Supplemental Information

Engineering Tunable GTP/TPi-Responsive Liposomes through Liposomal Membrane Modulation using a Bis-triphenylphosphonium Lipid Switch

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1. Experimental

1.1 General Experimental

Reagents and solvents were generally purchased from Sigma-Aldrich or Fisher Scientific and used without further purification. PC (L-α-Phosphatidylcholine, mixed isomers from chicken egg), PA (L-α-phosphatidic acid, mixed isomers from chicken egg), and 18:1 (Δ9-Cis) PE (1,2-dioleoyl-s*n*-glycero-3-phosphoethanolamine) were purchased from Avanti Polar Lipids, Inc (Alabaster, AL). Dry solvents were obtained from a Pure Solv MD-7 solvent purification system purchased from Innovative Technology, Inc (Newburyport, MA). Column chromatography was performed using 230-400 mesh silica gel purchased from Sorbent Technologies. NMR spectra were obtained using a Bruker 500 MHz NMR spectrometer. Mass spectra were obtained with JEOL DART-Accustom mass spectrometer. Liposome extruder and polycarbonate membranes were obtained from Avanti Polar Lipids, Inc (Alabaster, AL). Ultrapure water was purified via a Millipore water system (≥ 18 MW·cm triple water purification system). Small quantities (< 5 mg) were weighed on a Mettler Toledo XS105 dual range analytical balance. Osmotic pressure was measured with Wescor Vapor Pressure Osmometer 5520. Fluorescence studies were performed using a Cary Eclipse Fluorescence Spectrophotometer from Agilent Technologies. DLS analyses were carried out with a Malvern Zetasizer Nano ZS instrument equipped with a 4.0 mW laser operating at l = 633 nm. Plots were generated using Origin Pro 2025b. All error bars in plots indicate the standard errors of at least three experimental replicates. Compounds **1.2**-**1.5** were synthesized as previously reported.[1]

1.2 Synthetic Procedures and Characterization Data

4-Hydroxy-4'-nonyl-[1,1'-biphenyl]-3-carbaldehyde (1.2)

5-Bromo-2-hydroxybenzaldehyde (**1.1**, 0.5649 g, 2.81 mmol), 4-(*n*-nonylphenyl) boronic acid (1.0450 g 4.21 mmol), tetrakis(triphenylphosphine)palladium(0) (0.0325 g, 0.0281 mmol) and potassium carbonate (K_2CO_3, 0.5818 g, 4.21 mmol) were combined in a 100 mL RBF under argon. After being dissolved with 25 mL toluene/H_2O/MeOH (2/2/1, v/v), the reaction mixture was heated at 100 ºC overnight (10.5 hours). After completion, the reaction was cooled to rt and the solvent was removed using a rotary evaporator. The residue was re-dissolved in 25 mL DCM and washed with 10 mL of 0.5 M HCl, followed by the addition of 50 mL water. The aqueous layer was extracted with 3 x 25 mL DCM. The combined organic layer was further washed with 1 x 50 mL water and 1 x 50 mL brine, respectively, before being dried over Na_2SO_4 , filtered, and concentrated under reduced pressure. The crude was next loaded onto silica gel and subjected to column chromatography. Gradient elution from hexanes to 10% ethyl acetate in hexanes yielded the product as a slight yellow solid (0.7028 g, 2.17 mmol, 77% yield). Rf = 0.63 (10% EtOAc/hexanes).

^{1}H NMR (500 MHz, $CDCl_3$) δ 10.98 (s, 1H), 9.97 (s, 1H), 7.75 (d, J = 8.5 Hz, 2H), 7.46 (d, J = 8.0 Hz, 2H), 7.27 (d, J = 8.0 Hz, 2H), 7.06 (d, J = 8.5 Hz, 1H), 2.65 (t, J = 8.0 Hz, 2H), 1.65 (p, J = 7.5 Hz, 2H), 1.34 (m, 6H),1.27 (m, 6 H), 0.88 (t, J = 7.0 Hz, 3H).^{13}C NMR (126 MHz, $CDCl_3$) δ 196.70, 160.76, 142.33, 136.64, 135.64, 133.35, 131.64, 129.03, 126.42, 120.70, 118.04, 35.57, 31.88, 31.49, 29.51, 29.32, 22.66, 14.10. HRMS-DART: $[M+H]^+$ calcd for $C_{22}H_{29}O_2$: 325.2166, Found: 325.21463

4,4''-(Ethane-1,2-diylbis(oxy))bis(4'-nonyl-[1,1'-biphenyl]-3-carbaldehyde) (1.3)

Compound **1.2** (0.1095 g, 0.3364 mmol) and K_2CO_3 (0.1273 g, 0.9211 mmol) were combined in a 50-mL RBF under argon and were dissolved with 5 mL of dry DMF. After the addition of 1,2-dibromoethane (13.27 µL, 0.1535 mmol), the reaction was heated to 80 °C. Reaction progress was monitored via TLC (25% EtOAc-hexanes) until starting materials were consumed after 42 hours, with equivalent additions of 1,2-dibromoethane at the reaction times 18 and 24.5 hours to ensure reaction completion. After completion, the mixture was cooled to rt and the solvent was removed via rotary evaporator. The brown-orange mixture was added to 100 mL of 0.5 M HCl and extracted with 3 x 25 mL DCM. The combined organic layer was then washed with 5 x 50 mL H_2O and 1 x 50 mL brine. After being dried over Na_2SO_4, filtered and concentrated under reduced pressure, the crude orange product was purified via silica gel column chromatography with a gradient elution of hexanes to 25 % ethyl acetate in hexanes, yielding the product as a yellow-orange solid (**1.3**, 0.1068 g, 0.1581 mmol, 94% yield). Rf = 0.41 (25% EtOAc/hexanes).

^{1}H NMR (500 MHz, $CDCl_3$) δ 10.51 (s, 2H), 8.09 (d, J = 2.5 Hz, 2H), 7.81 (dd, J = 8.5, 2.5 Hz, 2H), 7.51 (d, J = 8.0 Hz, 4H), 7.26 (d, J = 7.0 Hz, 4H), 7.13 (d, J = 7.0 Hz, 2H), 4.58 (s, 4H), 2.56 (t, J = 8.0 Hz, 4H), 1.56 (p, J = 7.5 Hz, 4H), 1.31 (m, 24H), 0.89 (t, J = 7.0 Hz, 6H).^{13}C NMR (126 MHz, $CDCl_3$) δ 189.30, 159.88, 142.38, 136.59, 134.67, 134.10, 128.96, 126.75, 126.56, 125.27, 113.18, 67.21, 35.58, 31.89, 31.48, 29.55, 29.52, 29.34, 29.33, 22.67, 14.11. HRMS-DART: $[M+H]^+$ calcd for $C_{46}H_{59}O_4$: 675.441335, Found: 675.46768

((Ethane-1,2-diylbis(oxy))bis(4'-nonyl-[1,1'-biphenyl]-4,3-diyl))dimethanol (1.4)

Compound **1.3** (0.0803 g, 0.1189 mmol) was dissolved in 12 mL of DCM/*i*-PrOH (3/1 v/v) under argon in a 50 mL RBF at 0°C. Sodium borohydride ($NaBH_4$, 0.0135 g, 0.3567 mmol) was slowly added while stirring. The mixture was then warmed to room temperature, stirred for 6 hours, and then re-cooled to 0°C. To quench the reaction, 10% citric acid solution was added dropwise until no gas evolution was observed, followed by 20 minutes of stirring. The organic layer was collected and the aqueous was extracted with 2 x 25 mL DCM. The combined organic layer was then washed with 1 x 50 mL water, 1 x 50 mL brine, and dried over $MgSO_4$. After filtering and concentrating, the crude was purified via column chromatography with a gradient elution of hexanes to 25% EtOAc/hexanes. For larger scale reactions, solubility issues require the use of a DCM/hexanes eluent. The product was obtained as a yellow-white solid (0.06457 g, 0.09510 mmol, 80% yield). Rf = 0.30 (25% EtOAc/hexanes).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.54 (d, J = 2.5 Hz, 2H), 7.50 (dd, J = 8.5, 2.5 Hz, 2H), 7.48 (d, J = 8.0 Hz, 2H), 7.24 (d, J = 8.0 Hz, 4H), 7.00 (d, J = 8.5 Hz, 2H), 4.73 (s, 4H), 4.45 (s, 4H), 2.64 (t, J = 8.0 Hz, 4H), 1.65 (p, J = 7.5 Hz, 4h), 1.29 (m, 24H), 0.90 (t, J = 7.0 Hz, 6H).^{13}C NMR (126 MHz, $CDCl_3$) δ 155.70, 141.74, 137.82, 134.70, 130.00, 128.81, 128.19, 127.24, 126.65, 112.15, 67.04, 61.77, 35.60, 31.91, 31.53, 29.58, 29.55, 29.39, 29.35, 22.69, 14.12. HRMS-DART: $[M + H - H_2O]^+$ calcd for $C_{46}H_{61}O_3$: 661.46152, Found: 661.44322

1,2-bis((3-(bromomethyl)-4'-nonyl-[1,1'-biphenyl]-4-yl)oxy)ethane (1.5)

Compound **1.4** (0.0500 g, 0.0736 mmol) was dissolved in 20 mL of dry DCM under argon in a 100 mL RBF and cooled to 0°C. Phosphorus tribromide (PBr_3, 208 µL, 2.208 mmol) was added dropwise while stirring. The mixture was then warmed to rt and stirred for 6 hours, followed by a re-cooling to 0°C to quench any unreacted PBr_3 with dropwise additions of MeOH. After quenching, the mixture was added to a separatory funnel with 100 mL of water. The aqueous layer was extracted with 2 x 20 mL DCM, and the combined organic layer was washed with 1 x 50 mL water and 1 x 50 mL brine. The crude was then dried over $MgSO_4$, filtered, concentrated under pressure, and purified via a short silica column with a gradient elution of hexanes to 10% ethyl acetate/hexanes. The product was yielded as a yellow-white solid (0.0533 g, 0.0662 mmol, 90% yield). Rf = 0.80 (25% EtOAc/hexanes).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.57 (d, J = 2.5, 2H), 7.54 (dd, J = 8.5, 2.5 Hz, 2H), 7.48 (d, J = 8.0 Hz, 4H), 7.25 (d, J = 8.0 Hz, 4H), 7.06 (d, J = 8.5 Hz, 2H), 4.61 (s, 4H), 4.53 (s, 4H), 2.64 (t, J = 8.0 Hz, 4H), 1.64 (p, J = 7.5, 4H), 1.27 (m, 24H), 0.89 (t, J = 7.0, 6H).^{13}C NMR (126 MHz, $CDCl_3$) δ 155.83, 141.87, 137.47, 134.45, 129.61, 128.84, 128.52, 126.86, 126.59, 112.60, 67.29, 35.58, 31.90, 31.52, 29.70, 29.56, 29.36, 29.11, 22.68, 14.11. HRMS-DART: $[M - Br]^+$ calcd for $C_{46}H_{60}BrO_2$: 723.37712, Found: 723.50699

Methyl((4'-nonyl-4-(2-((4'-nonyl-3-((triphenylphosphonio)methyl)-[1,1'-biphenyl]-4-yl)oxy)ethoxy)-[1,1'-biphenyl]-3-yl)methyl)diphenylphosphonium (BPLS)

Compound **1.5** (0.0600 g, 0.0790 mmol) was dissolved in 50 mL of a 1:4 v/v water acetone mixture. While stirring, NaN_3 (0.0077 g, 0.1185 mmol) was added and the suspension was stirred vigorously for 24 hours. To separate the suspension, 10 mL of DCM and water were added. The aqueous layer was then extracted with 3 x 10 mL of DCM and the combined organic layers were washed with water and then dried over $MgSO_4$. The solution was filtered and concentrated under pressure, yielding the white crude Rf= 0.72 (25% EtOAc/hexanes). This crude was then dissolved in 8 mL of THF:H_2O (3:1, v/v) under Ar. While stirring, triphenylphosphine (PPh_3, 0.1094 g, 0.4163 mmol) was added and the mixture was allowed to stir at rt for 24 hours. The mixture was concentrated under reduced pressure and purified via flash chromatography on an alumina column with a 0 to 10% MeOH gradient in DCM, yielding a pale yellow solid (0.0876 g, 0.0749 mmol, 88%). Rf = 0.22 (10% MeOH/DCM).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.60 (t, J = 8.0 Hz, 6H), 7.44-7.50 (m, 16H), 7.42-7.44 (m, 12 H), 7.33 (t, J = 2.5 Hz, 4H), 7.13 (dd, J = 12.0, 8.5 Hz, 4H), 6.86 (d, J = 8.5 Hz, 2H), 5.14 (d, J = 14.0 Hz, 4H), 3.7 (s, 4H), 2.55 (t, J = 2.5 Hz, 4H), 1.55 (p, J = 8.0 Hz, 4H), 1.21 (m, 24 H), 0.81 (t, J = 7.0 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 156.39, 142.16, 136.48, 134.95, 134.27, 134.00, 133.92, 130.14, 130.04, 128.91, 126.33, 118.38, 117.70, 116.69, 113.61, 68.11, 35.56, 31.90, 31.50, 29.58, 29.54, 29.34, 26.24, 25.86, 22.68, 14.11. ESI-MS: [M/2] calcd for $C_{82}H_{90}O_2P_2^{2+}$: 584.3203, Found 584.3207

1.3 Liposome preparation and assays

Preparation of liposomes for NR release assays

Stock solutions of **BPLS** were prepared at 19.93 mM in $CHCl_3$/MeOH solution (1/1, v/v), 32.46 mM PC, 33.60 mM DOPE, and 5 mM Nile red (NR) solutions were prepared in chloroform. All the stock

solutions were kept in a -20 °C freezer after preparation. To prepare liposomes through thin film hydration, appropriate volumes of each stock solution were pipetted in 0.5 dr vials to reach a total lipid concentration of 2 mM, with desired percentages of each lipid type. NR was added as an extra 5% of the total lipid content. The organic solvents were then evaporated under a nitrogen stream, and the resulting lipid films were kept under vacuum for at least one hour. The films were then hydrated with proper volumes of 1 x TBS buffer, (pH = 7.4) in a 60 °C water bath for 1 hr. The vials were vortexed every 15 minutes while incubating. Next, 10 freeze-thaw cycles were performed in a dry ice-acetone bath and 60 °C water bath. Lastly, the liposome solutions were extruded through a 200 nm polycarbonate membrane for 21 passes with an extruder purchased from either Avestin or Avanti. The resulting liposomes were then studied immediately.

Phosphorylated and carboxylate metabolite selectivity screen for liposome NR release

50 mM stock solutions of each metabolite were prepared by dissolving the reagents (sodium phosphate dibasic heptahydrate (Pi, $Na_2HPO_4 \cdot 7H_2O$); sodium pyrophosphate dibasic (PPi, $Na_2H_2P_2O_7$); adenosine 5'-diphosphate sodium salt (ADP); adenosine 5'-triphosphate disodium salt, hydrate (ATP); D-fructose-6-phosphate disodium salt (FP); D-fructose-1,6-bisphosphate trisodium salt (FBP); sodium tripolyphosphate (TPi); cytidine-5'-triphosphate disodium salt (CTP); guanosine-5'- triphosphate trisodium salt (GTP); uridine 5'-triphosphate trisodium salt hydrate (UTP), adenosine 5'-monophosphate monohydrate (AMP), malic acid (Mal), fumaric acid (Fum), oxaloacetic acid (Oxa), succinic acid (Suc)); with MilliQ water. For each study, 56 µL of liposome solution encapsulating NR was first added into a sub-micro quartz cuvette. After an initial scan, 14 µL of 50 mM stock solution of the metabolite under study was added to the cuvette (final concentration = 10 mM). Samples were incubated for 16 hours at rt and fluorescence intensities were recorded after incubation (excitation wavelength = 552 nm, excitation slit = 5 nm, emission slit = 5 nm). Control experiments were run by adding 14 µL of 1x TBS. When processing the data, the fluorescence intensities at 635 nm were selected and converted to percentage of the initial fluorescence before metabolite addition. Experiments were run at least three times with different batches of liposomes. Averaged data were reported with error bars denoting standard error.

Nucleotide triphosphate and TPi metabolite selectivity screen for liposome NR release

300 mM stock solutions of each metabolite were prepared by dissolving the same reagents previously described (ATP, GTP, CTP, UTP, TPi) in MilliQ water. For each study, 68 µL of liposome solution encapsulating NR was first added into a sub-micro quartz cuvette. After an initial scan, 2.33 µL of the 300 mM stock solution of the metabolite under study was added to the cuvette (final concentration = 10 mM). Samples were incubated for 16 hours at rt and fluorescence intensities were recorded after incubation (excitation wavelength = 552 nm, excitation slit = 5 nm, emission slit = 5 nm). Control experiments were run by adding 2.33 µL of 1 x TBS buffer. When processing the data, the fluorescence intensities at 635 nm were selected and converted to percentage of initial fluorescence before metabolite addition. Experiments were run at least three times with different batches of liposomes. Averaged data were reported with error bars denoting standard error.

NR kinetic release assays

The same 300 mM stock solutions of TPi and GTP were prepared as previously mentioned. For each study, 68 µL of liposome solution encapsulating NR was first added into a sub-micro quartz cuvette. After an initial scan, 2.33 µL of the 300 mM stock solution of the metabolite under study was added to the cuvette (final concentration = 10 mM). Samples were then capped, sealed, and measured continuously for 16 hours with measurements every 15 or 30 minutes. Control experiments were run by adding 2.33 µL of 1x TBS buffer, or by treating 0% **BPLS** liposomes with 2.33 µL of the stock solutions. When processing the data, the fluorescence intensities at 635 nm were selected and converted to percentage of initial fluorescence before metabolite addition. Experiments were run at least three times with different batches of liposomes. Averaged data were reported with error bars denoting standard error.

Preparation of liposomes for sulforhodamine B release assays

The same stock solutions of **BPLS**, PC, and DOPE prepared for the NR release assay were used. An additional stock of PA was prepared at 5 mM in $CHCl_3$/MeOH solution (1/1, v/v). 30 mM sulforhodamine B (SRB) stock solutions were prepared by dissolving SRB with 25 mM Tris-HCl buffer (298 mOsm/L) and the pH was then adjusted to 7.4 by adding 1 N NaOH droplets. The osmolality of the solution was determined to be 291 mOsm/L. To prepare liposomes through thin film hydration, appropriate volumes of each stock solution were pipetted in 0.5 dr vials to reach a total lipid concentration of 5 mM, with desired percentages of each lipid type. The organic solvents were then evaporated under a nitrogen stream, and the resulting lipid films were kept under vacuum for at least one hour. The films were then hydrated with proper volumes of 30 mM SRB in a 60 °C water bath for 1 hr. The vials were vortexed every 15 minutes while incubating. Next, 10 freeze-thaw cycles were performed by cycling in a dry ice-acetone bath and 60 °C water bath. The liposome solutions were then extruded through a 200 nm polycarbonate membrane for 21 passes with an extruder purchased from either Avestin or Avanti. Lastly, the unencapsulated SRB dye was removed by size-exclusion chromatography. A micro-column (5 ¾" glass pipette) was packed with Sephadex G-50 (pre-saturated with isotonic 25 mM Tri-HCl buffer). The fractions were collected in 3 key color change transition (clear, pink, and magenta, Figure S1). Pink fractions with some turbidity were collected and used in the following release assays. Confirmation of liposome formation was assessed by treating liposomes with Triton X-100 detergent while measuring fluorescence before and after treatment, where an increase in fluorescence intensity confirmed liposome presence.

Sulforhodamine B kinetic release assays

A 68 µL aliquot of liposomes encapsulating SRB prepared as previously described was added into a sub-micro quartz cuvette. After an initial scan, 2.33 µL of the 300 mM stock solution of the metabolite under study was added to the cuvette (final concentration = 10 mM). Samples were capped and sealed, and then measured continuously for 16 hours with measurements every 15 or 30 minutes (excitation wavelength = 550 nm, excitation slit = 2.5 nm, emission slit = 2.5 nm). Control experiments were run by adding 2.33 µL of 25 mM Tris-HCl buffer, or by treating 0% **BPLS** liposomes with 2.33 µL of the stock solutions. To calibrate the assay, samples were treated with 2 µL of 20% Triton X-100

detergent to lyse liposomes and induce 100% dye release. When processing the data, the fluorescence intensities at 603 nm were selected and converted to percentage of the fluorescence after Triton X-100 treatment. Experiments were run at least three times with different batches of liposomes. Averaged data were reported with error bars denoting standard error.

DLS analysis of liposomes before and after triggered release

DLS measurements were performed with a Malvern Zetasizer Nano ZS instrument with a 4.0 mW laser at I = 633 nm. Samples were prepared by diluting liposomes by 10x (5 µL liposomes, 45 µL buffer). All measurements were taken at a scattering angle of 173° at 20 °C. Bar graphs were made by averaging at least three experiments, with error bars denoting standard error.

Transmission electron microscope analysis

Liposomes were prepared at 4 µg/mL without any dye encapsulation using the standard thin film hydration procedure described above. For TEM studies, droplets of the liposome solution were placed on a sheet of parafilm (10 µL). For treated liposomes, appropriate volumes of 300 mM GTP or TPi stocks were added to the liposome solutions before droplets were placed on the parafilm. Thin carbon films supported by 200 mesh copper grids were placed face down onto the droplets and allowed to soak for 1 minute. The grids were then removed from the droplet and blotted. Droplets of dH_2O were then placed on the parafilm, where the grids were briefly placed (10 seconds) to rinse before removal and blotting. Droplets of 1% uranyl acetate were next placed on the parafilm sheet, followed by another 1-minute soak of the grids and blotting. Grids were allowed to air dry for 2 hours. Images were taken using a JOEL 1400 Flash microscope with a beam energy of 80 keV. Images were taken with a Gaten One View camera and processed with ImageJ software.

Fluorescence titration BPLS-GTP and BPLS-TPi binding experiment

BPLS stock prepared in MeOH/$CHCl_3$ at 19.93 mM and 300 mM GTP and TPi stocks prepared in MilliQ water used for previous studies were utilized for these experiments. 7.53 uL of **BPLS** stock was diluted to 50 µM to 3 mL in DMSO. An additional reference solution was prepared with 7.53 uL of MeOH/$CHCl_3$ diluted to 3 mL in DMSO. Each solution was added to a quartz cuvette and 1 uL of either GTP or TPi stocks were added with spectra recorded after each addition. When processing the data, intensities at 335 nm were selected. The decreases in fluorescence were plotted against the concentrations of GTP or TPi. Binding of GTP to **BPLS** was quantified by fitting to a single-site Langmuir isotherm, $\theta = [L] / (K_d + [L])$, where [L] is the free GTP concentration and K_d is the equilibrium dissociation constant. Nonlinear least-squares fitting of the titration data yielded K_d = 0.150 mM (±0.010 mM). Fluorescence titration for the **BPLS**-TPi interaction was also fitted to a single-site Langmuir binding isotherm, $\theta = \theta_0 + \Delta\theta([L] / (K_d + [L])$, using nonlinear least-squares regression. Both unweighted and weighted fits (weighted by the standard error of each measurement) were performed. The unweighted fit, assuming uniform variance across data points, yielded K_d = 0.417 (±0.026 mM). The weighted fit, accounting for heteroscedasticity in the fluorescence data, provided a more statically robust estimate of K_d = 1.225 (±0.081 mM).

Kinetic Fluorescence Release Curve Fitting

Fluorescence kinetic release profiles in Figure 4 were analyzed using nonlinear regression in OriginPro 2025b. Data were expressed as % of initial fluorescence (P_0=100) over time and fit to kinetic models describing either biphasic or burst-type release. Initial evaluation using a bi-exponential model:

$$P(t) = P_\infty + (P_0 - P_\infty)\,[\phi e^{-k_1 t} + (1-\phi)e^{-k_2 t}]$$

revealed large uncertainties in the fast rate constant (k_1) due to the limited temporal resolution at early time points. As the fast release process occurred within the sampling interval, a simplified burst-plus-first-order model was adopted.

$$P(t) = P_\infty + (P_0 - P_\infty)(1-\beta)e^{-k_2 t}$$

where β represents the fraction of fluorescence quenched within the first measurement ("burst" release) and k_2 describes the subsequent slower first-order decay. Parameters P_0 and P_∞ were fixed to 100 and 0, respectively. The burst model provided statistically comparable fits to the bi-exponential with fewer free parameters and random residuals.

Fluorescence kinetic release profiles in Figure 7 were also analyzed using nonlinear regression in OriginPro 2025b and were fit to a single-exponential association model:

$$P(t) = F_\infty(1 - e^{-k\,t})$$

where F_∞ is the plateau fraction of released dye and $k$is the first-order rate constant.

Nile red encapsulation efficiency

Fluorescence scans were used to assess cargo encapsulation, where first a Nile red calibration curve in chloroform was generated. Liposomes used in NR release assays (35/35/30 and 50/20/30 **BPLS**/DOPE/PC) were prepared as previously described. After liposome formation, an aliquot of 300 µL of liposomes was added to a 4 dr vial and water was removed under reduced pressure. The film was then re-dissolved with 3 mL chloroform, followed by sonication and filtration through a 0.2 µm PTFE filter. The solution was then transferred to a quartz cuvette for fluorescent measurements and the fluorescent intensities at 600 nm were used to calculate the NR encapsulation efficiency.

Sulforhodamine B encapsulation efficiency

Fluorescence scans were used to assess cargo encapsulation, where first a sulforhodamine B calibration curve in MilliQ + 2% Triton X-100 was generated. Liposomes used in SRB release assays (37.5/32.5/2/28 and 37.5/36.5/2/24 **BPLS**/DOPE/PA/PC) were prepared as previously described. After liposome formation, samples were treated with Triton X-100 for a final concentration of 2%. All samples and stocks were diluted 10x to mitigate signal self-quenching. The solutions were then transferred to a quartz cuvette for fluorescent measurements and the fluorescent intensities at 585 nm were used to calculate the SRB encapsulation efficiency.

Preparation of liposomes for doxorubicin release assays

The same stock solutions of **BPLS**, PC, and DOPE prepared for the NR/SRB release assays were used. Fresh 10 mg/mL solutions of Dox were prepared in MilliQ water for each study and protected from light. Citrate buffer (150 mM, pH 4.0) and HEPES buffer (20 mM, pH 7.4) were prepared and their osmolarities corrected to 303 and 297 mOsm/L respectively. To prepare liposomes through thin film hydration, appropriate volumes of each stock solution were pipetted in 0.5 dr vials to reach a total lipid concentration of 5 mM, with desired percentages of each lipid type. The organic solvents were then evaporated under a nitrogen stream, and the resulting lipid films were kept under vacuum for at least one hour. The films were then hydrated with proper volumes of 150 mM citrate buffer in a 60 °C water bath for 1 hr. The vials were vortexed every 15 minutes while incubating. Next, 10 freeze-thaw cycles were performed by cycling in a dry ice-acetone bath and 60 °C water bath. The liposome solutions were then extruded through a 200 nm polycarbonate membrane for 21 passes with an extruder purchased from either Avestin or Avanti. Extruded liposome solutions were then loaded into Slide-A-Lyzer Mini cassettes (10 kDa, 0.1-0.5 mL) and dialyzed against 100 mL 20 mM HEPES buffer at 4°C while stirring. Buffer was changed every 30 minutes until 3 exchanges were completed. Liposomes were then added to a 1 dr vial with Dox added to a final concentration of 1 mM. Liposomes and Dox were incubated in a 60 °C water bath for 20 minutes with gentle mixing every 5 minutes. Solutions were then cooled on ice for 5 minutes before loading into an additional Slide-A-Lyzer Mini cassette (10 kDa, 0.1-0.5 mL) and exchanged against 100 mL 20 mM HEPES buffer at 4°C while stirring again. Buffer was changed every 30 minutes until 3 exchanges were completed. Liposomes were then recovered from cassettes and used for release studies. Confirmation of liposome formation was assessed by treating liposomes with Triton X-100 detergent while measuring fluorescence before and after treatment, where an increase in fluorescence intensity confirmed liposome presence.

Doxorubicin encapsulation efficiency

Fluorescence scans were used to assess cargo encapsulation, where first a doxorubicin (Dox) calibration curve in MilliQ + 2% Triton X-100 was generated. Liposomes used in Dox release assays (35/35/30 and 50/20/30 **BPLS**/DOPE/PC) were prepared as previously described. After liposome formation, samples were treated with Triton X-100 for a final concentration of 2%. The solutions were then transferred to a quartz cuvette for fluorescent measurements and the fluorescent intensities at 592 nm were used to calculate the Dox encapsulation efficiency.

Doxorubicin fluorescence release assays

Liposomes were prepared as previously described for Dox release assays. Initial liposome samples were lysed with 2% Triton X-100 and initial maximum fluorescence values were recorded. Fresh liposome samples were then left untreated or treated with 300 mM stock solution of TPi or GTP, or an equivalent volume of 20 mM HEPES buffer. Sample fluorescence was re-measured after 16 hours, followed by lysing with 2% Triton X-100 and final fluorescence values were recorded. When processing the data, the fluorescence intensities at 592 nm were selected and converted to percentage of Dox released after metabolite/buffer addition.

NR kinetic release assays in media

Liposomes were prepared as previously described for NR kinetic release assays, with concentration increased to 5 mM. Phenol red-free Dulbecco's modified eagle medium (DMEM) was supplemented with 10% (v/v) fetal bovine serum (FBS, Gibco) and 50 U mL^{-1} penicillin/streptomycin (pen/strep)Gibco). To each microplate well, 100 uL of DMEM and 40 uL of prepared liposome solution was added for a final liposome concentration of 1.42 mM per well. After an initial fluorescence scan, 4.66 µL of TPi, GTP or PBS (+Mg, +Ca) was added to the appropriate wells, and measurements were taken at sequential time points. When processing the data, the fluorescence intensities at 635 nm were selected and converted to percentage of initial fluorescence before metabolite/buffer addition.

NR release assays with mixed metabolite treatment

Liposomes were prepared as previously described for NR release assays at 2 mM concentration. Mixed metabolite solutions were made in MilliQ water with sodium phosphate (Pi), sodium pyrophosphate (PPi), adenosine 5'-diphosphate (ADP), adenosine 5'-triphosphate (ATP), D-fructose-6-phosphate (FP), D-fructose-1,6-bisphosphate (FBP), TPi, cytidine-5'-triphosphate (CTP), GTP, uridine 5'-triphosphate (UTP), adenosine 5'-monophosphate (AMP), malic acid (Mal), fumaric acid (Fum), and oxaloacetic acid (Oxa) for a total final concentration of 300 mM of all metabolites (25 mM for each metabolite). Alternate mixed metabolite solutions were made with the same metabolites at half the concentration (12.5 mM each metabolite, 150 mM total), with 150 mM TPi or GTP added. Prepared liposomes were treated with buffer, the 300 mM mixed metabolite solution, the TPi containing mixed metabolite solution or the GTP containing mixed metabolite solution for a final concentration of either 12 mM mixed metabolites, 6 mM mixed metabolites and 6 mM TPi, or 6 mM mixed metabolites and 6 mM GTP. When processing the data, the fluorescence intensities at 635 nm were selected and converted to percentage of initial fluorescence before metabolite/buffer addition.

NR release assays with phosphate-buffered saline hydrated liposomes

Liposomes were prepared as previously described for NR release assays at 2 mM concentration, but were hydrated with 1 x PBS (pH, 7.4) instead of TBS. Initial fluorescence was measured, then liposomes were left untreated, or treated with NaCl or an equivalent volume of PBS(+)(+) for a final concentration of 10 mM. After 48 hours, sample fluorescence was re-measured. When processing the data, the fluorescence intensities at 635 nm were selected and converted to percentage of initial fluorescence before salt/buffer addition.

Reference

(1) Lou, J.; Schuster, J. A.; Barrera, F. N.; Best, M. D. ATP-Responsive Liposomes via Screening of Lipid Switches Designed to Undergo Conformational Changes upon Binding Phosphorylated Metabolites. *J. Am. Chem. Soc.* **2022**, *144* (8), 3746–3756. https://doi.org/10.1021/jacs.2c00191.

2. Supplemental Figures

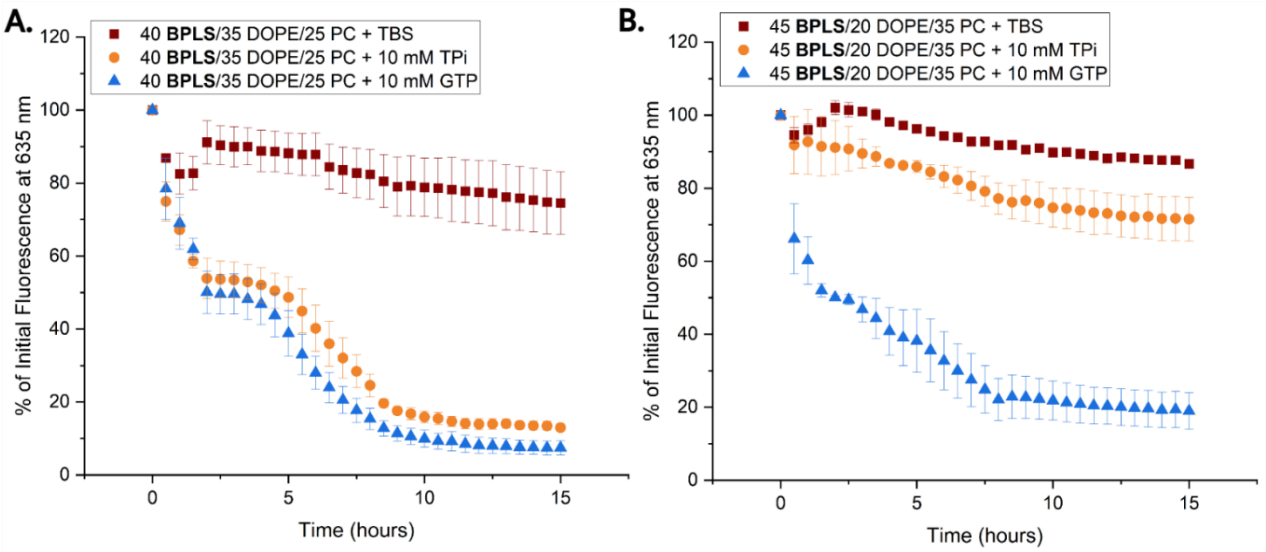


Figure S1. NR dye release comparison of two liposome formulations treated with 10 mM GTP or TPi. Significant triggered release from each metabolite was observed for 40/35/25 **BPLS**/DOPE/PC liposomes (A), but formulation adjustment to 45/20/35 **BPLS**/DOPE/PC (B) promoted metabolite triggering selectivity by GTP. Error bars denote standard errors from at least three biological replicates.

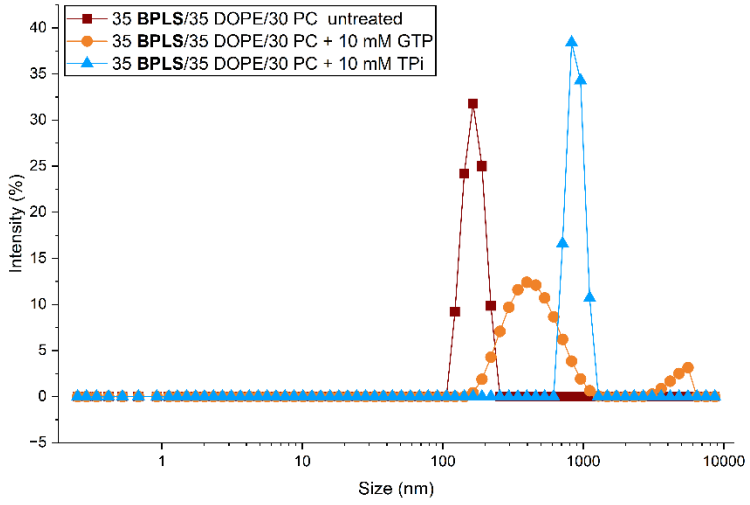


Figure S2. Representative raw DLS distribution curves for 35/35/30 **BPLS/**DOPE/PC liposomes before and after treatment with GTP or TPi. A mix of stable and aggregate liposomes was observed for GTP treated liposomes, whereas uniformly larger liposomes were observed with TPi treatment.

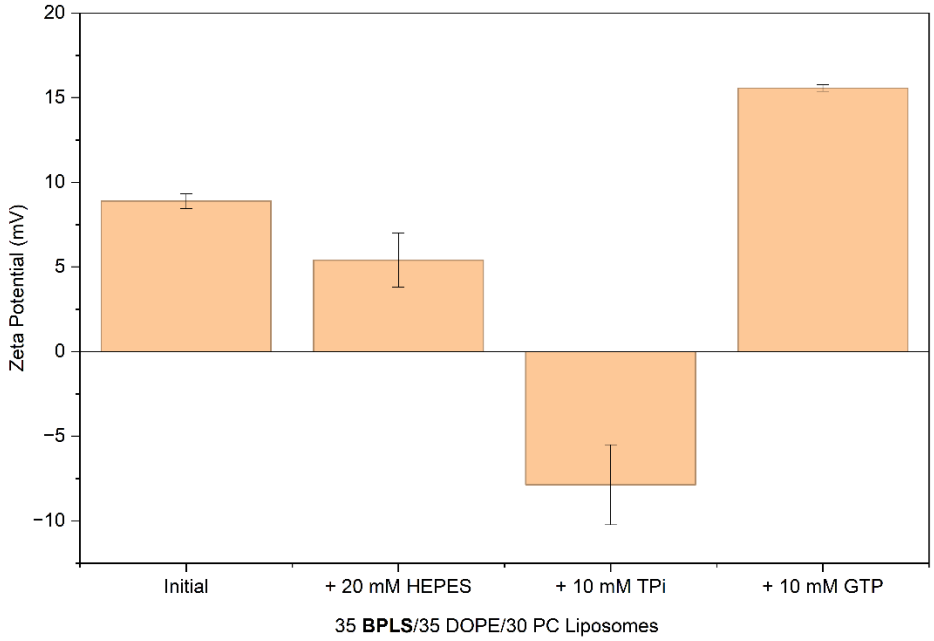


Figure S3. Zeta potential studies with 35/35/30 **BPLS**/DOPE/PC liposomes with initial measurements and samples treated with HEPES, TPi, or GTP. Treatment with 10 mM TPi resulted in an overall charge flip to negative, which is not observed in the other measured samples. Error bars denote standard errors from at least three biological replicates.

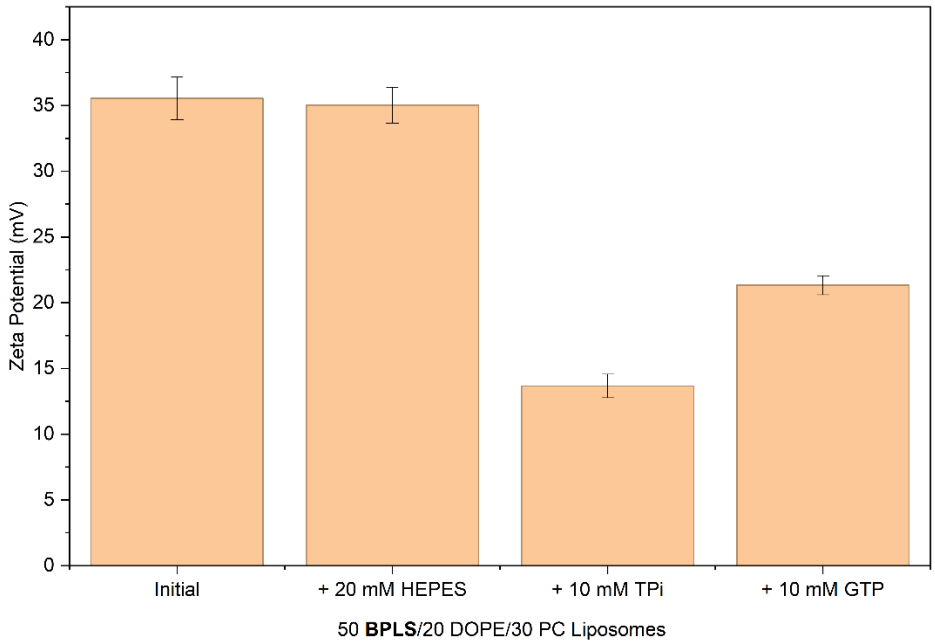


Figure S4. Zeta potential studies with 50/320/30 **BPLS**/DOPE/PC liposomes with initial measurements and samples treated with HEPES, TPi, or GTP. Treatment with 10 mM TPi resulted in a

larger charge decrease, but all samples remained positive overall. Error bars denote standard errors from at least three biological replicates.

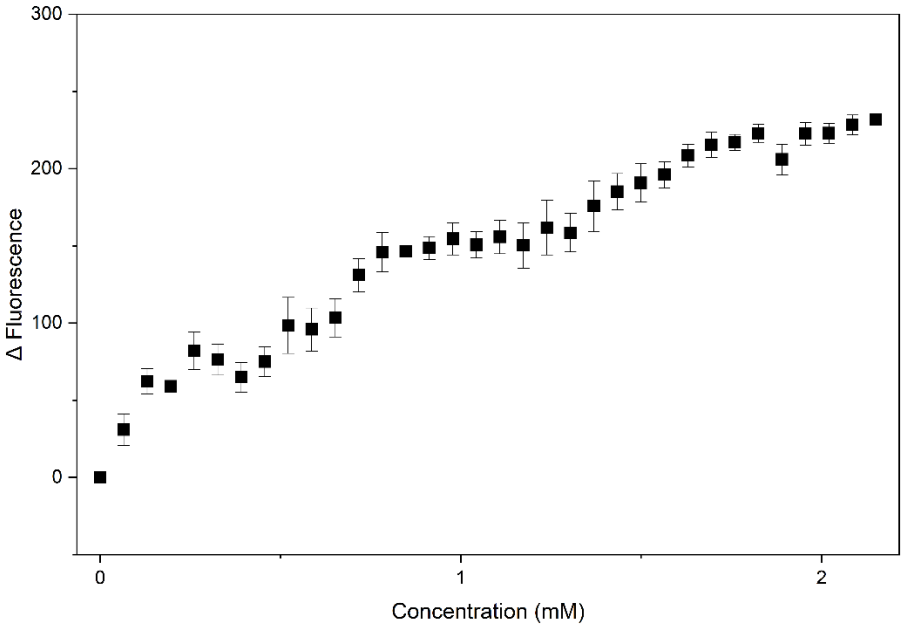


Figure S5. BPLS-TPi solution binding study was completed through fluorescence titration. Fluorescence titration for the **BPLS**-TPi interaction was fitted to a single-site Langmuir binding isotherm, $\theta = \theta_0 + \Delta\theta([L] / (K_d + [L])$, using nonlinear least-squares regression. Both unweighted and weighted fits (weighted by the standard error of each measurement) were performed. The unweighted fit, assuming uniform variance across data points, yielded K_d = 0.417 (±0.026 mM). The weighted fit, accounting for heteroscedasticity in the fluorescence data, provided a more statically robust estimate of K_d = 1.225 (±0.081 mM). Weighted regression reduced the influence of high-variance points and more accurately captured the mid-range curvature of the binding isotherm consistent with a single-site, non-cooperative binding model. Error bars denote standard errors from at least three biological replicates.

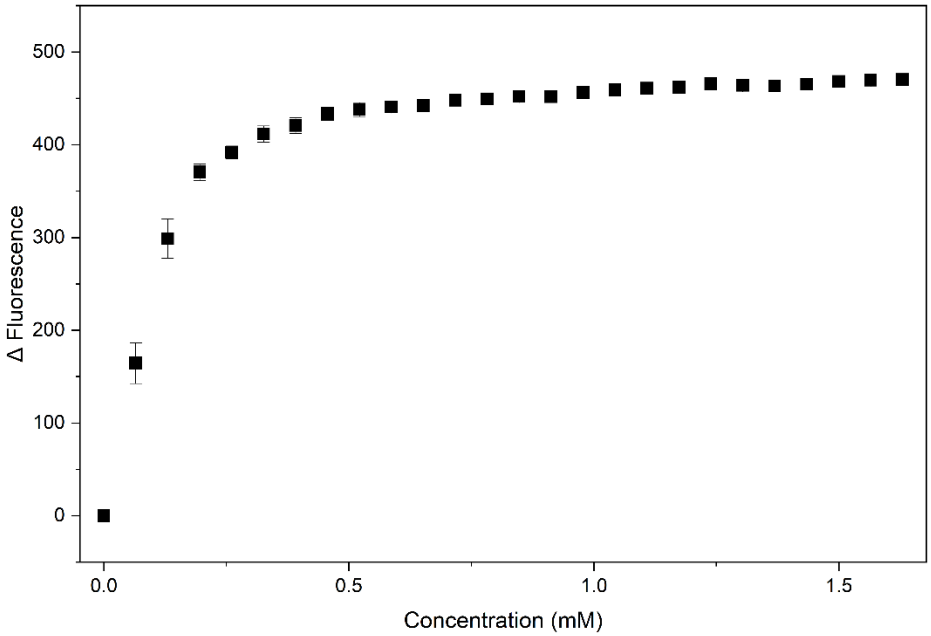


Figure S6. BPLS-GTP solution binding study was completed through fluorescence titration Binding of GTP to **BPLS** was quantified by fitting to a single-site Langmuir isotherm, $\theta = [L] / (K_d + [L])$, where [L] is the free GTP concentration and K_d is the equilibrium dissociation constant. Nonlinear least-squares fitting of the titration data yielded K_d = 0.150 mM (±0.010 mM). Error bars denote standard errors from at least three biological replicates.

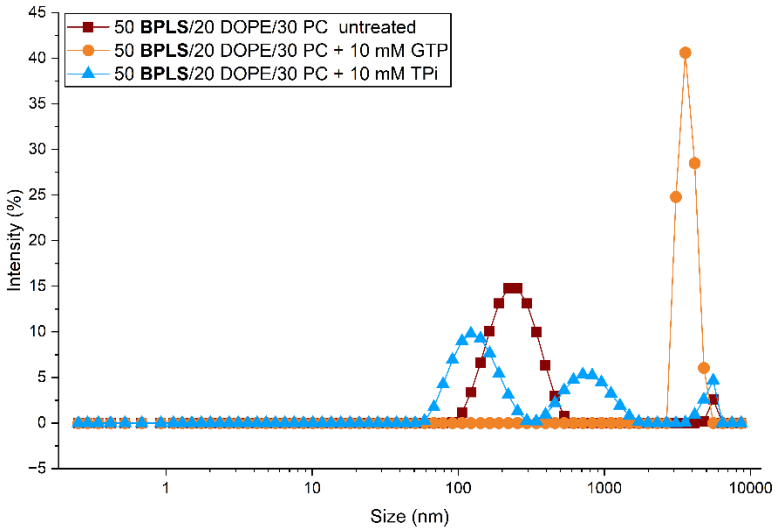


Figure S7. Representative raw DLS distribution curves for 50/20/30 **BPLS/**DOPE/PC liposomes before and after treatment with GTP or TPi. Mostly stable liposomes with smaller aggregates were observed for TPi treated liposomes, whereas more extensive perturbation and aggregation was observed for GTP treated liposomes.

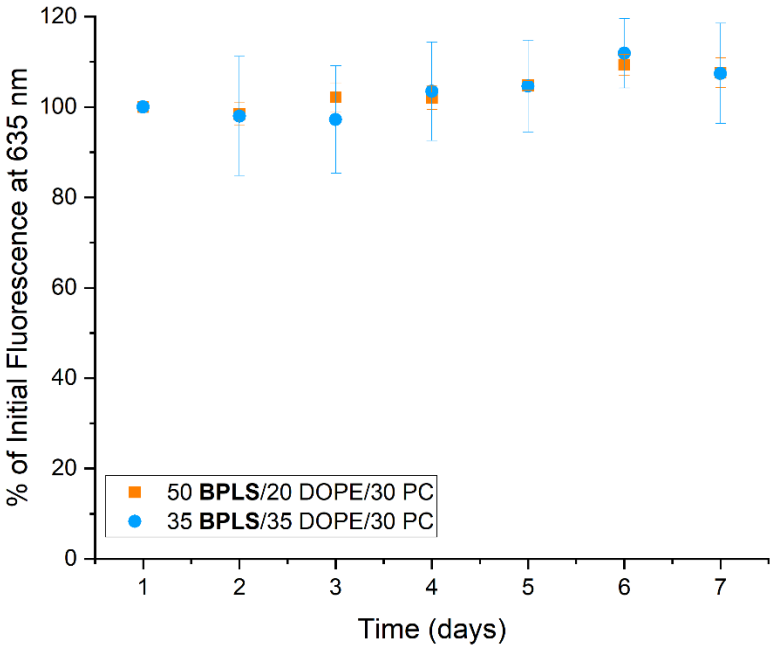


Figure S8. Liposomal stability test with untreated liposomes monitored over 7 days for any passive dye leakage. Error bars denote standard errors from at least three biological replicates.

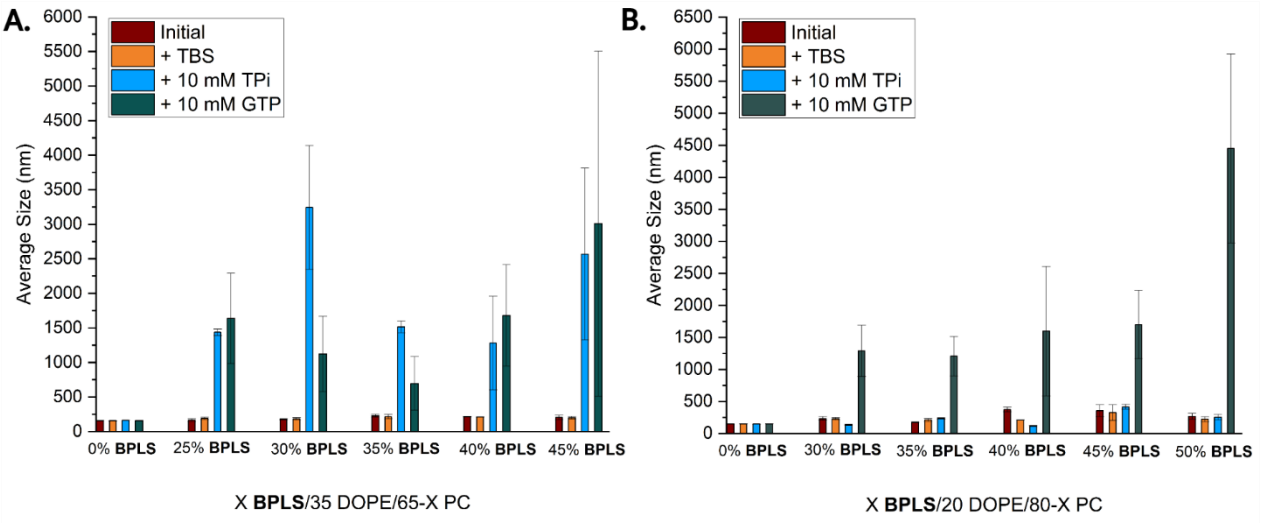


Figure S9. DLS analysis of liposomes with varying **BPLS** percentage and fixed DOPE, before and after (16 hours) treatment with TBS, 10 mM GTP, or 10 mM TPi. Liposomes showed uniform size in the expected range before metabolite treatment, with minimal changes after buffer treatment. Significant size increases were observed for **BPLS** liposomes treated with GTP (A-B) or TPi (A). Error bars denote standard errors from at least three biological replicates.

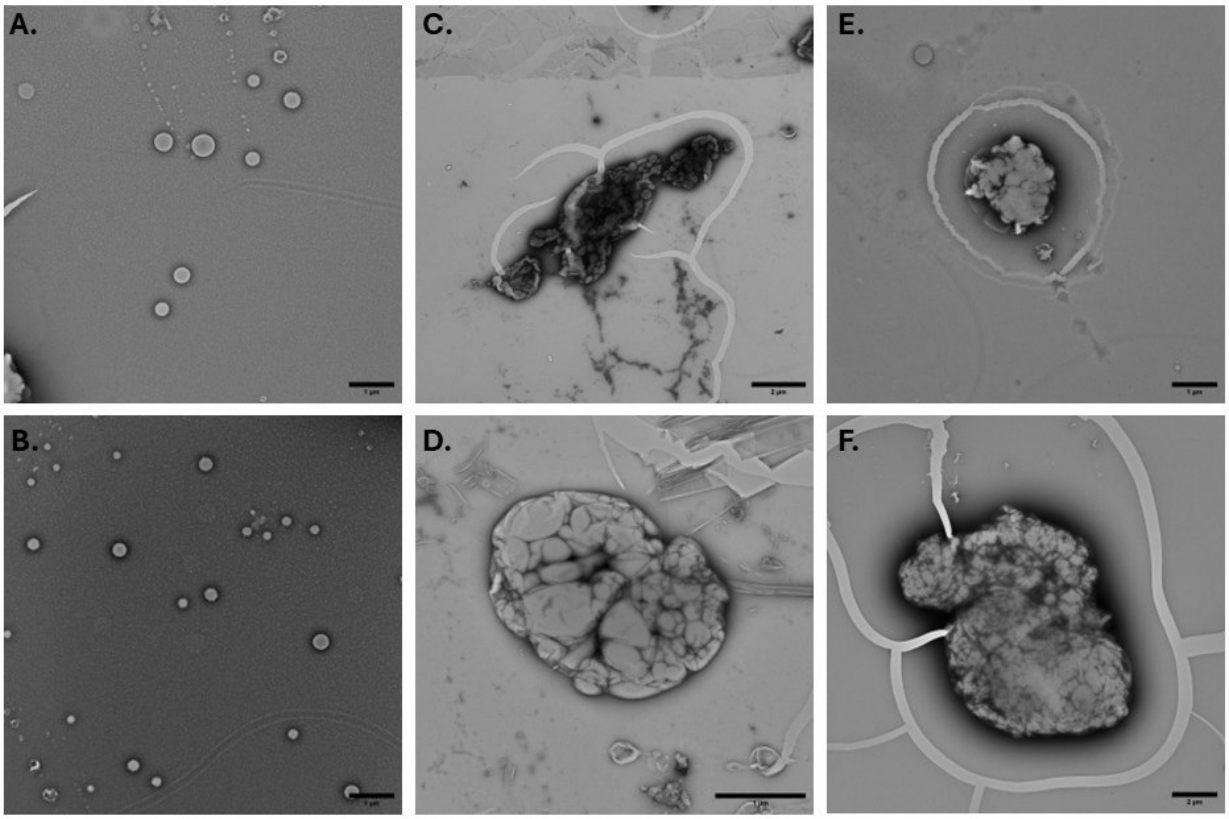


Figure S10. Negatively stained TEM images of 35/35/30 **BPLS**/DOPE/PC liposomes after treatment with TBS (A,B) highlighted unilamellar, uniform vesicles. After treatment with 10 mM GTP (C,D) or 10 mM TPi (E,F), multivesicular aggregates formed.

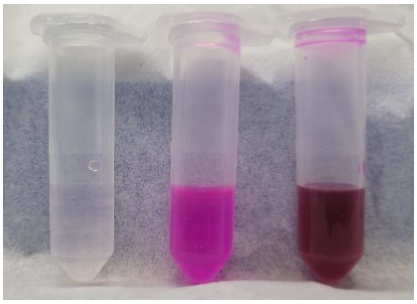


Figure S11. Fractions from SEC purification of sulforhodamine B loaded liposomes, with column purge (clear), dye-loaded liposomes (pink), and free dye (magenta) as the three color transitions collected.

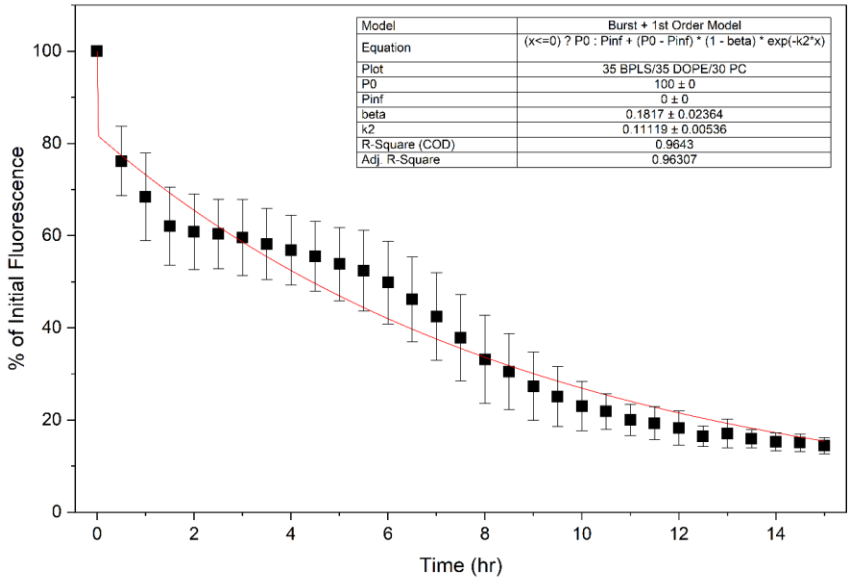


Figure S12. Fluorescence cargo release from 35 **BPLS**/35 DOPE/30 PC liposomes + 10 mM TPi fit to a burst + first-order model. An initial burst (β = 0.1817) is followed by slower release (k_2 = 0.11119, $t_{1/2}$ = 6.2339 hr). Error bars denote standard errors from at least three biological replicates.

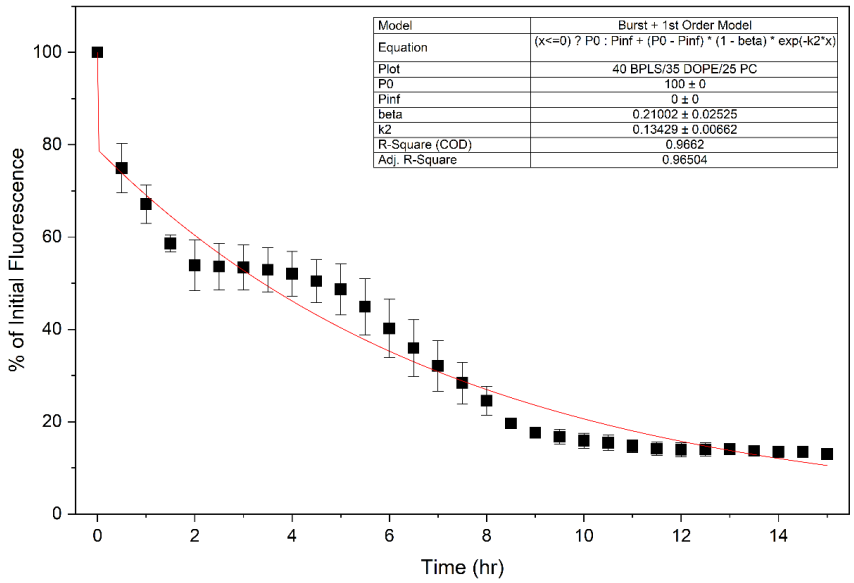


Figure S13. Fluorescence cargo release from 40 **BPLS**/35 DOPE/25 PC liposomes + 10 mM TPi fit to a burst + first-order model. An initial burst (β = 0.21002) is followed by slower release (k_2 = 0.13429, $t_{1/2}$ = 5.1616 hr). Error bars denote standard errors from at least three biological replicates.

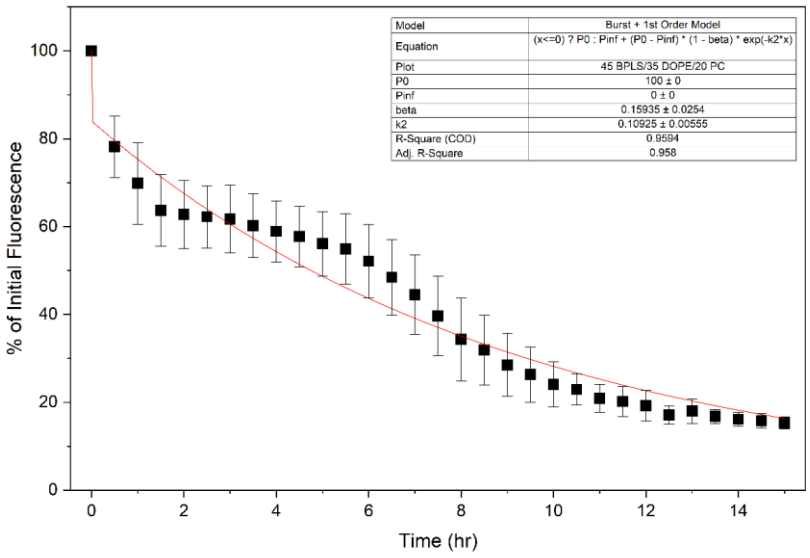


Figure S14. Fluorescence cargo release from 45 **BPLS**/35 DOPE/25 PC liposomes + 10 mM TPi fit to a burst + first-order model. An initial burst (β = 0.15935) is followed by slower release (k_2 = 0.10925, $t_{1/2}$ = 6.3446 hr). Error bars denote standard errors from at least three biological replicates.

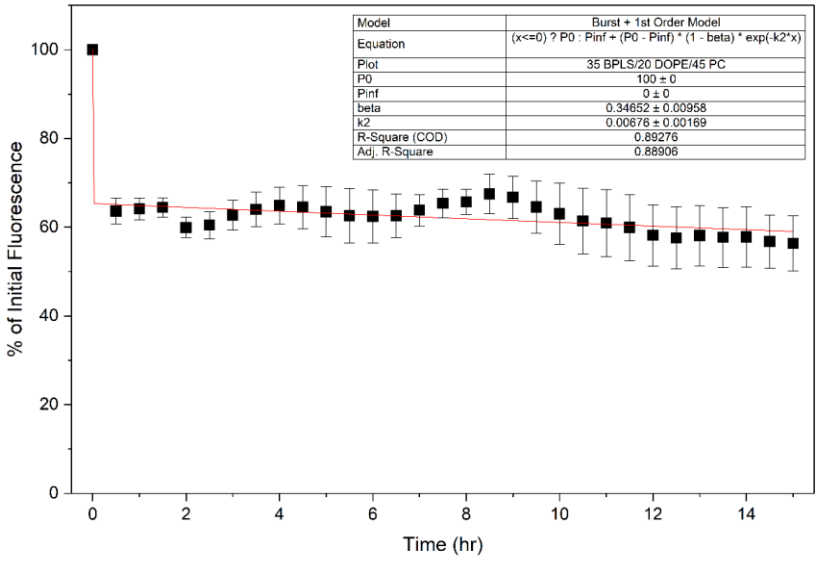


Figure S15. Fluorescence cargo release from 35 **BPLS**/20 DOPE/45 PC liposomes + 10 mM GTP fit to a burst + first-order model. An initial burst (β = 0.34652) is followed by slower release (k_2 = 0.00676, $t_{1/2}$ = 102.54 hr). The model indicates the majority of the release for this formulation occurs within the initial burst, with minimal subsequent change. Error bars denote standard errors from at least three biological replicates.

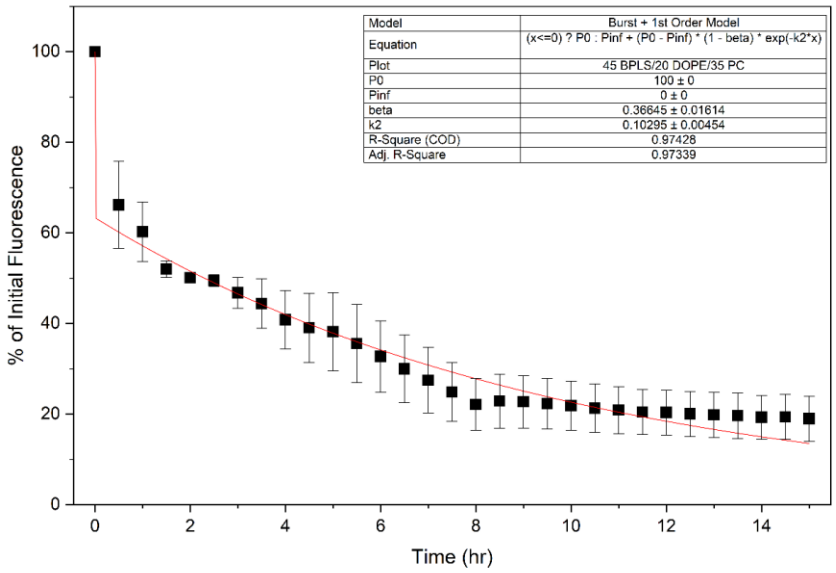


Figure S16. Fluorescence cargo release from 45 **BPLS**/20 DOPE/35 PC liposomes + 10 mM GTP fit to a burst + first-order model. An initial burst (β = 0.36645) is followed by slower release (k_2 = 0.10295, $t_{1/2}$ = 6.7329 hr). Error bars denote standard errors from at least three biological replicates.

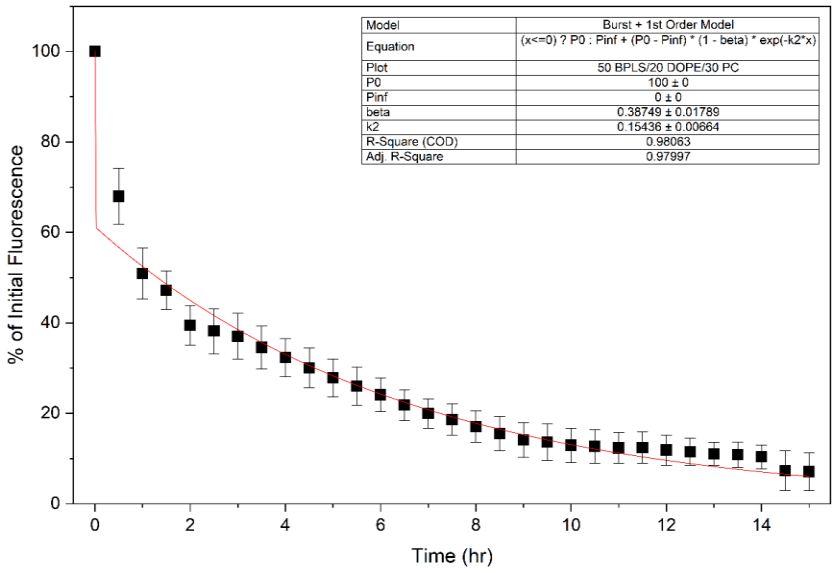


Figure S17. Fluorescence cargo release from 50 **BPLS**/20 DOPE/35 PC liposomes + 10 mM GTP fit to a burst + first-order model. An initial burst (β = 0.38749) is followed by slower release (k_2 = 0.15436, $t_{1/2}$ = 4.4905 hr). Error bars denote standard errors from at least three biological replicates.

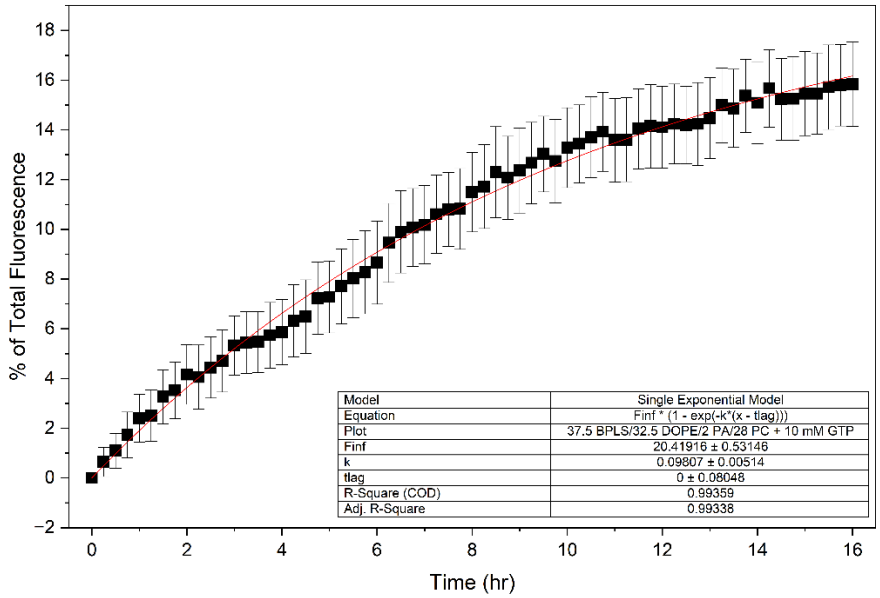


Figure S18. Fluorescence cargo release from 37.5 **BPLS**/32.5 DOPE/2 PA/28 PC liposomes + 10 mM GTP fit to a single-exponential model, where k = 0.09807, $t_{1/2}$ = 7.0679 hr. Error bars denote standard errors from at least three biological replicates.

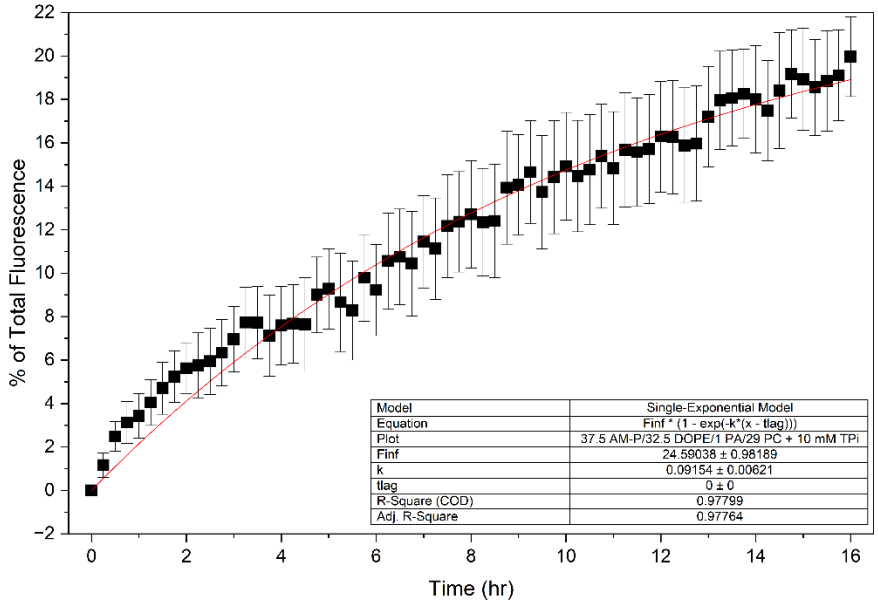


Figure S19. Fluorescence cargo release from 37.5 **BPLS**/32.5 DOPE/2 PA/28 PC liposomes + 10 mM TPi fit to a single-exponential model, where k = 0.09154, $t_{1/2}$ = 7.5721 hr. Error bars denote standard errors from at least three biological replicates.

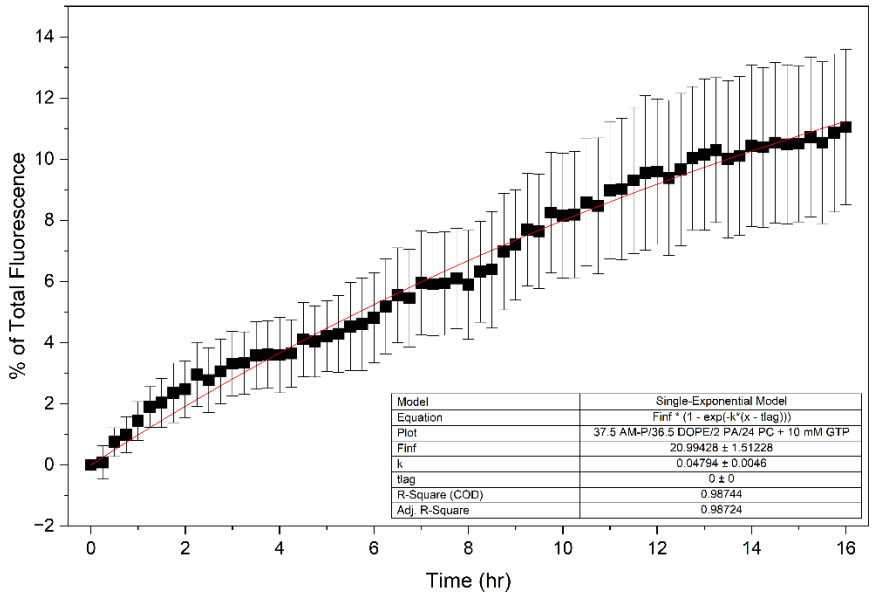


Figure S20. Fluorescence cargo release from 37.5 **BPLS**/36.5 DOPE/2 PA/24 PC liposomes + 10 mM GTP fit to a single-exponential model, where k = 0.04794, $t_{1/2}$ = 14.459 hr. Error bars denote standard errors from at least three biological replicates.

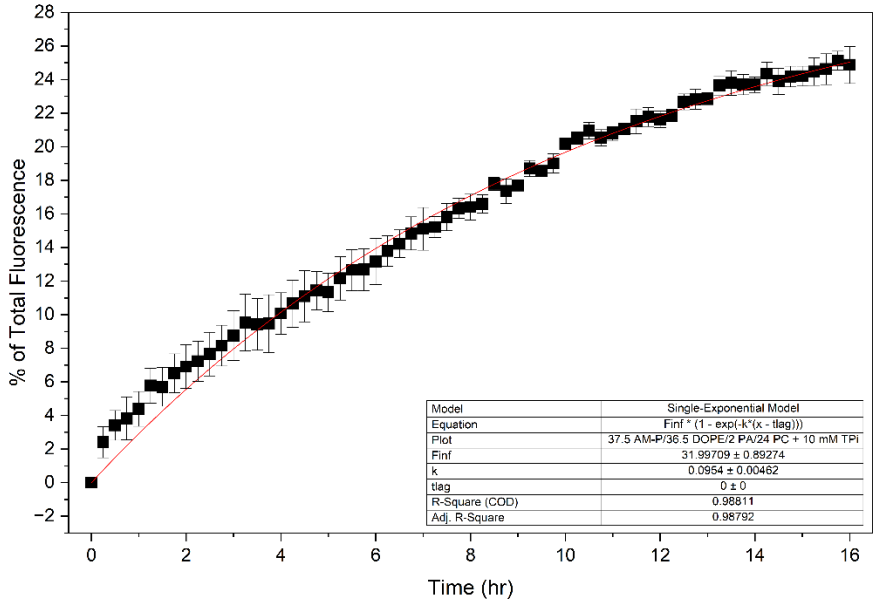


Figure S21. Fluorescence cargo release from 37.5 **BPLS**/36.5 DOPE/2 PA/24 PC liposomes + 10 mM TPi fit to a single-exponential model, where k = 0.0954, $t_{1/2}$ = 7.266 hr. Error bars denote standard errors from at least three biological replicates.

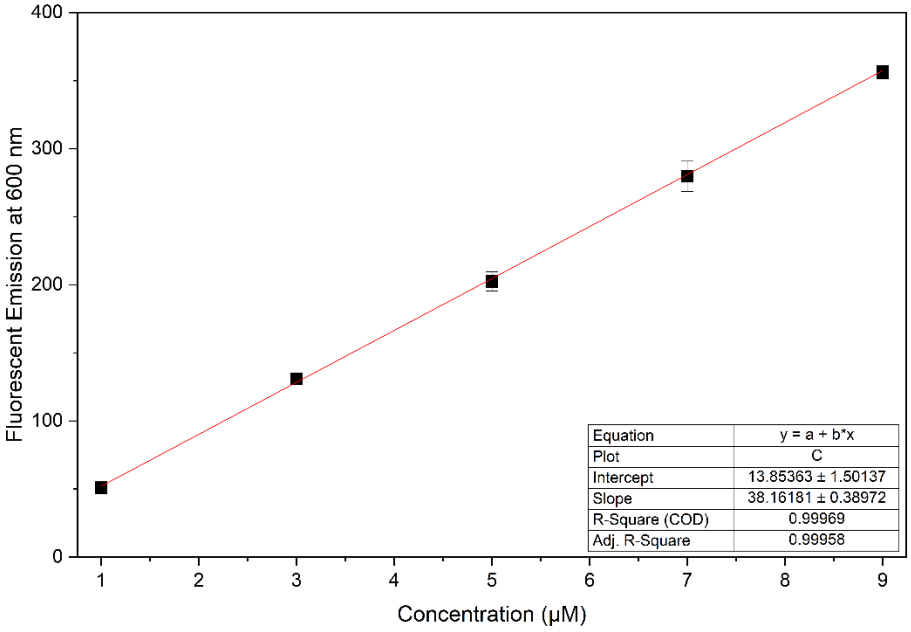


Figure S22. Fluorescence calibration curve for Nile red in chloroform used to measure encapsulation efficiency with hydrophobic liposomes. Error bars denote standard errors from at least three biological replicates. The linear fit equation was determined to be y = (38.16181± 0.38972)x + (13.85363 ± 1.50137).

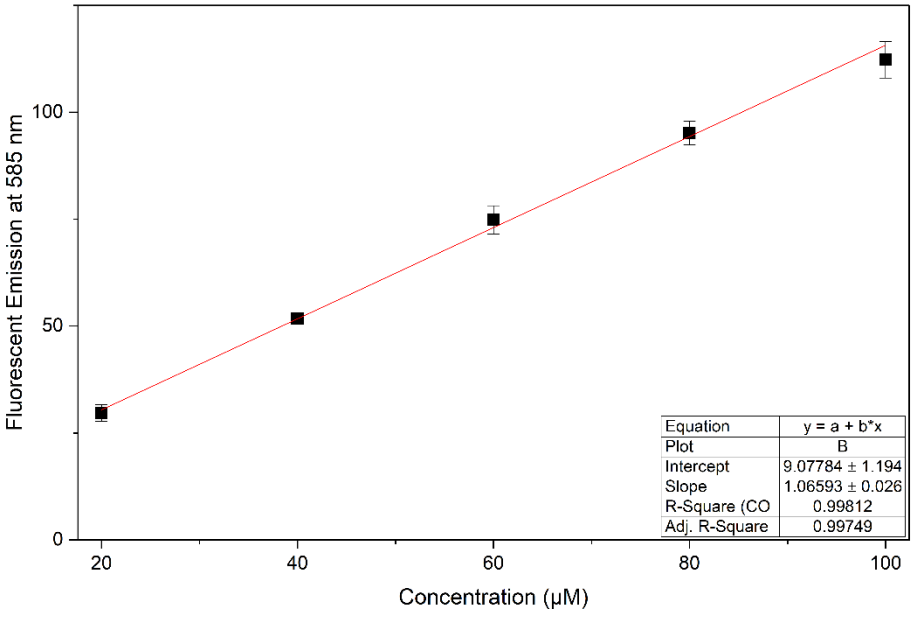


Figure S23. Fluorescence calibration curve for sulforhodamine in MilliQ water used to measure encapsulation efficiency with hydrophilic liposomes. Error bars denote standard errors from at

least three biological replicates. The linear fit equation was determined to be y = (1.06593 ± 0.0146)x + (9.07784 ± 1.194).

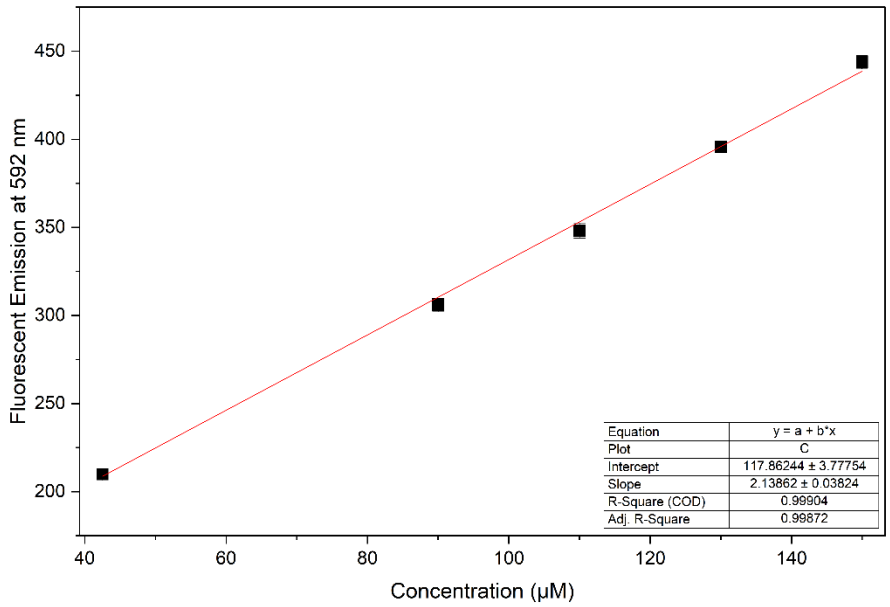


Figure S24. Fluorescence calibration curve for doxorubicin in MilliQ water used to measure encapsulation efficiency with Dox loaded liposomes. Error bars denote standard errors from at least three biological replicates. The linear fit equation was determined to be y = 2.13862 ± 0.03824)x + (117.86244 ± 3.77754).

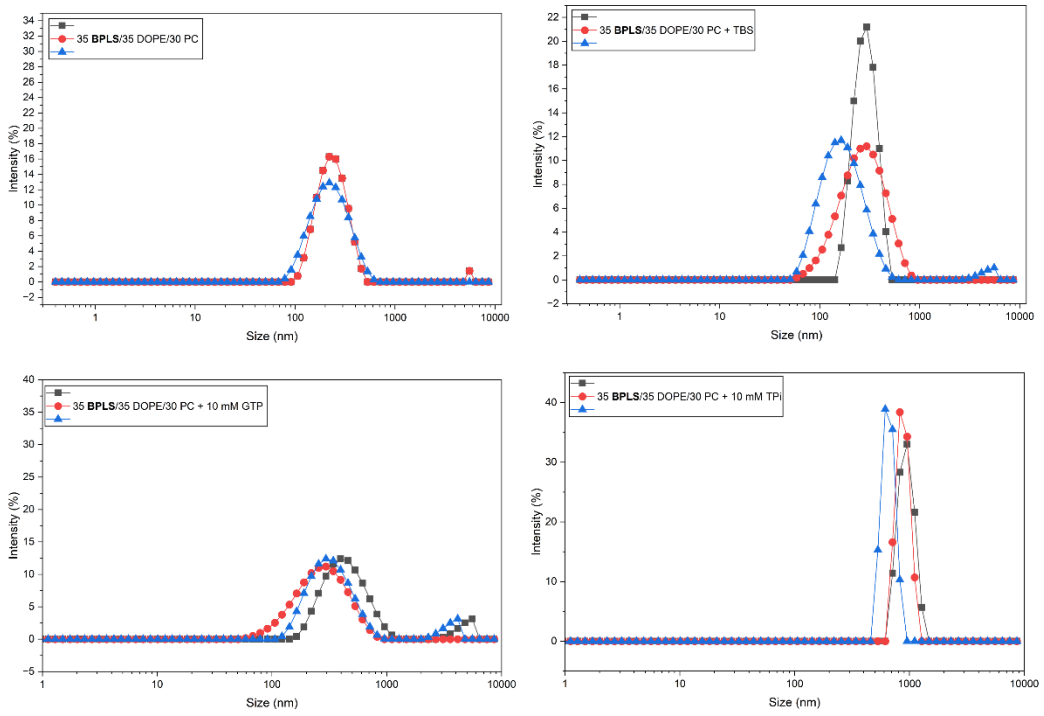


Figure S25. Raw DLS curves of 35/35/30 **BPLS**/DOPE/PC liposomes, before and after treatment.

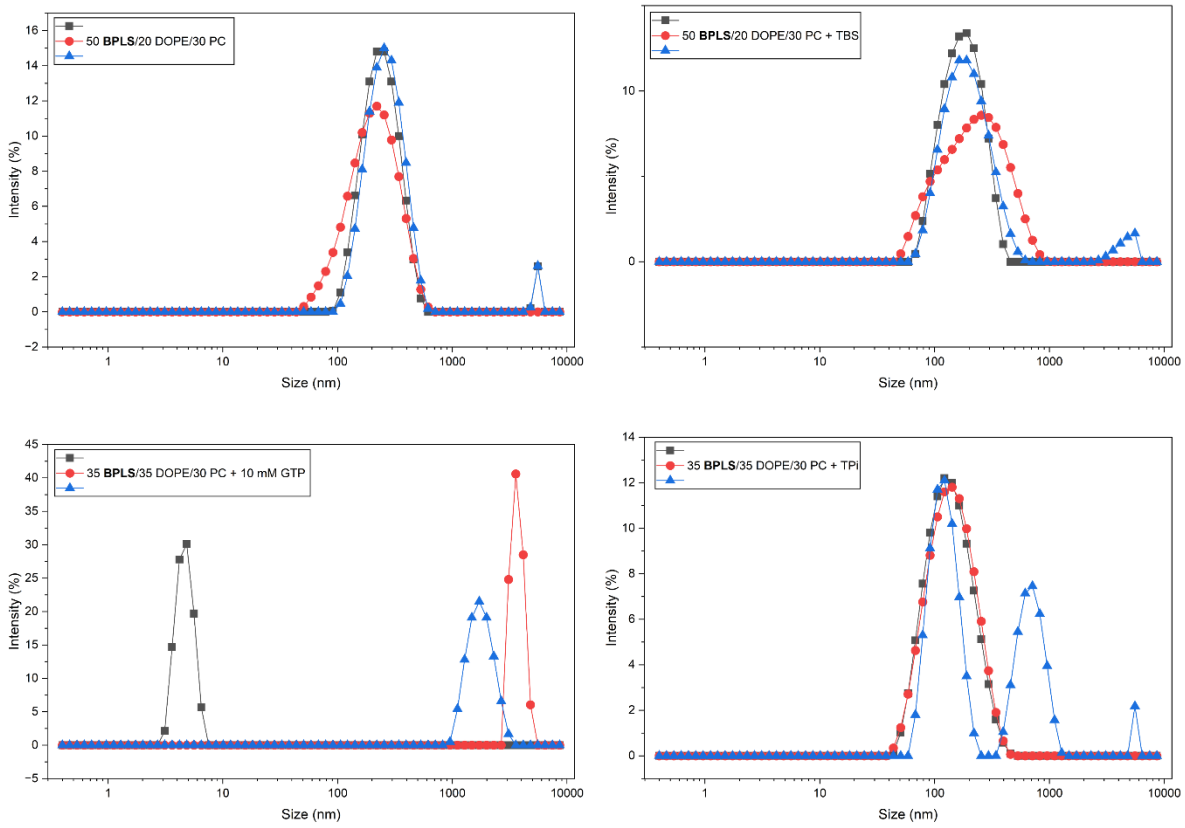


Figure S26. Raw DLS curves of 50/20/30 **BPLS**/DOPE/PC liposomes, before and after treatment.

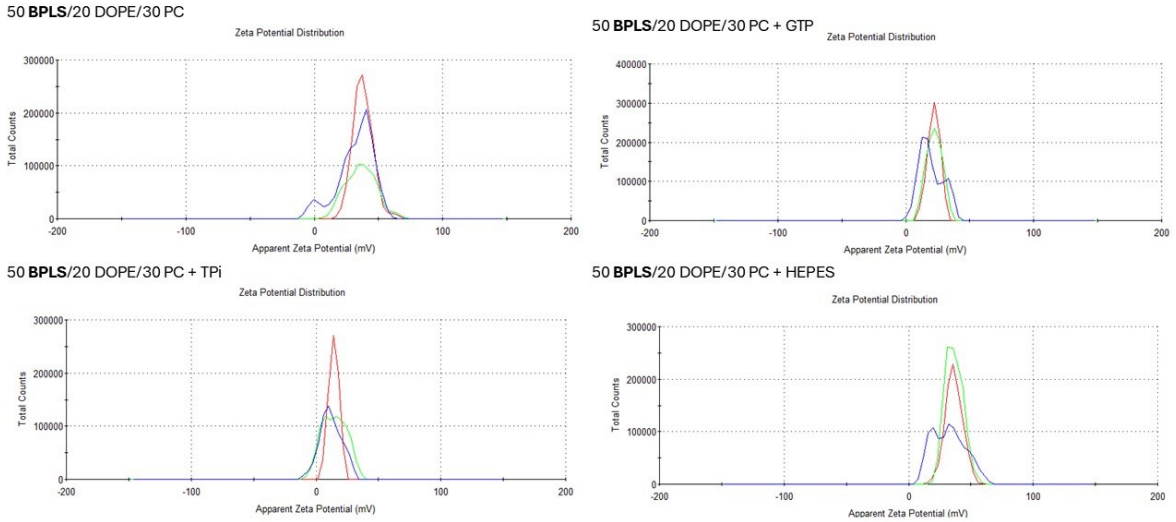


Figure S27. Raw zeta potential curves of 50/20/30 **BPLS**/DOPE/PC liposomes, before and after treatment.

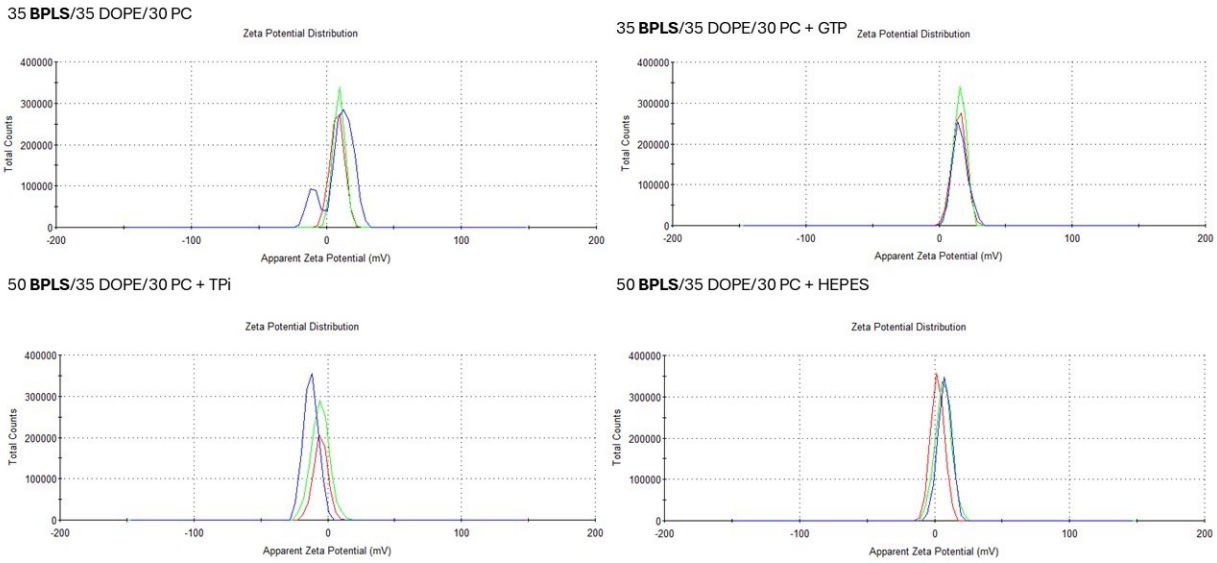


Figure S28. Raw zeta potential curves of 35/35/30 **BPLS**/DOPE/PC liposomes, before and after treatment.

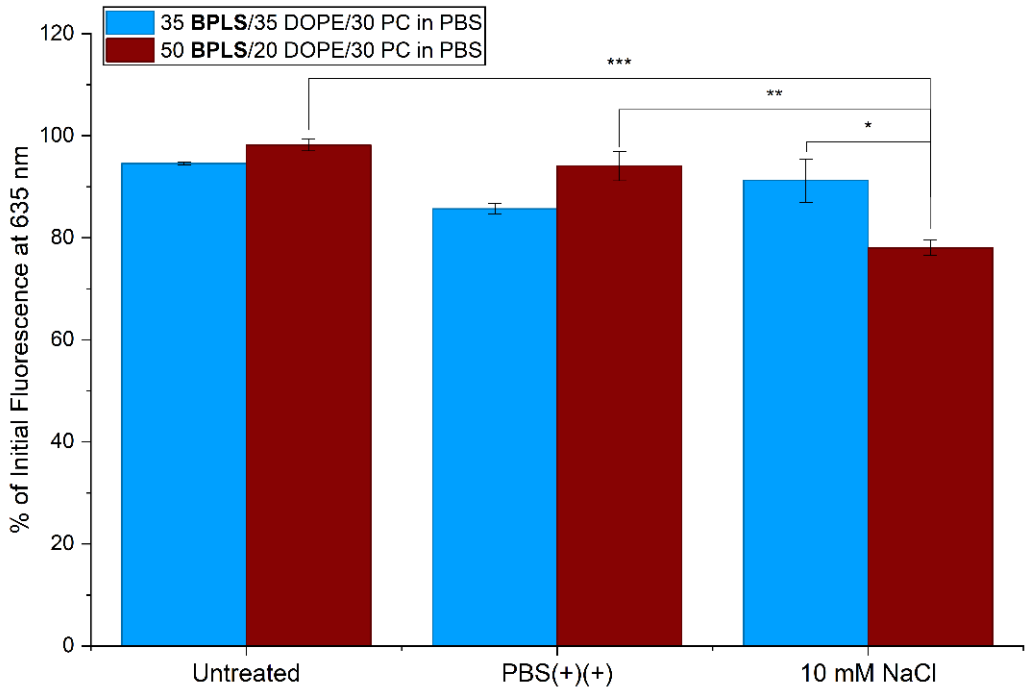


Figure S29. NR release studies were completed for liposomes hydrated with 1x PBS buffer in place of 1Xx TBS buffer, were treated with 10 mM NaCl or an equivalent volume of 1X PBS(+)(+), and measured after 48 hours. Error bars denote standard errors from at least three biological replicates. Statistical analysis was performed using a two-way ANOVA (factors: formulation and trigger) followed by Tukey's post-hoc multiple comparison test. Significance between triggers within each formulation is shown where n.s. ($p > 0.05$), * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$). The two-way ANOVA confirmed no statistical significance was observed between any treated 35/35/30 **BPLS**/DOPE/PC liposomes, whereas significance was observed for NaCl treated 50/20/30 **BPLS**/DOPE/PC liposomes when compared to buffer or no treatment of the same liposomes. This indicates some cargo leakage from liposomes occurs when osmolarity of the surrounding environment is increased, but minimal leakage is observed in the presence of increased phosphate from the buffer solution.

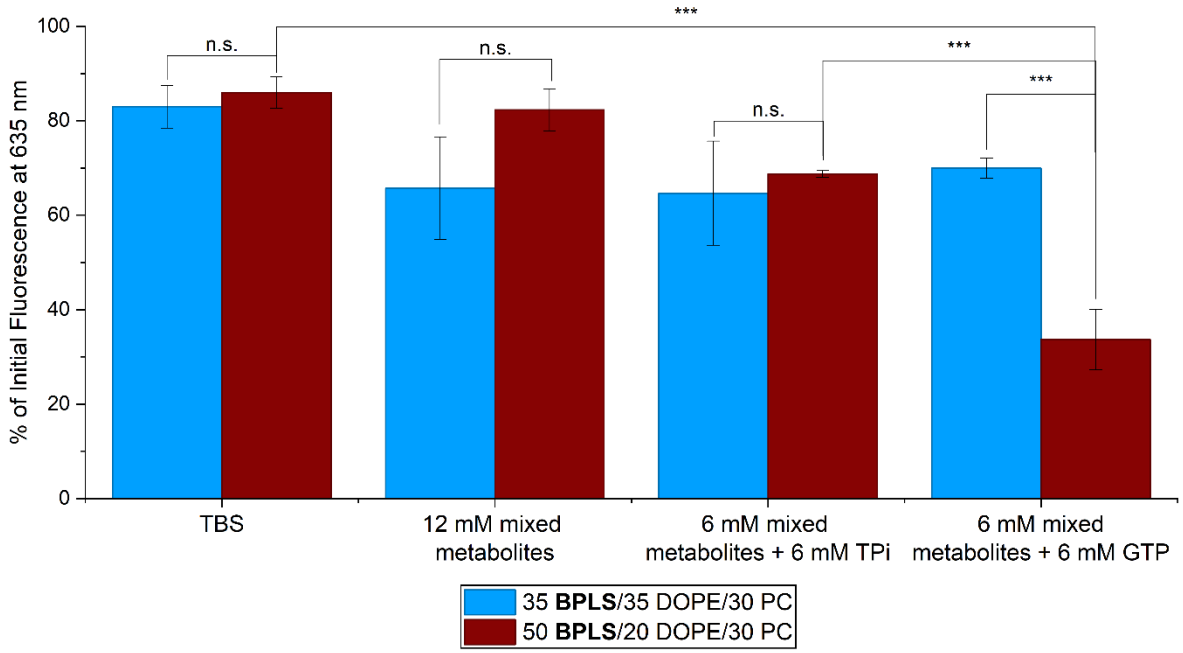


Figure S30. NR release studies were completed for liposomes treated with TBS buffer, a mixed metabolite solution for a final concentration of 12 mM, a mixed metabolite solution with a final concentration of 6 mM plus 6 mM TPi, and a mixed metabolite solution with a final concentration of 6 mM plus 6 mM of GTP. The mixed metabolite solution stock was made up of equal concentrations of Pi, PPi, ADP, ATP, FP, FBP, CTP, UTP, AMP, Mal, Fum, and Oxa for a total final concentration of 300 mM of all metabolites (25 mM for each metabolite), and diluted to a final concentration of either 6 mM or 12 mM when treating liposomes. Liposomes were treated and measured after 16 hours. Error bars denote standard errors from at least three biological replicates. Statistical analysis was performed using a two-way ANOVA (factors: formulation and trigger) followed by Tukey's post-hoc multiple comparison test. Significance between triggers within each formulation is shown where n.s. ($p > 0.05$), * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$). The two-way ANOVA confirmed no statistical significance was observed between any treated 35/35/30 **BPLS**/DOPE/PC liposomes, whereas significance was observed for GTP treated 50/20/30 **BPLS**/DOPE/PC liposomes when compared to buffer or TPi treatment of the same liposomes, and GTP treatment of the 35/35/30 liposomes. The 35/35/30 liposomes did not yield significant cargo release from exposure to control buffer treatments versus metabolites, whereas 50/20/30 liposomes exhibited significant cargo release from liposomes treated with 6 mM mixed metabolites plus 6 mM GTP.

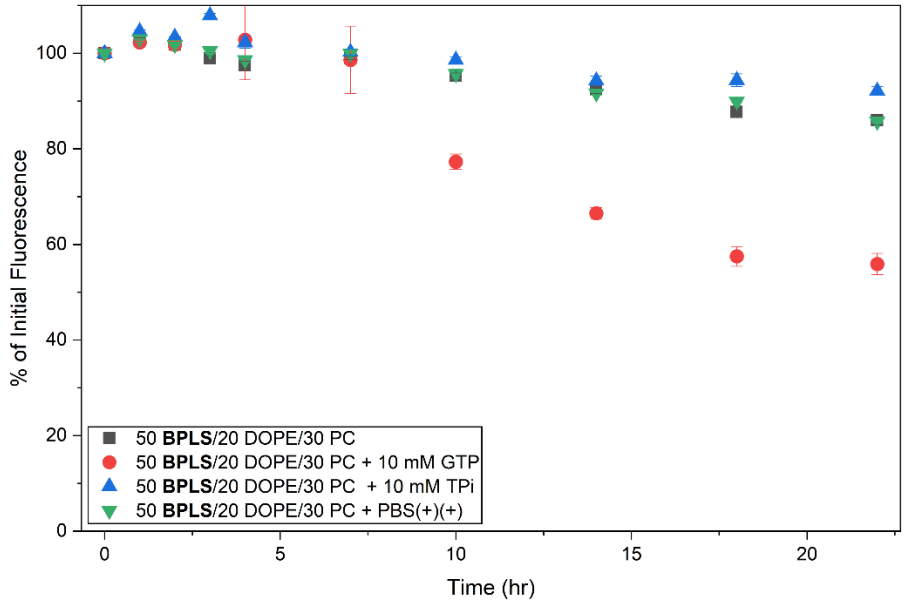


Figure S31. Fluorescence studies for release of NR from 50/20/30 **BPLS**/DOPE/PC liposomes were performed in phenol-red free DMEM media with +10% FBS, 50 U mL^{-1} pen/strep. GTP treated liposomes plateaued at 44% NR release while untreated, TPi treated, and PBS(+)(+) treated samples minimized passive leakage. Error bars denote standard errors from at least three biological replicates.

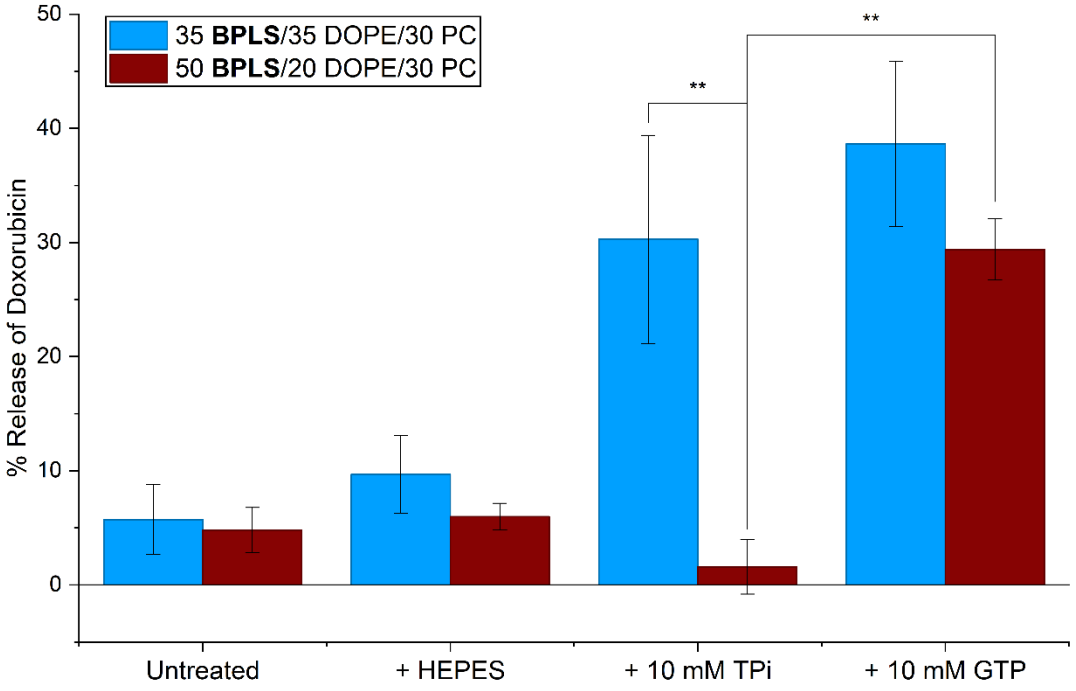


Figure S32. Fluorescence studies for release of Dox from 50/20/30 and 35/35/30 **BPLS**/DOPE/PC liposomes were performed where liposomes were left untreated, or treated with HEPES buffer, 10 mM TPi, or 10 mM GTP and measured after 16 hours. Error bars denote standard errors from at least three biological replicates. Statistical analysis was performed using a two-way ANOVA (factors: formulation and trigger) followed by Tukey's post-hoc multiple comparison test. Significance between triggers within each formulation is shown where n.s. ($p > 0.05$), * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$). 50% **BPLS** liposomes exhibited significant GTP selective release of Dox whereas the less selective 35% **BPLS** liposomes exhibited significant release of Dox with both TPi and GTP treatment. Passive cargo leakage from untreated and buffer treated samples was minimized and determined as statistically significant when compared to GTP treatment (50 and 35% **BPLS**) and TPi treatment (35% **BPLS**). Doxorubicin loading efficiencies were determined with a Dox calibration curve to be 5.411 ± 0.158% for 35/35/30 liposomes and 5.786 ± 0.162% for 50/20/30 liposomes.

% BPLS	% DOPE	% PC	Fluorescence Intensity for Dye Encapsulation (AU)	Cargo release after 10 mM GTP Treatment (%)	Passive Cargo Release (%)
25	0	75	456.587	37.651	38.1
50	0	50	372.343	64.417	39.836
75	0	25	344.867	86.467	64.105
100	0	0	212.111	94.306	82.268
30	20	50	521.147	59.464	3.192
30	25	45	715.701	34.412	29.131
30	30	40	786.063	57.316	39.884
30	35	35	1001.253	67.029	53.63
35	20	45	455.332	49.14	2.065
35	25	40	341.997	50.688	-3.511
35	30	35	424.055	61.696	1.816
35	35	30	362.236	94.821	1.016
40	20	40	383.981	95.405	22.474
40	25	35	333.625	91.177	11.789
40	30	30	388.229	93.163	6.065
40	35	25	373.523	97.647	20.878
45	20	35	380.592	92.091	0.551
45	25	30	483.852	93.836	9.281
45	30	25	516.105	95.489	16.719
45	35	20	471.885	96.451	26.314
50	20	30	392.503	94.659	12.022
50	25	25	439.322	95.581	15.321
50	30	20	432.84	96.59	15.573
50	35	15	351.183	96.995	25.877
55	20	25	308.921	96.799	8.518
55	25	20	300.966	95.931	25.567
55	30	15	265.128	95.766	53.31
55	35	10	192.878	94.568	80.499

Table S1. Examples from formulation testing of initial liposomes. Groups of four formulations with constant **BPLS** and varying DOPE/PC were tested and measured for initial dye encapsulation, cargo release after 24 hours of treatment with 10 mM GTP, and passive cargo release for untreated liposomes over 24 hours.

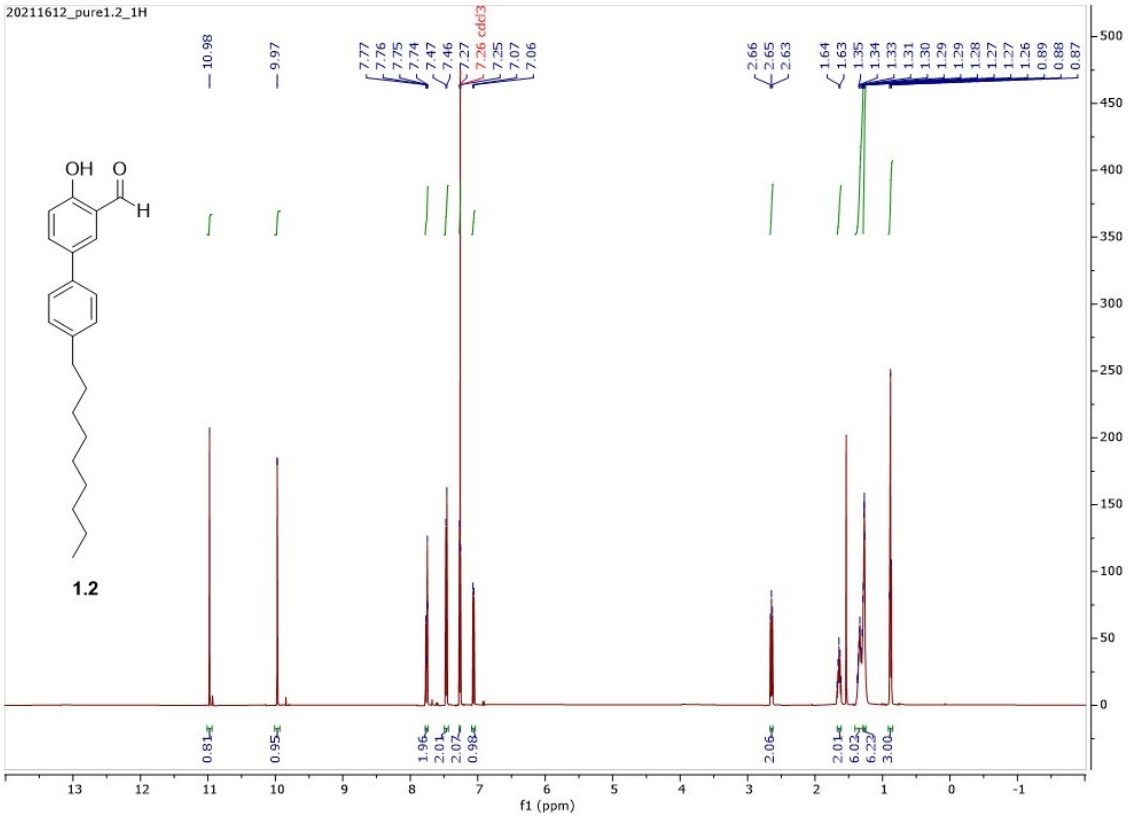
	0% BPLS		25% BPLS		30% BPLS		35% BPLS		40% BPLS		45% BPLS	
	Size	PDI	Size	PDI	Size	PDI	Size	PDI	Size	PDI	Size	PDI
Initial	156.9	0.228	150.9	0.199	192.2	0.35	192.1	0.32	215.3	0.461	158.6	0.353
	161.8	0.175	140	0.196	170.1	0.263	268.3	0.347	206.2	0.307	263.9	0.355
	159.8	0.218	205.4	0.316	165.3	0.272	219.9	0.258	226.2	0.299	187.2	0.416
TBS	157	0.187	177.5	0.308	214.3	0.274	156.9	0.235	211	0.341	168.7	0.382
	155.1	0.176	173.2	0.268	182.8	0.405	262.1	0.546	201	0.432	239	0.618
	153.9	0.162	221.3	0.45	154.8	0.217	229.4	0.213	214.8	0.439	193.8	0.461
TPi	158.3	0.182	1374	0.033	1784	0.332	1498	0.306	433.3	0.083	4549	0.083
	164.7	0.248	1533	0.278	4875	0.448	1672	0.579	2627	0.926	2878	0.926
	159.5	0.213	1403	0.47	3069	0.047	1380	0.624	784.2	0.616	276.8	0.616
GTP	158.9	0.199	2933	0.802	276.8	0.566	193.9	0.347	1548	1.000	824.7	0.72
	155.2	0.205	864.3	0.27	940.7	0.648	430.9	0.367	3008	1.000	209.1	0.636
	157.6	0.179	1113	0.144	2146	0.84	1464	0.816	484.6	0.568	7990	1.000

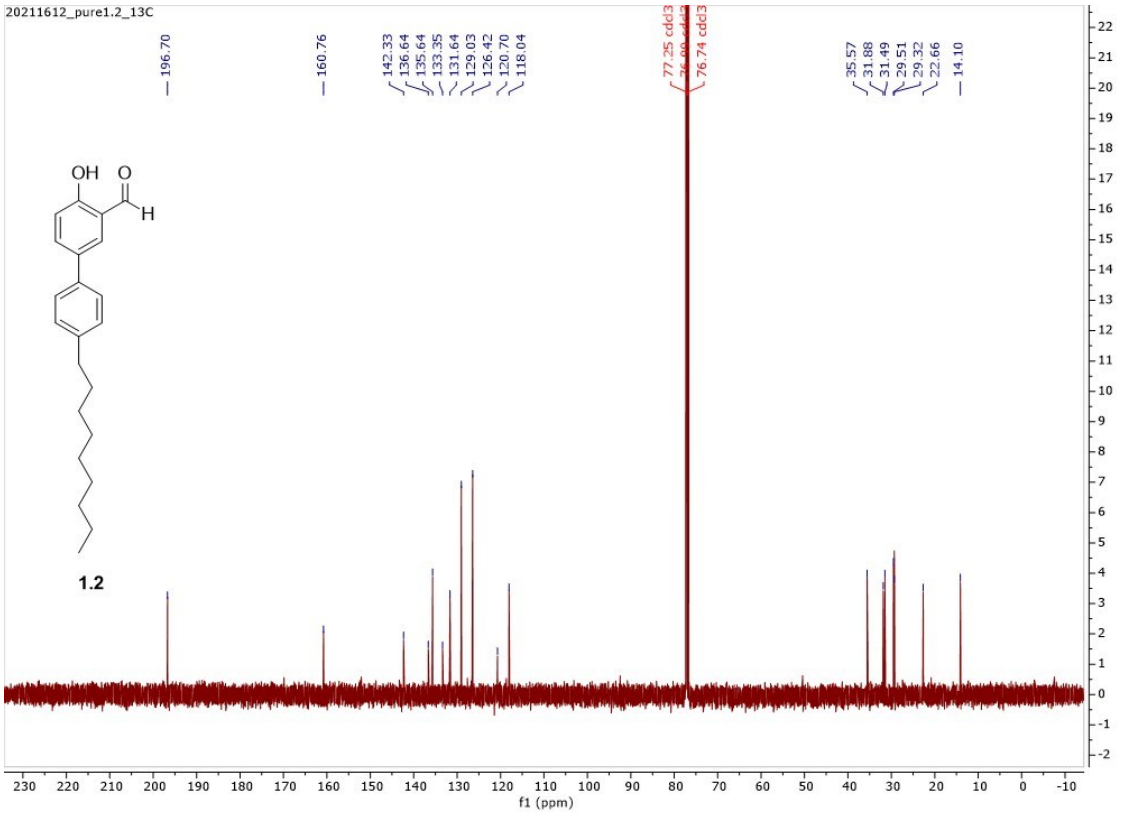
Table S2. X **BPLS**/35 DOPE/65-X PC liposomes size and PDI values from three separate batches.

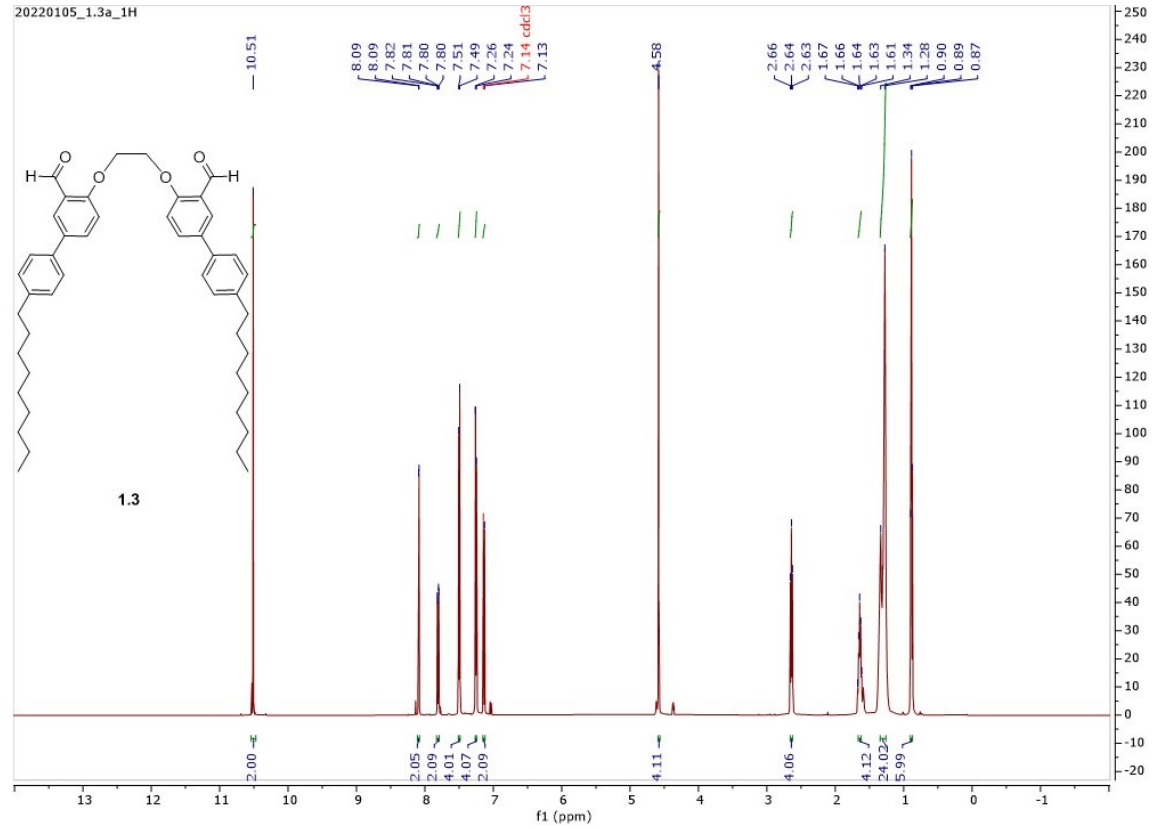
	0% BPLS		30% BPLS		35% BPLS		40% BPLS		45% BPLS		50% BPLS	
	Size	PDI	Size	PDI	Size	PDI	Size	PDI	Size	PDI	Size	PDI
Initial	147.5	0.123	201.9	0.311	224.8	0.374	308.4	0.335	198.5	0.255	296.1	0.419
	149.5	0.129	204.5	0.244	223.7	0.346	433.0	0.481	351.0	0.402	179.7	0.177
	148.9	0.130	289.0	0.396	147.9	0.218	377.0	0.361	514.2	0.480	325.7	0.445
TBS	152.5	0.164	213.2	0.396	187.3	0.211	210.2	0.336	207.1	0.320	297.1	0.357
	148.3	0.097	267.2	0.289	149.1	0.219	212.5	0.316	566.5	0.549	180.8	0.248
	149.0	0.098	211.2	0.289	150.0	0.178	206.6	0.290	195.3	0.255	187.3	0.246
TPi	147.0	0.131	148.9	0.189	260.2	0.449	109.0	0.198	488.3	0.491	258.7	0.586
	145.7	0.136	143.7	0.196	255.5	0.562	110.8	0.239	382.1	0.632	178.7	0.364
	146.4	0.118	110.9	0.228	295.2	0.412	132.1	0.357	377.9	0.728	323.9	0.462
GTP	149.5	0.111	1855.0	0.756	1941	1	594.8	0.495	670.2	0.533	5058.0	1.000
	148.8	0.107	1491.0	0.684	4680	0.685	3618.0	1.000	2464.0	0.369	6644.0	0.318
	153.0	0.102	518.7	0.539	154.6	0.201	581.0	0.601	1960.0	0.248	1642.0	0.128

Table S3. X **BPLS**/20 DOPE/80-X PC liposomes size and PDI values from three separate batches.

3. Characterization of Synthetic Compounds



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4.58
2.66
2.64
2.63
1.67
1.66
1.64
1.63
1.61
1.34
1.28
0.90
0.89
0.87
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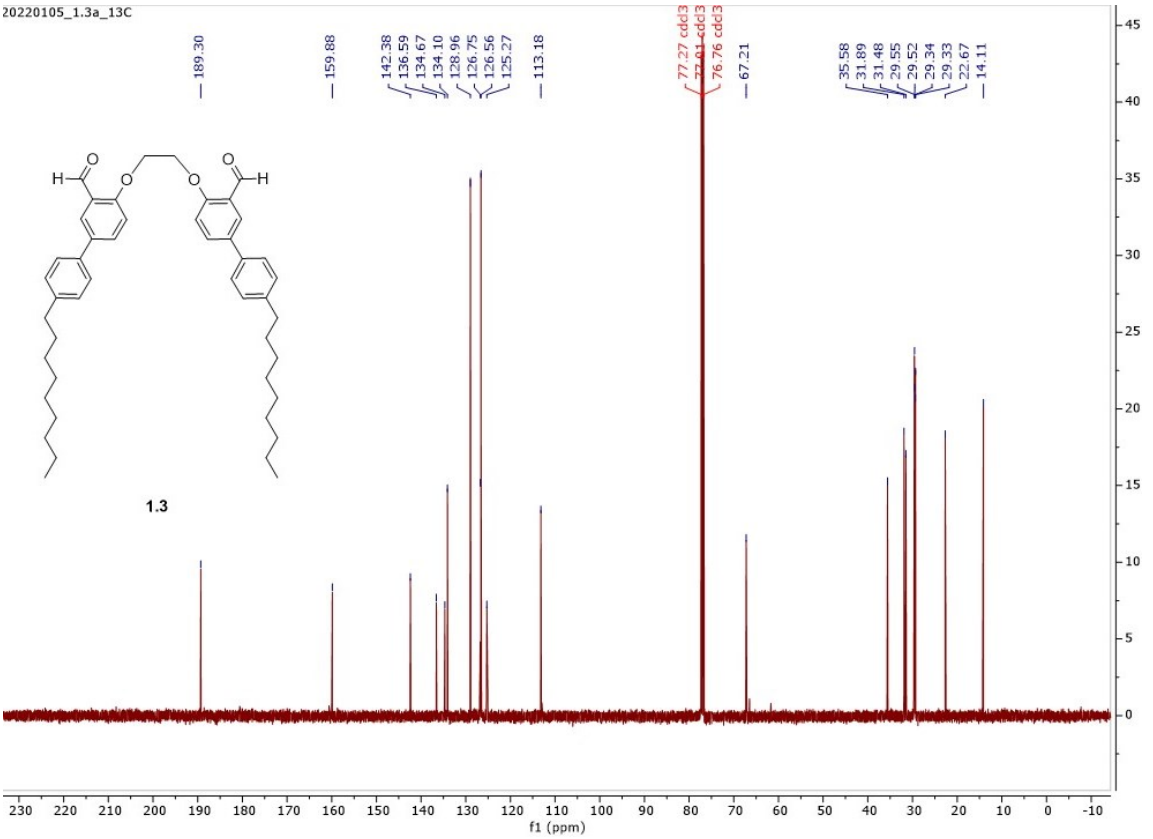
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76.76 cdcl3
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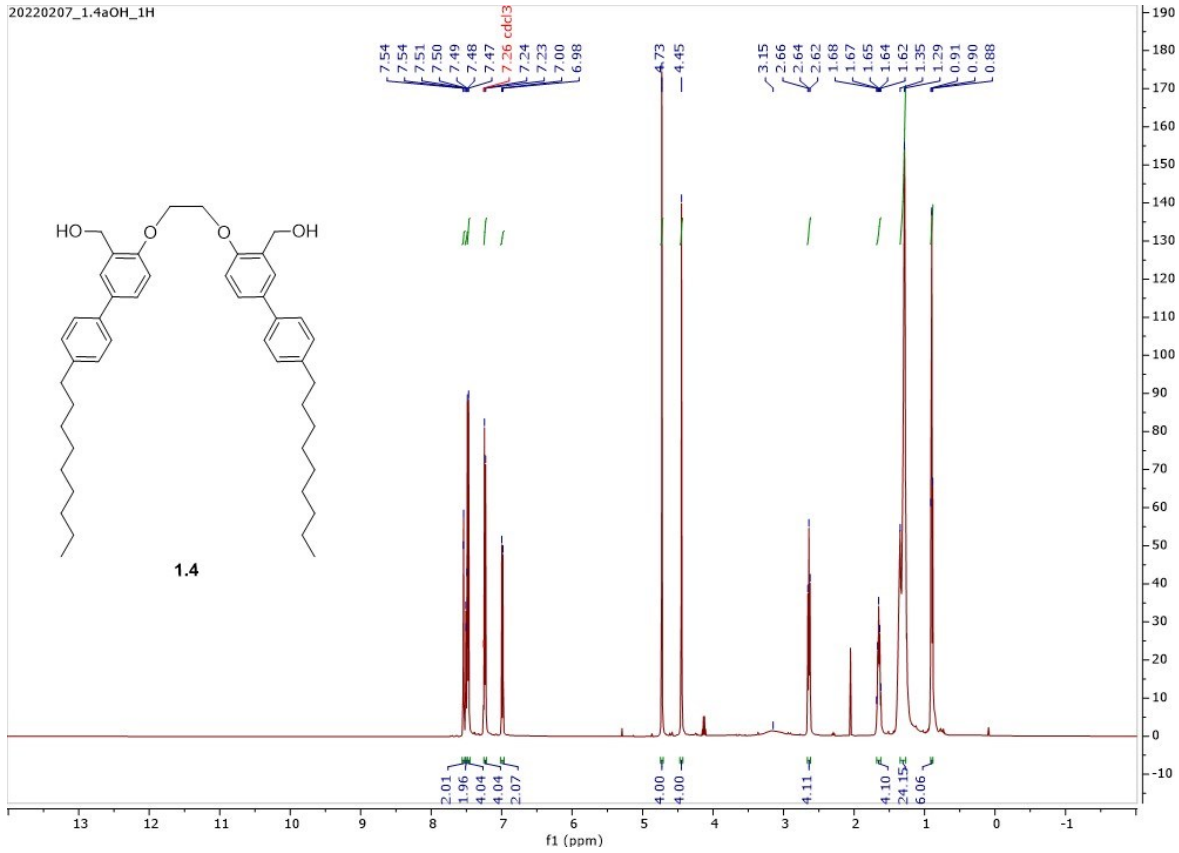
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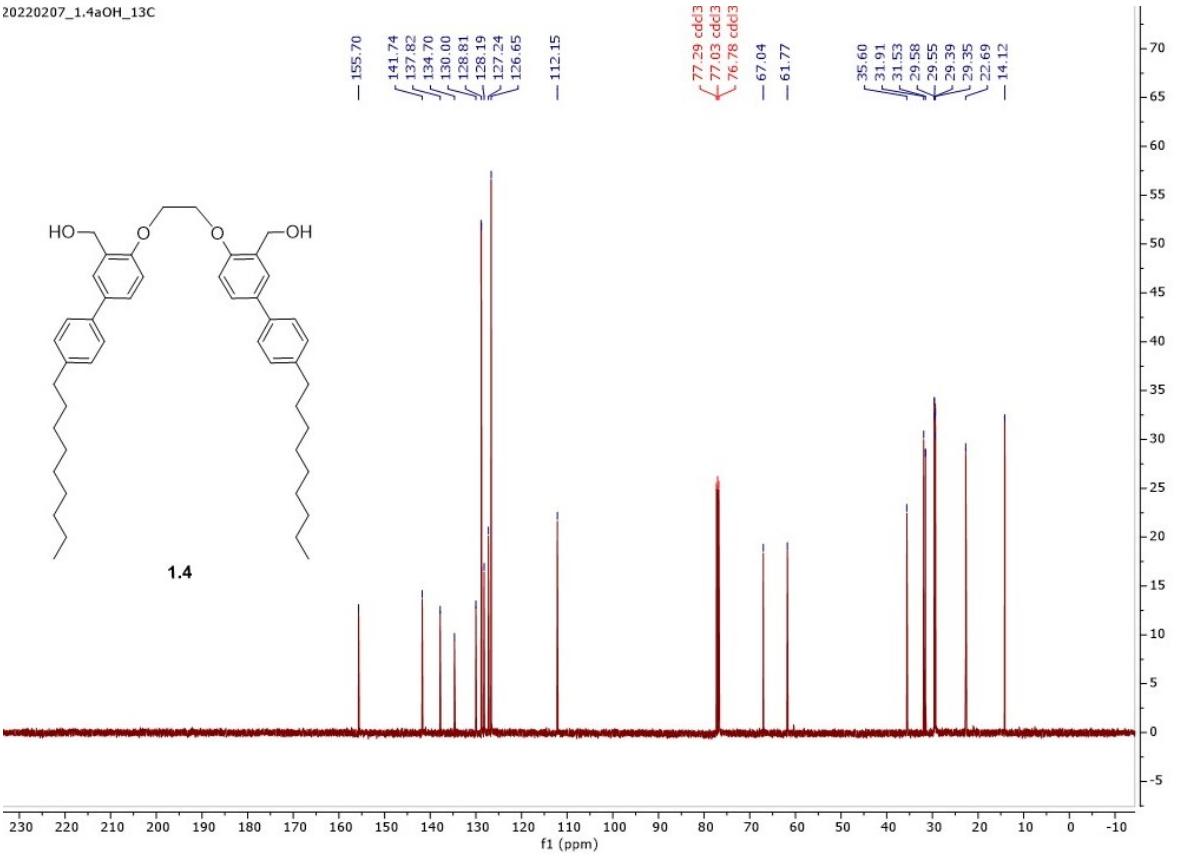
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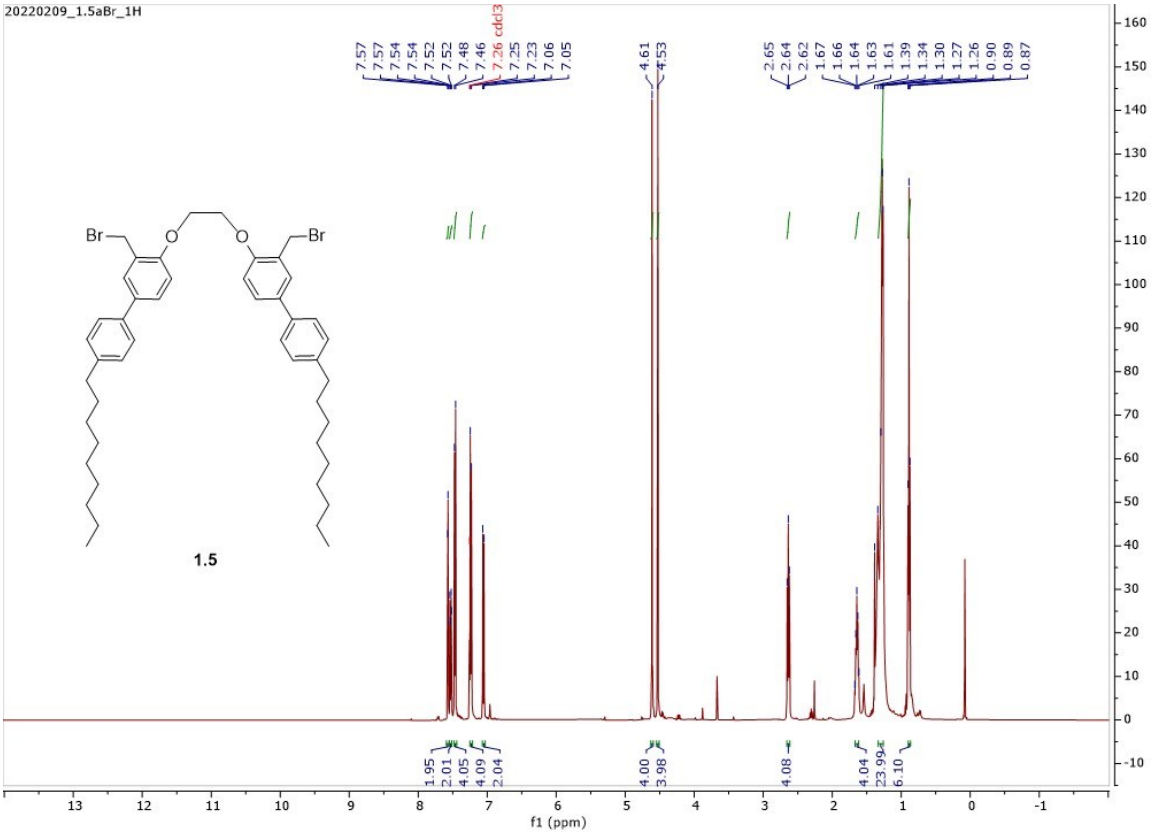
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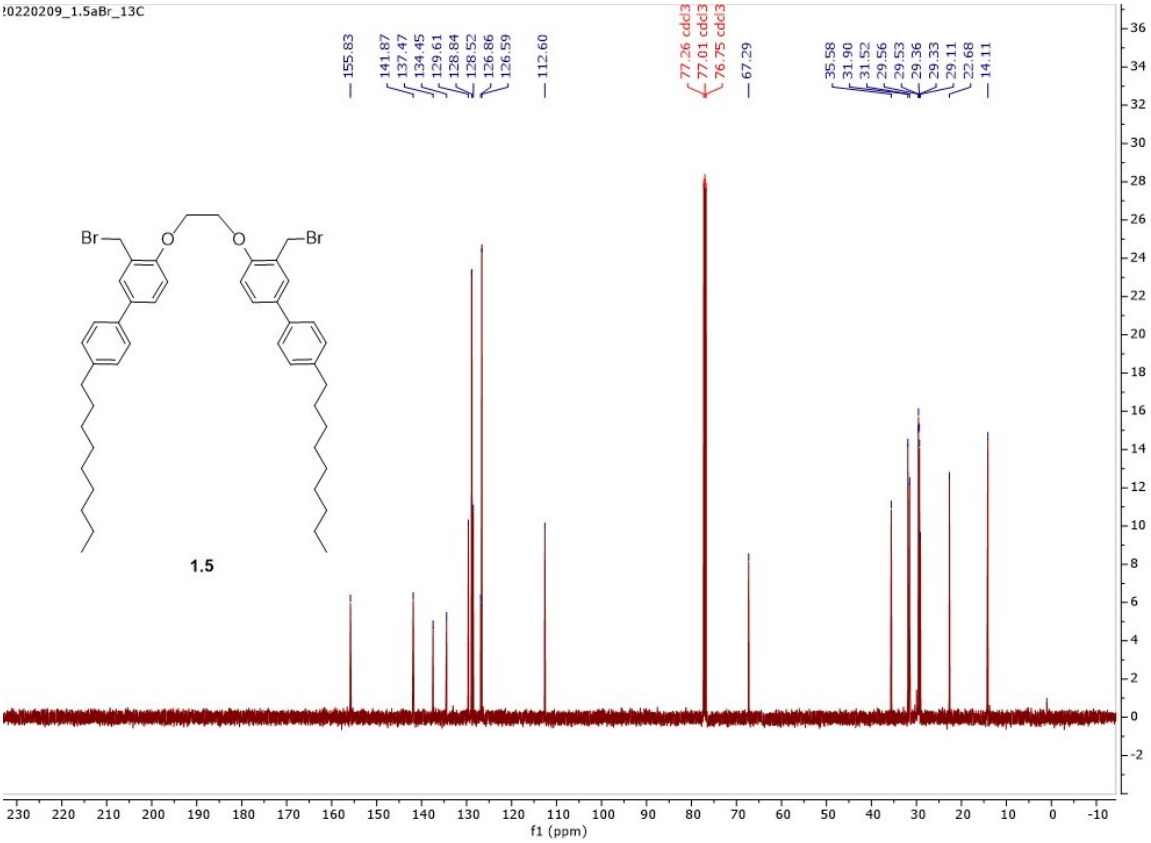
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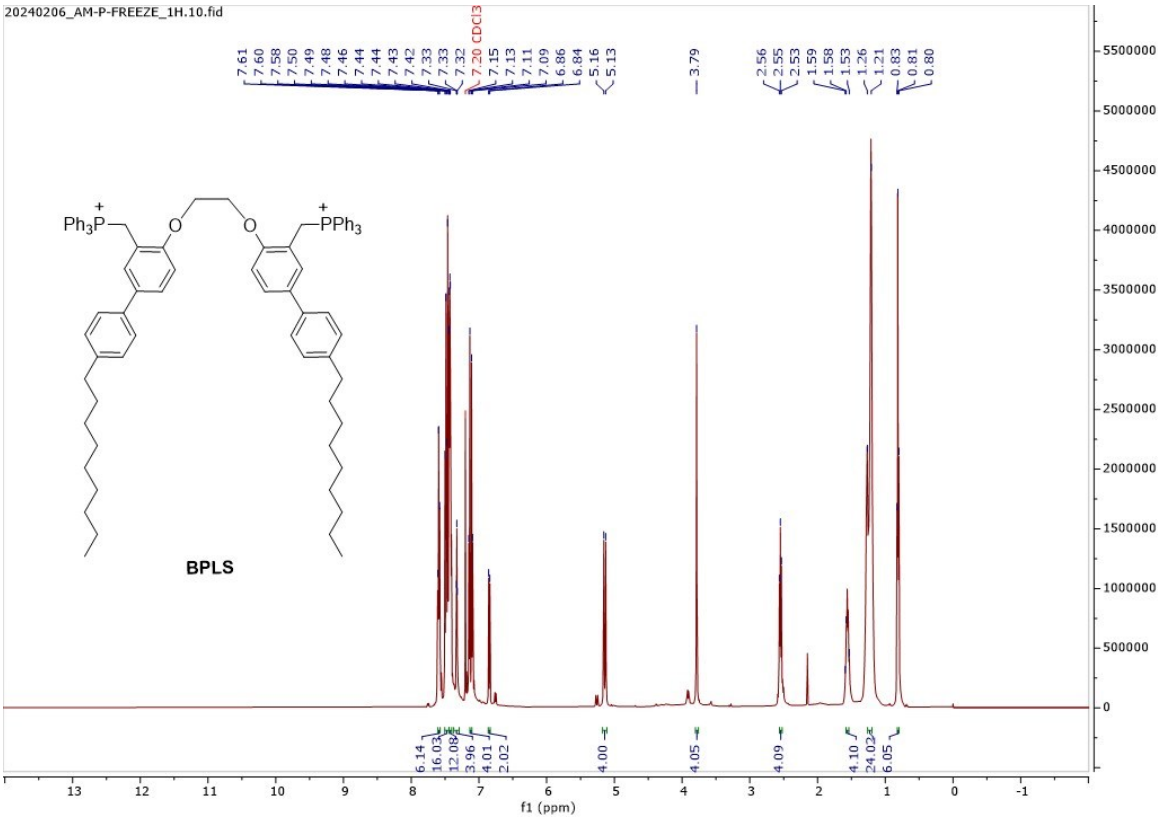
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5.13
3.79
2.56
2.55
2.53
1.59
1.58
1.53
1.26
1.21
0.83
0.81
0.80
Ph3P
O
O
PPh3
BPLS
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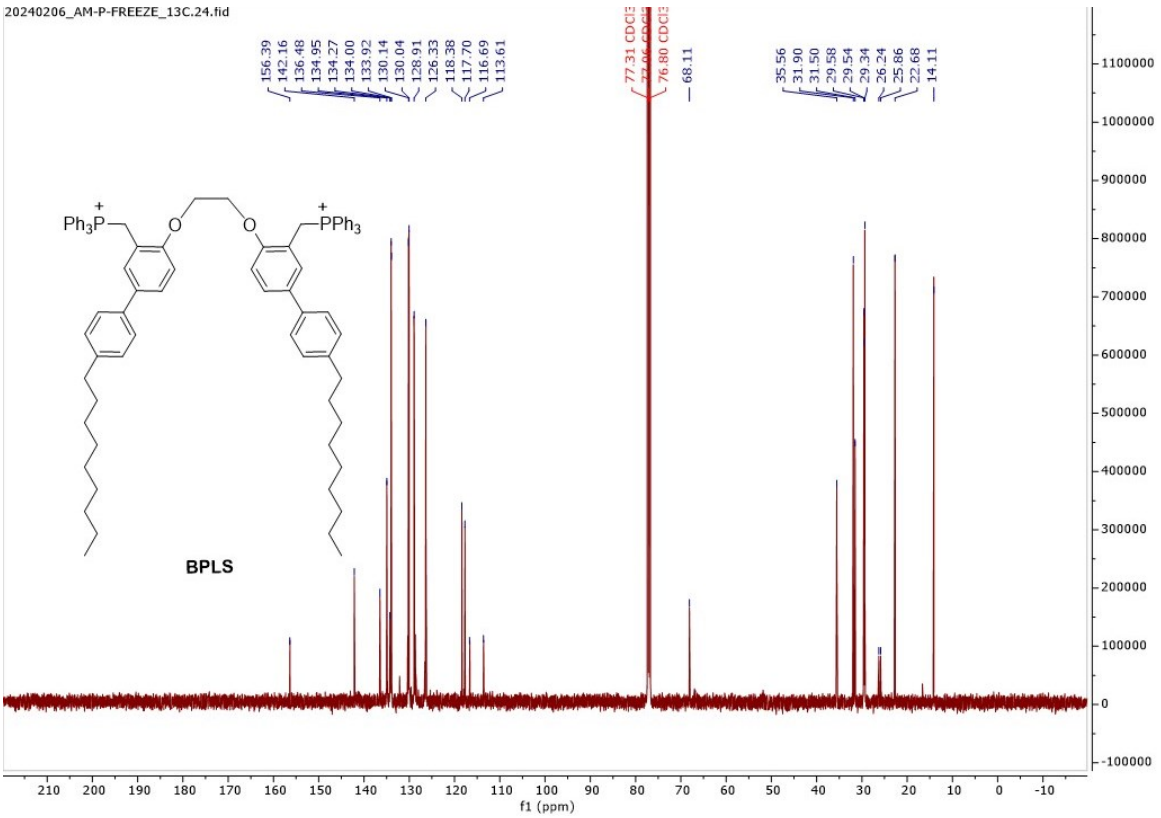
f1 (ppm)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68.11
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31.90
31.50
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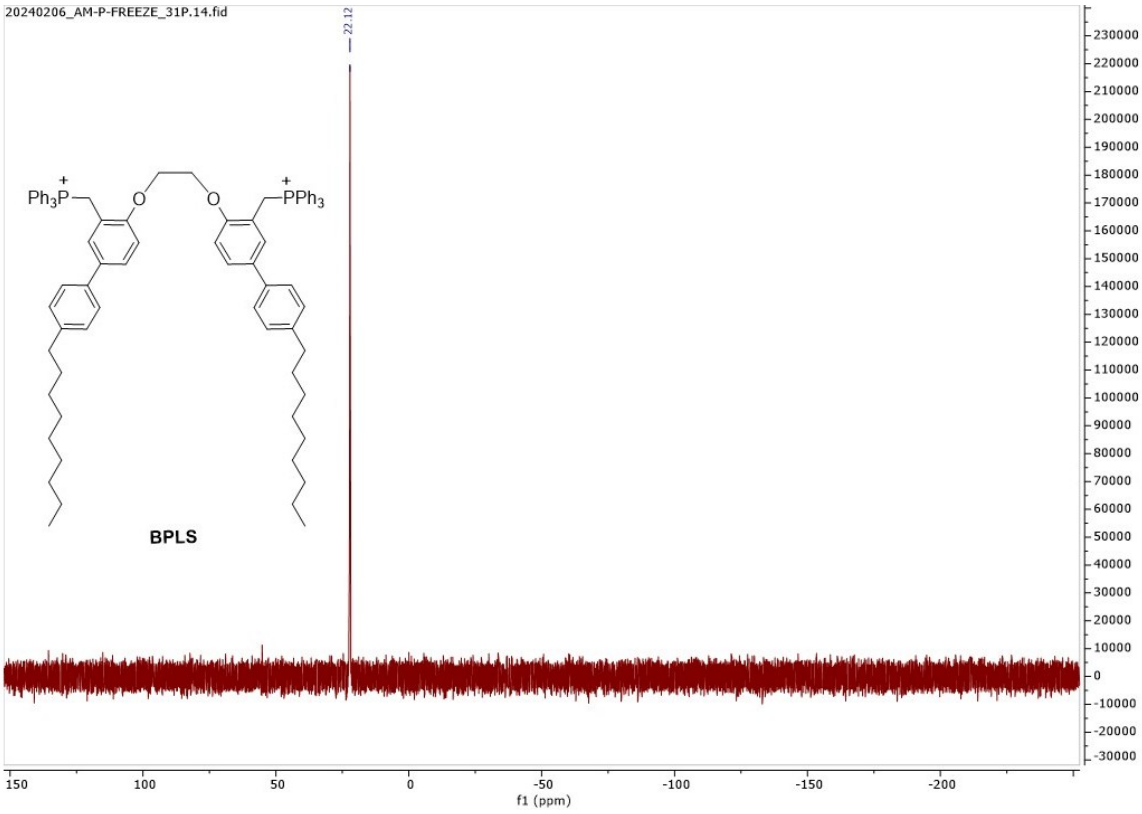
BPLS
f1 (ppm)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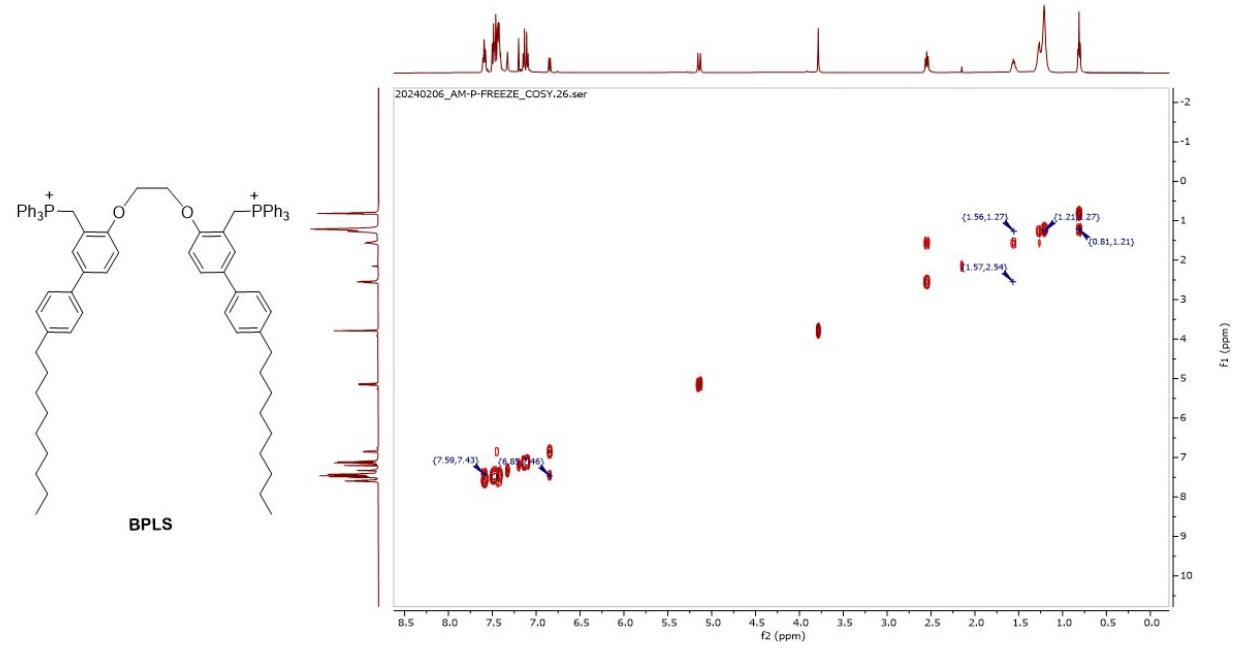
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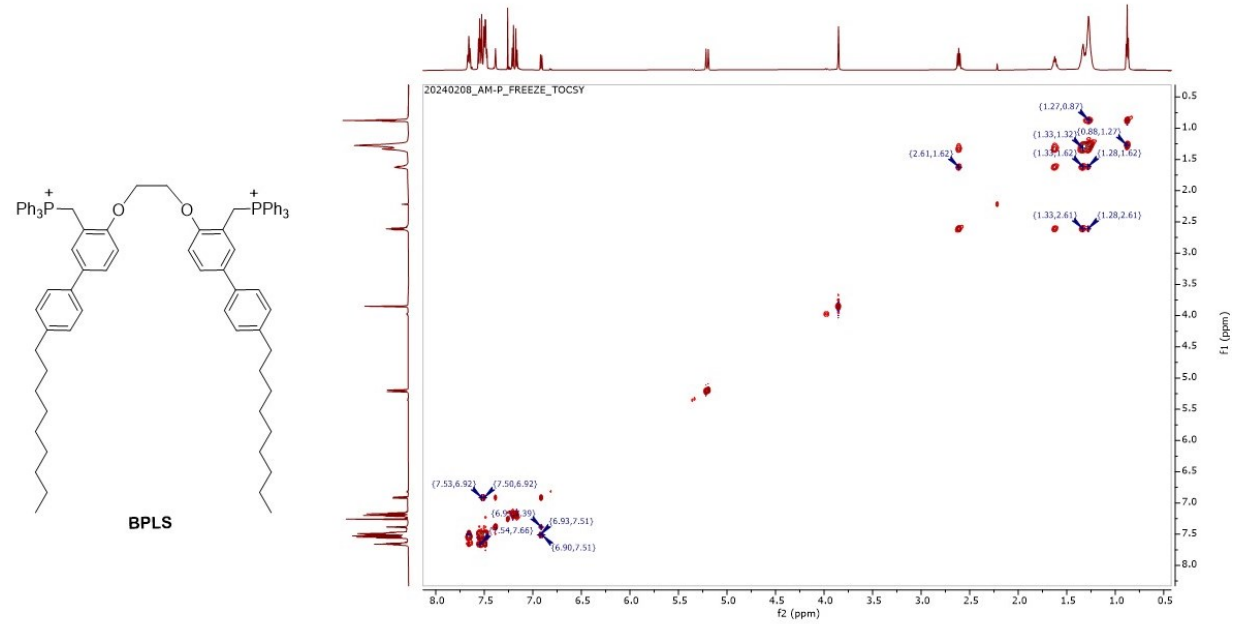
f2 (ppm)
f1 (ppm)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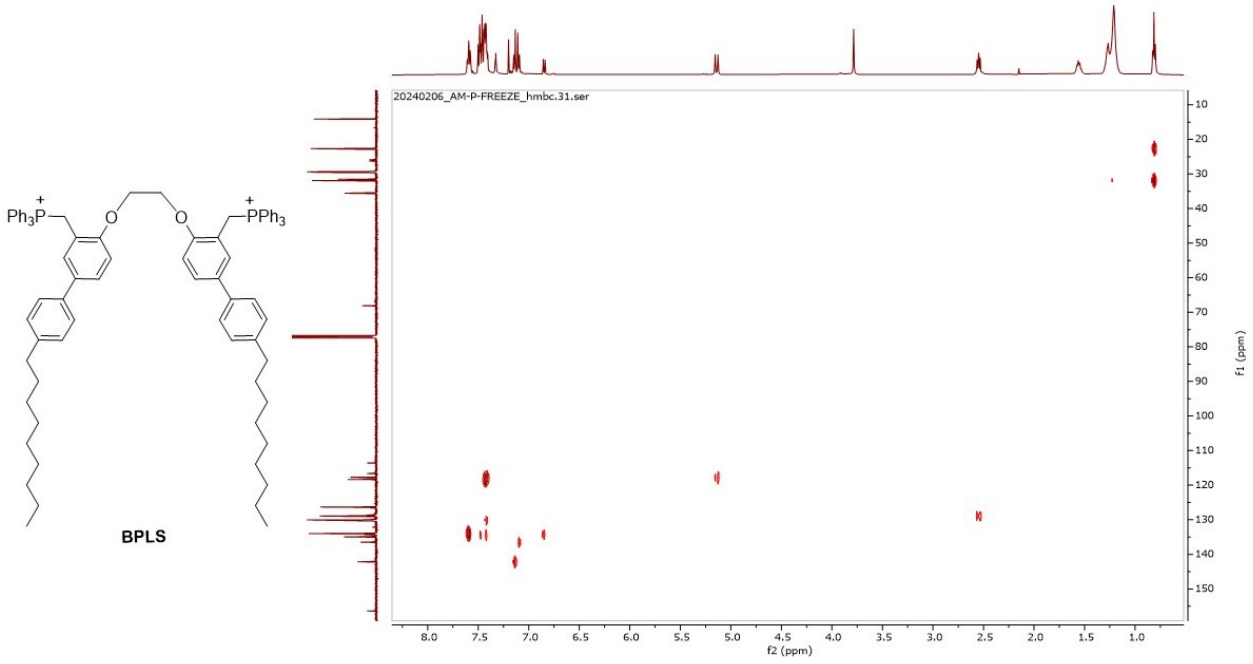
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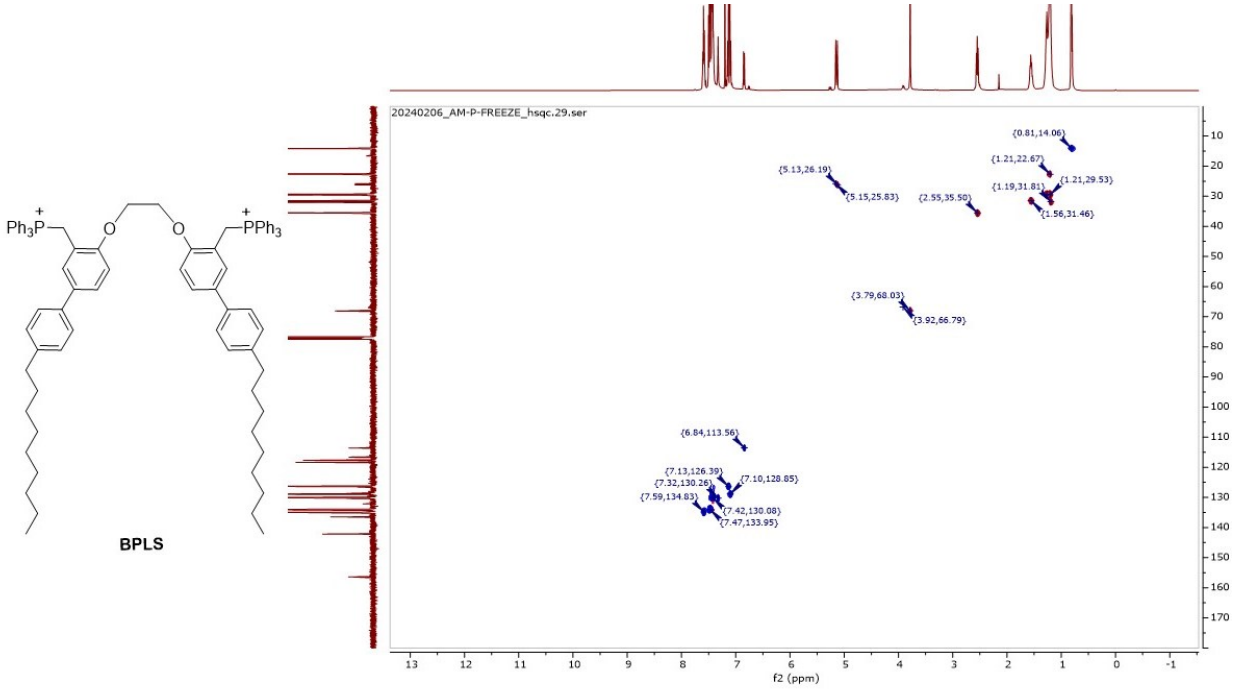
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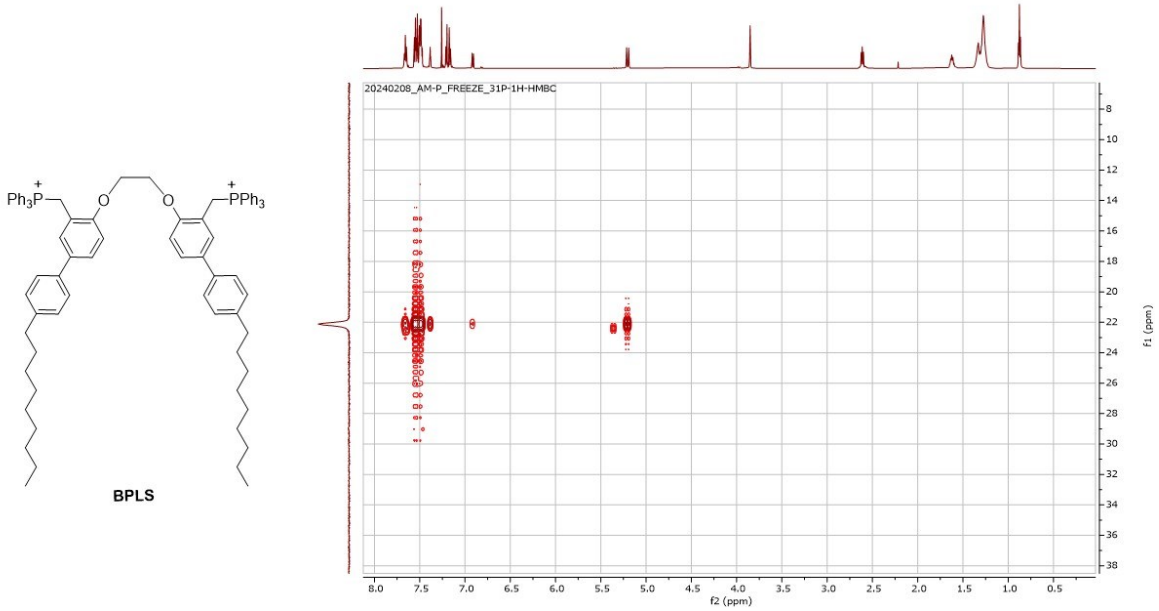
BPLS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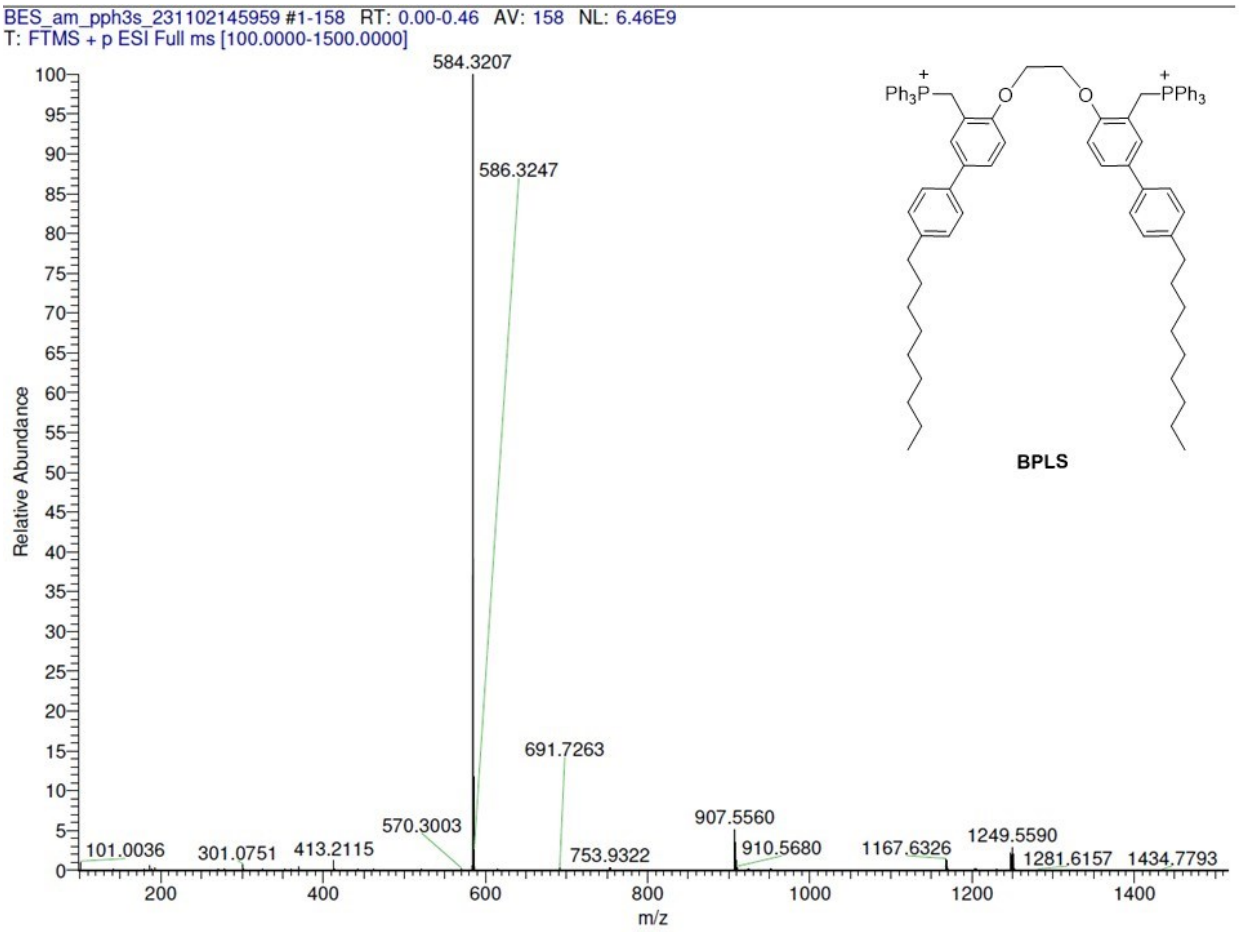
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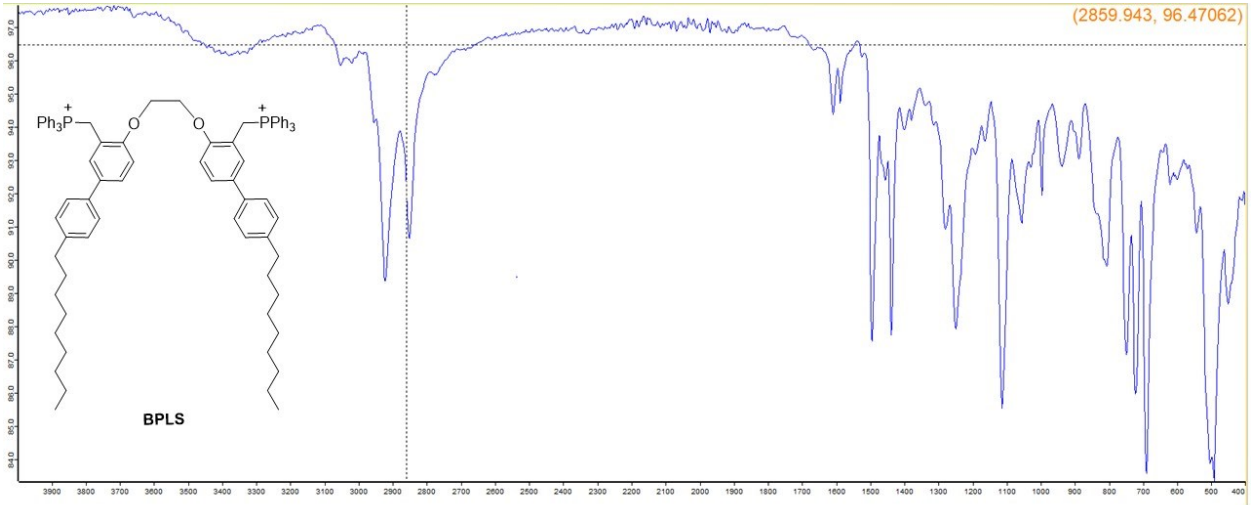
Relative Abundance
m/z
BPLS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