Synthesis of Modified Partial Structures of the Bacterial Cell Wall. 2. Retarded Metabolism of Lipopeptides by Insertion of α-Substituted α-Amino Acids

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The synthesis of the lipopeptide **1**, which exhibits both immunological activity (induction of the colony stimulating factor (CSF)) and stability against metabolic degradation, has been described. A detailed investigation of the course of the ene reaction between the dipeptide **21** and butyl glyoxylate enabled us to use this type of pericyclic reaction for the establishment of the essential pentenoic acid side chain in **23**. The required amino acid **15** was obtained by enzymatic hydrolysis of the corresponding *rac*-ester **19**. The absolute configuration of **1** was assigned by oxidative cleavage of the double bond in **24** and **25** followed by comparison of the degradation products with authentic samples.

Introduction

The development of potent and effective immunomodulating compounds for enhancement of the body's own immune defense has become an intensely pursued goal of contemporary medicinal chemistry.[1] In a previous paper[2] we reported the synthesis of the lipopeptide **2** whose structure was derived from the naturally occurring FK 156[3] and its synthetic analogue FK 565.[4] In contrast to these compounds **2** induced high titers of circulating colony stimulating factor (CSF),[1f] exhibiting no acute toxicity. The CSF levels in serum samples of treated mice were measured by the ability to support proliferation of murine bone marrow cells.[5] Unfortunately, this activity proved to be highly time dependent, indicating a rapid metabolism of **2** after oral application. This could be demonstrated by an *in vitro* experiment which showed rapid degradation of **2**: After 1-h incubation with freshly prepared murine stomach fluid (Scheme 1) mainly the inactive triacid **7** together with minor amounts of the hydroxy compound **6** which still exhibited CSF activity were obtained. In order to retard this rapid metabolic process, we considered the introduction of an additional methyl group into the α-position of the pimelic acid moiety of the lipopeptide **2**. Such a substitution pattern with maintenance of the required *S*-configuration[6] (**1**) should provide strong resistance against metabolic attack.[7] The first part of this paper deals with different approaches to the fully substituted amino acid **15** and with the difficulties in adjusting a hetero ene reaction to peptidic substrates. In the second part two alternative reaction sequences are described for the conversion of the dipeptide **21** to the lipopeptide **1**.

Results

Synthesis of the α,α′-Disubstituted Amino Acid 15 (Scheme 2). A number of routes[8,9] to α-alkyl-α-amino acids have appeared in the literature, but very few have addressed the synthesis of α-allylated α-amino acids.[9] For our purposes we needed an expedient route to multigram quantities of optically pure amino acid **15**. Initially we investigated known procedures which seemed to be most promising. Zydowsky et al.[9b] have prepared tricyclic versions of Seebach's oxazolidinones and used these synthons as a nucleophile equivalent of alanine. This method would have been appropriate for large-scale synthesis but in our hands the claimed enantiomeric excess (ee 95%) could not be reproduced. Belokon et al.[9a] used chiral Ni complexes of alanine which were said to be readily separable after conventional alkylation. The separation of the diastereoisomers, however, was found to be very tedious and time consuming. In view of this precedent, we tried to modify Seebach's oxazolidinone procedure[8a] for our requirements: The sodium salt of (*S*)-alanine was first condensed with pivalaldehyde to give the Schiff base **8** which in turn was cyclized by treatment with benzoyl chloride affording the easily separable diastereomeric mixture of the oxazolidinones **9** and **10**. By comparison of the NMR spectra with literature data and measurement of nuclear Overhauser effects (NOE),

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(1) (a) Review: Lederer, E. *J. Med. Chem.* **1980**, *23*, 819. (b) Homma, J. Y.; Matsuma, M.; Kumazawa, Y. *Drugs Future* **1989**, *14*, 645 and references cited therein. (c) Balreddy, K.; Dong, L.; Simpson, D. M.; Titmas, R. *Tetrahedron Lett.* **1993**, *34*, 3037. (d) Inamura, N.; Nakahara, K.; Kino, T.; Gotoh, T.; Kawamura, I.; Acki, H.; Imanaka, H.; Sono, S. *J. Biol. Response Modifiers* **1985**, *4*, 408. (e) Devlin, D. J.; Hangrave, K. D. *Tetrahedron* **1989**, *14* (report no. 256), 4327. (f) Metcalf, D. *The Hemopoietic Colony Stimulating Factors*; Elsevier: Amsterdam, 1984.

(2) Schneider, H.; Sigmund, G.; Schricker, B.; Thirring, K.; Berner, H. *J. Org. Chem.* **1993**, *58*, 683–689.

(3) Takeno, H.; Okada, S.; Hemmi, K.; Aratami, M.; Kitaura, Y.; Hashimoto, M. *Chem. Pharm. Bull.* **1984**, *32*, 2925.

(4) Mine, Y.; Yokota, Y.; Wakai, Y.; Fukuda, S.; Nishida, M.; Gotho, S.; Kuwahara, S. *J. Antibiot.* **1983**, *36*, 1045.

(5) Hildebrandt, J. Unpublished results.

(6) It would have been a much easier approach to merely change the configuration at this position but it turned out that (*R*)-configurated derivatives are completely lacking in immunological activity.

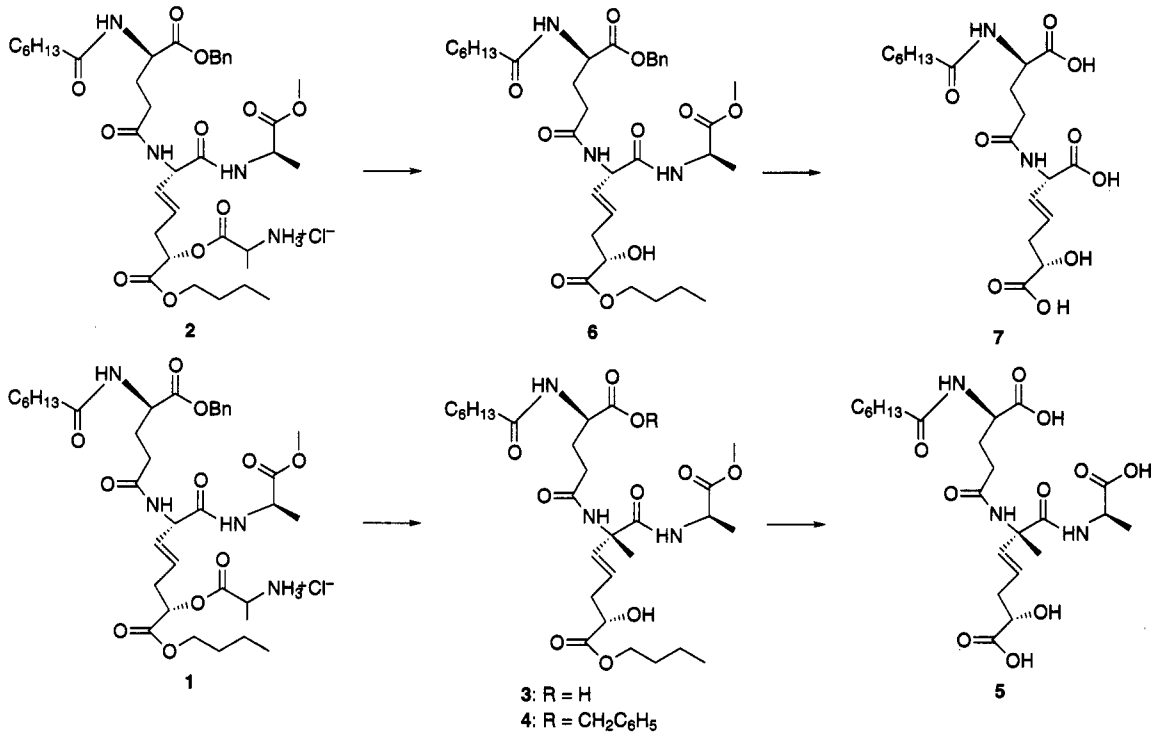
(7) (a) Bey, P. In *Enzymatic Activated Irreversible Inhibitors*; Seiler, N., Koch-Weser, J., Jung, M. J., Eds.; Elsevier: Amsterdam, 1978; p 27. Bey, P.; Metcalf, B.; Jung, M. J.; Forzard, J.; Koch-Weser, J. In *Strategy in Drug Research*; Keverling-Buisman, J. A., Ed.; Elsevier Scientific Publishing Company: Amsterdam, 1982; p 89.

(8) (a) Fitzi, R.; Seebach, D. *Tetrahedron* **1988**, *44*(17), 1988. (b) Seebach, D.; Fadel, A. *Helv. Chim. Acta* **1985**, *68*, 1243. (c) Fadel, A.; Salaun, J. *Tetrahedron Lett.* **1987**, *28*(20), 2243. (d) Ojima, I.; Chen, H. J. C. *Tetrahedron* **1988**, 5307. (e) Karady, S.; Amato, J. S.; Weinstoch, L. M. *Tetrahedron Lett.* **1984**, *25*(39), 4337. (f) Kruizinga, W. H.; Bolster, J.; Kellogg, R. M.; Kamphuis, J.; Boesten, W. H. J.; Meijer, E. M.; Schoemaker, H. E. *J. Org. Chem.* **1988**, *53*(8), 1826. (g) Schoellkopf, U.; Groth, U.; Westphalen, K. O.; Deng, C. *Synthesis* **1981**, 969. (h) Schmidt, U.; Respondek, M.; Liebknecht, A.; Werner, J.; Fischer, P. *Synthesis* **1989**, 256.

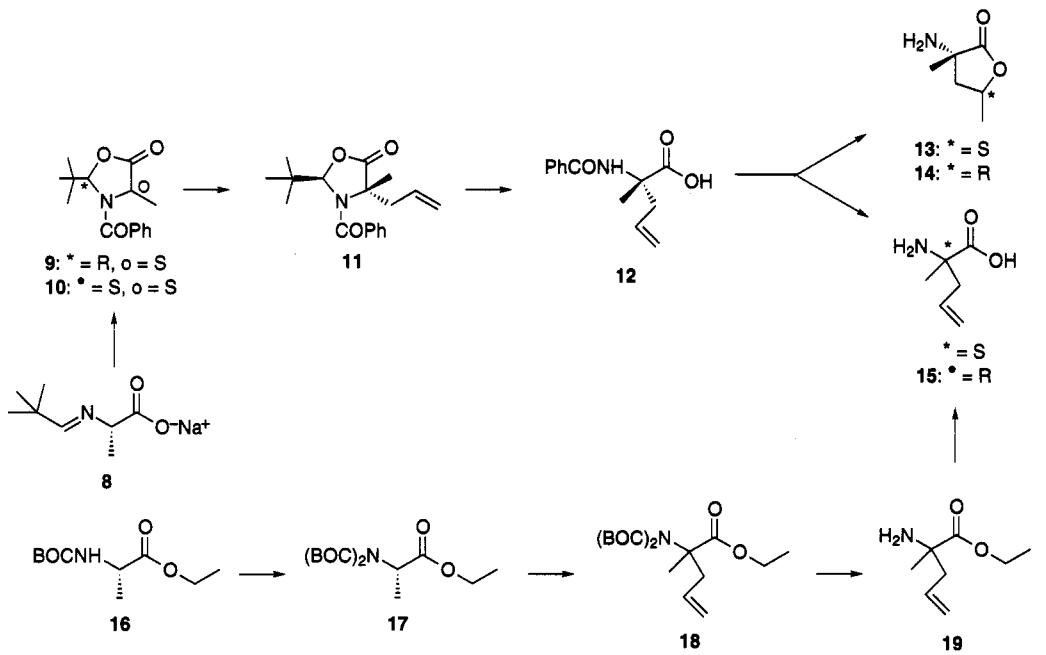
(9) (a) Belokon, Y. N.; Chernoglazova, N. I.; Kochetkov, C. A.; Garbalinskaya, N. S.; Belikov, V. M. *J. Chem. Soc., Chem. Commun.* **1985**, 171. (b) Zydowsky, T. M.; de Lara, E.; Spanton, S. G. *J. Org. Chem.* **1990**, *55*, 5437.

0022-3263/94/1959-4215$04.50/0

Scheme 1



Scheme 2

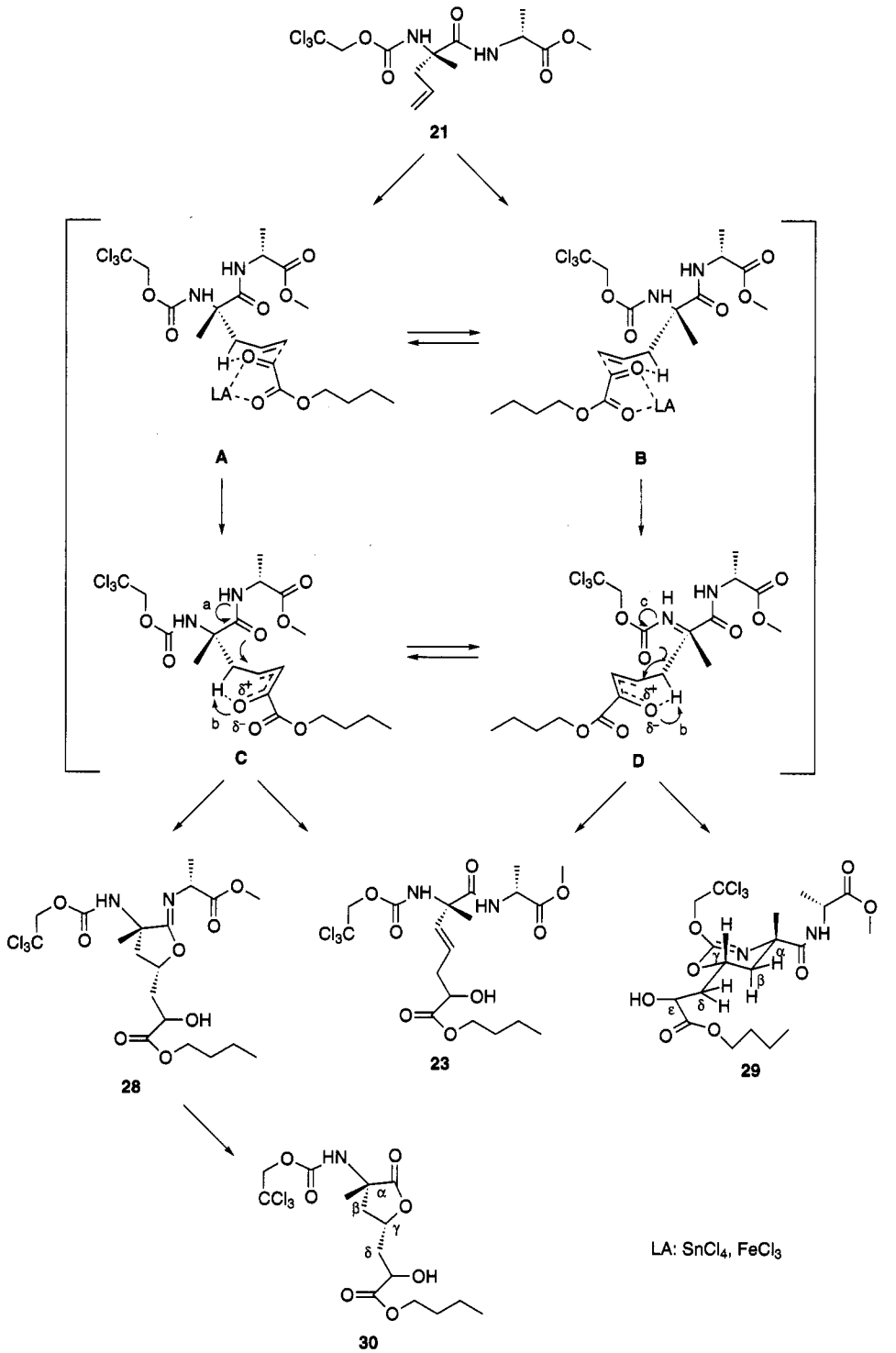


we assigned the cis configuration to the major isomer **10** and the trans configuration to **9**. After alkylation of **9** (LDA, allyl bromide) we obtained the geminally disubstituted oxazolidinone **11** with the desired chirality at the α-position (de 97%). The trifling amounts of trans product could be readily removed by crystallization. Although the acid hydrolysis of **11** to the free amino acid **15** can be performed in one step, it is advisable to isolate the intermediate benzoylated amino acid **12** after mild alkaline hydrolysis. Proceeding this way the subsequent acid hydrolysis of the benzoyl group takes a much smoother course compared to the one-step procedure. Owing to a preceding hydratization of the double bond which occurs to a minor extent during hydrolysis, one has to take account of the diastereomeric amino lactones **13/14** as side products. Due to high stereoselectivity and fair yields this approach seems very attractive, but the unfavorable ratio of **9** and **10** (1:4)—only the minor diastereomer **9** could be used for alkylation—precluded its application in large-scale synthesis.

An enzymatic approach to the amino acid **15** was next investigated. Contrary to N-protected amino acid esters with a fully substituted α-position,[10] those with a free N-terminus can be frequently hydrolyzed by esterases with high enantiomeric excess.[11] Thus, the readily available bis BOC-protected alanine ethyl ester **17** was

(10) Schricker, B.; Thirring, K.; Berner, H. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 387.
(11) Anantharamaia, G. M.; Roeske, R. W. *Tetrahedron Lett.* **1982**, *23*, 3335.

Scheme 3



allylated affording the racemic ester **18**. After deprotection of **18** with TFA, **19** proved to be an ideal substrate for a chymotrypsin-catalyzed hydrolysis. The *S*-configurated amino acid **15** could be isolated after ion-exchange chromatography with high chemical and stereochemical yield. A more favorable protocol, however, consisted in removing the unreacted (*R*)-ester of **18** from the aqueous reaction medium followed by intermediate treatment of **15** with trichloroethyl chloroformate under Schotten Baumann conditions[12] to give the protected amino acid **20**. Subsequent coupling with (*R*)-alanine methyl ester led finally to the dipeptide **21**[13] which was used for the ene reactio with butyl glyoxylate.

(12) Sonntag, N. O. V. *Chem. Rev.* **1953**, *52*, 272.

The Ene Reaction (Scheme 3). In order to synthesize the pentenoic acid side chain of the dipeptide **23** a hetero ene reaction was tried as in the former synthesis of the lipopeptide **2**.[2] Treatment of the protected dipeptide **21** with butyl glyoxylate catalyzed by $FeCl_3$ furnished the *E*-configurated ene product **23** together with the imino lactone **28**, the lactone **30**, and to a minor extent the oxazine **29**.

Our previous experience[2] with ene reactions using peptidic substrates made us aware of possible side

(13) Small amounts of (*R*)-configurated amino acid taken along from the enzymatic conversion gave rise to the (*R,R*)-configurated diastereoisomer **22**. This could be readily removed by crystallization or flash chromatography on silica gel.

reactions. As the amount of side products **28**, **29**, and **30** exceeded by far the amount of the ene product **23**, we investigated the entire mechanism in more detail in order to differentiate between the different reaction pathways.

Given the reasonable postulate that the Lewis acid is complexed to the glyoxylate in a *syn* fashion[14] we hypothesized two primary arrangements of ene and enophile which are visualized by **A** and **B**, respectively. In a first step a C—C bond is formed by a *re*- or *si*-side interaction of the glyoxylate carbonyl and the allylic double bond resulting in two different bipolar transition states[15] **D** and **C**, respectively. For the rate- and product-determining second step three different reaction pathways (a, b, and c) can be adopted: (b) the transfer of the allylic proton to the oxygen leading to the ene product **23** and (a and c) the nucleophilic attack of the peptide or carbamate carbonyl at the positive center, leading to iminolactone **28**, lactone **30**, or oxazine **29**. The assumption that the bipolar transition states **C** and **D** are the common transition states for all reaction products is based on two control experiments: (i) reaction of the isolated ene product **23** with a 3-fold excess of Lewis acid produced no detectable amounts of lactone, and (ii) reacting the allylated dipeptide **22** in a similar manner, no nucleophilic attack of the peptide carbonyl could be initiated after a 1-week reaction period. These findings provided strong evidence[16] for the postulated common intermediates (**C** and **D**) indicating that the nucleophilic attack has to occur prior to the proton transfer, the essential second step of the ene reaction.

The regio- and stereoselectivity observed in these reactions are consistent with the above reaction pathways. According to the alignment of the peptide moiety in the transition states **C** and **D**, ene product, iminolactone, or oxazine is formed preferentially. Owing to the configuration of the lactone moiety[17] in **28** the allylic double bond must be attacked from the *si*-side (transition states **A** and **C**), whereas the configuration of the oxazine moiety in **29** refers to a *re*-side attack (transition states **B** and **D**). We also found uniform configuration at the COH carbon of **28** and **29**, which implies in addition a specific alignment of the glyoxylic ester carbonyl in both transition states. Since the ene product **23** can be derived from both transition states (**C** and **D**) we found both *S* and *R* configurations at the COH position, *S* being the predominate (ee 86%).

At first we attributed this lack of product selectivity to an unfavorable alignment of the amide carbonyl whose nucleophilic attack interferes highly effectively with the proton transfer. Thus, dipeptides with a fully substituted α-position seemed to be impracticable for ene reactions. Fortunately, this scenario was changed by using a softer and more polarizable Lewis acid like $SnCl_4$[21] instead of $FeCl_3$, which can be classified as harder and smaller. In this case the proton transfer (b) proved to be the main reaction pathway whereas the nucleophilic attack (a and c) at the positive center occurred only to a minor extent.

It should be noted that the decrease of Lewis acid acidity[21] results in an increase in yield of ene product **28** linked to a decrease in stereoselectivity (ee 54%). The preponderance of pathway b in the $SnCl_4$-promoted reaction can be explained by a higher steric congestion of the $SnCl_4$ versus the $FeCl_3$ complexed transition state. Hence, it follows a restriction for the nucleophilic attack but obviously no significant decrease for the proton transfer to the oxygen.[18] These findings enabled us to suppress the above-mentioned side reactions and to make use of the ene reaction for the formation of the pentenoic side chain of the dipeptide **23**.

Synthesis and Properties of the Lipopeptide 1 (Scheme 4). Starting from the key intermediate **21** lipopeptide **1** was synthesized in two ways. The diastereomeric mixture of the dipeptide **23** was separated after esterification of the OH group with BOC-protected (*S*)-alanine to give the epimers **24** and **25**. The *S*-configurated epimer **24** was selectively deprotected at the N-terminus (**26**) and subsequently coupled with the acylated (*R*)-glutamic acid benzyl ester.[2] The BOC group of the resulting tripeptide was finally removed to give the hydrochloride of **1**.

The second pathway takes advantage of transferring the rather intricate ene reaction to a subsequent step: After deprotection **21** was first converted to the tripeptide **31** which thereupon was subjected to the hetero ene reaction with butyl glyoxylate. In contrast to the experience with the dipeptide **21** this reaction takes a highly stereospecific course, giving mainly *S*-configurated diester **3** and only minor amounts of *S*-configurated triester **4**. Thus, the advantage of stereospecificity was counterbalanced as even under carefully controlled conditions the hydrolysis of the benzyl ester could not be avoided.

The lipopeptide **1** exhibited stability against metabolic degradation showing also the required CSF activity.[5] By using the above-mentioned *in vitro* degradation with murine stomach fluid the metabolic stability could be demonstrated unambiguously: Besides the OH compound **3** which corresponds to **6** (Scheme 1) we found only the triacid **5** which still contains the (*R*)-alanine moiety that is essential for biological activity.

The Assignment of Configuration. A nuclear Overhauser effect of 4.5% between the ϵ-CH_3 and the γ-proton as well as the comparable H^1-NMR coupling patterns of the δ-protons in **30** with those in analogous compounds[19] indicate cis configuration of the lactone and thus *S*-configuration of the γ-carbon atom.

The configuration of the γ-carbon atom in **29** was assigned by means of NMR spectroscopy. Due to a 1,3-interaction with the lone pairs of oxygen and nitrogen the H-β_{ax} could be clearly assigned by its chemical shift (H-β_{ax} = 1.4 ppm, H-β_{equ} = 2.6 ppm). Besides the geminal coupling this proton shows only a trans-diaxial coupling with the γ-H, indicating in addition an equatorial position for the α- and γ-substituents. In addition, since we knew

(14) Whitesell, J. K.; Bhattacharya, A.; Buchanan, C. M.; Chen, H. H.; Deyo, D.; James, D.; Liu, C. L.; Minton, M. A. *Tetrahedron* **1986**, *40*, 2993.

(15) Inagaki, S.; Fujimoto, H.; Fukui, K. *J. Am. Chem. Soc.* **1976**, *98*, 4693.

(16) According to the findings in our previous paper[2] this mechanism cannot be generalized as the analogous ene product unsubstituted in the α-position can be converted subsequently to the lactone moiety. This indicates a highly structure-dependent mechanistic course.

(17) Due to an error during the preparation of the previous paper,[2] we assigned there a *trans*-configuration to the α-unsubstituted lactone **30**. According to the NMR data described in the paper it is clearly *cis* configurated, a finding which is also in full accordance with the literature data[19] for similar compounds.

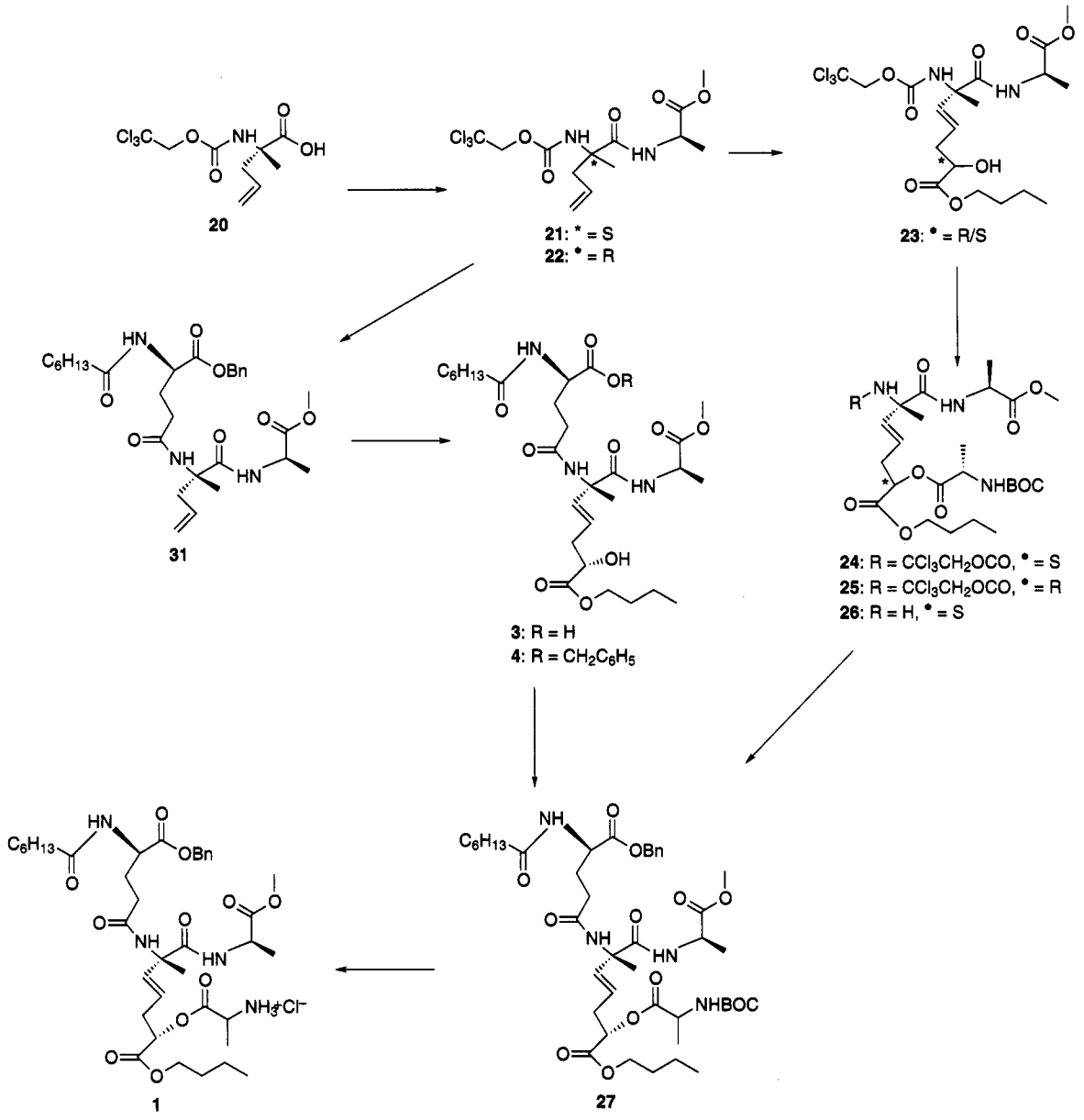
(18) We also tried to use chiral Lewis acids, especially BINOL derivatives,[20] but none of them proved to be sufficiently active to initiate the ene reaction.

(19) Matzinger, P.; Catalfono, Ph.; Eugster, C. H. *Helv. Chim. Acta* **1972**, *55*, 1478.

(20) Mikami, K.; Terada, M.; Narisawa, S.; Nakai, T. *Synlett* **1992**, 255.

(21) (a) Satchell, D. P. N. *Q. Rev. Chem. Soc.* **1971**, *25*, 171–199. (b) Pearson, R. G. *J. Chem. Educ.* **1987**, *64*, 561.

Scheme 4



the α-carbon is *S*-configurated, the γ-carbon has to be *R*-configurated.

As it was outlined in the first paper[2] the absolute configuration of C* in **24** and **25** was determined by oxidative degradation of these compounds with RuO_4 affording the appropriate malic acid derivatives. These were compared with authentic samples synthesized from (*R*)- and (*S*)-malic acid.[2] In addition, this result could be confirmed by comparison of the NMR data. The coupling patterns of the allylic protons of the (*R*)- and (*S*)-configurated lipopeptides **1** and **2**, respectively, are almost identical.

Conclusions. In this study, we have developed an efficient method for the preparation of the lipopeptide **1** which proved to exhibit both CSF activity and stability against metabolic peptide cleveage. The synthesis of the required α,α′-disubstituted amino acid **15** could be effected *via* enzymatic resolution of the *rac*-ester **19**. A hetero ene reaction was used to establish the pentenoic acid ester side chain. The course of this reaction was thoroughly investigated in order to suppress some inconvenient side reactions determined by the peptidic substrate **21**. Two different reaction sequences could be used (**21** → **23** → **24** → **27** → **1** or **21** → **31** → **4** → **27** → **1**) to synthesize **1**. The configuration of the α-carbon in the pentenoic acid side chain was assigned by comparison of the optical rotation of the degradation products of **24** and **25** with those of authentic samples.

Experimental Section

The general experimental procedures and the analytical instruments employed have been described in detail in a previous paper.[2] Cyclohexane is abbreviated CY.

***N*-[*N*-[*N*-Heptanoyl-(*R*)-γ-glutamyl-(α-benzyl ester)]-(*S*)-α-[5-butyl 2(*S*)-((*S*)-alanyloxy)-4-pentenoate)]-α-alanyl]-(*R*)-alanine Methyl Ester Hydrochloride (1).** To a solution of 200 mg of **27** in H_2O saturated CH_2Cl_2 was added under argon atmosphere (0 °C) 0.5 mL of TFA. After 15 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 with 2% ethereal HCl. After removal of the solvent (30 °C bath temperature) the hydrochloride was taken up in 15 mL of H_2O. The resultant solution was lyophilized to afford 126 mg (68%) of **1**: $[\alpha]^{20}_D$ +10.1° (*c* 0.8, CH_3OH); ^{1}H-NMR (CD_3OD) δ 7.42–7.34 (m, 5H), 6.04 (d, 1H, *J* = 15.7 Hz), 5.75 (ddd, 1H, *J* =

15.7, 7.6 Hz), 5.3 (dd, 1H, J = 7.8, 4.2 Hz), 5.21 (s, 2H,), 4.51 (dd, 1H, J = 9.9, 5 Hz), 4.42 (q, 1H, J = 7.3 Hz), 4.28 (q, 1H, J = 7.1 Hz), 4.22 (m, 2H), 3.74 (s, 3H), ABXY-system (ν_A = 2.78, ν_B = 2.66, J_{AB} = 15.6 Hz, J_{AY} = J_{BY} = 6.6 Hz, J_{BX} = 7.8 Hz, J_{AX} = 4.2 Hz), 2.36 (t, 2H, J = 6.7 Hz), 2.28 (t, 2H, J = 7.1 Hz), 1.64 (d, 3H, J = 7.1 Hz), 1.53 (s, 3H), 1.40 (d, 3H, J = 7.3 Hz), 0.99 (t, 3H, J = 7.4 Hz), 0.94 (t, 3H, J = 7.4 Hz); MS-FAB *m/e* 769 (100). Anal. Calcd for $C_{37}H_{57}ClN_4O_{11}$: C, 57.77; H, 7.47; N, 7.28; Cl, 4.61. Found: C, 57.51; H, 7.60; N, 6.98; Cl, 4.29.

***N*-[*N*-(*N*-Heptanoyl-(*R*)-γ-glutamyl)-(*S*)-α-[5-(butyl 2(*S*)-hydroxy-4-pentenoate)]-α-alanyl]-(*R*)-alanine Methyl Ester (3), *N*-[*N*-[*N*-Heptanoyl-(*R*)-γ-glutamyl-(α-benzyl ester)]-(*S*)-α-[5-(butyl 2(*S*)-hydroxy-4-pentenoate)]-α-alanyl]-(*R*)-alanine Methyl Ester (4), and *N*-[*N*-(*N*-Heptanoyl-(*R*)-γ-glutamyl)-(*S*)-α-[5-((2*S*)-hydroxy-4-pentenoic acid)]-α-alanyl]-(*R*)-alanine (5). Method A.** One g of **1** was suspended in 100 mL of 0.1 M phosphate buffer (pH 4.5) and treated with 2 mL of freshly prepared murine stomach fluid at 37 °C. After 2 h the reaction mixture was diluted with brine and repeatedly extracted with CH_2Cl_2. After gradient chromatography on silica gel (CH_2Cl_2/MEOH/H_2O (70:30:1 → 70:30:6)) 210 mg of **3**, 120 mg of **4**, and 380 mg of **5** were obtained.

Method B. The ene reaction with **31** and butyl glyoxylate was carried out according to the preparation of **23**; 42% of **3** and 15% of **4** were obtained. **3**: ^{1}H-NMR (CD_3OD) δ 6.2 (d, 1H, J = 15.6 Hz), 5.7 (ddd, 1H, J = 15.6, 6.4 Hz), 4.45 (q, 1 H, J = 7.3 Hz), 4.3 (dd, 1H, J = 9.9, 5 Hz), 4.2 (dd, 1H, J = 7.2, 4.5 Hz), 4.08 (m, 2H), 3.62 (s, 3H), 2.2–2.4 (m, 2H), 1.6 (s, 3H), 1.35 (d, 3H, J = 7.3 Hz), 0.9 (m, 6H); MS-FAB *m/e* 586 (MH^+, 100). Anal. Calcd for $C_{28}H_{47}N_3O_{10}$: C, 57.42; H, 8.09; N, 7.17. Found: C, 57.38; H, 8.20; N, 7.31. **4**: ^{1}H-NMR (CD_3OD) δ 7.3 (m, 5H), 5.2 (s, 2H), the other signals are identical with those in the ^{1}H-NMR of **3**. Anal. Calcd for $C_{35}H_{53}N_3O_{10}$: C, 62.20; H, 7.90; N, 6.22. Found: C, 62.05, H, 7.90; N, 6.12. **5**: ^{1}H-NMR (D_2O, 360 K) δ 5.5 (m, 2H), 4.2 (dd, 1H, J = 4.7, 9.3 Hz), 4.35 (q, 1H, J = 7.3 Hz), 4.0 (dd, 1H, J = 7, 4.8 Hz), 2.25–2.4 (m, 2H), 1.6 (s, 3H), 0.88 (t, 3H), 1.33 (d, 3H, J = 7.3 Hz); MS-FAB *m/e* 516 (MH^+, 100). Anal. Calcd for $C_{23}H_{37}N_3O_{10}$: C, 53.58; H, 7.23; N, 8.15. Found: C, 53.85; H, 7.37; N, 8.01.

***N*-[*N*-[*N*-Heptanoyl-(*R*)-γ-glutamyl-(α-benzyl ester)]-(*S*)-α-[5-(butyl 2(*S*)-hydroxy-4-pentenoate)]glycyl]-(*R*)-alanine Methyl Ester (6) and *N*-(*N*-Heptanoyl-(*R*)-γ-glutamyl)-(*S*)-α-[5-(2(*S*)-hydroxy-4-pentenoic acid)]glycine (7).** The enzymatic degradation was carried out according to the preparation of **3**, **4**, and **5**. One hundred-twenty mg of **6** and 420 mg of **7** were obtained. **6**: ^{1}H-NMR (CD_3OD/C_6D_6 (3:1)) δ 7.3 (m, 5H), 5.87 (ddd, 1H, J = 15.4, 7.1 Hz), 5.7 (dd, 1H, J = 15.4, 6.8 Hz), 5.12 (s, 2H), 4.98 (d, 1H, J = 6.8 Hz), 4.54, (dd, 1H, J = 4.8, 9.4 Hz), 4.45 (q, 1H, J = 7.3 Hz), 4.22 (dd, 1H, J = 7.1, 4.7 Hz), 4.1 (m, 2H), 3.63 (s, 3H), 2.4–2.55 (m, 2H), 1.35 (d, 3H, J = 7.3 Hz), 0.92 (t, 3H, J = 7.2 Hz), 0.85 (t, 3H, J = 7.2 Hz); MS-FAB *m/e* 662 (MH^+, 100). Anal. Calcd for $C_{34}H_{51}N_3O_{10}$: C, 61.71; H, 7.77; N, 6.35. Found: C, 61.57; H, 7.93; N, 6.10. **7**: ^{1}H-NMR (CD_3OD) δ 5.6 (m, 1H), 5.42 (m, 1H), 4.8 (d, 1H, J = 6.9 Hz), 4.5 (dd, 1H, J = 4.7, 9.4 Hz), 4.22 (dd, 1H, J = 7.2, 4.7 Hz), 2.6 (m, 2H), 0.9 (t, 3H); MS-FAB *m/e* 431 (MH^+ 100). Anal. Calcd for $C_{19}H_{30}N_2O_9$: C, 53.02; H, 7.12; N, 6.51. Found: C, 53.4; H, 7.31; N, 6.30.

Sodium Pivalylidene-(*S*)-alaninate (8). **8** was prepared according to the procedure of Seebach *et al.*[8b]

(2*S*/4*S*)- and (2*R*/4*S*)-3-Benzoyl-2-*tert*-butyl-4-methyl-1,3-oxazolidin-5-one (9 and 10). Following the procedure of Seebach *et al.*[8b] we obtained at 4:1 mixture of **9/10** (83%). The diastereomers were separated by chromatography (cyclohexane/dioxane (95:5)). It is possible as well to recrystallize the reaction mixture by seeding with a pure sample. **10**: mp 125–126 °C (CY/dioxane); $[\alpha]^{20}_D$ +77.8° (*c* 0.5, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 7.64–7.25 (m, 5H), 6.25 (s, 1H), 4.36 (q, 1H, J = 6.8 Hz), 1.1 (d, 3H, J = 6.8 Hz), 1.05 (s, 9H). Anal. Calcd for $C_{15}H_{19}NO_3$: C, 68.94; H, 7.33; N, 5.36. Found: C, 68.85; H, 7.10; N, 5.35. **9**: mp 93–94.5 °C (CY/dioxane); $[\alpha]^{20}_D$ −28.1° (*c* 0.5, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 7.52–7.25 (m, 5H), 6.14 (s, 1H), 4.06 (q, 1H, J = 7.1 Hz), 1.51 (d, 3H, J = 7.1 Hz), 1.05 (s, 9H). MS-FAB *m/e* 302 (MH^+, 76), 188 (100), 152 (38). Anal. Calcd for $C_{15}H_{19}NO_3$: C, 68.94; H; 7.33; N, 5.36. Found: C, 68.70; H, 7.44; N, 5.40.

(2*R*,4*S*)-4-Methyl-4-(2-propenyl)-2-*tert* butyl-3-benzoyl-1,3-oxazolidin-5-one (11). To a solution of LDA (15.2 mmol) in 30 mL of THF was added under argon atmosphere (−78 °C) a solution of 2 g (7.6 mmol) of **10** in 10 mL of THF (→ deep orange). After 30 min 0.76 mL of allyl bromide (9.12 mmol) was added, and the reaction mixture was allowed to warm to 20 °C within 2 h. After being stirred for an additional 5 h at rt, the resulting light yellow solution was poured into NH_4Cl solution and extracted twice with ethyl acetate. After usual workup and chromatography on silica gel (CY/ethyl acetate (3:1)) 1.48 g (64%) of **11** was obtained: $[\alpha]^{20}_D$ +48.4° (*c* 0.7, CH_3OH); ^{1}H-NMR (DMSO, 350 K) δ 7.55 (m, 5H), 6.05 (s, 1H), 5.37 (m, 1H), 5.12 (m, 2H), 2.31 (d(b), 2H), 1.56 (s, 3H), 0.88 (s, 9H); MS-FAB *m/e* (MH^+, 70), 188 (100). Anal. Calcd for $C_{18}H_{23}NO_3$: C, 71.73; H, 7.69; N, 4.65. Found: C, 71.60; H, 7.42; N, 4.31.

***N*-Benzoyl-(*S*)-α-(2-propenyl)-α-alanine (12).** A solution of 2.47 g (8.2 mmol) of **11** and 592 mg (24.6 mmol) of LiOH in 20 mL of CH_3OH (5% H_2O) was stirred at 40 °C for 20 h. The reaction mixture was adjusted with 1 N HCl to pH 3 and repeatedly extracted with ethyl acetate. After usual workup we obtained 1.8 g (94%) of **12**: $[\alpha]^{20}_D$ −15.5° (*c* 0.6, CH_3OH); ^{1}H-NMR ($CDCl_3$) δ 7.76–7.48 (m, 5H), 6.87 (s, 1H), 5.77 (m, 1H), 5.20 (m, 2H), ABX-system (ν_A = 2.99, ν_B = 2.79, J_{AB} = 13.9 Hz, J_{AX} = 7.6 Hz, J_{BX} = 7.2 Hz), 1.75 (s, 3H). MS-FAB *m/e* 234 (MH^+, 75), 105 (100). Anal. Calcd for $C_{13}H_{15}NO_3$: C, 66.94; H, 6.48; N, 6.00. Found: C, 67.23; H, 6.62; N, 6.20.

(2*S*,4*S*)-2-Amino-2,4-dimethylbutyrolactone (13), (2*S*,-4*R*)-2-Amino-2,4-dimethylbutyrolactone (14), and (*S*)-α-(2-Propenyl)-α-alanine (15). Method A. An emulsion of 1.8 g of **12** in 20 mL of 2 N HCl was heated for 2 h at 145 °C in a closed round-bottomed flask. The homogeneous reaction mixture was freed from benzoic acid by extraction with ethyl acetate and subsequently lyophilized. After chromatography on silica gel (ethyl ether (EE)/MeOH/H_2O (70:30:3)) 615 mg (61.6%) of amino acid **15** and 342 mg (34.3%) of the diastereomeric lactones **13/14** were obtained. For spectroscopic data, a small sample of **13/14** was separated by another chromatography on silica gel (EE/MeOH/H_2O (70:30:1)).

Method B. A 1.74-g (9 mmol) portion of *rac*-**19** was suspended in 200 mL of 0.1 M phosphate buffer (pH 8) and treated with 200 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 continuous addition of 1 N NaOH. After 20 h the unreacted (*R*)-ester of **19** was recovered by extracting the reaction mixture with ethyl acetate. Subsequently, the aqueous solution was lyophilized affording the crude amino acid **15** which was used without further purification. By HPLC analysis according to the method of Brückner et al.[22] an enantiomeric excess of ee 94% was determined. **13**: ^{1}H-NMR (CD_3OD) δ 3.9 (m, 1H), ABX-system (ν_A = 2.55, ν_B = 1.78, J_{AB} = 12.6 Hz, J_{AX} = 5.4 Hz, J_{BX} = 10.3 Hz), 1.53 (s, 3H), 1.43 (d, 3H, J = 5.6 Hz). Anal. Calcd for $C_6H_{11}NO_2$: C, 55.80; H, 8.58; N, 10.84. Found: C, 55.70; H, 8.60; N, 10.64. **14**: ^{1}H-NMR (CD_3OD) δ 4.2 (m, 1H), 2.15–1.85 (m, 2H), 1.53 (s, 3H), 1.24 (d, 3H, J = 6.2 Hz). Anal. Calcd for $C_6H_{11}NO_2$: C, 55.80; H, 8.58; N, 10.84. Found: C, 55.92; H, 8.72, N, 10.52. **15**: $[\alpha]^{20}_D$ −25.7° (*c* 0.8, CH_3OH); ^{1}H-NMR (D_2O) δ 5.77 (m, 1H), 5.29 (m, 2H), ABX-system (ν_A = 2.68, ν_B = 2.47, J_{AB} = 14.4 Hz, J_{AX} = 6.7 Hz, J_{BX} = 8.3 Hz), 1.5 (s, 3H); MS-FAB *m/e* 130 (MH^+, 100). Anal. Calcd for $C_6H_{11}NO_2$: C, 55.80; H, 8.58; N, 10.84. Found: C, 55.46; H, 8.37; N, 10.74.

***N,N*-[Bis(*tert*-butoxycarbonyl)]-(*S*)-α-alanine Ethyl Ester (17).** To a solution of 35 g (161 mmol) of **16** in 200 mL of CH_3CN were added 105.5 g (484 mmol) of BOC-anhydride and a catalytic amount of DMAP. The reaction mixture was kept at 50 °C for 8 h, subsequently concentrated to dryness, and purified by flash chromatography (CY/EE (4:1)). A total of 50.3 g (98%) of **17** was obtained: ^{1}H-NMR ($CDCl_3$) δ 4.93 (q, 1H, J = 6.9 Hz), 4.16 (q, 2H, J = 7.1 Hz), 1.49 (s, 18H),

(22) Brückner, H.; Wittner, R. *J. Chromatogr.* **1989**, *476*, 73.

1.48 (d, 3H, J = 6.9 Hz), 1.25 (t, 3H, J = 7.1 Hz); MS-FAB *m/e* 318 (MH^+, 53), 262 ($MH^+ - C_4H_8$, 90), 206 ($MH^+ - 2 \times C_4H_8$, 100), 162(95). Anal. Calcd for $C_{15}H_{27}NO_6$: C, 56.77; H, 8.57; N, 4.41. Found: C, 56.52; H, 8.40; N, 4.31.

***N,N*-[Bis(*tert*-butoxycarbonyl)]-*rac*-(*RS*)-α-(2-propenyl)-α-alanine Ethyl Ester (*rac*-18).** To a solution of LDA (102 mmol) in 100 mL of THF was added under argon atmosphere (−78 °C) a solution of 16.1 g (50 mmol) of **17** in 20 mL of THF (→ deep orange). After 30 min 5.49 mL (66 mmol) of allyl bromide was added and the temperature was allowed to warm to rt within 1 h. After being stirred for an additional 6 h at rt the reaction mixture was poured into half-saturated NH_4Cl solution. After usual workup and chromatography on silica gel (CY/EE (9:1)) 9.8 g (54%) of **18** was obtained: ^{1}H-NMR ($CDCl_3$) δ 5.82 (m, 1H), 5.09 (m, 2H), 4.18 (q, 2H, J = 7.1 Hz), ABX-system (ν = 2.83, ν = 2.60, J_{AB} = 13.1 Hz, J_{AX} = 6.7 Hz, J_{BX} = 7.1 Hz), 1.47 (s, 21H), 1.25 (t, 3H, J = 7.1 Hz); MS-FAB *m/e* 358 (MH^+, 5), 158 ($MH^+ - 2 \times C_4H_8 - 2 \times CO_2$, 100). Anal. Calcd for $C_{18}H_{31}NO_6$: C, 60.48; H, 8.74; N, 3.92. Found: C, 60.67; H; 8.44; N, 3.81.

***rac*-(*RS*)-α-(2-Propenyl)-α-alanine Ethyl Ester (*rac*-19).** To a cooled (0 °C) solution of 6 g of *rac*-**18** (16.8 mmol) and 3.2 g (16.8 mmol) of TSA in 25 mL of CH_2Cl_2 was added dropwise 25 mL of a 1:1 mixture of TFA and CH_2Cl_2. After 2 h the reaction mixture was concentrated to dryness, redissolved in 25 mL of CH_2Cl_2, and repeatedly extracted with saturated sodium bicarbonate solution. After usual workup 2.45 g (93%) of *rac*-**19** was obtained: ^{1}H-NMR ($CDCl_3$) δ 5.69 (m, 1H), 5.1 (m, 2H), 4.14 (q, 2H, J = 7.1 Hz), ABX-system (ν_A = 2.51, ν_B = 2.25, J_{AB} = 13.5 Hz, J_{AX} = 6.6 Hz, J_{BX} = 8.15 Hz), 1.31 (s, 3H), 1.24 (t, 3H, J = 7.1 Hz); MS-FAB *m/e* 158 (MH^+, 100). Anal. Calcd for $C_8H_{15}NO_2$: C, 61.12; H, 9.62; N, 8.91. Found: C, 61.28; H, 10.20; N, 8.71.

***N*-[(2,2,2-Trichloroethoxy)carbonyl]-(*S*)-α-(2-propenyl)-α-alanine (20).** Twenty-five mL of an aqueous solution of the crude amino acid **15** which was obtained after enzymatic hydrolysis of 1.74 g (9 mmol) of **19** was adjusted to pH 10 with 1 N NaOH. Under vigorous stirring 6.1 mL (45 mmol) of trichloroethyl chloroformate was added keeping the pH constant within the range of 9.5−10 by continuous addition of 1 N NaOH. When the pH did not change any more the reaction mixture was extracted with EE followed by acidification to pH 3 with 1 N HCl. After repeated extraction with EE the organic phase was concentrated under reduced pressure and chromatographed (EE/MeOH/H_2O (70:20:1)) on silica gel. 985 mg (58%) **20** were obtained: ^{1}H-NMR (DMSO) δ 7.07 (b, 1H), 5.61 (m, 1H, 4.93 (m, 2H), AB-system (ν_A = 4.78, ν_B = 4.7, J = 12.4 Hz), ABX-system (ν_A = 2.69, ν_B = 2.49, J_{AB} = 13.4 Hz, J_{AX} = 8.6 Hz, J_{BX} = 6.8 Hz), 1.36 (s, 3H); MS-FAB *m/e* 302, 304, 306 (MH^+ and isotope peaks, 25), 147 (100). Anal. Calcd for $C_9H_{12}Cl_3NO_4$: C, 35.49; H, 3.97; N, 4.60. Found: C, 35.77; H, 4.10; N, 4.51.

***N*-[*N*-[(2,2,2-Trichloroethoxy)carbonyl]-(*S* and *R*)-α-(2-propenyl)-α-alanyl]-(*R*)-alanine Methyl Ester (21) and (22).** To a cooled (0 °C) solution of 3.11 g (10.1 mmol) of **20**, 1.57 g (11.26 mmol) of (*R*)-alanine methyl ester, and 4.4 g (10.1 mmol) of (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP)[23] in 12 mL of CH_2Cl_2 were added 5.3 mL (30.3 mmol) of diisopropylethylamine. After 1 h the reaction mixture was concentrated to dryness, redissolved in EE, and extracted with 5% $KHSO_4$ solution, brine, saturated $NaHCO_3$ solution, and again brine twice. After the usual workup 7.42 g (93.2%) of *S,R*-configurated dipeptide **21** was obtained which contained 13% of the *R,R*-diastereoisomer **22**. After crystallization (CY, 15% EE) 5.9 g of pure **21** was obtained: mp 88−90 °C; HPLC Nucleosil 5 μm (CY/dioxane (88:12)), 0.9 mL/min, t_R (**21**) 15.9 min, t_R (**22**) 17.1 min; $[\alpha]^{20}_D$ +15.0° (c = 0.5, CH_3OH); ^{1}H-NMR (CD_3OD) δ 6.61 (d, 1H, J = 6.4 Hz), 5.81 (b, 1H), 5.74 (m, 1H), 5.19 (m, 2H), AB-system (ν_A = 4.75, ν_B = 4.70, J = 12.0 Hz), 4.58 (quin, 1H, J = 7.2 Hz), 3.76 (s, 3H), ABX-system (ν_A = 2.78, ν_B = 2.61, J_{AB} = 14.2 Hz, J_{AX} = 7.2 Hz, J_{BX} = 7.1 Hz), 1.61 (s, 3H), 1.40 (d, 3H, J = 5.1 Hz); MS-FAB *m/e* 389, 391, 393 (MH^+ and isotope peaks, 75), 258, 260, 262 (100). Anal. Calcd for $C_{13}H_{19}Cl_3N_2O_5$: C, 40.7; H, 4.91; N, 7.19. Found C, 40.3; H, 4.8; N, 6.95. **22**: ^{1}H-NMR ($CDCl_3$) δ 2.7 (m, 2H); the other signals are identical with those obtained in ^{1}H-NMR of **21**.

***N*-[*N*-[(2,2,2-Trichloroethoxy)carbonyl]-(*S*)-α-[5-(butyl 2(*R,S*)-hydroxy-4-pentenoate)]-α-alanyl]-(*R*)-alanine Methyl Ester (23), 2(*R*)-[3(*S*)-Methyl-3(*S*)-[[(2,2,2-trichloroethoxy)carbonyl]amino]-5(*S*)-[3-(butyl 2(*X*)-hydroxypropionate)]dihydrofuran-2-ylideneamino]-propionic Acid Methyl Ester (28), 2(*S*)-[(2,2,2-Trichloroethoxy)carbonyl]-2(*S*)-methyl-4(*S*)-[3-(butyl 2(*X*)-hydroxypropionate)]butyrolactone (30), and (4*S*,6*R*)-2-(2,2,2-Trichloroethoxy)-4-methyl-4-[[[2(*R*)-(methyl-propionate)]amino]carbonyl]-6-[3-(butyl 2(*X*)-hydroxy-propionate)]-4,5-dihydro-1,3,6-oxazine (29).** To a solution (0 °C, argon atmosphere) of 1.53 g (11.5 mmol) of freshly prepared butyl glyoxylate[24] and 1.65 mL (15.3 mmol) of $SnCl_4$ in 50 mL of CH_2Cl_2 was added 1.5 g (3.84 mmol) of **21**. After 2 h the reaction mixture was poured into 1 N HCl and repeatedly extracted with CH_2Cl_2. The combined extracts were concentrated to dryness and chromatographed on silica gel (CY/dioxane (3:1)) to afford 95 mg (4.7%) of **28**, 36 mg (2.1%) of **30**, 45 mg (2.2%) of **29**, and 1.3 g (65%) of **23**: HPLC Nucleosil 5 μm (CY/dioxane (10:3)), 0.9 mL/min, t_R (**23**) 5.5 min, t_R (**30**) 7 min, t_R (**29**) 5.3 min, t_R (**28**) 6.2 min. **23**: ^{1}H-NMR ($CDCl_3$) δ 6.0 (d, 1H, J = 15.8 Hz), 5.89 (ddd, 1H, J = 15.8, 8.0, 6.3 Hz), AB-system (ν_A = 4.76, ν_B = 4.65, J = 12.1 Hz), 4.47 (q, 1H, J = 7.2 Hz), 4.24 (dd, 1H, J = 7.2, 4.5 Hz), 4.10 (m, 2H), 3.61 (s, 3H), ABXY-system (ν_A = 2.57, ν_B = 2.42, J_{AB} = 14.0 Hz, J_{AX} = J_{AY} = 7.2 Hz, J_{BX} = J_{BY} = 5.2 Hz), 1.61 (s, 3H), 1.57 (m, 2H), 1.39 (d, 3H, J = 7.2 Hz), 1.35 (m, 2H), 0.9 (t, 3H, J = 7.4 Hz); MS-FAB *m/e* 519, 521, 523 (MH^+ and isotope peaks, 25), 328 (100), 197 (60). Anal. Calcd for $C_{19}H_{29}N_2Cl_3O_8$: C, 43.90; H, 5.62; N, 5.39. Found: C, 43.95; H, 5.70; N, 5.12.

28: ^{1}H-NMR (CD_3OD) δ 4.7 (m, 2H), 4.66 (m, 1H), 4.37 (q, 1H, J = 7.2 Hz), 4.29 (dd, 1H, J = 6.1, 5.5 Hz), 4.12 (m, 2H), 3.6 (s, 3H), ABX-system (ν_A = 2.39, ν_B = 2.33, J_{AB} = 14.3 Hz, J_{AX} = 6.4 Hz, J_{BX} = 10.2 Hz), ABXY-system (ν_A = 2.21, ν_B = 2.07, J_{AB} = 14.3 Hz, J_{AX} = J_{AY} = 6.9 Hz, J_{BX} = J_{BY} = 5.3 Hz), 1.44 (s, 3H), 1.32 (m, 2H), 1.3 (d, 3H, J = 7.2 Hz), 0.9 (t, 3H, J = 7.4 Hz); MS-FAB *m/e* 519, 521, 523 (MH^+ and isotope peaks, 100), 328 (34), 197 (57). Anal. Calcd for $C_{19}H_{29}N_2Cl_3O_8$: C, 43.90; H, 5.62; N, 5.39. Found: C, 43.95; H, 5.70; N, 5.12.

29: ^{1}H-NMR (CD_3Cl_3/330 K) δ 7.01 (d, 1H, J = 7.4 Hz), AB-system (ν_A = 5.07, ν_B = 4.74, J = 11.9 Hz), 4.51 (quin, 1H, J = 7.4 Hz), 4.46 (ddd, 1H, J = 10.2 Hz, 2.7 Hz and 4.4 Hz), 4.37 (dddd, 1H, γ-*H*, $J_{\gamma\beta ax}$ = 12.1 Hz, $J_{\gamma\beta equ}$ = 2.2 Hz, $J_{\gamma\delta 1}$ = 9.5 Hz, $J_{\gamma\delta 2}$ = 2.9 Hz), 4.2 (t, 2H, J = 6.7 Hz), 3.74 (s, 3H), 2.96 (d, 1H J = 4.4 Hz), ABX-system (ν_A = 2.73, ν_B = 1.38, J_{AB} = 13.8 Hz, $J_{equ\gamma}$ = 2.2 Hz, $J_{ax\gamma}$ = 12.2 Hz, β-H_{equ}, β-H_{ax}), ABXY-system (ν_A = 2.12, ν_B = 1.81, J_{AB} = 14.3 Hz, $J_{\delta 1\epsilon}$ = 9.5 Hz, $J_{\delta 2\gamma}$ = 3.0 Hz, $J_{\delta 1\gamma}$ = 9.5 Hz, $J_{\delta 2\epsilon}$ = 10.2 Hz, δ-CH_2), 1.64 (m, 2H), 1.4 (m, 2H), 1.4 (d, 3H, J = 7.4 Hz), 1.38 (s, 3H), 0.95 (t, 3H, J = 7.4 Hz); MS-FAB *m/e* 519, 521, 523 (MH^+ and isotope peaks, 100), 197 (52). Anal. Calcd for $C_{19}H_{29}Cl_3N_2O_8$: C, 43.90; H, 5.62; N, 5.39. Found: C, 43.59; H, 5.57; N, 5.10.

30: ^{1}H-NMR (CD_3OD) δ AB-system (ν_A = 4.8, ν_B = 4.71, J_{AB} = 12.2 Hz), 4.70 (m, 1H), 4.30 (dd, 1H, J = 6.1, 5.5 Hz), 4.17 (m, 2H), ABX-system (ν_A = 2.54, ν_B = 2.34, J = 12.5 Hz, 10.2 Hz, 6.4 Hz), ABXY-system (ν_A = 2.27, ν_B = 2.12, J_{AB} = 14.3 Hz, J_{AX} = J_{AY} = 6.9 Hz, J_{BX} = J_{BY} = 5.3 Hz), 1.45 (s, 3H), 0.96 (t, 3H); MS-FAB *m/e* 434, 436, 438 (MH^+ and isotope peaks, 50), 389 (99), 197 (82), 141 (100); IR ($CHCl_3$) 3520, 3400, 3440, 2980, 1780, 1740, 1500, 1110 cm^{-1}. Anal. Calcd for $C_{15}H_{22}Cl_3NO_7$: C, 41.45; H, 5.10; N, 3.22. Found: C, 41.6; H, 5.12; N, 3.46.

***N*-[*N*-[(2,2,2-Trichloroethoxy)carbonyl]-(*S*)-α-[5-[butyl 2-(*R* and *S*)-[(*tert*-butoxycarbonyl)-(*S*)-alanyl]-oxy]-4-pentenoate]]-α-alanyl]-(*R*)-alanine Methyl Ester (24) + (25).** To a cooled solution (5 °C) of 925 mg (1.78 mmol) of **23**, 500 mg (2.67 mmol) of BOC-(*S*)-alanine, and 15 mg of

(23) Corte, J.; Dufour, M.; Pantaloni, A.; Castro, B. *Tetrahedron* **1990**, *31*, 669.

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

DMAP in 20 mL of CH_2Cl_2 was added portionwise 549 mg (2.67 mmol) of DCC. After 10 h the precipitated urea was filtered and the reaction mixture concentrated and chromatographed on silica gel (CY/dioxane (10:1.5)) to afford 340 mg of **24**, 420 mg of **25**, and 350 mg of **24/25** (together 90.3%): HPLC Nucleosil 5 μm (CY/dioxane (10:0.5)), 1 mL/min, t_R (**24**) 5.17 min, t_R (**25**) 5.47 min. **24**: $[\alpha]^{20}_D$ −8.5° (*c* 0.5, CH_3OH); ^{1}H-NMR ($CDCl_3$/330 K) δ 6.68 (d, 1H, J = 7.1 Hz), 6.27 (s, 1H), 5.84 (m, 1H), 5.80 (d, 1H, J = 15.9 Hz), 5.17 (m, 1H), 5.0 (d, 1H, J = 8.1 Hz), AB-system (ν_A = 4.71, ν_B = 4.65, J = 12.1 Hz), 4.51 (quin, 1H, J = 7.4 Hz), 4.33 (quin, 1H, J = 7.3 Hz), 4.14 (m, 2H), 3.73 (s, 3H), ABXY-system (ν_A = 2.75, ν_B = 2.61, J_{AB} = 14.9 Hz, J_{AX} = J_{AY} = 4.4 Hz, J_{BX} = J_{BY} = 6.9 Hz), 1.7 (s, 3H), 1.44 (d, 3H, J = 7.3 Hz), 1.42 (d, 3H, J = 7.6 Hz), 1.44 (s, 9H), 0.94 (t, 3H); MS-FAB *m/e* 690, 692, 694 (MH^+ and isotope peaks, 25), 592 (100), 443 (30), 328 (15). Anal. Calcd for $C_{27}H_{42}Cl_3N_3O_{11}$: C, 46.93; H, 6.13; N, 6.08. Found: C, 46.81; H, 5.95; N, 6.12.

25: $[\alpha]^{20}_D$ −10° (*c* 0.8, CH_3OH); ^{1}H-NMR ($CDCl_3$/330 K) 6.7 (b, 1H), 6.26 (s, 1H), 5.86 (d, 1H, J = 15.9 Hz), 5.79 (ddd, 1H, J = 15.9, 6.9 Hz), 5.15 (dd, 1H, J = 6.6, 5.9 Hz), 5.09 (d, 1H, J = 7.3 Hz), AB-system (ν_A = 4.72, ν_B = 4.64, J = 12 Hz), 4.54 (quin, 1H, J = 7.3 Hz), 4.36 (b, 1H), 4.14 (m, 2H), 3.74 (s, 3H), 2.72−2.62 (m, 2H), 1.67 (s, 3H), 1.45 (s, 9H), 1.44−1.42 (m, 6H), 0.93 (t, 3H); MS-FAB *m/e* 690, 692, 694 (MH^+ and isotope peaks, 30), 592 (100). Anal. Calcd for $C_{27}H_{42}Cl_3N_3O_{11}$: C, 46.93; H, 6.13; N, 6.08. Found: C, 47.12; H, 6.25; N, 6.08.

(*S*)-α-[5-[Butyl2-(*S*)-[[(*tert*-butoxycarbonyl)-(*S*)-alanyl]-oxy]-4-pentenoate]]-α-alanyl]-(*R*)-alanine Methyl Ester (26). To a vigorously stirred solution of 340 mg (0.49 mmol) of **24** in 5 mL of THF and 5 mL of 0.5 M phosphate buffer (pH 4) was added portionwise 300 mg of zinc powder. After 2 h the reaction mixture was filtered, concentrated, and distributed between CH_2Cl_2 and saturated sodium bicarbonate solution. The organic phase was dried over Na_2SO_4 and concentrated to dryness to afford 210 mg (83%) **26** which was used for the following coupling reaction without any further purification: ^{1}H-NMR (CD_3OD) δ 5.85 (d, 1H, J = 15.7 Hz), 5.7 (ddd, 1H, J = 15.7, 6.7 Hz), 5.1 (m, 1H), 4.38 (q, 1H, J = 7.3 Hz), 4.2 (q, 1H, 7.4 Hz), 4.12 (t, 2H, J = 6.6 Hz), 3.73 (s, 3H), 2.65 (m, 2 H), 1.45 (s, 9H), 1.42 (m, 6 H), 0.95 (t, 3H, J = 7.4 Hz).

***N*-[*N*-[*N*-Heptanoyl-(*R*)-γ-glutamyl-(α-benzyl ester)]-(*S*)-α-[5-butyl-2-[[(*tert*-butoxycarbonyl)-(*S*)-alanyl]oxy]-4-pentenoate]]-α-alanyl]-(*R*)-alanine Methyl Ester (27).** To a cooled solution (0 °C, argon atmosphere) of 200 mg (0.29 mmol) of **24**, 125 mg (0.35 mmol) of *N*-heptanoyl-(*R*)-γ-glutamic acid α-benzyl ester, and 155 mg (0.35 mmol) of BOP[23] in 2.5 mL of CH_2Cl_2 were added 0.14 mL (0.8 mmol) of diisopropylethylamine. After 1 h the reaction mixture was worked up according to the prepare of **21**. After chromatography on silica gel (CY/EE (1:3)) 212 mg (64.8%) of **27** was obtained: $[\alpha]^{20}_D$ −10° (*c* 0.5, CH_3OH); ^{1}H-NMR ($CDCl_3$/330 K) δ 7.36−7.29 (m, 5H), 6.85 (b, 1H), 6.73 (b, 1H), 6.43 (d, 1H, J = 7.6 Hz), 5.82 (d, 1H, J = 15.8 Hz), 5.79−5.70 (b, 1H), AB-system (ν_A = 5.17, ν_B = 5.15, J = 12.2 Hz), 5.26 (b, 1H), 4.59 (ddd, 1H, J = 12.1, 8.9, 4.3 Hz), 4.5 (quin, 1H, J = 7.3 Hz), 4.33 (quin, 1H, J = 7.4 Hz), 4.16−4.10 (m, 3H), 3.70 (s, 3H), ABXY-system (ν_A = 2.70, ν_B = 2.56, J_{AB} = 14.9 Hz, J_{AX} = J_{AY} = 4.3 Hz, J_{BX} = J_{BY} = 7.5 Hz), 2.25−2.20 (m, 5H), 1.92 (b, 1H), 1.64 (s, 3H), 1.44 (s, 9H), 0.93 (t, 3H, J = 7.4 Hz), 0.88 (t, 3H, J = 7.4 Hz); MS-FAB *m/e* 8.47 (MH^+, 10), 747 (MH^+ − BOC, 100). Anal. Calcd for $C_{43}H_{66}N_4O_{13}$: C, 60.98; H, 7.85; N, 6.61. Found: C, 70.1; H, 7.73; N, 6.31.

***N*-[*N*-[*N*-Heptanoyl-(*R*)-γ-glutamyl-(α-benzyl ester)]-(*S*)-α-[(2-propenyl)alanyl]]-(*R*)-alanine Methyl Ester (31).** To a vigorously stirred suspension of 1.2 g of zinc powder in 30 mL of THF and 120 mL phosphate buffer (0.5 M, pH 4.5) was added a solution of 1.2 g (3 mmol) of **21** in 5 mL of THF. After 3 h the reaction mixture was concentrated and distributed between 50 mL of saturated $NaHCO_3$ solution and 50 mL of CH_2Cl_2. The organic phase was dried over Na_2SO_4 and concentrated to a volume of 5 mL. A 1.25-g (3.5 mmol) portion of *N*-heptanoyl-(*R*)-γ-glutamic acid α-benzyl ester and 1.15 g (3.5 mmol) of BOP[23] were added to the crude amine followed by the addition of 1.4 mL (0.8 mmol) of diisopropylethylamine. After 2 h the reaction mixture was worked up according to the preparation of **21**. After flash chromatography on silica gel (CY/EE (1:3)) 1.2 g (71.4%) of **31** was obtained: ^{1}H-NMR (CD_3OD) δ 5.78 (m, 1H), 5.1 (m, 2H), 4.55 (dd, 1H, J = 3.6, 9.8 Hz), 3.58 (s, 3H), 4.5 (q, 1H, J = 7.4 Hz), ABX-system (ν_A = 2.95, ν_B = 2.67, J_{AB} = 14 Hz, J_{AX} = 7.2 Hz, J_{BX} = 7.6 Hz), 1.48 (s, 3H), 1.35 (d, 3H, J = 7.4 Hz), 0.88 (t, 3H, J = 7.2 Hz); MS-FAB *m/e* 546 (MH^+, 100). Anal. Calcd for $C_{29}H_{43}N_3O_7$: C, 63.83; H, 7.94; N, 7.70. Found: C, 64.05; H, 7.60; N, 7.91.