An unexpected switch in the modulation of AI-2-based quorum sensing discovered through synthetic DPD analogs

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General Procedures. Unless otherwise stated, all reactions were performed under an inert atmosphere with dry reagents, solvents, and flame-dried glassware. All starting materials were purchased from Acros, Aldrich, Sigma, Fisher, ITN, or Lancaster and used as received. All flash column chromatography was performed using silica gel 60 (230- 400 mesh). Analytical and preparative thin-layer chromatography (TLC) was performed using Merck Kieselgel 60 F254 silica gel plates (0.25, 0.5, or 1 mm). ^{1}H NMR spectra were recorded on Bruker DRX-600 (600 MHz), DRX-500 (500 MHz), or DRX-400 (400 MHz) spectrometers, and ^{13}C NMR spectra were recorded on Bruker DRX-600 (150.7 MHz) or DRX-500 (125.7 MHz) spectrometers. Chemical shifts are reported in parts per million (ppm) on the δ scale from an internal standard. Mass spectra were recorded on a VG ZAB-VSE or an ABI /SCIEX API-150 EX single quadrupole electrospray mass spectrometer.

Preparation of (R)-1,2-Cyclohexylidenedioxybut-3-yne, 2. To a solution of diisopropyl amine (3.65 mL, 26.1 mmol) in THF (50 mL) at -78 °C was added n-BuLi (15.21 mL, 24.3 mmol). After 12 min., trimethylsilyl diazomethane (2.0 M in hexanes, 13.9 mL, 27.8 mmol) was added and stirring continued for 30 minutes. At this time, a prechilled (-78 °C) solution of aldehyde **1**[1] (2.96 g, 17.4 mmol) in THF (30 mL) was added to the reaction mixture. After 30 min., the solution was warmed to 23 °C and stirred for 6 h. The mixture was then cooled to 0 °C, quenched with H2O, dried with $MgSO_4$, filtered, and concentrated in vacuo. Purification via flash chromatography (SiO_2: 0.3:9.7 Et_2O:hexanes) gave **2** (2.05g, 71% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.71 (ddd, J = 6.5, 6.3, 2.0 Hz, 1H), 4.17 (dd, J = 8.0, 6.5 Hz, 1H), 3.95 (dd, J = 8.0, 6.3 Hz, 1H), 2.49 (d, J = 2.0 Hz, 1H), 1.76 (m, 2H), 1.63 (m, 6H), 1.42 (m, 2H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 112.1, 82.5, 74.6, 65.8, 36.5, 36.3, 25.9, 24.7. $[\alpha]_D^{20}$ -39.2 (*c* 0.774, $CHCl_3$). [Previously characterized by Yoshida, J.; Nakagawa, M.; Seki, H.; Hino, T. *J Chem Soc Perkin Trans* **1992**, *1*, 343-350.]

Representative procedure for the preparation of alkyl substituted alkyne 3. To a solution of alkyne **2** (1 equiv) in THF at -78 °C was added a solution of n-BuLi (2.5 M in hexane, 2 equiv) in one portion, and the mixture was allowed to stir for 30 min. At this

time, the desired alkyl iodide (2 equiv.) was added, and the mixture was allowed to warm to 23 °C and be stirred for 12 h. The reaction was then cooled to 0 °C and quenched with H_2O. Drying with $MgSO_4$, followed by concentration in vacuo, and purification via flash column chromatography (SiO_2, 0.3:9.7 EtOAc:Hexanes) provided alkyne **3**.

(R)-1,2-Cyclohexylidenedioxyhex-3-yne. ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.69 (tt, J = 6.9, 1.9 Hz, 1H), 4.10 (dd, J = 7.8, 6.2 Hz, 1H), 3.81 (dd, J = 7.0, 7.9, 1H), 2.21 (dq, J = 7.5, 1.9 Hz, 2H), 1.72 (m, 2H), 1.61 (m, 6H), 1.39 (m, 2H), 1.12 (t, J = 7.5 Hz, 3H). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 110.7, 88.4, 77.0, 70.2, 65.9, 36.2, 35.9, 25.5, 24.3, 24.2, 14.0, 12.9. HRMS (m/z): M^+ calcd. For $C_{12}H_{19}O_2$, 194.131; found, 195.1374. Yield: 75%.

(R)-1,2-Cyclohexylidenedioxyhept-3-yne. ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.69 (tt, J = 6.9, 1.9 Hz, 1H), 4.10 (dd, J = 7.8, 6.2 Hz, 1H), 3.81 (dd, J = 7.0, 7.9, 1H), 2.21 (dt, J = 7.1, 1.9 Hz, 2H), 1.72 (m, 2H), 1.65 (m, 6H), 1.60 (m, 2H), 1.39 (m, 2H), 0.98 (t, J = 7.4 Hz, 3H). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 110.9, 87.0, 77.9, 70.3, 65.9, 36.2, 35.9, 32.0, 25.4, 24.3, 22.3, 14.5, 13.8. HRMS (m/z): M^+ calcd. For $C_{13}H_{20}O_2$, 208.152; found, 209.2001. Yield: 72%.

(R)-1,2-Cyclohexylidenedioxyoct-3-yne. ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.69 (tt, J = 6.9, 1.9 Hz, 1H), 4.10 (dd, J = 7.8, 6.2 Hz, 1H), 3.81 (dd, J = 7.0, 7.9, 1H), 2.21 (dt, J = 7.1, 1.9 Hz, 2H), 1.72 (m, 2H), 1.65 (m, 6H), 1.60 (m, 2H), 1.39 (m, 2H), 1.31 (m, 4H), 1.04 (t, J = 7.4 Hz, 3H). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 110.9, 87.0, 77.9, 70.3, 65.9, 36.2, 35.9, 32.0, 30.9, 25.5, 24.3, 22.3, 14.5, 13.8. HRMS (m/z): M^+ calcd. For $C_{14}H_{22}O_2$, 222.161; found, 223.1689. Yield: 69%.

(R)-1,2-Cyclohexylidenedioxydec-3-yne. ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.69 (tt, J = 6.4, 1.8 Hz, 1H), 4.10 (dd, J = 7.8, 6.2 Hz, 1H), 3.81 (dd, J = 7.6, 7.0, 1H), 2.21 (dt, J = 7.2, 1.9 Hz, 2H), 1.72 (m, 2H), 1.61 (m, 6H), 1.48 (q, J = 14.4, 7.2 Hz, 2H), 1.39 (m, 4H), 1.27 (m, 4H), 0.88 (t, J = 7.1 Hz, 3H). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 110.7, 86.8, 77.3, 69.8, 65.5, 35.8, 35.5, 31.3, 28.5, 28.4, 25.1, 23.8, 22.5 (2C), 18.8, 14.0. HRMS (m/z): M^+ calcd. For $C_{16}H_{26}O_2$, 250.191; found, 251.2021. Yield: 55%.

(R)-1,2-Cyclohexylidenedioxyhex-3-yne-6-phenyl. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.36 (m, 2H), 7.30 (m, 2H), 4.69 (tt, J = 6.4, 1.8 Hz, 1H), 4.18 (dd, J = 6.2, 7.9 Hz, 1H), 3.88 (dd, J = 6.8, 7.8, 1H), 2.91 (t, J = 7.6 Hz, 2H), 2.59 (dt, J = 1.8, 7.6 Hz, 2H), 1.77 (m, 2H), 1.66 (m, 6H), 1.48 (m, 2H). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 140.9, 128.9, 128.8, 126.7, 111.6, 86.3, 78.7, 70.2, 65.9, 36.2, 35.9, 35.3, 25.5, 25.4, 24.3, 21.4. HRMS (m/z): M^+ calcd. For $C_{18}H_{22}O_2$, 270.194; found, 271.1700. Yield: 42%.

(R)-1,2-Cyclohexylidenedioxyoct-3-yne-8-azido. ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.68 (tt, tt, J = 6.4, 1.8 Hz, 1H), 4.19 (dd, J = 6.3, 7.7 Hz, 1H), 3.90 (dd, J = 7.0, 7.9, 1H), 3.37 (t, J = 6.7 Hz, 2H), 2.34 (dt, J = 1.8, 6.9 Hz, 2H), 1.72 (m, 4H), 1.65 (m, 8H), 1.39 (m, 2H). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 111.0, 86.0, 78.6, 70.2, 65.9, 51.4, 36.2, 35.9, 32.0, 28.4, 25.9, 25.5, 24.3, 18.8. HRMS (m/z): M^+ calcd. For $C_{14}H_{21}N_3O_2$, 263.163; found, 264.1698. Yield: 67%.

Preparation of alkylated (S)-4,5-Cyclohexylidenedioxy-2,3-pentadione, 4. To a solution of **3** (1 equiv) in CCl_4 (2.22 mL/mmol **3**) and MeCN (2.22 mL/mmol **3**) was added $NaIO_4$ (2.25 equiv) in H_2O (3.33 mL/mmol **3**). The mixture was vigorously stirred, and RuO_2•H_2O (0.025 equiv) was added. The mixture was vigorously stirred in air for 15 minutes. At this time, the reaction mixture was filtered through a celite and silica gel plug using CH_2Cl_2 as the eluant, dried with $MgSO_4$ and concentrated in vacuo to give a bright yellow oil. Purification via flash chromatography using SiO_2 eluted with 1:9 EtOAc:hexanes afforded **4**.

(*S*)-1,2-cyclohexylidenedioxy-3,4-hexadione (Ethyl Diketone, 4b) ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.20 (dd, J = 5.4, 7.9 Hz, 1H), 4.43 (dd, J = 8.0, 8.7 Hz, 1H), 4.09 (dd, J = 5.4, 8.9, 1H), 2.88 (m, 2H), 1.65 (m, 8H), 1.39 (m, 2H), 1.19 (t, J = 7.3 Hz, 3H). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 201.6, 196.0, 112.3, 77.1, 66.0, 35.9, 35.2, 30.9, 25.4, 24.3, 24.2, 7.0. HRMS (m/z): M^+ calcd. For $C_{12}H_{18}O_4$, 226.124; found, 227.2371. Yield: 53%.

(*S*)-1,2-cyclohexylidenedioxy-3,4-heptadione (Propyl Diketone, 4c) ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.17 (dd, J = 5.4, 7.9 Hz, 1H), 4.41 (dd, J = 8.0, 8.7 Hz, 1H), 4.09 (dd, J = 5.4, 8.9, 1H), 2.88 (m, 2H), 1.65 (m, 10H), 1.39 (m, 2H), 0.99 (t, J = 7.3 Hz, 3H). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 201.6, 196.5, 112.7, 77.5, 66.4, 39.6, 36.2, 35.6, 25.9, 24.7, 24.6, 19.0, 14.5. HRMS (m/z): M^+ calcd. For $C_{13}H_{20}O_4$, 240.136; found, 241.1431. Yield: 64%.

(*S*)-1,2- cyclohexylidenedioxy-3,4-octadione (Butyl Diketone, 4d) ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.10 (dd, J = 5.3, 7.9 Hz, 1H), 4.33 (dd, J = 8.0, 8.7 Hz, 1H), 3.99 (dd, J = 5.3, 8.7, 1H), 2.74 (m, 2H), 1.65 (m, 10H), 1.39 (m, 4H), 1.12 (t, J = 7.5 Hz, 3H). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 201.7, 196.5, 112.7, 77.5, 66.0, 37.1, 35.9, 35.2, 25.4, 25.2, 24.3, 24.2, 22.6, 14.2. HRMS (m/z): M^+ calcd. For $C_{14}H_{22}O_4$, 254.153; found, 255.5010. Yield: 58%.

(*S*)-1,2-cyclohexylidenedioxy-3,4-decadione (Hexyl Diketone, 4e) ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.18 (dd, J = 5.4, 7.9 Hz, 1H), 4.41 (dd, J = 8.0, 8.7 Hz, 1H), 4.09 (dd, J = 5.4, 8.9, 1H), 2.85 (m, 2H), 1.71 (m, 10H), 1.49 (m, 2H), 1.35 (m, 6H), 0.96 (t, J = 6.8 Hz, 3H). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 201.3, 196.1, 112.3, 77.1, 66.0, 37.3, 35.9, 35.2, 32.0, 29.2, 25.4, 24.4, 24.3, 23.1, 22.8, 14.4. HRMS (m/z): M^+ calcd. For $C_{16}H_{26}O_4$, 282.183; found, 283.1899. Yield: 61%.

(*S*)-1,2-cyclohexylidene-6-phenylhexane-3,4-dione (Phenyl Diketone, 4f) ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.30 (m, 2H), 7.21 (m, 3H), 5.10 (dd, J = 5.3, 7.9 Hz, 1H), 4.33 (dd, J = 8.0, 8.7 Hz, 1H), 3.95 (dd, J = 5.3, 8.7, 1H), 3.15 (m, 2H), 2.96 (m, 2H), 1.65 (m, 8H), 1.39 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$): 199.6, 195.2, 139.9, 128.6, 128.4, 128.3, 128.2, 126.4, 111.9, 76.6, 65.5, 38.4, 35.4, 34.7, 28.7, 25.0, 23.8, 23.7. HRMS (m/z): M^+ calcd. For $C_{18}H_{22}O_4$, 302.152; found, 303.1592. Yield: 44%.

(*S*)-8-azido-1,2-cyclohexylidenedioxy-3,4-octadione (Azido Diketone, 4g) ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.19 (dd, J = 5.3, 7.8 Hz, 1H), 4.42 (dd, J = 6.0, 10.7 Hz, 1H), 4.10

(dd, J = 5.3, 8.9, 1H), 3.39 (dt, J = 2.8, 6.5 Hz, 2H), 2.83 (m, 2H), 1.65 (m, 12H), 1.39 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$): 200.0, 195.6, 112.0, 76.6, 65.5, 51.0, 36.3, 35.5, 34.7, 28.2, 25.0, 23.9, 23.8, 19.9. HRMS (m/z): M^+ calcd. For $C_{14}H_{21}N_3O_4$, 295.153; found, 296.1071. Yield: 36%.

Representative procedure for characterization of analogs, 5b-g. To a 10 mM solution of **4b** (ethyl diketone) in D_2O (480 μL) and d_6-DMSO (120 μL) was added 0.6 μL of D_2SO_4. The reaction was allowed to continue for 6 h, at which point the compounds were analyzed by NMR to measure completion of the deprotection reaction. After analysis, the solutions were diluted with phosphate buffer to a final concentration of 1 mM in 2% DMSO, and used without further purification in the biological assays.[1]

(*S*)-1,2-dihydroxyhexane-3,4-dione (Ethyl-DPD, 5b) ^{1}H NMR (600 MHz, D_2O/d_6-DMSO, pD = 1.5): δ 4.36 (dd, J = 5.5, 7.0 Hz, 1H), 4.16 (m, 2H), 4.02 (dd, J = 3.3, 5.9 Hz, 1H), 3.93 (dd, J = 3.7, 7.0 Hz, 1H), 3.77 (m, 2H), 3.61 (dd, J = 7.2, 11.9 Hz, 1H), 3.52 (dd, J = 6.0, 9.4 Hz, 1H), 2.73 (m, 2H), 1.74 (m, 4H, obscured by cyclohexanone), 1.05 (t, J = 7.6 Hz, 3H), 1.00 (t, J = 6.8 Hz, 3H), 0.96 (t, J = 6.4 Hz, 3H). ^{13}C NMR (150.7 MHz, D_2O/d_6-DMSO): 105.9, 105.8, 100.8, 100.5, 97.9, 75.7, 75.2, 74.7, 71.9, 69.9, 62.4, 31.6, 27.3, 27.2, 8.1, 7.6, 7.3.

(*S*)-1,2-dihydroxyheptane-3,4-dione (Propyl-DPD, 4c) ^{1}H NMR (600 MHz, D_2O/d_6-DMSO, pD = 1.5): δ 4.37 (dd, J = 5.4, 7.1 Hz, 1H), 4.16 (m, 2H), 4.03 (dd, J = 3.3, 6.0 Hz, 1H), 3.96 (dd, J = 3.7, 7.4 Hz, 1H), 3.80 (m, 2H), 3.64 (dd, J = 7.4, 11.8 Hz, 1H), 3.54 (dd, J = 6.0, 9.3 Hz, 1H), 2.78 (m, 2H), 1.62 (m, 6H), 1.44 (m, 4H), 0.88 (m, 9H). ^{13}C NMR (150.7 MHz, D_2O/d_6-DMSO): 105.8, 105.7, 100.8, 100.2, 97.9, 75.9, 75.2, 74.8, 72.0, 69.4, 62.7, 37.2, 36.9, 36.7, 17.8, 17.5, 17.0, 15.4, 15.0, 14.5.

(*S*)-1,2-dihydroxyoctane-3,4-dione (Butyl-DPD, 4d) ^{1}H NMR (600 MHz, D_2O/d_6-DMSO, pD = 1.5): δ 4.37 (dd, J = 5.3, 8.0 Hz, 1H), 4.16 (m, 2H), 4.03 (dd, J = 3.3, 6.0 Hz, 1H), 3.95 (dd, J = 3.7, 7.3 Hz, 1H), 3.79 (m, 2H), 3.64 (dd, J = 7.4, 11.8 Hz, 1H), 3.53 (dd, J = 6.0, 9.3 Hz, 1H), 2.73 (m, 2H), 1.57 (m, 6H), 1.32 (m, 10H), 0.88 (m, 9H). ^{13}C NMR (150.7 MHz, D_2O/d_6-DMSO): 105.9, 105.8, 100.8, 100.3, 97.9, 75.9, 75.2, 74.8, 72.2, 69.4, 62.7, 37.7, 36.3, 34.1, 26.4, 26.1, 25.7, 23.9, 23.4, 23.0, 14.8, 14.7, 14.6.

(*S*)-1,2-dihydroxydecane-3,4-dione (Hexyl-DPD, 4e) ^{1}H NMR (600 MHz, D_2O/d_6-DMSO, pD = 1.5): δ 4.35 (dd, J = 4.5, 8.8 Hz, 1H), 4.12 (m, 2H), 4.00 (dd, J = 3.4, 5.9 Hz, 1H), 3.92 (dd, J = 3.8, 7.4 Hz, 1H), 3.76 (m, 2H), 3.60 (dd, J = 7.4, 11.8 Hz, 1H), 3.50 (dd, J = 6.0, 9.2 Hz, 1H), 2.75 (m, 2H) 1.46 (m, 4H), 1.20 (m, 24H), 0.73 (m, 9H). ^{13}C NMR (150.7 MHz, D_2O/d_6-DMSO): 105.9, 105.8, 100.5, 100.4, 98.0, 75.9, 75.3, 74.8, 72.3, 72.1, 62.7, 38.0, 36.7, 34.4, 32.5, 32.3, 32.2, 30.4, 29.8, 29.4, 23.5, 23.4, 23.35, 23.33, 23.31, 23.29, 15.1, 15.0, 14.9.

(*S*)-1,2-dihydroxy-6-phenylhexane-3,4-dione (Phenyl-DPD, 5f) ^{1}H NMR (600 MHz, D_2O/d_6-DMSO, pD = 1.5): δ 7.30 (m, 15H), 4.36 (dd, J = 6.0, 7.5 Hz, 1H), 4.17 (m, 2H),

4.04 (dd, J = 3.6, 5.9 Hz, 1H), 3.99 (dd, J = 5.7, 8.7 Hz, 1H), 3.89 (m, 2H), 3.70 (dd, J = 3.8, 11.8 Hz, 1H), 3.56 (dd, J = 7.3, 11.6 Hz, 1H), 3.11 (m, 2H), 2.94 (m, 4H), 2.71(t, J = 7.4 Hz, 2H), 1.61 (m, 4H). ^{13}C NMR (150.7 MHz, D_2O/d_6-DMSO): 142.5, 142.3, 142.1, 130.3, 130.2, 130.1, 129.9, 129.8, 128.0, 127.9, 127.8, 105.5, 105.3, 100.9, 100.6, 98.0, 75.9, 75.4, 74.8, 72.2, 70.3, 62.6, 37.2, 37.0, 36.8, 31.7, 30.0, 29.8.

(*S*)-8-azido-1,2-dihydroxyoctane-3,4-dione (Azidobutyl-DPD, 5g) ^{1}H NMR (600 MHz, D_2O/d_6-DMSO, pD = 1.5): δ 4.34 (dd, J = 5.0, 7.5 Hz, 1H), 4.14 (m, 2H), 4.01 (dd, J = 3.4, 5.9 Hz, 1H), 3.92 (dd, J = 4.0, 7.0 Hz, 1H), 3.77 (m, 2H), 3.61 (dd, J = 7.3, 11.8 Hz, 1H), 3.52 (dd, J = 5.9, 9.3 Hz, 1H), 3.36 (m, 6H), 2.70 (m, 2H), 1.63 (m, 12H), 1.52 (m, 4H). ^{13}C NMR (150.7 MHz, D_2O/d_6-DMSO): 105.6, 105.5, 100.8, 100.0, 97.9, 75.8, 75.3, 74.8, 72.1, 70.1, 62.6, 52.4, 52.2, 52.1, 37.4, 36.1, 34.7, 29.9, 29.4, 28.9, 21.3, 21.0, 20.7.

Preparation of 1-azido-4-chlorobutane. NaN_3 (1.0 g, 15.4 mmol) was added to a solution of 1-bromo-4-chlorobutane (2.63 g, 15.4 mmol) in 20 mL of DMF at room temperature. The reaction mixture was allowed to stir for 20 h, extracted with ether, and the organic layer was washed with water, dried over Mg_2SO_4 and concentrated to give 1-azido-4-chlorobutane (0.71 g, 94%). ^{1}H NMR (400 MHz, $CDCl_3$) d 1.76 (m, 2H), 1.87 (m, 2H), 3.36 (t, *J* = 6.6 Hz, 2H), 3.53 (t, *J* = 6.5 Hz, 2H) [Previously characterized by Yao, L.; Smith, B.T.; Aubé, J. *J. Org. Chem.* **2004**, *69*, 1720-1722.]

Preparation of 1-azido-4-iodobutane. NaI (768 mg, 5.12 mmol) was added to a solution of 1-azido-4-chlorobutane (341 mg, 2.56 mmol) in 15 mL of acetone and heated to reflux for 24 h. The reaction mixture was extracted with EtOAC and the combined organic layers were dried over Mg_2SO_4, concentrated, and purified via flash chromatography (1:9 EtOAc:hexane) to provide 1-azido-4-iodobutane (461 mg, 85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.73(m, 2H), 1.91 (m, 2H), 3.23 (t, *J* = 6.8 Hz, 2H), 3.36 (t, *J* = 6.6 Hz, 2H) [Previously characterized by Yao, L.; Smith, B.T.; Aubé, J. *J. Org. Chem.* **2004**, *69*, 1720-1722.]

Biological Assays

Antagonism of β-galactosidase in *S. typhimurium*

Evaluation of the structural analogs above was performed in *S. typhimurium* by measuring β-galactosidase activity as follows: an overnight culture was diluted 1:100 in LB broth and incubated with the test compound (0.05% DMSO) and 50 μM DPD at 37 °C for 4 hours. After incubation, the cells were centrifuged, resuspended in Z-buffer, and an aliquot was added to a 96 well plate to measure OD_{600}. 1% SDS and chloroform were added to solubilize the remaining cells, and aliquots of differing volumes were added to the plate. β-mercaptoethanol dissolved in Z-buffer was added to bring the final volume to 200μL per well, at which point 50 μL of substrate (*o*-nitrophenyl-β-D-galactopyranoside) in Z-buffer was added. The OD_{420} was read every 5 minutes for one hour. β-galactosidase activity was calculated according to the following equation:

Activity = $[OD_{420}/(OD_{600}{}^{x}\text{time}^{x}\text{cells})]^{x}(1\text{nmol}/0.0045\text{mL}\cdot\text{cm})^{x}$(total volume); where "time" is reaction time in minutes, and "cells" is the volume of bacterial cell culture used in mL. To measure agonistic activity of the test compounds, the above procedure was performed in the absence of DPD.

Modulation of bioluminescence in *V. harveyi*

The test compounds proposed above were evaluated for their (ant)agonistic activity in *V. harveyi* following the protocol reported. Briefly, *V. harveyi* strain MM32 (ATCC BAA-1121) was grown for 12 h at 30 °C in AB medium and then diluted 1:1000 into fresh AB medium. A 96-well microtiter plate was prepared with wells containing test compounds (120 mL/well) in 0.05% DMSO in AB medium, and 1 μM DPD. 30 μL of the diluted cells were added to each well, and luminescence was measured using a luminometer with an incubator set to 30^{o}C for 12 h, and measurements were recorded every 30 min. The luminescence was normalized to cell density. To measure agonistic activity of the test compounds, the above procedure was performed in the absence of DPD.

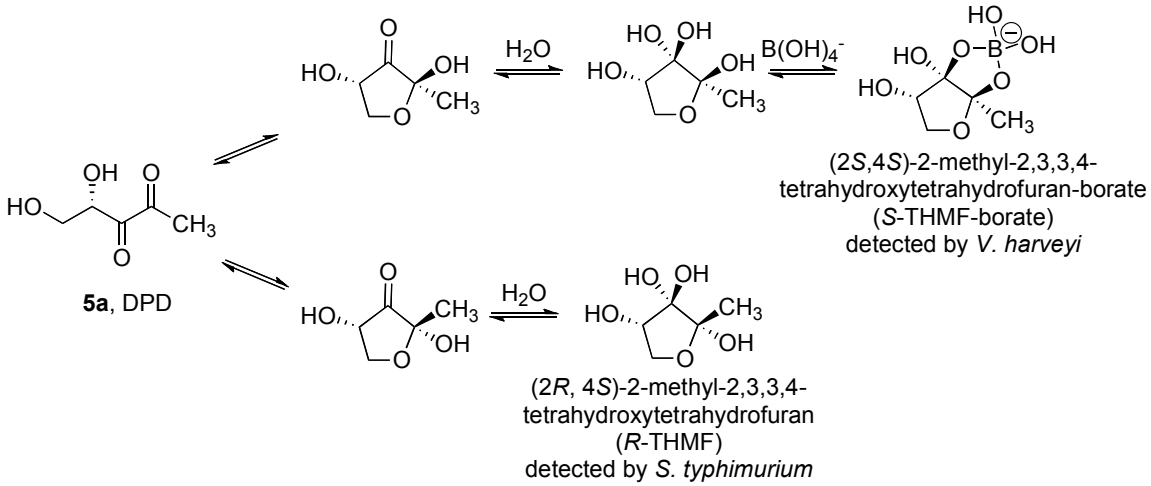

Figure S1. Structures of AI-2-derived autoinducers employed by *V. harveyi* and *S. typhimurium*.

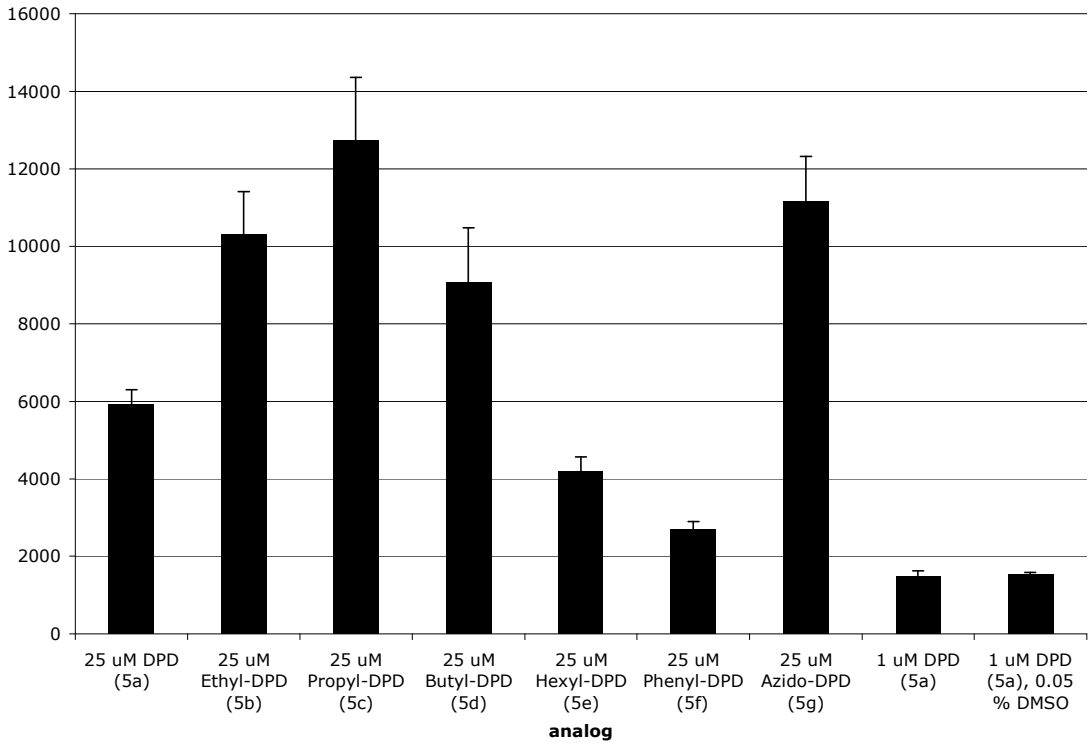

Figure S1. Synergy between DPD and DPD analogs in the presence of 25 μM analog and 1 μM DPD.

Compound	Relative Viability (%)
DPD (**5a**)	99.7 ± 5.0
Ethyl-DPD (**5b**)	100.5 ± 1.7
Propyl-DPD (**5c**)	91.3 ± 3.6
Butyl-DPD (**5d**)	95.2 ± 2.6
Hexyl-DPD (**5e**)	101.6 ± 1.6
Phenyl-DPD (**5f**)	90.6 ± 1.6
Azido-DPD (**5g**)	90.1 ± 4.1

Table S1. Toxicity of DPD and DPD analogs towards a mouse leukaemic monocyte macrophage cell line (RAW 264.7). The assay was performed according to the instructions provided by the manufacturer (Sigma) of the XTT based *in vitro* toxicology kit.

References:

(1) Semmelhack, M. F.; Campagna, S. R.; Federle, M. J.; Bassler, B. L. *Org Lett* **2005**, *7*, 569-72.