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Enzymatic resolution of a quaternary stereogenic centre as the key step in the synthesis of (*S*)-(+)-citalopram

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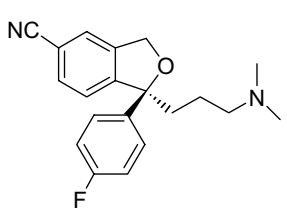
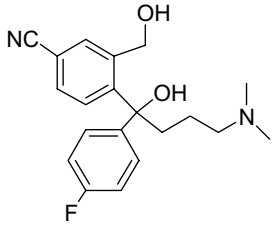
Abstract—The enzymatic resolution of 4-[(4-dimethylamino)-1-(4′-fluorophenyl)-1-hydroxy-1-butyl]-3-(hydroxymethyl)-benzonitrile, a useful intermediate in the synthesis of enantiomerically pure citalopram, has been studied. *Candida antarctica* lipase B (CAL-B) catalyzes the enzymatic acetylation of the primary benzylic alcohol with high enantioselectivity at the quaternary stereogenic centre. The enzymatic enantioselective hydrolysis of the 3-acetyloxymethyl derivative catalyzed by CAL-B is also possible.

1. Introduction

Citalopram, **1** is a very selective inhibitor of serotonin (5-HT) reuptake that has proved to be an efficient antidepressant in man.[1] It was shown that almost the entire inhibition activity resides in the (*S*)-(+)-enantiomer.[2]



(*S*)-(+)-Citalopram, 1



(±)-2

The 4-[(4-dimethylamino)-1-(4′-fluorophenyl)-1-hydroxy-1-butyl]-3-(hydroxymethyl)-benzonitrile, (±)-**2** is a useful intermediate in the synthesis of racemic citalopram.[3] The preparation of the single enantiomer has been carried out by the stereoselective crystallization of the diastereomeric salts of the diol (±)-**2** with (+)-di-*p*-toloyltartaric acid or by the formation of the diastereomeric esters with (+) and (−)-α-methoxy-α-trifluoro-methylphenylacetyl chloride and subsequent crystallization or chromatographic separation by HPLC.[2] The chromatographic separation of the enantiomers of citalopram or the intermediate (±)-**2** could also be carried out using a chiral stationary phase such as Chiralpak™ AD or Chiralcel™ OD.[4] These routes involve consumption of expensive enantiomerically pure reagents and, particularly the crystallization methods, give relatively low yields resulting in them being nonviable for industrial production. Hence, there is a desire for the development of new synthetic methods adequate to be carried out on a large scale; among them, the use of enzymes as catalysts is very suitable for this purpose.[5]

Moreover, the resolution of the diol (±)-**2** by enzymatic methods is an interesting challenge from a theoretical point of view. On the one hand, only a few hydrolases were shown to be active on tertiary alcohols, because of the adverse steric interactions caused by these substrates.[6] On the other hand, the enantioselective recognition at the primary hydroxyl group should be difficult because this reaction site is four bonds removed from the stereogenic centre.

2. Results and discussion

Taking into account the structure of substrate (±)-**2**, in a first approach we tested the capability of several lipases

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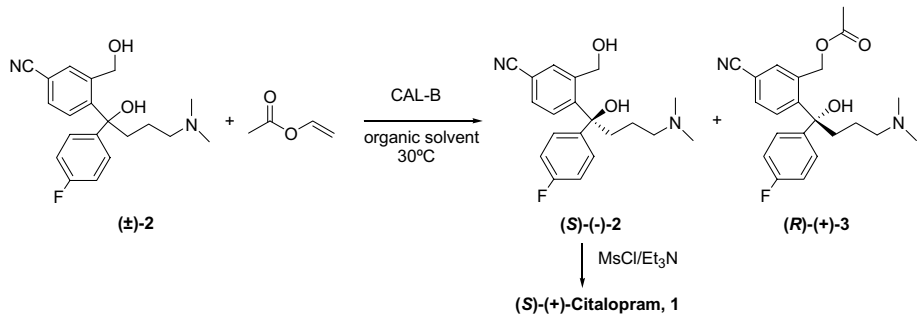


Scheme 1.

as catalysts in the acetylation process. In a first set of experiments, vinyl acetate was chosen as the acyl donor in dioxane. The enzymatic reactions were carried out at 30 °C (Scheme 1, Table 1). Only one of the tested enzymes catalyzed the acetylation of the primary alcohol: lipase B from *Candida antarctica* (CAL-B) showed a moderate enantioselectivity ($E = 17$),[7] at a low reaction rate. It is noteworthy that the remaining substrate (*S*)-(−)-**2** has the correct configuration to complete the synthesis of (+)-citalopram. In the processes catalyzed by other lipases from *C. antarctica* (CAL-A and CAL-B-L2), *Pseudomonas cepacia*, *Candida rugosa* or *Mucor miehei* and the proteases from *Aspergillus oryzae, Streptomyces griseus* or *Savinase* the unaltered starting material was recovered.

In order to improve the rate of the CAL-B catalyzed process we increased the concentration of the acylating agent from 10 to 20 equiv. Under these conditions, the reaction rate is moderated, 47% conversion is achieved after 60 h of reaction, but the enantioselectivity slightly decreased. Next we examined the effect of the organic solvent; entries 3, 4 and 9 (Table 1) summarize the results of the experiments carried out in *tert*-butyl methyl ether, toluene and acetonitrile, respectively. Under these conditions, it is apparent that toluene and acetonitrile are the best solvents for the CAL-B catalysis: after 24 h, a 58% conversion was achieved in toluene, with an enantioselectivity of $E = 27$. In acetonitrile the process is slower than in toluene but the enantioselectivity is higher ($E = 66$).

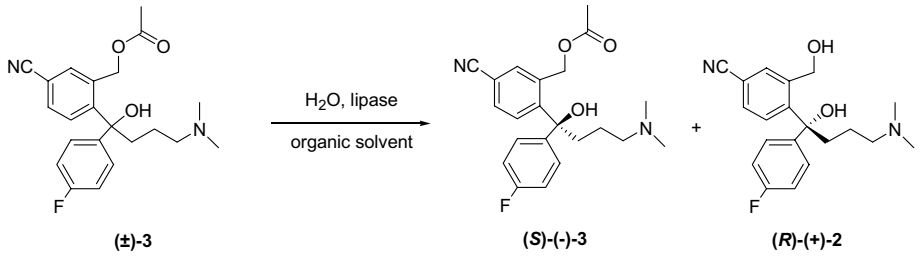
In order to improve the performance of the process, we studied the influence of the substrate, enzyme and the acylating agent concentrations. First, we increased the concentration of substrate and enzyme from 5 to 10 mg/mL in toluene (entry 5). Under these conditions we observed a significative improvement of the reaction rate but enantioselectivity decreased from $E = 27$ to $E = 13$. Then, using these last conditions (10 mg/mL of enzyme and substrate), we carried out the process using 5 or 1.5 equiv of vinyl acetate (entries 6 and 7). As one can see from the table, decreasing the amount of acylating agent increases the enantioselectivity of the enzymatic reaction without a significative loss of reaction rate. The best result ($E = 59$) was obtained using 1.5 equiv of vinyl acetate (entry 7). Even though one can expect that a better enantioselectivity can be obtained using a concentration of enzyme and substrate of 5 mg/mL and 1.5 equiv of acylating agent (entry 8), under these conditions, after 24 h, a 49% conversion was achieved, with lower enantioselectivity ($E = 13$).

A similar study was carried out in acetonitrile (entries 9–13). It is apparent that the influence of the concentration of the reagents and the enzyme is less dramatic in this

Table 1. CAL-B catalyzed acetylation of (±)-**2** with vinyl acetate, at 30 °C

Entry	Solvent	Substrate and enzyme concn (mg/mL)	Vinyl acetate concn (equiv)	Time (h)	c (%)[a]	Ee_s (%)[b]	Ee_p (%)[b]	E[c]
1	1,4-Dioxane	5	10	120	51	79	76	17
2	1,4-Dioxane	5	20	60	47	64	72	12
3	*t*-BuOMe	5	20	72	58	84	61	10
4	Toluene	5	10	24	58	>99	60	27
5	Toluene	10	10	17	74	>99	36	13
6	Toluene	10	5	14	60	>99	67	36
7	Toluene	10	1.5	9	56	>99	78	59
8	Toluene	5	1.5	24	49	71	73	13
9	Acetonitrile	5	10	39	53	99	85	66
10	Acetonitrile	10	5	17	54	>99	84	60
11	Acetonitrile	10	1.5	21	53	99	86	70
12	Acetonitrile	20	1.5	21	56	>99	79	62
13	Acetonitrile	5	1.5	26	50	75	74	15

[a] Conversion, $c = ee_s/(ee_s + ee_p)$.
[b] Determined by HPLC.
[c] Enantiomeric ratio, $E = \ln[(1 - c)(1 + ee_p)]/\ln[(1 - c)(1 - ee_p)]$.[7]



Scheme 2.

solvent compared to toluene. Again, we obtained the best enantioselectivity using 10 mg/mL of enzyme and substrate, and 1.5 equiv of acylating agent ($E = 70$, entry 11).

Next, we examined the influence of the acyl donor in the biocatalytic process. The reactions were carried out in acetonitrile using a concentration of enzyme and substrate 10 mg/mL and 1.5 equiv of acylating agent. Isopropenyl acetate or vinylpropionate led to lower enantioselectivities in our system ($E < 10$). When vinyl benzoate and methyl methoxyacetate were used the unaltered starting material was recovered after 5 days of reaction.

Other suitable acyl donors are the anhydrides but, in this case, all the attempts to use glutaric or succinic anhydride gave the racemic product, probably because of the significant amount of product formed through the parallel chemical reaction.

Another common approach for the resolution of racemic alcohols is the enzymatic hydrolysis. In order to study this reaction we prepared the racemic acetyl derivative (±)-**3** by treatment of the diol (±)-**2** with acetic anhydride. As in the case of acetylation, a first set of experiments were examined in order to find the most suitable enzyme for catalyzing the hydrolysis of the acetyl derivative. As the hydrolysis in aqueous media (phosphate buffer, pH 7) takes place spontaneously, we carried out the process in acetonitrile with a small amount of water (4 equiv) as nucleophile (Scheme 2, Table 2). Only two of the tested enzymes catalyzed the reaction, the lipases from *C. antarctica*, CAL-B and CAL-B-L2. As expected, the stereochemical preferences of these enzymes were the same as for the acetylation processes: the (*R*) enantiomer was preferentially hydrolyzed. The enantioselectivity of the CAL-B catalyzed hydrolysis was similar to that of the acetylation in the same solvent (entry 1, $E = 68$) but the reaction rate was much slower. The reaction catalyzed by CAL-B-L2 gave poorer results.

Next, we studied the effect of the reaction parameters on the enantioselectivity of the enzymatic hydrolysis. We observed a strong influence of the organic solvent (entries 3–5). Acetonitrile is the best of the tested solvents; when the processes were carried out in toluene, 1,4-dioxane or *tert*-butyl methyl ether the enantioselectivity showed by the lipase dramatically decreased. The attempt to improve the reaction rate increasing the temperature resulted in low enantioselectivities. Taking in account the influence of the pH of the aqueous solution employed in a enzymatic hydrolysis, 4 equiv of phosphate buffer 0.1 M, pH = 7 were used instead of H_2O (entry 8), but no improvement was observed in these conditions.

Finally, the reaction of enantiomerically pure substrate (*S*)-(−)-**2** with mesyl chloride and triethylamine was carried out to obtain the (*S*)-(+)-citalopram **4** with a yield higher than 90%.

3. Conclusions

The resolution of 4-[(4-dimethylamino)-1-(4′-fluorophenyl)-1-hydroxy-1-butyl]-3-(hydroxymethyl)-benzonitrile **2** via a CAL-B catalyzed acetylation is described. Good yields and high enantioselectivities can be achieved by an appropriate selection of the reaction parameters. The

Table 2. Lipase catalyzed hydrolysis[a] of (±)-**3** in organic solvents

Entry	Lipase[b]	Solvent	T (°C)	Time (h)	c (%)	Ee_s (%)	Ee_p (%)	E
1	CAL-B	Acetonitrile	30	89	40	63	95	68
2	CAL-B-L2	Acetonitrile	30	120	18	16	73	7
3	CAL-B	Toluene	30	65	26	11	31	2
4	CAL-B	1,4-Dioxane	30	65	25	8	5	1
5	CAL-B	*t*-BuOMe	30	48	74	73	28	4
6	CAL-B	Acetonitrile	50	145	50	67	67	10
7	CAL-B-L2	Acetonitrile	50	145	60	87	56	10
8	CAL-B	Acetonitrile[c]	30	120	36	44	78	13

[a] 4 equiv of H_2O.
[b] 10 mg/mL.
[c] 4 equiv of phosphate buffer 0.1 M, pH = 7.

best found conditions were the use of vinyl acetate as acyl donor in acetonitrile, at 30 °C. In these processes, the remaining alcohol (*S*)-(−)-**2** has the correct configuration to complete the synthesis of (*S*)-(+)-citalopram. On the other hand, the hydrolysis reaction catalyzed by CAL-B also achieved good enantioselectivity and a moderate reaction rate.

Taking into account the simplicity and easy scale-up of lipase catalyzed reactions, the applicability of this method to the industrial preparation of the antidepressant (+)-citalopram is noteworthy.[8]

It is remarkable that the enzymatic catalyzed resolution of a quaternary stereogenic centre is feasible by a transesterification or hydrolysis reaction in organic solvents. On the other hand, an enantioselective recognition can be achieved by the enzyme at a primary hydroxyl group four bonds removed from the stereogenic centre.

4. Experimental

Enzymatic reactions were carried out in a Gallenkamp incubatory orbital shaker. Immobilized *C. antarctica* lipase B Novozym 435 (CAL-B) was a gift from Novo Nordisk. *C. antarctica* lipase B Chirazyme-L2, cf., lyo. (CAL-B-L2) was purchased from Roche Diagnostics.

Melting points were taken using a Gallenkamp apparatus and were uncorrected. Optical rotations were measured using a Perkin–Elmer 241 polarimeter and specific rotations are quoted in units of 10^{-1} deg cm^2/g. ^{1}H and ^{13}C NMR spectra were obtained with TMS (tetramethylsilane) as internal standard using a Bruker AC-300 (^{1}H 300 MHz and ^{13}C 75.5 MHz) spectrometer. Mass spectra were recorded on a Hewlett-Packard 1100 LC/MSD. All reagents were purchased from Aldrich Chemie. Solvents were distilled over an adequate desiccant and stored under nitrogen. Flash chromatography was performed using Merck silica gel 60 (230–400 mesh).

The enantiomeric excesses were determined by chiral HPLC analysis on a Shimadzu LC liquid chromatograph, using a CHIRALCEL OD for the acetyl derivative **3**. The substrate (diol) **2** was first acetylated to convert it into the ester **3** and then analyzed.

4.1. Enzymatic acetylation of 4-[(4-dimethylamino)-1-(4′-fluorophenyl)-1-hydroxy-1-butyl]-3-(hydroxymethyl)-benzonitrile, (±)-2

The reaction mixture containing (±)-**2** (0.2 g), vinyl acetate and the lipase (0.2 g) in the corresponding organic solvent (see concentration of enzyme and substrate in Table 1) was shaken at 30 °C and 250 rpm in a rotatory shaker. The progress of the reaction was monitored by TLC using the solvent system ethyl acetate/Et_3N 10:1. When the reaction was terminated the enzyme was removed by filtration and washed with ethyl acetate. The solvent was evaporated under reduced pressure and the crude residue was purified by flash chromatography on silica gel (ethyl acetate/Et_3N 10:1) to afford the acetylated product (+)-**3** and the remaining substrate (−)-**2**.

4.1.1. (−)-4-[(4-Dimethylamino)-1-(4′-fluorophenyl)-1-hydroxy-1-butyl]-3-(hydroxymethyl)-benzonitrile, (–)-2. Colourless oil; $[\alpha]_D^{20}$ −98.5 (*c* 1, $CHCl_3$), ee > 99%. ^{1}H NMR ($CDCl_3$) δ (ppm) 1.63 (br s, 2H, OH), 2.24 (s, 6H, CH_3), 2.38 (m, 6H, CH_2), 4.30 (dd, 2H, CH_2), 6.98 (t, 2H, CH), 7.27 (m, 3H, CH), 7.59 (d, 2H, CH); ^{13}C NMR ($CDCl_3$) δ (ppm) 22.0 (CH_2), 43.8 (CH_2), 44.6 (CH_3), 59.8 (CH_2), 64.0 (CH_2), 111.3 (C), 114.6 (CH), 114.9 (CH), 118.5 (C), 127.0 (C), 127.4 (CH), 127.6 (CH), 128.1 (C), 128.9 (C), 130.8 (CH), 135.8 (CH), 142.1 (C), 151.1 (C); MS (ESI$^+$, *m*/*z*): 365 (M+Na)$^+$, 343 (M+H)$^+$.

4.1.2. (+)-3-Acetyloxymethyl-4-[(4-dimethylamino)-1-(4′-fluorophenyl)-1-hydroxy-1-butyl]-benzonitrile, (+)-3. Colourless oil; $[\alpha]_D^{20}$ +36.5 (*c* 1.2, $CHCl_3$), ee > 90%. ^{1}H NMR ($CDCl_3$) δ (ppm) 1.56 (br s, 1H, OH), 2.02 (s, 3H, CH_3), 2.18 (s, 6H, CH_3), 2.45 (m, 6H, CH_2), 5.19 (dd, 2H, CH_2), 6.96 (t, 2H, CH), 7.28 (m, 2H, CH), 7.65 (m, 3H, CH); ^{13}C NMR ($CDCl_3$) δ (ppm) 20.8 (CH_3), 22.1 (CH_2), 43.2 (CH_2), 44.6 (CH_3), 59.7 (CH_2), 63.5 (CH_2), 110.9 (C), 114.5 (CH), 114.8 (CH), 118.6 (C), 126.9 (CH), 127.6 (CH), 127.7 (CH), 130.3 (CH), 132.2 (CH), 137.5 (C), 142.3 (C), 149.9 (C), 170.3 (CO); MS (ESI$^+$, *m*/*z*): 407 (M+Na)$^+$, 385 (M+H)$^+$.

4.1.2.1. Determination of the ee by HPLC analysis. Two well resolved peaks were obtained for the racemic compound (1 mg in 1 mL mobile phase; 20 μL sample) at 36 °C in hexane/*i*-propanol (98:2), 0.5 cm^3/min, Rs 1.5 (*S*)-(−)-**3**, t_R 36.03 min; (*R*)-(+)-**3**, t_R 40.38 min.

4.2. Chemical acetylation of 4-[(4-dimethylamino)-1-(4′-fluorophenyl)-1-hydroxy-1-butyl]-3-(hydroxymethyl)-benzonitrile, (±)-2

Acetic anhydride (0.11 mL, 1.17 mmol) was added dropwise to a 0 °C solution of diol (±)-**2** (0.2 g, 0.58 mmol) in CH_2Cl_2 (6 mL) and Et_3N (0.16 mL, 1.17 mmol) and stirred for 8 h. The resulting mixture was washed with 1 N HCl. The organic fraction was dried over Na_2SO_4, filtered and evaporated under reduced pressure to afford the compound (±)-**3** as a yellow oil (189.5 mg, 85%).

4.3. Enzymatic hydrolysis of (±)-3-acetyloxymethyl-4-[(4-dimethylamino)-1-(4′-fluorophenyl)-1-hydroxy-1-butyl]-benzonitrile, (±)-3

The reaction mixture containing (±)-**3** (0.2 g), H_2O (4 equiv) and the lipase (0.2 g) in the corresponding organic solvent (20 mL) was shaken at 30 °C and 250 rpm in a rotatory shaker. The progress of the reaction was monitored by TLC using the solvent system ethyl acetate/Et_3N 10:1. When the reaction was terminated the

enzyme was removed by filtration and washed with ethyl acetate. The solvent was evaporated under reduced pressure and the crude residue was purified by flash chromatography on silica gel (ethyl acetate/Et_3N 10:1) to afford the hydrolyzed product (+)-**2** and the remaining substrate (−)-**3**.

4.4. Synthesis of (*S*)-(+)-1-[3-(dimethylamino)propyl]-1-(4′-fluorophenyl)-1,3-dihydro-5-isobenzofurancarbonitrile, (*S*)-(+)-citalopram, (*S*)-(+)-1

To a suspension of 4-[(4-dimethylamino)-1-(4′-fluorophenyl)-1-hydroxy-1-butyl]-3-(hydroxymethyl)-benzonitrile, (−)-**2** (0.5 g, 1.46 mmol) in dry dichloromethane (5 mL), under nitrogen atmosphere and 0 °C, was added dropwise a solution of mesyl chloride (0.14 mL, 1.81 mmol), in 3 mL of dry dichloromethane. The resulting mixture was allowed to warm up to 15 °C stirred for 1 h. The progress of the reaction was monitored by TLC using (ethyl acetate/Et_3N 10:1). After 1 h the resulting mixture was washed with NaOH 1 N. The organic fraction was dried over Na_2SO_4, filtered and evaporated under reduced pressure. No further purification was necessary to afford the compound (+)-citalopram as a yellow oil (426 mg, 90%). ^1H NMR ($CDCl_3$) δ (ppm) 2.14 (s, 6H, CH_3), 2.23 (m, 6H, CH_2), 5.16 (d, 2H, CH_2), 6.99 (t, 2H, CH), 7.44 (m, 3H, CH), 7.58 (d, 2H, CH); ^{13}C NMR ($CDCl_3$) δ (ppm) 22.0 (CH_2), 38.8 (CH_2), 45.2 (CH_3), 59.3 (CH_2), 71.2 (CH_2), 91.0 (C), 111.6 (C), 115.1 (CH), 115.4 (CH), 118.5 (C), 122.7 (CH), 125.1 (CH), 126.6 (CH), 126.7 (CH), 131.7 (CH), 139.5 (C), 140.2 (C), 149.4 (C); MS (ESI^+, m/z): 347 $(M+Na)^+$, 325 $(M+H)^+$.

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References and notes

1. Gravem, A.; Amthor, K. F.; Astrup, C.; Elgen, K.; Gjessing, L. R.; Gunby, B.; Pettersen, R. D.; Kyrdalen, L.; Vaadal, J.; Ofsti, E. *Acta Psych. Scand.* **1987**, *75*, 478–486.
2. Boegeso, K. P. EP Patent 0347066, 1989; *Chem. Abstr.* **1990**, *113*, 78150.
3. Boegeso, K. P. U.S. Patent 4,650,884, 1987; EP 171943, *Chem. Abstr.* **1986**, *105*, 42560.
4. Bech Sommer, M.; Nielsen, O.; Pedersen, H.; Ahmadian, H.; Pedersen, H.; Brosen, P.; Geiser, F.; Lee, J.; Cox, G.; Dapremont, O.; Steu, C.; Assenza, S. P.; Hariharan, S.; Nair, U. WO Patent 03/006449, 2003.
5. Schmid, A.; Dordick, J. S.; Hauer, B.; Kiener, A.; Wubbolts, M.; Witholt, B. *Nature* **2001**, *409*, 258–268.
6. Hari Krishna, S.; Persson, M.; Bornscheuer, U. T. *Tetrahedron: Asymmetry* **2002**, *13*, 2693–2696; Henke, E.; Pleiss, J.; Bornscheuer, U. T. *Angew. Chem., Int. Ed.* **2002**, *41*, 3211–3213.
7. Chen, C. S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294–7299.
8. Bayod, M.; Llorente, I.; Gotor, V.; Quirós, M.; Fernández-Solares, L.; Brieva, R. ES Patent application P200302215 to Astur Pharma.