SUPPORTING INFORMATION

An Ichip-Domesticated Sponge Bacterium Produces an *N*-Acyltyrosine Bearing an α-Methyl Substituent

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EXPERIMENTAL PROCEDURES

General experimental procedures. Optical rotation was measured on a Rudolph Autopol III polarimeter using a 50 mm microcell (1.2 mL). Infrared (IR) spectra were recorded using attenuated total reflectance on a Thermo Nicolet 6700 FT-IR spectrometer. All NMR spectra were acquired on a Bruker Avance III NMR spectrometer (^{1}H: 400 MHz, ^{13}C: 151 MHz) equipped with a 5 mm SmartProbe. All chemical shifts are reported in ppm and referenced to residual solvent signals [^{1}H (DMSO-d_6): 2.50 ppm, ^{13}C (DMSO-d_6): 39.51 ppm, ^{1}H ($CDCl_3$): 7.26 ppm, and ^{13}C ($CDCl_3$): 77.16 ppm]. All UHPLC-HRMS analyses (unless otherwise noted) were carried out using the following platform equipped with HRMS-ELSD-UV detection: Thermo Accela UHPLC Pump, Thermo Exactive HRMS fitted with an ESI source, Sedex 80 LT-ELSD, and Thermo PDA. A Kinetex core−shell 100 Å C_{18} column (2.1 × 50 mm, 1.7 μm, Phenomenex) was used with a mobile-phase flow rate of 0.5 mL/min and injection volume of 10 μL (all samples were prepared in CH_3OH). The following elution method was used [A = H_2O (0.1% formic acid), B = CH_3CN (0.1% formic acid)]: 5% B from 0.0 to 0.2 min, linear gradient from 5% B at 0.2 min to 99% B at 4.8 min, 99% B from 4.8 to 8.0 min, linear gradient from 99% B at 8.0 min to 5% B at 8.5 min and 5% B from 8.5 to 10.0 min. The following HRMS parameters were used: positive ionization mode, mass resolution of 30,000, mass range of m/z 190 to 2,000, spray voltage of 2.0 kV, capillary temperature of 300 °C, S-lens RF voltage of 60.0%, maximum injection time of 10 ms, and 1 microscan. The system was controlled by Thermo Xcalibur software modules. Automated flash chromatography was carried out on a Teledyne ISCO CombiFlash Rf 200 system equipped with UV detection. All reagents were purchased from commercial sources and used without further purification. All solvents used for purification were of HPLC grade or higher.

Ichip fabrication and validation. Twenty ichips were machined from hydrophobic plastic polyoxymethylene and each contains three components: a top and bottom plate (both 75.0 x 22.0 x 2.5 mm) and a center plate (63.5 x 11.5 x 1.0 mm) with 1.0 mm diameter (1.25 μL) through-holes. Each ichip was fitted with two polycarbonate membranes (Sterlitech; 0.03 μm pore diameter) that were cut with a scalpel to the exact dimensions of the center plate. The following procedure was used for aseptic assembly of the ichips in a laminar flow hood. Membranes were autoclaved and the center and outer plates were sanitized by submersion in isopropanol (70% v/v) for a minimum of 15 min and then the isopropanol was allowed to evaporate in the laminar flow hood before assembling the ichip. All components of the ichips were manipulated with sterilized tweezers. The center plates bearing the growth chamber were inoculated by submerging it in molten (45 °C) agar medium and then gently agitated to ensure all through-holes were filled. The center plates were then removed, the growth medium was allowed to solidify and then the excess agar on the surface of the plates was scraped off using a sterilize glass microscope slide. The ichip components were then assembled as indicated in Figure 1*A* and fastened with screws. To validate the ichips' seals and the effectiveness of our aseptic assembly protocol, three randomly chosen ichips were fully assembled such that they contain sterile LB agar with 0.2 mg/mL of 5-bromo-4-chloro-3-indolyl-β-D-galactosidase (X-gal). Each ichip was placed in a 50 mL

conical tube containing a 12 h culture (LB broth) of NEB 10-beta cells containing pUC19 (New England BioLabs) and allowed to incubate for 24 h. The ichips contained no visibly blue through-holes after this test indicating external bacteria did not infiltrate the membrane barrier and contaminate the agar plugs in the center plate. Subsequently, three ichips were fully assembled with LB agar inoculated with 3% (v/v) of a 12 h culture (LB broth) of *E. coli* C3019 containing pUC19 and then placed in a conical tube containing sterile LB broth for 24 h. After the incubation period no bacterial growth was visible in the surrounding culture medium indicating bacteria contained in the ichip are unable to migrate out of the ichip.

Ichip inoculation and incubation. An individual sponge (*X. muta*) was located at a depth of 10-12 m on Runway 10 Reef (24º03'57.2"N, 74º32'41.3"W) in San Salvador, The Bahamas. Sponge tissue was collected and processed according to a previously reported protocol.[1] A portion of sponge containing outer surface tissue and inner surface tissue was resected using a dive knife and then stored in a sterile plastic bag for transport to our field laboratory for further processing. In the laboratory, a piece of wet sponge tissue weighing approximately 1 g was excised with a sterilized blade, rinsed three times with filter-sterilized (0.2 µm) seawater and then added to 9 mL of filter-sterilized seawater. The tissue was homogenized with a rotor stator that was sterilized by sequentially soaking in 0.525% sodium hypochlorite and isopropanol (70% v/v) for 5 min each. The rotor stator was rinsed in sterile water to remove residual isopropanol before homogenizing the sponge tissue. The homogenate was poured through a sterile nylon filter (100 µm; autoclaved) to yield a bacterial suspension. We assumed a bacterial density of $8.2 \times 10^9 \pm 7.7 \times 10^8$ cells/mL as previously determined for a Floridian sample of *X. muta*.[1] The suspension was diluted with 45 ºC 1/10 R2Am medium [1.8 g/L R2A Agar (Difco), 12 g/L Nobel agar (Sigma), 33.3 g/L Instant Ocean (pH = 7.9)] to the following bacterial densities: 5000, 2500, 1000, 500 and 100 cells/mL. Each diluted bacterial suspension was used to inoculate duplicate ichips as described above. Fully assembled ichips were returned to Runway 10 Reef for implantation in *X. muta*. A dive knife was used to create slits in sponge tissue adjacent to the site of resection and one ichip was fully inserted into each slit. Coloured pins were placed next to each ichip to indicate its inoculum cell density. Tissue collection, inoculum preparation, ichip assembly and ichip insertion into sponge tissue all occurred within a period of 24 h. The ten ichips were allowed to incubate in the sponge for 7 d before they were removed, packaged into sterile WhirlPak™ plastic bags and transported at ambient temperature to Charlottetown, PE, Canada for microbial domestication.

Microbial domestication. Ichips were processed for microbial domestication according to the published protocol.[2] All ichips were disassembled aseptically and agar plugs from each through-hole were deposited using a sterile paper clip into separate wells of a 48-well plate (each well contained ~ 1 mL of 1/10 R2Am medium). The agar plugs were flattened using the tip of a sterile wooden stick and then incubated for eight weeks at room temperature (~ 22 ºC) in the dark. After eight weeks, wells that contained morphologically distinct colonies were purified by serial subculturing on 1/10 R2Am. To identify bacterial isolates, a small portion each single colony was first dispersed in 50 µL of DMSO (Sigma). The DMSO cell suspension was used as the template DNA in PCR

reactions utilizing the 16S rRNA gene primers pA (5′ – AGAGTTTGATCCTGGCTCAG – 3′) and pH (5′ – AAGGAGGTGATCCAGCC – 3′).[3] PCR reactions contained EconoTaq PLUS Green 2X master mix (Lucigen) at a 1X concentration, 1 µM of each primer and 5% DMSO (v/v) containing suspended cells. Amplicons were directly sequenced using the following primers: 530R (5′–GTATTACCGCGGCTGCTG–3′), 514F (5′–GTGCCAGCASCCGCGG– 3′), 936R (5′–GGGGTTATGCCTGAGCAGTTTG–3′) and 1114F (5′–GCAACGAGCGCAACCC– 3′).[4,5] Sequences were assembled using Geneious (v7.1). *Alteromonas* sp. RKMC-009 was most closely related to *Alteromonas aestuariivivens* JDTF-113T (KY497472) with 98.15% sequence similarity.[6]

The following procedure was carried out to determine the evolutionary relationship of *Atleromonas* sp. RKMC-009 to all validly described *Alteromonas* spp., as well as the type representative for all other genera in the *Alteromonadaceae*. A BlastN search of the GenBank non-redundant nucleotide database (excluding sequences from uncultured/environmental samples) was used to identified previously cultured *Alteromonas* strains closely related (>99.5% 16S rRNA gene sequence identity) to RKMC-009.[7] Sequences from strains fitting this criterion were included in the analysis. *Pseudoalteromonas haloplanktis* (X67024) was used as the outgroup. Phylogenetic analyses were conducted using MEGA X.[8] Sequences were aligned using the MUSCLE implementation in MEGA X using default parameters.[9] The alignment was manually corrected and trimmed. The analysis involved 38 nucleotide sequences and a total of 1365 positions. Model testing in MEGA X determined the Kimura 2-parameter model with a Gamma distribution and invariable sites (K2+G+I) model best described the dataset.[9] Phylogenetic reconstruction was performed using neighbor-joining, unweighted pair group method with arithmetic means, maximum likelihood, maximum parsimony methods.[10] Bootstrap analysis using 1000 replications was used to assess reproducibility of branches in the tree topologies.[11]

Fermentation and metabolite purification. Seed cultures of *Alteromonas* sp. RKMC-009 in culture tubes were cultivated overnight in 13 mL of Marine Broth (Difco) at 30 ºC with orbital shaking (200 rpm). Seed cultures (30 mL) were used to inoculate ten Fernbach flasks each containing 1 L of BFM4m broth (12 g/L ADM Baker's Soy Flour, 1 g/L NH_4Cl, 12 g/L dextrose, 0.4 g/L agar, 1 g/L $CaCO_3$, 3 g/L NZ-amine A (Sigma), 18 g/L Instant Ocean, pH = 6.8). Fermentations were incubated for 3 d at 30 ºC with shaking at 200 rpm. Fermentations were pooled and then extracted 3X with 1.5 L of EtOAc. Pooled EtOAc extracts were dried *in vacuo* to afford 1.38 g of crude extract. Compound **1** was found to elute between 32.5 min and 35.0 min. Compound **1** was further purified for bioassays by semi-preparative HPLC using a SunFire C_{18} column (10 x 250 mm, 5 µm, Waters) with the following elution method [A = H_2O (0.1% formic acid) and B = CH_3CN (0.1% formic acid)]: 90% B from 0 min to 20 min (flow rate = 3 mL/min, UV = 230, 275 nm).

*N-Palmitoyl-α,O-dimethyl-L-tyrosine (**1**).* : $[\alpha]^{26}_D$ –4.6 (*c* 0.12, CH_3OH); IR (film) ν_{max} 2923, 2852, 1723, 1649, 1613, 1513; ^1H and ^{13}C NMR located in Table S1; ESI+ HRMS *m/z* 448.3419 $[M + H]^+$ (calcd for $C_{27}H_{46}NO_4^+$, 448.3421).

Marfey's analysis. Marfey's method for determination of amino acid configuration was

carried out as follows. To separate vials containing dried **1** (5 mg, 0.01 mmol) and *O*,α-dimethyl-DL-tyrosine (Santa Cruz Biotech, 5 mg, 0.02 mmol) was added 6 M HCl (1 mL), and then the mixtures were heated under reflux overnight with stirring. The reaction mixtures were dried *in vacuo* and portions of these hydrolysates (1 mg) were transferred to separate vials, to which 150 µL of deionized H_2O, 300 µL of N_α-(2,4-dinitro-5-fluorophenyl)-L-alaninamide (L-FDAA; 10 mg/mL in acetone), and 70 µL of aqueous $NaHCO_3$ (1 M) were added. The reaction mixtures were heated to 37.0 °C for 2 h, quenched with 70 µL of HCl (1 M), and then dried *in vacuo*. The same process for L-FDAA-derivatization was carried out with α-methyl-L-tyrosine (Sigma-Aldrich; 1 mg, 0.01 mmol). Dried L-FDAA derivatization reactions were suspended in CH_3OH (10 mg/mL) UHPLC-HRMS analysis. These data were acquired on a the following platform: Thermo Accela UHPLC Pump coupled to a Thermo LTQ Orbitrap Velos mass spectrometer fitted with an ESI source and a Thermo Accela PDA. A Kinetex core−shell 100 Å C_{18} column (2.1 × 50 mm, 1.7 µm, Phenomenex) was used with a mobile-phase flow rate of 0.5 mL/min and injection volume of 10 µL (samples were prepared in CH_3OH). The following elution method was used [A = H_2O (0.1% formic acid), B = CH_3CN (0.1% formic acid)]: 5% B from 0 to 2 min, linear gradient from 5% B at 2 min to 25% B at 55 min, linear gradient from 25% B at 55 min to 99% B at 57 min, 99% B from 57 to 60 min, linear gradient from 99%B at 60 min to 5% B at 63 min and 5% B from 63 to 70 min. The following HRMS parameters were used: positive ionization mode, mass resolution of 30,000, mass range of m/z 190 to 2,000, spray voltage of 3.4 kV, capillary temperature of 320 °C, S-lens RF voltage of 70.0%, maximum injection time of 10 ms, and 1 microscan. The system was controlled by Thermo Xcalibur software modules.

Solvolysis artifact experiment. To determine whether OCH_3-7 in **1** arises from methanolysis, triplicate culture tubes containing 5 mL of BFM4m each were inoculated with overnight seed cultures of RKMC-009 (3% v/v) and incubated with orbital shaking (200 rpm) at 30 ºC. After 3 d, each culture was extracted with 5 mL of EtOAc. Organic layers from each culture were dried *in vacuo* and resuspended in CH_3CN (500 µg/mL) for UHPLC-HRMS analysis.

Chemical synthesis. Compounds **1**-**4** were synthesized using modified literature methodology by *N*-acylation of the corresponding amino acid with palmitoyl chloride.[12] Compound **3** was dimethylated using a large excess of CH_3I in the presence of Cs_2CO_3 to generate **5**, which was treated with LiOH to afford **1**.[13] NMR spectra of synthetic compounds **1**-**5** are located in the SI Appendix.

*N-Palmitoyl-O-methyl-L-tyrosine (**2**).* *O*-Methyl-L-tyrosine (ACROS Organics; 500 mg, 2.56 mmol, 1.00 eq) was stirred with palmitoyl chloride (Alfa Aesar; 7.04 g, 25.61 mmol, 10 eq) in 25 mL of DMF at room temperature. After 16 h, the reaction was diluted with 250 mL of 1N HCl (aq) and extracted with EtOAc (3 X 100 mL). The combined EtOAc extracts were washed with saturated NaCl (aq) and dried *in vacuo*. The dried EtOAc extract was resuspended in 250 mL of CH_3CN and extracted with hexanes (5 X 100 mL). The CH_3CN layer was dried *in vacuo* and fractionated by automated flash column chromatography using a 25 g silica-pentafluorophenyl column (Silicycle) with the following elution method (A = H_2O and B = CH_3OH): 50% B from 0 to 3 min, linear

gradient from 50% B at 3 min to 100% B 25 min and 100% B from 25 to 35 min (flow rate = 30 mL/min, UV = 230, 275 nm). Compound **2** (1.03 g, 2.38 mmol, 93%) was obtained as an amorphous white solid: $[\alpha]^{26}_D$ +17.1 (*c* 0.46, CH_3OH); IR (film) ν_{max} 3296, 2919, 2850, 1730, 1706, 1642, 1614, 1534, 1514 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.29 (1H, s, COO*H*), 7.07 (2H, d, *J* = 8.6 Hz, H-5, H-9), 6.83 (2H, d, *J* = 8.6 Hz, H-6, H-8), 5.97 (1H, d, *J* = 7.4 Hz, CON*H*), 4.82 (1H, dt, *J* = 7.4, 5.9 Hz, H-2), 3.78 (3H, s, OC*H*$_3$), 3.17 (1H, dd, *J* = 14.2, 5.8 Hz, H-3b), 3.07 (1H, dd, *J* = 14.2, 5.8 Hz, H-3a), 2.18 (2H, td, *J* = 7.7, 1.7 Hz, H-2'), 1.56 (2H, m, H-3'), 1.21-1.33 (24H, m, H-4', H-5', H-6', H-7', H-8', H-9', H-10', H-11', H-12', H-13', H-14', H-15'), 0.88 (3H, t, *J* = 7.0 Hz, H-16'); ^{13}C NMR ($CDCl_3$, 151 MHz) δ 175.09 (C, C-1), 174.1 (C, C-1'), 158.9 (C, C-7), 130.5 (CH, C-5, C-9), 127.7 (C, C-4), 114.2 (CH, C-6, C-8), 55.3 (CH_3, O*C*H3), 53.5 (CH, C-2), 36.6 (CH_2, C-2'), 36.5 (CH_2, C-3), 32.1 (CH_2, C-14'), 29.8 (CH_2), 29.8 (CH_2), 29.8 (CH_2), 29.8 (CH_2), 29.8 (CH_2), 29.8 (CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.5 (CH_2), 29.3 (CH_2, C-4'), 25.7 (CH_2, C-3'), 22.8 (CH_2, C-15'), 14.3 (CH_3, C-16'); ESI+ HRMS *m/z* 434.3262 $[M + H]^+$ (calcd for $C_{26}H_{44}NO_4^+$, 434.3265).

*N-Palmitoyl-α-methyl-L-tyrosine (**3**)*. α-Methyl-L-tyrosine (Sigma-Aldrich; 200 mg, 1.02 mmol, 1.00 eq) was stirred with palmitoyl chloride (Alfa Aesar; 2.82 g, 10.26 mmol, 10 eq) in 10 mL of DMF at room temperature. After 16 h, the reaction was diluted with 100 mL of 1N HCl (aq) and extracted with EtOAc (3 X 100 mL). The combined EtOAc extracts were washed with saturated NaCl (aq) and dried *in vacuo*. The dried EtOAc extract was resuspended in 250 mL of CH_3CN and extracted with hexanes (5 X 100 mL). The CH_3CN layer was dried *in vacuo* and fractionated using automated flash column chromatography (identical conditions as for **2**) to afford **3** (394 mg, 0.91 mmol, 89%) as an amorphous white solid: $[\alpha]^{26}_D$ –18.1 (*c* 0.20, CH_3OH); IR (film) ν_{max} 3326, 2923, 2853, 2156, 1716, 1646, 1615, 1516 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.95 (2H, d, *J* = 8.6 Hz, H-5, H-9), 6.72 (2H, d, *J* = 8.6 Hz, H-6, H-8), 6.10 (1H, s, CON*H*), 3.38 (1H, d, *J* = 13.7 Hz, H-3b), 3.17 (1H, d, *J* = 13.7 Hz, H-3a), 2.17 (2H, t, 7.9 Hz, C-2'), 1.63 (3H, s, C*H*$_3$-2), 1.58 (2H, m, H-3'), 1.21-1.32 (24H, m, H-4', H-5', H-6', H-7', H-8', H-9', H-10', H-11', H-12', H-13', H-14', H-15'), 0.88 (3H, t, *J* = 6.7 Hz, H-16'); ^{13}C NMR ($CDCl_3$, 151 MHz) δ 177.7 (C, C-1), 174.5 (C, C-1'), 155.1 (C, C-7), 131.3 (CH, C-5, C-9), 127.6 (C, C-4), 115.5 (CH, C-6, C-8), 61.5 (C, C-2), 40.6 (CH_2, C-3), 37.3 (CH_2, C-2'), 32.1 (CH_2, C-14'), 29.9 (CH_2), 29.9 (CH_2), 29.9 (CH_2), 29.8 (CH_2), 29.8 (CH_2), 29.8 (CH_2), 29.7 (CH_2), 29.5 (CH_2), 29.5 (CH_2), 29.4 (CH_2, C-4'), 25.7 (CH_2, C-3'), 23.3 (CH_2, *C*H_3-2), 22.8 (CH_2, C-15'), 14.3 (CH_3, C-16'); ESI+ HRMS *m/z* 434.3260 $[M + H]^+$ (calcd for $C_{26}H_{44}NO_4^+$, 434.3265).

*N-Palmitoyl-L-tyrosine (**4**).* L-Tyrosine (AMRESCO; 500 mg, 2.76 mmol, 1.00 eq) was stirred with palmitoyl chloride (Alfa Aesar; 7.59 g, 27.60 mmol, 10 eq) in 25 mL of DMF at room temperature. After 16 h, the reaction was diluted with 250 mL of 1N HCl (aq) and extracted with EtOAc (3 X 100 mL). The combined EtOAc extracts were washed with saturated NaCl (aq) and dried *in vacuo*. The dried EtOAc extract was resuspended in 250 mL of CH_3CN and extracted with hexanes (5 X 100 mL). The CH_3CN layer was dried *in vacuo* and fractionated using automated flash column chromatography (identical conditions as for **2**) to afford **4** (1.09 g, 2.59 mmol, 94%): $[\alpha]^{26}_D$ +17.4 (*c* 0.26, CH_3OH); IR (film) ν_{max} 3312, 3234, 2915, 2848, 2516, 1705, 1643, 1541, 1516 cm^{-1}; ^1H

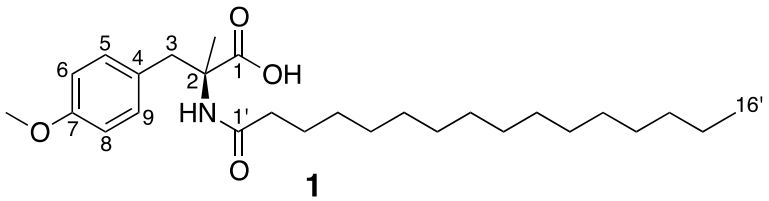
NMR ($CDCl_3$, 400 MHz) *δ* 12.54 (1H, s, COO*H*), 9.16 (1H, s, O*H*-7), 7.99 (1H, d, *J* = 8.2 Hz, CON*H*), 6.99 (2H, d, *J* = 8.5 Hz, H-5, H-9), 6.63 (2H, d, *J* = 8.5 Hz, H-6, H-8), 4.32 (1H, m, H-2), 2.90 (1H, dd, *J* = 14.1, 4.8 Hz, H-3b), 2.71 (1H, dd, *J* = 14.1, 9.6 Hz, H-3a), 2.03 (2H, t, *J* = 7.4 Hz, H-2'), 1.39 (2H, m, H-3'), 1.14-1.24 (24H, m, H-4', H-5', H-6', H-7', H-8', H-9', H-10', H-11', H-12', H-13', H-14', H-15'), 0.85 (3H, t, *J* = 7.0 Hz, H-16'); ^{13}C NMR ($CDCl_3$, 151 MHz) *δ* 173.4 (C, C-1), 172.1 (C, C-1'), 155.9 (C, C-7), 127.7 (C, C-4), 130.0 (CH, C-5, C-9), 114.9 (CH, C-6, C-8), 53.6 (CH, C-2), 36.0 (CH_2, C-2'), 35.1 (CH_2, C-3), 31.3 (CH_2, C-14'), 29.1 (CH_2), 29.1 (CH_2), 29.1 (CH_2), 29.1 (CH_2), 29.1 (CH_2), 29.0 (CH_2), 28.9 (CH_2), 28.8 (CH_2), 28.7 (CH_2), 28.5 (CH_2, C-4'), 25.2 (CH_2, C-3'), 22.1 (CH_2, C-15'), 14.0 (CH_3, C-16'); ESI+ HRMS *m/z* 420.3091 $[M + H]^+$ (calcd for $C_{25}H_{42}NO_4^+$, 420.3108).

*N-Palmitoyl-α,O-dimethyl-L-tyrosine methyl ester (**5**).* To a solution of **3** (75 mg, 0.17 mmol, 1.00 eq) in DMSO (5 mL) was added Cs_2CO_3 (Sigma-Aldrich; 118 mg, 0.36 mmol, 2.10 eq) and CH_3I (Sigma-Aldrich; 1.06 mL, 17.30 mmol, 100.00 eq), which were stirred for 16 h at room temperature. The reaction was diluted with 10 mL of H_2O and extracted with $CHCl_3$ (3 x 50 mL). The combined $CHCl_3$ extracts were washed with saturated NaCl (aq) and dried *in vacuo*. The dried $CHCl_3$ extract was fractionated using automated flash column chromatography (identical conditions as for **2**) to afford **5** (21 mg, 0.05 mmol, 27%): ^1H NMR ($CDCl_3$, 400 MHz) *δ* 6.95 (2H, d, *J* = 8.7 Hz), 6.79 (2H, d, *J* = 8.7 Hz), 6.01 (1H, s), 3.77 (3H, s), 3.77 (3H, s), 3.49 (1H, d, *J* = 13.5 Hz), 3.13 (1H, d, *J* = 13.5 Hz), 2.13 (2H, t, *J* = 7.09 Hz), 16.4 (3H, s), 1.60 (1H, m), 1.23-1.31 (24H, m), 0.89 (3H, t, *J* = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 151 MHz) *δ* 174.8, 172.6, 158.7, 131.0, 128.6, 113.8, 61.3, 55.3, 52.7, 40.6, 37.4, 32.1, 29.8, 29.8, 29.8, 29.8, 29.8, 29.8, 29.7, 29.5, 29.5, 29.4, 25.7, 23.4, 22.8, 14.3. ESI+ HRMS *m/z* 462.3582 $[M + H]^+$ (calcd for $C_{28}H_{48}NO_4^+$, 462.3578).

*N-Palmitoyl-α,O-dimethyl-L-tyrosine (**1**).* To a solution of **5** (10 mg, 0.02 mmol, 1.00 eq) in 75% THF (aq) (1 mL) was added LiOH (aq) (Sigma-Aldrich; 100 µL of 10 mg/mL solution, 0.04 mmol, 2.00 eq), which was stirred for 48 h at room temperature. The reaction was diluted with 10 mL of H_2O and extracted with $CHCl_3$ (3 x 50 mL). The combined $CHCl_3$ extracts were washed with saturated NaCl (aq) and dried *in vacuo*. The dried $CHCl_3$ extract was fractionated using automated flash column chromatography (identical conditions as for **2**) to afford **1** (9 mg, 0.02 mmol, 95%): $[\alpha]^{26}_D$ –5.1 (*c* 0.15, CH_3OH); IR (film) ν_{max} 2923, 2852, 1723, 1649, 1613, 1513; ^1H and ^{13}C NMR are located in Figure S9-10; ESI+ HRMS *m/z* 448.3425 $[M + H]^+$ (calcd for $C_{27}H_{46}NO_4^+$, 448.3421).

Evaluation of antimicrobial activity and cytotoxicity. The following five *Enterococcus* spp. were isolated from clinical specimens at the Atlantic Veterinary College (AVC) by the AVC Diagnostic Services Bacteriology Laboratory. Isolates were identified using the Bruker microflex LT MALDI-TOF with MBT Compass version 4.179. The direct colony transfer method was used with the Bruker Matrix HCCA (α-cyano-4-hydroxycinnamic acid) overlay, following manufacturer guidelines. Score values between 2.00 and 3.00 were considered high confidence identifications. The following isolates (with source species) were identified: *E. faecium* 15337 (feline), *E. faecalis*

16371 (canine), *E. gallinarum* 20993 (erinaceine), *E. casseliflavus* 15984 (equine) and *E. hirae* 17446 (avine). Antimicrobial activity of **1**-**4** was evaluated against all five clinical *Enterococcus* isolates in addition to methicillin-resistant *Staphylococcus aureus* ATCC 33591 (MRSA), *S. warneri* ATCC 17917, vancomycin-resistant *Enterococcus faecium* EF379 (VRE), *Pseudomonas aeruginosa* ATCC 14210, *Proteus vulgaris* ATCC 12454 and *Candida albicans* ATCC 14035. All testing was carried out in triplicate according to the Clinical Laboratory Standards Institute testing standards in a 96-well plate microbroth dilution assay as previously described.[14] Optical density was measured using a Thermo Scientific Varioskan Flash plate reader at 600 nm, recording at time zero and then again after incubation for 22 h (37 °C) to determine percent growth inhibition. Cytotoxicity was evaluated against Vero kidney cell line from African green monkey (*Cercopithecus aethiops*), MCF7 human breast adenocarcinoma cells (ATCC HTB-22), human breast adenocarcinoma cells (ATCC HTB-26) and HCT-116 human colorectal carcinoma cells (ATCC CCL-247). All assays were carried out as described previously.[14] Fluorescence was measured using a Thermo Scientific Varioskan Flash plate reader at 560/12 excitation, 590 nm emission both at time zero and 4 h after alamarBlue (Invitrogen) addition. For both antimicrobial and cytotoxicity data, growth inhibition was expressed as a percentage and plotted against the logarithm of concentration. Four-parameter dose-response curves were fit to these data using the variable slope model in GraphPad Prism 8.0.2.



1

Table S1. NMR spectroscopic data (^{1}H 400 MHz, ^{13}C 101 MHz, $CDCl_3$) of compound **1.**

Position		δ_C, Type		δ_H, mult.(*J* in Hz)	COSY	HMBC
1	COOH	177.3,	C	8.61, s(br)		
2		61.3,	C			
	CH_3	23.4,	CH_3	1.64, s		1, 2, 3a/3b
	NH			6.01, s		1, 2, 2-CH_3, 3, 1'
3a		40.3,	CH_2	3.26, d(13.7)	3b	1, 2, 2-CH_3 4, 5, 9
3b		40.3,	CH_2	3.39, d(13.7)	3a	1, 2, 2-CH_3 4, 5, 9
4		128.1,	C			
5		131.2,	CH_2	7.05, d(8.6)	6	2, 3, 6, 7, 9
6		113.9,	CH_2	6.80, d(8.6)	5	4, 5, 7
7		158.8,	C			
	OCH_3	55.3,	CH_3	3.77, s		7
8		113.9,	CH_2	6.80, d(8.6)	9	4, 7, 9
9		131.2,	CH_2	7.05, d(8.6)	8	2, 3, 5, 7, 8
1'		174.1,				
2'		37.2,	CH_2	2.17, t(8.1)	3'	1', 3', 4'
3'		25.7,	CH_2	1.59, m	2', 4'	1', 2', 4'
4'		29.4,	CH_2	1.26, m		
5'[a]		29.5,	CH_2	1.26, m		
6'[a]		29.6,	CH_2	1.26, m		
7'[a]		29.8,	CH_2	1.26, m		
8'[a]		29.8,	CH_2	1.26, m		
9'[a]		29.8,	CH_2	1.26, m		
10'[a]		29.8,	CH_2	1.26, m		
11'[a]		29.8,	CH_2	1.26, m		
12'[a]		29.8,	CH_2	1.26, m		
13'[a]		29.8,	CH_2	1.26, m		
14'		32.1,	CH_2	1.25, m		
15'		22.8,	CH_2	1.27, m		
16'		14.3,	CH_3	0.88, t(6.6)	15'	14', 15'

[a]Signals for 5'-13' are interchangeable.

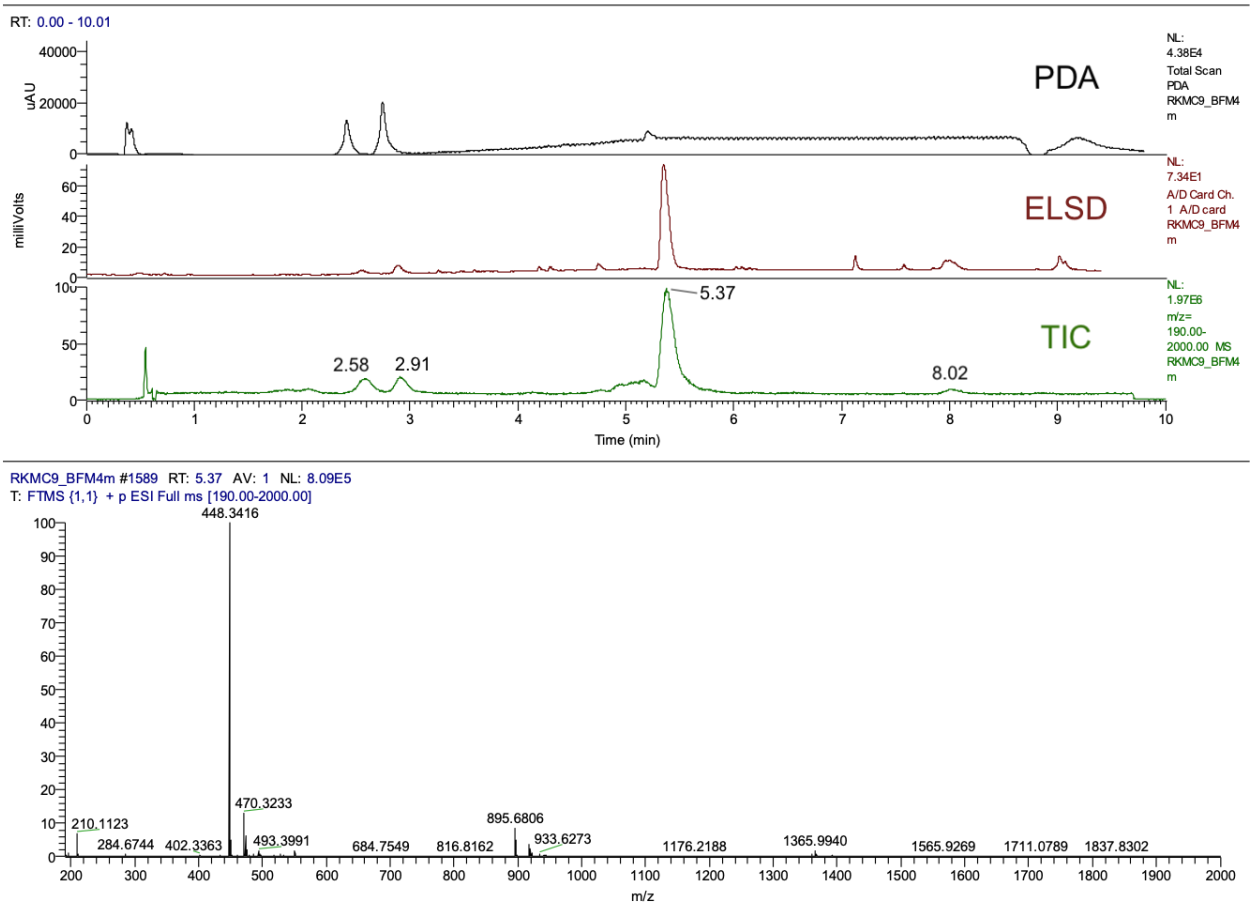


Figure S1. Ultra high pressure liquid chromatography-high resolution mass spectrometry (UHPLC-HMRS) analysis of the ethyl acetate extract of a 5 mL culture of *Alteromonas* sp. RKMC-009. Compound **1** elutes at 5.37 min (PDA = photodiode array, ELSD = evaporative light-scattering detector, TIC = total ion current).

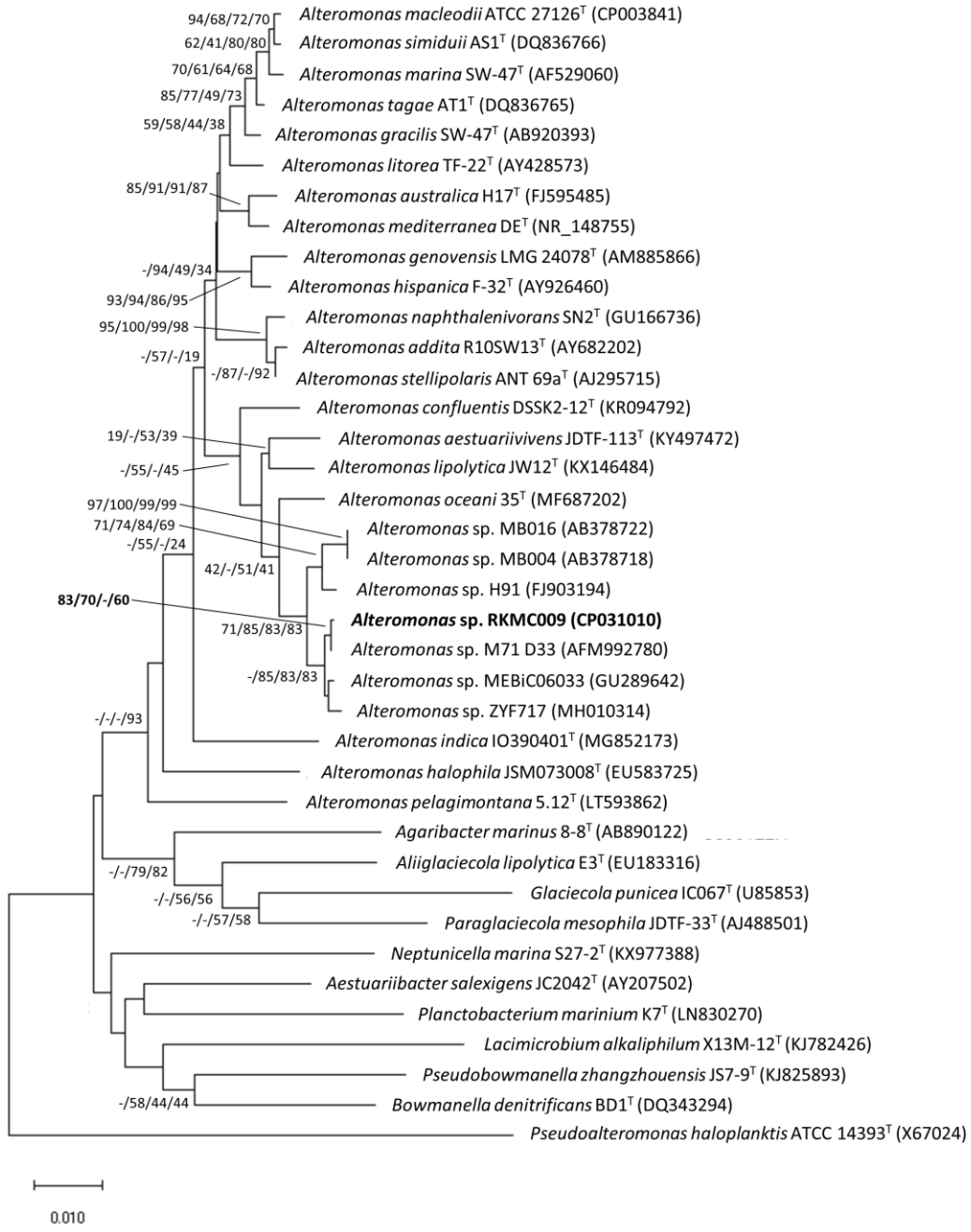


Figure S2. Neighbour-joining tree based on 38 16S rRNA gene sequences, which shows the phylogenetic relationships among *Alteromonas* sp. RKMC-009 and selected taxa from the *Alteromonadaceae*. Numbers at the nodes indicate bootstrap values (1000 replicates) for maximum parsimony, unweighted pair group method and arithmetic mean, maximum likelihood and neighbour-joining analyses (respectively). Only nodes with bootstrap values exceeding 50% in at least one analysis are shown. A dash indicates the node was not observed. *Pseudoalteromonas haloplanktis* was used as the outgroup. Scale bar = 0.01 changes per nucleotide position.

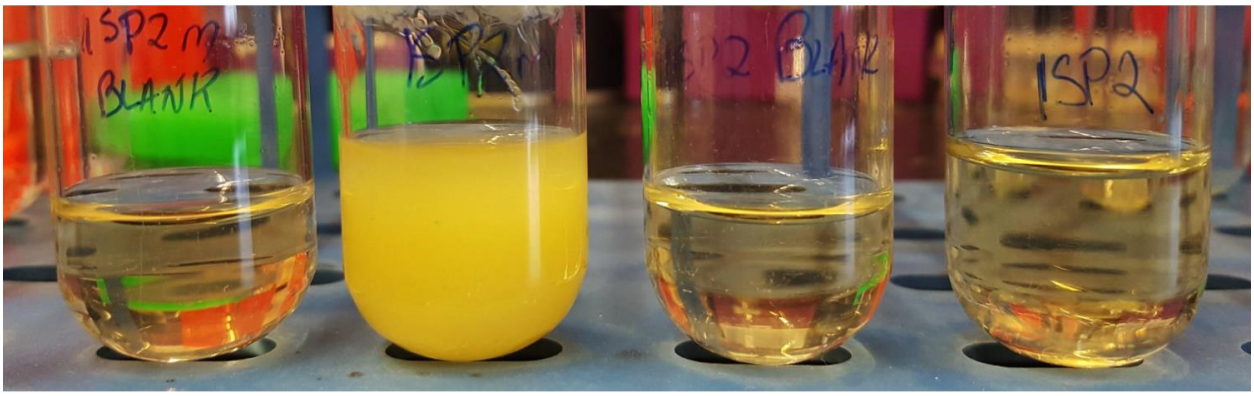


Figure S3. Small-scale fermentations of *Alteromonas* sp. RKMC-009 demonstrating the requirement of marine salts for this strain to grow. Growth media with (ISP2) and without marine salts [ISP2m; 18 g/L Instant Ocean (Spectrum Brands)] were inoculated with multiple colonies of RKMC-009. Growth was observed only in ISP2m. Blank cultures were not inoculated and are included for comparison to those that were inoculated with RKMC-009.

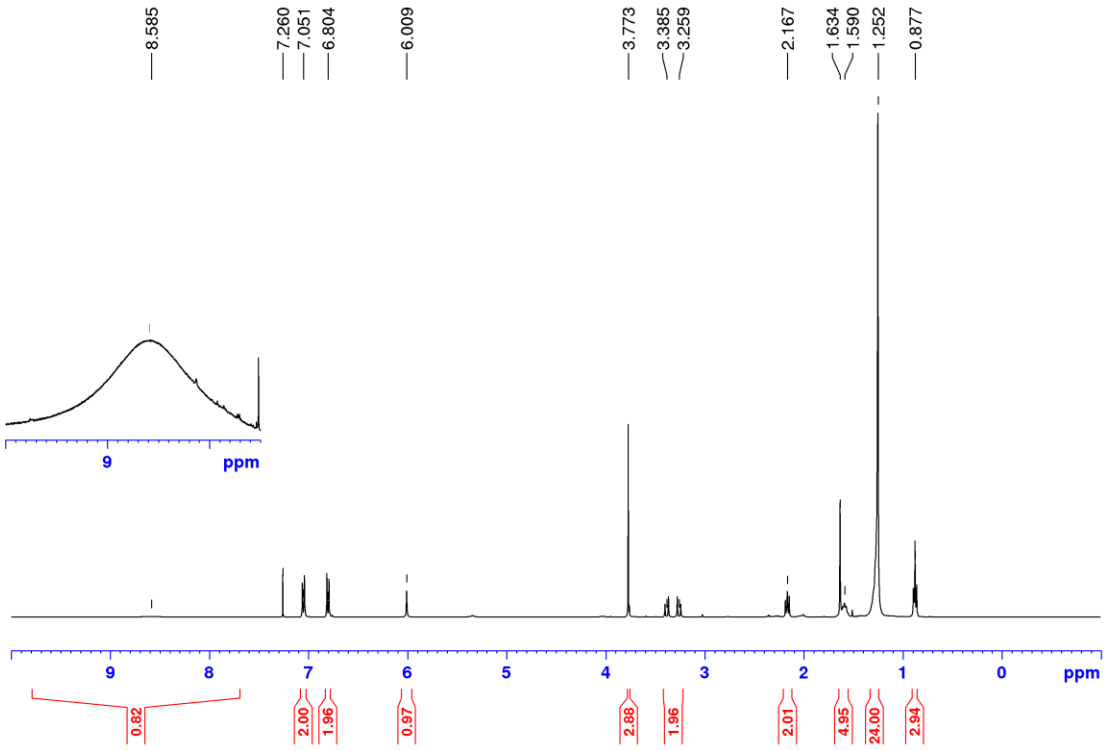


Figure S4. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound **1.**

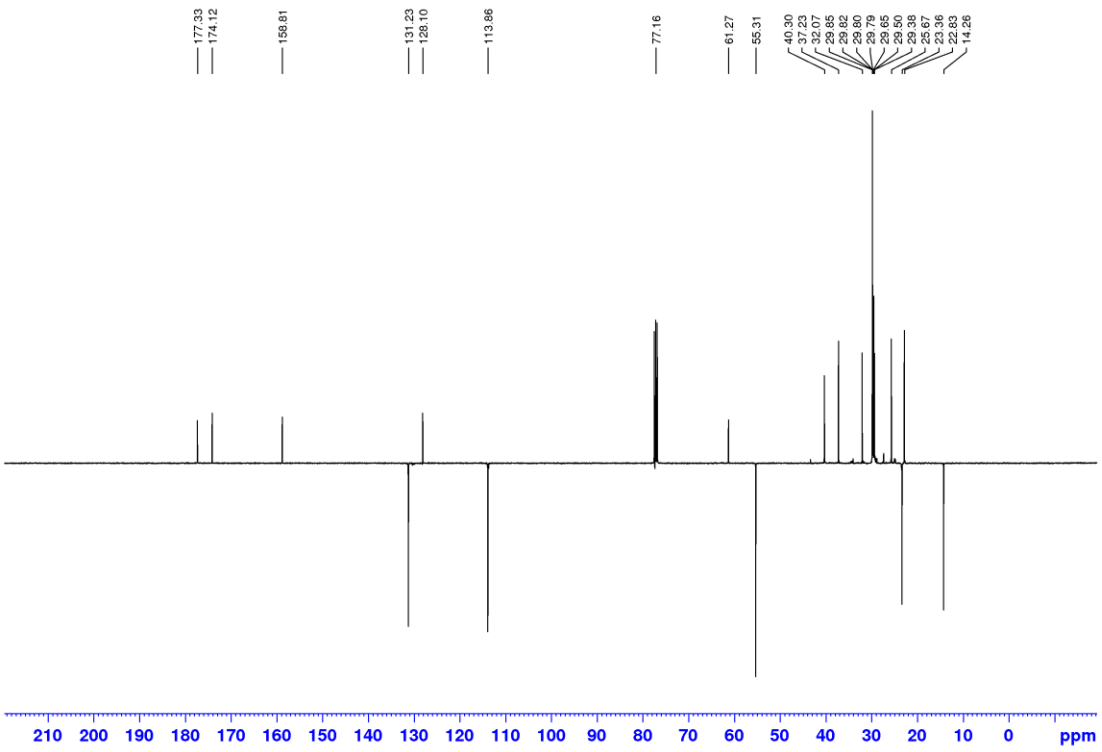


Figure S5. DEPTQ-135 NMR spectrum (101 MHz, $CDCl_3$) of compound **1.**

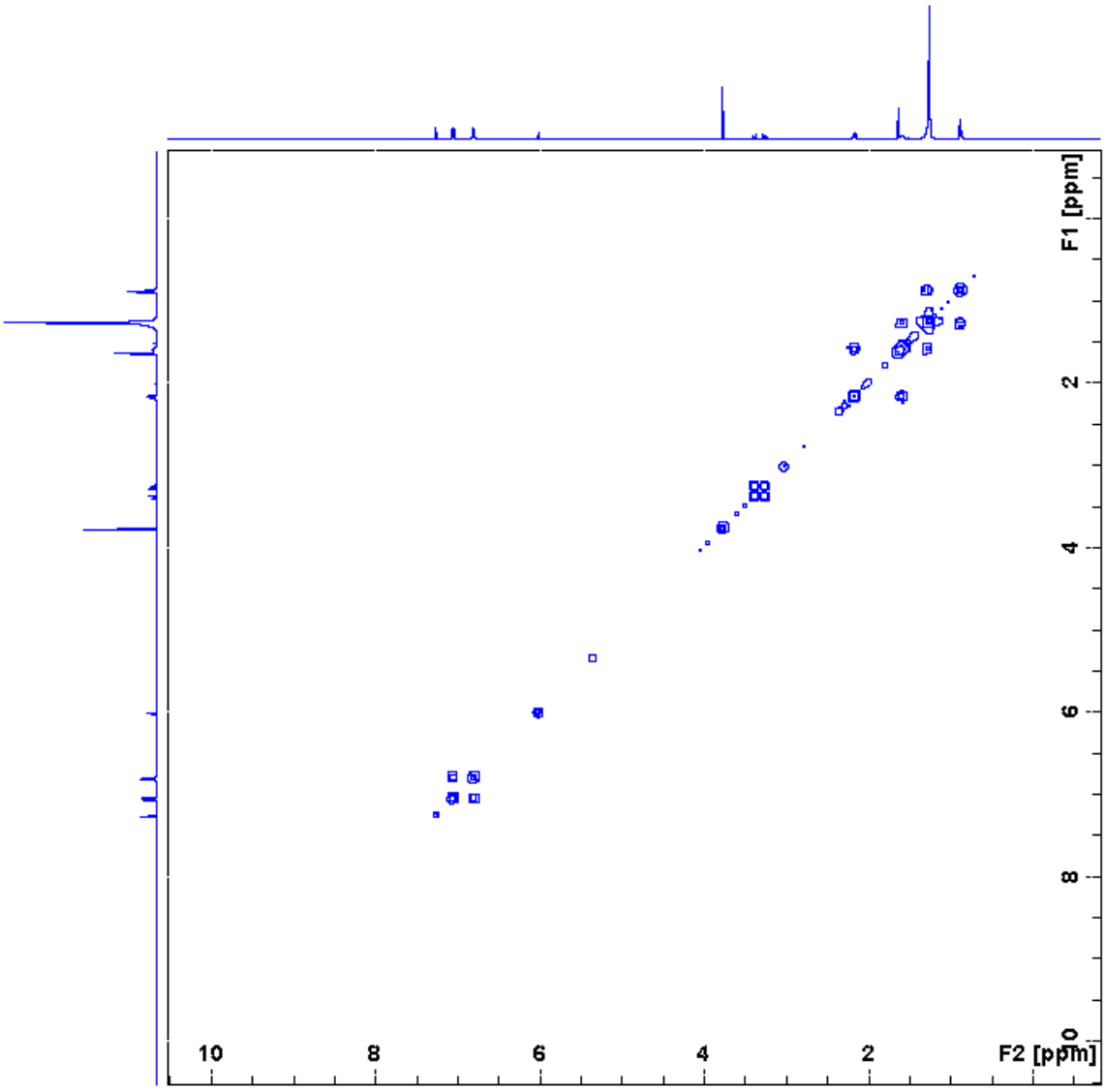


Figure S6. COSY NMR spectrum (400 MHz, $CDCl_3$) of compound **1.**

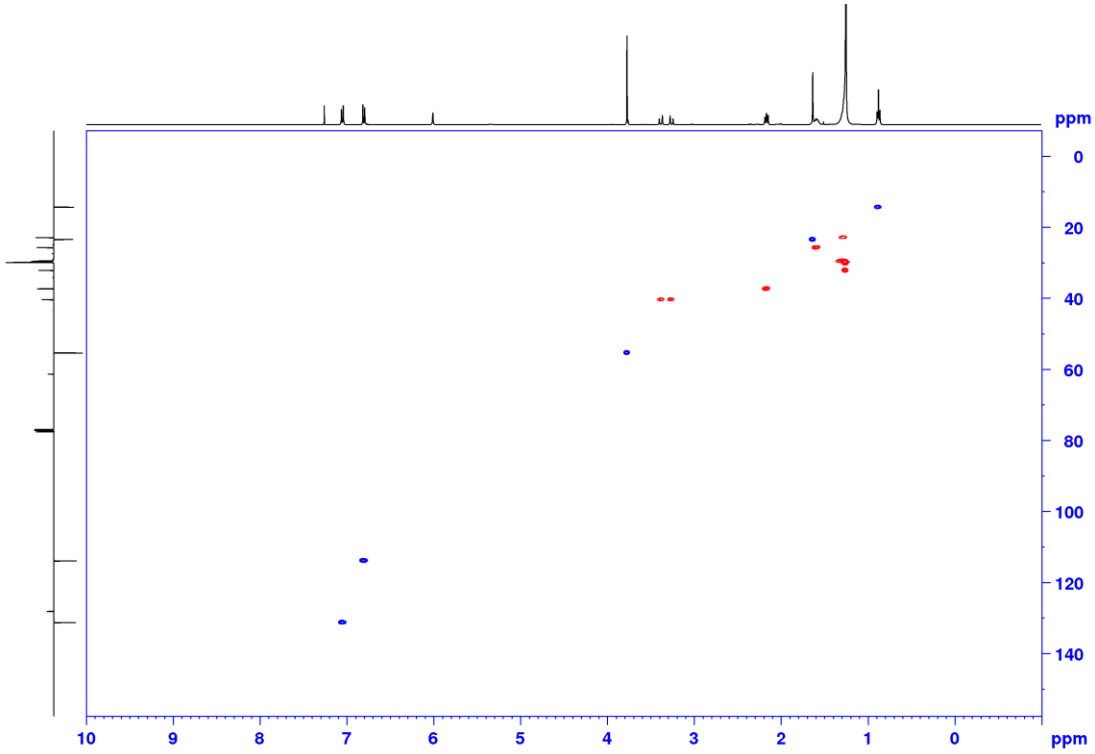


Figure S7. HSQC NMR spectrum (^{1}H 400 MHz, ^{13}C 101 MHz, $CDCl_3$) of compound **1**. Blue contours are positively phased (methyl and methine) and red contours are negatively phased (methylene).

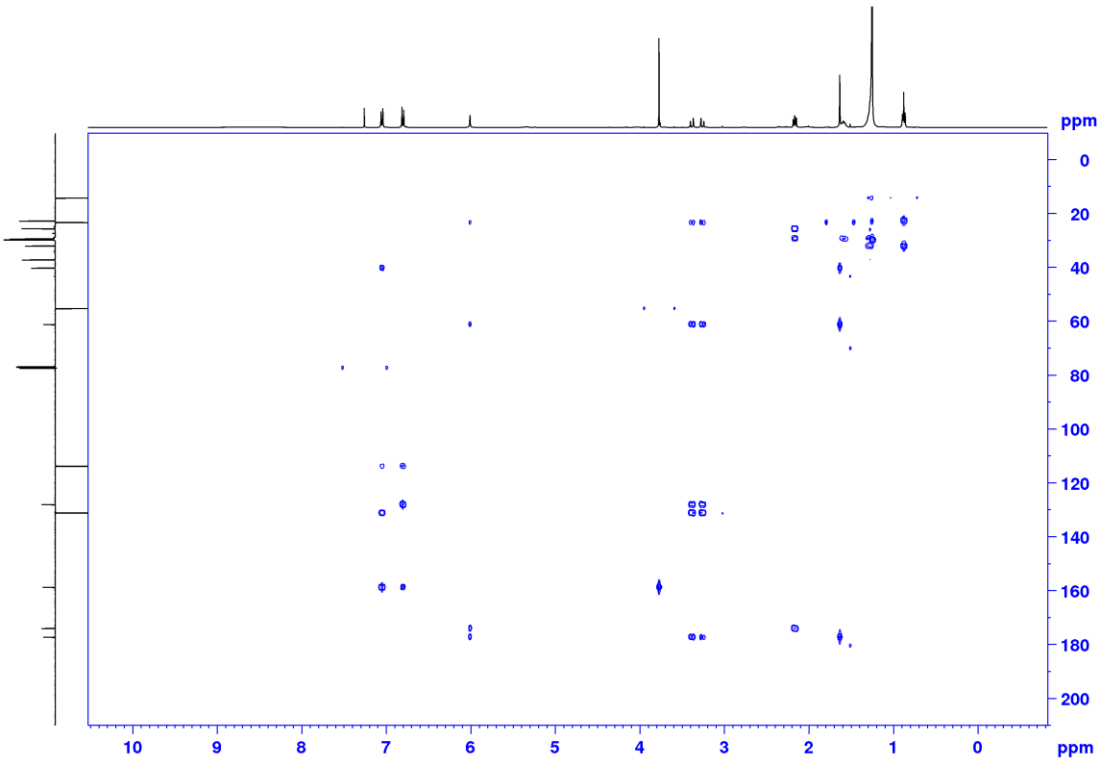


Figure S8. HMBC NMR spectrum (^{1}H 400 MHz, ^{13}C 101 MHz, $CDCl_3$) of compound **1.**

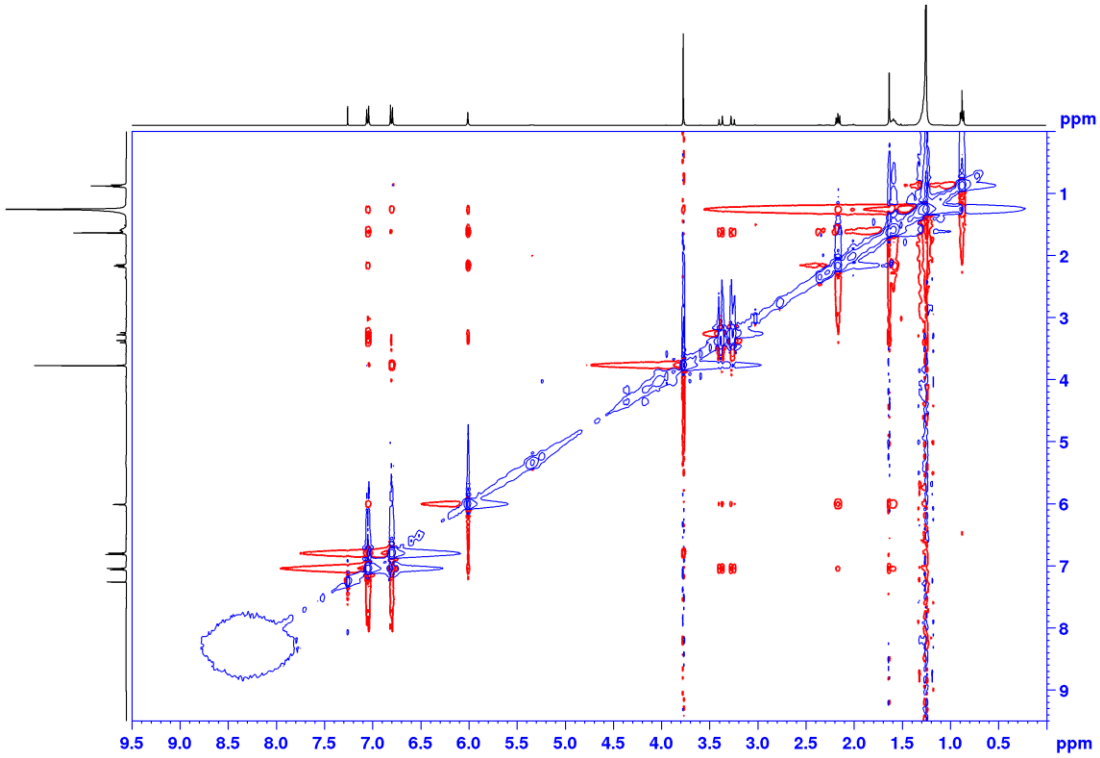


Figure S9. NOESY NMR spectrum (400 MHz, $CDCl_3$) of compound **1.**

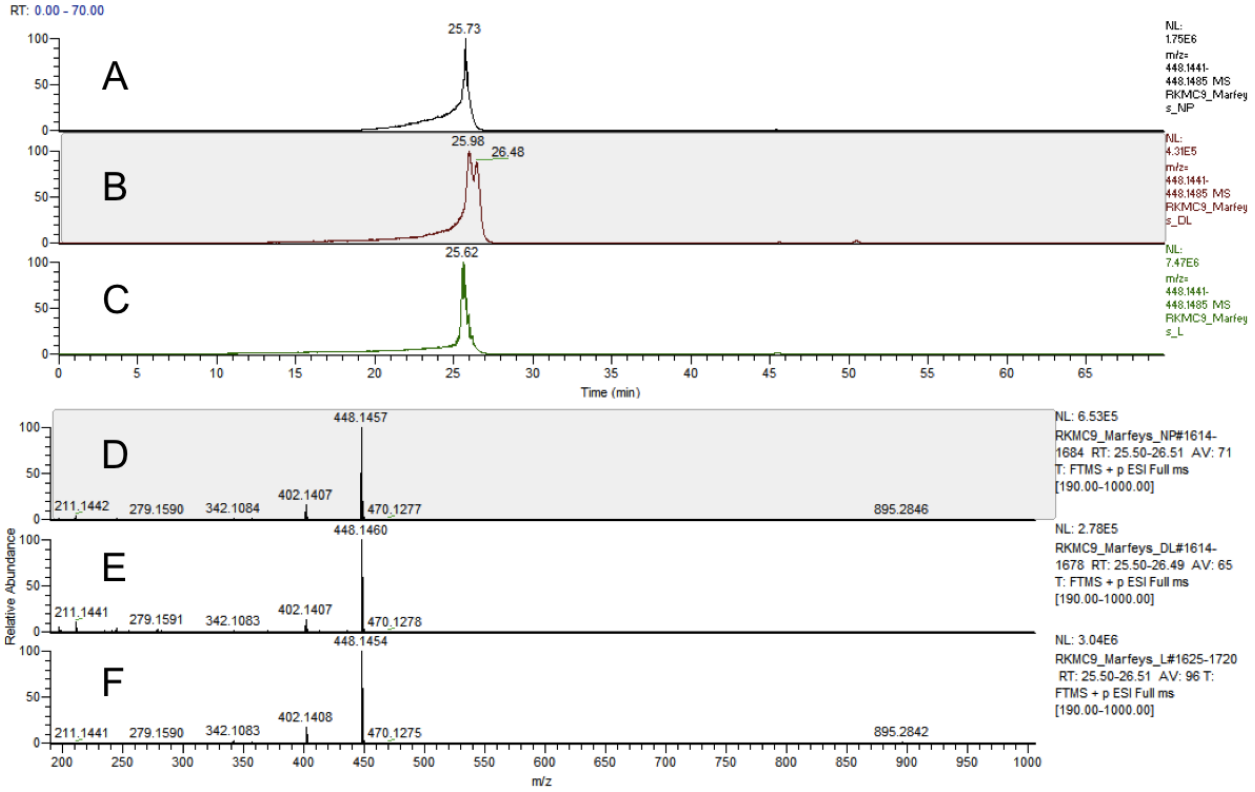


Figure S10. Marfey's analysis of compound **1**. Extracted ion chromatograms (*m/z* 448.1463) of L-FDAA-derivatized *(A)* **1** (hydrolysate), *(B)* α,*O*-dimethyl-DL-tyrosine (hydrolysate) and *(C)* α-methyl-L-tyrosine with the corresponding mass spectra in *(D)*-*(F)* (respectively).

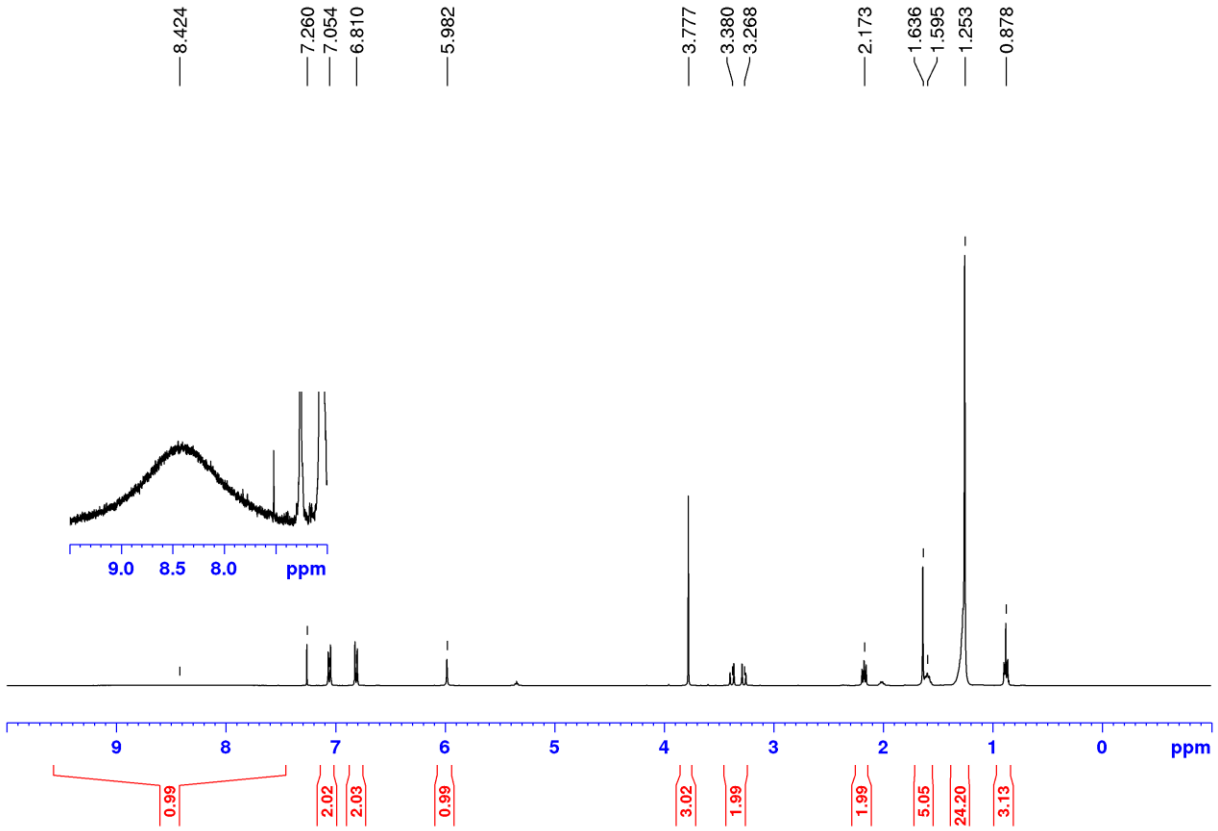


Figure S11. ^{1}H NMR spectrum of compound **1** (synthetic).

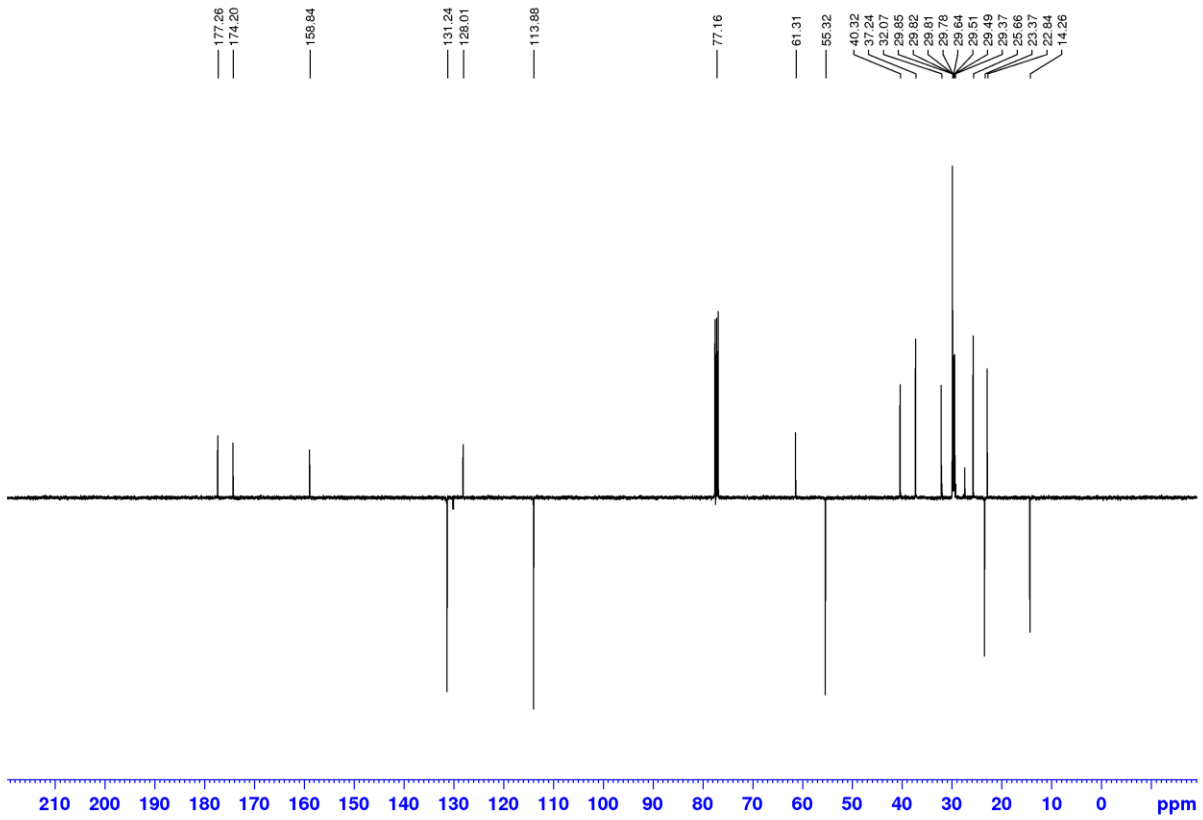


Figure S12. DEPTQ-135 NMR spectrum of compound **1** (synthetic).

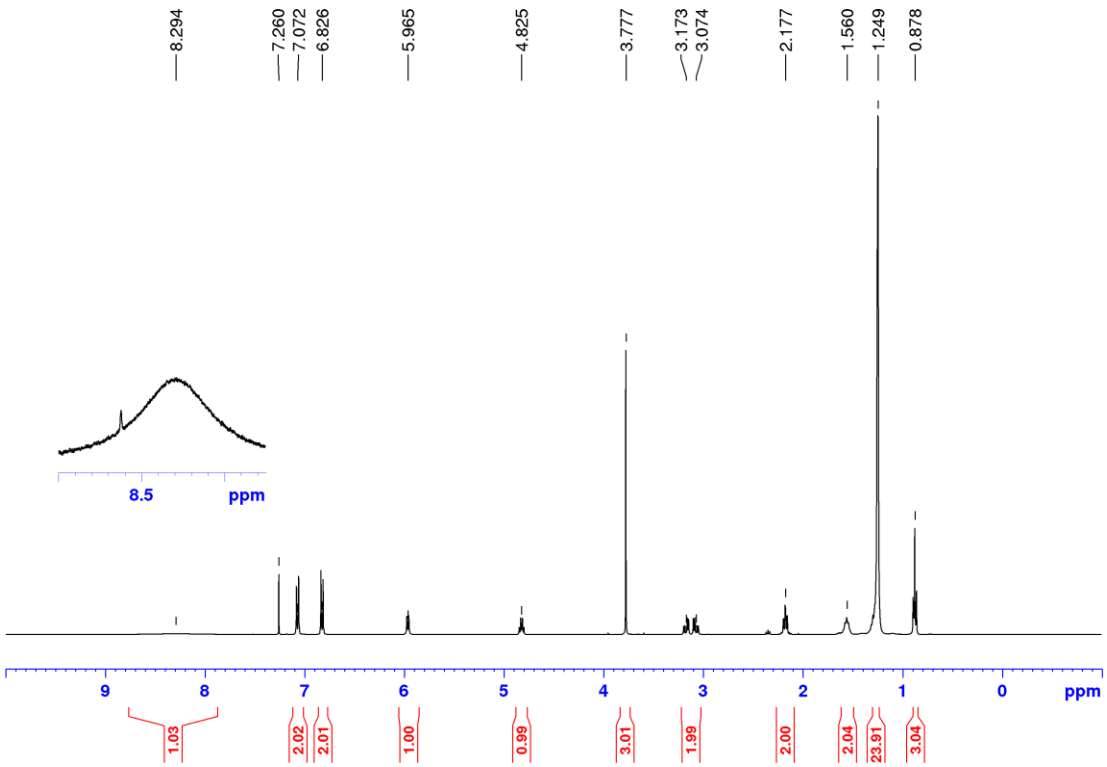


Figure S13. ^{1}H NMR spectrum of compound **2**.

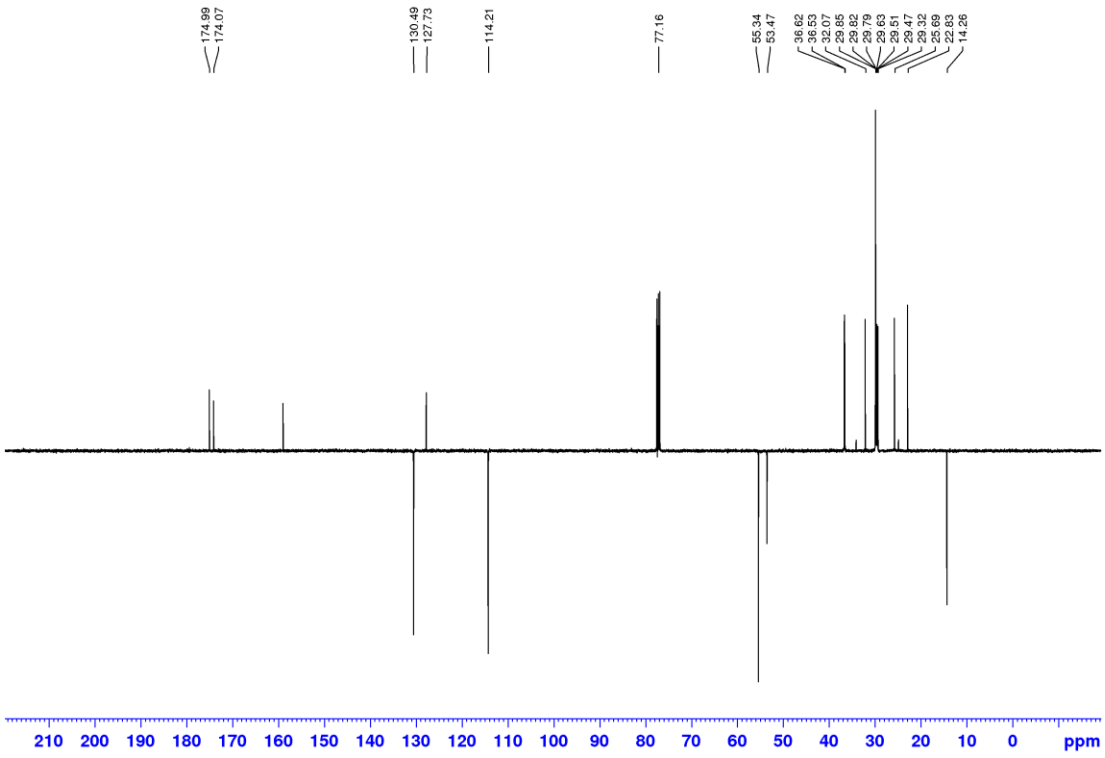


Figure S14. DEPTQ-135 NMR spectrum of compound **2**.

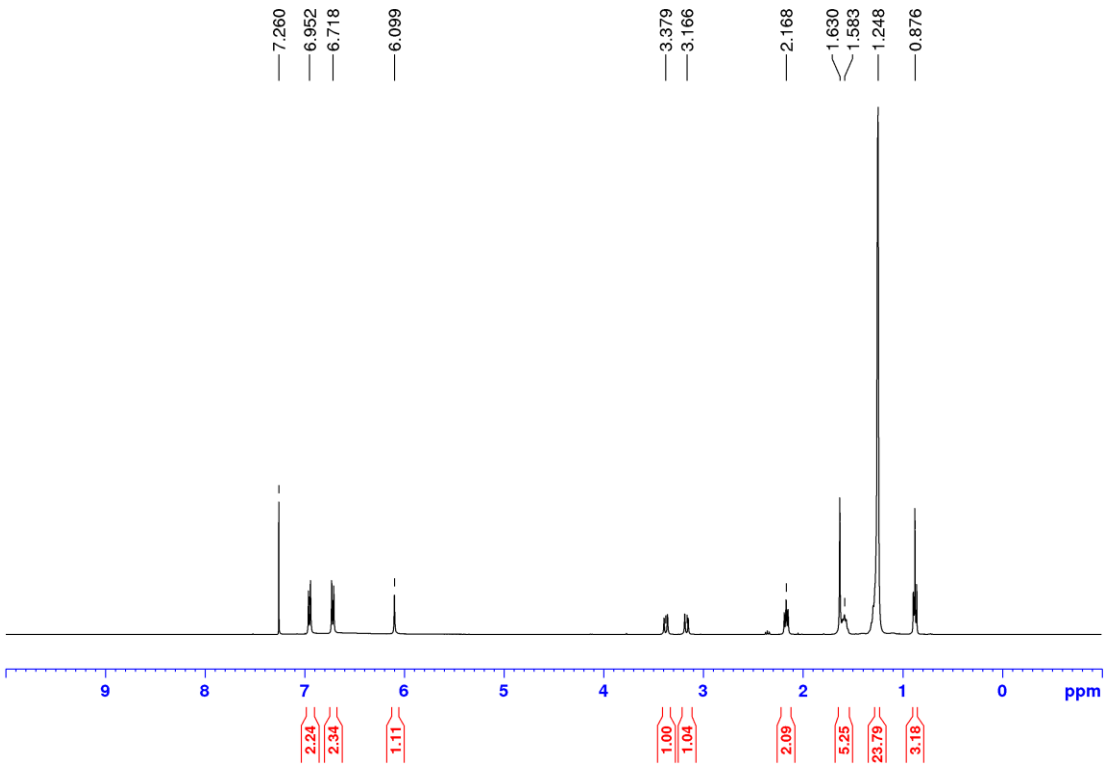


Figure S15. ^{1}H NMR spectrum of compound **3**.

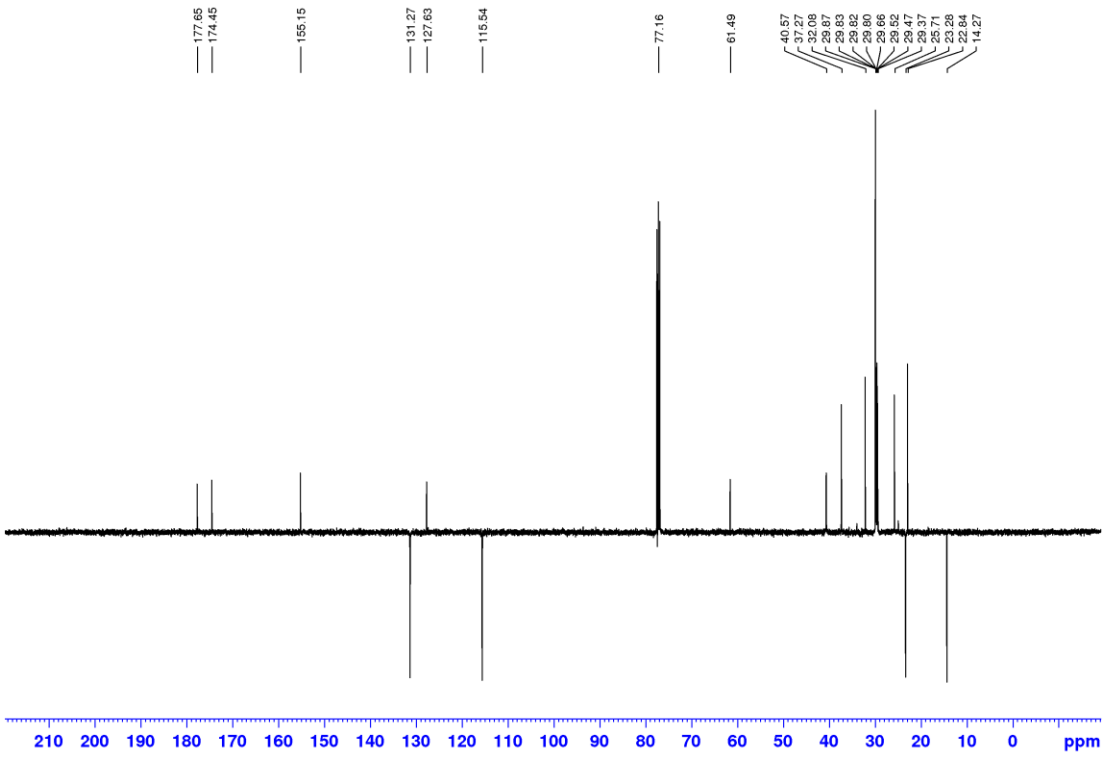


Figure S16. DEPTQ-135 NMR spectrum (101 MHz, $CDCl_3$) of compound **3**.

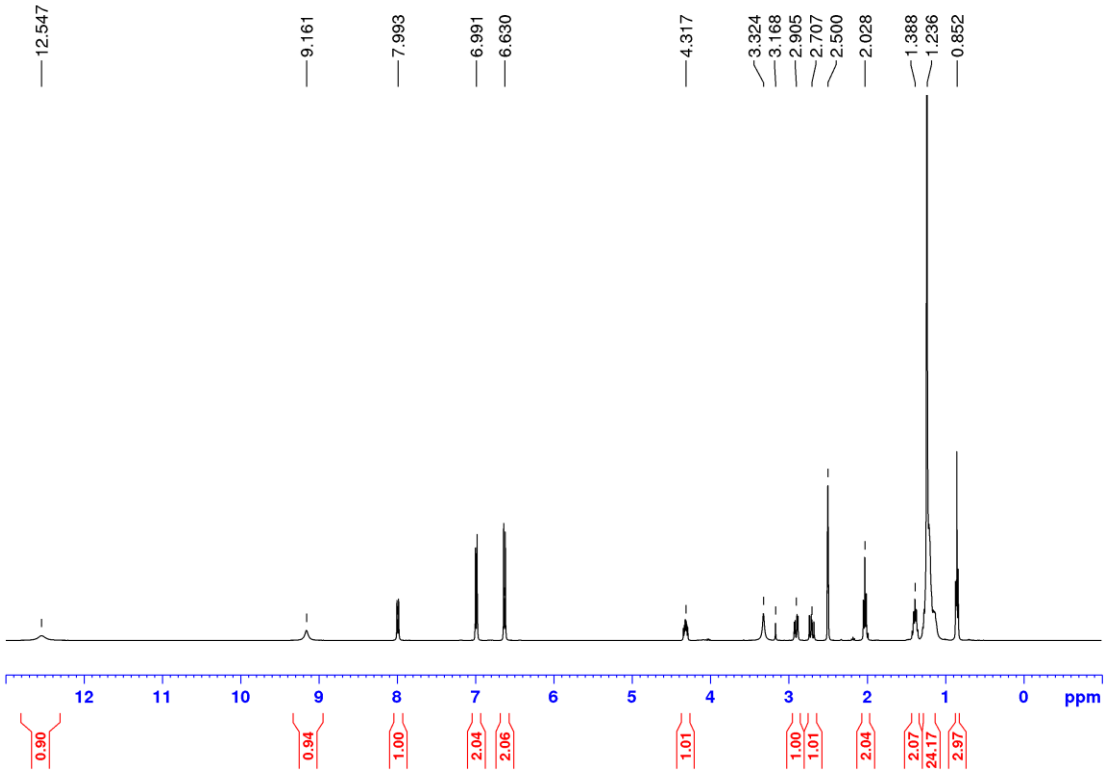


Figure S17. ^{1}H NMR spectrum (400 MHz, DMSO-d_6) of compound **4**.

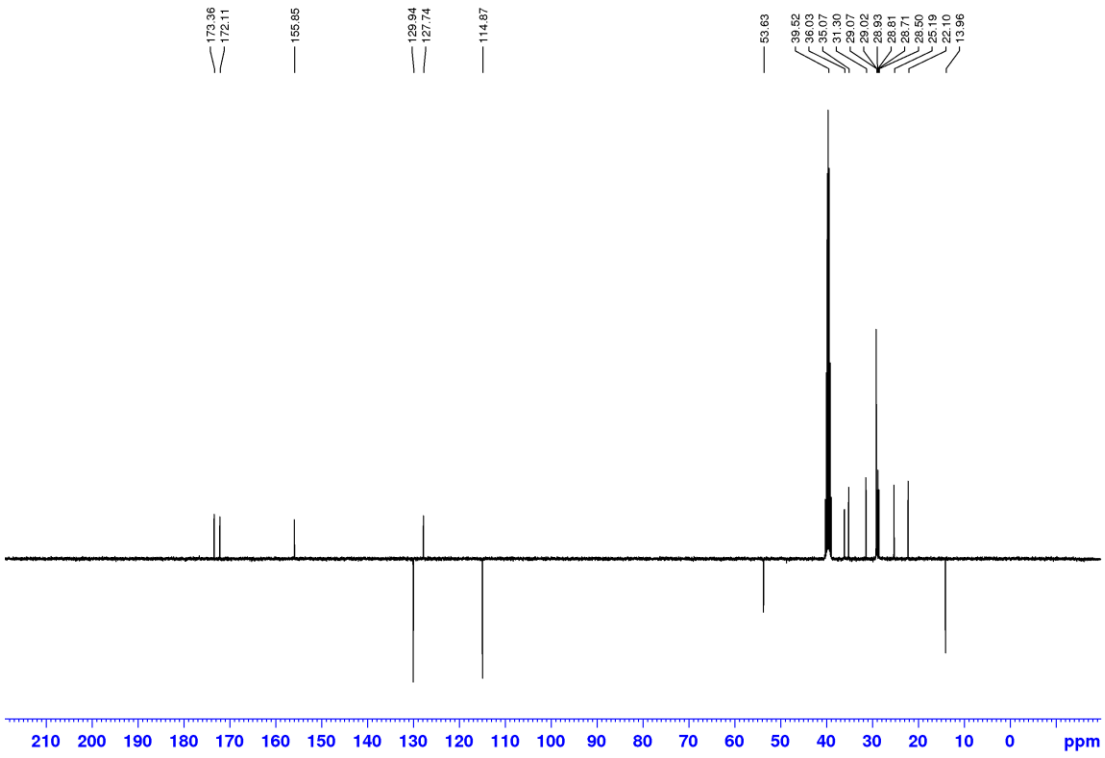


Figure S18. DEPTQ-135 NMR spectrum of compound **4**.

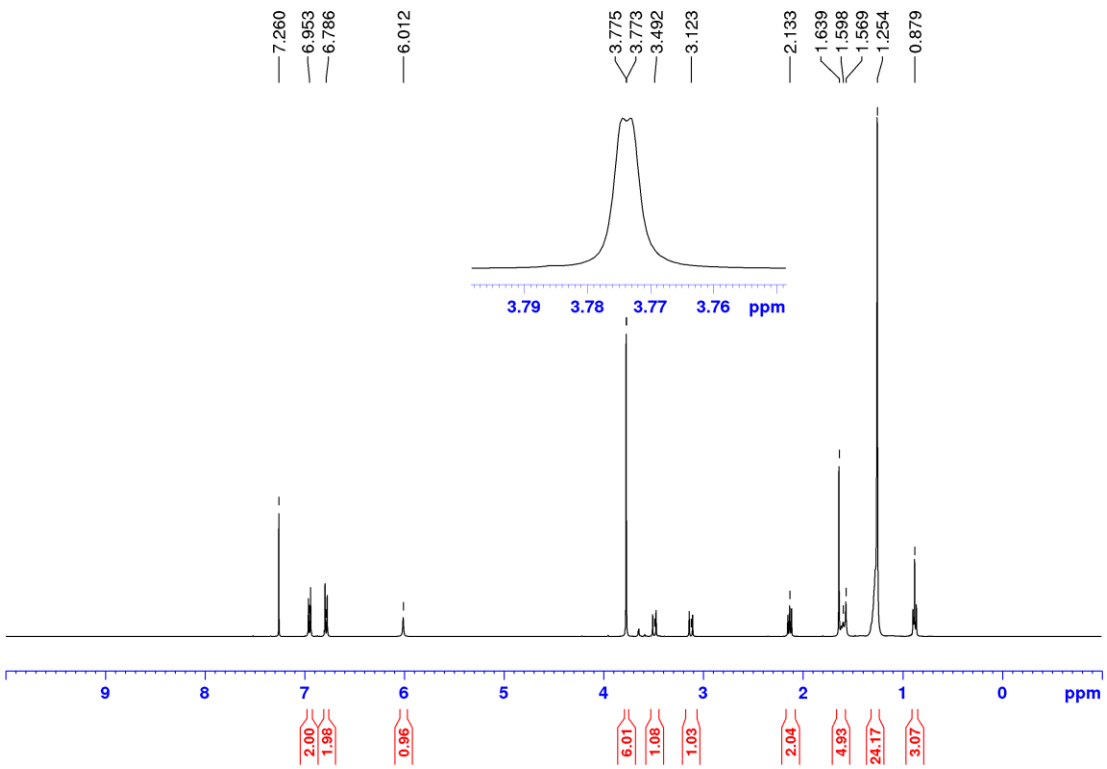


Figure S19. ^{1}H NMR spectrum of compound **5**.

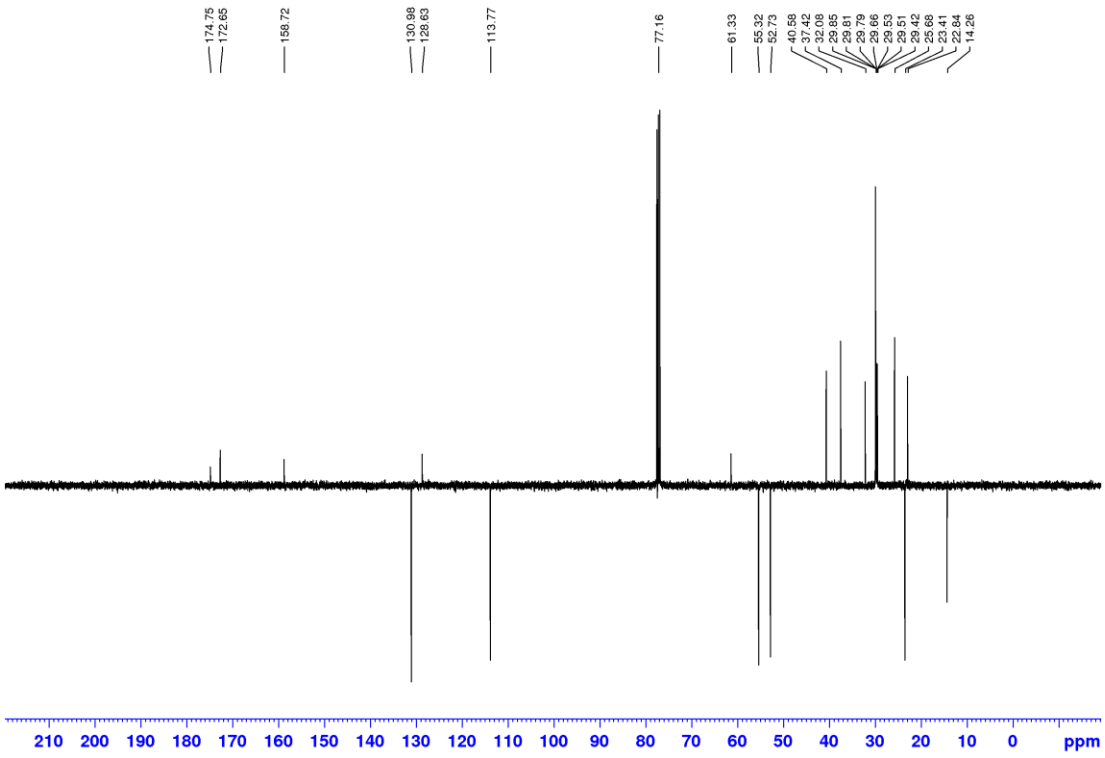


Figure S20. DEPTQ-135 NMR spectrum (101 MHz, $CDCl_3$) of compound **5**.

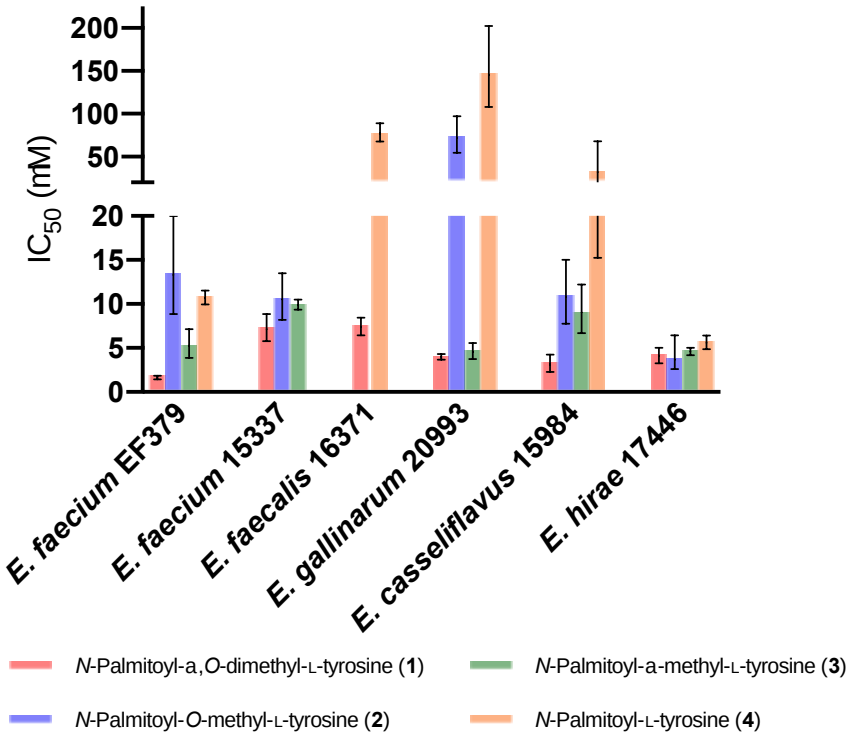


Figure S21. Antimicrobial activity of compounds **1**-**4** against six *Enterococcus* spp. *Enterococcus* inhibitory activity of compounds **1**-**4** expressed as 95% confidence intervals around mean IC_{50} values (N = 3). Data are not included for the following: *E. faecium* 15337 (**4**; inactive), *E. faecalis* 16371 (**2**; inactive) and *E. faecalis* 16371 (**3**; estimated IC_{50} = 9.2 – 18.4 µM).

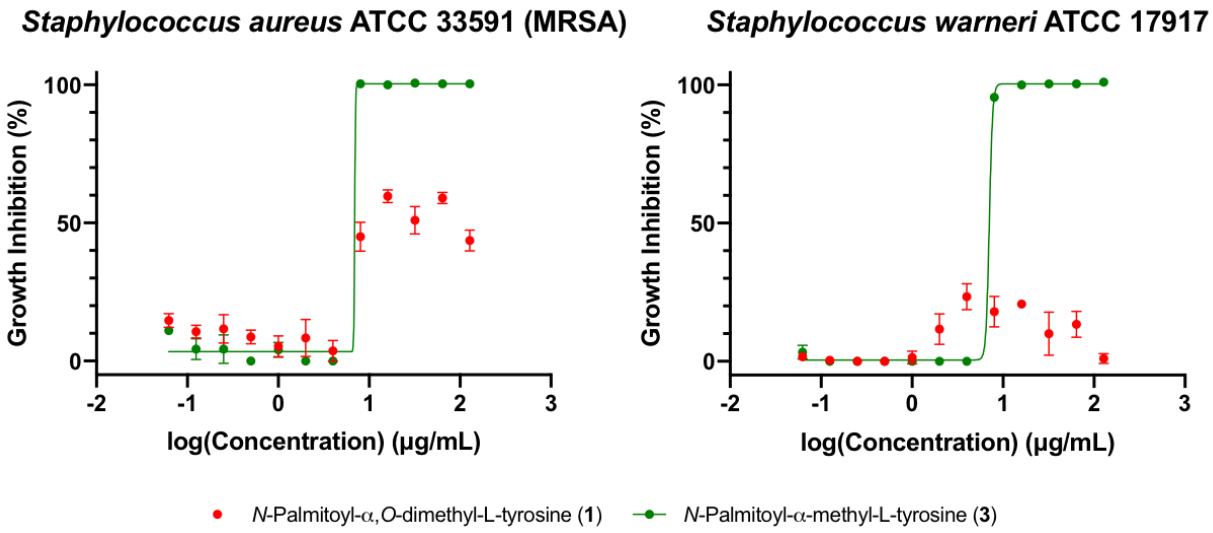


Figure S22. Dose-response curves for compounds **1** and **3** against two *Staphylococcus* spp.

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