Supporting Information

Nebraska Red: A Phosphinate-Based Near-Infrared Fluorophore Scaffold for Chemical Biology Applications

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General Experimental Details

Reagents and Instrumentation

Unless otherwise noted, reactions were done in oven-dried glassware under N_2. All reagents and solvents were used as commercially supplied. Tetrahydrofuran (THF) was dried using 3 Å molecular sieves.[1] Reaction progress was monitored using Thin Layer Chromatography (TLC) and products were purified by flash chromatography using Merck silica gel 60 (230-400 mesh). HPLC purification was conducted using a Waters 1525 Binary HPLC pump with a 2489 UV/Vis detector. Prep purification was done with a semi-prep column (YMC-Pack ODS-A, 5 μm, 250×20 mm) using a gradient of 30 – 95% acetonitrile containing 0.1% TFA over 30 mins in water containing 0.1% TFA. Mass spectra were recorded using electrospray ionization mass spectrometry (ESI, Thermo Finnigan LCQ Advantage). Mass data are reported in units of m/z for $[M+H]^+$ or $[M+Na]^+$. ^1H NMR and ^{13}C NMR experiments were performed in $CDCl_3$ at room temperature and the spectra were recorded on Bruker-DRX-Avance 300 or 400 MHz instruments. Chemical shifts are reported relative to $CDCl_3$ (7.26 ppm for ^1H NMR and 77.0 ppm for ^{13}C NMR). For ^{31}P NMR, phosphoric acid (85 wt. % in water, Sigma-Aldrich) was used as an external standard (0.00 ppm). UV-Vis spectra were recorded using a BioMate 3S UV-Visible Spectrophotometer (Thermo Scientific). Fluorescence spectra were recorded using a FluoroMax-4 Spectrofluorometer (Horiba Scientific) with 5 nm slit widths. All absorbance assays were conducted in 100 μL quartz cuvettes, and the fluorescence assays were conducted in either 100 μL or 3.5 mL quartz cuvettes. Near Infrared (NIR) fluorescence pictures were taken using a home-made full-spectrum DSLR camera (Canon Rebel XSi) and a 720 nm NIR filter (Hoya 67mm RM72 Infrared Filter). Confocal fluorescence imaging was performed using a Nikon A1 confocal system on a Nikon 90i upright fluorescence microscope. Laser lines were 641 nm (deep red) and emission filters were 663 nm – 738 nm.

Computational Calculation

Density functional theory (DFT) and time-dependent DFT (TDDFT) calculations were performed using the Quantum chemistry Polarizable force field program (QuanPol)[2] and the General Atomic and Molecular Electronic Structure System (GAMESS)[3] package. The B3LYP[4]/6-31++G(d,p)[5] method was used with the FixSol[6] solvation model (dielectric constant: 78.39).

Molar Extinction Coefficient Measurement

Stock solutions were made by dissolving a solid sample in DMSO. Increasing concentrations of the sample were prepared (1, 2, 3, 4, and 5 μM) as well as a blank. Sample absorbance was measured and the molar extinction coefficient was determined by a linear fit of the absorbance versus sample concentration.

Quantum Yield Measurement

Fluorescence quantum yield was determined by the following equation:

$$\Phi_X = (\Phi_{ST} \times A_{ST} \times F_X \times \eta_X^2)/(A_X \times F_{ST} \times \eta_{ST}^2)$$

Φ is the quantum yield; A is the absorbance at the excitation wavelength (A was kept at ≤0.05 during fluorescence measurements to avoid self-quenching), F is the fluorescence intensity at the excitation wavelength; η is the refractive index of the solvent. The subscripts $_{ST}$ and $_X$ refer to the standard and unknown respectively. For NR_{666}, NR_{698} and NR_{700}, sulfo-cyanine5.5 NHS ester (Lumiprobe) was used as the fluorescence reference standard (excitation: 670 nm, emission: 690 nm), which has a quantum yield of 0.23[7] in PBS at pH=7.4. For NR_{744}, HITCI (Sigma-Aldrich) was used as the fluorescence reference standard, which has the quantum yield of 0.283[8] in ethanol.

Photostability Tests

Samples were kept at the same absorbance (A = 0.1) at their excitation wavelength. Fluorescence intensity at the respective emission maxima was recorded with continuous exposure to excitation light for 1 hr with a slit width of 5 nm for each sample.

Selectivity Assay

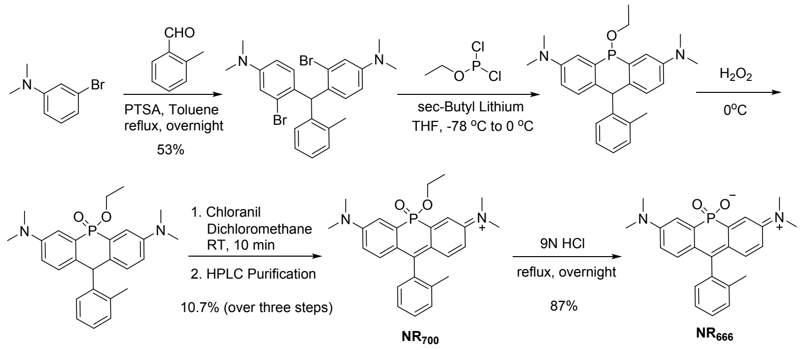
All ROS and RNS solutions were prepared by previously reported methods.[9] **NR-HOCl** was kept at 5 μM in PBS (10 mM, pH=7.4 with 0.5% DMF). The HOCl concentration was kept at 5 μM while all off-target species were assayed at 100 μM. **NR-HOCl** was incubated in different ROS and RNS for 15 min before fluorescence was measured.

Cell Imaging

For HeLa cell imaging: HeLa cells (ATCC, CCL-2) were grown to 80% confluency in DMEM (Life Tech, 11965092) with 10% FBS (Life Tech, 16000036), 1x Pen/Strep (Life Tech, 15140122), and 1x Anti-Anti (Life Tech, 15240062). Media was then removed and replaced with pre-warmed (37 ºC) DMEM with HEPES and no phenol red (Life Tech, 2106245) with 10% FBS, 1x Pen/Strep and 1x Anti-Anti for 1 hr prior to transport. The media was then removed and the cells were rinsed 3 times with prewarmed DPBS (Life Tech, 14040216). The cells were then incubated for 20 min in DPBS with 10 μM **NR-HOCl**. Following incubation the cells were rinsed 3 times with DPBS. The cells were further incubated with 20 μM NaOCl for 15 min before imaging.

For RAW 264.7 cell imaging: RAW 264.7 (ATCC, TIB-71) were grown to 80% confluency in DMEM with 10% FBS, 1x Pen/Strep and 1x Anti-Anti. Cells were then stimulated with LPS (1 μg/mL) for 12 hrs. Media was then removed and replaced with pre-warmed (37 ºC) DMEM with HEPES and no phenol red with 10% FBS, 1x Pen/Strep, and 1x Anti-Anti for 1 h. PMA (0.1 μg/mL or 1 μg/mL) was added and cells were further incubated for 30 min. All plates were rinsed 3 times with DPBS and 10 μM **NR-HOCl** was added. After 20 min, cells were washed with DPBS 3 times and imaged.

Synthesis of the Nebraska Red Dye Series and NR-HOCl.



4,4'-(o-tolylmethylene)bis(3-bromo-*N,N*-dimethylaniline)

3-bromo-*N,N*-dimethylaniline[10] (8 g, 39.98 mmol) and 2-methylbenzaldehyde (1.155 mL, 10 mmol) were dissolved in toluene (20 mL). *p*-Toluenesulfonic acid monohydrate (1.9 g, 10 mmol) was then added and the mixture solution was refluxed at 130 ºC using a Dean-Stark apparatus. After 3 hrs, another 20 mL of toluene was added into the reaction and the mixture was refluxed overnight. Extra toluene was removed by evaporation and the resulting oil mixture was dissolved in dichloromethane (DCM) and extracted using saturated sodium bicarbonate solution. The DCM layer was dried over sodium sulfate, filtered, and the solvent was evaporated. Flash column chromatography (50% DCM/hexane) was performed to give the product as a white solid (2.66 g, 53%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.15 – 7.02 (m, 3H), 6.92 (d, J = 2.7 Hz, 2H), 6.71 (d, J = 7.5 Hz, 1H), 6.61 (d, J = 8.6 Hz, 2H), 6.52 (dd, J = 8.7, 2.7 Hz, 2H), 5.95 (s, 1H), 2.90 (s, 12H), 2.19 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 150.11, 141.81, 137.18, 131.16, 130.43, 129.69, 128.92, 126.72, 126.41, 125.78, 116.66, 111.40, 51.59, 40.60, 19.75.

MS (ESI) m/z calculated for $C_{24}H_{26}Br_2N_2$ $[M+H]^+$ 503.3, found 504.0.

NR$_{700}$

4,4'-(o-tolylmethylene)bis(3-bromo-*N,N*-dimethylaniline (300 mg, 0.6 mmol) was dissolved in anhydrous THF (10 mL) and the solution was cooled to -78 ºC in an acetone/dry ice bath. After stirring for 10 min, *sec*-butyllithium (1.4 M in cyclohexane, 943 µL, 1.32 mmol) was added dropwise over 10 min and the resulting mixture was further stirred for 1 hr followed by dropwise addition of ethyl dichlorophosphite (75.4 µL, 0.66 mmol) over 20 min. The reaction was allowed to proceed for 2 hrs and then the dry ice bath was replaced by an ice/water bath. After stirring for another 2 hrs, hydrogen peroxide (50 wt. % in H_2O, 0.5 mL) was added dropwise and the mixture was further stirred for 1 hr at 0 ºC. The reaction was then quenched by saturated sodium sulfite solution and extracted using ethyl acetate. The organic layer was collected, dried over sodium sulfate, and the solvent was removed. The resulting yellow solid was dissolved in DCM (3 mL) and *p*-chloranil (295 mg, 1.2 mmol) was added. The mixture was stirred for 30 min at room temperature and then the temperature was lowered to 0 ºC. The solid was filtered the washed by cold dichloromethane (2 x 3 mL). The solvent was removed and to the resulting dark green solid was added to HPLC buffer (50% acetonitrile in water with 0.1% trifluoroacetic acid) and clarified by centrifugation. The product peak from HPLC was lyophilized to yield a dark green solid (35 mg, 10.7%). A racemic mixture was obtained using this method and it was used for further studies with no further purification.

^{1}H NMR (400 MHz, CD_3CN) δ 7.65 – 7.37 (m, 5H), 7.18 (t, *J* = 7.8 Hz, 1H), 7.04 (td, *J* = 10.3, 6.8 Hz, 2H), 6.83 (dd, *J* = 9.7, 2.8 Hz, 2H), 4.09 (dp, *J* = 9.6, 7.2 Hz, 2H), 3.38 (s, 12H), 2.06 (d, *J* = 21.9 Hz, 3H), 1.24 (dt, *J* = 18.1, 7.0 Hz, 3H).

^{13}C NMR (101 MHz, CD_3CN) δ 164.22, 164.13, 164.08, 164.00, 160.17, 159.81, 159.45, 159.09, 155.38, 155.33, 155.25, 155.20, 140.02, 140.00, 139.91, 139.90, 137.12, 137.00, 136.30, 136.10, 135.89, 135.78, 130.53, 130.47, 129.55, 129.46, 128.53, 125.99, 125.85, 124.06, 123.99, 123.79, 123.71, 118.44, 118.41, 118.38, 118.35, 115.65, 115.64, 115.56, 115.54, 114.91, 62.07, 62.01, 41.06, 18.65, 15.77, 15.71.

^{31}P NMR (162 MHz, CD_3CN) δ 12.06, 11.98.

MS (ESI) m/z calculated for $C_{28}H_{30}F_3N_2O_4P$ $[M-CF_3COO]^+$ 433.5, found 433.5.

NR$_{666}$

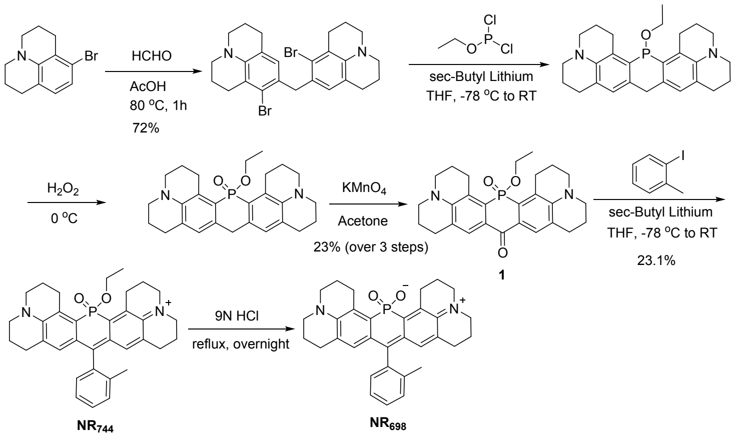
NR$_{700}$ (20 mg, 0.037 mmol) was dissolved in HCl (9 N, 2 mL) and the solution was refluxed overnight. After evaporating the solvent, the resulting blue solid was recrystallized in hot water, yielding a gold crystalline solid (14 mg, 87%). Phosphoric acid (85 wt. % in H_2O) was added as an internal standard when preparing NMR samples and stock solutions in order to increase solubility in DMSO.

^{1}H NMR (300 MHz, DMSO-d_6) δ 7.53 – 7.34 (m, 3H), 7.24 (d, J = 14.7 Hz, 2H), 7.12 (d, J = 7.3 Hz, 1H), 6.90 – 6.70 (m, 4H), 3.30 (s, 12H), 1.98 (s, 3H).

^{13}C NMR (75 MHz, DMSO-d_6) δ 155.48, 155.32, 138.76, 138.64, 137.41, 135.73, 130.61, 129.41, 129.22, 126.28, 123.56, 123.46, 115.89, 115.81, 114.67, 41.15, 19.41.

^{31}P NMR (121 MHz, DMSO-d_6) δ 0.67.

MS (ESI) m/z calculated for $C_{24}H_{25}N_2O_2P$ $[M+H]^+$ 405.5, found 405.8.



8-bromo-2,3,6,7-tetrahydro-*1H,5H*-pyrido[3,2,1-*ij*]quinoline

3-bromoaniline (20 mL, 1.83 mol) was added to 1-bromo-3-chloropropane (150 mL), followed by the addition of sodium carbonate (77.5 g, 7.32 mol). The mixture was refluxed at 140 ºC for 48 hrs before DCM (3x200 mL) was added to extract the product. The organic layer was dried over sodium sulfate and solvent was removed. The resulting brown oil was dissolved in DMF (40 mL) and stirred at 160 ºC for 24 hrs. Solvent was removed, water (200 mL) was added, and the mixture was extracted by DCM (3 x 200 mL). The organic layer was collected, dried over sodium sulfate, filtered, and solvent was removed. Flash chromatography was performed (1% ethyl acetate in hexanes) to give the pure product as a light yellow oil. Freezing the purified compound at -20 ºC overnight gives a light yellow waxy solid (28 g, 60.7%) which was kept in the dark at -20 ºC for long term storage.

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.90 (d, J = 8.0 Hz, 1H), 6.80 – 6.72 (d, 1H), 3.31 – 3.19 (m, 4H), 2.92 (t, J = 6.6 Hz, 2H), 2.83 (t, J = 6.4 Hz, 2H), 2.10 (dq, J = 12.6, 6.6 Hz, 4H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 144.80, 128.05, 123.19, 120.85, 120.69, 119.65, 50.27, 49.90, 28.83, 27.92, 22.25, 22.15.

MS (ESI) *m/z* calculated for $C_{12}H_{14}BrN$ $[M+H]^+$ 252.0, found 252.2.

bis(8-bromo-2,3,6,7-tetrahydro-1*H*,5*H*-pyrido[3,2,1-*ij*]quinolin-9-yl)methane

8-bromo-2,3,6,7-tetrahydro-*1H,5H*-pyrido[3,2,1-*ij*]quinoline (2.522 g, 10 mmol) was dissolved in acetic acid (35 mL). Formaldehyde solution (37 wt. % in H_2O, 1.5 g, 50 mmol) was added and the resulting mixture was heated at 80 ºC for 45 min. The precipitate was filtered and washed with acetic acid and methanol. The resulting white solid (1.859 g, 72%) was then dried under vacuum and was pure enough for the next step.

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.52 (s, 1H), 4.00 (s, 1H), 3.19 – 3.07 (m, 4H), 2.88 (t, J = 6.7 Hz, 2H), 2.69 (t, J = 6.6 Hz, 2H), 2.08 – 1.93 (m, 4H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 142.93, 128.51, 127.22, 125.68, 121.21, 120.78, 50.14, 49.60, 42.00, 29.45, 27.52, 22.36, 22.02.

MS (ESI) *m/z* calculated for $C_{25}H_{28}Br_2N_2$ $[M+H]^+$ 517.3, found 517.9.

1

In a flame dried round bottom flask charged with bis(8-bromo-2,3,6,7-tetrahydro-1*H*,5*H*-pyrido[3,2,1-*ij*]quinolin-9-yl)methane (2 g, 3.87 mmol), anhydrous THF (30 mL) was add and the temperature was lowered to -78 ºC using a dry ice/acetone bath. *Sec*-butyllithium (1.4 M in cyclohexane, 6.087 mL, 8.52 mmol) was then added dropwise into the reaction over 5 min and the mixture was stirred for 1 hr followed by dropwise addition of ethyl dichlorophosphite (487 µL, 4.26 mmol) over 20 min. The reaction was allowed to proceed for 3 hrs before the dry ice/acetone bath was removed, and the flask was slowly warmed to room temperature overnight. The next day, the flask was cooled to 0 ºC, hydrogen peroxide (50 wt. % in H_2O, 2.5 mL) was added dropwise into the reaction, and the reaction was stirred for 1 hr. Saturated sodium sulfite solution was then carefully added to quench the reaction and ethyl acetate was used for extraction. The organic layer was collected, dried over sodium sulfate, filtered, and the solvent was removed. The resulting solid was further dissolved in acetone and cooled to 0 ºC followed by portionwise addition of $KMnO_4$ (1.83 g, 11.61 mmol) over 2 hrs. After the last portion was added, the reaction was allowed to warm to room temperature and stirred for 1 hr. The mixture was then filtered and washed with DCM. Solvent was removed and flash chromatography (5-10% acetone in DCM) yielded the pure product as a yellow solid (410 mg, 23%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.86 (d, J = 6.5 Hz, 2H), 3.51 (p, J = 7.1 Hz, 4H), 3.26 (t, J = 5.9 Hz, 8H), 3.19 – 3.02 (m, 2H), 2.77 (t, J = 6.4 Hz, 4H), 1.95 (ddt, J = 24.9, 12.2, 6.0

Hz, 8H), 1.10 (t, J = 7.0 Hz, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 180.41, 180.26, 146.93, 146.75, 128.74, 128.58, 127.60, 126.00, 125.03, 124.92, 124.80, 124.77, 123.06, 122.97, 62.06, 61.99, 50.72, 50.08, 28.46, 25.77, 25.69, 21.39, 21.29, 16.34, 16.24.

^{31}P NMR (121 MHz, $CDCl_3$) δ 20.71.

MS (ESI) *m/z* calculated for $C_{27}H_{31}N_2O_3P$ $[M+H]^+$ 463.5, found 463.8.

NR$_{744}$

2-Iodotoluene (82.5 µL, 0.65 mmol) was added to a flame dried round bottom flask containing anhydrous THF (2 mL). The temperature was lowered to -78 ºC with a dry ice/acetone bath followed by dropwise addition of *sec*-butyllithium (1.4 M in cyclohexane, 464 µL, 0.65 mmol). After 1h, a solution of **1** (30 mg, 0.065 mmol) in anhydrous THF (2 mL) was added dropwise into the above reaction and the dry ice bath was removed, allowing the reaction to warm to room temperature. After 2 hrs the reaction was quenched by the addition of HCl at 0 ºC and stirred for another 30 min. Then the pH was adjusted to 7 by addition of a saturated sodium bicarbonate solution and ethyl acetate was used for extraction. The organic layer was collected, dried, and solvent was removed. The resulting dark green solid was dissolved in HPLC buffer (50% acetonitrile in water with 0.1% trifluoroacetic acid) and purified by HPLC. Lyophilization gave a green solid (10 mg, 23.1%) as the product.

^{1}H NMR (300 MHz, DMSO-d_6) δ 7.43 (dq, J = 14.2, 8.4, 7.2 Hz, 3H), 7.13 (d, J = 7.2 Hz, 1H), 6.39 (d, J = 6.5 Hz, 2H), 3.79 – 3.68 (m, 2H), 3.66 – 3.34 (m, 10H), 3.22 – 3.09 (m, 2H), 2.44 (d, J = 6.6 Hz, 4H), 2.00 (d, J = 9.2 Hz, 7H), 1.86 – 1.76 (m, 4H), 1.19 (t, J = 7.0 Hz, 3H).

^{13}C NMR (75 MHz, DMSO-d_6) δ 151.11, 150.92, 137.41, 135.72, 135.23, 135.09, 132.09, 132.00, 130.71, 129.53, 129.48, 129.36, 127.78, 126.46, 125.31, 123.42, 123.31, 118.70, 62.77, 52.23, 51.28, 27.43, 25.49, 20.17, 20.09, 19.32, 16.44, 16.34.

^{31}P NMR (121 MHz, DMSO-d_6) δ 17.29.

MS (ESI) *m/z* calculated for $C_{34}H_{38}IN_2O_2P$ $[M-I]^+$ 537.7, found 537.7.

NR$_{698}$

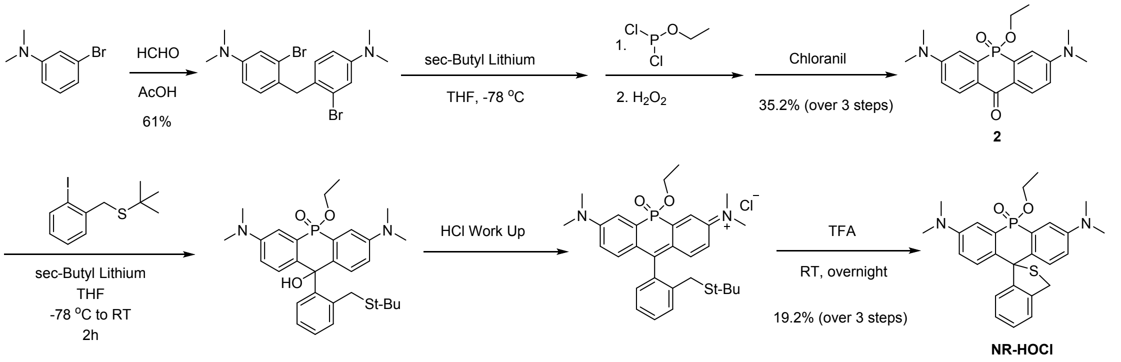
NR$_{744}$ was dissolved in HCl and the resulting orange solution was refluxed overnight. Further purification by HPLC yielded the product.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46 – 7.29 (m, 3H), 7.08 (d, J = 7.5 Hz, 1H), 6.43 (d, J = 6.1 Hz, 2H), 3.65 (s, 4H), 3.50 (q, J = 4.5 Hz, 8H), 2.47 (d, J = 6.1 Hz, 4H), 2.10 (d, J = 8.8 Hz, 7H), 1.97 – 1.88 (m, 4H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 151.55, 151.37, 136.37, 136.18, 136.05, 130.55, 129.97, 129.45, 128.57, 125.45, 123.99, 123.88, 123.28, 52.10, 51.53, 27.62, 25.76, 25.67, 20.54, 19.77.

^{31}P NMR (121 MHz, $CDCl_3$) δ 9.54.

MS (ESI) *m/z* calculated for $C_{32}H_{33}N_2O_2P$ $[M+H]^+$ 509.6, found 510.0.



4,4'-methylenebis(3-bromo-*N,N*-dimethylaniline)

3-bromo-*N,N*-dimethylaniline (10 g, 50 mmol) and formaldehyde (37 wt. % in H_2O, 7.5 g, 250 mmol) were dissolved in acetic acid (200 mL) and the mixture was stirred at 80 ºC for 1 hr. Solvent was removed by evaporation and saturated sodium bicarbonate was added. The resulting mixture was extracted by DCM and the organic layer was collected, dried over sodium sulfate, and filtered. Solvent was removed and flash chromatography (10% DCM in hexane) was performed to yield the product as a white solid (6.29 g, 61%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.98 (d, *J* = 2.7 Hz, 2H), 6.89 (d, *J* = 8.5 Hz, 2H), 6.62 (dd, *J* = 8.5, 2.7 Hz, 2H), 4.05 (s, 2H), 2.95 (s, 12H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 150.17, 130.91, 127.22, 125.75, 116.38, 111.99, 40.66, 40.02.

MS (ESI) *m/z* calculated for $C_{17}H_{20}Br_2N_2$ $[M+H]^+$ 413.2, found 413.8.

2

In a similar manner to the synthesis of **1**; to a flame dried round bottom flask charged with 4,4'-methylenebis(3-bromo-*N,N*-dimethylaniline) (5 g, 12.13 mmol), anhydrous THF (80 mL) was add and the temperature was lowered to -78 ºC with a dry ice/acetone bath. *Sec*-butyllithium (1.4 M in cyclohexane, 19.1 mL, 26.69 mmol) was then added dropwise into the reaction over 20 min and the mixture was stirred for an additional 1 hr followed by dropwise addition of ethyl dichlorophosphite (1.525 mL, 13.34 mmol) over 40 min. The reaction was allowed to proceed for 3 hrs and then the dry ice/acetone bath was replaced by an ice bath and allowed to proceed for an additional 2 hrs. Hydrogen peroxide (50 wt. % in H_2O, 8 mL) was added dropwise into the reaction and the reaction was stirred for 1 hr. Saturated sodium sulfite solution was then added to quench the reaction and ethyl acetate was used for extraction. The organic layer was collected, dried over sodium sulfate, filtered, and the solvent was removed. The resulting solid was further dissolved in DCM (50 mL) and chloranil (6 g, 24.26 mmol) was added. After stirring overnight, the reaction was filtered, washed with DCM, and the solvent was removed. Flash chromatography (5-15% acetone in DCM with 0.5% trimethylamine) yielded the pure product as a green-yellow solid (1.53 g, 35.2%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (tq, J = 6.8, 2.6, 2.0 Hz, 2H), 7.23 (ddd, J = 15.2, 4.1, 2.6 Hz, 2H), 6.91 (ddd, J = 9.1, 4.1, 2.6 Hz, 2H), 3.90 (ddd, J = 9.9, 5.6, 2.8 Hz, 2H), 3.21 – 3.09 (m, 12H), 1.24 – 1.11 (m, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 180.26, 152.47, 152.34, 132.52, 131.47, 131.36, 131.28,

125.42, 125.34, 114.82, 114.80, 110.69, 110.63, 61.78, 61.72, 40.26, 16.35, 16.28.
^{31}P NMR (162 MHz, CDCl3) δ 16.02.
MS (ESI) *m/z* calculated for $C_{19}H_{23}N_2O_3P$ [M+H]$^+$ 359.4, found 359.7.

NR-HOCl

Tert-butyl(2-iodobenzyl)sulfane (256 mg, 0.84 mmol) was added to a flame dried round bottom flask containing anhydrous THF (4 mL). The temperature was lowered to -78 ºC with a dry ice/acetone bath followed by dropwise addition of s*ec*-butyllithium (1.4 M in cyclohexane, 0.6 mL, 0.84 mmol). After 1 hr, a solution of **2** (100 mg, 0.28 mmol) in anhydrous THF (4 mL, partially dissolved by sonication) was added dropwise into the above reaction and the dry ice bath was replaced by an ice bath. The reaction was then allowed to proceed for 2 hrs. The reaction was quenched by 2 N HCl and stirred at 0 ºC for 30 min. The pH was then adjusted to 7 by adding a saturated sodium bicarbonate solution and the resulting mixture was extracted by ethyl acetate. After the organic layer was dried, filtered, and the solvent was removed, the resulting yellow oil was charged with trifluoroacetic acid (3 mL) and stirred overnight. The solvent was then removed and HPLC buffer (50% acetonitrile in water with 0.1% trifluoroacetic acid) was added. After purification by HPLC, a white-green solid was obtained (25 mg, 19.2%). NMR showed a mixture containing ~30% free thiol (ring-opened form), which is due to protonation during HPLC purification. Once dissolved in PBS (pH = 7.4) the solution showed no significant background fluorescence, indicating that **NR-HOCl** was regenerated.
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.58 – 7.40 (m, 3H), 7.32 (d, J = 7.4 Hz, 1H), 7.26 – 7.11 (m, 1H), 7.04 – 6.87 (m, 3H), 6.80 (q, J = 8.3 Hz, 2H), 4.45 (s, 2H), 4.10 (m, 2H), 3.06 (s, 12H), 1.34 (q, J = 6.9, 6.1 Hz, 3H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 147.65, 147.48, 146.52, 142.66, 139.95, 139.68, 139.56, 132.91, 132.75, 130.24, 130.08, 128.01, 127.96, 127.79, 127.51, 127.46, 126.77, 126.14, 125.04, 124.93, 124.83, 118.62, 118.29, 113.51, 113.42, 110.81, 110.73, 62.30, 62.21, 41.16, 40.87, 37.84, 16.39, 16.29.
^{31}P NMR (121 MHz, $CDCl_3$) δ 20.44.
MS (ESI) *m/z* calculated for $C_{26}H_{29}N_2O_2PS$ [M+H]$^+$ 465.6, found 465.1.

Computational Calculation Results

I. **NR**$_{700}$ calculation details

a. The optimized geometry from B3LYP/FixSol/6-31++G(d,p) for **NR**$_{700}$ is:

C	6.0	-0.7036367424109	-0.0046630611845	0.5350777150810
C	6.0	0.5356910933291	-0.5939337888211	0.6640300966447
C	6.0	0.7320969512868	-2.0026482582472	0.4718983532082
C	6.0	-0.4429158675526	-2.7524877758396	0.1542962625575
C	6.0	-1.6833112586263	-2.1783346130363	0.0146247176847
C	6.0	-1.8673746643670	-0.7726298727957	0.1970097798733
H	1.0	-0.7975698520608	1.0661810444291	0.7011934183130
H	1.0	-0.3579767803706	-3.8276105500815	0.0079397418917
H	1.0	-2.5230286820393	-2.8130330432951	-0.2470745403600
C	6.0	2.0016830350339	-2.6487842562134	0.5610385776151
P	15.0	1.8983035437105	0.4798250115526	1.1835578461652
C	6.0	3.2380611080406	-1.9931952008809	0.8365494520591
C	6.0	3.3589314162278	-0.5873958026462	1.1039382842734
C	6.0	4.5760756100170	0.0020873563493	1.3663953823063
C	6.0	4.4624809252344	-2.7326632977526	0.8452820935051
C	6.0	5.7915608223908	-0.7609064659206	1.3866719674562
C	6.0	5.6839175730446	-2.1571212882243	1.0972531166956
N	7.0	6.9731579865088	-0.1777265943967	1.6704791245907
C	6.0	7.0693603135855	1.2709311763888	1.8938186744951
C	6.0	8.2141618505672	-0.9615716728289	1.6853741094500
N	7.0	-3.0752738727097	-0.1883390532232	0.0649407781267
C	6.0	-3.2311111526852	1.2659791215734	0.2011575376802
C	6.0	-4.2616318145147	-0.9884680446712	-0.2644176857964
H	1.0	-2.5957811865888	1.7970997371119	-0.5212579031138
H	1.0	-2.9730115341699	1.5946012597125	1.2178290728127
H	1.0	-4.2752040715081	1.5218427022850	0.0049753205020
H	1.0	-5.1472800758543	-0.3590343169962	-0.1489911116965
H	1.0	-4.3495475347437	-1.8473202402141	0.4131155794371
H	1.0	-4.2145414577460	-1.3558056662137	-1.3007507161775
H	1.0	6.4400922785834	1.5822507464774	2.7387193401565
H	1.0	6.7654319779989	1.8297353141439	0.9970173902992
H	1.0	8.1090504447875	1.5170484539439	2.1225519940866
H	1.0	8.5203493588448	-1.2358549611312	0.6640056155196
H	1.0	8.9995488372066	-0.3561858080442	2.1441350705162
H	1.0	8.0867822948916	-1.8764257181924	2.2777562073104
H	1.0	4.6144770383747	1.0706566626230	1.5685815062400
H	1.0	6.5716736620256	-2.7810204235314	1.0630564604211
H	1.0	4.4301400273053	-3.8004837912474	0.6383856704017
O	8.0	1.6876779512793	1.1376918426796	2.5199977323748
O	8.0	2.0659847311375	1.6252395360563	0.0517779987396
C	6.0	2.0361538400230	-4.1410558956886	0.3731606430587

C	6.0	2.1604009564403	-4.7247495628192	-0.9064223037390
C	6.0	2.1731792362305	-6.1265318055153	-0.9946449005704
C	6.0	2.0779286179285	-6.9346726417594	0.1416022579091
C	6.0	1.9646853025509	-6.3456393859086	1.4045723135816
C	6.0	1.9423228036407	-4.9533135297625	1.5146135616754
H	1.0	2.2560168041936	-6.5848167790608	-1.9803396166009
H	1.0	2.0885680608513	-8.0200766583337	0.0402453558587
H	1.0	1.8972849810121	-6.9613977988282	2.3000779282841
H	1.0	1.8563319778039	-4.4886110661794	2.4969203801774
C	6.0	2.3072560462038	-3.8864611761374	-2.1564375113849
H	1.0	1.5975001640613	-3.0495795235957	-2.1754652290298
H	1.0	3.3177930424363	-3.4549819832615	-2.2248040730269
H	1.0	2.1452115746870	-4.4982746495671	-3.0519399975789
C	6.0	2.3621759482976	1.3322844111951	-1.3534522755144
C	6.0	2.1671388445191	2.6090034029470	-2.1475001500137
H	1.0	2.8177512293074	3.4073022583232	-1.7685898532342
H	1.0	2.4251229204162	2.4203210296485	-3.1987716724562
H	1.0	1.1243865147361	2.9476550860222	-2.1002211924245
H	1.0	3.3982971242667	0.9739285285498	-1.4125555547577
H	1.0	1.6885884786011	0.5368740572285	-1.7012838145122

b. The FixSol/TD-B3LYP/6-31++G(d,p) excitation energies for **NR_{700}** at S_0 ground state geometry. There are five transitions, S_0->S_1 at 1.910 eV with an oscillator strength 1.226, S_0->S_4 at 3.169 eV with an oscillator strength 0.229, S_0->S_7 at 3.736 eV with an oscillator strength 0.347, S_0->S_9 at 4.186 eV with an oscillator strength 0.221 and S_0->S_{14} at 4.740 eV with an oscillator strength 0.130:

```
  STATE            ENERGY      EXCITATION   TRANSITION DIPOLE, A.U.   OSCILLATOR
                  HARTREE         EV          X        Y        Z      STRENGTH
  0  A      -1609.2951600819    0.000
  1  A      -1609.2249668793    1.910     -5.0572 -0.0066 -0.7857     1.226
  2  A      -1609.1967153343    2.679      0.0148  0.2935  0.0545     0.006
  3  A      -1609.1886282485    2.899      0.2004  0.1965  0.0378     0.006
  4  A      -1609.1787130569    3.169     -0.0275  1.7036  0.2035     0.229
  5  A      -1609.1707763002    3.385     -0.8980 -0.0469 -0.1403     0.069
  6  A      -1609.1616416976    3.633      0.0344 -0.3142 -0.1098     0.010
  7  A      -1609.1578614274    3.736      1.9252  0.0177  0.2860     0.347
  8  A      -1609.1539839788    3.842     -0.6953  0.0166 -0.0980     0.046
  9  A      -1609.1413219273    4.186      0.0662 -1.4447 -0.2465     0.221
 10  A      -1609.1367188641    4.311     -0.0532  0.0122 -0.0600     0.001
 11  A      -1609.1339960655    4.385      0.0578 -0.1741 -0.2362     0.010
 12  A      -1609.1265839575    4.587     -0.4843  0.0024 -0.0900     0.027
 13  A      -1609.1256203122    4.613      0.0609  0.2508 -0.0231     0.008
 14  A      -1609.1209783694    4.740      0.0265 -1.0569 -0.0648     0.130
 15  A      -1609.1190505687    4.792     -0.0718  0.3333  0.0500     0.014
```

c. The $S_0 \rightarrow S_1$ transition for **NR$_{700}$** is mainly between MO115 (HOMO) and MO116 (LUMO):

```
STATE #     1    ENERGY =       1.910054 EV
OSCILLATOR STRENGTH =        1.225710
LAMBDA DIAGNOSTIC     =       0.680 (RYDBERG/CHARGE TRANSFER CHARACTER)
SYMMETRY OF STATE     =       A
                          EXCITATION    DE-EXCITATION
       OCC        VIR   AMPLITUDE          AMPLITUDE
         I           A        X(I->A)             Y(A->I)
       ---        ---     --------            --------
      108        116    -0.033321            0.001789
      111        116      0.035314            0.004872
      115        116    -0.999408            0.093683
      115        117    -0.031936           -0.004740
```

d. The FixSol/B3LYP/6-31++G(d,p) optimized S_1 state geometry of **NR$_{700}$** is:

C	6.0	-0.7015546943319	0.0016679133867	0.5397445557073
C	6.0	0.5493622829152	-0.5905463302334	0.6699879016907
C	6.0	0.7388028490596	-2.0056898579341	0.4666984226435
C	6.0	-0.4422796851571	-2.7518823819163	0.1461614426916
C	6.0	-1.6780714055664	-2.1700700526104	0.0104441702547
C	6.0	-1.8563939273234	-0.7585967544674	0.1985886871812
H	1.0	-0.7929014535584	1.0717282355185	0.7134600780266
H	1.0	-0.3597705247209	-3.8264221592755	-0.0044504347933
H	1.0	-2.5214375629726	-2.7992481148947	-0.2553933256470
C	6.0	2.0056854646845	-2.6737983497946	0.5507973498667
P	15.0	1.8975892209359	0.4789525285048	1.1876650552128
C	6.0	3.2362998845913	-1.9942136483536	0.8282769097290
C	6.0	3.3445155716367	-0.5832917829150	1.1114806945808
C	6.0	4.5716107176586	0.0094221465167	1.3835905425358
C	6.0	4.4700002533207	-2.7257925709121	0.8252384100753
C	6.0	5.7814762943801	-0.7426625928005	1.3913758574621
C	6.0	5.6843618600422	-2.1419389666089	1.0840221601656
N	7.0	6.9747785279136	-0.1641038947399	1.6780733660394
C	6.0	7.0672863657695	1.2768677996476	1.9169747282618
C	6.0	8.2158093449024	-0.9421379866457	1.6767945697582
N	7.0	-3.0760531556356	-0.1792347632654	0.0616528411795
C	6.0	-3.2309081720156	1.2704095313957	0.1914774258665
C	6.0	-4.2604545932273	-0.9781342501465	-0.2635477303177
H	1.0	-2.5839251657687	1.7969413885018	-0.5267930559159
H	1.0	-2.9707145852628	1.6010111692811	1.2093194061378
H	1.0	-4.2730940332582	1.5305747548584	-0.0093690080550

H	1.0	-5.1462929917212	-0.3495747449431	-0.1427834800062
H	1.0	-4.3433513268846	-1.8430196326511	0.4082942231550
H	1.0	-4.2172338812636	-1.3416897752983	-1.3034884290995
H	1.0	6.4351601343443	1.5760605087744	2.7665172685485
H	1.0	6.7493139323230	1.8438256916353	1.0276283494911
H	1.0	8.1064259258875	1.5280352232266	2.1429136011593
H	1.0	8.5179667511805	-1.2002010825881	0.6477364305324
H	1.0	9.0016702466900	-0.3396180711185	2.1390365868326
H	1.0	8.0960572977798	-1.8693193236855	2.2529282814093
H	1.0	4.6027428833943	1.0754215962096	1.6010448098489
H	1.0	6.5784046154038	-2.7575382971079	1.0400834177051
H	1.0	4.4440693072494	-3.7905056032307	0.6037213071410
O	8.0	1.6860230607713	1.1797563419550	2.5073318865711
O	8.0	2.0689315606203	1.6396166876207	0.0558211978346
C	6.0	2.0385848739063	-4.1629216363491	0.3724558727013
C	6.0	2.1484976887286	-4.7560934194108	-0.9059323869611
C	6.0	2.1588609712258	-6.1579701975484	-0.9924257860890
C	6.0	2.0759451988716	-6.9635846684145	0.1471559948468
C	6.0	1.9778785870047	-6.3707740031096	1.4095217772976
C	6.0	1.9567535086484	-4.9774845949013	1.5147096051805
H	1.0	2.2300100990658	-6.6193850910872	-1.9776728208858
H	1.0	2.0847247947163	-8.0492932575258	0.0484372364429
H	1.0	1.9207500816620	-6.9844154663209	2.3073507118021
H	1.0	1.8809802829715	-4.5108044546470	2.4970035475115
C	6.0	2.2808490718150	-3.9200555269068	-2.1591747114415
H	1.0	1.5614136483394	-3.0913701913575	-2.1770697897670
H	1.0	3.2852477703536	-3.4749732644762	-2.2314051807960
H	1.0	2.1236434840901	-4.5352588661843	-3.0532850918721
C	6.0	2.3617000633814	1.3405100375313	-1.3436903391039
C	6.0	2.1814240773654	2.6165042694378	-2.1443245331990
H	1.0	2.8383771441331	3.4103757757997	-1.7666920840840
H	1.0	2.4392154647732	2.4230863212159	-3.1947834612977
H	1.0	1.1418993316144	2.9658120070009	-2.0995015269093
H	1.0	3.3938575715265	0.9702266983042	-1.4063984826679
H	1.0	1.6810677953876	0.5515417884016	-1.6926305882553

e. The FixSol/TD-B3LYP/6-31++G(d,p) fluorescent de-excitation energy (S_1->S_0) for **NR$_{700}$** at the optimized S_1 state geometry is 1.833 eV with an oscillator strength 1.147:

```
STATE          ENERGY       EXCITATION  TRANSITION DIPOLE, A.U.  OSCILLATOR
               HARTREE          EV           X       Y       Z     STRENGTH
  0  A    -1609.2937031594    0.000
  1  A    -1609.2263521611    1.833    -4.9927 -0.0049 -0.7807     1.147
```

II. **NR$_{666}$** calculation details

a. The optimized geometry from B3LYP/FixSol/6-31++G(d,p) for **NR$_{666}$** is:

C	6.0	-0.7006236528455	0.0706509162633	0.4920862852496
C	6.0	0.5622602547146	-0.4696798408531	0.6320394144998
C	6.0	0.7937016653561	-1.8737469472296	0.4332387985033
C	6.0	-0.3516404872331	-2.6541034890465	0.0753518067566
C	6.0	-1.6090250722069	-2.1211110286109	-0.0677968265507
C	6.0	-1.8373060711646	-0.7254444622288	0.1444980867760
H	1.0	-0.8283783663707	1.1375409769657	0.6551639080928
H	1.0	-0.2323933023743	-3.7212741146250	-0.0951597179187
H	1.0	-2.4234531367144	-2.7770912152419	-0.3561713273797
C	6.0	2.0738807803366	-2.4938176288857	0.5623115004228
P	15.0	1.9053771396409	0.7012063000944	1.0611470725825
C	6.0	3.2812871250551	-1.8208921056414	0.9152703060541
C	6.0	3.3592930808929	-0.4100208417492	1.1746465176072
C	6.0	4.5583238106756	0.1812614669188	1.5141827209506
C	6.0	4.5080659181935	-2.5531975046282	1.0230286726486
C	6.0	5.7749641287215	-0.5618605044790	1.6273989062947
C	6.0	5.7038315737428	-1.9658432241979	1.3579693957019
N	7.0	6.9347933079407	0.0422860640190	1.9726870790280
C	6.0	7.0008766749391	1.4968634611205	2.1684275778140
C	6.0	8.1821050542606	-0.7243222434877	2.0681064297397
N	7.0	-3.0690420002226	-0.1817332505753	0.0212928578753
C	6.0	-3.2700548017822	1.2641076725347	0.1713251116946
C	6.0	-4.2283977780169	-1.0161173578724	-0.3141072020256
H	1.0	-2.6550677276324	1.8234880432761	-0.5476801203569
H	1.0	-3.0166442628989	1.5935883728867	1.1890646577749
H	1.0	-4.3224090214858	1.4916722041866	-0.0170696399405
H	1.0	-5.1344501977622	-0.4172861711669	-0.1924294664323
H	1.0	-4.2897018354601	-1.8836042099435	0.3555213084093
H	1.0	-4.1749315739663	-1.3741504737551	-1.3535134551766
H	1.0	6.3347786218817	1.8180243584018	2.9811210142201
H	1.0	6.7226515221742	2.0332215311282	1.2497758650838
H	1.0	8.0271772212692	1.7646745346359	2.4307013773114
H	1.0	8.5384163157633	-1.0302332379310	1.0722591671553
H	1.0	8.9405380241938	-0.0947612471855	2.5401231307265
H	1.0	8.0407502133384	-1.6207007079303	2.6852885685304
H	1.0	4.5782275396803	1.2528159862808	1.7010033458467
H	1.0	6.5954137743798	-2.5829450556093	1.4009746290778
H	1.0	4.4981984016187	-3.6238714321230	0.8297133933539
O	8.0	1.6256992655670	1.2945334571796	2.4416299320426
O	8.0	2.1146655569870	1.6559289714327	-0.1151418235912
C	6.0	2.1546022833679	-3.9791139918966	0.3224292900910
C	6.0	2.4547604527682	-4.4950586669808	-0.9571316664151
C	6.0	2.5074134520491	-5.8900082437947	-1.1143962911978

C	6.0	2.2796201824229	-6.7572904843136	-0.0425711446159
C	6.0	1.9883451762176	-6.2356826312004	1.2217500084372
C	6.0	1.9247351862867	-4.8509919302644	1.3979888080755
H	1.0	2.7311827975337	-6.2941019818455	-2.1020127511963
H	1.0	2.3216705470932	-7.8357921580599	-0.1949683180257
H	1.0	1.8073438536840	-6.8996183811744	2.0650966174696
H	1.0	1.6921388059818	-4.4406491077422	2.3806517387556
C	6.0	2.7373716660880	-3.5892266775442	-2.1352659871595
H	1.0	2.0573505052056	-2.7284171043829	-2.1599564343266
H	1.0	3.7632438067561	-3.1926997045417	-2.0901064419651
H	1.0	2.6348994916278	-4.1398424069258	-3.0780837284634

b. The FixSol/TD-B3LYP/6-31++G(d,p) excitation energies for **NR$_{666}$** at S_0 ground state geometry. There are four transitions, S_0->S_1 at 2.053 eV with an oscillator strength 1.398, S_0->S_7 at 3.370 eV with an oscillator strength 0.278, S_0->S_{10} at 4.004 eV with an oscillator strength 0.145 and S_0->S_{12} at 4.267 eV with an oscillator strength 0.180:

```
STATE              ENERGY      EXCITATION   TRANSITION DIPOLE, A.U.   OSCILLATOR
                   HARTREE          EV          X        Y        Z     STRENGTH
  0  A     -1530.2798020554      0.000
  1  A     -1530.2043644736      2.053    -5.1757 -0.1211 -0.9991      1.398
  2  A     -1530.1715506057      2.946    -0.0514 -0.0107  0.0042      0.000
  3  A     -1530.1688050362      3.020     0.0237 -0.1165 -0.0049      0.001
  4  A     -1530.1676033679      3.053     0.5920  0.0733  0.1238      0.028
  5  A     -1530.1651263272      3.120     0.2919 -0.0924  0.0503      0.007
  6  A     -1530.1613225602      3.224     0.3573 -0.3244  0.0184      0.018
  7  A     -1530.1559460111      3.370     0.0308 -1.8061 -0.3133      0.278
  8  A     -1530.1354138232      3.929    -0.0652 -0.0132  0.3137      0.010
  9  A     -1530.1339449107      3.969     0.0075  0.3055  0.0367      0.009
 10  A     -1530.1326578445      4.004    -1.1910 -0.0597 -0.2395      0.145
 11  A     -1530.1266614442      4.167    -0.5472 -0.0398 -0.1139      0.032
 12  A     -1530.1230084041      4.267    -0.1236  1.2873  0.2262      0.180
 13  A     -1530.1147787339      4.491     0.0711 -0.3652  0.0086      0.015
 14  A     -1530.1067066197      4.710    -0.0495 -0.3314  0.0122      0.013
 15  A     -1530.1061256816      4.726    -0.1191  0.4556 -0.0708      0.026
```

c. The $S_0 \rightarrow S_1$ transition for **NR$_{666}$** is mainly between MO107 (HOMO) and MO108 (LUMO):

```
STATE #    1   ENERGY =       2.052761 EV
OSCILLATOR STRENGTH =       1.398163
LAMBDA DIAGNOSTIC    =     0.681 (RYDBERG/CHARGE TRANSFER CHARACTER)
SYMMETRY OF STATE    =     A
                    EXCITATION   DE-EXCITATION
   OCC      VIR   AMPLITUDE         AMPLITUDE
    I        A      X(I->A)          Y(A->I)
```

---	---	--------	--------
104	108	-0.035768	-0.000881
107	108	-0.998572	0.085093

d. The FixSol/B3LYP/6-31++G(d,p) optimized S_1 state geometry of **NR$_{666}$** is:

C	6.0	-0.6969221124613	0.0758968537659	0.4892403020865
C	6.0	0.5735918020738	-0.4689827791028	0.6280198074396
C	6.0	0.7962552105231	-1.8785853564830	0.4328869383796
C	6.0	-0.3592410333831	-2.6569793222104	0.0890868805690
C	6.0	-1.6146711113446	-2.1151328065371	-0.0484528391885
C	6.0	-1.8326791031326	-0.7134345978688	0.1516427241719
H	1.0	-0.8183997579817	1.1446227076899	0.6487445672301
H	1.0	-0.2445738297133	-3.7261157339241	-0.0731839575481
H	1.0	-2.4357437668360	-2.7683358390917	-0.3264084062836
C	6.0	2.0795504358328	-2.5191284931100	0.5504250684247
P	15.0	1.9107225087957	0.7030726617627	1.0459192210273
C	6.0	3.2867117794418	-1.8226778021370	0.9017242967430
C	6.0	3.3538910660271	-0.4099774538281	1.1736189370747
C	6.0	4.5565132022627	0.1840442884249	1.5312773126949
C	6.0	4.5235445614425	-2.5466682788073	0.9995928883733
C	6.0	5.7716775340534	-0.5495536590457	1.6385100141387
C	6.0	5.7134758807870	-1.9518376306056	1.3455522035334
N	7.0	6.9381031467142	0.0550695850964	1.9995744024073
C	6.0	6.9928014097640	1.5000766942288	2.2261081210814
C	6.0	8.1896233966346	-0.7002383160841	2.0612404678102
N	7.0	-3.0726070662903	-0.1653647457998	0.0206732827968
C	6.0	-3.2630684296540	1.2796096569632	0.1403517973836
C	6.0	-4.2340063597479	-0.9965375114068	-0.2973090482231
H	1.0	-2.6377922326758	1.8187212269496	-0.5882009651885
H	1.0	-3.0008885618750	1.6279753269443	1.1517225258364
H	1.0	-4.3134744654254	1.5136208117577	-0.0510612763851
H	1.0	-5.1378108304157	-0.3940756893222	-0.1748016797277
H	1.0	-4.2906344617937	-1.8623513909449	0.3764634631199
H	1.0	-4.1885244635396	-1.3638755784528	-1.3362665382333
H	1.0	6.3021194556528	1.8010930760417	3.0279130102924
H	1.0	6.7247905057853	2.0537979905336	1.3118037555581
H	1.0	8.0108367678105	1.7704153811787	2.5163285314743
H	1.0	8.5323424605106	-0.9827934726744	1.0513163199479
H	1.0	8.9506992085071	-0.0742536873490	2.5339748104253
H	1.0	8.0658116955205	-1.6150261017140	2.6570026078084
H	1.0	4.5680938207434	1.2539162578601	1.7308616434654
H	1.0	6.6107984651104	-2.5622855919420	1.3815208058628
H	1.0	4.5213142252616	-3.6140304980852	0.7905702918921
O	8.0	1.6282891779008	1.3301951521337	2.4150187694787
O	8.0	2.1241289305999	1.6607740828863	-0.1323278723169

C	6.0	2.1593056014746	-3.9997106003983	0.3162183124869
C	6.0	2.4405013263871	-4.5248362555897	-0.9660303205285
C	6.0	2.4980250751489	-5.9198695230336	-1.1220960531573
C	6.0	2.2902155031692	-6.7858763957311	-0.0446833486075
C	6.0	2.0152340817678	-6.2616280648625	1.2221862418344
C	6.0	1.9498684459066	-4.8759166521335	1.3944856811779
H	1.0	2.7084135369420	-6.3261833625474	-2.1119124980901
H	1.0	2.3344875294161	-7.8646170641253	-0.1949913256392
H	1.0	1.8504347122935	-6.9240997925273	2.0701925348664
H	1.0	1.7315606639085	-4.4645448090131	2.3801797026843
C	6.0	2.6953913112073	-3.6205499589241	-2.1515737581785
H	1.0	1.9973111144426	-2.7744342894913	-2.1733968839291
H	1.0	3.7118553480164	-3.1995153511912	-2.1160221187219
H	1.0	2.5991825924406	-4.1773147481896	-3.0915263894554

e. The FixSol/TD-B3LYP/6-31++G(d,p) fluorescent de-excitation energy (S_1->S_0) for **NR$_{666}$** at the optimized S_1 state geometry is 2.001 eV with an oscillator strength 1.351:

```
STATE          ENERGY      EXCITATION   TRANSITION DIPOLE, A.U.   OSCILLATOR
               HARTREE         EV          X        Y        Z     STRENGTH
 0  A      -1530.2788518545   0.000
 1  A      -1530.2053177477   2.001    -5.1528 -0.1187 -0.9997     1.351
```

III. Tetramethylrhodamine (TMR) calculation details

a. The optimized geometry from B3LYP/FixSol/6-31++G(d,p) for TMR is:

C	6.0	-0.3369575952640	0.0496477959028	0.5974864177658
C	6.0	0.7998026364721	-0.7316259093951	0.6554307868221
C	6.0	0.7977792173890	-2.1374356864180	0.4051013107341
C	6.0	-0.4639348234568	-2.7119419764196	0.0622370527672
C	6.0	-1.6056076918764	-1.9587051881916	-0.0141726413349
C	6.0	-1.5848577411438	-0.5451479587026	0.2651188930808
H	1.0	-0.2458970273350	1.1133175053332	0.8000435536057
H	1.0	-0.5132850519109	-3.7790828415368	-0.1489263448099
H	1.0	-2.5369141792476	-2.4404750605898	-0.2920437040722
C	6.0	2.0074292497609	-2.8590402197185	0.4845453598683
C	6.0	3.1949375907781	-2.1665537836263	0.8023178288308
C	6.0	3.1473157880421	-0.7607068701704	1.0451960947320
C	6.0	4.2613271228014	-0.0114434489542	1.3683220081531
C	6.0	4.4811300456327	-2.7787540148494	0.9008672632000
C	6.0	5.5295409451440	-0.6446415793153	1.4792357465213
C	6.0	5.6033939805519	-2.0592872961108	1.2144505037507
N	7.0	6.6359265548188	0.0542305823036	1.8279848867016
C	6.0	6.5658137419653	1.5028960131681	2.0413295252512
C	6.0	7.9565444009359	-0.5851515276735	1.8532628616246
N	7.0	-2.7196904566988	0.1907091572977	0.2110456499376

C	6.0	-2.6828080505388	1.6267308583334	0.5015739724030
C	6.0	-3.9898265870614	-0.4041719084632	-0.2209246020443
H	1.0	-2.0911199985442	2.1708679636888	-0.2503541014727
H	1.0	-2.2466011572678	1.8071725904920	1.4930148917072
H	1.0	-3.7046460058477	2.0137413216234	0.4943959426520
H	1.0	-4.7734383510977	0.3518641971310	-0.1310880145165
H	1.0	-4.2604337139989	-1.2619624973292	0.4093688358197
H	1.0	-3.9376084054311	-0.7378394860539	-1.2677337031502
H	1.0	5.7758350889471	1.7520700478787	2.7614054295125
H	1.0	6.3641233286603	2.0368421181259	1.0995273453360
H	1.0	7.5228047002594	1.8442896606645	2.4434492789333
H	1.0	8.2968475233521	-0.8423734191776	0.8383193109886
H	1.0	8.6684779268363	0.1138853778214	2.2994011596954
H	1.0	7.9393284306386	-1.4967948474742	2.4634894144179
H	1.0	4.1431489898486	1.0554148791081	1.5289395114191
H	1.0	6.5597970993279	-2.5689953196435	1.2591801451632
H	1.0	4.5613977282797	-3.8483504816879	0.7172915227246
C	6.0	2.0231649741545	-4.3379732426023	0.2648667001025
C	6.0	2.3861606072598	-4.8922193528815	-0.9817499203030
C	6.0	2.3722925426144	-6.2910402871070	-1.1112733123767
C	6.0	2.0202884916672	-7.1228625334188	-0.0451238193856
C	6.0	1.6644023845111	-6.5627223304826	1.1855909302738
C	6.0	1.6639270777854	-5.1739041366287	1.3348703891422
H	1.0	2.6437094590942	-6.7268337344114	-2.0728763814245
H	1.0	2.0157864379510	-8.2051310668728	-0.1770117132311
H	1.0	1.3798534519455	-7.1983586400618	2.0221081111089
H	1.0	1.3805308236130	-4.7314723667679	2.2897613485389
C	6.0	2.7927836301809	-4.0290226118426	-2.1549732596509
H	1.0	2.1747219994864	-3.1252169366537	-2.2355315694855
H	1.0	3.8393903350992	-3.7011483625513	-2.0610587183515
H	1.0	2.7045385222217	-4.5908159914275	-3.0925743290626
O	8.0	1.9625078362132	-0.0876676993521	0.9676494977603

b. The FixSol/TD-B3LYP/6-31++G(d,p) excitation energies for TMR at S_0 ground state geometry. There are four transitions, S_0->S_1 at 2.351 eV with an oscillator strength 1.309, S_0->S_5 at 3.743 eV with an oscillator strength 0.161, S_0->S_7 at 4.182 eV with an oscillator strength 0.330, S_0->S_{10} at 4.730 eV with an oscillator strength 0.152 and S_0->S_{13} at 4.878 eV with an oscillator strength 0.794:

```
STATE          ENERGY        EXCITATION   TRANSITION DIPOLE, A.U.   OSCILLATOR
               HARTREE          EV          X         Y         Z     STRENGTH
 0  A      -1113.5893923230   0.000
 1  A      -1113.5029783417   2.351      4.6997 -0.0483   0.7954      1.309
 2  A      -1113.4774007206   3.047     -0.0106   0.5435   0.0748      0.022
 3  A      -1113.4686150373   3.287     -0.0412 -0.2067 -0.1094      0.005
```

```
  4   A          -1113.4611470740      3.490       0.2236 -0.7190 -0.0275      0.049
  5   A          -1113.4518239632      3.743       0.0373   1.3060   0.2274      0.161
  6   A          -1113.4361294513      4.170      -0.3251 -0.6963 -0.1719      0.063
  7   A          -1113.4357169568      4.182      -0.1357   1.7671   0.2905      0.330
  8   A          -1113.4253480925      4.464      -0.0437   0.2832   0.0078      0.009
  9   A          -1113.4212606908      4.575      -0.3644   0.2960 -0.0506      0.025
 10   A          -1113.4155700776      4.730       0.1410   1.1351   0.0628      0.152
 11   A          -1113.4149975649      4.746       0.0184 -0.3237 -0.1025      0.013
 12   A          -1113.4134838007      4.787      -0.1868   0.1458 -0.0739      0.007
 13   A          -1113.4101112663      4.878      -2.5429   0.0941 -0.4049      0.794
 14   A          -1113.4054424604      5.006      -0.0584   0.0218   0.7646      0.072
 15   A          -1113.4040262874      5.044       0.0978 -0.0022   0.0201      0.001
```

c. The $S_0 \rightarrow S_1$ transition for TMR is solely between MO95 (HOMO) and MO96 (LUMO):

```
STATE #    1   ENERGY =      2.351444 EV
 OSCILLATOR STRENGTH =       1.308985
 LAMBDA DIAGNOSTIC    =      0.655 (RYDBERG/CHARGE TRANSFER CHARACTER)
 SYMMETRY OF STATE    =      A
                         EXCITATION   DE-EXCITATION
     OCC        VIR   AMPLITUDE          AMPLITUDE
      I          A       X(I->A)            Y(A->I)
     ---       ---     --------           --------

     95         96     -0.997572          0.065580
```

d. The FixSol/B3LYP/6-31++G(d,p) optimized S_1 state geometry of TMR is:

C	6.0	-0.3440895173365	0.0455008053929	0.6051992370129
C	6.0	0.7924722272487	-0.7392916307334	0.6557220413066
C	6.0	0.7864574978684	-2.1411714108332	0.3955482148019
C	6.0	-0.4796500917619	-2.7015695723372	0.0511803152369
C	6.0	-1.6297888175771	-1.9427861060343	-0.0137017796041
C	6.0	-1.5988666262654	-0.5395969591000	0.2714996502119
H	1.0	-0.2477171730587	1.1080275731567	0.8145981764358
H	1.0	-0.5349058234087	-3.7661370728222	-0.1696818428276
H	1.0	-2.5626810955353	-2.4232136810499	-0.2906922334931
C	6.0	2.0035982778345	-2.8876316795737	0.4642731409375
C	6.0	3.1997683637322	-2.1685652607601	0.7764105265541
C	6.0	3.1455283489744	-0.7675850363480	1.0330881234595
C	6.0	4.2579522660656	-0.0155643161586	1.3623090285169
C	6.0	4.4921680927470	-2.7660769266702	0.8646864669959
C	6.0	5.5350808476454	-0.6381862317125	1.4604416283544
C	6.0	5.6206283632082	-2.0407275184476	1.1837102512904
N	7.0	6.6413818055167	0.0799658829404	1.8163451479259
C	6.0	6.5563002870424	1.5177507946460	2.0653181709312

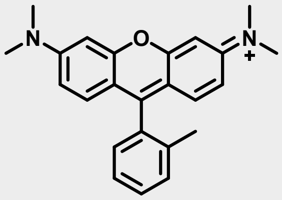
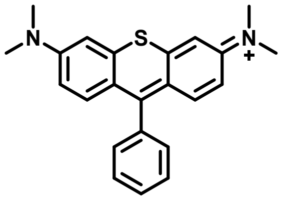
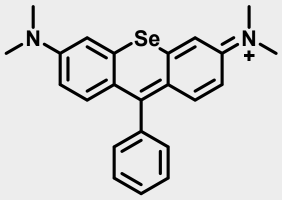
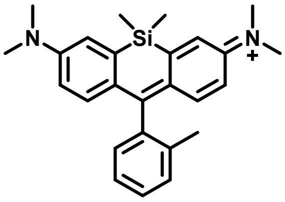
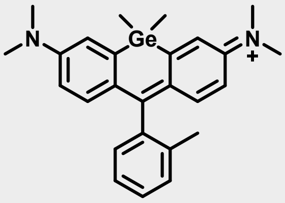
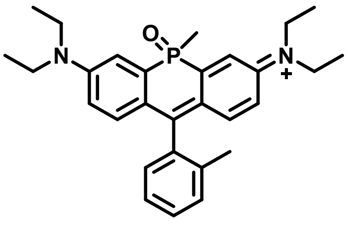
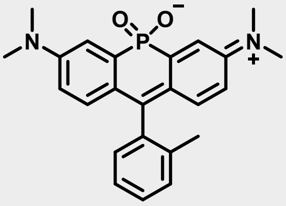
C	6.0	7.9618064546673	-0.5470219525288	1.8281735321920
N	7.0	-2.7338322073631	0.2183626947344	0.2249654754775
C	6.0	-2.6858962986692	1.6461158690816	0.5319693020828
C	6.0	-4.0047780417145	-0.3623357937224	-0.2044851353708
H	1.0	-2.0817905019139	2.1919084975821	-0.2115080820206
H	1.0	-2.2480312459428	1.8117393984493	1.5271218060183
H	1.0	-3.7038298434333	2.0433468477485	0.5225070700016
H	1.0	-4.7841122550752	0.3984759651881	-0.1179834445744
H	1.0	-4.2773967252527	-1.2234175775490	0.4242169449258
H	1.0	-3.9491347082709	-0.7025431496267	-1.2512202224551
H	1.0	5.7401428940848	1.7438414966352	2.7649081790124
H	1.0	6.3738310216387	2.0727398071519	1.1289576011578
H	1.0	7.4995245436453	1.8564762060811	2.5014329307527
H	1.0	8.2839448047710	-0.8104234655279	0.8064635258399
H	1.0	8.6779064756051	0.1572783253382	2.2584794048638
H	1.0	7.9549617589955	-1.4616274954487	2.4375940120228
H	1.0	4.1311943606243	1.0483630241668	1.5388714209229
H	1.0	6.5787897724937	-2.5487900665072	1.2229207830913
H	1.0	4.5803574963282	-3.8335091605520	0.6743080451720
C	6.0	2.0152282021470	-4.3628394001864	0.2548669894633
C	6.0	2.4240140933451	-4.9364713595751	-0.9720910270911
C	6.0	2.4146768756092	-6.3364097952243	-1.0937669143890
C	6.0	2.0149909618393	-7.1610592168975	-0.0385327421791
C	6.0	1.6053655822392	-6.5902087246891	1.1707671618558
C	6.0	1.6058853302724	-5.1998992243229	1.3095592052185
H	1.0	2.7258391781297	-6.7799283695998	-2.0399846525859
H	1.0	2.0149843114062	-8.2442651402489	-0.1629477284395
H	1.0	1.2818893526005	-7.2192297593014	1.9985836789573
H	1.0	1.2850394045976	-4.7518446430819	2.2501537422646
C	6.0	2.8705793805571	-4.0844054761150	-2.1389636502738
H	1.0	2.2623644514729	-3.1757414672330	-2.2372331246681
H	1.0	3.9168541725083	-3.7629247280749	-2.0207994172723
H	1.0	2.8014957942666	-4.6489432904189	-3.0766016243676
O	8.0	1.9566317461009	-0.0811411742565	0.9738060368747

e. The FixSol/TD-B3LYP/6-31++G(d,p) fluorescent de-excitation energy (S_1->S_0) for TMR at the optimized S_1 state geometry is 2.268 eV with an oscillator strength 1.266:

```
STATE          ENERGY      EXCITATION  TRANSITION DIPOLE, A.U.  OSCILLATOR
               HARTREE         EV          X       Y       Z     STRENGTH
0  A     -1113.5878528679   0.000
1  A     -1113.5044939006   2.268     -4.7093  0.0425 -0.7788     1.266
```

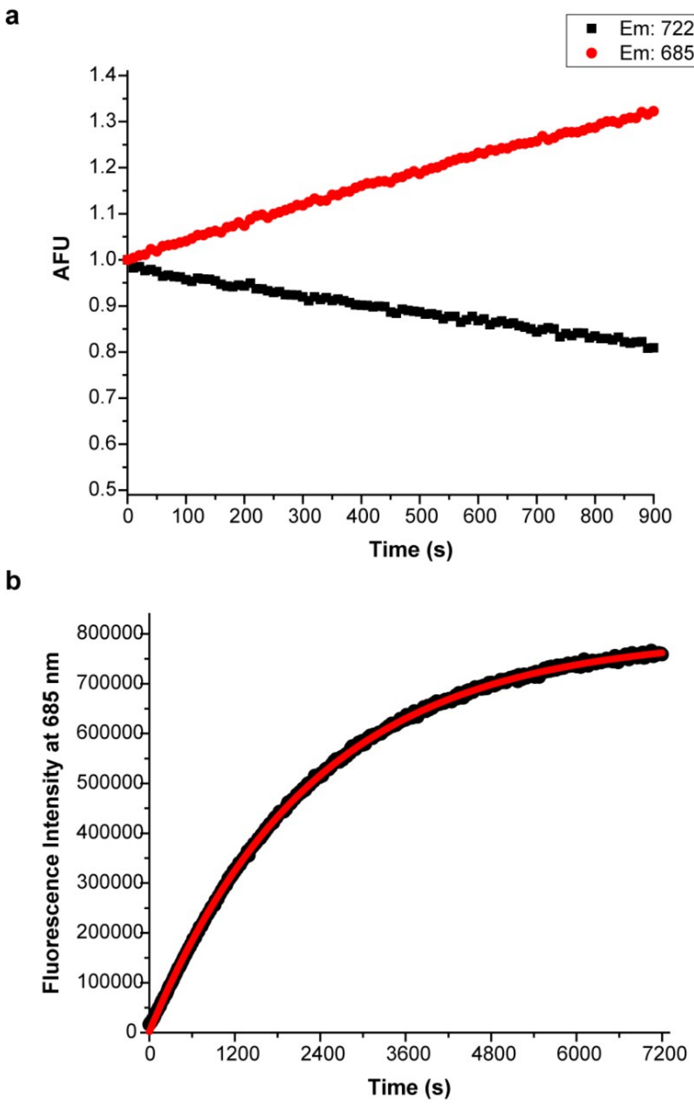
Table S1

TMR-based fluorophores

Fluorophores	Ex (nm)	Em (nm)	ε ($M^{-1}\cdot cm^{-1}$)	Φ	ε × Φ ($M^{-1}\cdot cm^{-1}$)	Ref.
	549	569	134,000	0.35	46,900	11
	571	599	62,600[a]	0.44[a]	27,544[a]	12
	581	608	44,000[a]	0.009[a]	396[a]	12
	646	660	110,000	0.31	34,100	11
	635	649	127,000	0.34	43,180	11
	694	712	92,000	0.11	10,120	13
	666	685	165,000	0.38	62,700	This work

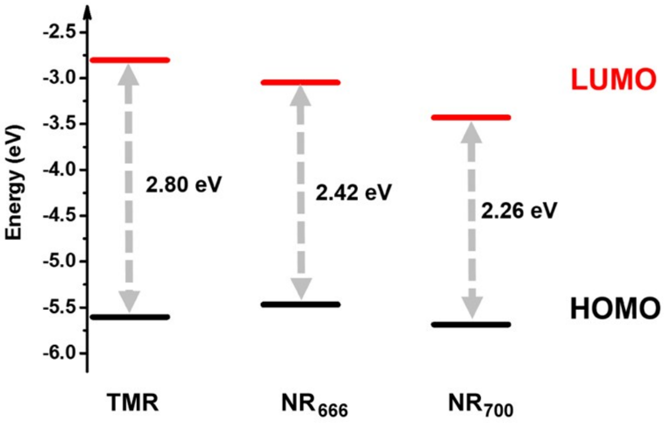
[a] Measured in methanol

Fig. S1



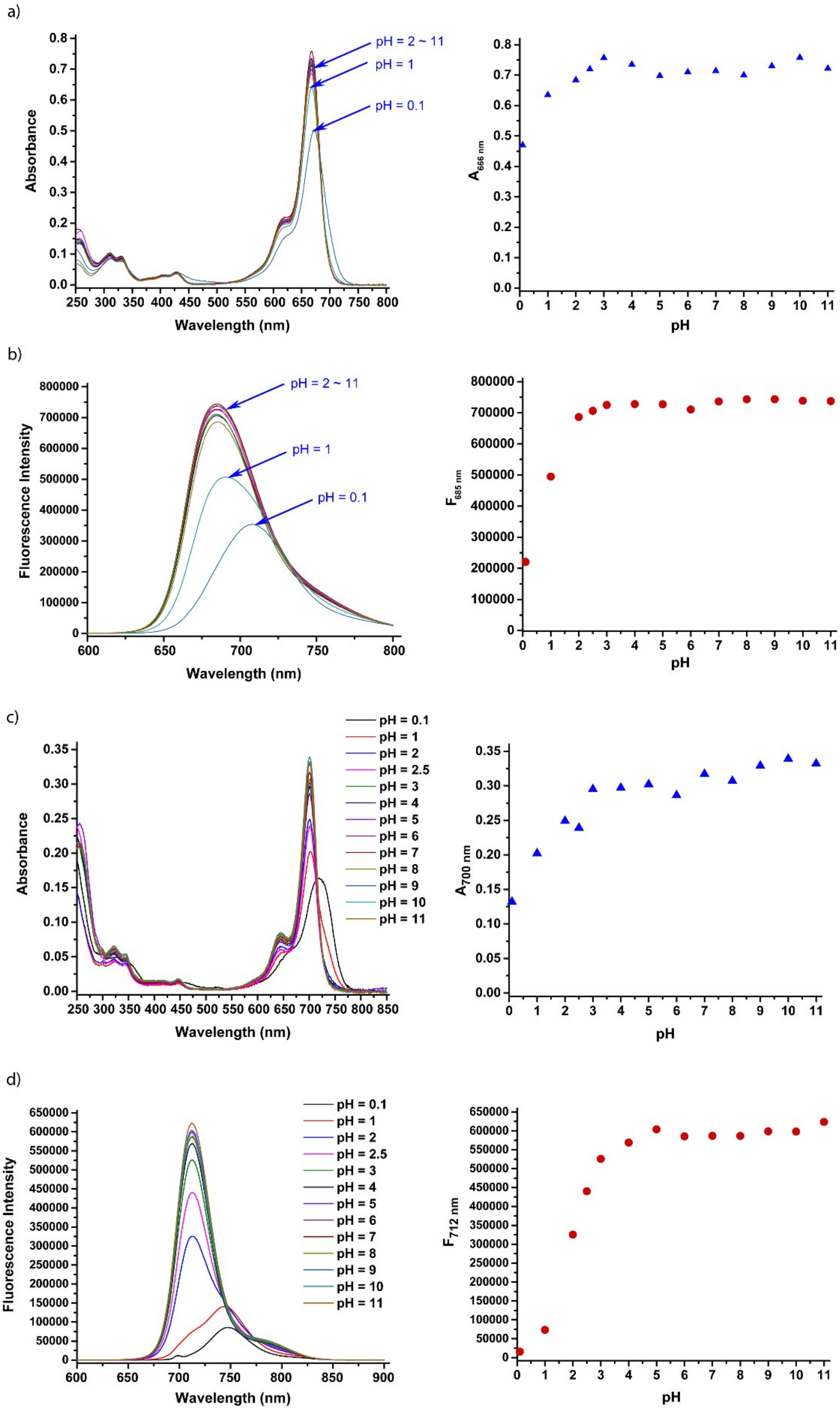
Autohydrolysis of NR700 in PBS (10 mM, pH=7.4) with 1% DMSO at 25°C. (a) Reactant consumption (black) and product formation (red) over time. (b) The rate of $\mathbf{NR_{666}}$ formation resulting from hydrolysis of $\mathbf{NR_{700}}$.

Fig. S2



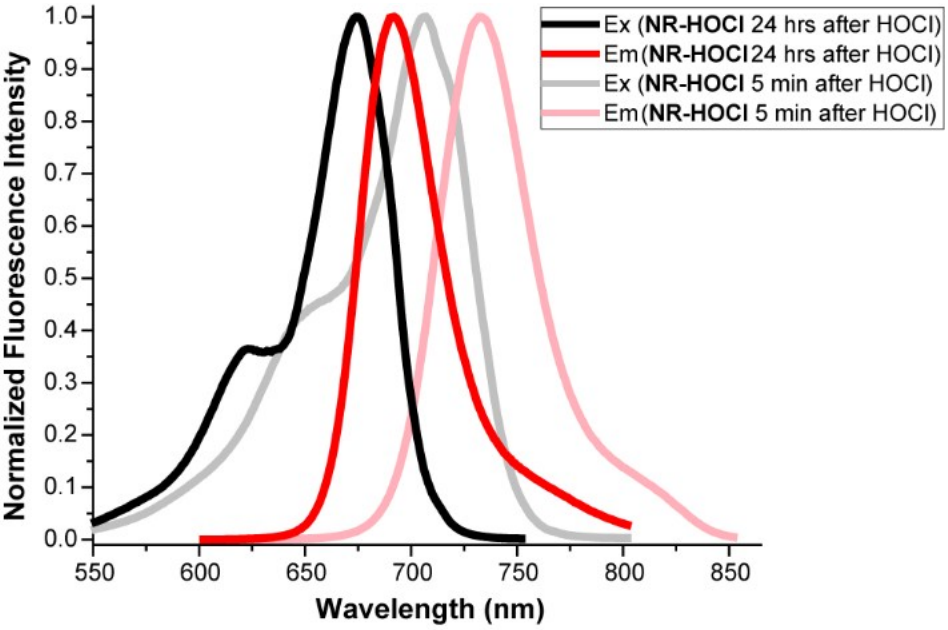
Computational calculation results of HOMO and LUMO energies of the S_0 state for **NR$_{666}$** and **NR$_{700}$** were compared to tetramethylrhodamine (TMR).Replacing the oxygen of TMR with a phosphinate or phosphinate ester functionality resulted in no appreciable difference in the HOMO energies of **NR$_{666}$** (-5.46 eV) or **NR$_{700}$** (-5.70 eV) compared to TMR (-5.59 eV). However, the LUMO energies of **NR$_{666}$** (-3.04 eV) and **NR$_{700}$** (-3.42 eV) were significantly lowered as compared to TMR (-2.81 eV). The more dramatic red-shift of **NR$_{700}$** is due to a greater stabilization of its LUMO relative to **NR$_{666}$**.

Fig. S3



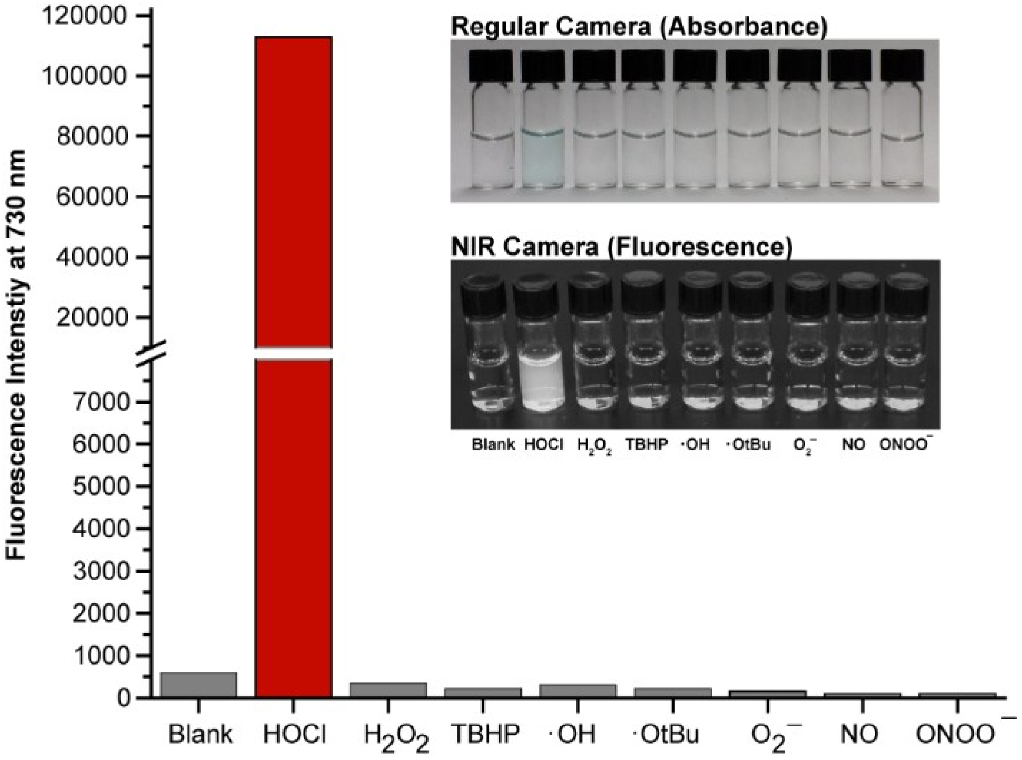
Absorbance and fluorescence of **NR**$_{666}$ (a and b) and **NR**$_{698}$ (c and d) with varying pH.

Fig. S4



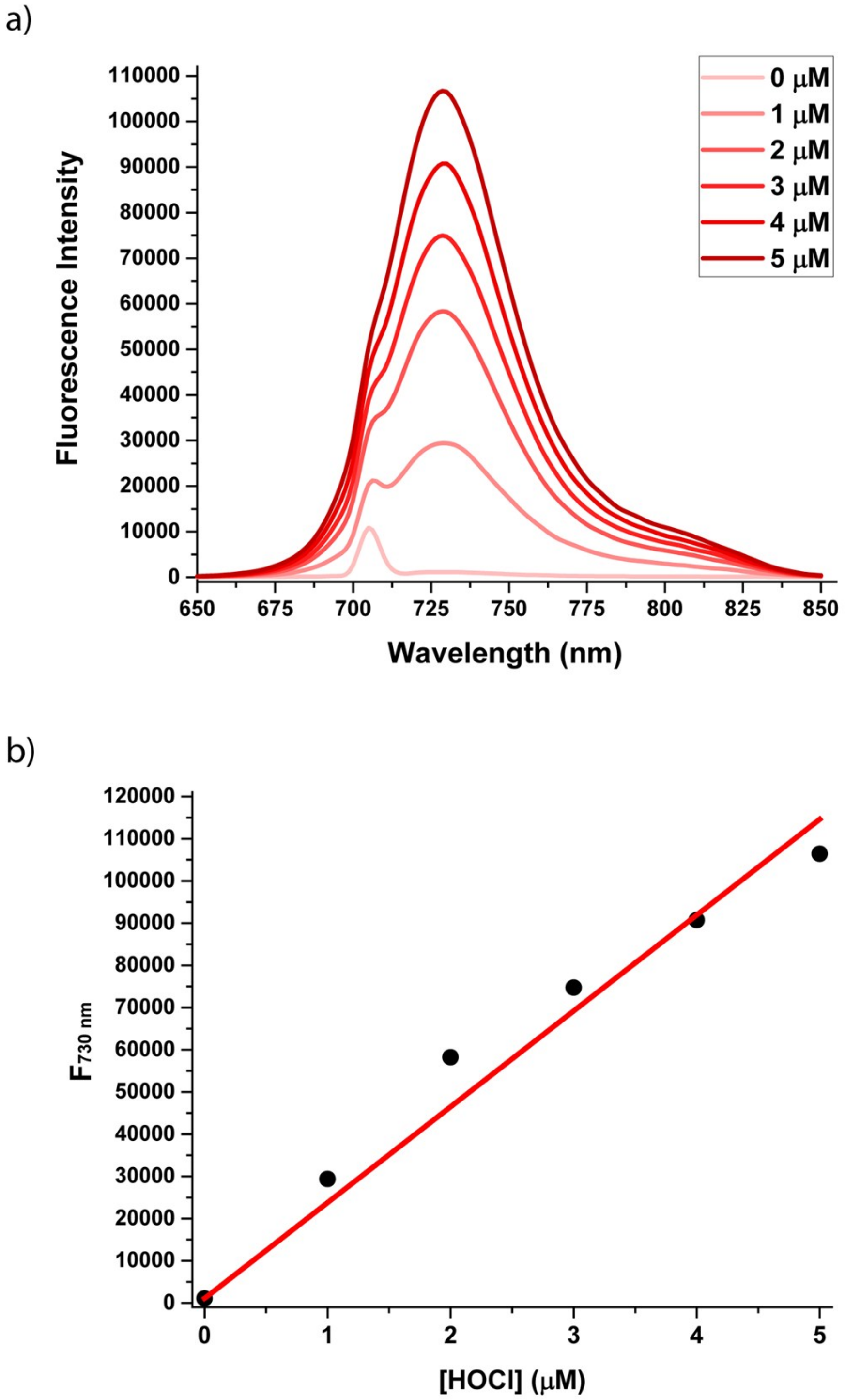
Excitation and emission spectrum of **NR-HOCl** (10 μM) 5 min (grey and pale red line) or 24 hrs (black and red line) after reaction with NaOCl (20 μM) in PBS (10 mM, pH=7.4 with 0.5% DMF). A blue-shifted excitation and emission is observed after 24 hrs, indicating the hydrolysis of the phosphinate ethyl ester group subsequent to reaction with NaOCl.

Fig. S5



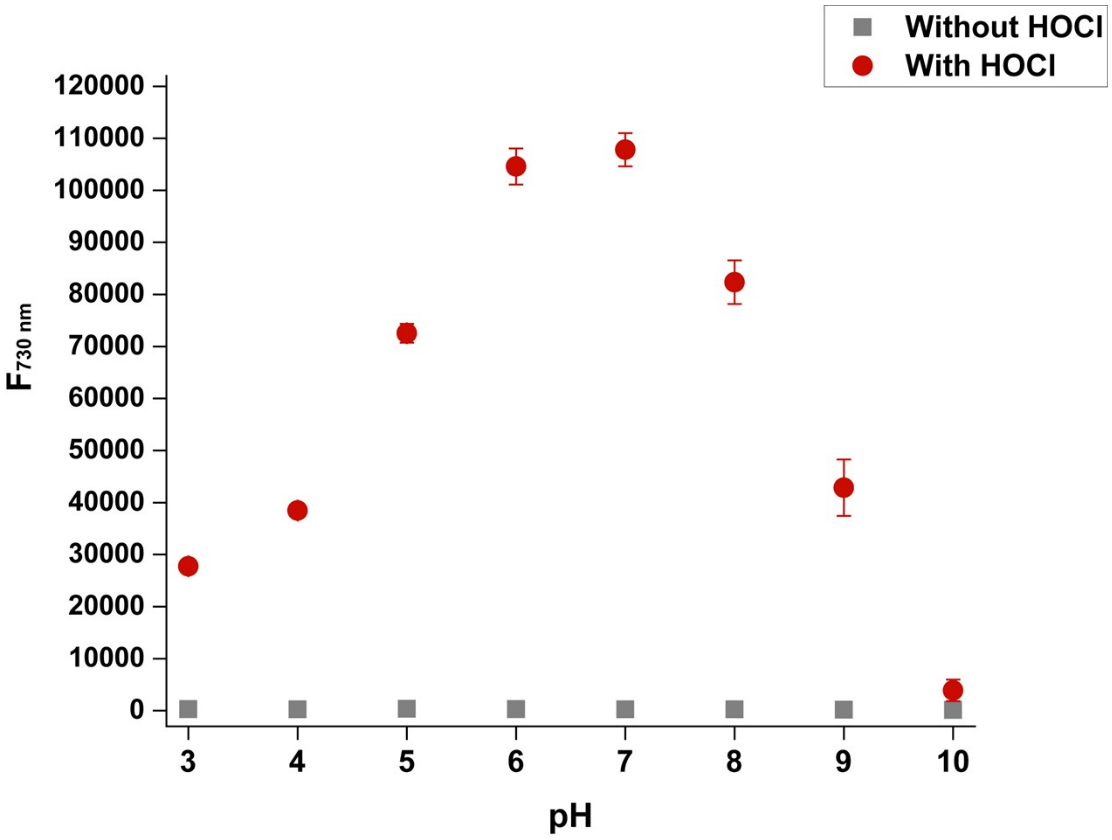
Selectivity of **NR-HOCl** for various biologically relevant ROS and RNS. **NR-HOCl** (5 µM) was incubated with HOCl (5 µM) for 15 minutes. Off-target species where assayed at a concentration of 100 µM. The insert shows the absorbance and fluorescence of **NR-HOCl** in the presence of the indicated analyte.

Fig. S6



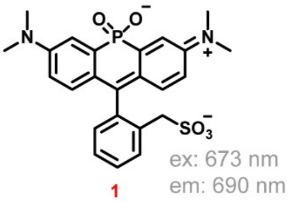
Limit of detection measurement of **NR-HOCl** for HOCl. The excitation wavelength was 705 nm. (Linear fit: $y = 22700x$, $R^2 = 0.989$)

Fig. S7



NR-HOCl exposed to HOCl at different pHs. Both the **NR-HOCl** and NaOCl concentration were kept at 5 μM.

Fig. S8



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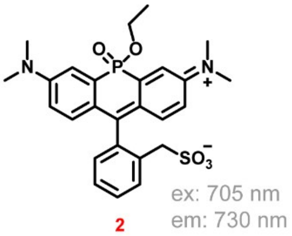

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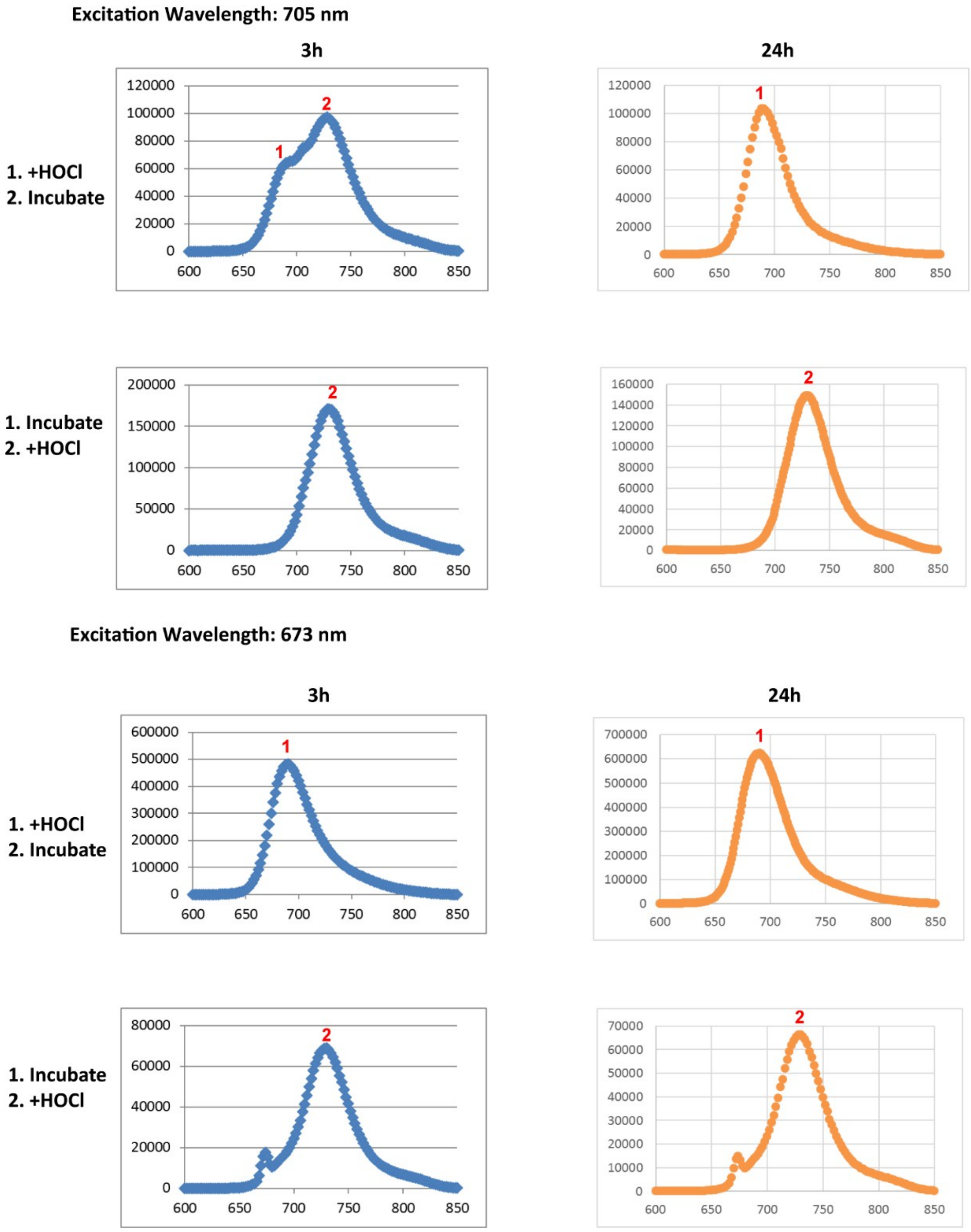
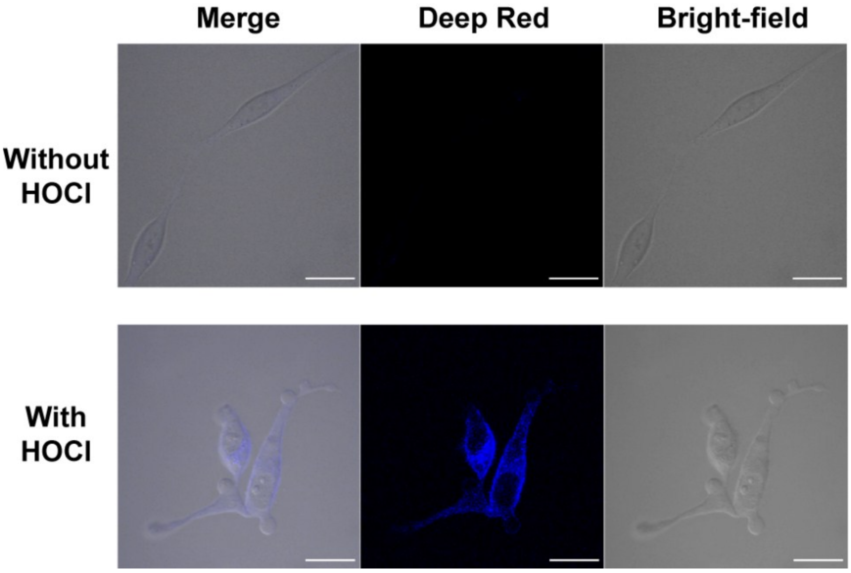


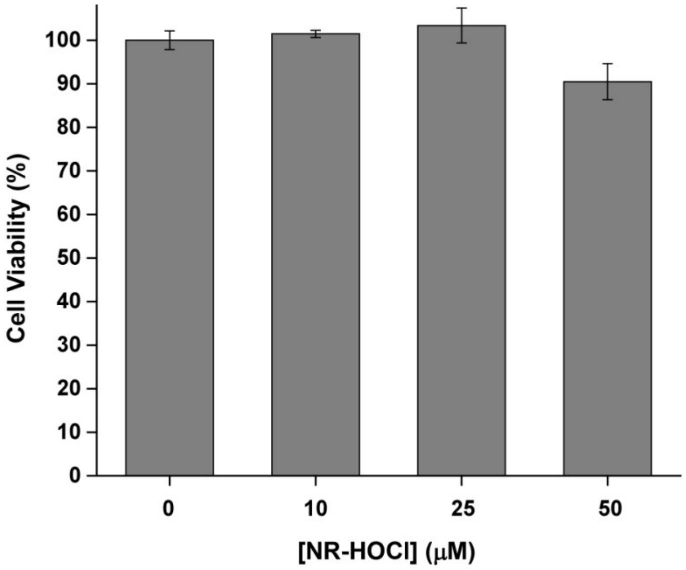
HOCl triggered phosphinate ester hydrolysis. **NR-HOCl** (10 μM) was incubated in PBS (10 mM, pH = 7.4) for 3 hrs or 24 hrs as indicated. NaOCl (20 μM) was added prior to incubation or after incubation as indicated. For all graphs above, x-axis = wavelength (nm), y-axis = fluorescence intensity. These data clearly demonstrate the initial appearance of the phosphinate ester, followed by hydrolysis. Furthermore, these data indicate that the phosphinate ester of **NR-HOCl** is stable prior to reaction with HOCl.

Fig. S9



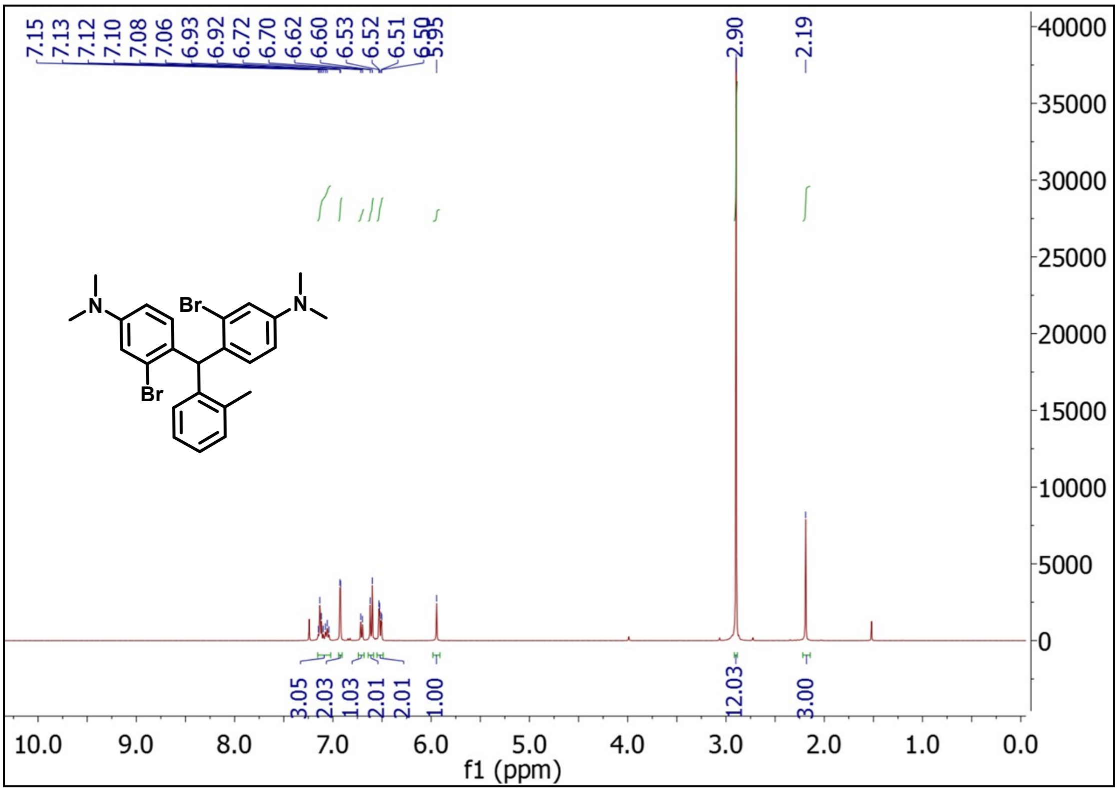
Confocal fluorescence microscopy imaging of living HeLa cells incubated with 10 μM **NR-HOCl** and without (top) or with (bottom) 20 μM HOCl. Scale bar: 25 μm.

Fig. S10

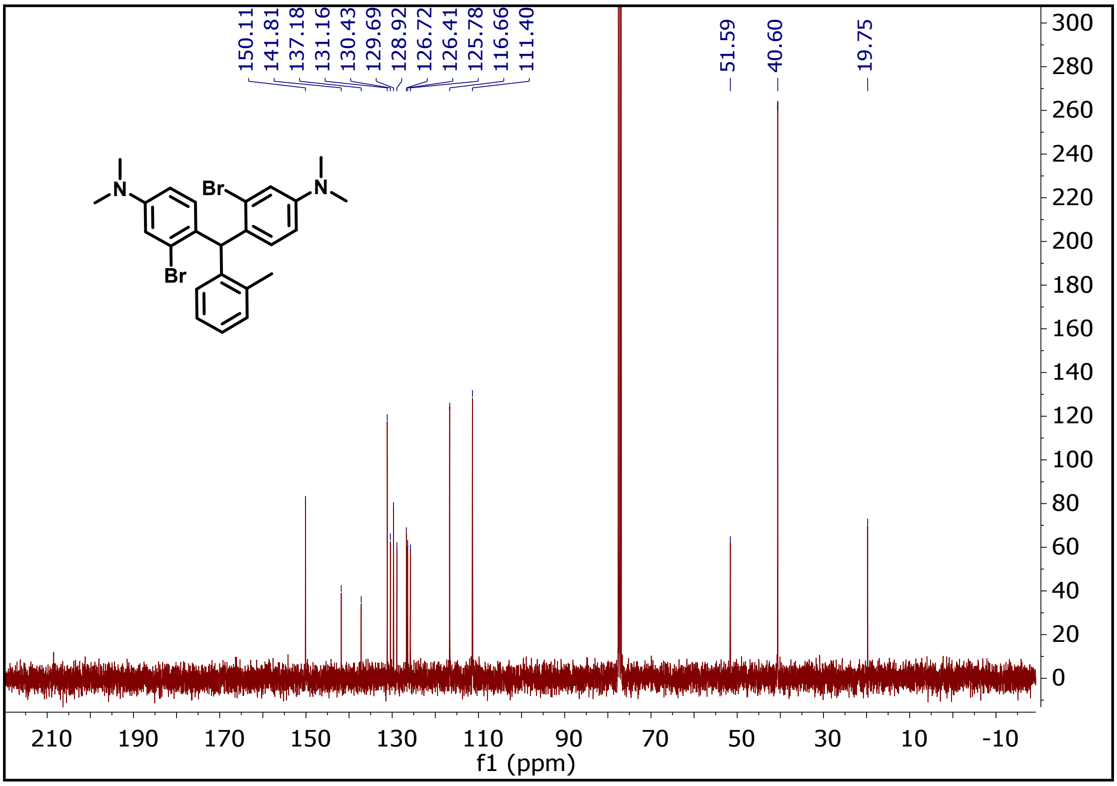


Toxicity assay of **NR-HOCl**. Living HeLa cells were incubated with the indicated concentration of **NR-HOCl** (containing 0.5% DMF) for 5 hrs. Cell viability was assessed using the CellTiter-Glo 2.0 Assay (Promega).

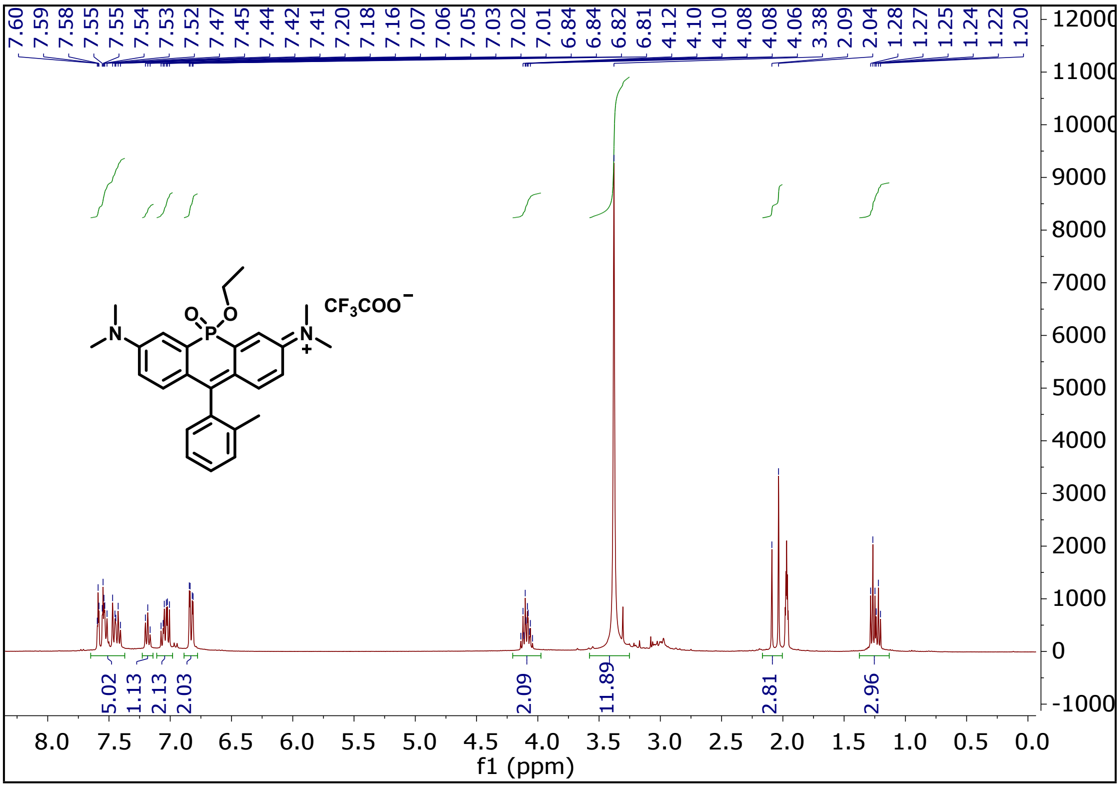
Copies of ^{1}H, ^{13}C, and ^{31}P NMR Spectra.



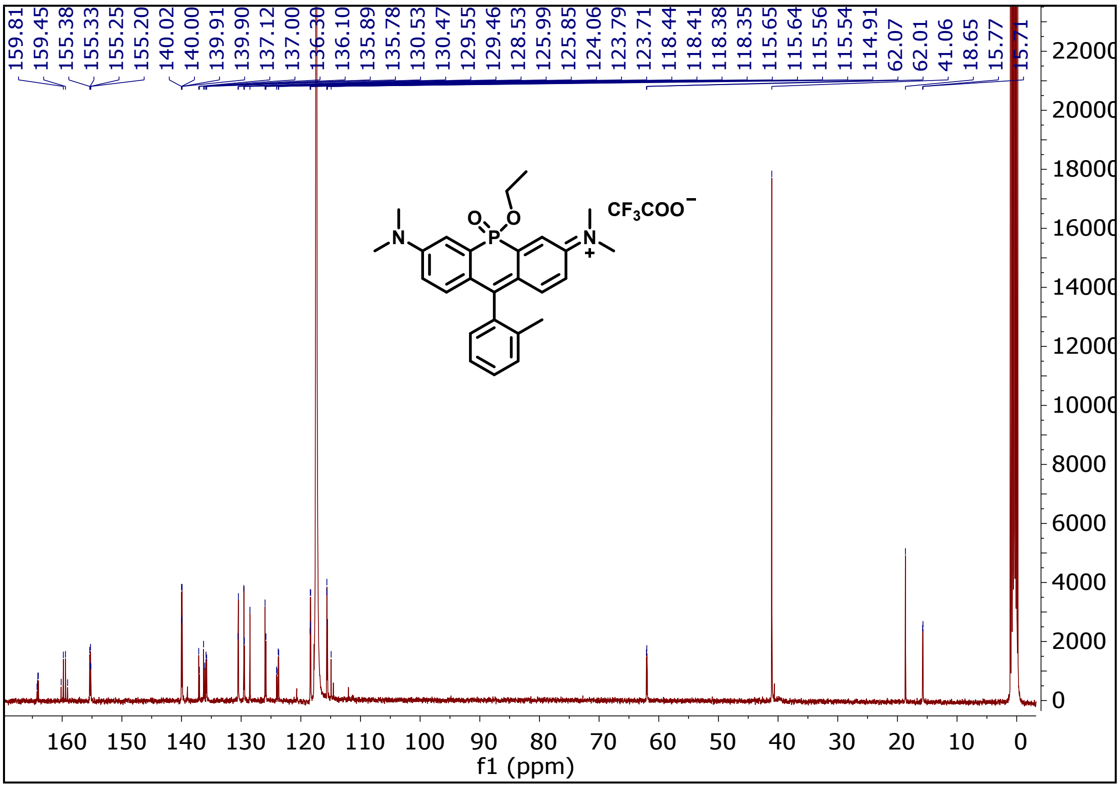
^{1}H NMR chart of **4,4'-(o-tolylmethylene)bis(3-bromo-*N,N*-dimethylaniline)** in $CDCl_3$ (400 MHz).



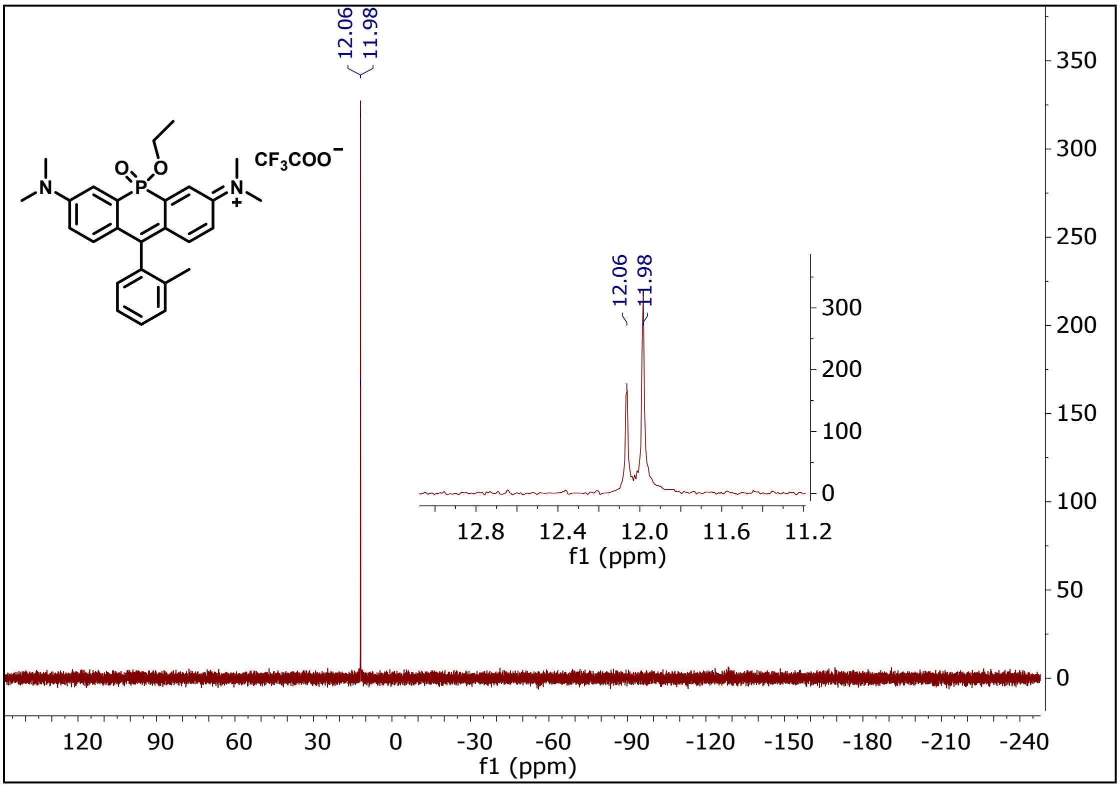
^{13}C NMR chart of **4,4'-(o-tolylmethylene)bis(3-bromo-*N,N*-dimethylaniline)** in $CDCl_3$ (101 MHz).



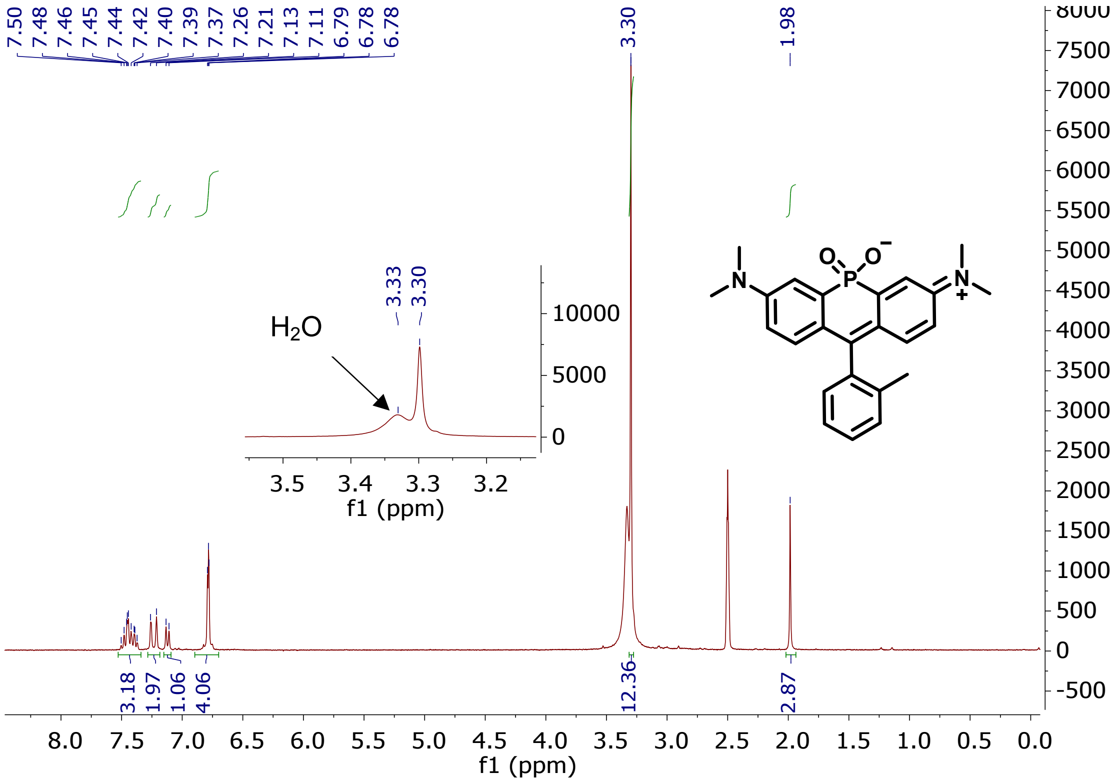
^{1}H NMR chart of **NR$_{700}$** in CD$_3$CN (400 MHz).



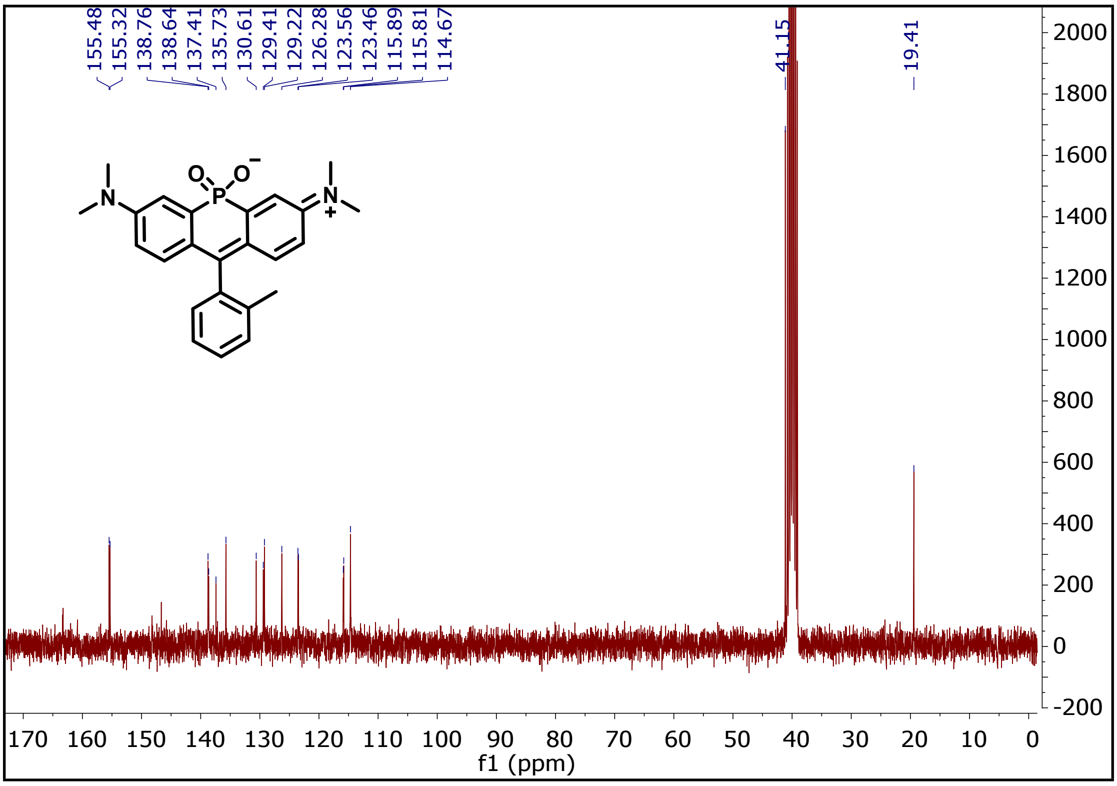
^{13}C NMR chart of **NR$_{700}$** in CD$_3$CN (101 MHz).



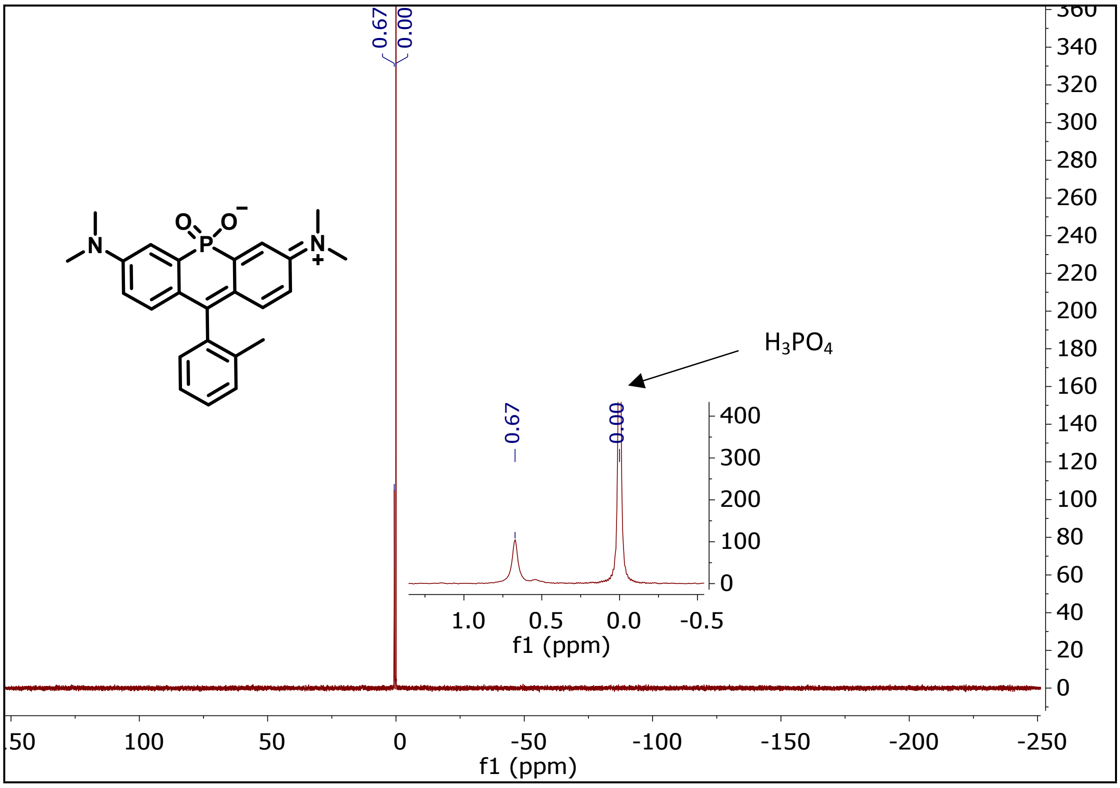
^{31}P NMR chart of **NR$_{700}$** in CD$_3$CN (162 MHz).



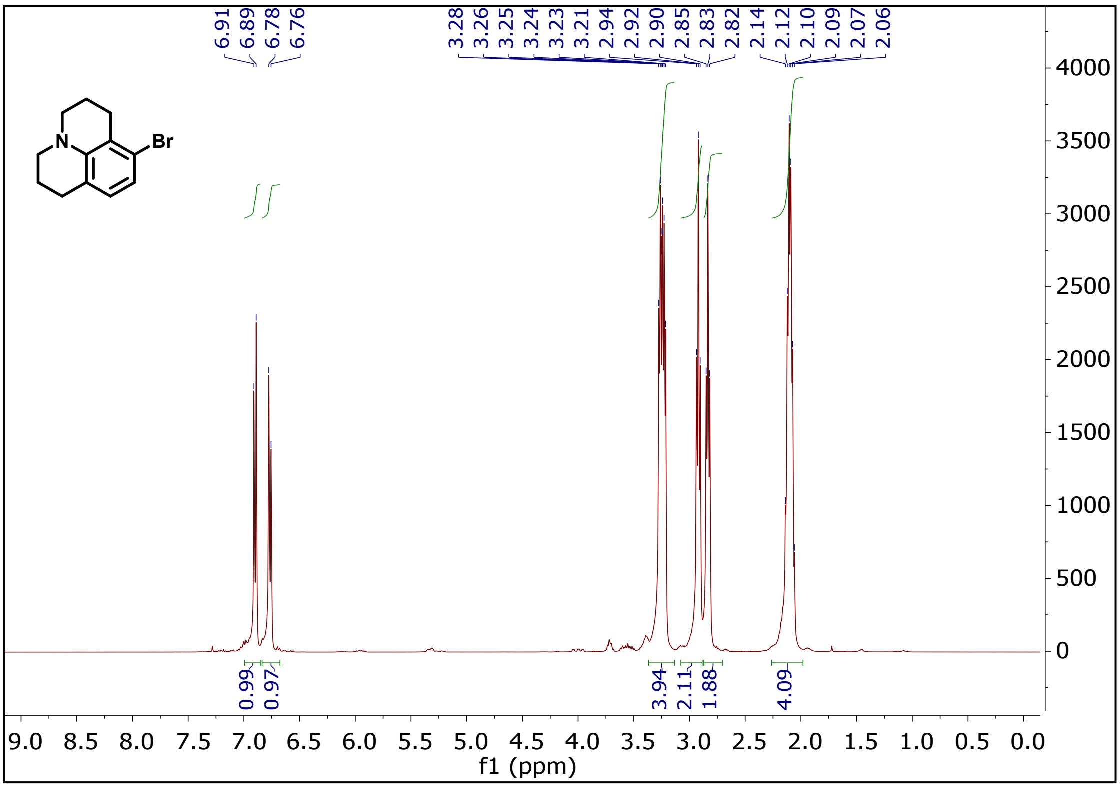
^{1}H NMR chart of **NR$_{666}$** in DMSO-d$_6$ (300 MHz).



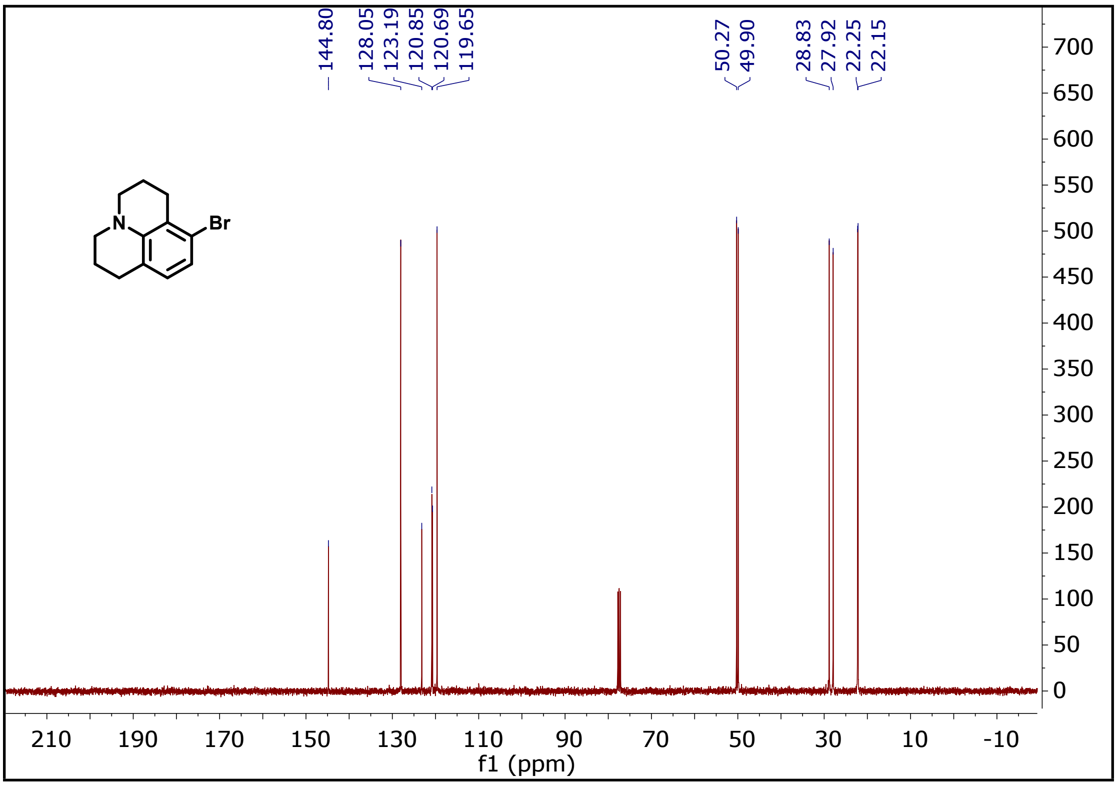
^{13}C NMR chart of **NR$_{666}$** in DMSO-d$_6$ (75 MHz).



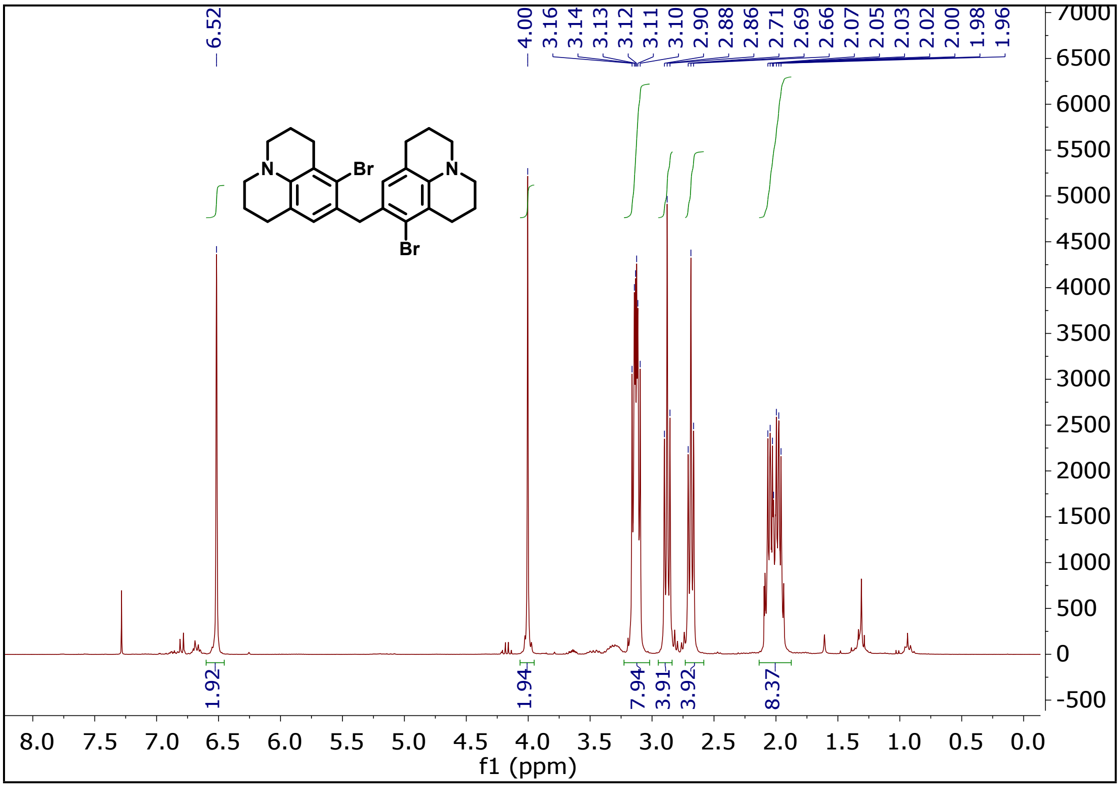
^{31}P NMR chart of **NR$_{666}$** in DMSO-d$_6$ (121 MHz).



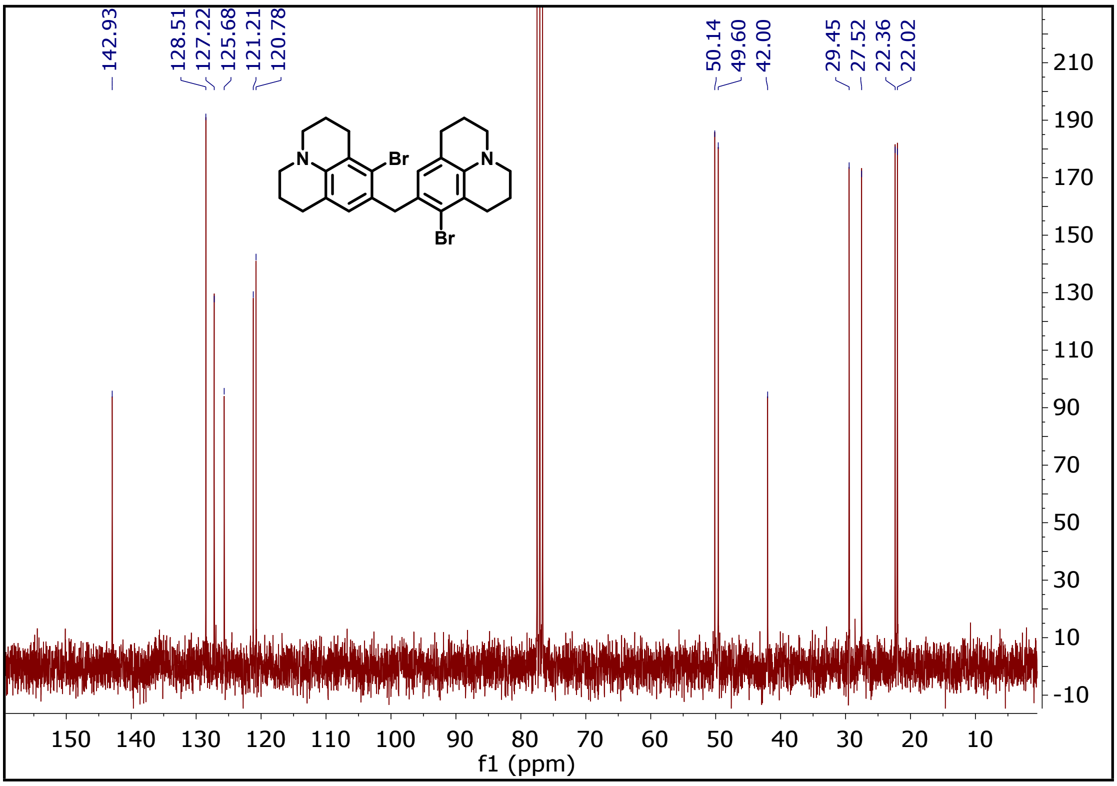
^{1}H NMR chart of **8-bromo-2,3,6,7-tetrahydro-*1H,5H*-pyrido[3,2,1-*ij*]quinoline** in $CDCl_3$ (400 MHz).



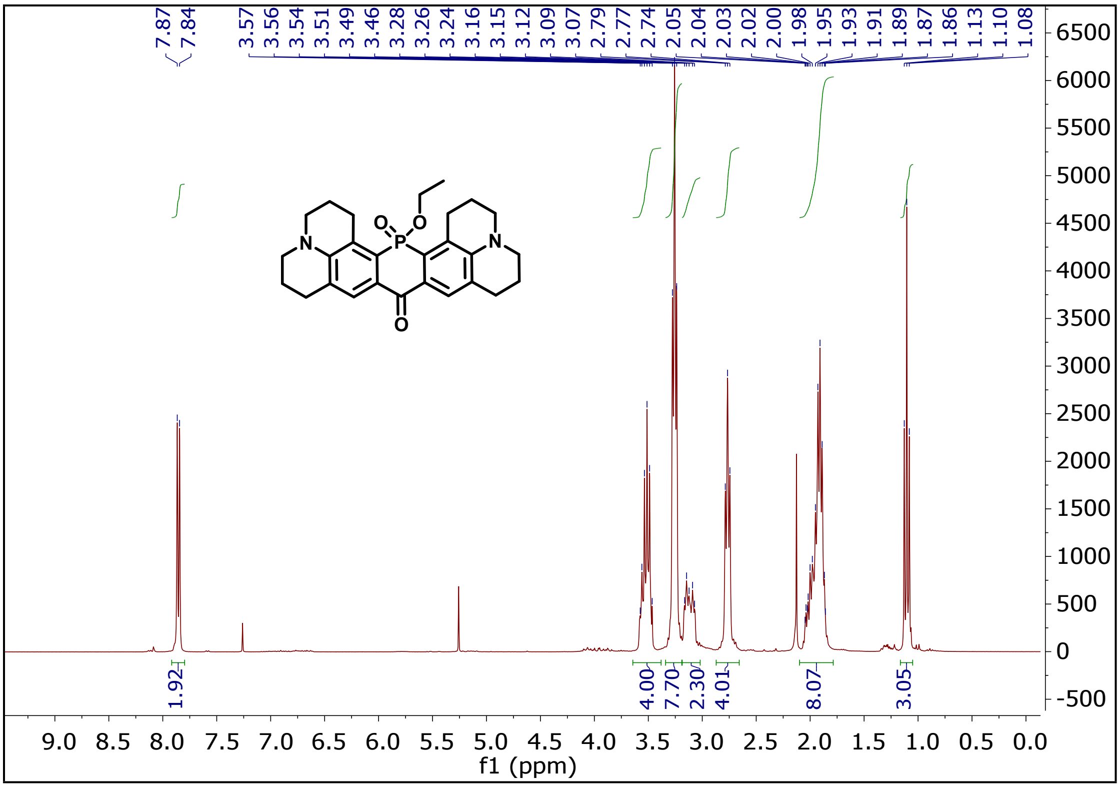
^{13}C NMR chart of **8-bromo-2,3,6,7-tetrahydro-*1H,5H*-pyrido[3,2,1-*ij*]quinoline** in $CDCl_3$ (101 MHz).



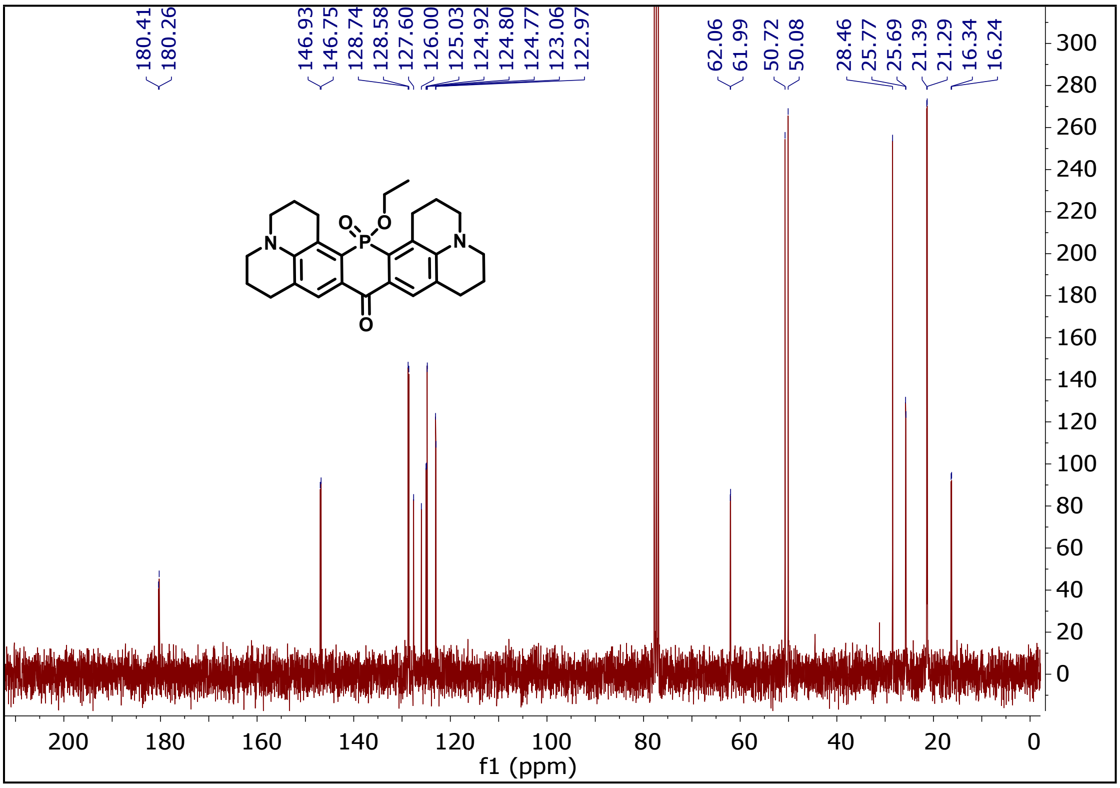
^{1}H NMR chart of **bis(8-bromo-2,3,6,7-tetrahydro-1*H*,5*H*-pyrido[3,2,1-*ij*]quinolin-9-yl)methane** in $CDCl_3$ (300 MHz)



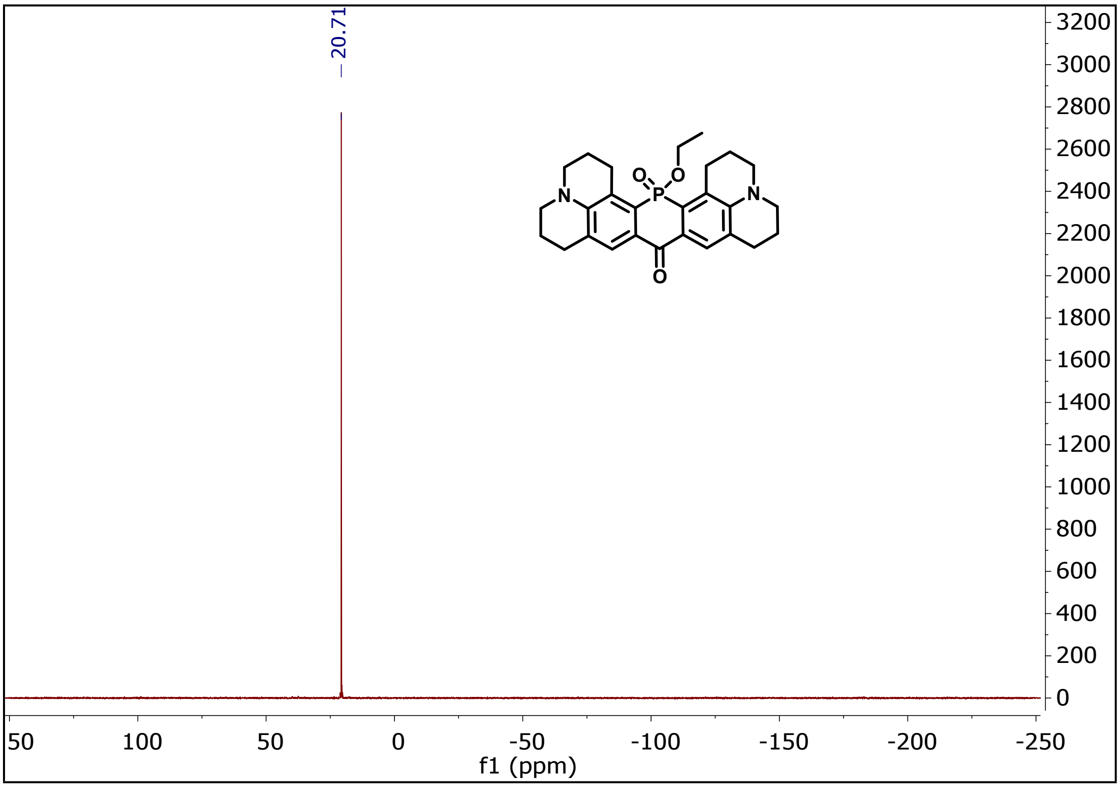
^{13}C NMR chart of **bis(8-bromo-2,3,6,7-tetrahydro-1*H*,5*H*-pyrido[3,2,1-*ij*]quinolin-9-yl)methane** in $CDCl_3$ (75 MHz)



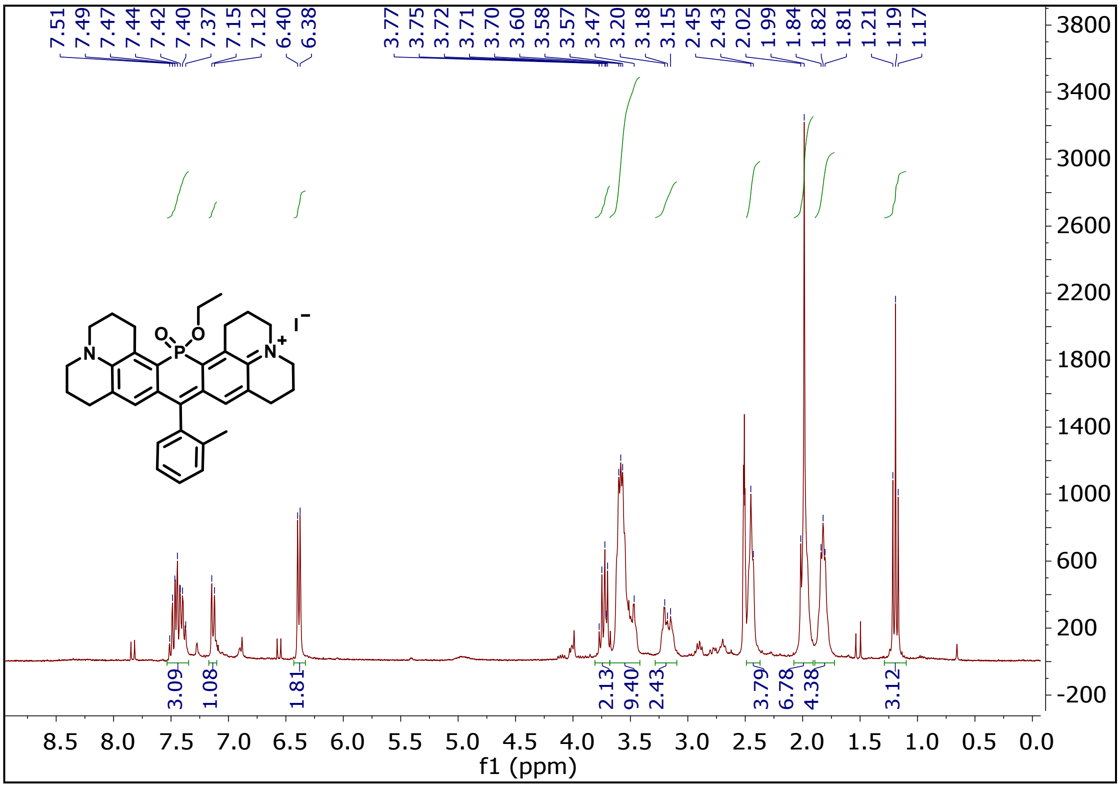
^{1}H NMR chart of **1** in CDCl$_3$ (300 MHz).



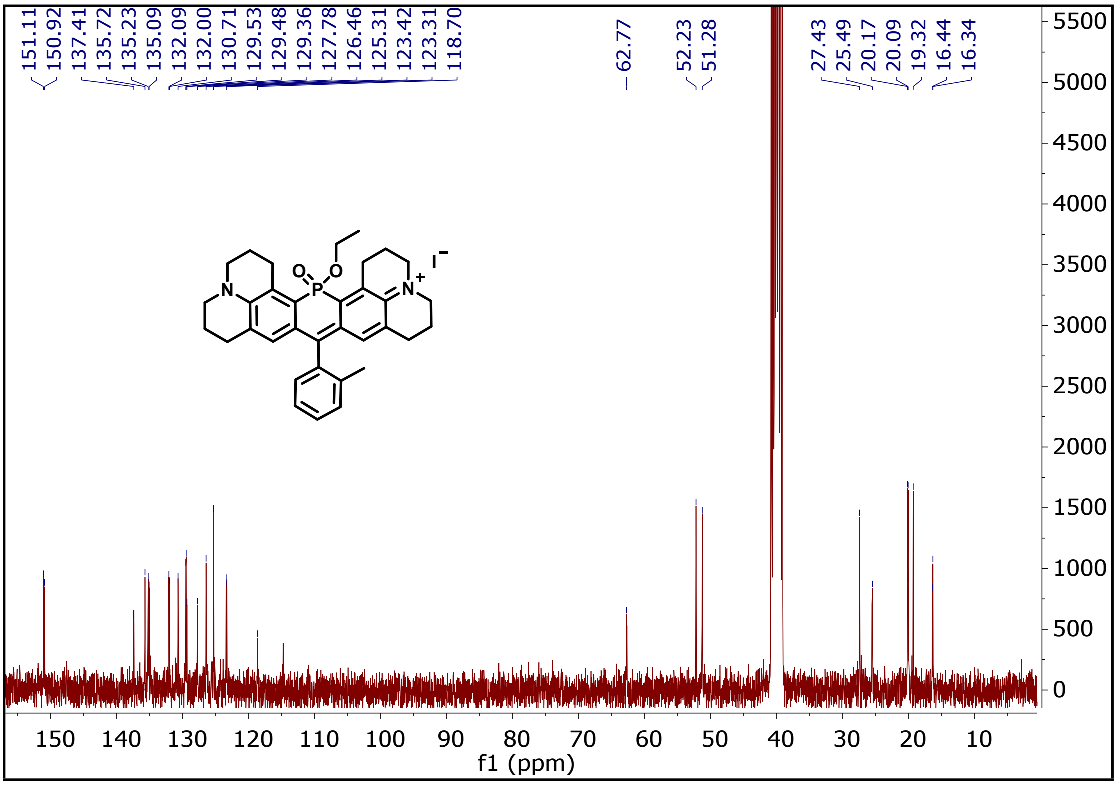
^{13}C NMR chart of **1** in CDCl$_3$ (75 MHz).



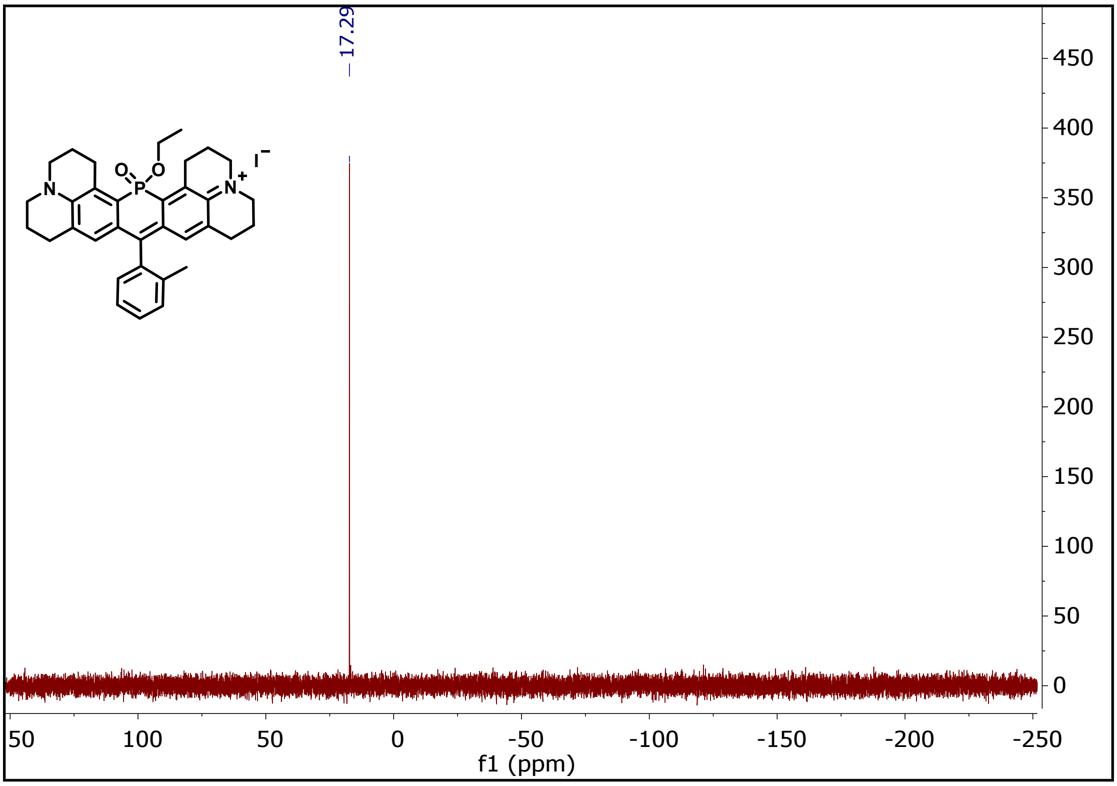
^{31}P NMR chart of **1** in $CDCl_3$ (121 MHz).



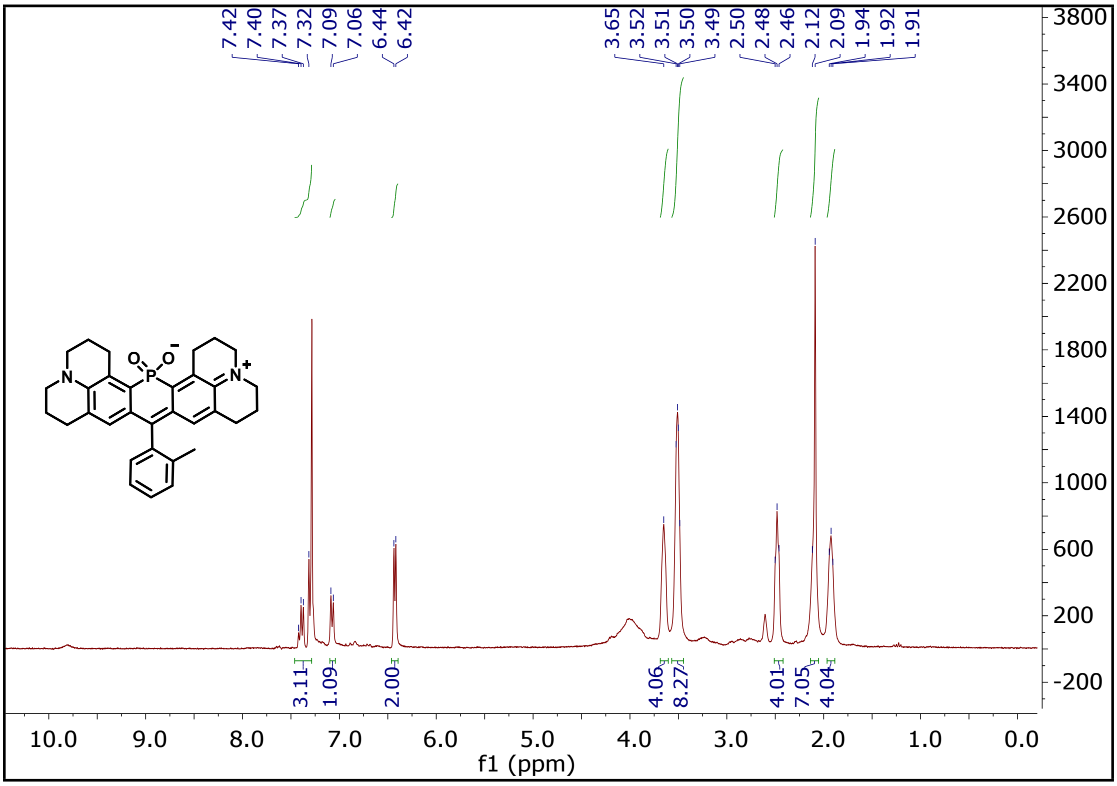
^{1}H NMR chart of **NR$_{744}$** in DMSO-d$_6$ (300 MHz).



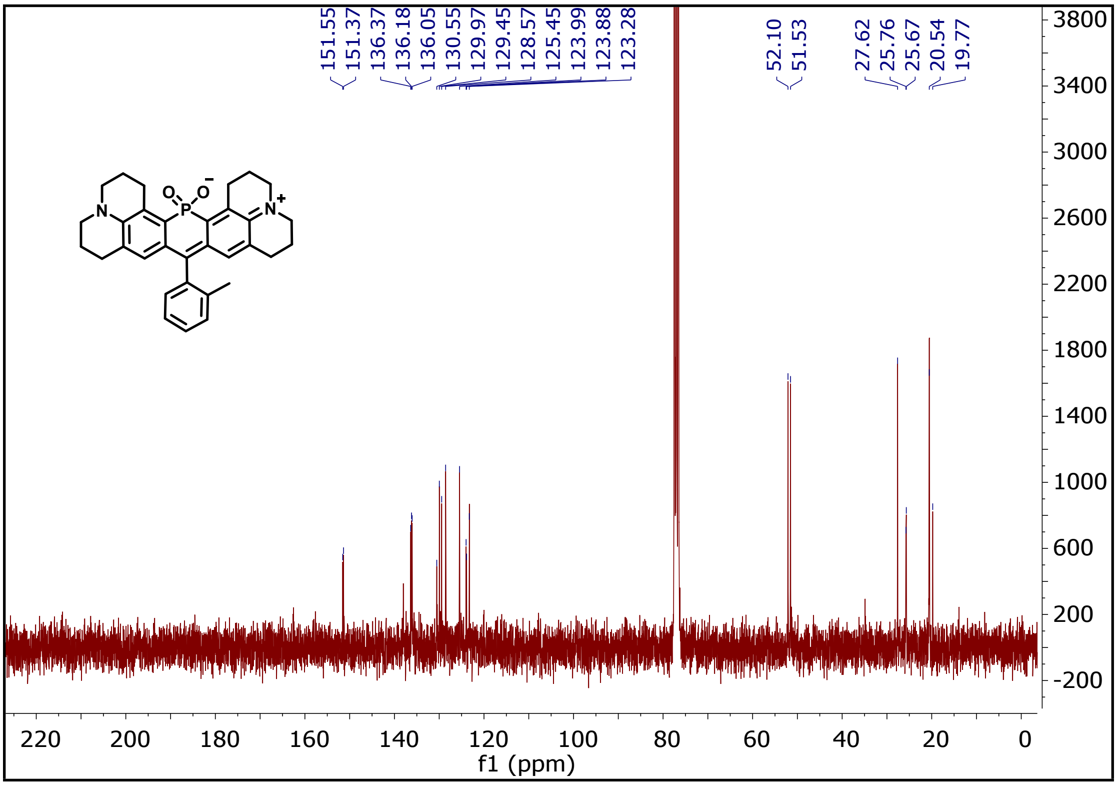
^{13}C NMR chart of **NR$_{744}$** in DMSO-d$_6$ (75 MHz).



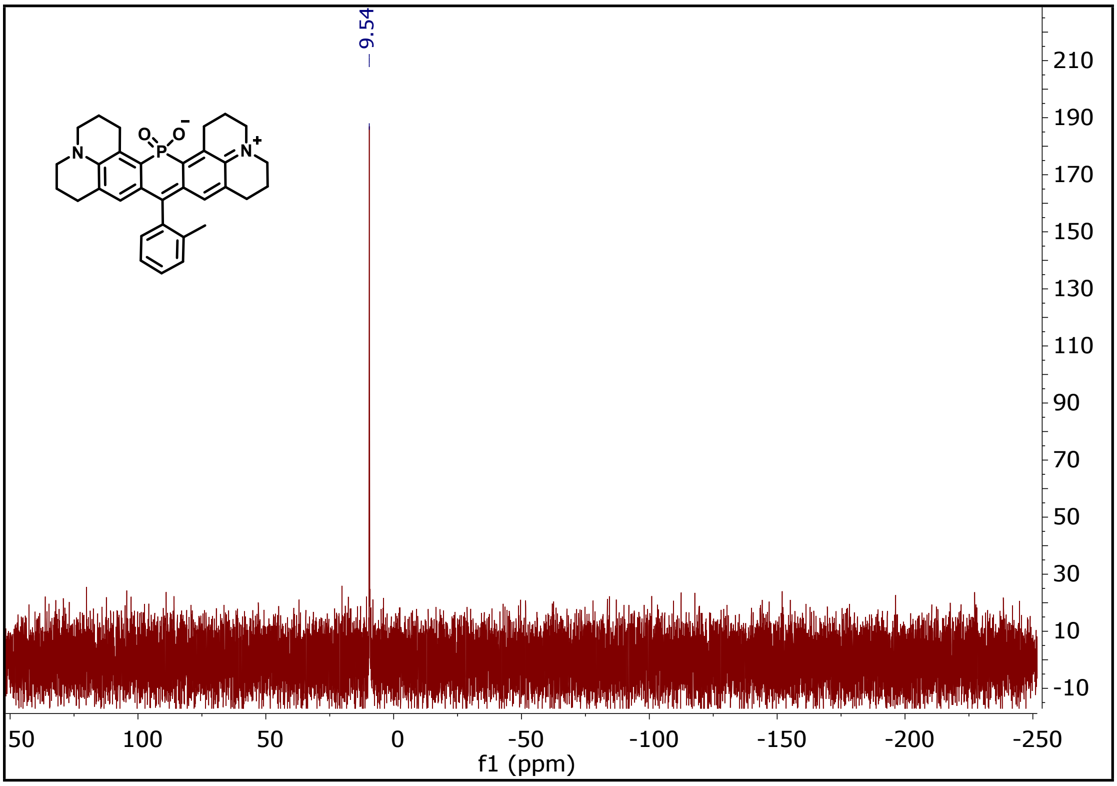
^{31}P NMR chart of **NR$_{744}$** in DMSO-d$_6$ (121 MHz).



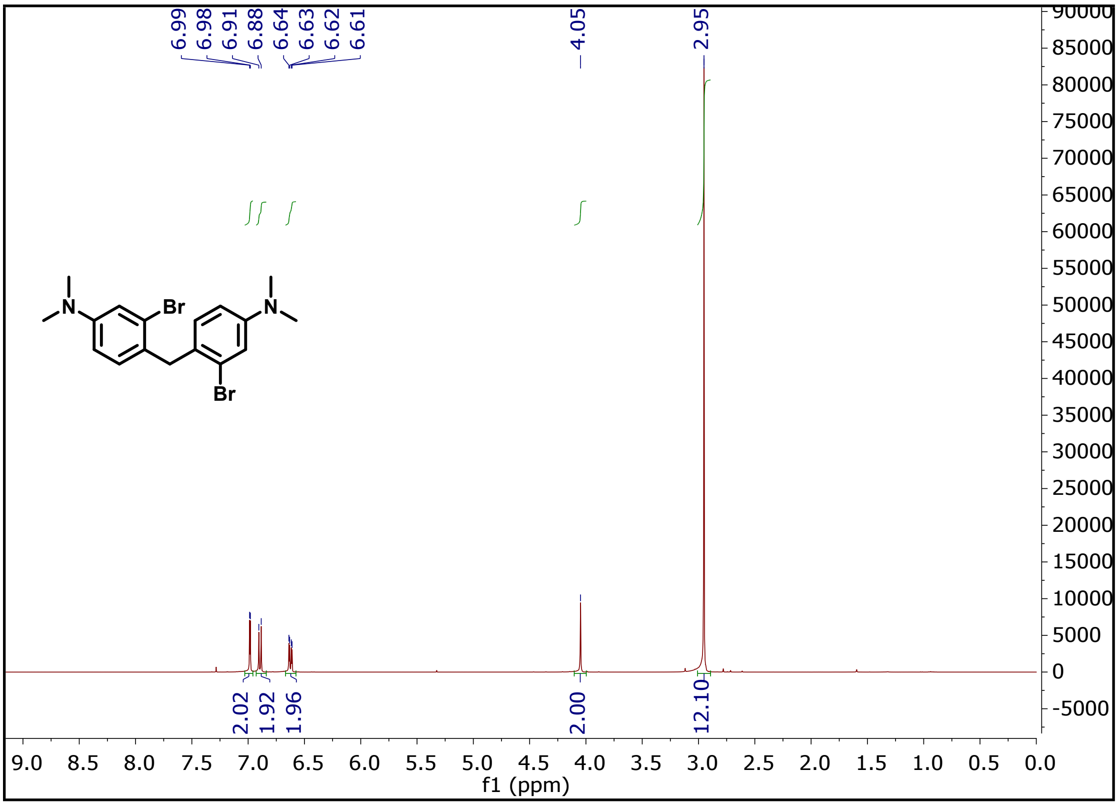
^{1}H NMR chart of **NR$_{698}$** in $CDCl_3$ (300 MHz).



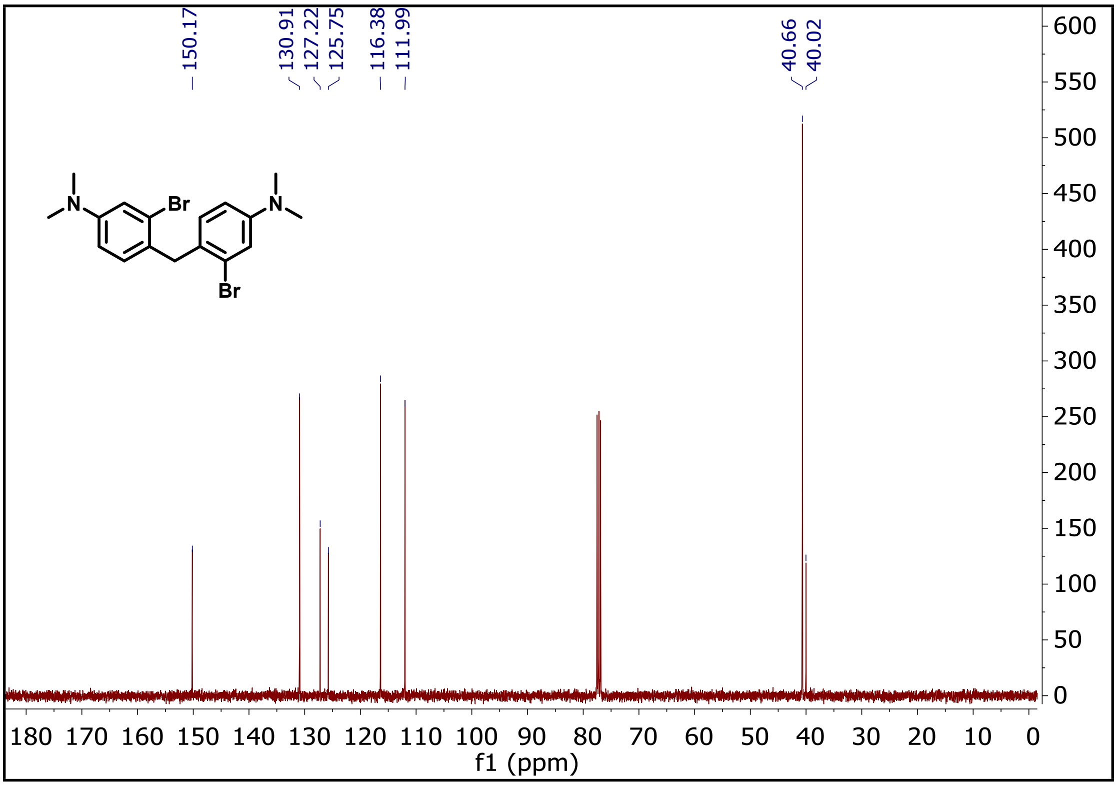
^{13}C NMR chart of **NR$_{698}$** in $CDCl_3$ (75 MHz).



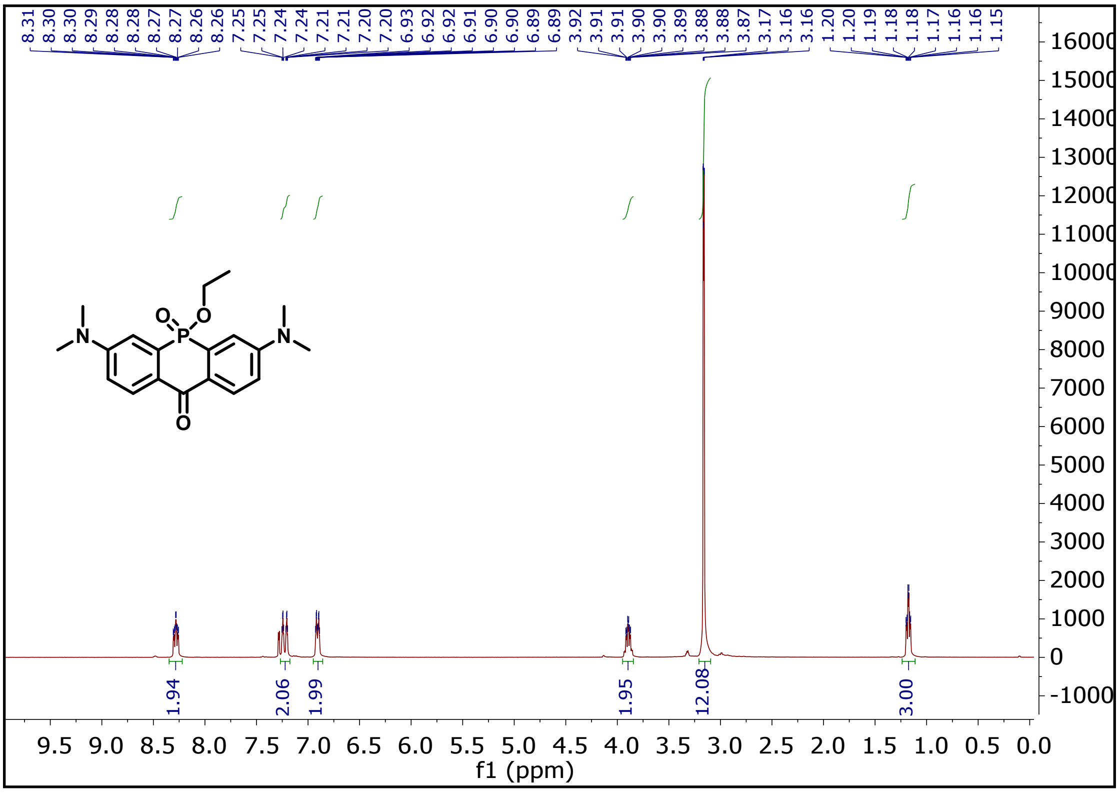
^{31}P NMR chart of **NR$_{698}$** in CDCl$_3$ (121 MHz).



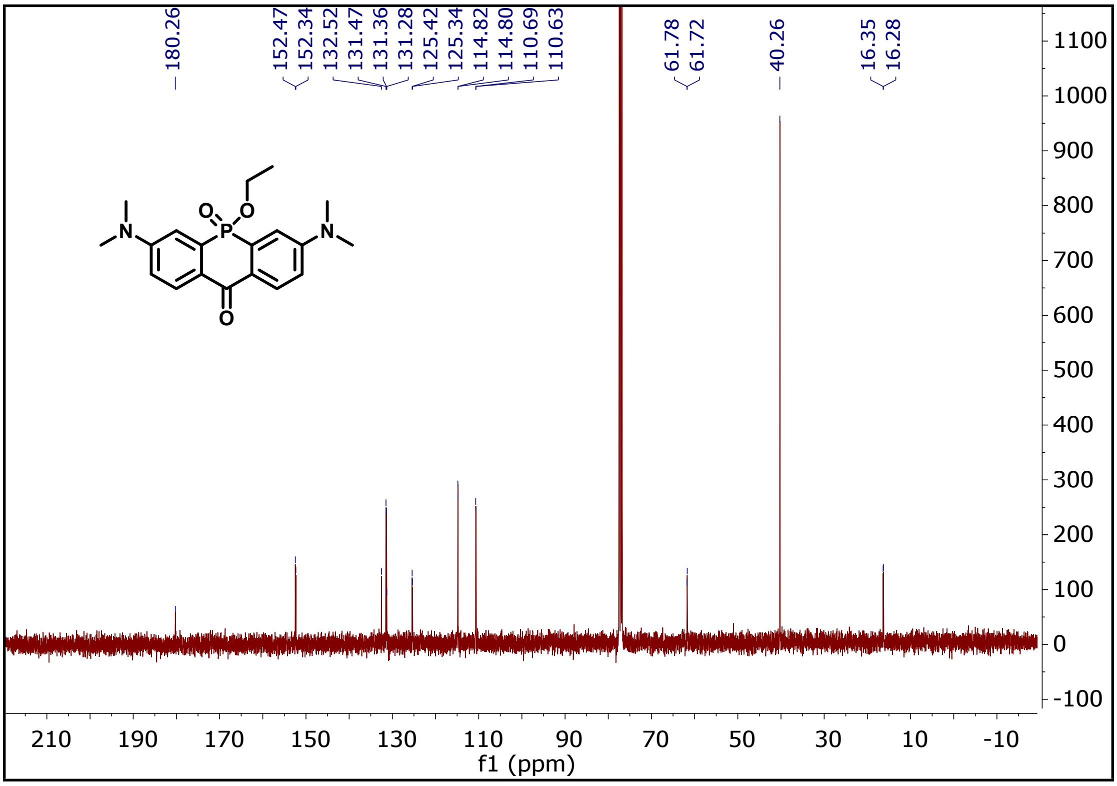
^{1}H NMR chart of **4,4'-methylenebis(3-bromo-*N,N*-dimethylaniline)** in $CDCl_3$ (400 MHz).



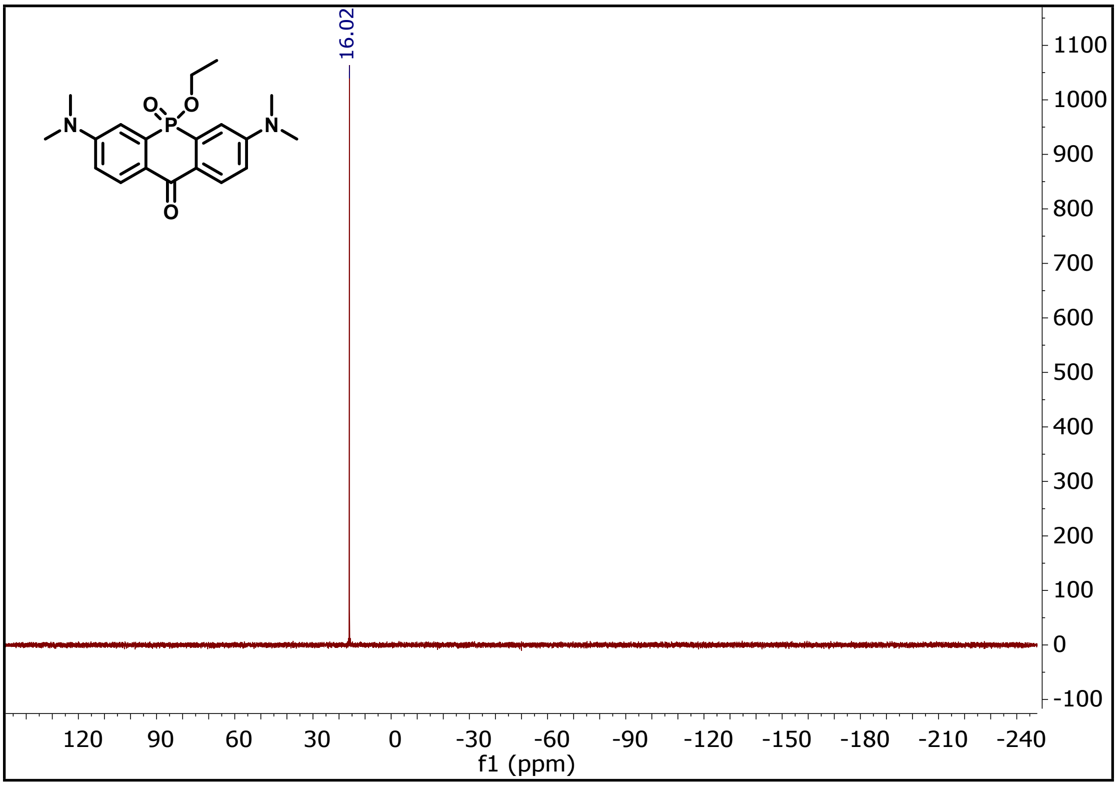
^{13}C NMR chart of **4,4'-methylenebis(3-bromo-*N,N*-dimethylaniline)** in $CDCl_3$ (101 MHz).



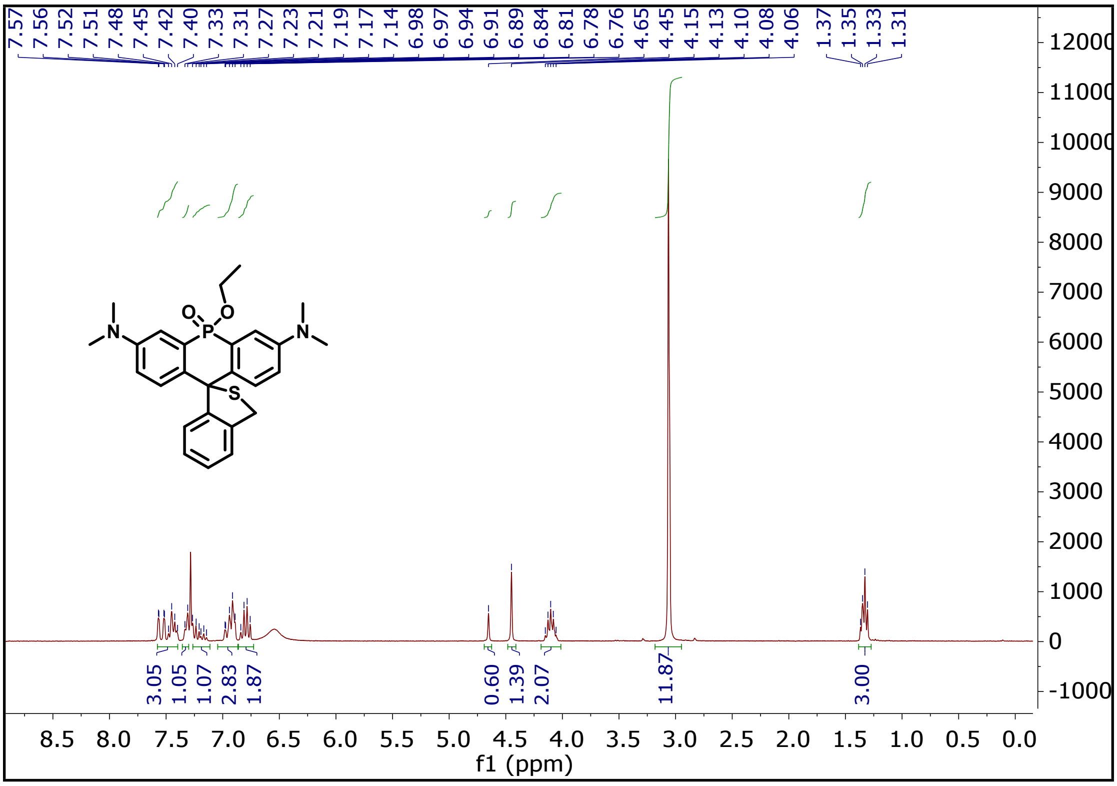
^{1}H NMR chart of **2** in $CDCl_3$ (400 MHz).



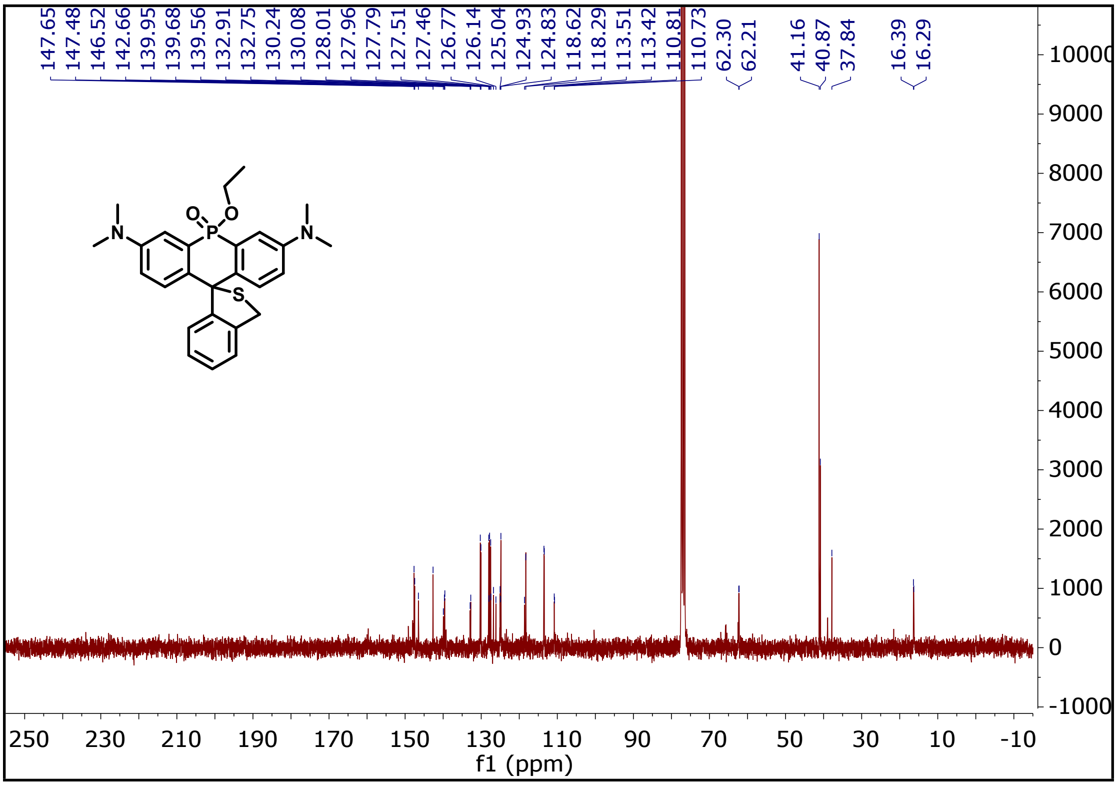
^{13}C NMR chart of **2** in $CDCl_3$ (101 MHz).



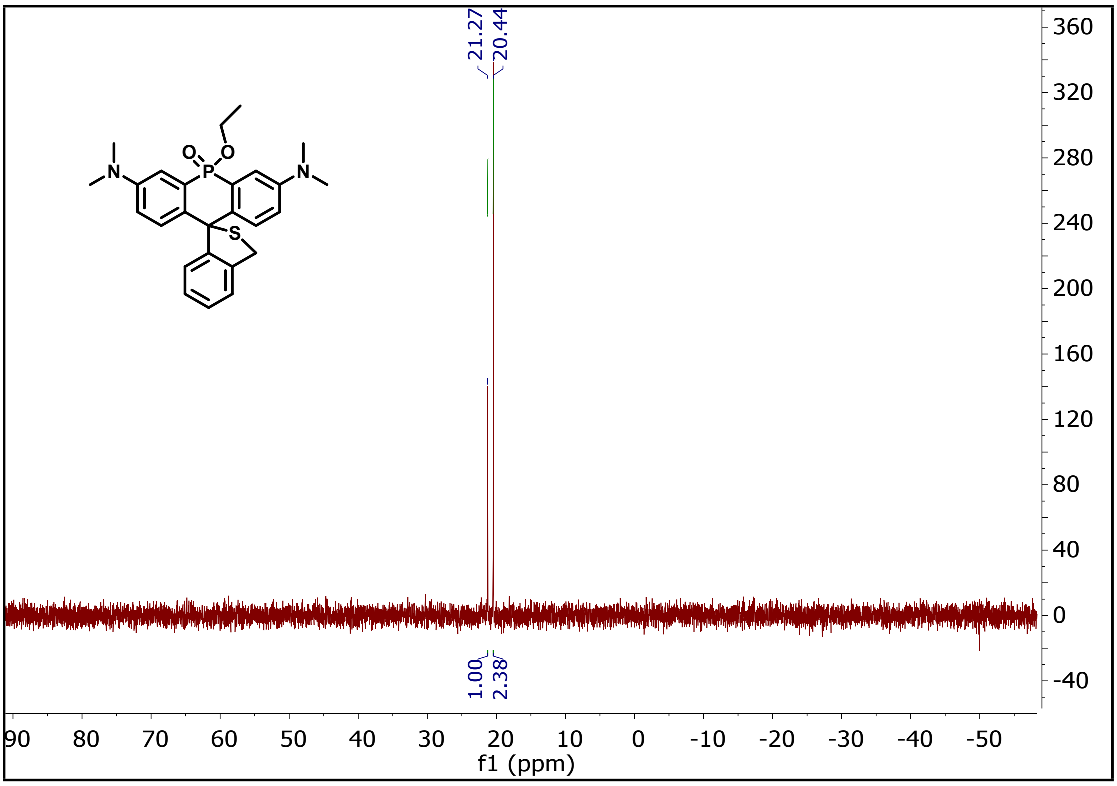
^{31}P NMR chart of **2** in $CDCl_3$ (162 MHz).



^{1}H NMR chart of **NR-HOCl** in $CDCl_3$ (300 MHz).



^{13}C NMR chart of **NR-HOCl** in $CDCl_3$ (75 MHz).



^{31}P NMR chart of **NR-HOCl** in $CDCl_3$ (121 MHz).

References

1 D. Bradley, G. Williams and M. Lawton, *J. Org. Chem.*, 2010, **75**, 8351-8354.

2 N. M. Thellamurege, D. Si, F. Cui, H. Zhu, R. Lai and H. Li, *J. Comput. Chem.*, 2013, **34**, 2816-2833.

3 (a) M. S. Gordon and M. W. Schmidt, *Theory and Applications of Computational Chemistry: the first forty years*, 2005, 1167-1189; (b) M. W. Schmidt, K. K. Baldridge, J. A. Boatz, S. T. Elbert, M. S. Gordon, J. H. Jensen, S. Koseki, N. Matsunaga, K. A. Nguyen and S. Su, *J. Comput. Chem.*, 1993, **14**, 1347-1363.

4 A. D. Becke, *J. Chem. Phys.*, 1993, **98**, 5648-5652.

5 M. M. Francl, W. J. Pietro, W. J. Hehre, J. S. Binkley, M. S. Gordon, D. J. DeFrees and J. A. Pople, *J. Chem. Phys.*, 1982, **77**, 3654-3665.

6 N. M. Thellamurege and H. Li, *J. Chem. Phys.*, 2012, **137**, 246101.

7 Y. Koide, Y. Urano, K. Hanaoka, W. Piao, M. Kusakabe, N. Saito, T. Terai, T. Okabe and T. Nagano, *J. Am. Chem. Soc.*, 2012, **134**, 5029-5031.

8 K. Rurack and M. Spieles, *Anal. Chem.*, 2011, **83**, 1232-1242.

9 (a) D. Srikun, E. W. Miller, D. W. Dornaille and C. J. Chang, *J. Am. Chem. Soc.*, 2008, **130**, 4596-4597; (b) X. Sun, Q. Xu, G. Kim, S. E. Flower, J. P. Lowe, J. Yoon, J. S. Fossey, X. Qian, S. D. Bull and T. D. James, *Chem. Sci.*, 2014, **5**, 3368-3373.

10 T. Pastierik, P. Sebej, J. Medalova, P. Stacko and P. Klan, *J. Org. Chem.*, 2014, **79**, 3374-3382.

11 Y. Koide, Y. Urano, K. Hanaoka, T. Terai and T. Nagano, *ACS Chem. Biol.*, 2011, **6**, 600-608.

12 T. Y. Ohulchanskyy, D. J. Donnelly, M. R. Detty and P. N. Prasad, *J. Phys. Chem. B*, 2004, **108**, 8668-8672.

13 X. Chai, X. Cui, B. Wang, F. Yang, Y. Cai, Q. Wu and T. Wang, *Chem.-Eur. J.*, 2015, **21**, 16754-16758.