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Probing Autoinducer-2 Based Quorum Sensing: The Biological Consequences of Molecules Unable To Traverse Equilibrium States

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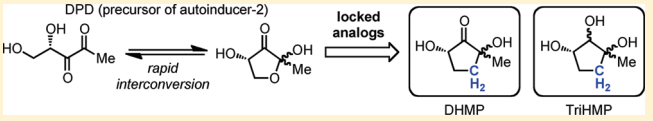
Supporting Information

ABSTRACT: Bacteria have developed a cell-to-cell communication system, termed quorum sensing (QS), which allows for the population-dependent coordination of their behavior via the exchange of chemical signals. Autoinducer-2 (AI-2), a class of QS signals derived from 4,5-dihydroxy-2,3-pentandione (DPD), has been revealed as a universal signaling molecule in a variety of bacterial species. In spite of considerable interest, the study of putative AI-2 based QS systems remains a challenging topic in part due to the rapid interconversion between the linear and cyclic forms of DPD. Herein, we report the design and development of efficient syntheses of carbocyclic analogues of DPD, which are locked in the cyclic form. The synthetic analogues were evaluated for the modulation of AI-2-based QS in *Vibrio harveyi* and *Salmonella typhimurium*. No agonists were uncovered in either *V. harveyi* or *S. typhimurium* assay, whereas weak to moderate antagonists were found against *V. harveyi*. On the basis of NMR analyses and DFT calculations, the heterocyclic oxygen atom within DPD appears necessary to promote hydration at the C3 position of cyclic DPD to afford the active tetrahydroxy species. These results also shed light on the interaction between the heterocyclic oxygen atom and receptor proteins as well as the importance of the linear form and dynamic equilibrium of DPD as crucial requirements for activation of AI-2 based QS circuits.



INTRODUCTION

Over the course of evolution, bacteria have developed a unique mechanism termed "quorum sensing (QS)", which allows for the coordination of their behavior in a population-dependent manner.[1,2] In this process, bacteria produce, release, and respond to small diffusible chemical signals called autoinducers. As the number of cells, and autoinducer concentration, reaches a threshold level, bacteria coordinate their gene expression to behave in a concerted, multicellular manner. This synchronized process represents an effective survival tactic in terms of bacterial population; however, this advantage often comes at the detriment of human health, as certain bacteria utilize QS to regulate pathogenic processes such as biofilm formation and virulence factor production. Consequently, the modulation of QS has emerged as a potential approach for the development of new antimicrobial therapeutics.[3−5]

Autoinducers have been categorized into three major classes based on structural differences:[6−10] (i) *N*-acylhomoserine lactones (AHLs) produced by Gram-negative bacteria, (ii) oligopeptides produced by Gram-positive bacteria, and (iii) autoinducer-2 (AI-2) produced by both Gram-negative and Gram-positive bacterial species.[11−13] Bacteria recognize AHLs and oligopeptides in a species-specific manner based on structural differences within the signaling molecules' chemical makeup. In contrast, AI-2 signaling molecules are derived from a common precursor, 4,5-dihydroxy-2,3-pentanedione (DPD), and the known signals vary only in stereochemistry at the C1 position or the chelation of boron. Interestingly, the gene that encodes the DPD synthase (*luxS*) has been identified in over 70 bacterial species.[14] The ubiquitous nature of the DPD synthase and the interconversion of AI-2 signals (via DPD, Figure 1) has led to the hypothesis that AI-2 functions as a universal signal for interspecies monitoring as well as intraspecies communication.[14,15] In addition to these distinct features, AI-2-based QS has been demonstrated to regulate detrimental events such as virulence factor production and biofilm formation in *Vibrio cholera*,[16] virulence expression in *Salmonella typhimurium*,[17] and mixed-species biofilm development in oral pathogens.[18] As a result, AI-2-based QS has become a target of particular interest in terms of the development of broad range therapeutics.

In spite of the considerable interest, the study of putative AI-2 based QS systems remains challenging largely due to the reactivity and rapid interconversion of DPD (Figure 1). In solution, DPD exists in an equilibrium that contains diastereomeric mixtures of dihydroxytetrahydrofurans (DHMF) and tetrahydroxytetrahydrofurans (THMF) through cyclization and hydration. Difficulties in the isolation and identification of AI-2 were exacerbated by this interconversion, and, in fact, the chemical identities of AI-2 have been solved exclusively via X-ray crystallography of the receptor protein in complex with the AI-2 signal in three species: *Vibrio harveyi*, *Salmonella typhimurium*, and *Sinorhizobium meliloti*. *V. harveyi* responds to

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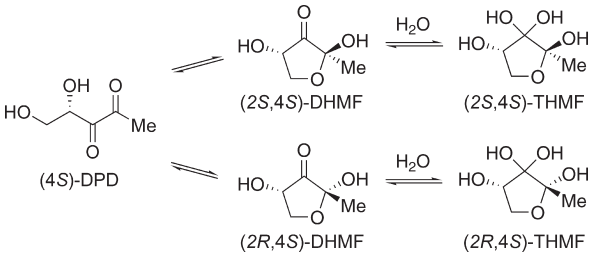


Figure 1. Equilibrium of DPD.

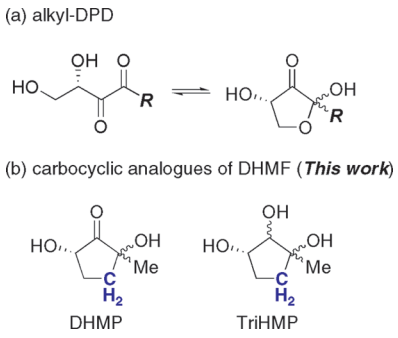


Figure 2. Strategies for the development of modulators of AI-2-based QS.

the borate diester derived from (2*S*,4*S*)-THMF,[19,20] whereas *S. typhimurium*[21] and *S. meliloti*[22] respond to (2*R*,4*S*)-THMF.

The elusive nature of AI-2 is in part created by this equilibrium, and we posit this has hindered the development of potent modulators of AI-2 based QS as well as mechanistic studies of this system.[23] Recently, our laboratory, followed later by other groups, reported the synthesis of a panel of alkyl-DPDs and disclosed that some of these analogues showed potent inhibitory activity against the QS of *S. typhimurium* and synergistic effects on the QS of *V. harveyi* (Figure 2a).[24−27] These results indicate that minor alterations at the C1 position of DPD can have a profound impact upon the function of AI-2 molecules. To complicate analogue development further, these analogues can also exist in multiplexed equilibrium states, thus providing little grounding concerning the roles of DPD acyclic and cyclic forms in QS. With this background in mind, we hypothesized that the use of DPD analogues that exist exclusively in the closed form could shed insights into the role of the DPD equilibrium in AI-2-based QS, as well as a rationale for the design of new modulators. We have focused on the closed forms based on crystallographic data of the AI-2 signals in complex with their cognate periplasmic binding proteins, which indicate that the form initially recognized by the bacteria is the closed form. As such, we hypothesized that the carbocyclic analogues would be effective mimics of these active forms of DPD. Herein, we report the design and syntheses of carbocyclic analogues of the DHMF forms of DPD, wherein the heterocyclic oxygen is replaced with a carbon atom, thus creating 2,5-dihydroxy-2-methylcyclopentanone (DHMP) and 1,2,3-trihydroxy-1-methylpentane (TriHMP) (Figure 2b). We also present the evaluation of these synthetic analogues for the modulation of AI-2-based QS in *V. harveyi* and *S. typhimurium* and scrutinize the structural requirements for agonist and antagonist activity.

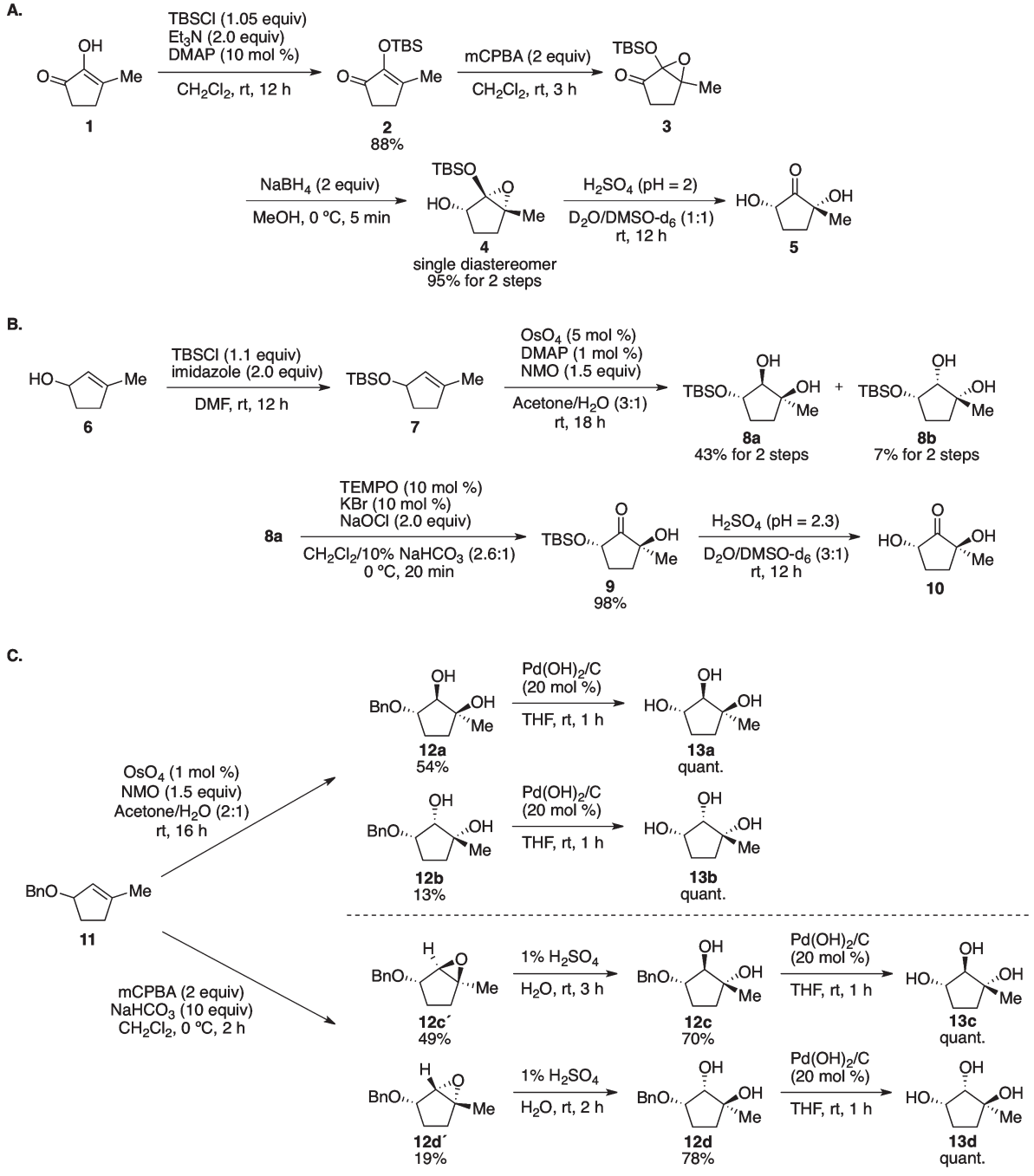
RESULTS

Synthesis of DHMPs and TriHMPs. In spite of the diminutive size of DPD, the chemical synthesis of DPD and its analogues has been a challenging issue because of their highly oxygenated frameworks, lability, and volatility.[28−32] Our target molecules have been no exception; indeed, there has been only one report of the synthesis of *cis*-DHMP and TriHMP that began from the asymmetric oxidation of 2-hydroxy-3-methylcyclopent-2-enone.[33] Thus, we attempted to prepare *cis*-DHMP according to this route; however, we were not able to replicate the reported procedure due to significantly low yields, and only a trace amount of the desired product was obtained at the final step. As such, we were faced with the need to develop a more efficient synthesis of racemic *cis*-DHMP (**5**), shown in Scheme 1a. Our synthesis was initiated with the protection of commercially available 2-hydroxy-3-methylcyclopent-2-enone (**1**) as a *tert*-butyldimethylsilyl (TBS) ether. Subsequently, epoxidation of the obtained 2-silyloxypentenone, **2**, using *m*-chloroperbenzoic acid (mCPBA) was accomplished. This was followed by the reduction of the silyloxy epoxide, **3**, using sodium borohydride to afford epoxide **4**, the precursor of *cis*-DHMP, as a single diastereomer in high yield. The relative configuration of the hydroxy group and the oxirane ring embedded within **4** was determined to be *syn* as analyzed by NOESY analysis. In addition to the high stereoselectivity, it is also noteworthy that the developed route did not require silica gel chromatography except at the silylation step of the starting material **1**. Finally, acidic deprotection of the TBS group provided racemic *cis*-DHMP, **5**, in quantitative yield. In pursuit of racemic *trans*-DHMP, we undertook a sequence engaging oxidation and debenzylation of diol **12a** (vide infra); however, hydrogenolysis or acidic deprotection of the obtained benzyl-protected *trans*-DHMP provided a complex mixture, and an analytically pure product could not be isolated. After further investigation, we discovered that TBS-protected *trans*-DHMP **9**, that could be prepared from 3-methylcyclopent-2-enol **6**[34] through a sequential silylation/osmium-catalyzed dihydroxylation/2,2,6,6-tetramethylpiperidine 1-oxyl (TEMPO) oxidation[35] process, could be converted to racemic *trans*-DHMP **10** quantitatively by acidic deprotection (Scheme 1b). Both *cis*-DHMP **5** and *trans*-DHMP **10** were stable under acidic conditions, and no decomposition was observed after the stock solutions were stored for two weeks at −20 °C. The stock solutions of *cis*-DHMP **5** and *trans*-DHMP **10** contained an equimolar amount of *tert*-butyldimethylsilanol, a side product of deprotection. Several attempts, including silica gel column chromatography, were undertaken to purify the final products from the silanol, but every trial failed due to a significant loss of yield and purity. This instability and degradation during silica gel column chromatography of *cis*-DHMP **5** were also described previously.[33]

The synthesis of the TriHMP analogues was achieved according to Scheme 1c. Benzyl-protected 3-methylcylopentenol **11** has been previously reported[36] and thus was converted into diols **12a** and **12b** under osmium-catalyzed oxidation conditions. At the same time, **11** was also derivatized to diols **12c** and **12d** through an epoxidation/ring-opening process. Notably, the two sets of diols were successfully separated in both routes. Finally, the benzyl groups of the diols **12a**−**d** were removed by palladium-catalyzed hydrogenolysis to give TriHMPs **13a**−**d**.

Evaluation of the Synthesized Analogues in *V. harveyi* and *S. typhimurium* QS Assays. The analogues were evaluated for modulation of QS using two established reporter assays:

Scheme 1. Synthesis of (a) *cis*-DHMP 5, (b) *trans*-DHMP 10, and (c) TriHMPs 13a−d



bioluminescence emission in *V. harveyi*[37] and induction of β-galactosidase activity in *S. typhimurium*.[38] These two reporter assays are ideal for the evaluation of DPD-derived analogues because of the rapid readout of each assay, along with the fact that the AI-2-based QS systems of these two species are among the best characterized AI-2 systems.

The effect of the analogues on bioluminescence was first examined in *V. harveyi* strain BB170 (ATCC BAA-1117, Δ*luxN*), a strain capable of producing bioluminescence through the AI-2 pathway but not through the AHL pathway because of the absence of the AHL receptor, LuxN. Thus, the use of this strain excludes the possibility of the analogues being recognized by the AHL receptor and exerting agonist or antagonist effects via this pathway. Although *V. harveyi* responds to a borate diester form of (2*S*,4*S*)-THMF,[19,20] boric acid was not added during these assays. This treatment was rationalized based on two facets of QS in *V. harveyi*: (1) boric acid itself induces QS activity, diminishing the sensitivity of the assay,[37] and (2) *V. harveyi* responds to DPD and produces bioluminescence without the addition of boric acid. *V. harveyi* was incubated in the presence of each analogue (25 μM), and no significant agonistic effect was observed; however, TriHMP **13a** exhibited a weak antagonistic effect (33% inhibition), and TriHMPs **13b** and **13c** exhibited moderate antagonistic effects (44 and 51% inhibition, respectively)

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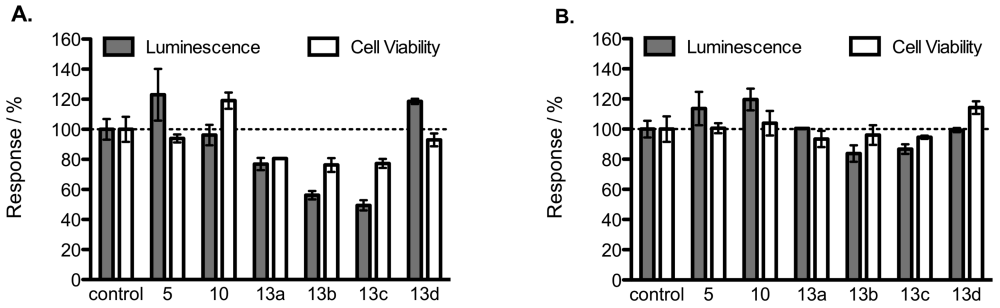

Figure 3. Bioluminescence and cell viability of *V. harveyi* using 25 μM analogue: (a) BB170 (Δ*luxN*); (b) MM32 (Δ*luxN*, Δ*luxS*) in the presence of 1 μM DPD. Bioluminescence was normalized to cell density. All assays were performed in triplicate, and error bars represent standard deviation.

(Figure 3a). However, these compounds also exerted slight cell toxicity resulting in a 23−28% decrease in cell viability of *cis*-DHMP **5** and TriHMPs **13a**−**c**. As a control, the cells were treated with 100 μM *tert*-butyldimethylsilanol, which is present in the stock solutions of DHMPs **5** and **10** from our synthetic protocols. Gratifyingly, neither modulation of QS nor cell toxicity was observed. We also investigated modulation in MM32 cells (ATCC BAA-1121, Δ*luxN*, Δ*luxS*), a strain that is incapable of producing its own DPD due to a lack of the synthase LuxS. To monitor antagonism, the cells were incubated with 1 μM DPD and 25 μM of each analogue and in general the analogues did not exert significant effects on bioluminescence or cell viability (Figure 3b). This observation may be contrasted with the results obtained using BB170 cells, in which the analogues demonstrated moderate effects on both bioluminescence and cell viability. We also monitored agonism by incubating MM32 cells in the absence of exogenous DPD; however, no agonists were uncovered (Supporting Information, Figure S1).

Next, the analogues were screened for QS modulation in *S. typhimurium* strain Met844, a Δ*luxS* strain with a *lacZ-lsr* fusion. The *lacZ-lsr* fusion, which encodes for the biosynthesis of β-galactosidase under the AI-2-regulated *lsr* promoter, enables the monitoring of AI-2-dependent *lsr* activation.[39] Assays were performed using the analogues (50 μM) in the absence of DPD (agonist assay) or in the presence of 50 μM DPD (antagonist assay). In both assays, however, neither agonists nor antagonists were discovered (Supporting Information, Figure S2). Additionally, as in the case of the *V. harveyi* assay, the use of 100 μM *tert*-butyldimethylsilanol did not affect β-galactosidase activity or cell viability.

***cis*-DHMP 5 and *trans*-DHMP 10 Predominantly Exist in Their Keto Forms rather than Hydrated Forms in Aqueous Conditions.** The two reporter assays revealed that the DHMP analogues, which possess a similar structural connectivity to the DHMF forms of DPD, did not significantly affect the QS of either *S. typhimurium* or *V. harveyi*. To investigate the structural features of these DHMP analogues in aqueous conditions, NMR spectra of *cis*-DHMP **5** and *trans*-DHMP **10** were measured in aqueous media (see the Supporting Information). Solutions were made in a mixture of D_2O containing 5% DMSO-d_6 to fully solubilize the compounds, ^{1}H NMR spectra were measured, and minor peaks were observed at 1.04 ppm in the spectrum of *cis*-DHMP **5** and at 1.05 ppm in the spectrum of *trans*-DHMP **10**. The ratios between the methyl peak and the minor peak of each analogue were approximately 8.4:1 and 4.9:1, respectively. Although the hydration forms were not observed using ^{13}C NMR, likely due to low concentration of the sample solutions and the according poor signal-to-noise ratio, we surmise the detected peaks in the ^{1}H spectrum to be the methyl protons of the hydrated forms of *cis*-DHMP **5** and *trans*-DHMP **10**. Working under this hypothesis, these results indicate that only 11% of *cis*-DHMP **5** and 18% of *trans*-DHMP **10** are hydrated in aqueous conditions. This is in contrast to DPD, which has been shown to exist almost exclusively hydrated in aqua.[29]

To obtain more information about the distinct difference between carbocyclic DHMPs **5** and **10** and the DHMF forms of DPD, density functional theory (DFT) calculations were performed using the B3LYP/6-31++(d,p) level of theory with a PCM solvation model to express an aqueous environment (Figure 4). Optimized structures of analogues **5** and **10**, along with (2*R*,4*S*)-DHMF and (2*S*,4*S*)-DHMF, were all found to possess a similarly shaped LUMO, and the oxygen atom within the ring of the DHMFs was not significantly engaged in orbital interactions at the LUMO level. However, the LUMO energies of *cis*-DHMP **5** and *trans*-DHMP **10** were higher than the LUMO energies of the corresponding DHMFs by 0.58 eV (= 15.8 kJ/mol) and 0.96 eV (= 26.2 kJ/mol), respectively. This energy difference is likely derived from the electronegative nature of the heterocyclic oxygen of the DHMF structures, and accordingly, the higher level of the LUMO of DHMPs should be unfavorable to accept nucleophiles such as water.

Equilibrium constants of hydration were also calculated according to the method developed by Casado and co-workers.[40] As a result, under standard conditions (298.15 K, 1 atm), the equilibrium constants of *cis*-DHMP **5** and *trans*-DHMP **10** were calculated to be 0.07 and 0.05, whereas those of (2*R*,4*S*)-DHMF and (2*S*,4*S*)-DHMF were calculated to be 12.34 and 4.60, respectively (Figure 5). These values suggest that DHMPs predominantly exist in their keto forms whereas DHMFs are predominantly hydrated in aqueous conditions, which is consistent with our NMR studies and a previous report on the hydration states of DPD.[29] Consequently, the tetrahydrofuran scaffolding seems to be crucial to promote hydration.

DISCUSSION

In the AI-2-based QS circuit of *V. harveyi*, the periplasmic sensor protein LuxP recognizes a borate diester derived from (2*S*,4*S*)-THMF, which triggers a phosphorylation cascade that ultimately leads to the destabilization of the repressor protein (LuxR) of the QS-regulated *lux* operon. As a result, the *lux* operon is expressed and light emission is initiated. In contrast, in the AI-2 QS circuit of *S. typhimurium*, (2*R*,4*S*)-THMF is recognized by the LsrB binding protein and transported into the cytoplasm. Subsequently, the internalized molecule is phosphorylated by the kinase (LsrK) and the phosphorylated AI-2

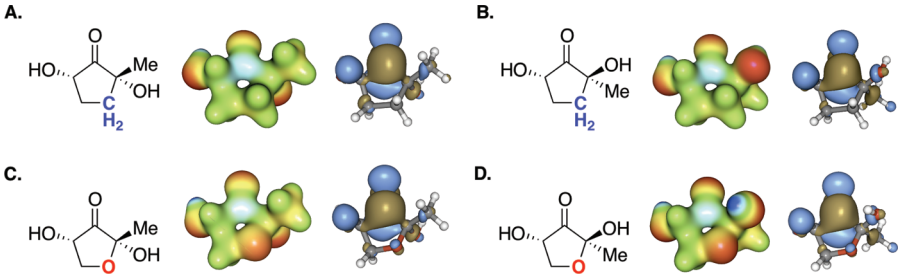

Figure 4. Calculated electrostatic potential maps and LUMO of (a) *cis*-DHMP **5**, (b) *trans*-DHMP **10**, (c) (2*R*,4*S*)-DHMF, and (d) (2*S*,4*S*)-DHMF. The DFT calculations were performed using the B3LYP/6-31++(d,p) level of theory with a PCM solvation model. The LUMO energies were calculated to be (a) −5.53 eV (b) −5.54 eV (c) −6.11 eV, and (d) −6.50 eV, respectively.

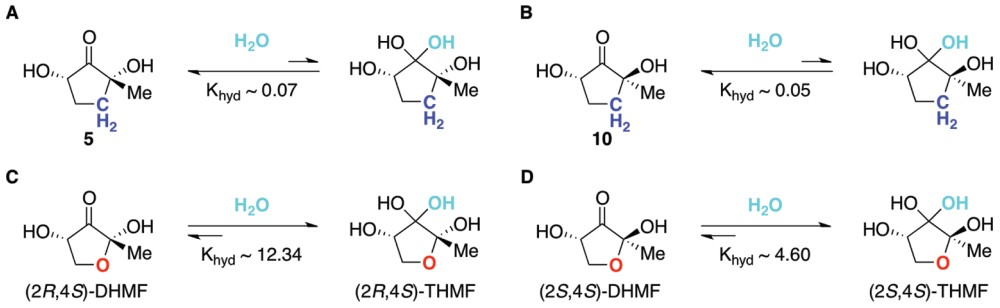

Figure 5. Calculated equilibrium constants (K_{hyd}) of the hydration of (a) *cis*-DHMP **5**, (b) *trans*-DHMP **10**, (c) (2*R*,4*S*)-DHMF, and (d) (2*S*,4*S*)-DHMF.

induces transcription of the *lsr* operon via inactivation of the repressor protein (LsrR).[41,42] In spite of these differences, the periplasmic receptor proteins of both bacterial species recognize a closed form of DPD in which the ketone at C3 is hydrated. On the other hand, the stereochemistry at C2 distinguishes the signals employed by each species: *V. harveyi* responds to the *S* isomer whereas *S. typhimurium* responds to the *R* isomer. Thus, we designed carbocyclic analogues of DPD that would serve as an effective mimic of the active closed forms of DPD while also allowing for the control of relative stereochemistry to tailor the analogues for each species. We hypothesized that these analogues could be recognized by the periplasmic receptor proteins LuxP (*V. harveyi*) and LsrB (*S. typhimurium*) and subsequently act as agonists or antagonists of the AI-2 systems. However, *cis*-DHMP **5** and *trans*-DHMP **10** did not exhibit significant modulation of the QS in either species. This lack of activity is likely the result of a combination of three factors: (1) the absence of the oxygen heteroatom in the cyclopentane scaffold, (2) insufficient hydration at C3, and (3) the elimination of the open-closed equilibrium inherent within the structure of DPD. The first two points may speak to the loss of several hydrogen-bonding interactions in the receptor−ligand complex. An examination of the crystal structures of AI-2 and the receptor proteins reveals that hydrogen bonds would be lost between the ligand and Gln77, Asn159, and Arg215 in LuxP and Asp116, Asp166, and Ala222 in LsrB. As such, these findings provide clues for future analogue design: for example, replacement of the tetrahydroxyfuran oxygen with a difluoromethylene group will serve as more effective mimic of oxygen than the methylene group, while also promoting hydration at the C3 position due to the increased electronegativity.

While the sum of the structural differences between the natural DPD signal and the carbocyclic analogues may account for the absence of observed QS activity, it is equally likely that the underlying reason for the lack of observed activity is the elimination of the dynamic equilibrium. Although crystallographic studies have implicated the closed form of DPD as the active form, we posit that the open form of DPD is initially recognized by the apo form of the receptor protein and subsequently cyclized upon receptor binding. Additionally, it has been demonstrated that the DPD equilibrium is particularly important to the AI-2 system of *S. typhimurium*. In this system, (2*R*,4*S*)-THMF is bound by LsrB and transported into the cytoplasm. It should be noted, however, that recent studies have revealed that this receptor-mediated transport is not crucial for the activity of DPD or alkyl-DPD analogues but these compounds may also enter the cell through diffusion or other unidentified transporters.[27,43] Once internalized, it is the linear form that undergoes phosphorylation at the primary alcohol to produce a molecule termed phospho-DPD, the intracellular agent responsible for regulating *lsr* expression.[41,44] In a similar vein, it has also been demonstrated that the alkyl-DPD analogues, established AI-2 antagonists, require phosphorylation at the primary alcohol to inhibit the *lsr* system.[27] The carbocyclic analogues, in contrast, are locked in the cyclopentane scaffolding, do not possess a primary alcohol for phosphorylation, and cannot achieve the linear conformation required for activity. As such, it is evident that the linear form of DPD and DPD analogues is indispensable for biological activity in *S. typhimurium*. The sum of these findings provides a new outlook on the importance of the equilibrium of DPD, which has always been regarded as the basis for interspecies signaling because of the rapid interconversion of the cyclic forms. While this certainly remains true, the presence of the acyclic form and the dynamic equilibrium may now also be considered as an important

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requirement for intraspecies signaling, at least in bacteria with purported *lsr*-type systems such as *B. anthracis*.[45] In this scenario, it is the cyclic isomer that is recognized by the cell but the linear isomer that is the agent directly responsible for regulating gene expression.

In contrast to the inactivity of DHMPs **5** and **10**, TriHMPs **13b** and **13c** exhibited a modest inhibitory activity on the bioluminescence of *V. harveyi* strain BB170. By replacing the C3 ketone of **5** and **10** with the hydrogen bond donating hydroxyl group, hydrogen bonding would be restored between the ligand and Gln77 in the LuxP complex. The restoration of this hydrogen-bonding interaction may account for the difference in activity between the TriHMP and DHMP analogues; however, although the reason for antagonism rather than agonism is still shrouded, we suggest it may in part be due to general cytotoxic effects of the TriHMP analogues. On the other hand, **13b** and **13c** did not exhibit significant inhibition of growth or bioluminescence in the Δ*luxS* mutant MM32. Given the shared *cis*-1,3-diol structure of **13b**, **13c**, and (2*S*,4*S*)-THMF, coupled with the restoration of QS activity in the Δ*luxS* mutant MM32, an interesting hypothesis is that the target protein of these analogues is the LuxS synthase rather than the receptor protein LuxP. In order to achieve these effects, it is plausible that the analogues enter the cytoplasm through simple diffusion or by previously undiscovered transporters. This hypothesis also dovetails with the results of the *S. typhimurium* assay in that the TriHMP analogues may have been inactive due to the absence of LuxS in the Met844 strain. However, it cannot be discounted that the BB170 cells are simply more sensitive to the toxicity of **13b** and **13c** than the Δ*luxS* strain MM32 via an unknown mechanism.

CONCLUSION

In conclusion, we have achieved an efficient synthesis of a panel of carbocyclic analogues of DHMF, in which the equilibrium forms of DPD were eliminated. These analogues enabled us to evaluate the structural effects of the cyclic forms of DPD on two different bacterial species with well-characterized AI-2 based QS systems: *V. harveyi* and *S. typhimurium*. The obtained results suggest that the dynamic equilibrium of DPD and the oxygen within the tetrahydroxyfuran ring of DHMF significantly affect the activation of AI-2-based QS in both bacterial species. In addition, TriHMPs with a *cis*-1,3-diol scaffold exhibited a moderate inhibitory activity, possibly via the targeting of the LuxS synthase. Although further investigation is necessary to elucidate the molecular mechanisms in detail, the results obtained herein establish the basics of designing more effective modulators and chemical probes for the identification of unknown proteins involved in AI-2-based QS.

EXPERIMENTAL SECTION

Preparation of 2-((*tert*-Butyldimethylsilyl)oxy)-3-methylcyclopent-2-enone (2). To a solution of *tert*-butyldimethylchlorosilane (1.58 g, 10.5 mmol), triethylamine (2.8 mL, 20.0 mmol), and 4-dimethylaminopyridine (0.12 g, 1.0 mmol) in CH_2Cl_2 (20 mL) was added 2-hydroxy-3-methylcyclopent-2-enone (**1**, 1.12 g, 10.0 mmol) at room temperature. After being stirred for 12 h, the resultant solution was quenched with H_2O (20 mL) and extracted with CH_2Cl_2 (×3). The organic layer was washed with brine (×1), dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude products were purified by column chromatography (hexanes/ethylacetate = 20:1) to give analytically pure **2** (2.0 g, 88% yield). White powder. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.49−2.36 (m, 2H), 2.34−2.21 (m, 2H), 1.96 (s, 3H), 0.96 (s, 9H), 0.19 (s, 6H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 202.7, 151.3, 149.4, 32.0, 26.8, 25.6, 18.2, 14.7, −4.2. HRMS (ESI$^+$, *m*/*z*): calcd for $C_{12}H_{23}O_2Si$ 227.1462, found 227.1461 (M + H).

Preparation of *rac*-(1*R*,5*S*)-1-((*tert*-Butyldimethylsilyl)oxy)-5-methyl-6-oxabicyclo[3.1.0]hexan-2-one (3). To a solution of the substrate **2** (226.4 mg, 1.0 mmol) in CH_2Cl_2 (5 mL) at room temperature was added mCPBA (70−75%, balanced with *m*-chlorobenzoic acid and water; 493.1 mg, 2.0 mmol) portionwise. After being stirred for 3 h, the resultant solution was quenched with saturated $NaHCO_3$ (10 mL) and 0.1 N $Na_2S_2O_3$ (10 mL). After the solution was stirred for 10 min, the organic layer was extracted with CH_2Cl_2 (×3) and washed with 0.1 N $Na_2S_2O_3$ (×1), saturated $NaHCO_3$ (×1), and brine (×1). The organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. The obtained crude product **3** (250 mg) was analytically pure and used without further purification. Colorless oil. ^{1}H NMR (400 MHz, CD_2Cl_2): δ 2.29−2.17 (m, 1H), 2.17−2.00 (m, 2H), 1.95−1.82 (m, 1H), 1.52 (s, 3H), 0.92 (s, 9H), 0.24 (s, 3H), 0.15 (s, 3H). ^{13}C NMR (100 MHz, CD_2Cl_2): δ 207.4, 86.1, 69.1, 30.6, 25.9, 25.1, 17.6, 14.8, −4.4, −4.7. HRMS (ESI$^+$, *m*/*z*): calcd for $C_{12}H_{23}O_3Si$ 243.1411, found 243.1400 (M + H).

Preparation of *rac*-(1*R*,2*S*,5*R*)-1-((*tert*-Butyldimethylsilyl)oxy)-5-methyl-6-oxabicyclo[3.1.0]hexan-2-ol (4). To a solution of the substrate **3** (250 mg, assumed as 1.0 mmol) in MeOH (10 mL) at 0 °C was added $NaBH_4$ (75.7 mg, 2.0 mmol). After being stirred for 5 min, the resultant mixture was quenched with acetone (5 mL) and concentrated in vacuo. Water (20 mL) was added to the residue, and then the residue was extracted with CH_2Cl_2 (×3) and washed with brine (×1). The organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. The obtained crude product **4** (231.0 mg, 95% yield for two steps) was analytically pure and used without further purification. White powder. ^{1}H NMR (400 MHz, CD_2Cl_2): δ 4.17 (dd, *J* = 17.1, 8.0 Hz, 1H), 1.93 (ddd, *J* = 13.0, 8.1, 8.1 Hz, 1H), 1.81 (dd, *J* = 14.2, 8.8 Hz, 1H), 1.75 (s, 1H), 1.55 (ddd, *J* = 14.2, 9.9, 8.4 Hz, 1H), 1.34 (s, 3H), 1.21−1.01 (m, 1H), 0.91 (s, 9H), 0.22 (s, 3H), 0.17 (s, 3H). The stereochemistry was determined by NOESY. ^{13}C NMR (100 MHz, CD_2Cl_2): δ 91.6, 73.9, 67.3, 28.5, 28.2, 25.1, 17.5, 14.3, −4.0, −4.2. HRMS (ESI$^+$, *m*/*z*): calcd for $C_{12}H_{24}O_3SiNa$ 267.1387, found 267.1391 (M + Na).

Preparation of *rac*-(2*R*,5*S*)-2,5-Dihydroxy-2-methylcyclopentanone (5). To a solution of the substrate **4** in DMSO-d_6/D_2O (1:1, 10 mM) at room temperature was added 0.5 M H_2SO_4 (1 vol %). The reaction was allowed to continue for 12 h, at which point the compounds were analyzed by NMR. The resultant solution was used in the biological assays without further purification. ^{1}H NMR (400 MHz, 50% DMSO-d_6 in D_2O, pH = 2): δ 4.23−4.07 (m, 1H), 2.17−2.03 (m, 1H), 1.90−1.78 (m, 1H), 1.78−1.55 (m, 2H), 1.11 (s, 3H, Me), 1.03 (s, 0.11H, a methyl group within the hydrated form), 0.75 (s, 9H, TBSOH), −0.09 (s, 6H, TBSOH). ^{13}C NMR (125 MHz, 50% DMSO-d_6 in D_2O, pH = 2): δ 220.1, 74.6, 74.3, 33.9, 28.3, 26.7 (TBSOH), 24.5, 18.8 (TBSOH), −2.7 (TBSOH). The solution was diluted with 1 M phosphate buffer (pH = 7, prepared with D_2O, KD_2PO_4, and K_2DPO_4) to a final concentration of 1 mM in 5% DMSO-d_6, and analyzed by ^{1}H NMR. ^{1}H NMR (400 MHz, 5% DMSO-d_6 in D_2O, pH = 7): δ 4.27 (dd, *J* = 9.5, 9.5 Hz, 1H), 2.18−2.12 (m, 1H), 1.89−1.84 (m, 1H), 1.80−1.73 (m, 1H), 1.68−1.59 (m, 1H), 1.13 (s, 3H, Me), 1.04 (s, a methyl group within the hydrated form), 0.72 (s, 9H, TBSOH), −0.09 (s, 6H, TBSOH). Keto form/hydrated form =8.4:1 (10.6% hydration).

Preparation of *tert*-Butyldimethyl((3-methylcyclopent-2-en-1-yl)oxy)silane (7). To a solution of *tert*-butyldimethylchlorosilane (420.0 mg, 2.79 mmol) and imidazole (347.2 mg, 5.10 mmol) in DMF (2.5 mL) was added a solution of 3-methylcyclopent-2-enol (**6**, 250.0 mg, 2.55 mmol) in DMF (2.5 mL) at room temperature. After being stirred for 12 h, the resultant solution was quenched saturated

NH_4Cl (20 mL) and extracted with diethyl ether (×3). The organic layer was washed with brine (×1), dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude products (517.7 mg) were immediately used without further purification.

Preparation of *rac*-(1*R*,2*S*,3*R*)-3-((*tert*-Butyldimethylsilyl)oxy)-1-methylcyclopentane-1,2-diol (8a) and *rac*-(1*R*,2*S*,3*S*)-3-((*tert*-Butyldimethylsilyl)oxy)-1-methylcyclopentane-1,2-diol (8b). To a solution of the substrate **7** (517.7 mg, 2.55 mmol), *N*-methylmorpholine oxide (448.1 mg, 3.83 mmol), and DMAP (3.1 mg, 0.025 mmol) in acetone (15 mL) and H_2O (5 mL) at room temperature was added OsO_4 (2.5 wt % in *t*-BuOH; 1.3 mL, 5 mol %). After being stirred for 18 h, the resultant mixture was quenched with 0.1 N $Na_2S_2O_3$ (10 mL) and stirred for 10 min. After addition of water (20 mL), the residue was extracted with CH_2Cl_2 (×3) and washed with saturated NH_4Cl (×1) and brine (×1). The organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. The obtained crude products were purified by PTLC (hexanes/ethyl acetate = 5:1 with 0.5% triethylamine) to afford analytically pure **8a** (270.0 mg, 43% yield for two steps) and **8b** (45.0 mg, 7% yield for two steps). **8a**. Off-white powder. ^{1}H NMR (400 MHz, CD_2Cl_2): δ 4.06 (dd, J = 13.9, 6.3 Hz, 1H), 3.42 (d, J = 6.0 Hz, 1H), 2.83 (bs, 1H), 2.56 (bs, 1H), 2.13−1.92 (m, 1H), 1.89−1.64 (m, 2H), 1.46−1.32 (m, 1H), 0.89 (s, 9H), 0.08 (s, 3H), 0.06 (s, 3H). ^{13}C NMR (100 MHz, CD_2Cl_2): δ 83.9, 78.0, 76.0, 34.7, 28.9, 26.1, 25.3, 17.6, −5.2, −5.2. HRMS (ESI$^+$, m/z): calcd for $C_{12}H_{26}O_3SiNa$, 269.1543; found 269.1544 (M + Na). **8b**: Yellow oil. ^{1}H NMR (500 MHz, CD_2Cl_2): δ 4.23−4.10 (m, 1H), 3.39 (dd, J = 8.1, 5.1 Hz, 1H), 2.94 (s, 1H), 2.80 (d, J = 8.1 Hz, 1H), 2.01−1.77 (m, 2H), 1.75−1.58 (m, 2H), 1.19 (s, 3H), 0.91 (s, 9H), 0.11, 0.11 (s × 2, 3H × 2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 77.2, 76.9, 74.0, 35.7, 29.6, 25.2, 24.4, 17.6, −5.4, −5.7. HRMS (ESI$^+$, m/z): calcd for $C_{12}H_{26}O_3SiNa$, 269.1543; found 269.1548 (M + Na).

Preparation of *rac*-(2*R*,5*R*)-5-((*tert*-Butyldimethylsilyl)oxy)-2-hydroxy-2-methylcyclopentanone (9). To a solution of the substrate **8a** (50.0 mg, 0.20 mmol) in CH_2Cl_2 (5.2 mL) and 10% $NaHCO_3$ solution (2 mL) at 0 °C were added TEMPO (3.1 mg, 0.02 mmol), KBr (2.4 mg, 0.02 mmol), and NaOCl (available Cl_2 = 10−15%, 0.28 mL, 0.40 mmol). After being stirred for 20 min, the resultant mixture was quenched with 0.1 N $Na_2S_2O_3$ (20 mL) and stirred for 5 min. The organic layer was extracted with CH_2Cl_2 (×3) and washed with saturated NH_4Cl (×1) and brine (×1). The organic layer was dried over Na_2SO_4, filtered through a silica pad, and concentrated in vacuo. The obtained crude product **9** (48.4 mg, 98% yield) was analytically pure and used without further purification. ^{1}H NMR (400 MHz, CD_2Cl_2): δ 4.17−4.10 (m, 1H), 2.32 (bs, 1H), 2.31−2.21 (m, 1H), 2.05 (dddd, J = 13.0, 6.8, 2.1, 0.6 Hz, 1H), 1.79 (dddd, J = 13.0, 12.4, 6.7, 0.6 Hz, 1H), 1.61 (dddd, J = 12.4, 10.0, 6.8 Hz, 1H), 1.26 (d, J = 0.7 Hz, 3H), 0.90 (s, 9H), 0.11, 0.11 (s × 2, 3H × 2). ^{13}C NMR (100 MHz, CD_2Cl_2): δ 216.5, 73.8, 73.3, 31.8, 27.3, 25.1, 23.8, 17.7, −5.4, −5.7. HRMS (ESI$^+$, m/z): calcd for $C_{12}H_{24}O_3SiNa$ 267.1387, found 267.1412 (M + Na).

Preparation of *rac*-(2*R*,5*R*)-2,5-Dihydroxy-2-methylcyclopentanone (10). To a solution of the substrate **9** in DMSO-d_6/D_2O (3:1, 15 mM) at room temperature was added 0.5 M H_2SO_4 (1 vol %). The reaction was allowed to continue for 12 h, at which point the compounds were analyzed by NMR. The resultant solution was used in the biological assays without further purification. ^{1}H NMR (400 MHz, 75% DMSO-d_6 in D_2O, pH = 2): δ 3.96 (dd, J = 11.1, 8.7 Hz, 1H), 2.14 (dddd, J = 12.5, 8.7, 6.6, 1.7 Hz, 1H), 1.87 (ddd, J = 12.8, 6.7, 1.7 Hz, 1H), 1.64 (ddd, J = 12.8, 6.7, 6.7 Hz, 1H), 1.38 (dddd, J = 12.5, 11.0, 6.7, 6.7 Hz, 1H), 1.05 (s, 3H), 0.78 (s, 9H, TBSOH), −0.08 (s, 6H, TBSOH). ^{13}C NMR (125 MHz, 75% DMSO-d_6 in D_2O, pH = 2): δ 220.7, 75.0, 73.8, 33.8, 27.4, 26.9 (TBSOH), 24.4, 19.0 (TBSOH), −2.4 (TBSOH). The solution was diluted with 1 M phosphate buffer (pH = 7, prepared with D_2O, KD_2PO_4, and K_2DPO_4) to a final concentration of 1 mM in 5% DMSO-d_6, and analyzed by ^{1}H NMR. ^{1}H NMR (500 MHz, 5% DMSO-d_6 in D_2O, pH = 7): δ 4.08 (dd, J = 11.3, 9.0 Hz, 1H), 2.21 (dddd, J = 12.5, 9.0, 6.7, 1.3 Hz, 1H), 1.95 (ddd, J = 13.2, 6.7, 1.3 Hz, 1H), 1.69−1.58 (m, 1H), 1.43−1.39 (m, 1H), 1.08 (s, 3H), 1.05 (s, a methyl group in the hydrated form), 0.70 (s, 9H, TBSOH), −0.10 (s, 6H, TBSOH). Keto form/hydrated form = 4.9:1 (17.9% hydration).

Preparation of *rac*-(1*R*,2*S*,3*R*)-3-(Benzyloxy)-1-methylcyclopentane-1,2-diol (12a) and *rac*-(1*R*,2*S*,3*S*)-3-(Benzyloxy)-1-methylcyclopentane-1,2-diol (12b). To a solution of the benzyl-protected 3-methylcylopentenol **11** (188.3 mg, 1.0 mmol) and *N*-methylmorpholine oxide (175.7 mg, 1.5 mmol) in acetone (10 mL) and H_2O (5 mL) at room temperature was added OsO_4 (2.5 wt % in *t*-BuOH; 130 μL, 1 mol %). After being stirred for 16 h, the resultant mixture was quenched with 0.1 N $Na_2S_2O_3$ (10 mL) and stirred for 10 min. After addition of water (20 mL), the residue was extracted with CH_2Cl_2 (×3) and washed with 1 N HCl (×1) and brine (×1). The organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. The obtained crude products were purified by PTLC (CH_2Cl_2/MeOH = 40:3) to afford analytically pure **12a** (119.5 mg, 54% yield) and **12b** (28.7 mg, 13% yield). **12a**. White powder. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.25−7.03 (m, 5H), 4.49 (d, J = 11.8 Hz, 1H), 4.46 (d, J = 11.8 Hz, 1H) 3.84 (ddd, J = 8.2, 6.1, 6.1 Hz, 1H), 3.57 (d, J = 6.1 Hz, 1H), 3.01 (bs, 1H), 2.45 (bs, 1H), 2.03 (ddd, J = 15.8, 13.4, 8.2 Hz, 1H), 1.80−1.56 (m, 2H), 1.50−1.33 (m, 1H), 1.17 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 138.5, 128.5, 127.9, 127.8, 85.1, 82.8, 77.5, 71.8, 35.0, 26.3, 26.0. HRMS (ESI$^+$, m/z): calcd for $C_{13}H_{18}O_3Na$ 245.1148, found 245.1153 (M + Na). **12b**. Colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.41−7.26 (m, 5H), 4.64 (d, J = 11.7 Hz, 1H), 4.54 (d, J = 11.7 Hz, 1H), 4.01−3.92 (m, 1H), 3.58 (d, J = 4.1 Hz, 1H), 2.96 (bs, 1H), 2.92 (bs, 1H), 2.03−1.76 (m, 3H), 1.72−1.57 (m, 1H), 1.26 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 137.7, 128.5, 127.9, 127.7, 79.9, 77.7, 77.4, 71.9, 35.9, 26.7, 24.9. HRMS (ESI$^+$, m/z): calcd for $C_{13}H_{18}O_3Na$ 245.1148, found 245.1149 (M + Na).

Preparation of *rac*-(1*R*,4*R*,5*S*)-4-(Benzyloxy)-1-methyl-6-oxabicyclo[3.1.0]hexane (12c′) and *rac*-(1*R*,4*S*,5*S*)-4-(Benzyloxy)-1-methyl-6-oxabicyclo[3.1.0]hexane (12d′). To a solution of the substrate **11** (188.3 mg, 1.0 mmol) and $NaHCO_3$ (840.0 mg, 10.0 mmol) CH_2Cl_2 (5 mL) at 0 °C was added mCPBA (70−75%, balanced with *m*-chlorobenzoic acid and water; 493.1 mg, 2.0 mmol) portionwise. After being stirred for 2 h, the resultant solution was quenched with saturated $NaHCO_3$ (10 mL) and 0.1 N $Na_2S_2O_3$ (10 mL). After being stirred 10 min, the organic layer was extracted with CH_2Cl_2 (×3) and washed with 0.1 N $Na_2S_2O_3$ (×1), saturated $NaHCO_3$ (×1), and brine (×1). The organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. The obtained crude products were purified by column chromatography (hexanes/ethyl acetate = 20:1 to 10:1) to afford analytically pure **12c′** (99.0 mg, 49% yield) and **12d′** (39.5 mg, 19% yield). **12c′**. Yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.39−7.09 (m, 5H), 4.51 (d, J = 11.9 Hz, 1H), 4.42 (d, J = 11.9 Hz, 1H), 3.98 (d, J = 5.5 Hz, 1H), 3.20 (s, 1H), 1.84−1.74 (m, 2H), 1.74−1.62 (m, 1H), 1.62−1.46 (m, 1H), 1.42 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 138.2, 128.4, 127.6, 127.6, 79.7, 71.4, 64.8, 63.4, 29.5, 27.7, 17.3. HRMS (ESI$^+$, m/z): calcd for $C_{13}H_{17}O_2$ 205.1229, found 205.1243 (M + H). **12d′**. Yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.38−7.12 (m, 5H), 4.53 (s, 2H), 3.96 (ddd, J = 7.6, 5.1, 1.2 Hz, 1H), 3.23 (d, J = 0.9 Hz, 1H), 1.96−1.81 (m, 1H), 1.80−1.70 (m, 1H), 1.57−1.37 (m, 2H), 1.34 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 138.4, 128.3, 127.7, 127.6, 79.7, 71.3, 62.5, 62.4, 29.7, 25.4, 18.0. HRMS (ESI$^+$, m/z): calcd for $C_{13}H_{17}O_2$ 205.1229, found 205.1133 (M + H).

Preparation of *rac*-(1*R*,2*R*,3*S*)-3-(Benzyloxy)-1-methylcyclopentane-1,2-diol (12c). To the substrate **12c′** (20.0 mg, 0.1 mmol) at room temperature was added 1% H_2SO_4 (2 mL). After being stirred for 3 h, the resultant mixture was extracted with diethyl ether (×3). The organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. The obtained crude products were purified by PTLC (hexanes/ethyl acetate = 1:1) to afford analytically pure **12c** (15.0 mg,

70% yield). Colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.35−7.27 (m, 5H), 4.59 (d, J = 11.8 Hz, 1H), 4.55 (d, J = 11.8 Hz, 1H), 3.92 (d, J = 4.1 Hz, 1H), 3.81 (ddd, J = 7.5, 3.8, 3.8 Hz, 1H), 2.65 (bs, 1H), 2.14 (bs, 1H), 2.11−1.97 (m, 1H), 1.94−1.64 (m, 4H), 1.27 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 138.0, 128.5, 127.7, 84.8, 83.1, 79.4, 71.6, 36.1, 27.1, 27.1, 21.9. HRMS (ESI$^+$, m/z): calcd for $C_{13}H_{18}O_3Na$ 245.1148, found 245.1152 (M + Na).

Preparation of *rac*-(1*R*,2*R*,3*R*)-3-(Benzyloxy)-1-methylcyclopentane-1,2-diol (12d). This compound was prepared from the substrate **12d′** (35.5 mg, 0.17 mmol) by the same method as the preparation of **12c** (30.0 mg, 78% yield). Colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.43−7.27 (m, 5H), 4.58 (d, J = 11.7 Hz, 1H), 4.52 (d, J = 11.7 Hz, 1H), 4.23 (dd, J = 8.1, 5.1 Hz, 1H), 3.75−3.66 (m, 1H), 2.76 (bs, 1H), 2.19−2.05 (m, 1H), 1.93 (ddd, J = 13.4, 10.6, 6.5 Hz, 1H), 1.79−1.67 (m, 1H), 1.63−1.47 (m, 2H), 1.36 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 137.9, 128.5, 127.9, 127.7, 80.5, 80.1, 78.5, 71.8, 35.4, 27.5, 23.4. HRMS (ESI$^+$, m/z): calcd for $C_{13}H_{18}O_3Na$ 245.1148, found 245.1152 (M + Na).

Preparation of *rac*-(1*R*,2*S*,3*R*)-1-Methylcyclopentane-1,2,3-triol (13a). To a solution of the substrate **12a** (34.0 mg, 0.153 mmol) in THF (10 mL) at room temperature was added $Pd(OH)_2/C$ (20 wt %, <50% water; 17.2 mg, 20 mol %). The mixture was bubbled using H_2 gas for 1 min, and the reaction was allowed to continue for 1 h with vigorous stirring. The resultant mixture was filtered through a Celite pad, rinsed with MeOH, and concentrated in vacuo. Azeotropic distillation of the obtained residue was conducted using dry toluene (10 mL) two times to afford **13a** containing water (37.8 mg). The purity of **13a** was ascertained by NMR, and the yield was determined to be quantitative. Colorless oil. ^{1}H NMR (400 MHz, CD_3OD): δ 4.07−4.01 (m, 1H), 3.37 (d, J = 6.9 Hz, 1H), 2.11−1.98 (m, 1H), 1.78 (ddd, J = 13.0, 10.8, 6.6 Hz, 1H), 1.69 (ddd, J = 14.0, 10.8, 6.6 Hz, 1H), 1.48−1.33 (m, 1H), 1.24 (s, 3H). ^{13}C NMR (100 MHz, CD_3OD): δ 85.2, 78.0, 77.5, 35.8, 29.1, 26.2. HRMS (ESI$^+$, m/z): calcd for $C_6H_{12}O_3Na$ 155.0679, found 155.0677 (M + Na).

Preparation of *rac*-(1*R*,2*S*,3*S*)-1-Methylcyclopentane-1,2,3-triol (13b). This compound was prepared from the substrate **12b** (11.3 mg, 0.051 mmol) by the same method as the preparation of **13a** (13.6 mg). The purity of **13b** was ascertained by NMR, and the yield was determined to be quantitative. Yellow solid. ^{1}H NMR (400 MHz, CD_3OD): δ 4.12−3.98 (m, 1H), 3.45 (d, J = 5.2 Hz, 1H), 2.00−1.81 (m, 2H), 1.81−1.66 (m, 1H), 1.66−1.50 (m, 1H), 1.23 (s, 3H). ^{13}C NMR (100 MHz, CD_3OD): δ 79.1, 78.6, 73.9, 36.7, 30.5, 25.8. HRMS (ESI$^+$, m/z): calcd for $C_6H_{12}O_3Na$ 155.0679, found 155.0686 (M + Na).

Preparation of *rac*-(1*R*,2*R*,3*S*)-1-Methylcyclopentane-1,2,3-triol (13c). This compound was prepared from the substrate **12c** (12.3 mg, 0.055 mmol) by the same method as the preparation of **13a** (14.0 mg). The purity of **13c** was ascertained by NMR, and the yield was determined to be quantitative. White solid. ^{1}H NMR (400 MHz, CD_3OD): δ 3.86−3.80 (m, 1H), 3.63−3.62 (m, 1H), 1.97−1.90 (m, 1H), 1.86−1.74 (m, 1H), 1.72−1.54 (m, 2H), 1.18 (s, 3H). ^{13}C NMR (100 MHz, CD_3OD): δ 86.6, 79.6, 78.6, 37.0, 29.9, 23.0. HRMS (ESI$^+$, m/z): calcd for $C_6H_{12}O_3Na$ 155.0679, found 155.0680 (M + Na).

Preparation of *rac*-(1*R*,2*R*,3*R*)-1-Methylcyclopentane-1,2,3-triol (13d). This compound was prepared from the substrate **12d** (11.0 mg, 0.049 mmol) by the same method as the preparation of **13a** (11.0 mg). The purity of **13d** was ascertained by NMR, and the yield was determined to be quantitative. Colorless oil. ^{1}H NMR (400 MHz, CD_3OD): δ 4.41−4.26 (m, 1H), 3.52 (d, J = 4.7 Hz, 1H), 2.14−1.96 (m, 1H), 1.93−1.75 (m, 1H), 1.60−1.54 (m, 2H), 1.27 (s, 3H). ^{13}C NMR (100 MHz, CD_3OD): δ 81.0, 73.9, 36.8, 30.7, 23.7. HRMS (ESI$^+$, m/z): calcd for $C_6H_{12}O_3Na$ 155.0679, found 155.0683 (M + Na).

Modulation of Bioluminescence in *V. harveyi*. Evaluation of the synthetic analogues was performed in *V. harveyi* BB170 or MM32 as follows: *V. harveyi* strain was grown for 14 h at 30 °C in AB medium and then diluted 1:2500 in fresh AB medium. A 96-well plate was prepared with wells containing test compounds and 1% DMSO in AB medium (100 μL/well). In the assay using MM32, DPD was also added to each well for a final concentration of 1 μM. The diluted cells (100 μL) were then added to each well and the plate was incubated with shaking at 30 °C for 7 h (MM32) or 9 h (BB170). After incubation, OD_{600} and bioluminescence were measured on a SpectraMax plate reader. The bioluminescence was normalized to cell density.

β-Galactosidase Activity Assay in *S. typhimurium* (*lsr* Expression). Evaluation of the synthetic analogues was performed in *S. typhimurium* Met844 by measuring β-galactosidase activity as follows: an overnight culture was diluted 1:100 in fresh Luria−Bertani (LB) medium and incubated with the test compound (0.5% DMSO) and 50 μM DPD at 37 °C for 4 h. After incubation, an aliquot (100 μL) was diluted 1:1 in LB medium and added to a 96-well plate to measure OD_{600}. Another aliquot (50 μL) was vigorously suspended in Z-buffer (500 μL, 40 mM Na_2HPO_4, 60 mM NaH_2PO_4, 10 mM KCl, and 1 mM $MgSO_4$, pH = 7), β-mercaptoethanol (1.78 μL), 1% SDS (2 μL), and $CHCl_3$ (20 μL). After settlement for 20 min, the resultant lysate (100 μL) was placed in a 96-well plate and *o*-nitrophenyl-β-D-galactopyranoside (ONPG, 4 mg/mL, 20 μL) was added to initiate the assay. The OD_{420} was read every 1 min for 15 min. β-Galactosidase activity was calculated according to the following equation: Activity = $[OD_{420}/(OD_{600} \times t \times V_{cell})] \times V_{total} \times C$; where "$t$" is reaction time in minutes, "V_{cell}" is the volume of bacterial cell culture used in mL (= 0.05 mL), "V_{total}" is the volume of solution used in measurement of OD_{420} in mL (= 0.12 mL), and C is a correction term including extinction coefficient of ONPG (= 1 (nmol)/0.0045 (mL cm^{-1})).

■ ASSOCIATED CONTENT

Supporting Information. Calculation data and characterization of new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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