SUPPORTING INFORMATION

Pentamethine Sulfobenzoindocyanine Dyes with Low Net Charge States and High Photostability

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Abbreviations:

AcOH = Acetic acid
Ac_2O = Acetic anhydride
ACN = Acetonitrile
c(RGDyK) = Cyclo(Arg-Gly-Asp-dTyr-Lys); targeting peptide
DIPEA = N,N-Diisopropylethylamine
DMF = N,N-Dimethylformamide
DMSO = Dimethyl sulfoxide
EtOH = Ethanol
EtOAc = Ethyl acetate
Et_2O = Diethyl ether
HRMS = High resolution mass spectrometry
IPA = Isopropyl alcohol
MeOH = Methanol
NMM = N-Methylmorpholine
NMP = N-Methyl-2-pyrrolidone
SWC = Single Wavelength Chromatogram
TEA = Triethylamine
TBTU = 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate
TSTU = N,N,N′,N′-Tetramethyl-O-(N-succinimidyl)uronium tetrafluoroborate

Materials and Methods:

Chemicals were purchased from VWR (Radnor, PA), Sigma Aldrich (St. Louis, MO), Alfa Aesar (Ward Hill, MA), TCI Chemicals (Tokyo, Japan), Acros Organics (Morris Plains, NY), CreoSalus (Louisville, KY), and Peptides International (Louisville, KY). All chemicals were used as received unless otherwise stated. All were of HPLC or ACS grade.

PBS buffer for conjugation reactions was 137 mM NaCl, 2.68 mM KCl, 10.1 mM Na_2HPO_4, 1.76 mM KH_2PO_3, and was adjusted to pH 8.5 using 0.1 M NaOH.

The HPLC instrument used was an Agilent 1100 with Diode Array Detector. For LC-MS, a Waters 2695 Alliance HPLC with a Waters 2998 Diode Array Detector and a Waters 3100 SQ Mass Spectrometer was used. For HPLC, the column used was a Phenomenex Synergi 4 µ Polar-RP 80 Å, 150 x 3.0 mm. For LC-MS the column used was an Agilent XDB C18 3.54 µ column, with dimensions of 3 mm x 100 mm. Mass spectra from this instrument were recorded at unit resolution with positive and negative switching mode at 35 or 50 V cone voltages. The flow rate for HPLC and LC-MS was 0.5 mL/min. HPLC/LC-MS solvents were purchased from VWR.

Preparative HPLC (prep-HPLC) was carried out with a Waters 600E system controller and a Waters 600 multi-solvent delivery system using a 30 mL/min flow rate. The column used for all purification of dyes and dye conjugates was a Phenomenex Synergi 10 µ Polar-RP 80 Å, 250 x 21.20 mm.

High resolution mass spectra (HRMS) were obtained on a Waters Synapt G2Si (School of Chemical Sciences, University of Illinois at Urbana-Champaign) using the following parameters: Flow injection at flow rate of 0.1 mL/min, H_2O/ACN/0.1% Formic Acid, positive and negative mode ESI, Cone voltage =25 V, capillary voltage = 3.0 V, ion source temperature = 100 °C, desolation temperature = 180 °C, nebulizing gas (N_2) flow = 200 L/h, cone gas (N_2) flow = 5 L/h.

Thin-Layer chromatography-mass spectrometry (TLC-MS) and flow injection-mass spectral analysis (FIA) was carried out on an Advion Expression Compact Mass Spectrometer, model L (CMS-L) equipped with a Plate Express module for TLC. The solvent for the TLC-MS and FIA-MS on the CMS was either methanol or acetonitrile containing 0.01 % ammonium acetate.

Chromatography gradients for HPLC, prep-HPLC, and LC-MS are noted as (method X: Y, t) where X is the organic mobile phase being either acetonitrile (ACN) or methanol (MeOH), Y is the starting percentage or overall range of the organic mobile phase, and t is the overall time in minutes of the gradient. For example, method (ACN: 50, 8 min) is a gradient that is initially 50% ACN and 50% H_2O, but gradually transitions to 100% ACN and 0% H_2O in 8 minutes. Method (MeOH: 20-70, 8 min) is a gradient that starts at 20% MeOH and 80% H_2O, and over 8 minutes the gradient transitions to 70% MeOH and 30% H_2O.

All aqueous mobile phases for HPLC, prep HPLC, and LC-MS are 0.1 M ammonium acetate unless otherwise noted. Ion exchange chromatography was carried out by using Amberlite IR-120(Na) ion exchange resin manufactured by Alfa Aesar.

The NMR (^1H, ^{13}C, 2D correlation) data were obtained using a Bruker Avance III 500 MHz NMR spectrometer using TopSpin 3.2 software. Chemical shifts (δ) are reported in ppm relative to TMS.

UV-Vis data was obtained using a Shimadzu UV-2600 UV-Vis Spectrophotometer with LabSolutions UV-VIS (version 1.03) software. Sample temperature was kept constant at 22.0 ± 0.1 °C in the instrument with the Shimadzu CPS-100 Cell Positioner. All UV-Vis data was collected under the spectrum tab with parameters as follows: Spectrum Type: absorbance, Start WL: 800 nm, End WL: 200 nm, Data Interval: 0.2 nm, Scan speed: medium, Slit Width: 1.0 nm. All samples and references were analyzed in capped 6Q quartz cuvettes with a 1 cm path length.

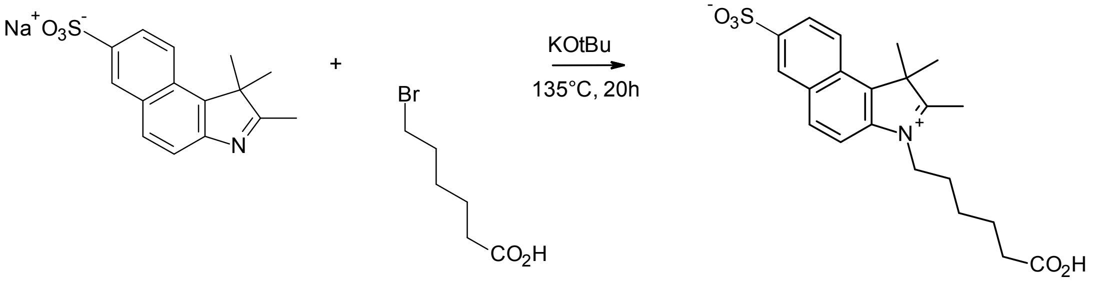
Emission spectra and dye fade data was obtained using a Shimadzu RF6000 Spectro Fluorophotometer with LabSolutions RF (version 1.12) software. Sample temperature was kept constant at 22.0 ± 0.1 °C in the instrument with a VWR 1160S Heated Refrigerated Circulating Bath. Samples were stirred in the instrument using a Scinics Co. Multistirrer CC-301. All emission spectra were collected under the spectrum tab with parameters as follows: Spectrum Type: emission, Excitation WL: 600 nm, Emission WL Start: 625 nm, Emission WL End: 800 nm, Data Interval: 0.2 nm, Scan speed: 60 nm/min, Excitation Slit Width: 3.0 nm, Emission Slit Width: 5.0 nm, Sensitivity: auto.

Dye fade data was collected under the time course tab with parameters as follows: Timing Mode: manual, Timing Unit: second, Cycle Time: 1 s. Number of Readings: 7200, Excitation Slit Width: 15.0 nm, Emission Slit Width: 5.0 nm, Sensitivity: low, Accumulation Time: 5 s. Excitation and emission wavelengths in the parameters were set to the compound's respective values where absorption and emission were at maximum. All samples were analyzed in capped 6Q quartz cuvettes with a 1 cm path length and stirred with a small magnetic stir bar during each entire experiment.

Relative quantum yields (RQY, Φ_R) were obtained using a Shimadzu RF6000 Spectro Fluorophotometer with LabSolutions RF (version 1.12) software. Sample temperature was kept constant at 22.0 ± 0.1 °C in the instrument while stirring. Relative quantum yields were determined using the Quantum Yield tab with the following parameters: Excitation WL: 645.0 nm, Emission WL Start: 660.0 nm, Emission WL End: 800 nm, Scan speed: 200 nm/min, Excitation Slit Width: 5.0 nm, Emission Slit Width: 5.0nm, Sensitivity: auto, Refractive Index (H_2O): 1.3333. The absorbance of the standard and unknowns were also entered. All samples for RQY measurement were in PBS Buffer (50 mM potassium phosphate, 150 mM NaCl, pH 7.2) and purged with Ar before acquisition. Alexa Fluor 680 (Invitrogen) with its published RQY was used as the reference sample.

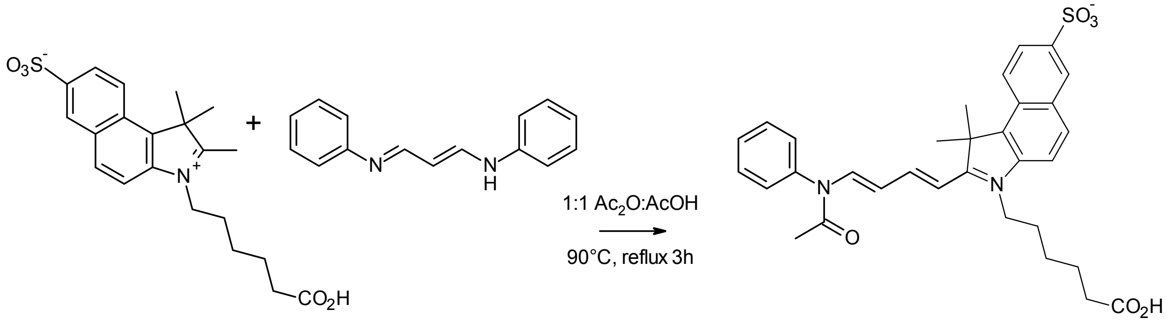
UV-Vis samples were not purged with Ar gas, or enriched with O_2 gas. They were placed in the instrument and allowed to equilibrate for 10 minutes to temperature before acquisition. For emission spectra and RQY, samples were purged with Ar for 10 minutes, sealed, and placed in the spectro - fluorophotometer while stirring to equilibrate to temperature for an additional 10 minutes. Data acquisition was started following the 10 minute equilibration. For dye fade, samples were purged with Ar gas or enriched with O_2 gas in the same manner for emission spectra and RQY. The concentration used for dye fade and emission spectra was 0.55 µM.

Experimental Procedures



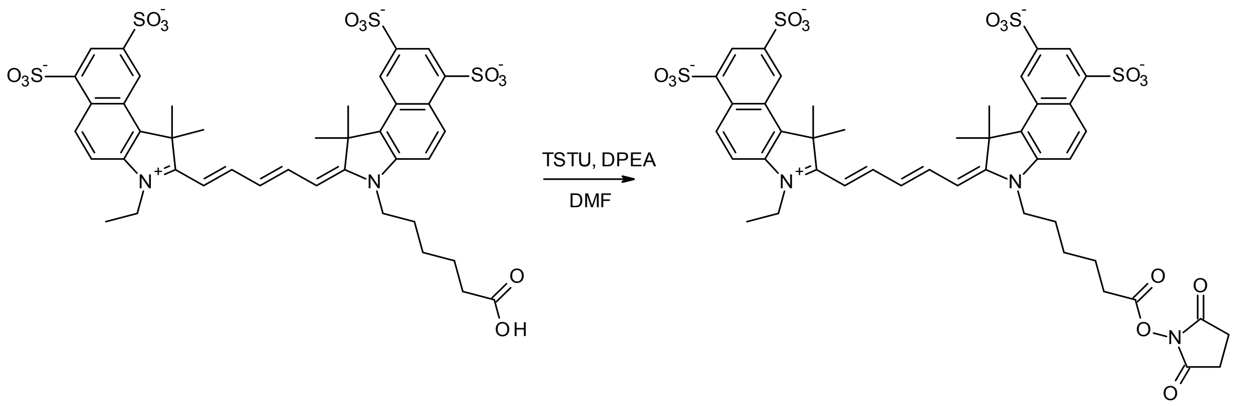
Scheme S1. *Reaction scheme for synthesis of compound* ***(i)****.*

(i); 3-(5-carboxypentyl)-1,1,2-trimethyl-1H-benzo[e]indol-3-ium-7-sulfonate; 1,1,2-trimethyl-1H-benzo[e]indole-7-sulfonate (2.50 g, 8.67 mmol, Carestream Molecular Imaging/Eastman Kodak, also available by synthesis[1]) was added to the reaction flask. Added in succession were bromohexanoic acid (12.1 g, 62.43 mmol, TCI Chemicals), and potassium tert-butoxide (2.92 g, 26.2 mmol, Alfa Aesar). The neat reaction was set to stir at 135 °C for 20 h. LC-MS was used to monitor reaction progress using method (ACN: 10, 8 min). Upon completion, the reaction mixture was triturated in triplicate with 25 mL additions of pure EtOH. The product was collected by vacuum filtration and dried under high vacuum. The yield was 3.14 g (89%). LC-MS (LR, ESI) = Calcd. For $C_{21}H_{26}NO_5S$: 403.14 (m/z), found: 402.32 $[M-H]^-$.



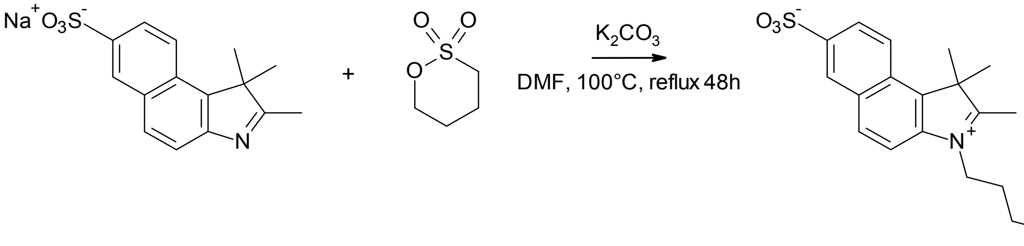
Scheme S2*. Reaction scheme for synthesis of compound* ***(ii)****.*

(ii); 3-(5-carboxypentyl)-1,1-dimethyl-2-((1E,3E)-4-(N-phenylacetamido)buta-1,3-dien-1-yl)-1H-benzo[e]indol-3-ium-7-sulfonate; Compound **(i)** (0.50 g, 1.228 mmol) and N-((1E,3E)-3-(phenylimino)prop-1-en-1-yl)aniline (0.350 g, 1.41 mmol, Sigma Aldrich) were added to the reaction flask. A mixture of 1:1 Ac_2O:glacial AcOH (5 mL) was added to the flask and used as the solvent. The flask was stirred and heated to 90°C for 3 h while being monitored every 0.5 h via LC-MS (method MeOH: 20, 8min). Upon completion, the reaction was triturated in triplicate with 25 mL additions of t-butanol. The product was collected by vacuum filtration and dried under high vacuum. The yield was 0.660 g (93%). LC-MS (LR, ESI) = Calcd. For $C_{32}H_{34}N_2O_6S$: 574.21 (m/z), found: 573.32 $[M-H]^-$.



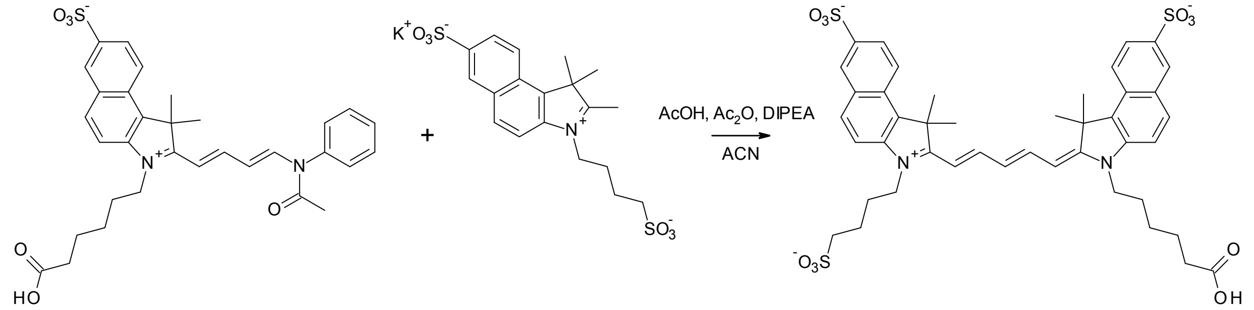
Scheme S3**. Reaction scheme for synthesis of compound **(1a), **Cy5.5 4S-NHS Ester***.*

(1a); Cy5.5 4S-NHS Ester; 2-((1E,3E,5E)-5-(3-(6-((2,5-dioxopyrrolidin-1-yl)oxy)-6-oxohexyl)-1,1-dimethyl-6,8-disulfonato-1,3-dihydro-2H-benzo[e]indol-2-ylidene)penta-1,3-dien-1-yl)-3-ethyl-1,1-dimethyl-1H-benzo[e]indol-3-ium-6,8-disulfonate; Cy5.5 4S (Compound **(1)**) (3.0 mg, 3.27 µmol, GEHealthcare) was dissolved in anhydrous DMF (2 mL). DIPEA (6.34 mg, 0.0491mmol, TCI), and TSTU (3.95 mg, 0.0131 mmol, CreoSalus) were then added to the flask. The reaction was stirred under an Ar atmosphere at r.t., and monitored every 1 h via LC-MS (method ACN: 10, 8 min) utilizing a 0.1 % butyl amine in water quench of a reaction aliquot, to ensure an accurate measure of conversion. The product was isolated by precipitation with 1:1 EtOAc:Et_2O. The precipitated product suspended in solution was centrifuged, decanted, and dried under high vacuum. The yield was 3.25 mg (98%). LC-MS (LR, ESI) = Calcd. For $C_{45}H_{53}N_3O_{13}S_4$ of the butyl amide (structure not shown): 919.32 (m/z), found: 971.25 (m/z), found: 484.43 $[M\text{-}2H]^-/2$.



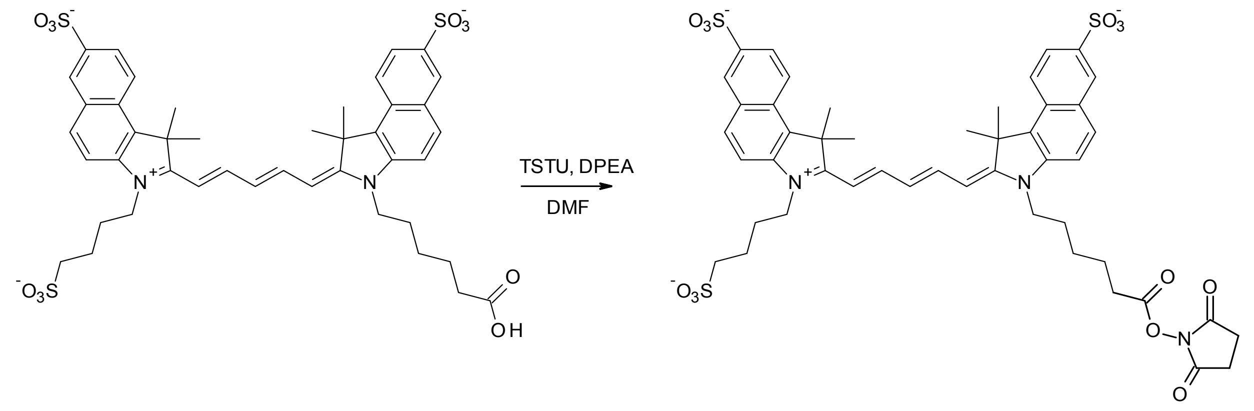
Scheme S4**. Reaction scheme for synthesis of compound **(iii).

(iii); 1,1,2-trimethyl-3-(4-sulfonatobutyl)-1H-benzo[e]indol-3-ium-7-sulfonate; 1,1,2-trimethyl-1H-benzo[e]indole-7-sulfonate (3.00 g, 9.64 mmol, Carestream Molecular Imaging/Eastman Kodak) was dissolved in DMF (10 mL). Added in succession were potassium carbonate (1.73 g, 12.5 mmol, VWR), and 1,4-butanesultone (5.67 g, 41.7 mmol, Alfa Aesar). The reaction was heated to 100 °C and stirred for 48 h. LC-MS was used to monitor reaction progress using (method ACN: 10, 8 min). Upon completion, the reaction was triturated in triplicate with 25 mL additions of 1:1 EtOH:IPA. The product was collected by vacuum filtration and dried under high vacuum. The yield was 3.05 g (69%). LC-MS (LR, ESI) = Calcd. For $C_{19}H_{25}NO_6S_2$: 427.10 (m/z), found: 428.38 $[M+H]^+$.



***Scheme S5**. Reaction scheme for synthesis of compound **(2)**. Final product was the Na salt form.*

(2); Cy5.5 3S; 4-(2-((1E,3E,5E)-5-(3-(5-carboxypentyl)-1,1-dimethyl-7-sulfo-1,3-dihydro-2H-benzo[e]indol-2-ylidene)penta-1,3-dien-1-yl)-1,1-dimethyl-7-sulfo-1H-benzo[e]indol-3-ium-3-yl)butane-1-sulfonate; Compound **(ii)** (0.200 g, 0.348 mmol) was dissolved in ACN (6 mL). In a separate flask, compound **(iii)** (0.120 g, 0.365 mmol) was dissolved in ACN (4 mL) with glacial AcOH (1.0 g, 16.7 mmol) and Ac$_2$O (35.0 mg, 0.347 mmol) added in quick succession. The solution containing the indole was added to the solution containing the half dye, and the reaction was placed in an ice bath. A solution of DIPEA (1.26 mL, 7.3 mmol, TCI Chemicals) in 5 mL ACN was then added to the reaction over 30 minutes while keeping the pH slightly acidic as monitored by a small drop quenched in water. The reaction was set to stir for 2 h, and was monitored via LC-MS (method ACN: 10, 8 min) every 0.5 h. Upon completion, the reaction was triturated with 0.1% AcOH in Et$_2$O. The precipitated product suspended in ethe was centrifuged, decanted, and dried under high vacuum. Purification was accomplished via prep-HPLC using (method ACN: 5-40, 60 min). Fractions were assayed via LC-MS with the previous method. Pure fractions were combined, rotary evaporated, and lyophilized three times. After the third lyophilization, the product was ion exchanged to the sodium salt and lyophilized a final time. The yield was 0.253 g (84%). LC-MS (LR, ESI) = Calcd. For $C_{43}H_{48}N_2O_{11}S_3$: 864.24 (m/z), found: 431.53 [M-2H]$^-$/2. LC-MS (HR, ESI) = Calcd. for $C_{43}H_{48}N_2O_{11}S_3$: 864.2420 (m/z), found: 865.2473[M+H]$^+$. ^{1}H NMR (500 MHz, D$_2$O) δ 8.27 (d, J = 2.3 Hz, 2H), 8.23 – 8.13 (m, 2H), 7.99 – 7.81 (m, 4H), 7.76 (d, J = 8.8 Hz, 2H), 7.39 (dd, J = 16.9, 8.9 Hz, 2H), 6.43 (t, J = 12.3 Hz, 1H), 6.10 (t, J = 12.9 Hz, 2H), 3.93 (dt, J = 42.2, 7.1 Hz, 4H), 2.93 (t, J = 7.0 Hz, 4H), 2.13 (t, J = 7.6 Hz, 4H), 1.92 – 1.73 (m, 2H), 1.74 – 1.47 (m, 14H), 1.33 (t, J = 8.1 Hz, 2H). ^{13}C NMR (126 MHz, D$_2$O) δ 183.51, 174.65, 174.11, 153.08, 141.13, 141.09, 138.31, 138.15, 133.77, 133.45, 131.47, 131.44, 130.25, 130.06, 128.40, 128.36, 127.42, 125.25, 123.54, 123.42, 123.35, 112.45, 112.20, 103.31, 102.85, 50.59, 50.56, 50.46, 43.85, 43.33, 37.43, 21.65.



Scheme S6**. Reaction scheme for synthesis of compound **(2a), **Cy5.5 3S-NHS Ester**.

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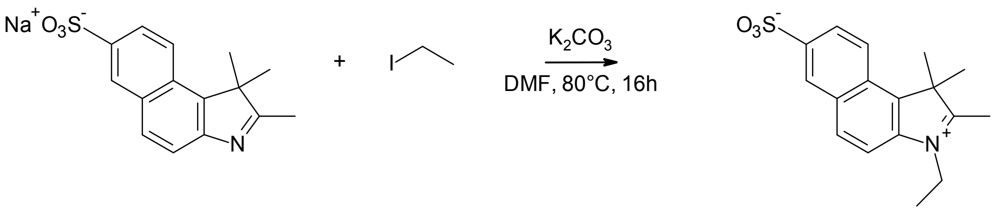
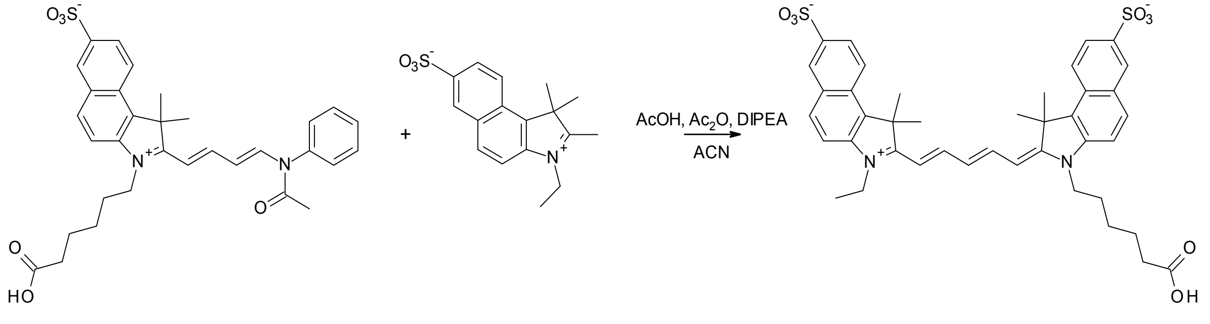
(2a); Cy5.5 3S-NHS Ester; 4-(2-((1E,3E,5E)-5-(3-(6-((2,5-dioxopyrrolidin-1-yl)oxy)-6-oxohexyl)-1,1-dimethyl-7-sulfo-1,3-dihydro-2H-benzo[e]indol-2-ylidene)penta-1,3-dien-1-yl)-1,1-dimethyl-7-sulfo-1H-benzo[e]indol-3-ium-3-yl)butane-1-sulfonate; Compound **(2)** (25.0 mg, 0.0289 mmol) was dissolved in anhydrous NMP (4 mL). DIPEA (44.9 mg, 0.347 mmol, TCI), and TSTU (17.5 mg, 0.0579 mmol, CreoSalus) were then added to the flask. The reaction was stirred under an Ar atmosphere at r.t., and monitored every 2 h via LC-MS (method ACN: 10, 8 min) utilizing a 0.1 % butyl amine in water quench of a reaction aliquot, to ensure an accurate measure of conversion. Upon full completion the reaction was quenched using a 1:1 EtOAc:Et_2O to precipitate the product. The wash was decanted, and the pure product was dried under high vacuum. The yield was 25.6 mg (92%). LC-MS (LR, ESI) = Calcd. For $C_{47}H_{57}N_3O_{10}S_3$ of the butyl amide (structure not shown): 919.32(m/z), found: 458.23 $[M-2H]^-/2$.



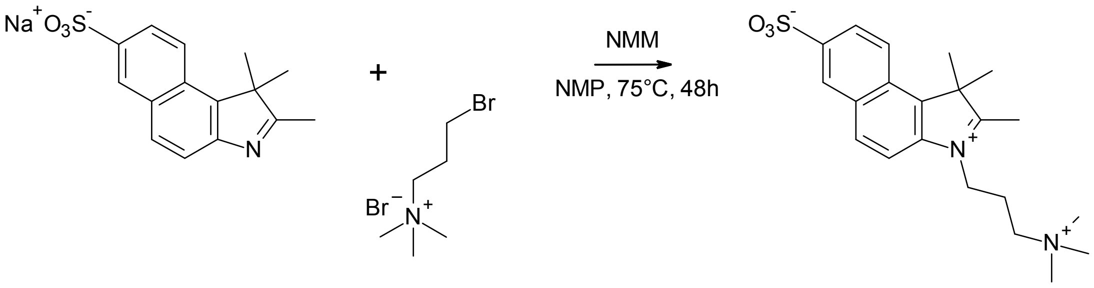
***Scheme S7**. Reaction scheme for synthesis of compound **(iv)**.*

(iv); 1,1,2-trimethyl-3-(4-sulfonatobutyl)-1H-benzo[e]indol-3-ium-7-sulfonate; 1,1,2-trimethyl-1H-benzo[e]indole-7-sulfonate (2.50 g, 8.67 mmol, Carestream Molecular Imaging/Eastman Kodak) was added to the reaction flask and dissolved in anhydrous DMF (5 mL). Added in succession were potassium carbonate (0.720 g, 62.43 mmol, VWR), and ethyl iodide (4.76 g, 30.6 mmol, Alfa Aesar). The reaction was set to stir at 80 °C for 16 h under reflux. LC-MS was used to monitor reaction progress using method (ACN: 10, 8 min). Upon completion, the reaction mixture was triturated in triplicate with 25 mL additions of pure EtOH. The product was collected by vacuum filtration and dried under high vacuum. The yield was 0.723 g (66%). LC-MS (LR, ESI) = Calcd. For $C_{17}H_{19}NO_3S$: 356.07 (m/z), found: 354.98 $[M-H]^-$.



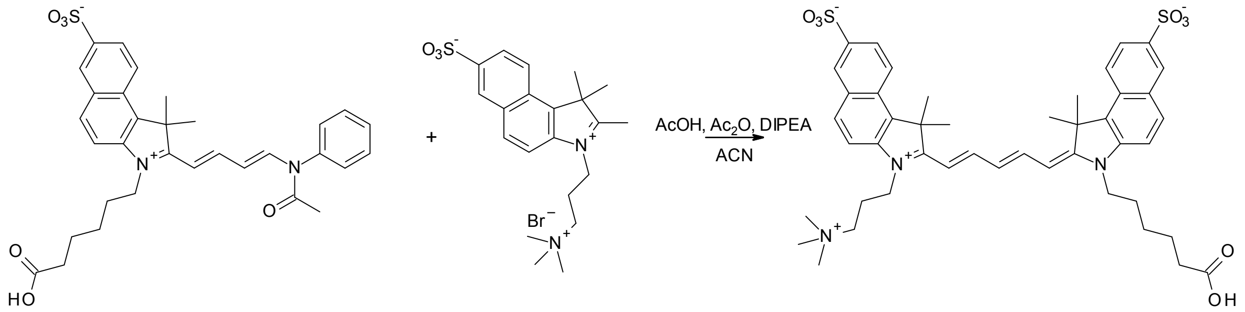
***Scheme S8**. Reaction scheme for synthesis of compound **(3)**. Final product was in the TEA salt form.*

(3); Cy5.5 2S; (E)-2-((2E,4E)-5-(3-(5-carboxypentyl)-1,1-dimethyl-7-sulfonato-1H-3l4-benzo[e]indol-2-yl)penta-2,4-dien-1-ylidene)-3-ethyl-1,1-dimethyl-2,3-dihydro-1H-benzo[e]indol-3-ium-7-sulfonate; Compound **(ii)** (0.412 g, 0.717 mmol) was dissolved in ACN (12 mL). In a separate flask, compound **(iv)** (0.273 g, 0.860 mmol) was dissolved in ACN (8 mL) with glacial AcOH (2.06 g, 34.5 mmol) and Ac_2O (1.96 74.0 mg, 0.717 mmol) added in quick succession. The solution containing the indole was added to the solution containing the half dye, and the reaction was placed in an ice bath. A solution of DIPEA (2.6 mL, 1.938 g, 15.0 mmol, TCI Chemicals) in 10 mL ACN was then added to the reaction over 30 minutes while keeping the pH slightly acidic as monitored by a small drop quenched in water. The reaction was and stirred for 1 h, and was monitored via LC-MS (method ACN: 10, 8 min) every 0.5 h. Upon completion, the reaction was quenched with 200 mL 0.1% AcOH in Et_2O. The precipitated product was centrifuged, decanted, and dried under high vacuum. Purification was accomplished via prep-HPLC using (method ACN: 5-40, 60 min). Fractions were assayed via LC-MS with the previous method. Pure fractions were combined, rotary evaporated, and lyophilized three times. After the third lyophilization, the product was again dissolved in H_2O (5 mL). TEA (5 µL) was subsequently added to ensure the TEA salt form of the dye, and the solution was lyophilized a final time. The yield was 0.448 g (83%). LC-MS (LR, ESI) = Calcd. For $C_{41}H_{44}N_2O_8S_2$: 756.25 (m/z), found: 757.32 $[M+H]^+$. LC-MS (HR, ESI) = Calcd. $C_{41}H_{44}N_2O_8S_2$: 756.2539 (m/z), found: 757.2606$[M+H]^+$. ^1H NMR (500 MHz, 1:1 D_2O:MeOD) δ 6.95 – 6.86 (m, 2H), 6.81 (t, J = 7.2 Hz, 2H), 6.71 (t, J = 13.3 Hz, 2H), 6.57 (q, J = 8.4 Hz, 2H), 6.47 (d, J = 8.8 Hz, 2H), 6.09 (q, J = 8.5 Hz, 2H), 5.15 (td, J = 12.5, 3.9 Hz, 1H), 4.88 – 4.74 (m, 2H), 2.80 – 2.52 (m, 4H), 0.77 (t, J = 7.4 Hz, 2H), 0.47 – 0.25 (m, 14H), 0.15 (t, J = 7.9 Hz, 2H), 0.02 – -0.17 (m, 5H). ^{13}C NMR (126 MHz, 1:1 D_2O:MeOD) δ 178.11, 173.00, 172.84, 151.49, 139.61, 139.15, 138.25, 138.21, 132.47, 132.20, 130.06, 129.15, 129.07, 127.00, 125.78, 123.98, 122.65, 121.48, 110.55, 110.38, 101.48, 49.39, 49.30, 46.97, 46.80, 46.63, 46.46, 46.29, 46.12, 45.94, 45.16, 42.16, 37.61, 33.71, 25.43, 24.99, 24.86, 24.40, 23.39, 10.23.



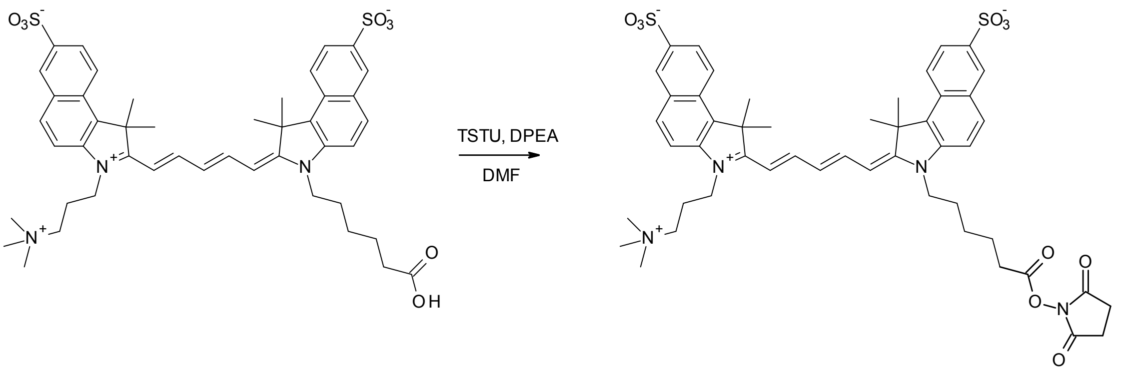
Scheme S9. *Reaction scheme for synthesis of compound* ***(v)****.*

(v); 1,1,2-trimethyl-3-(3-(trimethylammonio)propyl)-1H-benzo[e]indol-3-ium-7-sulfonate; 1,1,2-trimethyl-1H-benzo[e]indole-7-sulfonate (0.500 g, 161mmol, Carestream Molecular Imaging/Eastman Kodak) was dissolved in NMP (2 mL). Added in succession were NMM (0.382 g, 347 mmol, Alfa Aesar) and (3-Bromopropyl)trimethylammonium bromide) (1.860 g, 713 mmol, Sigma Aldrich). The solution was stirred at 75 °C for 48 h under reflux and an Ar atmosphere. LC-MS was used to monitor reaction progress using (method ACN: 10, 8 min). Upon completion, the reaction mixture was triturated with 25mL additions of Et_2O, EtOAc, and ACN respectively. Each solvent was decanted before the next was added. The product was collected via vacuum filtration of the ACN and followed by drying under high vacuum. The yield was 0.627 g (83%). LC-MS (LR, ESI) = Calcd. for $C_{21}H_{29}N_2O_3S$: 389.19 (m/z), found: 390.31 $[M+H]^+$.



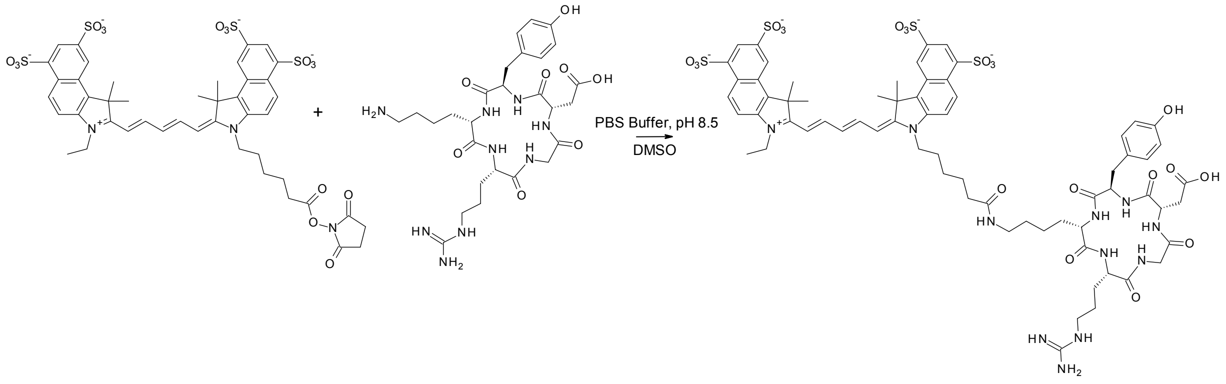
Scheme S10. Reaction scheme for synthesis of compound **(4)**. *Final product was in the TEA salt form.*

(4); **Cy5.5 ZW**; **2-((1E,3E,5E)-5-(3-(5-carboxypentyl)-1,1-dimethyl-7-sulfonato-1,3-dihydro-2H-benzo[e]indol-2-ylidene)penta-1,3-dien-1-yl)-1,1-dimethyl-3-(3-(trimethylammonio)propyl)-1H-benzo[e]indol-3-ium-7-sulfonate;** Compound **(ii)** (0.200 g, 0.348 mmol) was dissolved in ACN (6 mL). In a separate flask, compound **(v)** (0.154 g, 0.365 mmol) was dissolved in ACN (4 mL) with glacial AcOH (1.0 g, 16.7 mmol) and Ac$_2$O (35.0 mg, 0.347 mmol) added in quick succession. The solution containing the indole was added to the solution containing the half dye, and the reaction was placed in an ice bath. A solution of DIPEA (1.26 mL, 1.39 g, 7.3 mmol, TCI Chemicals) in 5 mL ACN was then added to the reaction over 30 minutes while keeping the pH slightly acidic as monitored by a small drop quenched in water. The reaction was and stirred for 1 h, and was monitored via LC-MS (method ACN: 10, 8 min) every 0.5 h. Upon completion, the reaction was quenched with 100 mL 0.1% AcOH in Et$_2$O. The precipitated product was centrifuged, decanted, and dried under high vacuum. Purification was accomplished via prep-HPLC using (method ACN: 40-80, 60 min) with 0.1% AcOH as the aqueous phase. Fractions were assayed via LC-MS with the previous method. Pure fractions were combined, rotary evaporated, and lyophilized three times. After the third lyophilization, the product was again dissolved in H$_2$O (5 mL). TEA (5 µL) was subsequently added to ensure the TEA salt form of the dye, and the solution was lyophilized a final time. The yield was 0.275 g (93%). LC-MS (LR, ESI) = Calcd. For $C_{45}H_{53}N_3O_8S_2$: 827.33 (m/z), found: 828.62 $[M+H]^+$. LC-MS (HR, ESI) = Calcd. for $C_{45}H_{53}N_3O_8S_2$: 827.3274 (m/z), found: 826.3181 $[M-H]^-$. ^{1}H NMR (500 MHz, 20% D$_2$O in MeOD) δ 8.53 – 8.24 (m, 6H), 8.15 (d, J = 8.9 Hz, 1H), 8.09 – 7.96 (m, 3H), 7.69 (dd, J = 26.6, 8.8 Hz, 2H), 6.81 (t, J = 12.5 Hz, 1H), 6.43 (dd, J = 36.3, 13.6 Hz, 2H), 4.38 – 4.21 (m, 4H), 3.71 – 3.62 (m, 2H), 3.23 (s, 9H), 2.44 – 2.30 (m, 2H), 2.23 (t, J = 7.4 Hz, 2H), 2.03 (d, J = 6.7 Hz, 12H), 1.92 (qd, J = 8.6, 7.7, 2.5 Hz, 2H), 1.75 (p, J = 7.5 Hz, 2H), 1.62 – 1.49 (m, 2H). ^{13}C NMR (126 MHz, 20% D$_2$O in MeOD) δ 176.28, 173.06, 154.15, 152.61, 141.32, 140.74, 140.70, 140.53, 134.63, 133.07, 131.49, 131.20, 130.60, 128.59, 128.30, 127.12, 126.51, 124.72, 124.59, 122.58, 122.39, 111.96, 111.13, 104.49, 102.19, 63.25, 52.71, 51.42, 50.58, 44.06, 40.19, 27.10, 26.50, 26.22, 26.14, 25.52, 21.05.



Scheme S11. Reaction scheme for synthesis of compound ***(4a),*** **Cy5.5 ZW-NHS Ester**

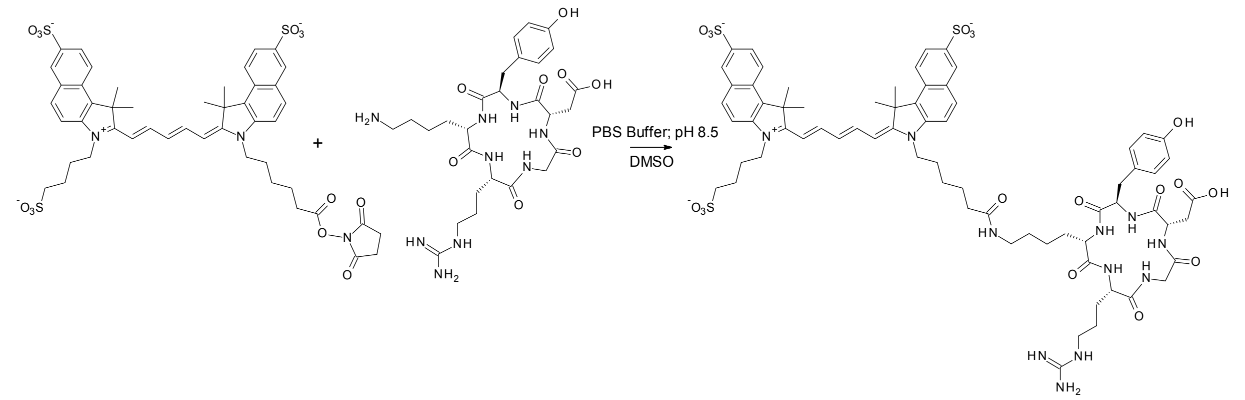
(4a); Cy5.5 ZW-NHS Ester; 2-((1E,3E,5E)-5-(1,1-dimethyl-7-sulfo-3-(3-(trimethylammonio)propyl)-1,3-dihydro-2H-benzo[e]indol-2-ylidene)penta-1,3-dien-1-yl)-3-(6-((2,5-dioxopyrrolidin-1-yl)oxy)-6-oxohexyl)-1,1-dimethyl-7-sulfo-1H-benzo[e]indol-3-ium; Compound **(4)** (14.1 mg, 0.0170 mmol) was dissolved in anhydrous NMP (4 mL). DIPEA (43.91 mg, 0.340 mmol, TCI), and TSTU (20.49 mg, 0.0679 mmol, CreoSalus) were then added to the flask. The reaction was stirred under an Ar atmosphere at r.t., and monitored every 2 h via LC-MS (method ACN: 10, 8 min) utilizing a 0.1 % butyl amine in water quench of a reaction aliquot, to ensure an accurate measure of conversion. Upon full completion the reaction was quenched using a 1:1 EtOAc:Et_2O to precipitate the product. The wash was decanted, and the pure product was dried under high vacuum. The yield was 15.50 mg (98%). LC-MS (LR, ESI) of the butyl amide (structure not shown) = Calcd. For $C_{49}H_{64}N_4O_7S_2$: 884.42 (m/z), found: 885.46 $[M+H]^+$.



Scheme S12. Reaction scheme for synthesis of compound ***(6)****. Final product was in the Na salt form.*

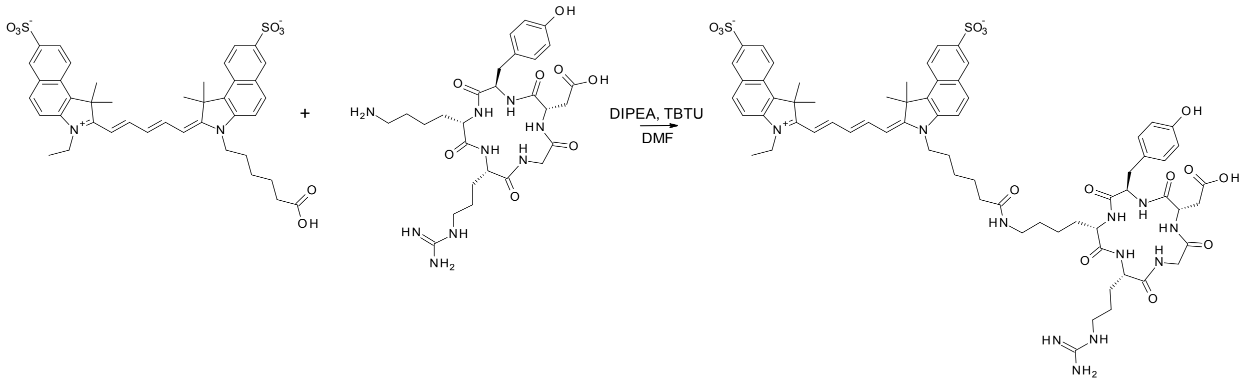
(6); Cy5.5 4S-c(RGDyK); 2-((1E,3E,5E)-5-(3-(6-((4-((2S,5S,11S,14R)-11-(carboxymethyl)-5-(3-guanidinopropyl)-14-(4-hydroxybenzyl)-3,6,9,12,15-pentaoxo-1,4,7,10,13-pentaazacyclopentadecan-2-yl)butyl)amino)-6-oxohexyl)-1,1-dimethyl-6,8-disulfonato-1,3-dihydro-2H-benzo[e]indol-2-ylidene)penta-1,3-dien-1-yl)-3-ethyl-1,1-dimethyl-1H-benzo[e]indol-3-ium-6,8-disulfonate;
c(RGDyK) (0.64 mg, 1.04 µmol, Peptides International) was dissolved in PBS buffer, pH 8.5 (1.0 mL). Compound **(1a)** (2.0 mg, 2.08 µmol) was dissolved in anhydrous DMSO (500 µL), and added to the reaction flask containing c(RGDyK). The solution was stirred for 0.5 h at r.t., and monitored via LC-MS (method ACN: 5-70, 8 min). Upon completion, the reaction was rotary evaporated and dried under high vacuum. Purification was accomplished via prep-HPLC using (method ACN: 5-40, 60 min). Fractions were assayed via LC-MS with the previous method. Pure fractions were combined, rotary evaporated, and lyophilized three times. After the third lyophilization, the product was again dissolved in H_2O (5 mL) and

treated to a sodium ion exchange column. The product was lyophilized a final time. The yield was 1.25 mg (79%). LC-MS (LR, ESI) = Calcd. For $C_{68}H_{83}N_{11}O_{21}S_4$: 1517.46 (m/z), found: 757.35 $[M-2H]^-/2$. LC-MS (HR, ESI) = Calcd. for $C_{68}H_{83}N_{11}O_{21}S_4$: 1517.4648 (m/z), found: 1518.4727 $[M+H]^+$.



***Scheme S13**. Reaction scheme for synthesis of compound **(7)**. Final product was in the Na salt form.*

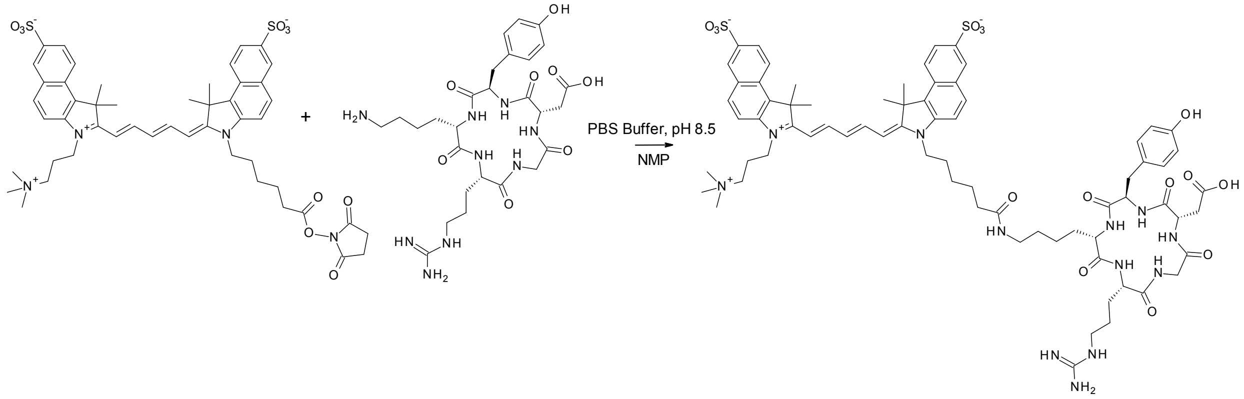
(7); Cy5.5 3S-c(RGDyK); 4-(2-((1E,3E,5E)-5-(3-(6-((4-((2S,5S,11S,14R)-11-(carboxymethyl)-5-(3-guanidinopropyl)-14-(4-hydroxybenzyl)-3,6,9,12,15-pentaoxo-1,4,7,10,13-pentaazacyclopentadecan-2-yl)butyl)amino)-6-oxohexyl)-1,1-dimethyl-7-sulfo-1,3-dihydro-2H-benzo[e]indol-2-ylidene)penta-1,3-dien-1-yl)-1,1-dimethyl-7-sulfo-1H-benzo[e]indol-3-ium-3-yl)butane-1-sulfonate; c(RGDyK) (0.64 mg, 1.04 µmol, Peptides International) was dissolved in PBS buffer, pH 8.5 (1.0 mL). Compound **(2a)** (2.0 mg, 1.04 µmol) was dissolved in anhydrous DMSO (500 µL) and added to the reaction flask containing c(RGDyK). The solution was stirred for 0.5 h at r.t., and monitored via LC-MS (method ACN: 10, 8 min). Upon completion, the reaction was rotary evaporated and dried under high vacuum. Purification was accomplished via prep-HPLC using (method ACN: 5-40, 60 min). Fractions were assayed via LC-MS with the previous method. Pure fractions were combined, rotary evaporated, and lyophilized three times. After the third lyophilization, the product was again dissolved in H_2O (5 mL) and treated to a sodium ion exchange column. The product was lyophilized a final time. The yield was 1.15 mg (76%). LC-MS (LR, ESI) = Calcd. For $C_{70}H_{87}N_{11}O_{18}S_3$: 1465.54 (m/z), found: 733.21 $[M+2H]^+/2$. LC-MS (HR, ESI) = Calcd. for $C_{70}H_{87}N_{11}O_{18}S_3$: 1465.5393 (m/z), found: 1466.5402 $[M+H]^+$.



***Scheme S14**. Reaction scheme for synthesis of compound **(8)**. Final product was in the TEA salt form.*

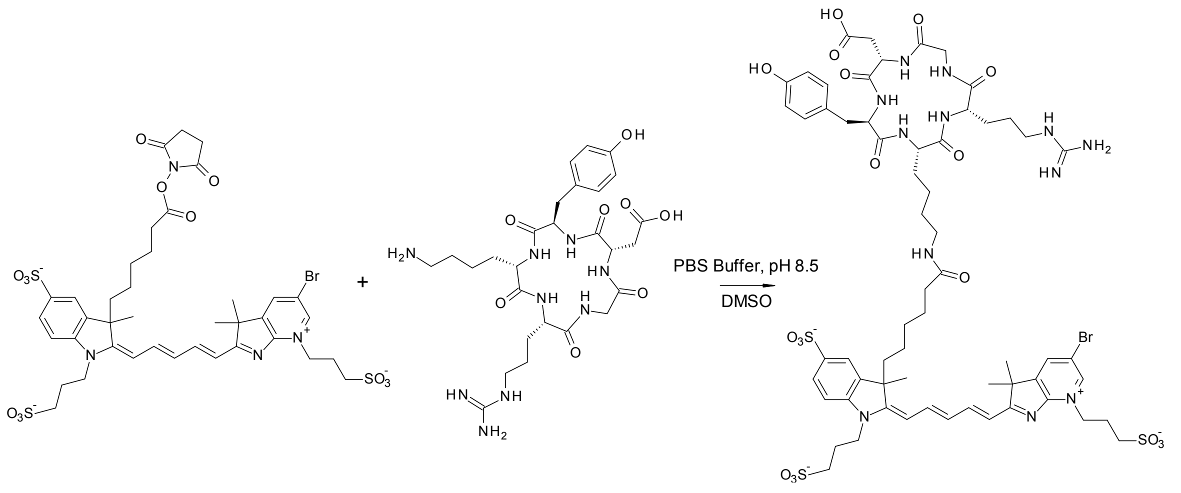
(8); Cy5.5 2S-c(RGDyK); (E)-2-((2E,4E)-5-(3-(6-((4-((2S,5S,11S,14R)-11-(carboxymethyl)-5-(3-guanidinopropyl)-14-(4-hydroxybenzyl)-3,6,9,12,15-pentaoxo-1,4,7,10,13-pentaazacyclopentadecan-2-

yl)butyl)amino)-6-oxohexyl)-1,1-dimethyl-7-sulfo-1H-3l4-benzo[e]indol-2-yl)penta-2,4-dien-1-ylidene)-3-ethyl-1,1-dimethyl-2,3-dihydro-1H-benzo[e]indol-3-ium-7-sulfonate; Compound **(3)** (5.0 mg, 5.82 µmol) was dissolved in anhydrous DMF (2 mL), and placed in a flask under an Ar atmosphere. Followed in quick succession was the addition DIPA (11.3 mg, 0.0871 mmol) and TBTU (1.87 mg, 5.82 µmol, CreoSalus). The reaction was stirred at r.t. and checked every 15min via LC-MS (method ACN: 5-70, 8min) utilizing a 0.1 % butyl amine in water quench of a reaction aliquot, to ensure an accurate measure of conversion. After stirring overnight, full conversion of compound (3) into the activated form was achieved, and c(RGDyK) (3.61 mg, 5.82 µmol, Peptides International) was added. The reaction then continued to stir for 2.5 h at r.t and monitored every 0.5 h via LC-MS using the previous method. The product was isolated by precipitation with 1:1 EtOAc:Et_2O. The precipitated product suspended in solution was centrifuged, decanted, and dried under high vacuum. Purification was accomplished via prep-HPLC using (method ACN: 5-40, 60 min). Fractions were assayed via LC-MS with the previous method. Pure fractions were combined, rotary evaporated, and lyophilized three times. After the third lyophilization, the product was again dissolved in H_2O (5 mL). TEA (5 µL) was subsequently added to ensure the TEA salt form of the dye, and the solution was lyophilized a final time. The yield was 4.25 mg (76%). LC-MS (LR, ESI) = Calcd. For $C_{68}H_{83}N_{11}O_{15}S_2$: 1357.55 (m/z), found: 677.53 $[M-2H]^-/2$. LC-MS (HR, ESI) = Calcd. $C_{68}H_{83}N_{11}O_{15}S_2$: 1357.5512 (m/z), found: 1356.5432 $[M-H]^-$.



***Scheme S15**. Reaction scheme for synthesis of compound **(9)**. Final product was in the TEA salt form.*

(9); Cy5.5 ZW-c(RGDyK); 3-(6-((4-((2S,5S,11S,14R)-11-(carboxymethyl)-5-(3-guanidinopropyl)-14-(4-hydroxybenzyl)-3,6,9,12,15-pentaoxo-1,4,7,10,13-pentaazacyclopentadecan-2-yl)butyl)amino)-6-oxohexyl)-2-((1E,3E,5E)-5-(1,1-dimethyl-7-sulfo-3-(3-(trimethylammonio)propyl)-1,3-dihydro-2H-benzo[e]indol-2-ylidene)penta-1,3-dien-1-yl)-1,1-dimethyl-7-sulfo-1H-benzo[e]indol-3-ium; c(RGDyK) (0.67 mg, 1.08 µmol, Peptides International) was dissolved in PBS buffer, pH 8.5 (2.0 mL). Compound **(4a)** (2.0 mg, 1.04 µmol) was dissolved in anhydrous NMP (1.5 mL) and added to the reaction flask containing c(RGDyK). The solution was stirred for 6 h at r.t., and monitored via LC-MS (method ACN: 10, 8min). Upon completion, the reaction was rotary evaporated and dried under high vacuum. Purification was accomplished via prep-HPLC using (method ACN: 5-40, 60 min). Fractions were assayed via LC-MS with the previous method. Pure fractions were combined, rotary evaporated, and lyophilized three times. After the third lyophilization, the product was again dissolved in H_2O (5 mL). TEA (5 µL) was subsequently added to ensure the TEA salt form of the dye, and the solution was lyophilized a final time. The yield was 1.35 mg (88%). LC-MS (LR, ESI) = Calcd. For $C_{72}H_{92}N_{12}O_{15}S_2$: 1428.6247 (m/z), found: 715.05 $[M+2H]^+/2$. LC-MS (HR, ESI) = Calcd. for $C_{72}H_{92}N_{12}O_{15}S_2$: 1428.6247 (m/z), found: 715.3201 $[M-2H]^-/2$.



Scheme S16. *Reaction scheme for synthesis of compound* ***(10)***. *Final product was in the TEA salt form.*

(10); Alexa Fluor 680-c(RGDyK); (E)-2-((2E,4E)-5-(5-bromo-3,3-dimethyl-7-(3-sulfonatopropyl)-3H-pyrrolo[2,3-b]pyridin-7-ium-2-yl)penta-2,4-dien-1-ylidene)-3-(6-((4-((2S,5S,11S,14R)-11-(carboxymethyl)-5-(3-guanidinopropyl)-14-(4-hydroxybenzyl)-3,6,9,12,15-pentaoxo-1,4,7,10,13-pentaazacyclopentadecan-2-yl)butyl)amino)-6-oxohexyl)-3-methyl-1-(3-sulfonatopropyl)indoline-5-sulfonate; c(RGDyK) (1.08 mg, 1.74 µmol, Peptides International) was dissolved in PBS buffer, pH 8.5 (1.0 mL). Alexa Flour 680 carboxylic acid, succinimidyl ester (compound **(5)**) (1.0 mg, 0.87 µmol, Invitrogen) was dissolved in anhydrous DMSO (500 µL), and added to the reaction flask containing c(RGDyK). The solution was stirred for 0.5 h at r.t., and monitored via LC-MS (method ACN: 10, 8min). Upon completion, the reaction was rotary evaporated and dried under high vacuum. Purification was accomplished via prep-HPLC using (method ACN: 5-40, 60 min). Fractions were assayed via LC-MS with the previous method. Pure fractions were combined, rotary evaporated, and lyophilized three times. After the third lyophilization, the product was again dissolved in H_2O (5 mL). TEA (5 µL, Alfa Aesar) was subsequently added to ensure the TEA salt form of the dye, and the solution was lyophilized a final time. The yield was 1.05 mg (73%). LC-MS (LR, ESI) = Calcd. For $C_{62}H_{83}N_{12}O_{18}S_3Br$: 1458.43 (m/z), found: 728.45 $[M-2H]^-/2$. LC-MS (HR, ESI) = Calcd. for $C_{62}H_{83}N_{12}O_{18}S_3Br$: 1458.4294 (m/z), found: 1457.4219 $[M-H]^-$.

<u>Photostability: Dye Fade Data:</u> All solutions were 0.55 µM in H_2O, pH 7.

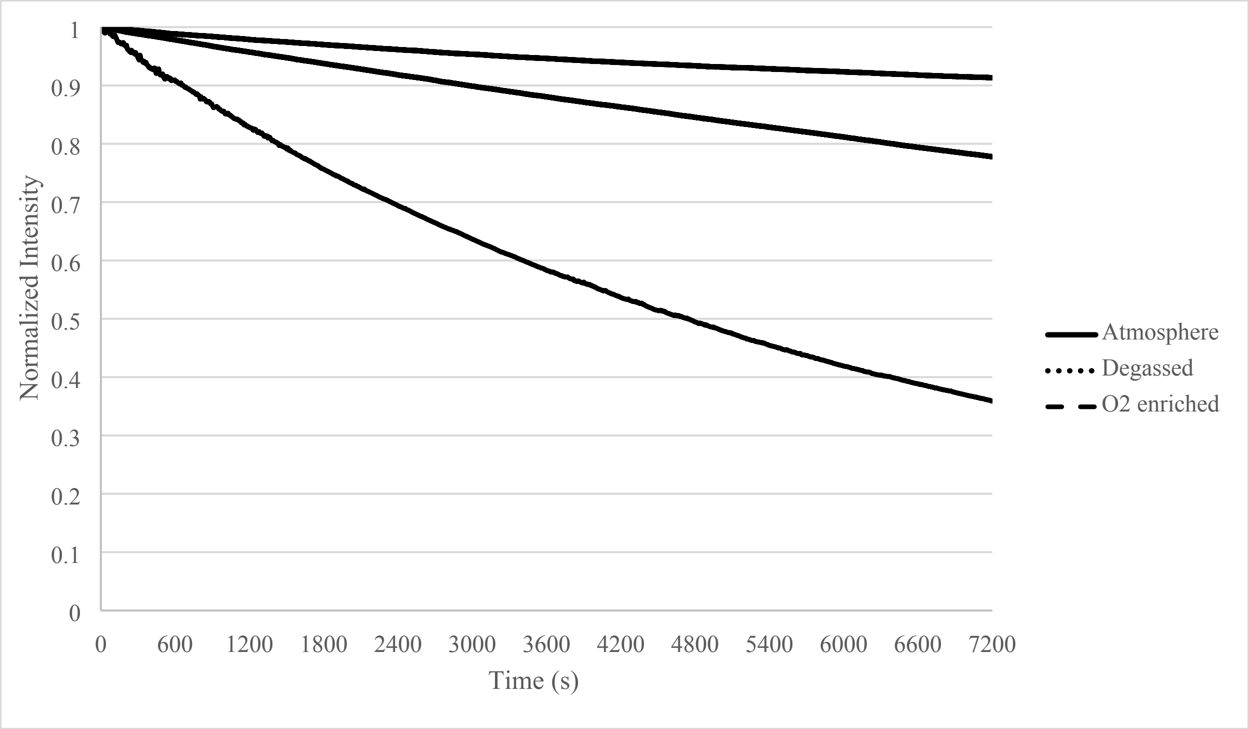


Figure S1. *NIR dye Cy5.5 4S,* ***(1)*** *photobleaching plot in H_2O.*

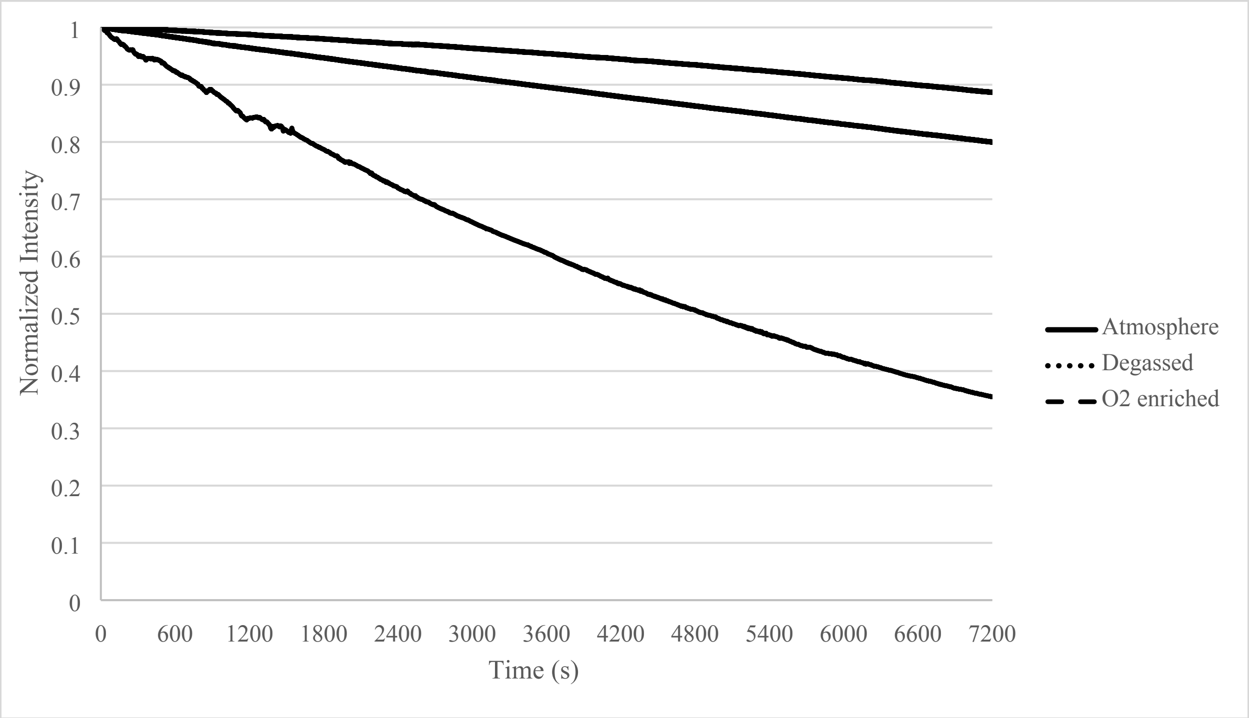


Figure S2. *NIR dye Cy5.5 3S,* ***(2)*** *photobleaching plot in H_2O.*

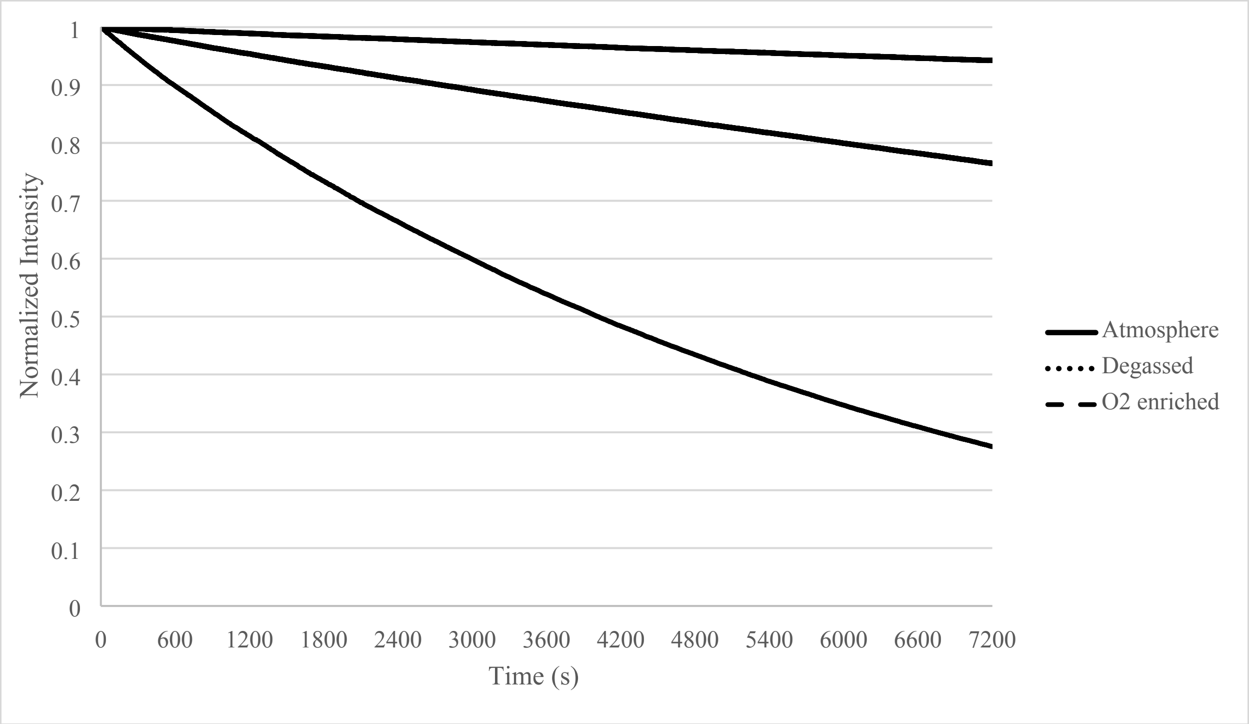


Figure S3. *NIR dye Cy5.5 2S,* ***(3)*** *photobleaching plot in* H_2O.

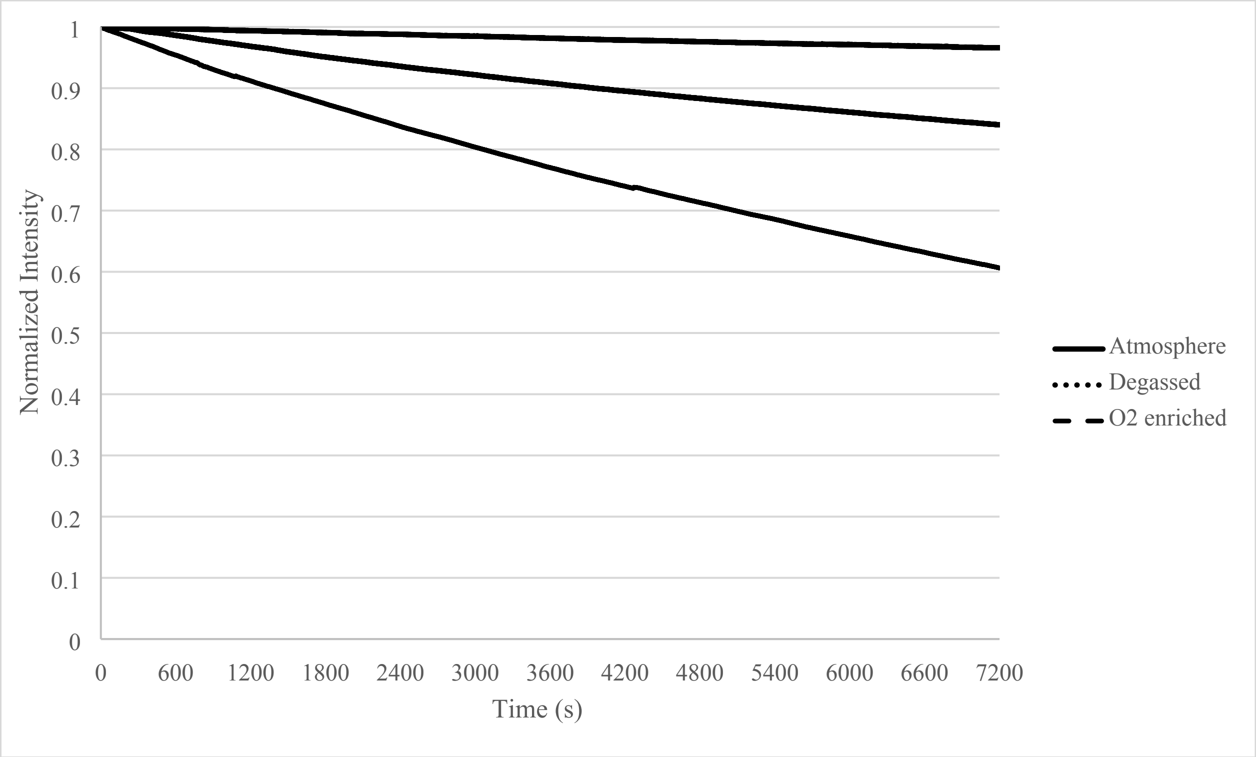


Figure S4. *NIR dye Cy5.5 ZW,* ***(4)*** *photobleaching plot in* H_2O.

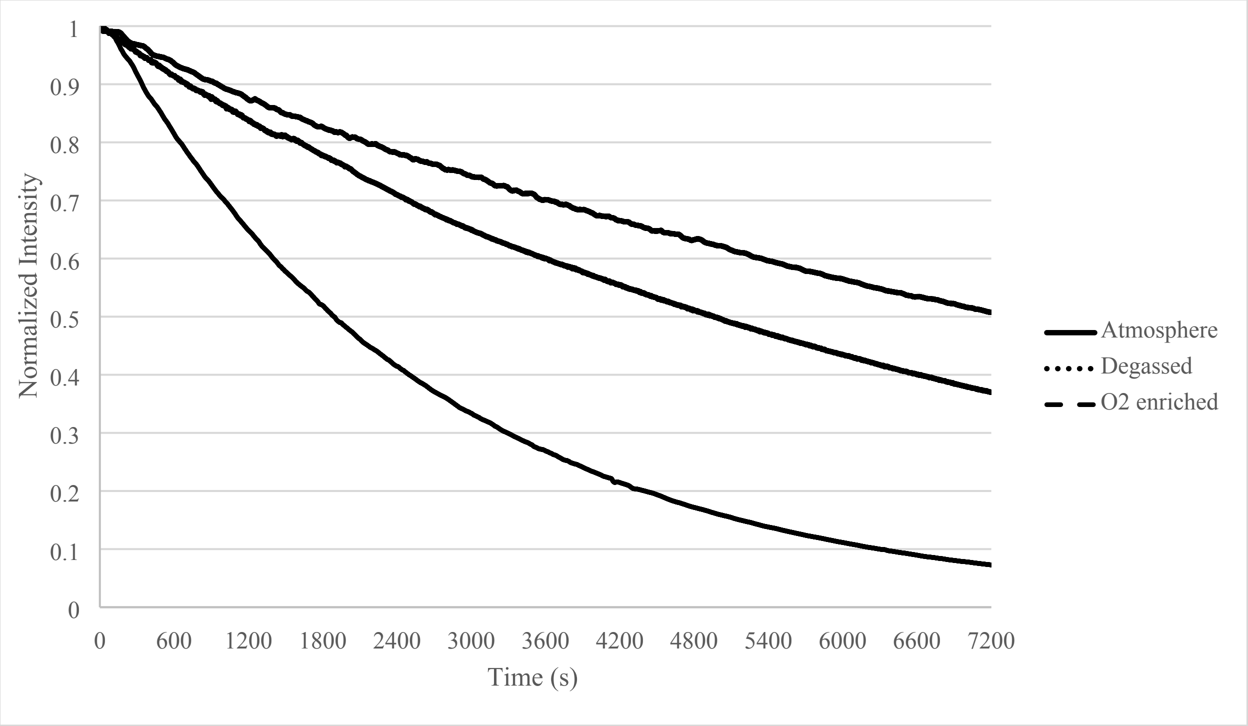


Figure S5. *NIR dye Alexa Fluor 680,* ***(5)*** *photobleaching plot in* H_2O.

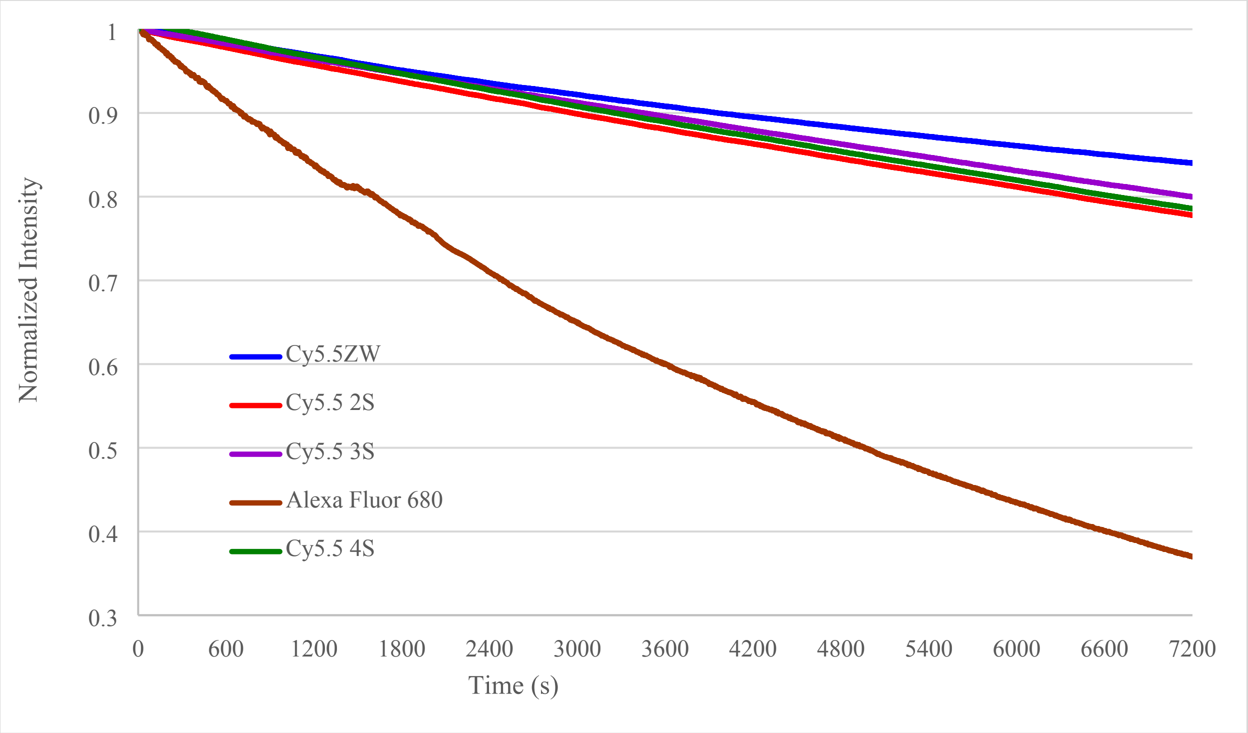


Figure S6. *Summary: Photobleaching plot of all 5 dyes as their free acids at ambient atmosphere (without Ar or* O_2 *bubbled into the solvent).*

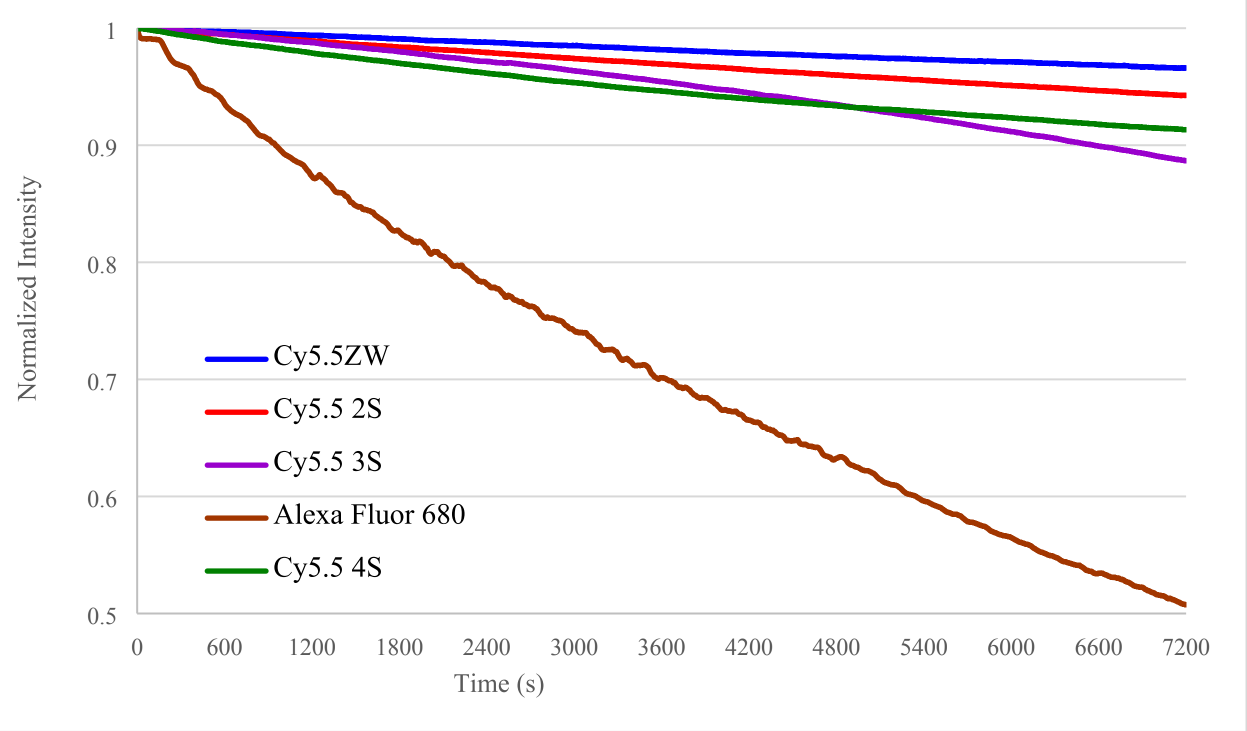


Figure S7. *Summary: Photobleaching plot of all 5 dyes as their free acids with solvents purged with Ar.*

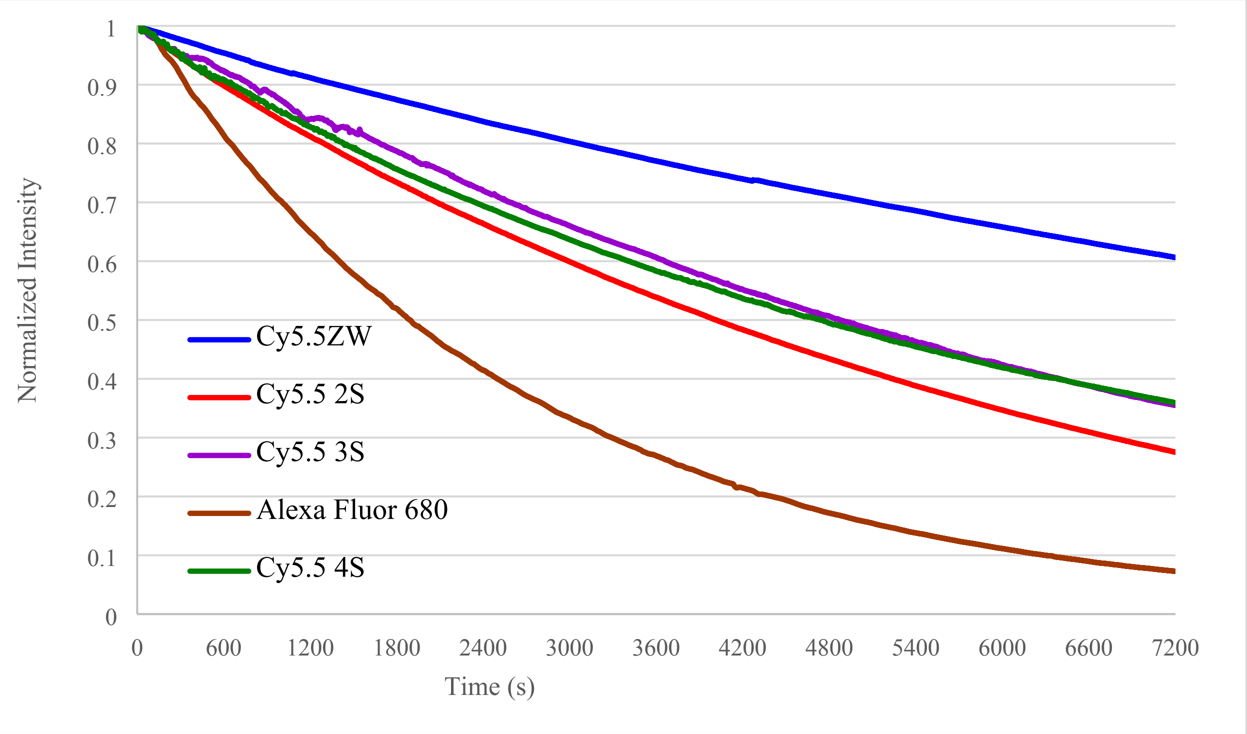


Figure S8. *Summary: Photobleaching plot of all 5 dyes free acids with solvents saturated with O_2 gas.*

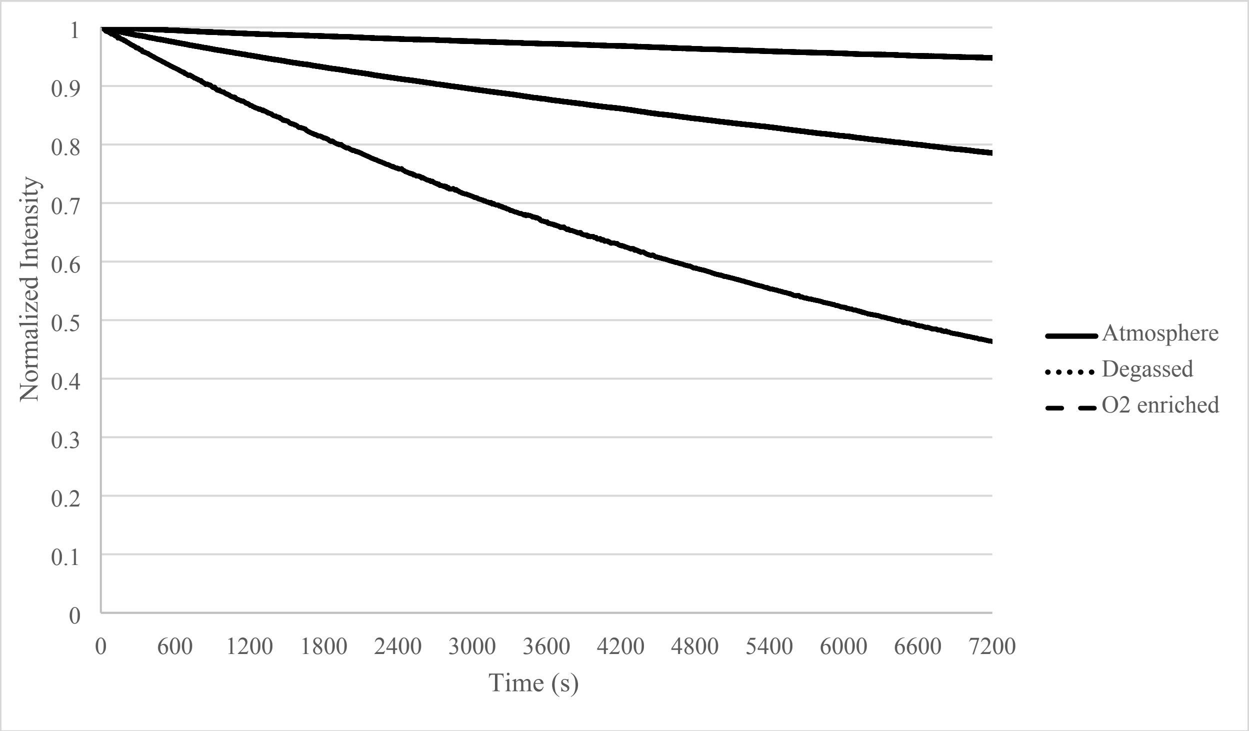


Figure S9. *Conjugate Cy5.5 4S-c(RGDyK),* ***(6)*** *photobleaching plots in* H_2O

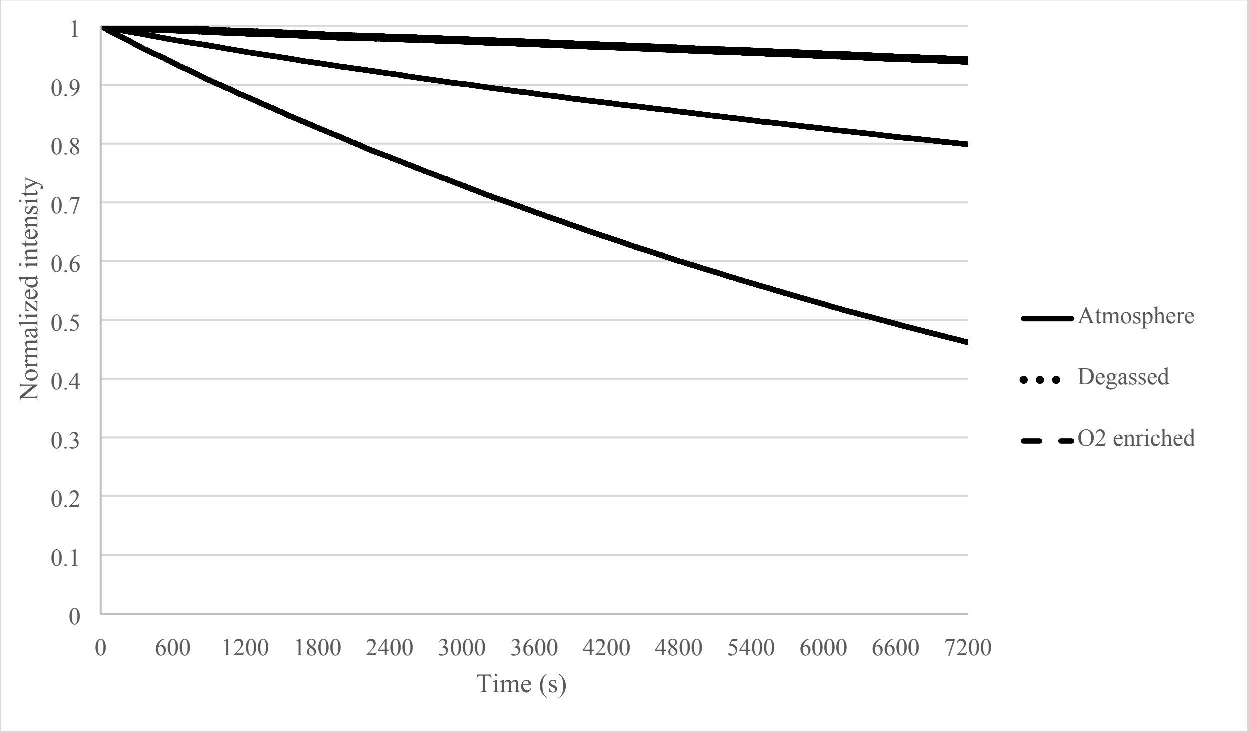


Figure S10. *Conjugate Cy5.5 3S-c(RGDyK),* ***(7)*** *photobleaching plots in* H_2O*.*

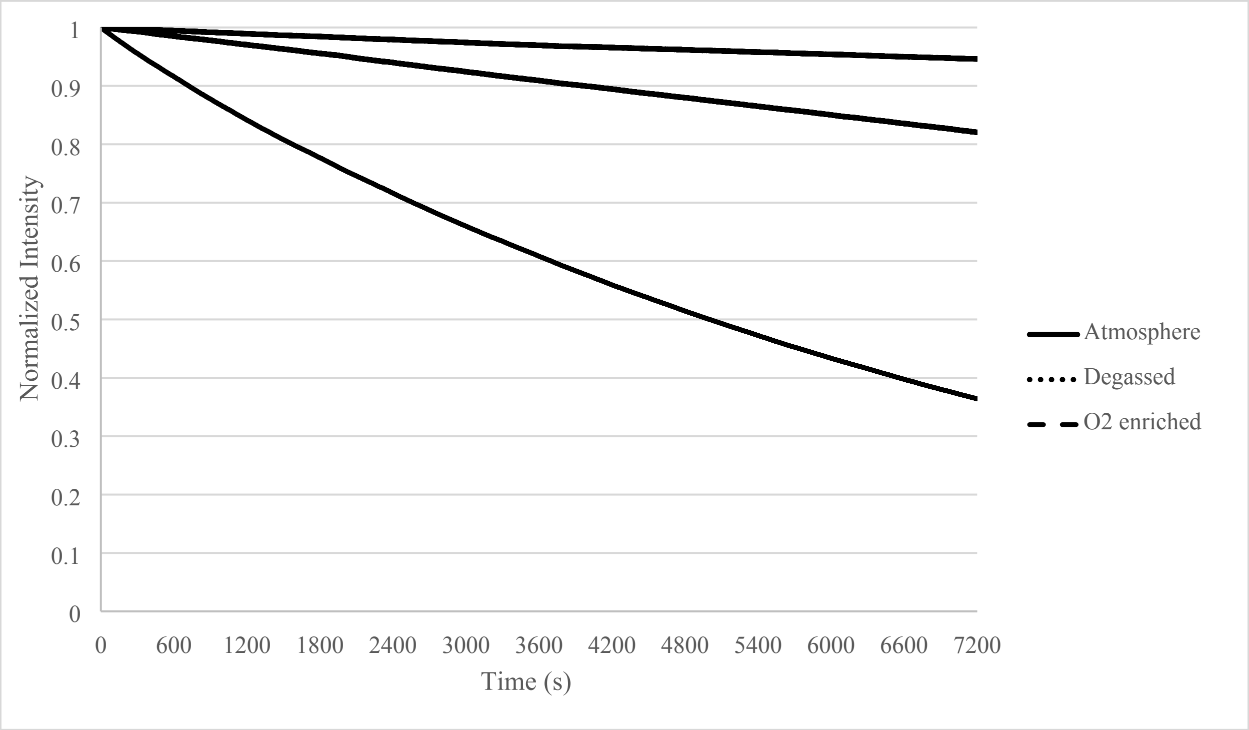


Figure S11. *Conjugate Cy5.5 2S-c(RGDyK),* ***(8)*** *photobleaching plots in* H_2O.

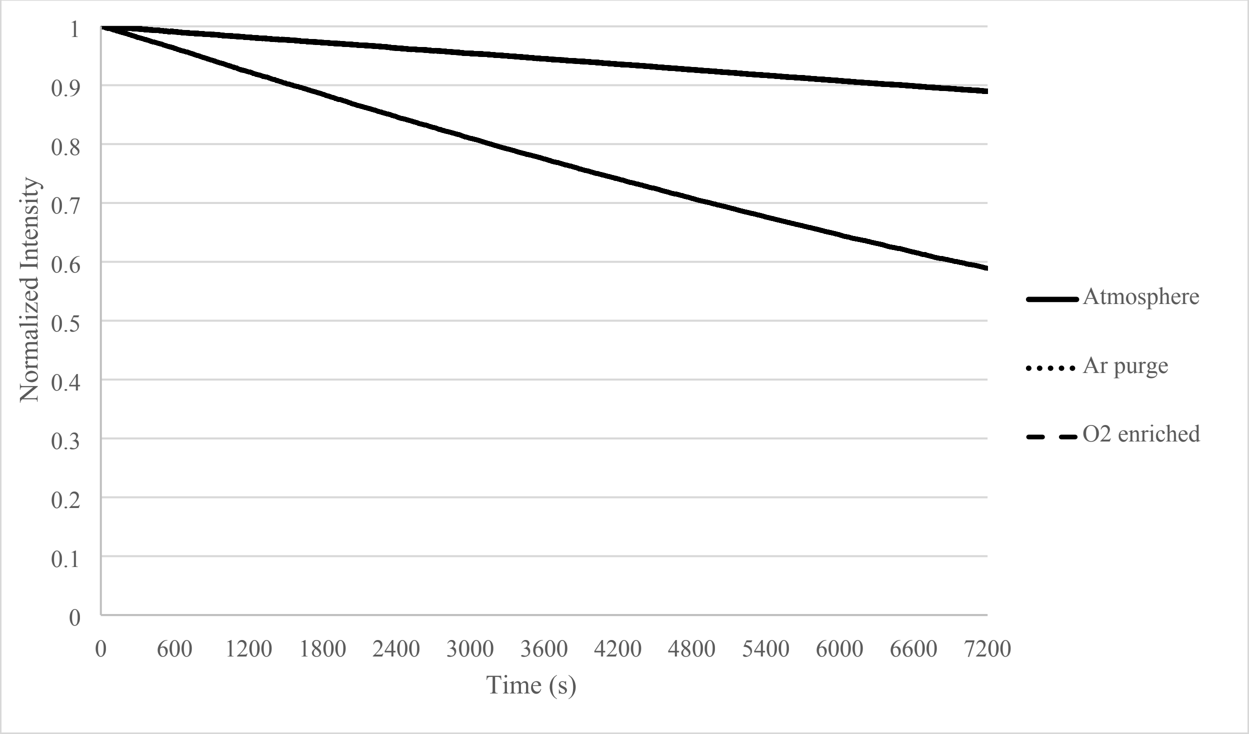


Figure S12. *Conjugate Cy5.5 ZW-c(RGDyK),* ***(9)*** *photobleaching plots in* H_2O.

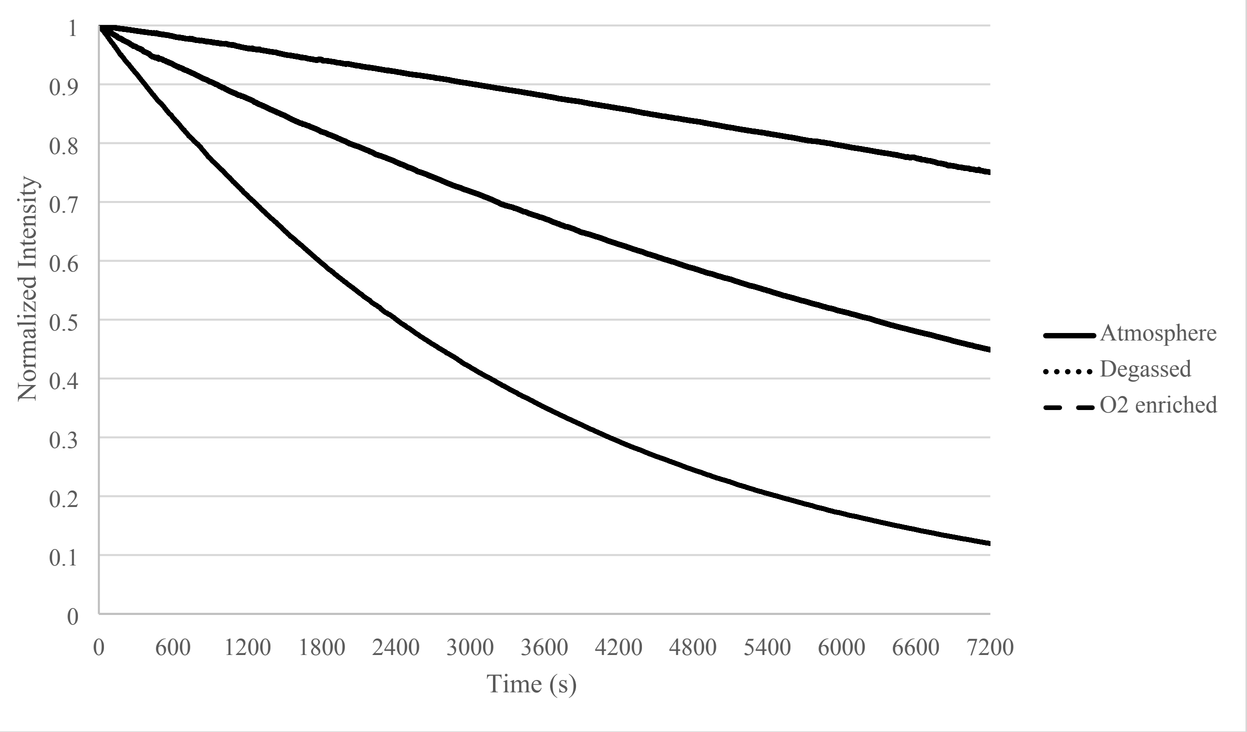


Figure S13. *Conjugate Alexa Fluor 680-c(RGDyK),* ***(10)*** *photobleaching plots in* H_2O*.*

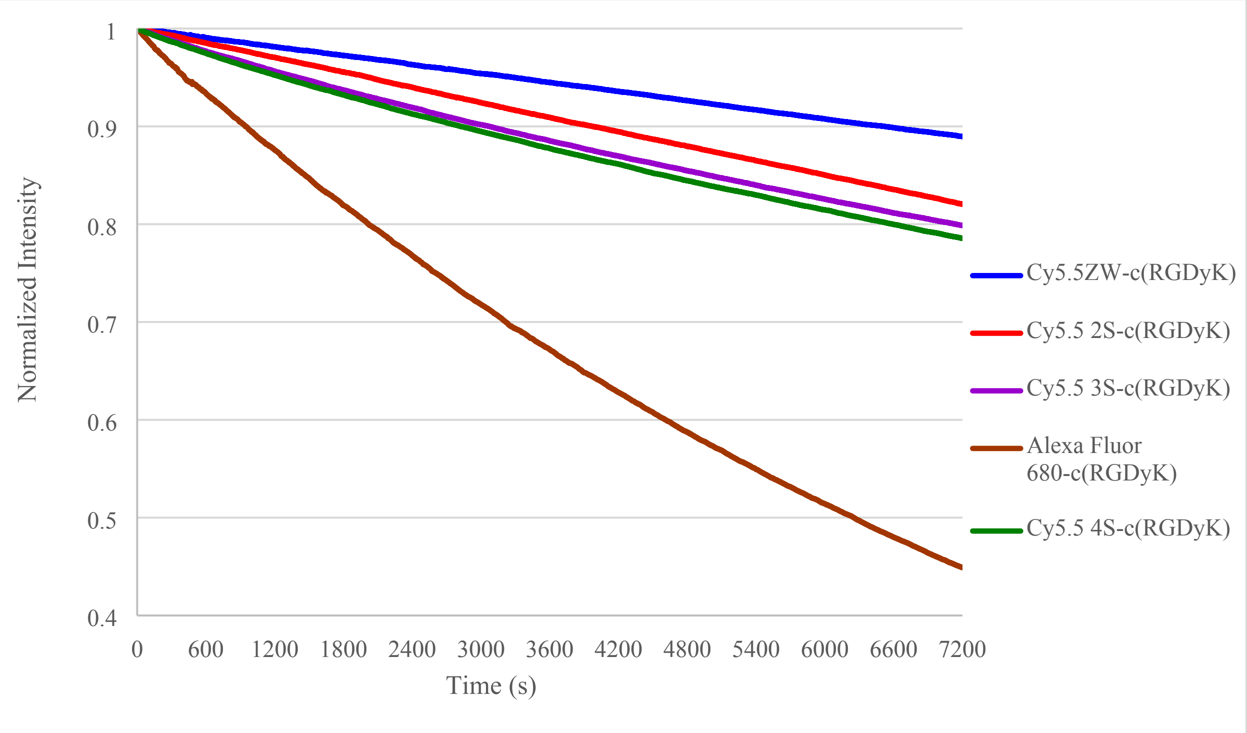


Figure S14. *Summary Photobleaching plot of all 5 dye conjugates at ambient atmosphere.*

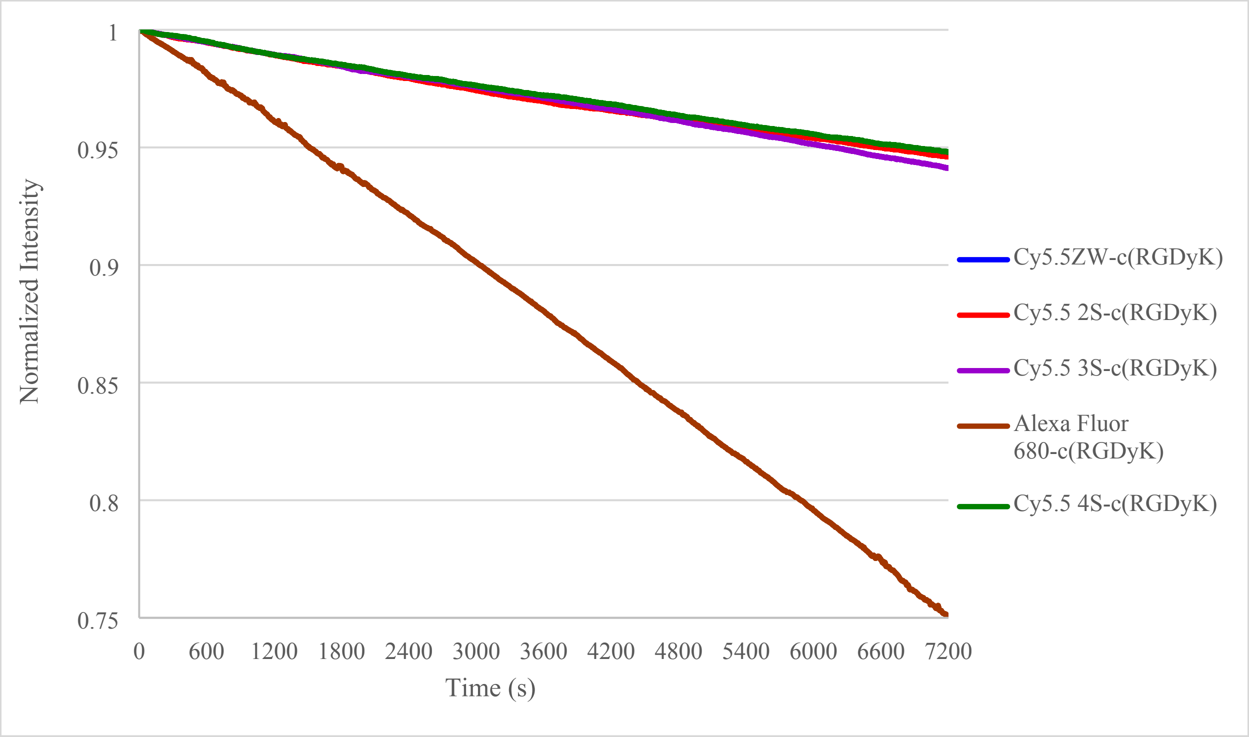


Figure S15. *Photobleaching plot of all 5 dye conjugates with their solvents purged with Ar.*

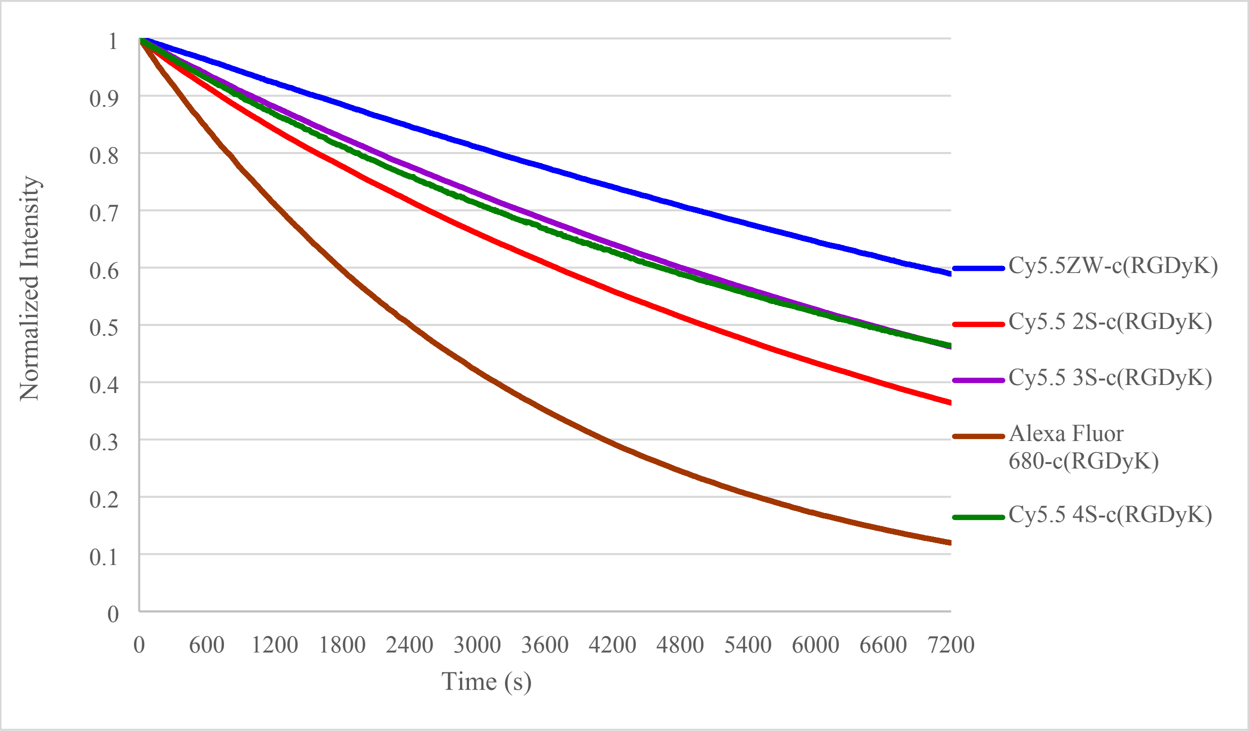


Figure S16. *Photobleaching of all 5 dye conjugate compounds with their solvents saturated with O_2 gas.*

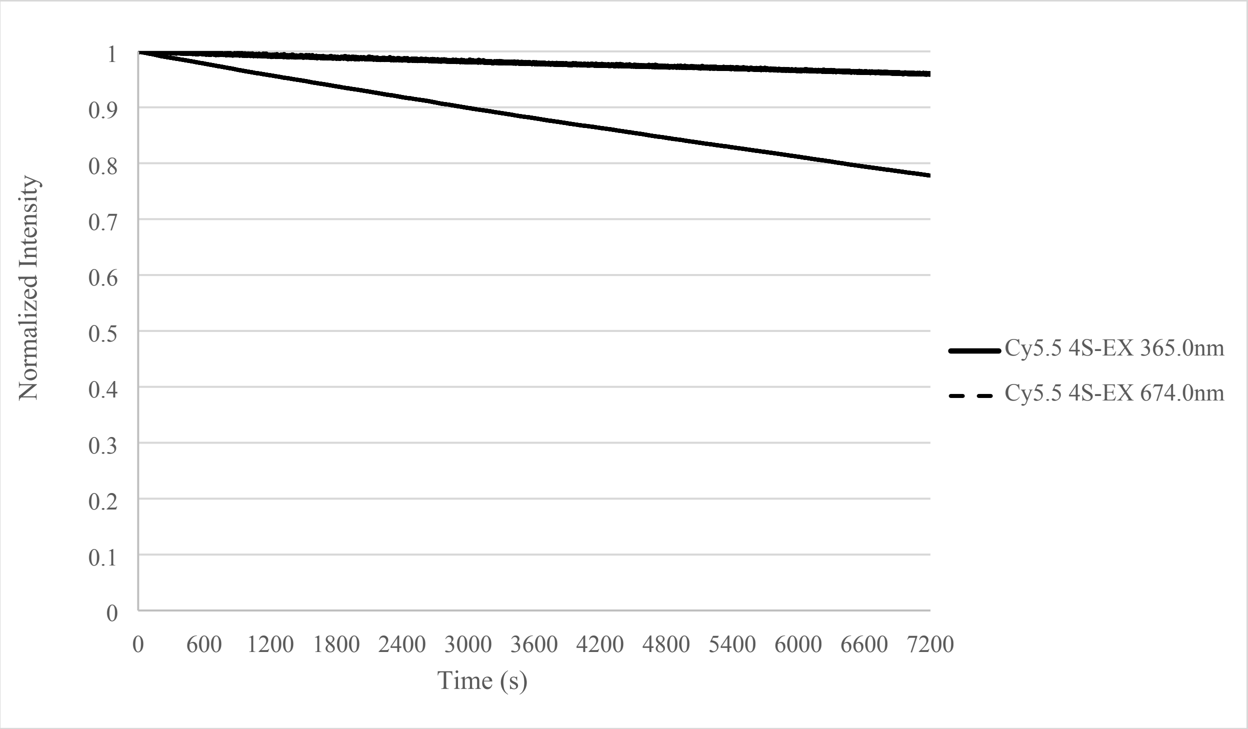


Figure S17. *Dependence of excitation wavelength (365nm, and λ_{max}) on photostability on Cy5.5 4S,* ***(1)****.*

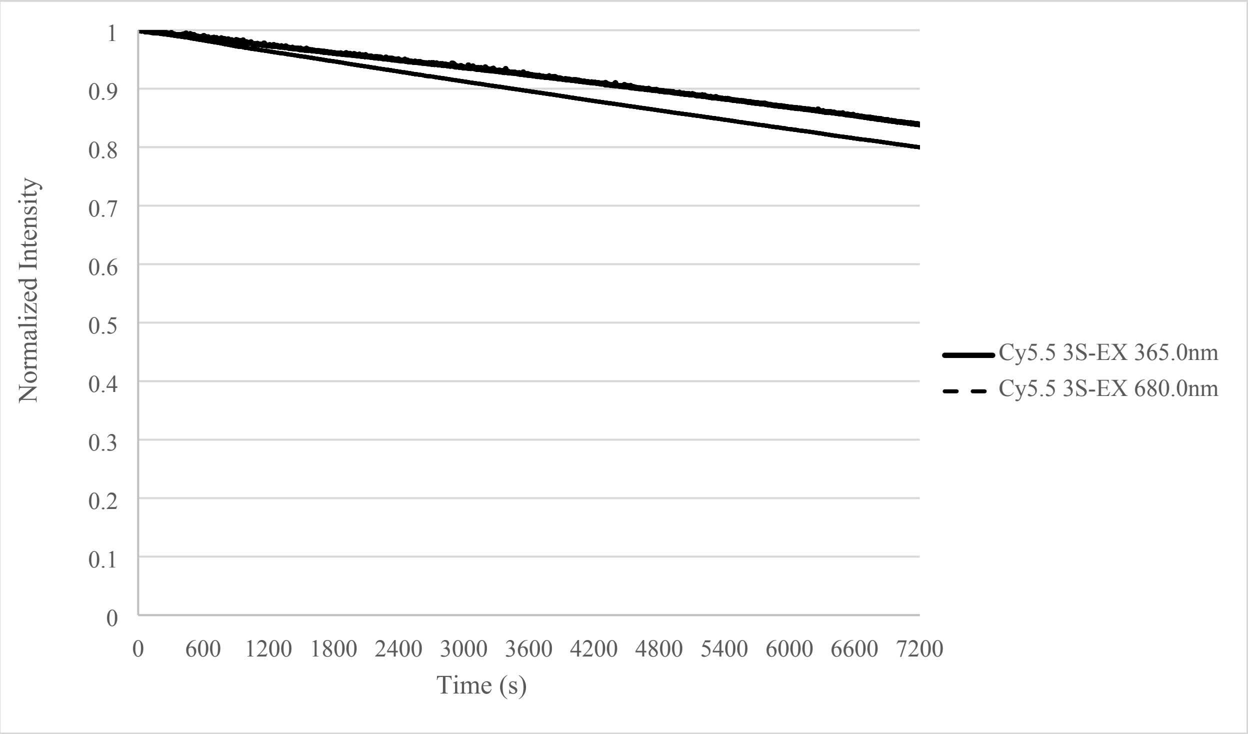


Figure S18. *Dependence of excitation wavelength (365nm, and λ_{max}) on photostability on Cy5.5 3S,* ***(2)****.*

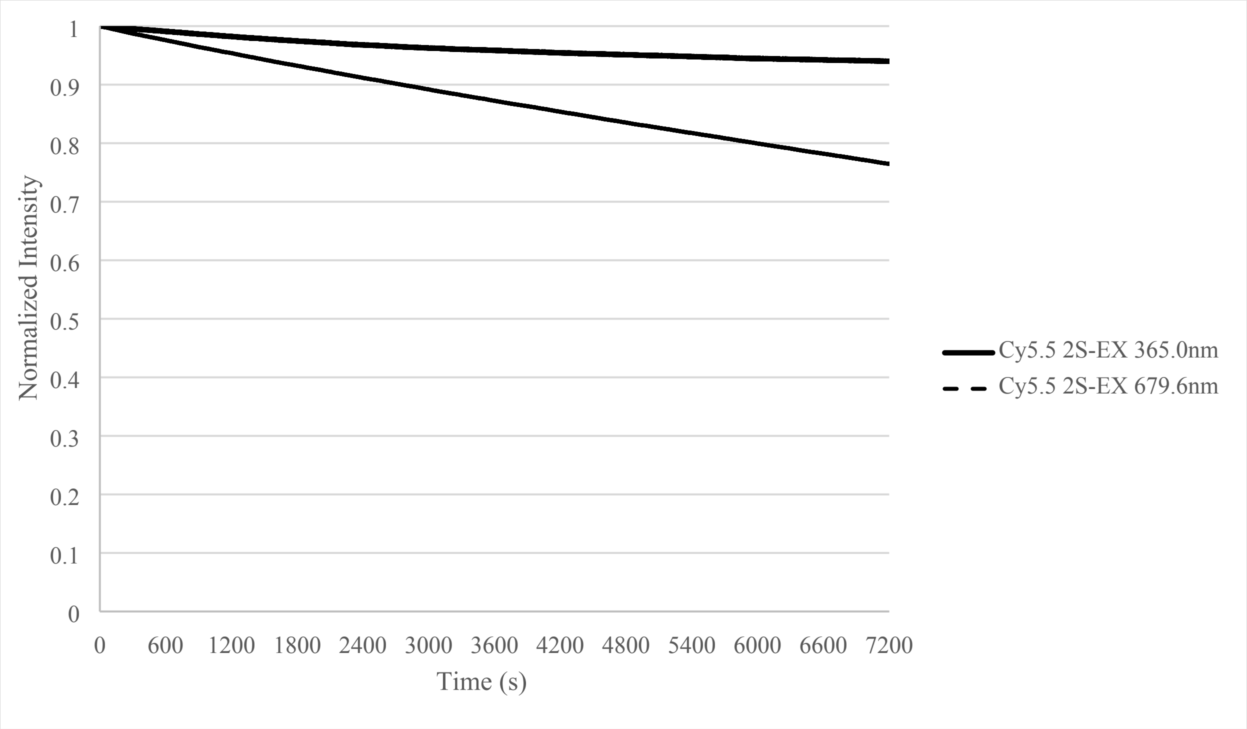


Figure S19. *Dependence of excitation wavelength (365nm, and λ_{max}) on photostability on Cy5.5 2S,* ***(3)****.*

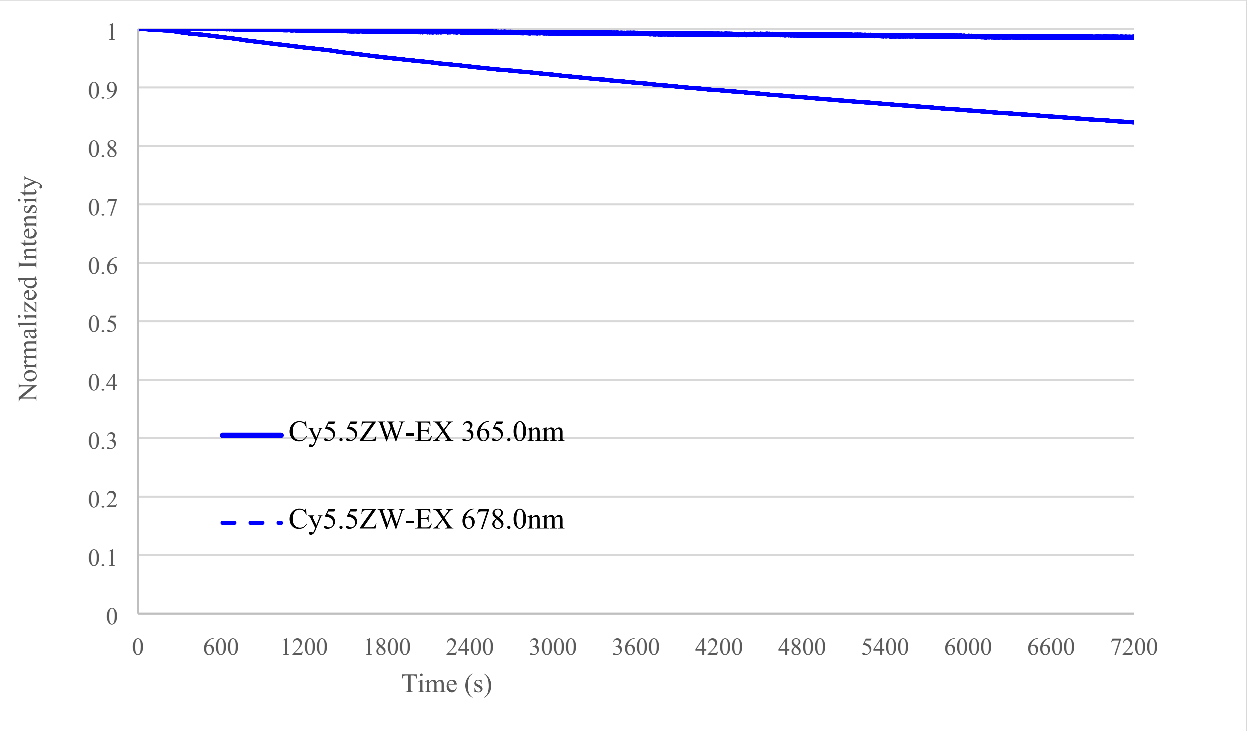


Figure S20. *Dependence of excitation wavelength (365nm, and λ_{max}) on photostability on Cy5.5 ZW,* ***(4)****.*

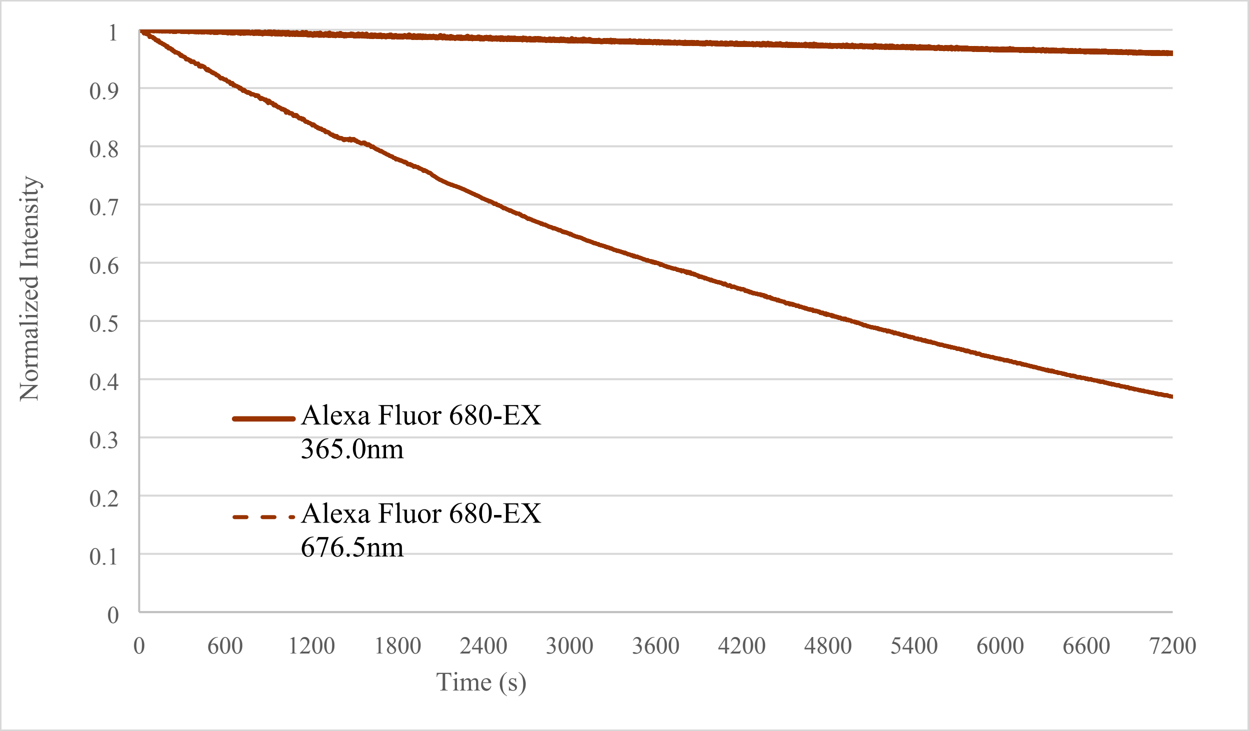


Figure S21. *Dependence of excitation wavelength (365nm, and λ_{max}) on photostability on compound Alexa Fluor 680,* ***(5)****.*

UV-VIS & Emission Spectra: Solutions for absorbance spectra not used for the calibration plot were brought close to Abs=1, and then normalized.

Compound	ε (L/mol*cm)	Stoke's Shift (nm)	λ_{EX} (nm)	λ_{EM} (nm)
(1) Cy5.5 4S	178,000	25	678	703
(2) Cy5.5 3S	213,000	25	683	708
(3) Cy5.5 2S	204,000	23	682	705
(4) Cy5.5 ZW	197,000	25	682	707
(5) AF 680	154,000	25	684	709

Table S1. *Spectral data for the 5 free acid dyes in methanol.*

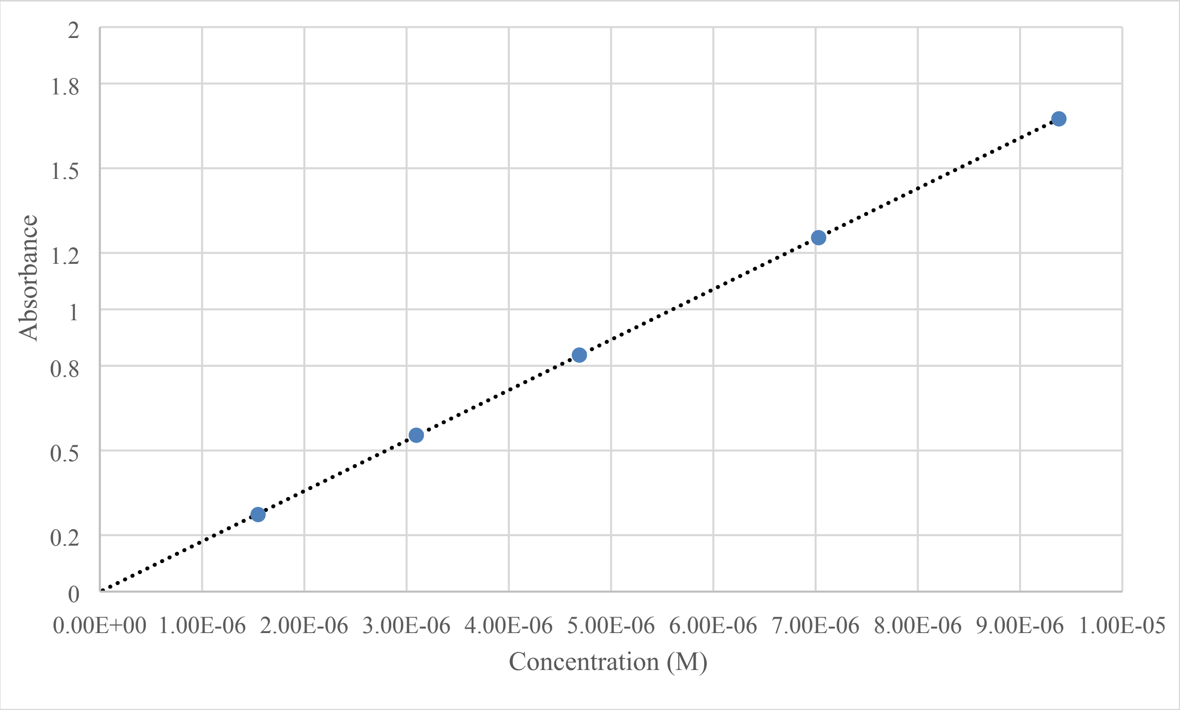


Figure S22a. *Beer's Law plot of compound Cy5.5 4S,* ***(1)*** *in H_2O. The extinction coefficient was determined from the slope of the data's trend line.*

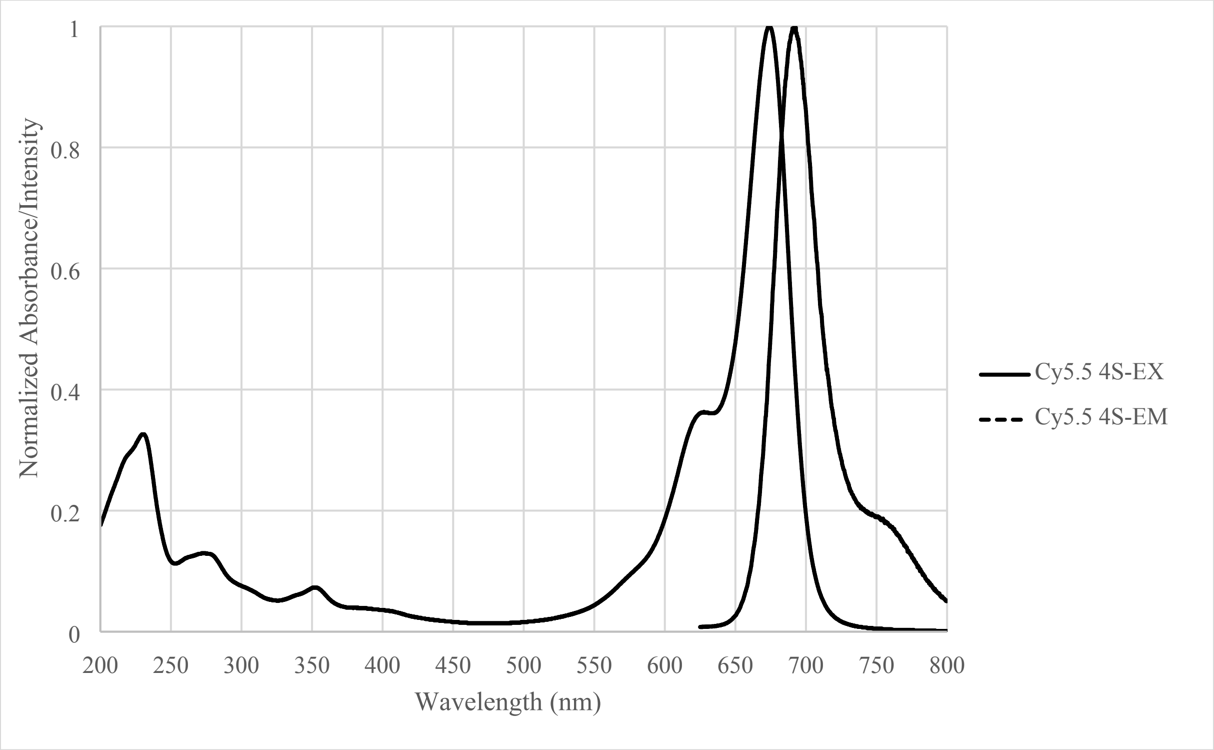


Figure S22b. *Normalized UV-VIS & emission spectrum of compound Cy5.5 4S,* ***(1)*** *in H_2O.*

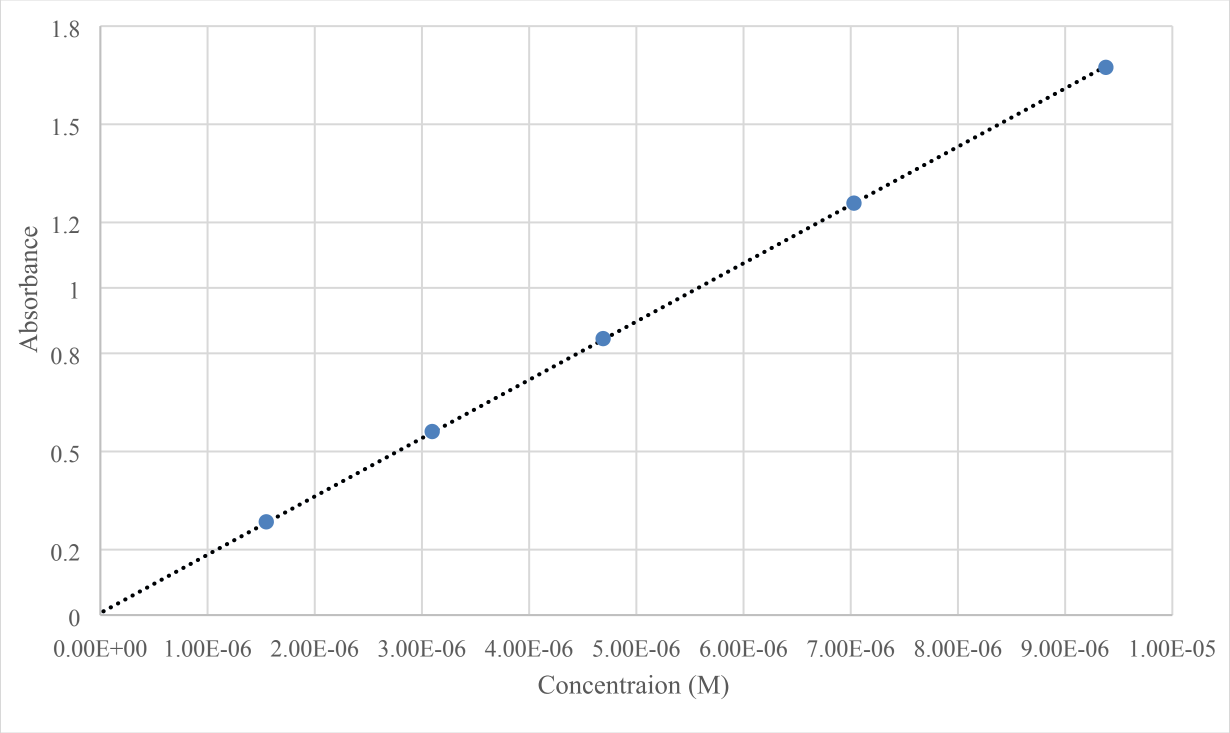


Figure S22c. *Beer's Law plot of compound Cy5.5 4S,* ***(1)*** *in methanol. The extinction coefficient was determined from the slope of the data's trend line.*

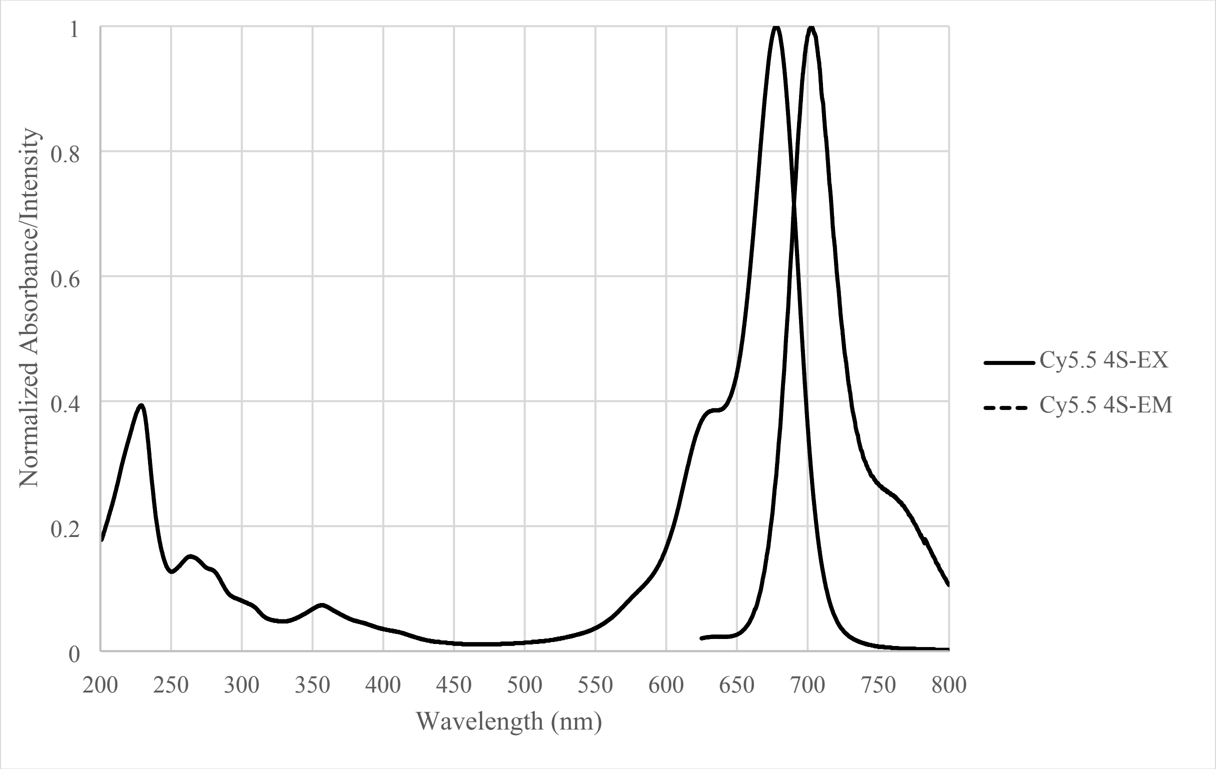


Figure S22d. *Normalized UV-VIS & emission spectrum of compound Cy5.5 4S,* ***(1)*** *in methanol.*

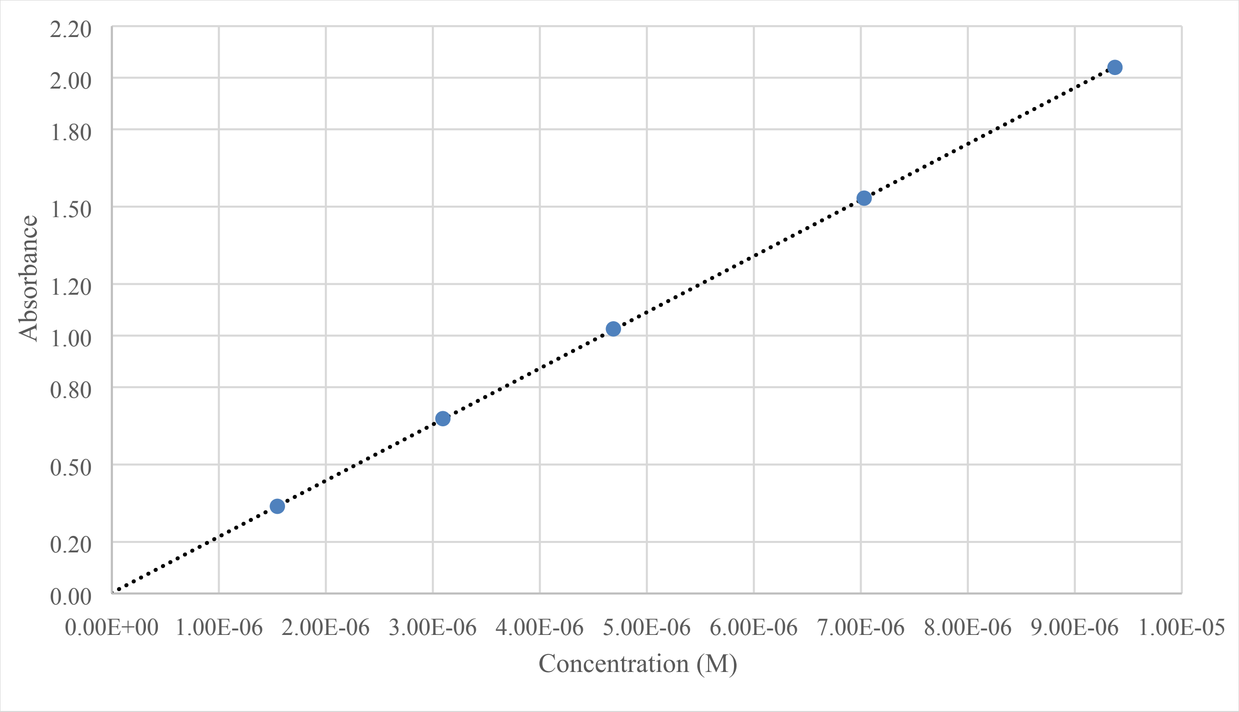


Figure S23a. *Beer's Law plot of compound Cy5.5 3S,* ***(2)*** *in H_2O. The extinction coefficient was determined from the slope of the data's trend line.*

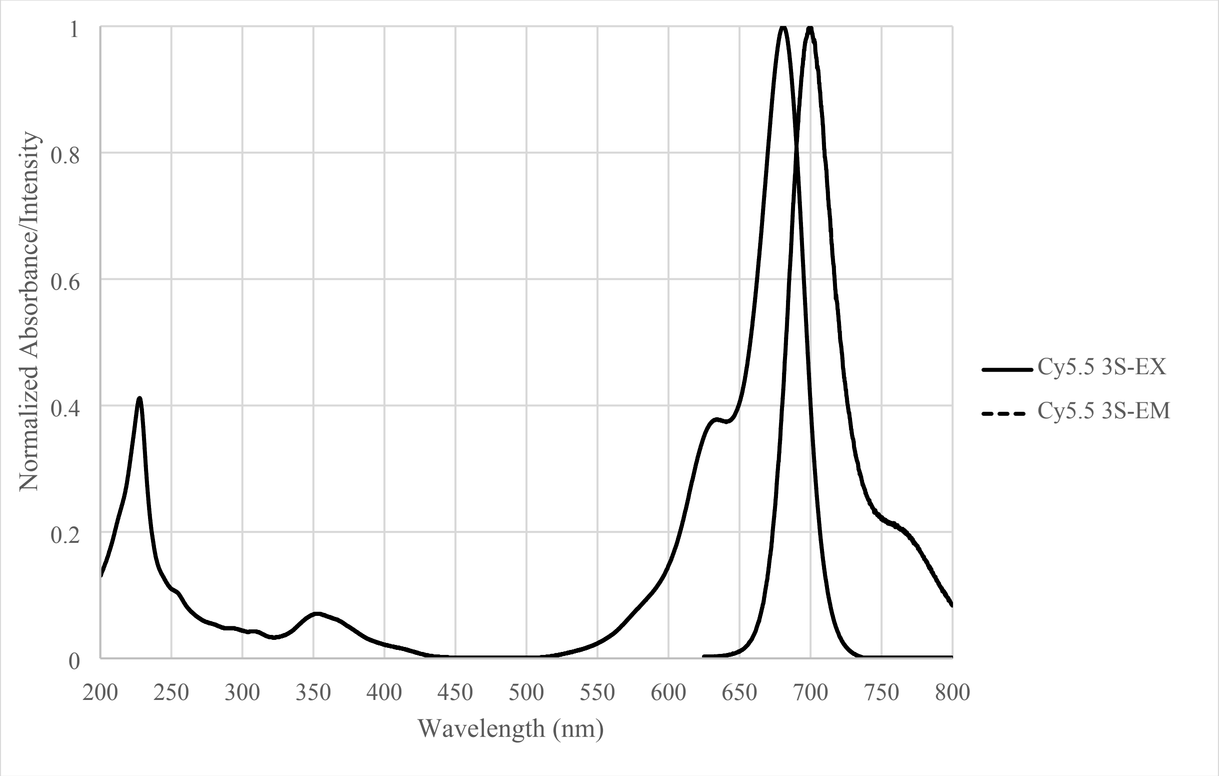


Figure S23b. *Normalized UV-VIS & emission spectrum of compound Cy5.5 3S,* ***(2)*** *in H_2O.*

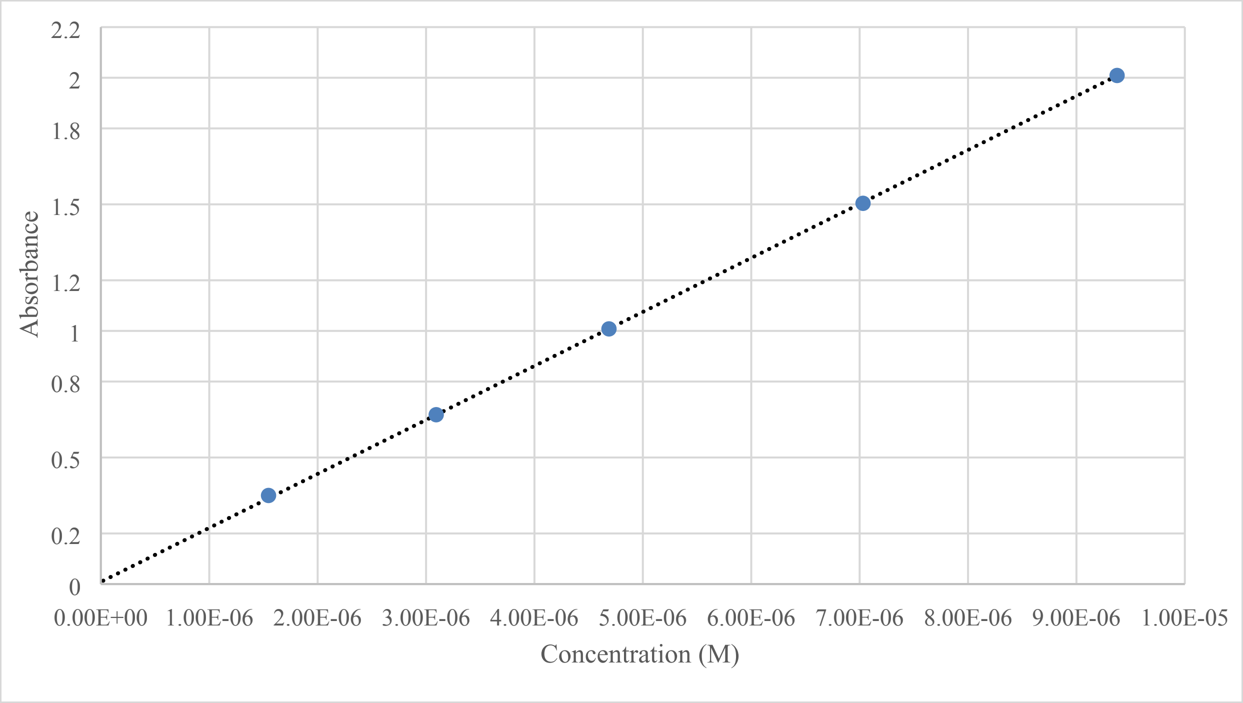


Figure S23c. *Beer's Law plot of compound Cy5.5 3S,* ***(2)*** *in methanol. The extinction coefficient was determined from the slope of the data's trend line.*

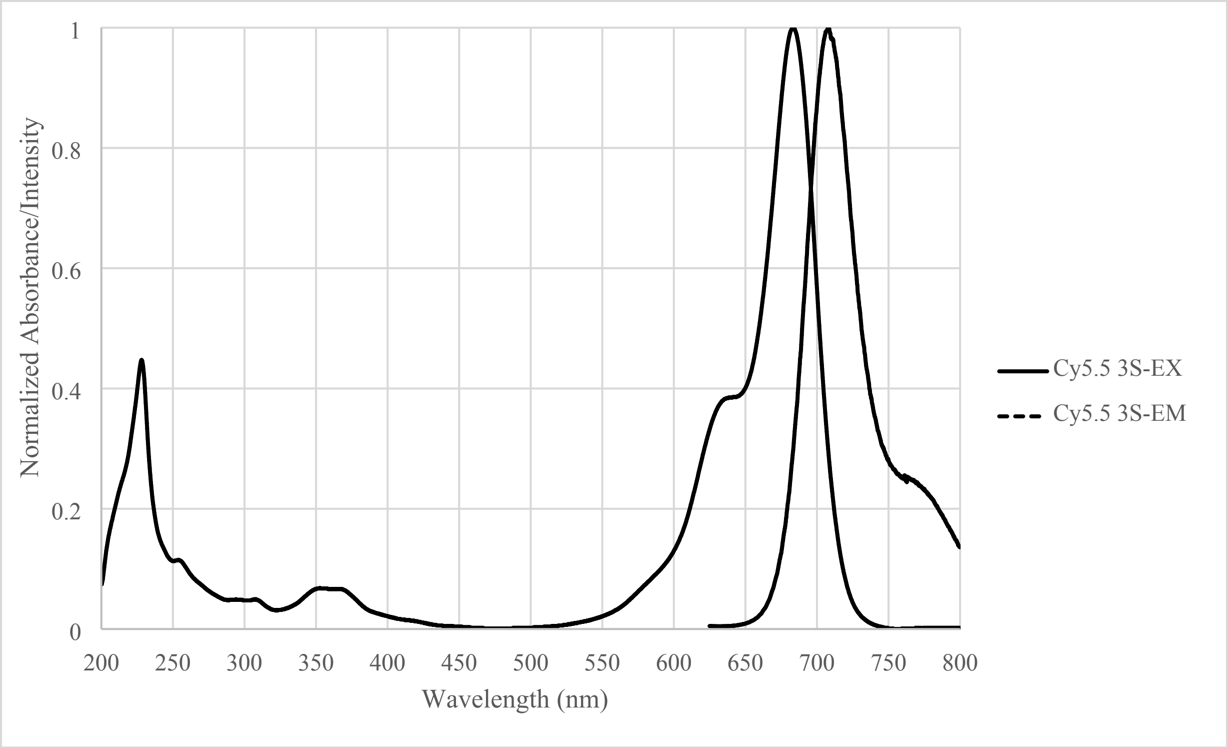


Figure S23d. *Normalized UV-VIS & emission spectrum of compound Cy5.5 3S,* ***(2)*** *in methanol.*

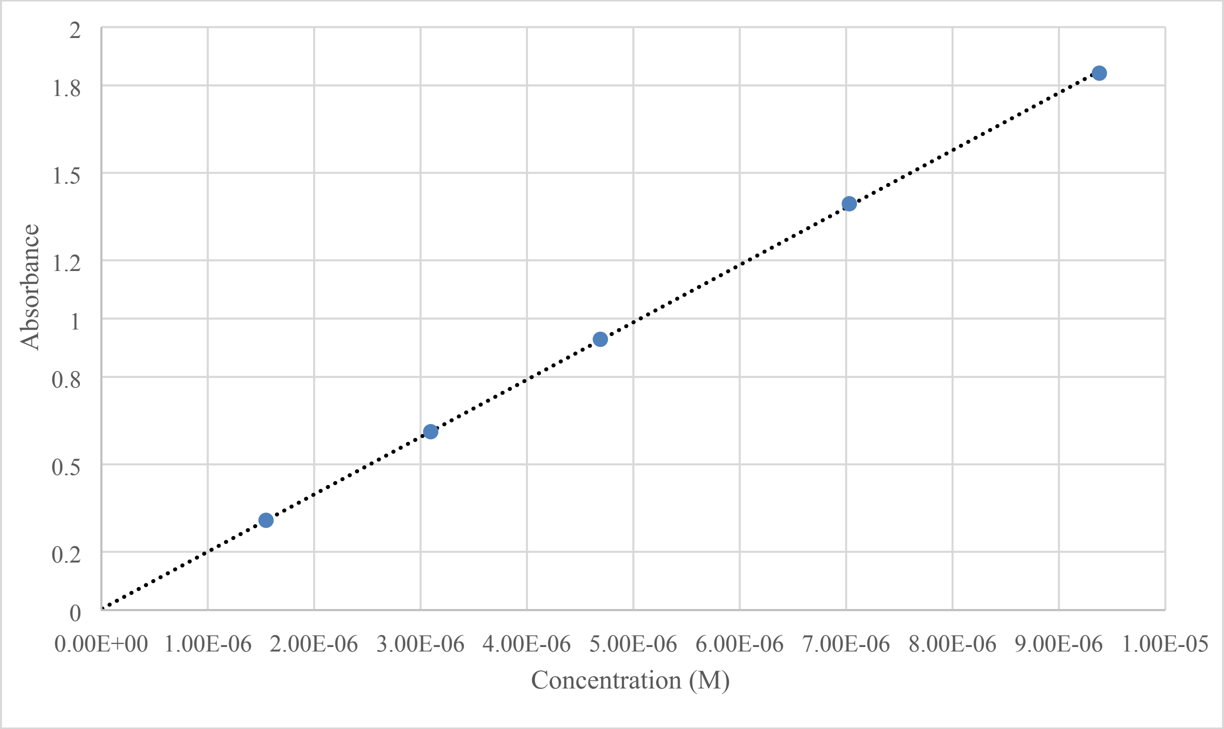


Figure S24a. *Beer's Law plot of compound Cy5.5 2S,* ***(3)*** *in H_2O. The extinction coefficient was determined from the slope of the data's trend line.*

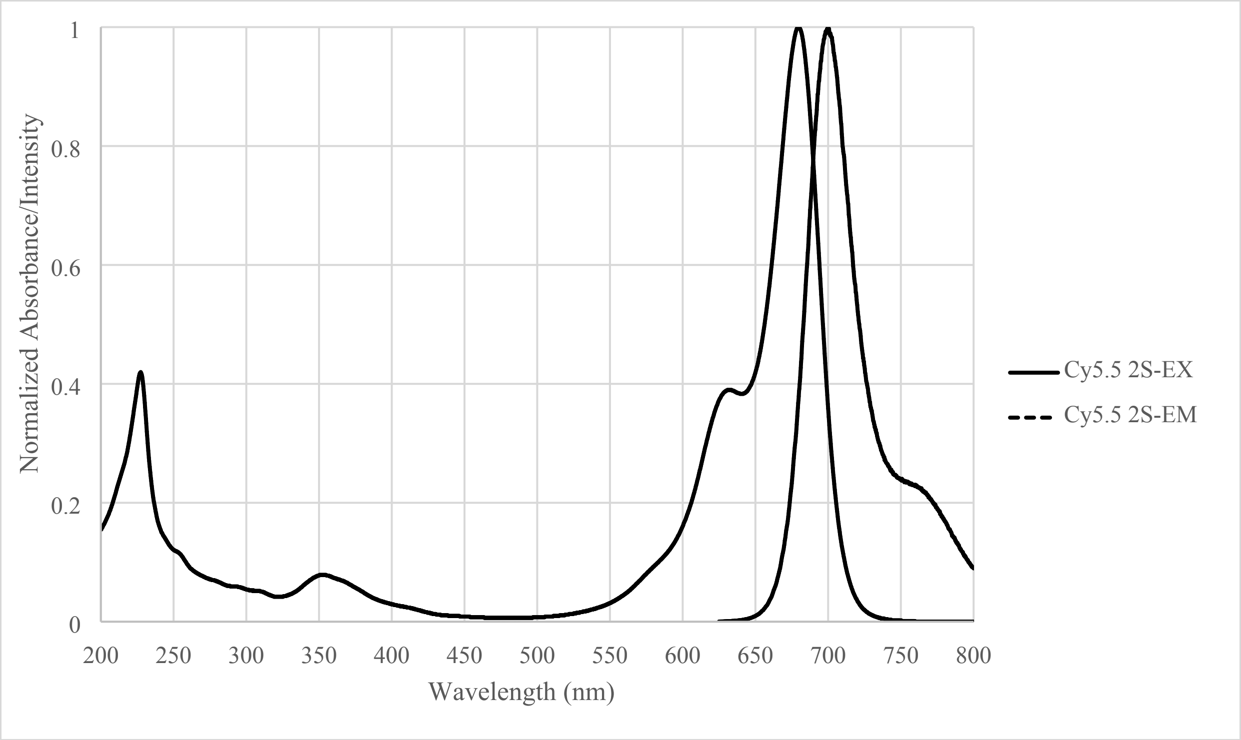


Figure S24b. *Normalized UV-VIS & emission spectrum of compound Cy5.5 2S,* ***(3)*** *in H_2O.*

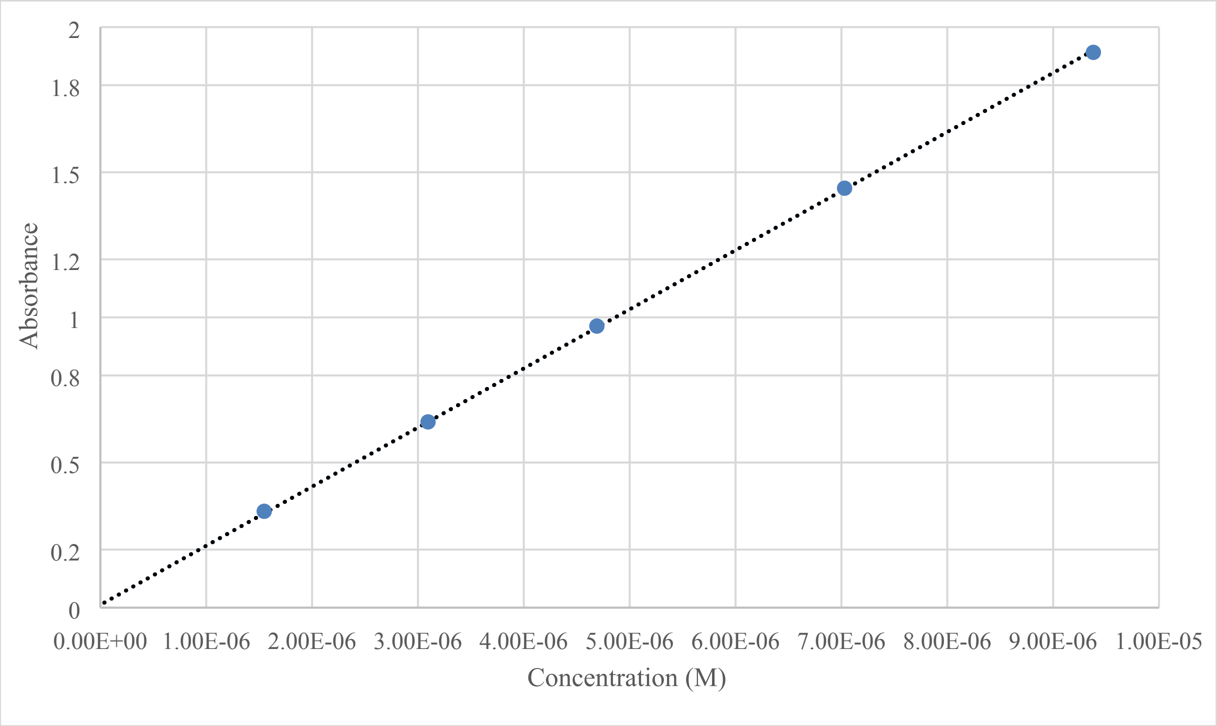


Figure S24c. *Beer's Law plot of compound Cy5.5 2S,* ***(3)*** *in methanol. The extinction coefficient was determined from the slope of the data's trend line.*

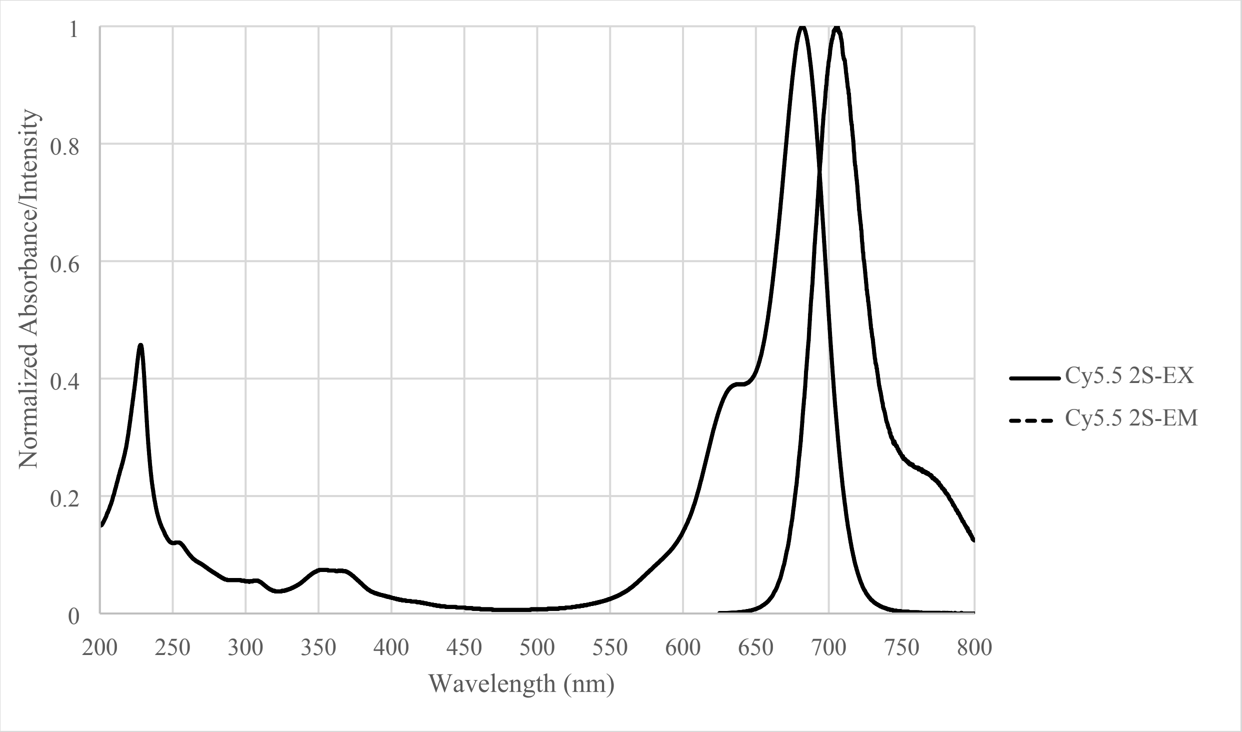


Figure S24d. *Normalized UV-VIS & emission spectrum of compound Cy5.5 2S,* ***(3)*** *in methanol.*

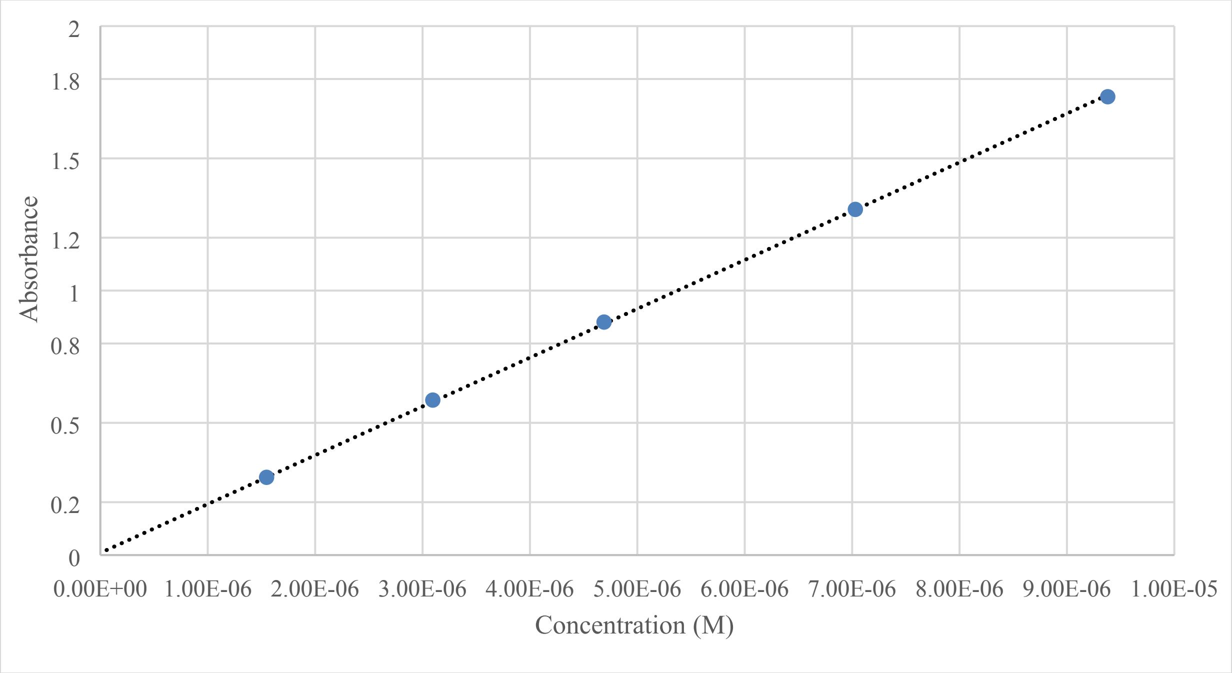


Figure S25a. *Beer's Law plot of compound Cy5.5 ZW,* ***(4)*** *in H_2O. The extinction coefficient was determined from the slope of the data's trend line.*

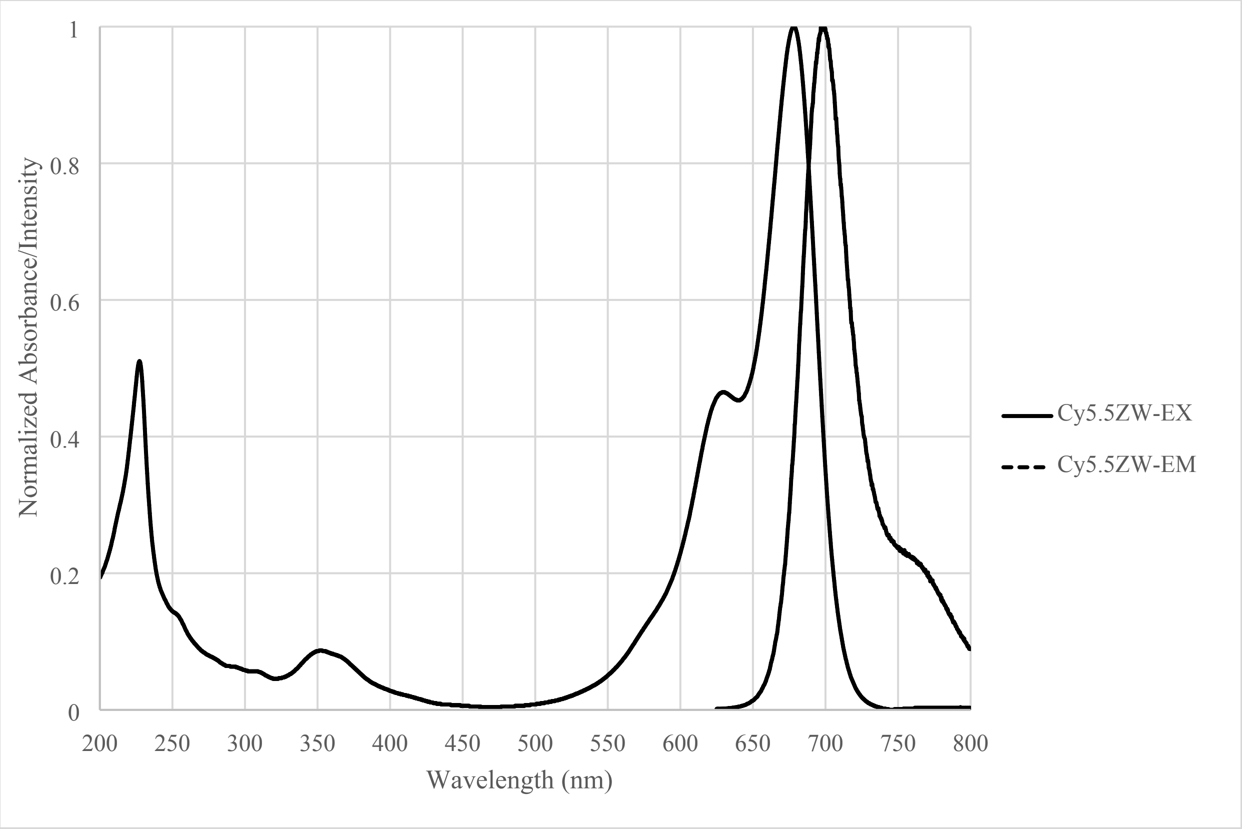


Figure S25b. *Normalized UV-VIS & emission spectrum of compound Cy5.5 ZW* ***(4)*** *in H_2O.*

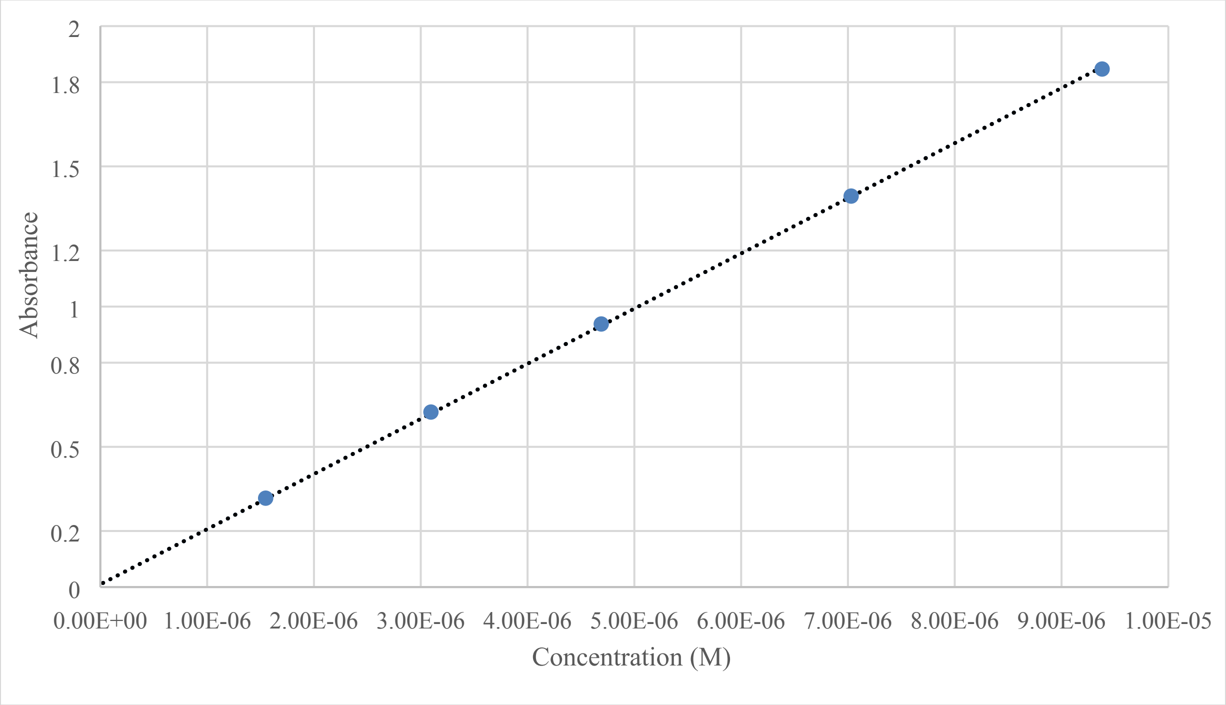


Figure S25c. *Beer's Law plot of compound Cy5.5 ZW,* ***(4)*** *in methanol. The extinction coefficient was determined from the slope of the data's trend line.*

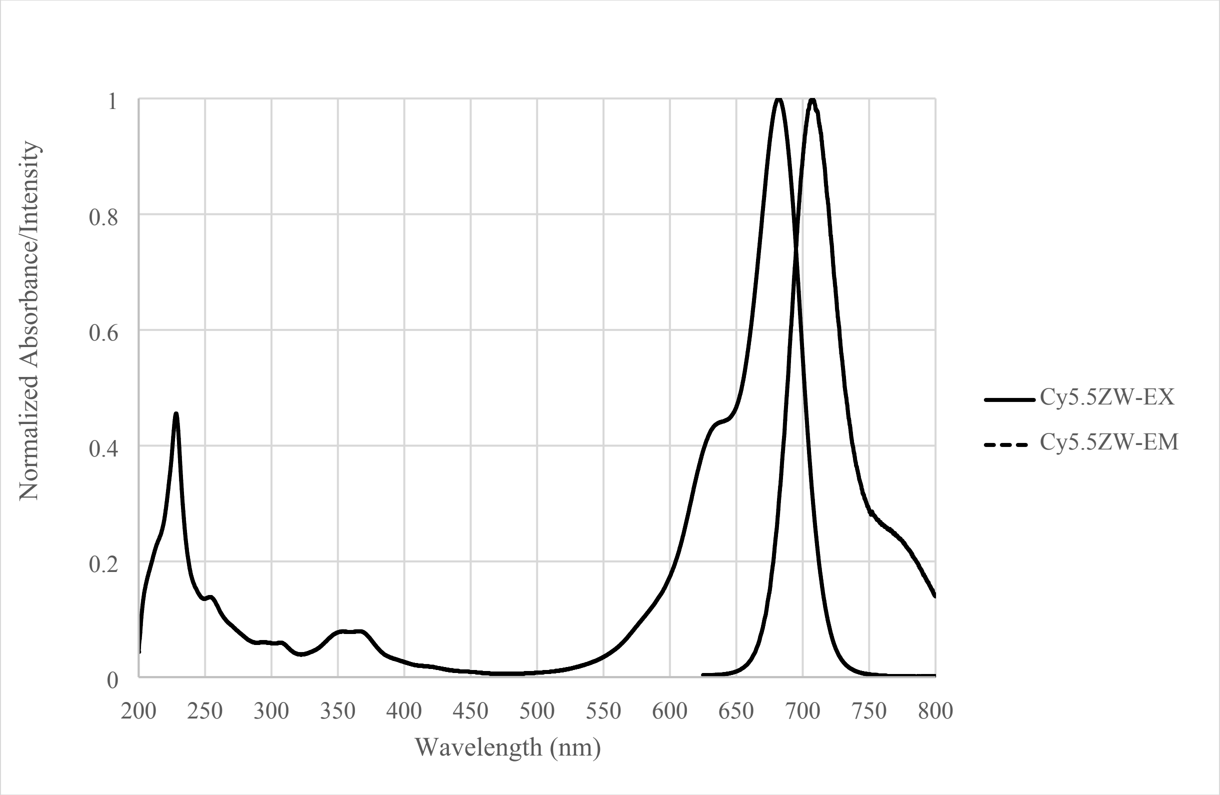


Figure S25d. *Normalized UV-VIS & emission spectrum of compound Cy5.5 ZW,* ***(4)*** *in methanol.*

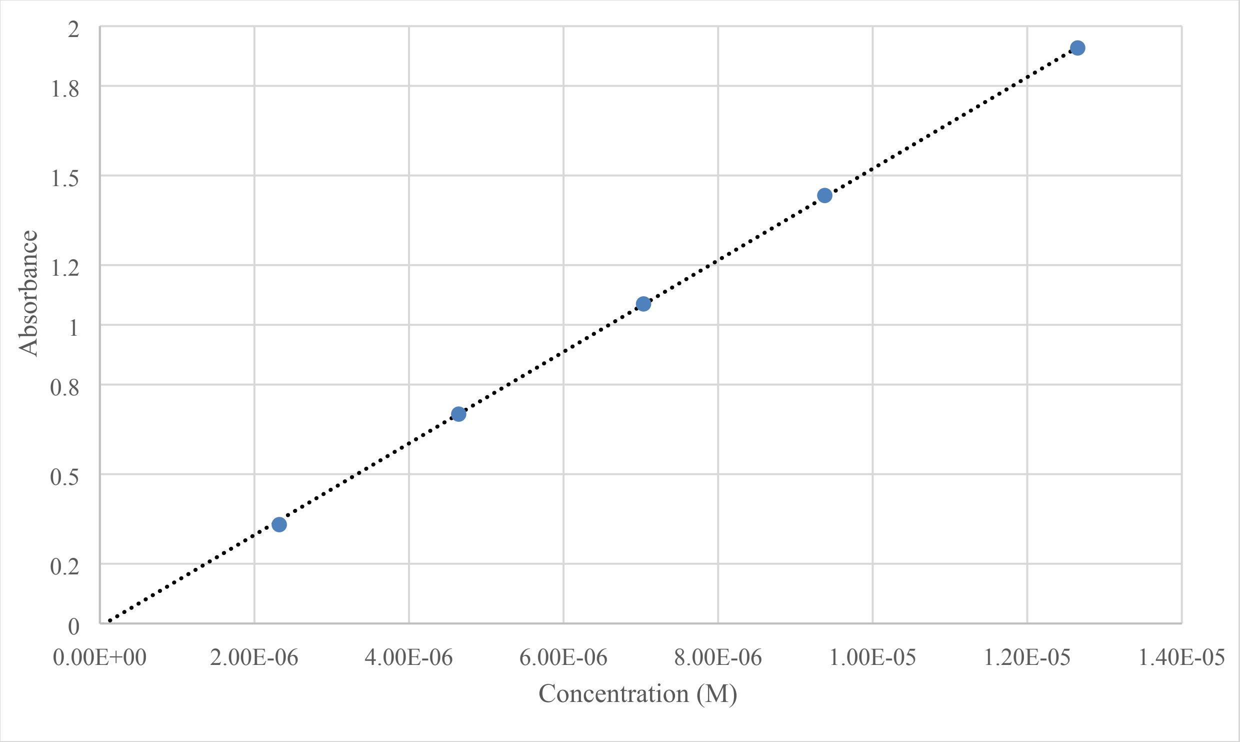


Figure S26a. *Beer's Law plot of compound Alexa Fluor 680,* ***(5)*** *in H_2O. The extinction coefficient was determined from the slope of the data's trend line.*

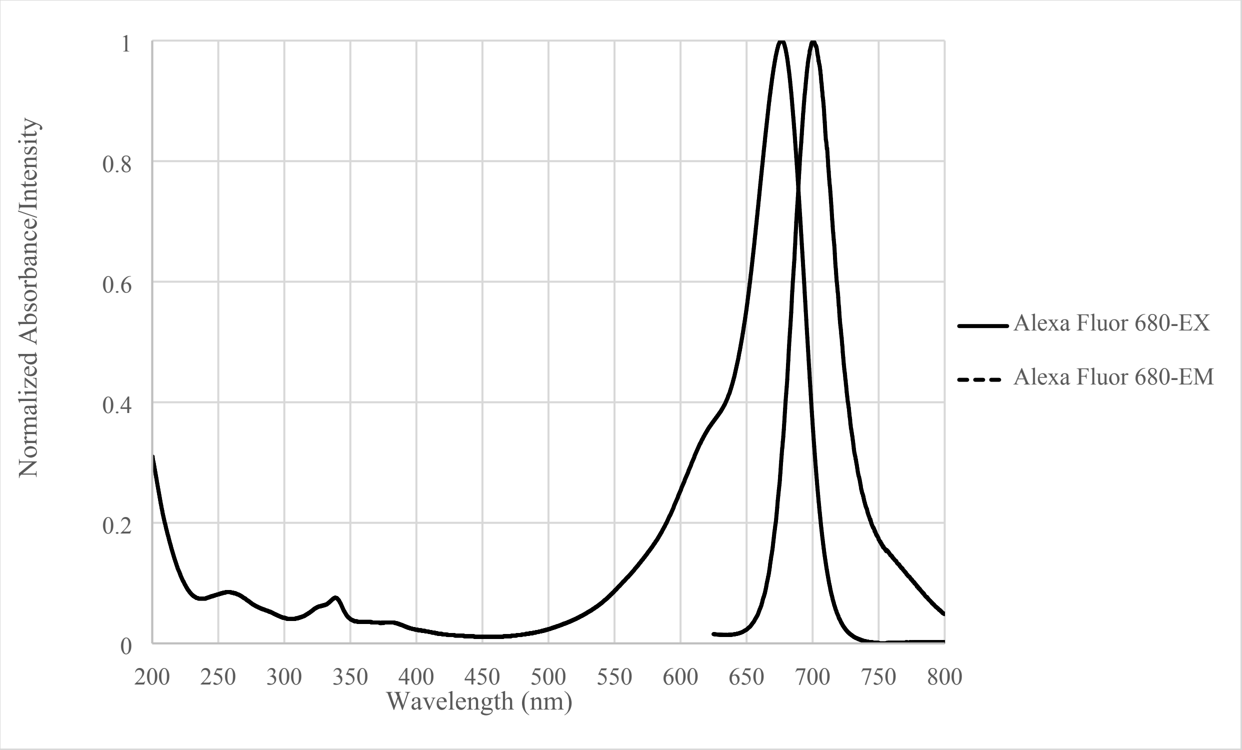


Figure S26b. *Normalized UV-VIS & emission spectrum of compound Alexa Fluor 680* ***(5)*** *in H_2O.*

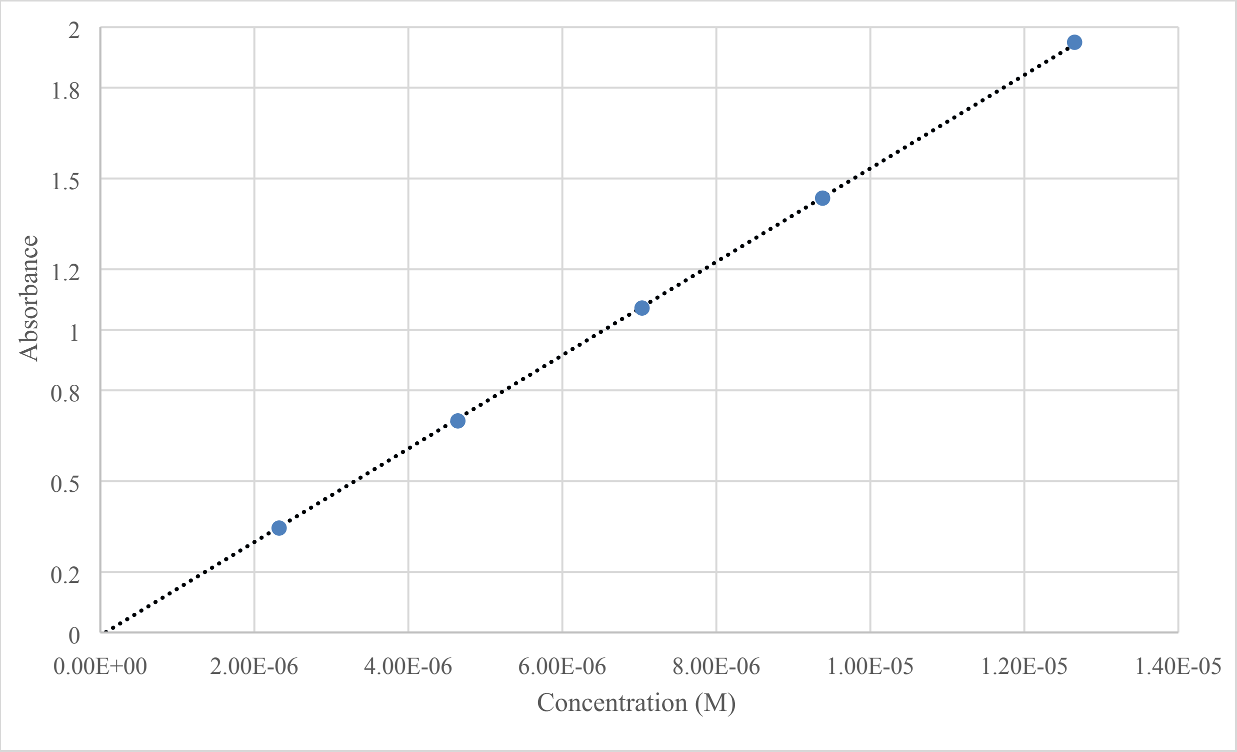


Figure S26c. *Beer's Law plot of compound Alexa Fluor 680,* ***(5)*** *in methanol. The extinction coefficient was determined from the slope of the data's trend line.*

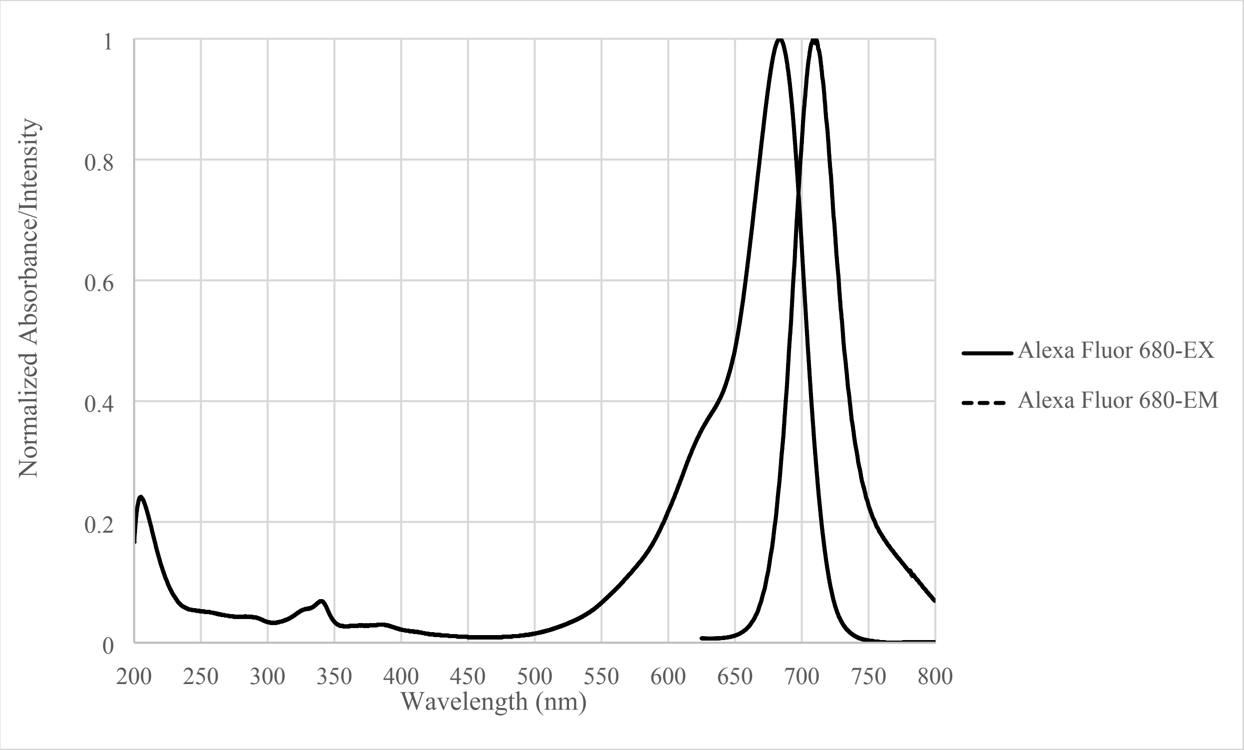


Figure S26d. *Normalized UV-VIS & emission spectrum of compound Alexa Fluor 680* ***(5)*** *in methanol.*

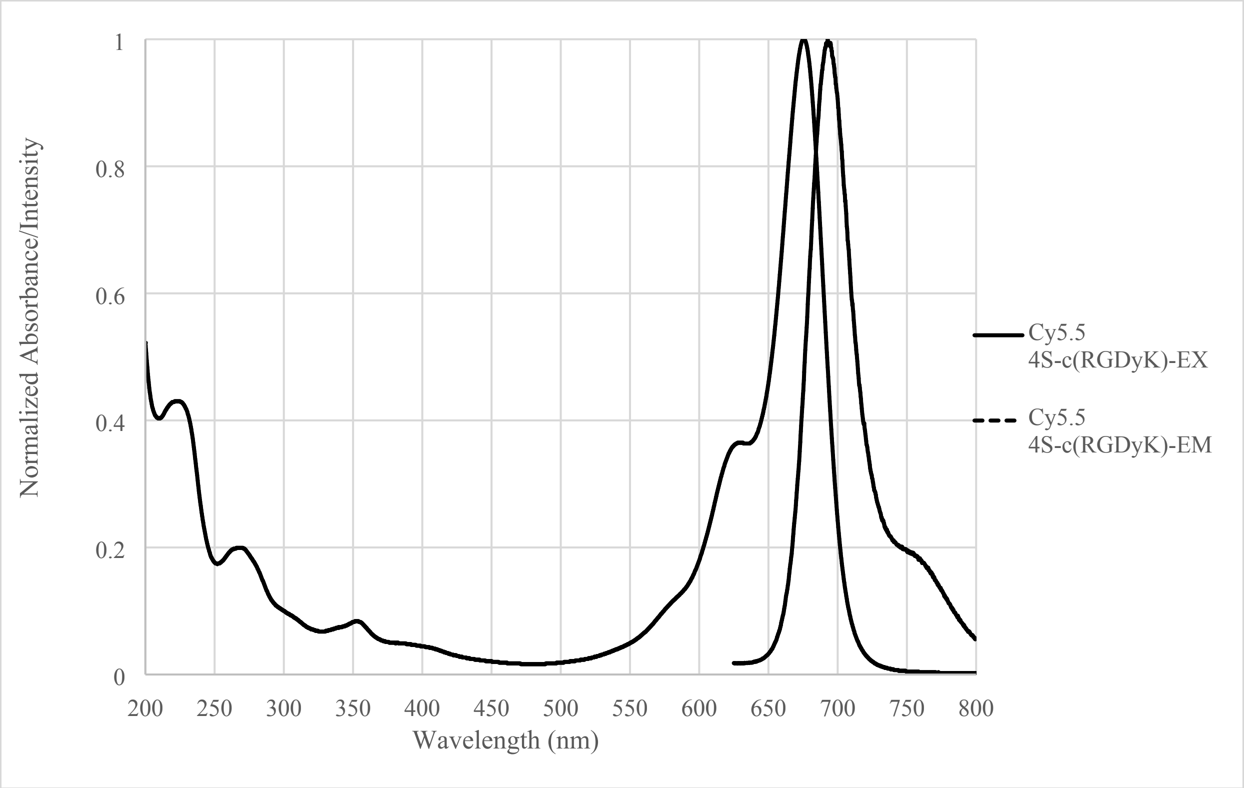


Figure S27. *Normalized UV-VIS & emission spectrum of compound Cy5.5 4S-c(RGDyK),* ***(6)*** *in* H_2O*.*

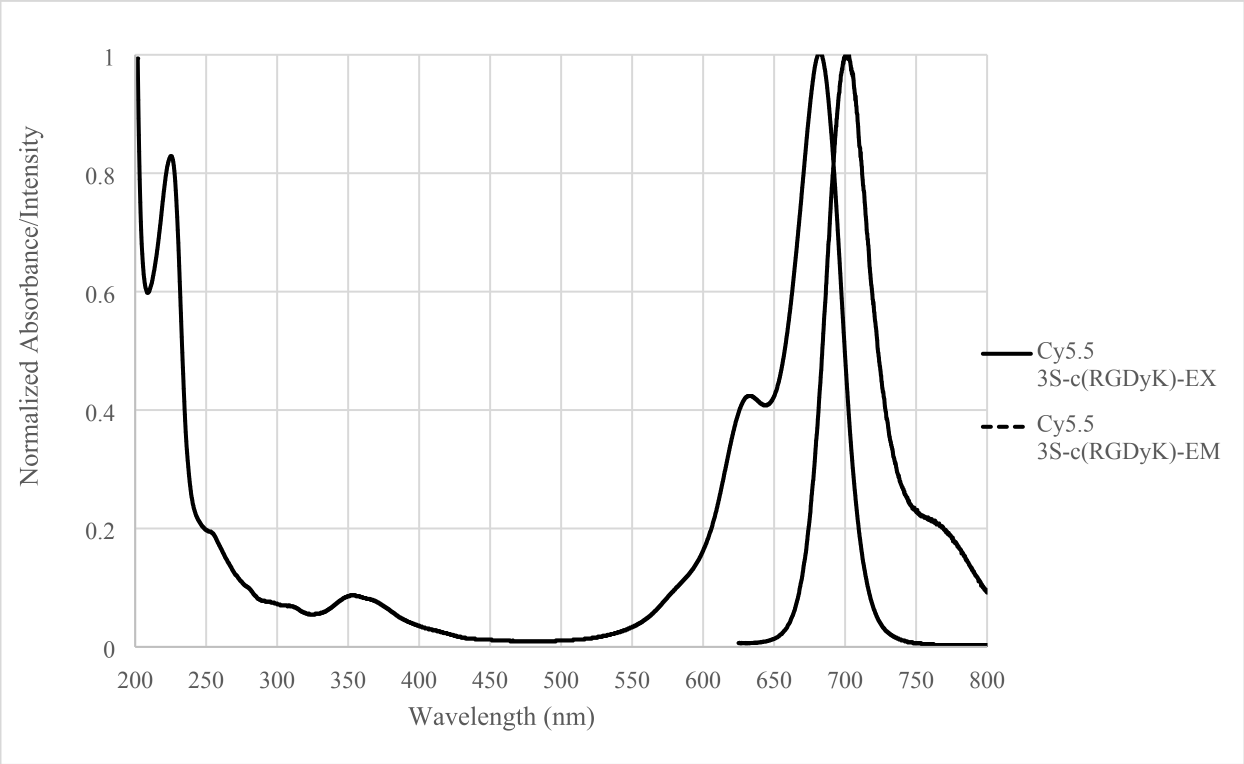


Figure S28. *Normalized UV-VIS & emission spectrum of compound Cy5.5 3S-c(RGDyK),* ***(7)*** *in* H_2O*.*

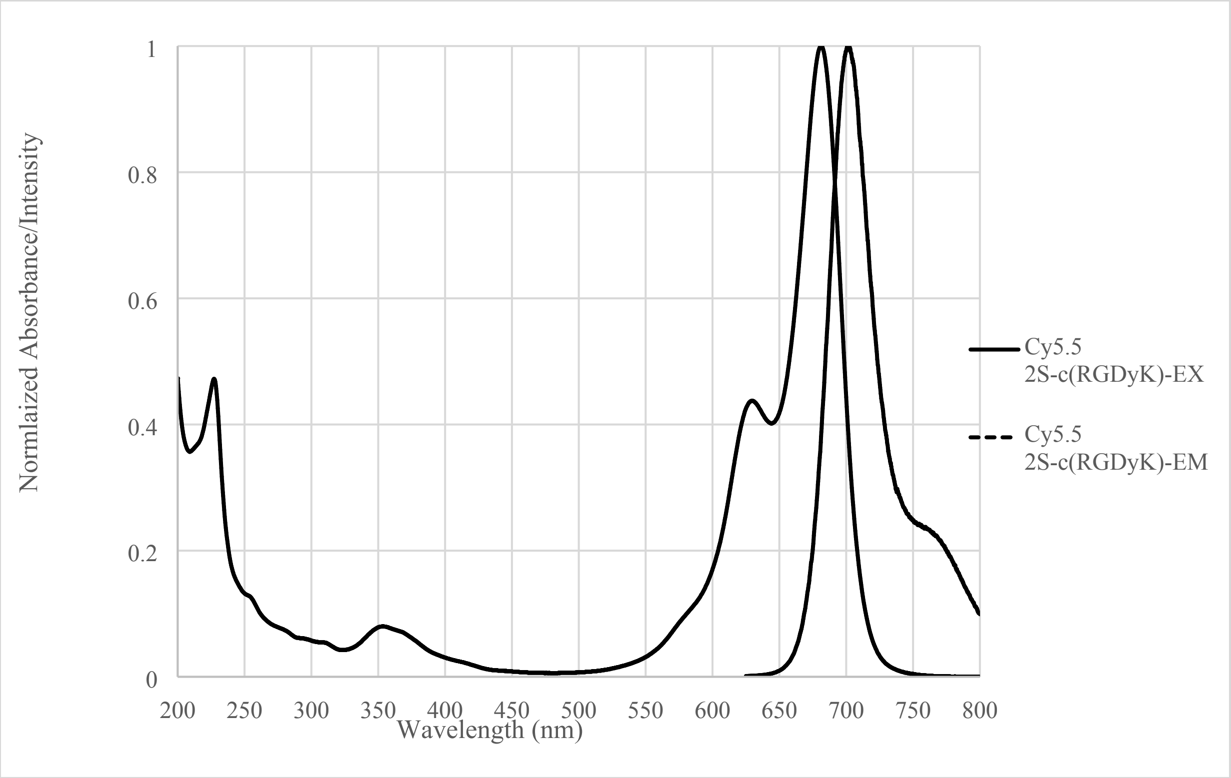


Figure S29. *Normalized UV-VIS & emission spectrum of compound Cy5.5 2S-c(RGDyK),* ***(8)*** *in* H_2O.

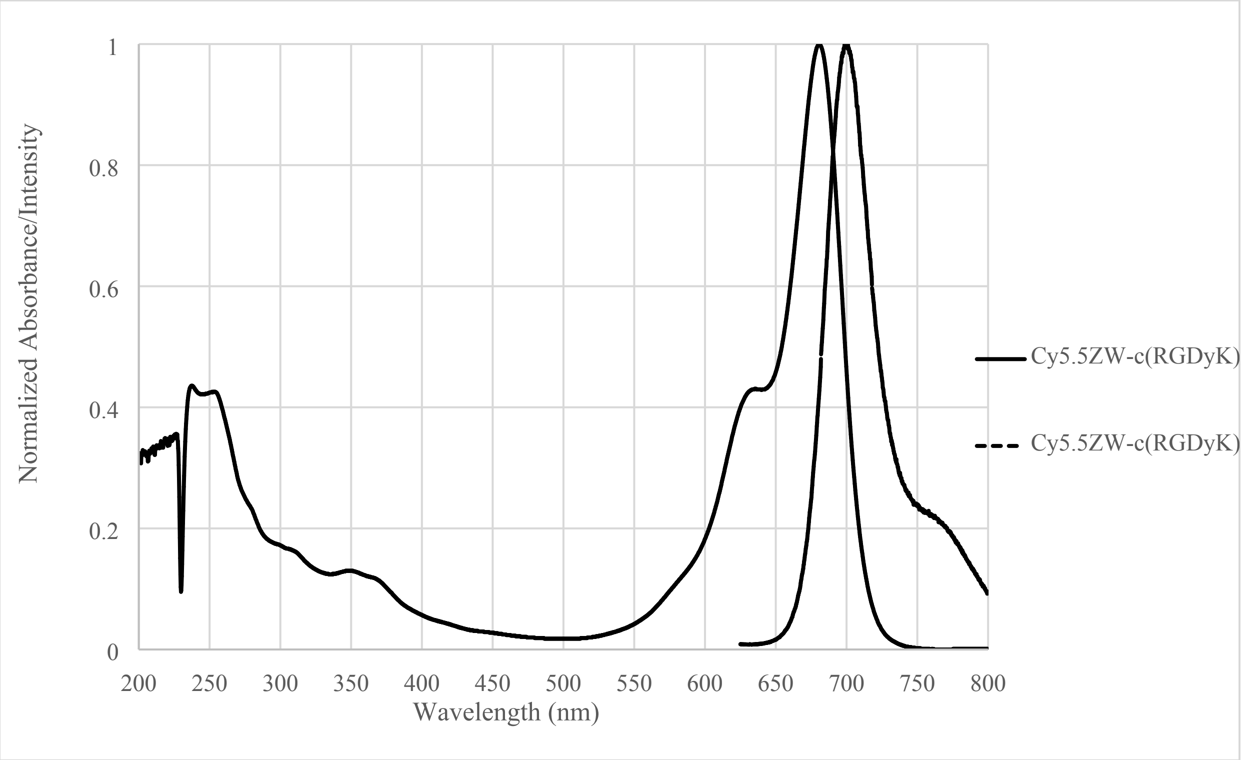


Figure S30. *Normalized UV-VIS & emission spectrum of compound Cy5.5 ZW-c(RGDyK),* ***(9)*** *in* H_2O.

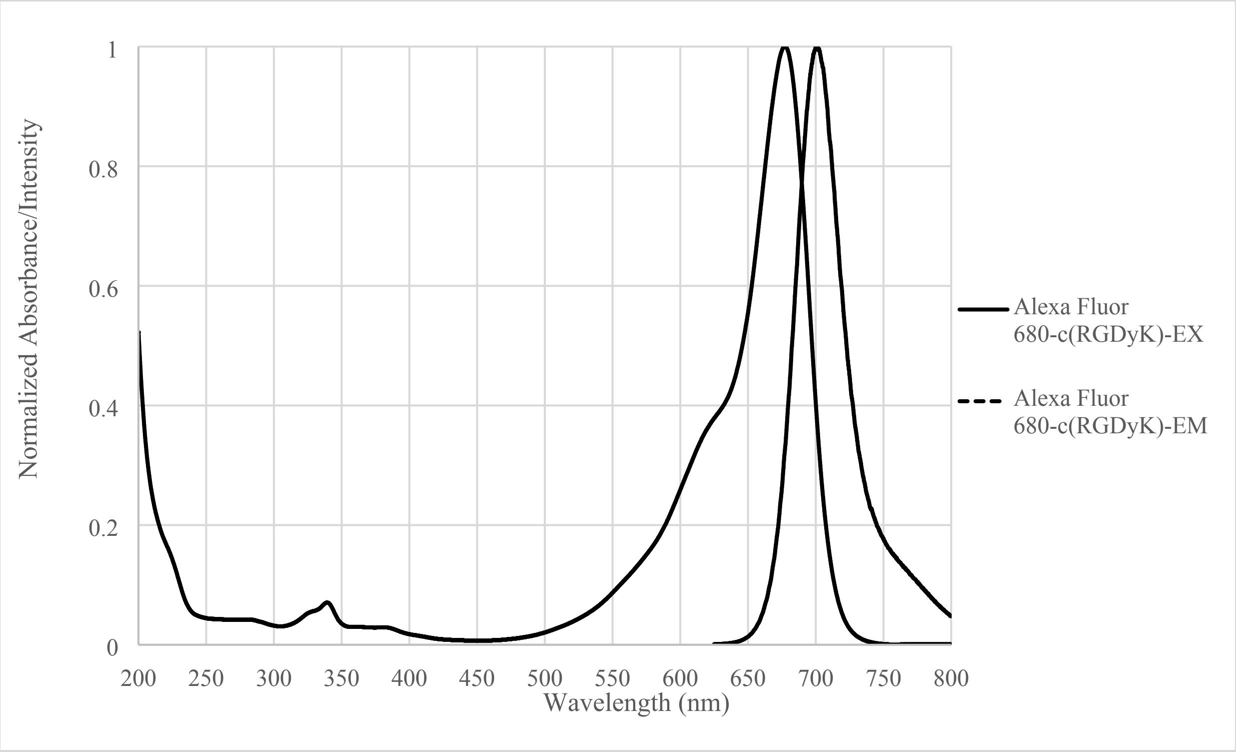


Figure S31. *Normalized UV-VIS & emission spectrum of compound Alexa Fluor 680-c(RGDyK),* ***(10)*** *in* H_2O.

HPLC Data:

*All samples were analyzed via a 12.5min ACN:H_2O gradient (10-70%) at 0.5mL/min. All SWCs (Single Wavelength Chromatograms) were monitored at 680.0nm.

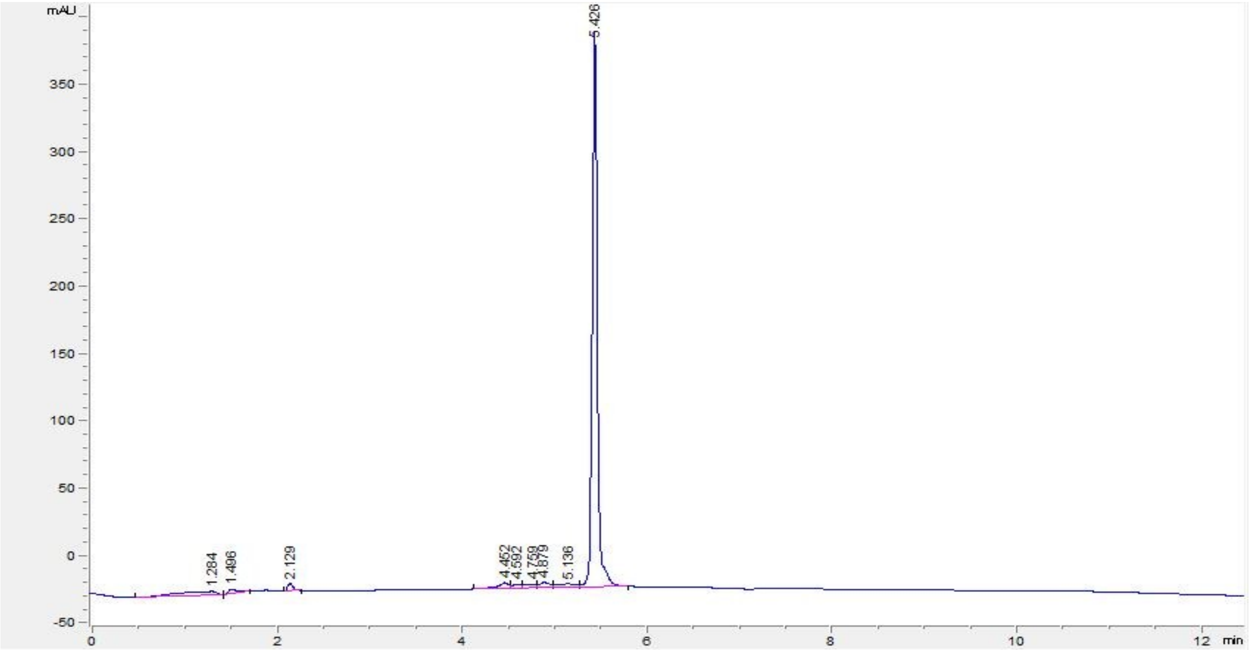


Figure S32a. *SWC of compound Cy5.5 4S,* ***(1)*** *H_2O stock solution (50 µM) used for all subsequent diluted solutions for photostability and spectral analysis.*

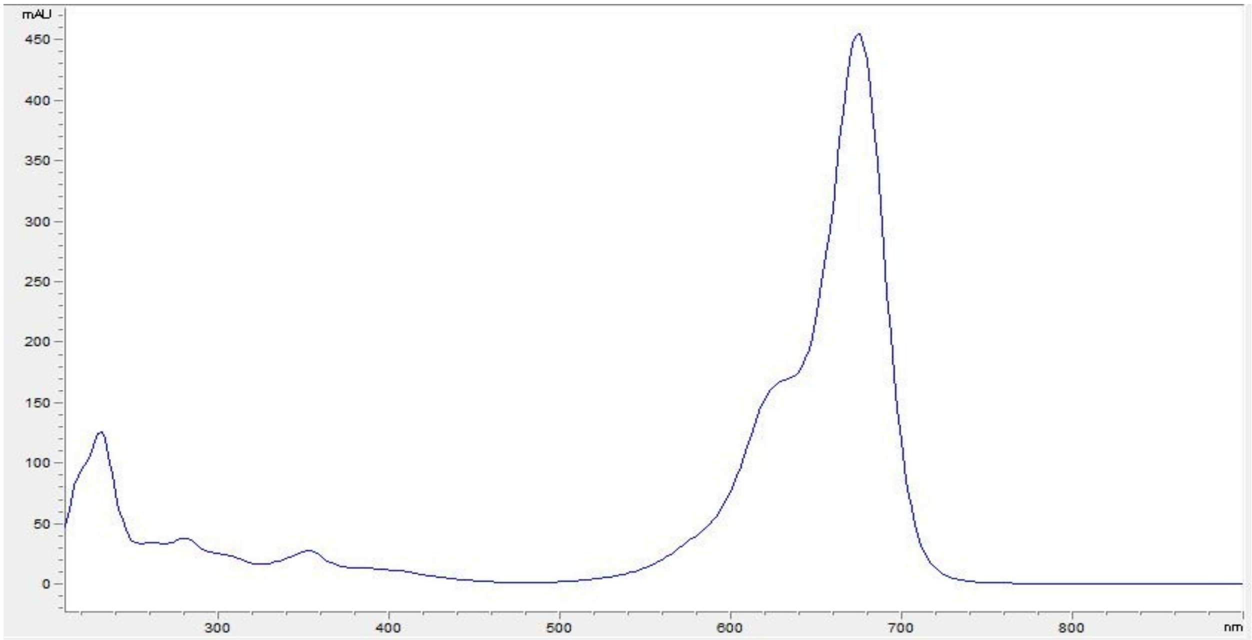


Figure S32b. *UV-Vis spectrum of compound Cy5.5 4S,* ***(1)*** *extracted from the peak at 5.426 min in Figure S32a.*

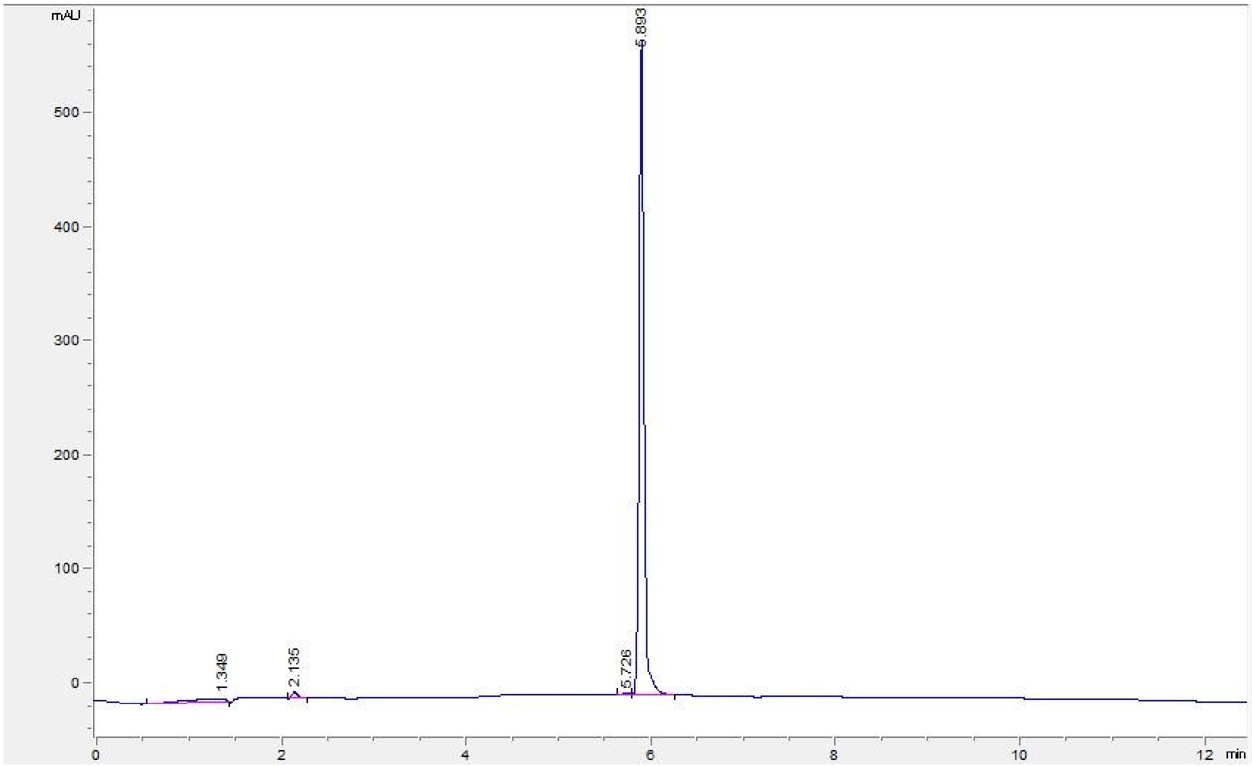


Figure S33a. *SWC of compound Cy5.5 3S,* ***(2)*** *H_2O stock solution (50 µM) used for all subsequent diluted solutions for photostability and spectral analysis.*

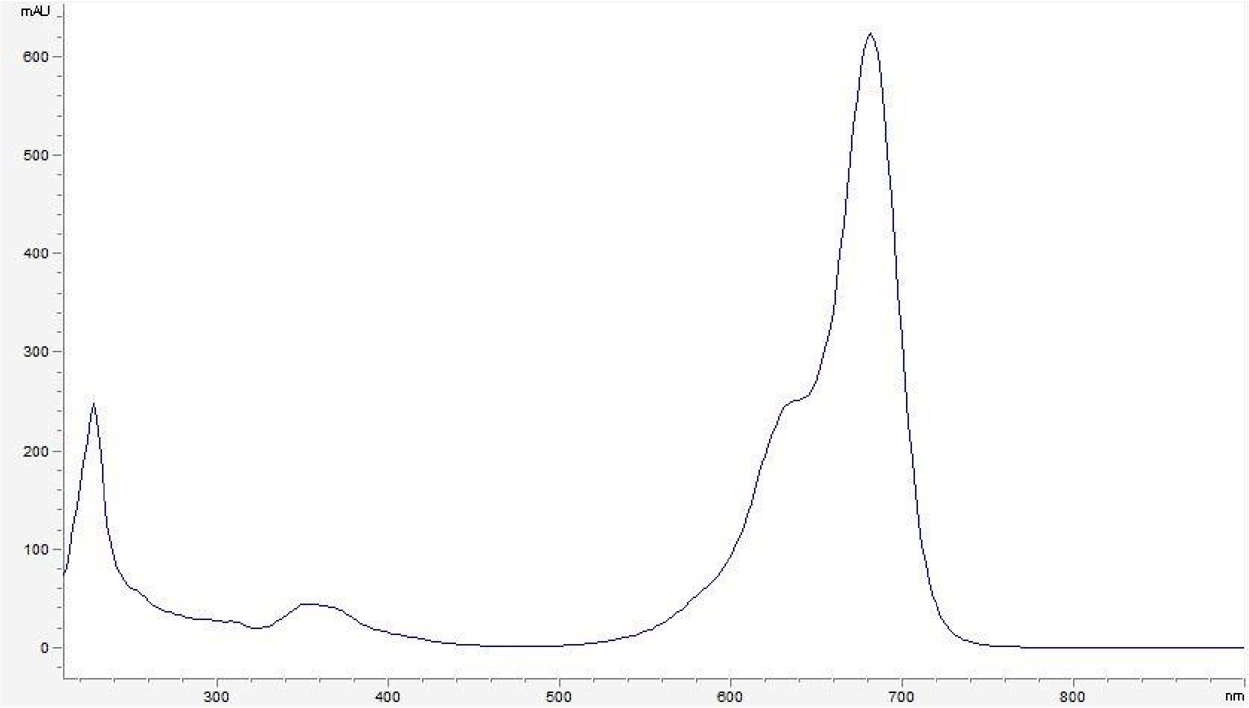


Figure S33b. *UV-Vis spectrum of compound Cy5.5 3S,* ***(2)*** *extracted from the peak at 5.893 min in Figure S33a.*

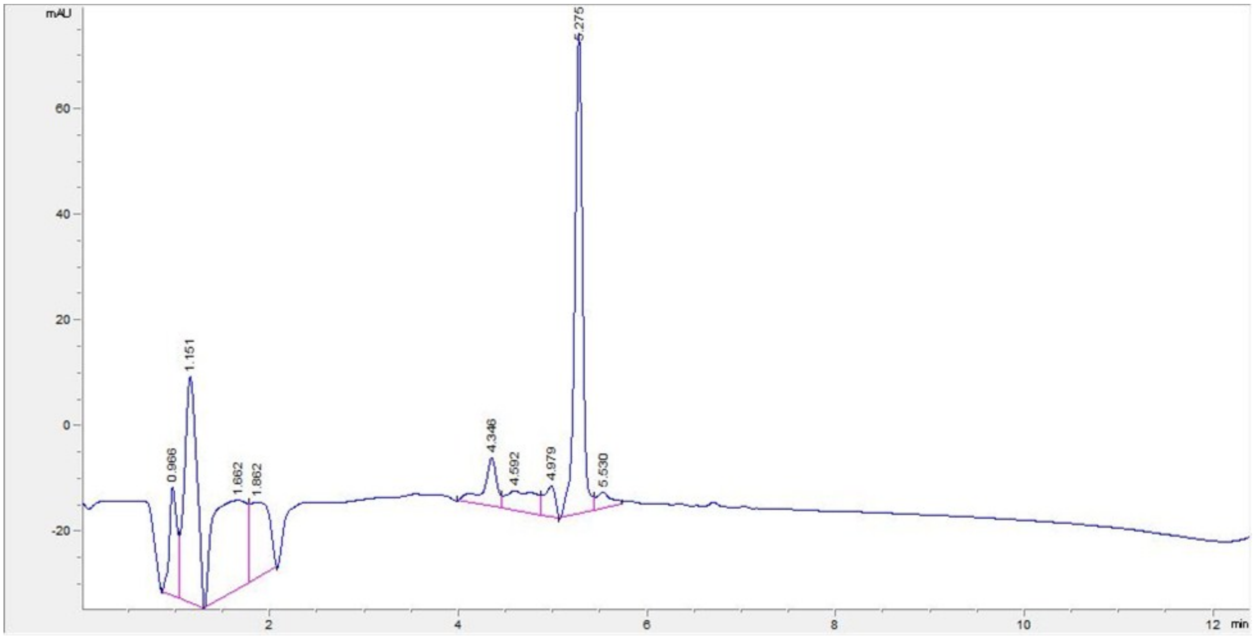


Figure S33c. *SWC of compound Cy5.5 3S,* ***(2)*** *(50 µM) left exposed to ambient laboratory light and temperature for 1 year.*

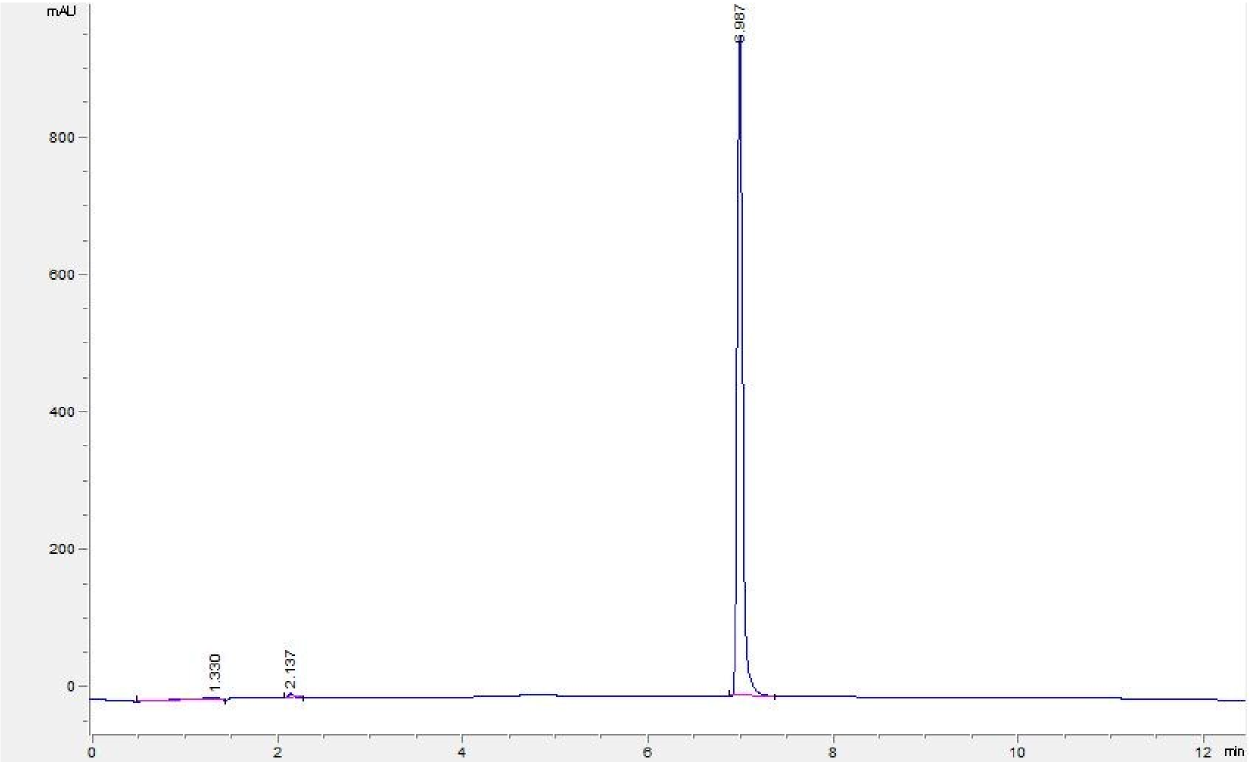


Figure S34a. *SWC of compound Cy5.5 2S,* ***(3)*** *H_2O stock solution (50 µM) used for all subsequent diluted solutions for photostability and spectral analysis.*

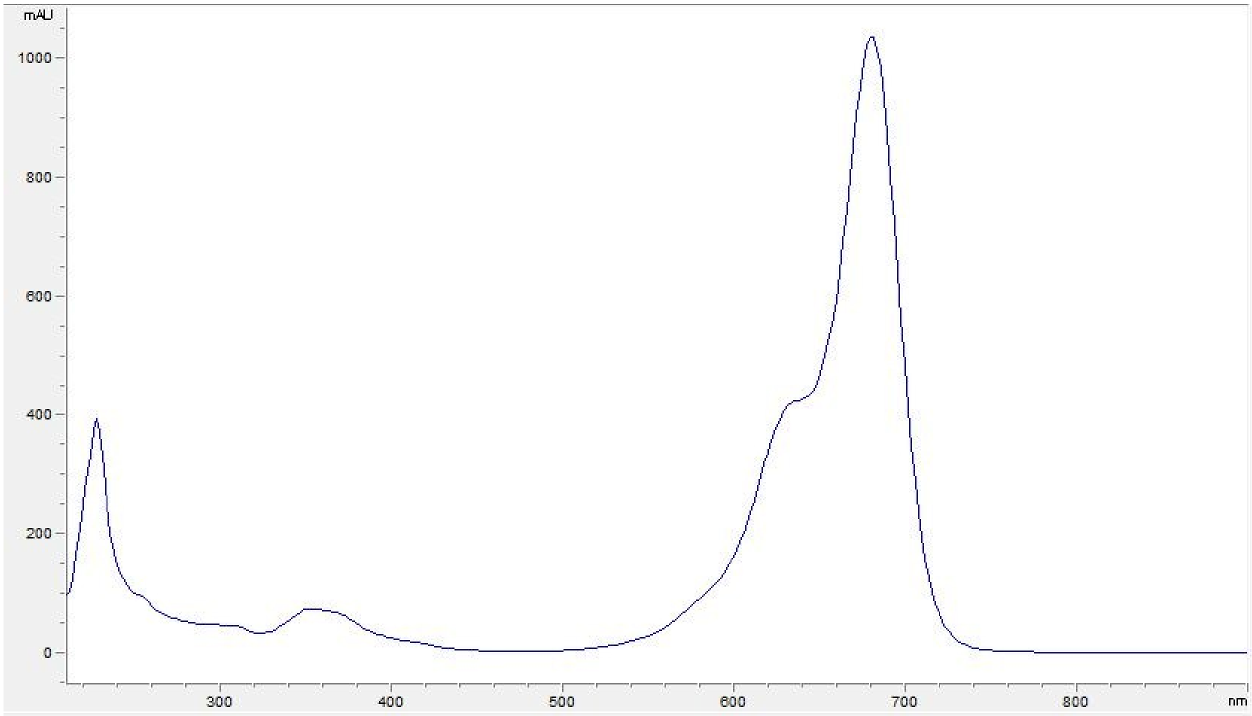


Figure S34b. *UV-Vis spectrum of compound Cy5.5 2S,* ***(3)*** *extracted from the peak at 6.987 min in Figure S34a.*

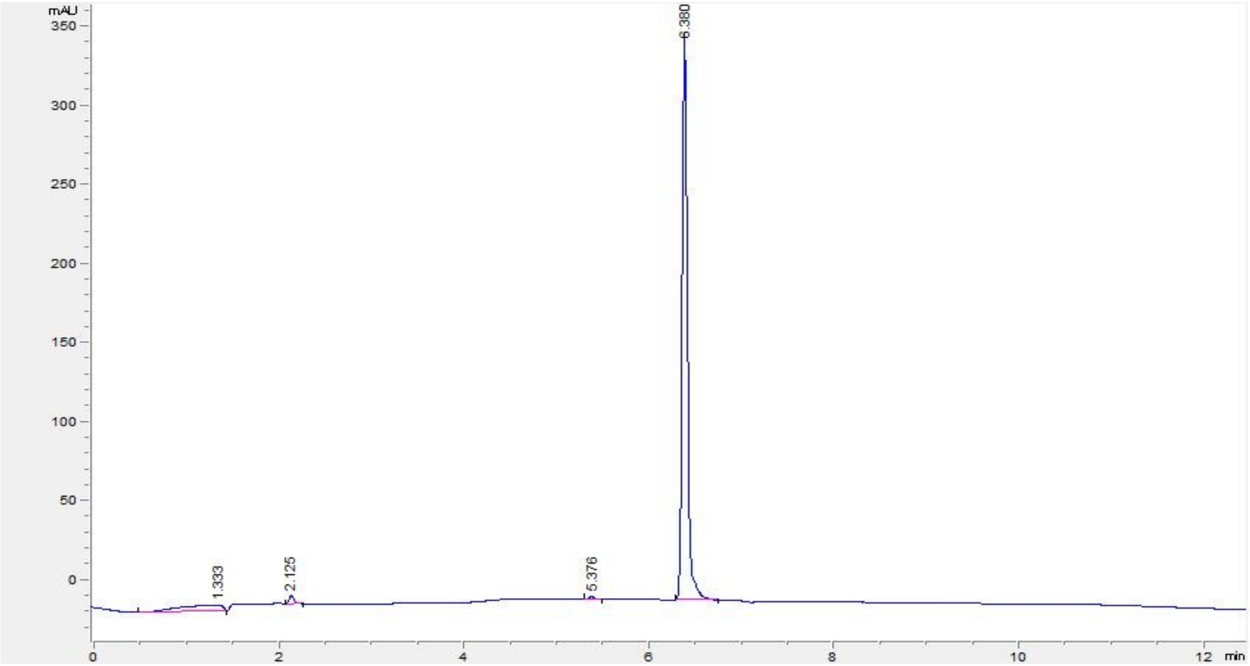


Figure S35a. *SWC of compound Cy5.5 ZW,* ***(4)*** *H_2O stock solution (50 µM) used for all subsequent diluted solutions for photostability and spectral analysis.*

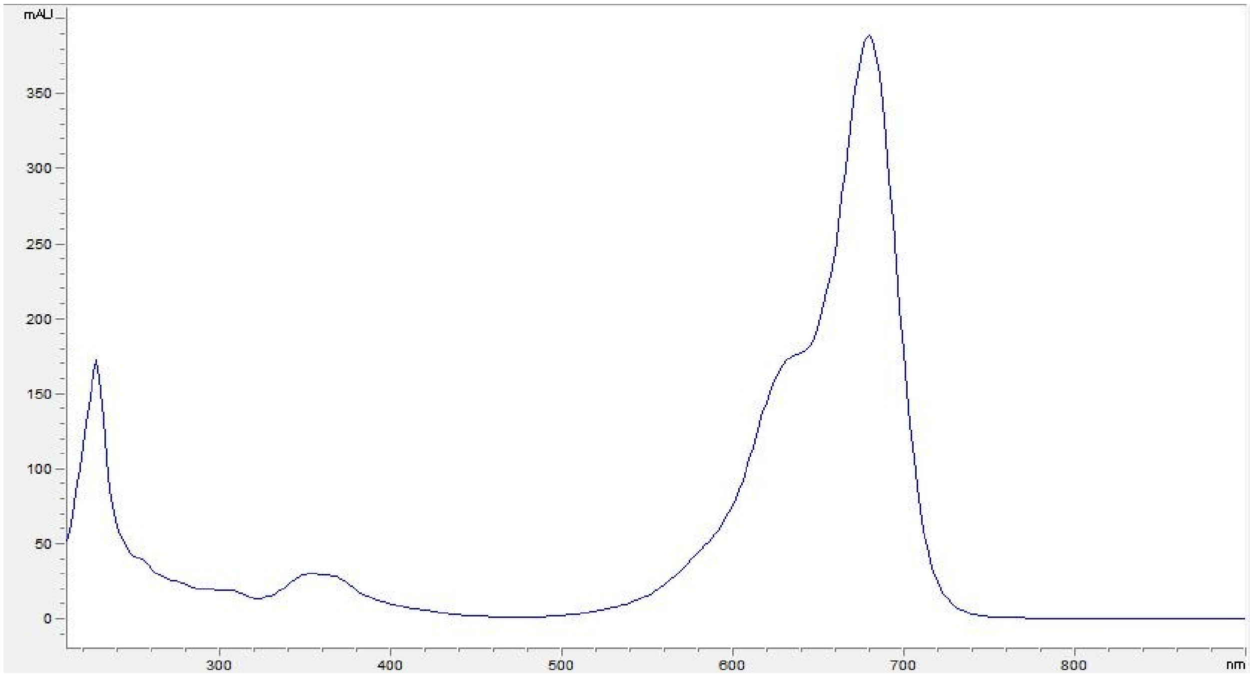


Figure S35b. *UV-Vis spectrum of compound Cy5.5 ZW,* ***(4)*** *extracted from the peak at 6.380 min in Figure S35a.*

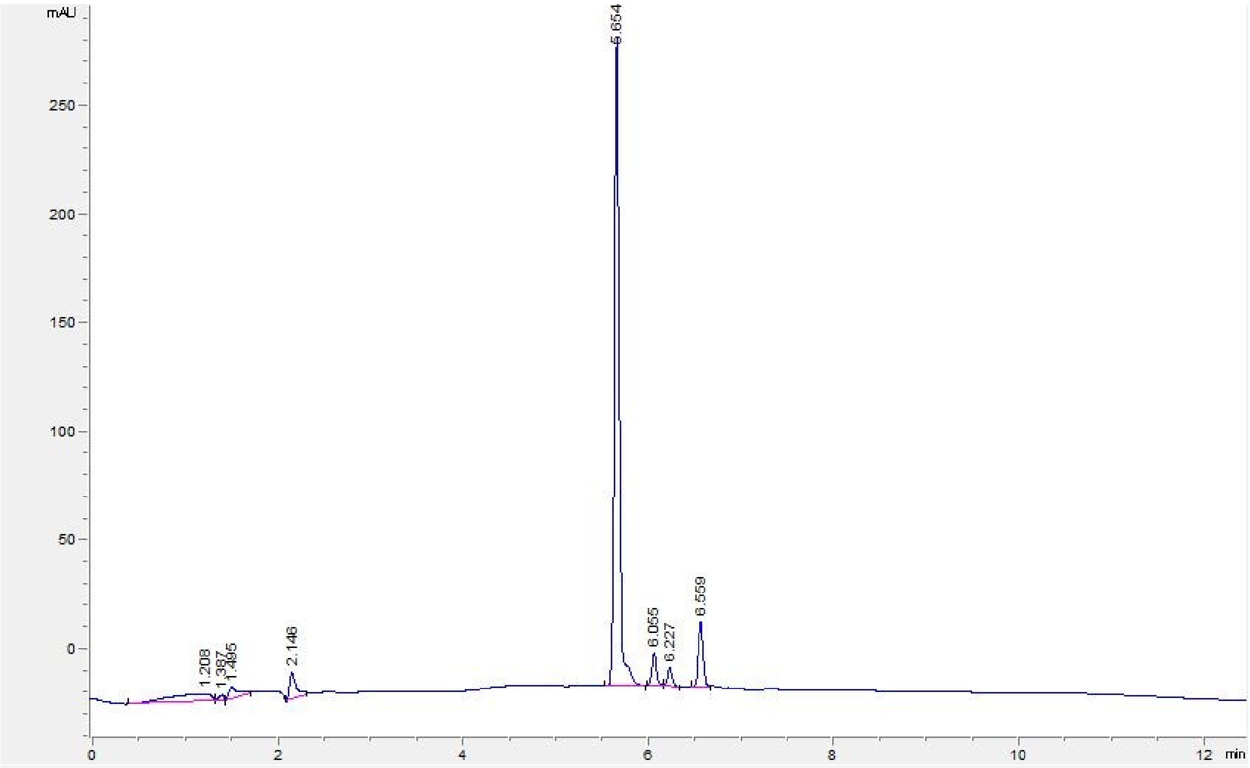


Figure S36a. *SWC of compound Alexa Fluor 680-succinimidyl ester,* ***(5)*** *H_2O stock solution (50 µM) used for all subsequent diluted solutions for photostability and spectral analysis.*

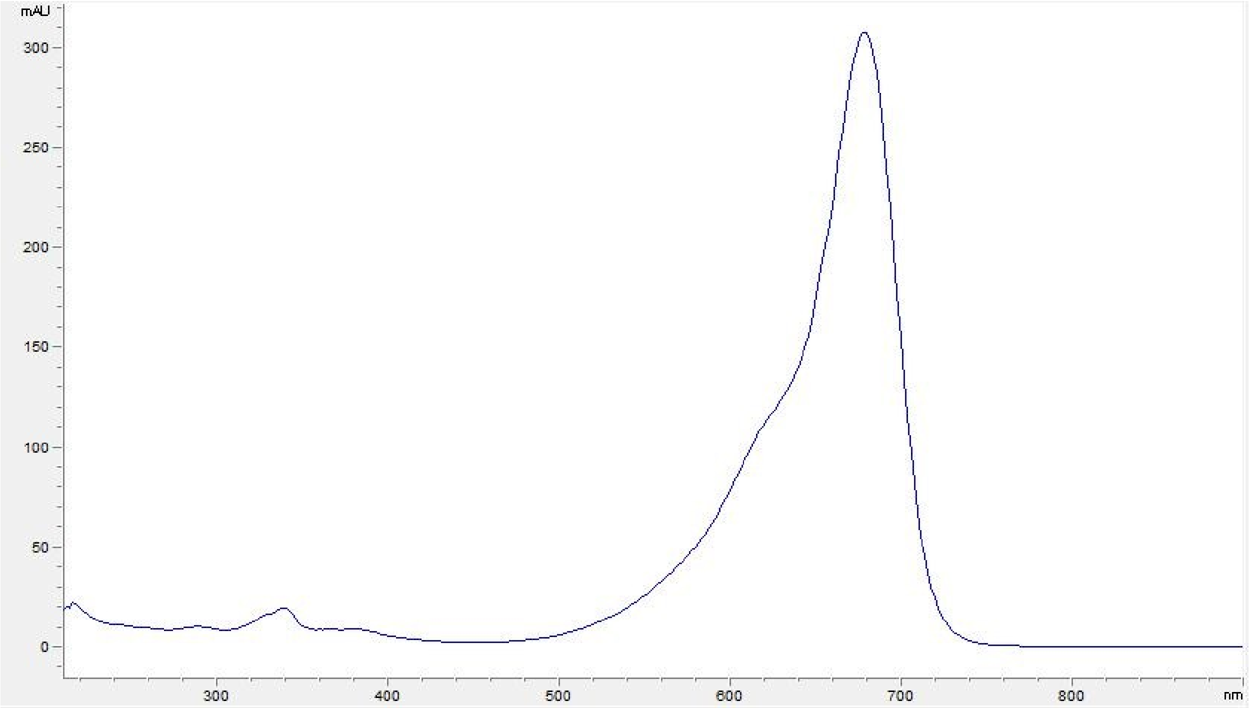


Figure S36b. *UV-Vis spectrum of compound Alexa Fluor 680-succinimidyl ester,* ***(5)*** *extracted from the peak at 5.654 min in Figure S36a.*

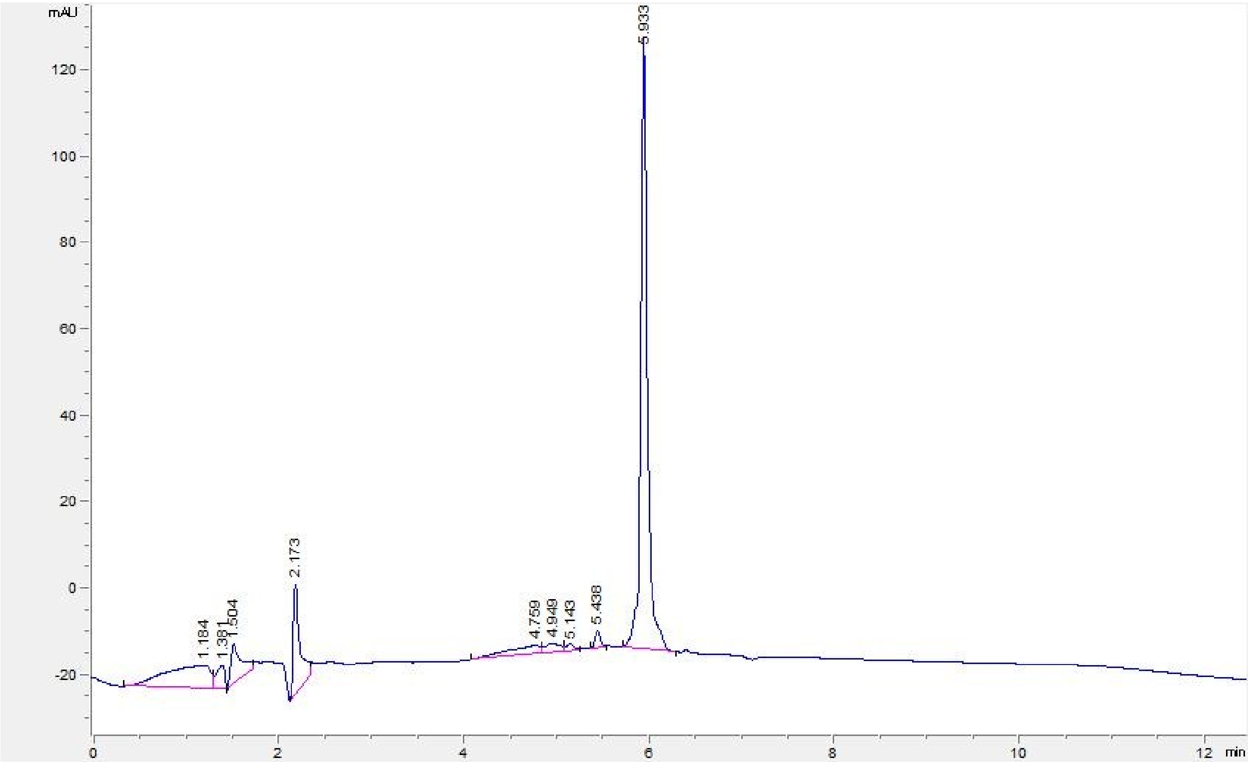


Figure S37a. *SWC of compound Cy5.5 4S-c(RGDyK),* ***(6)*** *H_2O stock solution (50 µM) used for all subsequent diluted solutions for photostability and spectral analysis.*

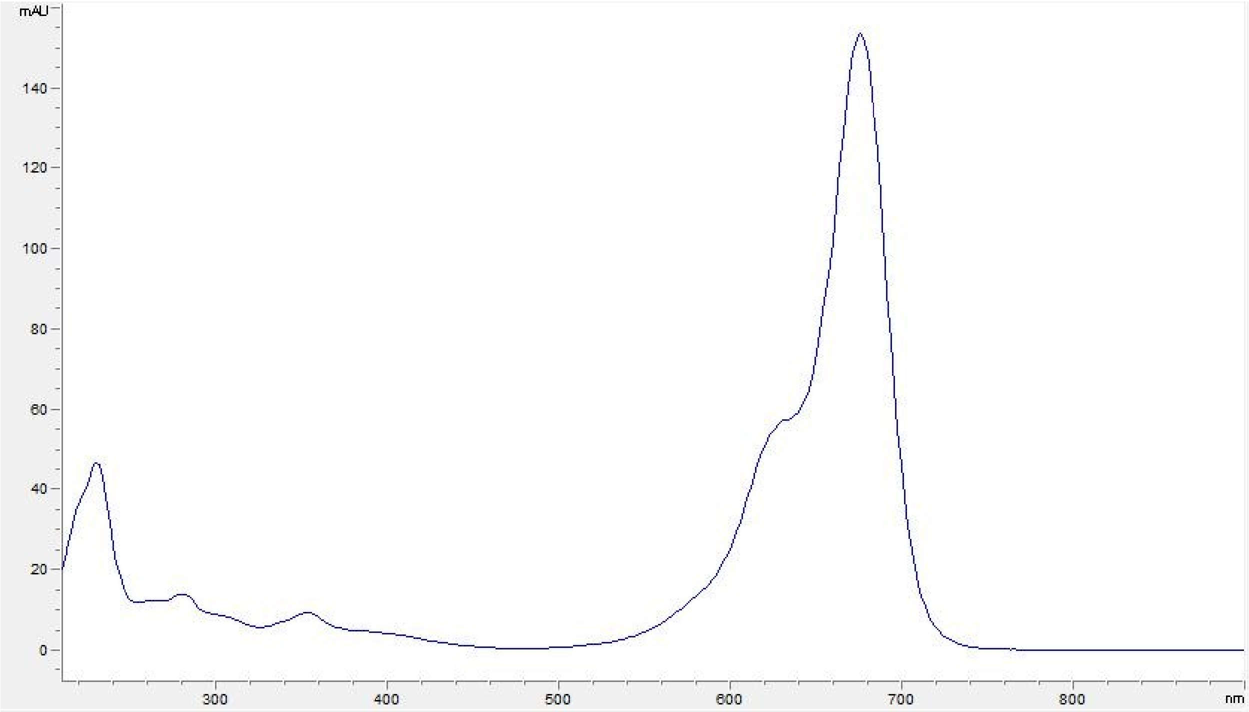


Figure S37b. *UV-Vis spectrum of compound Cy5.5 4S-c(RGDyK),* ***(6)*** *extracted from the peak at 5.933 min in Figure S37a.*

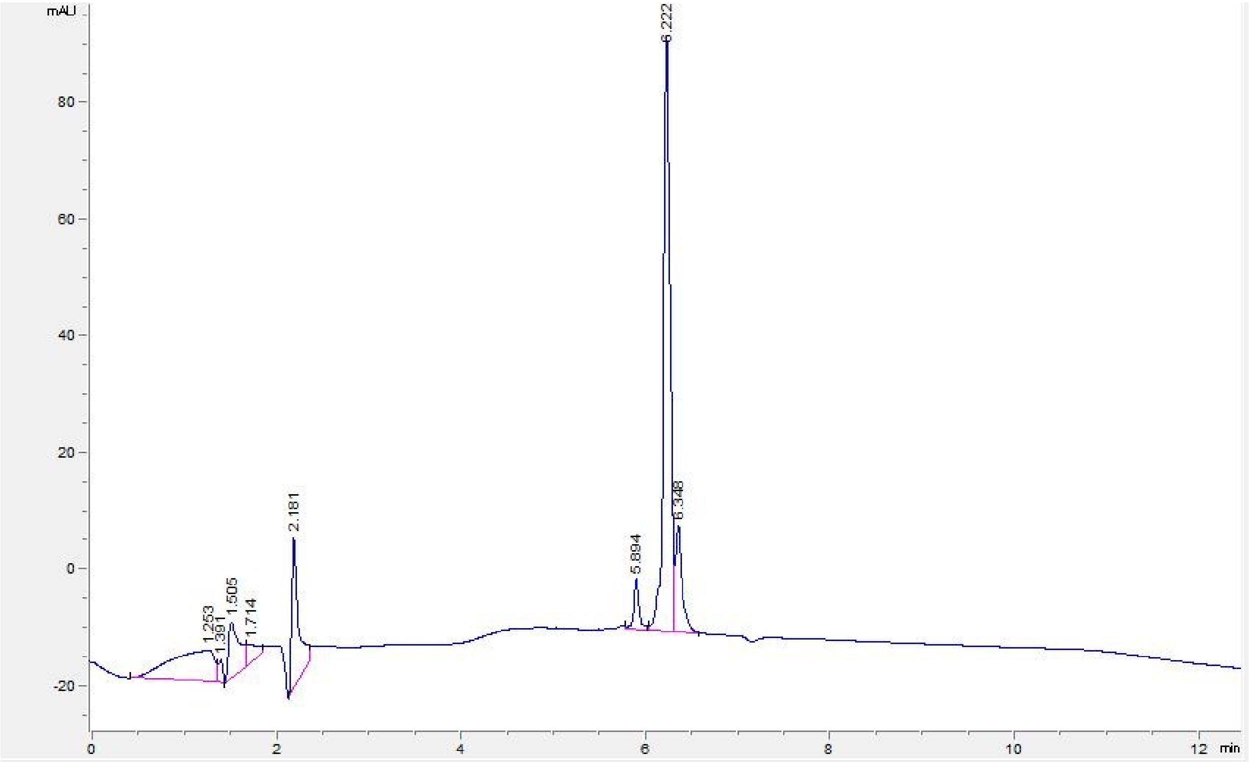


Figure S38a. *SWC of compound Cy5.5 3S-c(RGDyK),* ***(7)*** *H_2O stock solution (50 µM) used for all subsequent diluted solutions for photostability and spectral analysis.*

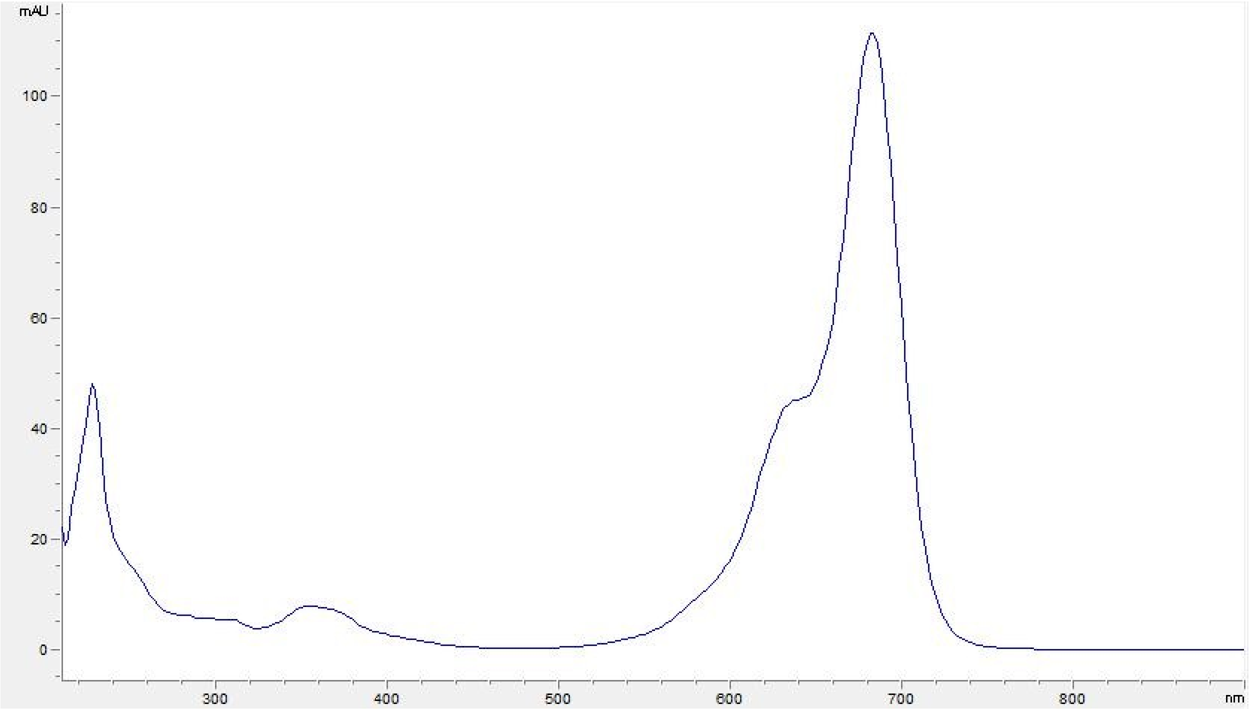


Figure S38b. *UV-Vis spectrum of compound Cy5.5 3S-c(RGDyK),* ***(7)*** *extracted from the peak at 6.222 min in Figure S38a.*

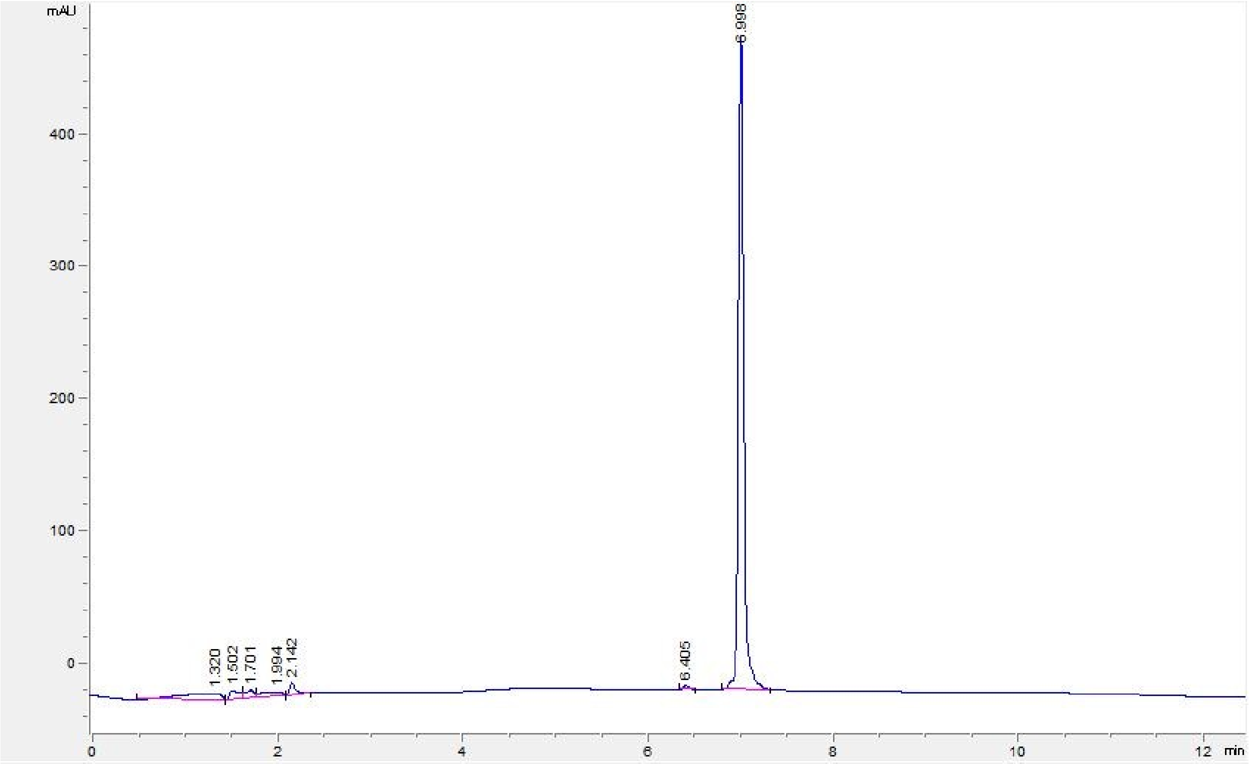


Figure S39a. *SWC of compound Cy5.5 2S-c(RGDyK),* ***(8)*** *H_2O stock solution (50 µM) used for all subsequent diluted solutions for photostability and spectral analysis.*

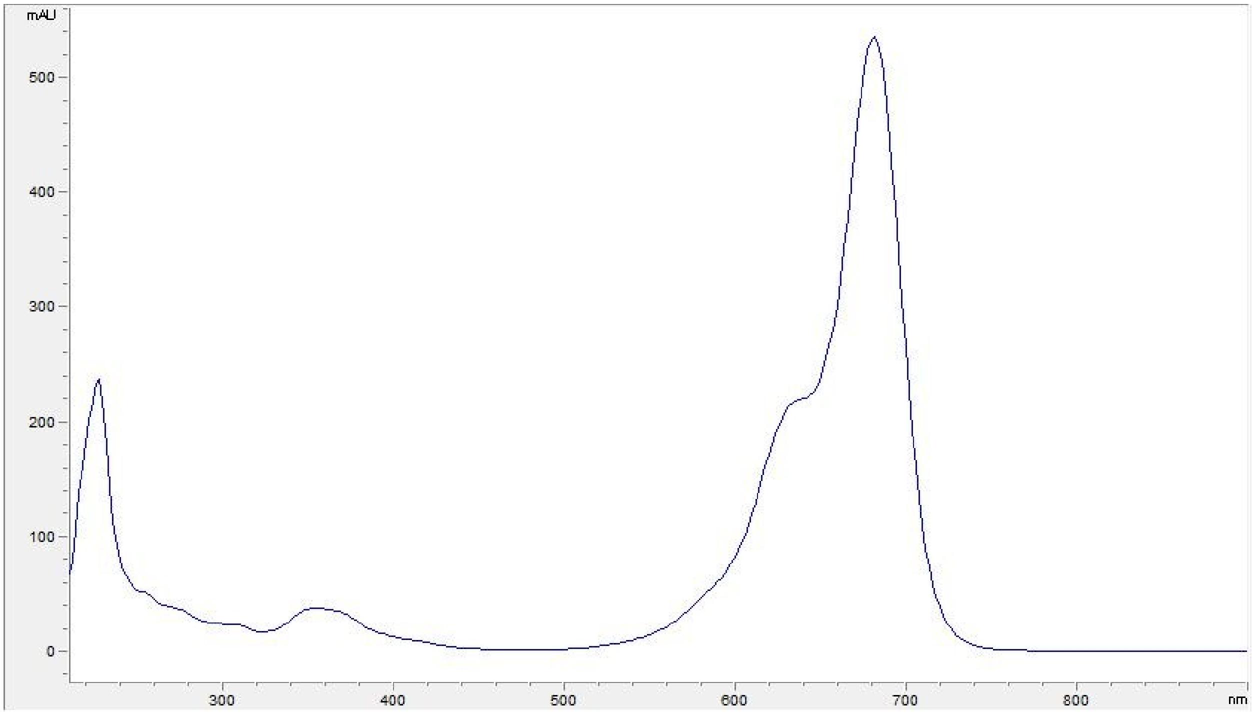


Figure S39b. *UV-Vis spectrum of compound Cy5.5 2S-c(RGDyK),* ***(8)*** *extracted from the peak at 6.998 min in Figure S39a.*

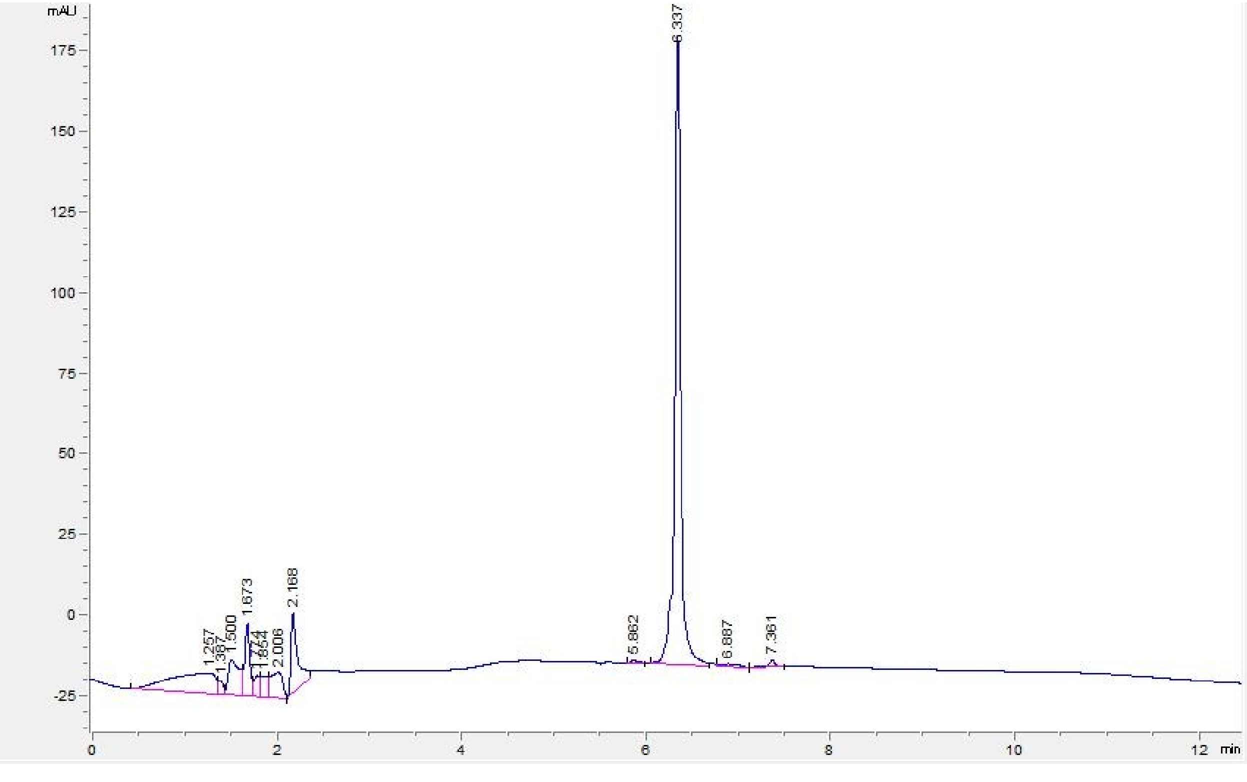


Figure S40a. *SWC of compound Cy5.5 ZW-c(RGDyK),* ***(9)*** *H_2O stock solution (50 µM) used for all subsequent diluted solutions for photostability and spectral analysis.*

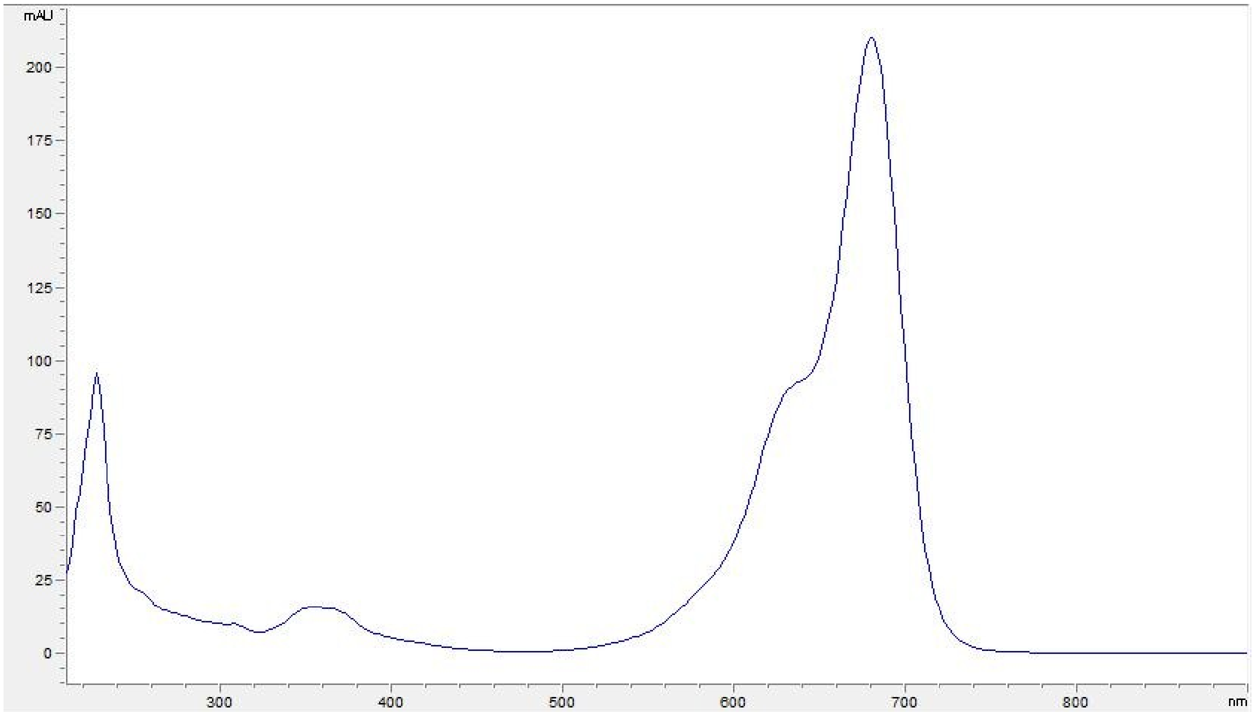


Figure S40b. *UV-Vis spectrum of compound Cy5.5 ZW-c(RGDyK),* ***(9)*** *extracted from the peak at 6.337 min in Figure S40a.*

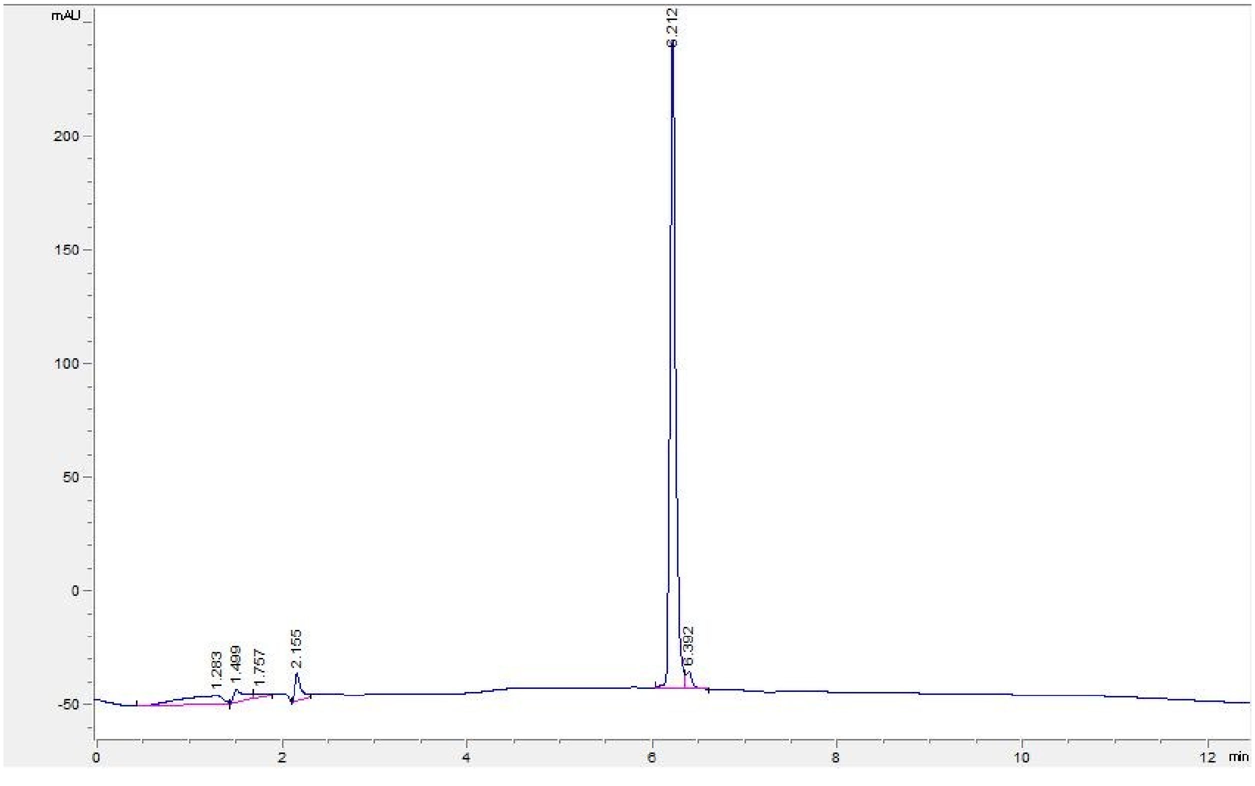


Figure S41a. *SWC of compound Alexa Fluor 680-c(RGDyK), (**10**) H_2O stock solution (50 µM) used for all subsequent diluted solutions for photostability and spectral analysis.*

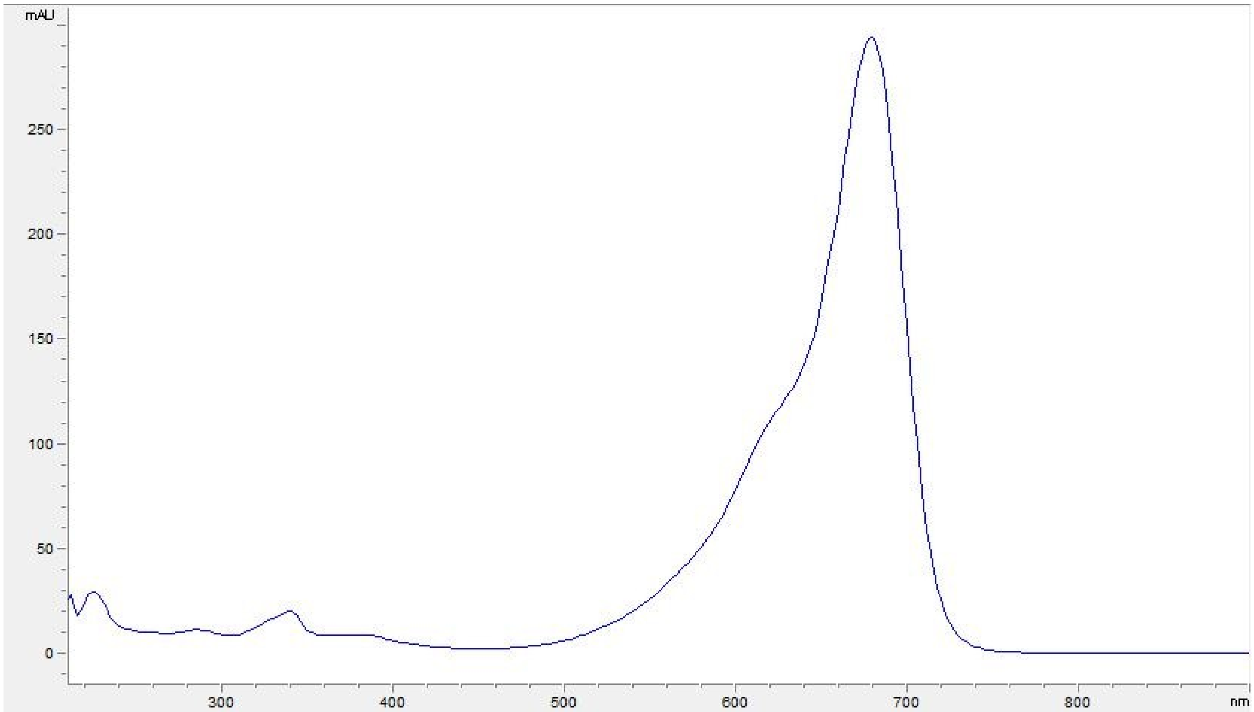


Figure S41b. *UV-Vis spectrum of compound Alexa Fluor 680-c(RGDyK),* ***(10)*** *extracted from the peak at 6.212 min in Figure S41a.*

MS Data HRMS Data:

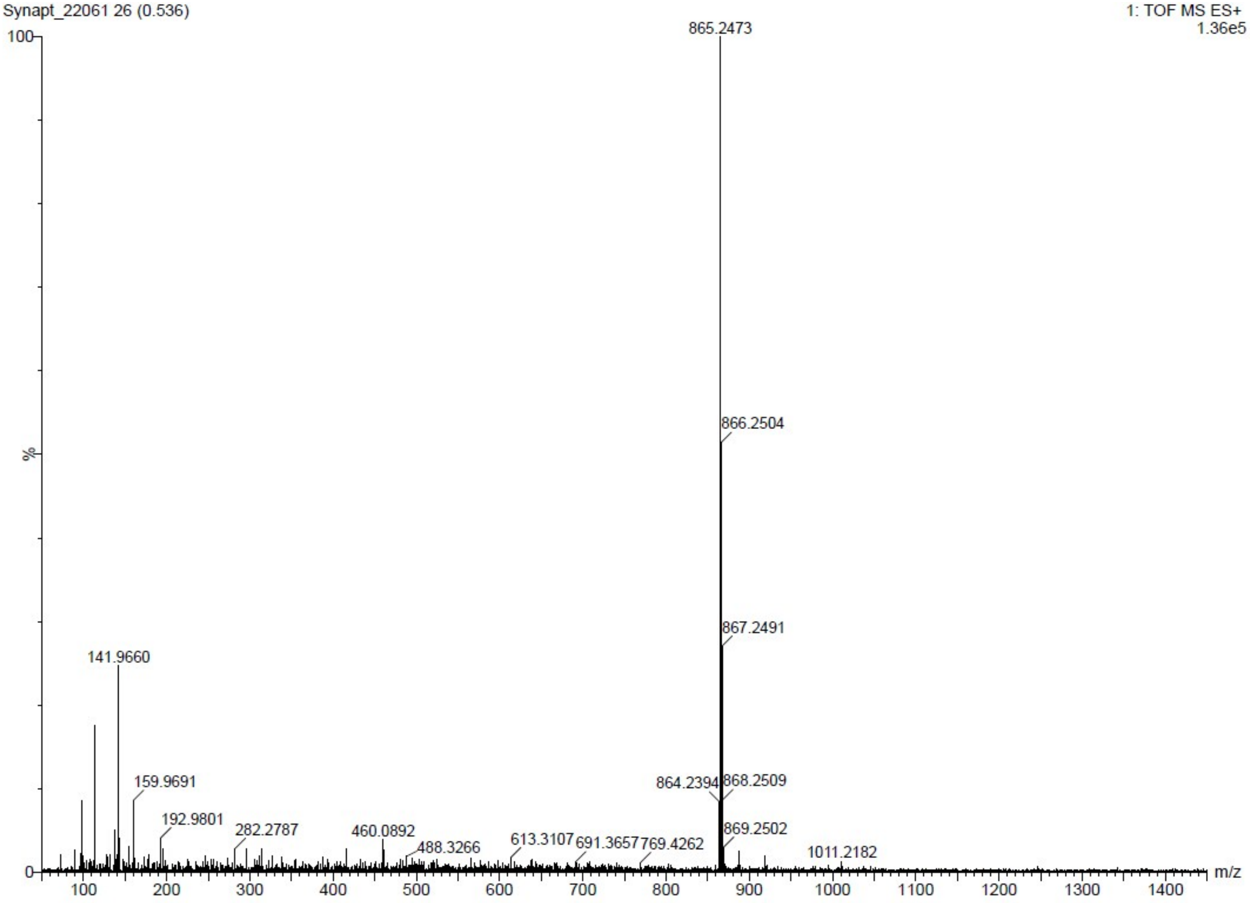


Figure S42. *Positive ion HR mass spectrum of compound Cy5.5 3S,* ***(2).***

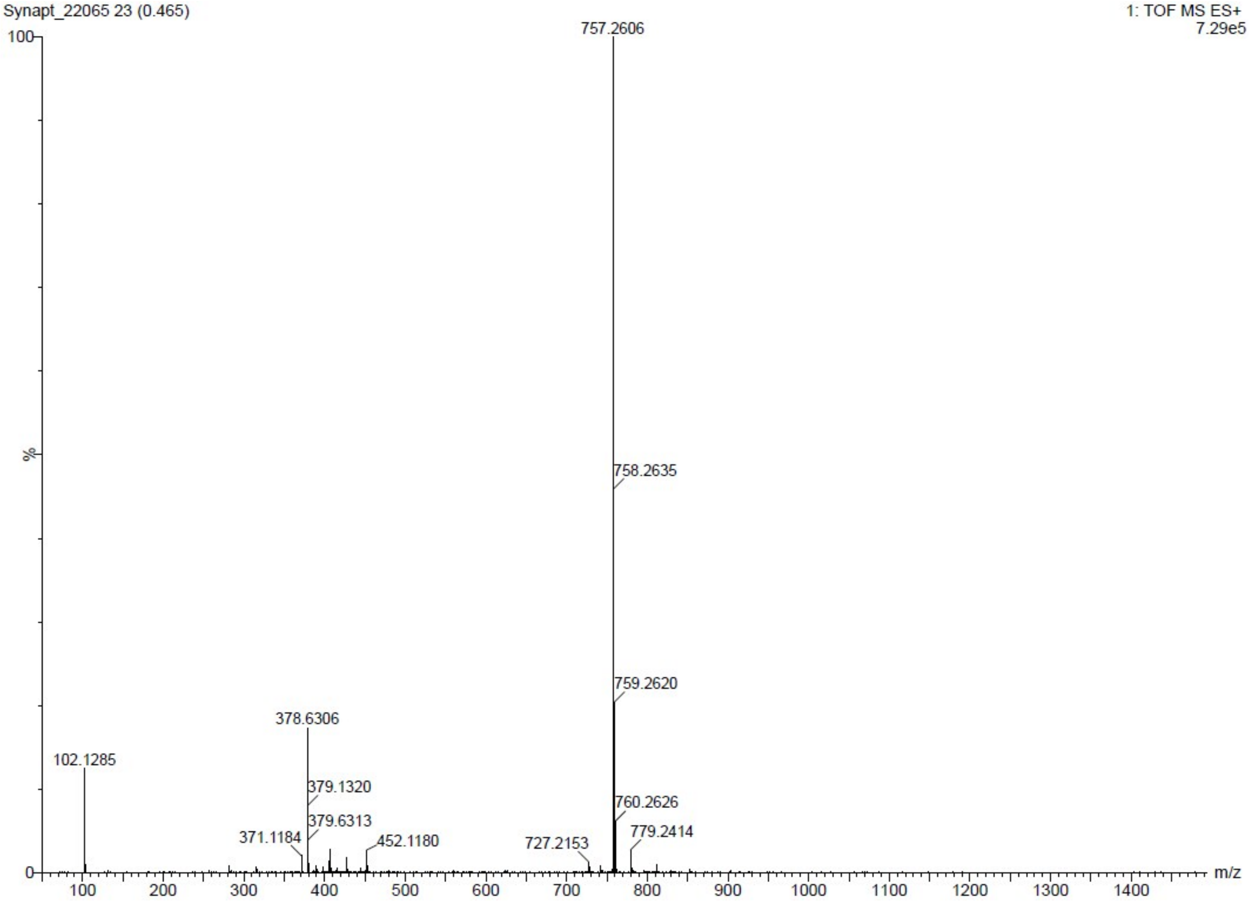


Figure S43. *Positive ion HR mass spectrum of compound Cy5.5 2S,* ***(3).***

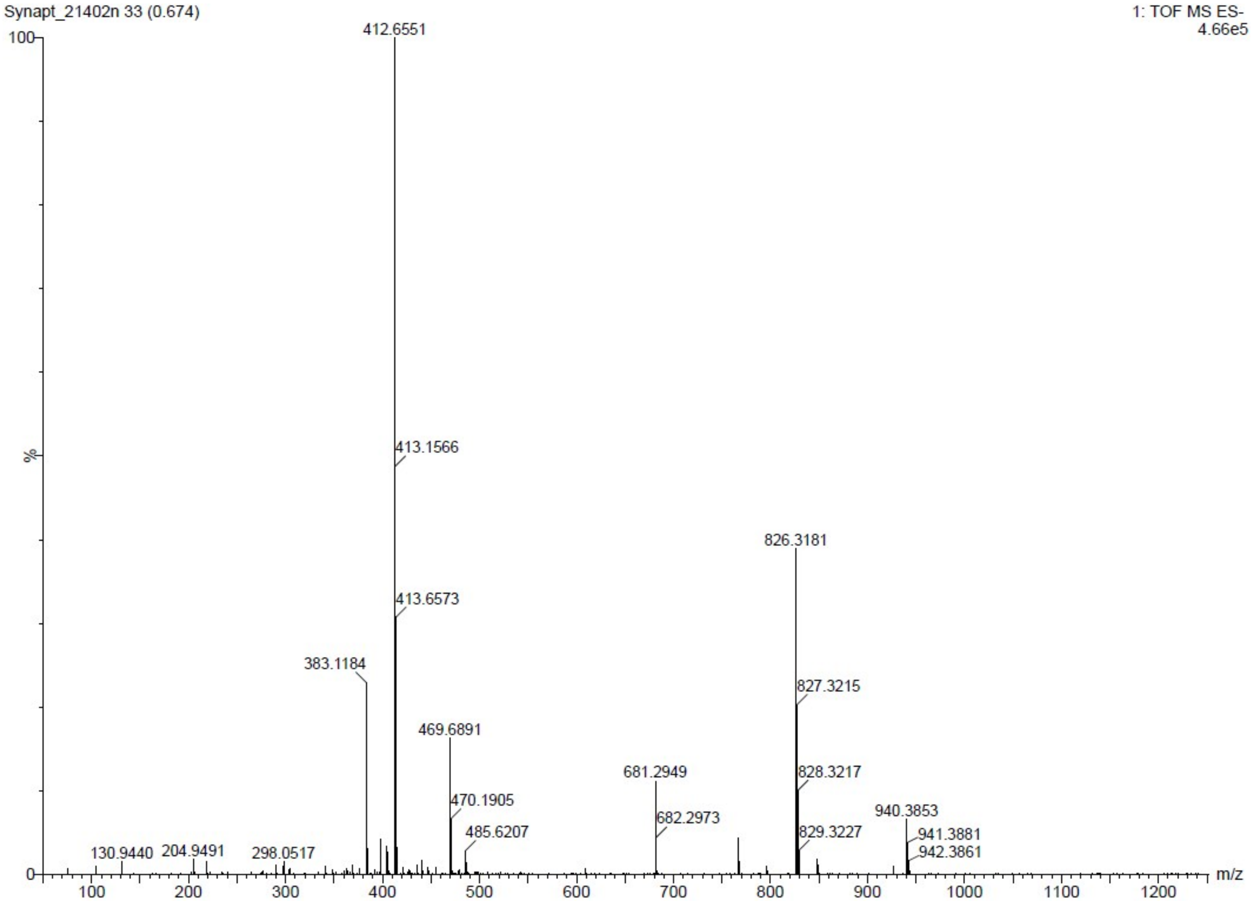


Figure S44. *Negative ion HR mass spectrum of compound Cy5.5 ZW,* ***(4).***

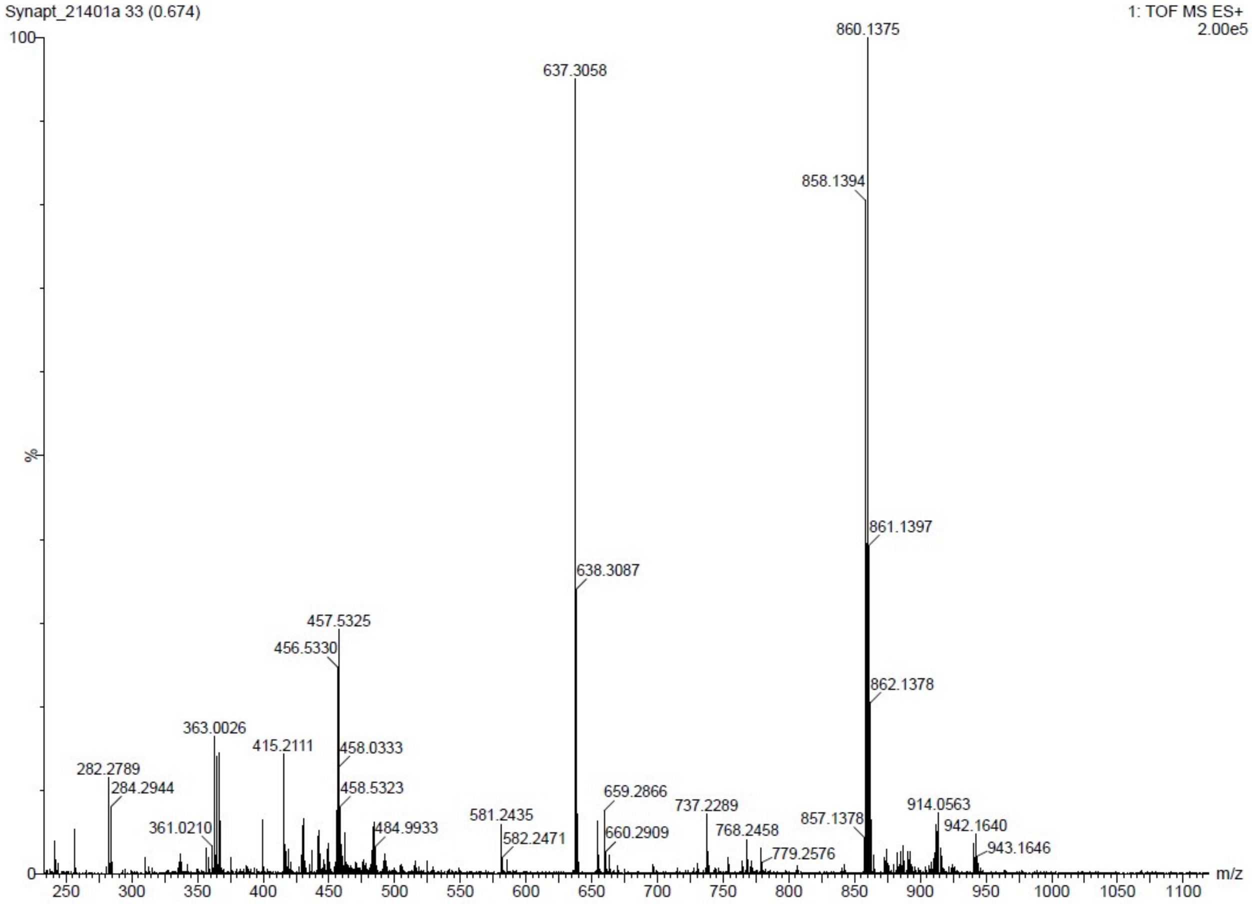


Figure S45a. *Positive ion HR mass spectrum of compound Alexa Fluor 680,* ***(5)****.*

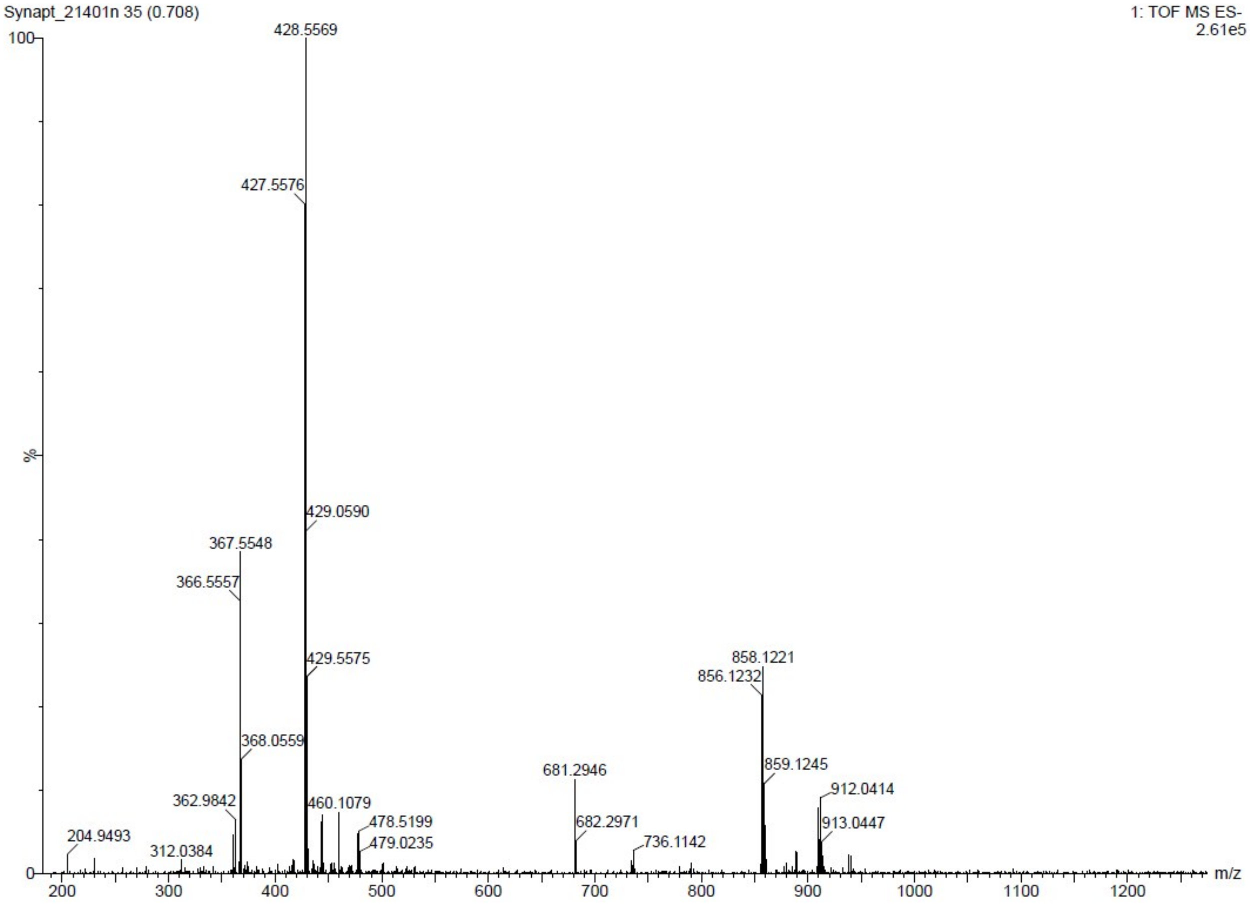


Figure S45b. *Negative ion HR mass spectrum of compound Alexa Fluor 680,* ***(5).***

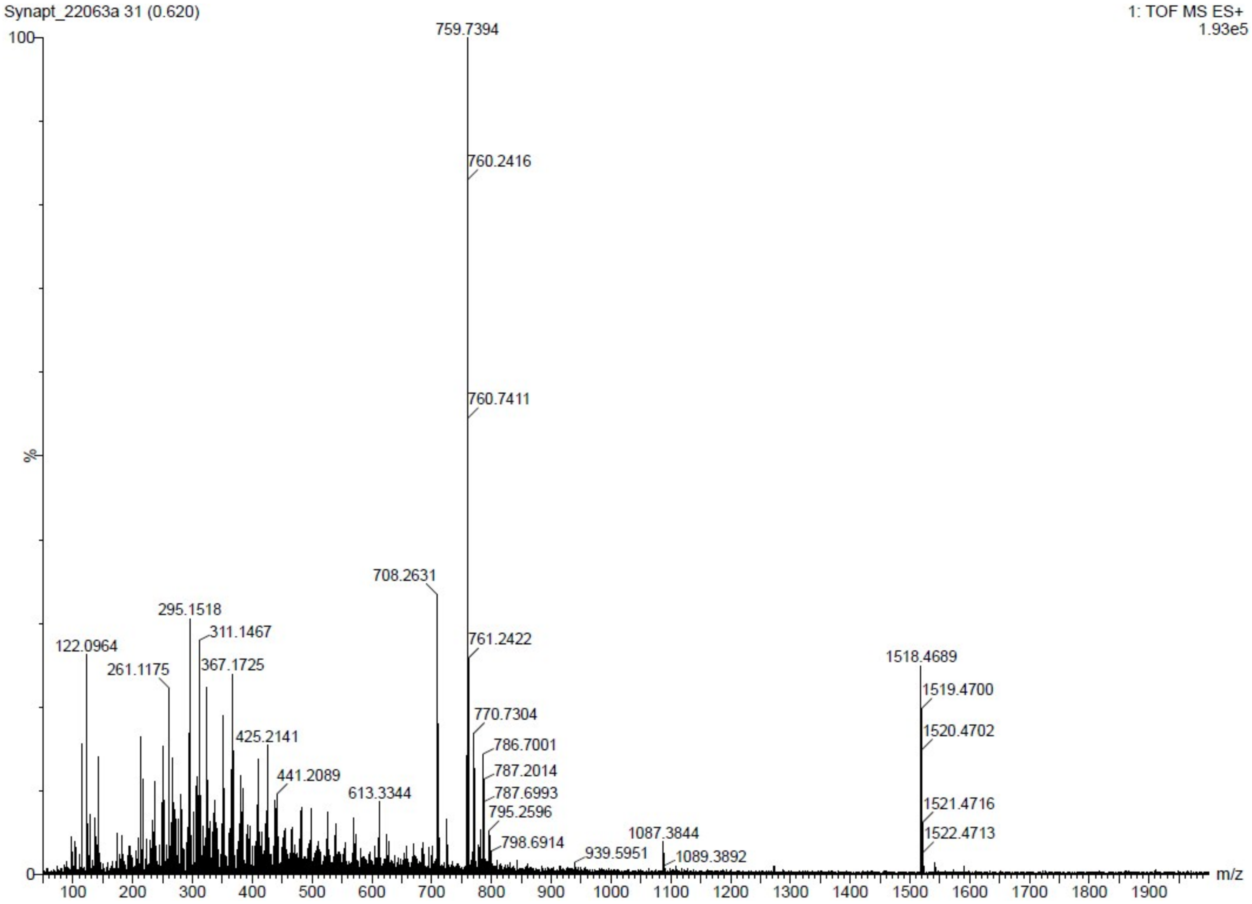


Figure S46. *Positive ion HR mass spectrum of compound Cy5.5 4S-c(RGDyK),* ***(6)***.

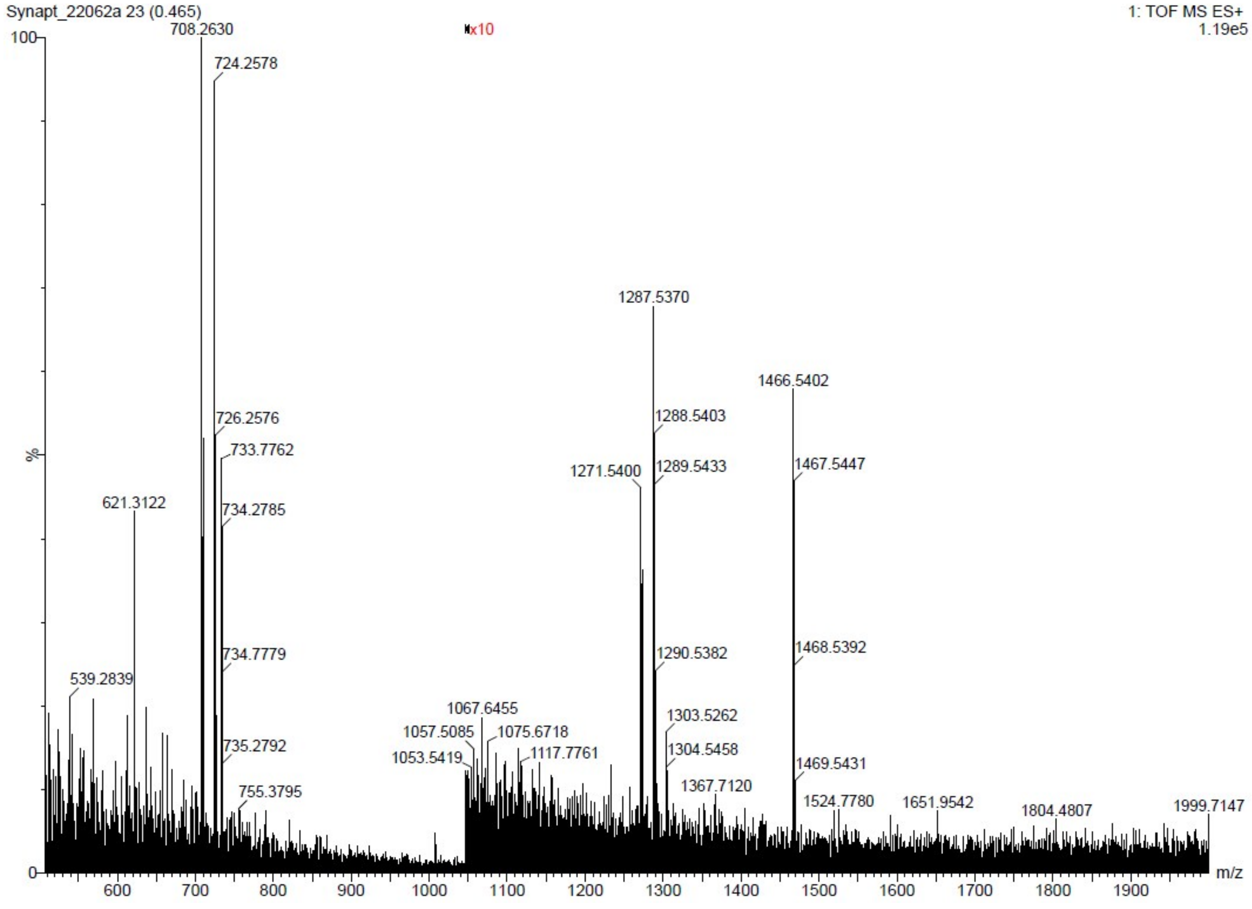


Figure S47. *Positive ion HR mass spectrum of compound Cy5.5 3S-c(RGDyK),* ***(7).***

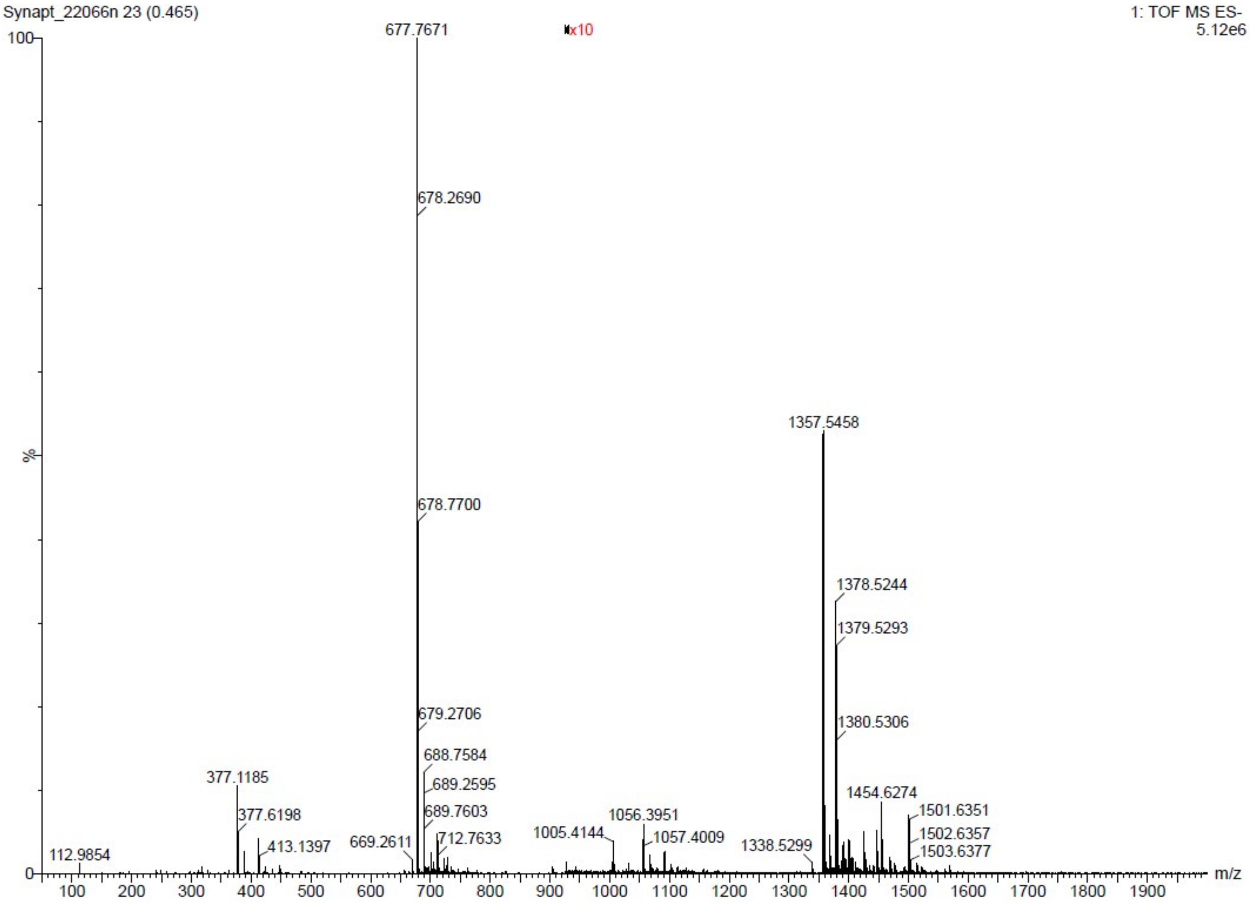


Figure S48. *Negative ion HR mass spectrum of compound Cy5.5 2S-c(RGDyK),* ***(8).***

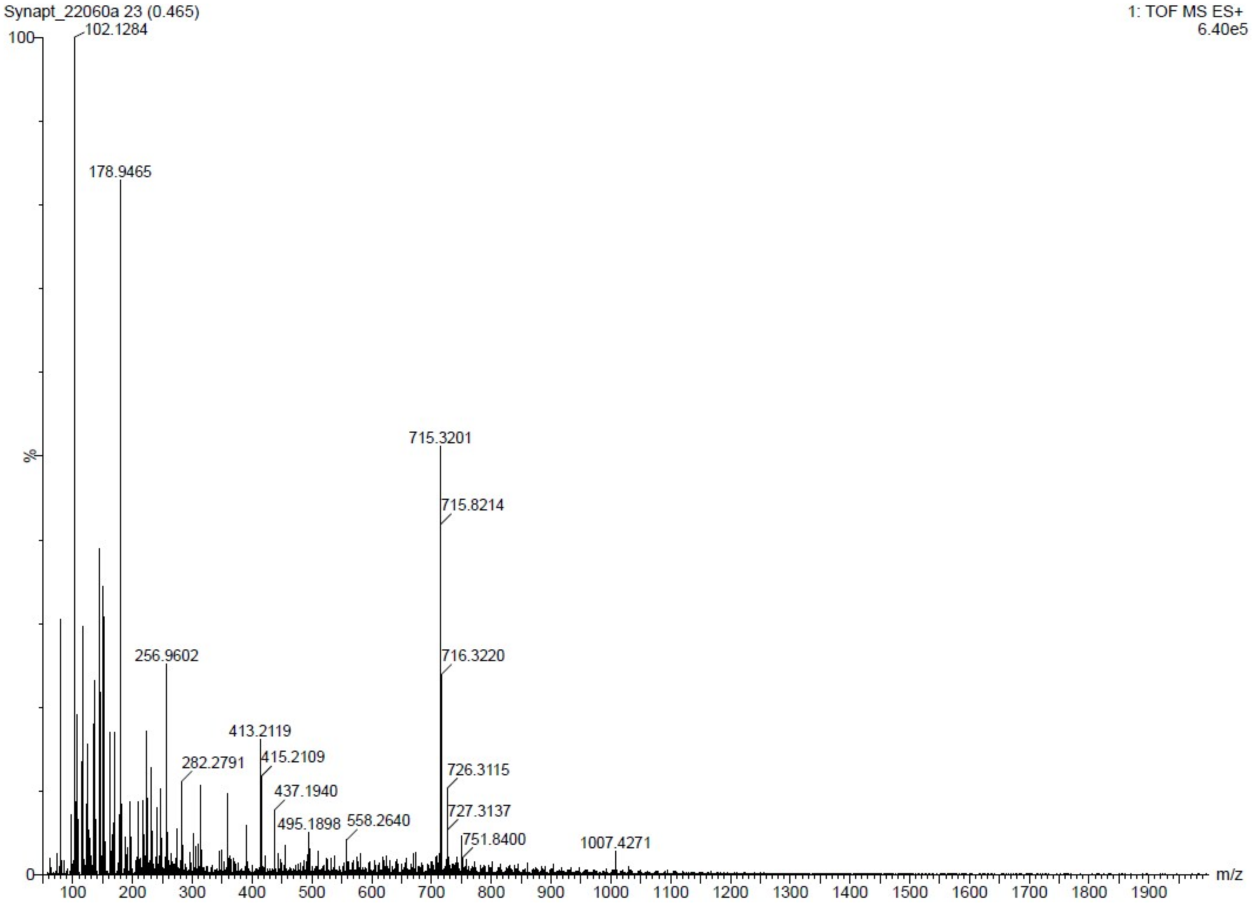


Figure S49. *Positive ion HR mass spectrum of compound Cy5.5 ZW-c(RGDyK),* ***(9).***

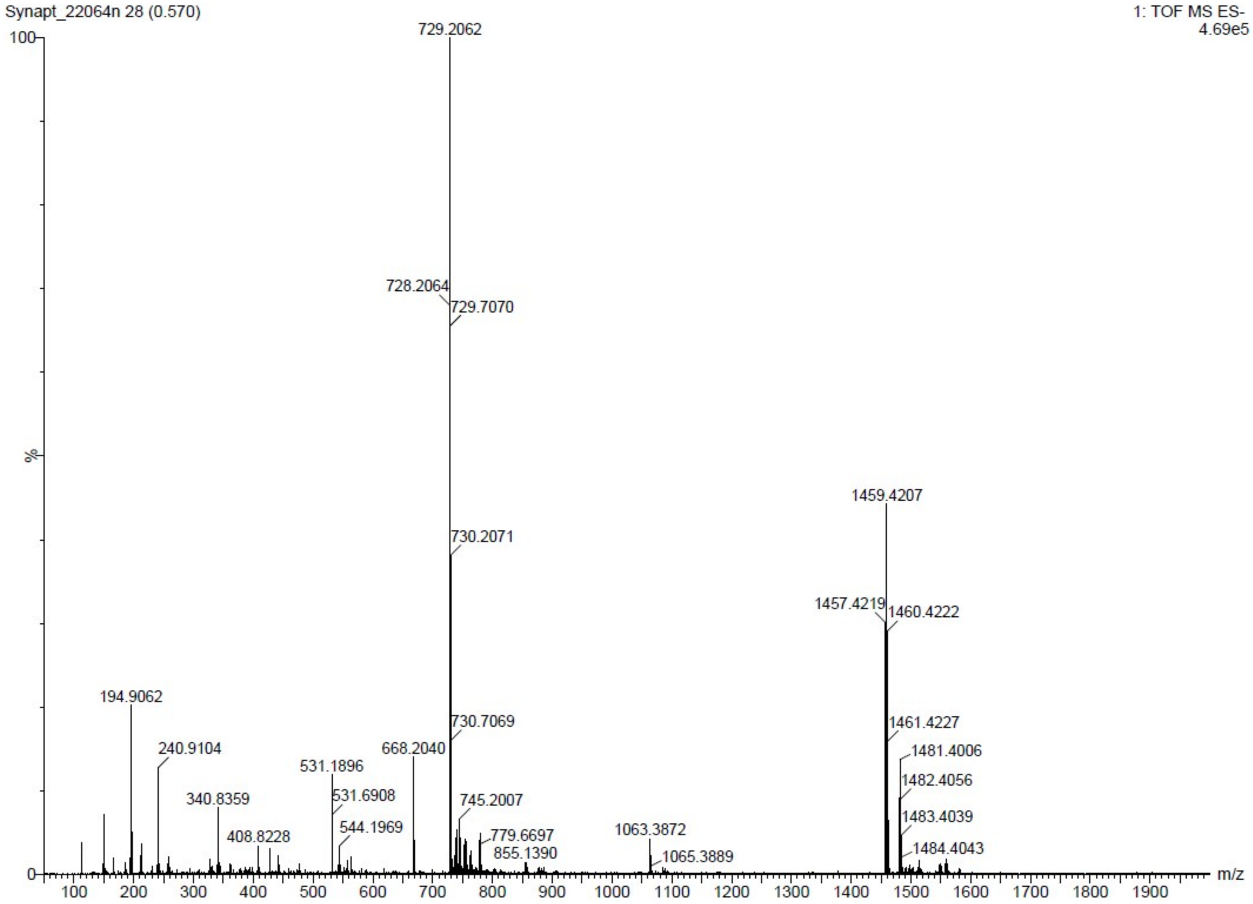


Figure S50. *Negative ion HR mass spectrum of compound Alexa Fluor 680-c(RGDyK),* ***(10).***

NMR Spectra:

*Impurity, solvent, and salt (TEA) peaks are not integrated or noted with a chemical shift label.

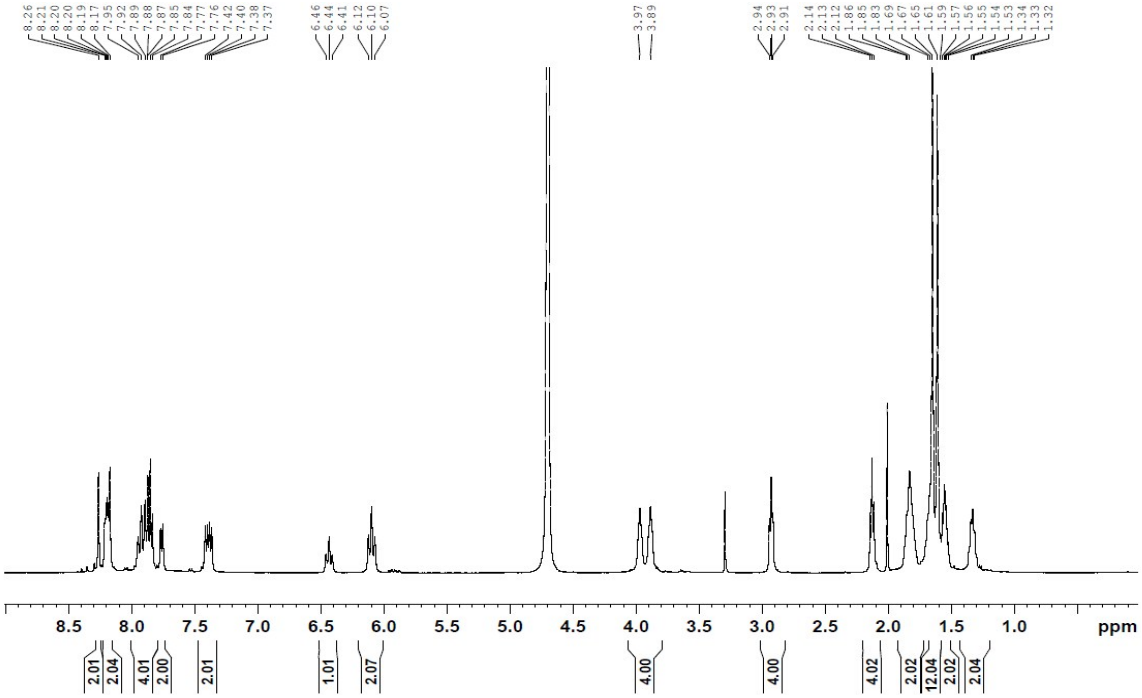


Figure S51a. *^{1}H spectrum of Cy5.5 3S,* ***(2)*** *in D_2O.*

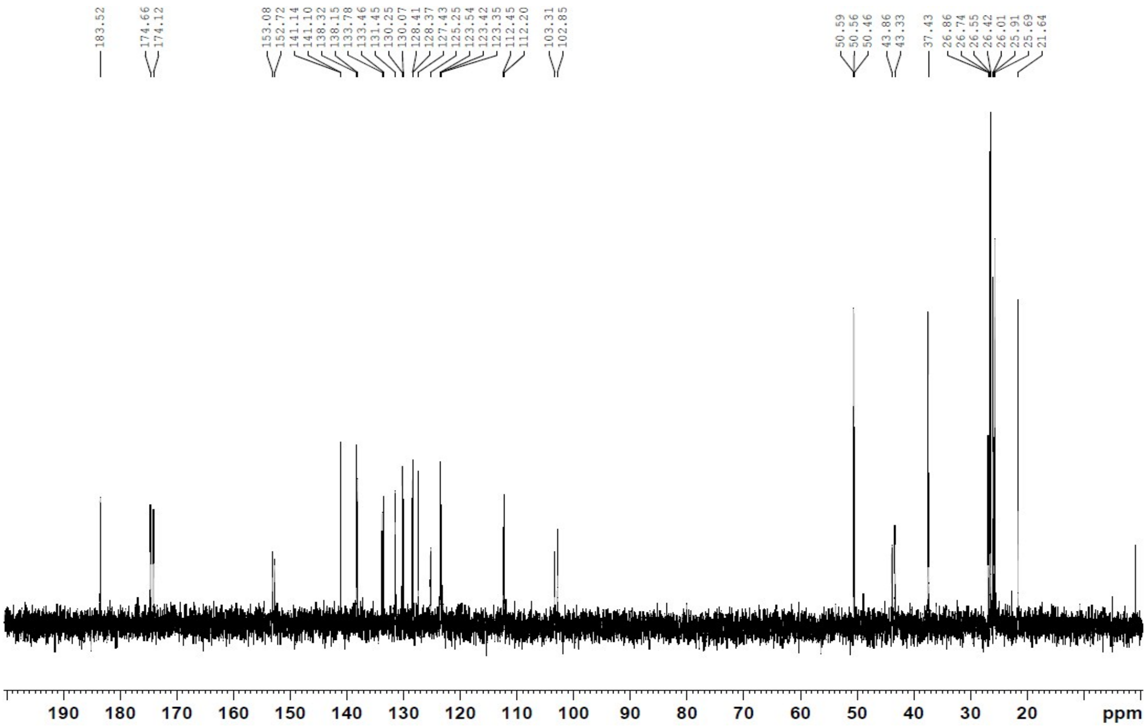
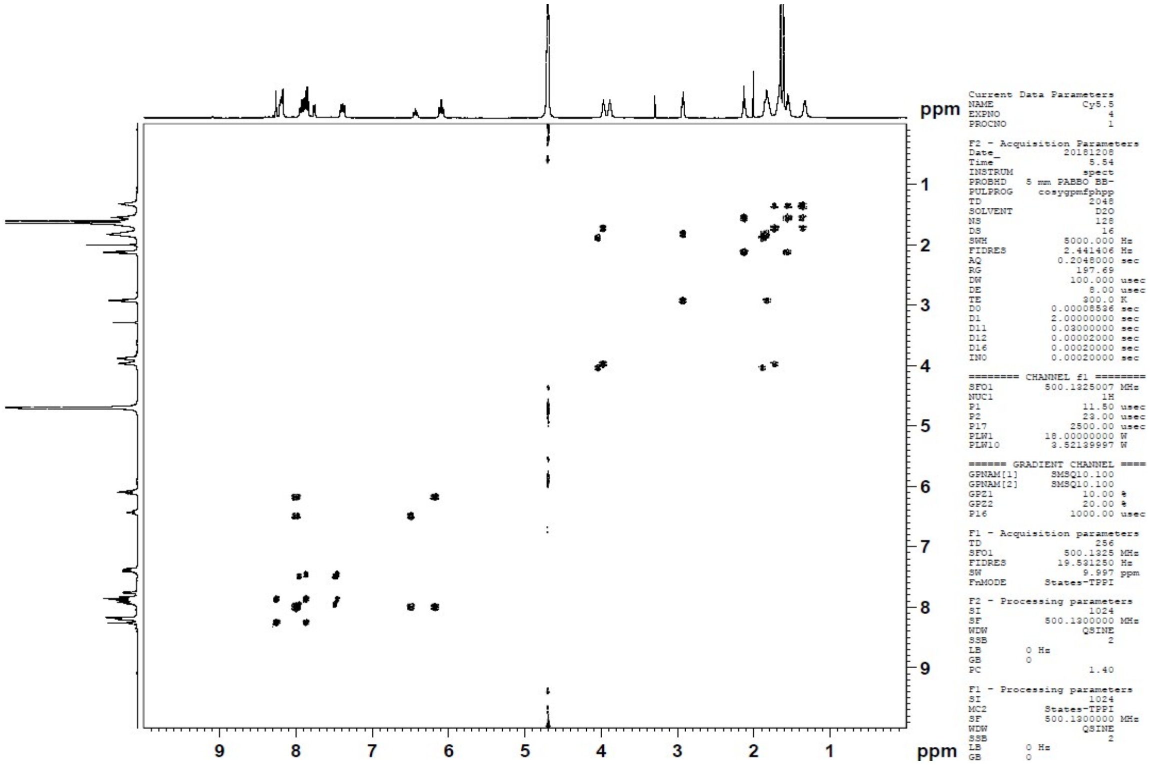
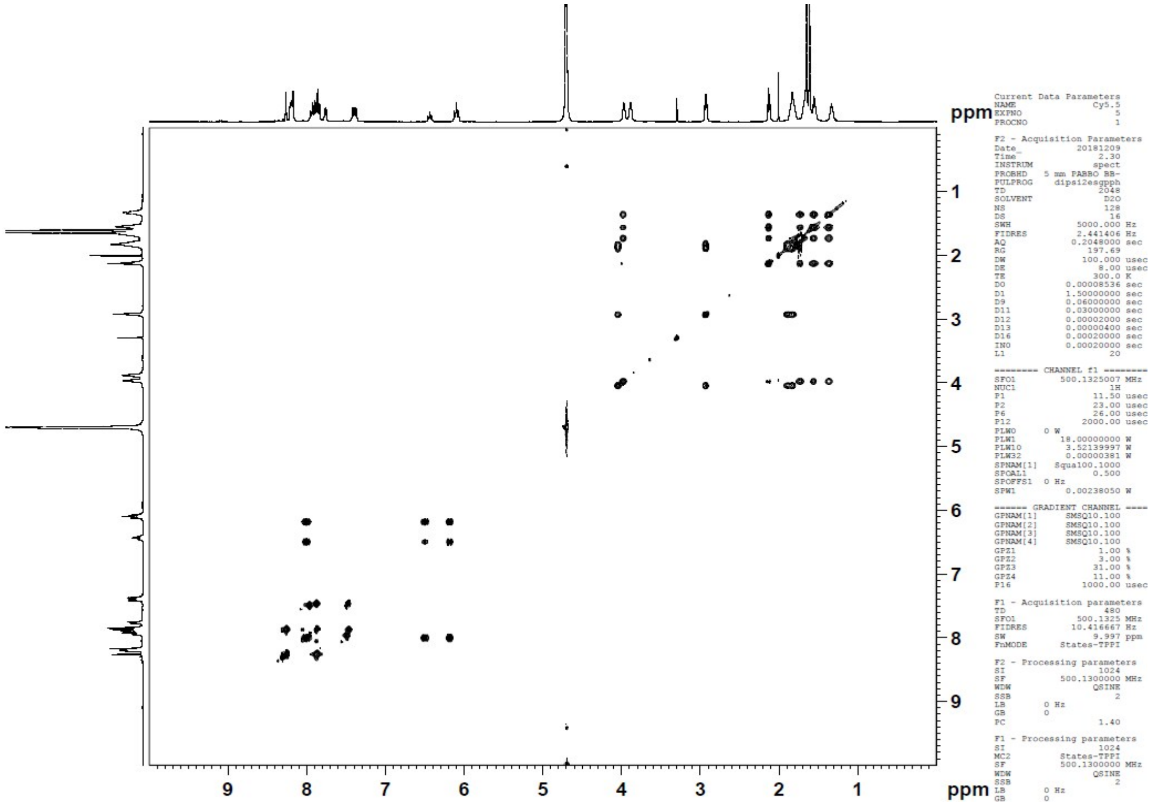


Figure S51b. *^{13}C spectrum of Cy5.5 3S,* ***(2)*** *in D_2O.*



```
Current Data Parameters
NAME                Cy5.5
EXPNO                   4
PROCNO                  1

F2 - Acquisition Parameters
Date_            20181208
Time                 5.54
INSTRUM             spect
PROBHD   5 mm PABBO BB-
PULPROG       cosygpmfphpp
TD                  2048
SOLVENT              D2O
NS                   128
DS                    16
SWH             5000.000 Hz
FIDRES          2.441406 Hz
AQ             0.2048000 sec
RG                197.69
DW               100.000 usec
DE                  8.00 usec
TE                 300.0 K
D0            0.00008536 sec
D1            2.00000000 sec
D11           0.03000000 sec
D12           0.00002000 sec
D16           0.00020000 sec
IN0           0.00020000 sec

======== CHANNEL f1 ========
SFO1         500.1325007 MHz
NUC1                  1H
P1                 11.50 usec
P2                 23.00 usec
P17              2500.00 usec
PLW1         18.00000000 W
PLW10         3.52139997 W

====== GRADIENT CHANNEL ====
GPNAM[1]      SMSQ10.100
GPNAM[2]      SMSQ10.100
GPZ1               10.00 %
GPZ2               20.00 %
P16              1000.00 usec

F1 - Acquisition parameters
TD                   256
SFO1            500.1325 MHz
FIDRES         19.531250 Hz
SW                 9.997 ppm
FnMODE       States-TPPI

F2 - Processing parameters
SI                  1024
SF           500.1300000 MHz
WDW                QSINE
SSB                    2
LB         0 Hz
GB         0
PC                  1.40

F1 - Processing parameters
SI                  1024
MC2          States-TPPI
SF           500.1300000 MHz
WDW                QSINE
SSB                    2
LB         0 Hz
GB         0
```

Figure S51c. *(^{1}H-^{1}H) DQF-COSY of Cy5.5 3S,* ***(2)*** *in D_2O.*



```
Current Data Parameters
NAME                Cy5.5
EXPNO                   5
PROCNO                  1

F2 - Acquisition Parameters
Date_            20181209
Time                 2.30
INSTRUM             spect
PROBHD   5 mm PABBO BB-
PULPROG       dipsi2esgpph
TD                  2048
SOLVENT              D2O
NS                   128
DS                    16
SWH             5000.000 Hz
FIDRES          2.441406 Hz
AQ             0.2048000 sec
RG                197.69
DW               100.000 usec
DE                  8.00 usec
TE                 300.0 K
D0            0.00008536 sec
D1            1.50000000 sec
D9            0.06000000 sec
D11           0.03000000 sec
D12           0.00002000 sec
D13           0.00000400 sec
D16           0.00020000 sec
IN0           0.00020000 sec
L1                    20

======== CHANNEL f1 ========
SFO1         500.1325007 MHz
NUC1                  1H
P1                 11.50 usec
P2                 23.00 usec
P6                 26.00 usec
P12              2000.00 usec
PLW0       0 W
PLW1         18.00000000 W
PLW10         3.52139997 W
PLW32         0.00000381 W
SPNAM[1]   Squa100.1000
SPOAL1             0.500
SPOFFS1    0 Hz
SPW1          0.00238050 W

====== GRADIENT CHANNEL ====
GPNAM[1]      SMSQ10.100
GPNAM[2]      SMSQ10.100
GPNAM[3]      SMSQ10.100
GPNAM[4]      SMSQ10.100
GPZ1                1.00 %
GPZ2                3.00 %
GPZ3               31.00 %
GPZ4               11.00 %
P16              1000.00 usec

F1 - Acquisition parameters
TD                   480
SFO1            500.1325 MHz
FIDRES         10.416667 Hz
SW                 9.997 ppm
FnMODE       States-TPPI

F2 - Processing parameters
SI                  1024
SF           500.1300000 MHz
WDW                QSINE
SSB                    2
LB         0 Hz
GB         0
PC                  1.40

F1 - Processing parameters
SI                  1024
MC2          States-TPPI
SF           500.1300000 MHz
WDW                QSINE
SSB                    2
LB         0 Hz
GB         0
```

Figure S51d. *(^{1}H-^{1}H) TOCSY of Cy5.5 3S,* ***(2)*** *in D_2O.*

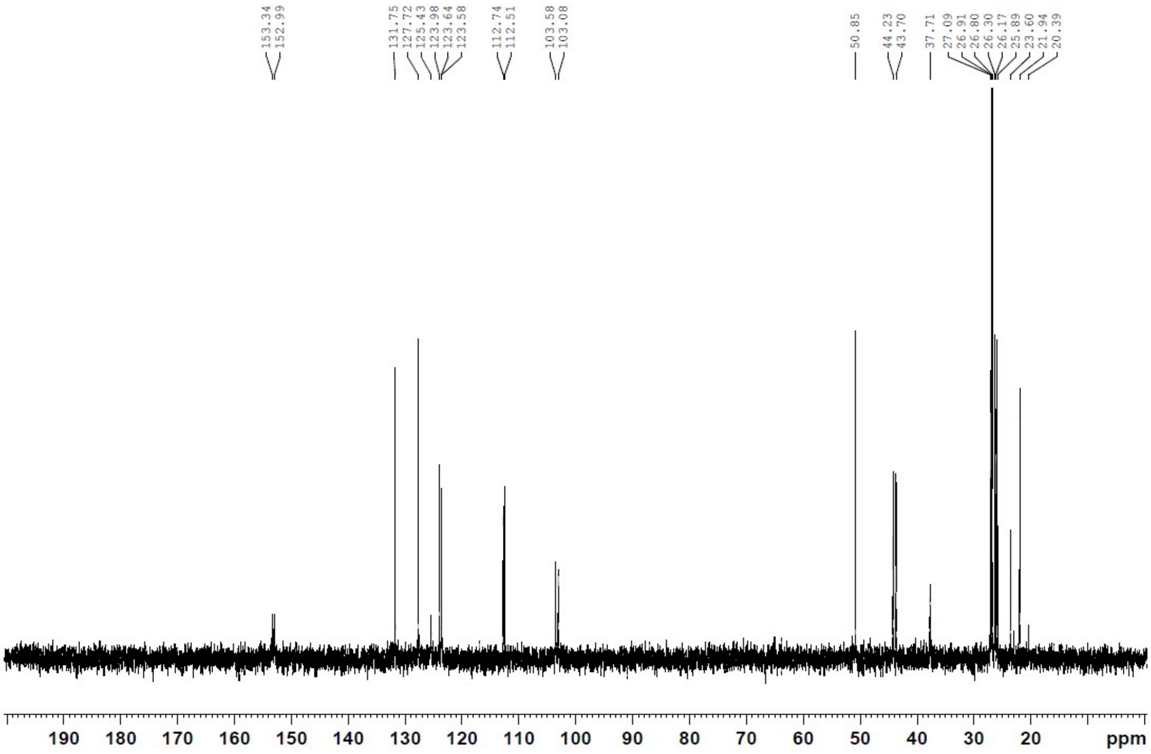


Figure S51e. *^{13}C DEPT45 (CH, CH_2, CH_3 signals) of Cy5.5 3S,* ***(2)*** *in D_2O.*

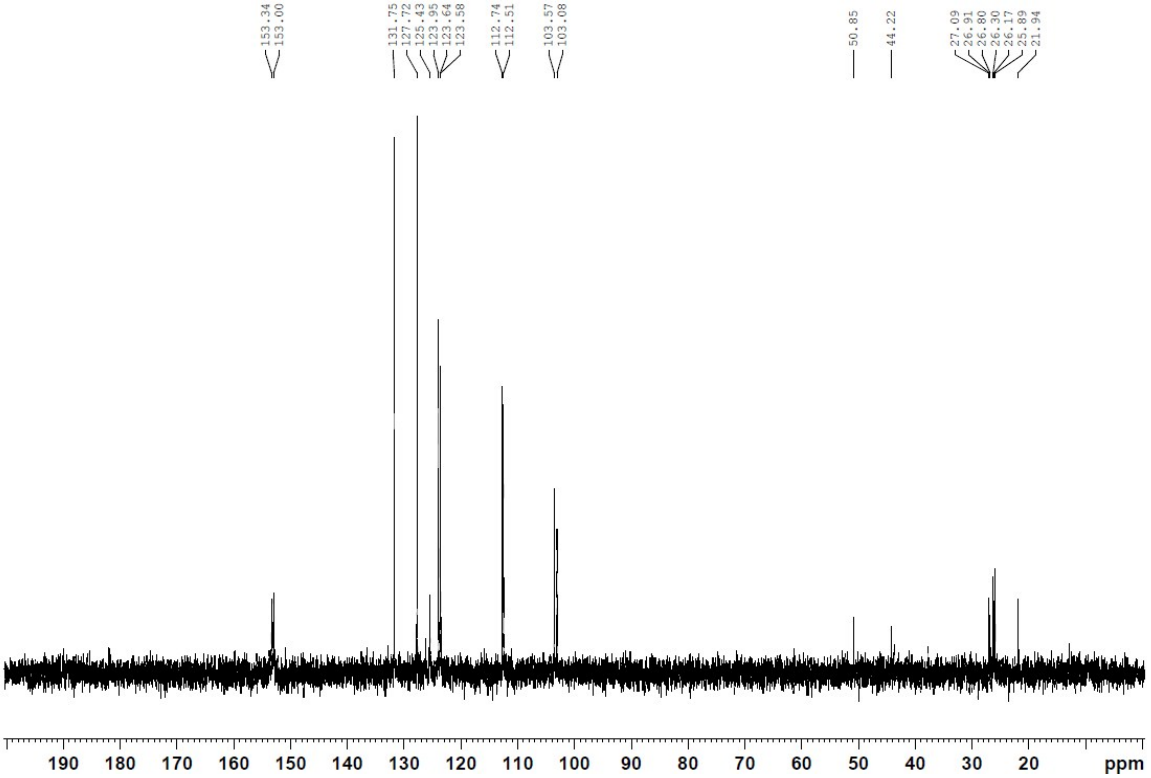


Figure S51f. *^{13}C DEPT90 (CH signals only) of Cy5.5 3S,* ***(2)*** *in D_2O.*

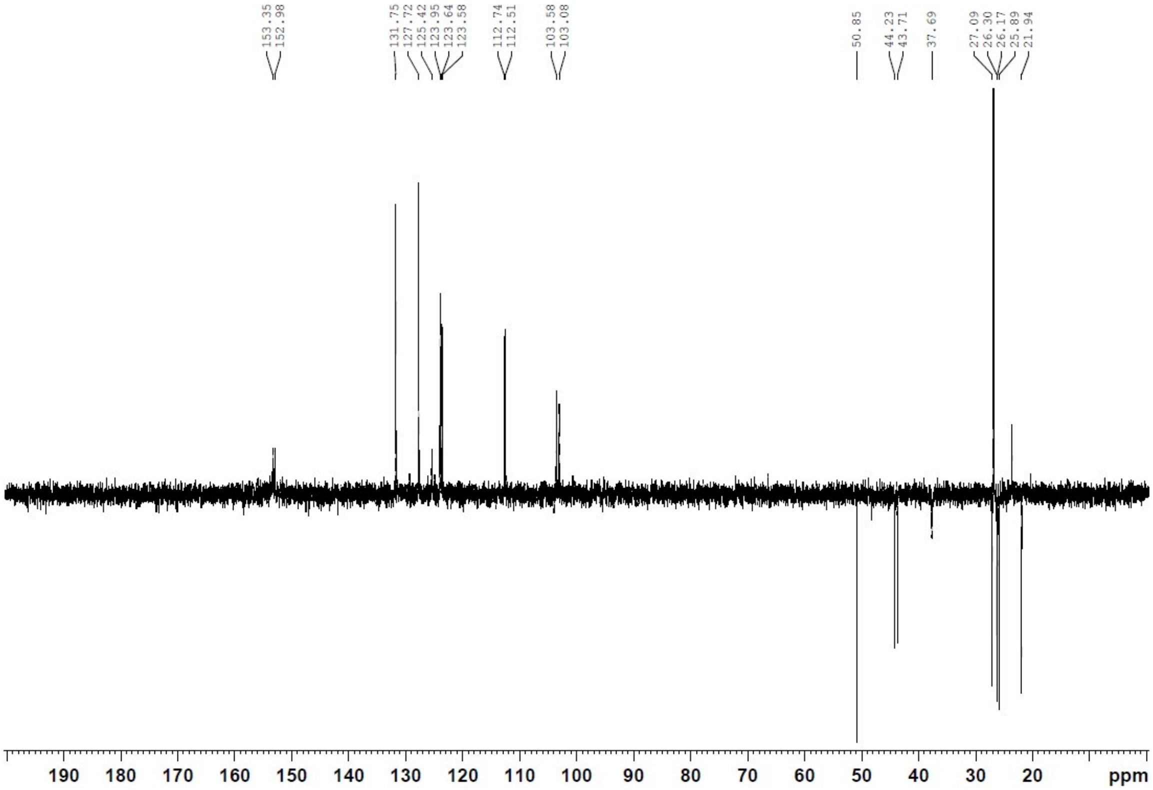
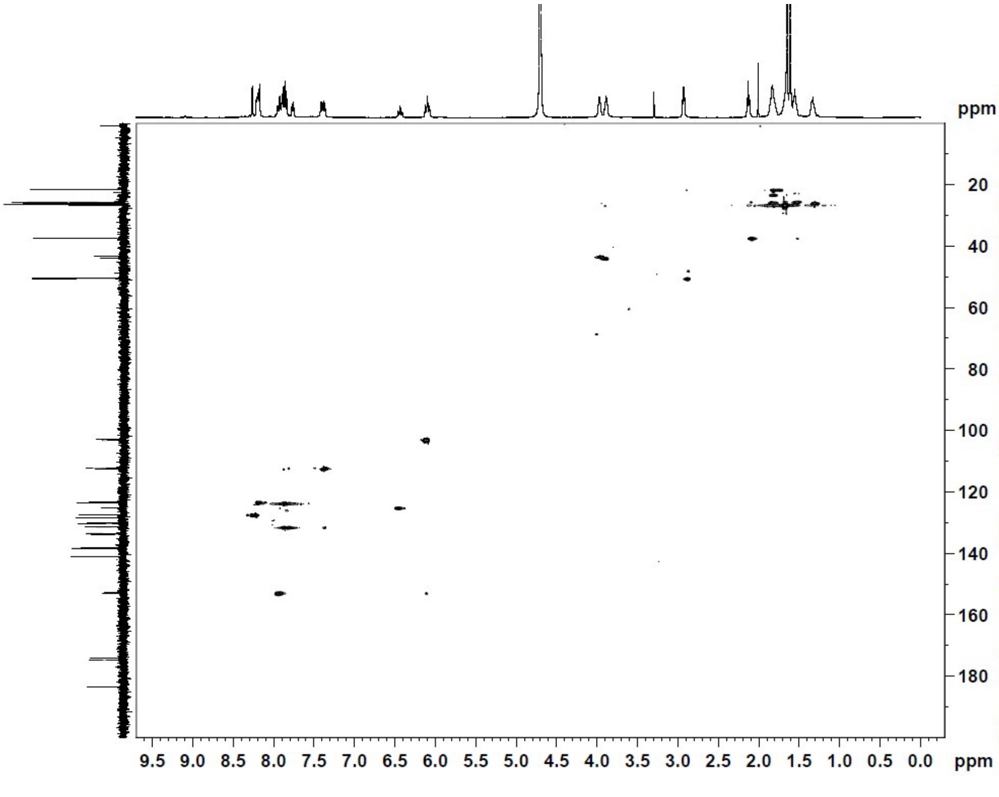
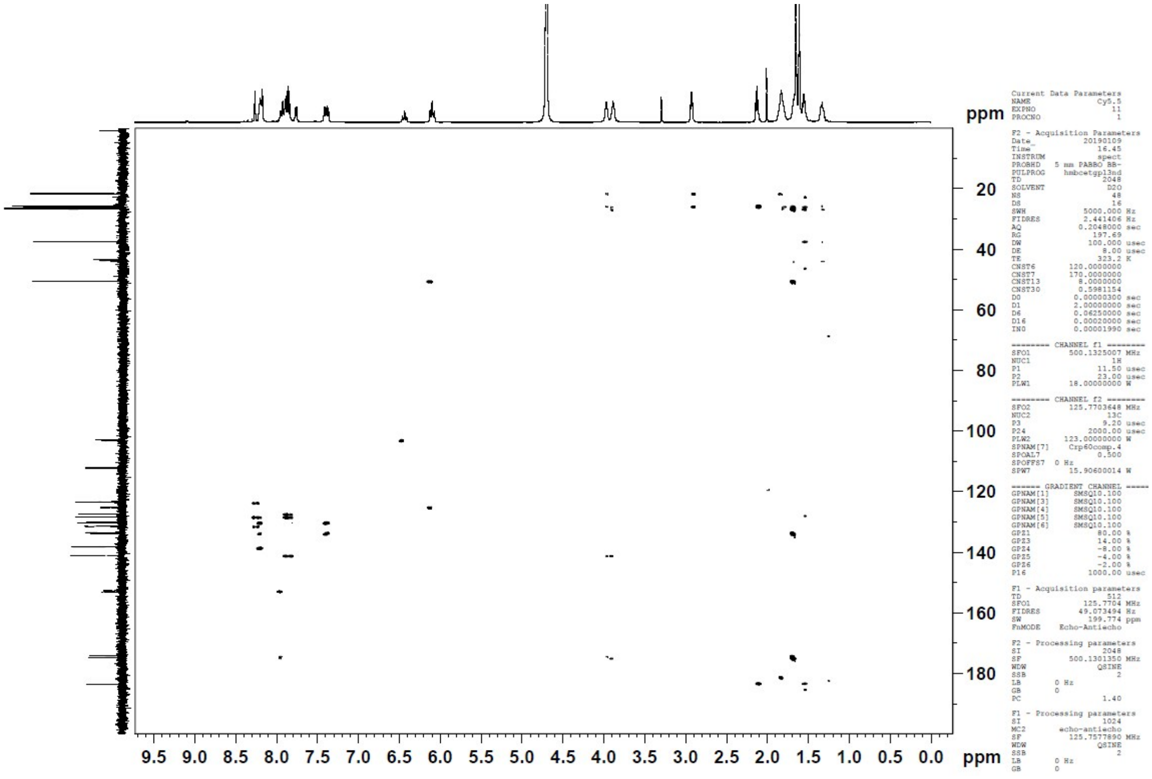


Figure S51g. *^{13}C DEPT135 (CH & CH_3 signals (+), CH_2 signals (-)) of Cy5.5 3S,* ***(2)*** *in D_2O.*



```
Current Data Parameters
NAME               Cy5.5
EXPNO                 10
PROCNO                 1

F2 - Acquisition Parameters
Date_           20190108
Time               12.09
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG        hsqcetgpsi
TD                  2048
SOLVENT              D2O
NS                    96
DS                    32
SWH             5000.000 Hz
FIDRES          2.441406 Hz
AQ             0.2048000 sec
RG                197.69
DW               100.000 usec
DE                  8.00 usec
TE                 323.1 K
CNST2        145.0000000
D0            0.00000300 sec
D1            1.50000000 sec
D4            0.00172414 sec
D11           0.03000000 sec
D16           0.00020000 sec
D24           0.00110000 sec
IN0           0.00001990 sec
ZGOPTNS

======== CHANNEL f1 ========
SFO1         500.1325007 MHz
NUC1                  1H
P1                 11.50 usec
P2                 23.00 usec
P28                 0.10 usec
PLW1         18.00000000 W

======== CHANNEL f2 ========
SFO2         125.7703648 MHz
NUC2                 13C
CPDPRG[2            garp
P3                  9.20 usec
P4                 18.40 usec
PCPD2              70.00 usec
PLW2        123.00000000 W
PLW12         1.89999998 W

====== GRADIENT CHANNEL =====
GPNAM[1]      SMSQ10.100
GPNAM[2]      SMSQ10.100
GPZ1               80.00 %
GPZ2               20.10 %
P16              1000.00 usec

F1 - Acquisition parameters
TD                   512
SFO1            125.7704 MHz
FIDRES         49.073494 Hz
SW               199.774 ppm
FnMODE    Echo-Antiecho

F2 - Processing parameters
SI                  1024
SF           500.1301500 MHz
WDW                QSINE
SSB                    2
LB          0 Hz
GB          0
PC                  1.40

F1 - Processing parameters
SI                  1024
MC2       echo-antiecho
SF           125.7577890 MHz
WDW                QSINE
SSB                    2
LB          0 Hz
GB          0
```

Figure S51h. *(^{13}C-^{1}H) HSQC of Cy5.5 3S,* ***(2)*** *in D_2O.*



```
Current Data Parameters
NAME                Cy5.5
EXPNO                  11
PROCNO                  1

F2 - Acquisition Parameters
Date_            20190109
Time                16.45
INSTRUM             spect
PROBHD   5 mm PABBO BB-
PULPROG      hmbcetgpl3nd
TD                   2048
SOLVENT               D2O
NS                     48
DS                     16
SWH              5000.000 Hz
FIDRES           2.441406 Hz
AQ              0.2048000 sec
RG                 197.69
DW                100.000 usec
DE                   8.00 usec
TE                  323.2 K
CNST6         120.0000000
CNST7         170.0000000
CNST13          8.0000000
CNST30          0.5981154
D0             0.00000300 sec
D1             2.00000000 sec
D6             0.06250000 sec
D16            0.00020000 sec
IN0            0.00001990 sec

======== CHANNEL f1 ========
SFO1          500.1325007 MHz
NUC1                   1H
P1                  11.50 usec
P2                  23.00 usec
PLW1          18.00000000 W

======== CHANNEL f2 ========
SFO2          125.7703648 MHz
NUC2                  13C
P3                   9.20 usec
P24               2000.00 usec
PLW2         123.00000000 W
SPNAM[7]      Crp60comp.4
SPOAL7              0.500
SPOFFS7  0 Hz
SPW7          15.90600014 W

====== GRADIENT CHANNEL =====
GPNAM[1]      SMSQ10.100
GPNAM[3]      SMSQ10.100
GPNAM[4]      SMSQ10.100
GPNAM[5]      SMSQ10.100
GPNAM[6]      SMSQ10.100
GPZ1                80.00 %
GPZ3                14.00 %
GPZ4                -8.00 %
GPZ5                -4.00 %
GPZ6                -2.00 %
P16               1000.00 usec

F1 - Acquisition parameters
TD                    512
SFO1             125.7704 MHz
FIDRES          49.073494 Hz
SW                199.774 ppm
FnMODE      Echo-Antiecho

F2 - Processing parameters
SI                   2048
SF            500.1301350 MHz
WDW                 QSINE
SSB                     2
LB           0 Hz
GB           0
PC                   1.40

F1 - Processing parameters
SI                   1024
MC2        echo-antiecho
SF            125.7577890 MHz
WDW                 QSINE
SSB                     2
LB           0 Hz
GB           0
```

Figure S51i. *(^{13}C-^{1}H) HMBC of Cy5.5 3S,* ***(2)*** *in D$_2$O. D6 was set to 41.7ms.*

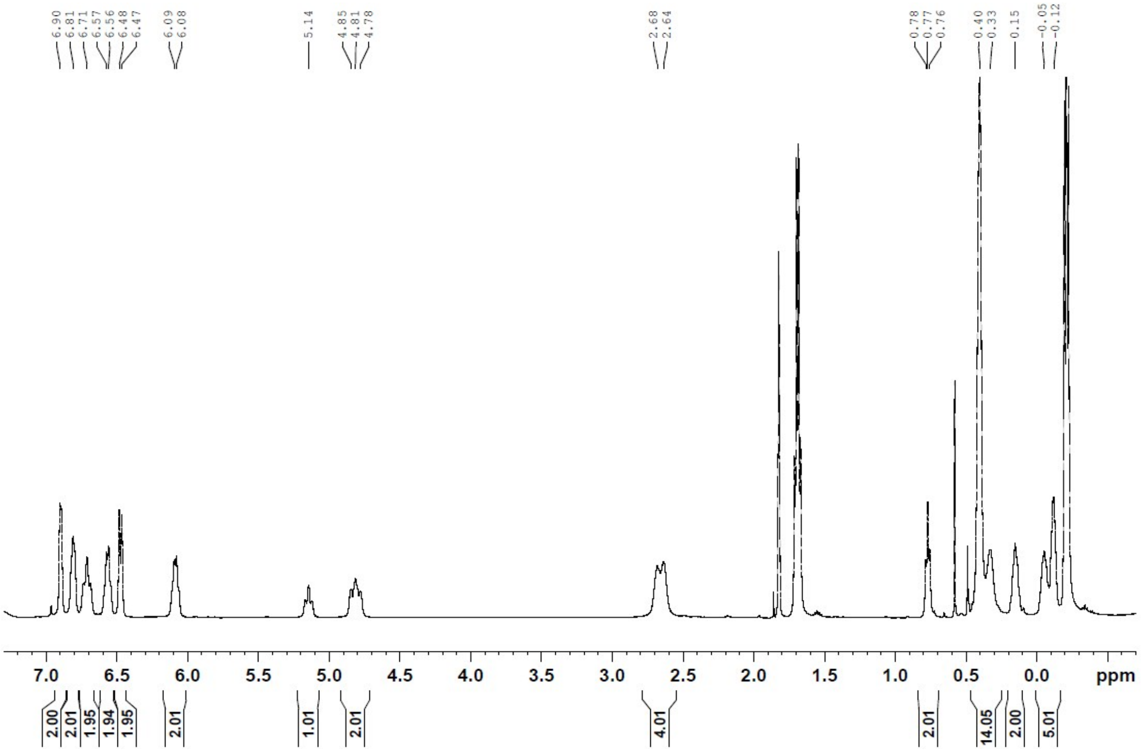


Figure S52a. *^{1}H Presaturation spectrum of Cy5.5 2S,* ***(3)*** *in 1:1 D$_2$O:MeOD. O1P was set to 1649.80 Hz.*

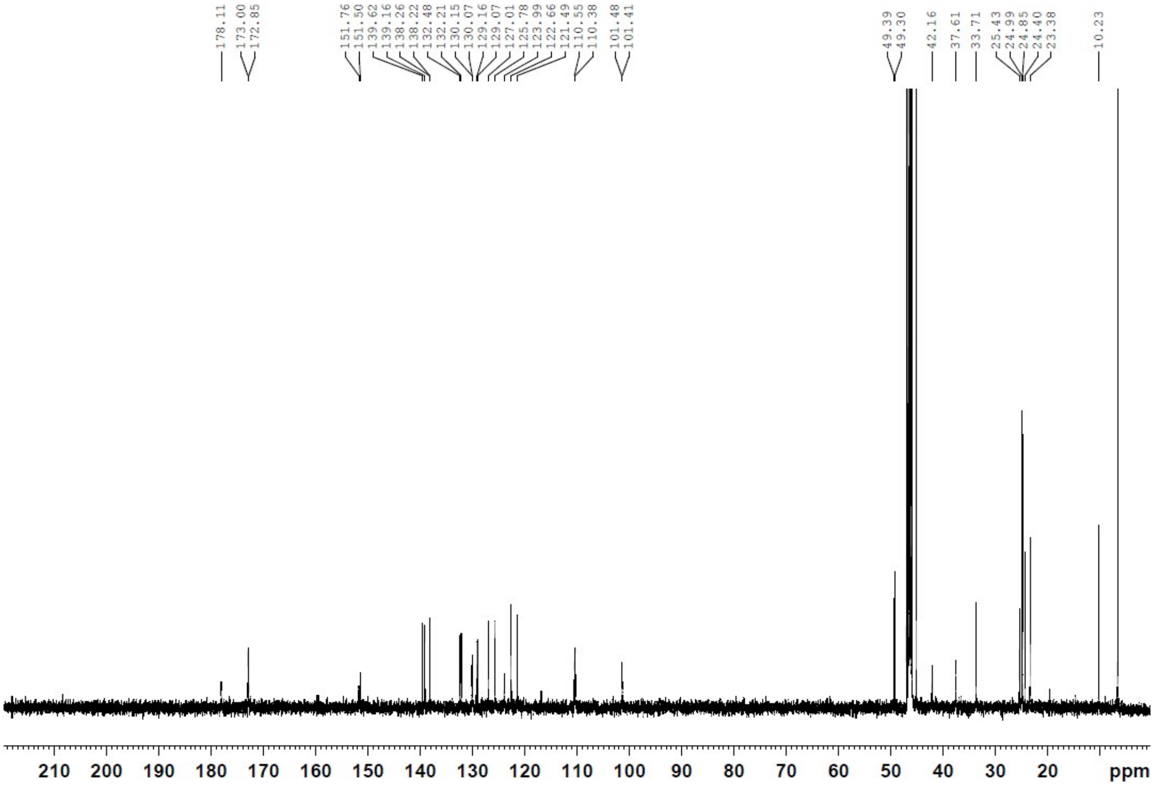
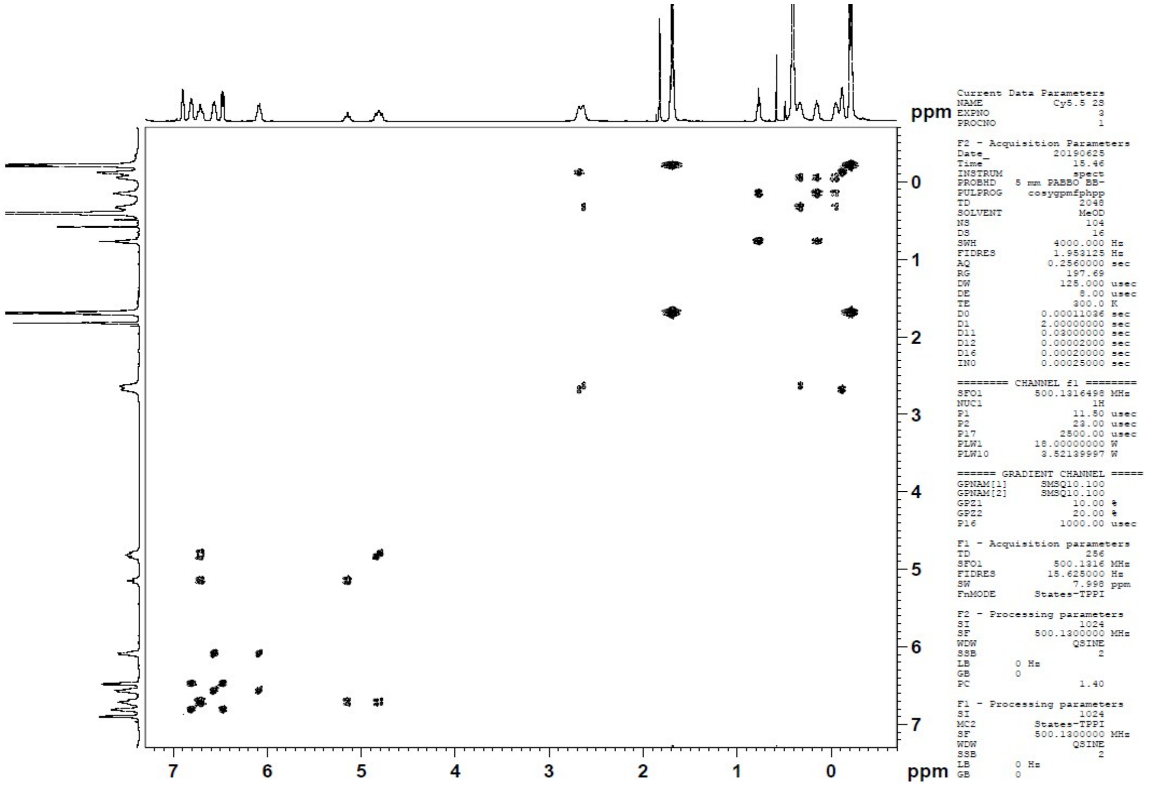
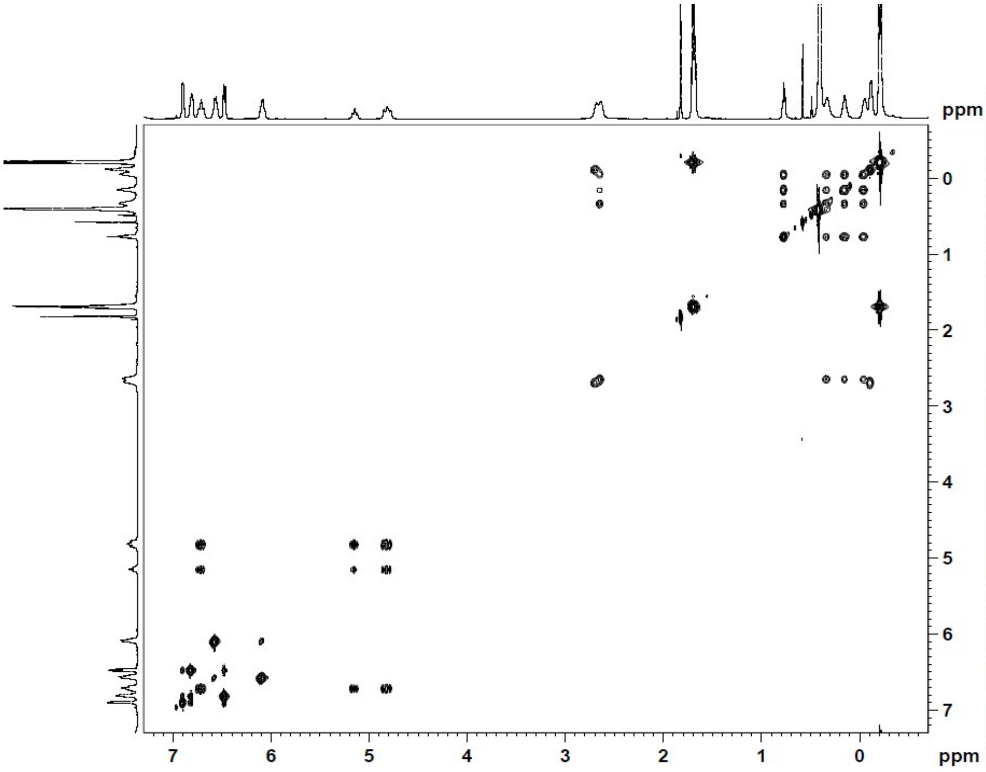


Figure S52b. *^{13}C spectrum of Cy5.5 2S,* ***(3)*** *in 1:1 D_2O:MeOD.*



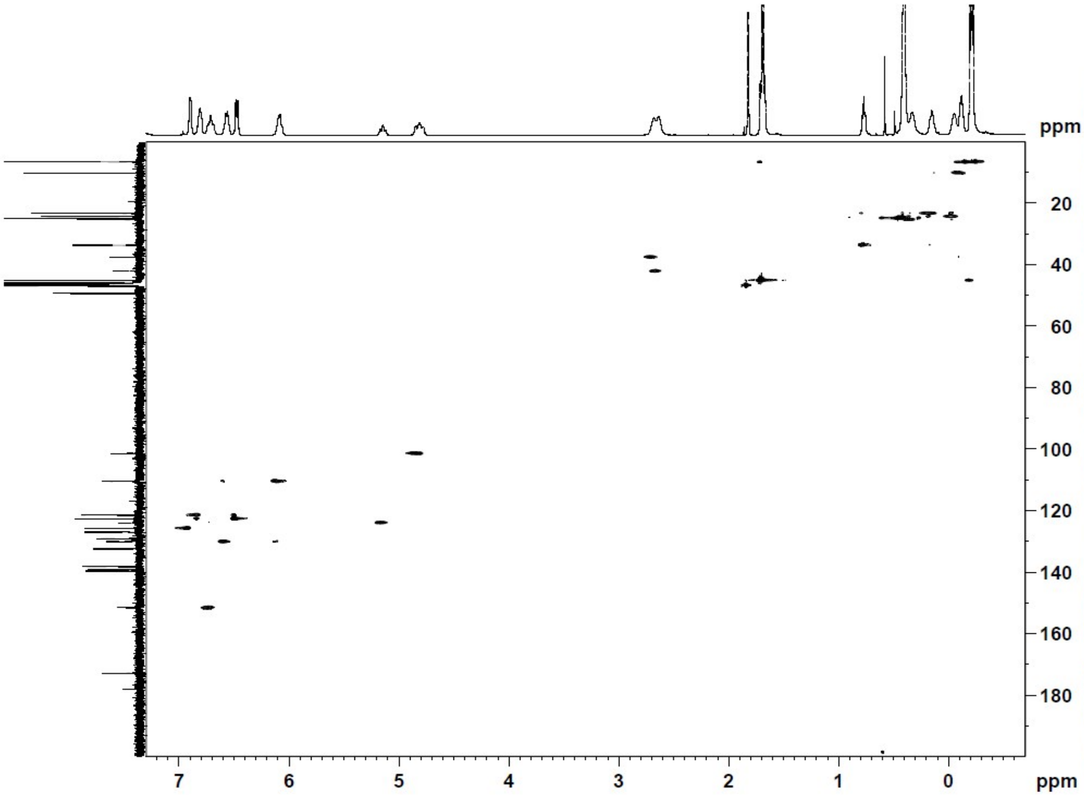
```
Current Data Parameters
NAME              Cy5.5 2S
EXPNO                    3
PROCNO                   1

F2 - Acquisition Parameters
Date_             20190625
Time                 15.46
INSTRUM              spect
PROBHD   5 mm PABBO BB-
PULPROG      cosygpmfphpp
TD                    2048
SOLVENT               MeOD
NS                     104
DS                      16
SWH               4000.000 Hz
FIDRES            1.953125 Hz
AQ               0.2560000 sec
RG                  197.69
DW                 125.000 usec
DE                    8.00 usec
TE                   300.0 K
D0              0.00011036 sec
D1              2.00000000 sec
D11             0.03000000 sec
D12             0.00002000 sec
D16             0.00020000 sec
IN0             0.00025000 sec

======== CHANNEL f1 ========
SFO1           500.1316498 MHz
NUC1                    1H
P1                   11.50 usec
P2                   23.00 usec
P17                2500.00 usec
PLW1           18.00000000 W
PLW10           3.52139997 W

====== GRADIENT CHANNEL =====
GPNAM[1]        SMSQ10.100
GPNAM[2]        SMSQ10.100
GPZ1                 10.00 %
GPZ2                 20.00 %
P16                1000.00 usec

F1 - Acquisition parameters
TD                     256
SFO1              500.1316 MHz
FIDRES           15.625000 Hz
SW                   7.998 ppm
FnMODE         States-TPPI

F2 - Processing parameters
SI                    1024
SF             500.1300000 MHz
WDW                  QSINE
SSB                      2
LB           0 Hz
GB           0
PC                    1.40

F1 - Processing parameters
SI                    1024
MC2            States-TPPI
SF             500.1300000 MHz
WDW                  QSINE
SSB                      2
LB           0 Hz
GB           0
```

Figure S52c. *(^{1}H-^{1}H) DQF-COSY of Cy5.5 2S,* ***(3)*** *in 1:1 D_2O:MeOD.*



```
Current Data Parameters
NAME            Cy5.5 2S
EXPNO                  4
PROCNO                 1

F2 - Acquisition Parameters
Date_           20190628
Time               14.53
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG     dipsi2esgpph
TD                  2048
SOLVENT             MeOD
NS                   128
DS                    16
SWH             4000.000 Hz
FIDRES          1.953125 Hz
AQ             0.2560000 sec
RG                197.69
DW               125.000 usec
DE                  8.00 usec
TE                 300.0 K
D0            0.00011036 sec
D1            1.50000000 sec
D9            0.06000000 sec
D11           0.03000000 sec
D12           0.00002000 sec
D13           0.00000400 sec
D16           0.00020000 sec
IN0           0.00025000 sec
L1                    20

======== CHANNEL f1 ========
SFO1         500.1316499 MHz
NUC1                  1H
P1                 11.50 usec
P2                 23.00 usec
P6                 26.00 usec
P12              2000.00 usec
PLW0       0 W
PLW1         18.00000000 W
PLW10         3.52139997 W
PLW32         0.00000381 W
SPNAM[1]    Squa100.1000
SPOAL1             0.500
SPOFFS1   0 Hz
SPW1          0.00238050 W

====== GRADIENT CHANNEL =====
GPNAM[1]      SMSQ10.100
GPNAM[2]      SMSQ10.100
GPNAM[3]      SMSQ10.100
GPNAM[4]      SMSQ10.100
GPZ1                1.00 %
GPZ2                3.00 %
GPZ3               31.00 %
GPZ4               11.00 %
P16              1000.00 usec

F1 - Acquisition parameters
TD                   256
SFO1            500.1316 MHz
FIDRES         15.625000 Hz
SW                 7.998 ppm
FnMODE       States-TPPI

F2 - Processing parameters
SI                  1024
SF           500.1300000 MHz
WDW                QSINE
SSB                    2
LB         0 Hz
GB         0
PC                  1.40

F1 - Processing parameters
SI                  1024
MC2          States-TPPI
SF           500.1300000 MHz
WDW                QSINE
SSB                    2
LB         0 Hz
GB         0
```

Figure S52d. *(^1H-^1H) TOCSY of Cy5.5 2S,* ***(3)*** *in 1:1 D_2O:MeOD.*



```
Current Data Parameters
NAME            Cy5.5 2S
EXPNO                  6
PROCNO                 1

F2 - Acquisition Parameters
Date_           20190702
Time               16.28
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG        hsqcetgpsi
TD                  2048
SOLVENT             MeOD
NS                    64
DS                    16
SWH             4000.000 Hz
FIDRES          1.953125 Hz
AQ             0.2560000 sec
RG                197.69
DW               125.000 usec
DE                  8.00 usec
TE                 300.2 K
CNST2        145.0000000
D0            0.00000300 sec
D1            1.50000000 sec
D4            0.00172414 sec
D11           0.03000000 sec
D16           0.00020000 sec
D24           0.00110000 sec
IN0           0.00001990 sec
ZGOPTNS

======== CHANNEL f1 ========
SFO1         500.1316499 MHz
NUC1                  1H
P1                 11.50 usec
P2                 23.00 usec
P28                 0.10 usec
PLW1         18.00000000 W

======== CHANNEL f2 ========
SFO2         125.7703648 MHz
NUC2                 13C
CPDPRG[2            garp
P3                  9.20 usec
P4                 18.40 usec
PCPD2              70.00 usec
PLW2        123.00000000 W
PLW12         1.89999998 W

====== GRADIENT CHANNEL =====
GPNAM[1]      SMSQ10.100
GPNAM[2]      SMSQ10.100
GPZ1               80.00 %
GPZ2               20.10 %
P16              1000.00 usec

F1 - Acquisition parameters
TD                   512
SFO1            125.7704 MHz
FIDRES         49.073494 Hz
SW               199.774 ppm
FnMODE     Echo-Antiecho

F2 - Processing parameters
SI                  1024
SF           500.1300000 MHz
WDW                QSINE
SSB                    2
LB         0 Hz
GB         0
PC                  1.40

F1 - Processing parameters
SI                  1024
MC2        echo-antiecho
SF           125.7577890 MHz
WDW                QSINE
SSB                    2
LB         0 Hz
GB         0
```

Figure S52e. *(^1H-^{13}C) HSQC of Cy5.5 2S,* ***(3)*** *in 1:1 D_2O:MeOD.*

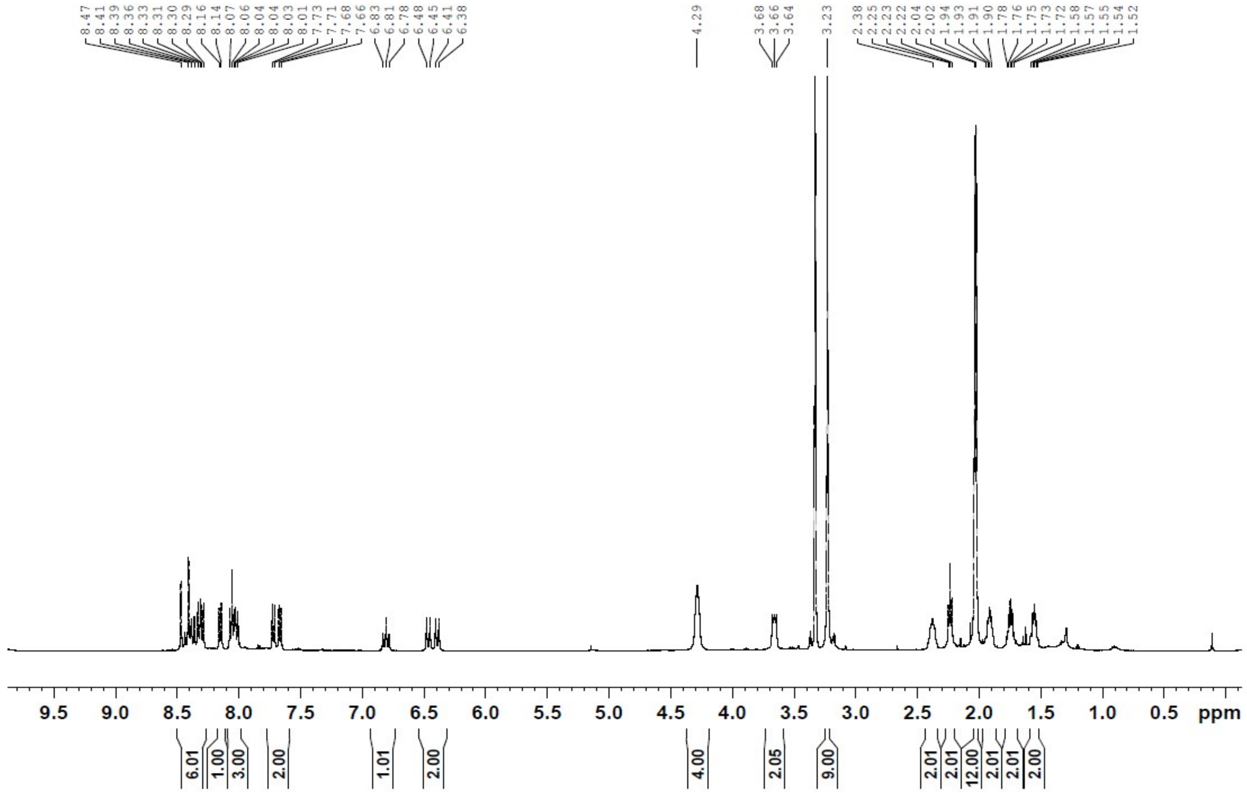


Figure S53a. ^{1}*H Presaturation spectrum of Cy5.5 ZW,* ***(4)*** *in 20%* D_2O *in MeOD. O1P was set to 2436.80 Hz.*

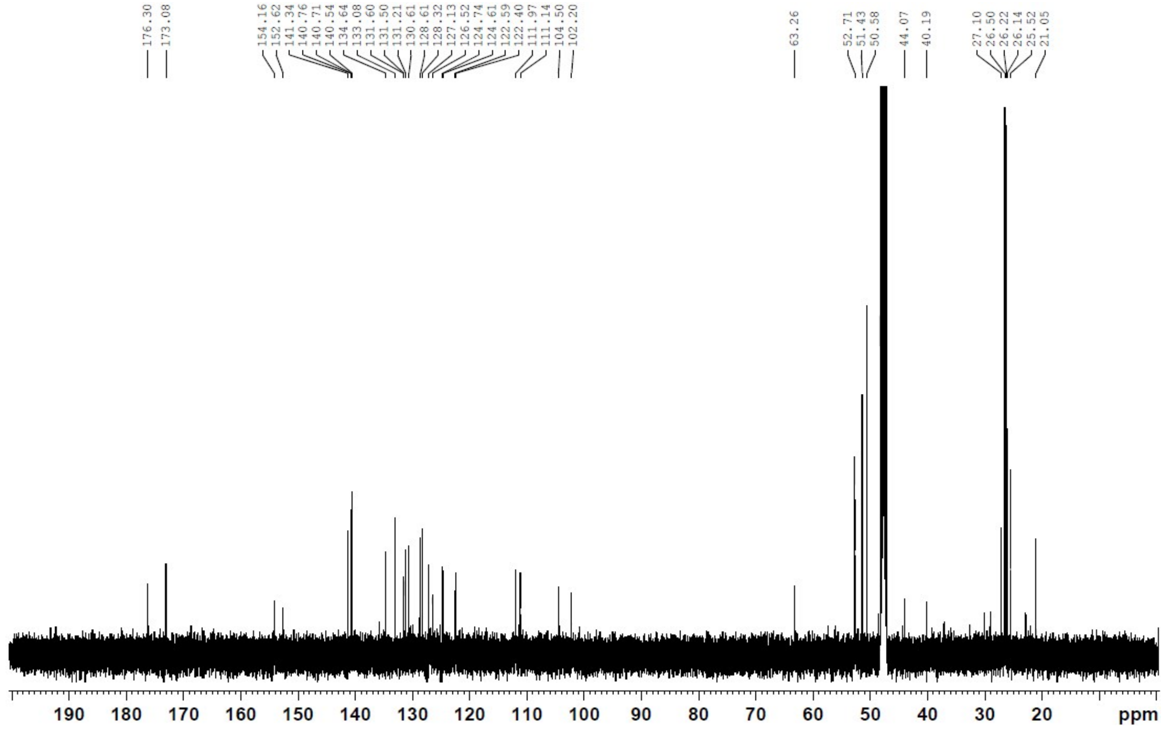
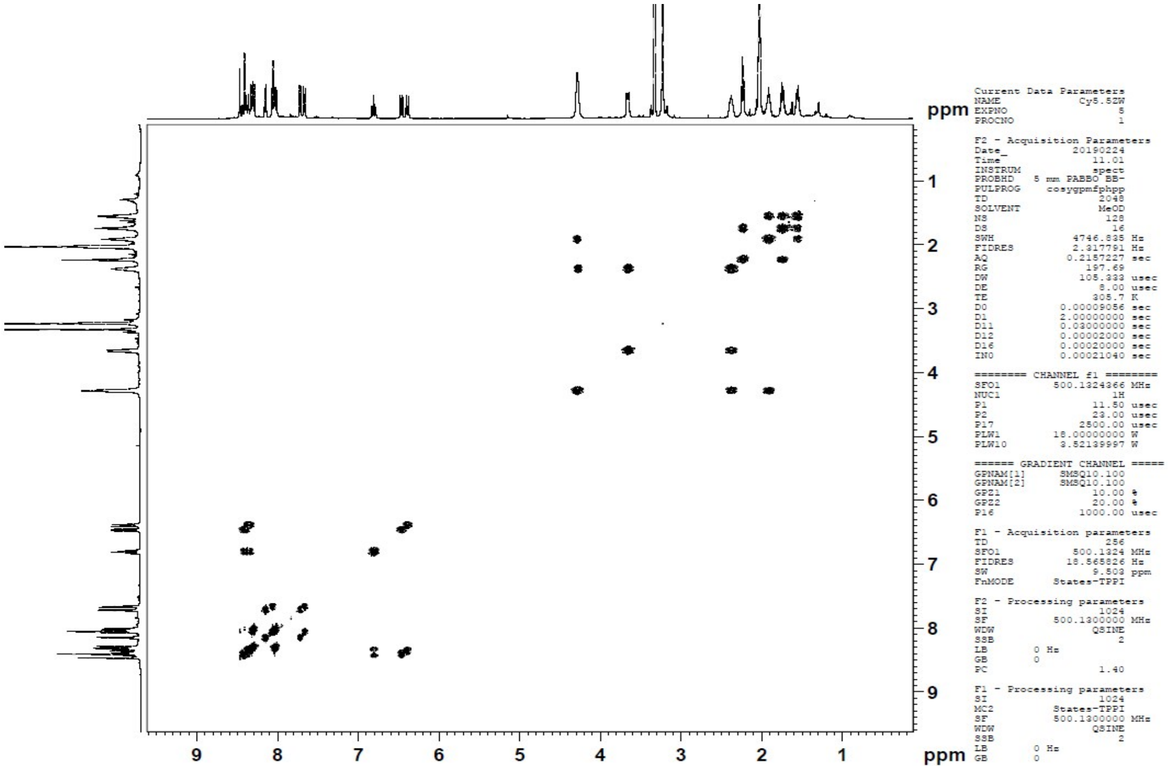
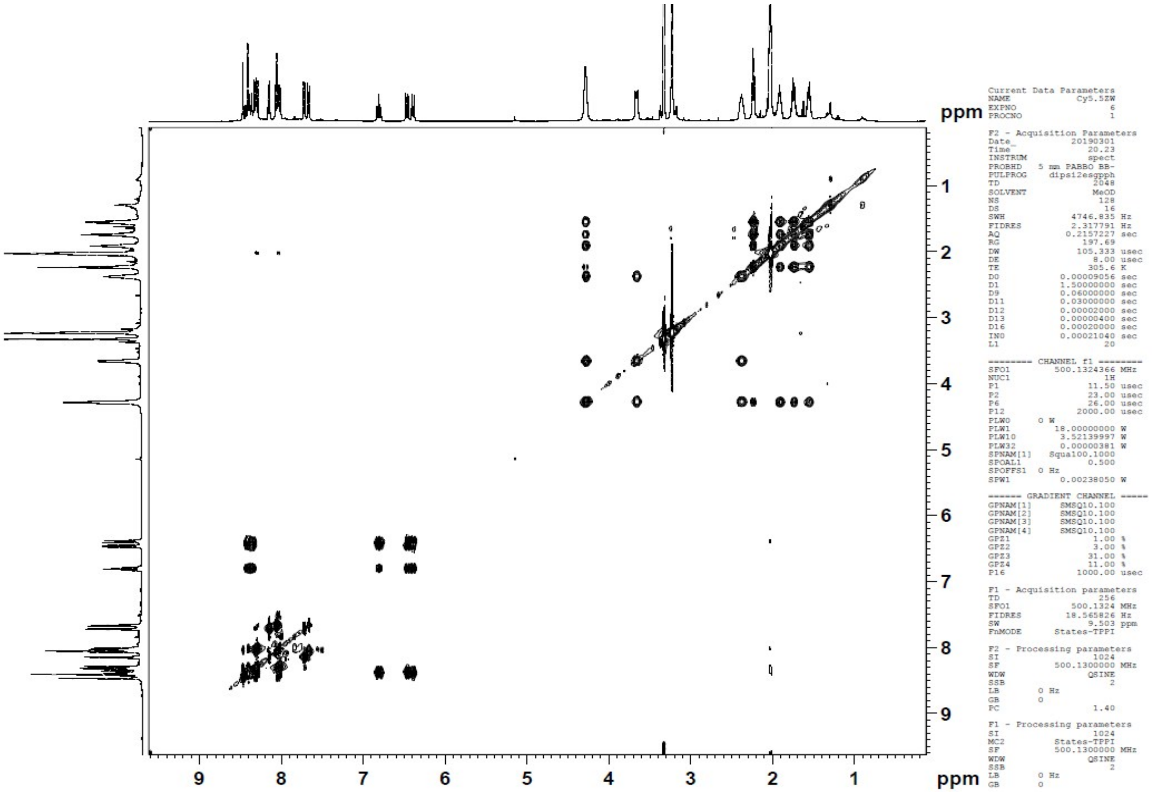


Figure S53b. ^{13}*C spectrum of Cy5.5 ZW,* ***(4)*** *in 20%* D_2O *in MeOD.*



```
Current Data Parameters
NAME              Cy5.5ZW
EXPNO                   5
PROCNO                  1

F2 - Acquisition Parameters
Date_            20190224
Time                11.01
INSTRUM             spect
PROBHD   5 mm PABBO BB-
PULPROG      cosygpmfphpp
TD                   2048
SOLVENT              MeOD
NS                    128
DS                     16
SWH              4746.835 Hz
FIDRES           2.317791 Hz
AQ              0.2157227 sec
RG                 197.69
DW                105.333 usec
DE                   8.00 usec
TE                  305.7 K
D0             0.00009056 sec
D1             2.00000000 sec
D11            0.03000000 sec
D12            0.00002000 sec
D16            0.00020000 sec
IN0            0.00021040 sec

======== CHANNEL f1 ========
SFO1          500.1324366 MHz
NUC1                   1H
P1                  11.50 usec
P2                  23.00 usec
P17               2500.00 usec
PLW1          18.00000000 W
PLW10          3.52139997 W

====== GRADIENT CHANNEL =====
GPNAM[1]       SMSQ10.100
GPNAM[2]       SMSQ10.100
GPZ1                10.00 %
GPZ2                20.00 %
P16               1000.00 usec

F1 - Acquisition parameters
TD                    256
SFO1             500.1324 MHz
FIDRES          18.565826 Hz
SW                  9.503 ppm
FnMODE        States-TPPI

F2 - Processing parameters
SI                   1024
SF            500.1300000 MHz
WDW                 QSINE
SSB                     2
LB             0 Hz
GB             0
PC                   1.40

F1 - Processing parameters
SI                   1024
MC2           States-TPPI
SF            500.1300000 MHz
WDW                 QSINE
SSB                     2
LB             0 Hz
GB             0
```

Figure S53c. *(^{1}H-^{1}H) DQF-COSY of Cy5.5 ZW,* ***(4)*** *in 20% D$_2$O in MeOD.*



```
Current Data Parameters
NAME              Cy5.5ZW
EXPNO                   6
PROCNO                  1

F2 - Acquisition Parameters
Date_            20190301
Time                20.23
INSTRUM             spect
PROBHD   5 mm PABBO BB-
PULPROG      dipsi2esgpph
TD                   2048
SOLVENT              MeOD
NS                    128
DS                     16
SWH              4746.835 Hz
FIDRES           2.317791 Hz
AQ              0.2157227 sec
RG                 197.69
DW                105.333 usec
DE                   8.00 usec
TE                  305.6 K
D0             0.00009056 sec
D1             1.50000000 sec
D9             0.06000000 sec
D11            0.03000000 sec
D12            0.00002000 sec
D13            0.00000400 sec
D16            0.00020000 sec
IN0            0.00021040 sec
L1                     20

======== CHANNEL f1 ========
SFO1          500.1324366 MHz
NUC1                   1H
P1                  11.50 usec
P2                  23.00 usec
P6                  26.00 usec
P12               2000.00 usec
PLW0           0 W
PLW1          18.00000000 W
PLW10          3.52139997 W
PLW32          0.00000381 W
SPNAM[1]     Squa100.1000
SPOAL1              0.500
SPOFFS1        0 Hz
SPW1           0.00238050 W

====== GRADIENT CHANNEL =====
GPNAM[1]       SMSQ10.100
GPNAM[2]       SMSQ10.100
GPNAM[3]       SMSQ10.100
GPNAM[4]       SMSQ10.100
GPZ1                 1.00 %
GPZ2                 3.00 %
GPZ3                31.00 %
GPZ4                11.00 %
P16               1000.00 usec

F1 - Acquisition parameters
TD                    256
SFO1             500.1324 MHz
FIDRES          18.565826 Hz
SW                  9.503 ppm
FnMODE        States-TPPI

F2 - Processing parameters
SI                   1024
SF            500.1300000 MHz
WDW                 QSINE
SSB                     2
LB             0 Hz
GB             0
PC                   1.40

F1 - Processing parameters
SI                   1024
MC2           States-TPPI
SF            500.1300000 MHz
WDW                 QSINE
SSB                     2
LB             0 Hz
GB             0
```

Figure S53d. *(^{1}H-^{1}H) TOCSY of Cy5.5 ZW,* ***(4)*** *in 20% D$_2$O in MeOD.*

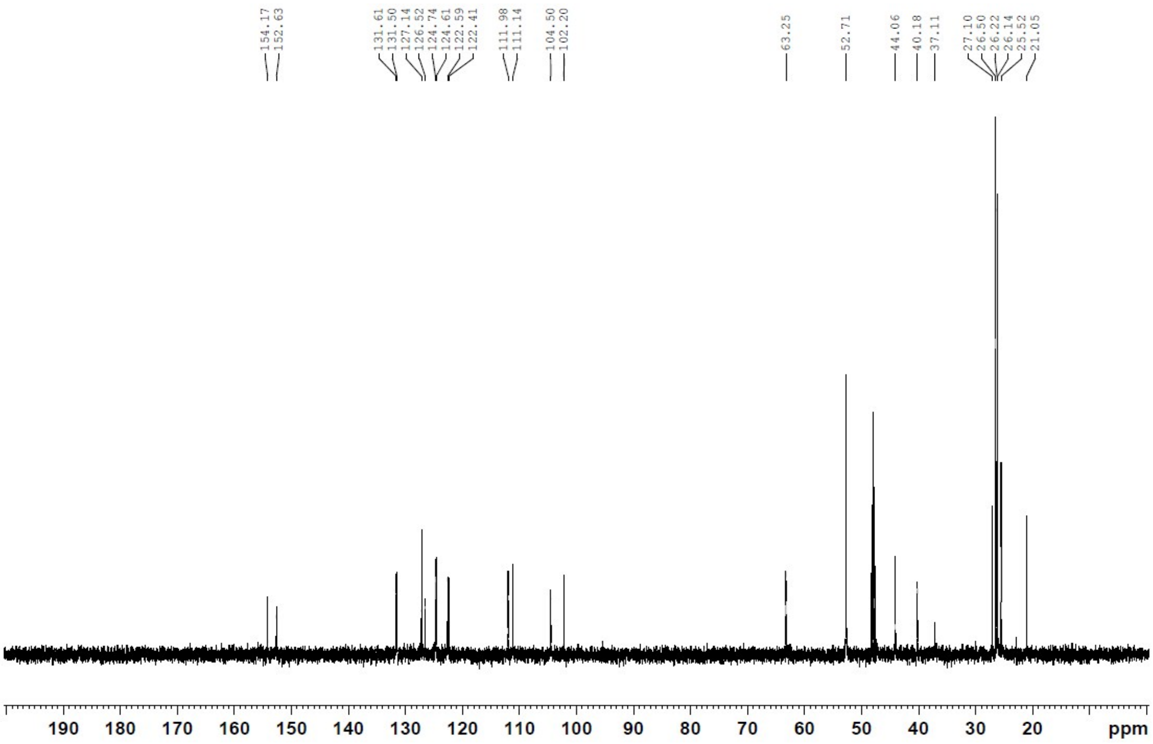


Figure S53e. ^{13}C *DEPT45 (CH, CH_2, CH_3 signals) of Cy5.5 ZW, **(4)** in 20% D_2O in MeOD.*

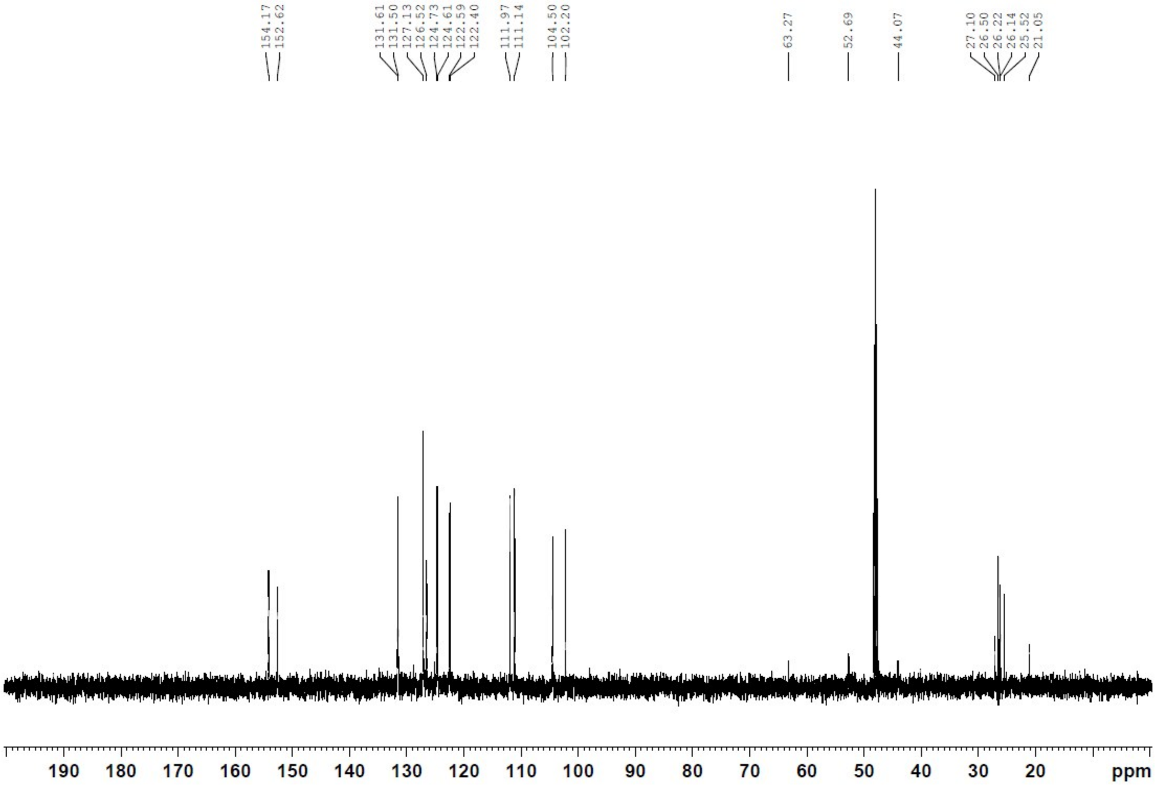


Figure S53f. ^{13}C *DEPT90 (CH signals only) of Cy5.5 ZW, **(4)** in 20% D_2O in MeOD.*

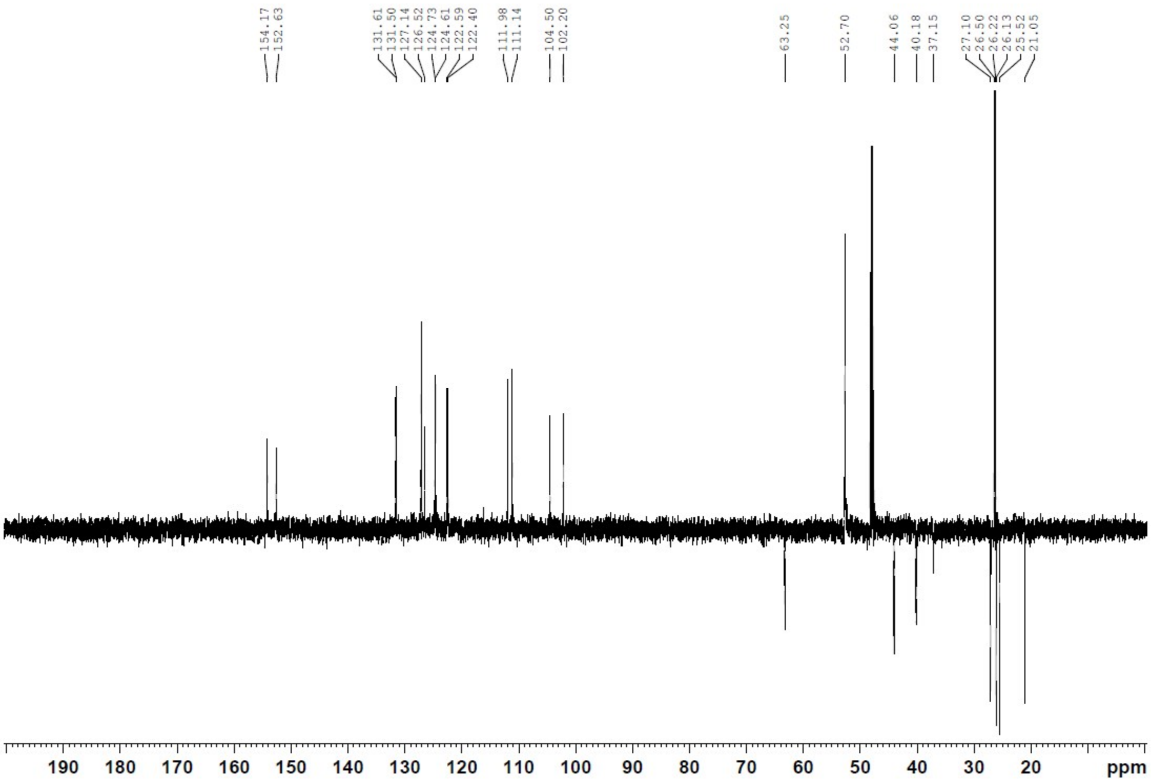
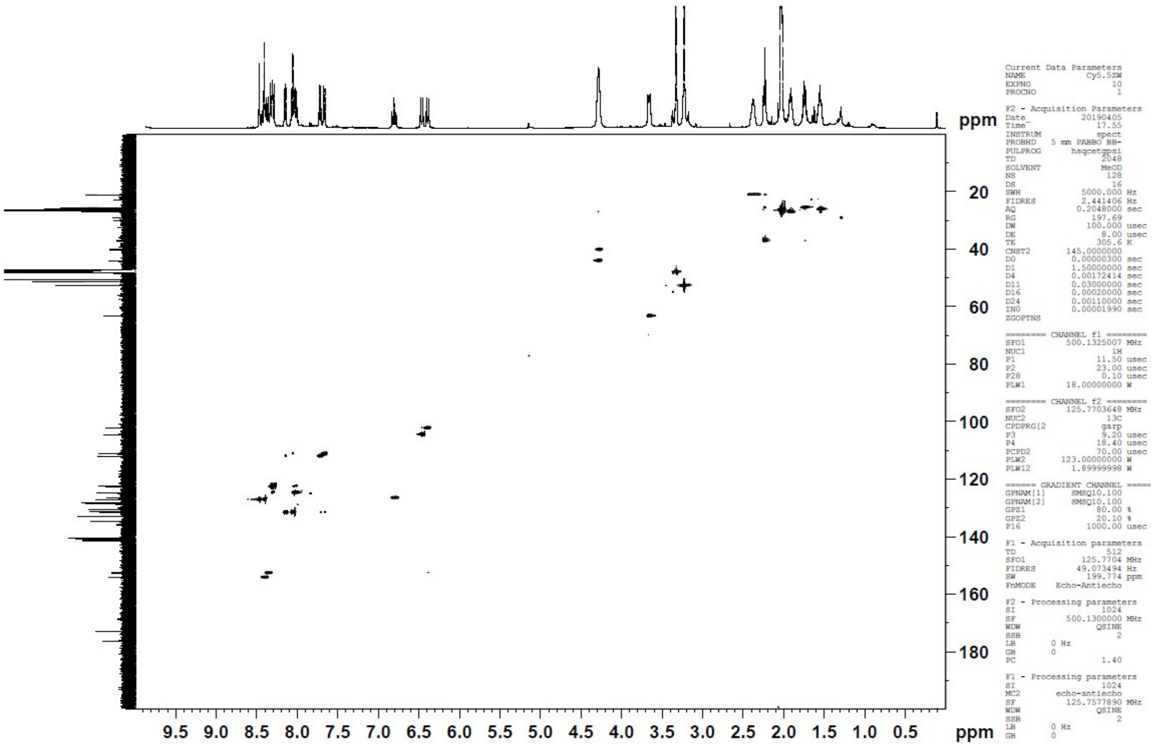
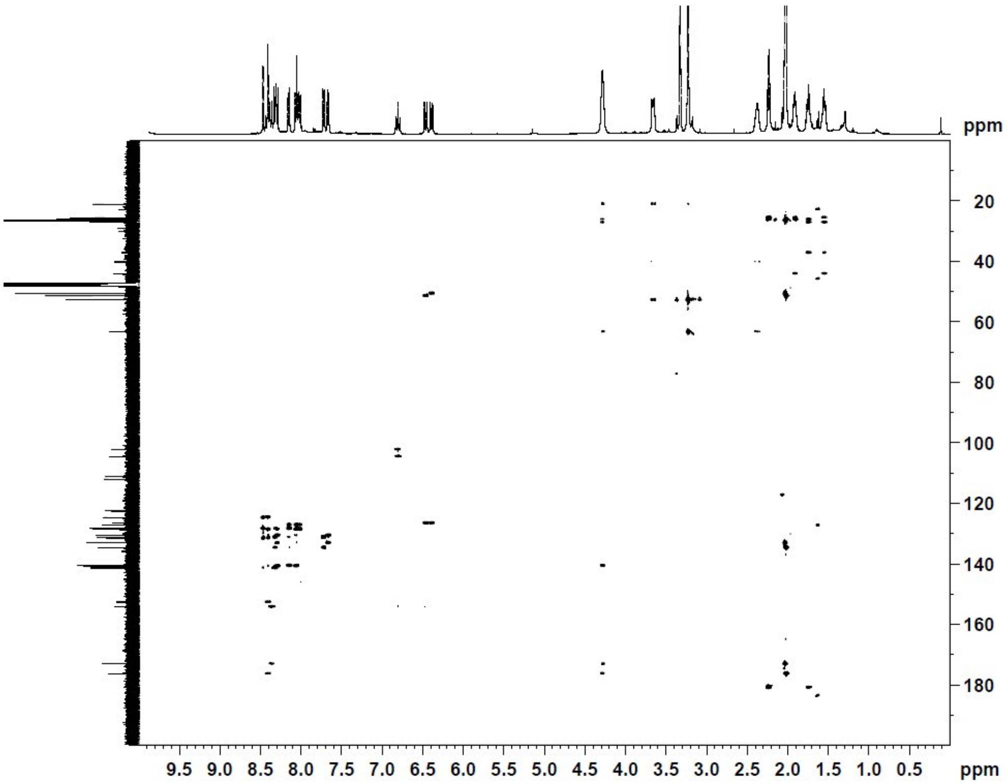


Figure S53g. *^{13}C DEPT135 (CH & CH_3 signals (+), CH_2 signals (-)) of Cy5.5 ZW,* ***(4)*** *in 20% D_2O in MeOD.*



```
Current Data Parameters
NAME              Cy5.5ZW
EXPNO                  10
PROCNO                  1

F2 - Acquisition Parameters
Date_            20190405
Time                17.55
INSTRUM             spect
PROBHD   5 mm PABBO BB-
PULPROG        hsqcetgpsi
TD                   2048
SOLVENT              MeOD
NS                    128
DS                     16
SWH              5000.000 Hz
FIDRES           2.441406 Hz
AQ              0.2048000 sec
RG                 197.69
DW                100.000 usec
DE                   8.00 usec
TE                  305.6 K
CNST2         145.0000000
D0             0.00000300 sec
D1             1.50000000 sec
D4             0.00172414 sec
D11            0.03000000 sec
D16            0.00020000 sec
D24            0.00110000 sec
IN0            0.00001990 sec
ZGOPTNS

======== CHANNEL f1 ========
SFO1          500.1325007 MHz
NUC1                   1H
P1                  11.50 usec
P2                  23.00 usec
P28                  0.10 usec
PLW1          18.00000000 W

======== CHANNEL f2 ========
SFO2          125.7703648 MHz
NUC2                  13C
CPDPRG[2             garp
P3                   9.20 usec
P4                  18.40 usec
PCPD2               70.00 usec
PLW2         123.00000000 W
PLW12          1.89999998 W

====== GRADIENT CHANNEL =====
GPNAM[1]       SMSQ10.100
GPNAM[2]       SMSQ10.100
GPZ1                80.00 %
GPZ2                20.10 %
P16               1000.00 usec

F1 - Acquisition parameters
TD                    512
SFO1             125.7704 MHz
FIDRES          49.073494 Hz
SW                199.774 ppm
FnMODE     Echo-Antiecho

F2 - Processing parameters
SI                   1024
SF            500.1300000 MHz
WDW                 QSINE
SSB                     2
LB          0 Hz
GB          0
PC                   1.40

F1 - Processing parameters
SI                   1024
MC2         echo-antiecho
SF            125.7577890 MHz
WDW                 QSINE
SSB                     2
LB          0 Hz
GB          0
```

Figure S53h. *(^{1}H-^{13}C) HSQC of Cy5.5 ZW,* ***(4)*** *in 20% D_2O in MeOD.*



```
Current Data Parameters
NAME               Cy5.5ZW
EXPNO                   11
PROCNO                   1

F2 - Acquisition Parameters
Date_             20190407
Time                  1.22
INSTRUM              spect
PROBHD   5 mm PABBO BB-
PULPROG     hmbcetgpl3nd
TD                    2048
SOLVENT               MeOD
NS                     100
DS                      16
SWH               5000.000 Hz
FIDRES            2.441406 Hz
AQ               0.2048000 sec
RG                  197.69
DW                 100.000 usec
DE                    8.00 usec
TE                   305.8 K
CNST6          120.0000000
CNST7          170.0000000
CNST13           8.0000000
CNST30           0.5981154
D0              0.00000300 sec
D1              2.00000000 sec
D6              0.06250000 sec
D16             0.00020000 sec
IN0             0.00001990 sec

======== CHANNEL f1 ========
SFO1           500.1325007 MHz
NUC1                    1H
P1                   11.50 usec
P2                   23.00 usec
PLW1           18.00000000 W

======== CHANNEL f2 ========
SFO2           125.7703648 MHz
NUC2                   13C
P3                    9.20 usec
P24                2000.00 usec
PLW2          123.00000000 W
SPNAM[7]       Crp60comp.4
SPOAL7               0.500
SPOFFS7  0 Hz
SPW7           15.90600014 W

====== GRADIENT CHANNEL =====
GPNAM[1]        SMSQ10.100
GPNAM[3]        SMSQ10.100
GPNAM[4]        SMSQ10.100
GPNAM[5]        SMSQ10.100
GPNAM[6]        SMSQ10.100
GPZ1                 80.00 %
GPZ3                 14.00 %
GPZ4                 -8.00 %
GPZ5                 -4.00 %
GPZ6                 -2.00 %
P16                1000.00 usec

F1 - Acquisition parameters
TD                     512
SFO1              125.7704 MHz
FIDRES           49.073494 Hz
SW                 199.774 ppm
FnMODE      Echo-Antiecho

F2 - Processing parameters
SI                    2048
SF             500.1300000 MHz
WDW                  QSINE
SSB                      2
LB       0 Hz
GB       0
PC                    1.40

F1 - Processing parameters
SI                    1024
MC2          echo-antiecho
SF             125.7577890 MHz
WDW                  QSINE
SSB                      2
LB       0 Hz
GB       0
```

Figure S53i. *(^{1}H-^{13}C) HMBC of Cy5.5 ZW,* ***(4)*** *in 20% D_2O in MeOD. D6 was set to 41.7ms.*