Supporting information

Photodynamic therapy for *ras*-driven cancers: targeting G-quadruplex RNA structures with bifunctional alkyl-modified porphyrins

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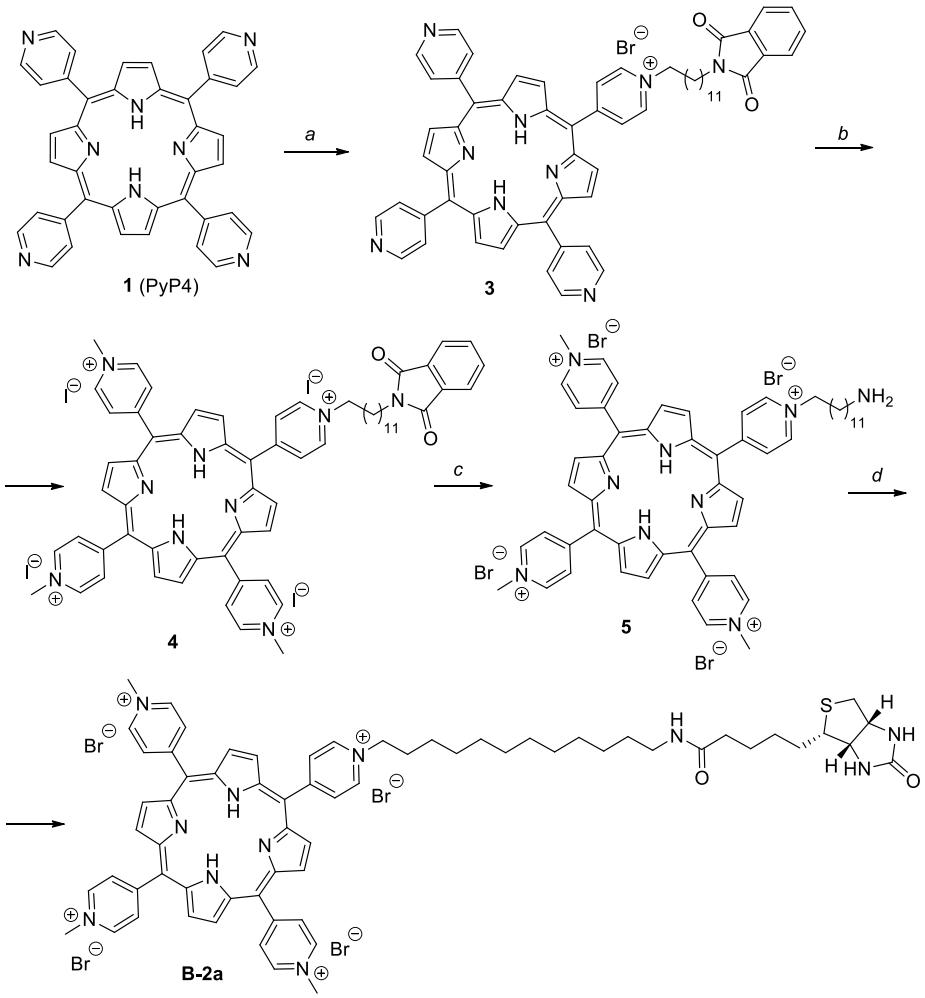
Fig. S1: Synthesis of biotinylated porphyrin **2a** (**B-2a**)



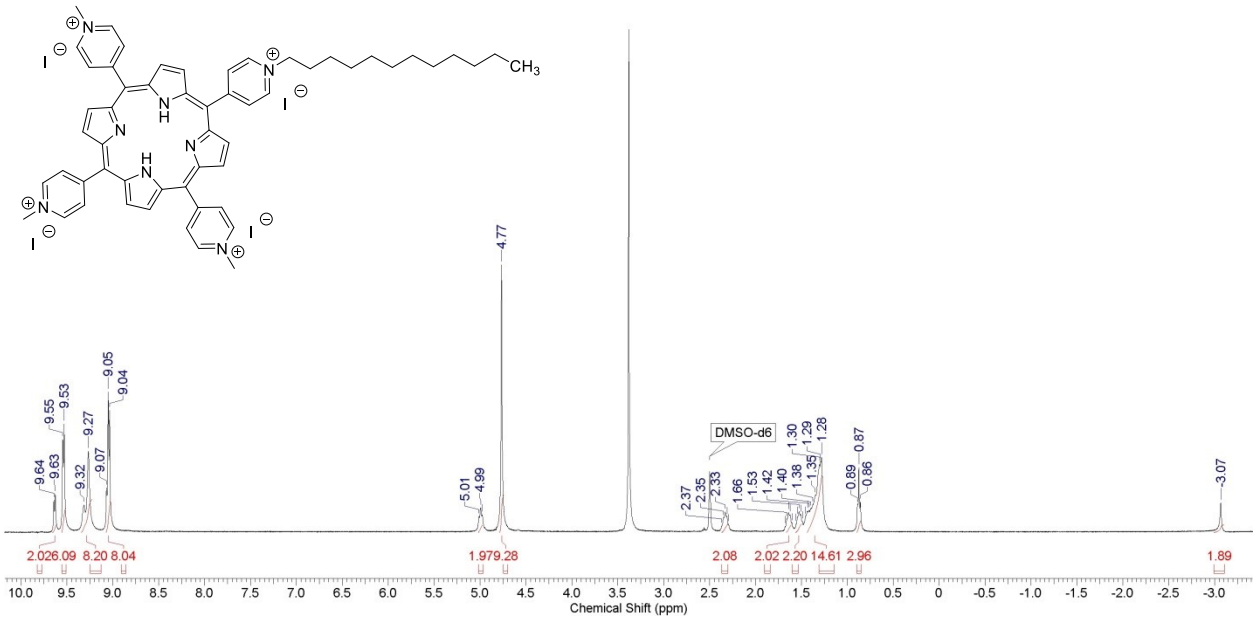
[a] Reagents and conditions: (a) 2-(12-bromododecyl)phthalimide, acetic acid, argon, reflux; 72 h; yield 33 %; (b) iodomethane, DMF, 140 °C; 3 h; yield 97 %; (c) hydrobromic acid, argon, boiling; 24 h; yield 80 %; (d) biotin, PyBOP, DIPEA, DMSO, rt; 1 h; yield 68 %.

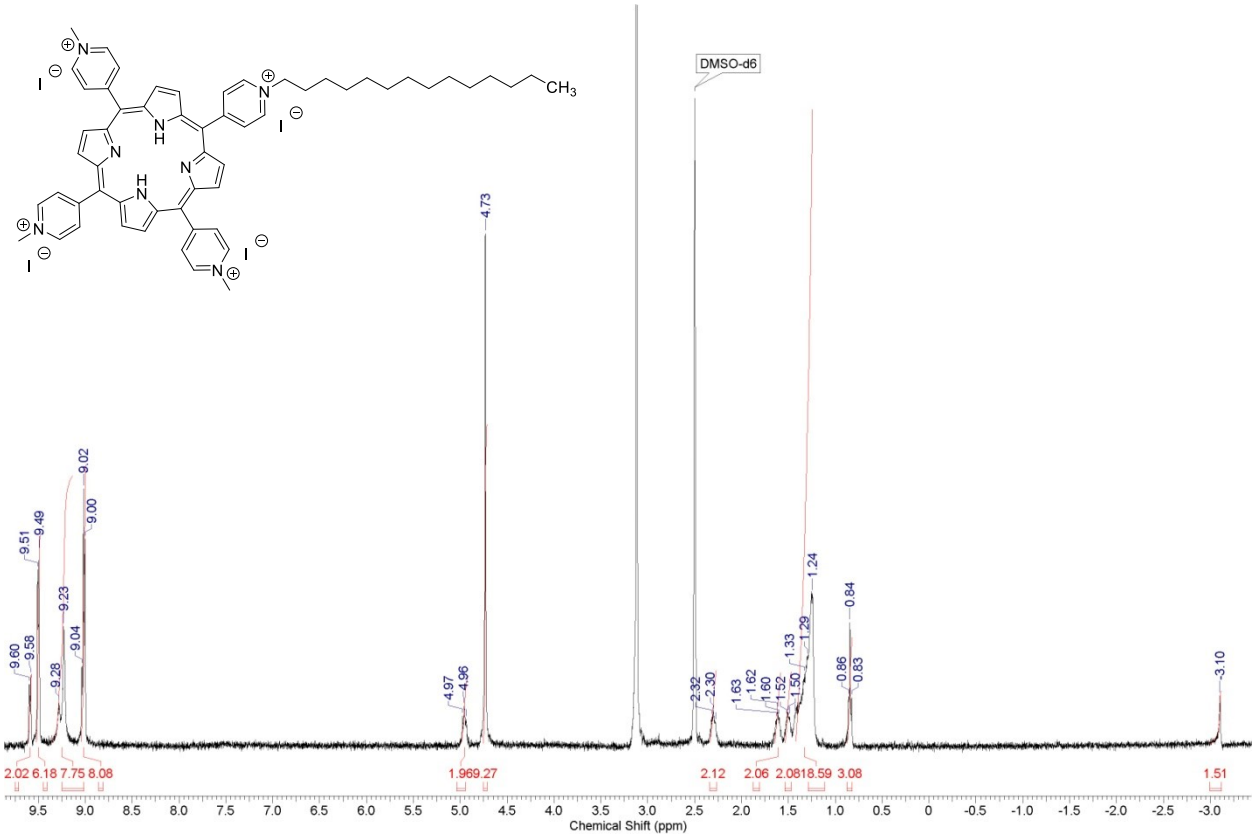
As shown in the above scheme, the alkylation of PyP4 (**1**) with 2-(12-bromododecyl) phthalimide in the same manner led to mono-cationic derivative **3** which was next methylated to produce 5-(4-*N*-(12-phtalimidododecyl)pyridyl)-10,15,20-tri(4-*N*-methylpyridyl)-21H,23H-porphyrine **4.** The cleavage of the phthalimide protecting group was carried out by boiling **4** in hydrobromic acid for 24 h to afford 12-aminododecyl porphyrin derivative **5**. Further coupling of the amino derivative **5** with biotin in presence of PyBOP resulted in cationic porphyrin **2a** conjugated to biotin (**B-2a**) in a good yield. The structures of synthesized compounds were

confirmed by ^{1}H and ^{13}C NMR, HRMS methods, and elemental analysis; the purity (≥95%) of final samples was estimated by HPLC (see Fig. S2). An absence or significant broadness of the pyrrole moiety signals at ^{13}C NMR spectra of obtained derivatives is typical for cationic porphyrins and can be explained by dynamic tautomerism (33).

Scheme 2. Synthesis of the biotinylated cationic porphyrin **B-2a**.[a]

Fig. S2: ^{1}H, ^{13}C NMR and UV spectra, HPLC of porphyrins **2a-d**

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9.60
9.58
9.51
9.49
9.28
9.23
9.04
9.02
9.00
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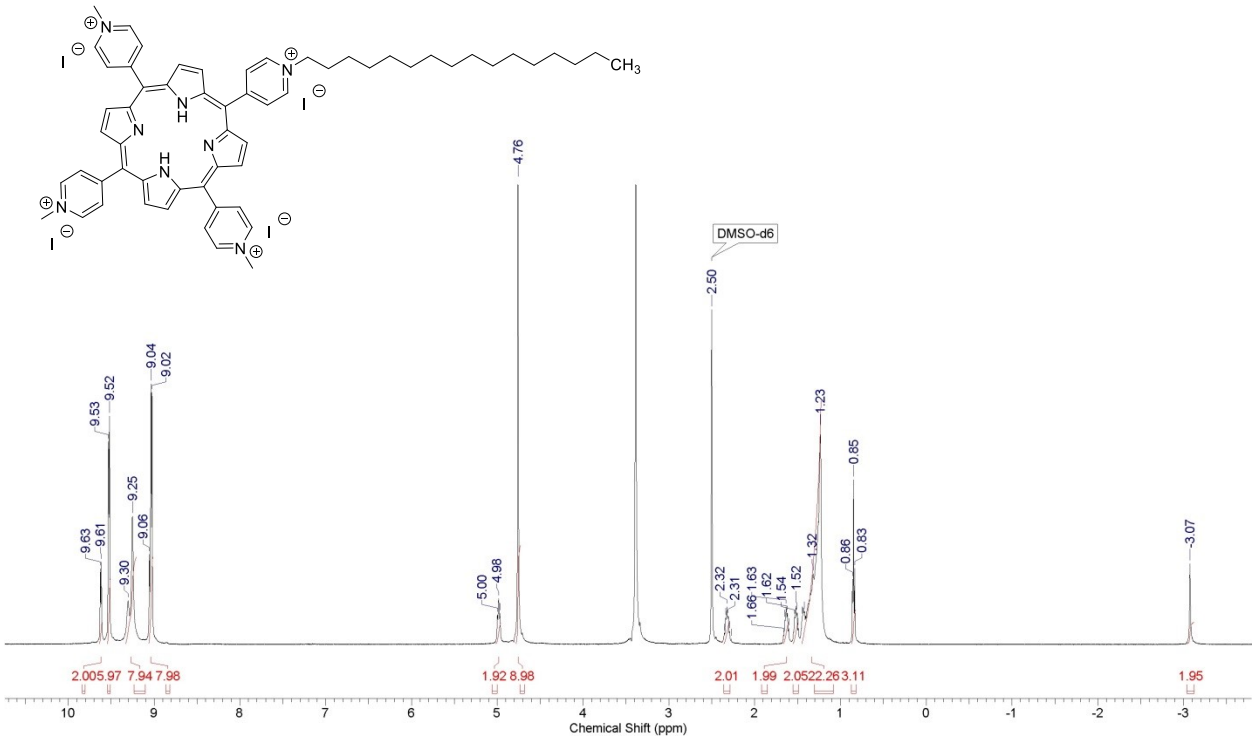
4.96
2.32
2.30
1.63
1.62
1.60
1.52
1.50
1.33
1.29
1.24
0.86
0.83
0.84
3.10
2.02 6.18 7.75 8.08
1.96 9.27
2.12
2.06 2.08 18.59 3.08
1.51
Chemical Shift (ppm)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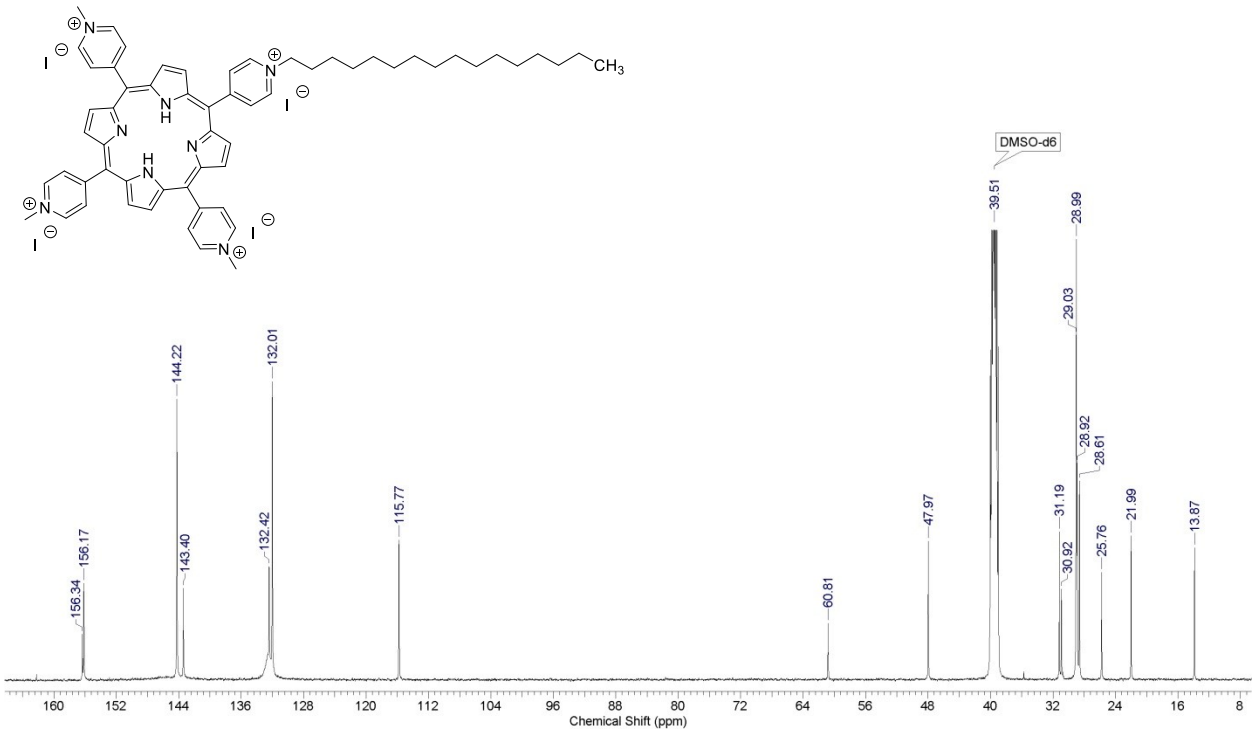
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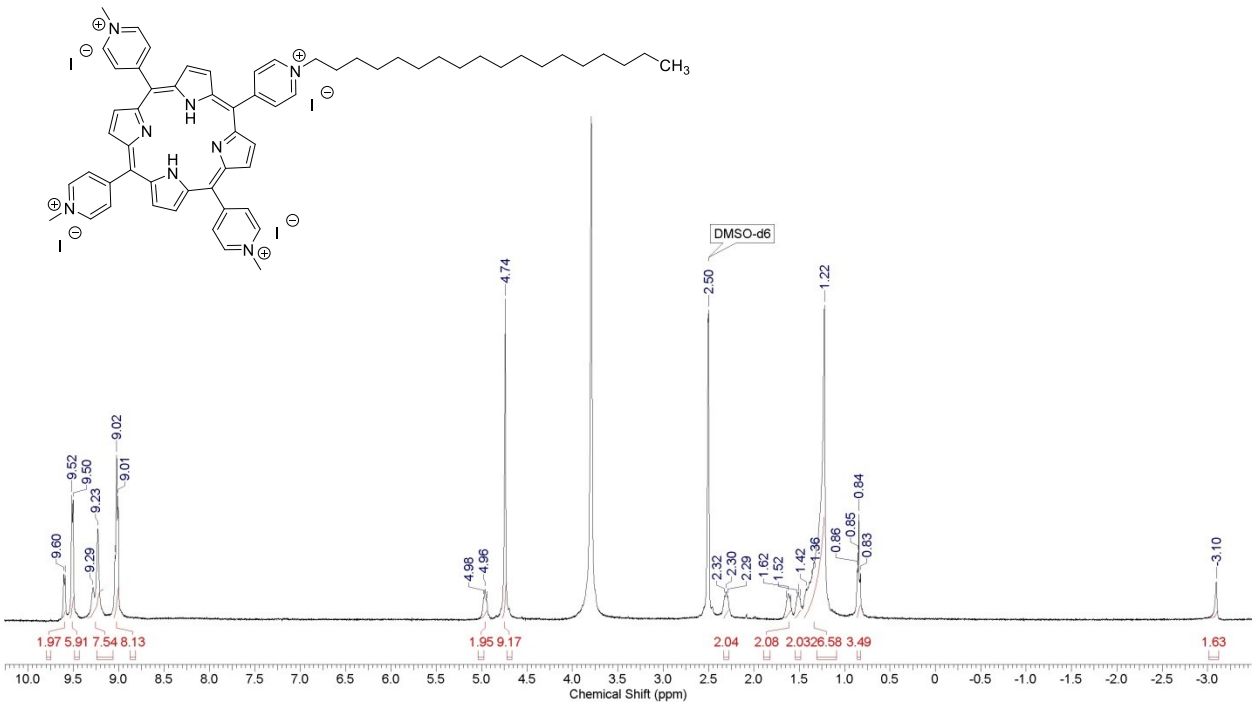
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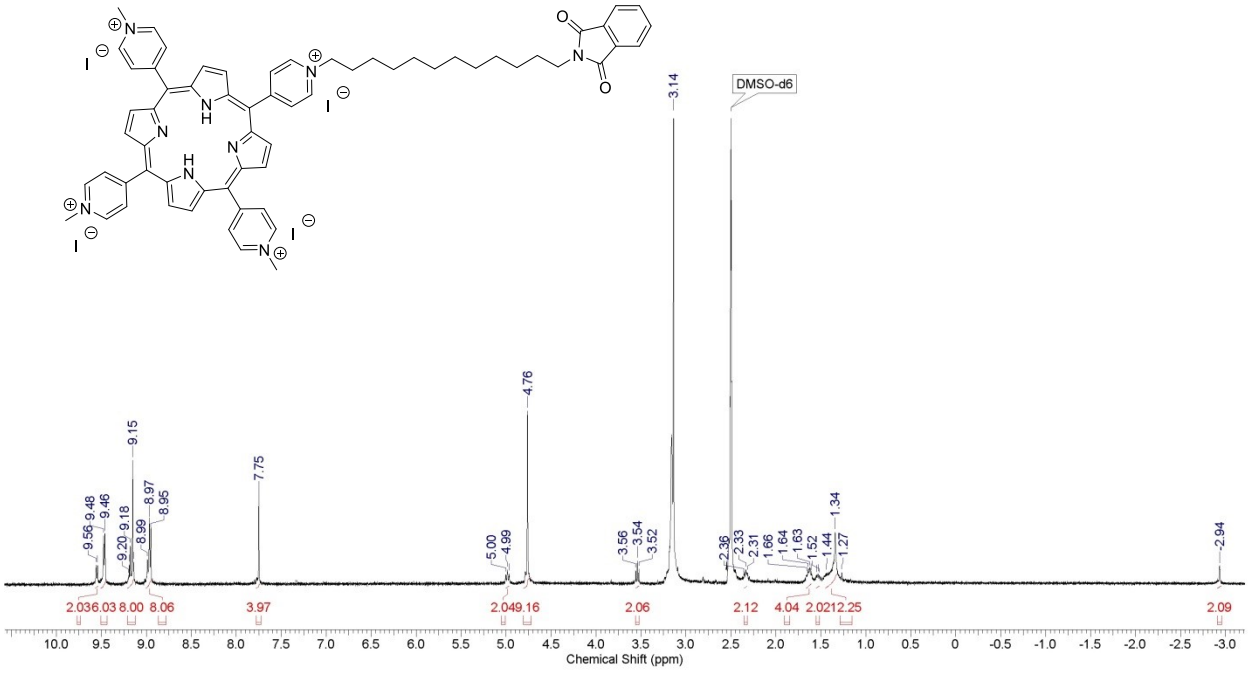
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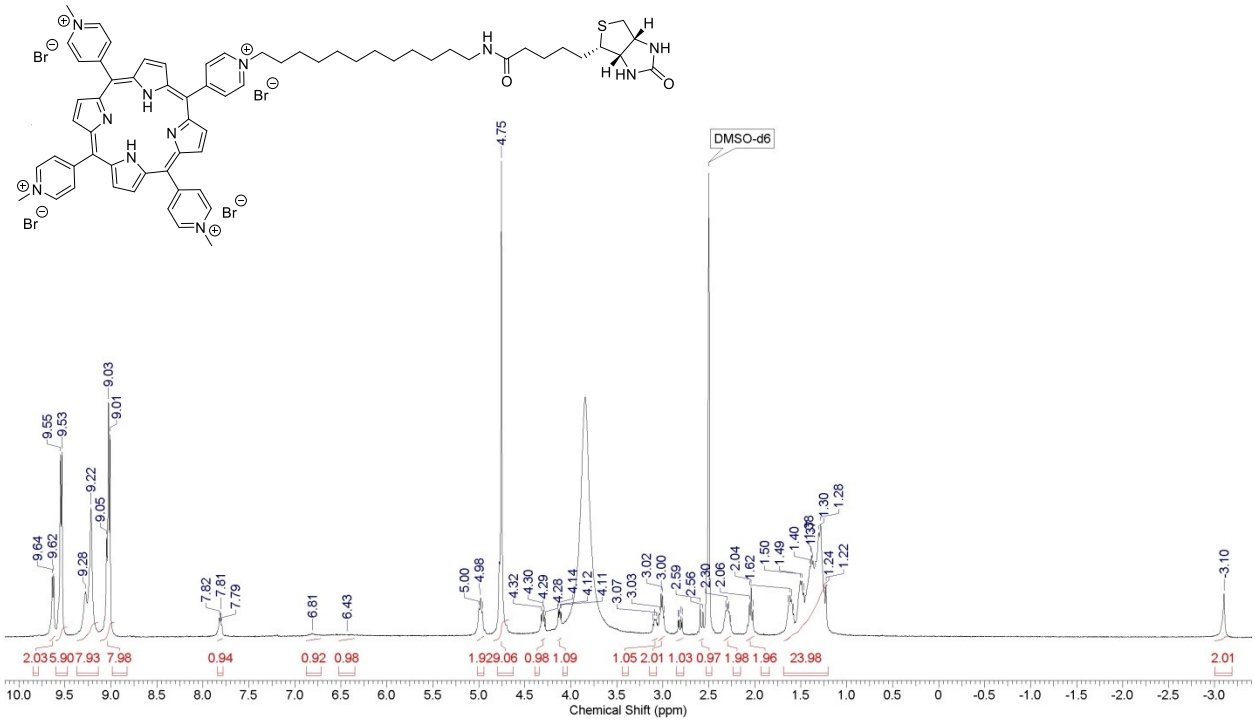
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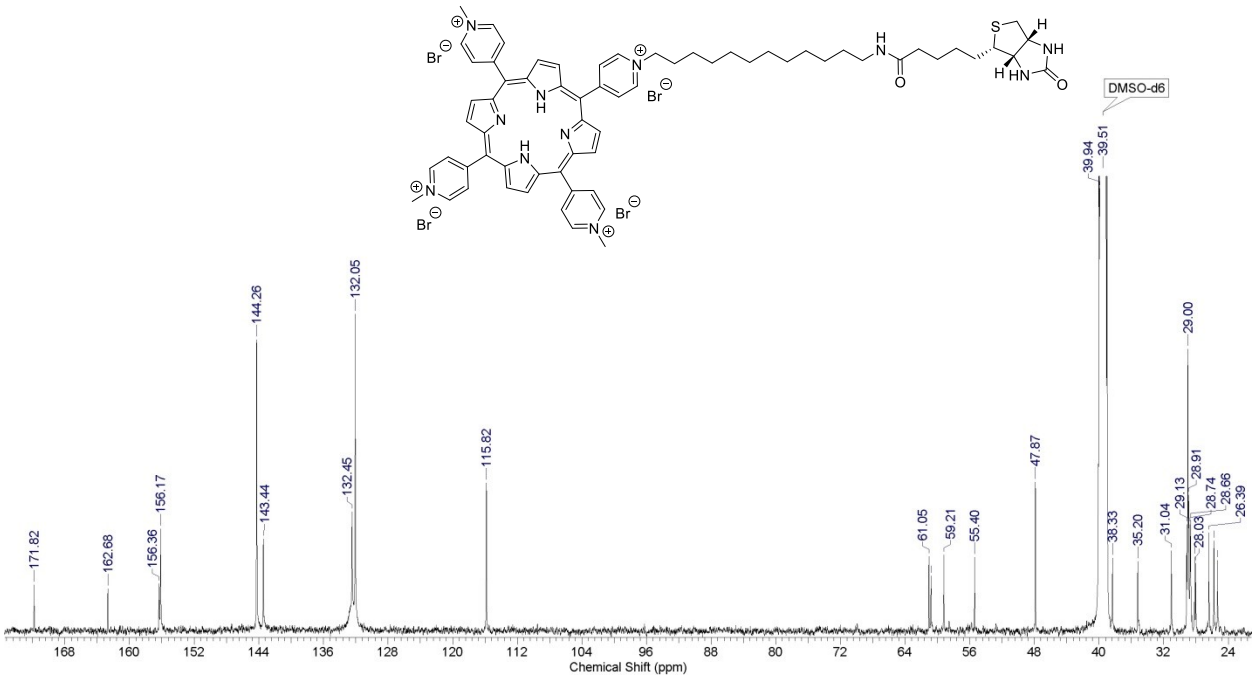
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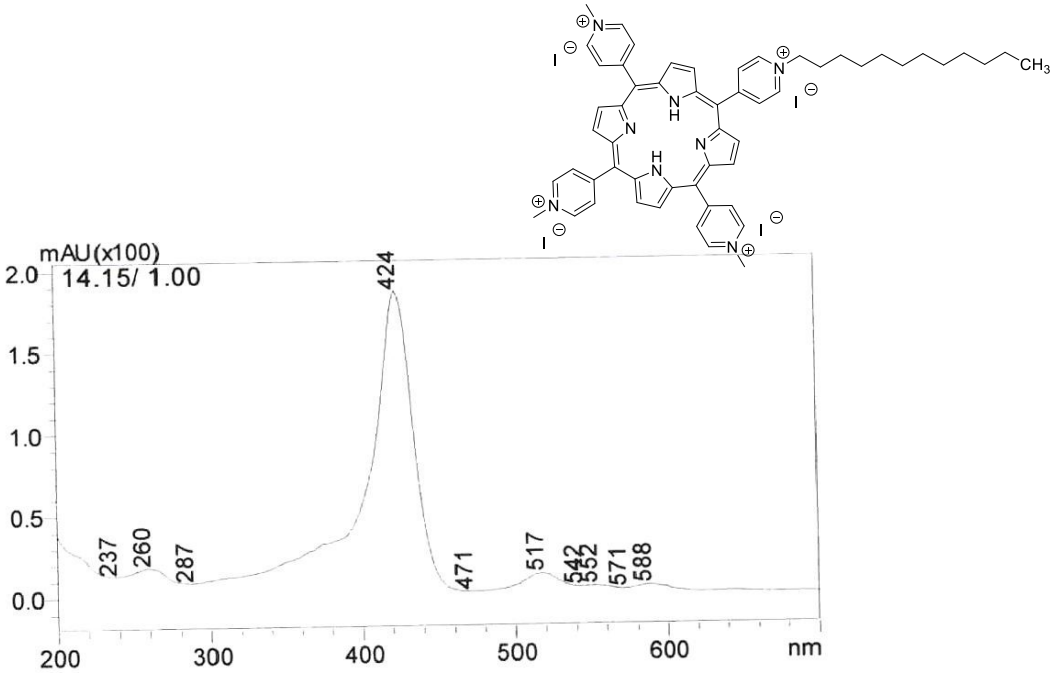
Chemical Shift (ppm)

UV Spectra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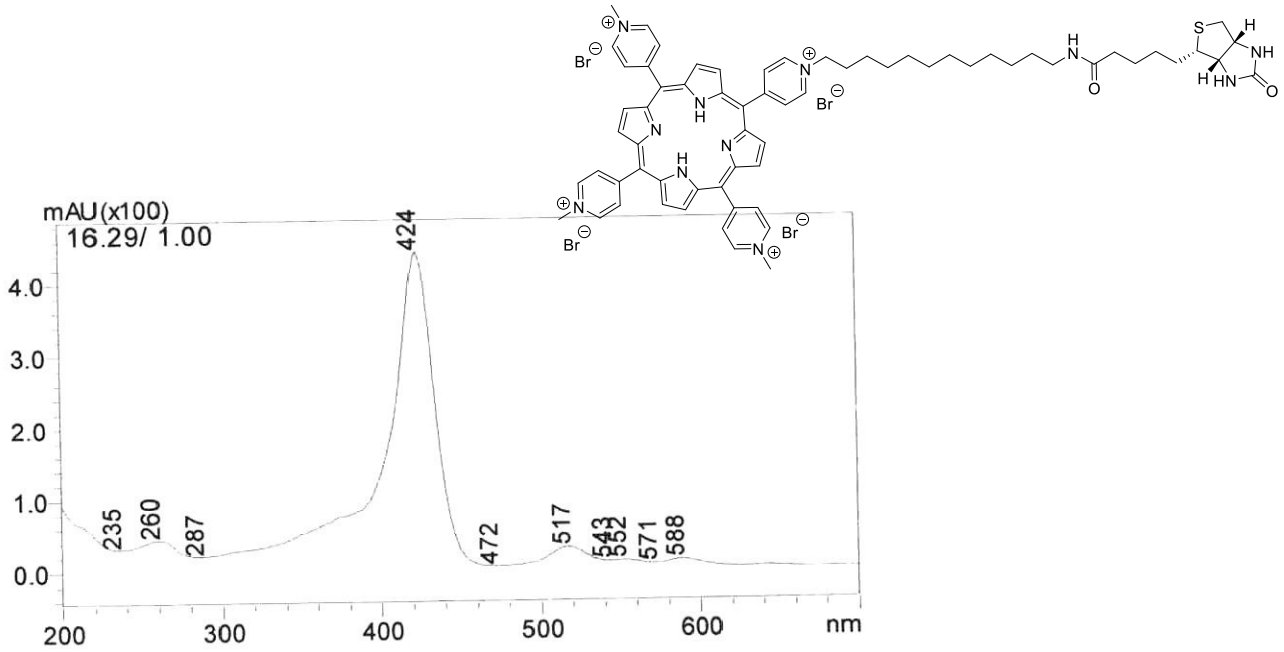
237
260
287
471
517
542
552
571
588
nm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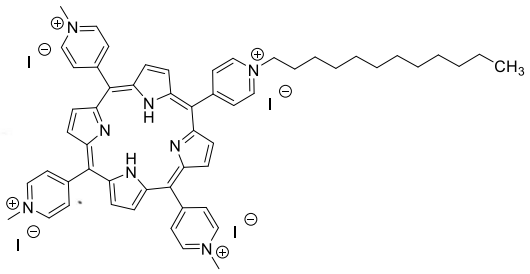
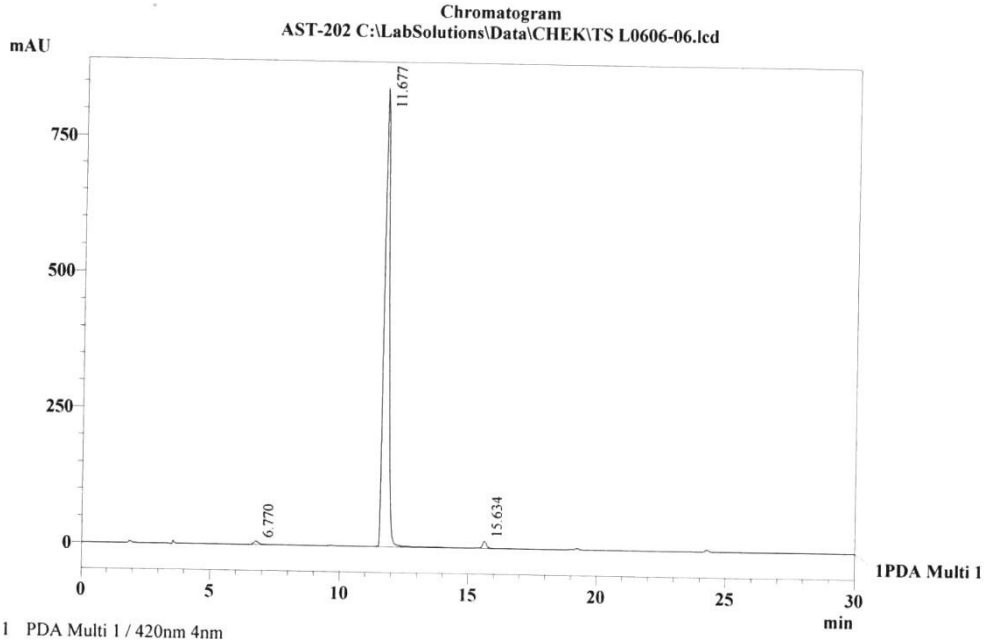
235
260
287
472
517
543
552
571
588
nm

HPLC Chromatograms





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PDA Ch1 420nm 4nm

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3	15.634	127137	12770	1.079
Total		11780555	862730	100.000

Method

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Time	Unit	Command	Value
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33.00	Pumps	B.Conc	25
45.00	Controller	Stop	

Method Filename : FOS C.lcm

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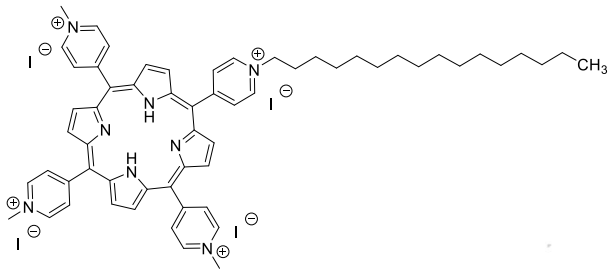

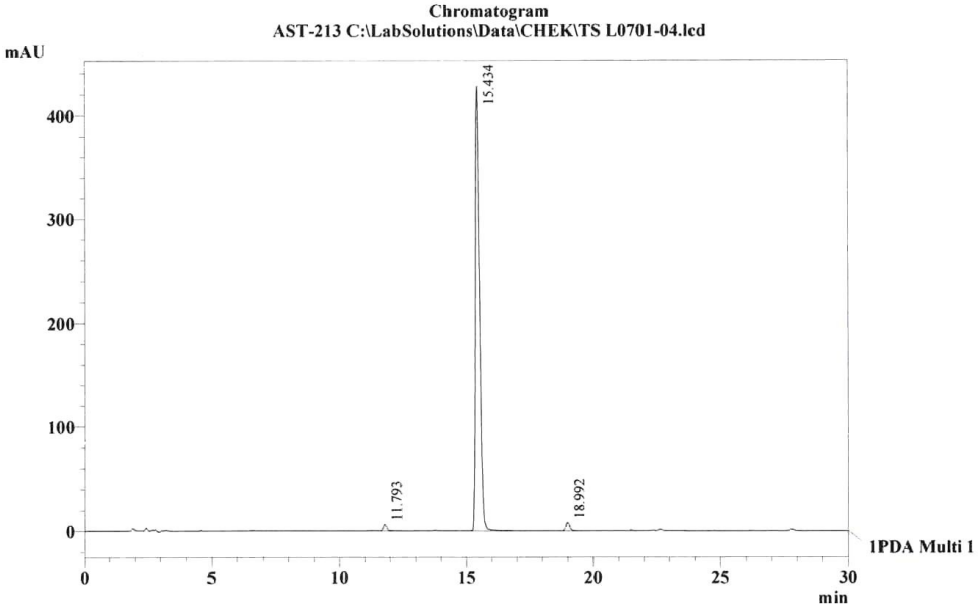

1 PDA Multi 1 / 424nm 4nm

PeakTable

PDA Ch1 424nm 4nm

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1	11.793	59646	6154	1.125
2	15.434	5158392	427698	97.291
3	18.992	84001	7989	1.584
Total		5302040	441842	100.000

Method

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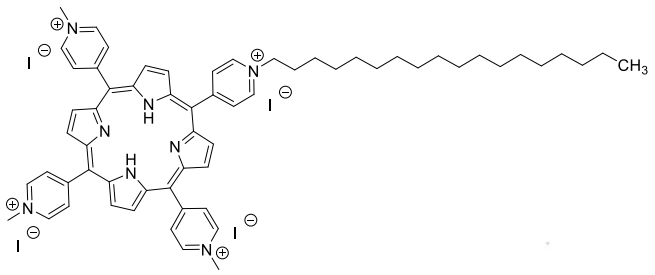

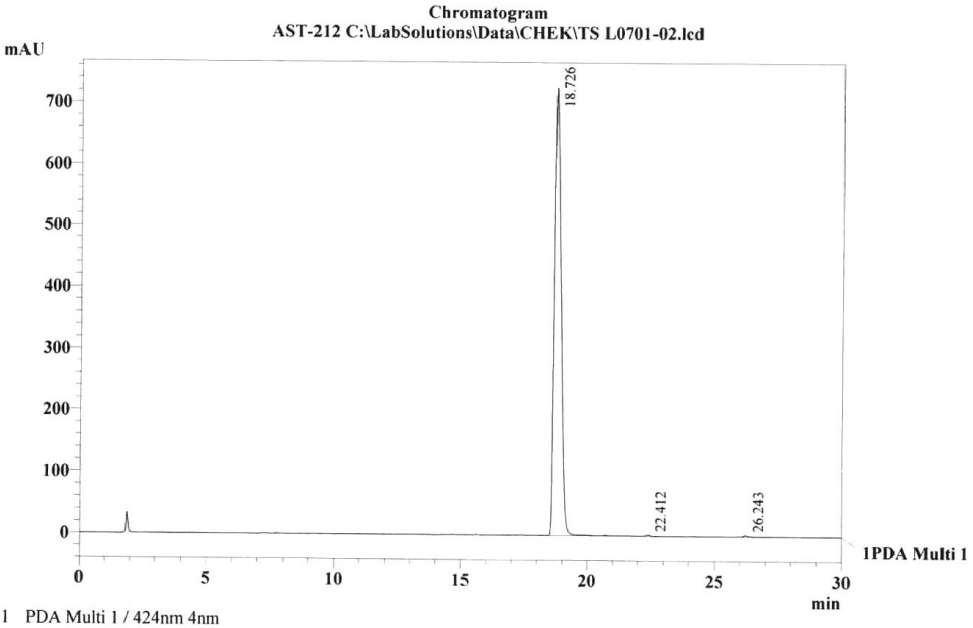

1 PDA Multi 1 / 424nm 4nm

PeakTable

PDA Ch1 424nm 4nm

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1	18.726	12571106	725863	99.629
2	22.412	21188	1664	0.168
3	26.243	25648	2324	0.203
Total		12617942	729851	100.000

Method

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Time	Unit	Command	Value
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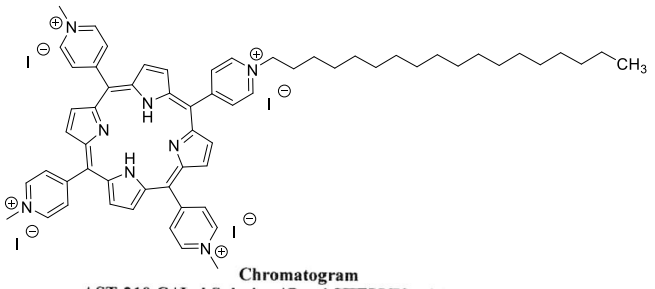
Method Filename : FOS Bv.lcm

Shimadzu LC-20AD; 2-System FOS, Colon Kromasil 100-C18,.size 5mkm, 4,6*250mm, N 86912
Elution: A - H3PO4 0.01M pH 2.6; B - MeCN, fl. 1,0 ml/min, loop 20mkl.



Chromatogram

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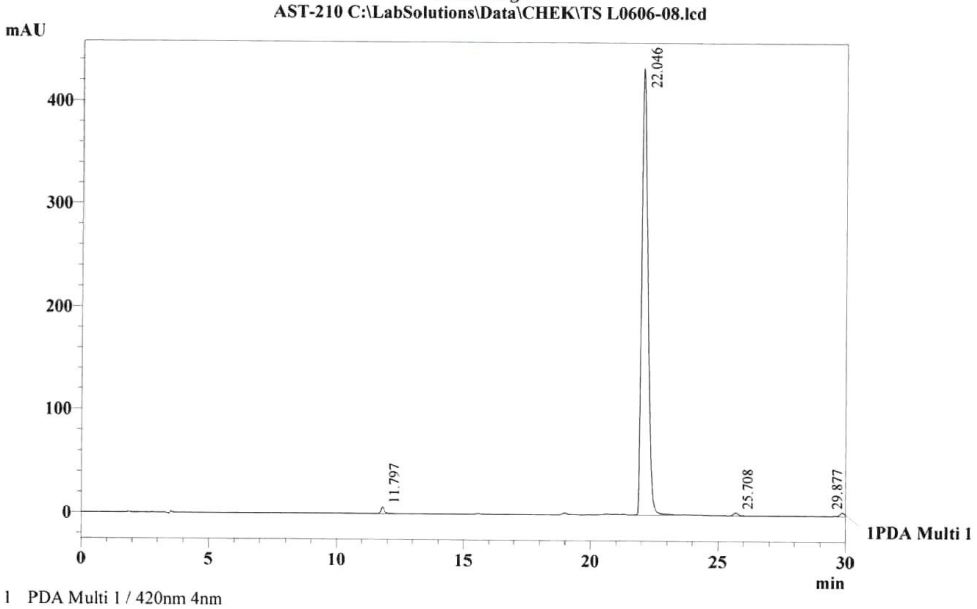

1 PDA Multi 1 / 420nm 4nm

PeakTable

PDA Ch1 420nm 4nm

Peak#	Ret. Time	Area	Height	Area %
1	11.797	60244	6095	0.788
2	22.046	7495108	432707	98.071
3	25.708	40684	3117	0.532
4	29.877	46463	3614	0.608
Total		7642500	445532	100.000

Method

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Time	Unit	Command	Value
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33.00	Pumps	B.Conc	25
45.00	Controller	Stop	

Method Filename : FOS C.lcm

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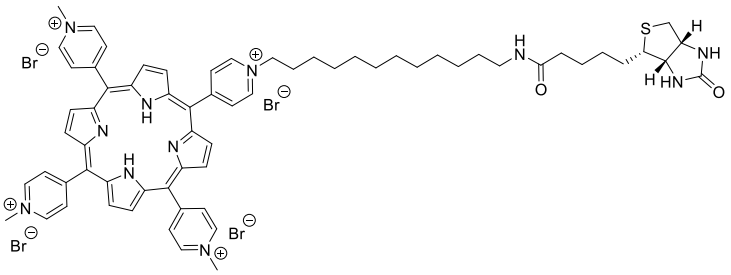
Elution: A - H3PO4 0.01M pH 2.6; B - MeCN, fl. 1,0 ml/min, loop 20mkl.



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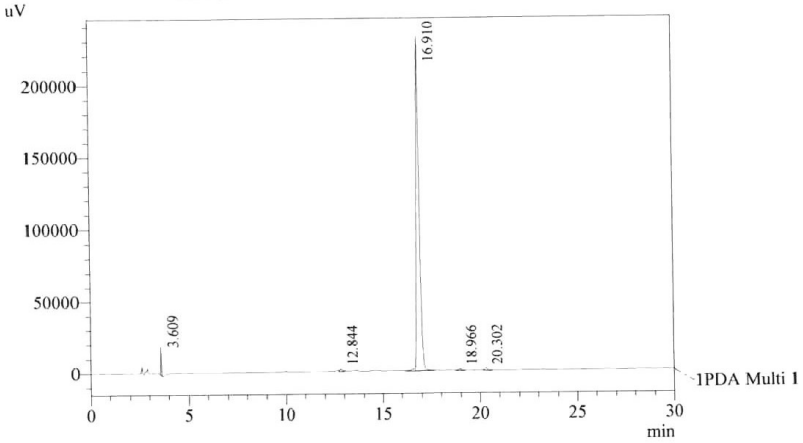

1 PDA Multi 1 / 425nm 4nm

PDA Ch1 425nm 4nm

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3	16.910	2722475	231547	96.806
4	18.966	12637	1232	0.449
5	20.302	13740	1651	0.489
Total		2812304	255715	100.000

Method Filename : FOS B.lcm 05.03.2018 15:37:54

Time	Unit	Command	Valu
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30.00	Pumps	B.Conc	60
33.00	Pumps	B.Conc	10
45.00	Controller	Stop	

Shimadzu LC-20 AD; System - FOS Colon- Kromasil-100-5mkm. C-18, 4,6x250 mm. N 62511
Elution: A - H3PO4 0,01M pH 2,6; B - MeCN, fl - 1.0 ml/min, loop 20 mkl

Fig. S3: 5'-UTR of NRAS. The G4 motif is highlighted in yellow. The CD of the G4 motif in 50 mM Tris-HCl pH 7.4, 20 mM KCl is also shown.

gaaacgtccc gtgtgggagg ggcgggtctg ggtgcggcct gccgcatgac tcgtggttcg
gaggcccacg tggccggggc ggggactcag gcgcctgggg cgccgactga ttacgtagcg
ggcggggccg gaagtgccgc tccttggtgg gggctgttca tggcggttcc ggggtctcca
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agtttatctc **atg**

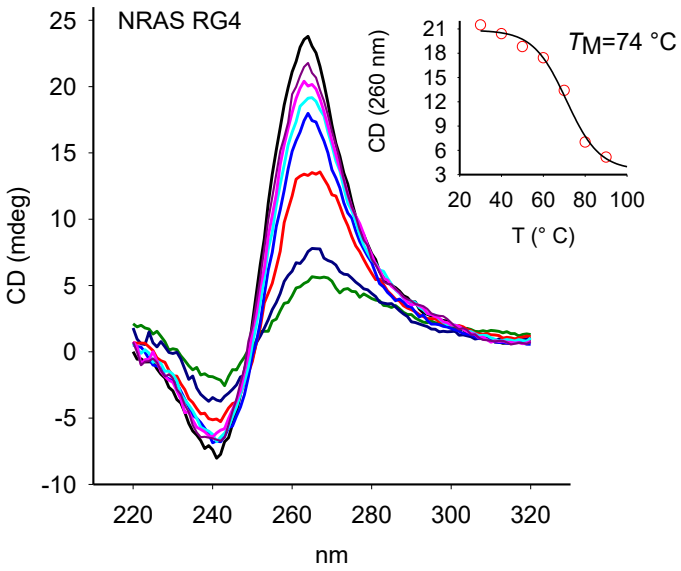


Fig. S4: Job plot relative to the interaction between porphyrin **2d** and utr-z RG4. At x=0.9, a stoichiometry of 9 porphyrins per RG4 is found.

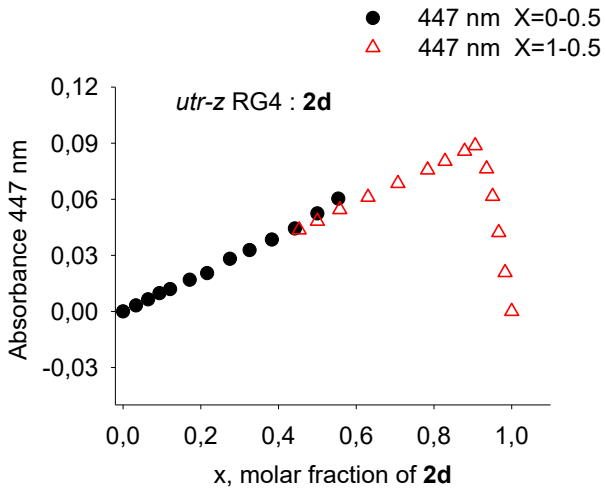


Fig. S5. Fluorescence titration of porphyrin 1 μM TMPyP2 (P2) with utr-z RG4 in 50 mM Tris-HCl, pH 7.4, 100 mm KCl

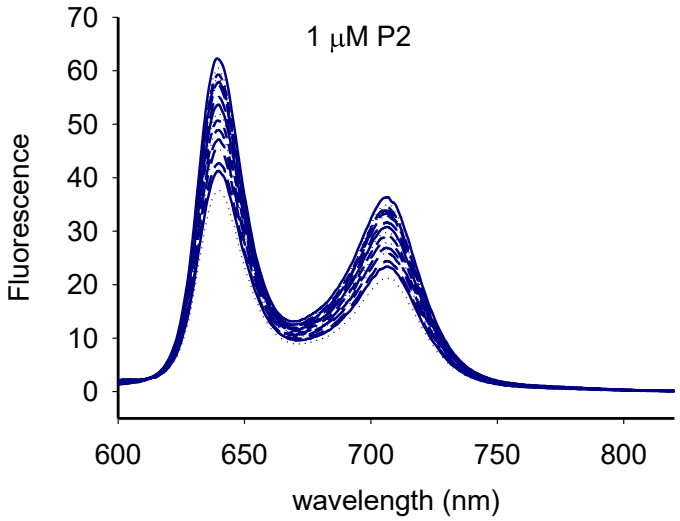
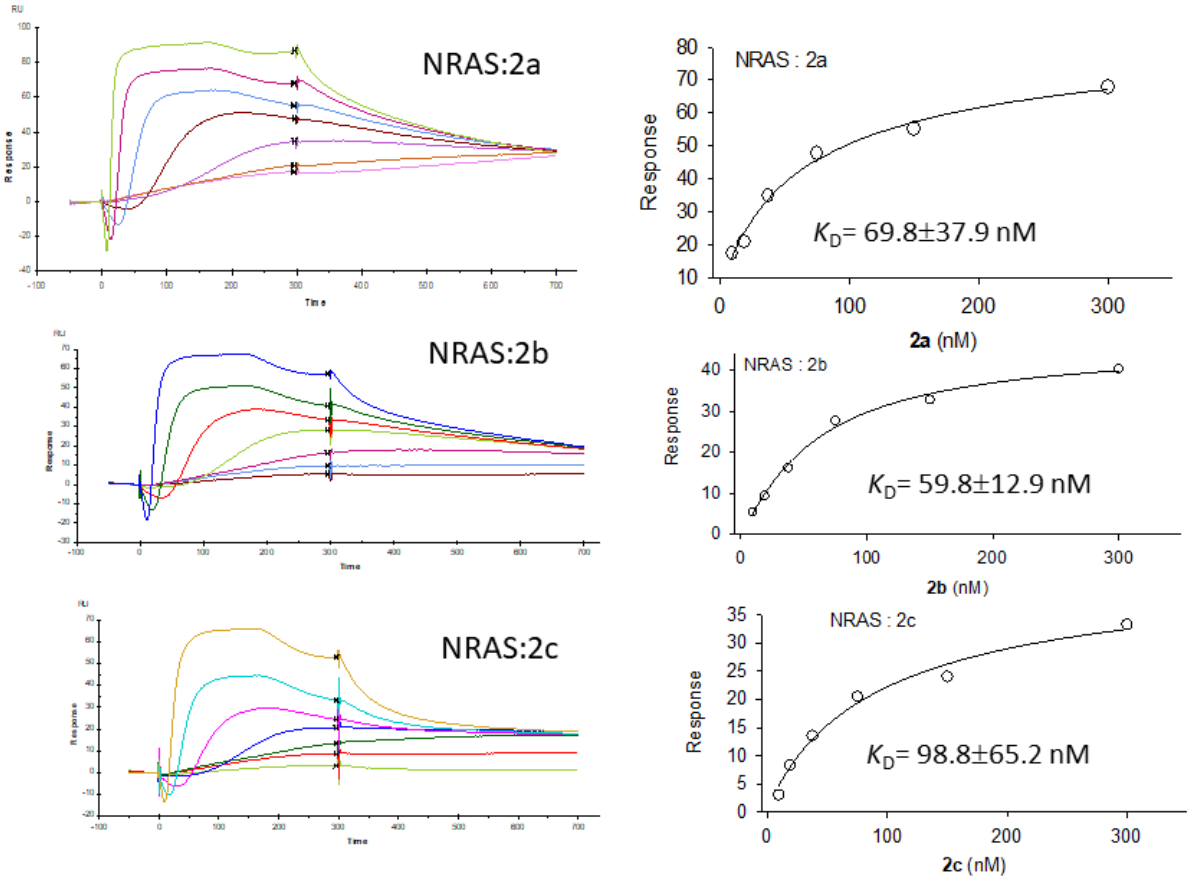
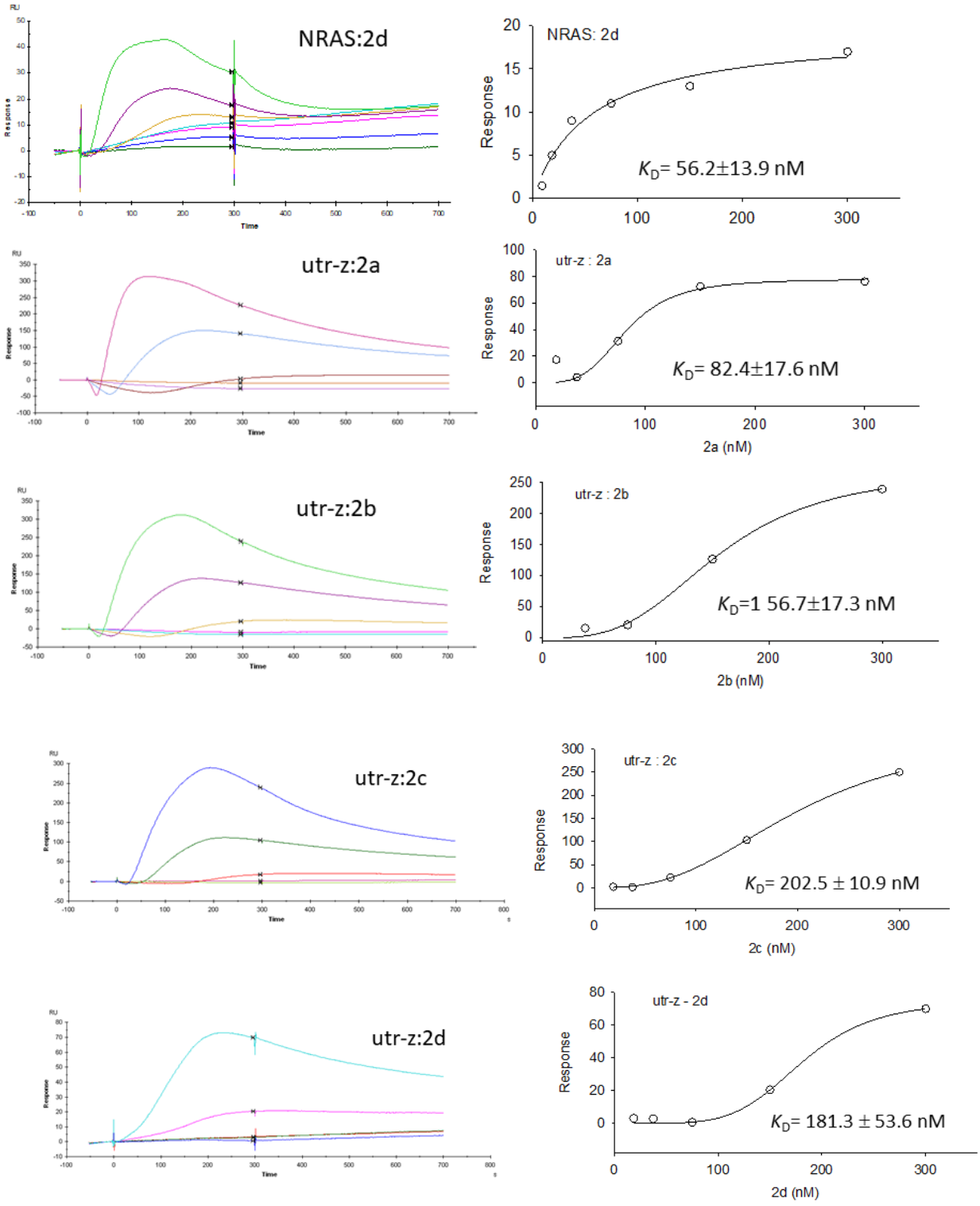


Fig. S6: Surface Plasmon Resonance experiments





SPR analysis was carried out on a Biacore T100 platform (Healthcare, Life Science, Milan, Italy). Biotinylated oligoribonucleotides (NRAS and utr-z), purchased form Microsynth (CH), were diluted to 80 nM and folded in HEPES-KCl buffer (10 mM HEPES pH 7.4,150 mM KCl and 3 mM EDTA). The folded oligonucleotides were immobilized on a streptavidin-coated sensor chip (SA sensor chip, Biacore, Healthcare) to reach a relative response of 500-1000 RU. Flow cell 1 was left

empty to allow reference subtraction. To avoid unspecific binding and aggregation, SPR analysis was performed at 40 °C using as running buffer HEPES KCl (10 mM HEPES, pH 7.4, 150 mM KCl, 3 mM EDTA) supplemented for the interaction with *NRAS* RG4 with 100 NaCl, 0.005 % Tween and 1 mg/ml BSA; the binding kinetic was monitored with 300 sec association time; 400 sec dissociation time; flow rate 25 µl/min. After each compound injection, the chip surface was regenerated with 2.5 M NaCl for 20 sec at 30 µl/min. Sensorgrams were obtained in the concentration range 600-9.37 nM and corrected for double subtraction of buffer injection response and reference flow cell. NaCl was removed from the running buffer to obtain more reliable sensorgrams of utr-z interaction. Sensorgrams were used to calculate steady state affinity according to Hill equation. For *NRAS* we excluded the sensorgram obtained at the highest porphyrin concentration, as it likely reflects high-order binding events (porphyrin aggregation on RG4), we obtained a steady-state binding curve saturating at a porphyrin concentration < 1 µM. The fit of the curve to the Hill equation gave the apparent K_D values reported in the plots. Most experiments were carried out in two replicates.

Fig. S7. Job plot relative to the interaction between porphyrin TMPyP4 and stem-loop hairpin. At x=0.67, a stoichiometry of 2 porphyrins per stem-loop hairpin is found.

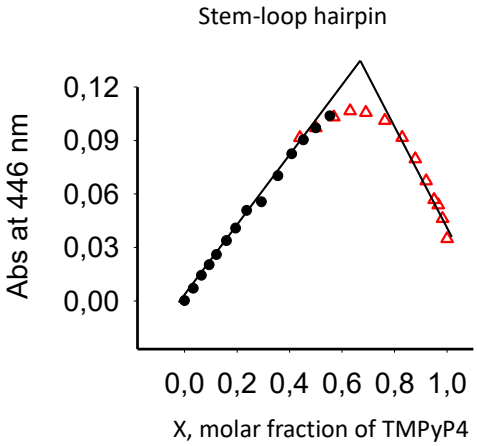


Fig. S8: FACS analyses of Panc-1, NIH 3T3 and HEK 293 cells incubated for 6 h with alkyl porphyrins **2b** and **2d** at 4 and 37 °C. Confocal microscopy shows that porphyrins **2b** and **2d** clearly delimit the cell surface suggests that they diffuse into the lipid bilayer and most likely penetrate the cell membrane also by passive diffusion. This is supported by the finding that in Panc-1 cell lines, the uptake at 4 °C is 25 % the uptake at 37 °C with **2b** and 35 % with **2d**. A similar behaviour was observed with NIH 3T3 and HEK 293 cells.

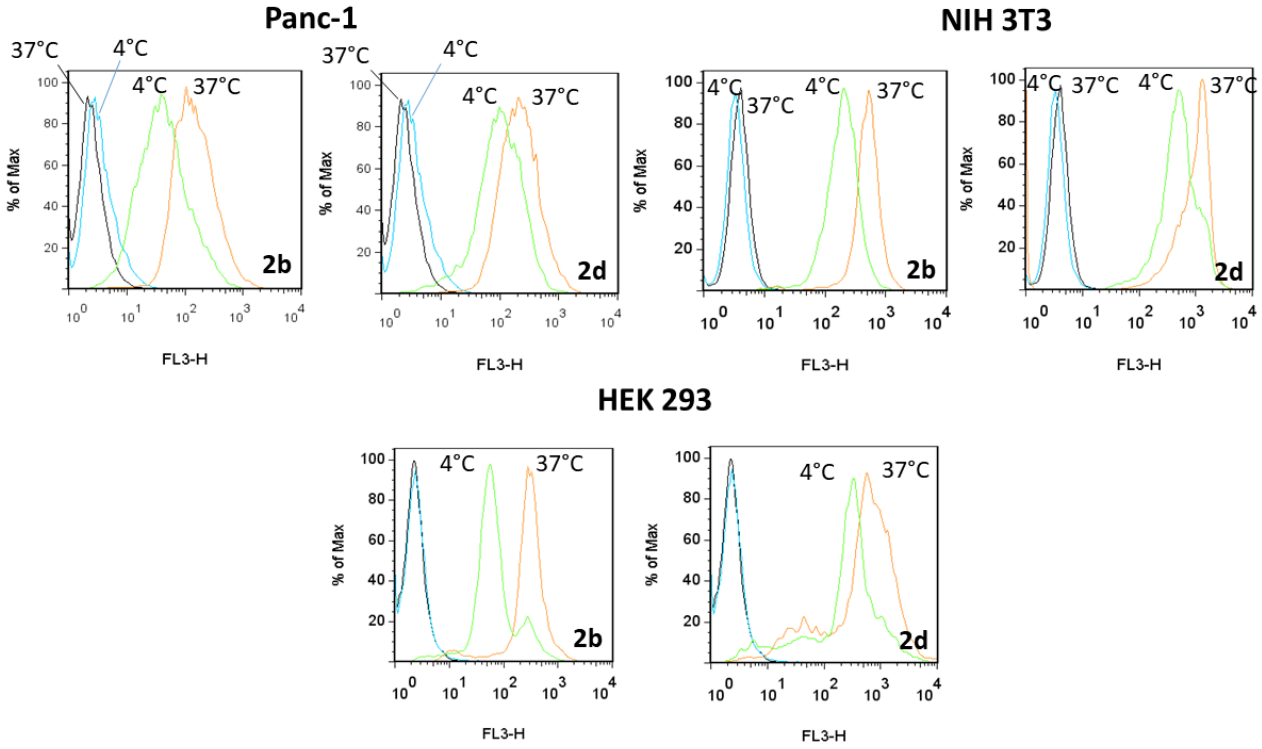


Fig. S9: melting curve of 95-mer template with the *htel* G4 sequence at the 3'-end. The profile is biphasic: the transition at ~ 63 °C is due to the htel G4 (ref 43).

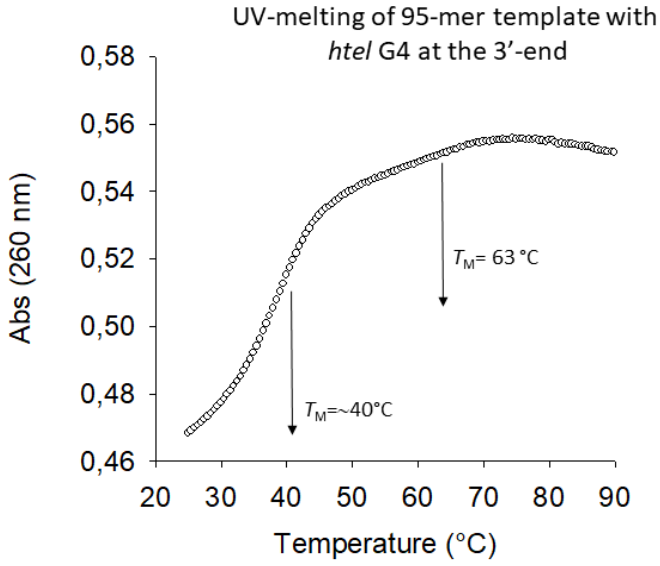
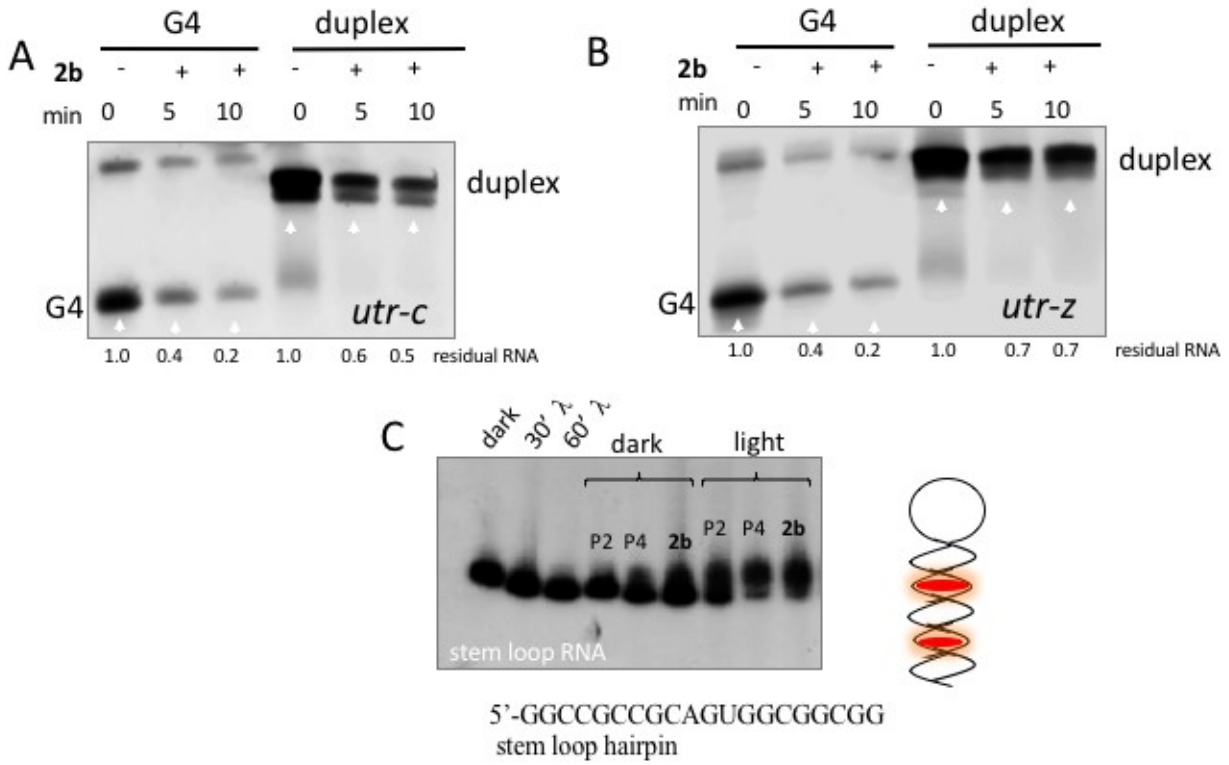


Fig. S10. Break down of RNA utr-c in G4 and duplex (A), RNA utr-z in G4 and duplex (B) by **2b** photoactivated for 5 and 10 min (7.2 J/cm^2).

Panel C shows the effect of photoirradiation on a 20 mer stem-loop hairpin treated with TMPyP2 (P2), TMPyP4 (P4) and **2b**. For all RNA analyzed, the RNA duplex or hairpin appears much more stable than the corresponding RG4 structure. The residual G4 or duplex RNA is reported for sequences *utr-c* and *utr-z*. The *utr-c* and *utr-z* duplexes are more resistant to photoirradiation than the corresponding RG4s. Stem loop RNA shows little degradation by photoirradiation after treatment with P2, P4 and **2b**.



Effect of photoactivated alkyl porphyrin 2b on TERRA RG4. TERRA RNA (5'-Cy5-UUAGGGUUAGGGUUAGGGUUAGGGUUAGGG) was folded in 50 mM Tris-HCl, pH 7.4, incubated for 30 min in ice with 2b and illuminated for 15 or 30 min with white light. After irradiation the RNA samples was loaded on a polyacrylamide gel. The gel shows the formation of two bands: one is due to

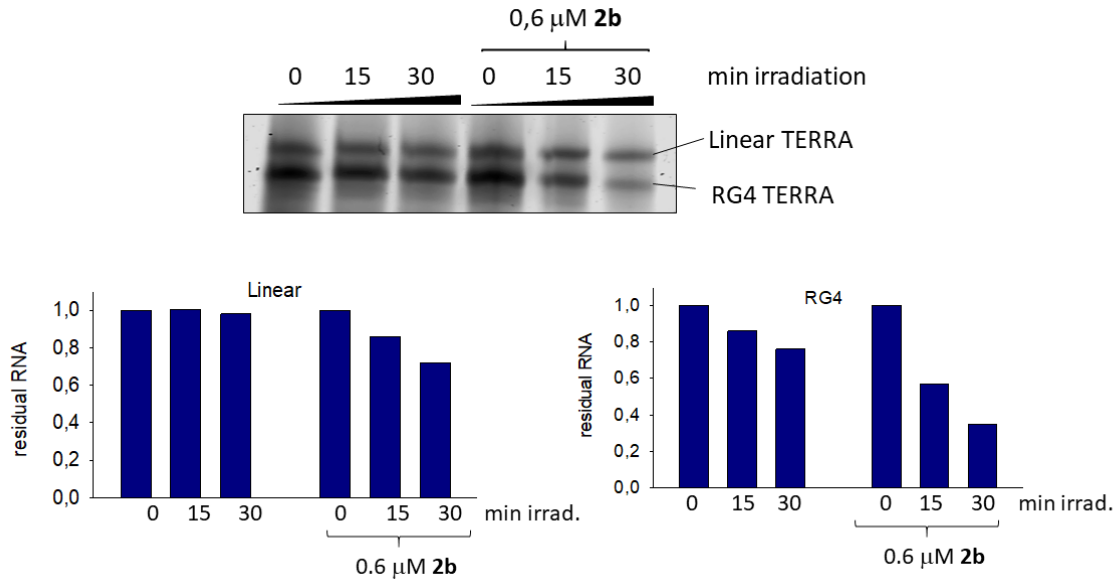
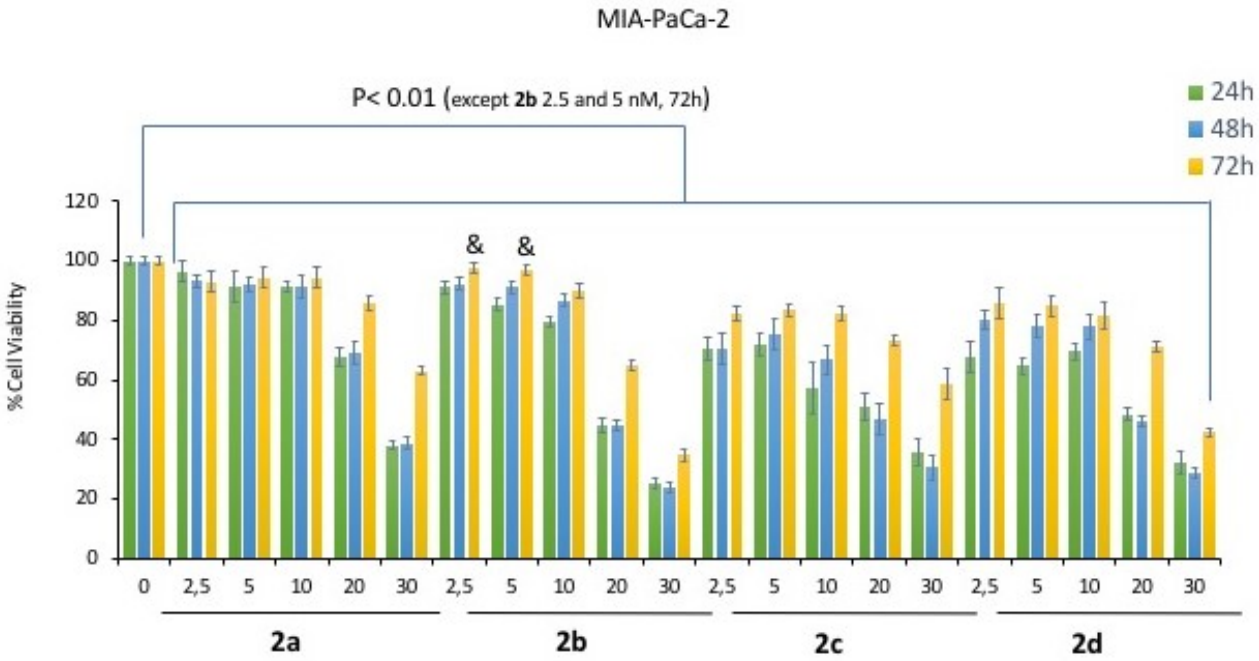


Fig S11: Cell viability of MIA-PaCa-2 cells treated with increasing amounts (0-30 nM) alkyl porphyrins and photoirradiated. Cell viability was measured 24, 48 and 72 h after photoirradiation. Compared to untreated cells, all treated cells (except samples 2.5 and 5 2b, 72 h) showed P<0.01. For comparison, a similar analysis was carried with non-cancer HEK 293 cells, which are not addicted to the *KRAS* proto-oncogene.



We have tested the effect of the designed alkyl porphyrins on the metabolic activity in non-cancer cell line HEK 293. This cell line is not addicted to oncogenic *KRAS* as pancreatic cancer cells is. This means that the survival of HEK 293 cells does not depend on the expression of *KRAS*. Indeed,

the effect of the porphyrins, which is expected to downregulate *KRAS* in these cells too, is weaker than the effect observed with pancreatic cancer cells.

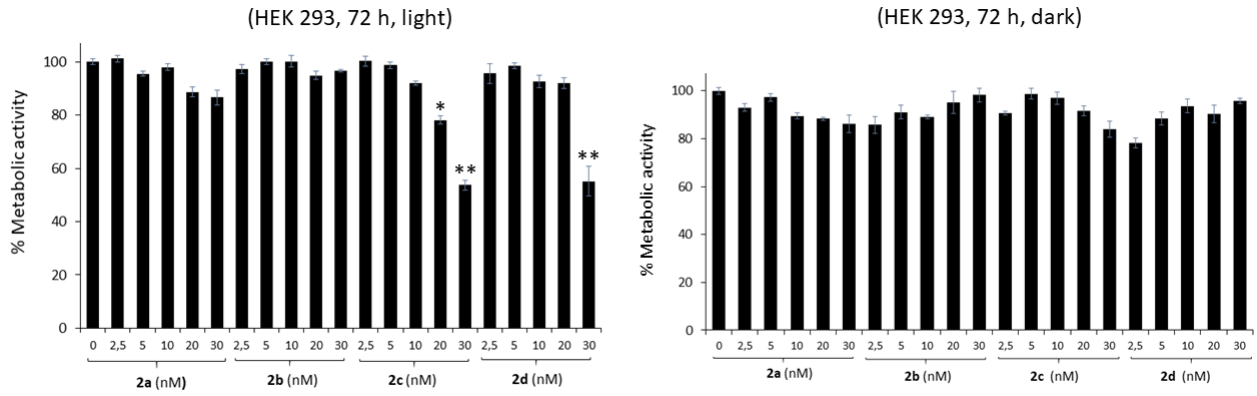


Fig. S12: Panc-1 and MIA-PaCa-2 cells treated with alkyl porphyrins (0-30 nM) in the dark. Resazurin assay carried out 96 h after treatment. No significant effects on cell viability are observed.

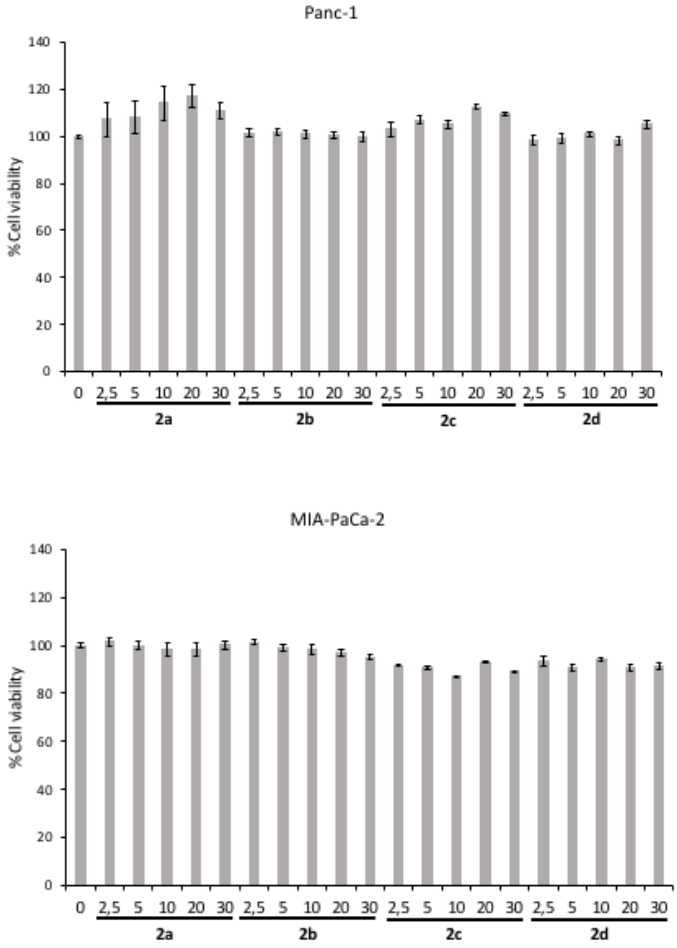


Table S13: Sequences of DNA primers and oligonucleotides used in the study

Name	Sequence (5'→3')
KRAS forward	CGAATATGATCCAACAATAGAG
KRAS reverse	ATGTACTGGTCCCTCATT
β2-microgobuline forward	CCCCACTGAAAAAGATGA
β2-microglobuline reverse	CCATGATGCTGCTTACAT
HPRT forward	CTTGATTGTGGAAGATATAATTG
HPRT reverse	TATATCCAACACTTCGTGG
*NRAS f*orward	CAG AGG CAG TGG AGC TTG
NRAS reverse	GCT TTT CCC AAC ACC ACC T
95-nt template for pull down	AGC TAC CTT GAT GAA TCC AAA CAA AGG CCT AAT CGC TAC CGT TAA GCA TCG ATC AGA TCA AGT GAT AGT ACT TAG GGT TAG GGT TAG GGT TAG GG
89-nt template for pull down	AGC TAC CTT GAT GAA TCC AAA CAA AGT CAT AGG GTC AGG ATG GTG CCT AAT CGC TAC CGT TAA GCA TCG ATC AGA TCA AGT GAT AGT AC
Primer template for (89- and 95-nt)	AGCTACCTTGATGAATCC
Primer template rev (89- and 95-nt)	GTACTATCACTTGATCTG AT
k-utr-Z, complementary sequence	CCGCCGACUGCCGCCGCC
k-utr-C, complementary sequence	AGCCGCCGCCACCUU
s-43 RNA fragment	CCGCGGCGGCGGAGGCAGCAGCGGCGGCGGCAGUGG CGGCGGC
Stem-loop hairpin	GGCCGCCGCAGUGGCGGC GG