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On the conversion of structural analogues of (*S*)-2-hydroxypropylphosphonic acid to epoxides by the final enzyme of fosfomycin biosynthesis in *S. fradiae*

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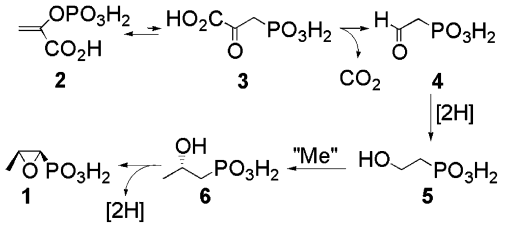
Abstract—2-Hydroxyethyl- and (*S*)-2-hydroxybutylphosphonic acid were prepared, starting in the latter case from (*S*)-2-aminobutyric acid. They were fed to cultures of *Streptomyces fradiae* producing fosfomycin. Only the latter (150 μg/mL of medium) was converted to the ethyl analogue of fosfomycin, isolated as 2-amino-1-hydroxybutylphosphonic acid (3%) in admixture with 2-amino-1-hydroxypropylphosphonic acid (97%) derived from fosfomycin.

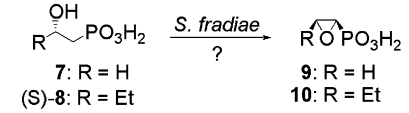
Fosfomycin [**1**, (1*R*,2*S*)-epoxypropylphosphonic acid] is a clinically utilized antibiotic of low toxicity, blocking bacterial cell wall biosynthesis by acting as an analogue of phosphoenolpyruvate (PEP).[1–3] It is one of the rare natural products containing a P–C bond,[4] produced by various species of *Streptomyces*,[5] *Pseudomonas syringae*, and *P. viridiflava*.[6,7] The biosynthesis of fosfomycin was unraveled by using feeding experiments with labeled precursors[8–12] and genetic[13] techniques. It comprises five steps, three of which are unique, starting from the primary metabolite PEP (**2**), which is rearranged reversibly by PEP mutase to give phosphonopyruvate (**3**) (Scheme 1).[14–16]

Decarboxylation[17] and reduction[18] produce 2-hydroxyethylphosphonic acid (**5**). The recently elucidated methylation of **5** producing (*S*)-2-hydroxypropylphosphonic acid (**6**, Hpp) follows a unique radical mechanism with SAM as methyl donor.[18,19] The final step is a dehydrogenative cyclization performed by a non-heme iron oxygenase [(*S*)-2-hydroxypropylphosphonic acid epoxidase, HppE].[20–24] Liu et al. found that the epoxidase converted (*R*)-2-hydroxypropylphosphonic and (*S*)-1,1-difluoro-2-hydroxypropylphosphonic acid to 2-oxopropylphosphonic acids, which has some bearing on the mechanism of the epoxide ring closure.[20]



Scheme 1. Biosynthesis of fosfomycin.

Inspired by the broad substrate specificity of isopenicillin *N* synthase,[25] also a non-heme iron dependent oxygenase, we decided to prepare analogues of Hpp, in the first place those with a hydrogen atom or an ethyl group replacing the methyl group in **6** to study the substrate specificity of the epoxidase. These homologues, **7** and **8**, were probed for their conversion to the analogous epoxides of fosfomycin by cultures of *Streptomyces fradiae* (Scheme 2). When this work was started, the epoxidase had not yet been purified and characterized. The cyclohexylammonium salt of 2-hydroxyethylphosphonic acid (**7**) was prepared by a literature procedure.[11] The



Scheme 2. Conversion of homologues of **6** to epoxides by *S. fradiae*.

Keywords: Fosfomycin; Biosynthesis; Phosphonic acids.
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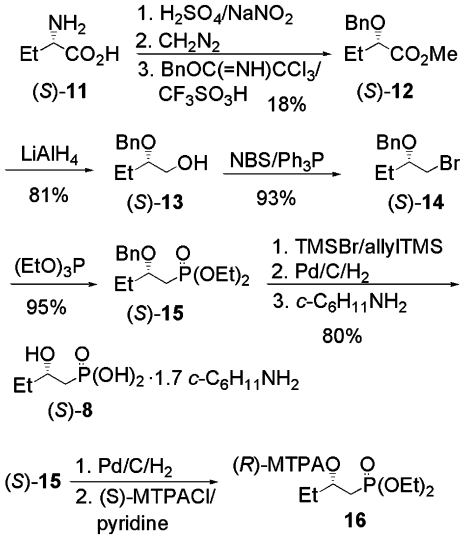
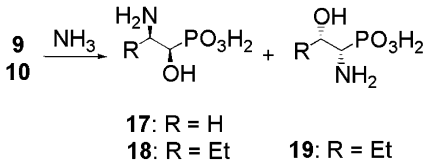


Scheme 3. Conversion of 2-aminobutyric acid to 2-hydroxyphosphonic acid (*S*)-**8** (as cyclohexylammonium salt).

synthesis of **8** is given in Scheme 3, starting from (*S*)-2-aminobutyric acid.[26,27] [(*S*)-**11**] as chiral precursor. The carboxyl group of the intermediate 2-hydroxyacid was esterified[26] and the hydroxyl group was benzylated[28] using Bundle's reagent to give ester (*S*)-**12** in an overall yield of 18%. Reduction of the ester (81%), conversion of the alcohol to the bromide (93%) followed by an Arbusov reaction with triethyl phosphite (95%) furnished 2-benzyloxyphosphonate (*S*)-**15**.[11,29] Removal of the protecting groups (TMSBr for Et on phosphorus, Pd/C/H_2 for Bn) gave phosphonic acid (*S*)-**8**, which was converted to the crystalline cyclohexylammonium salt for purification, containing 1.7 mol of amine as found by ^{1}H NMR spectroscopy. To establish the ee of the final product, a sample of phosphonate (*S*)-**15** was hydro-genolytically deprotected and esterified with (*S*)-MTPACl to yield Mosher ester **16**. Similarly, a reference sample of racemic **15**[30] was esterified. On the basis of the ^{1}H NMR spectra, the ee of (*S*)-**15** was found to be 98%. The salt of acid (*S*)-**8** should have the same ee, as the stereochemistry is not affected on deprotection of **15**.

Isolation of fosfomycin from the broth of *S. fradiae* was not possible because of its low concentration (up to 10 μg/mL) and its similar behavior to phosphoric acid (1 mg/mL of K_2HPO_4 in the medium) on ion exchange chromatography.[8] Therefore, the antibiotic was converted to two isomeric aminophosphonic acids by ring opening with ammonia, of which the (1*R*,2*R*)-2-amino-1-hydroxypropylphosphonic acid was amenable to isolation by ion exchange chromatography.[9] Assuming that the analogues **9** and **10** of fosfomycin are formed in smaller amounts than fosfomycin, if at all and then in admixture with it, we thought that treatment of the broth would yield amino-hydroxyphosphonic acids. The 2-amino-1-hydroxyphosphonic acids derived from fosfomycin and an analogue would behave similarly upon purification. NMR spectroscopy would allow the

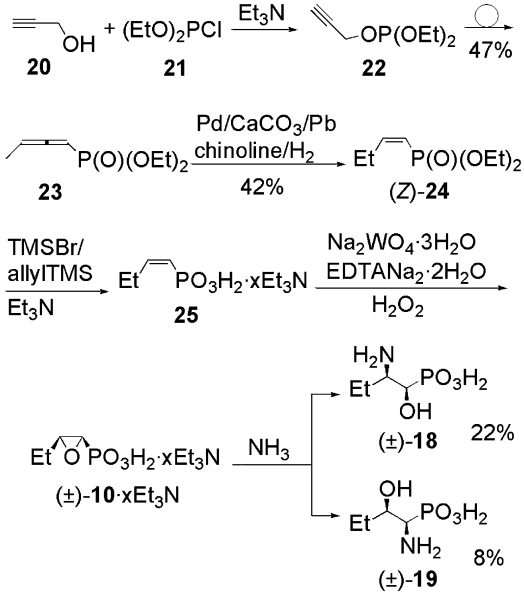


Scheme 4. Conversion of epoxides **9** and **10** to amino-hydroxyphosphonic acids by ammonia.

detection of the aminophosphonic acid **17** or **18** in the isolated mixture.

These arguments induced us to study the behavior of epoxides **9** and **10** toward ammonia and prepare reference compounds **17**–**19** (Scheme 4). Epoxide **9** will be opened by ammonia exclusively by attack at the less hindered site at C-2.[31] As 2-amino-1-hydroxyethylphosphonic acid[32] (**17**) was available from a previous project and its behavior was therefore known, it was not necessary to study the conversion of **9** to **17**.

However, the preparation of epoxide **10**, for convenience in the racemic form, and its conversion to amino-hydroxybutylphosphonic acids **18** and **19** had to be performed (Scheme 5). The used reactions are related to the ones used for the synthesis of fosfomycin, although some modifications had to be introduced.[33] Propargyl alcohol (**20**) was reacted with diethyl phosphorochloridite to phosphite **22** as intermediate, which underwent a smooth 2,3-sigmatropic rearrangement at ambient temperature to allenylphosphonate **23** in 47% yield.[34] The selective hydrogenation of one double bond was critical.[35] Using Pd/$CaCO_3$/Pb/H_2 (1 atm)/chinoline in dry ethanol furnished under optimized conditions at room temperature the desired (*Z*)-1-butenylphosphonate **23** (42%) and a mixture of (*E*)-**23** and possibly 2-butenylphosphonates (ratio 2:1, 20%) by flash chroma-



Scheme 5. Preparation of epoxide (±)-**10** and its ring opening with NH_3.