Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Amino-OPE Glycosides and Blue light: A Powerful Synergy in Photodynamic Therapy

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Supplementary material

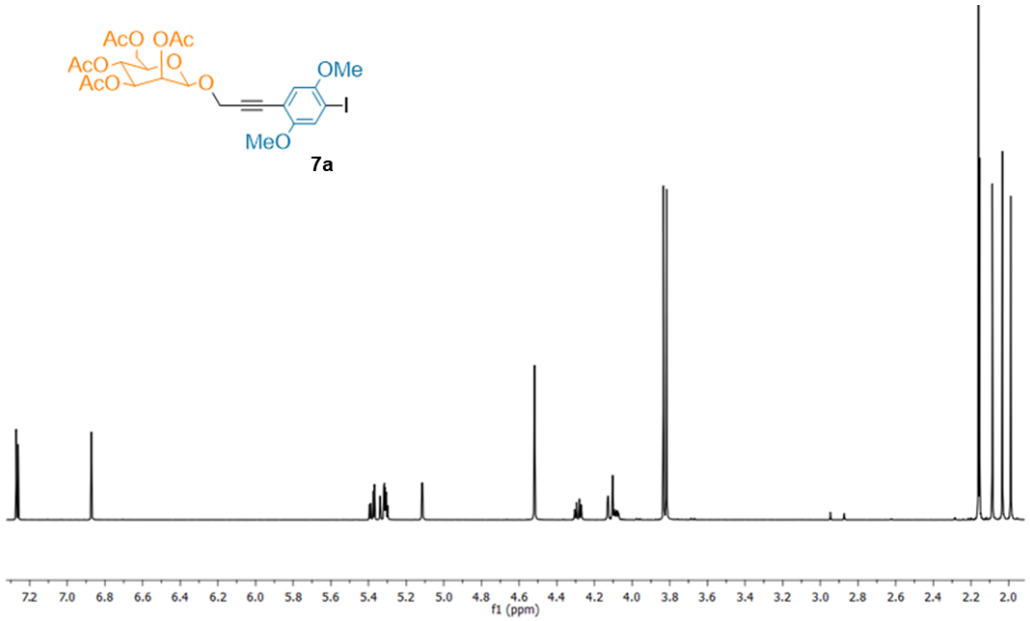

Fig. S1. ^{1}H NMR spectrum (500 MHz) of compound **7a** in $CDCl_3$. The reference signal of chloroform is set to δ = 7.26 ppm.

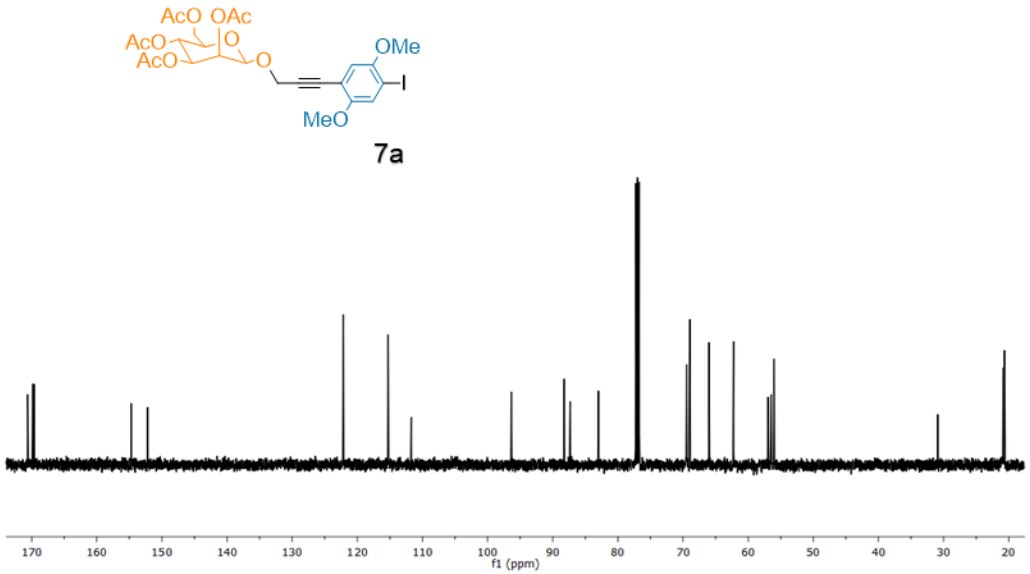

Fig. S2. ^{13}C NMR spectrum (500 MHz) of compound **7a** in $CDCl_3$. The reference signal of chloroform is set to δ = 77.0 ppm.

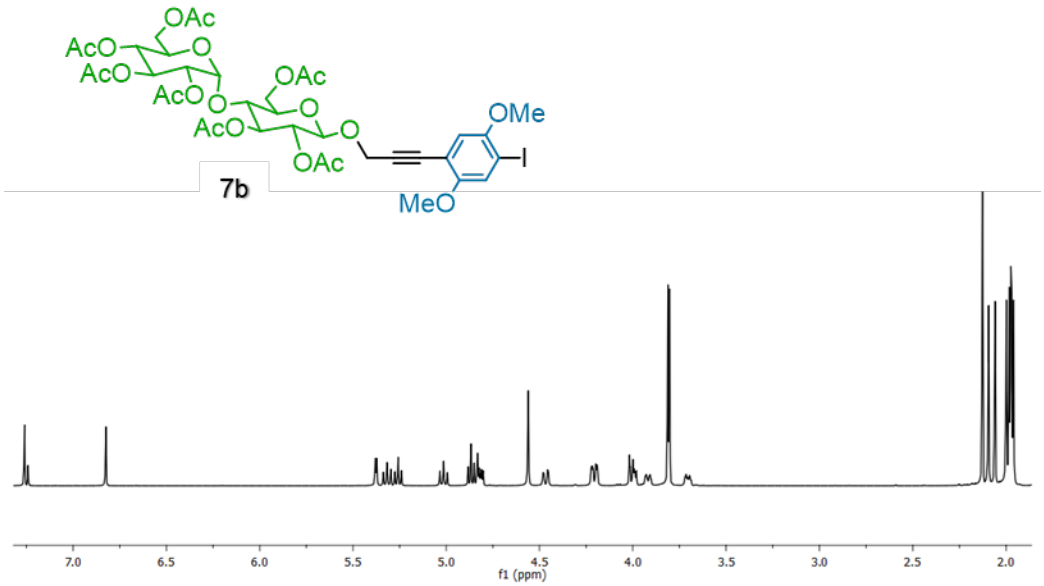

Fig. S3. ^{1}H NMR spectrum (500 MHz) of compound **7b** in $CDCl_3$. The reference signal of chloroform is set to δ = 7.26 ppm.

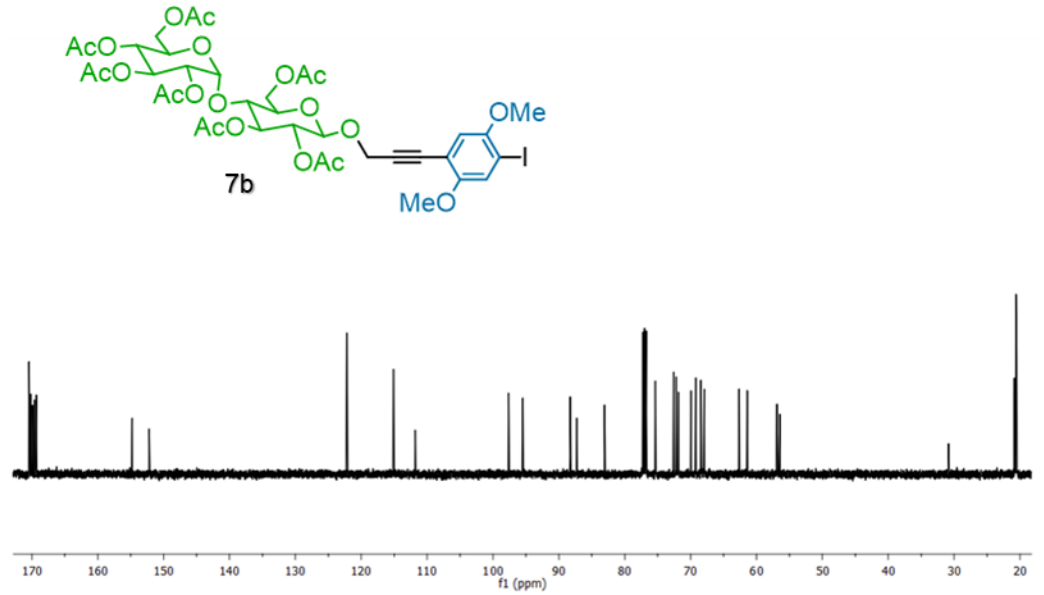

Fig. S4. ^{13}C NMR spectrum (500 MHz) of compound **7a** in $CDCl_3$. The reference signal of chloroform is set to δ = 77.0 ppm.

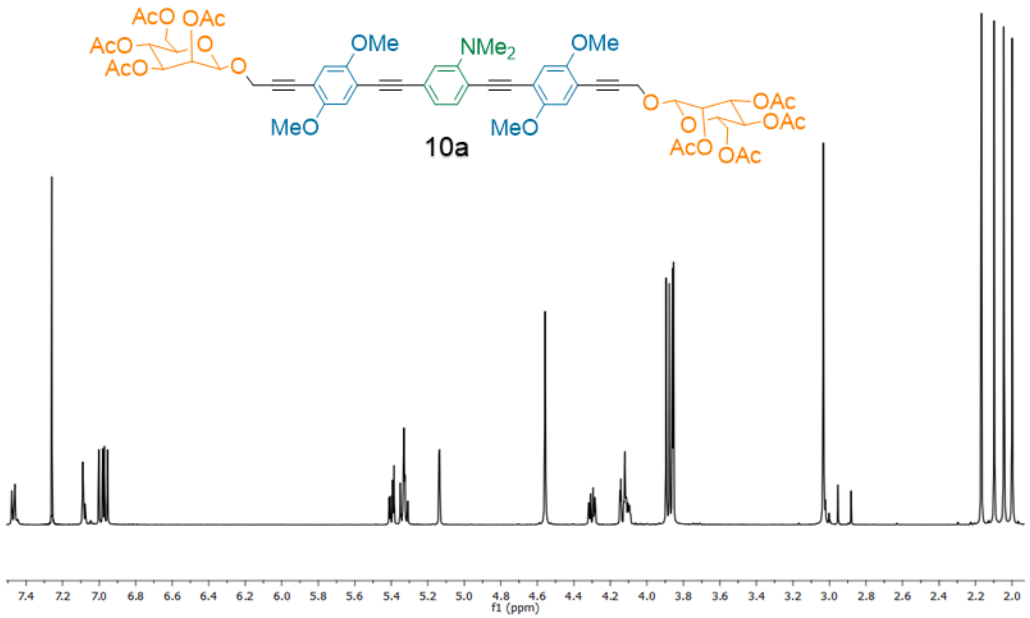

Fig. S5. ^{1}H NMR spectrum (500 MHz) of compound **10a** in $CDCl_3$. The reference signal of chloroform is set to δ = 7.26 ppm.

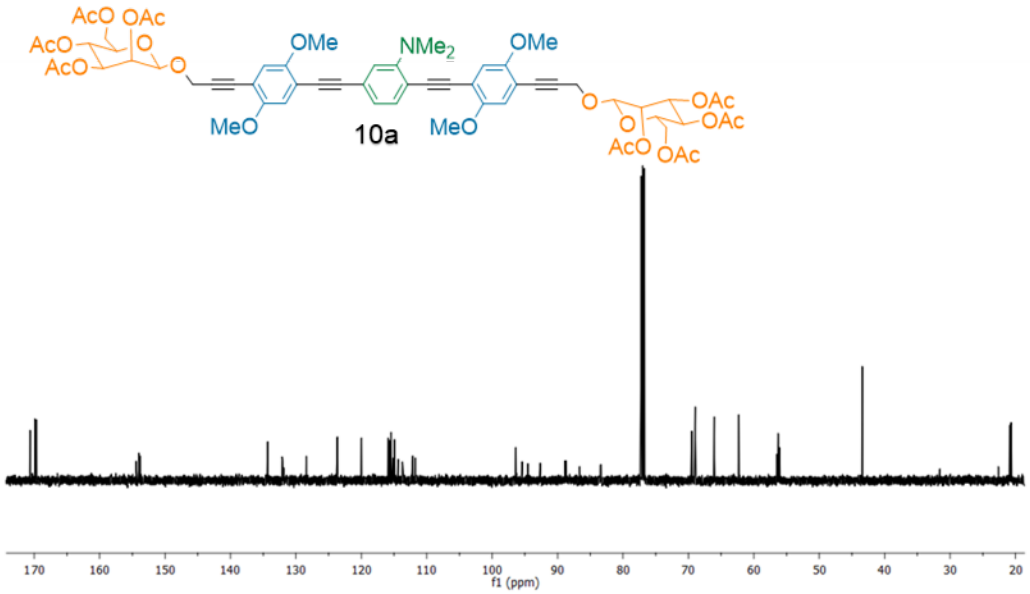

Fig. S6. ^{13}C NMR spectrum (500 MHz) of compound **7a** in $CDCl_3$. The reference signal of chloroform is set to δ = 77.0 ppm.

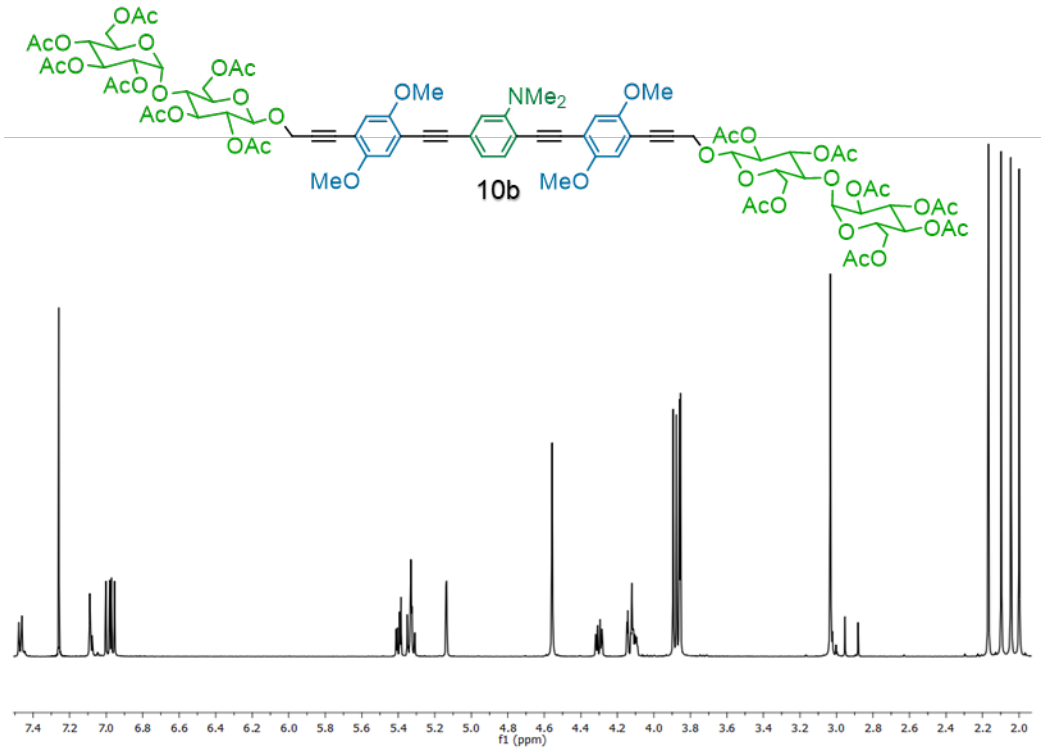

Fig. S7. ^{1}H NMR spectrum (500 MHz) of compound **10b** in $CDCl_3$. The reference signal of chloroform is set to δ = 7.26 ppm.

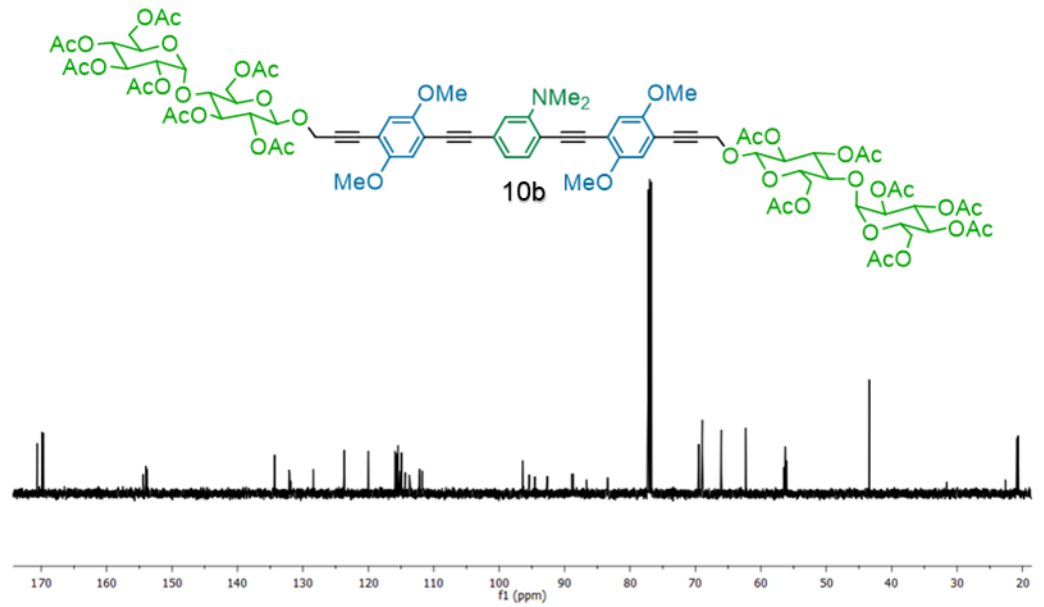

Fig. S8. ^{13}C NMR spectrum (500 MHz) of compound **7a** in $CDCl_3$. The reference signal of chloroform is set to δ = 77.0 ppm.

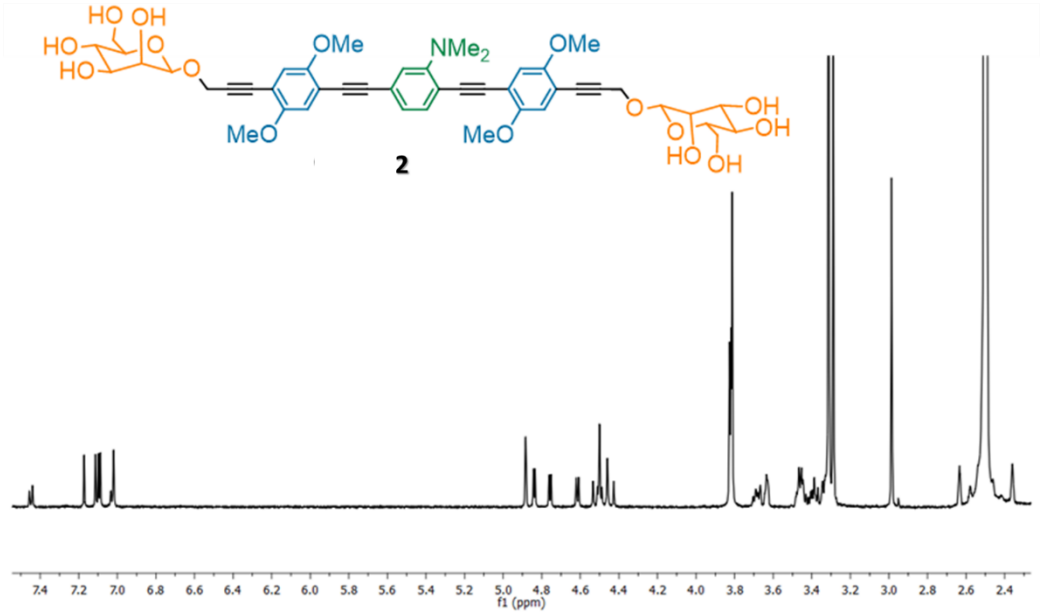

Fig. S9. [1]H NMR spectrum (500 MHz) of compound **2 OPE-Mannose** in dmso-d_6. The reference signal of dimethylsulfoxide is set to δ = 2.49 ppm.

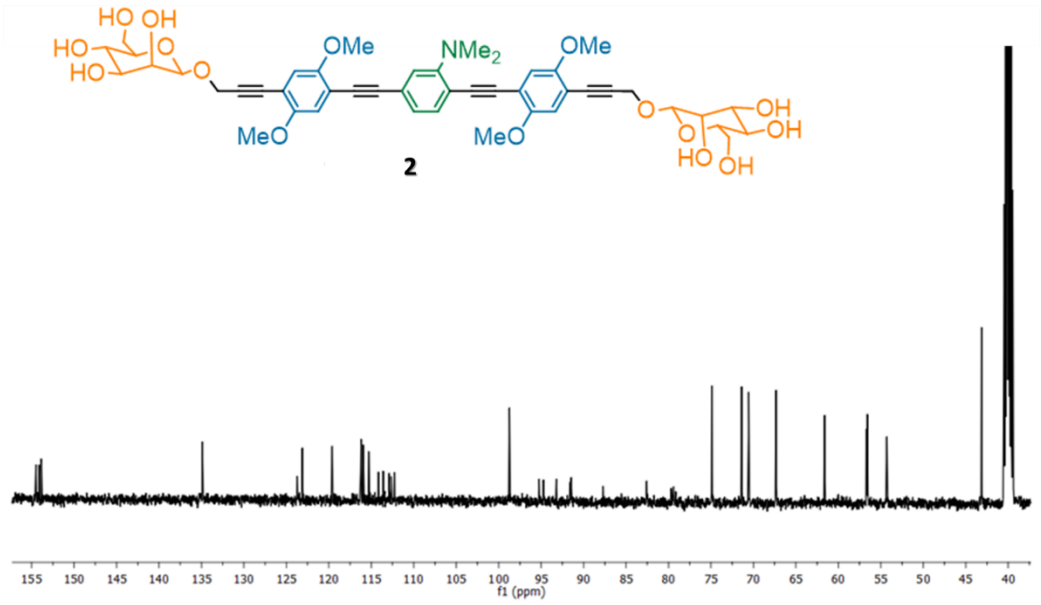

Fig. S10. [13] C NMR spectrum (500 MHz) of compound **2 OPE-Mannose** in dmso-d_6. The reference signal of dimethylsulfoxide is set to δ = 39.5 ppm.

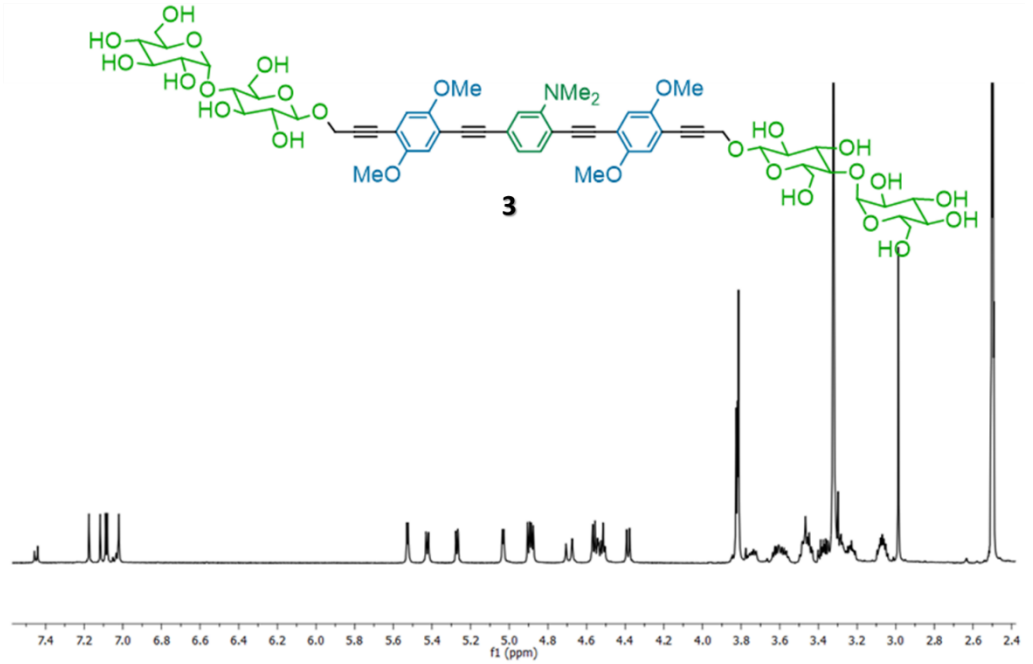

Fig. S11. [1]H NMR spectrum (500 MHz) of compound **3 OPE-Maltose** in dmso-d_6. The reference signal of dimethylsulfoxide is set to δ = 2.49 ppm.

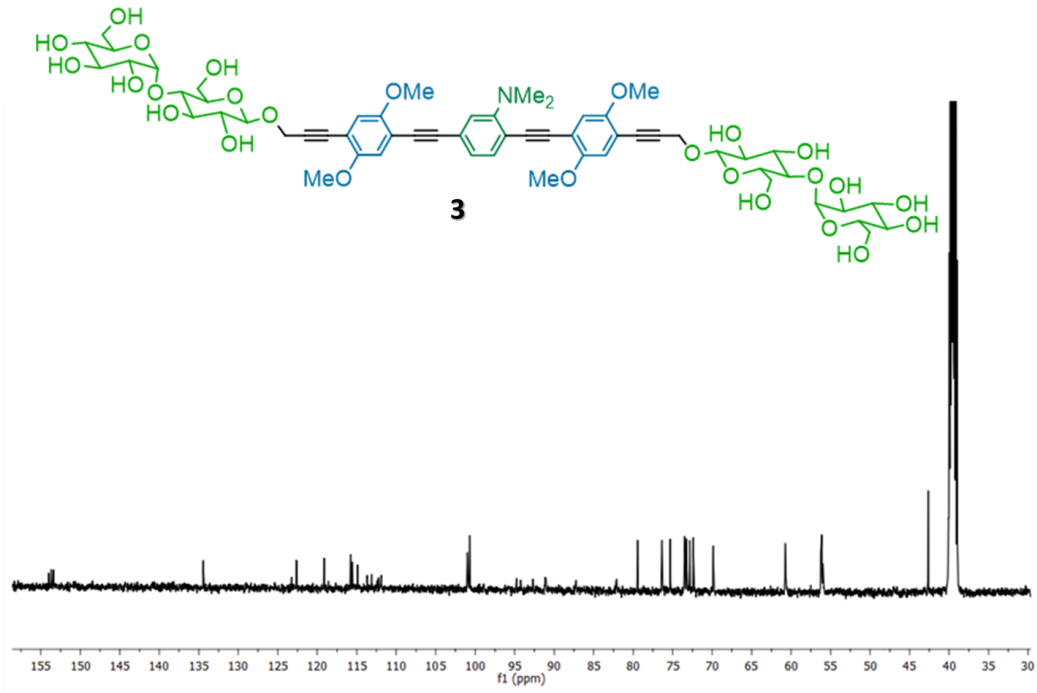

Fig. S10. [13] C NMR spectrum (500 MHz) of compound **3 OPE-Maltose** in *dmso*-d6. The reference signal of dimethylsulfoxide is set to δ = 39.5 ppm.

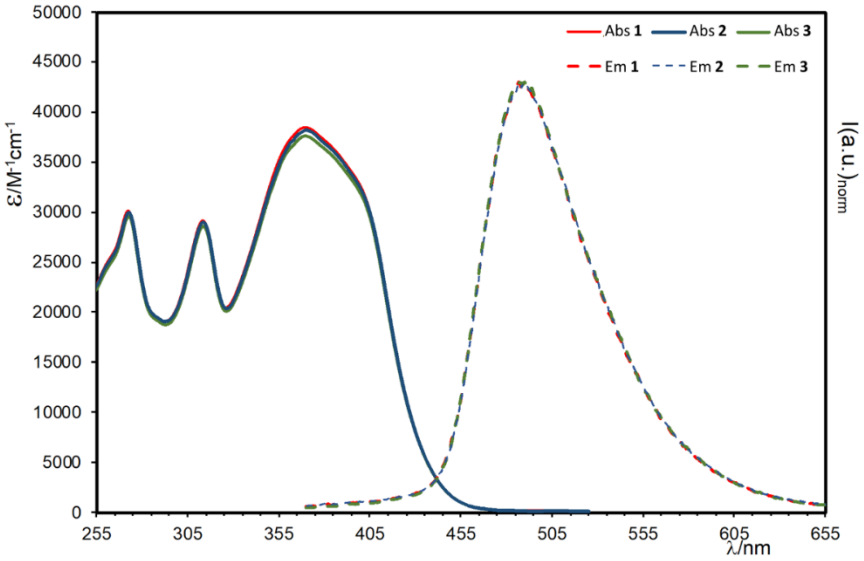

Fig. S11. Absorption (epsilon) and emission spectra of **1 OPE-Glucose**, **2 OPE-Mannose** and **3 OPE-Maltose** in dichloromethane.

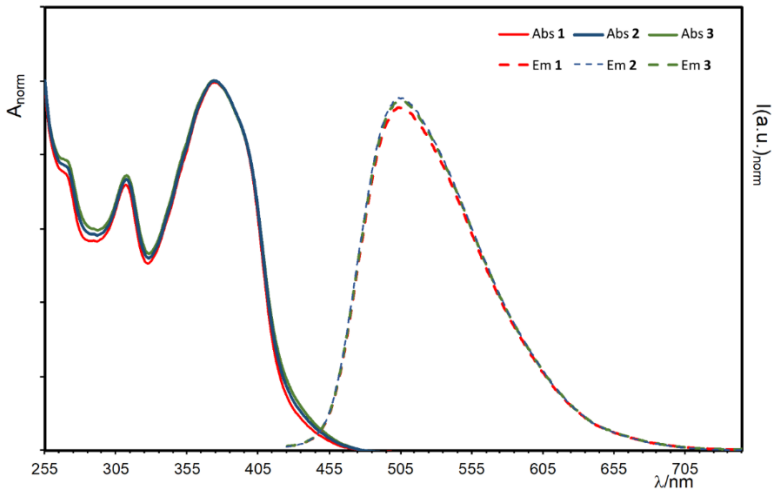

Fig. S12. Absorption and emission spectra of **1 OPE-Glucose**, **2 OPE-Mannose** and **3 OPE-Maltose** in aqueous solution

ROS/singlet oxygen quantification

A direct comparison between the oligomers and Methylene blue species in solution by monitoring the UA photooxidation is not possible, because of the different excitation wavelengths used for the photoproduction of ROS. Therefore, the efficiency of ROS deliver was calculated by normalizing for the photon flux of the lamp at the lambda used for excitation (380 nm for OPE Glycosides and 600 nm for the methylene blue).[1] At

neutral pH, even under irradiation, uric acid is stable and its absorption spectrum is dominated by an absorption band at 292 nm. If singlet oxygen (or ROS) is produced, uric acid is irreversibly oxidized and the corresponding absorption band decreases in intensity. UA absorption band decay curves as a function of irradiation time are shown in ESI. Using a standard (methylene blue)[2], the quantum yields of ROS production could be calculated. The obtained data together with the photophysical values are summarized in Table S1.

Table S1. Spectroscopic and photophysical data of OPE Glycosides in buffered aqueous solution.[a]

OPE Glycosides	Absorption[b]	Luminescence			Singlet oxygen/ROS
	λ_{max}/nm (ε/M^{-1} cm^{-1})	λ_{max}/nm	Φ	τ/ns	$\Phi^{1}_{O2/ROS}$
OPE-Glucose 1	389 (38500)	473	0.57	2.50	0.15
OPE-Mannose 2	389(38800)	473	0.58	2.49	0.15
OPE-Maltose 3	390 (38600)	473	0.56	2.51	0.16

a) Buffer phosphate aqueous solution ([OPE]= $5x10^{-5}$ M, pH=7.2).
b) For the absorption spectra, the maxima of lower energy bands are given.

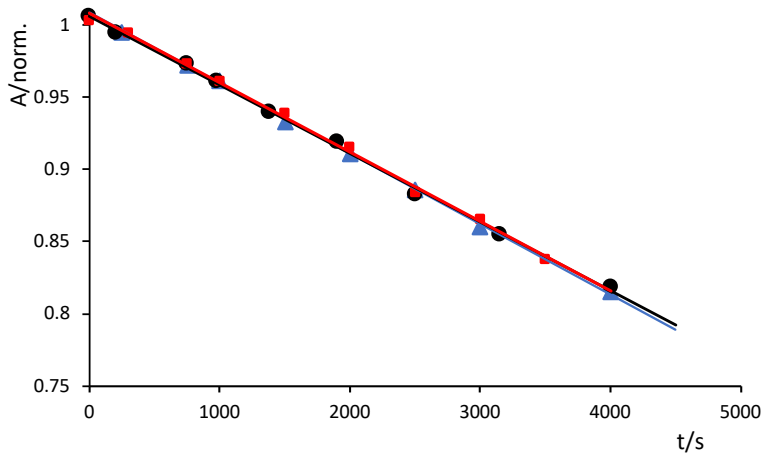

Fig. S13. Normalized absorbance variation (@ 292 nm) versus Time during degradation of uric acid using as sensitizer OPE-Glucose 1 (black circles, $5x10^{-5}$ M), OPE-Mannose 2 (red squares, $5x10^{-5}$ M) and OPE-Maltose 3 (blue triangles, $5x10^{-5}$ M), irradiated at 380 nm (photon flux: $2.98x10^{16}$photons/(s·cm^2).

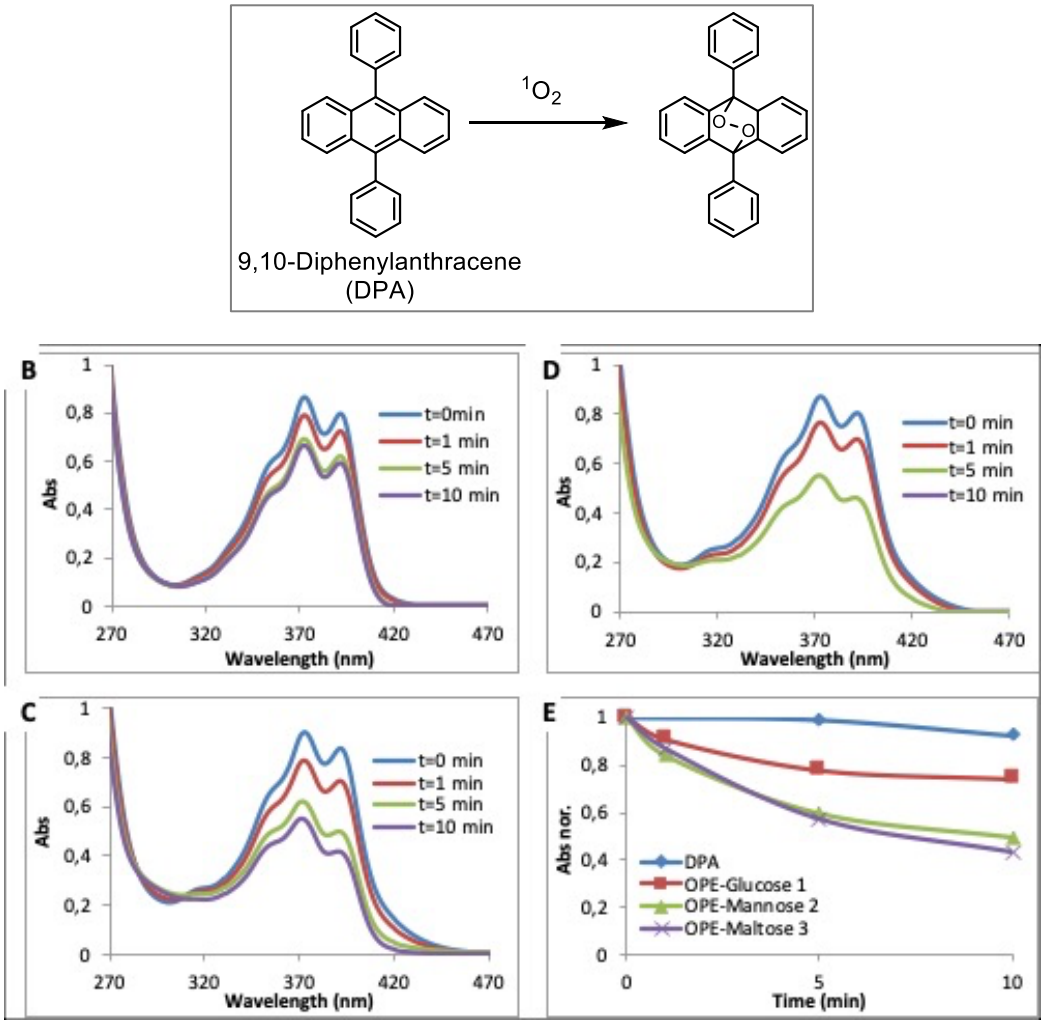

Fig. S14. Evaluation on singlet oxygen generating ability [3]. (A) Chemical reaction of DPA with singlet oxygen. UV-Vis absorbance spectra of DPA ($3.4 \cdot 10^{-4}$M in MeCN) upon irradiation of blue light (465nm) over different periods of time in presence of **(B)** OPE-Glucose 1 (10^{-6}M in MeCN). **(C)** OPE-Mannose 2 (10^{-6}M in MeCN) and **(D)** OPE-Maltose 3 (10^{-6}M in MeCN). **(E)** Photostability control of DPA (blue line) and absorbance changes of DPA in the presence of the corresponding OPE (red, green and purple lines), under 465 nm blue light irradiation over different periods of time. The OPE solutions (10^{-6}M in MeCN) were prepared from a stock DMSO solution (10^{-5}M).

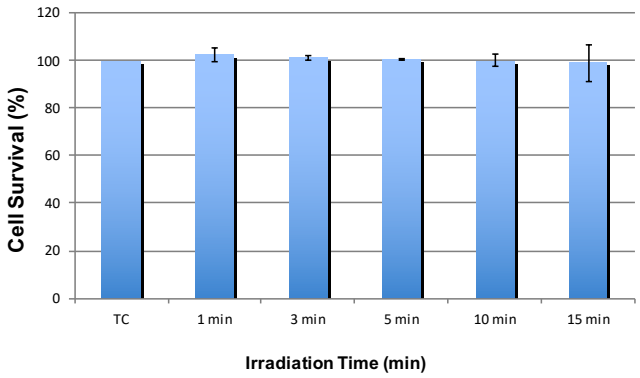

Fig. S15. Survival rates of HeLa cells after blue light irradiation. HeLa cells were plated in a 24-well plate. 48 h later, the cells were irradiated with blue light 1, 3, 5, 10 and 15 min. 24 h later, cell viability was measured by the MTT method. No substantial cytotoxic effect was observed for cells irradiated with blue light at all times used. Each point corresponds to the mean value ± SD of three different experiments.

Table S2 describes the IC50 obtained for HeLa cells after PDT mediated by OPE-conjugates 1-3 at each of the irradiation times used.

EC50			
Time (min)	**OPE-Glucose**	**OPE-Mannose**	**OPE-Maltose**
1	9.388×10^{-7} M	1.90×10^{-7} M	4.03×10^{-6} M
3	1.806×10^{-7} M	6.96×10^{-8} M	2.37×10^{-6} M
5	1.241×10^{-7} M	6.17×10^{-8} M	1.11×10^{-6} M
10	1.041×10^{-7} M	4.74×10^{-8} M	1.04×10^{-6} M

EC50 values were interpolated from a non-linear regression model (dose-response with variable slope) using GraphPad Prism 9 (GraphPad Software, San Diego, USA.

Table S3. The table shows the cell viability results obtained from interpolating the concentrations used in the phototoxicity tests, using the nonlinear regression model:

[OPE-Glu] M	**1 min**	**3 min**	**5 min**	**10 min**
3×10^{-8}	99.33%	95.96%	95.99%	90.88%
3×10^{-7}	83.94%	28.99%	12.19%	12.4%0
3×10^{-6}	15.65%	0.70%	0.08%	0.20%

[OPE-Man] M	1 min	3 min	5 min	10 min
3 x 10^{-8}	85.31%	81.78%	76.54%	65.05%
3 x 10^{-7}	39.29%	6.89%	6.95%	7.55%
3 x 10^{-6}	6.73%	0.12%	0.17%	0.36%

[OPE-Mal] M	1 min	3 min	5 min	10 min
3 x 10^{-8}	99.93%	99.95%	99.94%	99.92%
3 x 10^{-7}	97.93%	97.21%	93.78%	92.24%
3 x 10^{-6}	60.76%	39.96%	11.26%	10.66%

The concentrations that fulfill the requirements of 60 - 80 % viability at short times and high toxicity at longer times are the ones selected: OPE Glucose **3 x 10^{-7} M**, OPE Mannose **3 x 10^{-8} M** and OPE Maltose **3 x 10^{-6} M**.

Illumination source

Cells were irradiated using blue LEDs from Segainvex UAM (λmax 450 nm, 15,9 mW/cm^2), equipped with an internal fan to maintain the temperature at approximately 24 °C.

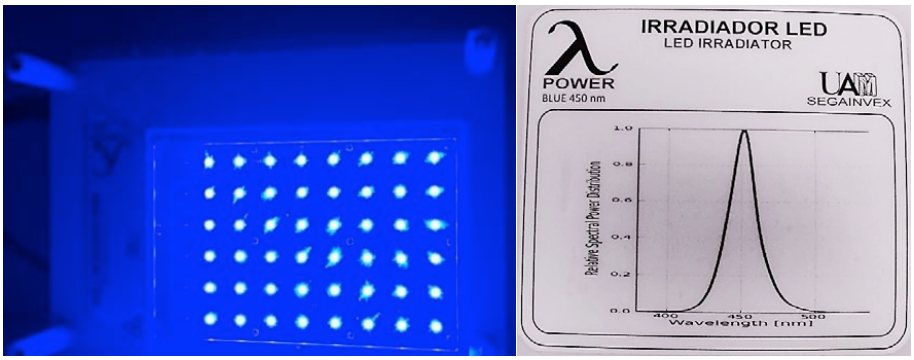

Fig. S16.- Relative Spectral Power Distribution of blue LEDs irradiator.

[1] A. Passarella Gerola, J. Semensato, D. Silva Pellosi, V. Roberto Batistela, B. Ribeiro Rabello, N. Hioka, W. Caetano, J. Photochem. Photobiol. A 2012, 232, 14-21.

[2] B. Ribeiro Rabello, A. Passarella Gerola, D. Silva Pellosi, A.L. Tessaro, J.L. Aparício, W. Caetano, N. Hioka, J. Photochem. Photobiol. A 2012, 238, 53-62.

[3] H. H. Wasserman, J. R. Scheffer, J. L. Cooper, J. Am. Chem. Soc. 1972, 94, 4991–4996.