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Asymmetric synthesis of (−)-fosfomycin and its *trans*-(1*S*,2*S*)-diastereomer using a biocatalytic reduction as the key step

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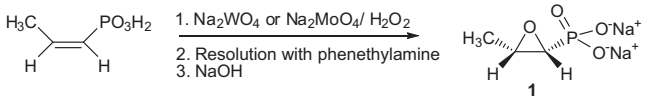
ABSTRACT

Fosfomycin is a gram positive and gram negative antibiotic that contains an asymmetric epoxide. An enzyme library was screened for its ability to reduce dimethyl(1-chloro-2-oxopropyl)phosphonate to the corresponding asymmetric chlorohydrin. Homology models were built in MOE, which were shown to accurately model the enzyme–substrate complex displaying the stereoselectivity that we observed. Two enzymes, YDR368w and YHR104w, were chosen for the scale up and synthesis of fosfomycin and its *trans*-(1*S*,2*S*)-diastereomer.

1. Introduction

Fosfomycin **1** is an antibiotic that targets gram positive and gram negative bacteria by inhibiting UDP-*N*-acetylglucosamine-3-*O*-enolpyruvyltransferase (MurA), an enzyme that plays an important role in peptidoglycan biosynthesis.[1] Historically it has been used as an effective treatment for urinary tract infections particularly since it is highly effective when applied as a single dose.[2] However, due to the increase in antibiotic resistance and because fosfomycin has a low toxicity for humans this antibiotic has reemerged for the treatment for a variety of infections such as Chronic Obstructive Pulmonary Disease (COPD),[3] cystic fibrosis,[4] Methicillin-Resistant *Staphylococcus aureus* (MRSA),[5] and is used intravenously for sepsis and soft tissue infections.[6]

Fosfomycin was originally isolated from *Streptomyces fradiae* and fully characterized by Christensen et al. who determined the absolute structure to be (−)-(1*R*,2*S*)-1,2-epoxypropylphosphonic acid.[7,8] It is part of a small class of biologically active compounds that contain the C–P bond, which are most commonly found in *Streptomyces*.[9] Presently, the large scale production of fosfomycin is achieved by the epoxidation of *cis*-propenylphosphonic acid to yield racemic fosfomycin. The racemate is then resolved by producing the diastereomeric salt with (+)-α-phenethylamine (Scheme 1).[10] The resulting yield is below 20% for the process and most of the loss of yield comes from the resolution step to obtain the pure enantiomer.[11]



Scheme 1. Popular large scale approach towards the fosfomycin sodium salt.

In an attempt to increase the yield, a recent strategy was able to utilize the (+)-enantiomer of fosfomycin and convert it to the biologically active isomer; however, this took seven steps and had an overall yield of less than 50%.[12] Thus it would be advantageous to develop alternative synthetic routes that did not rely on resolving the enantiomers. Asymmetric syntheses of fosfomycin have been developed utilizing chiral metal catalysts, chiral auxiliaries, or starting with a chiral molecule such as a sugar.[10,13–17] Many of these strategies still struggle to achieve high enantiomeric excess and the use of heavy metals for pharmaceutical synthesis can be problematic due to their toxicity. While there have been many investigations of how fosfomycin is biosynthesized naturally in a variety of organisms, the use of biocatalytic strategies for the asymmetric synthesis of fosfomycin has been relatively neglected. Wang et al. investigated the use of *Candida antartica* lipase B (CALB) to kinetically resolve the alcohol to obtain the correct stereoisomer, but once again when resolving the racemate, the reaction is limited to a 50% yield.[18]

Bakers' yeast (*Saccharomyces cerevisiae*) is a popular biocatalytic tool for organic chemist. It has been shown to catalyze a variety of reactions; however, it is most popularly used for its capability to reduce carbonyls to the enantiomerically enriched alcohol.[19] Its

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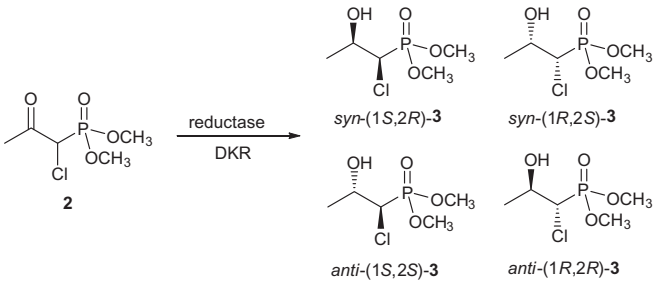
ability to reduce a collection of substrates can be explained by the many reductases it contains and that many of these enzymes have been characterized to be very promiscuous.[20] However, the use of whole cell bakers yeast often leads to a mixture of products often formed by competing enzymes. To circumvent this problem, GST-reductase chimeras were engineered and placed into *Escherichia coli* creating a bakers' yeast reductase library.[20–22] This system has been used to screen the stereospecificity of these reductases for a given substrate by use of the pure fusion protein or in whole-cells.[22–24]

Accurate enzyme homology models have become a useful tool in understanding the stereoselectivity of enzymes, particularly models that can predict the enantioselectivity of an enzyme for a specific substrate. Even if no crystal structure is known, it is suggested that most software programs can build an accurate homology model if it has at least 25% sequence similarity.[25] Recently, there have been models built that were successful in modeling reductases from *S. cerevisiae* to predict the location of the hydride source in relationship to the substrates carbonyl.[26,27] This is important because if an accurate model can be made, this can potentially remove the need for enzyme–substrate screening; more importantly this allows for a clear understanding of the intricate enzyme–substrate complex, which can assist in site-directed mutagenesis of the active site to achieve changes in enzyme stereoselectivity.[28]

2. Results and discussion

2.1. Enzyme screening

This manuscript describes the screening of 20 reductases from the aforementioned enzyme library for their ability to reduce dimethyl(1-chloro-2-oxopropyl)phosphonate **2** to the corresponding alcohol **3**. Due to the acidity of the α-hydrogen this single reaction is able to generate two stereogenic carbons through dynamic kinetic resolution (DKR) (Scheme 2).



Scheme 2. Dynamic kinetic resolution (DKR) of dimethyl(1-chloro-2-oxopropyl)phosphonate **2** and the corresponding four possible products.

The synthesis of **2** can be achieved by reacting sulfuryl chloride with dimethyl(2-oxopropyl)phosphonate which is commercially available. This ketone substrate was then screened for activity against the Bakers' yeast reductase library described above. For the sake of simplicity, the screening was achieved in whole-cells that overexpress each reductase. We discovered that 8 enzymes from the reductase library displayed activity for the reduction of **2** (Table 1). As seen in Table 1, only two of the four possible products were formed; *syn*-(1*R*,2*S*)-**3** and *anti*-(1*S*,2*S*)-**3** were both formed with 99% ee or better as shown by GC analysis. Bakers' yeast was also screened and while its two products were enantiomerically pure, it displayed poor diastereomeric excess. This illustrates the advantage of overexpressing and screening each reductase individually, which often gives better ee and de values.

Table 1
Enzyme library screening results for the reduction of **2**

Enzyme	*syn*-(1*R*,2*S*)-**3**	*anti*-(1*S*,2*S*)-**3**
YOL151w		64% de*
YGL157w	12% de	
YGL039w		56% de
YNL331c	79% de	
YOR120w	77% de	
YBR149w	95% de	
YHR104w		92% de
YDR368w	95% de	
Bakers yeast	18% de	

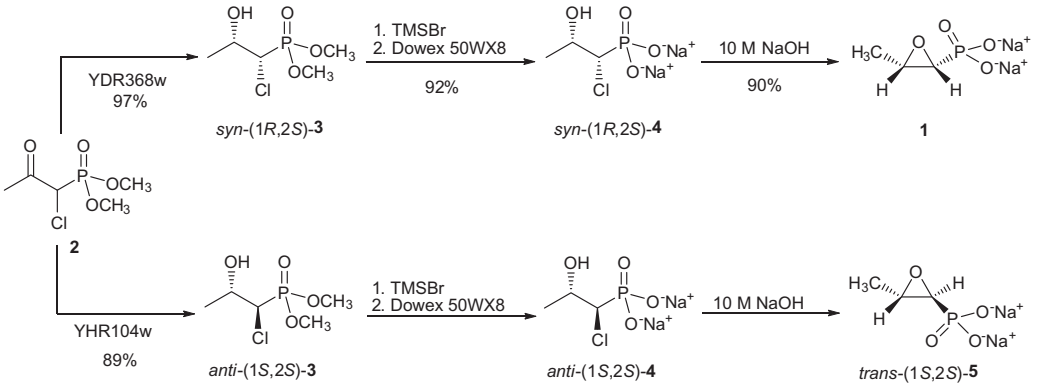
There was no detection of *syn*-(1*S*,2*R*)-**3** or *anti*-(1*R*, 2*R*)-**3** so the ee values for each enzyme was reported as 99% or better. Red coloured rows are members of the short chain dehydrogenase family while blue coloured rows belong to the aldose reductase family. The absolute configuration was determined by NMR.
* Percent de calculated by GC.

Additional analysis of this screening data gave some interesting results. First, this reductase library has enzymes that are a member of four possible enzyme families (aldose reductase, short chain dehydrogenase, medium chain dehydrogenase, and D-hydroxy acid dehydrogenase).[29] For this substrate **2**, we found that only enzymes from the short chain dehydrogenase or the aldose reductase family resulted in the product. Of these reduction products, enzymes in the aldose reductase family gave the best overall de values at 77% or better, whereas, the enzymes from the short chain dehydrogenase family gave lower de values. The only exception to this is YNL331c, which gives a 79% de. However, we recently proposed that due to similar reactivity and phylogenetic tree data that perhaps the reductase YNL331c may be part of the aldose reductase family.[30] Furthermore, while looking at the aldose reductase family, only enzymes with a long loop A region showed activity toward **2**. This trend is noteworthy since it suggested that the loop A region plays an important role in substrate binding.[31]

2.2. Synthesis of fosfomycin

We used enzymes YDR368 and YHR104w in our efforts to scale up the production of (−)-fosfomycin and its *trans*-(1*S*,2*S*)-diastereomer **5** since they displayed the best de values from our screening. Using previously optimized non-growing conditions,[32] we were able to achieve 3.4 and 3.1 g/L when using YDR368w and YHR104w, respectively. While the reactions resulted in complete conversion, we found that obtaining high yields was difficult due to the high polarity of product **3**. Thus, a continuous liquid–liquid extractor was used for a minimum of 24 h to pull the entire product out of the aqueous phase. The chiral alcohol **3** was then further purified by flash chromatography (solvent gradient from 1 Hex:1 EtOAc to 1 Hex:5 EtOAc), which was also able to separate the majority of the diastereomer impurity.

The phosphonate **3** was then treated with trimethylsilyl bromide (TMSBr) to give phosphonic acid **4** (Scheme 3). This reaction was purified by simply running the crude product through a Dowex 50WX8 resin preloaded with a sodium cation to afford the sodium salt **4**. The chlorohydrin **4** was then treated with a base. The solution was neutralized with HCl and the resulting NaCl was precipitated by the addition of methanol. It should be noted that this solution should be stirred for approximately 45 min to fully dissolve the fosfomycin sodium salt. The solution was gravity



Scheme 3. Asymmetric synthetic strategy for the synthesis of (−)-fosfomycin **1** and its diastereomer **5**.

filtered and the filtrate was dried over sodium sulfate and then concentrated under reduced pressure to result in (−)-fosfomycin **1** in near quantitative yields.

2.3. Computational methods

Homology models were built in MOE[33] for YDR368W and YHR104W. The models were constructed based on the crystal structure of *Sus Scrofa* aldose reductase[34] (Protein Data Bank code: 1AH0) and a mutant *Homo sapiens* Oxidoreductase[35] (Protein Data Bank code: 2AGT) respectively. The percent identity and percent similarity of the entire amino acid sequences were obtained from yeastgenome.org and were 43% identical and 26% similar for YDR368W/1AH0 and 43% identical and 24% similar for YDR104W/2AGT. The cofactor was added by superimposing the homology model with the crystal structure in which it was based. The cofactor was then minimized in the homology model while being tethered to its original coordinates. The homology models were solvated with a 5 Å layer of water; hydrogen atoms were added using the protanate3D algorithm in MOE with titration turned off. Lastly, the model was minimized with no constraints. Throughout this process the AMBER99 force field was used with the Rfield treatment of electrostatic interactions.

The two enantiomers of compound **2** were built and minimized using the Hartree–Fock method with a 6-31G* basis set as implemented in the Spartan 06 program.[36] The resultant structures were docked with MOE's docking algorithm using Alpha PMI as the placement method and The London dG scoring function followed by refinement using the AMBER99 with the Rfield treatment of electrostatic interactions. Both the ligand and the active site amino acid side chains were treated as flexible. Docked structures that were not hydrogen bonded to an amino acid that is part of the catalytic tetrad[37] were discarded along with complexes that had the prochiral carbonyl carbon more than 4 Å from the hydride source.

2.4. Computational results

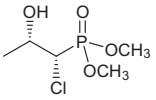
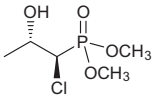
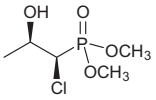
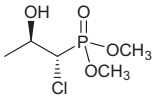
In order to explain the observed diastereoselectivity of YHR104W and YDR368w in the reduction of **2**, homology models were used to observe the orientation of **2** in the enzyme–substrate complex. It is known that the hydride transfer originates from the NADPH cofactor and the orientation of the carbonyl *re*- or *si*-face determines which enantiomer will be preferred. The two enantiomers of compound **2** were docked in the two enzymes. Low energy enzyme–substrate complexes were examined to determine what stereochemistry would occur. Table 2 lists the enzyme–substrate interaction energy for the lowest energy of each possible stereoisomer. These results agree with the experimentally determined values for the major and minor product of both enzymes.

The minimized structures corresponding to the major and minor product of the enzymes YHR104W (Fig. 1a and b) and YDR368W (Fig. 1c and d) are presented. In YHR104W the carbonyl group is held in place by hydrogen bonding to HIS 111 whereas in YDR368W the carbonyl group is hydrogen bonded to TYR 56; in both cases, these catalytic residues position the *re*-face of the carbonyl directly above the hydride source which results in the formation of the (*S*)-alcohol. The model predicts that the small differences in the active site geometry switch the catalytic protonation residue from the TYR in YDR368W to HIS in YHR104W resulting in a switching in the major and minor product between these two enzymes.

3. Conclusion

In conclusion, we have described the screening of dimethyl(1-chloro-2-oxopropyl)phosphonate **2** against a Bakers' yeast reductase library. Two enzymes, YDR368w and YHR104w, were chosen for the scale up and synthesis of fosfomycin and its *trans*-(1*S*,2*S*)-diastereomer due to their high ee and de values. Additionally, homology models were built in MOE and were able to accurately model the enzyme–substrate complex that would afford the stere-

Table 2
Energy of compound **2** docked into enzyme for the orientation that would result in the reduced compounds shown below

Enzyme	 *syn*-(1*R*,2*S*)-**3**	 *anti*-(1*S*,2*S*)-**3**	 *syn*-(1*S*,2*R*)-**3**	 *anti*-(1*R*,2*R*)-**3**
YDR368W	−94.54 kcal/mol	−89.78 kcal/mol	−87.06 kcal/mol	−50.01 kcal /mol
YHR104W	−51.64 kcal/mol	−53.51 kcal/mol	−37.34 kcal/mol	NS

NS indicates that the structure was not seen.

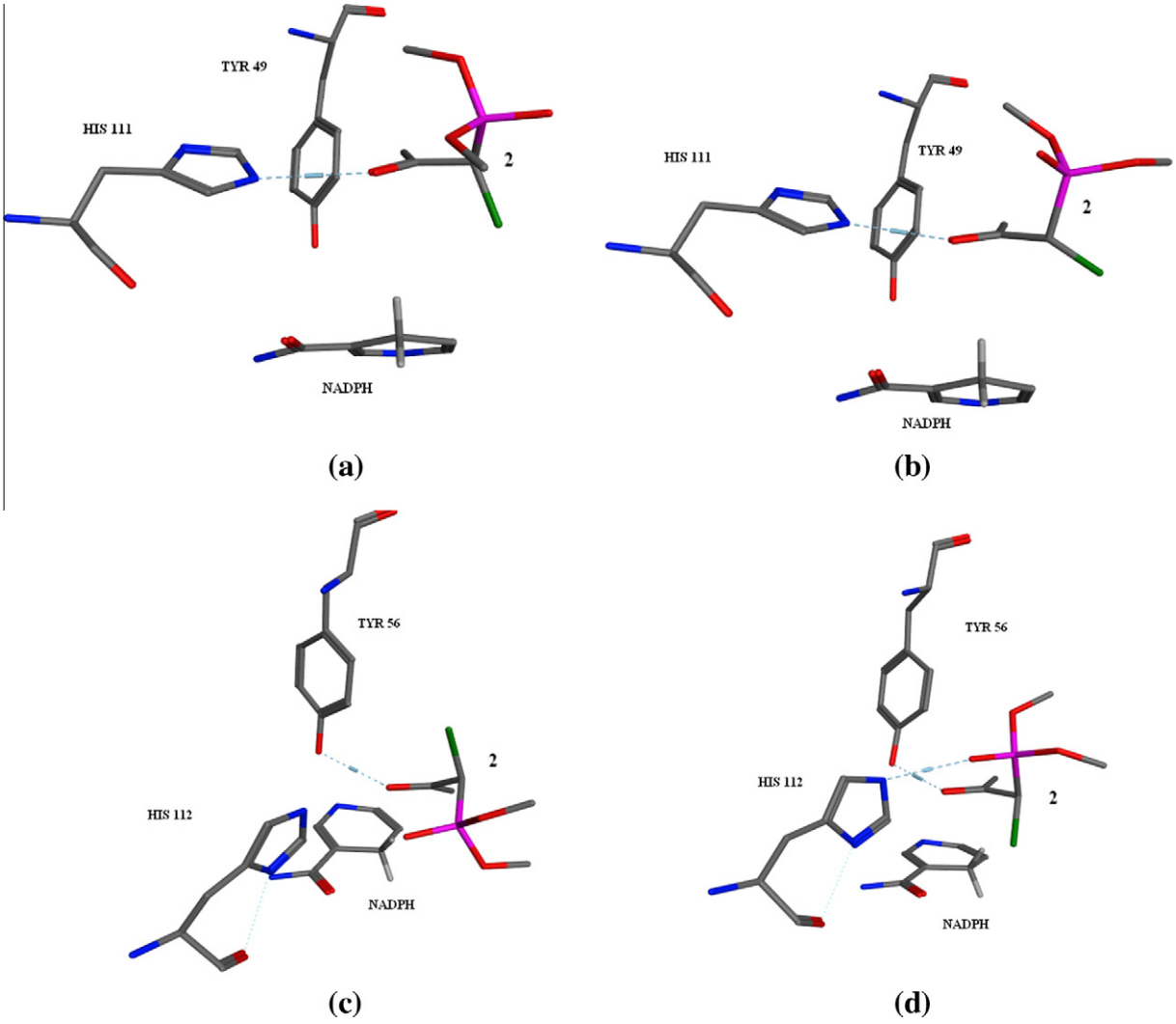


Figure 1. Low energy docked structure resulting in the observed products of the enzymes; (a) YHR104W minor product; (b) YHR104W major product; (c) YDR368w major product; (d) YDR368w minor product, are shown.

oselectivity that we observed. The enzyme products *syn*-(1*R*,2*S*)-**3** and *anti*-(1*S*,2*S*)-**3** were then used in a simple two-step synthesis to give fosfomycin and its diastereomer.

4. Experimental

4.1. General

Standard solvents and reagents were used unless otherwise specified. Commercially available β-keto nitriles were purchased from Aldrich. Protein overexpression was determined by SDS–PAGE and Western blotting. IR spectra were recorded on a Perkin Elmer Spectrum-100 FTIR. The absolute stereochemistry was determined on a Varian Inova spectrometer equipped with a 5 mm indirect detection probe, operating at 500 MHz for H1. Chemical shifts at 25 °C are reported in parts per million (ppm) relative to tetramethylsilane (TMS). The solvent was chloroform-*d*. NMR spectra were recorded on a JEOL JNM-ECP300 FT 300 MHz NMR. Peak positions are given in (ppm) using TMS as the internal standard and coupling constants (*J* values) are given in Hz. Gas chromatography mass spectra were recorded on an HP5973 MSD using a β-cyclodextrin column and optical rotations were measured using a Rudolph Research Analytical AUTOPOL IV polarimeter. HRMS was collected on an ABSciex Q-STAR Elite. Column chromatography purifications were performed using silica gel Sorbent Technology 40–63 μm.

4.2. Determination of the absolute configuration

For alcohols **3**, the absolute configurations were determined based on the difference in chemical shifts between the (*R*)- and (*S*)-α-methoxy-α-phenylacetic esters.[38,39] The esterification was carried out in the NMR tube, by adding 1 equiv of a mixture of 2p (*R*)-MPA and 1p (*S*)-MPA to the alcohol, followed by the addition of DCC (1.5 equiv) and DMAP (1 equiv). In some cases, the relevant signals overlapped with other signals from the reaction mixture. A TOCSY1D experiment, with selective excitation of the alpha proton in the ester (which showed up in a clear region) revealed the other protons in the coupling network.

4.3. Whole-cell screening procedure

An LB medium supplemented with 30 μg/mL kanamycin was inoculated with a single colony of *E. coli* (containing the appropriate overexpressed gene) and shaken 15 h at 37 °C. This preculture was diluted 1:100 into 30 mL of the same medium and shaken

2.5 h at 37 °C with a stir rate of 220 rpm. Upon reaching an OD_{600} ~0.5 the cell culture was cooled to 20 °C, and isopropylthio-β-D-galactoside was added to a final concentration of 100 μM and shaken for 2 h. The phosphonate substrate **2** was added to a final concentration of 3 mM using a concentrated ethanol solution. This reaction was allowed to shake at 220 rpm for 12 h. The reaction was then gently extracted with methylene chloride (2 mL) and analyzed by chiral GC–MS.

4.4. Dimethyl(1-chloro-2-oxopropyl)phosphonate 2

Dimethyl(2-oxopropyl)phosphonate (416 μL, 3.01 mmol, 1 equiv) was added to a stirred solution of chloroform (5 mL) at 0 °C. Sulfuryl chloride (205 μL, 3.01 mmol, 1 equiv) was added dropwise over 5 min. The reaction was allowed to stir for 1 h at 0 °C. Water (15 mL) was added and the reaction was extracted with methylene chloride (5 × 15 mL). The organic layers were combined and dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The crude oil was purified by flash chromatography (solvent gradient 3 Hex:1 EtOAc to 1 Hex:1 EtOAc) to give **2** in a 63% overall yield. IR (neat) 3476, 2961, 1732, 1450, 1360 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 4.53 (d, 1H, J = 17.58), 3.86 (d, 6H, J = 10.98), 2.42 (s, 3H); ^{13}C NMR (300 MHz $CDCl_3$) δ: 198.11, 57.79, 55.94, 54.97 (dd, J = 6.6, J = 16.4), 27.84; mass (EI): 187, 158, 128, 109, 79; HRMS (ESI) calcd for $C_5H_{10}O_4PCl$ $[M+H]^+$ 201.0078, found 201.0134.

4.5. *syn*-(1*R*,2*S*)-Dimethyl-1-chloro-2-hydroxypropanephosphonate 3

An LB medium supplemented with 30 μg/mL kanamycin was inoculated with a single colony of *E. coli* (engineered to overexpress the gene YDR368w) and shaken 15 h at 37 °C. This preculture was diluted 1:100 into 2 L of the same medium and shaken 2.5 h at 37 °C with a stir rate of 220 rpm. Upon reaching an OD_{600} = 0.5 the cell culture was cooled to 20 °C, and isopropylthio-β-D-galactoside was added to a final concentration of 100 μM and shaken for 12 h. Cells were collected by centrifugation (8000*g* for 10 min at 4 °C) and then resuspended in a phosphate buffer (750 mL, 100 mM, pH 7) containing 5 g/L glucose. The bioconversion was carried out in a 2 L baffled flask at 28 °C with a stir rate of 220 rpm. Portions of neat **2** were added in small increments (200 mg) and monitored by GC–MS for conversion until the reaction was complete. The cell solution was transferred to a continuous liquid–liquid extractor and extracted with methylene chloride for a minimum of 24 h to achieve complete extraction. The organic layer was dried with magnesium sulfate and then concentrated under reduced pressure. The resulting crude oil was then purified by flash chromatography (solvent gradient 1 Hex:1 EtOAc to 1 Hex: 5 EtOAc) to afford 2.5 g of *syn*-(1*R*,2*S*)-**3** in 97% yield. IR (neat) 3400, 2961, 1250, 1038; ^1H NMR (300 MHz $CDCl_3$) δ: 4.33 (m, 1H), 3.83 (dd, 6H, J = 10, J = 21), 3.28 (d, 1H, J = 5), 1.31 (dd, 3H, J = 10 Hz, J = 1.4); ^{13}C NMR (300 MHz $CDCl_3$) δ: 66.23, 58.19, 56.13, 54.29 (dd, J = 6.9, J = 111), 20.27 (d, J = 11); mass (EI) 187, 158, 128, 109, 79; HRMS (ESI) calcd for $C_5H_{12}O_4PCl$ $[M+H]^+$ 203.0235, found 203.0301. $[\alpha]_{365}$ = +2.8 (*c* 1, H_2O).

4.6. *anti*-(1*S*,2*S*)-Dimethyl-1-chloro-2-hydroxypropanephosphonate 3

An LB medium supplemented with 30 μg/mL kanamycin was inoculated with a single colony of *E. coli* (engineered to overexpress the gene YHR104w) and shaken for 15 h at 37 °C. This preculture was diluted 1:100 into 2 L of the same medium and shaken for 2.5 h at 37 °C with a stir rate of 220 rpm. Upon reaching an OD_{600} = 0.5 the cell culture was cooled to 20 °C, and isopropylthio-β-D-galactoside was added to a final concentration of 100 μM and shaken for 12 h. Cells were collected by centrifugation (8000*g* for 10 min at 4 °C) and then resuspended in a phosphate buffer (750 mL, 100 mM, pH 7) containing 5 g/L glucose. The bioconversion was carried out in a 2 L baffled flask at 28 °C with a stir rate of 220 rpm. Portions of neat **2** were added in small increments (200 mg) and monitored by GC–MS for conversion until the reaction was complete. The cell solution was transferred to a continuous liquid–liquid extractor and extracted with methylene chloride for a minimum of 24 h to receive complete extraction. The organic layer was dried over magnesium sulfate and then concentrated under reduced pressure. The resulting crude oil was purified by flash chromatography (solvent gradient 1 Hex:1 EtOAc to 1 Hex: 5 EtOAc) to afford 2.3 g of *anti*-(1*S*,2*S*)-**3** in a 89% yield. IR (neat) 3402, 2960, 1249, 1038; ^1H NMR (300 MHz $CDCl_3$) δ: 4.20 (m, 1H, J = 6, J = 8), 3.85 (dd, 6H, J = 11, J = 16), 3.56 (s, 1H), 1.38 (d, 3H, J = 6 Hz); ^{13}C NMR (300 MHz $CDCl_3$) δ: 67.84, 57.01, 55.00, 54.29 (dd, J = 6.6, J = 71), 20.05 (d, J = 7.5); mass (EI) 187, 158, 128, 109, 79; HRMS (ESI) calcd for $C_5H_{12}O_4PCl$ $[M+H]^+$ 203.0235, found 203.0279. $[\alpha]_{365}$ = −17 (*c* 1, H_2O).

4.7. *syn*-(1*R*,2*S*)-1-Chloro-2-hydroxypropane phosphonic acid 4

Trimethylsilyl bromide (2.09 mL, 15.84 mmol, 8 equiv) was added to a stirred solution of **3** (0.401 g, 1.98 mmol, 1 equiv) in methylene chloride (10 mL) with the atmosphere purged with argon. The reaction was stirred at room temperature for 2.5 h and then concentrated under reduced pressure. Methanol (4 mL) was added to the reaction flask, stirred for 1 h and then concentrated under reduced pressure. Water (5 mL) was added to the crude oil and then added to a Dowex 50WX8 column (resin was pretreated with 50 mL of 6 M HCl, 50 mL of 6 M NaOH, and then flushed with DI water until the water eluting the column had a pH 8–9) and the resin was flushed with DI water and fractions were collected. The initial fractions were collected (20 mL) in a round bottom flask and concentrated under reduced pressure. To remove any residual water, the white solid was dissolved in methanol (25 mL) dried over sodium sulfate, filtered, and then concentrated under reduced pressure to afford the white solid (0.398 g) in a 92% yield. ^1H NMR (300 MHz D_2O) δ: 4.23 (m, 1H, J = 4.5, J = 6), 3.78 (dd., 1H, J = 11.4, J = 4.3), 1.30 (d, 3H, J = 6); ^{13}C NMR (300 MHz D_2O) δ: 67.34, 60.51 (d, J = 141), 20.22 (d, J = 7.5); HRMS (ESI) calcd for $C_3H_7O_4PCl$ $[M^-]$ 172.9770, found 172.9838. $[\alpha]_{365}$ = +4 (*c* 1, H_2O).

4.8. *anti*-(1*S*,2*S*)-1-Chloro-2-hydroxypropane phosphonic acid 4

Procedure same as for *syn*-**4**. ^1H NMR (300 MHz D_2O) δ: 4.33 (m, 1H, J = 3), 4.01 (dd., 1H, J = 13, J = 3), 1.28 (d, 3H, J = 6); ^{13}C NMR (300 MHz D_2O) δ: 67.16 (d, J = 4.6), 60.99 (d, J = 139.5), 17.49.; HRMS (ESI) calcd for $C_3H_7O_4PCl$ $[M^-]$ 172.9770, found 172.9785. $[\alpha]_{365}$ = −31.5 (*c* 1, H_2O).

4.9. *syn*-(1*R*,2*S*)-1,2-Epoxypropylphosphonic acid 1

A 10 M NaOH solution (9 mL) was added to a round bottom flask containing **4** (814 mg, 3.72 mmol). The reaction was stirred for 1 h and then 6 M HCl was carefully added until the pH of the solution reached 8 or 9. The neutralized reaction was then concentrated under reduced pressure. Methanol (30 mL) was then added to the white solid and the suspension was allowed to stir for 1 h at 0 °C to ensure that the desired product dissolved. The suspension was then gravity filtered to remove the NaCl precipitate. Sodium sulfate was added to dry the methanol solution, which was filtered, and then concentrated under reduced pressure to yield the sodium

salt of **1** in 90% yield. Spectroscopic data matched that of the literature[18] ^{1}H NMR (300 MHz D_2O) δ: 3.30 (m, 1H, J = 5.5), 2.85 (dd, 1H, J = 5.22, J = 18.7), 1.49 (d, 3H, J = 5.5); ^{13}C NMR (300 MHz D_2O) δ: 55.27 (d, J = 175), 54.80, 13.85.; HRMS (ESI) calcd $C_3H_6O_4P$ [M^-] 137.0009, found 137.0066. $[\alpha]_{365} = -17$ (c 1, H_2O).

4.10. *trans*-(1*S*,2*S*)-1,2-Epoxypropylphosphonic acid 5

Procedure the same as for **1** (above) ^{1}H NMR (300 MHz D_2O) δ: 3.21 (m, 1H, J = 5.2, J = 3.3), 2.63 (dd, 1H, J = 21.4, J = 3.3), 1.36 (d, 3H, J = 5.2); ^{13}C NMR (300 MHz D_2O) δ: 57.24 (d, J = 174.6), 54.68, 17.52.; HRMS (ESI) calcd $C_3H_6O_4P$ [M^-] 137.0009, found 137.0080. $[\alpha]_{365} = +5.2$ (c 1, H_2O).

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