Boronic Acid: A Bio-Inspired Strategy to Increase the Sensitivity and Selectivity of Fluorescent NADH Probe

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Supporting Information

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1. Supplemental Figures and Tables

Table S1. Photophysical parameters of fluorophores.

Dye	$\lambda_{max\ ex}$ (nm) [a]	ε_{max} (10^4 $cm^{-1}mol^{-1}$) [b]	Φ (10^{-2}) [c]	$\lambda_{max\ em}$ (nm) [d]	Rate constant k (M^{-1} min^{-1}) [j]
BA-Resa	535 [f] /535 [g] /535 [h]	1.22 [f] /1.26 [g] /1.82 [h]	0.36 [f] /0.41 [g] /0.4 [h]	625 [f] /625 [g] /620 [h]	4.63 [f] /54.3 [g] /14.4 [h]
Con-Resa	530 [h]	1.78 [h]	0.40 [h]	615 [h]	3.36 [h]
BA-Resa-P	485 [g]	1.32 [g]	1.6 [g]	575 [g]	n.d. [g]
Resazurin	600 [g]	4.70 [g]	11 [g]	635 [g]	1.33 [g]
RA-Resa	535 [f]	1.2 [f]	0.37 [f]	625 [f]	38.6 [f]

All the photophysical parameters were measured in DMSO/PBS buffer ([f] 1/99, pH 7.4; [g] 1/99 pH 9.5; [h] 50/50, pH 9.5). [a] Maximum absorbance wavelength of fluorophores. [b] The molar extinction coefficients at λ_{max}. [c] Fluorescence quantum yield. Resazurin in PBS buffer (pH 9.5) was chosen as the reference fluorophore.[1] [d] Maximum emission wavelength. [j] The rate constant refer to reported methods.[2]

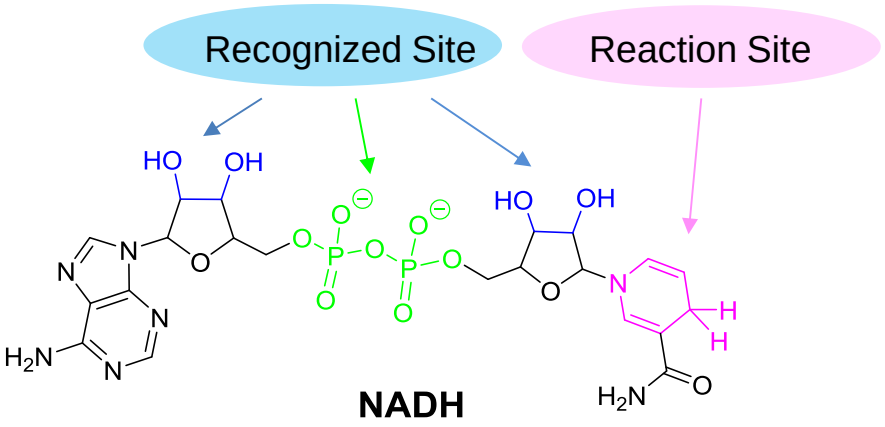
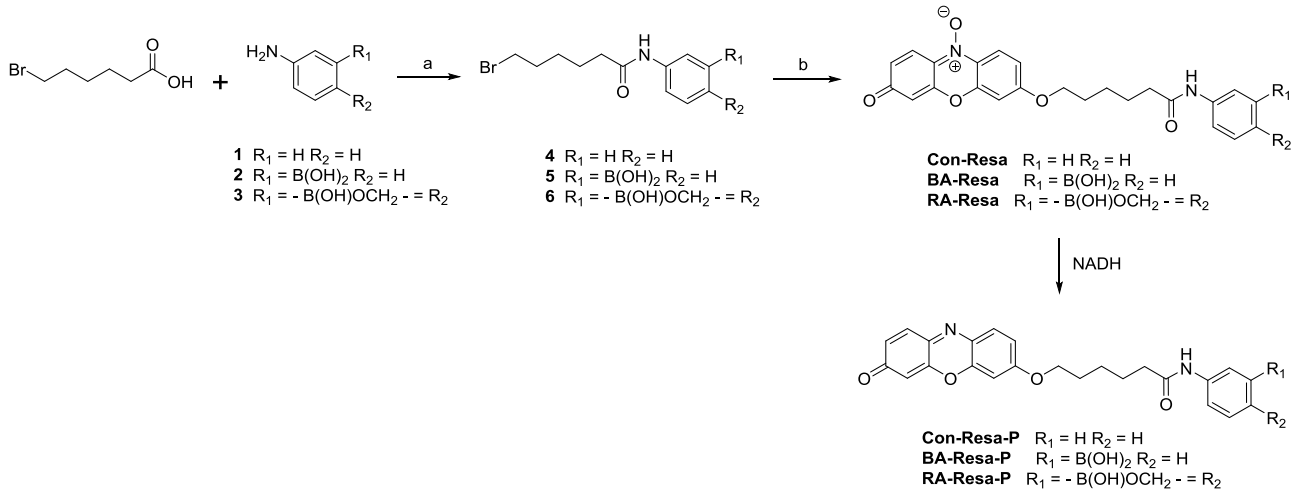


Figure S1. The structure of NADH.



Scheme S1. The synthesis of NADH probe (**BA-Resa** and **RA-Resa**) and control probe (**Con-Resa**). Synthetic conditions: a) EDC, HOBt, DCM, r.t. b) Resazurin, K_2CO_3, DMF, 75 ºC.

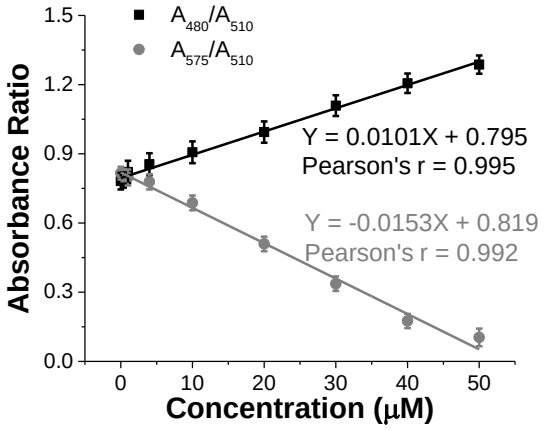


Figure S2. Plot of the UV-Vis absorbance ratio (A_{485}/A_{510} and A_{575}/A_{510}) in the NADH titration. Measurement condition: [**BA-Resa**] = 10 μM, [NADH] = 0 – 50 μM, 10 mM phosphate buffer saline (pH 9.5) solution, DMSO/PBS (1/99), 37 ˚C, and incubation time: 20 min. Results are expressed as mean ± standard deviation of three independent experiments.

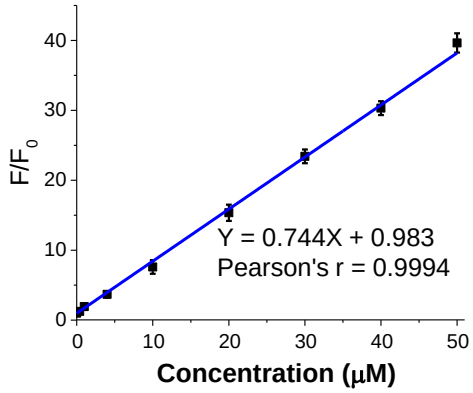


Figure S3. Curve-fitting analysis of the fluorescence intensity at 575 nm in the NADH titration. F/F_0 represents the fluorescence intensity ratio (575 nm) and F_0 is the initial fluorescence intensity (575 nm) of **BA-Resa** in the absence of NADH. Measurement condition: [**BA-Resa**] = 10 μM, [NADH] = 0 – 50 μM, 10 mM phosphate buffer saline (pH 9.5) solution, DMSO/PBS (1/99), λex = 480 nm, 37 ˚C, and incubation time: 20 min. Results are expressed as mean ± standard deviation of three independent experiments.

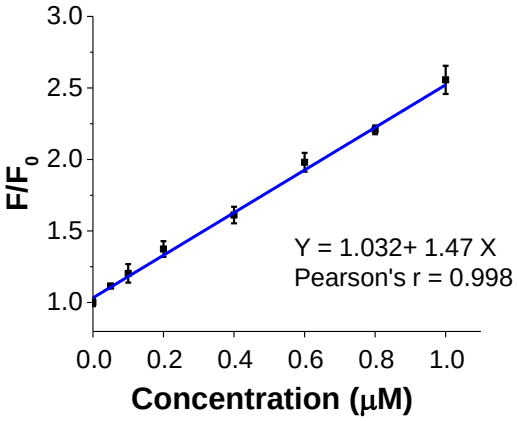


Figure S4. Curve-fitting analysis of the fluorescence intensity at 575 nm in the NADH titration (0 – 1 μM). F/F_0 represents the fluorescence intensity ratio (575 nm) and F_0 is the initial fluorescence intensity (575 nm) of **BA-Resa** in the absence of NADH. Measurement condition: [**BA-Resa**] = 2 μM, 10 mM phosphate buffer saline (pH 9.5) solution, DMSO/PBS (1/99), λex = 480 nm, 37 ˚C, and incubation time: 20 min. Results are expressed as mean ± standard deviation of three independent experiments.

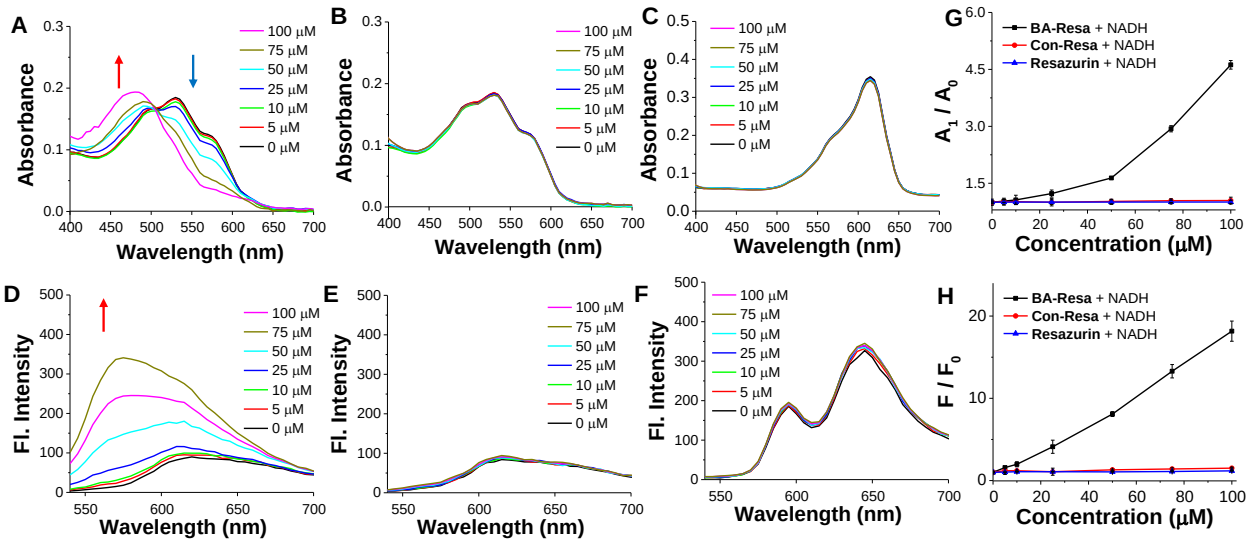


Figure S5. (A - C) UV-Vis absorption and (D - F) fluorescence spectra of **BA-Resa** (A, D), **Con-Resa** (B, E) and **Resazurin** (C, F) in the presence of NADH as a function of concentration (0 – 100 μM). (G,H) Plot of the UV absorbance ratiO (A_1/A_0) and fluorescence intensity ratio (F/F_0) of probe **BA-Resa, Con-Resa**, and **Resazurin** in the NADH titration (0−100 μM). BA-Resa: A_{480}/A_{575}, Resazurin: A575/A610. Measurement condition: [probe] = 10 μM, 10 mM phosphate buffer saline (pH 9.5) solution, DMSO/PBS buffer (v/v, 1/1), 37 ˚C, λ_{ex} = 480 nm, incubation time: 40 min.

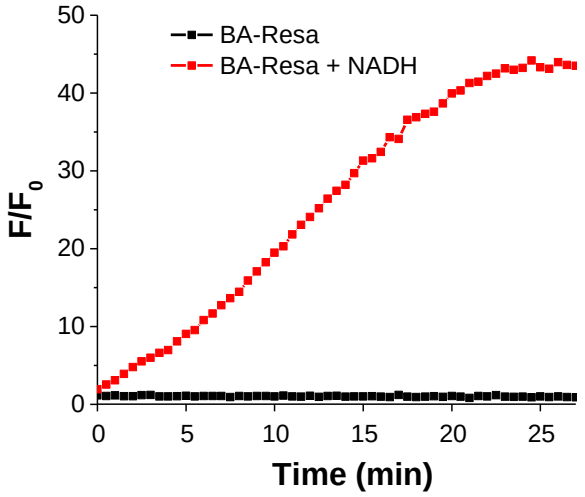


Figure S6. Time dependence of the fluorescence intensity change in the presence/absence of 50 μM NADH. F/F_0 represents the fluorescence intensity ratio (575 nm) and F_0 is the initial fluorescence intensity (575 nm) of **BA-Resa** in the absence of NADH. Measurement condition: [**BA-Resa**] = 10 μM, 10 mM phosphate buffer saline (pH 9.5) solution, DMSO/PBS (1/99), 37 ˚C, λex = 480 nm, time interval: 0.5 min.

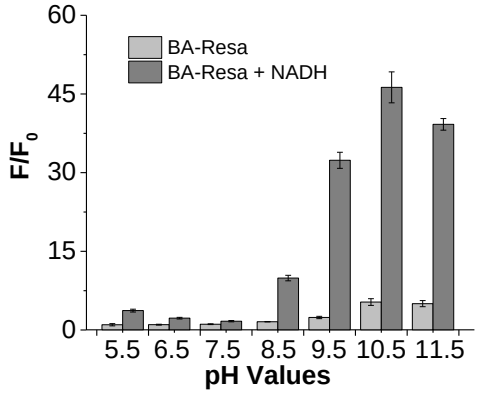


Figure S7. pH Effect to the fluorescent response of **BA-Resa** to NADH (50 μM). F/F_0 represents the fluorescence intensity ratio (575 nm) and F_0 is the initial fluorescence intensity (575 nm) of **BA-Resa** in the absence of NADH at pH 5.5. Measurement condition: [**BA-Resa**] = 10 μM, [NADH] = 50 μM, 10 mM phosphate buffer saline solution, DMSO/PBS (1/99), 37 ˚C, λex = 480 nm, and incubation time: 20 min. Results are expressed as mean ± standard deviation of three independent experiments.

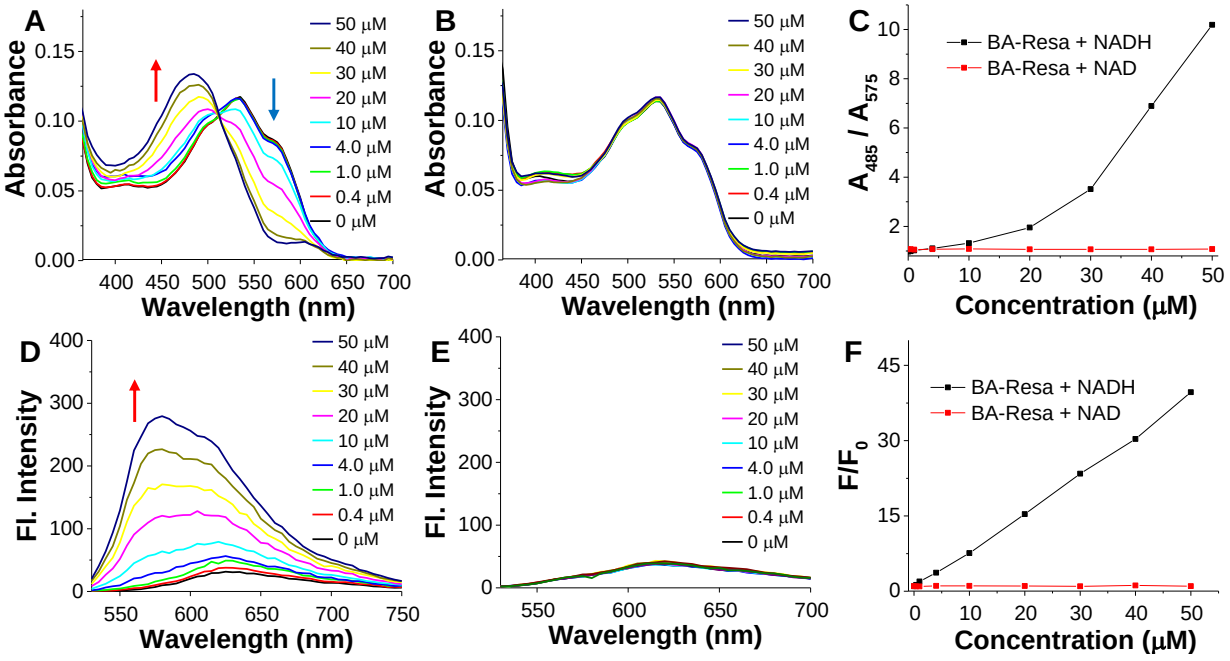


Figure S8. (A,B) UV-Vis absorption and (D,E) fluorescence spectra of **BA-Resa** in the presence of NADH (A,D) and NAD^+ (B,E) as a function of concentration (0 – 50 μM). C,F) Plots of the absorbance ratio (A_{485}/A_{575}) and fluorescence intensity ratio (F/F_0) in the NADH and NAD^+ titration (0 – 50 μM). F/F_0 represents the fluorescence intensity ratio (575 nm) and F_0 is the initial fluorescence intensity (575 nm) of BA-Resa in the absence of NADH or NAD^+. Measurement condition: [**BA-Resa**] = 10 μM, 10 mM phosphate buffer saline (pH 9.5) solution, DMSO/PBS (1/99), 37 ˚C, λ_{ex} = 480 nm, and incubation time: 20 min.

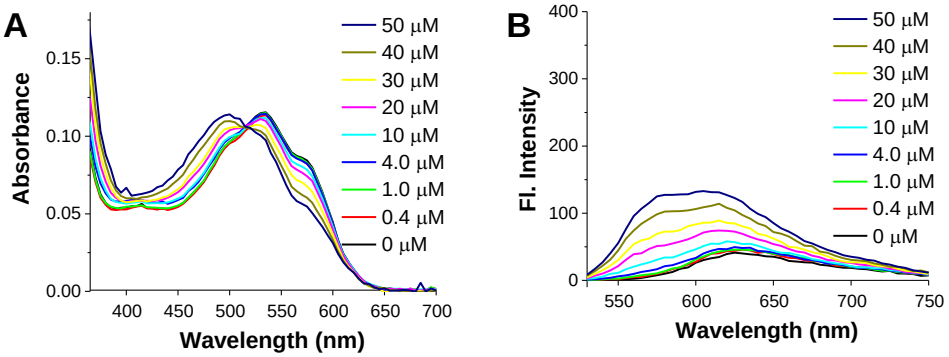


Figure S9. (A) UV absorption and (B) fluorescence spectra of **BA-Resa** in the presence of NADPH as a function of concentration (0 – 50 μM). Measurement condition: [**BA-Resa**] = 10 μM, 10 mM phosphate buffer saline (pH 9.5) solution, DMSO/PBS (1/99), 37 ˚C, λ_{ex} = 480 nm, and incubation time: 20 min.

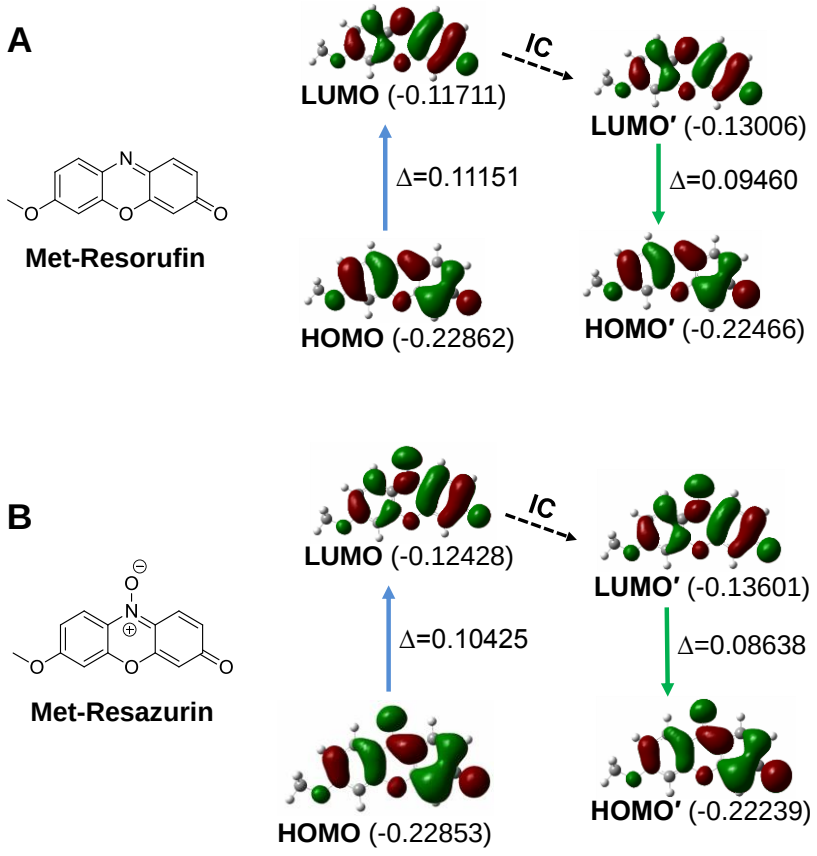


Figure S10. The frontier molecular orbitals (MOs) of **Met-Resorufin** (A) and **Met-Resazurin** (B) involved in the vertical excitation (i.e. Energy level, the left columns) and emission (right columns) shown in solid line. The vertical excitation related calculations were based on the optimized ground state (S_0 state), the emission related calculations were based on the optimized excited state (S_1 state), at the B3LYP/6- 31G (d)/level using Gaussian 09W. The electron distribution at HOMO and LUMO was shown in red and green color. IC stands for internal conversion (dash lines).

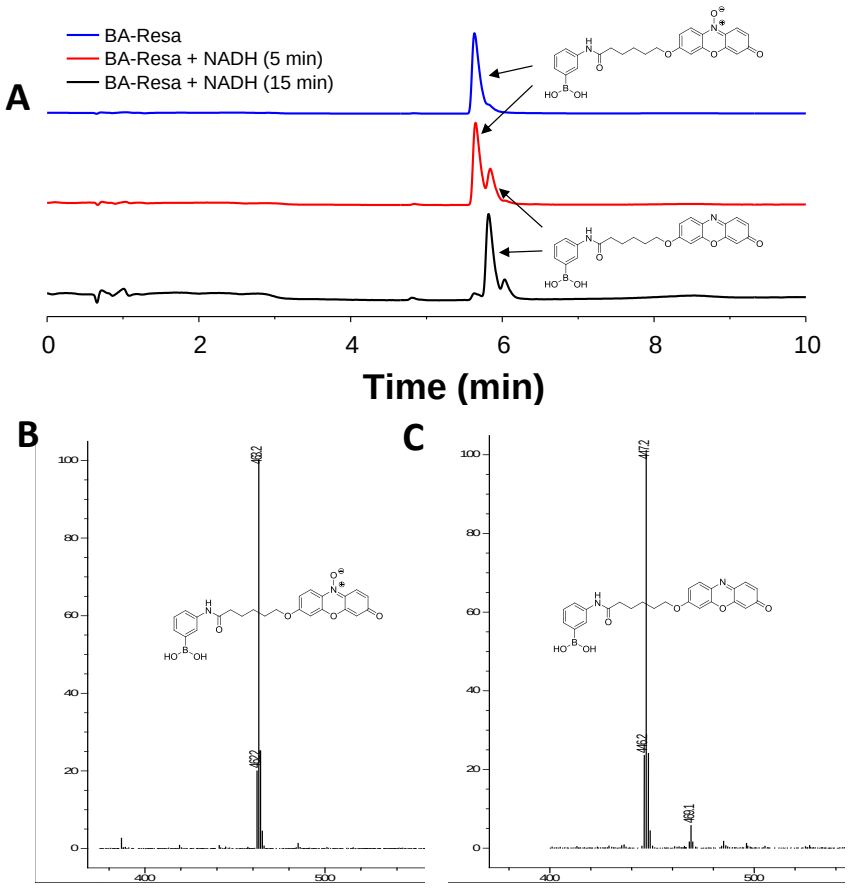


Figure S11. High Performance Liquid Chromatography (HPLC) spectrum indicating the reaction between probe **BA-Resa** and NADH. The absorbance signals was collected at 550 nm. Measurement condition: [**BA-Resa**] = 100 μM, [NADH] = 1 mM, 10 mM phosphate buffer saline (pH 9.5) solution, DMSO/PBS (1/99), 37 ˚C. Mobile phase condition (water/acetonitrile): 0 min, 95% water; 1 min, 90% water; 8 min, 5% water; 8.5 min, 5% water; 9 min, 95% water; 10 min, 95% water. Mobile phase contains 0.1% formic acid. B,C) the mass spectra of BA-Resa and **BA-Resa-P**. The boronic acid in the probe can work as catalyst to accelerate the reduction in sensing process rather than a binding group changing fluorescent intensity (Fig. 2-4). Specifically, once the boronic acid of probe binds with NADH, the spatial distance between them is significantly reduced, then the sensing process could be accelerated and achieved in a short time. Meanwhile, the reversible and fast reaction between boronic acid and diols of NADH promote the continuous sensing process (rate constant of esterification: 102-103 $M^{-1}s^{-1}$, Inorg. Chem. 1992, 31, 3243).

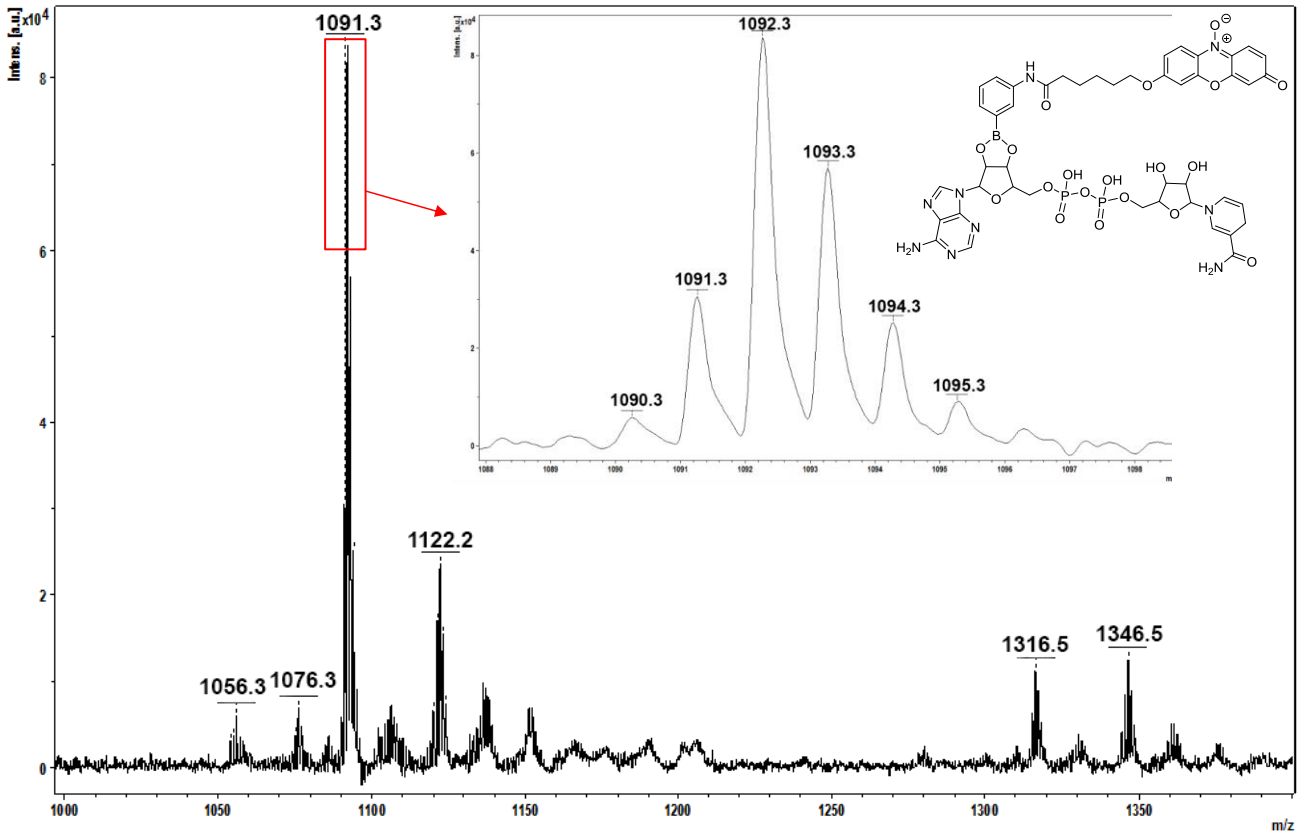


Figure S12. MALDI-TOF mass spectra of probe **BA-Resa** binding with NADH. Positive signals of the product of **BA-Resa** and NADH were indicated by peaks 1090.3 – 1095.3 (the top right enlarged figure). Probe and NADH were dissolved in methanol/water (1/1) mixture.

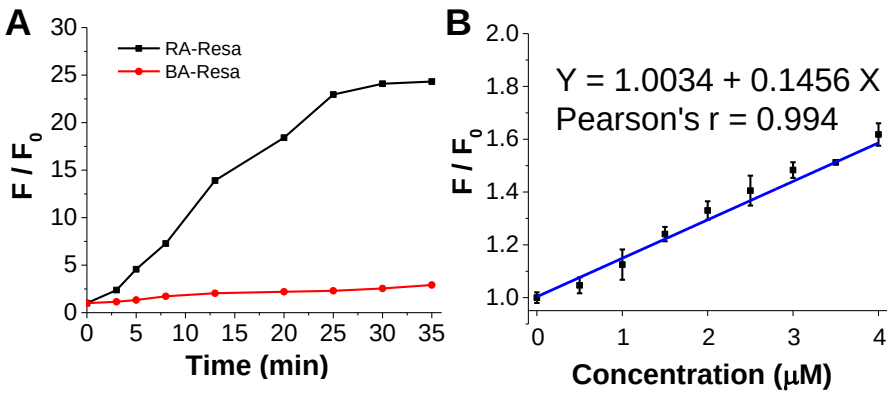


Figure S13. A) Time dependence of the fluorescence intensity change of **BA-Resa** and **RA-Resa** in the presence of 100 μM NADH at physiological pH. Measurement condition: [**BA-Resa**] = [**RA-Resa**] = 10 μM, 10 mM phosphate buffer saline (pH 7.4) solution, DMSO/PBS (1/99), λex = 480 nm, 37 ˚C. B) Curve-fitting analysis of

the fluorescence intensity at 575 nm in the NADH titration (0 – 4 μM). F/F_0 represents the fluorescence intensity ratio (575 nm) and F_0 is the initial fluorescence intensity (575 nm) of **RA-Resa** in the absence of NADH. Measurement condition: [**RA-Resa**] = 2 μM, 10 mM phosphate buffer saline (pH 7.4) solution, DMSO/PBS (1/99), λex = 480 nm, 37 ˚C, and incubation time: 20 min. Results are expressed as mean ± standard deviation of three independent experiments.

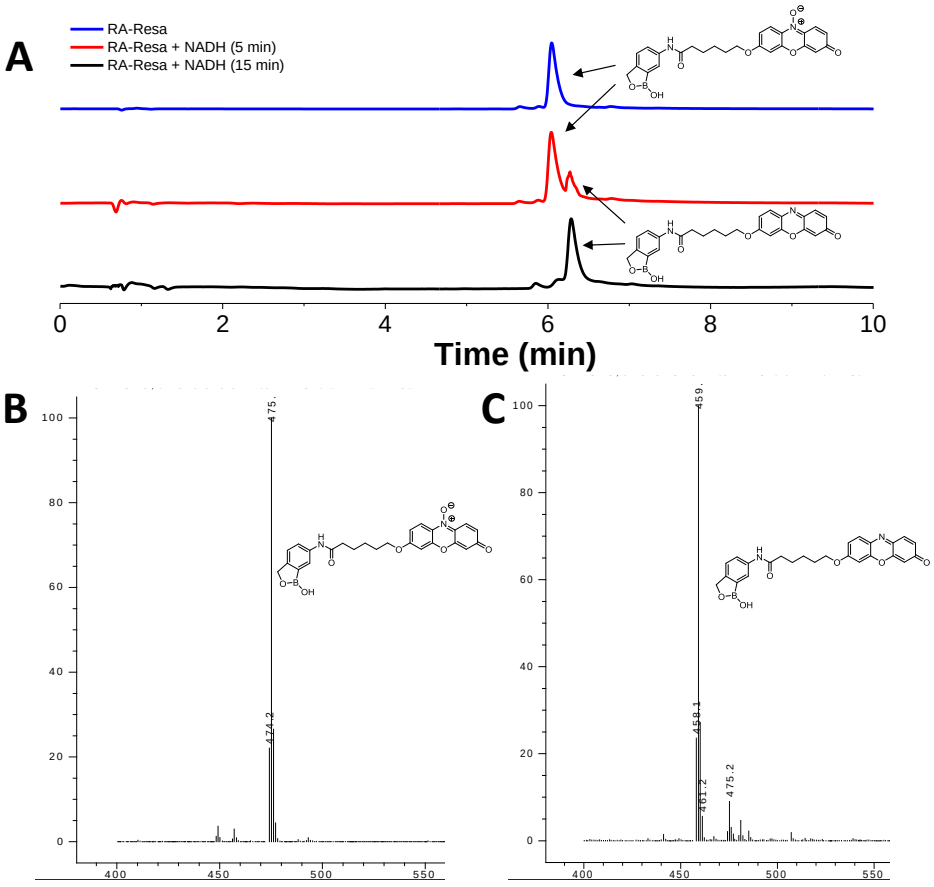


Figure S14. High Performance Liquid Chromatography (HPLC) spectrum indicating the reaction between probe **BA-Resa** and NADH. The absorbance signals was collected at 550 nm. Measurement condition: [**RA-Resa**] = 100 μM, [NADH] = 1 mM, 10 mM phosphate buffer saline (pH 7.4) solution, DMSO/PBS (1/99), 37 ˚C. Mobile phase condition (water/acetonitrile): 0 min, 95% water; 1 min, 90% water; 8 min, 5% water; 8.5 min, 5% water; 9 min, 95% water; 10 min, 95% water. Mobile phase contains 0.1% formic acid. B,C) the mass spectra of **RA-Resa** and **RA-Resa-P**.

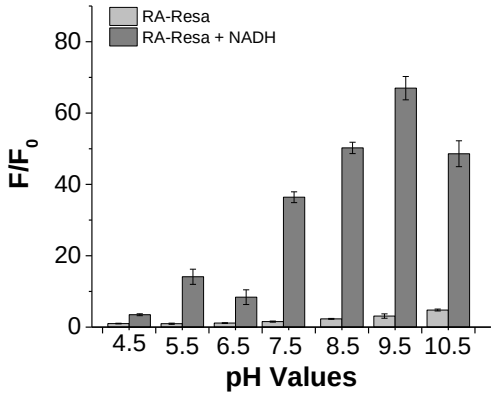


Figure S15. pH Effect on the fluorescence response of **RA-Resa** to NADH (100 μM). F/F_0 represents the fluorescence intensity ratio (575 nm) and F_0 is the initial fluorescence intensity (575 nm) of **RA-Resa** in the absence of NADH at pH 4.5. Measurement condition: [**RA-Resa**] = 10 μM, [NADH] = 100 μM, 10 mM phosphate buffer saline solution, DMSO/PBS (1/99), λex = 480 nm, 37 ˚C, and incubation time: 15 min. Results are expressed as mean ± standard deviation of three independent experiments.

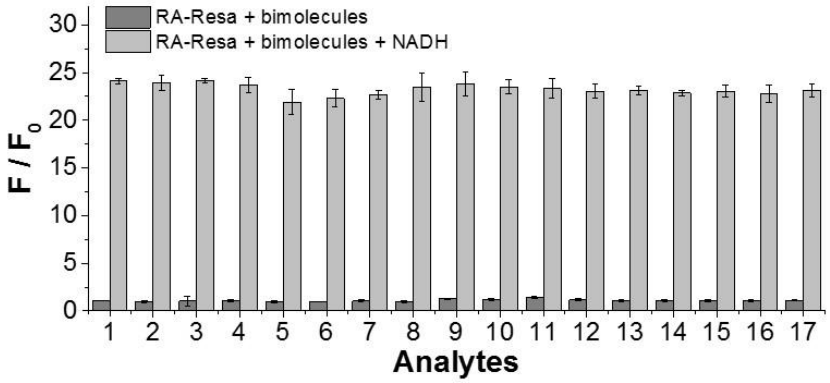


Figure S16. Fluorescence response of **RA-Resa** to NADH in the presence of various biomolecules. Analytes (2-7, 1 mM; 8-17, 100 μM): 1) buffer, 2) Ca^{2+}, 3) K^{+}, 4) Na^{+}, 5) Fe^{3+}, 6) Zn^{2+}, 7) Mg^{2+}, 8) Tyrosine, 9) Cysteine, 10) Methionine, 11) GSH, 12) Glucose, 13) Ribose, 14) Fructose, 15) ATP, 16) ADP, 17) NAD^{+}. F/F_0 represents the fluorescence intensity ratio and F_0 is the initial fluorescence intensity at 575 nm of **RA-Resa** in the absence of NADH. Measurement condition: [**RA-Resa**] = 10 μM, 10 mM phosphate buffer saline (pH 9.5) solution, λex = 480 nm. 37 ˚C, and incubation time: 20 min. Results are expressed as mean ± standard deviation of three independent experiments.

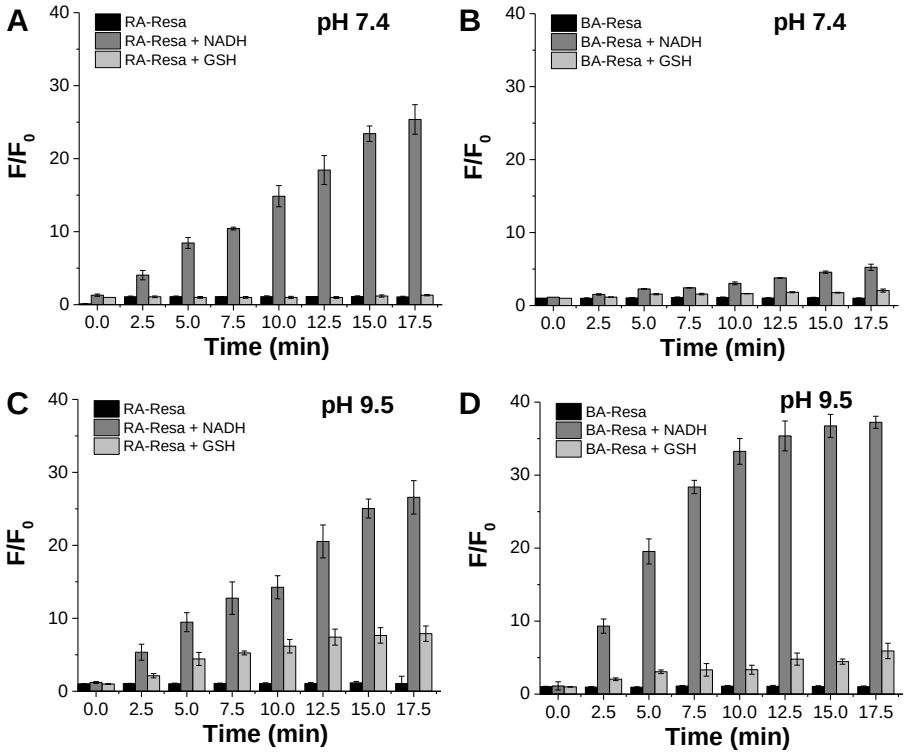


Figure S17. Time dependence of the fluorescence intensity change of **RA-Resa** (A,C) and **BA-Resa** (B,D) in the presence of NADH and GSH at pH 7.4 (A,B) or pH 9.5 (C,D) buffer solution respectively. F/F_0 represents the fluorescence intensity ratio (575 nm) and F_0 is the initial fluorescence intensity (575 nm) of **RA-Resa** or **BA-Resa** in the absence of NADH. Measurement condition: [**BA-Resa**] = [**RA-Resa**] = 10 μM, [NADH] = [GSH] = 100 μM, 10 mM phosphate buffer saline (pH 7.4 and 9.5) solution, DMSO/PBS (1/99), λex = 480 nm, 37 ˚C. Results are expressed as mean ± standard deviation of three independent experiments.

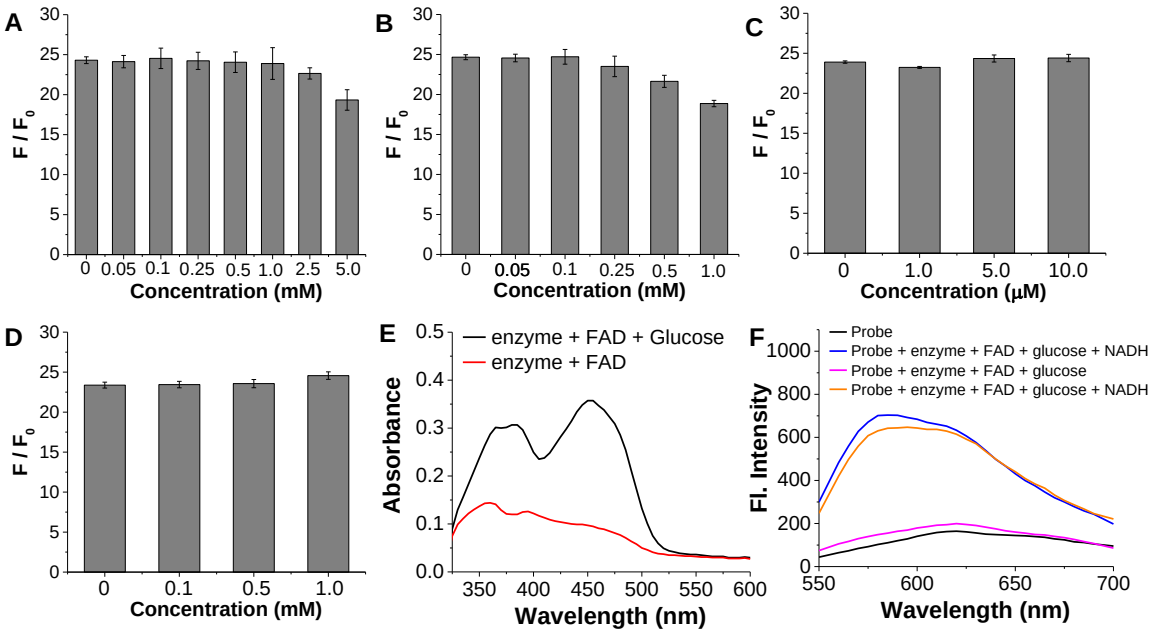


Figure S18. Fluorescence response of **RA-Resa** to NADH in the presence of Ribose (A), ATP (B), H_2O_2 (C), Tetrasodium pyrophosphate (D), and $FADH_2$ (E,F). E) The absorption spectrum of $FADH_2$ produced in the glucose oxidase enzyme system after incubation for 1 min. F) The fluorescence spectra of probe responding to NADH (50 μM) and $FADH_2$. Incubation time: 20 min F/F_0 represents the fluorescence intensity ratio (575 nm) and F_0 is the initial fluorescence intensity (575 nm) of **RA-Resa** in the absence of NADH. Measurement condition: [**RA-Resa**] = 10 μM, [NADH] = 100 μM, [Ribose] = 0.05-5.0 mM, [ATP] = 0.05-1.0 mM, [H_2O_2] = 1-10 μM, [Tetrasodium pyrophosphate] = 0.1-1.0 mM, [glucose oxidase] = 48 U/100 μL, [glucose] = 0.5 mM, [FAD+$FADH_2$] = 50 μM, 10 mM phosphate buffer saline (pH 7.4) solution, DMSO/PBS (1/99), λex = 480 nm, 37 ˚C, and incubation time: 30 min. Results are expressed as mean ± standard deviation of three independent experiments.

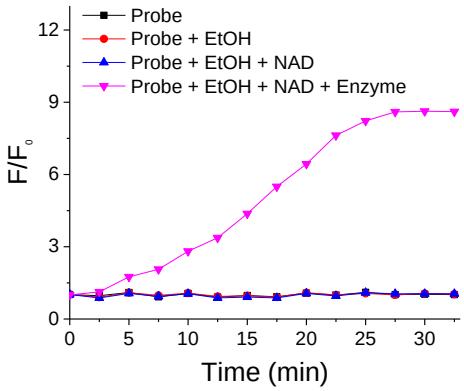
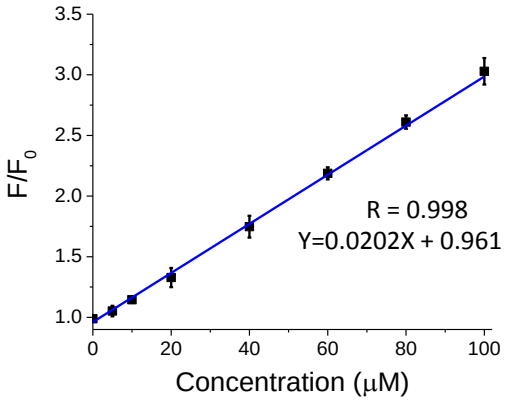


Figure S19. Plots of fluorescent intensity of **RA-Resa** in the absence/presence of alcohol dehydrogenase at physiological pH. F/F_0 represents the fluorescence intensity ratio and F_0 is the initial fluorescence intensity at 575 nm of **RA-Resa** in the absence of enzyme. Measurement condition: [Probes] = 10 μM, [EtOH] = 10 mM, [NAD^+] = 50 μM, 10 mM phosphate buffer saline (pH 7.4) solution, DMSO/PBS (1/99), λex = 480 nm, 37 ˚C.



	sample 1	sample 2	sample 3	sample 4
Y values	1.59673	1.62436	1.65713	1.57927
concentraion	31.47178	32.8396	34.46188	30.60743

5.12 mg ±0.25 close to the labeled 5 mg

Figure S20. The linearity of fluorescence intensity versus concentration in the NADH quantification of health care products. 11.2 mg was obtained from the 202 mg NADH tablet and dissolved in PBS (pH 7.4). The experiments were repeated four times. F/F_0 represents the fluorescence intensity ratio (575 nm) and F_0 is the initial fluorescence intensity (575 nm) of **RA-Resa** in the absence of NADH. Measurement condition: [Probes] = 10 μM, 10 mM phosphate buffer saline (pH 7.4) solution, DMSO/PBS (1/99), λex = 480 nm. 37 ˚C, incubation time: 5 min. Results are expressed as mean ± standard deviation of three independent experiments.

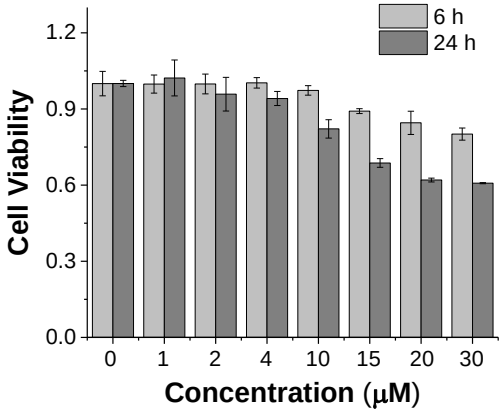


Figure S21. Cytotoxic effect of **RA-Resa**. OSCC cells were treated with each concentration of probe for 6 and 24 h. MTT assay was used to quantify the cell viability. Results are expressed as mean ± standard deviation of three independent experiments.

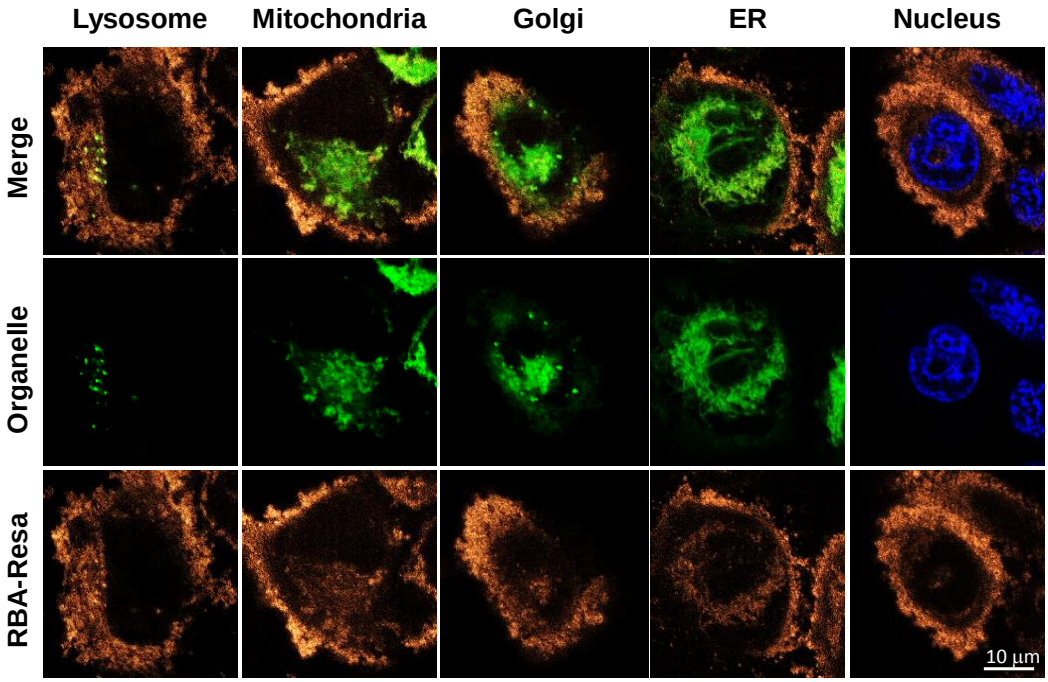


Figure 22. Confocal microscopic images of probe **RA-Resa** (5 μM, 30 min) and organelle markers stained live OSCC cells. Nucleus, Hoechst33342 (1ug/ml, 30 min), Mitochondria, MitoTracker (100 nM, 30 min), Lysosome LysoTracker (50 nM, 30 min), Golgi, BODIPY FL C5 ceramide (1 uM, 60 min under 4 °C), ER, ER-Tracker (100 nM, 30 min). Scale bar: 10 μm.

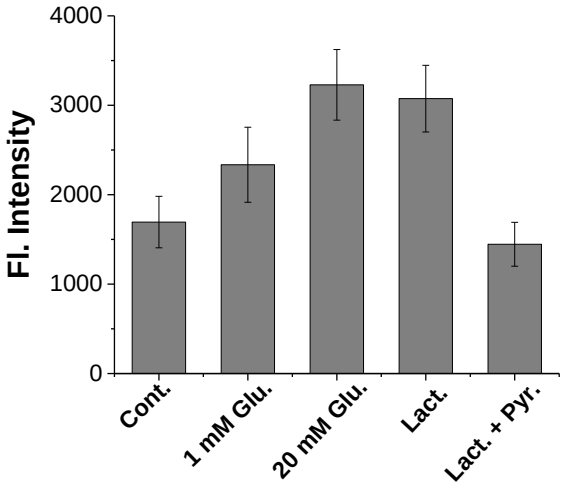


Figure S23. Quantification of fluorescence intensities of **RA-Resa** in live OSCC cells. After incubated with glucose (1 mM or 20 mM, 0.5 h) or lactate (10 mM, 10 min) or lactate (10 mM, 10 min) and pyruvate (10 mM, 10 min), the live OSCC cells were further treated with **RA-Resa** (5 μM) for another 15 min prior to imaging. In control group, the live OSCC cells were treated with **RA-Resa** (5 μM) for 15 min in starvation state (0.5 h). Error bars are standard deviation (SD). Each data point was obtained from ROIs (n = 5) inside the cells, and the experiments were repeated for 5 times.

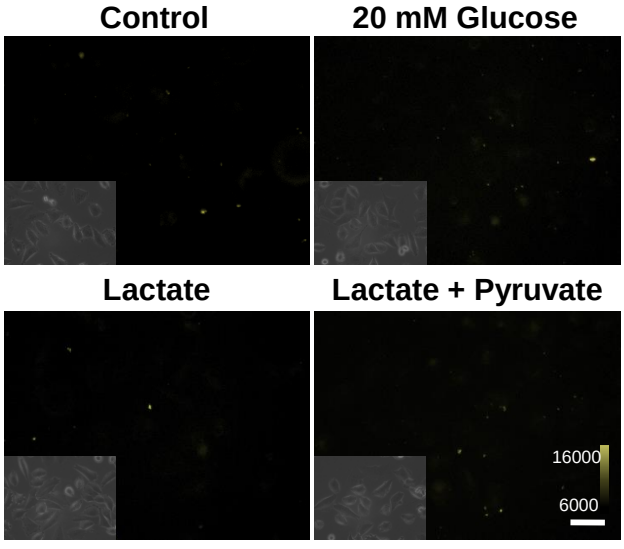


Figure S24. Microscopic images of probe **Con-Resa** (5 μM, 15 min)-stained live OSCC cells after incubation in following conditions. A) no glucose for 30 min; B) 20 mM glucose for 30 min; C) 10 mM lactate for 10 min; D) 10 mM lactate and 5 mM pyruvate for 10 min. Scale bar: 50 μm.

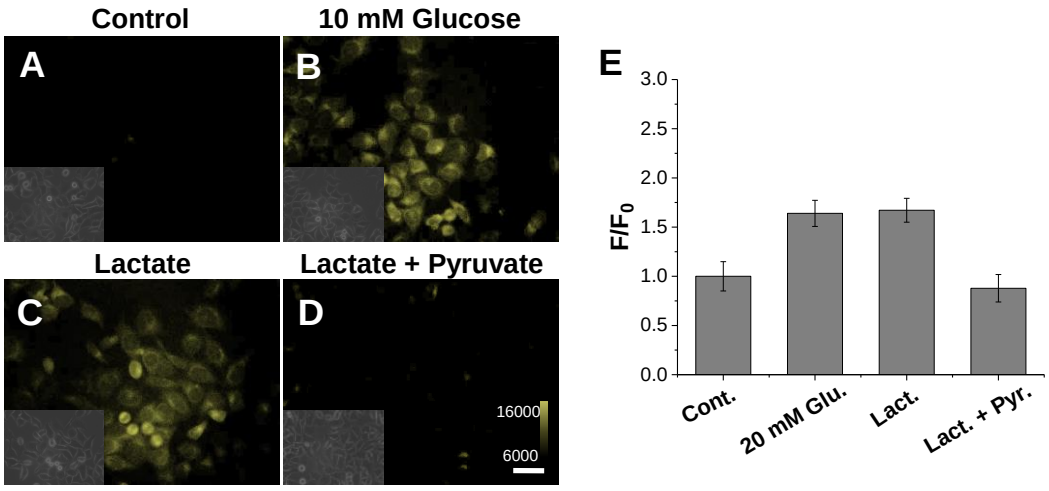


Figure S25. Microscopic images of probe **BA-Resa** (5 μM, 15 min)-stained live Hela cells after incubation in following conditions. A) no glucose for 30 min; B) 20 mM glucose for 30 min; C) 10 mM lactate for 10 min; D) 10 mM lactate and 5 mM pyruvate for 10 min. Scale bar: 50 μm. E) Quantification of fluorescence intensities of RA-Resa in live OSCC cells. Error bars are standard deviation (SD). Each data point was obtained from ROIs (n = 5) inside the cells, and the experiments were repeated for 3 times.

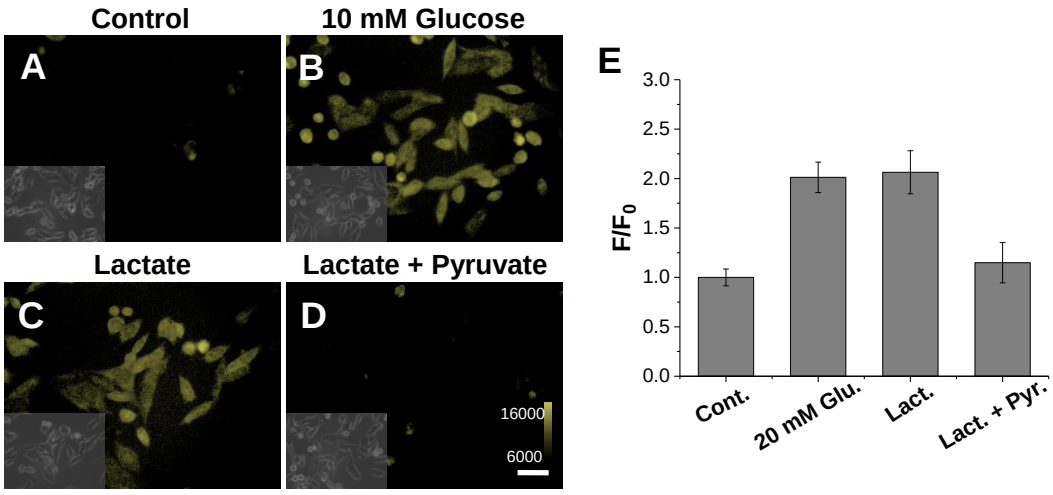


Figure S26. Microscopic images of probe **RA-Resa** (5 μM, 15 min)-stained live CHO cells after incubation in following conditions. A) no glucose for 30 min; B) 20 mM glucose for 30 min; C) 10 mM lactate for 10 min; D) 10 mM lactate and 5 mM pyruvate for 10 min. Scale bar: 50 μm. E) Quantification of fluorescence intensities of RA-Resa in live OSCC cells. Error bars are standard deviation (SD). Each data point was obtained from ROIs (n = 5) inside the cells, and the experiments were repeated for 3 times.

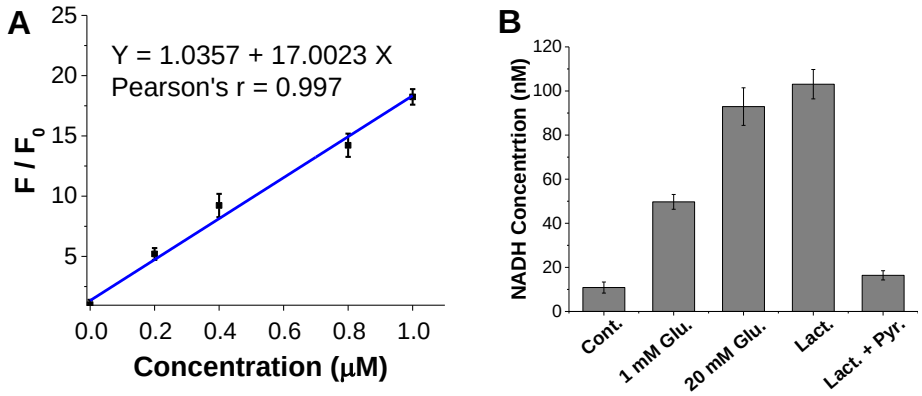


Figure S27. Quantification of NADH level in cells by NADH assay kit. 10^5 live OSCC cells in 1.5 mL tubes were incubated with the following conditions: A) no glucose for 30 min; B) 20 mM glucose for 30 min; C) 10 mM lactate for 10 min; D) 10 mM lactate and 5 mM pyruvate for 10 min. The cells were shook very 5 min. The NADH was extracted and measured based on the protocol in NADH kit (NAD/NADH Assay Kit, US Biological).

2. Materials and General Experimental Methods

All chemical reagents for the probe synthesis were obtained from Sigma Aldrich, Alfa Aesar, or MERCK, and used without further purification unless otherwise specified. The NADH dietary supplement was purchase from Ecological Formulas. NADH assay kit was purchased from US Biological (NAD/NADH Assay Kit). Column chromatography was carried out on Merck Silica Gel 60 (0.040-0.064 mm, 230–400 mesh). Synthetic reactions and analytical characterization were monitored by HPLC-MS (Agilient-1200 series) with a DAD detector and a single quadrupole mass spectrometer (6130 series) with an ESI probe. NMR spectra (^{1}H-300 or 500 MHz and ^{13}C-75 or 125 MHz) were recorded on Bruker Avance 300 or 500 NMR spectrometers. The high resolution electron spray ionization (HR-ESI) mass spectra were obtained on a Bruker micrOTOFQII. Spectroscopic and quantum yield data were measured on spectroscopic measurements, performed on a fluorometer and UV/VIS instrument, Spectra Max M2 by Molecular Device (The slit width: 1 nm). pH Value was determined by a Mettler Toledo S220 SEVENCOMPACT pH meter (Columbus, OH). Fluorescence microscopic images were obtained from a fluorescence Ti microscope (Nikon)

inverted microscope with epifluorescence and phase contrast optics using 20 × objective lenses. The orange fluorescence was collected by using an Ex 480 nm/40, Em 510nm/longpass filter. Confocal images were recorded from Nikon confocal microscope with 60x objective (3x zoom).

3. Synthesis and Characterization

Preparation of 4-6. 6-Bromohexanoic Acid (200 mg, 1.0 mmole), N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (229 mg, 1.2 mmole) and Hydroxybenzotriazole (183 mg, 1.2 mmole) were mixed and dissolved in 10 mL dichloromethane (DCM). After stirring for 5 min, anilines (1.0 mmole) were added and stirred for 3 h. The reaction was monitored by LC-MS and a product with reduced polarity was observed. Then 20 mL dichloromethane were added and extracted with water (3 x 30 mL). The organic phase was collected and evaporated under vacuum. The product was used in the next step without further purification.

Preparation of RA-Resa, BA-Resa and Con-Resa. To a stirred solution of Resazurin (114 mg, 0.5 mmole) and potassium carbonate (207 mg, 1.5 mmole) in 5 mL dimethylformamide (DMF), compound **4**, or **5**, or **6** (0.15 mmole) dissolved in 1 mL DMF was added respectively under argon atmosphere. The mixture was stirred at 75 ºC for 24 h. The reaction was monitored by LC-MS and a red product with reduced polarity was observed. After cooling to room temperature, 20 mL DCM was added and the mixture was extracted with water (3 x 50 mL). The organic phase was collected and removed under vacuum condition. Then the mixture was purified by preparative TLC with a solvent system (CH_2Cl_2 : MeOH = 100 : 6) and red solid products were obtained.

RA-Resa. Yield: 174.5 mg (73.5%) ^{1}H NMR (300 MHz, DMSO) δ 8.04 (d, *J* = 9.3 Hz, 1H), 8.00 – 7.90 (m, 2H), 7.58 (d, *J* = 8.0 Hz, 1H), 7.29 (d, *J* = 8.3 Hz, 1H), 7.17 (d, *J* = 2.3 Hz, 1H), 7.04 (dd, *J* = 9.2, 2.4 Hz, 1H), 6.65 (d, *J* = 10.1 Hz, 1H), 6.14 (d, *J* = 1.8 Hz, 1H), 4.91 (s, 2H), 4.15 (t, *J* = 6.3 Hz, 2H), 2.34 (t, *J* = 7.2 Hz, 2H), 1.86 – 1.74 (m, 2H), 1.71 – 1.60 (m, 2H), 1.48 (d, *J* = 7.0 Hz, 2H). ^{13}C NMR (126 MHz, DMSO) δ 184.29, 171.53, 163.77, 153.71, 149.28, 148.90, 138.60, 132.39, 130.95, 124.63, 123.37, 122.75, 121.83, 121.75, 121.46, 114.32, 114.31, 104.60, 101.62, 70.14, 69.45, 36.70, 28.59, 25.52, 25.33. ESI-MS $C_{25}H_{24}BN_2O_7^+$ [M + H^+], found 475.1674, calculated 475.1674.

BA-Resa. Yield: 160.3 mg (69.4%). ^{1}H NMR (300 MHz, MeOD) δ 8.29 – 8.14 (m, 2H), 7.85 (s, 1H), 7.70 (t, *J* = 6.8 Hz, 1H), 7.39 (d, *J* = 7.2 Hz, 2H), 7.14 (dd, *J* = 7.2, 2.8 Hz, 2H), 6.87 (dd, *J* =

10.0, 2.0 Hz, 1H), 6.39 (t, *J* = 4.8 Hz, 1H), 4.29 (q, *J* = 6.0 Hz, 2H), 2.51 (q, *J* = 7.5 Hz, 2H), 2.08 – 1.95 (m, 2H), 1.89 (dd, *J* = 15.1, 7.5 Hz, 2H), 1.78 – 1.62 (m, 2H). ^{13}C NMR (75 MHz, MeOD) δ 183.89, 171.45, 163.10, 152.62, 147.90, 129.53, 128.83, 126.22, 126.04, 123.94, 123.26, 121.55, 120.56, 119.90, 119.75, 112.97, 104.90, 102.51, 99.24, 67.52, 34.84, 26.73, 23.60, 23.51. ESI-MS $C_{24}H_{23}BN_2O_7Na^+$ [M + H^+], found 485.1496, calculated 485.1491.

Con-Resa. Yield: 143.7 mg (68.8%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.15 – 7.94 (m, 2H), 7.59 (dd, *J* = 8.3, 1.3 Hz, 2H), 7.28 (t, *J* = 7.8 Hz, 2H), 7.20 (d, *J* = 2.6 Hz, 1H), 7.12 – 6.96 (m, 2H), 6.68 (dd, *J* = 10.0, 1.9 Hz, 1H), 4.18 (t, *J* = 6.4 Hz, 2H), 2.35 (t, *J* = 7.3 Hz, 2H), 1.81 (p, *J* = 6.6 Hz, 2H), 1.69 (p, *J* = 7.3 Hz, 2H), 1.48 (t, *J* = 6.7 Hz, 2H). ^{13}C NMR (75 MHz, DMSO) δ 183.80, 171.09, 163.28, 153.24, 148.80, 139.28, 131.89, 130.47, 128.57, 124.16, 122.89, 121.26, 119.01, 118.91, 113.83, 112.53, 104.10, 101.14, 68.95, 36.25, 28.08, 25.01, 24.75. ESI-MS $C_{24}H_{22}N_2O_5Na^+$ [M + H^+], found 441.1423, calculated 441.1421.

Preparation of BA-Resa-P. BA-Resa (20 mg, 0.04 mmole) and reduced nicotinamide adenine dinucleotide (26 mg, 0.04 mmole) were dissolved in 10 mL DMSO/PBS buffer (v/v, 10/90, pH 9.5). After stirring for 30 min at 37 ºC, 20 mL DCM was added and extracted with water (3 x 50 mL). The organic phase was collected and removed under vacuum. The product was purified by preparative TLC with a solvent system (CH2Cl2 : MeOH = 100 : 6). Yield: 8.9 mg (50%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.94 (s, 1H), 7.87 – 7.76 (m, 1H), 7.74 – 7.58 (m, 2H), 7.49 – 7.40 (m, 1H), 7.30 – 7.12 (m, 1H), 7.10 – 6.90 (m, 2H), 6.72 (dd, *J* = 9.7, 2.1 Hz, 1H), 6.19 (s, 1H), 4.08 (t, *J* = 6.4 Hz, 2H), 2.30 (t, *J* = 7.2 Hz, 2H), 1.73 (d, *J* = 7.2 Hz, 2H), 1.62 (q, *J* = 7.9, 7.4 Hz, 2H), 1.49 – 1.35 (m, 2H). ^{13}C NMR (75 MHz, DMSO) δ 185.11, 170.89, 162.66, 149.52, 145.12, 144.82, 138.33, 134.71, 133.49, 131.15, 128.67, 128.67, 127.65, 127.45, 125.06, 121.03, 113.95, 105.49, 100.55, 68.64, 36.14, 28.09, 24.98, 24.78. ESI-MS $C_{24}H_{22}BN_2O_6Na^+$ [M + H^+], found 469.1559, calculated 469.1546.

4. Photophysical Studies

Measurement of photophysical properties. In general, the UV-Vis absorption and emission spectra were obtained on a Spectra Max M2 from Molecular Device. The excitation/emission slit width is 1 nm and the scan speed is medium. Stock solutions of the probes in DMSO (10 mM) were used to prepare the working solutions in buffered (PBS, pH 7.4 or 9.5) DMSO/water (v/v, 50/50 or

1/99) solution with a final concentration shown in Figure legend. Absorbance spectra were recorded from 350-700 nm while the emission spectra from 530-750 nm. 200 μL buffer solution each well was used in the 96-well plates. The work temperature is 310 K. The quantum yield was measured according the reported method and resazurin in PBS buffer (pH 9.5) was chosen as the reference fluorophore.[1]

5. *In Vitro* Fluorescence Microscopic Studies

Cell culture. Oral squamous cell carcinoma cell lines OSCC cells (American Type Culture Collection) were cultured as mono-layers in 75-cm^2 flasks with growth media containing DMEM (BSF) supplemented with 10% FBS (PAA), 1% penicillin streptomycin glutamine (GIBCO) at 37ºC in a humidified incubator of 5% CO_2. Cells were carefully harvested and split when they reached 80% confluence to maintain exponential growth.

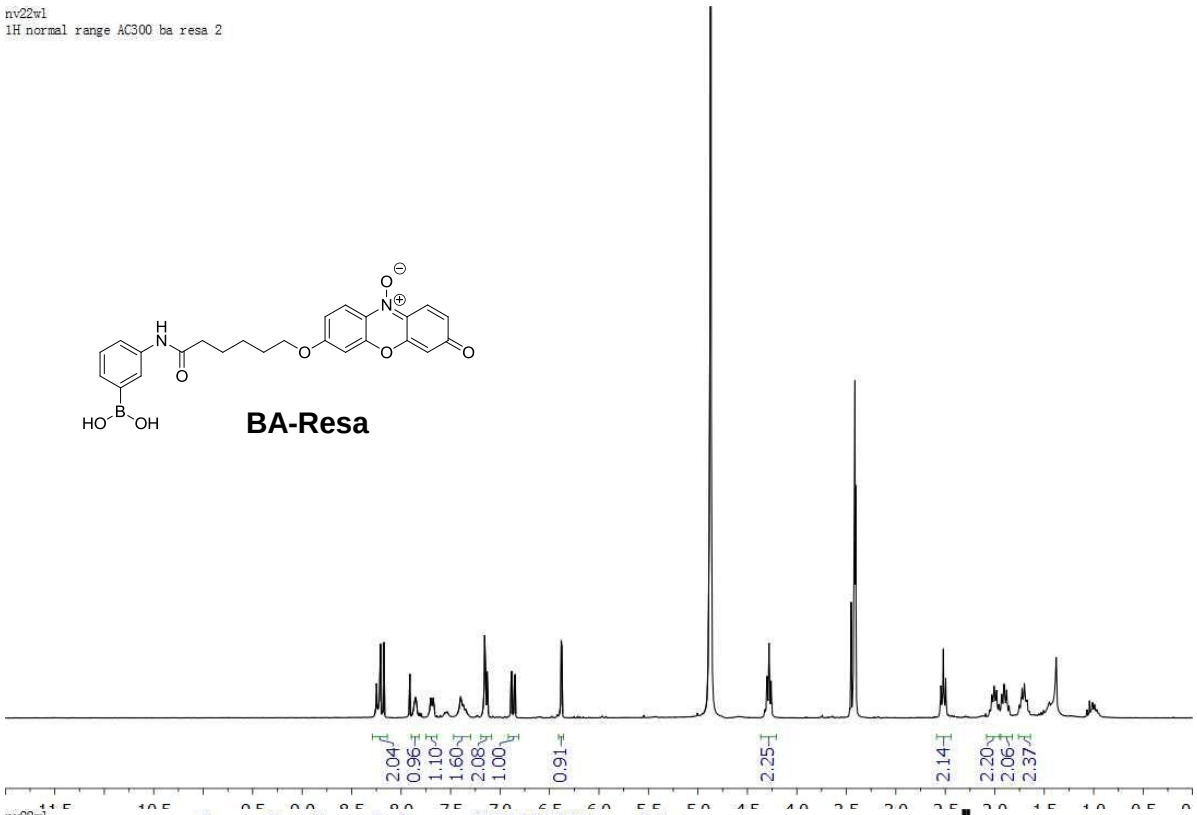
Cell cytotoxicity in MTT assay. OSCC cells were plated in 96-well flat-bottomed plates at 1×10^5 cells per well respectively and allowed to grow 6 or 24 h prior to exposure to the various concentrations of **RA-Resa**. Then MTT reagent was added for 4 h at 37 ^oC and DMSO (100 μL/well) was further incubated with cells for 15 min after removing the medium. The absorbance at 570 nm and 690 nm (background signal) was recorded in a Spectra Max M2 microplate reader. The following formula was used to calculate the viability of cell growth: Cell viability (%) = (mean of A value of treatment group / mean of A value of control) × 100. The untreated group was used as control group. Each concentration was tested with 6 wells and the experiment was repeated 3 times.

Fluorescence microscopic imaging. To avoid the artifacts that occur during fixation procedures, all the experiments were conducted in live cells. There are five groups in the cell experiment. Before treatment, the culture medium was replaced by the medium/PBS solution (5/95, pH 7.4). In the first group (A), live cells (OSCC cells or CHO cells or Hela cells) were treated with 5 μM **RA-Resa** for 15 min after incubation in the absence of glucose for 30 min. In the group B and C, live cells were incubated with 1 mM or 20 mM glucose for 0.5 h respectively and further treated with 5 μM **RA-Resa** for 15 min prior to imaging. In the fourth and fifth groups, live cells were cultured with 5 μM **RA-Resa** for 15 min after incubation with 10 mM lactate or 10 mM lactate and 5 mM pyruvate for 10 min. The background signal was removed by the software Nikon Analysis and the fluorescence intensity was ranged from 6000 to 16000.

Confocal microscopic imaging determining the subcellular location of probes.

OSCC cells stained with 5 μM RA-Resa and one of organelle markers for nucleus (Hoechst33342, 1 μg/ml), mitochondria (MitoTracker® Deep Red FM, 100 nM), lysosome (LysoTracker® Red DND-99, 50 nM) or ER (ER-TrackerTM Red, 100 nM) for 30 min under HBSS solution containing 10 mM glucose. Golgi (BODIPY FL C5 ceramide, 1 μM) stain were carried under 4oC for 1 hr prior to the staining of RA-Resa. Confocal images were acquired in 512x512 resolution using A1R+si Laser scanning confocal microscope (Nikon) with 60x objective (3x zoom) under 37oC supplemented with 5% CO2.

6. Supplemental Spectra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BA-Resa
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2.08
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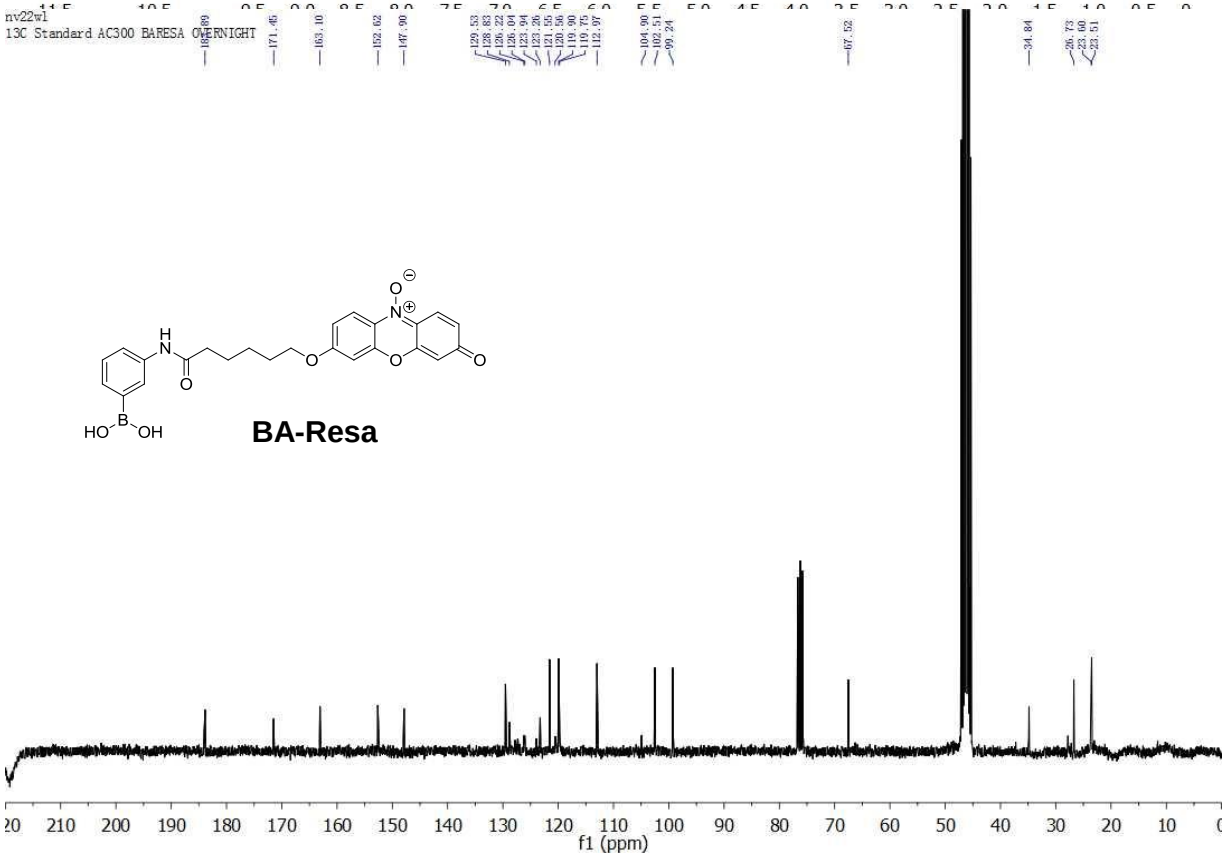
2.14
2.20
2.06
2.37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BA-Resa
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Scan End	2500 m/z	Set Collision Cell RF	300.0 Vpp	Set Divert Valve	Waste

Meas. m/z	#	Formula	m/z	err [ppm]	rdb	e⁻ Conf	N-Rule
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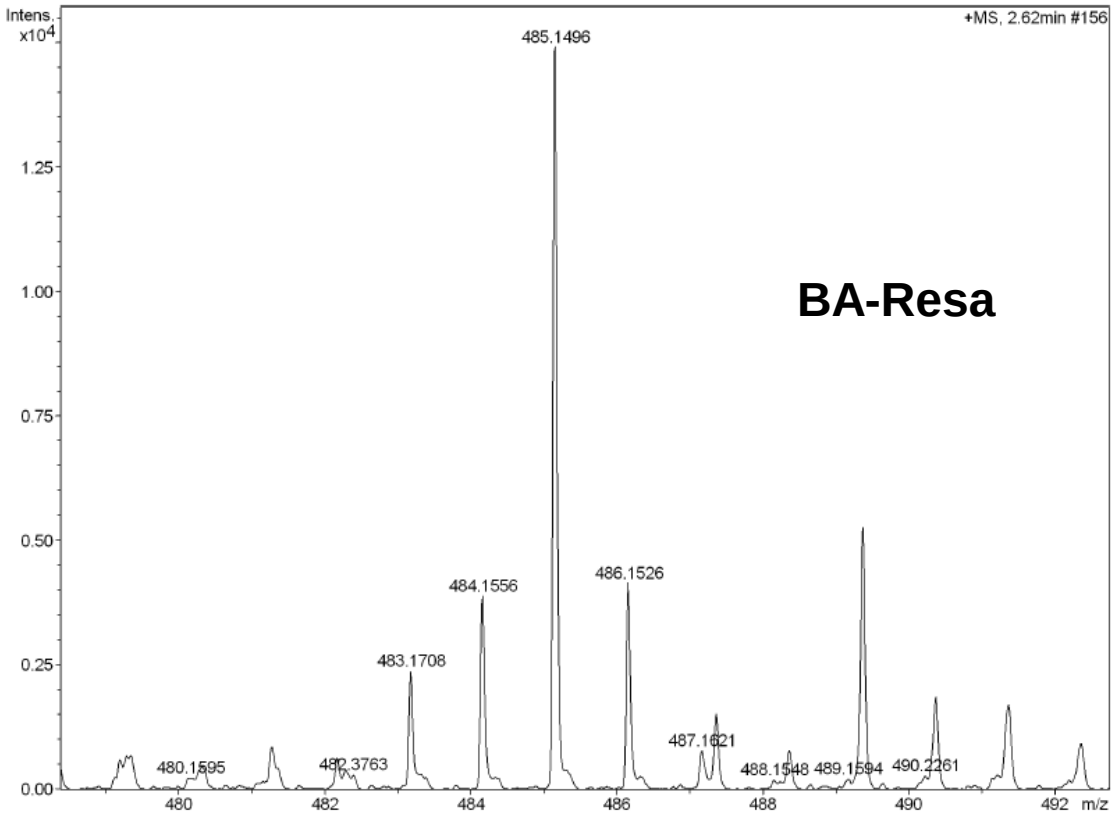

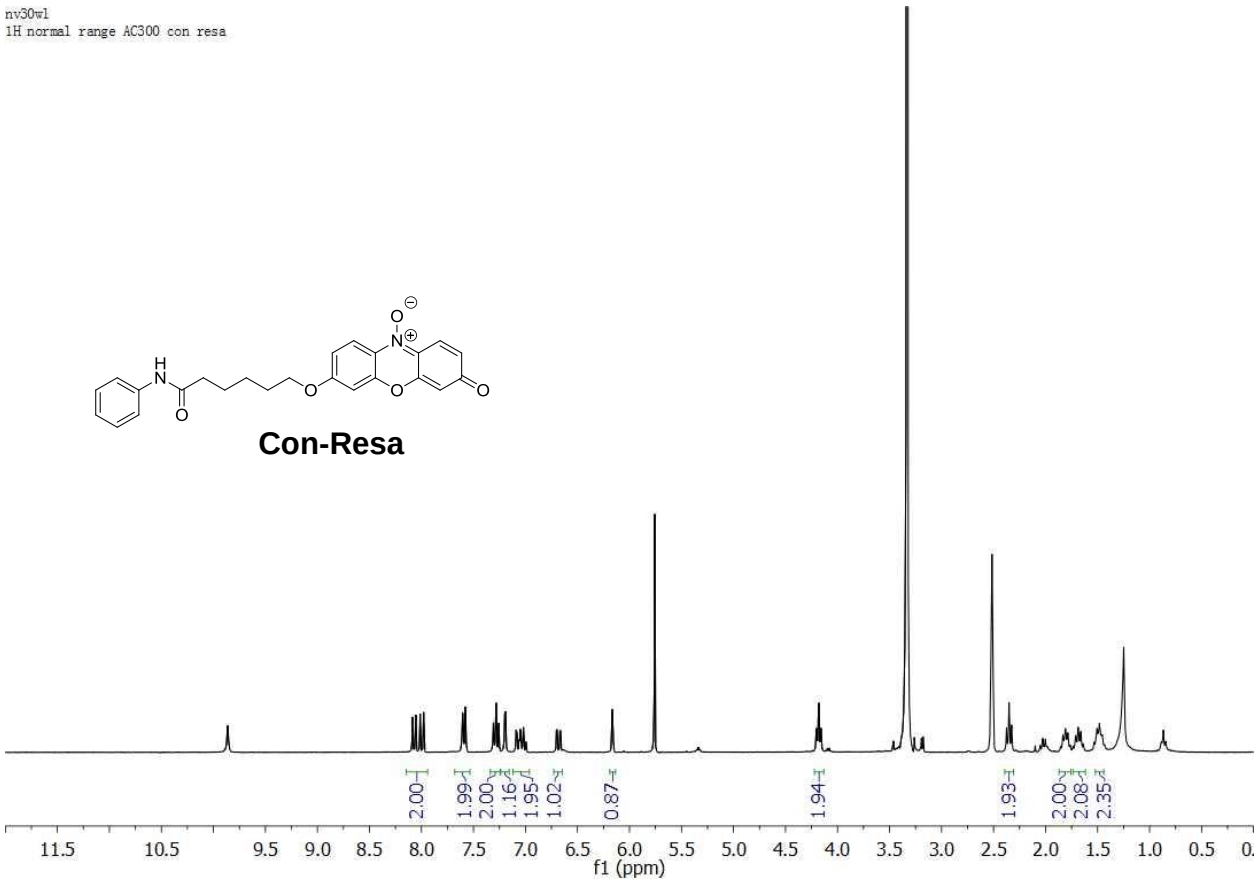
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1.95
1.02
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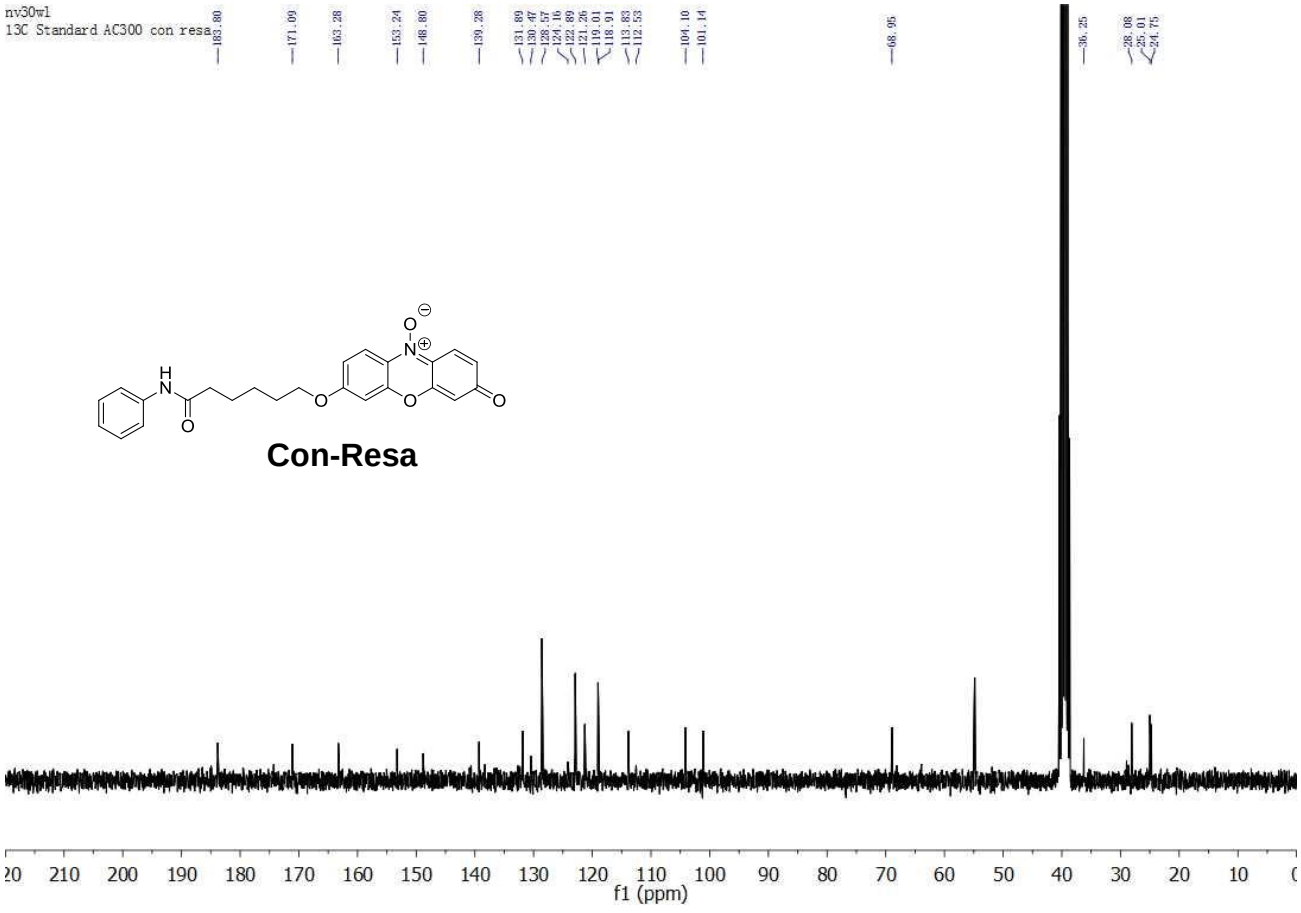
1.94
1.93
2.00
2.08
2.35
11.5
10.5
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)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13C Standard AC300 con resa
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112.53
104.10
101.14
68.95
36.25
28.08
25.01
24.75
H
N
O
O
N
O
O
O
Con-Resa
f1 (ppm)

Acquisition Parameter

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Scan End	2500 m/z	Set Collision Cell RF	300.0 Vpp	Set Divert Valve	Waste

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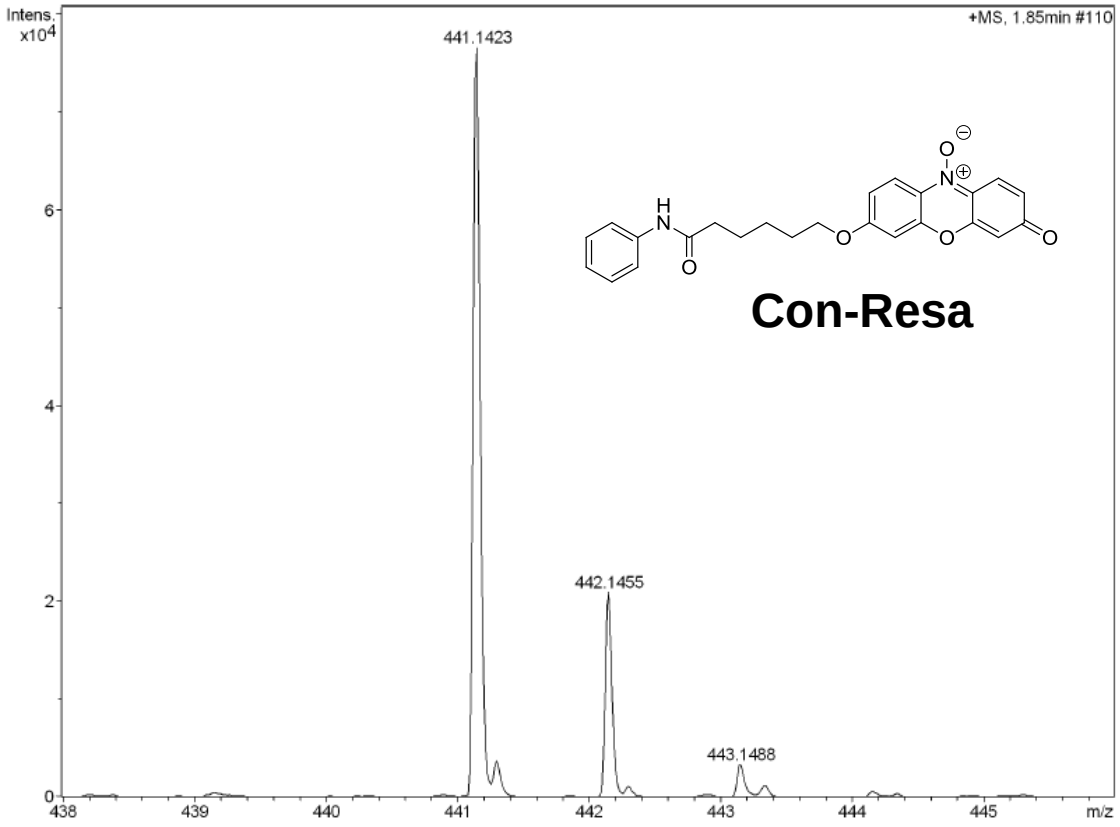

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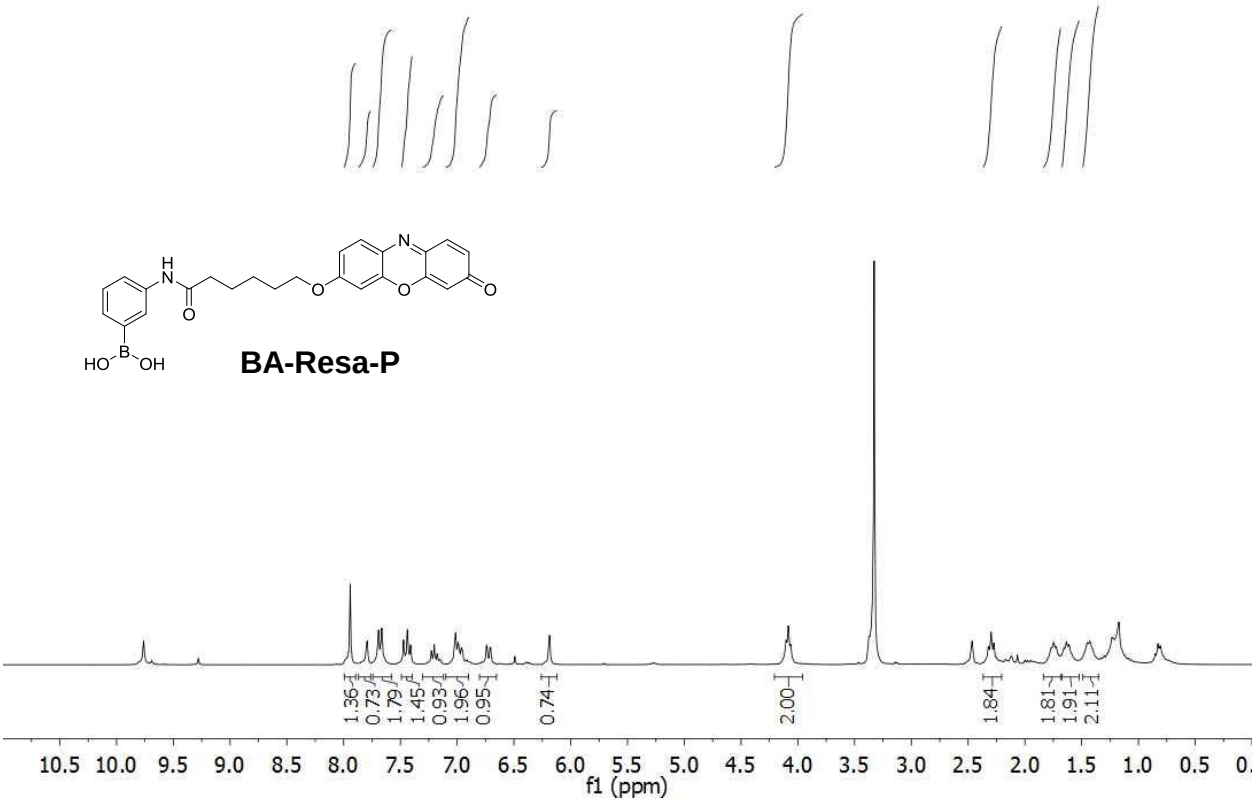

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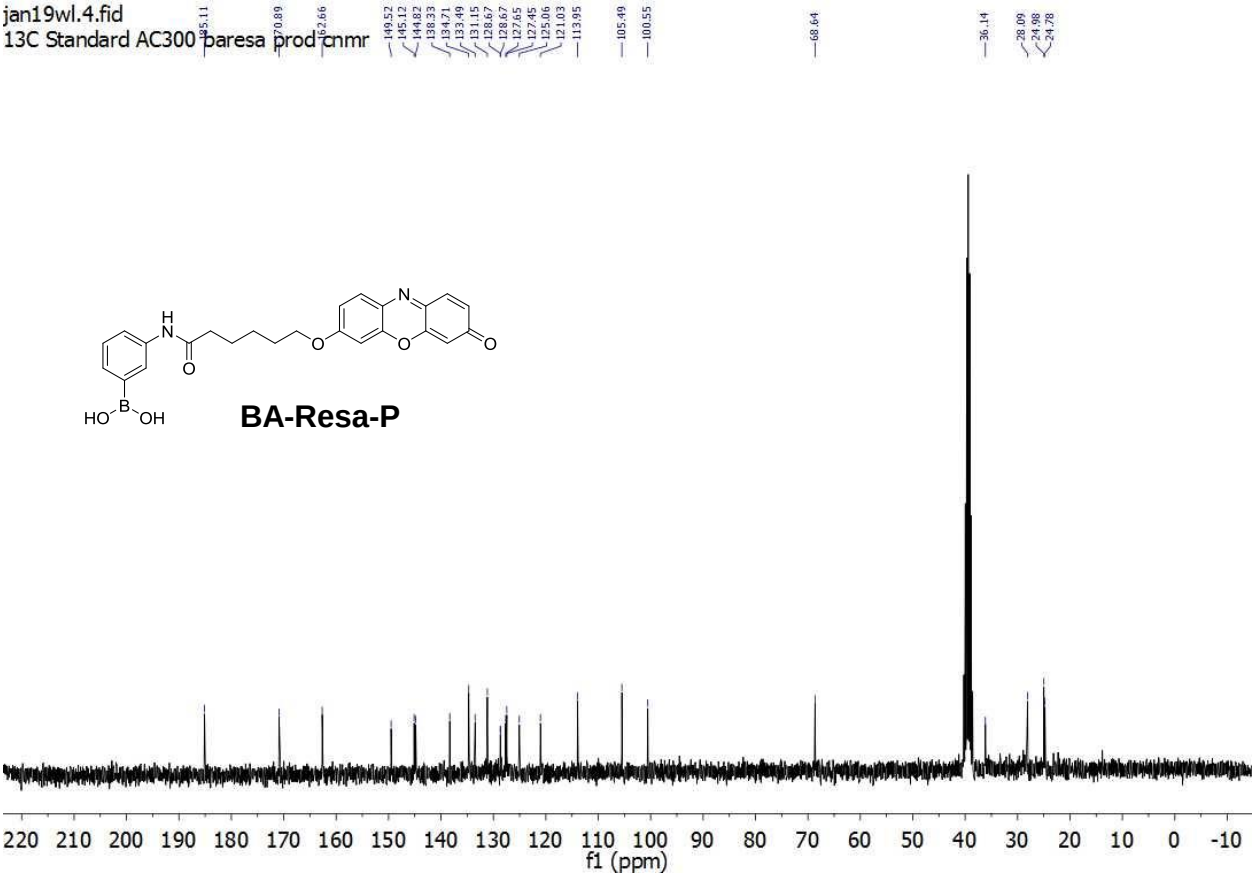

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Scan End	1800 m/z	Set Collision Cell RF	150.0 Vpp	Set Divert Valve	Waste

Meas. m/z	#	Formula	m/z	err [ppm]	rdb	e¯ Conf	N-Rule
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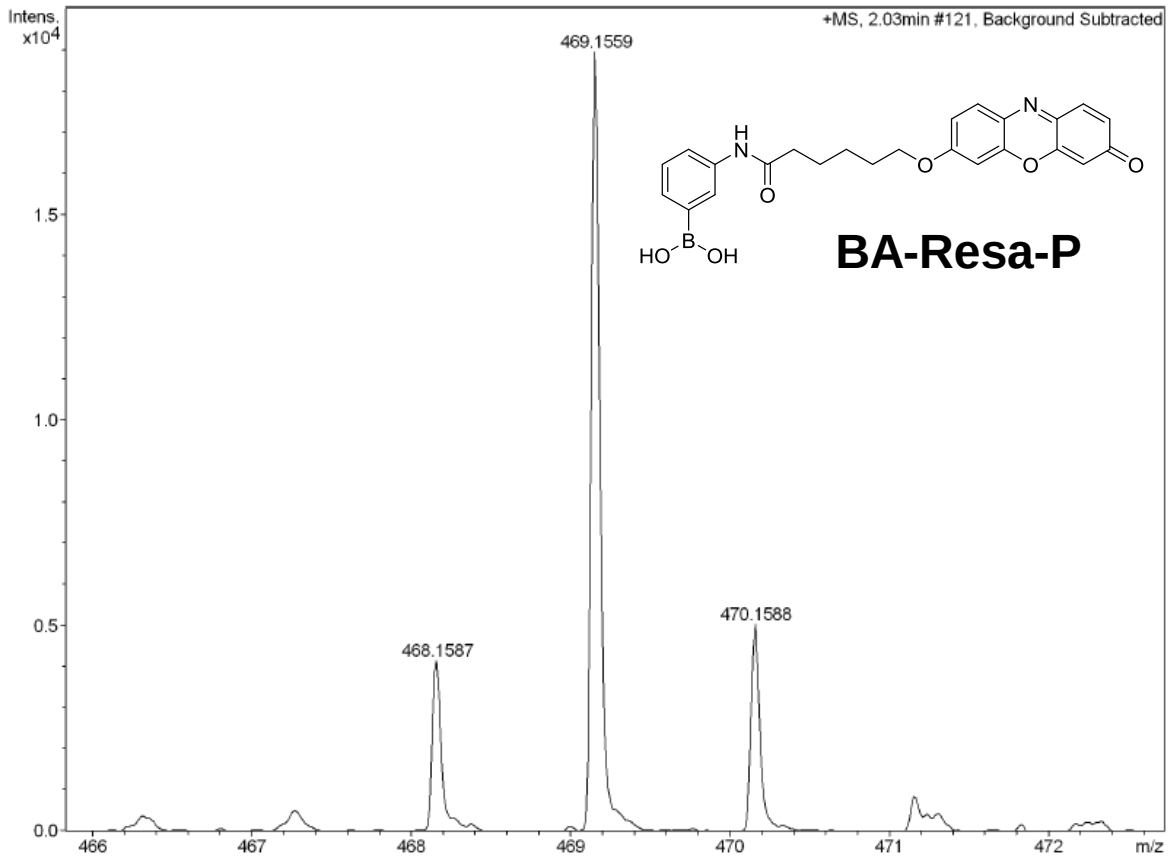

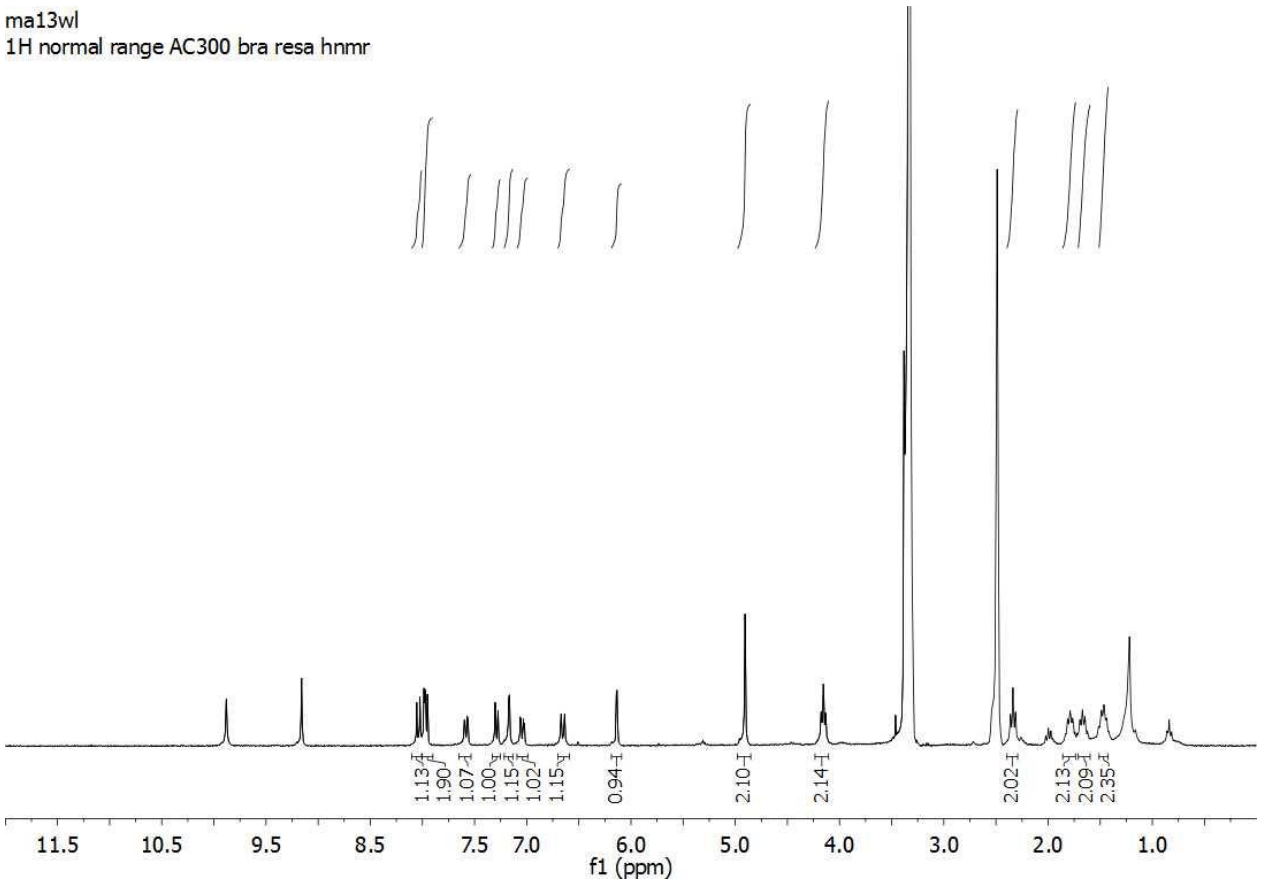
ma13wl
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1.90
1.07
1.00
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1.15
0.94
2.10
2.14
2.02
2.13
2.09
2.35
11.5
10.5
9.5
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7.5
7.0
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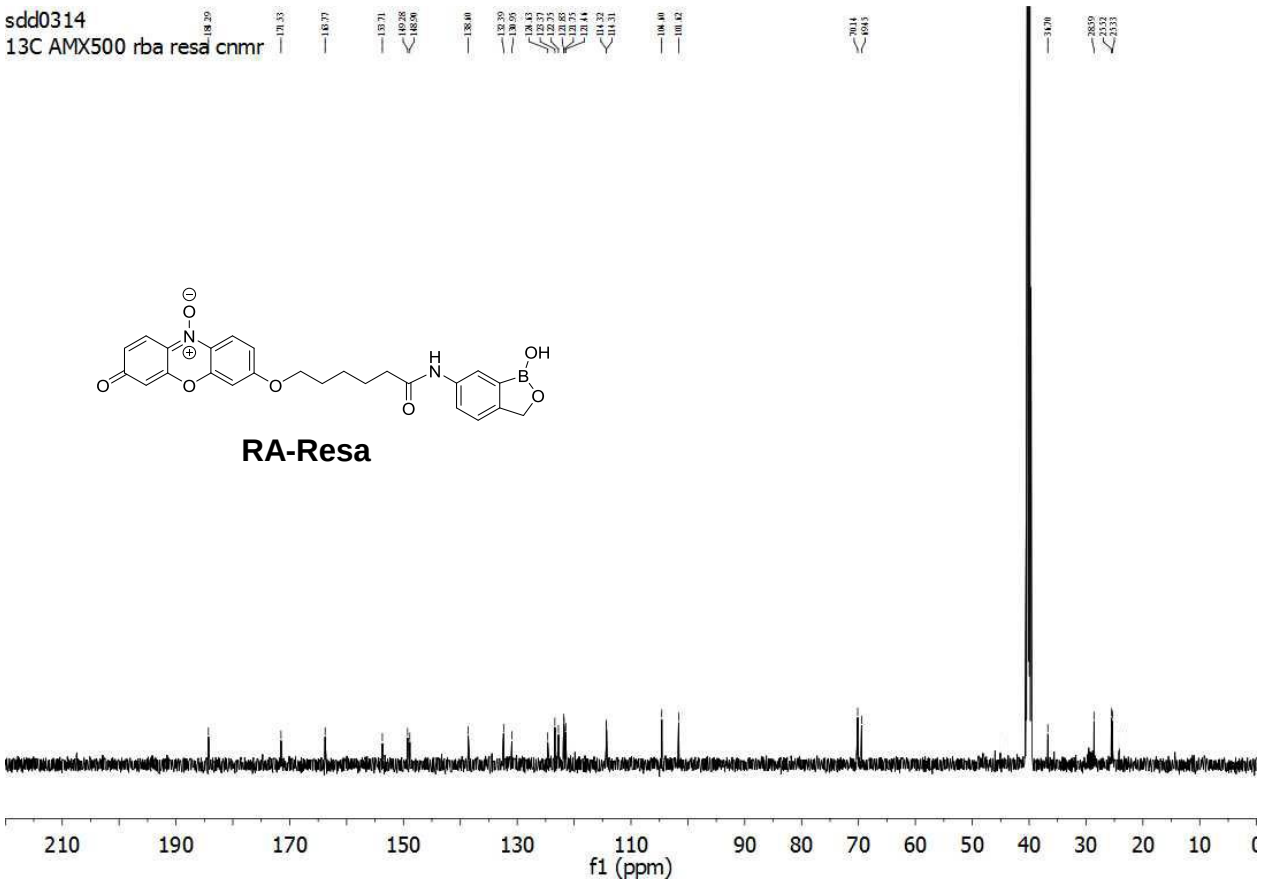
4.0
3.0
2.0
1.0
f1 (ppm)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13C AMX500 rba resa cnmr
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60
50
40
30
20
10
f1 (ppm)

O
N
O
O
O
H
N
OH
B
O
O

RA-Resa

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	2.0 Bar
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Scan End	1800 m/z	Set Collision Cell RF	200.0 Vpp	Set Divert Valve	Waste

Meas. m/z	#	Formula	m/z	err [ppm]	rdb	e⁻ Conf	N-Rule
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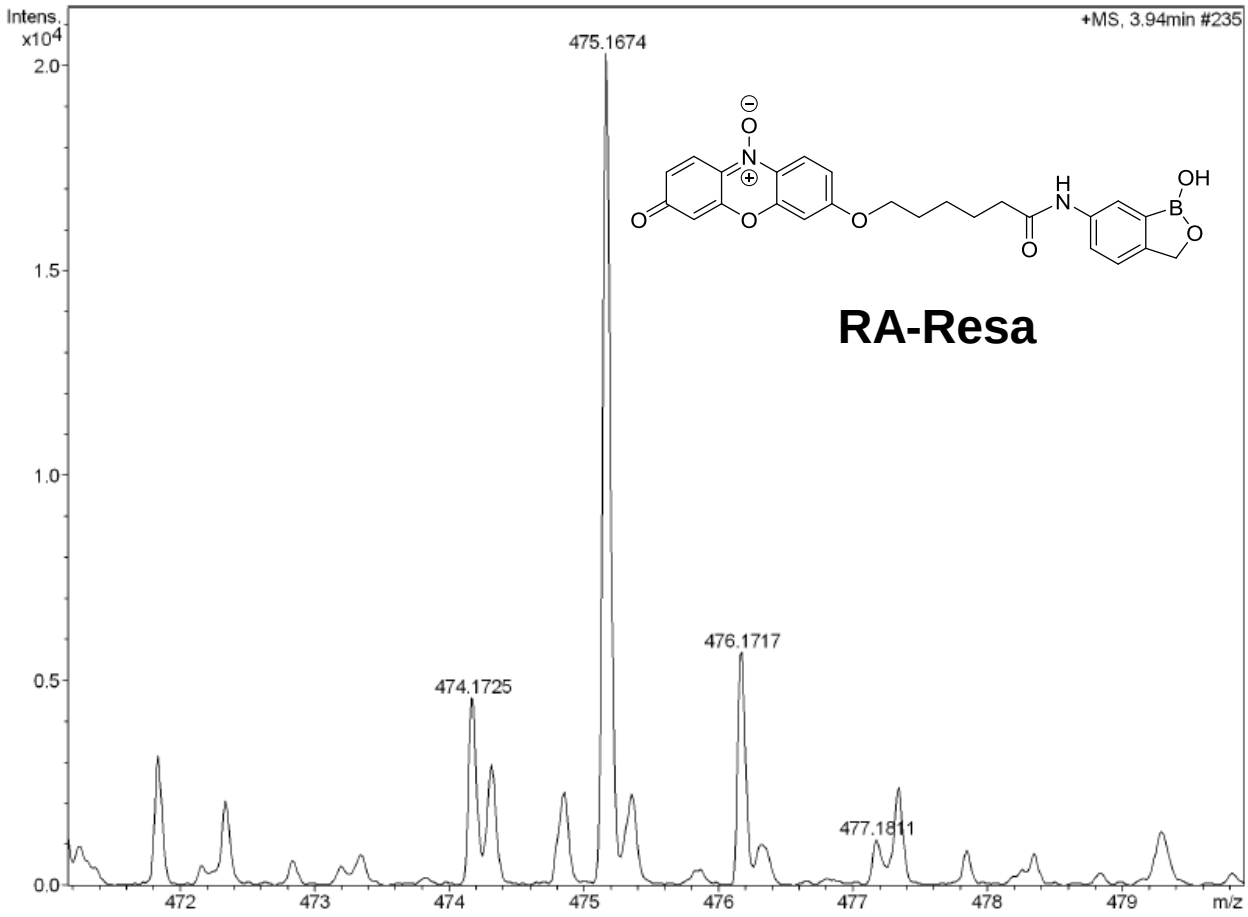

7. Reference:

(1) Bueno, C.; Villegas, M. L.; Bertolotti, S. G.; Previtali, C. M.; Neumann, M. G.; Encinas, M. V. *Photochemistry and photobiology* **2002**, *76*, 385.
(2) Yuan, L.; Lin, W.; Yang, Y. *Chemical communications* **2011**, *47*, 6275.