Synthesis of Lipidated eNOS Peptides by Combining Enzymatic, Noble Metal- and Acid-Mediated Protecting Group Techniques with Solid Phase Peptide Synthesis and Fragment Condensation in Solution

Rainer Machauer[b] and Herbert Waldmann*[a]

Abstract: Lipid-modified proteins play decisive roles in important biological processes such as signal transduction, organization of the cytoskeleton, and vesicular transport. Lipidated peptides embodying the characteristic partial structures of their parent lipidated proteins and semisynthetic proteins synthesized from such peptides are valuable tools for the study of these biological phenomena. We have developed an efficient synthesis strategy that allows for the synthesis of long multiply lipidated peptides embodying various side chain functional groups. The strategy was successfully applied in the synthesis of the N-terminal undetrigintapeptide of endothelial NO-synthase and related lipopeptides. Key elements of the synthesis strategy are the combined use of the enzyme-labile *para*-phenylacetoxybenzyloxycarbonyl (PhAcOZ) urethane as N-terminal blocking group, the Pd^0-sensitive allyl ester as C-terminal protecting function and acid-labile side chain protecting groups for solution-phase synthesis of labile *S*-palmitoylated building blocks under the mildest conditions with solid-phase techniques and solution-phase fragment condensations. The successful synthesis of the triply lipidated 29-mer eNOS peptide convincingly demonstrates the full capacity of the protecting group methods.

Keywords: enzymes · peptides · protecting groups · protein lipidation · NO synthase

Introduction

Numerous lipid modified proteins are involved in biological events of paramount importance, such as signal transduction, organization of the cytoskeleton, and vesicular transport.[1, 2] Lipidation of these proteins is a prerequisite to correct biological function, and the lipid groups are believed to participate in protein–protein and protein–membrane interactions, which, for instance, may determine the selective intracellular localization of lipid-modified proteins. Lipidated peptides embodying the characteristic lipidated structures of their parent proteins and semisynthetic lipoproteins obtained from such peptides have proven to be invaluable tools in the study of biological phenomena.[3] Thus, by means of such peptide and protein conjugates we have developed a general biological readout system that allows to determine and quantify the ability of a given lipidated peptide moiety to direct a given protein to the plasma membrane or the biological activity of a given protein depending on its plasma membrane localization.[4] In addition, the use of such conjugates has shed light on the mechanism by which farnesylated and palmitoylated Ras-proteins are selectively targeted to the plasma membrane,[5] on the precise orientation of the lipid groups in membranes,[6] the precise mode of action of lipidating proteins such as Rab geranylgeranyltransferase[7] and they yielded hints that biological signalling itself can be amplified with small lipidated peptide conjugates.[8]

The scope of this approach critically depends on the availability of efficient methods for the synthesis of multifunctional lipidated peptides and strategies for the correct orchestration of these methods in the context of the synthesis of large lipidated peptides embodying for instance several lipidated amino acid residues and amino acids with side chain functional groups that require temporary protection during the synthesis. In particular, these methods and strategies have to take into account that lipidated peptides are acid- and base-sensitive[3, 9] calling for sophisticated combinations of protecting groups that can be removed selectively under the mildest conditions yet are orthogonally stable to each other.

Here we describe a synthesis strategy that meets these demands. It relies on the combined use of enzyme-labile, acid-sensitive and noble-metal-sensitive protecting groups for solution-phase synthesis of labile *S*-palmitoylated building

[a] Prof. Dr. H. Waldmann
Max-Planck-Institut für molekulare Physiologie
Abteilung Chemische Biologie, Otto-Hahn-Strasse 11
44227 Dortmund (Germany) and
Universität Dortmund, Fachbereich 3, Organische Chemie
44227 Dortmund (Germany)
Fax: (+49)231-133-2499
E-mail: herbert.waldmann@mpi-dortmund.mpg.de

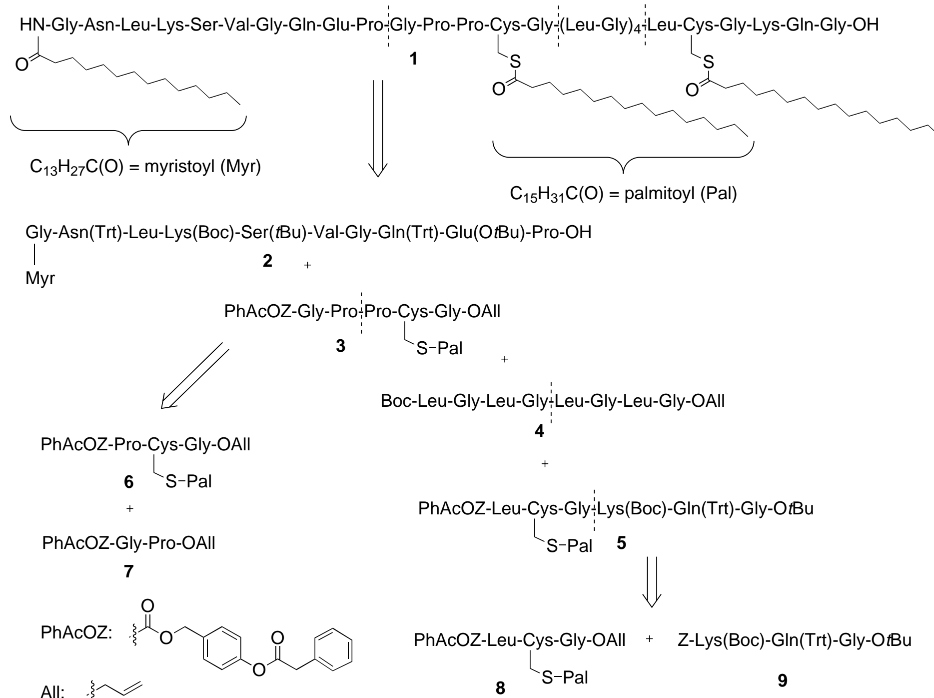
[b] Dr. R. Machauer
Universität Karlsruhe, Institut für Organische Chemie
Richard-Willstätter-Allee 2
76128 Karlsruhe (Germany)

 © WILEY-VCH Verlag GmbH, D-69451 Weinheim, 2001

blocks under very mild conditions with solid-phase and fragment-condensation techniques.[10]

As both biologically relevant and synthetically challenging target compound we chose the triply lipidated N-terminal undetrigintapeptide **1** of endothelial NO-synthase (eNOS; Scheme 1). In response to exogenous signals transduced, for example, by G-protein coupled receptors (GPCR), PKB or change of the intracellular Ca^{2+} this enzyme generates and releases NO from arginine (Figure 1). NO then diffuses across the endothelium to the surrounding smooth muscle cells of the blood vessel and activates soluble guanylate cyclase. Thereby a signal transduction cascade is triggered which leads to relaxation of the muscle cells. The correct orchestration of this sequence of events is paramount to the regulation of blood pressure.[11, 13]

Furthermore eNOS is involved in vascular remodelling and angiogenesis,[13] and contributes to the pathogenesis of blood vessel related disorders such as arteriosclerosis.[14] The localization of eNOS to the plasma membrane and its concentration in the caveolae, membrane microdomains highly enriched in various signal transducing proteins is crucial for its correct biological functioning.[15]



Scheme 1. Retrosynthetic analysis of the *N*-myristoylated and twice *S*-palmitoylated N-terminal 29-mer peptide of endothelial NO-synthase. Boc = *tert*-butoxycarbonyl, Trt = trityl = triphenylmethyl, Z = benzyloxycarbonyl.

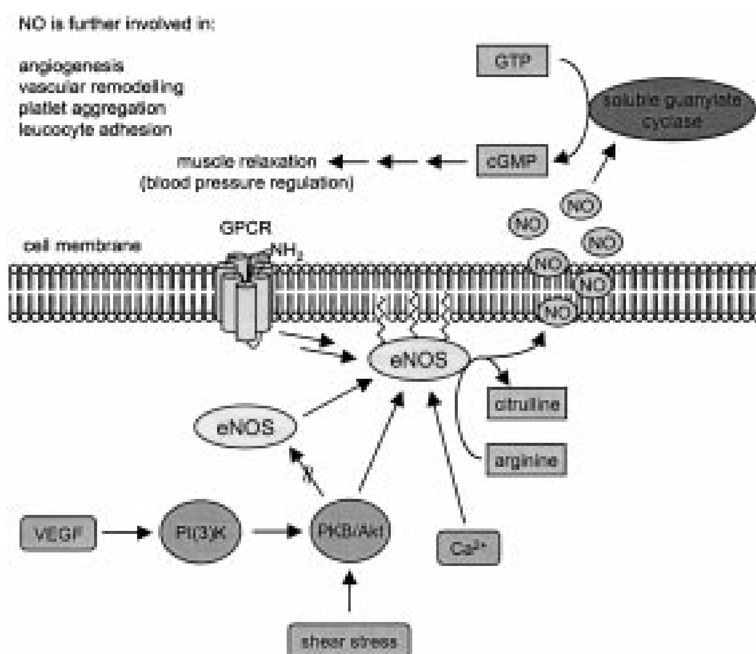


Figure 1. Membrane association and enzymatic activity of endothelial NO-synthase.

In contrast to the other isoforms of NO-synthase identified so far, the N-terminus of eNOS is *N*-myristoylated at the terminal glycine and twice *S*-palmitoylated at cysteines 14 and 25 (see Scheme 1).[16] The lipid groups are required for plasma membrane localization and biological activity.[15, 17] However, the precise biological roles fulfilled by the lipidated part of the protein are subject to various hypotheses. In particular, notions have been forwarded that the lipid groups might be responsible for selective targeting of eNOS at the plasma membrane and caveolae, for example, by mediation of protein/protein or protein/lipid interactions, and that palmitoylation/depalmitoylation might be involved in signalling through eNOS.[1, 2]

Results and Discussion

The synthesis of lipidated peptide **1** is complicated by several parameters.

1) The palmitic acid thioesters are sensitive to bases; that is, they spontaneously hydrolyze at pH >7 and are subject to base-induced β-elimination.[3] Therefore, base-labile protecting groups can not be employed in the synthesis of **1**.
2) For the synthesis of *S*-palmitoylated peptides solid-phase methods are not available.[3]

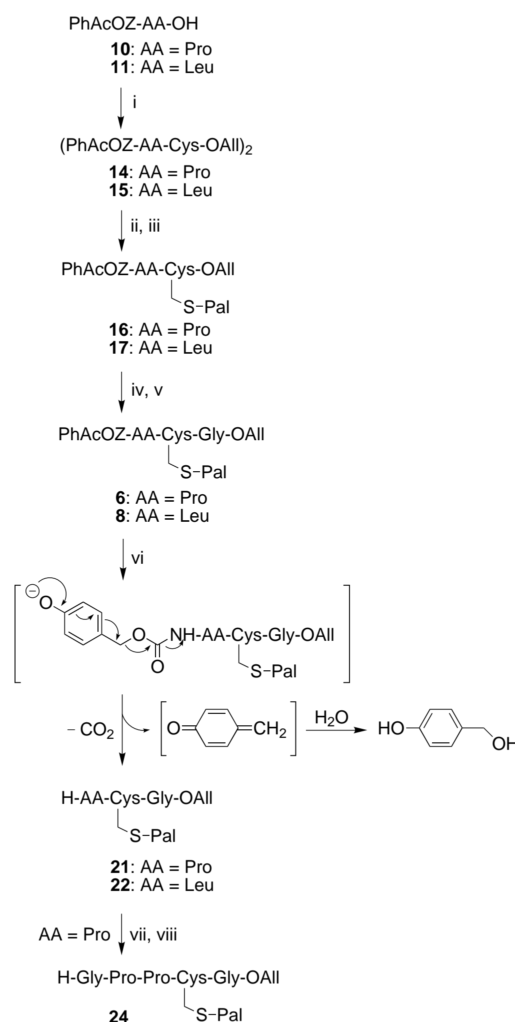
3) Peptide **1** embodies several amino acids with side chain functional groups that require protection during the synthesis and final deprotection under conditions mild enough to guarantee that the sensitive thioester bonds are not attacked.

In developing a plan for the synthesis of **1** we utitlized our experience in the synthesis of sensitive peptide conjugates.[3, 18] We chose a combination of enzyme-labile, acid-sensitive, and noble-metal-sensitive protecting groups and aimed to combine the use of solid-phase techniques with solution-phase fragment condensations. Thus, in a retrosynthetic sense, **1** was divided into *N*-myristoylated decapeptide **2**, *S*-palmitoylated pentapeptide **3**, octapeptide **4**, and *S*-palmitoylated hexapeptide **5** (Scheme 1). For the synthesis and selective deprotection of *S*-palmitoylated building blocks the enzyme-labile *para*-phenyl-acetoxybenzyloxycarbonyl (PhAcOZ) urethane group[19] and the Pd^0-labile allyl ester[20] were chosen as temporary N- and C-terminal protecting functions. The side chains of Asn, Lys, Ser, Gln, and Glu were masked with acid-labile protecting groups to be cleaved off simultaneously in the final step of the synthesis. We planned to build up the N-terminal decapeptide on the solid phase by means of an Fmoc/*tert*-butyl strategy and to assemble the entire 29-mer in solution by appropriate fragment condensations. The retrosynthetic cuts were placed at the C-termini of glycine and proline residues to exclude epimerization during the fragment condensations.

The base labile *S*-palmitoylated building blocks required for the fragment condensations were synthesized as shown in Scheme 2. PhAcOZ-protected amino acids **10** and **11** were synthesized according to the method described earlier[19] and then condensed with cystine bis(allyl ester) **13** to give homodimeric peptides **14** and **15** in high yields. After cleavage of the disulfide by treatment with dithiothreitol (DTT), the liberated mercapto groups were acylated with palmitoyl chloride to yield the fully masked dipeptides **16** and **17** in high overall yields. To elongate the peptide chain in the C-terminal direction, the allyl ester protecting group was selectively cleaved by means of Pd^0-catalyzed allyl transfer with *N,N'*-dimethylbarbituric acid (DMB) as the accepting C-nucleophile.[21] Coupling of the liberated carboxylic acids **18** and **19** with glycine allyl ester **20** gave masked intermediates **6** and **8** which had to be deblocked next at the N-terminus. Upon treatment of these PhAcOZ-protected compounds with immobilized penicillin G acylase in 0.2 M Na_3PO_4 buffer at pH 6.8, the phenylacetic acid ester incorporated into the urethane was saponified. Thereby a phenolate was generated which spontaneously fragmented into a quinone methide, CO_2, and the desired selectively deprotected lipidated tripeptides **21** or **22** (Scheme 2). Notably, the conditions of this enzyme-initiated blocking group fragmentation are so mild that the base-labile palmitic acid thioester, the C-terminal allyl ester and the peptide bonds remained completely intact.

In order to achieve reproducible, preparatively useful results in the enzymatic transformations, several reaction parameters had to be adjusted.

Firstly, to rule out undesired side reactions of the intermediary generated quinone methide (in particular with the liberated amino groups), this potent electrophile had to be trapped by a good nucleophile.



Scheme 2. Synthesis of the sensitive *S*-palmitoylated building blocks with a combination of the enzyme-labile PhAcOZ urethane and the Pd^0-sensitive allyl ester protecting groups. i) $[H\text{-}Cys\text{-}OAll]_2 \cdot 2\,p$TosOH (**13**), HOBt, EDC, NEt_3, CH_2Cl_2, **14**: 88%, **15**: 90%; ii) DTT, NEt_3, CH_2Cl_2; iii) $H_3C(CH_2)_{14}COCl$, NEt_3, 0°C, CH_2Cl_2, **16**: 72% (two steps), **17**: 85% (two steps); iv) $[Pd(PPh_3)_4]$, DMB, THF, **18**: 91%, **19**: 86%; v) H-Gly-OAll · *p*TosOH (**20**), HOBt, EDC, CH_2Cl_2, **6**: 91%, **8**: 94%; vi) penicillin G acylase, phosphate buffer pH 6.8, 0.1 M DMB or KI, dimethyl-β-cyclodextrin, 20% (*v*/*v*) MeOH, **21**: 53%, **22**: 56%; vii) PhAcOZ-Gly-Pro-OH **23**, HOAt, EDC, CH_2Cl_2, **3**: 82%; viii) penicillin G acylase CLEC, phosphate buffer pH 6.8, 0.1 M KI, dimethyl-β-cyclodextrin, 20% (*v*/*v*) MeOH, 39%. HOBt = 1-hydroxybenzotriazole, EDC=*N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide hydrochloride, DTT = 1,4-dithio-D,L-threitol, DMB = *N,N'*-dimethylbarbituric acid, *p*Tos = *para*-toluenesulfonyl, HOAt = 1-hydroxy-7-azabenzotriazole.

Thus, reagents were sought that are nucleophilic enough to trap the quinone methide efficiently but do not attack the thioesters at the same time. For this purpose several reagents were tested including NaI, KI, $NaHSO_3$, Na_2S, and DMB.

Surprisingly, in the case of leucine derivative **8** addition of KI proved to be best, whereas in the case of proline peptide **6** the rather weak nucleophile DMB gave the best results. We speculate that the reactivity of the additives is not only determined by the aqueous medium but also by the microenvironment in the active site of penicillin acylase which

 © WILEY-VCH Verlag GmbH, D-69451 Weinheim, 2001 0947-6539/01/0713-2942 $ 17.50+.50/0 *Chem. Eur. J.* **2001**, *7*, No. 13

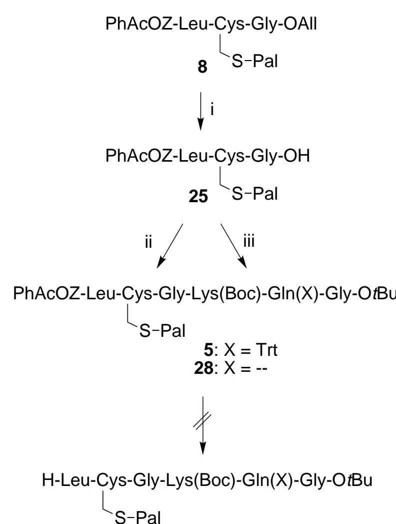
makes predictions on the right choice of nucleophile rather difficult.

Secondly, peptides **6** and **8** are only sparingly soluble in aqueous buffer so that solubilizing additives were required to render the substrates accessible to the biocatalysts. Initially, different cosolvents miscible with water were investigated. However, even in the presence of up to 50 vol% of methanol, ethanol, isopropanol, 1,4-dioxane, ethyleneglycol, DMF or DMSO a satisfying conversion could not be achieved. Therefore, cyclodextrins were additionally introduced, since these had already earlier served well in related problematic cases.[9] Finally, the combined use of 20 vol% methanol and 38 equivalents dimethyl-β-cyclodextrin gave the best results. The cyclodextrin most probably slips over the hydrophobic palmitoyl groups, thereby solubilizing the peptides and shielding the thioester from undesired hydrolysis.

Selectively deprotected lipotripeptide **21** was then condensed with PhAcOZ-masked dipeptide **23** and the resulting lipidated pentapeptide **3** was N-terminally deprotected by means of the enzyme-initiated fragmentation detailed above. In this case the use of cross-linked enzyme crystals (CLECs)[22] of penicillin G acylase proved to be superior to the use of soluble or immobilized enzyme. This preparation of the acylase is particularly stable in the presence of potentially denaturing cosolvents and gave access to the desired building block **24** in reasonable yield.

For the synthesis of lipid-modified fragment **5** tripeptide **8** was C-terminally deprotected by Pd^0-catalyzed allyl transfer to morpholine as accepting nucleophile (Scheme 3). Subsequent condensation of the resulting tripeptide carboxylic acid **25** with N-terminally unmasked tripeptide **26** yielded fully protected hexapeptide **5**. Enzymatic deprotection of **5** was attempted under the conditions and including the variations of the reaction conditions described above for tripeptides **6**, **8**, and pentapeptide **3**. However, due to the significantly higher hydrophobicity of hexapeptide **5** all attempts failed. In order to increase the hydrophilic character of the palmitoylated hexapeptide an analogue without trityl-masked glutamine side chain was synthesized by coupling of lipotripeptide **25** with tripeptide ester **27** (Scheme 3). Tripeptides **26** and **27** were obtained using standard methods of peptide chemistry (see the Experimental Section). Although hexapeptide **28** clearly is more polar than trityl-masked analogue **5** it could not be rendered accessible to the biocatalyst under all conditions described above. Since this variation of the protecting group pattern did not overcome the synthetic problem the reaction medium was varied.

Since the use of deoxytaurocholic acid improves the results in the lipase-catalyzed removal of the heptyl ester protecting group from phosphopeptides[23] in an initial series of experiments various detergents were investigated. However, neither in the presence of anionic (cholic acid, deoxycholic acid, taurocholic acid), cationic (cetyltrimethylammonium bromide), non-ionic (Triton X-100, Triton X-114, TWEEN 20, TWEEN 80, Nonidet P 40, *n*-octylglycoside, digitonin, Pril) detergents or other cyclodextrin derivatives (dimethyl α-cyclodextrin, hydroxypropyl β-cyclodextrin, hydroxypropyl γ-cyclodextrin) in different concentrations was the solubility of hexapeptides **5** and **28** significantly enhanced.



Scheme 3. Synthesis of the sensitive *S*-palmitoylated hexapeptides **5** and **28** by fragment condensation. i) $[Pd(PPh_3)_4]$, morpholine, THF, 80%; ii) H-Lys(Boc)-Gln(Trt)-Gly-O*t*Bu (**26**), HOBt, EDC, CH_2Cl_2, 62%; iii) H-Lys(Boc)-Gln-Gly-O*t*Bu (**27**), HOBt, EDC, $CHCl_3/CF_3CH_2OH$ 3:1 (*v*/*v*), 32%.

Finally, upon use of the zwitterionic detergents *N*-dodecyl-*N*,*N*-dimethyl-3-ammonium-1-propanesulfate (SB 3-12) and lauryldimethylamine N-oxide (LDAO) at 25–50 mM concentration and in the presence of 10–20 vol% DMSO as cosolvent clear solutions were obtained. Subsequent attempts to deprotect the lipopeptides with penicillin G acylase on Eupergit or with penicillin G acylase CLECs were not successful. Conversion of the starting materials could not be detected, and inhibition of the biocatalyst preparations by the detergents was ruled out by separate determination of its activity in the presence of the additives.

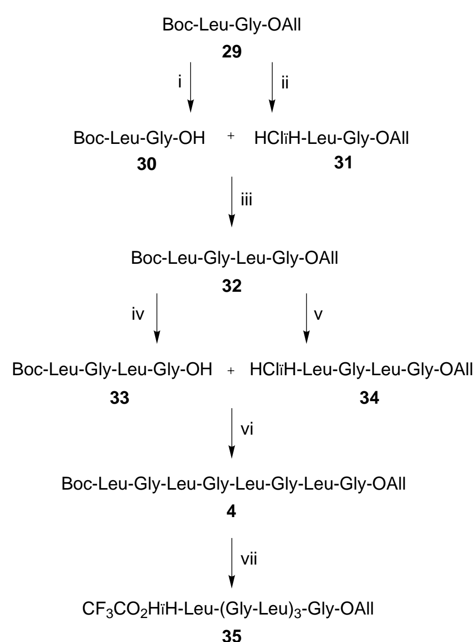
Furthermore, attempts to unmask hexapeptide **5** with penicillin G acylase CLEC in an organic solvent (ethyl acetate, toluene) saturated with water or in a reversed micelle system[24] were not successful. Finally, instead of hexapeptides **5** and **28** the corresponding octapeptides with the peptide chain elongated in N-terminal direction by dipeptide Gly-Leu were investigated. However, in these cases a successful enzymatic deprotection could not be achieved either.

These results shed light on the limitations of enzymatic protecting group techniques in lipopeptide synthesis. In order to guarantee that the substrates will be accessible to the biocatalyst their amino acid/lipid residue/protecting group composition must be chosen carefully. It appears that for the synthesis of longer lipidated peptides building blocks are preferable in which a combination of hydrophobic lipid residues with hydrophobic protecting groups and/or amino acids is kept minimal. We would like to stress that, in general, the enzymatic techniques are fairly tolerant of hydrophobic structures, allowing for instance for the successful removal of the PhAcOZ and the related AcOZ urethane from palmitoylated and farnesylated Ras peptides.[3, 4, 9] Obviously, however, the combination of the PhAcOZ urethane with a palmitoyl group, a Boc group, and a *tert*-butyl ester in **28** and even more so with a trityl group in **5** renders the substrates too

hydrophobic and prevents an enzymatic transformation. Reasons for this may be the low solubility of the lipidated peptides in aqueous systems or a tendency to form micells making the substrates inaccessible to the biocatalyst. In addition, we consider it possible that the hydrophobic peptides interact directly with hydrophobic patches on the surface of the protein thereby blocking its activity.

In the light of the difficulties encountered the strategy was changed and two tripeptide units were employed instead for the elongation of the peptide chain (see below), that is, palmitoylated tripeptide **22** (Scheme 2) and N-terminally deprotected non-lipidated tripeptide **26** (Scheme 3).

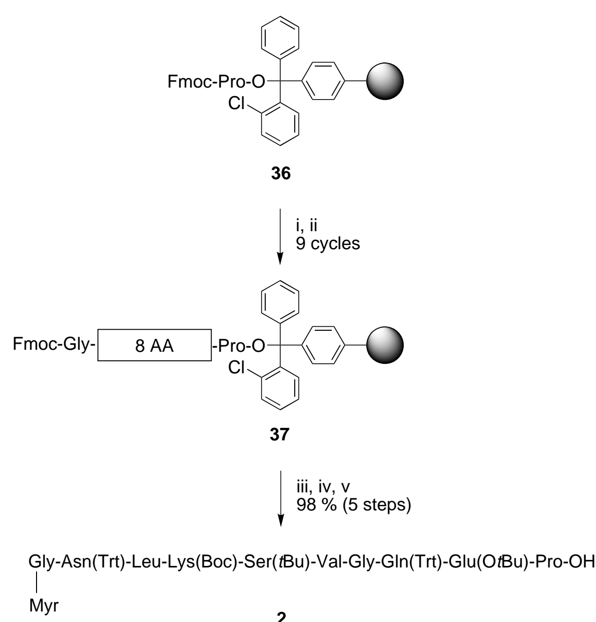
Octapeptide building block **4** was synthesized as shown in Scheme 4. Due to the fourfold repetition of the Leu-Gly dipeptide unit a highly convergent solution-phase synthesis



Scheme 4. Synthesis of the octapeptide **35**. i) $[Pd(PPh_3)_4]$, morpholine, THF, 99%; ii) HCl/Et_2O, CH_2Cl_2, 98%; iii) HOBt, EDC, NEt_3, CH_2Cl_2, 90%; iv) $[Pd(PPh_3)_4]$, morpholine, THF, 99%; v) HCl/Et_2O, CH_2Cl_2, 99%; vi) HOOBt, EDC, $CHCl_3$/CF_3CH_2OH 3:1 (*v*/*v*), 82%; vii) CF_3COOH, $CHCl_3$, 98%. HOOBt = 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine.

from dipeptide Boc-Leu-Gly-OAll (**29**) was developed. Thus, selective removal of the N- and the C-terminal blocking groups from **29** and condensation of the fragments gave tetrapeptide Boc-Leu-Gly-Leu-Gly-OAll (**32**) (90%) which was then subjected to the same sequence (82% coupling yield). Selective removal of the Boc group was carried out with HCl/ether and cleavage of the allyl ester was performed with $[Pd(PPh_3)_4]$/morpholine (98–99% yields in all cases). Finally, the Boc group was removed from octapeptide **4** by treatment with trifluoroacetic acid in $CHCl_3$ to yield building block $CF_3COOH \cdot$H-(Leu-Gly)$_4$-OAll (**35**) in 98% yield.

N-Myristoylated decapeptide **2** was synthesized on the solid phase (Scheme 5). For N-terminal protection the 9-fluorenylmethoxycarbonyl (Fmoc) group was employed and the side chains of the trifunctional amino acids were masked with acid labile trityl- or *tert*-butyl protecting groups. Attachment to the



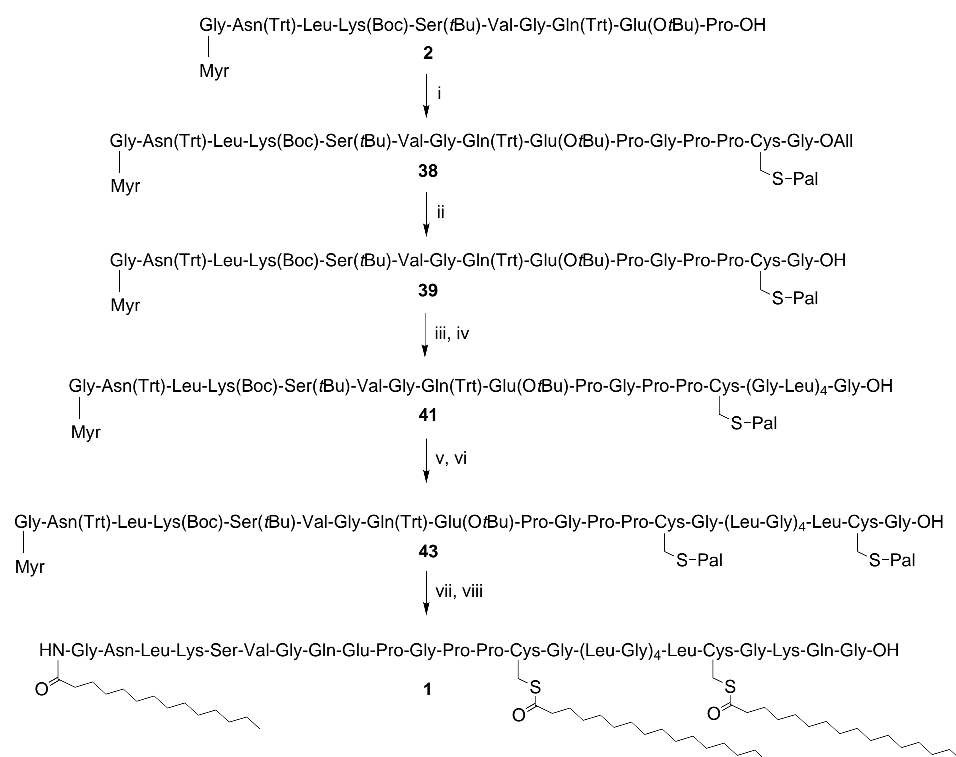
Scheme 5. Solid-phase synthesis of decapeptide **2**. i) piperidine/NMP 1:4 (*v*/*v*); ii) 4 equiv Fmoc-AA-OBt; (9 cycles); iii) piperidine/NMP 1:4 (*v*/*v*); iv) 4 equiv $H_3C(CH_2)_{12}COCl$, 8 equiv NEt(*i*Pr)$_2$; v) AcOH/CF_3CH_2OH/CH_2Cl_2 1:1:8 (*v*/*v*/*v*), 98% (all steps). NMP = *N*-methyl-pyrrolidinone.

solid support was achieved by means of the very acid-sensitive 2-chlorotrityl linker (see **36**).[25] After the entire peptide chain had been assembled, the N-terminal glycine residue was unmasked by removal of the Fmoc-urethane and then the myristic acid amide was formed on the solid support by treatment with myristoyl chloride (four equivalents) and Hünig's base (eight equivalents). The use of *N*-myristoylated glycine directly was problematic due to the low solubility of this building block. Finally, the desired lipidated and side-chain protected peptide **2** was released from the polymeric carrier **37** by treatment with acetic acid/2,2,2-trifluoroethanol/methylene chloride 1:1:8 (*v*/*v*/*v*) without any attack on the other side chain protecting groups. Peptide **2** was obtained in 98% overall yield.

N-Myristoylated decapeptide **2** turned out to be only sparingly soluble in most regular solvents and solvent systems employed usually for HPLC analysis such as CH_3CN/H_2O mixtures. Appreciable solubility could only be reached by using denaturing solvents such as DMSO or solvent mixtures containing fluorinated solvents such as $CHCl_3$/2,2,2-trifluoroethanol 3:1 (*v*/*v*) and CH_2Cl_2/hexafluoroisopropanol 95:5 (*v*/*v*). In accordance with earlier findings we assume that this is due to the formation of β-structures by intra- or intermolecular hydrogen bonding.[26, 27, 28]

With all required and selectively unmasked building blocks in hand, the assembly of the eNOS 29-mer peptide **1** by fragment condensation from the N- towards the C-terminus was approached (Scheme 6). Due to the limited solubility of myristoylated decapeptide **2** (see above) solvents that are usually used in peptide chemistry such as CH_2Cl_2 or DMF could not be employed in the coupling of **2** with palmitoylated building block **24**. However, by running the condensation reaction in $CHCl_3$/CF_3CH_2OH 3:1 (*v*/*v*) pentadecapeptide **38**

 © WILEY-VCH Verlag GmbH, D-69451 Weinheim, 2001 0947-6539/01/0713-2944 $ 17.50+.50/0



Scheme 6. Synthesis of the lipidated 29-mer target peptide **1** by fragment condensation. i) H-Gly-Pro-Pro-Cys(Pal)-Gly-OAll (**24**), HOOBt, EDC, $CHCl_3/CF_3CH_2OH$ 3:1 (*v*/*v*), 91%; ii) [Pd(PPh_3)$_4$], DMB, DMSO, 92%; iii: H-Leu-(Gly-Leu)$_3$-Gly-OAll · CF_3COOH (**35**), HOAt, EDC, NEt_3, DMSO, **40**: 72%; iv) [Pd(PPh_3)$_4$], DMB, DMSO, 87%; v) H-Leu-Cys(Pal)-Gly-OAll (**22**), HOAt, EDC, NMP, **42**: 86%; vi) [Pd(PPh_3)$_4$], DMB, DMSO, 69%; vii) H-Lys(Boc)-Gln(Trt)-Gly-O*t*Bu (**26**), HOAt, EDC, NMP, **44**: 86%; viii) CF_3COOH/ethanedithiol/H_2O 95:2.5:2.5 (*v*/*v*/*v*), 31%.

was obtained in 91% yield. Best results were obtained by employing a slight excess (1.3 equiv) of pentapeptide **24** and extraction of surplus palmitoylated peptide with methanol following precipitation of the product after the reaction was complete. This simple purification procedure was much more efficient than all attempts to purify doubly lipidated peptide **38** by HPLC on RP18 or RPC4 columns employing H_2O/CH_3CN or H_2O/*i*PrOH gradients or size-exclusion chromatography on Sephadex LH-20 or LH-60 using DMSO, $CF_3CH_2OH/CHCl_3/CH_3OH$ or $CHCl_3/CH_3OH$/0.1 N HCl mixtures as eluents.

In addition, analysis of the crude product mixture by MALDI-MS indicated that the coupling had proceeded efficiently since starting peptide **2** could not be detected. Therefore, in all subsequent fragment condensation steps the smaller peptide fragments were used in 1.3–1.5-fold excess and the products were purified by precipitation and washing with methanol. Selective removal of the allyl ester from fully masked peptide **38** with [Pd(PPh_3)$_4$]/*N*,*N'*-dimethylbarbituric acid initially was attempted in $CHCl_3/CF_3CH_2OH$ 3:1 (*v*/*v*) as well. However, mass spectrometrical analysis of the product mixture indicated that under these conditions the pentadecapeptide trifluoroethyl ester was formed as by-product.

Screening for alternative solvents revealed that in DMSO no undesired side reaction occurred. Under these conditions the C-terminal allyl ester was removed selectively and in high yield (92%). Therefore, all further C-terminal deprotections were carried out in DMSO.

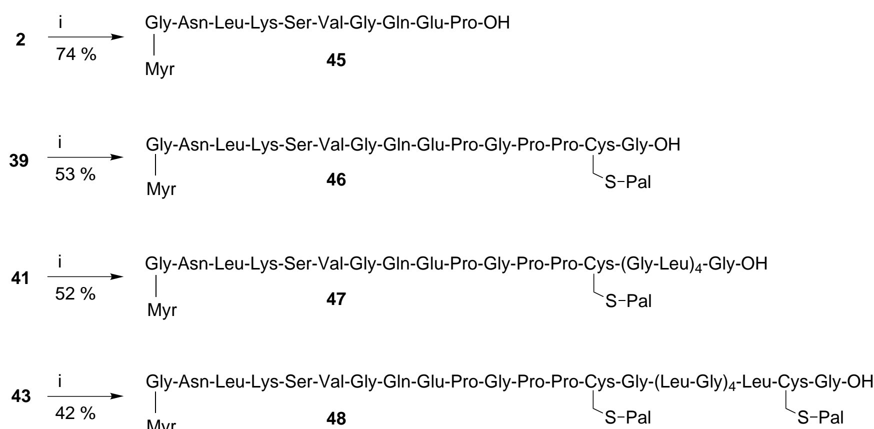
Coupling of pentadecapeptide **39** with octapeptide **35** initially was attempted in $CHCl_3/CF_3CH_2OH$ mixtures as described above for **2**, however, in this case conversion was incomplete. By analogy to the calcitonin synthesis by Schäfer et al.[29] DMSO was used as solvent and HOAt/EDC as coupling reagents. Under these conditions the fragment condensation proceeded with appreciable rate and resulted in a product mixture from which the desired 23-mer peptide **40** could be isolated readily by precipitation and washing. From this compound the allyl ester was removed selectively under the conditions described above to give carboxylic acid **41** in high yield. Elongation of the peptide chain with the third lipidated building block proceeded smoothly in *N*-methyl-pyrrolidinone (NMP) as solvent to yield 26-mer peptide **42**.

The selective removal of the allyl ester from the 26-mer intermediate **42** was considered to be particularly challenging since this peptide embodies two base-labile thioesters and several acid-labile groups. In addition, this fully masked compound is highly hydrophobic, so that formation of aggregates and poor solubility, which would render the compound poorly accessible to the Pd^0 catalyst, had to be anticipated. However, to our great pleasure this deprotection could also be effected without any undesired side reaction, and C-terminally deprotected 26-mer peptide **43** was obtained in high yield. Finally, the peptide chain was elongated with tripeptide **26** and, in the last step, all acid-labile side chain protecting groups were cleaved from the resulting undetrigintapeptide **44** by treatment with trifluoroacetic acid in the presence of ethanedithiol as cation scavenger.

All attempts to purify the completely masked lipopeptide intermediates by HPLC failed. However, deprotected target compound **1** could be purified by HPLC at elevated temperature employing RP C4 column material as the stationary phase and a water/acetonitrile gradient for product elution. Although this unmasked peptide is significantly more polar than its fully protected congener the influence of the three lipid residues remains dominant. Notably, appropriate product application to the column was crucial and best achieved after dissolving the peptide in pure formic acid and subsequent dilution with water to a 1:1 (*v*/*v*) mixture.

 © WILEY-VCH Verlag GmbH, D-69451 Weinheim, 2001 0947-6539/01/0713-2945 $ 17.50+.50/0

By analogy to the acid-mediated removal of the side chain protecting groups from 29-mer peptide **44**, also *N*-myristoylated decapeptide **2**, doubly lipidated pentadecapeptide **39**, doubly lipidated 23-mer peptide **41** and triply lipidated 26-mer peptide **43** were deprotected in appreciable yields (Scheme 7). Thereby, altogether a set of five peptides representing the N-terminus of eNOS but differing in size and lipidation pattern were accessible. These compounds may now be employed to determine the parameters (e.g. by surface plasmon resonance[4, 9]) that are responsible for permanent and/or reversible membrane binding of these model lipopeptides, and in extrapolation from the values to be obtained, of eNOS itself.



Scheme 7. Unmasking of the lipidated fragments **2**, **39**, **41**, and **43**. i) CF_3COOH/ethanedithiol/H_2O 95:2.5:2.5 (*v*/*v*/*v*).

Conclusion

We have developed a highly efficient synthesis of the *N*-myristoylated and twice *S*-palmitoylated N-terminus of endothelial NO-synthase and related peptides. The strategy relies on the combined use of enzyme-labile, acid-sensitive and noble-metal-sensitive protecting groups for solution-phase synthesis of labile *S*-palmitoylated building blocks under the mildest conditions with solid-phase and fragment-condensation techniques. The results demonstrate convincingly the full capacity of the protecting group methods for the synthesis of large and multiply lipidated peptides. Together with the recently developed methods for the synthesis of entire functional proteins by a combination of organic synthesis with molecular biology[4] they should open up new opportunities to study the chemical biology of endothelial NO-synthase in precise molecular detail, particularly the parameters determining its localization to the plasma membrane and caveolae of endothelial cells.

Experimental Section

General procedures: ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AC-250 and Bruker DRX-500 spectrometers. EI and FAB mass spectra were measured on a Finnigan MAT MS 70 and MALDI-TOF mass spectra on a Voyager BioSpectrometry Workstation. Specific rotations were measured with a Perkin – Elmer Polarimeter 241. UV spectra were recorded on a Perkin – Elmer Lambda 2 UV/VIS spectrometer. Elementary analyses were performed on a Hereaus CHN-Rapid apparatus. Melting points were determined in open capillaries using a Büchi 530 apparatus and are uncorrected. Solid-phase peptide synthesis was done on an Applied Biosystems 430A Peptide Synthesizer employing the Fmoc/*t*Bu-strategy, using the standard protocol. HPLC was performed on a Macherey&Nagel Nucleosil CC250/4.6 120-5 C4 analytical column, flow: 1 mL min^{-1} and Nucleosil CC250/8 120-5 C4 semi preparative column, flow: 3 mL min^{-1}.

Materials: Analytical chromatography was performed on E. Merck silica gel 60 F_{254} aluminum plates. Flash chromatography was performed on Baker silica gel (40 – 64 µm). Penicillin G acylase was obtained in immobilized form on Eupergit C from Roche (Diagnostics). Penicillin G acylase CLECs were obtained from Altus Biologics Inc. All solvents were distilled using standard procedures. Commercial reagents were used without further purification. Where indicated the reactions were performed under argon.

Synthesis of PhAcOZ-protected amino acids: Trimethylsilyl chloride (3.80 mL, 30 mmol) was added to stirred suspension of the amino acid (15 mmol) in CH_2Cl_2 (70 mL) under argon in one portion. The mixture was refluxed for 1 h and cooled to 0 °C and triethylamine (3.60 mL, 26 mmol) was added. Then a solution of 4-(phenylacetoxy)benzyl chloroformate[19] (3.04 g, 10 mmol) in CH_2Cl_2 (30 mL) was added dropwise (30 min) and the reaction mixture was stirred for 30 min at 0 °C and at room temperature for 2 h. The solvent was removed under reduced pressure and the resulting residue was dissolved in a mixture of $NaHCO_3$ (2.5 %, 125 mL) and diethyl ether (100 mL). The organic layer was separated and the aqueous phase was extracted with diethyl ether (2 × 40 mL). The combined organic layers were extracted with water (2 × 25 mL) and the pH of the combined aqueous layers was adjusted to pH 2 with HCl (2 N). The aqueous solution was extracted with ethyl acetate (3 × 40 mL). The combined organic layers were dried with $MgSO_4$ and concentrated under reduced pressure to give the desired amino acids.

PhAcOZ-Pro-OH (10): Colorless oil (3.42 g, 89 %); $[\alpha]_D^{22} = -40.1$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.37 - 7.26$ (m, 7 H; C_6H_5, 2 CH), 7.04 (d, $^3J = 8.5$ Hz, 2 H; 2 CH), 6.05 (brs, 1 H; COOH), 5.18 – 5.04 (m, 2 H; CCH_2O), 4.39 – 4.30 (m, 1 H; Pro α-CH), 3.85 (s, 2 H; $CCH_2C(O)$), 3.59 – 3.39 (m, 2 H; Pro δ-CH_2), 2.24 – 2.04 (m, 2 H; Pro β-CH_2), 1.98 – 1.85 (m, 2 H; Pro γ-CH_2); ^{13}C NMR (125.7 MHz, $CDCl_3$): $\delta = 177.1$ (COOH), 169.9 (COO), 156.0 (OCON), 150.6 (arom. C=O), 133.9 (arom. q), 133.4 (arom. q), 129.3, 128.7, 127.4, 121.6 (9 arom. CH), 66.9 (CH_2O), 59.5 (Pro α-CH), 46.7 (Pro δ-CH_2), 41.4 (CH_2) 29.1 (Pro β-CH_2), 24.3 (Pro γ-CH_2); MS (FAB, 3-NBA/TFA 10:1): *m*/*z* (%): 384.2 $[M+H]^+$; $C_{21}H_{21}NO_6$ (383.40).

PhAcOZ-Leu-OH (11): Colorless oil (3.30 g, 83 %); $[\alpha]_D^{2} = -2.1$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.38 - 7.29$ (m, 7 H; C_6H_5, 2 CH), 7.03 (d, $^3J = 8.5$ Hz, 2 H; 2 CH), 6.60 (brs, 1 H; COOH), 5.16 (d, $^3J = 8.6$ Hz, 1 H; OCONH), 5.08 (s, 2 H; CCH_2O), 4.40 – 4.35 (m, 1 H; Leu α-CH), 3.84 (s, 2 H; $CCH_2C(O)$), 1.74 – 1.65 (m, 2 H; Leu γ-CH, Leu β-CH_{2a}), 1.57 – 1.51 (m, 1 H; Leu β-CH_{2b}), 0.94 (m, 6 H; 2 Leu ω-CH_3); ^{13}C NMR (125.7 MHz, $CDCl_3$): $\delta = 177.5$ (COOH), 169.9 (COO), 156.1 (OCONH), 150.6 (arom. C=O), 133.9 (arom. q), 133.4 (arom. q), 129.3, 128.8, 127.4, 121.6 (9 arom. CH), 66.4 (CH_2O), 52.4 (Leu α-CH), 41.4 (CH_2, Leu β-CH_2), 24.8 (Leu γ-CH), 22.8, 21.7 (2 Leu ω-CH_3); HRMS (EI, 70 eV): *m*/*z* (%): calcd for $[M]^+$ 399.1682; found: 399.1696; $C_{18}H_{17}NO_6$ (399.44).

PhAcOZ-Gly-OH (12): Colorless solid (4.17 g, 81 %); m.p. 162 °C; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.38 - 7.26$ (m, 7 H; C_6H_5, 2 CH), 7.03 (d, $^3J = 8.5$ Hz, 2 H; 2 OCCH), 5.31 (brs, 1 H; OCONH), 5.08 – 5.04 (s, 2 H;

CCH_2O), 4.45 (brs, 1H; COOH), 3.98 (d, ^3J = 4.9 Hz, 2H; Gly α-CH_2), 3.85 (s, 2H; $CCH_2C(O)$); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 173.5 (COOH), 169.9 (COO), 156.3 (OCONH), 150.6 (arom. C=O), 133.8 (arom. q), 133.3 (arom. q), 129.3, 129.2, 128.7, 127.4, 121.6 (9 arom. CH), 66.5 (CH_2O), 42.5 (Gly α-CH_2), 41.4 (CCH_2CO); HRMS (EI, 70 eV, 165 °C): m/z (%): calcd for $[M]^+$ 343.1056; found: 343.1053; $C_{18}H_{17}NO_6$ (343.33).

(PhAcOZ-Pro-Cys-OAll)$_2$ (14): HOBt (505 mg, 3.3 mmol) was added to a solution of PhAcOZ-Pro-OH (**10**, 1.15 g, 3 mmol) and (pTosOH · H-Cys-OAll)$_2$ (**13**, 997 mg, 1.5 mmol) in CH_2Cl_2 (30 mL) and at 0 °C NEt_3 (0.43 mL, 3.1 mmol) and DIC (0.48 mL, 3.1 mmol). The mixture was stirred at 20 °C for 16 h. Then the solution was extracted with acetic acid (5%, 3 × 10 mL), $NaHCO_3$ (2.5%, 3 × 10 mL), and water and the organic layer was dried with $MgSO_4$. The solvent was removed under reduced pressure and the product **14** was purified by flash chromatography on silica gel using ethyl acetate/n-hexane 3:1 (v/v) as eluent to yield the title compound as a colorless oil (1.39 g, 88%). $[\alpha]_D^{22} = -22.4$ (c = 0.95 in $CHCl_3$); R_f = 0.32 (ethyl acetate/n-hexane 3:1 v/v); ^1H NMR (500 MHz, $CDCl_3$): δ = 8.02 (brs, 2H; 2CONH), 7.37–7.32 (m, 14H; 2C_6H_5, 4CH), 7.05 (d, ^3J = 8.5 Hz, 4H; 2OCCH), 5.90–5.85 (m, 2H; 2allyl CH=), 5.35–5.29 (m, 2H; 2allyl=CH_{2a}), 5.24 (m, 2allyl=CH_{2b}), 5.12–5.03 (m, 4H; 2CCH_2O), 4.79–4.75 (m, 2H; 2Cys α-CH), 4.61 (s, 4H; 2allyl OCH_2), 4.33–4.31 (m, 2H; 2Pro α-CH), 3.88 (s, 4H; 2$CCH_2C(O)$), 3.61–3.59 (m, 2H; 2Pro δ-CH_{2a}), 3.51–3.45 (m, 2H; 2Pro δ-CH_{2b}), 3.19–3.10 (m, 2H; 2Cys β-CH_{2a}), 3.01–2.97 (m, 2H; 2Cys β-CH_{2b}), 2.21–1.88 (m, 8H; 2Pro β-CH_2, 2Pro γ-CH_2); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 172.1, 169.9 (3C=O), 155.6 (OCON), 150.4 (arom. C=O), 134.2 (arom. q), 133.3 (arom. q), 131.4 (allyl CH), 129.3, 129.1, 128.7, 127.4, 121.5 (9 arom. CH), 119.1 (allyl CH_2), 66.6, 66.4 (CH_2O, allyl OCH_2), 60.3 (Pro α-CH), 52.3 (Cys α-CH), 47.5 (Pro δ-CH_2), 47.0 (Cys β-CH_2), 41.4 (CH_2), 29.0 (Pro β-CH_2), 24.4 (Pro γ-CH_2); MS (FAB, 3-NBA): m/z (%): 1051.0 $[M+H]^+$; elemental analysis calcd (%) for $C_{54}H_{58}N_4O_{14}S_2$ (1051.20): C 61.70, H 5.56, N 5.33; found: C 61.63, H 5.78, N 5.08.

(PhAcOZ-Leu-Cys-OAll)$_2$ (15): HOBt (322 mg, 2.1 mmol) was added to a solution of PhAcOZ-Leu-OH (**11**, 799 mg, 2 mmol) and (pTosOH · H-Cys-OAll)$_2$ (**13**, 665 mg, 1.0 mmol) in CH_2Cl_2 (30 mL) and at 0 °C NEt_3 (0.29 mL, 2.1 mmol) and EDC (384 mg, 2.0 mmol). The mixture was stirred at 20 °C for 16 h. Then the solution was extracted with acetic acid (5%, 3 × 10 mL), $NaHCO_3$ (2.5%, 3 × 10 mL), and water and the organic layer was dried with $MgSO_4$. The solvent was removed under reduced pressure and the product **15** was purified by crystallization from ethyl acetate/n-hexane to yield a colorless solid (991 mg, 91%). M.p. 126 °C; $[\alpha]_D^{22} = +32.0$ (c = 1.0 in $CHCl_3$); R_f = 0.43 (ethyl acetate/n-hexane 1:1 v/v); ^1H NMR (500 MHz, $CDCl_3$): δ = 7.64 (d, ^3J = 7.7 Hz, 2H; 2CONH), 7.38–7.26 (m, 14H; 2C_6H_5, 4CH), 7.00 (d, ^3J = 8.4 Hz, 4H; 2OCCH), 5.85 (ddt, $^3J_{trans}$ = 17.0, $^3J_{cis}$ = 10.8, $^3J_{vic}$ = 5.6 Hz, 2H; 2allyl CH=), 5.65 (d, ^3J = 8.7 Hz, 2H; 2OCONH), 5.29 (dd, $^3J_{trans}$ = 17.2, ^2J = 0.9 Hz, 2H; 2allyl=CH_{2a}), 5.21 (d, $^3J_{cis}$ = 10.5 Hz, 2H; 2allyl=CH_{2b}), 5.06 (d, J = 12.5 Hz, 2H; 2$CCH_{2a}O$), 5.00 (d, J = 12.5 Hz, 2H; 2$CCH_{2b}O$), 4.89–4.86 (m, 2H; 2Cys α-CH), 4.58 (d, ^3J = 5.6 Hz, 4H; 2allyl CH_2O), 4.49–4.44 (m, 2H; 2Leu α-CH), 3.84 (s, 4H; 2$CCH_2C(O)$), 3.02 (dd, ^2J = 4.1 Hz, ^3J = 14.0 Hz, 2H; 2Cys β-CH_{2a}), 2.86 (dd, ^2J = 7.2, ^3J = 14.0 Hz, 2H; 2Cys β-CH_{2b}), 1.72–1.59 (m, 4H; 2Leu γ-CH, 2Leu β-CH_{2a}), 1.56–1.51 (m, 2H; 2Leu β-CH_{2b}), 0.92 (d, ^3J = 6.5 Hz, 6H; 2Leu ω-CH_3), 0.88 (d, ^3J = 6.4 Hz, 6H; 2Leu ω-CH_3); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 172.9, 169.8, 169.7 (3C=O), 156.4 (OCONH), 150.4 (arom. C=O), 134.0 (arom. q), 133.3 (arom. q), 131.3 (allyl CH), 129.3, 128.7, 127.4, 121.5 (9 arom. CH), 119.0 (allyl CH_2), 66.3 (2OCH_2), 53.3 (Cys α-CH), 52.6 (Leu α-CH), 41.4 (CH_2, Leu β-CH_2), 39.9 (Cys β-CH_2), 24.6 (Leu γ-CH), 22.9, 21.9 (2Leu ω-CH_3); MS (FAB, 3-NBA): m/z: 1082.9 $[M+H]^+$; elemental analysis: calcd (%) for $C_{56}H_{66}N_4O_{14}S_2$ (1083.29): C 62.09, H 6.14, N 5.17; found: C 62.12, H 6.15, N 5.03.

PhAcOZ-Pro-Cys(Pal)-OAll (16): DTT (304 mg, 2.0 mmol) was added under argon to a solution of (PhAcOZ-Pro-Cys-OAll)$_2$ (**14**, 415 mg, 0.4 mmol) in CH_2Cl_2 (20 mL) and NEt_3 (110 µL, 0.8 mmol) and the mixture was stirred at 20 °C for 2 h. Then the solution was extracted with a degassed mixture of HCl (1N)/brine/water 1:1:1 ($v/v/v$) (3 × 10 mL) and the organic layer was dried with $MgSO_4$. At 0 °C to the solution was added NEt_3 (110 µL, 0.8 mmol) and palmitoyl chloride (596 µL, 2.0 mmol) and the mixture was stirred at 20 °C for 2 h. Then the solvent was removed under reduced pressure and product **16** was purified by flash chromatography on silica gel using ethyl acetate/n-hexane 2:3 (v/v) as eluent to yield a colorless wax (439 mg, 72%). M.p. 41 °C; $[\alpha]_D^{22} = -27.2$ (c = 0.5 in $CHCl_3$); R_f = 0.25 (ethyl acetate/n-hexane 2:3 v/v); ^1H NMR (500 MHz, $CDCl_3$): δ = 7.38–7.28 (m, 7H; C_6H_5, 2CH), 7.03 (brs, 2H; 2OCCH), 6.76 (brs, 1H; CONH), 5.88 (br, 1H; allyl CH=), 5.34 (d, $^3J_{trans}$ = 15.9 Hz, 1H; allyl=CH_{2a}), 5.24 (d, $^3J_{cis}$ = 9.9 Hz, 1H; allyl=CH_{2b}), 5.15 (brs, 2H; CCH_2O), 4.74 (brs, 1H; Cys α-CH), 4.62–4.56 (m, 2H; allyl OCH_2), 4.34–4.29 (m, 1H; Pro α-CH), 3.84 (s, 2H; $CCH_2C(O)$), 3.70–3.26 (m, 4H; Pro δ-CH_2, Cys β-CH_2), 2.49 (t, ^3J = 7.5 Hz, 2H; Pal α-CH_2), 2.31–2.15 (m, 1H; Pro β-CH_{2a}), 1.95–1.88 (m, 3H; Pro β-CH_{2b}, Pro γ-CH_2), 1.59 (t, ^3J = 7.1 Hz, 2H; Pal β-CH_2), 1.37–1.26 (brs, 24H; Pal $(CH_2)_{12}$), 0.88 (t, ^3J = 6.9 Hz, 3H; Pal ω-CH_3); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 198.5 (C=OS), 171.6, 169.8, 169.6 (3C=O), 155.7 (OCON), 150.5 (arom. C=O), 134.1 (arom. q), 133.4 (arom. q), 131.5 (allyl CH), 130.0, 129.4, 129.3, 129.2, 128.8, 128.6, 127.4, 121.5 (9 arom. CH), 119.0 (allyl CH_2), 66.7, 66.4 (CH_2O, allyl OCH_2), 60.5 (Pro α-CH), 52.2 (Cys α-CH), 47.0 (Pro δ-CH_2), 44.0 (Cys β-CH_2), 41.4 (CH_2), 32.0, 31.0, 30.4, 29.7, 29.6, 29.5, 29.4, 29.3 (10Pal CH_2), 29.0 (Pro β-CH_2), 28.5, 25.6 (2Pal CH_2), 24.5 (Pro γ-CH_2), 23.6, 22.7 (2Pal CH_2), 14.2 (Pal ω-CH_3); HRMS (EI, 70 eV, 195 °C): m/z (%): calcd for $[M]^+$ 764.4070; found: 764.4092; elemental analysis calcd (%) for $C_{43}H_{60}N_2O_8S$ (765.02): C 67.51, H 7.91, N 3.66; found: C 67.61, H 7.84, N 3.53.

PhAcOZ-Leu-Cys(Pal)-OAll (17): DTT (190 mg, 1.24 mmol) and NEt_3 (68 µL, 0.495 mmol) was added under argon to a solution of (PhAcOZ-Leu-Cys-OAll)$_2$ (**15**, 268 mg, 0.247 mmol) in CH_2Cl_2 (20 mL)and the mixture was stirred at 20 °C for 1 h. Then the solution was extracted with a degased mixture of HCl (1N)/brine/water 1:1:1 ($v/v/v$) (3 × 20 mL) and the organic layer was dried with $MgSO_4$. At 0 °C to the solution was added NEt_3 (68 µL, 0.495 mmol) and palmitoyl chloride (164 µL, 0.544 mmol) and the mixture was stirred at 20 °C for 1 h. Then the solvent was removed under reduced pressure and product **17** was purified by flash chromatography on silica gel using ethyl acetate/n-hexane 1:3 (v/v) as eluent to yield a colorless wax (330 mg, 85%). M.p. 74 °C; $[\alpha]_D^{22} = -12.0$ (c = 0.5 in $CHCl_3$); R_f = 0.19 (ethyl acetate/n-hexane 1:3 v/v); ^1H NMR (500 MHz, $CDCl_3$): δ = 7.38–7.29 (m, 7H; C_6H_5, 2CH), 7.04 (d, ^3J = 8.5 Hz, 2H; 2OCCH), 6.78 (d, ^3J = 7.5 Hz, 1H; CONH), 5.85 (ddt, $^3J_{trans}$ = 17.0, $^3J_{cis}$ = 10.8, $^3J_{vic}$ = 5.6 Hz, 1H; allyl CH=), 5.33 (d, $^3J_{trans}$ = 17.1 Hz, 1H; allyl=CH_{2a}), 5.25 (d, $^3J_{cis}$ = 10.7 Hz, 1H; allyl=CH_{2b}), 5.19 (d, ^3J = 8.2 Hz, 1H; OCONH), 5.11–5.07 (m, 2H; CCH_2O), 4.77–4.74 (m, 1H; Cys α-CH), 4.62 (d, ^3J = 5.5 Hz, 2H; allyl OCH_2), 4.20–4.18 (m, 1H; Leu α-CH), 3.84 (s, 2H; $CCH_2C(O)$), 3.37–3.34 (m, Cys β-CH_2), 2.52 (t, ^3J = 7.5 Hz, 2H; Pal α-CH_2), 1.69–1.60 (m, 4H; Pal β-CH_2, Leu γ-CH, Leu β-CH_{2a}), 1.51–1.48 (m, 1H; Leu β-CH_{2b}), 1.24 (brs, 24H; Pal $(CH_2)_{12}$), 0.94 (d, ^3J = 6.1 Hz, 6H; 2Leu ω-CH_3), 0.88 (t, ^3J = 7.0 Hz, 3H; Pal ω-CH_3); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 199.3 (C=OS), 172.0, 169.8, 169.5 (3C=O), 155.9 (OCONH), 150.6 (arom. C=O), 133.9 (arom. q), 133.3 (arom. q), 131.4 (allyl CH), 129.3, 129.2, 128.8, 127.4, 121.6 (9 arom. CH), 119.2 (allyl CH_2), 66.5, 66.3 (CH_2O, allyl OCH_2), 53.4 (Cys α-CH), 52.5 (Leu α-CH), 44.0 (Cys β-CH_2), 41.6, 41.4 (CH_2, Leu β-CH_2), 32.1, 31.9, 30.3, 29.7, 29.6, 29.4, 29.2, 29.0, 28.9, 25.7, 25.6 (13 Pal CH_2), 24.6 (Leu γ-CH), 22.9 (Leu ω-CH_3), 22.7 (Pal CH_2), 22.0 (Leu ω-CH_3), 14.2 (Pal ω-CH_3); HRMS (EI, 70 eV, 200 °C): m/z (%): calcd for $[M]^+$ 780.4383; found: 780.4407; elemental analysis calcd (%) for $C_{44}H_{64}N_2O_8S$ (781.07): C 67.66, H 8.26, N 3.59; found: C 67.85, H 8.06, N 3.32.

PhAcOZ-Pro-Cys(Pal)-OH (18): N,N'-Dimethylbarbituric acid (62 mg, 0.4 mmol) and a catalytic amount of $[Pd(PPh_3)_4]$ were added under argon to a solution of PhAcOZ-Pro-Cys(Pal)-OAll (**16**, 153 mg, 0.2 mmol) in tetrahydrofuran (20 mL) and the mixture was stirred at 20 °C for 1 h. The solvent was evaporated under reduced pressure, and the residue was dissolved in ethyl acetate (20 mL). Then the solution was extracted with phosphate buffer (0.1M, pH 6.8, 3 × 10 mL) and the organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. Recrystallization from CH_2Cl_2/n-hexane gave a colorless solid (132 mg, 91%). M.p. 113–114 °C; $[\alpha]_D^{22} = -28.4$ (c = 0.5 in $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$/CD_3OD 10:1 v/v): δ = 7.41–7.29 (m, 7H; C_6H_5, 2CH), 7.04 (brs, 2H; 2OCCH), 5.14 (brs, 2H; CCH_2O), 4.64 (brs, 1H; Cys α-CH), 4.31 (brm, 1H; Pro α-CH), 3.87 (s, 2H; $CCH_2C(O)$), 3.61–3.42 (m, 3H; Pro δ-CH_2, Cys β-CH_{2a}), 3.28–3.24 (m, 1H; Cys β-CH_{2b}), 2.51 (brs, 2H; Pal α-CH_2), 2.19 (brs, 1H; Pro β-CH_{2a}), 2.11–2.08 (m, 1H; Pro β-CH_{2b}), 1.90 (brs, 2H; Pro γ-CH_2), 1.60 (t, ^3J = 6.9 Hz, 2H; Pal β-CH_2), 1.32–1.26 (brs, 24H; Pal $(CH_2)_{12}$), 0.88 (t, ^3J = 6.9 Hz, 3H; Pal ω-CH_3); ^{13}C NMR (125.7 MHz, $CDCl_3$/CD_3OD 10:1): δ = 199.9 (C=OS), 173.2, 171.5, 170.7 (3C=O), 155.3 (OCON), 150.6 (arom. C=O), 134.3 (arom. q), 133.5 (arom. q), 129.7, 129.5, 129.4, 129.1, 129.0, 128.9, 128.8, 127.6, 121.7 (9 arom. CH), 67.0 (CH_2O), 60.9 (Pro α-CH), 52.4 (Cys α-CH), 47.6 (Pro δ-CH_2), 44.2 (Cys β-CH_2), 41.5

(CH_2), 32.1, 31.3, 30.4, 29.9, 29.8, 29.6, 29.4, 29.3 (10 Pal CH_2), 29.1 (Pro β-CH_2), 25.7 (Pal CH_2), 24.5 (Pro γ-CH_2), 23.7, 22.9 (2 Pal CH_2), 14.2 (Pal ω-CH_3); HRMS (FAB, 3-NBA/TFA 10:1): m/z (%): calcd for $[M+H]^+$ 725.3836; found: 725.3804; $C_{40}H_{56}N_2O_8S$ (724.95).

PhAcOZ-Leu-Cys(Pal)-OH (19): *N,N'*-Dimethylbarbituric acid (78 mg, 0.5 mmol) and a catalytic amount of $[Pd(PPh_3)_4]$ were added under argon to a solution of PhAcOZ-Leu-Cys(Pal)-OAll (**17**, 195 mg, 0.25 mmol) in tetrahydrofuran (20 mL) and the mixture was stirred at 20 °C for 1 h. The solvent was evaporated under reduced pressure, and the residue was dissolved in ethyl acetate (40 mL). Then the solution was extracted with phosphate buffer (0.1 M, pH 6.8, 3 × 10 mL) and the organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. Recrystallization from CH_2Cl_2/*n*-hexane gave a colorless solid (160 mg, 86 %). M.p. 115 °C; $[\alpha]_D^{22} = +8.3$ ($c = 0.5$ in $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3/CD_3OD$ 10:1): $\delta = 7.40 - 7.29$ (m, 7 H; C_6H_5, 2 CH), 7.05 (d, $^3J = 8.5$ Hz, 2 H; 2 OCCH), 5.08 (d, $J = 4.2$ Hz, 2 H; CCH_2O), 4.64 (dd, $^3J_1 = 6.9$ Hz, $^3J_2 = 4.7$ Hz, 1 H; Cys α-CH), 4.18 (dd, $^3J_1 = 9.3$ Hz, $^3J_2 = 5.2$ Hz, 1 H; Leu α-CH), 3.87 (s, 2 H; $CCH_2C(O)$), 3.47 (dd, $^2J = 14.0$, $^3J = 4.4$ Hz, 1 H; Cys β-CH_{2a}), 3.25 (dd, $^2J = 14.1$, $^3J = 7.3$ Hz, 1 H; Cys β-CH_{2b}), 2.53 (t, $^3J = 7.5$ Hz, 2 H; Pal α-CH_2), 1.69 – 1.58 (m, 4 H; Pal β-CH_2, Leu γ-CH, Leu β-CH_{2a}), 1.52 – 1.46 (m, 1 H; Leu β-CH_{2b}), 1.32 – 1.25 (br s, 24 H; Pal $(CH_2)_{12}$), 0.94 – 0.92 (m, 6 H; 2 Leu ω-CH_3), 0.88 (t, $^3J = 6.9$ Hz, 3 H; Pal ω-CH_3); ^{13}C NMR (125.7 MHz, $CDCl_3/CD_3OD$ 10:1): $\delta = 199.8$ (C=OS), 173.2, 171.7, 170.6 (3 C=O), 156.6 (OCONH), 150.6 (arom. C=O), 134.3 (arom. q), 133.4 (arom. q), 129.4, 129.2, 128.9, 127.6, 121.7 (9 arom. CH), 66.3 (CH_2O), 53.5 (Cys α-CH), 52.3 (Leu α-CH), 44.1 (Cys β-CH_2), 41.5, 41.4 (CH_2, Leu β-CH_2), 32.1, 30.3, 29.8, 29.7, 29.6, 29.5, 29.4, 29.1, 25.7 (13 Pal CH_2), 24.7 (Leu γ-CH), 22.9 (Leu ω-CH_3), 22.8 (Pal CH_2), 21.8 (Leu ω-CH_3), 14.1 (Pal ω-CH_3); HRMS (FAB, 3-NBA/TFA 10:1): m/z (%): calcd for $[M+H]^+$ 741.4149; found: 741.4179; elemental analysis calcd (%) for $C_{41}H_{60}N_2O_8S$ (740.99): C 66.46, H 8.16, N 3.78; found: C 66.07, H 7.99, N 3.73.

PhAcOZ-Pro-Cys(Pal)-Gly-OAll (6): HOBt (34 mg, 0.22 mmol) was added to a solution of PhAcOZ-Pro-Cys(Pal)-OH (**18**; 132 mg, 0.18 mmol) and *p*TosOH · H-Gly-OAll (**20**, 52 mg, 0.18 mmol) in CH_2Cl_2 (50 mL) and at 0 °C NEt_3 (25 µL, 0.12 mmol) and finally EDC (39 mg, 0.2 mmol). The mixture was stirred at 20 °C for 16 h and the solvent was washed with HCl (0.5 N, 3 × 10 mL) and water. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The product **6** was isolated from the residue by flash chromatography on silica gel using ethyl acetate/*n*-hexane 1:1 (*v*/*v*) as eluent to yield a colorless wax (137 mg, 91 %). M.p. 78 °C; $[\alpha]_D^{22} = +18.3$ ($c = 1.0$ in $CHCl_3$); $R_f = 0.36$ (ethyl acetate/*n*-hexane 3:2 *v*/*v*); ^1H NMR (500 MHz, $CDCl_3$): $\delta = 7.53$ (br s, 1 H; CONH), 7.40 – 7.28 (m, 7 H; C_6H_5, 2 CH), 7.18 (d, $^3J = 7.5$ Hz, 1 H; CONH), 7.06 (d, $^3J = 8.3$ Hz, 2 H; 2 OCCH), 5.88 (ddt, $^3J_{trans} = 16.4$, $^3J_{cis} = 10.8$, $^3J_{vic} = 5.6$ Hz, 1 H; allylCH=), 5.30 (dd, $^3J_{trans} = 17.2$, $^2J = 1.3$ Hz, 1 H; allyl=CH_{2a}), 5.21 (d, $^3J_{cis} = 10.4$ Hz, 1 H; allyl=CH_{2b}), 5.13 (s, 2 H; CCH_2O), 4.61 (br d, $^3J = 4.8$ Hz, 3 H; allyl OCH_2, Cys α-CH), 4.28 (dd, $^3J_1 = 8.5$, $^3J_2 = 4.6$ Hz, 1 H; Pro α-CH), 4.20 (dd, $^2J = 17.8$, $^3J = 6.5$ Hz, 1 H; Gly α-CH_{2a}), 3.89 – 3.85 (m, 3 H; Gly α-CH_{2b}, $CCH_2C(O)$), 3.61 – 3.56 (m, 1 H; Pro δ-CH_{2a}), 3.50 – 3.45 (m, 1 H; Pro δ-CH_{2b}), 3.37 – 3.27 (m, 2 H; Cys β-CH_2), 2.50 (t, $^3J = 7.4$ Hz, 2 H; Pal α-CH_2), 2.24 – 2.16 (m, 1 H; Pro β-CH_{2a}), 2.12 – 2.04 (m, 1 H; Pro β-CH_{2b}), 1.91 – 1.87 (m, 2 H; Pro γ-CH_2), 1.58 (t, $^3J = 6.6$ Hz, 2 H; Pal β-CH_2), 1.31 – 1.13 (br s, 24 H; Pal $(CH_2)_{12}$), 0.88 (t, $^3J = 6.9$ Hz, 3 H; Pal ω-CH_3); ^{13}C NMR (125.7 MHz, $CDCl_3$): $\delta = 202.2$ (C=OS), 172.1, 170.0, 169.8, 169.1 (4 C=O), 156.1 (OCON), 150.6 (arom. C=O), 133.8 (arom. q), 133.3 (arom. q), 131.6 (allyl CH), 129.4, 129.3, 129.0, 128.7, 127.4, 121.6 (9 arom. CH), 118.6 (allyl CH_2), 67.1, 65.8 (CH_2O, allyl OCH_2), 61.6 (Pro α-CH), 54.4 (Cys α-CH), 47.0 (Pro δ-CH_2), 43.9 (Cys β-CH_2), 41.4, 41.2 (CH_2, Gly α-CH_2), 31.9, 30.2, 29.7, 29.6, 29.4, 29.3 (10 Pal CH_2), 29.2 (Pro β-CH_2), 28.9, 25.6 (2 Pal CH_2), 24.6 (Pro γ-CH_2), 23.6, 22.7 (2 Pal CH_2), 14.1 (Pal ω-CH_3); HRMS (FAB, 3-NBA/TFA 10:1): m/z (%): calcd for $[M+H]^+$ 822.4363; found: 822.4343; elemental analysis calcd (%) for $C_{45}H_{63}N_3O_9S$ (822.07): C 65.75, H 7.72, N 5.11; found: C 65.34, H 7.66, N 5.02.

PhAcOZ-Leu-Cys(Pal)-Gly-OAll (8): HOBt (22 mg, 0.14 mmol) was added to a solution of PhAcOZ-Leu-Cys(Pal)-OH (**19**, 87 mg, 0.12 mmol) and *p*TosOH · H-Gly-OAll (**20**, 34 mg, 0.12 mmol) in CH_2Cl_2 (20 mL) and at 0 °C NEt_3 (16 µL, 0.12 mmol) and finally EDC (25 mg, 0.13 mmol). The mixture was stirred at 20 °C for 16 h and the solvent was washed with HCl (0.5 N, 3 × 10 mL) and water. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The product **8** was isolated from the residue by flash chromatography on silica gel using ethyl acetate/*n*-hexane 2:3 (*v*/*v*) as eluent to yield a colorless wax (92 mg, 94 %). M.p. 58 °C; $[\alpha]_D^{22} = -15.3$ ($c = 1.0$ in $CHCl_3$); $R_f = 0.23$ (ethyl acetate/*n*-hexane 2:3 *v*/*v*); ^1H NMR (500 MHz, $CDCl_3$): $\delta = 7.39 - 7.29$ (m, 7 H; C_6H_5, 2 CH), 7.20 (br s, 1 H; CONH), 7.08 (d, $^3J = 7.3$ Hz, 1 H; CONH), 7.05 (d, $^3J = 8.5$ Hz, 2 H; 2 OCCH), 5.86 (ddt, $^3J_{trans} = 16.4$, $^3J_{cis} = 10.8$, $^3J_{vic} = 5.7$ Hz, 1 H; allyl CH=), 5.31 (dd, $^3J_{trans} = 17.2$, $^2J = 1.4$ Hz, 1 H; allyl=CH_{2a}), 5.23 (dd, $^3J_{cis} = 10.3$, $^2J = 1.1$ Hz, 1 H; allyl=CH_{2b}), 5.12 – 5.04 (m, 3 H; OCONH, CCH_2O), 4.61 (d, $^3J = 5.6$ Hz, 3 H; allyl OCH_2, Cys α-CH), 4.15 – 4.09 (m, 2 H; Leu α-CH, Gly α-CH_{2a}), 3.93 (dd, $^2J = 18.0$ Hz, $^3J = 5.0$ Hz, 1 H; Gly α-CH_{2b}), 3.85 (s, 2 H; $CCH_2C(O)$), 3.35 – 3.25 (m, 2 H; Cys β-CH_2), 2.53 (t, $^3J = 6.6$ Hz, 2 H; Pal α-CH_2), 1.70 – 1.58 (m, 4 H; Pal β-CH_2, Leu γ-CH, Leu β-CH_{2a}), 1.49 – 1.43 (m, 1 H; Leu β-CH_{2b}), 1.33 – 1.24 (br s, 24 H; Pal $(CH_2)_{12}$), 0.93 (d, $^3J = 5.1$ Hz, 6 H; 2 Leu ω-CH_3), 0.88 (t, $^3J = 7.0$ Hz, 3 H; Pal ω-CH_3); ^{13}C NMR (125.7 MHz, $CDCl_3$): $\delta = 201.6$ (C=OS), 172.8, 169.9, 169.1 (4 C=O), 156.4 (OCONH), 150.7 (arom. C=O), 133.7 (arom. q), 133.3 (arom. q), 131.5 (allyl CH) 129.4, 129.3, 128.8, 127.4, 121.6 (9 arom. CH), 118.9 (allyl CH_2), 66.6, 65.9 (CH_2O, allyl OCH_2), 54.2 (Cys α-CH), 53.9 (Leu α-CH), 44.0 (Cys β-CH_2), 41.4, 41.3, 41.1 (CH_2, Leu β-CH_2, Gly α-CH_2), 31.9, 30.3, 29.7, 29.6, 29.4, 29.2, 29.0, 25.6 (13 Pal CH_2), 24.8 (Leu γ-CH), 23.0 (Leu ω-CH_3), 22.7 (Pal CH_2), 21.7 (Leu ω-CH_3), 14.1 (Pal ω-CH_3); HRMS (EI, 70 eV, 200 °C): m/z (%): calcd for $[M]^+$ 837.4598; found: 837.4637; elemental analysis calcd (%) for $C_{46}H_{67}N_3O_9S$ (838.46): C 65.92, H 8.06, N 5.01; found: C 66.17, H 8.01, N 4.86.

Enzymatic removal of the PhAcOZ group from the lipopeptides 6 and 8: A solution of the PhAcOZ-protected tripeptide (10 µmol) in methanol (0.4 mL) was added to a solution of dimethyl-β-cyclodextrin (500 mg, 0.37 mmol) in phosphate buffer (3.6 mL, 0.2 M, pH 6.8 and 0.2 M KI) (two Eppendorf vials, each 2 mL). The turbid solution was sonified until it became clear. To this solution penicillin G acylase (100 U) was added and the mixture was shaken at 25 °C for 16 h. After filtering off the enzyme and washing with methanol and water the organic solvent was removed under reduced pressure. The resulting aqueous phase was treated with benzyltriethylammonium bromide (1 g, 3.7 mmol) and extracted with diethyl ether (10 × 10 mL). The combined organic layers were dried with $MgSO_4$ and concentrated under reduced pressure. The product was isolated from the residue by flash chromatography on silica gel using $CHCl_3$/methanol 50:1 (*v*/*v*) as eluent.

H-Pro-Cys(Pal)-Gly-OAll (21): Colorless oil (3 mg, 53 %); $[\alpha]_D^{22} = -33.6$ ($c = 0.15$ in MeOH); $R_f = 0.35$ ($CHCl_3$/MeOH 10:1 *v*/*v*); ^1H NMR (500 MHz, CD_3OD): $\delta = 5.94$ (ddt, $^3J_{trans} = 16.1$, $^3J_{cis} = 10.9$, $^3J_{vic} = 5.5$ Hz, 1 H; allyl CH), 5.33 (dd, $^3J_{trans} = 17.2$, $^2J = 1.5$ Hz, 1 H; allyl=CH_{2a}), 5.23 (dd, $^3J_{cis} = 10.5$, $^2J = 1.3$ Hz, 1 H; allyl=CH_{2b}), 4.63 (d, $^3J = 5.6$ Hz, 2 H; allyl OCH_2), 4.62 – 4.59 (m, 1 H; Cys α-CH), 4.28 (dd, $^3J_1 = 8.4$, $^3J_2 = 6.4$ Hz, 1 H; Pro α-CH), 3.98 (s, 2 H; Gly α-CH_2), 3.45 – 3.38 (m, 2 H; Pro δ-CH_2), 3.36 – 3.32 (m, 1 H; Cys β-CH_{2a}), 3.17 (dd, $^2J = 13.9$, $^3J = 7.7$ Hz, 1 H; Cys β-CH_{2b}), 2.58 (t, $^3J = 7.5$ Hz, 2 H; Pal α-CH_2), 2.47 – 2.40 (m, 1 H; Pro β-CH_{2a}), 2.14 – 2.01 (m, 3 H; Pro β-CH_{2b}, Pro γ-CH_2), 1.64 (t, $^3J = 7.0$ Hz, 2 H; Pal β-CH_2), 1.32 – 1.24 (br s, 24 H; Pal $(CH_2)_{12}$), 0.89 (t, $^3J = 6.9$ Hz, 3 H; Pal ω-CH_3); ^{13}C NMR (125.7 MHz, CD_3OD): $\delta = 200.1$ (C=OS), 171.8, 170.5, 169.7 (3 C=O), 133.2 (allyl CH), 118.7 (allyl CH_2), 66.8 (allyl OCH_2), 61.0 (Pro α-CH), 54.3 (Cys α-CH), 47.5 (Pro δ-CH_2), 44.7 (Cys β-CH_2), 42.0 (Gly α-CH_2), 33.0, 31.0, 30.9, 30.7, 30.6, 30.5, 30.4, 30.3 (12 Pal CH_2, Pro β-CH_2), 26.6 (Pro γ-CH_2), 24.9, 23.7 (2 Pal CH_2), 14.5 (Pal ω-CH_3); HRMS (EI, 70 eV, 180 °C): m/z: calcd for $[M]^+$ 553.3549; found: 553.3524; $C_{45}H_{63}N_3O_9S$ (553.80).

H-Leu-Cys(Pal)-Gly-OAll (22): Colorless oil (3 mg, 56 %); $[\alpha]_D^{22} = -5.8$ ($c = 0.15$ in $CHCl_3$); $R_f = 0.29$ ($CHCl_3$/MeOH 10:1 *v*/*v*); ^1H NMR (500 MHz, CD_3OD): $\delta = 5.94$ (ddt, $^3J_{trans} = 16.2$, $^3J_{cis} = 10.6$, $^3J_{vic} = 5.6$ Hz, 1 H; allyl CH=), 5.33 (dd, $^3J_{trans} = 17.2$, $^2J = 1.3$, 1 H; allyl=CH_{2a}), 5.23 (dd, $^3J_{cis} = 10.5$, $^2J = 1.0$ Hz, 1 H; allyl=CH_{2b}), 4.63 (d, $^3J = 5.6$ Hz, 2 H; allyl OCH_2), 4.57 (t, $^3J = 6.8$ Hz, 1 H; Cys α-CH), 3.97 (d, $J = 2.0$ Hz, 2 H; Gly α-CH_2), 3.94 – 3.89 (m, 1 H; Leu α-CH), 3.39 (dd, $^2J = 13.8$, $^3J = 6.1$ Hz, 1 H; Cys β-CH_{2a}), 3.20 (dd, $^2J = 13.8$, $^3J = 7.7$ Hz, 1 H; Cys β-CH_{2b}), 2.59 (t, $^3J = 7.4$ Hz, 2 H; Pal α-CH_2), 1.76 – 1.62 (m, 5 H; Pal β-CH_2, Leu γ-CH, Leu β-CH_2), 1.29 (br s, 24 H; Pal $(CH_2)_{12}$), 1.00 (d, $^3J = 3.1$ Hz, 3 H; 1 Leu ω-CH_3), 0.99 (d, $^3J = 3.2$ Hz, 3 H; 1 Leu ω-CH_3), 0.90 (t, $^3J = 6.8$ Hz, 3 H; Pal ω-CH_3); ^{13}C NMR (125.7 MHz, CD_3OD): $\delta = 200.2$ (C=OS), 171.8, 170.6, 170.4 (3 C=O), 133.3 (allyl CH), 118.7 (allyl CH_2), 66.7 (allyl OCH_2), 54.2 (Cys α-CH), 52.9 (Leu α-CH), 44.8 (Cys β-CH_2), 42.0, 41.6 (Leu β-CH_2, Gly α-CH_2), 33.0, 31.0, 30.7, 30.6, 30.5, 30.4, 30.3, 30.0, 26.6 (13 Pal CH_2), 25.3 (Leu γ-CH), 23.7 (Pal CH_2), 23.2 (Leu ω-CH_3), 22.0 (Leu ω-CH_3), 14.5 (Pal

ω-CH$_3$); HRMS (EI, 70 eV, 220 °C): m/z (%): calcd for $[M]^+$ 569.3862; found: 569.3854; $C_{30}H_{55}N_3O_5S$ (569.84).

PhAcOZ-Gly-Pro-OAll: HOBt (367 mg, 2.4 mmol) was added to a solution of PhAcOZ-Gly-OH (**12**, 687 mg, 2.0 mmol) and pTosOH ·H-Pro-OAll (655 mg, 2.0 mmol) in CH_2Cl_2 (50 mL) and at 0 °C NEt_3 (277 µL, 2.0 mmol) and finally EDC (422 mg, 2.2 mmol). The mixture was stirred at 20 °C for 16 h and the solvent was washed with HCl (0.5 N, 3 × 20 mL), $NaHCO_3$ (2.5 %, 3 × 20 mL), water (3 × 20 mL) and brine (20 mL). The organic layer was dried with $MgSO_4$ and concentrated under reduced pressure. The dipeptide was isolated from the residue by flash chromatography on silica gel using ethyl acetate/n-hexane 3:2 (v/v) as eluent to yield a colorless oil (765 mg, 80 %). $[\alpha]_D^{22} = -52.2$ ($c = 1.05$ in $CHCl_3$); $R_f = 0.25$ (ethyl acetate/n-hexane 3:2 v/v); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.39-7.28$ (m, 7H; C_6H_5, 2CH), 7.04 (d, $^3J = 8.5$ Hz, 2H; 2OCCH), 5.88 (ddt, $^3J_{trans} = 16.2$, $^3J_{cis} = 10.6$, $^3J_{vic} = 5.6$ Hz, 1H; allyl CH=), 5.70 (brs, 1H; OCONH), 5.32 (dd, $^3J_{trans} = 15.7$, $^2J = 1.5$ Hz, 1H; allyl=CH$_{2a}$), 5.24 (dd, $^3J_{cis} = 10.5$, $^2J = 1.3$ Hz, 1H; allyl=CH$_{2b}$), 5.08 (s, 2H; CCH$_2$O), 4.61 (d, $^3J = 5.2$ Hz, 2H; allyl OCH$_2$), 4.53 (dd, $^3J_1 = 8.9$, $^3J_2 = 3.3$ Hz, 1H; Pro α-CH), 4.03 (dd, $^2J = 17.2$, $^3J = 4.8$ Hz, 1H; Gly α-CH$_{2a}$), 3.96 (dd, $^2J = 17.2$, $^3J = 4.0$ Hz, 1H; Gly α-CH$_{2b}$), 3.85 (s, 2H; CCH$_2$C(O)), 3.60–3.56 (m, 1H; Pro δ-CH$_{2a}$), 3.48–3.43 (m, 1H; Pro δ-CH$_{2b}$), 2.25–2.15 (m, 1H; Pro β-CH$_{2a}$), 2.10–1.99 (m, 3H; Pro β-CH$_{2b}$, Pro γ-CH$_2$); ^{13}C NMR (125.7 MHz, $CDCl_3$): $\delta = 171.4$, 169.9, 166.9 (3C=O), 156.1 (OCON), 150.4 (arom. C=O), 134.1 (arom. q), 133.4 (arom. q), 131.7 (allyl CH), 129.5, 129.3, 129.1, 128.7, 127.3, 121.5 (9 arom. CH), 118.6 (allyl CH$_2$), 66.1, 65.8 (CH$_2$O, allyl OCH$_2$), 58.9 (Pro α-CH), 45.9 (Pro δ-CH$_2$), 43.3 (Gly α-CH$_2$), 41.4 (CH$_2$), 30.0 (Pro β-CH$_2$), 24.6 (Pro γ-CH$_2$); HRMS (EI, 70 eV, 165 °C): m/z: calcd for $[M]^+$ 480.1897; found: 480.1875; $C_{26}H_{28}N_2O_7$ (480.51).

PhAcOZ-Gly-Pro-OH (23): Morpholine (44 µL, 0.5 mmol) was added under argon to a solution of PhAcOZ-Gly-Pro-OAll (240 mg, 0.5 mmol) in tetrahydrofuran (10 mL) and a catalytic amount of $[Pd(PPh_3)_4]$, and the mixture was stirred at 20 °C for 1 h. The solvent was evaporated under reduced pressure, and the residue was dissolved in ethyl acetate (50 mL). Then the solution was extracted with HCl (0.5 N, 3 × 10 mL), water (3 × 10 mL) and brine and the organic layer was dried over $MgSO_4$ and concentrated under reduced pressure to yield a pale yellow oil (126 mg, 98 %). $[\alpha]_D^{22} = -56.9$ ($c = 1.0$ in MeOH); ^{1}H NMR (500 MHz, $CDCl_3$/CD_3OD 1:1): $\delta = 7.38-7.23$ (m, 7H; C_6H_5, 2CH), 7.03 (d, $^3J = 8.5$ Hz, 2H; 2OCCH), 5.08 (s, 2H; CCH$_2$O), 4.45 (dd, $^3J_1 = 9.0$, $^3J_2 = 2.9$ Hz, 1H; Pro α-CH), 4.02 (d, $^2J = 17.1$ Hz, 1H; Gly α-CH$_{2a}$), 3.90 (d, $^2J = 17.1$ Hz, 1H; Gly α-CH$_{2b}$), 3.87 (s, 2H; CCH$_2$C(O)), 3.59–3.52 (m, 1H; Pro δ-CH$_2$), 2.22–2.19 (m, 1H; Pro β-CH$_{2a}$), 2.03–1.99 (m, 3H; Pro β-CH$_{2b}$, Pro γ-CH$_2$); ^{13}C NMR (125.7 MHz, $CDCl_3$/CD_3OD 1:1): $\delta = 175.0$, 171.5, 169.2 (3C=O), 158.2 (OCON), 151.5 (arom. C=O), 135.4 (arom. q), 134.5 (arom. q), 130.7, 130.1, 130.0, 129.9, 129.8, 129.4, 129.1, 128.0 (9 arom. CH), 66.8 (CH$_2$O), 60.0 (Pro α-CH), 46.9 (Pro δ-CH$_2$), 43.7 (Gly α-CH$_2$), 41.7 (CH$_2$), 29.7 (Pro β-CH$_2$), 25.3 (Pro γ-CH$_2$); HRMS (EI, 70 eV, 210 °C): m/z: calcd for $[M]^+$ 440.1584; found: 440.1590; $C_{23}H_{24}N_2O_7$ (440.45).

PhAcOZ-Gly-Pro-Pro-Cys(Pal)-Gly-OAll (3): HOAt (27 mg, 0.2 mmol) was added to a solution of PhAcOZ-Gly-Pro-OH (**23**, 74 mg, 0.17 mmol) and H-Pro-Cys(Pal)-Gly-OAll (**21**, 90 mg, 0.15 mmol) in CH_2Cl_2 (10 mL) and at 0 °C EDC (35 mg, 0.18 mmol). The mixture was stirred at 20 °C for 16 h and the solvent was extracted with HCl (0.5 N, 3 × 15 mL), water (3 × 15 mL), and brine (15 mL). The organic layer was dried with $MgSO_4$ and concentrated under reduced pressure. The product **3** was isolated from the residue by size-exclusion chromatography on Sephadex LH-20 using $CHCl_3$/methanol 1:1 (v/v) as eluent to yield a colorless oil (122 mg, 82 %). $[\alpha]_D^{22} = -56.2$ ($c = 1.0$ in $CHCl_3$); $R_f = 0.31$ ($CHCl_3$/methanol 10:1 v/v); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 8.10$ (d, $^3J = 7.9$ Hz, 1H; CONH), 7.49 (t, $^3J = 5.7$ Hz, 1H; CONH), 7.39–7.29 (m, 7H; C_6H_5, 2CH), 7.03 (d, $^3J = 8.4$ Hz, 2H; 2OCCH), 5.88 (ddt, $^3J_{trans} = 16.2$, $^3J_{cis} = 11.0$, $^3J_{vic} = 5.6$ Hz, 1H; allyl CH=), 5.64 (t, $^3J = 4.3$ Hz, 1H; OCONH), 5.32 (dd, $^3J_{trans} = 17.2$, $^2J = 1.1$ Hz, 1H; allyl=CH$_{2a}$), 5.29–5.20 (m, 1H; allyl=CH$_{2b}$), 5.08 (s, 2H; CCH$_2$O), 4.70–4.24 (m, 5H; allyl OCH$_2$, Cys α-CH, 2Pro α-CH), 4.10–3.78 (m, 6H; 2Gly α-CH$_2$, CCH$_2$C(O)), 3.68–3.39 (m, 4H; 2Pro δ-CH$_2$), 3.35–3.25 (m, 2H; Cys β-CH$_2$), 2.58–2.51 (m, 2H; Pal α-CH$_2$), 2.32–2.15 (m, 4H; 2Pro β-CH$_2$), 2.06–1.98 (m, 2H; 2Pro γ-CH$_{2b}$), 1.94–1.84 (m, 2H; Pro γ-CH$_{2b}$), 1.66–1.59 (m, 2H; Pal β-CH$_2$), 1.30–1.22 (brs, 24H; Pal (CH$_2$)$_{12}$), 0.88 (t, $^3J = 6.9$ Hz, 3H; Pal ω-CH$_3$); ^{13}C NMR (125.7 MHz, $CDCl_3$): $\delta = 202.9$ (C=OS), 172.5, 171.5, 170.0, 169.2, 167.0 (5C=O), 156.2 (OCON), 150.5 (arom. C=O), 134.1 (arom. q), 133.4 (arom. q), 131.7 (allyl CH), 129.3, 129.2, 129.1, 128.7, 127.4, 121.5 (9 arom. CH), 118.7 (allyl CH$_2$), 66.2, 65.7 (CH$_2$O, allyl OCH$_2$), 61.5, 58.5 (2Pro α-CH), 54.3 (Cys α-CH), 47.5, 46.3 (2Pro δ-CH$_2$), 44.1 (Cys β-CH$_2$), 44.0, 41.4 (2Gly α-CH$_2$), 41.2 (CH$_2$), 31.9, 30.4, 29.7, 29.6, 29.4, 29.3, 29.2, 29.0, 28.7, 28.5 (10Pal CH$_2$, 2Pro β-CH$_2$), 25.7, 25.5, 25.3, 25.1, 25.0, 22.7 (4Pal CH$_2$, 2Pro γ-CH$_2$), 14.1 (Pal ω-CH$_3$); HRMS (FAB, 3-NBA/TFA 10:1): m/z: calcd for $[M+H]^+$ 976.5106; found: 976.5134; elemental analysis calcd (%) for $C_{52}H_{73}N_5O_{11}S$ (976.25): C 63.98, H 7.54, N 7.17; found: C 63.66, H 7.49, N 7.21.

H-Gly-Pro-Pro-Cys(Pal)-Gly-OAll (24): A solution of PhAcOZ-Gly-Pro-Pro-Cys(Pal)-Gly-OAll (**3**, 10 mg, 10 µmol) in methanol (0.4 mL) was added to a solution of dimethyl-β-cyclodextrin (250 mg, 0.16 mmol) in phosphate buffer (3.6 mL, 0.2 M, pH 6.8; 0.1 M KI) (two Eppendorf vials, each 2 mL). The turbid solution was sonified until it became clear. To this solution penicillin G acylase CLEC slurry (200 µL) was added and the mixture was shaken at 25 °C for 16 h. After filtering off the enzyme and washing with methanol and water the organic solvent was removed under reduced pressure. The resulting aqueous phase was treated with benzyltriethylammonium bromide (1 g, 3.7 mmol) and extracted with diethyl ether (10 × 10 mL). The combined organic layers were dried with $MgSO_4$ and concentrated under reduced pressure. The product **24** was isolated from the residue by flash chromatography on silica gel using $CHCl_3$/methanol 50:1 (v/v) as eluent to yield a colorless oil (2.8 mg, 39 %). $[\alpha]_D^{22} = -98.4$ ($c = 0.125$ in methanol); $R_f = 0.17$ ($CHCl_3$/methanol 10:1 v/v); ^{1}H NMR (500 MHz, CD_3OD): $\delta = 5.94$ (ddt, $^3J_{trans} = 16.1$, $^3J_{cis} = 10.7$, $^3J_{vic} = 5.6$ Hz, 1H; allyl CH=), 5.32 (dd, $^3J_{trans} = 17.2$, $^2J = 1.4$ Hz, 1H; allyl=CH$_{2a}$), 5.23 (dd, $^3J_{cis} = 10.5$, $^2J = 1.2$ Hz, 1H; allyl=CH$_{2b}$), 4.75 (dd, $^3J_1 = 8.1$, $^3J_2 = 3.4$ Hz, 1H; Cys α-CH), 4.63 (d, 2H; $^3J = 5.6$ Hz, allyl OCH$_2$), 4.49 (dd, $^3J_1 = 8.3$, $^3J_2 = 5.3$ Hz, 1H; Pro α-CH), 4.42–4.40 (m, 1H; Pro α-CH), 4.00–3.81 (m, 4H; 2Gly α-CH$_2$), 3.70–3.49 (m, 4H; 2Pro δ-CH$_2$), 3.41 (dd, $^2J = 14.0$, $^3J = 5.3$ Hz, 1H; Cys β-CH$_{2a}$), 3.20 (dd, $^2J = 14.0$, $^3J = 8.4$ Hz, 1H; Cys β-CH$_{2b}$), 2.58 (t, $^3J = 7.4$ Hz, 2H; Pal α-CH$_2$), 2.45–1.95 (m, 8H; 2Pro β-CH$_2$, 2Pro γ-CH$_2$), 1.64 (t, $^3J = 7.0$ Hz, 2H; Pal β-CH$_2$), 1.28–1.22 (brs, 24H; Pal (CH$_2$)$_{12}$), 0.90 (t, $^3J = 6.9$ Hz, 3H; Pal ω-CH$_3$); ^{13}C NMR (125.7 MHz, CD_3OD): $\delta = 200.7$ (C=OS), 174.0, 172.9, 172.2, 170.5, 165.7 (5C=O), 133.3 (allyl CH), 118.7 (allyl CH$_2$), 66.7 (allyl OCH$_2$), 62.0, 59.9 (2Pro α-CH), 54.2 (Cys α-CH), 48.0, 47.7 (2Pro δ-CH$_2$), 44.7 (Cys β-CH$_2$), 42.0, 41.0 (2Gly α-CH$_2$), 33.0, 31.2, 30.7, 30.5, 30.4, 30.2, 30.0, 29.9, 29.6 (10 Pal CH$_2$, 2 Pro β-CH$_2$), 26.6, 26.0, 25.7, 23.7 (4Pal CH$_2$, 2Pro γ-CH$_2$), 14.5 (Pal ω-CH$_3$); HRMS (EI, 70 eV, 210 °C): m/z: calcd for $[M]^+$ 707.4292; found: 707.4260; $C_{36}H_{61}N_5O_7S$ (707.97).

PhAcOZ-Leu-Cys(Pal)-Gly-OH (25): Morpholine (11 µL, 0.13 mmol) and a catalytic amount of $[Pd(PPh_3)_4]$ were added to a solution of PhAcOZ-Leu-Cys(Pal)-Gly-OAll (**8**, 109 mg, 0.13 mmol) in tetrahydrofuran (10 mL) was added under argon and the mixture was stirred at 20 °C for 1 h. The solvent was evaporated under reduced pressure, and the residue was dissolved in ethyl acetate (20 mL). Then the solution was extracted with HCl (0.5 N, 3 × 10 mL), water (3 × 10 mL) and brine, and the organic layer was dried with $MgSO_4$ and concentrated under reduced pressure. The product **25** was isolated from the residue by flash chromatography on silica gel using ethyl acetate/n-hexane 2:1 (v/v) and then ethyl acetate/ethanol 1:1 (v/v) as eluent to yield a colorless wax (84 mg, 80 %). M.p. 76–78 °C; $[\alpha]_D^{22} = -10.8$ ($c = 0.5$ in $CHCl_3$); $R_f = 0.29$ (ethyl acetate/n-hexane 3:2 v/v); ^{1}H NMR (500 MHz, $CDCl_3$/CD_3OD 10:1 v/v): $\delta = 7.42-7.29$ (m, 7H; C_6H_5, 2CH), 7.05 (d, $^3J = 8.5$ Hz, 2H; 2OCCH), 5.11 (d, $^2J = 12.2$ Hz, 1H; CCH$_{2a}$O), 5.04 (d, $^2J = 12.4$ Hz, 1H; CCH$_{2b}$O), 4.58 (dd, $^3J_1 = 8.8$, $^3J_2 = 4.4$ Hz, 1H; Cys α-CH), 4.15–4.09 (m, 1H; Leu α-CH), 4.03 (d, $^2J = 18.0$ Hz, 1H; Gly α-CH$_{2a}$), 3.87–3.84(m, 3H; CCH$_2$C(O), Gly α-CH$_{2b}$), 3.41 (dd, $^2J = 14.2$, $^3J = 4.5$ Hz, 1H; Cys β-CH$_{2a}$), 3.22 (dd, $^2J = 14.2$, $^3J = 8.9$ Hz, 1H; Cys β-CH$_{2b}$), 2.53 (t, $^3J = 7.6$ Hz, 2H; Pal α-CH$_2$), 1.72–1.56 (m, 4H; Pal β-CH$_2$, Leu γ-CH, Leu β-CH$_{2a}$), 1.52–1.46 (m, 1H; Leu β-CH$_{2b}$), 1.32–1.22 (brs, 24H; Pal (CH$_2$)$_{12}$), 0.94 (m, 6H; 2Leu ω-CH$_3$), 0.88 (t, $^3J = 6.9$ Hz, 3H; Pal ω-CH$_3$); ^{13}C NMR (125.7 MHz, $CDCl_3$): $\delta = 200.7$ (C=OS), 173.9, 171.8, 170.7 (4C=O), 157.2 (OCONH), 150.7 (arom. C=O), 133.5 (arom. q), 132.2 (arom. q), 129.5, 129.4, 129.3, 129.0, 128.9, 128.8, 128.3, 127.6, 121.8 (9 arom. CH), 66.6 (CH$_2$O), 54.3 (Cys α-CH), 53.2 (Leu α-CH), 44.2 (Cys β-CH$_2$), 41.5, 41.3, 41.1 (CH$_2$, Leu β-CH$_2$, Gly α-CH$_2$), 32.1, 30.3, 29.9, 29.8, 29.6, 29.5, 29.4, 29.2, 29.0, 25.8, 24.9 (13 Pal CH$_2$), 23.1 (Leu γ-CH), 22.9 (Leu ω-CH$_3$), 21.6 (Pal CH$_2$), 21.1 (Leu ω-CH$_3$), 14.2 (Pal ω-CH$_3$); HRMS (FAB, 3-NBA/TFA 10:1): m/z: calcd for $[M+H]^+$ 798.4363; found: 798.4276; $C_{43}H_{63}N_3O_9S$ (798.05).

Z-Gln(Trt)-Gly-O*t*Bu: HOBt (562 mg, 3.67 mmol) was added to a solution of Z-Gln(Trt)-OH (1.60 mg, 3.06 mmol) and *p*TosOH · H-Gly-O*t*Bu (928 mg, 3.06 mmol) in CH_2Cl_2 (80 mL) and at 0 °C NEt_3 (424 µL, 3.06 mmol) and finally EDC (645 mg, 3.36 mmol). The mixture was stirred at 20 °C for 16 h and the solvent was washed with HCl (0.5 N, 3 × 20 mL), $NaHCO_3$ (saturated, 3 × 20 mL), water (3 × 20 mL), and brine (20 mL). The organic layer was dried with $MgSO_4$ and concentrated under reduced pressure. The product was isolated from the residue by flash chromatography on silica gel using ethyl acetate/*n*-hexane 1:1 (*v*/*v*) as eluent to yield a colorless solid (1.43 g, 74 %). M.p. 165 °C; $[\alpha]_D^{22} = +16.2$ ($c = 1.0$ in $CHCl_3$); $R_f = 0.34$ (ethyl acetate/*n*-hexane 1:1 *v*/*v*); ^1H NMR (500 MHz, $CDCl_3$): $\delta = 7.35 - 7.17$ (m, 21 H; CONH, Z, Trt), 6.40 (brs, 1 H; CONH), 5.83 (brs, 1 H; OCONH), 5.04 (s, 2 H; CCH_2O), 4.13 – 4.09 (m, 1 H; Gln α-CH), 3.83 – 3.79 (m, 1 H; Gly α-CH_{2a}), 3.72 – 3.68 (m, 1 H; Gly α-CH_{2b}), 2.62 – 2.52 (m, 2 H; Gln γ-CH_2), 2.08 – 2.04 (m, 2 H; Gln β-CH_2), 1.38 (s, 9 H; $C(CH_3)_3$); ^{13}C NMR (125.7 MHz, $CDCl_3$): $\delta = 172.3$, 171.5, 169.0 (3 C=O), 156.2 (OCONH), 144.5 (arom. q), 136.3 (arom. q), 128.5, 128.2, 128.0, 127.0 (20 arom. CH), 82.2 (*t*Bu q), 70.4 (Trt q), 66.9 (CH_2O), 54.3 (Gln α-CH), 41.8 (Gly α-CH_2), 33.0 (Gln γ-CH_2), 28.4 (Gln β-CH_2), 28.0 (*t*Bu); HRMS (EI, 70 eV, 175 °C): *m*/*z*: calcd for $[M]^+$ 635.2995; found: 635.3015; elemental analysis calcd (%) for $C_{38}H_{41}N_3O_6$ (635.76): C 71.79, H 6.50, N 6.61; found: C 71.70, H 6.48, N 6.54.

H-Gln(Trt)-Gly-O*t*Bu: Palladium on charcoal (10 %, 33 mg) was added to a solution of Z-Gln(Trt)-Gly-O*t*Bu (671 mg, 1.06 mmol) in methanol (30 mL) and the mixture was stirred under a hydrogen atmosphere at 20 °C for 16 h. Then the catalyst was filtered off and the solvent was removed under reduced pressure to yield a colorless solid (534 mg (quant.). $[\alpha]_D^{22} = +10.6$ ($c = 0.5$ in methanol); ^1H NMR (500 MHz, CD_3OD): $\delta = 7.27 - 7.18$ (m, 15 H; Trt), 6.40 (brs, 1 H; CONH), 3.88 (d, $^2J = 17.6$ Hz, 1 H; Gly α-CH_{2a}), 3.77 (d, $^2J = 17.6$ Hz, 1 H; Gly α-CH_{2b}), 3.35 – 3.32 (m, 1 H; Gln α-CH), 2.48 – 2.44 (m, 2 H; Gln γ-CH_2), 1.96 – 1.90 (m, 1 H; Gln β-CH_{2a}), 1.84 – 1.78 (m, 1 H; Gln β-CH_{2b}), 1.45 (s, 9 H; $C(CH_3)_3$); ^{13}C NMR (125.7 MHz, $CDCl_3$): $\delta = 177.1$, 174.7, 170.5 (3 C=O), 146.0 (arom. q), 130.0, 128.7, 127.8 (15 arom. CH), 83.0 (*t*Bu q), 71.5 (Trt q), 55.2 (Gln α-CH), 42.7 (Gly α-CH_2), 33.7 (Gln γ-CH_2), 31.9 (Gln β-CH_2), 28.3 (*t*Bu); HRMS (EI, 70 eV, 155 °C): *m*/*z*: calcd for $[M]^+$ 501.2628; found: 501.2609; elemental analysis calcd (%) for $C_{30}H_{35}N_3O_4 \cdot 0.5\ H_2O$: C 70.57, H 7.11, N 8.23; found: C 70.68, H 7.02, N 8.31; $C_{30}H_{35}N_3O_4$ (501.62).

Z-Lys(Boc)-Gln(Trt)-Gly-O*t*Bu: HOBt (196 mg, 1.28 mmol) was added to a solution of Z-Lys(Boc)-OH (404 mg, 1.06 mmol) and H-Gln(Trt)-Gly-O*t*Bu (534 mg, 1.06 mmol) in CH_2Cl_2 (70 mL) and at 0 °C EDC (225 mg, 1.17 mmol). The mixture was stirred at 20 °C for 16 h and the solvent was extracted with HCl (0.5 N, 3 × 20 mL), $NaHCO_3$ (saturated, 3 × 20 mL), water (3 × 20 mL) and brine (20 mL). The organic layer was dried with $MgSO_4$ and concentrated under reduced pressure. The product was isolated from the residue by flash chromatography on silica gel using ethyl acetate/*n*-hexane 3:2 (*v*/*v*) as eluent to yield a colorless solid (826 mg, 90 %). M.p. 89 °C; $[\alpha]_D^{22} = -21.2$ ($c = 1.0$ in $CHCl_3$); $R_f = 0.10$ (ethyl acetate/*n*-hexane 2:1 *v*/*v*); ^1H NMR (500 MHz, $CDCl_3$): $\delta = 7.41$ (d, $^3J = 6.7$ Hz, 1 H; CONH), 7.32 – 7.20 (m, 20 H; Z, Trt), 7.06 (brs, 2 H; 2 CONH), 5.52 (d, $^3J = 6.8$ Hz, OCONH), 5.05 (d, $^2J = 12.2$ Hz, 1 H; $CCH_{2a}O$), 4.94 (d, $^2J = 12.2$ Hz, 1 H; $CCH_{2b}O$), 4.75 (brs, 1 H; OCONH), 4.31 (m, 1 H; Lys α-CH), 4.06 (d, $^3J = 4.2$ Hz, 1 H; Gln α-CH), 3.87 (dd, $^2J = 17.9$, $^3J = 6.0$ Hz, 1 H; Gly α-CH_{2a}), 3.70 (dd, $^2J = 18.0$, $^3J = 5.0$ Hz, 1 H; Gly α-CH_{2b}), 3.05 (brs, 2 H; Lys ε-CH_2), 2.56 – 2.44 (m, 2 H; Gln γ-CH_2), 2.04 – 1.98 (m, 2 H; Gln β-CH_2), 1.76 – 1.74 (m, 1 H; Lys β-CH_{2a}), 1.57 – 1.54 (m, 1 H; Lys β-CH_{2b}), 1.42 (brs, 20 H; Lys δ-CH_2, 2 $C(CH_3)_3$), 1.33 – 1.24 (m, 2 H; Lys γ-CH_2); ^{13}C NMR (125.7 MHz, $CDCl_3$): $\delta = 172.3$, 171.9, 171.2, 168.6 (4 C=O), 156.3, 156.1 (2 OCONH), 144.4 (arom. q), 136.2 (arom. q), 128.6, 128.4, 128.1, 128.0, 127.0 (20 arom. CH), 81.9, 79.0 (2 *t*Bu q), 70.6 (Trt q), 66.9 (CH_2O), 55.0 (Gln α-CH), 52.6 (Lys α-CH), 41.9 (Gly α-CH_2), 39.8 (Lys ε-CH_2), 33.3 (Gln γ-CH_2), 32.0, 29.4 (2 Lys CH_2), 28.4 (*t*Bu), 28.3 (Gln β-CH_2), 28.0 (*t*Bu), 22.4 (Lys CH_2); HRMS (FAB, 3-NBA/TFA 10:1): *m*/*z*: calcd for $[M+H]^+$ 864.4548; found: 864.4493; elemental analysis calcd (%) for $C_{49}H_{61}N_5O_9$ (864.06): C 68.11, H 7.12, N 8.11; found: C 67.85, H 7.01, N 8.02.

H-Lys(Boc)-Gln(Trt)-Gly-O*t*Bu (26): Palladium on charcoal (10 %, 12 mg) was added to a solution of Z-Lys(Boc)-Gln(Trt)-Gly-O*t*Bu (120 mg, 0.14 mmol) in methanol (15 mL) and the mixture was stirred under a hydrogen atmosphere at 20 °C for 16 h. Then the catalyst was filtered off and the solvent was removed under reduced pressure. The product **26** was isolated from the residue by flash chromatography on silica gel using ethyl acetate/*n*-hexane 2:1 (*v*/*v*) and then ethyl acetate/ethanol 1:1 (*v*/*v*) as eluent to yield a colorless solid (88 mg, 87 %). M.p. 96 °C; $[\alpha]_D^{22} = -8.4$ ($c = 1.0$ in methanol); ^1H NMR (500 MHz, CD_3OD): $\delta = 7.30 - 7.18$ (m, 15 H; Trt), 5.52 (d, $^3J = 6.8$ Hz, OCONH), 5.05 (d, $^2J = 12.2$ Hz, 1 H; $CCH_{2a}O$), 4.94 (d, $^2J = 12.2$ Hz, 1 H; $CCH_{2b}O$), 4.75 (brs, 1 H; OCONH), 4.42 – 4.40 (m, 1 H; Lys α-CH), 3.92 (d, $^2J = 17.6$ Hz, 1 H; Gly α-CH_{2a}), 3.85 – 3.77 (m, 1 H; Gln α-CH), 3.73 (d, $^2J = 17.4$ Hz, Gly α-CH_{2b}), 3.03 – 3.00 (m, 2 H; Lys ε-CH_2), 2.57 – 2.48 (m, 2 H; Gln γ-CH_2), 2.10 – 2.05 (m, 1 H; Gln β-CH_{2a}), 1.97 – 1.91 (m, 1 H; Gln β-CH_{2b}), 1.86 – 1.76 (m, 2 H; Lys β-CH_2), 1.44 (brs, 22 H; 2 $C(CH_3)_3$, Lys δ-CH_2, Lys γ-CH_2); ^{13}C NMR (125.7 MHz, CD_3OD): $\delta = 174.3$, 173.4, 170.7, 170.2 (4 C=O), 158.4 (OCONH), 145.8 (arom. q), 129.9, 128.7, 127.8 (15 arom. CH), 82.9, 79.8 (2 *t*Bu q), 71.5 (Trt q), 54.4 (Gln α-CH), 54.1 (Lys α-CH), 42.7 (Gly α-CH_2), 40.8 (Lys ε-CH_2), 33.6 (Gln γ-CH_2), 32.4, 30.3 (2 Lys CH_2), 29.1 (Gln β-CH_2), 28.8 (*t*Bu), 28.3 (*t*Bu), 22.9 (Lys CH_2); HRMS (EI, 70 eV, 220 °C): *m*/*z*: calcd for $[M]^+$ 729.4101; found: 729.4093; $C_{41}H_{55}N_5O_7$ (729.91).

Z-Lys(Boc)-Gln-OH: *N*-Hydroxysuccinimide (231 mg, 2.0 mmol) and DCC (496 mg, 2.4 mmol) were added at 0 °C to a solution of Z-Lys(Boc)-OH (760 mg, 2.0 mmol) in tetrahydrofuran (30 mL) and the mixture was stirred at 20 °C for 16 h. The precipitated urea was filtered off and the solvent was removed under reduced pressure. The remaining *N*-hydroxysuccinimide ester was dissolved in dioxane (5 mL) and at 0 °C added dropwise to a suspension of H-Gln-OH (439 mg, 3.0 mmol) in NaOH (2 N, 1.5 mL, 3.0 mmol)/water (10 mL)/dioxane (5 mL). The mixture was stirred at 0 °C for 3 h and at 20 °C for 5 d. Then the dioxane was removed under reduced pressure and the solution was extracted several times with diethyl ether. At 0 °C the pH of the aqueous layer was adjusted to pH 2 with HCl (2 N) and extracted with ethyl acetate. The combined ethyl acetate extracts were dried with $MgSO_4$ and the solvent was removed under reduced pressure to yield a colorless solid (945 mg, 93 %). M.p. 147 °C; $[\alpha]_D^{22} = -5.0$ ($c = 1.0$ in $CHCl_3$/MeOH 1:1 *v*/*v*); ^1H NMR (500 MHz, CD_3OD): $\delta = 7.36 - 7.20$ (m, 5 H; C_6H_5), 5.08 (d, $^2J = 3.0$ Hz, 1 H; CCH_2O), 4.36 (t, $^3J = 4.1$ Hz, 1 H; Lys α-CH), 4.09 (dd, $^3J_1 = 8.3$, $^3J_2 = 5.3$ Hz, 1 H; Gln α-CH), 3.02 (t, $^3J = 6.4$ Hz, 2 H; Lys ε-CH_2), 2.33 – 2.22 (m, 3 H; Gln γ-CH_2, Gln β-CH_{2a}), 1.97 – 1.90 (m, 1 H; Gln β-CH_{2b}), 1.80 – 1.78 (m, 1 H; Lys β-CH_{2a}), 1.66 – 1.64 (m, 1 H; Lys β-CH_{2b}), 1.53 – 1.42 (m, 13 H; Lys δ-CH_2, Lys γ-CH_2, $C(CH_3)_3$); ^{13}C NMR (125.7 MHz, CD_3OD): $\delta = 177.9$, 175.3, 174.7 (3 C=O), 158.5, 158.4 (2 OCONH), 138.1 (arom. q), 129.4, 128.9, 128.7 (3 arom. CH), 79.8 (*t*Bu q), 67.6 (CH_2O), 56.4 (Lys α-CH), 53.6 (Gln α-CH), 41.3 (Gly α-CH_2), 40.9 (Lys ε-CH_2), 33.1 (Gln γ-CH_2), 32.7, 30.5 (2 Lys CH_2), 29.0 (Gln β-CH_2), 28.8 (*t*Bu), 24.0 (Lys CH_2); HRMS (FAB, 3-NBA/TFA 10:1): *m*/*z*: calcd for $[M+H]^+$ 509.2611; found: 509.2650; elemental analysis calcd (%) for $C_{24}H_{36}N_4O_8 \cdot H_2O$: C 54.74, H 7.27, N 10.64; found: C 54.47, H 6.74, N 10.26; $C_{24}H_{36}N_4O_8$ (508.57).

Z-Lys(Boc)-Gln-Gly-O*t*Bu: HOBt (144 mg, 0.94 mmol) and NEt_3 (108 µL, 0.78 mmol) were added to a solution of Z-Lys(Boc)-Gln-OH (397 mg, 0.78 mmol) and HCl · H-Gly-O*t*Bu (131 mg, 0.78 mmol) in dimethylformamide (2.5 mL) and CH_2Cl_2 (70 mL) was added and at 0 °C EDC (165 mg, 0.86 mmol). The mixture was stirred at 20 °C for 16 h. After addition of CH_2Cl_2 (30 mL) the solvent was extracted with HCl (0.5 N, 3 × 15 mL), $NaHCO_3$ (1 N, 3 × 15 mL), water (3 × 15 mL) and brine (20 mL). The precipitated product was filtered off and washed with water, CH_2Cl_2 and diethyl ether, the organic layer was dried with $MgSO_4$ and concentrated under reduced pressure to yield a colorless solid (406 mg, 84 %). M.p. 163 °C; $[\alpha]_D^{22} = -7.6$ ($c = 1.0$ in $CHCl_3$/methanol 1:1 *v*/*v*); ^1H NMR (500 MHz, $CDCl_3$/CD_3OD 1:1 *v*/*v*): $\delta = 7.36 - 7.29$ (m, 5 H; C_6H_5), 5.09 (d, $^2J = 4.8$ Hz, 1 H; CCH_2O), 4.42 (dd, $^3J_1 = 8.7$, $^3J_2 = 4.7$ Hz, 1 H; Lys α-CH), 4.07 (dd, $^3J_1 = 8.0$, $^3J_2 = 5.2$ Hz, Gln α-CH), 3.86 (d, $^3J = 3.5$ Hz, 2 H; Gly α-CH_2), 3.05 (t, $^3J = 5.9$ Hz, 2 H; Lys ε-CH_2), 2.38 – 2.28 (m, 2 H; Gln γ-CH_2), 2.19 – 2.15 (m, 1 H; Gln β-CH_{2a}), 2.00 – 1.96 (m, 1 H; Gln β-CH_{2b}), 1.82 – 1.76 (m, 1 H; Lys β-CH_{2a}), 1.69 – 1.66 (m, 1 H; Lys β-CH_{2b}), 1.53 – 1.34 (m, 22 H; Lys δ-CH_2, Lys γ-CH_2, 2 $C(CH_3)_3$); ^{13}C NMR (125.7 MHz, $CDCl_3$/CD_3OD 1:1): $\delta = 177.1$, 174.0, 172.7, 169.5 (4 C=O), 157.8, 157.5 (2 OCONH), 136.8 (arom. q), 128.9, 128.5, 128.4, 128.2 (5 arom. CH), 82.6, 79.5 (2 *t*Bu q), 67.4 (CH_2O), 56.0 (Lys α-CH), 53.1 (Gln α-CH), 42.2 (Gly α-CH_2), 40.2 (Lys ε-CH_2), 31.8 (Gln γ-CH_2), 29.7 (2 Lys CH_2), 28.6 (*t*Bu), 28.1 (*t*Bu), 28.0 (Gln β-CH_2), 23.2 (Lys CH_2); HRMS (FAB, 3-NBA/TFA 10:1): *m*/*z*: calcd for $[M+H]^+$ 622.3452; found: 622.3479; elemental analysis calcd (%) for $C_{30}H_{47}N_5O_9 \cdot H_2O$: C 56.32, H 7.72, N 10.95; found: C 56.10, H 7.24, N 10.66; $C_{30}H_{47}N_5O_9$ (621.73).

© WILEY-VCH Verlag GmbH, D-69451 Weinheim, 2001
0947-6539/01/0713-2950 $ 17.50+.50/0 *Chem. Eur. J.* **2001**, *7*, No. 13

H-Lys(Boc)-Gln-Gly-O*t*Bu (27): Palladium on charcoal (10%, 6 mg) was added to a solution of Z-Lys(Boc)-Gln-Gly-O*t*Bu (63 mg, 0.10 mmol) in methanol (10 mL) and the mixture was stirred under a hydrogen atmosphere at 20 °C for 16 h. Then the catalyst was filtered off and the solvent was removed under reduced pressure to yield a colorless solid (49 mg, 100%). M.p. 81 °C; $[\alpha]_D^{22} = -12.5$ ($c = 1.0$ in methanol); ^{1}H NMR (500 MHz, CD_3OD): $\delta = 4.43$ (dd, $^3J_1 = 8.4$ Hz, $^3J_2 = 5.6$ Hz, 1H; Lys α-CH), 3.92 (d, $^2J = 17.5$ Hz, 1H; Gly α-CH_{2a}), 3.77 (d, $^2J = 17.5$ Hz, 1H; Gly α-CH_{2b}), 3.55 (t, $^3J = 6.4$ Hz, 1H; Gln α-CH), 3.04 (t, $^3J = 6.9$ Hz, 2H; Lys ε-CH_2), 2.39–2.32 (m, 2H; Gln γ-CH_2), 2.17–2.10 (m, 1H; Gln β-CH_{2a}), 2.01–1.92 (m, 1H; Gln β-CH_{2b}), 1.80–1.74 (m, 1H; Lys β-CH_{2a}), 1.70–1.63 (m, 1H; Lys β-CH_{2b}), 1.52–1.33 (m, 22H; Lys δ-CH_2, Lys γ-CH_2, 2 $C(CH_3)_3$); ^{13}C NMR (125.7 MHz, CD_3OD): $\delta = 177.8$, 173.8, 170.2 (3 C=O), 158.5 (OCONH), 82.9, 79.9 (2 *t*Bu q), 55.3 (Lys α-CH), 53.9 (Gln α-CH), 42.7 (Gly α-CH_2), 41.0 (Lys ε-CH_2), 34.5 (Gln γ-CH_2), 32.4, 30.6 (2 Lys CH_2), 29.1 (Gln β-CH_2), 28.8 (*t*Bu), 28.3 (*t*Bu), 23.5 (Lys CH_2); HRMS (FAB, 3-NBA/TFA 10:1): *m*/*z*: calcd for $[M+H]^+$ 488.3084; found: 488.3052; $C_{22}H_{41}N_5O_7$ (487.59).

PhAcOZ-Leu-Cys(Pal)-Gly-Lys(Boc)-Gln(Trt)-Gly-O*t*Bu (5): HOBt (20 mg, 126 µmol), EDC (22 mg, 116 µmol) and H-Lys(Boc)-Gln(Trt)-Gly-O*t*Bu (**26**, 77 mg, 105 µmol) were added to a solution of PhAcOZ-Leu-Cys(Pal)-Gly-OH (**25**, 84 mg, 105 µmol) in CH_2Cl_2 (8 mL) were added at 0 °C. The mixture was stirred at 20 °C for 16 h. After addition of CH_2Cl_2 (30 mL) the solvent was extracted with HCl (0.5 N, 3 × 15 mL), water (3 × 15 mL) and brine (15 mL). The organic layer was dried with $MgSO_4$ and concentrated under reduced pressure. The product **5** was isolated from the residue by size-exclusion chromatography on Sephadex LH-20 using ethyl $CHCl_3$/methanol 1:1 (*v*/*v*) as eluent to yield a colorless solid (98 mg, 62%). $[\alpha]_D^{22} = -7.2$ ($c = 1.0$ in $CHCl_3$); $R_f = 0.60$ ($CHCl_3$/methanol 10:1 *v*/*v*); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.48$ (brs, 1H; CONH), 7.41 (brs, 1H; CONH), 7.36–7.18 (m, 23H; 3 CONH; Z, Trt, 2 CH), 7.01 (d, $^3J = 8.4$ Hz, 2H; 2 OCCH), 6.92 (brs, 1H; CONH), 6.31 (brs, 1H; OCONH), 5.04 (s, 2H; CCH_2O), 4.74 (brs, 1H; OCONH), 4.61 (d, $^3J = 6.5$ Hz, 1H; Cys α-CH), 4.24 (brs, 1H; Lys α-CH), 4.03 (brs, 1H; Leu α-CH), 3.92–3.84 (m, 5H; Gln α-CH, Gly α-CH_2, $CCH_2C(O)$), 3.71–3.57 (m, 2H; Gly α-CH_2), 3.04–2.93 (m, 4H; Lys ε-CH_2, Cys β-CH_2), 2.57–2.51 (m, 3H; Pal α-CH_2, Gln γ-CH_{2a}), 2.42–2.40 (m, 2H; Gln γ-CH_{2b}), 2.11–2.01 (m, 2H; Gln β-CH_2), 1.86–1.54 (m, 7H; Lys β-CH_2, Pal β-CH_2, Leu γ-CH, Leu β-CH_2), 1.49–1.40 (brs, 22H; 2 $C(CH_3)_3$, Lys δ-CH_2, Lys γ-CH_2), 1.30–1.25 (brs, 24H; Pal $(CH_2)_{12}$), 0.95–0.91 (m, 6H; 2 Leu ω-CH_3), 0.87 (t, $^3J = 6.9$ Hz, 3H; Pal ω-CH_3); ^{13}C NMR (125.7 MHz, $CDCl_3$): $\delta = 202.2$ (C=OS), 173.9, 172.5, 172.0, 171.6, 170.7, 170.4, 170.1, 168.9 (8 C=O), 156.8, 156.2 (2 OCONH), 150.7 (arom. C=O), 144.6 (arom. q), 133.7 (arom. q), 133.3 (arom. q), 129.4, 129.3, 128.8, 128.7, 128.0, 127.4, 127.1, 121.7 (24 arom. CH), 81.8, 79.1 (2 *t*Bu q), 70.5 (Trt q), 66.6 (CH_2O), 56.0, 54.3, 54.1 (Cys α-CH, Gln α-CH, Lys α-CH), 52.9 (Leu α-CH), 44.0 (Cys β-CH_2), 43.6, 42.1, 41.4, 40.3 (CH_2, Leu β-CH_2, 2 Gly α-CH_2), 39.9 (Lys ε-CH_2), 33.7 (Gln γ-CH_2), 31.9, 30.4, 29.7, 29.6, 29.5, 29.4, 29.3, 29.0 (13 Pal CH_2, 2 Lys CH_2, Gln β-CH_2), 28.4 (*t*Bu), 28.1 (*t*Bu), 27.5 (Pal CH_2), 25.7 (Lys CH_2), 24.7 (Leu γ-CH), 23.1 (Leu ω-CH_3), 22.7 (Pal CH_2), 21.5 (Leu ω-CH_3), 14.2 (Pal ω-CH_3); FAB-MS (FAB, 3-NBA/TFA 10:1): *m*/*z*: 1509.9 $[M+H]^+$, 1532.9 $[M+Na]^+$; elemental analysis calcd (%) for $C_{84}H_{116}N_8O_{15}S$ (1509.95): C 66.82, H 7.74, N 7.42; found: C 66.89, H 7.70, N 7.41.

PhAcOZ-Leu-Cys(Pal)-Gly-Lys(Boc)-Gln-Gly-O*t*Bu (28): HOBt (18 mg, 120 µmol) and EDC (20 mg, 110 µmol) were added at 0 °C to a solution of PhAcOZ-Leu-Cys(Pal)-Gly-OH (**25**, 80 mg, 100 µmol) and H-Lys(Boc)-Gln-Gly-O*t*Bu (**27**, 70 mg, 106 µmol) in $CHCl_3$/2,2,2-trifluoroethanol 3:1 (*v*/*v*) (4 mL) and the mixture was stirred at 20 °C for 16 h. Then the solvent was removed under reduced pressure and the product **28** was isolated from the residue by size flash chromatography on silica gel using $CHCl_3$/methanol 50:1 to 1:1 (*v*/*v*) as eluent to yield a colorless solid (40 mg, 32%). M.p. 194–195 °C (decomp); $[\alpha]_D^{22} = -21.5$ ($c = 0.4$ in $CHCl_3$/methanol 1:1 *v*/*v*); $R_f = 0.35$ ($CHCl_3$/methanol 10:1 *v*/*v*); ^{1}H NMR (500 MHz, $CDCl_3$/CD_3OD 1:1 *v*/*v*): $\delta = 7.40$–7.29 (m, 7H; C_6H_5, 2 CH), 7.06 (d, $^3J = 8.5$ Hz, 2H; 2 OCCH), 6.06 (brs, 1H; OCONH), 5.04 (d, $J = 4.4$ Hz, 2H; CCH_2O), 4.39–4.36 (m, 2H; Cys α-CH, Lys α-CH), 4.25 (dd, $^3J_1 = 8.1$, $^3J_2 = 5.0$ Hz, 1H; Leu α-CH), 4.11 (dd, $^3J_1 = 8.7$, $^3J_2 = 6.3$ Hz, 1H; Gln α-CH), 4.02 (d, $^2J = 16.9$ Hz, 1H; Gly α-CH_{2a}), 3.89 (s, 2H; $CCH_2C(O)$), 3.85 (s, 2H; Gly α-CH_2), 3.79 (d, $^2J = 16.8$ Hz, 1H; Gly α-CH_{2b}), 3.42 (dd, $^2J = 14.3$, $^3J = 4.4$ Hz, 1H; Cys β-CH_{2a}), 3.21 (dd, $^2J = 14.2$, $^3J = 9.0$ Hz, 1H; Cys β-CH_{2b}), 3.05–3.02 (m, 2H; Lys ε-CH_2), 2.57 (t, $^3J = 7.5$ Hz, 2H; Pal α-CH_2), 2.40–2.30 (m, 2H; Gln γ-CH_2), 2.19–2.13 (m, 1H; Gln β-CH_{2a}), 2.06–2.00 (m, 1H; Gln β-CH_{2b}), 1.87–1.84 (m, 1H; Lys β-CH_{2a}), 1.76–1.67 (m, 2H; Lys β-CH_{2b}, Leu γ-CH), 1.65–1.62 (m, 2H; Pal β-CH_2), 1.61–1.55 (m, 2H; Leu β-CH_2), 1.49–1.38 (2 brs, 22H; 2 $C(CH_3)_3$, Lys δ-CH_2, Lys γ-CH_2), 1.36–1.24 (brs, 24H; Pal $(CH_2)_{12}$), 0.96 (d, $^3J = 6.6$ Hz, 3H; Leu ω-CH_3), 0.94 (d, $^3J = 6.5$ Hz, 3H; Leu ω-CH_3), 0.89 (t, $^3J = 7.0$ Hz, 3H; Pal ω-CH_3); ^{13}C NMR (125.7 MHz, $CDCl_3$/CD_3OD): $\delta = 201.1$ (C=OS), 177.4, 175.3, 173.4, 173.0, 172.0, 171.5, 171.3, 169.6 (8 C=O), 158.1, 157.7 (2 OCONH), 151.3 (arom. C=O), 134.9 (arom. q), 134.1 (arom. q), 129.9, 129.7, 129.3, 128.0, 122.2 (9 arom. CH), 82.6 (*t*Bu q), 79.6 (*t*Bu q), 67.0 (CH_2O), 55.1, 54.9, 54.8 (Cys α-CH, Gln α-CH, Lys α-CH), 53.6 (Leu α-CH), 44.5 (Cys β-CH_2), 43.5, 42.5, 41.7, 41.2 (CH_2, Leu β-CH_2, 2 Gly α-CH_2), 40.6 (Lys ε-CH_2), 32.5 (Gln γ-CH_2), 31.9, 31.3, 30.3, 30.2, 30.1, 30.0, 29.9, 29.6 (13 Pal CH_2, 2 Lys CH_2, Gln β-CH_2), 28.7 (*t*Bu), 28.3 (*t*Bu), 27.9 (Pal CH_2), 26.2 (Lys CH_2), 25.3 (Leu γ-CH), 23.7 (Pal CH_2), 23.3 (Leu ω-CH_3), 23.2 (Pal CH_2), 21.9 (Leu ω-CH_3), 14.3 (Pal ω-CH_3); MS (FAB, 3-NBA/TFA 10:1): *m*/*z*: 1111.6 $[M - \text{Boc} - t\text{Bu} + 3H]^+$, 1167.6 $[M - \text{Boc} + 2H]^+$, 1267.6 $[M+H]^+$, 1289.6 $[M+Na]^+$; $C_{65}H_{102}N_8O_{15}S$ (1267.65).

Boc-Leu-Gly-OAll (29): HOBt (3.37 g, 22 mmol), NEt_3 (2.78 mL, 20 mmol) were added to a solution of Boc-Leu-OH (4.63 g, 20 mmol) and *p*TosOH · H-Gly-OAll (**20**, 5.75 g, 20 mmol) in CH_2Cl_2 (100 mL) was added and at 0 °C DIC (3.12 mL, 20 mmol). The mixture was stirred at 20 °C for 16 h, then solvent was extracted with acetic acid (5%, 3 × 100 mL), $NaHCO_3$ (1 N, 3 × 100 mL) and water (3 × 100 mL). The organic layer was dried with $MgSO_4$ and concentrated under reduced pressure. The product **29** was isolated from the residue by flash chromatography on silica gel using ethyl acetate/*n*-hexane 1:2 (*v*/*v*) as eluent to yield a colorless oil (5.75 g, 88%). $[\alpha]_D^{22} = -27.4$ ($c = 1.0$ in CH_2Cl_2); $R_f = 0.29$ (ethyl acetate/*n*-hexane 1:2 *v*/*v*); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 6.80$ (brs, 1H; CONH), 5.86 (ddt, $^3J_{trans} = 16.3$, $^3J_{cis} = 10.5$, $^3J_{vic} = 5.7$ Hz, 1H; allyl CH=), 5.32 (dd, $^3J_{trans} = 17.1$, $^2J = 1.5$ Hz, 1H; allyl=CH_{2a}), 5.26 (dd, $^3J_{cis} = 10.3$, $^2J = 1.2$ Hz, 1H; allyl=CH_{2b}), 5.00 (d, $^3J = 6.7$ Hz, 1H; OCONH), 4.64 (d, $^3J = 5.8$ Hz, 2H; allyl OCH_2), 4.20 (brs, 1H; Leu α-CH), 4.06 (t, $^3J = 5.2$ Hz, 2H; Gly α-CH_2), 1.70–1.66 (m, 2H; Leu γ-CH, Leu β-CH_{2a}), 1.52–1.47 (m, 1H; Leu β-CH_{2b}), 1.44 (s, 9H; $C(CH_3)_3$), 0.94 (d, $^3J = 6.1$ Hz, 6H; 2 Leu ω-CH_3); ^{13}C NMR (125.7 MHz, $CDCl_3$): $\delta = 173.0$, 169.4 (2 C=O), 155.8 (OCONH), 131.5 (allyl CH), 119.0 (allyl CH_2), 80.1 (*t*Bu q), 66.0 (allyl OCH_2), 52.9 (Leu α-CH), 41.3 (Leu β-CH_2, Gly α-CH_2), 28.3 (*t*Bu), 24.7 (Leu γ-CH), 23.0 (Leu ω-CH_3), 21.9 (Leu ω-CH_3); HRMS (EI, 70 eV, 90 °C): *m*/*z*: calcd for $[M]^+$ 328.1998; found: 328.1980; $C_{16}H_{28}N_2O_5$ (328.41).

Boc-Leu-Gly-OH (30): A catalytic amount of $[Pd(PPh_3)_4]$ and morpholine (800 µL, 9.2 mmol) were added to a solution of Boc-Leu-Gly-OAll (**29**, 2.71 g, 8.24 mmol) in CH_2Cl_2 (40 mL) under argon, and the mixture was stirred at 20 °C for 16 h. The solvent was evaporated under reduced pressure, and the residue was dissolved in $NaHCO_3$ (half saturated, 30 mL) and extracted with CH_2Cl_2 (5 × 10 mL). At 0 °C the solution was acidified to pH 2 with HCl (2 N) and extracted with ethyl acetate (3 × 10 mL). The combined organic layers were dried with $MgSO_4$ and concentrated under reduced pressure to yield a colorless solid (2.35 g, 99%). M.p. 106 °C; $[\alpha]_D^{22} = -18.6$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (500 MHz, CD_3OD): $\delta = 4.11$ (dd, $^3J = 10.0$, $^3J = 4.8$ Hz, 1H; Leu α-CH), 3.95 (dd, $^2J = 17.8$, $^3J = 4.3$ Hz, 1H; Gly α-CH_{2a}), 3.86 (dd, $^2J = 17.8$, $^3J = 4.7$ Hz, 1H; Gly α-CH_{2b}), 1.74–1.68 (m, 1H; Leu γ-CH), 1.61–1.47 (m, 2H; Leu β-CH_2), 1.44 (s, 9H; $C(CH_3)_3$), 0.95 (d, $^3J = 6.6$ Hz, 3H; Leu ω-CH_3), 0.93 (d, $^3J = 6.6$ Hz, 3H; Leu ω-CH_3); ^{13}C NMR (125.7 MHz, CD_3OD): $\delta = 176.1$, 172.6 (2 C=O), 157.8 (OCONH), 80.6 (*t*Bu q), 54.4 (Leu α-CH), 42.2, 41.7 (Leu β-CH_2, Gly α-CH_2), 28.7 (*t*Bu), 25.8 (Leu γ-CH), 23.5 (Leu ω-CH_3), 21.9 (Leu ω-CH_3); HRMS (EI, 70 eV, 135 °C): *m*/*z*: calcd for $[M+H]^+$ 288.1685; found: 288.1678; elemental analysis calcd (%) for $C_{13}H_{24}N_2O_5$ (288.35): C 54.15, H 8.39, N 9.72; found: C 54.08, H 8.21, N 9.52.

HCl · H-Leu-Gly-OAll (31): A saturated solution of HCl in diethyl ether (120 mL) was added at 0 °C to a solution of Boc-Leu-Gly-OAll (**29**, 2.71 g, 8.24 mmol) in CH_2Cl_2 (40 mL) and the mixture was stirred at 0 °C for 30 min and at 20 °C for 2 h. Then the excess HCl and the solvent was removed under reduced pressure to yield a colorless, hygroscopic solid (2.15 g, 98%). $[\alpha]_D^{22} = +33.1$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (500 MHz, CD_3OD): $\delta = 5.93$ (ddt, $^3J_{trans} = 16.1$, $^3J_{cis} = 10.5$, $^3J_{vic} = 5.7$ Hz, 1H; allyl CH=), 5.34 (dd, $^3J_{trans} = 17.2$, $^2J = 1.5$ Hz, 1H; allyl=CH_{2a}), 5.24 (dd, $^3J_{cis} = 10.5$, $^2J = 1.3$ Hz, 1H; allyl=CH_{2b}), 4.64 (dd, $^3J = 5.6$, $^2J = 2.8$ Hz, 2H; allyl OCH_2), 4.12 (d, $^2J = 17.6$ Hz, 1H; Gly α-CH_{2a}), 3.99–3.91 (m, 2H; Gly α-CH_{2b}, Leu α-CH), 1.82–1.77 (m, 2H; Leu γ-CH, Leu β-CH_{2a}), 1.75–1.67

(m, 1H; Leu β-CH_{2b}), 1.03–1.00 (m, 6H; 2Leu ω-CH_3); ^{13}C NMR (125.7 MHz, CD_3OD): δ = 171.2, 170.5 (2C=O), 133.2 (allyl CH), 118.8 (allyl CH_2), 66.9 (allyl OCH_2), 52.9 (Leu α-CH), 41.9, 41.7 (Leu β-CH_2, Gly α-CH_2), 25.3 (Leu γ-CH), 23.0 (Leu ω-CH_3), 22.3 (Leu ω-CH_3); HRMS (EI, 70 eV, 105 °C): *m*/*z*: calcd for $[M-HCl]^+$ 228.1474; found: 228.1461; $C_{11}H_{21}ClN_2O_3$ (264.75).

Boc-Leu-Gly-Leu-Gly-OAll (32): HOBt (1.38 g, 9.0 mmol), NEt_3 (1.04 mL, 7.5 mmol) were added to a solution of Boc-Leu-Gly-OH (**30**, 2.16 g, 7.5 mmol) and HCl·H-Leu-Gly-OAll (**31**, 1.99 g, 7.5 mmol) in CH_2Cl_2 (80 mL) was added and at 0 °C EDC (1.58 g, 8.25 mmol). The mixture was stirred at 20 °C for 16 h, then solvent was extracted with HCl (0.5 N, 3 × 20 mL), $NaHCO_3$ (1 N, 3 × 20 mL) and water (3 × 20 mL). The organic layer was dried with $MgSO_4$ and concentrated under reduced pressure. The product **32** was isolated from the residue by flash chromatography on silica gel using ethyl acetate/*n*-hexane 1:1 to 4:1 (*v*/*v*) as eluent to yield a colorless foam (3.36 g, 90%). M.p. 56 °C; $[\alpha]^{22}_D = -11.8$ (*c* = 1.0 in $CHCl_3$); R_f = 0.26 (ethyl acetate/*n*-hexane 4:1 *v*/*v*); ^1H NMR (500 MHz, $CDCl_3$): δ = 7.22 (m, 2H; 2CONH), 7.12 (brs, 1H; CONH), 5.89 (ddt, $^3J_{trans}$ = 16.3, $^3J_{cis}$ = 10.3, $^3J_{vic}$ = 5.7 Hz, 1H; allyl CH=), 5.32 (dd, $^3J_{trans}$ = 17.4, ^2J = 1.2 Hz, 1H; allyl=CH_{2a}), 5.26 (dd, $^3J_{cis}$ = 10.5, ^2J = 1.2 Hz, 1H; allyl=CH_{2b}), 5.19 (br, 1H; OCONH), 4.62 (d, ^3J = 5.8 Hz, 2H; allyl OCH_2), 4.55 (m, 1H; Leu α-CH), 4.18 (brs, 1H; Leu α-CH), 4.03 (m, 3H; Gly α-CH_2, Gly α-CH_{2a}), 3.89 (dd, ^3J = 16.6, ^2J = 4.9 Hz, 1H; Gly α-CH_{2b}), 1.76–1.62 (m, 4H; 2Leu γ-CH, 2Leu β-CH_{2a}), 1.59–1.48 (m, 2H; 2Leu β-CH_{2b}), 1.43 (s, 9H; $C(CH_3)_3$), 0.95–0.90 (m, 12H; 4Leu ω-CH_3); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 173.7, 172.5, 169.6, 169.3 (4C=O), 156.0 (OCONH), 131.6 (allyl CH), 118.8 (allyl CH_2), 80.4 (*t*Bu q), 65.9 (allyl OCH_2), 53.5, 51.9 (2 Leu α-CH), 43.3, 41.2, 40.6 (2Leu β-CH_2, 2Gly α-CH_2), 28.3 (*t*Bu), 24.8, 24.7 (2Leu γ-CH), 23.0, 22.8 (2Leu ω-CH_3), 21.9, 21.8 (2Leu ω-CH_3); HRMS (EI, 70 eV, 200 °C): *m*/*z*: calcd for $[M+H]^+$ 498.3053; found: 498.3067; elemental analysis calcd (%) for $C_{24}H_{42}N_4O_7$ (498.62): C 57.81, H 8.49, N 11.24; found: C 58.18, H 8.46, N 10.90.

Boc-Leu-Gly-Leu-Gly-OH (33): A catalytic amount of $[Pd(PPh_3)_4]$ and morpholine (44 µL, 0.5 mmol) were added to a solution of Boc-Leu-Gly-Leu-Gly-OAll (**32**, 250 mg, 0.5 mmol) in tetrahydrofuran (20 mL) under argon, and the mixture was stirred at 20 °C for 1 h. The solvent was evaporated under reduced pressure, and the residue was dissolved in $NaHCO_3$ (half saturated, 30 mL) and extracted with CH_2Cl_2 (5 × 10 mL). At 0 °C the solution was acidified to pH 2 with HCl (2 N) and extracted with ethyl acetate (3 × 10 mL). The combined organic layers were dried with $MgSO_4$ and concentrated under reduced pressure to yield a colorless solid (228 mg, 99%). M.p. 61 °C; $[\alpha]^{22}_D = -18.0$ (*c* = 1.0 in $CHCl_3$); ^1H NMR (500 MHz, CD_3OD): δ = 4.46 (m, 1H; Leu α-CH), 4.06 (m, 1H; Leu α-CH), 3.94–3.82 (m, 4H; 2Gly α-CH_2), 1.72–1.60 (m, 4H; 2Leu γ-CH, 2Leu β-CH_{2a}), 1.57–1.54 (m, 2H; 2Leu β-CH_{2b}), 1.45 (s, 9H; $C(CH_3)_3$), 0.96–0.91 (m, 12H; 4 Leu ω-CH_3); ^{13}C NMR (125.7 MHz, CD_3OD): δ = 176.3, 174.9, 172.5, 171.4 (4C=O), 157.9 (OCONH), 80.6 (*t*Bu q), 54.7, 52.9 (2 Leu α-CH), 43.6, 41.7, 41.6 (2Leu β-CH_2, 2Gly α-CH_2), 28.7 (*t*Bu), 25.8, 25.6 (2Leu γ-CH), 23.4 (2Leu ω-CH_3), 21.8 (2Leu ω-CH_3); HRMS (EI, 70 eV, 190 °C): *m*/*z*: calcd for $[M+H]^+$ 458.2740; found: 458.2750; elemental analysis calcd (%) for $C_{21}H_{38}N_4O_7$ (458.55): C 55.01, H 8.35, N 12.22; found: C 54.78, H 8.27, N 12.03.

H-Leu-Gly-Leu-Gly-OAll (34): A saturated solution of HCl in diethyl ether (35 mL) was added at 0 °C to a solution of Boc-Leu-Gly-Leu-Gly-OAll (**32**, 435 mg, 0.87 mmol) in CH_2Cl_2 (30 mL) and the mixture was stirred at 0 °C for 30 min and at 20 °C for 2 h. Then the excess HCl and the solvent was removed under reduced pressure to yield a colorless foam (381 g, 99%). M.p. 98 °C; $[\alpha]^{22}_D = +9.5$ (*c* = 0.75 in $CHCl_3$); ^1H NMR (500 MHz, CD_3OD): δ = 5.93 (ddt, $^3J_{trans}$ = 16.2, $^3J_{cis}$ = 10.6, $^3J_{vic}$ = 5.7 Hz, 1H; allyl CH=), 5.33 (dd, $^3J_{trans}$ = 17.2, ^2J = 1.5 Hz, 1H; allyl=CH_{2a}), 5.23 (dd, $^3J_{cis}$ = 10.5, ^2J = 1.3 Hz, 1H; allyl=CH_{2b}), 4.62 (d, ^3J = 5.6 Hz, 2H; allyl OCH_2), 4.45 (q, ^3J = 5.0 Hz, 1H; Leu α-CH), 4.04–3.89 (m, 5H; Leu α-CH, 2Gly α-CH_2), 1.77–1.58 (m, 6H; 2Leu γ-CH, 2Leu β-CH_2), 1.03–0.92 (m, 12H; 4Leu ω-CH_3); ^{13}C NMR (125.7 MHz, CD_3OD): δ = 175.1, 171.2, 170.9, 170.6 (4C=O), 133.2 (allyl CH), 118.6 (allyl CH_2), 66.6 (allyl OCH_2), 53.0, 52.9 (2Leu α-CH), 43.2, 41.9, 41.4 (2Leu β-CH_2, 2Gly α-CH_2), 25.7, 25.3 (2Leu γ-CH), 23.4, 22.9 (2Leu ω-CH_3), 22.1, 21.8 (2Leu ω-CH_3); HRMS (FAB, 3-NBA/TFA 10:1): *m*/*z*: calcd for $[M-Cl]^+$ 399.2607; found: 399.2630; elemental analysis calcd (%) for $C_{19}H_{35}ClN_4O_5\cdot 0.5H_2O$: C 51.40, H 8.17, N 12.62; found: C 51.50, H 8.25, N 12.21; $C_{19}H_{35}ClN_4O_5$ (434.96).

Boc-Leu-Gly-Leu-Gly-Leu-Gly-Leu-Gly-OAll (4): NEt_3 (14 µL, 0.1 mmol) and EDC (21 mg, 0.11 mmol) were added at 0 °C to a solution of Boc-Leu-Gly-Leu-Gly-OH (**33**, 46 mg, 0.1 mmol), HCl·H-Leu-Gly-Leu-Gly-OAll (**34**, 44 mg, 0.1 mmol), and HOOBt (16 mg, 0.1 mmol) in $CHCl_3$/2,2,2-trifluoroethanol 3:1 (*v*/*v*) (4 mL). The mixture was stirred at 20 °C for 16 h, then the solvent was removed under reduced pressure. The residue was washed with methanol (5 × 2 mL) and dried to yield a colorless solid (69 mg, 82%). $[\alpha]^{22}_D = -14.2$; (*c* = 1.0 in hexafluoroisopropanol/$CHCl_3$ 1:3); ^1H NMR (500 MHz, $[D_6]$DMSO): δ = 8.42 (t, ^3J = 5.4 Hz, 1H; CONH), 8.21 (t, ^3J = 6.3 Hz, 2H; CONH), 8.03 (brs, 1H; CONH), 7.91–7.87 (m, 3H; CONH), 6.97 (d, ^3J = 7.6 Hz, 1H; OCONH), 5.90 (ddt, $^3J_{trans}$ = 15.9, $^3J_{cis}$ = 10.5, $^3J_{vic}$ = 5.3 Hz, 1H; allyl CH=), 5.31 (dd, $^3J_{trans}$ = 17.1, ^2J = 1.3 Hz, 1H; allyl=CH_{2a}), 5.26 (d, $^3J_{cis}$ = 10.6 Hz, 1H; allyl=CH_{2b}), 4.57 (d, ^3J = 5.3 Hz, 2H; allyl OCH_2), 4.35–4.24 (m, 3H; 3Leu α-CH), 3.94 (m, 1H; Leu α-CH), 3.86 (d, ^3J = 5.9 Hz, 1H; Gly α-CH_{2a}), 3.83 (d, ^3J = 5.8 Hz, 1H; Gly α-CH_{2b}), 3.75–3.67 (m, 6H; 3Gly α-CH_2), 1.61–1.51 (m, 4H; 4Leu γ-CH), 1.47–1.42 (m, 8H; 4Leu β-CH_2), 1.38 (s, 9H; $C(CH_3)_3$), 0.88–0.83 (m, 24H; 8Leu ω-CH_3); ^{13}C NMR (125.7 MHz, $[D_6]$DMSO): δ = 172.9, 172.5, 172.2, 169.2, 168.7, 168.6, 168.4 (7C=O), 155.3 (OCONH), 132.2 (allyl CH), 117.7 (allyl CH_2), 78.0 (*t*Bu q), 64.6 (allyl OCH_2), 52.7, 51.0, 50.9, 50.6 (4Leu α-CH), 41.9, 41.8, 40.9, 40.7, 40.5 (4Leu β-CH_2, 4Gly α-CH_2), 28.1 (*t*Bu), 24.1, 23.9 (4Leu γ-CH), 22.9, 21.5, 21.4, 21.3 (8Leu ω-CH_3); MS (FAB, 3-NBA/TFA 10:1): *m*/*z*: 739.8 $[M-Boc+2H]^+$, 840.0 $[M+H]^+$, 862.1 $[M+Na]^+$; elemental analysis calcd (%) for $C_{40}H_{70}N_8O_{11}$ (839.04): C 57.26, H 8.41, N 13.36; found: C 57.07, H 8.62, N 13.31.

TFA·H-Leu-Gly-Leu-Gly-Leu-Gly-Leu-Gly-OAll (35): Trifluoroacetic acid (450 µL, 5.9 mmol) was added at 0 °C to a suspension of Boc-Leu-Gly-Leu-Gly-Leu-Gly-Leu-Gly-OAll (**4**, 55 mg, 66 µmol) in $CHCl_3$ (1 mL) and the mixture was stirred at 0 °C for 30 min and 20 °C for 1 h. The trifluoroacetic acid and the solvent were coevaporated with benzene under reduced pressure to yield a colorless solid (55 mg, 98%). $[\alpha]^{22}_D = -7.4$ (*c* = 1.0 in $CHCl_3$/2,2,2-trifluoroethanol 3:1 *v*/*v*); ^1H NMR (500 MHz, $[D_6]$DMSO): δ = 8.75 (t, ^3J = 5.6 Hz, 1H; CONH), 8.43 (t, ^3J = 5.9 Hz, 1H; CONH), 8.25–8.20 (m, 3H; CONH), 8.15 (brs, 1H; H_3N), 7.92 (t, ^3J = 8.0 Hz, 2H; CONH), 5.89 (ddt, $^3J_{trans}$ = 16.0, $^3J_{cis}$ = 10.6, $^3J_{vic}$ = 5.3 Hz, 1H; allyl CH), 5.31 (dd, $^3J_{trans}$ = 17.3, ^2J = 1.5 Hz, 1H; allyl=CH_{2a}), 5.26 (dd, $^3J_{cis}$ = 10.5, ^2J = 1.2 Hz, 1H; allyl=CH_{2b}), 4.56 (d, ^3J = 5.3 Hz, 2H; allyl OCH_2), 4.35–4.26 (m, 3H; 3Leu α-CH), 3.89–3.77 (m, 5H; Leu α-CH, 2Gly α-CH_2), 3.75–3.66 (m, 4H; 2Gly α-CH_2), 1.70–1.40 (m, 12H; 4Leu γ-CH, 4Leu β-CH_2), 0.90–0.82 (m, 24H; 8Leu ω-CH_3); ^{13}C NMR (125.7 MHz, $[D_6]$DMSO): δ = 172.6, 172.3, 172.2, 169.4, 169.3, 168.6, 168.5, 168.0 (8C=O), 132.4 (allyl CH), 117.9 (allyl CH_2), 64.8 (allyl OCH_2), 51.0, 50.9, 50.7 (4Leu α-CH), 41.9, 41.1, 41.0, 40.6, 40.3 (4Leu β-CH_2, 4Gly α-CH_2), 24.2, 24.1, 24.0 (4Leu γ-CH), 23.5, 23.1, 23.0, 22.6, 22.0, 21.6, 21.5 (8Leu ω-CH_3); HRMS (FAB, 3-NBA/TFA 10:1): *m*/*z*: calcd for $[M-CF_3COO]^+$: 739.4718; found: 739.4772; elemental analysis calcd (%) for $C_{37}H_{63}F_3N_8O_{11}\cdot 1.5H_2O$: C 50.50, H 7.56, N 12.73; found: C 50.62, H 7.56, N 12.57; $C_{37}H_{63}F_3N_8O_{11}$ (852.94).

Myr-Gly-Asn(Trt)-Leu-Lys(Boc)-Ser(*t*Bu)-Val-Gly-Gln(Trt)-Glu(O*t*Bu)-Pro-OH (2): The peptide chain was assembled on a polystyrene resin, starting with Fmoc-Pro-2-ClTrt-polystyrene **36** (479 mg, 0.53 mmol g^{-1}) using an Applied Biosystems 430A peptide synthesizer and the standard Fmoc/*t*Bu strategy in *N*-methyl-pyrrolidinone (deprotection with piperidine [20%, 5 and 20 min], couplings [4 equiv Fmoc-AA-OBt, 60 min]. Yield: 995 mg.

From a portion of the resin **37** (100 mg, 53 µmol), the Fmoc group was removed (piperidine 20%, 20 and 30 min) and the resin was washed with NMP and CH_2Cl_2 (3 × 5 mL). Then to the resin was added diisopropylethylamine (86 µL, 65 mg, 0.5 mmol) and myristoyl chloride (68 µL, 0.25 mmol) in NMP (3 mL) and the mixture was shaken for 4 h. The resin was filtered off, washed with NMP and CH_2Cl_2 (3 × 5 mL) and dried. To cleave off the product **2** a mixture of acetic acid/2,2,2-trifluoroethanol/CH_2Cl_2 1:1:8 (*v*/*v*/*v*) (5 mL) was added and the mixture was shaken for 1 h. The resin was filtered off, trifluroacetic acid (0.5%, 5 mL) was added and the resin was shaken for 100 min. After filtration the solvents were removed under reduced pressure and traces of acid were removed by addition of water and lyophilisation to yield a colorless solid (47.7 mg, 98%). M.p. 238–239 °C (decomp); $[\alpha]^{22}_D = -26.3$ (*c* = 1.0 in $CHCl_3$/2,2,2-trifluoroethanol 3:1 *v*/*v*); ^1H NMR (500 MHz, $[D_6]$DMSO): δ = 8.57 (s, 1H; CONH), 8.50 (s, 1H; CONH), 8.29 (d, ^3J = 7.6 Hz, 1H; CONH), 8.09–8.04 (m, 4H; CONH), 7.88 (d, ^3J = 7.8 Hz, 1H; CONH), 7.80 (d, ^3J = 7.5 Hz, 1H;

CONNH), 7.77 (d, ^3J = 7.7 Hz, 1H; CONH), 7.70 (d, ^3J = 7.7 Hz, 1H; CONH), 7.58 (d, ^3J = 8.7 Hz, 1H; CONH), 7.27–7.24 (m, 12H; C_6H_5), 7.19–7.15 (m, 18H; C_6H_5), 6.68 (brs, 1H; OCONH), 4.53–4.49 (m, 2H; 2α-CH), 4.34 (d, ^3J = 7.6 Hz, α-CH), 4.28–4.16 (m, 5H; 5α-CH), 3.82 (dd, ^2J = 16.5, ^3J = 5.7 Hz, 1H; Gly α-CH_{2a}), 3.75 (dd, ^2J = 16.5, ^3J = 5.1 Hz, 1H; Gly α-CH_{2b}), 3.68–3.39 (m, 6H; Ser β-CH_2, Gly α-CH_2, Pro δ-CH_2), 2.85 (dd, $^3J_1$ = $^3J_2$ = 6.6 Hz, 2H; Lys ε-CH_2), 2.66–2.63 (m, 2H; Asn β-CH_2), 2.29–2.26 (m, 4H; Gln γ-CH_2, Glu γ-CH_2), 2.19–2.09 (m, 5H; Myr α-CH_2, Val β-CH, Glx β-CH_{2a}, Glx β-CH_{2a}), 1.98–1.42 (m, 15H; Glx β-CH_{2b}, Glx β-CH_{2b}, Pro β-CH_2, Pro γ-CH_2, Lys β-CH_2, Myr β-CH_2, Myr γ-CH_2, Leu γ-CH, Leu β-CH_2), 1.39 (s, 9H; $C(CH_3)_3$), 1.36 (s, 9H; $C(CH_3)_3$), 1.33–1.27 (m, 4H; Lys δ-CH_2, Lys γ-CH_2), 1.23 (brs, 18H; Myr $(CH_2)_9$), 1.08 (s, 9H; $C(CH_3)_3$), 0.86–0.82 (m, 15H; Myr ω-CH_3, 2Leu ω-CH_3, 2Val ω-CH_3); ^{13}C NMR (125.7 MHz, [D6]DMSO): δ = 173.6, 173.1, 172.8, 171.8, 171.7, 171.3, 171.0, 170.9, 170.7, 169.4, 169.3, 169.2, 168.8, 168.2 (14=O), 155.4 (OCONH), 144.8, 144.6 (arom. q), 128.5, 128.4, 127.3, 126.2 (30 arom. CH), 79.6, 77.2, 72.8 (3tBu q), 69.3, 69.1 (Trt q), 61.4 (Ser β-CH_2), 58.3, 57.5, 53.0, 52.6, 51.8, 51.2, 50.2, 49.3 (Pro α-CH, Ser α-CH, Asn α-CH, Gln α-CH, Glu α-CH, Lys α-CH, Leu α-CH, Val α-CH), 52.9 (Leu α-CH), 48.4 (Pro δ-CH_2), 46.4, 42.2, 41.6 (Leu β-CH_2, 2 Gly α-CH_2), 40.3 (Lys ε-CH_2), 38.2 (Asn β-CH_2), 35.0, 32.5 (Glu γ-CH_2, Gln γ-CH_2), 31.2 (Myr CH_2), 30.6 (Val β-CH), 30.3, 30.0, 29.2, 29.0, 28.9, 28.8, 28.7, 28.6, 28.5, 28.4 (8Myr CH_2, Pro β-CH_2, 2 Lys CH_2, Gln β-CH_2, Glu β-CH_2), 28.2 (tBu), 27.7 (tBu), 27.0 (tBu), 26.4 (Myr CH_2), 25.0, 24.4 (Lys CH_2, Pro γ-CH_2), 24.0 (Leu γ-CH), 23.0 (Leu ω-CH_3), 22.6, 22.0 (2Myr CH_2), 21.4 (Leu ω-CH_3), 19.1 (Val ω-CH_3'), 17.9 (Val ω-CH_3'), 17.1 (Myr CH_2), 13.9 (Myr ω-CH_3); MS (FAB, 3-NBA/TFA 10:1): m/z: 1834.8 $[M-\text{Boc}+2H]^+$, 1935.6 $[M+H]^+$, 1957.4 $[M+Na]^+$; $C_{108}H_{151}N_{13}O_{19}$ (1935.48).

Myr-Gly-Asn-Leu-Lys-Ser-Val-Gly-Gln-Glu-Pro-OH (45): Myr-Gly-Asn(Trt)-Leu-Lys(Boc)-Ser(tBu)-Val-Gly-Gln(Trt)-Glu(OtBu)-Pro-OH (**2**, 3.9 mg, 0.9 µmol) was dissolved in a mixture of CF_3CO_2H/ethanedithiol/water 95:2.5:2.5 ($v/v/v$) (500 µL) and shaken at 20 °C for 20 min. Then the solvent was removed under reduced pressure and **45** was precipitated and washed with diethyl ether. After purification by HPLC the deprotected peptide was obtained as pale colorless solid (9.5 mg, 74%). M.p. 213–215 °C; $[\alpha]_D^{22}$ = +122 (c = 0.025 in $CHCl_3$/2,2,2-trifluoroethanol 3:1 v/v); HPLC: t_r = 11.9 min (50 °C, 25% to 100% B in 20 min, then 10 min B; A: H_2O+1% CH_3CN+0.1% TFA; B: CH_3CN+1% H_2O+0.1% TFA); ^{1}H NMR (500 MHz, $CDCl_3/CD_3OD$): δ = 4.56–4.21 (m, 8H; 8α-CH), 3.97–3.63 (m, 8H; Ser β-CH_2, 2Gly α-CH_2, Pro δ-CH_2), 2.94–2.90 (m, 2H; Lys ε-CH_2), 2.79–2.78 (m, 2H; Asn β-CH_2), 2.46–2.25 (m, 4H; Gln γ-CH_2, Glu γ-CH_2), 2.06–1.91 (m, 6H; Myr α-CH_2, Val β-CH, Glx β-CH_2, Glx β-CH_{2a}), 1.81–1.38 (m, 14H; Glx β-CH_{2b}, Pro β-CH_2, Pro γ-CH_2, Lys β-CH_2, Myr β-CH_2, Myr γ-CH, Leu γ-CH, Leu β-CH_2), 1.32–1.11 (brs, 22H; Lys δ-CH_2, Lys γ-CH_2, Myr $(CH_2)_9$), 1.02–0.85 (m, 15H; Myr ω-CH_3, 2Leu ω-CH_3, 2Val ω-CH_3); MS (MALDI-TOF, DHB/TFA): m/z: calcd for $[M+H]^+$ 1238.74; found: 1238.05, calcd for $[M+Na]^+$ 1261.49; found: 1262.07; $C_{57}H_{99}N_{13}O_{17}$ (1238.47).

Myr-Gly-Asn(Trt)-Leu-Lys(Boc)-Ser(tBu)-Val-Gly-Gln(Trt)-Glu(OtBu)-Pro-Gly-Pro-Pro-Cys(Pal)-Gly-OAll (38): EDC (2.9 mg, 15.1 µmol) was added at 10 °C to a solution of peptide **2**, 18.5 mg, 9.6 µmol), H-Gly-Pro-Pro-Cys(Pal)-Gly-OAll (**24**, 7.1 mg, 10.1 µmol) and HOOBt (2.5 mg, 15.1 µmol) in $CHCl_3$/2,2,2-trifluoroethanol 3:1 (v/v) (250 µL) and the mixture was stirred at 20 °C for 16 h. The solvent was removed under a stream of nitrogen and the residue was washed with methanol (5 × 1 mL) and dried to yield a colorless solid (23.0 mg, 91%). $[\alpha]_D^{22}$ = −73.2 (c = 0.25, $CHCl_3$/2,2,2-trifluoroethanol 3:1 v/v); ^{1}H NMR (500 MHz, [D6]DMSO): δ = 8.57 (s, 1H; CONH), 8.54 (s, 2H; CONH), 8.30–8.28(m, 2H; CONH), 8.08–8.01 (m, 4H; CONH), 7.95–7.87 (m, 4H; CONH), 7.80 (d, ^3J = 7.0 Hz, 1H; CONH), 7.77 (d, ^3J = 7.3 Hz, 1H; CONH), 7.69 (d, ^3J = 8.0 Hz, 1H; CONH), 7.58 (d, ^3J = 8.3 Hz, 1H; CONH), 7.27–7.24 (m, 12H; C_6H_5), 7.19–7.15 (m, 18H; C_6H_5), 6.68 (brs, 1H; OCONH), 5.93–5.87 (m, 1H; allyl CH=), 5.31 (d, $^3J_{trans}$ = 17.2 Hz, 1H; allyl=CH_{2a}), 5.21 (d, $^3J_{cis}$ = 10.6, 1H; allyl=CH_{2b}), 4.58–4.02 (m, 13H; 11α-CH, allyl OCH_2), 3.85–3.36 (m, 16H; 4Gly α-CH_2, Ser β-CH_2, 3Pro δ-CH_2), 3.17 (dd, ^2J = 13.7, ^3J = 8.5 Hz, 2H; Cys β-CH_2), 2.85 (d, ^3J = 5.8 Hz, 2H; Lys ε-CH_2), 2.64 (brs, 2H; Asn β-CH_2), 2.54 (t, ^3J = 7.3 Hz, 2H; Pal α-CH_2), 2.36–2.25 (m, 4H; Gln γ-CH_2, Glu γ-CH_2), 2.11–1.47 (m, 30H; Myr α-CH_2, Gln β-CH_2, Glu β-CH_2, 3Pro β-CH_2, 3Pro γ-CH_2, Lys β-CH_2, Myr β-CH_2, Myr γ-CH_2, Pal β-CH_2, Val β-CH, Leu γ-CH, Leu β-CH_2), 1.39 (s, 9H; $C(CH_3)_3$), 1.36 (s, 9H; $C(CH_3)_3$), 1.33–1.26 (m, 4H; Lys δ-CH_2, Lys γ-CH_2), 1.23 (brs, 42H; Myr $(CH_2)_9$, Pal $(CH_2)_{12}$), 1.08 (s, 9H; $C(CH_3)_3$), 0.86–0.82 (m, 18H; Myr ω-CH_3, Pal ω-CH_3', 2Leu ω-CH_3, 2 Val ω-CH_3); ^{13}C NMR (125.7 MHz, [D6]DMSO): δ = 198.2 (C=OS), 172.8, 171.8, 171.5, 171.3, 171.2, 171.0, 170.9, 170.7, 170.6, 169.9, 169.4, 169.2, 168.9, 168.8, 168.2, 166.4 (19C=O), 155.4 (OCONH), 144.8 (arom q), 144.6 (arom. q), 132.2 (allyl CH), 128.5, 128.4, 127.3, 126.2, 125.3 (30 arom. CH), 117.7 (allyl CH_2), 79.6, 77.2, 72.7 (3tBu q), 69.3, 69.1 (Trt q), 64.8, 64.7 (allyl OCH_2, Ser β-CH_2), 61.4, 59.5, 59.1, 57.5, 53.0, 52.6, 51.8, 51.5, 51.2 (3 Pro α-CH, Cys α-CH, Ser α-CH, Asn α-CH, Gln α-CH, Glu α-CH, Lys α-CH, Leu α-CH, Val α-CH), 50.2, 49.5 (3 Pro δ-CH_2), 46.7, 43.3, 42.2, 41.6 (Leu β-CH_2, Cys β-CH_2, 4Gly α-CH_2), 40.7 (Lys ε-CH_2), 38.1 (Asn β-CH_2), 34.9, 32.5 (Glu γ-CH_2, Gln γ-CH_2), 31.2 (Myr CH_2), 30.6 (Val β-CH), 30.0, 29.2, 28.9, 28.8, 28.7, 28.6 (8Myr CH_2, 10Pal CH_2, Pro β-CH_2, 2Lys CH_2, Gln β-CH_2, Glu β-CH_2), 28.2 (tBu), 27.9 (Pal CH_2), 27.7 (tBu), 27.0 (tBu), 26.4 (Myr CH_2), 25.0, 24.9, 24.4, 24.2 (Lys CH_2, 3 Pro γ-CH_2), 24.0 (Leu γ-CH), 23.0 (Leu ω-CH_3), 22.6, 22.0 (2Myr CH_2), 21.4 (Leu ω-CH_3), 19.1 (Val ω-CH_3'), 17.9 (Val ω-CH_3'), 15.1 (Pal ω-CH_3'), 13.8 (Myr ω-CH_3); MS (MALDI-TOF, DHB/TFA): m/z: calcd for $[M+Na]^+$ 2646.54; found: 2647.54, calcd for $[M+K]^+$ 2662.51; found: 2662.94; $C_{144}H_{210}N_{18}O_{25}S$ (2625.44).

Myr-Gly-Asn(Trt)-Leu-Lys(Boc)-Ser(tBu)-Val-Gly-Gln(Trt)-Glu(OtBu)-Pro-Gly-Pro-Pro-Cys(Pal)-Gly-OH (39): N,N'-Dimethylbarbituric acid (2.0 mg, 12.8 µmol) and a catalytic amount of $[Pd(PPh_3)_4]$ were added under argon to a solution allyl protected peptide **38** (16.7 mg, 6.4 µmol) in DMSO (400 µL), and the mixture was stirred at 20 °C for 2 h. Then citric acid (5%, 5 mL) was added at 0 °C and the precipitated 15-mer **39** was filtered off and washed several times with citric acid (5%, 1 mL), water, methanol. After drying in vacuo the peptide was isolated as colorless solid (15.1 mg, 92%). $[\alpha]_D^{22}$ = −76.0 (c = 0.25 in $CHCl_3$/2,2,2-trifluoroethanol 3:1 v/v); ^{1}H NMR (500 MHz, [D6]DMSO): δ = 8.57 (s, 1H; CONH), 8.54 (s, 2H; CONH), 8.29 (d, ^3J = 7.4 Hz, 1H; CONH), 8.09–8.03 (m, 4H; CONH), 7.92 (d, ^3J = 7.8 Hz, 1H; CONH), 7.88 (d, ^3J = 7.7 Hz, 2H; CONH), 7.81 (d, ^3J = 7.5 Hz, 1H; CONH), 7.77 (d, ^3J = 7.7 Hz, 1H; CONH), 7.72–7.68 (m, 2H; CONH), 7.59 (d, ^3J = 8.8 Hz, 2H; CONH), 7.27–7.24 (m, 12H; C_6H_5), 7.20–7.14 (m, 18H; C_6H_5), 6.68 (brs, 1H; OCONH), 4.57–4.43 (m, 3H; 3α-CH), 4.38–4.32 (m, 3H; 3α-CH), 4.26–4.01 (m, 5H; 5α-CH, 1Gly α-CH_{2a}), 3.85–3.39 (m, 15H; 1Gly α-CH_{2b}, 3 Gly α-CH_2, Ser β-CH_2, 3Pro δ-CH_2), 3.04 (d, ^2J = 13.3 Hz, 2H; Cys β-CH_2), 2.85 (dd, ^2J = 13.2, ^3J = 6.5 Hz, 2H; Lys ε-CH_2), 2.64 (brs, 2H; Asn β-CH_2), 2.54 (t, ^3J = 7.3 Hz, 2H; Pal α-CH_2), 2.29–2.24 (m, 4H; Gln γ-CH_2, Glu γ-CH_2), 2.13–2.08 (m, 3H; Myr α-CH_2, Val β-CH), 1.99–1.43 (m, 27H; Gln β-CH_2, Glu β-CH_2, 3Pro β-CH_2, 3Pro γ-CH_2, Lys β-CH_2, Pal β-CH_2, Myr β-CH_2, Myr γ-CH_2, Leu γ-CH, Leu β-CH_2), 1.39 (s, 9H; $C(CH_3)_3$), 1.36 (s, 9H; $C(CH_3)_3$), 1.32–1.27 (m, 4H; Lys δ-CH_2, Lys γ-CH_2), 1.22 (brs, 42H; Myr $(CH_2)_9$, Pal $(CH_2)_{12}$), 1.08 (s, 9H; $C(CH_3)_3$), 0.89–0.82 (m, 18H; Myr ω-CH_3, Pal ω-CH_3', 2Leu ω-CH_3, 2Val ω-CH_3); MS (MALDI-TOF, DHB/TFA): m/z: calcd for $[M+Na]^+$ 2606.50; found: 2605.94, calcd for $[M+K]^+$ 2622.48; found: 2621.25; $C_{141}H_{206}N_{18}O_{25}S$ (2585.37).

Myr-Gly-Asn-Leu-Lys-Ser-Val-Gly-Gln-Glu-Pro-Gly-Pro-Pro-Cys(Pal)-Gly-OH (46): The protected peptide **39** (5.9 mg, 2.3 µmol) was dissolved in a mixture of CF_3CO_2H/ethanedithiol/water 95:2.5:2.5 ($v/v/v$) (200 µL) and shakenfor 20 min at 20 °C. Then the solvent was removed under reduced pressure and **46** was precipitated and washed with diethyl ether. After separation by HPLC the deprotected peptide was obtained as colorless solid (2.3 mg, 53%). $[\alpha]_D^{22}$ = +37.4 (c = 0.02 in $CHCl_3$/2,2,2-trifluoroethanol 3:1 v/v); HPLC: t_r = 16.5 min (50 °C, 25% to 100% B in 20 min, then 10 min B; A: H_2O+1% CH_3CN+0.1% TFA; B: CH_3CN+1% H_2O+0.1% TFA); ^{1}H NMR (500 MHz, $CDCl_3/CD_3OD$ 1:1): δ = 4.64–3.36 (m, 27H; 11 α-CH, 4Gly α-CH_2, Ser β-CH_2, 3Pro δ-CH_2), 3.20–3.19 (m, 2H; Cys β-CH_2), 2.93 (d, ^3J = 7.1 Hz, 2H; Lys ε-CH_2), 2.77 (d, ^3J = 6.2 Hz, 2H; Asn β-CH_2), 2.54 (t, ^3J = 7.5 Hz, 2H; Pal α-CH_2), 2.44–1.39 (m, 34H; Gln γ-CH_2, Glu γ-CH_2, Myr α-CH_2, Gln β-CH_2, Glu β-CH_2, 3Pro β-CH_2, 3Pro γ-CH_2, Lys β-CH_2, Myr β-CH_2, Myr γ-CH_2, Pal β-CH_2, Val β-CH, Leu γ-CH, Leu β-CH_2), 1.36–1.20 (brs, 46H; Lys δ-CH_2, Lys γ-CH_2, Myr $(CH_2)_9$, Pal $(CH_2)_{12}$), 1.01–0.88 (m, 18H; Myr ω-CH_3, Pal ω-CH_3', 2Leu ω-CH_3, 2Val ω-CH_3); MS (MALDI-TOF, DHB/TFA): m/z: calcd for $[M+H]^+$ 1888.12; found: 1888.14, calcd for $[M+Na]^+$ 1910.11; found: 1909.82; calcd for $[M+K]^+$ 1926.08; found: 1926.45; $C_{90}H_{154}N_{18}O_{23}S$ (1888.39).

Myr-Gly-Asn(Trt)-Leu-Lys(Boc)-Ser(tBu)-Val-Gly-Gln(Trt)-Glu(OtBu)-Pro-Gly-Pro-Pro-Cys(Pal)-Gly-Leu-Gly-Leu-Gly-Leu-Gly-Leu-Gly-OAll (40): NEt_3 (0.7 µL, 6.9 µmol) and EDC (1.5 mg, 7.8 µmol) were added to a solution of peptide **39** (10.0 mg, 3.9 µmol), TFA·H-Leu-Gly-Leu-Gly-Leu-

 © WILEY-VCH Verlag GmbH, D-69451 Weinheim, 2001 0947-6539/01/0713-2953 $ 17.50+.50/0

Gly-Leu-Gly-OAll (**35**, 5.0 mg, 5.9 µmol) and HOAt (0.6 mg, 4.4 µmol) in dimethylsulfoxide (300 µL), and the mixture was stirred at 20 °C for 16 h. At 0 °C citric acid (5%, 5 mL) was added and the precipitated product was separated, washed with citric acid, water and methanol and dried to yield a colorless solid (9.2 mg, 72%). $[\alpha]_D^{22} = -27.4°$ ($c = 0.45$ in $CHCl_3$/2,2,2-trifluoroethanol 3:1 *v*/*v*); ^1H NMR (500 MHz, $[D_6]$DMSO): $\delta = 8.57$ (s, 1H; CONH), 8.53 (s, 1H; CONH), 8.40 (s, 2H; CONH), 8.29 (d, $^3J = 7.4$ Hz, 1H; CONH), 8.17 (brs, 2H; CONH), 8.08 – 8.04 (m, 5H; CONH), 7.87 – 7.81 (m, 8H; CONH), 7.80 (d, $^3J = 5.8$ Hz, 1H; CONH), 7.76 (d, $^3J = 8.0$ Hz, 1H; CONH), 7.69 (d, $^3J = 7.4$ Hz, 2H; CONH), 7.58 (d, $^3J = 6.5$ Hz, 1H; CONH), 7.27 – 7.24 (m, 12H; C_6H_5), 7.19 – 7.11 (m, 18H; C_6H_5), 6.68 (brs, 1H; OCONH), 5.93 – 5.86 (m, 1H; allyl CH=), 5.32 (d, $^3J_{trans} = 16.9$ Hz, 1H; allyl=CH_{2a}), 5.21 (d, $^3J_{cis} = 10.5$, 1H; allyl=CH_{2b}), 4.57 – 4.18 (m, 17H; 15 α-CH, allyl OCH_2), 3.89 – 3.38 (m, 24H; 8 Gly α-CH_2, Ser β-CH_2, 3 Pro δ-CH_2), 3.07 (m, 2H; Cys β-CH_2), 2.85 (d, $^3J = 5.4$ Hz, 2H; Lys ε-CH_2), 2.63 (brs, 2H; Asn β-CH_2), 2.54 (m, 2H; Pal α-CH_2), 2.29 – 2.25 (m, 4H; Gln γ-CH_2, Glu γ-CH_2), 2.11 – 2.09 (m, 3H; Myr α-CH_2, Val β-CH), 1.98 – 1.45 (m, 39H; Gln β-CH_2, Glu β-CH_2, 3 Pro β-CH_2, 3 Pro γ-CH_2, Lys β-CH_2, Myr β-CH_2, Myr γ-CH_2, Pal β-CH_2, 5 Leu γ-CH, 5 Leu β-CH_2), 1.39 (s, 9H; $C(CH_3)_3$), 1.35 (s, 9H; $C(CH_3)_3$), 1.33 – 1.29 (m, 4H; Lys δ-CH_2, Lys γ-CH_2), 1.23 (brs, 42H; Myr $(CH_2)_9$, Pal $(CH_2)_{12}$), 1.08 (s, 9H; $C(CH_3)_3$), 0.86 – 0.83 (m, 42H; Myr ω-CH_3, Pal ω-CH_3', 10 Leu ω-CH_3, 2 Val ω-CH_3); MS (MALDI-TOF, DHB/TFA): *m*/*z*: calcd for $[M-t\text{Bu}+H+Na]^+$ 3270.90; found: 3270.02, calcd for $[M+Na]^+$ 3326.96; found: 3326.15, calcd for $[M+K]^+$ 3342.93; found: 3341.91; $C_{176}H_{266}N_{26}O_{33}S$ (3306.29).

Myr-Gly-Asn(Trt)-Leu-Lys(Boc)-Ser(*t*Bu)-Val-Gly-Gln(Trt)-Glu(O*t*Bu)-Pro-Gly-Pro-Pro-Cys(Pal)-Gly-Leu-Gly-Leu-Gly-Leu-Gly-Leu-Gly-OH (41): *N*,*N*′-Dimethylbarbituric acid (1.0 mg, 6.4 µmol) and a catalytic amount of $[Pd(PPh_3)_4]$ were added under argon to a solution of peptide **40** (9.0 mg, 2.7 µmol) in DMSO (400 µL) and the mixture was stirred at 20 °C for 2 h. Then at 0 °C citric acid (5%, 5 mL) was added and the precipitated 23-mer **41** was separated and washed several times with citric acid (5%, 1 mL), water, methanol. After drying in vacuo the peptide was isolated as pale yellow solid (7.7 mg, 87%). $[\alpha]_D^{22} = -28.6$ ($c = 0.25$ in $CHCl_3$/2,2,2-trifluoroethanol 3:1 *v*/*v*); ^1H NMR (500 MHz, $[D_6]$DMSO): $\delta = 8.57$ (s, 1H; CONH), 8.54 (s, 1H; CONH), 8.51 (s, 1H; CONH), 8.28 (d, $^3J = 7.7$ Hz, 1H; CONH), 8.18 (brs, 4H; CONH), 8.08 – 8.01 (m, 5H; CONH), 7.88 – 7.85 (brs, 6H; CONH), 7.80 (d, $^3J = 7.3$ Hz, 1H; CONH), 7.76 (d, $^3J = 7.9$ Hz, 1H; CONH), 7.70 (d, $^3J = 7.6$ Hz, 1H; CONH), 7.64 – 7.55 (m, 3H; CONH), 7.27 – 7.15 (m, 30H; Trt), 6.69 (brs, 1H; OCONH), 4.58 – 4.47 (m, 3H; 3 α-CH), 4.35 – 4.17 (m, 12H; 12 α-CH), 3.84 – 3.38 (m, 24H; 8 Gly α-CH_2, Ser β-CH_2, 3 Pro δ-CH_2), 3.06 (dd, $^2J = 13.4$ Hz, $^3J = 8.4$ Hz, 2H; Cys β-CH_2), 2.85 (d, $^3J = 6.3$ Hz, 2H; Lys ε-CH_2), 2.63 (brs, 2H; Asn β-CH_2), 2.54 (t, $^3J = 7.4$ Hz, 2H; Pal α-CH_2), 2.36 – 2.26 (m, 4H; Gln γ-CH_2, Glu γ-CH_2), 2.12 – 2.09 (m, 3H; Myr α-CH_2, Val β-CH), 1.99 – 1.46 (m, 39H; Gln β-CH_2, Glu β-CH_2, 3 Pro β-CH_2, 3 Pro γ-CH_2, Lys β-CH_2, Myr β-CH_2, Myr γ-CH_2, Pal β-CH_2, 5 Leu γ-CH, 5 Leu β-CH_2), 1.39 (s, 9H; $C(CH_3)_3$), 1.36 (s, 9H; $C(CH_3)_3$), 1.32 – 1.28 (m, 4H; Lys δ-CH_2, Lys γ-CH_2), 1.23 (brs, 42H; Myr $(CH_2)_9$, Pal $(CH_2)_{12}$), 1.08 (s, 9H; $C(CH_3)_3$), 0.88 – 0.82 (m, 42H; Myr ω-CH_3, Pal ω-CH_3', 10 Leu ω-CH_3, 2 Val ω-CH_3); MS (MALDI-TOF, DHB/TFA): *m*/*z*: calcd for $[M+Na]^+$ 3286.93; found: 3284.57, calcd for $[M+K]^+$ 3302.90; found: 3302.22; $C_{173}H_{262}N_{26}O_{33}S$ (3266.22).

Myr-Gly-Asn-Leu-Lys-Ser-Val-Gly-Gln-Glu-Pro-Gly-Pro-Pro-Cys(Pal)-Gly-Leu-Gly-Leu-Gly-Leu-Gly-Leu-Gly-OH (47): Peptide **41** (3.9 mg, 1.2 µmol) was dissolved in a mixture of CF_3CO_2H/ethanedithiol/water 95:2.5:2.5 (*v*/*v*/*v*) (200 µL) and shaken at 20 °C for 20 min. Then the solvent was removed under reduced pressure and **47** was precipitated and washed with diethyl ether. After separation by HPLC the deprotected peptide was obtained as colorless solid (1.6 mg, 52%). $[\alpha]_D^{22} = -34.3$ ($c = 0.15$ in $CHCl_3$/2,2,2-trifluoroethanol 3:1 *v*/*v*); HPLC: $t_r = 18.4$ min (50 °C, 25% to 100% B in 20 min, then 10 min B; A: H_2O+1% CH_3CN+0.1% TFA; B: CH_3CN+1% H_2O+0.1% TFA); ^1H NMR (500 MHz, $[D_6]$DMSO): $\delta = 8.39$ (s, 2H; CONH), 8.21 – 8.17 (m, 10H; CONH), 7.88 – 7.78 (m, 10H; CONH), 7.43 (s, 1H; CONH), 7.27 (brs, 2H; CONH), 6.95 (s, 1H; CONH), 6.76 (s, 1H; CONH), 4.57 – 4.47 (m, 3H; 3 α-CH), 4.34 – 4.08 (m, 12H; 12 α-CH), 3.82 – 3.49 (m, 24H; 8 Gly α-CH_2, Ser β-CH_2, 3 Pro δ-CH_2), 3.08 (m, 2H; Cys β-CH_2), 2.75 (brs, 2H; Lys ε-CH_2), 2.47 (brs, 2H; Pal α-CH_2), 2.27 (brs, Myr α-CH_2), 2.14 – 2.01 (m, 7H; Asn β-CH_2, Val β-CH, Gln γ-CH_2, Glu γ-CH_2), 1.91 – 1.36 (m, 43H; Gln β-CH_2, Glu β-CH_2, 3 Pro β-CH_2, 3 Pro γ-CH_2, Lys β-CH_2, Myr β-CH_2, Myr γ-CH_2, Pal β-CH_2, 5 Leu γ-CH, 5 Leu β-CH_2, Lys δ-CH_2, Lys γ-CH_2), 1.24 (brs, 42H; Myr $(CH_2)_9$, Pal $(CH_2)_{12}$), 0.88 – 0.81 (m, 42H; Myr ω-CH_3, Pal ω-CH_3', 10 Leu ω-CH_3, 2 Val ω-CH_3); MS (MALDI-TOF, DHB/TFA): *m*/*z*: calcd for $[M+H]^+$ 2568.55; found: 2569.06, calcd for $[M+Na]^+$ 2590.53; found: 2591.02, calcd for $[M+K]^+$ 2606.50; found: 2608.24; $C_{122}H_{210}N_{26}O_{31}S$ (2569.24).

Myr-Gly-Asn(Trt)-Leu-Lys(Boc)-Ser(*t*Bu)-Val-Gly-Gln(Trt)-Glu(O*t*Bu)-Pro-Gly-Pro-Pro-Cys(Pal)-Gly-Leu-Gly-Leu-Gly-Leu-Gly-Leu-Gly-Leu-Cys(Pal)-Gly-OAll (42): EDC (0.8 mg, 4.2 µmol) was added at 0 °C to a solution of peptide **41** (6.5 mg, 2.0 µmol), H-Leu-Cys(Pal)-Gly-OAll (**22**, 2.4 mg, 4.0 µmol) and HOAt (0.6 mg, 4.4 µmol) in *N*-methyl-pyrrolidinone (300 µL), and the mixture was stirred at 20 °C for 16 h. At 0 °C citric acid (5%, 5 mL) was added and the precipitated product was separated, washed with citric acid, water and methanol and dried to yield a colorless solid (6.6 mg, 86%). $[\alpha]_D^{22} = -26.1$ ($c = 0.45$ in $CHCl_3$/2,2,2-trifluoroethanol 3:1 *v*/*v*); ^1H NMR (500 MHz, $[D_6]$DMSO): $\delta = 8.57$ (s, 1H; CONH), 8.53 (s, 1H; CONH), 8.30 – 8.28 (m, 2H; CONH), 8.18 (brs, 4H; CONH), 8.12 – 8.04 (m, 6H; CONH), 7.91 – 7.87 (m, 9H; CONH), 7.80 (d, $^3J = 7.1$ Hz, 1H; CONH), 7.76 (d, $^3J = 6.8$ Hz, 1H; CONH), 7.69 (d, $^3J = 7.7$ Hz, 2H; CONH), 7.58 (d, $^3J = 7.8$ Hz, 1H; CONH), 7.27 – 7.15 (m, 30H; Trt), 6.68 (brs, 1H; OCONH), 5.90 – 5.87 (m, 1H; allyl CH=), 5.31 (d, $^3J_{trans} = 16.8$ Hz, 1H; allyl=CH_{2a}), 5.20 (d, $^3J_{cis} = 10.2$ Hz, 1H; allyl=CH_{2b}), 4.58 – 4.45 (m, 4H; 2 α-CH, allyl OCH_2), 4.38 – 4.18 (m, 15H; 15 α-CH), 3.89 – 3.34 (m, 26H; 9 Gly α-CH_2, Ser β-CH_2, 3 Pro δ-CH_2), 3.04 (dd, $^2J = 13.2$ Hz, $^3J = 8.9$ Hz, 4H; 2 Cys β-CH_2), 2.85 (d, $^3J = 5.5$ Hz, 2H; Lys ε-CH_2), 2.63 (brs, 2H; Asn β-CH_2), 2.55 (m, 4H; 2 Pal α-CH_2), 2.28 – 2.25 (m, 4H; Gln γ-CH_2, Glu γ-CH_2), 2.17 (t, $^3J = 8.0$ Hz, 2H; Myr α-CH_2), 2.12 – 2.07 (m, 2H; Val β-CH, Glx β-CH_{2a}), 1.99 – 1.46 (m, 43H; Glx β-CH_{2b}, Glx β-CH_2, 3 Pro β-CH_2, 3 Pro γ-CH_2, Lys β-CH_2, Myr β-CH_2, Myr γ-CH_2, 2 Pal β-CH_2, 6 Leu γ-CH, 6 Leu β-CH_2), 1.39 (s, 9H; $C(CH_3)_3$), 1.36 (s, 9H; $C(CH_3)_3$), 1.32 – 1.31 (m, 4H; Lys δ-CH_2, Lys γ-CH_2), 1.23 (brs, 66H; Myr $(CH_2)_9$, Pal $(CH_2)_{12}$), 1.08 (s, 9H; $C(CH_3)_3$), 0.87 – 0.82 (m, 51H; Myr ω-CH_3, 2 Pal ω-CH_3', 11 Leu ω-CH_3, 2 Val ω-CH_3); MS (MALDI-TOF, DHB/TFA): *m*/*z*: calcd for $[M+Na]^+$ 3838.30; found: 3837.85, calcd for $[M+K]^+$ 3854.28; found: 3853.54; $C_{203}H_{315}N_{29}O_{37}S_2$ (3818.06).

Myr-Gly-Asn(Trt)-Leu-Lys(Boc)-Ser(*t*Bu)-Val-Gly-Gln(Trt)-Glu(O*t*Bu)-Pro-Gly-Pro-Pro-Cys(Pal)-Gly-Leu-Gly-Leu-Gly-Leu-Gly-Leu-Gly-Leu-Cys(Pal)-Gly-OH (43): *N*,*N*′-Dimethylbarbituric acid (0.5 mg, 3.2 µmol) and a catalytic amount of $[Pd(PPh_3)_4]$ were added under argon to a solution of peptide **42** (4.7 mg, 1.2 µmol) in DMSO (300 µL), and the mixture was stirred at 20 °C for 2 h. Then at 0 °C citric acid (5%, 5 mL) was added and the precipitated 26-mer **43** was separated and washed several times with citric acid (5%, 1 mL), water, methanol. After drying in vacuo the peptide was isolated as pale yellow solid. (3.2 mg, 69%). $[\alpha]_D^{22} = -23.5$ ($c = 0.15$, $CHCl_3$/2,2,2-trifluoroethanol 3:1 *v*/*v*); ^1H NMR (500 MHz, $[D_6]$DMSO): $\delta = 8.57$ (s, 1H; CONH), 8.53 (s, 1H; CONH), 8.30 – 8.27 (m, 2H; CONH), 8.18 (brs, 3H; CONH), 8.09 – 8.04 (m, 4H; CONH), 7.91 – 7.86 (m, 6H; CONH), 7.81 (brs, 1H; CONH), 7.76 (d, $^3J = 7.8$ Hz, 1H; CONH), 7.69 (brs, 1H; CONH), 7.58 – 7.41 (m, 8H; CONH), 7.27 – 7.15 (m, 30H; Trt), 6.69 (brs, 1H; OCONH), 4.53 – 4.46 (m, 3H; 3 α-CH), 4.34 – 4.18 (m, 15H; 15 α-CH), 3.88 – 3.37 (m, 26H; 9 Gly α-CH_2, Ser β-CH_2, 3 Pro δ-CH_2), 3.06 (m, 4H; 2 Cys β-CH_2), 2.85 (d, $^3J = 7.0$ Hz, 2H; Lys ε-CH_2), 2.63 (brs, 2H; Asn β-CH_2), 2.54 (m, 4H; 2 Pal α-CH_2), 2.37 – 2.25 (m, 4H; Gln γ-CH_2, Glu γ-CH_2), 2.11 – 2.09 (m, 3H; Myr α-CH_2, Val β-CH), 1.98 – 1.46 (m, 44H; Gln β-CH_2, Glu β-CH_2, 3 Pro β-CH_2, 3 Pro γ-CH_2, Lys β-CH_2, Myr β-CH_2, Myr γ-CH_2, 2 Pal β-CH_2, 6 Leu γ-CH, 6 Leu β-CH_2), 1.39 (s, 9H; $C(CH_3)_3$), 1.35 (s, 9H; $C(CH_3)_3$), 1.34 – 1.26 (m, 4H; Lys δ-CH_2, Lys γ-CH_2), 1.23 (brs, 66H; Myr $(CH_2)_9$, Pal $(CH_2)_{12}$), 1.08 (s, 9H; $C(CH_3)_3$), 0.86 – 0.83 (m, 51H; Myr ω-CH_3, 2 Pal ω-CH_3', 11 Leu ω-CH_3, 2 Val ω-CH_3); MS (MALDI-TOF, DHB/TFA): *m*/*z*: calcd for $[M+H]^+$ 3776.29; found: 3777.65, calcd for $[M+Na]^+$ 3798.27; found: 3797.32, calcd for $[M+K]^+$ 3814.25; found: 3815.12; $C_{200}H_{311}N_{29}O_{37}S_2$ (3777.99).

Myr-Gly-Asn-Leu-Lys-Ser-Val-Gly-Gln-Glu-Pro-Gly-Pro-Pro-Cys(Pal)-Gly-Leu-Gly-Leu-Gly-Leu-Gly-Leu-Gly-Leu-Cys(Pal)-Gly-OH (48): Peptide **43** (3.2 mg, 0.8 µmol) was dissolved in a mixture of CF_3CO_2H/ethanedithiol/water 95:2.5:2.5 (*v*/*v*/*v*) (200 µL) and shaken at 20 °C for 20 min. Then the solvent was removed under reduced pressure and **48** was precipitated and washed with diethyl ether. After separation by HPLC the deprotected peptide was obtained as colorless solid (1.1 mg, 42%). $[\alpha]_D^{22} = -17.8$ ($c = 0.15$ in $CHCl_3$/2,2,2-trifluoroethanol 3:1 *v*/*v*); HPLC: $t_r = 21.2$ min (50 °C, 25% to 100% B in 20 min, then 10 min B; A: H_2O+ 1% CH_3CN+0.1% TFA; B: CH_3CN+1% H_2O+0.1% TFA); ^1H NMR

 © WILEY-VCH Verlag GmbH, D-69451 Weinheim, 2001 0947-6539/01/0713-2954 $ 17.50+.50/0 *Chem. Eur. J.* **2001**, *7*, No. 13

(500 MHz, $[D_6]$DMSO): $\delta = 8.18-8.14$ (m, 6H; CONH), 8.09 (d, $^3J=6.1$ Hz, 1H; CONH), 8.02–7.86 (m, 9H; CONH), 7.70–7.55 (m, 5H; CONH), 7.42 (brs, 1H; CONH), 7.33–7.20 (m, 6H; CONH), 6.94 (s, 1H; CONH), 6.75 (s, 1H; CONH), 4.53–4.47 (m, 2H; 2 α-CH), 4.37–4.06 (m, 16H; 16 α-CH), 3.78–3.38 (m, 26H; 9 Gly α-CH_2, Ser β-CH_2, 3 Pro δ-CH_2), 3.05 (m, 4H; 2 Cys β-CH_2), 2.75 (brs, 2H; Lys ε-CH_2), 2.56 (brs, 4H; 2 Pal α-CH_2), 2.34–2.30 (m, 4H; Gln γ-CH_2, Glu γ-CH_2), 2.17–1.98 (m, 5H; Myr α-CH_2, Asn β-CH_2, Val β-CH), 1.91–1.33 (m, 48H; Gln β-CH_2, Glu β-CH_2, 3 Pro β-CH_2, 3 Pro γ-CH_2, Lys β-CH_2, Myr β-CH_2, Myr γ-CH_2, 2 Pal β-CH_2, 6 Leu γ-CH, 6 Leu β-CH_2, Lys δ-CH_2, Lys γ-CH_2), 1.23 (brs, 66H; Myr $(CH_2)_9$, 2 Pal $(CH_2)_{12}$), 0.86–0.82 (m, 51H; Myr ω-CH_3, 2 Pal ω-CH_3', 12 Leu ω-CH_3, 2 Val ω-CH_3); MS (MALDI-TOF, DHB/TFA): m/z: calcd for $[M+H]^+$ 3079.89; found: 3079.15, calcd for $[M+Na]^+$ 3101.88; found: 3103.02, calcd for $[M+K]^+$ 3117.85; found: 3115.49; $C_{149}H_{259}N_{29}O_{35}S_2$ (3081.01).

Myr-Gly-Asn(Trt)-Leu-Lys(Boc)-Ser(*t*Bu)-Val-Gly-Gln(Trt)-Glu(O*t*Bu)-Pro-Gly-Pro-Pro-Cys(Pal)-Gly-Leu-Gly-Leu-Gly-Leu-Gly-Leu-Gly-Leu-Cys(Pal)-Gly-Lys(Boc)-Gln(Trt)-Gly-O*t*Bu (44): EDC (0.6 mg, 3.2 μmol) was added at 0°C to a solution of peptide **43** (5.3 mg, 1.6 μmol), H-Lys(Boc)-Gln(Trt)-Gly-O*t*Bu **26** (1.8 mg, 2.4 μmol) and HOAt (0.5 mg, 3.2 μmol) in *N*-methylpyrolidinone (300 μL), and the mixture was stirred at 20°C for 16 h. At 0°C citric acid (5%, 5 mL) was added and the precipitated was separated, washed with citric acid, water and methanol and dried to yield a colorless solid (6.2 mg, 86%). $[\alpha]_D^{22} = -25.6$ ($c = 0.25$ in $CHCl_3$/2,2,2-trifluoroethanol 3:1 *v*/*v*); ^1H NMR (500 MHz, $[D_6]$DMSO): $\delta = 8.57$ (s, 1H; CONH), 8.54 (s, 1H; CONH), 8.52 (s, 1H; CONH), 8.30–8.28 (m, 2H; CONH), 8.18 (brs, 4H; CONH), 8.08–8.06 (m, 8H; CONH), 7.91–7.86 (m, 7H; CONH), 7.81–7.76 (m, 4H; CONH), 7.69 (m, 2H; CONH), 7.60–7.58 (m, 2H; CONH), 7.27–7.15 (m, 30H; Trt), 6.69 (brs, 2H; OCONH), 4.53–4.46 (m, 3H; 3 α-CH), 4.34–4.19 (m, 16H; 16 α-CH), 3.85–3.39 (m, 28H; 10 Gly α-CH_2, Ser β-CH_2, 3 Pro δ-CH_2), 3.06 (m, 4H; 2 Cys β-CH_2), 2.85 (d, $^3J = 6.5$ Hz, 4H; 2 Lys ε-CH_2), 2.63 (brs, 2H; Asn β-CH_2), 2.55 (m, 4H; 2 Pal α-CH_2), 2.36–2.25 (m, 6H; 2 Gln γ-CH_2, Glu γ-CH_2), 2.18–2.09 (m, 3H; Myr α-CH_2, Val β-CH), 1.99–1.46 (m, 48H; 2 Gln β-CH_2, Glu β-CH_2, 3 Pro β-CH_2, 3 Pro γ-CH_2, Lys β-CH_2, Myr β-CH_2, Myr γ-CH_2, 2 Pal β-CH_2, 6 Leu γ-CH, 6 Leu β-CH_2), 1.38 (s, 9H; $C(CH_3)_3$), 1.36 (s, 9H; $C(CH_3)_3$), 1.35 (s, 9H; $C(CH_3)_3$), 1.32–1.29 (m, 8H; 2 Lys δ-CH_2, 2 Lys γ-CH_2), 1.23 (brs, 66H; Myr $(CH_2)_9$, Pal $(CH_2)_{12}$), 1.08 (s, 9H; $C(CH_3)_3$), 0.86–0.82 (m, 51H; Myr ω-CH_3, 2 Pal ω-CH_3', 11 Leu ω-CH_3, 2 Val ω-CH_3); MS (MALDI-TOF, DHB/TFA): m/z: calcd for $[M+Na]^+$ 4509.67; found: 4510.12; $C_{241}H_{364}N_{34}O_{43}S_2$ (4489.90).

Myr-Gly-Asn-Leu-Lys-Ser-Val-Gly-Gln-Glu-Pro-Gly-Pro-Pro-Cys(Pal)-Gly-Leu-Gly-Leu-Gly-Leu-Gly-Leu-Gly-Leu-Cys(Pal)-Gly-Lys-Gln-Gly-OH (1): Protected peptide **44** (3.9 mg, 0.9 μmol) was dissolved in a mixture of CF_3CO_2H/ethanedithiol/water 95:2.5:2.5 (*v*/*v*/*v*) (200 μL) and shaken at 20°C for 20 min. Then the solvent was removed under reduced pressure and **1** was precipitated and washed with diethyl ether. After separation by HPLC the deprotected peptide was obtained as colorless solid (1.0 mg, 31%). $[\alpha]_D^{22} = -19.0$ ($c = 0.05$ in $CHCl_3$/2,2,2-trifluoroethanol 3:1 *v*/*v*); HPLC: $t_r = 22.2$ min (50°C, 25% to 100% B in 20 min, then 10 min B; A: H_2O+1% CH_3CN+0.1% TFA; B: CH_3CN+1% H_2O+s0.1% TFA); ^1H NMR (500 MHz, $[D_6]$DMSO): $\delta = 8.20-8.10$ (m, 9H; CONH), 7.95–7.84 (m, 9H; CONH), 7.62 (brs, 5H; CONH), 7.43 (s, 1H; CONH), 7.35–7.20 (m, 9H; CONH), 6.95 (s, 1H; CONH), 6.76 (s, 1H; CONH), 4.79–4.73 (m, 1H; 1 α-CH), 4.53–4.50 (m, 4H; 4 α-CH), 4.37–4.17 (m, 14H; 14 α-CH), 3.80–3.54 (m, 28H; 9 Gly α-CH_2, Ser β-CH_2, 3 Pro δ-CH_2), 3.07 (m, 4H; 2 Cys β-CH_2), 2.75 (brs, 4H; 2 Lys ε-CH_2), 2.56 (m, 4H; 2 Pal α-CH_2), 2.37–2.32 (m, 3H; 3 Glx γ-CH_{2a}), 2.14–1.98 (m, 8H; Myr α-CH_2, 3 Glx γ-CH_{2b}, Asn β-CH_2, Val β-CH), 1.91–1.27 (m, 52H; Gln β-CH_2, Glu β-CH_2, 3 Pro β-CH_2, 3 Pro γ-CH_2, Lys β-CH_2, Myr β-CH_2, Myr γ-CH_2, 2 Pal β-CH_2, 6 Leu γ-CH, 6 Leu β-CH_2, 2 Lys δ-CH_2, 2 Lys γ-CH_2), 1.23 (brs, 66H; Myr $(CH_2)_9$, 2 Pal $(CH_2)_{12}$), 0.88–0.84 (m, 51H; Myr ω-CH_3, 2 Pal ω-CH_3', 12 Leu ω-CH_3, 2 Val ω-CH_3); MS (ESI): m/z: calcd for $[M+2H]^{2+}$ 1697.04; found: 1697.9; MS (MALDI-TOF, DHB/TFA): m/z: calcd for $[M+H]^+$ 3393.07; found: 3394.16, calcd for $[M+Na]^+$ 3415.05; found: 3415.62, calcd for $[M+K]^+$ 3431.02; found: 3432.46; $C_{126}H_{282}N_{34}O_{39}S_2$ (3394.37).

Acknowledgement

This research was supported by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie.

[1] J. T. Dunphy, M. E. Linder, *Biochim. Biophys. Acta* **1998**, *1436*, 245–261.

[2] M. D. Resh, *Biochim. Biophys. Acta* **1999**, *1451*, 1–16.

[3] a) D. Kadereit, J. Kuhlmann, H. Waldmann, *ChemBioChem* **2000**, *1*, 144–169; b) D. Kadereit, J. Kuhlmann, H. Waldmann in *Stimulating Concepts in Chemistry* (Eds.: M. Shibaski, J. F. Stoddart, F. Vögtle), Wiley-VCH **2000**, pp. 369–382.

[4] B. Bader, K. Kuhn, D. J. Owen, H. Waldmann, A. Wittinghofer, J. Kuhlmann, *Nature* **2000**, *403*, 223–226.

[5] a) H. Waldmann, M. Schelhaas, E. Nägele, J. Kuhlmann, A. Wittinghofer, H. Schroeder, J. R. Silvius, *Angew. Chem.* **1997**, *109*, 2334–2337; *Angew. Chem. Int. Ed.* **1997**, *36*, 2238–2241; b) H. Schroeder, R. Leventis, S. Rex, M. Schelhaas, E. Nägele, H. Waldmann, J. R. Silvius, *Biochemistry* **1997**, *36*, 13102–13109.

[6] D. Huster, K. Kuhn, D. Kadereit, H. Waldmann, K. Arnold, *Angew. Chem.* **2001**, *113*, 1083–1085; *Angew. Chem. Int. Ed.* **2001**, *40*, 1056–1058.

[7] a) D. J. Owen, K. Alexandrov, E. Rostkova, A. J. Scheidig, R. S. Goody, H. Waldmann, *Angew. Chem.* **1999**, *111*, 570–573; *Angew. Chem. Int. Ed.* **1999**, *38*, 509–512; b) N. H. Thomä, A. Iakovenko, D. Owen, A. S. Scheidig, H. Waldmann, R. S. Goody, K. Alexandrov, *Biochemistry* **2000**, *39*, 12043–12052.

[8] M. Langer, C. Appelt, H. Waldmann, A. Beck-Sickinger, unpublished results.

[9] a) E. Nägele, M. Schelhaas, N. Kuder, H. Waldmann, *J. Am. Chem. Soc.* **1998**, *120*, 6889–6902; b) M. Schelhaas, E. Nägele, N. Kuder, H. B. Bader, J. Kuhlmann, A. Wittinghofer, H. Waldmann, *Chem. Eur. J.* **1999**, *5*, 1239–1252.

[10] Part of this work was published as a short communication: R. Machauer, H. Waldmann, *Angew. Chem.* **2000**, *112*, 1503–1507; *Angew. Chem. Int. Ed.* **2000**, *39*, 1449–1453.

[11] a) R. M. J. Palmer, D. S. Ashton, S. Moncada, *Nature* **1988**, *333*, 664–666; b) P. W. Shaul, E. J. Smart, L. J. Robinson, Z. German, I. S. Yuhanna, Y. Ying, R. G. W. Anderson, T. Michel, *J. Biol. Chem.* **1996**, *271*, 6518–6522.

[12] a) E. G. Shesely, N. Maeda, H. S. Kim, K. M. Desai, J. H. Krege, V. E. Laubach, P. A. Sherman, W. C. Sessa, O. Smithies, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 13176–13181; b) P. L. Huang, Z. H. Huang, H. Mashimo, K. D. Bloch, M. A. Moskowitz, J. A. Bevan, M. C. Fishman, *Nature* **1995**, *377*, 239–242.

[13] a) R. D. Rudic, E. G. Shesely, N. Maeda, O. Smithies, S. S. Segal, W. C. Sessa, *J. Clin. Invest.* **1998**, *101*, 731–736; b) T. Murohara, T. Asahara, M. Silver, C. Bauters, H. Masuda, C. Kalka, M. Kearney, D. Chen, D. Chen, J. F. Symes, M. C. Fishman, P. L. Huang, J. M. Isner, *J. Clin. Invest.* **1998**, *101*, 2567–2578.

[14] a) D. S. Bredt, S. H. Snyder, *Annu. Rev. Biochem.* **1994**, *63*, 175–195; b) C. Nathan, Q. Xie, *J. Biol. Chem.* **1994**, *269*, 13725–13728; c) S. Moncada, A. Higgs, *New Engl. J. Med.* **1993**, *329*, 2002–2012; d) P. W. Shaul in *Advances in Pediatrics* (Ed.: L. Barnes), Mosby Year Book Inc., Chicago, IL, **1995**, pp. 367–414.

[15] a) G. García-Cardeña, P. Oh, J. Liu, J. E. Schnitzler, W. C. Sessa, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 6448–6453; b) P. W. Shaul, E. J. Smart, L. J. Robinson, Z. German, I. S. Yuhanna, Y. Ying, R. G. W. Anderson, T. Michel, *J. Biol. Chem.* **1996**, *271*, 6518–6522; c) R. G. W. Anderson, *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 10909–10913; d) M. P. Lisanti, P. E. Scherer, Z. Tang, M. Sargiacomo, *Trends Cell Biol.* **1994**, *4*, 231–235; e) E. J. Smart, Y. S. Ying, R. G. W. Anderson, *J. Cell Biol.* **1995**, *131*, 929–938.

[16] a) J. Liu, W. C. Sessa, *J. Biol. Chem.* **1994**, *269*, 11691–11694; b) J. Liu, G. García-Cardeña, W. C. Sessa, *Biochemistry* **1995**, *34*, 12333–12340.

[17] L. J. Robinson, T. Michel, *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 11776–11780.

[18] K. Hinterding, D. Alonso-Diáz, H. Waldmann, *Angew. Chem.* **1998**, *110*, 716–780; *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 688–749.

 © WILEY-VCH Verlag GmbH, D-69451 Weinheim, 2001 0947-6539/01/0713-2955 $ 17.50+.50/0

[19] T. Pohl, H. Waldmann, *J. Am. Chem. Soc.* **1997**, *119*, 6702 – 6710.
[20] a) H. Kunz, H. Waldmann, *Angew. Chem.* **1984**, *96*, 49 – 50; *Angew. Chem. Int. Ed. Engl.* **1984**, *23*, 71 – 72; b) A. Cotté, B. Bader, J. Kuhlmann, H. Waldmann, *Chem. Eur. J.* **1999**, *5*, 922 – 953.
[21] H. Kunz, J. März, *Angew. Chem.* **1988**, *100*, 1424; *Angew. Chem. Int. Ed. Engl.* **1988**, *27*, 1375 – 1377.
[22] Reviews: a) D. Haring, P. Schreier, *Curr. Op. Chem. Bio.* **1999**, *3*, 35 – 38; b) T. Zelinski, H. Waldmann, *Angew. Chem.* **1997**, *109*, 746 – 748; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 722 – 724.
[23] D. Sebastian, A. Heuser, S. Schulze, H. Waldmann, *Synthesis* **1997**, *9*, 1098 – 1108.
[24] a) K. Martinek, A. V. Levashov, N. L. Klyachko, Y. L. Khmelnitski, I. V. Berezin, *Eur. J. Biochem.* **1986**, *155*, 453 – 468; b) K. Martinek, N. L. Klyachko, A. V. Kabanov, Y. L. Khmelnitski, A. V. Levashov, *Biochim. Biophys. Acta* **1989**, *981*, 161 – 172.
[25] K. Barlos, D. Gatos, J. Kallitsis, G. Papaphotiu, P. Sotiriu, Y. Wenquing, W. Schäfer, *Tetrahedron Lett.* **1989**, *30*, 3943 – 3946.
[26] S. Sakakibara, *Biopolymers* **1995**, *37*, 17 – 28.
[27] a) M. Narita, T. Fukunaga, A. Wakabayashi, K. Ishikawa, H. Nakano, *Int. J. Peptide Protein Res.* **1984**, *23*, 306 – 314; b) M. Natita, K. Ishikawa, J.-Y. Chen, Y. Kim, *Int. J. Peptide Protein Res.* **1984**, *24*, 580 – 587.
[28] a) H. Kuroda, Y.-N. Chen, T. Kimura, S. Sakakibara, *Int. J. Peptide Protein Res.* **1992**, *40*, 294 – 299; b) J. Bódi, H. Nishio, Y. Zhou, W. D. Branton, T. Kimura, S. Sakakibara, *Pept. Res.* **1995**, *4*, 228 – 235.
[29] K. Barlos, D. Gatos, G. Papaphotiu, W. Schäfer, *Liebigs Ann. Chem.* **1993**, 215 – 220.

Received: December 22, 2000 [F2959]

© WILEY-VCH Verlag GmbH, D-69451 Weinheim, 2001