Enantioselective Total Synthesis of the Antifungal Natural Products Chlorotetaine, Bacilysin, and Anticapsin and of Related Compounds: Revision of the Relative Configuration

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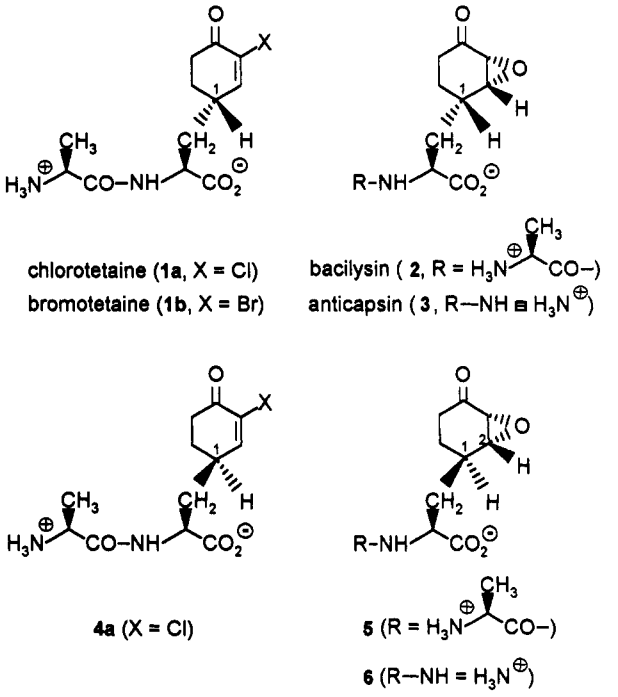
Enantioselective and diastereoselective syntheses of the title antifungal natural products and some of their diastereoisomers are described. Key steps include the diastereoselective 1,6-addition of bislactim ether **14** and a stereoselective deprotonation of ketone **17** using lithium (*S,S*)-bis(1-phenylethyl)amide as a chiral base. All natural products possess the (*S*)-configuration at C-1 of the substituted cyclohex(en)yl residues of the *C*-terminal amino acids, which contradicts the assignments in the literature. At physiological pH most of the dipeptides are instable and react by an intramolecular 1,4-addition with the formation of 6-oxoperhydroindoles.

Chlorotetaine **1a** and bromotetaine **1b** are natural products recently isolated from *Bacillus subtilis* strain BGSC 1E2.[1] These non-proteinogenic amino acid derivatives are closely related to the longer known dipeptide bacilysin (tetaine)[2] **2** and its *C*-terminal amino acid anticapsin[3] **3**. All compounds have a broad antifungal and at higher concentrations an additional antibacterial activity. For bacilysin and anticapsin it has been shown that they are irreversible inhibitors of glucosamine-6-phosphate synthetase and hence interfere with cell wall biosynthesis.[4] Originally, the latter two compounds had been assigned the structures **5** and **6**.[2,3] The absolute configuration of the epoxide was determined by CD and ORD measurements (positive Cotton effect) and the *trans*-relationship of the epoxide and the C-1 substituent was derived from the coupling constants of the adjacent protons (H-1 and H-2) in the ^{1}H NMR spectrum. By comparison with published data, coupling constants of less than 1 Hz should be typical for a *trans*-relationship, whereas *cis*-protons should have coupling constants of 2–4 Hz.[5] Three syntheses of anticapsin have already been published, however, none of them controls the stereochemistry at C-1 and all include separation of 1:1 diastereomeric mixtures.[6] Chlorotetaine had been assigned structure **4a**, also with an (*R*)-configuration at C-1 of the cyclohexenyl residue as determined by analysis of the CD spectrum (negative Cotton effect).[1]

We have developed an enantio- and diastereoselective synthesis of chlorotetaine in order to obtain larger quantities of the compound for complete evaluation of the biological properties and demonstrated that its structure had to be revised to **1a**.[7] Subsequently we could prove that bacilysin and anticapsin possess the structures **2** and **3**, all natural products having the (*S*)-configuration at C-1 of the cyclohex(en)yl residue in contradiction to the original assignments.[8] In the meantime, Baldwin and co-workers independently developed an enantiospecific synthesis of anticapsin and also revised the C-1 configuration to that in structure **3**.[9] In this article the full details of the synthesis of the four natural products together with most of their epimers **4–6** and of the related dipeptides **1c** and **4c** (X = H) are reported. The interesting differences in stability found in this class of compounds are discussed and reasons for the original misassignment of these structures are presented.



• Abstract published in *Advance ACS Abstracts,* April 15, 1994.

(1) (a) Chlorotetaine: Rapp, C.; Jung, G.; Katzer, W.; Loeffler, W. *Angew. Chem.* **1988**, *100*, 1801. *Angew. Chem. Int. Ed. Engl.* **1988**, *27*, 1733. (b) Bromotetaine: Jung, G. Personal communication.

(2) Walker, J. E.; Abraham, E. P. *Biochem. J.* **1970**, *118*, 557 and 563.

(3) Neuss, N.; Molloy, B. B.; Shah, R.; DeLaHiguera, N. *Biochem. J.* **1970**, *118*, 571.

(4) (a) Chmara, H.; Zähner, H.; Borowski, E.; Milewski, S. *J. Antibiot.* **1984**, *37*, 652. (b) Kenig, M.; Vandamme, E.; Abraham, E. P. *J. Gen. Microbiol.* **1976**, *94*, 46. (c) Buchanan, J. M. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1973**, *39*, 91. For further antifungal agents acting by this mechanism see: (d) Andruszkiewicz, R.; Chmara, H.; Milewski, S.; Zieniawa, T.; Borowski, E. *J. Med. Chem.* **1990**, *33*, 2755. (e) Rane, D. F.; Girijavallabhan, V. M.; Ganguly, A. K.; Pike, R. E.; Saksena, A. K.; McPhail, A. T. *Tetrahedron Lett.* **1993**, *34*, 3201.

(5) Tori, K.; Komeno, T.; Nakagawa, T. *J. Org. Chem.* **1964**, *29*, 1136.

(6) (a) Rickards, R. W.; Rodwell, J. L.; Schmalzl, K. J. *J. Chem. Soc., Chem. Commun.* **1977**, 849. (b) Laguzza, B. C.; Ganem, B. *Tetrahedron Lett.* **1981**, *22*, 1483. (c) Souchet, M.; Baillarge, M.; LeGoffic, F. *Tetrahedron Lett.* **1988**, *29*, 191.

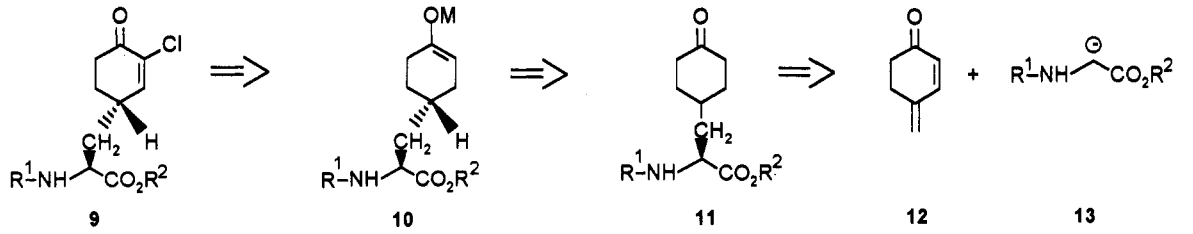
(7) Wild, H.; Born, L. *Angew. Chem.* **1991**, *103*, 1729. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1685.

(8) Wild, H. In *Antibiotics and Antiviral Compounds*; Krohn, K., Kirst, H. A., Maag, H., Eds.; VCH Publishers: Weinheim, New York, 1993; p 215. Proceedings of the 3rd International Symposium on the Chemical Synthesis of Antibiotics and Related Microbial Products; Kloster Banz: Germany; Sept 20–25, 1992.

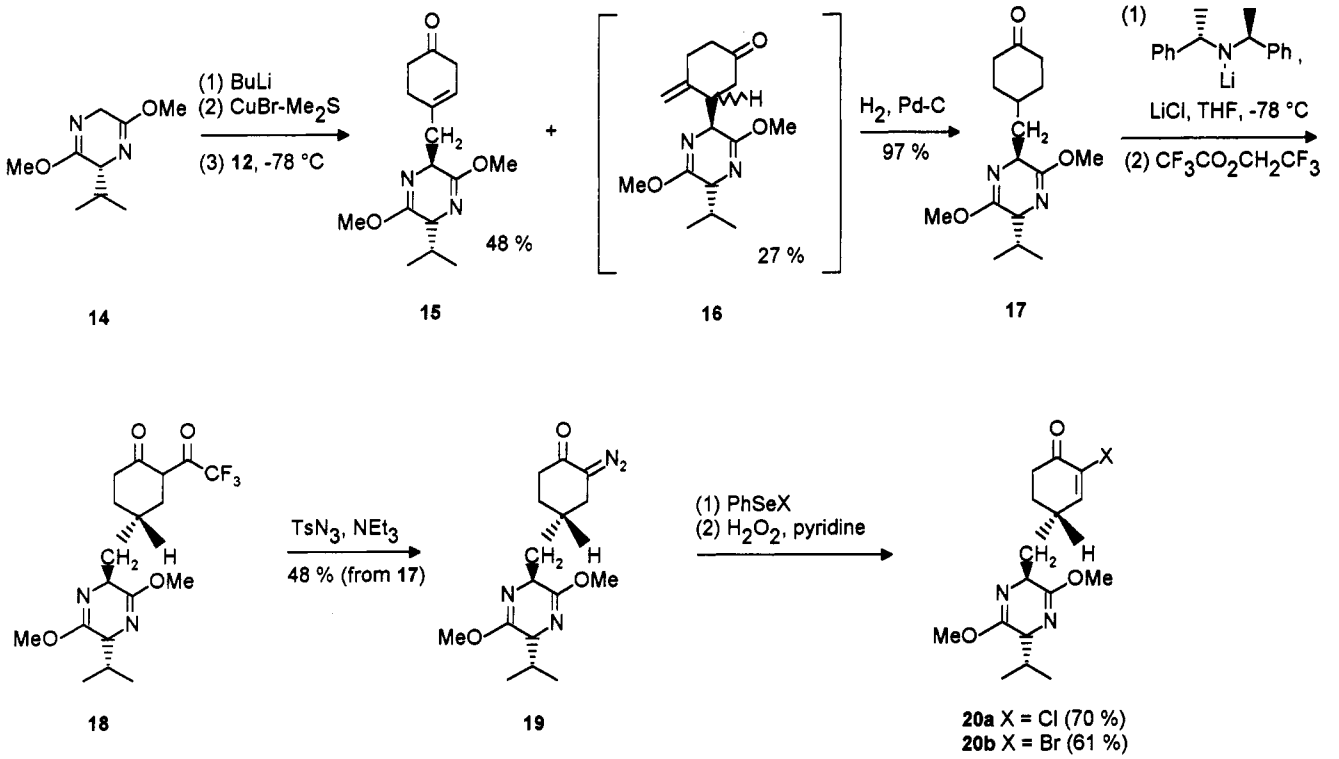
(9) Baldwin, J. E.; Adlington, R. M.; Mitchell, M. B. *J. Chem. Soc., Chem. Commun.* **1993**, 1332.

0022-3263/94/1959-2748$04.50/0

Scheme 1

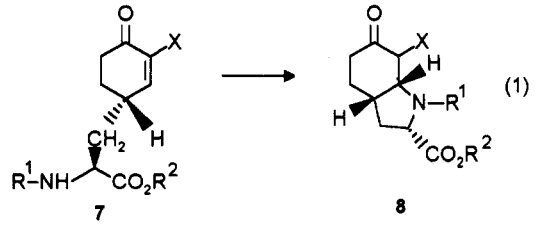


Scheme 2



Synthetic Strategy

In planning the synthesis of chlorotetaine, it should be noted that dipeptides such as **7** are stable in solution only at a pH between 3–5. Above pH 7 the biological activity decreases rapidly, especially on heating.[1] The main reaction in alkaline media is the intramolecular 1,4-addition of the amide to the enone system, with formation of a 6-oxoperhydroindole **8** (eq 1). Since the risk of this side reaction is present during all intermediate steps of the synthesis, the enone should not be formed until the latest possible moment. Retrosynthetically, the chloro



(1)

enone of chlorotetaine can be derived from an enolate **10**, which itself is the product of a known diastereoselective deprotonation[10] of a 4-substituted cyclohexanone **11** (Scheme 1). The α-amino acid is built up by an enantioselective 1,6-addition of a chiral amino acid synthon **13** to 4-methylene-2-cyclohexenone (**12**).

Results and Discussion

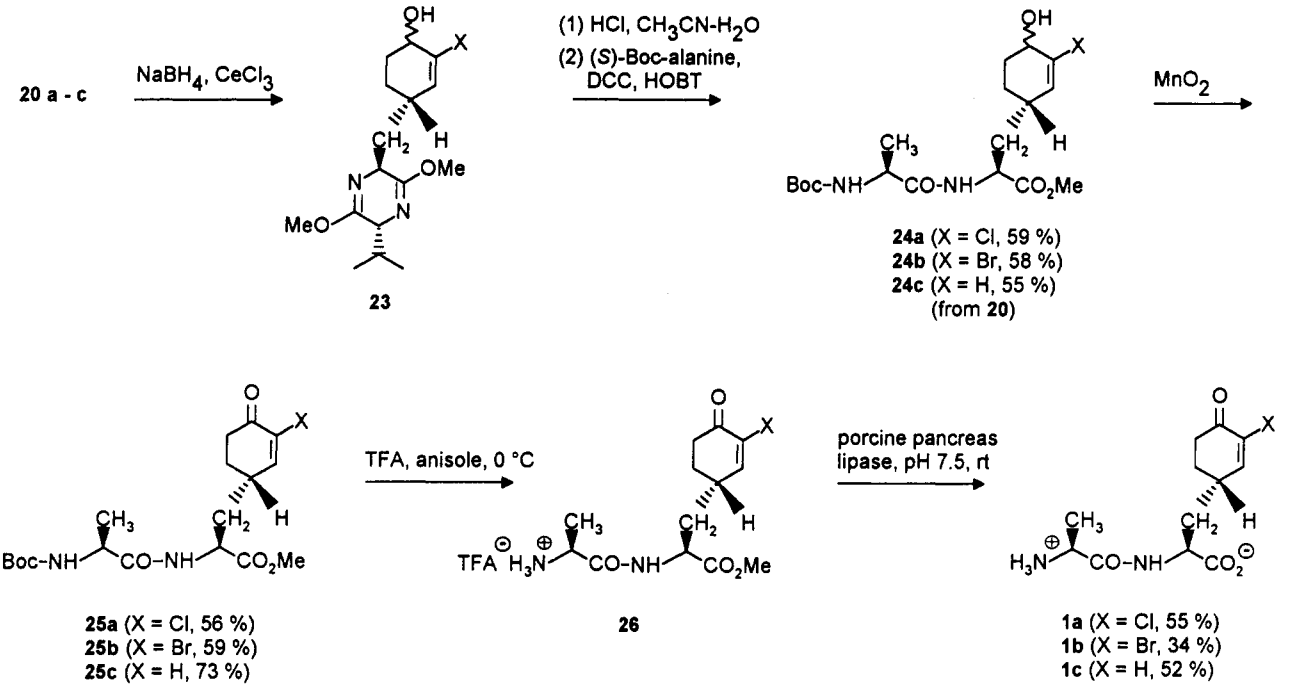
A. Preparation of Chlorotetaine, Bromotetaine, and Related Compounds. Bislactim ether **14** was chosen as an α-amino acid synthon.[11] 1,6-Addition of the cuprate of **14** to dienone **12** (readily available in two steps from *p*-methoxybenzyl alcohol)[12] was highly stereoselective to give the 3-enone **15** after kinetic protonation in moderate yield as a single diastereomer (Scheme 2). In addition, the undesired 1,4-adduct **16** was isolated as a 9:1 mixture of two diastereomers in 27% yield. The use of other lower order or higher order cuprates improved neither the yield nor the selectivity. After hydrogenation of the double bond of **15** the cyclohexanone **17** was obtained in 47% yield starting from **14**. This route was the most direct way to get gram quantities of **17**. However, for the production of **17** in 50-g batches it was more convenient to use a less elegant but higher yielding procedure, especially because the labile dienone **12** was difficult to handle in larger amounts (eq 2). Thus iodide **21**[13] reacted quantitatively with the lithium azaenolate of **14** (de = 70–80%) and after deprotection, oxidation of the alcohol,

(10) Cox, P. J.; Simpkins, N. S. *Tetrahedron: Asymmetry* **1991**, *2*, 1.

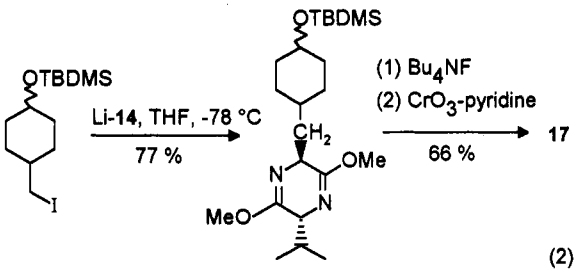
(11) (a) Schöllkopf, U.; Pettig, D.; Schulze, E.; Klinge, M.; Egert, E.; Benecke, B.; Noltemeyer, M. *Angew. Chem.* **1988**, *100*, 1238. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 1194. (b) E. Merck product number 818315.

(12) Birch, A. J. *J. Proc. R. Soc. N.S.W.* **1949**, *83*, 245.

Scheme 3

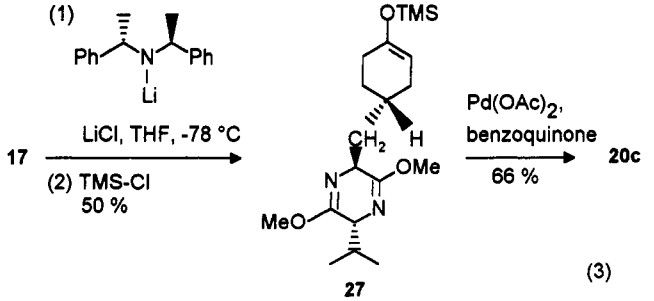


and chromatographic purification, ketone **17** was obtained as a single diastereoisomer in 51% overall yield.



The desired configuration at C-1 was established by a diastereoselective deprotonation with lithium (*S,S*)-bis-(1-phenylethyl)amide in the presence of lithium chloride following a modified procedure for this reaction described by Simpkins and co-workers.[14] The enolate was quenched with trifluoroethyl trifluoroacetate and the crude β-keto ester **18** reacted with *p*-tosyl azide to yield the α-diazo ketone **19** with a de of 80%.[15,16] This compound reacted smoothly with 1 equiv of phenylselenyl chloride to an intermediate which, after oxidative elimination, gave rise to the desired 2-chloro enone **20a**.[17] Before hydrolysis of the bislactim ether, the enone had to be reduced to the allylic alcohol, since acidic cleavage of **20a** with subsequent liberation of the amine led to the already mentioned intramolecular 1,4-addition. After reduction with sodium borohydride/cerium trichloride[18] the hydrolysis was uneventful. The product was coupled with (*S*)-Boc-alanine and the alcohol **24a** reoxidized by manganese dioxide (Scheme 3). At this stage, the small quantities of the undesired stereoisomer were easily removed by silica gel chromatography. After cleavage of the Boc protecting group, the methyl ester of **26a** was saponified with porcine pancreas lipase at pH 7.5 and ambient temperature. Decomposition products (mainly the 6-oxoperhydroindoles) were separated by reverse-phase chromatography to obtain pure chlorotetaine (**1a**), which was in every respect (NMR, HPLC, and most significantly CD) identical to the natural material.[19] By substituting phenylselenyl chloride with phenylselenyl bromide, diazo ketone **19** was converted by the same reaction sequence to bromotetaine (**1b**). In this case, the identity of the natural and the synthetic product was shown by comparison of their NMR spectra.[19] Most significantly, their ^{13}C NMR spectra were superimposable with differences of chemical shifts less than 0.1 ppm.

Using the same synthetic scheme, the unsubstituted cyclohexenyl derivative **1c** could be obtained as well starting from cyclohexenone **20c** (X = H). Compound **20c** was



available from cyclohexanone **17** by a diastereoselective

(13) Iodide **21** was prepared from ethyl 4-hydroxycyclohexanecarboxylate (Owen, L. N.; Robins, P. A. *J. Chem. Soc.* **1949**, 326.) in a four-step sequence, see Experimental Section.

(14) Bunn, B. J.; Simpkins, N. S. *J. Org. Chem.* **1993**, *58*, 533.

(15) Danheiser, R. L.; Miller, R. F.; Brisbois, R. G.; Park, S. Z. *J. Org. Chem.* **1990**, *55*, 1959.

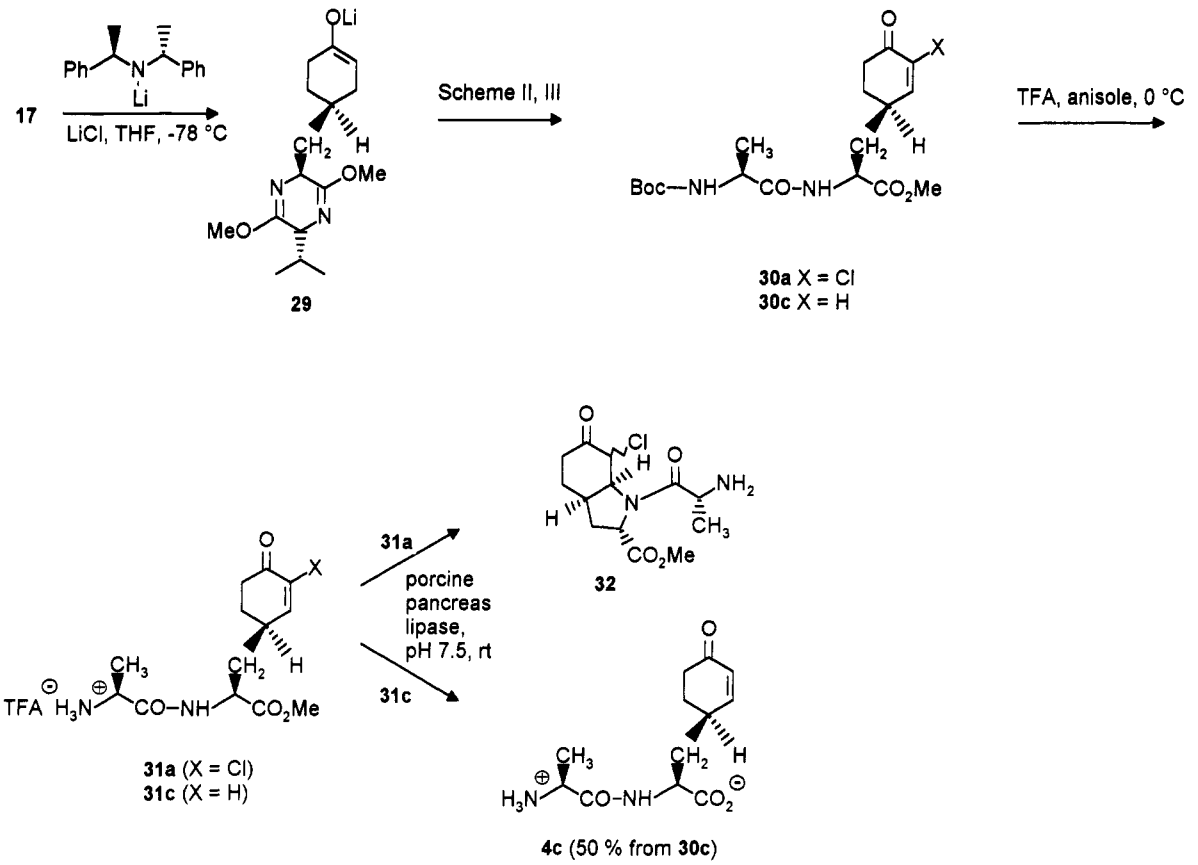
(16) The reaction of crude **18** with tosyl azide proceeded only to 15% completion. Workup followed by chromatography on silica gel provided product **19** and a mixture of tosyl azide and **18**. After addition of triethylamine, the starting materials now reacted completely to diazo ketone **19**. Purifying the β-keto ester **18** right from the start gave lower overall yields. The reason for this incomplete reaction remains unclear.

(17) Buckley, D. J.; McKervey, M. A. *J. Chem. Soc., Perkin Trans. 1* **1985**, 2193.

(18) Luche, J.-L.; Rodriguez-Hahn, L.; Crabbé, P. *J. Chem. Soc., Chem. Commun.* **1978**, 601.

(19) The natural sample of chlorotetaine and the NMR spectra of bromotetaine were kindly supplied by Prof. Jung, University of Tübingen.

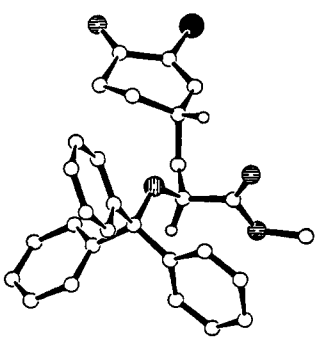
Scheme 4

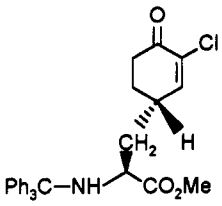


deprotonation–silylation sequence followed by palladium(II) oxidation of the silyl enol ether **27**[20,21] (eq 3).

To prove that the selective deprotonation of the cyclohexanone had actually followed the expected course, an X-ray structure analysis of the *N*-trityl derivative **28** of the *C*-terminal amino acid of chlorotetaine[22] was obtained. C-1 in the cyclohexene ring of **28**, and consequently in chlorotetaine as well, was shown by this analysis to possess the (*S*)-configuration. The amino acid residue in the crystal is in the axial position. However, this is a special effect occurring in this compound, because it was shown by ^{1}H NMR spectroscopy that the C-1 substituent of chlorotetaine in solution (D_2O) is equatorial.[23] Applying the reversed octant rule,[24] this conformation together with the (*S*)-configuration is completely in accord with the observed negative Cotton effect in the CD spectrum. The originally determined (*R*)-configuration resulted from a misinterpretation of the CD spectrum, conditioned by comparison of chlorotetaine with the published structure of bacilysin[2] and anticapsin.[3]

X-ray structure of compound **28**





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In the initial attempt to prepare compound **4a**, which at that time was thought to be natural chlorotetaine, the same reaction sequence which later was successful for the synthesis of the real chlorotetaine **1a** was used (Scheme 4). Starting from ketone **17**, deprotonation (this time with lithium (*R*,*R*)-bis(1-phenylethyl)amide) proceeded with comparable selectivity. The derived enolate **29** was then further converted to dipeptide **30a**. The cleavage of the Boc protecting group of **30a** proceeded cleanly; however, despite the extremely mild conditions of the following ester cleavage reaction (pH 7.5, rt, 3 h), the only product isolated was a diastereomeric mixture of the octahydroindole **32** arising from intramolecular 1,4-addition.[25] In contrast,

(20) Ito, Y.; Hirao, T.; Saegusa, T. *J. Org. Chem.* **1978**, *43*, 1011.

(21) Enone **20c** contained 11% of the saturated ketone. Interestingly, the de rose from 80% in **27** to 92% in **20c**, because the (1*R*)-diastereoisomer of **27** was converted to a higher extent to the saturated ketone than **27** itself.

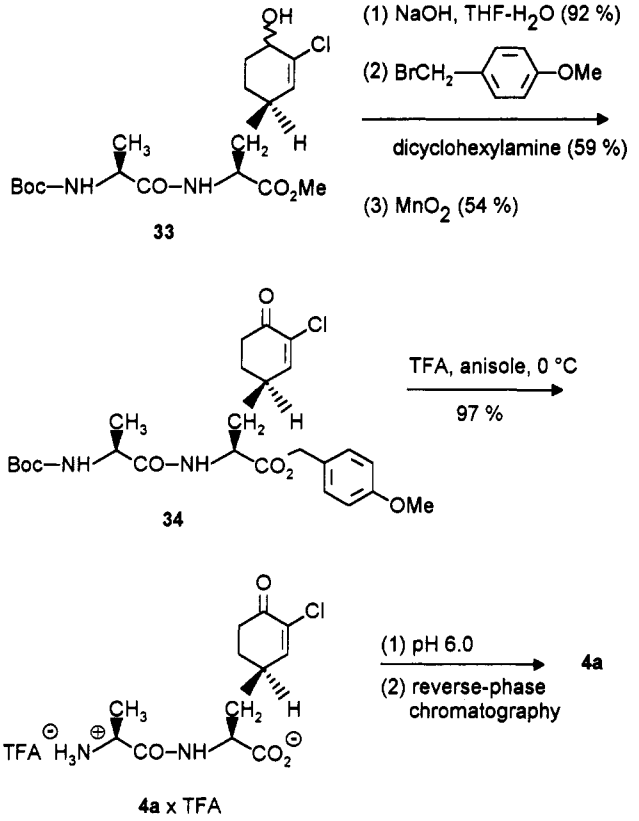
(22) Details of the crystal investigation can be obtained from the Fachinformationszentrum Karlsruhe, Gesellschaft für wissenschaftlich-technische Information mbH, D-76244 Eggenstein-Leopoldshafen, by quoting the file number CSD-55831 and ref 7.

(23) In the ^{1}H NMR spectrum of chlorotetaine **1a** in aqueous solution the proton H-1 at 2.75 ppm has three coupling partners in the cyclohexenone ring: the olefinic proton H-2 (J = 3.2 Hz), the equatorial proton H-6e (J = 5 Hz), and the axial proton H-6a (J = 9.1 Hz). This proves that H-1 is axial (one a–a and one a–e coupling) and that hence the residue at C-1 is in an equatorial position.

(24) (a) Djerassi, C.; Klyne, W.; Norin, T.; Ohloff, G.; Klein, E. *Tetrahedron* **1965**, *21*, 163. (b) Snatzke, G. *Tetrahedron* **1965**, *21*, 413.

(25) The presence of **32** was proven by comparison of the ^{1}H NMR spectrum of the crude reaction mixture with that of similar octahydroindoles, e.g. Souchet, M.; Guilhem, J.; LeGoffic, F. *Tetrahedron Lett.* **1987**, *28*, 2371. However, the crude reaction mixture contained further decomposition products. Probably **32** is not stable under the reaction conditions.

Scheme 5



the unsubstituted enone **31c** yielded the expected dipeptide **4c** under these conditions.[26]

Since the dipeptides were stable against cyclization under the deprotection conditions with TFA, the acid labile *p*-methoxybenzyl ester was used in place of the methyl ester in the next attempt to obtain betaine **4a** (Scheme 5). After oxidation, ketone **34** was obtained, which upon treatment with TFA at 0 °C was smoothly converted to the trifluoroacetate of **4a**. From this the betaine could be liberated and after purification by reverse-phase chromatography the desired dipeptide **4a** was obtained for the first time. **4a** was much more labile than chlorotetaine and had different spectroscopic characteristics, which further proved that the structure of the natural product had to be corrected to **1a**.

The interesting differences in stability between dipeptides **1** and **4** and the corresponding methyl esters **26** and **31** were investigated further by measuring their half-life in aqueous solution (Table 1).[27] Chlorotetaine **1a** has its maximum stability around pH 3 with a half-life of 190 h. Under physiological conditions (pH 7.5) the half-life is 12 h. Bromotetaine **1b** has a comparable stability, whereas the unsubstituted enone **1c** is much more stable, which demonstrates that the cyclization is governed at least in part by the inductive effect of the X-substituent. The epimers **4a** and **4c** have shorter half-lives, which is easy to explain (Scheme 6): Whereas the cyclization of **4** leads to an octahydroindole **36** with an *exo*-carboxyl substituent,

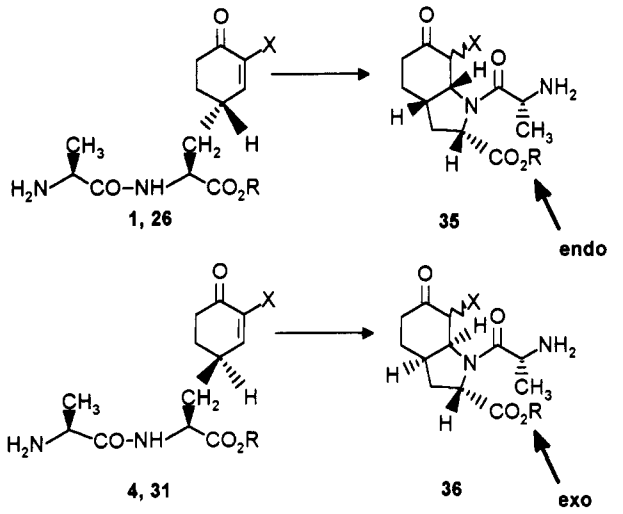
(26) Dipeptide **4c** was obtained with a de of only 30%. Because intermediate **31c** decomposed more readily under the conditions of the saponification reaction than epimer **26c**, the de of the product was much lower than the de of the starting material **31c** (80%).

(27) The half-life was measured by HPLC. The compounds were dissolved in a pH 7.5 phosphate buffer (1 mg/mL) and then brought to 28 °C, and at certain times the concentrations were determined by integrating the corresponding peaks in the HPLC plot.

Table 1. Half-Life of Dipeptides 1, 4, 26, and 31 at 28 °C

compd	X	R	pH	$t_{1/2}$ (h)
1a	Cl	H	1.5	111
1a	Cl	H	3.5	190
1a	Cl	H	5.5	92
1a	Cl	H	7.5	12
1b	Br	H	7.5	8.2
1c	H	H	7.5	>200
4a	Cl	H	7.5	1.2
4c	H	H	7.5	8.4
26a	Cl	Me	7.5	0.5
26b	Br	Me	7.5	0.3
26c	H	Me	7.5	6.5
31a	Cl	Me	7.5	<0.05
31c	H	Me	7.5	0.5

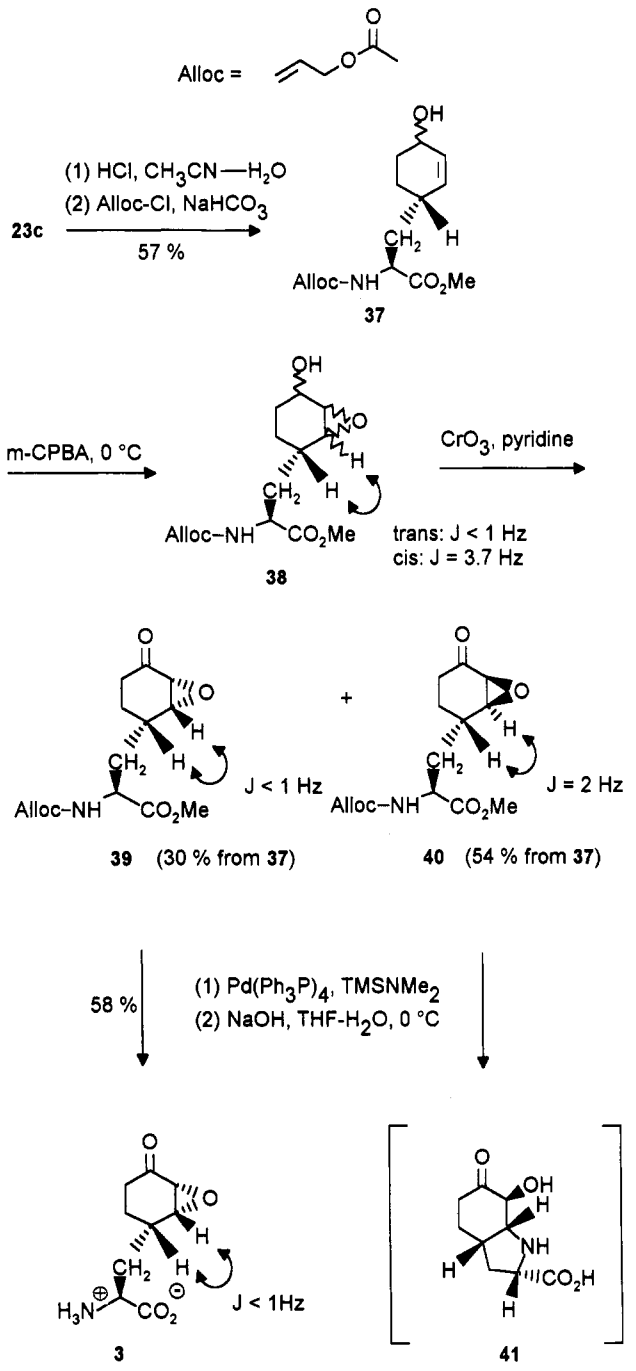
Scheme 6



the same reaction starting from **1** is made more difficult by the formation of a product with an *endo*-carboxyl residue. Between the methyl esters **26** and **31**, the same differences in stability are observed. Interestingly, the esters cyclize much more readily than the carboxylates. The chlorotetaine precursor **26a** has a half-life of only 30 min. Fortunately, the enzymatic saponification leading to chlorotetaine is slightly faster. However, the half-life of the epimeric methyl ester **31a** under these conditions is less than 3 min, which does explain that no trace of the free dipeptide **4a** could be isolated using this reaction sequence. The reason for this instability of the methyl esters in comparison to the carboxylates is not completely clear. The amide nitrogen of **1** should be more nucleophilic than that of **31**, because of the positive inductive effect of the carboxylate group as compared to the negative inductive effect of the carboxylic ester. Most probably there are differences in the preferred conformation and steric demand of the dipeptides in aqueous solution, which hinder cyclization in one case and facilitate it in the other.

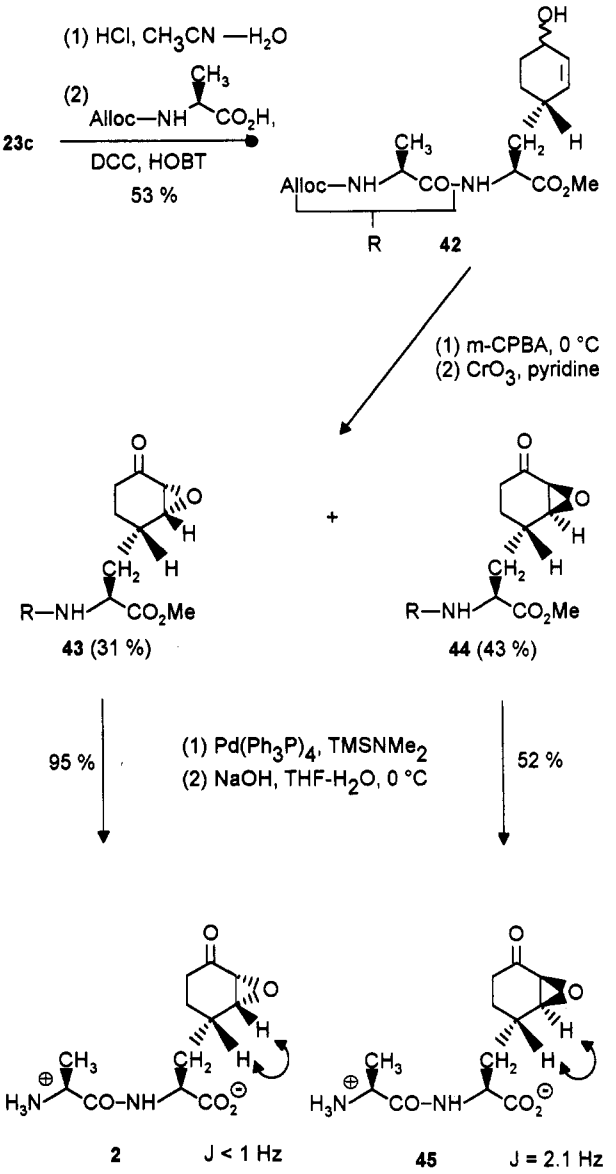
B. Preparation of Anticapsin and Bacilysin. With the structures of chlorotetaine and bromotetaine proven, it was suspected that bacilysin and anticapsin in contradiction to the literature assignments[2,3] also have the (*S*)-configuration at C-1 of the cyclohexanone ring. With the cyclohexanol **23c** as a favorable starting material in hand a synthesis of these two compounds was undertaken to answer this question (Scheme 7). **23c** had been obtained by reduction of the corresponding ketone **20c** with sodium borohydride/cerium trichloride and as expected for the reduction of 4-substituted cyclohexanones it was a 73:27

Scheme 7



mixture of *trans*/*cis*-diastereoisomers.[28] Acidic hydrolysis, protection of the amine as the allyl carbamate, and directed epoxidation with *m*-CPBA gave a 70:30 mixture of diastereoisomers. The major isomer with a *trans*-relationship of epoxide and C-1 substituent did not, as expected, show any coupling between the *trans*-protons at C-1 and C-2, whereas the minor isomer showed a small coupling, well in accord with the literature precedent.[5] However, after **38** had been oxidized with chromium trioxide to a separable mixture of diastereoisomeric ketones the situation changed. Now the *cis*-protons of the minor product **39** did not couple with each other, whereas the *trans*-protons of the major isomer **40** did. Palladium-catalyzed deprotection of the amine of **40**[29] followed by

Scheme 8



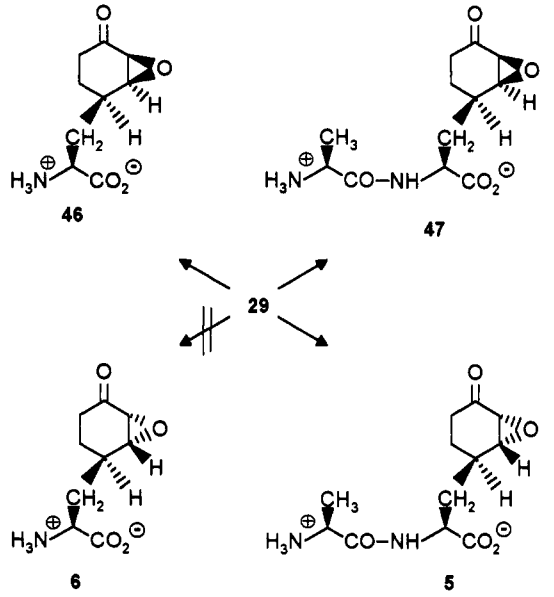
mild basic saponification did result in the isolation of a product mixture with the perhydroindole **41** as the major product. This reaction can only occur easily when the epoxide and the C-1 substituent are *trans*. **41** was not stable, but slowly deteriorated in solution. However, deprotection of the minor isomer **39** followed by saponification gave an amino acid **3** which was in every respect (NMR, α_D, CD) identical to a sample of natural anticapsin.[30] This proved that in fact anticapsin, like chlorotetaine and bromotetaine, has the (*S*)-configuration at the C-1 position. The relationship between the epoxide and the adjacent substituent is *cis* and not *trans*, which means that the originally determined absolute configuration of the epoxide, which is responsible for the positive Cotton effect in the CD spectrum, was correct.[3]

Using the same methodology, the dipeptide bacilysin was synthesized only by substituting the allyloxycarbonyl protecting group by a protected *L*-alanine (Scheme 8).

(28) Sucrow, W.; Rädecker, G. *Chem. Ber.* **1988**, *121*, 219.
(29) Merzouk, A.; Guibé, F. *Tetrahedron Lett.* **1992**, *33*, 477.

(30) The sample of natural anticapsin was kindly provided by E. Lilly and Co. There is some ambiguity about the optical rotation of anticapsin. In our hand natural anticapsin, which was slightly impure, showed $[\alpha]^{20}_D$ = +29.2° (*c* = 0.2, H_2O) and the synthetic material showed $[\alpha]^{20}_D$ = +32.3° (*c* = 0.3, H_2O); compare refs 3, 6, and 9, which give values between +21° (*c* = 0.2, H_2O) and 103° (*c* = 1, H_2O).

Scheme 9



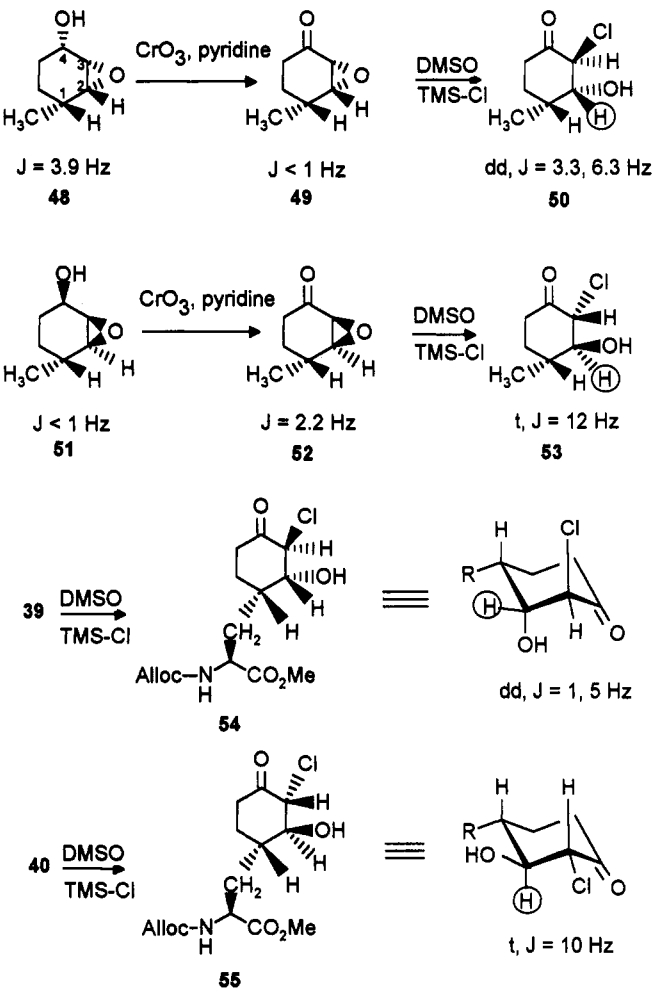
Again after oxidation and deprotection, the minor *cis*-isomer led to the natural product **2**. Compound **2** exhibited spectroscopic data (NMR, α_D, CD) which were in accord with those published for bacilysin.[2,31] In this case the *trans*-isomer **45** could be obtained as well, but it was somewhat labile against cyclization to the perhydroindole.

Starting from the enolate **29**, the remaining isomers of bacilysin and anticapsin were synthesized, but they were all clearly different from the natural products (Scheme 9). Again, the amino acid with the *trans*-substitution pattern, structure **6**, which was originally assigned to anticapsin,[3] could not be obtained, because it cyclized under the saponification conditions. However, the corresponding dipeptide **5**, whose structure had been assigned to bacilysin,[2] showed spectroscopic data clearly different from the natural product.[32]

Additional model studies were undertaken both to secure the relative stereochemistry of the epoxide in the cyclohexanone ring and to investigate further the differences in coupling constants between the epoxy alcohols and the epoxy ketones (Scheme 10). To this end the known 1,2-*trans*-epoxide **51**[33] and its *cis*-isomer **48**[34] were oxidized to the epoxy ketones **52** and **49**, respectively. Exactly parallel to the behavior in the anticapsin/bacilysin series, the alcohol of the *cis*-series (**48**) has an observable coupling between the protons H-1 and H-2 while the ketone **49** does not. In the *trans*-series, the epoxy alcohol and the epoxy ketone behave in the opposite manner. The original structure determination was mislead, because no data about comparable epoxy ketones had been available and anticapsin and bacilysin therefore had to be compared with epoxycyclohexanols and epoxycyclohexanes.

Opening the model epoxides **49** and **52** with chlorodimethylsulfonium generated *in situ* by mixing trimethylsilyl chloride and DMSO gave the chlorohydrins **50** and **53**, respectively.[35] In **50**, two substituents are axial, whereas in the diastereoisomer **53** all substituents are equatorial, as shown by the coupling constants in the ^{1}H NMR spectra. The same reactions performed with the anticapsin precursor **39** and its isomer **40** yielded chlorohydrins whose spectral data were perfectly in accord with the *cis*-stereochemistry for the anticapsin precursor **39** and the *trans*-relationship for the isomer **40**.

Scheme 10



A last point remains to be mentioned: In all ^{1}H NMR spectra of the free betaines of bacilysin, anticapsin, and their diastereoisomers which were recorded in D_2O, a second component was visible. This component could be seen as well in the original spectrum of anticapsin published in 1970.[36] Most probably this component, which is always present to an extent of about 20% even in highly purified material, is the hydrate (**56**, **57**) of the epoxy ketone (Scheme 11). This has also been suggested in the recent publication by Baldwin et al.[9] The change from an sp^2- to an sp^3-center in the cyclohexane ring again leads to a switch of the coupling constants of the *cis*- and the *trans*-protons, respectively, as already shown above for the epoxy alcohols. In the ^{13}C NMR spectrum of anticapsin **3** a second set of signals is observed for the hydrate form **56** with a very characteristic signal for C-4 at 93.3 ppm.

(31) As with anticapsin, the optical rotation of synthetic bacilysin was somewhat lower than the published value: reported (Rogers, H. J.; Lomakina, N.; Abraham, E. P. *Biochem. J.* **1965**, *97*, 579) $[\alpha]^{20}_D = +103°$ (c = 0.6, H_2O), found $[\alpha]^{20}_D = +63°$ (c = 0.45, H_2O).

(32) It was not possible to separate the dipeptides **5** and **47** or their protected precursors. However, in the ^{1}H NMR spectrum of the mixture of **5** and **47** the protons of the epoxide could cleanly be assigned and were different from natural bacilysin.

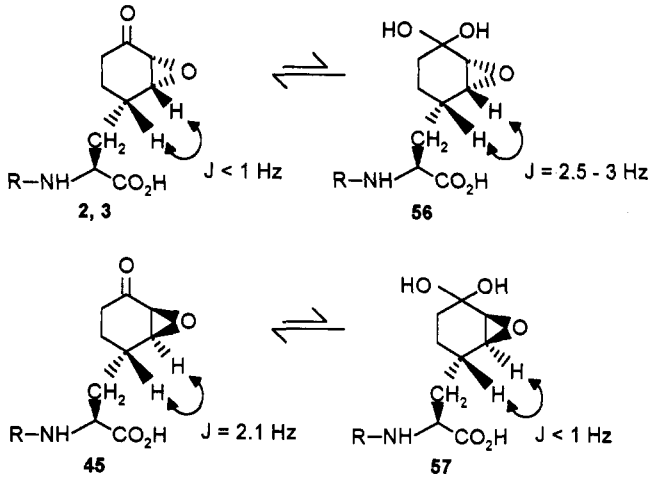
(33) Marino, J. P.; Hatanaka, N. *J. Org. Chem.* **1979**, *44*, 4467.

(34) Prepared from 4-methyl-2-cyclohexenone by reduction with sodium borohydride/cerium trichloride, separation of the *trans*- and *cis*-diastereoisomers and epoxidation of the *cis*-4-methyl-2-cyclohexenol.

(35) Ghelfi, F.; Grandi, R.; Pagnoni, U. M. *J. Chem. Res. (S)* **1988**, 200.

(36) Shah, R.; Neuss, N.; Gorman, M.; Boeck, L. D. *J. Antibiot.* **1970**, *23*, 613.

Scheme 11



Conclusion

This work for the first time proves the stereochemistry of the antifungal natural products chlorotetaine, bromotetaine, bacilysin, and anticapsin. The configuration at the chiral center at C-1 of the cyclohex(en)yl residue of all compounds has to be revised to (*S*). The epoxide in the cyclohexanone ring of bacilysin and anticapsin is *cis* to the C-1 substituent. The stereochemical assignments and the general and stereoselective access to these unusual non-proteinogenic amino acids may open new opportunities for further research in the field of antimycotic agents.

Experimental Section

All reactions were performed under an argon atmosphere. Analytical grade solvents were used unless otherwise stated. Fluka "anhydrous, stored over molecular sieves" solvents were used without further purification. Organic extracts from workup were dried over anhydrous magnesium sulfate and evaporated in vacuo. Silica gel columns for flash chromatography utilized E. Merck silica gel 60 (230–400 mesh ASTM) under a slightly positive pressure. Reverse-phase chromatography was performed using E. Merck LiChroprep RP-8 (40–63 μm) prepacked columns. For enzymatic ester hydrolysis, porcine pancreas lipase obtained from Sigma (L 3126) was used.

4-Methylene-2-cyclohexenone (12).[12] To *p*-methoxybenzyl alcohol (77 g, 0.56 mol) in liquid ammonia (1 L) and ethanol (700 mL) was added sodium metal (56 g, 2.4 mol) in portions. Ten minutes after the addition of the last piece of sodium, ammonium chloride (107 g, 2 mol) was added slowly, and then the solution was evaporated. The residue was taken up in methylene chloride (1 L), washed with water (2 × 1 L), dried ($MgSO_4$), and evaporated. The residual oil was distilled in vacuo (bp 72–74 °C, 0.01 mbar) to afford 53.8 g (69%) of 1-methoxy-4-(hydroxymethyl)-1,4-cyclohexadiene as a colorless liquid.

1 N Sulfuric acid (1.3 L) was warmed to 100 °C. The product of the above reaction (38 g, 0.27 mol) was added in one batch and the turbid solution was stirred at 100 °C for 15 min, by which time the solution became clear. The reaction mixture was cooled to 23 °C and extracted with ethyl acetate (1.5 L). The organic phase was dried ($MgSO_4$) and evaporated (10 mbar) to afford 32 g (quantitative) of a pale yellow liquid. It was found that this compound deteriorated rapidly at 23 °C and even slowly at −25 °C and was directly used for the next step: ^{1}H NMR ($CDCl_3$) δ 7.10 (d, *J* = 10.5 Hz, 1 H), 5.97 (d, *J* = 10.5 Hz, 1 H), 5.33 (s, 1 H), 5.30 (s, 1 H), 2.76 (t, *J* = 7 Hz, 2 H), 2.57 (t, *J* = 7 Hz, 2 H).

(3*S*,6*R*)-2,5-Dimethoxy-6-isopropyl-3-[(4-oxo-1-cyclohexenyl)methyl]-3,6-dihydro-1,4-pyrazine (15). To a −78 °C cold solution of bislactim ether 14[11] (50.4 mL, 0.28 mol) in anhydrous THF (0.5 L) was added a 1.6 N solution of *n*-BuLi in hexane (176 mL, 0.28 mol) over 15 min. After further 10 min this solution was added via double needle to a −30 °C mixture containing the cuprous bromide–dimethyl sulfide complex (29 g, 0.14 mol) dissolved in anhydrous THF (0.4 L) and dimethyl sulfide (0.28 L). The resulting brown solution was stirred at −30 °C for 30 min and recooled to −78 °C. Then a solution of dienone **12** (30.5 g, 0.28 mol) in anhydrous THF (0.2 L) was added over 15 min. The reaction was stirred for 1 h at −78 °C and quenched by the fast addition of AcOH (16 mL, 0.28 mol) in THF (0.12 L). The solution was diluted with ethyl acetate (2.5 L), washed with brine (2 L), dried, and evaporated. The crude product was purified twice on silica gel (10:1 toluene–ethyl acetate) to give as a first fraction 22.6 g (27%) of the 1,4-adduct **16** followed by 39.2 g (48%) of the desired 1,6-adduct **15**: ^{1}H NMR ($CDCl_3$) δ 5.48 (t, *J* = 3 Hz, 1 H), 4.15 (m, 1 H), 3.90 (t, *J* = 3 Hz, 1 H), 3.70 (s, 3 H), 3.68 (s, 3 H), 2.82 (d, *J* = 3 Hz, 2 H), 2.60 (dd, *J* = 4, 12 Hz, 1 H), 2.48–2.38 (m, 5 H), 2.28 (m, 1 H), 1.05 (d, *J* = 6.5 Hz, 3 H), 0.67 (d, *J* = 6.5 Hz, 3 H); MS (EI) *m/e* 292 (M), 277, 183, 141; IR (film) 1692, 1438, 1238 cm^{-1}; $[\alpha]^{20}_D$ +31.8° (*c* = 1, $CHCl_3$). Anal. Calcd for $C_{16}H_{24}N_2O_3$: C, 65.7; H, 8.3; N, 9.6. Found: C, 65.5; H, 8.1; N, 9.5.

(*cis/trans*)-1-[(*tert*-Butyldimethylsilyl)oxy]-4-(iodomethyl)cyclohexane (21). To a solution of ethyl (*cis/trans*)-4-hydroxycyclohexanecarboxylate[13] (100 g, 0.63 mol) in anhydrous DMF (630 mL) were added imidazole (86 g, 1.26 mol) and *tert*-butyldimethylsilyl chloride (104 g, 0.7 mol). The resulting solution was stirred at 23 °C for 4 h. Ether (2 L) was added and the solution was washed with 1 N HCl (2 × 1 L) and brine (1 L). The organic layer was dried ($MgSO_4$) and evaporated. The crude product was purified on silica gel (toluene) to provide 128 g (74%) of ethyl (*cis/trans*)-4-[(*tert*-butyldimethylsilyl)oxy]cyclohexanecarboxylate as a colorless liquid.

To this ester (125.6 g, 0.46 mol) dissolved in anhydrous methylene chloride (1.3 L) at −30 °C was added dropwise a 1 M solution of DIBALH in toluene (0.9 L). The solution was stirred an additional 30 min at −30 °C and then poured into ice-cold 1 N HCl (2 L). The aqueous layer was separated and extracted with methylene chloride (3 × 0.5 L). The combined organic layers were washed with brine (2 L), dried ($MgSO_4$), and evaporated to provide 109.7 g (98%) of (*cis/trans*)-4-[(*tert*-butyldimethylsilyl)oxy]cyclohexanemethanol as a pale yellow oil.

This compound was redissolved in anhydrous pyridine (1 L), and Mes-Cl (70 mL, 0.91 mol) was added over 5 min. The solution was kept at 23 °C for 1 h. Ethyl acetate (2 L) was added and the solution was extracted with 1 N HCl (3 × 2 L) and brine (2 L), dried ($MgSO_4$), and evaporated to provide 121.5 g (86%) of crude [(*cis/trans*)-4-[(*tert*-butyldimethylsilyl)oxy]-1-cyclohexyl]methyl mesylate.

The crude mesylate was redissolved in anhydrous acetone (1 L), sodium iodide (115 g, 0.76 mol) was added, and the mixture was refluxed for 16 h. The mixture was cooled to 23 °C and filtered over Celite. The red filtrate was evaporated, redissolved in ethyl acetate (1 L), and washed with saturated sodium dithionite solution (0.5 L). The resulting pale yellow solution was evaporated and the crude product was purified on silica gel (toluene) to provide 119.4 g (89%) of iodide **21** as a mixture of diastereoisomers (*trans:cis* = 6:4): ^{1}H NMR ($CDCl_3$) δ 3.88 and 3.5 (2 m, 1 H), 3.11 (m, 2 H), 1.85 (m, 2 H), 1.20–1.70 (m, 6 H), 1.02 (m, 1 H), 1.88 and 1.87 (2s, 9H), 0.05 and 0.01 (2s, 6 H); MS (EI) *m/e* 353 (M), 297, 95; IR (film) 1252, 1102, 1050, 1018, 833, 772 cm^{-1}. Anal. Calcd for $C_{13}H_{27}IOSi$: C, 44.1; H, 7.7. Found: C, 44.1; H, 7.5.

(3*S*,6*R*)-2,5-Dimethoxy-6-isopropyl-3-[[(*cis/trans*-)-4-[(*tert*-butyldimethylsilyl)oxy]-1-cyclohexyl]methyl]-3,6-dihydro-1,4-pyrazine (22). To a solution of bislactim ether 14[11] (61.8 mL, 322 mmol) in anhydrous THF (1 L) at −78 °C was added a 2.5 N solution of *n*-butyllithium in hexane (137 mL, 341 mmol) over 15 min. The solution was stirred at −78 °C for 30 min and then iodide **21** (115.4 g, 324 mmol) in anhydrous THF (380 mL) was added dropwise over 30 min. The solution was stirred in an ice-bath for 20 min and then quenched by the addition of acetic acid (29 mL, 520 mmol). Ethyl acetate (2 L) was added, and the solution was washed with brine (1 L), dried ($MgSO_4$), and evaporated. The crude product (de 70–80%) was purified on silica gel (50:1 toluene–ethyl acetate) to provide 95.2 g (77%) of dihydropyrazine **22** (*cis/trans*-mixture) as a pale yellow oil. The product was contaminated by less than 5% of the (3*R*,6*R*)-diastereoisomer: ^{1}H NMR ($CDCl_3$) δ 4.12 (m, 1 H), 3.90 (m, 1 H),

3.68, 3.67, and 3.66 (3 s, 6 H), 3.50 (m, 1 H), 2.25 (m, 1 H), 1.85–1.20 (m, 9 H), 1.05 (d, J = 6.5 Hz, 3 H), 0.95 (m, 2 H), 0.89 (s, 9 H × 1/2), 0.88 (s, 9 H × 1/2), 0.69 (d, J = 6.5 Hz), 0.05 (s, 6 H × 1/2), 0.02 (s, 6 H × 1/2); MS (EI) m/e 411, 409 (M), 279, 277, 141, 75, 73; IR (film) 1695, 1237 cm^{-1}. Anal. Calcd for $C_{22}H_{42}N_2O_3Si$: C, 64.3; H, 10.3; N, 6.8. Found: C, 64.5; H, 10.3; N, 7.0.

(3*S*,6*R*)-2,5-Dimethoxy-6-isopropyl-3-[(4-oxo-1-cyclohexyl)methyl]-3,6-dihydro-1,4-pyrazine (17). From **15**: Enone **15** (7.4 g, 25.3 mmol) in ethyl acetate (400 mL) was hydrogenated at 3 atm and 23 °C in the presence of 10% palladium on charcoal (3.7 g). After 4 h the catalyst was filtered off and the filtrate was evaporated to provide 14.4 g (97%) of ketone **17** as a crude yellow oil, which was pure as shown by TLC.

From **22**: The silyl ether **22** (95.1 g, 234 mmol) in THF (470 mL) was treated with a 1 N solution of tetrabutylammonium fluoride in THF (470 mL) and stirred for 3 h at 50 °C. Then saturated sodium carbonate solution (250 mL) was added and the mixture was extracted with methylene chloride (3 × 500 mL). The combined organic layers were dried ($MgSO_4$) and evaporated and the crude product was purified on silica gel (1:1 toluene–ethyl acetate) to provide 72.8 g of the intermediate alcohol.

To an ice-cold solution of pyridine (239 mL, 2.95 mol) in anhydrous methylene chloride (2.7 L) was added portionwise chromium trioxide (148.2 g, 1.48 mol) under mechanical stirring. The mixture was warmed to 23 °C and stirred for 1 h. To this mixture a solution of the alcohol obtained above (72.8 g) in methylene chloride (400 mL) was added over 15 min. The reaction mixture was stirred for further 20 min at 23 °C and decanted, and the black residue was washed with methylene chloride (2 × 0.5 L). The organic solvents were combined and washed with saturated sodium carbonate solution (2 × 1 L) and brine (1 L). The aqueous layers were combined and washed with methylene chloride (1 L) and the combined organic layers were dried ($MgSO_4$) and evaporated. The crude product was purified on silica gel (3:1 toluene–ethyl acetate) to afford 54 g (66%) of ketone **17** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 4.12 (m, 1 H), 3.97 (m, 1 H), 3.71 (s, 3 H), 3.70 (s, 3 H), 2.40–2.00 (m, 7 H), 1.85 (m, 1 H), 1.60–1.35 (m, 3 H), 1.07 (d, J = 6.5 Hz, 3 H), 0.70 (d, J = 6.5 Hz, 3 H); MS (EI) m/e 294 (M), 251, 183, 141; IR (film) 1693, 1237 cm^{-1}; $[\alpha]^{20}_D$ −5.7° (c = 0.8, $CHCl_3$). Anal. Calcd for $C_{16}H_{26}N_2O_3$: C, 65.3; H, 8.9; N, 9.5. Found: C, 65.3; H, 8.7; N, 9.4.

(3*S*,6*R*,1′*S*)-2,5-Dimethoxy-6-isopropyl-3-[(3-diazo-4-oxo-1-cyclohexyl)methyl]-3,6-dihydro-1,4-pyrazine (19). (*S*,*S*)-Bis(1-phenylethyl)amine[37] (29.1 g, 129 mmol) and lithium chloride (2.74 g, 64.6 mmol) were dissolved in anhydrous THF (1.2 L) and cooled to 0 °C. To this was added a 2.5 N solution of *N*-butyllithium (49.1 mL, 124 mmol) over 5 min. The solution was stirred for 30 min at 23 °C and then cooled to −78 °C. Ketone **17** (15 g, 51.2 mmol) dissolved in anhydrous THF (260 mL) was added over 15 min. The solution was stirred for an additional 30 min and then 2,2,2-trifluoroethyl trifluoroacetate (20.7 mL, 155.2 mmol) was added over 5 min. After an additional 15 min at −78 °C, the reaction mixture was partitioned between ethyl acetate (0.5 L) and saturated sodium bicarbonate solution (0.5 L). The organic layer was separated, washed with brine (0.5 L), dried ($MgSO_4$), and evaporated to give the crude β-keto ester **18**. The ester was dissolved in acetonitrile (180 mL) and treated with water (1 mL), triethylamine (9.7 mL, 63.1 mmol), and *p*-tosyl azide (12.4 g, 63.1 mmol). The mixture was stirred for 1 h at 23 °C, by which time no further reaction occurred. The solution was evaporated and chromatographed on silica gel (3:1 toluene–ethyl acetate) to provide as the first and major fraction a mixture of the educts **18** and *p*-tosyl azide followed by 2.28 g (14%) diazo ketone **19**. The educts were redissolved in acetonitrile (150 mL), and water (1 mL) and triethylamine (9 mL, 58.6 mmol) were added. The mixture was stirred at 23 °C for 1 h and purified as described above to provide additional 5.63 g (34%) of **19** as a yellow oil with a de of 80%: ^{1}H NMR ($CDCl_3$) δ 4.12 (m, 1 H), 3.96 (m, 1 H), 3.71 (s, 3 H), 3.65 (s, 3 H), 2.75 (dd, J = 1.7, 5 Hz, 1 H), 2.52–2.20 (m, 4 H), 2.10 (m, 1 H), 2.00–1.88 (m, 2 H), 1.70–1.45 (m, 2 H), 1.04 (d, J = 6.5 Hz, 3 H), 0.70 (d, J = 6.5 Hz, 3 H); MS (FAB) m/e 321 (M + H), 293, 141; IR (film) 2084, 1696, 1621, 1339, 1238 cm^{-1}; $[\alpha]^{20}_D$ +95.3° (c = 0.6, $CHCl_3$). HRMS calcd for $C_{16}H_{25}N_4O_3$ 321.1927, found 321.1917.

(3*S*,6*R*,1′*S*)-2,5-Dimethoxy-6-isopropyl3-[[4-[(trimethylsilyl)oxy]-3-cyclohexen-1-yl]methyl]-3,6-dihydro-1,4-pyrazine (27). Ketone **17** (7.31 g, 24.8 mmol) was deprotonated following the deprotonation method as described for the preparation of compound **19**. The reaction mixture was quenched by the addition of trimethylsilyl chloride (9.5 mL, 74.4 mmol), stirred for 15 min at −78 °C, and partitioned between hexane (0.5 L) and saturated sodium bicarbonate solution (0.5 L). The organic layer was washed with brine (0.5 L), dried ($MgSO_4$), and evaporated. The crude product was purified rapidly by chromatography on silica gel (20:1 toluene–ethyl acetate) to provide 4.54 g (50%) of silyl enol ether **27** as a colorless oil with a de of 80%: ^{1}H NMR ($CDCl_3$) δ 4.70 (m, 1 H), 4.03 (m, 1 H), 3.92 (m, 1 H), 3.68 (s, 3 H), 3.65 (s, 3 H), 2.25 (m, 1 H), 2.15–1.95 (m, 2 H), 1.90–1.70 (m, 4 H), 1.50–1.25 (m, 2 H), 1.60–1.35 (m, 3 H), 1.05 (d, J = 6.5 Hz, 3 H), 0.69 (d, J = 6.5 Hz, 3 H), 0.19 (s, 9 H); MS (FAB) m/e 367 (M + H), 365, 323, 183, 141, 73; IR (film) 1692, 1235, 1193 cm^{-1}; $[\alpha]^{20}_D$ +19.9° (c = 1, $CHCl_3$). Anal. Calcd for $C_{19}H_{34}N_2O_3Si$: C, 62.3; H, 9.3; N, 7.6. Found: C, 62.8; H, 9.0; N, 7.8.

(3*S*,6*R*,1′*S*)-2,5-Dimethoxy-6-isopropyl-3-[(3-chloro-4-oxo-2-cyclohexen-1-yl)methyl]-3,6-dihydro-1,4-pyrazine (20a). To an ice-cold solution of diazo ketone **19** (1.5 g, 4.68 mmol) in anhydrous methylene chloride (25 mL) was added a solution of phenylselenyl chloride (897 mg, 4.68 mmol) in anhydrous methylene chloride (20 mL). The resulting solution was cooled to −5 °C and pyridine (0.89 mL, 11.7 mmol) followed by 30% hydrogen peroxide (1.43 mL, 14 mmol) were added. The reaction mixture was stirred for 2 h at −5 °C and then washed with saturated sodium bicarbonate solution (50 mL). The aqueous layer was extracted with methylene chloride (50 mL) and the combined organic layers were washed with water (100 mL), dried ($MgSO_4$), and evaporated. The crude product was purified on silica gel (5:1 toluene–ethyl acetate) to provide 1.13 g (70%) of enone **20a** with a de of 80% as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.16 (d, J = 3 Hz, 1 H), 4.10–3.92 (m, 2 H), 3.71 (s, 3 H), 3.69 (s, 3 H), 2.95 (m, 1 H), 2.71 (td, J = 5.5, 1.5 Hz, 1 H), 2.51 (dd, J = 5, 15 Hz, 1 H), 2.35–2.15 (m, 2 H), 2.08 (dd, J = 4, 14 Hz, 1 H), 1.90–1.70 (m, 2 H), 1.05 (d, J = 7 Hz, 3 H), 0.71 (d, J = 7 Hz, 3 H); MS (FAB) m/e 329, 327 (M + H), 141; IR (film) 1694, 1240 cm^{-1}; $[\alpha]^{20}_D$ +39.2° (c = 0.5, $CHCl_3$). Anal. Calcd for $C_{16}H_{23}ClN_2O_3$: C, 58.8; H, 7.1; N, 8.6. Found: C, 58.8; H, 7.2; N, 8.6.

(3*S*,6*R*,1′*S*)-2,5-Dimethoxy-6-isopropyl-3-[(3-bromo-4-oxo-2-cyclohexen-1-yl)methyl]-3,6-dihydro-1,4-pyrazine (20b). Prepared like **20a** by using phenylselenyl bromide in 61% yield: ^{1}H NMR ($CDCl_3$) δ 7.39 (dd, J = 0.7, 3.4 Hz, 1 H), 4.00–3.90 (m, 2 H), 3.65 (s, 3 H), 3.63 (s, 3H), 2.85 (m, 1 H), 2.70 (td, J = 5, 15 Hz, 1 H), 2.48 (dd, J = 5, 15 Hz, 1 H), 2.30–2.10 (m, 2 H), 2.00 (dd, J = 4, 12 Hz, 1 H), 1.87–1.63 (m, 2 H), 0.98 (d, J = 7 Hz, 3 H), 0.62 (d, J = 7 Hz, 3 H); MS (FAB) m/e 373, 371 (M + H), 141; IR (film) 1686, 1239 cm^{-1}; $[\alpha]^{20}_D$ +47.6° (c = 0.5, $CHCl_3$); HRMS calcd for $C_{16}H_{24}BrN_2O_3$ 371.0970, found 371.0970.

(3*S*,6*R*,1′*S*)-2,5-Dimethoxy-6-isopropyl-3-[(4-oxo-2-cyclohexen-1-yl)methyl]-3,6-dihydro-1,4-pyrazine (20c). To silyl enol ether **27** (1.84 g, 5 mmol) in anhydrous acetonitrile (10 mL) were added palladium(II)acetate (0.56 g, 2.5 mmol) and benzoquinone (0.27 g, 2.5 mmol) and the mixture was stirred at 23 °C for 3.5 h. In some cases more palladium(II) acetate and benzoquinone had to be added to complete the reaction. The mixture was filtered through Celite and evaporated. The crude product was purified on silica gel (5:1 toluene–ethyl acetate) to provide 0.97 g (66%) of enone **20c** as a pale yellow oil. **20c** had a de of 92% and contained 11% of the cyclohexanone: ^{1}H NMR ($CDCl_3$) δ 6.88 (ddd, J = 1.2, 3, 10.2 Hz, 1 H), 5.97 (dd, J = 2.1, 10.2 Hz, 1 H), 4.10–3.93 (m, 2 H), 3.71 (s, 3 H), 3.70 (s, 3 H), 2.82 (m, 1 H), 2.53 (td, J = 5, 15 Hz, 1 H), 2.45–2.25 (m, 3 H), 2.00 (dd, J = 4, 12 Hz, 1 H), 1.85–1.68 (m, 2 H), 1.05 (d, J = 7 Hz, 3 H), 0.70 (d, J = 7 Hz, 3 H); MS (FAB) m/e 293 (M + H), 141; IR (film) 1687, 1238 cm^{-1}. HRMS calcd for $C_{16}H_{25}N_2O_3$ 293.1867, found 293.1865.

(*S*)-(*tert*-Butyloxycarbonyl)alanyl-(*S*)-3-[(1*S*,4*S*/*R*)-3-chloro-4-hydroxy-2-cyclohexen-1-yl]alanine Methyl Ester (24a). To an ice-cold solution of enone **20a** (1.13 g, 3.29 mmol) in methanol (10 mL) were added anhydrous cerium trichloride

(37) Overberger, C. G.; Marullo, N. P.; Hiskey, R. G. *J. Am. Chem. Soc.* **1961**, *83*, 1374.

(812 mg, 3.29 mmol) followed by sodium borohydride (125 mg, 3.29 mmol). The reaction mixture was stirred for 15 min without cooling, water (20 mL) was added, and the solution was extracted with ether (4 × 30 mL). The organic layers were combined, dried ($MgSO_4$), and evaporated to provide 1.02 g (90%) of the crude alcohol **23a** as a mixture of *cis*/*trans*-isomers.

Crude alcohol **23a** (1.01 g, 3.07 mmol) was dissolved in acetonitrile (16 mL), water (12.5 mL) and 1 N hydrochloric acid (6.14 mL) were added, and the resulting mixture was stirred for 2 h at 23 °C. The acetonitrile was distilled off under reduced pressure, 0.5 M sodium carbonate solution (50 mL) was added, and the resulting solution was extracted with methylene chloride (5 × 50 mL). The combined organic layers were dried ($MgSO_4$) and evaporated to provide 930 mg of a crude amine still containing larger amounts of (R)-valine methyl ester. To (*S*)-(*tert*-butyloxycarbonyl)alanine (805 mg, 4.25 mmol) in anhydrous THF (10 mL) were added 1-hydroxybenzotriazole (577 mg, 4.27 mmol) and DCC (945 mg, 4.58 mmol) at 0 °C. The mixture was stirred at 23 °C for 1 h, a solution of the crude amine (730 mg) obtained above in anhydrous THF (6 mL) was added, and the mixture was stirred for an additional 2 h at 23 °C. The mixture was filtered and the filtrate was partitioned between ethyl acetate (50 mL) and saturated sodium bicarbonate solution (50 mL). The aqueous layer was extracted with ethyl acetate (30 mL) and the combined organic layers were dried ($MgSO_4$) and evaporated. The crude product was purified on silica gel (1:1 toluene–ethyl acetate) to provide 688 mg (66%) of the dipeptide **24a** (de = 80%, major isomer **trans**) as a colorless oil: ^{1}H NMR ($CDCl_3$, *trans*-isomer) δ 6.65 (d, broad, J = 8.5 Hz, NH), 5.75 (d, J = 2.9 Hz, 1 H), 4.95 (broad, NH), 4.78 (m, 1 H), 4.13 (m, 2 H), 3.71 (s, 3 H), 2.32 (d, J = 4.5 Hz, OH), 2.20–1.60 (m, 7 H), 1.45 (s, 9 H), 1.35 (d, J = 7.5 Hz, 3 H); MS (FAB) m/e 407, 405 (M + H), 331, 287, 216, 57; IR (film) 3424, 1656, 1543, 1167 cm^{-1}; HRMS calcd for $C_{18}H_{30}ClN_2O_6$ 405.1792, found 405.1768.

(*S*)-(*tert*-Butyloxycarbonyl)alanyl-(*S*)-3-[(1*S*,4*S*/*R*)-3-bromo-4-hydroxy-2-cyclohexen-1-yl]alanine Methyl Ester (24b). Prepared as **24a** starting from ketone **20b** in 58% yield: ^{1}H NMR ($CDCl_3$, *trans*-isomer) δ 6.53 (broad, NH), 6.92 (m, 1 H), 4.85 (broad, NH), 4.60 (m, 1 H), 4.10 (m, 2 H), 3.68 (s, 3 H), 2.25–1.60 (m, 8 H), 1.48 (s, 9 H), 1.29 (d, J = 7.5 Hz, 3 H); MS (FAB) m/e 451, 449 (M + H), 377, 375, 333, 331, 57; IR (film) 3422, 1662, 1367, 1326, 1167 cm^{-1}; HRMS calcd for $C_{18}H_{30}BrN_2O_6$ 451.1267, found 451.1251.

(*S*)-(*tert*-Butyloxycarbonyl)alanyl-(*S*)-3-[(1*S*,4*S*/*R*)-4-hydroxy-2-cyclohexen-1-yl]alanine Methyl Ester (24c). Prepared as **24a** starting from ketone **20c** in 55% yield: ^{1}H NMR ($CDCl_3$, *trans*-isomer) δ 6.70 (broad, d, J = 7 Hz, NH), 5.73 (d, J = 10 Hz, 1 H), 5.57 (d, J = 10 Hz, 1 H), 4.97 (broad, NH), 4.68 (m, 1 H), 4.16 (m, 2 H), 3.72 (s, 3 H), 2.20–1.85 (m, 3 H), 1.75–1.55 (m, 5 H), 1.42 (s, 9 H), 1.33 (d, J = 7.5 Hz, 3 H); MS (FAB) m/e 371 (M + H), 353, 297, 253; IR (film) 3387, 1670, 1527, 1367, 1251, 1168 cm^{-1}; HRMS calcd for $C_{18}H_{31}N_2O_6$ 371.2182, found 371.2168.

(*S*)-(*tert*-Butyloxycarbonyl)alanyl-(*S*)-3-[(1*S*)-3-chloro-4-oxo-2-cyclohexen-1-yl]alanine Methyl Ester (25a). Alcohol **24a** (677 mg, 1.67 mmol) in anhydrous methylene chloride (35 mL) was stirred in the presence of activated manganese dioxide (6.8 g) for 3 h. The mixture was filtered through Celite and the solvent evaporated. To separate off residual amounts of the (1*R*)-diastereoisomer **30a**, the crude product was purified on silica gel (ether); 380 mg (56%) of ketone **25a** was obtained as a white foam with a de > 95%: ^{1}H NMR ($CDCl_3$) δ 6.92 (d, J = 3 Hz, 1 H), 6.70 (broad, d, J = 9 Hz, NH), 4.92 (broad, NH), 4.62 (m, 1 H), 4.12 (m, 1 H), 3.78 (s, 3 H), 2.80–2.60 (m, 2 H), 2.52 (dd, J = 5, 12 Hz, 1 H), 2.40–2.25 (m, 1 H), 2.10–1.70 (m, 3 H), 1.40 (s, 9 H), 1.37 (d, J = 7.5 Hz, 3 H); MS (FAB) m/e 405, 403 (M + H), 347, 305, 303; IR (film) 3365, 1700, 1524, 1249, 1169 cm^{-1}; $[\alpha]^{20}_D$ +6.8° (c = 0.5, $CHCl_3$). Anal. Calcd for $C_{18}H_{27}ClN_2O_6$: C, 53.7; H, 6.8; N, 7.0. Found: C, 53.7; H, 6.7; N, 6.9.

(*S*)-(*tert*-Butyloxycarbonyl)alanyl-(*S*)-3-[(1*S*)-3-bromo-4-oxo-2-cyclohexen-1-yl]alanine Methyl Ester (25b). Prepared as **25a** starting from alcohol **24b** in 59% yield: ^{1}H NMR ($CDCl_3$) δ 7.12 (d, J = 3 Hz, 1 H), 6.61 (broad, d, J = 8.5 Hz, NH), 4.83 (broad, NH), 4.66 (m, 1 H), 4.05 (m, 1 H), 3.71 (s, 3 H), 2.70–2.53 (m, 2 H), 2.45 (dd, J = 4, 13 Hz, 1 H), 2.40–2.20 (m, 1 H), 2.00–1.60 (m, 3 H), 1.38 (s, 9 H), 1.31 (d, J = 7.5 Hz, 3 H); MS (FAB) m/e 449, 447 (M + H), 393, 391, 349, 347; IR (film) 3423, 1689, 1327, 1166 cm^{-1}; $[\alpha]^{20}_D$ +11.0° (c = 0.5, $CHCl_3$); HRMS calcd for $C_{18}H_{27}BrN_2O_6$ 449.1111, found 449.1134. Anal. Calcd for $C_{18}H_{27}BrN_2O_6$: C, 48.3; H, 6.1; N, 6.3. Found: C, 49.0; H, 6.1; N, 6.2.

(*S*)-(*tert*-Butyloxycarbonyl)alanyl-(*S*)-3-[(1*S*)-4-oxo-2-cyclohexen-1-yl]alanine Methyl Ester (25c). Prepared as **25a** starting from alcohol **24c** in 73% yield: ^{1}H NMR ($CDCl_3$) δ 6.75 (d, J = 10 Hz, 1 H), 6.69 (broad, d, J = 8.5 Hz, NH), 5.59 (dd, J = 2, 9 Hz, 1 H), 4.95 (broad, NH), 4.73 (m, 1 H), 4.13 (m, 1 H), 3.76 (s, 3 H), 2.60–2.45 (m, 2 H), 2.38 (dd, J = 5, 13 Hz, 1 H), 2.32–2.22 (m, 1 H), 2.00–1.60 (m, 3 H), 1.43 (s, 9 H), 1.37 (d, J = 7.5 Hz, 3 H); MS (FAB) m/e 369 (M + H), 269, 57; IR (film) 3407, 1676, 1169 cm^{-1}; $[\alpha]^{20}_D$ +9.9° (c = 0.6, $CHCl_3$). Anal. Calcd for $C_{18}H_{28}N_2O_6$: C, 58.7; H, 7.7; N, 7.6. Found: C, 58.5; H, 7.3; N, 7.5.

(*S*)-Alanyl-(*S*)-3-[(1*S*)-3-chloro-4-oxo-2-cyclohexen-1-yl]alanine (1a, chlorotetaine). The protected dipeptide **25a** (250 mg, 0.62 mmol) was dissolved in anhydrous methylene chloride (0.6 mL) and anhydrous anisole (0.3 mL). At 0 °C, trifluoroacetic acid (3.1 mL) was added slowly and the resulting solution was stirred for 1 h at 0 °C. The solvent was evaporated and the residual oil was treated with ether (2 mL) until the residue became crystalline. Hexane (2 mL) was added, the solvent was decanted, and the crystals were washed with hexane (2 mL) and dried in vacuo to provide a quantitative yield of trifluoroacetate **26a**: ^{1}H NMR (DMSO-d_6) δ 8.87 (d, J = 9 Hz, NH), 8.12 (broad, NH_3^+), 7.20 (d, J = 3 Hz, 1 H), 4.46 (m, 1 H), 3.90 (m, 1 H), 3.67 (s, 3 H), 2.80–2.45 (m, 3 H), 2.15–1.62 (m, 4 H), 1.38 (d, J = 6.5 Hz, 3 H). Crude **26a** (240 mg) was dissolved in a pH 7.5 phosphate buffer (24 mL). Porcine pancreas lipase (24 mg) was added immediately. The mixture was stirred at 23 °C for 4.5 h and then lyophilized. The crude product was purified on reverse-phase (water) to provide 95 mg (55%) of pure chlorotetaine **1a** after lyophilization: ^{1}H NMR (D_2O, the chemical shifts of both α-protons of the amino acids are concentration dependent!) δ 7.23 (dd, J = 1.2, 3.2 Hz, H-2), 4.43 (dd, J = 7.0, 8.2 Hz, Hα), 4.09 (q, J = 7 Hz, 1 H), 2.75 (m, H-1a), 2.70 (ddd, J = 4.4, 5.6, 17.0 Hz, H-5e), 2.56 (ddd, J = 5, 11.9, 17.0 Hz, H-5a), 2.17 (dqd, J = 1.2, 5, 13.4 Hz, H-6e), 2.00 (t, J = 7.5 Hz, CH_2), 1.79 (dddd, J = 4.4, 9.1, 11.9, 13.4 Hz, H-6a), 1.53 (d, J = 7 Hz, CH_3); MS (FAB) m/e 291, 289 (M + H), 192; IR (film) 3424, 1685, 1603, 1389, 1116 cm^{-1}; $[\alpha]^{20}_D$ +47.7° (c = 0.3, H_2O); HRMS calcd for $C_{12}H_{18}ClN_2O_4$ 289.0941, found 289.0955.

(*S*)-Alanyl-(*S*)-3-[(1*S*)-3-bromo-4-oxo-2-cyclohexen-1-yl]alanine (1b, bromotetaine). Prepared as **1a** starting from dipeptide **25b** in 34% yield: ^{1}H NMR (D_2O, the chemical shifts of both α-protons of the amino acids are concentration dependent!) δ 7.56 (d, J = 2 Hz, H-2), 4.29 (dd, J = 6, 9 Hz, Hα), 4.11 (q, J = 7 Hz, 1 H), 2.82–2.70 (m, H-1a, H-5e), 2.62 (ddd, J = 5, 11.9, 17.0 Hz, H-5a), 2.21 (qd, J = 5, 13.4 Hz, H-6e), 1.98 (t, J = 7.5 Hz, CH_2), 1.72 (m, H-6a), 1.56 (d, J = 7 Hz, CH_3); ^{13}C NMR (D_2O) δ 197.5 (s), 179.3 (s), 171.4 (s), 159.5 (d), 123.0 (s), 54.3 (d), 50.2 (d), 37.3 (t), 37.2 (d), 36.6 (t), 28.1 (t), 17.6 (q); MS (FAB) m/e 335, 333 (M + H), 307, 192, 176; IR (film) 3424, 1686, 1596, 1390, 1134 cm^{-1}; $[\alpha]^{20}_D$ +29.3° (c = 0.5, H_2O); HRMS calcd for $C_{12}H_{18}BrN_2O_4$ 335.0430, found 335.0422.

(*S*)-Alanyl-(*S*)-3-[(1*S*)-4-oxo-2-cyclohexen-1-yl]alanine (1c). Prepared as **1c** starting from dipeptide **25c** in 52% yield: ^{1}H NMR (D_2O, the chemical shifts of both α-protons of the amino acids are concentration dependent!) δ 7.11 (ddd, J = 1, 2.6, 10.2 Hz, H-2), 6.06 (dd, J = 2.2, 10.2 Hz, H-3), 4.31 (dd, J = 6, 8.5 Hz, Hα), 4.09 (q, J = 7 Hz, 1 H), 2.70–2.40 (m, H-1a, H-5e, H-5a), 2.18 (m, H-6e), 1.93 (m, CH_2), 1.75 (m, H-6a), 1.57 (d, J = 7 Hz, CH_3); MS (FAB) m/e 255 (M + H), 192, 154, 136; IR (film) 3433, 1672, 1595, 1394, 1132 cm^{-1}; $[\alpha]^{20}_D$ +62.1° (c = 0.5, H_2O); HRMS calcd for $C_{12}H_{19}N_2O_4$ 255.1344, found 255.1345.

(*N*-(Triphenylmethyl)-(*S*)-3-[(1*S*)-3-chloro-4-oxo-2-cyclohexen-1-yl]alanine Methyl Ester (28). The bislactim ether of alcohol **23** was hydrolyzed in quantitative yield as described for the preparation of **24a**. To the crude amine (300 mg), still containing D-valine methyl ester, dissolved in anhydrous methylene chloride (3.5 mL) were added triphenylmethyl chloride (933 mg, 3.33 mmol) followed by triethylamine (0.66 mL, 4.75 mmol). The resulting mixture was stirred at 23 °C for 4 h, and then ethyl acetate (20 mL) was added. The solution was washed with brine (20 mL), dried ($MgSO_4$), and evaporated. The crude

product was purified on Lobar LiChroprep CN (toluene) to provide 148 mg (20%, two steps) of the intermediate trityl derivative. The intermediate (119 mg, 0.25 mmol) was stirred for 2 h in methylene chloride (3.4 mL) in the presence of activated manganese dioxide (1.7 g). The mixture was filtered through Celite and the filtrate was evaporated. The crude product was purified on silica gel (1:1 ether–hexane, saturated with water) to provide 48 mg (40.5%) of the trityl derivative **28**. For the X-ray analysis the compound was crystallized from ether–hexane by slow evaporation over 3 d: mp 157 °C; ^{1}H NMR ($CDCl_3$) δ 7.41 (d, J = 7 Hz, 6 H), 7.25–7.15 (m, 9 H), 6.99 (d, J = 3.2 Hz, 1 H), 3.34 (m, 1 H), 3.13 (s, 3 H), 2.70–2.52 (m, 3 H), 2.40 (ddd, J = 5, 11, 17 Hz, 1 H), 1.97 (m, 1 H), 1.82 (m, 1 H), 1.75–1.60 (m, 2 H); MS (SIMS) 582, 580 (M + Ag), 396, 243; IR (film) 1721, 1698, 1204, 1167, 743, 709, 700; $[\alpha]^{20}_D$ +138.9° (c = 0.5, $CHCl_3$). Anal. Calcd for $C_{29}H_{28}ClNO_3$: C, 73.5; H, 6.0; N, 3.0. Found: C, 73.7; H, 6.6; N, 2.6.

(*S*)-(*tert*-Butyloxycarbonyl)alanyl-(*S*)-3-[(1*R*)-3-chloro-4-oxo-2-cyclohexen-1-yl]alanine Methyl Ester (30a). Chloro enone **30a** was prepared starting from ketone **17** using the same sequence as described for the synthesis of the epimer **25a**:

1. **(3*S*,6*R*,1′*R*)-2,5-Dimethoxy-6-isopropyl-3-[3-diazo-4-oxo-1-cyclohexyl)methyl]-3,6-dihydro-1,4-pyrazine.** Ketone **17** was deprotonated with (*R*,*R*)-bis(1-phenylethyl)amine[37] in the presence of lithium chloride and further converted into the diazo ketone as described for the epimer **19** in 32% yield. The diazo ketone had a de of 80%: ^{1}H NMR ($CDCl_3$) δ 4.00–3.85 (m, 2 H), 3.62 (s, 3 H), 3.60 (s, 3H), 2.80 (ddd, J = 1.7, 5, 14 Hz, 1 H), 2.50–1.70 (m, 6H), 1.70–1.35 (m, 3 H), 0.96 (d, J = 6.5 Hz, 3 H), 0.62 (d, J = 6.5 Hz, 3 H); MS (FAB) m/e 293 (M − N_2), 183, 141; IR (film) 2082, 1692, 1630, 1337, 1236 cm^{-1}; $[\alpha]^{20}_D$ −89.7° (c = 0.8, $CHCl_3$). No HRMS of M$^+$ was possible.

2. **(3*S*,6*R*,1′*R*)-2,5-Dimethoxy-6-isopropyl-3-[3-chloro-4-oxo-2-cyclohexen-1-yl)methyl]-3,6-dihydro-1,4-pyrazine.** The diazo ketone prepared above was converted to the 2-chloro enone as described for the epimer **20a** in 58% yield: ^{1}H NMR ($CDCl_3$) δ 7.17 (dd, J = 1, 3.3 Hz, 1H), 4.10–3.90 (m, 2 H), 3.72 (s, 3 H), 3.69 (s, 3 H), 2.85 (m, 1 H), 2.63 (td, J = 5.5, 15 Hz, 1 H), 2.43 (dd, J = 5, 15 Hz, 1 H), 2.30–1.93 (m, 3 H), 1.90–1.60 (m, 2 H), 0.97 (d, J = 7 Hz, 3 H), 0.63 (d, J = 7 Hz, 3 H); MS (FAB) m/e 329, 327 (M + H), 141; IR (film) 1694, 1239 cm^{-1}; $[\alpha]^{20}_D$ −41.3° (c = 0.5, $CHCl_3$). Anal. Calcd for $C_{16}H_{23}ClN_2O_3$: C, 58.8; H, 7.1; N, 8.6. Found: C, 58.6; H, 6.9; N, 9.0.

3. **(*S*)-(*tert*-Butyloxycarbonyl)alanyl-(*S*)-3-[(1*R*,4*S*/*R*)-3-chloro-4-hydroxy-2-cyclohexen-1-yl]alanine Methyl Ester (33).** The chloro enone prepared above was converted to the dipeptide **33** as described for the epimer **24a** in 44% overall yield. Compound **33** was a mixture of *trans*/*cis*-diastereomers: ^{1}H NMR ($CDCl_3$, *trans*-isomer) δ 6.60 (d, broad, J = 8.5 Hz, NH), 5.89 (d, J = 3 Hz, 1 H), 4.90 (broad, NH), 4.60 (m, 1 H), 4.15–4.00 (m, 2 H), 3.68 (s, 3 H), 2.30 (m, 1 H), 2.20 (d, J = 4 Hz, OH), 2.10–1.50 (m, 6 H), 1.39 (s, 9 H), 1.29 (d, J = 7.5 Hz, 3 H); MS (FAB) m/e 407, 405 (M + H), 349, 331, 287, 225, 216, 154, 136, 57; IR (film) 3382, 1670, 1526, 1367, 1249, 1170 cm^{-1}. Anal. Calcd for $C_{18}H_{29}ClN_2O_6$: C, 53.4; H, 7.2; N, 6.9. Found: C, 53.1; H, 7.3; N, 7.2.

4. Alcohol **33** was oxidized by manganese dioxide to ketone **30a** as described for the epimer **25a** in 54% yield. After chromatographic purification, compound **30a** was obtained with a de of 93% as a pale yellow foam: ^{1}H NMR ($CDCl_3$) δ 7.09 (d, J = 3.2 Hz, 1 H), 6.76 (broad, d, J = 8.3 Hz, NH), 4.82 (broad, NH), 4.69 (m, 1 H), 4.04 (m, 1 H), 3.70 (s, 3 H), 2.70–2.57 (m, 2 H), 2.43 (ddd, J = 4, 9.5, 13 Hz, 1 H), 2.12–1.95 (m, 2 H), 1.80–1.63 (m, 2 H), 1.38 (s, 9 H), 1.30 (d, J = 6.5 Hz, 3 H); MS (FAB) m/e 405, 403 (M + H), 391, 347, 307, 305, 303, 259, 219; IR (film) 3312, 1743, 1700, 1523, 1249, 1169 cm^{-1}; $[\alpha]^{20}_D$ −49.2° (c = 1, $CHCl_3$). Anal. Calcd for $C_{18}H_{27}ClN_2O_6$: C, 53.7; H, 6.8; N, 7.0. Found: C, 53.5; H, 7.0; N, 6.9.

(*S*)-(*tert*-Butyloxycarbonyl)alanyl-(*S*)-3-[(1*R*)-4-oxo-2-cyclohexen-1-yl]alanine Methyl Ester (30c). Enone **30c** was prepared starting from ketone **17** using a similar sequence as described for the synthesis of the epimer **25**:

1. **(3*S*,6*R*,1′*R*)-2,5-Dimethoxy-6-isopropyl-3-[[4-[(trimethylsilyl)oxy]-3-cyclohexen-1-yl]methyl]-3,6-dihydro-1,4-pyrazine.** Ketone **17** was deprotonated with (*R*,*R*)-bis(1-phenylethyl)amine[37] in the presence of lithium chloride and further converted into the silyl enol ether as described for the epimer **27** in 58% yield. The silyl enol ether had a de of 80%: ^{1}H NMR ($CDCl_3$) δ 4.81 (m, 1 H), 4.02 (m, 1 H), 3.93 (m, 1 H), 3.69 (s, 3 H), 3.68 (s, 3 H), 2.29 (m, 1 H), 2.20–1.90 (m, 3 H), 1.85–1.65 (m, 4 H), 1.52 (m, 1 H), 1.35 (m, 1 H), 1.07 (d, J = 6.5 Hz, 3 H), 0.69 (d, J = 6.5 Hz, 3 H); MS (FAB) m/e 367 (M$^+$), 323, 183, 141, 73; IR (film) 1695, 1437, 1303, 1239, 1193, 1015, 892, 846 cm^{-1}; $[\alpha]^{20}_D$ −13.6° (c = 1.2, $CHCl_3$). Anal. Calcd for $C_{19}H_{34}N_2O_3Si$: C, 62.3; H, 9.3; N, 7.6. Found: C, 62.5; H, 9.5; N, 7.9.

2. **(3*S*,6*R*,1′*R*)-2,5-Dimethoxy-6-isopropyl-3-[4-oxo-2-cyclohexen-1-yl)methyl]-3,6-dihydro-1,4-pyrazine.** To the silyl enol ether prepared above (500 mg, 1.4 mmol) dissolved in anhydrous acetonitrile (10 mL) were added palladium(II) acetate (31 mg, 0.14 mmol) and allyl methyl carbonate (0.31 mL, 2.7 mmol).[38] The reaction mixture was held under reflux for 3.5 h. The mixture was cooled to 23 °C and filtered over Celite. The filtrate was evaporated and the crude product was purified on silica gel (10:1 toluene–ethyl acetate) to provide 238 mg (60%) of the enone as a pale yellow oil, which contained less than 5% of the saturated ketone **17**: ^{1}H NMR ($CDCl_3$) δ 6.99 (ddd, J = 1.2, 3, 10.2 Hz, 1 H), 5.98 (dd, J = 2.3, 10.2 Hz, 1 H), 4.15–3.92 (m, 2 H), 3.70 (s, 3 H), 3.68 (s, 3H), 2.77 (m, 1 H), 2.50 (td, J = 5, 15 Hz, 1 H), 2.45–1.95 (m, 4 H), 1.95–1.40 (m, 3 H), 1.06 (d, J = 7 Hz, 3 H), 0.70 (d, J = 7 Hz, 3 H); MS (FAB) m/e 293 (M + H), 141; IR (film) 1692, 1240 cm^{-1}; HRMS calcd for $C_{16}H_{25}N_2O_3$ 293.1865, found 293.1851.

3. **(*S*)-(*tert*-Butyloxycarbonyl)alanyl-(*S*)-3-[(1*R*,4*S*/*R*)-4-hydroxy-2-cyclohexen-1-yl]alanine Methyl Ester.** The enone prepared above was converted to the dipeptide in a three-step sequence as described for the dipeptide **24a** in 20% overall yield. The dipeptide was a mixture of *trans*/*cis*-diastereoisomers: ^{1}H NMR ($CDCl_3$, *trans*-isomer) δ 6.50 (broad, NH), 5.78–5.70 (m, 2 H), 4.95 (broad, NH), 4.65 (m, 1 H), 4.13 (m, 1 H), 3.73 (s, 3 H), 3.52 (m, 1 H), 2.00–1.45 (m, 7 H), 1.43 (s, 9 H), 1.35 (d, J = 7.5 Hz, 3 H); MS (FAB) m/e 371 (M + H), 317, 297, 273; IR (film) 3321, 1665, 1526, 1367, 1249, 1170 cm^{-1}. No HRMS of M$^+$ was possible.

4. The dipeptide prepared above was oxidized by manganese dioxide to ketone **30c** as described for the ketone **25a** in 20% yield. Compound **30c** was obtained with a de of 80% as a pale yellow foam, which was only about 85% pure: ^{1}H NMR ($CDCl_3$) δ 6.95 (dd, J = 3, 10 Hz, 1 H), 6.70 (broad, d, J = 8.4 Hz, NH), 6.00 (dd, J = 2.3, 10 Hz, 1 H), 4.92 (broad, NH), 4.73 (m, 1 H), 4.13 (m, 1 H), 3.77 (s, 3 H), 2.60–2.35 (m, 3 H), 2.32–1.62 (m, 4 H), 1.43 (s, 9 H), 1.37 (d, J = 7.5 Hz, 3 H); MS (FAB) m/e 369 (M + H), 271, 269, 198, 57; IR (film) 3330, 1736, 1676, 1508, 1212, 1168 cm^{-1}; $[\alpha]^{20}_D$ −42.2° (c = 1, $CHCl_3$). No HRMS of M$^+$ was possible.

(*S*)-(*tert*-Butyloxycarbonyl)alanyl-(*S*)-3-[(1*R*)-3-chloro-4-oxo-2-cyclohexen-1-yl]alanine *p*-Methoxybenzyl Ester (34). Methyl ester **33** (1.1 g, 2.33 mmol; preparation see under **30a**) was dissolved in THF (23 mL) and cooled to 0 °C. A 0.1 N NaOH solution was added and the solution was stirred for 30 min at 23 °C. The reaction mixture was partitioned between ethyl acetate (30 mL) and 1 N HCl (30 mL) and the aqueous layer was extracted with ethyl acetate (2 × 30 mL). The combined organic layers were dried ($MgSO_4$) and evaporated to provide 837 mg (92%) of crude (*S*)-(*tert*-butyloxycarbonyl)alanyl-(*S*)-3-[(1*R*,4*S*/*R*)-3-chloro-4-hydroxy-2-cyclohexen-1-yl]alanine. To a portion of this material (300 mg, 0.77 mmol) dissolved in anhydrous DMF (3 mL) were added dicyclohexylamine (140 mg, 077 mmol) followed by *p*-methoxybenzyl bromide (155 mg, 0.77 mmol) dissolved in anhydrous DMF (0.6 mL). The resulting mixture was stirred for 18 h at 23 °C. Ethyl acetate (10 mL) and water (10 mL) were added and the aqueous layer was extracted with ethyl acetate (2 × 10 mL). The combined organic layers were washed with saturated citric acid (10 mL), saturated sodium bicarbonate solution (10 mL), and brine (10 mL), dried ($MgSO_4$), and evaporated. The crude product was purified on silica gel (1:1 toluene–ethyl acetate) to provide 232 mg (59%) of (*S*)-(*tert*-butyloxycarbonyl)alanyl-(*S*)-3-[(1*R*,4*R*/*S*)-3-chloro-4-hydroxy-2-cyclohexen-1-yl]alanine *p*-methoxybenzyl ester as a white foam (70:30 mixture of *cis*/*trans* diastereoisomers): ^{1}H NMR ($CDCl_3$)

(38) Minami, I.; Takahashi, K.; Shimizu, I.; Kimura, T.; Tsuji, J. *Tetrahedron* **1986**, *42*, 2971.

δ 7.27 (d, J = 8 Hz, 2 H), 6.88 (d, J = 8 Hz, 2 H), 6.62 (broad, d, J = 8 Hz, NH), 5.89 and 5.83 (2d, J = 3 Hz, 1 H), 5.15 (d, J = 12 Hz, 1 H), 5.08 (d, J = 12 Hz, 1 H), 4.92 (broad, NH), 4.63 (m, 1 H), 4.20–4.00 (m, 2 H), 3.81 (s, 3 H), 2.30–1.50 (m, 7 H), 1.43 (s, 9 H), 1.34 (d, J = 6.5 Hz, 3 H); MS (FAB) m/e 511 (M + H), 411, 121; IR (film) 3330, 1694, 1617, 1451, 1367, 1250, 1173 cm^{-1}; HRMS calcd for $C_{25}H_{36}ClN_2O_7$ 511.2211, found 511.2218.

The dipeptide obtained above (217 mg, 0.42 mmol) in anhydrous methylene chloride (10 mL) was stirred for 4 h at 23 °C in the presence of manganese dioxide (1.7 g, 19.6 mmol). The reaction mixture was filtered over Celite and the filtrate was evaporated. The crude product was purified on silica gel (2:1 toluene–ethyl acetate) to provide 116 mg (54%) of ketone **34** as a white foam with a de of >90%: ^{1}H NMR ($CDCl_3$) δ 7.29 (d, J = 8 Hz, 2 H), 7.09 (dd, J = 1, 3 Hz, 1 H), 6.88 (d, J = 8 Hz, 2 H), 6.71 (broad, d, J = 8 Hz, NH), 5.18 (d, J = 12 Hz, 1 H), 5.10 (d, J = 12 Hz, 1 H), 4.90 (broad, NH), 4.72 (m, 1 H), 4.12 (m, 1 H), 3.81 (s, 3H), 2.70–2.52 (m, 2 H), 2.43 (m, 1 H), 2.10–1.95 (m, 2 H), 1.80–1.55 (m, 2 H), 1.43 (s, 9 H), 1.36 (d, J = 6.5 Hz, 3 H); MS (FAB) m/e 509 (M + H; to weak for HRMS) 459, 409, 121, 57; IR (film) 3386, 1702, 1514, 1458, 1367, 1249, 1171 cm^{-1}; $[\alpha]^{20}_D$ −39.9° (c = 1, $CHCl_3$). No HRMS of M^+ was possible.

(*S*)-Alanyl-(*S*)-3-[(1*R*)-3-chloro-4-oxo-2-cyclohexen-1-yl]-alanine (4a). Attempted preparation starting from methyl ester **30a**: Using the procedure described for the preparation of chlorotetaine **1a**, the Boc-protecting group of **30a** was cleaved to give trifluoroacetate **31a** in quantitative yield: ^{1}H NMR (DMSO-d_6) δ 8.85 (d, J = 9 Hz, NH), 8.11 (broad, NH_3^+), 7.30 (d, J = 3 Hz, 1 H), 4.60 (m, 1 H), 3.90 (m, 1 H), 3.67 (s, 3 H), 2.80–2.45 (m, 3 H), 2.15–1.97 1.62 (m, 2 H), 1.83–1.65 (m, 2 H), 1.38 (d, J = 6.5 Hz, 3 H). Reaction of **31a** with porcine pancreas lipase at pH 7.5 did not yield dipeptide **4a**, but a product mixture containing the octahydroindole diastereoisomers **32** and other decomposition products.

Preparation of **4a** starting from *p*-methoxybenzyl ester **34**: To an ice-cold solution of dipeptide **34** (95 mg, 0.187 mmol) in methylene chloride (2 mL) were added anisole (92 μL) and trifluoroacetic acid (0.94 mL). After 1 h at 0 °C the solution was evaporated and treated with ether (1 mL) until the residue became solid. The ether was decanted and the residue washed with ether (1 mL) and dried ($MgSO_4$) to provide 73 mg (97%) of the trifluoroacetate of **4a** as a white solid material. An amount of 35 mg of this salt were dissolved in a minimum amount of pH 6 phosphate buffer and chromatographed [reverse-phase (water)] to provide 17 mg of dipeptide **4a** after lyophilization at −20 °C (important: **4a** has to be kept cold during the whole lyophilization process!) as a white and very labile material: ^{1}H NMR (D_2O, the chemical shifts of both α-protons of the amino acids are concentration dependent!) δ 7.38 (broad s, H-2), 4.33 (m, Hα), 4.09 (q, J = 7 Hz, 1 H), 2.80–2.50 (m, 3 H), 2.19 (dd, J = 5, 10 Hz, 1 H), 2.09 (m, 1 H), 2.00–1.80 (m, 2 H), 1.56 (d, J = 6.5 Hz, CH_3); MS (FAB) m/e 291, 289 (M + H), 218, 192, 176; IR (film) 3389, 1686, 1598, 1389 cm^{-1}; $[\alpha]^{20}_D$ −24.0° (c = 0.3, H_2O); HRMS calcd for $C_{12}H_{18}ClN_2O_4$ 289.0941, found 289.0955.

(*S*)-Alanyl-(*S*)-3-[(1*R*)-4-oxo-2-cyclohexen-1-yl]alanine (4c). Prepared as **1c** starting from dipeptide **30c** in 50% yield. Because of the lower stability of intermediate **31c** compared to the epimer **26c** the de drops from 80% for dipeptide **30c** to 30% for betaine **4c**: ^{1}H NMR (D_2O, the chemical shifts of both α-protons of the amino acids are concentration dependent!) δ 7.19 (dd, J = 2.5, 10.5 Hz, H-2), 6.06 (dd, J = 2.5, 10.5 Hz, H-3), 4.33 (dd, J = 5, 9 Hz, Hα), 4.09 (q, J = 7 Hz, 1 H), 2.68–2.42 (m, 3 H), 2.18 (m, 1 H), 2.03 (m, 1 H), 1.95 (m, 1 H), 1.86–1.70 (m, 1 H), 1.57 (d, J = 7 Hz, CH_3); MS (FAB) m/e 255 (M + H); IR (film) 3386, 1671, 1590, 1390, 1123 cm^{-1}; $[\alpha]^{20}_D$ −6.3° (c = 0.3, H_2O, de = 30%!); HRMS calcd for $C_{12}H_{19}N_2O_4$ 255.1345, found 255.1355.

(Allyloxycarbonyl)-(*S*)-3-[(1*S*,4*S*/*R*)-4-hydroxy-2-cyclohexen-1-yl]alanine Methyl Ester (37). The bislactim ether of alcohol **23c** was hydrolyzed in quantitative yield as described for the preparation of dipeptide **24a**. To a solution of the crude amine (355 mg, 1.78 mmol) still containing D-valine methyl ester in methanol (9 mL) were added sodium bicarbonate (224 mg, 2.67 mmol) and allyl chloroformate (255 μL, 2.4 mmol). The mixture was stirred for 30 min at 23 °C. Water was added (25 mL) and the solution was extracted with ethyl acetate (2 × 25 mL). The organic layers were combined, dried ($MgSO_4$), and evaporated. The crude product was purified on silica gel (2:1 toluene–ethyl acetate) to provide 286 mg (57%) of amino acid **37** as a pale yellow oil (mixture of *cis*/*trans*-diastereoisomers): ^{1}H NMR ($CDCl_3$) δ 6.00–5.52 (m, 3 H), 5.30 (dd, J = 2, 18 Hz, 1 H), 5.23 (dd, J = 2, 12 Hz, 1 H), 5.15 (broad, d, J = 8 Hz, NH), 4.58 (d, J = 6 Hz, 2 H), 4.44 (m, 1 H), 4.20 (m, 1 H), 3.73 (s, 3H), 2.23 (m, 1 H), 2.03 (m, 1 H), 1.83–1.45 (m, 6 H); MS (SIMS) m/e 392, 390 (M + Ag), 266; IR (film) 3319, 1702, 1529, 1440, 1238 cm^{-1}. Anal. Calcd for $C_{14}H_{21}NO_5$: C, 59.4; H, 7.5; N, 4.9. Found: C, 59.1; H, 7.6; N, 4.4.

(Allyloxycarbonyl)-(*S*)-3-[(1*S*,2*R*,3*R*)-2,3-epoxy-4-oxo-1-cyclohexyl]alanine Methyl Ester (39) and (Allyloxycarbonyl)-(*S*)-3-[(1*S*,2*S*,3*S*)-2,3-epoxy-4-oxo-1-cyclohexyl]alanine Methyl Ester (40). Allyl alcohol **37** (286 mg, 1.01 mmol) and *m*-CPBA (218 mg, 1.01 mmol) in anhydrous methylene chloride (6.2 mL) were stirred at 0 °C for 11 h. A 0.5 N sodium carbonate solution (10 mL) and brine (10 mL) were added and the aqueous layer was extracted with methylene chloride (10 × 10 mL). The organic layers were combined, dried ($MgSO_4$), and evaporated to provide 301 mg (100%) of the crude epoxide **38** as a 70:30 mixture of *trans*- and *cis*-diastereoisomers. ^{1}H NMR ($CDCl_3$) Characteristic signals of the *trans*-epoxide: δ 3.30 (m, H-3), 3.06 (d, J = 4 Hz, H-2); characteristic signals of the *cis*-epoxide: δ 3.40 (t, J = 3.7 Hz, H-2), 3.27 (m, H-3).

Chromium trioxide (233 mg, 2.32 mmol) was added to an ice-cold solution of pyridine (0.38 mL, 4.65 mmol) in anhydrous methylene chloride (5 mL). The mixture was stirred for 15 min at 23 °C. A solution of crude epoxide **38** (116 mg, 0.39 mmol) in anhydrous methylene chloride (1 mL) was added and the mixture was stirred for an additional 15 min at 23 °C. The solvent was decanted from a black tar and the residue was washed with methylene chloride (2 × 5 mL). The solutions were combined and washed with 0.5 N sodium carbonate solution (25 mL) and brine (2 × 25 mL). The combined aqueous layers were reextracted with methylene chloride (2 × 25 mL) and the organic layers were combined, dried ($MgSO_4$), and evaporated. The crude product was purified on silica gel (2:1 ether–hexane) to provide as a first fraction 63 mg (54%) *trans*-epoxide **40** followed by 35 mg (30%) of the *cis*-epoxide **39** both as white foams. **39**: ^{1}H NMR ($CDCl_3$) δ 6.03–5.80 (m, 1 H), 5.33 (d, J = 18 Hz, 1 H), 5.23 (dd, J = 12 Hz, 1 H), 5.40–5.20 (hidden, NH), 4.60 (d, J = 6 Hz, 2 H), 4.46 (m, 1 H), 3.79 (s, 3 H), 3.42 (d, J = 4 Hz, 1 H), 3.24 (d, J = 4 Hz, 1 H), 2.54 (td, J = 5, 19 Hz, 1 H), 2.35 (m, 1 H), 2.26–1.65 (m, 5 H); MS (FAB) m/e 298 (M + H), 259, 219; IR (film) 3357, 1722, 1707, 1526 cm^{-1}; $[\alpha]^{20}_D$ +78.3° (c = 1, $CHCl_3$). Anal. Calcd for $C_{14}H_{19}NO_6$: C, 56.6; H, 6.4; N, 4.7. Found: C, 56.5; H, 6.4; N, 4.8. **40**: ^{1}H NMR ($CDCl_3$) δ 6.02–5.80 (m, 1 H), 5.33 (d, J = 18 Hz, 1 H), 5.23 (dd, J = 12 Hz, 1 H), 5.40–5.20 (hidden, NH), 4.60 (d, J = 6 Hz, 2 H), 4.46 (m, 1 H), 3.78 (s, 3 H), 3.40 (dd, J = 2, 3.8 Hz, 1 H), 3.22 (d, J = 3.8 Hz, 1 H), 2.60–2.43 (m, 2 H), 2.30–2.10 (m, 2 H), 1.90–1.60 (m, 3 H); MS (FAB) m/e 298 (M + H), 238, 219; IR (film) 3384, 1706, 1525, 1219 cm^{-1}; $[\alpha]^{20}_D$ −16.0° (c = 1, $CHCl_3$). Anal. Calcd for $C_{14}H_{19}NO_6$: C, 56.6; H, 6.4; N, 4.7. Found: C, 56.6; H, 6.6; N, 4.4.

(*S*)-3-[(1*S*,2*R*,3*R*)-2,3-Epoxy-4-oxo-1-cyclohexyl]alanine (3, anticapsin). To the protected amino acid **39** (100 mg, 0.34 mmol) dissolved in anhydrous methylene chloride (0.65 mL) were added *N*,*N*-dimethyl(trimethylsilyl)amine (55 μL, 0.34 mmol) and tetrakis(triphenylphosphine)palladium (6 mg, 6 μmol). The mixture was stirred for 15 min at 23 °C, evaporated, redissolved in THF (7.5 mL), and cooled to 0 °C. A 0.1 N NaOH solution (13 mL) was added slowly and the resulting solution was stirred at 0 °C for 30 min. The reaction mixture was brought to pH 7.5 with 1 N HCl and extracted with ether (3 × 20 mL). The aqueous layer was lyophilized and the crude product was purified [reverse-phase (water)] to provide after lyophilization 37 mg (58%) of anticapsin **3** as a white powder. In solution, **3** was in equilibrium with 23% of the corresponding hydrate **56**: ^{1}H NMR (D_2O, the chemical shift of the α-proton of the amino acid is concentration dependent!) δ 3.71 (t, J = 7 Hz, 1 H), 3.72 (d, J = 4 Hz, 1 H), 3.50 (dd, J = 3, 4 Hz, 1 H of the hydrate **56**), 3.39 (d, J = 4 Hz, 1 H), 3.28 (d, J = 4 Hz, 1 H of the hydrate **56**), 2.53 (td, J = 5, 16 Hz, 1 H), 2.35 (m, 1H), 2.10–1.90 (m, 3 H), 1.77 (m, 1 H), 1.60 (m, 1 H); ^{13}C NMR (D_2O) ketone δ 211.0, 177.2, 60.3, 56.9, 54.3, 36.3,

35.3, 31.3, 22.4; hydrate δ 177.1, 93.3, 59.7, 59.0, 54.6, 31.1, 22.7; IR (film) 3432, 1718, 1654 cm^{-1}; $[\alpha]^{20}_D$ +32.3° (c = 0.3, H_2O).

(S)-(Allyloxycarbonyl)alanine-(S)-3-[(1S,4S/R)-4-hydroxy-2-cyclohexen-1-yl]alanine Methyl Ester (42). The bislactim ether of alcohol **23c** was hydrolyzed and the resulting amine was coupled with (S)-(allyloxycarbonyl)alanine as described for the preparation of dipeptide **24a** to give **42** in 53% overall yield as a mixture of *cis*/*trans*-diastereoisomers: ^{1}H NMR ($CDCl_3$) δ 6.45 (broad, NH), 6.00–5.52 (m, 3 H), 5.30 (d, J = 18 Hz, 1 H), 5.23 (d, J = 12 Hz, 1 H), 5.25 (hidden, NH), 4.67 (m, 1 H), 4.55 (d, J = 6 Hz, 2 H), 4.30–4.10 (m, 2 H), 3.72 (s, 3 H), 2.20–1.90 (m, 2 H), 1.80–1.45 (m, 6 H), 1.40 (d, J = 6.5 Hz, 3 H); MS (FAB) *m/e* 355 (M + H), 337, 182, 154, 136; IR (film) 3298, 1714, 1665, 1535, 1449, 1250 cm^{-1}; HRMS calcd for $C_{17}H_{27}N_2O_6$ 355.1869, found 355.1899.

(S)-(Allyloxycarbonyl)alanine-(S)-3-[(1S,2R,3R)-2,3-epoxy-4-oxo-1-cyclohexyl]alanine Methyl Ester (43) and (S)-(Allyloxycarbonyl)alanine-(S)-3-[(1S,2S,3S)-2,3-epoxy-4-oxo-1-cyclohexyl]alanine Methyl Ester (44). Allyl alcohol **42** was first converted to the epoxide and then oxidized to the ketone as described for **39/40**. The crude product was purified on silica gel (2:1 ether–hexane) to provide as a first fraction *trans*-epoxide **44** (43% yield) followed by the *cis*-epoxide **43** (31% yield), both as white foams. **43**: ^{1}H NMR ($CDCl_3$) δ 6.48 (broad d, J = 8 Hz, NH), 6.00–5.80 (m, 1 H), 5.30 (d, J = 18 Hz, 1 H), 5.20 (dd, J = 12 Hz, 1 H), 5.18 (broad, NH), 4.72 (m, 1 H), 4.55 (d, J = 6 Hz, 2 H), 4.20 (m, 1H), 3.79 (s, 3 H), 3.42 (d, J = 3.9 Hz, 1 H), 3.24 (d, J = 3.9 Hz, 1 H), 2.50 (td, J = 5, 19 Hz, 1 H), 2.35 (m, 1 H), 2.23–1.92 (m, 3 H), 1.85–1.60 (m, 2 H); MS (FAB) *m/e* 369 (M + H); IR (film) 3329, 1714, 1670, 1528, 1241 cm^{-1}; $[\alpha]^{20}_D$ +39.5° (c = 0.8, $CHCl_3$); HRMS calcd for $C_{17}H_{25}N_2O_7$ 369.1662, found 369.1653. **44**: ^{1}H NMR ($CDCl_3$) δ 6.60 (broad d, J = 8 Hz, NH), 6.00–5.80 (m, 1 H), 5.32 (d, J = 18 Hz, 1 H), 5.23 (d, J = 12 Hz, 1 H), 5.17 (broad, NH), 4.70 (m, 1 H), 4.58 (d, J = 6 Hz, 2 H), 4.23 (m, 1H), 3.76 (s, 3 H), 3.39 (dd, J = 2, 3.8 Hz, 1 H), 3.21 (d, J = 3.8 Hz, 1 H), 2.60–2.45 (m, 3 H), 2.23–2.10 (m, 2 H), 1.95–1.70 (m, 2 H), 1.60 (m, 1 H), 1.40 (d, J = 6.5 Hz, 3 H); MS (FAB) *m/e* 369 (M + H), 154, 69, 55; IR (film) 3296, 1714, 1529, 1240 cm^{-1}; $[\alpha]^{20}_D$ −32.9° (c = 0.9, $CHCl_3$). HRMS calcd for $C_{17}H_{25}N_2O_7$ 369.1662, found 369.1645.

(S)-Alanine-(S)-3-[(1S,2R,3R)-2,3-epoxy-4-oxo-1-cyclohexyl]alanine (2, bacilysin). The protected amino acid **43** was converted to bacilysin **2** as described for the synthesis of anticapsin **3** (yield 95%). Bacilysin **2** was obtained as a white powder. In solution, **2** was in equilibrium with 20% of the corresponding hydrate **56**: ^{1}H NMR (D_2O, the chemical shifts of the α-protons of the amino acids are concentration dependent!) δ 4.32 (dd, J = 5.5, 9 Hz, 1 H), 3.80 (q, J = 7 Hz, 1 H), 3.68 (d, J = 4 Hz, 1 H), 3.45 (dd, J = 2.5, 4 Hz, 1 H of the hydrate **56**), 3.38 (d, J = 4 Hz, 1 H), 3.25 (d, J = 4 Hz, 1 H of the hydrate **56**), 2.50 (td, J = 4, 16 Hz, 1 H), 2.30 (m, 1 H), 2.10–1.95 (m, 2 H), 1.85 (m, 1 H), 1.73 (m, 1 H), 1.55 (m, 1 H), 1.37 (d, J = 7 Hz, 3 H); IR (film) 3422, 1602, 1396, 1120 cm^{-1}; $[\alpha]^{20}_D$ +63.0° (c = 0.45, H_2O).

(S)-Alanine-(S)-3-[(1S,2S,3S)-2,3-epoxy-4-oxo-1-cyclohexyl]alanine (45). The protected amino acid **44** was converted to betaine **45** as described for the synthesis of anticapsin **3** (yield 52%). **45** was obtained as a white powder containing smaller amounts of contaminants, probably cyclization products. In solution **45** was in equilibrium with 20% of the corresponding hydrate **57**: ^{1}H NMR (D_2O, the chemical shifts of the α-protons of the amino acids are concentration dependent!) δ 4.28 (dd, J = 5, 8 Hz, 1 H), 3.72 (q, J = 7 Hz, 1 H), 3.63 (dd, J = 2.1, 4 Hz, 1 H), 3.35 (d, J = 4 Hz, 1 H), 3.25 (d, J = 4 Hz, 1 H of the hydrate **57**), 3.20 (d, J = 4 Hz, 1 H of the hydrate **57**), 2.60–2.20 (m, 3 H), 2.10–1.95 (m, 2 H), 1.90 (m, 1 H), 1.62 (m, 1 H), 1.38 (d, J = 7 Hz, 3 H); $[\alpha]^{20}_D$ −4.3° (c = 0.3, H_2O).

(S)-3-[(1R,2R,3R)-2,3-epoxy-4-oxo-1-cyclohexyl]alanine (46). The amino acid **46** was prepared starting from (3S,6R,1′R)-2,5-dimethoxy-6-isopropyl-3-[4-oxo-2-cyclohexen-1-yl)methyl]-3,6-dihydro-1,4-pyrazine (preparation see under **30c**) using the same reaction sequence as described for the synthesis of the epimer **3** (anticapsin):

1. **(Allyloxycarbonyl)-(S)-3-[(1R,4S/R)-4-hydroxy-2-cyclohexen-1-yl]alanine Methyl Ester.** (3S,6R,1′R)-2,5-Dimethoxy-6-isopropyl-3-[4-oxo-2-cyclohexen-1-yl)methyl]-3,6-dihydro-1,4-pyrazine was converted into the title amino acid by a three-step sequence in 74% overall yield as described for the preparation of epimer **37**. The amino acid was a mixture of *cis*/*trans*-diastereoisomers: ^{1}H NMR ($CDCl_3$) δ 6.00–5.70 (m, 3 H), 5.30 (d, J = 18 Hz, 1 H), 5.21 (d, J = 12 Hz, 1 H), 5.15 (broad, NH), 4.59 (d, J = 6 Hz, 2 H), 4.45 (m, 1 H), 4.20 (m, 1 H), 3.72 (s, 3 H), 2.23 (m, 1 H), 2.13–1.40 (m, 7 H); MS (FAB) *m/e* 284 (M + H), 266; IR (film) 3349, 1705, 1530, 1442, 1326, 1219 cm^{-1}. No HRMS of M$^+$ was possible.

2. **(Allyloxycarbonyl)-(S)-3-[(1R,2S,3S)-2,3-epoxy-4-oxo-1-cyclohexyl]alanine Methyl Ester and (Allyloxycarbonyl)-(S)-3-[(1R,2R,3R)-2,3-epoxy-4-oxo-1-cyclohexyl]alanine Methyl Ester.** The allyl alcohol obtained above was epoxidized with *m*-CPBA as described for the preparation of the epoxy ketones **39/40** to provide the crude epoxide in 97% yield as a 70:30 mixture of *trans*- and *cis*-diastereoisomers. ^{1}H NMR ($CDCl_3$) Characteristic signals of the *trans*-epoxide: δ 3.30 (m, H-3), 3.12 (d, J = 4 Hz, H-2); characteristic signals of the *cis*-epoxide: δ 3.47 (m, H-3), 3.42 (t, J = 3.5 Hz, H-2).

The crude intermediate was further oxidized to the ketone as described for the preparation for the epimeric ketones **39/40**. The ketones were obtained in 59% yield as a 35:65 mixture of *cis*/*trans*-diastereoisomers, which could not be separated by chromatography. ^{1}H NMR ($CDCl_3$) (1*R*,2*S*,3*S*)-diastereoisomer: δ 6.03–5.80 (m, 1 H), 5.30 (d, J = 18 Hz, 1 H), 5.23 (dd, J = 12 Hz, 1 H), 5.40–5.20 (hidden, NH), 4.60 (d, J = 6 Hz, 2 H), 4.50 (m, 1 H), 3.79 (s, 3 H), 3.68 (d, J = 4 Hz, 1 H), 3.27 (d, J = 4 Hz, 1 H), 2.60–2.40 (m, 2 H), 2.30–1.90 (m, 3 H), 1.80–1.50 (m, 2 H); (1*R*,2*R*,3*R*)-diastereoisomer: 6.03–5.80 (m, 1 H), 5.30 (d, J = 18 Hz, 1 H), 5.23 (dd, J = 12 Hz, 1 H), 5.40–5.20 (hidden, NH), 4.60 (d, J = 6 Hz, 2 H), 4.50 (m, 1 H), 3.78 (s, 3 H), 3.54 (m, 1 H), 3.24 (d, J = 3.8 Hz, 1 H), 2.60–2.40 (m, 2 H), 2.30–1.90 (m, 3 H), 1.80–1.50 (m, 2 H); MS (FAB) *m/e* 298 (M + H), 289, 259, 219; IR (film) 3551, 1712, 1531, 1216 cm^{-1}; HRMS calcd for $C_{14}H_{20}NO_6$ 298.1291, found 298.1287.

3. The mixture of the ketones prepared above was deprotected as described for the synthesis of anticapsin **3**. Under these reaction conditions only the *cis*-(1*R*,2*S*,3*S*)-diastereoisomer was cleanly deprotected to amino acid **46**, whereas the *trans*-(1*R*,2*R*,3*R*)-diastereoisomer deteriorated. After purification, as described for epimer **3**, betaine **46** was obtained in 16% yield (46% calculated for the *cis*-diastereoisomer). In solution the ketone **46** was in equilibrium with 23% of the corresponding hydrate **56**: ^{1}H NMR (D_2O, the chemical shift of the α-proton of the amino acid is concentration dependent!) δ 3.80 (t, J = 7 Hz, 1 H), 3.71 (d, J = 4 Hz, 1 H), 3.48 (dd, J = 2.6, 4 Hz, 1 H of the hydrate **56**), 3.40 (d, J = 4 Hz, 1 H), 3.29 (d, J = 4 Hz, 1 H of the hydrate **56**), 2.50 (m, 1 H), 2.35 (m, 1H), 2.30–2.10 (m, 2 H), 1.92 (m, 1 H), 1.75 (m, 1 H), 1.60 (m, 1 H); ^{13}C NMR (D_2O) ketone δ 210.4, 175.3, 59.8, 56.3, 53.8, 35.4 (2 C), 34.4, 31.0, 21.8; hydrate δ 175.5, 92.7, 59.2, 58.4, 53.5, 30.7, 22.1; IR (film) 3424, 1708, 1638, 1406 cm^{-1}; $[\alpha]^{20}_D$ −42.4° (c = 0.6, H_2O).

(S)-Alanine-3-[(1R,2R,3R)-2,3-epoxy-4-oxo-1-cyclohexyl]-alanine (47)/(S)-Alanine-3-[(1R,2S,3S)-2,3-epoxy-4-oxo-1-cyclohexyl]alanine (5). The mixture of dipeptides **5** and **47** was prepared starting from (3S,6R,1′R)-2,5-dimethoxy-6-isopropyl-3-[4-oxo-2-cyclohexen-1-yl)methyl]-3,6-dihydro-1,4-pyrazine (preparation see under **30c**) using exactly the same reaction sequence as described for the epimer **2** (bacilysin):

1. **(S)-(Allyloxycarbonyl)alanine-(S)-3-[(1R,4S/R)-4-hydroxy-2-cyclohexen-1-yl]alanine Methyl Ester.** (3S,6R,1′R)-2,5-Dimethoxy-6-isopropyl-3-[4-oxo-2-cyclohexen-1-yl)methyl]-3,6-dihydro-1,4-pyrazine was converted into the title dipeptide by a three-step sequence in 38% overall yield as described for the preparation of epimer **42**. The amino acid was a mixture of *cis*/*trans*-diastereoisomers: ^{1}H NMR ($CDCl_3$) δ 6.60–5.90 (m, NH), 6.00–5.70 (m, 3 H), 5.32 (d, J = 18 Hz, 1 H), 5.30 (broad, NH), 5.22 (d, J = 12 Hz, 1 H), 4.70 (m, 1 H), 4.56 (d, J = 6 Hz, 2 H), 4.30–4.15 (m, 2 H), 3.72 (s, 3 H), 2.18 (m, 1 H), 2.10–1.20 (m, 7 H), 1.38 (d, J = 7 Hz, 3 H); MS (FAB) *m/e* 355 (M + H), 337, 226; IR (film) 3333, 1722, 1659, 1546, 1446, 1247 cm^{-1}. No HRMS of M$^+$ was possible.

2. **(S)-(Allyloxycarbonyl)alanine-(S)-3-[(1R,2S,3S-2,3-epoxy-4-oxo-1-cyclohexyl]alanine Methyl Ester and (S)-(Allyloxycarbonyl)alanine-(S)-3-[(1R,2R,3R)-2,3-epoxy-4-oxo-1-cyclohexyl]alanine Methyl Ester.** The allyl alcohol

obtained above was epoxidized with *m*-CPBA and then further oxidized to the ketone as described for the preparation of the diastereomeric ketones **43**/**44**. The ketones were obtained in 42% yield as a 30:70 mixture of *cis*/*trans*-diastereoisomers, which could not be separated by chromatography: ^{1}H NMR ($CDCl_3$) *cis*-(1*R*,2*S*,3*S*)-diastereoisomer δ 6.57 (broad, d, 8 Hz, NH), 5.92–5.75 (m, 1 H), 5.32 (d, J = 18 Hz, 1 H), 5.30 (broad, NH), 5.20 (dd, J = 12 Hz, 1 H), 4.77 (m, 1 H), 4.52 (d, J = 6 Hz, 2 H), 4.20 (m, 1 H), 3.70 (s, 3 H), 3.60 (d, J = 4 Hz, 1 H), 3.19 (d, J = 4 Hz, 1 H), 2.50–2.30 (m, 2 H), 2.20–1.90 (m, 3 H), 1.80–1.50 (m, 2 H), 1.33 (d, J = 6.5 Hz, 3 H); *trans*-(1*R*,2*R*,3*R*)-diastereoisomer 6.79 (broad, d, J = 8 Hz, NH), 6.92–5.75 (m, 1 H), 5.30 (broad, NH), 5.28 (d, J = 18 Hz, 1 H), 5.16 (dd, J = 12 Hz, 1 H), 4.65 (m, 1 H), 4.50 (d, J = 6 Hz, 2 H), 4.20 (m, 1 H), 3.70 (s, 3 H), 3.46 (dd, J = 2.2, 3.8 Hz, 1 H), 3.16 (d, J = 3.8 Hz, 1 H), 2.45–2.30 (m, 2 H), 2.20–1.90 (m, 3 H), 1.80–1.40 (m, 2 H), 1.33 (d, J = 6.5 Hz, 3 H); MS (FAB) *m*/*e* 369 (M + H), 307, 289, 154, 136; IR (film) 3321, 1714, 1689, 1538, 1445, 1241 cm^{-1}; HRMS calcd for $C_{17}H_{25}N_2O_7$ 369.1662, found 369.1668.

3. The mixture of ketones obtained above was deprotected and purified as described for the synthesis of bacilysin **2** in 33% yield. The 60:40 mixture of **5** and **47** could not be separated by chromatography. In solution **5** was in equilibrium with 25% of the corresponding hydrate **57** and **47** with 20% of the hydrate **56**. ^{1}H NMR (D_2O, the chemical shifts of the α-protons of the amino acids are concentration dependent!) Characteristic signals for **5**: δ 4.34 (dd, J = 5.5, 8 Hz, 1 H), 3.82 (q, J = 7 Hz, 1 H), 3.70 (dd, J = 2.1, 4 Hz, 1 H), 3.36 (d, J = 4 Hz, 1 H), 3.32 (d, J = 4 Hz, 1 H of the hydrate **57**), 3.20 (d, J = 4 Hz, 1 H of the hydrate **57**); characteristic signals for **47**: δ 4.40 (dd, J = 5, 9.5 Hz, 1 H), 3.81 (q, J = 7 Hz, 1 H), 3.73 (d, J = 4 Hz, 1 H), 3.46 (dd, J = 2.5, 4.2 Hz, 1 H of the hydrate **46**), 3.37 (d, J = 4 Hz, 1 H), 3.26 (d, J = 4.2 Hz, 1 H of the hydrate **56**).

(Allyloxycarbonyl)-(*S*)-3-[(1*S*,2*R*,3*S*)-3-chloro-2-hydroxy-4-oxo-1-cyclohexyl]alanine Methyl Ester (54). Epoxide **39** (20 mg, 67 μmol) in anhydrous acetonitrile (0.4 mL) was treated with trimethylsilyl chloride (26 μL, 0.2 mmol) followed by DMSO (20 μL, 0.28 mmol). The resulting solution was stirred at 23 °C for 20 min, water (5 mL) was added, and the mixture was extracted with ether (3 × 5 mL). The organic layers were combined, dried, ($MgSO_4$) and evaporated. The crude product was purified on Lobar LiChroprep CN (3:1 toluene–ethyl acetate) to provide 10 mg (50%) of the chlorohydrin **54** as a colorless film: ^{1}H NMR ($CDCl_3$) δ 5.90–5.73 (m, 1 H), 5.40 (broad d, J = 8 Hz, NH), 5.33 (d, J = 18 Hz, 1 H), 5.24 (d, J = 12 Hz, 1 H), 4.50 (d, J = 6 Hz, 2 H), 4.32 (m, 1H), 4.13 (m, H-3), 4.04 (dd, J = 1, 5 Hz, H-2), 3.71 (s, 3H), 2.81 (m, 1 H), 2.42 (m, 1 H), 2.33–2.20 (m, 2 H), 1.95–1.65 (m, 3 H); MS (FAB) *m*/*e* 336, 334 (M + H), 154, 136; IR (film) 3379, 1726, 1534, 1261, 1216, 1046 cm^{-1}; HRMS calcd for $C_{14}H_{21}ClNO_6$ 334.1057, found 334.1048.

(Allyloxycarbonyl)-(*S*)-3-[(1*S*,2*S*,3*R*)-3-chloro-2-hydroxy-4-oxo-1-cyclohexyl]alanine Methyl Ester (55). Prepared from epoxide **40** as described for chlorohydrin **54** (yield 59%): ^{1}H NMR ($CDCl_3$) δ 6.00–5.80 (m, 1 H), 5.47 (broad d, J = 8 Hz, NH), 5.30 (d, J = 18 Hz, 1 H), 5.20 (d, J = 12 Hz, 1 H), 4.58 (d, J = 6 Hz, 2 H), 4.45 (m, 1 H), 4.42 (d, J = 10 Hz, H-3), 3.73 (s, 3 H), 3.40 (t, J = 10 Hz, H-2), 2.62 (ddd, J = 2.5, 4.5, 14 Hz, 1 H), 2.47 (dd, J = 6, 12 Hz, 1 H), 2.35–2.15 (m, 2 H), 1.95 (m, 1 H), 1.70 (m, 1 H), 1.30 (m, 1 H); MS (FAB) *m*/*e* 336, 334 (M + H), 316, 154, 136, 57, 55; IR (film) 3392, 1727, 1527, 1270, 1219, 1048 cm^{-1}; HRMS calcd for $C_{14}H_{21}ClNO_6$ 334.1057, found 334.1051.

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Supplementary Material Available: ^{1}H-NMR spectra of compounds **1a–c**, **2**, **3**, **4a**, **4c**, **5**/**47**, **19**, **20b,c**, **24a–c**, **30c**, **34**, **42–46**, **54**, and **55** (23 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.