Electronic Supplementary Material (ESI) for New Journal of Chemistry.

Supplementary Information

Cyanine-based [^{18}F]F-*C*-glycosyl dual imaging probe: Synthesis, physico-chemical characterizations, in vitro binding evaluation and direct [^{18}F]fluorination.

Timothé Vucko, Julen Ariztia, Kamal Jouad, Dylan Chapeau, Benjamin Mourot, Valérie Jouan-Hureaux, Charlotte Collet, Cédric Boura, Katalin Selmeczi, Nadia Pellegrini-Moïse, Sandrine Lamandé-Langle

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1. Experimental section

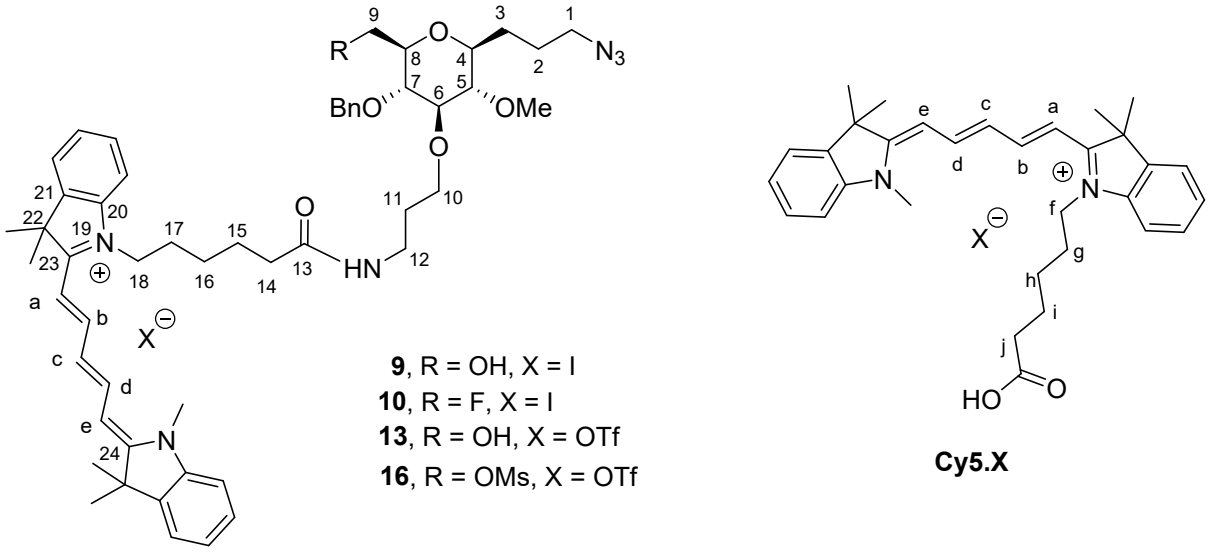


Figure S1. Atom numbering of compounds **9**, **10**, **13**, **16** and **Cy5.X**

Synthesis and physico-chemical characterization of 4,8-anhydro-1-azido-1,2,3-trideoxy-6-*O*-[3-(1,3-dihydro-1,3-dioxo-2H-isoindol-2-yl)propyl]-5-*O*-methyl-7,9-*O*-phenylmethylene-D-*glycero*-D-*gulo*-nonitol (3)[1]

To a solution of **2**[1] (150 mg, 0.4 mmol) in dry toluene (5 mL), was added under argon, dibutyltin oxide (130 mg, 0.68 mmol, 1.5 equiv.). The solution was stirred for 2 h at 110°C with a Dean-Stark apparatus. After evaporation under *vacuum*, the product was diluted in 3 mL of dry DMF, then CsF (100 mg, 0.68 mmol, 1.5 equiv.) and functionalized bromide (1 mmol, 2.5 equiv.) were added under argon at room temperature. The solution was then stirred for 48 h at 90°C. After evaporation under *vacuum*, the crude product was purified by flash chromatography on silica gel (eluent: cyclohexane/EtOAc 80/20 to 50/50). The phtaliminated derivatives was obtained in 31% yield. To a solution of phtaliminated compound (300 mg, 0.576 mmol) in DMF (6 mL), was added dropwise, under argon at 0°C, NaH (60% w/w) (60 mg, 1.04 mmol, 1.8 equiv.) and CH_3I (1.20 mL, 1.728 mmol, 30 equiv.). The solution was stirred for 1 h at room temperature, was then diluted with MeOH and evaporated *under vacuum*. The residue was diluted in EtOAc and washed with an aqueous saturated solution of $NaHCO_3$ and with water. The solution was dried over $MgSO_4$, filtered and evaporated *under vacuum*. The crude product was purified by column chromatography on silica gel (eluent: cyclohexane/EtOAc: 95/5 to 60/40) to give **3**.

Yield: 77%; White Gum; $[\alpha]_D^{25}$ -45.7 (*c* 0.1, $CHCl_3$); IR (film), ν 2924, 2866, 2093, 1769, 1703, 1466, 1449, 1391, 1368, 1342, 1312, 1260 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.44-1.51 (m, 1H, H-3a), 1.59-1.68 (m, 1H, H-2a), 1.73-1.90 (m, 2H, H-3b and H-2b), 1.94-2.03 (m, 2H, H-12), 2.78 (bt, 1H, $J_{5.4} = J_{5,6} = 9.5$ Hz, H-5), 3.23 (app td, 1H, $J_{4,5} = J_{4,3a} = 9.5$ Hz, $J_{4,3b} = 2.0$ Hz, H-4), 3.29 (t, 2H, $J_{1,2} = 7.0$ Hz, H-1), 3.29-3.34 (m, 1H, H-8), 3.44-3.50 (m, 2H, H-6 and H-7), 3.57 (s, 3H, CH_3), 3.65 (app t, 1H, $J_{9a,9b} = J_{9a,8} = 10.0$ Hz, H-9a), 3.72 (ddd, 1H, $J_{11a,11b} = 12.0$ Hz, $J_{11a,12a} = 9.5$ Hz, $J_{11a,12b} = 5.5$ Hz, H-11a), 3.79-3.86 (m, 2H, H-13), 4.02 (ddd, 1H, $J_{11b,12a} = 7.0$ Hz, $J_{11b,12b} = 5.5$ Hz, H-11b), 4.28 (dd, 1H, $J_{9b,8} = 4.5$ Hz, H-9b), 5.53 (s, 1H, H-10), 7.28-7.33 (m, 3H, H_{Ar}), 7.41-7.45 (m, 2H, H_{Ar}), 7.64-7.69 (m, 2H, H_{Ar}), 7.76-7.80 (m, 2H, H_{Ar}); ^{13}C NMR (100.6 MHz, $CDCl_3$,): δ 25.3 (C-2), 29.2 (C-3 or C-12), 29.4 (C-12 or C-3), 35.8 (C-13), 51.6 (C-1), 61.5 (C_{CH3}), 69.1 (C-9), 70.2 (C-8), 70.3 (C-11), 79.5 (C-4), 82.5 (C-6 or C-7), 83.6 (C-5 and C-6 or C-7), 101.0 (C-10), 123.2 (2 C_{Ar}), 126.0 (2C_{Ar}), 128.3 (2C_{Ar}), 128.9 (C_{qAr}), 132.4 (2 C_{qAr}), 133.9 (2C_{Ar}), 137.6 (C_{qAr}), 168.5 (2 C=O); HRMS (ESI): calcd for $C_{28}H_{32}N_4NaO_7$ $[M+H]^+$: 559.2169, found: 559.2215.

Synthesis of alkyne-modified c(RGDfK) (11)[2]

To a solution of commercially available c(RGDfK) (50 mg, 83 µmol, 1 eq.) and NEt_3 (34 µL, 250 µmol, 2.5 eq.) in DMF (1.5 mL) was added 1-(pent-4-ynoyloxy)pyrrolidine-2,5-dione (19.9 mg, 100 mmol, 1.2 eq.). The reaction was stirred for 16 h at room temperature and then evaporated under reduced pressure. MeOH (1 mL) was added to the reaction mixture and the peptide was precipitated in Et_2O (10 mL) and filtered to afford **11** (34 mg).

Yield 61%. Beige solid. ^1H NMR (D_2O, 400 MHz): δ = 1.26 (qt, 2H, $J_{12,13} = J_{12,11} = 7.5$ Hz, H12), 1.65 (qt, 2H, $J_{13,12} = J_{13,14} = 7.5$ Hz, H13), 1.70-1.86 (m, 3H, H11a, H2), 1.86-2.00 (m, 2H, H11b, H3a), 2.08-2.21 (m, 1H, H3b), 2.65-2.75 (brt, 2H, $J_{16,17} = 7.0$ Hz, H16), 2.79 (bt, 2H, H15), 2.94 (dd, 1H, $J_{7a,7b} = 16.5$ Hz, $J_{7a,6} = 7.0$ Hz, H7a), 3.07 (s, 1H, H18), 3.10 (dd, 1H, $J_{7b,7a} = 16.5$ Hz, $J_{7b,6} = 7.0$ Hz, H7b), 3.22-3.29 (m, 1H, H5a), 3.32-3.43 (m, 3H, H14, H5b), 3.43-3.54 (m, 2H, H1), 3.78 (d, 1H, *J* = 14.5 Hz, CH_2Bn), 4.16 (dd, 1H, *J* = 9.5 Hz, *J* = 3.5 Hz, H10), 4.50 (d, 1H, *J* = 14.5 Hz, CH_2Bn), 4.65 (app t, 1H, *J* = 7.0 Hz, H4), 4.77-4.87 (m, 1H, H8), 5.02 (appt, 1H, H6), 7.55 (d, 2H, *J* = 7.0 Hz, HAr), 7.58-763 (m, 1H, HAr), 7.67 (t, 2H, Har) ^{13}C NMR (D_2O, 100.6 MHz): δ = 14.9 (C16), 22.8 (C12), 24.7 (C2), 27.7 (C3 or C13), 27.8 (C13 or C3), 30.2 (C11), 34.8 (C15), 35.7 (C7), 37.2 (C5), 39.2 (C14), 40.9 (C1), 43.9 (CH_2Bn), 50.3 (C6), 52.7 (C4), 55.4 (C8 or C10), 55.7 (C10 or C8), 70.5 (C18), 84.0 (C17), 127.5 (CAr), 129.1 (2CAr), 129.5 (2CAr), 136.4 (CqAr), 157.1 (C=N), 171.5 (C=O), 172.0

(C=O), 173.0 (C=O), 173.3 (C=O), 174.6 (C=O), 174.7 (C=O), 176.6 (C=O). HRMS (ESI, $C_{32}H_{45}N_9O_8$ $[M]^+$) calcd 684.3469, found 684.3483.

Synthesis of compound Cy5.OTf and Cy5.TFA

Compound **Cy5.OTf** was synthesized using the same protocol than for compound **13** and was obtained quantitatively without further purification.

Compound **Cy5.TFA** was synthesized by elution of **Cy5.I** on silica gel column chromatography with CH_2Cl_2/MeOH (95/5 v/v) and 0.1% TFA. The compound **Cy5.TFA** was obtained quantitatively without further purification.

2-(5-(1-(5-Carboxypentyl)-3,3-dimethylindolin-2-ylidene)penta-1,3-dien-1-yl)-1,3,3-trimethyl-3H-indol-1-ium trifluoroacetate salt (Cy5.TFA)

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.52-1.63 (m, 2H, Hh), 1.69 (s, 12H, 4 CH_3), 1.79-1.87 (m, 4H, Hi and Hg), 2.51 (t, 2H, $J_{i,j}$ = 7.0 Hz, Hj), 3.64 (s, 3H, N-CH_3), 4.02 (t, 2H, $J_{f,g}$ = 7.5 Hz, Hf), 6.35 (d, 1H, $J_{d,e}$ = 13.5 Hz, He), 6.43 (d, 1H, $J_{a,b}$ = 13.5 Hz, Ha), 6.87 (app bt, 1H, $J_{b,c}$ = $J_{c,d}$ = 12.5 Hz, Hc), 7.06 (d, 1H, J = 7.5 Hz, H_{Ar}), 7.09 (d, 1H, H_{Ar}), 7.20-7.27 (m, 2H, H_{Ar}), 7.35 (d, 2H, J = 7.5 Hz, H_{Ar}), 7.36-7.41 (m, 2H, H_{Ar}), 7.76 (app t (dd), 1H, $J_{b,c}$ = $J_{b,a}$ = 13.5 Hz, Hb), 7.77 (app t (dd), 1H, $J_{d,e}$ = $J_{c,d}$ = 13.5 Hz, Hd).

^{1}H NMR (DMSO-d6, 400 MHz): δ = 1.35-1.42 (m, 2H, Hh), 1.50-1.59 (m, 2H, Hi), 1.68 (s, 12H, 4 CH_3), 1.65-1.74 (m, 2H, Hg), 2.20 (t, 2H, $J_{i,j}$ = 7.0 Hz, Hj), 3.60 (s, 3H, N-CH_3), 4.09 (t, 2H, $J_{f,g}$ = 7.5 Hz, Hf), 6.26 (d, 1H, $J_{d,e}$ = 13.5 Hz, He), 6.30 (d, 1H, $J_{a,b}$ = 13.5 Hz, Ha), 6.56 (app t, 1H, $J_{b,c}$ = $J_{c,d}$ = 12.5 Hz, Hc), 7.21-7.28 (m, 2H, H_{Ar}), 7.36-7.44 (m, 4H, H_{Ar}), 7.61 (d, 2H, J = 7.0 Hz, H_{Ar}), 8.32 (app t (dd), 2H, $J_{b,c}$ = $J_{b,a}$ = $J_{d,e}$ = $J_{c,d}$ = 13.5 Hz, Hb and Hd).

2-(5-(1-(5-Carboxypentyl)-3,3-dimethylindolin-2-ylidene)penta-1,3-dien-1-yl)-1,3,3-trimethyl-3H-indol-1-ium chloride (Cy5.Cl)

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.50-1.59 (m, 2H, Hh), 1.72 (s, 6H, 2 CH_3), 1.73 (s, 6H, 2 CH_3), 1.72-1.84 (m, 4H, Hi and Hg), 2.46 (t, 2H, $J_{i,j}$ = 7.0 Hz, Hj), 3.69 (s, 3H, N-CH_3), 4.04 (t, 2H, $J_{f,g}$ = 7.5 Hz, Hf), 6.32 (d, 1H, $J_{d,e}$ = 13.5 Hz, He), 6.43 (d, 1H, $J_{a,b}$ = 13.5 Hz, Ha), 6.90 (app bt, 1H, $J_{b,c}$ = $J_{c,d}$ = 12.5 Hz, Hc), 7.09 (d, 1H, J = 7.5 Hz, H_{Ar}), 7.11 (d, 1H, H_{Ar}), 7.16-7.22 (m, 2H, H_{Ar}), 7.31-7.37 (m, 4H, H_{Ar}), 8.05 (app t (dd), 1H, $J_{b,c}$ = $J_{b,a}$ = 13.5 Hz, Hb), 8.07 (app t (dd), 1H, $J_{d,e}$ = $J_{c,d}$ = 13.5 Hz, Hd).

^{1}H NMR (DMSO-d6, 400 MHz): δ = 1.34-1.41 (m, 2H, Hh), 1.50-1.58 (m, 2H, Hi), 1.68 (s, 12H, 4 CH_3), 1.65-1.75 (m, 2H, Hg), 2.17 (t, 2H, $J_{i,j}$ = 7.0 Hz, Hj), 3.60 (s, 3H, N-CH_3), 4.09

(t, 2H, $J_{f,g}$ = 7.5 Hz, Hf), 6.26 (d, 1H, $J_{d,e}$ = 13.5 Hz, He), 6.30 (d, 1H, $J_{a,b}$ = 13.5 Hz, Ha), 6.57 (app t, 1H, $J_{b,c}$ = $J_{c,d}$ = 12.5 Hz, Hc), 7.21-7.28 (m, 2H, H_{Ar}), 7.36-7.43 (m, 4H, H_{Ar}), 7.61 (d, 2H, J = 7.0 Hz, H_{Ar}), 8.32 (app t (dd), 2H, $J_{b,c}$ = $J_{b,a}$ = $J_{d,e}$ = $J_{c,d}$ = 13.5 Hz, Hb and Hd).

2-(5-(1-(5-Carboxypentyl)-3,3-dimethylindolin-2-ylidene)penta-1,3-dien-1-yl)-1,3,3-trimethyl-3H-indol-1-ium triflate salt (Cy5.OTf)

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.50-1.59 (m, 2H, Hh), 1.70 (s, 6H, 2 CH_3), 1.71 (s, 6H, 2 CH_3), 1.72-1.85 (m, 4H, Hi and Hg), 2.43 (t, 2H, $J_{i,j}$ = 7.0 Hz, Hj), 3.64 (s, 3H, N-CH_3), 4.00 (t, 2H, $J_{f,g}$ = 7.5 Hz, Hf), 6.23 (d, 1H, $J_{d,e}$ = 13.5 Hz, He), 6.35 (d, 1H, $J_{a,b}$ = 13.5 Hz, Ha), 6.79 (app bt, 1H, $J_{b,c}$ = $J_{c,d}$ = 12.5 Hz, Hc), 7.06 (d, 1H, J = 7.0 Hz, H_{Ar}), 7.11 (d, 1H, H_{Ar}), 7.19-7.25 (m, 2H, H_{Ar}), 7.33-7.40 (m, 4H, H_{Ar}), 7.89 (app t (dd), 1H, $J_{b,c}$ = $J_{b,a}$ = 13.5 Hz, Hb), 7.91 (app t (dd), 1H, $J_{d,e}$ = $J_{c,d}$ = 13.5 Hz, Hd).

^{1}H NMR (DMSO-d6, 400 MHz): δ = 1.34-1.41 (m, 2H, Hh), 1.50-1.59 (m, 2H, Hi), 1.68 (s, 12H, 4 CH_3), 1.65-1.75 (m, 2H, Hg), 2.19 (t, 2H, $J_{i,j}$ = 7.0 Hz, Hj), 3.60 (s, 3H, N-CH_3), 4.09 (t, 2H, $J_{f,g}$ = 7.5 Hz, Hf), 6.26 (d, 1H, $J_{d,e}$ = 13.5 Hz, He), 6.30 (d, 1H, $J_{a,b}$ = 13.5 Hz, Ha), 6.56 (app t, 1H, $J_{b,c}$ = $J_{c,d}$ = 12.5 Hz, Hc), 7.21-7.27 (m, 2H, H_{Ar}), 7.36-7.44 (m, 4H, H_{Ar}), 7.61 (d, 2H, J = 7.0 Hz, H_{Ar}), 8.32 (app t (dd), 2H, $J_{b,c}$ = $J_{b,a}$ = $J_{d,e}$ = $J_{c,d}$ = 13.5 Hz, Hb and Hd).

2-(5-(1-(5-Carboxypentyl)-3,3-dimethylindolin-2-ylidene)penta-1,3-dien-1-yl)-1,3,3-trimethyl-3H-indol-1-ium iodide (Cy5.I)

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.57-1.63 (m, 2H, Hh), 1.72 (s, 6H, 2 CH_3), 1.74 (s, 6H, 2 CH_3), 1.75-1.86 (m, 4H, Hi and Hg), 2.49 (t, 2H, $J_{i,j}$ = 7.0 Hz, Hj), 3.73 (s, 3H, N-CH_3), 4.06 (t, 2H, $J_{f,g}$ = 7.5 Hz, Hf), 6.40 (d, 1H, $J_{d,e}$ = 13.5 Hz, He), 6.58 (d, 1H, $J_{a,b}$ = 13.5 Hz, Ha), 7.05 (app bt, 1H, $J_{b,c}$ = $J_{c,d}$ = 12.5 Hz, Hc), 7.06 (d, 1H, J = 7.5 Hz, H_{Ar}), 7.10 (d, 1H, H_{Ar}), 7.19-7.25 (m, 2H, H_{Ar}), 7.33-7.40 (m, 4H, H_{Ar}), 7.98 (app t (dd), 1H, $J_{b,c}$ = $J_{b,a}$ = 13.5 Hz, Hb), 8.00 (app t (dd), 1H, $J_{d,e}$ = $J_{c,d}$ = 13.5 Hz, Hd).

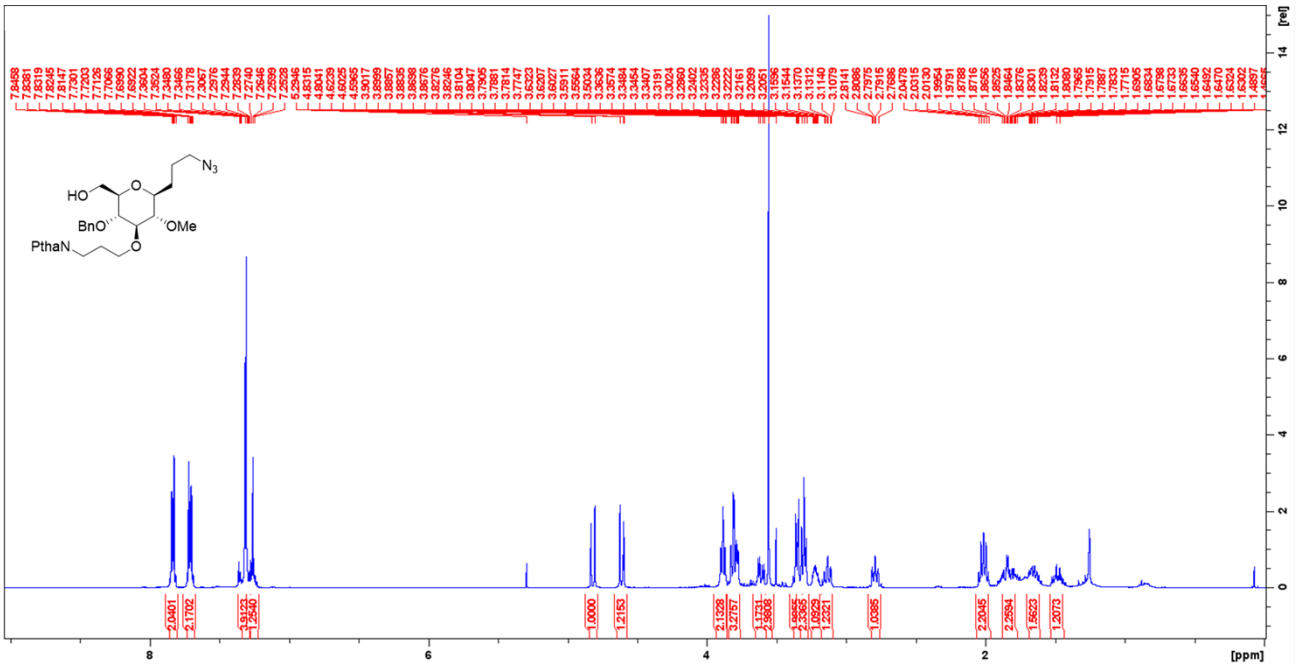
^{1}H NMR (DMSO-d6, 400 MHz): δ = 1.34-1.41 (m, 2H, Hh), 1.50-1.59 (m, 2H, Hi), 1.68 (s, 12H, 4 CH_3), 1.65-1.74 (m, 2H, Hg), 2.20 (t, 2H, $J_{i,j}$ = 7.0 Hz, Hj), 3.60 (s, 3H, N-CH_3), 4.09 (t, 2H, $J_{f,g}$ = 7.5 Hz, Hf), 6.26 (d, 1H, $J_{d,e}$ = 13.5 Hz, He), 6.30 (d, 1H, $J_{a,b}$ = 13.5 Hz, Ha), 6.56 (app t, 1H, $J_{b,c}$ = $J_{c,d}$ = 12.5 Hz, Hc), 7.21-7.28 (m, 2H, H_{Ar}), 7.36-7.44 (m, 4H, H_{Ar}), 7.61 (d, 2H, J = 7.0 Hz, H_{Ar}), 8.32 (app t (dd), 2H, $J_{b,c}$ = $J_{b,a}$ = $J_{d,e}$ = $J_{c,d}$ = 13.5 Hz, Hb and Hd).

Table 1. Comparison of the chemical shifts of the protons of the double bonds of **Cy5.X** depending of the cyanine-5 counter ion and the solvent

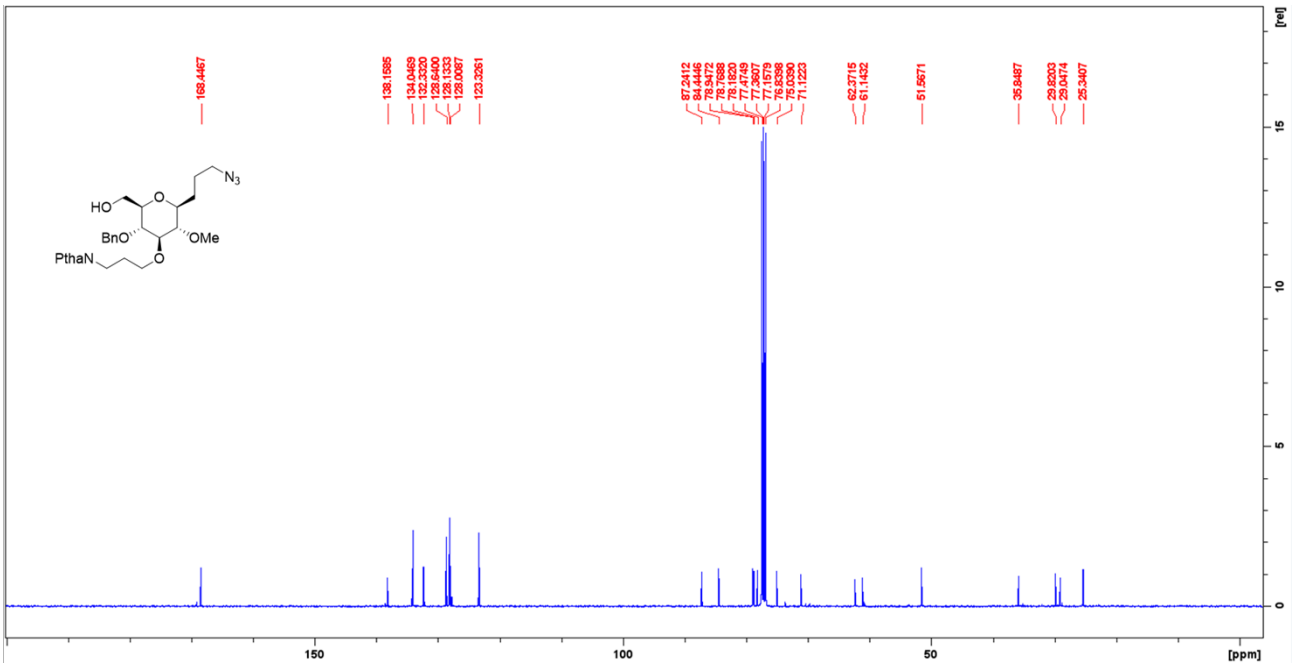
Protons	Solvent	Chemical shift depending on compounds (δ ppm)			
		Cy5-COOH.I	**Cy5-COOH.OTf**	**Cy5-COOH.Cl**	**Cy5-COOH.TFA**
He	$CDCl_3$	6.40	6.23	6.32	6.35
	DMSO-d6	6.26	6.26	6.26	6.26
Ha	$CDCl_3$	6.58	6.35	6.43	6.43
	DMSO-d6	6.30	6.30	6.30	6.30
Hc	$CDCl_3$	7.05	6.79	6.90	6.87
	DMSO-d6	6.56	6.56	6.57	6.56
Hb	$CDCl_3$	7.98	7.89	8.05	7.76
	DMSO-d6	8.32	8.32	8.32	8.32
Hd	$CDCl_3$	8.00	7.91	8.07	7.77
	DMSO-d6	8.32	8.32	8.32	8.32

2. NMR spectra

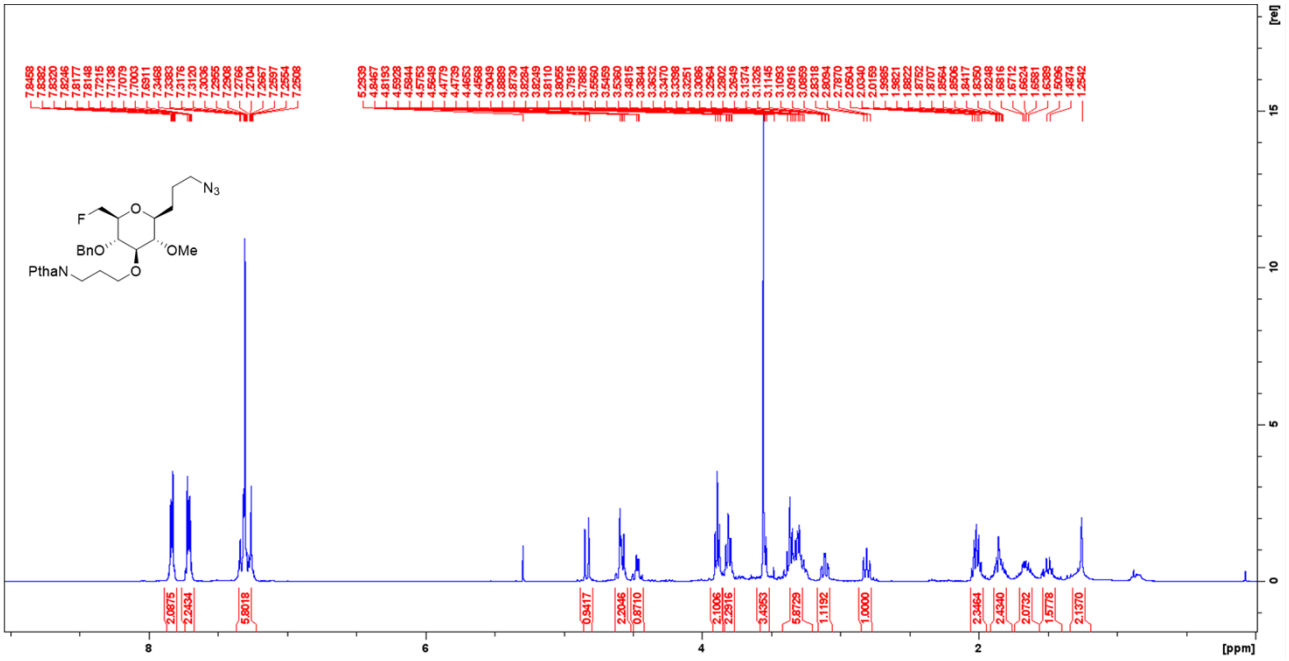
^{1}H of 4 (400 MHz, $CDCl_3$)



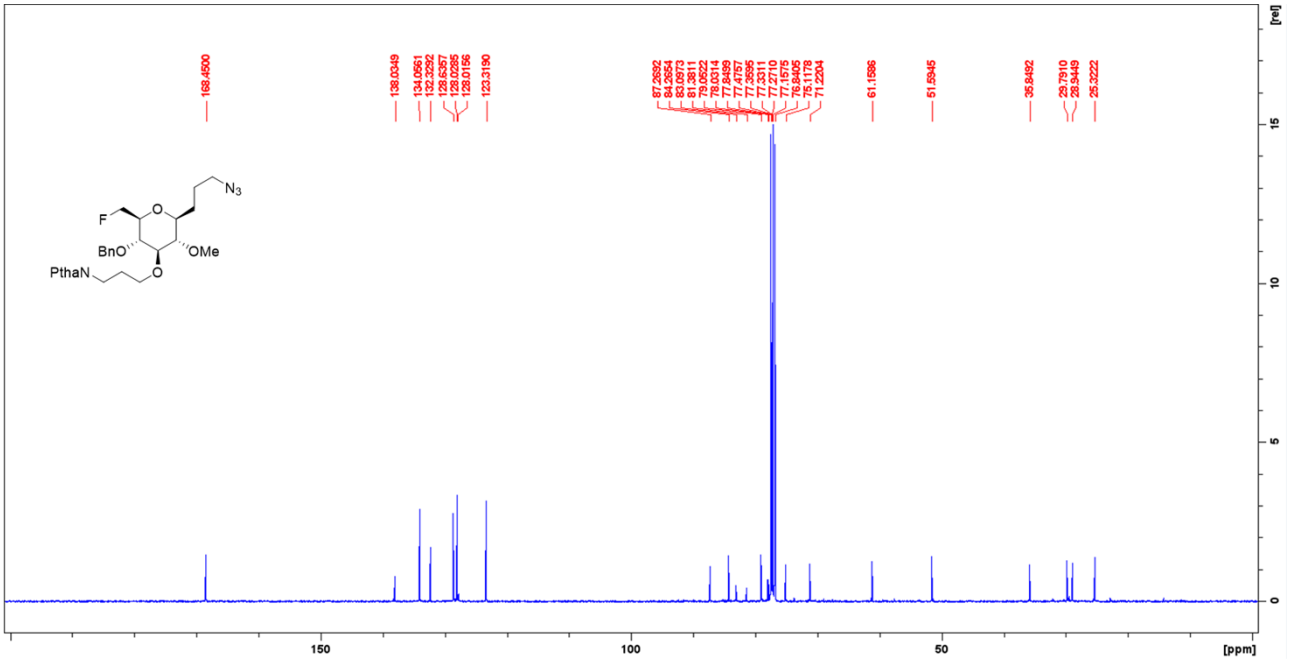
^{13}C of 4 (100.6 MHz, $CDCl_3$)



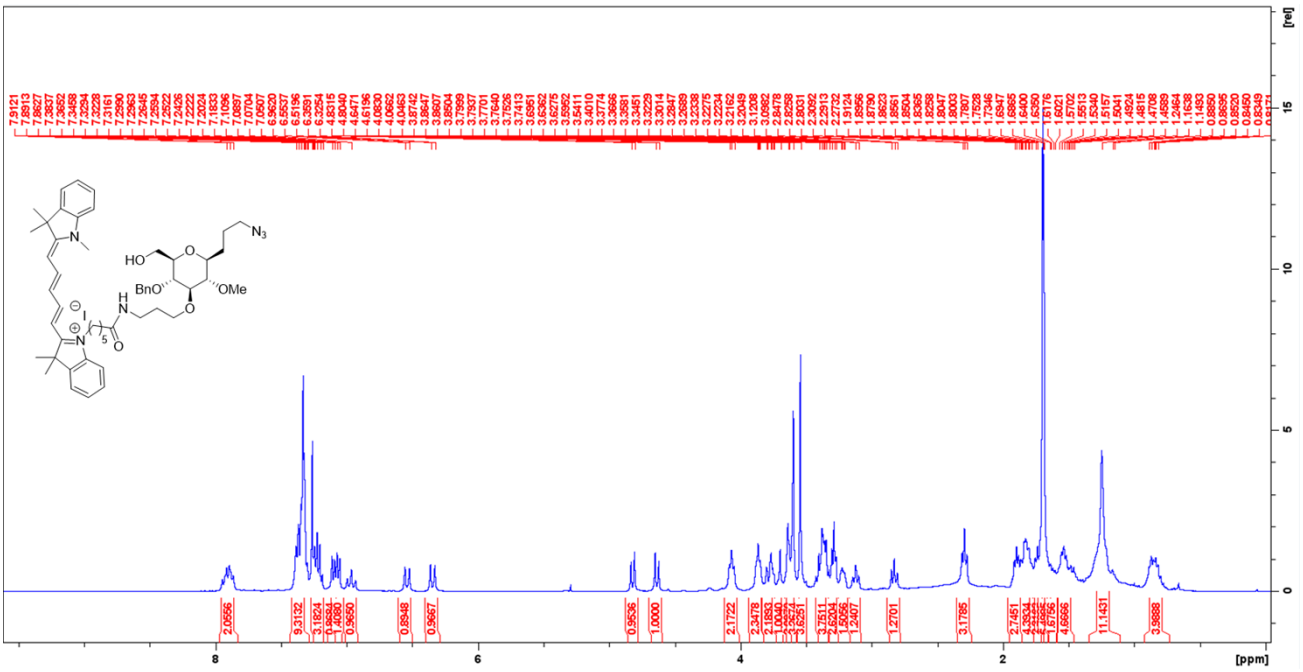
1H of 6 (400 MHz, $CDCl_3$)



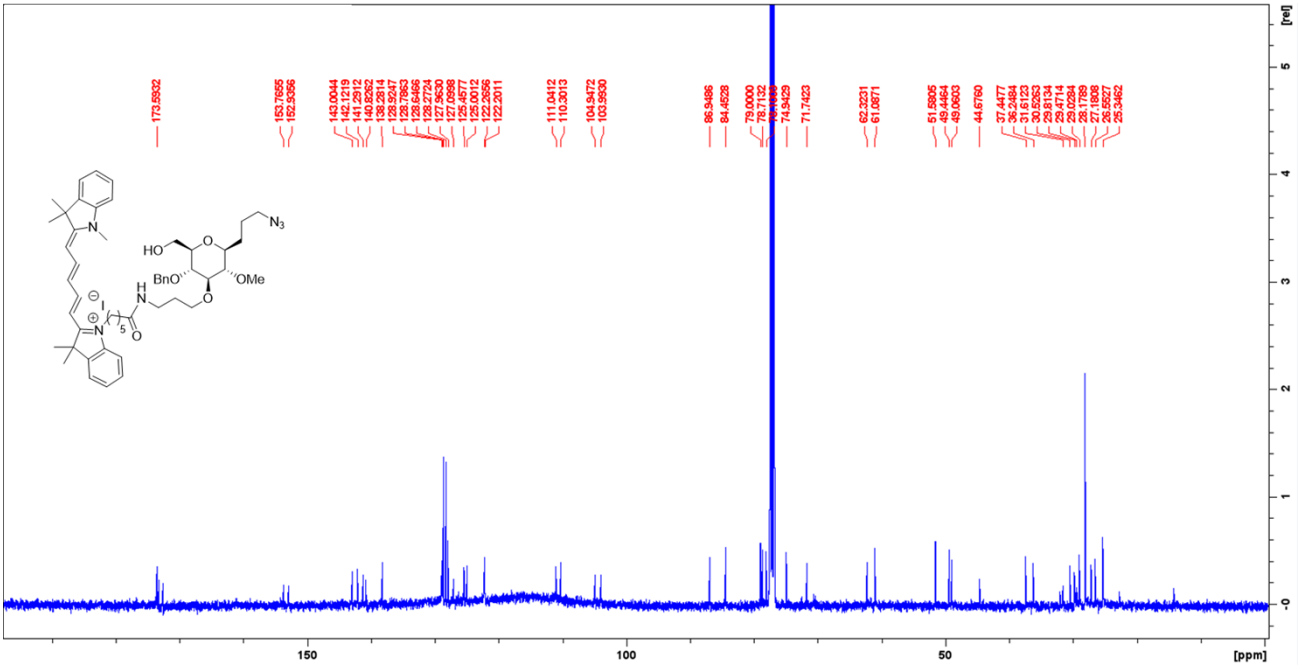
13C of 6 (100.6 MHz, $CDCl_3$)



1H of 9 (400 MHz, $CDCl_3$)



13C of 9 (100.6 MHz, $CDCl_3$)



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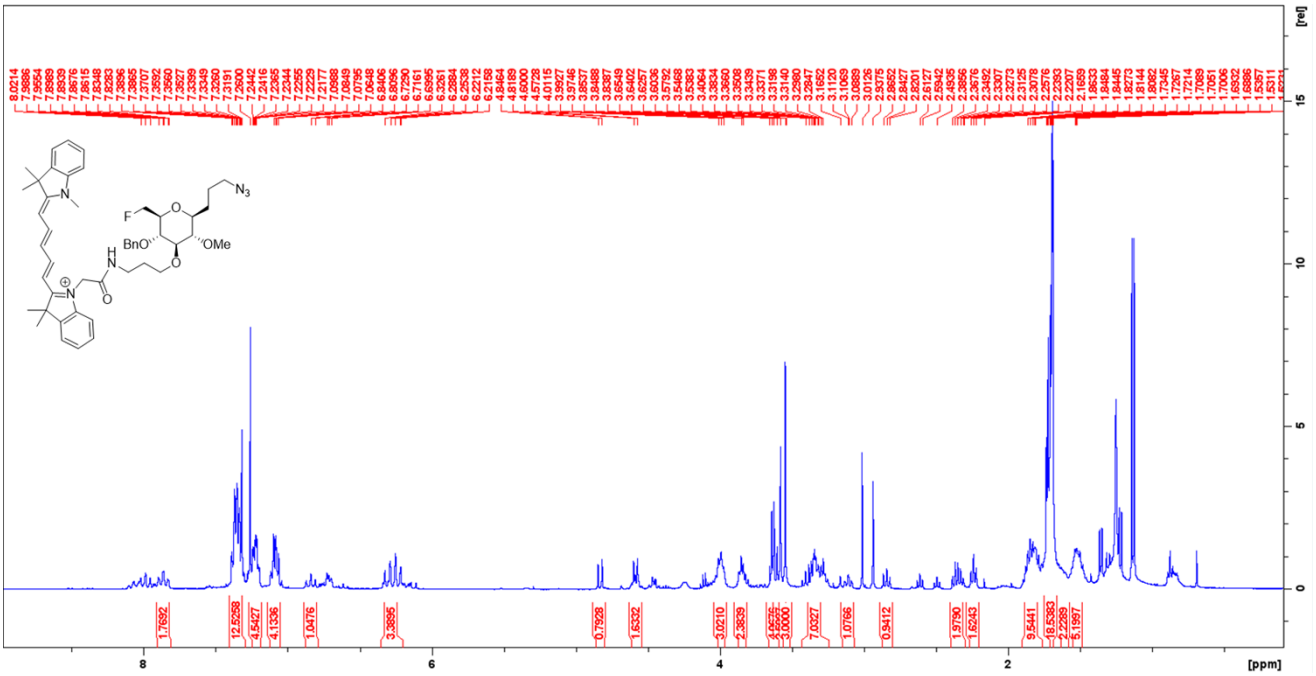

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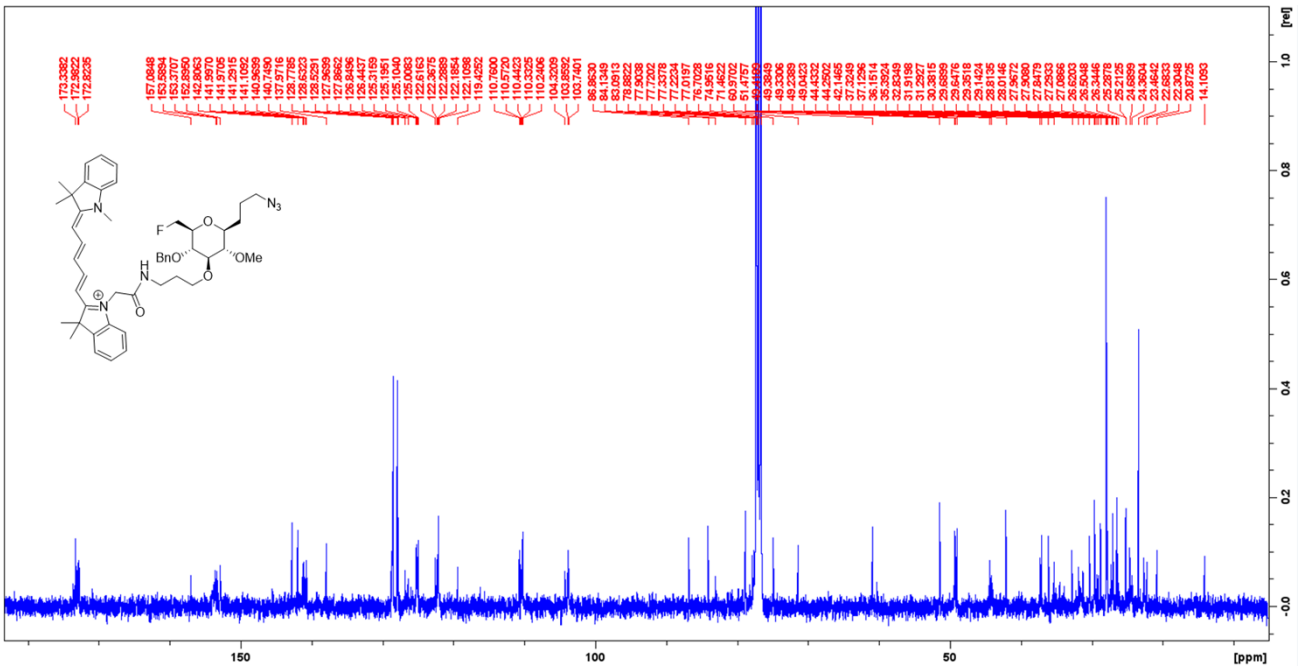

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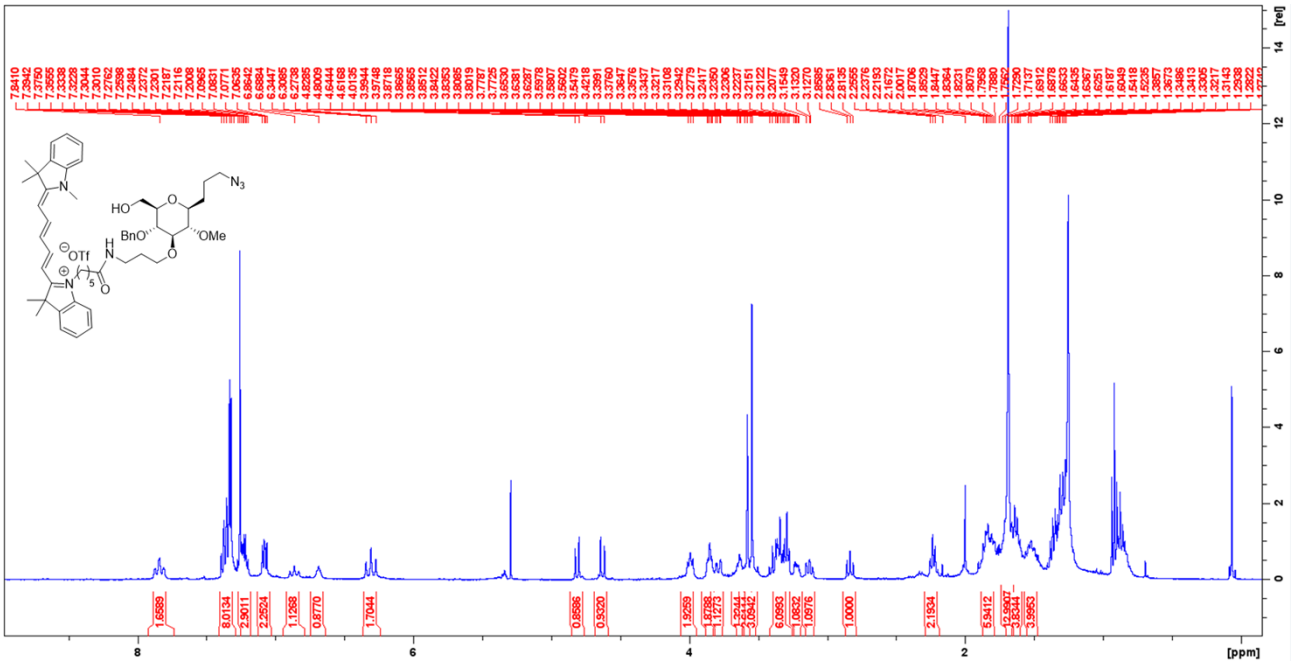
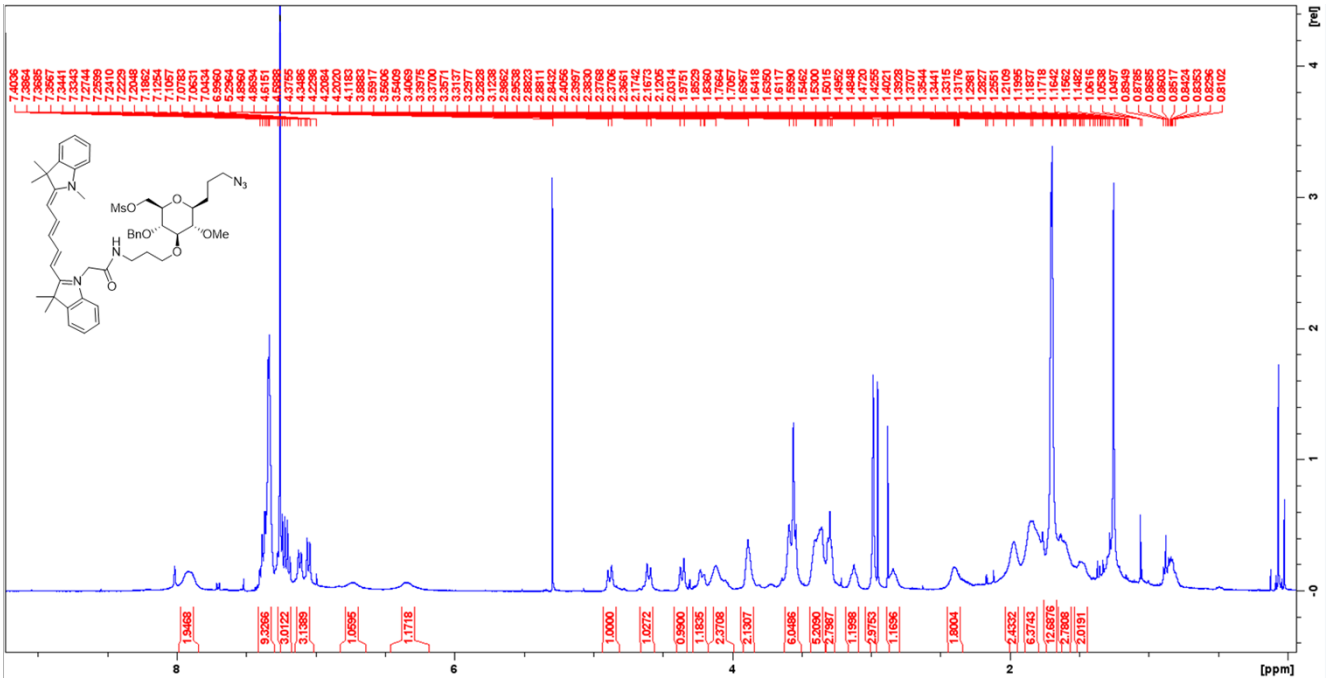
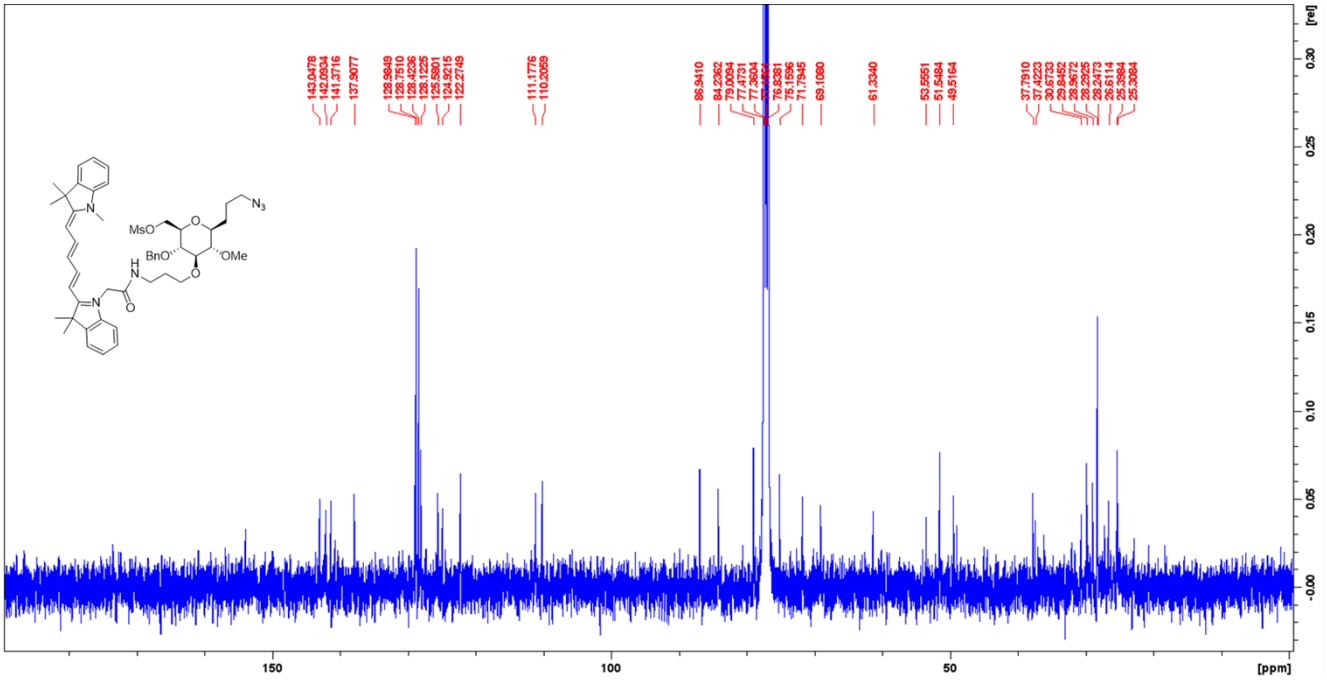


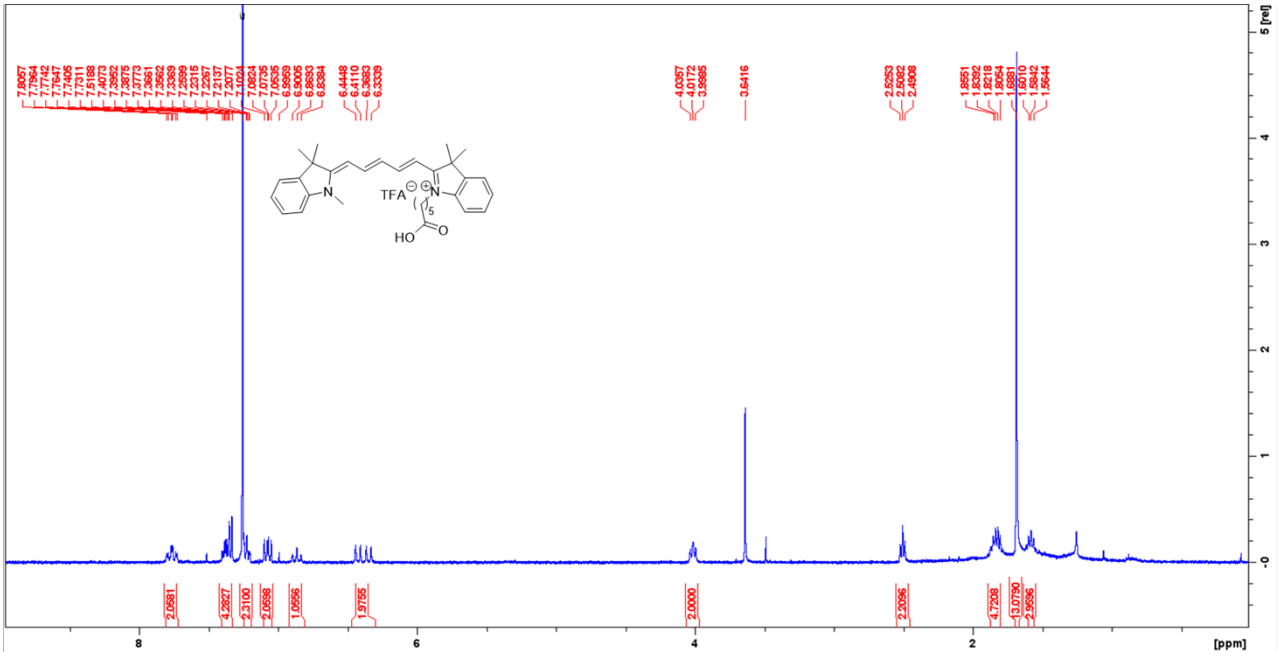
1H of 16 (400 MHz, $CDCl_3$)



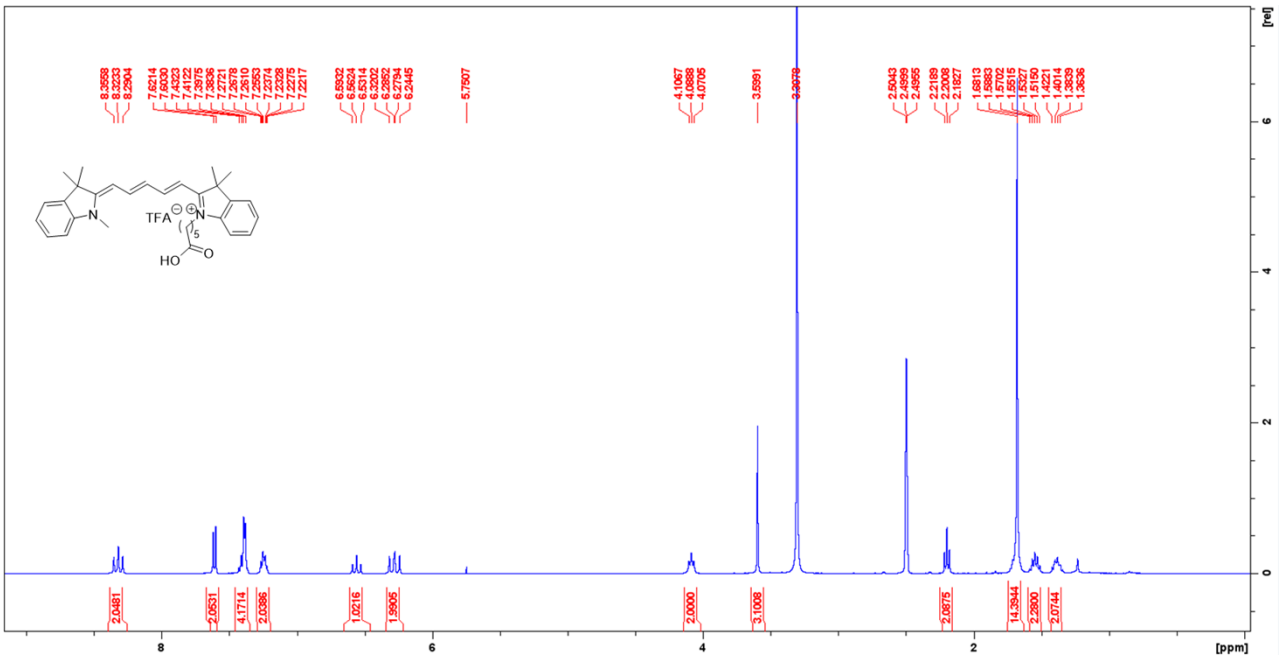
13C of 16 (100.6 MHz, $CDCl_3$)



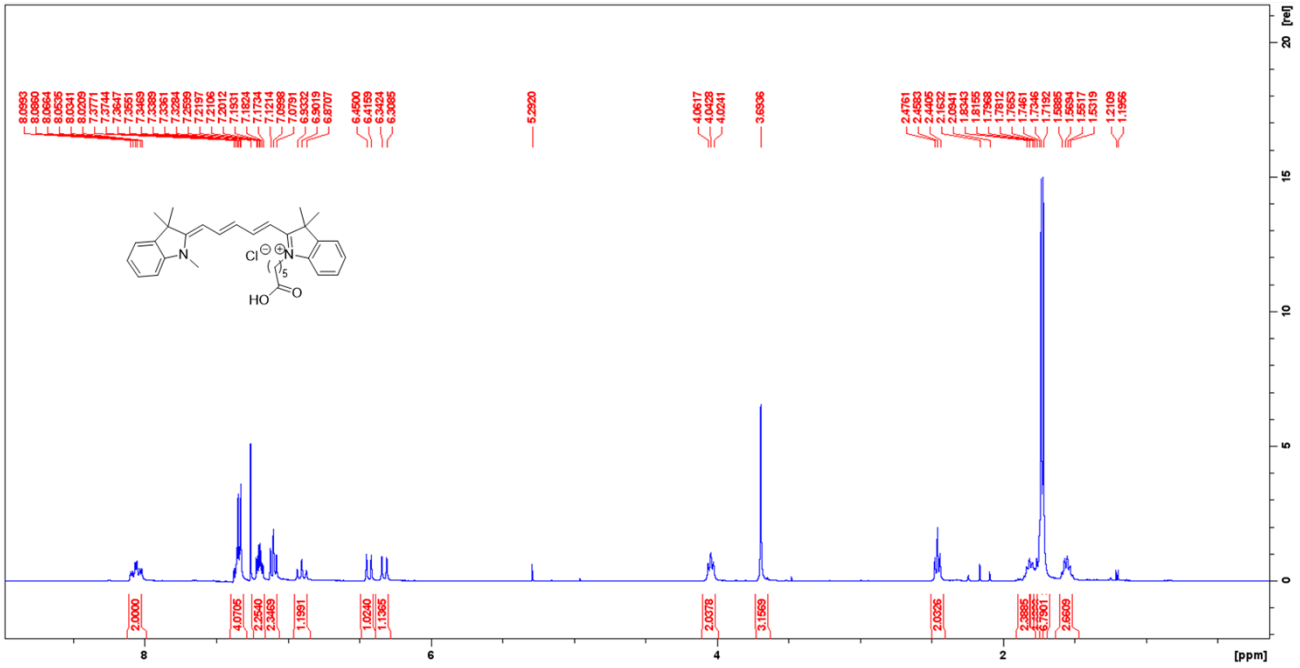
1H of Cy5.TFA (400 MHz, $CDCl_3$)



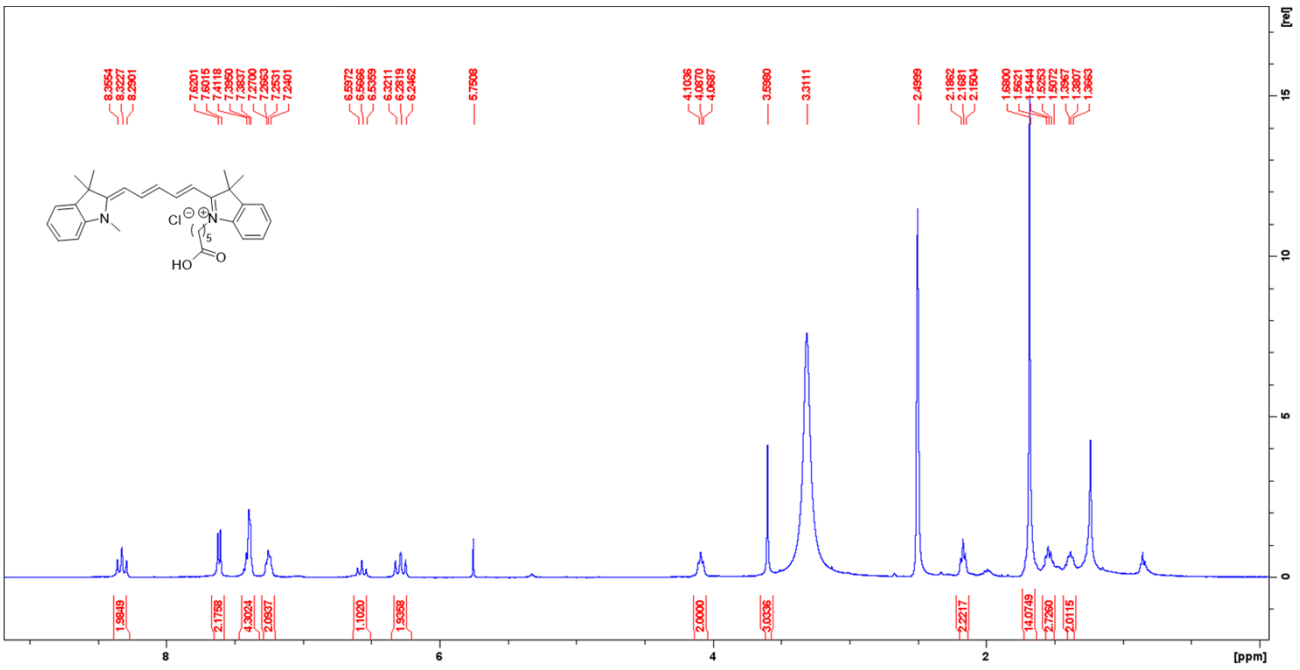
1H of Cy5.TFA (400 MHz, DMSO-d6)



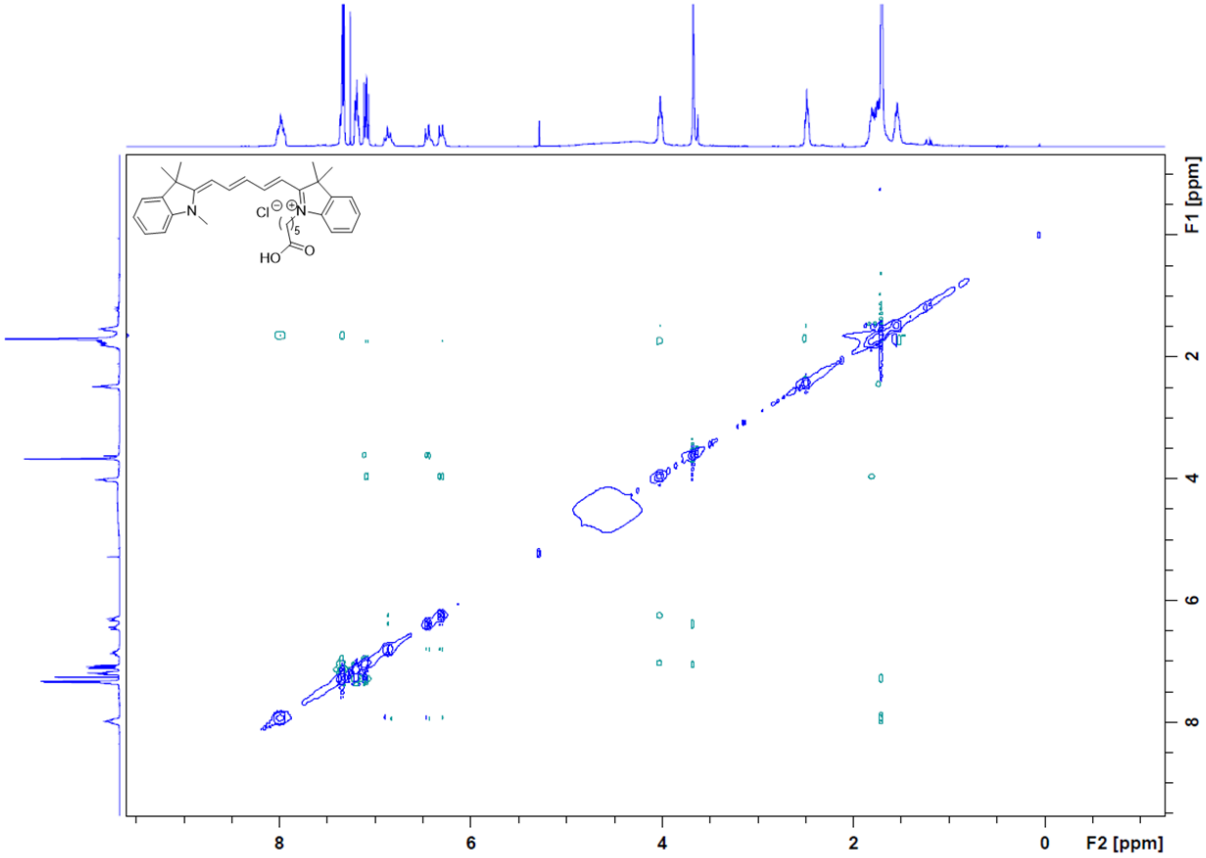
1H of Cy5.Cl (400 MHz, $CDCl_3$)



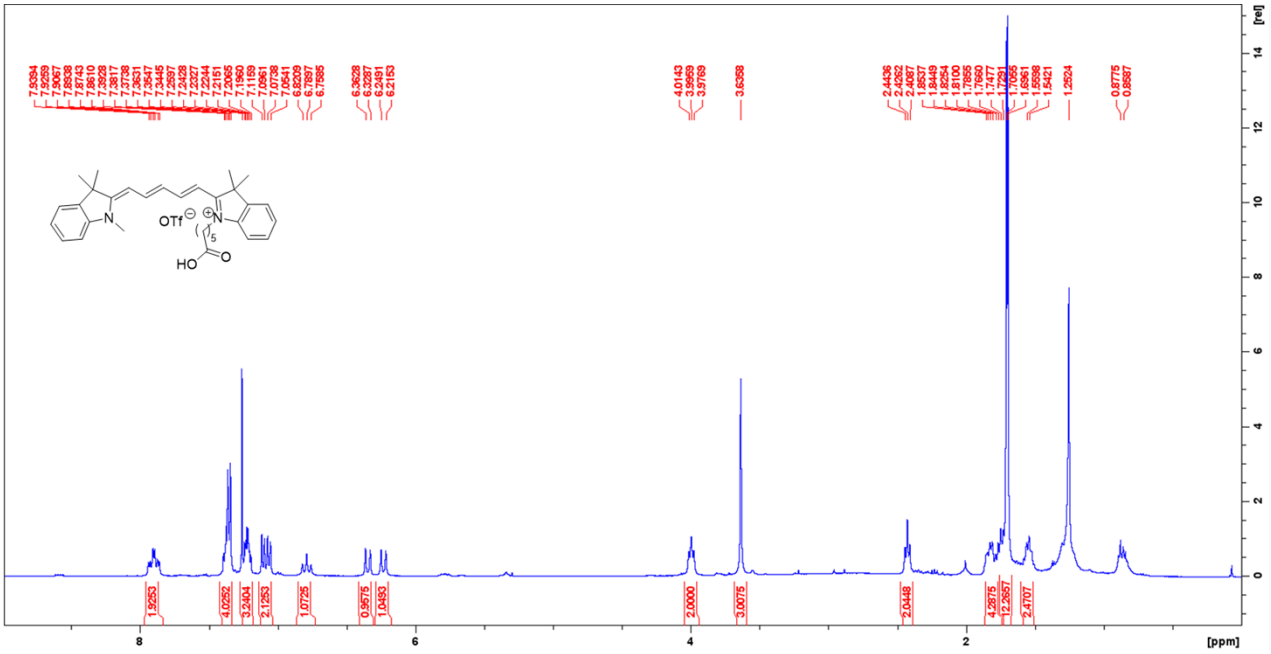
1H of Cy5.Cl (100.6 MHz, DMSO-d6)



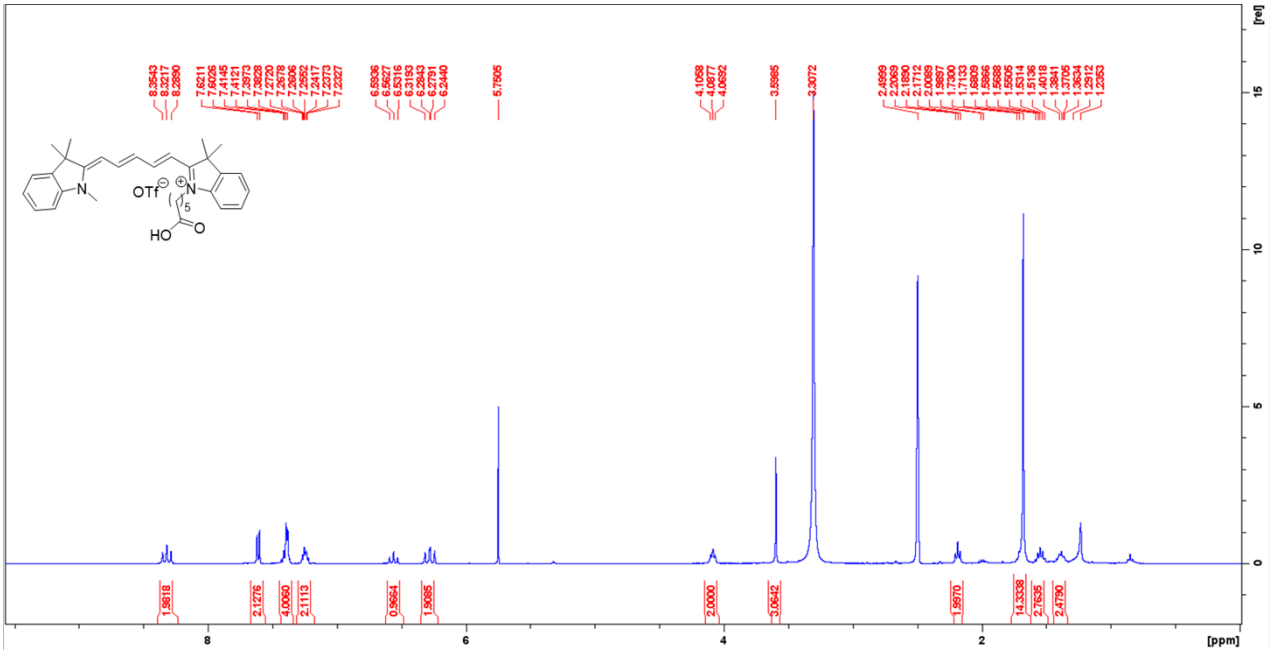
NOESY of Cy5.Cl (400 MHz, $CDCl_3$)



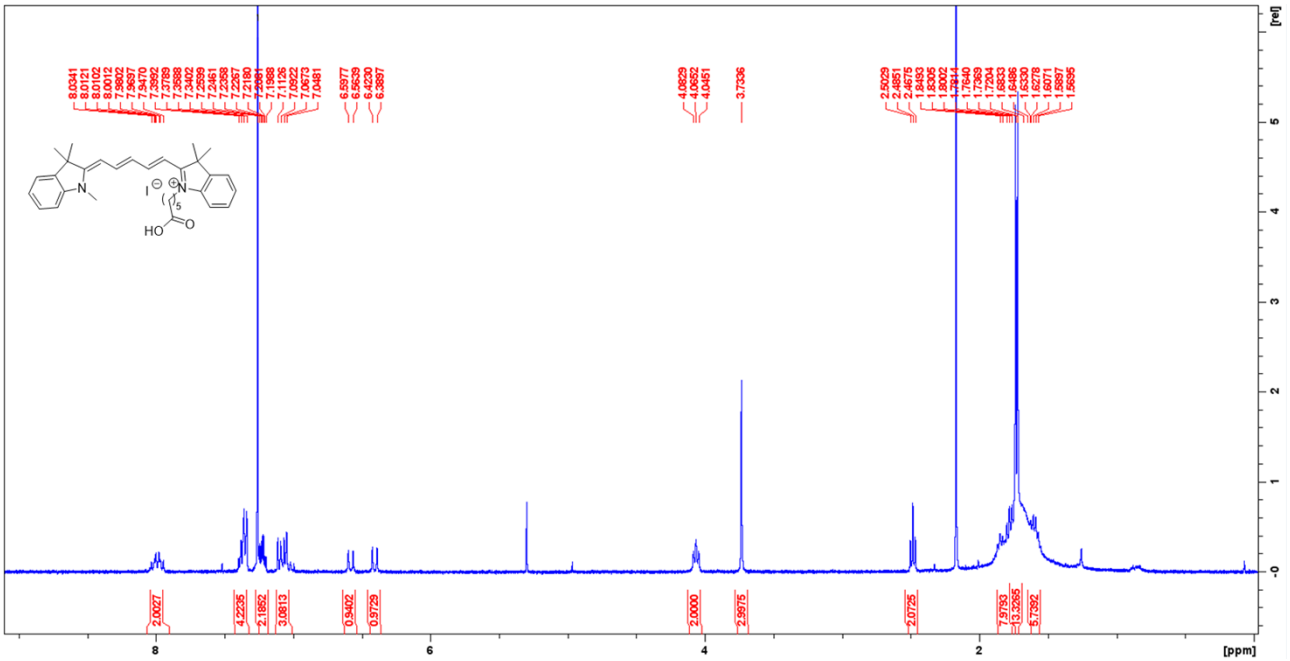
1H of Cy5.OTf (400 MHz, $CDCl_3$)



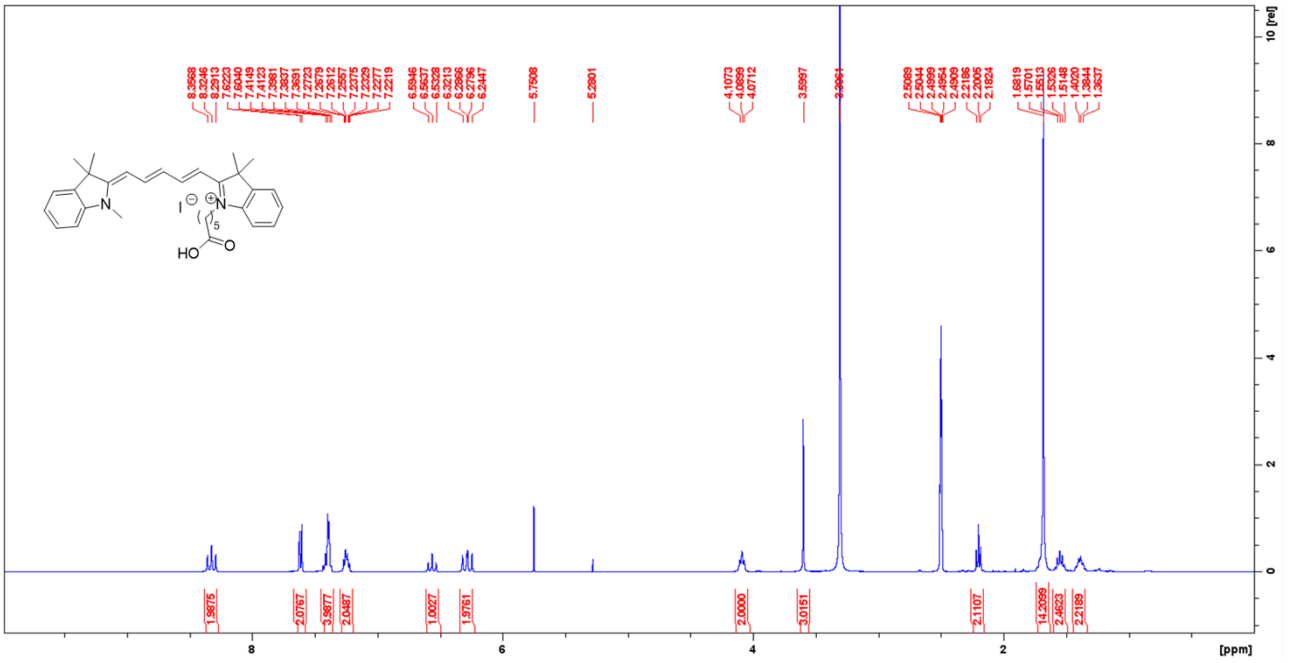
1H of Cy5.OTf (400 MHz, DMSO-d6)



1H of Cy5.I (400 MHz, $CDCl_3$)



1H of Cy5.I (400 MHz, DMSO-d6)



3. Mass spectrum of compound 12

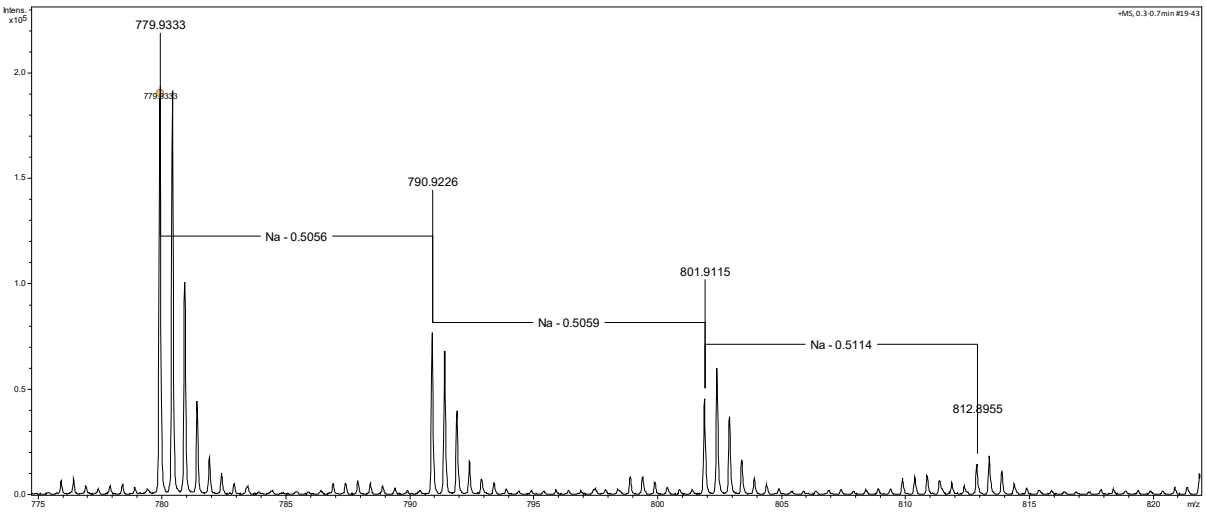


Figure S2. Mass spectrum of compound **12**

4. Normalized absorption and emission spectra for Cy5.Cl and compound 12

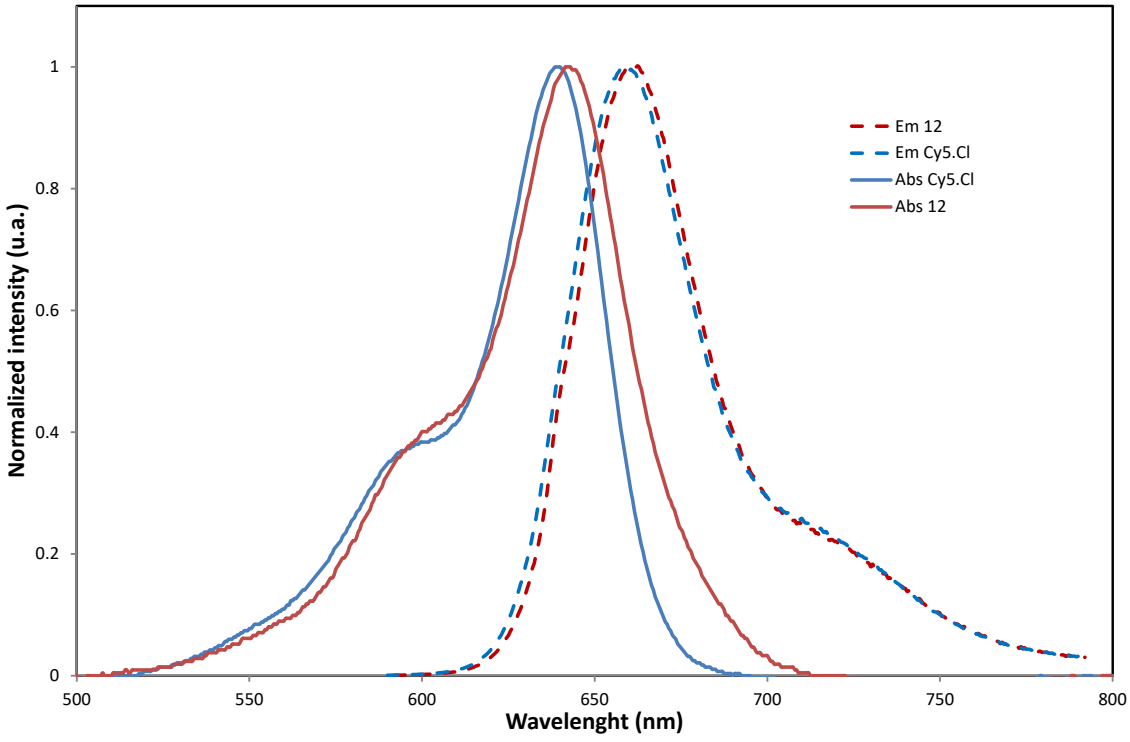


Figure S3. Normalized absorption (Abs, full lines) and emission (Em, dotted lines) spectra were recorded at 298 K for commercial Cy5.Cl (in blue) and for compound **12** (in red) in PBS (pH 7.4), λ_{ex} = 640 nm.

5. Radio-TLC of crude [^{18}F]16

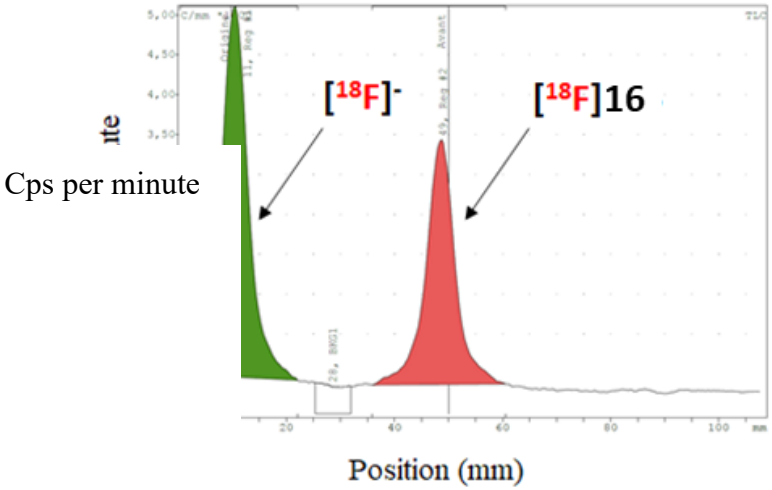


Figure S4. Radio-TLC of crude **[^{18}F]16**

6. References

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2 S. Passemarda, D. Staedlera, L. Ucnováa, G. S. Schneitera, P. Konga, L. Bonacinab, L. Juillerat-Jeanneretc, S. Gerber-Lemaire., *Bioorg. Med. Chem. Lett*., 2013, **23**, 5006.