Electronic Supplementary Information

Unsymmetrical β-Cyclodextrin-Conjugated Silicon(IV) Phthalocyanines as Highly Potent Photosensitisers for Photodynamic Therapy

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Contents

Experimental Section

Experimental details regarding the purification of solvents, spectroscopic and photophysical measurements and biological studies are described elsewhere unless otherwise stated.[1] Compounds **1**,[2] **5**,[3] **6**,[4] **7**[5] and **8**[1a] were prepared as described.

Phthalocyanine 2. A mixture of 1,2:3,4-di-*O*-isopropylidene-α-D-galacto-pyranose (**7**) (0.12 g, 0.46 mmol) and NaH (0.10 g, 4.17 mmol) in toluene (20 mL) was stirred at room temperature for 30 min. Phthalocyanine **5** (0.43 g, 0.70 mmol) was added and the mixture was refluxed for a further 1 h. Upon cooling, a mixture of mono-6-hydroxy permethylated β-cyclodextrin **6** (0.64 g, 0.45 mmol) and NaH (0.10 g, 4.17 mmol) in toluene (10 mL) was added into the mixture. It was heated at reflux for 2 days. After evaporating the solvent *in vacuo*, the residue was subject to silica gel column chromatography using $CHCl_3$ and then $CHCl_3$/MeOH (50:1) as the eluents. The product was obtained as a blue solid (0.14 g, 14%). R_f [$CHCl_3$/MeOH (20:1)] = 0.39. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.58-9.63 (m, 8 H, Pc-H_α), 8.31-8.35 (m, 8 H, Pc-H_β), 4.97-5.02 (m, 4 H, CD), 4.79 (d, J = 3.3 Hz, 1 H, CD), 4.37 (d, J = 4.8 Hz, 1 H, GAL-H_1), 2.18-3.78 (m, 98 H, CD, GAL-H_2 and GAL-H_3), 2.07 (t, J = 9.3 Hz, 1 H, CD-H_3), 1.94 (dd, J = 2.4, 9.6 Hz, 1 H, CD), 1.46-1.53 (m, 1 H, CD), 1.30-1.34 (m, 1 H, CD-H_2), 0.99 (d, J = 9.3 Hz, 1 H, CD-H_5), 0.79 (s, 4 H, GAL-Me

and GAL-H_4), 0.69 (s, 4 H, GAL-Me and GAL-H_5), 0.50 (s, 3 H, GAL-Me), 0.31 (s, 3 H, GAL-Me), 0.03-0.09 (m, 1 H, CD-H_4), -0.49 (d, J = 11.7 Hz, 1 H, CD-H_6), -1.70 (dd, J = 5.4, 8.4 Hz, 1 H, GAL-H_6), -2.36 (t, J = 8.4 Hz, 1 H, GAL-$H_{6'}$), -2.48 (d, J = 11.7 Hz, 1 H, CD-$H_{6'}$). The partial assignments were made according to the ^{1}H-^{1}H COSY spectrum and the data for the symmetrical analogues.[6] ^{13}C{^{1}H} NMR (75.4 MHz, $CDCl_3$): δ 149.4 (Pc), 136.1 (Pc), 130.8 (Pc), 123.6 (Pc), 107.3, 107.2, 99.0, 98.9, 98.7, 98.6, 97.3, 94.9, 94.0, 82.3, 82.1, 81.9, 81.8, 81.7, 81.6, 81.5, 81.1, 80.9, 80.8 79.9, 79.8, 79.7, 79.6, 79.3, 79.0, 71.3, 71.0, 70.9, 70.6, 70.3, 70.2, 69.8, 69.7, 69.3, 68.8, 67.3, 64.0, 61.8, 61.7, 61.3, 61.1, 61.0, 60.9, 60.6, 59.0, 58.9, 58.8, 58.6, 58.5, 58.4, 58.1, 56.4, 54.2, 53.0, 25.3, 24.4, 24.3, 22.8 (some of the signals are overlapped). MS (ESI): an isotopic cluster peaking at *m/z* 2237 {100%, $[M+Na]^+$}. HRMS (ESI): *m/z* calcd for $C_{106}H_{144}N_8NaO_{41}Si$ $[M+Na]^+$: 2235.9090; found: 2235.9073. Anal. Calcd for $C_{106}H_{144}N_8O_{41}Si$: C, 57.49; H, 6.55; N, 5.06. Found: C, 57.19; H, 6.66; N, 4.83.

Phthalocyanine 3. A mixture of protected glucose **8** (0.17 g, 0.39 mmol) and NaH (0.10 g, 4.17 mmol) in toluene (20 mL) was stirred at room temperature for 30 min. Phthalocyanine **5** (0.35 g, 0.57 mmol) was then added and the mixture was brought to reflux for 1 h. Upon cooling, a mixture of **6** (0.56 g, 0.40 mmol) and NaH (0.10 g, 4.17 mmol) in toluene (10 mL) was poured into the mixture. It was

heated at reflux for 2 days. After evaporating the solvent *in vacuo*, the residue was subject to column chromatography on neutral alumina using $CHCl_3$/MeOH (1:1) as the eluent, followed by gel permeation chromatography using THF as the eluent. The crude product was chromatographed again on neutral alumina using $CHCl_3$ and then $CHCl_3$/MeOH (1:1) as the eluents. The product was collected as a shiny blue solid (0.10 g, 10%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.59-9.62 (m, 8 H, Pc-H_α), 8.31-8.34 (m, 8 H, Pc-H_β), 5.75 (d, J = 3.6 Hz, 1 H, GLU-H_1), 4.96-5.01 (m, 4 H, CD), 4.77 (d, J = 3.3 Hz, 1 H, CD), 4.41 (d, J = 3.6 Hz, 1 H, GLU-H_2), 4.13-4.21 (m, 1 H, GLU-H_5), 4.03 (dd, J = 3.3, 7.8 Hz, 1 H, GLU-H_4), 3.88-3.98 (m, 2 H, GLU-H_6), 2.16-3.78 (m, 107 H, CD, CH_2 and GLU-H_3), 2.06 (t, J = 9.6 Hz, 1 H, CD-H_3), 1.91 (dd, J = 2.7, 9.6 Hz, 1 H, CD), 1.66 (t, J = 4.8 Hz, 2 H, CH_2), 1.46-1.48 (m, 1 H, CD), 1.42 (s, 3 H, GLU-Me), 1.35 (s, 3 H, GLU-Me), 1.28-1.32 (m, 1 H, CD-H_2) 1.23 (s, 3 H, GLU-Me), 1.20 (s, 3 H, GLU-Me), 0.98 (d, J = 9.3 Hz, 1 H, CD-H_5), 0.38 (t, J = 5.7 Hz, 2 H, CH_2), 0.04 (d, J = 9.3 Hz, 1 H, CD-H_4), -0.49 (d, J = 11.4 Hz, 1 H, CD-H_6), -1.92 (t, J = 5.7 Hz, 2 H, CH_2), -2.51 (d, J = 11.4 Hz, 1 H, CD-$H_{6'}$). The partial assignments were made according to the ^{1}H-^{1}H COSY spectrum and the data for the symmetrical analogues.[1a] ^{13}C{^{1}H} NMR (75.4 MHz, $CDCl_3$): δ 149.3 (Pc), 136.1 (Pc), 130.9 (Pc), 123.7 (Pc), 111.7, 108.9, 105.2, 99.1, 98.9, 98.7, 98.6, 97.3, 94.0, 82.7, 82.6, 82.3, 81.9, 81.8, 81.6, 81.1, 80.9, 79.8, 79.7, 79.4, 78.9, 72.6, 71.0,

70.9, 70.6, 70.3, 70.0, 69.9, 69.7, 69.5, 69.2, 68.7, 67.2, 61.7, 61.3, 61.0, 60.9, 60.6, 59.0, 58.9, 58.8, 58.7, 58.6, 58.4, 58.2, 56.4, 54.8, 54.1, 29.5, 26.9, 26.8, 26.3, 25.4 (some of the signals are overlapped). MS (ESI): an isotopic cluster peaking at *m/z* 2413 {92%, $[M+Na]^+$}. HRMS (ESI): *m/z* calcd for $C_{114}H_{160}N_8NaO_{45}Si$ $[M+Na]^+$: 2412.0139; found: 2412.0150. Anal. Calcd for $C_{114}H_{160}N_8O_{45}Si$: C, 57.28; H, 6.75; N, 4.68. Found: C, 56.96; H, 7.01; N, 4.47.

Phthalocyanine 4. A mixture of 1,3-bis(dimethylamino)-2-propanol (**9**) (0.10 g, 0.68 mmol) and NaH (0.12 g, 5.00 mmol) in toluene (20 mL) was stirred at room temperature for 30 min. Phthalocyanine **5** (0.61 g, 1.00 mmol) was added and the mixture was refluxed for a further 1 h. Upon cooling, a mixture of **6** (0.98 g, 0.69 mmol) and NaH (0.11 g, 4.58 mmol) in toluene (20 mL) was poured into the mixture. It was heated at reflux for 2 days. After evaporating the solvent *in vacuo*, the residue was subject to column chromatography on neutral alumina using $CHCl_3$ as the eluent, followed by gel permeation chromatography using THF as the eluent. It was then further purified by flash column chromatography on neutral alumina using $CHCl_3$/MeOH (20:1) and then $CHCl_3$/MeOH (10:1) as the eluents. The product was obtained as a shiny dark green solid (0.15 g, 10%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.59-9.61 (m, 8 H, Pc-H_α), 8.29-8.32 (m, 8 H, Pc-H_β), 4.98-5.02 (m, 4 H, CD), 4.80 (d, *J* = 3.3 Hz, 1 H, CD), 2.20-3.76 (m, 96 H, CD), 2.07 (t, *J* = 9.3 Hz, 1 H, CD-H_3), 1.93

(dd, J = 2.7, 9.6 Hz, 1 H, CD), 1.47 (d, J = 2.7 Hz, 1 H, CD), 1.33 (dd, J = 3.3, 9.9 Hz, 1 H, CD-H_2), 1.00 (d, J = 9.3 Hz, 1 H, CD-H_5), 0.49 (s, 12 H, NMe), 0.06 (t, J = 9.3 Hz, 1 H, CD-H_4), -0.50 (d, J = 11.4 Hz, 1 H, CD-H_6), -0.79 (dd, J = 5.1, 12.6 Hz, 2 H, CH_2), -1.50 (br s, 2 H, CH_2), -2.48 (d, J = 11.4 Hz, 1 H, CD-$H_{6'}$), -2.71 (virtual t, J = 5.1 Hz, 1 H, CH). The partial assignments were made according to the ^{1}H-^{1}H COSY spectrum and the data for the symmetrical analogues.[7] ^{13}C{^{1}H} NMR (300 MHz, $CDCl_3$): δ 149.5 (Pc), 135.6 (Pc), 131.4 (Pc), 123.9 (Pc), 99.1, 98.9, 98.7, 97.3, 94.1, 82.2, 81.8, 81.6, 80.9, 79.9, 79.6, 78.6, 71.1, 70.1, 69.7, 69.1, 61.7, 61.3, 61.0, 60.7, 60.2, 58.9, 58.7, 58.6, 58.3, 58.2, 56.4, 54.3, 44.3 (some of the signals are overlapped). MS (ESI): an isotopic cluster peaking at *m/z* 2101 {34%, $[M+H]^+$}. HRMS (ESI): *m/z* calcd for $C_{101}H_{143}N_{10}O_{36}Si$ $[M+H]^+$: 2099.9430; found: 2099.9420. Anal. Calcd for $C_{101}H_{142}N_{10}O_{36}Si$: C, 57.76; H, 6.81; N, 6.67. Found: C, 57.42; H, 6.76; N, 6.38.

ROS Measurements. ROS production was determined by using DCFDA (Molecular Probes) as a quencher. Approximately 3 x 10^5 HT29 or HepG2 cells were placed in a 96-well plate for 24 h before photodynamic treatment. The cells well then incubated with different concentrations of the phthalocyanines (two-fold dilution from 0.5 μM) for 2 h before illumination with red light. The light source consisted of a 300 W halogen lamp, a water tank for cooling and a colour glass filter (Newport) cut-on 610 nm. The fluence rate (λ > 610 nm) was 40 mW cm^{-2}. An

illumination of 20 min led to a total fluence of 48 J cm^{-2}. Immediately after illumination, the cells were washed with phosphate buffered saline (PBS) and then incubated with 100 μL of a 100 μM DCFDA solution in PBS at 37 °C for 60 min. Fluorescence measurements were made in a fluorescence plate reader (TECAN Polarion) with a 485 nm excitation filter and a 535 nm emission filter set at a gain of 60.

Subcellular Localisation Studies. About 6×10^4 HT29 cells in the culture medium (2 mL) were seeded on a coverslip and incubated overnight at 37 ^oC under 5% CO_2. The medium was then removed. The cells were incubated with a solution of phthalocyanines **1**-**4** (formulated with Cremophor EL) in the medium (0.5 μM, 2 mL) for 100 min under the same conditions. Lysotracker Green DND-26 [Molecular Probes, 1 mM in dimethylsulfoxide (DMSO), 4 μL] or Mitotracker Green FM (Molecular Probes, 2 mM in DMSO, 1 μL) was then added for incubation for a further 30 min. For both cases, the cells were then rinsed with PBS twice and viewed with a Leica SP5 confocal microscope as described previously.[1b]

Flow Cytometric Studies. Approximately 6×10^5 HT29 cells in the medium (2 mL) were seeded on a 35 mm dish and incubated for 24 h at 37 °C under 5% CO_2. The cells were then treated with **2** at various concentrations and incubated under the same conditions for 2 h. The cells were then rinsed thrice with PBS and refilled with

2 mL of the culture medium before being illuminated at ambient temperature using the halogen-lamp light source as described above. After 24 h of incubation, the cells were rinsed with PBS and then harvested by 0.25% trypsin-EDTA (Invitrogen, 500 μL) for 5 min, followed by centrifugation at 2400 rpm for 3 min. The pellet was then washed again by PBS and then subject to centrifugation. The cells were suspended in 1 mL of binding buffer (10 mM HEPES, 140 mM NaCl, 25 mM $CaCl_2$, pH 7.4) containing annexin V-GFP (5 μL) and PI (2 μg mL^{-1}). After incubation in darkness for 15 min at room temperature, the signals of annexin V-GFP and PI were measured by a BD FACSCanto flow cytometre (Becton Dickinson) with 10^4 cells counted in each sample. Both annexin V-GFP and PI were excited by a 488 nm argon laser. The emitted fluorescence was monitored at 500-560 nm for annexin V-GFP and at > 670 nm for PI. The data collected were analysed by using WinMDI 2.9.

***In vivo* Photodynamic Treatment.** Female Balb/c nude mice (20-25 g) were obtained from the Laboratory Animal Services Centre at The Chinese University of Hong Kong. All animal experiments had been approved by the Animal Experimentation Ethics Committee of the University. The mice were kept under a pathogen-free condition with free access of food and water. HT29 cells (1×10^7 cells in 200 μL PBS) were inoculated subcutaneously on the back of the mice. The length, width and thickness of tumour were measured by a micrometre digital caliper

(SCITOP Systems). The tumour volume (mm^3) was calculated by the formula: tumour volume = $\pi \times$ (length $\times$ width $\times$ thickness)/6. Once the tumours were grown up to the size of 80-100 mm^3, the mice were used for *in vivo* PDT. Firstly, phthalocyanine **2** was dissolved in DMF/Cremophor EL (Sigma-Aldrich, 1:1 v/v) to give a 10.2 mM solution, which was then diluted to 0.2 mM with deionised water. Then, 1 μmol of drug per kg body weight (100 μL) was intravenously injected into the tail vein of the tumour-bearing mice. After 12 h post-injection, the tumour was illuminated by a diode laser (Biolitec Ceralas) at 675 nm operated at 0.1 W. Illumination on a spot size of 1.0 cm^2 for 5 min led to a total fluence of 30 J cm^{-2}. The tumour sizes of the nude mice were monitored periodically for the next 15 days. The tumour volumes were compared with a control group of mice without the drug and light treatment.

References

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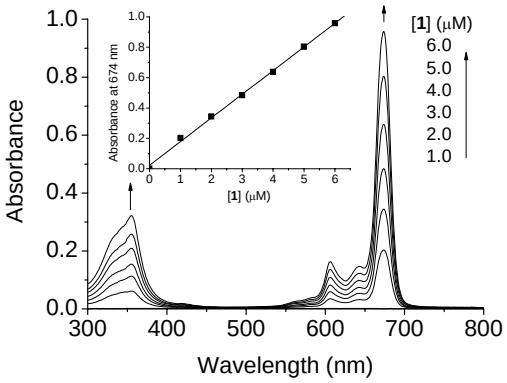
Table S1 Electronic absorption and fluorescence emission data for **1-4** in water with < 0.6% (v/v) THF.

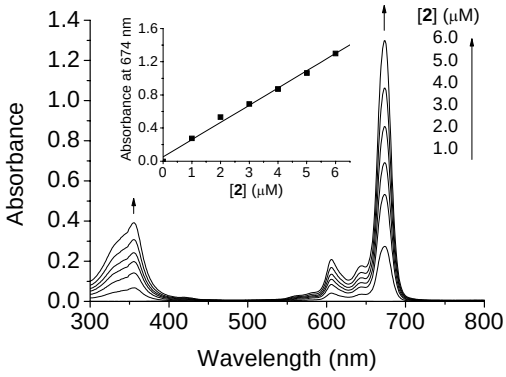
Compound	λ_{max} (nm) (log ε)	λ_{em}(nm)[a]	Φ_F[b]
1	354 (4.68), 611 (4.36), 679 (5.11)	683	0.28
2	359 (4.45), 617 (4.07), 682 (4.65)	686	0.09
3	355 (4.84), 613 (4.45), 681 (5.15)	683	0.22
4	353 (4.78), 615 (4.47), 683 (5.26)	689	0.24

[a] Excited at 610 nm. [b] Using ZnPc in DMF as the reference (Φ_F = 0.28).

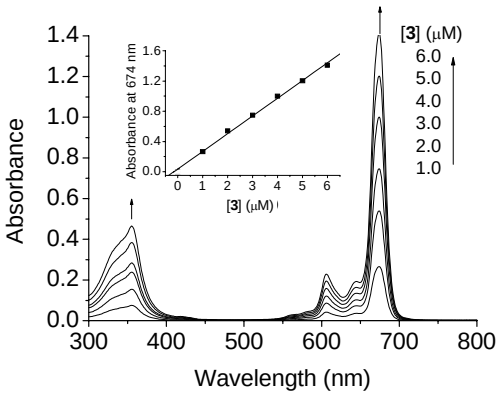
Compound **1**



Compound **2**



Compound **3**



Compound **4**

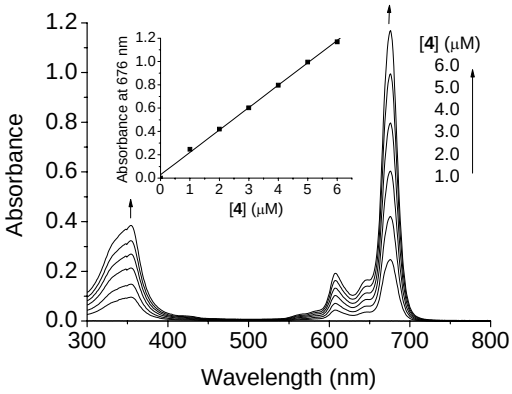
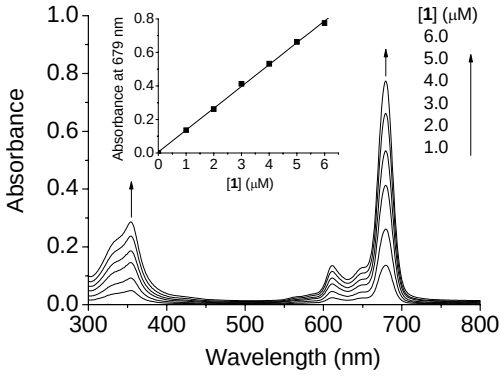
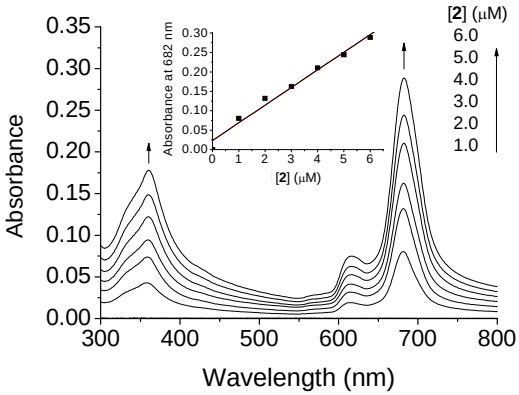


Fig. S1 Electronic absorption spectra of **1**-**4** in DMF in different concentrations. The inset of each spectrum plots the Q-band absorbance versus the concentration of the phthalocyanine, and the line represents the best-fitted straight line.

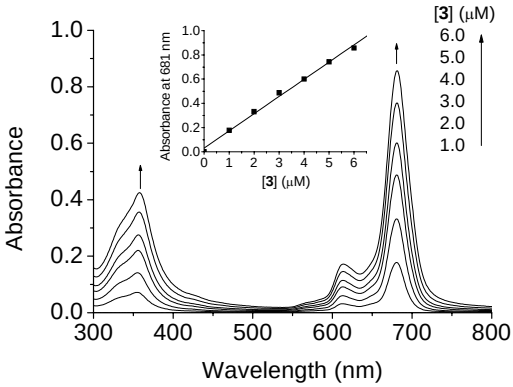
Compound **1**



Compound **2**



Compound **3**



Compound **4**

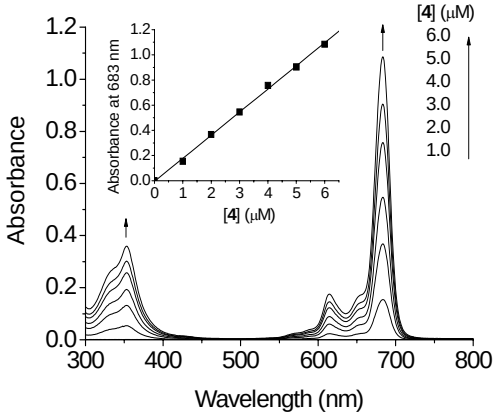


Fig. S2 Electronic absorption spectra of **1**-**4** in water with < 0.6% (v/v) THF in different concentrations. The inset of each spectrum plots the Q-band absorbance versus the concentration of the phthalocyanine, and the line represents the best-fitted straight line.

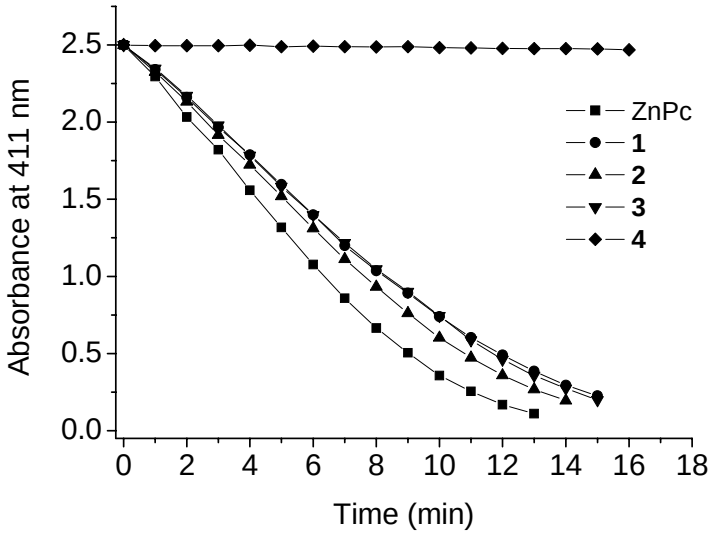


Fig. S3 Comparison of the rate of decay of DPBF sensitised by **1**-**4** and ZnPc in DMF as shown by the decrease in the absorbance at 411 nm. The data points are connected automatically by Origin 8.0 as a way of data plotting.

(a) For HT29 (b) For HepG2

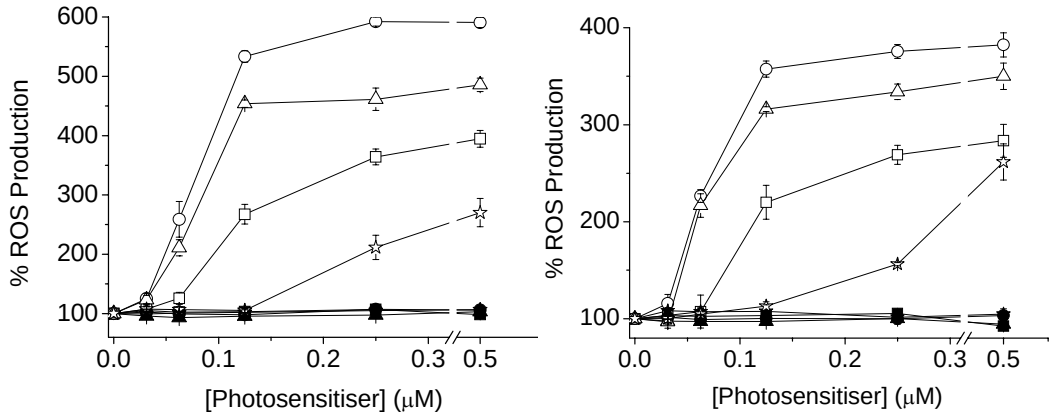


Fig. S4 ROS production in (a) HT29 and (b) HepG2 cells using **1** (stars), **2** (circles), **3** (triangles) and **4** (squares) as the photosensitisers in the absence (closed symbols) and presence (open symbols) of light (λ > 610 nm, 40 mW cm^{-2}, 48 J cm^{-2}). Data are expressed as mean value ± SEM of three independent experiments, each performed in quadruplicate. The data points are connected automatically by Origin 8.0 as a way of data plotting.

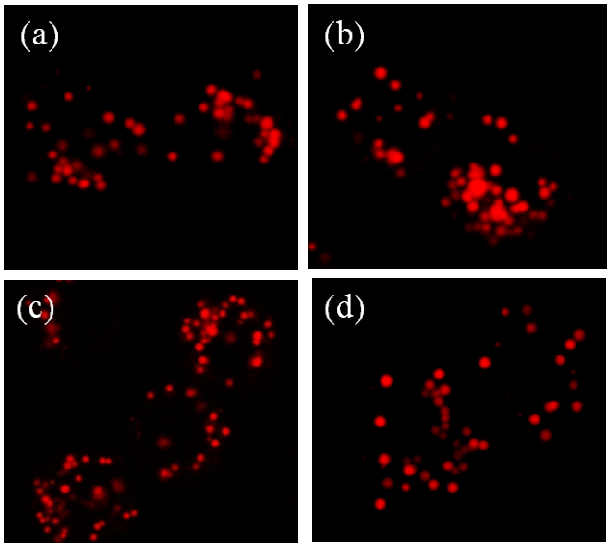


Fig. S5 Visualisation of intracellular fluorescence of HT29 cells after incubation with (a) **1**, (b) **2**, (c) **3** and (d) **4** for 2 h.

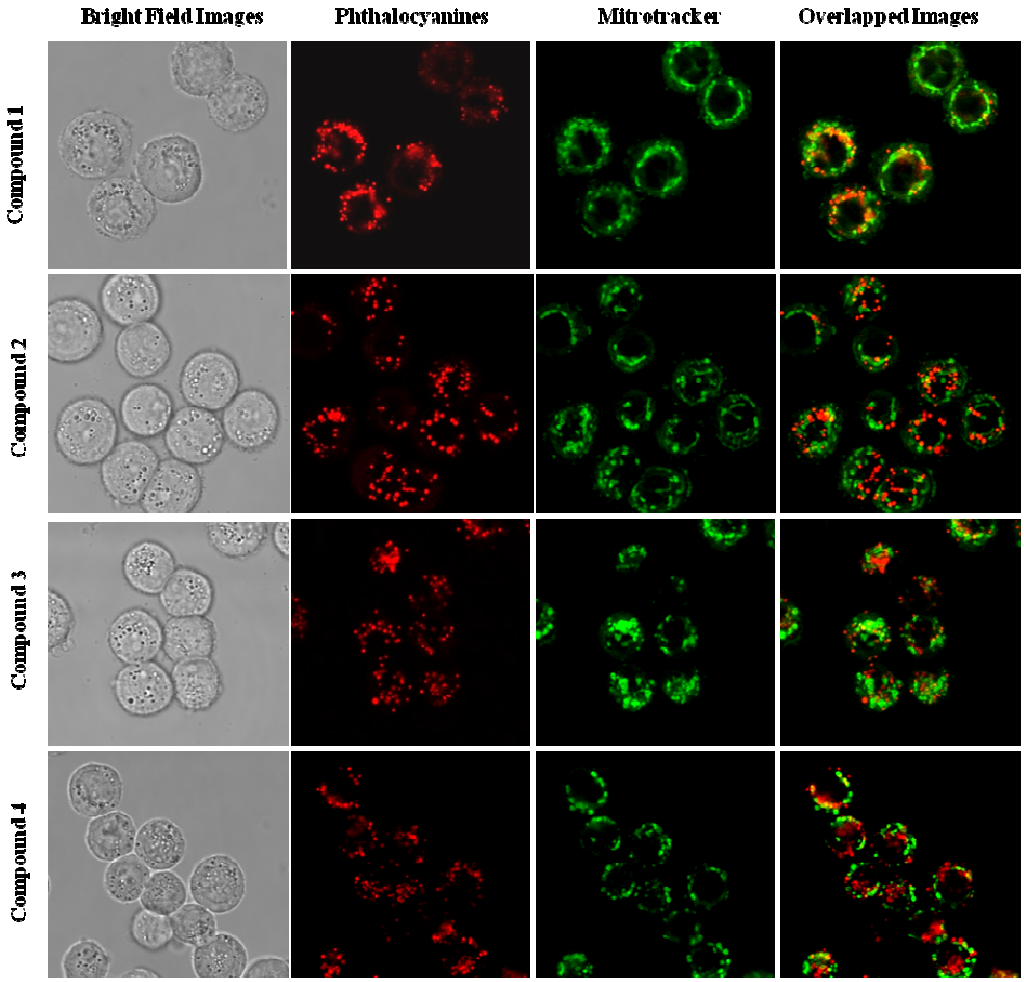


Fig. S6 Visualisation of the intracellular fluorescence of HT29 by using filter sets specific for **1-4** (in red, column 2) and Mitotracker Green (in green, column 3). The corresponding bright field and superimposed images are given in columns 1 and 4 respectively.

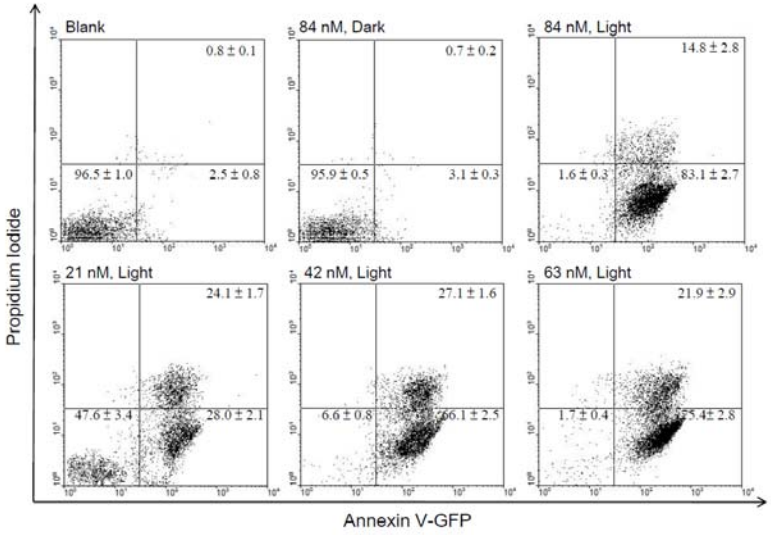


Fig. S7 Flow cytometric analysis of the cell death mechanism induced by **2** upon PDT treatment ($\lambda > 610$ nm, 40 mW cm^{-2}, 48 J cm^{-2}) on HT29 cells. Data are expressed as mean value ± standard deviation of three independent experiments.

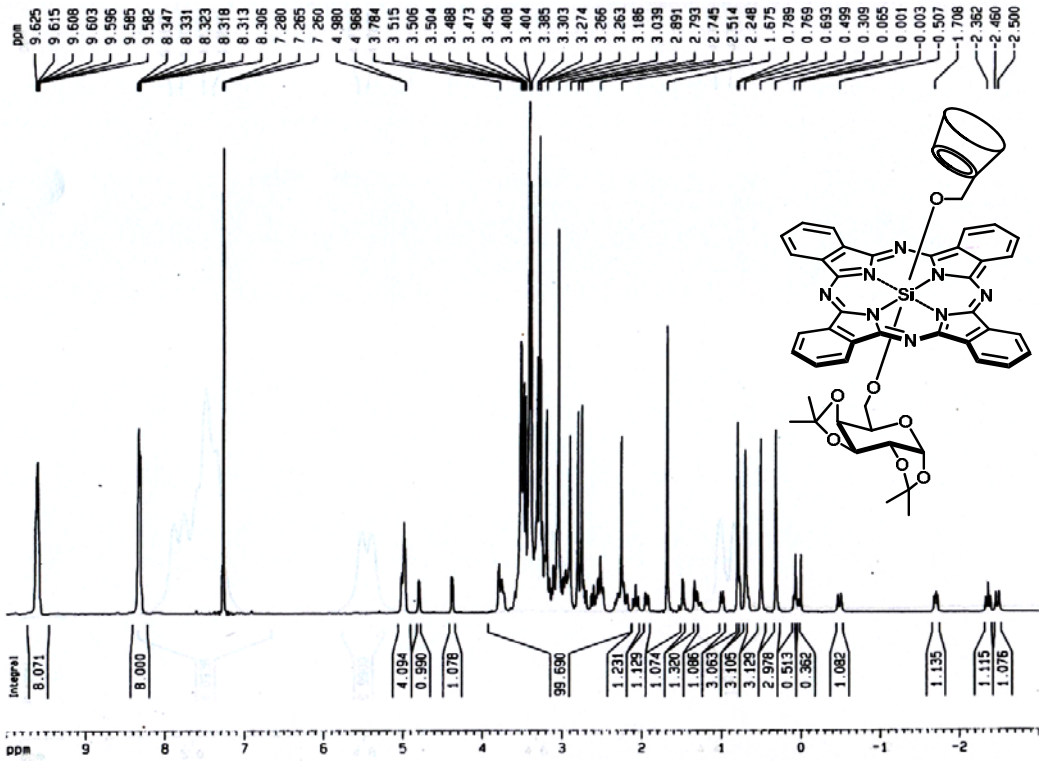


Fig. S8 ^{1}H NMR spectrum of **2** in $CDCl_3$.

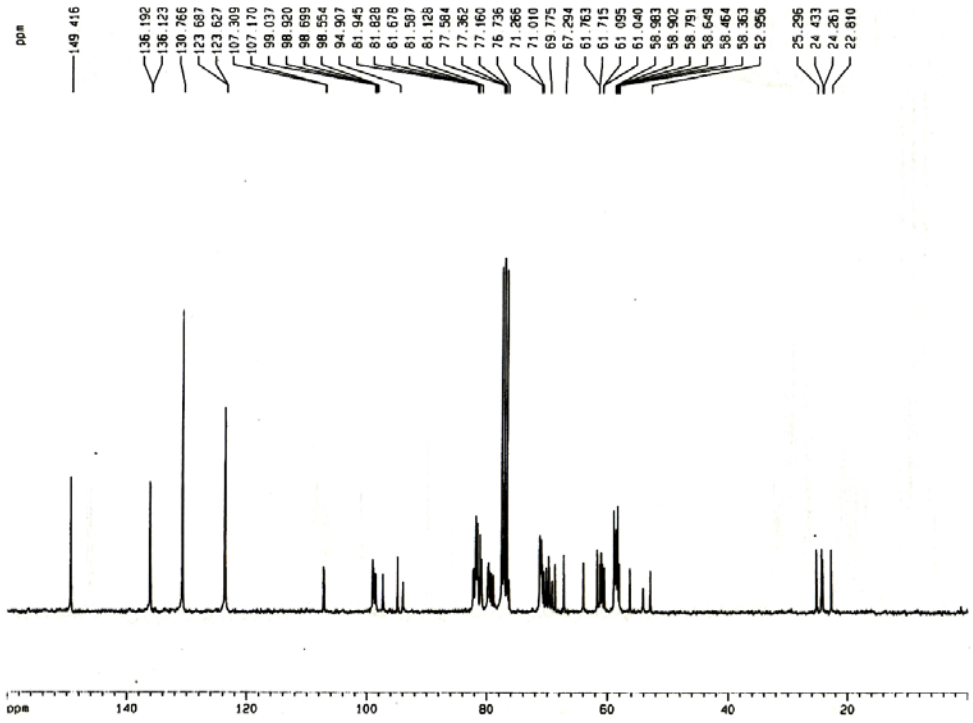


Fig. S9 $^{13}C\{^{1}H\}$NMR spectrum of **2** in $CDCl_3$.

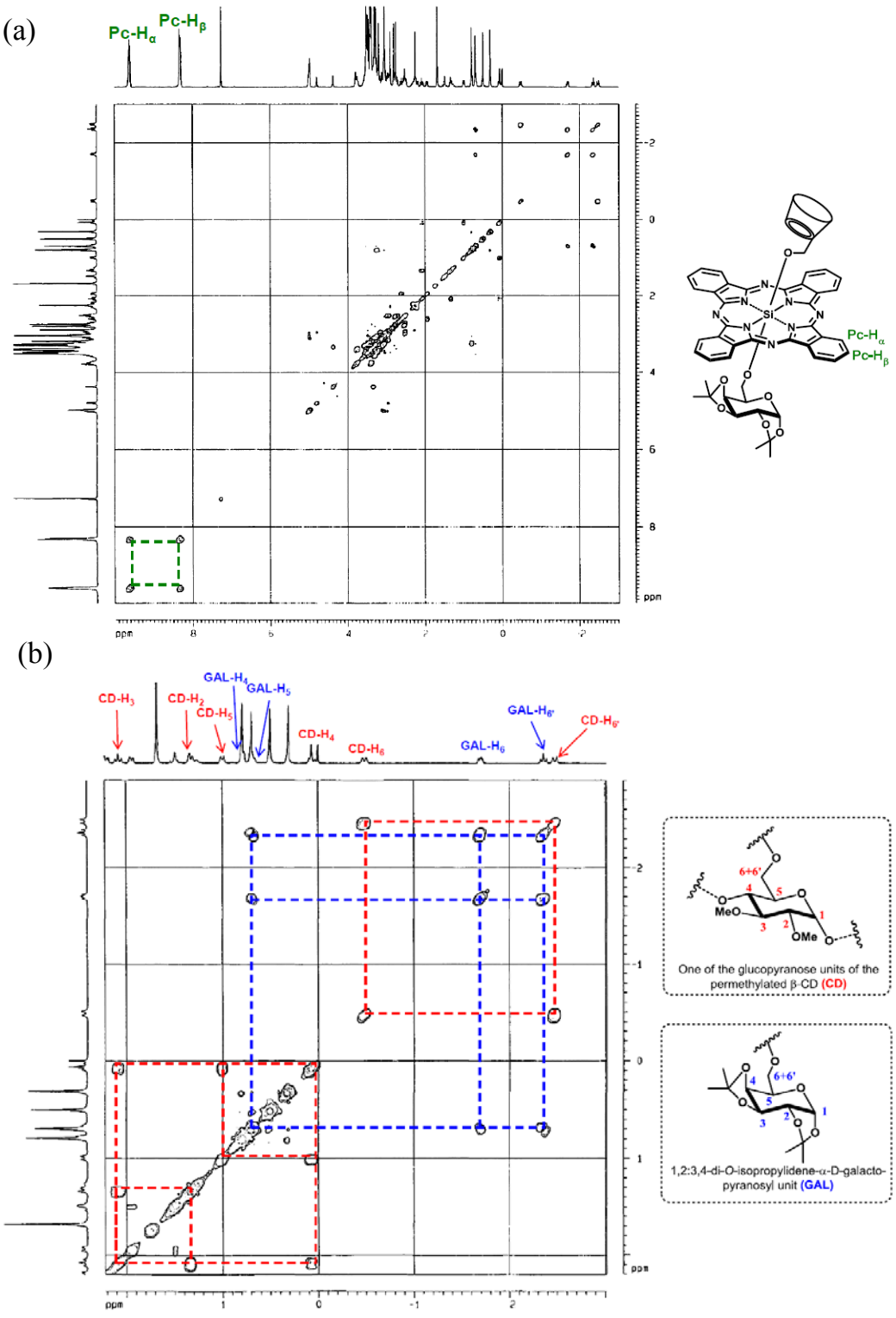


Fig. S10 (a) ^{1}H-^{1}H COSY spectrum of **2** in $CDCl_3$. An enlarged spectrum is shown in (b).

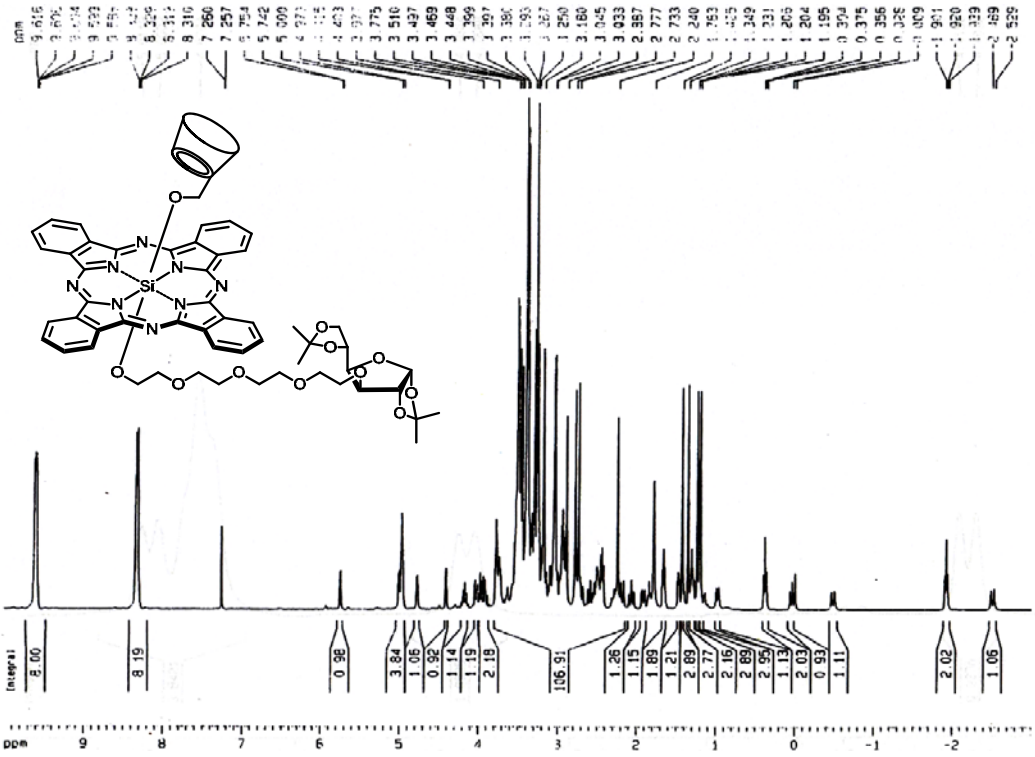


Fig. S11 ^{1}H NMR spectrum of **3** in $CDCl_3$.

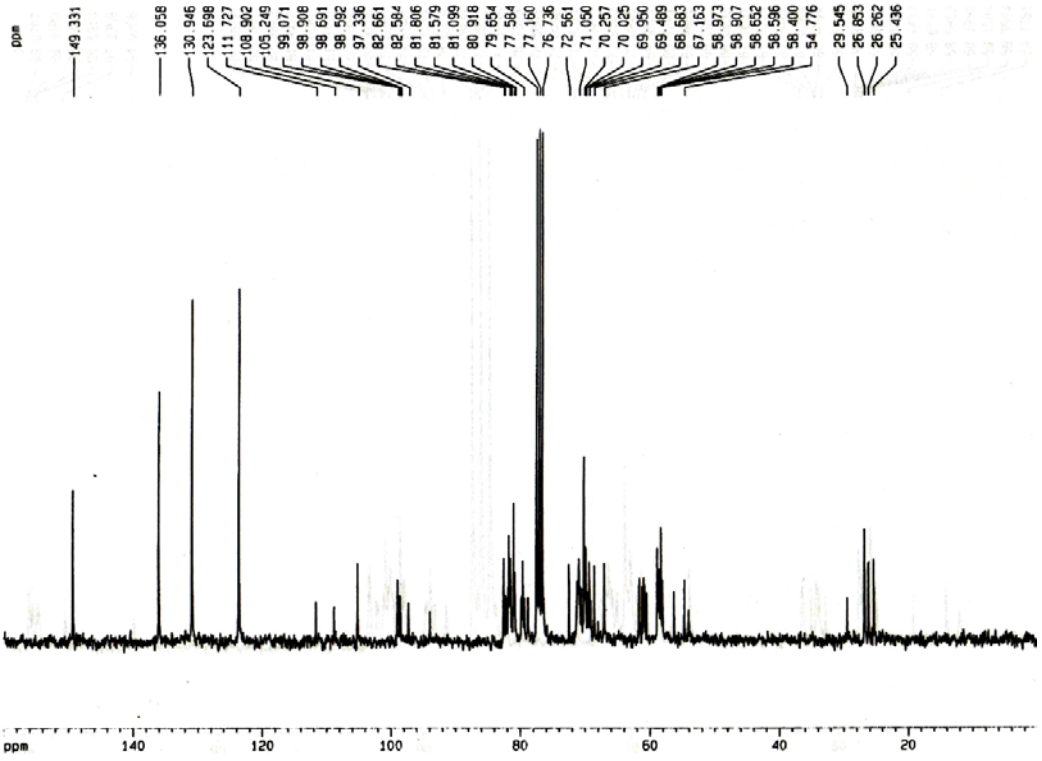


Fig. S12 $^{13}C\{^{1}H\}$NMR spectrum of **3** in $CDCl_3$.

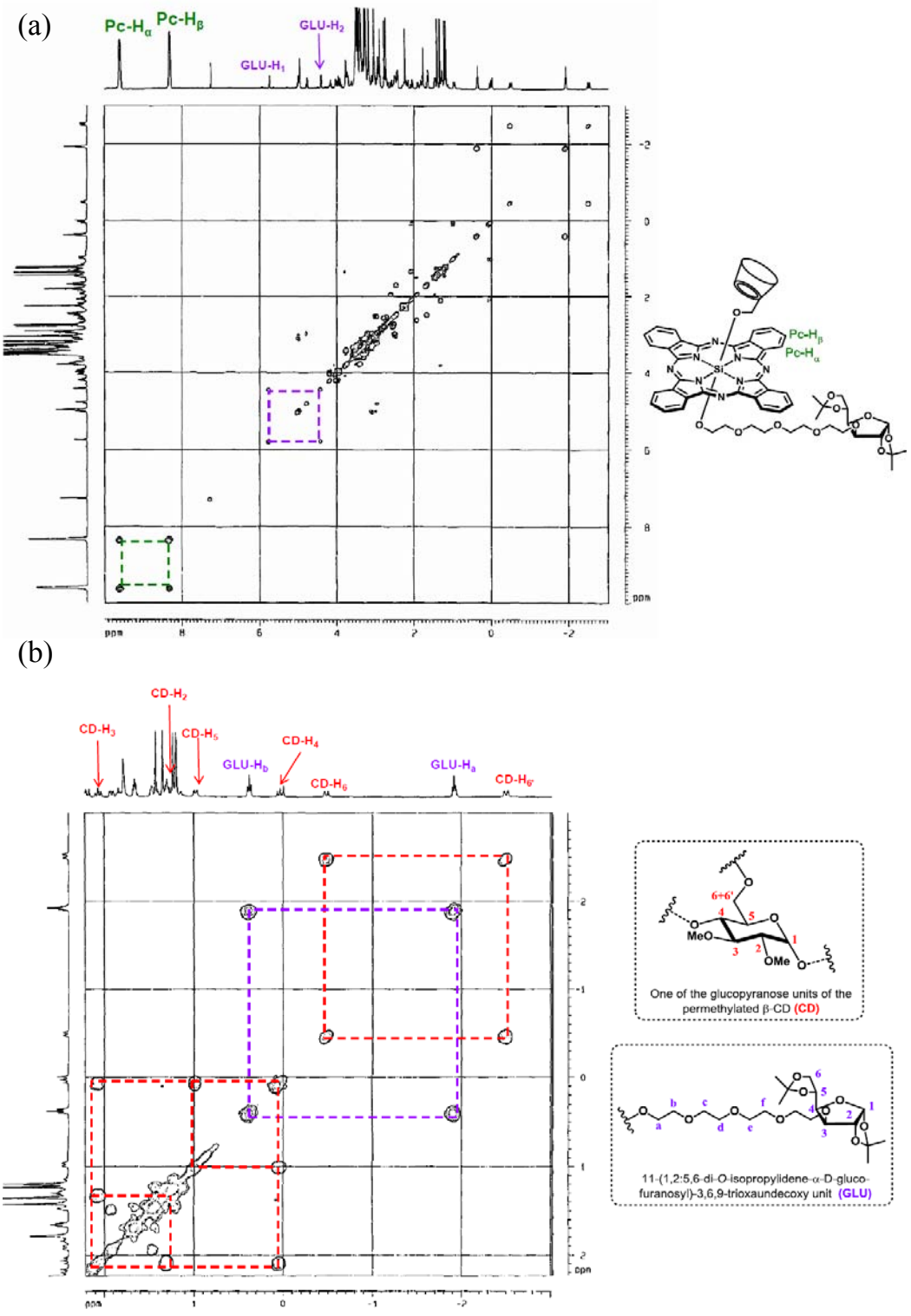


Fig. S13 (a) ^{1}H-^{1}H COSY spectrum of **3** in $CDCl_3$. An enlarged spectrum is shown in (b).

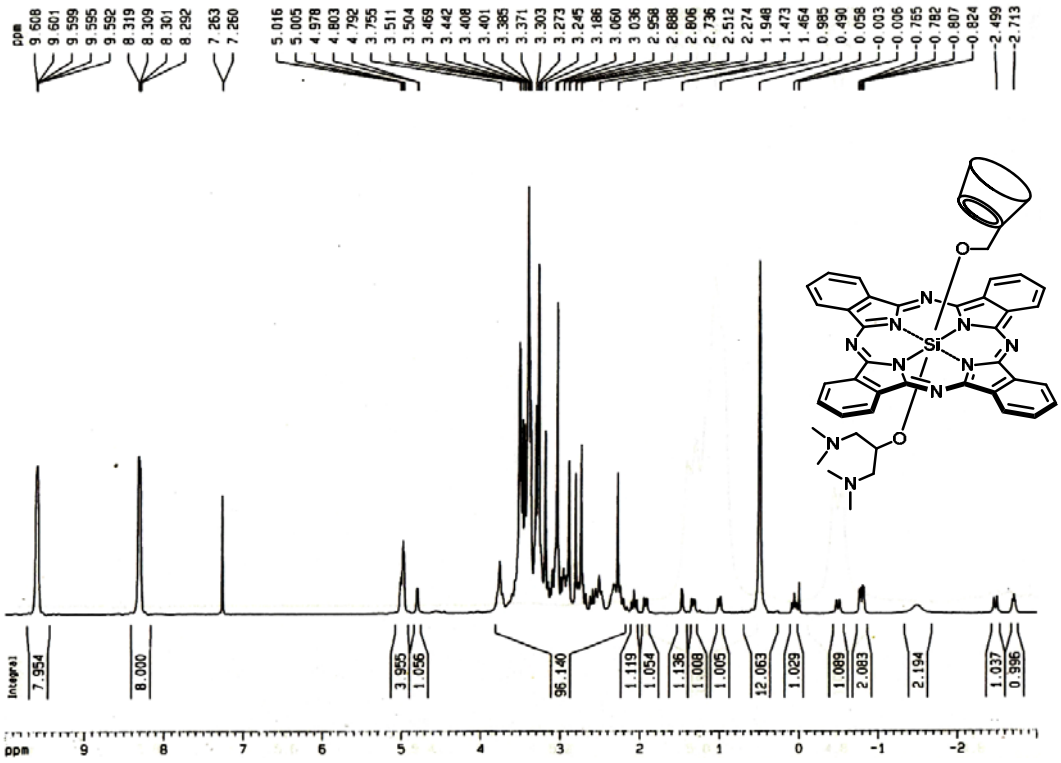


Fig. S14 ^{1}H NMR spectrum of **4** in $CDCl_3$.

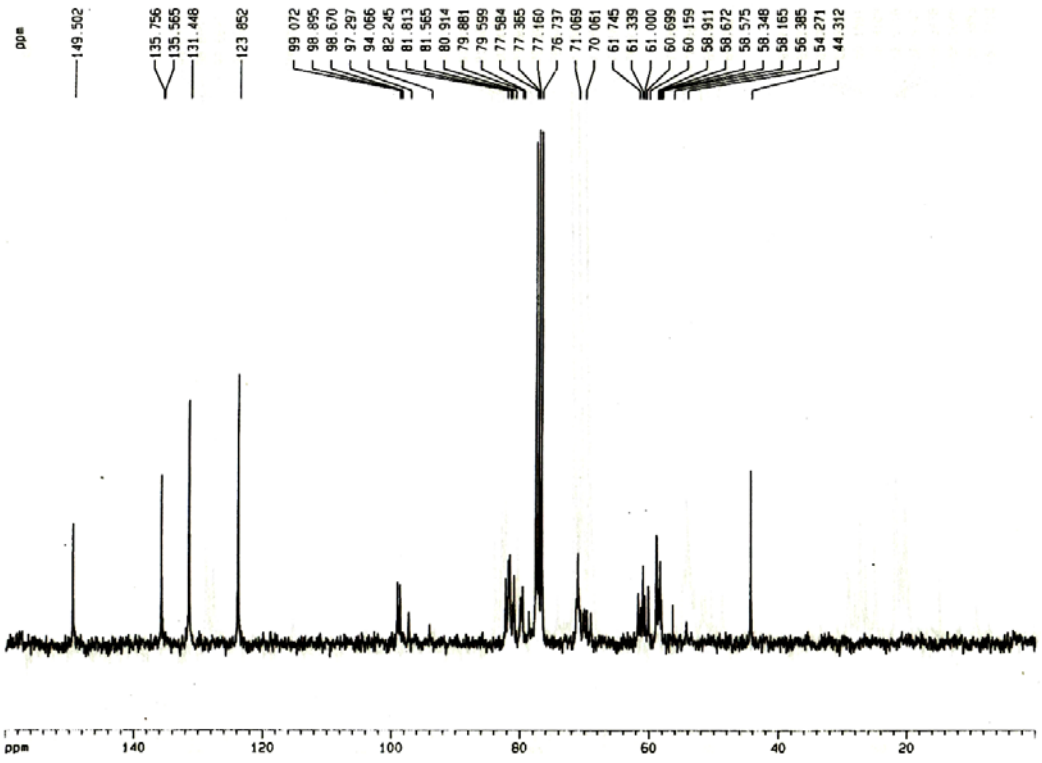


Fig. S15 ^{13}C{^{1}H}NMR spectrum of **4** in $CDCl_3$.

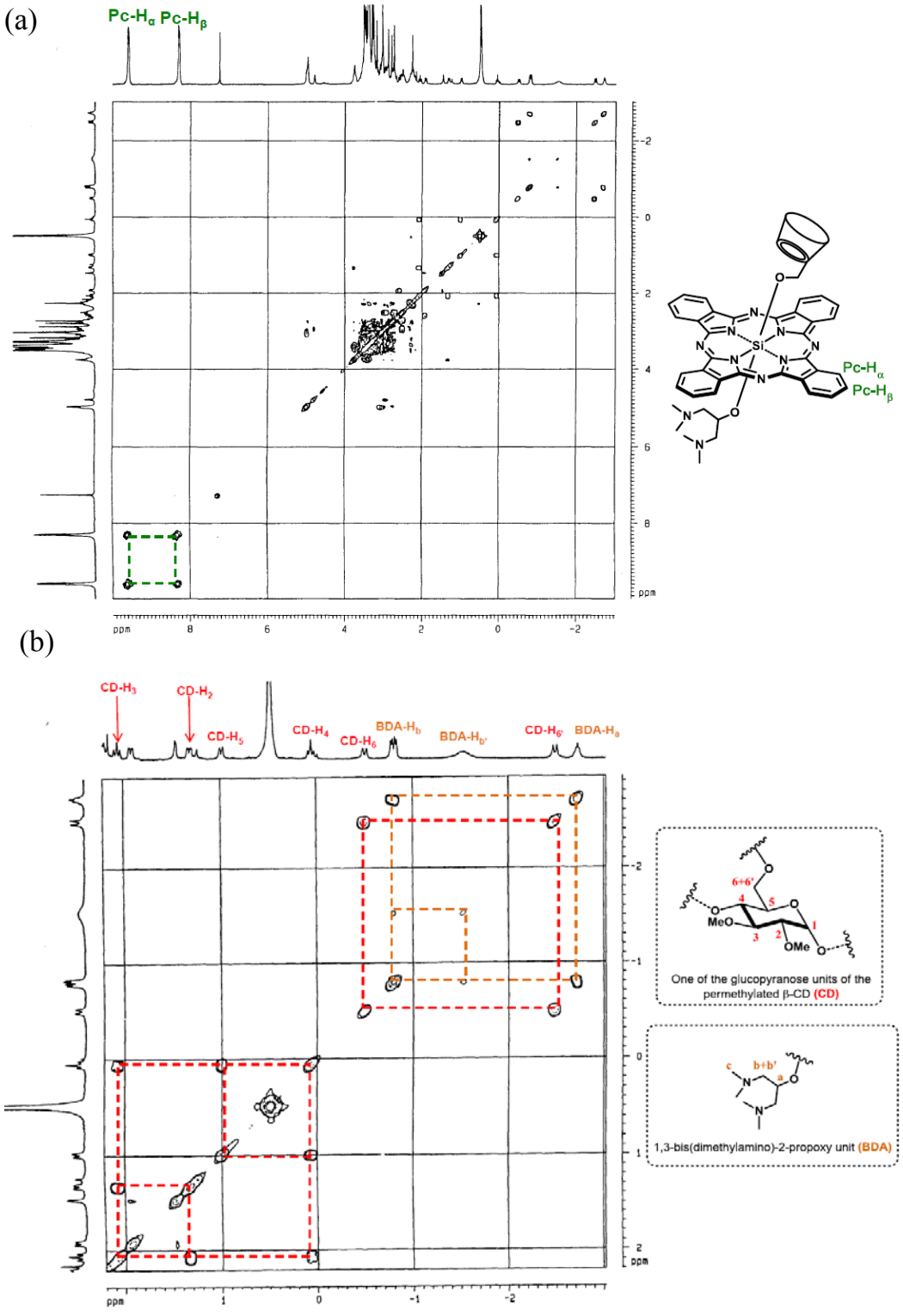


Fig. S16 (a) ^{1}H-^{1}H COSY spectrum of **4** in $CDCl_3$. An enlarged spectrum is shown in (b).

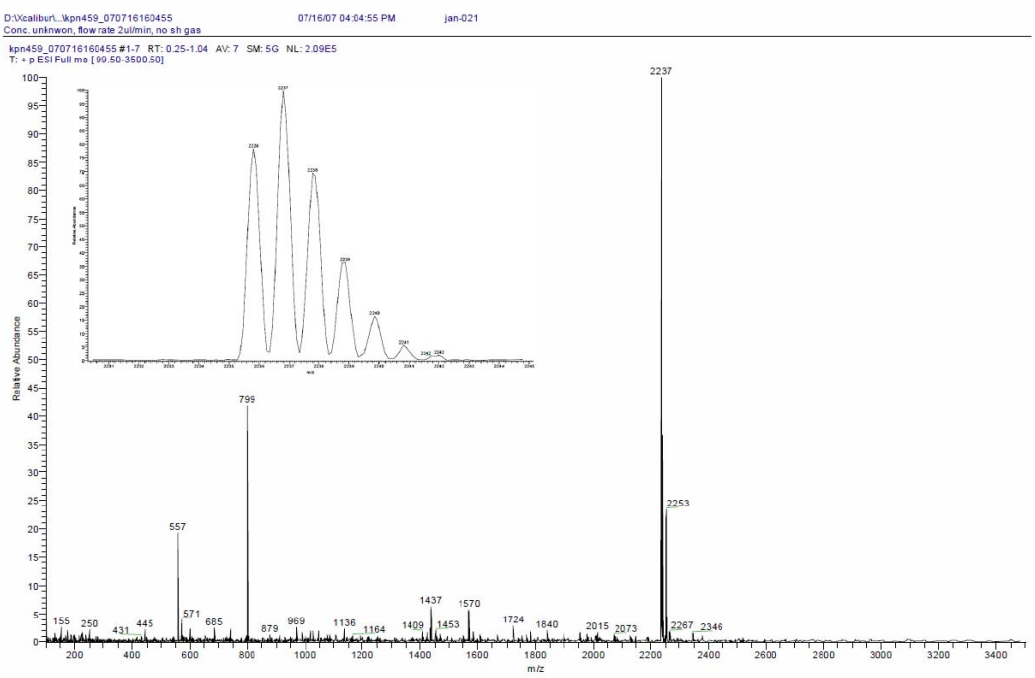


Fig. S17 ESI mass spectrum of **2**. The inset shows the enlarged molecular ion signal.

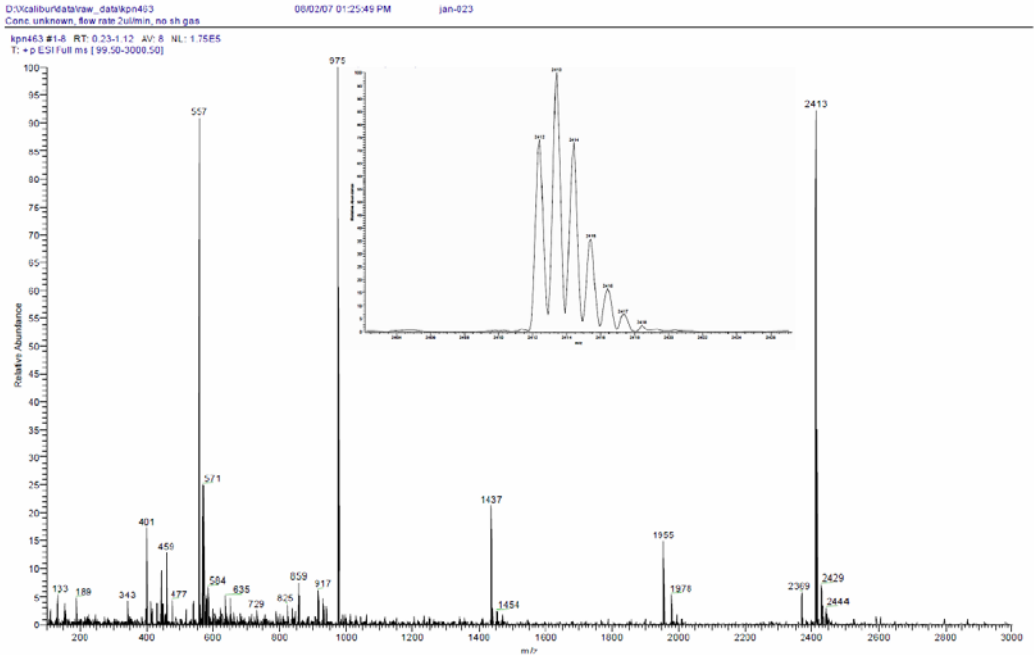


Fig. S18 ESI mass spectrum of **3**. The inset shows the enlarged molecular ion signal.

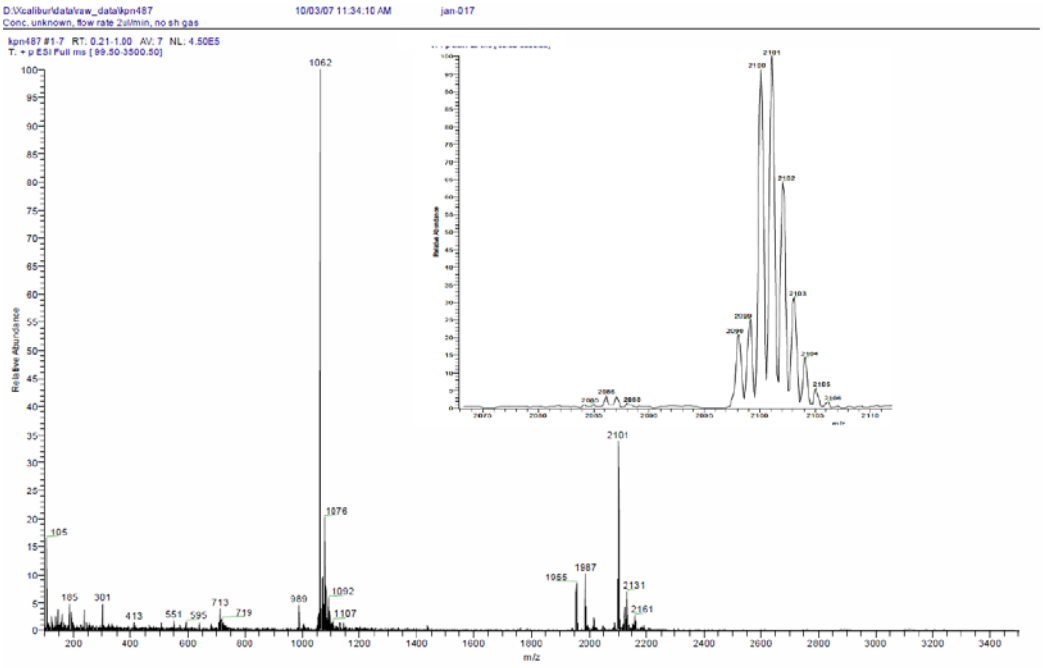


Fig. S19 ESI mass spectrum of **4**. The inset shows the enlarged molecular ion signal.