DOI: 10.1002/chem.200501128

# Synthesis and Evaluation of Acyl Protein Thioesterase 1 (APT1) Inhibitors

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**Abstract:** Lipid-modified proteins play decisive roles in important biological processes such as signal transduction, organisation of the cytoskeleton and vesicular transport. Lipidation of these proteins is essential for correct biological function. Among the modifications with lipids, prenylation and myristoylation are well understood. However, the machinery of palmitoylation is still under investigation. Recently, an enzyme, acyl protein thioesterase 1 (APT1), that may play a regulatory role in the palmitoylation cycle of H-Ras and G-protein α subunits, was purified. Motivated by this work, several inhibitors of APT1 were designed, synthesized and biologically evaluated leading to highly active compounds.

**Keywords:** palmitoylation · peptidomimetics · proteins · signal transduction

## Introduction

Numerous lipid-modified proteins are involved in important biological events, such as signal transduction, organisation of the cytoskeleton and vesicular transport.[1–3] Lipidation of these proteins is a prerequisite for a 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.

The most common lipid modifications are prenylation with farnesyl or geranylgeranyl moieties and acylation with myristoyl or palmitoyl residues. It was shown that peptides that resemble the dual lipidation motifs of Ras or G-protein α subunits are efficiently palmitoylated and localized at the plasma membrane.[4,5] The effect of palmitoylation on differentiation of cells was investigated by microinjection experiments. After microinjection of full-length recombinant oncogenic Ras(G12V) protein to the rat pheochromocytoma cell line PC12, nearly 80% of injected cells developed neurites.[6] When truncated Ras(G12V)ΔC181 protein lacking both the isoprenylation motif and one of two possible palmitoylation sites was microinjected, no neurites were formed. These findings strongly indicate that palmitoylation has regulatory functions in living cells.

Unlike myristoylation or farnesylation, palmitoylation is a dynamic process due to the reversibility of the thioester modification. Palmitoylated proteins cycle between acylated and deacylated states many times during their existence within a cell.[7] The regulation of reversible protein palmitoylation has been formally demonstrated for endothelial nitric oxide synthase, the β-adrenergic receptor, the m2 muscarinic receptor and the α subunit of the heterotrimeric G-protein $G_s$.[8–11]

Although protein-acyltransferase (PAT) activity was detected in membrane fractions of different cell types already 20 years ago, the identification of a first bona fide PAT has been difficult. The inherent instability of PAT activity during purification employing detergents like Triton-X-100 did not allow a final identification and isolation of the PAT enzyme.[12–14] Furthermore, enzymes of the lipid metabolism are able to mimic PAT activity. The amino acid sequencing of a protein, described as a H-Ras palmitoylating enzyme matched the sequence of a previously isolated peroxysomal thiolase A, an enzyme required for fatty acid β-oxidation.[15] In addition, several proteins such as G-protein α subunits are palmitoylated in vitro at the appropriate cysteine residues in a non enzymatic manner by incubation with palmitoyl-CoA.[16–18] This observation led to the concept of an autocatalytic acylation process in vivo.

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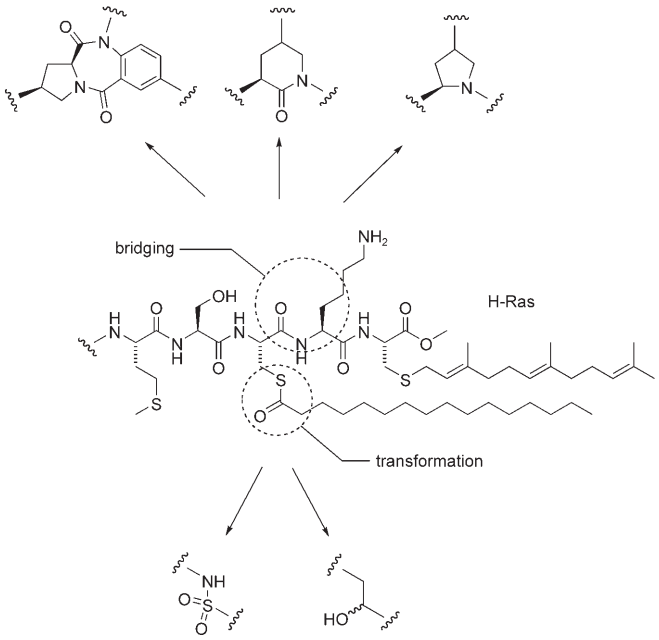
In a genetic approach using *Saccharomyces cerevisiae*, the protein complex Erf2/Erf4 could be identified as the Ras2p palmitoylating enzyme.[19] Although the ERF2-gene has orthologues in various eukaryotes, proteins with the corresponding S-palmitoylating activity have not been identified to date and the Erf2/Erf4 complex does not accept mammalian H-Ras as substrate. Furthermore homologues of the ERF4-gene exist only in closely related yeast. In contrast to the principle of biocatalysis, Erf2 mediated protein acylation in vitro is only performed at equimolar concentrations of substrate and enzyme, a finding which remains to be clarified.[20] Thus, palmitoylation in eukaryontes is still not well understood.

The enzymatic process which antagonizes acylation is protein deacylation. A palmitoyl protein thioesterase (PPT1) that is able to deacylate Ras and $G_\alpha$-proteins was purified and the cDNA was cloned.[21] Subsequent studies revealed that the enzyme is a lysosomal resident, and mutations in the PPT1 gene cause a fatal lysosomal storage disease, infantile neuronal ceroid lipofuscinosis.[22,23] Recently, a novel candidate for a regulatory enzyme of palmitoylated signalling proteins has been identified.[24] A cytosolic acyl protein thioesterase (APT1) was purified from rat liver which catalyzes the depalmitoylation of G-protein α subunits and H-Ras. Amino acid sequencing showed that APT1 was previously identified as a rat lysophospholipase,[25] but its affinity to acyl protein substrates is 250-fold higher compared with lysophosphatidylcholine, the substrate for lysophospholipase. In mammalian cells continuously expressing APT1, the rate of palmitate turnover is significantly faster compared with control cells.[24] Therefore, APT1 is proposed to be the first bona fide player in the regulated palmitoylation of intracellular signalling proteins in mammalian cells. Genetic or pharmacological inactivation of APT1 might serve to illuminate the role of the enzymes in reversible protein palmitoylation of $G_\alpha$ subunits or other palmitoylated proteins like H-Ras.

A strain of *Saccharomyces cerevisiae*, which lacks the APT1 gene, appears normal with regard to growth and sporulation when compared with the wild-type strain.[26] Although extracts, prepared from this strain have a significant reduction in biochemically detectable palmitoyl-$G_{i\alpha1}$ thioesterase activity, the yeast shows no obvious phenotypic alterations. Due to the significant differences in amino acid sequence and substrate selectivity between APT1, isolated from yeast and rat liver the development of potent inhibitors is important for the study of the physiological importance of the mammalian enzyme. In this paper we describe the design, synthesis and chemical evaluation of several different inhibitors of acyl protein thioesterase 1 (APT1).[27]

## Results and Discussion

The human H-Ras protein is a substrate for APT1 in vitro. Based on this fact, the possible inhibitors we designed to mimic the C-terminal end of the natural protein (Scheme 1).



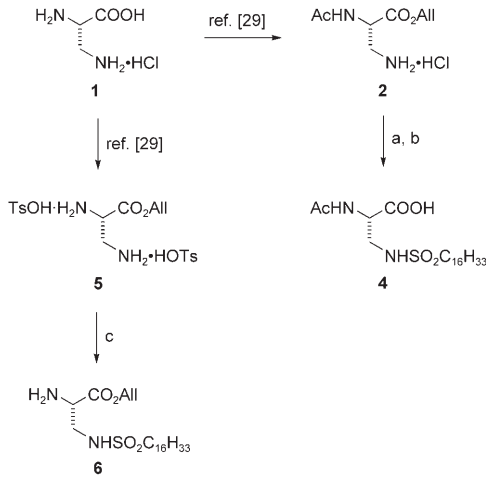

Scheme 1. Analysis for the possible inhibitors.

In a first step, several peptide analogues with intact peptide backbone consisting of three or five amino acids were planned to be synthesized. Therefore, the labile thioester group was transformed into either a sulfonamide moiety or a simple secondary alcohol function. In a second step, conformational rigidity should be introduced into the desired compounds. Therefore, we thought of bridging the peptide backbone and thus to synthesize proline and lactam derivatives. Finally, a peptide-imitating benzodiazepinedione core[28] was chosen as the underlying scaffold and equipped with a hydrolysis-stable sulfonamide to mimic the transition state of the hydrolysis of the thioester moiety. With this methodology we were able to generate different classes of possible inhibitors for APT1, and with those in hand it was possible to study structure–activity relationships.

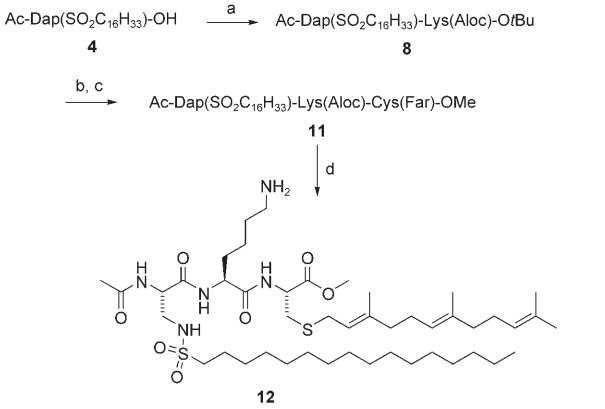
**Synthesis of tri- and pentapeptide analogues**: For the synthesis of the tri- and pentapeptide analogues, the two diaminopropionic acid derivatives **4** and **6** had to be generated (Scheme 2). Starting from (2*S*,3)-diaminopropionic acid hydrochloride (**1**), allyl ester **2** was prepared following a known procedure.[29] Then, the β-amino functionality was converted into a hexadecyl sulfonamide; subsequently the allyl group was removed leading to free acid **4**. Additionally, the known bishydrotosylate **5** could be easily transformed into amine **6**.

The tripeptide synthesis commenced with the coupling of Ac-Dap($SO_2C_{16}H_{33}$)-OH (**4**) to H-Lys(Aloc)-O*t*Bu (**7**) (Scheme 3). After cleavage of the *tert*-butyl ester, H-Cys-(Far)-OMe (**10**) was condensed with the resulting free acid to yield the protected tripeptide **11**. Finally, the Aloc group was removed leading to analogue **12**.




Scheme 2. Synthesis of the diaminopropionic acids **4** and **6**. a) $C_{16}H_{33}SO_2Cl$, NE*t*Pr$_2$, DMF, 0 °C → RT; 75 % (**3**); b) [Pd(PPh$_3$)$_4$], DMB, THF, 99 %; c) $C_{16}H_{33}SO_2Cl$, NEt$_3$, −70 °C → RT, 72 %. All = allyl, DMB = *N*,*N*′-dimethylbarbituric acid, TsOH = *para*-toluenesulfonic acid.

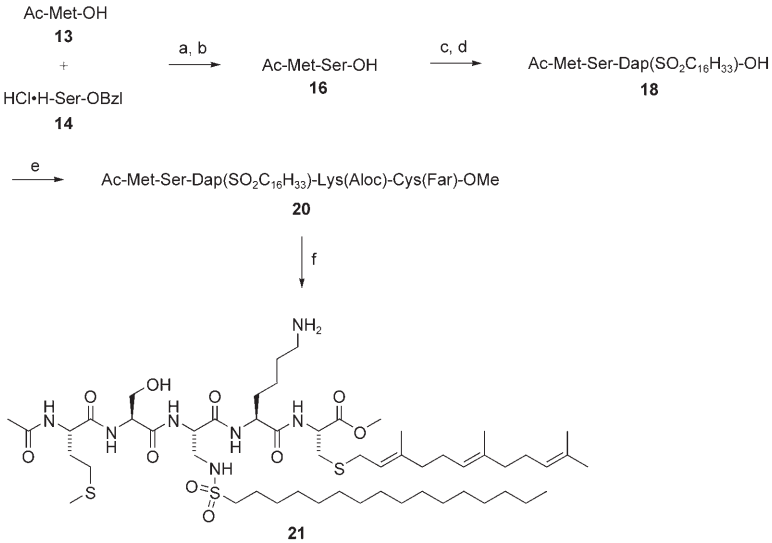



Scheme 3. Synthesis of the tripeptide analogue **12**. a) H-Lys(Aloc)-O*t*Bu (**7**), EDC, HOBt, $CH_2Cl_2$, 0 °C → RT, 82 %; b) TFA/$CH_2Cl_2$ 1:1, quant. (**9**); c) H-Cys(Far)-OMe (**10**), EDC, HOBt, $CH_2Cl_2$, 0 °C → RT, 90 %; d) [Pd(PPh$_3$)$_4$], DMB, THF, 73 %. Aloc = allyloxycarbonyl, Dap = diaminopropionic acid, EDC = *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride, Far = farnesyl, HOBt = 1-hydroxybenzotriazole, TFA = trifluoroacetic acid.

The synthesis of the first pentapeptide **21** started with the preparation of dipeptide **16** (Scheme 4). To this end, Ac-Met-OH (**13**) and HCl·H-Ser-OBzl (**14**) were coupled and the benzyl ester group was cleaved by hydrogenation. Condensation of H-Dap($SO_2C_{16}H_{33}$)-OAll (**6**) with free dipeptide **16** and subsequent deprotection of the allyl ester yielded unprotected tripeptide **18**. This intermediate was coupled with freshly prepared H-Lys(Aloc)-Cys(Far)-OMe (**19**) leading to protected pentapeptide **20**. Final Pd$^0$-catalyzed allyl transfer to *N*,*N*′-dimethylbarbituric acid gave the first pentapeptide analogue **21** in high yields.

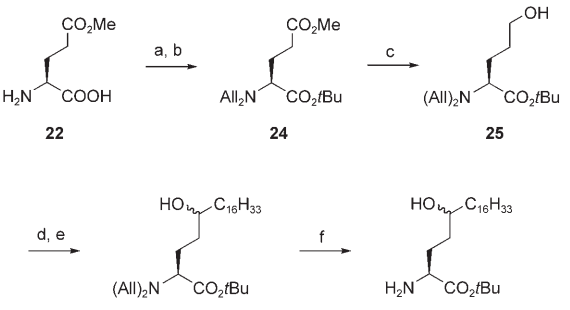
In order to prepare pentapeptide **30**, it was first necessary to synthesize the central $C_{21}$ amino acid **27** (Scheme 5). Therefore, H-L-Glu(OMe)-OH (**22**) was fully protected using the *tert*-butyl ester group for the carboxylic acid and a diallyl protection for the amino group. The two allyl groups were used to prevent the molecule from undesired deprotonation in a subsequent Grignard reaction. Glutamic acid **24** was then reduced at the methyl ester function with lithiumborohydride as reducing agent. Next, alcohol **25** was oxidized with Dess–Martin periodinane and the resulting aldehyde was subsequently treated with hexadecyl magnesium bromide at −78 °C to yield the secondary alcohol **26**. Final deprotection of the amine using Pd$^0$ chemistry at elevated temperatures,[30] converted diallyl amine **26** into the free amino acid **27**.

The synthesis of the second pentapeptide started with the condensation of Ac-Met-Ser-OH (**16**) with the $C_{21}$ amino acid **27** (Scheme 6). Attempts to deprotect the *tert*-butyl ester under acidic conditions resulted in lactone formation between the secondary alcohol of the $C_{21}$ acid and the C-terminal carboxylic acid in **28**. Therefore, basic saponification conditions were tried, and fortunately it was possible to cleave the *tert*-butyl group without any side reactions. Subsequently, H-Lys(Aloc)-Cys(Far)-OMe (**19**) was coupled to the resulting acid and the protected pentapeptide **29** could

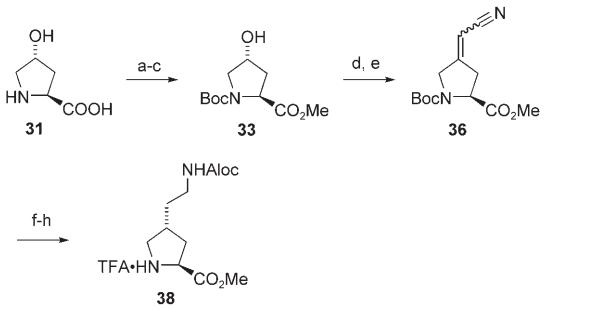



Scheme 4. Synthesis of the pentapeptide analogue **21**. a) EDC, HOBt, NEt$_3$, $CH_2Cl_2$, 0 °C → RT, 86 % (**15**); b) $H_2$, Pd/C (10 %), MeOH, 99 %; c) H-Dap($SO_2C_{16}H_{33}$)-OAll (**6**), EDC, HOBt, DMF, 0 °C → RT, 73 % (**17**); d) [Pd(PPh$_3$)$_4$], DMB, THF, 99 %; e) H-Lys(Aloc)-Cys(Far)-OMe (**19**), EDC, HOBt, $CH_2Cl_2$, 0 °C → RT, 50 %; f) [Pd(PPh$_3$)$_4$], DMB, THF, 82 %. Bzl = benzyl.

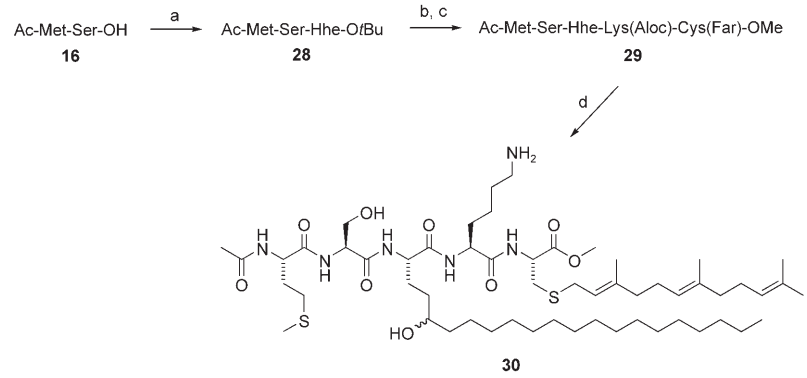
  




Scheme 5. Synthesis of the $C_{21}$ amino acid **27**. a) Isobutene, $H_2SO_4$, dioxane, 55% (**23**); b) AllBr, $NEt_3$, TBAI, THF, 80%; c) $LiBH_4$, THF, 71%; d) DMP, $CH_2Cl_2$; e) $C_{16}H_{33}MgBr$, THF, −78°C, 63%, two steps; f) [Pd($PPh_3$)$_4$], DMB, THF, 35°C, 75%. TBAI = tetrabutylammonium iodide, DMP = Dess–Martin periodinane.




Scheme 7. Synthesis of the central proline building block **38**. a) $Boc_2O$, NaOH, dioxane/$H_2O$ 2:1, 0°C → RT, 95% (**32**); b) $Cs_2CO_3$, MeOH; c) MeI, DMF, 88%, two steps; d) DMP, $CH_2Cl_2$, 81% (**34**); e) $(EtO)_2OPCH_2CN$ (**35**), NaH, THF, 85%; f) $H_2$, $PtO_2 \cdot H_2O$, EtOH, $CHCl_3$; g) AlocCl, $NEtiPr_2$, $CH_2Cl_2$, 95% (**37**), two steps; h) TFA/$CH_2Cl_2$ 1:1, quant. Boc = *tert*-butyloxycarbonyl.




Scheme 6. Synthesis of the pentapeptide analogue **30**. a) H-Hhe-O*t*Bu (**27**), EDC, HOBt, $CH_2Cl_2$, 0°C → RT, 69%; b) 1 M NaOH, MeOH; c) H-Lys(Aloc)-Cys(Far)-OMe (**19**), EDC, HOBt, $CH_2Cl_2$, 0°C → RT, 44%, two steps; d) [Pd($PPh_3$)$_4$], DMB, THF, 80%. Hhe = (2*S*)-amino-5-hydroxyheneicosanic acid.

be obtained in acceptable yield. Finally, Aloc deprotection led to the second pentapeptide analogue **30**.

**Synthesis of the proline derivatives**: The synthesis of proline derivatives **42** and **45** commenced with the protection of (4*R*)-hydroxy-L-proline (**31**) (Scheme 7). First, the amino function was blocked by a Boc group, and then the carboxylic acid was protected as a methyl ester. The resulting alcohol **33** was oxidized to the ketone by using Dess–Martin periodinane. A subsequent Horner–Wadsworth–Emmons reaction[31] yielded acrylic nitrile **36**. This intermediate was reduced via hydrogenation by using Adam's catalyst, and, without further purification, the resulting amino group was protected by an Aloc moiety to obtain proline derivative **37**. Cleavage of the Boc group yielded trifluoroacetate salt **38**, which is the central building block for the following syntheses of the two proline derivatives **42** and **45**.

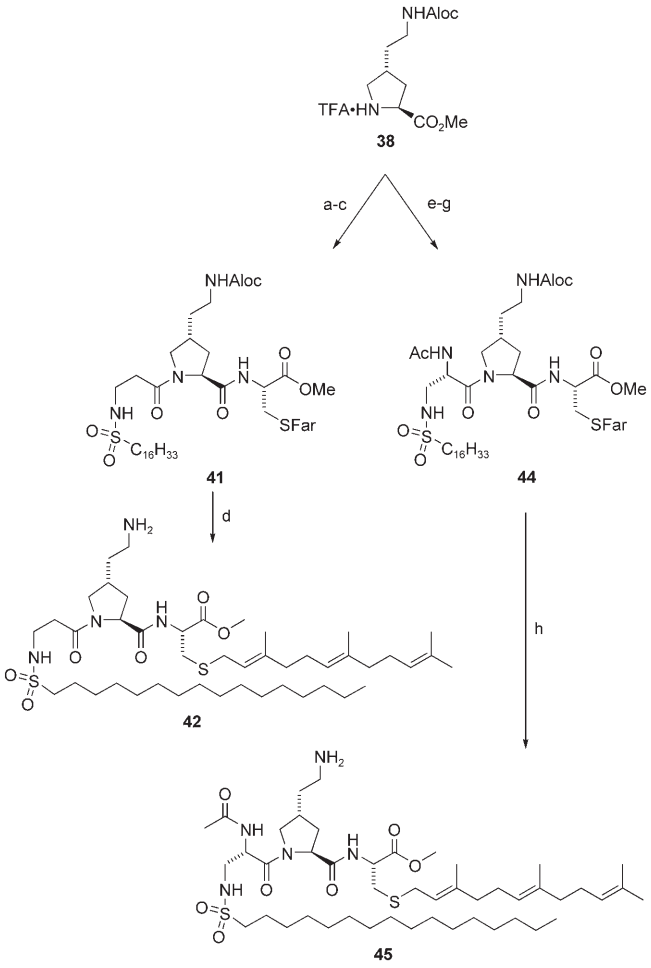
Compound **38** was coupled to $C_{16}H_{33}SO_2$-β-Ala-OH (**39**), and, after cleavage of the methyl ester with sodium hydroxide, H-Cys(Far)-OMe (**10**) was condensed to the C-terminus (Scheme 8). The resulting tripeptide **41** was finally deprotected to yield proline derivative **42**.

Coupling of Ac-Dap-($SO_2C_{16}H_{33}$)-OH (**4**) to compound **38** and again saponification and condensation of H-Cys(Far)-OMe (**10**) yielded tripeptide **44**. The Aloc group was then cleaved off leading to proline analogue **45**.

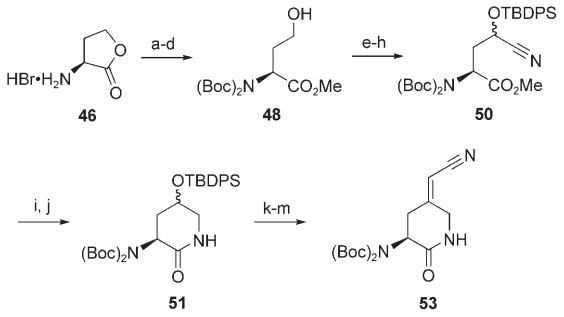
**Synthesis of the lactam derivatives**: For the synthesis of the lactam derivatives, (2*S*)-aminobutyrolactone hydrobromide (**46**) was doubly protected at the amino function with Boc groups (Scheme 9). The resulting lactone **47** was then opened by saponification by using CsOH and subsequently the obtained cesium salt of the carboxylic acid was esterified with methyl iodide to give homoserine **48**. After Swern oxidation[32] of the alcohol, TMSCN was used to generate a cyanohydrin from which the remaining TMS group was cleaved off by using ammonium fluoride. The free hydroxyl group of cyanohydrin **49** was then reprotected with the *tert*-butyldiphenylsilyl group to yield compound **50** in order to prevent the alcohol function from side reactions during ring closure. For this purpose, the cyano function was hydrogenated with 10% Pd on charcoal and the resulting amine was heated under reflux in toluene to give the lactam core structure **51** in good yield. The silyl group was cleaved, the resulting alcohol was oxidized with Dess–Martin periodinane, and a Horner–Wadsworth–Emmons reaction led to the *Z*-configured acrylic nitrile **53** as the single product. Structure determination was carried out by means of NOE spectroscopy. The coupling of the vinylic hydrogen with the β-hydrogens but not with the δ-hydrogens of the cyclic system is a strong argument for *Z* configuration of the double bond.

For the forthcoming alkylation of the lactam amide, the corresponding electrophile was needed. To this end, thioest-

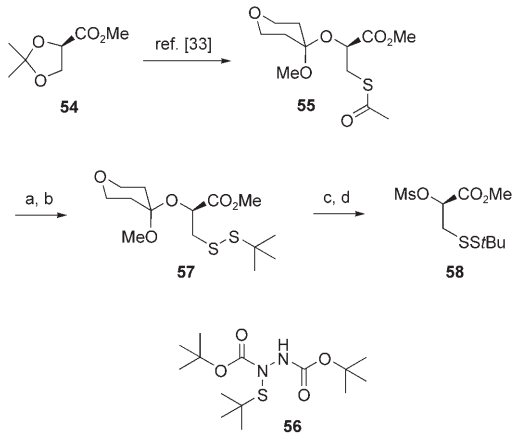
  




Scheme 8. Synthesis of the proline analogues **42** and **45**. a) $C_{16}H_{33}SO_2$-β-Ala-OH (**39**), EDC, HOBt, NEt*i*Pr$_2$, $CH_2Cl_2$, 0 °C → RT, 81 % (**40**); b) 1 M NaOH, MeOH; c) H-Cys(Far)-OMe (**10**), EDC, HOBt, $CH_2Cl_2$, 0 °C → RT, 87 %, two steps; d) [Pd(PPh$_3$)$_4$], DMB, THF, 79 %; e) Ac-Dap(S-$O_2C_{16}H_{33}$)-OH (**4**), EDC, HOBt, NEt*i*Pr$_2$, $CH_2Cl_2$, 0 °C → RT, 76 % (**43**); f) 1 M NaOH, MeOH; g) H-Cys(Far)-OMe (**10**), EDC, HOBt, $CH_2Cl_2$, 0 °C → RT, 87 %, two steps; h) [Pd(PPh$_3$)$_4$], DMB, THF, 64 %.




Scheme 9. Synthesis of the lactam building block **53**. a) $Boc_2O$, $NEt_3$, $CH_2Cl_2$, 0 °C → RT; b) $Boc_2O$, DMAP, $CH_3CN$, 94 % (**47**), two steps; c) CsOH, MeOH; d) MeI, DMF, 79 %, two steps; e) $(COCl)_2$, DMSO, −60 °C, NEt*i*Pr$_2$, −60 °C → RT; f) TMSCN, $CH_2Cl_2$; g) $NH_4F$, 90 % (**49**), three steps; h) TBDPSCl, imidazole, DMF, 90 %; i) $H_2$, Pd/C (10 %), MeOH, 0.1 % HOAc; j) toluene, reflux, 67 %, two steps; k) TBAF, THF, quant. (**52**); l) DMP, $CH_2Cl_2$; m) $(EtO)_2OPCH_2CN$ (**35**), NaH, THF, 90 %, two steps. DMAP = *N*,*N*-dimethylaminopyridine, TBAF = tetrabutylammonium fluoride, TBDPSCl = *tert*-butyldiphenylsilyl chloride, TMSCN = trimethylsilyl cyanide.



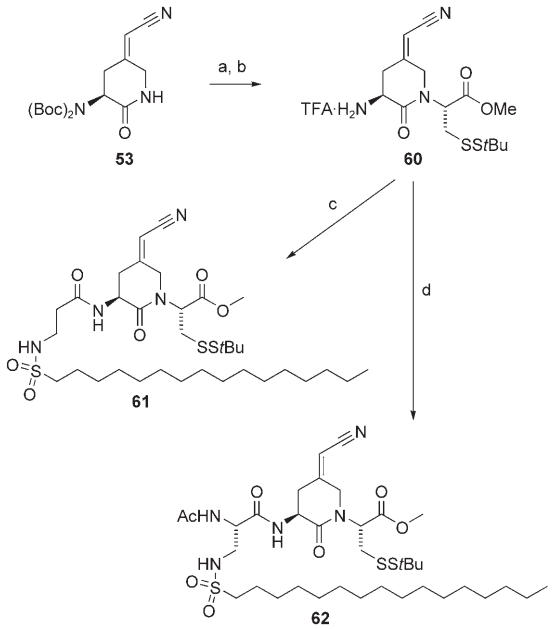

Scheme 10. Synthesis of the mesylate **58**. a) NaOMe, MeOH; b) **56**, DMF, 88 %, two steps; c) TsOH, MeOH; d) MsCl, pyridine, 0 °C → RT, quant., two steps.

er **55**, which was accessible following a literature procedure,[33] was treated with sodium methylate, and subsequently the free thiol group was protected as a *tert*-butyl disulfide using the methodology of Wünsch et al. (Scheme 10).[34] The methoxy tetrahydropyran group in **57** was cleaved and the resulting alcohol was then transformed into the desired mesylate **58**.

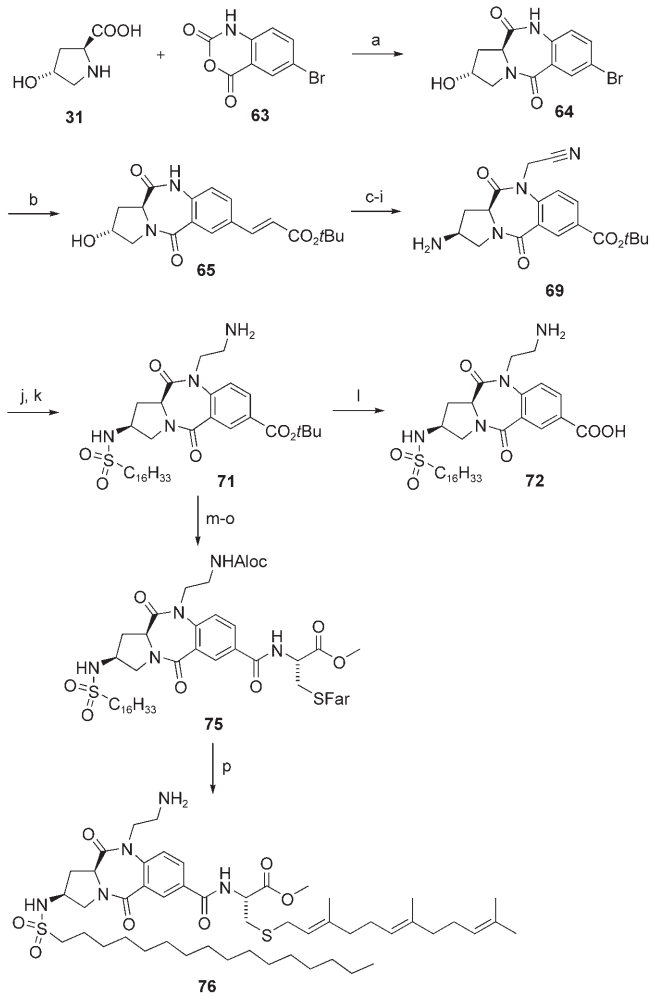
Several alkylation procedures were tried and the following proved to be the best: Mesylate **58** and lactam **53** were dissolved in THF and sodium hydride was added to deprotonate the lactam NH. After stirring for several hours the alkylated lactam **59** could be isolated in acceptable yield (Scheme 11). Deprotection of the Boc groups yielded the central structure **60**. Coupling of $C_{16}H_{33}SO_2$-β-Ala-OH (**39**) and Ac-Dap($SO_2C_{16}H_{33}$)-OH (**4**), respectively, gave the two lactam derivatives **61** and **62**. Unfortunately, the acrylic nitrile functionality in both compounds could not be reduced without complete loss of the disulfide moiety. However, the structures obtained had very interesting substitution patterns, so that a biological evaluation seemed worthwhile in any case. Therefore, further alternative syntheses were not investigated.

**Synthesis of the benzodiazepine derivatives**: The synthesis of the benzodiazepines started with the condensation of (4*R*)-hydroxy-L-proline (**31**) and 5-bromoisatoic acid anhydride (**63**) yielding the benzodiazepine core **64** (Scheme 12). A subsequent Heck reaction[35] with *tert*-butyl acrylate gave compound **65** in high yield. Then, the alcohol function was converted into a mesylate which was subsequently treated with $NaN_3$ to obtain azide **66**. Deprotonation of the amide NH and alkylation with bromoacetonitrile led to compound **67**, in which the acrylic double bond was cleaved by using $RuCl_3/NaIO_4$.[36] The resulting free carboxylic acid was protected with the *tert*-butyl group yielding benzodiazepine **68**



Scheme 11. Synthesis of the two lactams **61** and **62**. a) **58**, NaH, 0 °C → RT, 57 % (**59**); b) TFA/$CH_2Cl_2$ 1:1, 99 %; c) $C_{16}H_{33}SO_2$-β-Ala-OH (**39**), EDC, HOBt, NE*t*$Pr_2$, 0 °C → RT, 67 %; d) Ac-Dap($SO_2C_{16}H_{33}$)-OH (**4**), EDC, HOBt, NE*t*$Pr_2$, 0 °C → RT, 62 %.



Scheme 12. Synthesis of the benzodiazepines **72** and **76**. a) 140 °C, DMSO, 93 %; b) *tert*-butyl acrylate, [$Pd(OAc)_2$], P(*o*-tol)$_3$, $NEt_3$, $CH_3CN$, 100 °C, sealed tube, 97 %; c) MsCl, pyridine, 0 °C → RT; d) $NaN_3$, DMF, 45 °C, 89 % (**66**), two steps; e) NaH, THF, −40 °C; f) $BrCH_2CN$, −40 °C → RT, 95 % (**67**), two steps; g) $RuCl_3$, $NaIO_4$, $H_2O/CH_3CN/CCl_4$ 2:1:1; h) $Me_3CBr$, $K_2CO_3$, $Et_3(PhCH_2)NCl$, DMA, 55 °C, 90 % (**68**), two steps; i) $H_2$, Pd/$BaSO_4$, MeOH, $CHCl_3$, quant.; j) $C_{16}H_{33}SO_2Cl$, NE*t*$Pr_2$, DMF, 0 °C → RT, 82 % (**70**); k) $H_2$, $PtO_2$·$H_2O$, EtOH, $CHCl_3$, 81 %; l) HCl/$Et_2O$, quant.; m) AlocCl, $NEt_3$, $CH_2Cl_2$, 84 % (**73**); n) TFA/$CH_2Cl_2$ 1:1, quant. (**74**); o) H-Cys(Far)-OMe (**10**), EDC, HOBt, $CH_2Cl_2$, 0 °C → RT, 89 %; p) [$Pd(PPh_3)_4$], DMB, THF, 80 %. DMA = dimethylacetamide.

in very good yield. Selective hydrogenation of the azide using Pd on $BaSO_4$ gave amine **69**. The sulfonamide moiety was introduced by reaction with hexadecylsulfonic acid chloride leading to **70**; subsequent hydrogenation with $PtO_2$ catalysis generated amine **71**. From here, simple acidic cleavage of the *tert*-butyl ester gave the first benzodiazepine derivative **72**.

Alternatively, protection of amine **71** with the Aloc group, deprotection of the resulting compound **73** with trifluoroacetic acid and subsequent coupling of H-Cys(Far)-OMe (**10**) to the free acid **74** gave protected benzodiazepine **75** in very good yield. Finally, the Aloc group was removed leading to the dually lipidated benzodiazepine **76**.

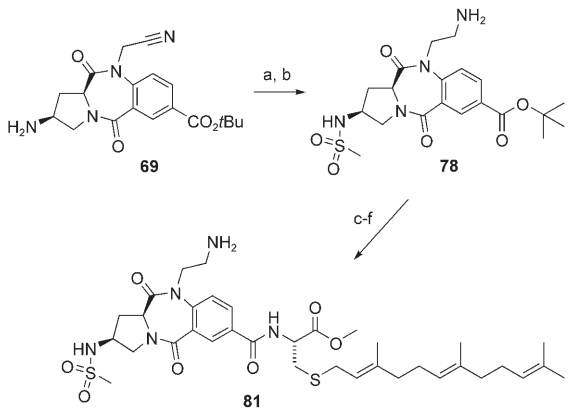
For the synthesis of monolipidated benzodiazepines, compound **69** was mesylated at the amino group to give **77**, and subsequently the nitrile function was hydrogenated by using Adam's catalyst (Scheme 13). The free amine in **78** was then blocked with an Aloc group yielding **79**; after the *tert*-butyl ester was cleaved off under acidic conditions, H-Cys(Far)-OMe (**10**) was condensed with the resulting free acid. Final deprotection of **80** using again $Pd^0$ chemistry led to the monolipidated benzodiazepine **81**.

**Results of the biological evaluation**: The syntheses detailed above had given access to 16 different compounds for biochemical evaluation. The results of the assay for inhibition of APT1 are shown in Table 1. A detailed procedure for the assay accompanied with the synthesis of a substrate peptide for APT1 is provided in the Experimental Section.

Comparison of the data given in Table 1 reveals that if a hexadecylsulfonamide moiety is present in the molecule, the $IC_{50}$ value is always in the low nanomolar range (see compounds **12**, **21**, **30**, **42**, **45**, **71**, **72**, **75** and **76**). This high activity of the compounds is important for their application in biological systems. The long fatty acid chains of the inhibitors are crucial for their inhibitory activity but they also decrease the polarity of the molecules and limit their solubility in aqueous solutions at high concentrations. Nevertheless, the high inhibitory activity of the compounds enables their application in several biological experiments.

The fact, that the tripeptide analogues are slightly more active than the corresponding pentapeptides, may result from a higher conformational flexibility of the pentapeptide backbone compared with the tripeptides. This may also explain the observation that the conformationally fixed proline




Scheme 13. Synthesis of the benzodiazepine **81**. a) MsCl, NEt*i*Pr$_2$, DMF, 0 °C → RT, 92 % (**77**); b) $H_2$, $PtO_2{\cdot}H_2O$, EtOH, $CHCl_3$, 85 %; c) AlocCl, $NEt_3$, $CH_2Cl_2$, 83 % (**79**); d) TFA/$CH_2Cl_2$ 1:1; e) H-Cys(Far)-OMe (**10**), EDC, HOBt, $CH_2Cl_2$, 0 °C → RT, 89 % (**80**), two steps; f) [Pd(PPh$_3$)$_4$], DMB, THF, 65 %.

and benzodiazepine derivatives are the most active inhibitors. If analogues with an Aloc group on the central amine are compared to those with a free amino group, the conclusion can be drawn, that the inhibitory potency of the protected compounds is about one order of magnitude lower (cf. **11**/**12**, **20**/**21**, **44**/**45** and **75**/**76**). The findings for lactam derivatives **61** and **62**, which have only a nitrile function, agree with this conclusion. Comparison of the benzodiazepines reveals that compounds **78** and **81**, which lack a hexadecyl group, are nearly inactive. The presence of a farnesyl group in **81** leads to an $IC_{50}$ value that is about one order of magnitude better than the corresponding *tert*-butyl ester **78**. Thus, at least one lipid group has to be present in the molecule for inhibition. In summary, a long chain sulfonamide moiety may be essential for nanomolar activity. If additionally a central free amine group is present, the activity is about one order of magnitude higher. Compounds with dual lipidation patterns are better inhibitors than monolipidated analogues, but the presence of a farnesyl group seems to be not essential for high activity.

## Conclusion

In conclusion, we have designed, synthesized, and evaluated different inhibitors of acyl protein thioesterase 1. Several inhibitors with an activity in the low nanomolar range could be found. A combinatorial approach starting from the highly active benzodiazepines may lead to even better inhibitors of APT1 with higher diversity. Following this approach, more precise structure–activity relationships could be obtained. Additionally, the application of the highly potent compounds in biophysical and biological experiments can serve to elucidate the physiological significance of APT1 in the reversible palmitoylation of signalling proteins.[27]

## Experimental Section

**General procedures**: $^1$H and $^{13}$C NMR spectra were recorded on a Bruker AC 250, AM 400 or DRX 500 and a Varian Mercury 400 spectrometer at room temperature. Mass spectra and high-resolution mass spectra (HRMS) were measured on a Finnigan MAT MS70 spectrometer. The optical rotation was determined with a Perkin–Elmer polarimeter 241.

**Materials**: When not otherwise indicated, all reactions were performed under argon atmosphere with freshly distilled and dried solvents. The solvents were dried following standard methods. Silica gel (40–60 μm) was used for column chromatography. Commercial reagents were used without further purification.

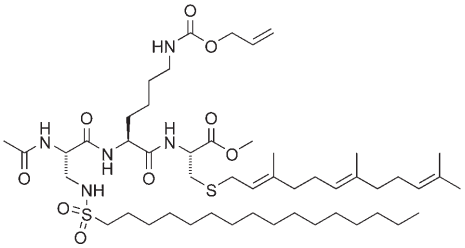
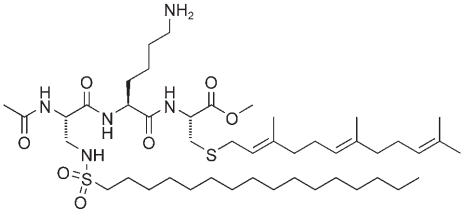
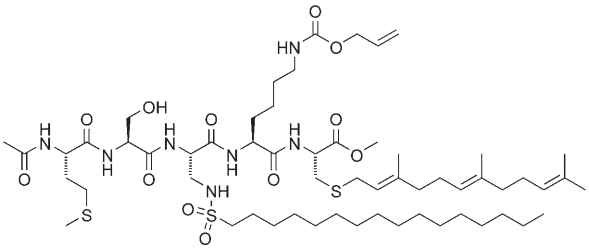
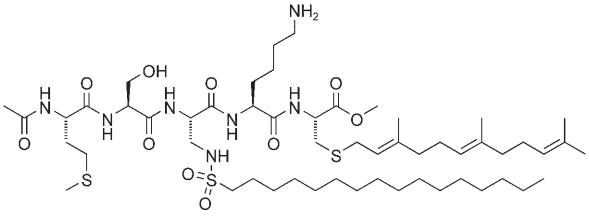
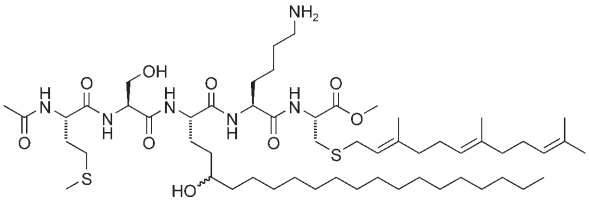
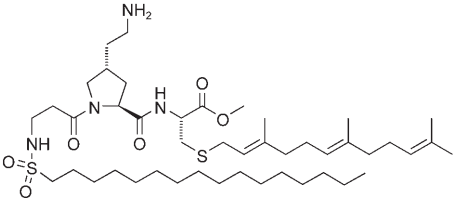
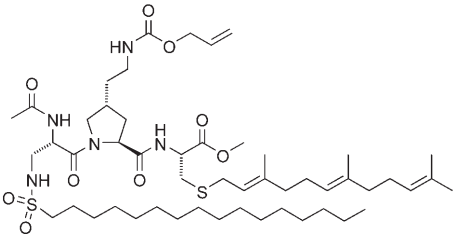
**(2*S*)-Acetylamino-3-(hexadecane-1-sulfonylamino)propionic acid allyl ester (3)**: At 0 °C, NEt*i*Pr$_2$ (1.04 mL, 6.06 mmol) and a solution of hexadecanesulfonic acid chloride (965 mg, 2.91 mmol) in DMF (10 mL) were subsequently added to a solution of hydrochloride **2** (540 mg, 2.43 mmol) in DMF (5 mL). The solution was warmed to room temperature and stirred for 20 h. Then, the reaction mixture was diluted with ethyl acetate (150 mL) and extracted with 1 N HCl (3×30 mL). The organic layer was dried over $Na_2SO_4$, and, after removal of the solvent under reduced pressure, the residue was purified by chromatography (*n*-hexane/ethyl acetate 1:1) to yield **3** as a colourless solid (865 mg, 75 %). M.p. 70 °C; $[\alpha]_D^{20}$ = +17.5 ($c$ = 1.0 in $CHCl_3$); $R_f$ = 0.20 (cyclohexane/ethylacetate 1:1); $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ = 0.84 (t, $J$ = 6.6 Hz, 3H, ω-$CH_3$ hexadecyl), 1.24 (m, 24H, 12×$CH_2$ hexadecyl), 1.38 (m, 2H, γ-$CH_2$ hexadecyl), 1.72 (m, 2H, β-$CH_2$ hexadecyl), 2.08 (s, 3H, $CH_3$ acetyl), 2.95 (m, 2H, α-$CH_2$ hexadecyl), 3.54 (m, 2H, β-$CH_2$ Dap), 4.62 (m, 1H, -OC$H_{2a}$CH=$CH_2$), 4.73 (m, 2H, α-CH Dap, -OC$H_{2b}$CH=$CH_2$), 5.23–5.36 (m, 2H, -OCH$_2$CH=C$H_2$), 5.66 (t, $J$ = 6.8 Hz, 1H, NH), 5.91 (m, 1H, -OCH$_2$C$H$=$CH_2$), 7.11 (d, $J$ = 7.6 Hz, 1H, NH); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta$ = 14.3 (ω-$CH_3$ hexadecyl), 22.8 ($CH_3$ acetyl), 23.1 ($CH_2$), 23.8 ($CH_2$), 28.4 ($CH_2$), 29.5 ($CH_2$), 29.6 ($CH_2$), 29.7 ($CH_2$), 29.8 ($CH_2$), 29.8 ($CH_2$), 29.8 ($CH_2$), 29.8 ($CH_2$), 29.9 ($CH_2$), 30.0 ($CH_2$), 30.2 ($CH_2$), 32.1 ($CH_2$), 44.6 (β-$CH_2$ Dap), 53.2 (α-$CH_2$ hexadecyl), 66.9 (OC$H_2$CH=$CH_2$), 119.5 (OCH$_2$CH=C$H_2$), 131.5 (OCH$_2$C$H$=$CH_2$), 170.2 (C=O), 171.0 (C=O); HRMS (FAB, 3-NBA): $m/z$: calcd for $C_{24}H_{47}N_2O_5S$: 475.3206, found: 475.3230 $[M+H]^+$.

**(2*S*)-Acetylamino-3-(hexadecane-1-sulfonylamino)propionic acid (4)**: [Pd(PPh$_3$)$_4$] (77 mg, 0.07 mmol) was added to a solution of allyl ester **3** (530 mg, 1.12 mmol) and *N*,*N*′-dimethylbarbituric acid (104 mg, 0.67 mmol) in THF (40 mL). The mixture was stirred at room temperature for 1.5 h. After removal of the solvent under reduced pressure, the residue was purified by chromatography ($CH_2Cl_2$/EtOH 10:1 + 1 % HOAc) to yield **4** as a colorless solid (480 mg, 99 %). M.p. 91 °C; $[\alpha]_D^{20}$ = +15.7 ($c$ = 1.0 in MeOH); $R_f$ = 0.22 ($CH_2Cl_2$/EtOH 10:1 + 1 % HOAc); $^1$H NMR ($CD_3OD$, 400 MHz): $\delta$ = 0.87 (t, $J$ = 6.6 Hz, 3H, ω-$CH_3$ hexadecyl), 1.30 (m, 24H, 12×$CH_2$ hexadecyl), 1.41 (m, 2H, γ-$CH_2$ hexadecyl), 1.79 (m, 2H, β-$CH_2$ hexadecyl), 2.03 (s, 3H, $CH_3$ acetyl), 3.04 (m, 2H, α-$CH_2$ hexadecyl), 3.52 (m, 2H, β-$CH_2$ Dap), 4.51 (m, 1H, α-CH Dap); $^{13}$C NMR ($CD_3OD$, 100.6 MHz): $\delta$ = 13.2 (ω-$CH_3$ hexadecyl), 21.6 ($CH_3$ acetyl), 23.1 ($CH_2$), 23.8 ($CH_2$), 28.4 ($CH_2$), 29.5 ($CH_2$), 29.6 ($CH_2$), 29.7 ($CH_2$), 29.8 ($CH_2$), 29.8 ($CH_2$), 29.8 ($CH_2$), 29.8 ($CH_2$), 29.9 ($CH_2$), 30.0 ($CH_2$), 30.2 ($CH_2$), 32.1 ($CH_2$), 43.3 (β-$CH_2$ Dap), 52.0 (α-$CH_2$ hexadecyl), 53.3 (α-CH Dap), 170.2 (C=O), 171.3 (C=O); HRMS (FAB, 3-NBA): $m/z$: calcd for $C_{21}H_{42}N_2NaO_5S$: 457.2714, found: 457.2682 $[M+Na]^+$.

**(2*S*)-Amino-3-(hexadecane-1-sulfonylamino)propionic acid allyl ester (6)**: At −70 °C, a solution of hexadecanesulfonic acid chloride (136 mg, 0.41 mmol) in $CH_2Cl_2$ (2 mL) was added to a solution of hydrotosylate **5** (200 mg, 0.41 mmol) and $NEt_3$ (174 μL, 2.37 mmol) in $CH_2Cl_2$ (2 mL). The solution was warmed to room temperature and stirred for 15 h. Then, the reaction mixture was diluted with $CH_2Cl_2$ (20 mL) and washed with $Na_2CO_3$ solution (10 %, 10 mL). The organic layer was dried over $Na_2SO_4$, and, after removal of the solvent under reduced pressure, the residue was purified by chromatography ($CHCl_3$/MeOH 5:1) to yield **6** as a colorless solid (129 mg, 72 %). M.p. 73 °C; $[\alpha]_D^{20}$ = +29.5 ($c$ = 1.0 in $CHCl_3$); $R_f$ = 0.58 ($CHCl_3$/MeOH 5:1); $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ =

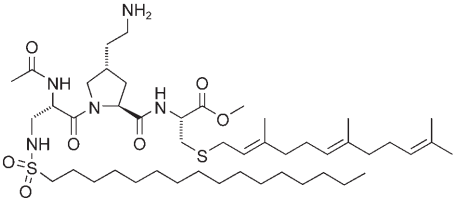
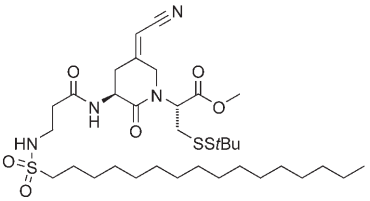
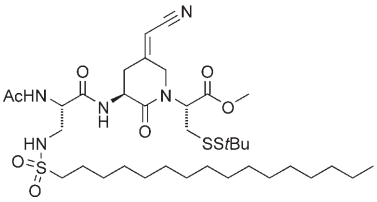
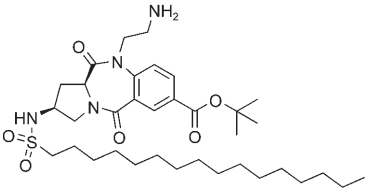
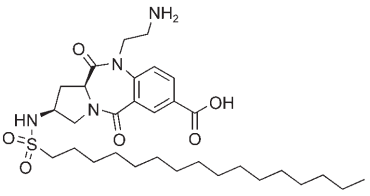
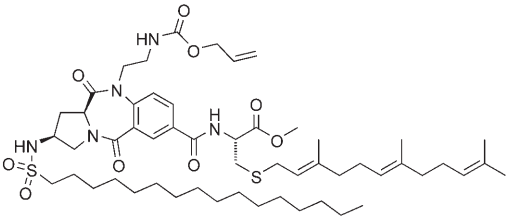
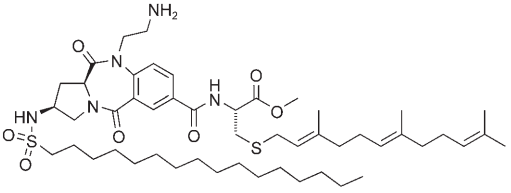
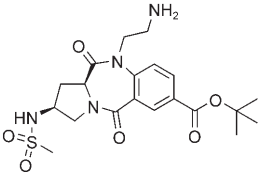
Table 1. Inhibition of APT1 by the synthesized compounds.[a]

| Entry | Compound | Structure | $IC_{50}$ [nM] |
|---|---|---|---|
| 1 | **11** |  | 153 ± 21 |
| 2 | **12** |  | 65 ± 3 |
| 3 | **20** |  | 1583 ± 240 |
| 4 | **21** |  | 291 ± 11 |
| 5 | **30** |  | 147 ± 20 |
| 6 | **42** |  | 61 ± 6 |
| 7 | **44** |  | 1223 ± 258 |

0.84 (t, $J$ = 6.5 Hz, 3H, ω-$CH_3$ hexadecyl), 1.22 (m, 24H, 12 × $CH_2$ hexadecyl), 1.36 (m, 2H, γ-$CH_2$ hexadecyl), 1.76 (m, 2H, β-$CH_2$ hexadecyl), 2.98 (m, 2H, α-$CH_2$ hexadecyl), 3.20 (dd, $J$ = 6.8, 13.1 Hz, 1H, β-$CH_{2a}$ Dap), 3.40 (dd, $J$ = 4.5, 13.1 Hz, 1H, β-$CH_{2b}$ Dap), 3.63 (dd, $J$ = 4.5, 6.8 Hz, 1H, α-CH Dap), 4.61 (m, 2H, -OC$H_2$CH=$CH_2$), 5.21–5.36 (m, 2H, -OCH$_2$CH=C$H_2$), 5.91 (m, 1H, -OCH$_2$C$H$=CH$_2$); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta$ = 14.3 (ω-$CH_3$ hexadecyl), 22.8 ($CH_2$), 23.7 ($CH_2$), 28.5 ($CH_2$), 28.6 ($CH_2$), 28.7 ($CH_2$), 28.9 ($CH_2$), 29.1 ($CH_2$), 29.2 ($CH_2$), 29.3 ($CH_2$), 29.3 ($CH_2$), 29.3 ($CH_2$), 29.5 ($CH_2$), 29.8 ($CH_2$), 32.1 ($CH_2$), 46.2 (β-$CH_2$ Dap), 53.0 (α-CH Dap), 54.5 (α-$CH_2$ hexadecyl), 66.3 (O$C$H$_2$CH=CH$_2$), 119.2 (OCH$_2$C$H$=$C$H$_2$), 131.7 (OCH$_2$C$H$=CH$_2$), 173.1 (C=O); HRMS (FAB, 3-NBA): $m/z$: calcd for $C_{22}H_{45}N_2O_4S$: 433.3101, found: 433.3120 $[M+H]^+$.

***N*$^{\alpha}$-[(2*S*)-Acetylamino-3-(hexadecane-1-sulfonylamino-propionyl]-*N*$^{\varepsilon}$-(allyloxycarbonyl)-L-lysine *tert*-butyl ester (8)**: At 0 °C, EDC (54 mg, 0.28 mmol) was added to a solution of diaminopropionic acid (**4**) (100 mg, 0.23 mmol), lysine **7** (66 mg, 0.23 mmol) and HOBt (72 mg, 0.46 mmol) in $CH_2Cl_2$ (8 mL). The solution was warmed to room temperature and stirred for 18 h. Then, the reaction mixture was diluted with ethyl acetate (50 mL) and subsequently washed with 1N HCl (20 mL) and $Na_2CO_3$ solution (10%, 20 mL). The organic layer was dried over $Na_2SO_4$, and, after removal of the solvent under reduced pressure, the residue was purified by chromatography (cyclohexane/ethyl acetate 1:3) to yield **8** as a colorless solid (132 mg, 82%). M.p. 80 °C; $[\alpha]_D^{20}$ = −12.1 ($c$ = 1.0 in $CHCl_3$); $R_f$ = 0.24 (cyclohexane/ethyl acetate 1:3); $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ = 0.81 (t, $J$ = 6.5 Hz, 3H, ω-$CH_3$ hexadecyl), 1.48 (s, 9H, C-($CH_3$)$_3$), 1.12–1.52 (m, 30H, 13 × $CH_2$ hexadecyl, 2 × $CH_2$ Lys), 1.54–1.85 (m, 4H, γ-$CH_2$ hexadecyl, $CH_2$ Lys), 2.02 (s, 3H, $CH_3$ acetyl), 2.96 (m, 2H, α-$CH_2$ hexadecyl), 3.02–3.29 (m, 3H, β-$CH_{2a}$ Dap, ε-$CH_2$ Lys), 3.56 (m, 1H, β-$CH_{2b}$ Dap), 4.30 (m, 1H, α-CH Lys), 4.52 (m, 3H, α-CH Dap, -OC$H_2$CH=CH$_2$), 4.95 (m, 1H, NH), 5.10–5.25 (m, 2H, -OCH$_2$CH=C$H_2$), 5.76 (m, 1H, NH), 5.88 (m, 1H, -OCH$_2$C$H$=CH$_2$), 6.70 (m, 1H, NH), 7.19 (m, 1H, NH); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta$ = 14.3 (ω-$CH_3$ hexadecyl), 22.9 ($CH_3$ acetyl), 23.1 ($CH_2$), 23.8 ($CH_2$), 28.3 (C($C$H$_3$)$_3$), 28.4 ($CH_2$), 28.5 ($CH_2$), 29.2 ($CH_2$), 29.3 ($CH_2$), 29.5 ($CH_2$), 29.6 ($CH_2$), 29.7 ($CH_2$), 29.7 ($CH_2$), 29.7 ($CH_2$), 29.8 ($CH_2$), 29.9 ($CH_2$),

Table 1. (Continued)

| Entry | Compound | Structure | $IC_{50}$ [nM] |
|---|---|---|---|
| 8 | **45** |  | 140 ± 18 |
| 9 | **61** |  | 725 ± 19 |
| 10 | **62** |  | 975 ± 7 |
| 11 | **71** |  | 148 ± 6 |
| 12 | **72** |  | 97 ± 8 |
| 13 | **75** |  | 149 ± 30 |
| 14 | **76** |  | 27 ± 5 |
| 15 | **78** |  | 252208 ± 159000 |

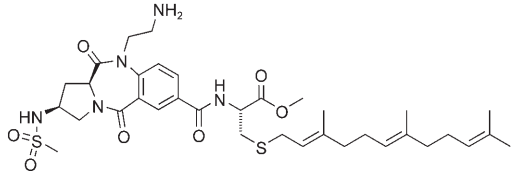
29.9 ($CH_2$), 30.0 ($CH_2$), 30.2 ($CH_2$), 32.1 ($CH_2$), 43.3 (β-$CH_2$ Dap), 44.6 (ε-$CH_2$ Lys), 53.5 (α-CH), 54.2 (α-$CH_2$ hexadecyl), 56.0 (α-CH), 67.3 ($OCH_2CH{=}CH_2$), 82.4 ($C(CH_3)_3$), 115.1 ($OCH_2CH{=}CH_2$), 137.4 ($OCH_2CH{=}CH_2$), 157.5 (C=O), 172.1 (C=O), 174.7 (C=O), 174.8 (C=O); HRMS (FAB, 3-NBA): *m*/*z*: calcd for $C_{35}H_{66}N_4NaO_8S$: 725.4501, found: 725.4512 $[M+Na]^+$.

***N*$^{\alpha}$-[(2*S*)-Acetylamino-3-(hexadecane-1-sulfonylamino)propionyl]-*N*$^{\varepsilon}$-allyloxycarbonyl-L-lysine (9)**: TFA (10 mL) was added to a solution of *tert*-butyl ester **8** (117 mg, 0.17 mmol) in $CH_2Cl_2$ (10 mL) at room temperature. The solution was stirred for 4 h. Then, the solvent was removed under reduced pressure. Coevaporation of the residue with toluene yielded **9** as a colorless solid (107 mg, 100%). M.p. 83°C; $[\alpha]_D^{20} = -14.0$ ($c = 1.0$ in MeOH); $R_f = 0.01$ (cyclohexane/ethyl acetate 1:3); $^1$H NMR ($CD_3OD$, 400 MHz): $\delta = 0.85$ (t, $J = 6.5$ Hz, 3H, ω-$CH_3$ hexadecyl), 1.12–1.52 (m, 30H, 13 × $CH_2$ hexadecyl, 2 × $CH_2$ Lys), 1.54–1.85 (m, 4H, γ-$CH_2$ hexadecyl, $CH_2$ Lys), 2.02 (s, 3H, $CH_3$ acetyl), 2.96 (m, 2H, α-$CH_2$ hexadecyl), 3.02–3.29 (m, 3H, β-$CH_{2a}$ Dap, ε-$CH_2$ Lys), 3.56 (m, 1H, β-$CH_{2b}$ Dap), 4.30 (m, 1H, α-CH Lys), 4.52 (m, 3H, α-CH Dap, -$OCH_2CH{=}CH_2$), 5.10–5.25 (m, 2H, -$OCH_2CH{=}CH_2$), 5.88 (m, 1H, -$OCH_2CH{=}CH_2$); $^{13}$C NMR ($CD_3OD$, 100.6 MHz): $\delta =$ 14.2 (ω-$CH_3$ hexadecyl), 22.9 ($CH_3$ acetyl), 23.1 ($CH_2$), 23.8 ($CH_2$), 28.3 ($CH_2$), 28.4 ($CH_2$), 28.5 ($CH_2$), 29.4 ($CH_2$), 29.5 ($CH_2$), 29.6 ($CH_2$), 29.7 ($CH_2$), 29.7 ($CH_2$), 29.8 ($CH_2$), 29.8 ($CH_2$), 29.9 ($CH_2$), 29.9 ($CH_2$), 30.0 ($CH_2$), 30.2 ($CH_2$), 32.1 ($CH_2$), 43.3 (β-$CH_2$ Dap), 44.6 (ε-$CH_2$ Lys), 53.5 (α-CH), 54.2 (α-$CH_2$ hexadecyl), 56.0 (α-CH), 67.3 ($OCH_2CH{=}CH_2$), 115.1 ($OCH_2CH{=}CH_2$), 137.4 ($OCH_2CH{=}CH_2$), 157.5 (C=O), 172.1 (C=O), 174.7 (C=O), 175.8 (C=O); HRMS (FAB, 3-NBA): *m*/*z*: calcd for $C_{31}H_{58}N_4NaO_8S$: 669.3875, found: 669.3887 $[M+Na]^+$.

***N*$^{\alpha}$-[(2*S*)-Acetylamino-3-(hexadecane-1-sulfonylamino)propionyl]-*N*$^{\varepsilon}$-allyloxycarbonyl-L-lysyl-*S*-farnesyl-L-cysteine methyl ester (11)**: At 0°C, EDC (36 mg, 0.19 mmol) was added to a solution of dipeptide **9** (100 mg, 0.16 mmol), cysteine **10** (53 mg, 0.16 mmol) and HOBt (72 mg, 0.46 mmol) in $CH_2Cl_2$ (10 mL). The solution was warmed to room temperature and stirred for 18 h. Then, the reaction mixture was diluted with ethyl acetate (50 mL) and subsequently washed with 1N HCl (20 mL) and $Na_2CO_3$ solution (10%, 20 mL). The organic layer was dried over $Na_2SO_4$,

Table 1. (Continued)

| Entry | Compound | Structure | $IC_{50}$ [nM] |
|---|---|---|---|
| 16 | **81** |  | 26975 ± 17000 |

[a] For the determination of $IC_{50}$ values, the described compounds were incubated with enzyme (APT1) and substrate (a palmitoylated C-terminal fragment of the H-Ras protein) at six different concentrations. The released palmitate, which directly correlates with the enzyme activity was determined according to the ADIFAB assay protocol in two independent measurements.

and, after removal of the solvent under reduced pressure, the residue was purified by chromatography (cyclohexane/ethyl acetate 1:5) to yield **11** as a colorless oil (135 mg, 90%). $[\alpha]_D^{20} = +5.8$ ($c = 0.5$ in $CHCl_3$); $R_f = 0.22$ (cyclohexane/ethyl acetate 1:5); $^1$H NMR ($CDCl_3/CD_3OD$ 1:1, 400 MHz): $\delta = 0.81$ (t, $J = 6.5$ Hz, 3H, ω-$CH_3$ hexadecyl), 1.24 (m, 24H, 12×$CH_2$ hexadecyl), 1.58 (s, 6H, 2×$CH_3$ Far), 1.67 (s, 6H, 2×$CH_3$ Far), 1.35–1.84 (m, 8H, 2×$CH_2$ Lys, 2×$CH_2$ hexadecyl), 2.04 (s, 3H, $CH_3$ acetyl), 1.85–2.14 (m, 10H, $CH_2$ Lys, 4×$CH_2$ Far), 2.68–3.28 (m, 10H, α-$CH_2$ hexadecyl, β-$CH_2$ Cys, -S-$CH_2$, β-$CH_2$ Dap, ε-$CH_2$ Lys), 3.71 (s, 3H, $OCH_3$), 4.40 (m, 1H, α-CH Lys), 4.52 (m, 3H, α-CH Dap, -OC*H*$_2$CH=$CH_2$), 4.72 (m, 1H, α-CH Cys), 5.12–5.30 (m, 5H, 3×CH Far, -$OCH_2$CH=C*H*$_2$), 5.91 (m, 1H, -$OCH_2$C*H*=$CH_2$), 6.10 (m, 1H, NH), 6.99 (m, 2H, NH), 7.21 (m, 1H, NH); $^{13}$C NMR ($CDCl_3/CD_3OD$ 1:1, 100.6 MHz): $\delta = 14.3$ (ω-$CH_3$ hexadecyl), 16.5 ($CH_3$ acetyl), 17.0 ($CH_3$ Far), 17.2 ($CH_3$ Far), 19.3 ($CH_3$ Far), 23.1 ($CH_2$), 23.8 ($CH_2$), 25.3 ($CH_3$ Far), 28.3 ($CH_2$), 28.4 ($CH_2$), 28.5 ($CH_2$), 29.4 ($CH_2$), 29.5 ($CH_2$), 29.5 ($CH_2$), 29.6 ($CH_2$), 29.7 ($CH_2$), 29.8 ($CH_2$), 29.8 ($CH_2$), 29.8 ($CH_2$), 29.8 ($CH_2$), 29.9 ($CH_2$), 30.0 ($CH_2$), 30.2 ($CH_2$), 32.1 ($CH_2$), 33.0 ($CH_2$), 33.3 ($CH_2$), 34.4 ($CH_2$), 39.8 ($CH_2$ Far), 40.5 ($CH_2$ Far), 42.0 (β-$CH_2$ Dap), 44.6 (ε-$CH_2$ Lys), 50.4 ($OCH_3$), 53.4 (α-CH), 54.2 (α-CH), 54.5 (α-$CH_2$ hexadecyl), 56.0 (α-CH), 67.3 (O*C*$H_2$CH=$CH_2$), 115.1 ($OCH_2$CH=*C*$H_2$), 117.1 (CH Far), 121.7 (CH Far), 122.5 (CH Far), 133.0 (C Far), 137.5 ($OCH_2$*C*H=$CH_2$), 138.8 (C Far), 139.3 (C Far), 157.5 (C=O), 170.9 (C=O), 172.0 (C=O), 174.7 (C=O), 174.8 (C=O); HRMS (FAB, 3-NBA): *m*/*z*: calcd for $C_{50}H_{89}N_5NaO_9S_2$: 990.6002, found: 990.6030 $[M+Na]^+$.

***N*$^{\alpha}$-[(2*S*)-Acetylamino-3-(hexadecane-1-sulfonylamino)propionyl]-L-lysyl-*S*-farnesyl-L-cysteine methyl ester (12)**: $[Pd(PPh_3)_4]$ (7 mg, 0.01 mmol) was added to a solution of tripeptide **11** (61 mg, 0.06 mmol) and *N*,*N*′-dimethylbarbituric acid (10 mg, 0.06 mmol) in THF (6 mL). The mixture was stirred at room temperature for 8 h. After removal of the solvent under reduced pressure, the residue was purified by chromatography ($CH_2Cl_2$/EtOH 5:1 + 1% $NEt_3$) to yield **12** as a colorless oil (41 mg, 73%). $[\alpha]_D^{20} = +8.1$ ($c = 0.5$ in MeOH); $R_f = 0.04$ ($CH_2Cl_2$/EtOH 1:1); $^1$H NMR ($CD_3OD$, 400 MHz): $\delta = 0.81$ (t, $J = 6.5$ Hz, 3H, ω-$CH_3$ hexadecyl), 1.24 (m, 24H, 12×$CH_2$ hexadecyl), 1.58 (s, 6H, 2×$CH_3$ Far), 1.67 (s, 6H, 2×$CH_3$ Far), 1.35–1.84 (m, 8H, 2×$CH_2$ Lys, 2×$CH_2$ hexadecyl), 2.04 (s, 3H, $CH_3$ acetyl), 1.85–2.14 (m, 10H, $CH_2$ Lys, 4×$CH_2$ Far), 2.68–3.28 (m, 10H, α-$CH_2$ hexadecyl, β-$CH_2$ Cys, -S-$CH_2$, β-$CH_2$ Dap, ε-$CH_2$ Lys), 3.71 (s, 3H, $OCH_3$), 4.20 (m, 1H, α-CH Lys), 4.25 (m, 1H, α-CH Dap), 4.45 (m, 1H, α-CH Cys), 4.90 (m, 2H, 2×CH Far), 5.02 (m, 1H, CH Far); $^{13}$C NMR ($CD_3OD$, 100.6 MHz): $\delta = 14.3$ (ω-$CH_3$ hexadecyl), 16.5 ($CH_3$ acetyl), 17.0 ($CH_3$ Far), 17.2 ($CH_3$ Far), 19.3 ($CH_3$ Far), 23.1 ($CH_2$), 23.8 ($CH_2$), 25.3 ($CH_3$ Far), 28.3 ($CH_2$), 28.4 ($CH_2$), 28.5 ($CH_2$), 29.4 ($CH_2$), 29.5 ($CH_2$), 29.5 ($CH_2$), 29.6 ($CH_2$), 29.7 ($CH_2$), 29.8 ($CH_2$), 29.8 ($CH_2$), 29.8 ($CH_2$), 29.8 ($CH_2$), 29.9 ($CH_2$), 30.0 ($CH_2$), 30.2 ($CH_2$), 32.1 ($CH_2$), 33.0 ($CH_2$), 33.3 ($CH_2$), 34.4 ($CH_2$), 39.8 ($CH_2$ Far), 40.5 ($CH_2$ Far), 42.0 (β-$CH_2$ Dap), 44.6 (ε-$CH_2$ Lys), 50.4 ($OCH_3$), 53.4 (α-CH), 54.2 (α-CH), 54.5 (α-$CH_2$ hexadecyl), 56.0 (α-CH), 117.1 (CH Far), 121.7 (CH Far), 122.5 (CH Far), 133.0 (C Far), 138.8 (C Far), 139.3 (C Far), 170.9 (C=O), 172.0 (C=O), 174.7 (C=O), 174.8 (C=O); HRMS (FAB, 3-NBA): *m*/*z*: calcd for $C_{46}H_{86}N_5O_7S_2$: 884.5969, found: 884.5936 $[M+H]^+$.

***N*-Acetyl-L-methionyl-L-serine benzyl ester (15)**: At 0°C, EDC (4.036 g, 20.63 mmol) was added to a solution of Ac-Met-OH **13** (3.288 g, 17.19 mmol), HCl·H-Ser-OBzl **14** (3.983 g, 17.19 mmol), HOBt (5.372 g, 34.38 mmol) and $NEt_3$ (3.6 mL, 25.79 mmol) in $CH_2Cl_2$ (100 mL). The solution was warmed to room temperature and stirred for 20 h. Then, the reaction mixture was subsequently washed with 1N HCl (50 mL) and $Na_2CO_3$ solution (10%, 50 mL). The organic layer was dried over $Na_2SO_4$, and, after removal of the solvent under reduced pressure, the residue was purified by chromatography ($CHCl_3$/MeOH 50:1) to yield **15** as a colorless solid (5.447 g, 86%). M.p. 57°C; $[\alpha]_D^{20} = -21.4$ ($c = 1.0$ in $CHCl_3$); $R_f = 0.17$ (cyclohexane/ethyl acetate 1:3); $^1$H NMR ($CD_3OD$, 400 MHz): $\delta = 1.92$ (m, 1H, β-$CH_{2a}$ Met), 2.01 (s, 3H, $CH_3$ acetyl), 2.06 (m, 1H, β-$CH_{2b}$ Met), 2.08 (s, 3H, $CH_3$ Met), 2.55 (m, 2H, γ-$CH_2$ Met), 3.85 (dd, $J = 3.7$, 11.3 Hz, 1H, β-$CH_{2a}$ Ser), 3.99 (dd, $J = 3.7$, 11.3 Hz, 1H, β-$CH_{2b}$ Ser), 4.54 (dd, $J = 6.1$, 8.0 Hz, 1H, α-CH Met), 4.61 (t, $J = 3.9$ Hz, 1H, α-CH Ser), 5.21 (s, 2H, -$OCH_2$-), 7.31–7.38 (m, 5H, arom. CH); $^{13}$C NMR ($CD_3OD$, 100.6 MHz): $\delta = 18.7$ ($CH_3$ acetyl), 25.9 ($CH_3$ Met), 33.9 (γ-$CH_2$ Met), 35.5 (β-$CH_2$ Met), 56.5 (α-CH Met), 59.0 (α-CH Ser), 65.7 (β-$CH_2$ Ser), 71.2 ($OCH_2$), 132.15 (arom. CH), 132.3 (arom.CH), 132.5 (arom. CH), 139.4 (arom. C), 174.1 (C=O), 176.0 (C=O), 176.3 (C=O); HRMS (FAB, 3-NBA): *m*/*z*: calcd for $C_{17}H_{25}N_2O_5S$: 369.1485, found: 369.1493 $[M+H]^+$.

***N*-Acetyl-L-methionyl-L-serine (16)**: Pd (10% on charcoal, 354 mg) was added to a degassed solution of dipeptide **15** (2 g, 5.43 mmol) in MeOH (125 mL) and acetic acid (12.5 mL). The reaction mixture was hydrogenated under hydrogen atmosphere at room temperature for 3 d. Then, the suspension was filtered through a pad of Celite. After removal of the solvents under reduced pressure, the residue was purified by chromatography ($CHCl_3$/MeOH 10:1) to yield **16** as a colorless solid (1.496 g, 99%). M.p. 93°C; $[\alpha]_D^{20} = -17.0$ ($c = 1.0$ in MeOH); $R_f = 0.19$ ($CHCl_3$/MeOH 5:1); $^1$H NMR ($CD_3OD$, 400 MHz): $\delta = 1.98$ (m, 1H, β-$CH_{2a}$ Met), 2.05 (s, 3H, $CH_3$ acetyl), 2.13 (m, 1H, β-$CH_{2b}$ Met), 2.14 (s, 3H, $CH_3$ Met), 2.60 (m, 2H, γ-$CH_2$ Met), 3.85 (dd, $J = 3.7$, 11.3 Hz, 1H, β-$CH_{2a}$ Ser), 3.99 (dd, $J = 3.7$, 11.3 Hz, 1H, β-$CH_{2b}$ Ser), 4.39 (dd, $J = 6.1$, 8.0 Hz, 1H, α-CH Met), 4.54 (t, $J = 3.9$ Hz, 1H, α-CH Ser); $^{13}$C NMR ($CD_3OD$, 100.6 MHz): $\delta = 18.7$ ($CH_3$ acetyl), 25.9 ($CH_3$ Met), 33.9 (γ-$CH_2$ Met), 35.5 (β-$CH_2$ Met), 56.5 (α-CH Met), 60.9 (α-CH Ser), 64.7 (β-$CH_2$ Ser), 174.1 (C=O), 176.0 (C=O), 176.3 (C=O); HRMS (FAB, 3-NBA): *m*/*z*: calcd for $C_{10}H_{19}N_2O_5S$: 279.1015, found: 279.1028 $[M+H]^+$.

***N*-Acetyl-L-methionyl-L-seryl-[(2*S*)-amino-3-(hexadecane-1-sulfonylamino)]-propionic acid allyl ester (17)**: At 0°C, EDC (43 mg, 0.22 mmol) was added to a solution of H-Dap($SO_2C_{16}H_{33}$)-OAll **6** (80 mg, 0.19 mmol), Ac-Met-Ser-OH **16** (62 mg, 0.22 mmol) and HOBt (58 mg, 0.37 mmol) in DMF (5 mL). The solution was warmed to room temperature and stirred for 18 h. After removal of the solvent under reduced pressure, the residue was dissolved in ethyl acetate and subsequently washed with 1N HCl (15 mL) and $Na_2CO_3$ solution (10%, 15 mL). The organic layer was dried over $Na_2SO_4$, and, after removal of the solvent under reduced pressure, the residue was purified by chromatography ($CH_2Cl_2$/EtOH 15:1, then 10:1) to yield **17** as a colorless solid (93 mg, 73%). M.p. 78°C; $[\alpha]_D^{20} = -9.4$ ($c = 1.0$ in MeOH); $R_f = 0.28$ ($CH_2Cl_2$/EtOH 10:1); $^1$H NMR ($CD_3OD$, 400 MHz): $\delta = 0.87$ (t, $J = 6.5$ Hz, 3H, ω-$CH_3$ hexadecyl), 1.25 (m, 24H, 12×$CH_2$ hexadecyl), 1.40 (m, 2H, γ-$CH_2$ hexadecyl), 1.75 (m, 2H, β-$CH_2$ hexadecyl), 1.95 (m, 1H, β-$CH_{2a}$ Met), 2.02 (s, 3H, $CH_3$ acetyl), 2.11 (s, 3H, $CH_3$ Met), 2.12 (m, 1H, β-$CH_{2b}$ Met), 2.56 (m, 2H, γ-$CH_2$ Met), 2.98 (m, 2H, α-$CH_2$ hexadecyl), 3.53 (m, 2H, β-$CH_2$ Dap), 3.78 (dd, $J = 3.7$, 11.3 Hz, 1H, β-$CH_{2a}$ Ser), 3.92 (dd, $J = 3.8$, 11.3 Hz, 1H, β-$CH_{2b}$ Ser), 4.40 (t, $J = 4.3$ Hz, 1H, α-CH Ser), 4.45 (dd, $J = 6.0$, 8.1 Hz, 1H, α-CH Met), 4.59 (t, $J = 5.4$ Hz, 1H, α-CH Dap), 4.66 (m, 2H, -O*C*$H_2$CH=$CH_2$), 5.24–5.36 (m, 2H, -$OCH_2$CH=C*H*$_2$), 5.93 (m, 1H, -$OCH_2$C*H*=$CH_2$); $^{13}$C NMR ($CD_3OD$, 100.6 MHz): $\delta = 14.2$ (ω-$CH_3$ hexa-

decyl), 22.6 ($CH_3$ acetyl), 22.9 ($CH_3$ Met), 23.4 ($CH_2$), 23.7 ($CH_2$), 28.5 ($CH_2$), 28.6 ($CH_2$), 28.7 ($CH_2$), 28.9 ($CH_2$), 29.1 ($CH_2$), 29.2 ($CH_2$), 29.3 ($CH_2$), 29.3 ($CH_2$), 29.3 ($CH_2$), 29.5 ($CH_2$), 29.8 ($CH_2$), 30.3 ($CH_2$), 31.2 (γ-$CH_2$ Met), 32.1 (β-$CH_2$ Met), 43.5 (β-$CH_2$ Dap), 53.2 (α-CH Dap), 53.3 (α-CH Met), 55.6 (α-$CH_2$ hexadecyl), 60.9 (α-CH Ser), 61.9 (β-$CH_2$ Ser), 66.8 (O*C*$H_2$CH=$CH_2$), 119.2 (OCH$_2$CH=*C*$H_2$), 131.6 (OCH$_2$*C*H=$CH_2$), 169.5 (C=O), 170.8 (C=O), 172.6 (C=O), 172.8 (C=O); HRMS (FAB, 3-NBA): *m*/*z*: calcd for $C_{32}H_{61}N_4O_8S_2$: 693.3932, found: 693.3944 $[M+H]^+$.

***N*-Acetyl-L-methionyl-L-seryl-[(2*S*)-amino-3-(hexadecane-1-sulfonylamino)]propionic acid (18)**: $[Pd(PPh_3)_4]$ (15 mg, 0.01 mmol) was added to a degassed solution of allyl ester **17** (90 mg, 0.13 mmol) and *N*,*N*′-dimethylbarbituric acid (12 mg, 0.08 mmol) in THF (5 mL). The mixture was stirred at room temperature for 1.5 h. After removal of the solvent under reduced pressure, the residue was purified by chromatography ($CH_2Cl_2$/EtOH 5:1 + 1% HOAc) to yield **18** as a colorless solid (85 mg, 99%). M.p. 82°C; $[\alpha]_D^{20} = -8.6$ ($c=1.0$ in $CHCl_3$); $R_f=0.09$ ($CHCl_3$/MeOH 5:1 + 1% HOAc); $^1$H NMR ($CD_3OD$, 400 MHz): $\delta=0.86$ (t, $J=6.5$ Hz, 3H, ω-$CH_3$ hexadecyl), 1.27 (m, 24H, 12×$CH_2$ hexadecyl), 1.43 (m, 2H, γ-$CH_2$ hexadecyl), 1.77 (m, 2H, β-$CH_2$ hexadecyl), 1.98 (m, 1H, β-$CH_{2a}$ Met), 2.09 (s, 3H, $CH_3$ acetyl), 2.12 (s, 3H, $CH_3$ Met), 2.13 (m, 1H, β-$CH_{2b}$ Met), 2.60 (m, 2H, γ-$CH_2$ Met), 3.01 (m, 2H, α-$CH_2$ hexadecyl), 3.51 (m, 2H, β-$CH_2$ Dap), 3.80 (dd, $J=3.7$, 11.3 Hz, 1H, β-$CH_{2a}$ Ser), 3.96 (dd, $J=3.8$, 11.3 Hz, 1H, β-$CH_{2b}$ Ser), 4.27 (m, 1H, α-CH Dap), 4.43 (t, $J=4.3$ Hz, 1H, α-CH Ser), 4.48 (dd, $J=6.0$, 8.1 Hz, 1H, α-CH Met); $^{13}$C NMR ($CD_3OD$, 100.6 MHz): $\delta=14.2$ (ω-$CH_3$ hexadecyl), 22.6 ($CH_3$ acetyl), 22.9 ($CH_3$ Met), 23.4 ($CH_2$), 23.7 ($CH_2$), 28.5 ($CH_2$), 28.6 ($CH_2$), 28.7 ($CH_2$), 28.9 ($CH_2$), 29.1 ($CH_2$), 29.2 ($CH_2$), 29.3 ($CH_2$), 29.3 ($CH_2$), 29.3 ($CH_2$), 29.5 ($CH_2$), 29.8 ($CH_2$), 30.3 ($CH_2$), 31.2 (γ-$CH_2$ Met), 32.1 (β-$CH_2$ Met), 43.5 (β-$CH_2$ Dap), 53.2 (α-CH Dap), 53.3 (α-CH Met), 55.6 (α-$CH_2$ hexadecyl), 60.9 (α-CH Ser), 61.9 (β-$CH_2$ Ser), 169.5 (C=O), 170.8 (C=O), 172.6 (C=O), 175.8 (C=O); HRMS (FAB, 3-NBA): *m*/*z*: calcd for $C_{29}H_{56}N_4NaO_8S_2$: 675.3440, found: 675.3469 $[M+Na]^+$.

***N*-Acetyl-L-methionyl-L-seryl-[(2*S*)-amino-3-(hexadecane-1-sulfonylamino)propionyl]-*N*$^\varepsilon$-allyloxycarbonyl-L-lysyl-*S*-farnesyl-L-cysteine methyl ester (20)**: At 0°C, EDC (19 mg, 0.10 mmol) was added to a solution of tripeptide **18** (52 mg, 0.08 mmol), freshly prepared H-Lys(Aloc)-Cys(Far)-OMe **19** (44 mg, 0.08 mmol) and HOBt (25 mg, 0.16 mmol) in $CH_2Cl_2$ (6 mL). The solution was warmed to room temperature and stirred for 16 h. Then, the reaction mixture was diluted with ethyl acetate (30 mL) and subsequently washed with 1N HCl (10 mL) and $Na_2CO_3$ solution (10%, 10 mL). The organic layer was dried over $Na_2SO_4$, and, after removal of the solvent under reduced pressure, the residue was purified by chromatography ($CH_2Cl_2$/EtOH 20:1, then 10:1) to yield **20** as colorless solid (47 mg, 50%). M.p. 101°C; $[\alpha]_D^{20} = +6.1$ ($c=0.5$ in $CHCl_3$); $R_f=0.30$ ($CH_2Cl_2$/EtOH 10:1); $^1$H NMR ($CDCl_3$/$CD_3OD$, 400 MHz): $\delta=$ 0.78 (t, $J=6.6$ Hz, 3H, ω-$CH_3$ hexadecyl), 1.15 (m, 24H, 12×$CH_2$ hexadecyl), 1.49 (s, 6H, 2×$CH_3$ Far), 1.57 (s, 6H, 2×$CH_3$ Far), 1.26–1.68 (m, 8H, 2×$CH_2$ Lys, 2×$CH_2$ hexadecyl), 1.93 (s, 3H, $CH_3$ acetyl), 2.00 (s, 3H, $CH_3$ Met), 1.76–2.05 (m, 12H, β-$CH_2$ Met, $CH_2$ Lys, 4×$CH_2$ Far), 2.46 (m, 2H, γ-$CH_2$ Met), 2.68–3.17 (m, 6H, α-$CH_2$ hexadecyl, β-$CH_2$ Cys, -SCH$_2$-), 3.26–3.46 (m, 4H, β-$CH_2$ Dap, ε-$CH_2$ Lys), 3.64 (s, 3H, $OCH_3$), 3.68 (m, 1H, β-$CH_{2a}$ Ser), 3.87 (m, 1H, β-$CH_{2b}$ Ser), 4.25–4.54 (m, 7H, 5×α-CH, -O*C*$H_2$CH=$CH_2$), 5.00 (m, 2H, 2×CH Far), 5.08–5.22 (m, 3H, CH Far, -OCH$_2$CH=C$H_2$), 5.74 (m, 1H, -OCH$_2$C*H*=$CH_2$); $^{13}$C NMR ($CDCl_3$/$CD_3OD$, 100.6 MHz): $\delta=14.0$ (ω-$CH_3$ hexadecyl), 16.5 ($CH_3$ acetyl), 16.8 ($CH_3$ Met), 17.0 ($CH_3$ Far), 17.2 ($CH_3$ Far), 19.3 ($CH_3$ Far), 19.3 ($CH_2$), 21.3 ($CH_2$), 24.0 ($CH_2$), 24.3 ($CH_2$), 25.3 ($CH_3$ Far), 28.6 ($CH_2$), 29.2 ($CH_2$), 29.3 ($CH_2$), 30.0 ($CH_2$), 30.0 ($CH_2$), 30.3 ($CH_2$), 30.3 ($CH_2$), 30.3 ($CH_2$), 30.3 ($CH_2$), 30.3 ($CH_2$), 30.3 ($CH_2$), 30.3 ($CH_2$), 30.3 ($CH_2$), 30.9 ($CH_2$), 31.8 ($CH_2$), 32.5 ($CH_2$), 33.8 ($CH_2$), 34.4 ($CH_2$), 39.8 ($CH_2$ Far), 40.5 ($CH_2$ Far), 42.0 (β-$CH_2$ Dap), 44.6 (ε-$CH_2$ Lys), 50.4 ($OCH_3$), 53.5 (α-CH), 54.2 (α-CH), 54.5 (α-$CH_2$ hexadecyl), 56.0 (α-CH), 56.1 (α-CH), 57.1 (α-CH), 64.8 (β-$CH_2$ Ser), 67.3 (O*C*H$_2$CH=$CH_2$), 115.1 (OCH$_2$CH=*C*$H_2$), 117.1 (CH Far), 121.7 (CH Far), 122.5 (CH Far), 133.9 (C Far), 137.5 (OCH$_2$*C*H=$CH_2$), 138.8 (C Far), 139.3 (C Far), 157.5 (C=O), 170.9 (C=O), 172.0 (C=O), 174.7 (C=O), 175.4 (C=O); HRMS (FAB, 3-NBA): *m*/*z*: calcd for $C_{58}H_{103}N_7NaO_{12}S_3$: 1208.6727, found: 1208.6708 $[M+Na]^+$.

***N*-Acetyl-L-methionyl-L-seryl-[(2*S*)-amino-3-(hexadecane-1-sulfonylamino)propionyl]-L-lysyl-*S*-farnesyl-L-cysteine methyl ester (21)**: $[Pd(PPh_3)_4]$ (7 mg, 0.01 mmol) was added to a desgassed solution of pentapeptide **20** (21 mg, 0.02 mmol) and *N*,*N*′-dimethylbarbituric acid (3 mg, 0.02 mmol) in THF (3 mL). The mixture was stirred at room temperature for 4 h. After removal of the solvent under reduced pressure, the residue was purified by chromatography ($CH_2Cl_2$/MeOH 1:1 + 1% $NEt_3$) to yield **21** as slightly yellow solid (16 mg, 82%). M.p. 86°C; $[\alpha]_D^{20} = +10.3$ ($c=0.5$ in MeOH); $R_f=0.01$ ($CH_2Cl_2$/EtOH 10:1); $^1$H NMR ($CD_3OD$, 400 MHz): $\delta=0.78$ (t, $J=6.6$ Hz, 3H, ω-$CH_3$ hexadecyl), 1.15 (m, 24H, 12×$CH_2$ hexadecyl), 1.49 (s, 6H, 2×$CH_3$ Far), 1.57 (s, 6H, 2×$CH_3$ Far), 1.26–1.68 (m, 8H, 2×$CH_2$ Lys, 2×$CH_2$ hexadecyl), 1.93 (s, 3H, $CH_3$ acetyl), 2.00 (s, 3H, $CH_3$ Met), 1.76–2.05 (m, 12H, β-$CH_2$ Met, $CH_2$ Lys, 4×$CH_2$ Far), 2.46 (m, 2H, γ-$CH_2$ Met), 2.68–3.17 (m, 6H, α-$CH_2$ hexadecyl, β-$CH_2$ Cys, -SCH$_2$-), 3.26–3.46 (m, 4H, β-$CH_2$ Dap, ε-$CH_2$ Lys), 3.64 (s, 3H, $OCH_3$), 3.68 (m, 1H, β-$CH_{2a}$ Ser), 3.87 (m, 1H, β-$CH_{2b}$ Ser), 4.25–4.54 (m, 5H, 5×α-CH), 5.00 (m, 2H, 2×CH Far), 5.14 (m, 1H, CH Far); $^{13}$C NMR ($CD_3OD$, 100.6 MHz): $\delta=14.0$ (ω-$CH_3$ hexadecyl), 16.5 ($CH_3$ acetyl), 16.8 ($CH_3$ Met), 17.0 ($CH_3$ Far), 17.2 ($CH_3$ Far), 19.3 ($CH_3$ Far), 19.3 ($CH_2$), 21.3 ($CH_2$), 24.0 ($CH_2$), 24.3 ($CH_2$), 25.3 ($CH_3$ Far), 28.6 ($CH_2$), 29.2 ($CH_2$), 29.3 ($CH_2$), 30.0 ($CH_2$), 30.0 ($CH_2$), 30.3 ($CH_2$), 30.3 ($CH_2$), 30.3 ($CH_2$), 30.3 ($CH_2$), 30.3 ($CH_2$), 30.3 ($CH_2$), 30.3 ($CH_2$), 30.3 ($CH_2$), 30.9 ($CH_2$), 31.8 ($CH_2$), 32.5 ($CH_2$), 33.8 ($CH_2$), 34.4 ($CH_2$), 39.8 ($CH_2$ Far), 40.5 ($CH_2$ Far), 42.0 (β-$CH_2$ Dap), 44.6 (ε-$CH_2$ Lys), 50.4 ($OCH_3$), 53.5 (α-CH), 54.2 (α-CH), 54.5 (α-$CH_2$ hexadecyl), 56.0 (α-CH), 56.1 (α-CH), 57.1 (α-CH), 64.8 (β-$CH_2$ Ser), 117.1 (CH Far), 121.7 (CH Far), 122.5 (CH Far), 133.9 (C Far), 138.8 (C Far), 139.3 (C Far), 170.9 (C=O), 172.0 (C=O), 174.7 (C=O), 175.4 (C=O); HRMS (FAB, 3-NBA): *m*/*z*: calcd for $C_{54}H_{100}N_7O_{10}S_3$: 1102.6695, found: 1102.6685 $[M+H]^+$.

**L-Glutamic acid 1-*tert*-butyl ester 5-methyl ester (23)**: Isobutene (75 mL) was added to a solution of H-L-Glu(OMe)-OH **22** (5 g, 31.03 mmol) in dioxane (45 mL) and conc. $H_2SO_4$ (4.5 mL) in a sealed tube. After stirring at room temperature for 3 d the reaction mixture was poured into a mixture of ethyl acetate (250 mL) and saturated $NaHCO_3$ solution (250 mL). The organic layer was separated and washed again with saturated $NaHCO_3$ solution (50 mL). The organic layer was dried over $Na_2SO_4$ and the solvent was removed under reduced pressure to yield **23** as a colorless oil (3.728 g, 55%). $[\alpha]_D^{20} = +25.7$ ($c=1.0$ in $CHCl_3$); $R_f=0.17$ (cyclohexane/ethyl acetate 1:1); $^1$H NMR ($CDCl_3$, 400 MHz): $\delta=1.44$ (br s, 2H, $NH_2$), 1.46 (s, 9H, C(CH$_3$)$_3$), 1.78 (m, 1H, β-$CH_{2a}$ Glu), 1.99 (m, 1H, β-$CH_{2b}$ Glu), 2.42 (t, $J=7.8$ Hz, 2H, γ-$CH_2$ Glu), 3.32 (dd, $J=5.3$, 8.2 Hz, 1H, α-CH Glu), 3.69 (d, 3H, $OCH_3$); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta=$ 28.1 (C(*C*H$_3$)$_3$), 30.0 (β-$CH_2$ Glu), 30.7 (γ-$CH_2$ Glu), 51.8 (α-CH Glu), 54.5 ($OCH_3$), 81.4 (*C*(CH$_3$)$_3$), 173.8 (C=O), 175.0 (C=O); HRMS (FAB, 3-NBA): *m*/*z*: calcd for $C_{10}H_{20}NO_4$: 218.1393, found: 218.1372 $[M+H]^+$.

***N*-Diallyl-L-glutamic acid 1-*tert*-butyl ester 5-methyl ester (24)**: A solution of H-Glu(OMe)-O*t*Bu **23** (8 g, 36.82 mmol), tetrabutylammoniumiodide (1.36 g, 3.68 mmol), $NEt_3$ (12.8 mL, 92.05 mmol) and allyl bromide (64.7 mL, 736.39 mmol) in THF (184 mL) was stirred under reflux for 2 d. The suspension was cooled down to room temperature and the solvent was removed under reduced pressure. The residue was dissolved in ethyl acetate (500 mL) and washed with $Na_2CO_3$ solution (10%, 2× 50 mL). The organic layer was dried over $Na_2SO_4$ and the solvent was removed under reduced pressure. The residue was purified by chromatography (cyclohexane/ethyl acetate 8:1) to yield **24** as a colorless oil (8.76 g, 80%). $[\alpha]_D^{20} = +17.3$ ($c=1.0$ in $CHCl_3$); $R_f=0.49$ (cyclohexane/ethyl acetate 2:1); $^1$H NMR ($CDCl_3$, 400 MHz): $\delta=1.42$ (s, 9H, C(CH$_3$)$_3$), 1.84 (m, 2H, β-$CH_2$ Glu), 2.32 (m, 2H, γ-$CH_2$ Glu), 2.97 (dd, $J=8.0$, 14.4 Hz, 2H, 2×-NC*H*$_{2a}$CH=$CH_2$), 3.28 (m, 3H, α-CH Glu, 2×-NC*H*$_{2b}$CH=$CH_2$), 3.60 (s, 3H, $OCH_3$), 4.98–5.12 (m, 4H, 2×-NCH$_2$CH=C$H_2$), 5.64 (m, 2H, 2×-NCH$_2$C*H*=$CH_2$); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta=24.7$ (β-$CH_2$ Glu), 28.4 (C(*C*H$_3$)$_3$), 30.8 (γ-$CH_2$ Glu), 51.5 (α-CH Glu), 53.4 ($OCH_3$), 61.3 (NCH$_2$CH=*C*H$_2$), 80.9 (*C*(CH$_3$)$_3$), 117.0 (NCH$_2$CH*C*H$_2$), 136.8 (NCH$_2$*C*H=CH$_2$), 171.9 (C=O), 173.8 (C=O); HRMS (FAB, 3-NBA): *m*/*z*: calcd for $C_{16}H_{28}NO_4$: 298.2019, found: 209.2027 $[M+H]^+$.

**(2*S*)-Diallylamino-5-hydroxypentanoic acid *tert*-butyl ester (25)**: A 2M solution of $LiBH_4$ in THF (12.2 mL, 24.48 mmol) was added to a solution of diester **24** (5.6 g, 18.83 mmol) in THF (90 mL). The solution was stir-

red at room temperature for 36 h. Then, saturated NaCl solution (50 mL) was added and the mixture was extracted with ethyl acetate (3×100 mL). The combined organic layers were dried over $Na_2SO_4$, and, after removal of the solvents under reduced pressure, the residue was purified by chromatography (cyclohexane/ethyl acetate 3:1) to yield **25** as a colorless oil (3.601 g, 71%). $[\alpha]_D^{20}$ = 24.0 ($c$ = 1.0 in $CHCl_3$); $R_f$ = 0.31 (cyclohexane/ethyl acetate 2:1); $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ = 1.48 (s, 9H, C-$(CH_3)_3$), 1.52–1.73 (m, 4H, β-$CH_2$, γ-$CH_2$), 3.21 (dd, $J$ = 8.0, 14.4 Hz, 2H, 2×-NC$H_{2a}$CH=$CH_2$), 3.32 (m, 1H, α-CH), 3.45 (m, 2H, 2×-NC$H_{2b}$CH=$CH_2$), 3.53 (m, 1H, δ-$CH_{2a}$), 3.62 (m, 1H, δ-$CH_{2b}$), 5.10–5.24 (m, 4H, 2×-$NCH_2$CH=C$H_2$), 5.80 (m, 2H, 2×-$NCH_2$C$H$=$CH_2$); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta$ = 27.7 (β-$CH_2$), 28.5 (C($CH_3$)$_3$), 30.7 (γ-$CH_2$), 53.7 (α-CH), 62.8 (N$CH_2$CH=$CH_2$), 63.6 (δ-$CH_2$), 81.2 ($C$($CH_3$)$_3$), 118.1 ($NCH_2$CH=$CH_2$), 136.0 ($NCH_2$C$H$=$CH_2$), 172.3 (C=O); HRMS (FAB, 3-NBA): $m/z$: calcd for $C_{15}H_{28}NO_3$: 270.2070, found: 270.2063 $[M+H]^+$.

**(2*S*)-Diallylamino-5-hydroxyheneicosanic acid *tert*-butyl ester (26)**: A solution of Dess–Martin periodinane in $CH_2Cl_2$ (15%, 9.59 mL, 4.51 mmol) was added to a solution of alcohol **25** (810 mg, 3.01 mmol) in $CH_2Cl_2$ (27 mL). The mixture was stirred at room temperature for 1 h. The solution was diluted with ethyl acetate (100 mL) and a mixture of saturated $Na_2S_2O_3$ solution (10 mL) and saturated $NaHCO_3$ solution (30 mL) was added. After the two phases had become clear, the separated organic layer was washed with saturated $NaHCO_3$ solution (30 mL). The organic layer was dried over $Na_2SO_4$, and, after removal of the solvents under reduced pressure, the residue was purified by chromatography (cyclohexane/ethyl acetate 4:1) to yield the aldehyde as a colorless oil (800 mg, 97%).

At −78 °C, a solution of hexadecyl magnesium bromide freshly prepared from magnesium (109 mg, 4.48 mmol) and hexadecyl bromide (1.41 mL, 4.48 mmol) in THF (1.5 mL) was added to a solution of the above aldehyde (800 mg, 2.92 mmol) in THF (15 mL). After 15 min, water (5 mL) was added to the reaction mixture. Then, the solution was diluted with ethyl acetate (50 mL) and washed with water (10 mL). The organic layer was dried over $Na_2SO_4$, and, after removal of the solvents under reduced pressure, the residue was purified by chromatography (cyclohexane/ethyl acetate 10:1) to yield **26** as a colorless oil (930 mg, 63%). $R_f$ = 0.44 (cyclohexane/ethyl acetate 3:1); $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ = 0.82 (t, $J$ = 6.6 Hz, 3H, ω-$CH_3$ Hhe), 1.13–1.38 (m, 32H, β-$CH_2$ Hhe, 15×$CH_2$ Hhe), 1.40 (s, 9H, C($CH_3$)$_3$), 1.52–1.80 (m, 2H, γ-$CH_2$ Hhe), 3.01 (m, 2H, 2×-NC$H_{2a}$CH=$CH_2$), 3.21 (m, 1H -CHOH), 3.24–3.46 (m, 3H, α-CH Hhe, 2×-NC$H_{2b}$CH=$CH_2$), 5.02–5.14 (m, 4H, 2×-$NCH_2$CH=C$H_2$), 5.86 (m, 2H, 2×-$NCH_2$C$H$=$CH_2$); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta$ = 14.2 (ω-$CH_3$ Hhe), 29.9 (C($CH_3$)$_3$), 24.3–37.6 (17×$CH_2$ Hhe), 53.5 (α-CH Hhe), 61.4 (N$CH_2$CH=$CH_2$), 71.3 (HCOH), 81.2 ($C$($CH_3$)$_3$), 117.5 ($NCH_2$CH=$CH_2$), 136.1 ($NCH_2$C$H$=$CH_2$), 172.3 (C=O); HRMS (FAB, 3-NBA): $m/z$: calcd for $C_{31}H_{60}NO_3$: 494.4574, found: 494.4540 $[M+H]^+$.

**(2*S*)-Amino-5-hydroxyheneicosanic acid *tert*-butyl ester (27)**: $[Pd(PPh_3)_4]$ (222 mg, 0.19 mmol) was added to a degassed solution of diallyl amine **26** (950 mg, 1.92 mmol) and *N*,*N*′-dimethylbarbituric acid (1.8 g, 11.59 mmol mmol) in $CH_2Cl_2$ (100 mL). The mixture was stirred for 6 h at 35 °C. Then, the solution was diluted with ethyl acetate (250 mL) and washed with $Na_2CO_3$ solution (10%, 50 mL). The organic layer was dried over $Na_2SO_4$, and, after removal of the solvent under reduced pressure, the residue was purified by chromatography (cyclohexane/ethyl acetate 3:1, then $CH_2Cl_2$/EtOH 10:1) to yield **27** as a yellowish solid (597 mg, 75%). M.p. 63 °C; $R_f$ = 0.29 ($CH_2Cl_2$/EtOH 10:1); $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ = 0.88 (t, $J$ = 6.6 Hz, 3H, ω-$CH_3$ Hhe), 1.21–1.47 (m, 28H, 14×$CH_2$ Hhe), 1.48–1.52 (m, 4H, δ-$CH_2$ Hhe, β-$CH_2$ Hhe), 1.49 (s, 9H, C($CH_3$)$_3$), 1.59–1.71 (m, 2H, γ-$CH_2$ Hhe), 3.30 (m, 1H, α-CH Hhe), 3.55 (m, 1H, -C$H$(OH)-); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta$ = 14.3 (ω-$CH_3$ Hhe), 29.5 (C($CH_3$)$_3$), 24.0–37.5 (17×$CH_2$ Hhe), 53.6 (α-CH Hhe), 71.6 (HCOH), 81.2 ($C$($CH_3$)$_3$), 172.5 (C=O); HRMS (FAB, 3-NBA): $m/z$: calcd for $C_{25}H_{52}NO_3$: 414.3948, found: 414.3929 $[M+H]^+$.

***N*-Acetyl-L-methionyl-L-seryl-[(2*S*)-amino-5-hydroxy]heneicosanic acid *tert*-butyl ester (28)**: At 0 °C, EDC (272 mg, 1.39 mmol) was added to a solution of dipeptide **16** (388 mg, 1.39 mmol), H-Hhe-O*t*Bu **27** (480 mg, 1.16 mmol) and HOBt (362 mg, 2.32 mmol) in $CH_2Cl_2$ (25 mL). The solution was warmed to room temperature and stirred for 20 h. Then, the reaction mixture was diluted with ethyl acetate (100 mL) and subsequently washed with 1 N HCl (50 mL) and $Na_2CO_3$ solution (10%, 50 mL). The organic layer was dried over $Na_2SO_4$, and, after removal of the solvent under reduced pressure, the residue was purified by chromatography ($CH_2Cl_2$/EtOH 15:1, then 10:1) to yield **28** as colorless oil (536 mg, 69%). $R_f$ = 0.23 ($CH_2Cl_2$/EtOH 10:1); $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ = 0.87 (t, $J$ = 6.6 Hz, 3H, ω-$CH_3$ Hhe), 1.24 (m, 28H, 14×$CH_2$ Hhe), 1.40 (m, 4H, δ-$CH_2$ Hhe, β-$CH_2$ Hhe), 1.48 (s, 9H, C($CH_3$)$_3$), 1.70–2.14 (m, 4H, γ-$CH_2$ Hhe, β-$CH_2$ Met), 2.01 (s, 3H, $CH_3$ acetyl), 2.10 (s, 3H, $CH_3$ Met), 2.53 (m, 2H, γ-$CH_2$ Met), 3.54 (m, 1H, -C$H$(OH)-), 3.72 (dd, $J$ = 3.7, 11.3 Hz, 1H, β-$CH_{2a}$ Ser), 3.95 (dd, $J$ = 3.8, 11.3 Hz, 1H, β-$CH_{2b}$ Ser), 4.44 (m, 1H, α-CH Hhe), 4.60 (m, 1H, α-CH Ser), 4.71 (dd, $J$ = 6.1, 8.0 Hz, 1H, α-CH Met), 7.02 (m, 1H, NH), 7.62 (m, 2H, 2×NH); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta$ = 14.3, (ω-$CH_3$ Hhe), 22.9 ($CH_3$ acetyl), 23.2 ($CH_3$ Met), 25.6–30.3 (17×$CH_2$ Hhe), 30.1 (C($CH_3$)$_3$), 37.6 (β-$CH_2$ Met), 53.0 (α-CH Hhe), 53.4 (α-CH Met), 54.9 (α-CH Ser), 62.9 (β-$CH_2$ Ser), 71.4 (CHOH), 82.5 ($C$($CH_3$)$_3$), 170.3 (C=O), 171.0 (C=O), 171.5 (C=O), 172.1 (C=O); HRMS (FAB, 3-NBA): $m/z$: calcd for $C_{35}H_{67}N_3NaO_7S$ $[M+Na]^+$: 696.4600, found: 696.4612.

***N*-Acetyl-L-methionyl-L-seryl-[(2*S*)-amino-5-hydroxy]heneicosanoyl-*N*$^\varepsilon$-(allyloxycarbonyl)-L-lysyl-*S*-farnesyl-L-cysteine methyl ester (29)**: A 1 M NaOH solution (16 mL) was added to a solution of tripeptide **28** (530 mg, 0.79 mmol) in MeOH (16 mL). The solution was stirred at room temperature for 3 h. Then, the solvent was removed under reduced pressure. The residue was dissolved in $CHCl_3$ (100 mL) and then washed with saturated $NH_4Cl$ solution (50 mL). The organic layer was dried over $Na_2SO_4$ and the solvent was removed under reduced pressure to yield the free acid as colorless solid (486 mg, 100%).

At 0 °C, EDC (32 mg, 0.16 mmol) was added to a solution of the above acid (67 mg, 0.11 mmol), freshly prepared H-Lys(Aloc)-Cys(Far)-OMe **19** (59 mg, 0.11 mmol) and HOBt (34 mg, 0.22 mmol) in $CH_2Cl_2$ (5 mL) and DMF (3 mL). The solution was warmed to room temperature and stirred for 15 h. Then, the reaction mixture was diluted with ethyl acetate (50 mL) and subsequently washed with 1 N HCl (15 mL) and $Na_2CO_3$ solution (10%, 15 mL). The organic layer was dried over $Na_2SO_4$, and, after removal of the solvent under reduced pressure, the residue was purified by chromatography (cyclohexane/ethyl acetate 1:1, then $CH_2Cl_2$/EtOH 20:1, then 10:1) to yield **29** as colorless oil (55 mg, 44%). $R_f$ = 0.11 ($CH_2Cl_2$/EtOH 10:1); $^1$H NMR ($CDCl_3$/$CD_3OD$, 400 MHz): $\delta$ = 0.80 (t, $J$ = 6.6 Hz, 3H, ω-$CH_3$ Hhe), 1.52 (s, 6H, 2×$CH_3$ Far), 1.61 (s, 6H, 2×$CH_3$ Far), 1.13–1.70 (m, 36H, 15×$CH_2$ Hhe, β-$CH_2$ Hhe, 2×$CH_2$ Lys), 1.93 (s, 3H, $CH_3$ acetyl), 2.00 (s, 3H, $CH_3$ Met), 1.72–2.05 (m, 14H, γ-$CH_2$ Hhe, β-$CH_2$ Met, $CH_2$ Lys, 4×$CH_2$ Far), 2.49 (m, 2H, γ-$CH_2$ Met), 2.72 (m, 1H, β-$CH_{2a}$ Cys), 2.86 (m, 1H, β-$CH_{2b}$ Cys), 3.00–3.18 (m, 4H, -$SCH_2$-, ε-$CH_2$ Lys), 3.50 (m, 1H, -C$H$(OH)-), 3.67 (s, 3H, $OCH_3$), 3.72 (m, 1H, β-$CH_{2a}$ Ser), 3.95 (m, 1H, β-$CH_{2b}$ Ser), 4.25–4.60 (m, 7H, 5×α-CH, -O$CH_2$CH=$CH_2$), 5.01 (m, 2H, 2×CH Far), 5.08–5.22 (m, 3H, CH Far, -$OCH_2$CH=C$H_2$), 5.80 (m, 1H, -$OCH_2$C$H$=$CH_2$); $^{13}$C NMR ($CDCl_3$/$CD_3OD$, 100.6 MHz): $\delta$ = 14.3 (ω-$CH_3$ Hhe), 17.0 ($CH_3$ Far), 17.2 ($CH_3$ Far), 19.3 ($CH_3$ Far), 22.9 ($CH_3$ acetyl), 23.2 ($CH_3$ Met), 25.3 ($CH_3$ Far), 23.6–34.2 (25×$CH_2$), 37.9 (β-$CH_2$ Met), 39.9 ($CH_2$ Far), 40.5 ($CH_2$ Far), 44.6 (ε-$CH_2$ Lys), 50.4 ($OCH_3$), 54.2 (α-CH), 55.9 (α-CH), 56.0 (α-CH), 57.1 (α-CH), 59.6 (α-CH), 64.9 (β-$CH_2$ Ser), 67.3 (O$CH_2$CH=$CH_2$), 82.2 (CHOH), 115.1 (OC$H_2$CH=C$H_2$), 117.1 (CH Far), 121.7 (CH Far), 122.5 (CH Far), 133.9 (C Far), 137.5 (OC$H_2$CH=C$H_2$), 139.3 (C Far), 142.4 (C Far), 157.5 (C=O), 170.9 (C=O), 172.1 (C=O), 174.7 (C=O), 175.1 (C=O); HRMS (FAB, 3-NBA): $m/z$: calcd for $C_{60}H_{106}N_6NaO_{11}S_2$: 1173.7261, found: 1173.7325 $[M+Na]^+$.

***N*-Acetyl-L-methionyl-L-seryl-[(2*S*)-amino-5-hydroxy]heneicosanoyl-L-lysyl-*S*-farnesyl-L-cysteine methyl ester (30)**: $[Pd(PPh_3)_4]$ (1 mg, 0.01 mmol) was added to a solution of pentapeptide **29** (7 mg, 0.01 mmol) and *N*,*N*′-dimethylbarbituric acid (1 mg, 0.01 mmol) in THF (2 mL). The mixture was stirred at room temperature for 6 h. After removal of the solvent under reduced pressure, the residue was purified by chromatography ($CH_2Cl_2$/EtOH 10:1, then $CH_2Cl_2$/MeOH 1:1 + 1% $NEt_3$) to yield **30** as a slightly yellow solid (5 mg, 80%). M.p. 71 °C; $R_f$ = 0.01 ($CH_2Cl_2$/EtOH 10:1); $^1$H NMR ($CDCl_3$/$CD_3OD$, 400 MHz): $\delta$ = 0.80 (t, $J$ = 6.6 Hz, 3H, ω-$CH_3$ Hhe), 1.52 (s, 6H, 2×$CH_3$ Far), 1.61 (s, 6H, 2×$CH_3$ Far),

1.13–1.70 (m, 36H, 15×$CH_2$ Hhe, β-$CH_2$ Hhe, 2×$CH_2$ Lys), 1.93 (s, 3H, $CH_3$ acetyl), 2.00 (s, 3H, $CH_3$ Met), 1.72–2.05 (m, 14H, γ-$CH_2$ Hhe, β-$CH_2$ Met, $CH_2$ Lys, 4×$CH_2$ Far), 2.49 (m, 2H, γ-$CH_2$ Met), 2.72 (m, 1H, β-$CH_{2a}$ Cys), 2.86 (m, 1H, β-$CH_{2b}$ Cys), 3.00–3.18 (m, 4H, -$SCH_2$-, ε-$CH_2$ Lys), 3.50 (m, 1H, -C*H*(OH)-), 3.67 (s, 3H, $OCH_3$), 3.72 (m, 1H, β-$CH_{2a}$ Ser), 3.95 (m, 1H, β-$CH_{2b}$ Ser), 4.25–4.60 (m, 5H, 5×α-CH), 5.01 (m, 2H, 2×CH Far), 5.14 (m, 1H, CH Far); $^{13}$C NMR ($CDCl_3$/$CD_3OD$, 100.6 MHz): $\delta$=14.2 (ω-$CH_3$ Hhe), 17.0 ($CH_3$ Far), 17.2 ($CH_3$ Far), 19.1 ($CH_3$ Far), 22.7 ($CH_3$ acetyl), 23.0 ($CH_3$ Met), 25.3 ($CH_3$ Far), 23.2–34.7 (25×$CH_2$), 37.9 (β-$CH_2$ Met), 39.9 ($CH_2$ Far), 40.5 ($CH_2$ Far), 44.6 (ε-$CH_2$ Lys), 50.4 ($OCH_3$), 54.2 (α-CH), 55.9 (α-CH), 56.2 (α-CH), 57.1 (α-CH), 59.6 (α-CH), 64.7 (β-$CH_2$ Ser), 82.0 (CHOH), 117.3 (CH Far), 121.7 (CH Far), 122.5 (CH Far), 133.9 (C Far), 139.3 (C Far), 142.4 (C Far), 170.7 (C=O), 172.0 (C=O), 174.7 (C=O), 175.3 (C=O); HRMS (FAB, 3-NBA): *m*/*z*: calcd for $C_{56}H_{103}N_6O_9S_2$: 1067.7229, found: 1067.7237 $[M+H]^+$.

***N-tert*-Butyloxycarbonyl-(4*R*)-hydroxy-L-proline (32)**: At 0°C, a 1M NaOH solution (24 mL) and a solution of $Boc_2O$ (4.118 g, 18.30 mmol) in dioxane (6 mL) were subsequently added to a solution of (4*R*)-hydroxy-L-proline **31** (2 g, 15.25 mmol) in dioxane/$H_2O$ (2:1, 54 mL). The solution was warmed to room temperature and stirred for 7 h. The solvents were removed to 30 mL and the residue was dissolved in ethyl acetate (200 mL). The solution was acidified with 1N HCl to pH 3 and extracted with ethyl acetate (3×50 mL). The combined organic layers were dried over $Na_2SO_4$ and the solvent was removed under reduced pressure to yield **32** as a colorless foam (3.36 g, 95%). M.p. 96°C; $[\alpha]_D^{20}$ = −54.1 (*c*= 1.0 in $CHCl_3$); $R_f$=0.56 ($CHCl_3$/MeOH 1:1); $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$=1.35 (s, 9H, $C(CH_3)_3$), 2.06–2.35 (m, 2H, β-$CH_2$ Pro), 3.36–3.58 (m, 2H, δ-$CH_2$ Pro), 4.30–4.43 (m, 2H, α-CH Pro, γ-CH Pro); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta$=20.9 ($C(CH_3)_3$), 28.5 (β-$CH_2$ Pro), 54.7 (δ-$CH_2$ Pro), 54.8 (α-CH Pro), 69.2 (γ-CH Pro), 81.2 ($C(CH_3)_3$), 154.2 (C=O), 178.4 (C=O); HRMS (FAB, 3-NBA): *m*/*z*: calcd for $C_{10}H_{18}NO_5$: 232.1186, found: 232.1175 $[M+H]^+$; elemental analysis calcd (%) for $C_{10}H_{17}NO_5$: C 51.94, H 7.41, N 6.06; found: C 51.77, H 7.35, N 6.00.

***N-tert*-Butyloxycarbonyl-(4*R*)-hydroxy-L-proline methyl ester (33)**: $Cs_2CO_3$ (2.56 g, 7.24 mmol) was added to a solution of acid **32** (3.35 g, 14.49 mmol) in MeOH (80 mL). After stirring at room temperature for 5 min, the solvent was removed under reduced pressure and the residue was coevaporated with toluene. The resulting salt was dissolved in DMF (100 mL). Methyl iodide (1.350 mL, 21.73 mmol) was added to this solution and the mixture was stirred at room temperature for 20 h. After removal of the solvent under reduced pressure, the residue was dissolved in ethyl acetate (200 mL) and washed subsequently with $Na_2CO_3$ solution (10%, 50 mL) and saturated NaCl solution (30 mL). The organic layer was dried over $Na_2SO_4$, and, after removal of the solvent, the residue was purified by chromatography (cyclohexane/ethyl acetate 1:1) to yield **33** as a yellowish oil (3.138 g, 88%). $[\alpha]_D^{20}$ = −50.6 (*c*=1.0 in $CHCl_3$); $R_f$=0.22 (cyclohexane/ethyl acetate 1:1); $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$=1.41 (s, 9H, $C(CH_3)_3$), 2.03 (m, 1H, β-$CH_{2a}$ Pro), 2.25 (m, 1H, β-$CH_{2b}$ Pro), 2.93 (br s, 1H, OH), 3.41–3.62 (m, 2H, δ-$CH_2$ Pro), 3.71 (s, 3H, $OCH_3$), 4.30–4.48 (m, 2H, α-CH Pro, γ-CH Pro); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta$= 28.6 ($C(CH_3)_3$), 39.2 (β-$CH_2$ Pro), 52.2 (δ-$CH_2$ Pro), 54.9 ($OCH_3$), 58.1 (α-CH Pro), 69.4 (γ-CH Pro), 80.6 ($C(CH_3)_3$), 154.2 (C=O), 173.8 (C=O); HRMS (FAB, 3-NBA): *m*/*z*: calcd for $C_{11}H_{19}NO_5$: 246.1342, found: 246.1338 $[M+H]^+$.

***N-tert*-Butyloxycarbonyl-4-oxo-L-proline methyl ester (34)**: A solution of Dess–Martin periodinane in $CH_2Cl_2$ (15%, 27.2 mL, 12.79 mmol) was added to a solution of alcohol **33** (2.6 g, 10.6 mmol) in $CH_2Cl_2$ (104 mL). The mixture was stirred at room temperature for 1 h. The solution was diluted with ethyl acetate (200 mL) and a mixture of saturated $Na_2S_2O_3$ solution (10 mL) and saturated $NaHCO_3$ solution (30 mL) was added. After the two phases had become clear, the separated organic layer was washed with saturated $NaHCO_3$ solution (30 mL). The organic layer was dried over $Na_2SO_4$, and, after removal of the solvents under reduced pressure, the residue was purified by chromatography (cyclohexane/ethyl acetate 1:1) to yield **34** as a colorless oil (2.1 g, 81%). $[\alpha]_D^{20}$ = −36.7 (*c*= 0.5 in $CHCl_3$); $R_f$=0.42 (cyclohexane/ethyl acetate 1:1); $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$=1.45 (s, 9H, $C(CH_3)_3$), 2.56 (m, 1H, β-$CH_{2a}$ Pro), 2.90 (m, 1H, β-$CH_{2b}$ Pro), 3.73 (s, 3H, $OCH_3$), 3.87 (m, 2H, δ-$CH_2$ Pro), 4.75 (m, 1H, α-CH Pro); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta$=28.4 (C-($CH_3$)$_3$), 40.9 (β-$CH_2$ Pro), 52.8 ($OCH_3$), 53.0 (α-CH Pro), 56.5 (δ-$CH_2$ Pro), 81.5 ($C(CH_3)_3$), 154.22 (C=O), 173.8 (C=O), 206.9 (C=O); HRMS (FAB, 3-NBA): *m*/*z*: calcd for $C_{11}H_{18}NO_5$: 244.1186, found: 244.1191 $[M+H]^+$.

***N-tert*-Butyloxycarbonyl-4-(cyanomethylen)-L-proline methyl ester (36)**: Cyanomethyldiethylphosphonate **35** (4.4 mL, 28.01 mmol) was added to a suspension of NaH (708 mg, 28.01 mmol) in THF (80 mL). After stirring at room temperature for 10 min, a solution of ketone **34** (1.974 g, 8 mmol) in THF (80 mL) was added. The reaction mixture was stirred for 1.5 h and then saturated $NH_4Cl$ solution (20 mL) was added. After removal of the solvent under reduced pressure, the residue was dissolved in ethyl acetate (200 mL) and washed subsequently with saturated $NH_4Cl$ solution (50 mL) and saturated NaCl solution (50 mL). The organic layer was dried over $Na_2SO_4$, and, after removal of the solvent under reduced pressure, the residue was purified by chromatography (cyclohexane/ethyl acetate 6:1) to yield **36** as a yellowish oil (1.812 g, 85%). $[\alpha]_D^{20}$ = −23.3 (*c*=1.0 in $CHCl_3$); $R_f$=0.40 (cyclohexane/ethyl acetate 1:1); $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$=1.45 (s, 9H, $C(CH_3)_3$), 3.10–3.22 (m, 2H, β-$CH_2$ Pro), 3.72 (s, 3H, $OCH_3$), 4.12–4.40 (m, 2H, δ-$CH_2$ Pro), 4.97 (m, 1H, α-CH Pro), 5.81 (m, 1H, -CHCN); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta$=28.5 ($C(CH_3)_3$), 36.2 (β-$CH_2$ Pro), 52.5 ($OCH_3$), 54.8 (δ-$CH_2$ Pro), 60.5 (α-CH Pro), 80.9 ($C(CH_3)_3$), 93.4 (C=*C*HCN), 115.7 (CN), 153.2 (C=O), 170.5 (*C*=CHCN), 171.2 (C=O); HRMS (FAB, 3-NBA): *m*/*z*: calcd for $C_{13}H_{18}N_2O_4$: 266.1267, found: 266.1265 $[M]^+$.

***N-tert*-Butyloxycarbonyl-(4*S*)-(*N'*-allyloxycarbonylaminoethyl)-L-proline methyl ester (37)**: $PtO_2$·$H_2O$ (63 mg, 0.25 mmol) was added to a degassed solution of nitrile **36** (670 mg, 2.52 mmol) in EtOH (114 mL) and $CHCl_3$ (2.2 mL). The reaction mixture was hydrogenated under hydrogen atmosphere at room temperature for 2.5 h. Then, the suspension was filtered through a pad of Celite. The solvents were removed under reduced pressure to yield the amine as a colorless oil (685 mg, 100%).

AlocCl (413 μL, 3.77 mmol) was then added to a solution of the above amine (685 mg, 2.52 mmol) and $NEt_iPr_2$ (861 μL, 5.03 mmol) in $CH_2Cl_2$ (27 mL). After stirring at room temperature for 18 h, the reaction mixture was diluted with ethyl acetate (100 mL) and washed with 1N HCl (30 mL). The organic layer was dried over $Na_2SO_4$, and, after removal of the solvents under reduced pressure, the residue was purified by chromatography (cyclohexane/ethyl acetate 1:1) to yield **37** as a colorless oil (852 mg, 95%). $[\alpha]_D^{20}$ = −17.6 (*c*=0.5 in $CHCl_3$); $R_f$=0.31 (cyclohexane/ethyl acetate 1:1); $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$=1.38 (s, 9H, $C(CH_3)_3$), 1.53 (m, 3H, γ-CH Pro, -C*H*$_2$CH$_2$NH-), 2.10 (m, 1H, β-$CH_{2a}$ Pro), 2.41 (m, 1H, β-$CH_{2b}$ Pro), 2.98 (m, 1H, δ-$CH_{2a}$ Pro), 3.14 (m, 2H, -$CH_2$C*H*$_2$NH-), 3.62 (s, 3H, $OCH_3$), 3.62–3.74 (m, 1H, δ-$CH_{2b}$ Pro), 4.08–4.20 (m, 1H, α-CH Pro), 4.48 (m, 2H, -OC*H*$_2$CH=CH$_2$), 4.63 (m, 1H, NH), 5.11–5.24 (m, 2H, $OCH_2CH$=C*H*$_2$), 5.84 (m, 1H, $OCH_2$C*H*=$CH_2$); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta$=27.1 (β-$CH_2$ Pro), 27.5 (γ-CH Pro), 28.7 ($C(CH_3)_3$), 33.1 (*C*H$_2$CH$_2$NH), 42.2 (CH$_2$*C*H$_2$NH), 48.5 (δ-$CH_2$ Pro), 50.4 ($OCH_3$), 57.5 (α-CH Pro), 66.1 (O*C*H$_2$CH=CH$_2$), 80.9 ($C(CH_3)_3$), 118.06 ($OCH_2CH$=*C*H$_2$), 132.8 ($OCH_2$*C*H=CH$_2$), 156.6 (C=O), 159.4 (C=O), 172.0 (C=O); HRMS (FAB, 3-NBA): *m*/*z*: calcd for $C_{17}H_{29}N_2O_6$: 357.2026, found: 357.2022 $[M+H]^+$.

**(4*S*)-(*N*-Allyloxycarbonylaminoethyl)-L-proline methyl ester hydrotrifluoroacetate (38)**: TFA (15 mL) was added to a solution of proline **37** (698 mg, 1.96 mmol) in $CH_2Cl_2$ (60 mL). After stirring at room temperature for 30 min the solvent was removed under reduced pressure and the residue was coevaporated with toluene to yield **38** as a colorless oil (725 mg, quant.). $[\alpha]_D^{20}$ = −39.8 (*c*=1.0 in MeOH); $R_f$=0.38 ($CH_2Cl_2$/EtOH 5:1); $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$=1.56–1.74 (m, 3H, γ-CH Pro, -C*H*$_2$CH$_2$NH-), 2.46 (m, 1H, β-$CH_{2a}$ Pro), 2.59 (m, 1H, β-$CH_{2b}$ Pro), 2.96 (m, 1H, δ-$CH_{2a}$ Pro), 3.14 (m, 2H, -$CH_2$C*H*$_2$NH-), 3.62 (s, 3H, $OCH_3$), 3.54 (m, 1H, δ-$CH_{2b}$ Pro), 4.36 (m, 1H, α-CH Pro), 4.48 (m, 2H, -OC*H*$_2$CH=CH$_2$), 5.02 (br d, 1H, NH), 5.11–5.23 (m, 2H, -$OCH_2CH$=C*H*$_2$), 5.83 (m, 1H, -$OCH_2$C*H*=CH$_2$); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta$= 32.7 (β-$CH_2$ Pro), 34.9 (γ-CH Pro), 36.2 (*C*H$_2$CH$_2$NH), 39.4 (CH$_2$*C*H$_2$NH), 50.7 (δ-$CH_2$ Pro), 53.7 ($OCH_3$), 59.1 (α-CH Pro), 65.8 (O*C*H$_2$CH=CH$_2$), 117.8 ($OCH_2CH$=*C*H$_2$), 128.4 (*C*F$_3$COOH), 132.9

($OCH_2CH{=}CH_2$), 161.8 (C=O), 169.5 (C=O), 176.0 ($CF_3COOH$); HRMS (FAB, 3-NBA): *m/z*: calcd for $C_{12}H_{21}N_2O_4$: 257.1502, found: 257.1512 $[M+H]^+$.

**3-(Hexadecane-1-sulfonylamino)propionic acid (39)**: TMSCl (651 μL, 5.13 mmol) was added to a suspension of β-alanine (229 mg, 2.56 mmol) in $CH_3CN$ (10 mL) and the reaction mixture was heated under reflux for 1 h. The solution was cooled down to room temperature and then $NEt_3$ (1.43 mL, 10.26 mmol) and a solution of hexadecanesulfonic acid chloride (1 g, 3.08 mmol) in $CH_3CN$ (10 mL) was subsequently added. After stirring at room temperature for 16 h, the reaction mixture was diluted with ethyl acetate (50 mL) and washed with 1 N HCl (20 mL). The organic layer was dried over $Na_2SO_4$, and, after removal of the solvents under reduced pressure, the residue was recrystallized from acetone to yield **39** as a colorless solid (900 mg, 93%). M.p. 124 °C; $^1$H NMR ($CDCl_3$, 400 MHz): $\delta = 0.72$ (t, $J = 6.6$ Hz, 3H, ω-$CH_3$ hexadecyl), 1.18 (m, 24H, 12×$CH_2$ hexadecyl), 1.24 (m, 2H, γ-$CH_2$ hexadecyl), 1.60 (m, 2H, β-$CH_2$ hexadecyl), 2.40 (t, $J = 6.4$ Hz, 2H, α-$CH_2$ β-Ala), 2.82 (m, 2H, α-$CH_2$ hexadecyl), 3.12 (t, $J = 6.4$ Hz, 2H, β-$CH_2$ β-Ala), 4.20 (br s, 1H, NH); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta = 17.9$ (ω-$CH_3$ hexadecyl), 26.7 ($CH_2$), 27.5 ($CH_2$), 32.3 ($CH_2$), 32.4 ($CH_2$), 32.6 ($CH_2$), 32.7 ($CH_2$), 33.0 ($CH_2$), 33.2 ($CH_2$), 33.7 ($CH_2$), 33.8 ($CH_2$), 34.6 ($CH_2$), 34.8 ($CH_2$), 35.2 ($CH_2$), 35.9 ($CH_2$), 38.8 (β-$CH_2$ β-Ala), 42.7 (α-$CH_2$ β-Ala), 56.5 (α-$CH_2$ hexadecyl), 178.0 (C=O); HRMS (FAB, 3-NBA): *m/z*: calcd for $C_{19}H_{40}NO_4S$: 378.2679, found: 378.2677 $[M+H]^+$.

***N*-[3-(Hexadecane-1-sulfonylamino)propionyl]-(4*S*)-(*N*′-allyloxycarbonyl-aminoethyl)-L-proline methyl ester (40)**: At 0 °C, EDC (279 mg, 1.43 mmol) was added to a solution of proline **38** (440 mg, 1.19 mmol), β-alanine **39** (538 mg, 1.43 mmol), HOBt (371 mg, 2.38 mmol) and NE*t*i$Pr_2$ (244 μL, 1.43 mmol) in $CH_2Cl_2$ (45 mL). The solution was warmed to room temperature and stirred for 18 h. Then, the reaction mixture was diluted with ethyl acetate (100 mL) and subsequently washed with 1 N HCl (30 mL) and $Na_2CO_3$ solution (10%, 30 mL). The organic layer was dried over $Na_2SO_4$, and, after removal of the solvent under reduced pressure, the residue was purified by chromatography (cyclohexane/ethyl acetate 1:2) to yield **40** as colorless oil (593 mg, 81%). $[\alpha]_D^{20} = -13.2$ ($c = 1.0$ in $CHCl_3$); $R_f = 0.30$ (cyclohexane/ethyl acetate 1:3); $^1$H NMR ($CDCl_3$, 400 MHz): $\delta = 0.86$ (t, $J = 6.6$ Hz, 3H, ω-$CH_3$ hexadecyl), 1.23 (m, 24H, 12×$CH_2$ hexadecyl), 1.37 (m, 2H, γ-$CH_2$ hexadecyl), 1.60 (m, 3H, γ-CH Pro, -C$H_2$$CH_2$NH-), 1.76 (m, 2H, β-$CH_2$ hexadecyl), 2.29 (m, 1H, β-$CH_{2a}$ Pro), 2.45 (m, 1H, β-$CH_{2b}$ Pro), 2.58 (m, 2H, α-$CH_2$ β-Ala), 2.97 (m, 2H, α-$CH_2$ hexadecyl), 3.19 (m, 3H, β-$CH_2$ β-Ala, δ-$CH_{2a}$ Pro), 3.36 (m, 3H, -$CH_2$C$H_2$NH-, δ-$CH_{2b}$ Pro), 3.71 (s, 3H, $OCH_3$), 4.34 (m, 1H, NH), 4.53 (m, 2H, -OC$H_2$CH=$CH_2$), 4.92 (m, 1H, α-CH Pro), 5.16–5.34 (m, 3H, NH, -$OCH_2$CH=C$H_2$), 5.88 (m, 1H, -$OCH_2$C$H$=$CH_2$); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta = 14.3$ (ω-$CH_3$ hexadecyl), 22.8 ($CH_2$), 24.7 ($CH_2$), 28.5 ($CH_2$), 28.6 ($CH_2$), 28.9 ($CH_2$), 29.1 ($CH_2$), 29.5 ($CH_2$), 29.6 ($CH_2$), 29.8 ($CH_2$), 30.3 ($CH_2$), 30.5 ($CH_2$), 30.7 ($CH_2$), 31.5 ($CH_2$), 32.3 ($CH_2$), 33.3 ($CH_2$), 35.2 (γ-CH Pro), 35.5 ($CH_2$), 36.7 (C$H_2$$CH_2$NH), 38.8 (β-$CH_2$ β-Ala), 39.4 ($CH_2$C$H_2$NH), 42.7 (α-$CH_2$ β-Ala), 52.5 (δ-$CH_2$ Pro), 52.8 ($OCH_3$), 59.1 (α-$CH_2$ hexadecyl), 59.2 (α-CH Pro), 65.8 (O$CH_2$CH=$CH_2$), 117.9 ($OCH_2$CH=C$H_2$), 133.0 ($OCH_2$C$H$=$CH_2$), 156.4 (C=O), 170.2 (C=O), 172.8 (C=O); HRMS (FAB, 3-NBA): *m/z*: calcd for $C_{31}H_{58}N_3O_7S$: 616.3996, found: 616.3976 $[M+H]^+$.

***N*-[3-(Hexadecane-1-sulfonylamino)propionyl]-(4*S*)-(*N*′-allyloxycarbonyl-aminoethyl)-L-prolyl-*S*-farnesyl-L-cysteine methyl ester (41)**: A 1 M NaOH solution (1.75 mL) was added to a solution of dipeptide **40** (539 mg, 0.88 mmol) in MeOH (3.5 mL). After stirring at room temperature for 3 h, the reaction mixture was diluted with $CHCl_3$ (30 mL), acidified with 1 N HCl to pH 3 and extracted with $CHCl_3$ (2×30 mL). The combined organic layers were dried over $Na_2SO_4$ and the solvents were removed under reduced pressure to yield the free acid (526 mg, 100%).

At 0 °C, EDC (205 mg, 1.05 mmol) was added to a solution of the above acid (562 mg, 0.87 mmol), H-Cys(Far)-OMe **10** (356 mg, 1.05 mmol) and HOBt (273 mg, 1.75 mmol) in $CH_2Cl_2$ (50 mL). The solution was warmed to room temperature and stirred for 20 h. Then, the reaction mixture was diluted with ethyl acetate (100 mL) and subsequently washed with 1 N HCl (30 mL) and $Na_2CO_3$ solution (10%, 30 mL). The organic layer was dried over $Na_2SO_4$, and, after removal of the solvent under reduced pressure, the residue was purified by chromatography (cyclohexane/ethyl acetate 1:1, then 1:5) to yield **41** as colorless oil (701 mg, 87%). $[\alpha]_D^{20} = -8.4$ ($c = 0.5$ in $CHCl_3$); $R_f = 0.29$ (cyclohexane/ethyl acetate); $^1$H NMR ($CDCl_3$, 400 MHz): $\delta = 0.86$ (t, $J = 6.6$ Hz, 3H, ω-$CH_3$ hexadecyl), 1.23 (m, 24H, 12×$CH_2$ hexadecyl), 1.37 (m, 2H, γ-$CH_2$ hexadecyl), 1.50 (s, 6H, 2×$CH_3$ Far), 1.55 (s, 3H, $CH_3$ Far), 1.57 (s, 3H, $CH_3$ Far), 1.60 (m, 3H, γ-CH Pro, -C$H_2$$CH_2$NH-), 1.76 (m, 2H, β-$CH_2$ hexadecyl), 1.82–2.08 (m, 8H, 4×$CH_2$ Far), 2.29 (m, 1H, β-$CH_{2a}$ Pro), 2.45 (m, 1H, β-$CH_{2b}$ Pro), 2.58 (m, 2H, α-$CH_2$ β-Ala), 2.72 (m, 2H, -$SCH_2$-), 2.96 (m, 3H, α-$CH_2$ hexadecyl, δ-$CH_{2a}$ Pro), 3.20 (m, 4H, β-$CH_2$ β-Ala, β-$CH_2$ Cys), 3.41 (m, 3H, -$CH_2$C$H_2$NH-, δ-$CH_{2b}$ Pro), 3.68 (s, 3H, $OCH_3$), 4.53 (m, 2H, -OC$H_2$CH=$CH_2$), 4.76 (m, 1H, α-CH Cys), 4.92 (m, 1H, α-CH Pro), 5.04–5.29 (m, 6H, 3×CH Far, NH, -$OCH_2$CH=C$H_2$), 5.54 (m, 1H, NH), 5.90 (m, 1H, -$OCH_2$C$H$=$CH_2$), 7.11 (m, 1H, NH); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta = 14.3$ (ω-$CH_3$ hexadecyl), 16.2 ($CH_3$ Far), 16.4 ($CH_3$ Far), 16.5 ($CH_3$ Far), 17.9 ($CH_3$ Far), 22.8 ($CH_2$), 22.9 ($CH_2$), 23.8 ($CH_2$), 24.7 ($CH_2$), 28.5 ($CH_2$), 28.6 ($CH_2$), 28.9 ($CH_2$), 29.1 ($CH_2$), 29.5 ($CH_2$), 29.6 ($CH_2$), 29.8 ($CH_2$), 30.3 ($CH_2$), 30.5 ($CH_2$), 30.7 ($CH_2$), 31.5 ($CH_2$), 32.1 ($CH_2$), 32.3 ($CH_2$), 33.3 ($CH_2$), 33.4 ($CH_2$), 35.2 (γ-CH Pro), 35.5 ($CH_2$), 36.7 (C$H_2$$CH_2$NH), 38.8 (β-$CH_2$ β-Ala), 39.2 ($CH_2$ Far), 39.4 ($CH_2$C$H_2$NH), 39.8 ($CH_2$ Far), 42.7 (α-$CH_2$ β-Ala), 52.5 (δ-$CH_2$ Pro), 52.9 ($OCH_3$), 57.4 (α-CH Cys), 59.1 (α-$CH_2$ hexadecyl), 59.2 (α-CH Pro), 65.8 (O$CH_2$CH=$CH_2$), 117.9 ($OCH_2$CH=C$H_2$), 119.7 (CH Far), 123.9 (CH Far), 124.5 (CH Far), 132.3 (C Far), 133.0 ($OCH_2$C$H$=$CH_2$), 135.5 (C Far), 140.8 (C Far), 156.4 (C=O), 170.2 (C=O), 171.0 (C=O), 172.8 (C=O); HRMS (FAB, 3-NBA): *m/z*: calcd for $C_{49}H_{87}N_4O_8S_2$: 923.5966, found: 923.5955 $[M+H]^+$.

***N*-[3-(Hexadecane-1-sulfonylamino)propionyl]-(4*S*)-(aminoethyl)-L-prolyl-*S*-farnesyl-L-cysteine methyl ester (42)**: $[Pd(PPh_3)_4]$ (38 mg, 0.03 mmol) was added to a degassed solution of tripeptide **41** (300 mg, 0.33 mmol) and *N,N*′-dimethylbarbituric acid (51 mg, 0.33 mmol) in THF (30 mL). The mixture was stirred at room temperature for 3 h. After removal of the solvent under reduced pressure, the residue was purified by chromatography ($CH_2Cl_2$/EtOH 20:1, then 10:1, then 5:1 + 1% $NEt_3$, then 1:1 + 1% $NEt_3$) to yield **42** as a slightly yellow foam (216 mg, 79%). $[\alpha]_D^{20} = -14.5$ ($c = 0.5$ in $CHCl_3$); $R_f = 0.07$ ($CH_2Cl_2$/EtOH 10:1); $^1$H NMR ($CD_3OD$, 400 MHz): $\delta = 0.86$ (t, $J = 6.6$ Hz, 3H, ω-$CH_3$ hexadecyl), 1.23 (m, 24H, 12×$CH_2$ hexadecyl), 1.37 (m, 2H, γ-$CH_2$ hexadecyl), 1.50 (s, 6H, 2×$CH_3$ Far), 1.55 (s, 3H, $CH_3$ Far), 1.57 (s, 3H, $CH_3$ Far), 1.60 (m, 3H, γ-CH Pro, -C$H_2$$CH_2$$NH_2$), 1.76 (m, 2H, β-$CH_2$ hexadecyl), 1.82–2.08 (m, 8H, 4×$CH_2$ Far), 2.29 (m, 1H, β-$CH_{2a}$ Pro), 2.45 (m, 1H, β-$CH_{2b}$ Pro), 2.58 (m, 2H, α-$CH_2$ β-Ala), 2.72 (m, 2H, -$SCH_2$-), 2.96 (m, 3H, α-$CH_2$ hexadecyl, δ-$CH_{2a}$ Pro), 3.20 (m, 4H, β-$CH_2$ β-Ala, β-$CH_2$ Cys), 3.41 (m, 3H, -$CH_2$C$H_2$$NH_2$, δ-$CH_{2b}$ Pro), 3.68 (s, 3H, $OCH_3$), 4.76 (m, 1H, α-CH Cys), 4.92 (m, 1H, α-CH Pro), 5.04–5.29 (m, 3H, 3×CH Far); $^{13}$C NMR ($CD_3OD$, 100.6 MHz): $\delta = 14.3$ (ω-$CH_3$ hexadecyl), 16.2 ($CH_3$ Far), 16.4 ($CH_3$ Far), 16.5 ($CH_3$ Far), 17.9 ($CH_3$ Far), 22.8 ($CH_2$), 22.9 ($CH_2$), 23.8 ($CH_2$), 24.7 ($CH_2$), 28.5 ($CH_2$), 28.6 ($CH_2$), 28.9 ($CH_2$), 29.1 ($CH_2$), 29.5 ($CH_2$), 29.6 ($CH_2$), 29.8 ($CH_2$), 30.3 ($CH_2$), 30.5 ($CH_2$), 30.7 ($CH_2$), 31.5 ($CH_2$), 32.1 ($CH_2$), 32.3 ($CH_2$), 33.3 ($CH_2$), 33.4 ($CH_2$), 35.2 (γ-CH Pro), 35.5 ($CH_2$), 36.7 (C$H_2$$CH_2$$NH_2$), 38.8 (β-$CH_2$ β-Ala), 39.2 ($CH_2$ Far), 39.4 ($CH_2$C$H_2$$NH_2$), 39.8 ($CH_2$ Far), 42.7 (α-$CH_2$ β-Ala), 52.5 (δ-$CH_2$ Pro), 52.9 ($OCH_3$), 57.4 (α-CH Cys), 59.1 (α-$CH_2$ hexadecyl), 59.2 (α-CH Pro), 119.7 (CH Far), 123.9 (CH Far), 124.5 (CH Far), 132.3 (C Far), 135.5 (C Far), 140.8 (C Far), 170.2 (C=O), 171.0 (C=O), 172.8 (C=O); HRMS (FAB, 3-NBA): *m/z*: calcd for $C_{45}H_{82}N_4NaO_6S_2$: 861.5576, found: 861.5562 $[M+Na]^+$.

***N*-[(2*S*)-Acetylamino-3-(hexadecane-1-sulfonylamino)propionyl]-(4*S*)-(*N*′-allyloxycarbonylaminoethyl)-L-proline methyl ester (43)**: At 0 °C, EDC (81 mg, 0.41 mmol) was added to a solution of proline **38** (150 mg, 0.35 mmol), Ac-Dap($SO_2C_{16}H_{33}$)-OH **4** (128 mg, 0.35 mmol), HOBt (108 mg, 0.69 mmol) and NE*t*i$Pr_2$ in $CH_2Cl_2$ (15 mL). The solution was warmed to room temperature and stirred for 18 h. Then, the reaction mixture was diluted with ethyl acetate (50 mL) and subsequently washed with 1 N HCl (15 mL) and $Na_2CO_3$ solution (10%, 15 mL). The organic layer was dried over $Na_2SO_4$, and, after removal of the solvent under reduced pressure, the residue was purified by chromatography (cyclohexane/ethyl acetate 1:5) to yield **43** as colorless solid (176 mg, 76%). M.p. 78 °C; $[\alpha]_D^{20} = -9.2$ ($c = 1.0$ in $CHCl_3$); $R_f = 0.09$ (cyclohexane/ethyl ace-

tate 1:5); $^1$H NMR ($CDCl_3$, 400 MHz): $\delta = 0.81$ (t, $J = 6.5$ Hz, 3H, ω-$CH_3$ hexadecyl), 1.18 (m, 24H, 12×$CH_2$ hexadecyl), 1.33 (m, 2H, γ-$CH_2$ hexadecyl), 1.55 (m, 3H, γ-CH Pro, -C$H_2$CH$_2$NH-), 1.72 (m, 2H, β-$CH_2$ hexadecyl), 1.94 (s, 3H, $CH_3$ acetyl), 2.25 (m, 1H, β-$CH_{2a}$ Pro), 2.45 (m, 1H, β-$CH_{2b}$ Pro), 2.93 (m, 2H, α-$CH_2$ hexadecyl), 3.14 (m, 3H, δ-$CH_{2a}$ Pro, -CH$_2$C$H_2$NH-), 3.30 (m, 3H, δ-$CH_{2b}$ Pro, β-$CH_2$ Dap), 3.68 (s, 3H, $OCH_3$), 3.99 (m, 1H, NH), 4.40 (m, 1H, α-CH Dap), 4.48 (m, 2H, -OC$H_2$CH=CH$_2$), 4.86 (m, 1H, α-CH Pro), 5.12–5.25 (m, 2H, -OCH$_2$CH=C$H_2$), 5.64 (m, 1H, NH), 5.85 (m, 1H, -OCH$_2$C$H$=CH$_2$), 6.70 (m, 1H, NH); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta = 14.3$ (ω-$CH_3$ hexadecyl), 22.8 ($CH_3$ acetyl), 22.9 ($CH_2$), 24.5 ($CH_2$), 28.6 ($CH_2$), 28.8 ($CH_2$), 29.2 ($CH_2$), 29.5 ($CH_2$), 29.6 ($CH_2$), 29.9 ($CH_2$), 30.2 ($CH_2$), 30.5 ($CH_2$), 30.6 ($CH_2$), 31.3 ($CH_2$), 32.0 ($CH_2$), 33.1 ($CH_2$), 35.1 (γ-CH Pro), 36.7 ($CH_2$), 36.9 (C$H_2$CH$_2$NH), 39.6 (CH$_2$C$H_2$NH), 45.0 (β-$CH_2$ Dap), 51.1 (δ-$CH_2$ Pro), 52.8 (α-CH Dap), 52.9 ($OCH_3$), 59.3 (α-$CH_2$ hexadecyl), 59.4 (α-CH Pro), 65.7 (OC$H_2$CH=CH$_2$), 117.8 (OCH$_2$CH=H$_2$), 133.0 (OCH$_2$C$H$=CH$_2$), 156.5 (C=O), 168.5 (C=O), 169.2 (C=O), 172.8 (C=O); HRMS (FAB, 3-NBA): *m*/*z*: calcd for $C_{33}H_{60}N_4NaO_8S$: 695.4032, found: 695.4054 $[M+Na]^+$.

***N*-[(2*S*)-Acetylamino-3-(hexadecane-1-sulfonylamino)propionyl]-(4*S*)-(*N*′-allyloxycarbonylaminoethyl)-L-prolyl-*S*-farnesyl-L-cysteine methyl ester (44)**: A 1 M NaOH solution (416 μL) was added to a solution of dipeptide **43** (140 mg, 0.21 mmol) in MeOH (832 μL). After stirring at room temperature for 3 h, the reaction mixture was diluted with $CHCl_3$ (30 mL), acidified with 1 N HCl to pH 3 and extracted with $CHCl_3$ (2× 20 mL). The combined organic layers were dried over $Na_2SO_4$ and the solvents were removed under reduced pressure to yield the free acid (137 mg, 100%).

At 0°C, EDC (49 mg, 0.25 mmol) was added to a solution of the above acid (137 mg, 0.21 mmol), H-Cys(Far)-OMe **10** (85 mg, 0.25 mmol) and HOBt (65 mg, 0.42 mmol) in $CH_2Cl_2$ (25 mL). The solution was warmed to room temperature and stirred for 20 h. Then, the reaction mixture was diluted with ethyl acetate (50 mL) and subsequently washed with 1 N HCl (15 mL) and $Na_2CO_3$ solution (10%, 15 mL). The organic layer was dried over $Na_2SO_4$, and, after removal of the solvent under reduced pressure, the residue was purified by chromatography (cyclohexane/ethyl acetate 1:8, then $CH_2Cl_2$/EtOH 10:1) to yield **44** as colorless oil (169 mg, 83%). $[\alpha]_D^{20} = -9.0$ ($c = 0.5$ in $CHCl_3$); $R_f = 0.13$ (cyclohexane/ethyl acetate 1:8); $^1$H NMR ($CDCl_3$, 400 MHz): $\delta = 0.81$ (t, $J = 6.6$ Hz, 3H, ω-$CH_3$ hexadecyl), 1.18 (m, 24H, 12×$CH_2$ hexadecyl), 1.34 (m, 2H, γ-$CH_2$ hexadecyl), 1.50 (s, 6H, 2×$CH_3$ Far), 1.55 (s, 3H, $CH_3$ Far), 1.57 (s, 3H, $CH_3$ Far), 1.59 (m, 3H, γ-CH Pro, -C$H_2$CH$_2$NH-), 1.72 (m, 2H, β-$CH_2$ hexadecyl), 1.94 (s, 3H, $CH_3$ acetyl), 1.82–2.08 (m, 8H, 4×$CH_2$ Far), 2.25 (m, 1H, β-$CH_{2a}$ Pro), 2.45 (m, 1H, β-$CH_{2b}$ Pro), 2.72 (m, 2H, -SCH$_2$-), 2.96 (m, 3H, α-$CH_2$ hexadecyl, δ-$CH_{2a}$ Pro), 3.20 (m, 4H, β-$CH_2$ Dap, β-$CH_2$ Cys), 3.41 (m, 3H, -CH$_2$C$H_2$NH-, δ-$CH_{2b}$ Pro), 3.71 (s, 3H, $OCH_3$), 4.53 (m, 2H, -OC$H_2$CH=CH$_2$), 4.76 (m, 1H, α-CH Cys), 4.92 (m, 1H, α-CH Pro), 5.04–5.29 (m, 6H, 3×CH Far, NH -OCH$_2$CH=C$H_2$), 5.54 (m, 1H, NH), 5.90 (m, 1H, -OCH$_2$C$H$=CH$_2$), 7.11 (m, 1H, NH); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta = 14.3$ (ω-$CH_3$ hexadecyl), 16.2 ($CH_3$ Far), 16.4 ($CH_3$ Far), 16.5 ($CH_3$ Far), 17.9 ($CH_3$ Far), 22.8 ($CH_3$ Acetyl), 22.8 ($CH_2$), 22.9 ($CH_2$), 23.8 ($CH_2$), 24.7 ($CH_2$), 28.5 ($CH_2$), 28.6 ($CH_2$), 28.9 ($CH_2$), 29.1 ($CH_2$), 29.5 ($CH_2$), 29.6 ($CH_2$), 29.8 ($CH_2$), 30.3 ($CH_2$), 30.5 ($CH_2$), 30.7 ($CH_2$), 31.5 ($CH_2$), 32.1 ($CH_2$), 32.3 ($CH_2$), 33.3 ($CH_2$), 33.4 ($CH_2$), 35.2 (γ-CH Pro), 35.5 ($CH_2$), 36.7 (C$H_2$CH$_2$NH), 39.2 ($CH_2$ Far), 39.4 (CH$_2$C$H_2$NH), 39.8 ($CH_2$ Far), 45.7 (β-$CH_2$ Dap), 52.5 (δ-$CH_2$ Pro), 52.8 (α-CH Dap), 53.0 ($OCH_3$), 57.4 (α-CH Cys), 59.1 (α-$CH_2$ hexadecyl), 59.2 (α-CH Pro), 65.8 (OC$H_2$CH=CH$_2$), 117.9 (OCH$_2$CH=C$H_2$), 119.7 (CH Far), 123.9 (CH Far), 124.5 (CH Far), 132.3 (C Far), 133.0 (OCH$_2$C$H$=CH$_2$), 135.5 (C Far), 140.8 (C Far), 156.4 (C=O), 170.2 (C=O), 171.0 (C=O), 171.2 (C=O), 172.8 (C=O); HRMS (FAB, 3-NBA): *m*/*z*: calcd for $C_{51}H_{90}N_5O_9S_2$: 980.6181, found: 980.6188 $[M+H]^+$.

***N*-[(2*S*)-Acetylamino-3-(hexadecane-1-sulfonylamino)propionyl]-(4*S*)-aminoethyl-L-prolyl-*S*-farnesyl-L-cysteine methyl ester (45)**: $[Pd(PPh_3)_4]$ (18 mg, 0.02 mmol) was added to a degassed solution of tripeptide **44** (149 mg, 0.15 mmol) and *N*,*N*′-dimethylbarbituric acid (24 mg, 0.15 mmol) in THF (15 mL). The mixture was stirred at room temperature for 24 h. After removal of the solvent under reduced pressure, the residue was purified by chromatography ($CH_2Cl_2$/EtOH 10:1, then 5:1, then 1:1, then 1:1 + 1% $NEt_3$) to yield **45** as a slightly yellow foam (87 mg, 64%). $[\alpha]_D^{20} = -4.9$ ($c = 0.5$ in $CHCl_3$); $R_f = 0.04$ ($CH_2Cl_2$/EtOH 1:1); $^1$H NMR ($CD_3OD$, 400 MHz): $\delta = 0.81$ (t, $J = 6.6$ Hz, 3H, ω-$CH_3$ hexadecyl), 1.18 (m, 24H, 12×$CH_2$ hexadecyl), 1.34 (m, 2H, γ-$CH_2$ hexadecyl), 1.50 (s, 6H, 2×$CH_3$ Far), 1.55 (s, 3H, $CH_3$ Far), 1.57 (s, 3H, $CH_3$ Far), 1.59 (m, 3H, γ-CH Pro, -C$H_2$CH$_2$NH$_2$), 1.72 (m, 2H, β-$CH_2$ hexadecyl), 1.94 (s, 3H, $CH_3$ acetyl), 1.82–2.08 (m, 8H, 4×$CH_2$ Far), 2.25 (m, 1H, β-$CH_{2a}$ Pro), 2.45 (m, 1H, β-$CH_{2b}$ Pro), 2.72 (m, 2H, -SCH$_2$-), 2.96 (m, 3H, α-$CH_2$ hexadecyl, δ-$CH_{2a}$ Pro), 3.20 (m, 4H, β-$CH_2$ Dap, β-$CH_2$ Cys), 3.41 (m, 3H, -CH$_2$C$H_2$NH$_2$, δ-$CH_{2b}$ Pro), 3.71 (s, 3H, $OCH_3$), 4.76 (m, 1H, α-CH Cys), 4.92 (m, 1H, α-CH Pro), 5.04–5.29 (m, 3H, 3×CH Far); $^{13}$C NMR ($CD_3OD$, 100.6 MHz): $\delta = 14.3$ (ω-$CH_3$ hexadecyl), 16.2 ($CH_3$ Far), 16.4 ($CH_3$ Far), 16.5 ($CH_3$ Far), 17.9 ($CH_3$ Far), 22.8 ($CH_3$ acetyl), 22.8 ($CH_2$), 22.9 ($CH_2$), 23.8 ($CH_2$), 24.7 ($CH_2$), 28.5 ($CH_2$), 28.9 ($CH_2$), 29.1 ($CH_2$), 29.5 ($CH_2$), 29.6 ($CH_2$), 29.8 ($CH_2$), 30.3 ($CH_2$), 30.5 ($CH_2$), 30.7 ($CH_2$), 31.5 ($CH_2$), 32.1 ($CH_2$), 32.3 ($CH_2$), 33.3 ($CH_2$), 33.4 ($CH_2$), 35.2 (γ-CH Pro), 35.5 ($CH_2$), 36.7 (C$H_2$CH$_2$NH$_2$), 39.2 ($CH_2$ Far), 39.4 (CH$_2$C$H_2$NH$_2$), 39.8 ($CH_2$ Far), 45.7 (β-$CH_2$ Dap), 52.5 (δ-$CH_2$ Pro), 52.8 (α-CH Dap), 53.0 ($OCH_3$), 57.4 (α-CH Cys), 59.1 (α-$CH_2$ hexadecyl), 59.2 (α-CH Pro), 119.7 (CH Far), 123.9 (CH Far), 124.5 (CH Far), 132.3 (C Far), 135.5 (C Far), 140.8 (C Far), 170.2 (C=O), 171.0 (C=O), 171.2 (C=O), 172.8 (C=O); HRMS (FAB, 3-NBA): *m*/*z*: calcd for $C_{47}H_{86}N_5O_7S_2$: 897.5969, found: 897.5938 $[M+H]^+$.

**(2*S*)-[Bis(*tert*-butyloxycarbonyl)amino]butyrolactone (47)**: At 0°C, a solution of $Boc_2O$ (29.666 g, 131.85 mmol) in $CH_2Cl_2$ (50 mL) was added to a solution of (2*S*)-aminobutyrolactone hydrobromide **46** (20 g, 109.88 mmol) and $NEtiPr_2$ (56.43 mL, 329.63 mmol) in $CH_2Cl_2$ (500 mL). The solution was warmed to room temperature and stirred for 20 h. Then, the reaction mixture was washed with 1 N HCl (100 mL). The organic layer was dried over $Na_2SO_4$ and the solvent was removed under reduced pressure to yield the monoprotected lactone as a colorless solid (22.1 g, 100%).

$Boc_2O$ (27.2 g, 120.86 mmol) was then added to a solution of the above lactone (22.1 g, 109.88 mmol) and DMAP (2.74 g, 21.98 mmol) in $CH_3CN$ (380 mL). After stirring at room temperature for 15 h, the solvent was removed under reduced pressure and the residue was purified by chromatography (cyclohexane/ethyl acetate 1:1) to yield **47** as a colorless solid (31.03 g, 94%). M.p. 75°C; $[\alpha]_D^{20} = -42.5$ ($c = 1.0$ in $CHCl_3$); $R_f = 0.51$ (cyclohexane/ethyl acetate 1:1); $^1$H NMR ($CDCl_3$, 400 MHz): $\delta = 1.52$ (s, 18H, C($CH_3$)$_3$), 2.53 (m, 2H, β-$CH_2$), 4.26 (dt, $J = 8.4$, 9.1 Hz, 1H, γ-$CH_{2a}$), 4.48 (dt, $J = 5.7$, 6.3 Hz, 1H, γ-$CH_{2b}$), 5.12 (t, $J = 9.9$ Hz, 1H, α-CH); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta = 26.8$ (β-$CH_2$), 28.2 (C($CH_3$)$_3$), 54.0 (α-CH), 65.4 (γ-$CH_2$), 84.3 (*C*($CH_3$)$_3$), 151.7 (C=O), 173.9 (C=O); HRMS (FAB, 3-NBA): *m*/*z*: calcd for $C_{14}H_{24}NO_6$: 302.1604, found: 302.1615 $[M+H]^+$.

**(2*S*)-[Bis(*tert*-butyloxycarbonyl)amino]-4-hydroxybutyric acid methyl ester (48)**: A 1 M CsOH solution (102.59 mL) was added to a solution of lactone **47** (30.915 g, 102.59 mmol) in MeOH (220 mL). After stirring at room temperature for 2 h, the solvent was removed under reduced pressure and the residue was coevaporated with toluene. The resulting salt was dissolved in DMF (834 mL). Methyl iodide (7.67 mL, 123.11 mmol) was added to this solution and the mixture was stirred at room temperature for 18 h. After removal of the solvent under reduced pressure, the residue was dissolved in ethyl acetate (500 mL) and washed subsequently with $Na_2CO_3$ solution (10%, 100 mL) and saturated NaCl solution (30 mL). The organic layer was dried over $Na_2SO_4$, and, after removal of the solvent, the residue was purified by chromatography (cyclohexane/ethyl acetate 4:1) to yield **48** as a colorless solid (27.019 g, 79%). M.p. 89°C; $[\alpha]_D^{20} = -37.8$ ($c = 1.0$ in $CHCl_3$); $R_f = 0.36$ (cyclohexane/ethyl acetate 1:1); $^1$H NMR ($CDCl_3$, 400 MHz): $\delta = 1.51$ (s, 18H, C($CH_3$)$_3$), 2.03 (m, 1H, β-$CH_{2a}$), 2.42 (m, 1H, β-$CH_{2b}$), 2.48 (br s, 1H, OH), 3.59 (m, 1H, γ-$CH_{2a}$), 3.73 (m, s, 4H, γ-$CH_{2b}$, $OCH_3$), 5.01 (dd, $J = 4.7$, 9.8 Hz, 1H, α-CH); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta = 28.1$ (C(*C*$H_3$)$_3$), 33.2 (β-$CH_2$), 52.5 ($OCH_3$), 55.6 (α-CH), 59.2 (γ-CH), 83.8 (*C*($CH_3$)$_3$), 152.6 (C=O), 171.5 (C=O); HRMS (FAB, 3-NBA): *m*/*z*: calcd for $C_{15}H_{28}NO_7$: 334.1867, found: 334.1886 $[M+H]^+$.

**(2*S*)-[Bis(*tert*-butyloxycarbonyl)amino]-4-cyano-4-hydroxybutyric acid methyl ester (49)**: At −60 °C, a solution of DMSO (9.352 mL, 131.78 mmol) in $CH_2Cl_2$ (28 mL) was added to a solution of oxalyl chloride (8.285 mL, 94.48 mmol) in $CH_2Cl_2$ (189 mL). The reaction mixture was stirred at −60 °C for 30 min. Then, a solution of alcohol **48** (13.5 g, 40.49 mmol) in $CH_2Cl_2$ (57 mL) was added. After stirring at −60 °C for 4 h, NEt*i*Pr$_2$ (39.293 mL, 281.91 mmol) was added and the reaction mixture was warmed to room temperature. The solution was poured into a 1 M $KH_2PO_4$ solution and extracted with ethyl acetate (100 mL). The organic layer was then subsequently washed with $H_2O$ (50 mL) and saturated NaCl solution (50 mL). The organic layers were dried over $Na_2SO_4$ and the solvents were removed under reduced pressure to yield the crude aldehyde as colorless oil (12.314 g, 92 %).

TMSCN (9.3 mL, 72.43 mmol) was added to a solution of the above aldehyde (12.314 g, 37.25 mmol) in $CH_2Cl_2$ (200 mL). The reaction mixture was stirred at room temperature for 20 h. Then, the solvent was removed under reduced pressure, the residue was dissolved in methanol and $NH_4F$ (2.7 g, 72.43 mmol) was added. After stirring for 5 min, the solvent was removed under reduced pressure. The residue was dissolved in ethyl acetate (500 mL) and subsequently washed with $H_2O$ (100 mL) and saturated NaCl solution (100 mL). The organic layer was dried over $Na_2SO_4$, and, after removal of the solvent under reduced pressure, the residue was purified by chromatography (cyclohexane/ethyl acetate 6:1) to yield **49** as a colorless oil (11.68 g, 90 %). $R_f$ = 0.40 (cyclohexane/ethyl acetate 1:1); $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ = 1.53 (s, 18H, $C(CH_3)_3$), 2.36 (m, 1H, β-$CH_{2a}$), 2.73 (m, 1H, β-$CH_{2b}$), 3.76 (s, 3H, $OCH_3$), 4.51 (m, 2H, γ-CH), 4.74 (br s, 1H, OH), 5.10 (m, 1H, α-CH); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta$ = 28.2 ($C(CH_3)_3$), 35.3 (β-$CH_2$), 55.0 ($OCH_3$), 58.7 (α-CH), 58.4 (γ-CH), 84.7 ($C(CH_3)_3$), 119.3 (CN), 152.5 (C=O), 170.4 (C=O); HRMS (FAB, 3-NBA): *m*/*z*: calcd for $C_{16}H_{27}N_2O_7$: 359.1819, found: 359.1846 $[M+H]^+$.

**(2*S*)-[Bis(*tert*-butyloxycarbonyl)amino]-4-(*tert*-butyldiphenylsilanyloxy)-4-cyanobutyric acid methyl ester (50)**: TBDPSCl (11.661 mL, 43.95 mmol) was added to a solution of cyanohydrin **49** (10.5 g, 29.3 mmol) and imidazole (6 g, 87.89 mmol) in DMF (30 mL). The solution was stirred at room temperature for 15 h. Then, the solvent was removed under reduced pressure, the residue was dissolved in ethyl acetate (500 mL) and washed subsequently with 1 N HCl (100 mL) and saturated NaCl solution (100 mL). The organic layer was dried over $Na_2SO_4$, and, after removal of the solvent under reduced pressure, the residue was purified by chromatography (cyclohexane/ethyl acetate 10:1) to yield **50** as a colorless oil (15.517 g, 90 %). $R_f$ = 0.13 (cyclohexane/ethyl acetate 7:1); $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ = 1.09 (s, 9H, $C(CH_3)_3$ silyl), 1.45 (s, 18H, $C(CH_3)_3$ Boc), 2.36 (m, 1H, β-$CH_{2a}$), 2.75 (m, 1H, β-$CH_{2b}$), 3.66 (s, 3H, $OCH_3$), 4.53 (dd, $J$ = 5.3, 7.4 Hz, 1H, γ-CH), 5.05, 5.19 (dd, $J$ = 4.9, 8.4 Hz, 1H, α-CH), 7.37–7.49 (m, 6H, arom. CH), 7.63–7.73 (m, 4H, arom. CH); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta$ = 19.4 ($C(CH_3)_3$ silyl), 26.9 ($C(CH_3)_3$ silyl), 28.1 ($C(CH_3)_3$ Boc), 37.3 (β-$CH_2$), 52.6 ($OCH_3$), 53.8 (α-CH), 60.5 (γ-$CH_2$), 83.7 ($C(CH_3)_3$ Boc), 119.0 (CN), 128.0 (arom. CH), 128.2 (arom. CH), 128.2 (arom. CH), 130.5 (arom. C), 131.6 (arom. C), 135.9 (arom. CH), 136.1 (arom. CH), 151.7 (C=O), 170.4 (C=O); HRMS (FAB, 3-NBA): *m*/*z*: calcd for $C_{32}H_{44}N_2NaO_7Si$: 619.2818, found: 619.2830 $[M+Na]^+$.

**(3*S*)-[Bis(*tert*-butyloxycarbonyl)amino]-5-(*tert*-butyldiphenylsilanyloxy)-piperidine-2-on (51)**: Pd (10 % on charcoal, 1.176 g) was added to a degassed solution of nitrile **50** (6.3 g, 19.56 mmol) in MeOH (630 mL) and acetic acid (3.5 mL). The reaction mixture was hydrogenated under hydrogen atmosphere at room temperature for 15 h. Then the suspension was filtered through a pad of Celite. After removal of the solvents under reduced pressure, the residue was dissolved in $CHCl_3$ (250 mL) and washed with $Na_2CO_3$ solution (10 %, 50 mL). The organic layer was dried over $Na_2SO_4$, and after removal of the solvent under reduced pressure, the residue was dissolved in toluene (500 mL). DMAP (0.270 g, 2.21 mmol) was added to this solution and the reaction mixture was refluxed for 42 h. After removal of the solvent under reduced pressure, the residue was purified by chromatography (cyclohexane/ethyl acetate 2:1) to yield **51** as a colorless foam (4.022 g, 67 %). $R_f$ = 0.27 (cyclohexane/ethyl acetate 1:1); $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ = 1.05 (s, 9H, $C(CH_3)_3$ silyl), 1.48 (s, 18H, $C(CH_3)_3$ Boc), 2.16–2.30 (m, 2H, β-$CH_2$), 3.11–3.30 (m, 2H, δ-$CH_2$), 3.99 (m, 1H, γ-CH), 4.57 (m, 1H, α-CH), 6.59 (m, 1H, NH), 7.36–7.46 (m, 6H, arom. CH), 7.62–7.71 (m, 4H, arom. CH); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta$ = 19.3 ($C(CH_3)_3$ silyl), 27.0 ($C(CH_3)_3$ silyl), 28.2 ($C(CH_3)_3$ Boc), 34.0 (β-$CH_2$), 52.7 (α-CH), 54.2 (δ-$CH_2$), 65.7 (γ-$CH_2$), 82.9 ($C(CH_3)_3$ Boc), 128.0 (arom. CH), 130.1 (arom. CH), 130.2 (arom. CH), 133.3 (arom. C), 133.5 (arom. C), 135.7 (arom. CH), 135.8 (arom CH), 151.9 (C=O), 169.5 (C=O); HRMS (FAB, 3-NBA): *m*/*z*: calcd for $C_{31}H_{44}N_2NaO_6Si$: 591.2869, found: 591.2888 $[M+Na]^+$.

**(3*S*)-[Bis(*tert*-butyloxycarbonyl)amino]-5-hydroxypiperidine-2-on (52)**: A 1 M TBAF solution in THF (9.7 mL, 9.7 mmol) was added to a solution of lactam **51** (1.1 g, 1.93 mmol) in THF (30 mL). After stirring at room temperature for 1.5 h, the solvent was removed under reduced pressure. The residue was purified by chromatography (cyclohexane/ethyl acetate 1:1) to yield **52** as a colorless solid (638 mg, quant.). M.p. 81 °C; $R_f$ = 0.19 (cyclohexane/ethyl acetate 1:10); $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ = 1.47 (s, 18H, $C(CH_3)_3$), 2.12–2.38 (m, 2H, β-$CH_2$), 3.18–3.41 (m, 2H, δ-$CH_2$), 3.44–3.61 (br s, 1H, OH), 4.05 (m, 1H, γ-CH), 4.81 (m, 1H, α-CH), 6.51 (m, 1H, NH); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta$ = 27.9 ($C(CH_3)_3$), 33.0 (β-$CH_2$), 52.3 (α-CH), 53.3 (δ-$CH_2$), 63.5 (γ-CH), 82.9 ($C(CH_3)_3$), 152.1 (C=O), 169.4 (C=O); HRMS (FAB, 3-NBA): *m*/*z*: calcd for $C_{15}H_{27}N_2O_6$: 331.1870, found: 331.1856 $[M+H]^+$.

**(3*S*)-[Bis(*tert*-butyloxycarbonyl)amino]-5-(cyanomethylene)piperidine-2-on (53)**: A solution of Dess–Martin periodinane in $CH_2Cl_2$ (15 %, 8.2 mL, 3.86 mmol) was added to a solution of alcohol **52** (638 mg, 1.93 mmol) in $CH_2Cl_2$ (20 mL). The mixture was stirred at room temperature for 1 h. The solution was diluted with ethyl acetate (100 mL) and a mixture of saturated $Na_2S_2O_3$ solution (10 mL) and saturated $NaHCO_3$ solution (30 mL) was added. After the two phases had become clear, the separated organic layer was washed with saturated $NaHCO_3$ solution (30 mL). The organic layer was dried over $Na_2SO_4$, and, after removal of the solvents under reduced pressure, the residue was purified by chromatography (cyclohexane/ethyl acetate 1:1) to yield the ketone as a colorless oil (445 mg, 70 %).

Cyanomethyldiethylphosphonate **35** (746 μL, 28.01 mmol) was added to a suspension of NaH (120 mg, 4.73 mmol) in THF (15 mL). After stirring at room temperature for 1 h, a solution of the above ketone (445 mg, 1.35 mmol) in THF (15 mL) was added. The reaction mixture was stirred for 1.5 h. After removal of the solvent under reduced pressure, the residue was dissolved in ethyl acetate (50 mL) and washed subsequently with $H_2O$ (10 mL), 1 N HCl (10 mL) and saturated $Na_2CO_3$ solution (10 mL). The organic layer was dried over $Na_2SO_4$, and, after removal of the solvent under reduced pressure, the residue was purified by chromatography (cyclohexane/ethyl acetate 2:1) to yield **53** as a colorless solid (428 mg, 90 %). M.p. 128 °C; $[\alpha]_D^{20}$ = +27.4 ($c$ = 1.0 in $CHCl_3$); $R_f$ = 0.18 (cyclohexane/ethyl acetate 1:1); $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ = 1.45 (s, 18H, $C(CH_3)_3$), 2.40 (dd, $J$ = 9.6, 16.8, 1H, β-$CH_{2a}$), 2.99 (m, 1H, β-$CH_{2b}$), 3.02 (m, 2H, δ-$CH_2$), 5.13 (m, 1H, α-CH), 6.05 (m, 1H, -CHCN), 7.10 (m, 1H, NH); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta$ = 22.1 (β-$CH_2$), 28.2 ($C(CH_3)_3$), 30.2 (δ-$CH_2$), 54.2 (α-CH), 83.7 ($C(CH_3)_3$, 105.4 (*C*HCN), 116.5 (CN), 122.6 (*C*=CHCN), 152.1 (C=O), 167.2 (C=O); HRMS (FAB, 3-NBA): *m*/*z*: calcd for $C_{17}H_{26}N_3O_5$: 352.1873, found: 352.1852 $[M+H]^+$.

**3-*tert*-Butyldisulfanyl-(2*R*)-(4-methoxytetrahydropyran-4-yloxy)propionic acid methyl ester (57)**: A 0.2 M NaOMe solution in MeOH (11.375 mL, 2.28 mmol) was added to a solution of thioester **55** (670 mg, 2.29 mmol) in MeOH (27 mL). After stirring at room temperature for 30 min, the solvent was removed under reduced pressure. The residue was dissolved in ethyl acetate (50 mL) and washed subsequently with saturated $NH_4Cl$ solution (10 mL) and saturated NaCl solution (10 mL). The organic layer was dried over $Na_2SO_4$, the solvent was removed under reduced pressure and the residue was dissolved in DMF (86 mL). This solution was added to a solution of **56** (2.200 g, 6.87 mmol) in DMF (43 mL). After stirring at room temperature for 18 h, the solvent was removed under reduced pressure. The residue was dissolved in ethyl acetate (100 mL) and washed with saturated NaCl solution (20 mL). The organic layer was dried over $Na_2SO_4$, and, after removal of the solvent under reduced pressure, the residue was purified by chromatography (cyclohexane/ethyl acetate 8:1) to yield **57** as a slightly yellow oil (682 mg, 88 %). $[\alpha]_D^{20}$ = +23.8 ($c$ = 1.0 in $CHCl_3$); $R_f$ = 0.27 (cyclohexane/ethyl acetate 3:1); $^1$H NMR ($CDCl_3$,

400 MHz): $\delta = 1.33$ (s, 9H, $C(CH_3)_3$). 1.78–1.88 (m, 4H, -$CH_2OCH_2$-), 3.00 (d, $J = 6.6$ Hz, 2H, β-$CH_2$), 3.23 (s, 3H, $CH_3O$-acetal), 3.61–3.78 (m, 4H, -$CH_2C(O)CH_2$-), 3.76 (s, 3H, $OCH_3$ ester), 4.53 (t, $J = 6.6$ Hz, 1H, α-CH); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta = 30.0$ ($C(CH_3)_3$), 34.2 ($CH_2C(O)_2CH_2$), 34.7 (β-$CH_2$), 43.7 ($C(CH_3)_3$), 48.3 ($OCH_3$ acetal), 49.0 ($OCH_3$ ester), 65.0 ($CH_2OCH_2$), 69.1 (α-CH), 100.0 (C acetal), 172.5 (C=O); GCMS (100 °C, 3 min, then 20 °C min$^{-1}$ to 300 °C): $t_R = 9.8$ min, MS: $m/z$: 338 $[M]^+$, 250 $[M+H-(CH_3)_3CS]^+$.

**3-*tert*-Butyldisulfanyl-(2*R*)-(methanesulfonyloxy)propionic acid methyl ester (58)**: TsOH (251 mg, 1.29 mmol) was added to a solution of acetal **57** (435 mg, 1.29 mmol) in MeOH (35 mL). After stirring at room temperature for 1 h, $Na_2CO_3$ solution (10%, 5 mL) was added and the solvent was removed under reduced pressure. The residue was dissolved in ethyl acetate and washed with saturated $NaHCO_3$ solution (10 mL). The organic layer was dried over $Na_2SO_4$ and the solvent was removed under reduced pressure to yield the free alcohol as a colorless oil (288 mg, quant.).

MsCl (120 μL, 1.54 mmol) was added to a solution of the above alcohol (288 mg, 1.29 mmol) in pyridine (9 mL). After stirring at room temperature for 15 h, the solution was diluted with ethyl acetate (50 mL) and washed with 1 N HCl (10 mL). The organic layer was dried over $Na_2SO_4$, and, after removal of the solvent under reduced pressure, the residue was purified by chromatography (cyclohexane/ethyl acetate 3:1) to yield **58** as a slightly yellow oil (388 mg, quant.). $[\alpha]_D^{20} = +12.9$ ($c = 0.5$, $CHCl_3$); $R_f = 0.27$ (cyclohexane/ethyl acetate 3:1); $^1$H NMR ($CDCl_3$, 400 MHz): $\delta = 1.33$ (s, 9H, $C(CH_3)_3$), 3.00 (dd, $J = 8.4$, 13.4 Hz, 1H, β-$CH_{2a}$), 3.15 (s, 3H, $CH_3SO_2$-), 3.19 (dd, $J = 4.1$, 13.9 Hz, 1H, β-$CH_{2b}$), 3.81 (s, 3H, $OCH_3$), 5.24 (dd, $J = 4.1$, 8.4 Hz, 1H, α-CH); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta = 30.0$ ($C(CH_3)_3$), 39.2 (β-$CH_2$), 41.8 ($CH_3SO_2$), 45.2 ($C(CH_3)_3$), 48.6 ($OCH_3$), 69.7 (α-CH), 172.4 (C=O); GCMS (100 °C, 3 min, then 20 °C min$^{-1}$ to 300 °C): $t_R = 8.6$ min, MS: $m/z$: 302 $[M]^+$, 246 $[M+H-(CH_3)_3C)]^+$.

**(2*S*)-{(3*S*)-[Bis(*tert*-butyloxycarbonyl)amino]-5-cyanomethylene-2-oxopiperidine-1-yl}-3-*tert*-butyldisulfanylpropionic acid methyl ester (59)**: At 0 °C, a solution of lactam **53** (235 mg, 0.67 mmol) and mesylate **58** (326 mg, 1.08 mmol) was added to a suspension of NaH (21 mg, 0.84 mmol) in THF (6 mL). The reaction mixture was warmed to room temperature and stirred for 20 h. Then, the solution was diluted with ethyl acetate (50 mL) and washed with $H_2O$ (10 mL). The organic layer was dried over $Na_2SO_4$, and, after removal of the solvents under reduced pressure, the residue was purified by chromatography (cyclohexane/ethyl acetate 4:1, then 1:2) to yield **59** as a colorless oil (213 mg, 57%). $[\alpha]_D^{20} = +46.8$ ($c = 0.5$ in $CHCl_3$); $R_f = 0.22$ (cyclohexane/ethyl acetate 1:1); $^1$H NMR ($CDCl_3$, 400 MHz): $\delta = 1.32$ (s, 9H, $C(CH_3)_3$ S*t*Bu), 1.49 (s, 18H, $C(CH_3)_3$ Boc), 2.38 (dd, $J = 9.6$, 16.8 Hz, 1H, β-$CH_{2a}$ lactam), 2.99–3.27 (m, 5H, β-$CH_{2b}$ lactam, β-$CH_2$ Cys, δ-$CH_2$ lactam), 3.72 (s, 3H, $OCH_3$), 5.17 (dd, $J = 8.4$, 15.6 Hz, 1H, α-CH lactam), 5.25 (dd, $J = 4.1$, 8.4 Hz, 1H, α-CH Cys), 6.20 (m, 1H, -C=CHCN); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta = 22.3$ (β-$CH_2$ lactam), 28.5 ($C(CH_3)_3$ Boc), 29.9 ($C(CH_3)_3$ S*t*Bu), 30.0 (δ-$CH_2$ lactam), 39.2 (β-$CH_2$ Cys), 48.8 ($C(CH_3)_3$ S*t*Bu), 49.7 ($OCH_3$), 52.7 (α-CH lactam), 69.7 (α-CH Cys), 83.4 ($C(CH_3)_3$ Boc), 105.3 (*C*HCN), 116.5 (CN), 128.9 (*C*=CHCN), 152.2 (C=O), 166.4 (C=O), 171.3 (C=O); HRMS (FAB, 3-NBA): $m/z$: calcd for $C_{25}H_{39}N_3NaO_7S_2$: 580.2129, found: 580.2104 $[M+Na]^+$.

**(2*S*)-[(3*S*)-Amino-5-cyanomethylene-2-oxopiperidine-1-yl]-3-*tert*-butyldisulfanylpropionic acid methyl ester hydrotrifluoroacetate (60)**: TFA (1.5 mL, 19.54 mmol) was added to a solution of lactam **59** (109 mg, 0.2 mmol) in $CH_2Cl_2$ (6 mL). After stirring at room temperature for 30 min, the solvent was removed under reduced pressure and the residue was coevaporated with toluene to yield **60** as a colorless oil (90 mg, 99%). $[\alpha]_D^{20} = +24.4$ ($c = 1.0$ in MeOH); $R_f = 0.01$ ($CH_2Cl_2$/EtOH 10:1); $^1$H NMR ($CDCl_3$, 400 MHz): $\delta = 1.31$ (s, 9H, $C(CH_3)_3$), 2.62–2.85 (m, 2H, β-$CH_2$ lactam), 3.16–3.28 (m, 4H, β-$CH_2$ Cys, δ-$CH_2$ lactam), 3.73 (s, 3H, $OCH_3$), 4.19 (m, 1H, α-CH lactam), 5.23 (m, 1H, α-CH Cys), 6.20 (m, 1H, -CHCN); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta = 30.1$ ($C(CH_3)_3$), 30.2 (δ-$CH_2$ lactam), 39.2 (β-$CH_2$ Cys), 40.4 (β-$CH_2$ lactam), 48.4 ($C(CH_3)_3$), 49.4 ($OCH_3$), 52.6 (α-CH lactam), 69.7 (α-CH Cys), 105.4 (*C*HCN), 116.6 (CN), 126.8 ($CF_3COOH$), 128.7 (*C*=CHCN), 166.5 (C=O), 171.11 (C=O), 176.0 ($CF_3COOH$); HRMS (FAB, 3-NBA): $m/z$: calcd for $C_{15}H_{24}N_3O_3S_2$: 358.1260, found: 358.1245 $[M-C_2F_3O_2]^+$.

**3-*tert*-Butyldisulfanyl-(2*S*)-{5-cyanomethylene-(3*S*)-[3-(hexadecane-1-sulfonylamino)-propionylamino]-2-oxopiperidine-1-yl}-propionic acid methyl ester (61)**: At 0 °C, EDC (20 mg, 0.1 mmol) was added to a solution of lactam **60** (39 mg, 0.08 mmol), $C_{16}H_{33}SO_2$-β-Ala-OH **39** (38 mg, 0.1 mmol), HOBt (26 mg, 0.16 mmol) and NE*t*i$Pr_2$ (17 μL, 0.1 mmol) in $CH_2Cl_2$ (6 mL). The solution was warmed to room temperature and stirred for 18 h. Then the reaction mixture was diluted with ethyl acetate (50 mL) and subsequently washed with 1 N HCl (10 mL) and $Na_2CO_3$ solution (10%, 10 mL). The organic layer was dried over $Na_2SO_4$, and, after removal of the solvent under reduced pressure, the residue was purified by chromatography (cyclohexane/ethyl acetate 1:2) to yield **61** as colorless foam (40 mg, 67%). $[\alpha]_D^{20} = 4.1$ ($c = 1.0$ in $CHCl_3$); $R_f = 0.16$ (cyclohexane/ethyl acetate 1:2); $^1$H NMR ($CDCl_3$, 400 MHz): $\delta = 0.86$ (t, $J = 6.5$ Hz, 3H, ω-$CH_3$ hexadecyl), 1.23 (m, 24H, 12 × $CH_2$ hexadecyl), 1.33 (s, 9H, $C(CH_3)_3$), 1.38 (m, 2H, γ-$CH_2$ hexadecyl), 1.76 (m, 2H, β-$CH_2$ hexadecyl), 2.55 (m, 3H, β-$CH_{2a}$ lactam, α-$CH_2$ β-Ala), 2.71 (m, 1H, β-$CH_{2b}$ lactam), 2.98 (m, 2H, α-$CH_2$ hexadecyl), 3.16 (m, 2H, β-$CH_2$ β-Ala), 3.36 (m, 2H, δ-$CH_2$ lactam), 3.73 (s, 3H, $OCH_3$), 4.58 (m, 1H, α-CH Lactam), 5.38 (m, 1H, NH), 5.48 (m, 1H, α-CH Cys), 6.18 (m, 1H, -C*H*CN), 6.66 (m, 1H, NH); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta = 14.3$ (ω-$CH_3$ hexadecyl), 22.1 ($CH_2$), 22.8 ($CH_2$), 23.8 ($CH_2$), 28.5 ($CH_2$), 28.7 ($CH_2$), 28.9 ($CH_2$), 29.1 ($CH_2$), 29.3 ($CH_2$), 29.5 ($CH_2$), 29.7 ($CH_2$), 29.8 ($CH_2$), 29.9 ($CH_2$), 29.9 ($CH_2$), 30.0 ($CH_2$), 30.1 ($C(CH_3)_3$), 31.2 (δ-$CH_2$ lactam), 32.1 (β-$CH_2$ Cys), 36.7 (β-$CH_2$ β-Ala), 39.6 (α-$CH_2$ β-Ala), 48.8 (β-$CH_2$ lactam), 49.1 ($C(CH_3)_3$), 49.4 ($OCH_3$), 52.3 (α-CH lactam), 52.8 (α-$CH_2$ hexadecyl), 52.8 (α-CH Cys), 108.1 (*C*HCN), 116.3 (CN), 128.5 (*C*=CHCN), 167.5 (C=O), 170.3 (C=O), 171.7 (C=O); HRMS (FAB, 3-NBA): $m/z$: calcd for $C_{34}H_{61}N_4O_6S_3$: 718.3754, found: 718.3727 $[M+H]^+$.

**(2*S*)-{(3*S*)-[(2*S*)-Acetylamino-3-(hexadecane-1-sulfonylamino)propionylamino]-5-cyanomethylene-2-oxopiperidine-1-yl}-3-(*tert*-butyldisulfanyl)-propionic acid methyl ester (62)**: At 0 °C, EDC (20 mg, 0.1 mmol) was added to a solution of lactam **60** (39 mg, 0.08 mmol), Ac-Dap-($SO_2C_{16}H_{33}$)-OH **4** (43 mg, 0.1 mmol), HOBt (26 mg, 0.16 mmol) and NE*t*i$Pr_2$ (17 μL, 0.1 mmol) in $CH_2Cl_2$ (3 mL). The solution was warmed to room temperature and stirred for 18 h. Then the reaction mixture was diluted with ethyl acetate (50 mL) and subsequently washed with 1 N HCl (10 mL) and $Na_2CO_3$ solution (10%, 10 mL). The organic layer was dried over $Na_2SO_4$, and, after removal of the solvent under reduced pressure, the residue was purified by chromatography (cyclohexane/ethyl acetate 1:10) to yield **62** as colorless foam (38 mg, 62%). $[\alpha]_D^{20} = +17.2$ ($c = 1.0$ in $CHCl_3$); $R_f = 0.07$ (cyclohexane/ethyl acetate 1:3); $^1$H NMR ($CDCl_3$, 400 MHz): $\delta = 0.81$ (t, $J = 6.5$ Hz, 3H, ω-$CH_3$ hexadecyl), 1.18 (m, 24H, 12 × $CH_2$ hexadecyl), 1.33 (s, 9H, $C(CH_3)_3$), 1.36 (m, 2H, γ-$CH_2$ hexadecyl), 1.71 (m, 2H, β-$CH_2$ hexadecyl), 2.01 (s, 3H, $CH_3$ acetyl), 2.40 (m, 1H, β-$CH_{2a}$ lactam), 2.60 (m, 1H, β-$CH_{2b}$ lactam), 2.94 (m, 2H, α-$CH_2$ hexadecyl), 3.11 (m, 1H, δ-$CH_{2a}$ lactam), 3.33 (m, 1H, δ-$CH_{2b}$ lactam), 3.52 (m, 2H, β-$CH_2$ Dap), 3.67 (s, 3H, $OCH_3$), 4.55 (m, 2H, α-CH lactam, α-CH Dap), 5.24 (m, 1H, α-CH Cys), 5.97 (m, 1H, NH), 6.13 (m, 1H, -C*H*CN), 6.86 (m, 1H, NH), 6.97 (m, 1H, NH); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta = 14.3$ (ω-$CH_3$ hexadecyl), 22.1 ($CH_2$), 22.8 ($CH_2$), 22.9 ($CH_3$ acetyl), 23.8 ($CH_2$), 28.5 ($CH_2$), 28.7 ($CH_2$), 28.9 ($CH_2$), 29.1 ($CH_2$), 29.3 ($CH_2$), 29.5 ($CH_2$), 29.7 ($CH_2$), 29.8 ($CH_2$), 29.9 ($CH_2$), 29.9 ($CH_2$), 30.0 ($CH_2$), 30.1 ($C(CH_3)_3$), 31.2 (δ-$CH_2$ lactam), 32.1 (β-$CH_2$ Cys), 44.7 (β-$CH_2$ Dap), 48.8 (β-$CH_2$ lactam), 49.1 ($C(CH_3)_3$), 49.4 ($OCH_3$), 52.3 (α-CH lactam), 52.8 (α-$CH_2$ hexadecyl), 52.8 (α-CH Cys), 53.3 (α-CH Dap), 107.1 (*C*HCN), 116.3 (CN), 128.5 (*C*=CHCN), 167.5 (C=O), 170.3 (C=O), 170.6 (C=O), 171.7 (C=O); HRMS (FAB, 3-NBA): $m/z$: calcd for $C_{36}H_{64}N_5O_7S_3$: 774.3969, found: 774.3989 $[M+H]^+$.

**7-Bromo-(2*R*)-hydroxy-1,2,3,11a-tetrahydro-10*H*-benzo[*e*]pyrrolo[1,2-*a*,1,4]diazepine-5,11-dione (64)**: A solution of 5-bromoisatoic acid anhydride **63** (5.219 g, 20.9 mmol) and (4*R*)-hydroxy-L-proline **31** (3.525 g, 26.8 mmol) in DMSO (30 mL) was stirred at 140 °C for 5 h. The reaction mixture was cooled down to room temperature, poured into ice cold water (30 mL) and extracted with ethyl acetate (4 × 50 mL). The combined organic layers were washed with ice cold water (3 × 30 mL) and dried over $Na_2SO_4$. The solvent was removed under reduced pressure

and the residue was purified by chromatography ($CH_2Cl_2/CH_3OH$ 10:1) to yield **64** as a slightly yellow solid (6.043 g, 93%). M.p. 128°C; $[\alpha]_D^{20}$ = +415 ($c$ = 1.0 in $CHCl_3$); $R_f$ = 0.22 ($CH_2Cl_2/CH_3OH$ 10:1); $^1$H NMR ($CD_3OD$, 400 MHz): $\delta$ = 2.00 (m, 1H, -$CH_{2a}$-), 2.83 (m, 1H, -$CH_{2b}$-), 3.61 (dd, $J$ = 4.9, 12.5 Hz, 1H, -$CH_{2a}$N-), 3.76 (ddd, $J$ = 1.4, 3.7, 12.3 Hz, 1H, -$CH_{2b}$N-), 4.31 (dd, $J$ = 5.9, 8.2 Hz, 1H, -C*H*OH), 4.50 (m, 1H, -CHC(O)-), 7.05 (d, $J$ = 8.6 Hz, 1H, arom. CH), 7.67 (dd, $J$ = 2.3, 8.6 Hz, 1H, arom. CH), 7.99 (d, $J$ = 2.3 Hz, 1H, arom. CH); $^{13}$C NMR ($CD_3OD$, 100.6 MHz): $\delta$ = 34.2 ($CH_2$), 54.0 ($CH_2$N), 55.8 (*C*HC(O)), 68.2 (CHOH), 117.1 (arom. C), 123.3 (arom. CH), 127.8 (arom. C), 132.9 (arom. CH), 135.4 (arom. CH), 135.7 (arom. C), 165.6 (C=O), 170.5 (C=O); HRMS (EI, 70 eV): $m/z$: calcd for $C_{12}H_{11}BrN_2O_3$: 309.9953, found: 309.9943 $[M]^+$; elemental analysis calcd (%) for $C_{12}H_{11}BrN_2O_3$: C 46.32, H 3.56, N 9.00; found: C 46.11, H 3.54, N 8.93.

**3-[(2*R*)-Hydroxy-5,11-dioxo-2,3,5,10,11,11a-hexahydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*,1,4]diazepine-7-yl]-acrylic acid *tert*-butyl ester (65)**: [Pd(OAc)$_2$] (145 mg, 0.64 mmol) was added to a solution of benzodiazepine **64** (4 g, 12.86 mmol), P(*o*-tol)$_3$ (404 mg, 1.29 mmol), $NEt_3$ (5.375 mL, 38.57 mmol) and *tert*-butyl acrylate (5.655 mL, 38.57 mmol) in $CH_3CN$ (16 mL). The solution was stirred at 100°C in a sealed tube for 20 h. The reaction mixture was cooled down to room temperature and the solvent was removed under reduced pressure. The residue was purified by chromatography ($CH_2Cl_2$/EtOH 20:1) to yield **65** as a yellowish solid (5.588 g, 97%). M.p. 88°C; $[\alpha]_D^{20}$ = +238.8 ($c$ = 1.0 in $CHCl_3$); $R_f$ = 0.11 ($CH_2Cl_2$/EtOH 20:1); $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ = 1.52 (s, 9H, -C($CH_3$)$_3$), 2.20 (m, 1H, -$CH_{2a}$-), 2.92 (m, 1H, -$CH_{2b}$-), 3.50 (br s, 1H, OH), 3.63 (dd, $J$ = 4.5, 12.7 Hz, 1H, -$CH_{2a}$N-), 4.02 (d, $J$ = 12.7 Hz, 1H, -$CH_{2b}$N-), 4.32 (m, 1H, -C*H*OH-), 4.61 (m, 1H, -CHC(O)-), 6.35 (d, $J$ = 16.0 Hz, 1H, -CH=C*H*C(O)-), 7.12 (d, $J$ = 8.4 Hz, 1H, arom. CH), 7.48 (d, $J$ = 16.0 Hz, 1H, -C*H*=CHC(O)-), 7.58 (dd, $J$ = 2.2, 8.6 Hz, 1H, arom CH), 8.00 (d, $J$ = 2.0 Hz, 1H, arom. CH), 9.27 (s, 1H, NH); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta$ = 28.3 (C(*C*H$_3$)$_3$), 34.8 ($CH_2$), 46.3 ($CH_2$N), 55.9 (*C*H$_2$C(O)), 68.9 ($CH_2$OH), 81.0 (*C*(CH$_3$)$_3$, 121.2 (CH=*C*HC(O)), 122.0 (arom. CH), 126.6 (arom. C), 131.3 (arom. CH), 131.6 (arom. C), 131.7 (arom. CH), 136.5 (arom. C), 141.6 (*C*H=CHC(O)), 165.9 (C=O), 166.2 (C=O), 169.2 (C=O); HRMS (EI, 70 eV): $m/z$: calcd for $C_{19}H_{22}N_2O_5$: 358.1529, found: 358.1547 $[M]^+$.

**3-[(2*S*)-Azido-5,11-dioxo-2,3,5,10,11,11a-hexahydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*,1,4]diazepine-7-yl]-acrylic acid *tert*-butyl ester (66)**: At 0°C, MsCl (570 μL, 7.37 mmol) was added to a solution of alcohol **65** (2.2 g, 6.14 mmol) in pyridine (20 mL). The reaction mixture was warmed to room temperature and stirred for 18 h. Then, the solution was diluted with $CH_2Cl_2$ (100 mL) and washed with 1N HCl (3×30 mL). The organic layer was dried over $Na_2SO_4$ and the solvent was removed under reduced pressure to yield the mesylate as a brownish solid (2.494 g, 93%).

$NaN_3$ (8.9 g, 137.46 mmol) was then added to a solution of the above mesylate (2.494 g, 5.71 mmol) in DMF (40 mL). The reaction mixture was stirred at 45°C for 4 d. Then, the solvent was removed under reduced pressure, the residue was dissolved in $H_2O$ (50 mL) and extracted with $CHCl_3$ (3×50 mL). The organic layer was dried over $Na_2SO_4$, and, after removal of the solvent under reduced pressure, the residue was purified by chromatography (cyclohexane/ethyl acetate 1:1, then $CHCl_3$) to yield **66** as a colorless solid (1.95 g, 89%). M.p. 211°C; $[\alpha]_D^{20}$ = +305.0 ($c$ = 1.0 in $CHCl_3$); $R_f$ = 0.53 ($CH_2Cl_2$/EtOH 10:1); $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ = 1.53 (s, 9H, -C($CH_3$)$_3$), 2.38 (m, 1H, -$CH_{2a}$-), 3.12 (m, 1H, -$CH_{2b}$-), 3.72 (d, $J$ = 12.9 Hz, 1H, -$CH_{2a}$N-), 3.82 (dd, $J$ = 5.3, 12.9 Hz, 1H, -$CH_{2b}$N-), 4.23 (dd, $J$ = 2.0, 9.0 Hz, 1H, -$CHN_3$), 4.39 (m, 1H, -CHC(O)-), 6.40 (d, $J$ = 15.8 Hz, 1H, -CH=C*H*C(O)-), 7.16 (d, $J$ = 8.4 Hz, 1H, arom. CH), 7.56 (d, $J$ = 15.8 Hz, 1H, -C*H*=CHC(O)-), 7.62 (dd, $J$ = 2.2, 8.4 Hz, 1H, arom CH), 8.15 (d, $J$ = 2.0 Hz, 1H, arom. CH), 9.63 (s, 1H, NH); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta$ = 28.3 (C(*C*H$_3$)$_3$), 31.0 ($CH_2$), 53.1 ($CH_2$N), 56.2 ($CHN_3$), 58.0 (*C*HC(O)), 80.9 (*C*(CH$_3$)$_3$, 121.3 (CH=*C*HC(O)), 122.0 (arom. CH), 125.8 (arom. C), 131.5 (arom. CH), 131.8 (arom. C), 131.9 (arom. CH), 136.7 (arom. C), 141.5 (*C*H=CHC(O)), 165.5 (C=O), 166.1 (C=O), 171.0 (C=O); HRMS (EI, 70 eV): $m/z$: calcd for $C_{19}H_{21}N_5O_4$: 383.1594, found: 383.1603 $[M]^+$.

**3-[(2*S*)-Azido-10-cyanomethyl-5,11-dioxo-2,3,5,10,11,11a-hexahydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*,1,4]diazepine-7-yl)-acrylic acid *tert*-butyl ester (67)**: At −40°C, a solution of the azide **66** (3.99 g, 10.41 mmol) in THF (20 mL) was added to a suspension of NaH (315 mg, 12.49 mmol) in THF (20 mL). After stirring for 30 min, bromoacetonitrile (897 μL, 12.49 mmol) was added. The reaction mixture was warmed to room temperature and stirred for 20 h. Then, the solvent was removed under reduced pressure, the residue was dissolved in $CHCl_3$ (200 mL) and washed with $H_2O$ (50 mL). The organic layer was dried over $Na_2SO_4$, and, after removal of the solvent, the residue was purified by chromatography ($CH_2Cl_2$/EtOH 50:1) to yield **67** as a colorless solid (4.176 g, 95%). M.p. 173°C; $[\alpha]_D^{20}$ = +468 ($c$ = 1.0 in $CHCl_3$); $R_f$ = 0.18 ($CH_2Cl_2$/EtOH 40:1); $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ = 1.54 (s, 9H, C($CH_3$)$_3$), 2.42 (m, 1H, -$CH_{2a}$-), 3.08 (m, 1H, -$CH_{2b}$-), 3.70 (d, $J$ = 12.9 Hz, 1H, -$CH_{2a}$N-), 3.83 (dd, $J$ = 5.3, 12.9 Hz, 1H, -$CH_{2b}$N-), 4.25 (m, 1H, -$CHN_3$), 4.30 (d, $J$ = 17.2 Hz, 1H, -$CH_{2a}$CN), 4.39 (m, 1H, -CHC(O)-), 4.96 (d, $J$ = 17.2 Hz, 1H, -$CH_{2b}$CN), 6.44 (d, $J$ = 16.0 Hz, 1H, -CH=C*H*C(O)-), 7.45 (d, $J$ = 8.4 Hz, 1H, arom. CH), 7.56 (d, $J$ = 16.0 Hz, 1H, -C*H*=CHC(O)-), 7.75 (dd, $J$ = 2.2, 8.4 Hz, 1H, arom. CH), 8.19 (d, $J$ = 2.2 Hz, 1H, arom. CH); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta$ = 28.2 (C(*C*H$_3$)$_3$), 31.4 ($CH_2$), 37.3 ($CH_2$CN), 52.6 ($CH_2$N), 56.5 ($CHN_3$), 58.2 (*C*HC(O)), 81.2 (*C*(CH$_3$)$_3$), 115.4 (CN), 121.3 (CH=*C*HC(O)), 122.9 (arom. CH), 129.6 (arom. C), 130.8 (arom. CH), 132.0 (arom. CH), 133.7 (arom. CH), 139.0 (arom.C), 140.6 (*C*H=CHC(O)), 164.7 (C=O), 165.7 (C=O), 169.7 (C=O); HRMS (EI, 70 eV): $m/z$: calcd for $C_{21}H_{22}N_6O_4$: 422.1703, found: 422.1707 $[M]^+$.

**(2*S*)-Azido-10-cyanomethyl-5,11-dioxo-2,3,5,10,11,11a-hexahydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*,1,4]diazepine-7-carboxylic acid *tert*-butyl ester (68)**: A solution of $RuCl_3$ in 1 mM HCl (2 mg $RuCl_3$ per mL 1 mM HCl, 73.56 mL) was added to a solution of $NaIO_4$ (10.229 g, 47.34 mmol) in $H_2O/CH_3CN/CCl_4$ (2:1:1, 150 mL). After stirring at room temperature for 5 min, a solution of benzodiazepine **67** (2 g, 4.73 mmol) in $CH_3CN/CCl_4$ (1:1, 150 mL) was added. After stirring for 18 h, the solvent was removed under reduced pressure, the residue was dissolved in 1N HCl (50 mL) and extracted with $CHCl_3$ (50 mL). The organic layer was dried over $Na_2SO_4$ and the solvent was removed under reduced pressure to yield the free acid as a colorless solid (1.604 g, quant.).

Then, $K_2CO_3$ (17.1 g, 122.29 mmol), and $Me_3CBr$ (26.2 mL, 225.69 mmol) were subsequently added to a solution of the above acid (1.604 g, 4.73 mmol) and $Et_3(PhCH_2)NCl$ (1.09 g, 4.7 mmol). The reaction mixture was stirred at 55°C for 16 h. Then, the solvent was removed under reduced pressure, the residue was dissolved in $H_2O$ (50 mL) and extracted with $CHCl_3$ (100 mL). The organic layer was dried over $Na_2SO_4$, and after removal of the solvent, the residue was purified by chromatography (cyclohexane/ethyl acetate 1:1) to yield **68** as a colorless solid (1.758 g, 90%). M.p. 140°C; $[\alpha]_D^{20}$ = +287.7 ($c$ = 1.0 in $CHCl_3$); $R_f$ = 0.28 (cyclohexane/ethyl acetate 1:1); $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ = 1.59 (s, 9H, C($CH_3$)$_3$), 2.40 (m, 1H, -$CH_{2a}$-), 3.14 (d, $J$ = 12.9 Hz, 1H, -$CH_{2b}$-), 3.68 (d, $J$ = 12.9 Hz, 1H, -$CH_{2a}$N-), 3.77 (dd, $J$ = 5.5, 12.9 Hz, 1H, -$CH_{2b}$N-), 4.20 (dd, $J$ = 2.3, 9.0 Hz, 1H, -$CHN_3$), 4.28 (d, $J$ = 17.4 Hz, 1H, -$CH_{2a}$CN), 4.36 (m, 1H, -CHC(O)-), 4.95 (d, $J$ = 17.4 Hz, 1H, -$CH_{2b}$CN), 7.48 (d, $J$ = 8.8 Hz, 1H, arom.CH), 8.18 (dd, $J$ = 2.2, 8.6 Hz, 1H, arom. CH), 8.49 (d, $J$ = 2.2 Hz, 1H, arom. CH); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta$ = 28.3 (C-(*C*H$_3$)$_3$), 31.5 ($CH_2$), 37.3 (*C*H$_2$CN), 52.6 ($CH_2$N), 56.5 ($CHN_3$), 58.2 (*C*HC(O)), 82.4 (*C*(CH$_3$)$_3$), 115.3 (CN), 120.7 (arom. CH), 128.7 (arom. C), 130.8 (arom. CH), 132.6 (arom. CH), 134.0 (arom. C), 141.2 (arom. C), 163.8 (C=O), 164.6 (C=O), 169.3 (C=O); HRMS (FAB, 3-NBA): $m/z$: calcd for $C_{19}H_{21}N_6O_4$: 397.1625, found: 397.1622 $[M+H]^+$; elemental analysis calcd (%) for $C_{19}H_{20}N_6O_4$: C 57.57, H 5.09, N 21.20; found: C 57.33, H 5.00, N 20.97.

**(2*S*)-Amino-10-cyanomethyl-5,11-dioxo-2,3,5,10,11,11a-hexahydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*,1,4]diazepine-7-carboxylic acid *tert*-butyl ester (69)**: Pd on $BaSO_4$ (354 mg) was added to a degassed solution of azide **68** (660 mg, 1.67 mmol) in MeOH (132 mL) and $CHCl_3$ (4.4 mL). The reaction mixture was hydrogenated under hydrogen atmosphere at room temperature for 4 h. Then the suspension was filtered through a pad of Celite and the solvent was removed under reduced pressure to yield **69** as a colorless solid (615 mg, quant.). M.p. 148°C; $[\alpha]_D^{20}$ = 216.2 ($c$ = 1.0 in $CHCl_3$); $R_f$ = 0.38 ($CH_2Cl_2$/EtOH 1:1); $^1$H NMR ($CD_3OD$, 400 MHz): $\delta$ = 1.63 (s, 9H, C($CH_3$)$_3$), 2.67 (m, 1H, -$CH_{2a}$-), 2.93 (br d, $J$ = 14.7 Hz, 1H, -$CH_{2b}$-), 3.86 (dd, $J$ = 6.3, 13.5 Hz, 1H, -$CH_{2a}$N-), 4.02 (dd, $J$ = 6.3,

13.5 Hz, 1H, -$CH_{2b}$N-), 4.13 (m, 1H, -CHC(O)-), 4.53 (br d, $J$=6.3 Hz, 1H, -C*H*$NH_2$), 4.94 (d, $J$=17.6 Hz, 1H, -$CH_{2a}$CN), 5.13 (d, $J$=17.6 Hz, 1H, -$CH_{2b}$CN), 7.62 (d, $J$=8.6 Hz, 1H, arom. CH), 8.21 (dd, $J$=2.2, 8.6 Hz, 1H, arom. CH), 8.43 (d, $J$=2.2 Hz, 1H, arom. CH); $^{13}$C NMR ($CD_3OD$, 100.6 MHz): $\delta$=27.1 (C(*C*$H_3$)$_3$), 30.4 ($CH_2$), 36.1 (*C*$H_2$CN), 49.4 ($CH_2$N), 56.8 (CH$NH_2$), 60.0 (*C*HC(O)), 82.3 (*C*(CH$_3$)$_3$), 115.5 (CN), 122.3 (arom. CH), 128.5 (arom. C), 130.3 (arom. CH), 131.6 (arom. CH), 133.5 (arom. C), 141.6 (arom. C), 164.0 (C=O), 165.2 (C=O), 169.8 (C=O); HRMS (FAB, 3-NBA): $m/z$: calcd for $C_{19}H_{23}N_4O_4$: 371.1720, found: 371.1702 [$M$+H]$^+$.

**10-Cyanomethyl-(2*S*)-(hexadecane-1-sulfonylamino)-5,11-dioxo-2,3,5,10,11,11a-hexahydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*,1,4]diazepine-7-carboxylic acid *tert*-butyl ester (70)**: At 0°C, NEt*i*Pr$_2$ (568 μL, 3.32 mmol) and hexadecanesulfonic acid chloride (674 mg, 1.99 mmol) were subsequently added to a solution of amine **69** (615 mg, 1.66 mmol) in DMF (20 mL). The solution was warmed to room temperature and stirred for 22 h. Then, the solvent was removed under reduced pressure, the residue was dissolved in ethyl acetate (50 mL) and washed with 1 N HCl (30 mL). The organic layer was dried over $Na_2SO_4$, and, after removal of the solvent under reduced pressure, the residue was purified by chromatography (*n*-hexane/ethyl acetate 2:1) to yield **70** as a colorless solid (887 mg, 82%). M.p. 96°C; $[\alpha]_D^{20}$ = +298.1 ($c$=1.0 in $CHCl_3$); $R_f$=0.32 (cyclohexane/ethyl acetate 1:1); $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$=0.88 (t, $J$=6.6 Hz, 3H, ω-$CH_3$ hexadecyl), 1.22–1.37 (m, 24H, 12×$CH_2$ hexadecyl), 1.43 (m, 2H, γ-$CH_2$ hexadecyl), 1.60 (s, 9H, C(CH$_3$)$_3$), 1.84 (m, 2H, β-$CH_2$ hexadecyl), 2.50 (m, 1H, -$CH_{2a}$-), 2.76 (d, $J$=14.5 Hz, 1H, -$CH_{2b}$-), 3.07 (m, 2H, α-$CH_2$ hexadecyl), 3.86 (dd, $J$=5.5, 13.1 Hz, 1H, -$CH_{2a}$N-), 3.97 (d, $J$=13.1 Hz, 1H, -$CH_{2b}$N-), 4.27 (d, $J$=8.8 Hz, 1H, -C*H*NH-), 4.29 (m, 1H, -CHC(O)-), 4.31 (d, $J$=17.2, 1H, -$CH_{2a}$CN), 5.05 (d, $J$=17.2, 1H, -$CH_{2b}$CN), 5.40 (d, $J$=7.4 Hz, 1H, NH), 7.51 (d, $J$=8.6 Hz, 1H, arom. CH), 8.23 (dd, $J$=2.1, 8.6 Hz, 1H, arom. CH), 8.53 (d, $J$=2.1 Hz, 1H, arom. CH); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta$=14.3 (ω-$CH_3$ hexadecyl), 22.9 ($CH_2$), 23.8 ($CH_2$), 28.3 (C(*C*H$_3$)$_3$), 28.5 ($CH_2$), 29.3 ($CH_2$), 29.5 ($CH_2$), 29.6 ($CH_2$), 29.7 ($CH_2$), 29.8 ($CH_2$), 29.8 ($CH_2$), 29.9 ($CH_2$), 29.9 ($CH_2$), 29.9 ($CH_2$), 29.9 ($CH_2$), 32.1 ($CH_2$), 33.0 ($CH_2$), 37.4 (*C*$H_2$CN), 51.9 ($CH_2$N), 54.2 (α-$CH_2$ hexadecyl), 55.4 (CHNH), 57.0 (*C*HC(O)), 82.6 (*C*(CH$_3$)$_3$), 114.9 (CN), 121.1 (arom. CH), 129.4 (arom. C), 131.4 (arom. CH), 132.7 (arom. CH), 134.1 (arom. C), 141.0 (arom. C), 163.7 (C=O), 164.1 (C=O), 170.3 (C=O); HRMS (FAB, 3-NBA): $m/z$: calcd for $C_{35}H_{55}N_4O_6S$: 659.3843, found: 659.3863 [$M$+H]$^+$.

**10-(2-Aminoethyl)-(2*S*)-(hexadecane-1-sulfonylamino)-5,11-dioxo-2,3,5,10,11,11a-hexahydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*,1,4]diazepine-7-carboxylic acid *tert*-butyl ester (71)**: $PtO_2$·$H_2O$ (25 mg, 0.1 mmol) was added to a degassed solution of nitrile **70** (670 mg, 1.02 mmol) in EtOH (45 mL) and $CHCl_3$ (900 μL). The reaction mixture was hydrogenated under hydrogen atmosphere at room temperature for 4.5 h. Then, the suspension was filtered through a pad of Celite. After removal of the solvents under reduced pressure, the residue was purified by chromatography ($CH_2Cl_2$/EtOH 10:1, then 1:1) to yield **71** as a colorless solid (543 mg, 81%). M.p. 109°C; $[\alpha]_D^{20}$ = +287.4 ($c$=1.0 in MeOH); $R_f$=0.51 ($CH_2Cl_2$/EtOH 1:1); $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$=0.88 (t, $J$=6.6 Hz, 3H, ω-$CH_3$ hexadecyl), 1.22–1.37 (m, 24H, 12×$CH_2$ hexadecyl), 1.42 (m, 2H, γ-$CH_2$ hexadecyl), 1.58 (s, 9H, C(CH$_3$)$_3$), 1.83 (m, 2H, β-$CH_2$ hexadecyl), 2.41 (m, 1H, -$CH_{2a}$-), 2.74 (br d, $J$=14.5 Hz, 1H, -$CH_{2b}$-), 2.92–3.13 (m, 4H, α-$CH_2$ hexadecyl, -NC$H_2$C$H_2$N$H_2$), 3.84 (dd, $J$=5.5, 13.0 Hz, 1H, -$CH_{2a}$N-), 3.91 (m, 2H, -$CH_{2b}$N-, NC$H_{2a}$C$H_2$N$H_2$), 4.17 (d, $J$=8.8 Hz, 1H, -C*H*NH-), 4.24 (m, 2H, -CHC(O)-, NC$H_{2b}$C$H_2$N$H_2$), 7.48 (d, $J$=8.6 Hz, 1H, arom. CH), 8.14 (dd, $J$=2.1, 8.6 Hz, 1H, arom. CH), 8.49 (d, $J$=2.0 Hz, 1H, arom. CH); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta$=14.3 (ω-$CH_3$ hexadecyl), 22.9 ($CH_2$), 23.7 ($CH_2$), 28.3 (C(*C*H$_3$)$_3$), 28.5 ($CH_2$), 29.3 ($CH_2$), 29.5 ($CH_2$), 29.6 ($CH_2$), 29.7 ($CH_2$), 29.8 ($CH_2$), 29.8 ($CH_2$), 29.9 ($CH_2$), 29.9 ($CH_2$), 30.0 ($CH_2$), 30.0 ($CH_2$), 32.1 ($CH_2$), 33.0 ($CH_2$), 51.8 ($CH_2$N), 54.1 (α-$CH_2$ hexadecyl), 55.2 (CHNH), 57.2 (*C*HC(O)), 58.1 (N*C*H$_2$CH$_2$NH$_2$), 60.5 (NCH$_2$*C*H$_2$NH$_2$), 82.4 (*C*(CH$_3$)$_3$), 121.0 (arom. CH), 129.3 (arom. C), 131.6 (arom. CH), 132.5 (arom. CH), 134.2 (arom. C), 141.2 (arom. C), 163.5 (C=O), 164.4 (C=O), 170.6 (C=O); HRMS (FAB, 3-NBA): $m/z$: calcd for $C_{35}H_{59}N_4O_6S$: 663.4156, found: 663.4177 [$M$+H]$^+$.

**10-(2-Aminoethyl)-(2*S*)-(hexadecane-1-sulfonylamino)-5,11-dioxo-2,3,5,10,11,11a-hexahydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*,1,4]diazepine-7-carboxylic acid (72)**: A saturated solution of HCl in $Et_2O$ (20 mL) was added to a solution of benzodiazepine **71** (178 mg, 0.27 mmol) in $CH_2Cl_2$. After stirring at room temperature for 4 h, the solvent was removed under reduced pressure and the residue was coevaporated with toluene to yield **72** as a colorless solid (164 mg, quant.). M.p. 166°C; $[\alpha]_D^{20}$ = +260.2 ($c$=0.5 in MeOH); $^1$H NMR ($CD_3OD$, 400 MHz): $\delta$=0.88 (t, $J$=6.6 Hz, 3H, ω-$CH_3$ hexadecyl), 1.22–1.37 (m, 24H, 12×$CH_2$ hexadecyl), 1.42 (m, 2H, γ-$CH_2$ hexadecyl), 1.83 (m, 2H, β-$CH_2$ hexadecyl), 2.41 (m, 1H, -$CH_{2a}$-), 2.74 (m, 1H, -$CH_{2b}$-), 3.13 (m, 2H, α-$CH_2$ hexadecyl), 3.35 (m, 1H, -$CH_{2b}$N-), 3.54 (dd, $J$=5.5, 13.0 Hz, 1H, -$CH_{2a}$N-), 4.04–4.44 (m, 6H, -NC$H_2$C$H_2$NH$_2$, NC$H_2$CH$_2$NH$_2$, -C*H*NH-, -CHC(O)-), 7.60 (d, $J$=8.6 Hz, 1H, arom. CH), 8.21 (dd, $J$=2.1, 8.6 Hz, 1H, arom. CH), 8.49 (d, $J$=2.0 Hz, 1H, arom. CH); $^{13}$C NMR ($CD_3OD$, 100.6 MHz): $\delta$=14.3 (ω-$CH_3$ hexadecyl), 22.9 ($CH_2$), 23.7 ($CH_2$), 28.5 ($CH_2$), 29.3 ($CH_2$), 29.5 ($CH_2$), 29.6 ($CH_2$), 29.7 ($CH_2$), 29.8 ($CH_2$), 29.8 ($CH_2$), 29.9 ($CH_2$), 29.9 ($CH_2$), 30.0 ($CH_2$), 30.0 ($CH_2$), 32.1 ($CH_2$), 33.0 ($CH_2$), 51.8 ($CH_2$N), 54.1 (α-$CH_2$ hexadecyl), 55.2 (CHNH), 57.2 (*C*HC(O)), 58.1 (N*C*H$_2$CH$_2$NH$_2$), 60.5 (NCH$_2$*C*H$_2$NH$_2$), 121.0 (arom. CH), 129.3 (arom. C), 131.6 (arom. CH), 132.5 (arom. CH), 134.2 (arom. C), 141.2 (arom. C), 164.4 (C=O), 170.6 (C=O), 173.5 (C=O); HRMS (FAB, 3-NBA): $m/z$: calcd for $C_{31}H_{51}N_4O_6S$: 607.3530, found: 607.3553 [$M$+H]$^+$.

**10-(2-Allyloxycarbonylaminoethyl)-(2*S*)-(hexadecane-1-sulfonylamino)-5,11-dioxo-2,3,5,10,11,11a-hexahydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*,1,4]diazepine-7-carboxylic acid *tert*-butyl ester (73)**: AlocCl (127 μL, 1.15 mmol) was added to a solution of amine **71** (510 mg, 0.77 mmol) and $NEt_3$ (214 μL, 1.54 mmol) in $CH_2Cl_2$ (20 mL). After stirring at room temperature for 18 h, the solution was diluted with ethyl acetate (100 mL) and washed with 1 N HCl (30 mL). The organic layer was dried over $Na_2SO_4$, and, after removal of the solvent under reduced pressure, the residue was purified by chromatography (cyclohexane/ethyl acetate 1:1) to yield **73** as a colorless solid (483 mg, 84%). M.p. 90°C; $[\alpha]_D^{20}$ = +269.3°C ($c$=1.0 in $CHCl_3$); $R_f$=0.31 (cyclohexane/ethyl acetate 1:1); $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$=0.88 (t, $J$=6.6 Hz, 3H, ω-$CH_2$ hexadecyl), 1.22–1.37 (m, 24H, 12×$CH_2$ hexadecyl), 1.42 (m, 2H, γ-$CH_2$ hexadecyl), 1.58 (s, 9H, C(CH$_3$)$_3$), 1.83 (m, 2H, β-$CH_2$ hexadecyl), 2.41 (m, 1H, -$CH_{2a}$-), 2.74 (br d, $J$=14.5 Hz, 1H, -$CH_{2b}$-), 3.04 (m, 2H, α-$CH_2$ hexadecyl), 3.37 (m, 1H, -NC$H_2$C$H_{2a}$NH-), 3.63 (m, 1H, -NC$H_2$C$H_{2b}$NH-), 3.82 (dd, $J$=5.8, 13.0 Hz, 1H, -$CH_{2a}$N-), 3.93 (m, 2H, -$CH_{2b}$N-, -NC$H_{2a}$CH$_2$NH-), 4.14 (m, 2H, -C*H*NH-, -NC$H_{2b}$CH$_2$NH-), 4.27 (m, 1H, -CHC(O)-), 4.50 (m, 2H, -OC$H_2$CH=CH$_2$), 5.00 (m, 1H, NH), 5.14–5.28 (m, 2H, -OCH$_2$CH=C$H_2$), 5.86 (m, 1H, -OCH$_2$C*H*=CH$_2$), 5.93 (m, 1H, NH), 7.44 (d, $J$=8.6 Hz, 1H, arom. CH), 8.15 (dd, $J$=2.0, 8.6 Hz, 1H, arom. CH), 8.48 (d, $J$=2.1 Hz, 1H, arom. CH); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta$=14.2 (ω-$CH_3$ hexadecyl), 22.9 ($CH_2$), 23.7 ($CH_2$), 28.3 (C(*C*H$_3$)$_3$), 28.4 ($CH_2$), 29.4 ($CH_2$), 29.5 ($CH_2$), 29.6 ($CH_2$), 29.7 ($CH_2$), 29.8 ($CH_2$), 29.8 ($CH_2$), 29.9 ($CH_2$), 29.9 ($CH_2$), 30.0 ($CH_2$), 30.1 ($CH_2$), 32.1 ($CH_2$), 33.1 ($CH_2$), 51.8 ($CH_2$N), 54.0 (α-$CH_2$ hexadecyl), 55.1 (CHNH), 57.2 (*C*HC(O)), 58.1 (N*C*H$_2$CH$_2$NH$_2$), 60.4 (NCH$_2$*C*H$_2$NH$_2$), 66.1 (OCH$_2$CH=CH$_2$), 82.3 (*C*(CH$_3$)$_3$), 118.0 (OCH$_2$CH=*C*H$_2$), 121.2 (arom. CH), 129.3 (arom. C), 131.5 (arom. CH), 132.4 (arom. CH), 132.6 (OCH$_2$*C*H=CH$_2$), 134.2 (arom. C), 141.2 (arom. C), 156.7 (C=O), 163.4 (C=O), 164.4 (C=O), 170.4 (C=O); HRMS (FAB, 3-NBA): $m/z$: calcd for $C_{39}H_{63}N_4O_8S$: 747.4367, found: 747.4379 [$M$+H]$^+$.

**10-(2-Allyloxycarbonylaminoethyl)-(2*S*)-(hexadecane-1-sulfonylamino)-5,11-dioxo-2,3,5,10,11,11a-hexahydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*,1,4]diazepine-7-carboxylic acid (74)**: TFA (8 mL) was added to a solution of benzodiazepine **73** (405 mg, 0.54 mmol) in $CH_2Cl_2$ (8 mL). After stirring at room temperature for 4 h, the solvent was removed under reduced pressure and the residue was coevaporated with toluene to yield **74** as a colorless solid (374 mg, quant.). M.p. 165°C; $[\alpha]_D^{20}$ = +229.8 ($c$=1.0, MeOH); $R_f$=0.64 ($CH_2Cl_2$/EtOH 1:1); $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$=0.87 (t, $J$=6.6 Hz, 3H, ω-$CH_2$ hexadecyl), 1.22–1.36 (m, 24H, 12×$CH_2$ hexadecyl), 1.44 (m, 2H, γ-$CH_2$ hexadecyl), 1.56 (s, 9H, C(CH$_3$)$_3$), 1.84 (m, 2H, β-$CH_2$ hexadecyl), 2.42 (m, 1H, -$CH_{2a}$-), 2.73 (br d, $J$=14.5 Hz, 1H, -$CH_{2b}$-), 3.03 (m, 2H, α-$CH_2$ hexadecyl), 3.35 (m, 1H, -NC$H_2$C$H_{2a}$NH-), 3.64 (m, 1H, -NC$H_2$C$H_{2b}$NH-), 3.82 (dd, $J$=5.8, 13.0 Hz, 1H, -$CH_{2a}$N-), 3.93 (m, 2H, -$CH_{2b}$N-, -NC$H_{2a}$CH$_2$NH-), 4.13 (m,

2H, -C*H*NH-, -NC$H_{2b}$CH$_2$NH-), 4.26 (m, 1H, -CHC(O)-), 4.50 (m, 2H, -OC$H_2$CH=CH$_2$), 5.28 (m, 1H, NH), 5.14–5.26 (m, 2H, -OCH$_2$CH=C$H_2$), 5.86 (m, 1H, -OCH$_2$C*H*=CH$_2$), 5.95 (m, 1H, NH), 7.45 (d, $J$=8.6 Hz, 1H, arom. CH), 8.14 (dd, $J$=2.0, 8.6 Hz, 1H, arom. CH), 8.46 (d, $J$=2.1 Hz, 1H, arom. CH); $^{13}$C NMR (CDCl$_3$, 100.6 MHz): $\delta$=14.2 (ω-CH3 hexadecyl), 22.8 (CH$_2$), 23.6 (CH$_2$), 28.4 (CH$_2$), 29.3 (CH$_2$), 29.5 (CH$_2$), 29.7 (CH$_2$), 29.7 (CH$_2$), 29.8 (CH$_2$), 29.9 (CH$_2$), 29.9 (CH$_2$), 29.9 (CH$_2$), 29.9 (CH$_2$), 30.1 (CH$_2$), 32.1 (CH$_2$), 33.3 (CH$_2$), 51.8 (CH$_2$N), 54.2 (α-CH$_2$ hexadecyl), 55.4 (CHNH), 57.5 (*C*HC(O)), 58.1 (N*C*H$_2$CH$_2$NH$_2$), 60.3 (NCH$_2$CH$_2$NH$_2$), 66.1 (O*C*H$_2$CH=CH$_2$), 118.2 (OCH$_2$CH=CH$_2$), 121.4 (arom. CH), 129.4 (arom. C), 131.6 (arom. CH), 132.5 (arom. CH), 132.6 (OCH$_2$*C*H=CH$_2$), 134.0 (arom. C), 141.0 (arom. C), 156.5 (C=O), 164.3 (C=O), 170.3 (C=O), 175.1 (C=O); HRMS (FAB, 3-NBA): $m/z$: calcd for C$_{35}$H$_{55}$N$_4$O$_8$S: 691.3741, found: 691.3755 [$M$+H]$^+$.

**10-(2-Allyloxycarbonylaminoethyl)-(2*S*)-(hexadecane-1-sulfonylamino)-5,11-dioxo-2,3,5,10,11,11a-hexahydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*,1,4]diazepine-7-yl-*S*-farnesyl-L-cysteine methyl ester (75)**: At 0°C, EDC (125 mg, 0.64 mmol) was added to a solution of acid **74** (367 mg, 0.53 mmol), H-Cys(Far)-OMe **10** (216 mg, 0.63 mmol) and HOBt (166 mg, 1.06 mmol) in CH$_2$Cl$_2$ (20 mL). The solution was warmed to room temperature and stirred for 18 h. Then the reaction mixture was diluted with ethyl acetate (50 mL) and subsequently washed with 1N HCl (30 mL) and Na$_2$CO$_3$ solution (10%, 30 mL). The organic layer was dried over Na$_2$SO$_4$, and, after removal of the solvent under reduced pressure, the residue was purified by chromatography (cyclohexane/ethyl acetate 1:1) to yield **75** as colorless solid (423 mg, 89%). M.p. 77°C; $[\alpha]_D^{20}$ = +194.1 ($c$=0.5 in CHCl$_3$); $R_f$=0.13 (cyclohexane/ethyl acetate 1:1); $^1$H NMR (CDCl$_3$, 400 MHz): $\delta$=0.88 (t, $J$=6.7 Hz, 3H, ω-CH$_3$ hexadecyl), 1.20–1.35 (m, 24H, 12×CH$_2$ hexadecyl), 1.43 (m, 2H, γ-CH$_2$ hexadecyl), 1.50 (s, 6H, 2×CH$_3$ Far), 1.55 (s, 3H, CH$_3$ Far), 1.57 (s, 3H, CH$_3$ Far), 1.73 (m, 2H, β-CH$_2$ hexadecyl), 1.82–2.08 (m, 8H, 4×CH$_2$ Far), 2.33 (m, 1H, -CH$_{2a}$-), 2.62 (m, 1H, -CH$_{2b}$-), 2.90–3.24 (m, 6H, α-CH$_2$ hexadecyl, β-CH$_2$ Cys, -SCH$_2$-), 3.38 (m, 1H, -NCH$_2$C$H_{2a}$NH-), 3.62 (m, 1H, -NCH$_2$C$H_{2b}$NH-), 3.76 (s, 3H, OCH$_3$), 3.83–3.99 (m, 3H, -CH$_2$N-, -NC$H_{2a}$CH$_2$NH-), 4.12–4.30 (m, 3H, -C*H*NH-, -CHC(O)-, -NC$H_{2b}$CH$_2$NH-), 4.50 (m, 2H, -OC$H_2$CH=CH$_2$), 4.96 (m, 1H, α-CH Cys), 5.04–5.29 (m, 6H, 3×CH Far, -OCH$_2$CH=C$H_2$, NH), 5.84 (m, 1H, OCH$_2$C*H*=CH$_2$), 5.96 (m, 1H, NH), 7.23 (m, 1H, NH), 7.49 (d, $J$=8.6 Hz, 1H, arom. CH), 8.08 (dd, $J$=2.1, 8.6 Hz, 1H, arom. CH), 8.30 (d, $J$=2.1 Hz, 1H, arom. CH); $^{13}$C NMR (CDCl$_3$, 100.6 MHz): $\delta$=14.3 (ω-CH$_3$ hexadecyl), 16.2 (CH$_3$ Far), 16.4 (CH$_3$ Far), 16.5 (CH$_3$ Far), 17.9 (CH$_3$ Far), 22.9 (CH$_2$), 23.8 (CH$_2$), 25.9 (CH$_2$), 26.6 (CH$_2$), 26.9 (CH$_2$), 27.0 (CH$_2$), 27.3 (CH$_2$), 27.5 (CH$_2$), 27.7 (CH$_2$), 27.9 (CH$_2$), 28.1 (CH$_2$), 28.2 (CH$_2$), 28.4 (CH$_2$), 28.7 (CH$_2$), 29.0 (CH$_2$), 29.4 (CH$_2$), 29.9 (CH$_2$), 32.1 (CH$_2$), 33.3 (CH$_2$), 39.2 (CH$_2$ Far), 39.8 (CH$_2$ Far), 39.9 (CHNH), 43.3 (NCH$_2$*C*H$_2$NH), 49.8 (CH$_2$N), 51.9 (OCH$_3$), 52.5 (N*C*H$_2$CH$_2$NH), 52.9 (*C*HC(O), 54.3 (α-CH$_2$ hexadecyl), 57.4 (α-CH Cys), 66.0 (O*C*H$_2$CH=CH$_2$), 118.08 (OCH$_2$CH=*C*H$_2$), 119.6 (CH Far), 123.4 (arom. CH), 123.9 (CH Far), 124.5 (CH Far), 129.2 (arom. C), 129.8 (arom. CH), 131.5 (arom. CH), 131.7 (arom. C), 132.4 (C Far), 132.9 (OCH$_2$*C*H=CH$_2$), 135.5 (C Far), 140.3 (C Far), 142.5 (arom. C), 156.7 (C=O), 164.5 (C=O), 165.1 (C=O), 170.2 (C=O), 171.6 (C=O); HRMS (FAB, 3-NBA): $m/z$: calcd for C$_{54}$H$_{85}$N$_5$NaO$_9$S$_2$: 1034.5689, found: 1034.5699 [$M$+Na]$^+$.

**10-(2-Aminoethyl)-(2*S*)-(hexadecane-1-sulfonylamino)-5,11-dioxo-2,3,5,10,11,11a-hexahydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*,1,4]diazepine-7-yl-*S*-farnesyl-L-cysteine methyl ester (76)**: [Pd(PPh$_3$)$_4$] (23 mg, 0.02 mmol) was added to a degassed solution of benzodiazepine **75** (200 mg, 0.2 mmol) and *N*,*N*′-dimethylbarbituric acid (31 mg, 0.2 mmol) in THF (20 mL). The mixture was stirred for 3 h at room temperature. After removal of the solvent under reduced pressure, the residue was purified by chromatography (CH$_2$Cl$_2$/EtOH 30:1, then 15:1, then 5:1 + 1% NEt$_3$) to yield **76** as a slightly yellow foam (147 mg, 80%). $[\alpha]_D^{20}$ = +165.2 ($c$=0.5 in CHCl$_3$); $R_f$=0.16 (CH$_2$Cl$_2$/EtOH); $^1$H NMR (CD$_3$OD, 400 MHz): $\delta$=0.88 (t, $J$=6.7 Hz, 3H, ω-CH$_3$ hexadecyl), 1.20–1.35 (m, 24H, 12×CH$_2$ hexadecyl), 1.43 (m, 2H, γ-CH$_2$ hexadecyl), 1.50 (s, 6H, 2×CH$_3$ Far), 1.55 (s, 3H, CH$_3$ Far), 1.57 (s, 3H, CH$_3$ Far), 1.73 (m, 2H, β-CH$_2$ hexadecyl), 1.82–2.08 (m, 8H, 4×CH$_2$ Far), 2.33 (m, 1H, -CH$_{2a}$-), 2.62 (m, 1H, -CH$_{2b}$-), 2.90–3.20 (m, 8H, α-CH$_2$ hexadecyl, β-CH$_2$ Cys, -SCH$_2$-, -NCH$_2$C$H_2$NH$_2$), 3.70 (m, 1H, -NC$H_{2a}$CH$_2$NH$_2$), 3.74 (s, 3H, OCH$_3$), 3.99 (m, 1H, -NC$H_{2b}$CH$_2$NH-), 4.12–4.30 (m, 2H, -CHC(O)-, -C*H*NH-), 4.80 (m, 1H, α-CH Cys), 5.01 (m, 2H, 2×CH Far), 5.15 (m, 1H, CH Far), 7.49 (d, $J$=8.6 Hz, 1H, arom. CH), 8.08 (dd, $J$=2.1, 8.5 Hz, 1H, arom. CH), 8.30 (d, $J$=2.1 Hz, 1H, arom. CH); $^{13}$C NMR (CD$_3$OD, 100.6 MHz): $\delta$=14.3 (ω-CH$_3$ hexadecyl), 16.2 (CH$_3$ Far), 16.5 (CH$_3$ Far), 16.5 (CH$_3$ Far), 17.9 (CH$_3$ Far), 22.7 (CH$_2$), 23.7 (CH$_2$), 25.9 (CH$_2$), 26.5 (CH$_2$), 26.8 (CH$_2$), 27.1 (CH$_2$), 27.3 (CH$_2$), 27.4 (CH$_2$), 27.7 (CH$_2$), 27.7 (CH$_2$), 28.2 (CH$_2$), 28.2 (CH$_2$), 28.3 (CH$_2$), 28.6 (CH$_2$), 29.0 (CH$_2$), 29.3 (CH$_2$), 29.9 (CH$_2$), 32.1 (CH$_2$), 33.2 (CH$_2$), 39.1 (CH$_2$ Far), 39.8 (CH$_2$ Far), 39.9 (CHNH), 43.5 (NCH$_2$*C*H$_2$NH$_2$), 49.7 (CH$_2$N), 51.9 (OCH$_3$), 52.7 (N*C*H$_2$CH$_2$NH$_2$), 52.7 (*C*HC(O), 54.3 (α-CH$_2$ hexadecyl), 57.4 (α-CH Cys), 119.5 (CH Far), 123.5 (arom. CH), 123.9 (CH Far), 124.5 (CH Far), 129.4 (arom. C), 129.7 (arom. CH), 131.5 (arom. CH), 131.6 (arom. C), 132.4 (C Far), 135.5 (C Far), 140.3 (C Far), 142.5 (arom. C), 164.4 (C=O), 165.4 (C=O), 170.4 (C=O), 171.6 (C=O); HRMS (FAB, 3-NBA): $m/z$: calcd for C$_{50}$H$_{82}$N$_5$O$_7$S$_2$: 928.5656, found: 928.5638 [$M$+H]$^+$.

**10-Cyanomethyl-(2*S*)-(methanesulfonylamino)-5,11-dioxo-2,3,5,10,11,11a-hexahydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*,1,4]diazepine-7-carboxylic acid *tert*-butyl ester (77)**: At 0°C, MsCl (37 μL, 0.48 mmol) was added to a solution of amine **71** (118 mg, 0.32 mmol) and NEt*i*Pr$_2$ (161 μL, 0.64 mmol) in DMF (15 mL). The reaction mixture was warmed to room temperature and stirred for 22 h. Then, the solvent was removed under reduced pressure, the residue was dissolved in ethyl acetate (50 mL) and washed with 1N HCl (30 mL). The organic layer was dried over Na$_2$SO$_4$, and, after removal of the solvent under reduced pressure, the residue was purified by chromatography (cyclohexane/ethyl acetate 1:5) to yield **77** as a colorless solid (132 mg, 92%). M.p. 114°C; $[\alpha]_D^{20}$ = +378.4 ($c$=1.0 in CHCl$_3$); $R_f$=0.34 (cyclohexane/ethyl acetate 1:5); $^1$H NMR (CDCl$_3$, 400 MHz): $\delta$=1.52 (s, 9H, C(CH$_3$)$_3$), 2.42 (m, 1H, -CH$_{2a}$-), 2.61 (m, 1H, -CH$_{2b}$-), 2.95 (s, 3H, CH$_3$SO$_2$-), 3.70 (dd, $J$=2.5, 12.9 Hz, 1H, -CH$_{2a}$N-), 3.90 (dd, (5.9, 12.9 Hz, 1H, -CH$_{2b}$N-), 4.18 (m, 2H, -C*H*NH-, -CHC(O)-), 4.34 (d, $J$=17.4, 1H, -CH$_{2a}$CN), 4.93 (d, $J$=17.4, 1H, -CH$_{2b}$CN), 7.42 (d, $J$=8.6 Hz, 1H, arom. CH), 8.16 (dd, $J$=2.1, 8.6 Hz, 1H, arom. CH), 8.42 (d, 2.1 Hz, 1H, arom. CH); $^{13}$C NMR (CDCl$_3$, 100.6 MHz): $\delta$=28.1 (C(*C*H$_3$)$_3$), 32.6 (CH$_2$), 37.2 (*C*H$_2$CN), 41.5 (CH$_2$N), 51.4 (CH$_3$SO$_2$), 54.0 (CHNH), 56.7 (*C*HC(O)), 82.7 (*C*(CH$_3$)$_3$), 115.1 (CN), 121.2 (arom. CH), 129.0 (arom. C), 132.0 (arom. CH), 132.4 (arom. CH), 134.0 (arom. C), 141.1 (arom. C), 163.9 (C=O), 164.6 (C=O), 169.8 (C=O); HRMS (FAB, 3-NBA): $m/z$: calcd for C$_{20}$H$_{25}$N$_4$O$_6$S: 449.1496, found: 449.1486 [$M$+H]$^+$.

**10-(2-Aminoethyl)-(2*S*)-(methanesulfonylamino)-5,11-dioxo-2,3,5,10,11,11a-hexahydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*,1,4]diazepine-7-carboxylic acid *tert*-butyl ester (78)**: PtO$_2$·H$_2$O (8 mg, 0.03 mmol) was added to a degassed solution of nitrile **77** (123 mg, 0.27 mmol) in EtOH (9 mL) and CHCl$_3$ (180 μL). The reaction mixture was hydrogenated under hydrogen atmosphere at room temperature for 4.5 h. Then, the suspension was filtered through a pad of Celite. After removal of the solvents under reduced pressure, the residue was purified by chromatography (CH$_2$Cl$_2$/EtOH 10:1, then 1:1 + 1% NEt$_3$) to yield **78** as a colorless solid (105 mg, 85%). M.p. 123°C; $[\alpha]_D^{20}$ = +310.1 ($c$=1.0 in CHCl$_3$); $R_f$=0.39 (CH$_2$Cl$_2$/EtOH 1:1); $^1$H NMR (CDCl$_3$, 400 MHz): $\delta$=1.58 (s, 9H, C(CH$_3$)$_3$), 2.41 (m, 1H, -CH$_{2a}$-), 2.74 (br d, $J$=14.5 Hz, 1H, -CH$_{2b}$-), 2.95 (s, 3H, CH$_3$SO$_2$-), 3.03 (m, 2H, -NCH$_2$C$H_2$NH$_2$), 3.84 (dd, $J$=5.5, 13.0 Hz, 1H, -CH$_{2a}$N-), 3.91 (m, 2H, -CH$_{2b}$N-, NC$H_{2a}$CH$_2$NH$_2$), 4.17 (d, $J$=8.8 Hz, 1H, -C*H*NH-), 4.24 (m, 2H, -CHC(O)-, NC$H_{2b}$CH$_2$NH$_2$), 7.48 (d, $J$=8.6 Hz, 1H, arom. CH), 8.14 (dd, $J$=2.1, 8.6 Hz, 1H, arom. CH), 8.49 (d, $J$=2.0 Hz, 1H, arom. CH); $^{13}$C NMR (CDCl$_3$, 100.6 MHz): $\delta$=28.3 (C(*C*H$_3$)$_3$), 33.0 (CH$_2$), 41.8 (CH$_2$N), 51.4 (CH$_3$SO$_2$), 55.2 (CHNH), 57.2 (*C*HC(O)), 58.1 (N*C*H$_2$CH$_2$NH$_2$), 60.5 (NCH$_2$*C*H$_2$NH$_2$), 82.4 (*C*(CH$_3$)$_3$), 121.0 (arom. CH), 129.3 (arom. C), 131.6 (arom. CH), 132.5 (arom. CH), 134.2 (arom. C), 141.2 (arom. C), 163.5 (C=O), 164.4 (C=O), 170.6 (C=O); HRMS (FAB, 3-NBA): $m/z$: calcd for C$_{20}$H$_{29}$N$_4$O$_6$S: 453.1809, found: 453.1793 [$M$+H]$^+$.

**10-(2-Allyloxycarbonylaminoethyl)-(2*S*)-(methanesulfonylamino)-5,11-dioxo-2,3,5,10,11,11a-hexahydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*,1,4]diazepine-7-carboxylic acid *tert*-butyl ester (79)**: AlocCl (40 μL, 0.36 mmol) was added to a solution of amine **78** (107 mg, 0.24 mmol) and NEt$_3$ (66 μL, 0.47 mmol) in CH$_2$Cl$_2$ (4 mL). After stirring at room temperature for 18 h, the solution was diluted with ethyl acetate (50 mL) and washed

with 1 N HCl (10 mL). The organic layer was dried over $Na_2SO_4$, and, after removal of the solvent under reduced pressure, the residue was purified by chromatography (cyclohexane/ethyl acetate 1:5) to yield **79** as a colorless solid (105 mg, 83%). M.p. 111 °C; $[\alpha]_D^{20} = +288.3$ ($c = 1.0$ in $CHCl_3$); $R_f = 0.28$ (cyclohexane/ethyl acetate 1:5); $^1$H NMR ($CDCl_3$, 400 MHz): $\delta = 1.58$ (s, 9H, $C(CH_3)_3$), 2.41 (m, 1H, -$CH_{2a}$-), 2.74 (br d, $J =$ 14.5 Hz, 1H, -$CH_{2b}$-), 2.95 (s, 3H, $CH_3SO_2$-), 3.37 (m, 1H, -$NCH_2CH_{2a}NH$-), 3.63 (m, 1H, -$NCH_2CH_{2b}NH$-), 3.82 (dd, $J = 5.8$, 13.0 Hz, 1H, -$CH_{2a}N$-), 3.93 (m, 2H, -$CH_{2b}N$-, -$NCH_{2a}CH_2NH$-), 4.14 (m, 2H, -$CH$NH-, -$NCH_{2b}CH_2NH$-), 4.27 (m, 1H, -CHC(O)-), 4.50 (m, 2H, -$OCH_2CH{=}CH_2$), 5.00 (m, 1H, NH Urethan), 5.14–5.28 (m, 2H, -$OCH_2CH{=}CH_2$), 5.86 (m, 1H, -$OCH_2CH{=}CH_2$), 5.93 (m, 1H, NH), 7.44 (d, $J = 8.6$ Hz, 1H, arom. CH), 8.15 (dd, $J = 2.0$, 8.6 Hz, 1H, arom. CH), 8.48 (d, $J = 2.1$ Hz, 1H, arom. CH); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta =$ 28.3 ($C(CH_3)_3$), 33.1 ($CH_2$), 41.8 ($CH_2N$), 51.4 ($CH_3SO_2$), 55.1 (CHNH), 57.2 (CHC(O)), 58.1 ($NCH_2CH_2NH_2$), 60.4 ($NCH_2CH_2NH_2$), 66.1 ($OCH_2CH{=}CH_2$), 82.3 ($C(CH_3)_3$), 118.0 ($OCH_2CH{=}CH_2$), 121.2 (arom. CH), 129.3 (arom. C), 131.5 (arom. CH), 132.4 (arom. CH), 132.6 ($OCH_2CH{=}CH_2$), 134.2 (arom. C), 141.2 (arom. C), 156.7 (C=O), 163.4 (C=O), 164.4 (C=O), 170.4 (C=O); HRMS (FAB, 3-NBA): $m/z$: calcd for $C_{24}H_{33}N_4O_8S$: 537.2020, found: 537.2007 $[M+H]^+$.

**10-(2-Allyloxycarbonylaminoethyl)-(2*S*)-(methanesulfonylamino)-5,11-dioxo-2,3,5,10,11,11a-hexahydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*,1,4]diazepine-7-yl-*S*-farnesyl-L-cysteine methyl ester (80)**: TFA (5 mL) was added to a solution of benzodiazepine **79** (50 mg, 0.09 mmol) in $CH_2Cl_2$ (8 mL). After stirring at room temperature for 4 h, the solvent was removed under reduced pressure and the residue was coevaporated with toluene to yield the acid as a colorless solid (45 mg, quant.).

At 0 °C, EDC (22 mg, 0.11 mmol) was added to a solution of the above acid (45 mg, 0.09 mmol), H-Cys(Far)-OMe **10** (32 mg, 0.09 mmol) and HOBt (29 mg, 0.19 mmol) in $CH_2Cl_2$ (10 mL). The solution was warmed to room temperature and stirred for 18 h. Then the reaction mixture was diluted with ethyl acetate (50 mL) and subsequently washed with 1 N HCl (30 mL) and $Na_2CO_3$ solution (10%, 30 mL). The organic layer was dried over $Na_2SO_4$, and, after removal of the solvent under reduced pressure, the residue was purified by chromatography (cyclohexane/ethyl acetate 1:5) to yield **80** as colorless solid (56 mg, 75%). M.p. 92 °C; $[\alpha]_D^{20} =$ +196.6 ($c = 0.5$ in $CHCl_3$); $R_f = 0.13$ (cyclohexane/ethyl acetate 1:5); $^1$H NMR ($CDCl_3$, 400 MHz): $\delta = 1.50$ (s, 6H, 2×$CH_3$ Far), 1.55 (s, 3H, $CH_3$ Far), 1.57 (s, 3H, $CH_3$ Far), 1.82–2.08 (m, 8H, 4×$CH_2$ Far), 2.33 (m, 1H, -$CH_{2a}$-), 2.62 (m, 1H, -$CH_{2b}$-), 2.95 (s, 3H, $CH_3SO_2$-), 2.90–3.24 (m, 4H, β-$CH_2$ Cys, -$SCH_2$-), 3.38 (m, 1H, -$NCH_2CH_{2a}NH$-), 3.62 (m, 1H, -$NCH_2CH_{2b}NH$-), 3.76 (s, 3H, $OCH_3$), 3.83–3.99 (m, 3H, -$CH_2N$-, -$NCH_{2a}CH_2NH$-), 4.12–4.30 (m, 3H, -$CH$NH-, -CHC(O)-, -$NCH_{2b}CH_2NH$-), 4.50 (m, 2H, -$OCH_2CH{=}CH_2$), 4.96 (m, 1H, α-CH Cys), 5.04–5.29 (m, 6H, 3×CH Far, -$OCH_2CH{=}CH_2$, NH), 5.84 (m, 1H, $OCH_2CH{=}CH_2$), 5.96 (m, 1H, NH), 7.23 (m, 1H, NH), 7.49 (d, $J =$ 8.6 Hz, 1H, arom. CH), 8.08 (dd, $J = 2.1$, 8.6 Hz, 1H, arom. CH), 8.30 (d, $J = 2.1$ Hz, 1H, arom. CH); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta = 16.2$ ($CH_3$ Far), 16.4 ($CH_3$ Far), 16.5 ($CH_3$ Far), 17.9 ($CH_3$ Far), 22.9 ($CH_2$ Far), 23.3 ($CH_2$ Far), 32.1 ($CH_2S$), 33.3 ($CH_2$), 39.2 ($CH_2$ Far), 39.4 ($CH_2$), 39.8 ($CH_2$ Far), 39.9 (CHNH), 43.3 ($NCH_2CH_2NH$), 49.8 ($CH_2N$), 51.4 ($CH_3SO_2$), 51.9 ($OCH_3$), 52.5 ($NCH_2CH_2NH$), 52.9 (CHC(O), 57.4 (α-CH Cys), 66.0 ($OCH_2CH{=}CH_2$), 118.0 ($OCH_2CH{=}CH_2$), 119.6 (CH Far), 123.4 (arom. CH), 123.9 (CH Far), 124.5 (CH Far), 129.2 (arom. C), 129.8 (arom. CH), 131.5 (arom. CH), 131.7 (arom. C), 132.4 (C Far), 132.9 ($OCH_2CH{=}CH_2$), 135.5 (C Far), 140.3 (C Far), 142.5 (arom. C), 156.7 (C=O), 164.5 (C=O), 165.1 (C=O), 170.2 (C=O), 171.6 (C=O); HRMS (FAB, 3-NBA): $m/z$: calcd for $C_{39}H_{56}N_5O_9S_2$ $[M+H]^+$: 802.3520, found: 802.3527.

**10-(2-Aminoethyl)-(2*S*)-(methanesulfonylamino)-5,11-dioxo-2,3,5,10,11,11a-hexahydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*,1,4]diazepine-7-yl-*S*-farnesyl-L-cysteine methyl ester (81)**: $[Pd(PPh_3)_4]$ (11 mg, 0.01 mmol) was added to a degassed solution of benzodiazepine **80** (37 mg, 0.05 mmol) and *N*,*N*′-dimethylbarbituric acid (7 mg, 0.05 mmol) in THF (5 mL). The mixture was stirred for 3 h at room temperature. After removal of the solvent under reduced pressure, the residue was purified by chromatography ($CH_2Cl_2$/EtOH 20:1, then 15:1, then 5:1 + 1% $NEt_3$) to yield **81** as a slightly yellow foam (21 mg, 65%). $[\alpha]_D^{20} = +180.5$ ($c = 0.5$ in $CHCl_3$); $R_f = 0.16$ ($CH_2Cl_2$/EtOH); $^1$H NMR ($CD_3OD$, 400 MHz): $\delta = 1.50$ (s, 6H, 2×$CH_3$ Far), 1.55 (s, 3H, $CH_3$ Far), 1.57 (s, 3H, $CH_3$ Far), 1.82–2.08 (m, 8H, 4×$CH_2$ Far), 2.33 (m, 1H, -$CH_{2a}$-), 2.62 (m, 1H, -$CH_{2b}$-), 2.95 (s, 3H, $CH_3SO_2$), 2.90–3.20 (m, 6H, β-$CH_2$ Cys, -$SCH_2$-, -$NCH_2CH_2NH_2$), 3.70 (m, 1H, -$NCH_{2a}CH_2NH_2$), 3.74 (s, 3H, $OCH_3$), 3.99 (m, 1H, -$NCH_{2b}CH_2NH_2$), 4.12–4.30 (m, 2H, -CHC(O)-, -$CH$NH-), 4.80 (m, 1H, α-CH Cys), 5.01 (m, 2H, 2×CH Far), 5.15 (m, 1H, CH Far), 7.49 (d, $J =$ 8.6 Hz, 1H, arom. CH), 8.08 (dd, $J = 2.1$, 8.5 Hz, 1H, arom. CH), 8.30 (d, $J = 2.1$ Hz, 1H, arom. CH); $^{13}$C NMR ($CD_3OD$, 100.6 MHz): $\delta = 16.2$ ($CH_3$ Far), 16.5 ($CH_3$ Far), 16.5 ($CH_3$ Far), 17.9 ($CH_3$ Far), 22.9 ($CH_2$ Far), 23.9 ($CH_2$ Far), 33.2 ($CH_2$), 32.1 ($CH_2S$), 39.1 ($CH_2$ Far), 39.4 ($CH_2$), 39.8 ($CH_2$ Far), 39.9 (CHNH), 43.5 ($NCH_2CH_2NH_2$), 49.7 ($CH_2N$), 51.4 ($CH_3SO_2$), 51.9 ($OCH_3$), 52.7 ($NCH_2CH_2NH_2$), 52.7 (CHC(O), 57.4 (α-CH Cys), 119.5 (CH Far), 123.5 (arom. CH), 123.9 (CH Far), 124.5 (CH Far), 129.4 (arom. C), 129.7 (arom. CH), 131.5 (arom. CH), 131.6 (arom. C), 132.4 (C Far), 135.5 (C Far), 140.3 (C Far), 142.5 (arom. C), 164.4 (C=O), 165.4 (C=O), 170.4 (C=O), 171.6 (C=O); HRMS (FAB, 3-NBA): $m/z$: calcd for $C_{35}H_{51}N_5NaO_7S_2$: 740.3130, found: 740.3144 $[M+Na]^+$.

**Synthesis of the pentapeptide substrate for the inhibition studies of APT1**

**Bis-[(*N*-Acetyl-L-methionyl)-L-seryl]-L-cystine-di-*tert*-butyl ester (82)**: At 0 °C, EDC (253 mg, 1.29 mmol) was added to a solution of Ac-Met-Ser-OH **16** (300 mg, 1.08 mmol), (HCl·H-Cys-O*t*Bu)$_2$ (252 mg, 0.59 mmol), HOBt (337 mg, 2.16 mmol) and NE*t*i$Pr_2$ (221 μL, 1.29 mmol) in DMF (25 mL). The reaction mixture was warmed to room temperature and stirred for 18 h. After removal of the solvent under reduced pressure, the residue was dissolved in $CHCl_3$ (100 mL) and subsequently washed with 1 N HCl (20 mL) and $Na_2CO_3$ solution (10%, 20 mL). The organic layer was dried over $Na_2SO_4$, and, after removal of the solvent under reduced pressure, the residue was purified by chromatography ($CH_2Cl_2$/EtOH 10:1, then 5:1) to yield **82** as a colorless oil (336 mg, 65%). $[\alpha]_D^{20} = -16.4$ ($c = 1.0$ in MeOH); $R_f = 0.10$ ($CH_2Cl_2$/ethanol 10:1); $^1$H NMR ($CD_3OD$, 400 MHz): $\delta = 1.48$ (s, 18H, 2×$C(CH_3)_3$), 2.01 (s, 6H, 2×$CH_3$ acetyl), 2.09 (s, 6H, 2×$CH_3$ Met), 1.91–2.14 (m, 4H, 2×β-$CH_2$ Met), 2.55 (m, 4H, 2×γ-$CH_2$ Met), 3.06 (dd, $J = 7.8$, 13.8 Hz, 2H, 2×β-$CH_{2a}$ Cys), 3.20 (dd, $J = 5.3$, 13.9 Hz, 2H, 2×β-$CH_{2b}$ Cys), 3.82 (m, 4H, 2×β-$CH_2$ Ser), 4.51 (m, 4H, 4×α-CH Ser, Cys), 4.68 (m, 2H, 2×α-CH Met); $^{13}$C NMR ($CD_3OD$, 100.6 MHz): $\delta = 14.2$ ($CH_3$ acetyl), 21.4 ($CH_3$ Met), 27.1 (C-$(CH_3)_3$), 29.9 (β-$CH_2$ Met), 31.6 (γ-$CH_2$ Met), 39.9 (β-$CH_2$ Cys), 53.0 (α-CH Cys), 53.6 (α-CH Met), 55.4 (α-CH Ser), 61.7 (β-$CH_2$ Ser), 82.6 (*C*-$(CH_3)_3$), 169.5 (C=O), 170.8 (C=O), 172.4 (C=O), 172.8 (C=O); HRMS (FAB, 3-NBA): $m/z$: calcd for $C_{34}H_{61}N_6O_{12}S_4$: 873.3231, found: 873.3268 $[M+H]^+$.

***N*-Acetyl-L-methionyl-L-seryl-*S*-palmitoyl-L-cysteine-*tert*-butyl ester (83)**: 1,4-Dithiothreitol (208 mg, 1.35 mmol) and $NEt_3$ (75 μL, 0.54 mmol) were added to a degassed solution of (Ac-Met-Cys-O*t*Bu)$_2$ **82** (0.236 g, 0.27 mmol) in $CH_2Cl_2$ (10 mL). After stirring at room temperature for 2 h, the solution was washed with 1 N HCl (2×2 mL). The organic layer was dried over $Na_2SO_4$ and cooled down to 0 °C. $NEt_3$ (75 μL, 054 mmol) and palmitoyl chloride (418 μL, 1.35 mmol) were added to this solution. The solution was warmed to room temperature and stirred for 3.5 h. Then, the reaction mixture was diluted with ethyl acetate (100 mL) and washed with 1 N HCl (20 mL). The organic layer was dried over $Na_2SO_4$, and, after removal of the solvents under reduced pressure, the residue was purified by chromatography ($CH_2Cl_2$/EtOH 30:1, then, 20:1, then, 10:1) to yield **83** as a colorless solid (273 mg, 75%). M.p. 53 °C; $[\alpha]_D^{20} = -15.0$ ($c = 1.0$ in $CHCl_3$); $R_f = 0.31$ ($CH_2Cl_2$/EtOH); $^1$H NMR ($CDCl_3$, 400 MHz): $\delta = 0.88$ (t, $J = 6.6$ Hz, 3H, ω-$CH_3$ Pal), 1.20–1.40 (m, 24H, 12×$CH_2$ Pal), 1.48 (s, 9H, $C(CH_3)_3$), 1.65 (m, 2H, β-$CH_2$ Pal), 2.05 (s, 3H, $CH_3$ acetyl), 2.12 (s, 3H, $CH_3$ Met), 1.94–2.18 (m, 2H, β-$CH_2$ Met), 2.59 (m, 4H, α-$CH_2$ Pal, γ-$CH_2$ Met), 3.30 (dd, $J = 7.8$, 13.9 Hz, 1H, β-$CH_{2a}$ Cys), 3.48 (dd, $J = 5.3$, 13.9 Hz, 1H, β-$CH_{2b}$ Cys), 3.50 (dd, $J = 5.4$, 11.5 Hz, 1H, β-$CH_{2a}$ Ser), 4.03 (dd, $J = 3.9$, 11.7 Hz, 1H, β-$CH_{2b}$ Ser), 4.52 (m, 1H, α-CH Ser), 4.68 (m, 2H, α-CH Cys, α-CH Met), 6.62 (m, 1H, NH), 7.21 (m, 1H, NH), 7.25 (m, 1H, NH); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta = 14.3$ (ω-$CH_3$ Pal), 22.9 ($CH_3$ acetyl), 23.2 ($CH_3$ Met),

23.3 ($CH_2$), 25.7 ($C(CH_3)_3$), 28.1 ($CH_2$), 28.8 ($CH_2$), 28.4 ($CH_2$), 28.6 ($CH_2$), 28.9 ($CH_2$), 29.1 ($CH_2$), 29.3 ($CH_2$), 29.4 ($CH_2$), 29.5 ($CH_2$), 29.6 ($CH_2$), 29.7 ($CH_2$), 29.8 ($CH_2$), 29.9 ($CH_2$), 30.1 ($CH_2$), 30.2 ($CH_2$), 30.3 ($CH_2$), 32.1 ($CH_2$), 44.3 (α-$CH_2$ Pal), 52.7 (α-CH Cys), 53.4 (α-CH Met), 54.8 (α-CH Ser), 63.0 (β-$CH_2$ Ser), 83.6 ($C(CH_3)_3$), 169.1 (C=O), 170.3 (C=O), 170.6 (C=O), 171.9 (C=O), 197.6 (C=O); HRMS (FAB, 3-NBA): $m/z$: calcd for $C_{33}H_{62}N_3O_7S_2$: 676.4030, found: 676.4008 $[M+H]^+$.

***N*-Acetyl-L-methionyl-L-seryl-*S*-palmitoyl-L-cysteine (84)**: TFA (10 mL) was added to a solution of Ac-Met-Ser-Cys(Pal)-O*t*Bu **83** (100 mg, 015 mmol) in $CH_2Cl_2$ (10 mL). After stirring at room temperature for 2 h, the solvent was removed under reduced pressure and the residue was coevaporated with toluene to yield **84** as a colorless solid (92 mg, quant.). M.p. 64 °C; $[\alpha]_D^{20} = -17.0$ ($c = 1.0$ in MeOH); $R_f = 0.04$ ($CH_2Cl_2$/EtOH 10:1); $^1$H NMR ($CD_3OD$, 400 MHz): $\delta = 0.88$ (t, $J = 6.6$ Hz, 3H, ω-$CH_3$ Pal), 1.20–1.40 (m, 24H, 12×$CH_2$ Pal), 1.65 (m, 2H, β-$CH_2$ Pal), 2.05 (s, 3H, $CH_3$ acetyl), 2.12 (s, 3H, $CH_3$ Met), 1.94–2.18 (m, 2H, β-$CH_2$ Met), 2.59 (m, 4H, α-$CH_2$ Pal, γ-$CH_2$ Met), 3.30 (dd, $J = 7.8$, 13.9 Hz, 1H, β-$CH_{2a}$ Cys), 3.48 (dd, $J = 5.3$, 13.9 Hz, 1H, β-$CH_{2b}$ Cys), 3.50 (dd, $J = 5.4$, 11.5 Hz, 1H, β-$CH_{2a}$ Ser), 4.03 (dd, $J = 3.9$, 11.7 Hz, 1H, β-$CH_{2b}$ Ser), 4.38 (m, 1H, α-CH Ser), 4.49 (m, 2H, α-CH Cys, α-CH Met); $^{13}$C NMR ($CD_3OD$, 100.6 MHz): $\delta = 14.3$ (ω-$CH_3$ Pal), 22.9 ($CH_3$ acetyl), 23.2 ($CH_3$ Met), 23.3 ($CH_2$), 28.1 ($CH_2$), 28.8 ($CH_2$), 28.4 ($CH_2$), 28.6 ($CH_2$), 28.9 ($CH_2$), 29.1 ($CH_2$), 29.3 ($CH_2$), 29.6 ($CH_2$), 29.7 ($CH_2$), 29.8 ($CH_2$), 29.9 ($CH_2$), 30.1 ($CH_2$), 30.2 ($CH_2$), 30.3 ($CH_2$), 32.1 ($CH_2$), 44.3 (α-$CH_2$ Pal), 52.7 (α-CH Cys), 53.4 (α-CH Met), 54.8 (α-CH Ser), 63.0 (β-$CH_2$ Ser), 169.1 (C=O), 170.3 (C=O), 170.6 (C=O), 174.9 (C=O), 197.6 (C=O); HRMS (FAB, 3-NBA): $m/z$: calcd for $C_{29}H_{53}N_3O_7S_2Na$: 642.3225, found: 642.3218 $[M+Na]^+$.

***N*-Acetyl-L-methionyl-L-seryl-*S*-palmitoyl-L-cysteyl-$N^{\varepsilon}$-(allyloxycarbonyl)-L-lysyl-*S*-farnesyl-L-cysteine methyl ester (85)**: At 0 °C, EDC (23 mg, 0.12 mmol) was added to a solution of Ac-Met-Ser-Cys(Pal)-OH **84** (60 mg, 0.10 mmol), freshly prepared H-Lys(Aloc)-Cys(Far)-OMe **19** (53 mg, 0.10 mmol) and HOBt (30 mg, 0.19 mmol) in $CH_2Cl_2$ (10 mL). The solution was warmed to room temperature and stirred for 16 h. Then the reaction mixture was diluted with ethyl acetate (30 mL) and subsequently washed with 1N HCl (10 mL) and $Na_2CO_3$ solution (10%, 10 mL). The organic layer was dried over $Na_2SO_4$, and, after removal of the solvent under reduced pressure, the residue was purified by chromatography ($CH_2Cl_2$/EtOH 20:1, then 10:1) to yield **85** as colorless solid (55 mg, 65%). M.p. 71 °C; $[\alpha]_D^{20} = -11.4$ ($c = 0.5$ in $CHCl_3$); $R_f = 0.31$ ($CH_2Cl_2$/EtOH 10:1); $^1$H NMR ($CDCl_3$/$CD_3OD$ 1:1, 400 MHz): $\delta = 0.78$ (t, $J = 6.6$ Hz, 3H, ω-$CH_3$ Pal), 1.15 (m, 24H, 12×$CH_2$ Pal), 1.49 (s, 6H, 2×$CH_3$ Far), 1.57 (s, 6H, 2×$CH_3$ Far), 1.26–1.68 (m, 8H, 2×$CH_2$ Lys, 2×$CH_2$ Pal), 1.93 (s, 3H, $CH_3$ acetyl), 2.00 (s, 3H, $CH_3$ Met), 1.76–2.05 (m, 12H, β-$CH_2$ Met, $CH_2$ Lys, 4×$CH_2$ Far), 2.46 (m, 2H, γ-$CH_2$ Met), 2.68–3.17 (m, 8H, α-$CH_2$ Pal, 2×β-$CH_2$ Cys, -$SCH_2$-), 3.26–3.46 (m, 2H, ε-$CH_2$ Lys), 3.64 (s, 3H, $OCH_3$), 3.68 (m, 1H, β-$CH_{2a}$ Ser), 3.87 (m, 1H, β-$CH_{2b}$ Ser), 4.25–4.54 (m, 7H, 5×α-CH, -O$CH_2$CH=$CH_2$), 5.00 (m, 2H, 2×CH Far), 5.08–5.22 (m, 3H, CH Far, -$OCH_2CH$=C$H_2$), 5.74 (m, 1H, -$OCH_2CH$=$CH_2$); $^{13}$C NMR ($CDCl_3$/$CD_3OD$ 1:1, 100.6 MHz): $\delta = 14.0$ (ω-$CH_3$ Pal), 16.5 ($CH_3$ acetyl), 16.8 ($CH_3$ Met), 17.0 ($CH_3$ Far), 17.2 ($CH_3$ Far), 19.3 ($CH_3$ Far), 19.3 ($CH_2$), 21.3 ($CH_2$), 24.0 ($CH_2$), 24.3 ($CH_2$), 25.3 ($CH_3$ Far), 28.6 ($CH_2$), 29.2 ($CH_2$), 29.3 ($CH_2$), 30.0 ($CH_2$), 30.0 ($CH_2$), 30.3 ($CH_2$), 30.3 ($CH_2$), 30.3 ($CH_2$), 30.3 ($CH_2$), 30.3 ($CH_2$), 30.3 ($CH_2$), 30.9 ($CH_2$), 31.8 ($CH_2$), 32.5 ($CH_2$), 33.8 ($CH_2$), 34.4 ($CH_2$), 34.5 ($CH_2$), 39.8 ($CH_2$ Far), 40.5 ($CH_2$ Far), 44.6 (ε-$CH_2$ Lys), 50.4 ($OCH_3$), 53.5 (α-CH), 54.2 (α-CH), 54.5 (α-$CH_2$ Pal), 56.0 (α-CH), 56.1 (α-CH), 57.1 (α-CH), 64.8 (β-$CH_2$ Ser), 67.3 (O$CH_2$CH=$CH_2$), 115.1 ($OCH_2CH$=$CH_2$), 117.1 (CH Far), 121.7 (CH Far), 122.5 (CH Far), 133.9 (C Far), 137.5 ($OCH_2CH$=$CH_2$), 138.8 (C Far), 139.3 (C Far), 157.5 (C=O), 170.9 (C=O), 172.0 (C=O), 174.7 (C=O), 175.4 (C=O), 197.6 (C=O); HRMS (FAB, 3-NBA): $m/z$: calcd for $C_{58}H_{100}N_6O_{11}S_3Na$: 1175.6512, found: 1175.6524 $[M+Na]^+$.

***N*-Acetyl-L-methionyl-L-seryl-*S*-palmitoyl-L-cysteyl-L-lysyl-*S*-farnesyl-L-cysteine methyl ester (86)**: $[Pd(PPh_3)_4]$ (7 mg, 0.01 mmol) was added to a degassed solution of pentapeptide **85** (50 mg, 0.04 mmol) and *N*,*N*′-dimethylbarbituric acid (7 mg, 0.04 mmol) in THF (6 mL). The mixture was stirred for 4 h at room temperature. After removal of the solvent under reduced pressure, the residue was purified by chromatography ($CH_2Cl_2$/EtOH 10:1, then 5:1, then 1:1 + 1% $NEt_3$) to yield **4** as a colorless solid (34 mg, 75%). M.p. 69 °C; $[\alpha]_D^{20} = -9.0$ ($c = 0.5$ in MeOH); $R_f = 0.01$ ($CH_2Cl_2$/EtOH 10:1); $^1$H NMR ($CD_3OD$, 400 MHz): $\delta = 0.78$ (t, $J = 6.6$ Hz, 3H, ω-$CH_3$ Pal), 1.15 (m, 24H, 12×$CH_2$ Pal), 1.49 (s, 6H, 2×$CH_3$ Far), 1.57 (s, 6H, 2×$CH_3$ Far), 1.26–1.68 (m, 8H, 2×$CH_2$ Lys, 2×$CH_2$ Pal), 1.93 (s, 3H, $CH_3$ acetyl), 2.00 (s, 3H, $CH_3$ Met), 1.76–2.05 (m, 12H, β-$CH_2$ Met, $CH_2$ Lys, 4×$CH_2$ Far), 2.46 (m, 2H, γ-$CH_2$ Met), 2.68–3.17 (m, 8H, α-$CH_2$ Pal, 2×β-$CH_2$ Cys, -$SCH_2$-), 3.26–3.46 (m, 2H, ε-$CH_2$ Lys), 3.64 (s, 3H, $OCH_3$), 3.68 (m, 1H, β-$CH_{2a}$ Ser), 3.87 (m, 1H, β-$CH_{2b}$ Ser), 4.25–4.54 (m, 5H, 5×α-CH), 5.00 (m, 2H, 2×CH Far), 5.14 (m, 1H, CH Far); $^{13}$C NMR ($CD_3OD$, 100.6 MHz): $\delta = 14.0$ (ω-$CH_3$ Pal), 16.5 ($CH_3$ acetyl), 16.8 ($CH_3$ Met), 17.0 ($CH_3$ Far), 17.2 ($CH_3$ Far), 19.3 ($CH_3$ Far), 19.3 ($CH_2$), 21.3 ($CH_2$), 24.0 ($CH_2$), 24.3 ($CH_2$), 25.3 ($CH_3$ Far), 28.6 ($CH_2$), 29.2 ($CH_2$), 29.3 ($CH_2$), 30.0 ($CH_2$), 30.0 ($CH_2$), 30.3 ($CH_2$), 30.3 ($CH_2$), 30.3 ($CH_2$), 30.3 ($CH_2$), 30.3 ($CH_2$), 30.3 ($CH_2$), 30.3 ($CH_2$), 30.9 ($CH_2$), 31.8 ($CH_2$), 32.5 ($CH_2$), 33.8 ($CH_2$), 34.4 ($CH_2$), 34.5 ($CH_2$), 39.8 ($CH_2$ Far), 40.5 ($CH_2$ Far), 44.6 (ε-$CH_2$ Lys), 50.4 ($OCH_3$), 53.5 (α-CH), 54.2 (α-CH), 54.5 (α-$CH_2$ Pal), 56.0 (α-CH), 56.1 (α-CH), 57.1 (α-CH), 64.8 (β-$CH_2$ Ser), 117.1 (CH Far), 121.7 (CH Far), 122.5 (CH Far), 133.9 (C Far), 138.8 (C Far), 139.3 (C Far), 170.9 (C=O), 172.0 (C=O), 174.7 (C=O), 175.4 (C=O), 197.6 (C=O); HRMS (FAB, 3-NBA): $m/z$: calcd for $C_{54}H_{97}N_6O_9S_3$: 1069.6480, found: 1069.6503 $[M+H]^+$.

**Assay for inhibition of APT1**: The assay for inhibition of APT1 is based on the free fatty acid indicator Acrylodan labeled Intestinal Fatty Acid Binding Protein (ADIFAB). Our system has been adapted from an assay described in the literature.[37,38]

**ADIFAB storage solution**: 200 μg ADIFAB (from Molecular Probes, Oregon) were dissolved in 1000 μL storage buffer (50 mM HEPES, 1 mM EDTA, 0.5 mM phenylmethylsulfonylfluoride, 0.05% $NaN_3$, pH 8.0).

**Substrate solution in sample buffer**: Ac-Met-Ser-Cys(Pal)-Lys-Cys(Far)-OMe (**86**; 4 mg, 3.74 μmol) was dissolved in DMSO (500 μL). 5 μL of this solution were added to 50 μL with sample buffer (20 mM HEPES, 150 mM NaCl, 5 mM KCl, 1 mM $Na_2HPO_4$, pH 7.4).

**Inhibitor solutions in sample buffer**: According to their molecular mass all inhibitors were treated like the above substrate leading to the desired concentrations of the inhibitor solutions.

**APT1 solution**: The applied APT1 solution was the result of the isolation of the enzyme from rat liver following a protocol of Duncan et al.[24] and consisted of 30 μg APT1 in 10 mL buffer (50 mM HEPES, 2 mM $MgCl_2$, 1 mM EDTA, 7.5 mM CHAPS, pH 8.0).

**Determination of the maximal palmitate concentration**: Substrate solution (2 μL) were pipetted into a quarz cuvette with sample buffer (1.5 mL) resulting a substrate concentration of 1 μM. Then, after excitation at 386 nm the fluorescence emissions at 432 and 505 nm were measured at 25 °C ($I_{505}^{blank}$, $I_{432}^{blank}$). Subsequently, ADIFAB solution (10 μL) was added, and again the fluorescence emissions at 432 and 505 nm were measured ($I_{505}^{0}$, $I_{432}^{0}$). The $R_0$ value was then calculated as follows:

$$R_0 = \frac{I_{505}^{0} - I_{505}^{blank}}{I_{432}^{0} - I_{432}^{blank}}$$

Now, APT1 solution (20 μL) was added, and, after incubation at 25 °C for 15 min, the fluorescence emissions at 432 and 505 nm were measured at least eight times and averaged ($I_{505}$, $I_{432}$). The $R$ value was then calculated as follows:

$$R = R_0 = \frac{I_{505} - I_{505}^{blank}}{I_{432} - I_{432}^{blank}}$$

The free palmitate concentration was calculated as follows:

$$[Pal] = K_d \times 19.5 \times \frac{R - R_0}{11.5 - R} + \frac{[ADIFAB]_{total} \times 19.5 \times (R - R_0)}{11.5 - R + 19.5 \times (R - R_0}$$

In this equation $K_d = 0.28$ and $[ADIFAB]_{total} = 100$ nM according to the literature.[37]

**Determination of the $IC_{50}$ values**: The free palmitate concentration when inhibitors are present, was measured with the method described above. The determination of the blank values ($I_{505}^{blank}$, $I_{432}^{blank}$) was measured after the appropriate amount of inhibitor was added. In order to calculate the $IC_{50}$ values, the relative palmitate concentration in percent (palmitate with inhibitor/palmitate without inhibitor) were used in a curve fitting with the program Origin.

## Acknowledgements

This research was supported by the Deutsche Forschungsgemeinschaft and the Landesgraduiertenförderung Baden-Württemberg.

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Received: September 12, 2005
Published online: March 10, 2006

  