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Synthesis and bioluminescence-inducing properties of autoinducer (*S*)-4,5-dihydroxypentane-2,3-dione and its enantiomer

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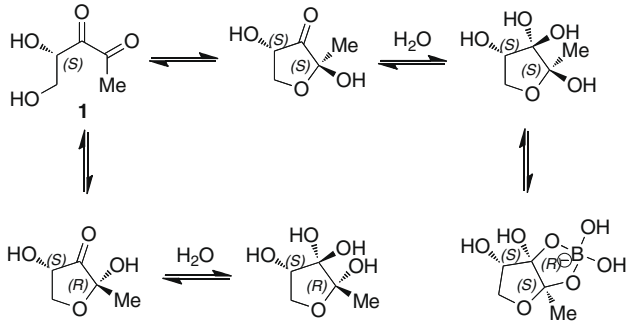
ABSTRACT

The autoinducer (4*S*)-4,5-dihydroxypentane-2,3-dione ((*S*)-DPD, AI-2) facilitates chemical communication, termed 'quorum sensing', amongst a wide range of bacteria, The synthesis of (*S*)-DPD is challenging in part due to its instability. Herein we report a novel synthesis of (*S*)-DPD via (2*S*)-2,3-*O*-isopropylidene glyceraldehyde, through Wittig, dihydroxylation and oxidation reactions, with a complimentary asymmetric synthesis to a key precursor. Its enantiomer (*R*)-DPD, was prepared from D-mannitol via (2*R*)-2,3-*O*-isopropylideneglyceraldehyde. The synthesized enantiomers of DPD have AI-2 bioluminescence-inducing properties in the *Vibrio harveyi* BB170 strain.

The AI-2 class of inducer, used by both Gram-positive and Gram-negative bacteria,[1] has been proposed to be an universal signal for *inter*species communication (quorum sensing). In contrast, the acyl homoserine lactones (AHLs) and autoinducing peptides (AIPs) are believed to control intraspecies communication.[2] (4*S*)-4,5-Dihydroxypentane-2,3-dione (**1**) (DPD), an unstable diketone biosynthesised by bacteria, is the penultimate precursor of autoinducer AI-2. AI-2 coordinates bacterial behaviour and function to enable adaptation to changing environments and possibly to facilitate competition with multicellular organisms.[3] The receptor for the AI-2 inducer, activates gene transcription, including those for its biosynthesis.[4] The biosynthesis of DPD involves *S*-adenosylmethionine in three enzymatic steps, the last step catalyzed by LuxS.[5] DPD is proposed to exist as an equilibrium mixture of cyclic, hydrated and borate complexes, collectively known as autoinducer-2 (AI-2). *Vibrio harveyi*, an indicator bacterium which forms the basis of the bioluminescence assay, recognizes the 2,3-borate diester of the hydrated α-anomer of DPD[6] (Scheme 1). The role of borate complexation in structurally-analogous sugars suggests this can play a key role in signalling efficacy.[7]

Despite its relatively simple structure, the synthesis of DPD is complicated by its instability at high concentrations giving rise to oligomerisation. In the published syntheses, DPD was obtained as dilute aqueous solutions, either from acidic hydrolysis of an orthoester[8] or a ketal,[9] or by reductive ozonolysis of an α-methylene ketone.[10] In addition, the di-*O*-acetylated derivative of (*S*)-DPD was prepared as a enzyme-labile, chemically stable precursor of DPD.[11]



Scheme 1. Autoinducer AI-2: Biological forms of (*S*)-DPD (**1**).[9]

Here we report a novel synthesis of the unnatural enantiomer, (*R*)-DPD (**2**), starting from D-mannitol (**3**). We also describe a novel synthesis of the natural (*S*)-DPD (**1**) to provide samples of both enantiomers to investigate bacterial coordination of gene expression, biofilm formation and other AI-2 quorum sensing-regulated process.

For the synthesis of the unnatural *R* enantiomer, D-mannitol (**3**) is an inexpensive starting material which possesses the absolute stereochemistry at C-2/C-5 required to give an enantiospecific synthesis of (*R*)-DPD (**2**).[12] Using published procedures[12] D-mannitol

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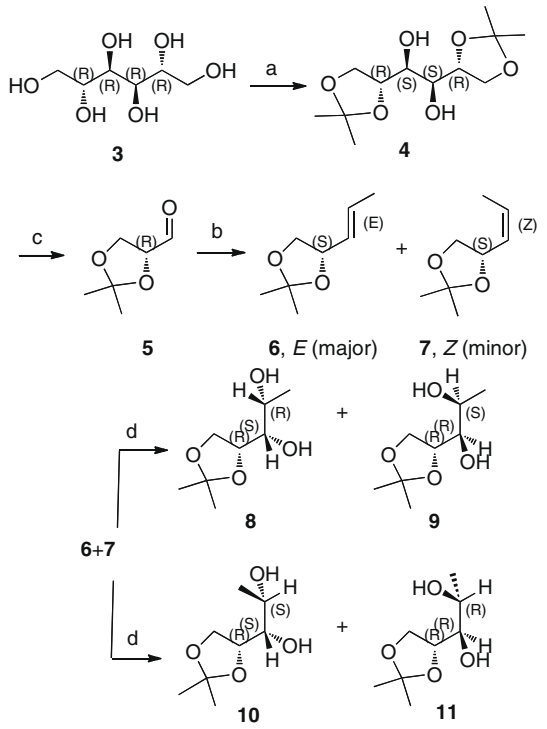


Scheme 2. Synthesis of intermediate diols. Reagents and conditions: (a) 2,2-dimethoxypropane, *p*-TSA·H_2O, 57%; (b) $NaIO_4$, $NaHCO_3$, 75%; (c) (ethyl)triphenyl phosphonium bromide, *n*-BuLi, 70%; (d) 4%OsO_4, NMO·H_2O, 70%.

(**3**) was reacted with 2,2-dimethoxypropane and catalytic *p*-TSA to give 1,2:5,6-di-*O*-isopropylidene-D-mannitol (**4**), with subsequent oxidative cleavage with $NaIO_4$ to give known (2*R*)-2,3-*O*-isopropylideneglyceraldehyde (**5**).[13] Reaction of (**5**) with (ethyl)triphenylphosphonium bromide/*n*-BuLi[14] provided the novel alkenes (**6**) and (**7**) in 70% combined yield with a geometrical isomer ratio of >20:1 *E*:*Z* by ^{1}H NMR spectroscopy (Scheme 2). Subsequent preparations of alkenes using *n*-BuLi which had been stored for even moderate periods led to formation of alkenes with a geometrical isomer ratio of 2:1 *E*:*Z* (^{1}H NMR). Dihydroxylation of alkenes (**6**) and (**7**) using catalytic OsO_4 and stoichiometric *N*-methyl morpholine-*N*-oxide,[15] afforded the diastereoisomeric diols (**8**)/(**9**) and (**10**)/(**11**), respectively in a 70% yield.

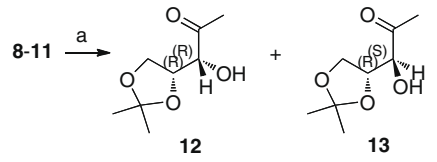
Whilst dihydroxylation of such alkene substrates is known to proceed with good substrate diasterecontrol or matching double diastereocontrol using AD, in the current synthesis both the newly-introduced chiral secondary alcohols are to be converted into ketones, so that the mixture of diols (**8**)-(**11**) from achiral osmylation can be taken forwards. However, oxidation of (**8**)–(**11**) using Swern oxidation[16] led to the formation of monoketones (**12**) and (**13**) (Scheme 3), and not the desired α-diketone (**14**). There are literature precedents for the difficulty of oxidising vicinal diols,[17] under Swern conditions, reporting mixtures of 1,2-diketone and monoketones products.[18]

However, oxidation of (**8**)–(**11**) with PCC led to the formation of α-diketone (**14**) in 30% yield.[19] Subsequent acid-catalysed deprotection of the acetonide group of (**14**) in D_2O (to allow monitoring by ^{1}H NMR spectroscopy) led to the formation of (*R*)-DPD (**2**), with a complex ^{1}H NMR spectrum as described in the literature for (*S*)-DPD (**1**).[9] Further evidence for the formation of (*R*)-DPD (**2**) was obtained by addition of 1,2-phenylenediamine, forming a single quinoxaline derivative (**15**) (Scheme 4), also consistent with the data reported for (*S*)-DPD (**1**).[9]

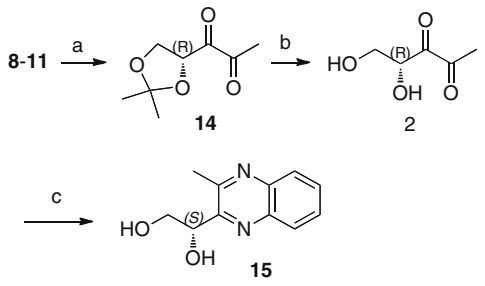
Whilst this synthesis establishes a simple entry to the (*R*)-DPD system, storage of the acetal **14** (and certainly **2**) long term may be subject to potential degradation. Thus, the bis-*O*-benzoyl and bis-*O*-acetyl derivatives of (*R*)-DPD, namely (**16**) and (**17**), were prepared (Scheme 5) as stable bioprecursors of (*R*)-DPD, which could then be stored for prolonged periods, prior to use in biological assays. Thus, acetals (**6**) and (**7**) were deprotected to give diols (**18**)/(**19**) and then esterified with either benzoyl or acetyl chloride to give alkene diesters (**20**)/(**21**) and (**22**)/(**23**) in 69% and 58% yield, respectively. The alkenes were dihydroxylated as previously with OsO_4 to give the corresponding diols, which were subsequently oxidised with PCC to give (**16**) and (**17**) in 41% and 30% yield, respectively.

We also report here a novel synthesis of the natural (*S*)-DPD (**1**) from (*R*)-2,2-dimethyl-1,3-dioxolane-4-methanol (**24**) (Scheme 6), employing the same Wittig-osmylation strategy applied above in the synthesis of unnatural (*R*)-DPD. Thus, alcohol (**24**) was oxidised to aldehyde (**25**) using PCC oxidation.[20] Reaction of (**25**) with (ethyl)triphenylphosphonium bromide/*n*-BuLi in a Wittig reaction provided the novel alkenes (**26**) and (**27**), in the same 2:1 ratio as observed for Wittig reaction of **5**. Subsequent dihydroxylation of these alkenes provided (**28**)-(**31**), oxidation to give (**32**) and deprotection to give (*S*)-DPD (**1**) (Scheme 6). All substrates and products were characterised by NMR spectroscopy, IR spectrometry, mass and high resolution mass spectroscopy, with the (*R*) and (*S*) enantiomers of DPD having identical spectroscopic characterisation.

An alternative strategy to the DPD backbone was also explored in which the 2,3-chiral centres are introduced by asymmetric synthesis and these are then used to direct the establishment of the natural product 4*S* centre by diastereoselective osmylation. Whilst the asymmetrically-introduced chiral centres are then of course lost in final oxidations, they have transferred the key chiral information to the C4 chiral centre of the target. This route thus starts from an achiral precursor, rather than a chiral pool source as employed in the prior routes. Benzyl (*E*)-2-butenoate (**33**) underwent asymmetric dihydroxylation to give **34**, which was then elaborated via acetonide protection of the new diol followed by ester reduction and oxidation-Wittig to give **35** (Scheme 7). A second dihydroxylation then proceeded with essentially complete diastereocontrol followed by benzylation to give the fully-protected DPD precursor tetraol **37**, with C4 and C5 protected orthogonally to C2 and C3. This serves both as a precursor dibenzyl analogue and natural stereochemical antipode of **16**, as well as precursor to **38**, which is the direct dibenzyl analogue of intermediate **28**, and can thus be converted into the dibenzyl protected variant of **32**.



Scheme 3. Reagents and conditions: (a) Swern oxidation: oxalyl chloride, dry DMSO, Et_3N, 49%.



Scheme 4. Reagents and conditions: (a) PCC, 30%; (b) H_2O, H_2SO_4; (c) 1,2-phenylenediamine.