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An Expeditious Synthesis of DPD and Boron Binding Studies

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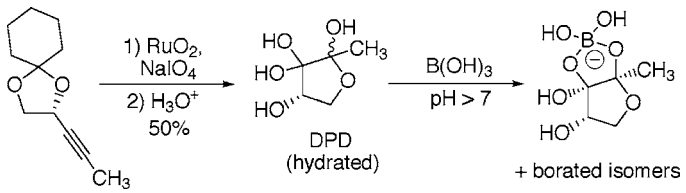
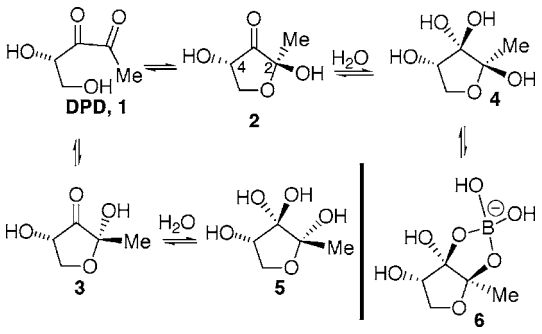
ABSTRACT



A practical synthesis has been developed for DPD (4,5-dihydroxypentane-2,3-dione), an unstable small molecule that is proposed to be the source of universal signaling agents for quorum sensing in bacteria. The synthesis allows preparation of isotopically labeled DPD and ent-DPD as well as detailed studies of spontaneous binding to borate to give the unusual borate complex 6, the signal for marine bacteria such as *Vibrio harveyi*.

DPD (**1**) is an enigmatic molecule. It has been known since 1971 as the product of catabolism of *S*-adenosylhomocysteine in many bacteria,[1] and more recently, it is proposed to be the core molecule from which all bacterial AI-2 signaling molecules are derived.[2] These molecules are widely used in inter-species communication in the bacterial world.[3] It is a simple molecule but was reported to be quite unstable toward rearrangement[4] and oligomerization[5,6] and has only recently been synthesized and tentatively characterized in dilute solution.[6] DPD can exist as an equilibrium mixture of three isomers (**1**, **2**, and **3**), hydrated versions (**4** and **5**), and borate complexes (e.g., **6**), collectively known as autoinducer 2 (AI-2).[2] *V. harveyi* recognizes the 2,3-borate diester (**6**) of the hydrated α-anomer (**4**) of DPD as AI-2.[7] *Salmonella Typhimurium* recognizes the hydrated *β*-anomer (**5**) of DPD without borate.[2] The incorporation of borate into the AI-2 signal may be a function of the natural habitat of the bacteria. *V. harveyi* is a marine bacterium that lives in an environment

Scheme 1. Biological Forms of DPD



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(1) Duerre, J. A.; Baker, D. J.; Salisbury, L. *Fed. Proc.* **1971**, *30*, 1067.
(2) Miller, S. T.; Xavier K.; Campagna, S. R.; Taga, M. E.; Semmelhack, M. F.; Bassler, B. L.; and Hughson, F. M. *Mol. Cell* **2004**, *15*, 677−687. This paper also presents a nomenclature system for the structures in Scheme 1.
(3) Federle, M. J.; Bassler, B. L. *J. Clin. Invest.* **2003**, *112*, 1291−1299.
(4) (a) Winzer, K.; Hardie, K. R.; Burgess, N.; Doherty, N.; Kirke, D.; Holden, M. T. G.; Linforth, R.; Cornell, K. A.; Taylor, A. J.; Hill, P. J.; Williams, P. *Microbiology* **2002**, *148*, 909−922. (b) Slaughter, J. C. *Biol. Rev.* **1999**, *74*, 259−276.
(5) Semmelhack, M. F.; Campagna, S. R.; Hwa, C.; Federle, M. J.; Bassler, B. L. *Org. Lett.* **2004**, 6, 2635−2637.
(6) Meijler, M. M.; Hom, L. G., Kaufmann, G. F.; McKenzie, K. M.; Sun, C.; Moss, J. A.; Matsushita, M.; Janda, K. D. *Angew. Chem., Int. Ed.* **2004**, *43*, 2106−2108.
(7) Chen, X.; Schauder, S.; Potier, N.; Van Dorsselaer, A.; Pelczer, I.; Bassler, B. L.; Hughson, F. M. *Nature* **2002**, *415*, 545−549.

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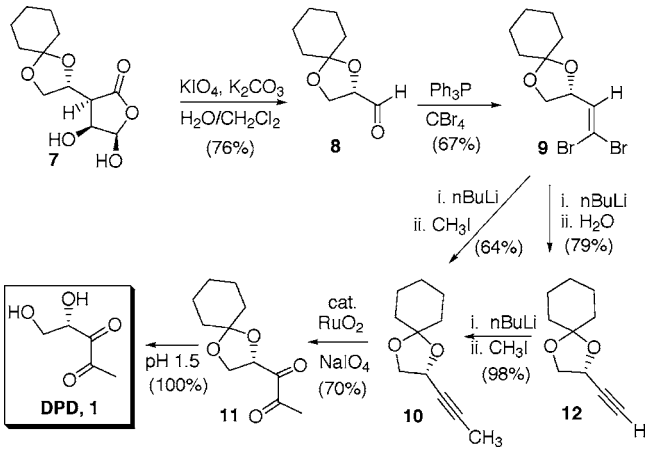
with high borate concentration compared to *S. typhimurium*, an enteric bacterium. Here we report an efficient synthesis of DPD that allows characterization of DPD isomers, hydration processes, and boron complexation; and that facilitates the preparation of isotopically labeled versions. Our synthesis strategy (Scheme 2) parallels that recently

Scheme 2. Synthesis Strategy for DPD



reported[6] but employs different tactics which significantly improve the efficiency and make DPD readily available in multigram quantities. The previous synthesis of DPD made use of a labile methyl ortho ester, which could be removed in dilute aqueous solution to produce DPD in situ for biological evaluation. The delicacy of that protecting group required that it be introduced at a late stage as a replacement for the acetonide group. However, this protection strategy led to complications with the oxidation to form the α-diketone (analogue of **11**; 10% yield). In addition, the acetonide series involves two intermediates of inconveniently high volatility. With access to DPD prepared enzymatically,[8] we showed that it is stable at pH 1.5−2 in dilute aqueous solution for extended periods (no significant decrease in bioactivity[9] after 16 h at 20 °C). While enantio-pure glyceraldehyde equivalents provide a convenient conceptual starting point for the synthesis of DPD and other small molecules, the handling of these molecules can be difficult.[10]

The cyclohexylidene group (Scheme 2) offers lower volatility and efficient isolation, and can be removed rapidly at pH 1.3−1.5 and 20 °C. Importantly, the cyclohexanone byproduct from deprotection does not inhibit cell growth at concentrations under 1 M. The monocyclohexylidene derivative (**7**) of L-gulonic acid γ-lactone was prepared in 75% yield as a white solid.[11] Oxidative cleavage with KIO_4 led to aldehyde **8** (76%). Following a literature procedure,[12] the Corey-Fuchs protocol gave rise to the dibromoalkene **9**, which was purified (67% yield) prior to treatment with ^{n}BuLi; the intermediate alkyne anion was quenched with methyl iodide to give alkyne **10** in 64% yield after careful chromatography. Alternative quenching with water gave the alkyne **12** (79%); then methylation proceeded in 98% yield to **10**. The critical oxidation process followed the protocol of Seebach[13] with RuO_2/$NaIO_4$ and produced the α-diketone **11** in 70% yield as a bright yellow crystalline solid. The yield is ca. 20% overall from gulonic acid γ-lactone via **12**. Compound **11** showed a strong tendency to hydrate at the C-3 carbonyl group to give **13** (Figure 1), which complicates

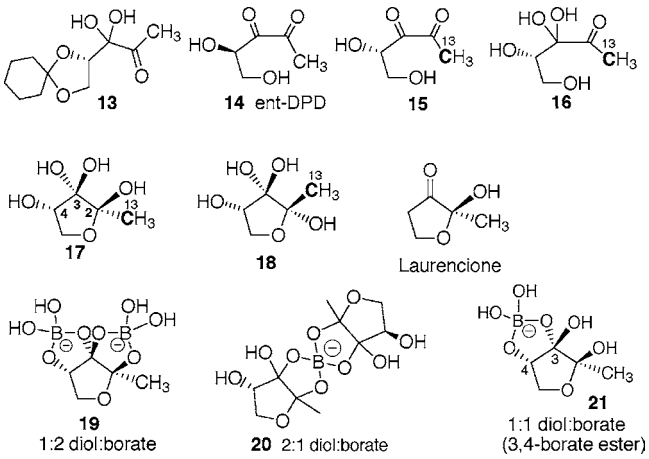


Figure 1. Derivatives of DPD.

the characterization.[9] When **11** is placed in H_2O or D_2O at pH 1.5 and 20 °C at concentrations up to 30 mM, deprotection was >95% complete after 2.0 h with no detectable byproducts (^{1}H NMR). After adjusting the buffer (0.5 M potassium phosphate, pH 7.3), synthetic DPD was observed to be equal in activity to enzymatically prepared DPD at equal concentration in the *V. harveyi* bioassay.[9] Using this route, *ent*-DPD (**14**) and DPD labeled with ^{13}C at C-1 (**15**) were synthesized.[9] *ent*-DPD (**14**) had only 1% of the activity of DPD (**1**) in the *V. harveyi* bioassay.[9]

Synthetically produced DPD was stable under acidic conditions; a 30 mM sample of the molecule was monitored for decomposition via ^{1}H NMR for 5 h at 20 °C and pH 1.5. Under these conditions, no decomposition products were observed. A further examination of a 100 mM sample stored at 20 °C and pH 1.3 for 16 h showed no loss in activity as monitored by the *V. harveyi* bioassay.[9] The purity of the synthetic DPD was further established by reaction with

(8) Schauder, S.; Shokat, K.; Surette, M. G.; Bassler, B. L. *Mol. Microbiol.* **2001**, *41*, 463−476.

(9) For full data, see Supporting Information.

(10) Isopropylidene glyceraldehyde can be generated as either enantiomer in a few steps, but it is quite volatile and dimerizes readily. These drawbacks have led to the development of other protecting groups for glyceraldehyde. (a) For a general discussion, see: Schmid, C. R.; Bradley, D. A. *Synthesis* **1992**, 587−590. (b) For discussion and elaborate solutions, see: Michel, P.; Ley, S. V. *Synthesis* **2003**, *10*, 1598−1602. Aube, J.; Mossman, C. J.; Dickey, S. *Tetrahedron* **1992**, *48*, 45, 9819−9826. (c) First reported by: Grauert, M.; Schollkopf, U. *Liebigs, Ann. Chem.* **1985**, 1817−1824

(11) First reported by: Vekemans, J. A. J. M.; Boerekamp, J.; Godefroi, E. F.; Chittenden, G. J. F. *Recl. Trav. Chim. Pays-Bas* **1985**, *104*, 266−272.

(12) Yoshida, J.; Nakagawa, M.; Seki, H.; Hino, T. *J. Chem. Soc., Perkin Trans. 1* **1992**, 343−350.

(13) Zibuck, R.; Seebach, D. *Helv. Chim. Acta* **1988**, *71*, 237−240.

o-phenylenediamine to produce (*S*)-1-(3-methylquioxalin-2-yl)-ethane-1,2-diol in >98% purity.[9]

Hydration of the carbonyl group at C-3 of the cyclic form (**2**) of DPD is important for borate binding in the *V. harveyi* signal, **6**. Initially, it was not known whether hydration and subsequent borate addition were intrinsically favorable or required the presence of the protein receptor.[14] We showed that laurencione, which is 4-deoxy-DPD, hydrates spontaneously and associates strongly with borate in aqueous media at pH 7.8.[5] It forms complexes with both 1:1 and 2:1 laurencione:borate stoichiometry. For synthetic DPD, the appearance of five ^{13}C NMR signals in the spectrum at pH 1.5 (30 mM DPD, aq) in the region from 90 to 110 ppm assigned to the hemiacetal and hydrated carbons strongly suggests that the majority of DPD, both open and closed isomers, is hydrated at C3. It is less stable at pH >8 unless bound to borate. Unlike laurencione, which is lacking the C-4 hydroxyl group and therefore in the hydrated form contains only one site for borate complexation, hydrated DPD (**4**) has two sites for borate complexation. Since both 2,3- and 3,4-DPD borates are possible for each of the two anomers, a complex mixture of borate species is expected.

Borate binding was followed with ^{13}C-labeled DPD, **15**, by both ^{11}B and ^{13}C NMR spectroscopy. As shown in Figure 2a, in H_2O (5% D_2O) and in the absence of borate, 15 mM **15** produces three main peaks from the incorporated label, assigned to the hydrated open form **16** (δ 24.9 ppm), and the two hydrated cyclic forms, **17**/**18** (δ 19.9 or 20.4 ppm). As borate is added, the complexity of the ^{13}C NMR spectrum increases, consistent with the hypothesis that a number of 1:1 and 2:1 DPD−borate complexes are present in solution; with 15 mM **15** and 45 mM $B(OH)_3$ (Figure 2b) the signals for **16**−**18** are gone, confirming a high affinity of these isomers for boron. When the solution is saturated with boric acid (~0.9 M) and the concentration of **15** is kept at 15 mM, the ^{13}C NMR spectrum is dominated by a peak at δ 22.3 ppm to which we assign the 2:1 borate complex, **19** (Figure 2c).[10] Although **19** appears crowded and possesses two negatively charged groups, precedence for this structural type exists in work showing that small polyhydroxylated molecules can complex multiple tetrahedral phenyboranate ions in close proximity.[15]

When the identical titration is followed by ^{11}B NMR, no signal was observed initially with 15 mM **1** and no $B(OH)_3$ (Figure 2**d**), but as the $B(OH)_3$ concentration was increased to 40 mM while holding the concentration of **1** constant (Figure 2**e**), new peaks appeared: two broad peaks at δ 4.7 and 5.8 ppm and a family of peaks at δ 8−11 ppm (excess borate at δ 18.3 ppm not shown). The peak at δ 5.8 ppm is assigned to the natural product, **6** (and its anomer), based on analogy with **6** bound to the *V. harveyi* receptor LuxP (6.1 ppm),[7] **6** released from the LuxP receptor (δ 5.8 ppm),[2] **6** (δ 5.8 ppm) observed when **17** is released into borate from its receptor, LsrB, from *S. typhimurium*,[2] and the 1:1 borate complex of laurencione (δ 5.9 ppm).[5]

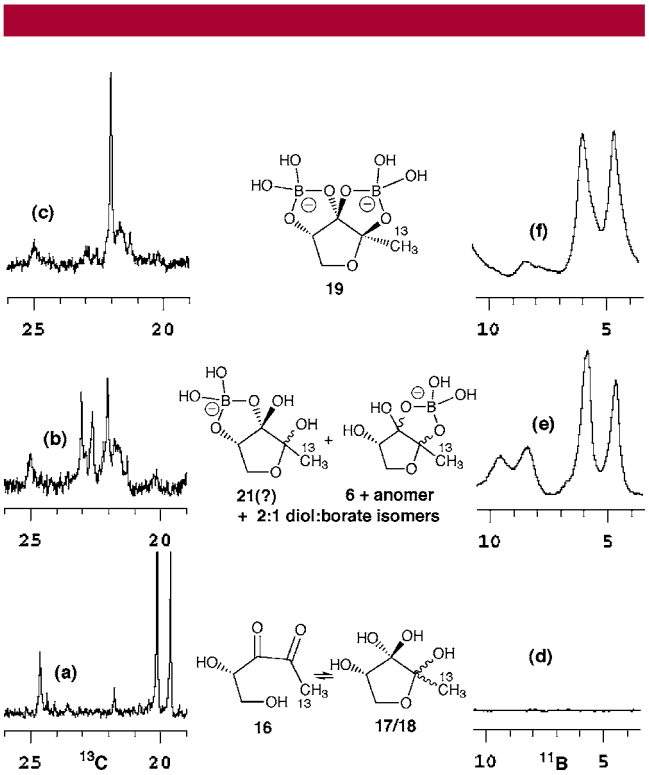


Figure 2. Titrating borate into 15 mM DPD at pH 7.8. ^{13}C and ^{11}B NMR monitoring.[10] ^{13}C NMR titration: (a) no added $B(OH)_3$; (b) 45 mM $B(OH)_3$; (c) saturated $B(OH)_3$. ^{11}B NMR titration: (d) no added $B(OH)_3$; (e) 40 mM $B(OH)_3$; (f) saturated $B(OH)_3$.

The peak at δ 4.7 ppm is at a position typical of 1:1 sugar−borate complexes[16] and is tentatively assigned to the 3,4-borate complex, **21**. The family of peaks from δ 8−11 ppm, have positions consistent with a mixture of 2:1 DPD−borate complexes (e.g., **20**) with borate bound at either the 2,3 or 3,4 position.[5] As the concentration of borate is increased to saturation while keeping the concentration of **1** constant at 15 mM, the intensity of the peaks from δ 8−11 ppm decrease while the peaks at δ 4.7 and 5.8 ppm increase and become almost equal in intensity (Figure 2f).

These data are consistent with the conversion of 2:1 DPD−borate complexes to 1:1 DPD−borate complexes (e.g., **6**), followed by further conversion to 1:2 DPD−borate complex **19** as the borate concentration is increased. The inherently large number of borated DPD species formed coupled with the difficulties associated with accurately measuring the equilibrium constant for sugar borate binding[17] has not allowed quantitation of the equilibrium constant for the association of DPD with borate.

To explore the binding of DPD with borate under conditions that more closely resemble those in the natural habitat of *V. harveyi* and to correlate this study with previous work using DPD produced in vivo and released from the *V. harveyi* receptor protein, LuxP, the ^{11}B NMR spectrum of a 100 μM DPD solution in 400 μM $B(OH)_3$[18] at pH 7.5 was

(14) Pei and co-workers proposed that the C-3 carbonyl of DPD (enzymatically prepared) was hydrated in aqueous media: Zhu, J. G.; Hu, X. B.; Dizin, E.; Pei, D. H. *J. Am. Chem. Soc.* **2003**, *125*, 13379−13381.

(15) Lorand, J. P.; Edwards, J. O. *J. Org. Chem.* **1959**, *24*, 769−774.

(16) Van Duin, M.; Peters, J. A.; Kieboom, A. P. G.; Van Bekkum, H. *Tetrahedron Lett.* **1985**, *41*, 3411−3412.

(17) Springsteen, G.; Wang, B. *Tetrahedron* **2002**, *58*, 5291−5300.

recorded. Under these conditions, one peak at δ 5.8 ppm corresponding to 1:1 DPD−borate complexes with borate bound at the 2,3-positions (**6** and its anomer) was observed significantly above noise level. Based on the ratio of integrals for the $B(OH)_3$ peak and the DPD−borate peak, the concentration of DPD−borate complex was calculated to be ca. 10 μM, consistent with ca. 10% DPD bound at this dilution. We were unable to observe conclusively the presence of complexes with a 2:1 DPD−borate stoichiometry due to sensitivity limitations of the ^{11}B NMR experiment, although a peak with a S/N of 2 was recorded at δ 9.8 ppm.

We have provided an effective path to DPD that is amenable to preparation of isotopically labeled versions and allows a detailed study of the isomerization, hydration, and boron complexation phenomena.

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Supporting Information Available: Procedures and spectra related to Scheme 2, bioassay data, and borate titration data. This material is available free of charge via the Internet at http://pubs.acs.org.

(18) Dickson, A. G. *Deep-Sea Res.* **1990**, *37*, 755−766.

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