Jockers_Figure S1

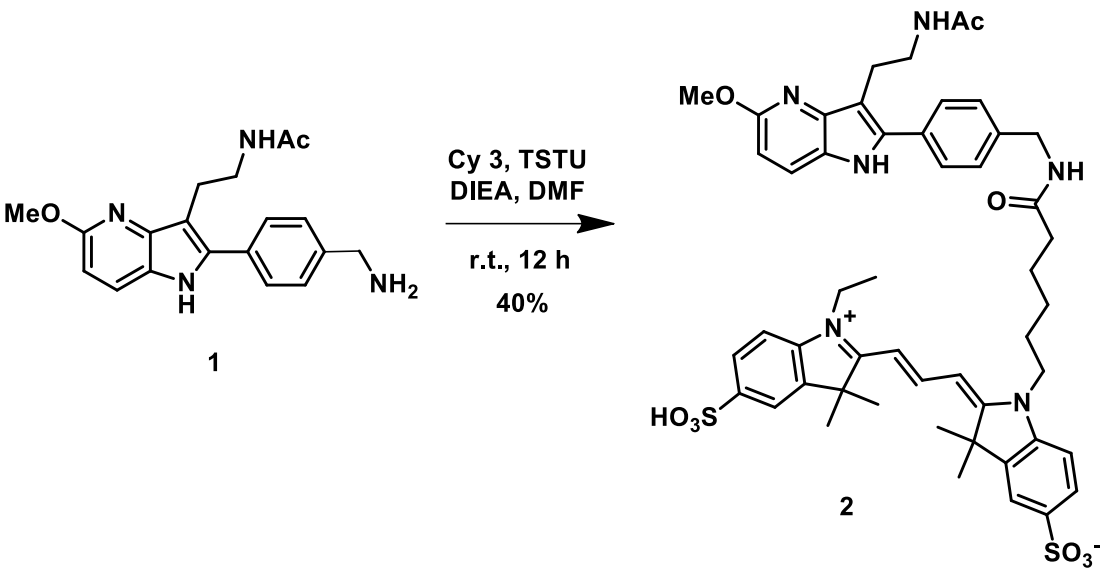

Figure S1: Synthesis of probe **2** "C2 tagging".

Jockers_Figure S2

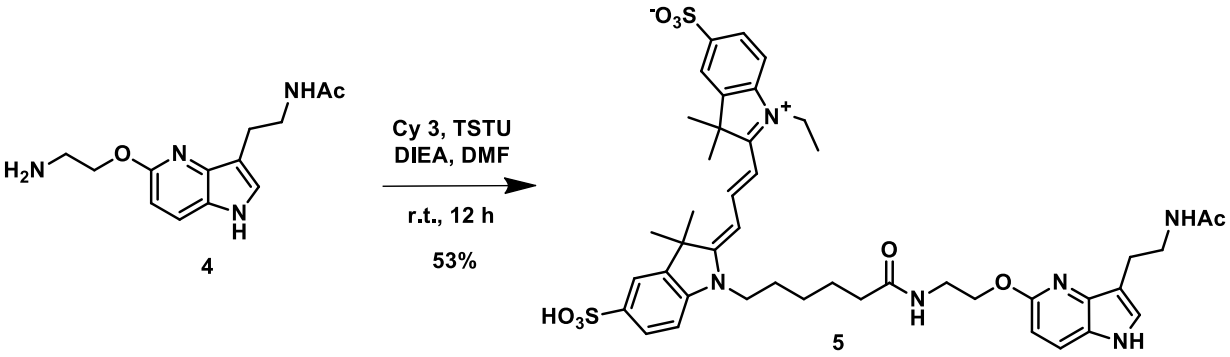

Figure S2: Synthesis of probes 5 “C5 tagging”.

Jockers_Figure S3

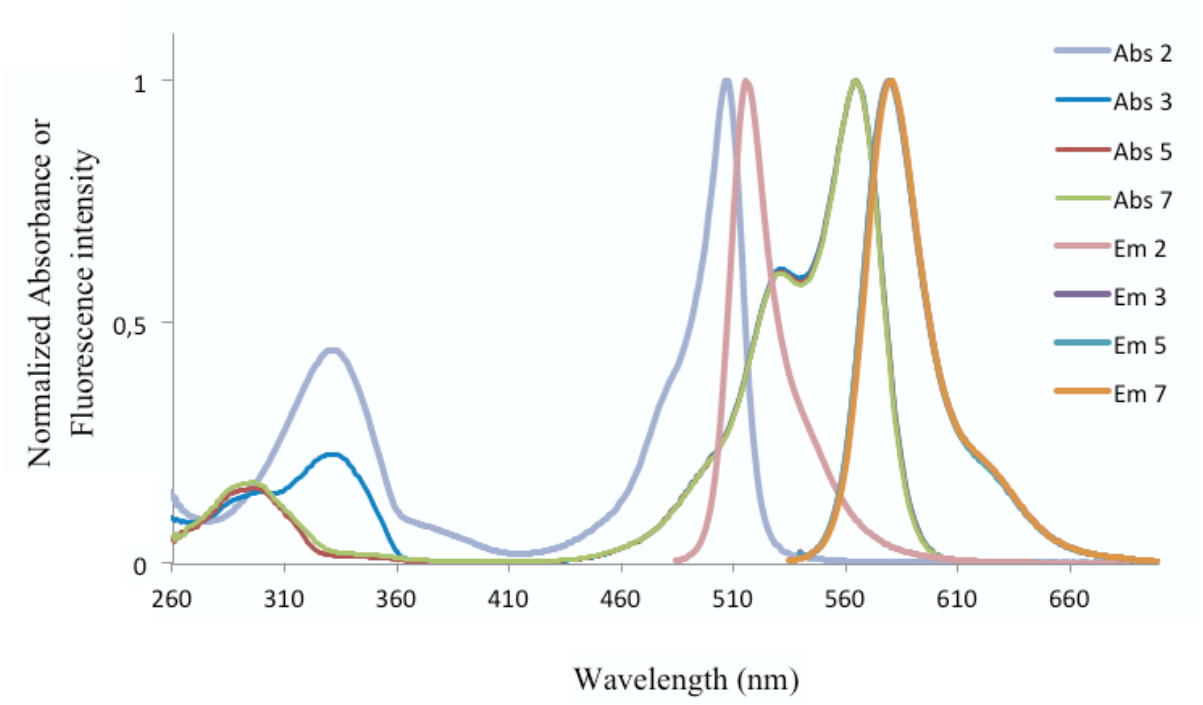

Figure S3: Absorption (Abs) and emission (Em) spectra of compounds **2**, **3, 5** and **7** recorded in DMSO at 25 °C.

Jockers_Figure S4

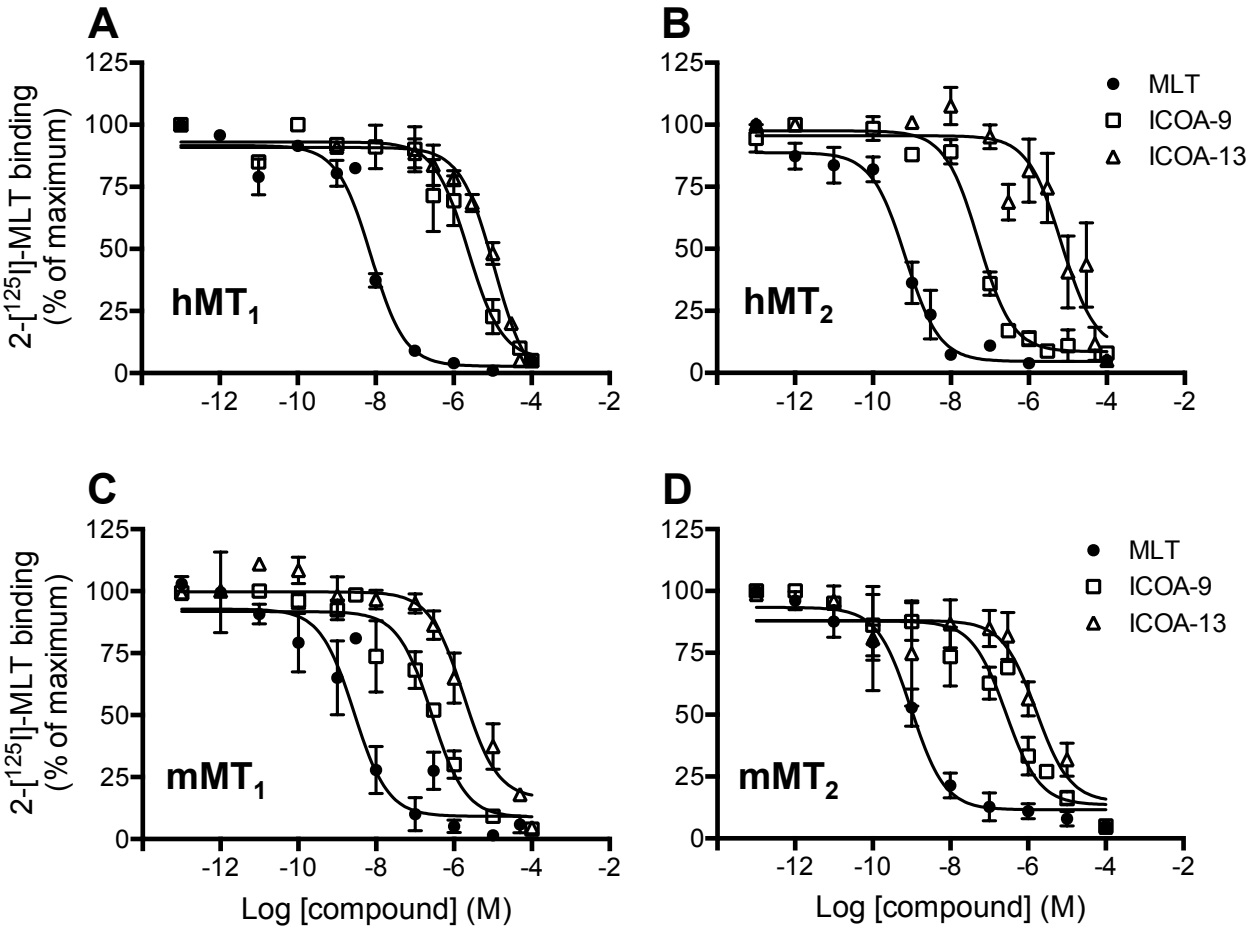

Figure S4: Competition of 2-[^{125}I]-MLT binding by melatonin (MLT), ICOA-9 and ICOA-13. Crude membranes of HEK293 cells expressing hMT_1 **(A)**, hMT_2 **(B),** mMT_1 **(C)** or mMT_2 **(D)** receptors were incubated with 0.1nM 2-[^{125}I]-MLT and increasing concentrations of the indicated compounds. Data are expressed as mean ± S.E.M. from 3-8 independent experiments (A. melatonin n=4, ICOA-9 n=4, ICOA-13 n=3; B. all groups n=4; C. melatonin n=8, ICOA-9 n=7, ICOA-13 n=6; D. melatonin n=8, ICOA-9 and ICOA-13 n=7). Data are represented as percentage of maximal binding in the absence of compounds and normalized to melatonin maximum effect.

Jockers_Figure S5

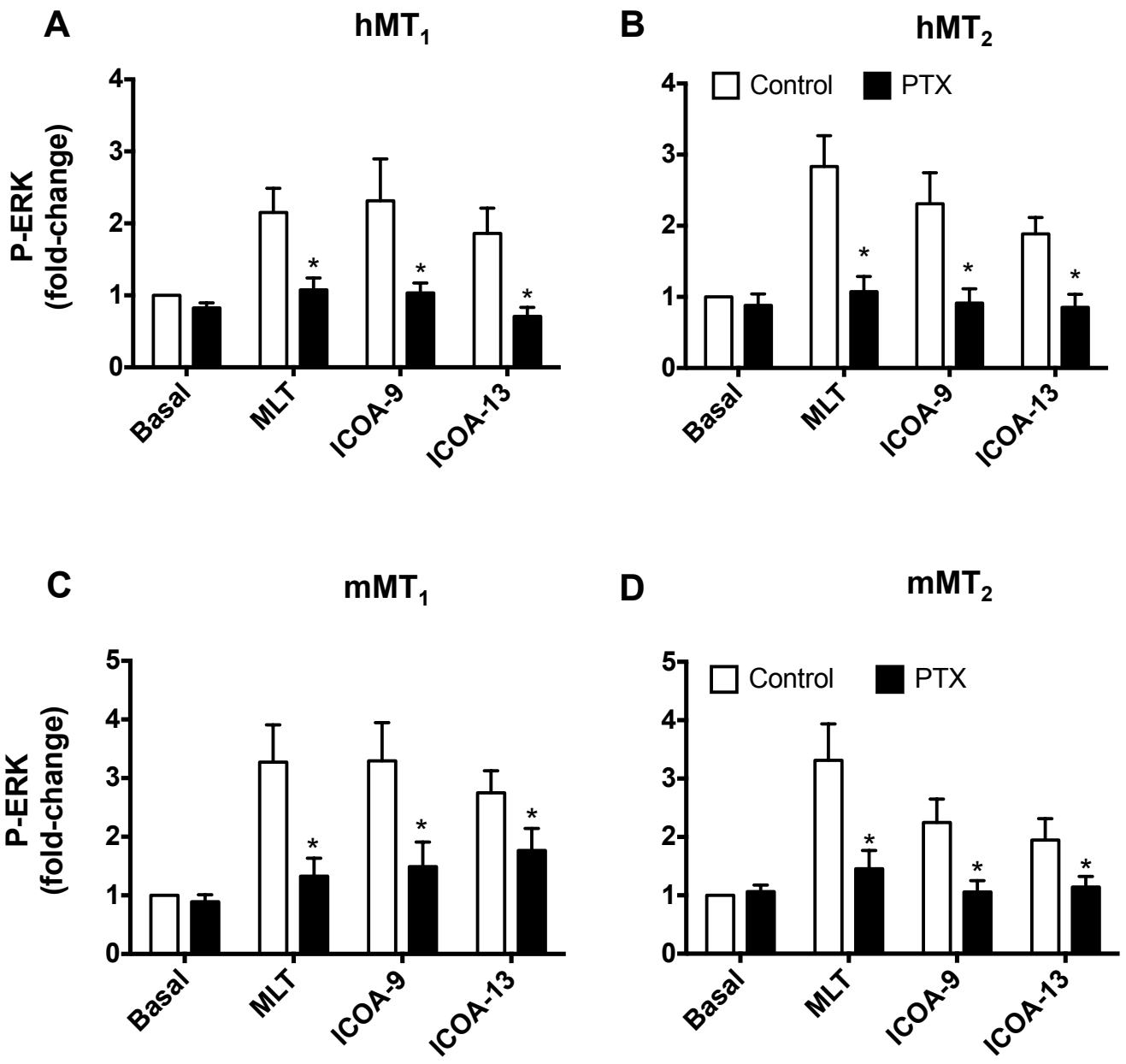

Figure S5: ERK1/2 activation in the absence or presence of PTX. Detection of phospho-ERK1/2 in HEK293 cells expressing hMT_1 **(A)**, hMT_2 **(B),** mMT_1 **(C)** or mMT_2 **(D)** receptors incubated for 5 min with MLT (100 nM), ICOA-9 (1 µM), ICOA-13 (1 µM) in the absence or presence of PTX (10 ng/µl, overnight). Data are expressed as mean ± S.E.M. from 5 independent experiments. *$p < 0.05$ compared to respective control by Student t test, one-tail analysis.

Jockers_Figure S6

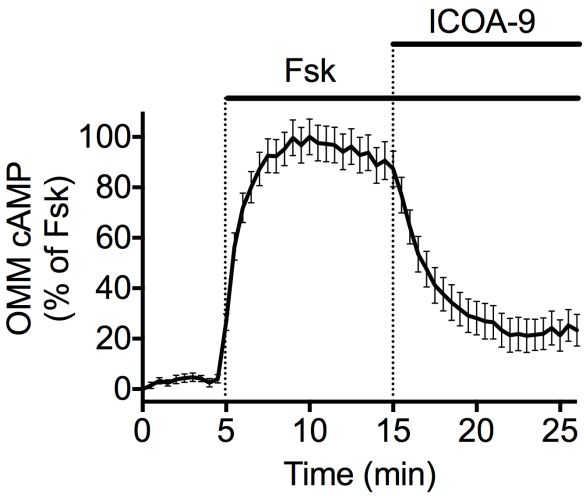

Figure S6: Effect of ICOA-9 on cAMP production in neuroblastoma cells. Averaged time courses of Fsk-mediated (10μM) cAMP production in N2a cells expressing MT_1 and the FRET-based cAMP biosensors localized at the outer mitochondrial membrane (OMM) and challenged by ICOA-9 (1 μM). Data expressed as the mean value ± s.d. from n = 48.

Jockers_Figure S7

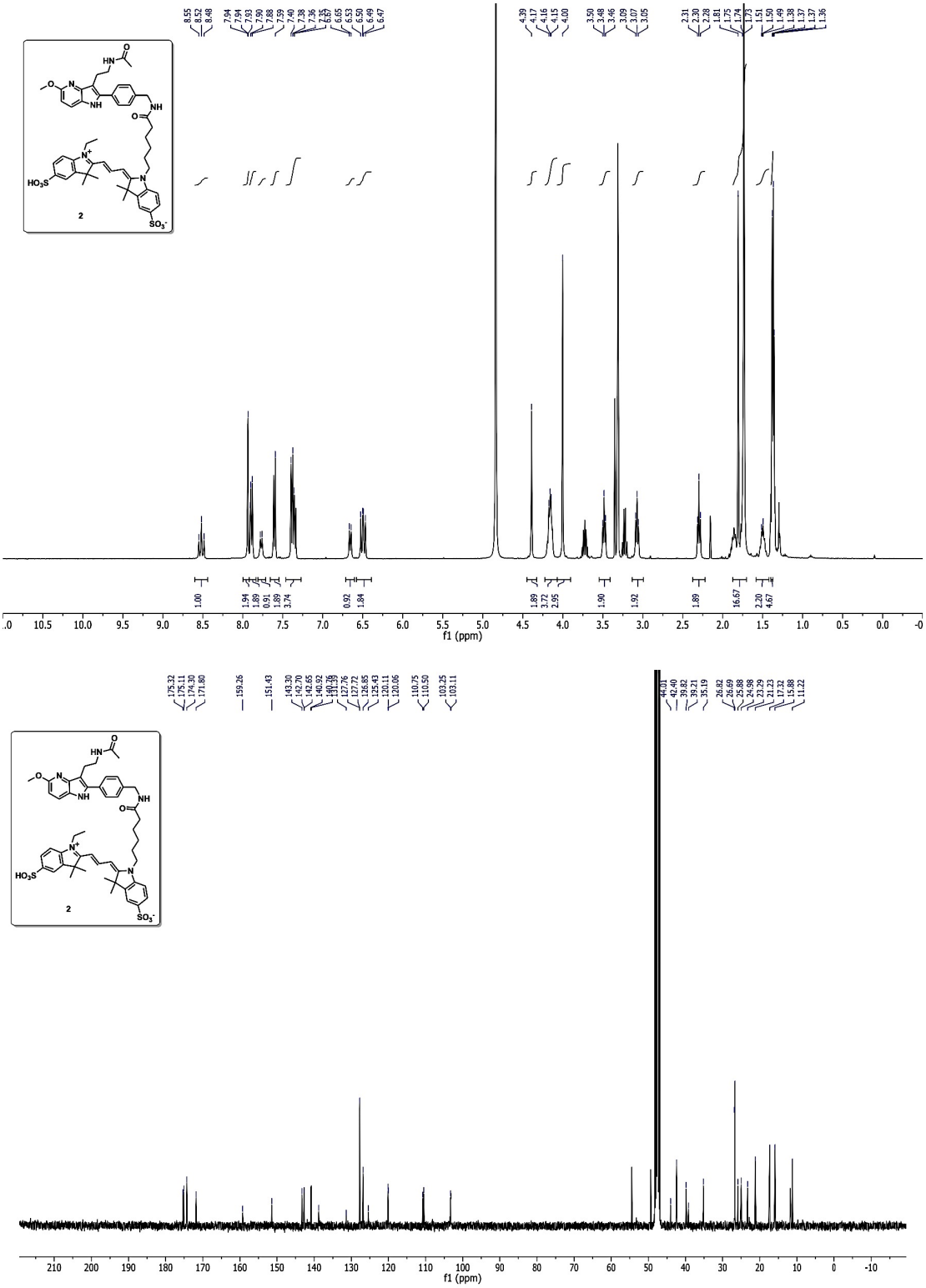

Figure S7: ^{1}H and ^{13}C NMR spectra of compound **2**.

Jockers_Figure S8

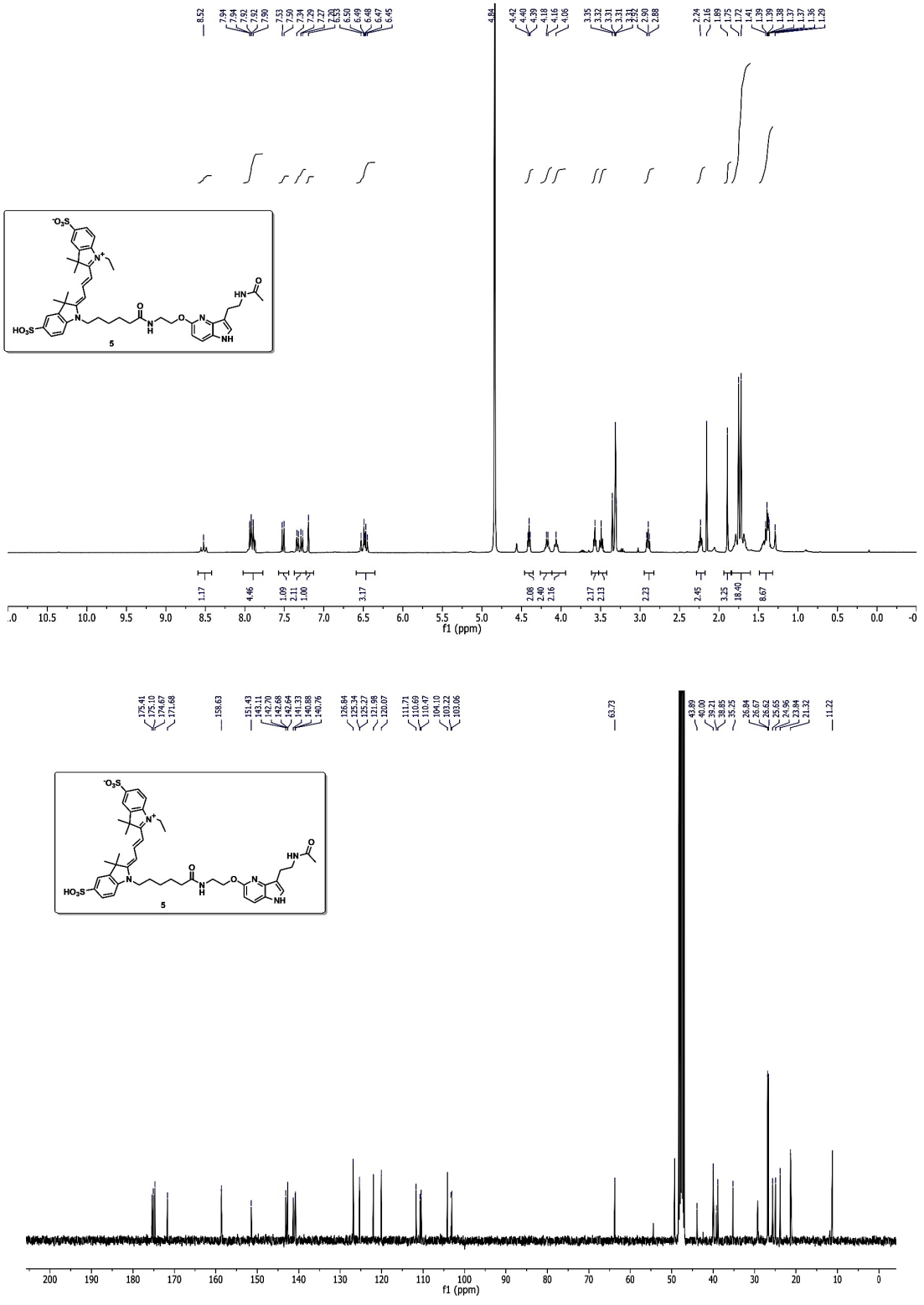

Figure S8: ^{1}H and ^{13}C NMR spectra of compound **5**.

Jockers_Figure S9

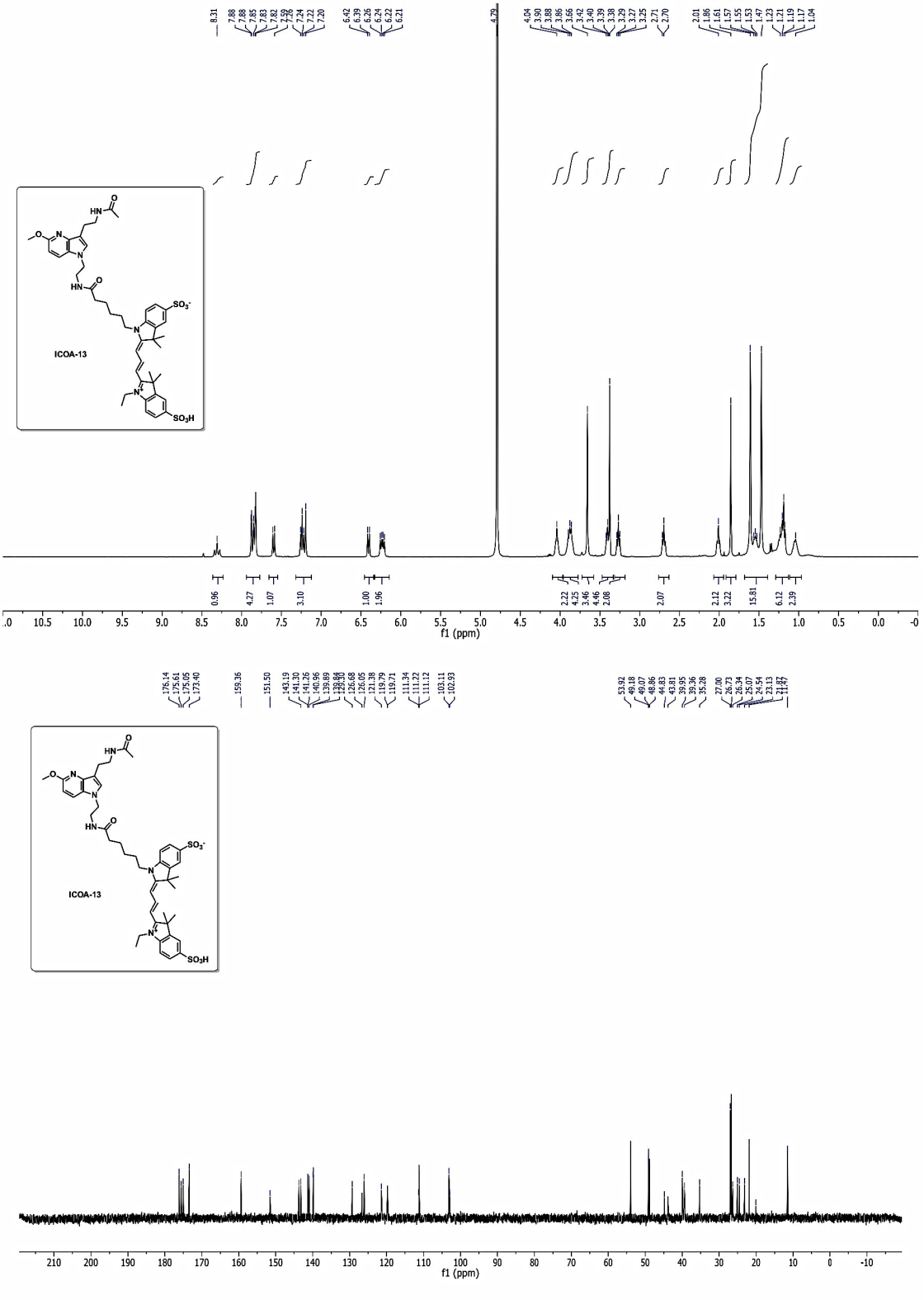

Figure S9: ^{1}H and ^{13}C NMR spectra of compound **7** (ICOA-13).

Jockers_Table S1

Table S1: Photophysical and physicochemical properties of compounds **2**, **3**, **5** and **7**.

Compound	λ_{max} (nm)	λ_{em} (nm)	$\varepsilon_{(\lambda max)}$ (L.mol-1.cm-1)	Φ^{*}	$\varepsilon \Phi^{\#}$
2	565	580	126126	0.15	18919
3	508	516	89177	0.46	41021
5	565	580	99234	0.16	15877
7	565	580	63125	0.17	10731

* Quantum yield; # Brightness

Jockers_Table S2

Table S2: $\Delta\Delta\log(\tau/K_A)$ ratios and bias factors for ICOA-9 and ICOA-13 at the hMT_1, hMT_2, mMT_1 and mMT_2

Receptor	Ligand	cAMP-βarr		cAMP-ERK		βarr-ERK	
		ΔΔlog	BF	ΔΔlog	BF	ΔΔlog	BF
hMT_1	Melatonin	0	1.00	0	1.00	0	1.00
	ICOA-9	1.40 ± 0.44	25.64*	1.76 ± 0.47	57.54*	0.35 ± 0.29	2.24
	ICOA-13	-0.06 ± 0.57	0.86	-0.15 ± 0.52	0.70	-0.09 ± 0.40	0.81
hMT_2	Melatonin	n.d.		n.d.		0	1.00
	ICOA-9	n.d.		n.d.		0.24 ± 0.47	1.72
	ICOA-13	n.d.		n.d.		0.81 ± 0.60	6.47
mMT_1	Melatonin	N/A		0	1.00	N/A	
	ICOA-9	N/A		1.47 ± 0.68	29.24	N/A	
	ICOA-13	N/A		1.03 ± 0.69	10.81	N/A	
mMT_2	Melatonin	N/A		0	1.00	N/A	
	ICOA-9	N/A		0.43 ± 0.64	2.70	N/A	
	ICOA-13	N/A		0.07 ± 0.68	1.18	N/A	

$\Delta\log(\tau/K_A)$ ratios were used to calculate $\Delta\Delta\log(\tau/K_A)$ ratios and expressed as mean $\Delta\Delta\log(\tau/K_A)$ ± S.E.M of three to six independent experiments with repeats in duplicate or triplicate. Ligand bias factors (BF), relative to melatonin, were determined and data were analyzed in a pairwise manner using a two-tailed unpaired Student's *t* test [on the $\Delta\log(\tau/K_A)$ ratios] to determine the significance of the ligand biases. βarr: β-arrestin2; n.d.: could not be determined as EC_{50} and E_{max} values could not be determined for the cAMP assay; N/A: Not Applicable; $^*P < 0.05$.