Supporting information for:

Synthesis, characterization, and cellular investigations of porphyrin– and chlorin–indomethacin conjugates for Photodynamic Therapy of cancer

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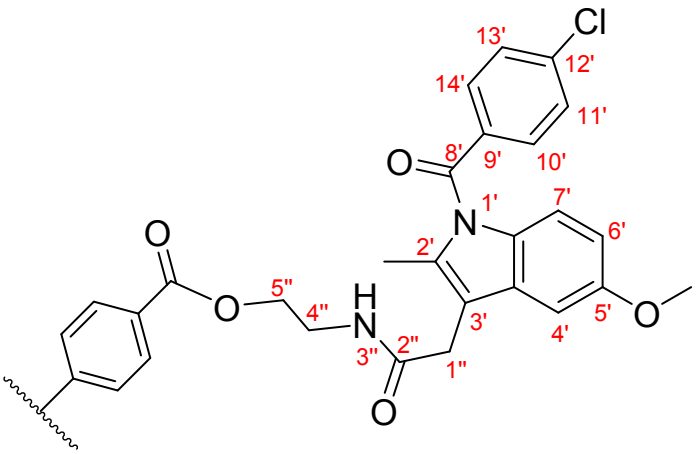
Content

Optimization reactions yielding P2-Ind or C2-Ind

Table S1. Reaction conditions for the optimization of **P2** or **C2** coupling with **Ind-OH**, using standard Steglich reactants (entry 1) and Steglich-type reactants (entry 2-4).

Entry	P2/C2 acid	Ind-OH (eq)	Carbodiimide	Base	Additive	Solvent	Temp.	Time	Comments
1	P2	2.5	DCC (2 eq)	DMAP (cat.)	-	DMF	r.t.	20 hours	Approximately 50% yield for P2-Ind. Difficult purification due to the presence of dicyclohexylurea (observed by ^{1}H NMR)
2	P2/C2	2.5	EDC·HCl (2 eq)	K_2CO_3 (2 eq)	HOBt (2 eq)	CH_2Cl_2	0 °C	5 hours	Very low yield for indomethacin conjugates.
3	P2/C2	0.5	EDC·HCl (2 eq)	K_2CO_3 (2 eq)	HOBt (2 eq)	CH_2Cl_2	0 °C	24 hours	Starting porphyrin or chlorin were totally consumed (by TLC), but it was also observed a significant amount of side products (by TLC). Reaction not reproducible.
4	P2/C2	1	EDC·HCl (1 eq)	DMAP (cat.)	-	CH_2Cl_2	0 °C	4 hours	88% yield for P2-Ind and 95% yield for C2-Ind. Reaction reproducible for at least 4 times

Numbering scheme for indomethacin fragment of the conjugates



NMR spectra of conjugate P2-Ind

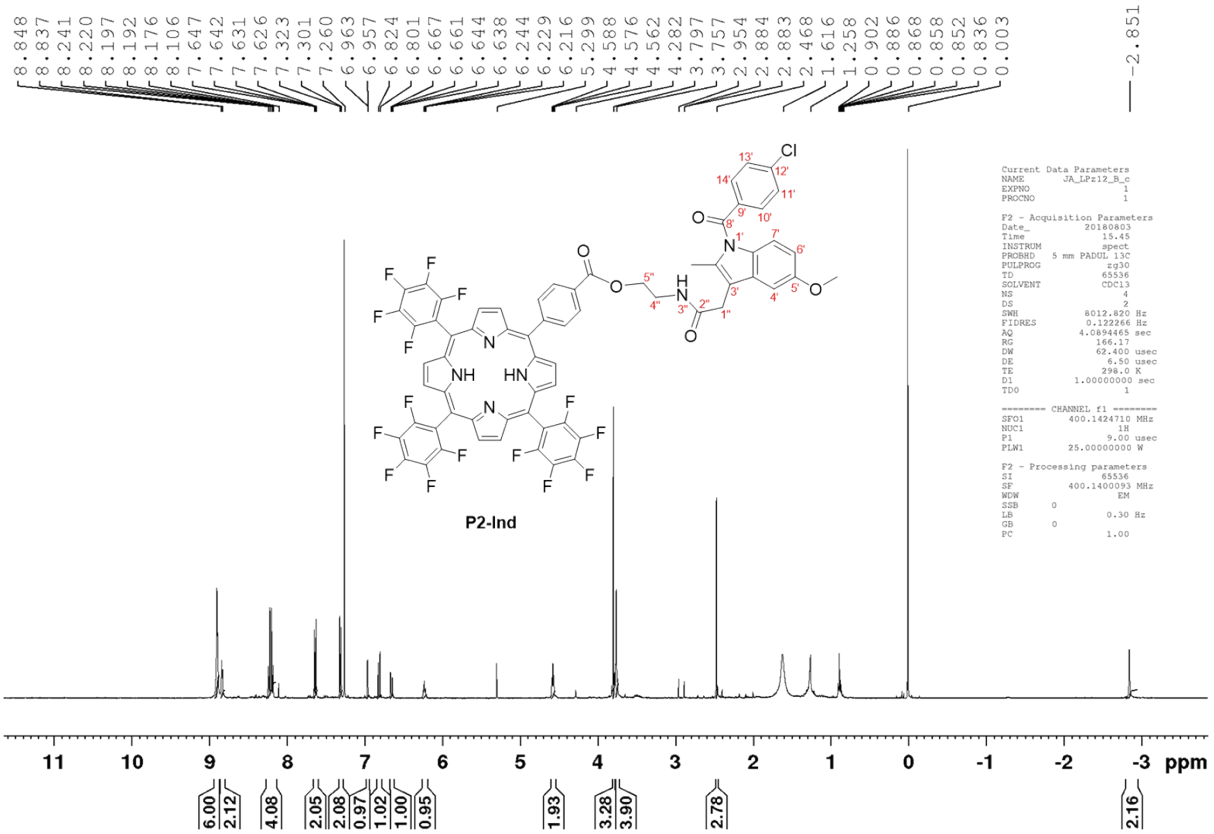


Figure S1. ^{1}H NMR spectrum of porphyrin-indomethacin conjugate **P2-Ind** in $CDCl_3$.

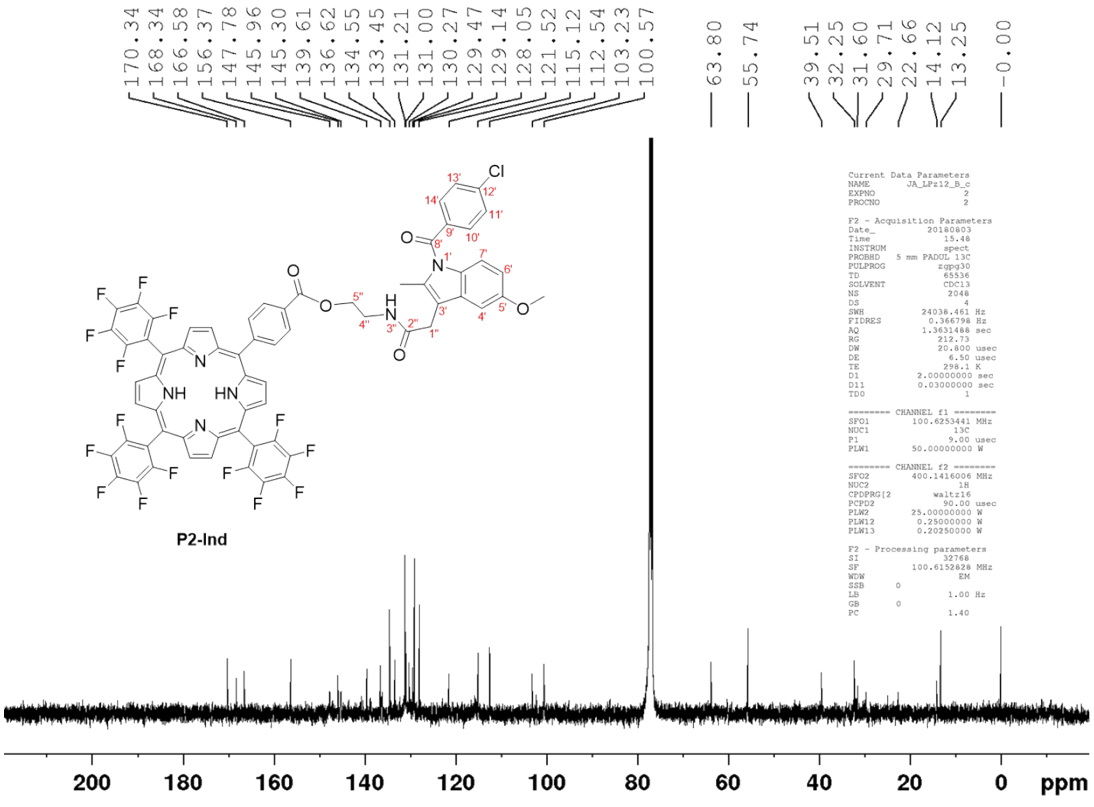


Figure S2. $^{13}C\{^{1}H\}$ NMR spectrum of porphyrin-indomethacin conjugate **P2-Ind** in $CDCl_3$.

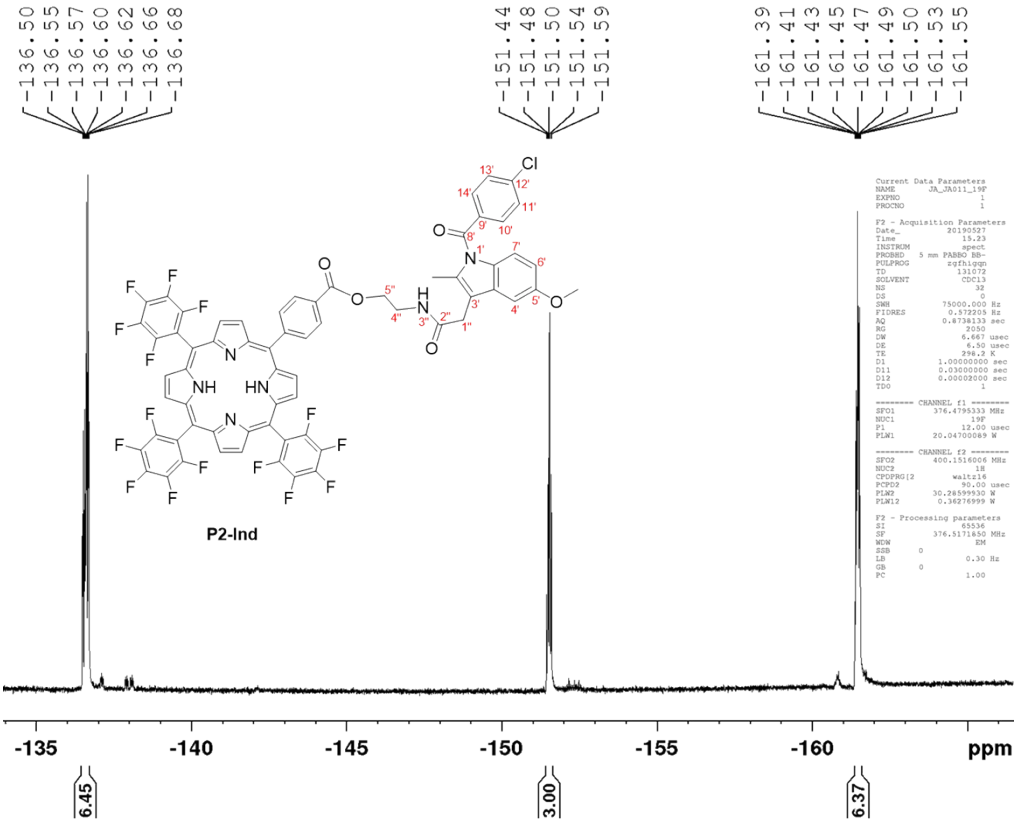


Figure S3. $^{19}F\{^{1}H\}$ NMR spectrum of porphyrin-indomethacin conjugate **P2-Ind** in $CDCl_3$.

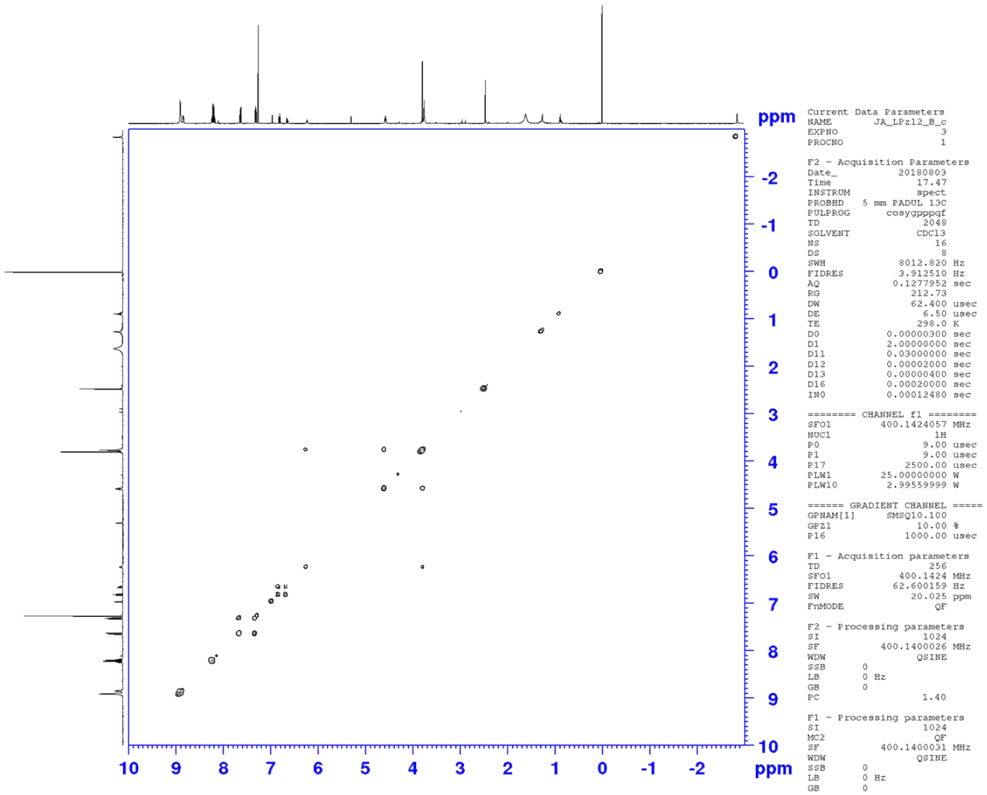


Figure S4. COSY spectrum of conjugate **P2-Ind**.

ESI mass spectrum of P2-Ind

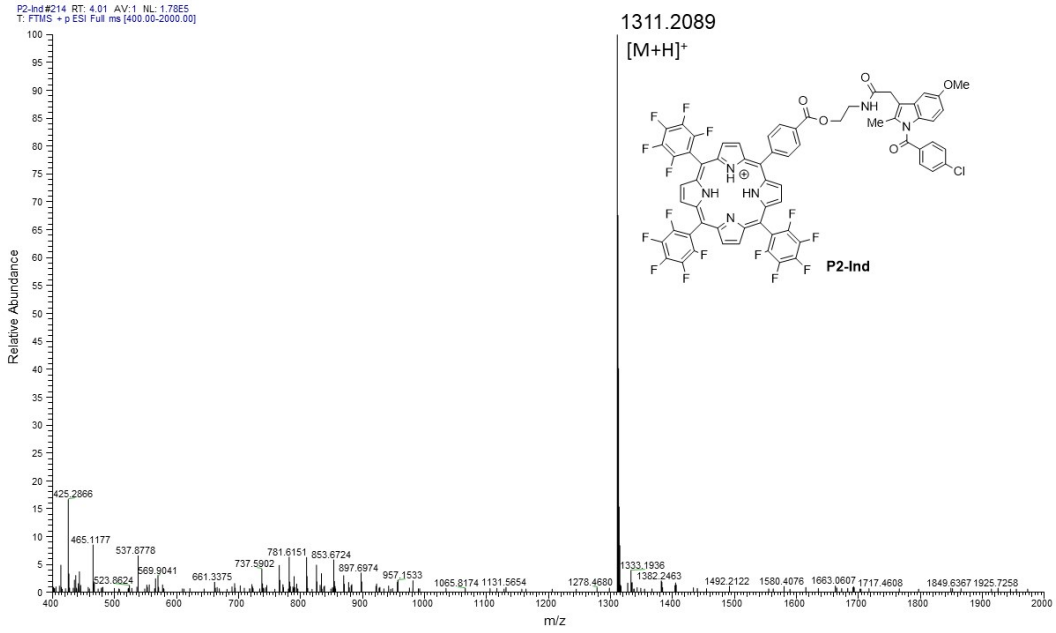
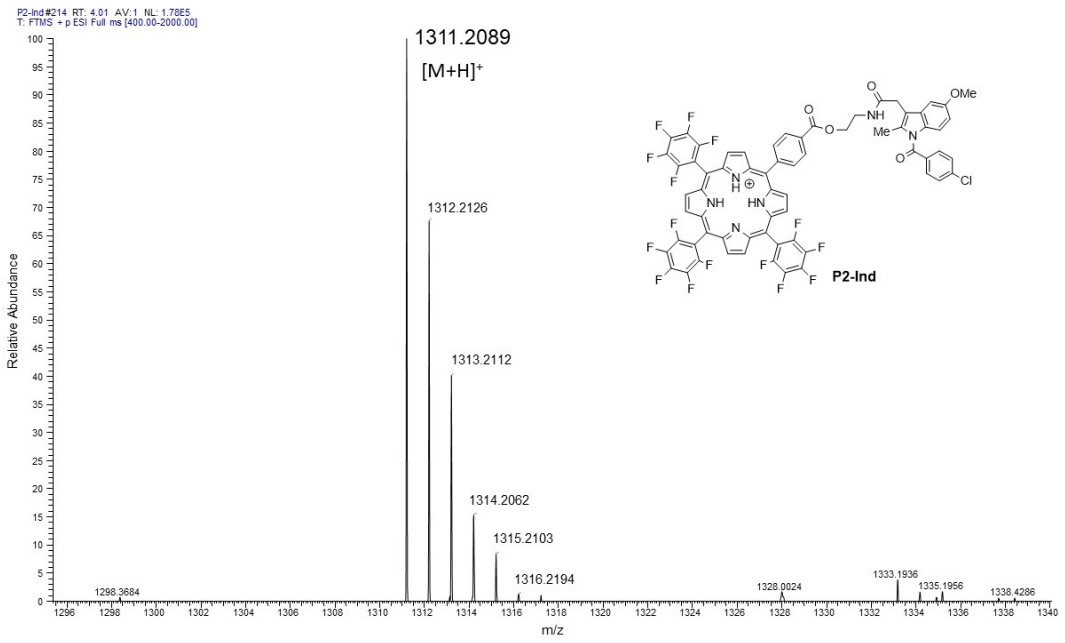


Figure S5. ESI mass spectrum of conjugate **P2-Ind**.

Figure S6. Zoomed-in section of the mass ion peak 1311.2089 of the ESI mass spectrum of conjugate **P2-Ind**.



NMR spectra of conjugate C2-Ind

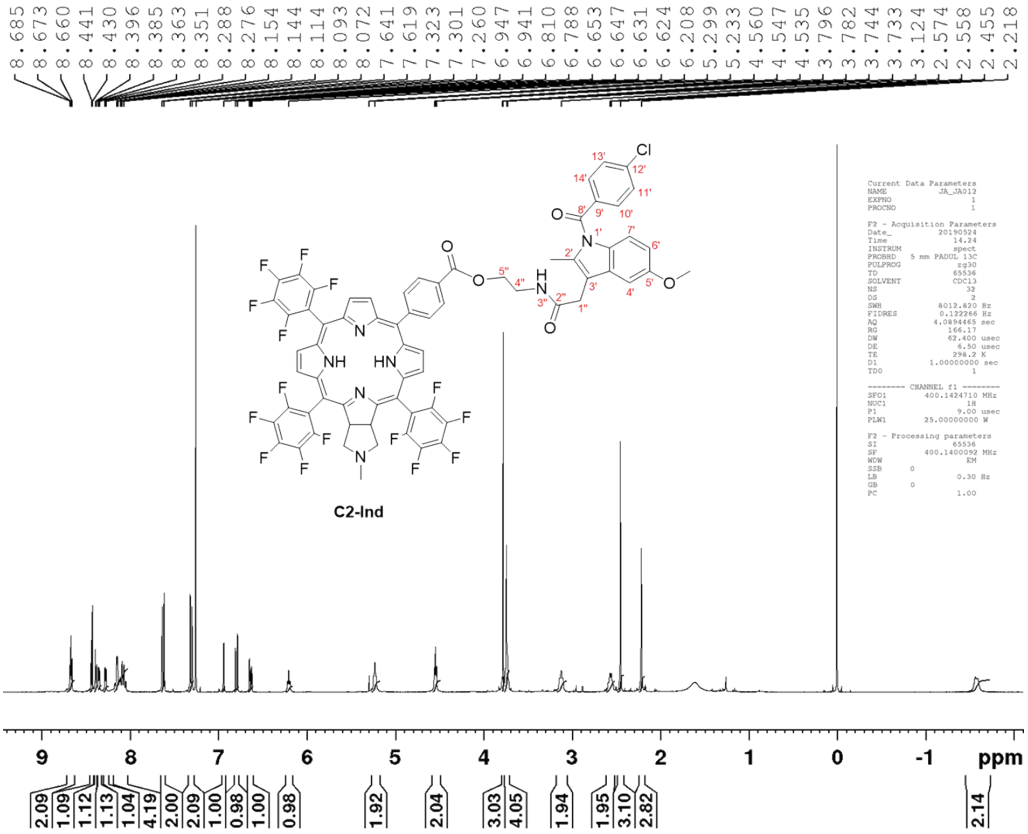


Figure S7. ^{1}H NMR spectrum of conjugate **C2-Ind** in $CDCl_3$.

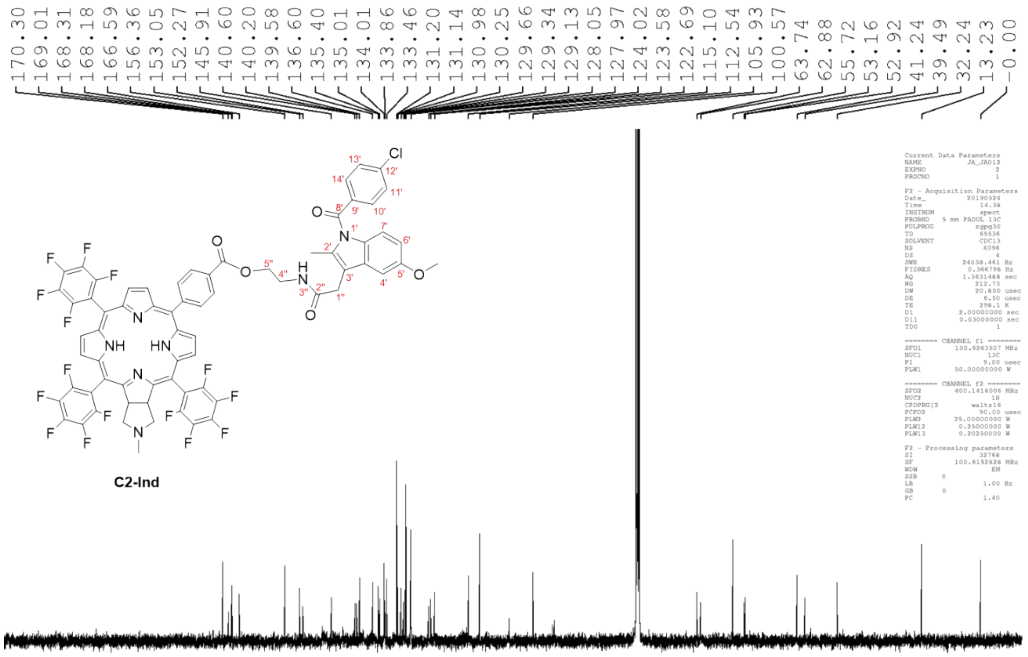


Figure S8. $^{13}C\{^{1}H\}$ NMR spectrum of conjugate **C2-Ind** in $CDCl_3$.

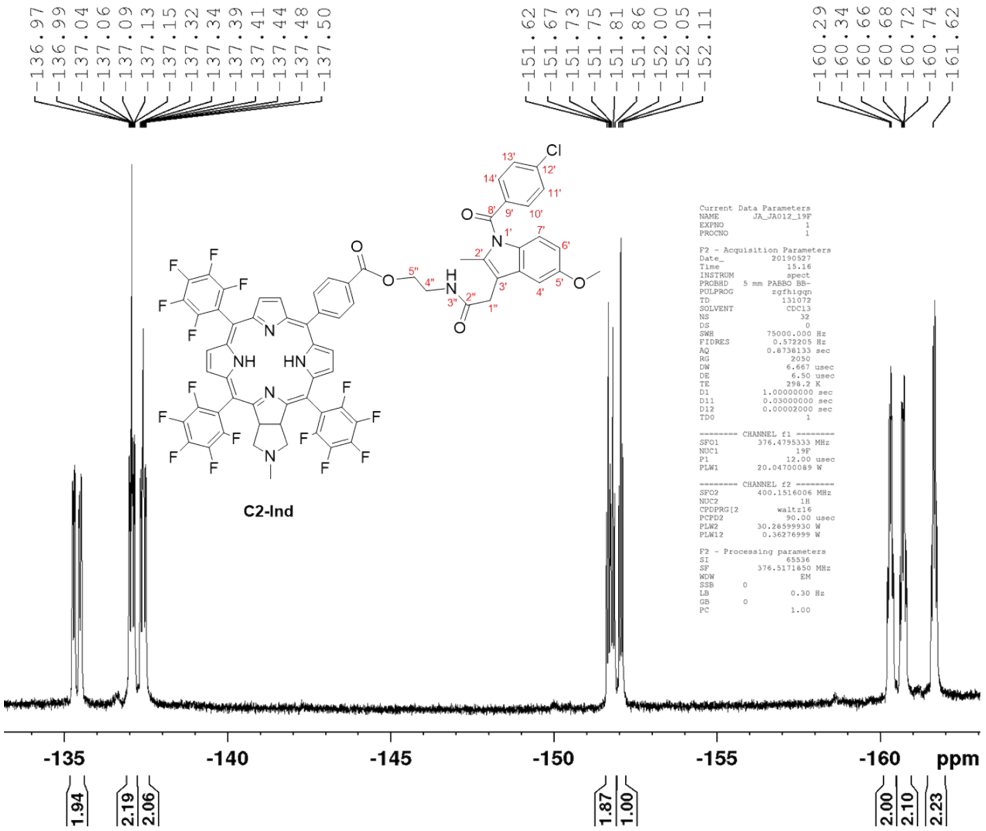


Figure S9. $^{19}F\{^{1}H\}$ NMR spectrum of conjugate **C2-Ind** in $CDCl_3$.

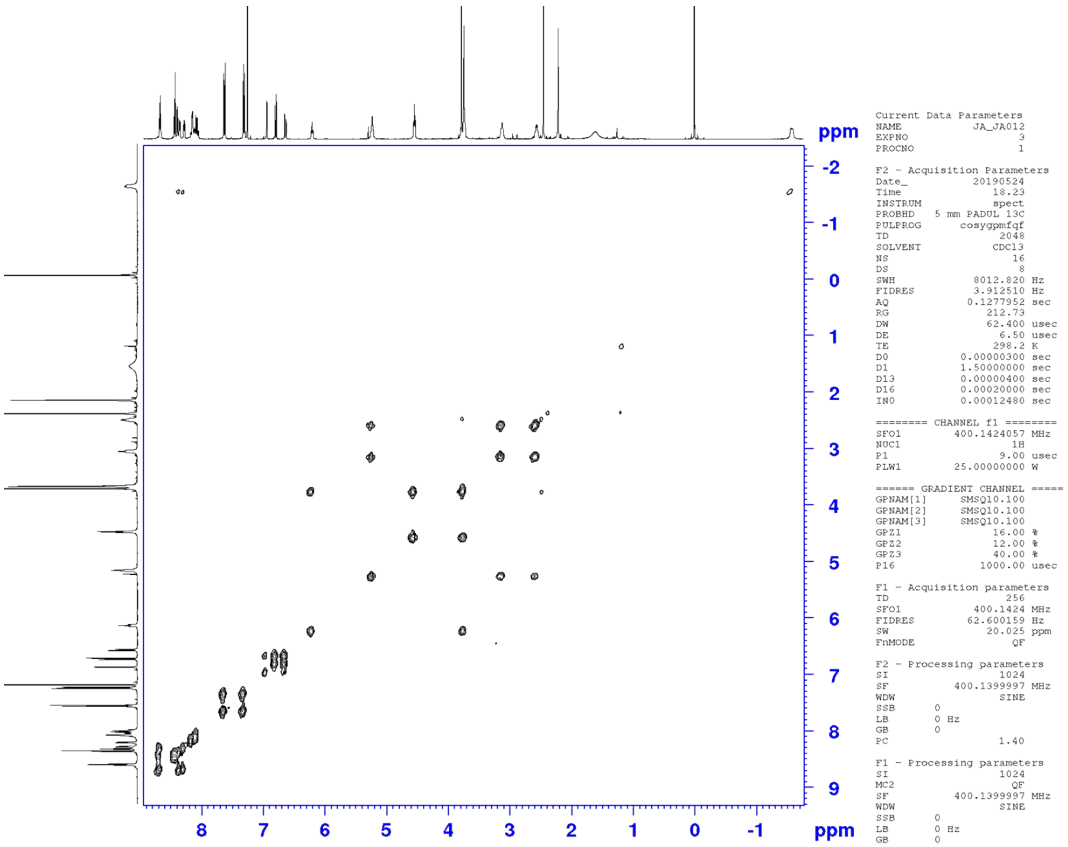


Figure S10. COSY spectrum of conjugate **C2-Ind**.

ESI mass spectrum of C2-Ind

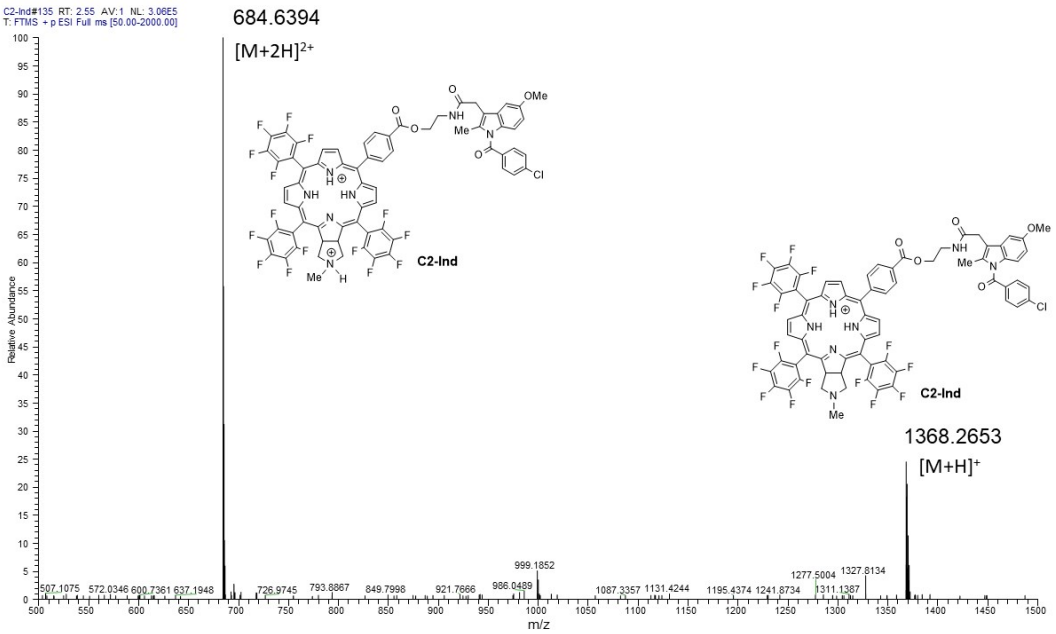
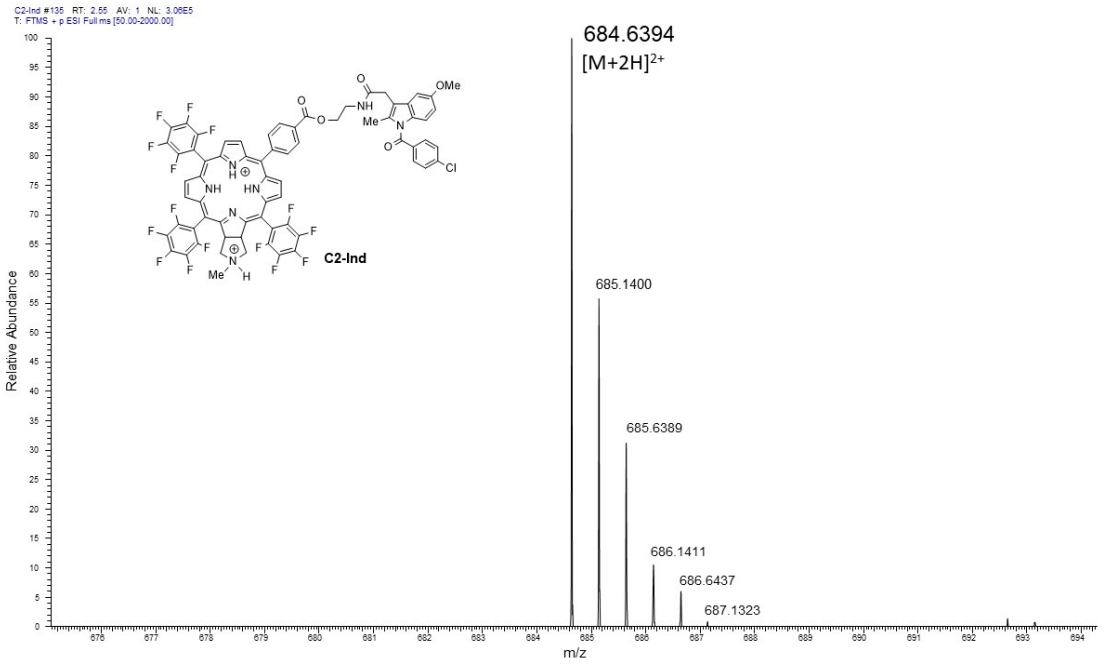


Figure S11. ESI mass spectrum of conjugate **C2-Ind**

Figure S12. Zoom-in of the ESI mass spectrum of conjugate **C2-Ind** at [573 and 694 *m/z*]



Additional information on compounds phototoxicity

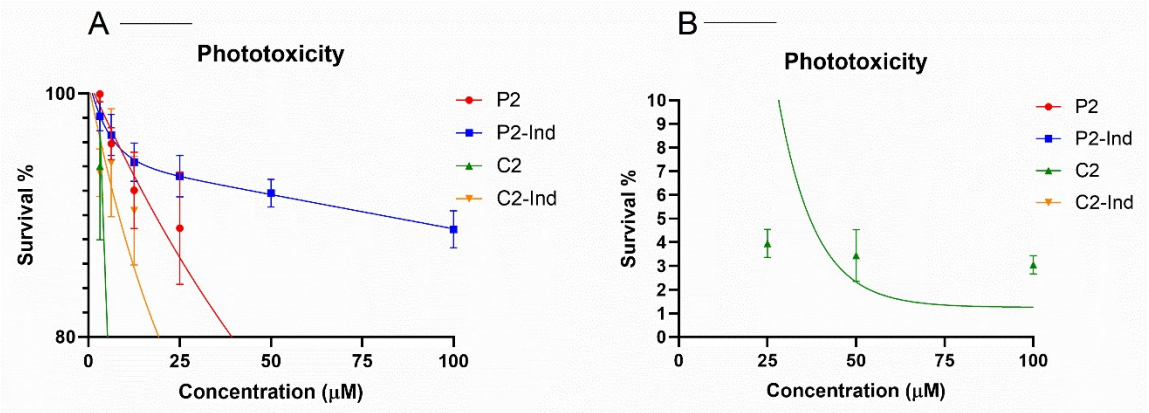


Figure S13.A) Zoom-in section (80-100% cell survival) of all compounds and B) chlorin **C2** (0-10% cell survival) phototoxicity towards HEp2 cells using 1.5 J/cm^2 light dose and the Cell Titer Blue assay.

DFT calculations

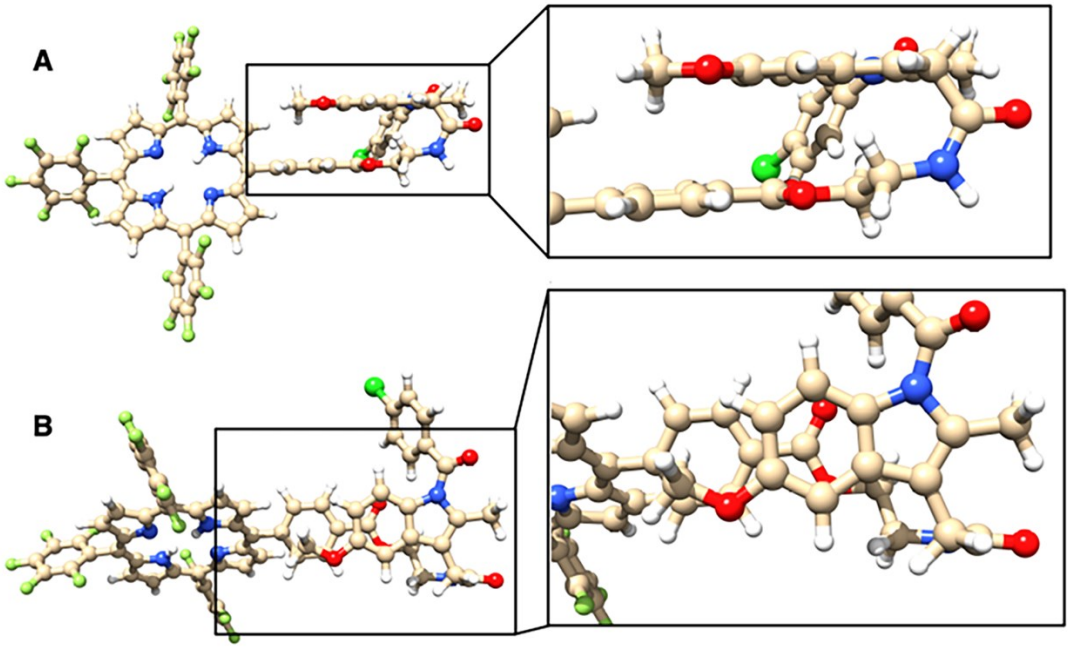


Figure S14. Side (A) and top (B) views of the indomethacin moiety in **P2-Ind-1**, with a zoom on the right, showing the almost parallel arrangement of the two aromatic groups in the most stable conformation.

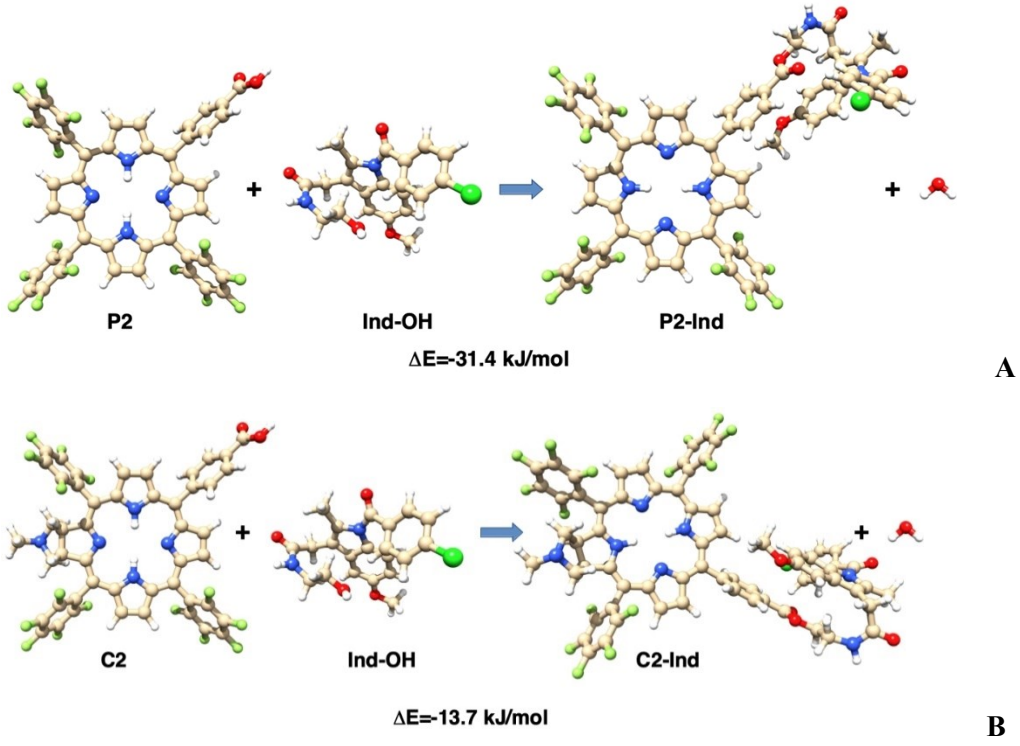


Figure S15. Structures and reaction energy, in DMF, obtained by DFT calculations to produce the most stable isomers of **P2-Ind (A)** and **C2-Ind (B)**.

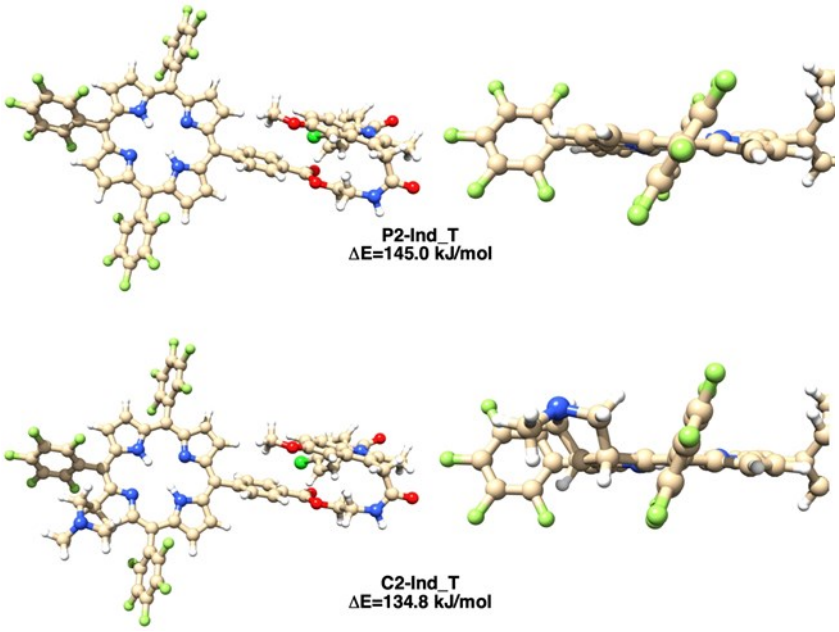


Figure S16. Structures of the excited triplet state of **P2-Ind** and **C2-Ind** found by DFT calculations. The enlarged pictures on the right show the larger distortion from planarity of the porphyrin ring of **P2-Ind**.

Photophysical properties of the conjugates in DMF, MeOH and DMSO

Table S2. Spectral data of porphyrin **P2**, chlorin **C2** and indomethacin conjugates **P2-Ind** and **C2-Ind** in *N.N*-dimethylformamide. methanol and DMSO at 25 °C

		Absorption					Fluorescence
		λ_{max}/nm (log $\varepsilon_{\lambda max}$)					λ_{max}/nm (ϕ_F)
Solvent	Compound	Soret Band	Q Bands				
DMF	**P2**	413 (5.44)	507 (4.23)	536 (3.46)	581 (3.73)	652 (3.35)	640, 652, 705 (0.085 ± 0.001)
	C2	408 (5.14)	503 (4.08)	530 (3.79)	594 (3.68)	648 (4.64)	650, 712 (0.32)
	P2-Ind	413 (5.58)	507 (4.40)	536 (3.61)	581 (3.91)	652 (3.52)	640, 654, 704 (0.077 ± 0.003)
	C2-Ind	408 (5.23)	503 (4.15)	530 (3.76)	594 (3.70)	648 (4.61)	652, 717 (0.288 ± 0.002)
MeOH	**P2**	409 (5.24)	505 (4.08)	536 (2.90)	582 (3.60)	653 (3.30)	642, 708 (0.051 ± 0.001)
	C2	400 (5.19)	503 (4.08)	530 (3.74)	594 (3.86)	647 (4.54)	648, 714 (0.157 ± 0.001)
	P2-Ind	409 (5.74)	505 (4.54)	538 (3.72)	582 (4.02)	652 (3.64)	641, 653, 706 (0.050 ± 0.001)
	C2-Ind	402 (5.06)	504 (3.99)	530 (3.65)	594 (3.53)	648 (4.44)	651, 714 (0.256)
DMSO	**P2**	415 (5.38)	508 (4.24)	537 (3.45)	582 (3.76)	653 (3.16)	642, 705 (0.079 ± 0.001)
	C2	410 (5.20)	507 (4.16)	534 (3.84)	596 (3.72)	648 (4.61)	652, 713 (0.306)
	P2-Ind	414 (5.12)	507 (4.18)	538 (3.39)	582 (3.70)	653 (3.29)	641, 654, 705 (0.080 ± 0.001)
	C2-Ind	411 (5.01)	505 (3.93)	532 (3.59)	597 (3.50)	649 (4.37)	652, 714 (0.279)