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## Dissecting AI-2-mediated quorum sensing through C5-analogue synthesis and biochemical analysis

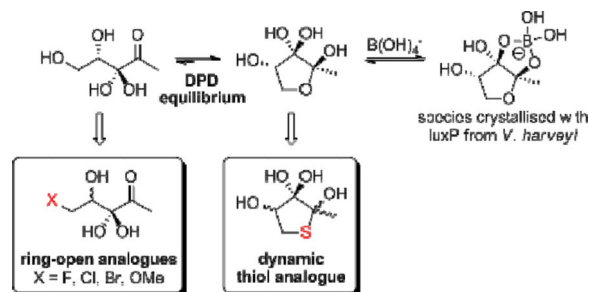
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### Abstract

Autoinducer-2 (AI-2)-mediated quorum sensing (QS) is utilised for both intra- and inter-species communication by a wide variety of bacteria. An understanding of the mechanism of this communication has the potential to elucidate new targets for antibacterial therapeutics. Herein, we report the synthesis of DPD analogues with modified dynamic equilibria and the evaluation of their behaviour in Gram-negative bacteria. None of the compounds showed modulation of QS in *S. Typhimurium*, and although no antagonism of *V. harveyi* was observed, chloro-analogue **C5-Cl-DPD** showed modest agonism in this marine bacterium. This raises the possibility that access to a cyclic form of DPD may not be required for AI-2-mediated QS in *V. harveyi*.

### Graphical abstract



## 1. Introduction

Quorum sensing (QS) is communication between bacteria through recognition of small molecule modulators. QS is responsible for the orchestration of bacterial behaviour in a density-dependent manner, including events that increase bacterial defences such as biofilm formation and virulence factor expression, and thus would be a powerful/important target for

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### Supplementary Material

Additional tables and figures containing DFT calculations, numerical antagonism data and  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectra.

future therapeutics. Autoinducer-2 (AI-2) is a prolific modulator, recognised by over 70 species of both Gram-negative and - positive bacteria.<sup>1</sup> However, AI-2-mediated QS has proved challenging to study given the propensity of the precursor, (4*S*)-DPD, to polymerisation at high concentration, and its existence as a complex equilibrium with recognition of different equilibrium species by different bacteria (Figure 1).<sup>2</sup> Crystallisation of the AI-2 signal with LuxP, the AI-2 sensor protein in the marine organism *Vibrio harveyi*, suggests the requirement of a cyclic DPD species coordinating to boron, (2*S*, 4*S*)-THMF-borate.<sup>3</sup> The corresponding sensor protein in the enteric bacterium *Salmonella* Typhimurium, LsrB, was also crystallised with a cyclic form of DPD, albeit recognising an alternative stereoisomer that lacked boron coordination, (2*R*, 4*S*)-THMF.<sup>4</sup>

Although AI-2 is not internalised by *V. harveyi*, it appears this is not the case for all species. Binding to LsrB is the primary route through which AI-2 can enter *S. Typhimurium*, but an alternative, currently unknown, pathway is believed to exist.<sup>5</sup> Thus the cyclic form may not be crucial for activity in the latter species. Downstream biological recognition of AI-2 in this species includes phosphorylation of the C5 alcohol by kinase LsrK, requiring a ring-opened form of DPD,<sup>6</sup> before binding of the resulting phospho-(4*S*)-THP to transcriptional regulator LsrR.<sup>5</sup>

Given the effects of cyclisation on signalling activity have not been fully elucidated, we sought to explore the importance of linear and cyclic forms of DPD to investigate the requirements for the AI-2 structure in these systems. Analysis of the NMR spectra has shown that DPD predominantly exists as a mixture of ring-closed stereoisomers under physiological pH.<sup>2</sup> We have previously investigated the use of carbocyclic analogues to mimic fixed ring-closed conformations of DPD (DHMP and triHMP, Figure 2).<sup>7</sup> However, the direct analogue DHMP had no significant effect against either *V. harveyi* or *S. Typhimurium*, and triHMP showed only limited antagonism in *V. harveyi*. DFT calculations suggested limited hydration at the C3 ketone of DHMP, a requirement for formation of the borate diester. These compounds also lack the dynamic equilibrium that DPD utilises for multi-species recognition. We were therefore interested in designing new analogues that would exhibit both a predominantly ring-closed form and hydration of the C3 ketone. We identified **C5-SH-DPD** as a suitable target as DFT calculations suggested that the ring-closed form of this compound should exist mainly as the C3-hydrate (see Table S1, Supplementary material).

In a parallel, complementary approach we proposed the installation of a C5-halide in order to mimic the (4*S*)-THP open-chain form of DPD. Fluoride is considered a classical bioisostere for oxygen, being of similar size and able to (weakly) participate as a hydrogen-bond acceptor.<sup>8</sup> The larger, less electronegative halides have been shown to form *halogen*-bonding interactions,<sup>9</sup> potentially mimicking the hydrogen-bond donor character of the hydroxyl group. Indeed, this strategy has been utilised in replacement of the hydrated ketone in C3-dihalo-DPD analogues, with both the dichloro and dibromo analogues displaying comparable agonism to DPD itself.<sup>10</sup> Although non-fluoro alkyl halides have the potential to undergo nucleophilic displacement and are widely considered to be genotoxic fragments, drugs containing alkyl chloride motifs are generally stable *in vivo* and are relatively common.<sup>11</sup> There are also examples of alkyl bromides being used in medicinal chemistry

with apparent stability (cannabinoid receptor agonists O-806 and O-1236<sup>12</sup>), although electrophilic reactivity is anticipated. However, alkyl iodides are scarcely reported in this context due to their instability; in our context, **C5-I-DPD** would almost certainly act as an alkylating agent or simply hydrolyse to form DPD. We therefore proposed the synthesis of the C5-fluoro, -chloro and -bromo analogues to allow for investigation of both hydrogen-bond donors and acceptors in place of the C5-hydroxy group. We also investigated the biological activity of previously synthesised open-chain analogue C5-MeO-DPD,<sup>2</sup> for its ability to act as a hydrogen-bond acceptor.

## 2. Results and Discussion

Three of our targets, **rac-C5-SH-DPD**, **rac-C5-Cl-DPD** and **rac-C5-F-DPD** could be readily synthesised in a similar manner to **rac-C5-MeO-DPD** (Scheme 1).<sup>2</sup> Alkynyl Grignard addition to Weinreb amide **1**<sup>13</sup> and alkynylation of chloroacetyl chloride afforded ketones **rac-2a** and **rac-2b** in high yield, which could be readily reduced by Luche reduction. Displacement of the chloride in **rac-3b** allowed the introduction of a thioacetate group; acetate protection was chosen as this was stable to the subsequent reaction conditions but would ultimately allow for facile deprotection either under mild conditions or *in situ* as was the case for diacetylated-DPD<sup>14</sup>; in the latter case, the absence of base should render the resulting thiol resistant to oxidation. Subsequent TBS-protection and alkyne oxidation<sup>15</sup> proceeded smoothly to give the three DPD precursors **5a–c** in low to moderate yields. As with all  $\alpha$ -diketone DPD analogues, the final acid-catalysed deprotection was carried out without purification or concentration of the resulting solution; it has previously been shown that the *tert*-butyldimethylsilanol by-product is non-toxic and inactive up to 100  $\mu$ M against both *V. harveyi* and *S. Typhimurium*.<sup>7</sup>

The final target **C5-Br-DPD** was synthesised in enantiopure form via an alternative route. Tosylate **3d** could be prepared in 6 steps from L-gulonic acid  $\gamma$ -lactone,<sup>15–17</sup> subsequent TBS-protection of the alcohol and oxidation of the alkyne provided diketone **7**. The bromide was then installed by displacement of the tosylate in good yield, before acidic deprotection to give **C5-Br-DPD**.

Deprotection of the resulting C5-DPD analogues was monitored by NMR. As was previously observed, **rac-C5-MeO-DPD** gave a clean spectrum with one compound, the linear hydrated form. The same was observed for both **rac-C5-F-DPD** and **rac-C5-Cl-DPD**; detailed analysis of the spectra of these derivatives showed no evidence of instability. However, **C5-Br-DPD** was found to slowly hydrolyse to form DPD under the deprotection conditions, and therefore this compound was not further analysed. TBS deprotection of thioacetate analogue **rac-5c**, initially formed intermediate **rac-C5-SAc-DPD**, and neutralisation subsequently initiated deprotection of the thiol (to give **rac-C5-SH-DPD**). As predicted, the spectra of this compound supported the presence of a complex equilibrium, analogous to that seen upon deprotection of DPD.

All four stable C5-DPD analogues were then tested for both agonism and antagonism of AI-2-mediated QS in Gram negative bacteria *S. Typhimurium* and *V. harveyi* using previously established reporter assays.<sup>18,19</sup> Although one might expect that the linear

analogues cannot bind LsrB, which is believed to require cyclisation for recognition, another pathway for DPD uptake has been postulated, albeit, much slower than LsrB-modulated transport for AI-2 and analogues.<sup>5</sup>

*S. Typhimurium* strain Met844 ( *luxS*), a knockout of the gene encoding DPD synthase *luxS*, was chosen for these assays. This strain also contains a *lacZ-lsr* fusion, coupling activation of the AI-2-dependent *lsr* promoter to the biosynthesis of  $\beta$ -galactosidase, enabling quantification of AI-2-based QS. The strain can be used in the absence of DPD to identify AI-2 QS agonists, and DPD can be added for the detection of antagonists or synergistic agonists, which have previously been reported.<sup>20,21</sup> All four compounds were tested at two concentrations (50 and 200  $\mu$ M) in the presence or absence of DPD (50  $\mu$ M), using DPD itself as an agonism control and C1-Pr-DPD<sup>20</sup> as an antagonism control (Figure 3a–b). No significant agonism or antagonism was observed for any of the tested compounds, which aligned with our expectations for the open-chain analogues. The lack of activity shown by *rac*-C5-SH-DPD is harder to interpret due to the complexity of the AI-2 pathway in this bacterium. There are multiple steps involved in the processing of DPD; binding to LsrB, phosphorylation by LsrK and subsequent binding to transcriptional regulator LsrR. Further work is required to identify what effect if any, *rac*-C5-SH-DPD has on the individual stages of AI-2 processing.

The compounds were subsequently tested against *V. harveyi* strain MM32 ( *luxN*; *luxS*) to look for agonism and strain BB170 ( *luxN*) to identify antagonism; the inherent bioluminescence of this bacterium allows facile quantification of AI-2 mediated QS. Both strains lack the gene encoding AHL receptor *luxN*, excluding the detection of QS modulation through this pathway, and strain MM32 has also had the AI-2 synthase, *luxS*, removed. No antagonism was observed for any of the species, but *rac*-C5-Cl-DPD and *rac*-C5-SH-DPD exhibited moderate and weak agonism, respectively (Figure 3c). Direct comparison with DPD showed a reduced response of *ca.* 10-fold in the case of *rac*-C5-Cl-DPD and *ca.* 100-fold in the case of *rac*-C5-SH-DPD. It should be noted that (*4R*)-DPD has negligible activity compared with (*4S*)-DPD,<sup>22</sup> and thus this assay likely underestimates the true potency of the active enantiomer of these racemic analogues. Furthermore, although partial agonism was indicated by the lower comparative maximum bioluminescence, some or all of this decrease resulted from toxicity of the compounds at higher concentrations, as evidenced both by the decrease in bioluminescence and cell density at higher concentrations of all compounds, including DPD. To rule out the possibility of non-AI-2-mediated QS being responsible for the resulting agonism, *rac*-C5-Cl-DPD was tested against *V. harveyi* strain BB886 ( *luxPQ*), wherein no agonism was observed at concentrations up to 200  $\mu$ M, likely owing to the absence of the AI-2 receptor protein.

As discussed previously, activity of DPD is believed to result from (2*S*,4*S*)-THMF-borate, requiring cyclisation and borate complexation, as observed in a crystal structure of DPD bound to the receptor protein, LuxP. However, the observed agonism of *rac*-C5-Cl-DPD, which is locked in the linear form in *V. harveyi* directly counters this binding requirement.

Given the instability of *rac*-C5-Br-DPD, it was proposed that the chloro analogue may also form small amounts of DPD that could conceivably account for the agonism observed in *V.*

*harveyi*. Although no DPD was observed by  $^1\text{H}$ - or  $^{13}\text{C}$ -NMR, the characteristic signals of the complex DPD equilibrium are much smaller in magnitude than those of the chloride, which exists as a single, hydrated species; the 20% *rac*-DPD required to account for the observed agonism may not be detectable *via* this method. Direct LCMS analysis is not possible on the DPD analogues due to their inherent instability, but derivatisation with 1,2-phenylenediamine allowed for analysis of the resulting quinoxaline products.<sup>23,24</sup> Reaction of *rac*-C5-CI-DPD did in fact show evidence of DPD derivative **10**, suggesting that a small quantity of DPD may have been present, potentially explaining the agonism observed in the *V. harveyi* assay (Scheme 2). Analysis of the supernatant from an assay of the compound against *V. harveyi* does not indicate increased amounts of DPD after an 8-hour incubation at 30 °C, ruling out the possibility of slow DPD formation at neutral pH. It is possible that DPD is produced upon reaction of *rac*-C5-CI-DPD with 1,2-phenylenediamine, *via* formation of the corresponding epoxide derivative **11**, which was also detected. However, this does not rule out the possibility that both *rac*-DPD and *rac*-epoxy-DPD formation occurred during the acidic deprotection step or the subsequent neutralization.

Strong evidence for the absence of a DPD impurity present in the sample of *rac*-C5-CI-DPD can be inferred from the *S. Typhimurium* assays, wherein no agonism was observed at concentrations up to 200  $\mu\text{M}$  compared to the DPD positive control at 50  $\mu\text{M}$ . This combination of assay results suggests that AI-2-mediated QS may not be dependent upon access to a cyclic form of DPD, despite analytical challenges preventing a definitive characterisation.

### 3. Conclusion

A series of DPD analogues were synthesised and evaluated for their ability to modulate QS in two gram-negative bacteria. Neither dynamic DPD analogue *rac*-C5-SH-DPD nor the ring opened analogues showed any activity in *S. Typhimurium*, but linear chloride, *rac*-C5-CI-DPD, was found to be a moderate agonist of AI-2 mediated QS in *V. harveyi* in spite of the presumed requirement for a cyclic DPD signal. As such, the activity of this molecule may hinge on the presence of an unidentified alternative mechanism or binding mode to the LuxP receptor protein, and ultimately may suggest a broader lexicon for *V. harveyi* communication than currently understood.

### 4. Experimental section

Reactions were performed under an inert atmosphere at rt using flame-dried glassware with dry solvents. Reagents were used as commercially supplied, unless otherwise specified. TLC was performed on glass-backed plates pre-coated with silica (EMD 60 F<sub>254</sub>, 0.25–1 mm) and developed using standard visualising agents: UV fluorescence (254 nm) and  $\text{KMnO}_4$ , cerium ammonium nitrate or ninhydrin with appropriate heating.  $^1\text{H}$  and  $^{13}\text{C}$ -NMR were performed on Bruker spectrometers, with the reference from the residual solvent peak for  $^1\text{H}$ -NMR (7.26 ppm for  $\text{CDCl}_3$ , 3.31 ppm for  $\text{CD}_3\text{OD}$ ), and the solvent peak for  $^{13}\text{C}$ -NMR (77.1 ppm for  $\text{CDCl}_3$ , 49.0 ppm for  $\text{CD}_3\text{OD}$ ), coupling constants (*J* values) are given in Hz. HRMS (ESI) were performed on an Agilent 1100 Series LC/MSD-TOF.

#### 4.1. General Method for Luche Reduction

CeCl<sub>3</sub>·7H<sub>2</sub>O (1.1 eq) was added to a solution of crude ketone (1.0 eq) in MeOH (*ca.* 70 mM) at 0 °C. After 5 min, NaBH<sub>4</sub> (1.2 eq) was added portionwise over 5 min, before the solution was stirred at 0 °C (1 h). The reaction was quenched with sat. aq. NH<sub>4</sub>Cl, the solution extracted with CH<sub>2</sub>Cl<sub>2</sub> (× 3), the combined organic layers dried (MgSO<sub>4</sub>), filtered and the solvent removed *in vacuo*. Purification by column chromatography eluting with EtOAc/hexane (gradient, 0:1 → 1:2) gave the title compound.

#### 4.2. General Method for TBS Protection

TBSCl (2.5 eq) was added to a solution of alcohol (1.0 eq) and imidazole (5.0 eq) in CH<sub>2</sub>Cl<sub>2</sub> (*ca.* 200 mM) and the solution stirred at rt (17 h). Sat. aq. NH<sub>4</sub>Cl and CH<sub>2</sub>Cl<sub>2</sub> were added, the aqueous layer was extracted with CH<sub>2</sub>Cl<sub>2</sub> (× 2), the combined organic layers were dried (MgSO<sub>4</sub>), filtered and the solvent removed *in vacuo*. Purification by column chromatography eluting with EtOAc/hexane (gradient, 0:1 → 1:19) gave the title compound.

#### 4.3. General Method for Alkyne Oxidation

A solution of NaIO<sub>4</sub> (2.3 eq) in H<sub>2</sub>O (*ca.* 700 mM) was added to a solution of alkyne (1.0 eq) in CCl<sub>4</sub>/MeCN (1:1, *ca.* 225 mM) and stirred vigorously at rt under air for 3 min before the addition of RuO<sub>2</sub>·xH<sub>2</sub>O (0.02 eq) and the solution stirred vigorously (15 min) before being filtered through a silica pad, washing with CH<sub>2</sub>Cl<sub>2</sub>. Purification by column chromatography eluting with EtOAc/hexane (gradient, 0:1 → 1:9) gave the title compound.

#### 4.4. General Method for TBS-Deprotection

D<sub>2</sub>SO<sub>4</sub> (0.82 μL) was added to a solution of silyl ether (10 μmol) in 4:1 D<sub>2</sub>O/d<sub>6</sub>-DMSO (500 μL) and the solution incubated at rt (24 h). Upon NMR confirmation of complete deprotection, the sample was carefully neutralised to pH 7 from *ca.* pH 1 by the addition of NaOD.

#### 4.5. 1-Fluoropent-3-yn-2-one (2a)

1-propynylmagnesium bromide (52 mL, 0.5M in THF, 26.0 mmol) was added to a solution of Weinreb amide **1** (2.10 g, 17.3 mmol) in THF (165 mL) at 0 °C and the solution stirred at 0 °C (4.5 h). The reaction was quenched with sat. aq. NH<sub>4</sub>Cl (120 mL) and H<sub>2</sub>O (30 mL) was added. The organic layer was separated and the aqueous layer extracted with Et<sub>2</sub>O (2 × 100 mL), the combined organic layers were dried (MgSO<sub>4</sub>), filtered and the solvent removed *in vacuo* to give the ketone **2a** as a yellow oil, which was used without further purification (1.54 g, 89%), δ<sub>H</sub> (500 MHz, CDCl<sub>3</sub>) 4.82 (2H, d, *J*47.2, CH<sub>2</sub>), 2.02 (3H, d, *J*1.2, CH<sub>3</sub>); δ<sub>C</sub> (126 MHz, CDCl<sub>3</sub>) 181.5 (d, *J*22.0), 95.9 (d, *J*2.2), 84.83 (d, *J*188.1), 76.58, 4.19; δ<sub>F</sub> (377 MHz, CDCl<sub>3</sub>) -225.0. Found: 101.0402. C<sub>5</sub>H<sub>6</sub>FO requires 101.0397.

#### 4.6. 1-Chloropent-3-yn-2-one, (2b)

A solution of chloroacetyl chloride (478 mL, 60 mmol) and 1-(trimethylsilyl)propyne (8.88 mL, 60 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (30 mL) was cannulated into a suspension of AlCl<sub>3</sub> (8.80 g, 66 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (120 mL) at 0 °C over *ca.* 30 min. The solution was stirred at 0 °C (1 h) and rt (1 h) before being poured into ice/conc. HCl (200 mL). The aqueous layer was



extracted with CH<sub>2</sub>Cl<sub>2</sub> (2 × 100 mL), the combined organic layers were washed with brine (2 × 150 mL), dried (MgSO<sub>4</sub>), filtered and the solvent removed *in vacuo* to give ketone **2b** as a brown liquid, which was used without further purification (6.8 g, 97%), δ<sub>H</sub> (400 MHz, CDCl<sub>3</sub>) 4.17 (2H, s, CH<sub>2</sub>), 2.05 (3H, s, CH<sub>3</sub>); δ<sub>C</sub> (101 MHz, CDCl<sub>3</sub>) 178.7, 95.1, 77.7, 59.7, 4.3.

#### 4.7. 1-Fluoropent-3-yn-2-ol (**rac-3a**)

Synthesised using the general method for Luche reduction, giving alcohol **rac-3a** as a colourless oil (321 mg, 37%), R<sub>f</sub>0.22 (1:19 EtOAc/hexane); δ<sub>H</sub> (500 MHz, CDCl<sub>3</sub>) 4.62-4.52 (1H, br m, CHOH), 4.42 (1H, ddd, *J*46.9, 9.3, 3.3, CHHF), 4.33 (1H, ddd, *J*47.8, 9.3, 7.2, CHHF), 2.89 (1H, br s, OH), 1.82 (3H, d, *J*2.4, ≡-CH<sub>3</sub>); δ<sub>C</sub> (101 MHz, CDCl<sub>3</sub>) 85.8 (d, *J*175.5), 83.4 (d, *J*1.8), 75.1 (d, *J*12.6), 61.8 (d, *J*23.9), 3.55.

#### 4.8. 1-Chloropent-3-yn-2-ol (**rac-3b**)

Synthesised using the general method for Luche reduction, giving alcohol **rac-3b** as a yellow oil (2.50 g, 66% over 2 steps), R<sub>f</sub>0.33 (1:4 EtOAc/hexane); δ<sub>H</sub> (500 MHz, CDCl<sub>3</sub>) 4.57-4.53 (1H, m, CHOH), 3.69 (1H, dd, *J*11.1, 3.9, CHHCl), 3.61 (1H, dd, *J*11.1, 6.8, CHHCl), 2.27 (1H, br s, OH), 1.87 (3H, d, *J*2.0, CH<sub>3</sub>); δ<sub>C</sub> (101 MHz, CDCl<sub>3</sub>) 83.2, 76.6, 62.8, 49.6, 3.7.

#### 4.9. S-(2-Hydroxypent-3-yn-1-yl) ethanethioate (**rac-3c**)

A solution of KSAc (21 mg, 0.186 mmol) in DMF (1 mL) was added dropwise to a solution of chloride **rac-3b** (20 mg, 0.169 mmol) in DMF (1 mL) at 0 °C, and the solution allowed to warm to rt and stirred (72 h). EtOAc (20 mL) and H<sub>2</sub>O (20 mL) were added and the aqueous layer extracted with EtOAc (2 × 20 mL), washed combined organic layers with brine (2 × 30 mL), dried (MgSO<sub>4</sub>), filtered and the solvent removed *in vacuo*. Purification by column chromatography eluting with EtOAc/hexane (gradient, 0:1 → 1:2) gave alcohol **rac-3c** as a pale yellow oil (16 mg, 60%), R<sub>f</sub>0.40 (1:2 EtOAc/hexane); δ<sub>H</sub> (400 MHz, CDCl<sub>3</sub>) 4.49-4.43 (1H, m, CHOH), 3.24 (1H, dd, *J*13.8, 4.9, CHHS), 3.14 (1H, dd, *J*13.8, 7.0 Hz, CHHS), 2.37 (3H, s, COCH<sub>3</sub>), 1.84 (3H, d, 2.1, ≡-CH<sub>3</sub>); δ<sub>C</sub> (101 MHz, CDCl<sub>3</sub>) 196.0, 82.4, 78.5, 61.9, 36.9, 30.7, 3.7. Found: MH<sup>+</sup>, 159.0471. C<sub>7</sub>H<sub>11</sub>O<sub>2</sub>S requires 159.0474.

#### 4.10. tert-Butyl((1-fluoropent-3-yn-2-yl)oxy)dimethylsilane (**rac-4a**)

Synthesised using the general method for TBS protection, giving silyl ether **rac-4a** as a pale yellow oil (768 mg, 77%), R<sub>f</sub>0.60 (1:19 EtOAc/hexane); δ<sub>H</sub> (500 MHz, CDCl<sub>3</sub>) 4.61-4.54 (1H, m, CHOSi), 4.33 (1H, ddd, *J*46.8, 9.1, 3.8, CHHF), 4.28 (1H, ddd, *J*47.9, 9.1, 7.5, CHHF), 1.82 (3H, d, *J*2.1, ≡-CH<sub>3</sub>), 0.91 (9H, s, C(CH<sub>3</sub>)<sub>3</sub>), 0.13 (3H, s, SiCH<sub>3</sub>), 0.12 (3H, s, SiCH<sub>3</sub>); δ<sub>C</sub> (126 MHz, CDCl<sub>3</sub>) 86.1 (d, *J*178.3), 82.4 (d, *J*2.4), 76.2 (d, *J*13.1), 62.7 (d, *J*24.9), 28.9, 18.4, 3.60, -4.6, -4.9; δ<sub>F</sub> (377 MHz, CDCl<sub>3</sub>) -219.6.

#### 4.11. tert-Butyl((1-chloropent-3-yn-2-yl)oxy)dimethylsilane (**rac-4b**)

Synthesised using the general method for TBS protection, giving silyl ether **rac-4b** as a pale yellow oil (73 mg, 74%), R<sub>f</sub>0.67 (1:19 EtOAc/hexane); δ<sub>H</sub> (500 MHz, CDCl<sub>3</sub>) 4.48-4.44 (1H, m, CHOH), 3.55 (1H, ddd, *J*10.8, 5.0, 0.6, CHHCl), 3.51 (1H, ddd, *J*10.8, 7.3, 0.7,

CH<sub>2</sub>Cl), 1.83 (3H, dd, *J* 2.0, 0.7, ≡-CH<sub>3</sub>), 0.91 (9H, d, *J* = 0.8 Hz, C(CH<sub>3</sub>)<sub>3</sub>), 0.14 (3H, s, SiCH<sub>3</sub>), 0.13 (3H, s, SiCH<sub>3</sub>); δ<sub>C</sub> (126 MHz, CDCl<sub>3</sub>) 82.1, 77.9, 64.1, 49.1, 25.9, 18.4, 3.6, -4.5, -4.9.

#### 4.12. S-(2-((tert-Butyldimethylsilyloxy)pent-3-yn-1-yl) ethanethioate (rac-4c)

Synthesised using the general method for TBS protection, giving silyl ether *rac-4c* as a pale yellow oil (84 mg, 81%), *R<sub>f</sub>* 0.43 (1:9 EtOAc/hexane); δ<sub>H</sub> (400 MHz, CDCl<sub>3</sub>) 4.39-4.34 (1H, m, CHOSi), 3.15 (1H, dd, 13.4, 5.9 Hz, CHHS), 3.09 (1H, dd, *J* 13.4, 7.0, CHHS), 2.32 (3H, s, COCH<sub>3</sub>), 1.81 (3H, d, *J* 2.1, ≡-CH<sub>3</sub>), 0.89 (9H, s, C(CH<sub>3</sub>)<sub>3</sub>), 0.11 (3H, s, SiCH<sub>3</sub>), 0.09 (3H, s, SiCH<sub>3</sub>); δ<sub>C</sub> (101 MHz, CDCl<sub>3</sub>) 195.4, 81.2, 79.3, 62.4, 37.5, 30.6, 25.9, 18.4, 3.6, -4.6, -4.9. Found: MH<sup>+</sup>, 273.1345. C<sub>13</sub>H<sub>25</sub>O<sub>2</sub>SSi requires 273.1339.

#### 4.13. 4-((tert-Butyldimethylsilyloxy)-5-fluoropentane-2,3-dione (rac-5a)

Synthesised using the general method for alkyne oxidation, giving diketone *rac-5a* as a yellow oil (162 mg, 40%), *R<sub>f</sub>* 0.20 (1:19 EtOAc/hexane); δ<sub>H</sub> (400 MHz, CDCl<sub>3</sub>) 5.02 (1H, ddd, *J* 22.9, 5.1, 3.5, CHOSi), 4.67 (1H, ddd, *J* 47.2, 9.8, 5.1, CHHF), 4.56 (1H, ddd, *J* 46.9, 9.8, 3.5, CHHF), 2.36 (3H, s, COCH<sub>3</sub>), 0.89 (9H, s, C(CH<sub>3</sub>)<sub>3</sub>), 0.10 (3H, s, SiCH<sub>3</sub>), 0.09 (3H, s, SiCH<sub>3</sub>); δ<sub>C</sub> (151 MHz, CDCl<sub>3</sub>) 199.3, 196.7 (d, *J* 6.9), 84.3 (d, *J* 174.4), 73.5 (d, *J* 20.9), 25.7, 24.9, 18.3, -4.9, -5.0; δ<sub>F</sub> (377 MHz, CDCl<sub>3</sub>) -228.9. Also recovered alkyne *rac-4a* as a colourless oil (126 mg, 36%).

#### 4.14. 4-((tert-Butyldimethylsilyloxy)-5-chloropentane-2,3-dione (rac-5b)

Synthesised using the general method for alkyne oxidation, giving diketone *rac-5b* as a yellow oil (364 mg, 56%), δ<sub>H</sub> (400 MHz, CDCl<sub>3</sub>) 5.06 (1H, t, *J* 5.0, CHOSi), 3.84-3.79 (1H, m, CHHCl), 3.79-3.74 (1H, m, CHHCl), 2.41 (3H, s, COCH<sub>3</sub>), 0.92 (9H, s, C(CH<sub>3</sub>)<sub>3</sub>), 0.13 (3H, s, SiCH<sub>3</sub>), 0.12 (3H, s, SiCH<sub>3</sub>); δ<sub>C</sub> (126 MHz, CDCl<sub>3</sub>) 199.1, 196.1, 73.2, 45.0, 25.7, 24.9, 18.3, -4.8, -4.9.

#### 4.15. S-(2-((tert-Butyldimethylsilyloxy)-3,4-dioxopentyl) ethanethioate (rac-5c)

Synthesised using the general method for alkyne oxidation, giving diketone *rac-5c* as a yellow oil (33 mg, 38%), *R<sub>f</sub>* 0.50 (1:9 EtOAc/hexane); δ<sub>H</sub> (400 MHz, CDCl<sub>3</sub>) 5.10 (1H, dd, *J* 6.8, 3.9, CHOSi), 3.38 (1H, dd, *J* 13.9, 3.9, CHHS), 3.08 (1H, dd, *J* 13.9, 6.8, CHHS), 2.35 (3H, s, SCOCH<sub>3</sub>), 2.29 (3H, s, OCOCH<sub>3</sub>), 0.87 (9H, s, C(CH<sub>3</sub>)<sub>3</sub>), 0.09 (3H, s, SiCH<sub>3</sub>), 0.06 (3H, s, SiCH<sub>3</sub>); δ<sub>C</sub> (126 MHz, CDCl<sub>3</sub>) 198.6, 195.9, 195.4, 71.1, 71.0, 32.7, 30.5, 25.8, 24.6, 18.3, -4.9. Also recovered alkyne *rac-4c* as a colourless oil (18 mg, 21%).

#### 4.16. 5-Fluoro-4-hydroxypentane-2,3-dione (rac-C5-F-DPD, rac-6a)

Synthesised using the general method for *in situ* TBS-deprotection, giving a colourless solution of *rac-C5-F-DPD*, *rac-6a*, δ<sub>H</sub> (400 MHz, 4:1 D<sub>2</sub>O/d<sub>6</sub>-DMSO) 4.64 (1H, ddd, *J* 46.2, 10.2, 3.3, CHOH), 4.48 (1H, ddd, *J* 47.9, 10.1, 6.6, CHHF), 4.09 (1H, ddd, *J* 19.8, 6.6, 3.3, CHHF), 2.30 (3H, s, COCH<sub>3</sub>), 0.85 (4.5H, s, C(CH<sub>3</sub>)<sub>3</sub>), 0.03 (3H, s, SiCH<sub>3</sub>); δ<sub>C</sub> (151 MHz, 4:1 D<sub>2</sub>O/d<sub>6</sub>-DMSO) 210.8, 97.4 (d, *J* 7.5), 84.9 (d, *J* 164.8), 73.7 (d, *J* 18.6), 26.6 (TBS-OH), 25.8 (TBS-OH), 18.8, -3.0 (TBS-OH); δ<sub>F</sub> (377 MHz, 4:1 D<sub>2</sub>O/d<sub>6</sub>-DMSO) -232.1.



**4.17. 5-Chloro-4-hydroxypentane-2,3-dione (rac-C5-Cl-DPD, rac-6b)**

Synthesised using the general method for *in situ* TBS-deprotection, giving a colourless solution of **rac-C5-Cl-DPD, rac-6b**,  $\delta_{\text{H}}$  (600 MHz, 4:1 D<sub>2</sub>O/d<sub>6</sub>-DMSO) 4.11 (1H, dd, *J* 9.4, 2.5, *CHOH*), 3.92 (1H, dd, *J* 11.8, 2.5, *CHHCl*), 3.61 (1H, dd, *J* 11.8, 9.4, *CHHCl*), 2.39 (3H, s, COCH<sub>3</sub>), 0.93 (9H, s, C(CH<sub>3</sub>)<sub>3</sub>), 0.12 (6H, s, 2 × SiCH<sub>3</sub>);  $\delta_{\text{C}}$  (151 MHz, 4:1 D<sub>2</sub>O/d<sub>6</sub>-DMSO) 210.7, 97.8, 75.6, 46.0, 26.6 (TBS-OH), 25.7, 18.8 (TBS-OH), -3.0 (TBS-OH).

**4.18. 4-Hydroxy-5-mercaptopentane-2,3-dione (rac-C5-SAc-DPD, rac-6c precursor)**

Synthesised using the general method for *in situ* TBS-deprotection, giving a colourless solution of **rac-C5-SAc-DPD, rac-6c precursor**,  $\delta_{\text{H}}$  (400 MHz, 4:1 D<sub>2</sub>O/d<sub>6</sub>-DMSO) 3.88 (1H, dd, *J* 10.0, 2.6, *CHOH*), 3.30 (1H, dd, *J* 14.1, 2.6, *CHHS*), 2.86 (1H, dd, *J* 14.1, 10.0, *CHHS*), 2.36 (3H, s, COCH<sub>3</sub>), 2.31 (3H, s, COCH<sub>3</sub>), 0.87 (9H, s, C(CH<sub>3</sub>)<sub>3</sub>), 0.05 (6H, s, 2 × SiCH<sub>3</sub>);  $\delta_{\text{H}}$  (151 MHz, 4:1 D<sub>2</sub>O/d<sub>6</sub>-DMSO) 211.2, 201.6, 98.1, 73.7, 31.6, 31.5, 26.6 (TBS-OH), 25.7, 18.8 (TBS-OH), -3.0 (TBS-OH).

**4.19. (R)-2-((tert-Butyldimethylsilyloxy)pent-3-yn-1-yl 4-methylbenzenesulfonate (7)**

Synthesised using the general method for TBS protection, giving silyl ether **7** as a colourless oil (209 mg, 81%), *R<sub>f</sub>* 0.34 (1:9 EtOAc/hexane);  $[\alpha]_{\text{D}}^{25}$  -46.1 (*c* 1.0, CHCl<sub>3</sub>);  $\delta_{\text{H}}$  (400 MHz, CDCl<sub>3</sub>) 7.79 (2H, d, *J* 7.8, Ar-H), 7.33 (2H, d, *J* 7.8, Ar-H), 4.55-4.49 (1H, m, CHOSi), 4.01 (1H, dd, *J* 9.6, 3.6, *CHHOS*), 3.91 (1H, dd, *J* 9.8, 7.9, *CHHOS*), 2.44 (3H, s, CH<sub>3</sub>Ar), 1.77 (3H, s, ≡-CH<sub>3</sub>), 0.85 (9H, s, C(CH<sub>3</sub>)<sub>2</sub>), 0.09 (3H, s, SiCH<sub>3</sub>), 0.06 (3H, s, SiCH<sub>3</sub>);  $\delta_{\text{C}}$  (101 MHz, CDCl<sub>3</sub>) 144.9, 135.1, 129.9, 128.1, 82.7, 76.3, 73.0, 61.6, 25.8, 21.8, 18.3, 3.6, -4.7, -4.9. Found: MH<sup>+</sup>, 369.1551. C<sub>18</sub>H<sub>29</sub>O<sub>4</sub>SSi requires 359.1556.

**4.20. (S)-2-((tert-Butyldimethylsilyloxy)-3,4-dioxopentyl 4-methylbenzenesulfonate (8)**

Synthesised using the general method for alkyne oxidation, giving diketone **5d** as a yellow oil (232 mg, 53%), *R<sub>f</sub>* 0.54 (1:2 EtOAc/hexane);  $\delta_{\text{H}}$  (500 MHz, CDCl<sub>3</sub>) 7.76 (2H, d, *J* 8.3, Ar-H), 7.35 (2H, d, *J* 7.9, Ar-H), 5.00-4.98 (1H, m, CHOSi), 4.27 (1H, ddd, *J* 10.4, 5.2, 0.8 Hz, *CHHOS*), 4.22 (1H, ddd, *J* 10.5, 4.1, 0.8, *CHHOS*), 2.45 (3H, s, CH<sub>3</sub>Ar), 2.33 (3H, s, COCH<sub>3</sub>), 0.83 (9H, s, C(CH<sub>3</sub>)<sub>3</sub>), 0.05 (3H, s, SiCH<sub>3</sub>), 0.04 (3H, s, SiCH<sub>3</sub>);  $\delta_{\text{C}}$  (126 MHz, CDCl<sub>3</sub>) 198.8, 195.4, 145.3, 132.6, 130.1, 128.2, 71.7, 70.2, 25.7, 24.8, 21.8, 18.3, -4.9. Found: MH<sup>+</sup>, 401.1454. C<sub>18</sub>H<sub>29</sub>O<sub>6</sub>SSi requires 401.1449. Also recovered alkyne **8** as a colourless oil (71 mg, 18%).

**4.21. (R)-5-Bromo-4-((tert-butyldimethylsilyloxy)pentane-2,3-dione (5d)**

LiBr (6.5 mg, 0.050 mmol) was added to a solution of tosylate **8** (20 mg, 0.050 mmol) in acetone (1.5 mL) and stirred at rt (24 h), whereupon the solution was filtered and washed through with acetone before the solvent was removed *in vacuo*. Purification by column chromatography eluting with EtOAc/hexane (gradient, 0:1 → 1:24) gave bromide **5d** as a yellow oil (12 mg, 78%), *R<sub>f</sub>* 0.24 (1:19 EtOAc/hexane);  $[\alpha]_{\text{D}}^{25}$  +22.3 (*c* 0.8, CHCl<sub>3</sub>);  $\delta_{\text{H}}$  (500 MHz, CDCl<sub>3</sub>) 5.09 (1H, t, *J* 5.0, CHOSi), 3.63 (1H, dd, *J* 10.5, 4.7, *CHHBr*), 3.58 (1H, dd, *J* 10.5, 5.3, *CHHBr*), 2.39 (3H, s, COCH<sub>3</sub>), 0.90 (9H, s, C(CH<sub>3</sub>)<sub>3</sub>), 0.11 (3H, s, SiCH<sub>3</sub>), 0.09 (1H, s, SiCH<sub>3</sub>);  $\delta_{\text{C}}$  (101 MHz, CDCl<sub>3</sub>) 199.0, 195.9, 72.6, 32.6, 25.7, 24.9, 18.3, -4.8, -4.8.

#### 4.22. 5-Bromo-4-hydroxypentane-2,3-dione (C5-Br-DPD, 6d)

Synthesised using the general method for *in situ* TBS-deprotection, giving a colourless solution of C5-Br-DPD, 6d,  $\delta_{\text{H}}$  (600 MHz, 4:1 D<sub>2</sub>O/d<sub>6</sub>-DMSO) 4.10 (1H, d, *J*9.9, C $\text{HOH}$ ), 3.74 (1H, d, *J*11.0, C $\text{HHBr}$ ), 3.42-3.37 (1H, m, C $\text{HBr}$ ), 2.33 (3H, s, CH<sub>3</sub>);  $\delta_{\text{C}}$  (151 MHz, 4:1 D<sub>2</sub>O/d<sub>6</sub>-DMSO) 210.7, 97.9, 75.6, 34.5, 26.6, 25.6, 18.8, -3.0.

#### 4.23. Modulation of bioluminescence in *V. harveyi*

Agonistic and antagonistic activity was evaluated in *V. harveyi* using the procedure of Taga.<sup>25</sup> Briefly, *V. harveyi* strain BB170 (ATCC BAA-1117), MM32 (ATCC BAA-1121) or BB886 (ATCC BAA-1118) was grown in AB medium (14 h, 30 °C). The cells were diluted in fresh AB medium (1/2,500) and added (100  $\mu\text{L}$ /well) to the serially diluted test compounds (previously adjusted to pH 7 using NaOD) in AB medium (100  $\mu\text{L}$ /well) at a final concentration of 0.5% DMSO. The plate was incubated (30 °C, *ca.* 8 h), whereupon the bioluminescence (OD<sub>490</sub>) and cell density (OD<sub>600</sub>) were measured. EC<sub>50</sub> values were calculated using GraphPad Prism 5 from a plot of the bioluminescence (normalised to cell density) versus log(concentration) as the concentration corresponding to 50% maximum bioluminescence.

#### 4.24. Modulation of bioluminescence in *S. Typhimurium*

Agonistic and antagonistic activity was evaluated in *S. Typhimurium* using the procedure of Wolf.<sup>26</sup> Briefly, *S. Typhimurium* strain Met844<sup>5</sup> was grown in LB medium (16 h, 37 °C). The cells were diluted in fresh LB medium (1/25) and added (50  $\mu\text{L}$ /well) to the serially diluted test compounds (previously adjusted to pH 7 using NaOD) with or without DPD in LB medium (150  $\mu\text{L}$ /well) at a final concentration of 0.5% DMSO in deep-well polypropylene plates. The plates were incubated (37 °C, *ca.* 4 h), whereupon an aliquot (50  $\mu\text{L}$ ) was diluted 1/4 and the cell density (OD<sub>600</sub>) measured. A second aliquot (50  $\mu\text{L}$ ) was added to Z buffer (500  $\mu\text{L}$ , containing 0.35% BME), whereupon CHCl<sub>3</sub> (20  $\mu\text{L}$ ) was added and the solution thoroughly mixed. ONPG (20  $\mu\text{L}$ , 4 mg/mL in Z buffer) was added to an aliquot of the aqueous top layer (100  $\mu\text{L}$ ) and the resulting luminescence (OD<sub>420</sub>) was recorded every 1 min for 20 min.  $\beta$ -Galactosidase activity was calculated according to the following equation: "activity =  $V_{\text{max}}/\text{OD}_{600}$ ", where " $V_{\text{max}}$  = maximum slope of kinetic display of OD<sub>420</sub>/min".

### Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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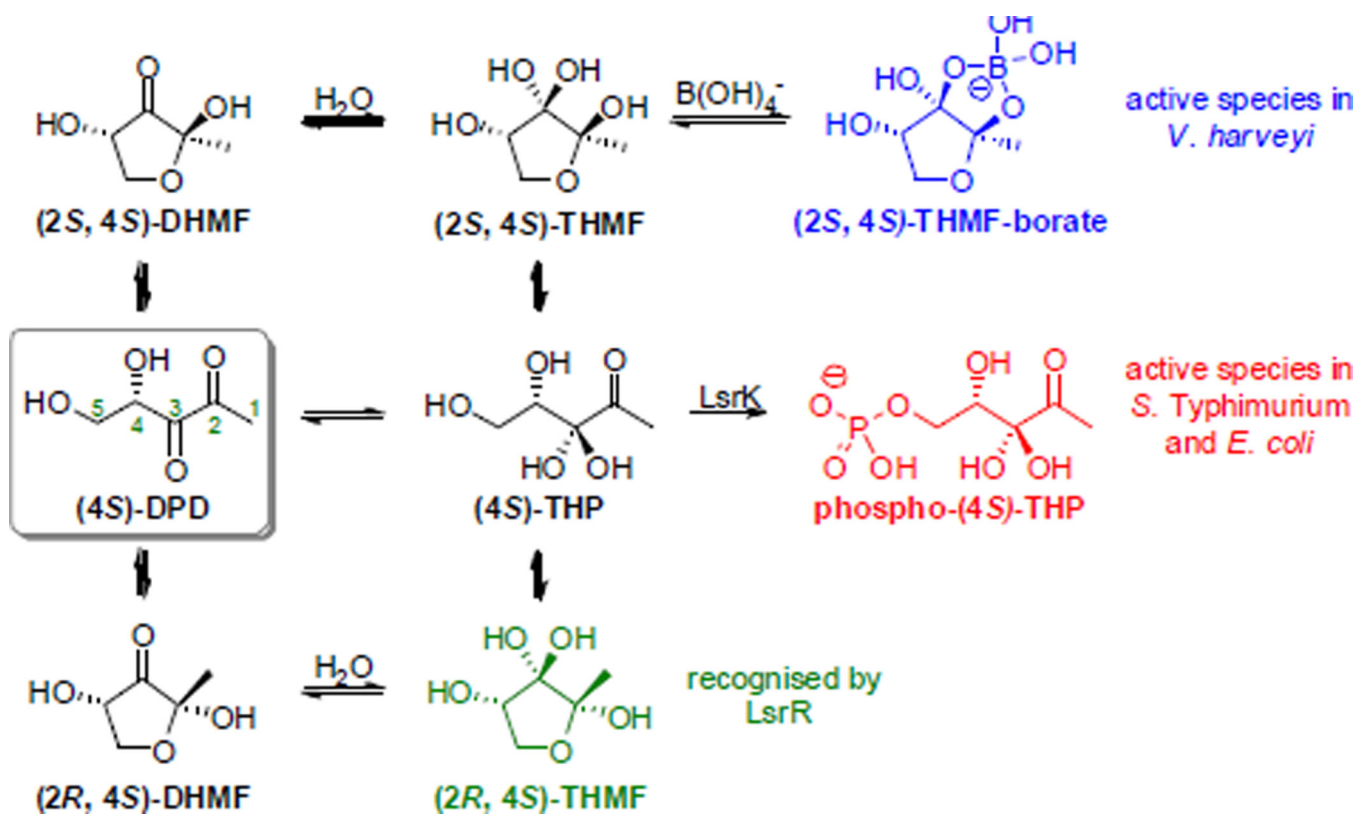
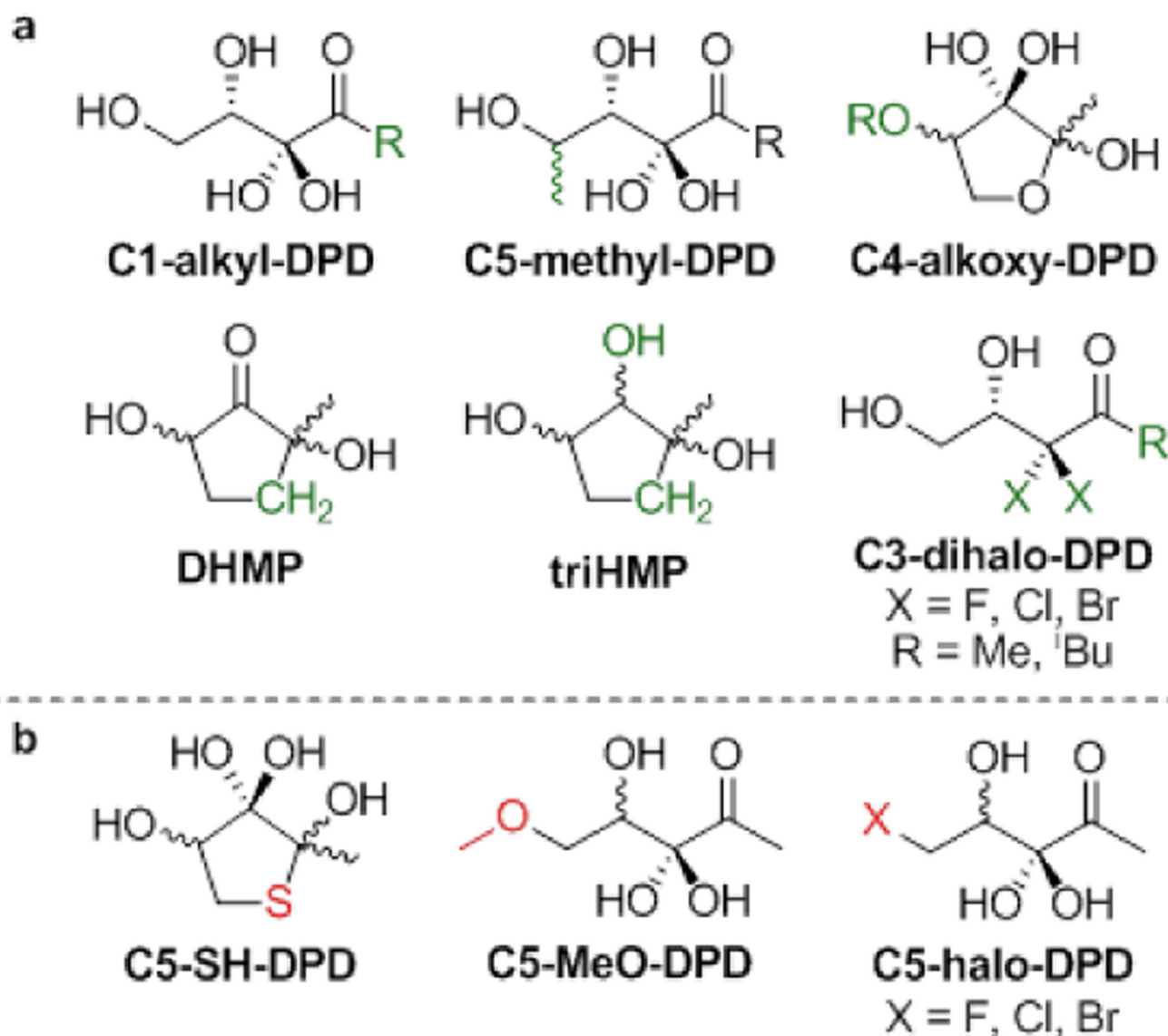
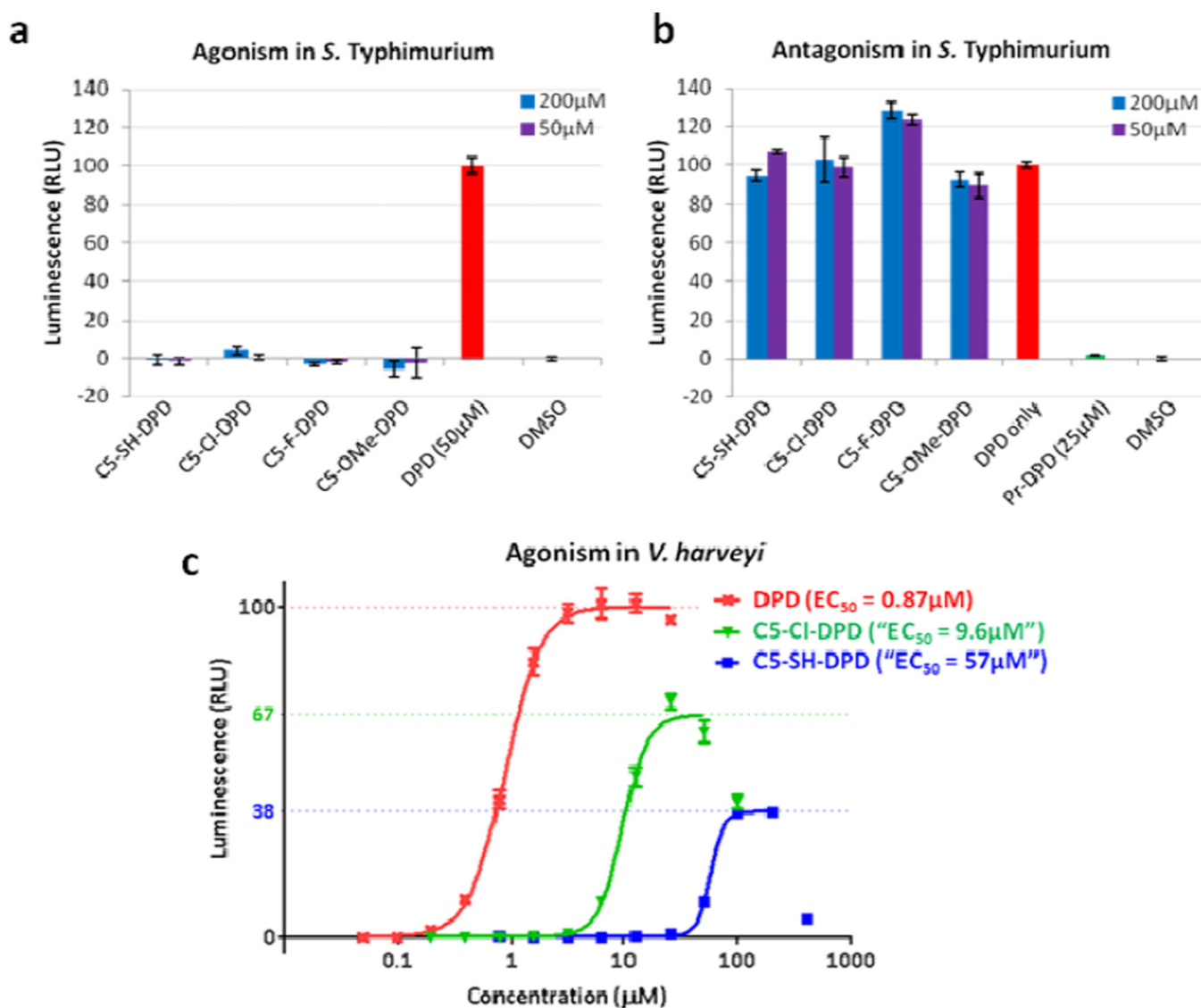


Figure 1.  
Structures of DPD analogues.



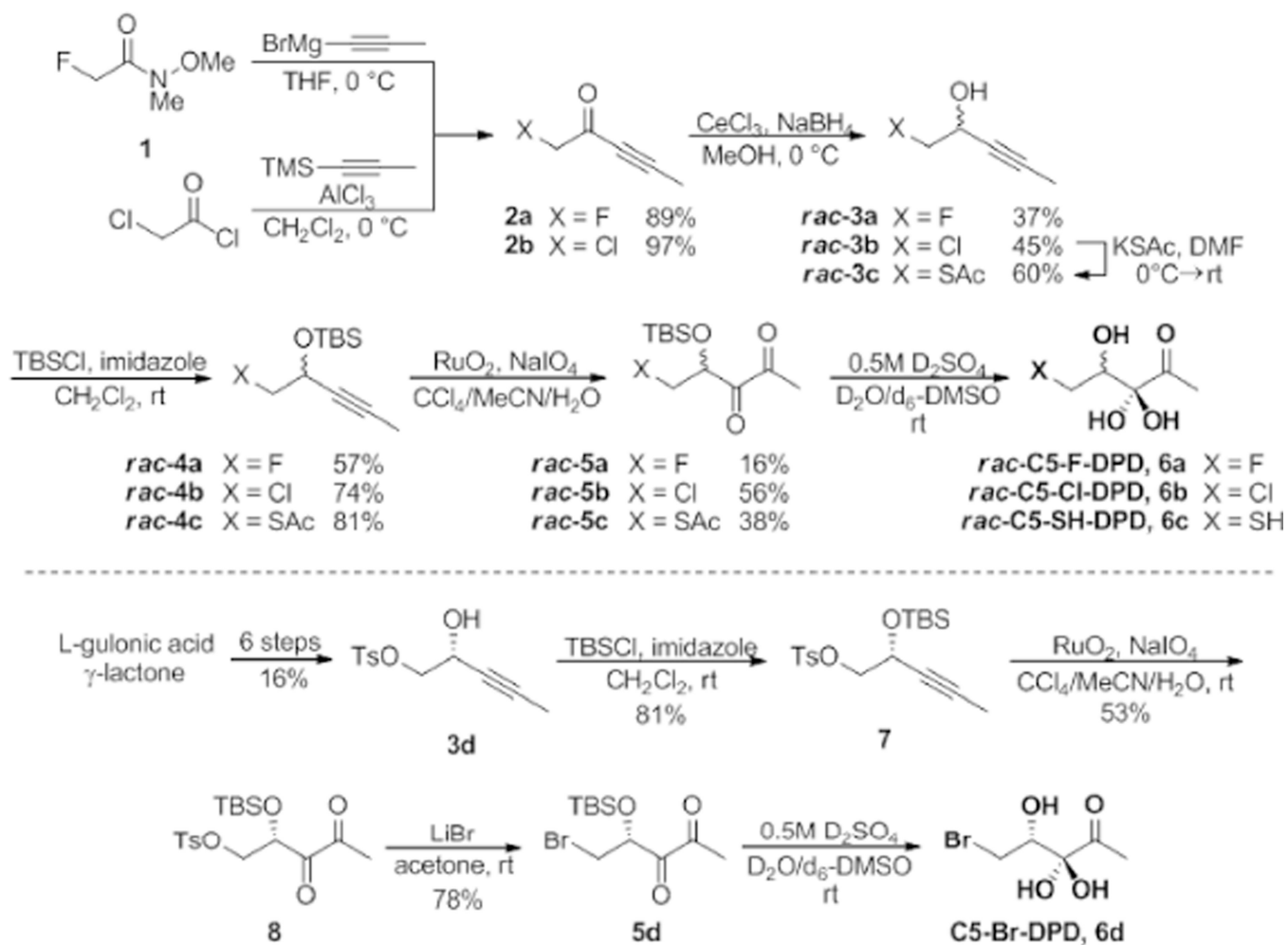
**Figure 2.**  
 Complex equilibrium of AI-2 including the known biologically recognised species.  
 a. Existing DPD analogues. b. Targets of this research.

**Figure 3.**

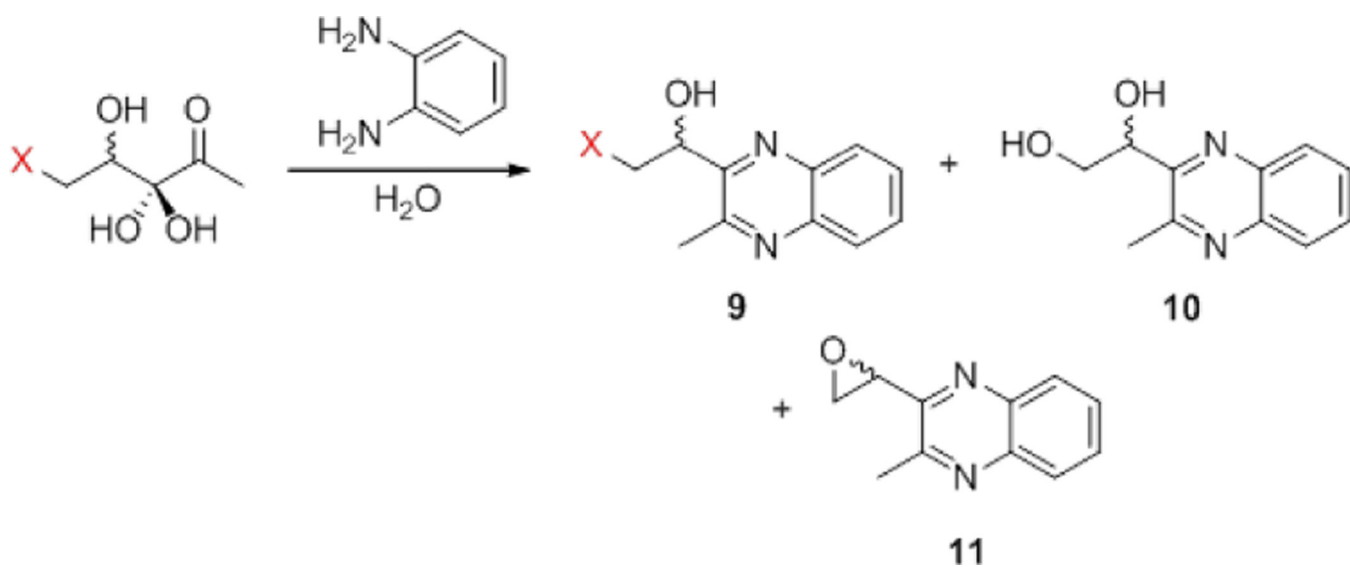
Agonism and antagonism of C5-DPD analogues in *V. harveyi* and *S. Typhimurium*.

**a.** and **b.** Agonism and antagonism, respectively, of C5-DPD analogues in *S. Typhimurium* strain Met844 in the absence and presence of DPD (50  $\mu\text{M}$ ); Pr-DPD used as a positive antagonism control. Bioluminescence in and  $\beta$ -galactosidase activity in *S. Typhimurium* were normalised to cell density. **c.** Agonism of C5-DPD analogues using *V. harveyi* strain MM32. All data was performed in triplicate, errors represent SEM.





**Scheme 1.**  
Synthesis of open- and closed-ring DPD analogues.

**Scheme 2.**

Reaction of C5-halo-DPD analogues and DPD with 1,2-phenylenediamine.