Activating a silver lipoate nanocluster with a penicillin backbone induces a synergistic effect against *S. aureus* biofilm.

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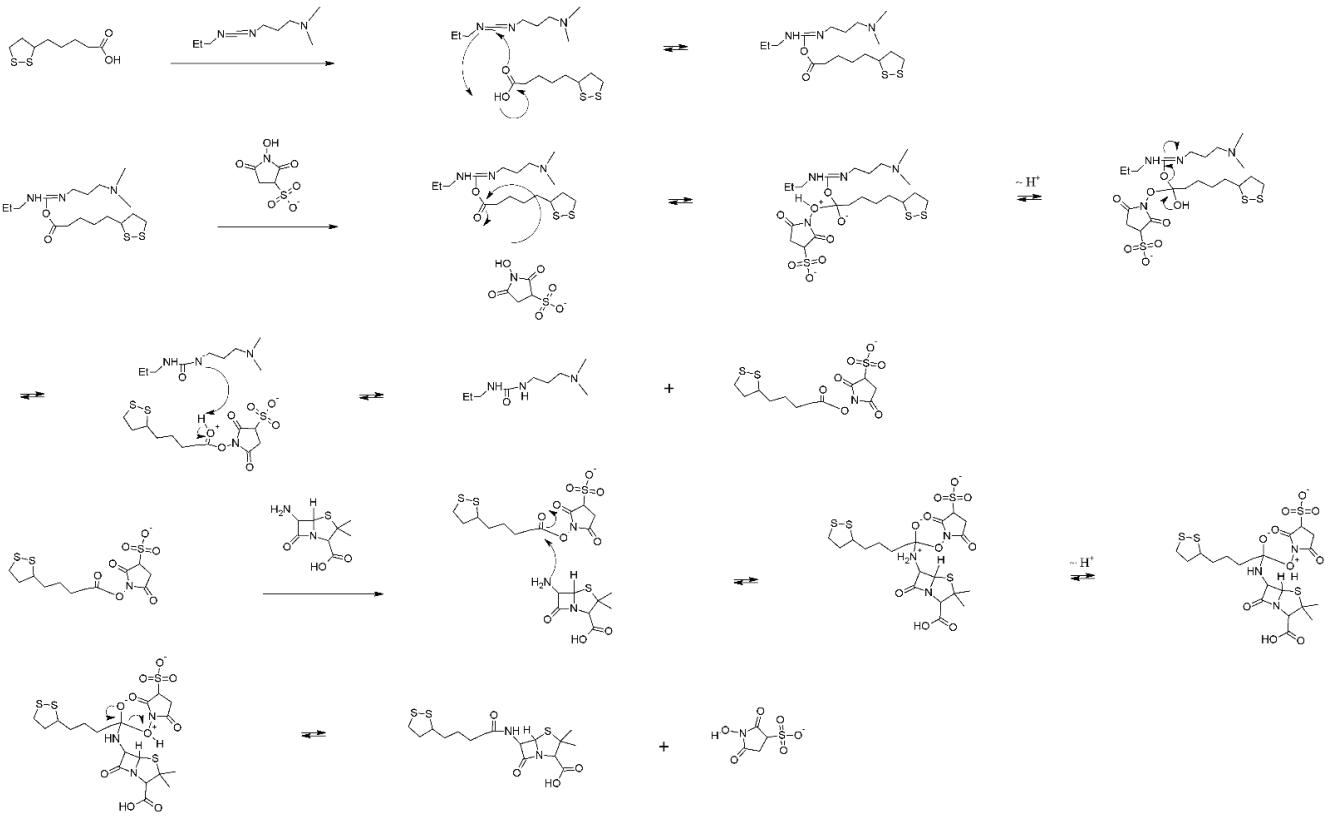
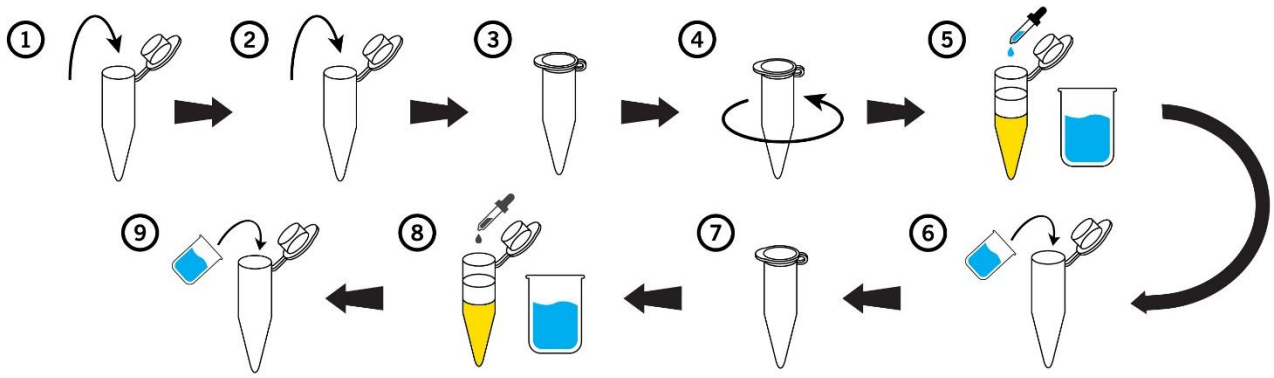
Summary of Conjugation Reaction & Reaction Workup

Key mechanistic steps are summarized in Scheme S1.



Scheme S1: Key mechanistic steps of conjugation. The presence of the cluster has been omitted for clarity.

In the first step, carried out in pH 6 buffer, an EDC ester intermediate is formed but not isolated. Simultaneous addition of sNHS results in the formation of a sNHS ester that precipitates out of solution. The sNHS ester is cleaned and then allowed to react with 6-APA in pH 7 buffer, resulting in the formation of the conjugated cluster. Scheme S2, summarizes various reaction steps performed in a 2 mL microcentrifugation tube.



Scheme S2: Key steps in cluster conjugation.

Step 1. Start with 1,0 mL of 500 mM MES buffer solution.

Step 2: Add
a) 50 µL of cluster solution (1.4 mg Ag/mL concentration estimated to contain a maximum of 3 µmol RALA in total)
b) 20 mg free-base EDC (130 µmol)
c) 2 mg sNHS (13 µmol)

Step 3: React for 60 minutes

Step 4: Centrifuge at 2000 rpm for five minutes to precipitate less soluble sNHS- cluster. Discard supernatant.

Step 5: Wash precipitate twice with 500 µL of distilled water.

Step 6: Dissolve precipitate in 1 mL of 1 M TEAA solution or 250 mM MES buffer

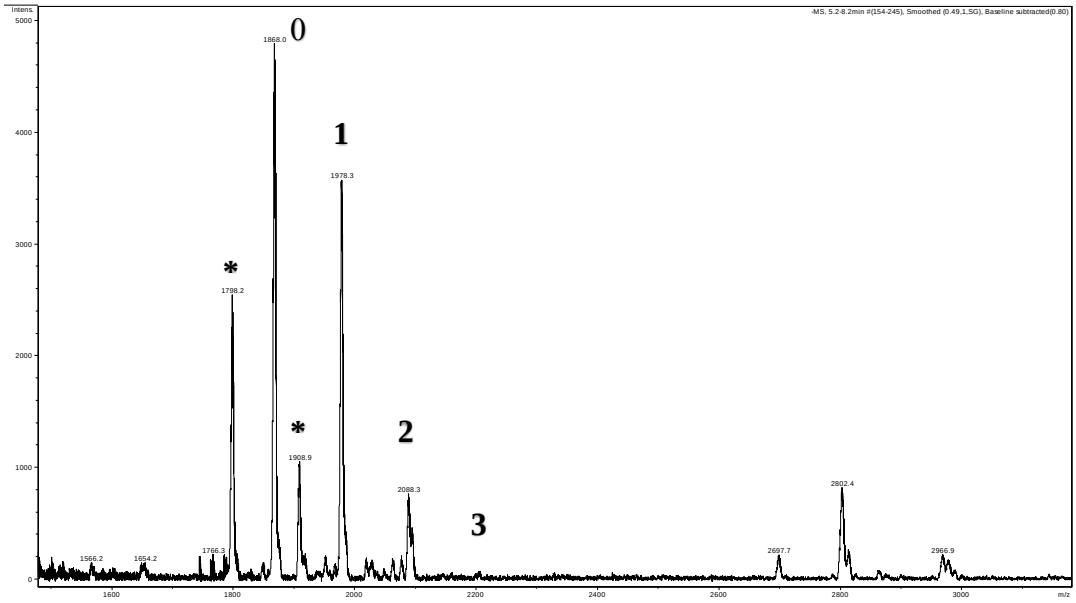
Step 7: React for 60 minutes.

Step 8: Centrifuge at 2000 rpm for five minutes to precipitate less soluble conjugated cluster. Discard supernatant. Wash precipitate four times with 500 µL of distilled water.

Step 9: Dissolve conjugated product in 500 µL 50 mM TEA solution for ESI-MS analysis or suitable buffer for antibiotic testing.

Ampicillin Conjugation

Results of conjugating the $(Ag_{29}LA_{12})^{[3-]}$ cluster to ampicillin are summarized in Figure S1. The conjugate was obtained by substituting molar equivalents of ampicillin for 6-APA in the synthetic protocol.



m/z

Figure S1: ESI-MS evidence of conjugation of Ampicillin to the $(Ag_{29}LA_{12})^{[3-]}$ Cluster. The spectrometer was operated in negative mode. In addition to the triply charged signals, fragmentation products (*) of the electro-spray ionization process are apparent.

Glycine ethyl ester Conjugation

Liquid chromatography (LC) experiments were performed on an Eksigent nanoLC 2D system coupled to a Bruker micrOTOF time-of-flight mass spectrometer (MS). All separations were carried out using an Ace 300Å C18 HPLC column (0.5 mm x 150 mm, 3 µm particle size)

(Advanced Chromatography Technologies Limited, Aberdeen, UK) maintained at ambient laboratory temperature. Mobile phases were prepared 400 mM hexfluoroisopropanol (HFIP) -

15 mM triethylamine (TEA) in ddH2O (mobile phase A) and neat methanol (mobile phase B). All solvents for direct infusion and LC-MS were obtained from Fisher Scientific (Fairlawn, NJ). The flow rate used for all experiments was ten microliters per minute (µL/min). Injections – 5.0 µL – were carried out by an Eksigent AS-1 autosampler configured with a 20-µL sample loop.

All reaction mixture samples were diluted 20x in mobile phase A. Direct infusion was carried out by loop injection (i.e., no column between autosampler and mass spectrometer) using a mobile phase composition of 95% MP A: 5% MP B.HPLC experiments were carried out using twenty-minute linear gradient methods with varied starting and ending mobile phase conditions.

After completion of the twenty-minute gradient, 100% methanol was rinsed through the column to remove any non-polar components for five minutes. This was then followed by a twenty minute re-equilibration at initial method conditions. Mass spectrometer acquisition settings were identical for both direct infusion and LC-MS experiments. Data was acquired from m/z 100 -

6,000. Ten-thousand spectra were summed per spectrum acquired. Nebulizer pressure was set to 4.0 bar. Nitrogen sheath gas was set to zero L/min. The endplate offset and capillary potentials were held at -1000 V and 3500 V, respectively. Capillary exit and skimmer voltage settings were -100 V and -33 V respectively. Lens 1 pre-pulse storage and transfer times were 35 µs and 140 µs, respectively. MCP detector voltage was increased to 2350 V (from 2100 V standard) for improved detection.

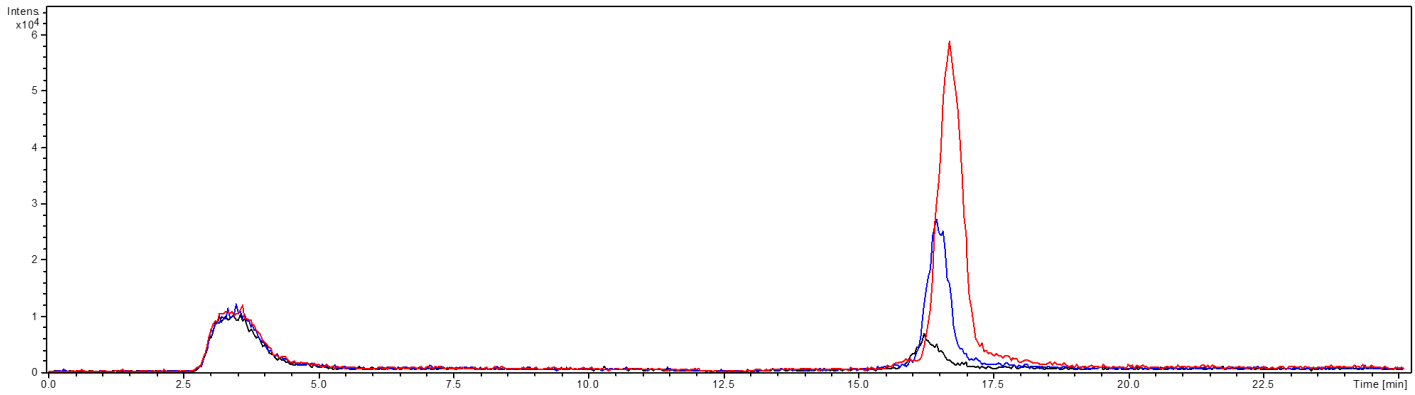


Figure S2: Overlaid LC-MS Base Peak Chromatogram Traces – Analysis of the Ag29LA12 reaction mixture. Red Trace= Ag29(LA)12. Blue Trace = Ag29(Lipoic Acid)11(Lipoic Acid-Glycine)1. Black Trace = Ag29(Lipoic Acid)10(Lipoic Acid-Glycine)2

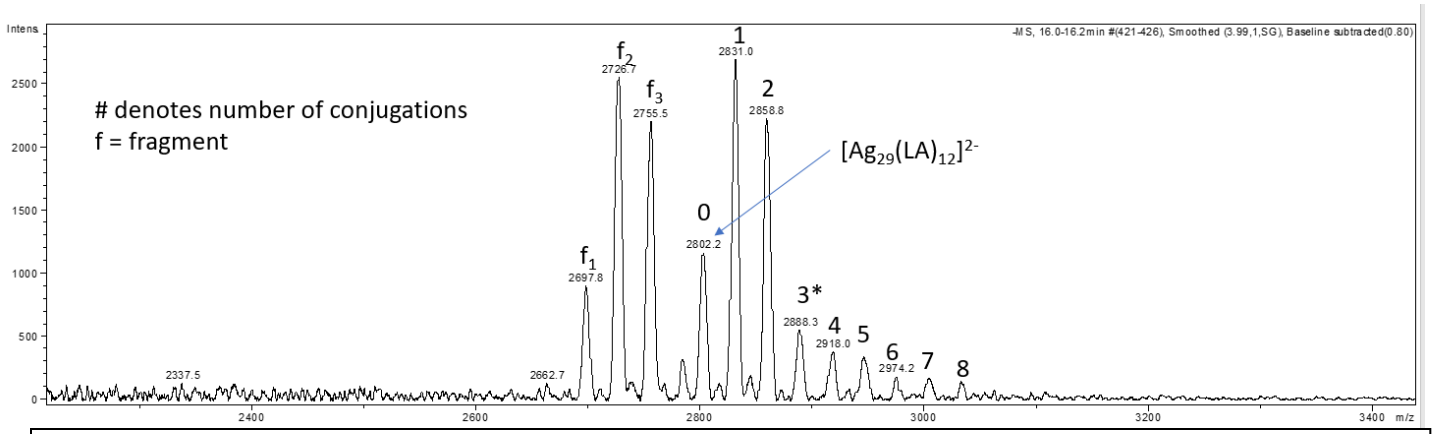


Figure S3: Averaged mass spectrum from under Ag29(Lipoic Acid)10(Lipoic Acid-Glycine)2 chromatographic peak (black trace in Figure S2)

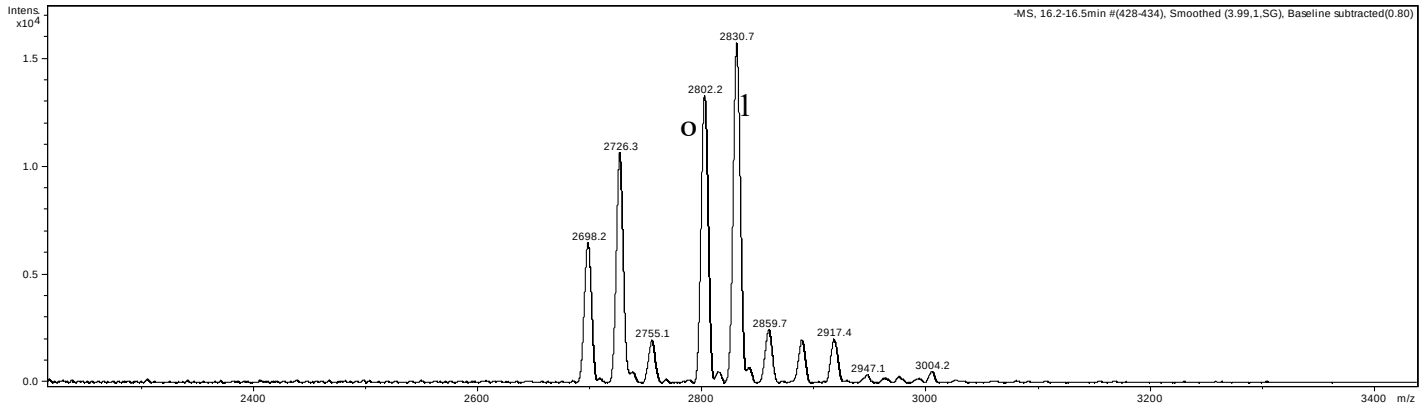


Figure S4: Averaged mass spectrum from under Ag29(Lipoic Acid)11(Lipoic Acid-Glycine)1 chromatographic peak (blue trace in Figure S2)

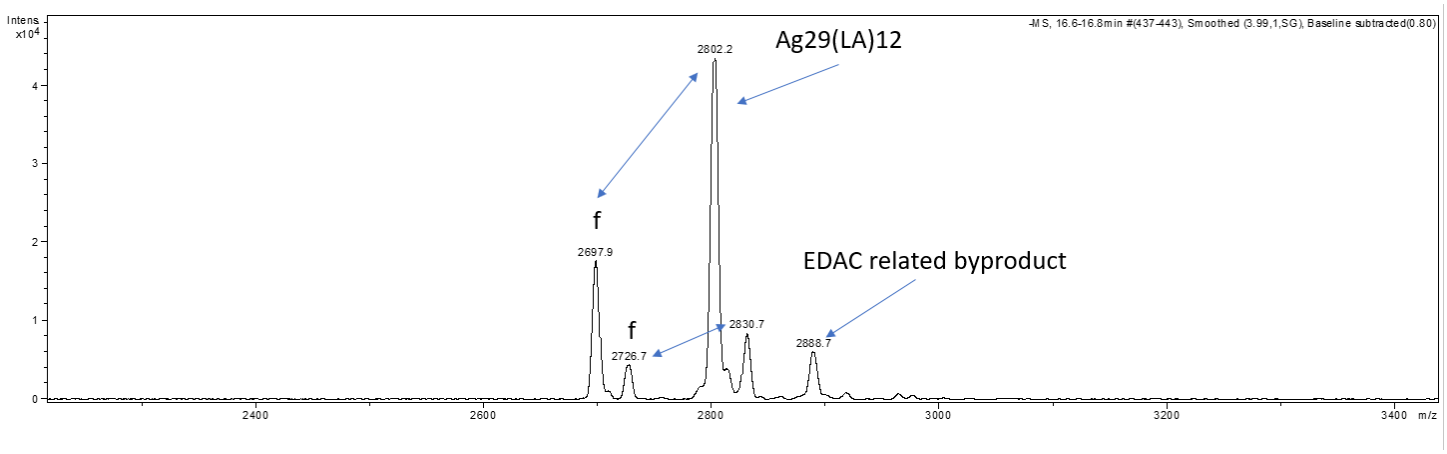


Figure S5: Averaged mass spectrum from under Ag29(LA)12chromatographic peak (red trace in Figure S2)

Summary of Synergy Evaluation

Table S1. Synergistic or antagonistic effect based on the dose effect curves.

Dose 6-APA µM	Biofilm Inhibition 6-APA (%)*	Dose Ag29 µM	Biofilm Inhibition Ag29 (%)*	Dose µM 6-APA + Ag29 = Conjugate µM	Biofilm Inhibition Conjugate (%)*	Fa	CI[b]
93	0	0.6	0	0.023 + 0.0086 = **0.032**	**3**	0.03	4.43E-04
185	0.1	1.6	0	0.047 + 0.0173 = **0.064**	**6**	0.06	5.89E-04
370	2	3.2	0	0.117 + 0.0432 = **0.160**	**13**	0.13	9.15E-04
741	9	6.4	1	0.233 + 0.0864 = **0.320**	**17**	0.17	0.00153
1481	11	12.8	1	0.466 + 0.1727 = **0.639**	**22**	0.22	0.00257
3009	16	25.6	4	0.933 + 0.3455 = **1.278**	**33**	0.33	0.00376
6019	28	51.1	15	1.866 + 0.6910 = **2.557**	**40**	0.40	0.00635
12037	54	102.3	43	3.731 + 1.3820 = **5.113**	**61**	0.61	0.00788
24074	75	207.7	74	7.463 + 2.7639 = **10.227**	**81**	0.81	0.00904
48148	87	415.5	81	15.158 + 5.6143 **=20.773**	**90**	0.90	0.01213

[a] The data of the dose–effect inhibition (%) was obtained from a phenotypic luciferase assay and were generated by the Origin software. Data are presented as the mean of three independent assays in duplicates. [b]Fraction affected CI (Fa-CI) plots was generated using CompuSyn software. CI=1 means additive effect, CI >1 is antagonistic effect, and CI <1 means synergy (red numbers).

Table S2. Median dose (IC50) and other parameters reported by curve-fitting the phenotypic assay results (by the CompuSyn program)

Drug/Combo	Median Dose µM	Kinetic Parameter, m	Quality of Fit R^2
6-APA	9989	1.8	0.99
Ag29	142	1.4	0.96
Conjugated Cluster	2.31	0.81	0.99