# Phosphinatophenylporphyrins tailored for high photodynamic efficacy

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**Content**

Figure S1. $^{1}H$ NMR ($CDCl_3$) spectrum of compound **2a**.

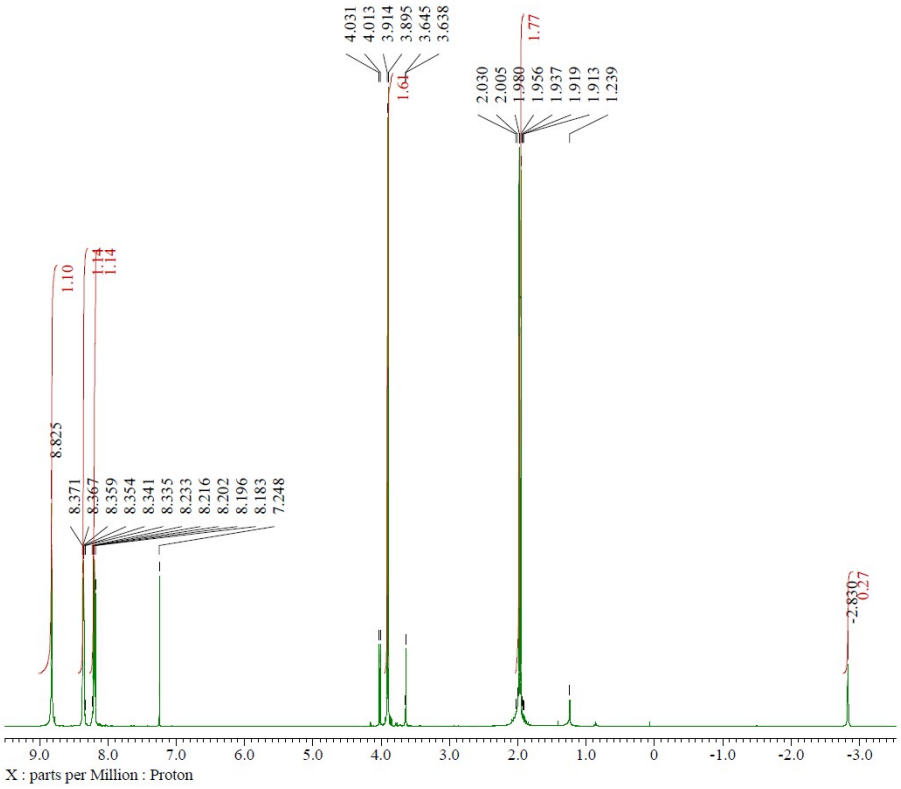



Figure S2. $^{31}P\{^{1}H\}$ NMR ($CDCl_3$) spectrum of compound **2a**.

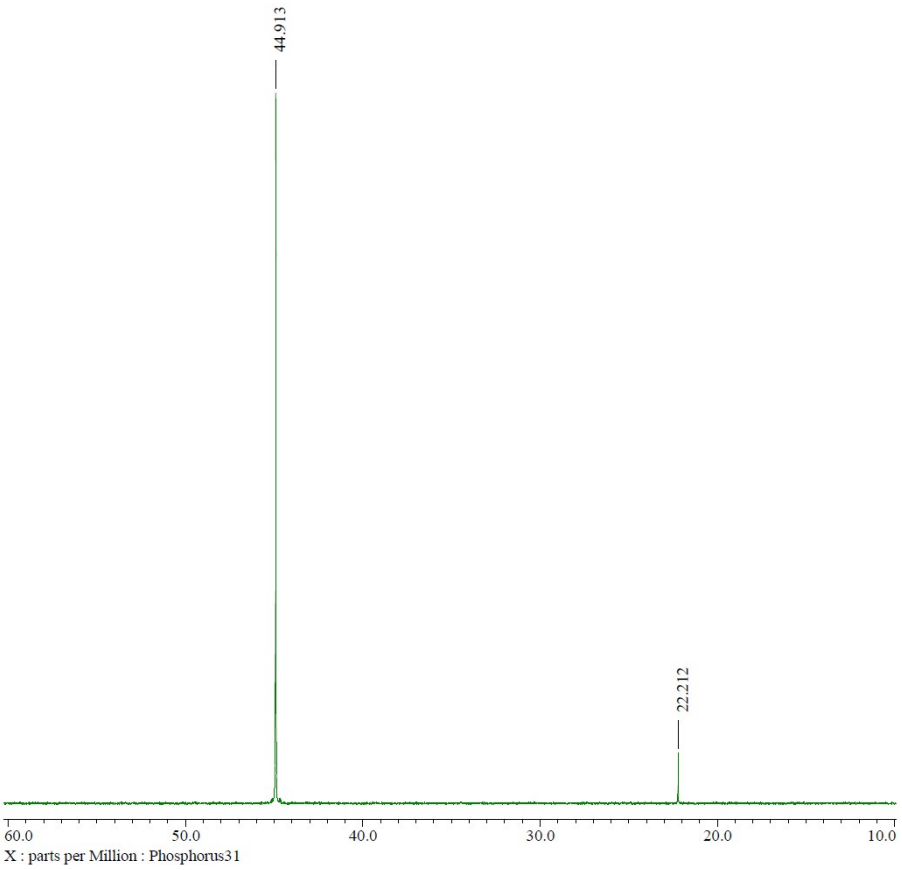


Figure S3. $^{13}C\{^{1}H\}$ NMR ($CDCl_3$) spectrum of compound **2a**.

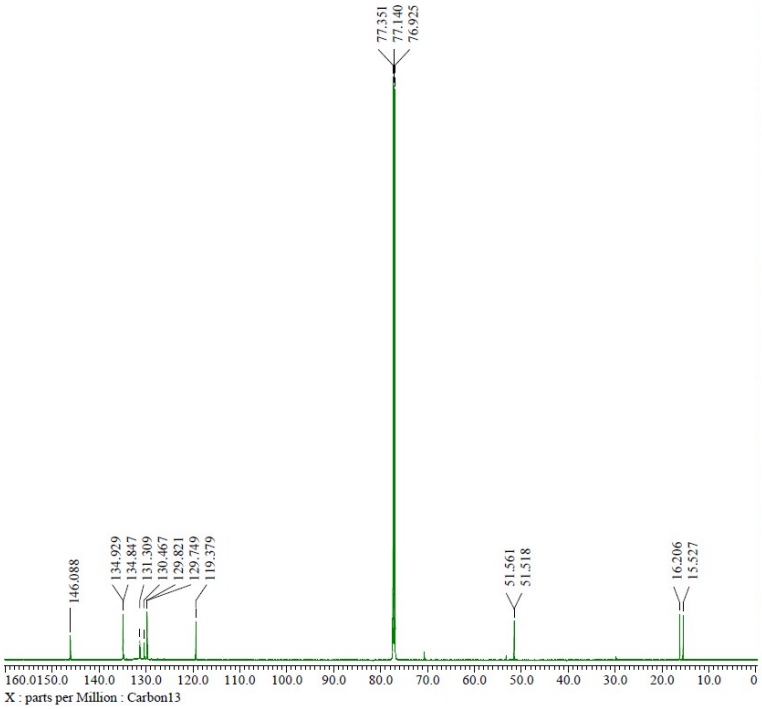



Figure S4. $^{1}H$ NMR ($CDCl_3$) spectrum of compound **2b**.

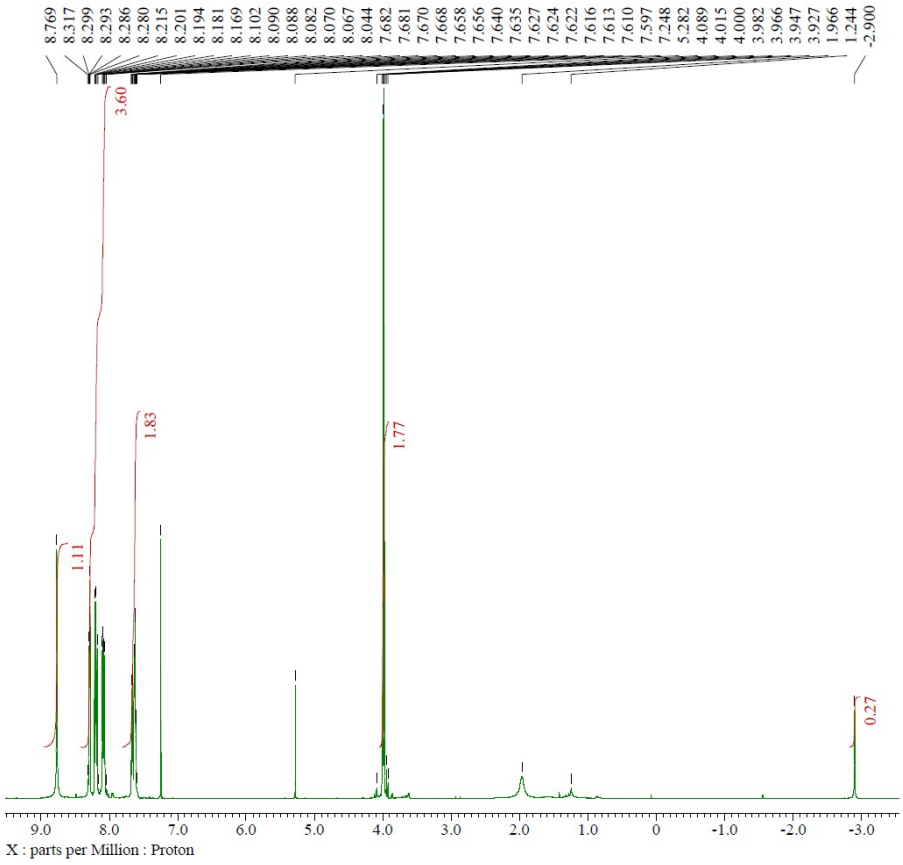


Figure S5. $^{31}P\{^{1}H\}$ NMR ($CDCl_3$) spectrum of compound **2b**.

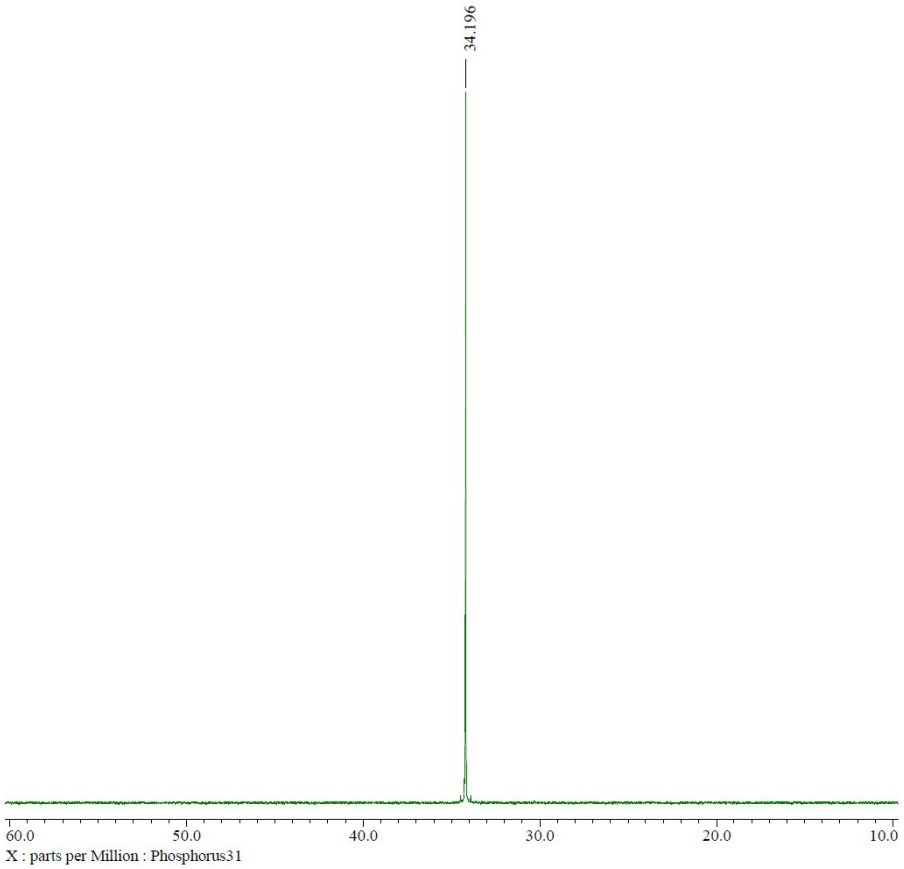



Figure S6. $^{13}C\{^{1}H\}$ NMR ($CDCl_3$) spectrum of compound **2b**.

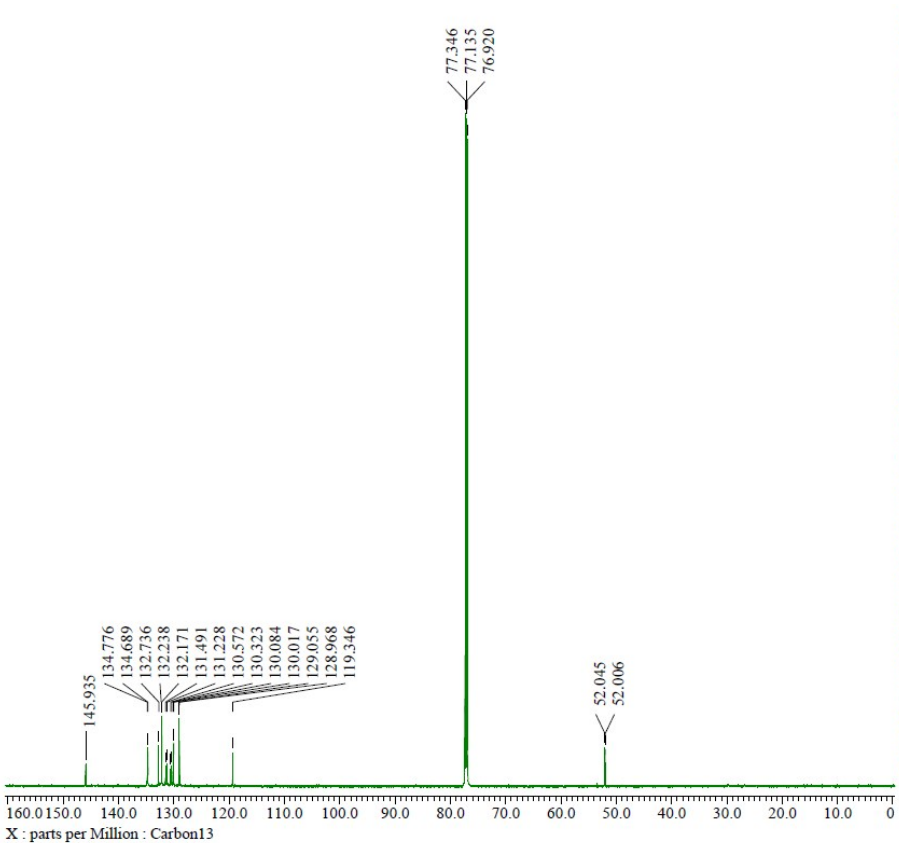


Figure S7. $^{1}H$ NMR ($CDCl_3$) spectrum of compound **2c**.

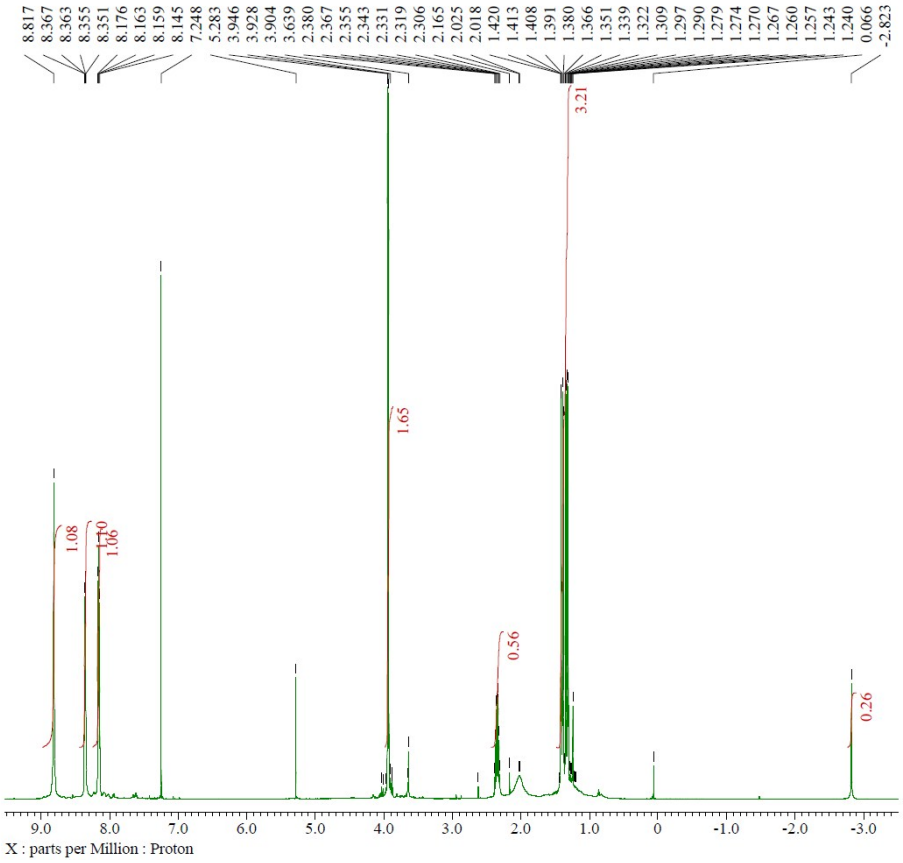



Figure S8. $^{31}P\{^{1}H\}$ NMR ($CDCl_3$) spectrum of compound **2c**.

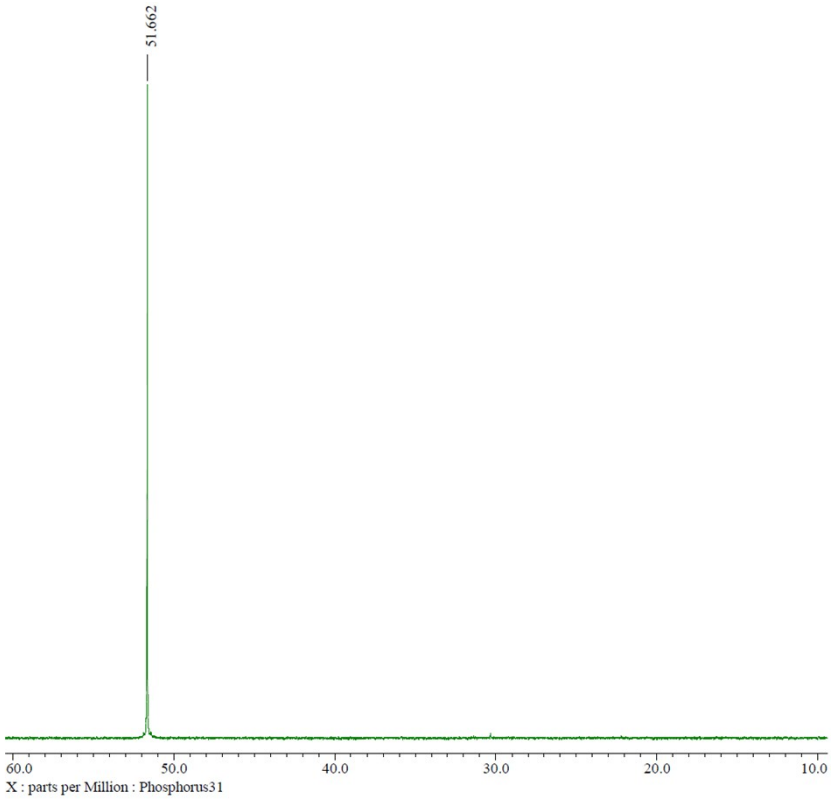


Figure S9. $^{13}C\{^{1}H\}$ NMR ($CDCl_3$) spectrum of compound **2c**.

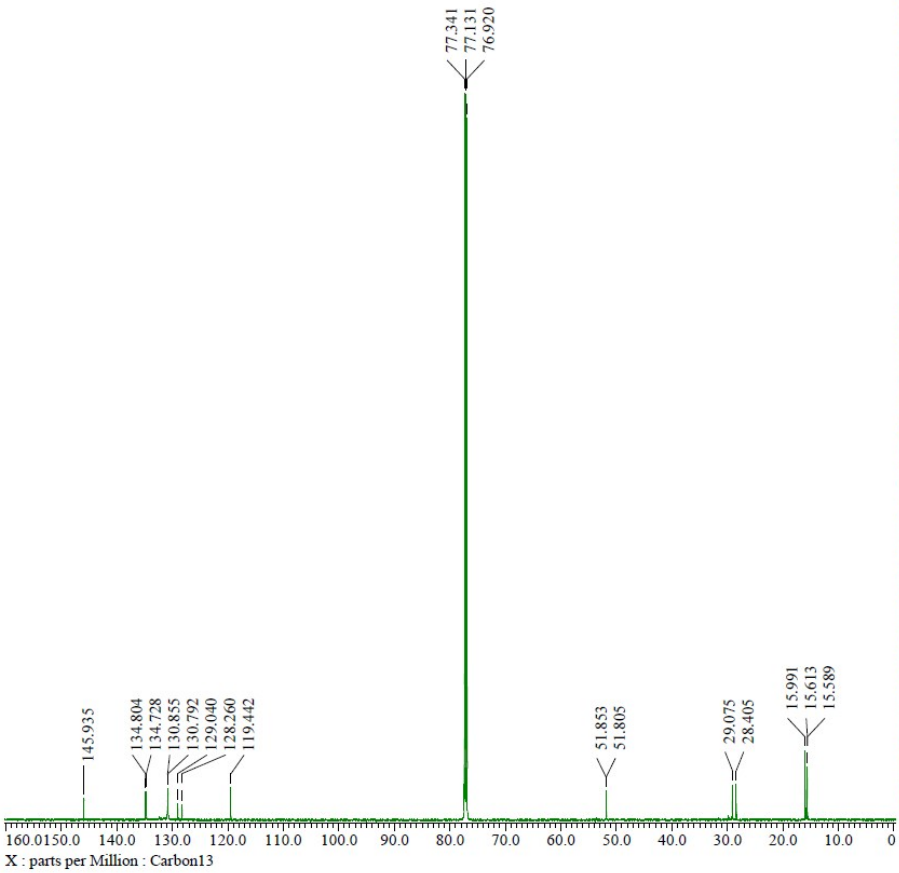


Figure S10. Electrospray ionization-mass spectrum of **2a** in the positive mode (top) and high-resolution mass spectrum (bottom).

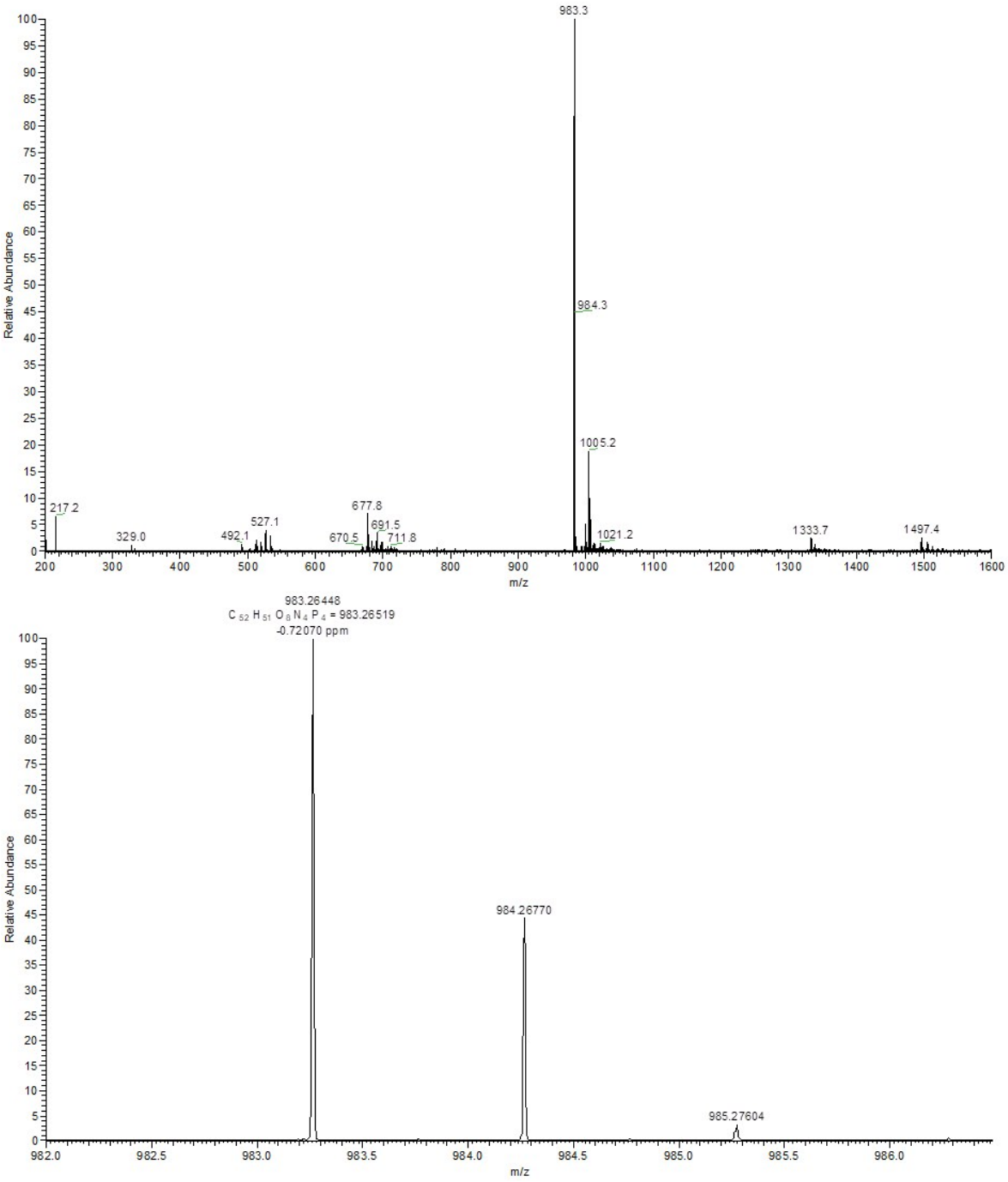


Figure S11. Electrospray ionization-mass spectrum of **2b** in the positive mode (top) and high-resolution mass spectrum (bottom).

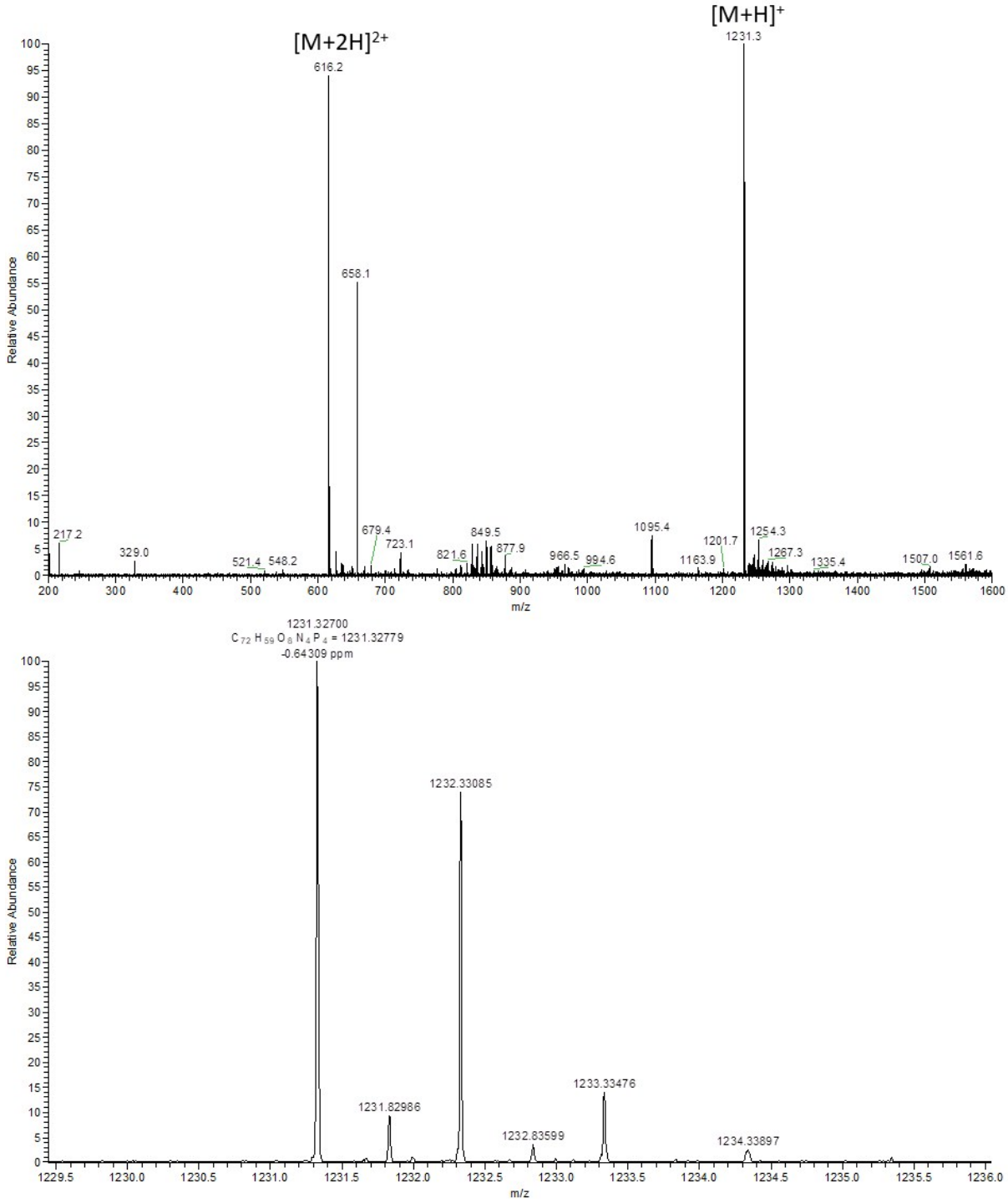


Figure S12. Electrospray ionization-mass spectrum of **2c** in the positive mode (top) and high-resolution mass spectrum (bottom).

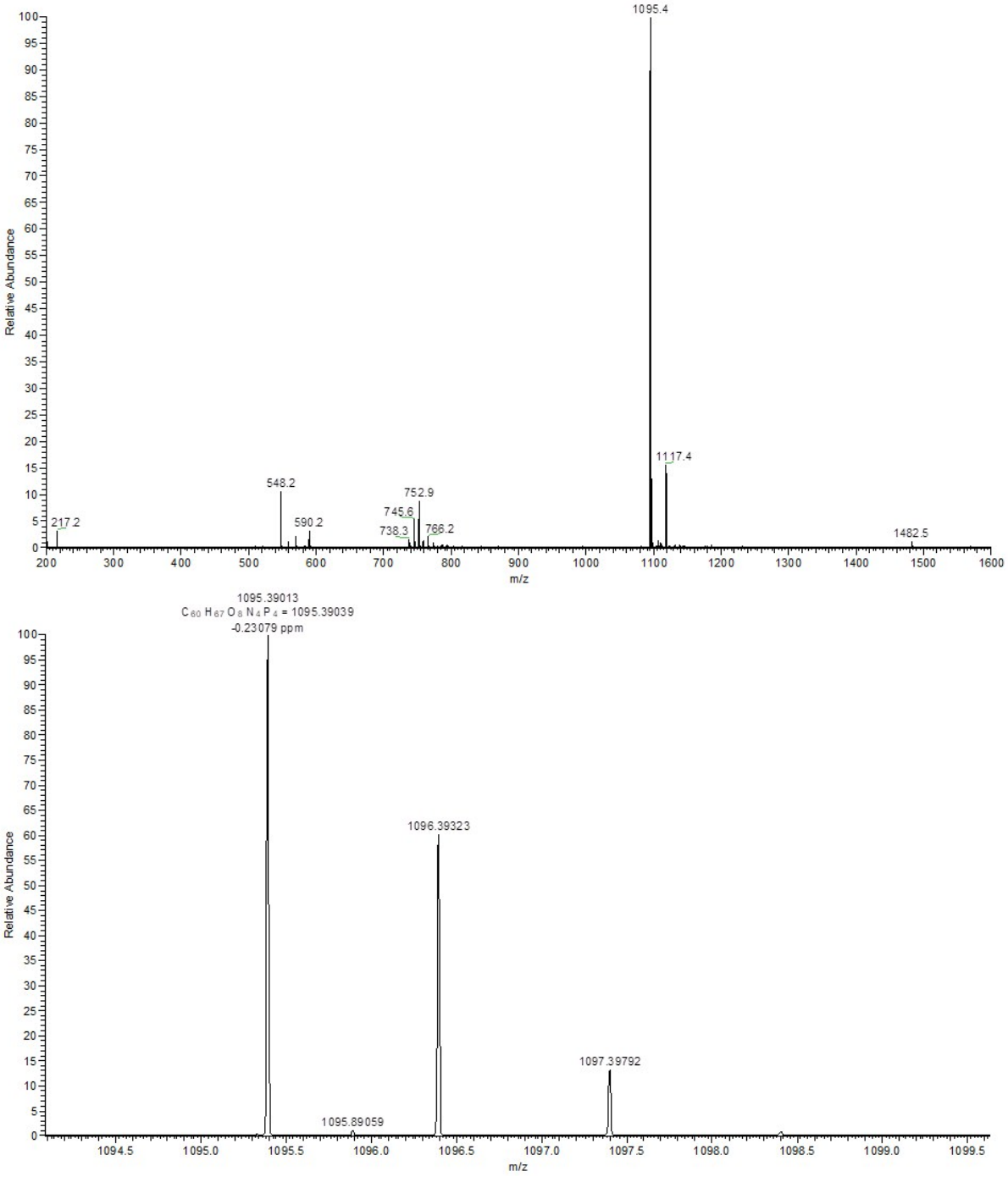


Figure S13. Electrospray ionization-mass spectrum of **3a** in the positive mode (top) and high-resolution mass spectrum (bottom).

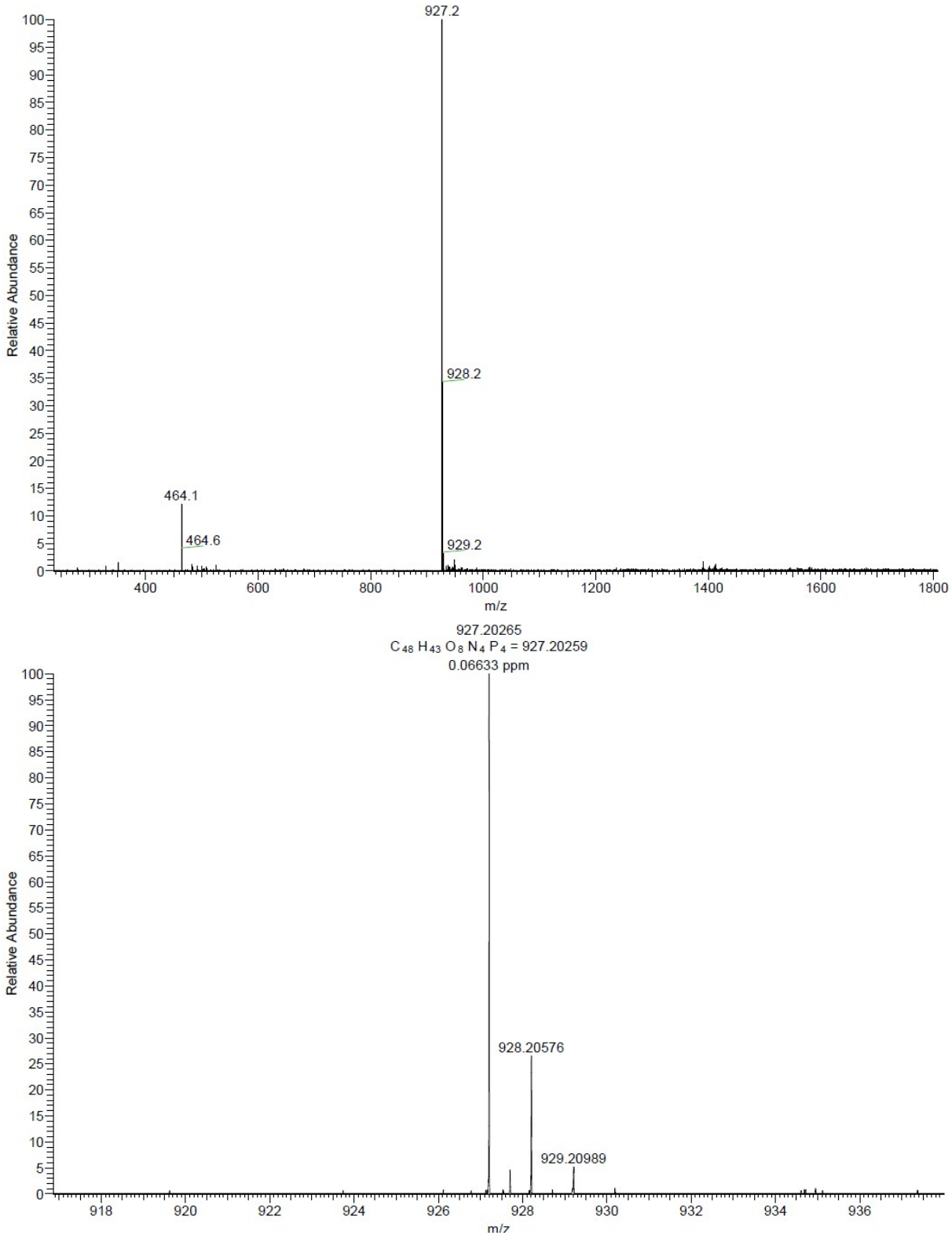


Figure S14. Electrospray ionization-mass spectrum of **3b** in the positive mode (top) and high-resolution mass spectrum (bottom).

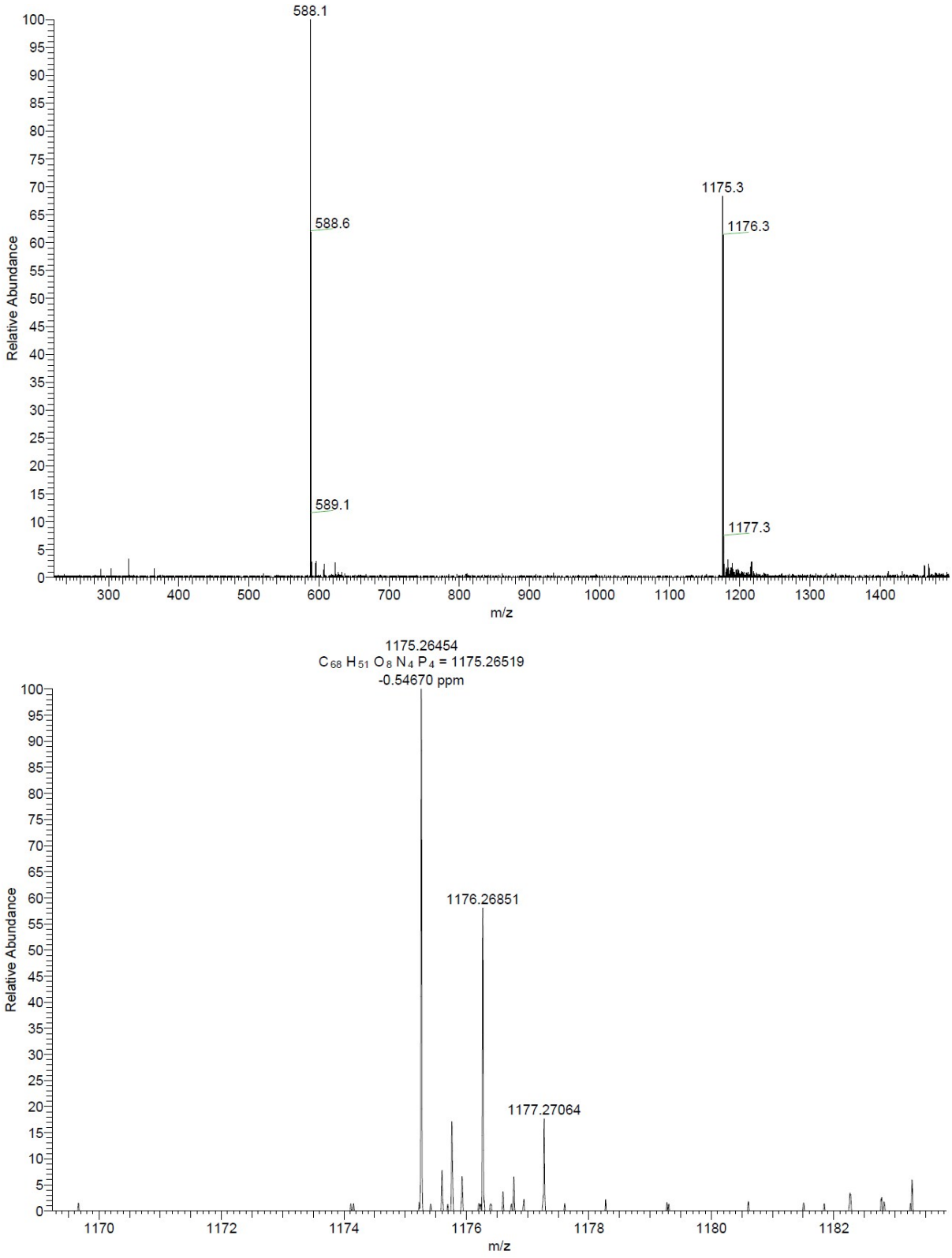


Figure S15. Electrospray ionization-mass spectrum of **3c** in the positive mode (top) and high-resolution mass spectrum (bottom).

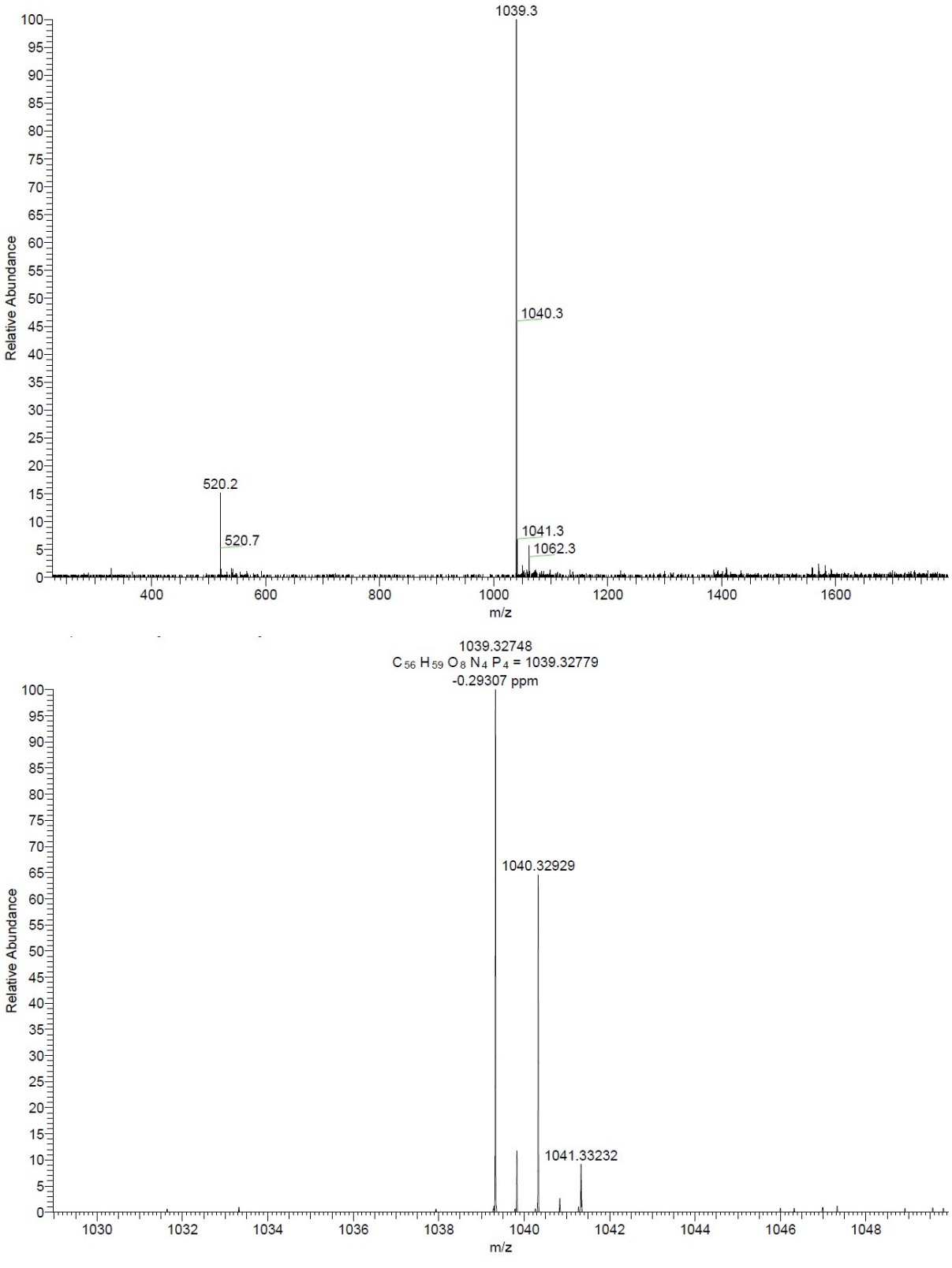


Figure S16. Absorption spectra of **3a**, **3b** and **3c** in PBS.

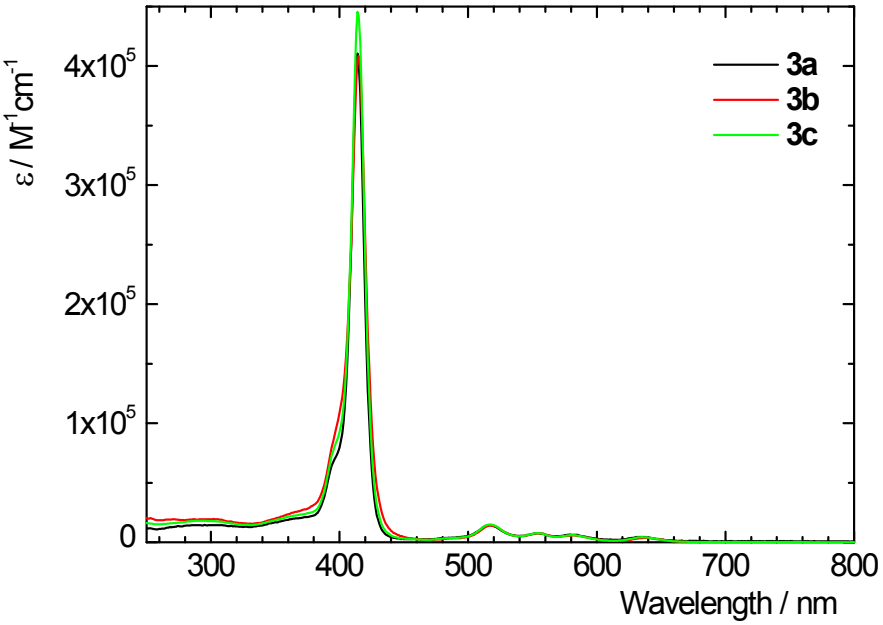



Figure S17. Titration of TPPS and **3a-c** with HSA in PBS: A) Changes of the Soret bands of porphyrins after addition of HSA; B) Difference absorption spectra; C) Corresponding binding isotherms at 422 nm. Arrows indicate absorption changes after the addition of HSA.

In a simple binding equilibrium assuming 1:1 stoichiometry, the relationship between the observed absorption changes and experimental parameters is as follows:

$$\Delta A = \frac{\Delta A_{max} K_b\,[HSA]}{1 + K_b[HSA]},$$

where $\Delta A$ is the absorbance change after the addition of HSA at a selected wavelength, $\Delta A_{\max}$ is the maximum absorbance change, i.e., all porphyrin molecules are bound to HSA, $K_b$ is the binding constant, and [HSA] is the equilibrium molar concentration of free HSA given by the equation:

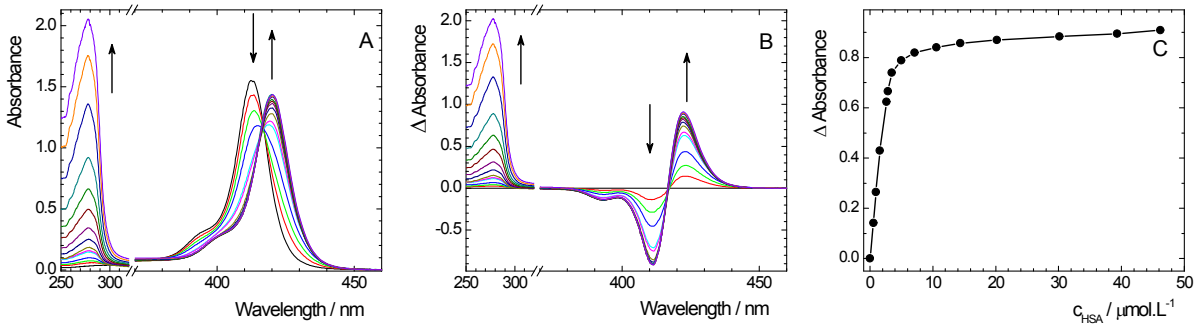
$$[HSA] = (c_{HSA}K_b - c_{porph}\,K_b - 1)/2K_b + \sqrt{(c_{porph}\,K_b - c_{HSA}K_b + 1)^2 + 4\,c_{HSA}K_b}\;/2K_b ,$$

where $c_{HSA}$ is the total HSA concentration and $c_{porph}$ is the total porphyrin concentration.

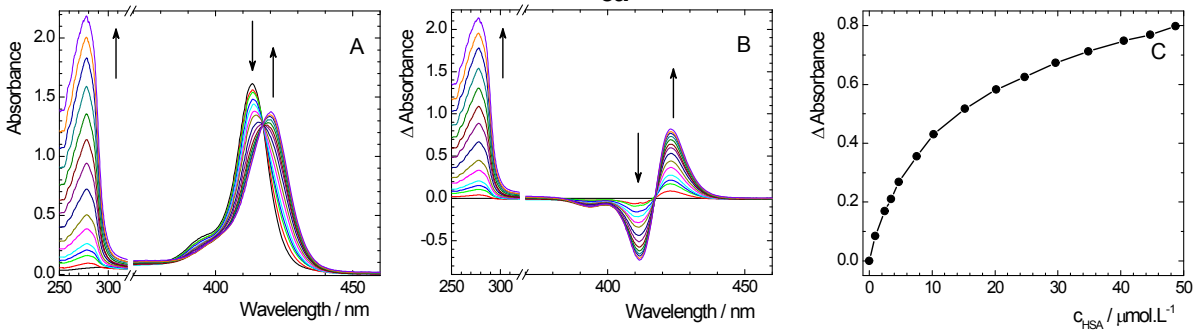
A nonlinear fit to the binding isotherms affords binding constant $K_b$. The estimated error is 15%.

Reference: K. A. Connors, *Binding Constants*, John Wiley & Sons, New York, 1987.

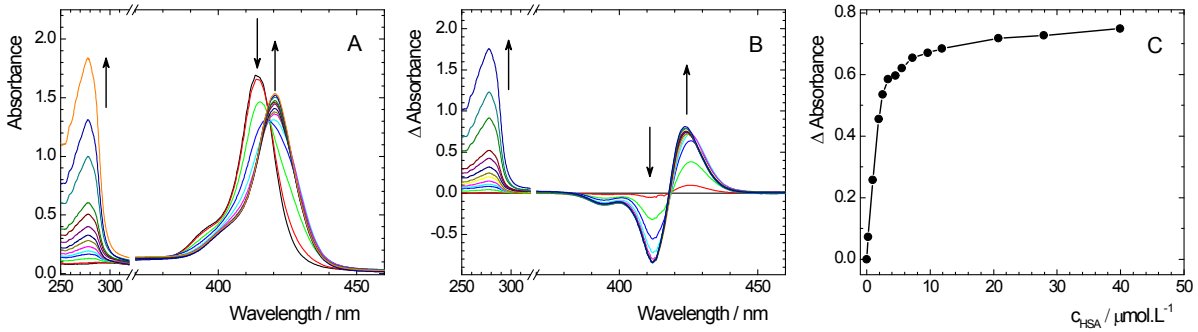
TPPS


A
Absorbance
Wavelength / nm
B
Δ Absorbance
Wavelength / nm
C
Δ Absorbance
c_HSA / μmol.L^-1

**3a**


A
Absorbance
Wavelength / nm
B
Δ Absorbance
Wavelength / nm
C
Δ Absorbance
c_HSA / μmol.L^-1

**3b**


A
Absorbance
Wavelength / nm
B
Δ Absorbance
Wavelength / nm
C
Δ Absorbance
c_HSA / μmol.L^-1

**3c**

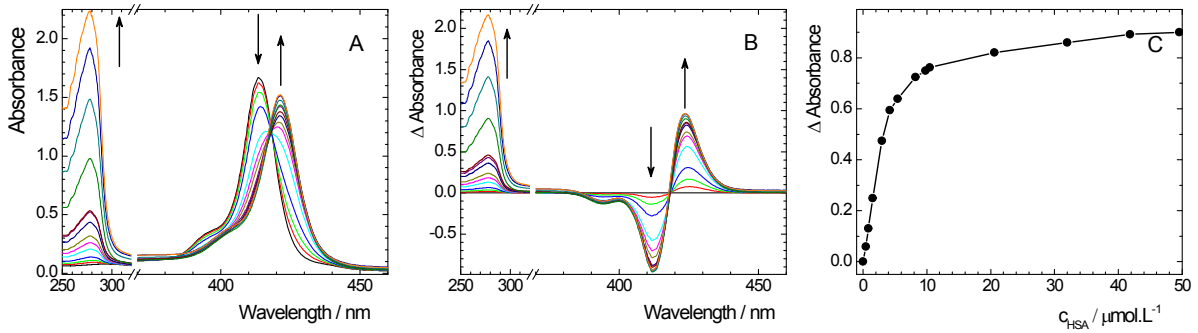

A
Absorbance
Wavelength / nm
B
Δ Absorbance
Wavelength / nm
C
Δ Absorbance
c_HSA / μmol.L^-1

Figure S18. Kinetics of the porphyrins triplet states of **3a-c** monitored by transient absorption at 460 nm in oxygen-, air- and argon-saturated PBS. Excited at 420 nm.

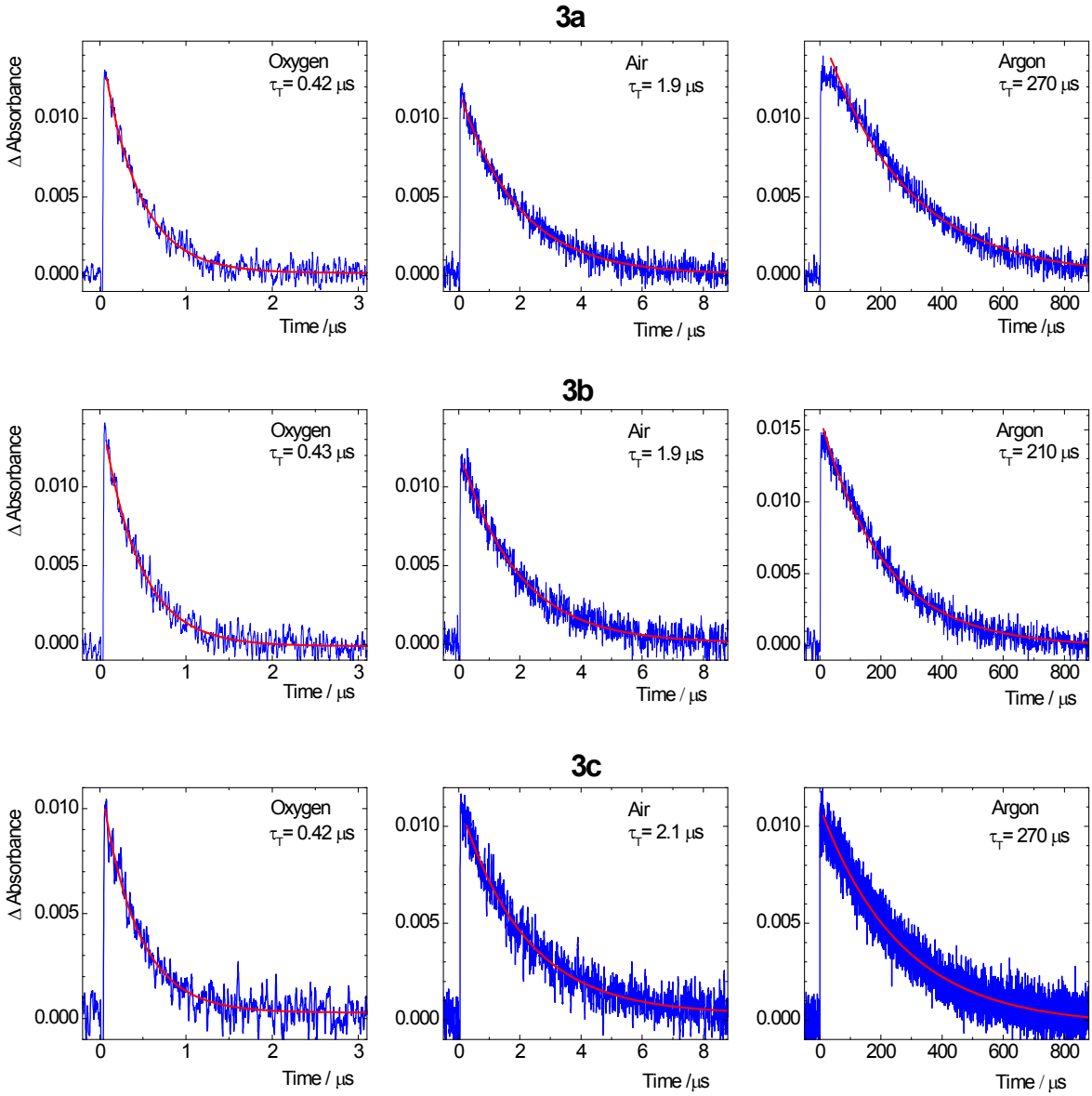


Figure S19. Phosphorescence of $O_2(^1\Delta_g)$ produced by **3a-c** and TPPS in $D_2O$ after excitation at 420 nm (solutions with matched absorbance, A = 0.400). The sharp signal at the beginning of kinetic traces is due to porphyrin short-lived fluorescence (fluorescence lifetime of ~10 ns). Red lines represent single exponential fits to experimental data.

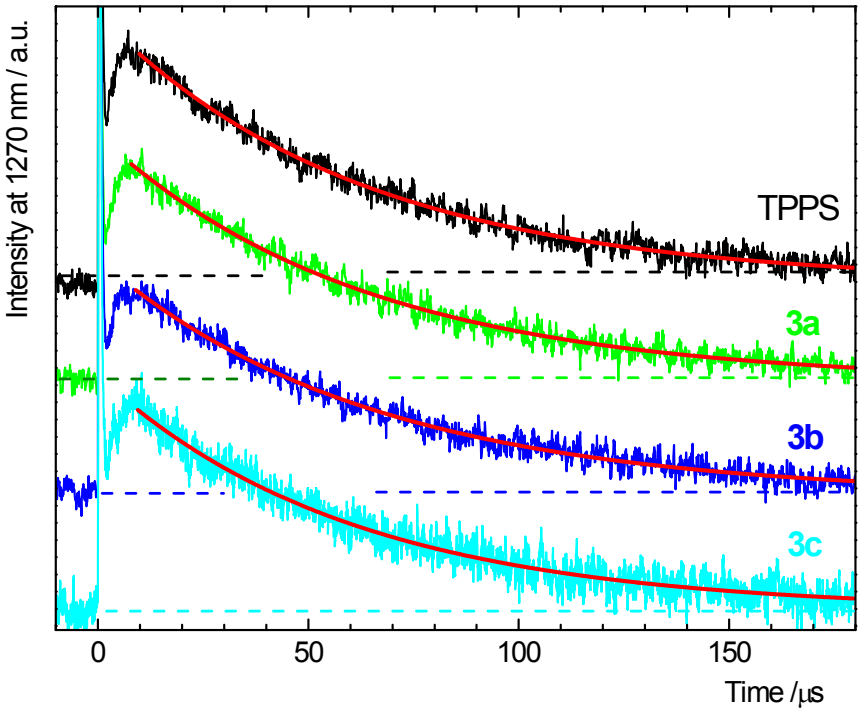
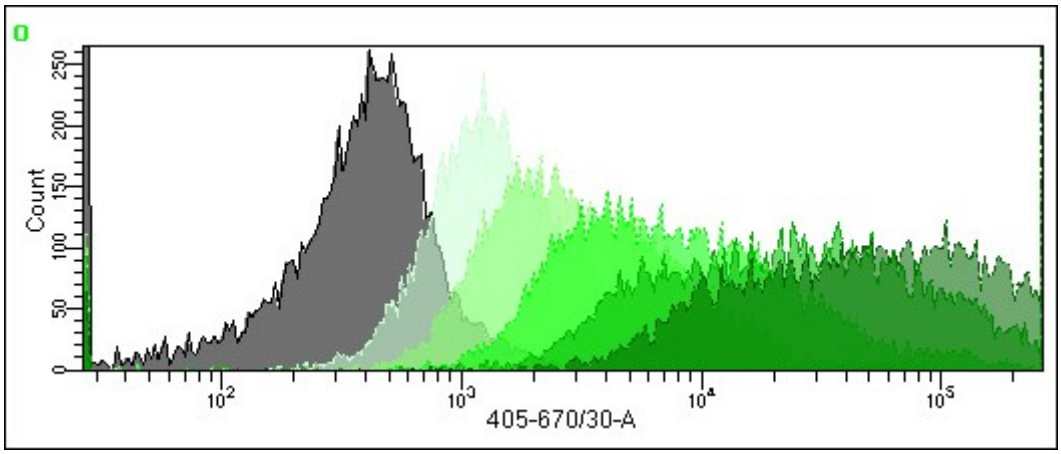
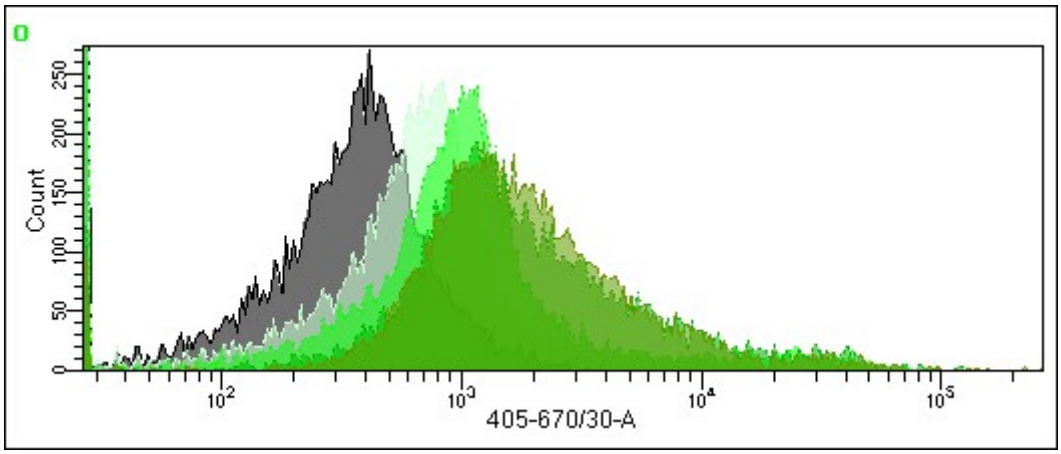


Figure S20. Flow cytometry histograms: (A) HeLa cells incubated with 0.625, 1.25, 2.5, 5 or 10 µM **3c** for 24 h (darkening tones of green); (B) HeLa cells incubated with 0.625µM **3c** for 0, 2, 18 or 24 h (from light green to dark green). (C) HeLa cells incubated with porphyrins **3a** (grey), **3b** (orange), **3c** (green), TPPS (red) for 24 hours (porphyrins concentrations are 1.25 µM). Black color is a control.

A




B




C

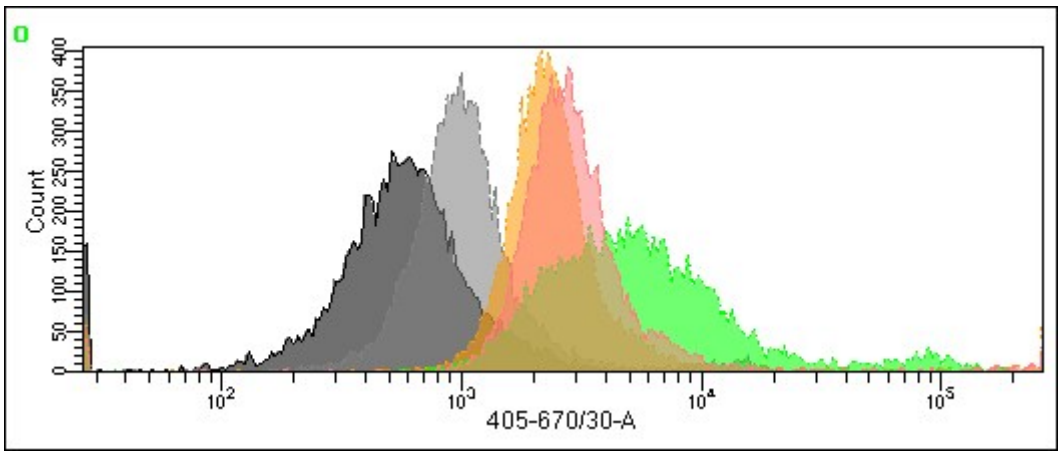


Table S1. $IC_{50}$ values of **3a-c** and TPPS for HeLa and MRC-5 cells irradiated with a 150 W halogen lamp (45 mW $cm^{-2}$) or 525 nm light (9 mW $cm^{-2}$).

| Cell line | Light | Porphyrin | $IC_{50}$/μM | Cell line | Light | Porphyrin | $IC_{50}$/μM |
|---|---|---|---|---|---|---|---|
| HeLa | Halogen lamp | TPPS | 8.82 | MRC-5 | Halogen lamp | TPPS | 1.86 |
| | | TPPC | 6.35 | | | TPPC | 1.67 |
| | | **3c** | 0.63 | | | **3c** | 0.69 |
| | | **3b** | 3.19 | | 525 nm | TPPS | 0.85 |
| | | **3a** | > 10 | | | TPPC | 1.59 |
| | 525 nm | TPPS | 2.50 | | | **3c** | 0.43 |
| | | TPPC | 7.64 | | | | |
| | | **3c** | 0.45 | | | | |
| | | **3b** | 2.51 | | | | |
| | | **3a** | > 10 | | | | |