Pergamon

Tetrahedron: Asymmetry Vol. 6, No. 7, pp. 1765-1772, 1995

0957-4166/95 $9.50+0.00

0957-4166(95)00222-7

A Chemoenzymatic Synthesis of Both Enantiomers of 2-Phenyl-3-hydroxypropylcarbamate, a Metabolite of Felbamate

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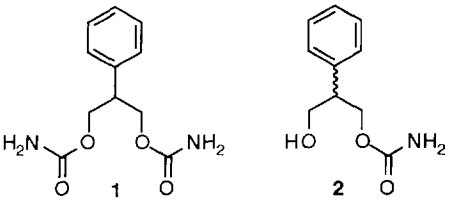
Abstract: PPL catalyzed desymmetrization of 2-phenyl-1,3-propanediol (**3**) was the key step in the chemoenzymatic synthesis of both enantiomers of 2-phenyl-3-hydroxypropylcarbamate (**2**). Unsuccessful attempts at enzyme catalyzed (PPL, Novo SP435) transcarbamoylation, aminolysis and ammonolysis are also described.

Introduction

2-Phenyl-3-hydroxypropylcarbamate (**2**) has been identified as a minor metabolite of the antiepileptic drug felbamate (Felbatol) (**1**). To carry out further biological testing, quantities of both enantiomers of monocarbamate **2** were required.

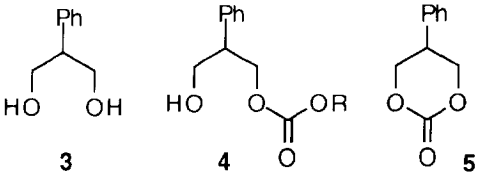


Herein we describe convenient unoptimized syntheses of both enantiomers of the monocarbamate **2** in 3-6 steps, not requiring chromatography. The syntheses use a biotransformation at two stages: enzyme catalyzed desymmetrization of 2-phenyl-1,3-propanediol (**3**) to introduce the chiral center at position C-2, and an enzymaticdeacylation of the ester/carbamate **7**.

Results and Discussion

A. Transcarbamoylation, Aminolysis and Ammonolysis

Several approaches to a chemoenzymatic synthesis of both enantiomers of **2** were investigated. Both the enzyme catalyzed transcarbamoylation of diol **3** with methyl carbamate,[1] and the alcoholysis of felbamate (**1**) with nBuOH [2] were screened without success.[3]



We then considered the aminolysis or ammonolysis of selected carbonates. Following the work of Gotor,[3b,4] we examined enzymatic alkoxycarbonylation. We anticipated that enzymatic desymmetrization of diol **3** to a chiral carbonate **4**, followed by treatment with ammonia or an amine would yield the optically

enriched monocarbamate **2** or a suitable precursor. However, the PPL catalyzed[5] alkoxycarbonylation of diol **3** with diethyl carbonate or the activated carbonylating agents, vinyloxime carbonate and vinylbenzyloxy carbonate provided the monocarbonate in only low yield and low ee (60-70% ee).

Using the conditions described by de Zoote[6] for the enzymatic ammonolysis of esters, we also examined the Novo SP435 catalyzed ammonolysis of racemic monoethyl carbonate **4** (R=Et) in tBuOH[7] and tAmylOH.[8] Only racemic monocarbamate **2** was observed. Similarly, attempted SP435 catalyzed ammonolysis of the cyclic carbonate **5** using NH_3 gas in THF, or Et_3N/NH_4Cl in tBuOH[9] resulted in racemic **2**.

B. Transesterification and Hydrolysis

Following our lack of success in enzymatic transcarbamoylation, alkoxycarbonylation or ammonolysis, we investigated a 3 step route to chiral monocarbamate (S)-**2** via enzymatic acylation of diol **3** followed by chemical carbamoylation and deacylation.

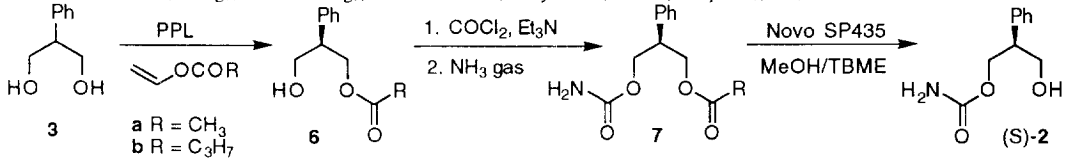
From a screen of 53 lipases, Porcine Pancreatic Lipase (PPL)(Sigma Type II) emerged as the best candidate for the enzymatic acetylation of diol **3** under transesterification conditions;[10] a similar result has been reported previously.[11]

In a study of 10 solvents, the best results were obtained with vinyl acetate, THF, TBME and acetone (Table 1). The reactions were slower in dioxane, acetonitrile and t-amyl alcohol, and very slow in toluene, in which the diol **3** did not completely dissolve. Reactions in isopropenyl acetate were extremely slow compared to vinyl acetate.

Table 1. Acylation of Diol **3** with PPL in Various Solvents

Solvent	% Diol	% MonoOAc	% DiOAc
Vinyl Acetate	0	78.9	19.5
THF	0	83.2	16.8
TBME	0.4	82.7	16.9
Acetone	0.7	86.5	12.9
Acetonitrile	17.0	76.9	6.1
t-Amyl Alcohol	9.6	75.6	14.8
1,4-Dioxane	30.0	67.9	2.2
Toluene	49.3	48.8	1.9
isoPropenyl Acetate	94.9	5.1	0

Conditions: Diol **3** (50 mg), PPL (200 mg), Solvent 1.0 mL, VinylOAc (0.1 mL, 3 equivs.), RT, 3.5 h.



R-(+)-monoacetate **6a** (97% ee) was prepared on a 50 g scale using vinyl acetate in THF, but a subsequent attempt to prepare the (S)-acetoxycarbamate **7a** using isocyanic acid resulted in complete racemization, presumably due to 1,3 acyl migration under acidic conditions. Carbamoylation with phosgene/NH_3 followed by deacylation with K_2CO_3/MeOH did provide the desired monocarbamate (S)-**2a**, but with significant racemization during the deacylation step (66-80% ee). To avoid this problem, enzymatic

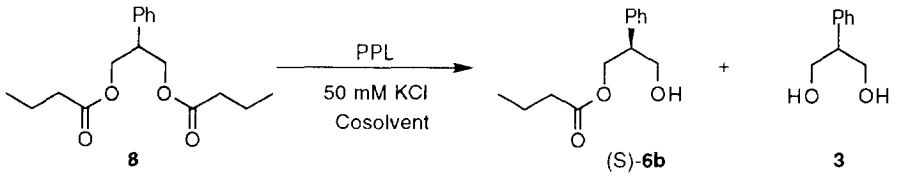
methanolysis of **7a** was examined using the 9 enzymes[12] which showed highest reactivity in the screen for acetylation of diol **3**. After 68 h Novo SP435 showed the highest conversion. Significantly, PPL showed only sluggish methanolysis when carried out under similar conditions.[12] This combination of an enzymatic desymmetrization and chemical carbamoylation provided a convenient route to both enantiomers of the monocarbamate **2**.

For the initial acylation reaction, the enzymatic butyrylation of diol **3** was preferred to acetylation because: (i) the monobutyrate **6b** showed better resolution on chiral HPLC than the monoacetate **6a**, facilitating ee determination and, (ii) since the reaction does not show complete prochiral selectivity, a significant amount of diester has to be formed to ensure complete consumption of diol **3** and to enhance the ee of the remaining monoester **6**[13], thus lowering the overall yield. The use of butyrate rather than acetate esters allowed the subsequent separation of dibutyrate and unreacted diol by differing solubility in nonpolar solvents.[14]

Diol **3** (40 g) was acylated with 1.5 equivalents of vinyl butyrate in THF at 0°C in the presence of PPL (80 g). After 23.5 h the composition of the isolated crude product was 24.3% dibutyrate, 74.4% monobutyrate (96.8% ee) and 1.3% diol. The absolute stereochemistry of the monobutyrate **6b** was initially designated as (R) by analogy with the monoacetate **6a** formed under similar conditions. However, since the optical rotation of the crude product was reversed in different solvents, the stereochemistry was established as (R) by conversion to (S)-(-)-Tropic acid by Jones' oxidation and enzymatic deacylation.[15]

Carbamoylation was carried out using phosgene/NH_3 (20 g scale) and the butyroxycarbamate **7b** was isolated as a white waxy solid by addition of hexane to the crude isolated product (15.5 g, 79%). Enzymatic deacylation was carried out in 10% MeOH/TBME using an equal weight of Novo SP435 to provide monocarbamate (S)-**2** (97.6% ee). The crude product was recrystallized from EtOAc/hexane (1:1) to yield fine white needles (1st crop 5.05 g, 44.8%; 2nd crop 2.50 g, 22.2%). Both crops showed a single enantiomer on chiral HPLC.

Preparation of monocarbamate (R)-**2** required diol **3** to be derivatized with opposite chirality at the C-2 position. This could be achieved either by PPL catalyzed hydrolysis of the dibutyrate **8**,[11a,16] or by inverting the chiral centre of the monobutyrate (R)-**6b** by a protection/deacylation sequence.



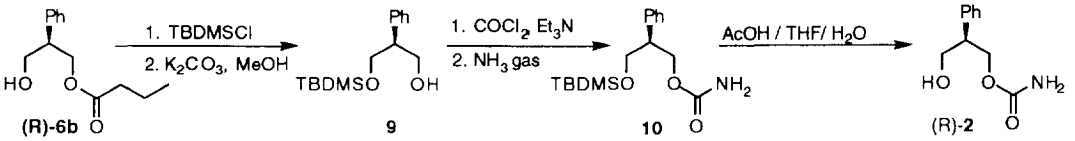
8 (S)-**6b** **3**

Table 2. PPL Hydrolysis of Dibutyrate **8**

Cosolvent	Volume 0.2M NaOH	Initial Rate mmol/h.g enzyme	% Diol **3**	% MonoOBu **S-6b**	% DiOBu **8**	% ee
None[a]	3.90	2.14	41.1	14.0	44.9	92.8
10% THF[b]	3.81	2.65	16.2	59.7	24.1	98.6
20% TBME[c]	4.30	3.29	29.3	48.0	22.7	99.3

Conditions: DiOBu (0.25 g), PPL (0.25 g). [a] 50 mM KCl (15 mL). [b] 50 mM KCl (15 mL), THF (1.5 mL). [c] 50 mM KCl (12 mL), TBME (3 mL), pH 7.0 pHstat.

Hydrolysis of **8** in 50 mM KCl in the absence of cosolvent showed poor prochiral selectivity and poor chemoselectivity (Table 2). In the presence of a miscible (10% THF) or immiscible (20% TBME) cosolvent excellent prochiral selectivity was observed (98-99% ee), but the chemoselectivity remained poor; after addition of one equivalent of base, substantial amounts of diol **3** and dibutyrate **8** were present.



Because of the lack of chemoselectivity under hydrolytic conditions, the protection/deacylation sequence was used to invert the chiral center. The free hydroxyl of (R)-**6b** (20 g, ~17% dibutyrate) was protected as the TBDMS ether (TBDMS-Cl/imidazole in DMF). The crude product was deacylated with K_2CO_3/MeOH and the isolated material was purified by the addition of hexane: the insoluble diol **3** crystallized and was removed by filtration while the soluble monoTBDMS ether **9** was isolated from the mother liquor (17.4 g, 88.0%).

The carbamate was introduced using phosgene/ammonia and the TBDMS group removed under aqueous acidic conditions (AcOH/THF/H_2O 3:1:1), without racemization. The crude product (R)-**2** (96.4% ee) was recrystallized from EtOAc/hexane (5.91 g, 50.9%; 99.5% ee).

Experimental

General: Porcine Pancreatic Lipase (PPL) (lot# 41H0954) was obtained from Sigma Chemical Co., and Novo SP435 (lot# LCC0002) from Novo Nordisk. SP435 is the Type B lipase of *Candida antarctica*, cloned and expressed in *Aspergillus oryzae*, and immobilized on macroporous acrylic beads. This is now commercially available as Novozyme 435. All enzymes and chemicals were used as received. HPLC was carried out on a Waters 600 system equipped with a Daicel Chiralcel OD column (0.46 x 25 cm) (5% or 10% EtOH/hexane; 1.0 mL/min; RT; detection at 215 nm). GC was performed on a Shimadzu GC-14A equipped with a J&W DB-1 capillary column (15 m x 0.25 mm x 0.25 μm). Optical rotations were determined on a Perkin-Elmer 243-B Polarimeter. Flash chromatography was carried out with Sorbisil C60 (40/60A).

(R)-2-Phenyl-3-hydroxypropyl butyrate (**6b**). A mixture of 2-phenyl-1,3-propanediol (**3**) (40.0 g, 0.26 mol), vinyl butyrate (50.0 mL, 0.39 mol) and porcine pancreatic lipase (80.0 g) in THF (200 mL) was stirred at 0°C for 6 h, and then stored in a freezer for 23.5 h . The reaction mixture was then filtered through a Celite plug and the filter cake washed with EtOAc (2 x 100 mL). The combined filtrate was evaporated on a rotary evaporator (bath temperature 30°C). The residue was redissolved in EtOAc (200 mL) and washed with water (2 x 100 mL), satd. $NaHCO_3$ (100 mL), water (100 mL) and satd. NaCl (50 mL), dried ($MgSO_4$), filtered and evaporated (bath temperature 30°C) to yield a yellow oil (64.42 g, theoretical yield 57.79 g;[17] 96.8% ee)($[\alpha]_D^{17}$ = -4.17 (c 0.888, EtOH), $[\alpha]_D^{23}$ = +14.22 (c 1.118, $CHCl_3$). A sample (10 g) of the crude reaction product was purified by column chromatography and the monobutyrate isolated (6.85 g, 94.9% ee): $[\alpha]_D^{25}$ = -6.07 (c 2.241, EtOH), $[\alpha]_D^{25}$ = +18.56 (c 2.952, $CHCl_3$); IR (neat) 3446 (b), 1735 cm^{-1}; CI-MS (CH_4): *m/z* 223 (M+1)(53%), 117 (100%); ^1H NMR ($CDCl_3$) δ 0.90 (t, 3H), 1.60 (sex, 2H), 2.19 (bs, 1H; D_2O exch.), 2.26 (t, 2H), 3.14 (quin, 1H), 3.82 (bm, 2H; d D_2O exch.), 4.38 (7.2-7.4 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 13.62,

18.39, 36.13, 47.33, 63.81, 64.79, 127.26, 128.13, 128.69, 139.08, 173.94. Anal. Calcd. for $C_{13}H_{18}O_3$: C, 70.24; H, 8.16. Found: C, 70.09; H, 7.95.

(S)-2-Phenyl-3-butyroxypropylcarbamate (**7b**). A solution of crude (R)-**6b** (20.0 g, 90.0 mmol) in toluene (300 mL) was stirred under N_2 and cooled to -20°C, and then Et_3N (20.0 mL, 143 mmol) added. A solution of phosgene in toluene (50 mL, 96.5 mmol) was added dropwise to the reaction mixture over a period of 12 minutes, maintaining the temperature at -28 to -16°C. The reaction mixture was stirred for 15 minutes, allowed warm to -10°C and then recooled to -22°C. Ammonia gas was passed through the reaction mixture for a period of 20 minutes at -20°C. Gas addition was halted, the reaction cooled to -60°C and stirred for 85 minutes during which time the reaction warmed to -5°C. The reaction was quenched by addition of water (200 mL), and the organic layer was separated and washed with water (100 mL), 1.2 M HCl (100 mL), water (100 mL), satd. NaCl (50 mL), dried ($MgSO_4$), filtered and evaporated to obtain a yellow liquid (23.78 g). Hexane (100 mL) was added to the crude product, the mixture vigorously stirred, and the waxy solid which precipitaed triturated. The solid was filtered and washed with hexane (2 x 50 mL), air dried (15.54 g) and used without further purification (90-92% pure by GC/HPLC peak areas). A sample was purified by column chromatography: $[\alpha]_D^{17}$ = -6.18 (c 1.084, EtOH), $[a]_D^{25}$ = +1.10 (c 0.907, $CHCl_3$); IR (Nujol) 3455, 3351, 1702 cm^{-1}; CI-MS (CH_4): *m/z* 266 (M+1)(2%), 205 (100%); ^1H NMR ($CDCl_3$) δ 0.89 (t, 3H), 1.59 (sex, 2H), 2.25 (t, 2H), 3.32 (quin, 1H), 4.34 (m, 4H), 4.77 (bs, 2H), 7.22-7.33 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 13.61, 18.35, 36.08, 44.06, 64.66, 65.45, 127.37, 128.00, 128.63, 138.43, 156.62, 173.52. Anal. Calcd. for $C_{14}H_{19}NO_4$: C, 63.48; H, 7.87; N, 5.28. Found: C, 63.41; H, 7.87; N, 5.35.

(S)-2-Phenyl-3-hydroxypropylcarbamate (S)-(**2**). A mixture of crude (S)-**7b** (15.31g, 57.7 mmol) and Novo SP435 (15.95 g) in TBME (200 mL) and MeOH (25 mL) was stirred at room temperature for 25 h. EtOAc (200 mL) was added to the reaction mixture, which was stirred for 15 minutes and then filtered. The enzyme beads were washed on the filter with MeOH (4 x 50 mL) to redissolve precipitated product. The combined filtrates were evaporated (30°C) and then dried under vacuum (13.03 g). EtOAc (200 mL) was added to the crude product, the mixture heated to reflux and then filtered through a bed of Celite while still warm to remove a small amount of insoluble material. Hexane (200 mL) was added to the filtrate, and the solid which crystallized was filtered and air-dried (7.25 g). Recrystallization from EtOAc/hexane (100:150 mL) yielded a white solid (1.26 g).[18] The mother liquors were evaporated and the residue recrystallized from EtOAc/hexane (50:75 mL) to give white needles (5.05 g, 44.8%; single enantiomer by chiral HPLC). A second crop was obtained by evaporating the mother liquors and recrystallizing the residue from EtOAc/hexane (50:75 mL) (2.50 g, 22.2%; single enantiomer by chiral HPLC): $[\alpha]_D^{25}$ = -19.84 (c 0.373, $CHCl_3$); IR (Nujol) 3440, 3302, 3218, 1667, 1618 cm^{-1}; CI-MS (CH_4): *m/z* 196 (M+1)(2%), 91 (100%); ^1H NMR (DMSO-d_6) δ 3.00 (quin, 1H), 3.59 (m, 2H), 4.13 (dd, 1H), 4.25 (dd, 1H), 4.74 (t, 1H; D_2O exch.); 6.43 (bs, 2H), 7.18-7.32 (m, 5H); ^{13}C NMR (DMSO-d_6) δ 47.34, 62.56, 64.44, 126.31, 128.06, 140.71, 156.69. Anal. Calcd. for $C_{10}H_{13}NO_3$: C, 61.52; H, 6.71; N, 7.18. Found: C, 61.47; H, 6.96; N,7.30.

(S)-2-Phenyl-3-tertbutyldimethylsilyloxypropyl Butyrate. (R)-**6b** (20.12 g, 90.5 mmol) and TBDMSCl (14.0 g, 92.9 mmol) were dissolved in DMF (100 mL), stirred under N_2 and cooled to -20°C. Imidazole (12.01 g, 176 mmol) was added in 10 portions over a period of 50 minutes, maintaining the temperature at ~15°C. After 1 h the rection flask was sealed and stored in a freezer overnight . The reaction was quenched by the addition of

water (100 mL) and then partitioned between water (200 mL) and EtOAc (250 mL). The water layer was separated, diluted with water (300 mL) and back-extracted in three portions with EtOAc (200 mL). The combined organic extracts were washed with water (100 mL), 1.2 M HCl (100 mL), water (100 mL), and satd. NaCl (100 mL), then dried ($MgSO_4$), filtered and evaporated (bath temperature 30°C), and used immediately without purification.

(S)-2-Phenyl-3-tertbutyldimethylsilyloxypropanol (**9**). The crude TBDMSbutyrate (*vide supra*) was dissolved in MeOH (100 mL) and anhydrous K_2CO_3 (20.0 g, 145 mmol) added. The mixture was stirred at room temperature for 5 h. Most of the MeOH was removed (rotary evaporator, bath temperature 30°C), and the residue was partitioned between water (100 mL) and EtOAc (100 mL). The organic layer was then washed with 1.2 M HCl (100 mL), water (100 mL) and satd. NaCl (50 mL), then dried ($MgSO_4$), filtered and evaporated. Hexane (100 mL) was added to the crude product, and the solution seeded with 2-phenylpropanediol crystals and kept at 4°C. After 18 h the precipitated crystals were removed and the filtrate evaporated to obtain a yellowish liquid (17.40 g, 92% pure by HPLC) which was used without further purification.

(S)-2-Phenyl-3-tertbutyldimethylsilyloxypropylcarbamate (**10**) . A solution of **9** (17.14g, 64.3 mmol) in toluene (300 mL) was stirred under N_2, cooled to -20°C and Et_3N (13.0 mL, 93.3 mmol) added. A solution of phosgene in toluene was added dropwise over period of 22 minutes, maintaining the temperature at -22 to -9°C. Stirred for a further 6 minutes then cooled to -57°C and stirred for 1.75 h, during which time the reaction warmed to -2°C. The reaction was cooled to -25°C, and ammonia gas bubbled through the reaction for 15 minutes, maintaining temperature at -28 to -16°C. The reaction was quenched by addition of water (200 mL), and the separated organic layer was further washed with water (100 mL), 1.2 M HCl (100 mL), water (100 mL) and satd. NaCl (50 mL), then dried ($MgSO_4$), filtered and evaporated (bath temperature 30°C) to obtain a yellow liquid (19.31 g).

(R)-2-Phenyl-3-hydroxypropylcarbamate (R)-(**2**). Crude **10** (18.32 g, 59.2 mmol) was dissolved in a AcOH/THF/water mixture (100 mL; 3:1:1) and stirred at room temperature. After 23.3 h the reaction mixture was concentrated to ~20 mL (bath temperature 40°C), partitioned between water (150 mL) and EtOAc (200 mL). The organic layer was washed with water (100 mL), satd. Na_2CO_3 (4 x 50 mL), water (50 mL) and satd. NaCl (50 mL) and dried ($MgSO_4$). The filtrate was evaporated to obtain a liquid which solidified under vacuum (9.14 g). The crude product was dissolved in hot EtOAc (100 mL), hexane (200 mL) added, and stood in the fridge overnight. The white solid which crystallized was filtered and dried (5.91 g, 47.1%; 99.5% ee). A second crop (0.54 g) was obtained from the mother liquors: $[\alpha]_D^{25}$ = +23.78 (c 0.349, $CHCl_3$); IR (Nujol) 3445, 3300, 3214, 1646 cm^{-1}; CI-MS (C_4H_8): *m/z* 196 (M+1)(16%), 117 (100%); ^1H NMR (DMSO-d_6) δ 3.02 (quin, 1H), 3.59 (m, 2H), 4.13 (dd, 1H), 4.25 (dd, 1H), 4.74 (t, 1H; D_2O exch.), 6.45 (bs, 2H), 7.18-7.32 (m, 5H); ^{13}C NMR (DMSO-d_6) δ 47.33, 62.55, 64.43, 126.30, 128.04, 140.70, 156.67. Anal. Calcd. for $C_{10}H_{13}NO_3$: C, 61.52; H, 6.71; N, 7.18. Found: C, 61.53; H, 6.99; N, 7.33.

*Structure Correlation of (R)-2-Phenyl-3-hydroxy propyl butyrate (**6b**) and (S)-(-)-Tropic Acid.* Crude **6b** (4.73 g, ~17.4 mmol) was disssolved in acetone (30 mL) and cooled in an ice bath. Added Jones' reagent until an orange color persisted, then quenched by addition of iPrOH. The reaction mixture was partitioned between EtOAc (150 mL) and H_2O (100 mL) and the organic layer was further washed with H_2O (2X100 mL) and then extracted with satd Na_2CO_3 (2X50 mL) and H_2O (50 mL). The combined basic extracts were acidified with 6

M HCl (90 mL) and extracted with EtOAc (150 mL), and the organic layer washed with H_2O (50 mL), satd. NaCl (50 mL), dried ($MgSO_4$), filtered and evaporated to obtain a colorless liquid. This was dissolved in MeOH (20 mL), anhydrous K_2CO_3 (1.5 g, 11.5 mmol) added, and the mixture stirred at RT for 16.5 h.[19] Quenched by addition of 2.0 M HCl (50 mL), extracted with EtOAc (100 mL) and washed the organic layer with satd. NaCl (50 mL), dried ($MgSO_4$), filtered and evaporated to get a pinkish solid (1.36 g, 46.9% crude yield). A sample was recrystallized from hot toluene: $[\alpha]_D^{24}$ = -68.1 (c 0.844, EtOH), lit.[14] $[\alpha]_D^{20}$ = +74.4 (c 2.057, EtOH) for (R)-(+) enantiomer.

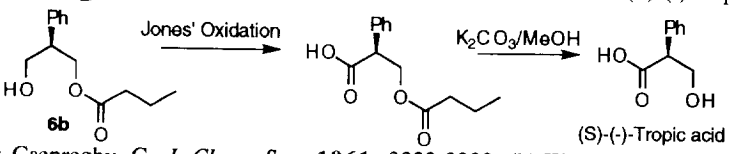
Acknowledgements: We would like to thank Drs. David Tsai and Albert Kwok for providing samples of diol **3**, felbamate **1** and the monocarbamate **2**, for helpful discussions, and for carrying out the reaction of (S)-**7a** with isocyanic acid.

REFERENCES AND NOTES

1. The reaction of diol **3** (50.5 mg, 0.33 mmol) with methyl carbamate (0.26 g, 3.4 mmol) in MeCN (1.0 mL) at RT was screened using 39 commercially available solid lipases. No product was detected after 64 h with any enzyme.
2. The reaction of felbamate (**1**) (50.0 mg, 0.21 mmol) with nBuOH (10 equivs.) in THF (2.0 mL) at 30°C was screened using 138 hydrolases commercially available as dry solids. No significant reaction was observed after 48-72 h. Cf. Bevinakatii, H.S.; Newadkar, R.V. *Tetrahedron: Asymm.* **1990**, *1*, 583-586.
3. Some carbamates have been reported to inhibit lipases: (a) Hosie, L.; Quinn, D.M.; Sutton, L.D. *J. Biol. Chem.* **1987**, *262*, 260-264. (b) García-Alles, L.F.; Morís, F.; Gotor, V. *Tetrahedron Lett.* **1993**, *34*, 6337-6338.
4. The preparation of chiral N-alkyl carbamates via the enzymatic alkoxycarbonylation of amines was recently reported, although the enantioselectivity was generally low. Pozo, M.; Gotor, V. *Tetrahedron* **1993**, *49*, 4321-4326.
5. Since a screen of the enzyme catalyzed acetylation of diol (**3**) identified PPL as the best enzyme, further studies were confined to PPL and Novo SP435.
6. (a) de Zoote, M.C.; Kock-van Dalen, A.C.; van Rantwijk, F.; Sheldon, R.A. *J. Chem. Soc. Chem. Commun.* **1993**, 1831-1832. (b) Garcia, M.J.; Rebolledo, F.; Gotor, V. *Tetrahedron* **1993**, *50*, 6935-6940. de Zoete et al., ruled out PPL as a suitable catalyst for enzymatic ammoniolysis of esters.
7. NH_3 gas was bubbled through a solution of **4** (R=Et) (140 mg, 0.67 mmol) in tBuOH (5 mL) for 4 h; only ~2% monocarbamate was formed. Added SP435 (143 mg) and continued NH_3 addition for 9 h. Only racemic monocarbamate was observed.
8. NH_3 gas was bubbled through a mixture of **4** (R=Et) (165 mg, 0.79 mmol) and SP435 (155 mg) in tAmylOH. After 9 h only racemic monocarbamate was observed.
9. Chen, S.-T.; Jeng, M.-K.; Wang, K.-T. *Synthesis* **1993**, 858-860.
10. A mixture of diol (**3**) (0.35 M), vinylOAc (5 equivs.) and enzyme (typically 20-60 mg) was shaken in EtOAc (1.0 mL) at 250 rpm at RT. Reactions were examined by TLC (30% EtOAc/hexane) after 2 h and 19 h, and promising reactions were analyzed by chiral HPLC (Chiralcel OD).
11. For the previous use of crude, fractionated or immobilized PPL under transesterification conditions, see: (a) Ramos Tombo, G.M.; Schar, H.-P.; Fernandez i Busquets, X.; Ghisalba, O. *Tetrahedron Lett.* **1986**, *27*, 5707-5710. (b) Guanti, G.; Banfi, L.; Riva, R. *Tetrahedron: Asymm.* **1994**, *5*, 9-12.
12. **7a** (45 mg, 0.19 mmol), 10% MeOH/iPr_2O (2 mL), 225 rpm at RT for 65 h, enzyme (mg, % conversion): Amano PS 30 (58.3, no reaction), Meito Sangyo Lipase PL (43.7, 30.5), Biocatalyst *Alcaligenes sp.* (37.6, 48.9), Scientific PEC High Lipase (199.6, 65.7), Toyobo Lipoprotein Lipase (17.9, 74.2), Sawa LIP-301 (54.9, no reaction), Sawa LPL-701 (52.5, 92.4), Novo SP435 (56.9, 97.4). Using **7a** (104 mg) and PPL (590 mg) in 10% MeOH/iPr_2O (6 mL) showed only 10% reaction after 19 h.
13. Wang, Y.-F.; Chen, C.-S.; Girdaukas, G.; Sih, C.J. *J. Am. Chem. Soc.* **1984**, *106*, 3695-3696.
14. Vinyl butyrate, trifluoroethyl isobutyrate (TFEiBu) and trifluroethyl 2-methylbutyrate (TFE2MeBu) were compared: **3** (0.05 g), PPL (0.25 g), acylating agent (3 equivs.), THF (1.0 mL), RT. VinylOBu showed

almost complete consumption of diol after 2 h (ee 0.984), TFEiBu showed 73% conversion after 48 h (ee 0.943) while TFE2MeBu showed essentially no reaction after 48 h.

15. Pure monoOBu **6b**, isolated by column chromatography, showed the following rotations: $[\alpha]_D^{25}$ = -6.07 (c 2.241, EtOH) and $[\alpha]_D^{25}$ = +18.56 (c 2.952, $CHCl_3$). **6b** was correlated with (S)-(-)-tropic acid as shown:

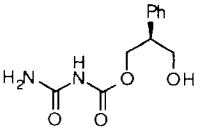


(a) Fodor, G.; Csepreghy, G. *J. Chem. Soc.* **1961**, 3222-3223. (b) Watson & Youngson *J. Chem. Soc. Perkin Trans. I*, **1972**, 1597-1598.

16. (a) Guanti, G.; Narisano, E.; Podgorski, T.; Thea, S.; Williams, A. *Tetrahedron* **1990**, *46*, 7081-7082.(b) Didier, E.; Loubineux, B.; Ramos Tombo, G.M.; Rihs, G. *Tetrahedron* **1991**, *47*, 4941-4958. (c) Boland, W.; Froβl, C.; Lorenz, M. *Synthesis* **1991**, 1049-1072. (d) Faber, K.; Riva, S. *Synthesis* **1992**, 895-910. (e) L. Poppe, L. Novak, *Selective Biocatalysis: a Synthetic Approach*, VCH, New York, 1992. p 139-142. (f) Danieli, B.; Lesma, G.; Passerella, D.; Riva, S. *Advances in the Use of Synthons in Organic Chemistry*. **1993**, *1*, 143-219.

17. HPLC indicated a Diol **3**:MonoOBu **6b**:DiOBu **8** ratio of 1.32 : 74.41 : 24.27.

18. On the basis of ^1H, ^{13}C NMR and FAB and high resolution mass spectrometry, this compound was identified as:



19. An attempted enzymatic deacylation with Novo SP435 in TBME/MeOH was very slow, and no reaction was observed with PPL.

(*Received in USA* 19 *May* 1995)