Ketomethylenebestatin: Synthesis and Aminopeptidase Inhibition

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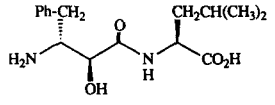
The synthesis of (6*R*,5*S*,2*RS*)-6-amino-5-hydroxy-2-isobutyl-4-oxo-7-phenylheptanoic acid (**9**), a carbaanalogue of the aminopeptidase (AP) inhibitor bestatin (**1**) is described. This synthesis was carried out by a malonic ester alkylation with the suitably protected halomethyl ketone of (2*S*,3*R*)-AHPBA*), followed by a second alkylation with isobutyl bromide of the resulting 4-ketodiester, and subsequent decarboxylation and deprotection. The inhibitory potencies of the 1:1 diastereomeric mixture **9** against AP-B, AP-M and Leu-AP were approximately 10-fold lower than those of bestatin.

Ketomethylenbestatin: Synthese und Aminopeptidase Hemmwirkung

Die Synthese von (6*R*,5*S*,2*RS*)-6-Amino-5-hydroxy-2-isobutyl-4-oxo-7-phenylheptansäure (**9**), eines Carbaanalogen des natürlichen Aminopeptidase (AP) Inhibitors Bestatin (**1**), wird beschrieben. Diese Synthese wurde über Malonesteralkylierung mit dem entspr. geschützten Halomethylketon des (2*S*,3*R*)-AHPBA*) durchgeführt, gefolgt von einer zweiten Alkylierung des resultierenden 4-Ketodiesters mit Isobutylbromid und anschließender Decarboxylierung und Abspaltung der Schutzgruppe. Der Hemmeffekt der 1:1 Diastereoisomermischung **9** gegenüber AP-B, AP-M und Leu-AP war annähernd zehn mal niedriger als der von Bestatin.



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Bestatin, *N*-[(2*S*,3*R*)-3-amino-2-hydroxy-4-phenylbutyryl]-L-leucine (**1**, (2*S*,3*R*)-AHPBA-L-Leu-OH) is a dipeptide isolated from *Streptomyces olivoreticuli*[1], which inhibits cytosolic leucine aminopeptidases (Leu-AP; EC 3.4.11.1)[2], microsomal or membrane bound leucine aminopeptidase (AP-M; EC 3.4.11.2)[3] and cell surface-associated aminopeptidase B (AP-B; EC 3.4.11.6)[1,2,4] and acts as immunomodifier[5-11]. Bestatin, in clinical use in Japan[12], also shows antitumor activities[6,13-15] or enhances the antitumor effects of other cytotoxic agents[15,16]. It is believed that these activities are the result of the interaction of bestatin with membrane bound APs to activate macrophages and T cells[17]. Metabolism studies[18] have shown that **1** is mainly metabolized to N-[(2*S*,3*R*)-3-amino-2-hydroxy-4-(4'-hydroxy)-phenylbutyryl]-L-leucine (*p*-hydroxybestatin), with similar activities to those of bestatin, and to (2*S*,3*R*)-3-amino-2-hydroxy-4-phenylbutyric acid [(2*S*,3*R*)-AHPBA], which is inactive[19]. With the aim of increasing the stability to enzymatic degradation and, in turn, leading to a longer acting bestatin analogue, we have replaced its scissile peptide bond by the isosteric, and non-hydrolyzable, ketomethylene ψ[$COCH_2$] group[20]. This replacement has been successfully used to prepare metabolic resistant pseudopeptides[21] and enzyme inhibitors[22]. Additionally, this type of peptide bond surrogate is present in arphamenines [Argψ[$COCH_2$]Phe and Argψ[$COCH_2$]Tyr], two naturally occurring ketomethylene dipeptides, which inhibit AP-B and also enhance immune response[23]. Here, the synthesis of ketomethylenebestatin (**9**) and its AP inhibition (AP-B, AP-M and Leu-AP) are described.

Results and Discussion

Synthesis

The route leading to ketomethylenebestatin (**9**) was based on the general procedure for the synthesis of ketomethylene dipeptide analogues[24,25], which involves, as key steps, a malonic ester alkylation of a suitably protected amino acid halomethyl ketone, followed by a second alkylation of the resulting 4-ketodiester. As shown in the scheme, the synthetic sequence began with the *O*-protection of (2*S*,3*R*)-*N*-Z-AHPBA methyl ester (**2**), prepared according to the method for the stereoselective synthesis of (2*S*,3*R*)-3-amino-2-hydroxy acids[26], by acid catalyzed reaction with 2,2-dimethoxypropane, to give the *N*-*O*-acetonide **3**. Saponification of the methyl ester **3** gave the acid **4**, which was transformed into the chloromethyl ketone **5** through the corresponding diazoketone, following the usual procedure[24] (attempts to prepare the *O*-unprotected chloromethyl ketone derived of **2** were unsuccessful, due to the reactivity of the free 2-OH group in the formation of the intermediate mixed anhydride with isobutyl chloroformate). Conversion of the chloromethyl ketone **5** to the corresponding iodomethyl ketone *in situ*, followed by reaction with the Na salt of dimethyl malonate in 1,2-dimethoxyethane gave the 4-ketodiester **6**. Alkylation of the Na salt of **6** with isobutyl bromide in dry DMSO led to the 2-isobutyl-4-ketodiester **7**, which, after saponification and subsequent decarboxylation and hydrogenolysis of the protecting groups, yielded (6*R*,5*S*,2*RS*)-6-amino-5-hydroxy-2-isobutyl-4-oxo-7-phenylheptanoic acid (**9**, ketomethylenebestatin) as its hydrochloride. Due to the lack of stereoselectivity in the decarboxylation of the 4-ketodiester **7**, ketomethylenebestatin **9** was obtained as a 1:1 mixture of diastereoisomers in C-2. Neither the two diastereoisomers of **9**, nor the corresponding *N*-*O*-protected methyl esters **10**, obtained by reaction of the acids **8** with diazomethane, could be separated by reverse-phase HPLC. Taking into account the influence of the *S*-configuration of the Leu residue on the AP inhibition by bestatin[2,5] the enzymatic resolution of **10** was attempted using pig liver esterase, pig pancreas lipase and

*) (2*S*,3*R*)-3-amino-2-hydroxy-4-phenylbutyric acid

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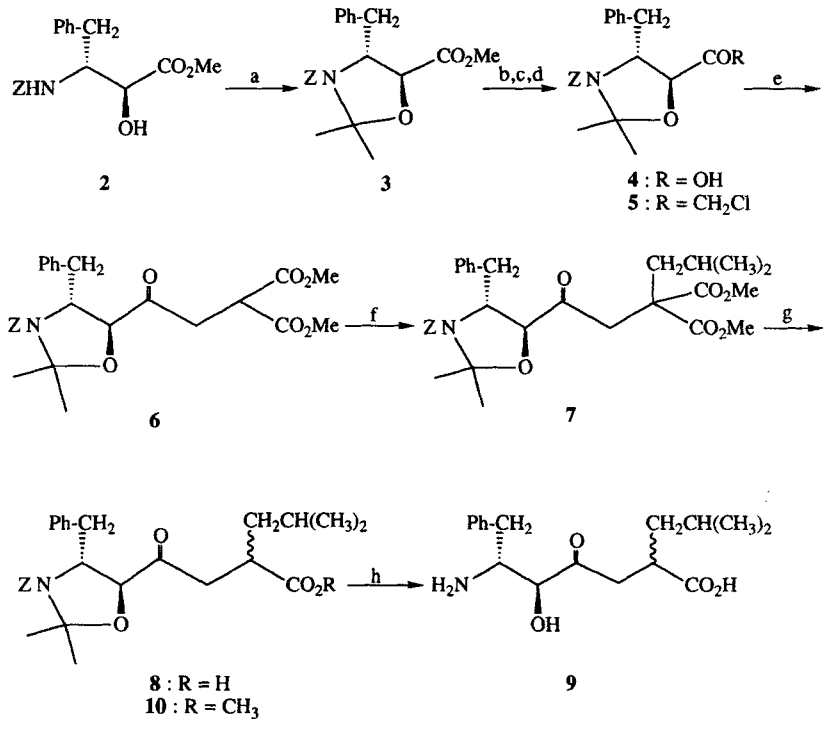

(a) 2,2-Dimethoxypropane, *p*-toluenesulfonic acid; (b) N NaOH, H_2O/Dioxane (1:1); (c) *N*-methylmorpholine, isobutyl chloroformate, CH_2N_2,THF 0°C; (d) HCl/MeOH, THF; (e) NaI, sodium dimethyl malonate, 1,2-dimethoxyethane; (f) NaH, isobutyl bromide, DMSO; (g) N NaOH, H_2O/Dioxane (1:1), then H^+, 100°C; (h) H_2, Pd/C, MeOH.

Candida cylindracea, *Mucor mihiei*, *Pseudomonas fluorescens*, *Aspergillus niger*, *Rhizopus arrhizus*, and *Chromobacterium viscosum* lipases, which have been used for optical resolution of amino acids[27,28]. However, none of these enzymes hydrolyzed the methyl esters **10**. Due to the difficulties in the resolution of ketomethylenebestatin **9**, we tested the AP inhibition by this diastereomeric mixture.

AP Inhibition

Table 1: Inhibition of AP-B, AP-M and Leu-AP by bestatin (**1**) and Ketomethylenebestatin (**9**)

	IC_{50} (μM)[a]		
Compound	AP-B	AP-M	Leu-AP
1	6.5	19.4	0.03
9	56	752	0.39

[a] Values are the mean of 4-5 experiments with 3-5 different concentrations of inhibitor. S.E. were less than 10% of the mean.

The inhibitory potencies of **9** against AP-B (associated with the surface of murine L cells), AP-M and Leu-AP are shown in table 1. For comparative purposes bestatin (**1**) was included in the assay. The results indicate that ketomethylenebestatin (**9**) is approximately 10-fold less potent than bestatin (**1**) against these aminopeptidases. This decrease could be due to the fact of having tested the diastereomeric mixture **9**, since (2*S*,3*R*)-AHPBA-D-Leu is approximately 10- and 350-times less potent against AP-B and Leu-AP, respectively, than bestatin (**1**).

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Experimental Part

Elemental analyses: Heraeus CHN-O-RAPID instrument.- ^{1}H-NMR spectra: Varian XL-300 (300 MHz), TMS int. stand.- FAB^+ mass spectra: VG Autospec, 3% polyethylene glycol in nitrobenzylalcohol as matrix.- IR spectra: Shimadzu IR 435.- Analytical TLC: Al-sheets coated with 0.2 mm silica gel (60 F_{254}, Merck).- Column chromatography: silica gel 60 (230-400 mesh, Merck).- Reverse-phase HPLC: μ-Bondapak C-18 stainless-steel column (3.9 x 300 mm) in a Waters Associates instrument fitted with a Waters 484 tunable absorbance UV-detector; flow rate 1 ml/min, 3:1 0.05% TFA in H_2O:CH_3-CN mixture as mobile phase. Pig liver esterase and pig pancreas lipase: Sigma (U.K.).- Lipase AY from *Candida cylindracea*, lipase P from *Pseudomonas fluorescens* and lipase AP from *Aspergillus niger*: Amano (Japan).- *Rizopus arrhizus* and *Chromobacterium viscosum* lipases: Fluka (Switzerland).- Lypozyme Y from *Mucor mihiei*: Novo Industries (Denmark).- Bestatin, Lys-NA, Leu-NA, and Fast Garnet GBC: Sigma (U.K.).- Dulbecco's modified Eagle's medium and 10% fetal calf serum: Flow Labs (U.K.).- Enzymatic bioassays: performed as described (L cell surface-associated AP-B[29], AP-M[3], Leu-AP[2]).

(4R,5S)-4-Benzyl-3-benzyloxycarbonyl-2,2-dimethyl-oxazolidine-5-carboxylic acid methyl ester (**3**)

p-Toluenesulfonic acid (3.44 g, 2 mmol) and 2,2-dimethoxypropane (40 ml) were added to a solution of (2*S*,3*R*)-*N*-Z-AHPBA methyl ester[26] (6.88 g, 20 ml) in dry benzene (150 ml), and the mixture was stirred under argon at room temp. for 72 h, evaporated to dryness and the residue was purified by flash chromatography with hexane-ethyl acetate (7:1) to afford **3** as a white solid (3.83 g, 50%), m.p. 68-70°C.- IR (KBr): $\tilde{\nu}$ = 1720 and 1700 (C=O) cm^{-1}.- ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.45 and 1.53 (2s; 6H, $C(CH_3)_2$), 2.60-3.30 (m; 2H, 4-CH_2), 3.60 (s; 3H, OCH_3), 4.47 (d, 1H, J = 2.1 Hz, 5-H), 4.53 (dd; 1H, J = 2.1 and 11 Hz, 4-H), 5.16 (s; 2H, OCH_2Ph), 7.30 (m; 10 H aromat.).- $C_{22}H_{25}NO_5$ (383.4) Calcd. C 68.9 H 6.57 N 3.7 Found C 69.1 H 6.61 N 3.9.

(4R,5S)-4-Benzyl-3-benzyloxycarbonyl-2,2-dimethyl-oxazolidine-5-carboxylic acid (**4**)

To a solution of ester **3** (3.83 g, 10 mmol) in 1:1 dioxane-water (100 ml), N NaOH (10 ml, 10 mmol) was added, and the mixture was stirred at room temp. for 8 h. Then, the mixture was concentrated (≈ 30 ml) and washed with CH_2Cl_2 (3 x 30 ml). The aqueous phase was acidified to pH 3-4 with Dowex 50W-X4 resin. The resin was filtered and washed with ethyl acetate (50 ml). The aqueous phase was extracted with ethyl acetate (3 x 50 ml), and the combined org. extracts, after being washed with brine and dried over Na_2SO_4, were evaporated to give acid **4** as a white solid (3.50 g, 95%), m.p. 98-100°C.- IR (KBr): $\tilde{\nu}$ = 3000 (OH), 1740, 1710, and 1690 (C=O) cm^{-1}.- ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.30 and 1.50 (2s; 6H, $C(CH_3)_2$), 3.10 (m; 2H, 4-CH_2), 4.30-4.60 (m; 2H, 4-H and 5-H), 5.35 (s; 2H, OCH_2Ph), 7.30 (m; 10 H aromat.), 8.30 (s, 1H, COOH).- $C_{21}H_{23}NO_5$ (369.4) Calcd. C 68.3 H 6.28 N 3.8 Found C 68.3 H 6.27 N 4.0.

(4R,5S)-4-Benzyl-3-benzyloxycarbonyl-5-(chloromethyl)carbonyl-2,2-dimethyl-oxazolidine (**5**)

N-Methylmorpholine (0.9 ml, 8 mmol) and isobutyl chloroformate (1.1 ml, 9.5 mmol) were added to a cooled solution (-20°C) of acid **4** (2.95 g, 8 mmol) in dry THF (50 ml). The mixture was stirred at this temp. for 30 min, and then filtered. An ethereal solution of CH_2N_2, prepared from nitrosomethyl urea (0.98 g, 9.5 mmol), was added to the filtrate and, after stirring at 0°C for 30 min, the reaction mixture was evaporated. The residue was redissolved in dry THF (30 ml) at 0°C and N methanolic HCl (8.5 ml) was slowly added at 0°C. After stirring for 1 h, the excess of HCl was neutralized with triethylamine and the reaction mixture evaporated to dryness. The residue was dissolved in CH_2Cl_2, washed with water and brine, dried over Na_2SO_4, and evaporated. The residue was purified by flash chromatography (hexane-ethyl acetate (7:1)), affording the chloromethyl ketone **5** as a white solid (2.41 g, 75%), m.p. 46-48°C.- IR (KBr): $\tilde{\nu}$ = 1740 and 1700 (C=O) cm^{-1}.- ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.35 and 1.55 (2s; 6H, $C(CH_3)_2$), 3.10 (m; 2H, 4-CH_2), 4.16 and 4.40 (2d; 2H, J = 15 Hz, CH_2Cl), 4.40 (d; 1H, J = 4 Hz, 5-H), 4.60 (m; 1H, 4-H), 5.23 (s; 2H, OCH_2Ph), 7.25 (m; 10 H aromat.).- $C_{22}H_{24}ClNO_4$ (401.9) Calcd. C 65.7 H 6.02 N 3.5 Cl 8.8 Found C 66.0 H 6.32 N 3.3 Cl 8.7.

(4'R,5'S)-4-(4'-Benzyl-3'-benzyloxycarbonyl-2',2'-dimethyl-oxazolidine-5'-yl)-2-methoxycarbonyl-4-oxobutanoic acid methyl ester (**6**)

A mixture of the chloromethylketone **5** (2.01 g, 5 mmol) and NaI (0.75 g, 5 mmol) in dry 1,2-dimethoxyethane (20 ml) was stirred at room temp. for 15 min, followed by the addition of the Na salt of dimethyl malonate (0.86 g, 5.5 mmol), freshly prepared from the corresponding diester and $NaOCH_3$ in 1,2-dimethoxyethane (10 ml). Stirring was continued at room temp. for 1 h, the solvent was removed, and the residue, dissolved in ethyl acetate (40 ml), was washed with water (20 ml) and brine (20 ml). After drying over Na_2SO_4 and evaporation, the residue was purified by flash chromatography (hexane-ethyl acetate (7:1)) to provide the 4-ketodiester **6** as a white solid (1.24 g, 50%), m.p. 58-61°C.- IR (KBr): $\tilde{\nu}$ = 1750; 1715; 1705, and 1690 (C=O) cm^{-1}.- ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.28 and 1.56 (2s; 6H, $C(CH_3)_2$), 2.88-3.12 (m; 4H, 4'-CH_2 and 3-CH_2), 3.64 and 3.67 (s; 6H, OCH_3), 3.72 (m; 1H, 2-H), 4.23 (d; 1H, J = 3.5 Hz, 5'-H), 4.46 (m; 1H, 4'-H), 5.12 (s; 2H, OCH_2Ph), 7.00-7.35 (m; 10 H aromat.).- $C_{27}H_{31}NO_8$ (497.5) Calcd. C 65.2 H 6.28 N 2.8 Found C 65.4 H 6.40 N 2.9.

(4'R,5'S)-4-(4'-Benzyl-3'-benzyloxycarbonyl-2',2'-dimethyl-oxazolidine-5'-yl)-2-isobutyl-2-methoxycarbonyl-4-oxobutyric acid methyl ester (**7**)

The mixture of the 4-ketodiester **6** (1.19 g, 2.4 mmol) in dry DMSO (5 ml) and of 80% NaH (78 mg, 2.6 mmol) was stirred at room temp. for 1 h. Then, isobutyl bromide (0.74 ml, 7.2 mmol) was added and stirring was continued for 24 h. The mixture was diluted with ethyl acetate (40 ml), and washed with N citric acid, saturated $NaHCO_3$ and brine. After drying over Na_2SO_4 and removal of the solvent under reduced pressure, the residue was purified by flash chromatography with hexane-ethyl acetate (10:1) to yield the 4-ketodiester **7** as an oil (0.40 g, 30%).- IR (KBr): $\tilde{\nu}$ = 1720 and 1710 (C=O) cm^{-1}.- ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.75 and 0.78 (2d; 6H, J = 6.5 Hz, CH_3 (isobutyl)), 1.41-1.60 (m; 7H, $C(CH_3)_2$ and $CH(CH_3)_2$), 1.87 (d; 2H, J = 6.5 Hz, CH_2 (isobutyl)), 2.97-3.16 (m; 4H, 4'-CH_2 and 3-CH_2), 3.63 and 3.65 (2s; 6H, OCH_3), 4.32 (d; 1H, J = 3 Hz, 5'-H), 4.52 (m; 1H, 4'-H), 5.16 (s; 2H, OCH_2Ph), 7.04-7.40 (m; 10 H aromat.).- $C_{31}H_{39}NO_8$ (553.7) Calcd. C 67.3 H 7.10 N 2.5 Found C 67.5 H 7.34 N 2.8.

(4'R,5'S,2RS)-4-(4'-Benzyl-3'-benzyloxycarbonyl-2',2'-dimethyl-oxazolidine-5'-yl)-2-isobutyl-4-oxobutyric acid (**8**)

A solution of the 4-ketodiester **7** (0.38 g, 0.7 mmol) in dioxane-water (1:1, 10 ml) was stirred with N NaOH (1.4 ml, 1.4 mmol) at room temp. for 1 h. After concentration (≈ 5 ml), the mixture was diluted with water (5 ml) and washed with ethyl acetate (2 x 10 ml). The aqueous phase was acidified to pH 3-4 with Dowex 50W-X4 resin. The resin was filtered off and washed with ethyl acetate (2 x 10 ml). The aqueous phase was extracted with ethyl acetate (3 x 10 ml), and the combined org. extracts, after washing with brine and drying over Na_2SO_4, were evaporated. The resulting diacid was dissolved in dioxane (20 ml) and heated under reflux for 30 min. Removal of the solvent left a residue which was purified by flash chromatography (hexane - ethyl acetate (1:3)) to give the butyric acid derivative **8** as a foam (0.24 g, 70%).- IR (KBr): $\tilde{\nu}$ = 3300-2985 (OH); 1730; 1720 and 1695 (C=O) cm^{-1}.- ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.77 (m; 6H, CH_3 (isobutyl)), 1.10-1.60 (m; 9H, $C(CH_3)_2$ and CH_2-CH (isobutyl)), 2.60-3.20 (m; 4H, 3-CH_2 and 4'-CH_2), 4.00-4.60 (m; 2H, 5'-H and 4'-H), 5.20 (s; 2H, OCH_2Ph), 7.00-7.60 (m; 10 H aromat.), 8.20 (s; 1H, COOH).- $C_{28}H_{35}NO_6$ (481.6) Calcd. C 69.8 H 7.33 N 2.9 Found C 69.7 H 7.43 N 3.0.

(6R,5S,2RS)-6-Amino-5-hydroxy-2-isobutyl-4-oxo-7-phenylheptanoic acid (Ketomethylenebestatin, **9**)

To a solution of the butanoic acid derivative **8** (192 mg, 0.4 mmol) in MeOH (30 ml), N HCl (0.4 ml, 0.4 mmol) and 10% Pd/C (20 mg) were added, and the mixture was hydrogenated at room temp. and 1 atm of H_2 for 1 h. The catalyst was filtered off, and the solution evaporated to dryness. The residue was dissolved in water (3 ml), and lyophilized to yield ketomethylenebestatin (**9**) hydrochloride as a white solid (96 mg, 70%).- IR (KBr): $\tilde{\nu}$ = 3400; 3150; 3025 (OH and NH_2); 1720 and 1640 (C=O) cm^{-1}.- ^{1}H-NMR [$(CD_3)_2CO$ + D_2O]: δ (ppm) = 0.86, 0.87, 0.91 and 0.92 (4d; 6H, J = 6.5 Hz, CH_3 (isobutyl)), 1.35-1.76 (m; 3H, CH_2-CH (isobutyl)), 2.84-3.02 (m; 3H, 2-H, 3-CH_2), 3.21 (m; 2H, 7-CH_2), 4.36 and 4.42 (2d;

1H, J = 4 Hz, 5-H), 4.65 (m; 1H, 6-H), 7.33 (m; 5H, Ph).- ^{13}C-NMR (CD_3OD): δ (ppm) = 204.50 (C=O), 180.23 and 179.82 (CO_2H), 136.88, 136.83, 130.48, 130.13 and 128.59 (Ph), 73.54 (C-5), 54.74 and 54.30 (C-6), 42.14, 42.05, 39.70 and 39.58 (C-3 and CH_2 isobutyl), 37.16 and 37.09 (C-7), 27.06, 23.07 and 22.44 ($CH(CH_3)_2$ isobutyl).- $C_{17}H_{25}NO_4 \cdot HCl$ (343.9) Calcd. C 59.4 H 7.62 N 4.1 Cl 10.3 Found C 59.2 H 7.73 N 3.9 Cl 10.2.- MS: Calc. for $C_{17}H_{26}NO_4$ (MH^+) m/z = 308.1862, Found m/z = 308.1833.

(4'R,5'S,2RS)-4-Amino-(4'-benzyl-3'-benzyloxycarbonyl-2',2'-dimethyl-oxazolidine-5'-yl)-2-isobutyl-4-oxo-butyric acid methyl ester (**10**)

An ethereal solution of diazomethane, prepared from nitrosomethylurea (0.20 g, 1.93 mmol), was added to a cooled solution (0°C) of the protected acid **8** (0.34 g, 0.7 mmol) in THF (50 ml) and the mixture was stirred at room temp. for 1 h. After evaporation, the residue was purified by flash chromatography (hexane-ethyl acetate (6:1)) to afford the methyl ester **10** (0.24 g, 70%).- IR (KBr): $\tilde{\nu}$ = 1725 and 1705 (C=O) cm^{-1}.- ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.85, 0.87, and 0.91 (3d; 6H, J = 6.5 Hz, CH_3 (isobutyl)), 1.20-1.50 (m; 3H, CH_2-CH (isobutyl)), 1.55 and 1.57 (2s; 6H, $C(CH_3)_2$), 2.50-3.20 (m; 5H, 2-H, 3-CH_2, and 4'-CH_2), 3.57 and 3.60 (2s; 3H, OCH_3), 4.25 (m; 1H, 5'-H), 4.49 and 4.54 (m; 1H, 4'-H), 5.23 (m; 2H, OCH_2Ph), 7.00-7.50 (m; 10 H aromat.).- $C_{29}H_{37}NO_6$ (495.6) Calcd. C 70.3 H 7.52 N 2.8 Found C 70.0 H 7.70 N 3.0.- MS: Calcd. for $C_{29}H_{38}NO_6$ (MH^+) m/z = 496.2699, Found m/z = 496.2650.

Assay of the enzymatic resolution of the diastereoisomeric methyl esters **10**

Pig liver esterase or a lipase (from pig pancreas, *Candida cylindracea*, *Mucor mihiei*, *Pseudomonas fluorescens*, *Aspergillus niger*, *Rhizopus arrhizus* or *Chromobacterium viscosum*) (2 mg) was added to a suspension of the diastereomeric mixture of the methyl esters **10** (2.5 mg, 5 μmol) in 0.15 M phosphate buffer pH 7 (1 ml) with 10% of acetone as cosolvent. After 64 h of stirring at 25°C, acetonitrile was added until complete solution of **10**, and the mixture was filtered through a 0.5 μm filter. The solution was analyzed by reverse-phase HPLC with 0.05% TFA in H_2O-CH_3CN (2:8). Hydrolysis of the methyl esters **10** was not detected in any assay.

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