

Supporting Information

for

Synthesis and Application of Fluorescent Ras-Proteins for Live Cell Imaging

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General procedures: ^1H and ^{13}C spectra were recorded on Bruker AC-250, Bruker AM 400, Varian Mercury 400 and Bruker DRX-500 spectrometers. The signal of the residual protonated solvent (CDCl_3 or CD_3OD) was taken as reference (^1H : $\delta = 7.24$ (CHCl_3) or 3.31 (CH_3OH), ^{13}C : $\delta = 77.0$ (CHCl_3) or 49.0 (CH_3OH)). EI and FAB mass spectra were measured on a Finnigan MAT MS 70 workstation (FAB: 3-nitrobenzyl alcohol (NBA) as matrix). ESI and HPLC/ESI mass spectra were measured on a HPLC/ESI-MS system with a Finnigan Thermoquest LCG spectrometer and a Hewlett Packard (Agilent, 1100 series) HPLC. CC 250/4 NUCLEOSIL 120-5 C4 RP-analytical columns were purchased from Macherey-Nagel. The following elution and detection conditions were used: 1 mL/min eluent gradient ($\text{CH}_3\text{CN}/\text{H}_2\text{O}/\text{HCO}_2\text{H}$): 19.95/79.95/0.1 to 89.85/9.95/0.1 in 40 min, detection: 210, 310, 468, 515 nm. Specific rotations were measured with a Perkin-Elmer polarimeter 241.

Materials: Analytical chromatography was performed on E. Merck silica gel 60F₂₅₄ coated plates. Flash chromatography was performed on Baker silica gel (40-65 μm). Size-exclusion chromatography was performed on Pharmacia Sephadex LH20. All solvents were distilled using standard procedures. Commercial reagents were used without further purification. All peptide synthesis reactions were performed under argon. Several compounds were prepared according to literature methods: 2-(3,7-dimethyl-octa-2,6-dienyloxy)-tetrahydropyrane (Ger-OTHP),^[S1] 2-(3,7,11-trimethyl-dodeca-2,6,10-trienyloxy)-tetrahydropyrane (Far-OTHP),^[S1] 2,6-dimethyl-8-(tetrahydropyrane-2-yloxy)octa-2,6-dien-1-ol (HO-Ger-OTHP) (**1a**),^[8d,16] 2,6,10-trimethyl-12-(tetrahydropyrane-2-yloxy)dodeca-

2,6,10-trien-1-ol (HO-Far-OTHP) (**1b**),^[8d,16,17] 2-[2,6-dimethyl-8-(tetrahydropyran-2-yloxy)octa-2,6-dienyl]-isoindol-1,3-dion (Pht-Ger-OTHP) (**2a**),^[8d] 2,6-dimethyl-8-(tetrahydropyran-2-yloxy)octa-2,6-dienylamine (H₂N-Ger-OTHP) (**3a**),^[8d] MIC-Gly-Cys(S*t*Bu)-Met-Gly-Leu-Pro-OH (**8**),^[6c,d] *N*-fluorenylmethoxycarbonyl-L-cysteine, *N*-fluorenylmethoxycarbonylethanediamine trifluoro-acetate^[S2,S3] and S-Hexadecyl-L-cysteine-*tert*-butylester^[S4].

2-[2,6,10-Trimethyl-12-(tetrahydropyran-2-yloxy)-dodeca-2,6,10-trienyl]-isoindol-1,3-dione (Pht-Far-OTHP) (2b): To a solution of phthalimide (204 mg, 1.39 mmol), triphenylphosphine (364 mg, 1.39 mmol), and HO-Far-OTHP **1b** (448 mg, 1.39 mmol) in THF (1 mL) was added DEAD (242 mg, 1.39 mmol). The reaction mixture was left stirring for 12 hours and the solvent was removed *in vacuo*. The residue was taken up in diethyl ether (50 mL), filtered and the solvent was removed *in vacuo*. Purification of the resulting oil by flash chromatography using *n*-hexane/ethyl acetate (6:1) as eluent, obtained 497 mg (1.10 mmol, 79%) of the desired product **2b** as a colorless oil. *R*_f 0.30 (*n*-hexane/ethyl acetate (3:1); ¹H NMR (400 MHz, CDCl₃): δ = 1.55 (s, 3H, CH₃ Far); 1.63 (s, 3H, CH₃ Far); 1.64 (s, 3H, CH₃ Far); 1.49-1.70 (m, 6H, CH₂ THP); 1.94-2.15 (m, 4H, CH₂ Far); 3.48-3.50 (m, 1H, OCH(R)-O-CH_{2a}-CH₂); 3.85-3.89 (m, 1H, OCH(R)-O-CH_{2b}-CH₂); 3.90-4.08 (m, 1H, CH_{2a}-O-CH(R)-O-CH₂); 4.17 (s, 2H, R₂N-CH₂); 4.10-4.30 (m, 1H, CH_{2b}-O-CH(R)-O-CH₂); 4.61 (t, *J* = 3.5 Hz, 1H, O-CH(R)-O); 5.03-5.08 (m, 1H, CH Far); 5.32-5.34 (m, 2H, CH Far); 7.70 (m, 2H, CH arom); 7.83 (m, 2H, CH arom); ¹³C NMR (100.6 MHz, CDCl₃): δ = 14.6 (CH₃ Far); 16.0 (CH₃ Far); 16.4 (CH₃ Far); 19.6 (CH₂ THP); 25.5, 26.2, 26.4 (2*CH₂ Far, CH₂ THP); 30.7 (CH₂ THP); 39.1 (CH₂ Far); 39.5 (CH₂ Far); 44.9 (R₂N-CH₂); 62.2-63.6 (CH₂ Far, CH₂ THP); 97.7 (CH THP); 120.5 (CH Far); 123.2 (CH Far); 124.2 (CH Far); 127.4 (CH arom); 129.1 (Cq arom); 132.1 (Cq Far); 133.9 (CH arom); 134.7 (Cq Far); 140.2 (Cq Far); 168.2 (C=O); MS (FAB, 3-NBA): m/z: calcd for [M+Na]⁺: 474.2723; found: 474.2682; C₂₈H₃₇NO₄ (451.6).

2,6,10-Trimethyl-12-(tetrahydropyran-2-yloxy)-dodeca-2,6,10-trienylamine (H₂N-Far-OTHP) (3b): To a solution Pht-Far-OTHP **2b** (3.84 g, 10 mmol) in ethanol (30 mL) was added hydrazine (3.00 g, 60 mmol). The reaction mixture was left stirring for 12 hours, filtered and the solvent was removed *in vacuo*. Purification of the resulting oil by flash chromatography using ethyl acetate/triethylamine (99:1) as eluent obtained 3.05 g (9.50 mmol, 95%) of the desired product **3b** as a yellowish oil. *R*_f 0.24 (ethyl acetate/triethylamine (99:1)); ¹H NMR (250 MHz, CDCl₃): δ = 1.61 (s, 3H, CH₃ Far); 1.64

(s, 3H, CH₃ Far); 1.68 (s, 3H, CH₃ Far); 1.40-1.90 (m, 6H, CH₂ THP); 2.07 (m, 8H, CH₂ Far); 3.16 (s, 2H, H₂N-CH₂); 3.52 (m, 1H, O-CH(R)-O-CH_{2a}-CH₂); 3.90 (m, 1H, O-CH(R)-O-CH_{2b}-CH₂); 4.03 (dd, J = 11.8 Hz, J = 7.4 Hz, 1H, CH_{2a}-O-CH(R)-O-CH₂); 4.24 (dd, J = 11.8 Hz, J = 6.4 Hz, 1H, CH_{2b}-O-CH(R)-O-CH₂); 4.63 (t, J = 3.3 Hz, 1H, O-CH(R)-O); 5.12 (t, J = 6.2, 1H, CH Far); 5.26 (t, J = 6.8, 1H, CH Far); 5.36 (t, J = 6.9, 1H, CH Far); ¹³C NMR (100.6 MHz, CDCl₃): δ = 14.4 (CH₃ Far); 15.9 (CH₃ Far); 16.3 (CH₃ Far); 19.5 (CH₂ THP); 25.4, 26.1, 26.2 (2*CH₂ Far, CH₂ THP); 30.6 (CH₂ THP); 39.4 (CH₂ Far); 39.5 (CH₂ Far); 44.9 (H₂N-CH₂); 62.1-63.5 (CH₂ Far, CH₂ THP); 97.6 (CH THP); 120.5 (CH Far); 123.4 (CH Far); 123.9 (CH Far); 135.1 (Cq Far); 137.2 (Cq Far); 140.2 (Cq Far); MS (FAB, 3-NBA): m/z: calcd for [M+H]⁺: 322.2688; found: 322.2725.; C₂₀H₃₅NO₂ (321.5).

7-Nitrobenzo[1,2,5]oxadiazol-4-yl-1-[2,6,10-trimethyl-12-(tetrahydro-pyran-2-yloxy)-dodeca-2,6,10-trienyl]-amine (NBD-NH-Far-OTHP) (4b): Compound **4b** was prepared using H₂N-Far-OTHP **3b** (322 mg, 1.00 mmol), by means of the procedure described for the synthesis of **4a**. Purification of the resulting oil by flash chromatography using methylene chloride as eluent obtained 315 mg (0.65 mmol, 65%) of the desired product **4b** as a reddish brown oil. R_f 0.10 (methylene chloride); ¹H NMR (250 MHz, CDCl₃): δ = 1.54 (s, 3H, C(CH₃)-CH-CH₂-OR); 1.61 (s, 3H, C(CH₃)-CH-(CH₂)₂-C(CH₃)-CH-CH₂-OR); 1.69 (s, 3H, NH-CH₂-C(CH₃)); 1.46-1.81 (6H, m, CH₂ THP); 1.96-2.20 (m, 8H, CH₂ Far); 3.47 (m, 1H, OCH(R)-O-CH_{2a}-CH₂); 3.86 (m, 1H, OCH(R)-O-CH_{2b}-CH₂); 3.98 (dd, J = 11.9 Hz, J = 4.8 Hz, 1H, CH_{2a}-O-CH(R)-O-CH₂); 4.02 (d, J = 6.0 Hz, 2H, NH-CH₂); 4.19 (dd, J = 11.9 Hz, J = 6.5 Hz, 1H, CH_{2b}-O-CH(R)-O-CH₂); 4.58 (dd, J = 2.2 Hz, J = 2.2 Hz, 1H, O-CH(R)-O), 5.04 (tq, J = 6.4 Hz, J = 0.9 Hz, 1H, CH-(CH₂)₂-C(CH₃)-CH-CH₂-O); 5.29 (ddq, J = 6.5 Hz, J = 4.8 Hz, J = 1.0 Hz, 1H, CH-CH₂-O); 5.45 (tq, J = 6.5 Hz, J = 1.0 Hz, 1H, NH-CH₂-C(CH₃)-CH); 6.16 (d, J = 8.7 Hz, 1H, CH arom); 6.81 (t, J = 6.0, 1H, NH); 8.42 (d, J = 8.7 Hz, 1H, CH arom); ¹³C NMR (100.6 MHz, CDCl₃): δ = 14.5 (CH₃ Far); 15.9 (CH₃ Far); 16.3 (CH₃ Far); 19.6 (CH₂ THP); 25.4, 26.1, 26.2 (2*CH₂ Far, CH₂ THP); 30.6 (CH₂ THP); 38.9 (CH₂ Far); 39.4 (CH₂ Far); 51.4 (H₂N-CH₂); 62.3-63.6 (CH₂ Far, CH₂ THP); 97.8 (CH THP); 98.5 (CH arom); 120.6 (CH Far); 123.5 (CH Far); 124.4 (CH Far); 128.9 (Cq Far); 136.5 (CH arom); 128.6, 134.4, 139.9, 143.8, 144.2 (3*Cq Far, 4*Cq arom); MS (EI): m/z: calcd for [M+H]⁺: 484.2686; found: 484.2707; C₂₆H₃₆N₄O₅ (484.6).

Dansyl[2,6,10-trimethyl-12-(tetrahydro-pyran-2-yloxy)-dodeca-2,6,10-trienyl]-amide (Dansyl-NH-Far-OTHP) (4c): To a solution of H₂N-Far-OTHP **3b** (240 mg, 0.75 mmol) and sodium carbonate (79 mg, 0.75 mmol) in methanol/THF 1:1 (5 mL) was slowly added

dansylchloride (202 mg, 0.75 mmol) in THF (1 mL). The reaction mixture was left stirring for 2 hours and poured into a separation funnel containing dichloromethane and brine. The layers were separated, dried over Na_2SO_4 , filtered and the solvent was removed *in vacuo*. Purification of the resulting oil by flash chromatography using c-hexane/ ethyl acetate (5:1) as eluent obtained 325 mg (0.63 mmol, 85%) of the desired product **4c** as a yellowish oil. R_f 0.35 (c-hexane/ ethyl acetate (5:1)); ^1H NMR (250 MHz, CDCl_3): δ = 1.40 (s, 3H, $\text{C}(\text{CH}_3)\text{-CH-CH}_2\text{-OR}$); 1.50 (s, 3H, $\text{C}(\text{CH}_3)\text{-CH-(CH}_2)_2\text{-C(CH}_3\text{)-CH-CH}_2\text{-OR}$); 1.64 (s, 3H, $\text{NH-CH}_2\text{-C(CH}_3\text{)}$); 1.45-1.75 (6H, m, CH_2 THP); 1.78-2.10 (m, 8H, CH_2 Far); 2.87 (s, 6H, NCH_3); 3.33 (d, J = 6.3 Hz, 2H, NH-CH_2); 3.50 (m, 1H, $\text{O-CH(R)-O-CH}_{2a}\text{-CH}_2$); 3.88 (m, 1H, $\text{O-CH(R)-O-CH}_{2b}\text{-CH}_2$); 4.00 (dd, J = 11.9 Hz, J = 4.8 Hz, 1H, $\text{CH}_{2a}\text{-O-CH(R)-O-CH}_2$); 4.19 (dd, J = 11.9 Hz, J = 6.5 Hz, 1H, $\text{CH}_{2b}\text{-O-CH(R)-O-CH}_2$); 4.58 (dd, J = 2.2 Hz, J = 2.2 Hz, 1H, O-CH(R)-O), 5.00 (tq, J = 6.4 Hz, J = 0.9 Hz, 1H, $\text{CH-(CH}_2)_2\text{-C(CH}_3\text{)-CH-CH}_2\text{-O}$); 5.12 (ddq, J = 6.5 Hz, J = 4.8 Hz, J = 1.0 Hz, 1H, $\text{CH-CH}_2\text{-O}$); 5.32 (tq, J = 6.5 Hz, J = 1.0 Hz, 1H, $\text{NH-CH}_2\text{-C(CH}_3\text{-CH)}$; 7.21 (d, J = 8.4 Hz, 1H, CH arom), 7.54 (dd, J = 8.4 Hz, J = 7.4 Hz, 1H, CH arom), 7.58 (dd, J = 8.7 Hz, J = 8.1 Hz, 1H, CH arom); 8.26 (d, J = 7.4 Hz, 1H, CH arom); 8.34 (d, J = 8.7 Hz, 1H, CH arom); 8.56 (d, J = 8.1 Hz, 1H, CH arom); ^{13}C NMR (100.6 MHz, CDCl_3): δ = 14.4 (CH_3 Far); 16.2 (CH_3 Far); 16.6 (CH_3 Far); 19.8 (CH_2 THP); 25.7, 26.4, 27.1 (2 $^*\text{CH}_2$ Far, CH_2 THP); 30.9 (CH_2 THP); 39.2 (CH_2 Far); 39.8 (CH_2 Far); 45.6 (N- CH_3 dansyl); 51.4 (R-HN- CH_2); 62.5, 63.6 (CH_2 Far, CH_2 THP); 98.0 (CH THP); 115.3 (CH dansyl); 119.0 (CH dansyl); 120.9 (CH Far); 123.4 (CH Far); 124.4 (CH Far); 128.5 (CH dansyl); 128.6 (CH dansyl); 129.9 (CH dansyl); 129.9 (C dansyl); 130.1 (C dansyl); 130.4 (C dansyl); 130.5 (CH dansyl); 134.8 (Cq Far); 135.3 (Cq Far); 140.3 (Cq Far); 152.2 (C dansyl); MS (FAB, 3-NBA): m/z: calcd for $[M+\text{H}]^+$: 555.3256; found: 555.3242; $\text{C}_{32}\text{H}_{47}\text{N}_2\text{O}_4\text{S}$ (555.3).

Bodipy®FL-N-[2,6,10-trimethyl-12-(tetrahydro-pyran-2-yloxy)-dodeca-2,6,10-trienyl]-amide (Bodipy-NH-Far-OTHP) (4d): To a solution of $\text{H}_2\text{N-Far-OTHP}$ **3b** (27 mg, 86 μmol) and Bodipy®FL (25 mg, 86 μmol) in CH_2Cl_2 (5-10 ml) at 0°C was added HOBr (17 mg, 129 μmol), followed by EDC (20 mg, 103 μmol). The reaction mixture was left stirring at room temperature for 12h, diluted with ethyl acetate (50 ml) and extracted with 0.5 M HCl (2 x 10 mL), 1M NaHCO_3 (2 x 10 mL) and finally with brine (2 x 10 ml). The combined organic layers were dried over MgSO_4 , filtered and concentrated *in vacuo*. Purification of the resulting oil by flash chromatography using a gradient from c-hexane/ ethyl acetate (5:1) to c-hexane/ ethyl acetate (1:1) as eluent obtained 47 mg (79 μmol , 92%) of the desired product **4d** as a orange oil. R_f 0.35 (c-hexane/ethyl acetate (1:1)); ^1H NMR (250 MHz,

CDCl₃): δ = 1.45 (s, 3H, C(CH₃)-CH-CH₂-OR); 1.50 (s, 3H, C(CH₃)-CH-(CH₂)₂-C(CH₃)-CH-CH₂-OR); 1.60 (s, 3H, NH-CH₂-C(CH₃)); 1.4-1.8 (6H, m, CH₂ THP); 1.9-2.1 (m, 8H, CH₂ Far); 2.18 (s, 3H, CH₃ Bodipy®FL; 2.49 (s, 3H, CH₃ Bodipy®FL); 2.58 (tr, J = 7.5 Hz, 2H, CH₂ Bodipy®FL); 3.21 (tr, J = 7.4 Hz, 2H, CH₂ Bodipy®FL); 3.43 (m, 1H, O-CH(R)-O-CH_{2a}-CH₂); 3.66 (d, J = 6.0 Hz, 2H, NH-CH₂); 3.81 (m, 1H, O-CH(R)-O-CH_{2b}-CH₂); 3.95 (dd, J = 11.9 Hz, J = 4.8 Hz, 1H, CH_{2a}-O-CH(R)-O-CH₂); 4.16 (dd, J = 11.9 Hz, J = 6.5 Hz, 1H, CH_{2b}-O-CH(R)-O-CH₂); 4.55 (dd, J = 2.2 Hz, J = 2.2 Hz, 1H, O-CH(R)-O), 5.01 (tq, J = 6.5 Hz, J = 0.9 Hz, 1H, CH₂-CH₂-O); 5.12 (ddq, J = 6.7 Hz, J = 4.8 Hz, J = 1.0 Hz, 1H, CH₂-C(CH₃)-CH-CH₂-O); 5.28 (tq, J = 6.5 Hz, J = 1.0 Hz, 1H, NH-CH₂-C(CH₃)-CH₂); 5.63 (br tr, J = 4.9 Hz, 1H, NH); 6.04 (s, 1H, CH Bodipy®FL); 6.22 (d, J = 3.9 Hz, 1H, CH Bodipy®FL); 6.80 (d, J = 3.9 Hz, 1H, CH Bodipy®FL); 7.00 (s, 1H, CH Bodipy®FL); ¹³C NMR (100.6 MHz, CDCl₃): δ = 11.5 (CH₃ Bodipy®FL); 14.6 (CH₃ Far); 15.1 (CH₃ Bodipy®FL); 16.2 (CH₃ Far); 16.6 (CH₃ Far); 19.9 (CH₂ THP); 25.2 (CH₂ Bodipy®FL); 25.7, 26.5, 26.7 (2*CH₂ Far, CH₂ THP); 30.9 (CH₂ THP); 36.3 (CH₂ Bodipy®FL); 39.5 (CH₂ Far); 39.8 (CH₂ Far); 47.1 (R-NH-CH₂); 62.5, 63.9 (CH₂ Far, CH₂ THP); 98.0 (CH THP); 117.8; 120.6; 120.9; 124.0; 124.3; 126.7; 128.5 (4*CH Bodipy®FL, 3*CH Far); 131.7; 133.1; 135.1; 140.3; 144.2; 157.3; 160.0 (3*Cq Far, 5*Cq Bodipy); 171.8 (CO Bodipy®FL); MS (FAB, 3-NBA): m/z: calcd for [M+H]⁺: 596.3757; found: 596.3858; C₆₄H₄₈BF₂N₃O₃ (595.6).

3,7,11-Trimethyl-12-(7-nitro-benzo[1,2,5]oxadiazolo-4-ylamino)-dodeca-2,6,10-trien-1-ol (NBD-NH-Far-OH) (5b): Compound **5b** was prepared using NBD-NH-Far-OTHP **4b** (417 mg, 0.86 mmol), by means of the procedure described for the synthesis of **5a**. Purification of the resulting oil by flash chromatography using *n*-hexane/ ethyl acetate (1.5:1) as eluent obtained 324 mg (0.80 mmol, 94%) of the desired product **5b** as a reddish brown oil. R_f 0.23 (*n*-hexane/ ethyl acetate (1.5:1)); ¹H NMR (250 MHz, CDCl₃): δ = 1.59 (br, 3H, C(CH₃)-CH-CH₂-OH); 1.67 (br, 3H, C(CH₃)-CH-(CH₂)₂-C(CH₃)-CH-CH₂-OH); 1.73 (br, 3H, NH-CH₂-C(CH₃)); 1.95-2.30 (m, 8H, CH₂ Far); 4.02 (d, J = 5.5 Hz, 2H, NH-CH₂); 4.18 (d, J = 6.8 Hz, 2H, CH₂-OH); 5.08 (tq, J = 6.9 Hz, J = 1.1 Hz, 1H, CH₂-C(CH₃)-CH-CH₂-OH); 5.38 (tq, J = 7.4 Hz, J = 1.2 Hz, 1H, CH₂-OH); 5.46 (tq, J = 7.1 Hz, J = 1.2 Hz, 1H, NH-CH₂-C(CH₃)-CH₂); 6.17 (d, J = 8.7 Hz, 1H, CH arom); 6.84 (br, 1H, NH); 8.46 (d, J = 8.7 Hz, 1H, CH arom); ¹³C NMR (100.6 MHz, CDCl₃): δ = 14.7 (NH-CH₂-C(CH₃)); 15.9 (C(CH₃)-CH-CH₂-OH); 16.3 (C(CH₃)-CH-(CH₂)₂-C(CH₃)-CH-CH₂-OH); 26.0, 26.1 (C(CH₃)-CH-CH₂); 38.9-39.4 (CH₂-C(CH₃)); 51.3 (H₂N-CH₂); 59.5 (CH₂-OH); 99.2 (CH arom); 123.4 (CH-CH₂-OH); 124.5 (CH₂-C(CH₃)-CH-CH₂-OH); 136.5 (CH arom); 123.8,

128.6, 134.4, 139.3, 143.9, 144.2, 144.3 (3^*Cq Far, 4^*Cq arom); MS (EI): m/z: calcd for $[M+\text{H}]^+$: 400.2111; found: 400.2099; $\text{C}_{21}\text{H}_{28}\text{N}_4\text{O}_4$ (400.5).

Dansyl (12-hydroxy-2,6,10-trimethyl-dodeca-2,6,10-trienyl)-amide (Dansyl-NH-Far-OH) (5c): Compound **5c** was prepared using Dansyl-NH-Far-OTHP **4c** (478 mg, 0.86 mmol), by means of the procedure described for the synthesis of **5a**. Purification of the resulting oil by flash chromatography using *c*-hexane/ ethyl acetate (3:1) as eluent obtained 304 mg (0.65 mmol, 75%) of the desired product **5c** as a yellowish oil. R_f 0.20 (*c*-hexane/ ethyl acetate (3:1)); ^1H NMR (250 MHz, CDCl_3): δ = 1.32 (br, 3H, $\text{C}(\text{CH}_3)\text{-CH-CH}_2\text{-OH}$); 1.45 (br, 3H, $\text{C}(\text{CH}_3)\text{-CH-(CH}_2)_2\text{-C(CH}_3\text{)-CH-CH}_2\text{-OH}$); 1.63 (br, 3H, $\text{NH-CH}_2\text{-C}(\text{CH}_3)$); 1.7-2.1 (m, 8H, CH_2 Far); 2.92 (s, 6H, NCH_3); 3.33 (d, J = 6.3 Hz, 2H, NH-CH_2); 4.18 (d, J = 7.8 Hz, 2H, $\text{CH}_2\text{-OH}$); 4.83 (t, J = 6.2 Hz, 1H, NH); 4.92 (tq, J = 6.6 Hz, J = 1.1 Hz, 1H, $\text{CH}(\text{CH}_2)_2\text{-C(CH}_3\text{)-CH-CH}_2\text{-OH}$); 5.06 (tq, J = 6.9 Hz, J = 1.2 Hz, 1H, $\text{CH-CH}_2\text{-OH}$); 5.34 (tq, J = 8.0 Hz, J = 1.2 Hz, 1H, $\text{NH-CH}_2\text{-C}(\text{CH}_3)\text{-CH}$); 7.12 (d, J = 7.6 Hz, 1H, CH arom), 7.44 (tr, J = 7.5 Hz, J = 7.4 Hz, 1H, CH arom), 7.49 (tr, J = 7.8 Hz, 1H, CH arom); 8.16 (dd, J = 7.2 Hz, J = 1.2 Hz, 1H, CH arom); 8.26 (d, J = 8.6 Hz, 1H, CH arom); 8.49 (d, J = 8.6 Hz, 1H, CH arom); ^{13}C NMR (100.6 MHz, CDCl_3): δ = 14.4 ($\text{NH-CH}_2\text{-C}(\text{CH}_3)$); 16.2 ($\text{C}(\text{CH}_3)\text{-CH-CH}_2\text{-OH}$); 16.2 ($\text{C}(\text{CH}_3)\text{-CH-(CH}_2)_2\text{-C(CH}_3\text{)-CH-CH}_2\text{-OH}$); 26.2; 26.5 ($\text{C}(\text{CH}_3)\text{-CH-CH}_2$); 39.1; 39.5 ($\text{CH}_2\text{-C(CH}_3\text{)}$); 45.7 (N-CH_3 dansyl); 51.4 (R-HN-CH₂); 62.9 ($\text{CH}_2\text{-OH}$); 115.5; 120.6; 123.5; 123.9; 123.9; 128.5; 128.6; 129.9; 130.4 (6 $^*\text{CH}$ dansyl; 3 $^*\text{CH}$ Far); 129.9; 129.9; 130.4; 135.2; 135.4; 142.8; 152.0 (4 $^*\text{Cq}$ dansyl; 5 $^*\text{Cq}$ Far); 176.2 (CONH); MS (FAB, 3-NBA): m/z: calcd for $[M+\text{H}]^+$: 471.2681; found: 471.2675; $\text{C}_{27}\text{H}_{38}\text{N}_2\text{O}_3\text{S}$ (470.7).

Bodipy®FL -N-(12-hydroxy-2,6,10-trimethyl-dodeca-2,6,10-trienyl)-amide 17d (Bodipy®FL-NH-Far-OH) (5d): Compound **5d** was prepared using Bodipy®FL-N-Far-OTHP **4d** (47 mg, 79 μmol), by means of the procedure described for the synthesis of **5a**. Purification of the resulting oil by flash chromatography using a gradient *c*-hexane/ ethyl acetate (from 5:1 to 1:1) as eluent obtained 37 mg (73 μmol , 92%) of the desired product **5d** as a orange oil. R_f 0.23 (*c*-hexane/ ethyl acetate (1:1)); ^1H NMR (250 MHz, CDCl_3): δ = 1.46 (br, 3H, $\text{C}(\text{CH}_3)\text{-CH-CH}_2\text{-OH}$); 1.51 (br, 3H, $\text{C}(\text{CH}_3)\text{-CH-(CH}_2)_2\text{-C(CH}_3\text{)-CH-CH}_2\text{-OH}$); 1.59 (br, 3H, $\text{NH-CH}_2\text{-C}(\text{CH}_3)$); 1.85-2.30 (m, 8H, CH_2 Far); 2.18 (s, 3H, CH_3 Bodipy®FL); 2.49 (s, 3H, CH_3 Bodipy®FL); 2.57 (tr, J = 7.5 Hz, 2H, CH_2 Bodipy®FL); 3.21 (tr, J = 7.4 Hz, 2H, CH_2 Bodipy®FL); 3.66 (d, J = 5.7 Hz, 2H, NH-CH_2); 4.07 (d, J = 6.8 Hz, 2H, $\text{CH}_2\text{-OH}$); 5.01 (tq, J = 6.5 Hz, J = 1.1 Hz, 1H, $\text{CH}(\text{CH}_2)_2\text{-C(CH}_3\text{)-CH-CH}_2\text{-OH}$); 5.12 (tq, J = 6.7

Hz, $J = 1.2$ Hz, 1H, $\text{CH}-\text{CH}_2-\text{OH}$); 5.32 (tq, $J = 6.5$ Hz, 1H, $\text{NH}-\text{CH}_2-\text{C}(\text{CH}_3)-\text{CH}$); 5.73 ($J = 4.9$ Hz, 1H, NH); 6.04 (s, 1H, CH Bodipy®FL); 6.23 (d, $J = 3.9$ Hz, 1H, CH Bodipy®FL); 6.80 (d, $J = 3.9$ Hz, 1H, CH Bodipy®FL); 7.01 (s, 1H, CH Bodipy®FL); ^{13}C NMR (100.6 MHz, CDCl_3): $\delta = 11.5$ (CH_3 Bodipy®FL); 14.4 ($\text{NH}-\text{CH}_2-\text{C}(\text{CH}_3)$); 15.1 (CH_3 Bodipy®FL); 16.2 ($\text{C}(\text{CH}_3)-\text{CH}-\text{CH}_2-\text{OH}$); 16.5 ($\text{C}(\text{CH}_3)-\text{CH}-(\text{CH}_2)_2-\text{C}(\text{CH}_3)-\text{CH}-\text{CH}_2-\text{OH}$); 25.2 (CH_2 Bodipy®FL); 26.4, 26.5 ($2^*\text{C}(\text{CH}_3)-\text{CH}-\text{CH}_2$); 36.4 (CH_2 Bodipy®FL); 39.4, 39.7 ($2^*\text{CH}_2-\text{C}(\text{CH}_3)$); 47.1 (R-NH-CH₂); 59.6 (CH_2 -OH); 117.9, 120.6, 123.8, 124.0, 124.3, 126.5, 128.5 (3^{*}CH Far, 4^{*}CH Bodipy®FL); 131.8; 133.7; 135.0; 135.1; 139.5; 144.0; 157.8; 160.3 (5^{*} C_q Bodipy, 3^{*}C_q Far); 171.8 (CO Bodipy®FL); MS (FAB, 3-NBA): m/z: calcd for [M+Na]⁺: 534.31; found: 534.04; $\text{C}_{29}\text{H}_{40}\text{BF}_2\text{N}_3\text{O}_2$ (511.5).

(12-Chloro-2,6,10-trimethyl-dodeca-2,6,10-trienyl)-(7-nitro-benzo[1,2,5]oxadiazol-4-yl)amine (NBD-NH-Far-Cl) (6b): Compound **6b** was prepared using NBD-NH-Ger-OH **5b** (172 mg, 0.43 mmol), by means of the procedure described for the synthesis of **6a**. 182 mg (0.43 mmol, quant.) NBD-NH-Far-Cl **6b** were obtained as a reddish brown oil. R_f 0.45 (*n*-hexane/ ethyl acetate (3:1)); ^1H NMR (250 MHz, CDCl_3): $\delta = 1.55$ (br, 3H, $\text{C}(\text{CH}_3)-\text{CH}-\text{CH}_2-\text{Cl}$); 1.67 (d, $J = 1.0$, 3H, $\text{C}(\text{CH}_3)-\text{CH}-(\text{CH}_2)_2-\text{C}(\text{CH}_3)-\text{CH}-\text{CH}_2-\text{Cl}$); 1.70 (br, 3H, $\text{NH}-\text{CH}_2-\text{C}(\text{CH}_3)$); 1.96-2.16 (m, 8H, CH₂ Far); 4.03 (d, $J = 7.8$ Hz, 2H, $\text{NH}-\text{CH}_2$); 4.05 (d, $J = 8.0$ Hz, 2H, CH_2-Cl); 5.04 (tq, $J = 6.4$ Hz, $J = 1.0$ Hz, 1H, $\text{CH}-(\text{CH}_2)_2-\text{C}(\text{CH}_3)-\text{CH}-\text{CH}_2-\text{Cl}$); 5.38 (tq, $J = 8.0$ Hz, $J = 1.2$ Hz, 1H, $\text{CH}-\text{CH}_2-\text{Cl}$); 5.47 (tq, $J = 6.6$ Hz, $J = 0.9$ Hz, 1H, $\text{NH}-\text{CH}_2-\text{C}(\text{CH}_3)-\text{CH}$); 6.18 (d, $J = 8.7$ Hz, 1H, CH arom); 6.78 (d, $J = 5.8$, 1H, NH); 8.41 (d, $J = 8.7$ Hz, 1H, CH arom); ^{13}C NMR (100.6 MHz, CDCl_3): $\delta = 14.5$ (CH_3 Far); 15.9 (2^*CH_3 Far); 25.9- 26.2 ($\text{C}(\text{CH}_3)-\text{CH}-\text{CH}_2$); 38.9-39.2 ($\text{CH}_2-\text{C}(\text{CH}_3)$); 41.1 (CH_2-Cl); 51.4 ($\text{H}_2\text{N}-\text{CH}_2$); 99.3 (CH arom); 120.2 (CH₂ Far); 123.9 (CH Far); 129.1 (CH Far); 136.6 (CH arom); 123.4, 128.6, 134.7, 142.5, 143.8, 144.2 (3^{*}C_q Far, 4^{*}C_q arom); MS (FAB, 3-NBA): m/z: calcd for [M+H]⁺: 419.1772; found: 419.1760; $\text{C}_{21}\text{H}_{27}\text{N}_4\text{O}_3\text{Cl}$ (418.9).

Dansyl (12-chloro-2,6,10-trimethyl-dodeca-2,6,10-trienyl)-amide (Dansyl-NH-Far-Cl) (6c): Compound **6c** was prepared using Dansyl-NH-Far-OH **5c** (82 mg, 173 μmol), by means of the procedure described for the synthesis of **6a**. 60 mg (130 μmol , 75%) NBD-NH-Far-Cl **6c** were obtained as a yellowish oil. R_f 0.45 (c-hexane/ ethyl acetate (1:1)); ^1H NMR (250 MHz, CDCl_3): $\delta = 1.35$ (br, 3H, $\text{C}(\text{CH}_3)-\text{CH}-\text{CH}_2-\text{Cl}$); 1.48 (s, 3H, $\text{C}(\text{CH}_3)-\text{CH}-(\text{CH}_2)_2-\text{C}(\text{CH}_3)-\text{CH}-\text{CH}_2-\text{Cl}$); 1.59 (br, 3H, $\text{NH}-\text{CH}_2-\text{C}(\text{CH}_3)$); 1.70-2.10 (m, 8H, CH₂ Far); 2.83 (s, 6H, NCH₃); 3.37 (d, $J = 6.2$ Hz, 2H, $\text{NH}-\text{CH}_2$); 4.09 (d, $J = 7.8$ Hz, 2H, CH_2-Cl); 4.95 (t, 1H, $J = 6.2$ Hz, NH); 5.08 (tq, $J = 6.6$ Hz, $J = 1.0$ Hz, 1H, $\text{CH}-(\text{CH}_2)_2-\text{C}(\text{CH}_3)-\text{CH}$ -).

CH₂-Cl); 5.16 (tq, *J* = 6.9 Hz, *J* = 1.2 Hz, 1H, CH-CH₂-Cl); 5.33 (tq, *J* = 8.0 Hz, *J* = 1.0 Hz, 1H, NH-CH₂-C(CH₃)-CH); 7.13 (d, *J* = 7.4 Hz, 1H, CH arom), 7.46 (dd, *J* = 8.4 Hz, *J* = 7.6 Hz, 1H, CH arom), 7.50 (dd, *J* = 8.8 Hz, *J* = 7.8 Hz, 1H, CH arom); 8.19 (dd, *J* = 7.3 Hz, *J* = 1.1 Hz, 1H, CH arom); 8.31 (d, *J* = 8.7 Hz, 1H, CH arom); 8.49 (d, *J* = 8.4 Hz, 1H, CH arom); ¹³C NMR (100.6 MHz, CDCl₃): δ = 14.3 (CH₃ Far); 16.1; 16.4 (2*CH₃ Far); 26.3; 27.2 (C(CH₃)-CH-CH₂); 39.1; 39.6 (2*CH₂-C(CH₃)); 45.6 (N-CH₃ dansyl); 51.3 (R-HN-CH₂); 59.5 (CH₂Cl); 115.3; 119.2; 123.4; 123.8; 124.3; 128.3; 128.4; 129.7; 130.4 (6* CH dansyl, 3* CH Far); 129.9; 130.0; 130.4; 134.8; 135.4; 139.2 (3* Cq Far; 4* Cq dansyl); 171.4 (CONH); MS (FAB, 3-NBA): m/z: calcd for [M+H]⁺: 488.2264; found: 488.2289; C₂₇H₃₇ClN₂O₂S (489.1).

N-(12-Chloro-2,6,10-trimethyl-dodeca-2,6,10-trienyl)-Bodipy®FL-amide (Bodipy®FL-NH-Far-Cl) (6d): Compound **6d** was prepared using Bodipy®FL-NH-Far-OH **5d** (47 mg, 73 μmol), by means of the procedure described for the synthesis of **6a**. 39 mg (73 μmol, quant.) Bodipy®FL-NH-Far-Cl **6d** were obtained as an orange oil. *R*_f 0.45 (*n*-hexane/ ethyl acetate (1:1)); ¹H NMR (250 MHz, CDCl₃): δ = 1.46 (br, 3H, C(CH₃)-CH-CH₂-Cl); 1.51 (d, *J* = 1.0, 3H, C(CH₃)-CH-(CH₂)₂-C(CH₃)-CH-CH₂-Cl); 1.64 (br, 3H, NH-CH₂-C(CH₃)); 1.86-2.05 (m, 8H, CH₂ Far); 2.18 (s, 3H, CH₃ Bodipy®FL); 2.48 (s, 3H, CH₃ Bodipy®FL); 2.55 (tr, *J* = 7.4 Hz, 2H, CH₂ Bodipy®FL); 3.21 (tr, *J* = 7.4 Hz, 2H, CH₂ Bodipy®FL); 3.66 (d, *J* = 6.0 Hz, 2H, NH-CH₂); 4.01 (d, *J* = 8.0 Hz, 2H, CH₂-Cl); 4.99 (tq, *J* = 6.4 Hz, *J* = 1.0 Hz, 1H, CH-(CH₂)₂-C(CH₃)-CH-CH₂-Cl); 5.12 (tq, *J* = 8.0 Hz, *J* = 1.2 Hz, 1H, CH-CH₂-Cl); 5.36 (tq, *J* = 6.6 Hz, *J* = 0.9 Hz, 1H, NH-CH₂-C(CH₃)-CH); 5.73 (br s, 1H, NH); 6.04 (s, 1H, CH Bodipy®FL); 6.23 (d, *J* = 4.0 Hz, 1H, CH Bodipy®FL); 6.90 (d, *J* = 4.0 Hz, 1H, CH Bodipy®FL); 7.02 (s, 1H, CH Bodipy®FL); ¹³C NMR (100.6 MHz, CDCl₃): δ = 10.3 (CH₃ Bodipy®FL); 13.4 (CH₃ Far); 13.9 (CH₃ Bodipy®FL); 15.0; 15.1 (2*CH₃ Far); 23.9 (CH₂ Bodipy®FL); 25.1, 25.4 (2*C(CH₃)-CH-CH₂); 35.0 (CH₂ Bodipy®FL); 38.2; 38.4; 40.2(2*CH₂-C(CH₃,CH₂-Cl); 45.9 (R-NH-CH₂); 116.6; 119.3; 122.6; 122.7; 122.8; 125.4; 127.3 (4*CH Bodipy, 3*CH Far); 130.5; 132.4; 134.1; 134.3; 141.7; 142.8; 156.5; 159.1 (5*C_q Bodipy, 3*C_q Far); 171.8 (CO Bodipy®FL); MS (FAB, 3-NBA): m/z: calcd for [M+H]⁺: 530.2843; found: 530.2745; C₂₉H₃₉BClF₂N₃O (529.9).

{S-[3,7,11-trimethyl-12-(7-nitro-benzo[1,2,5]oxadiazolo-4-ylamino)-dodeca-2,6,10-trienyl]-L-cysteine-methyl ester (Cys-(S-Far-NH-NBD)-OMe) (7b): Compound **7b** was prepared using NBD-NH-Far-Cl **6b** (172 mg, 0.41 mmol), by means of the procedure described for the synthesis of **7a**. 312 mg (0.41 mmol, quant.) Cys-(S-Far-NH-NBD)-OMe

7b were obtained as a reddish brown oil. R_f 0.38 (methylene chloride/methanol (46:1)); $[\alpha]^{20}_D = -30.5^\circ$ ($c = 1$, CHCl_3); ^1H NMR (250 MHz, CDCl_3): $\delta = 1.51$ (br, 3H, CH_3 Far); 1.58 (br, 3H, CH_3 Far); 1.65 (br, 3H, CH_3 Far); 1.84-2.14 (m, 8H, CH_2 Far); 2.63 (dd, $J = 13.6$ Hz, $J = 7.7$ Hz, 1H, β - CH_{2a} Cys); 2.86 (dd, $J = 13.6$ Hz, $J = 7.7$ Hz, 1H, β - CH_{2b} Cys); 3.07 (d, $J = 3.7$ Hz, 1H, Far CH_{2a} -S); 3.10 (d, $J = 4.5$ Hz, 1H, Far CH_{2b} -S); 3.69 (s, 3H, CH_3 -Cys); 3.42-3.66 (m, 1H, α -CH Cys); 3.97 (m, 2H, NH- CH_2); 5.00 (m, 1H, CH Far); 5.12 (m, 1H, CH Far); 5.40 (tr, $J = 6.8$ Hz, 3H, CH Far); 6.11 (d, $J = 8.7$ Hz, 1H, CH arom); 8.37 (d, $J = 8.7$ Hz, 1H, CH arom); ^{13}C NMR (100.6 MHz, CDCl_3): $\delta = 14.5$ (CH_3 Far); 15.9 (CH_3 Far); 16.0 (CH_3 Far); 26.1; 29.7; 36.2; 38.9; 39.4; 43.1 (β - CH_2 Cys, 4* CH_2 Far); 51.3 (HN- CH_2); 52.3 (CH_3 Cys), 53.6 (α -CH Cys); 99.2 (CH arom); 119.9; 124.2; 128.7 (3*CH Far); 123.2; 128.6; 134.5; 139.3; 143.8; 144.2 ($\beta^*\text{C}_q$ Far, 4* C_q arom); 136.6 (CH arom); MS (FAB, 3-NBA): m/z: calcd for $[M+\text{H}]^+$: 517.2359; found: 518.2455; $\text{C}_{25}\text{H}_{35}\text{N}_5\text{O}_5\text{S}$ (517.7).

{S-[3,7,11-trimethyl-12-(Dansyl-amino)-dodeca-2,6,10-trienyl]-L-cysteinemethyl ester (Cys-(S-Far-NH-Dansyl)-OMe) (7c): Compound **7c** was prepared using Dansyl-NH-Far-Cl **6c** (201 mg, 0.41 mmol), by means of the procedure described for the synthesis of **7a**. 229 mg (0.39 mmol, 95%) Cys-(S-Far-NH-Dansyl)-OMe **1c** were obtained as a yellowish oil. R_f 0.35 (c-hexane/ ethyl acetate (1:1)); $[\alpha]^{20}_D = -2.1^\circ$ ($c = 1$, CHCl_3); ^1H NMR (250 MHz, CDCl_3): $\delta = 1.37$ (br, 3H, CH_3 Far); 1.49 (br, 3H, CH_3 Far); 1.61 (br, 3H, CH_3 Far); 1.75-2.05 (m, 8H, CH_2 Far); 2.66 (dd, $J = 13.6$ Hz, $J = 7.7$ Hz, 1H, β - CH_{2a} Cys); 2.83 (dd, $J = 13.6$ Hz, $J = 7.7$ Hz, 1H, β - CH_{2b} Cys); 2.84 (s, 6H, NCH_3); 3.11 (d, $J = 3.7$ Hz, 1H, Far CH_{2a} -S); 3.13 (d, $J = 4.5$ Hz, 1H, Far CH_{2b} -S); 3.37 (d, $J = 6.2$ Hz, 2H, NH- CH_2); 3.69 (s, 3H, CH_3 -Cys); 3.60 (m, 1H, α -CH Cys); 4.97 (m, 1H, NH); 5.09 (m, 2H, CH Far); 5.17 (tr, $J = 6.8$ Hz, 3H, CH Far); 7.14 (d, $J = 8.4$ Hz, 1H, CH arom), 7.47 (dd, $J = 8.4$ Hz, $J = 7.4$ Hz, 1H, CH arom), 7.51 (dd, $J = 8.7$ Hz, $J = 8.1$ Hz, 1H, CH arom); 8.19 (d, $J = 7.4$ Hz, 1H, CH arom); 8.28 (d, $J = 8.7$ Hz, 1H, CH arom); 8.49 (d, $J = 8.1$ Hz, 1H, CH arom); ^{13}C NMR (100.6 MHz, CDCl_3): $\delta = 14.4$ (CH_3 Far); 16.2 (CH_3 Far); 16.3 (CH_3 Far); 26.4; 26.5 (CH_2 Far); 30.1 (CH_2S Far); 36.6 (β - CH_2 Cys); 39.1; 39.7 (CH_2 Far); 45.6 (NCH_3 dansyl); 51.3 (NH CH_2); 52.4 (CH_3 Cys), 54.4 (α -CH Cys); 115.3; 119.1; 120.3; 123.4; 124.3; 128.4; 128.5; 129.8; 130.4 (6*CH arom, 3*CH Far); 129.8; 129.9; 130.0; 135.0; 135.2; 135.4; 139.6 (4* C_q dansyl; 3* C_q Far); 174.7 (CONH); MS (FAB, 3-NBA): m/z: calcd for $[M+\text{H}]^+$: 588.2850; found: 588.2880; $\text{C}_{31}\text{H}_{45}\text{N}_3\text{O}_4\text{S}_2$ (587.4).

{S-[3,7,11-trimethyl-12-(Bodipy®FL-amino)-dodeca-2,6,10-trienyl]}-L-cysteinmethylester (Cys-(S-Far-NH-Bodipy®FL)-OMe) (7d): Compound **7d** was prepared using Bodipy-NH-Far-Cl **6d** (39 mg, 74 μmol), by means of the procedure described for the synthesis of **7a**. 25 mg (40 μmol , 55%) Cys-(S-Far-NH-Bodipy®FL)-OMe **1d** were obtained as a orange oil. R_f 0.25 (c-hexane/ ethyl acetate (1:10)); ^1H NMR (250 MHz, CDCl_3): δ = 1.47 (br, 3H, CH_3 Far); 1.51 (br, 3H, CH_3 Far); 1.59 (br, 3H, CH_3 Far); 1.80-2.10 (m, 8H, CH_2 Far); 1.97 (s, 3H, CH_3 Bodipy®FL); 2.48 (s, 3H, CH_3 Bodipy®FL); 2.57 (tr, J = 7.4 Hz, 2H, CH_2 Bodipy®FL); 2.63 (dd, J = 13.6 Hz, J = 7.7 Hz, 1H, β - CH_{2a} Cys); 2.80 (dd, J = 13.6 Hz, J = 7.7 Hz, 1H, β - CH_{2b} Cys); 3.09 (d, J = 3.7 Hz, 1H, Far CH_{2a} -S); 3.10 (d, J = 4.5 Hz, 1H, Far CH_{2b} -S); 3.21 (tr, J = 7.4 Hz, 2H, CH_2 Bodipy®FL); 3.55 (d, J = 6.0 Hz, 2H, $\text{NH}-\underline{\text{CH}}_2$); 3.62 (s, 3H, CH_3 -Cys); 3.53 (m, 1H, α -CH Cys); 5.00 (m, 1H, CH Far); 5.13 (m, 1H, CH Far); 5.63 (tr, J = 6.8 Hz, 3H, CH Far); 5.63 (br s, 1H, NH); 6.04 (s, 1H, CH Bodipy®FL); 6.23 (d, J = 4.0 Hz, 1H, CH Bodipy®FL); 6.80 (d, J = 4.0 Hz, 1H, CH Bodipy®FL); 7.01 (s, 1H, CH Bodipy®FL); ^{13}C NMR (100.6 MHz, CDCl_3): δ = 11.6 (CH_3 Bodipy®FL); 14.5 (CH_3 Far); 15.1 (CH_3 Bodipy®FL); 16.2 (CH_3 Far); 16.3 (CH_3 Far); 25.2 (CH_2 Bodipy®FL); 26.6; 26.7 (CH_2 Far); 29.9; 30.1 (β - CH_2 Cys, $\text{S}-\text{CH}_2$ Far); 36.5 (CH_2 Bodipy®FL); 39.5; 39.8 (CH_2 Far); 47.1 (R-NH- CH_2); 52.4 (CH_3 Cys), 54.5 (α -CH Cys); 117.8; 120.2; 124.0; 124.2; 124.3; 126.7; 128.6 (4* CH Bodipy®FL, 3* CH Far); 131.8; 133.6; 135.3; 139.7; 141.0; 144.0; 157.8; 160.4 (5* Cq Bodipy®FL, 3*Cq Far); 171.8 (CONH); MS (FAB, 3NBA): m/z: calcd for $[M+\text{H}]^+$: 629.3430; found: 629.3508; $\text{C}_{33}\text{H}_{47}\text{BF}_2\text{N}_4\text{O}_3\text{S}$ (628.6).

Maleimidocaproyl-glycyl-(S-tert-butyl)-L-cysteyl-L-methionyl-glycyl-L-leucyl-L-prolinyl-{S-[3,7,11-tri-methyl-12-(7-nitro-benzo[1,2,5]oxadiazolo-4-ylamino)-dodeca-2,6,10-trienyl]}-L-cysteinemethyl ester (MIC-Gly-Cys(S*t*Bu)-Met-Gly-Leu-Pro-Cys(Far-NH-NBD)OMe) (9b): Compound **9b** was prepared using MIC-Gly-Cys(S*t*Bu)-Met-Gly-Leu-Pro-OH **8**^[6c,d] (65 mg, 75.8 μmol) and Cys-(S-Far-NH-NBD)-OMe **7a** (39 mg, 75.8 μmol), by means of the procedure described for the synthesis of **9a**. Purification of the resulting oil by flash chromatography using a gradient from c-hexane/ ethyl acetate (1:1) to ethyl acetate/methanol (1:1) as eluent obtained 66 mg (48.6 μmol , 64%) of the desired product **9b** as a reddish brown oil. t_{R} : 26.12 min; $[\alpha]^{20}_{\text{D}} = 1.1^\circ$ ($c = 1$, CHCl_3); ^1H NMR (250 MHz, CDCl_3): δ = 0.95 (m, 6H, 2*? $-\text{CH}_3$ Leu); 1.32 (m, 11 H, CH_2 MIC, S*t*Bu); 1.50-1.75 (m, 13 H, 2* CH_2 MIC, β - CH_2 Leu, γ -CH Leu, 3* CH_3 Far); 1.90-2.30 (m, 19H, β - CH_2 Met, β - CH_2 Pro, γ - CH_2 Pro, -SMe, 4* CH_2 Far, α - CH_2 MIC); 2.45-2.80 (m, 2H, γ - CH_2 Met); 2.95-3.25 (m,

6H, 2^{*} β -CH₂ Cys, Far CH₂-S); 3.47 (m, 2H, NCH₂ MIC); 3.71 (s, 3H, OCH₃ Cys); 3.60-3.90 (m, 6H, α -CH₂ Gly, δ -CH₂ Pro, α -CH₂ Gly'); 4.10 (m, 3H, NH-CH₂Far, α -CH Pro); 4.40-4.75 (m, 4H, α -CH Leu, α -CH Met, 2^{*} α -CH Cys); 5.03 (m, 2H, CH Far); 5.12 (m, 1H, CH Far); 5.47 (m, 2H, Far NH); 6.28 (d, J = 8.7 Hz, 1H, CH NBD), 6.79 (s, 2H, -CH=CH- MIC); 7.45-7.80 (m, 6H, NH); 8.58 (d, J = 8.7 Hz, 1H, CH NBD).; MS (FAB, 3-NBA): m/z: calcd for [M+H]⁺: 1357.6; found: 1357.1; MS (HPLC/ESI, water/acetonitrile/formic acid): m/z: calcd for [M+H]⁺: 1357.6; found: 1357.3; C₆₂H₉₂N₁₂O₁₄S₄ (1357.7).

Maleimidocaproyl-glycyl-(S-tert-butyl)-L-cysteinyl-L-methionyl-glycyl-L-leucyl-L-prolinyl-{S-[3,7,11-tri-methyl-12-(dansyl-amino)-dodeca-2,6,10-trienyl]}-L-cysteinemethyl ester (MIC-Gly-Cys(S*t*Bu)-Met-Gly-Leu-Pro-Cys(Far-NH-Dansyl)OMe) (9c**):** Compound **9c** was prepared using MIC-Gly-Cys(S*t*Bu)-Met-Gly-Leu-Pro-OH **8**^[6c,d] (38 mg, 44.3 μ mol) and Cys-(S-Far-NH-Dansyl)-OMe **7c**(26 mg, 44.3 μ mol), by means of the procedure described for the synthesis of **9a**. Purification of the resulting oil by flash chromatography using a gradient from *c*-hexane/ ethyl acetate (1:1) to ethyl acetate/methanol (1:1) as eluent obtained 53 mg (37.1 mol, 84%) of the desired product **9c** as a yellowish oil. t_R = 26.81 min; $[\alpha]^{20}_D$ = -12.9 ° (c = 1, CHCl₃); ¹H NMR (250 MHz, CDCl₃): δ = 0.87 (m, 6H, ? -CH₃ Leu); 1.20 (m, 11 H, CH₂ MIC, S*t*Bu); 1.35-1.75 (m, 13 H, 2^{*}CH₂ MIC, β -CH₂ Leu, γ -CH Leu, 3^{*}CH₃ Far); 1.80-2.15 (m, 19H, β -CH₂ Met, β -CH₂ Pro, γ -CH₂ Pro, -SMe, 4^{*}CH₂Far, α -CH₂ MIC); 2.56 (m, 2H, γ -CH₂ Met); 2.78 (s, 6H, NMe₂ dansyl); 3.00-3.15 (m, 6H, 2^{*} β -CH₂ Cys, Far CH₂-S); 3.38 (m, 2H, NCH₂ MIC); 3.61 (s, 3H, OCH₃ Cys); 3.45-3.95 (m, 9 H, α -CH₂ Gly, δ -CH₂ Pro, α -CH₂ Gly', NH-CH₂Far, α -CH Pro); 4.10-4.75 (m, 4H, α -CH Leu, α -CH Met, 2^{*} α -CH Cys); 4.90 (m, 1H, CH Far); 4.94 (m, 1H, CH Far); 5.08 (m, 1H, CH Far); 6.69 (s, 2H, -CH=CH- MIC); 7.45-7.71 (m, 6H, NH); 7.14 (d, J = 8.4 Hz, 1H, CH arom), 7.47 (dd, J = 8.4 Hz, J = 7.4 Hz, 1H, CH arom), 7.51 (dd, J = 8.7 Hz, J = 8.1 Hz, 1H, CH arom); 8.19 (d, J = 7.4 Hz, 1H, CH arom); 8.28 (d, J = 8.7 Hz, 1H, CH arom); 8.49 (d, J = 8.1 Hz, 1H, CH arom); MS (FAB, 3-NBA): m/z: calcd for [M+Na]⁺: 1449.6; found: 1450.0; MS (HPLC/ESI, water/acetonitrile/formic acid): m/z: calcd for [M+H]⁺: 1427.6; found: 1427.3; C₆₈H₁₀₂N₁₀₀O₁₃S₅ (1427.9).

Maleimidocaproyl-glycyl-(S-tert-butyl)-L-cysteinyl-L-methionyl-glycyl-L-leucyl-L-prolinyl-{S-[3,7,11-tri-methyl-12-(Bodipy®FL-amino)-dodeca-2,6,10-trienyl]}-L-cysteine-methyl ester (MIC-Gly-Cys(S*t*Bu)-Met-Gly-Leu-Pro-Cys(Far-NH-Bodipy®FL)OMe) (9d**):** Compound **9d** was prepared using MIC-Gly-Cys(S*t*Bu)-Met-Gly-

Leu-Pro **8** [6c,d] (16 mg, 18.6 μ mol) and Cys-(S-Far-NH-Bodipy®FL)-OMe **7d** (12 mg, 18.6 mmol), by means of the procedure described for the synthesis of **9a**. Purification of the resulting oil by flash chromatography using a gradient from *c*-hexane/ ethyl acetate (1:1) to ethyl acetate/methanol (1:1) as eluent obtained 21 mg (14.3 mol, 77%) of the desired product **9d** as a orange oil. t_R = 29.26 min; 1 H NMR (250 MHz, CDCl₃): δ = 0.95-1.15 (m, 6H, $\text{?}-\text{CH}_3$ Leu); 1.20-1.50 (m, 20 H, CH₂ MIC, SiBu, 3*CH₃ Far); 1.65-2.05 (m, 15H, 2*CH₂ MIC, β -CH₂ Leu, γ -CH Leu, 4*CH₂Far); 1.95-2.35 (m, 11H, β -CH₂ Met, β -CH₂ Pro, γ -CH₂ Pro, -SMe, α -CH₂ MIC); 2.16 (s, 3H, CH₃ Bodipy®FL); 2.43 (s, 3H, CH₃ Bodipy®FL); 2.70 (m, 2H, γ -CH₂ Met); 2.57 (m, 2H, CH₂ Bodipy®FL); 2.96 (m, 6H, 2* β -CH₂ Cys, Far CH₂-S); 3.15 (m, 2H, CH₂ Bodipy®FL); 3.05-3.70 (m, 5H, NCH₂ MIC, δ -CH_{2b} Pro, NH-CH₂Far); 3.63 (s, 3H, OCH₃ Cys); 3.85-4.55 (m, 10H, δ -CH_{2a} Pro, α -CH₂ Gly, α -CH₂ Gly', α -CH Leu, α -CH Met, 2* α -CH Cys, α -CH Pro); 4.90-5.10 (m, 3H, CH Far); 6.04 (s, 1H, CH Bodipy®FL); 6.23 (m, 1H, CH Bodipy®FL); 6.53 (s, 2H, -CH=CH- MIC); 6.80 (m, 1H, CH Bodipy®FL); 7.01 (s, 1H, CH Bodipy®FL); 7.45-7.80 (m, 6H, NH); MS (HPLC/ESI, water/acetonitrile/formic acid): m/z: calcd for [M+H]⁺: 1468.4; found: 1468.7; C₇₀H₁₀₄BF₂N₁₁₀O₁₂S₄ (1468.7).

Maleimidocaproyl-glycyl-(S-hexadecyl)-L-cysteinyl-L-methionyl-glycyl-L-leucyl-L-prolyl-(S-Farnesyl)-L-cysteinyl-ethylenediamine (MIC-Gly-Cys(HD)-Met-Gly-Leu-Pro-Cys(Far)-NH-Et-NH₂) (11b): Compound **11b** was prepared using Fmoc-NH-Et-NH-MeTrt-resin (14 mg, 0.0119 mmol, loading 0.87 mM/g), by means of the procedure described for the synthesis of **11a**. Cleavage was accomplished by addition of 1% TFA in dichloromethane and consecutive shaking for 0.5 hours to obtain 15.3 mg (0.0114 mmol, 96%) of the desired product **11b** as yellow oil. t_R : (gradient (CH₃CN/H₂O/HCO₂H): 44.95/54.95/0.1 to 89.85/9.95/0.1 in 15 min) 7.6 min; $[\alpha]_D^{20}$ = -0.20 (c= 1, CHCl₃); 1 H NMR (500 MHz, CDCl₃: δ / ppm): 0.88 (t, J = 6.8, 3H, $\text{?}-\text{CH}_3$ HD), 0.82-1.00 (m, 6H, $\text{?}-\text{CH}_3$ Leu), 1.25-1.63 (m, 28H, 13*CH₂ HD, CH₂ MIC), 1.42-1.73 (m, 21H, 2 CH₂ MIC, β -CH₂ Leu, γ -CH Leu, 4*CH₃ Far, β -CH₂ HD), 1.83-2.15 (m, 15H, β -CH_{2b} Met, β -CH_{2b} Pro, γ -CH₂ Pro, SMe, 4*CH₂ Far), 2.22-2.30 (m, 4H, α -CH₂ MIC, β -CH_{2a} Met, β -CH_{2a} Pro), 2.48-2.63 (m, 4H, γ -CH₂ Met, α -CH₂ HD), 2.89-3.00 (m, 4H, β -CH₂ Cys, NH-CH₂-CH₂-NH), 3.15 (m, 4H, β -CH₂ Cys, Far CH₂-S), 3.42-3.70 (m, 8H, δ -CH_{2a} Pro, δ -CH_{2b} Pro, NCH₂ MIC, NH-CH₂-CH₂-NH), 3.73-4.00 (m, 3 H, α -CH Pro, α -CH₂ Gly, α -CH₂ Gly'), 4.32-4.55 (m, 4H, 2* α -CH Cys, α -CH Leu, α -CH Met), 5.09 (t, J = 6.7, 2H, 2*C=CH Far), 5.17-5.22 (m, 1H, C=C_H-CH₂-S Far), 6.69 (s, 2H, -CH=CH- MIC), 7.15-8.25 (m, 7H, NH); MS (FAB, 3-NBA):

m/z: calcd for $[M+H]^+$: 1343.8195., found 1343.8247; MS (ESI): m/z: calcd for $[M+H]^+$: 1343.8, found 1343.9; $C_{69}H_{118}N_{10}O_{10}S_3$ (1343.93).

Maleimidocaproyl-glycyl-(O-trityl)-L-serinyl-L-methionyl-glycyl-L-leucyl-L-prolyl-(S-Farnesyl)-L-cysteinyl-ethylenediamine (MIC-Gly-Ser(OTrt)-Met-Gly-Leu-Pro-Cys(Far)-NH-Et-NH₂) (11c): Compound **11c** was prepared using Fmoc-NH-Et-NH-MeTrt-resin (269 mg, 0.24 mmol, loading 0.87 mM/g), by means of the procedure described for the synthesis of **11a**. Cleavage was accomplished with methylene chloride/trifluoroethanol/acetic acid (3:1:1) and yielded 79 mg (0.06 mol, 25%) of the desired product **11c** as a colorless oil. t_R : 35.00 min; $[\alpha]^{20}_D = 2.23^\circ$ (c = 1, $CHCl_3$); 1H NMR (250 MHz, $CDCl_3$): δ = 0.86 (m, 6H, ?-CH₃ Leu); 1.20 (m, 2H, CH₂ MIC, S*t*Bu); 1.42-1.70 (m, 19H, 2 CH₂ MIC, β -CH₂ Leu, γ -CH Leu, 4*CH₃ Far); 1.80-2.20 (m, 19H, β -CH₂ Met, β -CH₂ Pro, γ -CH₂ Pro, SMe, 4*CH₂ Far, α -CH₂ MIC); 2.55 (m, 2H, γ -CH₂ Met); 3.11 (m, 8H, NH-CH₂-CH₂-NH, β -CH₂ Cys, Far CH₂-S); 3.45 (m, 4H, NCH₂ MIC, NH-CH₂-CH₂NH); 3.60 (m, 1H, δ -CH_{2b} Pro); 3.65-4.00 (m, 8H, α -CH₂ Gly, δ -CH_{2a} Pro, α -CH₂ Gly', α -CH Pro, β -CH₂ Ser); 4.25-4.75 (m, 4H, α -CH Met, α -CH Cys, α -CH Ser, α -CH Leu); 5.07 (m, 2H, CH Far); 5.19 (m, 1H, CH Far); 6.65 (s, 2H, -CH=CH- MIC); 7.00-8.20 (m, 8H, NH); 7.15-7.40 (m, 15H, CH arom); MS (FAB, 3-NBA): m/z: calcd for $[M+H]^+$: 1345.70; found: 1345.29; MS (HPLC/ESI, water/acetonitrile/formic acid): m/z: calcd for $[M+H]^+$: 1345.7; found: 1345.7; $C_{72}H_{100}N_{10}O_{11}S_2$ (1345.8).

Maleimidocaproyl-glycyl-(S-*tert*-butyl)-L-cysteinyl-L-methionyl-glycyl-L-leucyl-L-prolyl-(S-Farnesyl)-L-cysteinyl-ethylenediamino-Bodipy TR (MIC-Gly-Cys(S*t*Bu)-Met-Gly-Leu-Pro-Cys(Far)-NH-Et-NH-Bodipy TR) (12b): Compound **12b** was prepared using MIC-Gly-Cys(S*t*Bu)-Met-Gly-Leu-Pro-Cys(Far)-NH-Et-NH₂ **11a** (9.5 mg, 7.9 μ mol) and Bodipy TR (5 mg, 7.9 μ mol), by means of the procedure described for the synthesis of **9a**. Purification of the resulting oil by flash chromatography using a gradient from c-hexane/ ethyl acetate (1:1) to ethyl acetate/methanol (1:1) as eluent obtained 11.5 mg (6.6 μ mol, 84%) of the desired product **12b** as a blue oil. t_R = 30.04 min; $[\alpha]^{20}_D = 4.4^\circ$ (c = 1, $CHCl_3$); 1H NMR (250 MHz, $CDCl_3$): δ = 0.89 (m, 6H, ?-CH₃ Leu); 1.30-1.45 (m, 11H, CH₂ MIC, S*t*Bu); 1.30-1.65 (m, 19H, 2 CH₂ MIC, β -CH₂ Leu, γ -CH Leu, 4*CH₃ Far); 1.70-2.20 (m, 27H, β -CH₂ Met, β -CH₂ Pro, γ -CH₂ Pro, SMe, 4*CH₂ Far, α -CH₂ MIC, 4*CH₂ Bodipy TR); 2.51 (m, 2H, γ -CH₂ Met); 3.05-3.15 (m, 8H, 2* β -CH₂ Cys, Far CH₂-S, NH-CH₂-CH₂NH); 3.20-3.50 (m, 6H, NCH₂ MIC, NH-CH₂-CH₂NH, NCH₂ Bodipy TR); 3.65-3.80 (m, 6H, α -CH₂ Gly, δ -CH₂

Pro, α -CH₂ Gly'); 4.10-4.60 (m, 7H, α -CH Met, 2* α -CH Cys, α -CH Leu, α -CH Pro, OCH₂ Bodipy TR); 5.07 (m, 2H, CH Far); 5.21 (m, 1H, CH Far); 6.65 (s, 2H, -CH=CH- MIC); 6.70- 8.00 (m, 20H, 12*CH arom Bodipy TR, 8*NH); MS (HPLC/ESI, water/acetonitrile/formic acid): m/z: calcd for [M+H]⁺: 1726.8; found: 1726.8; C₈₄H₁₁₈BF₂N₁₃O₁₃S₅ (1727.1).

Maleimidocaproyl-glycyl-(S-Hexadecyl)-L-cysteYL-L-methionyl-glycyL-leucyl-L-proYL-(S-Farnesyl)-L-cysteYL-ethylenediamino-Bodipy®FL (MIC-Gly-Cys(HD)-Met-Gly-Leu-Pro-Cys(Far)-NH-Et-NH-Bodipy® FL) (12c): To a solution of Bodipy®FL (6.7 mg, 22.8 μ mol), HBTU (7.8 mg, 20.5 μ mol), HOBr (3.5 mg, 22.8 μ mol) and DIPEA (7.8 μ l, 45.6 μ mol) dissolved in 0.5 ml dry dichloromethane was added under argon atmosphere MIC-Gly-Cys(HD)-Met-Gly-Leu-Pro-Cys(Far)-NH-Et-NH₂ **11c** (15.3 mg, 11.4 μ mol). The reaction mixture was left stirring at room temperature for 18 hours in the dark, concentrated *in vacuo* and redisolved in acetonitrile/water (1:1). Purification over a Chromafix C4 column using 50-100% acetonitrile in water obtained 4.3 mg (2.7 μ mol, 23%) of the desired product **12c** as a red oil. t_R (gradient (CH₃CN/H₂O/HCO₂H): 44.95/54.95/0.1 to 89.85/9.95/0.1 in 35 min) = 17.78 min; ¹H NMR (400 MHz, CDCl₃: δ /ppm): 0.82-1.00 (m, 9H, ?-CH₃ Leu, ?-CH₃ HD), 1.25-1.63 (m, 28H, 13*CH₂ HD, CH₂ MIC), 1.42-1.73 (m, 21H, 2 CH₂ MIC, β -CH₂ Leu, γ -CH Leu, 4*CH₃ Far, β -CH₂ HD), 1.83-2.15 (m, 17H, CH₂- Bodipy®FL, β -CH_{2b} Met, β -CH_{2b} Pro, γ -CH₂ Pro, SMe, 4*CH₂ Far), 2.22-2.30 (m, 7H, α -CH₂ MIC, β -CH_{2a} Met, β -CH_{2a} Pro, CH₃- Bodipy®FL), 2.48-2.63 (m, 7H, γ -CH₂ Met, α -CH₂ HD, CH₃- Bodipy®FL), 2.83-3.70 (m, 16H, δ -CH_{2a} Pro, δ -CH_{2b} Pro, NCH₂ MIC, NH-CH₂-CH₂-NH, CH₂ Bodipy®FL, β -CH₂ Cys, Far CH₂-S, β -CH₂ Cys), 3.72-4.00 (m, 3H, α -CH Pro, α -CH₂ Gly, α -CH₂ Gly'), 4.55-4.32 (m, 4H, 2* α -CH Cys, α -CH Leu, α -CH Met), 5.09 (t, J = 6.7, 2H, 2*C=CH Far), 5.17-5.22 (m, 1H, C=CH-CH₂-S Far), 6.13 (s, 1H, H Pyrrol), 6.27-6.29 (m, 1H, H Pyrrol), 6.67 (s, 2H, -CH=CH- MIC), 6.87-6.92 (m, 1H, H Pyrrol), 7.11 (s, 1H, H Pyrrol), 7.15-8.25 (m, 7H, NH); MS (HPLC/ESI, water/acetonitrile/formic acid): m/z: calcd for [M+H]⁺: 1617.9, found 1617.8; C₈₃H₁₃₂BF₂N₁₂O₁₁S₃ (1619.01).

Maleimidocaproyl-glycyl-(O-trityl)-L-serinyl-L-methionyl-glycyl-L-leucyl-L-proYL-(S-Farnesyl)-L-cysteYL-ethylenediamino-Bodipy®FL (MIC-Gly-Ser(Trt)-Met-Gly-Leu-Pro-Cys(Far)-NH-Et-NH-Bodipy®FL) (12d): Compound **12d** was prepared using MIC-Gly-Ser(Trt)-Met-Gly-Leu-Pro-Cys(Far)-NH-Et-NH₂ **11c** (11.5 mg, 8.6 μ mol) and Bodipy®FL

(2.5 mg, 8.6 μ mol), by means of the procedure described for the synthesis of **9a**. Purification of the resulting oil by flash chromatography using a gradient from *c*-hexane/ethyl acetate (1:1) to ethyl acetate/methanol (1:1) as eluent obtained 12.5 mg (7.7 μ mol, 90%) of the desired product **12d** as a red oil; t_R = 30.43; ^1H NMR (250 MHz, CDCl_3): δ = 0.80 (m, 6H, -CH_3 Leu); 1.10 (m, 2H, CH_2 MIC); 1.45-1.60 (m, 19H, 2 CH_2 MIC, $\beta\text{-CH}_2$ Leu, $\gamma\text{-CH}$ Leu, 4^*CH_3 Far); 1.75-2.00 (m, 21H, $\beta\text{-CH}_2$ Met, $\beta\text{-CH}_2$ Pro, $\gamma\text{-CH}_2$ Pro, SMe, 4^*CH_2 Far, $\alpha\text{-CH}_2$ MIC, NH- $\text{CH}_2\text{-CH}_2\text{NH}$); 2.28 (s, 3H, CH_3 Bodipy®FL); 2.42 (s, 3H, CH_3 Bodipy®FL); 2.58 (m, 2H, CH_2 Bodipy®FL); 2.88 (m, 2H, $\gamma\text{-CH}_2$ Met); 3.10 (m, 4H, $\beta\text{-CH}_2$ Cys, Far $\text{CH}_2\text{-S}$); 3.20 (m, 2H, CH_2 Bodipy®FL); 3.40 (m, 4H, NCH₂ MIC, NH- $\text{CH}_2\text{-CH}_2\text{NH}$); 3.60-3.90 (m, 8H, $\alpha\text{-CH}_2$ Gly, $\delta\text{-CH}_2$ Pro, $\alpha\text{-CH}_2$ Gly', $\beta\text{-CH}_2$ Ser); 4.10-4.60 (m, 4H, $\alpha\text{-CH}$ Met, $\alpha\text{-CH}$ Cys, $\alpha\text{-CH}$ Ser, $\alpha\text{-CH}$ Leu, $\alpha\text{-CH}$ Pro); 5.00 (m, 2H, CH Far); 5.10 (m, 1H, CH Far); 6.04 (s, 1H, CH Bodipy®FL); 6.23 (m, 1H, CH Bodipy®FL); 6.59 (s, 2H, -CH=CH- MIC); 6.79 (m, 1H, CH Bodipy®FL); 7.00-7.80 (m, 8H, NH); 7.01 (s, 1H, CH Bodipy®FL); 7.10-7.35 (m, 15H, CH arom); 7.45-7.80 (m, 6H, NH); MS (HPLC/ESI, water/acetonitrile/formic acid): m/z: calcd for [M-H]⁻: 1617.8; found: 1617.7; $\text{C}_{86}\text{H}_{113}\text{BF}_2\text{N}_{12}\text{O}_{12}\text{S}_2$ (1619.8).

Maleimidocaproyl-glycyl-L-serinyl-L-methionyl-glycyl-L-leucyl-L-prolyl-(S-Farnesyl)-L-cysteyl-ethylene-diamino-BodipyFL (MIC-Gly-Ser-Met-Gly-Leu-Pro-Cys(Far)-NH-Et-NH-Bodipy®FL) (12e): To a solution of MIC-Gly-Ser(Trt)-Met-Gly-Leu-Pro-Cys(Far)-NH-Et-NH-Bodipy®FL **12d** (12.5 mg, 7.7 μ mmol) in methylene chloride (10 mL) under an argon atmosphere was added trifluoroacetic acid (100 μ L). The reaction mixture was left stirring for 10 minutes and after addition of methanol (1 mL) the solvent was removed by evaporation in vacuo under repeated addition of toluene for azeotropic removal of the acid. Purification of the resulting oil by flash chromatography using a gradient from *c*-hexane/ethyl acetate (1:1) to ethyl acetate/methanol (1:1) as eluent obtained 9.7 mg (7.0 μ mol, 91%) of the desired product **12e** as a red oil. t_R = 30.23 min; ^1H NMR (400 MHz, CDCl_3): δ = 0.80 (m, 6H, -CH_3 Leu); 1.20 (m, 2H, CH_2 MIC); 1.45-1.70 (m, 19H, 2 CH_2 MIC, $\beta\text{-CH}_2$ Leu, $\gamma\text{-CH}$ Leu, 4^*CH_3 Far); 1.85-2.15 (m, 21H, $\beta\text{-CH}_2$ Met, $\beta\text{-CH}_2$ Pro, $\gamma\text{-CH}_2$ Pro, SMe, 4^*CH_2 Far, $\alpha\text{-CH}_2$ MIC, NH- $\text{CH}_2\text{-CH}_2\text{NH}$); 2.25 (s, 3H, CH_3 Bodipy®FL); 2.45 (s, 3H, CH_3 Bodipy®FL); 2.58 (m, 2H, CH_2 Bodipy®FL); 2.85 (m, 2H, $\gamma\text{-CH}_2$ Met); 3.05-3.35 (m, 6H, $\beta\text{-CH}_2$ Cys, Far $\text{CH}_2\text{-S}$, CH_2 Bodipy®FL); 3.40 (m, 4H, NCH₂ MIC, NH- $\text{CH}_2\text{-CH}_2\text{NH}$); 3.65 (m, 8H, $\alpha\text{-CH}_2$ Gly, $\delta\text{-CH}_2$ Pro, $\alpha\text{-CH}_2$ Gly', $\beta\text{-CH}_2$ Ser); 4.20-4.70 (m, 4H, $\alpha\text{-CH}$ Met, $\alpha\text{-CH}$ Cys, $\alpha\text{-CH}$ Ser, $\alpha\text{-CH}$ Leu, $\alpha\text{-CH}$ Pro); 5.00 (m, 2H, CH Far); 5.10 (m, 1H, CH Far); 6.08

(s, 1H, CH Bodipy®FL); 6.18 (m, 1H, CH Bodipy®FL); 6.65 (s, 2H, -CH=CH- MIC); 6.85 (m, 1H, CH Bodipy®FL); 7.10 (s, 1H, CH Bodipy®FL); 7.25-7.95 (m, 8H, NH); MS (HPLC/ESI, water/acetonitrile/formic acid): m/z: calcd for $[M+H]^+$: 1377.7; found: 1377.6; $C_{67}H_{99}BF_2N_{12}O_{12}S_2$ (1377.5).

N-Fluorenylmethoxycarbonyl-S-farnesyl-L-cysteine (Fmoc-Cys(Far)-OH)(14):

Compound **14** was prepared using *N*-fluorenylmethoxycarbonyl-L-cysteine (2.07 g, 6.03 mmol) and farnesyl bromide (2.20 ml, 8.04 mmol), by means of the procedure described for the synthesis of **7a**. Purification of the resulting oil by flash chromatography using *c*-hexane/ ethyl acetate (gradient from 5:1 to 1:5) as eluent obtained 1.27 g (2.32 mol, 39%) of the desired product **14** as a colorless oil. R_f 0.10 (*c*-hexane/ ethyl acetate (1:1)); $[\alpha]^{20}_D = -2.5^\circ$ ($c = 1$, $CHCl_3$); 1H NMR (250 MHz, $CDCl_3$): $\delta = 1.60$ (s, 3H, CH_3 Far); 1.62 (s, 3H, CH_3 Far); 1.65 (s, 3H, CH_3 Far); 1.70 (s, 3H, CH_3 Far); 1.98-2.13 (m, 8H, CH_2 Far); 2.94 (dd, $J = 14.0$ Hz, $J = 7.0$ Hz, 1H, β - CH_{2a} Cys); 3.04 (dd, $J = 14.0$ Hz, $J = 7.0$ Hz, 1H, β - CH_{2b} Cys); 3.21 (tr, $J = 6.6$ Hz, 2H, Far CH_2 -S); 4.22 (t, $J = 7.0$ Hz, 1H, CH Fmoc); 4.39 (m, 2H, CH_2 Fmoc); 4.63 (m, 1H, α -CH Cys); 5.11 (m, 2H, CH Far); 5.23 (t, $J = 7.0$ Hz, 1H, CH Far); 5.97 (m, 1H, NH); 7.29 (dd, $J = 7.2$ Hz, $J = 7.2$ Hz, 2H, CH Fmoc); 7.37 (dd, $J = 7.2$ Hz, $J = 7.2$ Hz, 2H, CH Fmoc); 7.61 (d, $J = 7.2$ Hz, 2H, CH Fmoc); 7.73 (d, $J = 7.2$ Hz, 2H, CH Fmoc); ^{13}C NMR (100.6 MHz, $CDCl_3$): $\delta = 16.3$; 16.4; 18.0; 26.0 (4* CH_3 Far); 26.7; 27.2 (2* CH_2 Far); 30.4 (β - CH_2 Cys); 33.7 (S- CH_2 Far); 39.9; 40.0 (2* CH_2 Far); 47.3 (CH Fmoc); 54.2 (α -CH Cys); 67.7 (CH₂ Fmoc); 119.9 (CH Fmoc); 120.2; 124.1; 124.7 (CH Far); 125.4 (CH Fmoc); 127.4 (CH Fmoc); 127.9 (CH Fmoc); 131.4; 135.5 (2*Cq Far); 140.2 (Cq Fmoc); 141.5 (Cq Far); 144.0 (Cq Fmoc); 156.5 (C=O Fmoc); 171.9 (C=O Cys); MS (FAB, 3-NBA): m/z: calcd for $[M+H]^+$: 547.27; found: 546.11; $C_{33}H_{41}NO_4S$ (547.7).

N-Fluorenylmethoxycarbonyl-S-hexadecyl-L-Cysteine-tert-butylester (Fmoc-Cys(HD)-OtBu) (15):

To a solution of S-Hexamethyl-L-cysteine-*tert*-butylester (1.85 g, 4.60 mmol) in a solution of 10% aqueous Na_2CO_3 (4 ml) and dioxane (2 mL), stirring vigorously at 0°C under an argon atmosphere, was added a solution of 9-Fluorenylmethoxycarbonylchloride (1.19 g, 4.60 mmol) in dioxane (3 mL). The reaction mixture was left stirring at room temperature for 8 hours, poured into a separation funnel containing diethyl ether (10 mL) and water (40 mL). The layers were separated and the aqueous layer was washed once more with diethyl ether. The pH of the aqueous phase was adjusted with concentrated HCl to pH 1 and extracted with ethyl acetate (3 x 10 mL).

The combined organic phases were washed with water, dried over MgSO₄, filtered and the solvent was removed *in vacuo*. Purification of the resulting oil by flash chromatography using *c*-hexane/ ethyl acetate (gradient from 15:1 to 2:1) as eluent obtained 1.90 g (3.05 mol, 66%) of the desired product 15 as a colorless oil. *R*_f 0.5 (*c*-hexane/ethyl acetate (5:1)); [α]_D²⁰ = -2.8 ° (c = 1, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ = 0.94 (t, *J* = 6.8, 3H, ? -CH₃ HD); 1.53 (s, 3H, CH₃ tBu); 1.25-1.63 (m, 28H, CH₂ HD); 2.58 (t, *J* = 7.2 , 3H, α-CH₂ HD); 2.98 (dd, *J* = 14.0 Hz, *J* = 7.0 Hz, 1H, β-CH_{2a} Cys); 3.05 (dd, *J* = 14.0 Hz, *J* = 7.0 Hz, 1H, β-CH_{2b} Cys); 4.25 (t, *J* = 7.0 Hz, 1H, CH Fmoc); 4.40 (m, 2H, CH₂ Fmoc); 4.56 (m, 1H, α-CH Cys); 5.86 (m, 1H, NH); 7.32 (dd, *J* = 7.2 Hz, *J* = 7.2 Hz, 2H, CH Fmoc); 7.39 (dd, *J* = 7.2 Hz, *J* = 7.2 Hz, 2H, CH Fmoc); 7.63 (d, *J* = 7.2 Hz, 2H, CH Fmoc); 7.74 (d, *J* = 7.2 Hz, 2H, CH Fmoc); ¹³C NMR (100 MHz, CDCl₃) δ = 14.5; 23.0; 27.2; 28.2; 29.1; 29.5; 29.7; 29.8; 29.9; 30.0; 32.2; 33.3; 34.9 (15 CH₂ HD, CH₃ tBu, β-CH₂ Cys); 47.2 (CH Fmoc); 54.6 (α-CH Cys); 67.4 (CH₂ Fmoc); 82.7 (C_q tBu); 120.3; 125.4; 127.4; 128.1 (CH Fmoc); 141.5 (C_q Fmoc); 144.1 (C_q Fmoc); 155.8 (C=O Fmoc); 170.1 (C=O Cys); MS (FAB, 3-NBA): m/z: calcd for [M+Na]⁺ 623.4008, found 623.4023; C₃₈H₅₇NO₄S (623.9).

N-Fluorenylmethoxycarbonyl-S-hexadecyl-L-cysteine (Fmoc-Cys(HD)-OH) (16): To a solution of *N*-Fluorenylmethoxycarbonyl-S-hexadecyl-L-cystein-*tert*-butylester 15 (1.9 g, 3.1 mmol) in 2 mL dichloromethane was added TFA (5 mL) and stirred for 1 hour at room temperature. After addition of thioanisole (1 mL) the solvent was evaporated *in vacuo* using toluene as cosolvent to obtain 1.62 g (2.85 mol, 94%) of the desired product 16 as a colorless oil. *R*_f 0.2 (*c*-hexane/ethyl acetate (5:1)); [α]_D²⁰ = 6.2 ° (c = 1, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ = 0.92 (t, *J* = 6.8, 3H, ? -CH₃ HD); 1.24-1.39 (m, 26H, CH₂ HD); 1.51-1.63 (m, 2H, β-CH₂ HD); 2.56 (t, *J* = 7.2 , 3H, α-CH₂ HD); 3.05 (m, 2H, β-CH₂ Cys); 4.24 (t, *J* = 7.0 Hz, 1H, CH Fmoc); 4.42 (m, 2H, CH₂ Fmoc); 4.67 (m, 1H, α-CH Cys); 5.82 (m, 1H, NH); 7.31 (dd, *J* = 7.2 Hz, *J* = 7.2 Hz, 2H, CH Fmoc); 7.40 (dd, *J* = 7.2 Hz, *J* = 7.2 Hz, 2H, CH Fmoc); 7.61 (d, *J* = 7.2 Hz, 2H, CH Fmoc); 7.76 (d, *J* = 7.2 Hz, 2H, CH Fmoc); ¹³C NMR (100 MHz, CDCl₃) δ = 14.5; 23.1; 27.2; 28.2; 29.1; 29.5; 29.7; 29.8; 29.9; 30.0; 32.2; 33.3; 34.5 (15 CH₂ HD, β-CH₂ Cys); 47.4 (CH Fmoc); 53.8 (α-CH Cys); 67.7 (CH₂ Fmoc); 119.8; 125.0; 127.0; 127.6 (CH Fmoc); 141.3 (C_q Fmoc); 143.8 (C_q Fmoc); 156.2 (C=O Fmoc); 175.3 (C=O Cys); MS (FAB, 3-NBA): m/z: calcd for [M+Na]⁺ 568.3382, found 568.3452; C₃₄H₄₉NO₄S (567.3).

Generation of semisynthetic neo-Ras-proteins:^[6] Prior to coupling the N-RasΔ181 protein was passed through a HiTrap® gel filtration column (Amersham Pharmacia Biotech) in order to remove any excess of salts and DTE required for storage of the protein. At all stages of the coupling reaction, all samples containing protein, unless otherwise stated, were kept by or below 4°C. To a solution of the peptide dissolved in methanol (50 µL) in a 1.5 ml Eppendorf tube a buffer of 11% Triton X-114 (Fluka) containing 30 mM Tris/HCl, 100 mM NaCl (1 mL) was added. The detergent solution was cooled to 0°C and 1 mL of an aqueous solution (20 mM Tris/HCl, 5 mM MgCl₂, pH 7.4) containing 20 mg of the Ras protein was added. The coupling reaction was performed with stoichiometric amounts of peptide and protein under argon and incubated at 4°C for 16 h. The soluble supernatant was diluted with 3 mL buffer (containing 2 mM DTE), and heated to 37°C resulting in a phase separation of the detergent phase from the aqueous phase after centrifugation at room temperature. The aqueous phase was removed and extracted two more times with a 11% Triton X-114 detergent solution (2 x 1 mL). The detergent phases were combined and washed three times with fresh buffer (3 x 7 mL). The protein extract was diluted to 2% Triton with fresh buffer A, and applied on a DEAE-sepharose column. Bound protein was eluted with a sodium chloride gradient (0 M to 1 M NaCl) and concentrated. The product was analyzed by MALDI-TOF MS (Perseptive Biosystems) and SDS-PAGE.

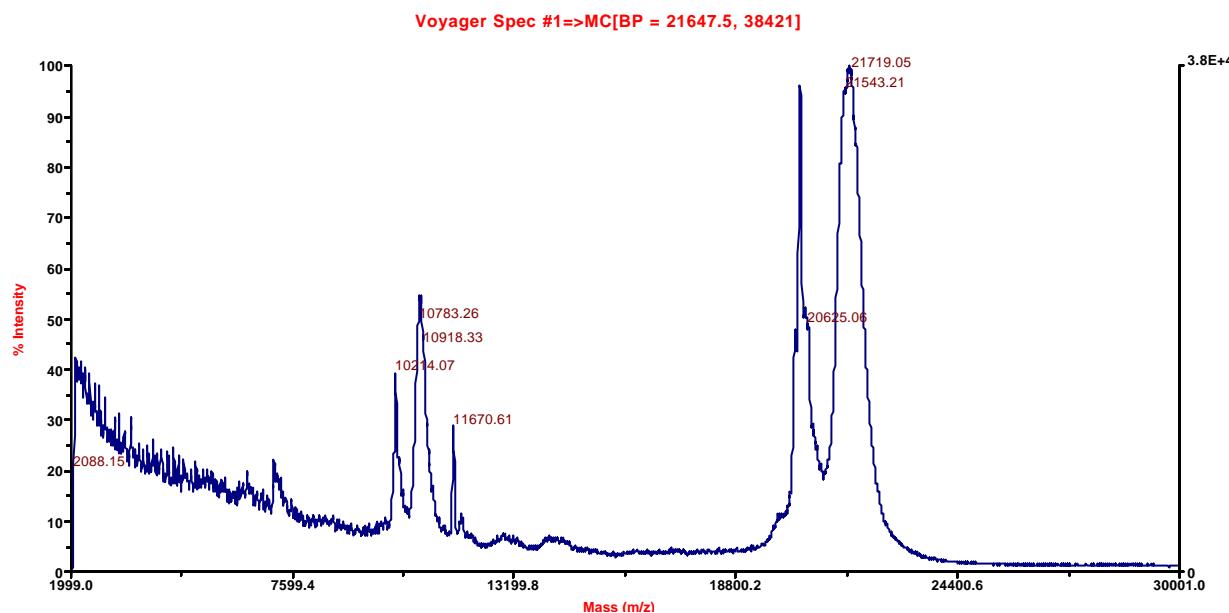
The MALDI-TOF MS show the mass of the N-RasΔ181 (20440 Da) and a broad peak in the range of the semisynthetic neo-Ras-proteins due to decomposition of the Bodipy.

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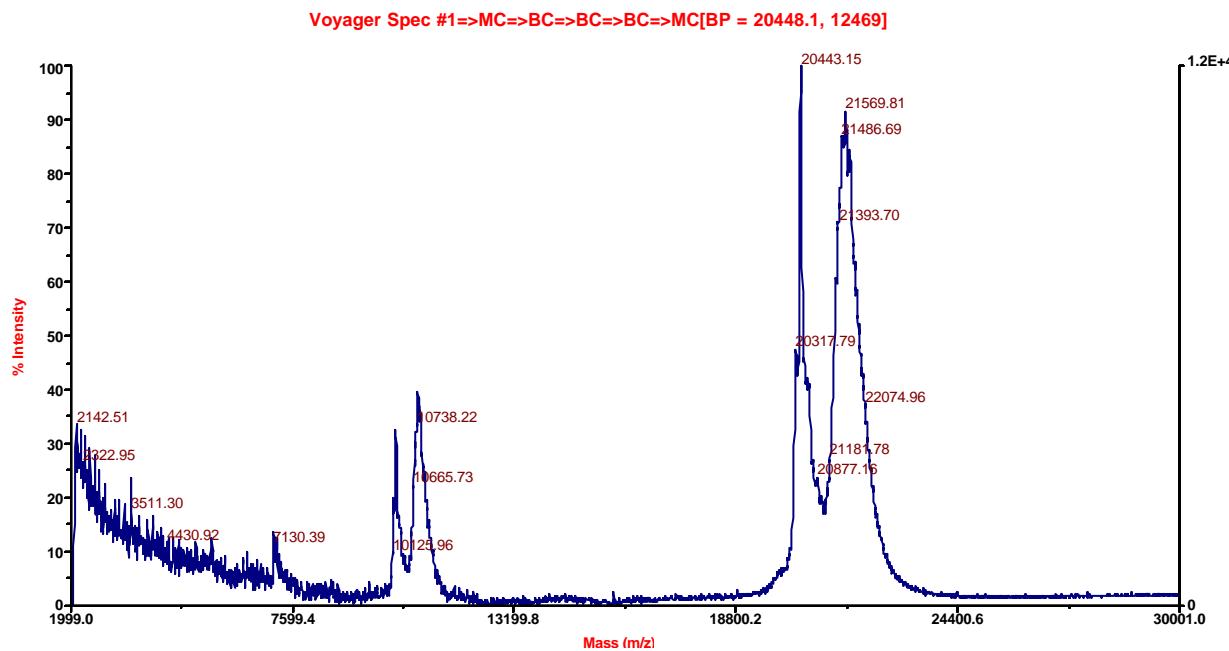
N-RasD181 MIC-Gly-Cys-Met-Gly-Leu-Pro-Cys(Far)-NH-Et-NH-Bodipy®FL (13a):

21835 Da



N-RasD181 MIC-Gly-Cys-Met-Gly-Leu-Pro-Cys(Far)-NH-Et-NH-Bodipy®TR (13b):

22080 Da



N-RasD181 MIC-Gly-Cys(Hd)-Met-Gly-Leu-Pro-Cys(Far)-NH-Et-NH-Bodipy®FL (13c):

22060 Da

