Supplemental Information

Targeting Tumor Hypoxia: A Third Generation 2-Nitroimidazole–Indocyanine Dye–Conjugate with Improved Fluorescent Yield

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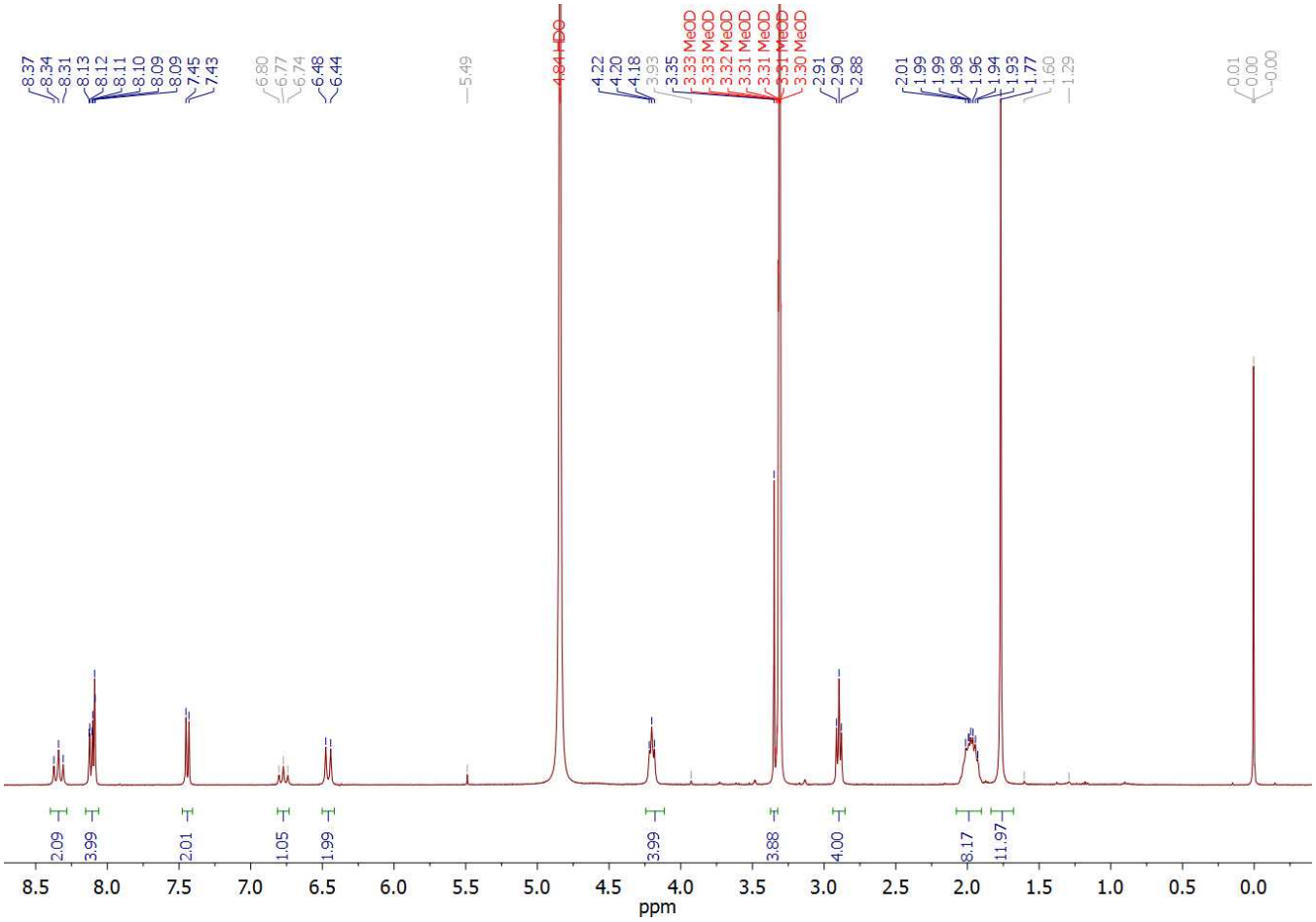
^{1}H NMR data for new compounds, **8** and **12**. Repeated efforts to obtain the ^{13}C NMR failed to give a spectrum. We observed this problem with dye conjugate **4**.[18] Although the dye (**2** and **8**) showed reasonable ^{13}C NMR spectra, **4** and **12** did not. We examined the possibility of aggregation, rotamers, relaxation time, and low concentration due to solubility issues. All of these issues can lead to poor ^{13}C NMR spectra. Attempts to obtain ^{13}C NMR spectra at 25 °C failed, and we heated the samples to 55 °C to promote deaggregation. We also explored different solvents, including CD_3OD, $(CD_3)_2CO$, D_2O. We examined extended delay (relaxation) times up to 10 to 15 sec, as well as long acquisition times (up to 12 hours). None of these experiments led to a ^{13}C NMR. We have attempted indirect C^{13} experiments, including HSQC, HMBC, and CIGAR. The problem is likely due to low solubility of **4** and **12**, coupled with the low isotope percentage of ^{13}C relative to ^{12}C versus the high percentage of ^{1}H, but we do not have a definitive answer to this problem. No experiments have been successful, so only ^{1}H NMR data is provided.

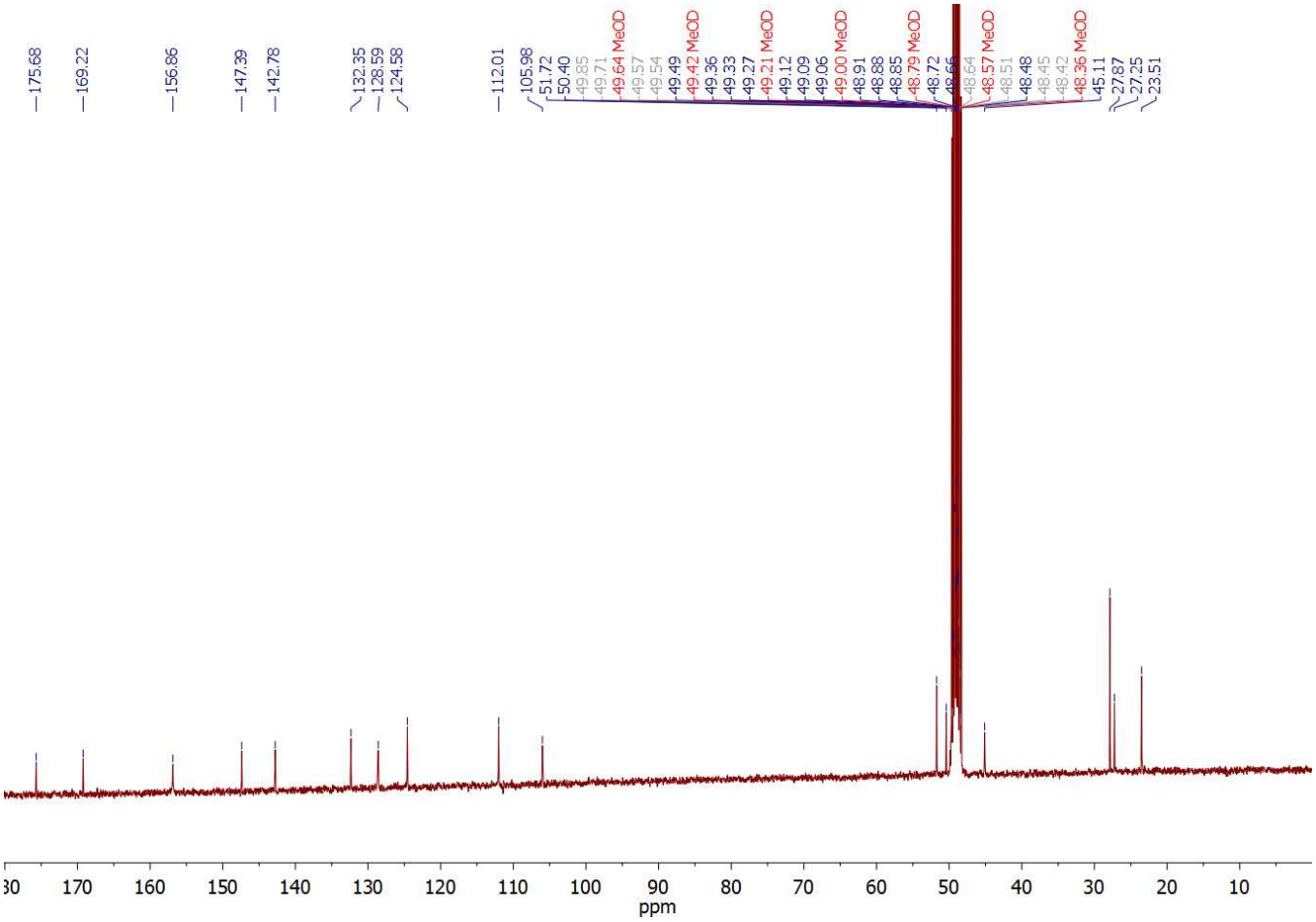
Note that the spectra for compound **8** were previously reported in another publication: "Targeting Tumor Hypoxia with 2-Nitroimidazole-ICG Dye Conjugates" Yan Xu, Saeid Zanganeh, Innus Mohammad, Andres Aguirre, Tianheng Wang, Yi Yang, Michael Smith and Quing Zhu *J. Biomed. Optics, **2013**, 18(6), 066009*-1-11.

We also provide the data for the solubility studies in Figure 7.

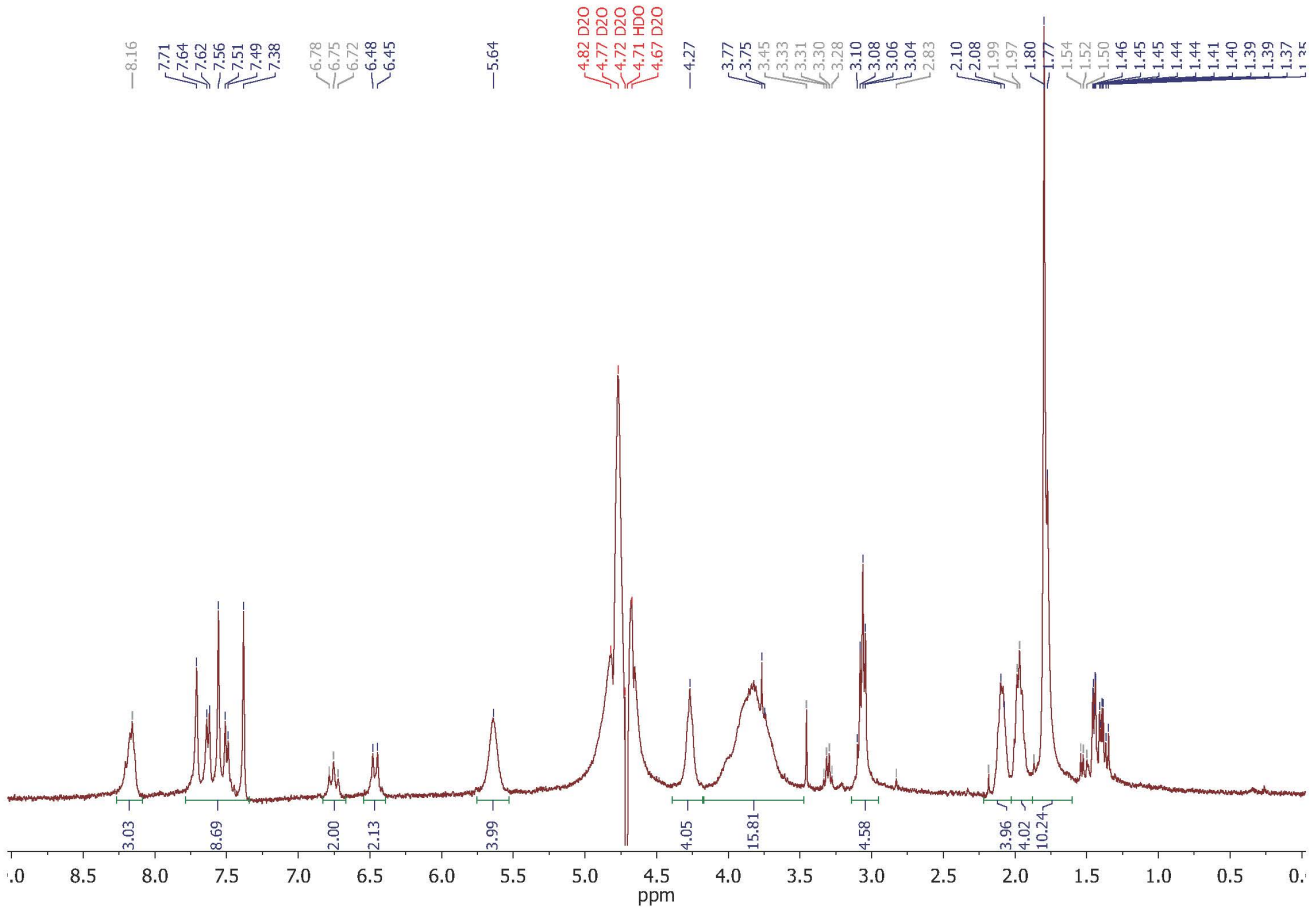
^{1}H NMR. Sodium 4-[2-[(1*E*,3*E*,5*Z*)-7-[1,1-dimethyl-3-(4-sulfonatobutyl)indol-2-ylidene]penta-1,3-dienyl]-1,1-dimethylindol-3-ium-3-yl]butane-1-sulfonate, **8.**

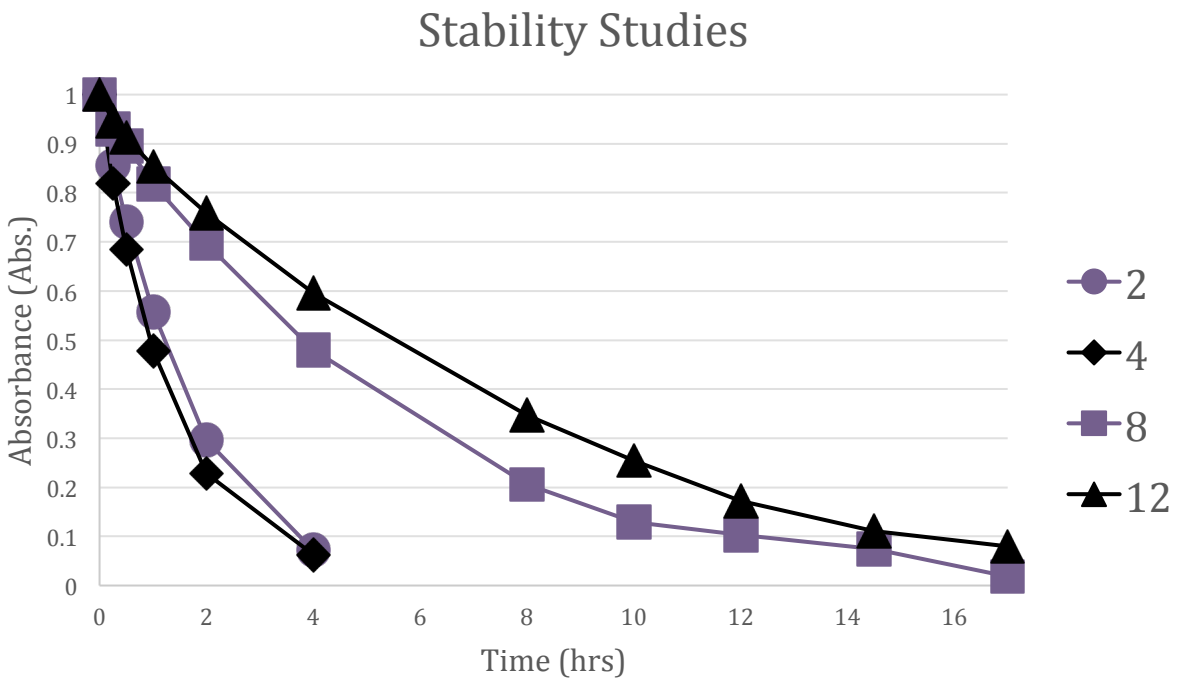


^{13}C NMR. Sodium 4-[2-[(1*E*,3*E*,5*Z*)-7-[1,1-dimethyl-3-(4-sulfonatobutyl)indol-2-ylidene]penta-1,3-dienyl]-1,1-dimethylindol-3-ium-3-yl]butane-1-sulfonate, **8.**



^{1}H NMR. Sodium 4-((*Z*)-2-((2*E*,4*E*)-5-(3,3-dimethyl-5-(4-(2-(2-nitro-1*H*-imidazol-1-yl)acetyl)-piperazine-1-carbonyl)-1-(4-sulfonatobutyl)-3*H*-indol-1-ium-2-yl)penta-2,4-dien-1-ylidene)-3,3-dimethyl-5-(4-(2-(2-nitro-1*H*-imidazol-1-yl)acetyl)piperazine-1-carbonyl)indolin-1-yl)butane-1-sulfonate, **12.**





Time (hrs)	Absorbance (Abs.)	Time (hrs)	Absorbance (Abs.)	Time (hrs)	Absorbance (Abs.)	Time (hrs)	Absorbance (Abs.)
2		4		8		12	
0	1.00	0	1.00	0	1.00	0	1.00
0.25	0.855	0.25	0.819	0.25	0.930	0.25	0.946
0.5	0.740	0.5	0.685	0.5	0.896	0.5	0.913
1	0.557	1	0.477	1	0.818	1	0.854
2	0.296	2	0.228	2	0.696	2	0.759
4	0.072	4	0.063	4	0.480	4	0.595
				8	0.206	8	0.347
				10	0.129	10	0.254
				12	0.103	12	0.172
				14.5	0.074	14.5	0.111
				17	0.018	17	0.080