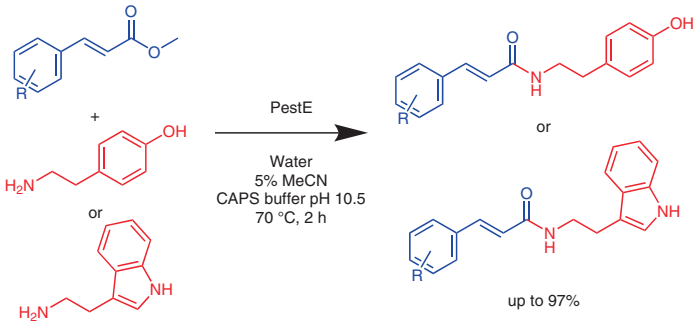
Enzymatic Synthesis of Hydroxycinnamic Acid Amides in Water Using the Promiscuous Hydrolase/Acyltransferase PestE from *Pyrobaculum calidifontis* VA1

Benjamin Baumert[a]
Henrik Terholsen[a]
Clemens Cziegler[a]
Isabel Thier[b]
Christoffel P. S. Badenhorst[a]
Karsten Siems[b]
Uwe T. Bornscheuer*[a]

[a] Department of Biotechnology and Enzyme Catalysis, Institute of Biochemistry, University of Greifswald, Felix-Hausdorff-Str. 4, 17487 Greifswald, Germany
uwe.bornscheuer@uni-greifswald.de
[b] AnalytiCon Discovery GmbH, Herrmanswerder 17, 14473 Potsdam, Germany

Published as part of the Cluster
Chemical Synthesis and Catalysis in Germany



Received: 12.10.2023
Accepted after revision: 25.01.2024
Published online: 14.02.2024 (Accepted Manuscript), 01.03.2024 (Version of Record)
DOI: 10.1055/a-2268-8035; Art ID: ST-2024-01-0003-A

Abstract Hydroxycinnamic acid amides are believed to have antioxidant, antidiabetic, cytotoxic, anticancer, neuroprotective, and antiinflammatory properties, making them interesting target molecules for potential applications in the food, cosmetics, and pharmaceutical industries. Here, we describe the synthesis of hydroxycinnamic acid amides starting from hydroxycinnamic acid esters and the corresponding amines by using variants of the promiscuous hydrolase/acyltransferase from *Pyrobaculum calidifontis* VA1 (PestE) in water as a solvent. Up to 97% conversion within two hours at 60 °C was achieved with methyl ferulate and tyramine as substrates. This is a promising, environmentally friendly alternative strategy to established chemical synthesis routes or enzymatic methods using lipases in nonaqueous organic solvents.

Key words acyltransferase, amides, biocatalysis, carboxylesterase, hydroxycinnamic amides

Hydroxycinnamic acid amides (HCAAs) are a class of naturally occurring secondary plant metabolites with presumptive roles in plant growth, development, and senescence.[1] In addition to their role in plants, in vivo and in vitro studies have revealed potent antioxidant, antidiabetic, antiinflammatory, antimelanogenic, and cytotoxic properties, making these compounds interesting candidates for applications in the food, cosmetics, and pharmaceutical industries.[2] HCAAs are naturally synthesized by the conversion of CoA esters of hydroxycinnamic acids and aliphatic or aromatic amines into amides.[3] Although they occur in many plants, direct extraction is not economically feasible due to the small quantities available.[2]

Therefore, methods for the synthesis of HCAAs are necessary. There is a wide range of chemical strategies for amide-bond formation.[4,5] For large-scale applications, stoichiometric coupling reagents are commonly used. These activate the carboxylic acid partner for the condensation reaction with the amine. The most common methods are the formation of activated esters with carbodiimides such as 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide (EDC) or *N*,*N*′-dicyclohexylcarbodiimide (DCC). Another possibility is the formation of acid chlorides with thionyl chloride or the preparation of mixed anhydrides by using reagents such as pivaloyl chloride.[6] However, these reagents are used in stoichiometric amounts; moreover, some are very atom-inefficient, and many of the solvents used are not environmentally friendly. Consequently, the American Chemical Society Green Chemistry Institute Pharmaceutical Roundtable has requested the development of more environmentally friendly and efficient methods for amide-bond formation as one of their top priorities.[7]

A more environmentally friendly approach to the synthesis of amides could involve the use of enzymes. Several enzymatic methods for amide-bond formation have been summarized in two recent reviews.[8,9] Enzymes that catalyze this reaction can be roughly divided into two groups. The first group consists of ATP-dependent enzymes that use ATP to activate the carboxylic acid. Due to the cost of ATP, these enzymes are not suitable for industrial applications. The second group consists of hydrolases, including, for example, lipases, esterases, penicillin G acylases, and proteases.[10] Most of these enzymes still need activated car-

boxylic acids in the form of esters, but they do not need any cofactors, and many of them are very robust, making them attractive candidates for industrial applications. Lipases are the most studied enzymes for amide-bond formation. In the absence of water, transacylation reactions are favorable, thus promoting the aminolysis of esters, leading to amides. Unfortunately, these reactions must be catalyzed in non-aqueous organic solvents in the presence of molecular sieves to prevent hydrolysis. According to the CHEM21 solvent-selection guide,[11] which is based on safety, health, and environmental criteria, most solvents used in these applications are considered hazardous or highly hazardous.[9] For example, the HCAAs *N-trans*-caffeoyltyramine and *N-trans*-feruloyltyramine were previously synthesized by using the *Candida antarctica* lipase B (CAL-B) in methyl *tert*-butyl ether with methyl esters as acyl donors (molar ratio 1:2 with 78% and 73% conversion, respectively),[12] and, in another approach *N-trans*-feruloyltyramine was made by using Lipozyme TL IM and ferulic acid as an acyl donor in acetonitrile as the solvent (70% conversion with a molar ratio of 1:1).[13]

A promising greener alternative involves the so-called promiscuous hydrolases/acyltransferases. Usually, hydrolases prefer hydrolysis with water over acyl transfer in bulk water; however, some promiscuous acyltransferases can catalyze acylation in aqueous solution.[14] Land et al.[15] showed that a promiscuous hydrolase/acyltransferase from *Mycobacterium smegmatis* (MsAcT), which had previously been shown to catalyze the synthesis of esters, is able to synthesize amides. They used MsAcT in an amine transaminase/acyltransferase cascade for the formation of amides from the corresponding aldehydes and ketones in aqueous solution. This enzyme was further studied by Contente et al.,[16] who demonstrated a synthesis of simple amides by using vinyl acetate or ethyl acetate and, more recently, the synthesis of more structurally complex molecules, such as vanillamides, in water.[17] We had discovered that EstCE1, a member of the Family VIII carboxylesterases, is another promiscuous hydrolase/acyltransferase that catalyzes amide condensation. By a synthesis of the antidepressant moclobemide, we showed that the catalytic potential of EstCE1 extends beyond structurally simple amides.[18] In that study, we used a promiscuous hydrolase/acyltransferase from *Pyrobaculum calidifontis* VA1 (PestE). This enzyme was originally discovered by Hotta et al. as an extremely stable carboxylesterase from a hyperthermophilic archaeon.[19] Its acyltransferase activity for the synthesis of esters was discovered by Müller et al.[20] and further improved by enzyme engineering for the acetylation of monoterpene alcohols[21] and hydroxytyrosol.[22]

Here, we describe the ability of PestE to synthesize hydroxycinnamic acid amides in aqueous solution. In an initial screening using ultra-high-pressure liquid chromatography (UHPLC) analysis, multiple promiscuous hydrolases/acyltransferases were studied for amide-bond formation with tyramine and vinyl ferulate as substrates.[23] Of all the candidates, PestE showed the highest *N-trans*-feruloyltyramine formation, which is why we chose this enzyme for further optimization to increase the efficiency of the reaction. In this case, a more efficient reaction means that the ratio of aminolysis (attack by the nucleophile tyramine) increases relative to the hydrolysis of the acyl donor vinyl ferulate (attack by the nucleophile water). As already observed by Land et al., the pH has an influence on the efficiency of the reaction.[15] They determined a pH optimum of 11 for MsAcT and speculated about a connection with the protonation status of the amine benzylamine (pK_a = 9.34). Therefore, the optimal pH for the synthesis of *N-trans*-feruloyltyramine from vinyl ferulate and tyramine was determined. Given that tyramine has a pK_a value of 9.22 for the amine,[24] which is comparable to that of benzylamine, and the optimal pH of PestE for the hydrolysis of *p*-nitrophenyl caproate was determined to be pH 7, the range between pH 7 and pH 11 was investigated. To select the most suitable buffer system, primary alcohols and primary amines were avoided, as they are potential nucleophiles and could unintentionally influence the reaction. For this reason, potassium phosphate buffer was used from pH 7 to 8, *N*-cyclohexyl-2-aminoethanesulfonic acid (CHES) buffer from pH 8.5 to 10, and *N*-cyclohexyl-3-aminopropanesulfonic acid (CAPS) buffer from pH 10 to 11 (reaction conditions: 5 mM tyramine, 5 mM vinyl ferulate, 0.21 mg/ml PestE wild-type and PestE_I208A_L209F_N288A, 25 °C).[25] As expected, the reaction appeared to be strongly pH-dependent. At pH 7 or 7.5, marginal conversions were detected, and, starting from pH 8, *N-trans*-feruloyltyramine was formed, with an optimum pH of 10.5 (Figure 1). At pH 7, mostly ferulic acid is formed by hydrolysis of the substrate, vinyl ferulate, by water. This

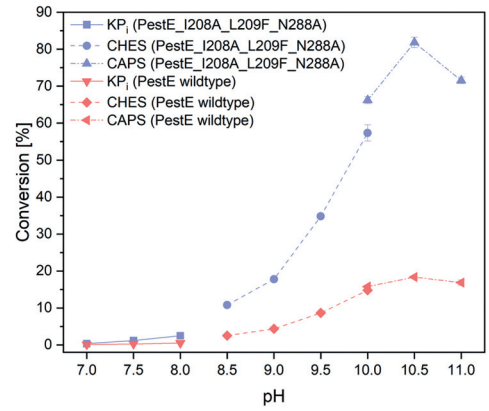


Figure 1 pH-profile of PestE_I208A_L209F_N288A and PestE wild-type for the synthesis of *N-trans*-feruloyltyramine. *Reaction conditions*: enzyme (0.21 mg/mL), vinyl ferulate (5 mM), tyramine (5 mM), buffer (178 mM), 25 °C. Conversions were determined by UHPLC and represent the mean of three independent experiments with the standard deviations displayed.

could be explained by the fact that tyramine is mainly protonated at pH 7 and is not available as a nucleophile for the aminolysis reaction. Measurements were taken at various time points. The conversions were determined after the acyl donor had been consumed either by aminolysis or hydrolysis. Even after prolonged incubation, no amidase activity was detected. A control with the buffer instead of the enzyme was performed to exclude chemical background reactions as a cause of the amide formation. After a comparable incubation time to that with PestE, a conversion of 1% was measured with the vinyl ester as the substrate. In contrast, wild-type PestE gave 14% conversion (reaction conditions: 5 mM tyramine, 5 mM vinyl ferulate, CHES buffer, pH 10, 25 °C),[25] indicating that the reaction is catalyzed by the enzyme, despite the background activity.

The next step was to investigate variants of PestE that had previously been developed in our group (Figure 2).[21,22,26] The PestE variant I208A_L209F_N288A showed the highest conversion of 57% (reaction conditions: 5 mM tyramine, 5 mM vinyl ferulate, CHES buffer, pH 10, 25 °C),[25] so further experiments were conducted with this variant, which had already shown the highest conversion for the acetylation of hydroxytyrosol.[22] Because vinyl esters violate many principles of green chemistry, the methyl ester and ethyl ester were also investigated and these showed similar conversions (63% and 58%, respectively).[25] The conversions are also comparable to those in previously published approaches using lipases in organic solvents.[12,13] In the case of these esters, no background reaction was detected when the buffer was used as a control. Ferulic acid was studied under the same reaction conditions, but there was no indication that a reaction was occurring. The disadvantage of these esters is that they are not as reactive as the vinyl ester; consequently, the reaction times were prolonged (90% conversion with the vinyl ester after 2 h at 25 °C compared with 13% at 20 °C or 32% at 30 °C with the methyl ester).

To accelerate the reaction, the temperature profile was determined by measuring the conversions of PestE wild-type and PestE_I208A_L209F_N288A with the methyl ester as substrate after two hours at temperatures between 20 and 90 °C (Figure 3) (reaction conditions: 5 mM tyramine, 10 mM methyl ferulate, CHES buffer, pH 10).[25] After two hours, the highest conversion for the wild-type enzyme was achieved at 90 °C but, for the best variant PestE_I208A_L209F_N288A, this occurred at 60 °C. Lower conversions at higher temperatures indicated a slower reaction. The optimum temperature of 90 °C for the wild-type enzyme corresponds to that for the highest activity of the wild-type enzyme in the hydrolysis of *p*-nitrophenyl caproate, which had been previously determined.[19] An increase in the molar ratio of methyl ferulate to tyramine to 2:1 yielded a conversion of 97% at 60 °C (Figure 3).

In the reaction mixture, 5% (v/v) acetonitrile as a water-miscible cosolvent was used. According to the CHEM21 solvent-selection guide, acetonitrile is classified as 'problematic'.[11] Unfortunately, most of the recommended water-miscible solvents are primary alcohols or esters, which, as nucleophiles or acyl donors, might affect the reaction. For example, ethanol is not a suitable cosolvent for this reaction, as it lowered the conversion from 63% to 47% when using methyl ferulate and tyramine in a molar ratio of 1:1.[25] However, at lower substrate concentrations, a cosolvent might not be necessary. Other hydroxycinnamic acids and amines are also acceptable substrates for PestE. For example, the methyl esters of coumaric acid and caffeic acid were

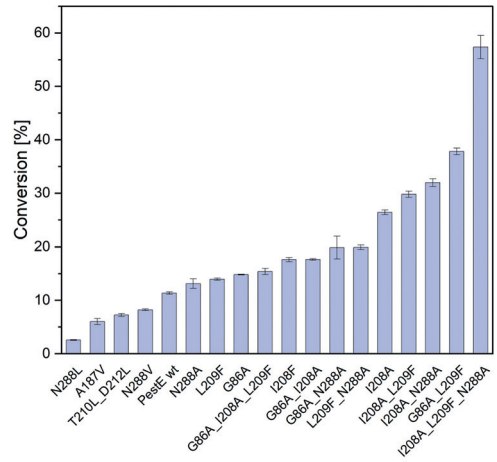


Figure 2 Synthesis of *N-trans*-feruloyltyramine with PestE variants. *Reaction conditions*: PestE (0.21 mg/mL), vinyl ferulate (5 mM), tyramine (5 mM), CHES buffer (pH 10; 178 mM), 25 °C. Conversions were determined by UHPLC and represent the mean of three independent experiments with the standard deviations displayed.

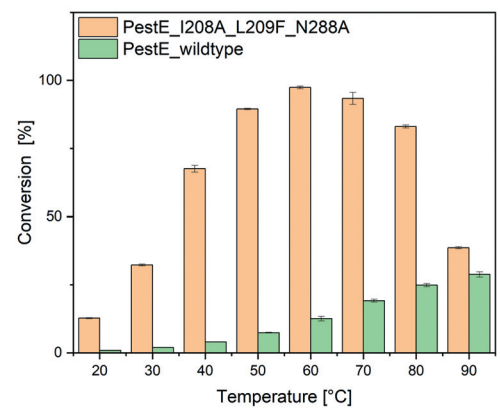


Figure 3 Temperature profile of PestE_I208A_L209F_N288A and PestE wild-type for the synthesis of *N-trans*-feruloyltyramine. Conversions measured after incubation for 2 h. *Reaction conditions*: enzyme (0.21 mg/mL), methyl ferulate (10 mM), tyramine (5 mM), CHES buffer (pH 10; 178 mM). Conversions were determined by UHPLC and represent the mean of three independent experiments with the standard deviations displayed.

Table 1 Reactions studied with PestE_I208A_L209F_N288A[a]

Acyl donor	Amine	Product	Conversion[b] (%)
vinyl ferulate	tyramine	*N-trans*-feruloyltyramine	57
methyl ferulate	tyramine	*N-trans*-feruloyltyramine	63
ethyl ferulate	tyramine	*N-trans*-feruloyltyramine	58
methyl caffeate[c]	tyramine	*N-trans*-caffeoyltyramine	54
methyl coumarate	tyramine	*N-trans*-coumaroyltyramine	(TLC)
methyl ferulate	tryptamine	*N-trans*-feruloyltryptamine	(TLC)
methyl ferulate	octopamine	*N-trans*-feruloyloctopamine	(TLC; trace)

[a] Reaction conditions: PestE_I208A_L209F_N288A (0.21 mg/mL), acyl donor (5 mM), amine (5 mM), CHES buffer (pH 10; 178 mM), 25 °C.
[b] Determined by UHPLC.
[c] With ascorbic acid (50 mM).

studied as alternative acyl donors. However, the methyl ester of caffeic acid oxidizes very rapidly at pH 10 and 70 °C, as indicated by a brown coloring of the solution, preventing product formation. Alkaline conditions and temperatures around 70 °C promote the oxidation of caffeic acid, but since these conditions cannot be changed without affecting the efficiency of the reaction, the antioxidant ascorbic acid was used instead (5 mM tyramine, 5 mM methyl caffeate, 50 mM ascorbic acid, CHES buffer, pH 10, 70 °C).[25] Ascorbic acid effectively prevented oxidation of the substrate, leading to the synthesis of *N-trans*-caffeoyltyramine with 54% conversion when using a molar ratio of 1:1. Because ascorbic acid contains a primary alcohol group, the efficiency of the reaction could be compromised. Therefore, the influence of ascorbic acid on the synthesis of *N-trans*-feruloyltyramine was investigated. The product formation was only marginally affected, leading to 58% conversion compared with 63% without ascorbic acid. This means that ascorbic acid can be added to the reactions without strongly affecting the conversion, although ascorbic acid is only strictly necessary when methyl caffeate is used as substrate. In addition to methyl ferulate and methyl caffeate, methyl coumarate was also acceptable as a substrate and showed product formation on TLC.

Other naturally occurring aromatic amines were also studied as substrates. Tryptamine was converted, but octopamine, with an additional hydroxy group compared with tyramine, showed only traces of the product on TLC analysis (Table 1).

Based on these results, a synthesis of *N-trans*-feruloyltyramine was performed at a 200 mg scale. After TLC analysis indicated full conversion of the starting materials, the crude product was isolated by extraction with ethyl acetate, yielding 191 mg (96% yield). After purification by column chromatography, 168 mg (84% yield) of a white, slightly pale-brown solid was obtained, and its structure was confirmed by NMR spectroscopy.[27] The ^{1}H NMR spectrum was in accordance with that reported in the literature (see the Supporting Information).[28] This demonstrated that this method is also suitable for preparative-scale synthesis.

In conclusion, the ability of the promiscuous hydrolase/acyltransferase PestE to catalyze the formation of amide bonds in aqueous solution for the synthesis of hydroxycinnamic acid amides has been demonstrated. This method provides a more sustainable alternative to established chemical synthesis strategies, which often require the use of coupling reagents in stoichiometric amounts. Unlike lipases, environmentally harmful organic solvents can be avoided.

Conflict of Interest

A patent application has been filed. I.T. and K.S. are employees of AnalytiCon Discovery.

Funding Information

This work was financially supported by the European Union's Horizon 2020 research and innovation programme RADICALZ (Grant number: 101000560). H.T. was funded by the Leibniz Association's strategic networking funding program, Leibniz ScienceCampus ComBioCat.

Acknowledgment

The authors would like to thank Ina Menyes for help with the HPLC analyses.

Supporting Information

Supporting information for this article is available online at https://doi.org/10.1055/a-2268-8035.

References and Notes

(1) Facchini, P. J.; Hagel, J.; Zulak, K. G. *Can. J. Bot.* **2002**, *80*, 577.
(2) Leonard, W.; Zhang, P.; Ying, D.; Fang, Z. *Crit. Rev. Food Sci. Nutr.* **2022**, *62*, 1608.

(3) Macoy, D. M.; Kim, W.-Y.; Lee, S. Y.; Kim, M. G. *Plant Biotechnol. Rep.* **2015**, *9*, 269.
(4) De Figueiredo, R. M.; Suppo, J.-S.; Campagne, J.-M. *Chem. Rev.* **2016**, *116*, 12029.
(5) Massolo, E.; Pirola, M.; Benaglia, M. *Eur. J. Org. Chem.* **2020**, 4641.
(6) Dunetz, J. R.; Magano, J.; Weisenburger, G. A. *Org. Process Res. Dev.* **2016**, *20*, 140.
(7) Bryan, M. C.; Dunn, P. J.; Entwistle, D.; Gallou, F.; Koenig, S. G.; Hayler, J. D.; Hickey, M. R.; Hughes, S.; Kopach, M. E.; Moine, G.; Richardson, P.; Roschangar, F.; Steven, A.; Weiberth, F. J. *Green Chem.* **2018**, *20*, 5082.
(8) Petchey, M. R.; Grogan, G. *Adv. Synth. Catal.* **2019**, *361*, 3895.
(9) Lubberink, M.; Finnigan, W.; Flitsch, S. L. *Green Chem.* **2023**, *25*, 2958.
(10) Wu, S.; Snajdrova, R.; Moore, J. C.; Baldenius, K.; Bornscheuer, U. T. *Angew. Chem. Int. Ed.* **2021**, *60*, 88.
(11) Prat, D.; Wells, A.; Hayler, J.; Sneddon, H.; McElroy, C. R.; Abou-Shehada, S.; Dunn, P. J. *Green Chem.* **2016**, *18*, 288.
(12) Zeng, F.; Zhang, H.; Xu, M.; Huang, K.; Zhang, T.; Duan, J. *J. Biotechnol.* **2021**, *334*, 51.
(13) Basri, M.; Alrub, M. A.; Malek, E. A.; Ainliah, S.; Salleh, A. B.; Abdul Rahman, M. B. *Chem. Eng. Commun.* **2014**, *201*, 1582.
(14) Müller, H.; Terholsen, H.; Godehard, S. P.; Badenhorst, C. P. S.; Bornscheuer, U. T. *ACS Catal.* **2021**, *11*, 14906.
(15) Land, H.; Hendil-Forssell, P.; Martinelle, M.; Berglund, P. *Catal. Sci. Technol.* **2016**, *6*, 2897.
(16) Contente, M. L.; Pinto, A.; Molinari, F.; Paradisi, F. *Adv. Synth. Catal.* **2018**, *360*, 4814.
(17) Pinna, C.; Martino, P. A.; Meroni, G.; Sora, V. M.; Tamborini, L.; Dallavalle, S.; Contente, M. L.; Pinto, A. *J. Agric. Food Chem.* **2022**, *70*, 223.
(18) Müller, H.; Godehard, S. P.; Palm, G. J.; Berndt, L.; Badenhorst, C. P. S.; Becker, A.; Lammers, M.; Bornscheuer, U. T. *Angew. Chem. Int. Ed.* **2021**, *60*, 2013.
(19) Hotta, Y.; Ezaki, S.; Atomi, H.; Imanaka, T. *Appl. Environ. Microbiol.* **2002**, *68*, 3925.
(20) Müller, H.; Becker, A.-K.; Palm, G. J.; Berndt, L.; Badenhorst, C. P. S.; Godehard, S. P.; Reisky, L.; Lammers, M.; Bornscheuer, U. T. *Angew. Chem. Int. Ed.* **2020**, *59*, 11607.
(21) Staudt, A.; Terholsen, H.; Kaur, J.; Müller, H.; Godehard, S. P.; Itabaiana, I.; Leal, I. C. R.; Bornscheuer, U. T. *Microorganisms* **2021**, *9*, 1790.
(22) Terholsen, H.; Kaur, J.; Kaloudis, N.; Staudt, A.; Müller, H.; Pavlidis, I. V.; Bornscheuer, U. T. *ChemBioChem* **2022**, *23*, e202200254.
(23) **Initial Screening of Starting Enzyme Candidates**
A 2 M solution of vinyl ferulate in acetonitrile was prepared. Tyramine was dissolved in 200 mM potassium phosphate buffer (pH 8). Due to the addition of tyramine, the pH shifted to pH 10. Final reaction concentrations: tyramine (44.5 mM), potassium phosphate buffer (178 mM), vinyl ferulate (100 mM), enzyme (0.3 mg/mL), 5% (v/v) MeCN. Potassium phosphate buffer served as a control. Incubation was performed for 16 h at 25 °C and 1000 rpm. Samples were analyzed by using TLC, and *N-trans*-feruloyltyramine formation was quantified on a UHPLC system by using a calibration curve.
(24) Tuckerman, M. M.; Mayer, J. R.; Nachod, F. C. *J. Am. Chem. Soc.* **1959**, *81*, 92.
(25) **Enzyme Assays**
Amines were dissolved in 200 mM potassium phosphate buffer, CHES buffer, or CAPS buffer and, by using HCl or NaOH, the pH was adjusted to pH 7, 7.5, and 8 for the phosphate buffer; pH 8.5, 9, 9.5, and 10 for the CHES buffer; and pH 10, 10.5, and 11 for the CAPS buffer. If ascorbic acid was used, this was dissolved in the respective buffer together with the amine, and the pH was adjusted accordingly. Ascorbic acid was used in a fivefold molar excess compared with the combined concentration of the amine and hydroxycinnamic acid ester. The hydroxycinnamic acid esters were dissolved in MeCN at concentrations such that 5% (v/v) of the cosolvent was present in the reactions. In all reactions, final concentrations of 5 mM amine, 178 mM buffer, 5% (v/v) MeCN, and 0.21 mg/mL of PestE variant were used. Samples were incubated at 1000 rpm. The reactions were stopped by the addition of MeCN to a final concentration of 50% (v/v) after full consumption of the hydroxycinnamic acid ester. Samples were analyzed by TLC, and *N-trans*-feruloyltyramine and *N-trans*-caffeoyltyramine formation was quantified by using a UHPLC system with a calibration curve. For temperature profiles, conversions after 2 h of incubation were measured.
(26) Expression of PestE variants was performed according to a protocol previously described.[21]
(27) **Preparative-Scale Synthesis of *N-trans*-Feruloyltyramine with PestE**
Ethyl ferulate (567.3 mg), tyramine (88 mg; molar ratio 4:1), ascorbic acid (2.81 g), CHES (5.29 g), MeCN (6.383 mL) and H_2O (127 mL) were mixed, and the pH was adjusted to pH 10 by using NaOH. The reaction was started by using PestE_I208A_L209F_N288A (26.8 mg) and the mixture was incubated at 70 °C. When the ethyl ferulate was completely consumed (TLC), the product was extracted with EtOAc (×3). The organic phase was washed with 2 M HCl and brine, dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography [silica gel, hexane–EtOAc (2:3)] to give a white, slightly pale-brown solid; yield: 168 mg (84%).
^{1}H NMR (300 MHz, DMSO) δ = 9.40 (s, 1 H), 9.16 (s, 1 H), 7.30 (d, *J* = 15.7 Hz, 1 H), 7.11 (d, *J* = 1.7 Hz, 1 H), 7.04–6.93 (m, 3 H), 6.79 (dd, *J* = 8.1, 1.6 Hz, 1 H), 6.68 (d, *J* = 8.5 Hz, 2 H), 6.43 (d, *J* = 15.7 Hz, 1 H), 3.80 (s, 3 H), 3.33–3.26 (m, 2 H), 2.64 (t, *J* = 7.3 Hz, 2 H). The ^{1}H NMR was in accordance with the reported NMR spectrum (see the Supporting Information).[28]
(28) Takao, K.; Toda, K.; Saito, T.; Sugita, Y. *Chem. Pharm. Bull.* **2017**, *65*, 1020.