Electronic Supporting Information for

Supramolecular Antibiotics: A Strategy for Conversion of Broad-Spectrum to Narrow-Spectrum Antibiotics for Staphylococcus aureus

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1. Supplementary table and figures

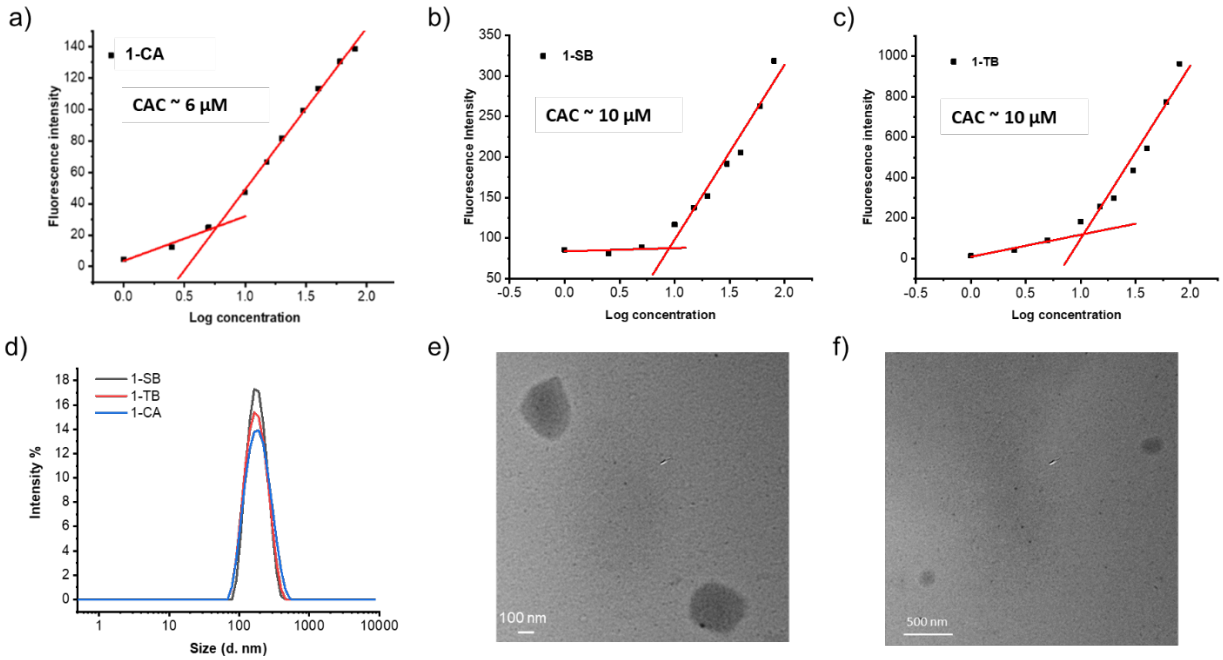


Fig. S1 Self-assembly study of amphiphiles. Critical aggregation concentration (CAC) measurements of a) 1-CA b) 1-SB c) 1-TB. d) Hydrodynamic diameter of nanoassemblies formed by oligomers determined by dynamic light scattering (DLS). TEM images of e) 1-SB and f) 1-TB.

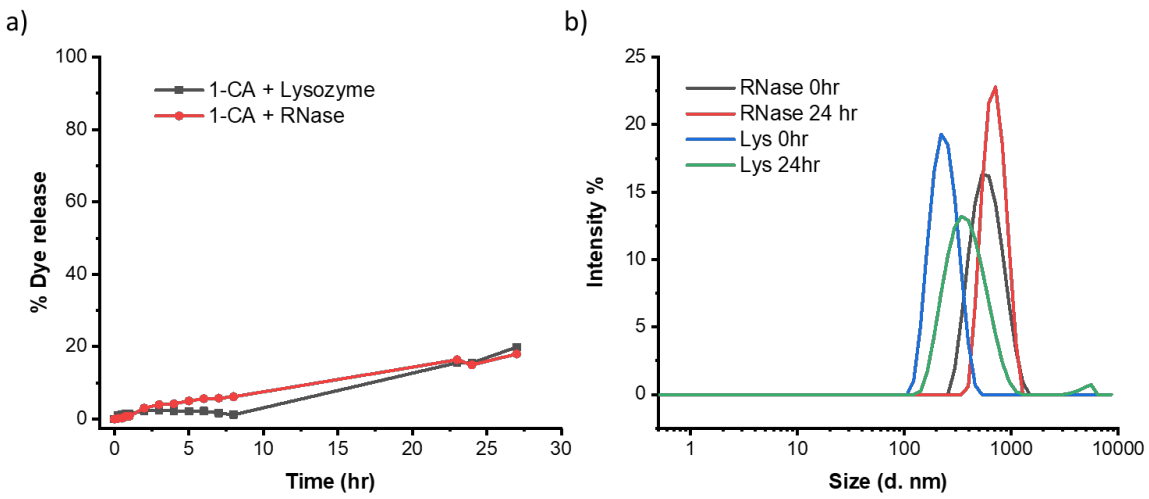


Fig. S2 a) Plot of % release of encapsulated Nile red in presence of nonspecific enzymes b) DLS profiles of 1-CA nanoassembly before and after incubation of nonspecific enzymes. Lys – lysozyme

Nanoassembly	Enzyme	Temperature	Concentration of enzyme	Results
1-CA 30 µM	β-lactamase from *Enterobacter cloacae*	25° C	1 unit	No release
1-CA 30 µM	β-lactamase from *Enterobacter cloacae*	25° C	5 units	No release
1-CA 30 µM	β-lactamase from *Bacillus cereus*	25° C	700-1500 units	No release
1-CA 30 µM	β-lactamase from *Bacillus cereus*	37° C	700-1500 units	No release

Table S1 Encapsulated dye release study from 1-CA nanoassembly with different β-lactamases. One unit - hydrolyzes 1 µmole of benzylpenicillin per min at pH 7 and 25 °C.

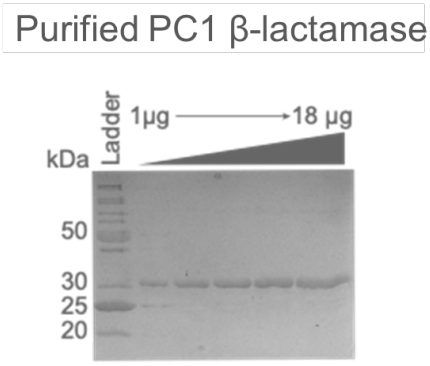


Fig. S3 SDS-PAGE demonstrating pure PC1 β-lactamase expressed and purified, ~31 kDa.

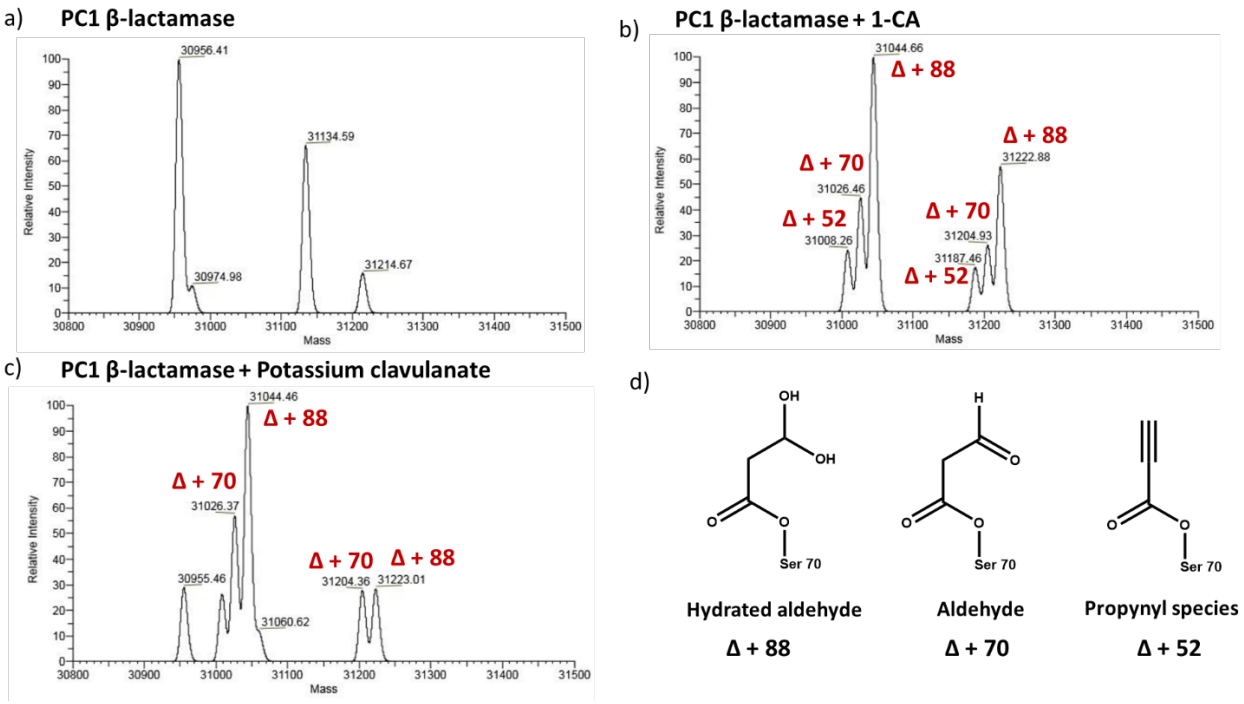


Fig. S4 Mass spectrometry analysis of a) Intact PC1 β-lactamase b) PC1 β-lactamase incubated with clavulanate modified with oligomer, 1-CA and c) PC1 β-lactamase with potassium clavulanate (CA). The marked peaks show the presence of new enzyme inactivated adducts indicated in d. d) Proposed mass adducts corresponding to the observed inactivated PC1 β-lactamase.[1, 2]

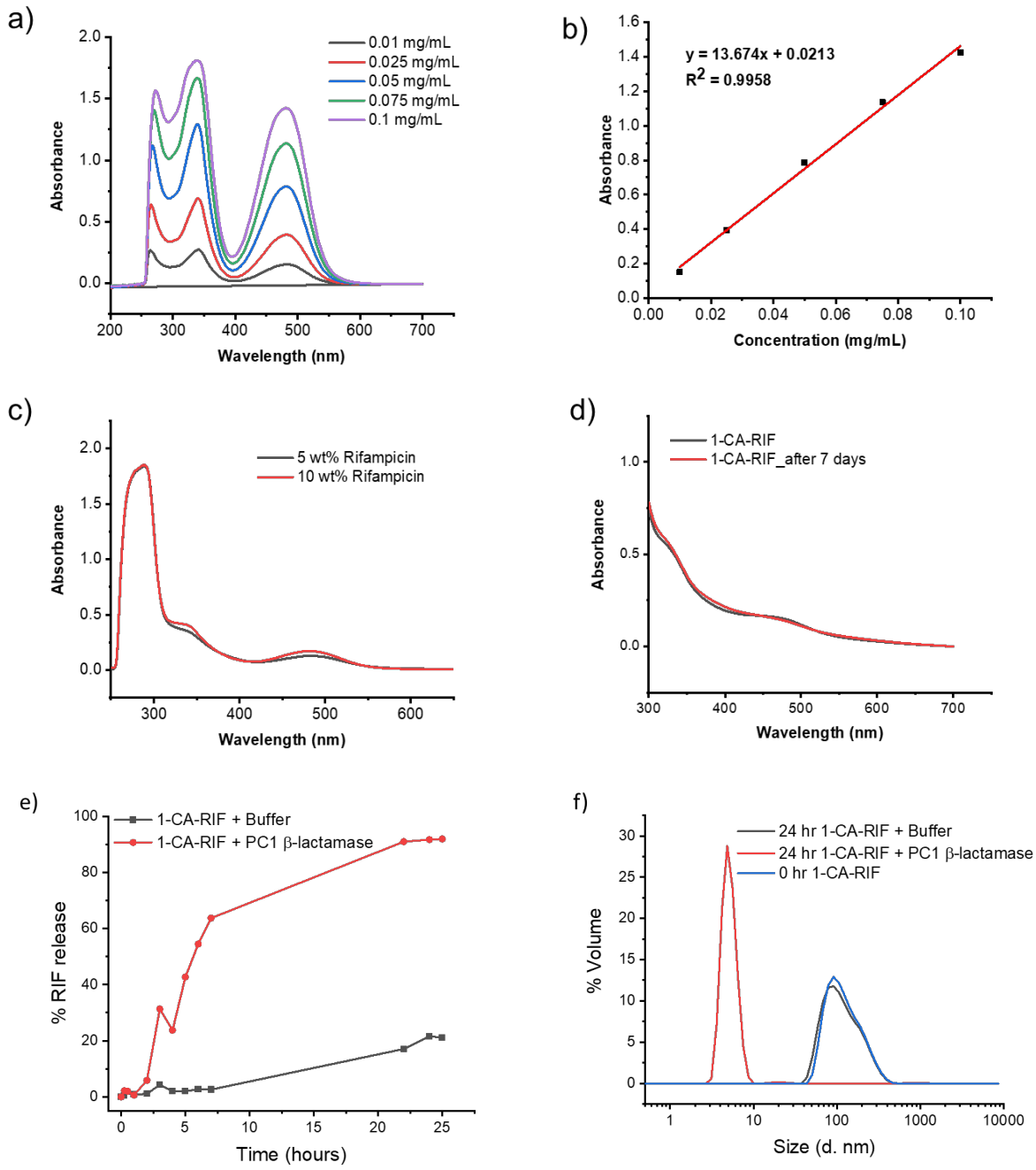


Fig. S5 Encapsulation and quantification of rifampicin drug. a) UV-Vis spectra of different concentrations of rifampicin in DMSO. b) Calibration curve for rifampicin in DMSO. c) Different weight % drug loading in to the 1-CA nanoassembly. d) Stability of encapsulation of rifampicin in 1-CA. e) Plot of percentage release of RIF from 1-CA nanoassemblies over time. f) DLS profiles of 1-CA nanoassembly before and after incubation with PC1 β-lactamase.

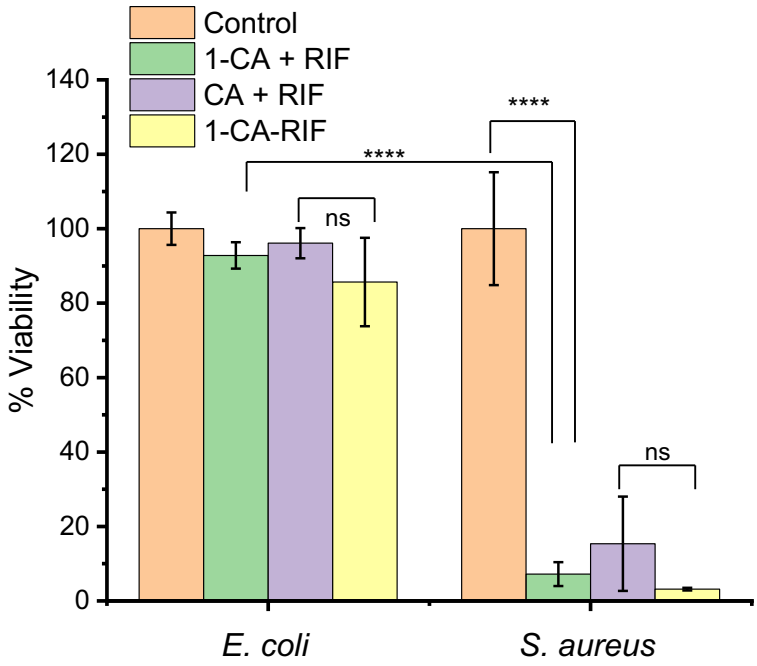


Fig. S6 *E. coli* and *S. aureus* were grown independently and challenged with free RIF and 1-CA, RIF and clavulanate (CA), and 1-CA-RIF overnight. Then, alamarBlue was used to assess the metabolic activity of the cultures. Control is the bacteria without any treatment. ns, no statistically significant differences; ****p<0.0001, Tukey's test.

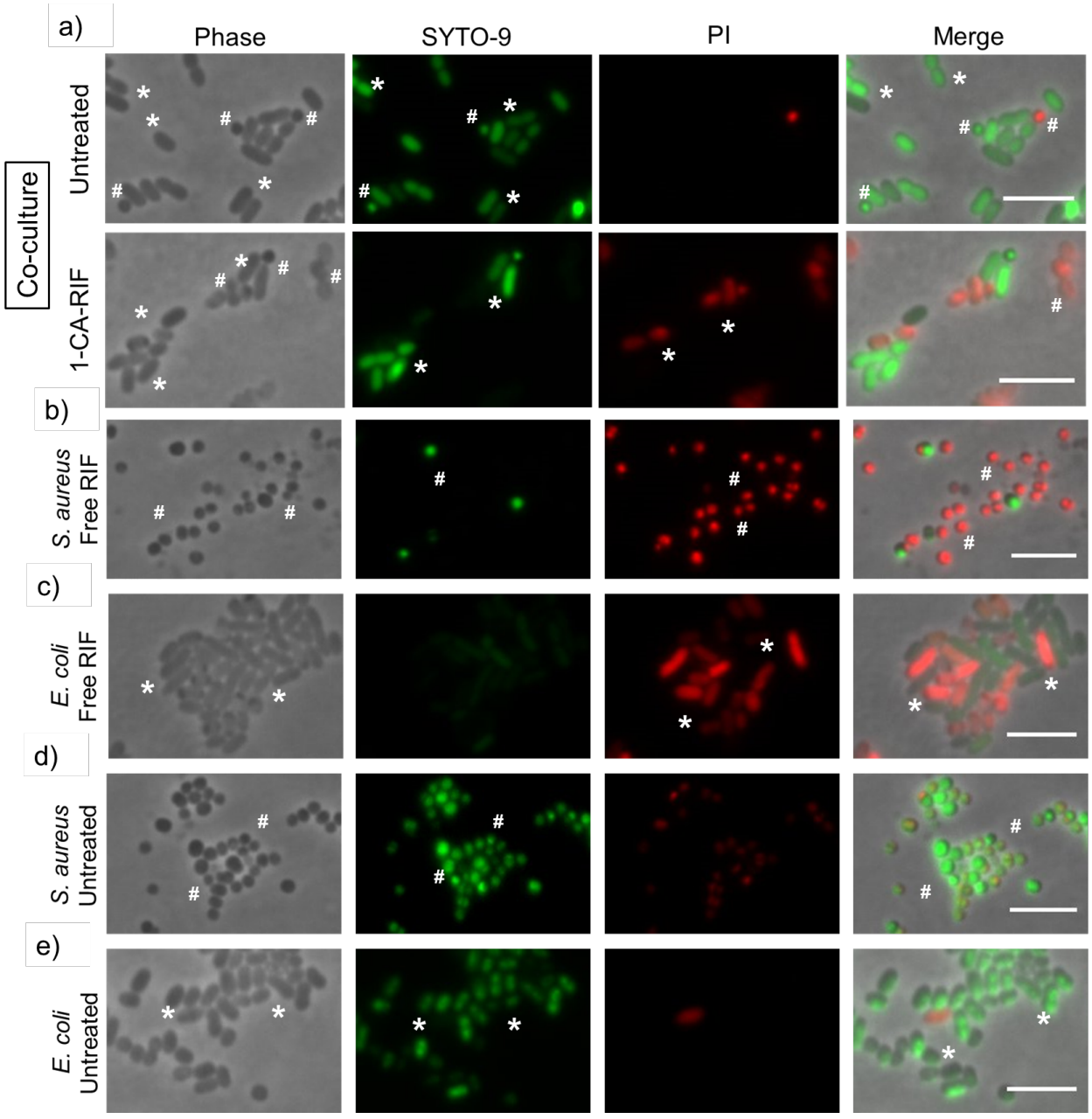


Fig. S7 Fluorescence microscopy imaging using SYTO-PI co-staining in a) *S. aureus-E. coli* coculture with and without the treatment of 1-CA-RIF; Free RIF treatment in b) *S. aureus* and c) *E. coli*; Without any treatment d) *S. aureus* and e) *E. coli*. 1-CA-RIF was incubated with the bacterial cultures for 6 hours, followed by the staining using SYTO-9 and PI dyes. Scale bar: 5 µm; a) *S. aureus* b) *E. coli* and without any treatment c) *S. aureus* d) *E. coli.*

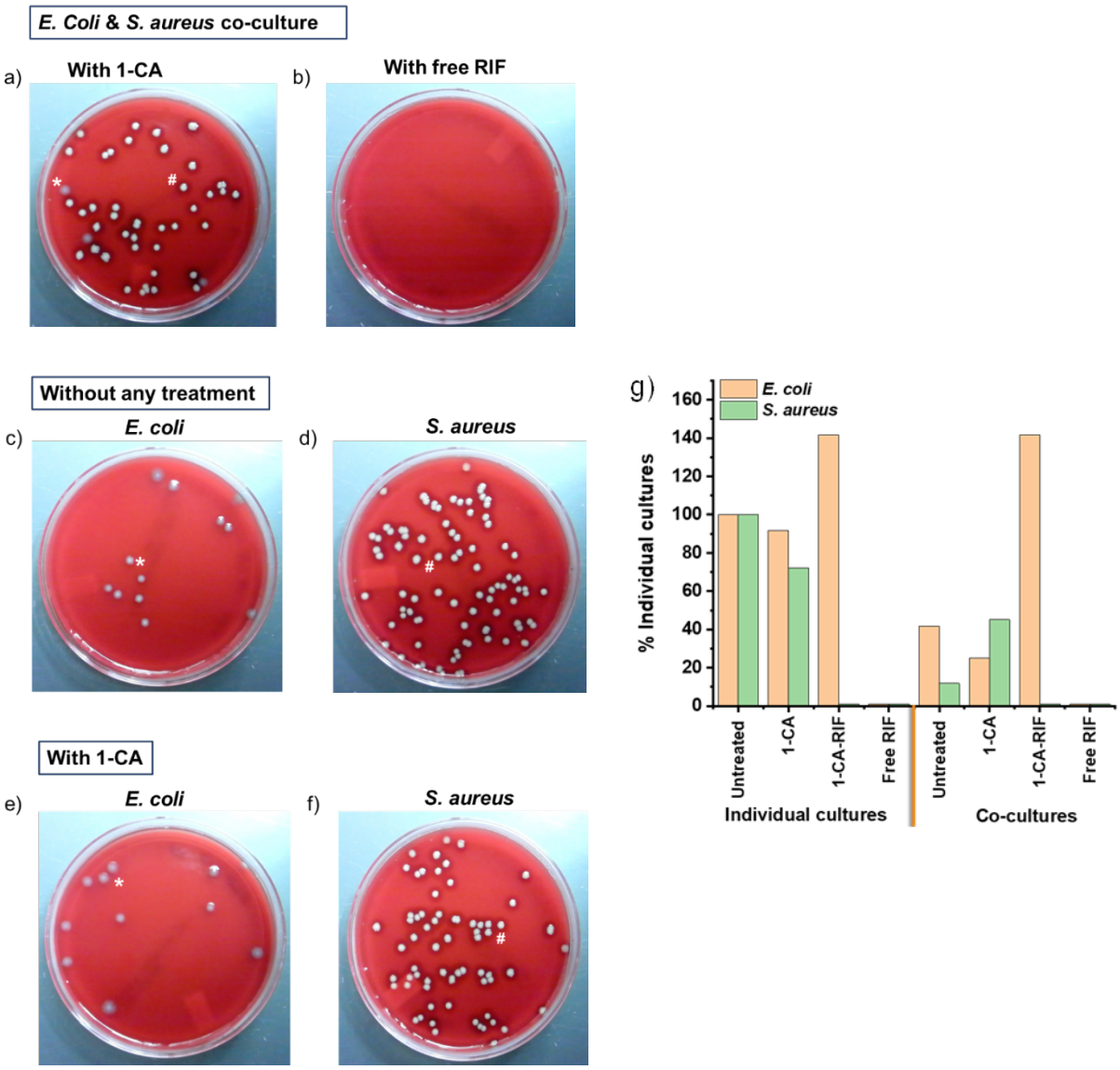


Fig. S8 Growth of bacteria on blood agar media with the treatment of free rifampicin or 1-CA (nanoparticle alone). a) *E. coli* and *S. aureus* treated with 1-CA b) *E. coli* and *S. aureus* treated with free rifampicin c) *E. coli* without any treatment d) *S. aureus* without any treatment e) *E. coli* treated with 1-CA and f) *S. aureus* treated with 1-CA g) Representative graph showing bacterial colonies count, normalized to individual cultures in both homogenous and co-culture conditions with different treatments. Where applicable, values that are plotted as 0 were undetectable in our dilutions and therefore correspond to $<10^5$ CFU/mL.

- *S. aureus* colonies, * - *E. coli* colonies.

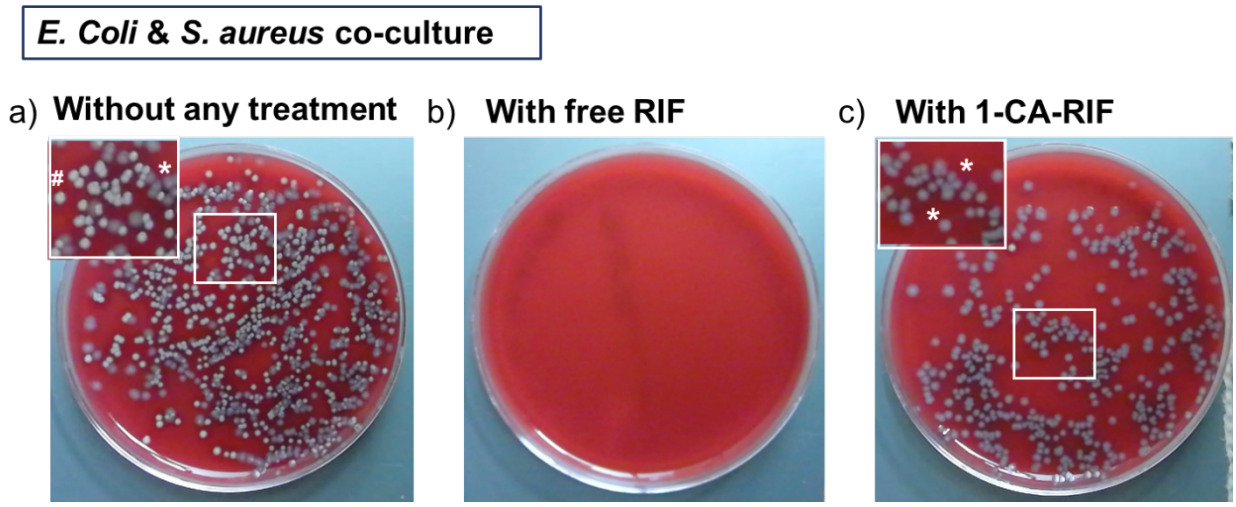


Fig. S9 Co-culture experiment of *S. aureus-E. coli* on blood agar media with high colony density of bacteria. a) *E. coli* and *S. aureus* treated with 1-CA b) *E. coli* and *S. aureus* treated with free rifampicin c) *E. coli* with 1-CA-RIF.

- *S. aureus* colonies, * - *E. coli* colonies.

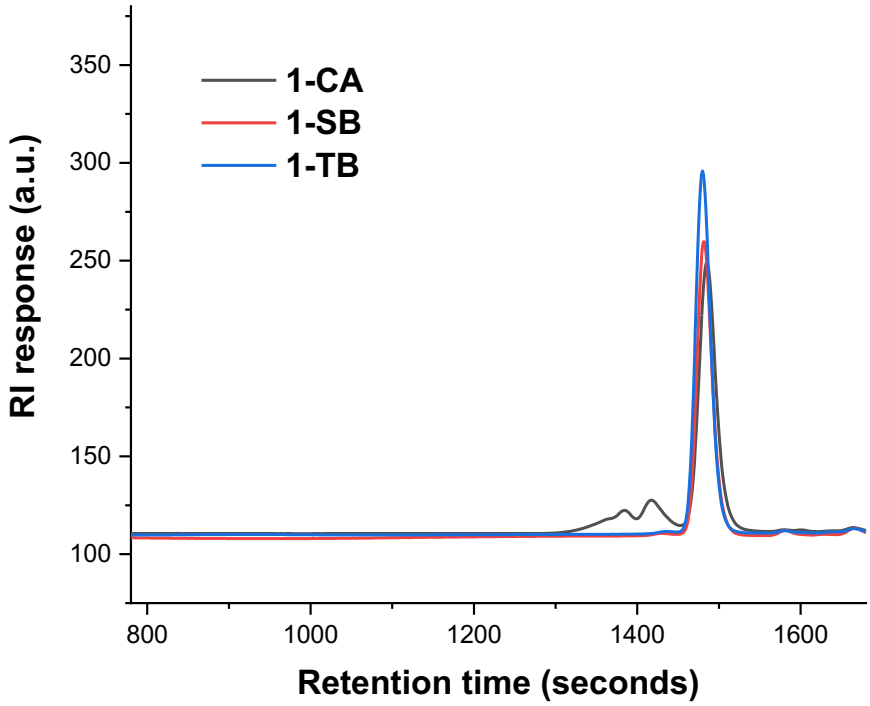


Fig. S10 GPC profiles of the amphiphiles modified with different β-lactamase inhibitors.

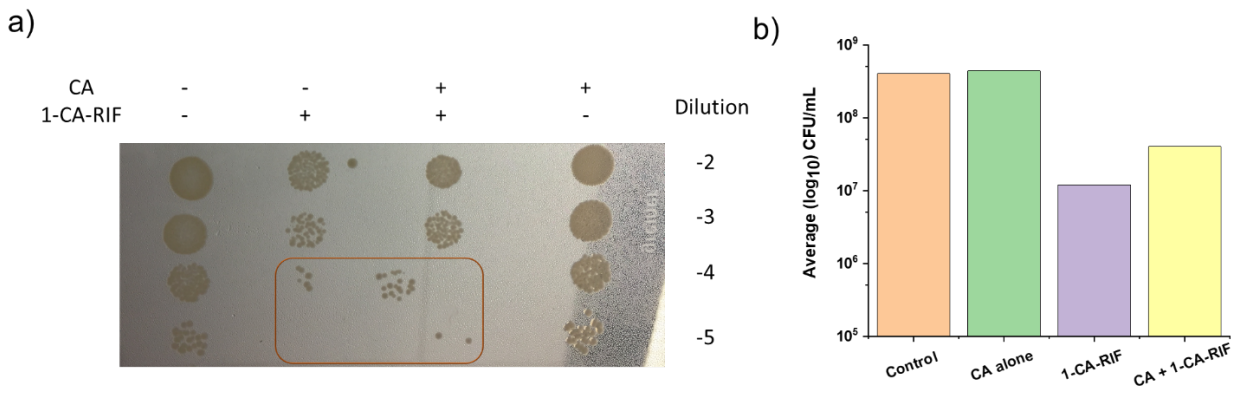


Fig. S11 Competitive experiment with clavulanate (CA). a) Treatment with CA protects bacteria against 1-CA-RIF nanoassemblies. *S. aureus* was grown with or without CA and incubated for 30 min. Further, the cultures were incubated with 1-CA-RIF. After spotting the cultures in a plate, a three-fold increased viability of *S. aureus* was observed when cells were pretreated with CA compared to bacteria incubated without CA; b) Representative experiment showing the number of colonies in a).

1-CA-RIF = Rifampicin encapsulated 1-CA.

2. Materials and methods

Materials and instrumentation: All the reagents were purchased and used as received from commercial sources unless otherwise stated. Potassium clavulanate was purchased from AK Scientific, sulbactam and tazobactam were purchased from Alfa Aeser. 4-Dimethylaminopyridine (DMAP), α,α'-Dibromo-p-xylene, sodium hydride (60 % dispersion in mineral oil), β-lactamase from *Bacillus cereus*, β-lactamase from *Enterobacter cloacae* and rifampicin were purchased from Sigma Aldrich. TEM-1 β-lactamase was purchased from Thermofisher Scientific. Azido-PEG4-alcohol and DBCO-acid were purchased from BroadPharm. 3-(3-Dimethylaminopropyl)-1-ethyl-carbodiimide hydrochloride (EDC.HCl) was purchased from Chem-Impex and sodium bicarbonate ($NaHCO_3$) from Fisher Scientific.

^1H NMR and ^{13}C NMR spectra were recorded on a 400 MHz Bruker NMR spectrometer using the residual proton resonance of the solvent as the internal standard. UV-Vis absorption spectra were obtained by a PerkinElmer Lambda 35 UV-Vis Spectrometer. Fluorescence spectra were recorded on a PerkinElmer LS 55 spectrofluorimeter. Dynamic light scattering (DLS) measurements were performed using a Malvern Nanozetasizer ZS90 with a 637 nm laser source with non-invasive backscattering technology detected at 173^o using disposable sizing cuvette at 25 ºC. Morphology of the nanoassemblies were analyzed by transmission electron microscopy (TEM) by drop casting sample onto carbon-coated copper grid. The grid was then dried by slow evaporation in air for overnight and then images were recorded on a JEOL-2000FX electron microscopy operated at 200kV and at a nominal magnification of 5000X. At least 10 locations on the TEM grid were examined.

Self-assembly of nanoassemblies: 1-CA, 1-SB or 1-TB were self-assembled using co-solvent method. In order to obtain 100 μM solution of 2a, 1.1 mg of 2a was dissolved in 50 μL of acetone. To this stirring solution, 4.66 mL of milli-Q water was added dropwise and let it stir in open air at room temperature for overnight.

Guest encapsulation in to nanoassemblies: 100 μM amphiphile solutions in milli-Q water were stirred at room temperature and Nile red/DiI stock solution (1 mg/mL in acetone, 5 wt.% to **1-CA, 1-SB or 1-TB**) was added in each solution. The solutions were stirred for 8 hours in open atmosphere allowing the organic solvent to evaporate at room temperature, and then filtered through hydrophilic membranes with pore size of 0.45 μm.

Determination of critical aggregation concentration (CAC): The fluorescence probe method was used to determine the CAC values of nanoassemblies (**1-CA, 1-SB or 1-TB**). Fixed concentration of DiI solution (20 μL from 1 mM) in acetone was added into a series of vials. The varying concentration (1 μM to 100 μM) of oligomeric amphiphiles were added to the vials. All the micelle solutions were kept uncapped and stirred for overnight to evaporate acetone. The emission spectra were recorded for the solutions and emission maxima of each spectra were plotted as a function of the concentration of nanoassemblies. The inflection point of the plot was taken as CAC of the nanoassemblies.

Guest release study: Nile red encapsulated nanoassemblies 25 μM (**1-CA**) were incubated with 50 μM PC1 β-lactamase in 0.1M Tris- HCl buffer at pH 7. The Fluorescence spectra of Nile red at 612 nm were monitored overtime and the changes in emission intensities were used to calculate the percentage dye release.

% Dye release = $(I_0-I)/I_0*100$

Where I_0 = Initial emission intensity and I = emission intensity after the incubation with β-lactamase enzyme at each time point.

PC1 β-Lactamase expression and purification: Plasmid encoding β-lactamase PC1 with an N-terminal His-tag, within pET-15b, was purchased from Biomatik. This PC1 variant contains an N-terminal truncation (residues 1-24) of the signal peptide (Protein Eng. 1995 Dec;8(12):1275-85 ; Uniprot P00807 (BLAC_STAAU)). The plasmid was transformed into BL21(DE3) *E.coli* cells via electroporation and plated on agar plates containing ampicillin. Single colony cultures were grown in 50 mL LB media with ampicillin at 37 °C overnight. The following day, 8L of LB were inoculated with ~5 mL of the overnight culture per liter and grown at 37 °C in the presence of antibiotic until an OD_{600} of ~0.6-0.8 was achieved. The incubation temperature was then reduced to 30 °C and cells were induced with 1.0 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) and left to express for ~6 hours (16-hour induction at 16 °C). Cells were then harvested via centrifugation for 10 minutes at 5k RPM and 4L pellets were individually stored at -80 °C. 4L cell pellets were thawed and lysed using a microfluidizer (Microfluidics, Inc.) in a buffer containing 50 mM Na_3PO_4, 100 mM NaCl, 2 mM imidazole, pH 6.8. Lysed cells were centrifuged at ~27k RCF for 50 minutes to remove cellular debris. The lysate supernatant was then loaded onto a precharged 5 mL HiTrap Ni-affinity column (GE Healthcare) and the column was subsequently washed with lysis buffer. Following the lysis wash, the column was further washed with an identical buffer supplemented with a medium imidazole concentration, 50 mM. The bound protein was finally eluted using a linear gradient to 300 mM imidazole over 60 mL in 4 mL fractions. The fractions were analyzed for purity via SDS-PAGE (MW ~31,088 kDa). Fractions were pooled and buffer exchanged using 10k MWCO spin columns to 1X PBS, pH 7 with 10% v/v glycerol to avoid precipitation. To remove any possible precipitate before storage, the final solution was filtered using a 0.22 μm filter and concentration achieved via A_{280} absorbance, using a molar extinction coefficient of 19,370 M^{-1} cm^{-1}. The final solution was aliquoted, and aliquots were subsequently stored at -80 °C. All protein chromatography was executed at 4 °C.

PC1 β-Lactamase amino acid sequence used:
MGSSHHHHHHSSGLVPRGSHMKELNDLEKKYNAHIGVYALDTKSGKEVKFNSDKRFAYASTS
KAINSAILLEQVPYNKLNKKVHINKDDIVAYSPILEKYVGKDITLKALIEASMTYSDNTANNKIIKEIG
GIKKVKQRLKELGDKVTNPVRYEIELNYYSPKSKKDTSTPAAFGKTLNKLIANGKLSKENKKFLL
DLMLNNKSGDTLIKDGVPKDYKVADKSGQAITYASRNDVAFVYPKGQSEPIVLVIFTNKDNKSD
KPNDKLISETAKSVMKEF

β-lactamase activity assay: The activity of purified PC1 β-lactamase was checked using amplite™ colorimetric β-lactamase activity assay kit (AATBioquest). PC1 β-lactamase was incubated with Nitrocefin substrate from assay kit for 30 minutes and followed the instructions from the manufacturer. Monitored the absorbance increase with an Epoch microplate spectrophotometer reader (BioTek, Winooski VT, USA) at OD ratio of 490/380 nm. A standard curve was made using the β-lactamase standards given with the kit.

Preparation of rifampicin (antibiotic) encapsulated nanoassemblies: Rifampicin stock solution (11 mg/mL) was prepared in DMSO. 5 or 10 wt.% of rifampicin was added to 250 μM nanoassemblies (**1-CA, 1-SB or 1-TB**) and sonicated for 10 minutes. This solution was further dialyzed against milli-Q water to remove DMSO and nonencapsulated rifampicin using 8 kDa cut-

off dialysis membrane for overnight. During dialysis, milli-Q water was replaced 2-3 times. The samples were lyophilized and re-dissolved in DMSO to quantify the drug encapsulation. A standard curve was made with different concentrations of rifampicin drug in DMSO to quantify the amount of rifampicin encapsulation using UV-Vis absorption spectrometer.

Mass spectrometry: For intact protein mass spectrometry to determine intermediates and products of inactivation, 40 μM of PC1 β-lactamase were incubated for 30 min with and without 4 mM potassium clavulanate and with 4 mM **1-CA** independently. Each reaction was terminated by the addition of 0.1% trifluoroacetic. 5 μL of sample was loaded on to a C4 protein trap column (Waters Corp), desalted for 3 mins at 0.2 mL/min with 95% solvent B (A: 0.1% formic acid in H_2O, B: 0.1% formic acid in acetonitrile) and then eluted by ramping to 100% B over 2 minutes. The chromatographic data was summed for the peak eluting 6.0 – 6.5 min to obtain the spectrum. Spectra were generated on a Thermo Ultimate 3000 UHPLC system coupled to a Thermo Orbitrap Fusion Mass Spectrometer.

Bacterial strains and growth conditions: *Staphylococcus aureus*, *E. coli* K12, *Bacillus subtilis* JH642 and *Pseudomonas aeruginosa* were grown in LB broth (VWR, Radnor PA, USA); *C. crescentus,* in peptone yeast extract (BD Difco, Franklyn Lakes NJ, USA); *Lactococcus lactis,* in Man, Ragosa & Sharpe (MRS, Oxoid, Basingstoke, Hampshire UK); and *Mycobacterium smegmatis* was grown in Middlebrook 7H9 growth medium (BD Difco, Franklin Lakes, NJ) supplemented with 0.4% glycerol, 0.05% Tween-80 (Sigma-Aldrich, St. Louis, MO USA) and 10% ADC (albumin-dextrose-catalase). All bacteria were grown with shaking at 37ºC, except for *C. crescentus*, which was grown at 30ºC. See the table below for more information.

Bacterial species	Designation	Source	Additional information
Strain (*B. subtilis* JH642)	*B. subtilis*	NZ_CP007800 in GeneBank	
Strain (*C. crescentus*)	*C. crescentus*	NA 1000	Obtained from Dr. Peter Chien (UMass Amherst)
Strain (*E. coli* K12)	*E. coli K12*	MG1655	
Strain (*S. aureus*)	*S. aureus*	ATCC BA-1718	
Strain (*L. lactis*)	*L. lactis lactis*	NRRL B633	
Strain (*P. aeruginosa*)	*P. aeruginosa*	PAK	Obtained from Dr. Alejandro Heuck (UMass Amherst)
M. smegmatis mc2155	*M. smegmatis*	NC_008596 in Genebank	

Determination of minimum inhibitory concentration (MIC): To calculate the minimum concentration needed to inhibit growth of bacteria, serial dilutions were made in sterile 96-well microtiter plates (Genesee Scientific, El Cajon CA, USA) to have 100 μL media containing different concentrations of either free rifampicin or drug encapsulated nanoparticles. Afterwards, 1% of bacteria (OD 0.6-0.8) were inoculated and incubated overnight at 37 ºC with shaking, except for *C. crescentus*, which was incubated for at 30 ºC with shaking. The MIC was regarded as the minimum concentration of rifampicin or rifampicin-encapsulated nanoparticle that inhibited growth of bacteria.

AlamarBlue assay: Wherever necessary, 10 µL of alamarBlue reagent (ThermoFisher Scientific) was added to the 96-well plates and incubated at 37 ºC for 60 minutes in the dark to evaluate active metabolism in the bacteria that were treated with 1-CA-RIF (0.215 µg/mL) or 1-CA (25 µM). The absorbance of reagent was monitored at 570 nm, using 600 nm as a reference wavelength by an Epoch microplate spectrophotometer reader (BioTek, Winooski VT, USA).

Coculture experiments: To test for selective targeting of *S. aureus* by the 1-CA-RIF, we performed a coincubation assay in blood agar media. Briefly, *E. coli* and *S. aureus* were grown separately and normalized to OD ~0.5. Cells were washed with sterile PBS and resuspended in LB broth. Cells were combined, which corresponded to a ratio of 1:8 of *E. coli* : *S. aureus* cells in a tube containing LB broth with and without 0.95 µg/mL of 1-CA-RIF (rifampicin encapsulated 1-CA) and incubated overnight at 37 ºC with shaking. In parallel, nanoparticles devoid of rifampicin (25 µM) and free rifampicin (20 µg/mL) were incubated. Afterwards, to distinguish the cells from *E. coli* and *S. aureus*, 10-fold dilutions were made in sterile PBS and plated in blood agar media plates (Fisher Scientific) and left overnight at 37 ºC to allow growth of surviving bacteria. In this media, colonies from *S. aureus* are distinguishable due to their white color and β-hemolysis, which produces a clear halo around the colonies and contrasts with that of *E. coli* colonies, which do not breakdown the erythrocytes in blood and display a grey color in this media.[3]

Bacterial SYTO9-PI co-staining. *E. coli* and *S. aureus* were grown individually on LB broth to OD 0.3, then the cultures were split to either be combined in a coculture in a 1:1 ratio or handled separately as monocultures. Then, the combined and individual cultures (which contained $3x10^9$ cells in the *S. aureus* cultures, $7x10^7$ cells in the *E. coli* culture; and $1x10^9$ and $7x10^7$ of *S. aureus* and *E. coli* cells in the coculture, respectively) were incubated with 0.5x MIC of 1-CA-RIF, 1-CA, 2x MIC free rifampicin, or vehicle for 6 hours at 37ºC with shaking. Afterwards, to discriminate live from dead bacteria, the cells were labeled with propidium iodide and SYTO-9 by using the BacLight kit (ThermoFisher Scientific, Waltham MA). Cells were then imaged in a Nikon 562 Eclipse E600, Nikon Eclipse Ti microscope with 100x objectives.

Competitive binding experiment with clavulanate: *S. aureus* was grown individually, and their optical density was adjusted to ~0.5-0.7. Later, cultures were preincubated with or without 5 µg/mL clavulanate for 30 min at 37ºC with shaking. Afterwards, 1-CA-RIF (0.95 µg/mL) nanoassemblies were added or not to the mixture and incubated for overnight. We performed serial dilutions in sterile PBS for each condition and spotted aliquots on LB plates, which were left for overnight incubation at 37ºC.

Statistical analysis: GraphPad Prism (version 8.4.3) was the software used for statistical analysis.

3. Synthetic procedures

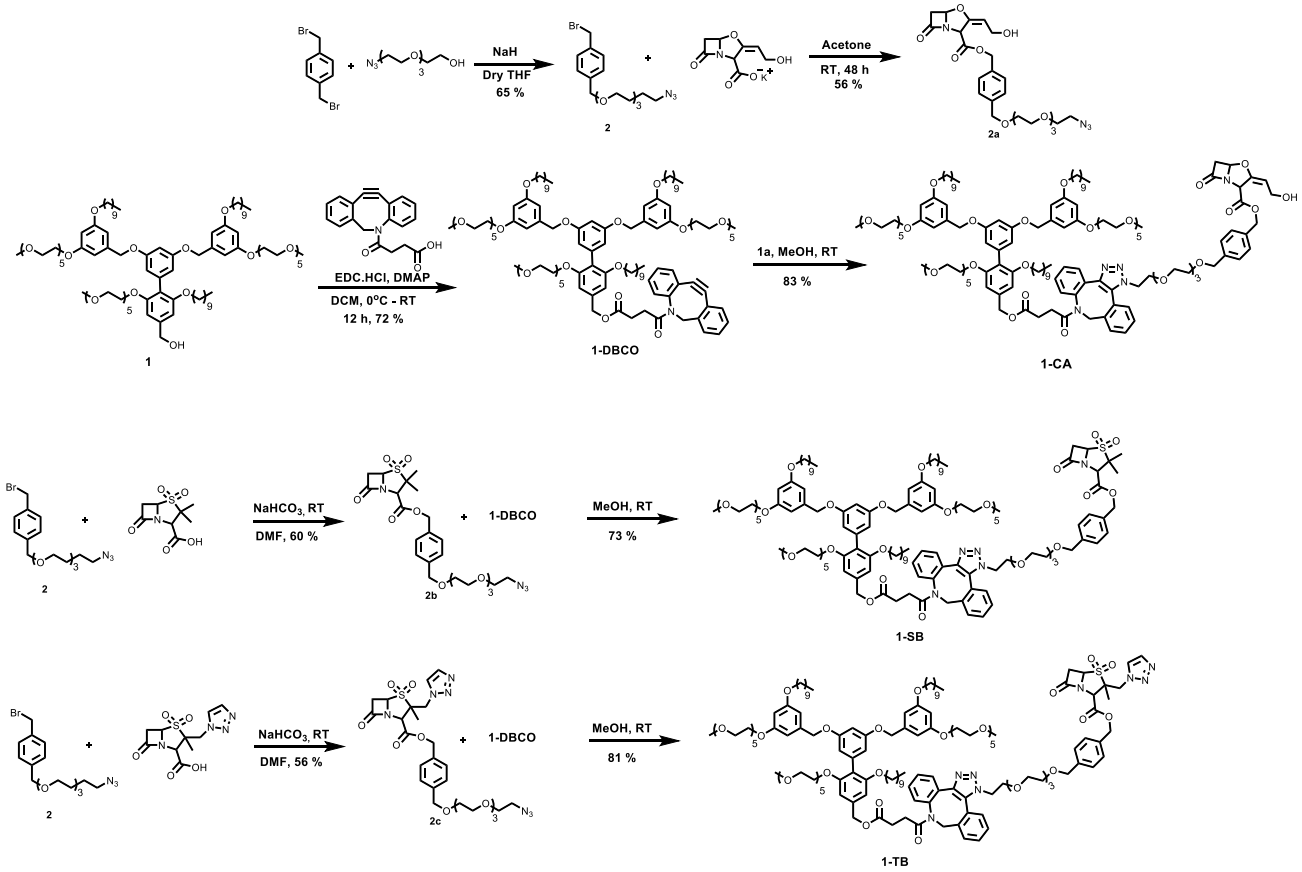


Fig. S12 Synthetic scheme for oligomeric amphiphiles bearing β-lactamase inhibitors.

Synthesis of compound 2: To a solution of α,α'-dibromo-p-xylene (4.2 g, 15.9 mmol) and sodium hydride 60% in mineral oil (318 mg, 7.98 mmol) in dry THF (50 mL), azido-PEG3-alcohol (1.75 g, 7.98 mmol) in dry THF (10 mL) was added in a dropwise manner. The reaction mixture was stirred at room temperature for 12 hours under argon. THF was evaporated and the reaction mixture was dissolved in ethyl acetate (50 mL) and washed with 10% NH_4Cl solution (2x30 mL) and water (2x30 mL). The organic layer was dried over anhydrous Na_2SO_4, concentrated and purified by silica gel chromatography using hexane/ethyl acetate (65:35 v/v) to obtain compound **2** (2.1 g, 65%). ^{1}H NMR (400 MHz, $CDCl_3$)(δ ppm): δ 7.30-7.37 (m, 4H), 4.55 (s, 2H), 4.48 (s, 2H), 3.62-3.68 (m, 14H), 3.35-3.37 (t, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) (δ ppm): δ 138.71, 137.06, 129.08, 128.04, 72.74, 70.68, 70.64, 70.61, 70.01, 69.58, 50.66, 33.35. ESI-MS (m/z) Expected: 401.10 and 403.09 Obtained: $[M+Na]^+$ 424.0681, 426.3602, $[M+K]^+$ 440.1043, 442.0389.

Synthesis of compound 2a: Potassium clavulanate (0.137 g, 0.56 mmol) and compound **2** (75 mg, 0.19 mmol) were dissolved in acetone and stirred the reaction mixture at room temperature for 72 hours. Acetone was concentrated and purified by silica gel chromatography using hexane/ethyl acetate (30:70 v/v) to obtain **2a** (53 mg, 56%). ^{1}H NMR (400 MHz, $CDCl_3$)(δ ppm): δ 7.33-7.37 (m, 4H), 5.68-5.69 (d, 1H), 5.14-5.24 (m, 2H), 5.07 (d, 1H), 4.83-4.86 (td, 1H), 4.57 (s, 2H), 3.62-3.70 (m, 14) , 4.18-4.22 (m, 2H), 3.46-3.51 (dd, 1H), 3.36-3.39 (m, 2H), 3.09 (s, 1H), 1.60 (s, 1H), 1.48-1.51 (t, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) (δ ppm): 174.41, 166.94, 152.25, 139.02, 134.06, 128.59, 128.02, 100.44, 87.97, 72.81, 70.70, 70.66, 70.64, 70.03, 69.63, 67.65,

60.54, 57.27, 50.69, 46.39. ESI-MS (m/z) Expected: 520.22 Obtained: $[M+H]^+$ - 521.0655, $[M+Na]^+$ - 543.1727.

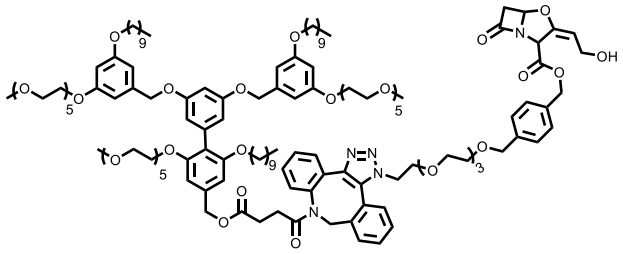
Synthesis of 2b: To a solution of **2** (100 mg, 0.42 mmol) and sodium bicarbonate (43 mg, 0.54mmol) in DMF (1 mL), sulbactam (185 mg, 0.46 mmol) in DMF (1 mL) was added. The reaction was stirred at room temperature and monitored the progress by TLC. Reaction mixture was poured into cold water (10 mL), extracted the mixture using ethyl acetate (3x20 mL). Aqueous layer was saturated using NaCl then re-extracted the mixture using ethyl acetate (2x20 mL). The combined organic layers were concentrated and purified using silica-gel column chromatography (97:3 DCM/MeOH v/v) to obtain **2b** (140 mg, 60%). ^{1}H NMR (400 MHz, $CDCl_3$)(δ ppm): δ 7.34-7.38 (m, 4H), 5.14-5.28 (m, 2H), 4.57-4.60 (m, 3H), 4.40 (s, 1H), 3.63-3.70 (m, 14H), 3.44-3.47 (m, 2H), 3.36-3.39 (t, 2H), 1.55 (s, 3H), 1.29 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) (δ ppm): δ 170.82, 166.96, 139.56, 133.72, 129.10, 128.61, 128.16, 72.89, 70.86, 70.82, 70.81, 70.79, 70.19, 69.87, 68.12, 63.33, 62.91, 61.21, 50.83, 38.45, 20.32, 18.75. ESI-MS (m/z) Expected: m/z – 554.20 Obtained: $[M+Na]^+$ - 577.1638.

Synthesis of 2c: To a solution of **2** (100 mg, 0.33 mmol) and sodium bicarbonate (35 mg, 0.41 mmol) in DMF (1 mL), tazobactam (147 mg, 0.36 mmol) in DMF (1 mL) was added. The reaction was stirred at room temperature and monitored the progress by TLC. Reaction mixture was poured into cold water (10 mL), extracted the mixture using ethyl acetate (3x20 mL). Aqueous layer was saturated using NaCl then re-extracted the mixture using ethyl acetate (2x20 mL). The combined organic layers were concentrated and purified using silica-gel column chromatography (97:3 DCM/MeOH v/v) to obtain **2c** (115 mg, 56%). ^{1}H NMR (400 MHz, $CDCl_3$)(δ ppm): δ 7.72-7.76 (dd, 2H), 7.39 (s, 4H), 5.25 (s, 2H), 4.99-5.09 (m, 2H), 4.62-4.64 (m, 1H), 4.58 (s, 2H), 4.56 (s, 1H), 3.62-3.70 (m, 14H), 3.47-3.56 (m, 2H), 3.36-3.38 (t, 2H), 1.65 (s, 1H), 1.24 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) (δ ppm): δ 169.78, 165.89, 162.56, 139.67, 134.32, 133.07, 129.22, 128.16, 125.69, 72.71, 70.70, 70.65, 70.62, 70.02,69.70, 68.71, 65.21, 62.59, 60.44, 50.69, 50.39, 39.12, 36.50, 31.45, 15.94. ESI-MS (m/z) Expected: m/z – 621.22 Obtained: $[M+H]^+$ - 622.1918, $[M+Na]^+$ - 644.1735.

Synthesis of 1-DBCO: Compound **1** was synthesized according to our previous report.[4] DBCO-acid (45 mg, 0.149 mmol), EDC.HCl (28 mg, 0.149 mmol) and 4-dimethylaminopyridine (1.5 mg, 0.025 mmol) were dissolved in dichloromethane (DCM, 2 mL) and stirred at 0 ^{o}C for 30 minutes. To this reaction mixture, compound **1** (200 mg, 0.124 mmol) in DCM (2 mL) was added and stirred for 24 hours at room temperature under argon. The reaction mixture was diluted with 50 mL DCM and washed with 3x30 mL H_2O and 3x30 mL brine solution. The organic layer was collected and dried over anhydrous Na_2SO_4, concentrated and purified by column chromatography using acetone/DCM (20:80 v/v) solvent system as eluant to obtain compound **1-DBCO** (168 mg, 72% yield). ^{1}H NMR (400 MHz, $CDCl_3$)(δ ppm): δ 7.30-7.69 (m, DBCO aromatic 8H), 6.41-6.60 (oligomer aromatic protons, 11H), 4.95-5.19 (m, 4H), 4.91 (s, 4H), 3.35-4.11 (m, ethylene glycol 76H), 2.70-2.79 (m, 2H), 2.37-2.43 (m, 1H), 1.95-2.01 (m, 1H), 0.83-1.77 (m, aliphatic 61H). ^{13}C NMR (100 MHz, $CDCl_3$) (δ ppm): 172.70, 171.61, 160.44, 160.04, 159.06, 139.37, 132.23, 129.33, 128.55, 128.28, 128.15, 127.76, 127.10, 125.50, 110.16, 106.18, 105.68, 100.80, 71.93, 70.80, 70.62, 70.59, 70.54, 70.52, 70.44, 69.95, 69.70, 69.44, 68.85, 68.08, 67.43, 59.03, 31.90, 29.60, 29.58, 29.43, 29.33, 29.29, 26.08, 22.68, 14.13. ESI-MS (m/z) Expected: m/z – 1903.15 Obtained: $[M+Na]^+$ 1925.7262, $[M+2Na]^{2+}$ 974.3537.

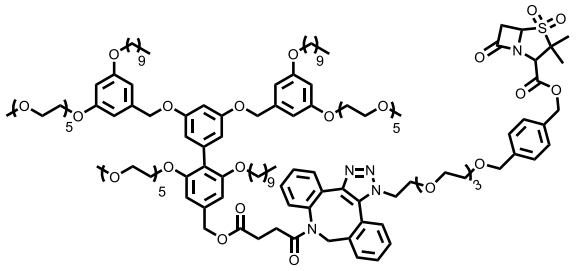
General procedure for strain promoted azide alkyne click (SPAAC) reaction

Compound **1-DBCO** (1 eq) and azide (1.2 eq) were dissolved in methanol and stirred at room temperature under inert condition. The reaction progress was monitored by TLC (typically 2-4 hours). The reaction mixture was concentrated and purified by silica-gel column chromatography.



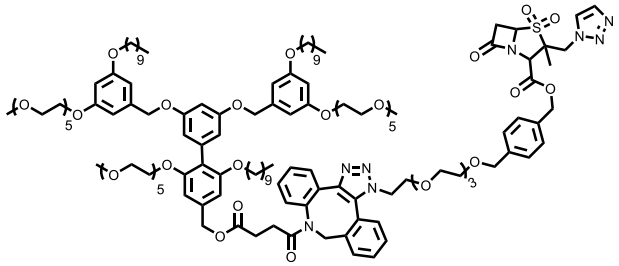
1-CA

Synthesis of 1-CA: According to the general procedure for SPAAC, compound **1-DBCO** (87 mg, 0.05 mmol) was treated with azide **2a** (29 mg, 0.06 mmol) in methanol (1 mL). Compound **1-CA** (102 mg, 83%) was obtained by purifying by column chromatography using acetone/DCM (20:80 v/v). ^{1}H NMR (400 MHz, $CDCl_3$)(δ ppm): δ 7.18-8.07 (m, 12H), 6.42-6.62 (m, 11H), 6.02-6.10 (t, 1H), 5.68-5.69 (d, 1H), 4.84-5.23 (m, 10H), 4.39-4.67 (m, 5H), 3.36- 4.22 (m, 98H), 3.05-3.09 (d, 1H), 2.15-2.71 (m, 5H), 1.19-1.80 (m, 58H), 0.84-0.91 (m, 10H). ^{13}C NMR (100 MHz, $CDCl_3$) (δ ppm): 172.63, 160.44, 160.04, 159.05, 157.22, 156.91, 139.36, 135.86, 129.87, 128.56, 127.99, 127.01, 126.92, 120.04, 118.74, 110.17, 107.75, 106.20, 105.70, 100.80, 71.93, 70.80, 70.62, 70.58, 70.52, 70.44, 69.96, 69.70, 69.43, 68.87, 68.08, 67.43, 59.03, 31.90, 29.59, 29.58, 29.43, 29.33, 29.31, 29.11, 26.08, 22.08, 22.68, 14.13. ESI-MS (m/z) Expected: m/z – 2423.37, Obtained: $[M+2Na]^{2+}$ 1234.4921.



1-SB

Synthesis of 1-SB: According to the general procedure for SPAAC, compound **1-DBCO** (60 mg, 0.03 mmol) was treated with azide **2b** (21 mg, 0.038 mmol) in methanol (1 mL). Compound **1-SB** (76 mg, 73%) was obtained by purifying by column chromatography using acetone/DCM (20:80 v/v). ^{1}H NMR (400 MHz, $CDCl_3$)(δ ppm): 7.17-7.84 (m, 12H), 6.43-6.63 (m, 11H), 6.07-6.11 (t, 1H), 5.03-5.30 (m, 4H), 4.93 (s, 4H), 4.31-4.62 (m, 7H), 3.37-4.14 (m, 95H), 2.16-2.66 (m, 6H), (m, 66H), 0.85-0.92 (m, 10H). ^{13}C NMR (100 MHz, $CDCl_3$) (δ ppm): 172.61, 171.41, 170.63, 166.82, 160.44, 160.04, 159.06, 139.36, 128.95, 128.01, 110.17, 106.20, 105.70, 100.80, 71.94, 70.63, 70.59, 70.54, 70.52, 70.47, 70.44, 69.71, 63.18, 62.75, 61.07, 59.01, 53.79, 51.77, 38.30, 31.90, 29.60, 29.58, 29.33, 29.31, 26.08, 22.68, 20.18, 18.60,14.13. ESI-MS (m/z) Expected: m/z – 2457.35, Obtained: $[M+Na]^{+}$ 2480.1468, $[M+2Na]^{2+}$ 1251.5754.



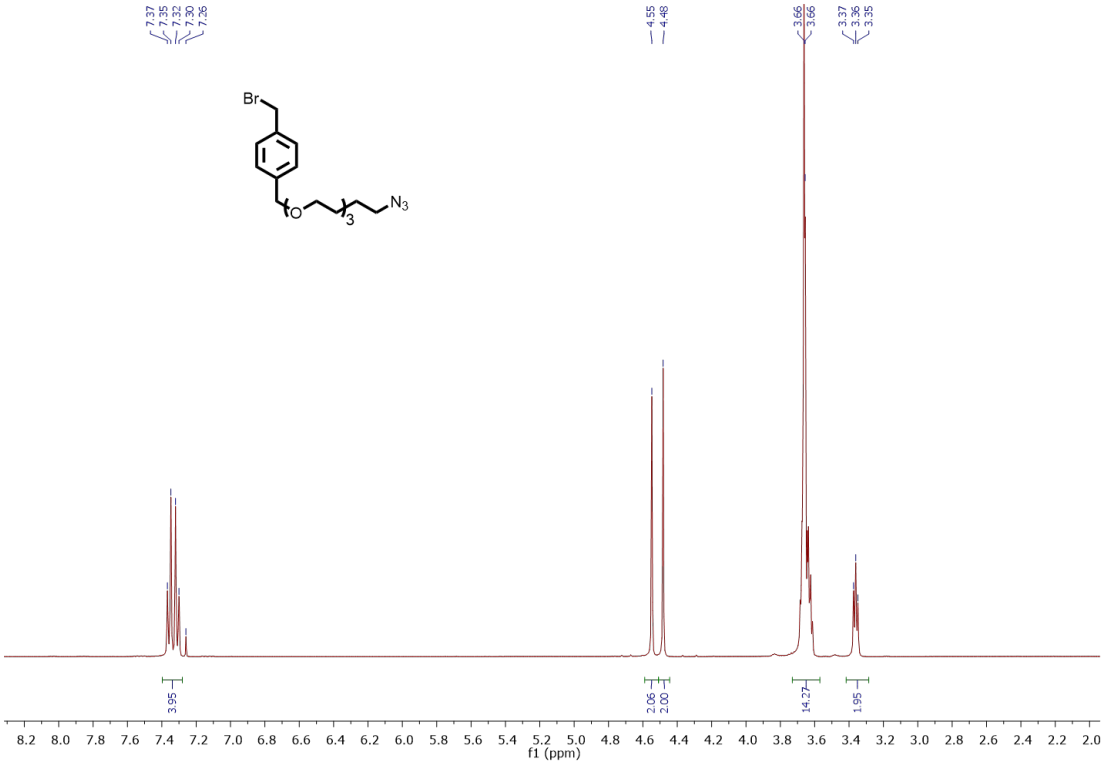
1-TB

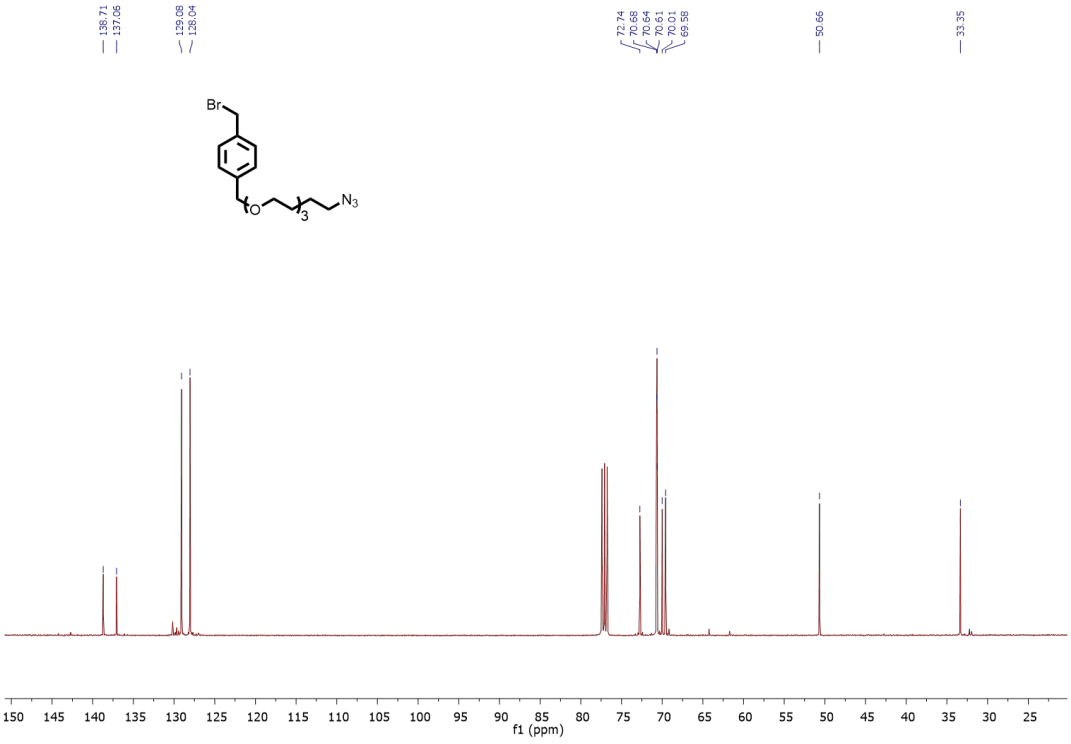
Synthesis of 1- TB: According to the general procedure for SPAAC, compound **1-DBCO** (67 mg, 0.035 mmol) was treated with azide **2c** (26 mg, 0.042 mmol) in methanol (1 mL). Compound **1-TB** (72 mg, 81%) was obtained by purifying by column chromatography using acetone/DCM (25:75 v/v). ^{1}H NMR (400 MHz, $CDCl_3$)(δ ppm): 7.19-7.85 (m, 12H), 6.43-6.63 (m, 11H), 6.03-6.10 (t, 1H), 5.27 (s, 2H), 5.01-5.11 (m, 4H), 4.93 (s, 4H), 4.28- 4.66 (m, 8H), 3.37-4.14 (m, 98H), 2.91-2.98 (d, 1H), 2.16-2.66 (m, 5H), 1.2-1.81 (m, 69H), 0.85-0.92 (m, 10H). ^{13}C NMR (100 MHz, $CDCl_3$) (δ ppm): 172.85, 171.48, 169.70, 165.88, 160.44, 160.04, 159.05, 156.91, 139.36, 134.32, 129.20,128.13, 125.68, 110.17, 106.20, 105.70, 100.80, 72.69, 71.94, 70.80, 70.62, 70.59, 70.47, 70.06, 69.96, 69.71, 69.44, 68.08, 68.69, 65.19, 62.58, 60.43, 59.01, 50.38, 39.11, 31.90, 29.60, 29.58, 29.44, 29.33, 29.31, 29.11, 26.08, 22.68, 15.93, 14.13. ESI-MS (m/z) Expected: m/z – 2523.37, Obtained: $[M+Na]^+$ 2546.8369, $[M+2Na]^{2+}$ 1284.9467.

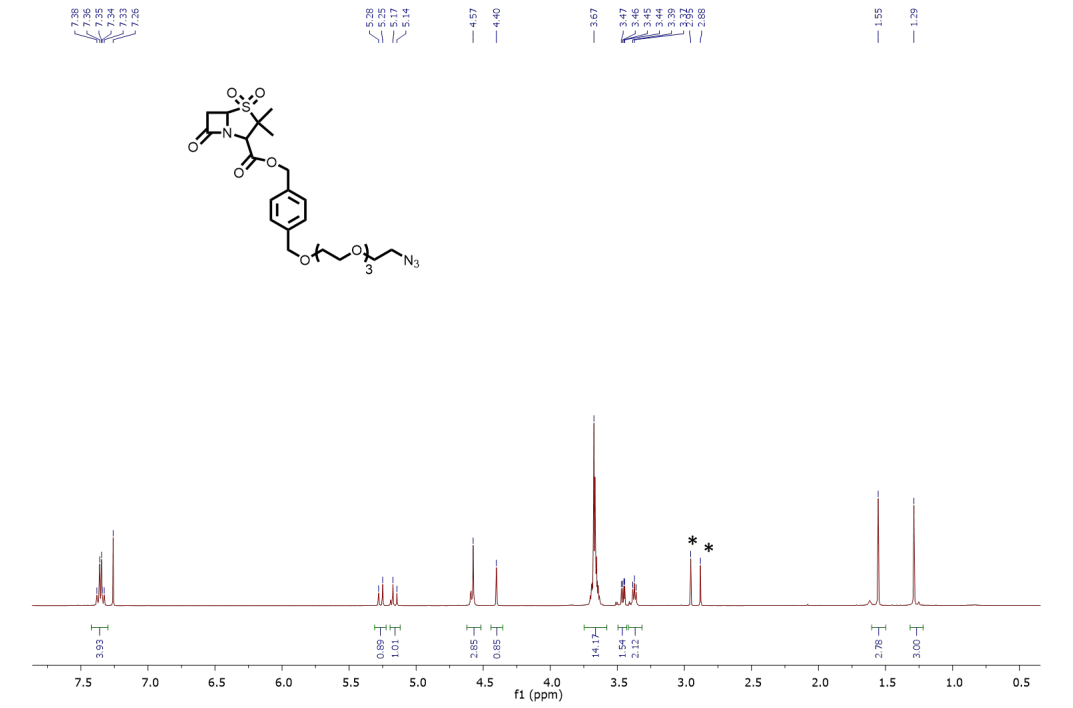
4. References

1. Y. Yang, K. Janota, K. Tabei, N. Huang, M. M. Siegel, Y. I. Lin, B. A. Rasmussen and D. M. Shlaes, *J. Biol. Chem.*, 2000, **275**, 26674-26682.
2. R. P. A. Brown, R. T. Aplin and C. J. Schofield, *Biochemistry*, 1996, **35**, 12421-12432.
3. R. U. Haque and J. N. Baldwin, *J. bacteriol.*, 1964, **88**, 1442-1447.
4. S. V. Aathimanikandan, E. N. Savariar and S. Thayumanavan, *J. Am. Chem. Soc.*, 2005, **127**, 14922-14929.

5. Spectral data



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1.29
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O
O
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N3
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0.85
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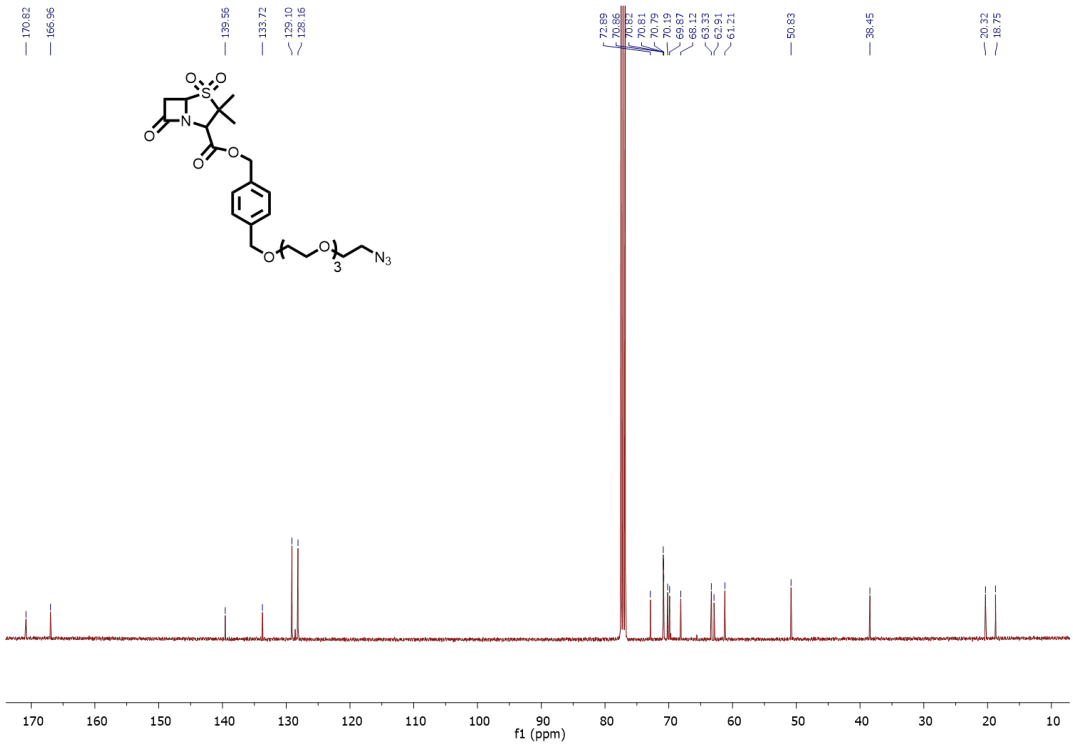
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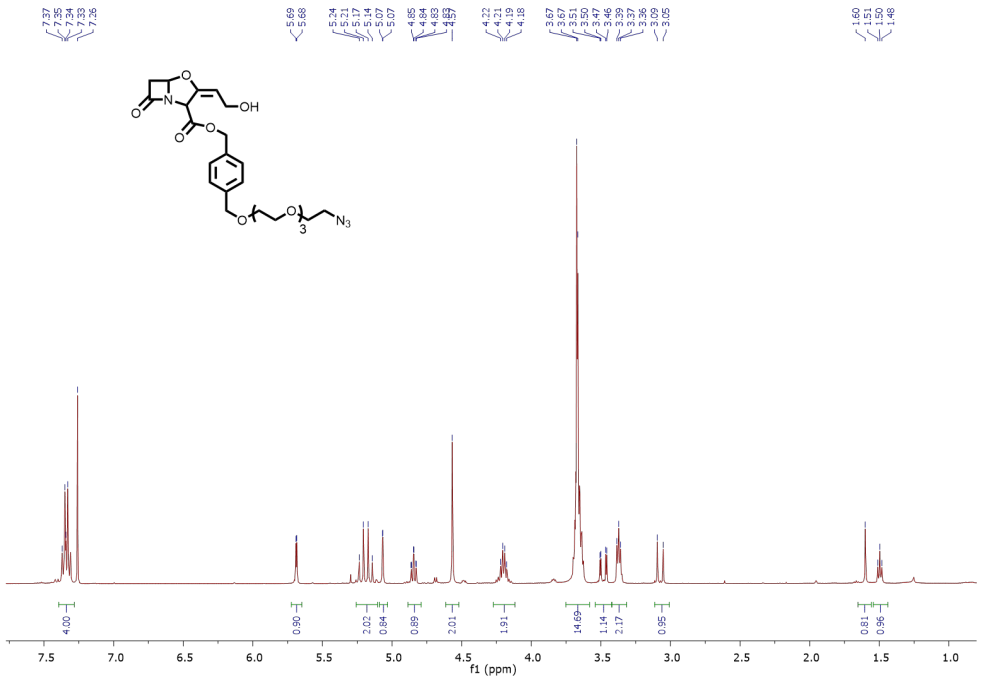
f1 (ppm)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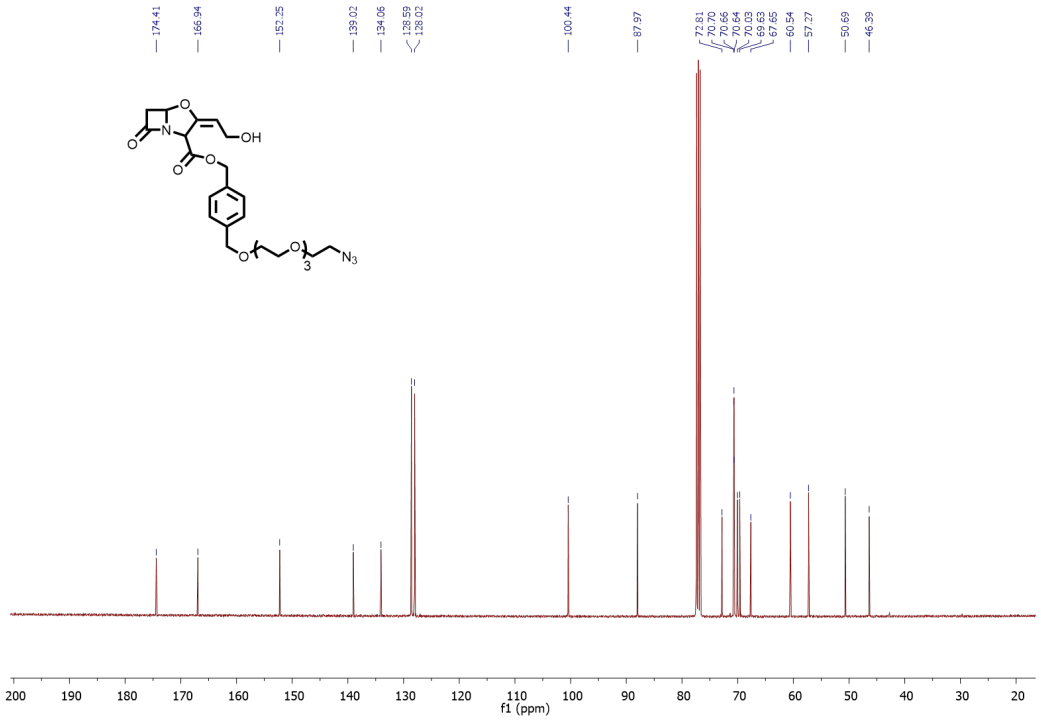
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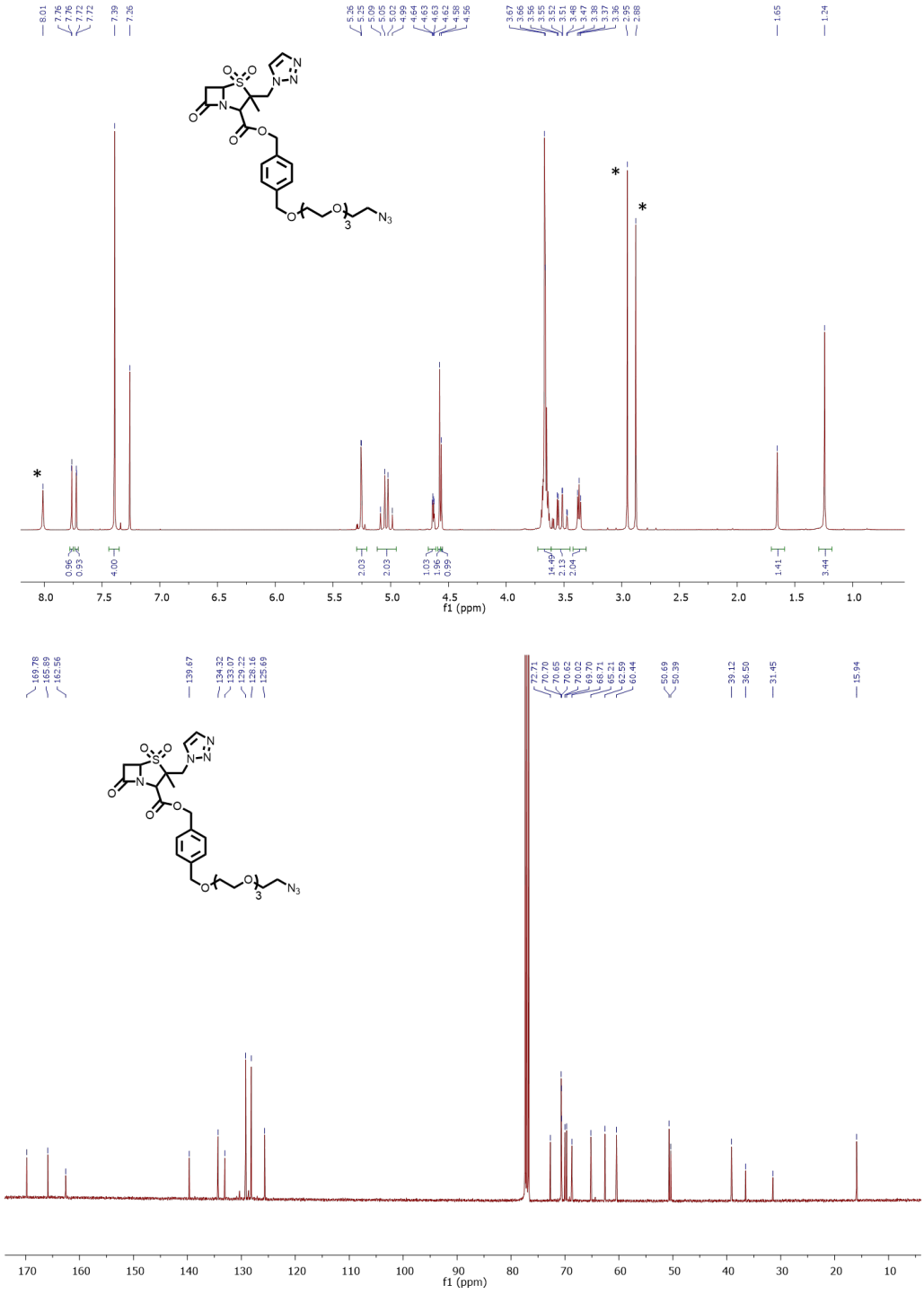

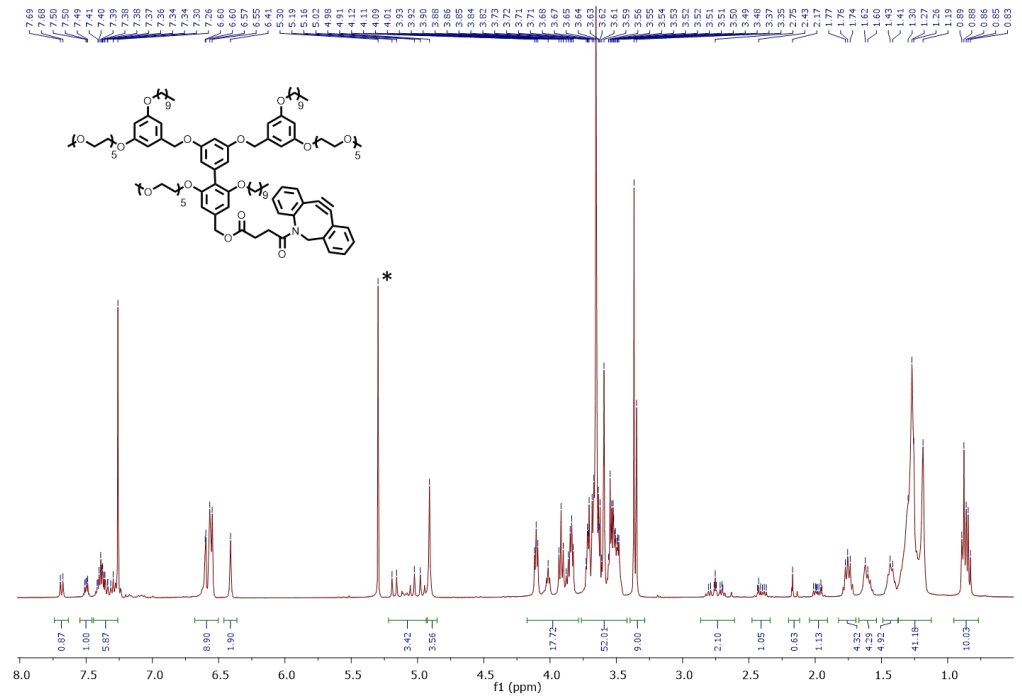
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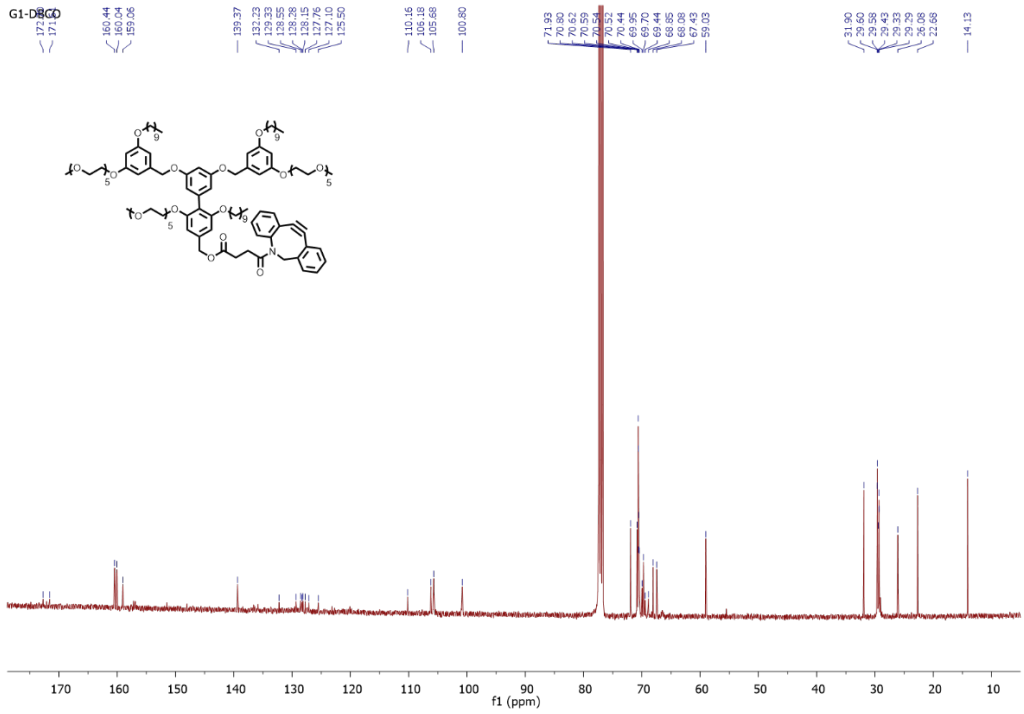
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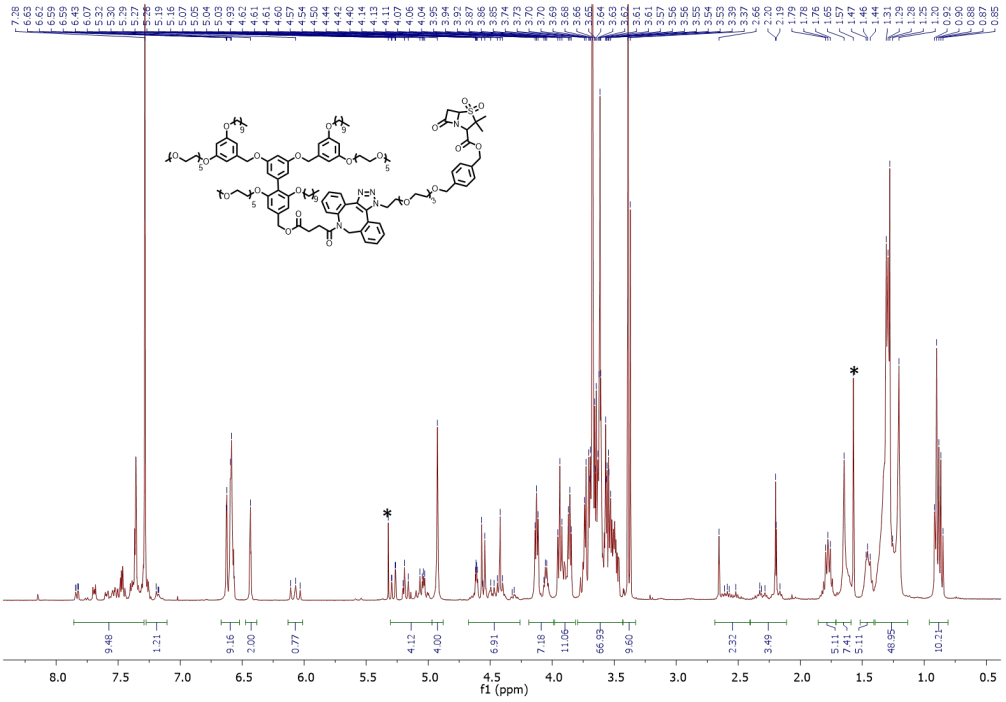
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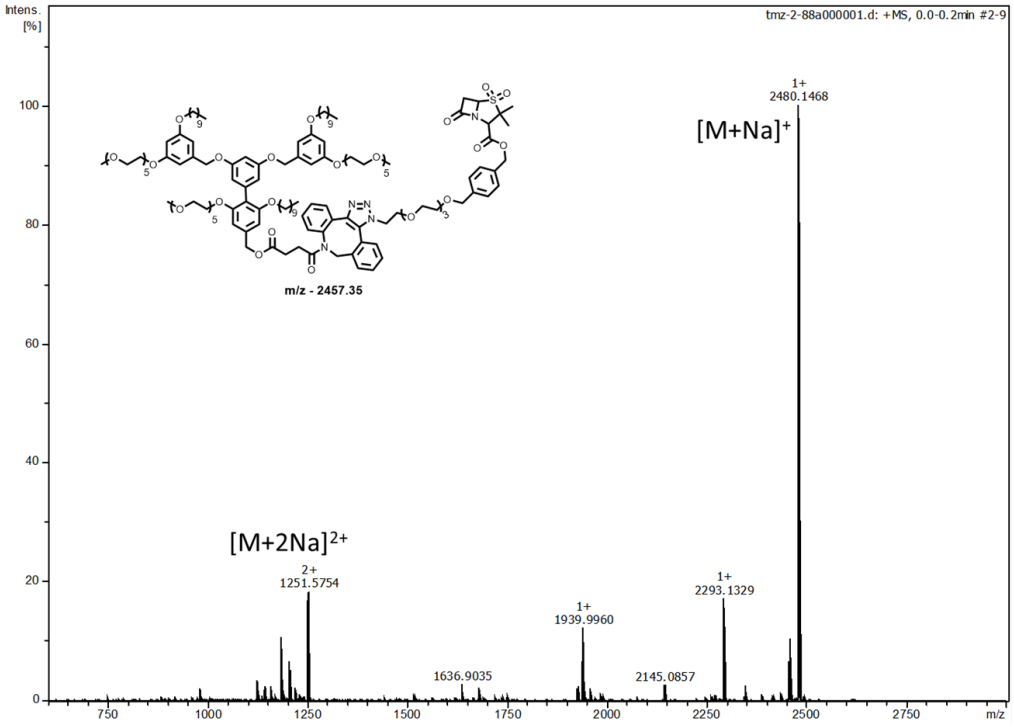
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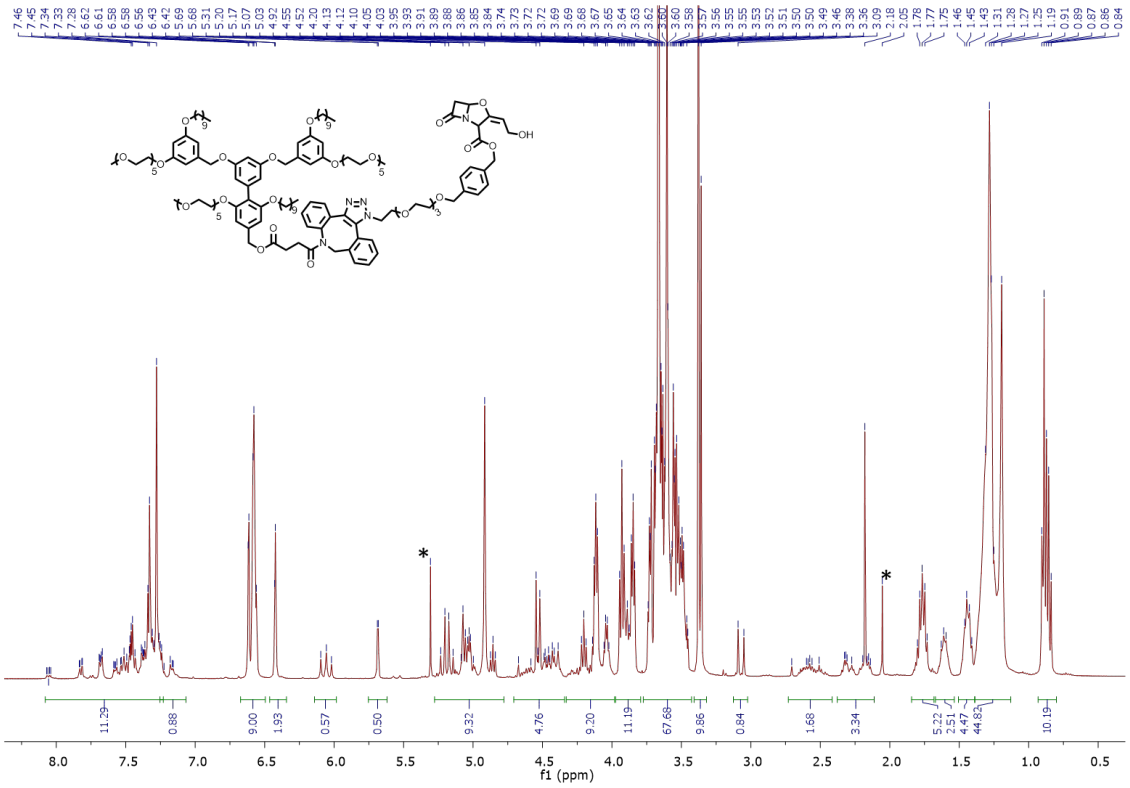
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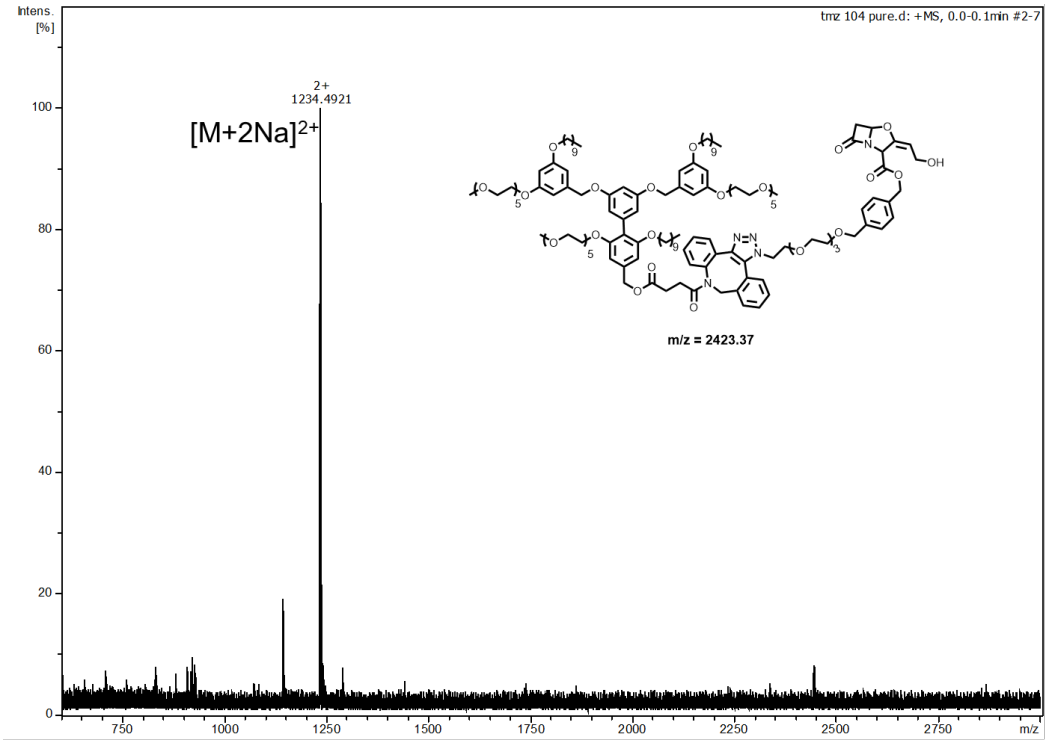
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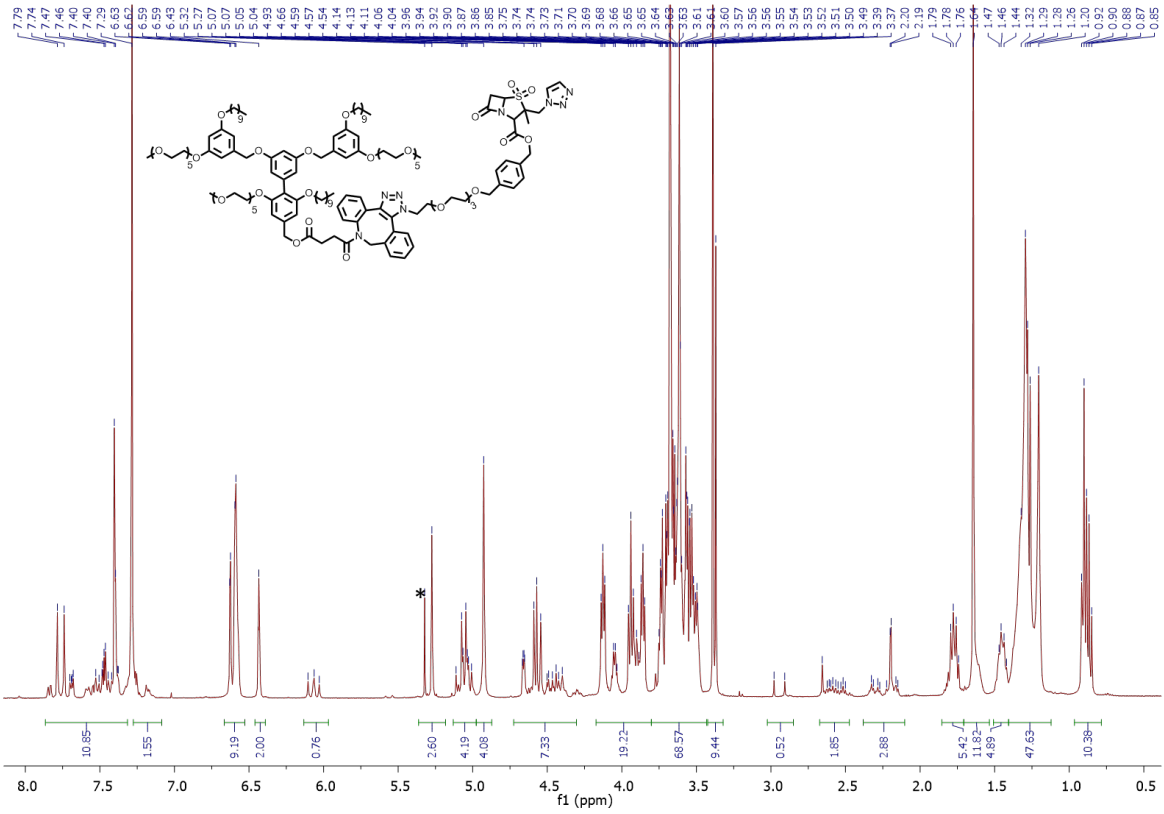
m/z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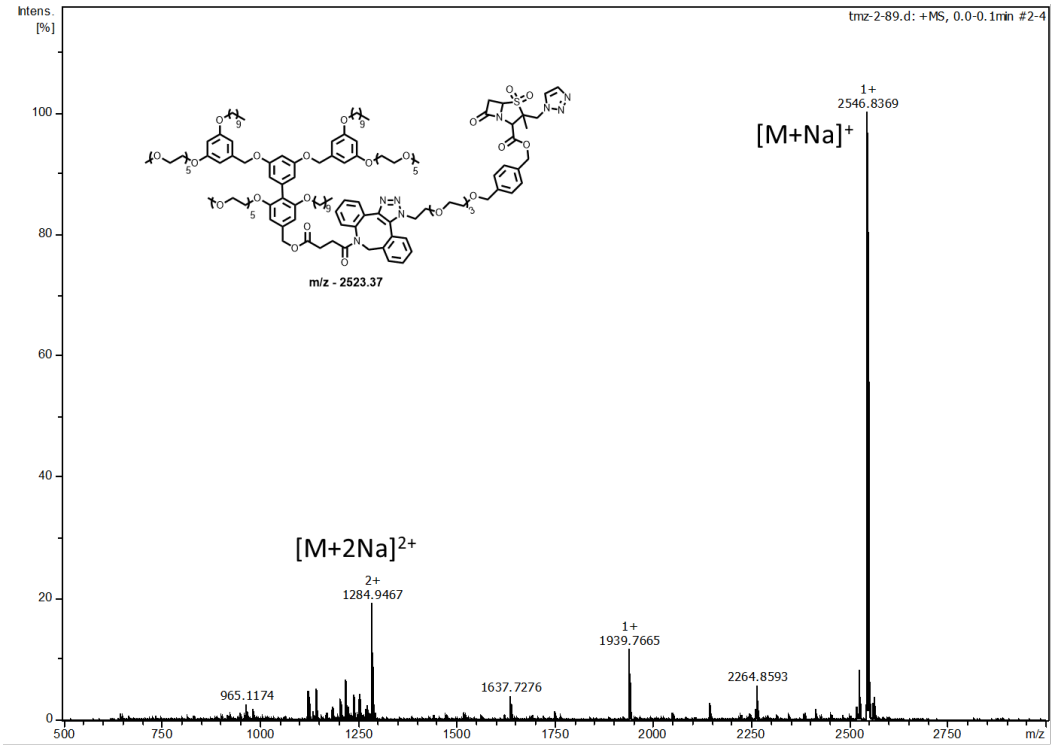
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