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Ac_2-DPD, the bis-(*O*)-acetylated derivative of 4,5-dihydroxy-2,3-pentanedione (DPD) is a convenient stable precursor of bacterial quorum sensing autoinducer AI-2

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Abstract—Ac_2-DPD, the bis-(*O*)-acetylated derivative of 4,5-dihydroxy-2,3-pentanedione (DPD), was prepared both as a racemic mixture and in the optically active form found in naturally occurring DPD. It was shown to exhibit the same ability as DPD to induce bioluminescence in *Vibrio Harveyi* and β-galactosidase activity in *Salmonella enterica* Typhimurium, both Gram-negative bacteria. Likewise, it was also shown to inhibit biofilm formation in Gram-positive *Bacillus cereus*. The most likely hypothesis is that Ac_2-DPD activity is due to the release of DPD by in situ hydrolysis of the ester groups. Importantly, by contrast with DPD, Ac_2-DPD proved to be a stable compound which can be purified and stored.

Quorum sensing (QS) is a cell to cell communication system used by bacteria to regulate gene expression in a concerted way dependent upon the population density.[1] This process is based on the synthesis of small, diffusible signalling molecules called autoinducers (AI) which are able to activate transcriptional regulators. In Gram-negative bacteria, AI are mainly acylated homoserine lactone (AHLs), while linear and cyclic peptides are used by Gram-positive bacteria. Besides these two families, it has been recently shown that, either the hydrate **1** or the furanosyl borate diester **2**, mediates QS communication in both Gram-negative and Gram-positive bacteria (Scheme 1).[2] Compound **1** is issued from the hemiketalic ring-closed form of the (4*S*)-4,5-dihydroxy-2,3-pentanedione (DPD) derived from (*S*)-adenosyl methionine. Since DPD, **1** and **2** are in equilibrium, autoinducer of type 2 (AI-2) has been suggested as a collective term for DPD-derived compounds promoting bacterial cross-communication.[3]

In the last few years, AI-2 has received a great deal of interest not only from microbiologists but also from chemists as an interesting synthetic target. Indeed, despite its very simple structure, the synthesis of DPD is not an easy task owing to its instability at high concentrations. As a consequence, in the reported synthesis of DPD this compound was not purified and was obtained as dilute aqueous solutions, either from acidic hydrolysis of a orthoester[4] or a ketal,[5] or by reductive ozonolysis of an α-methylene ketone.[6,7]

We envisaged to overcome this lack of practical availability of DPD by preparing a precursor with enhanced stability that could be stored and used in biological assays, without prior chemical treatment. With this objective in mind, we decided to prepare and test the corresponding mono and di-*O*-acetylated derivatives. The acetyl was chosen as the OH masking group because of its moderate stability at physiological pH and its known enzymo-lability.[8]

We first synthesised the 5-*O*-acetyl-4,5-dihydroxy-2,3-pentanedione **3**.[7] This compound proved to be hardly more stable than DPD and could not be concentrated

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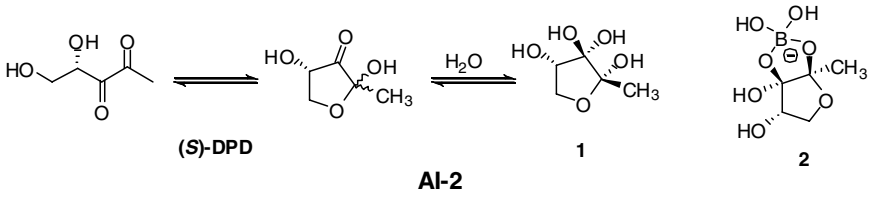
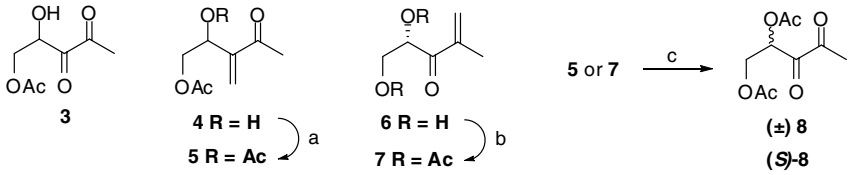


Scheme 1. Structures of bacterial QS autoinducers of type-2 (AI-2).



Scheme 2. Synthesis of **8**. Reagents and conditions: (a) acetic anhydride, 4-dimethylaminopyridine (DMAP), CH_2Cl_2, 70%; (b) acetic anhydride, pyridine, Et_3N (0.1 equiv), CH_2Cl_2, 0 °C, 90%; (c) O_3, MeOH, −78 °C then dimethyl sulfide (DMS), −78 °C–rt.

without decomposition. We then prepared the bis-*O*-acetyl-4,5-dihydroxy-2,3-pentanedione **8** (Scheme 2). The synthesis of racemic (±) **8** was achieved, using our methodology,[7] by acetylation of the β-hydroxy enone **4** to give **5** and subsequent ozonolysis. To prepare (***S***)-**8**, we used the methodology reported by Vanderleyden et al. for the synthesis of ***(S)*-DPD** from the dihydroxy enone ***(S)*-6**.[6] The latter compound was first acetylated to give **7** which was then submitted to reductive ozonolysis. Both crude (±) **8** and (***S***)-**8** were purified using column chromatography on silica gel without notable degradation and were obtained as yellow oils (Scheme 2).

The diacetate (**8**) was first evaluated for its ability to induce bioluminescence in *Vibrio harveyi* bacteria known to use borate **2** as autoinducer.[9] As shown by results depicted in Figure 1, (***S***)-**8** strongly induces light with about the same activity as (***S***)-**DPD**, with IC_{50} values being of 2.1 and 2.6 μM, respectively. Similar results were obtained for **(±) 8** compared with (±) DPD with IC_{50} values of 2.4 and 3.3 μM, respectively (data not shown).[10]

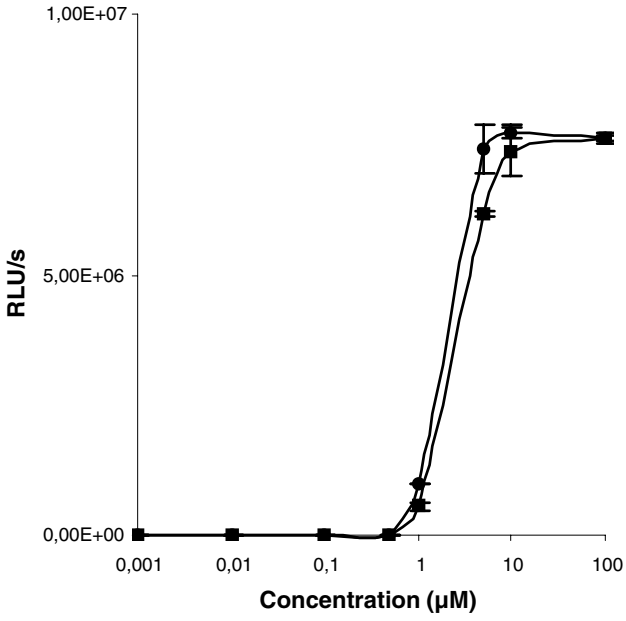


Figure 1. Bioluminescence induction in *V. harveyi* after 4 h of incubation at 30 °C with increasing concentration of (***S***)-**8** (●) and (***S***)-**DPD** (■).

We anticipated that Ac_2-DPD would have the same activity as DPD by releasing the latter compound after in situ hydrolysis of the ester groups. To support this hypothesis, we studied the chemical behaviour of (***S***)-**8** in an aqueous medium at room temperature and at the pH of biological tests (100 mM phosphate buffer, pH 7.4) by recording ^1H NMR spectra every 10 min (Fig. 2).[11] The monoacetylated compound **3** resulting from hydrolysis of the secondary 4-OAc group of (***S***)-**8** is rapidly observed in the medium (Fig. 2). The concentration of **3** increases to reach a maximum after about 1 h and then slowly decreases due to the further hydrolysis of the primary acetate function giving rise to DPD. After about 280 min of reaction side products arising from DPD degradation are also detected preventing an accurate analysis of the mixture.

Comparison of the rate of this in vitro formation of **DPD** from (***S***)-**8** is not fully consistent with the

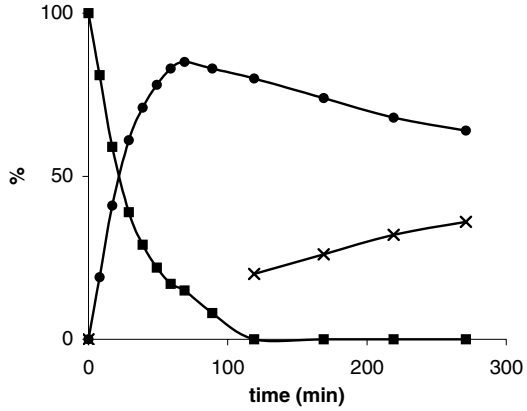


Figure 2. Hydrolysis of diacetate (***S***)-**8** (■) into monoacetate **3** (●) and DPD (×) in buffered (pH 7.4) aqueous medium.

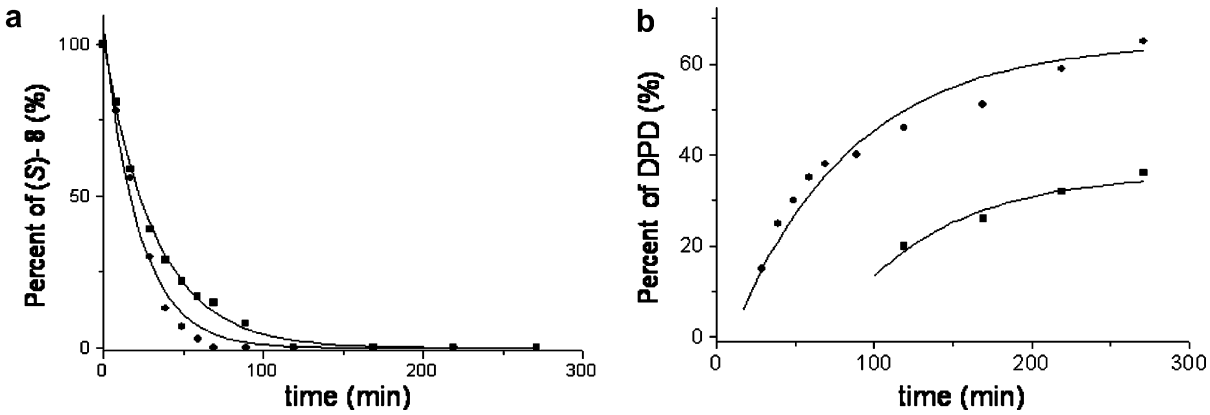


Figure 3. (a) Hydrolysis of the secondary acetyl group of (***S***)-**8**, in the absence (■) or in the presence (●) of PLE. (b) (***S***)-**DPD** release from (***S***)-**8** in the absence (■) or in the presence (●) of PLE.

biological activity of the latter compound on *V. harveyi* bacterium. Indeed, after 4 h only about 30% of (***S***)-**8** has been transformed into **DPD** (Fig. 2) while, after a same 4 h period of incubation, the activity of **8** is identical to that of pure DPD (Fig. 1). A combination of different factors can explain this discrepancy such as the presence of mineral components in the biological medium increasing the rate of hydrolysis, or the acceleration of DPD formation at the temperature of incubation. Also, in the case of biological assays using DPD, concomitant degradation all over the incubation period might lower its actual concentration. Finally, bacterial hydrolytic enzymes could also participate in the acceleration of the hydrolysis of acetate groups during the biological assay. In order to detect the eventual contribution of an enzymatic catalysis, we studied the hydrolysis of **8** in the presence of pig liver esterase (PLE), a common used hydrolytic enzyme.[12] As shown by results depicted in Figure 3, this enzyme is able to increase significantly the formation of (***S***)-**DPD** though resulting from moderate rate enhancements of both the first and second hydrolysis of acetyl groups of (***S***)-**8**.[13]

Compound (***S***)-**8** was then tested for its ability to induce β-galactosidase production in *Salmonella enterica* Typhimurium bacteria which use hydrate **1** as autoinducer.[2b,15] As shown by results depicted in Figure 4, this compound proved to be a potent inducer of β-galactosidase production.[16] The higher activity of (***S***)-**8** compared to DPD observed in this case is likely due to partial degradation of this latter during the biological assays.

Finally, the effect of (***S***)-**8** on biofilm formation was assayed in the strain 407 of the *Bacillus cereus* which is a Gram-positive bacterium.[17] At 8 μM and after 24 h of incubation, both (***S***)-**8** and (***S***)-**DPD** inhibited strongly and significantly ($p < 0.01$, *t*-test) biofilm formation (Fig. 5).[18]

In conclusion, we have observed that Ac_2-DPD induces the same biological effects as DPD on two Gram-negative and one Gram-positive bacteria most probably through in situ release of DPD. Indeed, these bacteria using two different derivatives of DPD as natural quorum sensing autoinducers (either a hydrate or a borate), it would be very unlikely that Ac_2-DPD be active by itself or after hydrolysis of only one of its ester groups. The fact that the same behaviour of Ac_2-DPD is observed in three different microorganisms comforts us in the idea that it could be a precursor of DPD of general use for biochemical, microbiological or biotechnological

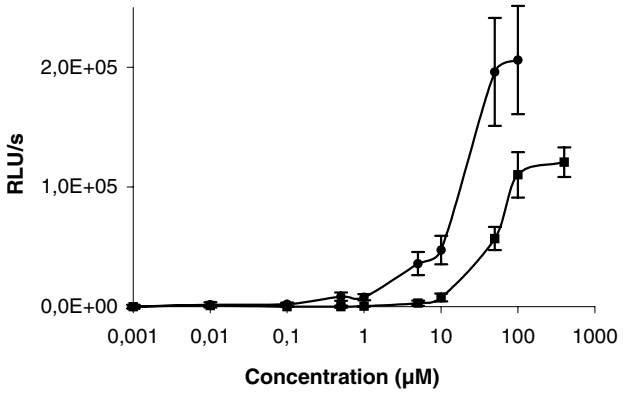


Figure 4. β-Galactosidase production in *Salmonella enterica* Typhimurium with increasing concentration of (***S***)-**8** (●) and (***S***)-**DPD** (■).

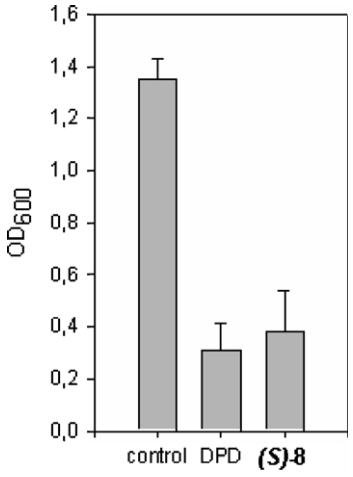


Figure 5. Biofilm inhibition in *B. cereus* after 24 h of incubation with (***S***)-**8** or (S)-**DPD** at 8μM.

applications. Also, this work opens new perspectives for the development of prodrug type derivatives as quorum sensing modulators.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.11.076.

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11. NMR samples were prepared as follow: (***S***)-**8** (1.8 mg, 8.33 μmol) was dissolved in a 100 mM Na_2HPO_4 buffer in D_2O (0.6 mL, pH 7.4). ^1H NMR spectra were recorded on a Bruker spectrometer DRX 500 MHz each 10 min (64 scans) for 10 h at room temperature. The proportion of each compound, (***S***)-**8**, **3**, and DPD, was determined on the basis of integration of the signals (in D_2O) corresponding to the 2 diastereotopic H of the methylene group (C-5) at 4.24 and 4.44 ppm for (***S***)-**8** and at 4.14 ppm for**3** or to the hemiketalic methyl groups (2 diasteroisomers) at 1.39 and 1.42 ppm for DPD. The precision for integration measurements is about 5%. At low concentrations of DPD (ca. 10%) no accurate values can be given by this method because the intensity of DPD signals is weak due to its equilibrium between three species.
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