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Resolution of 1-(4-amino-3-chloro-5-cyanophenyl)-2-bromo-1-ethanol by lipase mediated enantioselective alcoholysis, hydrolysis and acylation

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Abstract

Resolution of 1-(4-amino-3-chloro-5-cyanophenyl)-2-bromo-1-ethanol has been achieved by ethanol enantioselective lipase-catalysed alcoholysis, hydrolysis and acylation. Although a good enantioselectivity was observed in the three reactions, the best results were obtained by hydrolysis. This α-bromohydrin is an intermediate in the synthesis of a new adrenergic agent.

1. Introduction

Many 2-amino-1-arylethanols derived from (*R*)-adrenaline **1a** and (*R*)-noradrenaline **1b** (Fig. 1) as the parent compounds, are important adrenergic drugs. Studies on these drugs showed that the biological activity resides mostly in their (*R*)-enantiomer while the (*S*)-isomer is usually less active or may even cause undesired side effects.[1] This fact and the current US Food & Drug Administration guidelines[2] for the marketing of chiral drugs have stimulated the development of new biocatalytic methods to obtain pure enantiomers. In this regard, lipases are widely used in organic chemistry in the resolution of racemic mixtures because of their low cost and high stereoselectivity combined with a high catalytic activity in organic media under very mild experimental conditions.[3] These outstanding catalytic features have also been used for obtaining enantiomerically pure adrenergic agents by acylation[4] of the hydroxy group of the intermediate halohydrin or by hydrolysis[5] or alcoholysis[6] of its esters. In this paper we present our studies aimed at obtaining the enantiomerically pure intermediate bromohydrin **2a** in the synthesis of LAS 32.521[7] **2b**, a new molecule whose (*R*)-enantiomer displays interesting bronchial β_2-adrenergic agonist properties, by enantioselective enzyme-catalysed reactions. Enantiopure **2a** was obtained chemically by

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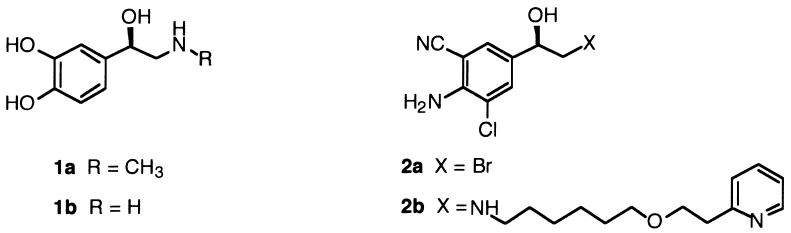


Fig. 1.

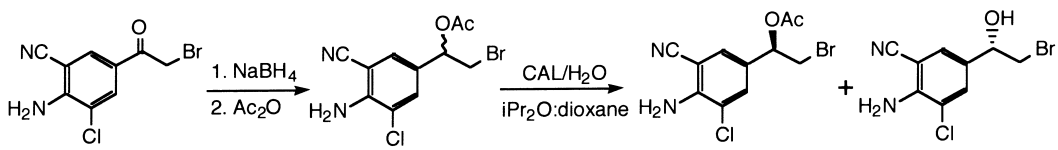
asymmetric reduction of the corresponding α-bromoketone **3** with a D-proline-borane agent,[8,9] a chemo- and enantioselective but highly expensive reactant.

Results of the enzymatic reaction were not predictable because, in spite of the above-mentioned previous use of lipases in this field, to our knowledge, there are no examples of the resolution of these racemic alcohols when the aromatic ring is trisubstituted or when one substituent, even a unique one, is an unprotected amino group. A double strategy of enantioselective acylation and alcoholysis has been developed.

2. Enantioselective hydrolysis of the racemic ester (±)-4 (Scheme 1)

2.1. Chemoselective reduction of the α-bromoketone 3

This reaction must be carried out under controlled experimental conditions in order to reduce the keto group while the bromine atom remains untouched. $NaBH_4$ (1 mmol) was slowly added to a stirred suspension of the α-bromoketone **3** (1 mmol) in ice-cooled anhydrous MeOH (5 mL). The reaction mixture become clear when the ketone disappeared (TLC, hexane:EtAcO=4:1) in about 15 min. A small amount of silica gel was then put into the flask and stirred while the solvent was evaporated under vacuum. The remaining solid was eluted in a silica gel column (hexane:EtAcO=6:1) to yield the racemic bromoalcohol **2a** (68%).



Scheme 1.

The experimental conditions of this reaction are rather critical: insufficient anhydrous MeOH, higher temperature or a standard extracting–washing process afforded a mixture of countless by-products, with the predominant one (up to 15%) isolated and identified[10] as the α-methoxy derivative **5** (Fig. 2).

2.2. Acetylation of the bromohydrin (±)-2a

A nearly quantitative yield of (±)-**4**[11] was obtained when a solution of (±)-**2a** (300 mg) in pyridine (2 mL) was treated with Ac_2O (3 mL) at room temperature for 24 h (TLC-control, hexane:EtAcO=4:1).

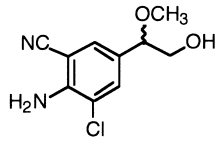


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Fig. 2.

2.3. Enantioselective lipase-catalysed cleavage of (±)-*4*

2.3.1. Alcoholysis

Our search was initially directed towards the alcoholysis. Several commercially available lipases (20 mg/mL) were checked in an *i*Pr_2O solution of (±)-**4** (10 mM) and *n*BuOH (50 mM) in screw-cap 2 mL vials, sealed, shaken for 24 h at 45°C and then analyzed by TLC (hexane:AcOEt=4:1). A low conversion, as judged visually, had taken place with the lipase of *Candida rugosa* and Amano's *Pseudomonas* lipases P and PS while a great transformation appeared in the lipase B of *Candida antarctica*[12] (CAL from here on) reaction. This reaction was repeated and analysed by chiral HPLC:[13] after 4 days and at 53.5% conversion, (−)-(*R*)-**4**, 42%, ee=99%, and (+)-(*S*)-**2a**, 50%, ee=86% (E=75) were afforded.[14,15]

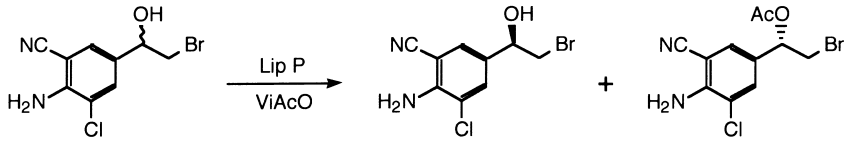
2.3.2. Hydrolysis

Hydrolytic resolution of (±)-**4** was also studied. The four above-mentioned lipases were checked in three different media: *t*BuOH:phosphate buffer (pH 7.0)=9:1, buffer-saturated *i*Pr_2O and neat buffer suspension. Very good results were obtained when CAL (20 mg/mL) was added to a solution of (±)-**4** (20 mM) in buffer-saturated *i*Pr_2O in 2 mL screw-cap vials, sealed and stirred at 60°C: after 24 h and at 49.6% conversion, (−)-(*R*)-**4**, 49%, ee=96%, and (+)-(*S*)-**2a**, 47%, ee=98% (E≥200) were afforded.

From an economical and easy-to-handle point of view, it is desirable to work at the highest possible substrate concentration in order to reduce the volume of solvent. Five concentrations (20, 50, 100, 250 and 500 mM) were tested with increasing amounts of enzyme (25, 50 and 125 mg/mL) and, from the results obtained, the reaction was carried out on a preparative scale: CAL (250 mg) was added to a (±)-**4** (250 mM) *i*Pr_2O:dioxane:buffer solution (90:9:1) (2 mL) and stirred for 2 days (conversion about 52%) at 60°C. The enzyme was filtered off and washed with EtAcO. The combined organic extracts were evaporated to dryness and the resulting residue eluted (hexane:EtAcO=6:1) in a silica gel column. (−)-(*R*)-**4** was obtained in 42% yield, ee=99%, $[\alpha]_D$=−68.8 (c=0.5, MeOH). (+)-(*S*)-**2a** was also obtained, 41%, ee=96%, $[\alpha]_D$=+23.3 (c=0.5, MeOH). The recovered enzyme displayed a remaining activity of 93% when it was measured with a standard procedure[16] of alcoholysis of tributyrin.

3. Enantioselective acylation of the racemic alcohol (±)-2a (Scheme 2)

Resolution of the racemic α-bromohydrin (±)-**2a** via enantioselective lipase-catalysed acylation was also investigated. The four above-mentioned lipases were checked under acylating conditions. They were added (20 mg/mL) to solutions of (±)-**2a** (20 mM) and trifluoroethyl butyrate or vinyl acetate (50 mM) in anhydrous *i*Pr_2O or in neat vinyl acetate as the acyl donor and organic medium, in screw cap 2 mL vials, sealed and shaken for 24 h at 40°C. Results appeared promising by TLC when vinyl acetate was used as the acyl donor and solvent in reactions catalysed by CAL and both lipases PS and P. When these three reactions were repeated and analysed by chiral HPLC, the best results were obtained in the lipase P reaction: conversion after 5 days was 53%, 47.1% of (−)-(*R*)-**2a**, ee=96%, E=85.



(±)-**2a** (–)-(*R*)-**2a** (+)-(*S*)-**4**

Scheme 2.

On a preparative scale, lipase P (400 mg) was added to a solution of the racemic alcohol (±)-**2a** (110 mg, 100 mM) in vinyl acetate (4 mL) and the resulting suspension was incubated at 40°C for 5 days (conversion 55%). The enzyme was filtered off and washed with EtOAc. The combined organic extracts were evaporated to dryness and the resulting residue was eluted (hexane:EtOAc=6:1) on a silica gel column. (−)-(*R*)-**2a** was obtained in 44% yield, ee=97%, $[\alpha]_D$=−27 (c=0.5, MeOH). (+)-(*S*)-**3** was also obtained, 46%, ee=90%, $[\alpha]_D$=+69.2 (c=0.5, MeOH). The recovered enzyme retained 89% of its original activity.

Acknowledgements

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References

1. Ruffolo Jr., R. R. *Tetrahedron* **1991**, *47*, 9953–9980.
2. Stinson, S. D. *Chem. Eng. News* **1992**, *70*, 46–79.
3. de Zoete, M. C.; van Rantwijk, F.; Sheldon, R. A. *Catal. Today* **1994**, *22*, 563–590.
4. Ader, U.; Schneider, M. P. *Tetrahedron: Asymmetry* **1992**, *3*, 521–524.
5. Ader, U.; Schneider, M. P. *Tetrahedron: Asymmetry* **1992**, *3*, 201–204.
6. Bevinakatti, H. S.; Thakkar, N. V.; Banerji, A. A. *Biotechnol. Lett.* **1995**, *17*, 217–218.
7. Puig, C.; Pujol, F.; Crespo, M. I.; Moragues, J. PCT Int. Appl. WO 96 31,466 [CA 125(1996):328273v].
8. Nishizawa, M.; Noyori, R. In *Reduction of C=X to CHXH by Chirally Modified Hydride Reagents*; Trost, B. M.; Fleming, I., Eds. Comprehensive Organic Synthesis. Reductions. Volume 8. Pergamon Press: Oxford, 1991; pp. 170–173.
9. Corey, E. J.; Bakshi, R. K.; Shibata, S. *J. Am. Chem. Soc.* **1987**, *109*, 5551–5553.
10. Compound **5**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.40 (d, 1H, *J*=1.8 Hz), 7.27 (d, 1H, *J*=1.8 Hz), 4.84 (s br, 2H, disappears with D_2O), 4.14 [t, 1H, *J*=6.2 Hz], 3.58 [dd, 2H, *J*=5.0, 6.2 Hz], 3.28 (s, 3H), 2.24 [t, 1H, *J*=5.0 Hz, disappears with D_2O]. ^{13}C NMR (75 MHz, $CDCl_3$): δ 145.9, 132.4, 129.4, 128.6, 119.7, 116.6, 96.8, 82.9, 66.6, 56.9. Anal. calcd for $C_{10}H_{11}ClN_2O_2$ (226.66): C, 52.99; H, 4.89; N, 12.36; Cl, 15.64. Found: C, 52.70; H, 4.90; N, 12.40; Cl, 15.50.
11. Compound (±)-**4**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.44 (d, 1H, *J*=2.0 Hz), 7.33 (d, 1H, *J*=2.0 Hz), 5.79 [dd, 1H, *J*=5.7, 7.0 Hz], 4.92 (s br, 2H, disappears with D_2O), 3.59 [dd, 1H, *J*=7.0, 10.7 Hz], 3.51 [dd, 1H, *J*=5.7, 10.7 Hz], 2.12 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.5, 146.3, 132.3, 129.4, 127.7, 119.6, 116.2, 97.0, 73.1, 33.4, 20.8. MS (70 eV) m/z (rel. intensity): 43 (100), 316 (5, M^+), 318 (6, M^++2), 320 (1, M^++4). Anal. calcd for $C_{11}H_{10}BrClN_2O_2$ (317.57): C, 41.60; H, 3.17; N, 8.82. Found: C, 41.65; H, 3.06; N, 9.03.
12. The lipase B of *Candida antarctica* used was Novozym 435, a Novo Nordisk's commercial immobilized preparation of that lipase.
13. We used a 25 cm Spherisorb S5 chiral column and hexane:*i*PrOH (95:5) as eluent.
14. Chen, C.-S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294–7299.
15. Straathof, A. J. J.; Jongejan, J. A. *Enzyme Microb. Technol.* **1997**, *21*, 559–571.
16. Conde, S.; Dorronsoro, I.; Fierros, M.; Rodríguez-Franco, M. I. *Tetrahedron* **1997**, *53*, 2907–2914.