# Chemistry of Natural Compounds and Bioorganic Chemistry

## A new approach to the synthesis of cilastatin, an inhibitor of renal dipeptidase

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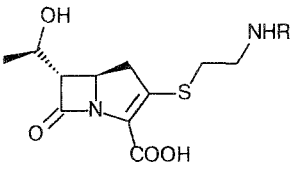
A convenient preparative synthesis of cilastatin, an inhibitor of renal dipeptidase used in drugs with the antibiotic imipenem, has been elaborated. The key intermediate in this synthesis is 2-amino-7-chloroheptanoic acid prepared by oxidative cleavage of cycloheptanone followed by bromination of 7-chloroheptanoyl chloride with subsequent amination of the 2-bromo-7-chloroheptanoic acid thus formed. All of the stages of the new synthesis are easily performed, as is the isolation of the intermediate products, and they do not require any organometallic reagents.

**Key words:** cilastatin, (*R*)-cysteine, 7-chloroheptanoic acid, 2-amino-7-chloro-2-heptenoic acid, 2,2-dimethylcyclopropanecarbonyl chloride; oxidation, bromination, amination, cyclopropanation.

Thienamycin (**1a**) in the form of its *N*-formimidoyl derivative, *viz.*, imipenem (**1b**), is one of the most universal antibiotics.[1] Nevertheless, it is readily metabolized by the enzyme renal dipeptidase and its pharmacologic activity is thus dramatically decreased. In order to prevent the destruction of the antibiotic, a dehydroaminoacid that inhibits the enzyme[2] is added to its pharmaceutic formulation.

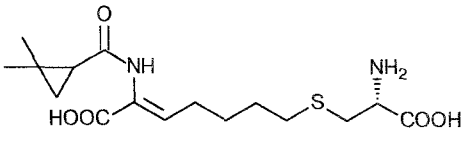
Among the many aminoacids tested for their inhibiting activity,[2] cilastatin (**2**) was found to be practical for use in the form of its mono Na-salt.[2,3]

According to the route developed by Merck,[4] the key intermediate in the synthesis of cilastatin is 7-bromo- or 7-chloro-2-oxoheptanoic acid (**3**), which is condensed




**1a,b**

R = H (**a**), CH=NH (**b**)




**2**

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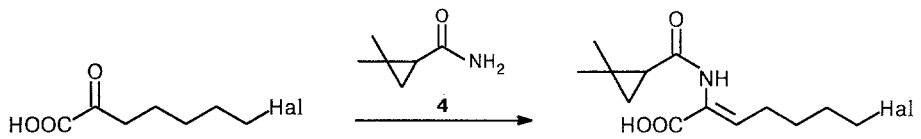
**Scheme 1**




**3** **5**

Hal = Br, Cl

with dimethylcyclopropanecarboxamide according to Eliel's and Hartmann's method[5] (Scheme 1) with the subsequent replacement of the halogen atom with the (*R*)-cysteinic moiety. Racemic dimethylcyclopropanecarboxylic acid is the starting material for the production of compound **2**, which is a part of the pharmaceutic formulation of the antibiotic primaxin[6] based on imipenem (**1b**). By this route, cilastatin (**2**) is obtained as a mixture of two diastereomers.

The synthesis of compound **2** through ketoacid **3** has serious disadvantages. One of them is the instability of aliphatic α-ketocarboxylic acids with more than 5 carbon atoms resulting from their tendency to undergo the decarboxylation.[7] To produce acid **3**, one has to use such "inconvenient to handle" substances as 1,3-propanedithiol and 5-chloro-1-pentylmagnesium bromide.[4] In addition, the condensation of acid **3** with amide **4** (see Scheme 1) affords *N*-acylated dehydroaminoacid **5** in a low yield (30—35 %), which is typical of Eliel's and Hartmann's method.[2,5]

We have developed a new approach to the synthesis of cilastatin. Its characteristic feature is the use of 2-amino-7-chloroheptanoic acid (**8**) as the key intermediate (Scheme 2). Acid **8** is synthesized starting from cycloheptanone. According to our method, cycloheptanone is oxidized into 7-chloroheptanoic acid (**6**) with $H_2O_2/CuCl_2$ (see Ref. 8). The transformation of acid **6** into the corresponding acyl chloride followed by bromination and subsequent replacement of the bromine atom with an amino group leads to aminoacid **8**. Esterification of acid **8** by MeOH and acylation of the resulting ester **9** by dimethylcyclopropanecarbonyl chloride (**10**) affords amide **11**, which is then transformed into *N*-acylated dehydroaminoacid **14** (see Ref. 9). Since our conditions for the replacement of the chlorine atom with the cisteinic fragment in compound **14** were different from those reported earlier,[4] it was necessary to make certain that the chiral center of (*R*)-cysteine did not racemize at the stage of cilastatin formation (see Scheme 2, stage *n*). The control experiment showed that the (*R*)-configuration of cysteine is completely retained under the conditions of the formation of cilastatin (**2**) from acid **14**.

Acyl chloride **10** used for acylation of ester **9** was obtained by cyclopropanation of isobutylene by methyl diazoacetate in the presence of a rhodium catalyst[10] with subsequent saponification and chlorination of the resultant acid with $SOCl_2$.

The developed method is a useful preparative route to cilastatin. A feature of this method is that all of the intermediate compounds are obtained in reasonable yields and are stable, and can be isolated either by distillation or by recrystallization. All of the stages are accomplished using easily available substances without any organometallic reagents.

All of the compounds obtained were identified by their $^1H$ and $^{13}C$ NMR spectra. The configurational purity of compound **13**, *i.e.*, the absence of *E*-isomer, is confirmed by the presence of the clear triplet at δ 6.6 (CH=C) in its $^1H$ NMR spectrum as well as by the presence in its $^{13}C$ NMR spectrum of only two signals in the 120—140 ppm region, which correspond to the quarternary (δ 125.35) and tertial (δ 136.85) carbon atoms of the double bond. The diastereomeric composition of cilastatin has not been analyzed.

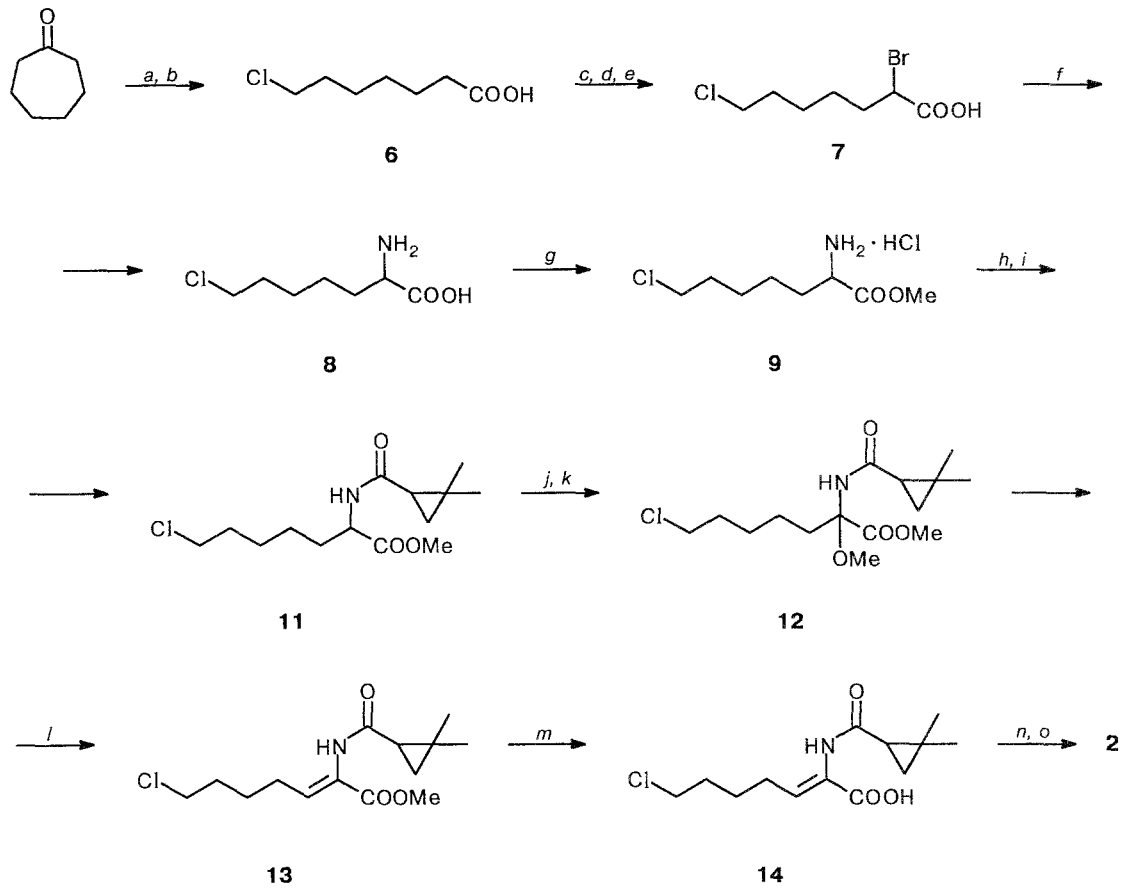
## Experimental

$^1H$ and $^{13}C$ {$^1H$} NMR spectra were recorded on a Bruker AC-200P instrument. Reaction mixtures and reaction products were analyzed by TLC on Silufol UV254 plates using a 4:1:1 *n*-butanol—acetic acid—water mixture as the eluent.

**7-Chloroheptanoic acid (6).** 28 % $H_2O_2$ (400 mL) and MeOH (400 mL) were added to cycloheptanone (112 g, 1 mol), and the mixture was kept for 24 h at room temperature, during which time it formed two layers. The lower oily layer containing cycloheptanone hydroperoxide was then isolated and used for the second stage of the synthesis, *i.e.*, the decomposition of hydroperoxide, without any further purification. $CuCl_2 \cdot 2H_2O$ (20 g), NaCl (120 g), and water (600 mL) were placed in a 4-L three-necked flask equipped with a mechanical stirrer, thermometer, and a dropping funnel. The solution of cycloheptanone hydroperoxide obtained in the first stage of the reaction (the lower layer) was added dropwise over a period of 3 h, while the temperature of the reaction mixture was maintained at 25—30 °C (the reaction is exothermic). Then concentrated HCl (40 mL) was added, and the reaction mixture was stirred for an additional 1 h and then extracted with ether (4×200 mL). The combined organic layers were washed with aqueous $Na_2CO_3$ solution and evaporated. The residue contained 57 g of unreacted cycloheptanone.

The aqueous layer was acidified with HCl to pH ~2, the acid formed was extracted with ether (4×100 mL), the solvent was then removed, and the residue (~40 g) was extracted with petroleum ether (b.p. 40—60 °C; 3×100 mL). Evaporation of the extract afforded 34 g (40 % yield from the starting ketone) of 7-choroheptanoic acid, b.p. 171 °C (12 Torr) (*cf.* Ref. 8: b.p. 171 °C (12 Torr)). $^1H$ NMR ($CCl_4$), δ: 1.7—2.5 (m,

**Scheme 2**




**Reagents and conditions:** *a*. $H_2O_2$, $H_2O$—MeOH, 25 °C. *b*. $CuCl_2$ + NaCl, $H_2O$, 25 °C (40 % yield from the starting ketone). *c*. $SOCl_2$, 30—40 °C. *d*. $Br_2$, 80—90 °C. *e*. $H_2O$ (74 % yield from acid **6**). *f*. $NH_3$, EtOH—$H_2O$, 35 °C (75 %). *g*. MeOH, HCl(gas), 20 °C (90 %). *h*. $Et_3N$, MeOH—ether. *i*. Acyl chloride **10**, $Et_3N$, ether, 0→30 °C (75 %). *j*. Bu$^t$OCl, MeOH, 0 °C. *k*. MeONa, MeOH, 0 °C (88 %). *l*. HCl, ether, 0→20 °C (91 %). *m*. NaOH, MeOH, 25 °C (87 %). *n*. (*R*)-Cystine, Na/$NH_3$, MeOH, −33→25 °C. *o*. Zeocarb-225 ($H^+$-form), Dowex 1-X2 (50 %).

4 $CH_2$); 2.27 (t, $CH_2COO$, $J$ = 6.2 Hz); 3.38 (t, $CH_2Cl$, $J$ = 6.3 Hz); 10.95 (s, COOH).

**2-Bromo-7-chloroheptanoic acid (7).** $SOCl_2$ (35 g, 0.293 mol) was added to 7-chloroheptanoic acid (40 g, 0.243 mol), and the reaction mixture was kept for 3 h at 30—40 °C. The excess $SOCl_2$ was removed *in vacuo*. $Br_2$ (40 g, 0.25 mol) was added dropwise at 80—90 °C to the acyl chloride thus obtained, and the heating was continued for an additional 4 h. The reaction mixture was treated with a saturated aqueous $Na_2CO_3$ solution, and the resultant aqueous solution was washed with ether (3×50 mL). The aqueous layer was acidified with HCl to pH ~2 and then extracted with ether (3×50 mL). Removal of the ether afforded 49 g of crude 2-bromo-7-chloroheptanoic acid containing 7 % of the starting acid (according to $^1H$ and $^{13}C$ NMR spectra). The yield of the pure product from acid **6** was 74 %. The product obtained was used for the subsequent amination without any further purification.

**2-Amino-7-chloroheptanoic acid (8).** Gaseous ammonia was passed through a mixture of 95 % EtOH (238 mL) and 25 % aqueous $NH_3$ (22 mL) until the reaction mixture increased in weight by ~10 g. Then 2-bromo-7-chloroheptanoic acid (5 g, 0.02 mol) of 93 % purity was added at 35 °C, and the stirring was continued at the same temperature in a weak flow of ammonia. The reaction was monitored using TLC and $^{13}C$ NMR. After the reaction was completed (~72 h), the reaction mixture was concentrated *in vacuo* to 50 mL, the precipitate was filtered off, washed subsequently with small amounts of ethanol and hexane, and dried in a desiccator over NaOH to afford 2.73 g (75 %) of the desired product as light-colored crystals, m.p. 207—208 °C (*cf.* Ref. 11: m.p. 208—209 °C (decomp.)). $^1H$ NMR ($CF_3COOD$), δ: 1.7—2.6 (m, 4 $CH_2$); 3.6 (t, $CH_2Cl$); 4.4 (t, CHN). $^{13}C$ NMR ($CF_3COOD$), δ: 28.45, 30.35, 34.45, 36.1 (4 $CH_2$); 48.95 ($CH_2Cl$); 58.85 (CHN). Found (%): C, 46.96; H, 7.76; Cl, 19.74; N, 7.43. $C_7H_{14}ClNO_2$. Calculated (%): C, 47.1; H, 7.85; Cl, 19.8; N, 7.89.

**Methyl 2-amino-7-chloroheptanoate hydrochloride (9).** A flow of dry HCl was passed through a stirred suspension of 2-amino-7-chloroheptanoic acid (**8**) (2 g, 0.01 mol) in absolute MeOH (40 mL). When the acid dissolved, the reaction mixture was ice-cooled and dry HCl was passed through it

with cooling for an additional 4 h. Twenty-four hours later, the reaction mixture was concentrated to afford 2.56 g (90 %) of hydrochloride **9** as a gradually crystallizing oil. $^1$H NMR ($CF_3COOD$), δ: 1.7—2.6 (m, 4 $CH_2$); 3.8 (t, $CH_2Cl$); 4.2 (s, $CH_3O$); 4.65 (t, CHN). $^{13}$C NMR ($CF_3COOD$), δ: 28.45, 30.35, 34.45, 36.1 (4 $CH_2$); 48.95 ($CH_2Cl$); 58.85 (CHN). Found (%): C, 40.92; H, 7.2; Cl, 30.4; N, 6.02. $C_8H_{17}Cl_2NO_2$. Calculated (%): C, 41.8; H, 7.43; Cl, 30.8; N, 6.08.

**2,2-Dimethylcyclopropanecarbonyl chloride (10).** $CH_2Cl_2$ (500 mL) distilled over $P_2O_5$ was placed into a 2-L two-necked flask equipped with a reflux condenser and cooled to −20 to −10 °C by an acetone-dry ice mixture. Isobutylene (3 mol) was condensed into the flask with cooling. After that, $Rh_2(OAc)_4$ (0.88 g, 2 mmol) was added with energetic stirring, and then a solution of methyl diazoacetate (100 g, 1 mol) in $CH_2Cl_2$ (250 mL) was added to the boiling mixture over a period of 10 h by means of a controlled-volume pump. Five hours after the beginning of the addition of the diazo ester, more isobutylene (1 mol) was added to the flask, and the reaction mixture was stirred for an additional 0.5 h. The solvent was removed under atmospheric pressure using a 40 cm column; the residue was filtered to remove the catalyst, and the filtrate was diluted with pentane (200 mL) and filtered again. The pentane was evaporated and the residue was distilled to afford 90 g (70 %) of methyl 2,2-dimethylcyclopropanecarboxylate, b.p. 130—133 °C (*cf.* Ref. 12: b.p. 69—71 °C (64 Torr)). $^1$H NMR ($CDCl_3$), δ: 1.08 and 1.13 (both s, 2 $CH_3$); 0.77 (dd); 1.00 (m); 1.41 (dd, 3 CH); 3.57 (s, $OCH_3$). $^{13}$C NMR ($CDCl_3$), δ: 18.6 ($CH_2$); 21.9 ($CH_3$); 2.28 (C); 26.6 (CH); 26.7 ($CH_3$); 51.2 ($OCH_3$).

Alkaline hydrolysis of the methyl ester followed by the interaction of the resultant 2,2-dimethylcyclopropanecarboxylic acid with excess $SOCl_2$ (Ref. 13) afforded acyl chloride **10**, b.p. 84—85 °C (15 Torr) (*cf.* Ref. 12: b.p. 85 °C (15 Torr)). $^1$H NMR ($CDCl_3$), δ: 1.25 and 1.26 (both s, 2 $CH_3$); 1.15, 1.32, and 2.12 (all dd, 3 CH). $^{13}$C NMR ($CDCl_3$), δ: 18.7 ($CH_2$); 26.5 ($CH_3$); 26.8 ($CH_3$); 29.5 (C); 38.2 (CH).

**Methyl 7-chloro-2-(2,2-dimethylcyclopropylcarbonyl)aminoheptanoate (11).** A solution of triethylamine (3.84 g, 0.038 mol) in absolute ether (50 mL) was added to a solution of methyl 2-amino-7-chloroheptanoate hydrochloride (**9**) (5.85 g, 0.025 mol) in absolute methanol (40 mL). The reaction mixture was stirred for 20 min, then it was filtered and the solvents were removed. The residue was dissolved in ether (50 mL). The solution was ice-cooled to 0—5 °C, and a solution of triethylamine (3.84 g, 0.038 mol) in ether (50 mL) was added quickly with stirring. Afterwards, a solution of dimethylcyclopropanecarbonyl chloride (**10**) (3.63 g, 0.028 mol) in ether (50 mL) was added dropwise over a period of 40 min with cooling. The reaction mixture was stirred for an additional 1 h at 0 °C, then for 24 h at room temperature, and finally for 2 h at 30—35 °C. After the reaction was completed, the precipitate was filtered off and washed with ether. The ether solution was subsequently washed with 0.1 *N* HCl (2×50 mL), with water to pH ~7, with 0.1 *N* $NaHCO_3$, and again with water. The ether solution was dried over $MgSO_4$ and concentrated to afford 5.7 g (75 %) of chromatographically pure (TLC) ester **11** as a clear yellow oil. $^1$H NMR ($CDCl_3$), δ: 0.7 and 1.05 (both dd, CH(a)H(b), cyclopropane fragment); 1.1 (s, 2 $CH_3$); 1.2—1.5 (m, CHCON + 2 $CH_2$); 1.5—1.9 (m, 2 $CH_2$); 3.45 (t, $CH_2Cl$); 3.7 (s, $OCH_3$); 4.6 (m, CHN); 6.2 (br.s, NH). $^{13}$C NMR ($CDCl_3$), δ: 18.55 ($CH_2$, cyclopropane fragment); 20.05 and 20.2 (*cis*-$CH_3$, diastereomers); 21.65 and 21.8 (C(Me)$_2$); 24.45 and 24.5 ($CH_2$); 26.3 ($CH_2$); 26.9 (*trans*-$CH_3$); 28.65 and 28.75 (CHCONH); 32.2 ($CH_2$); 32.3 and 32.5 ($CH_2$); 44.7 ($CH_2Cl$); 51.9 ($OCH_3$); 52.0 and 52.15 (CHN); 171.0 and 171.2 (NHC=O); 173.2 (OC=O). Found (%): C, 55.8; H, 8.12; Cl, 11.1; N, 4.2. $C_{15}H_{26}ClNO_4$. Calculated (%): C, 56.3; H, 8.2; Cl, 11.1; N, 4.4.

**Methyl 7-chloro-2-(2,2-dimethylcyclopropylcarbonyl)amino-2-methoxyheptanoate (12).** *tert*-Butyl hypochlorite (2.68 g, 2.95 mL, 24.7 mmol) was added dropwise to a stirred solution of ester **11** (5.35 g, 18.5 mmol) in absolute methanol (20 mL) cooled to 0 °C. A 0.75 *M* solution of Na methylate in absolute methanol (33 mL, 25.1 mmol) was added dropwise with stirring for ~20 min at 0 °C to the solution obtained. The mixture was stirred for an additional 40 min (TLC monitoring, hexane—ethyl acetate, 4 : 1). The solvent was removed, the residue was treated with water and extracted with ether, and the extract was dried over $MgSO_4$. After the evaporation of the ether, the oily product was treated with petroleum ether, and the thick white precipitate was filtered off and dried to afford 5.15 g (88 %) of ester **12**, m.p. 103—103.5 °C. $^1$H NMR ($CDCl_3$), δ: 0.76 (dd, 1 H, $CH_2$, cyclopropane fragment); 1.12 (s, 2 $CH_3$); 1.05—1.52 (m, 2 H, cyclopropane fragment + 2 $CH_2$); 1.67—2.03 (m, $CH_2$ + CH(a)CNH); 2.32—2.6 (m, CH(b)CNH); 3.23 and 3.26 (both s, $CH_3OCN$, diastereomers); 3.51 (t, $CH_2Cl$, $J$ = 7 Hz); 3.81 and 3.82 (both s, $CH_3OOC$); 6.43 and 6.88 (both s, NH). $^{13}$C NMR ($CDCl_3$), δ: 18.55 and 18.65 ($CH_2$, cyclopropane fragment, diastereomers); 20.3 (*cis*-$CH_3$); 22.2 and 22.5 (CMe$_2$); 22.8 and 22.9 ($CH_2$); 26.45 ($CH_2$); 27.03 (*trans*-$CH_3$); 29.15 (CHCONH); 32.2 ($CH_2$); 35.25 and 35.75 ($CH_2CNH$); 44.75 ($CH_2Cl$); 51.4 ($CH_3OC$); 51.5 (MeOC); 52.95 ($CH_3OO$); 187.8 and 187.9 (2 C=O). Found (%): C, 55.8; H, 8.0; Cl, 11.1; N, 4.2. $C_{15}H_{26}ClNO_4$. Calculated (%): C, 56.3; H, 8.2; Cl, 11.1; N, 4.4.

**Methyl (*Z*)-7-chloro-2-(2,2-dimethylcyclopropylcarbonyl)amino-2-heptenoate (13).** Absolute ether (40 mL) saturated with dry HCl was added dropwise to a stirred solution of ester **12** (3.75 g, 11.7 mmol) in absolute ether (40 mL) at 0 °C. The reaction mixture was then stirred for 2 h at room temperature (TLC monitoring, hexane—ethyl acetate, 4 : 1). After reaction ended, the ether was evaporated *in vacuo*, the residue was dissolved in ether, and the precipitate was filtered off. The filtrate was subsequently washed with a saturated aqueous solution of $NaHCO_3$ and water and dried over $MgSO_4$. Removal of the solvent afforded 3.06 g (91 %) of chromatographically pure (TLC) ester **13** as a clear yellow oil. $^1$H NMR ($CDCl_3$), δ: 0.7 (dd, 1 H, $CH_2$, cyclopropane fragment); 1.2 (s, 2 $CH_3$); 1.4—1.9 (m, 3 $CH_2$); 2.2 (q, $CH_2$—C=C); 3.53 (t, $CH_2Cl$); 3.77 (s, $CH_3OOC$); 6.62 (t, HC=); 7.27 (br.s, NH). $^{13}$C NMR ($CDCl_3$), δ: 18.5 ($CH_2$, cyclopropane fragment); 20.5 (*cis*-$CH_3$); 22.0 (CMe$_2$); 25.3 ($CH_2$); 26.9 (*trans*-$CH_3$); 28.05 ($CH_2$); 29.0 (CHCONH); 32.0 ($CH_2$); 44.5 ($CH_2Cl$); 52.2 ($OCH_3$); 125.35 (HNC=); 136.85 (HC=); 165.15 (HNC=O); 169.75 (OC=O). Found (%): C, 58.15; H, 7.58; Cl, 12.1; N, 4.82. $C_{14}H_{22}ClNO_3$. Calculated (%): C, 58.5; H, 7.66; Cl, 12.34; N, 4.88.

**(*Z*)-7-Chloro-2-(2,2-dimethylcyclopropylcarbonyl)amino-2-heptenoic acid (14).** 1 *N* NaOH (12.5 mL, 12.5 mmol) was added to a solution of ester **13** (2.78 g, 9.77 mmol) in methanol (15 mL) , and the reaction mixture was stirred for 2 h at room temperature (TLC monitoring, toluene—acetic acid, 4 : 1). After the end of the hydrolysis, the reaction mixture was made slightly alkaline with 0.1 *N* HCl (~2.5 mL), and the main portion of the methanol was then evaporated *in vacuo*. The residue was treated with cold water (30 mL) and extracted with ether (2×25 mL). The aqueous layer was acidified with

2.5 *N* HCl to weakly acidic reaction and extracted with ether (2×30 mL). The combined ether layers were dried over $MgSO_4$. The solvent was removed, and the residue was washed with petroleum ether and dried to afford 2.27 g (87 %) of acid **14**, m.p. 110—112 °C. $^1$H NMR ($CDCl_3$), δ: 0.78 (dd, 1 H, $CH_2$, cyclopropane fragment); 1.2 (s, 2 $CH_3$); 1.0—2.0 (m, 2 CH, cyclopropane fragment + 2 $CH_2$); 2.23 (q, $CH_2C$=C); 3.53 (t, $CH_2Cl$); 6.78 (t, HC=C); 7.32 (s, NH). $^{13}$C NMR ($CDCl_3$), δ: 18.65 ($CH_2$, cyclopropane fragment); 20.7 (*cis*-$CH_3$); 22.8 ($\underline{C}Me_2$); 25.25 ($CH_2$); 26.95 (*trans*-$CH_3$); 28.35 ($CH_2$); 29.1 ($\underline{C}HCONH$); 32.1 ($CH_2$); 44.55 ($CH_2Cl$); 125.0 (HNC=); 139.6 (HC=); 168.75 (HNC=O); 170.55 (OC=O). Found (%): C, 57.16; H, 6.92; Cl, 12.8; N, 5.02. $C_{13}H_{20}ClNO_3$. Calculated (%): C, 57.31; H, 7.06; Cl, 12.95; N, 5.13.

**7-[(*R*)-2-Amino-2-carboxyethylthio-2-(2,2-dimethylcyclopropylcarbonyl)amino]-(*Z*)-2-heptenoic acid (cilastatin) (2).** Na metal (0.2 g, 8.7 mmol) weighed in absolute toluene was added in small portions to a stirred suspension of (*R*)-cystine (0.44 g, 1.83 mmol) in liquid ammonia (18 mL) under a nitrogen atmosphere. When the blue coloring of the solution had weakened, a solution of acid **14** (1 g, 3.66 mmol) in MeOH (6 mL), was added quickly, followed by more MeOH (9 mL) and KI (30 mg). After the temperature of the reaction mixture reached ~20 °C, the stirring was continued for ~10 h (TLC monitoring). The reaction mixture was evaporated *in vacuo* and the residue was dissolved in water (20 mL). The solution was passed through a cationite Zeocarb 225 ($H^+$) column (2.7×10 cm) and the column was washed with water (~300 mL) until the negative probe for the presence of an acid was obtained. Amine-containing components were eluted with 1 % aqueous ammonia (~300 mL) until the reaction to ninhydrin became negative. The eluate was removed *in vacuo* and the residue was dissolved in water (15 mL). The solution was passed through an anionite Dowex 1-X2 ($AcO^-$) column (2.7×10 cm), the column was washed with water (300 mL), and the product eluted with 2.5 *N* AcOH (~400 mL) up to the negative reaction to ninhydrin. Evaporation of the eluate gave 0.66 g (50 %) of a chromatographically pure (according to TLC analysis) amorphous solid. $^1$H NMR ($CDCl_3$), δ: 0.78 and 1.05 (both dd, CH(a)H(b), cyclopropane fragment); 1.18 and 1.20 (both s, 2 $CH_3$); 1.25—1.75 (m, 2 $CH_2$); 2.18 (dt, $CH_2$—C=C); 2.62 (m, $CH_2S$); 2.8—3.2 (m, NC—$CH_2S$); 3.85 (dd, CHN); 6.67 (t, HC=). $^{13}$C NMR ($CD_3OD$), δ: 19.3 ($CH_2$, cyclopropane fragment); 20.85 (*cis*-$CH_3$); 23.15 ($\underline{C}Me_2$); 27.3 (*trans*-$CH_3$); 28.2 ($CH_2$); 28.7 ($\underline{C}HCONH$); 29.55 and 30.1 (2 $CH_2$); 32.55 and 33.5 (2 $CH_2S$); 171.0 and 173.6 (3 C=O). Found (%): C, 53.4; H, 7.18; N, 7.62; S, 8.7. $C_{16}H_{26}N_2O_5S$. Calculated (%): C, 53.7; H, 7.26; N, 7.8; S, 8.94.

**Study of the stability of (*R*)-cysteine under the conditions of the synthesis of cilastatin (2) from acid 14.** Na metal (0.467 g, 20 mmol) was added portionwise in an argon flow to a stirred solution of (*R*)-cystine (1 g, 4.17 mmol; $[\alpha]_D^{20}$ −225° (*c* 1, 1 *N* HCl)) in liquid ammonia (50 mL). 20 min later, absolute MeOH (30 mL) was added. The solution was stirred for 42 h at 25 °C, then it was concentrated and water (25 mL) and 2.5 *N* HCl (up to pH ~7) were added. To transform cysteine into cystine,[14] a 5 % aqueous solution of $FeCl_3$ (3 drops) was added and the reaction mixture was aerated until the coloration probe with Na nitroprusside became negative (~2.5 h). The precipitate was filtered off, washed sequentially with ethanol and ether and dried *in vacuo* to give 0.85 g (85 %) of pure cystine (NMR data), $[\alpha]_D^{20}$ −223° (*c* 1, 1 *N* HCl).

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