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An efficient synthesis of the precursor of AI-2, the signalling molecule for inter-species quorum sensing

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ABSTRACT

Autoinducer-2 (AI-2) is a signalling molecule for bacterial inter-species communication. A synthesis of (*S*)-4,5-dihydroxypentane-2,3-dione (DPD), the precursor of AI-2, is described starting from methyl glycolate. The key step was an asymmetric reduction of a ketone with (*S*)-Alpine borane. This new method was highly reproducible affording DPD for biological tests without contaminants. The biological activity was tested with the previously available assays and compared with a new method using an *Escherichia coli* reporter strain thus avoiding the use of the pathogenic *Salmonella* reporter.

1. Introduction

(*S*)-4,5-Dihydroxypentane-2,3-dione (DPD) **1** is the uncyclized precursor of AI-2, a signalling molecule for bacterial inter-species communication.[1,2] DPD is a reactive 1,2-diketone which in aqueous medium forms an equilibrium mixture of the linear form and two anomeric cyclic forms **2** and **3**, their hydrated versions **4** and **5** (Scheme 1).[3] The hydrated isomer **4** can exist also as its 2,3-borate diester **6**. It has been shown that distinct bacteria can detect different forms of this molecule and thus all these forms are known collectively as AI-2. Specifically, many *Vibrio* have the LuxP-type of AI-2 receptor which recognizes **6**,[4] whereas members belonging to phylogenetically distinct families such as the human pathogens *Salmonella typhimurium* and *Bacillus anthracis* as well as the plant symbiont *Sinorhizobium meliloti* contain the LsrB-type of receptors and recognize the non-borated diastereoisomer **5**.[3,5]

Bacterial populations use cell–cell communication in order to coordinate their behaviour and function in such a way that they can adapt to changing environments. Chemical communication among bacteria is called 'quorum sensing'. Examples of quorum sensing behaviours are biofilm formation, virulence-factor expression, antibiotic production and bioluminescence.[6] AI-2 is unique in that it is produced and detected by a wide variety of bacteria.[2,7] Thus, using AI-2 bacteria are able to detect the presence of other bacterial species in their vicinity and regulate gene expression according to the species composition in the environment.[8] Ultimately, the understanding of the molecular mechanisms that bacteria use to regulate their behaviours can lead to the development of new therapies to control bacterial infections, and also to develop biotechnological applications for the control of industrial scale production of beneficial bacterial products, such as antibiotics or recombinant proteins.

Although a small molecule, DPD is highly functionalised, optically active and highly reactive. This presents a significant synthetic challenge and the production of reasonable quantities presents many problems. The absence of an accessible synthesis of DPD has been a major drawback in studies aimed at the understanding of AI-2 quorum sensing.[7] In this work we describe an efficient and economic method to synthesise DPD on a reasonable scale. We have also developed a simple procedure to remove the last protective group thus furnishing uncontaminated DPD solutions.

2. Results and discussion

Currently, there are five published[9–13] syntheses of DPD but all have problems and hence were considered unsuitable for the routine production of larger quantities required for biological studies. These routes generally use optically active starting materials and in

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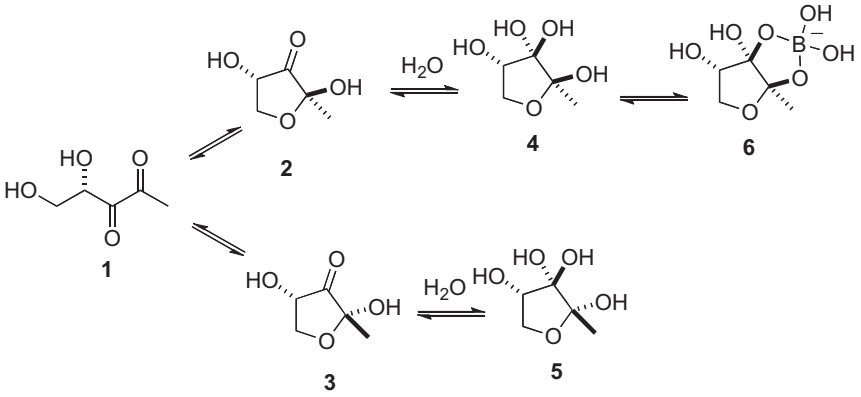


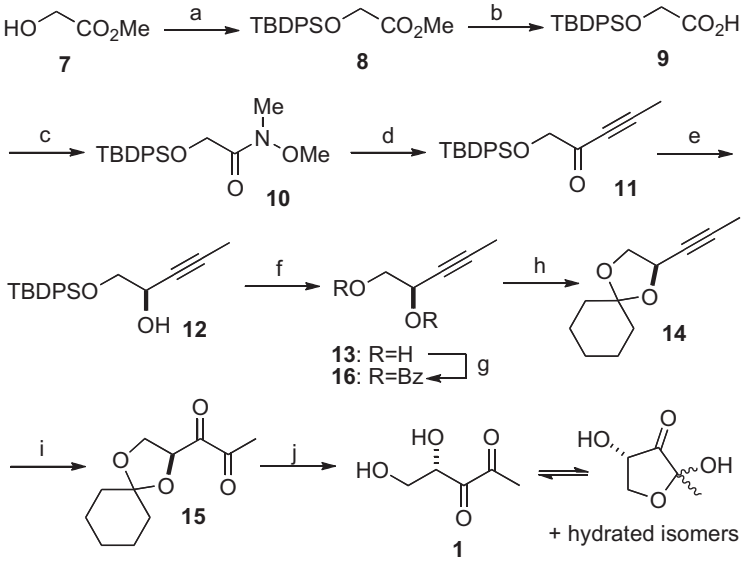
Scheme 1. Forms of DPD/AI-2.

most cases glyceraldehyde, derived from sugars. Each presented chemical or practical problems and we reasoned that a route in which the asymmetric centre was produced by the asymmetric reduction of a ketone could resolve many of them and would allow us to build up the carbon skeleton from two simple units (Scheme 2), making the synthesis efficient in terms of atom economy, and flexible for the production of analogues and isomers.

Protection of the hydroxyl group of methyl glycolate with *tert*-butyldiphenylsilyl chloride (TBDPSCl),[14] followed by hydrolysis of the methyl ester with LiOH in THF/H_2O afforded carboxylic acid **9**[14] in 94% yield. Without purification **9** was converted into amide **10**[14] using *N,O*-dimethylhydroxylamine hydrochloride and DCC in dichloromethane, in 85% yield. Direct formation of the Weinreb amide **10** from the glycolate ester afforded low yields of the product. Conversion of amide **10** to the acetylenic ketone **11** was easily accomplished by using 1-propynyl lithium (85% yield).[15]All of these intermediates could be prepared on a large scale and were stable compounds.

Treatment of **11** with (*S*)-Alpine borane[16] afforded alcohol **12** with the required (*R*) configuration in 67% yield. The enantiomeric excess (ee) of **12** was 86%, $[\alpha]_D^{20}$ +8.85 (*c* 1.57, CH_2Cl_2), as determined by esterification with (*S*)-MTPCl and proton NMR analysis of the two diastereomers formed. Diol **13** was obtained as a crystalline solid in 86% yield after cleavage of the silyl ether with TBAF in THF. Recrystallisation from hexane/ethyl acetate increased the ee of the product to >98%, $[\alpha]_D^{20}$ −18.4 (*c* 0.62, CH_2Cl_2), mp 73.4–74.6 °C, as determined by chiral HPLC analysis of the dibenzoate **16** (Scheme 2).

Reprotection of diol **13** with a cyclohexylidene group afforded alkyne **14**, $[\alpha]_D^{20}$ −41.4 (*c* 1.16, $CHCl_3$), Lit.[10] $[\alpha]_D^{20}$ −39.2 (*c* 0.767, $CHCl_3$). Oxidation of **14** with $RuO_2/NaIO_4$ as described by Semmelhack[10] afforded protected DPD **15** in 81% yield, $[\alpha]_D^{20}$ −11.2 (*c* 1.45, $CHCl_3$). Lit.[10] $[\alpha]_D^{20}$ −11.8 (*c* 0.900, $CHCl_3$). This method had the very important advantage that the acetylenic compound **14** was obtained very cleanly. In our hands, methylation of the terminal acetylene with MeI (Semmelhack)[10] was not always complete, particularly when scaling up, and the desired product was contaminated by the unreacted terminal alkyne, which had the same R_f and was not separable by normal chromatography. Furthermore, oxidation of this mixture afforded a product containing acidic material and after purification low yields of the required product **14** were obtained. Ruthenium mediated oxidation of the pure alkyne **14**, after a flash filtration through a small pad of silica gel to remove ruthenium residues, cleanly afforded dione **15**.



Scheme 2. Synthesis of DPD. Reagents and conditions: (a) TBDPSCl, Pyr, DMAP, rt, 97%; (b) LiOH, THF/H_2O, rt, 94%; (c) HNMeOMe·Cl, DCC, CH_2Cl_2, rt, △ 85%; (d) BuLi, propyne, THF, −78 °C/0 °C, 95%; (e) (*S*)-Alpine borane, THF, rt, 67%; (f) TBAF, THF, rt, 86%; (g) BzCl, (*i*Pr)$_2$NEt, DMAP, CH_2Cl_2, 93%; (h) 1,1-dimethoxycyclohexanone, H_2SO_4, DMF, 91%; (i) $NaIO_4$, RuO_2, CCl_4/MeCN, rt, 86%; (j) Dowex 50WX8, H_2O, pH 3, rt.

ent-DPD was obtained by the same route using (*R*)-Alpine borane to reduce the acetylenic ketone **11**. Oxidation of ***Ent*-14**: $[\alpha]_D^{20}$ +40.5 (*c* 1.68, $CHCl_3$), Lit.[10] $[\alpha]_D^{20}$ +39.4 (*c* 0.796, $CHCl_3$) afforded ***Ent*-15**: $[\alpha]_D^{20}$ +9.7 (*c* 1.83, $CHCl_3$), Lit.[10] $[\alpha]_D^{20}$ +11.5 (*c* 0.733, $CHCl_3$). A rotation lower than that previously reported is explained by partial hydration of the dione. The preparation of ^{13}C-labelled DPD by this method using 3-^{13}C propyne[17] is also envisioned. Up to 0.5 g batches of **15** could be obtained by this method and stored neat in this form. Scale up problems were not encountered and larger batches should be possible.

A hydrolysis protocol to prepare aqueous solutions of DPD **1** from the acetal **15** was developed for the generation of small quantities for NMR and biological studies. This consisted of exposing an aqueous solution of acetal **15** to an aqueous suspension of acidic Dowex 50WX8 resin (pH 3) for a short period followed by a simple filtration to remove the resin. Washing the resulting aqueous solution of DPD **1** with chloroform removed the cyclohexanone. This important step facilitates enzyme studies and NMR analysis of DPD **1,** DPD was thus obtained uncontaminated by inorganic salts and the only salts present during the biological studies were 10 mM phosphate buffer.

The biological activity of the synthetic DPD was compared to that of the enzymatically produced compound (Figs. 1 and 2).

Two methods that detect the borated form of AI-2 **6** were used: the in vivo method using the *Vibrio harveyi* reporter strain which is the most commonly used method, and an in vitro method with LuxP-FRET.[18] By both methods activation by DPD synthesized by the new synthesis was the same as for enzymatic DPD.

To test the biological activity of DPD with an assay for the un-borated isomer of AI-2 **5** we engineered an *Escherichia coli* strain to be used as reporter because this bacterium contains the LsrB receptor which recognizes **5**[5] and induction of the expression of the Lsr system is AI-2-dependent.[19,20] As shown in Figure 2A using this assay the synthetic and enzymatic DPD had the same activities. The use of *E. coli* for assaying this activity in vivo instead of the *S. typhimurium* reporter strain used in previous studies[3] has the advantage of not requiring the use of a pathogen.

Previous studies have shown that the unnatural *ent*-DPD is a weak agonist in the assays for the borated-AI-2 form **6** (Fig. 1),[10,21] we have observed that the same is true for the assay for the isomer **5** (Fig. 2B). To induce the *E. coli* reporter, six times more *ent*-DPD than DPD was necessary in order to obtain the same activity.

3. Conclusion

In conclusion, (*R*)- and (*S*)-DPD were obtained in 8 steps with a 33% overall yield and >98% ee using a common strategy. Synthetic (*S*)-DPD proved to have the same biological activity as that of the enzymatically produced DPD. All intermediates were easy to handle and presented low volatility and excellent stability, the reactions were very reproducible and afforded high yields. Liberation of DPD from acetal **15** with Dowex resin and washing with chloroform allowed us to obtain a final DPD preparation devoid of inorganic salts and organic impurities. With slight modification to this scheme 5-[^{13}C]-labelled DPD could be produced. This synthesis also allows the production of novel DPD analogues.

4. Experimental

4.1. General

^1H NMR spectra were obtained at 400 MHz in $CDCl_3$ or D_2O with chemical shift values (δ) in ppm downfield from tetramethylsilane in the case of $CDCl_3$, and ^{13}C NMR spectra were obtained at 100.61 MHz in $CDCl_3$ or D_2O. Assignments are supported by 2D correlation NMR studies. Flash column chromatography: silica gel Merck 60, 0.040–0.063 mm (230–400 mesh ASTM). Analytical TLC: Aluminium-backed silica gel Merck 60 F_{254}. Specific rotations ($[\alpha]_D^{20}$) were measured using an automatic polarimeter. Reagents and solvents were purified and dried according to Ref. 22. All the reactions were carried out in an inert atmosphere (argon), except when the solvents were undried. The enantiomeric excesses were determined by HPLC on a Waters 600E/U6K instrument using a Daicel Chiralpack AD-H column.

4.1.1. Methyl 2-(*tert*-butyldiphenylsilyloxy)acetate 8

To a solution of methyl glycolate (2.5 g, 27.7 mmol) in pyridine (10 mL) was added *tert*-butyldiphenylsilyl chloride (8.4 g, 30.5 mmol). A catalytic amount of DMAP was added at 0 °C, and the reaction was stirred overnight at room temperature. H_2O was then added and the resulting mixture was extracted with CH_2Cl_2 (3×10 mL), the organic phase was dried with $MgSO_4$, concentrated under vacuum and purified by flash chromatography (5:95 EtOAc/hexane) to give **8** as a colourless oil (8.86 g, 97% yield).

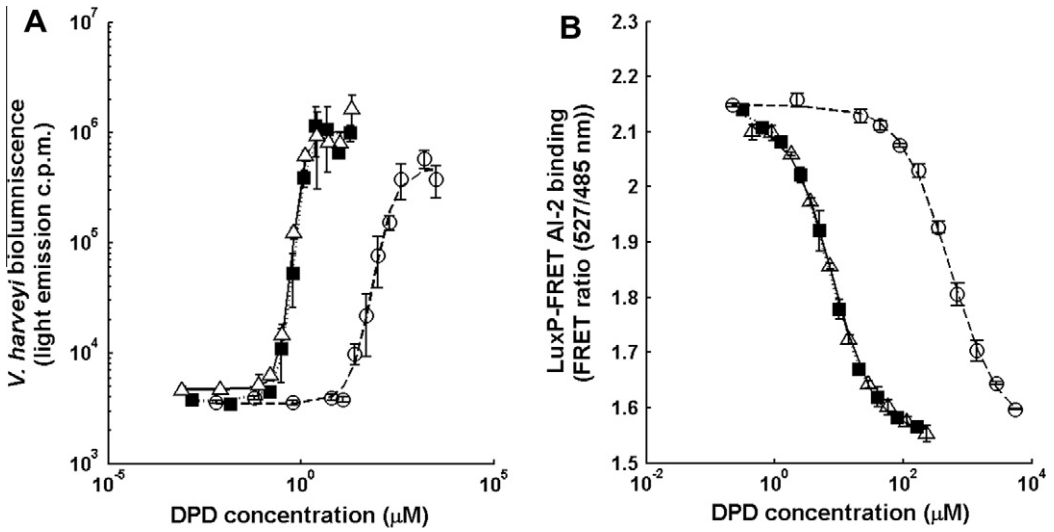


Figure 1. Dose–response curves for the borated form of AI-2 **6** mediated by LuxP. Bioluminescence produced by *V. harveyi* strain MM32 (A) and FRET ratio of the LuxP-FRET protein (B) were assayed following the addition of DPD produced by the new synthesis (squares), DPD produced enzymatically (triangles), and the *ent*-DPD (circles). Error bars represent the standard deviation of three independent trials.

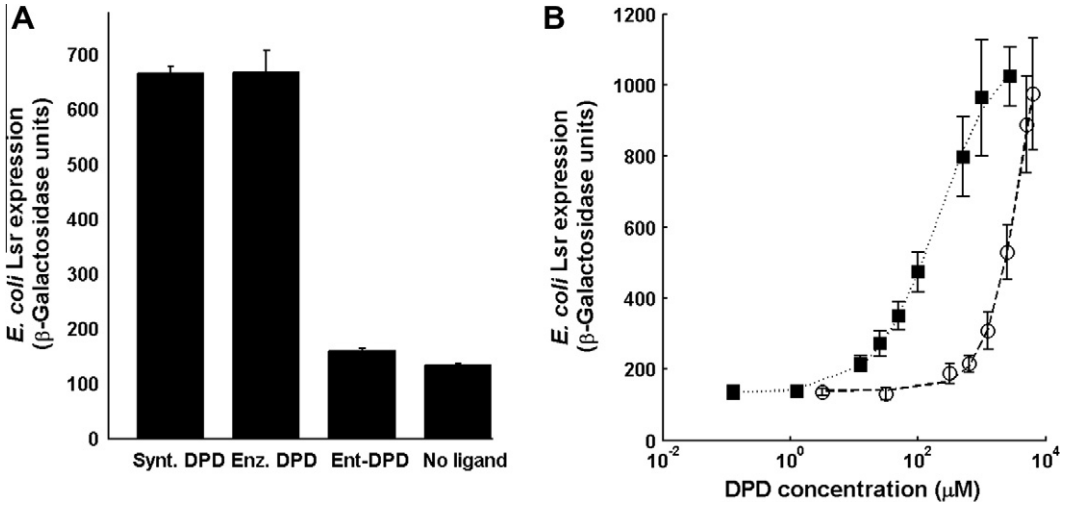


Figure 2. Bioactivity of the non-borated form of AI-2 **5** mediated by LsrB. Expression of the *lsr-lacZ* promoter fusion in *E. coli* strain KX1446 was assayed by determining the β-galactosidase activity following the addition of synthetic or enzymatically produced DPD, the *ent*-DPD (sample concentration of 40 μM was used), and no ligand as negative control (A). A dose–response curve with DPD (squares) and *ent*-DPD (circles) for the synthetic compound is shown in (B). Error bars represent the standard deviation of three independent cultures.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.69–7.68 (m, 4H), 7.43–7.37 (m, 6H), 4.25 (s, 2H), 3.68 (s, 3H), 1.09 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.7, 135.6, 132.8, 129.9, 127.8, 62.1, 26.7, 19.3. FT-IR (film): 1760 (C═O). *M/z* 271.0 (M^+-tBu), 251.1 (M^+-Ph). Elemental Anal. Calcd: C, 69.47; H, 7.36. Obtained: C, 69.50; H, 7.08.

4.1.2. 2-(*tert*-Butyldiphenylsilyloxy)acetic acid 9

To ester **8** (8.86 g, 27.0 mmol) in THF/H_2O (4:1) (33 ml), was added LiOH 0.75 M (97 mL, 72.9 mmol) and the mixture was stirred until all starting material was consumed (TLC). H_2O was added and the resulting mixture was extracted with ethyl ether (3 × 40 mL). The aqueous phase was acidified with HCl 10% (pH ⩾ 3), extracted with ethyl acetate (3 × 50 mL), the combined organic phases dried with $MgSO_4$, filtered and concentrated under vacuum to give **9** which was pure by NMR (7.97 g, 94% yield) as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.68–7.65 (m, 4H), 7.46–7.38 (m, 6H), 4.25 (s, 2H), 1.10 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.9, 135.5, 132.9, 130.2, 128.0, 61.9, 26.7 19.2. *M/z* 237.1 (M^+-Ph). FT-IR (film): 1737 (C═O). Elemental Anal. Calcd: C, 68.75; H, 7.05. Obtained: C, 68.70; H, 6.91.

4.1.3. *N*-Methoxy-*N*-methyl-2-(*tert*-butyldiphenylsilyloxy)acetamide 10

Acid **9** (7.34 g, 23.3 mmol) and *N,O*-dimethylhydroxylamine hydrochloride (6.83 g, 69.9 mmol) were dissolved in CH_2Cl_2 (20 mL) at room temperature. To this mixture was added, slowly, DCC (14.4 g, 69.9 mmol) in CH_2Cl_2 (23 mL) and then refluxed until all the starting material had been consumed (TLC). After cooling to room temperature, H_2O was added, the resulting mixture was extracted with ethyl acetate (3 × 30 mL), the extracts dried with anhydrous $MgSO_4$, concentrated under vacuum and purified by flash chromatography (30:70 EtOAc/hexanes) to afford **10** as white crystals (7.10 g, 85% yield). Mp = 53.4–54.8 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.74–7.72 (m, 4H), 7.42–7.37 (m, 6H), 4.43 (s, 2H), 3.43 (s, 3H), 3.13 (s, 3H), 1.10 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 180.4, 135.5, 133.1, 129.8, 127.7, 62.0, 61.2, 32.5, 26.7, 19.4. FT-IR (KBr): 1691 (C═O). *M/z* 300.4 (M^+-tBu). Elemental Anal. Calcd: C, 67.19; H, 7.61; N, 3.92. Obtained: C, 67.30; H, 7.23; N, 4.30.

4.1.4. 1-(*tert*-Butyldiphenylsilyloxy)pent-3-yne-2-one 11

To a solution of 1.45 M propyne in THF (16 mL, 22.5 mmol) was added Buli 1.6 M in hexanes (13.3 mL, 21.2 mmol) dropwise from a syringe at −78 °C and the mixture stirred at 0 °C over 30 min. At −78 °C, Weinreb amide **10** (6.5 g, 18.2 mmol), previously dissolved in THF (10 mL), was added. The mixture was stirred for 20 min at −78 °C and then at 0 °C until all starting material had been consumed (TLC). Saturated NH_4Cl solution (3 × 30 mL) was added and the resulting mixture was extracted with dichloromethane, dried with anhydrous $MgSO_4$, concentrated under vacuum and purified by flash chromatography (30:70 EtOAc/hexanes) to afford **11** as white crystals (5.79 g, 95% yield). Mp = 50–51 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.68–7.67 (m, 4H), 7.44–7.37 (m, 6H), 4.30 (s, 2H), 1.99 (s, 3H), 1.10 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 186.0, 135.6, 132.8, 129.9, 127.9, 93.2, 78.3, 70.5, 26.7, 19.3, 4.2. FT-IR (film); 1695 (C═O), 2219 (C≡C). *M/z* 279.0 (M^+-tBu), 259.1 (M^+-Ph). Elemental Anal. Calcd: C, 74.96; H, 7.19. Obtained: C, 74.90; H, 7.45.

4.1.5. (*R*)-1-(*tert*-Butyldiphenylsilyloxy)pent-3-yn-2-ol 12

Ketone **11** (3 g, 2.97 mmol) was dissolved in THF (15 mL) at room temperature. (*S*)-Alpine Borane 0.5 M in THF (26.7 mL, 4.45 mmol) was added and the mixture was stirred for 48 h. Saturated NH_4Cl solution (14 mL) was added and the resulting mixture was extracted once with ethyl ether (15 mL) and then with dichloromethane (2 × 15 mL), dried with anhydrous $MgSO_4$, concentrated under vacuum and purified by flash chromatography (5:95 AcOEt/hexane) to afford **12** (2.02 g, 67% yield, 86% ee). $[\alpha]_D^{20}$ +8.85 (*c* 1.57, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.70–7.65 (m, 4H), 7.44–7.36 (m, 6H), 4.45–4.42 (m, 1H), 3.77 (dd, *J* = 3.7, 10.1 Hz, 1H), 3.68 (dd, *J* = 7.5, 10.3 Hz, 1H), 1.81 (d, *J* = 2.2, 3H), 1.07 (s, 9H). ^{13}C NMR (100 MHz. $CDCl_3$): δ 135.6, 135.5, 133.0, 132.9, 129.9, 129.8, 127.8, 127.7, 82.0, 76.9, 67.9, 63.4, 41.9, 26.8, 19.3, 3.6. FT-IR (film): 3571 (OH), 2244 (C≡C). Elemental Anal. Calcd: C, 74.51; H, 7.74. Obtained: C, 74.70; H, 7.69.

To determine the enantioselectivity of the reaction, a small sample of alcohol **12** was treated with (*R*)-MTPA-Cl (3 equiv) and DMAP (3 equiv) in CH_2Cl_2 at room temperature. Integration of ^{1}H NMR (400 MHz, $CDCl_3$) peaks at δ 1.79 (d, *J* = 2.1 Hz, major) and 1.75 (d, *J* = 1.9 Hz, minor) ppm indicated a dr of 93:7 corresponding to 86% ee.

The same procedure using (*R*)-Alpine Borane afforded ***ent*-12**: $[\alpha]_D^{20}$ −8.55 (*c* 1.38, CH_2Cl_2).

4.1.6. (*R*)-Pent-3-yne-1,2-diol 13

To the alcohol **12** (2 g, 5.91 mmol) in THF (10 mL) was added TBAF 1 M in THF (7.09 mL, 7.09 mmol) at room temperature.

The reaction mixture was stirred for 1 h after which all the starting material had been consumed (TLC). The resulting mixture was extracted with ethyl acetate (3 × 10 mL), concentrated under vacuum and purified by flash chromatography (70:30 AcOEt/hexane) to give **13** as white crystals, after recrystallisation from AcOEt/hexane 10 mL/5 mL (0.509 g, 86% yield, 98% ee). $[\alpha]_D^{20}$ −18.4 (*c* 0.62, CH_2Cl_2). Mp = 73.4–74.6 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.44–4.40 (m, 1H), 3.71 (dd, *J* = 3.8, 11.3, 1H), 3.64 (dd, *J* = 6.5, 11.3, 1H), 1.80 (d, *J* = 2.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 82.4, 76.9, 66.7, 63.4, 3.5. FT-IR (KBr): 3603 (OH), 2305 (C≡C). Elemental Anal. Calcd: C, 59.98; H, 8.05. Obtained: C, 59.80; H, 8.15. The same procedure afforded ***ent*-13**: $[\alpha]_D^{20}$ +17.7 (*c* 1.37, CH_2Cl_2). In order to determine the improved ee of the crystallised diol **13** (0.008 g, 0.08 mmol) it was converted to its dibenzoate **16** by treatment with *N*-ethyldiisopropylamine (0.055 mL, 0.32 mmol) and benzoyl chloride (0.027 mL, 0.24 mmol), in the presence of a catalytic amount of DMAP, in CH_2Cl_2 (0.5 mL). The optical purity was determined by HPLC chromatography, using a Chiralcel AD-H column (0.5 mL/min flow in 5:95 isopropanol/hexane) to be 98% (***ent*-13** R_f = 19.0 min, **13** R_f = 20.2 min). Dibenzoate **16**: $[\alpha]_D^{20}$ −49.3 (*c* 0.98, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.07–8.00 (m, 4H), 7.56–7.52 (m, 2H), 7.45–7.39 (m, 4H), 5.96–5.94 (m, 1H), 4.64 (d, *J* = 5.8 Hz, 2H), 1.88 (d, *J* = 2.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.0, 165.5, 133.3, 133.2, 129.9, 129.7, 129.63, 129.61, 128.4, 83.9, 73.1, 65.4, 62.9, 3.7. Dibenzoate ***ent*-16**: $[\alpha]_D^{20}$ +48.9 (*c* 1.55, CH_2Cl_2).

4.1.7. (*R*)-1,2-Cyclohexylidenedioxypent-3-yne 14

To the diol **13** (189 mg, 1.89 mmol) in DMF (2.5 mL) at room temperature, was added cyclohexanone dimethyl ketal (0.567 mL, 3.78 mmol), and two drops of H_2SO_4 and the reaction mixture was stirred overnight. A saturated aqueous solution of $NaHCO_3$ (3 mL) was added (pH ⩾ 8). The mixture was extracted with ethyl ether (3 × 3 mL), concentrated under vacuum and purified by flash chromatography (5:95 AcOEt/hexane) to afford colorless oil **14** (310 mg, 91% yield). $[\alpha]_D^{20}$ −41.4 (*c* 1.16, $CHCl_3$); Lit.[10] $[\alpha]_D^{20}$ −39.2 (*c* 0.767, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.70–4.65 (m, 1H), 4.11 (dd, *J* = 6.1, 7.8 Hz, 1H), 3.82 (dd, *J* = 7.8, 7.0 Hz, 1H), 1.86 (d, *J* = 2.1, 3H), 1.74–1.58 (m, 10H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 110.5, 82.3, 76.4, 69.7, 65.5, 35.8, 35.4, 25.1, 23.9, 23.8, 3.7. FT-IR (film): 2243 (C≡C). *M/z* 181.0 (M^++H), 180.2 (M^+). The same procedure afforded ***ent*-14**: $[\alpha]_D^{20}$ +40.5 (*c* 1.68, $CHCl_3$); Lit.[10] $[\alpha]_D^{20}$ +39.4 (*c* 0.796, $CHCl_3$).

4.1.8. (*S*)-4,5-Cyclohexylidenedioxy-2,3-pentadione 15

To compound **14** (306 mg, 1.7 mmol) dissolved in CCl_4 (9.18 mL) and MeCN (9.18 mL) was added a solution of $NaIO_4$ (826 mg, 3.86 mmol) in H_2O (14 mL) and $RuO_2{\cdot}H_2O$ (5.6 mg, 0.042 mmol) and the reaction mixture was stirred vigorously until all starting material had been consumed (TLC). The mixture was extracted with CH_2Cl_2 (3 × 15 mL), filtered by a very short pad of silica for medium pressure chromatography and concentrated under vacuum to give the bright yellow oil **15** that crystallised as a low melting yellow solid (310 mg, 86% yield). $[\alpha]_D^{20}$ −11.2 (*c* 1.45, $CHCl_3$). Lit.[10] $[\alpha]_D^{20}$ −11.8 (*c* 0.90, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.14 (dd, *J* = 5.3, 7.9 Hz, 1H), 4.35 (dd, *J* = 8.0, 8.9 Hz, 1H), 4.00 (dd, *J* = 5.3, 8.9 Hz, 1H), 2.39 (s, 3H), 1.75–1.56 (m, 10H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 198.2, 194.9, 111.9, 76.5, 65.6, 35.4, 34.7, 25.0, 24.5, 23.9, 23.8. FT-IR (film): 1795 (C═O), 1725 (C═O). The same procedure afforded ***ent*-15**: $[\alpha]_D^{20}$ +9.7 (*c* 1.83, $CHCl_3$); Lit.[10] $[\alpha]_D^{20}$ +11.5 (*c* 0.733, $CHCl_3$).

4.1.9. (*S*)-4,5-Dihydroxy-2,3-pentadione 1. Preparation of DPD and *ent*-DPD samples for bio-assays

Cyclohexylidene DPD **15** was dissolved in water to 10 mM and the pH lowered with acidic Dowex 50WX8 resin (100 mg per mL of sample). After stirring overnight at rt the resin was removed by filtration. Unprotected DPD was then neutralized with 10 mM potassium phosphate buffer, pH 7.0. The same procedure was used to obtain *ent*-DPD ***ent*-1**. To determine the concentration of these samples DPD and *ent*-DPD were quantified by ^{1}H NMR using formate as an internal reference. Spectra were acquired with full relaxation. To remove the released cyclohexanone liquid–liquid extraction with an equal volume of deuterated chloroform was carried out.

DPD obtained enzymatically was produced and quantified as previously described.[23]

4.2. Bacterial strains and media

V. harveyi MM32 (*luxN*::Cm, *luxS*::Tn*5*Kan) was obtained from the laboratory of Bonnie Bassler.[3] *E. coli* strain KX1446 (*lsr-lacZ, ΔluxS, lsrFG*::Cm) was constructed by replacing the *lsrFG* genes in KX1290[17] by chloramphenicol resistance cassettes as described previously[24] using primers with 50 bp of homology to the flanking regions of *lsrF* and *lsrG*.

4.3. DPD biological activity

V. harveyi in vivo response was measured using MM32 *V. harveyi* reporter strain grown in AB (autoinducer bioassay medium) as described previously.[3] Light emission was measured in a Wallac Model 1450 Microbeta scintillation counter after 4 h incubation at 30 °C. Bioluminescence is reported as the light emitted by the cultures as counts per minute (cpm). In vitro response of LuxP-FRET protein was measured based on the method described previously[17] optimized for 96 well plate reading using a multilabel counter (1420 Victor 3, Perkin Elmer). Serial dilution of sample was added to 12.5 μg/ml CFP-LuxP-YFP chimeric protein in 25 mM Sodium Phosphate buffer (pH 8), 35 mM NaCl, and 1 mM borate. Samples (2.5 μl) were added to 280 μl reaction volume and AI-2 concentrations of the samples was calculated from FRET ratio (527/485 nm). Binding of AI-2 to the CFP-LuxP-YFP protein causes a dose-dependent decrease in the FRET signal. *E. coli* in vivo response was measured with the new reporter strain KX1446 grown in LB (Luria-Bertani) using the methodology previously described for the *S. typhimurium* reporter strain.[3] β-Galactosidase activity of the *lsr-lacZ* promoter fusion in *E. coli* was measured with a Bio-Rad 3550-UV microplate Reader. β-Galactosidase units are defined as $[(OD_{420}\ min^{-1} \times \text{dilution factor})/OD_{600})]$. All dose–response curves were fit to variable-slope sigmoidal curves using non-linear least squares analysis.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.12.036.

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