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Synthesis and Biological Evaluation of the Trifluoromethyl Analog of (4*S*)-4,5-Dihydroxy-2,3-pentanedione (DPD)

Marine Frezza,[a] Damien Balestrino,[b] Laurent Soulère,[a] Sylvie Reverchon,[c] Yves Queneau,[a] Christiane Forestier,[b] and Alain Doutheau*[a]

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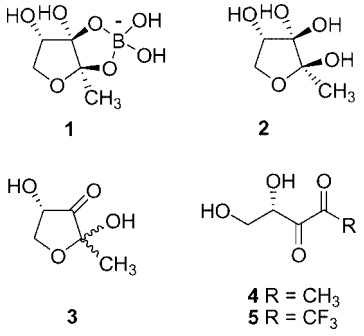
The asymmetric synthesis of the trifluoromethyl analogue of (4*S*)-4,5-dihydroxy-2,3-pentanedione [(4*S*)-DPD], the metabolic precursor of the bacterial quorum sensing (QS) autoinducer AI-2, is described. The trifluoromethyl group was introduced by treating (trifluoromethyl)trimethylsilane with an α-methylene ester resulting from a Baylis–Hillman reaction between (*tert*-butyldimethylsilyloxy)acetaldehyde and a vinylamide derived from Oppolzer's sultam reagent. The α-diketonic moiety is formed during the last step by reductive ozonolysis of the hemiketal form of an α-methylene trifluoromethyl ketone. Biological evaluation shows that this analogue is a weaker inducer of QS in *Vibrio harveyi* bacteria than DPD.

Introduction

Many bacteria regulate their gene expression by using a population-monitoring system, known as quorum sensing (QS), which involves the synthesis, secretion and detection of diffusible small signal molecules called autoinducers (AI).[1] Since several pathogenic bacteria use QS systems to regulate the expression of their virulence genes[2] or to coordinate biofilm formation,[3] this process has become a target for exploring new prophylactic strategies.[4] Among the different classes of AIs that have been identified so far, both the furanosyl borate diester **1**[5] or the hydrated furanone **2**[6] (Scheme 1) have been shown to mediate QS communication in both Gram-negative and Gram-positive bacteria. Compound **2** results from the hemiketal ring-closed form (**3**) of (4*S*)-4,5-dihydroxy-2,3-pentanedione (DPD, **4**) derived from (*S*)-adenosyl methionine. Since **4**, **3**, **2** and **1** are in equilibrium, type-2 AI (AI-2) has been suggested as a collective term for DPD-derived compounds that promote bacterial cross-communication.[7]



Scheme 1.

As part of an ongoing program aimed at preparing QS AI analogues displaying agonist or antagonist activity,[8] we recently became interested in obtaining analogues of AI-2. Considering that the substitution of hydrogen atoms by fluorine atoms often results in a modulation of biological activity,[9] we decided to prepare the trifluoromethyl-substituted DPD **5** (Scheme 1). We report here the synthesis of this compound, both in the racemic and optically active form found in natural DPD, together with the biological evaluation of the latter compound.

Results and Discussion

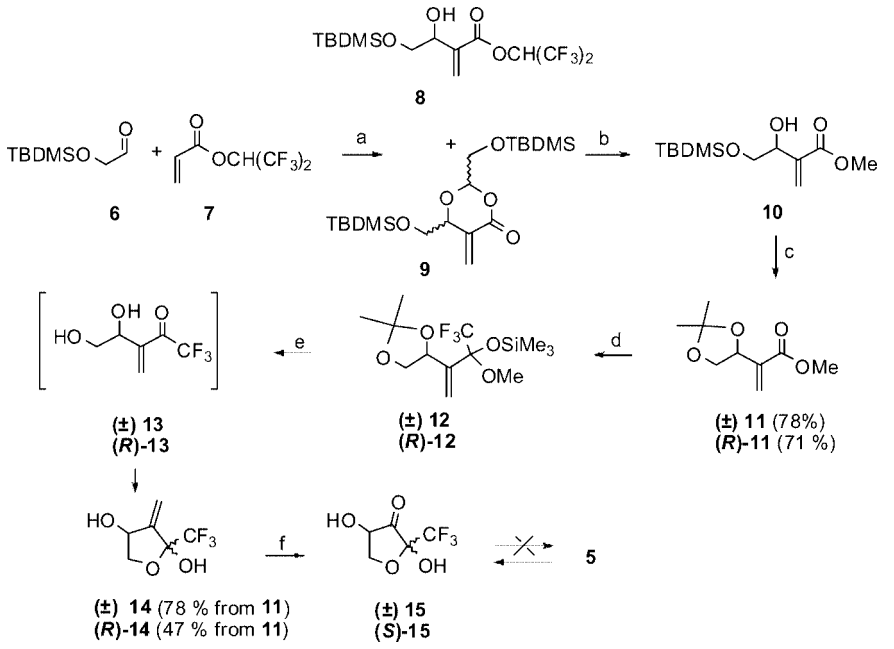
The synthetic route used to prepare (±)-**5** (Scheme 2) is derived from the three-step sequence that we recently reported for the synthesis of DPD and some analogues.[10] The reaction of (*tert*-butyldimethylsilyloxy)acetaldehyde (**6**)[11] with 1,1,1,3,3,3-hexafluoroisopropyl acrylate (**7**),[12] in the presence of 1,4-diazabicyclo[2.2.2]octane (DABCO),

[a] Laboratoire de Chimie Organique, UMR 5181 CNRS, Université Lyon 1, INSA, Institut National des Sciences Appliquées,
Bât. Jules Verne, 20 avenue A. Einstein, 69621 Villeurbanne, France
Fax: +33-4-72438896
E-mail: alain.doutheau@insa-lyon.fr
[b] Laboratoire de Bactériologie Faculté de Pharmacie, Université d'Auvergne Clermont-Ferrand,
28 Place Henri Dunant, B. P. 38, 63001 Clermont-Ferrand cedex 1, France
[c] Unité de Microbiologie et Génétique, UMR 5122 CNRS, INSA, Université Lyon 1,
Bât. A. Lwoff, 69622 Villeurbanne, France

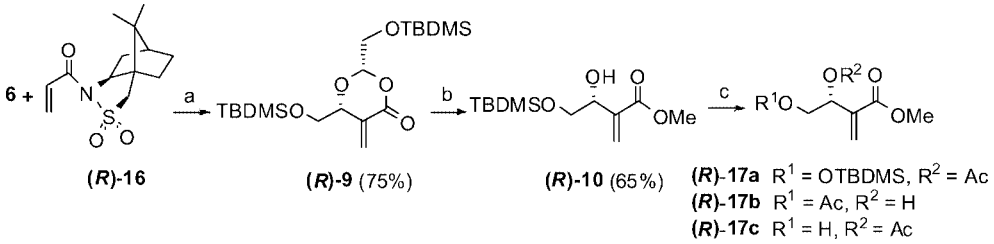
gives rise to a mixture of compounds **8** and **9**, which was further transformed into α-methylene β-hydroxy ester (±)-**10** in 45% overall yield. This compound was then converted into the acetonide (±)-**11** in 78% yield and further treated with (trifluoromethyl)trimethylsilane ($TMSCF_3$) in the presence of CsF[13] to give (±)-**12**, which was then converted into the diol (±)-**13**, which was isolated as the hemiketal (±)-**14** (2:1 mixture of diastereoisomers) in 78% overall yield from (±)-**11**. Finally, reductive ozonolysis of (±)-**14** gave a solution of the hemiketal (±)-**15** of the target compound (±)-**5** in D_2O.[14]

The synthetic sequence to (±)-**5** having been established, we then turned to the preparation of (*S*)-**5**. Among the several asymmetric approaches involving Baylis–Hillman reactions that have been reported,[15] use of the acrylamide prepared from the camphor-derived Oppolzer's sultam reagent has proved to be particularly efficient in terms of yield and enantiomeric excess. We thus decided to select this approach, particularly because it has been applied successfully with *O*-protected 2-hydroxyacetaldehydes and, therefore, we knew which enantiomer of the acrylamide to start from in order to get the desired (*S*) stereochemistry for **5**.[16] Reaction of **6** with vinylamide (*R*)-**16**[17] in the presence of DABCO led to (*R*)-**9** (75%), which was further converted into (*R*)-**10** in 65% yield (Scheme 3).

To confirm its configuration and determine its *ee*, we converted **10** into known **17b**. For this purpose **10** was first transformed into the corresponding acetate **17a** and then further treated with tetrabutylammonium fluoride (TBAF; Scheme 3).[18] After column chromatography **17b** was isolated contaminated by inseparable trace amounts (<10%) of its isomer **17c**. Since the optical rotatory power of impure **17b** was found to be $[a]_D^{20} = -22$ (c = 0.95, $CHCl_3$) whilst the reported value[16b] for the pure (*S*) enantiomer is $[a]_D^{20} = +25$ (c = 2.4, $CHCl_3$), this result confirms the (*R*) absolute stereochemistry of **17b** and, consequently, of **10**. However, the presence of a small amount of **17c** in **17b** prevented the calculation of the *ee* of the latter compound based on its optical rotatory power. We were able to determine the *ee* of (*R*)-**10** by converting **10**, both in racemic and non-racemic forms, into the corresponding diastereoisomeric esters of *O*-methyl-D-mandelic acid (**18**; Scheme 4). In the ^1H NMR spectrum of **18** prepared from racemic **10**, we observed two signals at δ = 3.74 and 3.68 ppm with the

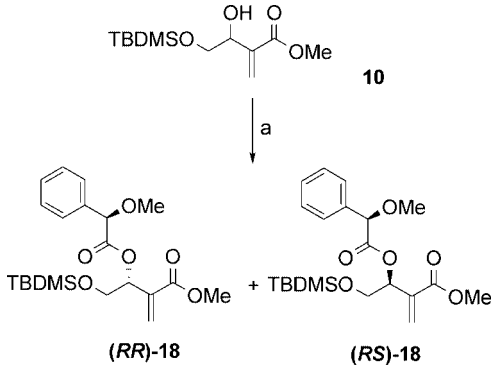


Scheme 2. Synthesis of compound **5**. Reagents and conditions: (a) DABCO, THF, 0 °C; (b) Et_3N, MeOH, room temp.; (c) camphorsulfonic acid, MeOH then 2,2-dimethoxypropane, room temp.; (d) $TMSCF_3$, CsF, room temp.; (e) aq. HCl (3 N), THF, room temp.; (f) O_3, CD_3OD, –78 °C then dimethyl sulfide, –78 °C to room temp.



Scheme 3. Synthesis of compounds (*R*)-**10** and (*R*)-**17**. Reagents and conditions: (a) DABCO, THF, 0 °C; (b) Et_3N, MeOH, room temp.; (c) Ac_2O, DMAP, CH_2Cl_2, room temp., 6 h, then TBAF, THF, 1 h.

same integration corresponding to the methoxy group of the ester group (CO_2Me) of the two diastereoisomers. For the same ester prepared from (*R*)-**10** only the former signal was observed. Similar observations were made when examining the *tert*-butyldimethylsilyl group (see Experimental Section). These ^1H NMR spectroscopic data allowed us to confirm that the enantiomeric excess of (*R*)-**10** is ≥95%.



Scheme 4. Derivatisation of **10** into diastereoisomeric esters **18**. Reagents and conditions: (a) *O*-methyl-D-mandelic acid, DCC, DMAP, $CDCl_3$, room temp.

The ester (*R*)-**10** was then submitted to the same experimental conditions as described for the racemic ester **10** (Scheme 2) to give (*R*)-**11**, which was subsequently transformed into (*R*)-**14** (2:1 mixture of stereoisomers) in 47% yield (unoptimised). (*R*)-**14** was finally submitted to reductive ozonolysis to provide a solution, in D_2O, of the target compound (*S*)-**5** in its hemiketal form (*S*)-**15**. It turned out that, like DPD,[19] its analogue (*S*)-**5** could not be isolated due to its instability during concentration of the solution resulting from ozonolysis. Consequently, the determination of its *ee* was not possible.

The AI-2-like activity of (*S*)-**5** was then compared with that of (±)-**DPD**.[10] For this purpose, a *V. harveyi* bioluminescence assay was used, with the levels of activity monitored by a sensor BB170 reporter strain (*lux*N::Tn5).[20] These BB170 cells were exposed to an exogenous compound and the resulting induced bioluminescence was quantified. As shown by the results depicted in Figure 1, (*S*)-**5** displays an agonist activity although it is 10-fold less active than (*S*)-**DPD**, with an IC_{50} value of around 30 μM compared to about 3 μM for (*S*)-**DPD**.[21] The graphs for the two compounds are very similar. However, a very low level of luminescence is induced by (*S*)-**5**, compared to **DPD**, for concentrations up to 10 μM, thus indicating a weaker affinity of the former compound for the receptor. At concentrations higher than 100 μM the luminescence induced by both (*S*)-**5** and DPD[19b,19c,22] decreases rapidly, probably because of a toxic effect of the molecules on the bacterial cells.

In *Vibrio harveyi*, the QS inducer is the furanosyl borate diester **1**, which results from the reaction between the hydrated furanone **2** and the $B(OH)_4^-$ anion.[7] The crystal structure of **1** bound to its receptor protein, LuxP, has been solved.[5] Only a few analogues of AI-2 have been tested as agonists or antagonists of these QS signals. Very recently, Janda et al. prepared the (*R*) enantiomer of DPD, epimers,

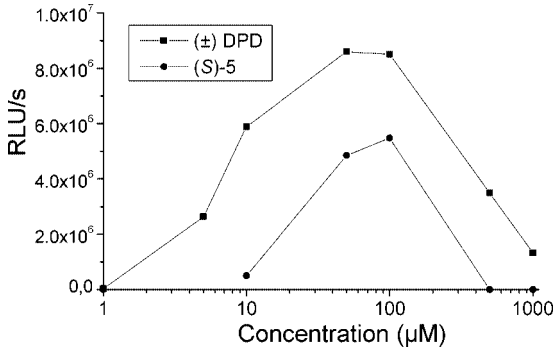
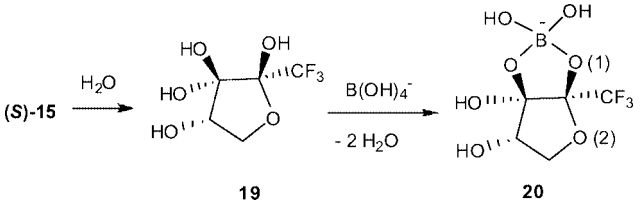


Figure 1. Induced luminescence in *Vibrio harveyi* by (*S*)-**5** compared to (±)-**DPD**.

carbon-chain-elongated analogues and analogues deprived of one hydroxyl function.[23] Some of these compounds proved to be agonists with IC_{50} values 25- to 15000-times higher than (*S*)-**DPD**. The authors concluded that their results show that both the chelation of boron and the hydroxyl functions in **1** are critical in the binding event.

In the case of (*S*)-**5**, the replacement of the CH_3 group by its CF_3 counterpart would modify the conformation of the corresponding hydrate **19**, as compared to **2**, and consequently would affect both the facility of formation and the conformation of the trifluoroborate diester **20** (Scheme 5). However, molecular modelling (data not shown) indicates that the calculated preferred conformations for **2** and **19** are identical. From an electronic point of view, one could hypothesise that the strong electron-withdrawing effect of the CF_3 group would facilitate the formation of **19** by rendering the carbonyl function more electrophilic[14] and thus increase the AI activity of (*S*)-**5** compared to (*S*)-**DPD**. This is not the case, however, and a possible explanation for this is suggested by analysis of the crystal structure of the ligand–protein complex.[5] In **20**, the CF_3 group would make the two adjacent oxygen atoms less prone to developing hydrogen bonds with Arg 310 and Arg 215 for the upper oxygen atom [O(1)] and Arg 215 and Asn 159 for the lower oxygen [O(2)], thus resulting in a decreased affinity.



Scheme 5. Structures of **19** and **20**.

Conclusion

In conclusion, we have described the synthesis, both in racemic and enantiomerically pure forms, of the trifluoromethyl analogue of (*S*)-**DPD**, the precursor of the furanosyl borate diester that induces QS in *Vibrio harveyi*. These compounds were obtained using our Baylis–Hillman/ozonolysis approach and its asymmetric extension, which has again

proved to be efficient for the preparation of these very sensitive molecules. The trifluoromethyl-DPD displays the highest agonist activity of any DPD analogues reported to date.

Experimental Section

General: All chemicals were purchased from Aldrich. Organic solutions were dried with anhydrous sodium sulfate. The reactions were performed under a constant flow of nitrogen and were monitored by TLC on Silica Gel 60 F_{254} (Merck); detection was carried out by charring with a 5% phosphomolybdic acid solution in ethanol containing 10% of H_2SO_4. Silica gel (Kieselgel 60, 70–230 mesh ASTM, Merck) was used for flash column chromatography. The ^1H (200 MHz or 300 MHz) and ^{13}C NMR (50 MHz or 75 MHz) spectra were recorded with a Bruker AC200, ALS300 or DRX300 spectrometer. The signal of the residual protonated solvent was taken as a reference. Chemical shifts (δ) and coupling constants (J) are reported in ppm and Hz, respectively. The HR mass spectra were recorded with a ThermoFinnigan spectrometer. A Berthold, Sirius luminometer was used to measure luminescence.

2,2,2-Trifluoro-1-(trifluoromethyl)ethyl 4-(*tert*-Butyldimethylsilyloxy)-3-hydroxy-2-methylenebutyrate (8) and 2,6-Bis(*tert*-butyldimethylsilyloxymethyl)-5-methylene-1,3-dioxan-4-one (9): DABCO (0.100 g, 0.9 mmol) and 1,1,1,3,3,3-hexafluoroisopropyl acrylate **7** (1.17 mL, 7.0 mmol) were added, at 0 °C, to a solution of (*tert*-butyldimethylsilyloxy)acetaldehyde (**6**; 0.610 g, 3.5 mmol) in dry THF (3 mL). After 2 h of stirring at 0 °C, the reaction mixture was evaporated. The residue was purified by flash column chromatography (90:10, pentane/Et_2O) to give **8** (0.516 g, 1.3 mmol) and **9** (0.248 g, 0.6 mmol) as colourless oils.

8: R_f = 0.46. ^1H NMR (300 MHz, $CDCl_3$): δ = 0.05 (s, 3 H), 0.07 (s, 3 H), 0.89 (s, 9 H), 2.95 (d, J = 5.1 Hz, 1 H), 3.49 (dd, J = 6.0, 9.8 Hz, 1 H), 3.88 (dd, J = 3.4, 9.8 Hz, 1 H), 4.60 (m, 1 H), 5.84 (septuplet, J = 6.0 Hz, 1 H), 6.30 (dd, J = 0.75, 1.5 Hz, 1 H), 6.61 (d, J = 0.75 Hz, 1 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = –4.9, 16.4, 26.6, 67.7 (sept, J = 34 Hz), 67.9, 71.5, 119.7 (q, J = 277 Hz), 131.0, 140.3, 163.7 ppm. HR-MS (CI): [MH^+] calcd. 397.1270; found 397.1273.

9: R_f = 0.32. ^1H NMR (300 MHz, $CDCl_3$): major stereoisomer: δ = 0.08 (m, 12 H), 0.89 (m, 18 H), 3.75 (dd, J = 4.8, 10.9 Hz, 1 H), 3.76 (dd, J = 5.3, 11.3 Hz, 1 H), 3.82 (dd, J = 5.3, 10.9 Hz, 1 H), 3.83 (dd, J = 3.8, 1.3 Hz, 1 H), 4.66 (m, 1 H), 5.30 (dd, J = 3.8, 5.3 Hz, 1 H), 5.78 (dd, J = 0.75, 2.2 Hz, 1 H), 6.48 (dd, J = 0.75, 2.2 Hz, 1 H) ppm; minor stereoisomer: δ = 0.08 (m, 12 H), 0.89 (m, 18 H), 3.77 (dd, J = 4.9, 11.3 Hz, 1 H), 3.83 (dd, J = 3.8, 11.3 Hz, 1 H), 3.94 (dd, J = 4.9, 11.3 Hz, 1 H), 4.00 (dd, J = 3.8, 11.3 Hz, 1 H), 4.75 (m, 1 H), 5.74 (dd, J = 3.8, 4.9 Hz, 1 H), 5.69 (d, J = 1.9 Hz, 1 H), 6.57 (d, J = 2.2 Hz, 1 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): major stereoisomer: δ = –5.5, –5.2, 18.4, 25.9, 64.3, 65.8, 78.2, 100.4, 126.8, 134.5, 163.7 ppm; minor stereoisomer: δ = –5.5, –5.2, 18.3, 18.4, 25.8, 25.9, 64.6, 65.8, 75.7, 98.7, 127.1, 133.2, 163.0 ppm. HR-MS (CI): [MH^+] calcd. 403.2336; found 403.2337.

Methyl 4-(*tert*-Butyldimethylsilyloxy)-3-hydroxy-2-methylenebutyrate (10): Et_3N (0.5 mL, 3.6 mmol) was added to a solution of **8** (0.510 g, 1.3 mmol) and **9** (0.216 g, 0.5 mmol) in MeOH (15 mL). After 2 h of stirring at room temp., the reaction mixture was evaporated. The residue was purified by flash column chromatography (pentane/EtOAc, 90:10) to give **10** (0.405 g, 84%) as a colourless oil. ^1H NMR (300 MHz, $CDCl_3$): δ = 0.05 (s, 3 H), 0.06 (s, 3 H), 0.89 (s, 9 H), 2.98 (br. s, 1 H), 3.46 (dd, J = 6.4, 9.8 Hz, 1 H), 3.75 (s, 3 H), 3.86 (dd, J = 3.8, 9.8 Hz, 1 H), 4.55 (m, 1 H), 5.99 (t, J = 1.5 Hz, 1 H), 6.35 (dd, J = 1.1, 1.5 Hz, 1 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = –5.3, –5.2, 18.4, 25.6, 51.9, 66.6, 71.0, 126.6, 139.2, 166.6 ppm. HR-MS (CI): [MH^+] calcd. 261.1522; found 261.1524.

Methyl (±)-2-(2,2-Dimethyl-1,3-dioxolan-4-yl)acrylate [(±)-(11)]: A catalytic amount of camphorsulfonic acid was added to a solution of **10** (0.385 g, 1.48 mmol) in MeOH (15 mL). After 25 min of stirring the removal of the TBDMS group was complete (TLC) and 2,2-dimethoxypropane (15 mL) was added. After 35 min, the reaction mixture was evaporated and further 2,2-dimethoxypropane (15 mL) was added. After 1 h of stirring at room temp., the reaction mixture was evaporated. The residue was purified by flash column chromatography (pentane/Et_2O, 90:10) to give (±)-**11** (0.215 g, 78%) as a colourless oil. ^1H NMR (300 MHz, $CDCl_3$): δ = 1.43 (s, 3 H), 1.45 (s, 3 H), 3.61 (dd, J = 6.8, 8.3 Hz, 1 H), 3.76 (s, 3 H), 4.36 (dd, J = 6.8, 8.3 Hz, 1 H), 4.89 (tt, J = 1.5, 6.8 Hz, 1 H), 6.05 (t, J = 1.5 Hz, 1 H), 6.30 (t, J = 1.5 Hz, 1 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 25.6, 26.3, 51.9, 70.2, 74.1, 109.5, 126.6, 139.2, 166.1 ppm. HR-MS (CI): [MH^+] calcd. 187.0970; found 187.0972.

(±)-[2-(2,2-Dimethyl-1,3-dioxolan-4-yl)-1-methoxy-1-(trifluoromethyl)allyloxy]trimethylsilane [(±)-(12)]: Cesium fluoride (4 mg, 0.026 mmol) was added to a solution of **11** (0.170 g, 0.91 mmol) in (trifluoromethyl)trimethylsilane (0.174 mL, 0.94 mmol). The progress of the reaction was followed by ^1H and ^{19}F NMR spectroscopy. After 8 h, because some starting material was still present in the reaction mixture, further (trifluoromethyl)trimethylsilane (0.070 mL, 0.45 mmol) was added. After 26 h of stirring at room temp., the excess of (trifluoromethyl)trimethylsilane was evaporated to give (±)-**12** (3:1 mixture of stereoisomers). ^1H NMR (300 MHz, $CDCl_3$): δ = 0.24 (m, 9 H), 1.43 (s, 3 H), 1.45 (s, 3 H), 3.23 (s, 3 H, minor stereoisomer), 3.31 (s, 3 H, major stereoisomer), 3.57 (t, J = 8.1 Hz, 1 H), 4.17 (dd, J = 6.2, 8.1 Hz, 1 H), 4.67 (dd, J = 6.2, 8.1 Hz, 1 H), 5.55 (s, 1 H, minor stereoisomer), 5.58 (s, 1 H, major stereoisomer), 5.88 (s, 1 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): major stereoisomer: δ = 1.1, 25.9, 26.3, 50.4, 70.6, 74.9, 97.9 (q, J = 31 Hz), 108.7, 118.4, 122.4 (q, J = 288 Hz), 142.4 ppm; minor stereoisomer: δ = 1.9, 23.8, 26.3, 49.8, 71.2, 74.4, 98.4 (q, J = 35 Hz), 108.9, 118.5, 122.0 (q, J = 264 Hz), 142.2 ppm. ^{19}F NMR (280 MHz, $CDCl_3$): major stereoisomer: δ = –80.9 ppm; minor stereoisomer: δ = –82.3 ppm.

(±)-3-Methylene-2-(trifluoromethyl)tetrahydrofuran-2,4-diol [(±)-(14)]: Aqueous HCl (3 N, 2 mL) was added to a solution of the mixture of **12** in THF (2 mL). After 18 h of stirring at room temp., the reaction mixture was evaporated. The residue was dissolved in acetone and the resulting solution was filtered. The filtrate was evaporated and the residue was purified by flash column chromatography (pentane/EtOAc, 40:60) to give (±)-**14** (0.130 g, 78%) as a white solid. ^1H NMR (300 MHz, $CDCl_3$): major stereoisomer: δ = 3.90 (dd, J = 6.4, 9.0 Hz, 1 H), 4.24 (dd, J = 6.8, 9.0 Hz, 1 H), 4.77–4.80 (m, 1 H), 5.70 (d, J = 2.3 Hz, 1 H), 5.77 (d, J = 2.3 Hz, 1 H) ppm; minor stereoisomer: δ = 4.02 (dd, J = 2.1, 10.0 Hz, 1 H), 4.27 (dd, J = 4.5, 10.0 Hz, 1 H), 4.74–4.77 (m, 1 H), 5.82 (s, 2 H) ppm. ^{13}C NMR (50 MHz, CD_3OD): major stereoisomer: δ = 69.1, 72.9, 101.4 (q, J = 33 Hz), 115.4, 124.1 (q, J = 283 Hz), 150.2 ppm; minor stereoisomer: δ = 71.9, 74.8, 101.2 (q, J = 33 Hz), 117.2, 124.5 (q, J = 283 Hz), 150.5 ppm. ^{19}F NMR (280 MHz, CD_3OD): major stereoisomer: δ = –86.2 ppm; minor stereoisomer: δ = –84.5 ppm. HR-MS (CI): [MH^+] calcd. 185.0426; found 185.0425.

(±)-2,4-Dihydroxy-2-(trifluoromethyl)dihydrofuran-3-one [(±)-(15)]: Ozone was bubbled through a solution of **14** (4.8 mg, 26 μmol) in

CD_3OD (1.7 mL) cooled to –78 °C until it turned blue. The solution was then purged with oxygen and dimethyl sulfide (19 μL, 260 μmol) was added. The reaction mixture was warmed to room temperature and was then stirred for 24 h. After the addition of D_2O (2 mL), CD_3OD and the excess of dimethyl sulfide were removed by evaporation to give a solution of (±)-**15** (3:2 mixture of stereoisomers). ^1H NMR (300 MHz, D_2O): major stereoisomer: δ = 3.87 (dd, J = 4.9, 9.0 Hz, 1 H), 4.18 (dd, J = 4.9, 5.7 Hz, 1 H), 4.25 (dd, J = 6.0, 9.0 Hz, 1 H) ppm; minor stereoisomer: δ = 3.76 (dd, J = 5.3, 8.7 Hz, 1 H), 4.25 (dd, J = 6.7, 8.7 Hz, 1 H), 4.30 (dd, J = 5.3, 6.4 Hz, 1 H) ppm. ^{19}F NMR (280 MHz, D_2O): major stereoisomer: δ = –80.2 ppm; minor stereoisomer: δ = –79.7 ppm.

(*R*)-2,6-Bis(*tert*-butyldimethylsilyloxymethyl)-5-methylene-1,3-dioxan-4-one [(*R*)-9]: (*tert*-Butyldimethylsilyloxy)acetaldehyde (**6**; 2.64 g, 15.2 mmol) and DABCO (0.215 g, 1.9 mmol) were added, at 0 °C, to a solution of (*R*)-**16**[17] (1.02 g, 3.8 mmol) in CH_2CL_2 (10 mL). After 5 d of stirring at 0 °C, the reaction mixture was evaporated. The residue was purified by flash column chromatography (pentane/Et_2O, 95:5) to give (*R*)-**9** (1.15 g, 75%) as a colourless oil contaminated with about 5% of residual aldehyde **6**. NMR spectroscopic data: see (±)-**9** major stereoisomer. $[\alpha]_D^{20}$ = –32 (c = 2.0, CH_2Cl_2).

Methyl (*R*)-4-(*tert*-Butyldimethylsilyloxy)-3-hydroxy-2-methylenebutyrate [(*R*)-10]: Triethylamine (306 μL, 2.20 mmol) was added dropwise to a solution of (*R*)-**9** (0.442 g, 1.10 mmol) in dry MeOH (4 mL). After 10 min of stirring at room temp., the reaction mixture was evaporated. The residue was purified by flash column chromatography (pentane/CH_2Cl_2, 50:50, then 100% CH_2Cl_2) to give (*R*)-**10** (0.185 g, 65%) as a colourless oil. $[\alpha]_D^{20}$ = –9.6 (c = 1.05, $CHCl_3$).

Methyl (*R*)-2-(2,2-Dimethyl-1,3-dioxolan-4-yl)acrylate [(*R*)-11]: A catalytic amount of camphorsulfonic acid was added to a solution of (*R*)-**10** (0.180 g, 0.69 mmol) in MeOH (5 mL). When the deprotection was complete (45 min, TLC), 2,2-dimethoxypropane (5 mL) was added. After 1.5 h of stirring at room temp., the reaction mixture was evaporated and 2,2-dimethoxypropane (5 mL) was added. After 1 h, the solvent was evaporated. The residue was purified by flash column chromatography (pentane/Et_2O, 90:10) to give (*R*)-**11** (0.091 g, 71%) as a colourless oil. $[\alpha]_D^{20}$ = –44.6 (c = 2.3, $CHCl_3$).

(*S*)-3-Methylene-2-(trifluoromethyl)tetrahydrofuran-2,4-diol [(*R*)-14]: Cesium fluoride (5 mg, 0.033 mmol) was added to a solution of (*R*)-**11** (0.088 g, 0.47 mmol) in (trifluoromethyl)trimethylsilane (89 μL, 0.57 mmol). The reaction was followed by ^1H and ^{19}F NMR spectroscopy. After 21 h of stirring at room temp., the excess of (trifluoromethyl)trimethylsilane was evaporated to give (*R*)-**12**. Then, 3 N aq. HCl (0.5 mL) was added to a solution of the mixture of (*R*)-**12** in THF (0.5 mL). After 24 h of stirring at room temp., the reaction mixture was evaporated. The residue was dissolved in acetone and the resulting solution was filtered. The filtrate was evaporated and the residue was purified by flash column chromatography (pentane/EtOAc, 60:40) to give (*R*)-**14** (0.040 g, 47%) as a white solid.

(*S*)-2,4-Dihydroxy-2-(trifluoromethyl)dihydrofuran-3-one [(*S*)-15]: A solution of (*R*)-**14** (1.7 mg, 9.2 μmol) in CD_3OD (0.9 mL) was cooled to –78 °C and ozone was bubbled through the solution until it turned blue. The solution was then purged with oxygen and dimethyl sulfide (19 μL, 260 μmol) was added. The reaction mixture was warmed to room temperature and was stirred for 24 h. After the addition of 2 mL D_2O, CD_3OD and the excess of dimethyl sulfide were removed by evaporation to give an aqueous solution of (*S*)-**15** (3:2 mixture of stereoisomers).

Methyl 3-Acetoxy-4-(*tert*-butyldimethylsilyloxy)-2-methylenebutyrate (17a): Acetic anhydride (116 μL, 1.23 mmol) and DMAP (0.019 g, 0.15 mmol) were added to a solution of (*R*)-**10** (0.145 g, 0.56 mmol) in dry CH_2Cl_2 (5 mL). After 6 h of stirring at room temp., water (10 mL) was added and the aqueous phase was extracted with CH_2Cl_2 (3 × 15 mL). The organic phase was washed with brine (15 mL) and dried with Na_2SO_4. The solvent was evaporated and the residue was purified by flash column chromatography (pentane/EtOAc, 90:10) to give **17a** (0.1 g, 60%) as a colourless oil. ^1H NMR (300 MHz, $CDCl_3$): δ = 0.04 (s, 3 H), 0.05 (s, 3 H), 0.87 (s, 9 H), 2.10 (s, 3 H), 3.70 (dd, J = 6.4, 10.9 Hz, 1 H), 3.78 (s, 3 H), 3.83 (dd, J = 3.4, 10.9 Hz, 1 H), 5.70 (m, 1 H), 5.84 (d, J = 1.1 Hz, 1 H), 6.36 (s, 1 H) ppm. $[\alpha]_D^{20}$ = +6.5 (c = 2.1, CH_2Cl_2).

Methyl 4-Acetoxy-3-hydroxy-2-methylenebutyrate (17b) and Methyl 3-Acetoxy-4-hydroxy-2-methylenebutyrate (17c): A 1 M THF solution of tetrabutylammonium fluoride (0.3 mL, 0.3 mmol) was added to a solution of **17a** (0.083 g, 0.27 mmol) in dry THF (1 mL). After 45 min of stirring at room temp., the reaction mixture was evaporated. The residue was then dissolved in CH_2Cl_2 (10 mL). The organic phase was washed with water (2 × 1 mL) and dried with Na_2SO_4. The solvent was evaporated and the residue was purified by flash column chromatography (pentane/Et_2O, 40:60) to give **17b** (0.016 g, 32%) as a colourless oil. This compound was contaminated by a small amount (<10%) of **17c**. ^1H NMR (300 MHz, $CDCl_3$): δ = 2.07 (s, 3 H), 3.78 (s, 3 H), 4.17 (dd, J = 6.8, 11.5 Hz, 1 H), 4.28 (dd, J = 3.8, 11.5 Hz, 1 H), 4.71 (m, 1 H), 5.98 (s, 1 H), 6.37 (s, 1 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 20.8, 52.1, 67.4, 69.7, 127.3, 138.6, 166.2, 171.4 ppm. $[\alpha]_D^{20}$ = –21.2 (c = 0.95, $CHCl_3$).

Methyl 4-(*tert*-Butyldimethylsilyloxy)-3-(2-methoxy-2-phenylacetoxy)-2-methylenebutyrate (18): *O*-Methyl-D-mandelic acid (3 equiv.) and DMAP (5 equiv.) were added to a solution of racemic ester **10** (1 equiv.) in $CDCl_3$ (0.5 mL) and DCC was then added gradually until the reaction was complete (disappearance of the TBDMS signals at δ = 0.05, 0.06 and 0.89 ppm). ^1H NMR (300 MHz, $CDCl_3$): (*R*,*R*)-**18**: δ = –0.07 (s, 3 H), –0.06 (s, 3 H), 0.79 (s, 9 H), 3.40 (s, 3 H), 3.65–3.75 (m, 2 H), 3.74 (s, 3 H), 4.81 (s, 1 H), 5.70 (m, 2 H), 6.28 (s, 1 H), 7.23–7.43 (m, 5 H); (*S*,*R*)-**18**: δ = 0.03 (s, 3 H), 0.04 (s, 3 H), 0.86 (s, 9 H), 3.43 (s, 3 H), 3.60–3.85 (m, 2 H), 3.68 (s, 3 H), 4.81 (s, 1 H), 5.14 (s, 1 H), 5.70 (m, 1 H), 6.03 (s, 1 H), 7.23–7.43 (m, 5 H) ppm. When the same experiment was carried out with (*R*)-**10** only the signals of the (*R*,*R*) stereoisomer were observed. ^1H NMR (300 MHz, $CDCl_3$): (*R*,*R*)-**18** [from (*R*)-**10**]: δ = –0.07 (s, 3 H), –0.06 (s, 3 H), 0.79 (s, 9 H), 3.40 (s, 3 H), 3.65–3.75 (m, 2 H), 3.74 (s, 3 H), 4.81 (s, 1 H), 5.70 (m, 2 H), 6.28 (s, 1 H), 7.23–7.43 (m, 5 H) ppm.

Determination of Bioluminescence-Inducing Activity: The bioluminescence-inducing activity of (±)-**DPD** or (*S*)-**5** was measured using a *V. harveyi* bioluminescence assay.[24] The luminescent reporter strain *V. harveyi* BB170 was grown for 18 h at 30 °C, with aeration, in AB medium and was diluted 1:5000 into fresh AB medium. Next, (±)-**DPD** or (*S*)-**5** was added to the diluted BB170 cells at a 10% (v/v) final concentration, and then shaken at 30 °C for 4 h. The luminescence background was determined by the addition of sterile AB medium to *V. harveyi* BB170 diluted cells. After the incubation period, the resulting light production of each sample was measured with a luminometer, and activity was expressed as relative light units of luminescence (RLU) per second.

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