

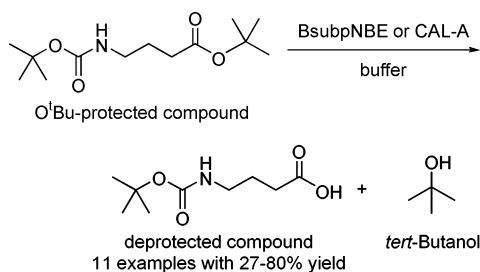
Enzymatic Removal of Carboxyl Protecting Groups. 1. Cleavage of the *tert*-Butyl Moiety

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A recent discovery that a certain amino acid motif (GGG(A)X-motif) in lipases and esterases determines their activity toward tertiary alcohols prompted us to investigate the use of these biocatalysts in the mild and selective removal of *tert*-butyl protecting groups in amino acid derivatives and related compounds. An esterase from *Bacillus subtilis* (BsubpNBE) and lipase A from *Candida antarctica* (CAL-A) were identified as the most active enzymes, which hydrolyzed a range of *tert*-butyl esters of protected amino acids (e.g., Boc-Tyr-O^tBu, Z-GABA-O^tBu, Fmoc-GABA-O^tBu) in good to high yields and left Boc, Z, and Fmoc-protecting groups intact.

The synthesis of complex, polyfunctional organic molecules, such as oligonucleotides, oligosaccharides, and in general natural products, requires the proper introduction and removal of protecting groups. For this purpose, a large number of protecting groups as well as chemical methods for protection and deprotection of various functionalities have been developed and are widely used in synthetic organic chemistry.¹ A major problem for the

synthesis of polyfunctional molecules is that a given functional group has to be protected or deprotected selectively in the presence of functionalities of similar reactivity as well as functionalities sensitive to acids, bases, oxidation, or reduction. The application of biocatalysts in the protecting group chemistry may offer excellent alternatives to chemical methods because enzymes (i) carry out highly chemo- and regioselective transformations, (ii) usually operate at neutral pH values, and (iii) combine a high selectivity for the reactions they catalyze and the structure they recognize with a broad substrate tolerance.²

Among the biocatalysts, hydrolases have attracted special interest for their chemo-, regio-, and enantioselectivities and have found interesting applications in organic synthesis, in particular for the production of enantiopure organic molecules.³ Indeed, some hydrolases were already found to be suitable for the selective removal of protecting groups.² Lipases were shown to hydrolyze heptyl esters of peptides,⁴ and an enzyme from *Sphingomonas paucimobilis* SC16113 was able to enantioselectively cleave *N*-benzyloxycarbonyl (Cbz) groups from protected amino acids and related compounds.⁵ For *tert*-butyl esters, an alkaline serine protease (thermitase) could be used to cleave differently *N*-protected peptides,⁶ and a lipase from *Burkholderia* sp. YY62 was able to hydrolyze *tert*-butyl octanoate.⁷

Recently, we discovered that a certain amino acid motif (GGG(A)X-motif, in single-letter amino acid code where G denotes glycine and X denotes any amino acid; in a few enzymes, one glycine is replaced by an alanine, A) located in the oxyanion binding pocket of lipases and esterases determines activity toward tertiary alcohols.⁸ All enzymes bearing this motif (e.g., lipase from *Candida rugosa*, pig liver esterase, acetyl choline esterases, and a *p*-nitrobenzyl esterase from *Bacillus subtilis* (BsubpNBE)) were active toward several acetates of tertiary alcohols, while enzymes bearing the more common GX-motif did not hydrolyze the model compounds. Consequently, the search for a hydrolase active toward the sterically demanding tertiary alcohol moiety is considerably facilitated, as it can be simply based on the existence of the GGG(A)X-motif. Thus, the trial and error approach in testing all available lipases and esterases is not necessary any more.

(2) (a) Kadereit, D.; Waldmann, H. *Chem. Rev.* **2001**, *101*, 3367. (b) Waldmann, H.; Sebastian, D. *Chem. Rev.* **1994**, *94*, 911.

(3) Bornscheuer, U. T.; Kazlauskas, R. *J. Hydrolases in Organic Synthesis – Regio- and Stereoselective Biotransformations*; Wiley-VCH: Weinheim, 1999.

(4) (a) Braun, P.; Waldmann, H.; Vogt, W.; Kunz, H. *Synthesis* **1990**, 105. (b) Braun, P.; Waldmann, H.; Vogt, W.; Kunz, H. *Liebigs Ann. Chem.* **1991**, 165. (c) Braun, P.; Waldmann, H.; Kunz, H. *Synlett* **1992**, 39.

(5) (a) Nanduri, V. B.; Goldberg, S.; Johnston, R.; Patel, R. N. *Enzyme Microb. Technol.* **2004**, *34*, 304. (b) Patel, R. N.; Nanduri, V. B.; Brzozovski, D. B.; McNamee, C.; Banerjee, A. *Adv. Synth. Catal.* **2003**, *345*, 830.

(6) Schultz, M.; Hermann, P.; Kunz, H. *Synlett* **1992**, 37. (7) Yeo, S. H.; Nihira, T.; Yamada, Y. *Biosci. Biotechnol. Biochem.* **1998**, *62*, 2312.

(8) (a) Henke, E.; Pleiss, J.; Bornscheuer, U. T. *Angew. Chem., Int. Ed.* **2002**, *41*, 3211. (b) Henke, E.; Bornscheuer, U. T.; Schmid, R. D.; Pleiss, J. *ChemBioChem* **2003**, *4*, 485.

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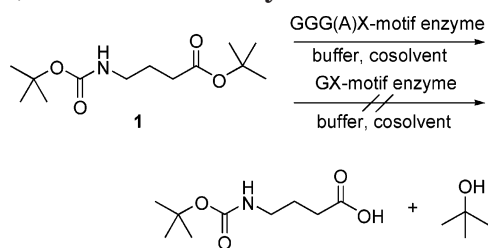
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^{||} Agricultural University of Athens.

(1) (a) Kocienski, P. J. *Protecting Groups in Organic Synthesis*, 3rd ed.; Wiley and Sons: New York, 1999. (b) Greene, T. W.; Wuts, P. G. M. *Protecting Groups*; Georg Thieme Verlag: Stuttgart, 1994.

SCHEME 1. Hydrolysis of *tert*-Butyl γ -(*tert*-Butoxycarbonylamino)butyrate (1) Using GGG(A)- and GX-Motif Enzymes



In this paper, we have therefore selected enzymes bearing the GGG(A)X-motif and investigated their applicability in the selective removal of the *tert*-butyl protecting group in amino acid derivatives and related compounds.

A variety of hydrolases containing either the GGG(A)X-motif (BsubpNBE,⁹ CAL-A, PLE, PCE¹⁰) or the GX motif (CAL-B, PFE, BstE, RMIM,⁹ serving as negative control) were used in an initial screening for activity toward *tert*-butyl γ -(*tert*-butoxycarbonylamino) butyrate **1** serving as model substrate (Scheme 1). Compound **1** was chosen as model, as both the *C*-*tert*-butyl and *N*-*tert*-butoxycarbonyl protecting groups may be removed by chemical means under acidic conditions.¹ The *tert*-butoxycarbonyl group may be selectively removed in the presence of *tert*-butyl esters by using 1 M HCl in EtOAc,¹¹ TBAF in refluxing THF,¹² 4 M HCl in anhydrous dioxane,¹³ and concentrated H₂SO₄ in *t*-BuOAc or MeSO₃H.¹⁴ The selective removal of *C*-*tert*-butyl esters in the presence of the Boc group may be achieved by using the CeCl₃·7H₂O–NaI system¹⁵ or ZnBr₂ in CH₂Cl₂.¹⁶ *C*-*tert*-Butyl esters may also be cleaved selectively, in the presence of *C*-*tert*-butyl ethers, by using montmorillonite KSF clay.¹⁷

The results of the screening are summarized in Table 1. Two enzymes containing the GGG(A)X-motif, BsubpNBE⁹ and lipase A from *Candida antarctica* (CAL-A, Novozymes), hydrolyzed the substrate totally or partly, respectively. However, two other enzymes containing the GGG(A)X motif, PLE and PCE, did not show activity, presumably because their specific activity was too low or they do not accept amino acid derivatives in general. Next, the reaction conditions for the enzymatic hydrolysis

TABLE 1. Hydrolysis of *tert*-Butyl γ -(*tert*-Butoxycarbonylamino)butyrate (1) by Various Hydrolases

enzyme	motif	conversion
BsubpNBE	GGG(A)X	+
CAL-A	GGG(A)X	+
PLE	GGG(A)X	–
PCE	GGG(A)X	–
PFE	GX	–
CAL-B	GX	–
BstE	GX	–
RMIM	GX	–

of *tert*-butyl esters were optimized using BsubpNBE and CAL-A by the addition of various cosolvents. Toluene, *n*-hexane, diethyl ether, and to a smaller extent methanol, chloroform, and dimethyl sulfoxide facilitated the enzymatic hydrolysis. Acetonitrile, acetone, ethyl acetate, ethanol, *n*-propanol, and 2-propanol turned out to be inappropriate cosolvents (data not shown).

To expand the applicability of this method, a variety of *tert*-butyl esters were prepared by conventional chemical methods. The results of their hydrolyses by BsubpNBE and CAL-A are summarized in Table 2. BsubpNBE hydrolyzed substrates **1–4**, **10**, and **11** (Table 2) in good to high yields. A variety of urethane-type *N*-protecting groups (Boc, Z, Fmoc), usually used in protecting group chemistry, remained intact under the conditions of the enzymatic hydrolysis. Thus, we demonstrated that the *tert*-butyl moiety of substrate **1** may be selectively removed in the presence of the Boc group by enzymatic hydrolysis. In addition, BsubpNBE selectively hydrolyzes the *tert*-butyl ester of substrate **11**, leaving the *tert*-butyl ether intact. However, BsubpNBE is unable to remove the *tert*-butyl ester group from the dipeptide substrate **5** (Boc-Val-Gly-O^tBu). Substrates **6** and **7**, based on phenylalanine and tyrosine moieties, were easily hydrolyzed by BsubpNBE and the products of the enzymatic hydrolysis were isolated in satisfactory yields. In contrast, substrates **8** and **9** were hydrolyzed in low yields, indicating that BsubpNBE rather prefers the aromatic side chains of phenylalanine and tyrosine than the small aliphatic chains of alanine and valine. Under similar conditions CAL-A seems to hydrolyze effectively only substrates **2** and **6**. However, when we used for the CAL-A hydrolysis only buffer at elevated temperature (50 °C), in the absence of any cosolvent, better yields of isolated products were observed (substrates **1–4**).

Compared to the earlier work using thermitase-catalyzed deprotection of the *tert*-butyl moiety from peptides,⁶ the use of these esterases has the advantage that esterases in general are more stable toward organic solvents. In addition, the thermitase was only used for the deprotection of peptides, and it is unclear whether nonpeptidic substrates are accepted by this protease. Although it appears that our methodology requires relatively large amounts of enzyme, it must be noted that these are crude preparations (in case of BsubpNBE the protein content of the lyophilisate is approximately 40% and the overall esterase content is estimated to approximately 15%). In addition, the reactivity of esterases toward the sterically demanding esters of tertiary alcohols is approximately 100–1000-fold lower compared to natural triglycerides.^{8a} Despite this aspect, we

(9) BsubpNBE, recombinant *p*-nitrobenzyl esterase from *Bacillus subtilis* produced in *E. coli*; CAL-A, lipase A from *Candida antarctica* (Novozymes); PLE, pig liver esterase (Roche); PCE, recombinant esterase from *Pyrobaculum calidifontis* produced in *E. coli*; CAL-B, lipase B from *Candida antarctica* (Novozymes); PFE, recombinant esterase from *Pseudomonas fluorescens* produced in *E. coli*; BstE, recombinant esterase from *Bacillus stearothermophilus* produced in *E. coli*; RMIM, immobilized lipase from *Rhizomucor miehei* (Novozymes).

(10) Hotta, Y.; Ezaki, S.; Atomi, H.; Imanaka, T. *Appl. Environm. Microbiol.* **2002**, *68*, 3925.

(11) Gibson, F. S.; Bergmeier, S. C.; Rapoport, H. *J. Org. Chem.* **1994**, *59*, 3216.

(12) Routier, S.; Sauge, L.; Ayerbe, N.; Coudert, G.; Merour, J. Y. *Tetrahedron Lett.* **2002**, *43*, 589.

(13) Han, G.; Tamaki, M.; Hruby, V. J. *J. Pept. Res.* **2001**, *58*, 338.

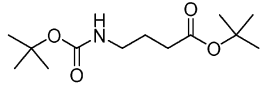
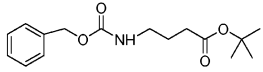
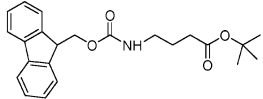
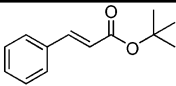
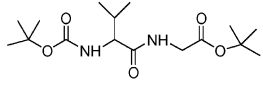
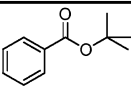
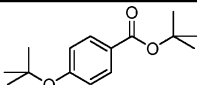
(14) Lin, L. S.; Lanza, T., Jr.; de Lazlo, S. E.; Truong, Q.; Kame-necka, T.; Hagmann, W. K. *Tetrahedron Lett.* **2000**, *41*, 7013.

(15) Marcantoni, E.; Massaccesi, M.; Torregiani, E. *J. Org. Chem.* **2001**, *66*, 4430.

(16) Wu, Y.; Limburg, D. C.; Wilkinson, D. E.; Vaal, M. J.; Hamilton, G. S. *Tetrahedron Lett.* **2000**, *41*, 2847.

(17) Yadav, J. S.; Subba Reddy, B. V.; Sanjeeva Rao, K.; Harikishan, K. *Synlett* **2002**, *5*, 826.

TABLE 2. Hydrolysis of Various Esters by BsubpNBE and CAL-A

Number	Substrate	BsubpNBE		CAL-A			
		Time [h]	Yield ^a [%]	Time [h]	Yield ^{a,b} [%]	Time [h]	Yield ^{a,c} [%]
1		48	77	48	11	48	50
2		48	54	48	69	24	80
3		48	61	48	31	48	40
4		48	66	48	7	48	21
5		48	–	48	–	n.d.	n.d.
6	Boc-Phe-O ^t Bu	48	62	48	68	n.d.	n.d.
7	Boc-Tyr-O ^t Bu	24	74	n.d.	n.d.	n.d.	n.d.
8	Boc-Ala-O ^t Bu	48	16	48	27	n.d.	n.d.
9	Boc-Val-O ^t Bu	48	29	n.d.	n.d.	n.d.	n.d.
10		48	65	48	12	n.d.	n.d.
11		60	61	60	–	n.d.	n.d.

^a Yield of isolated product. ^b Mixture of buffer and *n*-hexane (37 °C). ^c Buffer (50 °C); n.d., not determined.

have demonstrated that two enzymes, BsubpNBE and CAL-A, can be used in synthetic organic chemistry to cleave *tert*-butyl esters from various substrates.

Experimental Section

Melting points were determined on a Buchi apparatus and are uncorrected. Specific rotations were measured at 25 °C on a Perkin-Elmer polarimeter using a 10 cm cell. NMR spectra were recorded on a 200 MHz spectrometer. All amino acid derivatives were purchased from Fluka and Bachem. TLC plates (silica gel 60 F254) and silica gel 60 (70–230 or 230–400 mesh) for column chromatography were purchased from Merck. Visualization of spots was effected with UV light and/or phosphomolybdic acid and/or ninhydrin, both in EtOH stain. Esterase BsubpNBE was produced recombinantly (see below), but is also available from Jülich Fine Chemicals (Esterase BS2, www.juelich-chemicals.com), CAL-A was purchased from Novozymes (Denmark).

Synthesis of Substrates. 4-(9*H*-Fluoren-9-ylmethoxycarbonylamino)butyric Acid *tert*-Butyl Ester (3). To a stirred solution of Fmoc-GABA (0.33 g, 1 mmol) and *tert*-butyl alcohol (0.3 mL, 3 mmol) in CH₂Cl₂ (2 mL), 4-(dimethylamino)pyridine (0.01 g, 0.1 mmol), and subsequently *N,N'*-dicyclohexylcarbodiimide (0.25 g, 1.2 mmol) were added at 0 °C. The reaction mixture was stirred for 1 h at 0 °C and overnight at room temperature. After filtration, the solvent was evaporated under reduced pressure, and EtOAc (20 mL) was added. The organic layer was washed consecutively with brine, 1 N HCl, brine, 5% NaHCO₃, and brine, dried over Na₂SO₄, and evaporated under reduced pressure. The residue was purified by column chromatography using CHCl₃ as eluent: yield 0.17 g (43%); oil; ¹H NMR (CDCl₃) δ 7.68 (m, 2H), 7.60 (m, 2H), 7.35 (m, 4H), 5.03 (m, 1H), 4.41 (d, 2H, *J* = 6.6 Hz), 4.22 (t, 1H, *J* = 6.6 Hz), 3.24 (m, 2H), 2.28 (t, 2H, *J* = 7.0 Hz), 1.81 (m, 2H), 1.47 (s, 9H); ¹³C NMR (CDCl₃) δ 172.6, 156.4, 143.9, 141.2, 127.8, 127.6, 127.1, 126.9, 125.3, 125.0, 119.9, 80.4, 66.5, 47.2, 40.4, 32.7, 28.0, 25.1. Anal.

Calcd for $C_{23}H_{27}NO_4$: C, 72.42; H, 7.13; N, 3.67. Found: C, 72.25; H, 7.23; N, 3.72.

Substrates **1**, **2**, **4**, and **6–9** were prepared according to the above-described procedure. Their analytical data were in accordance with those reported in the literature (substrate **1**,¹⁵ substrate **2**,¹⁸ substrate **4**,¹⁹ substrate **6**,^{15,20} substrate **7**,^{15,21} substrate **8**,^{15,22} substrate **9**,^{15,23}).

(S)-(2-tert-Butoxycarbonylamino-3-methylbutyrylamino)acetic Acid tert-Butyl Ester (5). To a stirred solution of H-Gly-O^tBu·HCl (0.42 g, 2.5 mmol) and Boc-Val-OH (0.54 g, 2.5 mmol) in CH_2Cl_2 (25 mL) were added Et₃N (0.76 mL, 5.5 mmol) and subsequently 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide (0.53 g, 2.8 mmol) and 1-hydroxybenzotriazole (0.34 g, 2.5 mmol) at 0 °C. The reaction mixture was stirred for 1 h at 0 °C and overnight at room temperature. The solvent was evaporated under reduced pressure, and EtOAc (20 mL) was added. The organic layer was washed consecutively with brine, 1 N HCl, brine, 5% NaHCO₃, and brine, dried over Na₂SO₄, and evaporated under reduced pressure. The residue was purified by column chromatography using $CHCl_3$ as eluent: yield 0.59 g (71%); oil; $[\alpha]_D^{25} = -7.3$ (c 1, $CHCl_3$); ¹H NMR ($CDCl_3$) δ 6.61 (t, 1H, *J* = 4.8 Hz), 5.14 (d, 1H, *J* = 8.8 Hz), 4.00–3.92 (m, 3H), 2.15 (m, 1H), 1.43 (br s, 18H), 0.96 (d, 3H, *J* = 6.6 Hz), 0.91 (d, 3H, *J* = 6.8 Hz); ¹³C NMR ($CDCl_3$) δ 171.7, 168.7, 155.8, 82.2, 79.8, 59.7, 41.9, 30.9, 28.2, 27.4, 19.2, 17.6. Anal. Calcd for $C_{16}H_{30}N_2O_5$: C, 58.16; H, 9.15; N, 8.48. Found: C, 58.47; H, 8.95; N, 8.36.

tert-Butyl 4-tert-Butoxybenzoate (11). To a stirred solution of benzoic acid (1.0 g, 7.2 mmol) in dioxane (29 mL) was added concentrated H₂SO₄ (1.44 mL) dropwise under vigorous stirring at 0 °C. Isobutylene was bubbled through the mixture for 1 h at 0 °C. The reaction mixture was stirred for 1 h at 0 °C and overnight at room temperature. The mixture was cooled at 0 °C, and aqueous sodium hydroxide (1 N, 50 mL) was added slowly, followed by ethyl acetate (30 mL). The organic layer was separated and the aqueous phase washed with ethyl acetate (2 × 30 mL). The combined organic layers were washed with brine, dried over Na₂SO₄, and evaporated under reduced pressure. The residue was purified by column chromatography using petroleum ether (60–80 °C)/EtOAc as eluent: yield 0.68 g (38%); oil; ¹H NMR ($CDCl_3$) δ 7.91 (dd, 2H, *J* = 8.8, 2.2 Hz), 6.99 (dd, 2H, *J* = 8.8, 2.2 Hz), 1.58 (s, 9H), 1.38 (s, 9H); ¹³C NMR ($CDCl_3$) δ 165.5, 159.5, 130.6, 122.6, 80.6, 79.3, 28.8, 28.1. Anal. Calcd for $C_{15}H_{22}O_3$: C, 71.97; H, 8.86. Found: C, 71.72; H, 9.06.

Substrate **10** was prepared according to above-described procedure. Its analytical data were in accordance with those reported in the literature.²⁴

(18) Muller, D.; Zeltser, I.; Bitan, G.; Gilon, C. *J. Org. Chem.* **1997**, *62*, 411.

(19) Parrish, J. P.; Chun Jung, Y.; Il Shin, S.; Woon Jung, K. *J. Org. Chem.* **2002**, *67*, 7127.

(20) Konda, Y.; Takahashi, Y.; Arima, S.; Sato, N.; Takeda, K.; Dobashi, K.; Baba, M.; Harigaya, Y. *Tetrahedron* **2001**, *57*, 4311.

(21) Doherty, G. A.; Kamenecka, T.; McCauley, E.; Van Riper, G.; Mumford, R. A.; Tong, S.; Hagmann, W. K. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 5, 729.

(22) Hayashida, O.; Sebo, L.; Rebek, J., Jr. *J. Org. Chem.* **2002**, *67*, 8291.

(23) Barrett, A. G. M.; Pilipauskas, D. *J. Org. Chem.* **1990**, *55*, 5170.

Expression of Recombinant Esterases in *Escherichia coli*. Clones containing the expression vector encoding genes for esterases (BsubpNBE, BstE, PCE, and PFE) were used to inoculate 5 mL of an overnight culture (LB-media supplemented with 100 μg/mL of ampicillin). Five hundred microliters of the overnight culture was used to inoculate 500 mL LB-Amp. The culture was incubated at 37 °C and 200 rpm to a cell density of OD₆₀₀ 0.4–0.6, and enzyme expression was induced by addition of L-rhamnose solution (end concentration 0.2% w/v). After 4 h of further incubation at 37 °C, cells were harvested by centrifugation (15 min, 4 °C, 8000 g) and washed twice with 50 mL sodium phosphate buffer (50 mM, pH 7.5). Cells were resuspended in 20 mL phosphate buffer and disrupted by sonification with cooling on ice. Cell debris was removed by centrifugation (15 min, 4 °C, 8000 g), the supernatant was frozen at –80 °C and then lyophilized. Specific activities of crude extracts were determined spectrophotometrically using *p*-nitrophenyl acetate for activity and the Bradford reagent for protein content.

General Method for Enzymatic Hydrolysis. To a stirred solution of the substrate (0.15–0.20 mmol) in *n*-hexane (1 mL) and CH₃OH (100 μL) was added a solution of the enzyme (50 mg) in phosphate buffer (9 mL, 50 mM pH 7.4). The reaction mixture was stirred for 24–60 h at 37 °C. After acidification until pH 6 and extraction with EtOAc (3 × 5 mL), the organic layers were combined and washed with 5% NaHCO₃ (3 × 5 mL). The aqueous layer was acidified until pH 6 and extracted with EtOAc (3 × 10 mL). The combined organic layers were dried over Na₂SO₄, and the organic solvent was removed under reduced pressure to give the product.

4-tert-Butoxybenzoic Acid. To a stirred solution of *tert*-butyl 4-*tert*-butoxybenzoate **11** (45 mg, 0.18 mmol) dissolved in 1 mL of *n*-hexane and 100 μL CH₃OH was added a solution of 50 mg BsubpNBE (dissolved in 9 mL sodium phosphate buffer, 50 mM pH 7.4). The reaction mixture was stirred for 60 h at 37 °C followed by workup as described above for the general method: yield 15 mg (61%); white solid; mp 132–135 °C; ¹H NMR ($CDCl_3$) δ 8.04 (d, 2H, *J* = 5.6 Hz), 7.05 (d, 2H, *J* = 5.6 Hz), 1.44 (s, 9H); ¹³C NMR ($CDCl_3$) δ 168.3, 161.4, 130.7, 127.1, 122.7, 79.3, 28.3. Anal. Calcd for $C_{11}H_{14}O_3$: C, 68.02; H, 7.27. Found: C, 67.76; H, 7.46.

The other products of the enzymatic hydrolysis were identified by their analytical data in comparison with authentic samples.

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(24) Hans, J. J.; Driver, R. W.; Burke, S. D. *J. Org. Chem.* **2000**, *65*, 2114.