Expression and purification of ψ-b* and Aha-ψ-b*

Methionine auxotrophic cells *E. coli* B834 were transformed with expression plasmid pQE80L-ψ-b*. An overnight culture in LB with ampicillin was prepared and 1 mL of this culture was used to inoculate 1 L of NMM defined medium (7.5 mM NH_4SO_4, 8.5 mM NaCl, 22 mM KH_2PO_4, 50 mM K_2HPO_4, 1 mM $MgSO_4$, 20 mM D-Glucose, 0.5 mM of every amino acid but methionine, 1 μg mL^{-1} $CaCl_2$, 1 μg mL^{-1} $FeCL_2$, 0.01 μg mL^{-1} of each $CuCl_2$, $ZnCl_2$, $MnCl_2$, $MoOHCl_2$, 10 μg mL^{-1} biotin, 10 μg mL^{-1} thiamine) supplemented with ampicillin and 45 μM methionine. Cultures were grown for approximately 12 h, during which methionine was depleted. OD_{600} was confirmed to be 0.7 and 0.5 mM met or aha were added, followed by induction of expression with 1 mM IPTG. Proteins were expressed for 4 h at 30 °C. The inclusion body isolation and refolding protocol was identical for both congeners. Cells were first harvested and lysed in lysis buffer (50 mM Tris-HCl pH 8) and lysozyme (0.5 mg mL^{-1}) was added. After 30 min at 0 °C the suspension was sonicated and subsequently spun down at 18500 x g, 4 °C for 60 min. The pellet with inclusion bodies was resuspended and homogenized in urea buffer (7.5 M urea, 50 mM Tris-HCl pH 8). Insoluble material was removed by centrifugation at 18500 x g, 4 °C for 60 min. The supernatant was dialyzed three times against 50 mM Tris-HCl pH 8 and 0.1 M NaCl at 4 °C. After a final centrigugation as described above, the supernatant containing the desired renatured ψ-b* congener and residual cellular proteins was loaded in batches of 10 mL onto a ResourceQ anion exchange column (GE Healthcare Bio-Sciences AB, Uppsala, Sweden), washed with 50 mM Tris-HCl pH 8.0 with 0.1 M NaCl and eluted with a gradient of 0.1 M NaCl in 50 mM Tris-HCl pH 8 to 1 M NaCl in 50 mM Tris-HCl pH 8. Eluates containing ψ-b* were pooled.

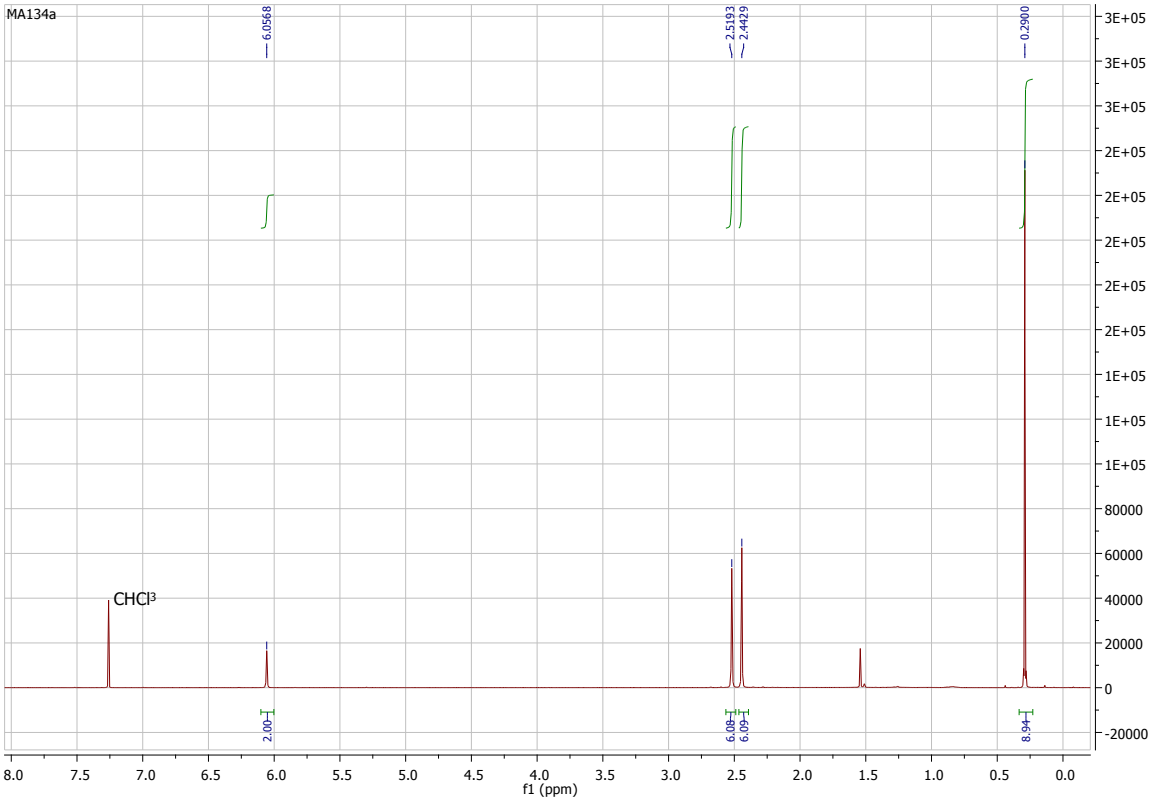

Figure S1: ^{1}HNMR (400 MHz) of **1** in $CDCl_3$.

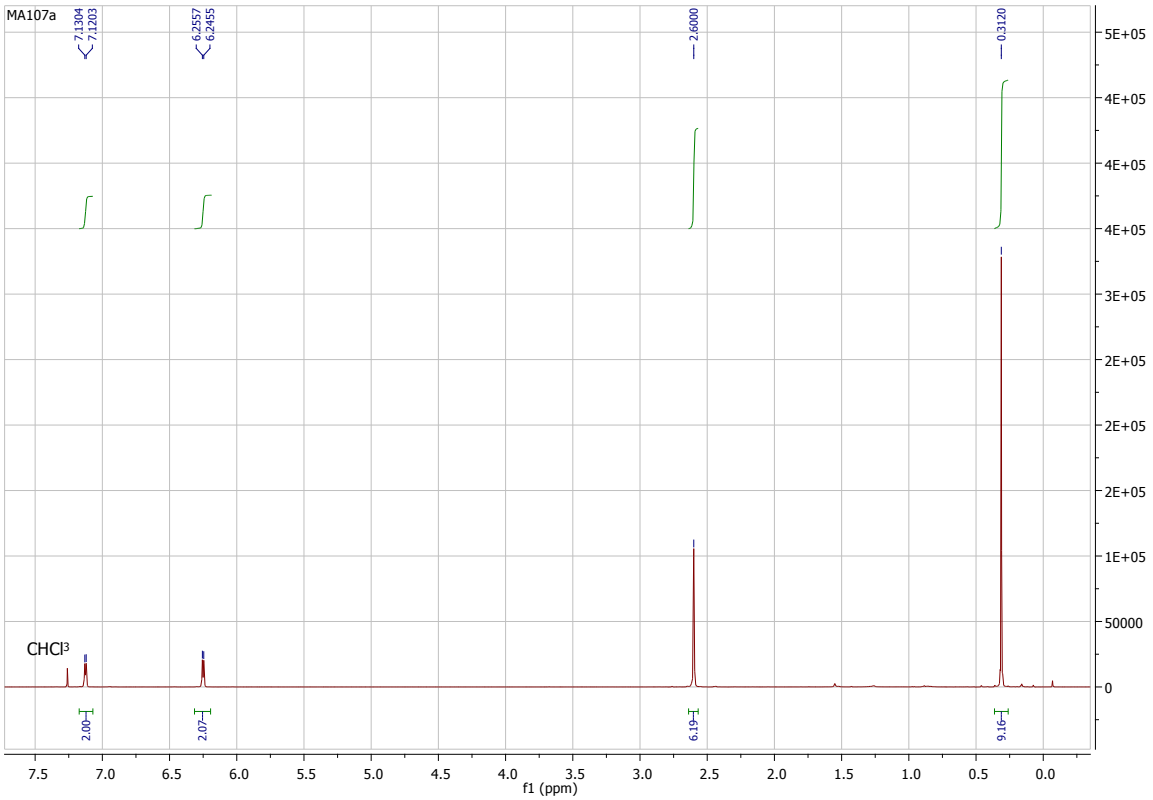

Figure S2: ^{1}HNMR (400 MHz) of **2** in $CDCl_3$.

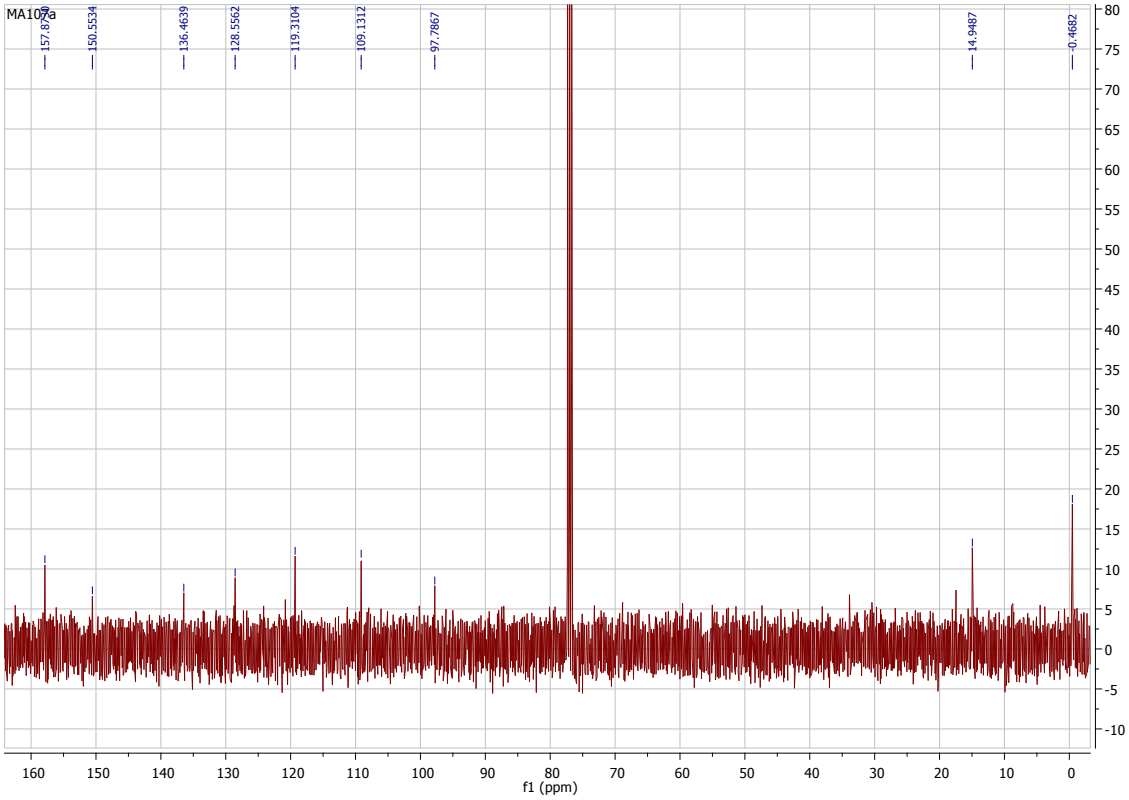

Figure S3: ^{13}CNMR (100 MHz) of **2** in $CDCl_3$.

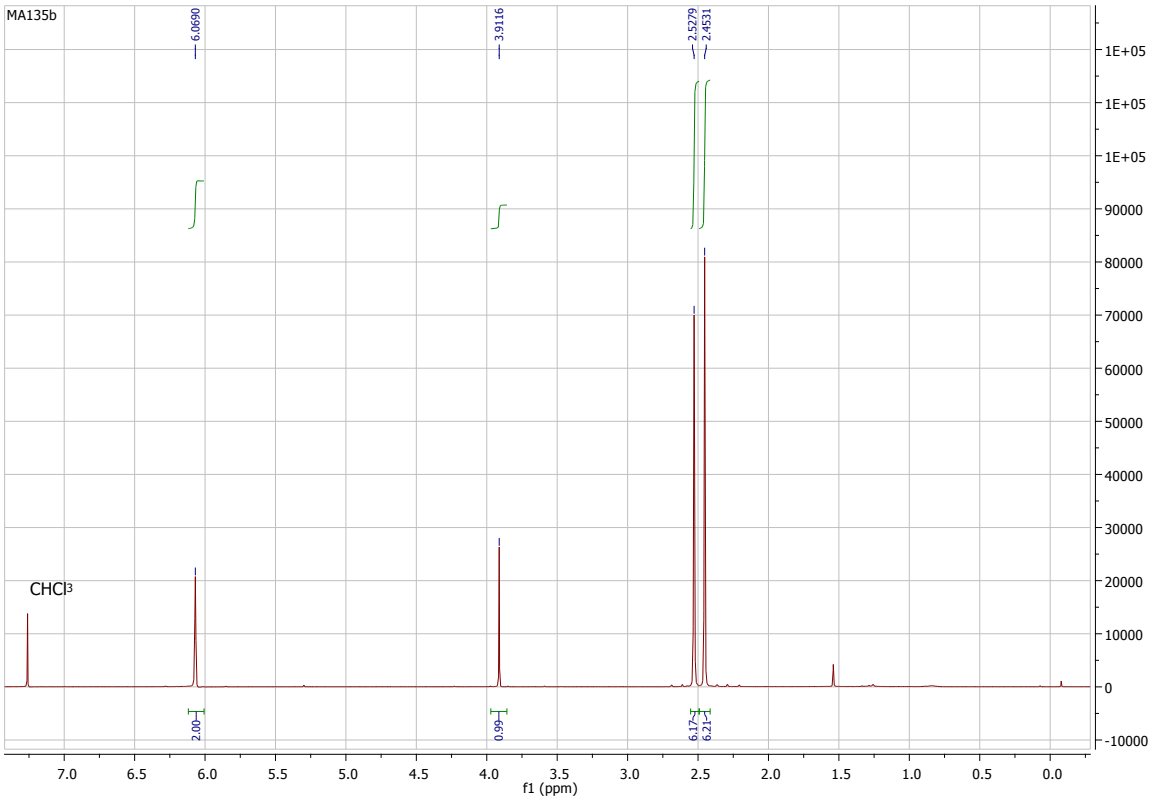

Figure S4: ^{1}HNMR (400 MHz) of **3** in $CDCl_3$.

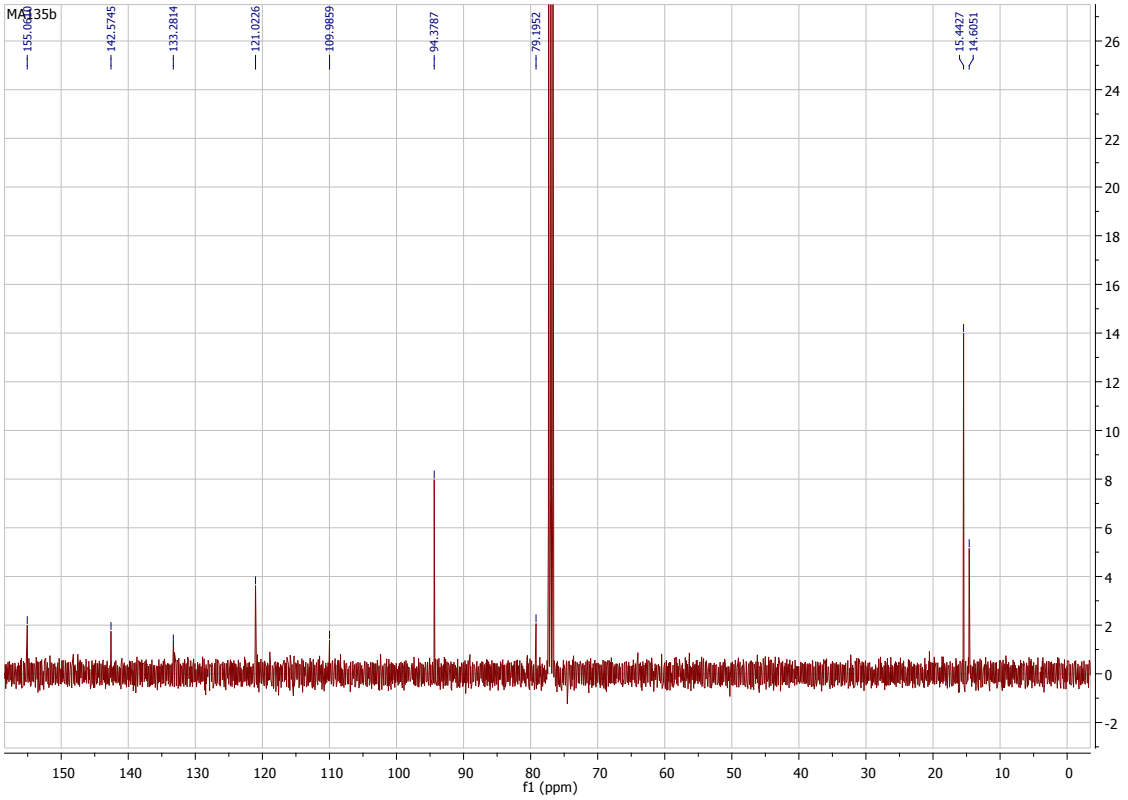

Figure S5: ^{13}CNMR (100 MHz) of **3** in $CDCl_3$.

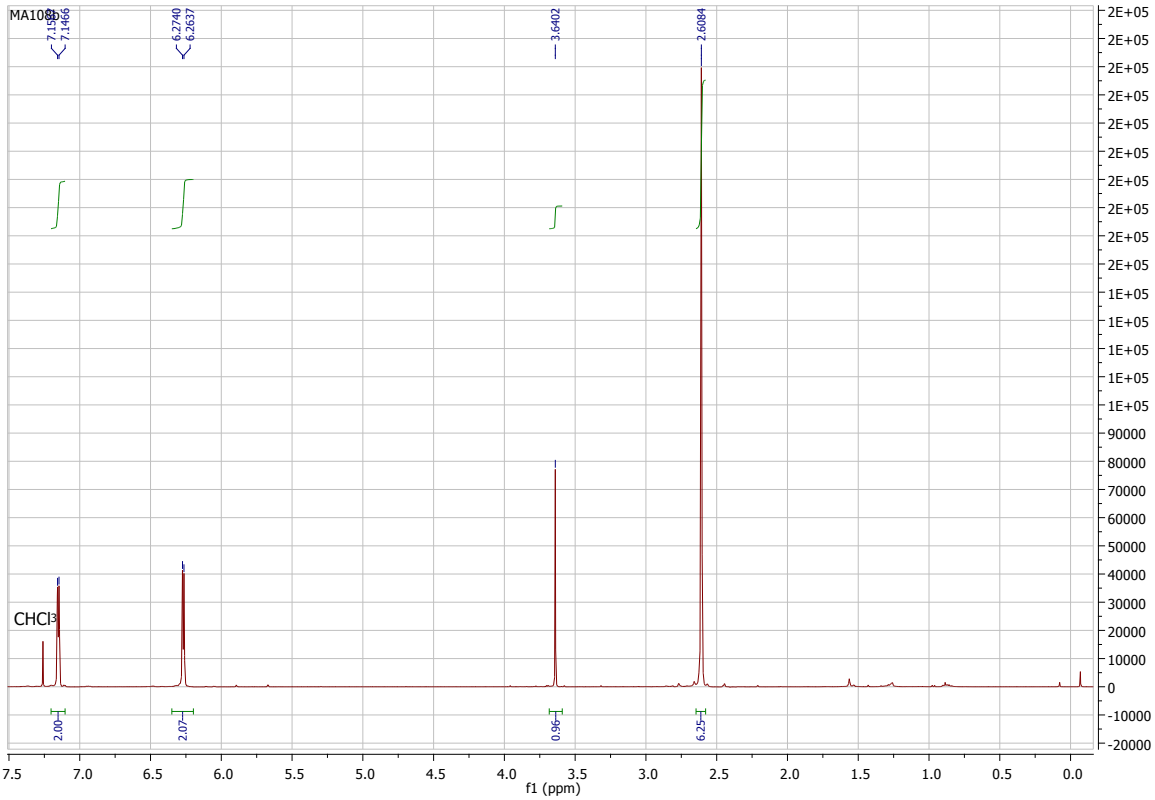

Figure S6: ^{1}HNMR (400 MHz) of **4** in $CDCl_3$.

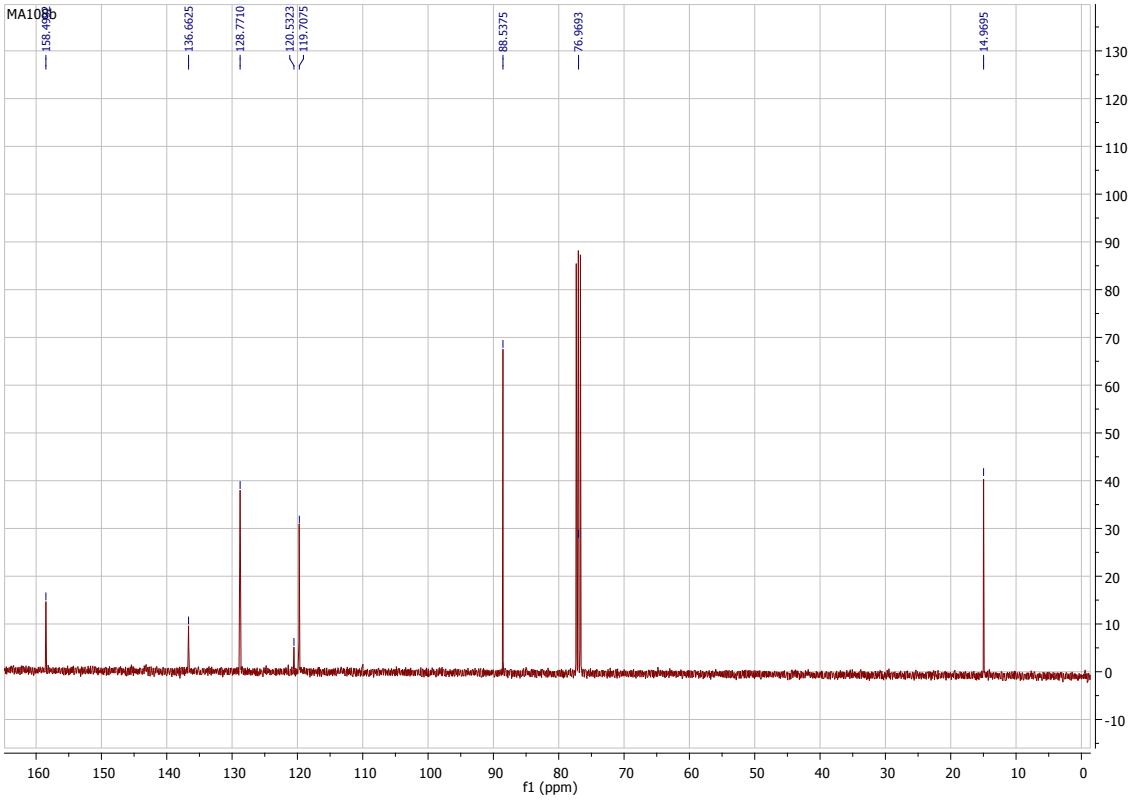

Figure S7: ^{13}CNMR (100 MHz) of **4** in $CDCl_3$.

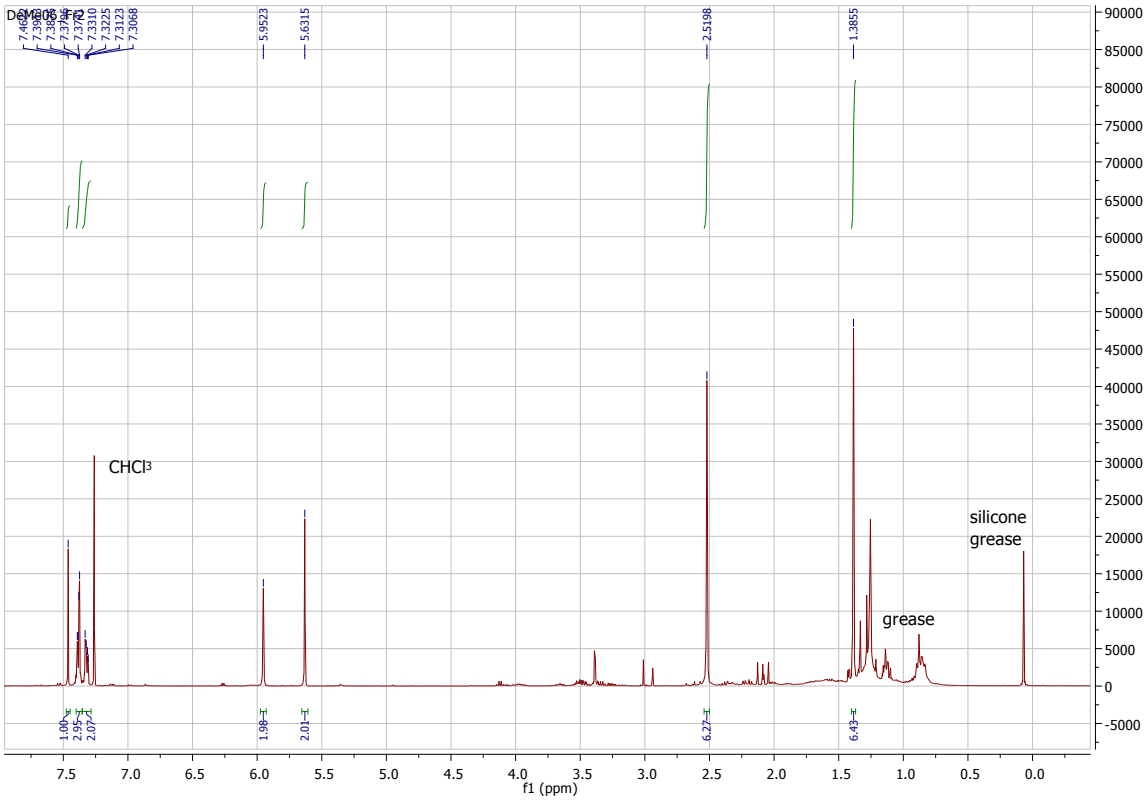

Figure S8: ^{1}HNMR (400 MHz) of **5** in $CDCl_3$.

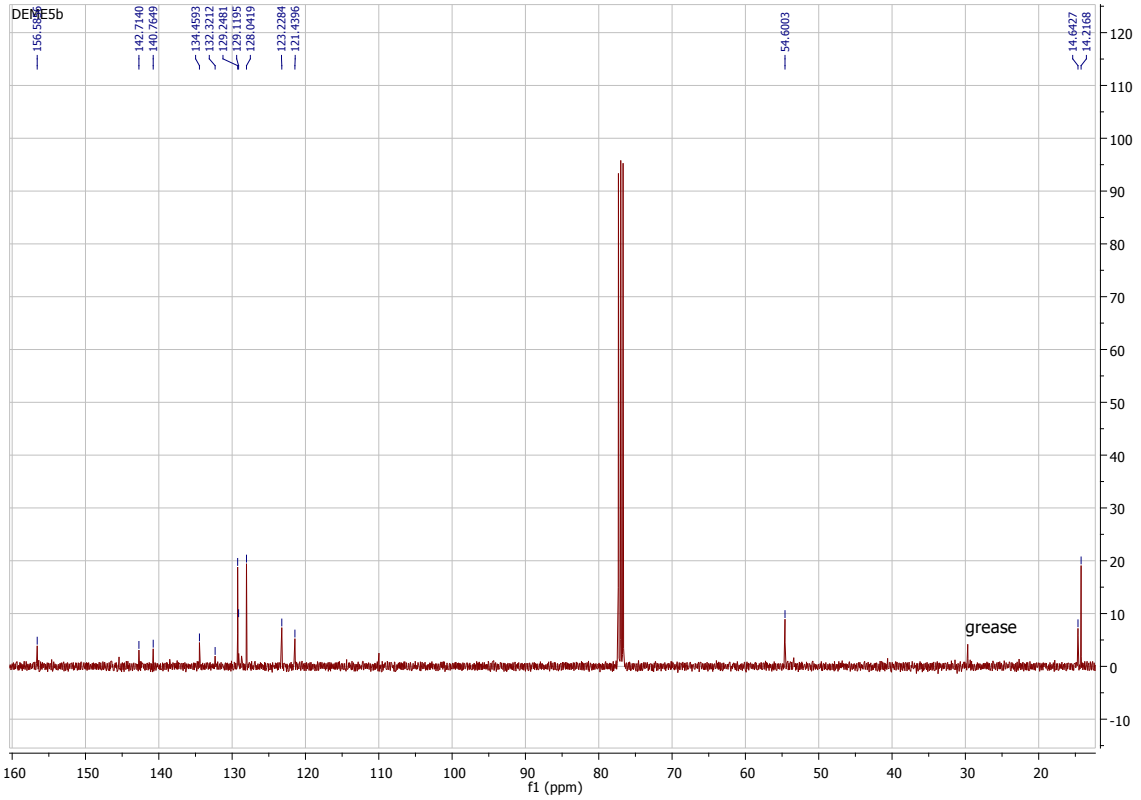

Figure S9: ^{13}CNMR (100 MHz) of **5** in $CDCl_3$.

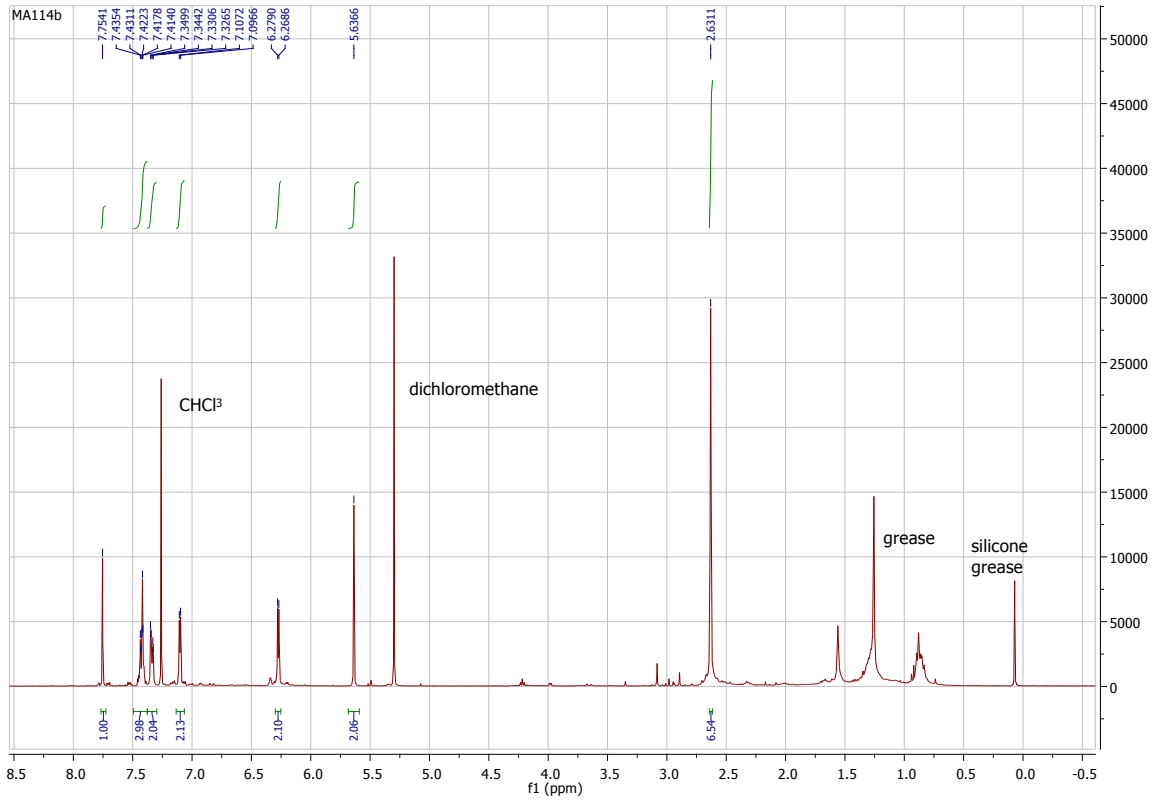

Figure S10: ^{1}HNMR (400 MHz) of **6** in $CDCl_3$.

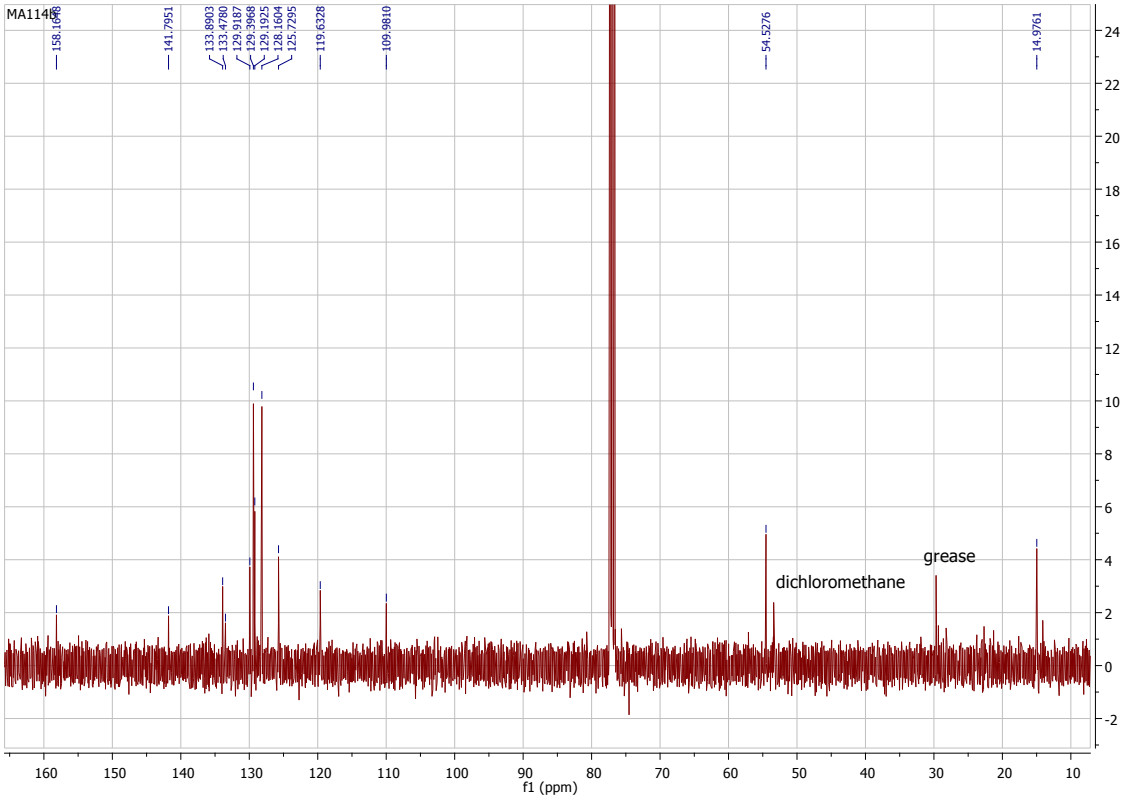

Figure S11: ^{13}CNMR (100 MHz) of **6** in $CDCl_3$.

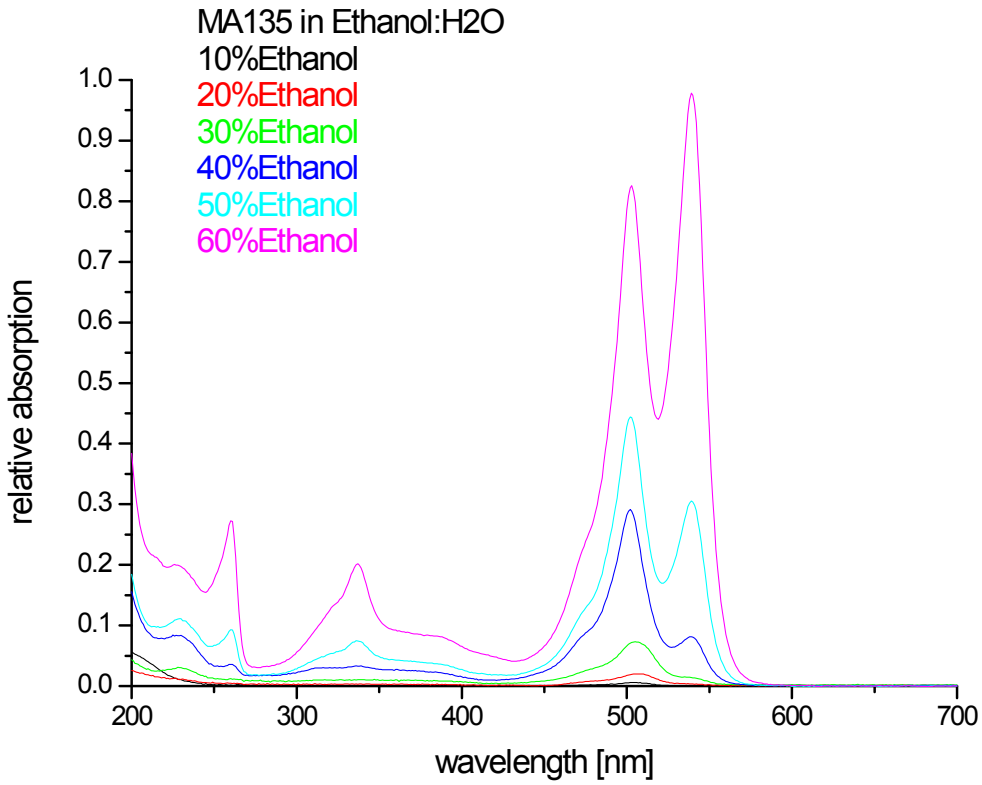

Figure S12: Absorption spectra of **3** in various ethanol/water mixtures.

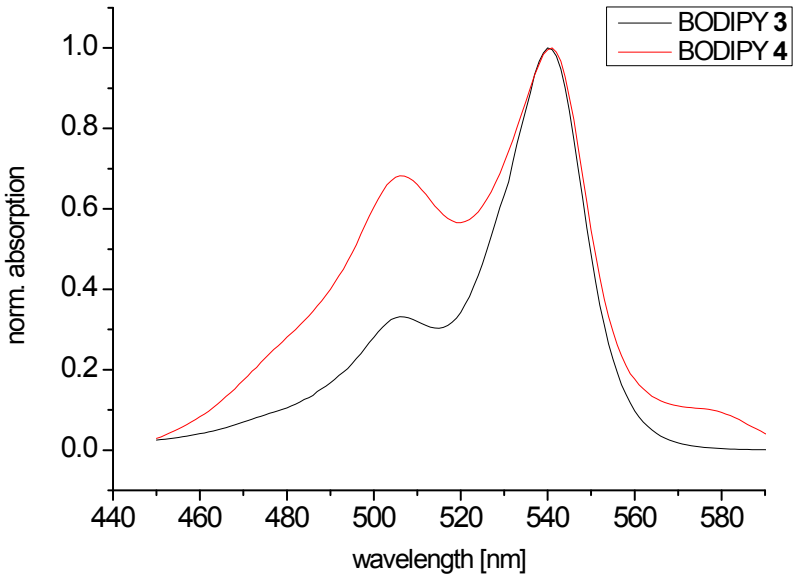

Figure S13: Normalized absorption spectra of BODIPYs **3**/**4** in ethanol/water (2/1 v/v).