Electronic Supplementary Material (ESI) for RSC Advances.

Near-infrared asymmetrical heptamethine cyanines specifically imaging cancer cells by sensing their acidic lysosomal lumen

Rui Xi[a,1], Jingye Zhang[a,1], Yunfei Zhang[a], Sihan Li[a], Yang Li[a], Xinwei Li[a], Liang Chen[b], Cong Li[a,*]

[a]Key Laboratory of Smart Drug Delivery, Ministry of Education, School of Pharmacy, Fudan University, Shanghai 201203, China; [b]Department of Neurosurgery, Huashan Hospital, Fudan University, Shanghai 200040, China

Supporting Information

Table of content

1. Supplemental Figures

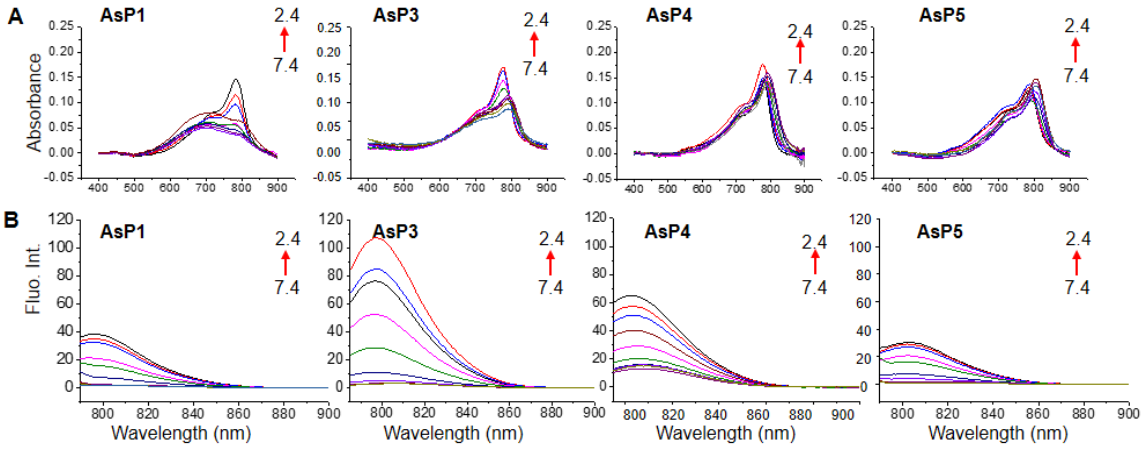


Figure S1. pH dependent absorption (A) and emission (B) of **AsP1**, **AsP3**−**AsP5**. pH changed with an interval of 0.5 units from 2.4 to 7.4. All the fluorophores (1.0 μM, λ_{ex} = 765 nm) were measured in disodium hydrogenphosphate buffer containing 0.1% DMSO. The pH was modulated by adding 0.1 M HCl or NaOH solution.

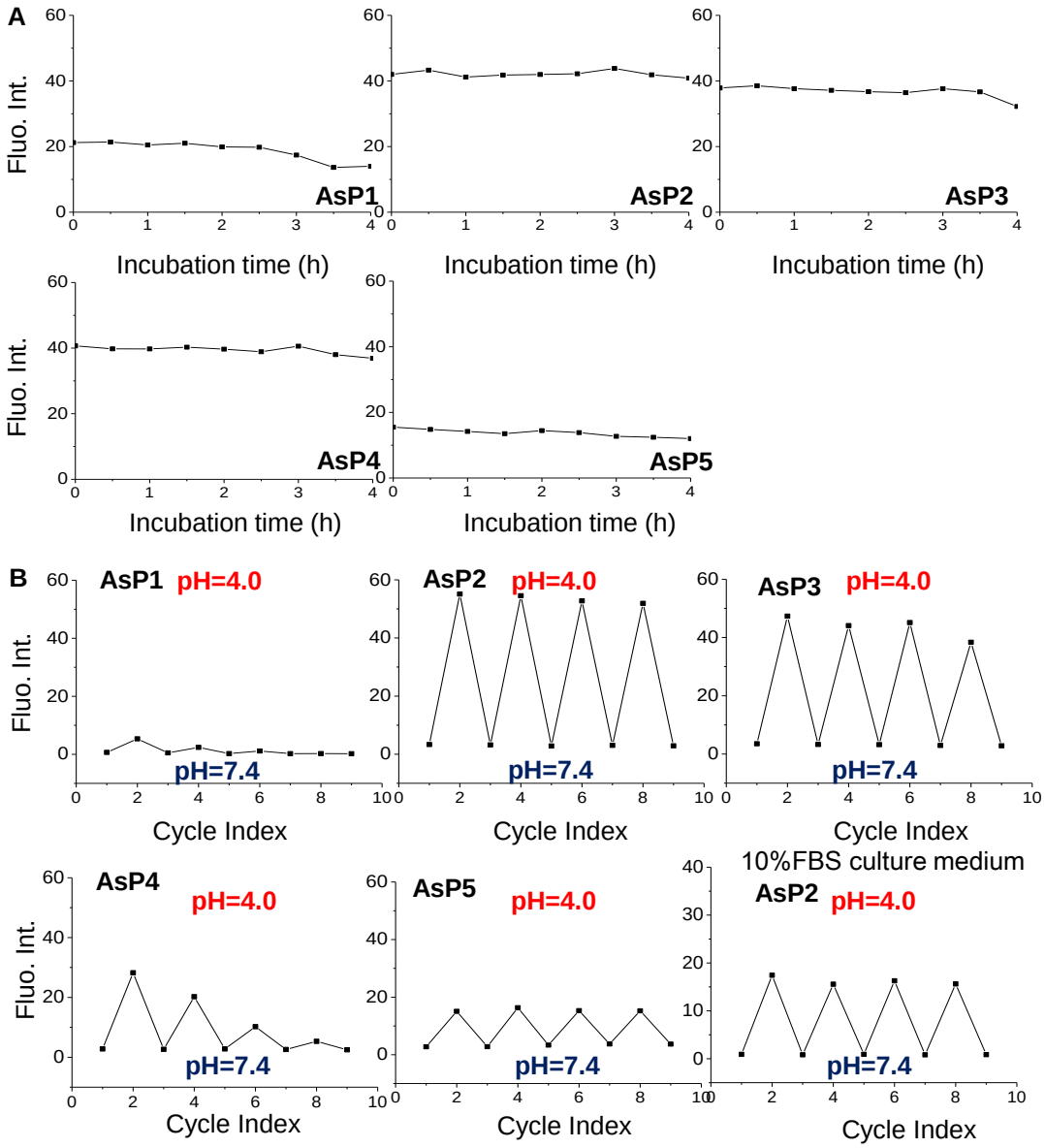


Figure S2. Stability of **AsP1–5** (1.0 μM, λ_{ex} = 765 nm) under physiologically acidic environment. (A) Fluorescence maximums of **AsP1–5** in buffered solution (pH 4.0) as a function of incubation time. Fluorescence intensities were measured every 30 min for 4 h. (B) Fluorescence maximums of **AsP1–5** with pH reversibly changed between 4.0 and 7.4 for four cycles in buffered solutions. The fluorescence maximum of **AsP2** was further investigated in culture medium contained 10% FBS.

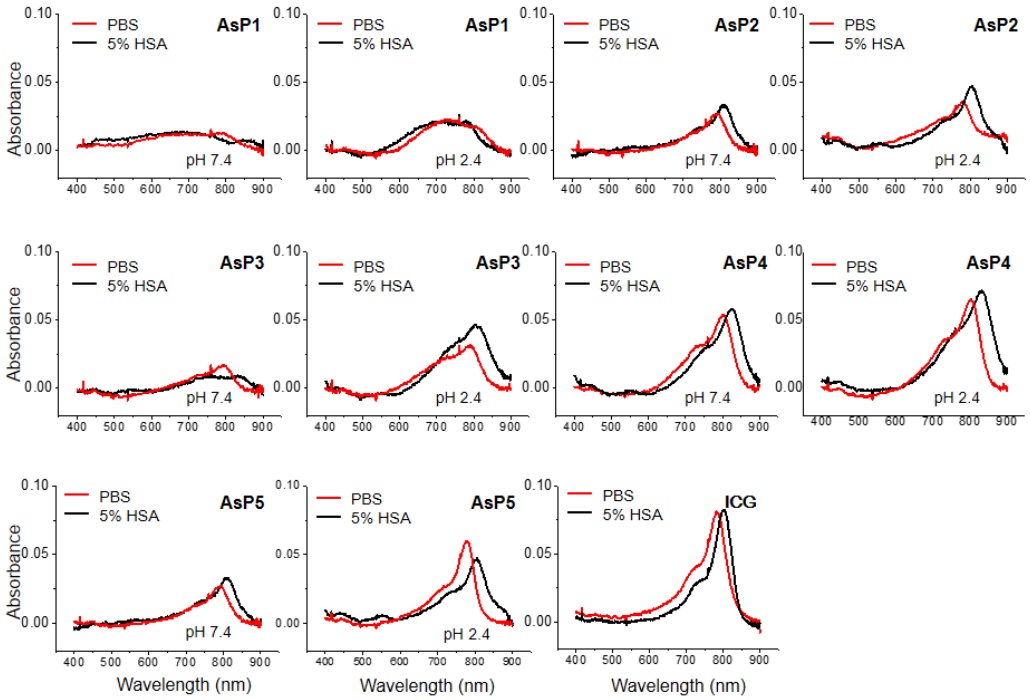


Figure S3. Absorption of **AsP1**−**AsP5** (1.0 μM) measured in PBS in presence or absence of 5% HSA. All the fluorophores (1.0 μM, λ_{ex} = 765 nm) were measured in disodium hydrogen phosphate buffer.

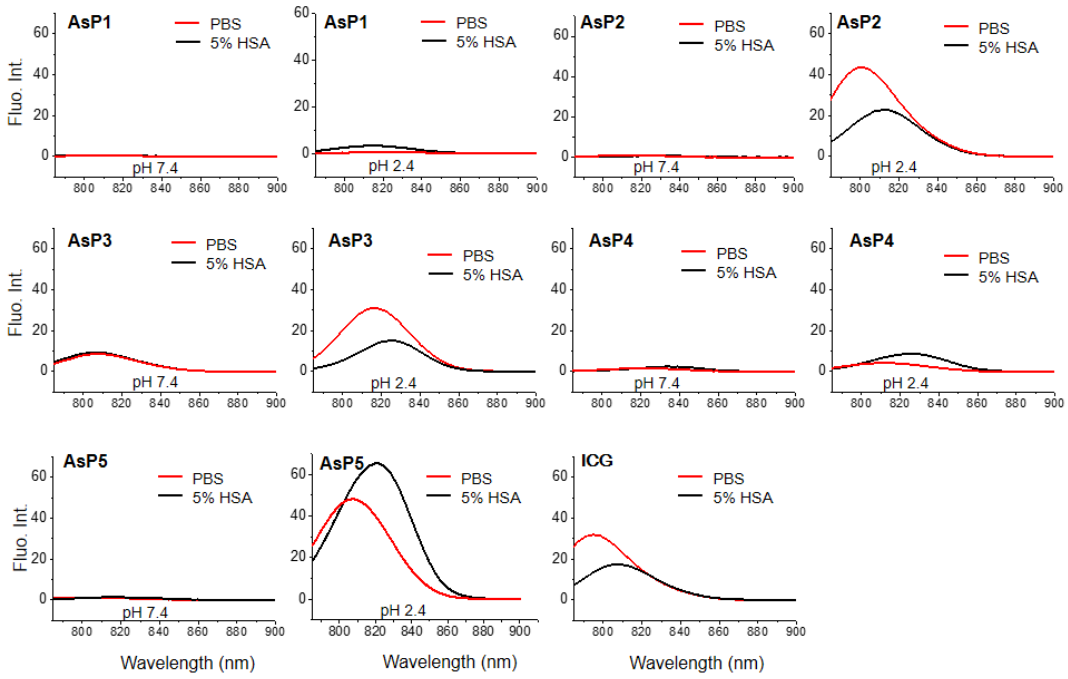


Figure S4. Emission of **AsP1**−**AsP5** (1.0 μM) measured in PBS in presence or absence of 5% HSA. All the fluorophores (1.0 μM, λ_{ex} = 765 nm) were measured in disodium hydrogen phosphate buffer.

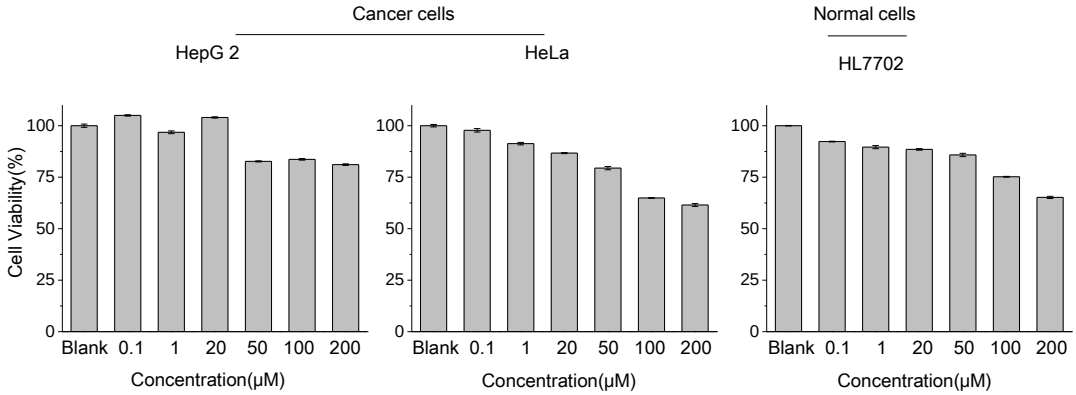


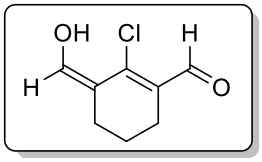
Figure S5. **AsP2** showed low cytotoxicity in both cancer and normal cells. Concentration dependent cytotoxicities of **AsP2** in hepatocellular carcinoma HepG2, adenocarcinoma HeLa and normal hepatic HL-7702 cell lines after incubation for 24 h. The relative cell viabilities were determined by CCK-8 assay. The data were presented as mean ± SD, n = 5 wells of 96-well plates for each group.

2. General Materials and Instruments

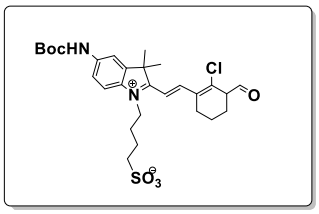
All chemical reagents and solvents for synthesis were obtained from Aladdin Reagent (China) unless otherwise specified. Thin layer chromatography was recorded on alumina based silica gel plates (Merck, Germany). 2,3,3-trimethyl-3H-indolium, 5-Cl-2,3,3-trimethyl-3H-indolium, 5-nitro-2,3,3-tetramethyl-3H-indolium, 2,3,3,5-tetramethyl-3H-indolium and 2,3,3-trimethyl-5-methoxy-3H-indolium were purchased from Beijing Chengyu Specialty Chemical Co. Ltd. (China). ^{1}H and ^{13}C NMR spectra were recorded on a 400 MHz (Varian, USA) or 600 MHz (Bruker, USA) NMR spectrometer. High resolution electron spray ionization (HR-ESI) mass spectra were obtained on Q-TOF 2 (Micromass, USA) or AB 5600+ Q-TOF mass spectrometer (AB Sciex, USA). All pH measurements were performed with a Mettler Toledo (Switzerland) MP220 pH meter. Absorption spectra were performed on a SHIMADZU (Japan) UV-2550 spectrophotometer. Fluorescence spectra were collected on a SHIMAZDU (Japan) RF-5301PC fluorophotometer. Fluorescence microscopic images were performed Zeiss (Germany) LSM 710 or 880 confocal microscope using a using a 63 X oil objective lenses and collected by using 488 nm or 633 nm lasers. Filter set: Band pass (BP) 495–550 nm, Long pass (LP) 645 nm. Dulbecco's Modified Eagle Medium (DMEM), Roswell Park Memorial Institute 1640 (RPMI 1640) and GIBCO fetal bovine serum (FBS) were purchased from Thermo Fisher Scientific (USA). LysoTracker Green DND-26

were purchased from Invitrogen (USA). Human hepatic HepG2 cancer cell line and human cervical HeLa cancer cell line were purchased from ATCC (USA). The normal human hepatic HL-7702 cell line was purchased from the Cell Bank of the Chinese Academy of Sciences (China).

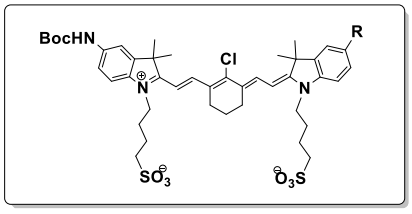
3. Synthesis and Characterization



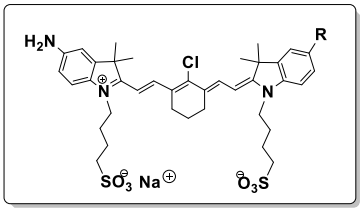
Compound 2. A mixture of 40 mL dimethylformamide (0.5 mol) and 40 mL of methylene chloride was chilled in an ice bath for 30 min. 37 mL phosphorus oxychloride (0.4 mol) and cyclohexanone (10.0 g, 0.1 mol) was added dropwise to the mixture with stirring. The solution was refluxed for 3 h, cooled, poured onto 200.0 g of ice, and allowed to stand overnight. The yellow solid was collected with a yield of 7.9 g (45.9%).



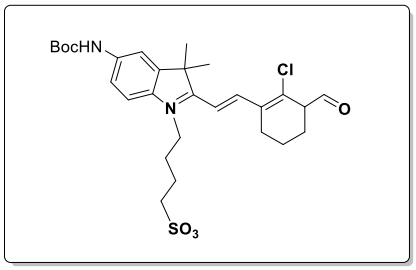
Compound 3. 2,3,3-trimethyl-5-tert-butoxycarbonylamino-indole sulfonate (419.1 mg, 1.0 mmol), sodium acetate (41.1 mg, 0.5 mmol) and 43.0 mg (1.0 mmol) of compound 2 were dissolved in 50 mL butanol/methylbenzene (V:V=7:3). The reaction flask was constantly stirring for 1.5 h under room temperature and the solvent was removed under vacuum. The crude was purified by column chromatography on silica gel with a mixture of CH_2Cl_2 and CH_3OH as eluent. Removal of solvent under vacuum afforded pure claret powder (394.9 mg, 70.2%).



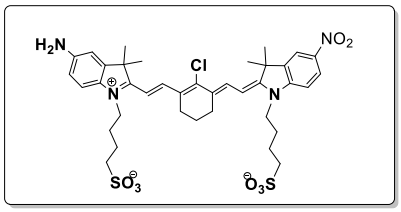
Compound 5a–5e. Compound **4a–4e** (1.0 mmol) and Compound 3 (1.0 mmol ,564.2 mg) were dissolved in 6 mL acetic anhydride in a flask. The reaction flask was heated at 70 ^oC refluxing with constant stirring for 45 min. The reaction solution was cooled to room temperature and the mixture was dropped into the methyl ether in an ice bath and rude green powder was filtrated after precipitation. The crude was purified by column chromatography on silica gel with a mixture of CH_2Cl_2 and CH_3OH as eluent. Removal of solvent under vacuum afforded pure deep green powder. The yields of **5a–5e** were determined in a range of 48.4-65.6%.



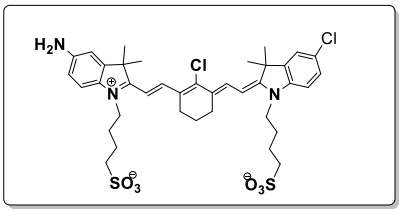
AsP1–5. De-protection of compound **5a–5e** (0.1 mmol) in a mixture of TFA and CH_2Cl_2 (V:V = 1:1) offered compound **AsP1–5**. The crude product was purified by silica gel based column chromatography with an eluting solvent system in which the gradient of CH_2Cl_2/CH_3OH (containing 0.1% trimethylamine). Removal of solvent and dehydration under vacuum afforded the purified product as green powder.



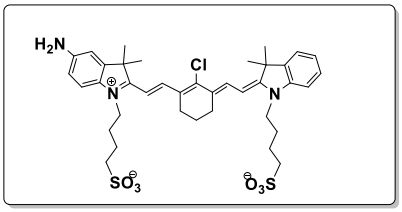
Characterization of Compound 3. ^{1}H NMR (400 MHz, CD_3OD) δ 10.08 (s, 1H), 7.91 (d, J = 12.5 Hz, 1H), 7.51 – 7.35 (m, 1H), 6.82 (d, J = 8.4 Hz, 1H), 6.39 (d, J = 8.2 Hz, 1H), 5.62 (d, J = 12.1 Hz, 1H), 2.98–2.79 (m, 2H), 2.60 (s,1H), 2.42 (s, 1H), 1.51 (s,5H), 1.49 (s,5H), 1.28 (d, J=9.6Hz, 6H). ^{13}CNMR (151MHz, DMSO) δ 188.72 (CH), 161.98 (C), 152.79 (C), 152.56 (C), 148.19 (CH), 144.80 (C), 135.84 (C), 132.01 (C), 131.46 (C), 113.24 (CH), 112.18 (CH), 111.11 (CH), 105.92 (CH), 91.40 (C), 67.84 (CH,CH_2), 52.21 (CH_2), 49.70 (C), 49.35 (2×CH_3), 24.76 (CH_2), 22.72 (CH_2), 20.87 (3×CH_3), 20.56 (CH_2), 19.29 (2×CH_2) TOF-MS calculated for $C_{28}H_{37}ClN_2O_6S$ - [M-]: 587.1953 (100%), found 587.1948.



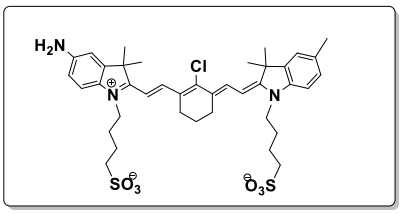
Characterization of AsP1. (yield:44.4%) ^{1}H NMR (400 MHz, CD3OD) δ 8.48 (d, J = 14.3 Hz, 1H), 8.21 (s, 1H), 7.89 – 7.75 (m, 1H), 7.4648 (d, J = 7.8 Hz, 2H), 7.35 (d, J = 7.8 Hz, 2H), 7.11 (s, 1H), 6.93 (s, 1H), 6.80 (s, 2H), 2.82 (d, J = 50.3 Hz, 3H), 2.70 (s, 2H), 1.84 (d, J = 66.6 Hz, 5H), 1.84 – 1.61 (m, 5H), 1.31 (dd, J = 15.8, 8.6 Hz, 56H), 0.89 (s, 3H). ^{13}C NMR (151 MHz, MeOD) δ 173.72 (C), 160.98 (C), 150.19 (C), 148.83 (C), 144.98 (C), 144.37 (C), 141.14 (CH), 139.51 (C), 134.17 (C), 132.20 (C), 129.06 (CH), 126.67 (CH), 125.02 (C), 124.27 (CH), 123.91 (CH), 118.13 (CH), 116.77 (CH), 114.61 (C), 113.79 (CH), 108.88 (CH), 107.05 (CH), 106.66 (C), 50.38 (C), 49.98 (CH_2), 49.63 (CH_2), 41.85 (CH_2), 41.47 (CH_2), 31.15 (CH_2), 29.82 (CH_2), 28.77 (2×CH_3), 28.71 (2×CH_3,CH_2), 28.42 (CH_2), 26.49 (CH_2), 25.80 (CH_2), 21.70 (CH_2). TOF-MS calculated for $C_{38}H_{46}N_4O_8S_2Cl$ - [M-]: 808.2338 (100%), found 808.2328.



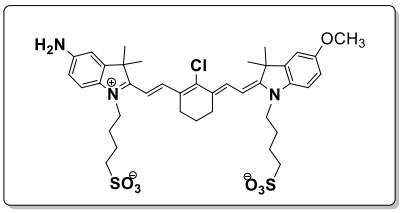
Characterization of AsP2. (yield:47.2%) ^{1}H NMR (400 MHz, CD_3OD) δ 8.46 (d, *J* = 14.7 Hz, 1H), 7.99 (d, *J* = 13.1 Hz, 1H), 7.31 (d, *J* = 34.6 Hz, 2H), 7.03 (s, 1H), 6.83 (d, *J* = 38.4 Hz, 2H), 6.59 (s, 1H), 5.89 (d, *J* = 13. 2 Hz, 1H), 4.36 (s, 2H), 3.93 (s, 2H), 3.19 (s, 2H), 2.87 (s, 3H), 2.71 (s, 3H), 1.91 (s, 4H), 1.68 (d, *J* = 20.3 Hz, 6H), 1.28 (s, 6H). ^{13}C NMR (15 1 MHz, MeOD) δ 173.03 (C), 164.33 (C), 148.38 (C), 146.00 (C), 144.64 (C), 144.39 (C), 141.02 (CH), 140.64 (C), 135.81 (C), 131.24 (C), 127.29 (CH), 126.62 (CH), 125.48 (C), 121.55 (CH), 115.22 (CH), 113.77 (CH), 113.09 (CH),113.65 (CH), 108.93 (C), 107.00 (C H), 106.02 (CH), 50.00 (C), 49.85 (C), 49.56 (CH_2), 47.83 (CH_2), 45.80 (CH_2), 44.48 (CH $_2$), 28.71 (CH_2), 26.49 (2×CH_3), 26.04 (CH_2), 25.97 (2×CH_3), 24.69 (CH_2), 21.64 (CH_2), 21. 49 (CH_2), 21.12(CH_2), 20.21 (CH_2). TOF-MS calculated for $C_{38}H_{46}N_3O_6S_2Cl_2$ - [M-]: 797.2 097 (100%), found 797.2100.



Characterization of AsP3. (yield:49.5%) ^{1}H NMR (400 MHz, CD3OD) δ 8.44 (d, J = 15.0 Hz, 1H), 8.09 (d, J = 13.0 Hz, 1H), 7.43 (s, 1H), 7.40 – 7.27 (m,2H), 7.08 (d d, J = 11.2, 7.5 Hz, 2H), 6.76 (d, J = 6.9 Hz, 1H), 6.53 (d, J = 14.5 Hz, 1H), 6.01 – 5.88 (m, 1H), 4.67 (s, 2H), 4.32 (s, 1H), 3.99 (s, H), 2.87 (s,2H), 2.70 (s,2H), 2.10 – 1.84 (m, 6H), 1.79 – 1.61 (m, 6H), 1.29 (d, J = 5.0 Hz, 14H), 0.88 (d, J = 6.9 Hz, 2H). ^{13}C NMR (151 MHz, MeOD) δ 172.40 (C), 166.01 (C), 148.65 (C), 144.04 (C), 142.51 (C), 139.34 (CH), 137.25 (C), 13 1.27 (C), 129.39 (C), 129.02 (CH), 128.83 (CH), 127.47 (CH), 124.37 (CH), 122.04 (CH), 121.08 (CH), 113.75 (CH), 113.20 (CH), 112.45 (C), 109.19 (C), 108.25 (CH), 107.42 (CH), 52.58 (CH_2), 50.03 (C), 49.62 (CH_2), 47.84 (CH_2), 44.33 (C), 42.00 (CH_2), 31.03 (CH_2), 26.62 (CH_2), 26.08 (2×CH_3,CH_2), 25.96 (CH_2), 25.40 (CH_2), 25.30 (CH_2), 24.91 (CH_2), 21.6 9 (2×CH_3), 21.52 (CH_2). TOF-MS calculated for $C_{38}H_{47}N_3O_6S_2Cl$ - [M-]: 763.2487 (100%), found 763.2489.



Characterization of AsP4. (yield:48.6%) ^{1}H NMR (400 MHz, CD_3OD) δ 8.85 (s, 1H), 8.61 (t, *J* = 7.4 Hz, 1H), 8.07 (s, 1H), 7.60 – 7.17 (m, 1H), 3.31 (d, *J* = 15.0 Hz, 2H), 2.77 (d, *J* = 86.3 Hz, 2H), 2.32 (d, *J* = 68.7 Hz, 1H), 1.91 (s, 2H), 1.65 (t, *J* = 30.4 Hz, 3H), 1.24 (s, 3 H), 0.86(s,1H). ^{13}C NMR(151MHz,MeOD): δ171.80 (C), 171.63 (C), 160.77 (C), 153.31 (C), 143.10 (C), 141.539 (C), 139.46 (CH), 134.89 (C), 129.04 (C), 128.36 (C), 125.72 (C H), 123.05 (CH), 122.09 (CH), 114.22 (CH), 110.75 (C), 110.075 (CH), 109.88 (CH), 107. 25 (C),106.02 (CH), 100.68 (CH), 100.18 (CH), 49.80 (CH_3), 49.14 (C), 48.82 (CH_2), 48.5 2 (CH_2), 48.38 (C), 47.84 (CH_2), 31.03 (CH_2), 28.70 (2×CH_3), 26.70 (2×CH_3, CH_2), 26.35 (CH_2), 25.46 (CH_2), 25.34 (CH_2), 21.69 (CH_2), 21.58 (CH_2), 20.26 (CH_2). TOF-MS calculat ed for $C_{39}H_{49}N_3O_6S_2Cl$ - [M-]: 777.2644 (100%), found 777.2650.



Characterization of AsP5. (yield:34.7%) ^{1}H NMR (400 MHz, CD_3OD) δ 8.37 (d, *J* = 14.4 Hz, 1H), 8.16 (d, *J* = 13.0 Hz, 1H), 7.23 (d, *J* = 8.6 Hz, 1H), 7.12 (d, *J* = 8.5 Hz, 1H), 7.05 (s, 1H), 6.94 – 6.82 (m, 1H), 6.75 (d, *J* = 8.4 Hz, 1H), 6.38 (d, *J* = 14.6 Hz, 1H), 6.01 (d, *J* = 14.0 Hz, 1H), 4.67 (s, 3H), 4.23 (s, 2H), 4.03 (s, 1H), 2.88 (s, 3H), 2.70 (s, 3H), 1.92 (s, 5H), 1.68 (d, *J* = 2.9 Hz, 6H), 1.27 (d, *J* = 7.1 Hz, 11H), 0.88 (d, *J* = 6.8 Hz, 2H). ^{13}C NMR (151 MHz, MeOD) δ171.43 (C), 168.02 (C), 156.87 (C), 147.66 (C), 143.35 (C), 143. 11 (C), 141.47 (CH), 138.98 (C), 135.75 (C),131.92 (C), 129.01 (C), 126.43 (CH), 125.74 (CH), 118.02 (CH), 113.79 (C), 112.47 (CH), 112.36 (CH), 109.67 (CH), 108.11 (CH), 107. 79 (CH), 102.62 (CH), 54.42 (CH_3), 49.96 (C), 49.73 (CH_2), 49.16 (CH_2), 47.84 (CH_2), 43. 67 (C), 42.52 (CH_2), 31.04 (CH_2), 29.94 (CH_2), 29.82 (2×CH_3), 26.52 (CH_2), 26.35 (CH_2), 26.23 (2×CH_3), 25.75 (CH_2), 21.81 (CH_2), 20.23 (CH_2). TOF-MS calculated for $C_{39}H_{49}N_3O_7S_2Cl$ - [M-]: 793.2593 (100%), found 793.2600.

4. Photospectroscopic Studies

Preparation of the stock solution. Stock solutions of **AsP 1–5** were prepared in dimethyl sulfoxide (DMSO) with a concentration of 10 mM. The solutions were then sub-packaged and stored at -40 ºC.

Absorption spectra. Absorption spectra of various samples at room temperate were obtained in the 400–900-nm spectral range using a SHIMADZU (Japan) UV-2550 spectrophotometer. Working solutions (1.0 μM) were prepared by diluting the stock solution in disodium hydrogen phosphate-citric acid buffer solution with different pH values (2.4, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.4). All absorption spectra were recorded in a quartz cuvette (10 × 10 mm) and scanning speed is 1.0 nm/s (slit width: 5 nm).

Emission spectra. Working solutions (1.0 μM) were prepared under the same procedures as absorption test. All emission spectra were performed with a photomultiplier tube at r.t. Fluorescence emission spectra of all samples were collected at a 90-degree angle relative to the excitation light path. All fluorophores were excited at 765 nm recorded from 780 nm to 900 nm.

Quantum yield measurements. The fluorescence quantum yields of **AsP1–5** in disodium hydrogen phosphate-citric acid buffer solution at pH 7.4 and pH 2.4 were determined with reference of that of indocyanine Green (ICG) in DMSO solution (Q.Y. = 0.12). The absorption spectra of each compound were measured with a concentration of 1.0 μM and the emission spectra with same concentration were excited at 765 nm wavelength. The fluorescence of each fluorescence spectrum was integrated, and the quantum yield was determined according to Equation 1:

$$\Phi_S=\Phi_R\times\frac{I_S}{I_R}\times\frac{A_R}{A_S}\times\frac{\eta_S^2}{\eta_R^2} \qquad \text{Equation 1}$$

In Equation 1, subscripts S and R refer to sample and reference respectively, Φ represents the fluorescence quantum yield, I stands for the measured integratedemission intensity, η is the refractive index, A is the optical density.

Measurements of pKa. pH dependent NIR fluorescent intensities of **AsP1–5** at their maximal emission wavelength were obtained from the emission spectra. All the values are normalized to the values under pH 2.4 respectively. The normalized fluorescent intensities were plotted against pHs in GraphPad Prism software. The plots were fitted into nonlinear regression curves (log(agonist) vs. response – Variable slope) and the pKa values were determined as the logEC50 values.

Photostability evaluation. Working solutions (1.0 μM) of **AsP1–5** were prepared in pH 4.0 disodium hydrogen phosphate-citric acid buffer solution. All fluorophores were excited at 765 nm and recorded at maximal emission wavelengths (796 nm, 802 nm, 797 nm, 802 nm and 815 nm for **AsP1–5** respectively). Fluorescence intensities were measured every 30 min for 4 h at r.t.

Reversibility evaluation. Working solutions (1.0 μM) of **AsP1–5** were prepared in disodium hydrogen phosphate-citric acid buffer solution. pH values of working solutions were reversibly adjusted between 7.4 and 2.4 by adding hydrochloric acid (5.0 M) and sodium hydroxide (5.0 M) solutions. All fluorophores were excited at 765 nm and recorded at maximal emission wavelengths (same as mentioned above). Fluorescence signals were measured for four rounds.

5. *In Vitro* Microscopic studies

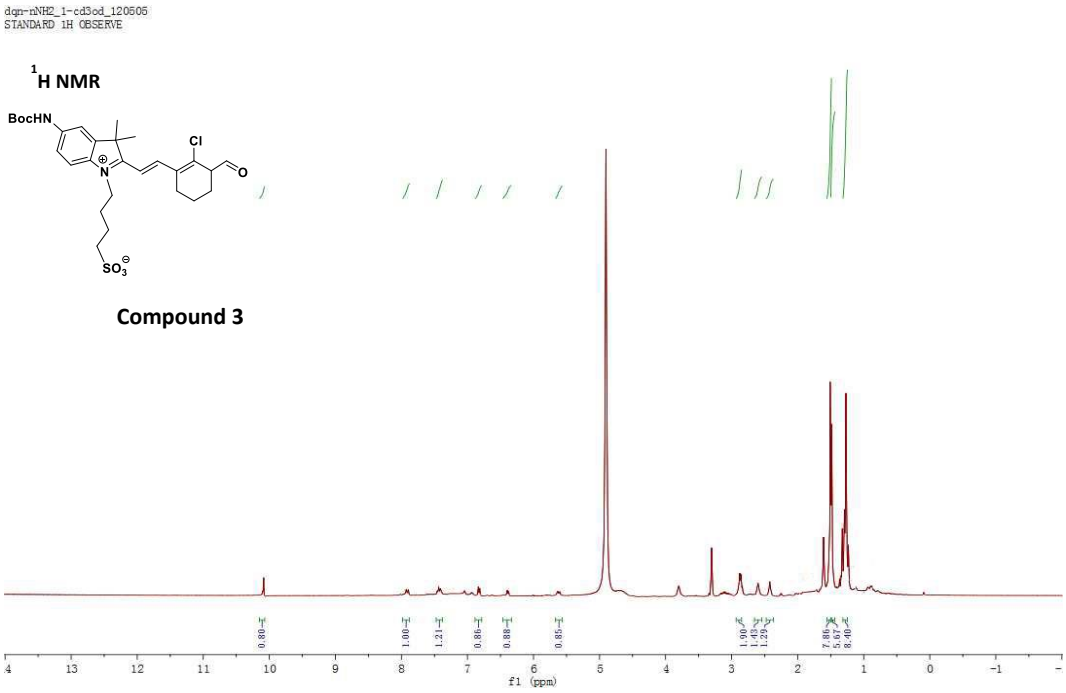
Cell culture. Human hepatocellular liver carcinoma HepG2 cell line, human adenocar cinoma HeLa cell line, and human normal liver HL-7702 cell line were used for *in vitro* st udies. Cells were grown in plastic tissue culture flasks added DMEM (for HeLa and Hep-G2) and RMPI 1640 (for HL-7702) culture medium containing 10% fetal bovine serum and 1% penicillin and streptomycin (PS) at 37 ^{o}C in a humidified incubator of 5% CO_2. Cells were carefully harvested and split. Experiments were carried out with cells cultured in ac-idic (pH 6.8) or normal (pH 7.4) medium for 6.0 h. The pH of the medium was adjusted with hydrochloric acid and sodium hydrate.

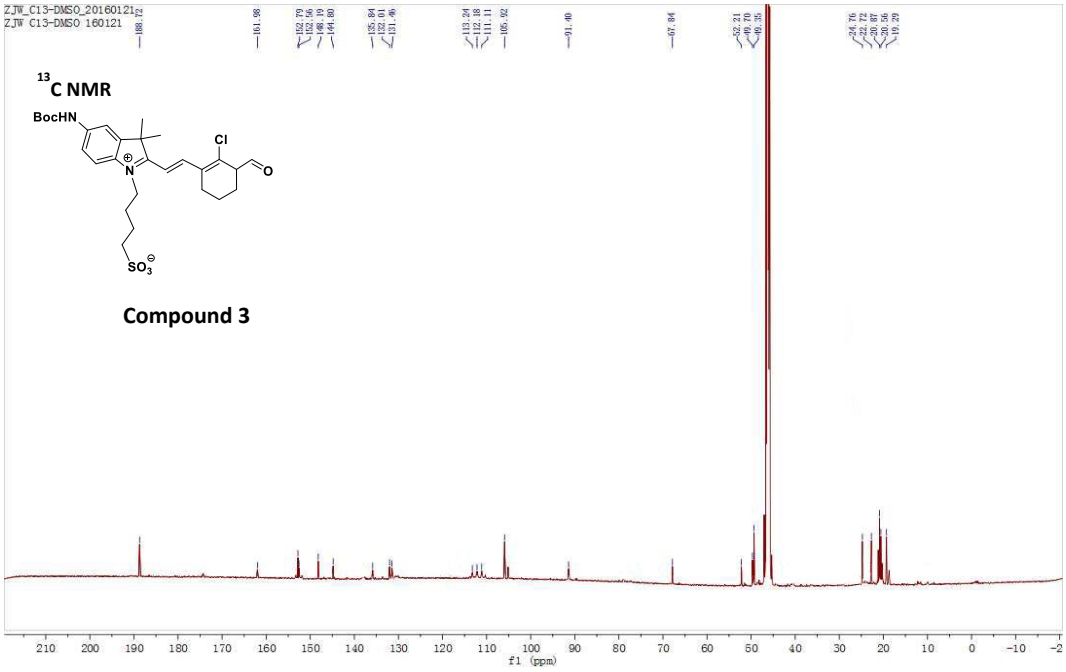
Cytotoxicity studies. The CCK-8 cell proliferation assay was applied to determine t-he viabilities of the cells treated with **AsP2**. A cell monolayer in exponential growth was harvested using 0.25% trypsin, and a single-cell suspension was obtained. Cell suspensi-ons with a density of 1.0 × 10^4 cells/well in 100 μL cell culture medium were added to 9 6-well plates. After cell attachment, the cells were treated by **AsP2** with different concent rations (0.01–200 μM) that was sterile filtered through MILLEX®-HV 0.22 μm syringe filter in DMEM/RMPI 1640. After another 24 h incubation, cells were washed twice with phos-phate buffer saline (PBS) and incubated with fresh medium containing 10 μL CCK-8. The cell viabilities were measured in an iMark Microplate Absorbance Reader according to th-e protocol. Cell viability (%) = mean of absorbance value of treatment group/mean of ab-sorbance value of control × 100%.

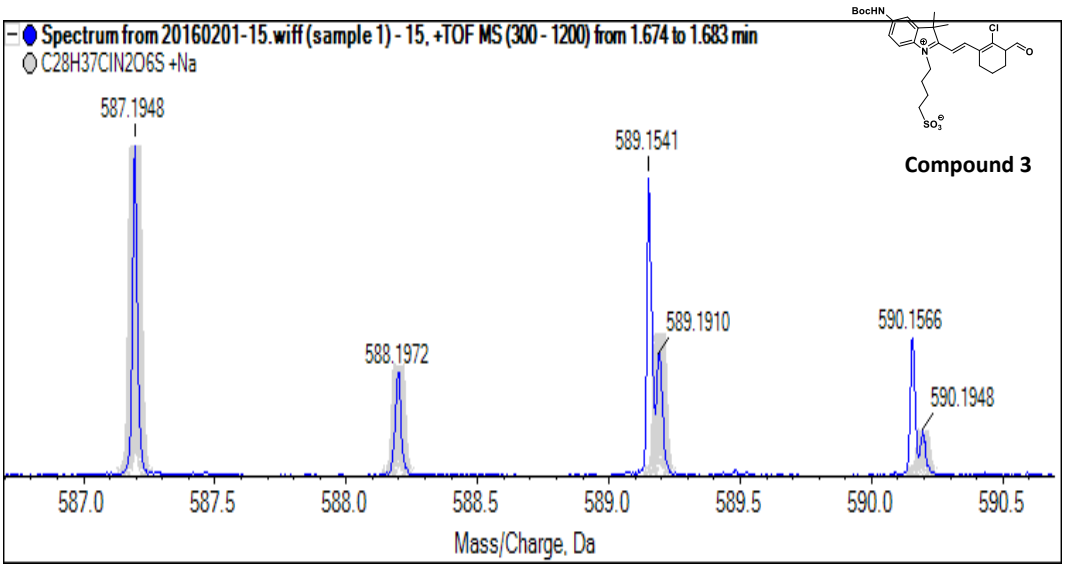
***In vitro* cellular uptake**. HepG2 cells (2 × 10^5 cells/well) were seeded into 35 mm glass bottom cell culture dishes (φ20 mm, NEST Biotechnology) in 2.0 mL culture medium. The culture medium was replaced by the DMEM containing **AsP2** (10 μM) after cell attachment and incubated for another 6 h. LysoTracker Green DND-26 (500 nM for 30 min) was added before cellular imaging. The cells were washed twice with PBS and observed by Zeiss LSM 880 confocal laser scanning microscope using a 63X oil objective lenses. A portion HL-7702 cells pre-treated with **AsP2** and LysoTracker were incubated with PBS (pH 4.0) for another 10 min before cellular imaging. The green fluorescence of LysoTracker Green DND-26 was collected by using a 488

nm laser. Filter set: Band pass (BP) 495–550 nm. The red fluorescence of **AsP2** were obtained by using a 633 nm laser. Filter set: Long pass (LP) 645 nm.

6. Supplemental spectra

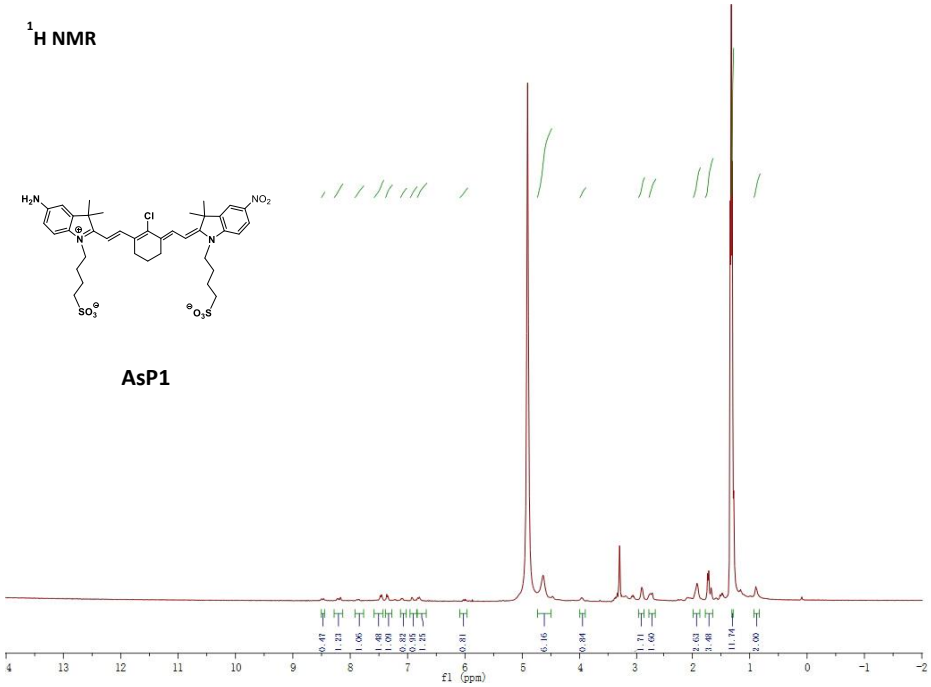




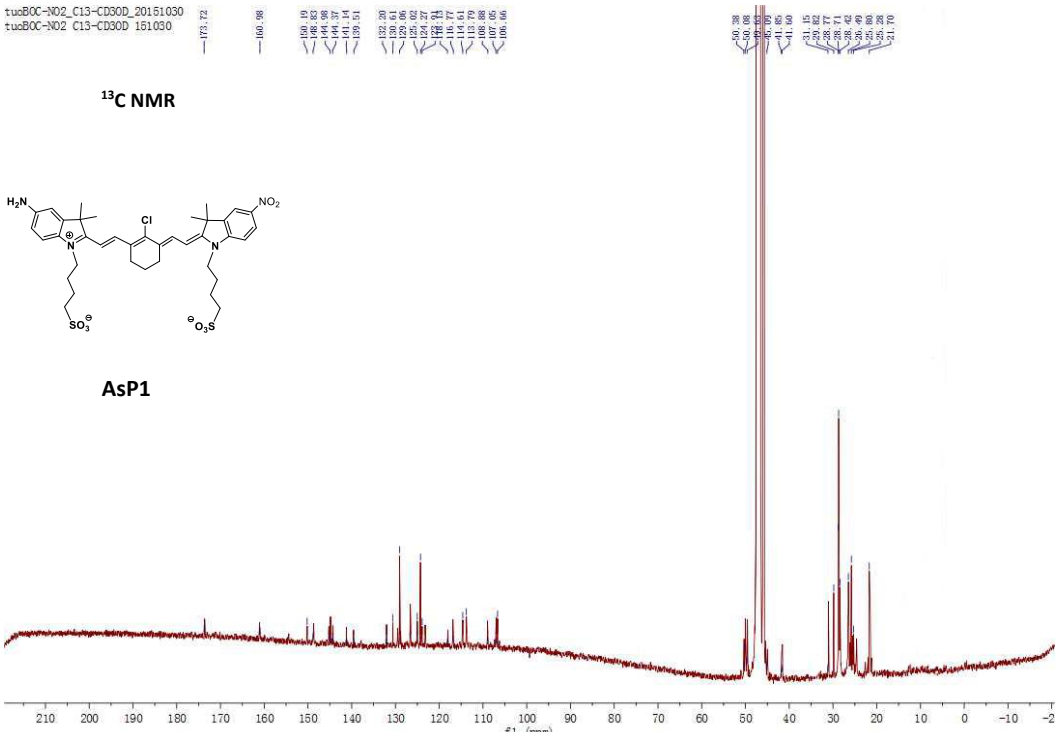


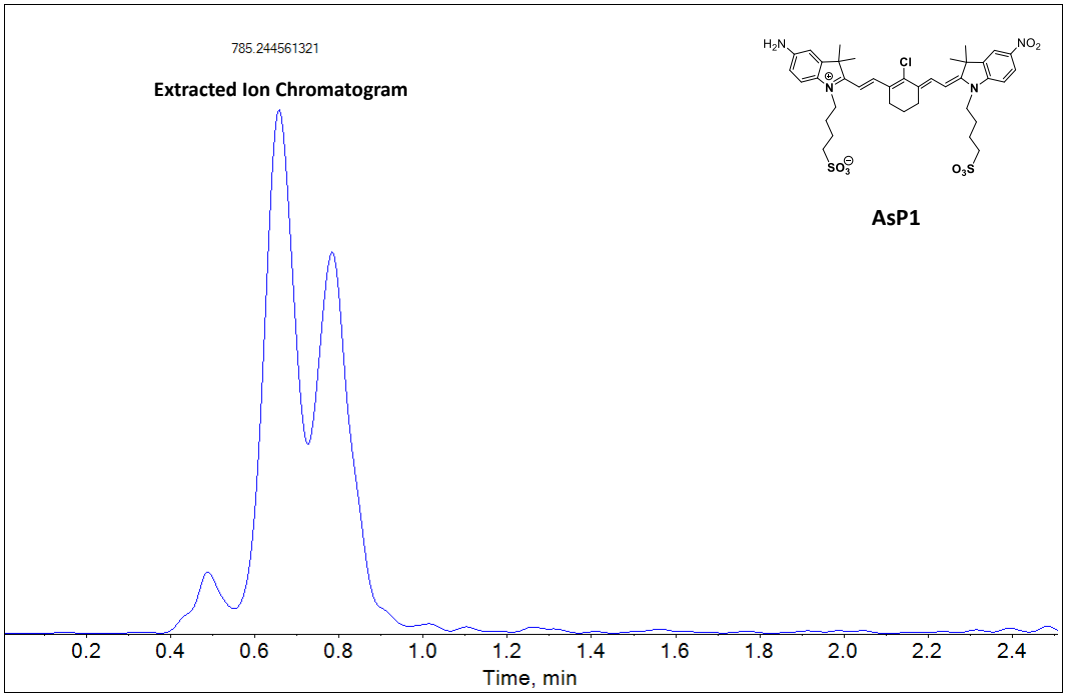
Compound 3

^{1}H NMR



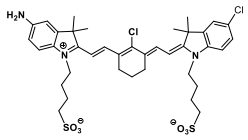
AsP1





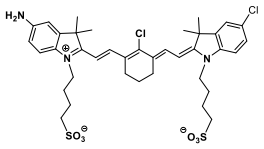
	Compound Name (Library Hit)	Formula	Intensity	Threshold	Expected m/z	Found at m/z	Error (ppm)	Expected RT (min)	Found RT (min)	RT Delta (min)	Isotope Diff (%)	Purity (%
✓●●●●	(1) 785.244561321	C38H46N4O8S2Cl	2037	100	808.2338	808.2328	-1.2	0.00	0.66	0.66	680.1%	N/A

^{1}H NMR

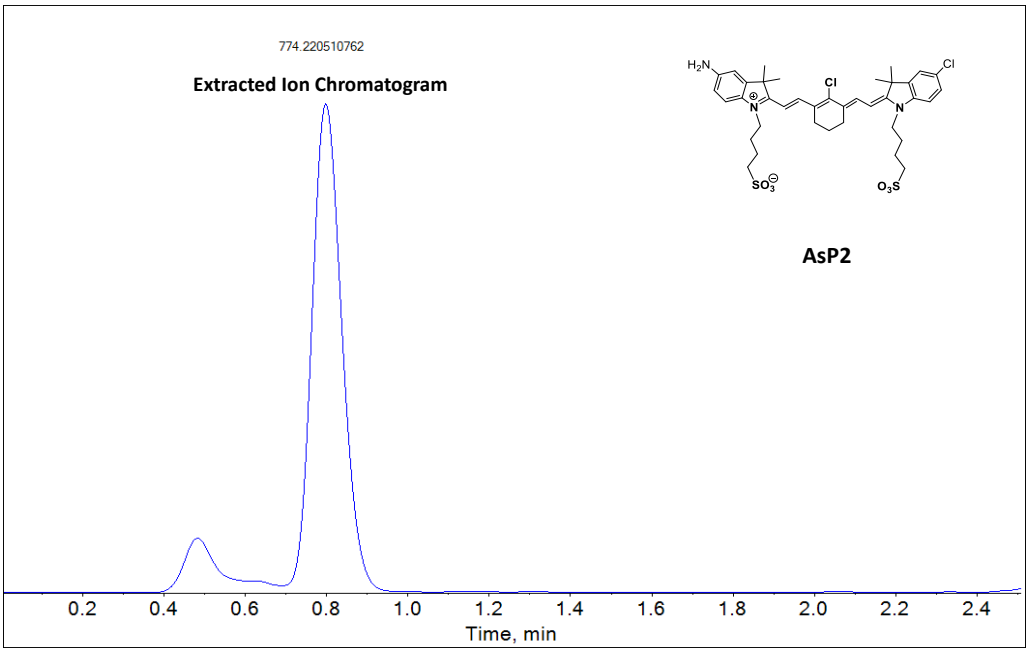


AsP2

^{13}C NMR



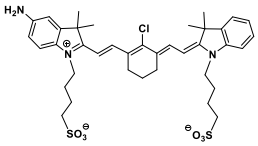
AsP2



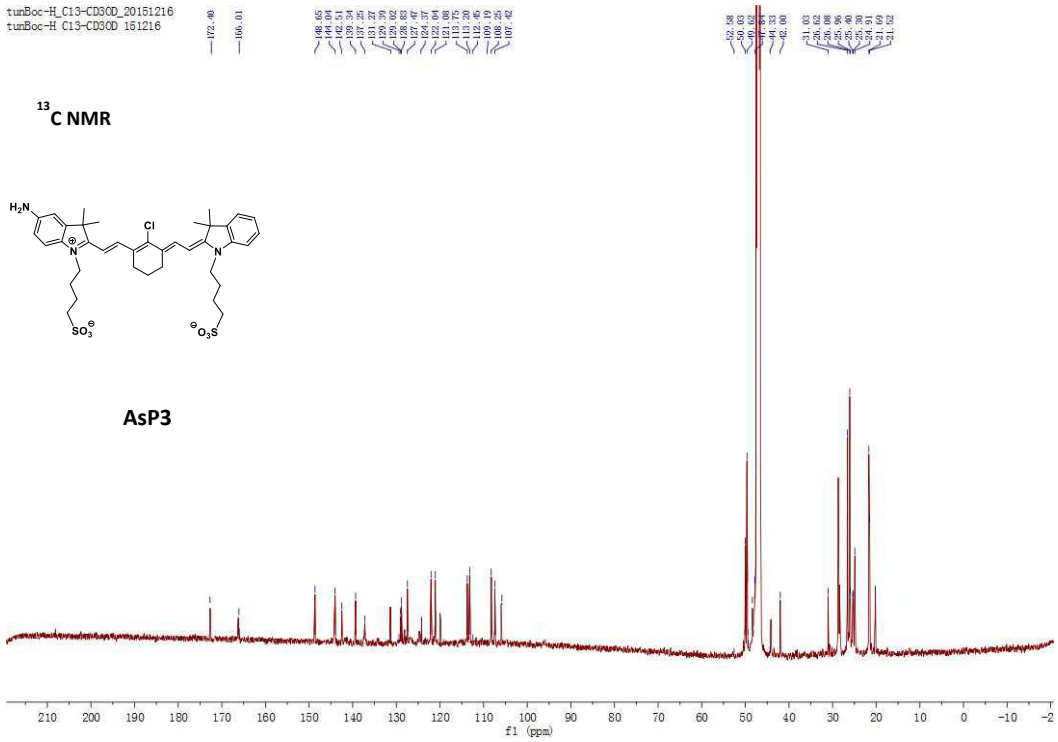
AsP2

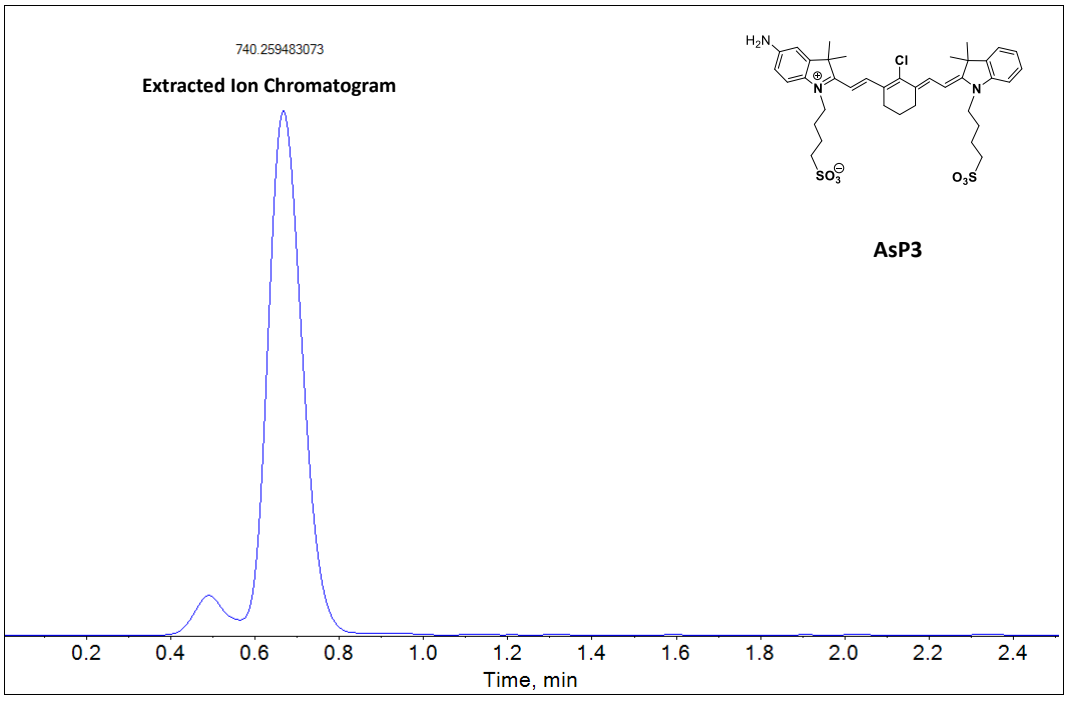
	Compound Name (Library Hit)	Formula	Intensity	Threshold	Expected m/z	Found at m/z	Error (ppm)	Expected RT (min)	Found RT (min)	RT Delta (min)	Isotope Diff (%)	Purity (%)
✓●●●●	(2) 774.220510762	C38H46N3O6S2Cl2	21868	100	797.2097	797.2100	0.3	0.00	0.80	0.80	45.1%	N/A

^{1}H NMR



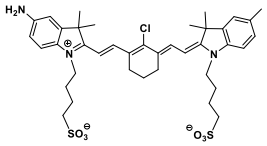
AsP3





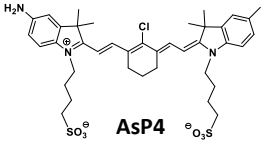
	Compound Name (Library Hit)	Formula	Intensity	Threshold	Expected m/z	Found at m/z	Error (ppm)	Expected RT (min)	Found RT (min)	RT Delta (min)	Isotope Diff (%)	Purity (%
✓●●●●	(3) 740.259483073	C38H47N3O6S2Cl	49825	100	763.2487	763.2489	0.2	0.00	0.67	0.67	55.1%	N/A

^{1}H NMR

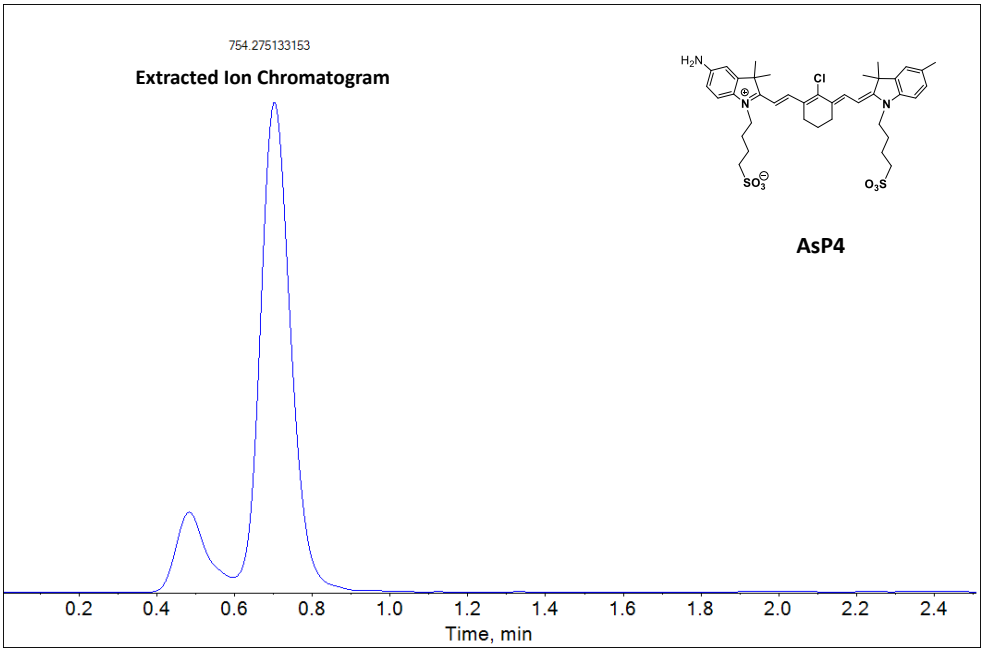


AsP4

^{13}C NMR



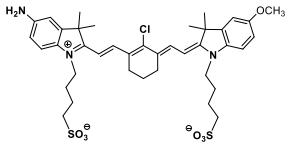
AsP4



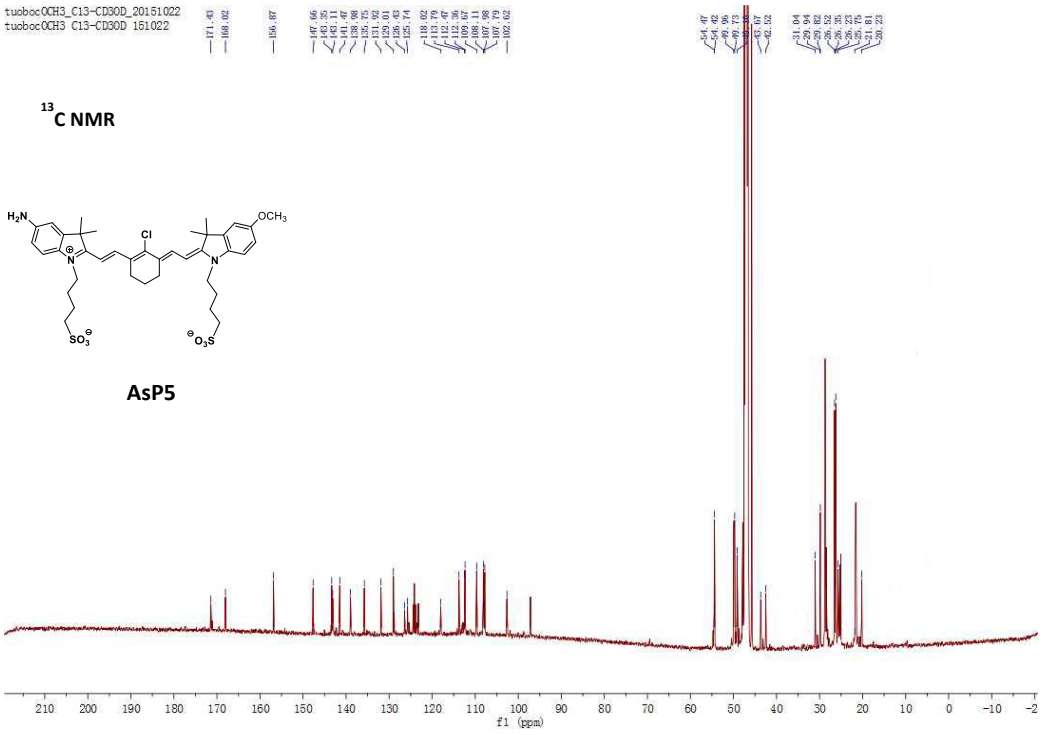
AsP4

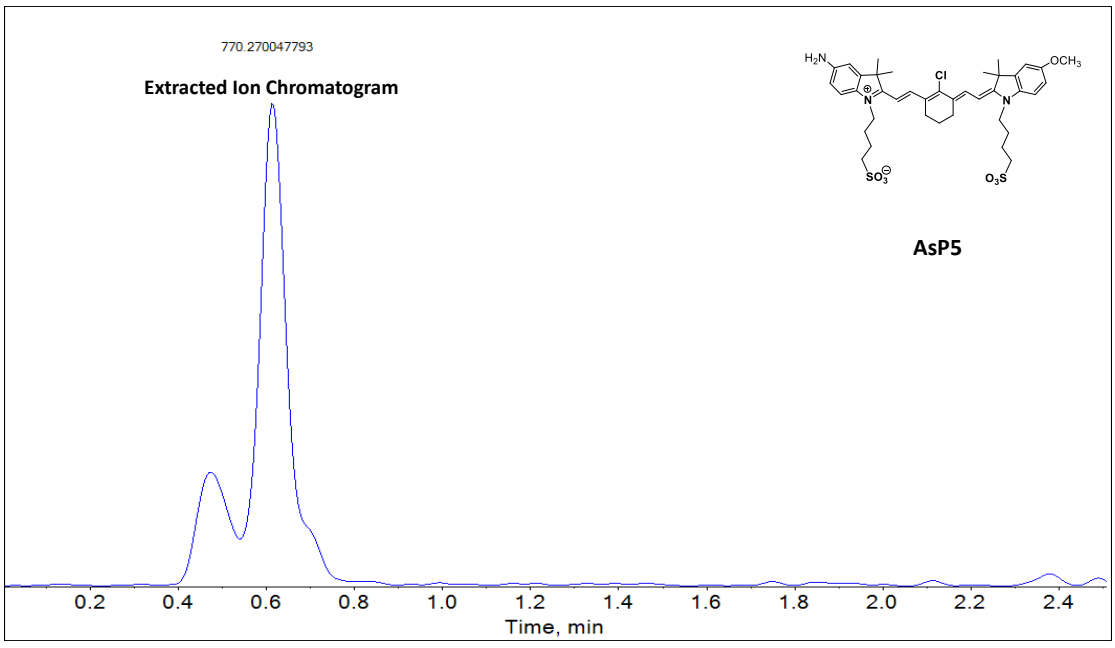
	Compound Name (Library Hit)	Formula	Intensity	Threshold	Expected m/z	Found at m/z	Error (ppm)	Expected RT (min)	Found RT (min)	RT Delta (min)	Isotope Diff (%)	Purity (%
✓	(4) 754.275133153	C39H49N3O6S2Cl	36181	100	777.2644	777.2650	0.9	0.00	0.70	0.70	92.7%	N/A

^{1}H NMR



AsP5





	Compound Name (Library Hit)	Formula	Intensity	Threshold	Expected m/z	Found at m/z	Error (ppm)	Expected RT (min)	Found RT (min)	RT Delta (min)	Isotope Diff (%)	Purity (%
✓●●●●	(5) 770.270047793	C39H49N3O7S2Cl	2189	100	793.2593	793.2600	0.9	0.00	0.61	0.61	236.5%	N/A