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Facile synthesis of chlorin bioconjugates by a series of click reactions

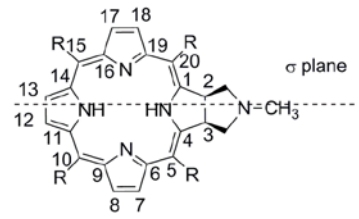
Junior Gonzales,[ab] N. V. S. Dinesh K. Bhupathiraju,[a] William Perea,[a] Huong Chu,[ab] Naxhije Berisha,[a] Veronica Bueno,[a] Naser Dodic,[a] Julia Rozenberg,[a] Nancy L. Greenbaum[ab] and Charles Michael Drain*[abc]

[a] Department of Chemistry, Hunter College of the City University of New York. 695 Park Avenue, New York, New York,10065 United States

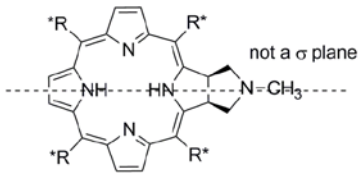
[b] Chemistry Program, Graduate Center of the City University of New York, 365 5th Avenue, New York, New York 10016, United States

[c] Rockefeller University, 1230 York Avenue, New York, New York 10065, United States

Email: cdrain@hunter.cuny.edu



(2*R*,3*S*) - meso when R is achiral



(2*R*,3*S*) - optically active
diastereomer when R* is chiral

Figure S1. Diastereomers result from chlorins bearing chiral R groups on the meso positions.

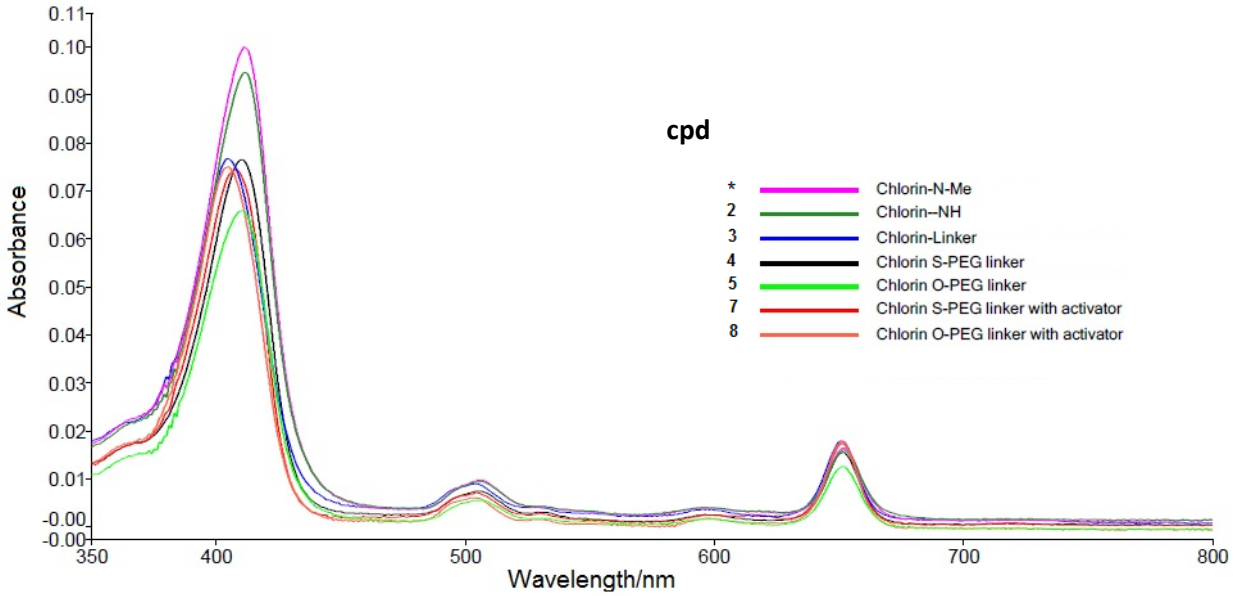


Figure S2. UV-visible spectra of published chlorin N-Me and chlorin NH compound 2, the S-PEG-chlorin 4, and the O-PEG-chlorin compound 5 and the corresponding chlorin NHS esters 7 and 8 at ca. 1 µM CH_2Cl_2. Taken on a Perkin-Elmer Lambda 35 UV-visible spectrometer.

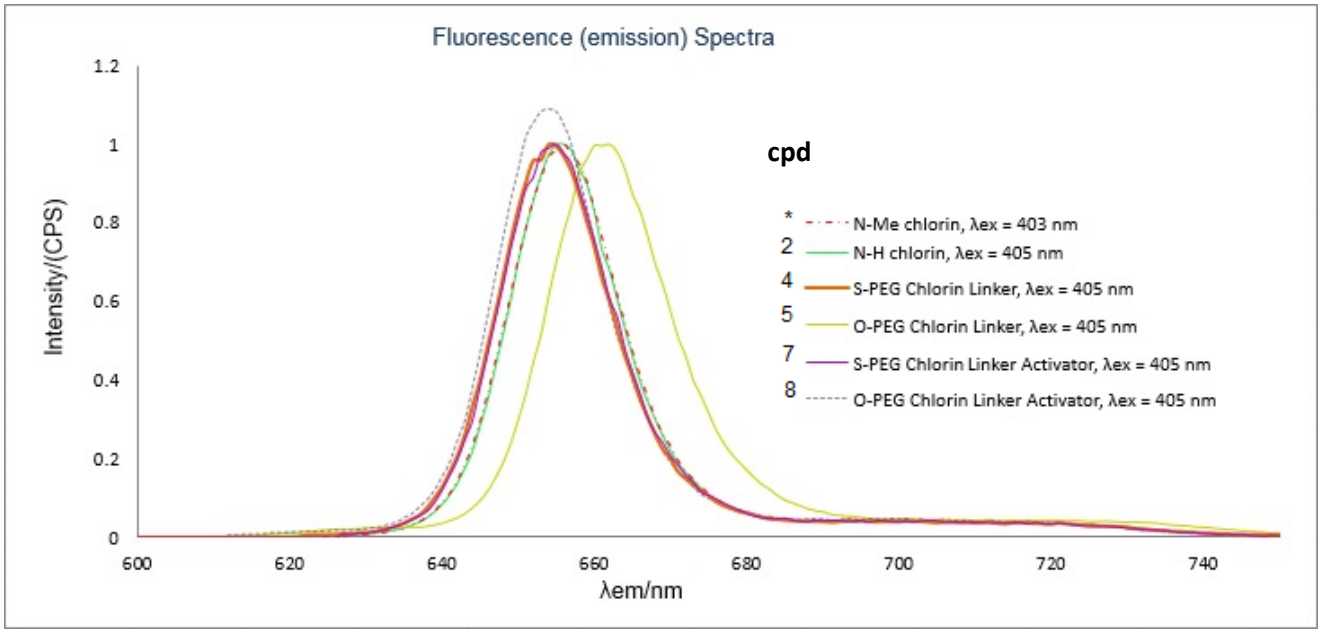


Figure S3. Fluorescence emission of published chlorin N-Me and chlorin NH compound 2, the S-PEG-chlorin 4, and the O-PEG-chlorin compound 5 and the corresponding chlorin NHS esters 7 and 8 in CH_2Cl_2 spectra normalized to λ_{max} Taken on a Horiba FL-1065 fluorimeter.

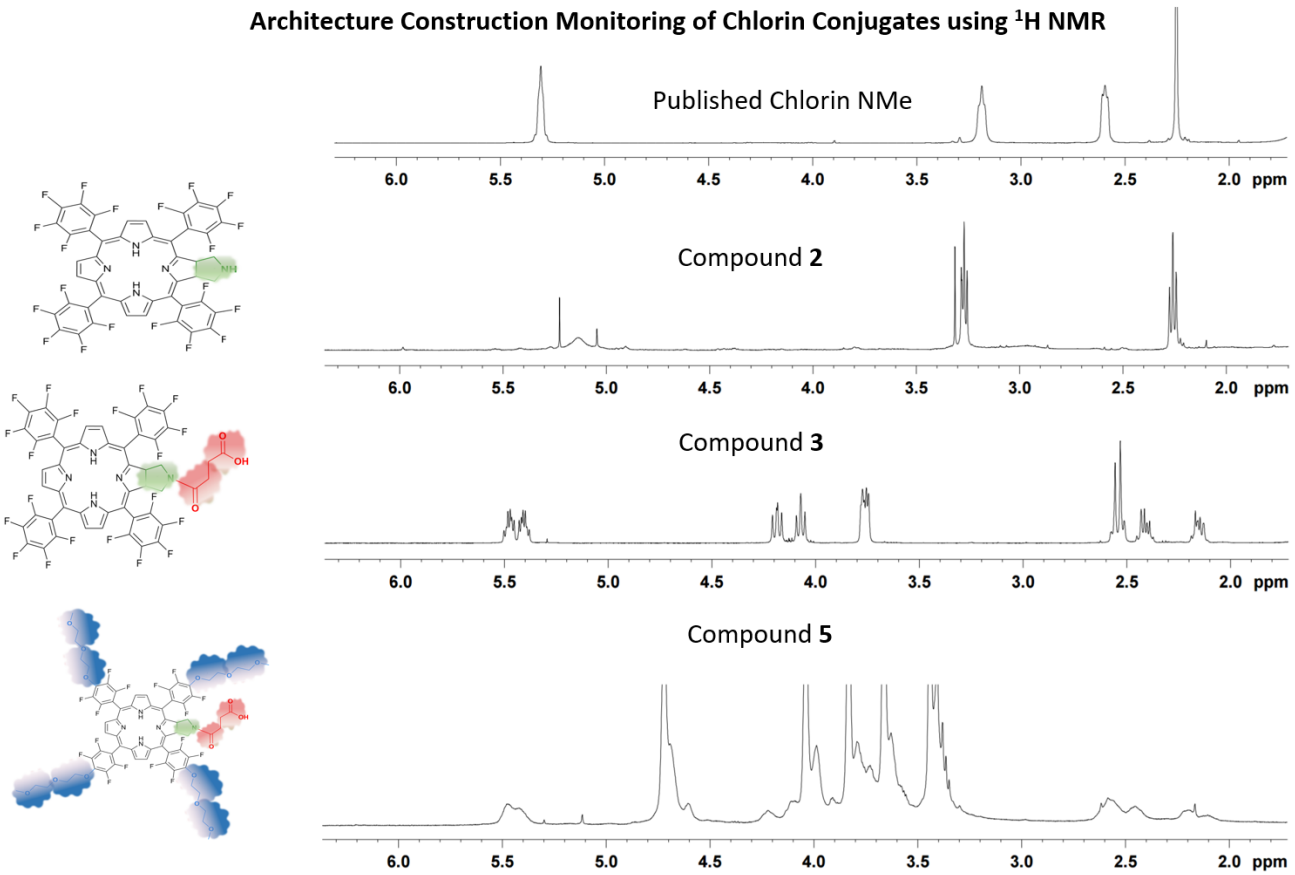


Figure S3a. Overview of the transformation of an N-H chlorin to include a tether and PEG groups to tune solubility.

Detailed Synthesis
Experimental Section

General
^{1}H and ^{13}C solution NMR spectra were recorded using Bruker 500 MHz (spectrometer operating at 500 MHz for ^{1}H; 125 MHz, for ^{13}C), and ^{19}F solution NMR spectra was recorded using Bruker 400 MHz. $CDCl_3$ was used as solvent and TMS as internal reference; the chemical shifts are expressed in (ppm) and the coupling constants (*J*) in Hertz (Hz). Trace solvent impurities (eg. water at approx. δ 1.6 ppm, CH_2Cl_2 at approx. δ 5.2 ppm in ^{1}H NMR spectra) are indicated. High Resolution Electrospray Mass Spectra (HRESIMS) were obtained using an Agilent 6520 Q-TOF instrument and using CH_2Cl_2 as a solvent. The UV-vis spectra were recorded using CH_2Cl_2 as solvent. Reagent grade chemicals and solvents were purchased from Sigma-Aldrich Inc. or Fisher Scientific Inc. Reactions were monitored by TLC with Analtech Uniplate silica gel G/UV 254 precoated plates (0.2 mm). TLC plates were visualized by UV (254 nm), and by iodine vapour. Preparative thin-layer chromatography was carried out on 20x20 cm glass plates coated with silica gel (1 mm thick).

Outline
5, 10, 15, 20-tetrakis-(2, 3, 4, 5, 6-pentafluorophenyl)-porphyrin $TPPF_{20}$ (100 mg, 0.102 mmol) dissolved in chlorobenzene (10 mL) was reacted with 10 equivalents of an azomethine ylide prepared by grinding a mixture of glycine (77 mg, 1.05 mmol) and paraformaldehyde (30.8 mg, 1.05 mmol). The prepared azomethine ylide (25 mg) was added in 4 aliquots every 2 hours and allowed to react at 145 ^{o}C, under a N_2 atmosphere. Chlorin **2** (37.5 mg, 0.036 mmol), triethylamine (0.5 mL, 0.004 mmol) and succinic anhydride (36 mg, 0.367 mmol) were added and stirred for 4 hr. in a reaction vessel at room temperature (r.t.) under a N_2 atmosphere. A solution of compound **3** (100 mg, 0.09 mmol) in acetone (12 mL) was added K_2CO_3 (74 mg, 0.54 mmol) and stirred for 10 min. Next, 2(2-methoxyethoxy)ethane thiol (54.00 µL, 0.40 mmol) was added to the reaction mixture and allowed to react at room temperature under N_2 to afford the tetra S-PEG compound **4**. Similarly, to a solution of **3** (34 mg, 0.0306 mmol), dissolved acetone (5 mL) was added K_2CO_3 (74 mg, 0.54 mmol) and stirred for 10 min. Subsequently, 2-(2-methoxyethoxy)ethanol (48 µL, 0.40 mmol) was added to the reaction mixture and allowed to reflux under N_2 to yield the tetra O-PEG compound **5**. The O-PEG-chlorin or the S-PEG-chlorin was dissolved in dioxane or THF (6 mL) and activated with N-hydroxysuccinimide (5.06 mg, 0.04 mmol) and dicyclohexylcarbodiimide DCC (9.9 mg, 0.05 mmol) at r.t. under N_2 to yield compounds **7** and **8**, respectively.

Either the S-PEG chlorin **7** or the O-PEG-chlorin **8** in DMSO and lysozyme from chicken egg white (SIGMA L6876) in DMSO (final concentration 100µM) were conjugated at room temperature in the dark with constant shaking to afford S-PEG-chlorin or O-PEG-chlorin-lysozyme conjugates. After the reaction, samples were centrifuged at 16300 xg for 5 min at 4 °C under which condition no precipitates were observed, and stored at -20 °C for further analysis. Analysis by SDS PAGE shows that that the best reaction conditions were at 1:1 ratio (100 µM each) and the reaction was nearly complete after 16 h.

Either the S-PEG-chlorin **7** or the O-PEG-chlorin **8** were coupled to the 5' end of 14 nt DNA via 6 carbon amine linker (5'-NH_2-$(CH_2)_6$-NH-TTCTTCTCCTTTCT-3') in phosphate buffer 0.1 M, pH 7.4 with 10:1 ratio at 37 ^{o}C and the precipitate under these conditions affords S-PEG-chlorin or O-PEG-chlorin DNA conjugates. After the reaction, the sample was centrifuged at 16300 xg for 5 min, whereupon the supernatant and the pellet dissolved in DMF were run on 20% polyacrylamide gel as shown in the ESI. The gels for S-PEG-chlorin and for O-PEG-chlorin conjugates to DNA

confirmed the coupling. A smeared band towards the top of the gel indicates some contribution of higher ordered labelled DNA.

Chlorin (2). Glycine (77 mg, 1.05 mmol) and paraformaldehyde (30.8 mg, 1.05 mmol) were mixed *in situ* for 5 min to afford the azomethine ylide adduct (107 mg). To a stirred solution of 5, 10, 15, 20 -tetrakis-(2, 3, 4, 5, 6-pentafluorophenyl)-porphyrin $TPPF_{20}$ (100 mg, 0.102 mmol) dissolved in chlorobenzene (8 mL) the prepared azomethine ylide (25 mg) was added in 4 aliquots every 2 hours and allowed to react at 145 ^{o}C, under nitrogen atmosphere for a total of 8h. After cooling to room temperature, chlorobenzene was evaporated under reduced pressure and dissolved in 4 mL of CH_2Cl_2 for purification by silica gel using CH_2Cl_2 to get the unreacted $TPPF_{20}$ followed by 7% EtOAc/ CH_2Cl_2 as eluant to furnish 36 % pure **2** (37.5 mg, 0.0367 mmol) as a dark green solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.64 (d, *J*=5 Hz, 2H), 8.41 (s, 2H), 8.30 (d, *J*=4 Hz, 2H), 5.14 (s, 2H), 3.28-3.25 (dd, J_1=5, J_2=10 Hz, 2H), 2.27-2.24 (dd, J_1=5, J_2=10 Hz, 2H), -1.95 (s, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ; 151.77, 146.31, 145.59, 144.31, 143.59, 142.21, 140.16, 139.26, 138.17, 137.45, 136.12, 135.46, 134.17, 131.34, 126.98, 122.84, 114.69, 114.24, 105.30, 95.67, 51.32, 27.99; ^{19}F NMR (376 MHz, $CDCl_3$): δ -135.29 to -136.80 (m, 8F, Ar-m-F), -150.96 to -151.64 (m, 4F, Ar-p-F), -157.21 to -161.28 (m, 8F, Ar-o-F). HRMS (ESI) *m/z* calcd for $C_{46}H_{15}F_{20}N_5$ ($[M+H]^+$), 1018.1081, found 1018.1091; calcd for $C_{46}H_{15}F_{20}N_5$ ($[M+Na]^+$), 1040.0900, found 1040.0908.

Synthesis of (3). To a 10 mL round bottom flask, compound **2** (37.5 mg, 0.036 mmol) was dissolved in 5 mL CH_2Cl_2 and 0.5 mL NEt_3 then succinic anhydride (36 mg, 0.367 mmol) was added and stirred overnight at room temperature under N_2 atmosphere. Then 20 mL of CH_2Cl_2 is added the resulting mixture is washed with water followed by brine. The organic layer was dried using sodium sulfate to remove trace amount of water. CH_2Cl_2 was removed through a rotator evaporator and the crude product was purified by flash chromatography (silica gel) using 3% Methanol/ CH_2Cl_2 as eluent to afford 85 % pure **3** (34 mg, 0.0306 mmol) as a dark green solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.74 (d, *J*=4.85 Hz, 2H), 8.50 (s, 2H), 8.38-8.42 (dd, J_1=4.65, J_2=4.7 Hz, 2H), 5.40-5.48 (m, 2H), 4.17 (t, J=10.15 Hz, 1H), 4.07 (t, J=9.95 Hz, 1H), 3.74-3.77 (m, 2H), 2.50-2.54 (m, 2H), 2.12-2.17 (m, 2H), -1.82 (s, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ; 168.57, 168.51, 153.18, 152.95, 147.26, 146.49, 145.31, 144.82, 143.34, 141.29, 140.59, 140.15, 139.15, 138.56, 137.22, 136.52, 135.59, 135.29, 132.69, 132.64, 128.37, 128.08, 124.32, 123.80, 115.52, 115.24, 106.79, 106.50, 96.94, 96.37, 53.68, 52.28, 52.17, 52.07, 51.34, 51.22, 51.20, 50.48, 31.76, 30.99, 29.72, 29.69, 29.21, 28.83, 28.67, 28.55; ^{19}F NMR (376 MHz, $CDCl_3$): δ -134.37 (d, 1F, Ar-m-F), -135.39 (d, 1F, Ar-m-F), -136.74 to 136.98 (m, 5F, Ar-m-F), -137.50 to -137.74 (d, 1F, Ar-m-F), -150.10 to -150.42 (m, 2F, Ar-p-F), -151.38 to -151.49 (m, 2F, Ar-p-F), -159.02 to -159.08 (m, 2F, Ar-o-F), -159.82 to -159.94 (m, 2F, Ar-o-F), -161.35 (s, 4F, Ar-o-F). HRMS (ESI) *m/z* calcd for $C_{50}H_{19}F_{20}N_5O_3$ ($[M+H]^+$), 1118.1241, found 1118.1238; calcd for $C_{50}H_{19}F_{20}N_5O_3$ ($[M+Na]^+$), 1140.1061, found 1140.1048.

Synthesis of (4). To a solution of chlorin acid **3** (100 mg, 0.09 mmol, 1 equiv.) dissolved in acetone (12 mL) was added K_2CO_3 (74 mg, 0.54 mmol, 6 equiv.) and stirred for 10 min. 2(2-methoxy ethoxy)ethane thiol (54.00 µL, 0.40 mmol, 4.5 equiv.) was added to the reaction mixture and allowed to react at r.t. under inert conditions for 4h. Acetone was removed under reduced pressure, the crude mixture was dissolved in ethyl acetate (15 mL) and washed with water (12 mL). The organic layer was evaporated and concentrated in vacuo. The crude product was purified by flash column chromatography over deactivated silica gel using 0.7% MeOH/ CH_2Cl_2 as eluent to furnish pure **4** as a green solid (78 mg, 60 % yield from **3**). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.77-8.72 (m, 2H), 8.53-8.38 (m, 4H), 5.46 (bs, 2H), 4.25-4.20 (m, 2H), 3.94-3.85 (m, 8H), 3.75-3.72 (m, 8H), 3.68 (bs, 2H), 3.63-3.61 (m, 8H), 3.44-3.35 (m, 20H), 2.64-2.57 (m, 1H), 2.48-2.43

(m, 1H), 2.17-2.12 (m, 2H), -1.82 (s, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ; 173.90, 170.07, 152.83, 150.09, 148.16, 146.09, 144.75, 144.01, 142.23, 140.75, 139.86, 138.45, 137.34, 135.78, 133.11, 132.68, 128.51, 128.21, 123.39, 122.13, 114.12, 105.45, 96.03, 71.96, 71.91, 71.81, 70.92, 70.86, 70.77, 70.67, 70.59, 70.43, 70.37, 59.0, 53.42, 52.40, 30.36; ^{19}F NMR (376 MHz, $CDCl_3$): δ -136.84 (s, 1F, Ar-m-F) -137.78 (s, 1F, Ar-m-F) -138.75 to -139.25 (m, 5F, Ar-m-F), -140.07 (s, 1F, Ar-m-F), -155.01 to -158.03 (m, 8F, Ar-o-F). HRMS (ESI) *m/z* calcd for $C_{70}H_{63}F_{16}N_5O_{11}S_4$ ($[M+H]^+$), 1581.3151, found 1582.3214

Synthesis of (5). To a solution of **3** (34 mg, 0.0306 mmol), dissolved in dry acetone (5 mL) was added K_2CO_3 (74 mg, 0.54 mmol) and stirred for 10 min. 2-(2-methoxyethoxy)ethanol (48 µL, 0.40 mmol) was added to the reaction mixture and allowed to reflux under nitrogen overnight. Acetone was removed under reduced pressure. The crude product was purified by silica gel using 7% MeOH/ CH_2Cl_2 as eluent to furnish 85% pure **5** (39.45 mg, 0.026 mmol**)** as a green solid**.** Note: If acetone is not dry the reaction takes longer (48 h) and excess 2-(2-methoxyethoxy)ethanol is required. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.74 (bs, 2H), 8.52 (bs, 2H), 8.42 (bs, 2H), 5.41-5.47 (m, 2H), 4.69-4.72 (bs, 8H, OCH_2), 3.98-4.03 (m, 8H), 3.72-3.83 (m, 10H), 3.62-3.66 (m, 8H), 3.34-3.44 (m, 14H), 2.58-2.61 (bs, 1H), 2.45 (bs, 1H), 2.10-2.19 (m, 2H), -1.86 (s, 2H) ; ^{13}C NMR (125 MHz, $CDCl_3$) δ; 168.57, 168.51, 153.22, 153.18, 153.07, 153.00, 147.41, 146.63, 145.43, 144.68, 142.52, 142.25, 141.94, 141.91, 141.82, 141.79, 140.72, 140.54, 140.35, 140.32, 139.95, 139.93, 139.84, 139.81, 139.15, 138.91, 138.81, 135.62, 135.44, 132.59, 128.35, 128.15, 124.26, 123.85, 113.65, 113.50, 107.43, 107.29, 97.57, 97.02, 74.49, 72.05, 71.92, 70.96, 70.83, 70.75, 70.59, 70.46, 59.20, 59.08, 52.32, 51.33, 50.53, 29.74.; ^{19}F NMR (376 MHz, $CDCl_3$): δ -136.86 (bs, 1F, Ar-m-F), -137.84 (bs, 1F, Ar-m-F), -139.21 to -139.36 (m, 5F, Ar-m-F), -140.16 (bs, 1F, Ar-m-F) -154.98 to -155.80 (m, 4F, Ar-o-F), -157.02 (bs, 4F, Ar-o-F). HRMS (ESI) *m/z* calcd for $C_{70}H_{63}F_{16}N_5O_{15}$ ($[M+H]^+$), 1518.4138, found 1518.4101; calcd. for $C_{70}H_{63}F_{16}N_5O_{15}$ ($[M+Na]^+$), 1540.3957, found 1540.3905.

Synthesis of (6). To a solution of **3** (50 mg, 0.04 mmol, 1 equiv.) dissolved in THF (6 mL) was added N-hydroxysuccinimide (5.06 mg, 0.04 mmol, 1.1 equiv.) and DCC (9.9 mg, 0.05 mmol, 1.2 equiv.), and allowed to react at r.t under inert conditions for 45 min. THF was evaporated under reduced pressure and the crude dissolved in CH_2Cl_2 (5 mL). The organic layer was washed with $NaHCO_3$ (6 mL) followed an additional wash with water (6 mL). The organic layer was concentrated in vacuo to furnish compound **6** as a green solid (35 mg 72 % yield from **3**). HRMS (ESI) *m/z* calcd. for $C_{54}H_{22}F_{20}N_6O_5$ ($[M+H]^+$), 1214.1332, found 1215.1397.

Synthesis of (7). To a solution of **4** (50 mg, 0.03 mmol, 1 equiv.) dissolved in THF (6 mL) was added N-hydroxysuccinimide (4.0 mg, 0.03 mmol, 1.1 equiv.) and DCC (7.3 mg, 0.04 mmol, 1.2 equiv.) and allowed to react at r.t under inert conditions for 45 min. THF was evaporated under reduced pressure and the crude dissolved in CH_2Cl_2 (5 mL). The organic layer was washed with $NaHCO_3$ (6 mL) followed an additional wash with water (6 mL). The organic layer was concentrated *in vacuo*. The organic layer was concentrated *in vacuo* to furnish compound **7** as a green solid (32 mg 67 % yield from **4**). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.62-8.48 (bs x2, 6H), 4.77 (bs, 2H), 4.11-4.07 (m, 10H), 3.89-3.85 (m, 10H), 3.70-3.61 (m, 18H), 3.52-3.43 (m, 8H), 3.41 (bs, 1H), 3.35 (bs, 1H), 3.30 (bs, 1H), 2.92 (bs, 2H), 2.36-2.33 (m, 2H), 2.31-2.29 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ; 147.37, 145.02, 135.42, 132.87, 116.41, 106.61, 71.95, 71.87, 71.82, 70.92, 70.78, 70.64, 70.59, 70.39, 70.31, 69.73, 59.09, 53.45, 38.34, 30.36; HRMS (ESI) *m/z* calcd. for $C_{74}H_{66}F_{16}N_6O_{13}S_4$ ($[M+H]^+$), 1678.3315, found 1679.3343.

Synthesis of (8). To compound **5** (39.45 mg, 0.026 mmol**)** in dry dioxane (3 mL) was added N-hydroxysuccinimide (4.0 mg, 0.03 mmol, 1.1 equiv.) and DCC (7.6 mg, 0.04 mmol, 1.2 equiv.) and allowed to react at r.t. under nitrogen overnight. The resulting mixture is centrifuged at 13000

rpm for 10 min and the supernatant was evaporated under reduced pressure to furnish compound **8** (41 mg, 0.025 mmol) in 98% yield as a green solid. The compound is used for conjugation without further purification as it results in free acid (compound **5**). HRMS (ESI) *m/z* calcd. for $C_{74}H_{66}F_{16}N_6O_{17}$ ($[M+H]^+$), 1615.4362, found 1615.4230; $C_{74}H_{66}F_{16}N_6O_{17}$ ($[M+Na]^+$), 1637.4121, found 1637.4084.

Synthesis of 10b (Lysozyme Conjugate with OPEG-Chlorin in DMSO)

A mixture of chlorin-NHS (compound **8**) in DMSO and lysozyme from chicken egg white (SIGMA L6876) in DMSO (final concentration 100 μM) at a chlorin:lysozyme molar ratio 1:1 was adjusted to a final volume of 100 μL with DMSO. Another two reactions were set at a chlorin:lysozyme 1:10 (10 μM Chlorin Vs 100 μM Lysozyme) and at a chlorin:lysozyme 1:100 (1 μM Chlorin Vs 100 μM Lysozyme). The conjugation lasted overnight at room temperature in the dark with constant shaking. After reaction, samples were centrifuged at 16300xg for 5 min at 4 °C (no precipitates were observed) and stored at -20 °C for further analysis. The SDS-PAGE analysis shows that the best conditions were at 1:1 ratio (100 μM each) and reaction was almost complete after 16 h.

Spectroscopy of new compounds

NMR

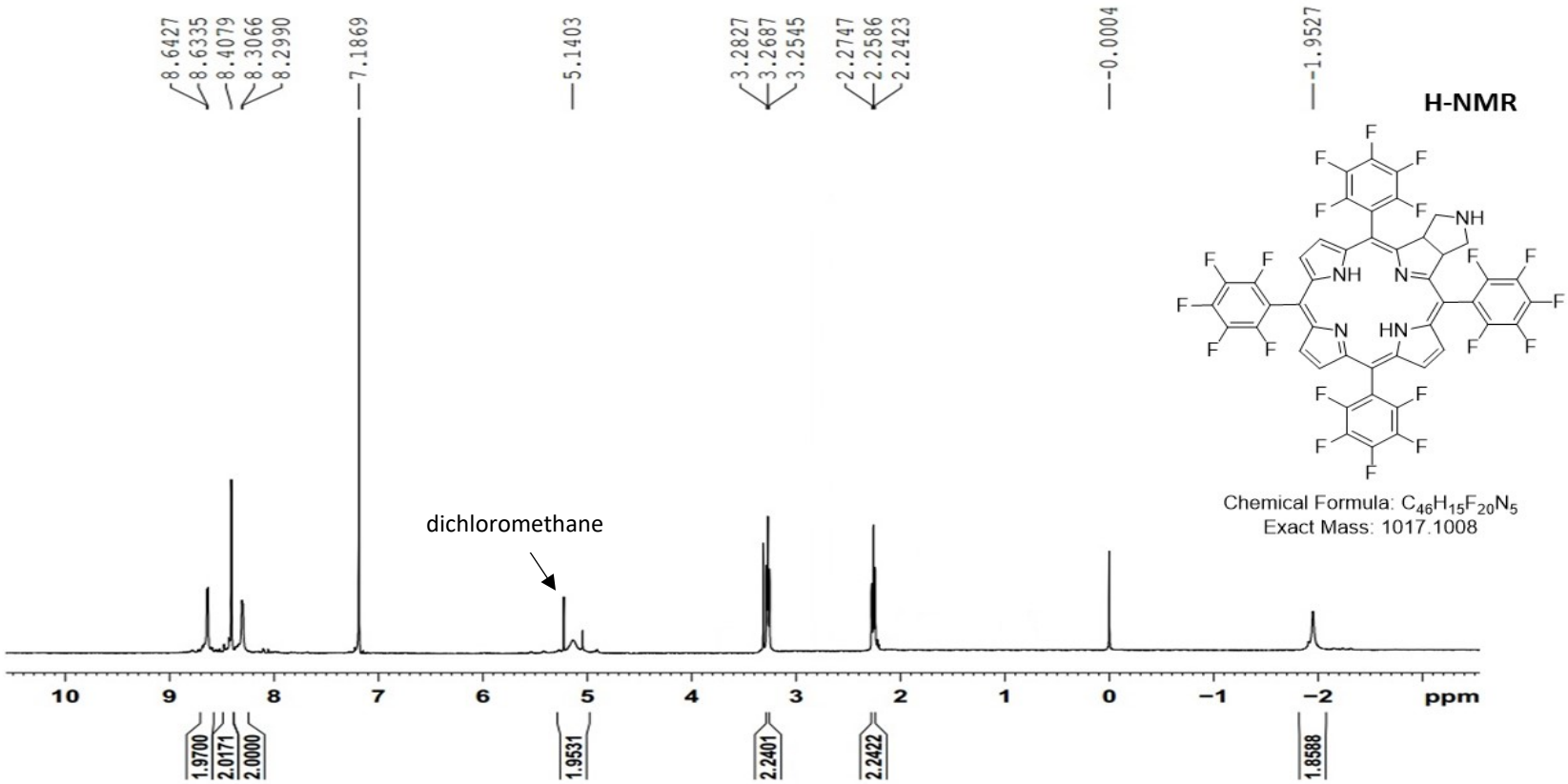


Figure S4. Compound **2**, Solvent used: Deuterated chloroform ($CDCl_3$, 7.19 ppm) was used as solvent and tetramethylsilane as internal reference (TMS, 0.00 ppm).

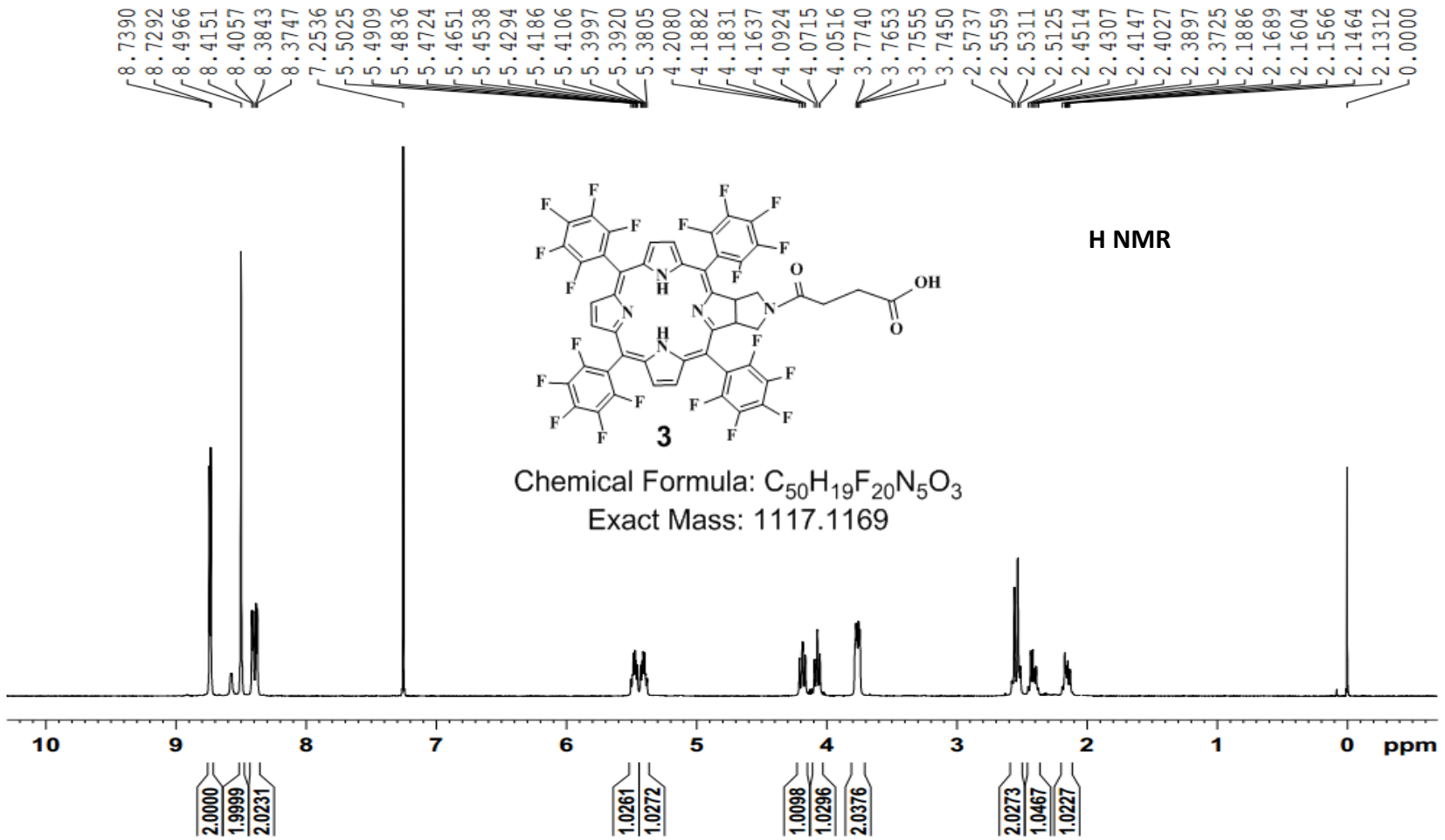


Figure S5. Compound **3**, Solvent used: Deuterated chloroform ($CDCl_3$, 7.25 ppm) was used as solvent and tetramethylsilane as internal reference (TMS, 0.00 ppm).

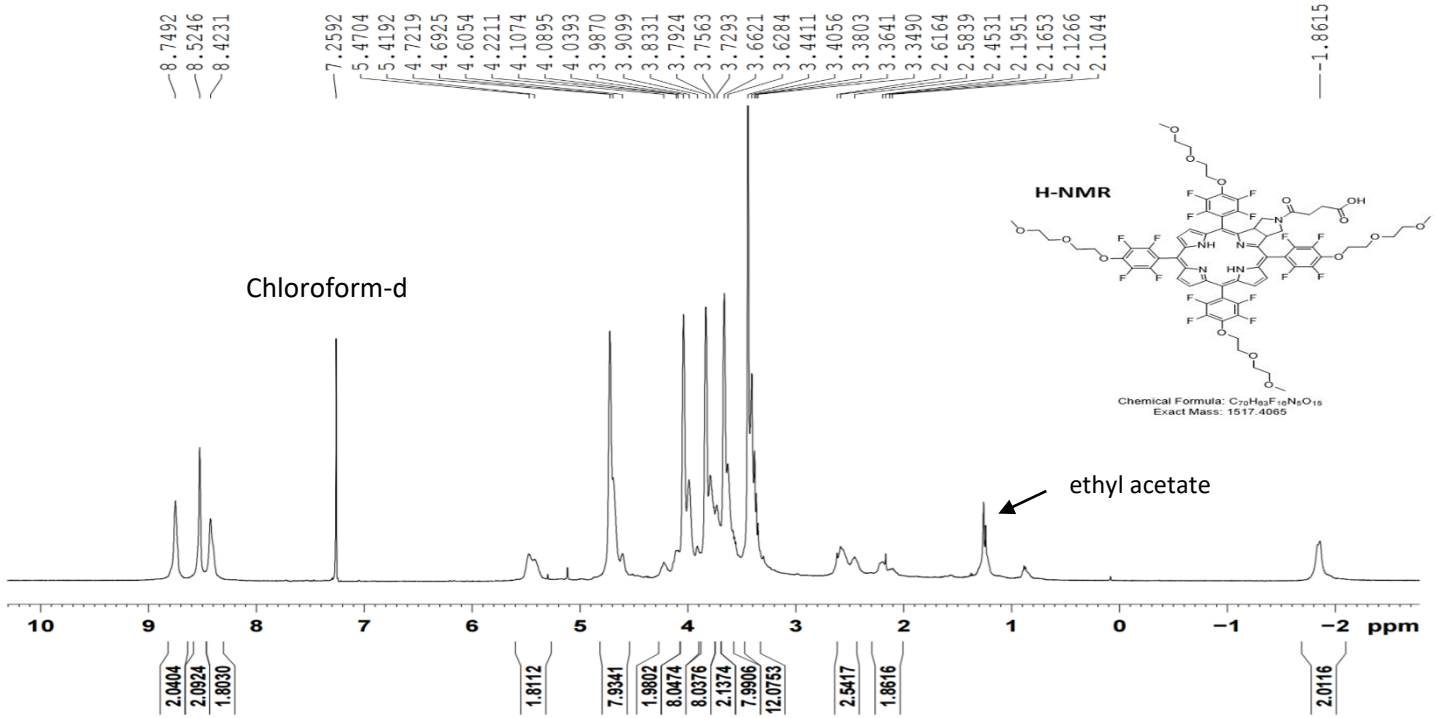


Figure S6. Compound **5** in $CDCl_3$ was used as solvent.

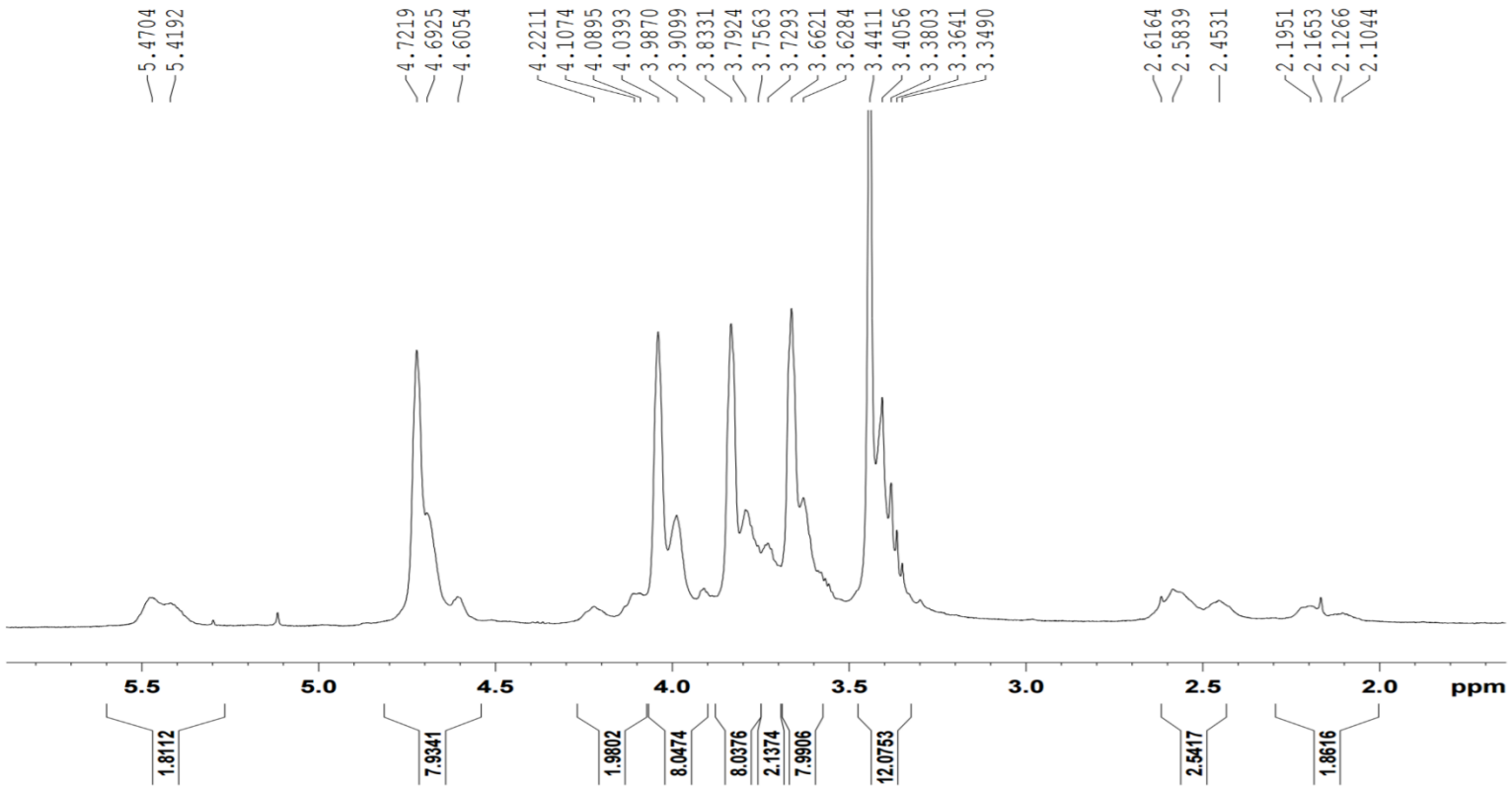


Figure S6a. Compound **5** in $CDCl_3$ expanded.

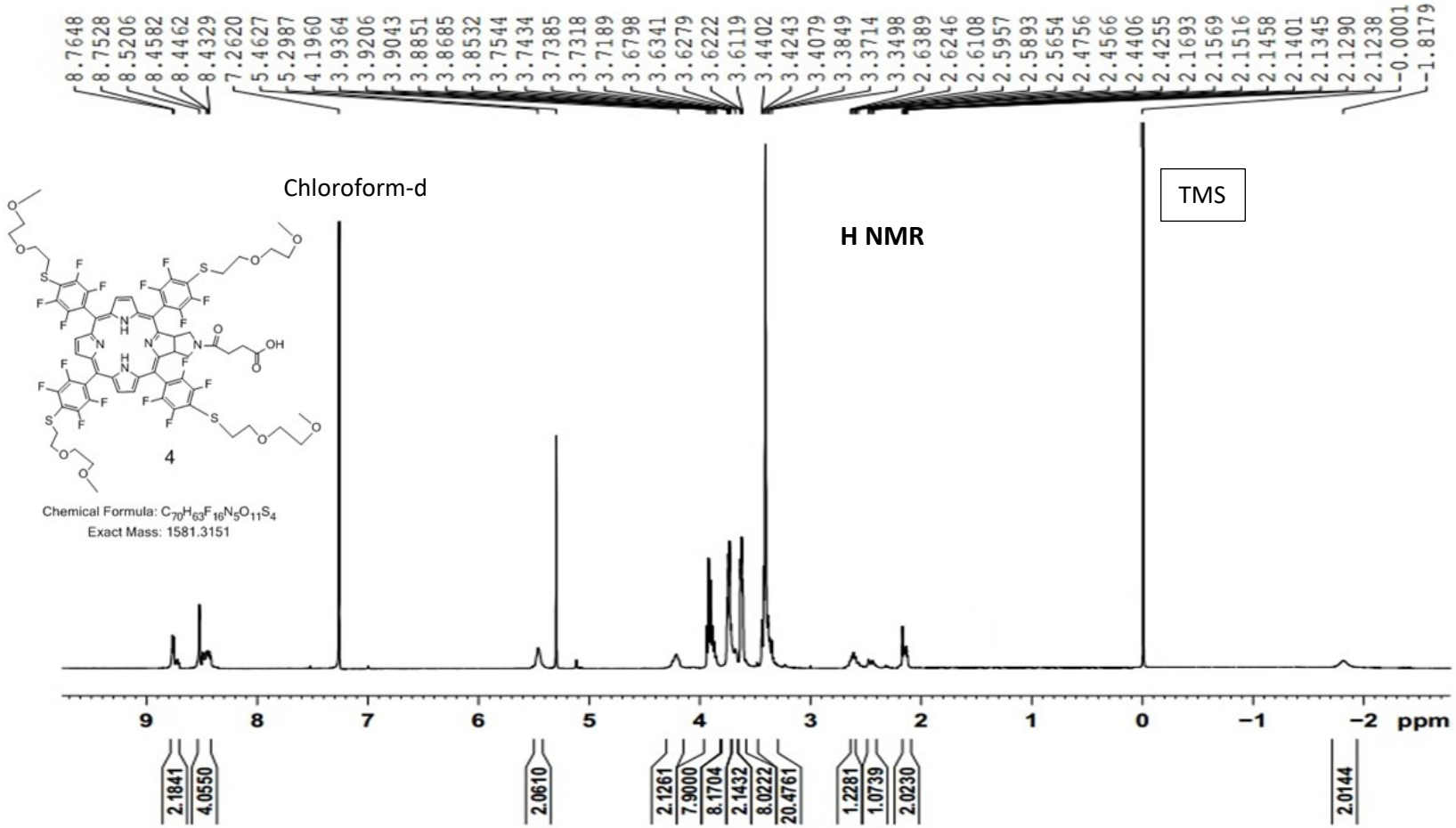


Figure S7. Compound **4** in $CDCl_3$, 7.26 ppm (TMS, 0.0001 ppm).

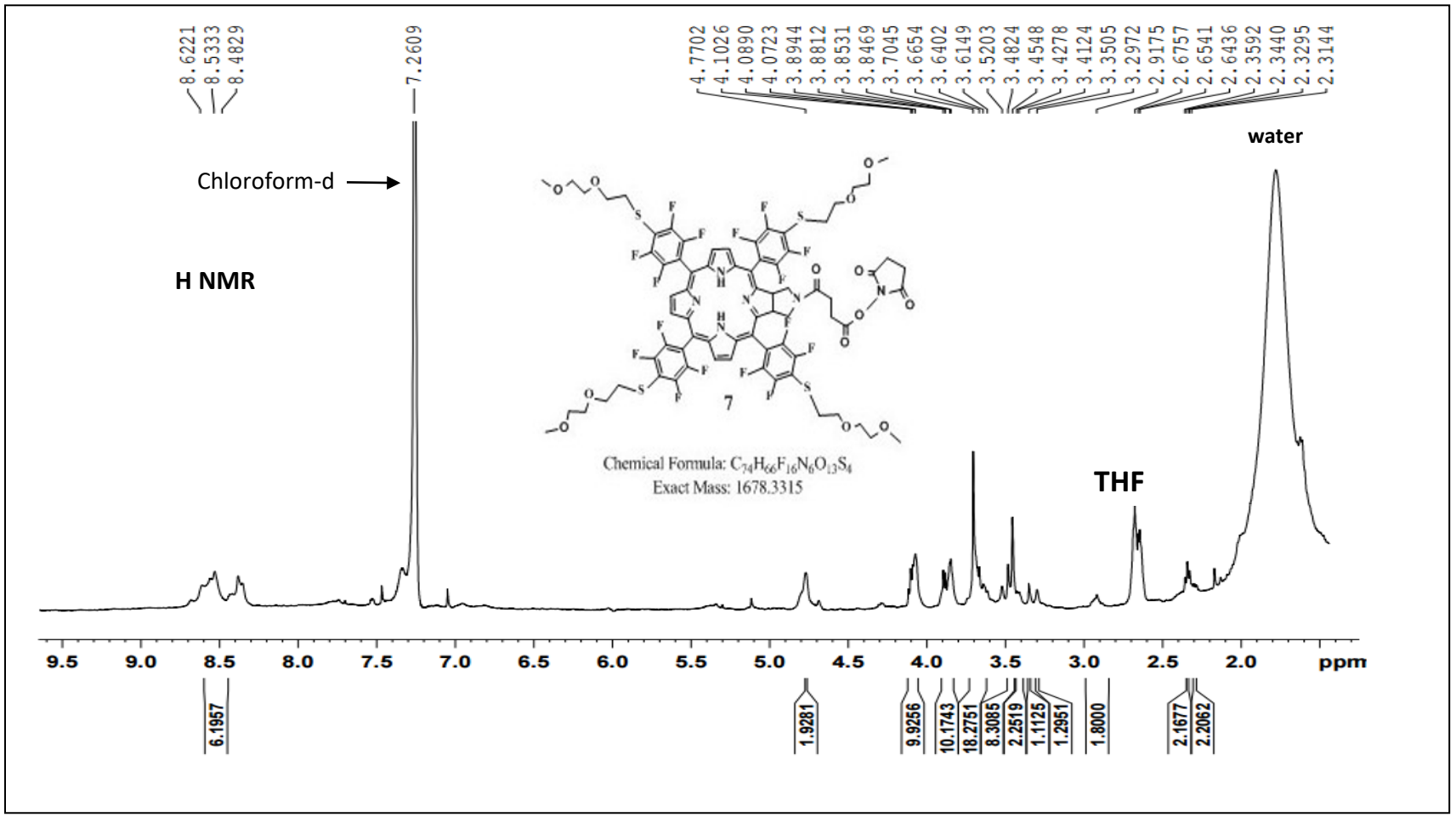


Figure S8. 1H NMR of a crude mixture of compound **7** and **4** in $CDCl_3$, 7.26 ppm; internal reference TMS.

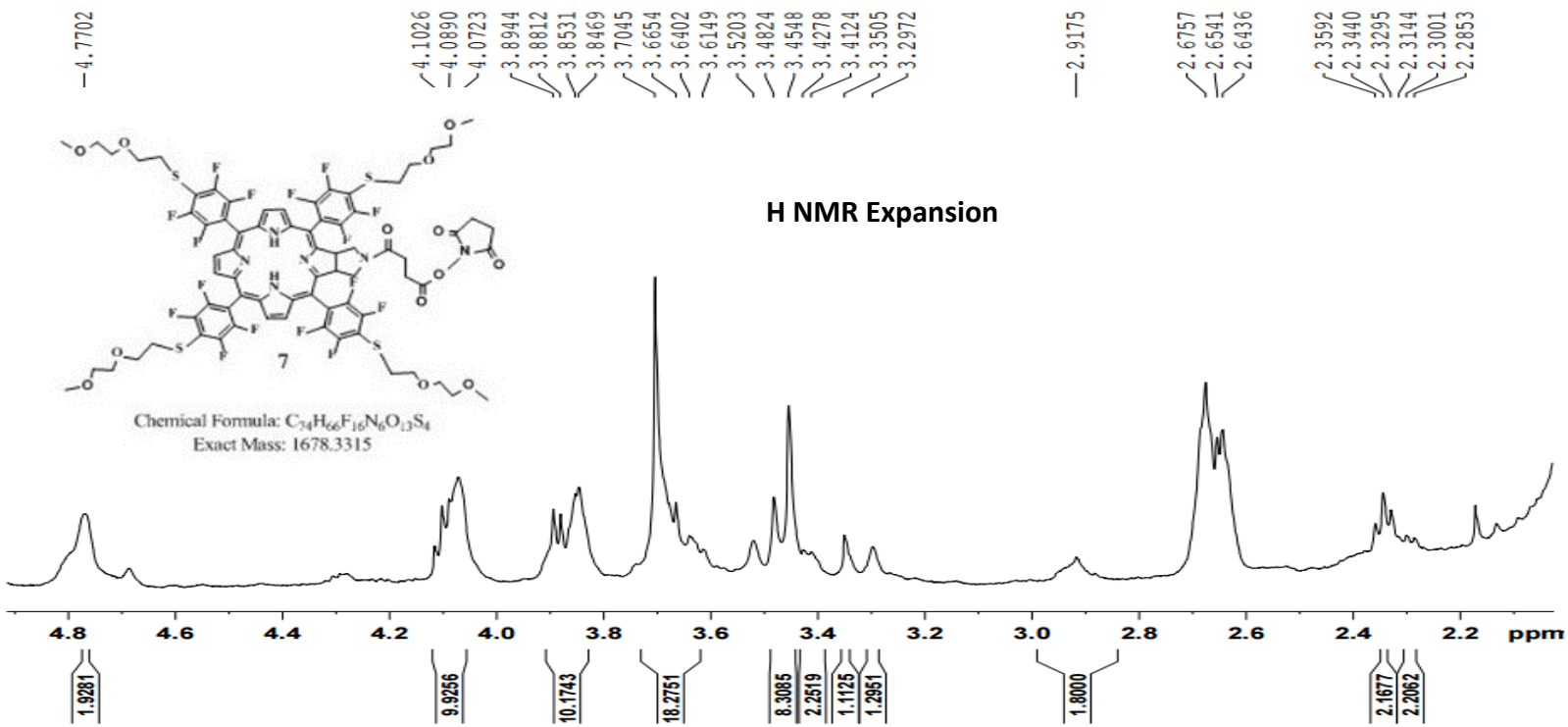


Figure S9. 1H NMR expansion of a crude mixture of compound **4** and **7**, showing the bridgehead hydrogens and SPEGs. THF appears at 2.69-2.64 ppm.

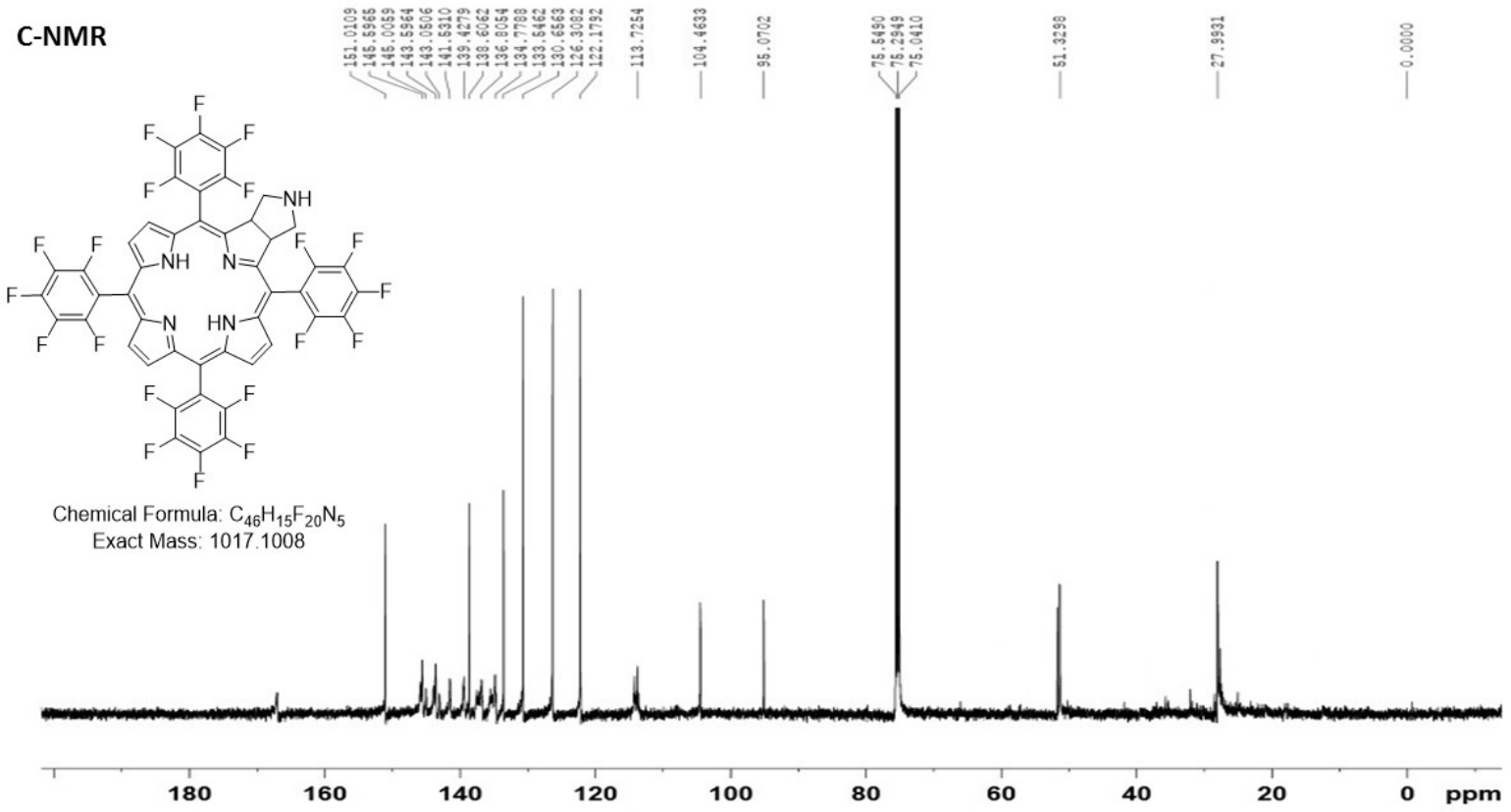


Figure S10. Compound **2** in $CDCl_3$, approx. 77.16 ppm

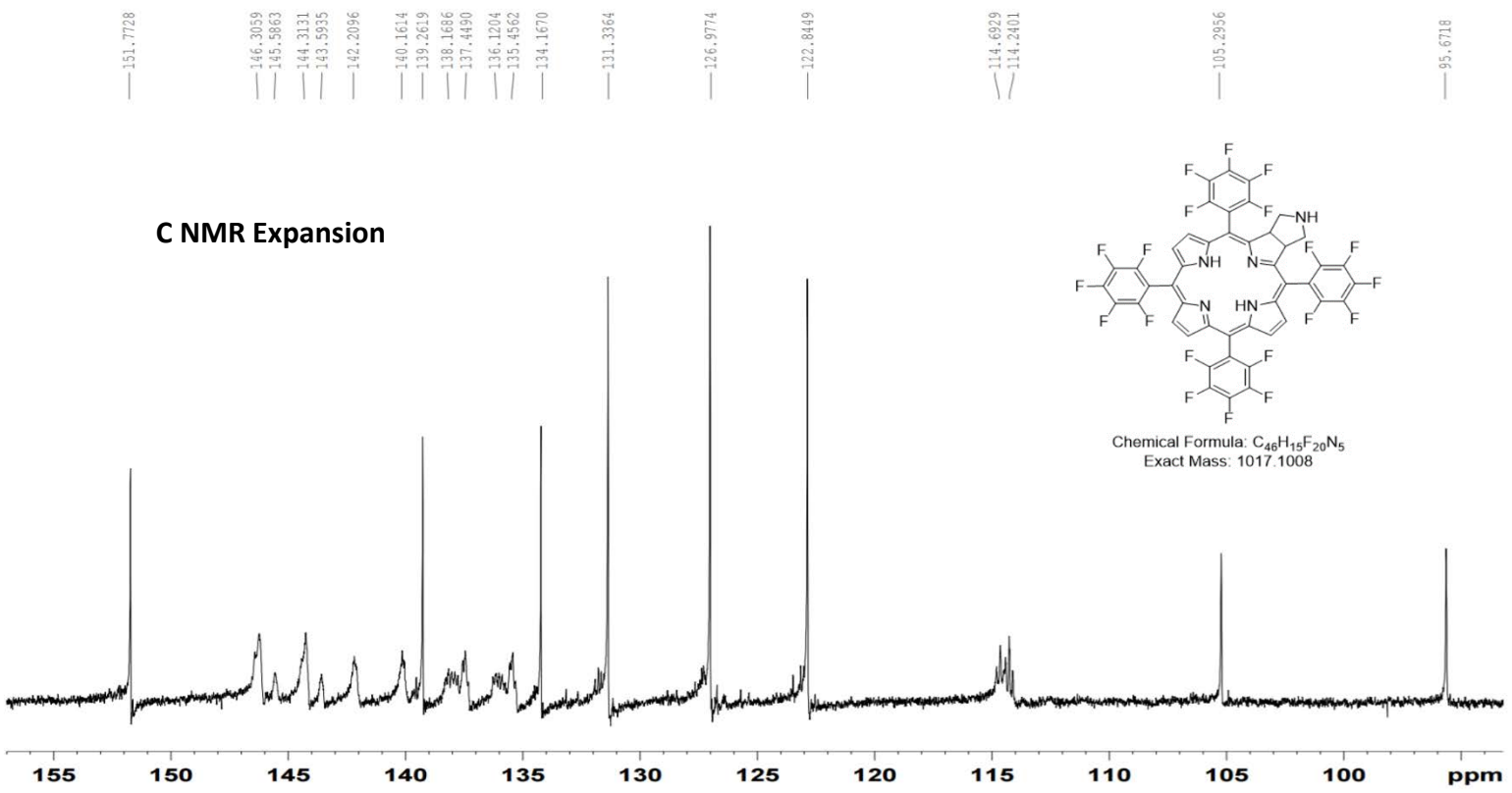


Figure S11. Compound **2** in $CDCl_3$, approx. 77.16. Expansion (see tables S1-3 for assignments and comparison to the literature).

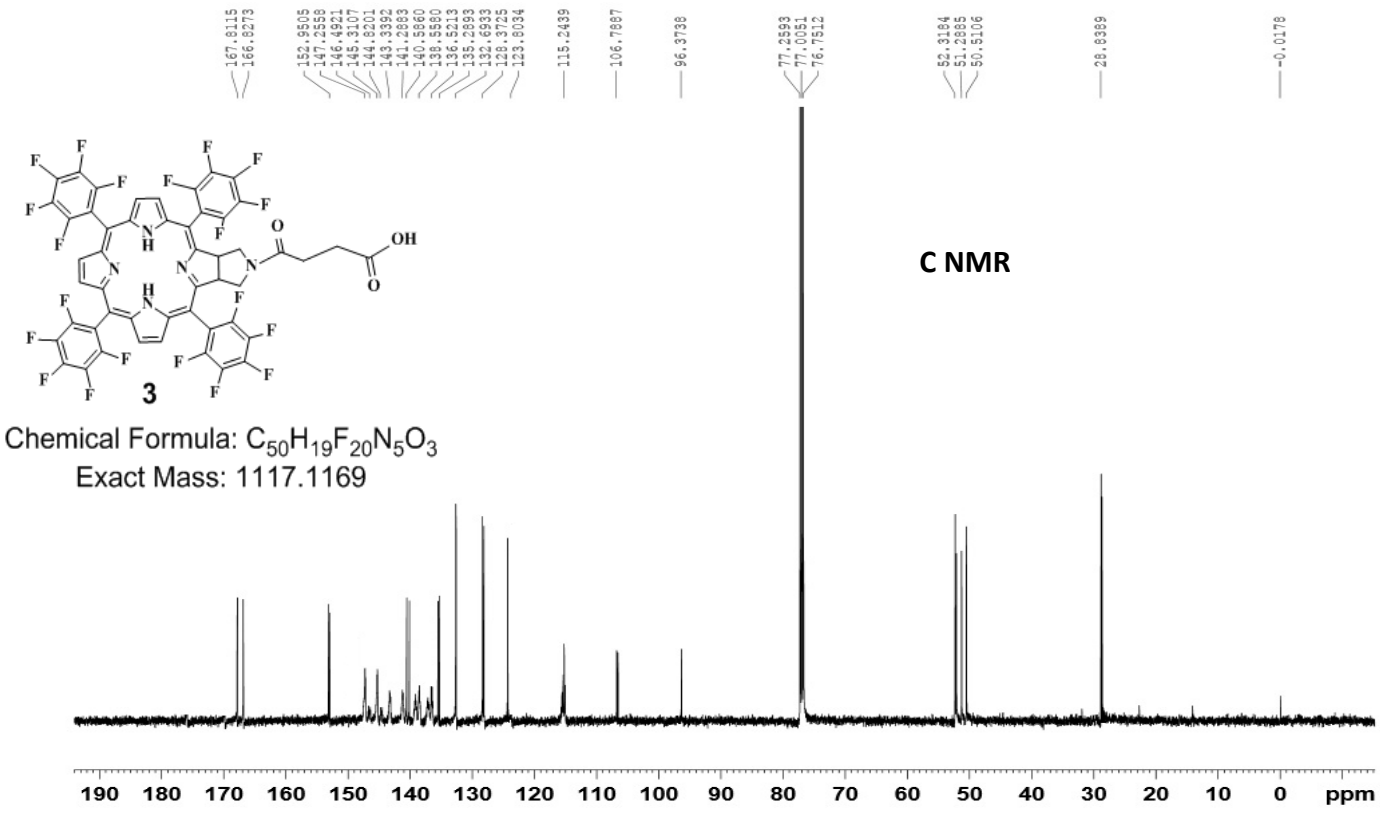


Figure S12. Compound **3** in $CDCl_3$ (approx. 77.16).

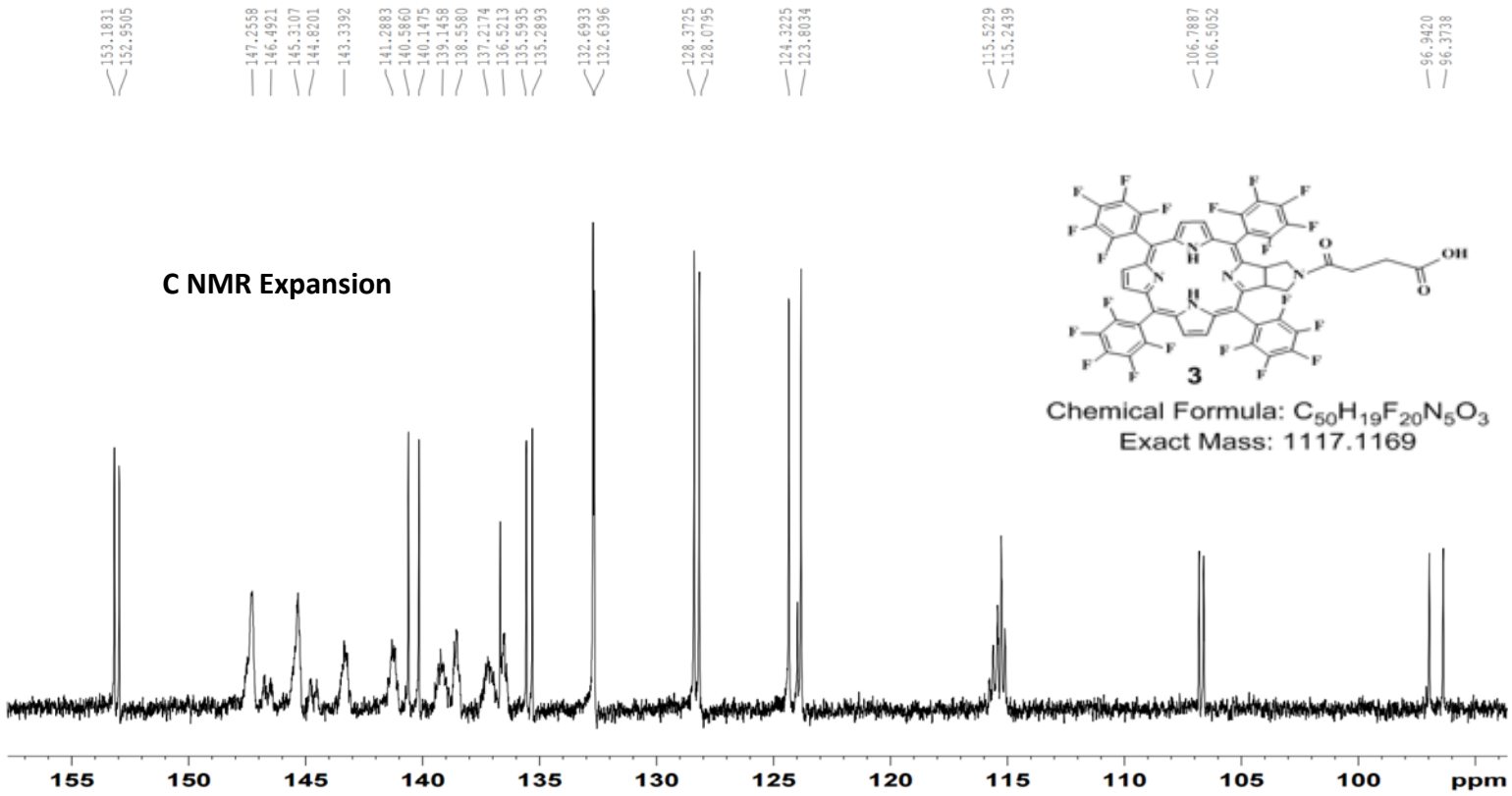


Figure S13. Compound **3** in $CDCl_3$ (approx. 77.16). See tables S1-3 for assignments and comparison to literature.

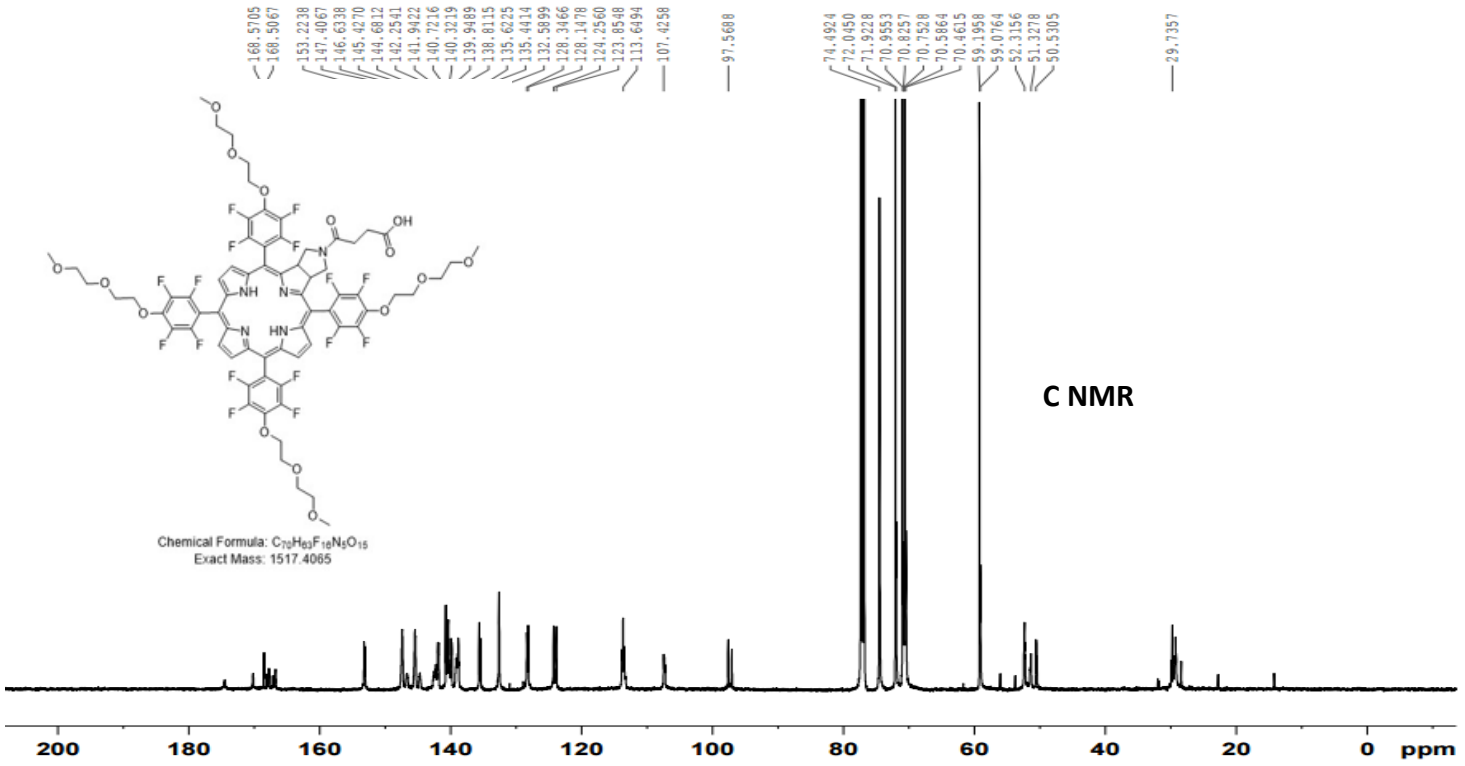


Figure S14. Compound **5** in $CDCl_3$. See tables S1-3 for assignments and comparison to the literature.

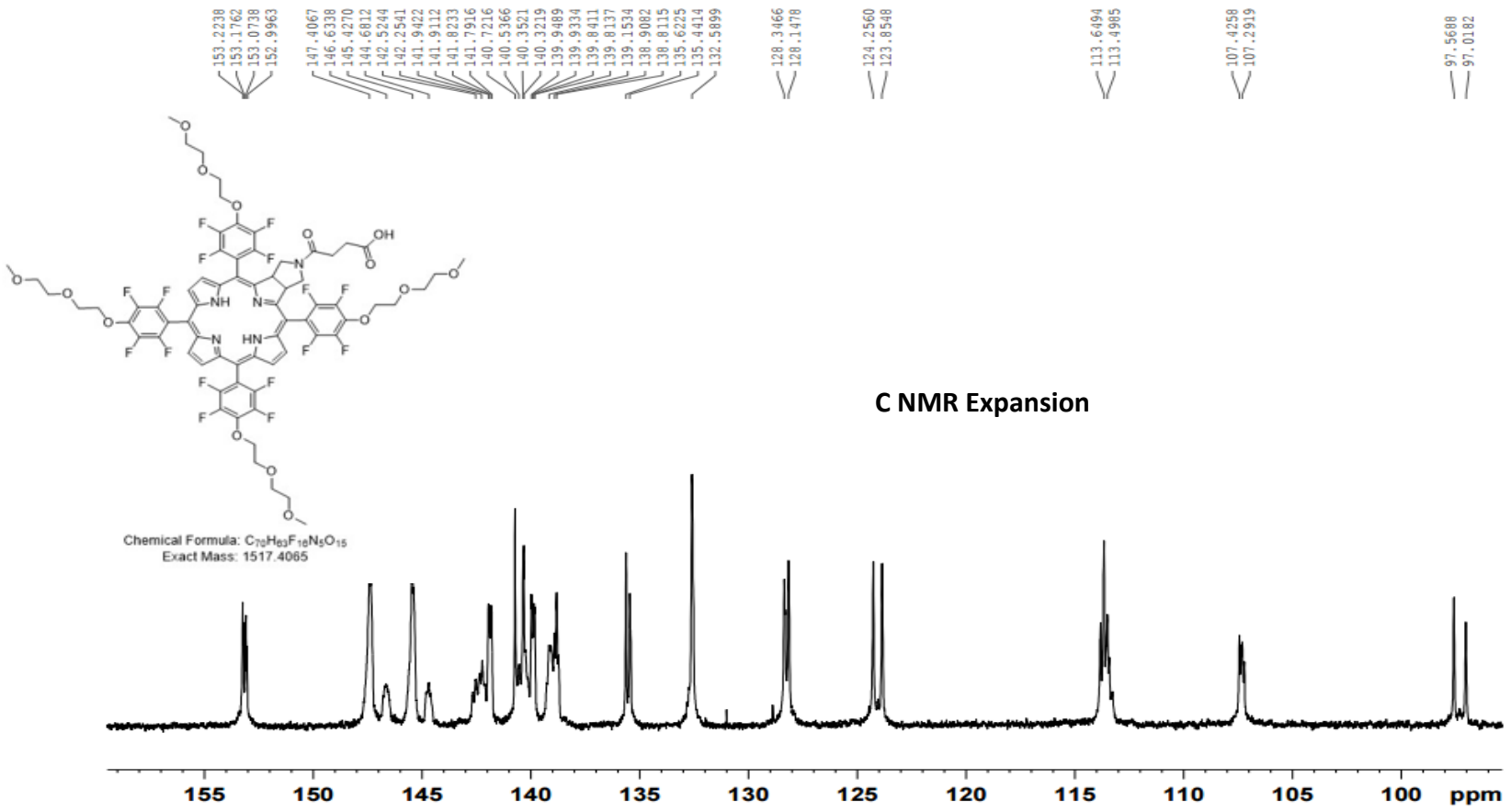


Figure S15. Compound **5** in $CDCl_3$ expanded.

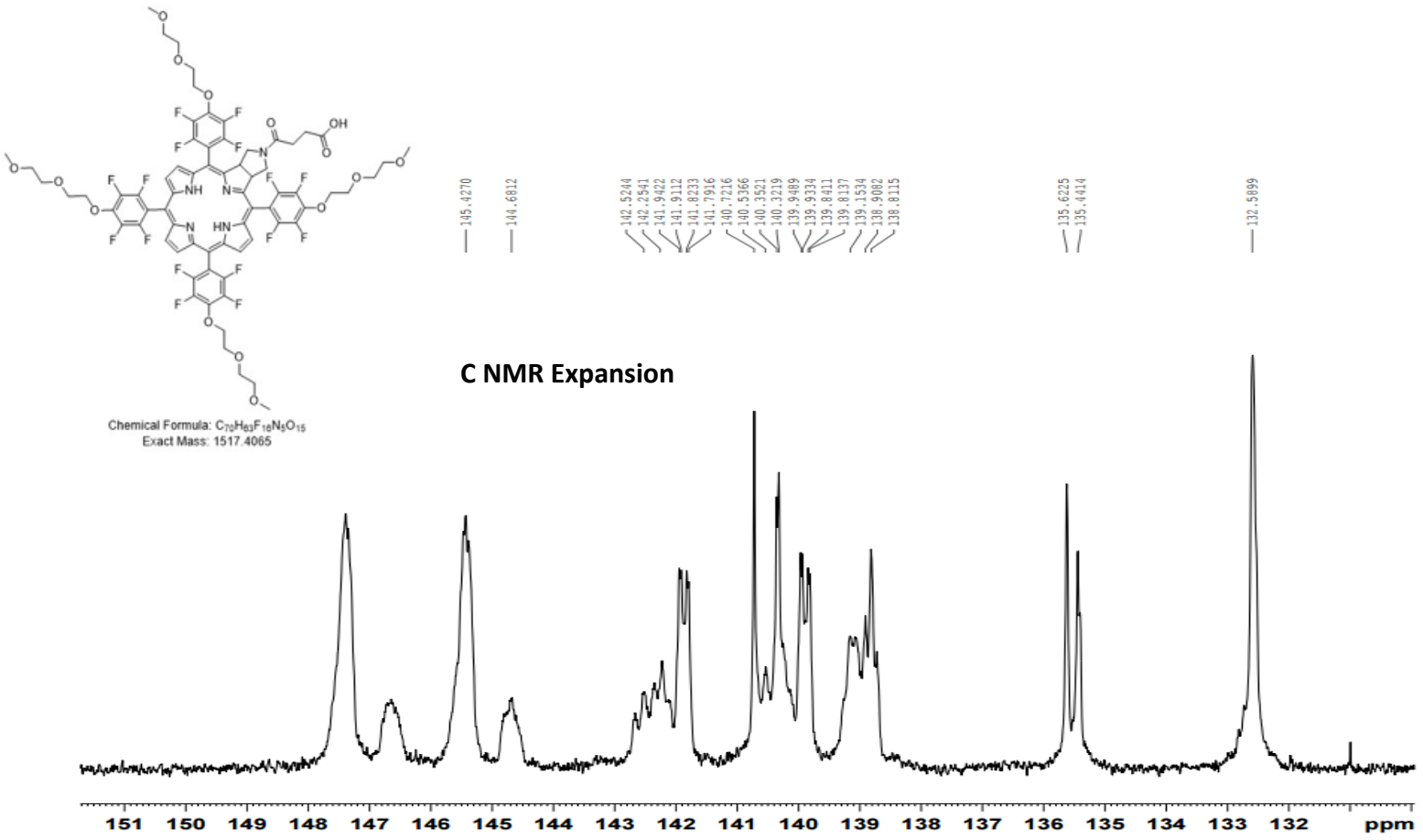


Figure S16. Compound **5** in $CDCl_3$ expanded.

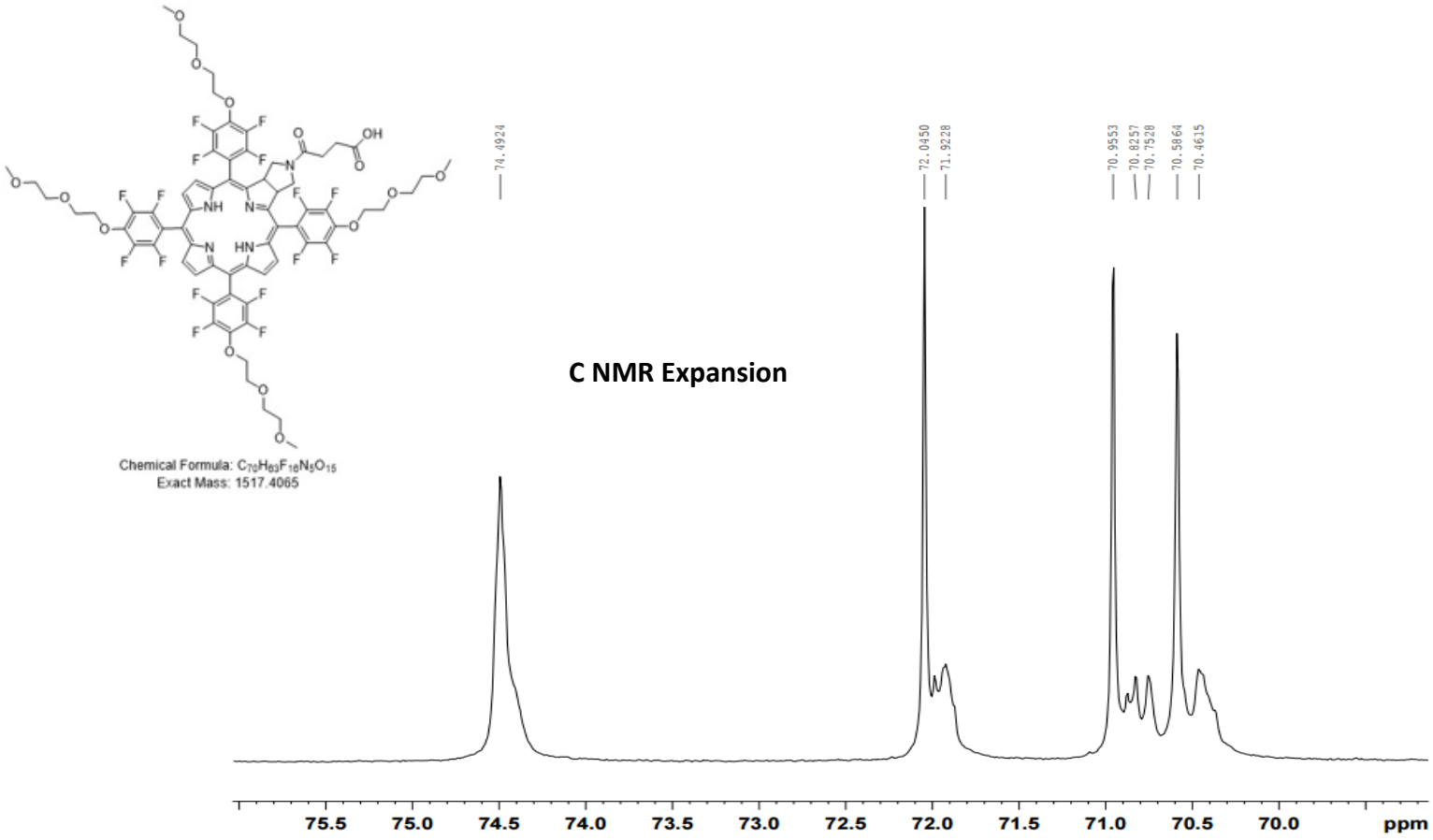


Figure S17. Compound **5** in $CDCl_3$ (approx. 77.16) expanded.

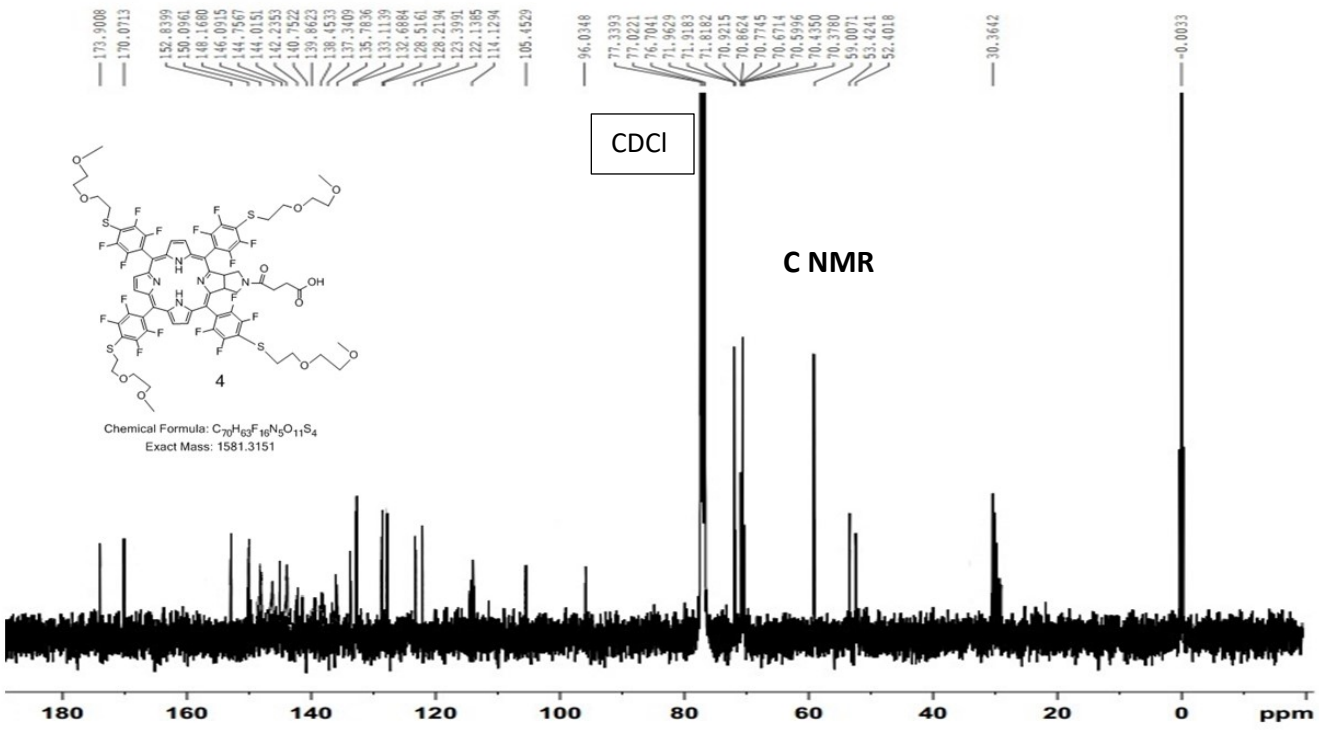


Figure S18. Compound **4** in $CDCl_3$ (approx. 77.16).

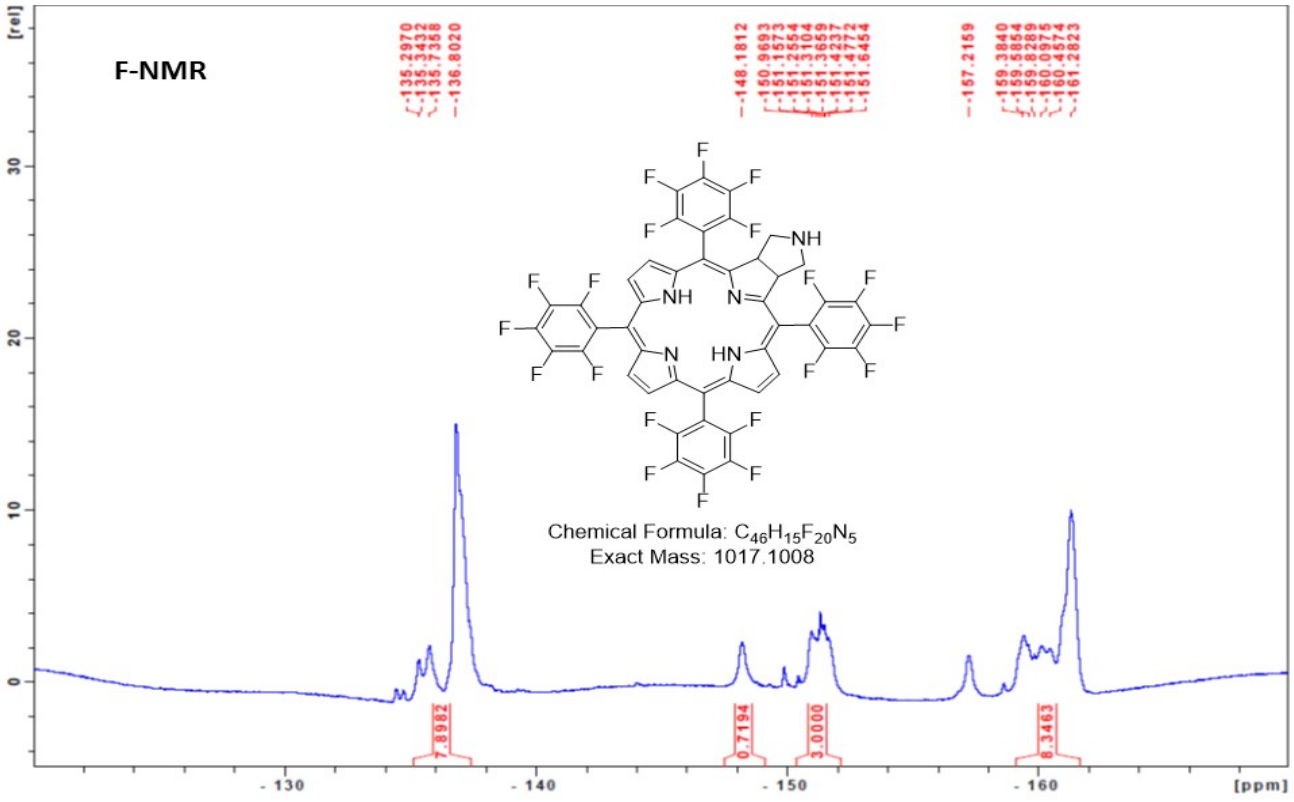


Figure S19. Compound **2** F-19 NMR in $CDCl_3$ with trifluoroacetic acid standard.

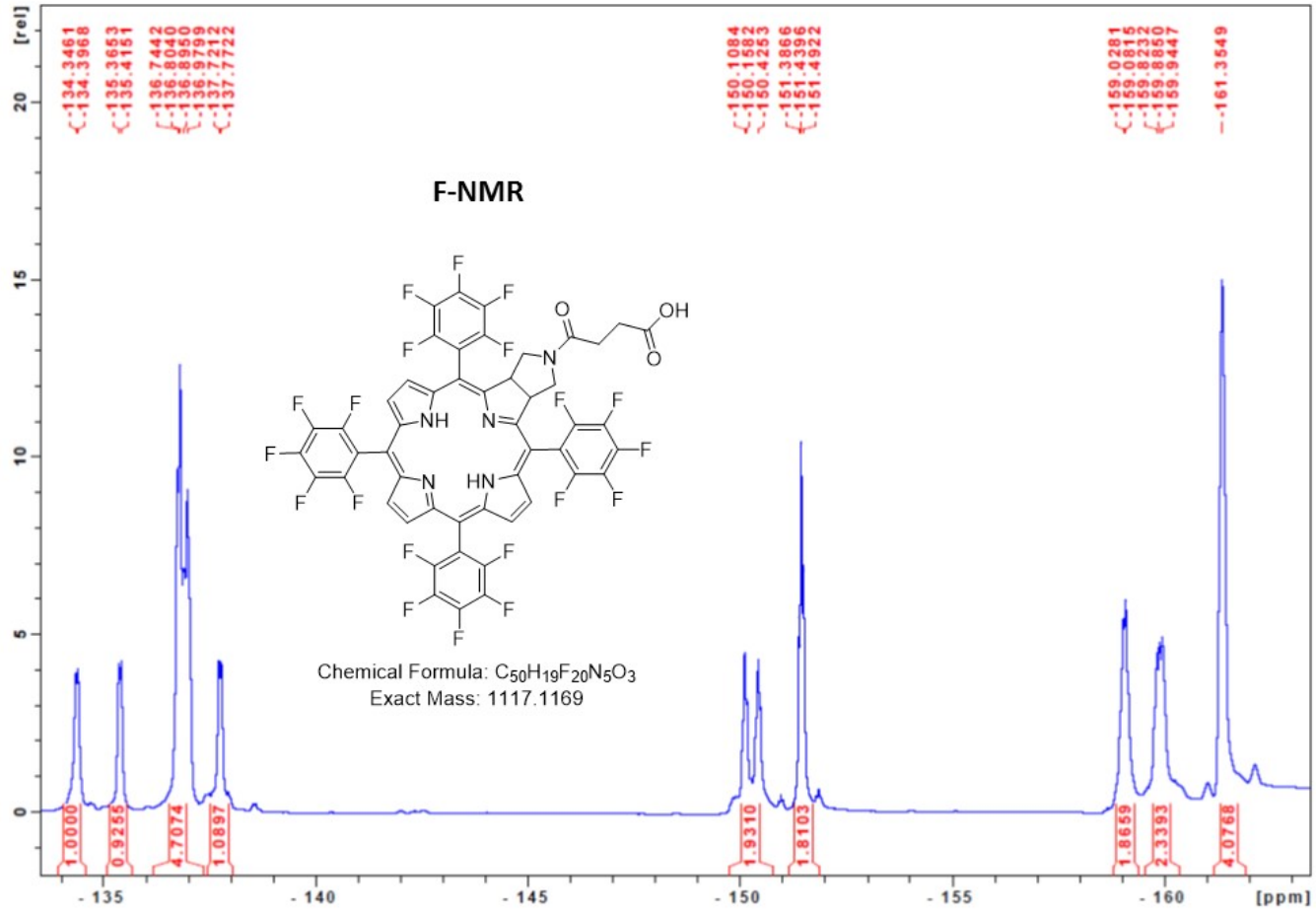


Figure S20. Compound **3** F-19 NMR in $CDCl_3$ with trifluoroacetic acid standard.

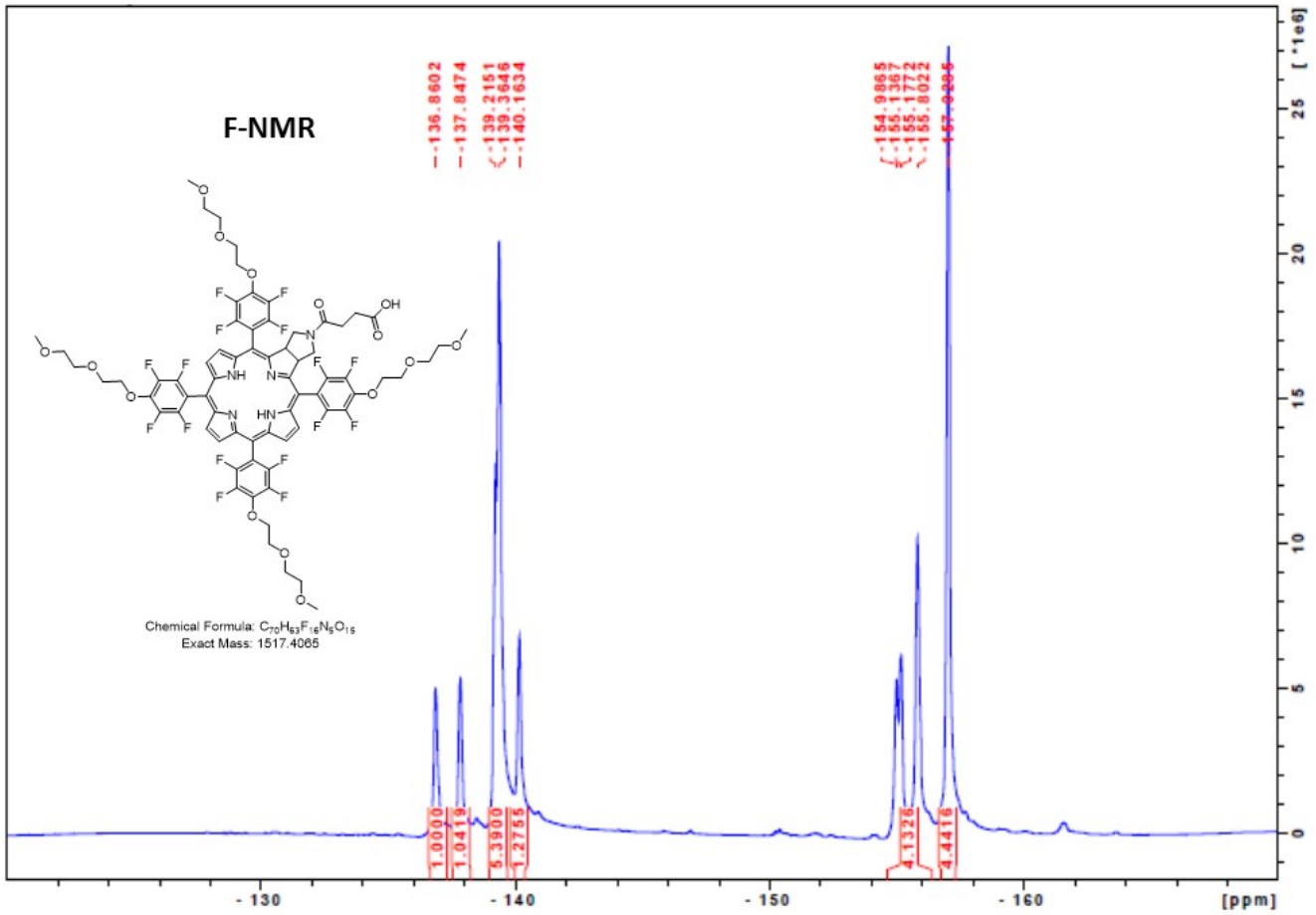


Figure S21. Compound **5** F-19 NMR in $CDCl_3$ with trifluoroacetic acid standard.

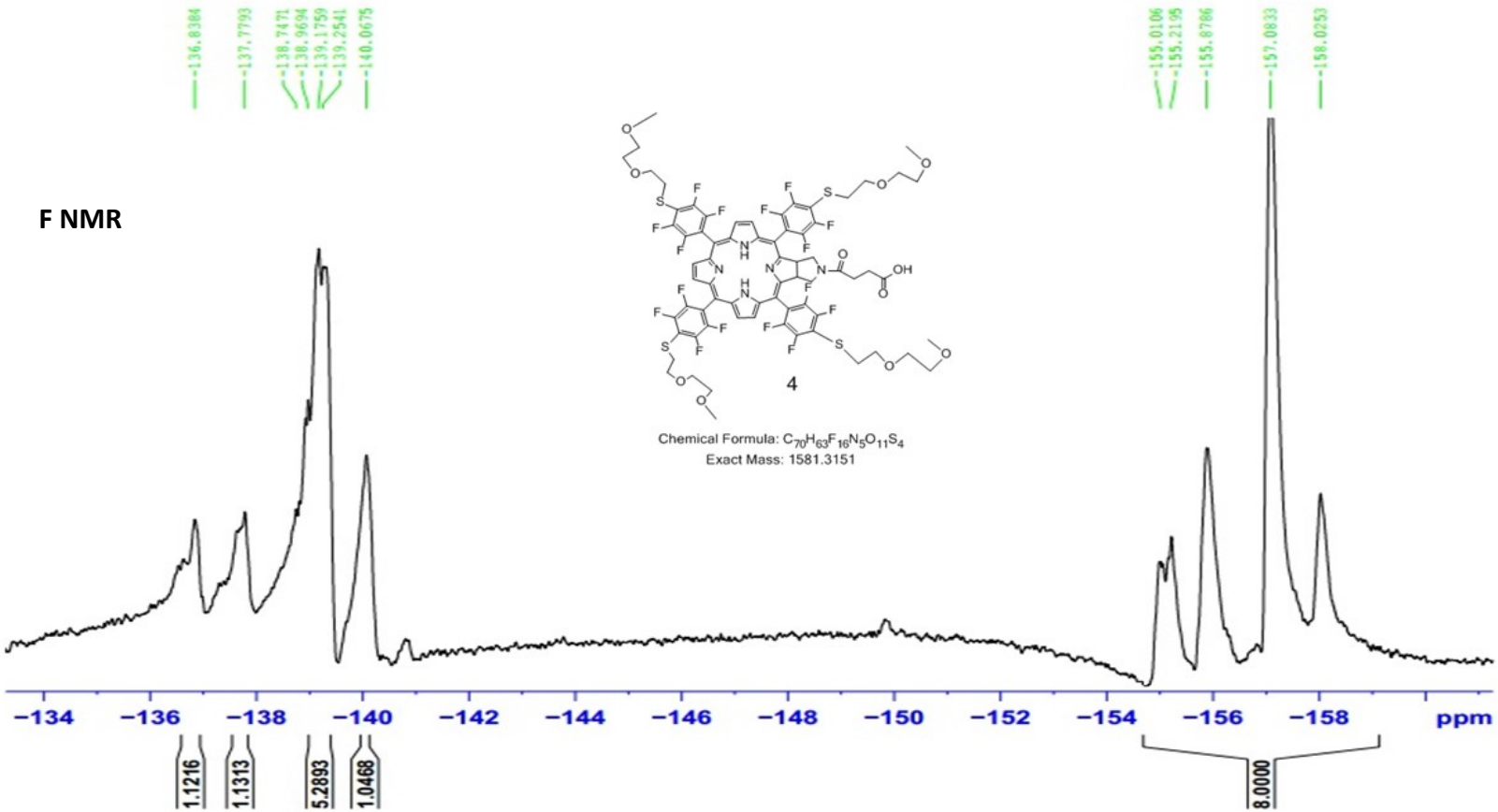
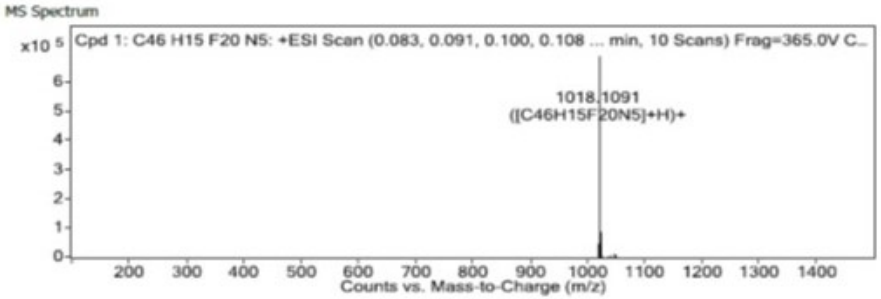


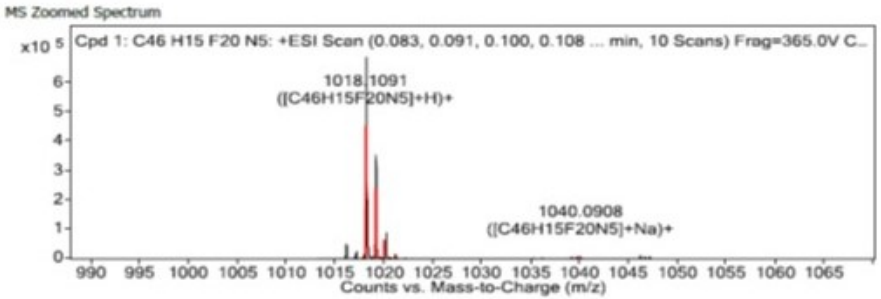
Figure S22. Compound **4** F-19 NMR in $CDCl_3$ with trifluoroacetic acid standard.

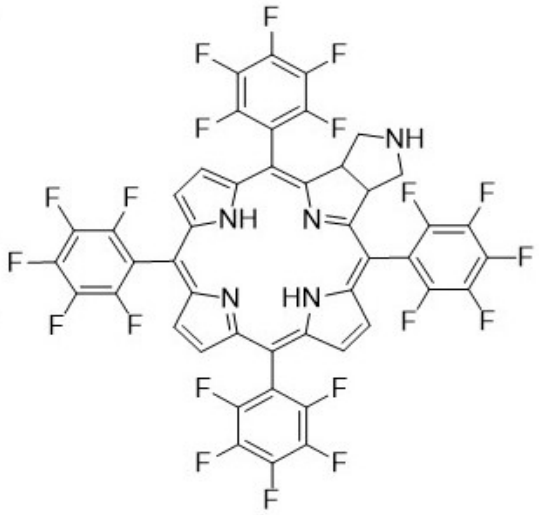
Compound Table

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)	MFG Formula	DB Formula
Cpd 1: C46 H15 F20 N5	0.091	1017.1017	451436	C46 H15 F20 N5	1017.1008	0.83	C46 H15 F20 N5	C46 H15 F20 N5

Compound Label	*m/z*	RT	Algorithm	Mass
Cpd 1: C46 H15 F20 N5	1018.1091	0.091	Find By Formula	1017.1017







MS Spectrum Peak List

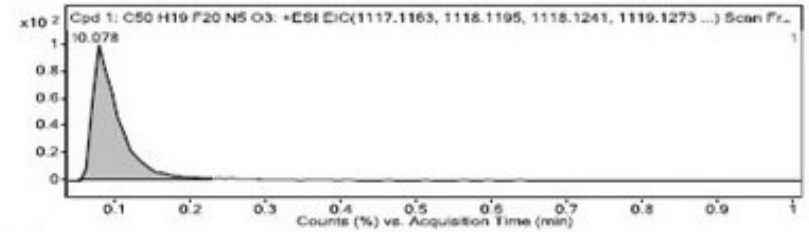
m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
1018.1091	1018.1081	-1	1	451435.5	C46H15F20N5	(M+H)+
1019.1122	1019.1112	-0.95	1	228535.92	C46H15F20N5	(M+H)+
1020.1142	1020.1144	0.16	1	55347.31	C46H15F20N5	(M+H)+
1021.1149	1021.1175	2.5	1	8688.63	C46H15F20N5	(M+H)+
1022.1074	1022.1206	12.89	1	1315.94	C46H15F20N5	(M+H)+
1023.113	1023.1237	10.46	1	290.33	C46H15F20N5	(M+H)+
1040.0908	1040.09	-0.74	1	2817.51	C46H15F20N5	(M+Na)+
1041.0927	1041.0932	0.47	1	589.93	C46H15F20N5	(M+Na)+
1042.1218	1042.0963	-24.41	1	133.49	C46H15F20N5	(M+Na)+

Figure S23. High resolution mass spectrum of compound **2**.

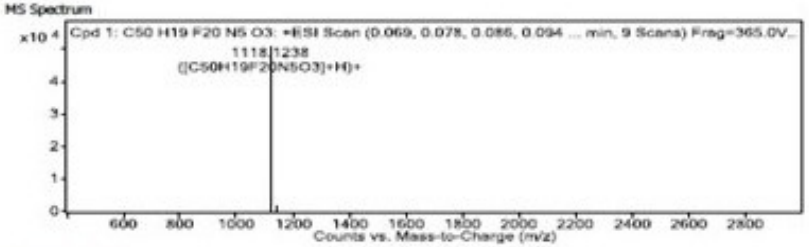
Compound Table

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)	MFG Formula	DB Formula
Cpd 1: C50 H19 F20 N5 O3	0.078	1117.1163	51138	C50 H19 F20 N5 O3	1117.1169	-0.46	C50 H19 F20 N5 O3	C50 H19 F20 N5 O3

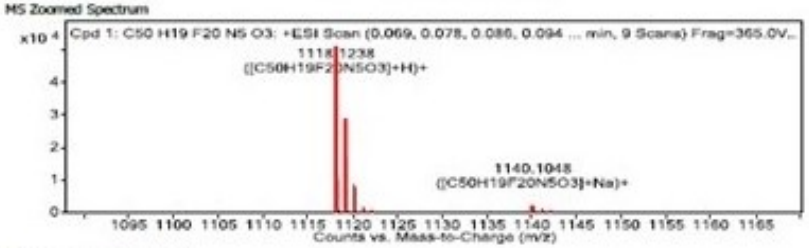
Compound Label	*m/z*	RT	Algorithm	Mass
Cpd 1: C50 H19 F20 N5 O3	1118.1238	0.078	Find By Formula	1117.1163



MS Spectrum



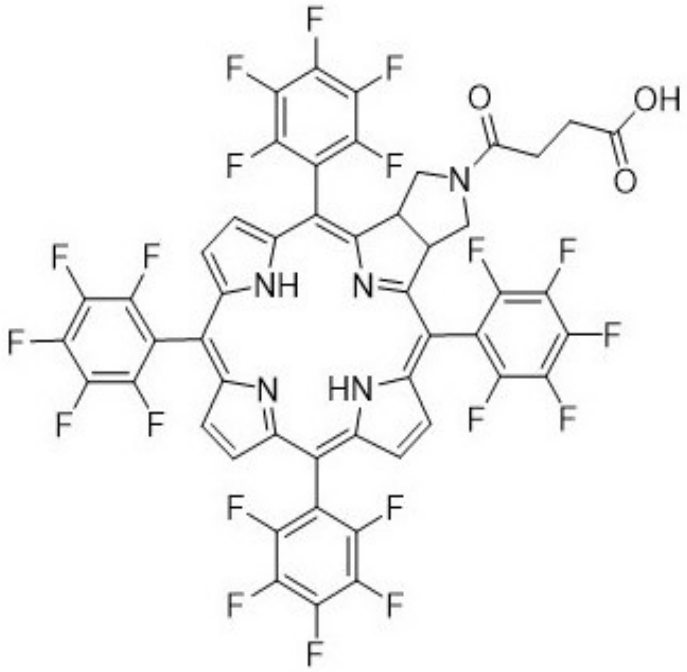
MS Zoomed Spectrum



MS Spectrum Peak List

m/z	*Calc m/z*	Diff(ppm)	z	Abund	Formula	Ion
1118.1238	1118.1241	0.28	1	51138.2	C50H19F20N5O3	(M+H)+
1119.1267	1119.1273	0.49	1	28621.47	C50H19F20N5O3	(M+H)+
1120.1294	1120.1304	0.9	1	7873.74	C50H19F20N5O3	(M+H)+
1121.1315	1121.1334	1.68	1	1547.92	C50H19F20N5O3	(M+H)+
1122.1348	1122.1363	1.4	1	141.66	C50H19F20N5O3	(M+H)+
1140.1048	1140.1061	1.12	1	2019.59	C50H19F20N5O3	(M+Na)+
1141.1079	1141.1092	1.18	1	1121.33	C50H19F20N5O3	(M+Na)+
1142.1072	1142.1123	4.45	1	208.39	C50H19F20N5O3	(M+Na)+

--- End Of Report ---



Chemical Formula: $C_{50}H_{19}F_{20}N_5O_3$
Exact Mass: 1117.1169

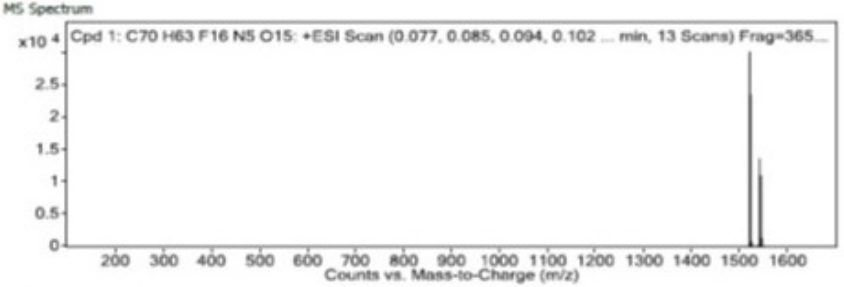
Figure S24. High resolution mass spectrum of compound **3**.

Compound Table

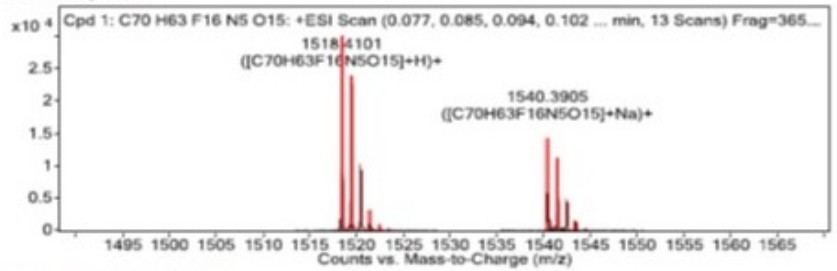
Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)	MFG Formula	DB Formula
Cpd 1: C70 H63 F16 N5 O15	0.094	1517.402	30345	C70 H63 F16 N5 O15	1517.4065	-2.96	C70 H63 F16 N5 O15	C70 H63 F16 N5 O15

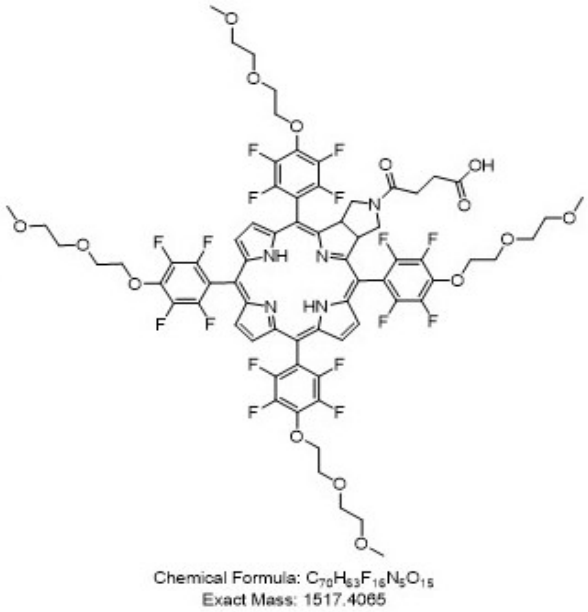
Compound Label	*m/z*	RT	Algorithm	Mass
Cpd 1: C70 H63 F16 N5 O15	1518.4101	0.094	Find By Formula	1517.402

MS Spectrum



MS Zoomed Spectrum





Chemical Formula: $C_{70}H_{63}F_{16}N_5O_{15}$
Exact Mass: 1517.4065

MS Spectrum Peak List

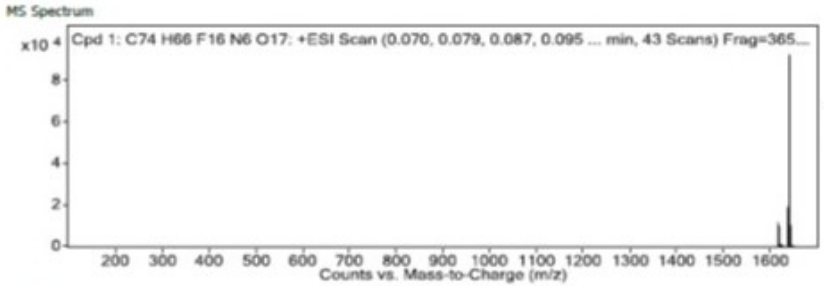
m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
1518.4101	1518.4138	2.43	1	30345.12	C70H63F16N5O15	(M+H)+
1519.4131	1519.417	2.6	1	23575.3	C70H63F16N5O15	(M+H)+
1520.4139	1520.4201	4.09	1	9639.04	C70H63F16N5O15	(M+H)+
1521.4151	1521.423	5.2	1	3250.95	C70H63F16N5O15	(M+H)+
1522.4222	1522.4258	2.4	1	873.28	C70H63F16N5O15	(M+H)+
1540.3905	1540.3957	3.41	1	13907	C70H63F16N5O15	(M+Na)+
1541.3943	1541.399	3.03	1	11243.95	C70H63F16N5O15	(M+Na)+
1542.3968	1542.402	3.38	1	4721.96	C70H63F16N5O15	(M+Na)+
1543.403	1543.4049	1.23	1	1601.81	C70H63F16N5O15	(M+Na)+
1544.4084	1544.4078	-0.43	1	410.34	C70H63F16N5O15	(M+Na)+

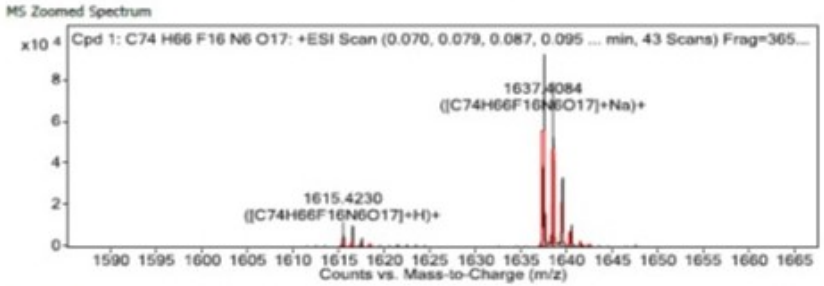
Figure S25. High resolution mass spectrum of compound **5**.

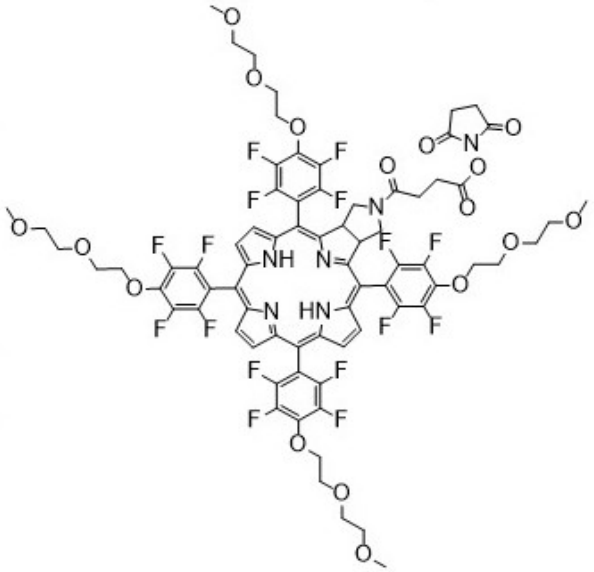
Compound Table

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)	MFG Formula	DB Formula
Cpd 1: C74 H66 F16 N6 O17	0.079	1614.4186	55016	C74 H66 F16 N6 O17	1614.4229	-2.68	C74 H66 F16 N6 O17	C74 H66 F16 N6 O17

Compound Label	*m/z*	RT	Algorithm	Mass
Cpd 1: C74 H66 F16 N6 O17	1637.4084	0.079	Find By Formula	1614.4186







Chemical Formula: $C_{74}H_{66}F_{16}N_6O_{17}$
Exact Mass: 1614.42
Molecular Weight: 1615.34

MS Spectrum Peak List

m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
1615.423	1615.4302	4.44	1	2040.35	C74H66F16N6O17	(M+H)+
1616.4294	1616.4334	2.49	1	1782.41	C74H66F16N6O17	(M+H)+
1617.4272	1617.4364	5.68	1	906.68	C74H66F16N6O17	(M+H)+
1618.4318	1618.4393	4.66	1	378.68	C74H66F16N6O17	(M+H)+
1637.4084	1637.4121	2.27	1	55016.47	C74H66F16N6O17	(M+Na)+
1638.4114	1638.4153	2.39	1	46229.29	C74H66F16N6O17	(M+Na)+
1639.4131	1639.4184	3.22	1	19886.59	C74H66F16N6O17	(M+Na)+
1640.4142	1640.4213	4.28	1	6340.03	C74H66F16N6O17	(M+Na)+
1641.4161	1641.4241	4.87	1	1762.66	C74H66F16N6O17	(M+Na)+
1642.4251	1642.4268	1.06	1	666.64	C74H66F16N6O17	(M+Na)+

Figure S26. High resolution mass spectrum of compound **8**.

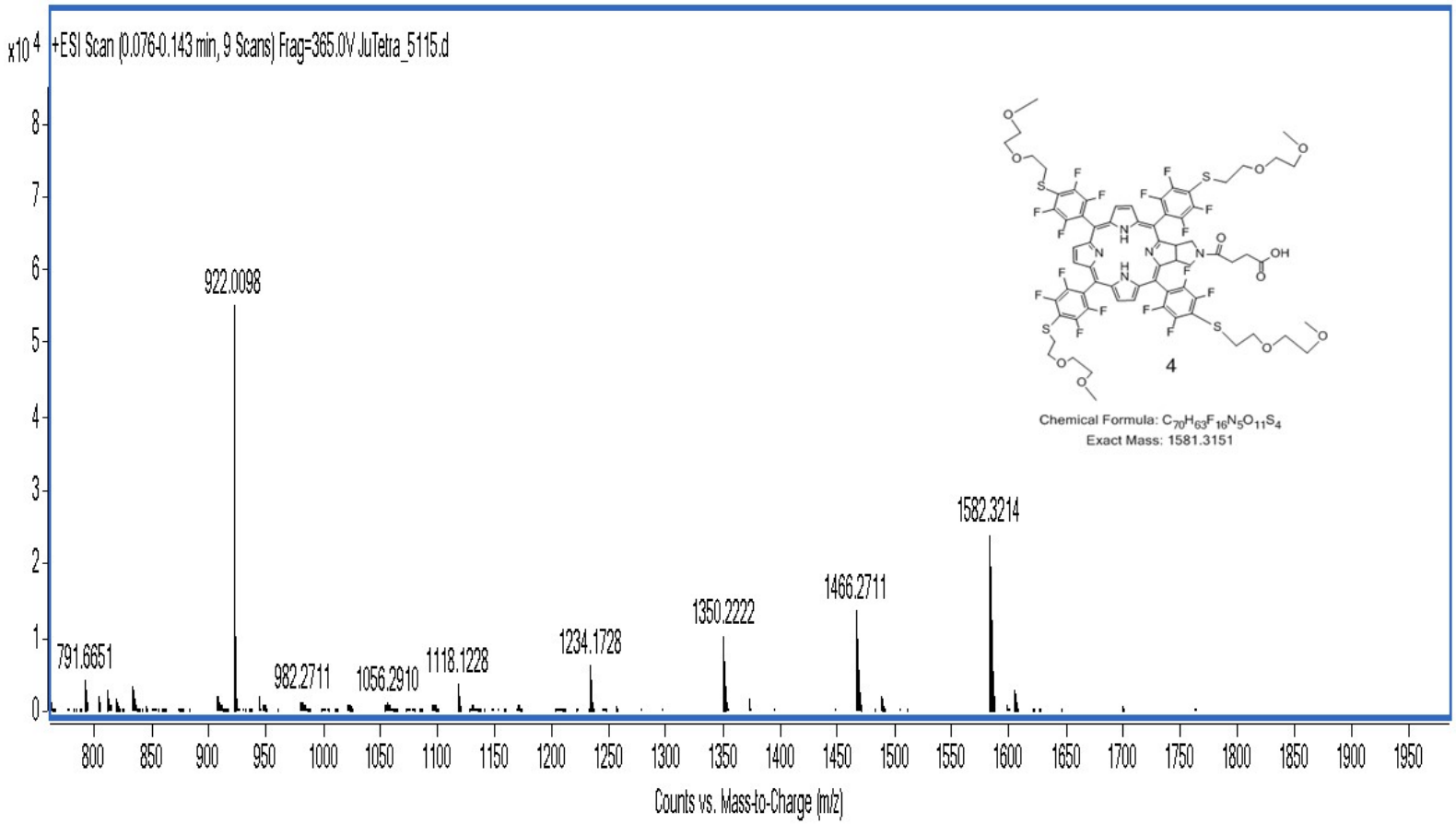


Figure S27. High resolution mass spectrum of compound **4**.

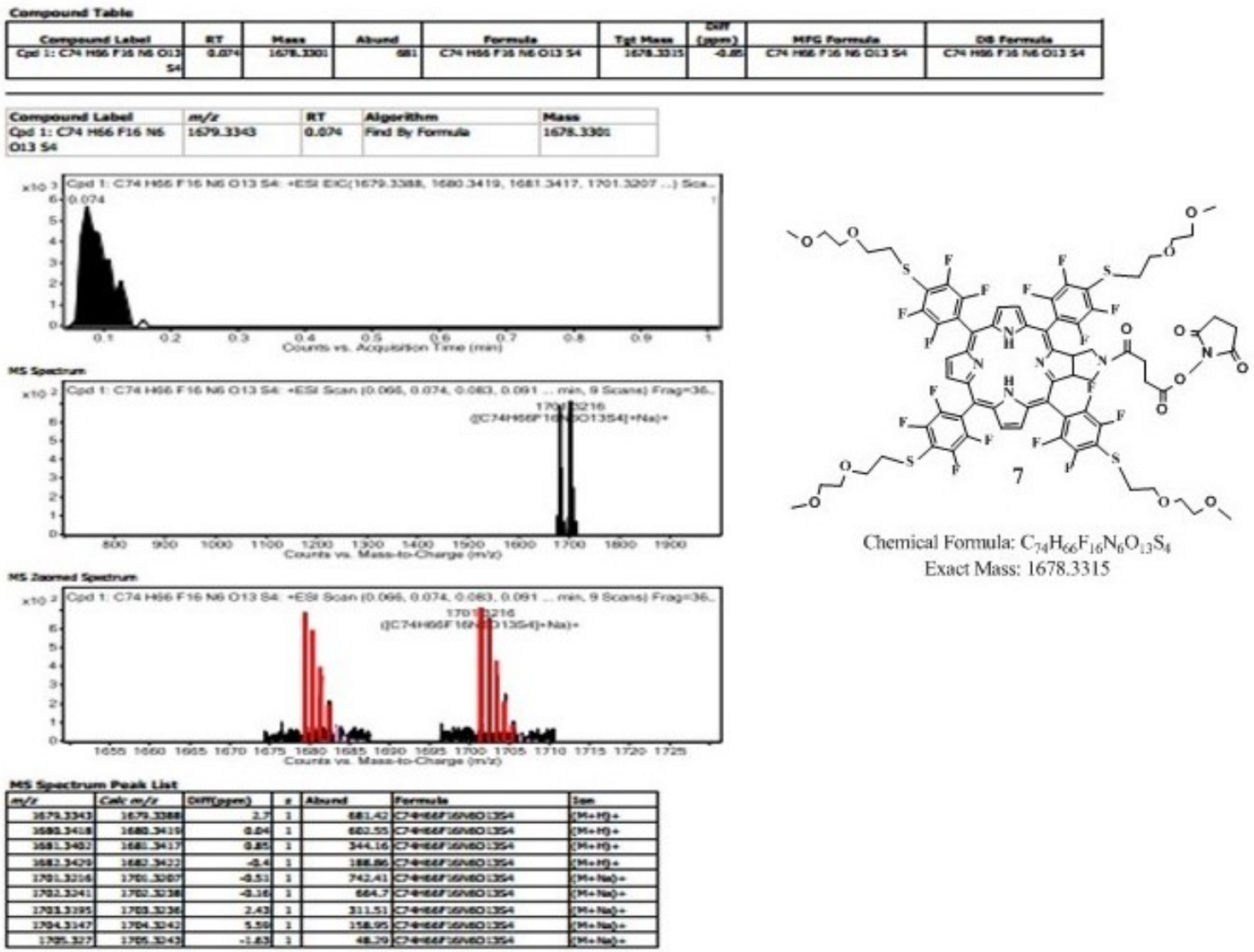
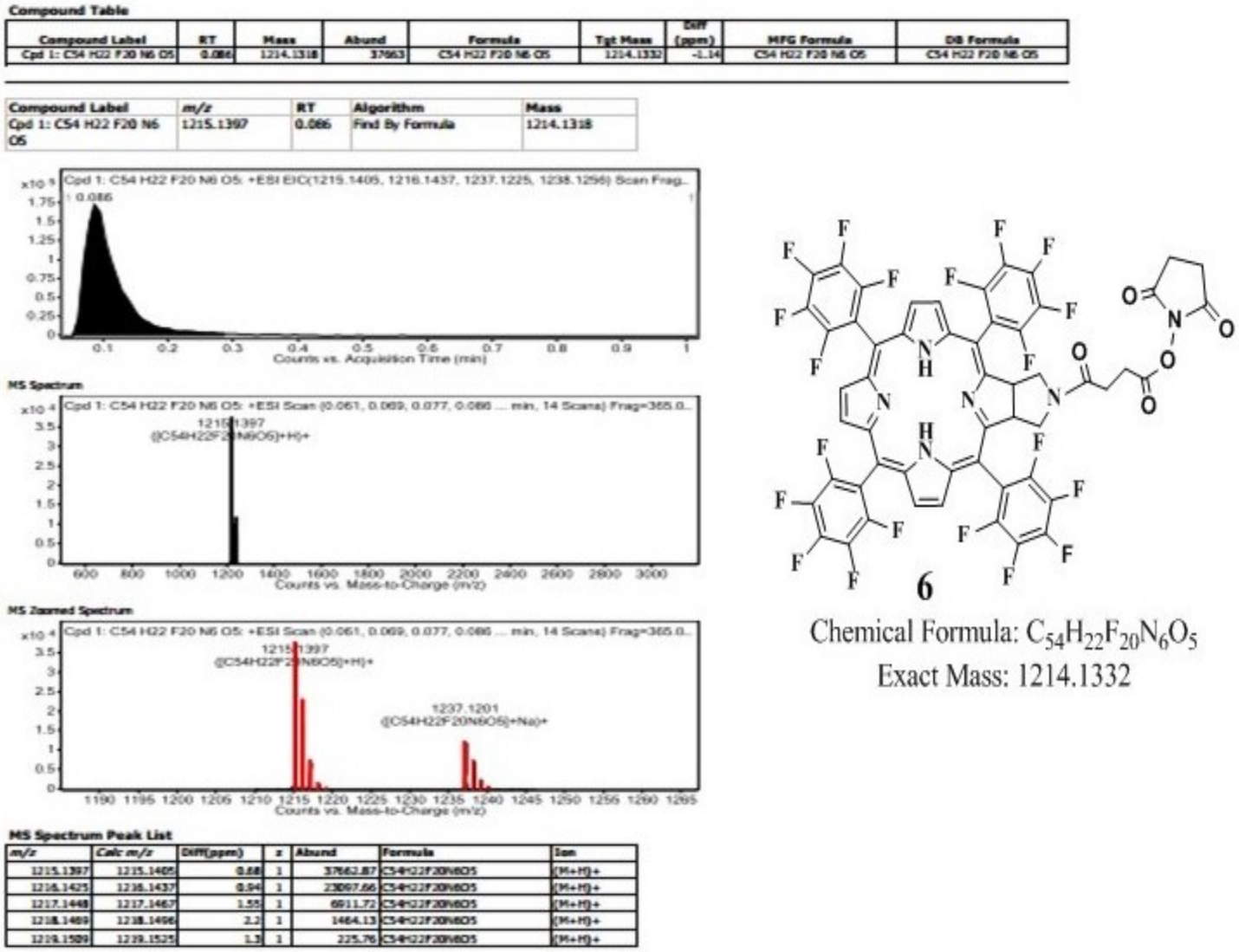


Figure S28. High resolution mass spectrum of compound **7**.



Compound Table

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)	MFG Formula	DB Formula
Cpd 1: C54 H22 F20 N6 O5	0.086	1214.1318	37663	C54 H22 F20 N6 O5	1214.1332	-1.14	C54 H22 F20 N6 O5	C54 H22 F20 N6 O5

Compound Label	m/z	RT	Algorithm	Mass
Cpd 1: C54 H22 F20 N6 O5	1215.1397	0.086	Find By Formula	1214.1318

MS Spectrum Peak List

m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
1215.1397	1215.1405	0.68	1	37662.87	C54H22F20N6O5	(M+H)+
1216.1425	1216.1437	0.94	1	23097.66	C54H22F20N6O5	(M+H)+
1217.1448	1217.1467	1.55	1	6911.72	C54H22F20N6O5	(M+H)+
1218.1469	1218.1496	2.2	1	1464.13	C54H22F20N6O5	(M+H)+
1219.1509	1219.1525	1.3	1	225.76	C54H22F20N6O5	(M+H)+

6

Chemical Formula: $C_{54}H_{22}F_{20}N_6O_5$

Exact Mass: 1214.1332

Figure S29. High resolution mass spectrum of compound **6**.

Bioconjugates

Synthesis of Lysozyme Conjugate

A mixture of S-PEG-chlorin NHS ester (compound **7**) or an O-PEG-chlorin NHS ester (compound **8)** in DMSO (final concentration 100 μM) and aqueous lysozyme from chicken egg white (SIGMA L6876) at a PEG-chlorin:lysozyme molar ratio 1:1 was adjusted to a final volume of 1 mL with DMSO. The conjugation lasted overnight at room temperature in the dark with constant shaking. After reaction, samples were centrifuged at 16300 xg for 5 min at 4 °C (no precipitates were observed) and stored at -20 °C. Crude product was purified by gel filtration on a 5 mL column of Sephadex™ G-25 Superfine (GE Healthcare 17-0031-01). Conjugates were eluted with distilled deionized water and 2 mL fractions were collected. The fluorescence of the chlorins allows direct visualization of the gels with or without staining by standard Coomassie Blue protocols. Fractions 2 and 3 (V_e 3-6 mL) luminesced upon excitation with 365 nm UV light. Fractions were dried in a Speed-Vac concentrator overnight and analysed by SDS-PAGE (ESI S30, S31), non-denaturing PAGE (ESI S32) and UV-Vis (ESI S26) after dissolving in 50 μL distilled deionized water. Lysozyme contains lysine amino acids that can potentially react with the NHS ester.

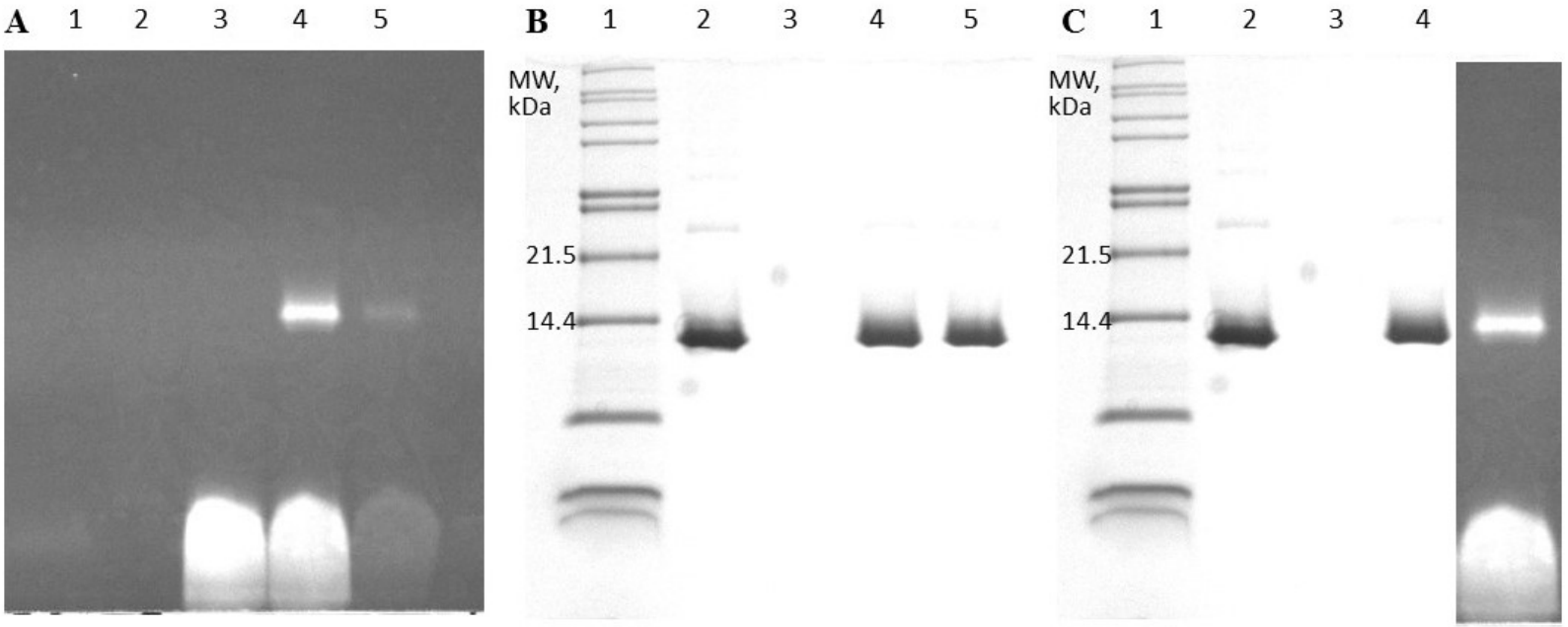


Figure S30. SDS-PAGE for conjugation of S-PEG-Chlorin to lysozyme in DMSO. Lane **1**: Molecular weight marker. **2**: Free lysozyme. **3:** Free S-PEG-chlorin. **4**: Crude product reaction 1:1. **5:** Crude product reaction 1:10. **A**: Visualization of chlorin fluorescence after excitation at 365 nm **B**: Visualization of protein after staining with Coomassie Blue **C**: Overlay UV/Coomassie.

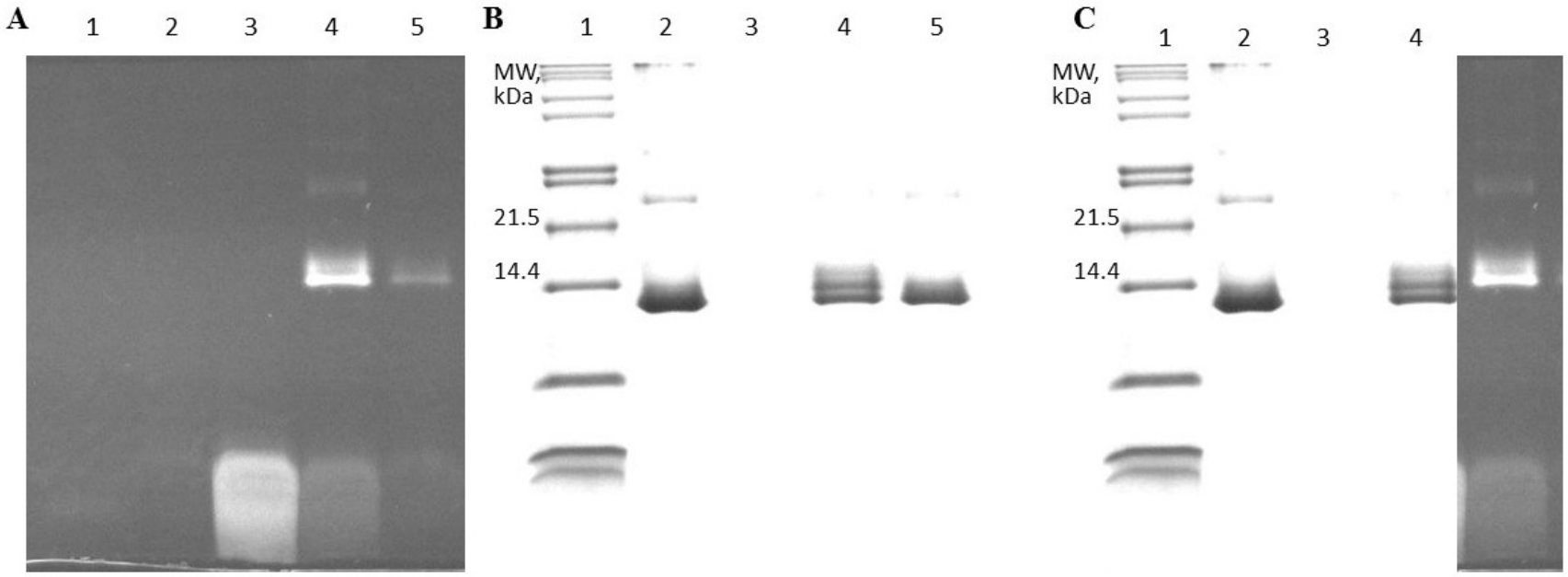


Figure S31. SDS-PAGE for conjugation of O-PEG-chlorin to lysozyme in DMSO. Lane **1**: molecular weight marker. **2**: Free lysozyme. **3:** Free Chlorin. **4**: Crude product reaction 1:1. **5:** Crude product reaction 1:10. **A**: Visualization of chlorin fluorescence after excitation at 365 nm **B**: Visualization of protein after staining with Coomassie Blue. **C:** Overlay UV/Coomassie.

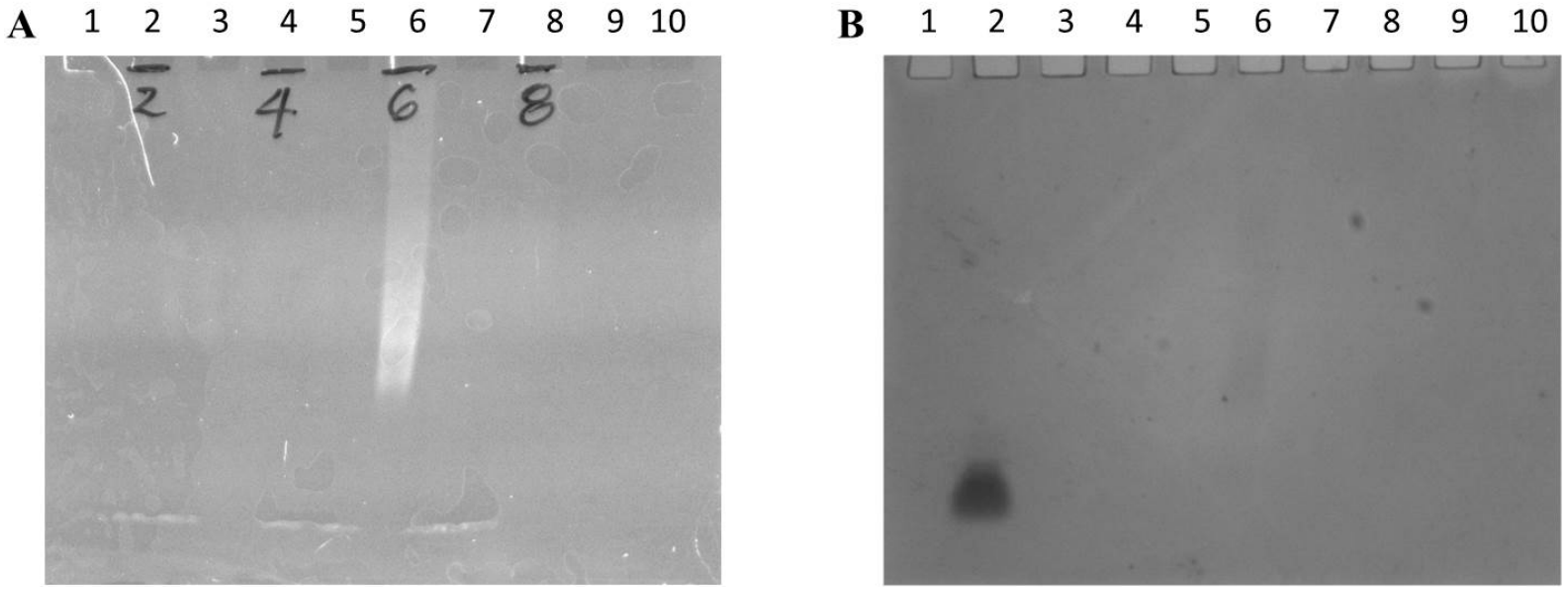
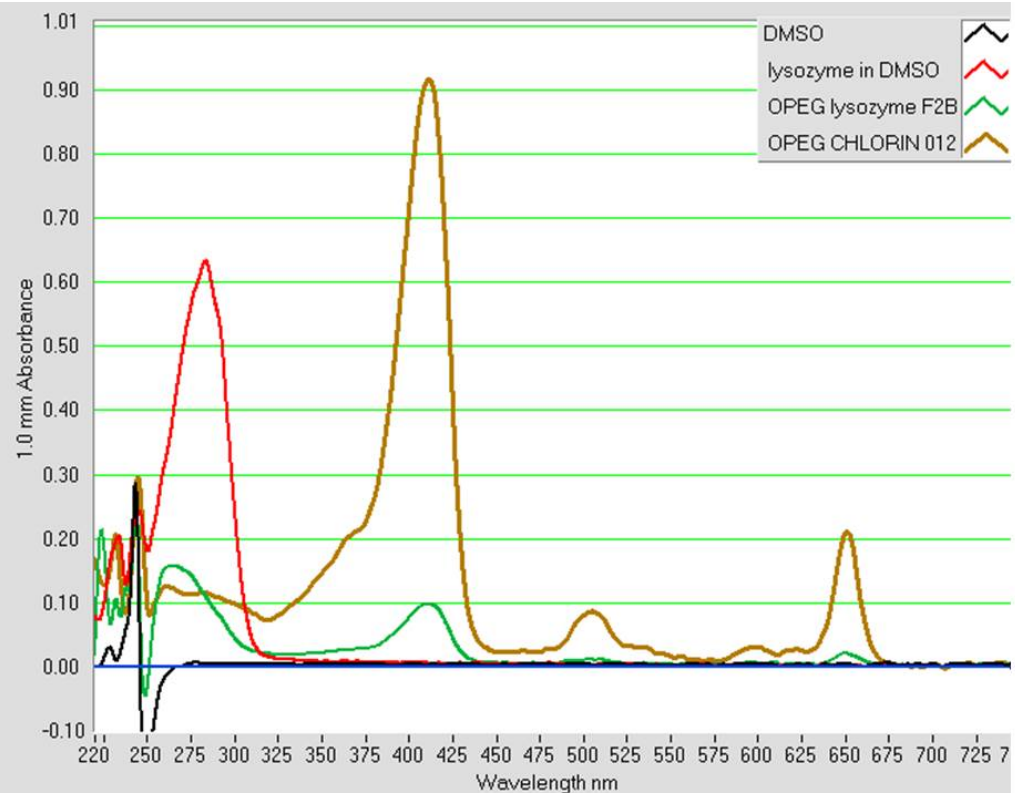


Figure S32. Nondenaturing PAGE for bioconjugation of O-PEG-Chlorin to lysozyme in DMSO after SEC. Lane 2: Free lysozyme. Lane 4: Fraction-1. Lane 6: Fraction-2. Lane 8: Fraction-3. **A**: Visualized with UV light at 365nm before staining with Coomassie Blue. **B**: After staining with Coomassie Blue.



UV-visible λ_{max} (nm)	
O-PEG Chlorin	O-PEG Chlorin Lysozyme
411	409
504	503
592	601
651	655

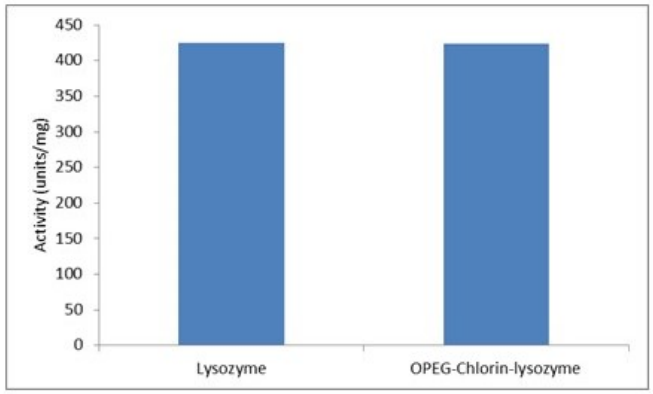
Figure S33. UV-Vis absorption spectra of OPEG-chlorin **8** (brown) and OPEG-chlorin-lysozyme conjugate after SEC (green). Red: lysozyme in DMSO; black DMSO. Spectra recorded in a NanoDrop 1000 spectrophotometer.

Conjugation to ssDNA. Chlorin was coupled to the 5' end of a 14 nt DNA via 6 carbon amine linker (5'-NH_2-$(CH_2)_6$-NH-dTTCTTCTCCTTTCT-3'). Chlorin was added to 100 μM DNA containing phosphate buffer 0.1 M, pH 7.4 with 10:1 ratio. The reaction was carried out overnight at 37 °C; aggregation was observed under these conditions. After reaction, sample was centrifuged at top speed for 5 min, the supernatant and pellet dissolved in DMF were electrophoresed on a 20% polyacrylamide gel as shown in Figure 1. The same position on the gel for both chlorin and DNA confirmed the coupling of chlorin and DNA. A smeared band from top of the gel in lane 3 might be the contribution of free dye and higher ordered labelled DNA.

Figure S34. Enzymatic activity assay. **A**: lysozyme in DMSO; **B**: O-PEG-chlorin-lysozyme conjugate in DMSO, *M. lysodeikticus* cells (SIGMA M3770 ATCC No 4698) (1.25 mL, 0.15 mg/mL) in 66 mM potassium phosphate pH 6.2 was mixed with 0.05 mL lysozyme or lysozyme conjugate in DMSO. The change in absorbance at 450 nm was monitored for five minutes in a Cary 50 Scan UV-Vis spectrophotometer (Varian). The activity was calculated as:

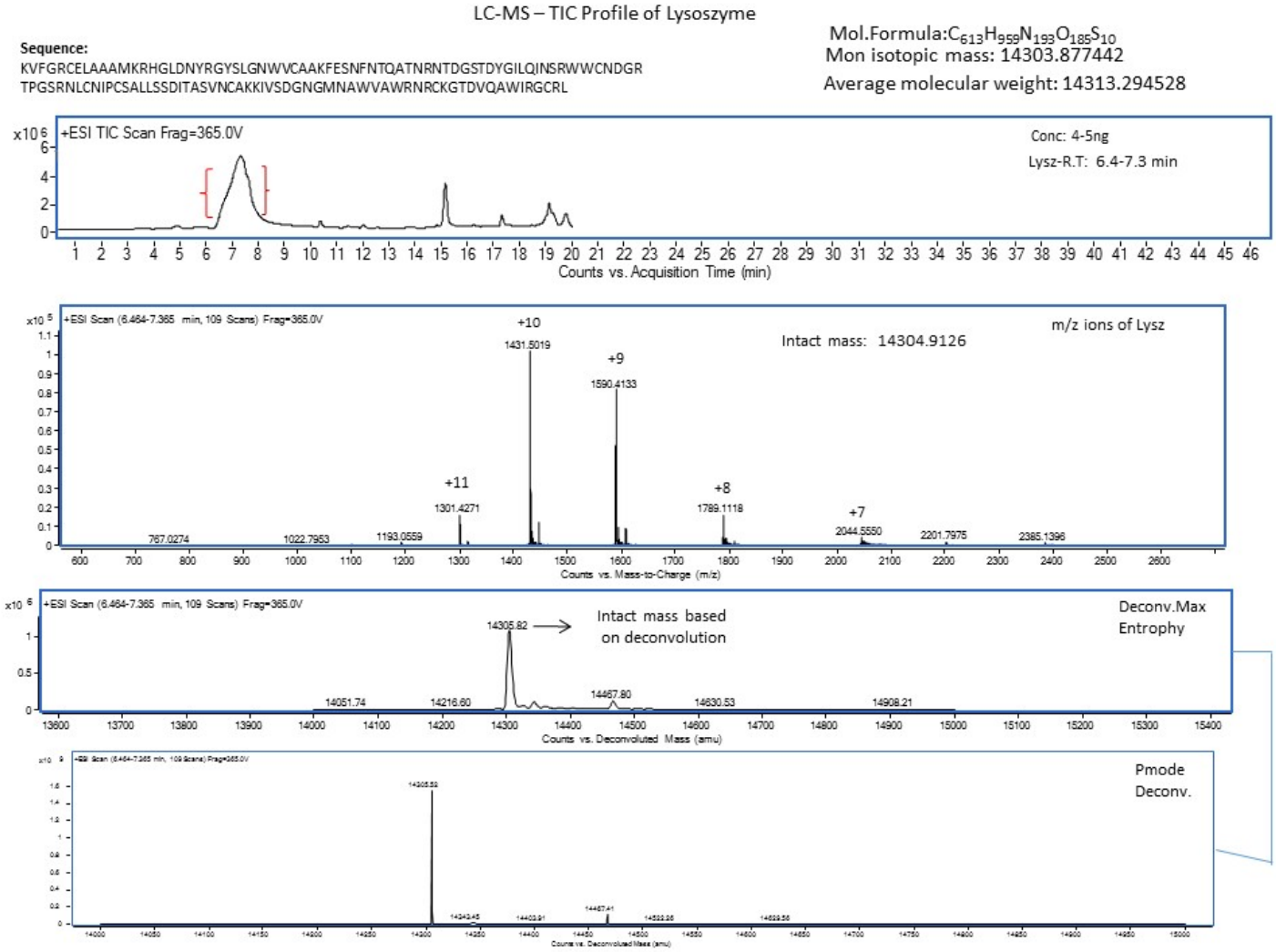
$$\text{Units/mg} = \frac{\Delta A_{450}/\text{min} * 1000}{\text{mg enzyme}}$$

$$\text{Units/mL} = \frac{\Delta A_{450}/\text{min}}{(0.001)*(0.05)}$$



Activity (units/mg) in DMSO	
Lysozyme	OPEG-Chlorin-lysozyme
425	424

Mass spectrometry of the bioconjugates


LC-MS – TIC Profile of Lysozyme
Sequence:
KVFGRCELAAAMKRHGLDNYRGYSLGNWVCAAKFESNFNTQATNRNTDGSTDYGILQINSRWWCNDGR
TPGSRNLCNIPCSALLSSDITASVNCAKKIVSDGNGMNAWVAWRNRCKGTDVQAWIRGCRL
Mol.Formula:C613H959N193O185S10
Mon isotopic mass: 14303.877442
Average molecular weight: 14313.294528
+ESI TIC Scan Frag=365.0V
Conc: 4-5ng
Lysz-R.T: 6.4-7.3 min
Counts vs. Acquisition Time (min)
+ESI Scan (6.464-7.365 min, 109 Scans) Frag=365.0V
m/z ions of Lysz
Intact mass: 14304.9126
+10
1431.5019
+9
1590.4133
+11
1301.4271
+8
1789.1118
+7
2044.5550
767.0274
1022.7953
1193.0559
2201.7975
2385.1396
Counts vs. Mass-to-Charge (m/z)
Intact mass based on deconvolution
Deconv.Max Entrophy
14305.82
14051.74
14216.60
14467.80
14630.53
14908.21
Counts vs. Deconvoluted Mass (amu)
Pmode Deconv.

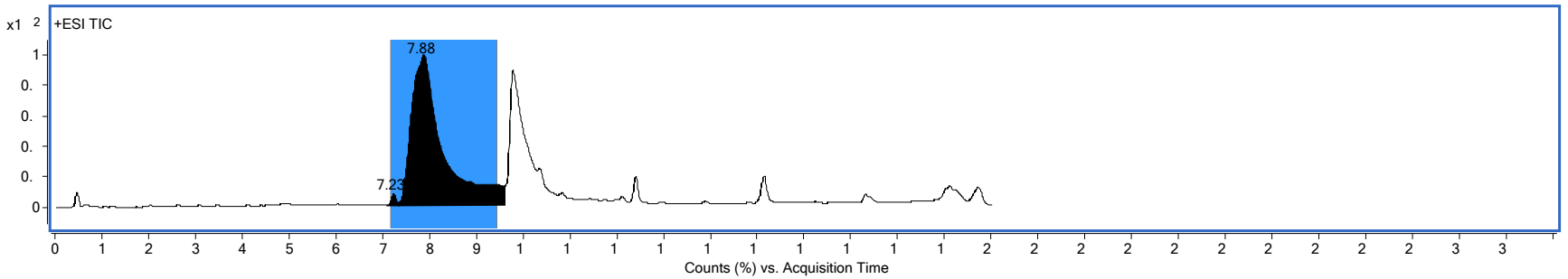
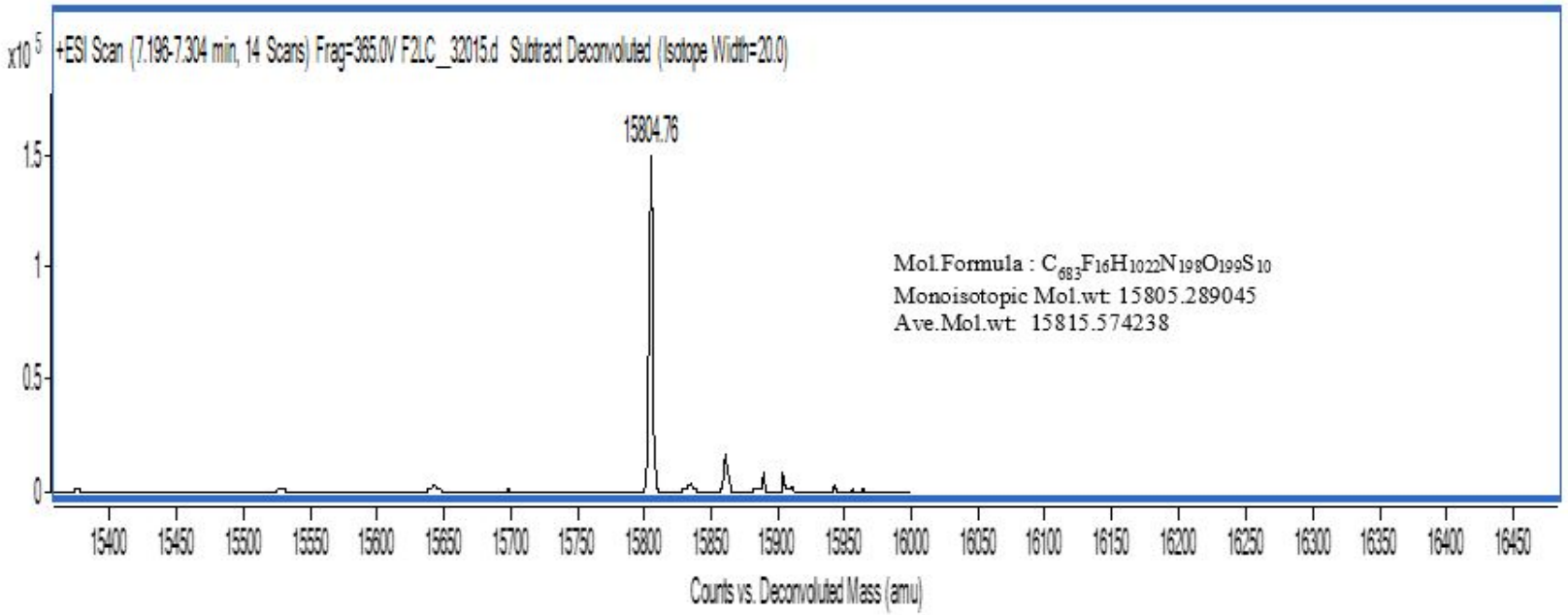


Figure S35. HRESI-MS of the conjugate from HPLC. The second peak in the HPLC is the DCU by-product resulting from PEG-chlorin synthesis by DCC.



The above intact mass corresponds to the Lysozyme-OPEG-Chlorin conjugate attached to one lysine reside.

KVFGRCELAAAMKRHGLDNYRGYSLGNWVCAAKFESNFNTQATNRNTDGSTDYGILQIN
SRWWCNDGRTPGSRNLCNIPCSALLSSDITASVNCAKKIVSDGNGMNAWVAWRNRCKGT
DVQAWIRGCRL;13:OPEGChlorin,

Figure S36. The deconvoluted HRESI-MS of lysozyme is 14,305.82 from pH 7.2 buffer. Deconvolution of the HRESI-MS of the fractions from the conjugation reaction using the O-PEG-chlorin include 15804.76 for the lysozyme-OPEG chlorin conjugate

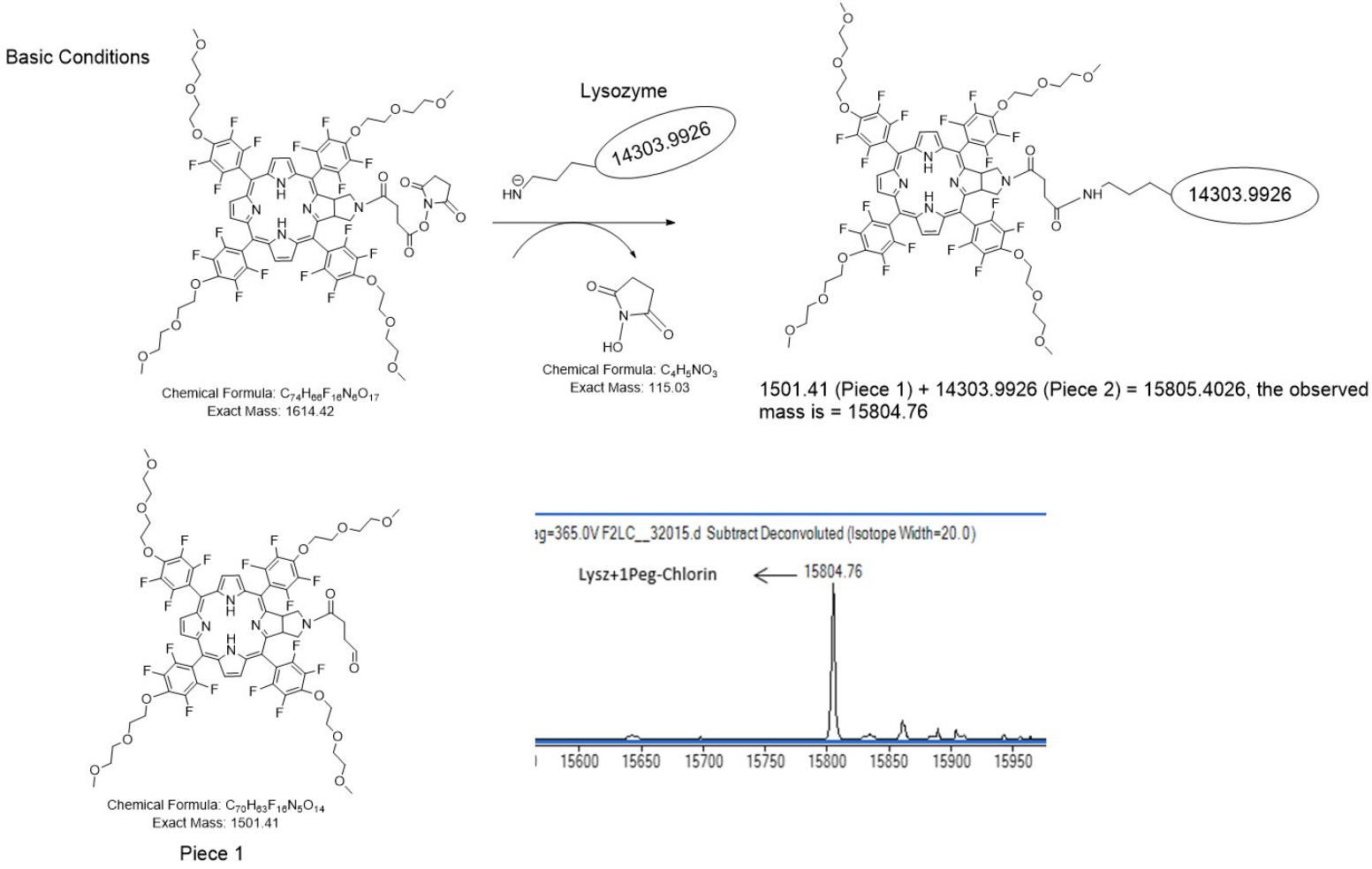


Table S1. Compound 2, N-H chlorin compared to the N-CH_3 derivative, proton NMR assignments and comparison to literature.

	Hirohara[a]		Drain[b]		Tome[c]		Silva[d]	
C8-H, C17H, β-pyrrole*H*	8.54	dd, *J*=5.8 Hz, *J*=9.9 Hz, 2H	8.64	d, *J*=5 Hz, 2H	Aromatic = four of the six b-pyrrolic protons appear as two doublets and there is a singlet at 8.48	8.40 (2H) and 8.71 (J 4.9 Hz, 2H) 8.48 (2H)	Pyrrolic β-H[c]	8.31 d (1) 8.37 d (1) 8.42 d (1) 8.46 d (1) 8.68 d (2)
C12-H, C13-H, β-pyrrole*H*	8.51	s, 2H	8.41	s, 2H				
C7-H, C18-H, β-pyrrole*H*	8.26, 8.23	dd, *J*=5.0 Hz, *J*=4.8 Hz, 2H	8.30	d, *J*=4 Hz, 2H				
C2-H, C3-H, β-pyrrole*H*	5.34	m, 2H	5.14	s, 2H	1 m, two b-pyrrolic protons of the reduced ring	5.24–5.27	Pyrrolic β-C(sp3)-H	5.24-5.25 m (2)
CHH	3.18	brs, 2H	3.28-3.25	dd, J_1=5, J_2=10 Hz, 2H	2 m, methylene protons	2.52–2.56 and 3.11–3.16	H-methylene	2.53-2.60 m (2) 3.09-3.14 m (2)
CHH	2.25-2.06	brs, 2H	2.27-2.24	dd, J_1=5, J_2=10 Hz, 2H				

a ^{1}H NMR (600.07 MHz, $CDCl_3$, Si(CH_3) = 0 ppm), Hirohara and coworkers.[1]

b ^{1}H NMR (500 MHz, $CDCl_3$) this work.

c chemical shifts (ppm) in CDCl3 , multiplicity (number of protons), Tome and coworkers.[2]

d chemical shifts (ppm) in $CDCl_3$, Cavaleiro and coworkers.[3]

Table S2. C-13 NMR assignments and comparison to literature.

Hirohara[a]	PPM	Drain[b]	PPM
C1, C4, C11, C14-α-pyrroleC	156.04-155.83	C-F pentafluorophenyl	151.77 (ortho)
C2C6-PhC, C3C5-PhC	148.97-144.55	C15	146.31
C9, C16-α-pyrroleC	138.46	Quaternary, pentafluorophenyl	145.59
C6, C19-α-pyrroleC	137.69	C10	144.31
C8, C17-β-pyrroleC	131.92	Quaternary, pentafluorophenyl	143.59
C12, C13-β-pyrroleC	127.85	C14	142.21
C7, C18-β-pyrroleC	127.34, 127.14	C16	140.16
C4-PhC	121.44	C-F pentafluorophenyl	139.26 (para)
C1-PhC	111.67	C5, meso	138.17
C10, C15, mesoC	109.49	C19	137.45
C5, C20, mesoC	98.22	C20, meso	136.12
N-CH2	63.60	C1	135.46
C2, C3-β-pyrroleC	50.74	C-F pentafluorophenyl	134.17 (para)
		C-F pentafluorophenyl	131.34 (meta)
		C-F pentafluorophenyl	126.31 (ortho)
		C-F pentafluorophenyl	122.84 (meta)
		C12, C13	114.69, 114.24
		C17	105.30
		C18	96.07
		N-CH2	27.99
		C2, C3-β-pyrrole (bridge head)	51.33

a ^{13}C NMR ($CDCl_3$, 100.40 MHz, $CDCl_3$ = 77 ppm)[1]

b a^{13}C NMR ($CDCl_3$, 125 MHz, $CDCl_3$ = 77 ppm)

Table S3. F-19 NMR assignments and comparison to literature.

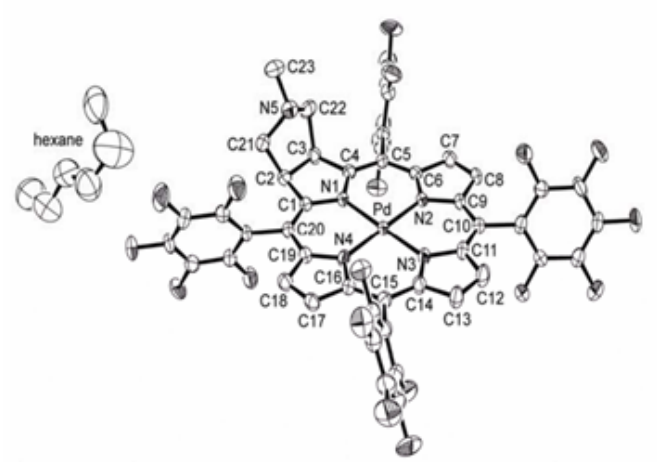
	Hirohara[a]		Drain[b]		
	-131.38	1F, brs, 3,5-Ph*F*	-135.29 to -136.80	(m, 8F, Ar-m-F)	
	-131.74, -131.97	1F, m, 3,5-Ph*F*	-150.96 to -151.64	(m, 4F, Ar-p-F)	
	-132.67	2F, s, 3,5-Ph*F*	-157.21 to -161.28	(m, 8F, Ar-o-F)	
	-135.31	1F, brs, 2,6-Ph*F*			
	-137.26	2F, m, 2,6-Ph*F*			
	-137.79, -137.83	1F, m, 2,6-Ph*F*			

a ^{19}F NMR (376 MHz, $CDCl_3$, CF_3CO_2H = -76.50 ppm)

b ^{19}F NMR (376 MHz, $CDCl_3$)

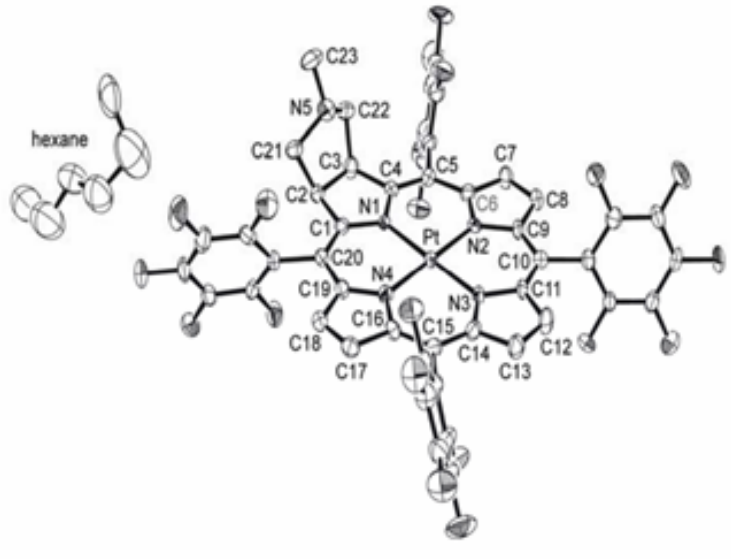
Relaxation of ^{13}C by ^{19}F (measured by NOE) is not efficient; J coupling of ^{13}C-^{19}F = 1-25 Hz, resulting in weak multiplets in the carbon spectrum.[4]

Doubling of the peaks for chlorins is due to the two tautomeric form of the pyrrole NH, but these are not well resolved in all ^{13}C spectra.[1,5,6]



. ORTEP drawing of **4b**·C_6H_{14} asymmetric unit with thermal ellipsoids shown at the 50 % probability level. Hydrogen atoms are omitted for clarity.

Figure S37. Crystal structure of the Pd(II) complex of the N-CH_3 chlorin.



ORTEP drawing of **4c**·C_6H_{14} asymmetric unit with thermal ellipsoids shown at the 50 % probability level. Hydrogen atoms are omitted for clarity.

Figure S38. Crystal structure of the Pt(II) N-CH_3 chlorin.

The crystal structures show the distortion of the dye and the out-of-plane orientation of the pyrrolidine group.[7]

Cell studies with DNA Conjugate

Confocal Microscopy. MDA-MB-231 cells were plated onto cell culture dishes. O-PEG-chlorin-DNA conjugate **9b** were prepared at final concentration of 1, 2.5, 5, 7.5 and 10 nM in DMEM (Dulbecco's Modified Eagle's medium). After incubation for 24 h, the cells were rinsed three times with phosphate buffered saline (PBS), and incubated with a 4% paraformaldehyde solution for 15 min at 37 °C under cell growth conditions. Cells were then washed three times with PBS and visualized using a Nikon Eclipse Ti microscope in Hunter's Bio-Imaging Facility. Excitation was 572nm+/-35nm and emission was 630+/-60nm for all samples.

Observations:

The images were taken at five different concentrations (1, 2.5, 5, 7.5 and 10 nM). After overnight incubation of the cells with concentrations over ca. 5 nM of **9b**, we observed decreases in cell density, changes in cell morphology, and cell clumping. Neither the DNA by itself nor the chlorin platform cause these morphological changes in the cells at these concentrations.[8] We expected the amphipathic chlorin moiety to diffuse passively into the cell membrane due to the PEG groups; however, the DNA part of the conjugate inhibits uptake into the cell due to the high negative charge of the phosphate backbone. Although the mechanism for the changes in cell morphology and necrosis induced by **9b** is under investigation, we postulate that aggregates of the compound cause a disruption of the cancer cell membrane. The fluorescence microscopy studies were conducted on the same day the cells were fixed using paraformaldehyde and 7 days after fixing. We observed a substantial increase in the fluorescence on day 7 compared to day 1, suggesting that **9b** initially was aggregated in/on the cells, and then slowly disaggregates after fixing. This increase in fluorescence upon disaggregation is similar to what is observed for glycosylated phthalocyanines.[9] We also observed aggregates of the compound mostly on the surface or in the cytoplasm, which is in agreement with our assumption that the DNA inhibits the conjugate from entering the cell and cell organelles.

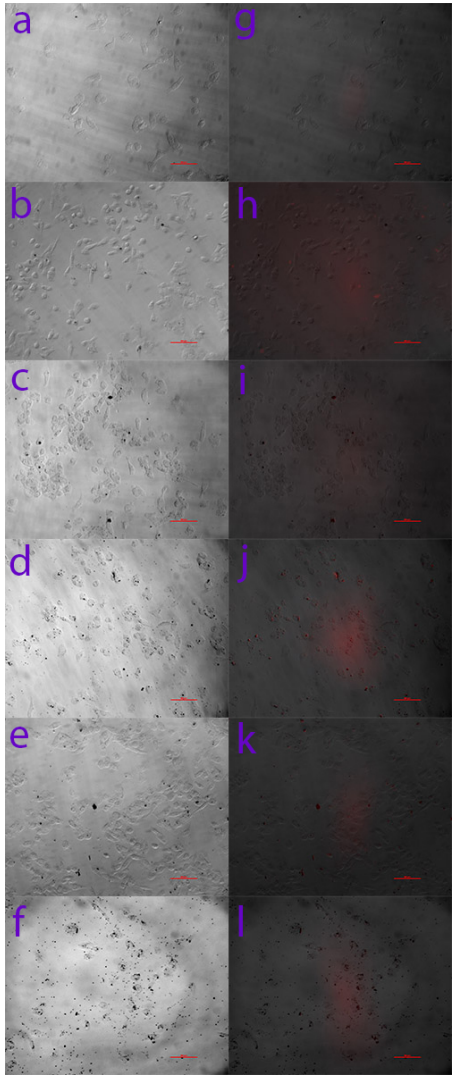


Figure S39. Fluorescence images of DNA-chlorin conjugate on the day of fixing MDA-MB-231 cells using 4% paraformaldehyde. **a-f** are bright field images and **g-l** are overlays at 0, 1, 2.5, 5, 7.5 and 10 nM concentration of the conjugate.

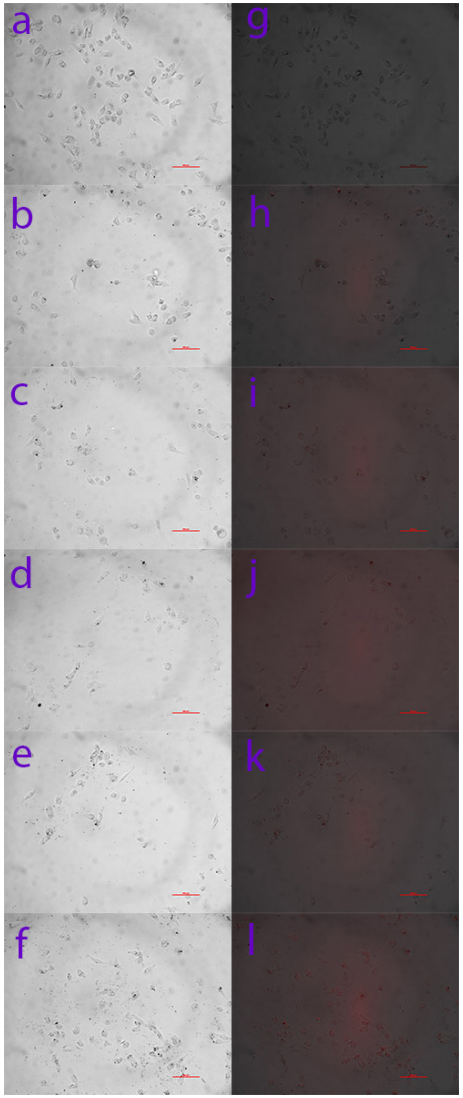


Figure S40. Fluorescence images of DNA-chlorin conjugate after 7 days of fixing MDA-MB-231 cells using 4% paraformaldehyde. **a-f** are bright field images and **g-l** are overlays at 0, 1, 2.5, 5, 7.5 and 10 nM concentration of the conjugate.

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