

Electronic Supplementary Information

# **Novel Bis-Cyclic Guanidines as Potent Membrane-Active Antibacterial Agents with Therapeutic Potential**

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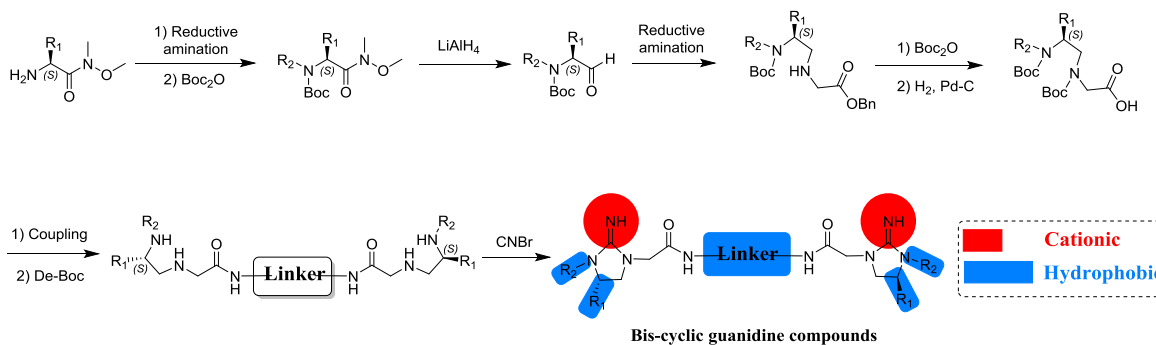
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## 1. General information.

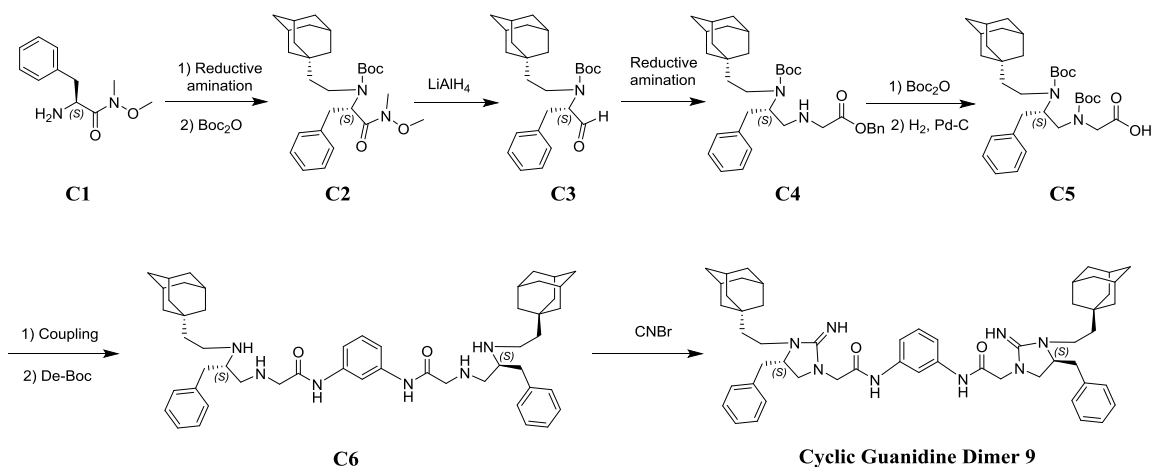
$\alpha$ -Phenylalanine was purchased from Chem-Impex International, Inc. Solvents and other reagents were purchased from either Sigma-Aldrich or Fisher Scientific and were used without further purification. The final products were purified on a Waters Breeze 2 HPLC system, and lyophilized on a Labcono lyophilizer. The purity of the compounds was determined to be >95% by analytical HPLC (1 mL/min flow, 5% to 100% linear gradient of solvent B (0.1% TFA in acetonitrile) in A (0.1% TFA in water) over 50 min was used). NMR data of compound **7** were collected on a Varian Inova 600 instrument, and others were obtained on a Varian Inova 500 instrument.

## 2. Synthesis of desired compounds



Scheme S1. The general synthetic scheme of cyclic guanidine dimers

The typical procedure for the synthesis of **9** is shown below. The other compounds were synthesized according to the same procedure as compound **9**. Different aldehydes were used at the first step to give different compounds with various side chains.



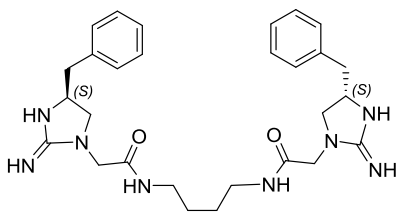
Scheme S2. Typical synthetic route for compound **9**

**2.1 Synthesis of the intermediate build block C5.** Compound **C1** (TFA salt, 10.8g, 33.6 mmol) was dissolved in MeOH and treated with TFA (5.1 mL, 33.6 mmol) before adding to a solution of 2-((3R,5R,7R)-adamantan-1-yl) acetaldehyde (6g, 33.6mmol) in MeOH and acetic acid (2 mL, 67.2 mmol). After stirring for 10 min under ice/H<sub>2</sub>O bath, NaBH<sub>3</sub>CN (3.2g, 50.4 mmol) was added portion wise. The reaction was stirred for 3 h at room temperature before solvent was removed. The crude mixture was treated with NaHCO<sub>3</sub> (aq.) and extracted with EtOAc, and the organic layer was separated and evaporated to give an oil crude, which was purified by silica gel column chromatography to give 8.2 g of the desired secondary amine. Boc<sub>2</sub>O (7g, 32.4 mmol) was added in the THF/H<sub>2</sub>O (1:1, v/v) solution of this intermediate containing NaHCO<sub>3</sub> (3.6g, 43.2 mmol) and allowed to react for 5 h, after which EtOAc was added and the organic layer was collected. The solvent was removed in reduced pressure to give the colorless crude, which was purified by flash column chromatography to give 8.5 g of compound **C2**. Next, compounds **C2** was taken in THF and reduced by LiAlH<sub>4</sub> (687 mg, 18 mol) for 30 min at -20 °C, then water was added to quench the reaction. The mixture was extracted with EtOAc, and the organic layer was separated and the solvent was removed in vacuo to give the crude **C3**, which was used in the next reaction without any further purification. Compound **C3** was converted into compound **C4** with the same procedure

for the synthesis of compound **C2**. BOC protecting group was attached as the same procedure for attaching BOC onto compound **C2**, followed by hydrogenation to remove benzyl protecting group in MeOH to give the building block **C5** as a white solid after filtration and concentration.

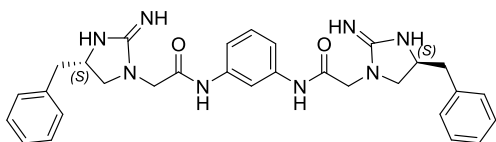
**2.2 Synthesis of compound 9.** Building block **C5** (300 mg, 0.52 mmol), HOBt (159 mg, 1.04 mmol), DIPEA (129  $\mu$ L, 1.04 mmol), and *m*-Phenylenediamine (34 mg, 0.32 mmol) was dissolved in DMF (2 mL) and then DCC (214 mg, 1.04 mmol) was added. The reaction mixture was stirred at room temperature for 24 h. The afforded byproduct DCU was filtered off and the filtration was added into water and extracted with EtOAc ( $\times 3$ ). The organic phase was combined and washed with 1M HCl ( $\times 2$ ), dried over anhydrous  $\text{Na}_2\text{SO}_4$ , filtered, and concentrated under reduced pressure. The crude oil compound was treated with TFA in DCM (1:1, v/v) for 2 h to completely remove BOC protecting groups to yield crude compound **C6**. Subsequently, **C6** was dissolved in acetonitrile (3 mL), to which CNBr (4 eq.) was added carefully (caution: very toxic). The reaction was stirred for 12 h at room temperature. 1M NaOH solution was added carefully, followed by proper amount of bleach to deactivate excessive CNBr. The mixture was filtered through a millipore filter and purified by HPLC purification on Waters HPLC system, and the desired fraction was lyophilized to give the pure product **9**.

### 2.3 $^1\text{H}$ NMR and $^{13}\text{C}$ NMR of compounds 1–9 and building block C5.



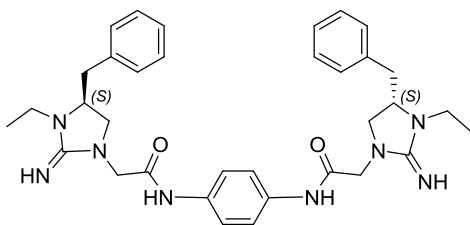
**Compound 1**

$^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  7.31–7.34 (m, 4H), 7.22–7.26 (m, 6H), 4.22–4.28 (m, 2H), 3.94, 3.98 (ABq,  $J_{\text{AB}} = 12.0$  Hz, 4H), 3.72 (t,  $J = 9.5$  Hz, 2H), 3.42 (dd,  $J = 9.0, 6.0$  Hz, 2H), 3.22 (t,  $J = 5.5$  Hz, 4H), 2.93 (ddd,  $J = 15.5, 14.0, 7.0$  Hz, 4H), 1.54 (dt,  $J = 9.0, 6.0, 3.5$  Hz, 4H).  $^{13}\text{C}$  NMR (125 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  167.0, 159.2, 136.1, 128.9 (2C), 128.4 (2C), 126.7, 54.5, 53.2, 46.2, 40.2, 38.7, 26.2. HRMS (ESI)  $\text{C}_{28}\text{H}_{39}\text{N}_8\text{O}_2$   $[\text{M}+\text{H}]^+$  calcd = 519.3190; found = 519.3193.



**Compound 2**

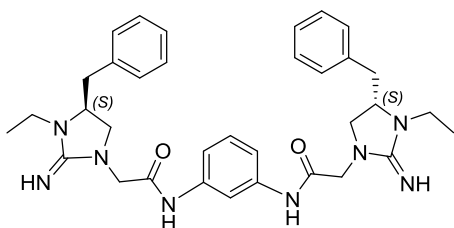
$^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  8.05 (s, 1H), 7.31–7.34 (m, 4H), 7.22–7.27 (m, 9H), 4.25–4.30 (m, 2H), 4.14, 4.19 (ABq,  $J_{\text{AB}} = 18.0$  Hz, 4H), 3.79 (t,  $J = 9.0$  Hz, 2H), 3.50 (dd,  $J = 9.5, 5.5$  Hz, 2H), 2.93 (ddd,  $J = 15.0, 13.5, 6.5$  Hz, 4H), 1.54 (dt,  $J = 9.0, 6.0, 3.5$  Hz, 4H).  $^{13}\text{C}$  NMR (125 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  165.2, 159.3, 138.5, 136.1, 128.9 (2C), 128.4 (2C), 126.7, 115.4, 111.2, 54.6, 53.3, 46.5, 40.3. HRMS (ESI)  $\text{C}_{30}\text{H}_{35}\text{N}_8\text{O}_2$   $[\text{M}+\text{H}]^+$  calcd = 539.2877; found = 539.2877.



**Compound 3**

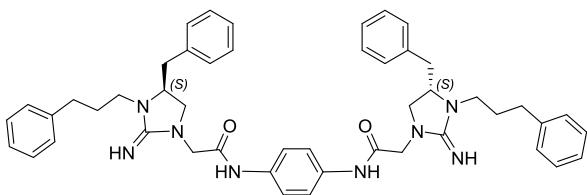
$^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  7.52 (brs, 4H), 7.31–7.33 (m, 4H), 7.22–7.28 (m, 6H), 4.29–4.34 (m, 2H), 4.14, 4.10 (ABq,  $J = 18.0$  Hz, 4H), 3.65 (t,  $J = 11.5$  Hz, 2H), 3.53–3.61 (m, 2H), 3.38–3.45 (m, 4H), 3.21 (dd,  $J = 13.5, 4.5$  Hz, 2H), 2.86 (dd,  $J = 13.5, 8.5$  Hz, 2H), 1.26 (t,  $J = 7.5$  Hz,

6H).  $^{13}\text{C}$  NMR (125 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  165.1, 157.9, 135.7, 134.3, 128.9 (2C), 128.5 (2C), 126.8, 120.1 (2C), 57.8, 51.8, 37.8, 37.5, 11.2. HRMS (ESI)  $\text{C}_{34}\text{H}_{43}\text{N}_8\text{O}_2$   $[\text{M}+\text{H}]^+$  calcd = 595.3503; found = 595.3488.



**Compound 4**

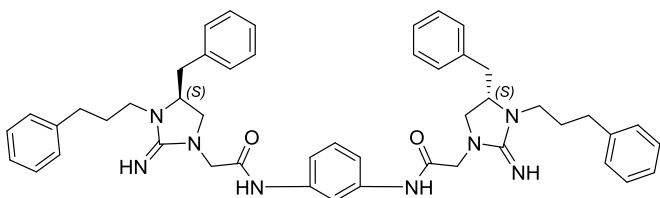
$^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  8.05 (d,  $J = 1.5$  Hz, 1H), 7.31–7.34 (m, 4H), 7.23–7.28 (m, 9H), 4.28–4.34 (m, 2H), 4.15, 4.11 (ABq,  $J = 17.5$  Hz, 4H), 3.63 (t,  $J = 9.5$  Hz, 2H), 3.56 (quintet,  $J = 6.5$  Hz, 2H), 3.36–3.44 (m, 4H), 3.21 (dd,  $J = 13.5, 4.5$  Hz, 2H), 2.85 (dd,  $J = 13.5, 9.0$  Hz, 2H), 1.26 (t,  $J = 7.0$  Hz, 6H).  $^{13}\text{C}$  NMR (125 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  165.2, 157.9, 138.5, 135.7, 129.0 (2C), 128.4 (2C), 126.8, 115.4, 111.2, 57.8, 51.7, 37.8, 37.6, 11.2. HRMS (ESI)  $\text{C}_{34}\text{H}_{43}\text{N}_8\text{O}_2$   $[\text{M}+\text{H}]^+$  calcd = 595.3503; found = 595.3490.



**Compound 5**

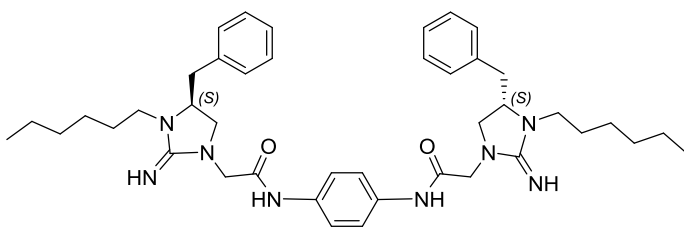
$^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  7.53 (s, 4H), 7.17–7.32 (m, 20H), 4.22–4.27 (m, 2H), 4.0 (s, 4H), 3.60 (t,  $J = 9.5$  Hz, 2H), 3.50–3.57 (m, 2H), 3.40 (dd,  $J = 9.5, 5.0$  Hz, 2H), 3.33 (dd,  $J = 8.5, 5.5$  Hz, 2H), 3.08 (dd,  $J = 13.5, 5.0$  Hz, 2H), 2.83 (dd,  $J = 13.5, 8.0$  Hz, 2H), 2.61–2.73 (m, 4H), 1.91–

2.05 (m, 4H).  $^{13}\text{C}$  NMR (125 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  165.0, 158.0, 140.9, 135.7, 134.3, 128.9 (2C), 128.4 (2C), 128.2 (2C), 128.0 (2C), 126.8, 125.8, 120.0, 58.0, 51.6, 42.4, 37.4, 32.1 (2C), 28.3. HRMS (ESI)  $\text{C}_{48}\text{H}_{55}\text{N}_8\text{O}_2$   $[\text{M}+\text{H}]^+$  calcd = 775.4442; found = 775.4443.



**Compound 6**

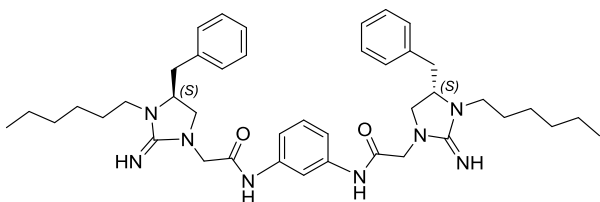
$^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  8.05–8.12 (m, 1H), 7.17–7.32 (m, 23H), 4.19–4.25 (m, 2H), 4.12, 4.09 ((ABq,  $J_{\text{AB}} = 18.0$  Hz, 4H), 3.60 (t,  $J = 8.5$  Hz, 2H), 3.52 (quintet,  $J = 7.5$  Hz, 2H), 3.39 (dd,  $J = 9.5, 5.0$  Hz, 2H), 3.27–3.33 (m, overlapped with  $\text{CD}_3\text{OD}$ , 2H), 3.07 (dt,  $J = 13.5$  Hz, 5.4 Hz, 2H), 2.82 (ddd,  $J = 13.0, 9.0, 3.0$  Hz, 2H), 2.60–2.72 (m, 4H), 1.92–2.02 (m, 4H).  $^{13}\text{C}$  NMR (125 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  165.2, 158.0, 140.8, 138.5, 135.6, 129.0 (2C), 128.4 (2C), 128.2 (2C), 128.0 (2C), 126.8, 125.8, 115.3, 58.0, 51.6, 42.3, 37.4, 32.1 (2C), 28.3. HRMS (ESI)  $\text{C}_{48}\text{H}_{55}\text{N}_8\text{O}_2$   $[\text{M}+\text{H}]^+$  calcd = 775.4442; found = 775.4438.



**Compound 7**

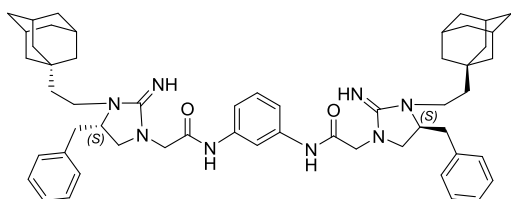
$^1\text{H}$  NMR (600 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  7.50 (brs, 4H), 7.29–7.32 (m, 4H), 7.22–7.26 (m, 6H), 4.26–4.31 (m, 2H), 4.08 (s, 4H), 3.65 (t,  $J = 9.6$  Hz, 2H), 3.45 (ddd,  $J = 15.0, 9.0, 6.6$  Hz, 2H), 3.40 (dd,  $J =$

9.6, 5.4 Hz, 2H), 3.24–3.27 (m, 2H), 3.14 (dd,  $J = 13.8, 4.8$  Hz, 2H), 2.87 (dd,  $J = 13.2, 8.4$  Hz, 2H), 1.58–1.69 (m, 4H), 1.32–1.37 (m, 16H), 0.91 (t,  $J = 7.2$  Hz, 6H).  $^{13}\text{C}$  NMR (150 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  165.0, 158.0, 135.8, 134.3, 129.0 (2C), 128.4 (2C), 126.7, 120.0 (2C), 57.9, 51.7, 42.8, 37.5, 31.1, 26.6, 25.8, 22.1, 12.9. HRMS (ESI)  $\text{C}_{42}\text{H}_{59}\text{N}_8\text{O}_2$   $[\text{M}+\text{H}]^+$  calcd = 707.4755; found = 707.4748.



**Compound 8**

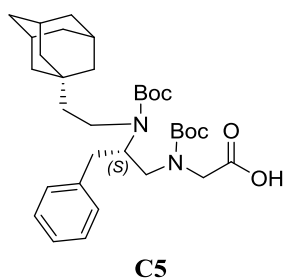
$^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  8.05 (s, 1H), 7.31–7.34 (m, 4H), 7.24–7.28 (m, 9H), 4.27–4.32 (m, 2H), 4.14, 4.10 (ABq,  $J = 18.0$  Hz, 4H), 3.65 (t,  $J = 8.0$  Hz, 2H), 3.47 (ddd,  $J = 15.0, 9.0, 6.5$  Hz, 2H), 3.42 (dd,  $J = 9.5, 5.5$  Hz, 2H), 3.24–3.27 (m, 2H), 3.16 (dd,  $J = 13.5, 5.0$  Hz, 2H), 2.89 (dd,  $J = 13.5, 8.0$  Hz, 2H), 1.59–1.69 (m, 4H), 1.32–1.37 (m, 12H), 0.92 (t,  $J = 7.0$  Hz, 6H).  $^{13}\text{C}$  NMR (125 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  165.2, 158.0, 138.5, 135.8, 129.0 (2C), 128.5 (2C), 126.8, 115.3, 111.1, 62.9, 58.0, 42.9, 37.5, 31.1, 26.6, 25.8, 22.1, 12.9. HRMS (ESI)  $\text{C}_{42}\text{H}_{59}\text{N}_8\text{O}_2$   $[\text{M}+\text{H}]^+$  calcd = 707.4755; found = 707.4751.



**Compound 9**



$^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  8.12 (t,  $J = 1.5$  Hz, 1H), 7.34 (t,  $J = 7.5$  Hz, 4H), 7.22–7.29 (m, 9H), 4.28–4.33 (m, 2H), 4.15, 4.12 (ABq,  $J = 18.5$  Hz, 4H), 3.70 (t,  $J = 9.5$  Hz, 2H), 3.53 (qd,  $J = 7.0, 5.5$  Hz, 2H), 3.42 (dd,  $J = 10.0, 5.5$  Hz, 2H), 3.16–3.19 (m, 2H), 3.12 (dd,  $J = 14.0, 5.5$  Hz, 2H), 2.90 (dd,  $J = 14.0, 7.5$  Hz, 2H), 1.95 (brs, 6H), 1.67–1.77 (m, 12H), 1.52–1.54 (m, 12H), 1.44 (td,  $J = 13.0, 4.5$  Hz, 2H), 1.35 (td,  $J = 12.0, 5.5$  Hz, 2H).  $^{13}\text{C}$  NMR (125 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  165.2, 158.0, 138.6, 136.0, 129.0 (2C), 128.5 (3C), 126.8, 115.3, 111.2, 57.4, 51.9, 41.7 (3C), 39.6, 38.1, 38.0, 36.6 (2C), 31.3, 28.6 (3C), 28.6. HRMS (ESI)  $\text{C}_{54}\text{H}_{71}\text{N}_8\text{O}_2$   $[\text{M}+\text{H}]^+$  calcd = 863.5694; found = 863.5670.



$^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  7.25–7.29 (m, 2H), 7.19–7.21 (m, 3H), 3.73–3.97 (m, 3H), 3.59 (s, 1H), 3.43–3.50 (m, 1H), 2.95–3.11 (m, 2H), 2.77–2.81 (m, 2H), 1.88 (brd, 4H), 1.71 (brd, 4H), 1.60 (brd, 4H), 1.28–1.52 (m, 21H), 0.87–0.92 (m, 3H).  $^{13}\text{C}$  NMR (125 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  171.7, 155.8, 155.7, 128.9 (2C), 128.0 (2C), 126.0, 80.5, 80.3, 79.5, 79.2, 49.3, 42.0 (3C), 41.8, 36.8 (3C), 36.5, 31.2, 28.6 (4C), 28.5, 27.6, 27.5 (2C), 27.4, 27.3, 27.2. HRMS (ESI)  $\text{C}_{33}\text{H}_{51}\text{N}_2\text{O}_6$   $[\text{M}+\text{H}]^+$  calcd = 571.3742; found = 571.3763.

### 3. Minimum inhibitory concentrations (MICs) against bacteria.

We test the antimicrobial activity of the 8 compounds on six bacteria strains: MRSA (ATCC 33591), MRSE (RP62A), KP (ATCC 13383), PA (ATCC27853), *E. coli* (ATCC 25922), VER

(ATCC 700802) according to the same procedures reported previously.<sup>1</sup> Two-fold serial dilutions of compounds (0.1–25 µg/mL) were used in the assay. The experiments were repeated at least three times with duplicates each time. The absorption at 600 nm wavelength was read on a Biotek Synergy HT microtiter plate reader.

#### **4. Hemolytic assays.**

The freshly drawn rat red blood cells (hRBCs) were washed with 1× PBS buffer, and centrifuged at 3500 rpm for 10 min. The rest procedures were followed as reported previously.<sup>1</sup> The experiment was repeated at least three times with duplicates each time. The hemolysis activity was calculated by the formula % hemolysis =  $(Abs_{\text{sample}} - Abs_{\text{SPBS}}) / (Abs_{\text{Triton}} - Abs_{\text{SPBS}}) \times 100\%$ .

#### **5. Fluorescence microscopy.**<sup>2</sup>

The PF assay was used to assess the ability of the compound **8** to compromise bacterial membranes. The compound **8** was incubated with the bacteria at 37 °C for 2 h after MRSA and *E. Coli* grew to mid-logarithmic phase. After centrifuged at 5000 rpm for 15 min, the cell pellets were washed with the PBS buffer for three times, and incubated with DAPI (10 µg/mL) or PI (5 µg/mL) for 15 min sequentially on ice in the dark. The mixture was then centrifuged and the pellets were washed with the PBS buffer. Next, 10 µL of the samples were placed on chamber slides and observed under Zeiss Axio Image Zloptical microscope using 100× oil-immersion objective.

#### **6. Time kill study.**

The kinetics of bacteria killing by the lead compounds **7** and **8** were also tested. The bacteria *E. Coli* were grown to mid-logarithmic phase in TSB medium to make the suspension of 10<sup>6</sup> CFU/ml.

The suspension was incubated with different concentrations of **7** or **8** (12.5 µg/mL, 25 µg/mL, and 50 µg/mL) for 10 min, 30 min, 1 h and 2 h respectively. The mixtures were diluted by 10<sup>2</sup> to 10<sup>4</sup> fold and spread on TSB agar plates. After incubation at 37 °C 12 h, the colonies on the plates were counted and plotted against the incubation time.

### **7. Drug resistance study.**<sup>3</sup>

Bacteria in wells containing concentration of 1/2 MIC of the compounds **7** and **8** were used to make bacterial suspension (10<sup>6</sup> CFU/ml) for the next measurement of MICs. The experiment was repeated each day for 14 passages.

### **8. Inhibition of biofilms.**<sup>4</sup>

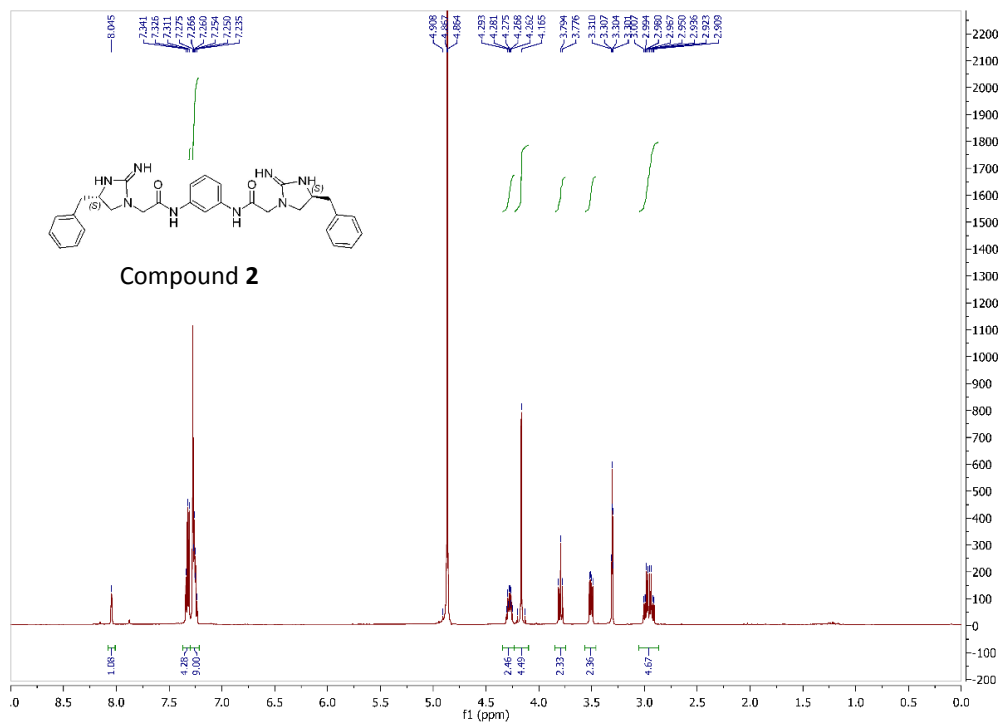
Overnight grown bacteria MRSA and *E. Coil* were inoculated into fresh 10% of MIII broth at a ratio of 1:100. Afterwards, 100 µL of inoculated culture was incubated with appropriated amounts of compounds **7** and **8** in 96-well plate, which was then incubated at 37 °C for overnight. Optical density of each wells was recorded (wavelength 600 nm) and then the biofilm biomass were recorded by the crystal violet (CV) method. Relative biofilm biomass values were normalized by the biomass value of control (no compound added). Data were presented in mean value of three replicates.

### **9. In vivo study of mouse thigh burden infection model.**

All protocols and methods associated with animal experiments were approved by University of South Florida (USF) Institutional Animal Care and Use Committee. The in vivo experiment on the mouse model of the thigh burden infection with MRSA was conducted adapted from previously reported protocol.<sup>5</sup> The CD-1 female mice which were 6 to 8 weeks old and around 25 g in weights

were used for the study. Neutropenic Mice were induced by injecting cyclophosphamide (150 mg/kg) intraperitoneally twice at 4 and 1 days before bacterial inoculation. One MRSA colony from tryptic soy agar (TSA) cultures was allowed to grow in tryptic soy broth (TSB) overnight at 37 °C, then 100 µL culture was withdrawn and diluted with TSB to a total volume of 4 mL, which was subsequently incubated at 37 °C for another 6 h. The bacterial culture was then diluted in sterile PBS to give the final inoculum concentration of approximate 10<sup>6</sup> CFU/mL. The thigh burden infection model was established by injecting both posterior thighs of mice with 100 µL of inoculums. Two doses of the compounds **7** and **8** were given at 1 h and 7 h by i.v. bolus injection in the tail vein at 5 mg/kg per dose of drugs after bacterial infection. Thighs were harvested at 25 h for both groups after bacterial inoculation. Thigh muscles were collected in a sterile tared tube, to which 5 mL sterile PBS was added. The mixture was then homogenized with a tissue homogenizer (BioSpec product tissue tearor 985-370) for approximately 30 sec. 100 µL of serial diluted aliquots were plated on tryptic soy agar plates, which were incubated for 24 h at 37 °C. The formed colonies were counted to calculate CFU per thigh.





TP-F-112-1\_500MHz

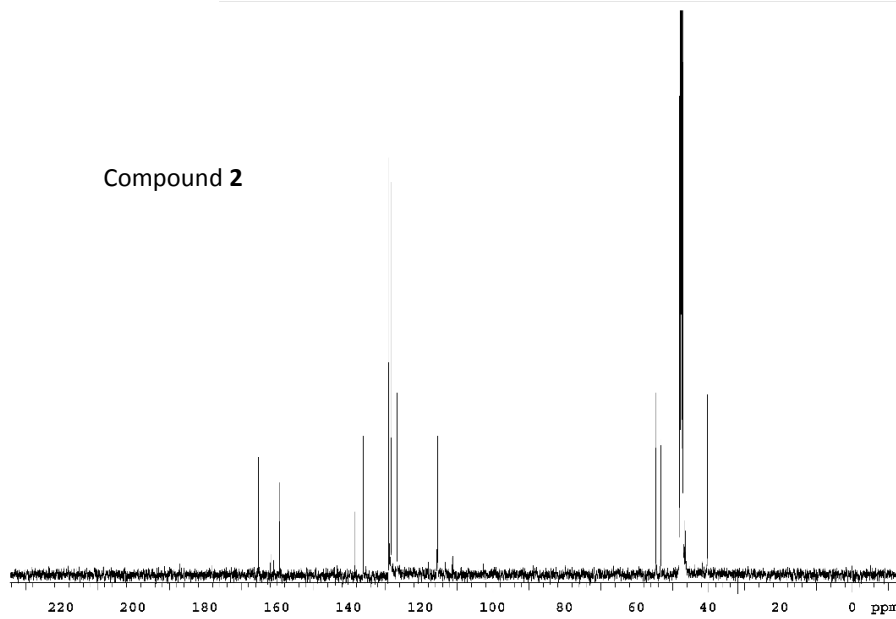
Sample Name TP-F-112-1\_500MHz  
Date collected 2016-12-24

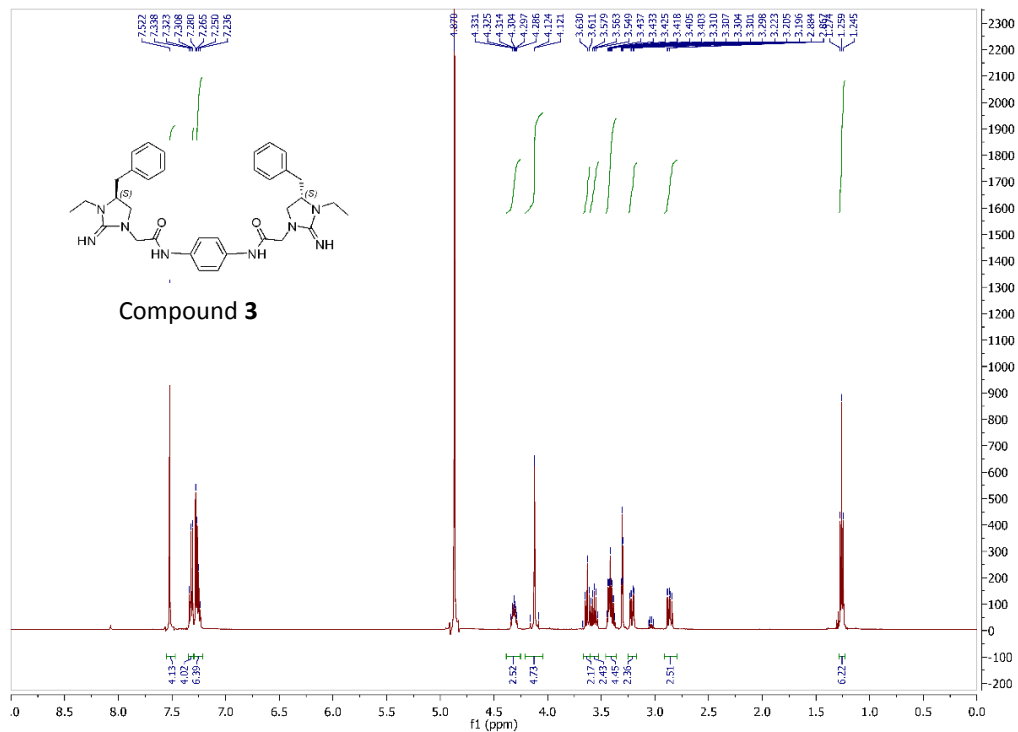
Pulse sequence CARBON  
Solvent cd3od

Temperature 26  
Spectrometer cd600-1.cas.usf.edu-vnmrs600erator

Study owner tpeing  
Operator tpeing

**Compound 2**





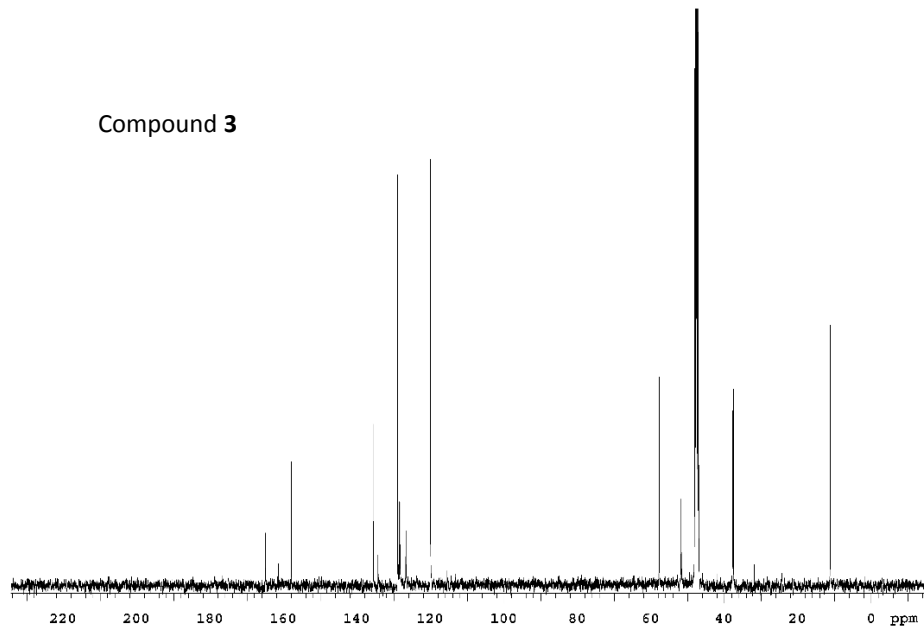
TP-G-064-3\_500MHz

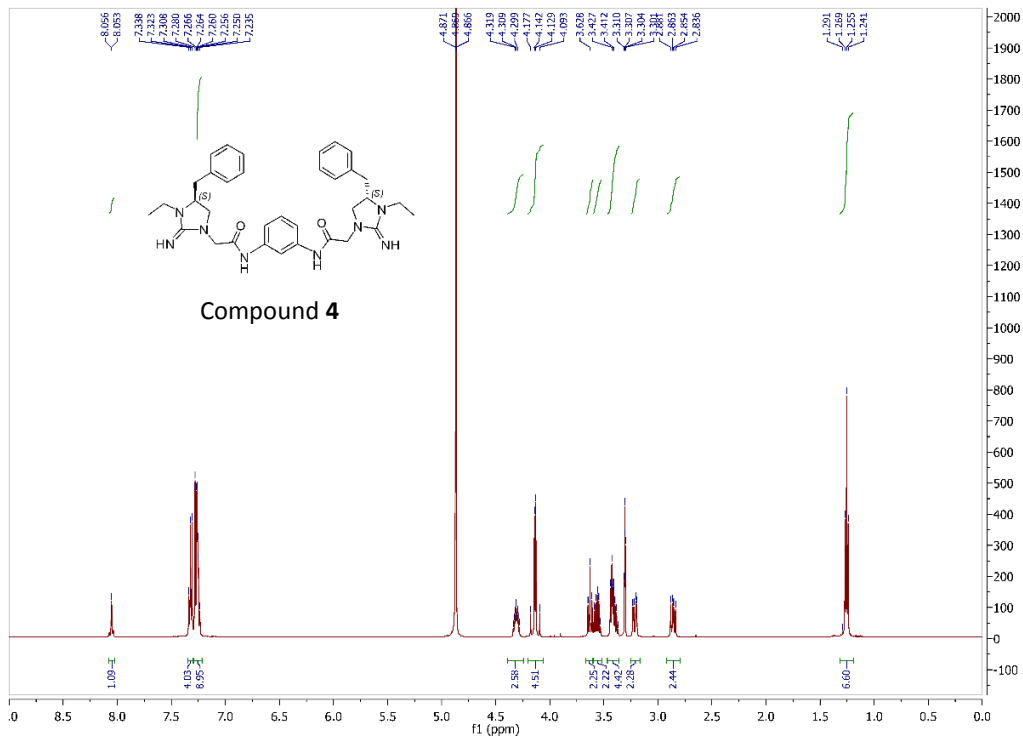
Sample Name TP-G-064-3\_500MHz  
Date collected 2016-12-23

Pulse sequence CARBON  
Solvent cd3od

Temperature 26  
Spectrometer dd500-1.cas.usf.edu-vnmrs500 Operator tpeng

**Compound 3**





TP-G-065-3\_500MHz

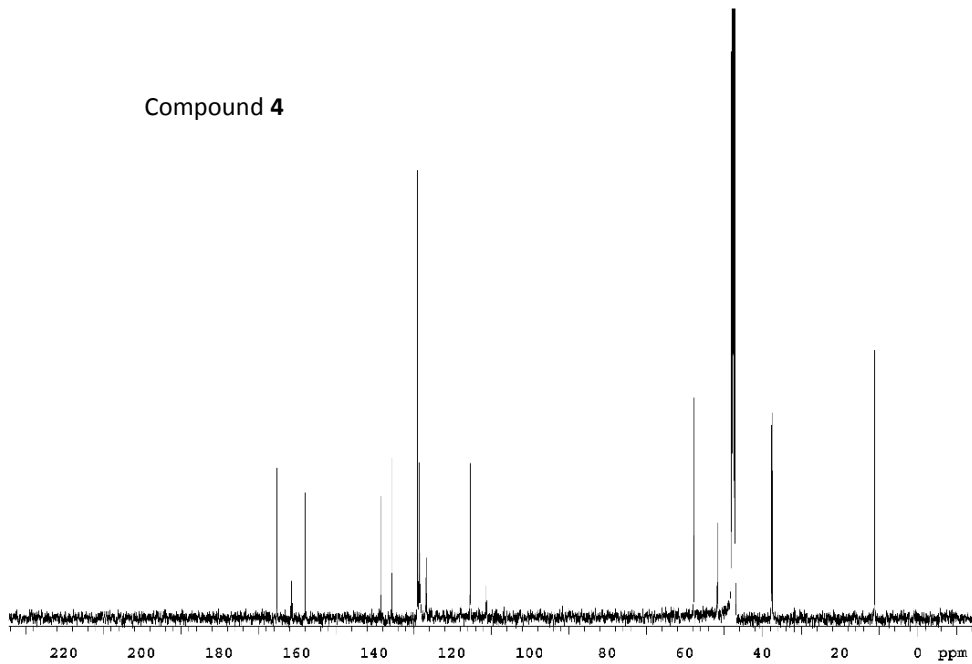
Sample Name TP-G-065-3\_500MHz  
Date collected 2016-12-23

Pulse sequence CARBON  
Solvent cd3od

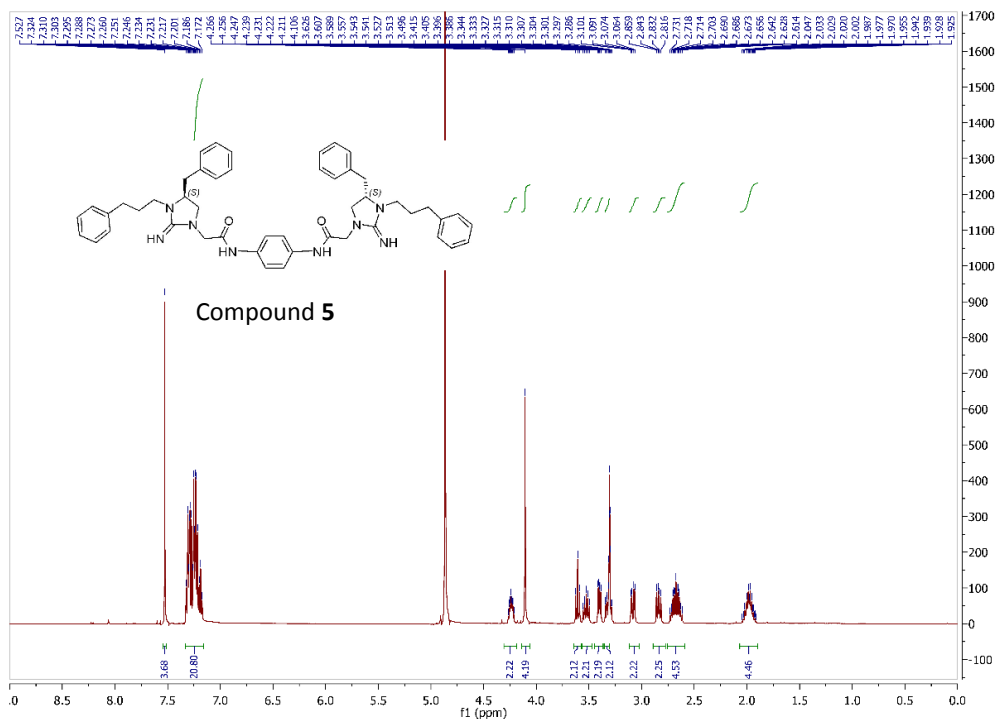
Temperature 26  
Spectrometer dd000-1.cas.usf.edu-vnmr560

Study owner tpeng  
Operator tpeng

**Compound 4**







TP-G-019-3\_500MHz

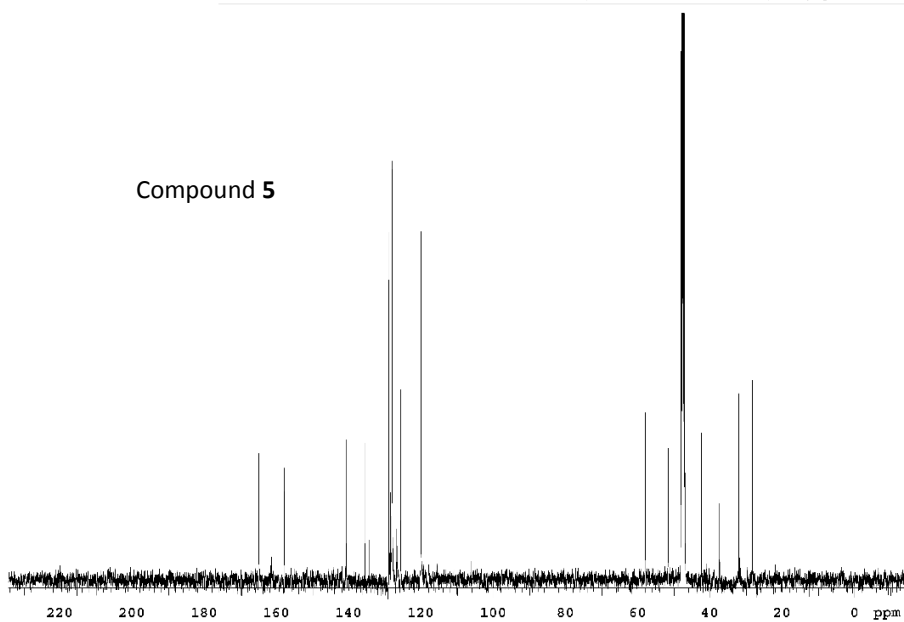
Sample Name: TP-G-019-3\_500MHz  
Date collected: 2016-12-23

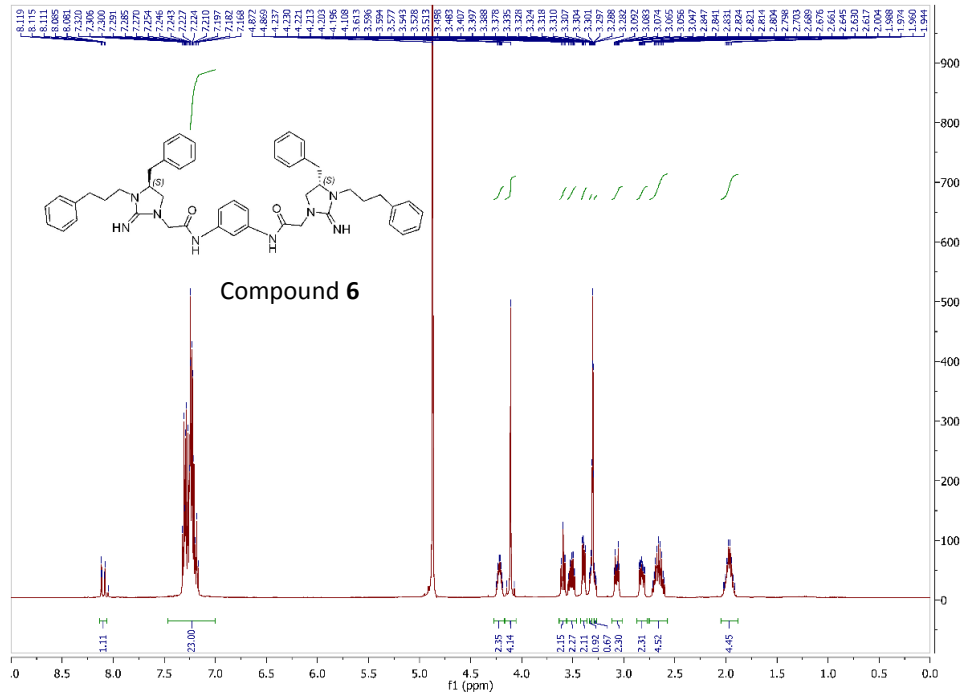
Pulse sequence: CARBON  
Solvent: cd3od

Temperature: 26  
Spectrometer: dd500-1.cas.usf.edu-vnmrs500

Study owner: tpeng  
Operator: tpeng

**Compound 5**





TP-G-028-3\_500MHz

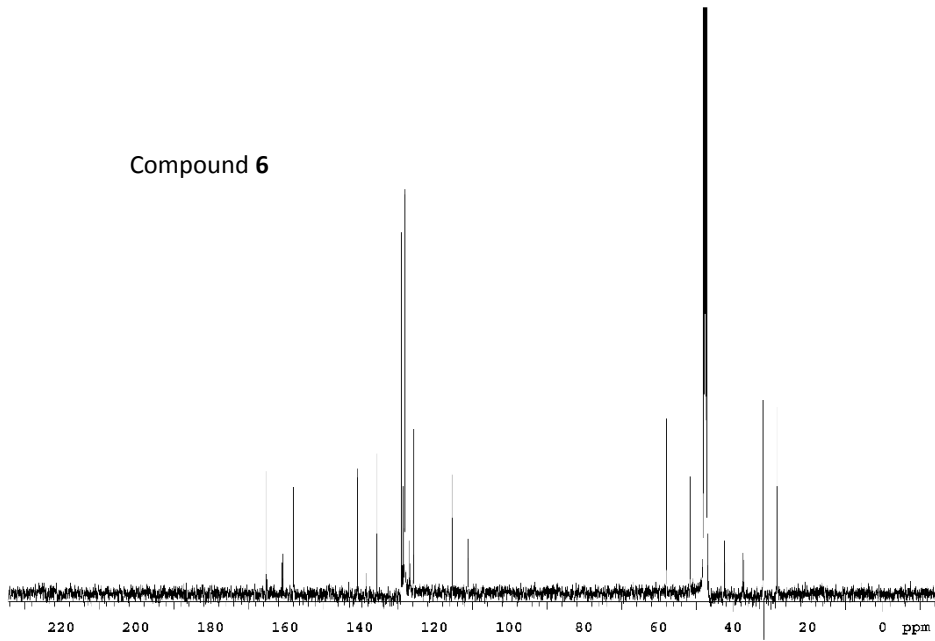
Sample Name: TP-G-028-3\_500MHz  
Date collected: 2016-12-24

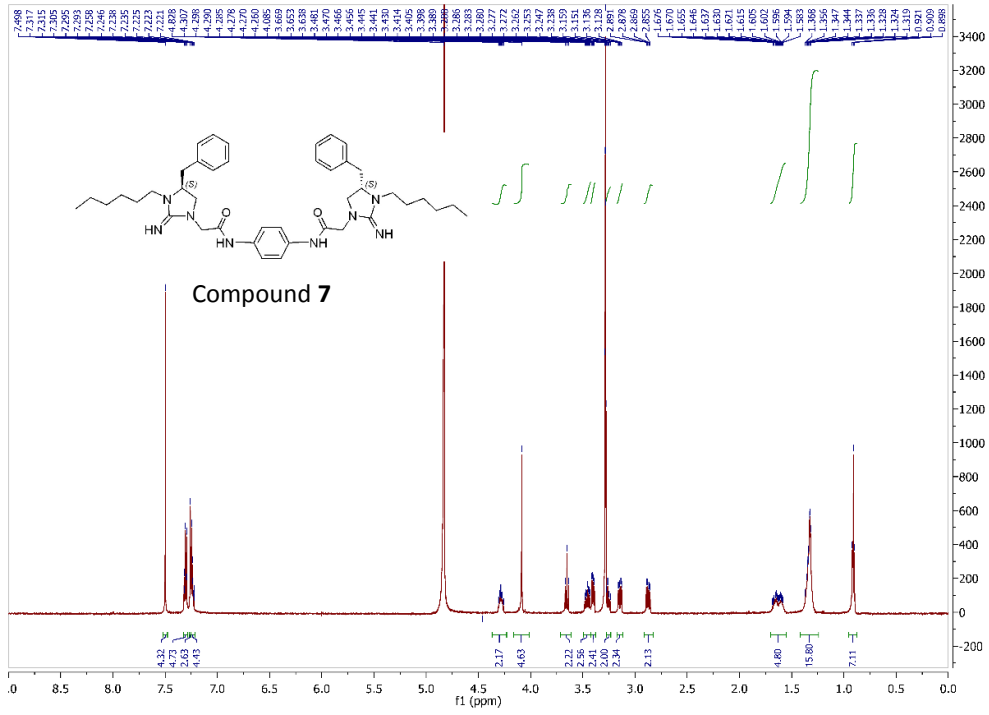
Pulse sequence: CARBON  
Solvent: cd3od

Temperature: 26  
Spectrometer: dd500-1.cas.usf.edu/vnmrs500operator tpng

Study owner: tpng

**Compound 6**





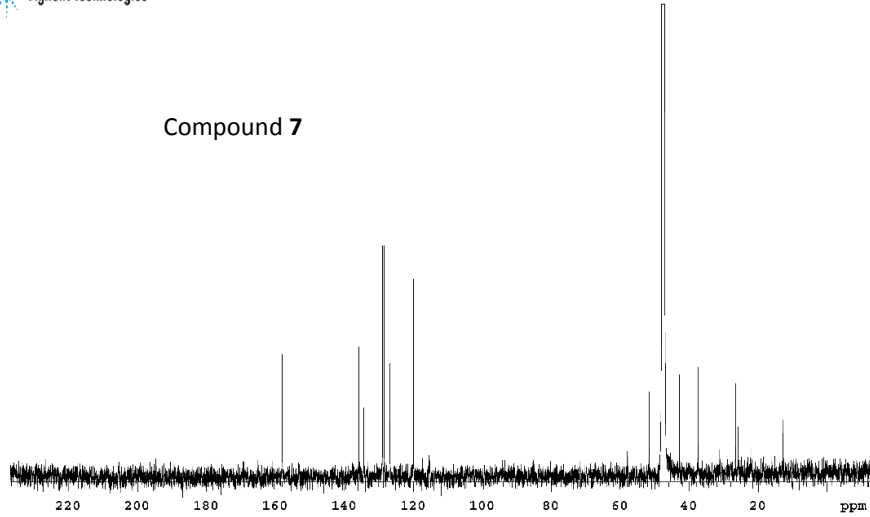
Sample Name: 2016-12-22  
Date collected: 2016-12-22

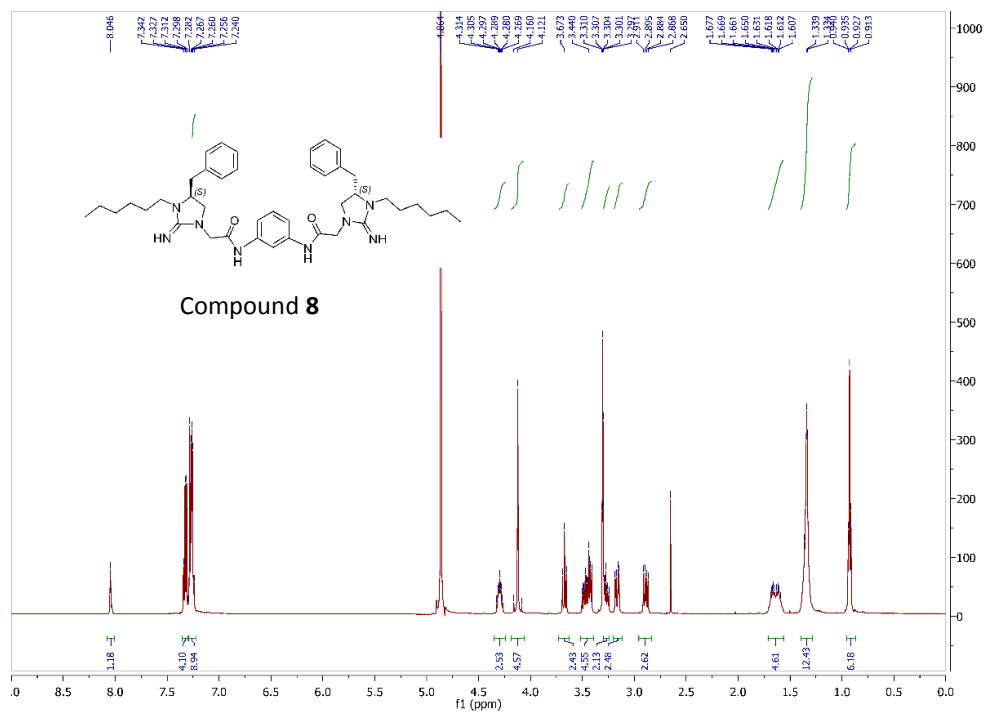
Pulse sequence: CARBON  
Solvent: cd3od

Temperature: 25  
Spectrometer: Inova600-1.cas.usf.edu-thova600

Study owner: pteng  
Operator: pteng

**Compound 7**





TP-G-044-3\_500MHz

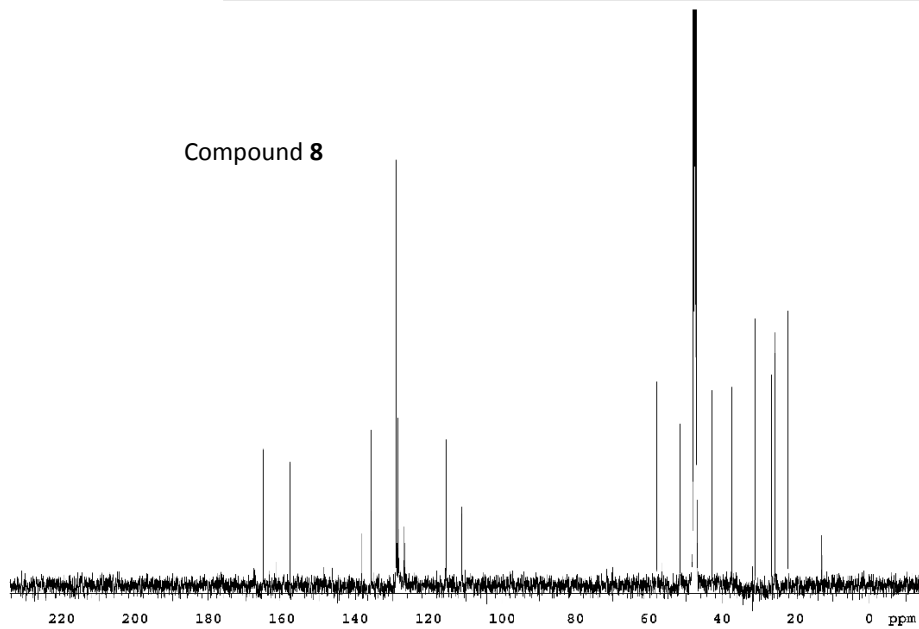
Sample Name TP-G-044-3\_500MHz  
Date collected 2016-12-23

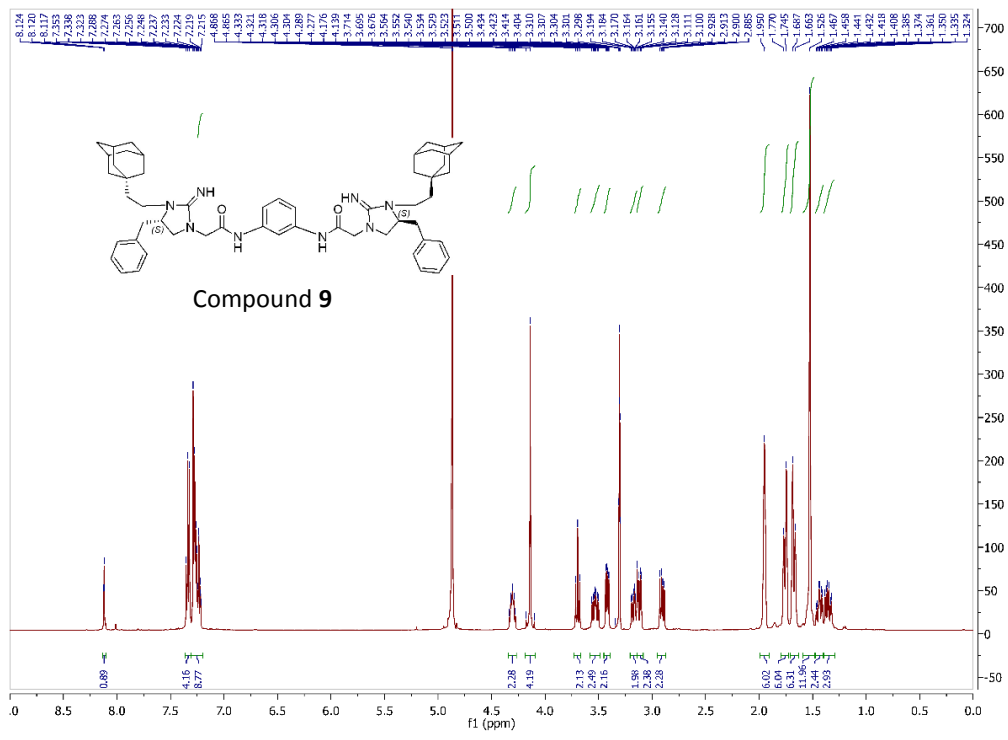
Pulse sequence CARBON  
Solvent cd3cod

Temperature 28  
Spectrometer dd600-1.cas.usf.edu-vnmrs56

Study owner tpeng  
Operator tpeng

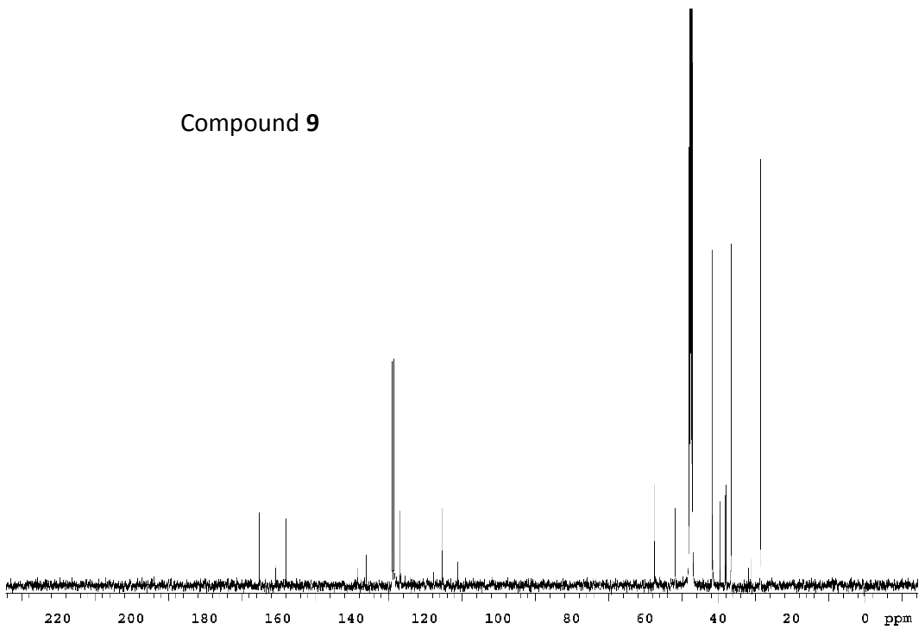
**Compound 8**

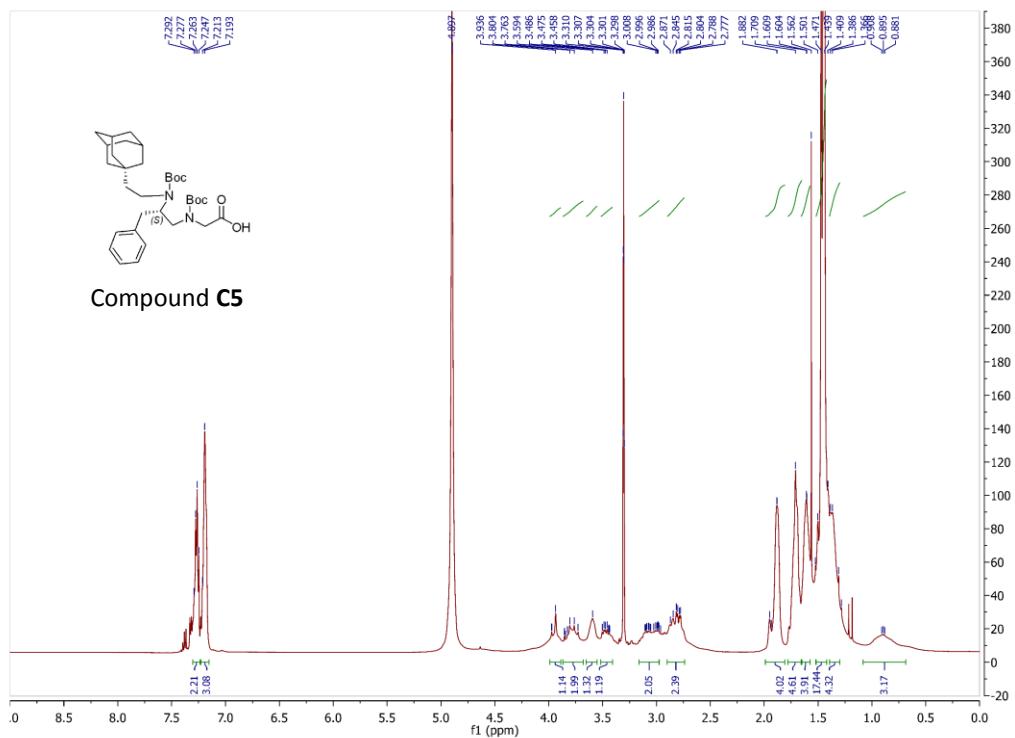




TP-G-141-3\_500MHz  
 Sample Name: TP-G-141-3\_500MHz  
 Date collected: 2016-12-24  
 Pulse sequence: CARBON  
 Solvent: cd3od  
 Temperature: 25  
 Spectrometer: dd600-1.cas.usf.edu/vnmrs500  
 Study owner: tpeng  
 Operator: tpeng

**Compound 9**





TP-G-136-2B\_500MHz

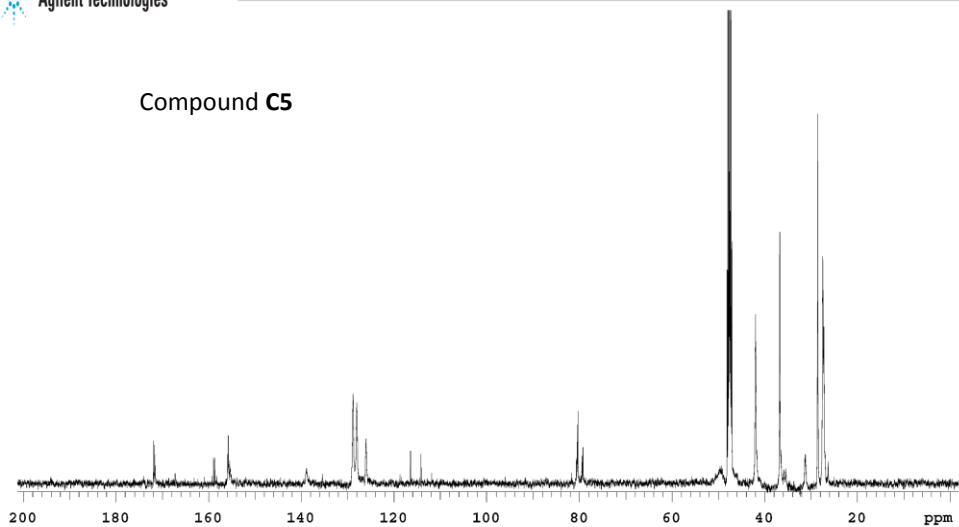
Sample Name TP-G-136-2B\_500MHz  
Date collected 2017-05-05

Pulse sequence CARBON  
Solvent cd3od

Temperature 26  
Spectrometer dd500-1.cas.usf.edu-vnmrs500

Study owner tpeng  
Operator tpeng

**Compound C5**

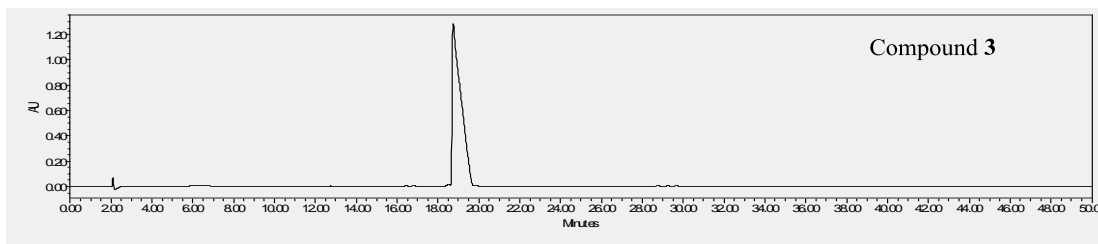
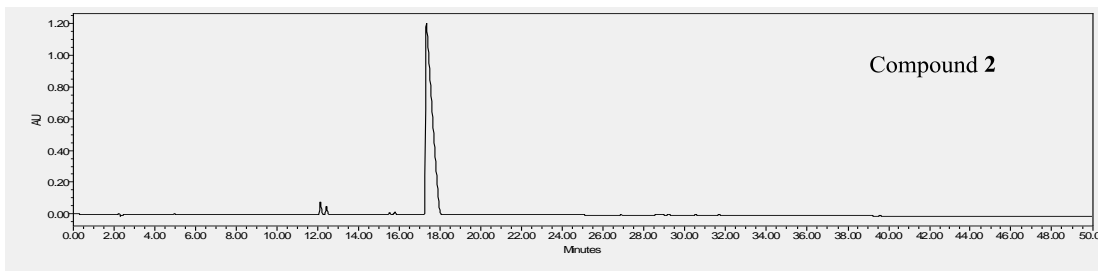
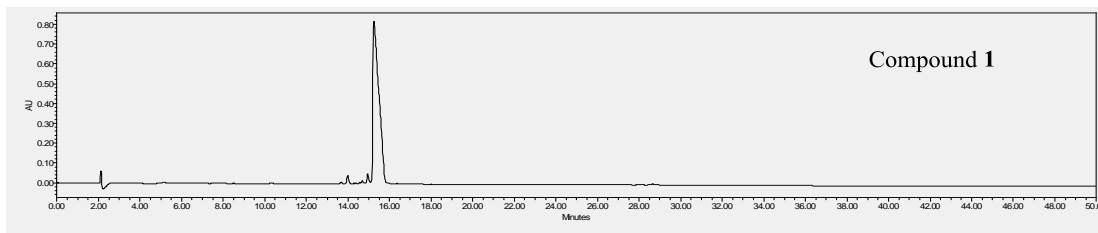


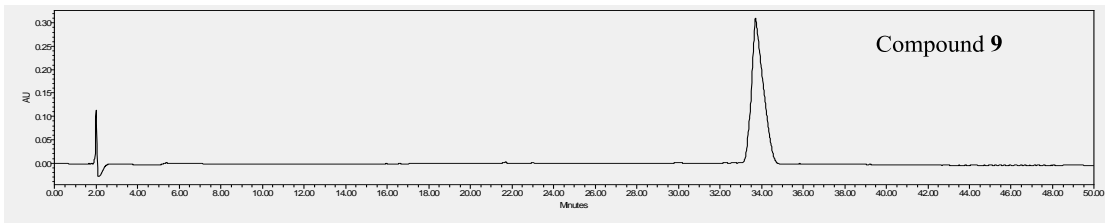
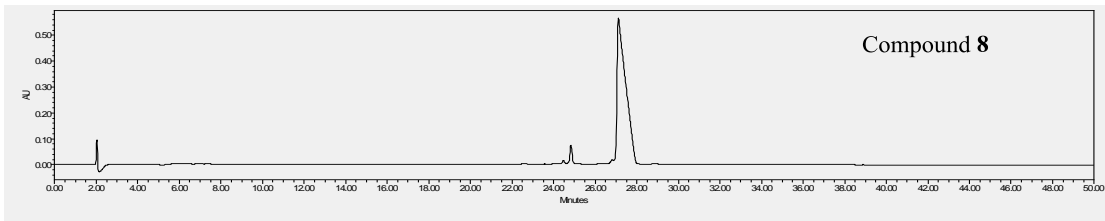
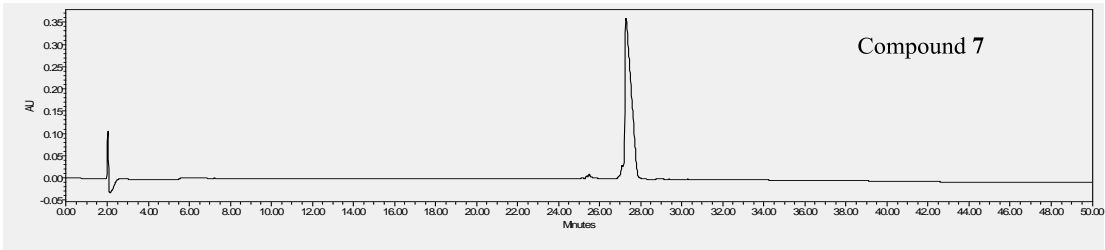
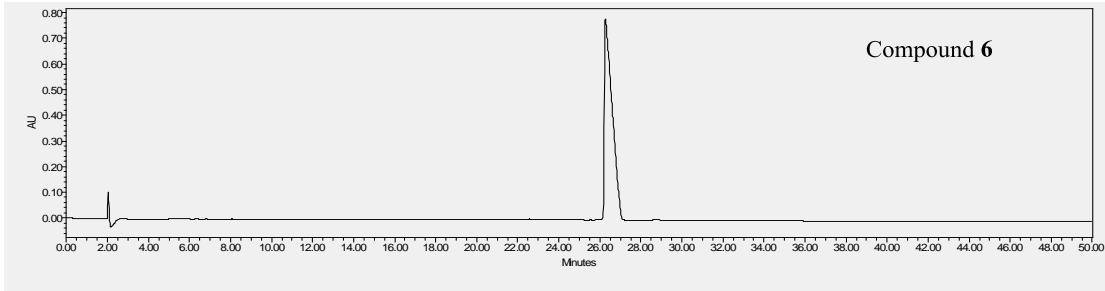
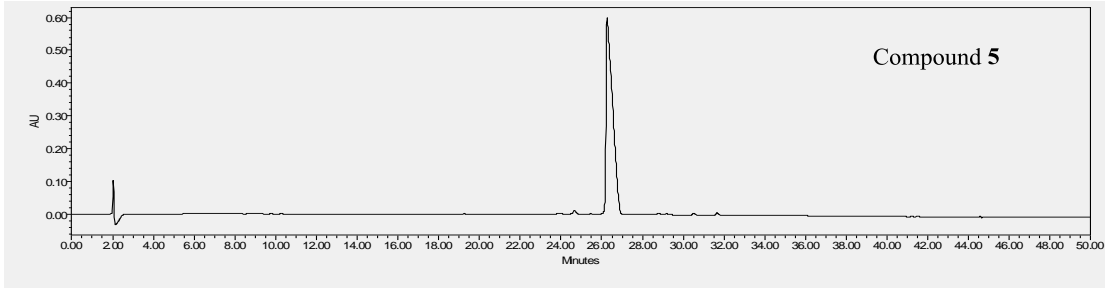
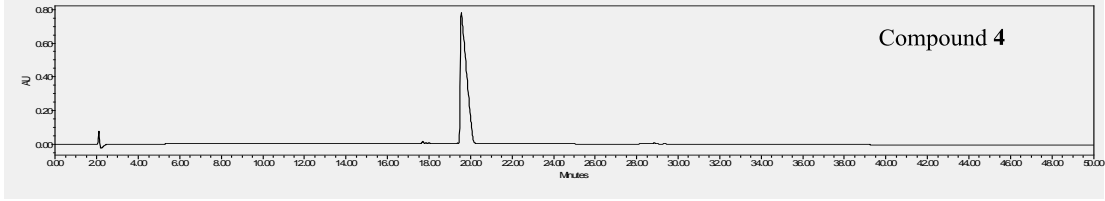
## HPLC analysis of compounds 1–9

Table S1. HPLC purities and retention time of compounds 1–9

Compound Name	Purity (based on HPLC) (%)	Retention Time (min)
1	97.68	15.24
2	96.88	17.32
3	99.82	18.74
4	98.82	19.51
5	99.79	26.23
6	99.81	26.33
7	98.55	27.25
8	96.31	27.15
9	99.30	33.72

## HPLC spectra of compounds 1–9







## References:

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