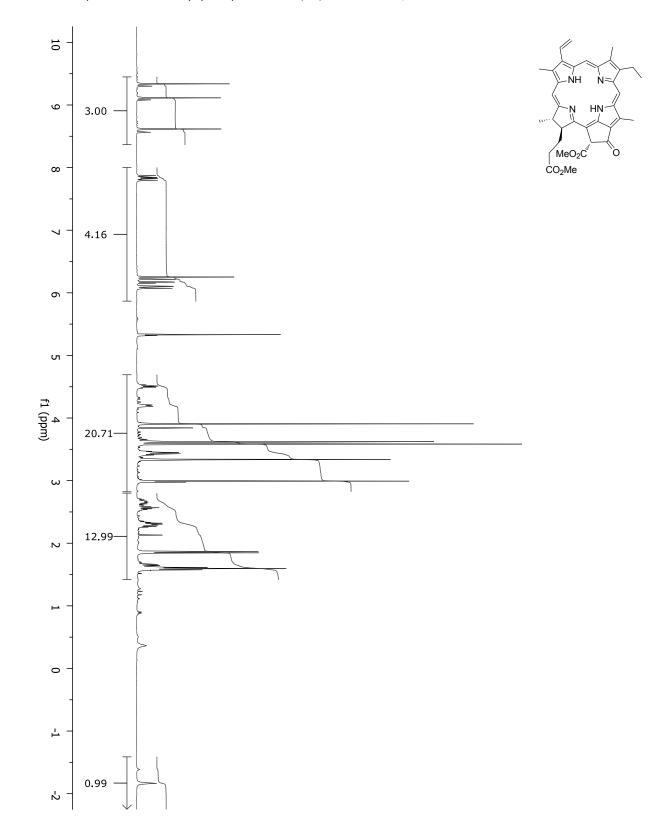
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## Syntheses and Cellular Investigations of Di(aspartate) and Aspartate-lysine Chlorin e<sub>6</sub> Conjugates

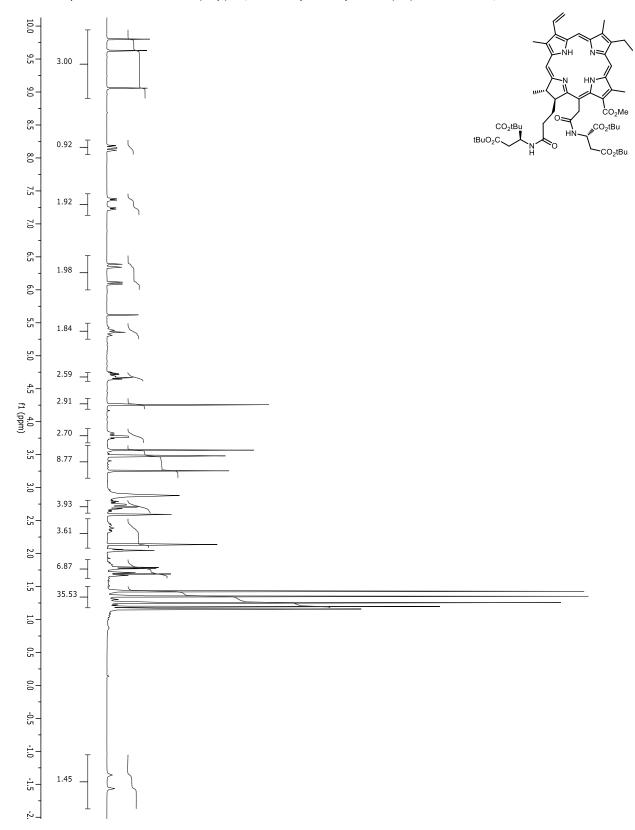
R. G. Waruna Jinadasa, Zehua Zhou, M. Graça H. Vicente and Kevin M. Smith\*

## **ELECTRONIC SUPPLEMENTARY INFORMATION**

Copies of <sup>1</sup> H NMR spectra	S2 – S14
Copies of <sup>13</sup> C NMR spectra	S15 – S18
Dark and phototoxicity curves	S19-S20
Subcellular localization for chlorin $e_6$	S21

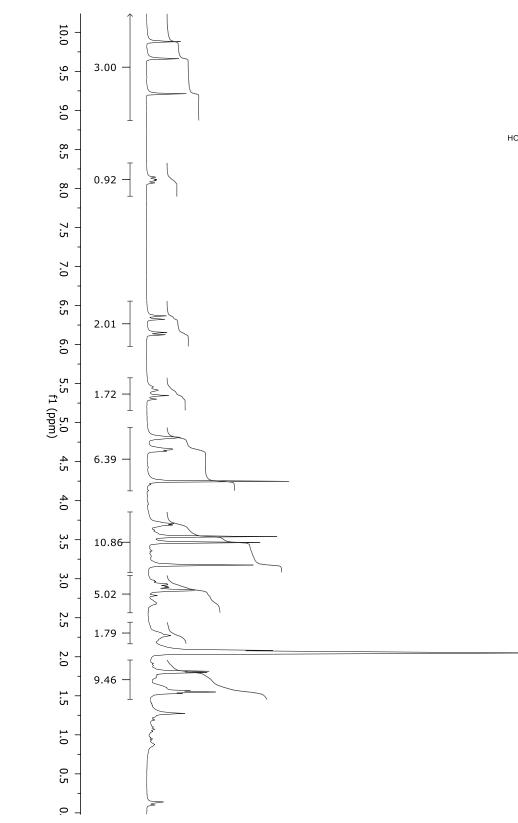


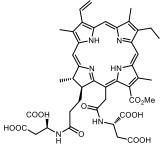
<sup>1</sup>H NMR spectrum of methyl pheophorbide a (**21**) in acetone- $d_6$  at 400 MHz

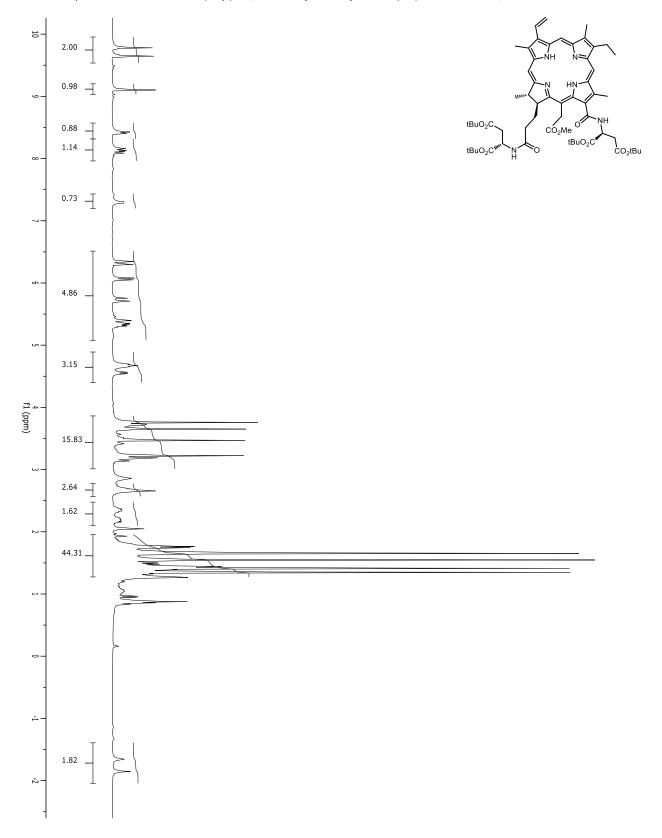


<sup>1</sup>H NMR spectrum of  $15^2$ ,  $17^3$ -di(Asp)Ce<sub>6</sub> tert-butyl methyl ester (**11**) in acetone- $d_6$  at 400 MHz

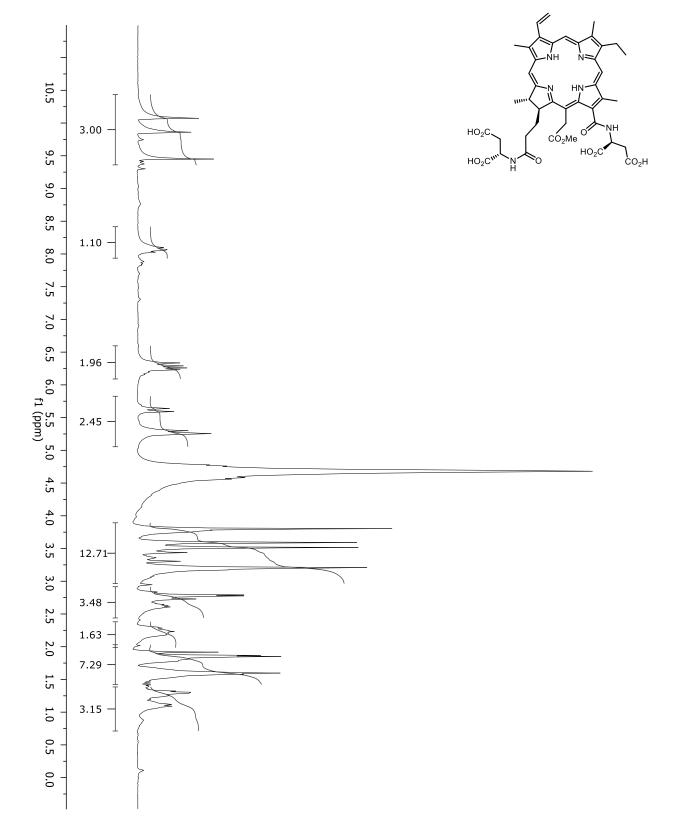
<sup>1</sup>H NMR spectrum of  $15^2$ ,  $17^3$ -di(Asp)Ce<sub>6</sub> methyl ester (**12**) in acetone- $d_6$  at 400 MHz







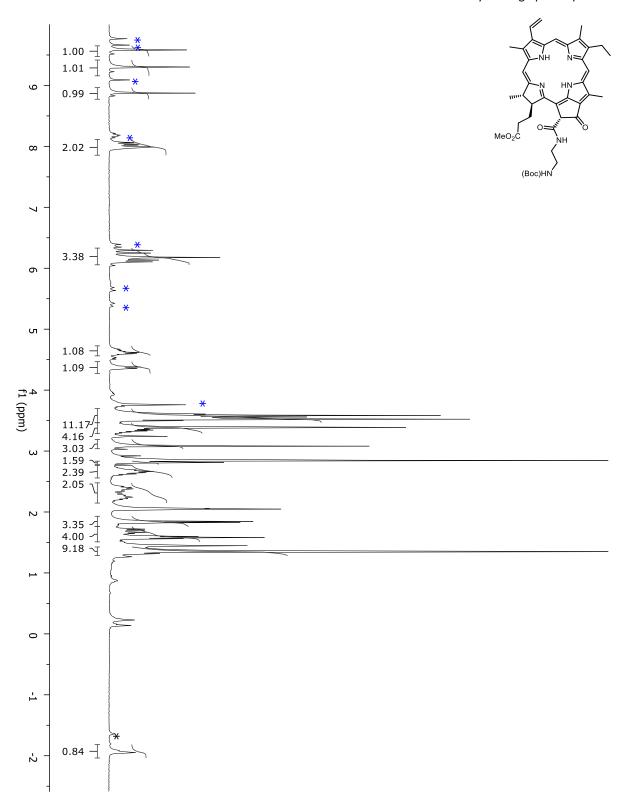
<sup>1</sup>H NMR spectrum of  $13^{1}$ ,  $17^{3}$ -di(Asp)Ce<sub>6</sub> *tert*-butyl methyl ester (**20**) in acetone- $d_{6}$  at 400 MHz

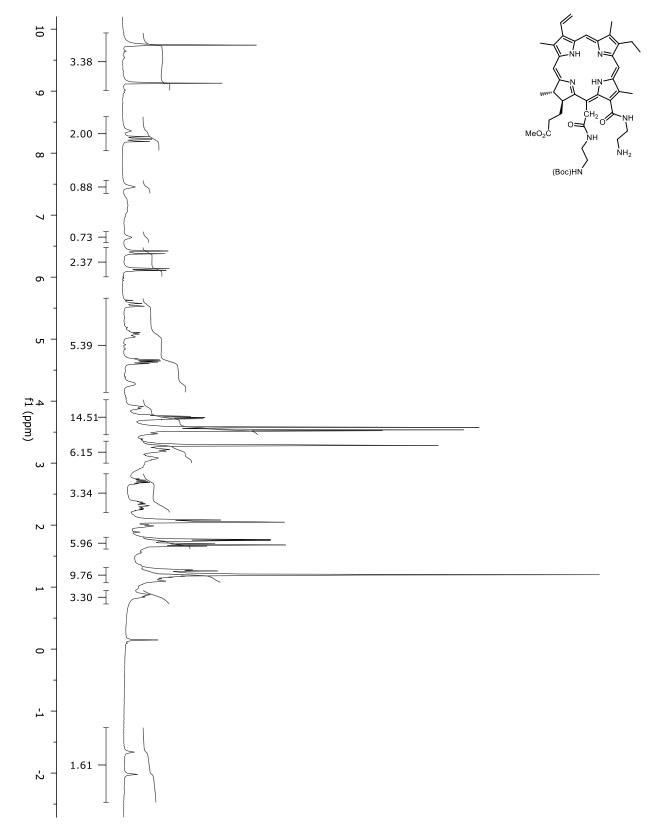


 $^{1}$ H NMR spectrum of 13 $^{1}$ ,17 $^{3}$ -diaspartylchlorin e<sub>6</sub> methyl ester (**13**) in MeOD at 400 MHz

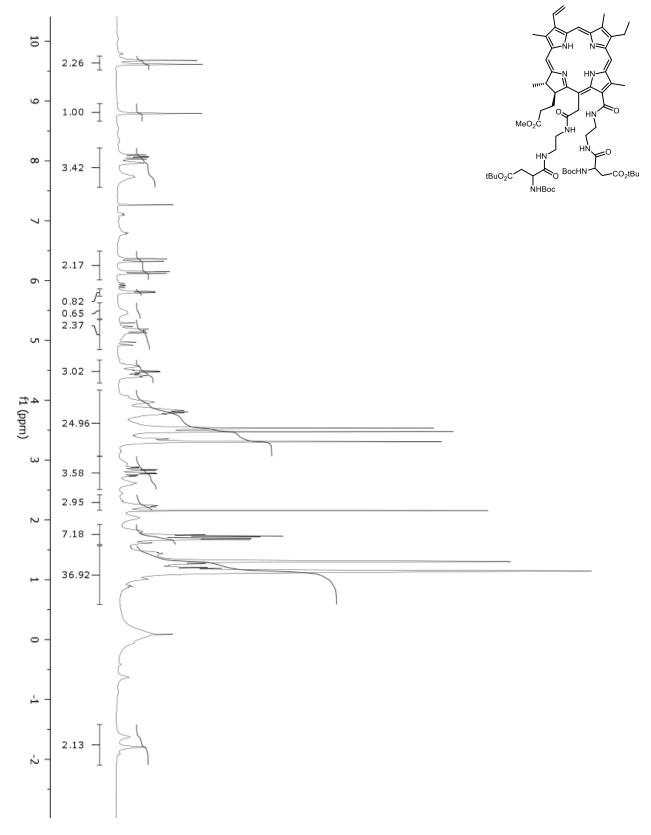
## <sup>1</sup>H NMR spectrum of ethylenediaminyl(boc) pheophorbide a (**25**) in acetone- $d_6$ at 400 MHz

\*Contain 10% isocyclic ring opened product 24

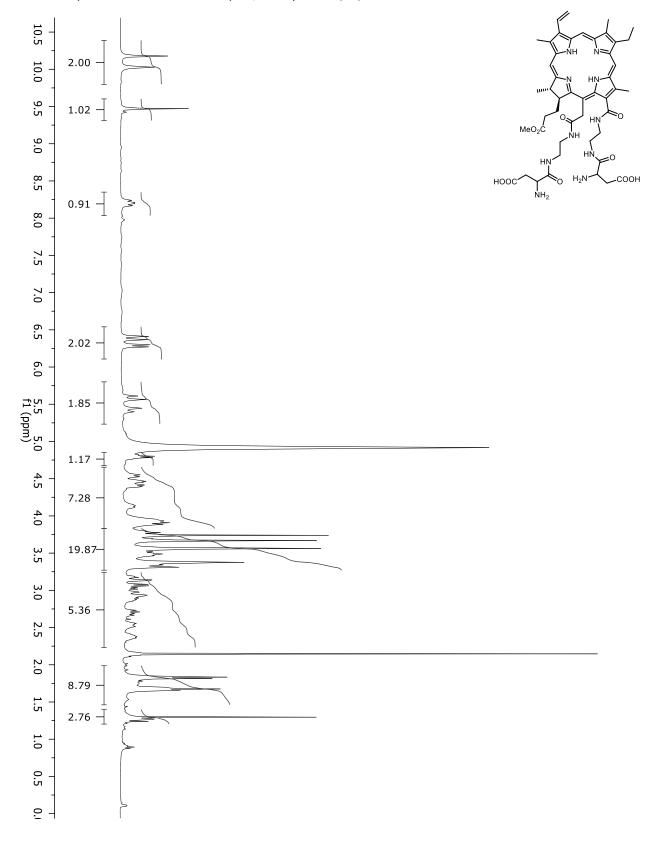




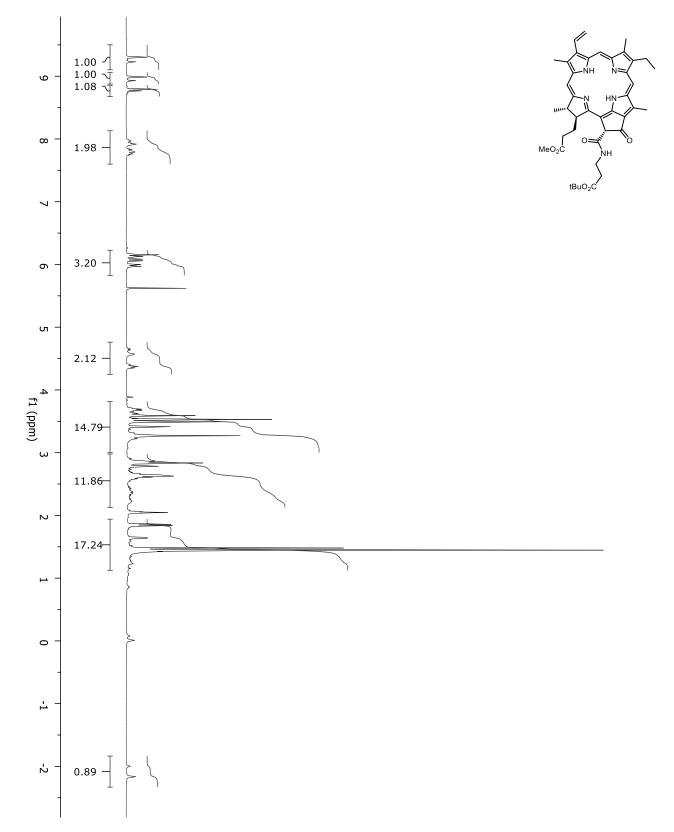
<sup>1</sup>H NMR spectrum of  $13^{1}$ -ED  $15^{2}$ -ED(boc)Ce<sub>6</sub> methyl ester (**26**) in acetone- $d_{6}$  at 400 MHz



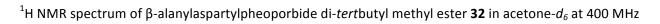
<sup>1</sup>H NMR spectrum of  $13^{1}$ ,  $15^{2}$ -ED-AspCe<sub>6</sub> *tert*-butyl boc methyl ester (**28**) in chloroform-*d* at 400 MHz

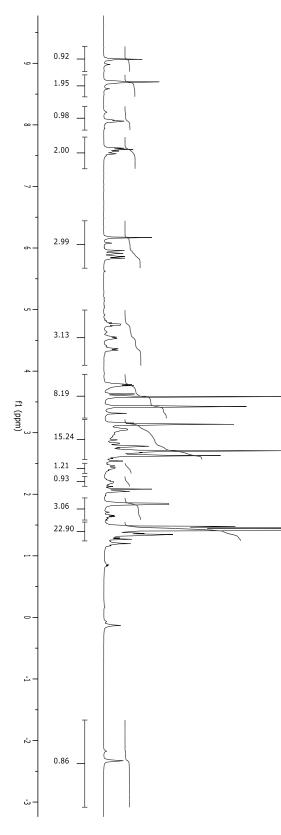


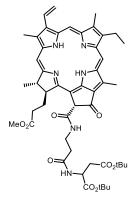
<sup>1</sup>H NMR spectrum of  $13^{1}$ ,  $15^{2}$ -ED-AspCe<sub>6</sub> methyl ester (**29**) in methanol- $d_{4}$  at 400 MHz

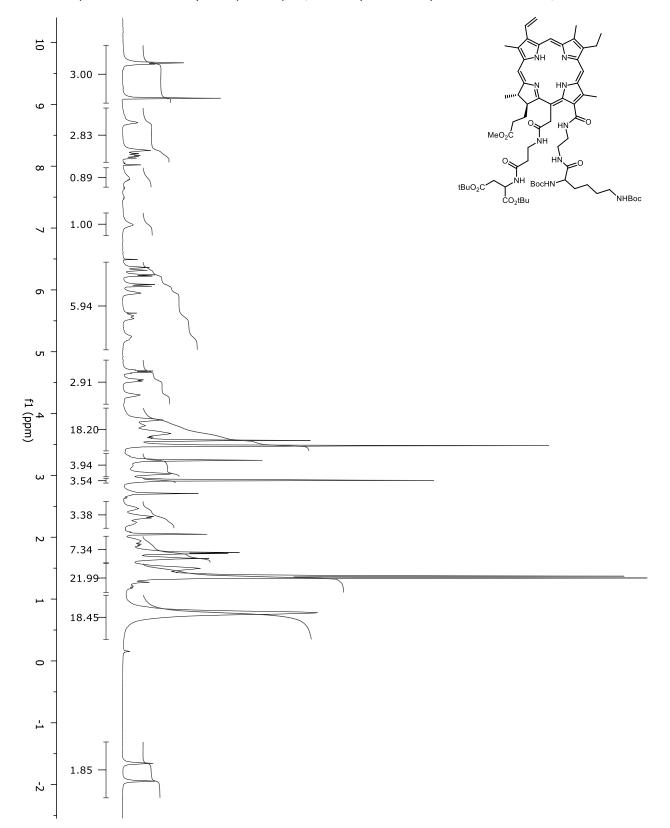


<sup>1</sup>H NMR spectrum of  $\beta$ -alanylpheoporbide *tert*-butyl methyl ester (**30**) in acetone- $d_6$  at 400 MHz

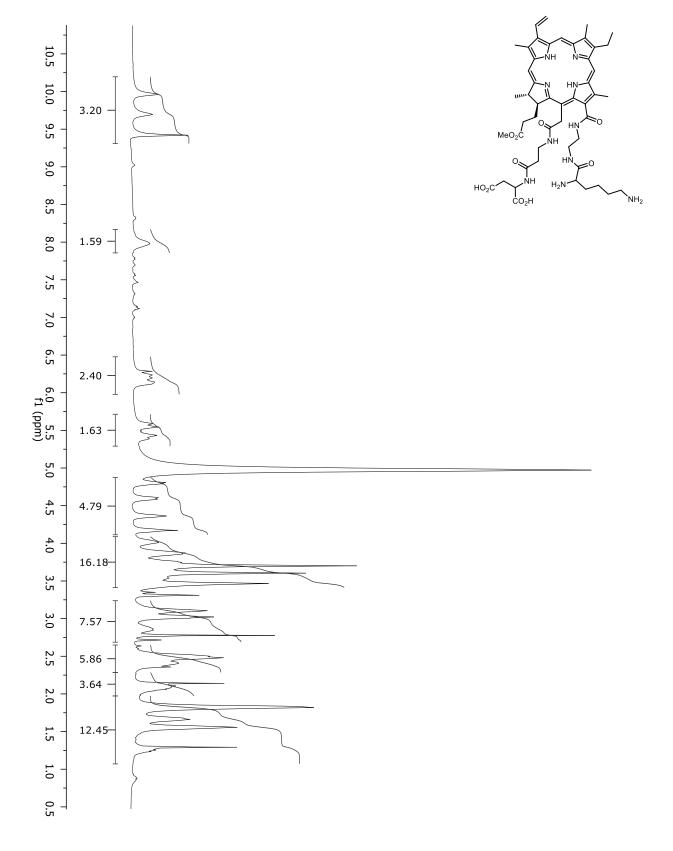






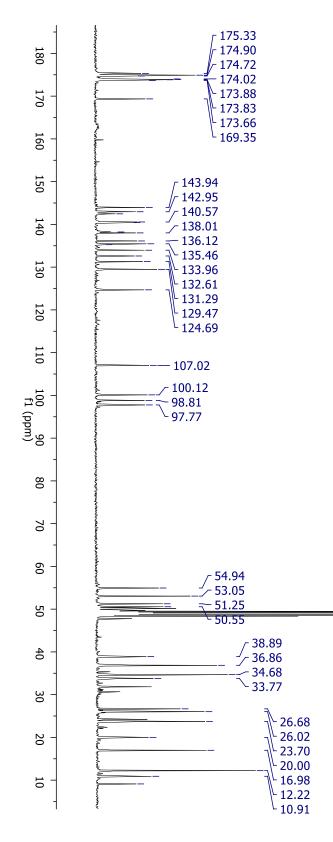


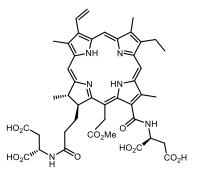
<sup>1</sup>H NMR spectrum of  $13^2$ -EDLys- $15^2$ - $\beta$ -AlaAspCe<sub>6</sub> *tert*-butyl boc methyl ester in acetone- $d_6$  at 400 MHz

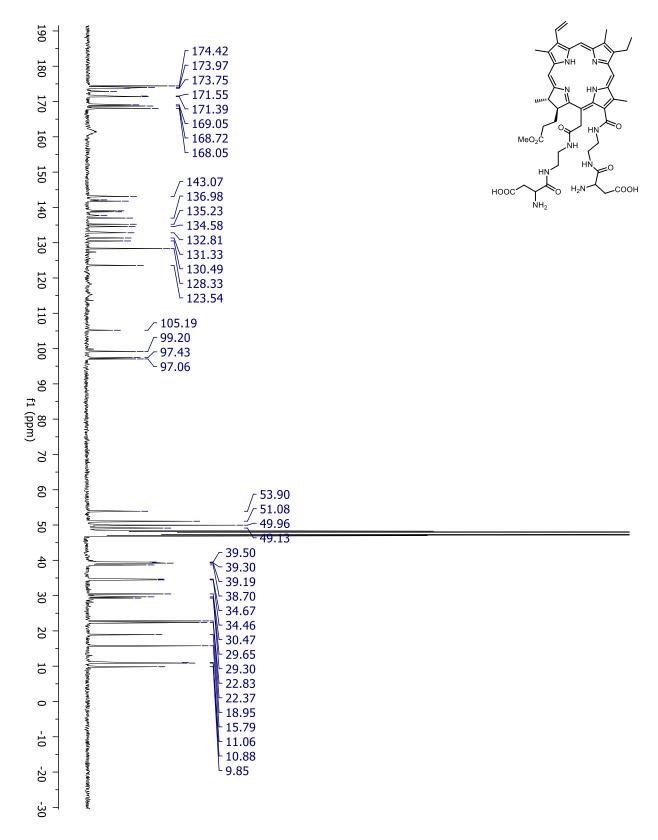


<sup>1</sup>H NMR spectrum of  $13^2$ -EDLys- $15^2$ - $\beta$ -AlaAspCe<sub>6</sub> methyl ester (**34**) in acetone- $d_6$  at 400 MHz

 $^{1}$ H NMR spectrum of 13 $^{1}$ ,17 $^{3}$ -diaspartylchlorin e<sub>6</sub> methyl ester (**13**) in MeOD at 400 MHz



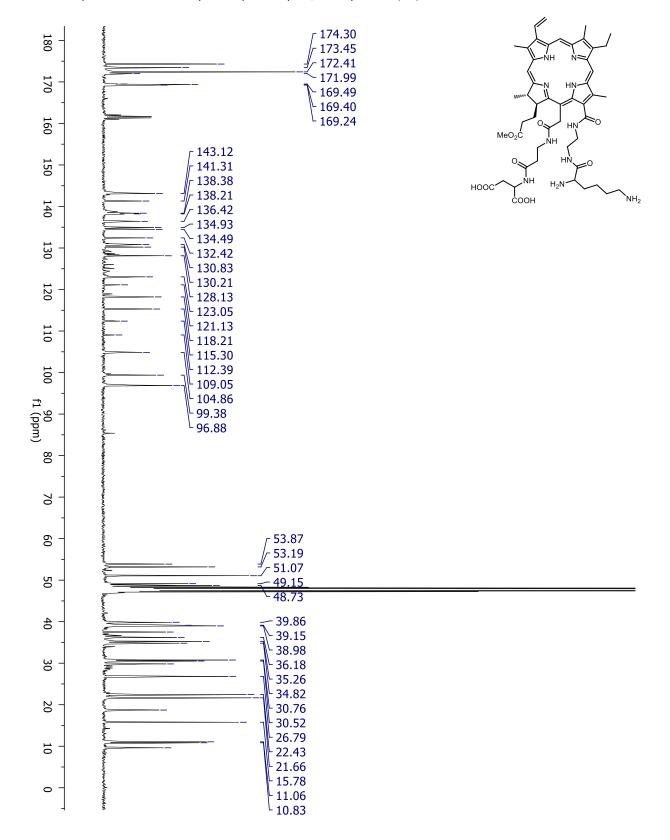




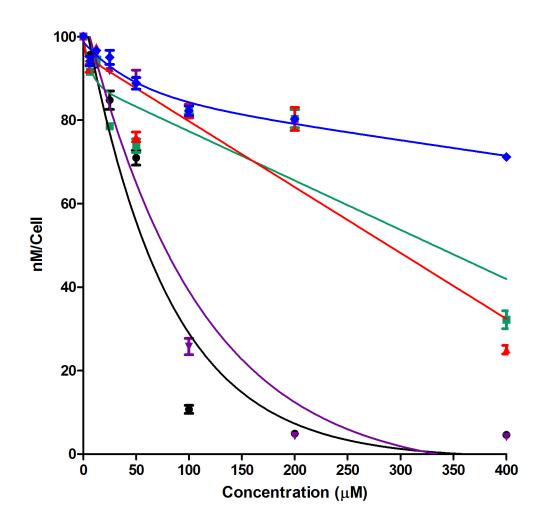
<sup>13</sup>C NMR spectrum of  $13^{1}$ ,  $15^{2}$ -ED-AspCe<sub>6</sub> methyl ester (**29**) in methanol- $d_{4}$  at 400 MHz

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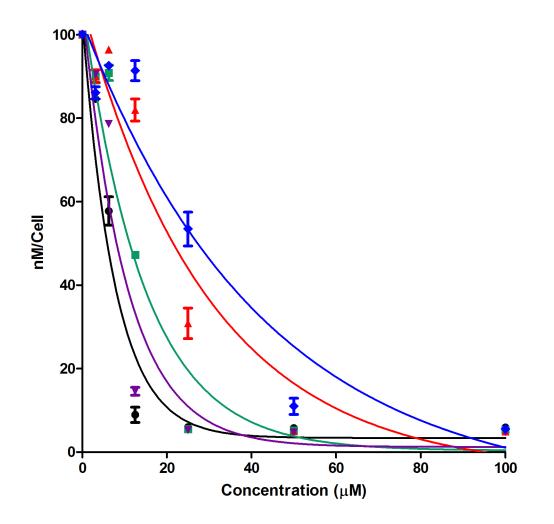
 $^{13}$ C NMR spectrum of  $15^2$ ,  $17^3$ -di(Asp)Ce<sub>6</sub> methyl ester (**12**) in methanol- $d_4$  at 100 MHz



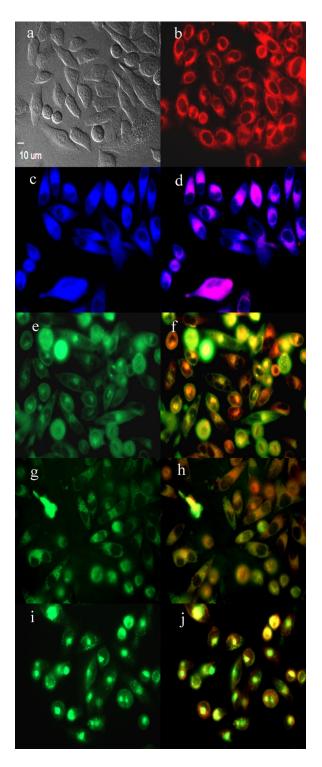
<sup>13</sup>C NMR spectrum of  $13^2$ -EDLys - $15^2$ -β-AlaAspCe<sub>6</sub> methyl ester (**34**) in methanol- $d_4$  at 100 MHz



Dark toxicity of chlorin  $e_6$  (**1**, green) and its derivatives  $15^2$ ,  $17^3$ -di(Asp)Ce<sub>6</sub> MME (**12**, blue),  $13^1$ ,  $17^3$ -di(Asp)Ce<sub>6</sub> MME (**13**, red),  $13^1$ ,  $15^2$ -di(EDAsp)Ce<sub>6</sub> MME (**28**, purple) and  $13^1$ -EDLys- $15^2$ - $\beta$ -AlaAspCe<sub>6</sub> MME (**33**, black), toward HEp2 cells using the Cell Titer Blue assay.



Phototoxicity of chlorin  $e_6$  (**1**, green) and its derivatives  $15^2$ ,  $17^3$ -di(Asp)Ce<sub>6</sub> MME (**12**, blue),  $13^1$ ,  $17^3$ -di(Asp)Ce<sub>6</sub> MME (**13**, red),  $13^1$ ,  $15^2$ -di(EDAsp)Ce<sub>6</sub> MME (**28**, purple) and  $13^1$ -EDLys- $15^2$ - $\beta$ -AlaAspCe<sub>6</sub> MME (**33**, black), toward HEp2 cells using 1J/cm<sup>2</sup> light dose and the Cell Titer Blue assay.



Subcellular localization of chlorin  $e_6$  in HEp2 cells at 10  $\mu$ M for 6h, (a) phase contrast, (b) overlay of chlorin  $e_6$  and phase contrast, (c) ER Tracker Blue, (d) overlay of chlorin  $e_6$  and ER Tracker Blue, (e) BODIPY Ceramide, (f) overlay of chlorin  $e_6$  and BODIPY Ceramide, (g) MitoTracker Green, (h) overlay of chlorin  $e_6$  and MitoTracker Green, (i) LysoSensor Green, (j) overlay of chlorin  $e_6$  and LysoSensor Green. Scale bar: 10  $\mu$ m