Pergamon

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Tetrahedron: *Asymmetry* 14 (2003) 603–609

TETRAHEDRON: *ASYMMETRY*

Desymmetrization of dimethyl 3-substituted glutarates through enzymatic ammonolysis and aminolysis reactions

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Received 29 November 2002; accepted 16 December 2002

Abstract—The desymmetrization of differently 3-substituted glutarates through enzymatic aminolysis and ammonolysis has been studied. The effect of the diester and nucleophile structures, the enzymatic preparation as well as the reaction conditions have been compared in terms of both the chemical yield and enantiomeric excess of the corresponding monoamide products.

1. Introduction

Enzymatic ammonolysis and aminolysis reactions have been widely used for the preparation of chiral synthons in high enantiomeric excesses. The mild reaction conditions and high enantioselectivities often obtained, make these biocatalytic processes very useful.[1] By far the most common approach has been the resolution of racemic mixtures of either acyl donor,[2] nucleophile[3] or even both.[4] The major disadvantage of this methodology is the inherent 50% upper limit for theoretical conversion. Two ways to overcome this problem have been the desymmetrization of *meso* and prochiral compounds[5] or the dynamic kinetic resolution of substrates able to racemize in the reaction media.[6] These elegant approaches allow theoretically complete conversion and avoid difficult separation steps, as they usually lead to very clean crude reaction products. Enzymatic hydrolysis, transesterification or lactonization of prochiral diesters and diols have been applied to the preparation of optically active compounds in high enantiomeric excesses.[5] However, there are only few reported examples detailing the enzymatic aminolysis and ammonolysis reactions of prochiral substrates. We have recently reported the desymmetrization of dimethyl 3-hydroxyglutarate[7] and dimethyl 3-(benzylamino)glutarate.[8] In both cases, monoamides were obtained in high chemical yield and in enantiopure form. The synthetic value of the reaction products have been demonstrated by their conversion into the corresponding enantiopure γ-amino acid derivatives, such as (*R*)-4-amino-3-hydroxybutanoic acid[7] and (*R*)-3,4-diaminobutanoic acid,[8] respectively. Encouraged by these results, we envisioned to generalize this methodology to different 3-substituted glutarates and to study the scope of this biocatalytic process as well as the effect of such substitution on the efficiency and enantioselectivity of the reaction. The monoamides thus obtained are interesting chiral synthons for the preparation of biologically active amino acids,[9] β-lactams[10] and other pharmaceuticals.[11]

2. Results and discussion

Lipase B from *Candida antarctica* (CAL-B) has demonstrated its ability to catalyze amide bond formation from non-activated esters in organic solvents under very mild reaction conditions.[1] As previously pointed out, we found its utility in the desymmetrization of some 3-substituted glutarates **1a**–**b** through both aminolysis and ammonolysis reactions.[7,8] To test the generality of lipase-catalyzed desymmetrization, a series of dimethyl 3-substituted glutarates **1c**–**f** were prepared[11d,12] and subjected to reaction (see Scheme 1). Their enzymatic aminolysis (R^2 = Bn, Bu) and ammonolysis (R^2 = H), catalyzed by CAL-B (Novozyme 435) in 1,4-dioxane at 30°C, led to the corresponding monoamides **2g**–**u**. The reaction times, chemical yields and enantiomeric excesses for **2g**–**u** are shown in Table 1 (entries 7–21) as well as the previously

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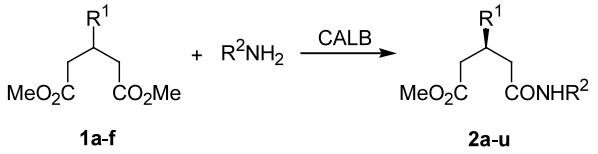
0957-4166/03/$ - see front matter
doi:10.1016/S0957-4166(02)00869-8



1a-f 2a-u

Scheme 1. Enzymatic aminolysis and ammonolysis of **1**.

reported results for the 3-hydroxy[7] (Table 1, entries 1–3) and 3-benzylamino[8] derivatives (Table 1, entries 4–6) for comparison. In all of the cases studied, CAL-B catalyzed the exclusive formation of the monoamide **2**, although the chemical yield and the enantioselectivity of the process mainly depends on the structure of diester (R^1), and, to a lesser extent, on the nucleophile (R^2). The reaction always stopped at the monoamide stage, and the differences in yields correspond to cases where the reaction was incomplete. For diester **1d**, the deacylated product **1a** was formed during the reaction, probably due to enzymatic aminolysis/ammonolysis of the acetate group in C-3 position. The formation of this side product lowered the chemical yield of the desired product but did not affect the enantiomeric purity (Table 1, entries 10–12).

As a general trend, the derivatives bearing a heteroatom in R^1 (**1a–d**) yielded better results both in conversion and enantioselectivity (e.e. $\geq$97%). The aliphatic (**1e**) and aromatic (**1f–g**) derivatives led to longer reaction times, lower chemical yields and poorer enantiomeric excesses (Table 1, entries 13–21). Very slow reactions are obtained with aromatic R^1 diesters (**1f–g**, Table 1, entries 16–21). All these results suggest that more hydrophobic C-3 substituents are less suitable for the enzymatic reaction. On the other hand, the successful desymmetrization obtained with derivatives **1a–d** can be explained by hydrogen bonding stabilization of the substrate in the enzyme active site.

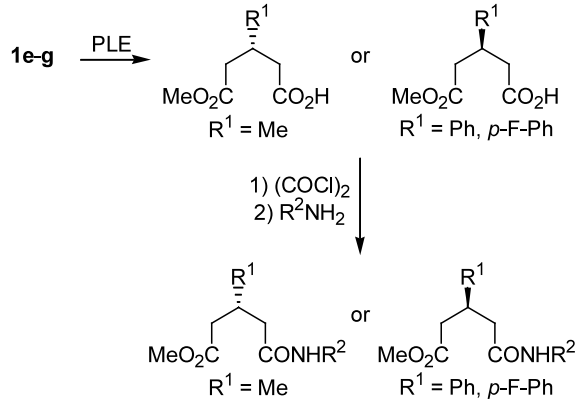
Related to the nucleophiles, there are no big differences both in reactivity and enantioselectivity if we compare the most reactive diesters (Table 1, entries 1–15). When the aromatic substrates are considered (Table 1, entries 16–21) the obtained e.e.s also depend on the nucleophile structure, being benzylamine (R^2=Bn) the less selective for these ones (**1f–g**, Table 1, entries 16 and 19).

It is especially interesting to note that although the degree of enantioselection changes for different substrates, monoamides **2a–u** are always obtained with the same absolute configuration (*S*). This means the enzyme always showed preference for the *pro-R* ester group in **1**. The absolute configuration of the final compounds was determined by comparison of at least one product of the series for every diester, and the ones obtained as described in the literature. For compounds **2g–l**, we compared chiral HPLC retention times (t_R) and the sign of the specific rotation (α) between the title compounds and those obtained by simple methylation (**2g–i**) or acylation (**2j–l**) of **2a–c**. For the 3-aliphatic and 3-aromatic derivatives **2m–u**, again the specific rotation and HPLC retention times were compared with the monoamides obtained as depicted in Scheme 2. Enzymatic (PLE) hydrolysis of the diesters **1** followed by conventional transformation of the monoacid into the monoamide, allowed us to unequivocally assign the stereochemistry of monoamides **2m–u**. We must point

Table 1. CAL-B 'Novozyme 435' catalyzed desymmetrization of dimethyl 3-substituted glutarates (1,4-dioxane, 30°C)

Entry	Substrate	Product	R^1	R^2	Time (h)	Yield (%)[c]	e.e. (%)[d]	Abs. config.
1[a]	**1a**	**2a**	OH	Bn	9	98	>99	*S*
2[a]	**1a**	**2b**	OH	Bu	9	96	>99	*S*
3[a]	**1a**	**2c**	OH	H	5	98	>99	*S*
4[b]	**1b**	**2d**	NHBn	Bn	48	92	>99	*S*
5[b]	**1b**	**2e**	NHBn	Bu	48	79	>99	*S*
6[b]	**1b**	**2f**	NHBn	H	72	85	>99	*S*
7	**1c**	**2g**	OMe	Bn	36	90	99	*S*
8	**1c**	**2h**	OMe	Bu	36	84	97	*S*
9	**1c**	**2i**	OMe	H	60	80	>99	*S*
10	**1d**	**2j**	OAc	Bn	36	40	>99	*S*
11	**1d**	**2k**	OAc	Bu	48	52	>99	*S*
12	**1d**	**2l**	OAc	H	72	69	>99	*S*
13	**1e**	**2m**	Me	Bn	72	67	76	*S*
14	**1e**	**2n**	Me	Bu	72	60	71	*S*
15	**1e**	**2o**	Me	H	96	63	72	*S*
16	**1f**	**2p**	Ph	Bn	240	12	88	*S*
17	**1f**	**2q**	Ph	Bu	240	17	92	*S*
18	**1f**	**2r**	Ph	H	240	12	92	*S*
19	**1g**	**2s**	*p*-F-Ph	Bn	240	8	68	*S*
20	**1g**	**2t**	*p*-F-Ph	Bu	240	13	90	*S*
21	**1g**	**2u**	*p*-F-Ph	H	240	7	91	*S*

[a] Taken from Ref. 7.
[b] Taken from Ref. 8.
[c] After column chromatography.
[d] Determined by HPLC.



Scheme 2.

out that CAL-B-catalyzed aminolysis and ammonolysis shows increasing stereoselectivity as the size of the 3-substituent increases, which is different to that seen with PLE in the hydrolysis of the same substrates.[5j] This difference is reflected in the lower e.e.s obtained for the methyl derivative **1e** (Table 1, entries 13–15).

In an attempt to improve the results for derivatives **1e–g**, we tested other reaction conditions such as different preparations of the same lipase, different solvents, reaction temperature, diester:amine molar ratio and overall concentration. From this screening, the best results were obtained with CAL-B 'Chirazyme L-2' and *tert*-butylmethyl ether as solvent at 30°C (Table 2). In this case, a clear improvement in both reactivity and enantioselectivity was observed for the aromatic derivatives (Table 2, entries 4–9) the e.e.s being $\geq 96\%$. In spite of the higher enantiomeric purity and chemical yield, the reaction is still slow, requiring 6 days to reach more than 20% conversion. Addition of molecular sieves and faster orbital shaking did not improve the results. For the methyl derivative **1e**, lower yields were obtained for both ammonolysis and aminolysis processes (Table 2, entries 1–3). From these results we can conclude that compound **1e** is not a suitable substrate for the desymmetrization with CAL-B, probably due to the small difference between the size and polarity of the substituents at C-3. On the other hand, this lipase can show high stereoselectivity for the aromatic derivatives **1f–g** but the desymmetrization reaction is still very slow.

3. Conclusions

CAL-B-catalyzed aminolysis and ammonolysis of dimethyl 3-substituted glutarates has been studied. Different reaction conditions were tested and the effect of diester and nucleophile structures was studied. The reaction always stops at the monoamide stage, allowing the desymmetrization of the *meso* starting material. In all the examples studied, the enzyme showed a clear preference toward the *pro-R* ester group, leading to the monoamide with *S* configuration. Both chemical yield and stereoselectivity strongly depends on the substrate structure. Derivatives with a heteroatom with possible hydrogen bonding acceptor behaviour at C-3 of the diester showed higher chemical yields and e.e.s. Dimethyl 3-methylglutarate gave poorer results under all of the reaction conditions tested. Aromatic derivatives are less reactive for this enzymatic process although high e.e.s can be obtained when the reaction conditions are optimized.

4. Experimental

Lipase B from *C. antarctica* (CAL-B) 'Novozym 435' was donated by Novo Nordisk Co. and the preparations 'Chirazyme L-2, c-f, lyo' and 'Chirazyme L-2, c-f, C3, lyo' were obtained from Roche Diagnostics. All reagents were purchased from commercial suppliers and used without further purification. Solvents were freshly distilled on the suitable desicant and stored under nitrogen atmosphere. Thin layer chromatography was performed on aluminum plates coated with Merck silica gel $60F_{254}$. Column chromatography was carried out on Merk silica gel 60/230–400 mesh at increased pressure (hand-held bellows). Melting points were taken using a Gallenkamp apparatus and are uncorrected. Optical rotations were measured with a Perkin–Elmer 241 polarimeter. IR spectra were recorded on a 'UNICAM Mattson 3000 FT' IR spectrometer. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker AC-300 (^{1}H 300 MHz), Bruker 300-DPX (^{1}H 300 MHz) and Bruker

Table 2. CAL-B 'Chirazyme L-2' catalyzed desymmetrization of dimethyl 3-substituted glutarates (*tert*-butylmethyl ether, 30°C)

Entry	Substrate	Product	R^1	R^2	Time (days)	Yield (%)[a]	e.e. (%)[b]	Abs. config.
1	**1e**	**2m**	Me	Bn	3	21	75	*S*
2	**1e**	**2n**	Me	Bu	3	31	75	*S*
3	**1e**	**2o**	Me	H	4	26	78	*S*
4	**1f**	**2p**	Ph	Bn	6	20	98	*S*
5	**1f**	**2q**	Ph	Bu	6	35	98	*S*
6	**1f**	**2r**	Ph	H	6	42	96	*S*
7	**1g**	**2s**	*p*-F-Ph	Bn	6	23	99	*S*
8	**1g**	**2t**	*p*-F-Ph	Bu	6	42	99	*S*
9	**1g**	**2u**	*p*-F-Ph	H	6	34	99	*S*

[a] After column chromatography.
[b] Determined by HPLC.

AC-200 (^{1}H 200 MHz) spectrometer. Chemical shifts are quoted in ppm on the scale using tetramethylsilane as internal standard. Coupling constants are given in hertz. Mass spectra were recorded on a HP-MS 1100, using electrospray ionization. Microanalyses were performed on a Perkin–Elmer 2400 elemental analyzer. The enantiomeric excesses were determined by chiral HPLC analysis on a 'Shimadzu LC-10AD'.

4.1. General procedure for the aminolysis of dimethyl 3-substituted glutarates

The corresponding amine (0.5 mmol) was added to a suspension of the dimethyl 3-substituted glutarate (0.5 mmol) and CAL-B (60 mg) in 1,4-dioxane or *tert*-butylmethyl ether (2 ml) under nitrogen atmosphere. The solvents were dried previously in order to avoid the competitive enzymatic hydrolysis. The mixture was orbitally shaken at 30°C at 250 rpm. At times indicated in Tables 1 and 2, the enzyme was filtered off, washed with dichloromethane and the combined organics were evaporated under reduced pressure. Products **2g**–**u** were purified by column chromatography on silica gel.

4.1.1. Methyl (*S*)-(+)-3-methoxy-4-(*N*-benzylcarbamoyl)butanoate, 2g. Colorless oil; yield, 90%; $[\alpha]_D^{20}=+5.5$ (*c* 1.0, $CHCl_3$); IR (neat): 3303, 1738, 1651 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.52 (m, 4H), 3.34 (s, 3H), 3.66 (s, 3H), 4.04 (q, $J=5.8$, 1H), AB portion of an ABX system (δ_A 4.37, δ_B 4.45, $J_{AB}=14.6$, $J_{AX}=5.9$, $J_{BX}=6.2$, 2H), 6.65 (br, 1H), 7.20–7.30 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 38.4 (CH_2), 40.7 (CH_2), 43.2 (CH_2), 51.6 (CH_3), 57.1 (CH_3), 74.7 (CH), 127.1 (CH), 127.4 (CH), 128.4 (CH), 138.1 (C), 170.1 (C=O), 171.2 (C=O); ESI MS *m*/*e* (%): 266 [(M+H)$^+$, 15], 288 [(M+Na)$^+$, 100], 304 [(M+K)$^+$, 15]. Anal. calcd for $C_{14}H_{19}NO_4$: C, 63.38; H, 7.22; N, 5.28. Found: C, 63.5; H, 7.1; N, 5.2%. HPLC analysis [Chiralcel OD, hexane:ethanol 92:8, 0.8 ml/min, 20°C, 210 nm, (−)-(*R*): 18.3 min, (+)-(*S*): 20.4 min] 99% e.e.

4.1.2. Methyl (*S*)-(−)-3-methoxy-4-(*N*-butylcarbamoyl)butanoate, 2h. Colorless oil; yield, 84%; $[\alpha]_D^{20}=-1.5$ (*c* 1.0, $CHCl_3$); IR (neat): 3306, 1739, 1645 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.85 (m, 3H), 1.22–1.54 (m, 4H), AB portion of an ABX system (δ_A 3.39, δ_B 2.48, $J_{AB}=14.6$, $J_{AX}=6.7$, $J_{BX}=5.4$, 2H), 2.56 (d, $J=5.9$, 2H), 3.22 (c, $J=6.6$, 2H), 3.37 (s, 3H), 3.67 (s, 3H), 4.04 (m, 1H), 6.21 (br, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.5 (CH_3), 19.9 (CH_2), 31.4 (CH_2), 38.4 (CH_2), 39.0 (CH_2), 40.7 (CH_2), 51.6 (CH_3), 57.2 (CH_3), 74.7 (CH), 170.0 (C=O), 171.2 (C=O); ESI MS *m*/*e* (%): 232 [(M+H)$^+$, 19], 254 [(M+Na)$^+$, 100], 270 [(M+K)$^+$, 23]. Anal. calcd for $C_{11}H_{21}NO_4$: C, 57.12; H, 9.15; N, 6.06. Found: C, 57.3; H, 9.1; N, 5.9%. HPLC analysis [Chiralcel OD, hexane:ethanol 97:3, 0.8 ml/min, 20°C, 215 nm, (+)-(*R*): 21.2 min, (−)-(*S*): 23.1 min] 97% e.e.

4.1.3. Methyl (*S*)-(+)-3-acetoxy-4-(*N*-benzylcarbamoyl)butanoate, 2j. White solid; yield, 40%; mp 52–53°C; $[\alpha]_D^{20}=+1.3$ (*c* 1.1, $CHCl_3$); IR (KBr): 3282, 1727, 1642 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.00 (s, 3H), 2.60–2.87 (m, 4H), 3.64 (s, 3H), 4.42 (d, $J=5.8$, 2H), 5.47 (m, 1H), 6.09 (br, 1H), 7.21–7.33 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.9 (CH_3), 38.0 (CH_2), 40.4 (CH_2), 43.6 (CH_2), 51.9 (CH_3), 67.8 (CH), 127.6 (CH), 127.7 (CH), 128.7 (CH), 137.9 (C), 168.6 (C=O), 170.1 (C=O), 170.5 (C=O); ESI MS *m*/*e* (%): 316 [(M+Na)$^+$, 100], 332 [(M+K)$^+$, 48]. Anal. calcd for $C_{15}H_{19}NO_5$: C, 61.42; H, 6.53; N, 4.78. Found: C, 61.5; H, 6.8; N, 4.5%. HPLC analysis [Chiralcel OD, hexane:ethanol 95:5, 0.6 ml/min, 20°C, 210 nm, (−)-(*R*): 63.9 min, (+)-(*S*): 68.5 min] >99% e.e.

4.1.4. Methyl (*S*)-(−)-3-acetoxy-4-(*N*-butylcarbamoyl)butanoate, 2k. Colorless oil; yield, 52%; $[\alpha]_D^{20}=-2.1$ (*c* 1.1, $CHCl_3$); IR (neat): 3305, 1740, 1647 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, $J=7.2$, 3H), 1.30 (m, 2H), 1.44 (m, 2H), 2.00 (s, 3H), 2.53 (m, 2H), 2.73 (m, 2H), 3.20 (c, $J=6.9$, 2H), 3.65 (s, 3H), 5.42 (m, 1H), 5.90 (br, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.6 (CH_3), 19.8 (CH_2), 20.9 (CH_3), 31.3 (CH_2), 37.9 (CH_2), 39.1 (CH_2), 40.4 (CH_2), 51.7 (CH_3), 67.7 (CH), 168.7 (C=O), 170.1 (C=O), 170.5 (C=O); ESI MS *m*/*e* (%): 260 [(M+H)$^+$, 90], 282 [(M+Na)$^+$, 37], 298 [(M+K)$^+$, 100]. Anal. calcd for $C_{12}H_{21}NO_5$: C, 55.58; H, 8.16; N, 5.40. Found: C, 55.3; H, 8.1; N, 5.7%. HPLC analysis [Chiralcel OB-H, hexane:ethanol 95:5, 0.4 ml/min, 30°C, 215 nm, (+)-(*R*): 32.0 min, (−)-(*S*): 34.3 min] >99% e.e.

4.1.5. Methyl (*S*)-(+)-3-methyl-4-(*N*-benzylcarbamoyl)butanoate, 2m. White solid; yield, 67%; mp 48–50°C; $[\alpha]_D^{20}=+2.7$ (*c* 1.1, $CHCl_3$); IR (KBr): 3307, 1732, 1641 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.96 (d, $J=6.0$, 3H), 2.03–2.43 (m, 5H), 3.60 (s, 3H), 4.35 (d, $J=5.8$, 2H), 6.52 (br, 1H), 7.20–7.30 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 19.7 (CH_3), 28.0 (CH), 40.3 (CH_2), 42.8 (CH_2), 43.2 (CH_2), 51.3 (CH_3), 127.2 (CH), 127.5 (CH), 128.4 (CH), 138.2 (C), 170.3 (C=O), 172.8 (C=O); APCI MS *m*/*e* (%): 250 [(M+H)$^+$, 100]. Anal. calcd for $C_{14}H_{19}NO_3$: C, 63.45; H, 7.68; N, 5.62. Found: C, 63.5; H, 7.4; N, 5.8%. HPLC analysis [Chiralcel OB-H, hexane:ethanol 9:1, 0.5 ml/min, 30°C, 215 nm, (−)-(*R*): 23.0 min, (+)-(*S*): 25.1 min] 76% e.e.

4.1.6. Methyl (*S*)-(+)-3-methyl-4-(*N*-butylcarbamoyl)butanoate, 2n. Colorless oil; yield, 60%; $[\alpha]_D^{20}=+3.3$ (*c* 1.0, $CHCl_3$); IR (neat): 3302, 1740, 1643 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.87–0.94 (t, $J=5.9$, 3H), 1.01 (d, $J=5.9$, 3H), 1.25–1.52 (m, 4H), 2.03–2.49 (m, 5H), 3.24 (c, $J=6.0$, 2H), 3.67 (s, 3H), 5.66 (br, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.5 (CH_3), 19.5 (CH_3), 19.9 (CH_2), 27.9 (CH), 31.4 (CH_2), 38.9 (CH_2), 40.2 (CH_2), 42.8 (CH_2), 51.2 (CH_3), 171.4 (C=O), 172.9 (C=O); APCI MS *m*/*e* (%): 216 [(M+H)$^+$, 100]. Anal. calcd for $C_{11}H_{21}NO_3$: C, 61.37; H, 9.83; N, 6.51. Found: C, 61.2; H, 9.9; N, 6.2%. HPLC analysis [Chiralcel OB-H, hexane:ethanol 97:3, 0.4 ml/min, 20°C, 215 nm, (−)-(*R*): 34.8 min, (+)-(*S*): 36.9 min] 71% e.e.

4.1.7. Methyl (*S*)-(+)-3-phenyl-4-(*N*-benzylcarbamoyl)butanoate, 2p. Colorless oil; yield, 20%; $[\alpha]_D^{20}=+2.0$ (*c* 0.6, $CHCl_3$); IR (neat): 3300, 1740, 1649 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.45–2.81 (m, 4H), 3.59 (s, 3H), 3.65 (m, 1H), AB portion of an ABX system (δ_A 4.27, δ_B 4.35, $J_{AB}=14.6$, $J_{AX}=5.9$, $J_{BX}=6.2$, 2H), 5.68

(br, 1H), 6.99 (m, 2H), 7.20–7.25 (m, 8H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 38.9 (CH), 40.2 (CH_2), 42.9 (CH_2), 43.3 (CH_2), 51.6 (CH_3), 126.9 (CH), 127.1 (CH), 127.2 (CH), 127.4 (CH), 128.5 (CH), 128.6 (CH), 137.8 (C), 142.4 (C), 170.5 (C=O), 172.1 (C=O); ESI MS m/e (%): 312 [(M+H)$^+$, 5], 334 [(M+Na)$^+$, 100], 350 [(M+K)$^+$, 23]. Anal. calcd for $C_{19}H_{21}NO_3$: C, 73.29; H, 6.80; N, 4.50. Found: C, 73.5; H, 7.1; N, 4.8%. HPLC analysis [Chiralcel OB-H, hexane:ethanol 9:1, 0.5 ml/min, 30°C, 215 nm, (−)-(*R*): 21.6 min, (+)-(*S*): 23.6 min] 98% e.e.

4.1.8. Methyl (*S*)-(−)-3-phenyl-4-(*N*-butylcarbamoyl)-butanoate, 2q. Colorless oil; yield, 35%; $[\alpha]_D^{20} = -6.3$ (*c* 0.9, $CHCl_3$); IR (neat): 3300, 1740, 1643 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.85 (t, $J = 7.0$, 3H), 1.11–1.34 (m, 4H), 2.39–2.85 (m, 4H), 3.08–3.18 (m, 2H), 3.59 (s, 3H), 3.64 (m, 1H), 5.35 (br, 1H), 7.18–7.34 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.6 (CH_3), 19.8 (CH_2), 31.4 (CH_2), 39.0 (CH), 39.1 (CH_2), 40.1 (CH_2), 43.1 (CH_2), 51.6 (CH_3), 126.9 (CH), 127.1 (CH), 128.6 (CH), 142.6 (C), 170.6 (C=O), 172.2 (C=O); APCI MS m/e (%): 278 [(M+H)$^+$, 100]. Anal. calcd for $C_{16}H_{23}NO_3$: C, 69.29; H, 8.36; N, 5.05. Found: C, 69.5; H, 8.1; N, 5.3%. HPLC analysis [Chiralcel OB-H, hexane:ethanol 93:7, 0.4 ml/min, 30°C, 215 nm, (+)-(*R*): 19.4 min, (−)-(*S*): 21.6 min] 98% e.e.

4.1.9. Methyl (*S*)-(+)-3-(4-flouorophenyl)-4-(*N*-benzyl-carbamoyl)butanoate, 2s. White solid; yield, 23%; mp 58–60°C; $[\alpha]_D^{20} = +5.0$ (*c* 1.5, $CHCl_3$); IR (neat): 3285, 1734, 1639 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.38–2.82 (m, 4H), 3.58 (s, 3H), 3.62–3.77 (m, 1H), 4.17–4.49 (m, 2H), 5.89 (br, 1H), 6.91–7.30 (m, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 38.1 (CH), 40.2 (CH_2), 42.9 (CH_2), 43.2 (CH_2), 51.6 (CH_3), 115.3 (CH, $^2J_{CF} = 21.0$), 127.3 (CH, $^3J_{CF} = 5.2$), 128.4 (CH), 128.7 (CH), 128.8 (CH), 137.8 (C), 138.0 (C), 161.6 (C, $^1J_{CF} = 245.2$), 170.3 (C=O), 171.9 (C=O); APCI MS m/e (%): 330 [(M+H)$^+$, 100]. Anal. calcd for $C_{19}H_{20}FNO_3$: C, 69.29; H, 6.12; N, 4.25. Found: C, 69.5; H, 6.3; N, 4.4%. HPLC analysis [Chiralcel OD,: hexane:ethanol 9:1, 0.5 ml/min, 20°C, 215 nm, (+)-(*S*): 24.0 min, (−)-(*R*): 27.1 min] 99% e.e.

4.1.10. Methyl (*S*)-(−)-3-(4-fluorophenyl)-4-(*N*-butylcarbamoyl)butanoate, 2t. Colorless oil; yield, 42%; $[\alpha]_D^{20} = -8.9$ (*c* 1.2, $CHCl_3$); e.e. 99%; IR (neat): 3307, 1735, 1639 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.85 (t, $J = 7.0$, 3H), 1.08–1.40 (m, 4H), 2.34–2.82 (m, 4H), 3.06–3.21 (m, 2H), 3.57 (s, 3H), 3.65 (m, 1H), 5.53 (br, 1H), 6.93–7.22 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.5 (CH_3), 19.8 (CH_2), 31.4 (CH_2), 38.2 (CH), 39.0 (CH_2), 40.1 (CH_2), 43.0 (CH_2), 51.5 (CH_3), 115.3 (CH, $^2J_{CF} = 21.0$), 128.6 (CH, $^3J_{CF} = 7.6$), 138.2 (C), 161.6 (CH, $^1J_{CF} = 244.6$), 170.4 (C=O), 172.0 (C=O); APCI MS m/e (%): 296 [(M+H)$^+$, 100]. Anal. calcd for $C_{16}H_{22}FNO_3$: C, 65.07; H, 7.51; N, 4.74. Found: C, 65.1; H, 7.8; N, 4.5%. HPLC analysis [Chiralcel OD, hexane:ethanol 97:3, 0.5 ml/min, 20°C, 215 nm, (−)-(*S*): 43.3 min, (+)-(*R*): 48.4 min] 99% e.e.

4.2. General procedure for the ammonolysis of dimethyl 3-substituted glutarates

Ammonia was bubbled through 1,4-dioxane or *tert*-butylmethyl ether at 5–10°C for 30 min under nitrogen. Then, 2 ml of this solution were added to a mixture of the corresponding 3-substituted glutarate (0.5 mmol) and CAL-B (60 mg). The reaction mixture was shaken at 30°C and 250 rpm several days (Tables 1 and 2), whereupon the enzyme was filtered, washed with dichloromethane and the combined organic solvents were evaporated. Purification by column chromatography on silica gave the title compound.

4.2.1. Methyl (*S*)-(−)-3-methoxy-4-carbamoylbutanoate, 2i. White solid; yield, 80%; mp 45–47°C; $[\alpha]_D^{20} = -4.4$ (*c* 0.7, $CHCl_3$); e.e. 97%; IR (KBr): 3346, 3192, 1736, 1667 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.42–2.67 (m, 4H), 3.39 (s, 3H), 3.68 (s, 3H), 4.02 (m, 1H), 5.96 (br, 1H), 6.24 (b, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 38.3 (CH_2), 40.1 (CH_2), 51.7 (CH_3), 57.2 (CH_3), 74.5 (CH), 171.3 (C=O), 173.1 (C=O); ESI MS m/e (%): 198 [(M+Na)$^+$, 100]. Anal. calcd for $C_7H_{13}NO_4$: C, 47.99; H, 7.48; N, 8.0. Found: C, 47.6; H, 7.1; N, 7.6%. HPLC analysis [Chiralcel OD, hexane:propan-2-ol 92:8, 0.8 ml/min, 30°C, 215 nm, (+)-(*R*): 28.6 min, (+)-(*S*): 32.3 min] >99% e.e.

4.2.2. Methyl (*S*)-(−)-3-acetoxy-4-carbamoylbutanoate, 2l. White solid, yield, 69%; mp 85–86°C; $[\alpha]_D^{20} = -4.5$ (*c* 1.1, $CHCl_3$); IR (KBr): 3404, 3218, 1729, 1714, 1626 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.04 (s, 3H), 2.56–2.86 (m, 4H), 3.68 (s, 3H), 5.41–5.54 (m, 1H), 6.07 (br, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.0 (CH_3), 38.0 (CH_2), 39.7 (CH_2), 51.8 (CH_3), 67.5 (CH), 170.2 (C=O), 170.5 (C=O), 171.3 (C=O); ESI MS m/e (%): 204 [(M+H)$^+$, <5], 226 [(M+Na)$^+$, 100], 242 [(M+K)$^+$, 30]. Anal. calcd for $C_8H_{13}NO_5$: C, 47.29; H, 6.45; N, 6.89. Found: C, 47.5; H, 6.2; N, 6.7%. HPLC analysis [Chiralcel OB-H, hexane:ethanol 9:1, 0.5 ml/min, 30°C, 215 nm, (+)-(*R*): 28.1 min, (+)-(*S*): 32.3 min] >99% e.e.

4.2.3. Methyl (*S*)-(+)-3-methyl-4-carbamoylbutanoate, 2o. White solid; yield, 26%; mp 46–48°C; $[\alpha]_D^{20} = +2.3$ (*c* 1.2, $CHCl_3$); IR (KBr): 3425, 3356, 1734, 1668, 1617 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.01 (d, $J = 6.2$, 3H), 2.05–2.47 (m, 5H), 3.65 (s, 3H), 6.03 (br, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 19.7 (CH_3), 27.8 (CH), 40.3 (CH_2), 42.2 (CH_2), 51.4 (CH_3), 173.0 (C=O), 174.5 (C=O); ESI MS m/e (%): 182 [(M+Na)$^+$, 100]. Anal. calcd for $C_7H_{13}NO_3$: C, 52.82; H, 8.23; N, 8.80. Found: C, 52.6; H, 8.5; N, 8.6%. HPLC analysis [Chiralcel OB-H, hexane:ethanol 93:7, 0.5 ml/min, 30°C, 215 nm, (+)-(*R*): 21.6 min, (+)-(*S*): 22.9 min] 72% e.e.

4.2.4. Methyl (*S*)-(−)-3-phenyl-4-carbamoylbutanoate, 2r. White solid; yield, 42%; mp 85–87°C; $[\alpha]_D^{20} = -7.0$ (*c* 1.1, $CHCl_3$); IR (KBr): 3432, 3182, 1731, 1655, 1629 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.49–2.81 (m, 4H), 3.58 (s, 3H), 3.63 (m, 1H), 5.43 (br, 2H), 7.19–7.23 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 38.6 (CH), 40.2 (CH_2), 42.0 (CH_2), 51.6 (CH_3), 127.0 (CH), 127.1 (CH), 128.7 (CH), 142.5 (C), 172.2 (C=O), 173.1 (C=O);

ESI MS m/e (%): 222 [(M+H)$^+$, 7], 244 [(M+Na)$^+$, 100], 260 [(M+K)$^+$, 21]. Anal. calcd for $C_{12}H_{15}NO_3$: C, 65.14; H, 6.83; N, 6.33. Found: C, 65.4; H, 7.1; N, 6.3%. HPLC analysis [Chiralcel OB-H, hexane:propan-2-ol 9:1, 0.5 ml/min, 30°C, 215 nm, (+)-(*R*): 22.5 min, (−)-(*S*): 26.4 min] 96% e.e.

4.2.5. Methyl (*S*)-(−)-3-(4-fluorophenyl)-4-carbamoyl)butanoate, 2u. White solid; yield, 34%; mp 78–80°C; $[\alpha]_D^{20} = -7.5$ (c 1.1, $CHCl_3$); IR (neat): 3415, 3196, 1733, 1655 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.41–2.81 (m, 4H), 3.56 (s, 3H), 3.65 (m, 1H), 5.78 (br, 1H), 6.02 (br, 1H), 6.92–7.22 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 37.8 (CH), 40.2 (CH_2), 41.9 (CH_2), 51.5 (CH_3), 115.3 (CH, $^2J_{CF} = 21.0$), 128.6 (CH, $^3J_{CF} = 7.6$), 138.2 (C), 161.6 (CH, $^1J_{CF} = 245.2$), 172.1 (C=O), 173.3 (C=O); ESI MS m/e (%): 240 [(M+H)$^+$, 5], 262 [(M+Na)$^+$, 100], 278 [(M+K)$^+$, 17]. Anal. calcd for $C_{12}H_{14}FNO_3$: C, 60.24; H, 5.90; N, 5.85. Found: C, 60.1; H, 5.8; N, 5.5%. HPLC analysis [Chiralcel OB-H, hexane:ethanol 9:1, 0.5 ml/min, 30°C, 215 nm, (+)-(*R*): 22.4 min, (−)-(*S*): 25.1 min] 99% e.e.

Acknowledgements

We express our appreciation to Novo Nordisk Co. for the generous gift of the CA lipase. Financial support of this work by the Spanish Ministerio de Ciencia y Tecnología (Project PPQ-2001-2683) and by Principado de Asturias (Project GE-EXP01-03) is gratefully acknowledged. M.L.-G. thanks the Ministerio de Educación, Cultura y Deporte for a predoctoral fellowship.

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