)-isomer]; rt = 21.15 min, 0.7% [(*R*)-isomer].

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