Supplementary materials

An easy tool to monitor the elemental steps of *in vitro* translation via gel electrophoresis of fluorescently labelled small peptides

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Supplementary Table 1. Sequences of primers used to generate templates for coupled *in vitro* transcription-translation.

Primer	sequence*
MF	ATGCATAATACGACTCACTATAGGGCTTAAGTATAAGGAGGAAAACAT**ATGTTCTAA**GAGACGGACGAGAGCGGC
MF_2	ATGCATAATACGACTCACTATAGGGCTTAAGTATAAGGAGGAAAACAT**ATGTTCTTCTAA**GAGACGGACGAGAGCGGC
ML_2	ATGCATAATACGACTCACTATAGGGCTTAAGTATAAGGAGGAAAACAT**ATGTTGTTGTAA**GAGACGGACGAGAGCGGC
MF_4	ATGCATAATACGACTCACTATAGGGCTTAAGTATAAGGAGGAAAACAT**ATGTTCTTCTTCTTCTAA**GAGACGGACGAGAGCGGC
MF_6	ATGCATAATACGACTCACTATAGGGCTTAAGTATAAGGAGGAAAACAT**ATGTTCTTCTTCTTCTTCTTCTAG**GAGACGGACGAGAGCGGC
MF (UAA)	ATGCATAATACGACTCACTATAGGGCTTAAGTATAAGGAGGAAAACAT**ATGTTCTAA**GAGACGGACGAGAGCGGC
MF (UAG)	ATGCATAATACGACTCACTATAGGGCTTAAGTATAAGGAGGAAAACAT**ATGTTCTAG**GAGACGGACGAGAGCGGC
MF (UGA)	ATGCATAATACGACTCACTATAGGGCTTAAGTATAAGGAGGAAAACAT**ATGTTCTGA**GAGACGGACGAGAGCGGC
reverse	GCGTTAAGGCTATGTACGGAACAGCTCCTCGCCCTTG

*coding parts are shown in bold.

Supplementary Table 2. Sequences of mRNAs for *in vitro* translation on the basis of individually purified components.

mRNA	sequence*
MF	GGAAUUCAAAAAUUUAAAAGUUAACAGGUAUACAUACU**AUGUUU**ACGAUUACUACGAUCUUCUUCACUUAAUGCGUCUGCAGGCAUGCAAGC
MF_6	GGCACUUAAGGAGGUAUACU**AUGUUCUUCUUCUUCUUCUUC**UCUUCUUCUUCUUCUUCUUCUUCUUCUUCUUCUUCUUCTUCGUUUGAAGCUU

*coding parts are shown in bold.

SUPPLEMENTARY MATERIAL AND METHODS

The synthesis of the sulfosuccinimide ether BODIPY FL

The synthesis of the sulfosuccinimide ether BODIPY FL (Fig. S1, 1) (in the form of a sodium salt) was performed from 3-[4,4-difluoro-5,7-dimethyl-4-boron-3a,4a-diase-s-indacene-3-yl] propionic acid (2), which in turn can be obtained from pyrrol-2-carboxyaldehyde according to the methods described previously (1). Activation of the carboxyl group 2 was performed using N,N-dicyclohexylcarbodiimide (DCC), with subsequent interaction with the sodium salt of N-hydroxysulfosuccinimide in dry N,N-dimethylformamide (Fig. S1). Filtration of by-product (N,N-dicyclohexyl urea), followed by removal of the solvent in vacuum at room temperature, and trituration of the residue with ethyl acetate leads to the production of 1 in the form of an orange powder.

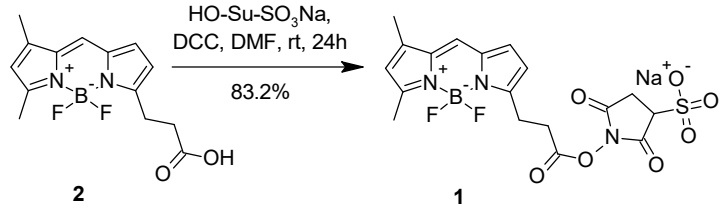

Figure S1. Synthesis of BODIPY FL SSE (1)

1.47 g (5.0 mmol; 1.0 eq) of 3-[4,4-difluoro-5,7-dimethyl-4-boron-3a,4a-diase-s-indacene-3-yl]propionic acid (2), 1.08 g (5.0 mmol; 1.0 eq) sodium salt of N-hydroxysulfosuccinimide and 40 ml of freshly distilled N,N-dimethylformamide (DMF) were added to a dry argon-filled flask with a volume of 250 ml. Then, 1.34 g (6.5 mmol; 1.30 eq) of N,N-

dicyclohexylcarbodiimide (DCC) was added to the flask at a time, and a dark orange suspension was stirred at room temperature for 24 hours. The suspension was cooled to +3 °C, the precipitated N, N-dicyclohexylurea was filtered out and washed on a filter with a 2x10 ml of dimethylformamide. After by-product removal, the solvent was removed from the reaction mixture using the vacuum dryer (~0.5 mmHg) fitted with a liquid nitrogen trap. The viscous residue in the flask was rubbed with dry ethyl acetate. Finally, the product was separated by centrifugation, washed with dry ethyl acetate (3x30 ml) containing no acetic acid and dried in vacuum (0.05 mmHg) at room temperature for 3 hours. The yield is 1.95 g (83.2%). The product is a bright orange powder, easily soluble in water.

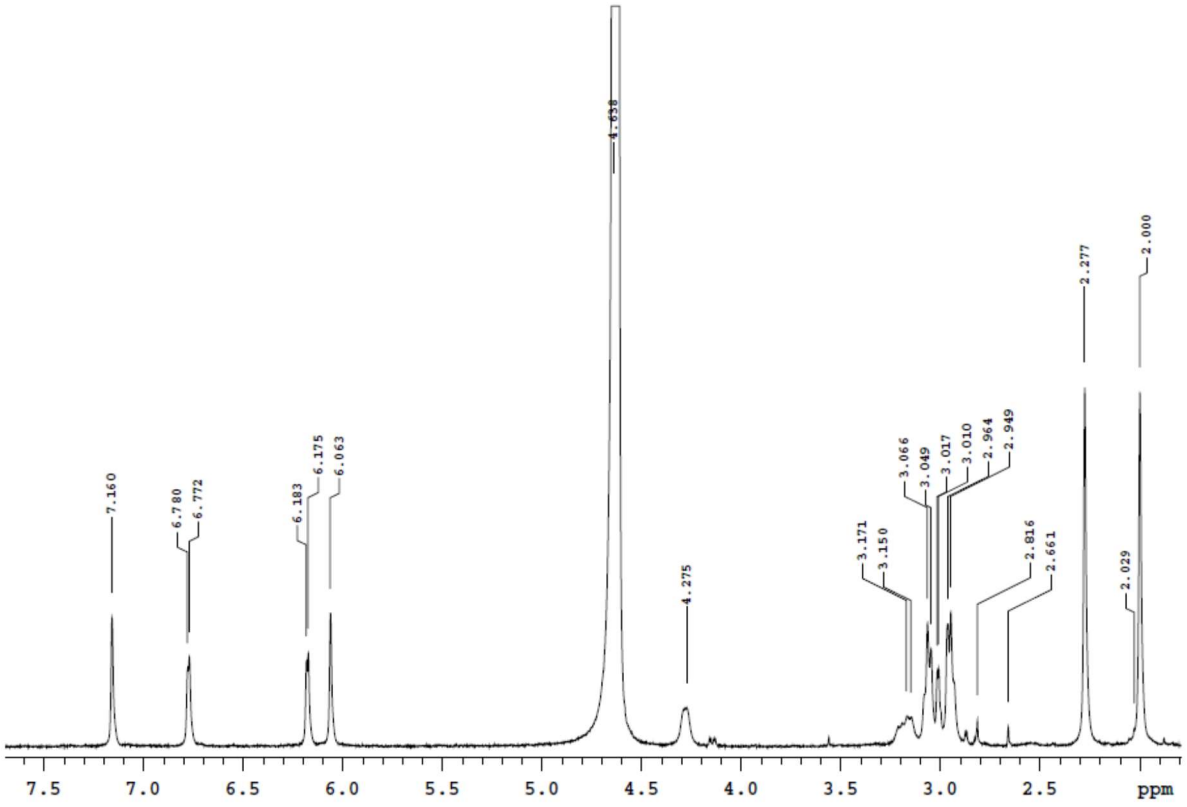

Figure S2. 1H NMR spectra (D_2O; 400 MHz)

Preparation of BODIPY-Met-tRNA^{fMet}.

Initiator tRNA from E. coli (tRNA^{fMet}) was incubated in aminoacylation buffer (25 mM Tris-acetate, pH 7.5, 10 mM $MgCl_2$, 100 mM NH_4Cl, 30 mM KCl) with 2 mM DTT, 3 mM ATP, 60 µM Methionine, 20 U/ml tRNA^{fMet} and 5% vol/vol of *E. coli* crude extract S100 containing all tRNA synthetases for 30 min at 37 °C. After incubation, 1/10 volume of 20% KOAc pH 5.0 was added to stop the reaction, followed by phenol extraction and ethanol precipitation of nucleic acids. Aminoacylated Met-tRNA^{fMet} was separated by HPLC using LiChrosphere WP 300 column (250-10 RP-18 5 mkM, Merck) equilibrated in buffer A (20 mM NH_4OAc, 10 mM $MgCl_2$, 400 mM NaCl, 5% EtOH). Met-tRNA^{fMet} containing fractions were collected on a linear gradient from 0 to 100% buffer B (20 mM NH_4OAc, 10 mM $MgCl_2$, 400 mM NaCl, 15% EtOH), pooled and precipitated with ethanol. The obtained pellets were dried and dissolved in H_2O. The concentration of the Met-tRNA^{fMet} was determined by measuring the OD at 260 nm. To modify the α-amino group of the Met-tRNA^{fMet} with BODIPY-FL, SSE (Invitrogen, D6140, or prepared as described previously), the aminoacylated tRNA (30 µM final) was incubated with a 100-fold excess of BODIPY-FL, SSE (3 mM) in 50 mM HEPES buffer (pH 8.5) for 10 min at 0 °C. 1/10 volume of 20% KOAc pH 5.0 was added to stop the reaction, followed by the ethanol precipitation of tRNA.

Purification of BODIPY-Met-tRNA^{fMet}.

Four additional ethanol precipitation steps were done to remove the dye excess. BPY-Met-tRNA^{fMet} was separated by HPLC. LiChrosphere WP 300 (Merck) column was equilibrated in buffer A (20 mM NH_4OAC, 10 mM $MgCl_2$, 400 mM NaCl, 5% EtOH). BODIPY-Met-tRNA^{fMet} was eluted on a linear gradient from 0 to 100% buffer B (20 mM NH_4OAC, 10 mM $MgCl_2$, 400 mM NaCl, 40% EtOH). BODIPY-Met-tRNA^{fMet} containing fractions were pooled and precipitated with ethanol. The obtained pellets were dried and dissolved in H_2O. BODIPY-Met-tRNA^{fMet} concentration was determined by measuring the OD at 260. Small aliquots were shock-frozen in liquid nitrogen and stored at -80C.

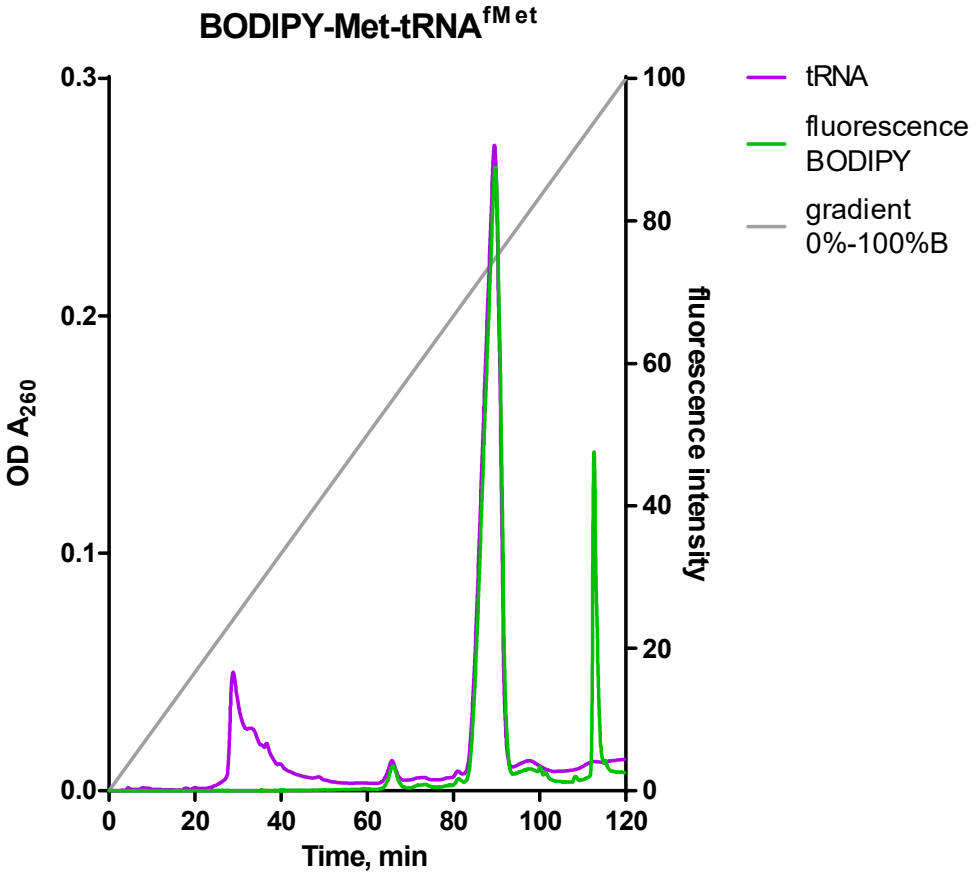

Figure S3. Separation of modified BPY-Met-tRNA^{fMet} by HPLC.

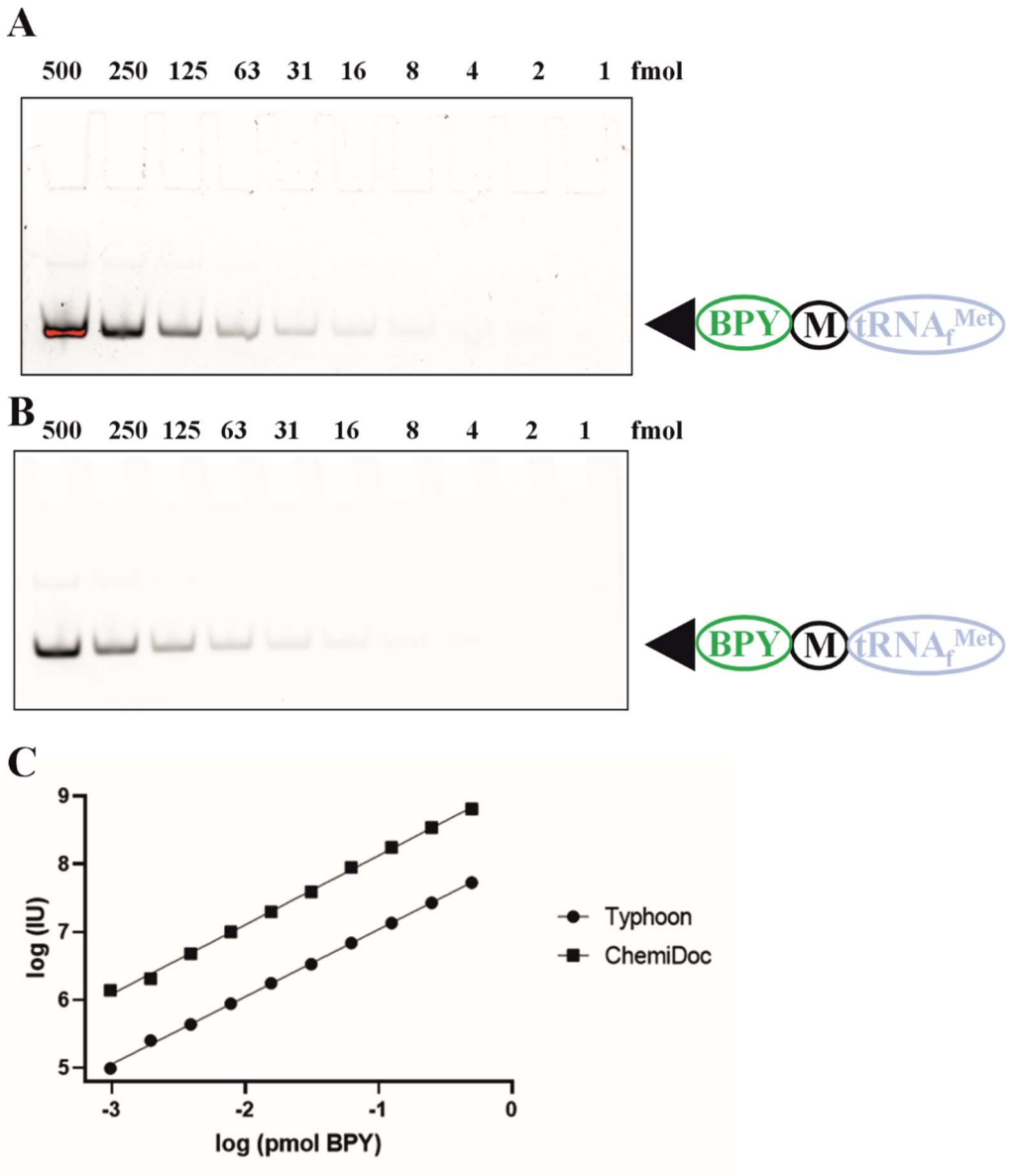

Figure S4. Sensitivity of single band BPY-Met-tRNA^{fMet} on regular UREA-PAGE visualized by A) ChemiDoc (Bio-Rad) and (B) Typhoon FLA 9500 (GE Healthcare). Serial dilutions of BPY-Met-tRNA^{fMet} from 0.5 pmol (left lane) to 1 fmol (right lane) were loaded on a gel. Down to 1 fmol ($1*10^{-15}$ mol) can be detected. (C). On both examples of detection, all dilutions were in the linear range.

The synthesis of BODIPY-Met-(Phe)$_2$ (BPY-MF$_2$) and BODIPY-Met-(Phe)$_4$ (BPY-MF$_4$)

Peptides Fmoc-Met-$(Phe)_2$-OH and Fmoc-Met-$(Phe)_4$-OH were synthesized according to the standard Fmoc solid-phase peptide synthesis protocol using a 2-chlorotrityl resin (1.5 mmol Cl^-/g) (2) and HBTU as an activating agent. Preparation of 2-chlorotrityl resin for synthesis was carried out in the following way. In a solid phase peptide synthesis reactor, 400 mg of resin was soaked in DMF for 10-15 min. The solvent was removed by filtration and the resin was washed sequentially with: dioxane (1×4 min), DMF (1×4 min), CH_2Cl_2 (1×4 min) using 10 ml of solvent per 1 g of resin. Next, the first amino acid was attached: Fmoc-Phe-OH (232 mg, 0.6 mmol, 2 eq. relative to resin loading) and DIPEA (104 µl, 0.6 mmol, 2 eq.) were dissolved in CH_2Cl_2 and added to the reactor with resin, then shaken for 10 min. After that, another 3 eq. of DIPEA was added and shaken for an hour. Then 0.4 ml of methanol was added and shaken for another 10 minutes. The solvent was separated by filtration, the resin was washed sequentially with CH_2Cl_2 (3×4 ml), DMF (3×4 ml), methanol (3×4 ml). The resin was then dried in a vacuum desiccator and loading was calculated. The rest of the amino acids were attached according to the following protocol: (1). Washing resin with DMF (3×4 ml). (2). Removal of Fmoc protection with 2 ml of 20% piperidine solution in DMF (1×5 min, 1×15 min). (3). Control of loading using Fmoc test (3). (4). Washing with DMF (2×4 ml). (5). Preactivation: Fmoc-amino acid (3 eq.), HBTU (3 eq.), DIPEA (3 eq.) were dissolved in DMF (1×5 min). (6). Addition of the mixture from point 5 to the resin. Coupling (30-60 min.). 0.6 eq. DIPEA was added 10-20 min after the beginning of coupling. (7). Solvent removal by filtering. Washing with alternating DMF (3×4 ml) and isopropanol (3×4 ml). (8).

Coupling control using the Kaiser test (4). In case of a negative result, we moved on to the next step. In case of a positive result, stages 5-7 were repeated. (9). Capping with 4 ml of a freshly prepared solution of acetic anhydride-DIPEA-DMF (5: 6: 89, v/v) (1×5 min, 1×30 min).

Steps 1-9 were repeated until the peptide reached the required length. In the final stage, after the removal of Fmoc-group (step 2), the resin was washed sequentially with DMF (1×5 min), CH_2Cl_2 (1×5 min) and a half of the Fmoc-Met-$(Phe)_2$-polymer was separated. The remaining resin was used to continue synthesis of Fmoc-Met-$(Phe)_4$-OH. The peptides were cleaved from the resin using a 50% solution of HFIP in CH_2Cl_2, then the solvents were removed on a vacuum evaporator and residues were crystallized under ether to give H-Met-$(Phe)_2$-OH and H-Met-$(Phe)_4$-OH, which were used in the next steps of synthesis without further purification.

H-Met-$(Phe)_2$-OH. Yield: 35 mg (53%). LC-MS, t_R = 1.30 min, m/z calculated for $[C_{23}H_{29}N_3O_4S + H]^+$ 444.20; found 445.11.

H-Met-$(Phe)_4$-OH. Yield: 61 mg (55%). LC-MS, t_R = 1.77 min, m/z calculated for $[C_{41}H_{47}N_5O_6S + H]^+$ 738.33; found 738.26.

BODIPY-Met-$(Phe)_2$-OH (BODIPY-MF_2). MF_2 (3.4 mg, 7.7 μmol) and DIPEA (2.6 μl, 15.5 μmol) were dissolved in 0.5 ml of DMF and BODIPY-C3-SE (1.5 mg, 3.85 μmol) was added. The mixture was stirred at 0 °C for 18 h. Then 10 ml of H_2O was added, the pH was adjusted to a value of 3 using 0.1 M HCl, and the mixture was extracted with ethyl acetate (3×10 ml). The organic fraction was dried over molecular cites (4Å) and evaporated on a vacuum evaporator, and the product was crystallized from the residue with petroleum ether. The precipitate was separated on a silica gel column

using a mixture of CH_2Cl_2 and methanol (6: 1, v/v) as eluent to give 2.38 mg (86%) of BODIPY-MF_2. LC-MS: t_R = 2.65 min, m/z calculated for $[C_{37}H_{42}BF_2N_5O_5S + Na]^+$ 740.29; found 740.65. ^{1}H NMR, *COSY*, *HMBC*, *HSQC* (DMSO-d_6, 600 MHz) δ (ppm): 12.76 (s, 1H, $F3_{COOH}$), 8.07 (d, J = 8.1 Hz, 1H, $M1_{HN}$), 8.01 (d, J = 8.3 Hz, 1H, $F2_{HN}$), 7.68 (s, 1H, $B0_{Hζ2}$), 7.28-7.20 (m, 8H, $F2_{Hδ}$, $F2_{Hε}$, $F3_{Hδ}$, $F3_{Hε}$), 7.19-7.14 (m, 2H, $F2_{Hζ}$, $F3_{Hζ}$), 7.06 (d, J = 4.0 Hz, 1H, $B0_{Hε3}$), 6.33 (d, J = 4.0 Hz, 1H, $B0_{Hδ2}$), 6.30 (s, 1H, $B0_{Hθ2}$), 4.50 (td, J = 9.0, 4.3 Hz, 1H, $F2_{Hα}$), 4.39-4.34 (m, 1H, $F3_{Hα}$), 4.32 (td, J = 8.4, 5.0 Hz, 1H, $M1_{Hα}$), 3.11-3.03 (m, 3H, $B0_{Hβ}$, $F3_{Hβ1}$), 3.00 (dd, J = 14.0, 4.6 Hz, 1H, $F2_{Hβ1}$), 2.93 (dd, J = 13.8, 7.9 Hz, 1H, $F3_{Hβ2}$), 2.76 (dd, J = 14.0, 9.6 Hz, 1H, $F2_{Hβ2}$), 2.55 (dt, J = 15.4, 7.9 Hz, 1H, $B0_{Hα1}$), 2.50-2.45 (m, 1H, $B0_{Hα2}$), 2.46 (s, 3H, $B0_{Hθ1}$), 2.32 (dd, J = 9.1, 6.7 Hz, 2H, $M1_{Hγ}$), 2.26 (s, 3H, $B0_{Hμ}$), 1.98 (s, 3H, $M1_{Hε}$), 1.84-1.75 (m, 1H, $M1_{Hβ1}$), 1.72-1.61 (m, 1H, $M1_{Hβ2}$). ^{13}C NMR, *HMBC*, *HSQC* (DMSO-d_6, 151 MHz) δ (ppm): 173.29 ($F3_C$), 171.45 (2C, $B0_C$, $M1_C$), 171.29 ($F2_C$), 159.61 ($B0_{Cη1}$), 158.32 ($B0_{Cγ}$), 144.62 ($B0_{Cη2}$), 138.22 (2C, $F2_{Cγ}$, $F3_{Cγ}$), 134.96 ($B0_{Cθ3}$), 133.53 ($B0_{Cε2}$), 129.72 (4C, $F2_{Cδ}$, $F3_{Cδ}$), 129.46 ($B0_{Cε3}$), 128.61 (2C, $F3_{Cε}$), 128.58 (2C, $F2_{Cε}$), 126.81 ($F2_{Cζ}$), 126.79 ($F3_{Cζ}$), 125.94 ($B0_{Cζ2}$), 120.84 ($B0_{Cθ2}$), 117.14 ($B0_{Cδ2}$), 54.33 ($F3_{Cα}$), 54.24 ($F2_{Cα}$), 52.33 ($M1_{Cα}$), 37.97 ($F2_{Cβ}$), 37.37 ($F3_{Cβ}$), 33.98 ($B0_{Cα}$), 32.49 ($M1_{Cβ}$), 30.03 ($M1_{Cγ}$), 24.37 ($B0_{Cβ}$), 15.13 ($M1_{Cε}$), 15.10 ($B0_{Cθ1}$), 11.54 ($B0_{Cμ}$).

BODIPY-Met-(Phe)$_4$-OH (BODIPY-MF$_4$). *BODIPY-MF$_4$* was synthesized according to the procedure described for *BODIPY-Met-(Phe)$_2$-OH (BODIPY-MF$_2$)* starting with MF_4 (5.7 mg, 7.7 μmol), DIPEA (2.6 μl, 15.5 μmol) and BODIPY-C3-SE (1.5 mg, 3.85 μmol). The crude product was purified by isocratic HPLC (45% acetonitrile in 10 mM water ammonium

acetate) to give 1.40 mg (36%) of BODIPY-MF_4. LC-MS: t_R = 3.15 min, m/z calculated for $[C_{55}H_{60}BF_2N_7O_7S]^+$ 1011.43; found 1010.39. ^{1}H NMR, *COSY*, *ROESY, HSQC* (DMSO-d_6, 600 MHz) δ (ppm): 12.78 (s, 1H, $F5_{COOH}$), 8.30 (br s, 1H, $F5_{HN}$), 8.10 (br s, 1H, $F4_{HN}$), 8.06 (br s, 1H, $M1_{HN}$), 8.02 (d, J = 7.7 Hz, 1H, $F3_{HN}$), 7.93 (br s, 1H, $F2_{HN}$), 7.68 (s, 1H, $B0_{Hζ2}$), 7.29-7.11 (m, 20H, F2-$F5_{Hδ}$, F2-$F5_{Hε}$, F2-$F5_{Hζ}$), 7.05 (d, J = 3.7 Hz, 1H, $B0_{Hε3}$), 6.32 (d, J = 3.7 Hz, 1H, $B0_{Hδ2}$), 6.30 (s, 1H, $B0_{Hθ2}$), 4.60-4.52 (m, 1H, $F4_{Hα}$), 4.49 (td, J = 8.8, 4.9 Hz, 1H, $F3_{Hα}$), 4.45 (td, J = 9.0, 4.3 Hz, 2H, $F2_{Hα}$, $F5_{Hα}$), 4.34-4.26 (m, 1H, $M1_{Hα}$), 3.12-2.99 (m, 4H, $B0_{Hβ}$, $F4_{Hβ1}$, $F5_{Hβ1}$), 2.97-2.91 (m, 2H, $F3_{Hβ1}$, $F5_{Hβ2}$), 2.89 (dd, J = 14.0, 3.7 Hz, 1H, $F2_{Hβ1}$), 2.78 (dd, J = 14.2, 9.0 Hz, 1H, $F4_{Hβ2}$), 2.73 (dd, J = 14.0, 9.0 Hz, 1H, $F3_{Hβ2}$), 2.69 (dd, J = 14.0, 9.4 Hz, 1H, $F2_{Hβ2}$), 2.52-2.47 (m, 2H, $B0_{Hα}$), 2.46 (s, 3H, $B0_{Hθ1}$), 2.30 (t, J = 7.8 Hz, 2H, $M1_{Hγ}$), 2.26 (s, 3H, $B0_{Hμ}$), 1.96 (s, 3H, $M1_{Hε}$), 1.80-1.71 (m, 1H, $M1_{Hβ1}$), 1.70-1.61 (m, 1H, $M1_{Hβ2}$).

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