Solid Phase Synthesis of Lipidated Peptides

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General. Unless otherwise noted, reagents and chemicals were obtained from Acros, Chimica, Advanced Chemtech, Aldrich, AppliChem, Avocado, Biosolve, Fluka, Novabiochem or Senn Chemicals and used without further purification. Dichloromethane and piperidine were refluxed under argon over CaH_2 , and freshly distilled prior to usage. ^1H and ^{13}C NMR data were recorded on a Varian Mercury 400 or a Bruker DRX 500 spectrometer at room temperature. NMR spectra were afterwards calibrated to the solvent signals of CDCl_3 (7.26 ppm and 77.16 ppm). ESI-MS was carried out using a Agilent 1100 series binary pump together with a reversed face HPLC column (Macherey-Nagel) and a Finnigan Thermoquest LCQ. FAB measurements were taken with a Jeol SX 102A using a 3-nitrobenzyl alcohol (3-NBA) matrix. Optical rotations were measured with a Perkin-Elmer Polarimeter 341. Flash chromatography was performed using Merck Silicalgel 60. TLC was performed with aluminium-backed silica gel 60 F₂₅₄ plates (Merck). MALDI was carried out with a Voyager-DE Pro BioSpectrometerTM from PerSeptive Biosystems using a 2,5-dihydroxybenzoic acid (DHB) matrix. Yield and scale of the solid phase reactions is given with respect to the amount of the first amino acid coupled onto the resin and its loading determined with the UV-Fmoc method. After cleavage from resin no major byproducts could be detected (example HPLC plots below). However, column chromatography was carried out to remove copper salts.

General Conditions for the Synthesis of Lipopeptides using the Phenylhydrazide Linker.

For all reactions except for product **4**, commercially available Fmoc-4-hydrazinobenzoyl NovaGelTM resin from Novabiochem was used. All reactions were carried out under an argon atmosphere in a 50 mL solid phase peptide synthesis (SPPS) reactor. Agitation was achieved by bubbling argon gas through the glass sinter or by using an orbital shaker. After liberation

of the thiol group of cysteine the following reaction was carried out without any delay. Loading of the resin was determined using the UV-Fmoc method. To this end, a small amount of dried resin (5 to 9 mg) was treated with freshly prepared piperidine/DMF 1:4 solution (18 mL) for 10 minutes and the UV absorption of the solution at 301 nm ($\epsilon = 7800 \text{ M}^{-1}\text{cm}^{-1}$) was determined.

Fmoc cleavage. Fmoc cleavage was achieved using a solution of 50 % piperidine in DMF for two times 7 minutes.

Peptide coupling. Unless stated otherwise all amino acid were coupled using HBTU/HOBt chemistry. Typically 4 eq of amino acid were reacted for 2-3 min with 3.6 eq of HBTU, 4 eq of HOBt and 8 eq of DIPEA in DMF. The solution was added to the resin and agitated for 2 h at room temperature. Cysteine derivatives were coupled using DIC/HOBt chemistry in order to avoid extensive racemisation. Typically 4 eq of amino acid were reacted with 4 eq of DIC and 6 eq of HOBt in DMF for 2-3 min and then added to the resin. Reaction times varied from 3 to 4 h at room temperature.

Trt deprotection from cysteine. The Trt group was cleaved using 3 % TES and 50 % TFA in dichloromethane for 1 h. The resin was washed several times with dichloromethane and then with DMF.

Trt deprotection from nitrogen / Mmt deprotection from cysteine. Cleavage was achieved using 1% TFA, 2% TES in dichloromethane. In case of deprotection from nitrogen the procedure was repeated two times. The resin was washed 6 times with dichloromethane and then with DMF.

SStBu deprotection from Cysteine. The SStBu group was cleaved using 100 eq of PBU_3 and 400 eq of H_2O in a 1:1 mixture of DMF and dichloromethane for 12 h. Typically 6.4 mL PBU_3 together with 2 mL H_2O were used in 40 mL solvent. The resin was washed 6 times with dichloromethane and then with DMF.

Farnesylation. The farnesyl group was introduced onto the freshly deprotected thiol using 5 eq Far-Br, 12 eq. DIPEA in DMF for 4 h. The resin was washed 6 times with DMF.

Palmitoylation. The palmitoyl group was introduced using 20 eq of Pal-Cl, 20 eq of HOBt and 22 eq of Et₃N in a 1:3 mixture of DMF and dichloromethane for 15 h. The resin was washed 6 times with dichloromethane and then with DMF.

Synthesis of Fmoc-Cys(Far)-OMe 4

In contrast to all other compounds Fmoc-Cys(Far)-OMe **4** was synthesized using Fmoc-4-hydrazinobenzoyl polystyrene resin. Fmoc-Cys(Trt)-OH was coupled to the resin using DIC/HOBt chemistry with additional 0.4 eq of triethylamine to give 131 mg cysteine bound resin with a loading of 0.42 mmol/g (0.055 mmol). The Trt group was cleaved and the thiol was farnesylated. The resin was treated with a solution of 5 mg (0.028 mmol) Cu(OAc)₂ and 23 μ L (0.28 mmol) pyridine in THF/methanol for 3 h under oxygen. The resin was filtered off, the solvent was evaporated under reduced pressure and the crude reaction mixture was dried *in vacuo*. Flash chromatography with ethyl acetate/hexane 1:4 as eluent furnished 15 mg (0.027 mmol, yield 49%) of **4**.

TLC: 0.35 (hexane/ethyl acetate 4:1); $[\alpha]_D^{22} = -48.3$ (c = 0.5, CHCl₃); **¹H-NMR** (500 MHz, CDCl₃; δ / ppm): 7.56 (d, ³J = 7.5 Hz, 2H, aromatic), 7.62-7.60 (m, 2H, aromatic), 7.40 (t, ³J = 7.5 Hz, 2H, aromatic), 7.32 (t, ³J = 7.4 Hz, 2H, aromatic), 5.59 (d, ³J = 8.1 Hz, 1H, NH), 5.21 (t, ³J = 7.5 Hz, 1H, C=CH-CH₂-S Far), 5.10-5.07 (m, 2H, 2 x C=CH Far), 4.62-4.58 (m, 1H, α -CH Cys), 4.42 (dd, ²J = 10.6 Hz, ³J = 7.0 Hz, 1H, CH_{2a} Fmoc), 4.38 (dd, ²J = 10.6 Hz, ³J = 7.0 Hz, 1H, CH_{2b} Fmoc), 4.24 (t, ³J = 7.0 Hz, 1H, CH Fmoc), 3.78 (s, 3H, OCH₃), 3.21 (dd, ²J = 13.0 Hz, ³J = 8.2 Hz, 1H, α -CH_{2a} Far), 3.16 (dd, ²J = 13.0 Hz, ³J = 7.5 Hz, 1H, α -CH_{2b} Far), 2.96 (dd, ²J = 14.0 Hz, ³J = 4.8 Hz, 1H, β -CH_{2a} Cys), 2.89 (dd, ²J = 14.0 Hz, ³J = 5.9 Hz, 1H, β -CH_{2b} Cys), 2.12-1.95 (m, 8H, CH₂ Far), 1.68 (s, 3H, CH₃ Far), 1.66 (s, 3H, CH₃ Far), 1.59 (s, 6H, CH₃ Far); **¹³C-NMR** (125 MHz, CDCl₃; δ / ppm): 171.4, 155.8, 143.9, 143.8, 141.33, 140.2, 135.4, 131.4, 127.8, 127.1, 125.2, 120.0, 124.34, 123.7, 119.6, 67.3, 53.7, 52.7, 47.1, 39.7, 39.7, 33.6, 30.1, 26.8, 26.44, 25.7, 17.7, 16.2, 16.1; **HR-FAB** (3-NBA): calc. for C₃₄H₄₄NO₄S [M+H] 561.2930, found 561.2961

Synthesis of Fmoc-Leu-Pro-Cys(Far)-OMe 5

Fmoc-Cys(Trt)-OH was coupled to deprotected Fmoc-4-hydrazinobenzoyl NovaGelTM resin **1** to give 119 mg cysteine bound resin with a loading of 0.35 mmol/g (0.042 mmol). After deprotection of Fmoc, Fmoc-Pro-OH was coupled. The Trt group was removed and the dipeptide farnesylated followed by deprotection and coupling of Fmoc-Leu-OH to give **3**. The resin was treated with a solution of 6.7 mg (0.037 mmol) Cu(OAc)₂, 119 μ L (1.47 mmol)

pyridine, 170 μ L (2.97 mmol) acetic acid and 374 μ L (9.2 mmol) MeOH in 15 mL dry dichloromethane for 2 h under oxygen. The resin was filtered off, the solvent was evaporated under reduced pressure and the crude reaction mixture was dried *in vacuo*. Flash chromatography with ethyl acetate/cyclohexane 1:1 as eluent furnished 15.8 mg (0.021 mmol, yield 50%) of **5**.

TLC: 0.5 (cyclohexane/ethyl acetate 1:1); **¹H-NMR** (400 MHz, CDCl₃: δ / ppm): 7.75 (d, 2H, ³J=7.6 Hz, Fmoc), 7.59 (m, 2H, Fmoc), 7.39 (t, 2H, ³J=7.4 Hz, Fmoc), 7.31 (t, 2H, ³J=7.4 Hz, Fmoc), 5.48 (d, ³J=9.0 Hz, 1H, NH, Urethan), 5.19, (t, ³J=7.6 Hz, 1H, C=CH-CH₂-S Far), 5.08 (m, 2H, 2 x C=CH Far), 4.70-4.65 (m, 1H, α -CH), 4.60-4.56 (m, 1H, α -CH), 4.37-4.34 (m, 2H, CH₂ Fmoc), 4.22-4.19 (m, 1H, CH Fmoc), 3.50-3.80 (m, 5H, OMe, CH₂ Pro), 3.18-3.08 (m, 2H, CH₂ Far), 2.94-2.97 (m, 1H, β -CH_{2a} Cys), 2.80-2.75 (m, 1H, β -CH_{2b} Cys), 2.20-1.10 (m, 27H, γ -CH Leu, β -CH₂ Leu, 4 x CH₂ Far, 2 x CH₂ Pro, 4 x CH₃ Far), 1.01-0.94 (m, 2 x δ -CH₃ Leu); **¹³C-NMR** (100 MHz, CDCl₃: δ / ppm): 173.2, 171.3, 171.0, 156.5, 144.1, 141.5, 140.2, 135.6, 131.5, 129.7, 127.9, 127.3, 125.4, 124.5, 124.0, 120.2, 119.8, 67.3, 60.0, 52.7, 52.0, 51.1, 47.4, 42.7, 42.4, 39.9, 33.5, 30.0, 27.4, 27.0, 26.7, 25.9, 25.2, 24.9, 23.7, 21.8, 17.9, 16.4, 16.2 **ESI-MS** + m/z: calc. for C₄₅H₆₂N₃O₆S ([M+H]) 772.4, found 772.3; **HR-FAB** (3-NBA): calc. for [M+H] 772.4359, found 772.4387

Synthesis of Aloc-Gly-Cys(Pal)-Met-Gly-Leu-Pro-Cys(Far)-OMe **8**

From 302 mg of the resin bound Fmoc-Cys(Trt)-OH (loading 0.35 mmol/g, 0.106 mol) resin bound tripeptide was synthesized as described above. Fmoc-group was deprotected followed by subsequent HBTU/HOBt mediated coupling with Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-Cys(Mmt)-OH and Aloc-Gly-OH. The Mmt group was removed and the thiol palmitoylated. The resin was treated with a solution of 20 mg (0.11 mmol) Cu(OAc)₂, 280 μ L (3.47 mmol) pyridine, 400 μ L (6.99 mmol) acetic acid and 882 μ L (21.7 mmol) MeOH in 20 mL dry dichloromethane for 2 h under oxygen. The resin was filtered off, the solvent was evaporated under reduced pressure and the crude reaction mixture was dried *in vacuo*. Flash chromatography with dichloromethane/methanol 20:1 furnished 53.3 mg (0.044 mmol, yield 42%) of **8**.

TLC: 0.2 (dichloromethane/MeOH 20:1); $[\alpha]_D^{22}$ = - 52.8 (c= 0.74, CHCl₃); **¹H-NMR** (500 MHz, CDCl₃: δ / ppm): 7.96 (s, 1H, NH), 7.60 (s, 1H, NH), 7.51 (s, 1H, NH), 7.34 (s, 1H, NH), 7.05 (s 1H, NH), 5.88-5.84 (s, 1H, CH=CH₂ Aloc), 5.31 (d, 1H, ³J=17.4 Hz, CH=CH_{2a} Aloc), 5.21 (d, 1H, ³J=10.4 Hz, CH=CH₂ Aloc), 5.19-5.16 (m, 1H C=CH-CH₂-S Far), 5.08

(m, 2H, 2 x C=CH Far), 4.8-4.36 (m, 11H, 5 x α -CH, O-CH₂ Allyl, 2 x CH₂ Gly), 3.5-4.0 (m, 7H, CH₂ Pro, OMe, β -CH₂ Cys), 3.36-2.76 (m, 4H, CH₂, Far, β -CH₂ Cys), 2.54 (m, 4H, γ -CH₂ Met, α -CH₂ Pal), 2.3-1.8 (m, 17H, 4 x CH₂ Far, 2 x CH₂ Pro, γ -CH₂ Met, SCH₃), 1.70-1.30 (m, 17H, γ -CH Leu, β -CH Leu, 4 x CH₃, Far, β -CH₂ Pal), 0.90-1.20 (m, 24H, 12 x CH₂ Pal), 0.88-0.70 (m, 9H, ω -CH₃ Pal, 2 x δ -CH₃ Leu); **¹³C-NMR** (100 MHz, CDCl₃: δ / ppm): 200.5, 173.2, 172.6, 171.6, 171.2, 170.5, 169.6, 196.0, 157.4, 140.3, 135.6, 132.8, 131.5, 124.5, 123.9, 119.8, 118.1, 66.3, 60.1, 53.9, 53.0, 52.7, 52.0, 49.3, 47.6, 45.0, 44.3, 43.4, 42.0, 41.2, 33.3-24.0 (several signals), 17.9, 16.4, 16.2, 16, 14.3; **ESI-MS** + m/z: calc for C₆₂H₁₀₆N₇O₁₁S₃ [M+H] 1220.7, found 1220.5; **MALDI** (DHB) calc. for [M+Na] 1242.7, [M+K] 1258.8, found 1243.1, 1259.0

Synthesis of NBDaca-Ser(Trt)-Cys(SStBu)-Lys(Aloc)-Cys(Far)-OMe 11

Fmoc-Cys(Trt) resin with a loading of 0.38 mmol/g (269 mg, 0.102 mmol) was deprotected and coupled with Fmoc-Lys(Aloc)-OH. The Trt group was removed and the liberated thiol group was farnesylated. Subsequent Fmoc-Cys(SStBu)-OH, Fmoc-Ser(Trt)-OH and NBDaca were coupled. The resin was treated with a solution of 7.8 mg (0.043 mmol) Cu(OAc)₂, 140 μ L (1.74 mmol) pyridine and 441 μ L (10.9 mmol) MeOH in 20 mL dry dichloromethane for 80 min under oxygen. The resin was filtered off and the crude mixture was directly subjected to flash chromatography with ethyl acetate/cyclohexane 1:5 and 2 % Et₃N as eluent to give 64.5 mg (0.048 mmol, yield 47%) of **11**.

TLC: 0.5 (dichloromethane/MeOH 20:1); [α]_D²² = -55.1 (c= 0.118, CHCl₃); **¹H-NMR** (400 MHz, CDCl₃: δ / ppm): 8.36 (d, ³J=8.6 Hz, 1H, CH NBD), 7.45-7.06 (m, 19H, Trt, 4 x NH), 6.28 (m, 1H, CH NBD), 6.07 (s, 1H, NH), 5.79 (m, 1H, CH=CH₂ Aloc), 5.34 (s, 1H, NH), 5.19-5.00 (m, 7H, CH=CH₂ Aloc, C=CH-CH₂-S, 2 x C=CH Far, 2 x α -CH), 4.72-4.59 (m, 2H, α -CH), 4.45-4.35 (m, 4H, α -CH, O-CH₂ Alloc), 3.80-3.55 (m, 4H, OMe, β -CH_{2a} Ser), 3.4-3.6 (m, 2H, α -CH₂ Aca), 3.35-3.04 (m, 7H, 1H, β -CH_{2b} Ser, α -CH₂ Far, β -CH₂ Cys, ϵ -CH₂ Lys), 2.85-2.84 (m, 1H, β -CH₂ Cys), 2.76-2.71 (m, 1H, β -CH₂ Cys), 2.18-2.16 (m, 2H, ϵ -CH₂ Aca), 1.99-1.86 (m, 8H, 4 x CH₂ Far), 1.71-1.30 (m, 24H, β -CH₂ Lys, γ -CH₂ Lys, δ -CH₂ Lys, 4 x CH₃ Far, β -CH₂ Aca, γ -CH₂ Aca, δ -CH₂ Aca), 1.21 (s, 9H, SSStBu); **¹³C-NMR** (100 MHz, CDCl₃: δ / ppm): 174.0, 171.5, 171.4, 170.6, 170.0, 156.8, 144.5, 144.3, 143.6, 143.4, 140.2, 136.8, 135.6, 133.2, 131.5, 128.7, 128.4, 127.7, 124.5, 123.9, 119.8, 117.6, 98.7, 87.7, 65.7, 63.0, 54.9, 54.9, 53.9, 52.8, 52.1, 49.1, 41.6, 40.7, 39.9, 36.0, 33.1, 31.2, 30.0,

29.8, 29.6, 28.2, 27.0, 26.7, 26.6, 25.9, 24.9, 24.8, 23.0, 17.9, 16.4, 16.2; **ESI-MS** calc. for $C_{70}H_{92}N_9O_{12}S_3$ [M-H] 1346.6, [M+Cl] 1382.6 found [M-H] 1346.5, [M+Cl] 1382.5

Synthesis of MantAca-Met-Gly-Leu-Pro-Cys(Far)-OMe 12

Fmoc-Cys(Trt) resin with a loading of 0.38 mmol/g (517 mg, 0.196 mmol) was deprotected and coupled with Fmoc-Pro-OH. The Trt group was removed and the liberated thiol group was farnesylated. Subsequently Fmoc-Leu-OH, Fmoc-Gly, Fmoc-Met-OH and MantAca were coupled using standard HBTU/HOBt chemistry. The resin was treated with a solution of 7.8 mg (0.043 mmol) $Cu(OAc)_2$, 140 μ L (1.74 mmol) pyridine and 441 μ L (10.9 mmol) MeOH in 20 mL dry dichloromethane for 2 h under oxygen. The resin was filtered off, the solvent was evaporated under reduced pressure and the crude reaction mixture was dried *in vacuo*. Flash chromatography with dichloromethane/methanol 20:1 furnished 95 mg (0.097 mmol, yield 49 %) of **12**.

TLC: 0.3 (dichloromethane/MeOH 20:1); $[\alpha]_D^{22} = -37.6$ (c= 2.5, $CHCl_3$); **1H -NMR** (400 MHz, $CDCl_3$: δ / ppm): 7.81 (m, 1H, NH), 7.53 (m, 1H, NH), 7.43-7.39 (m, 2H, NH), 7.34 (d, $^3J=7.8$ Hz, 1H, Aromat), 7.22 (t, $^3J=7.8$ Hz, 1H, Aromat), 6.87-6.85 (m, 1H, NH), 6.73 (m, 1H, NH), 6.59 (d, $^3J=8.4$ Hz, 1H, Aromat), 6.50 (t, $^3J=7.0$ Hz, 1H, Aromat), 5.12 (t, $^3J=7.4$ Hz, 1H, CH=C, Far), 5.06-5.03 (m, 2H, CH=C, Far), 4.79-4.76 (m, 1H, α -CH), 4.65-4.58 (m, 4H, 4 x α -CH), 4.00 (m, 2H, α -CH, Gly), 3.76-3.73 (m, 1H, CH_{2a} , Pro), 3.67 (s, 3H, OMe), 3.61-3.57 (m, 1H, CH_{2b} , Pro), 3.33-3.29 (m, 2H, α -CH₂, Aca), 3.16-3.11 (m, 1H, α -CH_{2a}, Far), 3.04-2.98 (m, 1H, α -CH_{2b}, Far), 2.85-2.89 (m, 1H, β -CH_{2a}, Cys), 2.79 (d, $^3J=4.9$ Hz, 3H, NCH₃), 2.67-2.64 (m, 3H, β -CH_{2b}, Cys, γ -CH₂, Met), 2.48-2.45 (m, 2H, ϵ -CH₂, Aca), 2.20-2.16 (m, 3H, CH₂, Pro, CH_{2a}, Pro), 2.10-1.90 (m, 15H, β -CH₂, Met, CH₃-S, Met, γ -CH, Leu, 4 x CH₂, Far, CH_{2b}, Pro), 1.63-1.30 (m, 20H, β -CH₂, Aca, γ -CH₂, Aca, δ -CH₂, Aca, 4 x CH₃, Far, β -CH₂, Leu), 0.90-0.87 (m, 6H, 2 x CH₃, Leu); **^{13}C -NMR** (100 MHz, $CDCl_3$: δ / ppm): 173.5, 172.4, 172.0, 171.5, 171.3, 170.1, 168.6, 150.6, 140.1, 135.5, 132.7, 131.4, 127.6, 124.4, 123.8, 119.7, 115.6, 114.5, 111.0, 60.0, 52.5, 52.3, 52.1, 49.1, 47.5, 43.1, 41.7, 39.8, 39.5, 36.1, 32.9, 31.8, 30.1, 29.8, 29.7, 29.2, 28.4, 26.8, 26.6, 26.5, 25.8, 25.2, 24.9, 24.7, 23.4, 22.0, 17.8, 16.2, 16.1, 15.4; **ESI-MS** + m/z : calc. for $C_{51}H_{82}N_7O_8S_2$ [M+H] 984.6, found 984.6; **HR-FAB** (3-NBA): calc. for [M+H] 984.5666, found 984.5728

Synthesis of MantAca-Met-Gly-Leu-Pro-Cys(Far)-OH **13**

Synthesis was carried out as described above for compound **12** but 239 mg of the Fmoc-Cys(Trt)-resin with a loading of 0.39 mmol/g (0.093 mmol) were used and the MantAca was coupled using DIC/HOBt chemistry. The resin was treated with a solution of 10 mg (0.055 mmol) Cu(OAc)₂, 280 μ L (3.47 mmol) pyridine and 400 μ L (6.99 mmol) acetic acid in 10 mL THF containing 350 μ L (19.4 mmol) H₂O for 2 h under oxygen. The resin was filtered off, the solvent was evaporated under reduced pressure and the crude reaction mixture was dried *in vacuo*. A solution of the crude product in dichlormethane was absorbed in 200 mg trisaminoethyl HL resin (novabiochem) and shaken for 2h. The resin was washed with dichlormethane /H₂O then the product was carefully eluted with THF/H₂O (20:1) and followed by THF/H₂O/AcOH (20:1:0.5) and dried *in vacuo* to give 28 mg (0.029 mmol, yield 31%) of **13**.

$[\alpha]_D^{22} = -31.5$ (c = 1.3, CHCl₃); **¹H-NMR** (400 MHz, CDCl₃: δ / ppm): 7.38 (d, ³J=7.8 Hz, 1H, Aromat), 7.29-7.26 (m, 1H, Aromat), 7.06 (m, 1H, NH), 6.75 (m, 1H, NH), 6.62 (d, ³J=8.4 Hz, 1H, Aromat), 6.54 (t, ³J=7.2 Hz, 1H, Aromat), 5.15 (m, 1H, CH=C, Far), 5.07 (m, 2H, CH=C, Far), 4.76-4.31 (m_{br}, 5H, α -CH), 4.0-4.38 (m_{br}, 2H, 4 x α -CH, Gly), 3.72 (m, 1H, CH_{2a}, Pro), 3.56 (m, 1H, CH_{2b}, Pro), 3.33 (m, 2H, α -CH₂, Aca), 3.31-2.91 (m_{br}, 4H, α -CH₂, Far, β -CH₂, Cys), 2.86-2.66 (m_{br}, 5H, NCH₃, γ -CH₂, Met), 2.50 (m, 2H, ϵ -CH₂, Aca), 2.26-1.86 (m, 15H, 2 x CH₂, Pro, CH₃-S, Met, 4 x CH₂, Far), 1.66-1.57 (m, 21H, β -CH₂, Aca, γ -CH₂, Aca, δ -CH₂, Aca, 4 x CH₃, Far, β -CH₂, Leu, γ -CH, Leu), 0.87 (m, 6H, 2 x CH₃, Leu); **ESI-MS** + m/z : calc. for C₅₀H₈₀N₇O₈S₂ [M+H] 970.5, found 970.5; - m/z : calc. for C₅₀H₇₈N₇O₈S₂ [M-H] 968.5, found 968.5; **HR-FAB** (3-NBA): calc. for [M+Na] 992.5329, found 992.5376.

Synthesis of MantAca-Gly-Cys(Pal)-Lys(Aloc)-Cys(Far)-OMe **16**

Fmoc-Cys(Trt) resin with a loading of 0.43 mmol/g (249 mg, 0.107 mmol) was deprotected and coupled with Fmoc-Lys(Aloc)-OH. The Trt group was removed and the liberated thiol group was farnesylated. Subsequently Fmoc-Cys(SStBu)-OH and and Trt-Gly were coupled using standard HBTU/HOBt chemistry. The StBu group was cleaved and the liberated thiol palmitoylated. The Trt group on glycine was cleaved and MantAca-OH was coupled using 111 mg (0.42 mmol) MantAca-OH, 61.9 μ L (0.40 mmol) DIC, 97 mg (0.64 mmol) HOBt and 15 μ L (0.11 mmol) Et₃N for 4 h. The resin was treated with a solution of 18 mg (0.10 mmol) Cu(OAc)₂, 280 μ L (3.47 mmol) pyridine, 400 μ L (6.99 mmol) acetic acid and 882 μ L (21.7

mmol) MeOH in 20 mL dry dichlormethane for 2 h under oxygen. The resin was filtered off, the solvent was evaporated under reduced pressure and the crude reaction mixture was dried *in vacuo*. Flash chromatography with dichlormethane/methanol 30:1 furnished 36.7 mg (0.031 mmol, yield 29 %) of **16**.

TLC: 0.3 (dichlormethane/MeOH 20:1); $[\alpha]_D^{22} = -19.3$ (c= 1.6, CHCl₃); **¹H-NMR** (400 MHz, CDCl₃: δ / ppm): 7.35 (d, ³J=6.6 Hz, 1 H, Aromat), 7.28 (d, ³J=8.6 Hz, 1 H, Aromat), 6.62 (m, 1H, Aromat), 6.56-6.52 (m, 1H, Aromat), 5.88-5.81 (m, 1H, CH=CH₂, Aloc), 5.25-5.07 (m, 5H, CH=CH₂, Allyl, C=CH-CH₂-S, 2 x C=CH Far), 4.80-4.39 (m, 5H, 3 x α-CH, OCH₂, Allyl), 4.08-3.96 (m, 2H, α-Gly), 3.72 (s, 3H, OMe), 3.37-3.09 (m, 8H, β-CH₂, Cys, ε-CH₂, Lys, α-CH₂, Far, α-CH₂, Aca), 2.92-2.80 (m, 2H, β-CH₂, Cys), 2.82 (s, CH₃, Mant), 2.54 (t, ³J=7.1 Hz, 2H, α-CH₂, Pal), 2.31-2.27 (m, CH₂, ε-CH₂, Aca), 2.07-1.95 (m, 8H, 4 x CH₂ Far), 1.90-1.37 (m, 24H, β-CH₂, Aca, γ-CH₂, Aca, δ-CH₂, Aca, 4 x CH₃ Far, β-CH₂, Lys, δ-CH₂, Lys, γ-CH₂, Lys), 1.23 (s, 24 H, Pal), 0.87 (t, ³J=6.6 Hz, 3H, CH₃-Pal); **¹³C-NMR** (100 MHz, CDCl₃: δ / ppm): 200.4, 174.2, 171.8, 171.4 170.2, 169.8, 169.6, 156.7, 150.7, 140.3, 135.6, 133.3, 132.8, 131.5, 127.6, 124.7, 123.9, 119.7, 117.6, 115.7, 114.6, 111.2, 65.6, 53.2, 52.7, 52.5, 44.3, 43.9, 40.8, 39.9, 39.6, 35.9, 32.8-22.5 (several signals), 17.9, 16.4, 16.2, 14.3; **ESI-MS** + m/z : calc. for C₆₄H₁₀₆N₇O₁₀S₂ [M+H] 1196.7; found [M+H] 1196.7.

Synthesis of MIC-Gly-Cys(Pal)-Lys(Aloc)-Cys(Far)-OMe **17**

The synthesis was carried out as described above for compound **16** but 128 mg (0.055 mmol) of resin-bound Fmoc-Cys(Trt) were used. After cleavage of the Trt group MIC-OH was coupled using 73.9 mg (0.35 mmol) MIC-OH, 31 μL (0.20 mmol) DIC, 46 mg (0.30 mmol) HOBt and 34 μL (0.20 mmol) Et₃N for 3 h. The resin was treated with a solution of 19 mg (0.10 mmol) Cu(OAc)₂, 140 μL (1.74 mmol) pyridine, 200 μL (3.50 mmol) acetic acid and 441 μL (10.9 mmol) MeOH in 20 mL dry dichlormethane for 2 h under oxygen. The resin was filtered off, the solvent was evaporated under reduced pressure and the crude reaction mixture was dried *in vacuo*. Flash chromatography with dichlormethane/methanol 20:1 furnished 13.9 mg (0.012 mmol, yield 22 %) of **17**.

TLC: 0.3 (dichlormethane/MeOH 20:1); $[\alpha]_D^{22} = -12.2$ (c= 0.85, CHCl₃); **¹H-NMR** (400 MHz, CDCl₃: δ / ppm): 7.40 (s, 1H, NH), 7.18 (s, 1H, NH), 6.67 (s, 2H, CH=CH, MIC), 6.52 (s, 1H, NH), 5.90 (m, 1H, CH=CH₂, Aloc), 5.29-5.08 (m, 5H, CH=CH₂, C=CH-CH₂-S, 2 x C=CH Far), 4.68-4.52 (m, 5H, 3 x α-CH, OCH₂, Allyl), 4.00-3.85 (m, 2 H, α-Gly), 3.73 (s, 3H, OMe), 3.52-3.48 (m, 2H, NCH₂, MIC), 3.27-3.09 (m, 6H, β-CH₂, Cys, ε-CH₂, Lys, α-CH₂,

Far), 2.95-2.78 (m, 2H, β -CH₂, Cys), 2.57 (t, $^3J=6.0$ Hz, 2H, α -CH₂, Pal), 2.28-2.24 (m, 2H, MIC), 2.1- 1.90 (m, 8H, 4 x CH₂ Far), 1.8-1.1 (m, 48H, β -CH₂, Aca, γ -CH₂, Aca, δ -CH₂, Aca, β -CH₂, Lys, δ -CH₂, Lys, γ -CH₂, Lys, 4 x CH₃ Far, 24 H, Pal), 0.87-0.85 (m, 3H CH₃-Pal). **ESI-MS** + m/z : calc. for C₆₀H₉₉N₆O₁₁S₂ [M+H] 1143.7; found 1143.6; **MALDI** (DHB): calc. for [M+Na] 1165.7, found 1165.9.

HPLC chromatograms of compounds 11 and 17.

HPLC method: RP- C4-column, 25 °C, A= H₂O + 0.1 % formic acid; B= acetonitrile + 0.1 % formic acid; time 0 min, 50 % B; time 30 min, 100 % B; time 40 min, 100 %B

Compound **11**: detection at 210 nm, the detected peak showed the correct ESI-mass.

Compound **17**: detection at 210 nm. The detected peak showed the correct ESI-mass.