A Methyl 4-Oxo-4-phenylbut-2-enoate with *in Vivo* Activity against MRSA that Inhibits MenB in the Bacterial Menaquinone Biosynthesis Pathway

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Table S1: Comparison of theoretical vs observed ion isotopic distribution of **8**.

Table S2: Thermodynamic constants for the binding of CoA and CoA adducts **7** and **8** to *sa*MenB.

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Figure S2: 4-Oxo-4-phenylbutanoic acids are able to mimic the transition state of the MenB reaction by forming CoA adducts through a Michael addition reaction.

Figure S3: Menaquinone quantification in MRSA treated with ½ MIC of anti-bacterial agents.

Figure S4: Isothermal titration calorimetry (ITC) binding thermographs.

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Figure S6: LC/ESI^+ chromatograms for DHNA standard.

Figure S7: NMR and mass spectrum of compound **8**.

Table S1: Comparison of theoretical vs observed ion isotopic distribution of **8.** Molecular formula: $C_{32}H_{45}ClN_7O_{19}P_3S$. $[M-H]^-$ m/*z* = 990.1315 and $[M-2H]^{-2}$ m/*z* = 494.5608.

Theoretical		Observed		
m/*z*	Abundance (%)	m/*z*	Abundance	Abundance (%)
990.1320	100	990.1315	1014.79	100.00%
991.1349	39.19	991.1335	347.95	34.29%
992.1308	47.86	992.1301	392.43	38.67%
993.1329	16.71	993.1325	37.73	3.72%
994.1325	6.03			
Theoretical		**Observed**		
m/*z*	Abundance (%)	m/*z*	Abundance	Abundance (%)
494.5623	100	494.5609	2451.61	100.00%
495.0638	39.18	495.0630	1003.83	40.95%
495.5618	47.85	495.5604	1145.04	46.71%
496.0628	16.71	496.0626	386.26	15.76%
496.5626	6.03	496.5613	28.86	1.18%

Table S2: Thermodynamic constants for the binding of CoA and CoA adducts **7** and **8** to *sa*MenB

	CoA	**7**	**8**
K_d (μM)	25.4	2.8	2.8
ΔH^1	-12.9	-25.6	-2.55
$T\Delta S^1$	-6.7	-18.1	4.93
ΔG^1	-6.2	-7.5	-7.5
$\Delta\Delta G^{1a}$		1.3	1.3
H-bond[b]		+ 1	+1

[1]kcal/mol. [a]Relative to **CoA** ΔG. [b]Predicted hydrogen bond formed relative to CoA binding where 1 H bond = 1.4 kcal/mol.

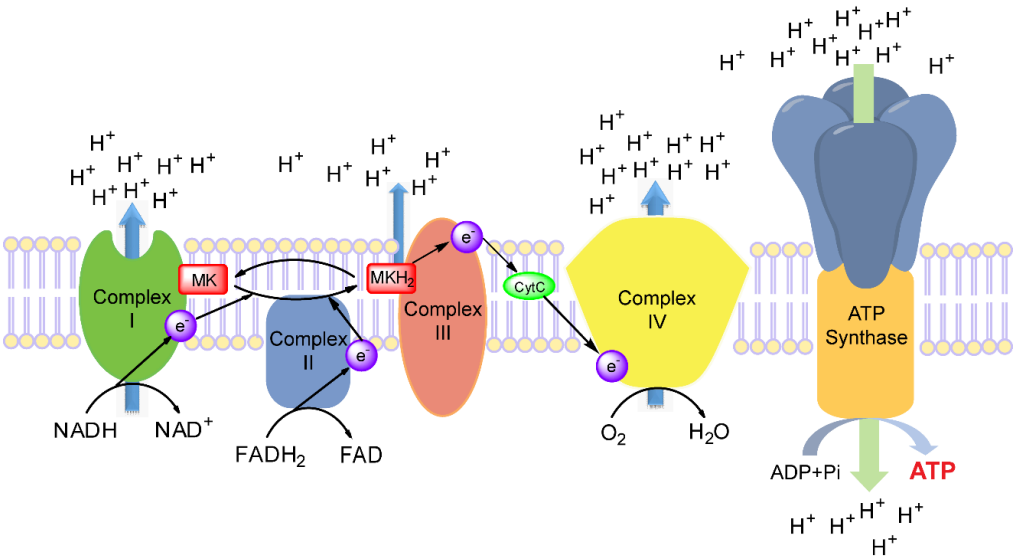

Figure S1: Role of Menaquinone (MK) in the Electron Transport Chain. MK (vitamin K2) is a lipid soluble redox active cofactor that shuttles reducing equivalents between components of the electron transport chain in human pathogens such as *S. aureus*. In *S. aureus*, MK functions as an electron carrier during both oxidative and fermentative respiration.

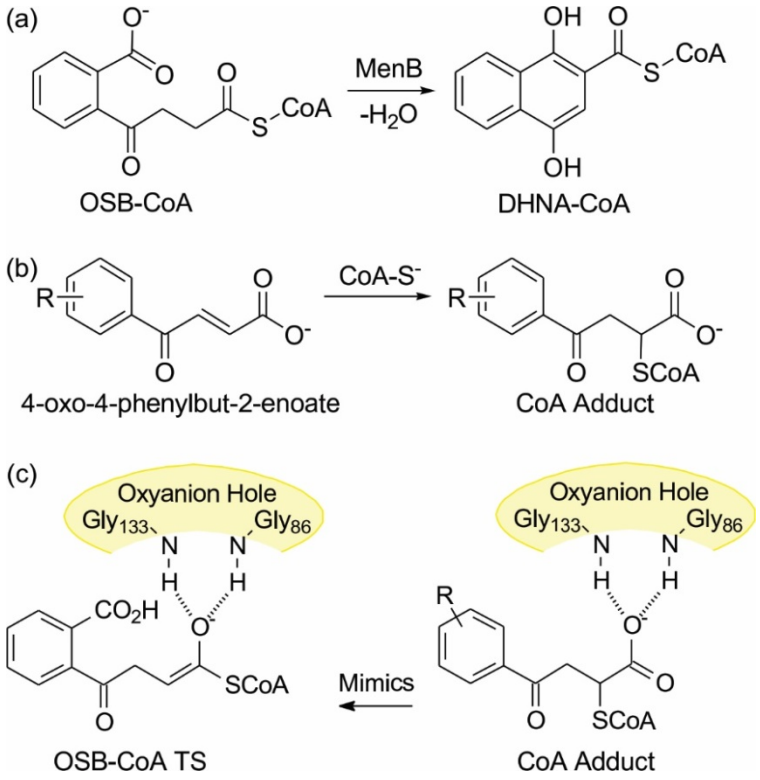

Figure S2: 4-Oxo-4-phenylbutanoic acids are able to mimic the transition state of the MenB reaction by forming CoA adducts through a Michael addition reaction. (**a**) MenB (1,4-dihydroxynaphthoyl-CoA synthase) catalyzes an intramolecular Dieckmann condensation leading to the formation of 1,4-dihydroxynaphthoyl-CoA (DHNA-CoA) from o-succinyl-benzoyl-CoA (OSB-CoA). (**b**) 4-oxo-4-phenylbut-2-enoates react with CoA to form adducts. (**c**) Adducts are transition state (TS) analogs of the MenB reaction in which the carboxylate binds in the MenB oxyanion hole (*E. coli* MenB numbering). This figure also shows the general structure of CoA adduct **7**.[46]

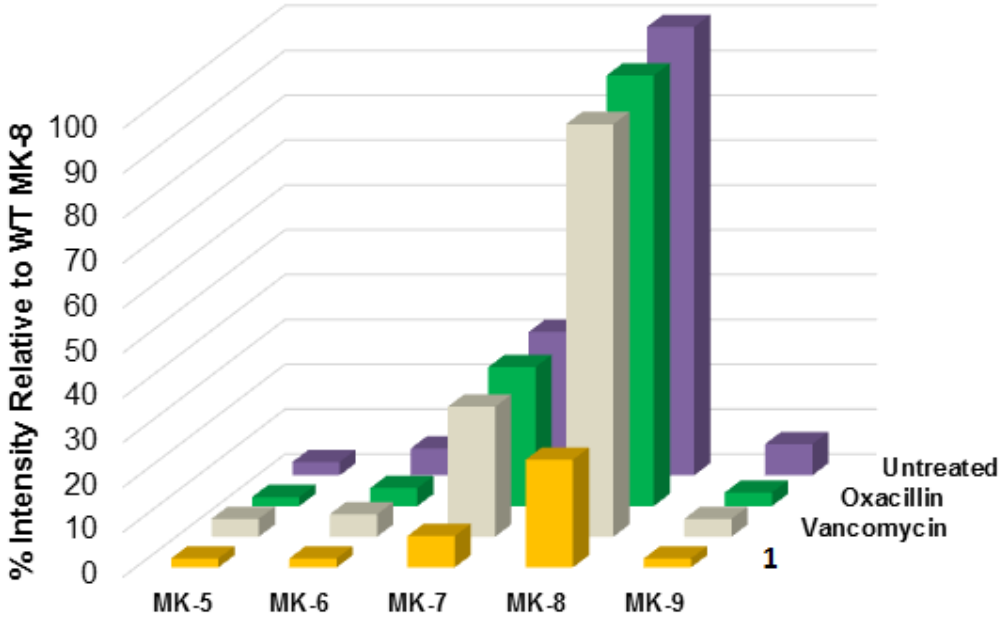

Figure S3: Menaquinone quantification in MRSA with or without anti-bacterial agents: 0.38 µg/mL of **1**, 2 µg/mL vancomycin, and 100 µg/mL oxacillin. 10^9 cells per sample were analyzed for their change in MK levels. Each MK species is presented as a percentage (y-axis) where MK-8 = 100% in untreated MRSA. Vancomycin and oxacillin were also evaluated to determine if MK levels change upon treatment with these drugs.

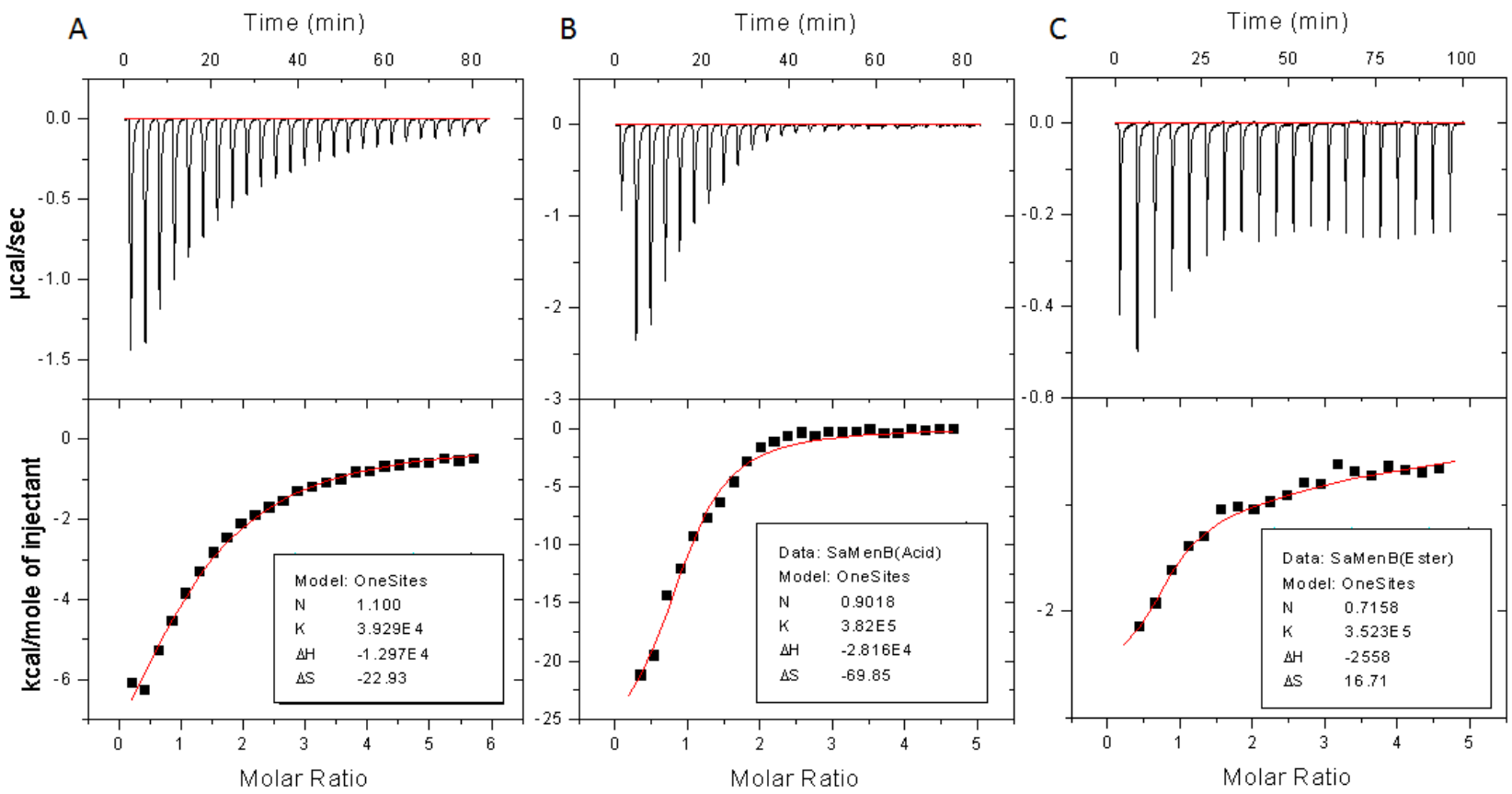

Figure S4: Isothermal titration calorimetry (ITC) binding thermographs. *sa*MenB was titrated with **(A)** CoA, **(B) 7**, and **(C) 8** at 22 °C. Data were analyzed using a 1-site binding model. Ligands were prepared as a stock solution (2 mM) in the same buffer as that used for the protein (20 mM sodium phosphate pH 8.5, 250 mM NaCl, and 1 mM $MgCl_2$). In each case 4-8 µL aliquots of ligand were titrated into the ITC cell containing 1.8 mL of 25-50 µM purified *sa*MenB. The titration period was 8-16 s with 300 s between each titration.

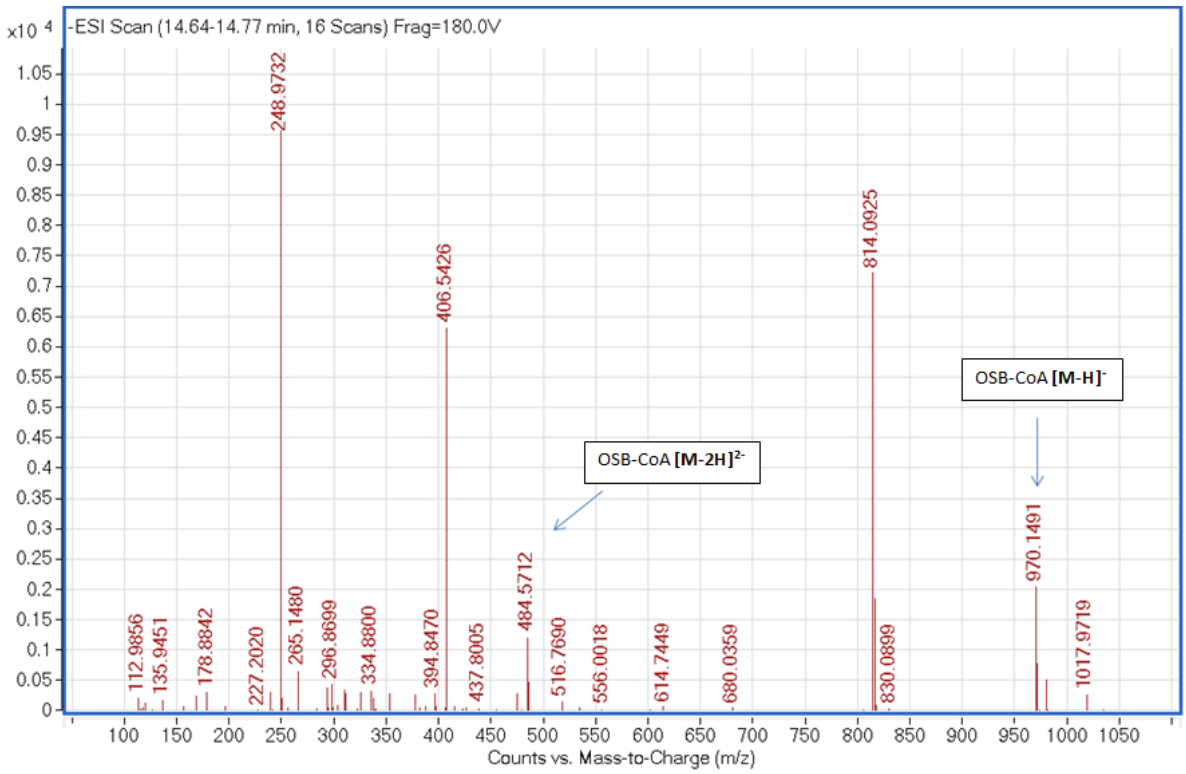

Figure S5: High Resolution LC/UV/MS ESI$^-$ of OSB-CoA. OSB-CoA m/*z* = 971.1491 was enzymatically made by incubating MenE with OSB, ATP, and CoA for 1hour and filtering the product out. Observed peaks correspond to OSB-CoA EIC: $[M-2H]^{2-}$ m/*z* = 494.5711, $[M-H]^-$ m/*z* = 970.1490, 971.1525. Extracts from **1** treated MRSA did not show accumulation of OSB-CoA.

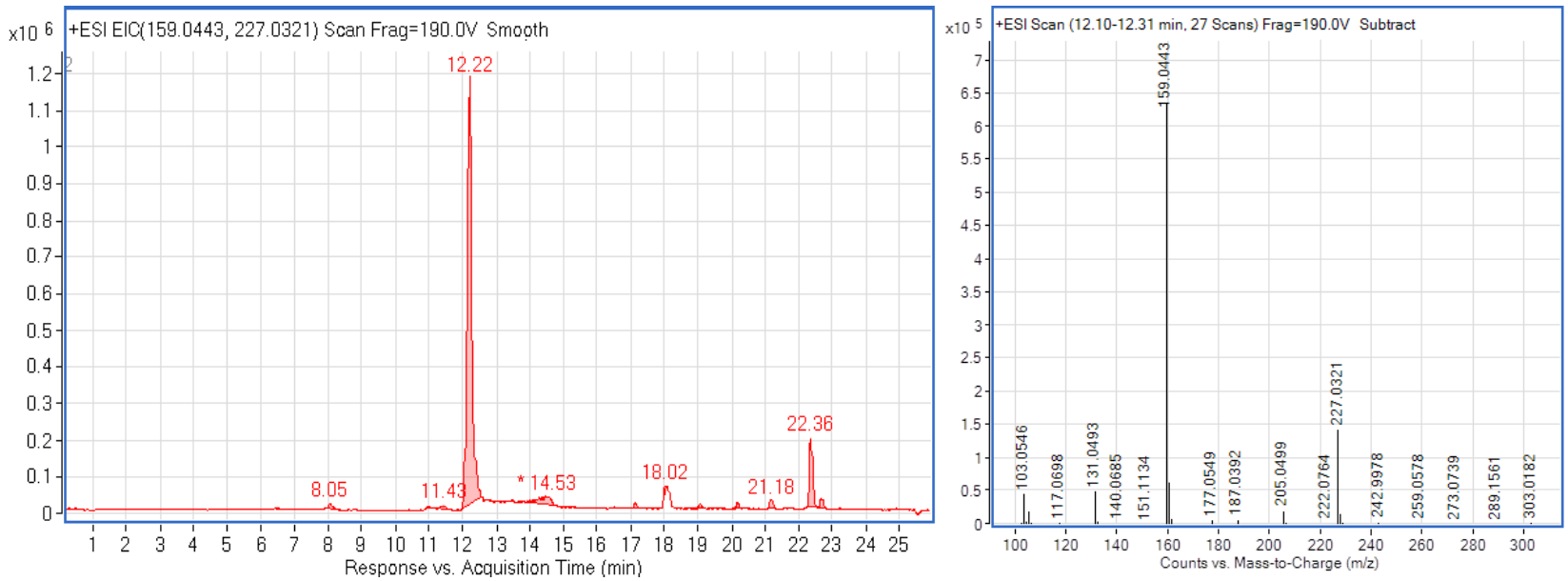

Figure S6: LC/ESI$^+$ chromatograms for DHNA standard. The extracted mass spectra from the peak with retention time of 12.22 min shows that DHNA undergoes facile MS fragmentation with the loss of CO_2 to give a m/*z* =159.0442 ion. Other indicative ions are m/*z* = 227.0321, $[M+Na]^+$ and fragment ions, m/*z*=131.0493 (CO loss) and 103.0546 (CO loss). The extracted UV spectra show an UV max of 275nm.

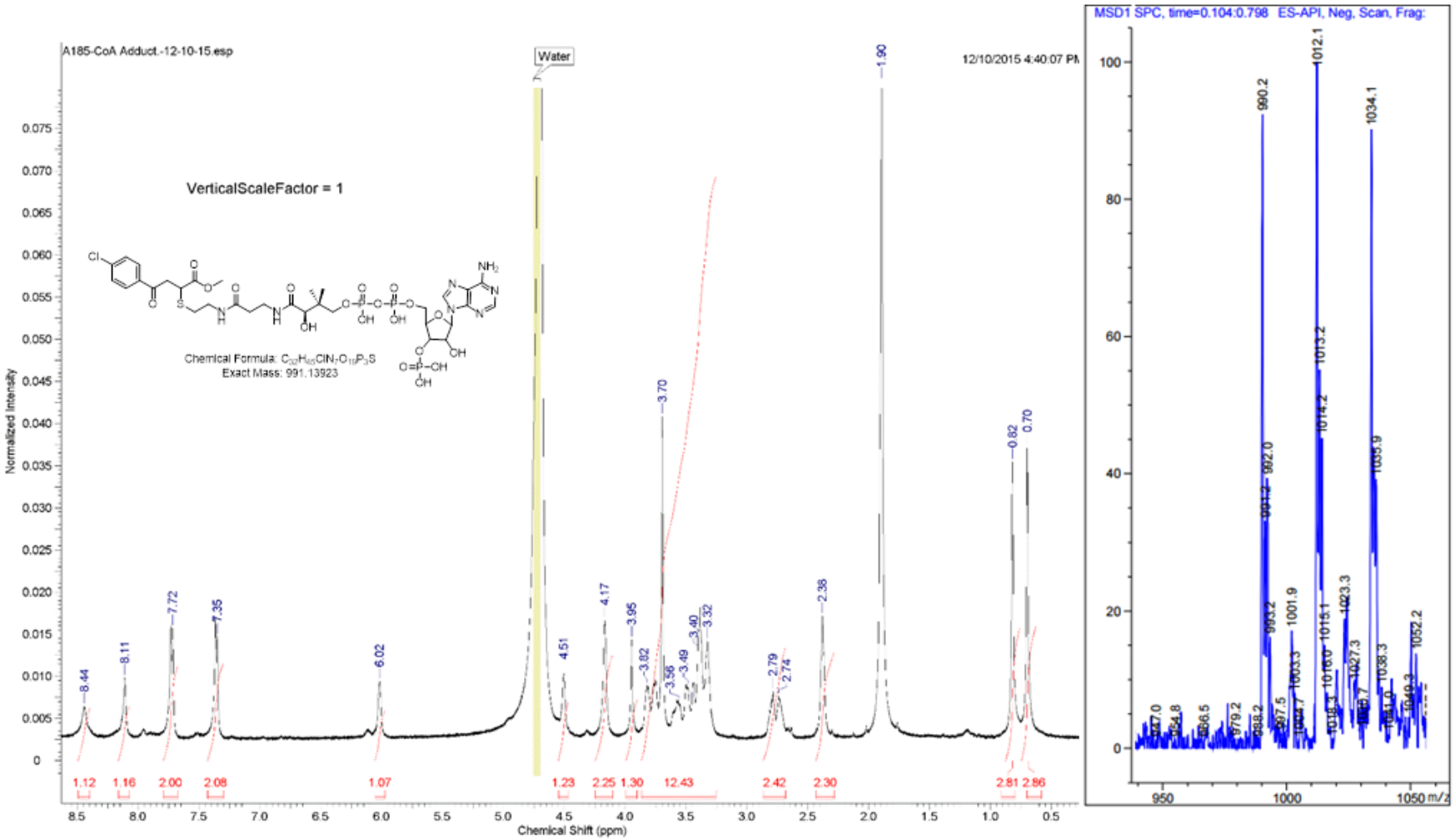

Figure S7: NMR (left) and MS ESI$^-$ (right) of compound **8**. ^{1}H NMR (500 MHz, D_2O) d ppm 0.70 (br. s., 3 H) 0.82 (br. s., 3 H) 2.38 (br. s., 2 H) 2.74 (br. s., 1 H) 2.79 (br. s., 1 H) 3.32 (br. s., 2 H) 3.36 - 3.52 (m, 4 H) 3.56 (br. s., 2 H) 3.63 - 3.77 (m, 4 H) 3.82 (br. s., 2 H) 3.95 (br. s., 1 H) 4.17 (br. s., 2 H) 4.51 (br. s., 1 H) 6.02 (br. s., 1 H) 7.36 (d, *J*=7.63 Hz, 2 H) 7.72 (d, *J*=7.93 Hz, 2 H) 8.11 (br. s., 1 H) 8.44 (br. s., 1 H). ESI$^-$ calculated for [M-H]$^-$ $C_{32}H_{45}ClN_7O_{19}P_3S$ (compound **8**) is 990.13 observed 990.2.