Anhydrides as Acylating Agents in the Enzymatic Resolution of an Intermediate of (−)-Paroxetine

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Abstract: A new chemoenzymatic method for the preparation of an intermediate of (−)-Paroxetine is reported. Cyclic anhydrides are used as acylating agents in the lipase-catalyzed esterification of *trans*-4-(4′-fluorophenyl)-3-hydroxymethyl-*N*-phenyloxycarbonylpiperidine in organic solvents. The best enantioselectivities are obtained with two different lipases from *Candida antarctica*. These two lipases show opposite stereochemical preference in these processes, so that both enantiomers can be obtained in their optically pure forms. The (3*S*,4*R*) isomer is an intermediate for the synthesis of (−)-Paroxetine.

Optically pure (−)-Paroxetine **5** (Scheme 1) is a potent and selective inhibitor of 5-hydroxytryptamine reuptake, and is used in the treatment of a variety of human diseases such as depression, obsessive compulsive disorder, and panic disorder.[1] The interest of the pharmaceutical industry in the preparation of this drug requires the development of new synthetic methods suitable to be carried out at large scale. As a part of our research on the enzymatic preparation of optically active drugs by chemoenzymatic methods,[2] we have focused on the resolution of *N*-substituted *trans*-4-(4′-fluophenyl)-3-hydroxymethylpiperidines **1**, key intermediates in the synthesis of (−)-Paroxetine. In a previous report, we described the resolution of these intermediates via a lipase-catalyzed acylation.[3] In this procedure, the enantiopure acylated product and the remaining nonacylated alcohol are separated by chromatography after the enzymatic reaction. Although the availability, the low cost, and the low environmental impact of the lipase-catalyzed resolution of the racemates are important advantages for the large-scale production of the optically pure Paroxetine, the need for a chromatographic separation is a major drawback for the scaling up of that procedure.

The enzymatic transesterification in the resolution of racemic alcohols is well documentated.[4] However, the use of anhydrides instead of esters in the enzyme-catalyzed acylation of alcohols in organic solvent has been less applied and was first described by Cesti et al.[5] The reverse reaction involves the nucleophilic attack of a carboxylic acid to an ester, and is therefore thermodynamically unfavored. The utilization of cyclic anhydrides as acylating agents for the enzymatic esterification is especially advantageous, because it leads to the formation of a monoester of a diacid that can be easily separated from the unreacted alcohol by extraction with base. For instance, Terao et al.[6] have described the lipase-catalyzed resolution of racemic alcohols in organic solvents using succinic anhydride as an acylating agent. Recently, this anhydride has also been used for the resolution of a hydroxymethylpiperidine,[7] even though only moderate enantioselectivities were obtained. Here, we study the enzymatic esterification of alcohol (±)-*trans*-**1** using several commercially available cyclic anhydrides as acyl donors.[8]

In a first set of experiments, several lipases were tested in the esterification reaction of alcohol (±)-*trans*-**1** with 2 equiv of succinic anhydride in toluene at 30 °C. Table 1 (entries 1−4) shows the results obtained with the immobilized lipases from *Candida antarctica* (CAL-A, CAL-B, and CAL-B-L2) and *Pseudomonas cepacia* (PS-C). With other lipases—*Pseudomonas cepacia* (PS), *Candida rugosa* (CRL), or porcine pancreatic (PPL)—the unaltered starting material was recovered. CAL-A showed the highest enantioselectivity ($E = 51$, entry 1),[9] and a high reaction rate. In an attempt to optimize this reaction, we studied the effect of other reaction parameters on the enantioselectivity of the esterification of (±)-*trans*-**1** catalyzed by CAL-A. First, we studied the influence of the organic solvent. The reaction in *t*-BuOMe (entry 5) was slower and less enantioselective than that in toluene (entry 1). On the other hand, the reaction rate was enhanced in *i*-Pr_2O but again the enantioselectivity was lower than in toluene. There was no reaction when acetonitrile, acetone, or 1,4-dioxane were used. Lowering the temperature to 15 °C in toluene (entry 6) did not significantly affect the enantioselectivity. Additional experiments showed that the amount of succinic anhydride affects both the reaction rate and the enantioselectivity. As expected, the reaction rate decreased when 1 equiv of anhydride was employed (entry 7). By contrast, the reaction rate was not significantly enhanced in the presence of 4 equiv of anhydride (compare entries 8 and 1). In both experiments, lower enantioselectivities were

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(1) (a) Dechant, K. L.; Clissold, S. P. *Drugs* **1991**, *41*, 225. (b) Mathis, C. A.; Gerdes, J. M.; Enas, J. D.; Whitney, J. N.; Taylor, S. E.; Zahang, Y.; Mckenna, D. J.; Havlik, S.; Peroutka, S. J. *J. Pharm. Pharmacol.* **1992**, *44*, 801−805.
(2) Gotor, V. *Org. Proc. Res. Dev.* **2002**, *6*, 420−426.
(3) (a) De Gonzalo, G.; Brieva, R.; Sánchez, V. M.; Bayod, M.; Gotor, V. *J. Org. Chem.* **2001**, *66*, 8947−8953. (b) Bayod, M.; Sánchez, V. M.; de Gonzalo, G.; Brieva, R.; Gotor, V. U.S. Patent 2003/0018048 to Astur Pharma.
(4) Santaniello, E.; Reza-Elahi, S.; Ferraboschi, P. Chiral Synthons by Enzymatic Acylation and Esterification Reactions. In *Topics in Current Chemistry*; Fessner, W.D., Ed.; Springer-Verlag: Berlin, Germany, 1999; Vol. 200, pp 415−460.
(5) Bianchi, D.; Cesti, P.; Battistel, E. *J. Org. Chem.*, **1988**, *53*, 5531−5534.
(6) Terao, Y.; Tsuji, K.; Murata, M.; Achiwa, K.; Nishio, T.; Watanabe, N.; Seto, K. *Chem. Pharm. Bull.* **1989**, *37*, 1653.
(7) Goswami, A.; Howell, J. M.; Hua, E. Y.; Mirfakhrae, K. D.; Soumeillant, C. M.; Swaminathan, S.; Qian, X.; Quiroz, F.; Vu, T. C.; Wang, X.; Zheng, B. *Org. Proc. Res. Dev.* **2001**, *5*, 415−420.
(8) Bayod, M.; Sánchez, V. M.; de Gonzalo, G.; Brieva, R.; Gotor, V. ES Patent P200202916 to Astur Pharma.
(9) Chen, C. S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294.

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SCHEME 1. Lipase-Catalyzed Acylation of (±)-*trans*-1 in Organic Solvents with Cyclic Anhydrides

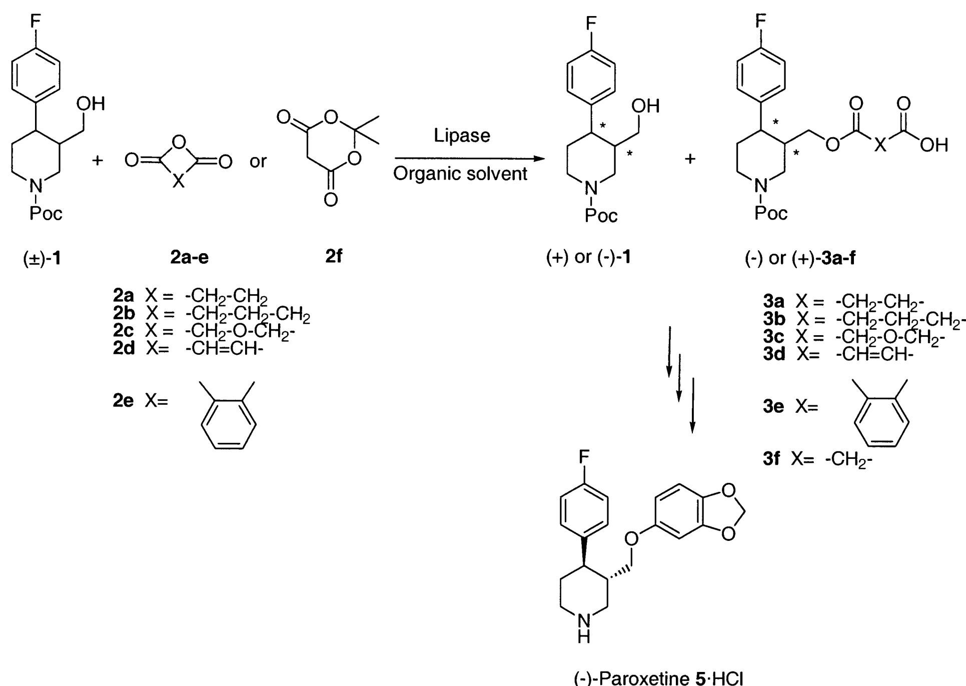

TABLE 1. Lipase-Catalyzed Esterification of (±)-*trans*-1 in Organic Solvents

entry	lipase	anh.	equiv of anh.	solvent	T (°C)	time (h)	c (%)[a]	remaining alcohol *trans*-**1** config	ee$_s$ (%)[b]	product *trans*-**3a**–**f** yield (%)[c]	ee$_p$ (%)[b]	E[e]
1	CAL-A	**2a**	2	toluene	30	2	40	(3*R*,4*S*)	61	72	93	51
2	CAL-B	**2a**	2	toluene	30	7	33	(3*S*,4*R*)	46	56	92	37
3	PS–C	**2a**	2	toluene	30	168	7	(3*S*,4*R*)	3	10	40	2.4
4	CAL-B–L2	**2a**	2	toluene	30	7	30	(3*S*,4*R*)	37	50	85	17
5	CAL-A	**2a**	2	*t*-BuOMe	30	2	24	(3*R*,4*S*)	29	32	91	28
6	CAL-A	**2a**	2	toluene	15	5	42	(3*R*,4*S*)	67	74	93	55
7	CAL-A	**2a**	1	toluene	30	2	26	(3*R*,4*S*)	32	43	94	44
8	CAL-A	**2a**	4	toluene	30	2	42	(3*R*,4*S*)	59	69	91	38
9	CAL-A	**2b**	2	toluene	30	2	32	(3*R*,4*S*)	35	50	73	9.0
10	CAL-B	**2b**	2	toluene	30	20	46	(3*S*,4*R*)	74	75	95	84
11	CAL-B	**2b**	2	*t*-BuOMe	30	10	53	(3*S*,4*R*)	93	79[d]	83	37
12	CAL-B	**2b**	1	toluene	30	20	36	(3*S*,4*R*)	54	61	96	80
13	CAL-B	**2b**	4	toluene	30	20	44	(3*S*,4*R*)	70	75	89	36
14	CAL-A	**2c**	2	toluene	30	168	12	(3*R*,4*S*)	3.0	10	43	2.6
15	CAL-B	**2c**	2	*t*-BuOMe	30	96	52	(3*S*,4*R*)	33	74[d]	30	2.5
16	CAL-A	**2f**	2	*i*-Pr$_2$O	30	120	29	(3*R*,4*S*)	16	39	39	2.6
17	CAL-B	**2f**	2	*t*-BuOMe	30	144	48	(3*S*,4*R*)	31	71	34	2.7

[a] Conversion, $c = ee_s/(ee_s + ee_p)$. [b] Determined by HPLC. [c] Yield referred to the maximum theoretical conversion (50%). [d] Yield referred to the conversion of the resolution. [e] Enantiomeric ratio, $E = \ln[(1 - c)(1 + ee_p)]/\ln[(1 - c)(1 - ee_p)]$.

obtained than in those reactions with 2 equiv of anhydride.

CAL-A catalyzes the esterification of the enantiomer (3*S*,4*R*)-**1**, thus giving (3*S*,4*R*)-**3**. As the intermediate needed to complete the synthesis of (−)-Paroxetine is alcohol (3*S*,4*R*)-**1**, the acylated compound (3*S*,4*R*)-**3** has to be deacylated. Reaction of (3*S*,4*R*)-**3** with 2.0 M sodium hydroxide affords (3*S*,4*R*)-**1** in 76% yield. With the other lipases, the remaining alcohol has the correct configuration (3*S*,4*R*)-**1** and no further transformations are required.

When glutaric anhydride **2b** is used instead of succinic anhydride **2a**, different effects are observed for CAL-A and CAL-B. With glutaric anhydride, the reaction catalyzed by CAL-A (entry 9) is slightly slower and much less enantioselective. By contrast, the reaction catalyzed by CAL-B becomes slower but much more enantioselective (entry 10, $E = 84$). As in the case of the esterification with succinic anhydride and CAL-A, a strong influence of the organic solvent on the CAL-B-catalyzed reaction was observed. The use of *t*-BuOMe (entry 11) or *i*-Pr$_2$O (not shown) as solvents resulted in lower enantioselectivities, even though the reaction rate was considerably enhanced. No improvement was observed in the enantioselectivity when the process was carried out at 15 °C. Finally, changing the amount of glutaric anhydride in the reaction media gave similar results to succinic anhydride: lower reaction rate and almost unchanged enantioselectivity in the process carried out with 1 equiv of anhydride (entry 12), but a high decrease in enantioselectivity with 4 equiv of anhydride (entry 13).

In view of the excellent results obtained in the esterification with the glutaric anhydride, we decided to study this process with the more reactive diglycolic anhydride **2c**, in the belief that it would give a higher reaction rate (because the oxygen atom makes the anhydride more reactive) and comparable enantioselectivity (because its geometry is similar to that of glutaric anhydride). Surprisingly, the enzymatic esterification of alcohol (±)-*trans*-**1** with diglycolic anhydride gave much lower enantioselectivities and reaction rates in all the tested conditions with either CAL-A or CAL-B (entries 14 and 15). These results might be explained by inhibition of the enzyme due to changes in the pH during the course of the reaction. This may happen because the products **3a**−**f** bear a carboxylic acid group. From the comparison of the pK_a value for the first ionization of the corresponding diacids (diglycolic acid, pK_a 4.15;[10] glutaric acid, pK_a 5.27[11]), we can assume that **3c** is more acidic than **3b**. We hypothesize that, due to its higher acidity, **3c** protonates some key positions of the enzyme, thus causing its inhibition. To prove this hypothesis, and taking as reference the experiment of entry 10 (Table 1), we carried out the reaction catalyzed by CAL-B in toluene at 30 °C in the presence of 1 equiv of glutaric anhydride and 1 equiv of diglycolic anhydride. After 20 h, the two possible products were obtained with only 18% overall conversion instead of the 46% obtained with 2 equiv of glutaric anhydride. The enzyme used in this process was washed and reused for a new reaction in the presence of 2 equiv of glutaric anhydride to afford the product with 44% conversion and enantioselectivity $E = 83$, which means that the activity and selectivity have been completely recovered (compare to entry 10). We also carried out the process with fresh enzyme and 2 equiv of glutaric anhydride in the presence of 0.1 equiv of diglycolic acid. Conversion (25%) was lower than that in the absence of diglycolic acid (46%), indicating inhibition by the diglycolic acid. Addition of base might compensate this change in the acidity. Unfortunately, we cannot prove the effect of a small amount of triethylamine as additive in the reaction carried out with the diglycolic anhydride as an acyl donor. In these conditions, the spontaneous nonenzymatic reaction occurs in competition with the enzymatic process. Nevertheless, it can be concluded from these results that the inhibition is reversible and is due to the acidity of the hemiglycidate that is formed in the reaction. However, inhibition by the diglycolic anhydride itself cannot be ruled out by these experiments.

Other anhydrides such as maleic **2d** or phthalic **2e** afforded the unaltered starting material. Finally, we studied the Meldrum's acid as a synthetic equivalent of the malonic anhydride. After 7 days, no reaction was observed in toluene at 30 °C with 2 equiv of the Meldrum's acid. When the process was carried out in toluene at 50 °C, or in solvents such as *t*-BuOMe or *i*-Pr_2O (entries 16 and 17), moderate to good conversions were obtained, but enantioselectivities were too low.

To prove the economic efficiency of these biocatalytic methods, we also have investigated the feasibility of reusing the immobilized lipase CAL-B in the best conditions found for this process: toluene as organic solvent, 2 equiv of glutaric anhydride as acylating agent, and 20 h reaction at 30 °C. As shown in Table 2, the reuse of the immobilized lipase afforded almost the same enantioselectivity in the second and third cycles, with only a very small decrease in reaction rate. A moderate loss of enzyme activity was observed in the fourth and fifth cycles, as indicated by the lower conversions and enantioselectivities. An appreciable loss of enzyme activity was observed only when the enzyme was recycled for the sixth time.

TABLE 2. Recycling of the Enzyme CAL-B in the Esterification of (±)-*trans*-1[a]

cycle no.	*c* (%)	remaining alcohol *trans*-**1** ee_s (%)	product *trans*-**3a**−**f** ee_p (%)	*E*
1	34	49.1	96.1	82.0
2	32	44.9	96.3	82.6
3	29	40.4	96.4	81.1
4	27	37.0	96.4	78.4
5	25	32.8	96.1	69.3
6	17	20.2	94.6	43.9

[a] CAL-B catalyzed esterification of (±)-*trans*-**1** with 1 equiv of glutaric anhydride in toluene at 30 °C and 20 h reaction time.

In conclusion, we have been able to resolve the *trans*-4-(4′-fluorophenyl)-3-hydroxymethyl-*N*-phenyloxycarbonilpiperidine [(±)-*trans*-**1**] by a lipase-catalyzed esterification with cyclic anhydrides. Good yields and high enantioselectivities can be achieved by an appropriate selection of the experimental conditions. It is noteworthy that CAL-A and CAL-B have complementary enantiopreference for opposite enantiomers. Taking into account the advantages of the lipase-catalyzed reactions, the easy separation of the reaction products by extraction, and the feasibility of reusing the immobilized lipase, this procedure is expected to be suitable for the multigram scale preparation of the antidepressant (−)-Paroxetine.

Experimental Section[12]

General Methods. *Candida antarctica* lipase B (CAL-B, Novozym 435, 7300 PLU/g) was a gift from Novo Nordisk Co. Lipases from *Pseudomonas cepacia* lipase (PS-C, CHIRAZYME L-1, >10 kU/g), *Candida antarctica* lipase B (CAL-B-L2, CHIRAZYME L-2, c−f, C3, ≥400 U/g), *Candida rugosa* lipase (CRL, CHIRAZYME L-3, >250 U/mg), and *Candida antarctica* lipase A (CAL-A, CHIRAZYME L-5, 1 kU/g) were supplied by Roche Molecular Biochemicals. *Porcine pancrea*s lipase (PPL, type II, 308 U/mg of solid) was supplied by Sigma. Immobilized *Pseudomonas cepacia* lipase (PSL-C, 1019 U/g) was a product of Amano Co. All these commercial lipases were carrier-fixed products except CHIRAZYME L-3. All other chemicals or solvents were of the highest quality grade available.

Optical rotations were measured on a Perkin-Elmer 241 polarimeter and are quoted in units of 10^{-1} deg cm^2 g^{-1}. IR spectra were recorded on a Perkin-Elmer 1720-X FT Infrared spectrophotometer. ^{1}H and ^{13}C NMR were obtained with TMS (tetramethylsilane) as internal standard, using Bruker AC-200 (^{1}H, 200.13 MHz and ^{13}C, 50.3 MHz), AC-300 (^{1}H, 300.13 MHz and ^{13}C, 75.5 MHz), or DPX-300 (^{1}H, 300.13 MHz and ^{13}C, 75.5 MHz) spectrometers. Mass spectra were recorded on a Hewlett-

(10) Kiso, Y.; Hirokawa, T. *Chem. Lett.* **1980**, 323−326.
(11) Dubey, S. N.; Baweia, R. K.; Puri, D. M. *J. Indian Chem. Soc.* **1982**, *59*, 1133−1135.

(12) Compound **1**[2] has been previously reported. ^{1}H and ^{13}C NMR data are given in the Supporting Information. For compounds **3a**−**f** and **4a**−**f**, full spectra data and copies of ^{1}H and ^{13}C NMR spectra are given in the Supporting Information. The degree of purity is indicated by the inclusion of elemental analysis.

Packard 1100 Series spectrometer. Microanalyses were performed on a Perkin-Elmer 240B elemental analyzer. Flash chromatography was performed on Merck silica gel 60 (230–400 mesh). The ee values were determined by chiral HPLC analysis on a Shimazdu LC liquid chromatograph, using a CHIRALCEL OD column (4.5 × 250 mm). Two well-resolved peaks were obtained for all the racemic compounds (1 mg in 4 mL of mobile phase; 20-μL sample).

Enzymatic Resolution of (±)-*trans*-4-(4′-Fluorophenyl)-3-hydroxymethyl-*N*-phenyloxycarbonylpiperidine [(±)-*trans*-1] with Cyclic Anhydrides (2a–f). The lipase (125 mg) and the corresponding anhydride (2 equiv, unless stated otherwise)—or the Meldrum's acid—were added to a solution of (±)-*trans*-**1** (100 mg, 1 equiv) in the corresponding solvent (15 mL). The mixture was shaken at the selected temperature and 250 rpm in a rotatory shaker. The progress of the reaction was monitored by TLC, using the solvent system hexane/ethyl acetate 1:2. The enzyme was removed by filtration and washed with ethyl acetate, and the solvent was evaporated under reduced pressure. After this, chloroform (20 mL) was added to the mixture and the reaction was extracted with a 5% aqueous solution of sodium bicarbonate (3 × 20 mL). The organic layer containing the unreacted alcohol (+)- or (−)-*trans*-**1** was dried over Na_2SO_4 and the solvent was evaporated under reduced pressure.

The aqueous layer was adjusted to pH 4–5 by slow addition of 1.0 N HCl. After acidification, this layer was extracted with MTBE (3 × 50 mL). The MTBE extract was washed with a 10% aqueous solution of sodium chloride (3 × 15 mL) and dried over Na_2SO_4, and the solvent was removed to give the corresponding enantiomerically enriched carboxylic acid (+)- or (−)-*trans*-**3a–f**.

Enzymatic Resolution of (±)-*trans*-4-(4′-Fluorophenyl)-3-hydroxymethyl-*N*-phenyloxycarbonylpiperidine [(±)-*trans*-1] with Glutaric Anhydride (2b). CAL-B (125 mg) and glutaric anhydride (68 mg, 2 equiv) were added to a solution of (±)-*trans*-**1** (100 mg, 1 equiv) in toluene (15 mL). The mixture was shaken at 30 °C and 250 rpm in a rotatory shaker for 20 h. The enzyme was removed by filtration and washed with ethyl acetate, and the solvent was evaporated under reduced pressure. After this, chloroform (20 mL) was added to the mixture and the reaction was extracted with a 5% aqueous solution of sodium bicarbonate (3 × 20 mL). The organic layer was dried over Na_2SO_4 and the solvent was evaporated under reduced pressure to obtain (−)-*trans*-**1** as a white solid (39.5 mg, 73%). $[\alpha]_D^{18}$ −2.19 (*c* 0.60, MeOH), ee 74%.

The aqueous layer was adjusted to pH 4–5 by slow addition of 1.0 N HCl. After acidification, this layer was extracted with MTBE (3 × 50 mL). The MTBE extract was washed with a 10% aqueous solution of sodium chloride (3 × 15 mL) and dried over Na_2SO_4, and the solvent was removed to give the corresponding enantiomerically enriched carboxylic acid (+)-*trans*-**3b** as an hygroscopic solid (50.4 mg, 75%). $[\alpha]_D^{18}$ +2.84 (*c* 0.56, MeOH), ee 95%.

Methylation of the Carboxylic Acids for HPLC Analysis. To a solution of the corresponding carboxylic acid *trans*-**3a–f** (100 mg, 1 equiv) in anhydrous THF (4 mL) were added dry MeOH (200 μL) and a 2.0 M solution of trimethylsilyldiazomethane in hexane (200 μL). The mixture was stirred for 2 h at room temperature. Then, the solvents were evaporated under reduced pressure and the crude residue was purified by flash cromathography on silica gel with CH_2Cl_2/diethyl ether 9:1 to afford the esters *trans*-**4a–f**.

Chemical Hydrolysis of (3*S*,4*R*)-(−)-*trans*-3-[(3-Carboxypropanoyl)oxymethyl]-4-(4′-fluorophenyl)-*N*-phenyloxycarbonylpiperidine. (3*S*,4*R*)-*trans*-**3a** (100 mg, 0.23 mmol) was dissolved in a 2.0 M aqueous solution of NaOH (5 mL). The mixture was stirred for 4 h at room temperature. The reaction was extracted with toluene (4 × 10 mL) and then the organic layer was washed with a 10% aqueous solution of sodium chloride (3 ×10 mL), dried over Na_2SO_4, filtered, and evaporated under reduce pressure to afford (3*S*,4*R*)-*trans*-**1** as a white solid (57.5 mg, 76%).

Study of Enzyme Recycling. To a solution of (±)-*trans*-**1** (100 mg, 0.3 mmol) in toluene (6 mL) were added 125 mg of Novozym 435 and glutaric anhydride (34 mg, 0.3 mmol). The reaction was stirred at 30 °C for 20 h in a rotatory shaker. After this, the enzyme was filtered off and washed with dry toluene and stored under nitrogen atmosphere. This enzyme was used repeatedly in the subsequent enzymatic resolutions, following in all cases the same procedure.

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Supporting Information Available: Complete ^{1}H and ^{13}C NMR spectral data in addition to mp, IR, microanalysis, and MS data. This material is available free of charge via the Internet at http://pubs.acs.org.

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