# Synthesis and In Vitro Evaluation of the Farnesyltransferase Inhibitor Pepticinnamin E

**Klaus Hinterding, Patrizia Hagenbuch, Janos Rétey,* and Herbert Waldmann*[a]**

**Abstract:** The farnesyltransferase inhibitor pepticinnamin E was synthesized and shown to have the *S* configuration at the central, non-proteinogenic amino acid. Using a recombinant yeast farnesyltransferase the biological activity of the natural product and structural analogues was determined. It was shown that pepticinnamin E is a bisubstrate inhibitor. Furthermore, several structural parameters were identified that decisively influence inhibition of the farnesyl transfer.

**Keywords:** bioorganic chemistry · enzyme inhibitors · pepticinnamin E · signal transduction

## Introduction

Ras proteins are critically involved in the transduction of mitogenic signals given by external growth factors to the cell nucleus.[1] Point mutations in the corresponding *ras* genes are found in approximately 40 % of all human tumors, particularly in over 90 % of human pancreatic carcinomas and 50 % of human colon cancers.[2] These mutations affect the guanosine phosphate binding site and lock the protein in its active GTP-bound form, thereby generating a permanent signal.[2b] Ras proteins are located within the plasma membrane; this is achieved by posttranslational modification of the C-terminal CAAX motif of corresponding precursor proteins (C: cysteine, A: aliphatic amino acid, X: methionine or serine), which includes cysteine *S*-farnesylation, removal of the AAX tripeptide, and transformation of the cysteine to the methyl ester. The farnesylation of the CAAX cysteine, which is catalyzed by the enzyme protein-farnesyltransferase (PFT), is essential to proper functioning of the Ras proteins as transducers of signals in the normal as well as in the transformed state; unlipidated Ras is cytosolic and inactive.[2, 3] Thus, the inhibition of this covalent protein modification has become a promising target in the development of new classes of antitumor agents.[2, 4, 5]

Recent results[5] suggest that inhibitors of farnesyltransferase reduce the growth of transformed cells[6] by preventing not only the lipidation of Ras proteins but also other cellular targets,[7] which hitherto have not been unequivocally identified. Alternative PFT inhibitors are of pivotal interest in the study of these biological phenomena and in the development of new therapeutic agents. Modular inhibitors allow the efficient and rapid variation of their structure and their biological activity and are therefore particularly important.[8] Furthermore, bisubstrate inhibitors, which mimic both the peptide and the farnesyl substrate, are expected to exhibit better affinity and specificity for the enzyme than compounds mimicking either substrate alone.[9]

Pepticinnamin E (**1**) (see Scheme 1) was isolated from *Streptomyces* species and identified as a potent PFT-inhibitor[10] that could fulfil these requirements: it consists of five fragments that can be assembled by peptide coupling. The N-terminal pentenyl acrylic acid may resemble the farnesyl moiety and is connected to an N-methylated peptide part that may mimic the C-terminal CAAX motif of the Ras precursor proteins. Here we describe in full the synthesis of two diastereomers of this unusual peptidic natural product (the absolute configuration of the central, non-proteinogenic amino acid was unknown), as well as the evaluation of their PFT inhibitory activity using a recombinant yeast farnesyltransferase.[11]

## Results and Discussion

A retrosynthetic analysis of pepticinnamin E is shown in Scheme 1. It leads to the pentenyl phenyl acrylic acid **2**,[12] the amino acids **3**, **4**, and **5**, and the diketopiperazine **6**. Central to the synthesis is the sequence of the fragment coupling. Three possible routes were considered: sequential addition of segments to the C-terminal diketopiperazine **6** (route a), synthesis of the central tripeptide **7** followed by C- and N-terminal modification (route b), and the more convergent coupling of two fragments **8** and **9** (route c). The last-mentioned strategy is hampered by the fact that an amide is

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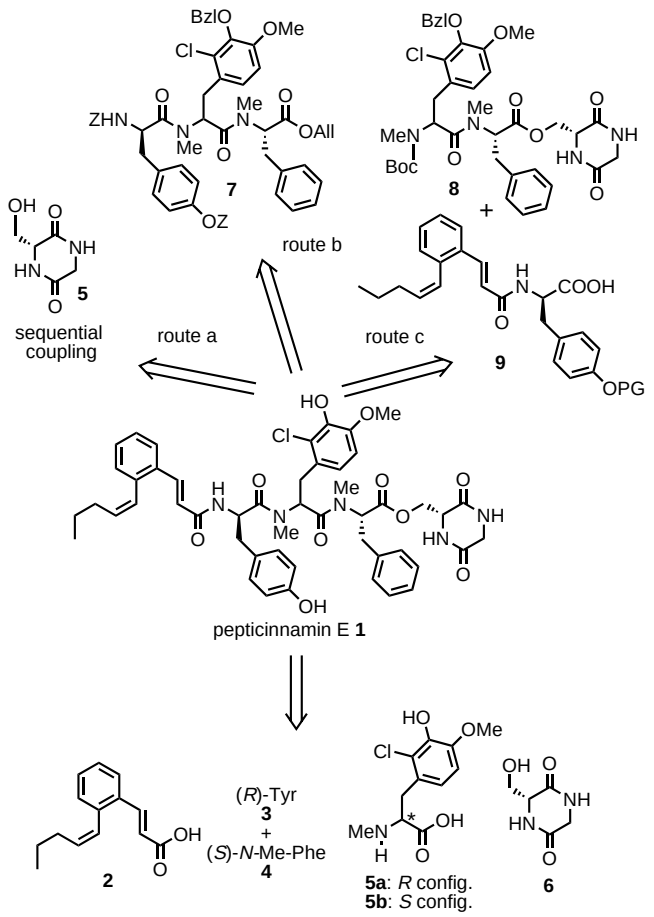
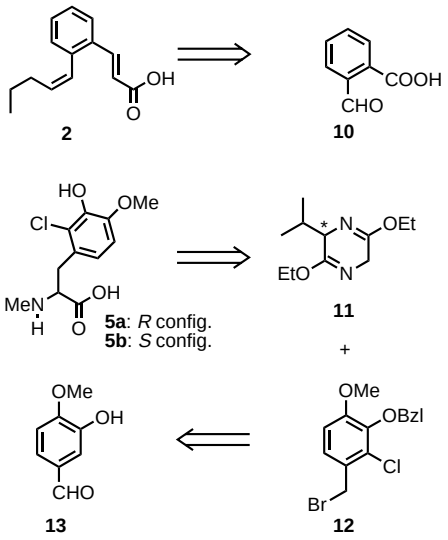



Scheme 1. Structure and retrosynthetic analysis of pepticinnamin E (**1**).

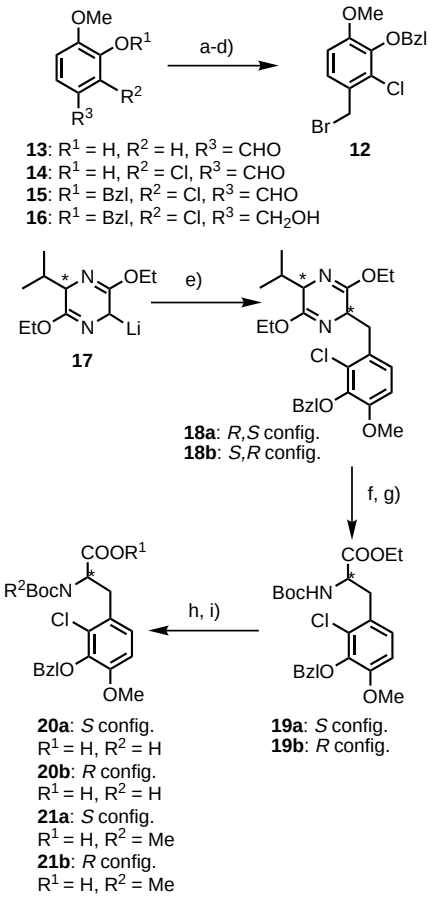
formed in the central step in which racemization of the tyrosine derivative may occur. Scheme 2 shows the retrosynthetic analysis of the fragments **2**, **5a**, and **5b**. The pentenyl side chain of acid **2** was to be synthesized by using a *cis*-selective Wittig reaction, whereas the acrylic acid should be formed by a *trans*-selective Knoevenagel condensation from




Scheme 2. Retrosynthetic analysis of pentenyl phenyl acrylic acid **2** and amino acids **5a** and **5b**.

the disubstituted aromatic compound **10**. In order to determine the absolute configuration of the central stereogenic center in the natural product **1** it was planned to synthesize both enantiomers of a suitably protected amino acid derivative (**5a** and **5b**) by using the Schöllkopf method.[13] The retrosynthetic analysis therefore led to the bis ethyl lactim ether **11** and the appropriately substituted benzylic bromide **12**, to be synthesized from aromatic aldehyde **13**.

Thus chlorination of aldehyde **13** with liquid chlorine in $CH_2Cl_2$[14] gave **14** in 68% yield (Scheme 3). A directing effect of the free hydroxyl function in **13** may be responsible for this highly regioselective aromatic substitution. The phenol was




Scheme 3. Synthesis of benzyl bromide **12** and amino acids **21a** and **21b** in both enantiomeric forms; a) $Cl_2$, $CH_2Cl_2$, 68%; b) BzlBr, $K_2CO_3$, DMF, 85%; c) $NaBH_4$, then $H_2O$, 99%; d) $CBr_4$, $PPh_3$, $Et_2O$, 85%; e) **12**, THF, 83%, 90% de; f) 0.5 N HCl, THF, $H_2O$; g) $Boc_2O$, MeOH, $NEt_3$, 88%; h) $LiOH \cdot H_2O$, THF, $H_2O$, 99% (→ **20a, b**); i) NaH, MeI, THF, 96% (→ **21a, b**).

protected as benzyl ether **15** (93% yield) by standard methodology and reduced to benzyl alcohol **16** (99% yield) by using sodium borohydride. The addition of mineral acid during the workup had to be avoided, otherwise unwanted side products, for example, the benzyl chloride, were formed. The alcohol **16** was transformed to benzyl bromide **12** in 85% yield by using $PPh_3$ and $CBr_4$ in diethyl ether.[15] Addition of this alkylating reagent to either one of the enantiomers of the lithiated bis ethyl lactim ether **17** yielded the enantiomeric



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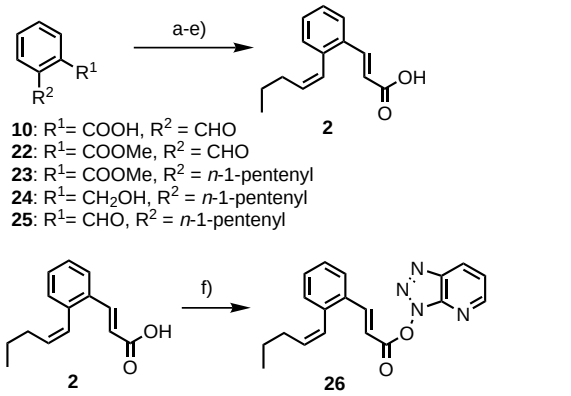
adducts **18a** (*R,S* configuration) and **18b** (*S,R* configuration) in 83% yield with a diastereomeric excess of 90%. The desired stereoisomers were easily separated from unwanted diastereomers by flash chromatography. The removal of the chiral auxiliary (**19a** and **19b**) with one equiv of $LiOH \cdot H_2O$ (99% yield) gave acids **20a** (*S* configuration) and **20b** (*R* configuration). N-Methylated amino acids **21a** (*S* configuration) and **21b** (*R* configuration) were formed by using NaH and MeI in THF in 96% yield.[16] In DMF, however, the corresponding methyl ester was obtained as the main product. By this five-step sequence both amino acids **21a** (*S* configuration) and **21b** (*R* configuration) were obtained in enantiomerically pure form from benzylic bromide **12** with an overall yield of 69%.

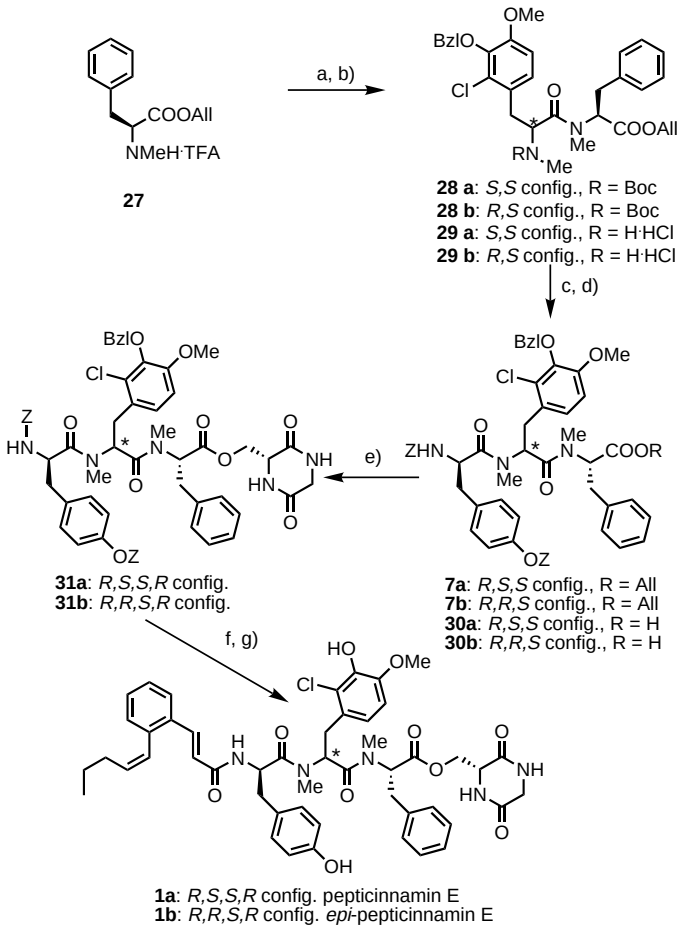
The synthesis of pentenyl phenyl acrylic acid **2** (Scheme 4) started with the esterification of benzoic acid **10 by** using MeI in the presence of $K_2CO_3$ as a base.[17] Reaction of aldehyde **22** with *n*PrCH=$PPh_3$ gave diastereomerically pure *cis*-olefin **23**




Scheme 4. Synthesis of pentenyl phenyl acrylic acid **2** and derivative **26**; a) MeI, $K_2CO_3$, acetone, 76%; b) *n*PrCH=$PPh_3$, THF, $-100\,^{\circ}C$, 77%; c) $LiAlH_4$, $Et_2O$, 97%; d) PCC, $CH_2Cl_2$, 96%; e) $CH_2(COOH)_2$, piperidine, pyridine, 70%; f) HOAt, EDC, $CH_2Cl_2$, 75%.

after distillation in 77% yield (*de* = 84%). To achieve this result the addition had to be performed at $-100\,^{\circ}C$ and the ylide had to be formed by deprotonation with $NaNH_2$ and $((CH_3)_3Si)_2NH$ to avoid the use of lithium salts. Transformation of ester **23** to aldehyde **25** was performed in a two-step sequence involving reduction with $LiAlH_4$ to the benzyl alcohol **24** and selective oxidation with pyridinium chlorochromate (PCC). The synthesis of acid **2** was completed by a *trans*-selective Knoevenagel condensation of aldehyde **25** with malonic acid and decarboxylation in the presence of a catalytic amount of piperidine. By using this five-step sequence diastereomerically pure acrylic acid **2** was synthesized on a multigramm scale with an overall yield of 38%. Furthermore, this acid could be transformed into activated esters such as hydroxyazobenzotriazole (HOAt) ester **26**.

Initial efforts to sequentially add further fragments to the C-terminal diketopiperazine **6**[18] (route a) were unsuccessful. Therefore, in accord with route b, the central tripeptide **7** was synthesized in both diastereomeric forms by using hydroxyazobenzotriazole (HOAt) and *N,N*-dimethylaminopropyl ethyl carbodiimide (EDC) as coupling reagents (Scheme 5).



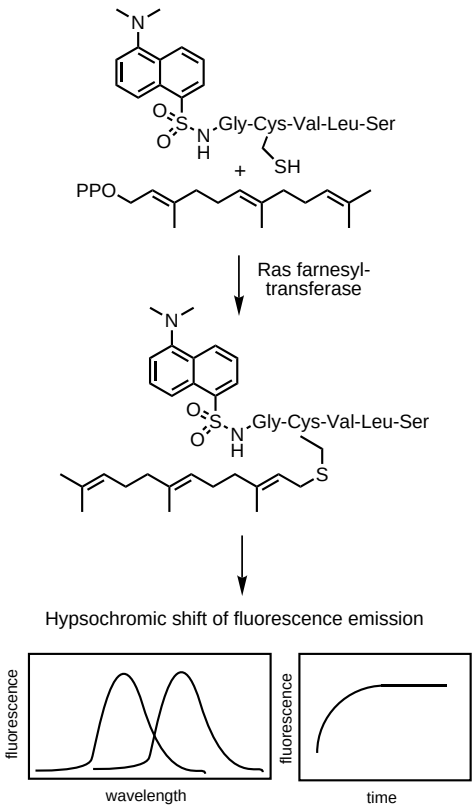

Scheme 5. a) **18a** or **18b**, EDC, HOAt, DMF, 77% (*S,S*), 81% (*R,S*); b) HCl, $Et_2O$, quant.; c) Z-(*R*)-Tyr(Z), EDC, HOAt, DMF, 77% (*R,S,S*), 75% (*R,R,S*); d) $Pd(PPh_3)_4$, morpholine, $CH_2Cl_2$, 89% (*R,S,S*), 88% (*R,R,S*); e) **6**, DEAD, $PPh_3$, DMF, 55% (*R,S,S,R*), 53% (*R,R,S,R*); f) $H_2$, Pd/C, EtOH, EtOAc,HOAc; g) for *epi*-pepticinnamin E: **2**, EDC, HOAt, $NEt_3$, DMF, 23%; for pepticinnamin E: **26**, $NEt_3$, DMF, 33%.

To this end, the suitably protected N-methylated *S*-configured Phe derivative **27** was coupled with both enantiomers **21a** (*S* configuration) and **21b** (*R* configuration) to give the diastereomeric dipeptides **28a** (*S,S* configuration) and **28b** (*R,S* configuration) in a yield of about 80%. The Boc group was cleaved quantitatively with a saturated solution of HCl in dry diethyl ether to yield **29a** (*S,S* configuration) and **29b** (*R,S* configuration). Subsequently, the fully protected diastereomeric tripeptides **7a** (*R,S,S* configuration) and **7b** (*R,R,S* configuration) were built up by coupling of the corresponding secondary amine with *bis*-Z-protected (*R*)-Tyr. To complete the synthesis of the natural product, the C-terminal allyl ester protecting group had to be removed first by $Pd^0$-catalyzed transfer of the allyl group to morpholine as an accepting nucleophile to give **30a** (*R,S,S* configuration) and **30b** (*R,R,S* configuration). Attempts to form the esters **31a** (*R,S,S,R* configuration) and **31b** (*R,R,S,R* configuration) by using DMAP-mediated activation of the carboxylic acids (e.g., the use of DMAP and carbodiimide[19a] or the alcoholysis of mixed anhydrides[19b]) failed or gave yields below 20%. In these transformations DMF had to be used as a solvent for the highly polar diketopiperazine **6**, but DMAP is catalytically

inactive in polar solvents.[19a] However, activation of the alcohol by means of the Mitsunobu reagent[20] yielded an electrophilic species, which was attacked by the nucleophilic carboxylate to give the desired esters **31a** (*R,S,S,R* configuration) and **31b** (*R,R,S,R* configuration) in yields higher than 50%. Finally all three benzyl protecting groups present in peptide **31** could be removed by Pd/C-catalyzed hydrogenolysis in the presence of acetic acid. The compounds formed thereby were immediately transformed into both epimers of the natural product to avoid decomposition. *epi*-Pepticinnamin E was thus synthesized by treatment of the deprotected amine **31b** (*R,R,S,R* configuration) with pentenyl phenyl acrylic acid **2** in the presence of EDC and HOAt in 23% yield. In an improved procedure, the natural product pepticinnamin E was obtained by coupling of the deprotected amine **31a** (*R,S,S,R* configuration) with the preactivated HOAt ester **26** in 33% yield. Pepticinnamin E embodies the *S*-configured enantiomer of the central amino acid. The use of the active ester thus reduced the formation of an unwanted acetamide and simplified the purification of the final product. The identity of compound **1** (*R,S,S,R* configuration) as the natural product was confirmed by comparison of HPLC retention times in four different solvents, specific optical rotation values, and 500 MHz $^1$H NMR spectra with that of an authentic sample.[21]

Inhibition of PFTase by the pepticinnamin E isomers was studied by using an in vitro assay described by Pompliano et al.[22] In this analytical procedure the enzyme-catalyzed reaction between a fluorescence-labeled substrate peptide (Dans-GCVLS) and farnesyl pyrophosphate (FPP) is monitored by taking advantage of the hypsochromic shift of the fluorescence emission of the dansylated substrate peptide upon enzyme catalyzed farnesylation (Scheme 6). A recombinant Ras-farnesyltransferase from *S. cerevisiae*,[23] that was expressed in the pT7-7/*E. coli* BL21 system, was used as target enzyme.[24]

The Michaelis–Menten analysis of the kinetic data yielded $K_M$ (the concentration of substrate at which the reaction rate $v$ is half that of the maximum value) of the enzyme with respect to the FPP substrate ($K_M = 14\,\mu M$) and the Dans-GCVLS substrate ($K_M = 9\,\mu M$).[25] A Lineweaver–Burk plot (see Figure 1 for variation of peptide substrate and Figure 2 for variation of FPP substrate) gave straight lines crossing in one point on the $y$ axis and therefore indicating that the natural product is a competitive inhibitor with respect to both the peptide and FPP substrate. Table 1 shows the values of $K_M$ and $K_I$ ($K_I$: concentration of inhibitor at which the $K_M$ value of the enzyme for the corresponding substrate is doubled). A rationalization for the observation that the natural product is a bisubstrate analogue is depicted in Figure 3: the pentenyl phenyl acrylic acid part of pepticinnamin E may mimic the FPP moiety of the transition state of farnesyl transfer, whereas the CAAX-peptide part is imitated by the N-methylated peptidic fragment of the natural product. To examine which structural features are necessary for inhibitory activity the $IC_{50}$ values (concentration of inhibitor at which 50% activity of the enzyme is observed) of pepticinnamin E (**1a**), *epi*-pepticinnamin E (**1b**), allyl esters **7a** and **7b**, and acids **30a** and **30b** were determined. The data compiled in Table 2




Scheme 6. In vitro farnesylation assay: upon farnesylation the fluorescence emission of the dansylated substrate peptide shifts to shorter wavelengths.

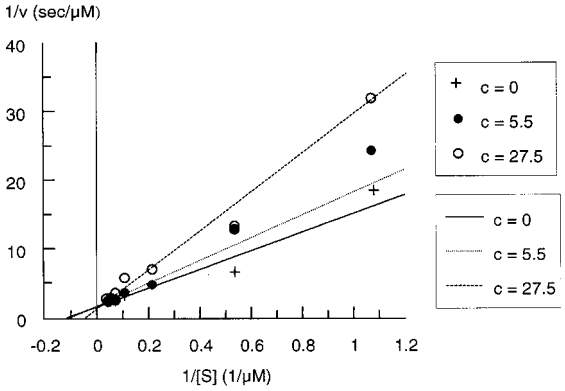



Figure 1. Lineweaver–Burk plot of reciprocal activity versus reciprocal peptide substrate concentration; the different straight lines correspond to variing inhibitor concentrations ($c_{Inh} = 0\,\mu M$, $5.5\,\mu M$, $27.5\,\mu M$).

demonstrate that the absolute configuration of the central amino acid is essential for biological activity. On the one hand the natural product **1a** is six times more active than its unnatural epimer **1b**. On the other hand, only the (*R,S,S*)-configured acid **30a** is an inhibitor, whereas the diastereomeric acid **30b** is not active under the assay conditions. Furthermore, the C- and N-terminal modifications, namely the pentenyl phenyl acrylic acid and the diketopiperazine, are not necessary for inhibitory activity (Table 2, entry 2) and that the exact aromatic substitution pattern of the central amino acid does not play a key role for inhibition. Based on this



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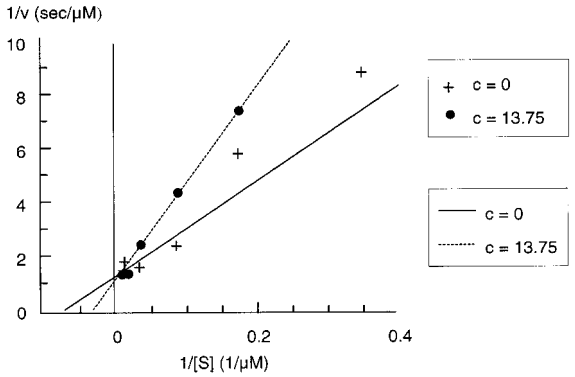



Figure 2. Lineweaver–Burk plot of reciprocal activity versus reciprocal FPP substrate concentration; the different straight lines correspond to different inhibitor concentrations ($c_{Inh} = 0\,\mu M$, $13.75\,\mu M$).

Table 1. $K_M$ values of the recombinant yeast PFTase for the Dans-GCVLS-peptide and the FPP-substrate as well as $K_I$ values of pepticinnamin E with respect to both substrates.

| $K_M$ [μM] for Dansyl-GCVLS | $K_M$ [μM] for FPP | $K_I$ [μM] with regard to Dansyl-GCVLS | $K_I$ [μM] with regard to FPP |
|---|---|---|---|
| 9 | 14 | 30 | 8 |

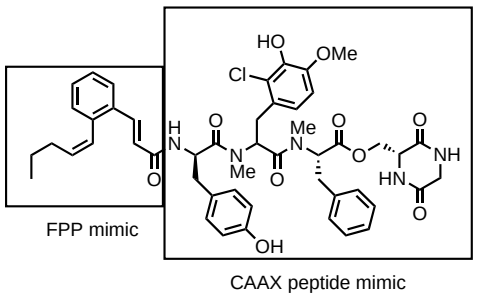
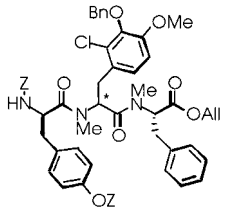
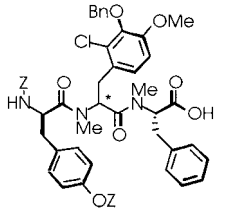



Figure 3. Pepticinnamin E is a bisubstrate inhibitor. As a rationalization the pentenyl phenyl acrylic acid may imitate FPP and the peptide part may mimic the CAAX motif.

Table 2. $IC_{50}$ values of pepticinnamin E (**1a**), the unnatural epimer **1b**, and further analogues.

| Entry | Structure | Compound | $IC_{50}$ value |
|---|---|---|---|
| 1 |  | **7a** (*R,S,S*) | no inhibition |
| | | **7b** (*R,R,S*) | no inhibition |
| 2 |  | **30a** (*R,S,S*) | 67 μM |
| | | **30b** (*R,R,S*) | no inhibition |
| 3 | pepticinnamin E | **1a** | 42 μM |
| 4 | *epi*-pepticinnamin E | *epi*-**1** | 237 μM |

information a more detailed investigation of the structure–activity relationships, for example, by combinatorial synthesis of pepticinnamin E analogues, will be possible.

## Experimental Section

**General**: Melting points were determined in open capillaries using a Büchi 535 apparatus and are uncorrected. $^1H$ and $^{13}C$ NMR spectra were recorded on a Bruker AC 250, AM 400, or DRX 500 spectrometer at room temperature. IR spectra were recorded on a Bruker IFS 88 spectrometer. Mass spectra and high-resolution mass spectra (HRMS) were measured on a Finnigan MAT MS70 spectrometer. Elemental analyses were performed on a Heraeus CHN-Rapid apparatus. An LS50B spectrometer from Perkin-Elmer was used for the fluorimetric observation of the enzymatic reaction. The rections were run in precision cuvettes 115F-OS from Hellma, Germany.

**Materials**: Solvents were dried by standard methods and stored over molecular sieves. For column chromatography silica gel (40–60 μm, Baker) was used. Commercial reagents were used without further purification. FPP (Sigma) was used as a 2.3 mM solution in MeOH. The Dans-GCVLS substrate peptide was obtained from ZMBH, Heidelberg. Where indicated reactions were performed under nitrogen or argon.

Several compounds were prepared according to literature methods: 2-chloro-3-hydroxy-4-methoxy benzaldehyde (**14**);[14] (3*R*)- and (3*S*)-bis lactim ether **11**;[13] 2-methoxycarbonylbenzaldehyde (**22**);[26] (*S*)-*N*-*tert*-butyloxycarbonyl-*N*-methylphenylalanine;[29] *N*-benzyloxycarbonyl-(*O*-benzyloxycarbonyl)-(*R*)-tyrosine:[30] m.p. 88 °C; 117 °C [30]; $[\alpha]_D^{20} = -2.1$ ($c = 0.5$, HOAc); $[\alpha]_D^{20} = +5$ ($c = 10$, HOAc).[30] Various values for specific rotation and melting points are described in the literature; *cyclo*-glycyl-(*R*)-serine **6**;[18] m.p. 225 °C; ref. [18] 227 °C; $[\alpha]_D^{20} = -44.8$ ($c = 0.58$, MeOH).

**3-Benzyloxy-2-chloro-4-methoxy benzaldehyde** (**15**):[28] Benzyl bromide (4.5 mL, 38 mmol) was added to a solution of phenol **14** (5 g, 27 mmol) and $K_2CO_3$ (5.19 g, 52 mmol) in DMF (50 mL). After stirring for 2 d at room temperature water (500 mL) was added. The precipitate was filtered off and recrystallized from ethyl acetate/hexane to yield colorless crystals. Yield: 6.97 g, 25 mmol, 93 %; m.p. 88 °C, ref. [28]: 86–87 °C; $^1H$ NMR ($CDCl_3$, 250 MHz): $\delta = 3.95$ (s, 3H, $OCH_3$), 5.08 (s, 2H, $PhCH_2O$), 6.93 (d, $^3J = 9$ Hz, 1H, arom. CH), 7.39 (m, 3H, arom. CH), 7.53 (m, 2H, arom. CH), 7.75 (d, $^3J = 9$ Hz, 1H, arom. CH), 10.35 (s, 1H, CHO).

**3-Benzyloxy-2-chloro-4-methoxybenzyl alcohol** (**16**):[28] $NaBH_4$ (400 mg, 10.6 mmol) was added to a solution of aldehyde **15** (1 g, 3.6 mmol) in a mixture of THF (40 mL) and MeOH (60 mL). After stirring for 5 h at 40 °C water (100 mL) was added and stirring was continued for another 30 min The reaction mixture was extracted with $CH_2Cl_2$ ($3 \times 100$ mL) and the combined organic layers were dried with $Na_2SO_4$. Evaporation of the solvent in vacuo yielded a slowly crystallizing oil, which was used without further purification. Yield: 1 g, 3.6 mmol, 99 %; $R_f = 0.30$ (ethyl acetate/hexane = 1/1 (v/v)); m.p. 54 °C (ref. [28]: 55–57 °C); $^1H$ NMR ($CDCl_3$, 250 MHz): $\delta = 1.92$ (t, $^3J = 7$ Hz, 1H, OH), 3.89 (s, 3H, OMe), 4.72 (d, $^3J = 7$ Hz, 2H, $CH_2OH$), 5.05 (s, 2H, $PhCH_2O$), 6.85 (d, $^3J = 8$ Hz, 1H, arom. CH), 7.17 (d, $^3J = 8$ Hz, 1H, arom. CH), 7.38 (m, 3H, arom. CH), 7.55 (m, 2H, arom. CH).

**3-Benzyloxy-2-chloro-4-methoxy benzyl bromide** (**12**): $PPh_3$ (1.62 g, 6.2 mmol) was added to a solution of alcohol **16** (1 g, 3.6 mmol) in diethyl ether (30 mL) followed by $CBr_4$ (2.05 g, 6.2 mmol). After 20 h at room temperature the precipitate was filtered off, the solvent was evaporated in vacuo and the residue was purified by chromatography on silica gel (ethyl acetate/hexane = 1/4 (v/v), $R_f = 0.35$) to yield white crystals. Yield: 1.04 g, 3.05 mmol, 85 %; m.p. 76 °C; $^1H$ NMR ($CDCl_3$, 250 MHz): $\delta = 3.88$ (s, 3H, OMe), 4.61 (s, 2H, $CH_2Br$), 5.05 (s, 2H, $CH_2O$), 6.82 (d, $^3J = 8$ Hz, 1H, arom. CH), 7.17 (d, $^3J = 8$ Hz, 1H, arom. CH), 7.37 (m, 3H, arom. CH), 7.54 (m, 2H, arom. CH); $^{13}C$ NMR ($CDCl_3$, 100.5 MHz): $\delta = 31.59$ ($CH_2Br$), 56.15 ($OCH_3$), 74.87 ($OCH_2Ph$), 110.54, 126.25 (arom. CH), 128.19 (quart. C), 128.35, 128.40, 128.45, 128.71 (arom. CH), 129.39, 137.05, 144.79, 154.29 (quart.C); IR (KBr): $\tilde{\nu} = 3421, 3035, 2946, 2887, 2844, 1863, 1592, 1491, 1367, 1278, 1047, 809, 696\ cm^{-1}$; MS (70 eV): *m/z* (%): 340/342/344 (7/10/5) [$M^+$], 261/263 (37/12) [$M^+ - Br$], 170/172 (23/7) [$M^+ - Br - CH_2Ph$], 141 (6), 107 (10), 91 (100), 65 (8); HRMS (70 eV): calcd for $C_{15}H_{14}BrClO_2$: 339.9866,

found: 339.9857; elemental analysis: calcd C 52.83, H 4.14, found C 53.09 H 4.18.

**(3*S*,6*R*)- and (3*R*,6*S*)-3-(3-Benzyloxy-2-chloro-4-methoxy)benzyl-6-isopropyl-2,5-bisethyl lactim ether (18a,b)**: To a solution of the (3*R*)- or (3*S*)-bislactim ether **11**[13] (1 g, 4.71 mmol) in dry THF (15 mL) was added dropwise at −78 °C a solution of *n*BuLi in hexane (2.2 M, 2.26 mL, 4.95 mmol). After stirring for 20 min a cooled solution (−78 °C) of benzyl bromide **12** (1.61 g, 4.71 mmol) in dry THF (15 mL) was added dropwise. The mixture was stirred for 5 h at −78 °C and then allowed to warm to room temperature. Addition of water (10 mL) was followed by extraction with diethyl ether (3 × 30 mL). The combined organic layers were dried with $Na_2SO_4$ and after evaporation of the solvent in vacuo the product was purified by chromatography on silica gel (ethyl acetate/hexane = 1/8 (v/v), $R_f = 0.25$ (main diastereomer), $R_f = 0.19$ (side product)). Overall yield: 1.93 g, 4.08 mmol, 87%; diastereomeric ratio = 95/5; $[\alpha]_D^{20} = -12.06$ ($c = 0.94$, EtOH for the (3*R*,6*S*) enantiomer); $[\alpha]_D^{20} = +12.05$ ($c = 0.94$, EtOH for the (3*S*,6*R*) enantiomer); $^1$H NMR ($CDCl_3$, 250 MHz): $\delta = 0.67$ (d, $^3J = 7$ Hz, 3H, Val-$CH_3$), 1.00 (d, $^3J = 7$ Hz, 3H, Val-$CH_3$), 1.24 (t, $^3J = 7$ Hz, 3H, Et-$CH_3$), 1.30 (t, $^3J = 7$ Hz, 3H, Et-$CH_3$), 2.22 (m, 1H, Val-CH), 2.99 (dd, $^2J = 14$ Hz, $^3J = 6$ Hz, 1H, benz. $CH_2$), 3.38 (dd, $^2J = 14$ Hz, $^3J = 5$ Hz, 1H, benz. $CH_2$), 3.54 (dd, $^3J = 6$ Hz, $^3J = 5$ Hz, 1H, NCH), 3.84 (s, 3H, $OCH_3$), 3.95–4.32 (m, 5H, 2Et-$CH_2$, Val-CH), 5.00 (s, 2H, $PhCH_2O$), 6.75 (d, $^3J = 8$ Hz, 1H, arom. CH), 6.93 (d, $^3J = 8$ Hz, 1H, arom. CH), 7.37 (m, 3H, arom. CH), 7.55 (m, 2H, arom. CH); $^{13}$C NMR ($CDCl_3$, 100.5 MHz): $\delta = 14.38$ (2Val-$CH_3$), 16.55 (Et-$CH_3$), 19.14 (Et-$CH_3$), 31.33 (Val-CH), 37.22 (benz. $CH_2$), 56.06 ($OCH_3$), 56.24 (NCH), 60.27 (NCH), 60.56 (Et-$CH_2$), 60.63 (Et-$CH_2$), 74.68 ($PhCH_2O$), 110.05, 126.34, 127.98, 128.28, 128.40 (arom. CH), 129.18, 129.71, 137.38, 144.12, 152.33 (quart.C), 162.67 (C=N), 163. 43 (C=N); IR (KBr): $\tilde{\nu} = 2972$, 2899, 2870, 2838, 1692, 1597, 1490, 1238, 1046, 698 $cm^{-1}$; MS (70 eV): *m*/*z* (%): 474/472 (12/38) [$M^+$], 437 (12) [$M^+ - Cl$], 429 (8) [$M^+ - CH(CH_3)_2$], 381 (3), 263 (25) [arom. fragment], 261 (80) [arom. fragment], 211/210 (60/37) [*bis*-lactim ether fragment], 170 (12), 169 (93) [arom. fragment], 141 (14) [arom. fragment], 91 (100) [$PhCH_2^+$], 43 (3) [$CH(CH_3)_2$]; HRMS (70 eV): calcd for $C_{26}H_{33}ClN_2O_4$: 472.2129, found.: 472.2111; elemental analysis: calcd C 66.01, H 7.05, N 6.92, found: C 66.28, H 7.08, N 6.70.

**(*R*)- and (*S*)-*N*-*tert*-Butyloxycarbonyl-(3-benzyloxy-2-chloro-4-methoxy)-phenylalanine ethyl ester (19a, 19b)**: A solution of bis lactim ether adduct **18a** (3*S*, 6*R*-config.) or **18b** (3*R*, 6*S*-config.)(1.82 g, 3.82 mmol) in a mixture of THF (15 mL) and 0.5 M HCl (25 mL, 12.5 mmol) was stirred for 16 h at room temperature. After evaporation of the solvent in vacuo the residue was dissolved in MeOH (100 mL). $Boc_2O$ (2.50 g, 11.46 mmol) and $NEt_3$ (1.07 mL, 7.64 mmol) were added and the solution was stirred for another 15 h at room temperature. Addition of diethyl ether (200 mL) was followed by washing with 0.5 M HCl (2 × 20 mL). The organic layer was dried with $Na_2SO_4$ and the solvent was removed in vacuo. Chromatography on silica gel (ethyl acetate/hexane = 1/2 (v/v), $R_f = 0.51$) yielded white crystals. Yield: 1.56 g, 3.36 mmol, 88%; $[\alpha]_D^{20} = -12.4$ ($c = 0.5$, $CH_2Cl_2$ for the *R* enantiomer); $[\alpha]_D^{20} = +12.5$ ($c = 0.6$, $CH_2Cl_2$ for the *S* enantiomer; m.p. 80 °C; $^1$H NMR ($CDCl_3$, 250 MHz): $\delta = 1.20$ (t, $^3J = 7$ Hz, 3H, Et-$CH_3$), 1.39 (s, 9H, Boc-$CH_3$), 3.07 (dd, $^2J = 14$ Hz, $^3J = 6$ Hz, 1H, $\beta$-$CH_2$), 3.21 (dd, $^2J = 14$ Hz, $^3J = 6$ Hz, 1H, $\beta$-$CH_2$), 3.82 (s, 3H, $OCH_3$), 4.14 (q, $^3J = 7$ Hz, 2H, Et-$CH_2$), 4.55 (t, $^3J = 6$ Hz, 1H, $\alpha$-CH), 5.00 (s, 2H, $PhCH_2$), 6.76 (d, $^3J = 8$ Hz, 1H, arom. CH), 6.91 (d, $^3J = 8$ Hz, 1H, arom. CH), 7.36 (m, 3H, arom. CH), 7.51 (m, 2H, arom. CH); $^{13}$C NMR ($CDCl_3$, 100.5 MHz): $\delta = 14.08$ (Et-$CH_3$), 28.28 (Boc-$CH_3$), 35.71 (Et-$CH_2$), 53.74 ($\alpha$-CH), 56.09 ($OCH_3$), 61.38 ($\beta$-$CH_2$), 74.73 ($PhCH_2$), 79.77 (quart. C, Boc), 110.47, 125.91 (arom. CH), 127.31 (quart. C), 128.06, 128.23, 128.42 (arom. CH), 129.47, 137.17, 144.37, 152.92, (quart. C), 155.03 (C=O), 172.03 (C=O); MS (70 eV): *m*/*z* (%): 463 (3) [$M^+$], 407 (5) [$M^+ - C(CH_3)_3$], 390 (3), 311 (2), 263 (5), 261 (18), 256 (26), 221 (15), 171 (23), 91 (100), 57 (28); HRMS (70 eV): calcd for $C_{24}H_{30}NClO_6$: 463.1762, found: 463.1774; elemental analysis: calcd C 62.12, H 6.53, N 3.02 found C 61.96, H 6.56, N 2.86.

**(*R*)- and (*S*)-*N*-*tert*-Butyloxycarbonyl-(3-benzyloxy-2-chloro-4-methoxy)-phenylalanine (20a, 20b)**: $LiOH \cdot H_2O$ (185 mg, 4.4 mmol) was dissolved in water (35 mL) and added to a solution of the enantiomeric ethyl ester **19a** or **19b** (1.96 g, 4.2 mmol) in THF (80 mL). After stirring for 3 h at room temperature the pH was adjusted to 4–5 by addition of 0.5 M HCl. Extraction with $CH_2Cl_2$ (3 × 80 mL) and drying of the combined organic layers with $Na_2SO_4$ was followed by evaporation of the solvent in vacuo. Chromatography on silica gel (ethyl acetate/hexane = 2/1 (v/v), $R_f = 0.32$) yielded a white foam. Yield: 1.86 g, 4.1 mmol, 99%; m.p. 46 °C; $[\alpha]_D^{20} = +15.7$ ($c = 0.94$, EtOH for the *R* enantiomer); $[\alpha]_D^{20} = -15.3$ ($c = 0.7$, EtOH for the *S* enantiomer); $^1$H NMR ($CDCl_3$, 250 MHz): $\delta = 1.40$ (s, 9H, Boc-$CH_3$), 2.80–3.15 (m, 1H, $\beta$-$CH_2$), 3.28–3.48 (m, 1H, $\beta$-$CH_2$), 3.84 (s, 3H, $OCH_3$), 4.59 (m, 1H, $\alpha$-CH), 5.02 (s, 2H, $PhCH_2$), 6.80 (d, $^3J = 8$ Hz, 1H, arom. CH), 6.96 (d, $^3J = 8$ Hz, 1H, arom. CH), 7.36 (m, 3H, arom. CH), 7.54 (m, 2H, arom. CH); MS (70 eV): *m*/*z* (%): 435 (2) [$M^+$], 379 (17) [$M^+ - C(CH_3)_3$], 261 (16), 225 (3), 171 (45), 91 (100), 57 (20); HRMS (70 eV): calcd for $C_{22}H_{26}NClO_6$: 435.1449, found: 435.1437; elemental analysis: calcd C 60.31, H 6.02, N 3.21 found C 60.11, H 6.04, N 2.93.

**(*R*)- and (*S*)-*N*-*tert*-Butyloxycarbonyl-*N*-methyl-(3-benzyloxy-2-chloro-4-methoxy)phenylalanine (21a, 21b)**: NaH (320 mg, 13.2 mmol) was added under nitrogen at 0 °C to a solution of the enantiomeric urethane **20a** or **20b** (1.92 g, 4.4 mmol) and MeI (1.12 mL, 17.6 mmol) in dry THF (100 mL). Within 16 h the mixture was allowed to warm to room temperature. After addition of ethyl acetate (20 mL) and $NH_3$ (25%, 3 mL) it was stirred for another 30 min The pH was adjusted to 3–4 by addition of 2 M HCl and extraction with ethyl acetate (3 × 80 mL) was followed by washing of the combined organic layers with a concentrated solution of $Na_2SO_3$ in water (30 mL). Drying of the organic layer with $Na_2SO_4$, evaporation of the solvent in vacuo, and chromatography (ethyl acetate/hexane = 3/2 (v/v), $R_f = 0.33$) yielded a white solid. Yield: 1.90 g (4.2 mmol), 96%; m.p. 48 °C; $[\alpha]_D^{20} = +82.3$ ($c = 0.35$, $CH_2Cl_2$ for the *R* enantiomer); $[\alpha]_D^{20} = -82.0$ ($c = 0.35$, $CH_2Cl_2$ for the *S* enantiomer); $^1$H NMR ($CDCl_3$, 250 MHz): $\delta = 1.37$, 1.45 (s, 9H, Boc-$CH_3$), 2.70 (s, 3H, N-$CH_3$), 3.05 (m, 1H, $\beta$-$CH_2$), 3.35 (m, 1H, $\beta$-$CH_2$), 3.85 (s, 3H, $OCH_3$), 4.62 (m, 1H, $\alpha$-CH), 5.04 (s, 2H, $PhCH_2$), 6.78 (d, $^3J = 8$ Hz, 1H, arom. CH), 6.86 (d, $^3J = 8$ Hz, 1H, arom. CH), 7.38 (m, 3H, arom. CH), 7.51 (m, 2H, arom. CH); $^{13}$H NMR ($CDCl_3$, 100.5 MHz): $\delta = 28.20$ (Boc-$CH_3$), 28.30 (N-$CH_3$), 33.17 ($\beta$-$CH_2$), 56.08 ($OCH_3$), 59.59 ($\alpha$-CH), 74.71 ($PhCH_2$), 80.76 (Boc-quart. C), 110.59, 126.16, 128.10, 128.30, 128.48 (arom. CH), 137.07, 144.22, 152.75, 152.87 (quart. C), 154.98 (COOH), 176.26 (C=O); MS (70 eV): *m*/*z* (%): 449 (2) [$M^+$], 393 (8) [$M^+ - C(CH_3)_3$], 320 (3) [$M^+ - N(CH_3)COOC(CH_3)_3$], 318 (8), 261 (16), 178 (6), 171 (22), 91 (100), 57 (56); HRMS (70 eV): calcd for $C_{23}H_{28}NClO_6$: 449.1605, found: 449.1587; elemental analysis: calcd C 60.48, H 6.29, N 3.11, found C 60.74, H 6.27, N 2.99.

**Methyl 2-(1-*Z*-pentenyl)benzoate (23)**:[27] $NaNH_2$ (2.1 g, 53.8 mmol) and $((CH_3)_3Si)_2NH$ (850 mg, 5.3 mmol) were added unter nitrogen to a solution of $nBu_3PPh_3Br$ (21.4 g, 53.6 mmol) in dry THF (170 mL). The suspension was stirred at 40 °C for 6 h, filtered under nitrogen, and the residue washed with dry THF (2 × 15 mL). The mixture was cooled to −100 °C and a solution of aldehyde **22** (8 g, 48.7 mmol) in dry THF (40 mL) was added dropwise within 1 h. After stirring for another 30 min at −100 °C the reaction mixture was poured into warm THF (200 mL, 40 °C). Evaporation of the solvent in vacuo was followed by the addition of hexane (100 mL). The white precipitate was filtered off and after evaporation of the solvent in vacuo distillation under reduced pressure yielded the diastereomerically pure product as colorless oil. Yield: 7.35 g, 35.9 mmol, 74%; b.p. 62 °C/ $2.7 \times 10^{-1}$ mbar) (ref. [27]: b.p. 66 °C ($p = 3 \times 10^{-1}$ mbar)); $^1$H NMR ($CDCl_3$, 250 MHz): $\delta = 0.88$ (t, $^3J = 7$ Hz, 3H, $CH_3$), 1.43 (sext, $^3J = 7$ Hz, 2H, $CH_2CH_3$), 2.10 (quart, $^3J = 7$ Hz, 2H, $CHCH_2$), 3.88 (s, 3H, OMe), 5.72 (dt, $^3J = 7$ Hz, $^3J_{cis} = 12$ Hz, 1H, $CHCH_2$), 6.87 (d, $^3J_{cis} = 12$ Hz, 1H, $CHCHCH_2$), 7.30 (m, 2H, arom. CH), 7.45 (m, 1H, arom. CH), 7.94 (m, 1H, arom. CH).

**2-(1-*Z*-Pentenyl)-benzyl alcohol (24)**:[27] $LiAlH_4$ (1.85 g, 50 mmol) was added to a solution of ester **23** (6.62 g, 30.6 mmol) in dry THF (250 mL). After stirring at room temperature for 3 h water (20 mL) was added carefully followed by 1 M HCl (30 mL). The solution was extracted with diethyl ether (3 × 100 mL) and the combined organic layers were dried with $MgSO_4$. Evaporation of the solvent in vacuo yielded a colorless oil which was used without further purification. Yield: 5.24 g (29.7 mmol), 97%; $^1$H NMR ($CDCl_3$, 250 MHz): $\delta = 0.88$ (t, $^3J = 7$ Hz, 3H, $CH_3$), 1.41 (sext, $^3J = 7$ Hz, 2H, $CH_2CH_3$), 1.77 (br, 1H, OH), 2.11 (quart, $^3J = 7$ Hz, 2H, $CHCH_2$), 4.66 (d, $^3J = 6$ Hz, 2H, $CH_2OH$), 5.78 (dt, $^3J = 7$ Hz, $^3J_{cis} = 12$ Hz, 1H, $CHCH_2$), 6.55 (d, $^3J_{cis} = 12$ Hz, 1H, $CHCHCH_2$), 7.18–7.45 (m, 4H, arom. CH).

**2-(1-*Z*-Pentenyl)-benzyl aldehyde (25)**: PCC (2.45 g, 11.4 mmol) was added at room temperature to a solution of alcohol **24** (1 g, 5.7 mmol) in $CH_2Cl_2$ (20 mL). After stirring for 2 h the suspension was filtered over celite and



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the solvent was evaporated in vacuo. Chromatography on silica gel (ethyl acetate/hexane = 1/10 (v/v), $R_f = 0.33$) yielded a colorless oil. Yield: 948 mg, 5.4 mmol, 96 %; $^1$H NMR ($CDCl_3$, 250 MHz): $\delta = 0.87$ (t, $^3J =$ 7 Hz, 3 H, $CH_3$), 1.41 (sext, $^3J = 7$ Hz, 2 H, $CH_3CH_2$), 2.05 (qd, $^3J = 7$ Hz, $^3J = 1$ Hz, 2 H, $CH_3CH_2CH_2$), 5.95 (dt, $^3J_{cis} = 13$ Hz, $^3J = 7$ Hz, 1 H, $CH_2CH$=), 6.82 (dd, $^3J_{cis} = 13$ Hz, $^3J = 1$ Hz, 1 H, $CH_2CH$=C*H*), 7.28 (d, $^3J = 6$ Hz, 1 H, arom.CH), 7.40 (t, $^3J = 6$ Hz, 1 H, arom.CH), 7.55 (q, $^3J =$ 6 Hz, 1 H, arom.CH), 7.90 (d, $^3J = 6$ Hz, 1 H, arom.CH), 10.28 (s, 1 H, CHO); $^{13}$C NMR ($CDCl_3$, 100.5 MHz): $\delta = 13.90$ ($CH_3$), 22.78 ($CH_2$), 30.64 ($CH_2$), 125.51 (CH), 127.38 (CH), 128.67 (CH), 130.62 (CH), 132.32 (quart.C), 133.72 (CH), 136.33 (CH), 141.20 (quart.C), 192.64 (CHO); IR (KBr): $\tilde{\nu} = 2959$, 2931, 2872, 1696, 1597, 1198, 765 cm$^{-1}$; MS (70 eV): *m*/*z* (%): 174 (9) [$M^+$], 145 (17) [$M^+ -$ CHO], 132 (30), 131 (100) [$M^+ -$ $CH_3CH_2CH_2$)], 117 (10) [$M^+ - CH_3CH_2CH_2CH$], 115 (21), 103 (9) [$M^+ -$ $CH_3CH_2CH_2 -$ CHO], 91 (11), 77 (7); HRMS (70 eV) calcd for $C_{12}H_{14}O$ : 174.1045, found: 174.1029.

**3-(2-(1-*Z*-Pentenyl)phenyl)-*E*-acrylic acid (2)**:[12] Malonic acid (3.18 g, 31 mmol) was dissolved in pyridine (15 mL) and the solution was stirred for 10 min at room temperature. Aldehyde **25** (4.31 g, 25 mmol) and piperidine (215 μL) were added and the solution was heated for 4 h at 100 °C. After cooling to room temperature the mixture was poured onto a mixture of ice (10 mL) and concentrated HCl (5 mL) and diluted with water (30 mL). The precipitate was filtered off, dissolved in diethyl ether (100 mL), and washed with 1N HCl (2 × 20 mL). The aqueous filtrate was extracted with diethyl ether (3 × 70 mL) and the combined organic layers were dried with $Na_2SO_4$. The solvent was evaporated in vacuo and the residue crystallized from hexane. Yield: 3.75 g, 17 mmol, 70 %, white crystals; m.p. 72 °C; $^1$H NMR ($CDCl_3$, 250 MHz): $\delta = 0.87$ (t, $^3J = 7$ Hz, 3 H, $CH_3$), 1.41 (sext, $^3J = 7$ Hz, 2 H, $CH_2CH_3$), 2.02 (quart, $^3J = 7$ Hz, 2 H, $CH_2CH$), 5.88 (dt, $^3J = 7$ Hz, $^3J_{cis} = 12$ Hz, 1 H, $CHCH_2$), 6.40 (d, $^3J_{trans} =$ 15 Hz, 1 H, *CH*COOH), 6.56 (d, $^3J_{cis} = 12$ Hz, 1 H, *CH*CHCH$_2$), 7.20–7.41 (m, 3 H, arom. CH), 7.65 (d, $^3J = 6$ Hz, 1 H, arom. CH), 8.02 (d, $^3J_{trans} =$ 15 Hz, 1 H, C*H*CHCOOH).

**3-(2-(1-*Z*-Pentenyl)phenyl)-*E*-acrylic acid hydroxyazo benzotriazolyl ester (26)**: Ethyl dimethylaminopropyl carbodiimide (170 mg, 0.92 mmol) was added to a solution of acid **2** (100 mg, 0.46 mmol) and hydroxyazobenzotriazole (124 mg, 0.92 mmol) in $CH_2Cl_2$ (10 mL). After stirring at room temperature for 3 h the solvent was evaporated in vacuo. Chromatography on silica gel (ethyl acetate/hexane = 1/2 (v/v), $R_f = 0.29$) yielded a colorless oil. Yield: 123 mg, 0.35 mmol, 75 %; $^1$H NMR ($CDCl_3$, 250 MHz): $\delta = 0.89$ (t, $^3J = 8$ Hz, 3 H, $CH_3$), 1.44 (sext., $^3J = 8$ Hz, 2 H, $CH_3CH_2$), 2.04 (dq, $^3J =$ 8 Hz, $^3J = 0.7$ Hz, 2 H, $CH_3CH_2CH_2$), 5.90 (dt, $^3J = 6$ Hz, $^3J_{cis} = 12$ Hz, 1 H, $CH_2CH$=CH), 6.59 (d, $^3J_{cis} = 12$ Hz, 1 H, *CH*=CHCH$_2$), 6.76 (d, $^3J_{trans} =$ 16 Hz, 1 H, COC*H*=CH), 7.28 (d, $^3J = 8$ Hz, 1 H, arom. CH), 7.38–7.46 (m, 3 H, arom. CH), 7.76 (d, $^3J = 8$ Hz, 1 H, arom. CH), 8.32 (d, $^3J_{trans} =$ 16 Hz, 1 H, COCH=C*H*), 8.46 (d, $^3J = 7$ Hz, 1 H, arom. CH), 8.75 (d, $^4J =$ 2 Hz, 1 H, arom. CH); MS (70 eV): *m*/*z* (%): 334 (0.1) [$M^+$], 200 (14), 199 (100) [$M^+ - C_5H_3N_4O$], 171 (7) [$M^+ - C_5H_3N_4O - CH_3CH_2$], 157 (20) [$M^+ - C_5H_3N_4O - CH_3CH_2CH_2$], 128 (22), 115 (16), 55 (18); HRMS (70 eV) calcd for $C_{19}H_{18}N_4O_2$: 334.1430, found: 334.1442.

**(*S*)-*N*-*tert*-Butyloxycarbonyl-*N*-methylphenylalanine allyl ester**: Diisopropylcarbodiimide (0.67 mL, 8.6 mmol) was added to a solution of (*S*)-*N*-*tert*-butyloxycarbonyl-*N*-methylphenylalanine (1.14 g, 4.3 mmol), allyl alcohol (1.2 mL, 17.2 mmol), and DMAP (105 mg, 0.86 mmol) in dry $CH_2Cl_2$ (30 mL). After stirring for 2 h at room temperature the solvent was evaporated in vacuo and the residue was purified by chromatography (ethyl acetate/hexane = 1/5 (v/v), $R_f = 0.30$). Yield: 1.13 g, 3.7 mmol, 85 %, colorless oil; $[\alpha]_D^{20} = -67.7$ ($c = 0.75$, $CH_2Cl_2$); $^1$H NMR ($CDCl_3$, 250 MHz): $\delta =$ 1.30 [1.34] (s, 9 H, Boc-$CH_3$), 2.70 [2.75] (s, 3 H, $NCH_3$), 3.05 (m, 1 H, $\beta$-$CH_2$), 3.30 (m, 1 H, $\beta$-$CH_2$), 4.64 (m, 2 H, allyl. $OCH_2$), 4.94 (m, 1 H, $\alpha$-CH), 5.29 (m, 2 H, allyl. CH=C*H*$_2$), 5.90 (m, 1 H, allyl. C*H*=CH$_2$), 7.10–7.35 (m, 5 H, arom. CH); MS (70 eV): *m*/*z* (%): 319 (7) [$M^+$], 246 (8) [$M^+ - (CH_3)_3CO$], 188 (23) [$M^+ - (CH_3)_3COCONCH_3$], 178 (52), 134 (63), 128 (100), 57 (89); HRMS (70 eV): calcd for $C_{18}H_{25}NO_4$: 319.1784, found: 319.1772.

**(*S*)-*N*-Methylphenylalanine allyl ester hydrotrifluoro acetate (27)**: $CF_3COOH$ (3 mL) was added dropwise to a solution of (*S*)-*N*-*tert*-butyloxycarbonyl-*N*-methylphenylalanine allyl ester (1.02 g, 3.3 mmol) in dry $CH_2Cl_2$ (20 mL). After stirring for 1 h at room temperature the solvent was evaporated and the residue was dried for 3 d in vacuo. The slowly formed crystals were used without further purification. Yield: quantitative. $[\alpha]_D^{20} = +28$ ($c = 0.8$, EtOH); $^1$H NMR ($CD_3OD$, 250 MHz): $\delta = 2.75$ (s, 3 H, $NCH_3$), 3.20 (dd, $^2J = 15$ Hz, $^3J = 7$ Hz, 1 H, $\beta$-$CH_2$), 3.48 (dd, $^2J = 15$ Hz, $^3J = 7$ Hz, 1 H, $\beta$-$CH_2$), 4.34 (t, $^3J = 7$ Hz, 1 H, $\alpha$-CH), 4.65 (d, $^3J = 8$ Hz, 2 H, allyl. $OCH_2$), 5.25 (d, $^3J_{trans} = 10$ Hz, 1 H, allyl. CH=C*H*$_2$), 5.30 (d, $^3J_{cis} =$ 8 Hz, 1 H, allyl. CH=C*H*$_2$), 5.82 (m, 1 H, allyl. C*H*=CH$_2$), 7.25–7.40 (m, 5 H, arom. CH); MS (70 eV): *m*/*z* (%): 220 (0.1) [$M^+$], 199 (0.3), 136 (6), 135 (64) [$M^+ -$ COOAll], 129 (100), 120 (4), 92 (7), 42 (12); HRMS (70 eV): calcd for $C_{13}H_{18}NO_2$: 220.1338, found: 220.1347.

***N*-*tert*-Butyloxycarbonyl-*N*-methyl-(3-benzyloxy-2-chloro-4-methoxy)-(*R*)-phenylalanyl-*N*-methyl-(*S*)-phenylalanine allyl ester (28b)**: To a cooled (0 °C) solution of amine **27** (54 mg, 0.21 mmol), acid **21b** (100 mg, 0.21 mmol), HOAt (50 mg, 0.32 mmol), and $NEt_3$ (28 μL, 0.21 mmol) in dry DMF (2 mL) was added EDC (70 mg, 0.32 mmol). After 15 min the mixture was allowed to warm to room temperature and was stirred for 14 h. After addition of ethyl acetate (50 mL) and washing with 0.5 M HCl (2 × 10 mL) and water (10 mL) the organic layer was dried with $Na_2SO_4$. The solvent was evaporated in vacuo and the residue was purified by chromatography (ethyl acetate/hexane = 1/2 (v/v), $R_f = 0.30$) to yield a colorless oil. Yield: 110 mg, 0.17 mmol, 81 %; $[\alpha]_D^{20} = +113$ ($c = 0.5$, $CH_2Cl_2$); $^1$H NMR ($CDCl_3$, 400 MHz): $\delta = 1.15$ [1.30] (s, 9 H, Boc-$CH_3$), 2.75–3.08 (m, 11 H, 2 $NCH_3$, 3 × $\beta$-$CH_2$), 3.48 (m, 1 H, $\beta$-$CH_2$), 3.81 [3.82] (s, 3 H, $OCH_3$), 4.63 (d, $^3J = 7$ Hz, 2 H, allyl. $OCH_2$), 5.03 (m, 3 H, $PhCH_2$, $\alpha$-CH), 5.24 (d, $^3J_{cis} = 9$ Hz, 1 H, allyl-CH=C*H*$_2$), 5.25 (m, 1 H, $\alpha$-CH), 5.30 (d, $^3J_{trans} = 16$ Hz, 1 H, allyl-CH=C*H*$_2$), 5.88 (m, 1 H, allyl. CH), 6.65–6.85 (m, 2 H, arom. CH), 7.25 (m, 8 H, arom. CH), 7.55 (m, 2 H, arom. CH); $^{13}$C NMR ($CDCl_3$, 100.5 MHz): $\delta = 28.00$ [28.21] (Boc-$CH_3$), 28.99 ($NCH_3$), 31.89 ($NCH_3$), 32.95 [34.63] ($\beta$-$CH_2$), 37.79 ($\beta$-$CH_2$), 54.77 ($\alpha$-CH), 56.15 ($OCH_3$), 58.52 ($\alpha$-CH), 66.00 [65.88] (allyl. $OCH_2$), 74.73 ($PhCH_2$), 80.09 [79.82] (Boc-quart. C), 110.44 [110.29] (arom. CH), 118.96 (allyl. $CH_2$), 125.74, 126.25, 128.08, 128.34, 128.43, 128.53, 128.82, 129.25 (arom. CH), 131.62 (allyl. CH), 135.52, 136.80, 137.31, 144.35, 152.62, 152.84, 154.52 (quart. C), 170.28 (C=O), 170.37 [170.91] (C=O); MS (FAB, NBA) *m*/*z* (%): 651 (1) [$M^+$], 633 (5), 611 (7) [$M^+ - CH_2CH$=$CH_2$], 597 (8) [$M^+ -$ $OCH_2CH$=$CH_2$], 555 (20) [$M^+ - COOC(CH_3)_3$], 511 (60) [$M^+ -$ $OCH_2CH$=$CH_2$-COO-$C(CH_3)_3$], 304 (63), 154 (69), 91 (100); HRMS (FAB): calcd for $C_{36}H_{43}ClN_2O_7$ 651.2837, found: 651.2560; elemental analysis: calcd C 66.39, H 6.67, N 4.30, found C 66.05, H 6.59, N 3.99. The *S,S* diastereomer was prepared following the same protocol in 77 % yield.

***N*-*tert*-Butyloxycarbonyl-*N*-methyl-(3-benzyloxy-2-chloro-4-methoxy)-(*S*)-phenylalanyl-*N*-methyl-(*S*)-phenylalanine allyl ester (28a)**: $[\alpha]_D^{20} = -306$ ($c = 0.1$, $CH_2Cl_2$); $^1$H NMR ($CDCl_3$, 250 MHz): $\delta = 1.13$ [1.25] (s, 9 H, Boc-$CH_3$), 2.31 (s, 3 H, $NCH_3$), 2.50–3.25 (m, 6 H, 3 × $\beta$-$CH_2$), 2.75 (s, 3 H, $NCH_3$), 3.40 (m, 1 H, $\beta$-$CH_2$), 3.81 [3.82] (s, 3 H, $OCH_3$), 4.62 (m, 2 H, allyl-$OCH_2$), 4.75–5.39 (m, 6 H, 2 × $\alpha$-CH, allyl-$CHCH_2$, $PhCH_2O$), 5.87 (m, 1 H, allyl. CH), 6.68 (d, $^3J = 8$ Hz, 1 H, arom. CH), 6.78 (d, $^3J = 8$ Hz, 1 H, arom. CH), 7.05–7.45, 7.53 (m, 10 H, arom. CH).

**(*R*)-*N*-Methyl-(3-benzyloxy-2-chloro-4-methoxy)phenyl-alanyl-*N*-methyl-(*S*)-phenylalanine allyl ester hydrochloride (29b)**: The Boc-protected dipeptide **28b** (280 mg, 0.43 mmol) was dissolved in a saturated solution of HCl in dry diethyl ether (30 mL) and stirred for 90 min at room temperature. The solvent was evaporated under reduced pressure and the residue was used without further purification. Yield: 252 mg, 0.43 mmol, quantitative; m.p. > 105 °C, decomp.; $[\alpha]_D^{20} = -53.7$ ($c = 0.36$, MeOH); $^1$H NMR ($CDCl_3$, 400 MHz): $\delta = 2.58$ (s, 3 H, $NCH_3$), 2.67 (s, 3 H, $NCH_3$), 2.68–2.94 (m, 3 H, $\beta$-$CH_2$), 3.35 (m, 1 H, $\beta$-$CH_2$), 3.88 [3.90] (s, 3 H, $OCH_3$), 4.55 (t, $^3J = 8$ Hz, $\alpha$-CH), 4.66 (d, $^3J = 7$ Hz, 2 H, allyl-$OCH_2$), 5.03 [5.04] (s, 2 H, $PhCH_2$), 5.20–5.98 (m, 3 H, allyl-$CH_2$, $\alpha$-CH), 5.94 (m, 1 H, allyl-CH), 6.78 [7.00] (s, 2 H, arom. CH), 7.30 (m, 8 H, arom. CH), 7.45 (m, 2 H, arom. CH); $^{13}$C NMR ($CDCl_3$, 100.5 MHz): $\delta = 31.30$ ($NCH_3$), 31.86 ($NCH_3$), 34.36 ($\beta$-$CH_2$), 34.66 ($\beta$-$CH_2$), 56.09 ($OCH_3$), 57.55 ($\alpha$-CH), 58.18 ($\alpha$-CH), 65.92 (allyl-$OCH_2$), 74.71 ($PhCH_2$), 111.06 (arom. CH), 118.96 (allyl-$CH_2$), 127.00, 128.10, 128.28, 128.48, 128.74, 129.05, 129.11 (arom. CH), 131.51 (allyl-CH), 128.59, 129.23, 136.33, 137.02, 144.30, 153.58 (quart. C), 167.77 (C=O), 169.04 (C=O); MS (70 eV): *m*/*z* (%): 551 (100) [$M^+$], 517 (5), 304 (45), 289 (8), 220 (15), 214 (8), 134 (18), 91 (46); elemental analysis: calcd C 63.47, H 6.03, N 4.78, found C 63.24, H 6.26, N 4.46; $C_{31}H_{35}Cl_2N_2O_5$. The *S,S* diastereomer was prepared following the same protocol in quantitative yield.

**(*S*)-*N*-Methyl-(3-benzyloxy-2-chloro-4-methoxy)phenyl-alanyl-*N*-methyl-(*S*)-phenylalanine allyl ester hydrochloride (29a)**: $[\alpha]_D^{20} = -9.5$ ($c = 1.05$, MeOH); $^1$H NMR ($CDCl_3$, 250 MHz): $\delta = 2.32$ (s, 3 H, $NCH_3$), 2.65-3.50

(m, 6H, $NCH_3$, 3 × β-$CH_2$), 3.52 (m, 1H, β-$CH_2$), 3.78 [3.81] (s, 3H, $OCH_3$), 4.58 (m, 3H, allyl-$OCH_2$, α-CH), 5.00 [4.92] (s, 2H, $PhCH_2O$), 5.25 (m, 3H, allyl-$CHCH_2$, α-CH), 5.82 (m, 1H, allyl. CH), 6.32 (d, $^3J = 7$ Hz, 1H, arom. CH), 6.45 (d, $^3J = 7$ Hz, 1H, arom. CH), 7.10 – 7.55 (m, 10H, arom. CH).

***N*-Benzyloxycarbonyl-(*O*-benzyloxycarbonyl)-(*R*)-tyrosyl-*N*-methyl-(3-benzyloxy-2-chloro-4-methoxy)-(*R*)-phenylalanyl-*N*-methyl-(*S*)-phenylalanine allyl ester (7b)**: EDC (146 mg, 0.76 mmol) was added under nitrogen at 0 °C to a solution of hydrochloride **29b** (220 mg, 0.38 mmol), *N*-benzyloxycarbonyl-(*O*-benzyloxycarbonyl)-(*R*)-tyrosine (255 mg, 0.56 mmol), HOAt (103 mg, 0.76 mmol), and $NEt_3$ (53 μL, 0.38 mmol) in dry DMF (10 mL). The solution was stirred for 16 h and allowed to warm to room temperature. After addition of ethyl acetate (60 mL) the mixture was washed with 0.5 M HCl (2 × 10 mL), and water (10 mL). The organic layer was dried with $Na_2SO_4$ and after evaporation of the solvent in vacuo the residue was purified by chromatography (ethyl acetate/hexane = 2/3 (v/v), $R_f = 0.35$). Yield: 280 mg, 0.29 mmol, 75%, colorless oil; $[\alpha]_D^{20} = +62.2$ ($c = 0.05$, $CH_2Cl_2$); $^1$H NMR ($CDCl_3$, 400 MHz): $\delta = 2.38$ (s, 3H, $NCH_3$), 2.70 – 3.01 (m, 8H, 5 × β-$CH_2$, $NCH_3$), 3.38 (dd, $^2J = 14$ Hz, $^3J = 7$ Hz, 1H, β-$CH_2$), 3.61 (s, 3H, $OCH_3$), 4.58 (d, $^3J = 8$ Hz, 2H, allyl. $OCH_2$), 4.80 (dd, $^3J = 13$ Hz, $^3J = 6$ Hz, 1H, α-CH), 4.95 (s, 2H, $PhCH_2$), 5.05 (d, $^2J = 3$ Hz, 2H, $PhCH_2$), 5.22 (d, $^3J_{cis} = 8$ Hz, 1H, allyl-CH=C$H_2$), 5.25 (s, 2H, $PhCH_2$), 5.28 (d, $^3J_{trans} = 14$ Hz, 1H, allyl-CH=C$H_2$), 5.37 (dd, $^3J = 7$ Hz, $^3J = 11$ Hz, 1H, α-CH), 5.53 (dd, $^3J = 7$ Hz, $^3J = 9$ Hz, 1H, α-CH), 5.81 (m, 1H, allyl. C*H*=$CH_2$), 6.50 (d, $^3J = 8$ Hz, 1H, arom. CH), 6.65 (d, $^3J = 8$ Hz, 1H, arom. CH), 7.00 (d, $^3J = 8$ Hz, 2H, Tyr-arom. CH), 7.13 (d, $^3J = 8$ Hz, 2H, Tyr-arom. CH), 7.10 – 7.55 (m, 20H, arom. CH); $^{13}$C NMR ($CDCl_3$, 100.5 MHz): $\delta = 30.58$ ($NCH_3$), 31.53 ($NCH_3$), 32.28 (β-$CH_2$), 34.18 (β-$CH_2$), 38.10 (β-$CH_2$), 51.72 (α-CH), 52.40 (α-CH), 55.78 ($OCH_3$), 57.86 (α-CH), 65.91 (allyl-$OCH_2$), 67.07 ($PhCH_2$), 70.29 ($PhCH_2$), 74.55 ($PhCH_2$), 109.90 (arom. CH), 118.78 (allyl-CH=C$H_2$), 121.08, 125.57, 126.70, 127.57, 127.93, 128.21, 128.28, 128.35, 128.44, 128.53, 128.62, 128.69, 128.78 (arom. CH), 129.45 (quart. C), 130.46 (arom. CH), 131.71 (allyl-CH), 134.05, 134.76, 136.12, 136.67, 137.29, 144.23, 149.99 (quart. C), 152.65, 153.56, 155.32, 170.00, 170.32 (C=O); MS (FAB, NBA), *m/z* (%): 898 (1) [$M^+$ – COOAll], 746 (1) [$M^+$ – COOAll – BnOCOO], 599 (7), 447 (5), 403 (16), 256 (23), 120 (11), 91 (100); elemental analysis: calcd for $C_{56}H_{55}ClN_3O_{11}$ : C 68.52, H 5.66, N 4.28, found: C 68.52, H 5.93, N 4.41. The *R,S,S* diastereomer was prepared following the same protocol in a yield of 77%.

***N*-Benzyloxycarbonyl-(*O*-benzyloxycarbonyl)-(*R*)-tyrosyl-*N*-methyl-(3-benzyloxy-2-chloro-4-methoxy)-(*S*)-phenylalanyl-*N*-methyl-(*S*)-phenylalanine allyl ester (7a)**: $[\alpha]_D^{20} = -72.4$ ($c = 0.21$, $CH_2Cl_2$); $^1$H NMR ($CDCl_3$, 250 MHz): $\delta = 2.32$ [2.40](s, 3H, $NCH_3$), 2.63 (s, 3H, $NCH_3$), 2.65 – 2.95 (m, 5H, 5 × β-$CH_2$), 3.32 (dd, $^2J = 14$ Hz, $^3J = 5$ Hz, 1H, β-$CH_2$), 3.80 (s, 3H, $OCH_3$), 4.60 (m, 3H, allyl-$OCH_2$, α-CH), 5.00 [4.95] (s, 2H, $PhCH_2O$), 5.10 [5.05] (d, $^2J = 2$ Hz, 2H, $PhCH_2O$), 5.21 (d, $^3J_{cis} = 8$ Hz, 1H, allyl-CH=C$H_2$), 5.25 (m, 3H, $PhCH_2O$, α-CH), 5.28 (d, $^3J_{trans} = 15$ Hz, 1H, allyl-CH=C$H_2$), 5.81 (m, 1H, allyl-C*H*=$CH_2$), 6.63 (d, $^3J = 8$ Hz, 1H, arom. CH), 6.71 (d, $^3J = 8$ Hz, 1H, arom. CH), 7.00 (d, $^3J = 8$ Hz, 2H, Tyr-arom. CH), 7.13 (d, $^3J = 8$ Hz, 2H, Tyr-arom. CH), 7.10 – 7.55 (m, 20H, arom. CH).

***N*-Benzyloxycarbonyl-(*O*-benzyloxycarbonyl)-(*R*)-tyrosyl-*N*-methyl-(3-benzyloxy-2-chloro-4-methoxy)-(*S*)-phenylalanyl-*N*-methyl-(*S*)-phenylalanine (30a)**: To a solution of allyl ester **7a** (210 mg, 0.21 mmol) in dry $CH_2Cl_2$ (20 mL) were added under nitrogen successively $Pd(PPh_3)_4$ (5 mg) and morpholine (22 μL, 0.25 mmol). After stirring for 4 h at room temperature the solvent was evaporated in vacuo and the residue was purified by chromatography (ethyl acetate/hexane = 2/1 (v/v), $R_f = 0.33$) to yield a light yellow solid. Yield: 176 mg, 0.19 mmol), 89%; $[\alpha]_D^{20} = -88.2$ ($c = 0.10$, $CH_2Cl_2$); $^1$H NMR ($CDCl_3$, 400 MHz): $\delta = 2.35$ (s, 3H, $NCH_3$), 2.65 – 3.05 (m, 8H, 5 × β-$CH_2$, $NCH_3$), 3.35 (dd, $^2J = 14$ Hz, $^3J = 7$ Hz, 1H, β-$CH_2$), 3.66 (s, 3H, $OCH_3$), 4.92 (m, 3H, $PhCH_2$, α-CH), 5.05 (s, 2H, $PhCH_2$), 5.30 (m, 3H, $PhCH_2$, α-CH), 5.48 (m, 1H, α-CH), 6.58 (d, $^3J = 8$ Hz, 1H, arom. CH), 6.70 (d, $^3J = 8$ Hz, 1H, arom. CH), 7.00 – 7.53 (m, 24H, arom. CH); $^{13}$C NMR ($CDCl_3$, 100.5 MHz): $\delta = 30.76$ ($NCH_3$), 31.45 ($NCH_3$), 32.24 (β-$CH_2$), 33.88 (β-$CH_2$), 38.24 (β-$CH_2$), 51.86 (α-CH), 52.52 (α-CH), 55.82 ($OCH_3$), 57.15 (α-CH), 67.12 ($PhCH_2$), 70.23 ($PhCH_2$), 74.61 ($PhCH_2$), 110.04, 121.18, 125.49, 126.72, 127.33, 128.00, 128.22, 128.28, 128.42, 128.45, 128.60, 128.65, 128.73, 128.88 (arom. CH), 129.24 (quart. C), 130.49 (arom. CH), 134.15, 134.50, 134.83, 136.15, 136.69, 137.29, 144.26, 149.87, 152.74 (quart. C), 154.16, 155.56, 169.95, 171.33, 172.94 (C=O); MS (FAB, NBA): *m/z* (%): 942 (6) [$M^+$], 763 (15) [$M^+$ – N(Me) – Phe], 391 (18), 307 (30), 154 (100), 91 (70); HRMS (FAB): calcd for $C_{53}H_{51}ClN_3O_{11}$: 942.3369, found: 942.3090; elemental analysis: calcd C 67.61, H 5.47, N 4.46, found: C 67.90, H 5.67, N 4.25. The *R,S,S* diastereomer was prepared following the same protocol in a yield of 88%.

***N*-Benzyloxycarbonyl-(*O*-benzyloxycarbonyl)-(*R*)-tyrosyl-*N*-methyl-(3-benzyloxy-2-chloro-4-methoxy)-(*R*)-phenylalanyl-*N*-methyl-(*S*)-phenylalanine (30b)**: $[\alpha]_D^{20} = +28.7$ ($c = 0.12$, $CH_2Cl_2$); $^1$H NMR ($CDCl_3$, 250 MHz): $\delta = 2.50$ (s, 3H, $NCH_3$), 2.67 – 3.00 (m, 8H, 5 β-$CH_2$, $NCH_3$), 3.31 (dd, $^2J = 14$ Hz, $^3J = 6$ Hz, 1H, β-$CH_2$), 3.59 (s, 3H, $OCH_3$), 4.88 (m, 1H, α-CH), 4.98 (s, 2H, $PhCH_2O$), 5.09 (s, 2H, $PhCH_2O$), 5.27 (s, 2H, $PhCH_2O$), 5.35 – 5.58 (m, 2H, 2 α-CH), 6.49 (d, $^3J = 8$ Hz, 1H, arom. CH), 6.65 (d, $^3J = 8$ Hz, 1H, arom. CH), 7.00 (d, $^3J = 8$ Hz, 2H, Tyr-arom. CH), 7.21 (d, $^3J = 8$ Hz, 2H, Tyr-arom. CH), 7.08 – 7.55 (m, 20H, arom. CH).

***N*-Benzyloxycarbonyl-(*O*-benzyloxycarbonyl)-(*R*)-tyrosyl-*N*-methyl-(3-benzyloxy-2-chloro-4-methoxy)-(*S*)-phenylalanyl-*N*-methyl-(*S*)-phenylalanine-(*R*)-(piperazine-2,5-dione)-methyl ester (31a)**: DEAD (54 μL, 0.34 mmol) was added under nitrogen to a solution of acid **30a** (160 mg, 0.17 mmol), alcohol **6** (49 mg, 0.34 mmol), and $PPh_3$ (89 mg, 0.34 mmol) in dry DMF (5 mL). After stirring for 24 h at room temperature the solvent was evaporated in vacuo and the residue was purified by chromatography (ethyl acetate/hexane = 2/1 (v/v), $R_f = 0.06$). Yield: 97 mg, 0.09 mmol, 53%, white crystals; $[\alpha]_D^{20} = -85.4$ ($c = 0.5$, $CH_2Cl_2$); $^1$H NMR ($CDCl_3$, 400 MHz): $\delta = 2.46$ [2.52] (s, 3H, $NCH_3$), 2.86 [2.99] (s, 3H, $NCH_3$), 2.86 – 3.00 (m, 4H, β-$CH_2$), 3.07 (dd, $^2J = 14$ Hz, $^3J = 6$ Hz, 1H, β-$CH_2$), 3.23 (dd, $^2J = 14$ Hz, $^3J = 6$ Hz, 1H, β-$CH_2$), 3.76 [3.77] (s, 3H, $OCH_3$), 3.90 (d, $^2J = 9$ Hz, 1H, Gly-$CH_2$), 4.05 (d, $^2J = 9$ Hz, 1H, Gly-$CH_2$), 4.26 (m, 1H, Ser-α-CH), 4.43 (dd, $^2J = 11.4$ Hz, $^3J = 3$ Hz, 1H, Ser-$OCH_2$), 4.50 (m, 1H, α-CH), 4.53 (dd, $^2J = 11.4$ Hz, $^3J = 5$ Hz, 1H, Ser-$OCH_2$), 4.98 (m, 5H, 2 × $PhCH_2$, α-CH), 5.23 [5.24] (s, 2H, $PhCH_2$), 5.69 (dd, $^3J = 9$ Hz, $^3J = 5$ Hz, 1H, α-CH), 6.73 (d, $^3J = 8$ Hz, 1H, arom. CH), 6.80 (d, $^3J = 8$ Hz, 1H, arom. CH), 6.87 (d, $^3J = 8$ Hz, 2H, Tyr-arom-CH), 7.00 – 7.75 (m, 22H, arom. CH); $^{13}$C NMR ($CDCl_3$, 100.5 MHz): $\delta = 30.93$ ($NCH_3$), 33.33 (β-$CH_2$), 34.51 ($NCH_3$), 35.13 (β-$CH_2$), 37.69 (β-$CH_2$), 45.44 (Gly-β-$CH_2$), 53.64 (α-CH), 55.57 (α-CH), 56.65 ($OCH_3$), 62.95 (α-CH), 67.48 (Ser-$OCH_2$), 71.31 (2 $PhCH_2$), 75.81 ($PhCH_2$), 111.86, 122.17, 127.89, 128.11, 128.67, 128.99, 129.05, 129.28, 129.32, 129.46, 129.69, 129.93, 130.02, 130.32, 131.42 (arom. CH), 132.25 (quart. C), 133.12, 133.79 (arom. CH), 136.09, 136.69, 138.24, 138.44, 138.68, 145.44, 151.56, 154.31, 155.11 (quart. C), 158.01, 167.67, 168.52, 171.21, 171.58, 173.45 (C=O); elemental analysis: calcd for $C_{58}H_{57}ClN_5O_{13}$: C 65.24, H 5.39, N 6.56, found C 65.11, H 5.57, N 6.36. The *R,R,S,R* diastereomer was prepared following the same protocol in a yield of 55%.

***N*-Benzyloxycarbonyl-(*O*-benzyloxycarbonyl)-(*R*)-tyrosyl-*N*-methyl-(3-benzyloxy-2-chloro-4-methoxy)-(*R*)-phenylalanyl-*N*-methyl-(*S*)-phenylalanine-(*R*)-(piperazine-2,5-dione)-methyl ester (31b)**: $[\alpha]_D^{20} = +56.3$ ($c = 0.32$, $CH_2Cl_2$); $^1$H NMR ($CDCl_3$, 250 MHz): $\delta = 2.37$[2.30] (s, 3H, $NCH_3$), 2.98 (s, 3H, $NCH_3$), 2.70 – 3.00 (m, 6H, β-$CH_2$), 3.67 (s, 3H, $OCH_3$), 3.87 (d, $^3J = 8$ Hz, 1H, Gly-$CH_2$), 4.02 (d, $^2J = 8$ Hz, 1H, Gly-$CH_2$), 4.21 (m, 1H, Ser-α-CH), 4.37 (dd, $^2J = 11.5$ Hz, $^3J = 3$ Hz, 1H, Ser-$OCH_2$), 4.47 (dd, $^2J = 11.5$ Hz, $^3J = 5$ Hz, 1H, Ser-$OCH_2$), 4.57 (m, 1H, α-CH), 4.92 [4.93] (s, 2H, $PhCH_2$), 5.03 (s, 2H, $PhCH_2$), 5.25 (m, 1H, α-CH), 5.27 (s, 2H, $PhCH_2$), 5.44 (m, 1H, α-CH), 6.61 (d, $^3J = 8$ Hz, 1H, arom. CH), 6.75 (d, $^3J = 8$ Hz, 1H, arom. CH), 7.03 (d, $^3J = 9$ Hz, 2H, Tyr-arom-CH), 7.15 – 7.50 (m, 22H, arom. CH).

**3-(2-(1-*Z*-Pentenyl)phenyl)-*E*-acryloyl-(*R*)-tyrosyl-*N*-methyl-(3-benzyloxy-2-chloro-4-methoxy)-(*R*)-phenylalanyl-*N*-methyl-(*S*)-phenylalanine-(*R*)-(piperazine-2,5-dione)-methyl ester, *epi*-pepticinnamin E (1b)**: Pd/C (10 mg, 10%) was added to a solution of Z-protected peptide ester **31b** in a mixture of ethyl acetate (10 mL), EtOH (1 mL), and HOAc (1 mL). The suspension was stirred for 18 h under an atmosphere of hydrogen. Filtration over celite, washing with EtOH, and evaporation of the solvent in vacuo yielded a yellowish solid (ca. 14 mg), which was dissolved together with acid **2** (4.3 mg, 0.02 mmol), HOAt (4 mg, 0.022 mmol), and $NEt_3$ (2.8 μL, 0.02 mmol) in dry DMF (1.5 mL). EDC (6 mg, 0.03 mmol) was added under nitrogen at 0 °C and the mixture was stirred for 16 h at room temperature. After addition of ethyl acetate (20 mL) and successive washing with 1M HCl (5 mL), concentrated $NaHCO_3$ solution (5 mL), and water (5 mL), the organic layer was dried with $MgSO_4$. Evaporation of the solvent in vacuo was followed by chromatography (ethyl acetate/EtOH = 10/1 (v/v)) to yield a white solid. Yield: 4 mg, 0.004 mmol, 23%; $[\alpha]_D^{20} = +57$ ($c = 0.20$, MeOH); TLC: $R_f = 0.22$ (ethyl acetate/EtOH = 25/1 (v/v)); $R_f = 0.33$



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($CHCl_3$/MeOH = 20/3 (v/v)); $R_f$ = 0.50 (ethyl acetate/EtOH = 10/1 (v/v)); $R_f$ = 0.13 (ethyl acetate); HPLC (Bischoff, Spherisorb ODS II, RP 18, 5 μm; flow rate: 0.6 mL min$^{-1}$): $t_R$ = 15.45 min ($CH_3CN$/water = 50/50); $t_R$ = 10.44 min (MeOH/water = 75/25); separation of synthetic product and authentic natural product was observed after coinjection; the UV spectra are identical; $^1$H NMR ($CD_3OD$, 500 MHz): $\delta$ = 0.86 (t, $^3J$ = 7.5 Hz, 3H, $CH_2CH_3$), 1.41 (sext., $^3J$ = 7.5 Hz, 2H, $CH_2CH_3$), 2.03 ( dq, $^3J$ = 7.5 Hz, $^3J$ = 1.5 Hz, 2H, $CH_2CH_2CH_3$), 2.38 [2.55] (s, 3H, $NCH_3$), 3.04 [3.00] (s, 3H, $NCH_3$), 2.70–3.08 (m, 6H, $\beta$-$CH_2$), 3.60 (s, 3H, $OCH_3$), 3.91 (d, $^2J$ = 17 Hz, 1H, Gly-$CH_2$), 4.05 (d, $^2J$ = 17 Hz, 1H, Gly-$CH_2$), 4.26 (m, 1H, Ser-$\alpha$-CH), 4.43 (dd, $^2J$ = 11.4 Hz, $^3J$ = 3 Hz, 1H, Ser-$OCH_2$), 4.53 (dd, $^2J$ = 11.4 Hz, $^3J$ = 5 Hz, 1H, Ser-$OCH_2$), 5.18 (t, $^3J$ = 7.5 Hz, 1H, $\alpha$-CH), 5.36 (dd, $^3J$ = 5.6 Hz, $^3J$ = 10.5 Hz, 1H, $\alpha$-CH), 5.48 (dd, $^3J$ = 6.1 Hz, $^3J$ = 8.7 Hz, 1H, $\alpha$-CH), 5.87 (dt, $^3J_{cis}$ = 11.4 Hz, $^3J$ = 4 Hz, 1H, $CH_2CH$=CH), 6.45 (d, $^3J$ = 8.5 Hz, 1H, arom. CH), 6.51 (d, $^3J$ = 8.5 Hz, 1H, arom. CH), 6.52 (d, $^3J_{trans}$ = 15.7 Hz, 1H, CH=CHC=O), 6.68 (d, $^3J$ = 8.4 Hz, 2H, Tyr-arom. CH), 7.03 (d, $^3J$ = 8.4 Hz, 2H, Tyr-arom. CH), 7.18-7.36 (m, 8H, arom. CH), 7.71 (d, $^3J$ = 8.2 Hz, 1H, arom. CH), 7.78 (d, $^3J_{trans}$ = 15.7 Hz, 1H, CH=CHCO); MS (FAB, glycerol): *m*/*z* (%): 908 (2) [$M^+$], 737 (1.5), 645 (2), 603 (5), 547 (8), 461 (10), 185 (100); HRMS-FAB: calcd for $C_{49}H_{55}ClN_5O_{10}$: 908.3637, found: 908.3419.

**3-(2-(1-*Z*-Pentenyl)phenyl)-*E*-acryloyl-(*R*)-tyrosyl-*N*-methyl-(3-benzyloxy-2-chloro-4-methoxy)-(*S*)-phenylalanyl-*N*-methyl-(*S*)-phenylalanine-(*R*)-(piperazine-2,5-dione)-methyl ester, pepticinnamin E (1)**: Pd/C (5 mg, 10%) was added to a solution of Z-protected peptide ester **31a** in a mixture of ethyl acetate (1 mL), EtOH (1 mL), and HOAc (0.5 mL). This suspension was stirred for 18 h under an atmosphere of hydrogen. Filtration over celite, washing with EtOH, and evaporation of the solvent in vacuo yielded a yellowish solid (ca. 5 mg), which was dissolved in dry DMF (0.5 mL). At 0 °C a mixture of $NEt_3$ and dry DMF (10 μL $NEt_3$ and 90 μL DMF, 12 μL thereof, 0.008 mmol $NEt_3$) was added followed by a solution of ester **26** in dry DMF (27 mg **26** in 50 μL dry DMF; 5 μL thereof, 2.7 mg, 0.008 mmol). The mixture was allowed to warm to room temperature and after 16 h the solvent was evaporated in vacuo and the residue purified by preparative TLC (Merck, PSC plates, silica gel 60 $F_{254}$, 1 mm). Yield: 2.3 mg, 0.003 mmol, 33%, white solid; TLC: $R_f$ = 0.33 ($CHCl_3$/MeOH = 20/3 (v/v)); $R_f$ = 0.50 (ethyl acetate/EtOH = 10/1 (v/v)); $R_f$ = 0.13 (ethyl acetate); HPLC (Bischoff, Spherisorb ODS II, RP 18, 5 μM; flow rate: 0.6 mL min$^{-1}$): $t_R$ = 18.10 min ($CH_3CN$/water = 50/50); $t_R$ = 11.15 min ($CH_3CN$/water = 55/45); $t_R$ = 5.95 min (MeOH/water = 80/20); $t_R$ = 7.95 min (MeOH/water = 75/25); no separation of synthetic and authentic natural product was observed after coinjection; the UV spectra are identical; $[\alpha]_D^{20} = -156$ ($c$ = 0.05, MeOH), authentic natural product: $[\alpha]_D^{20} = -164$ ($c$ = 0.05, MeOH); $^1$H NMR ($CD_3OD$, 500 MHz): $\delta$ = 0.84 (t, $^3J$ = 7.4 Hz, 3H, $CH_2CH_3$), 1.39 (sext., $^3J$ = 7.4 Hz, 2H, $CH_2CH_3$), 2.01 (dq, $^3J$ = 7.4 Hz, $^3J$ = 1.1 Hz, 2H, $CH_2CH_2CH_3$), 2.29 (s, 3H, $NCH_3$), 2.46 (s, 3H, $NCH_3$), 2.62 (dd, $^2J$ = 13 Hz, $^3J$ = 7 Hz, 1H, $\beta$-$CH_2$), 2.72-3.02 (m, 4H, $\beta$-$CH_2$), 3.09 (dd, $^2J$ = 15 Hz, $^3J$ = 4.8 Hz, 1H, $\beta$-$CH_2$), 3.57 (s, 3H, $OCH_3$), 3.91 (d, $^2J$ = 17.9 Hz, 1H, Gly-$CH_2$), 4.03 (d, $^2J$ = 17.9 Hz, 1H, Gly-$CH_2$), 4.27 (m, 1H, Ser-$\alpha$-CH), 4.40 (dd, $^2J$ = 11.4 Hz, $^3J$ = 3.2 Hz, 1H, Ser-$OCH_2$), 4.55 (dd, $^2J$ = 11.4 Hz, $^3J$ = 4.3 Hz, 1H, Ser-$OCH_2$), 5.01 (t, $^3J$ = 8.2 Hz, 1H, $\alpha$-CH), 5.20 (dd, $^3J$ = 4.5 Hz, $^3J$ = 12.1 Hz, 1H, $\alpha$-CH), 5.56 (dd, $^3J$ = 4.6 Hz, $^3J$ = 9.9 Hz, 1H, $\alpha$-CH), 5.87 (dt, $^3J_{cis}$ = 11.4 Hz, $^3J$ = 7.6 Hz, 1H, $CH_2CH$=CH), 6.44 (d, $^3J_{trans}$ = 15.7 Hz, 1H, CH=CHC=O), 6.50 (s, 1H, arom. CH), 6.60 (d, $^3J_{cis}$ = 11.4 Hz, 1H, $CH_2CH$=CH), 6.61 (d, $^3J$ = 7.1 Hz, 1H, arom. CH), 6.72 (d, $^3J$ = 8.4 Hz, 2H, Tyr-arom. CH), 7.03 (d, $^3J$ = 8.4 Hz, 2H, Tyr-arom. CH), 7.04 (m, 2H, arom. CH), 7.18–7.36 (m, 6H, arom. CH), 7.67 (d, $^3J$ = 7.7 Hz, 1H, arom. CH), 7.77 (d, $^3J_{trans}$ = 15.7 Hz, 1H, CH=CHCO); MS (FAB, glycerol): *m*/*z* (%): 909 (0.3) [$M^+$+H], 908 (0.5) [$M^+$], 737 (0.2), 645 (0.5), 603 (2), 547 (1.5), 461 (2), 369 (5), 277 (17), 185 (100), 93 (95); HRMS-FAB: calcd for $C_{49}H_{55}ClN_5O_{10}$: 908.3637, found: 908.3450.

**Determination of biological activity**: A buffer solution (1 mL) was prepared using aqueous solutions of Tris HCl (pH = 7.5, 1M, 0.5 mL), dithiothreitol (DTT) (1M, 0.05 mL), $MgCl_2$ (1M, 0.05 mL), $ZnCl_2$ (1 mM, 0.1 mL), and water (0.3 mL). The enzymatic reactions were run in a total volume of 0.4 mL. Therefore the buffer solution (0.04 mL) was placed in a cuvette, followed by the calculated amount of water, the enzyme preparation (0.002 mL), and the calculated amount of methanolic inhibitor solution. After preincubation at 25 °C for 10 min the calculated amounts of aqueous Dans-GCVLS solution and methanolic FPP solution were added and the fluorescence emission was observed spectroscopically for 10 min at 30 °C. No preincubation was performed in cases where no inhibitor was added (see below); in these cases the enzyme preparation was added last.

**Determination of $K_M$ for Dans-GCVLS**: The final concentration of peptide in the reaction mixture was varied between 0.9 μM and 23.4 μM, that of FPP was constant at 75.0 μM. The value of $K_M$ was determined as $K_M$ = 9 μM.

**Determination of $K_I$ with respect to Dans-GCVLS**: The final concentration of peptide in the reaction mixture was varied between 0.9 μM and 28.1 μM, that of FPP was constant at 75.0 μM. The concentration of inhibitor was constant at 5.5 μM and 27.5 μM. The $K_I$ value was determined as $K_I$ = 31.9 μM and $K_I$ = 28.6 μM.

**Determination of $K_M$ for FPP**: The final concentration of FPP in the reaction mixture was varied between 2.9 μM and 115.1 μM, that of Dans-GCVLS was constant at 84.3 μM. The $K_M$ value was determined as $K_M$ = 14 μM.

**Determination of $K_I$ with respect to FPP**: The final concentration of FPP in the reaction mixture was varied between 2.9 μM and 115.1 μM, that of Dans-GCVLS was constant at 84.3 μM. The concentration of inhibitor was constant at 13.8 μM. The $K_I$ value was determined as $K_I$ = 7.64 μM.

**Determination of $IC_{50}$ values**: The final concentration of FPP in the reaction mixture was constant at 75 μM, that of the substrate peptide constant at 42.1 μM.

**Determination of $IC_{50}$ value of pepticinnamin E (1a)**: The concentration of inhibitor was varied between 0 and 68.8 μM. The enzymatic activity varied between 100% and 40%. The $IC_{50}$ value was determined by extrapolation as 42 μM.

**Determination of $IC_{50}$-value of *epi*-pepticinnamin E (1b)**: The concentration of inhibitor was varied between 0 and 264 μM. The enzymatic activity varied between 100% and 29%. The $IC_{50}$ value was determined by extrapolation as 237 μM.

**Determination of $IC_{50}$ value of acid 30a**: The concentration of inhibitor was varied between 0 and 89 μM. The enzymatic activity varied between 100% and 31%. The $IC_{50}$ value was determined by extrapolation as 67 μM.

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