Synthesis of Modified Partial Structures of the Bacterial Cell Wall. 1. Lipopeptides Containing Nonproteinogenic Amino Acids

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Received July 13, 1992

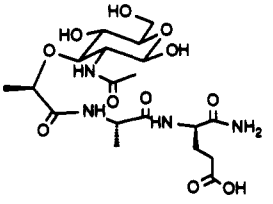
Two stereoisomeric lipopeptides **1** and **2** which can be regarded as modified peptidoglycans have been synthesized by using three different reaction sequences. The ene reaction of the α-allylated dipeptide **12** with butyl glyoxylate was used as a key step. The required enantiomerically pure substrates **9**, **10**, and **23** were obtained by enzymatic hydrolysis of the corresponding racemic α-allylated esters. The absolute configuration of both stereoisomers **1** and **2** was assigned by oxidative cleavage of the double bond in **18** and **19** followed by comparison of the esterified degradation products **28** and **29** with samples of authentic configuration, derived from (*R*)- and (*S*)-malic acid.

Introduction

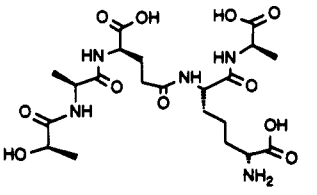
The chemistry of peptidoglycans[1] which constitute the bacterial cell wall has been the subject of much interest recently because of the unique immunostimulating activity of these compounds. Muramyldipeptide (MDP, Chart I) was considered to be the minimal structural unit of peptidoglycans capable of eliciting all immunostimulatory activities. Contrary to this long prevailing view, it has been found that the lipopeptide FK-156[2,3] and its synthetic analog FK 565[4] (Chart I), which both lack the muramyldipeptide residue, exhibit activities similar to those of MDP. It was thus considered that the (α,α′-*meso*-diaminopimelyl)alanine moiety in FK-156 and FK-565, like the muramyl moiety in MDP, might play an important role in the unique biological activity of these compounds. Considerable attention has therefore been focused on the synthesis of new lipopeptides related to FK-565.[5] Most of these compounds containing the unaltered α,α′-*meso*-diaminopimelyl residue are associated with undesirable side effects which preclude their therapeutic use.[6]

For our study we hypothesized that the α,α′-diaminopimelyl residue might be responsible not only for the immunological activity but also for the toxic effects of FK-565 derivatives. In order to differentiate between activity and toxicity it seemed reasonable to alter just that part of the molecule with cautious structural modifications: On the basis of molecular modeling considerations[7] the α′-amino group of the α,α′-diaminopimelyl residue was dislocated by means of (*S*)-alanine as a spacer, while its former position was substituted for a hydroxy group. In addition, the alignment of the C-7 chain was altered by introduction of a trans double bond at the β-position. In the present work we describe the synthesis of the lipopeptides **1** and **2** (Chart I) comprising the above structural modifications.[8]

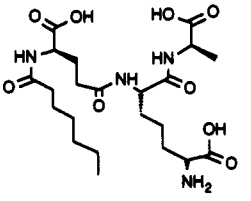
Chart I



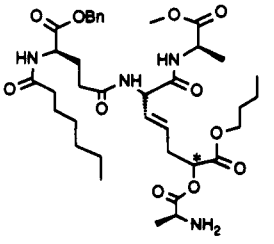
MDP



FK 156



FK 565



1 * = (S)
2 * = (R)

Results

The Ene Reaction (Scheme I). We focused primarily on methodologies to synthesize the chiral unsaturated hydroxypimelic acid residue as it seemed to be the central problem of this synthesis. The most promising procedure appeared to be the Lewis acid catalyzed ene reaction between a glyoxylic acid ester and an appropriate N-protected, enantiomerically pure, α-allylated glycine derivative: On the basis of our previous results related to the

(1) Review: Lederer, E. *J. Med. Chem.* **1980**, *23*, 819.
(2) Takeno, H.; Okada, S.; Hemmi, K.; Aratami, M.; Kitaura, Y.; Hashimoto, M. *Chem. Pharm. Bull.* **1984**, *32*, 2925.
(3) Kitama, Y.; Nakguchi, O.; Takeno, H.; Okada, S.; Yonishi, S.; Hemmi, K.; Mori, J.; Senoh, H.; Mine, Y.; Hashimoto, M. *J. Med. Chem.* **1982**, *25*, 335.
(4) Mine, Y.; Yokota, Y.; Wakai, Y.; Fukuda, S.; Nishida, M.; Goto, S.; Kuwahara, S. *J. Antibiot.* **1983**, *36*, 1045.
(5) Yokota, Y.; Mine, Y.; Wakai, Y.; Fukuda, S.; Nishida, M. *J. Antibiot.* **1983**, *36*, 1051.
(6) Hildebrandt, H.; Thirring, K.; Berner, H. Unpublished results.
(7) By using Marshall's "Active Analog Approach" (Marshall, G. R.; Motoc, I. Naturo, S. *Eur. J. Med. Chem.* **1985**, *20*, 529) it could be shown (Hecht, P.; Mehlführer, M.; Thirring, K.; Berner, H., to be published) that this type of compound can be superimposed with FK-565 at three presumable pharmacophoric positions: The carboxylic groups of glutamic acid and (*R*)-alanine and the α′-amino group of the diaminopimelic acid. With respect to this pharmacophoric pattern the number of possible conformations within the range of 10 kcal was drastically reduced (factor of 20) compared to the number of conformations FK-565 can adopt. Assuming that the pharmacophore responsible for toxicity differs from the biologically active one, enhancement of rigidity reduces the probability of encountering "toxic conformations", still maintaining a sufficient proportion of biologically active conformations.
(8) In this study only esterified derivatives were considered since in contrast to the free acids these compounds exhibit their immunological activity after peroral administration as well.

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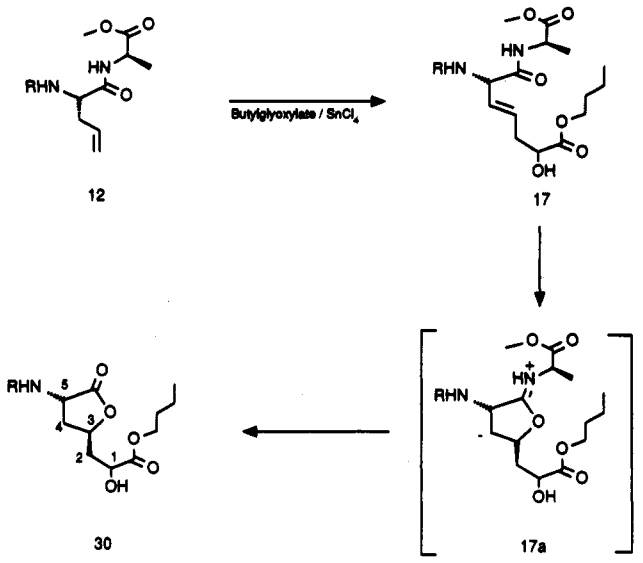
Scheme I



synthesis of enantiomerically pure, nonproteinogenic amino acids by enzymatic hydrolysis,[9] a high-yield access to a variety of substituted α-alkenylated glycine derivatives was at our disposal. In addition, the 2,2,2-trichloroethyl carbamate was found to be a stable N-protecting group in the presence of strong Lewis acids. These advantageous prerequisites encouraged us to study the course of the ene reaction with peptidic substrates.[10]

Treatment of the dipeptide **12** with butyl glyoxylate[11] catalyzed by $SnCl_4$[12] furnished the appropriate *E*-configurated ene product **17** together with the lactone **30** (Scheme I). This lactonization can be explained by a Lewis acid catalyzed followup reaction of **17**. A carbonium ion intermediate generated by $SnCl_4$ at the olefinic center invites participation of the amide carbonyl, forming the iminolactone **17a** which hydrolyzes to the lactone **30** and (*R*)-alanine methyl ester.[13,14] These mechanistic considerations are supported by the facile conversion of the intermediate **17** to the lactone **30** with only catalytic amounts of Lewis acid. Contrary to the ene reaction which showed only modest stereocontrol, the ensuing cyclization to the lactone proceeded with high induction, showing uniform trans-configuration.[15] As under carefully controlled conditions the undesired consecutive reaction (**17** → **30**) could be suppressed almost completely, we were able to employ the ene reaction as a key step in the synthesis of the lipopeptides **1** and **2**.

Syntheses of the Lipopeptides 1 and 2 (Scheme II). The racemic α-allylated glycine derivatives **7** and **8** were synthesized in excellent yields by treating the protected diethylamino malonates **3** and **4** with allyl bromide in ethanol/sodium ethoxide leading to **5** and **6** followed by hydrolysis and decarboxylation. The enzymatic hydrolysis of **7** and **8** with α-chymotrypsin[9] afforded an easily separable mixture of the (*R*)-configurated esters and (*S*)-configurated acids **9** and **10** which in turn were coupled to (*R*)-alanine methyl ester using DCC to give the dipeptides **11** and **12**. **12** was subsequently treated with butyl glyoxylate and $SnCl_4$ to yield the diastereomeric mixture **17** with only weak induction (de 25%).

A different approach was used to obtain **17**. Treatment of **8** with butyl glyoxylate analogous to the reaction of **12** resulted in the diastereomeric mixture of **22** in fair yield. The low substrate specificity of α-chymotrypsin[9] allowed stereospecific hydrolysis of **22** to give the (*S*)-acid **23**, which in turn was coupled to (*R*)-alanine methyl ester furnishing the diastereomeric mixture **17** in good yield (de 28%). This procedure proved to be more favorable than the previous one since the rather sensitive ene reaction was accomplished at an earlier stage. An additional advantage of this pathway was that the ester carbonyl in **8** attacked the olefinic center much slower than an amide carbonyl.[13] The undesired lactonization was thus suppressed more effectively by using **8** as a substrate for the ene reaction instead of **12**.

By means of DCC and DMAP the secondary hydroxy group of **17** was esterified with BOC-(*S*)-alanine furnishing the diasteromeric mixture **18/19**, which was readily separated by crystallization or chromatography. After selective cleavage of the trichloroethyl carbamate proceeding smoothly in a pH 4 buffer at 25 °C, the corresponding amines **20/21** were coupled to the γ-carboxygroup of the *N*-heptanoyl-(*R*)-glutamic acid α-benzyl ester[4] which was preactivated with isobutyl chloroformate.[16] The BOC-protected lipopeptides **15** and **16** were obtained in good yield to give the hydrochlorides **1** and **2** after deprotection with TFA and treatment with HCl/ether.

A third pathway was followed to obtain **1** and **2**: In this case the dipeptide **11** was coupled after deprotection with TFA to the γ-carboxy group of *N*-heptanoyl-(*R*)-glutamic acid α-benzyl ester similar to the preparation of **18** and **19**. The acylated tripeptide **13**, in turn, was submitted to the ene reaction with butyl glyoxylate affording the diastereomeric mixture **14**. After esterification with BOC-(*S*)-alanine a mixture of the already described lipopeptides **15** and **16** was obtained. However, this pathway suffered from a big disadvantage as the required separation of the diastereomeric lipopeptides **15** and **16** at this stage was very tedious.

The Assignment of the Configuration at C* (Scheme III). In order to determine the absolute configuration of the asymmetric carbon (C*) formed during the ene reaction we envisaged an oxidative degradation of the intermediates **18** and **19**. This would ultimately lead to malic acid derivatives of type **28** or **29** which in turn could be compared with samples of authentic configuration derived from (*R*)- or (*S*)-malic acid (**26/27**). Thus, compound **18** was treated with $RuO_4/NaIO_4$ in acetone[17] to give after chromatography the malic acid monoester **24** (62% yield) which was

(9) Schricker, B.; Thirring, K.; Berner, H. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 387.

(10) Agouridas et al. (Agouridas, K.; Girodeau, J. M.; Pineau, R. *Tetrahedron. Lett.* **1985**, *26*, 3115) have already synthesized racemic aminoacids using this methodology but their methods were not general and have not been widely adopted.

(11) Butyl glyoxylate was synthesized according to the procedure of: Hook, J. M. *Synth. Commun.* **1984**, *14*, 83.

(12) Among the various Lewis acids tested (BF_3, $ZnCl_2$, $ZnBr_2$, $AlCl_3$, $Al(CH_3)_2Cl$, $TiCl_4$) $SnCl_4$ and $FeCl_3$ emerged to be suited best for this type of ene reaction.

(13) Winstein, S.; Boschan, R. *J. Am. Chem. Soc.* **1950**, *72*, 4669.

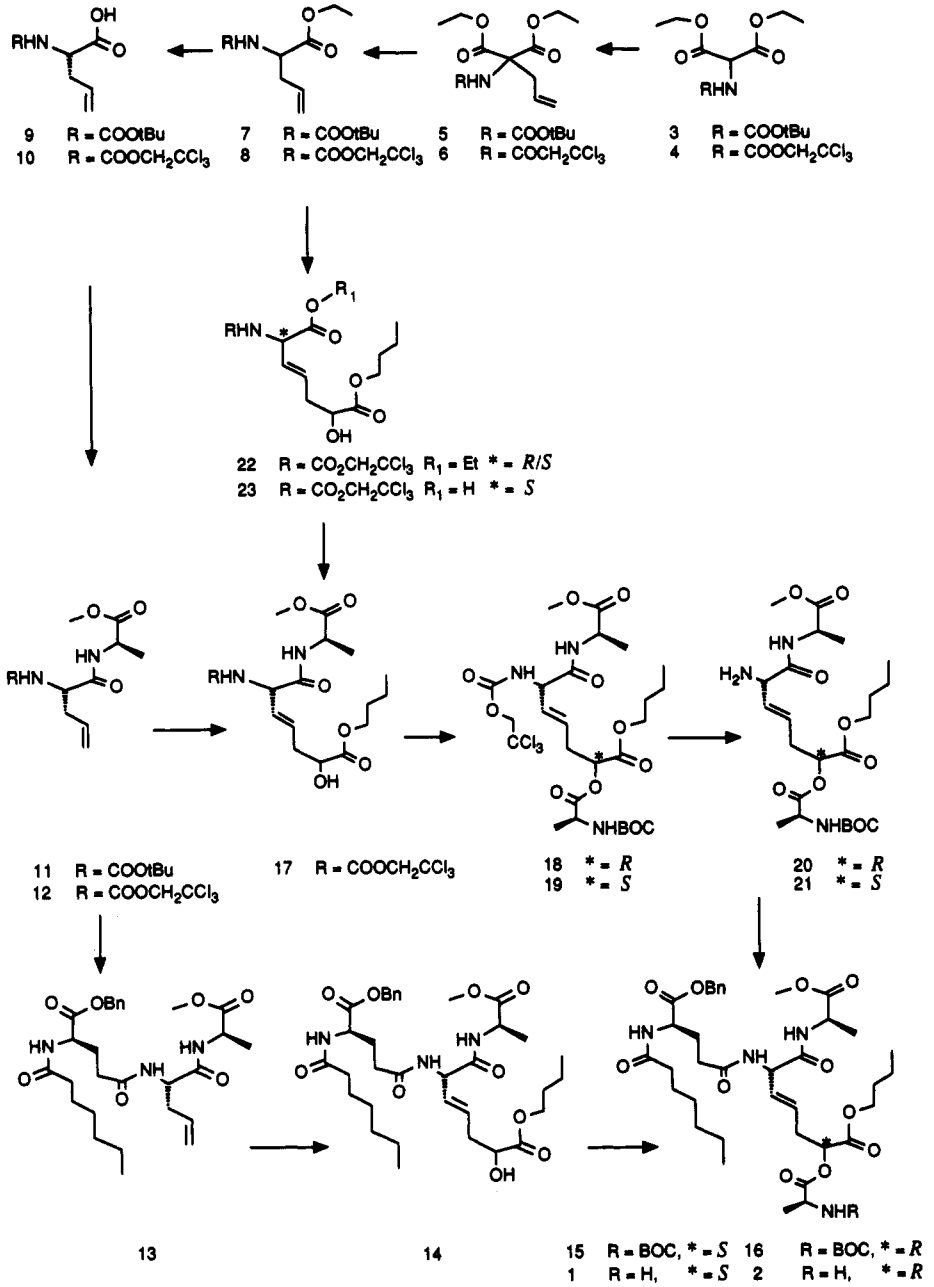
(14) Witkop, B. *Advances in Protein Chemistry*; Academic Press: New York, 1961; Vol. 16, p 239.

(15) The trans configuration was assigned by NOE measurements. The detailed investigation on the steric course and the synthetic use of the lactonization will be subject of a forthcoming publication: Thirring, K.; Berner, H. Manuscript in preparation.

(16) Anderson, G. W.; Zimmermann, F. M.; Callahan, F. M. *J. Am. Chem. Soc.* **1967**, *89*, 5012.

(17) Sorel, S.; Yanuka, Y. *J. Org. Chem.* **1959**, *24*, 2018.

Scheme II



esterified with *n*-butanol (DCC/DMAP)[18] to afford the diester **28** (57% yield). Diester **28** and the corresponding diastereomer **29** was readily synthesized by acid-catalyzed esterification of (*R*)- and (*S*)-malic acid to give **26**/**27** followed by acylation of the hydroxy group with BOC-(*S*)-alanine using DCC/DMAP.[18] Compounds **28** and **29** were easily distinguished from each other by TLC, HPLC, optical rotation, and NMR spectroscopy. Compound **28**, obtained by degradation of **18**, proved to be identical to the derivative synthesized from (*R*)-malic acid, confirming the (*R*)-configuration at C*. Alternatively, **19** was subjected to the same oxidative degradation leading to compound **29** which, as expected, corresponded to the (*S*)-malic acid derivative.

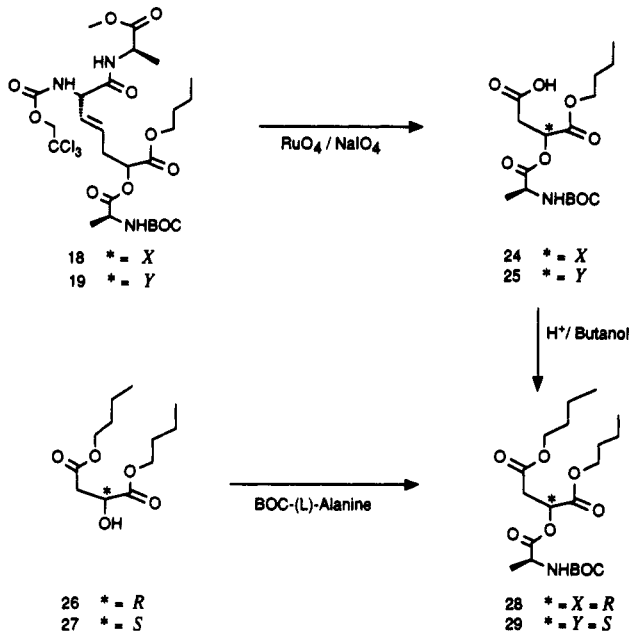
Summary

As outlined in Scheme II, three different synthetic pathways were employed to synthesize the immunostimulating[19] lipopeptides **1** and **2**. The key step in our synthesis involved the pericyclic ene reaction of enantiomerically pure α-allylated glycine derivatives **7**, **11**, **13** and butyl glyoxylate. The synthetic pathway via the intermediates **8** → **22** → **23** → **17** emerged as the most favorable because the ene reaction could be accomplished at the earliest possible stage and the undesired lactonization was more effectively suppressed. The required enantiomerically pure substrates were obtained by a stereospecific hydrolysis of the corresponding racemic esters giving the (*S*)-acids (96% ee, 97% yield). The configuration of the new asymmetric center formed during the ene reaction could be assigned through oxidative cleavage of the intermediates **18** and **19** and by comparison of the degradation products with authentic samples derived from (*R*)- or (*S*)-malic acid.

(18) Boden, E. P.; Keck, G. E. *J. Org. Chem.* **1985**, *50*, 2394.

(19) The immunological and toxicological investigations of **1** and **2** will be the subject of a forthcoming publication: Hildebrandt, H.; Thirring, K.; Berner, H. Manuscript in preparation.

Scheme III



Experimental Section

Melting points were determined using a Kofler apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 421 spectrometer. ^{1}H-NMR spectra were recorded on a Bruker WM 250 and AMX 500 spectrometer. Chemical shifts are quoted in parts per million downfield from TMS. Splitting patterns were designated as s (singlet), d (doublet), q (quartet), quin (quintet), dd (doubled doublet), ddd (twice doubled doublet), b (broad), bd (broad doublet), dt (doubled triplet), m (multiplet). Optical rotations were obtained on a Perkin-Elmer 241 polarimeter at wavelength 589 nm (sodium line) in a 10-cm cell with a total volume of 1 mL. Specific rotations, $[\alpha]_D$, are reported in deg/dm at the specified temperature and the concentration (c) given in grams per 100 mL in the specified solvent. Mass spectra were run on a VG 70 SE spectrometer operating in the FAB mode using xenon atoms and a matrix of thioglycerol. Microanalyses were carried out by Mikroanalytisches Institut der Universität Wien and are accurate to within the calculated values by 0.4%. Chromatography refers to medium-pressure column chromatography using silica gel (Merck 0.05–0.2 mm) and Merck columns of type A, B, and C. Visualization of TLC was done by iodine vapor, phosphomolybdic stain, a vanillin spray regent containing 0.5% H_2SO_4, and the TDM[20] reagent. HPLC analyses were carried out with a Hewlett-Packard HP1050 apparatus with a variable-wavelength UV detector set at 225 nm. Polygosil, Lichrosorb RP8, and Nucleosil columns (5 μm, 125 × 4 mm) were used. "Usual workup" means the reaction mixture was poured into water and the aqueous layer was extracted repeatedly with ethyl acetate. The combined organic extracts were washed subsequently with water and brine, dried over sodium sulfate, and evaporated under reduced pressure to dryness. The abbreviations DMAP (dimethylaminopyridine), TFA (trifluoroacetic acid), TSA (toluene-4-sulfonic acid), DCC (dicyclohexylcarbodiimide), EE (ethyl acetate), ala (alanine), gly (glycine), and glu (glutamic acid) were used.

[*N*-Heptanoyl-(*R*)-γ-glutamyl(α-benzyl ester)-(*S*)-α-[5-(butyl 2-(*R*)-((*S*)-alanyloxy)-4-pentenoate)]glycyl]-(*R*)-alanine Methyl Ester Hydrochloride (1). Under argon atmosphere (0 °C) 200 mg **15** was added to 5 mL of TFA. After 5 min the reaction mixture was concentrated to dryness and redissolved in CH_2Cl_2. The organic phase was washed with 10% $NaHCO_3$ solution and brine, dried over anhydrous sodium sulfate, concentrated to dryness, and redissolved again in 20% ethereal HCl. After removal of the solvent under reduced pressure (30 °C bath temperature) the hydrochloride was taken up in 20 mL of H_2O. The resultant solution was lyophilized to afford 180 mg (89%) **1**: $[\alpha]^{20}_D$ +38.4° (c = 0.8, CH_3OH); ^{1}H-NMR (CD_3OD) 5.75 (m, 2 H, olefin), 5.2 (dd, 1 H, CH_2CHOCO, J = 3.75 and 8.75 Hz), 5.15 (s, 2 H, $OCH_2C_6H_5$), 4.87 (d, 1 H, α-H-gly, J = 5 Hz), 4.45 (dd, 1 H, α-H-glu, J = 5 and 8.75 Hz), 4.38 (q, 1 H, α-H-ala, J = 6.25 Hz), 4.18 (m, 3 H, $OCH_2C_3H_7$, α-H-ala), 3.7 (s, 3 H, OCH_3), 1.55, 1.35 (2 × d, 6 H, 2 × CH_3-ala, J = 6.25 Hz); MS-FAB m/e 733.8 (MH^+, 100); HPLC Lichrosorb RP8 (EtOH/H_2O (88:12)), 1.5 mL/min, t_R 5.33. Anal. Calcd for $C_{37}H_{57}N_4O_{11}Cl$: C, 57.7; H, 7.41; N, 7.28; Cl, 4.60. Found: C, 57.43; H, 7.02; N, 6.92; Cl, 4.22.

[*N*-Heptanoyl-(*R*)-γ-glutamyl(α-benzyl ester)-(*S*)-α-[5-(butyl 2-(*R*)-((*S*)-alanyloxy)-4-pentenoate)]glycyl]-(*R*)-alanine Methyl Ester Hydrochloride (2). According to the preparation of **1**, yield 92%: mp 85–87 °C; $[\alpha]^{20}_D$ +23.64° (c = 0.7, CH_3OH); ^{1}H-NMR (CD_3OD) 5.8 (m, 2 H, olefin), 5.27 (dd, 1 H, CH_2CHOCO, J = 3.75 and 8.75 Hz), 5.15 (s, 2 H, $OCH_2C_6H_5$), 4.94 (d, 1 H, α-H-gly, J = 5 Hz), 4.57 (dd, 1 H, α-H-glu, J = 5 and 8.75 Hz), 4.48 (q, 1 H, α-H-ala, J = 6.25 Hz), 4.28 (m, 3 H, $OCH_2C_3H_7$, α-H-ala), 3.56 (s, 3 H, OCH_3), 1.54, 1.32 (2 × d, 6 H, 2 × CH_3-ala, J = 6.25 Hz); MS-FAB m/e 733.8 (MH^+, 100); HPLC Lichrosorb RP8 (EtOH/H_2O (88:12)), 1.5 mL/min, t_R 5.66. Anal. Calcd for $C_{37}H_{57}N_4O_{11}Cl$: 57.7; H, 7.41; N, 7.28; Cl, 4.60. Found: C, 57.72; H, 7.32; N, 6.90; Cl, 4.82.

Diethyl 2-[(*tert*-Butyloxycarbonyl)amino]malonate (3). To a stirred and cooled (5 °C) solution of 250 g (1.18 mol) of diethyl 2-aminomalonate hydrochloride in 1190 mL (1.19 mol) of 1 N NaOH and 2 L of dioxane was added a solution of 256 g (1.17 mol) of BOC anhydride in 500 mL of dioxane. After 24 h the reaction mixture was concentrated under reduced pressure and poured into ethyl acetate. The organic layer was washed with 1 L of 1 N HCl and brine and dried over anhydrous sodium sulfate. Removal of the solvent under reduced pressure gave 262 g (81.4%) of crude **3** which was used for the alkylation without further purification: ^{1}H-NMR ($CDCl_3$) 5.58 (d, 1 H, N*H*, J = 7.5 Hz), 4.95 (d, 1 H, α-H, J = 7.5 Hz), 4.3 (m, 4 H, OCH_2CH_3), 1.46 (s, 9 H, *t*-Bu), 1.31 (t, 6 H, OCH_2CH_3).

Diethyl 2-[[(2,2,2-Trichloroethoxy)carbonyl]amino]-malonate (4). To a stirred and cooled (0 °C) solution of 42.4 g (0.2 mol) of diethyl 2-aminomalonate hydrochloride and 44 mL (0.4 mol) of *N*-methylmorpholine in 500 mL of THF was added dropwise 42.2 g (0.2 mol) of 2,2,2-trichloroethyl chloroformate. The reaction mixture was allowed to warm within 2 h to 25 °C and was subsequently poured into 1 L of water. After usual workup 64.1 g (91.6%) of crude **4** was obtained and used for the subsequent alkylation without further purification: ^{1}H-NMR ($CDCl_3$) 6.05 (d, 1 H, N*H*, J = 7.3 Hz), 5.0 (d, 1 H, α-H, J = 7.3 Hz), 4.72 (s, 2 H, CCl_3CH_2O), 4.3 (m, 4 H, OCH_2CH_3), 1.15 (t, 6 H, OCH_2CH_3).

Diethyl 2-[(*tert*-Butyloxycarbonyl)amino]-2-(2-propenyl)-malonate (5). To a solution of 1.8 g (90 mmol) of sodium in 100 mL of ethanol was added dropwise a solution of 20.5 g (75 mmol) of **3** in 20 mL of ethanol. After 30 min 9 g (75 mmol) of 2-propenyl bromide was added and the reaction mixture was subsequently refluxed for 4 h. After removal of the precipitated sodium bromide and usual workup 18.9 g (80.5%) of crude **5** was isolated and used for the following reaction without further purification: ^{1}H-NMR ($CDCl_3$) 5.9 (b, 1 H, N*H*), 5.65 (m, 1 H, olefin), 5.13 (m, 2 H, olefin), 4.25 (m, 4 H, OCH_2CH_3), 3.04 (d, 2 H, $CH_2CH{=}CH_2$, J = 7 Hz), 1.44 (s, 9 H, *t*-Bu), 1.26 (t, 6 H, OCH_2CH_3).

Diethyl 2-[[(2,2,2-Trichloroethoxy)carbonyl]amino]-2-(2-propenyl)malonate (6). The alkylation was carried out according to the preparation of **5**. The crude material was purified by filtration over a short column of silica gel (cyclohexane/ethyl acetate (4:1)): yield 85%; ^{1}H-NMR ($CDCl_3$) 6.4 (s, 1 H, N*H*), 5.6 (m, 1 H, olefin), 5.15 (m, 2 H, olefin), 4.75 (s, 2 H, CCl_3CH_2OCO), 4.24 (m, 4 H, OCH_2CH_3), 3.1 (d, 2 H, $CH_2CH{=}CH_2$, J = 7.3 Hz), 1.3 (t, 3 H, OCH_2CH_3).

***rac*-(2*RS*)-*N*-(*tert*-Butyloxycarbonyl)-2-(2-propenyl)glycine Ethyl Ester (*rac*-7).** A solution of 14.5 g (46 mmol) of **5** in 30 mL of ethanol and 46 mL (46 mmol) of 1 N NaOH was allowed to stand under an argon atmosphere for 16 h at 20 °C. After the reaction was complete (TLC analysis), reaction mixture was acidified with 1 N HCl to pH 3.5 to afford after usual workup 11 g (83.3%) of crude material which was subsequently decarboxylated in xylene (2 h, reflux). After removal of the solvent under reduced pressure and filtration over a short column of

(20) Arx, E.; Faupel, M.; Brugger, M. *J. Chromatogr.* **1976**, *120*, 224.

silica gel (hexane/ethyl acetate (6:1)) 8.3 g (89.8%) of **7** was isolated: ^{1}H-NMR ($CDCl_3$) 5.65 (m, 1 H, olefin), 5.12 (m, 3 H, N*H*, olefin), 4.35 (m, 1 H, α-H), 4.2 (q, 2 H, OCH_2CH_3), 2.53 (m, 2 H, $CH_2CH{=}CH_2$), 1.45 (s, 9 H, *t*-Bu), 1.28 (t, 3 H, OCH_2CH_3). Anal. Calcd for $C_{12}H_{21}NO_4$: C, 59.24; H, 8.70; N, 5.76. Found: C, 59.01; H, 8.43; N, 5.81.

***rac*-(2*RS*)-*N*-[(2,2,2-Trichloroethoxy)carbonyl]-2-(2-propenyl)glycine Ethyl Ester (*rac*-8).** The saponification and decarboxylation were carried out according to the preparation of **7**. The crude material was filtered over a short column of silica gel (cyclohexane/ethyl acetate (3:1)), yield 81.5%: ^{1}H-NMR ($CDCl_3$) 5.7 (m, 1 H, olefin), 5.2 (m, 2 H, olefin), AB-system (ν_A = 4.75, ν_B = 4.65, 2 H, CCl_3CH_2O, J = 12 Hz), 4.5 (m, 1 H, α-H-gly), 4.25 (m, 2 H, OCH_2CH_3), 2.6 (m, 2 H, $CH_2CH{=}CH_2$), 1.3 (t, 3 H, OCH_2CH_3). Anal. Calcd for $C_{10}H_{14}Cl_3NO_4$: C, 37.40; H, 4.43; N, 4.40; Cl, 33.38. Found: C, 37.46; H, 4.32; N, 4.12; Cl, 32.98.

(2*S*)-*N*-(*tert*-Butoxycarbonyl)-2-(2-propenyl)glycine (9). 50 g (0.19 mol) of *rac*-**7** were suspended in 3 L of 0.1 M phosphate buffer (pH 8) and treated with 250 mg of α-chymotrypsin (75 units/mg, Fluka 27270) with gentle stirring at 37 °C. The pH was kept constant within the range of 7.5–8 by continous addition of 1 N NaOH. After 24 h, when 171 mL (ca. 1 equiv) of 1 N NaOH had been consumed, the unreacted *R*-ester of **7** was recovered by extracting the reaction mixture with ethyl acetate. Subsequently, the aqueous solution was acidified to pH 2.5 with 1 N HCl and continously extracted with ethyl acetate. After usual workup 21.37 g (95.8%) of *S*-configurated acid **9** was obtained. The configuration of **9** was verified after deprotection and comparison of its optical rotation with an authentic sample of (*S*)-α-(2-propenyl)glycine.[21] Acid **9** was used for the following coupling reaction without any further purification. A small sample was purified by chromatography (CH_2Cl_2/methanol/isopropyl ether/H_2O/acetic acid (40:10:3:1.5:1)) for spectroscopic data: $[\alpha]^{20}_D$ +7.1° (*c* = 1.15, CH_3OH); ^{1}H-NMR (CD_3OD) 5.82 (m, 1 H, olefin), 5.15 (m, 2 H, olefin), 4.2 (dd, 1 H, α-H, J = 4.81, 7.64 Hz), 2.4–2.7 (m, 2 H, $CH_2CH{=}CH_2$), 1.43 (s, 9 H, *t*-Bu). Anal. Calcd for $C_{10}H_{17}NO_4$: C, 55.80; H, 7.96; N, 6.51. Found: C, 55.56; H, 8.21; N, 6.38.

(2*S*)-*N*-[(2,2,2-Trichloroethoxy)carbonyl]-2-(2-propenyl)glycine (10). The enzymatic saponification was carried out according to the preparation of **9**, yield 84%. **10** was used for the following coupling reaction without any further purification. For spectroscopic data, a small sample of **10** was purified by chromatography (CH_2Cl_2/methanol/isopropyl ether/H_2O/acetic acid (40:10:3:1.5:1)): $[\alpha]^{20}_D$ −7.53° (*c* = 1, CH_3OH); ^{1}H-NMR (CD_3OD/C_6D_6 (3:1)) 5.8 (m, 1 H olefin), 5.0–5.2 (m, 2 H, olefin), AB-system (ν_A = 4.7, ν_B = 4.8, 2 H, CCl_3CH_2O, J = 12 Hz), 4.3 (dd, 1 H, α-H-gly, J = 6, 8.1 Hz), 2.4–2.7 (m, 2 H, $CH_2CH{=}CH_2$). Anal. Calcd for $C_8H_{10}Cl_3NO_4$: C, 33.07 H, 3.47; N, 4.82. Found: C, 33.12; H, 3.48; N, 4.72.

[*N*-(*tert*-Butoxycarbonyl)-2(*S*)-(2-propenyl)glycyl]-(*R*)-alanine Methyl Ester (11). To a cooled (5 °C) solution of 4.3 g (20 mmol) **9**, 2.75 g (20 mmol) of (*R*)-alanine methyl ester hydrochloride, and 2 g (20 mmol) of *N*-methylmorpholine in 100 mL of CH_2Cl_2 was added portionwise 4.1 g (20 mmol) of DCC. After 6 h the precipitated urea was filtered and the reaction mixture concentrated and chromatographed on silica gel (hexane/EE = 5:1), yield 83.5%: mp 71–72 °C; $[\alpha]^{20}_D$ +13.9° (*c* = 0.4, CH_3OH); ^{1}H-NMR ($CDCl_3$) 6.75 (d, 1 H, N*H*, J = 7.5), 5.75 (m, 1 H, olefin), 5.18 (m, 2 H, olefin), 4.6 (quin, 1 H, α-H-ala, J = 7.5 Hz), 4.2 (q, 1 H, α-H-gly, J = 7.2 Hz), 3.6 (s, 3 H, OCH_3), 2.52 (m, 2 H, $CH_2CH{=}CH_2$), 1.45 (s, 9 H, *t*-Bu), 1.4 (d, 3 H, CH_3-ala, J = 7.5 Hz); HPLC Polygosil, cyclohexane/2-propanol (100:5), 1.5 mL/min, t_R 7.13. Anal. Calcd for $C_{14}H_{24}N_2O_5$: C, 55.99; H, 8.05; N, 9.33. Found: C, 56.31; H, 8.22; N, 9.38.

[*N*-[(2,2,2-Trichloroethoxy)carbonyl]-2(*S*)-(2-propenyl)glycyl]-(*R*)-alanine Methyl Ester (12). The coupling reaction was carried out according to the preparation of **11**, yield 79%: $[\alpha]^{20}_D$ +13.8° (*c* = 0.56, CH_3OH); ^{1}H-NMR(CD_3OD/C_6D_6 (3:1)) 5.8 (m, 1 H, olefin), 5.15 (m, 2 H, olefin), AB-system (ν_A = 4.75, ν_B = 4.85, 2 H, CCl_3CH_2O, J = 12 Hz), 4.48 (q, 1 H, α-H-ala, J = 6.3 Hz), 4.36 (dd, 1 H, α-H-gly, J = 5.8, 8.2 Hz), 3.68 (s, 3 H, OCH_3), 1.38 (d, 3 H, CH_3-ala, J = 6.3 Hz). Anal. Calcd for $C_{12}H_{17}Cl_3N_2O_5$: C, 38.37; H, 4.56, N, 7.46. Found: C, 38.38; H, 4.58; N, 7.65.

[*N*-Heptanoyl-(*R*)-γ-glutamyl-(α-benzyl ester)-(*S*)-(2-propenyl)glycyl]-(*R*)-alanine Methyl Ester (13). A solution of 1.5 g (1.5 mmol) of **11** and 950 mg (5 mmol) of 4-toluenesulfonic acid in 20 mL of aqueous CF_3COOH was allowed to stand at 20 °C for 30 min. The reaction mixture which was subsequently concentrated to dryness and redissolved in a mixture of 20 mL of CH_2Cl_2 and 1.5 g (15 mmol) of *N*-methylmorpholine was added to a cooled (0 °C) solution of 1.75 g (5 mmol) of *N*-heptanoyl-(*R*)-γ-glutamic acid α-benzyl ester, 0.5 g (5 mmol) of *N*-methylmorpholine, and 0.6 g (5 mmol) of isobutyl chloroformate in 20 mL of CH_2Cl_2. After 2 h during which the reaction mixture warmed to room temperature it was worked up as usual and the crude material chromatographed on silica gel ($CHCl_3/CH_3OH$ (100:7)) to afford 1.92 g (72%) of **13**: $[\alpha]^{20}_D$ +19.3° (*c* = 0.94, CH_3OH); ^{1}H-NMR (d_6-DMSO) 8.37 (d, 1 H, N*H*, J = 6 Hz), 8.21 (d, 1 H, N*H*, J = 6 Hz), 7.95 (d, 1 H, N*H*, J = 7 Hz), 7.37 (s, 5 H, C_6H_5), 5.12 (s, 2 H, $OCH_2C_6H_5$), 5.7 (m, 1 H, olefin), 5.05 (m, 2 H, olefin), 4.27 (quin, 1 H, α-H-ala, J = 6 Hz), 4.26 (m, 1 H, α-H-gly), 3.61 (s, 3 H, OCH_3), 1.25 (d, 3 H, CH_3-ala, J = 6 Hz), 0.86 (t, 3 H, $(CH_2)_5CH_3$); MS-FAB *m/e* 532 (MH^+, 50), 332 (48), 220 (100). Anal. Calcd for $C_{28}H_{41}N_3O_7$: C, 63.26; H, 7.77; N, 7.90. Found: C, 63.41; H, 7.82; N, 7.76.

[*N*-Heptanoyl-(*R*)-γ-glutamyl(α-benzyl ester)-(*S*)-α-[5-(butyl 2(*RS*)-hydroxy-4-pentenoate)]glycyl]-(*R*)-alanine Methyl Ester (14). To a cooled solution (0 °C) of 1.17 g (9 mmol) of freshly distilled butyl glyoxalate and 3.12 g (12 mmol) of $SnCl_4$ in 20 mL of CH_2Cl_2 was added dropwise via syringe a solution of 1.06 g (2 mmol) of **13** in 5 mL of CH_2Cl_2. After 3 h the reaction mixture was poured into 0.1 N HCl and repeatedly extracted with CH_2Cl_2. The combined extracts were concentrated to dryness under reduced pressure and chromatographed on silica gel (CH_2Cl_2/2-propanol/cyclohexane (3:2:7)) to afford 780 mg (59.2%) of **14**: ^{1}H-NMR (DMSO) 4.68–4.72 (dt, 1 H, olefin, J = 12, 6 Hz), 5.5 (dd, 1 H, olefin, J = 12, 6 Hz), 5.44 (d, 1 H, OH, J = 5 Hz), 5.12 (s, 2 H, $OCH_2C_6H_5$), 4.89 (t, 1 H, α-H-gly), 4.25 (quin, 1 H, α-H-ala, J = 6 Hz), 4.26 (m, 1 H, α-H-glu), 4.03 (m, 3 H, $OCH_2C_3H_7$, *C*HOH), 3.62 (s, 3 H, OCH_3), 1.25 (d, 3 H, CH_3-ala, J = 6 Hz), 1.38 (t, 3 H, OCH_2CH_3); MS-FAB *m/e* 662 (MH^+, 100), 557 (15), 559 (18), 332 (24), 220 (48), 183 (54). Anal. Calcd for $C_{34}H_{51}N_3O_{10}$: C, 61.71; H, 7.77; N, 6.35. Found: C, 61.93; H, 7.73; N, 6.40.

[*N*-Heptanoyl-(*R*)-γ-glutamyl(α-benzyl ester)-(*S* and *R*)-α-[5-[butyl 2(*S*)-[[(*tert*-butoxycarbonyl)-(*S*)-alanyl]oxy]-4-pentenoate]]glycyl]-(*R*)-alanine Methyl Ester (15) + (16). Method A. To a cooled (5 °C) solution of 0.99 g (1.5 mmol) of **14**, 0.28 g (1.5 mmol) of BOC-(*S*)-alanine, and 15 mg of DMAP in 15 mL of CH_2Cl_2 were added portionwise 0.31 g (1.5 mmol) of DCC. After 10 h the precipitated urea was filtered and the reaction mixture concentrated and chromatographed on silica gel (cyclohexan/2-propanol/CH_2Cl_2 (10:1:3)) to afford 475 mg of **15** and 515 mg of **16** (total yield 80%).

Method B. According to the preparation of **11**, the amines **20** (*R*-configurated) and **21** (*S*-configurated) are reacted with *N*-heptanoyl-(*R*)-(α-benzyl ester)-γ-glutamic acid to afford **15** (76%) and **16** (83%). **15**: mp 121–122 °C (methanol/ether); $[\alpha]^{20}_D$ +28.5° (*c* = 1, CH_3OH); ^{1}H-NMR (CD_3OD/C_6D_6 (3:1)) 5.83 (dt, 1 H, olefin, J = 7.7, 15 Hz), 5.7 (dd, 1 H, olefin, J = 5, 15 Hz), 5.1 (s, 2 H, $OCH_2C_6H_5$), 5.07 (dd, 1 H, CH_2CHOCO, J = 4, 7.5 Hz), 5.0 (d, 1 H, α-H-gly, J = 7.7 Hz), 4.54 (dd, 1 H, α-H-glu, J = 3.75, 5 Hz), 4.4 (q, 1 H, α-H-ala, J = 6.25 Hz), 4.32 (q, 1 H, α-H-ala, J = 6.25 Hz), 4.08 (t, 2 H, $OCH_2C_3H_7$), 3.60 (s, 3 H, OCH_3), 1.37 (d, 6 H, 2 × CH_3-ala, J = 6.25 Hz), 0.87 (t, 3 H, $C_5H_{11}CH_3$); HPLC Lichrosorb RP18 (CH_3CN/EtOH (99.2:0.8)), 1.5 mL/min, t_R 4.80. Anal. Calcd for $C_{42}H_{64}N_4O_{13}$: C, 60.56; H, 7.74; N, 6.73. Found: C, 60.77; H, 7.64; N, 6.63. **16**: $[\alpha]^{20}_D$ +16.5° (*c* = 1, CH_3OH); ^{1}H-NMR (CD_3OD/C_6D_6 (3:1)) 5.86 (dt, 1 H, olefin, J = 7.5, 15 Hz), 5.71 (dd, 1 H, olefin, J = 5, 15 Hz), 5.1 (s, 2 H, $OCH_2C_6H_5$), 4.98 (dd, 1 H, CH_2CHOCO, J = 4, 7.5 Hz), 5.05 (d, 1 H, α-H-gly, J = 5 Hz), 4.54 (dd, 1 H, α-H-glu, J = 3.75, 5 Hz), 4.5 (q, 1 H, α-H-ala, J = 6.25 Hz), 4.32 (q, 1 H, α-H-ala, J = 6.25 Hz), 4.05 (t, 2 H, $OCH_2C_3H_7$), 3.60 (s, 3 H, OCH_3), 1.36 (d, 3 H, CH_3-ala, J = 6.25 Hz), 1.4 (d, 3 H, CH_3-ala, J = 6.25 Hz), 0.87 (t, 3 H, $C_5H_{11}CH_3$); HPLC Lichrosorb RP18 (CH_3CN/EtOH

(21) Schöllkopf, U.; Hartwig, W.; Pospischil, K. H.; Kehne, H. *Synthesis* **1981**, 966.

(99.2:0.8)), 1.5 mL/min, t_R 4.33. Anal. Calcd for $C_{42}H_{64}N_4O_{13}$: C, 60.56; H, 7.74; N, 6.73. Found: C, 60.30; H, 7.50; N, 6.92.

[*N*-[(2,2,2-Trichloroethoxy)carbonyl]-(*S*)-α-[5-(butyl 2(*RS*)-hydroxy-4-pentenoate)]glycyl]-(*R*)-alanine Methyl Ester (17). Method A. To a cooled solution of 4.11 g (30 mmol) of freshly distilled butyl glyoxylate and 7.6 g (45 mmol) of dry $FeCl_3$ in 100 mL of CH_2Cl_2 was added dropwise via syringe a solution of 5.93 g (15 mmol) of **12** in 20 mL of CH_2Cl_2. The reaction mixture was allowed to warm to room temperture, stirred for an additional 3 h, and subsequently poured into cooled 0.1 N HCl. After repeated extraction with CH_2Cl_2 the combined extracts were evaporated to dryness and chromatographed on silica gel (CH_2Cl_2/cyclohexane/2-propanol (3:5:2)) to afford 7.9 g (61.2%) of **17** and small amounts of lactone **30**.

Method B. To a cooled (5 °C) solution of 500 mg (10 mmol) of **23**, 1.4 g (10 mmol) of (*R*)-alanine methyl ester, and 1.08 mL (10 mmol) of *N*-methylmorpholine in 25 mL of CH_2Cl_2 was added portionwise 2.04 g (10 mmol) of DCC. After 3 h the precipitated urea was filtered and the reaction mixture washed with 0.1 N HCl and brine. Chromatography on silica gel (cyclohexane/ethyl acetate (1:1)) yielded 510 mg (84.4%) of **17**: ^{1}H-NMR (CD_3OD/C_6D_6 (3:1)) 5.95 (m, 1 H, olefin), 5.7 (m, 1 H, olefin), 4.83 (d, 1 H, α-H-gly, J = 5 Hz), AB-system (ν_A = 4.72, ν_B = 4.82, 2 H, CCl_3CH_2OCO, J = 12 Hz), 4.48 (q, 1 H, α-H-ala, J = 7.2 Hz), 4.27 (m, 1 H, CH_2CHOCO), 3.64 (s, 3 H, OCH_3), 2.5 (m, 2 H, $CH_2CH{=}CH_2$), 0.82 (t, 3 H, $C_3H_7CH_3$). Anal. Calcd for $C_{17}H_{25}Cl_3N_2O_8$: C, 41.52; H, 5.12; N, 5.70; Cl, 21.63. Found: C, 41.63; H, 5.28; N, 5.65; Cl, 21.73.

[*N*-[(2,2,2-Trichloroethoxy)carbonyl]-(*S*)-α-[5-[butyl 2(*R* and *S*)-[[(*tert*-butoxycarbonyl)-(*S*)-alanyl]oxy]-4-pentenoate]]glycyl]-(*R*)-alanine Methyl Ester (18) + (19). Alcohol **17** was esterified with BOC-(*S*)-alanine according to the preparation of **15** and **16**. The crude reaction mixture was chromatographed on silica gel (cyclohexane/ethyl acetate (2:1)) to afford both diastereoisomers **18** and **19** in the ratio of 3:4 (total yield 82%). **18**: ^{1}H-NMR (CD_3OD/C_6D_6 (3:1)) 5.85 (m, 1 H, olefin), 5.7 (dd, 1 H, olefin, J = 6.25, 15 Hz), 5.08 (t, 1 H, CH_2CHOCO, J = 5.2 Hz), 5.0 (d, 1 H, α-H-gly, J = 5 Hz), AB-system (ν_A = 4.78, ν_B = 4.69, 2 H, CCl_3CH_2OCO, J = 12 Hz), 4.45 (q, 1 H, α-H-ala, J = 7.25 Hz), 4.32 (q, 1 H, α-H-ala, J = 7.25 Hz), 4.08 (t, 2 H, $OCH_2C_3H_7$), 3.60 (s, 3 H, OCH_3), 2.58 (m, 2 H, $CH_2CH{=}CH_2$), 1.43 (s, 9 H, *t*-Bu), 0.9 (t, 3 H, $C_3H_7CH_3$). Anal. Calcd for $C_{26}H_{40}Cl_3N_3O_{11}$: C, 46.13; H, 5.97; N, 6.21; Cl, 15.71. Found: C, 46.36; H, 6.18; N, 6.22; Cl, 15.42. **19**: mp 106–107 °C (isopropyl ether); $[\alpha]^{20}_D$ +15.8 (c = 0.8, CH_3OH); ^{1}H-NMR (CD_3OD/C_6D_6 (3:1)) AB-system (ν_A = 5.75, ν_B = 5.92, 2 H, olefin, J = 6.75, 15.3 Hz), 5.13 (t, 1 H, CH_2CHOCO, J = 5.2 Hz), 4.88 (d, 1 H, α-H-gly, J = 7.2 Hz), AB-system (ν_A = 4.72, ν_B = 4.82, 2 H, CCl_3CH_2OCO, J = 12.2 Hz), 4.48 (q, 1 H, α-H-ala, J = 7.4 Hz), 4.3 (q, 1 H, α-H-ala, J = 7.2 Hz), 4.08 (t, 2 H, $OCH_2C_3H_7$), 3.63 (s, 3 H, OCH_3), 2.61 (m, 2 H, $CH_2CH{=}CH_2$), 1.42 (s, 9 H, *t*-Bu), 0.9 (t, 3 H, $C_3H_7CH_3$); ^{1}H-NMR (DMSO-d_6) 8.37, 7.94, 7.28 (3 × d, 9 H, 3 × NH, J = 7 Hz), AB-system (ν_A = 5.75, ν_B = 5.6, 2 H, olefin, J = 6.8, 15.4 Hz), 4.98 (t, 1 H, CH_2CHOCO, J = 5.4 Hz), 4.68 (t, 1 H, α-H-gly, J = 7.3 Hz), AB-system (ν_A = 4.78, ν_B = 4.83, 2 H, CCl_3CH_2OCO, J = 12.4 Hz), 4.24 (q, 1 H, α-H-ala, J = 7.3 Hz), 4.08 (q, 1 H, α-H-ala, J = 7.3 Hz), 3.63 (s, 3 H, OCH_3), 1.37 (s, 9 H, *t*-Bu), 1.27 (d, 3 H, CH_3-ala, J = 7.3 Hz), 0.87 (t, 3 H, $C_3H_7CH_3$); MS-FAB m/e 678 (MH^+, 12), 620 ($(MH - C_4H_8)^+$, 11), 576 ($(MH - BOC)^+$, 100). Anal. Calcd for $C_{26}H_{40}Cl_3N_3O_{11}$: C, 46.13; H, 5.97; N, 6.21; Cl, 15.71. Found: C, 46.16; H, 6.01; N, 6.01; Cl, 15.35.

(*S*)-α-[[5-[Butyl 2(*S*)-[[(*tert*-butoxycarbonyl)-(*S*)-alanyl]oxy]-4-pentenoate]]glycyl]-(*R*)-alanine Methyl Ester (21). To a vigorously stirred suspension of 1.5 g of zinc powder in 30 mL of THF and 3 mL of phosphate buffer (0.5 M, pH 4.5) was added a solution of 1.94 g (15 mmol) of **19** in 10 mL of THF. After 2 h the reaction mixture was concentrated and distributed between 40 mL of 5% $NaHCO_3$ solution and 40 mL of CH_2Cl_2. The organic phase was dried over sodium sulfate and concentrated to dryness under reduced pressure to afford 1.1 g (78%) of **21**, which can be used for the following reaction without any further purification. A small sample was purified for spectroscopic data: ^{1}H-NMR (CD_3OD/C_6D_6 (3:1)) 5.8 (m, 2 H, olefin), 5.15 (dd, 1 H, CH_2CHOCO, J = 3.75 Hz), 3.95 (d, 1 H, α-H-gly, J = 5 Hz), 4.48 (q, 1 H, α-H-ala, J = 6.25 Hz), 4.3 (q, 1 H, α-H-ala, J = 6.25 Hz), 3.64 (s, 3 H, OCH_3), 2.5 (m, 2 H, $CH_2CH{=}CH_2$), 1.45, 1.41 (2 × d, 6 H, 2 × CH_3-ala, J = 6.25 Hz), 1.4 (s, 9 H, *t*-Bu), 0.82 (t, 3 H, $C_3H_7CH_3$).

(*S*)-α-[[5-[Butyl 2(*R*)-[[(*tert*-butoxycarbonyl)-(*S*)-alanyl]oxy]-4-pentenoate]]glycyl]-(*R*)-alanine Methyl Ester (20). The deprotection of compound **18** was carried out according to the preparation of **21** to give compound **20** in 82% yield.

***N*-[(2,2,2-Trichloroethoxy)carbonyl]-(*RS*)-α-[5-(butyl 2(*RS*)-hydroxy-4-pentenoate)]glycine Methyl Ester (22).** The ene reaction of **8** with butyl glyoxylate was carried out according to the preparation of **17**, yield 61%: ^{1}H-NMR (CD_3OD/C_6D_6 (3:1)) 5.9 (dt, 1 H, olefin, J = 18.5, 6.5 Hz), 5.75 (dd, 1 H, olefin, J = 6, 15.8 Hz), 4.85 (m, 1 H, α-H-gly), AB-system (ν_A = 4.72, ν_B = 4.82, 2 H, CCl_3CH_2OCO, J = 12.2 Hz), 4.25 (dd, 1 H, CH_2OCO, J = 5.1, 6.7 Hz), 4.15 (m, 2 H, $OCH_2C_3H_7$, OCH_2CH_3), 2.5 (m, 2 H, $CH_2CH{=}CH_2$), 1.25 (t, 3 H, OCH_2CH_3), 0.95 (t, 3 H, $OC_3H_7CH_3$). Anal. Calcd for $C_{16}H_{24}Cl_3NO_7$: C, 42.83; H, 5.39; N, 3.12; Cl, 23.70. Found: C, 42.90; H, 5.45; N, 3.01; Cl, 23.50.

***N*-[(2,2,2-Trichloroethoxy)carbonyl]-(*S*)-α-[5-(butyl 2(*RS*)-hydroxy-4-pentenoate)]glycine (23).** The enzymatic hydrolysis of **22** with chymotrypsin was carried out according to the preparation of **9**, yield 75%: ^{1}H-NMR (CD_3OD/C_6D_6 (3:1)) 5.9 (m, 1 H, olefin), 5.7 (m, 1 H, olefin), 4.75 (m, 1 H, α-H-gly), 4.65 (m, 2 H, CCl_3CH_2OCO), 4.17 (m, 1 H, CH_2CHOCO), 4.0 (m, 2 H, $OCH_2C_3H_7$), 0.82 (t, 3 H, $C_3H_7CH_3$). Anal. Calcd for $C_{14}H_{20}Cl_3NO_7$: C, 39.97; H, 4.79; N, 3.33; Cl, 25.28. Found: C, 40.02; H, 4.76; N, 3.10; Cl, 25.02.

Oxidative Cleavage of 18 to 24. 26 mg of RuO_4 was added to 5 mL of a 5% aqueous solution of $NaIO_4$ and then added to a solution of 420 mg (0.62 mmol) of **18** in 100 mL of acetone. A black precipitate formed, and during the next 30 min 840 mg (0.62 mmol) of $NaIO_4$ was added. After 1 h the reaction mixture was filtered over Celite and concentrated in vacuo. The residue was dissolved in water and extracted with ether. The aqueous phase was acidified with 0.1 N HCl (pH 2.5) and extracted with ether (3×). The organic phase was dried and concentrated to give 500 mg of a brown oil which was subjected to column chromatography (CH_2Cl_2/MeOH (50:1 → 12:1)) to give 140 mg (62.5%) of **24**: $[\alpha]^{25}_D$ +7.0° (c = 2, CH_2Cl_2); ^{1}H NMR($CDCl_3$) 5.50 (m, 1 H, OC*H*), 5.18 (b, 1 H, N*H*), 4.42 (m, 1 H, α-H-ala), 4.15 (m, 2 H, OCH_2), 2.93 (m, 2 H, $COCH_2$), 1.62 (m, 2 H, CH_2), 1.42 (s, 9 H, *t*-Bu), 1.39 (d, 3 H, α-H-ala, J = 7 Hz), 1.33 (m, 2 H, CH_2), 0.92 (t, 3 H, CH_3, J = 7 Hz). Anal. Calcd for $C_{16}H_{27}NO_8$: C, 53.18; H, 7.53; N, 3.88. Found: C, 53.26; H, 7.58; N, 3.84.

Oxidative Cleavage of 19 to 25. In analogy to the cleavage of **18**, 700 mg (1.03 mmol) of **19** was subjected to the above conditions to yield 198 mg (53%) of **25**: $[\alpha]^{25}_D$ −16.0° (c = 1, CH_2Cl_2); ^{1}H NMR($CDCl_3$) 5.50 (m, 1 H, OC*H*), 5.28 (b, 1 H, N*H*), 4.42 (m, 1 H, α-H-ala), 4.14 (t, 2 H, OCH_2, J = 7 Hz), 2.85 (m, 2 H, $COCH_2$), 1.60 (m, 2 H, CH_2), 1.42 (s, 9 H, *t*-Bu), 1.38 (d, 3 H, α-H-ala, J = 7 Hz), 1.33 (m, 2 H, CH_2), 0.92 (t, 3 H, CH_3, J = 7 Hz). Anal. Calcd for $C_{16}H_{27}NO_8$: C, 53.18; H, 7.53; N, 3.88. Found: C, 53.32; H, 7.30; N, 3.70.

Dibutyl 2(*R*)-[[(*tert*-Butoxycarbonyl)-(*S*)-alanyl]oxy]-1,4-butanedioate (28). 5 g (37 mmol) of (*R*)-(+)-malic acid (Fluka 2300) was suspended together with a catalytic amount of toluenesulfonic acid monohydrate in 200 mL of toluene. The mixture was heated to 120 °C, and after dropwise addition of 6.77 mL (74 mmol) of 1-butanol in 20 mL of toluene the reaction was stirred for 3 h at 120 °C with azeotropic removal of water until a clear solution had formed. Toluene was removed under reduced pressure, and the residue was dissolved in ethyl acetate and washed with $NaHCO_3$ (3×). Concentration in vacuo gave 7.62 g (84%) of **26** as a colorless, oily liquid which was carried on without further purification: $[\alpha]^{25}_D$ +8.0° (c = 1, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$) 4.47 (m, 1 H, OC*H*), 4.22 (m, 2 H, OCH_2), 4.12 (t, 2 H, OCH_2, J = 7 Hz), 3.22 (m, 1 H, O*H*), 2.81 (m, 2 H, CH_2), 1.62 (m, 4 H, 2 × CH_2), 1.35 (m, 4 H, 2 × CH_2), 0.90 (t, 6 H, 2 × CH_3, J = 7 Hz).

7.62 g (31 mmol) of **26** and 5.86 g (31 mmol) of BOC-L-alanine were dissolved in 150 mL of dry CH_2Cl_2, and a catalytic amount of DMAP was added and stirred for 20 min. 6.4 g (31 mmol) of DCC was added, and the reaction was stirred for 15 h at room temperature. The precipitated urea was filtered off, and the

filtrate was concentrated in vacuo and purified by column chromatography (cyclohexane:EE = 4:1) to give 10.8 g (83.5%) of **28** as a colorless oil: $[\alpha]^{25}_D$ +6.0° (c = 1, CH_2Cl_2); ^{1}H NMR-($CDCl_3$) 5.52 (dd, 1 H, OC*H*, J = 7.5, 5 Hz), 5.05 (bd, 1 H, N*H*), 4.43 (m, 1 H, α-H-ala), 4.18 (m, 2 H, OCH_2), 4.12 (m, 2 H, OCH_2), 2.92 (m, 2 H, COCH_2), 1.62 (m, 4 H, 2 × CH_2), 1.45 (s, 9 H, t-Bu), 1.40 (d, 9 H, CH_3-ala, J = 7 Hz), 1.38 (m, 4 H, 2 × CH_2), 0.95 (t, 3 H, CH_3, J = 7 Hz), 0.94 (t, 3 H, CH_3, J = 7 Hz); HPLC Polygosil (cyclohexane/1% 2-propanol), 1 mL/min, t_R 5.79 min; TLC (Merck 60 F254, cyclohexane/EtOAc 4:1)) R_f = 0.23; MS-FAB m/e 419 (MH^+, 6), 318 ((MH - BOC)$^+$, 100). Anal. Calcd for $C_{20}H_{35}NO_8$: C, 57.54; H, 8.45; N, 3.35. Found: C, 57.43; H, 8.23; N, 3.01.

Esterification of 24 to 28. 74 mg (0.2 mmol) of **24** were dissolved in 2 mL of CH_2Cl_2; 187 μL of n-BuOH (2 mmol), 45 mg of DCC (0.22 mmol), and a catalytic amount of DMAP were added. The reaction was stirred at room temperature overnight, the precipitated urea was filtered off, and after concentration in vacuo the residue was subjected to column chromatography (CH_2Cl_2 → CH_2Cl_2/MeOH (100:1)) to give 49 mg (57%) of **28**: $[\alpha]^{25}_D$ +4.8° (c = 3, CH_2Cl_2).

Dibutyl 2(*S*)-[[(*tert*-Butoxycarbonyl)-(*S*)-alanyl]oxy]-1,4-butanedioate (29). **27** was prepared from 3 g of (*S*)-(−)-malic acid (Fluka 2290) in analogous manner to **26** to yield 4.81 g (89%) of **27**: $[\alpha]^{25}_D$ −8.2° (c = 1, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$) 4.43 (dd, 1 H, OC*H*, J = 5 and 10 Hz), 4.17 (m, 2 H, OCH_2), 4.12 (t, 2 H, OCH_2, J = 7 Hz), 3.42 (d, 1 H, O*H*), 2.73 (m, 2 H, CH_2), 1.56 (m, 4 H, 2 × CH_2), 1.29 (m, 4 H, 2 × CH_2), 0.85 (t, 6 H, 2 × CH_3, J = 7 Hz).

Compound **29** was prepared from 2 g of **27** in an analogous manner to **28** to yield 2.7 g (81%) of **29**: $[\alpha]^{25}_D$ −14.8° (c = 1, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$) 5.53 (dd, 1 H, OC*H*, J = 6.5, 5.5 Hz), 5.05 (bd, 1 H, N*H*), 4.39 (m, 1 H, α-H-ala), 4.17 (m, 2 H, OCH_2), 4.14 (t, 2 H, OCH_2, J = 7 Hz), 2.92 (m, 2 H, COCH_2), 1.62 (m, 4 H, 2 × CH_2), 1.47 (d, 9 H, CH_3-ala, J = 7 Hz), 1.44 (s, 9 H, t-Bu), 1.37 (m, 4 H, 2 × CH_2), 0.94 (t, 3 H, CH_3, J = 7 Hz), 0.92 (t, 3 H, CH_3, J = 7 Hz); HPLC Polygosil (cyclohexane/1% 2-propanol, 1 mL/min): t_R 5.44 min; TLC (Merck 60 F254, cyclohexane/EE (4:1)) R_f = 0.27; MS-FAB m/e 419 (MH^+, 6), 318 ((MH - BOC)$^+$, 100). Anal. Calcd for $C_{20}H_{35}NO_8$: C, 57.54; H, 8.45, N, 3.35. Found: C, 57.63; H, 8.43; N, 3.38.

Esterification of 25 to 29. In analogy to the esterification of **24**, 150 mg (0.4 mmol) of **25** was subjected to the above conditions to yield 95 mg (55%) of **29**: $[\alpha]^{25}_D$ −14.9° (c = 3, CH_2Cl_2).

2(*S*)-[[(2,2,2-Trichloroethoxy)carbonyl]amino]-4(*R*)-[3-(butyl 2(*X*)-hydroxypropionate)]butyrolactone (30). Method A. A solution of 500 mg of **17** and catalytic amounds of dry $FeCl_3$ in 15 mL of CH_2Cl_2 was allowed to stand at 25 °C for 10 h. After usual workup and chromatography (cyclohexane/EE (6:4)) the ene product **17** and the lactone **30** were obtained in the ratio 5:3 (total yield 74%).

Method B. If the reaction mixture of the ene reaction **12** → **17** was allowed to stand at 25 °C for 15 h a mixture of **17** and **30** was obtained in the ratio of almost 1:1. **30**: mp 96–97 °C; $[\alpha]^{20}_D$ +12.6° (c = 0.75, CH_3OH); ^{1}H-NMR (CD_3OD/C_6D_6 (3:1)) 4.75 (s, 2 H, CCl_3CH_2OCO), 4.7 (m 1 H, H_3, $J_{3,4}$ = 5.5 Hz), 4.53 (dd, 1 H, H_5, $J_{5,4}$ = 8.8 and $J_{5,4'}$ = 12 Hz), 4.35 (dd, 1 H, H_1, $J_{1,2}$ = 6.4 and $J_{1,2'}$ = 5.4 Hz), 4.19 (m, 2 H, OC$H_2C_3H_7$), 2.62 (ddd, 1 H, H_4, $J_{4,5}$ = 8.8, $J_{4,3}$ = 5.5, and $J_{4,4'}$ = 12.2 Hz), 2.25 (m, 2 H, H_2, $J_{2,2'}$ = 14.2, $J_{2,1}$ = 6.4, $J_{2',1}$ = 5.4, $J_{2,3}$ = 6.4, and $J_{2',3}$ = 5.4 Hz), 0.9 (t, 3 H, $C_3H_7CH_3$); MS-FAB m/e 420, 422, 424 (MH^+ + isotope peaks, 100); IR (KBr) 3500, 3390, 1795, 1780, 1720, 1695, 1535 cm^{-1}; HPLC Nucleosil (cyclohexane/2-propanol (9:1)), 0.8 mL/min, t_R 4.6. Anal. Calcd for $C_{14}H_{20}Cl_3NO_7$: C, 39.97; H, 4.79; N, 3.33; Cl, 25.28. Found: C, 40.08; H, 4.77; N, 3.12; Cl, 25.02.