

On the Transformation of (S)-2-Hydroxypropylphosphonic Acid into Fosfomycin in *Streptomyces fradiae*—A Unique Method of Epoxide Ring Formation**

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(1*S*,2*S*)- and (1*R*,2*S*)-2-hydroxy-[1-*D*₁]propylphosphonic acid were synthesised from (1*S*,2*S*)-2-benzyloxy-[1-*D*₁]propanol, which was obtained by horse liver alcohol dehydrogenase catalysed reduction of the corresponding aldehyde. When (1*S*,2*S*)-2-hydroxy-[1-*D*₁]propylphosphonic acid was fed to *Streptomyces fradiae*, the deuterium was retained to the same extent in fosfomycin (cis-epoxide) and its co-metabolite trans-epoxide. Removal of the hydrogen (deuterium) atom from the C-1 atom of deuterated 2-hydroxypro-

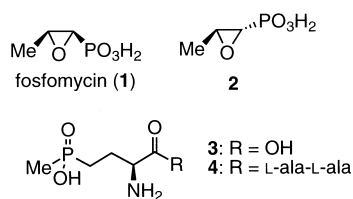
pylphosphonic acids is a stereospecific process (the hydrogen atom of (S)-2-hydroxypropylphosphonic acid is pro-R). The formation of the O–C-1 bond of fosfomycin occurs with net inversion of configuration, the formation of the O–C-1 bond of the trans-epoxide with net retention.

KEYWORDS:

epoxides · isotopic labelling · oxidases · phosphonic acids · reaction mechanisms

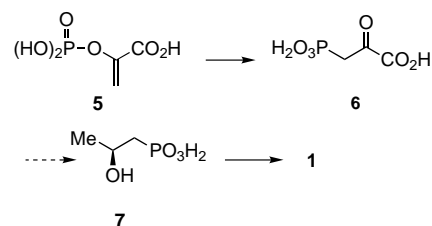
Introduction

The number of known natural products that contain a P–C bond is steadily growing.^[1] Three of them, fosfomycin (**1**; the *trans* isomer **2** is discussed below), phosphinothricin (**3**) and the tripeptide bialaphos (**4**; Scheme 1), are produced by various strains of *Streptomyces* and are of commercial importance. Fosfomycin is marketed as a clinical antibiotic that interferes with cell wall biosynthesis in bacteria.^[2] Phosphinothricin, which blocks glutamine synthase, and bialaphos are sold as herbicides.^[3]



Scheme 1. Natural products with a P–C bond.

Biosynthetic studies have shown that all natural compounds with a P–C bond are generated from phosphonopyruvic acid (**6**), which is formed by a mutase-catalysed intramolecular rearrangement of phosphoenolpyruvate (**5**; Scheme 2).^[4] Decarboxylation^[5] of **6**, followed by methylation,^[6–8] gives (S)-2-hydroxypropylphosphonic acid (**7**).^[9] which is used as the precursor for biosynthesis of fosfomycin in *Streptomyces fradiae*. β-Hydroxyphosphonic acid **7** is oxidatively cyclised (loss of two hydrogen



Scheme 2. Biosynthesis of fosfomycin (**1**) from phosphoenolpyruvate in *Streptomyces fradiae*.

atoms) to give fosfomycin.^[9] The oxygen of the oxirane ring is derived from the hydroxy group.^[9–11] Furthermore, the O–C-1 bond is formed with inversion of configuration at C-1, as shown by feeding experiments with chirally labelled (*R*)- and (*S*)-2-hydroxy-[1-*D*₁]ethylphosphonic acids.^[6] This unique method for

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the biosynthesis of an oxirane is reminiscent of the biosynthesis of the plant alkaloid scopolamine, where 6 β -hydroxyhyoscyamine is dehydrogenated to scopolamine, an epoxide.^[12] Contrary to the biosynthesis of fosfomycin, the carbon–oxygen bond is formed with retention of configuration in the scopolamine synthesis. Normally, epoxides are biosynthesised by monooxygenases from olefins.^[13]

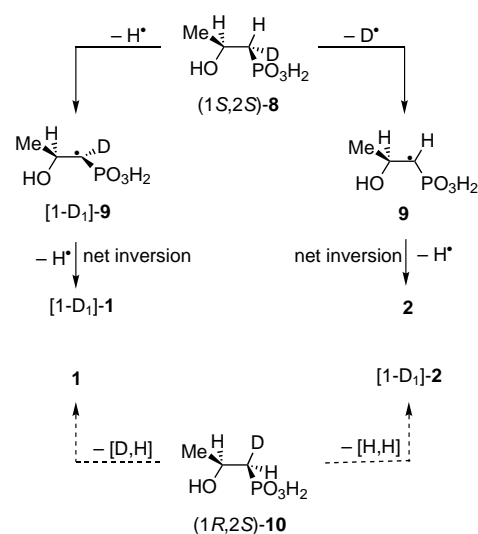
We found recently that the culture broth of *Streptomyces fradiae* contains, in addition to the *cis*-epoxide fosfomycin, 3% stereoisomeric *trans*-epoxide **2** as a co-metabolite.^[14] We assume that this compound gives a clue to the mechanism of the oxirane ring formation. Very recently, Liu and co-workers reported the purification of the iron-dependent protein that catalyses the formation of fosfomycin.^[11] They also proposed a radical mechanism for the formation of the C-1–O bond.

Results and Discussion

The absolute configuration of phosphonic acids **1** and **2** is identical at C-2, but opposite at C-1.^[14] We therefore hypothesise that a single monooxygenase performs a stepwise oxidative cyclisation of **7** that involves intermediate radicals and produces natural products **1** and **2**. Formally, two bonds have to be broken (carbon–hydrogen and oxygen–hydrogen) and one bond (carbon–oxygen) has to be formed. The stereochemically intriguing steps are the removal of the hydrogen atom from C-1 and the formation of a bond between the oxygen atom and C-1. In principle each step could be stereospecific or non-stereospecific, a situation amenable to experimental verification by use of stereospecifically deuterated 2-hydroxypropylphosphonic acids. Whether removal of the hydrogen atom from the hydroxy group precedes or follows formation of the oxygen–C-1 bond does not have any bearing on the stereochemical outcome of the cyclisation and is therefore not considered further. Additionally, oxidation of the radical to a carbocation before ring closure followed by loss of a proton from the hydroxy group is feasible. Rotation about the C-1–C-2 bond is more likely for a radical-type than carbocation-type intermediate. For simplicity, only a combination of a stereospecific and a nonstereospecific step for the oxirane ring formation is necessary initially. The mechanistic proposal must account for the observation that the formation of the oxirane ring of fosfomycin occurs with inversion of configuration at C-1. The major pathway must be the one that leads to fosfomycin.

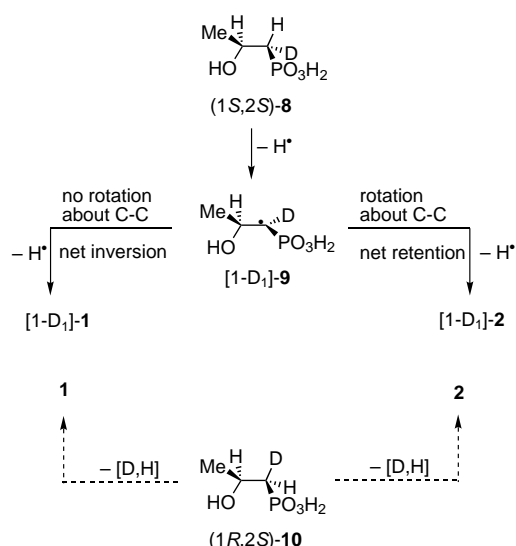
We first studied the consequences for the labelling of **1** and **2** if a combination of nonstereospecific removal of a hydrogen (deuterium) atom from C-1 and stereospecific formation of the carbon–oxygen bond takes place with (1*S*,2*S*)-2-hydroxy-[1- D_1]propylphosphonic acid [(1*S*,2*S*)-**8**] as substrate (Scheme 3). Removal of the hydrogen or deuterium atom gives radical intermediates **9** and [1- D_1]-**9**, respectively. Stereospecific ring closure of both radicals with inversion of configuration gives a mixture of deuterated fosfomycin and nondeuterated **2**. Analogously, (1*R*,2*S*)-2-hydroxy-[1- D_1]propylphosphonic acid [(1*R*,2*S*)-**10**] should yield nondeuterated fosfomycin **1** and deuterated **2**.

Secondly, we considered the consequences of stereospecific hydrogen atom abstraction and nonstereospecific bond forma-



Scheme 3. Possible mechanism for formation of **1** and **2** from (*S*)-2-hydroxypropylphosphonic acid stereospecifically deuterated at C-1: a combination of nonstereospecific removal of the hydrogen (deuterium) atom from C-1 and stereospecific formation of the C–O bond with inversion of configuration.

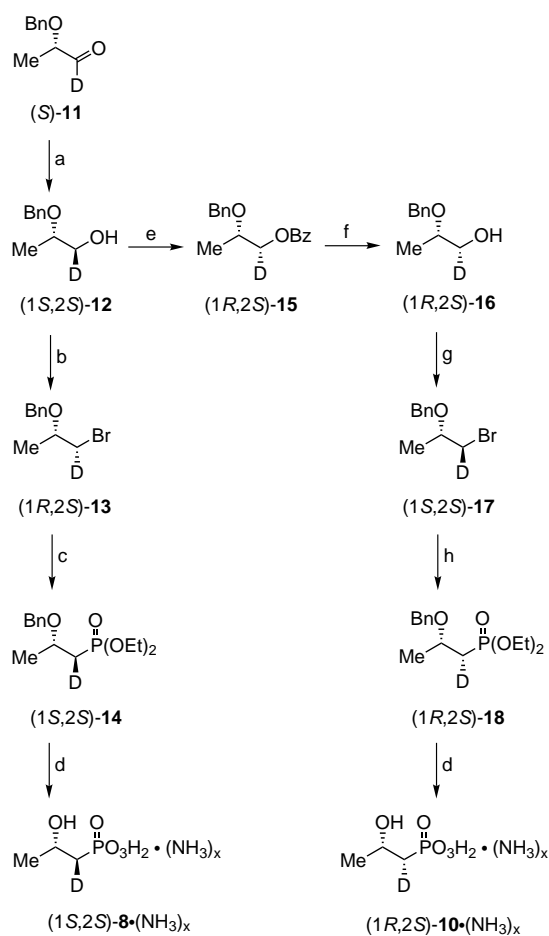
tion for the labelling of **1** and **2** (Scheme 4). Loss of a hydrogen atom from (1*S*,2*S*)-**8** generates radical [1- D_1]-**9**, which results either in deuterated fosfomycin with net inversion of configuration, or deuterated **2** after rotation about the C-1–C-2 bond by 180°, which constitutes net retention of configuration. Similarly, (1*R*,2*S*)-**10** will result in the formation of unlabelled **1** and **2**.



Scheme 4. Possible mechanism for formation of **1** and **2** from (*S*)-2-hydroxypropylphosphonic acid stereospecifically deuterated at C-1: a combination of stereospecific removal of the hydrogen (deuterium) atom from C-1 and nonstereospecific formation of the C–O bond, with either inversion or retention of configuration.

These two pathways can easily be studied by use of stereospecifically deuterated 2-hydroxypropylphosphonic acids (1*S*,2*S*)-**8** and (1*R*,2*S*)-**10**. If a combination of two stereospecific processes results in the formation of only one product, either **1** or **2**, then a second oxygenase must be postulated for the biosynthesis of **2**. A combination of two nonstereospecific processes will yield mixtures of labelled and unlabelled **1** and **2** from precursors (1*S*,2*S*)-**8** and (1*R*,2*S*)-**10**, respectively.

To test the mechanisms presented in Scheme 3 and 4 experimentally, we synthesised deuterated (*S*)-2-hydroxypropylphosphonic acids (1*S*,2*S*)-**8** and (1*R*,2*S*)-**10** (Scheme 5). The preparation of these two precursors is in general terms similar

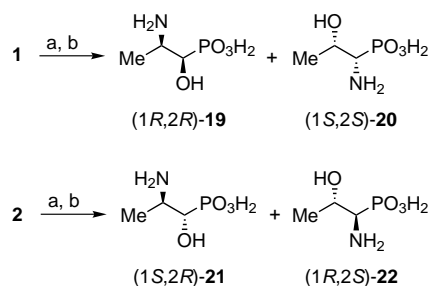


Scheme 5. a) HLADH/(nicotinamide adenine dinucleotide)⁺/EtOH/phosphate buffer, pH 6.9, 94%; b) Ph₃P/NBS, 92%; c) (EtO)₃P, bath temperature 170 °C, 81%; d) TMSBr/allylTMS, 50 °C, 2.5 h, then H₂O; Pd/C/H₂; NH₃/H₂O, 90%; e) Ph₃P/diethyl azodicarboxylate/C₆H₅CO₂H, 93%; f) MeOH/MeONa, 91%; g) Ph₃P/NBS, 96%; h) (EtO)₃P, bath temperature 170 °C, 70%. NBS = *N*-bromosuccinimide, TMS = trimethylsilane.

to the synthesis of (*R*)- and (*S*)-2-hydroxy-[1-*D*]₂ethylphosphonic acid.^[6] Surprisingly, the starting alcohol (1*S*,2*S*)-**12** can be prepared easily with a yield of 94% by reduction of deuterated *O*-benzyl lactaldehyde (*S*)-**11**, catalysed by horse liver alcohol dehydrogenase (HLADH).^[15] This procedure is more convenient than the laborious preparation of (*S*)-lactaldehyde from α -threonine, its reduction by HLADH/[1,1-*D*₂]ethanol and isolation

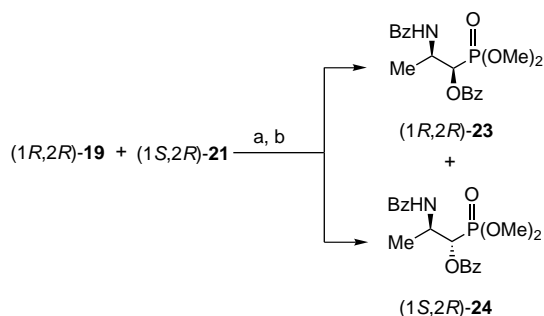
of the 1,2-[1-*D*]₂propandiol, as reported by Abeles and co-workers.^[16] The selective protection of the secondary alcohol would possibly add another three steps to the sequence. The configuration of the protected diol at C-1 was assigned on the basis of precedence.^[17] The diastereomeric excess (de) and enantiomeric excess of (1*S*,2*S*)-**12** were $\geq 98\%$ and $\geq 99\%$, as determined by ¹H NMR spectroscopy of the (*R*)-Mosher ester.^[18] Monoprotected diol (1*S*,2*S*)-**12** was used to prepare the C-1 epimer (1*R*,2*S*)-**16**. The configuration was inverted by Mitsunobu esterification with benzoic acid followed by a sodium methoxide catalysed transesterification in methanol as solvent.^[17] Comparison of the ¹H NMR spectra of the (*R*)-Mosher esters of (1*R*,2*S*)-**16** and (1*S*,2*S*)-**12** revealed that the esterification was effected with clean inversion. The hydroxy groups of (1*S*,2*S*)-**12** and (1*R*,2*S*)-**16** were replaced by bromide by treatment with triphenylphosphane/NBS, with inversion of configuration.^[18] Bromide (1*R*,2*S*)-**13** was diastereomerically pure (de = 99%), but bromide (1*S*,2*S*)-**17** had a de of only 96%, which indicates that a small amount of double inversion by bromide took place. The next step, the Arbuzov reaction of bromide (1*R*,2*S*)-**13**, was the stereochemically critical step^[6, 17] (see also the Experimental Section) in the preparation of the ammonium salt of (1*S*,2*S*)-**8** because of the high reaction temperature (bath temperature 170 °C). Bromides (1*R*,2*S*)-**13** and (1*S*,2*S*)-**17** gave phosphonates (1*S*,2*S*)-**14** and (1*R*,2*S*)-**18** with diastereomeric excesses of 88% and 95%, respectively. The de of the phosphonates could be determined easily because the hydrogen resonances of the CHDP groups of the phosphonates (1*S*,2*S*)-**14** and (1*R*,2*S*)-**18** are well separated in ¹H NMR spectra ($\delta = 2.18$ and 1.89 ppm, respectively). These experiments show that partial racemisation occurs under the conditions of the Arbuzov reaction^[6, 17] but this racemisation can be suppressed by suitable silylation of the glass surface. Substitution of the hydroxy group for a bromide moiety can bring about a trace of racemisation. The phosphonates were deblocked to give the free phosphonic acids (1*S*,2*S*)-**8** and (1*R*,2*S*)-**10**, which were stored and used as their ammonium salts for the feeding experiments.

The first four feeding experiments^[10] were carried out with deuterated phosphonic acid (1*S*,2*S*)-**8**·(NH₃)_x (corresponding to 30 mg L⁻¹ free acid). For each experiment six 1-L Erlenmeyer flasks, each containing 220 mL of corn starch medium, were used to grow *Streptomyces fradiae*. The cells were removed by centrifugation and the supernatants were pooled and saturated with ammonia. The epoxides **1** and **2** were transformed into a mixture of 1-amino-2-hydroxypropylphosphonic acids (1*S*,2*S*)-**20** and (1*R*,2*S*)-**22** and 2-amino-1-hydroxypropylphosphonic acids (1*R*,2*R*)-**19** and (1*S*,2*R*)-**21** by heating the solution to 60 °C for 3 days (Scheme 6).^[10, 14] The mixture of 2-aminophosphonic acids (1*R*,2*R*)-**19** and (1*S*,2*R*)-**21** was isolated by ion exchange chromatography (Dowex 50, H⁺; Dowex 1, AcO⁻; Dowex 50, H⁺); the less acidic 1-aminophosphonic acids are not retained on Dowex 50, H⁺. The mixtures of 2-aminophosphonic acids from the four feeding experiments were pooled and gave 22 mg impure material. The ³¹P NMR (242.94 MHz, D₂O) spectrum of the mixture showed that the sample was a mixture of 2-aminophosphonic acid (1*R*,2*R*)-**19** (96.2%) and (1*S*,2*R*)-**21** (3.8%). The ¹H NMR spectrum (600 MHz) demonstrated that (1*R*,2*R*)-**19**



Scheme 6. Transformation of epoxides **1** and **2** into amino-hydroxypropyl-phosphonic acids in a culture broth of *Streptomyces fradiae*. a) Gaseous NH_3 , 0°C ; b) heating at 60°C for 3 days.

contained deuterium at C-1, but exact determination was not possible because of overlapping impurities. Crystallisation from water yielded 8 mg pure (1*R*,2*R*)-**19**, with 24% of the molecules deuterated at C-1, as determined by ^1H NMR spectroscopy (400 MHz, D_2O). The remaining mixture of phosphonic acids in the mother liquor, in which (1*S*,2*R*)-**21** was enriched relative to (1*R*,2*R*)-**19**, was transformed into benzamides to facilitate purification (Scheme 7). At first, the procedure was optimised



Scheme 7. Derivatisation of 2-aminophosphonic acid (1*R*,2*R*)-**19**: a) TBDMSCl/pyridine/acetone nitrile, reflux, 5 h; $\text{C}_6\text{H}_5\text{COCl}$, room temperature, 16 h; H_2O ; Dowex 50, H^+ ; b) CH_2N_2 ; overall yield 23%. Derivatisation of (1*S*,2*R*)-**21** and mixtures of (1*R*,2*R*)-**19** and (1*S*,2*R*)-**21**: a) TMSCl/pyridine, reflux, 1.5 h; $\text{C}_6\text{H}_5\text{COCl}$, 50°C , 3 h; H_2O ; Dowex 50, H^+ ; b) CH_2N_2 ; overall yield up to 36%. TBDMSCl = tert-butyl dimethylsilyl chloride.

for samples of pure (1*R*,2*R*)-**19** and (1*S*,2*R*)-**21** and then mixtures of both were used (racemic **21** was also used instead of (1*S*,2*R*)-**21**). The yields were low (up to 36%) despite extensive efforts to increase them, but were sufficient for samples of biological origin for NMR spectroscopic investigation. The aminophosphonic acids of the mother liquor were derivatised and yielded a mixture (1 mg, 7%) of benzamides (1*R*,2*R*)-**23** and (1*S*,2*R*)-**24** purified by preparative TLC to give these compounds in a ratio of 87:13, respectively, as determined by ^{31}P NMR (243 MHz, $\text{DMSO}-d_6$) and ^1H NMR spectroscopy (400 MHz). The ratio of labelled to unlabelled benzamide was 21:79 for (1*R*,2*R*)-**23** and 20:80 for (1*S*,2*R*)-**24**. This result shows that epoxides **1** and **2** are deuterated and that the extent of labelling is equal.

Feeding was repeated with (1*S*,2*S*)-**8** (50 mg L^{-1}). The combined yield from four runs was 20 mg crude 2-aminophosphonic acids (1*R*,2*R*)-**19** and (1*S*,2*R*)-**21** in a ratio of 97:3 (determined by

^{31}P NMR spectroscopy, 243 MHz). Crystallisation of the mixture was carried out as previously described and gave 12 mg pure (1*R*,2*R*)-**19** (30% D at C-1, measured by 600 MHz ^1H NMR spectroscopy). The aminophosphonic acids of the mother liquor were first investigated by ^{13}C NMR spectroscopy (201.2 MHz, 128 000 scans) and showed isotope-induced satellite doublets^[9] for C-2 and C-3 (Figure 1). The resonances of C-1 for the two

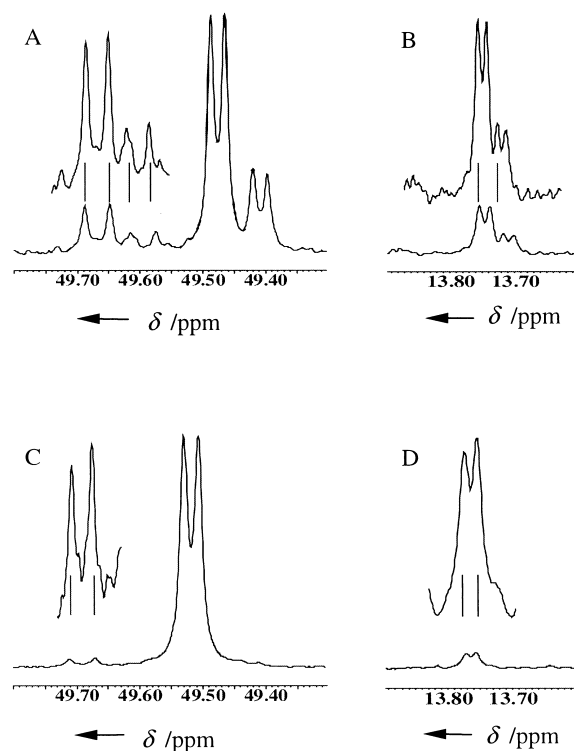


Figure 1. Sections from ^{13}C NMR spectra (201.2 MHz, D_2O) of mixtures of aminophosphonic acids (1*R*,2*R*)-**19** and (1*S*,2*R*)-**21** obtained from feeding experiments. A, B: Sections of the spectrum of the mother liquor obtained from crystallisation of a sample isolated after feeding *Streptomyces fradiae* with (1*S*,2*S*)-**8**; A: C-2 of (1*R*,2*R*)-**19** and (1*S*,2*R*)-**21**; B: C-3 of (1*S*,2*R*)-**21**. C, D: The same signals as in (A) and (B) from a sample isolated when (1*R*,2*S*)-**10** was fed to *Streptomyces fradiae*.

aminophosphonic acids overlap. Part A of the spectrum shows two sets of resonances at $\delta = 49.41$ and 49.48 ppm (2d, $J(\text{C},\text{P}) = 4.6$ Hz), and 49.59 and 49.67 ppm (2d, $J(\text{C},\text{P}) = 8.2$ Hz). The lower field peak of each set corresponds to unlabelled (1*R*,2*R*)-**19** and (1*S*,2*R*)-**21** and that at higher field to the species deuterated at C-1. The C-3 resonance of (1*S*,2*R*)-**21** is also given (part B). The doublet at 13.75 ppm ($J(\text{C},\text{P}) = 2.7$ Hz) corresponds to the C-3 atom of unlabelled (1*S*,2*R*)-**21** and that at 13.71 ppm to the labelled compound. The ratio of (1*R*,2*R*)-**19** to (1*S*,2*R*)-**21** is estimated to be 87:13 and the deuterium content of the two aminophosphonic acids appears to be equal (about 25%). The mother liquor was concentrated and the residue was derivatised to give a mixture of amides (1*R*,2*R*)-**23** and (1*S*,2*R*)-**24** (ratio 83:17 by ^{31}P NMR spectroscopy at 243 MHz). The difference indicated by the ratio is possibly caused by differences in yield of derivatisation. The ^1H NMR spectrum recorded in $\text{DMSO}-d_6$ at 400 MHz revealed that 30% (1*R*,2*R*)-**22** derived from fosfomicin

contained deuterium at C-1 and 27% (1*S*,2*R*)-**23** derived from the *trans*-epoxide contained deuterium.

Lastly, a feeding experiment was carried out with 50 mg L⁻¹ (1*R*,2*S*)-**10** in the same way as with (1*S*,2*S*)-**8**. The combined yield from four runs was 23 mg crude 2-aminophosphonic acids (1*R*,2*R*)-**19** and (1*S*,2*R*)-**21** in a ratio of 97:3 (determined by ³¹P NMR spectroscopy at 242.9 MHz). In this case, (1*R*,2*R*)-**19** was not crystallised to enrich the sample in (1*S*,2*R*)-**21**. The ¹³C NMR spectrum (201.2 MHz, 128 000 scans) was recorded directly. The same parts of the spectrum as described above are shown in Figure 1 C and D. It is evident that neither aminophosphonic acid contains deuterium. Half the crude mixture of these aminophosphonic acids was also derivatised. The mixture of amides (1*R*,2*R*)-**23** and (1*S*,2*R*)-**24** was investigated by ¹H NMR spectroscopy (400 MHz, DMSO-*d*₆). None of the amides contained deuterium.

In summary, the feedings demonstrate that (1*S*,2*S*)-**8** is transformed into a mixture of epoxides **1** and **2**, each of which contains the same amount of deuterium; 25% or 30% depending on the concentration of precursor (30 or 50 mg L⁻¹) in the medium. When the diastereomeric phosphonic acid (1*R*,2*S*)-**10** was fed to *Streptomyces fradiae*, the deuterium was lost to the medium and only unlabelled 2-aminophosphonic acids were isolated. These findings are in agreement with a mechanism as outlined in Scheme 4. The hydrogen atom is removed from C-1 of (1*S*,2*S*)-**8** and the deuterium atom from (1*R*,2*S*)-**10** to give either a deuterated or nondeuterated intermediate, respectively. The major pathway (about 97%) leads to fosfomycin with net inversion of configuration, in agreement with an earlier finding.^[9] The minor pathway (3%) leads to *trans*-epoxide **2**. A rotation about the C-1–C-2 bond must take place before the formation of the C-1–O bond to give net retention of configuration. Consequently, the enzyme carries out a stereospecific removal of the hydrogen (deuterium) atom and a nonstereospecific ring closure. This implies that the pro-*R* hydrogen atom of (*S*)-2-hydroxypropylphosphonic acid, the intermediate in the biosynthesis of fosfomycin, is removed for the formation of the O–C(1) bond.

Oxygenases are a large group of enzymes involved in hydroxylations, epoxidations, desaturations and other biotransformations.^[19, 20] For example, a cytochrome P-450 is involved in the biodegradation of camphor in *Pseudomonas putida*.^[21] This enzyme removes one hydrogen atom (either *exo* or *endo*) from C-5 and produces 5-*exo* hydroxycamphor. Baldwin et al. have shown that isopenicillin N synthase, a nonhaem iron protein, uses dioxygen, δ-(*L*-α-amino adipoyl)-*L*-cysteinyll-*D*-valine and a variety of structural analogues to make β-lactams.^[22] It is assumed that radical-type, partly configurationally labile intermediates are involved in certain cases. As reported by Liu and coworkers, the enzyme that catalyses the formation of the epoxide ring of fosfomycin from (*S*)-2-hydroxypropylphosphonic acid is a nonheme iron protein.^[11] They proposed a mechanism based on the assumption that the hydroxy group of the substrate is first ligated to the iron. An electron is then transferred to reduce the Fe³⁺ ion to an Fe²⁺ ion, dioxygen is bound and a hydrogen is removed from C-1 to generate a radical. Transfer of a second electron induces a collapse of the

reactive species to fosfomycin. We think that this mechanism does not easily accommodate the inversion of configuration for the formation of fosfomycin. One would expect preferential formation of co-metabolite **2** with retention of configuration. Any mechanism for the formation of the O–C(1) bond of fosfomycin has to take into account the stereochemical findings reported here and the concomitant formation of *trans*-epoxide **2** as a co-metabolite.

Interestingly, (1*S*,2*S*)-1,2-dihydroxypropylphosphonic acid blocks the biosynthesis of fosfomycin.^[9] The hydroxy group on C-1 occupies the same position as the deuterium atom of (1*R*,2*S*)-**10**. It is likely that this inhibitor becomes firmly bound to the enzyme by ligation of the OH group at the C-1 atom to iron. The results of feeding experiments with structural analogues of (*S*)-2-hydroxypropylphosphonic acids will be published separately.

Experimental Section

General: TLC: Merck precoated TLC plates (0.25 mm), silica gel 60, F₂₅₄; detection: UV and/or spraying with a 2% solution of Ce^{IV}SO₄·4H₂O in 2*N* H₂SO₄ and heating on a hot plate; flash chromatography: Merck silica gel 60, 0.040–0.063 mm; IR: Perkin-Elmer FT 1600 IR Spectrometer; liquid samples were measured as films and solids as nujol mulls between NaCl plates; ¹H NMR, ¹³C NMR (*J*-modulated), and ³¹P NMR spectroscopy: Bruker spectrometers AC 250F, AM 400, WB 600, and DRX 800 MHz. The latter instrument was equipped with TXI HCN z-grad or QXI HCNP z-grad probes; samples were dissolved in D₂O (about 0.3 mL) in a Shigemi microcell tube. TMS was used as internal standard; Optical rotation: Perkin-Elmer polarimeter 241 (1 dm cell); melting points were measured with a Reichert Thermovar instrument and are uncorrected.

(1*S*,2*S*)-(+)-2-Benzoyloxy-[1-*D*]₂propanol [(1*S*,2*S*)-12**]:** Aldehyde (*S*)-**11** was prepared from (*S*)-2-benzoyloxy-[1,1-*D*]₂propanol^[18] ($[\alpha]_D^{20} = +44.8$ ($c = 0.945$, CHCl₃)) by Swern oxidation.^[23] The crude aldehyde was purified by flash chromatography (CH₂Cl₂) instead of bulb-to-bulb distillation and used immediately for the next step. This aldehyde (1.53 g, 9.27 mmol) was reduced according to a literature procedure^[17] and purified by flash chromatography (hexane/EtOAc, 3:1) to give alcohol (1*S*,2*S*)-**12** (1.45 g, 94%) as a colourless oil. $R_f = 0.23$ (hexane/EtOAc, 3:1); $[\alpha]_D^{20} = +46.8$ ($c = 0.9$, CH₂Cl₂); Ref.[24]: $\alpha_D^{28} = +28.6$ (neat) for alcohol dideuterated at C-1; diastereomeric ($\geq 98\%$) and enantiomeric excess ($\geq 99\%$) were determined by ¹H NMR spectroscopy (400 MHz, CDCl₃) of the (*R*)-Mosher ester, diagnostic resonance at 4.20 ppm (d, $J = 5.8$ Hz, CHDO); The (*R*)-Mosher ester of alcohol (1*R*,2*S*)-**16** was identical to that of (1*S*,2*S*)-**12** except for a resonance at 4.32 ppm (d, $J = 3.8$ Hz, CHDO) in the (1*R*,2*S*)-**16** spectrum; IR (NaCl): $\tilde{\nu} = 3418, 2972, 2872, 2164, 1454, 1374, 1110, 1069, 1028$ cm⁻¹; ¹H NMR (400 MHz, CDCl₃): $\delta = 1.16$ (d, $J = 6.1$ Hz, 3H, Me), 2.08 (d, $J = 4.3$ Hz, 1H, OH), 3.47 (m, 1H, CHDO), 3.66 (quint, $J = 6.4$ Hz, 1H, CHO), 4.55 (AB system, $J = 11.6$ Hz, 2H, OCH₂Ph), 7.30 (m, 5H, H_{arom}) ppm; ¹³C NMR (100.6 MHz, CDCl₃): $\delta = 15.81$ (Me), 66.00 (t, $J(C,D) = 21.8$ Hz, CHDO), 70.77 (OCH₂), 75.46 (CHO), 127.69 (3 × CH_{arom}), 128.44 (2 × CH_{arom}), 138.43 (C_{arom}) ppm.

(1*R*,2*S*)-(+)-2-Benzoyloxy-1-bromo-[1-*D*]₂propane [(1*R*,2*S*)-13**]:** Alcohol (1*S*,2*S*)-**12** (686 mg, 4.1 mmol) was transformed into bromide (1*R*,2*S*)-**13** by a literature procedure.^[18] The crude product was purified by flash chromatography (hexane/CH₂Cl₂, 1:1) to give bromide (1*R*,2*S*)-**13** (865 mg, 92%) as a colourless liquid. $R_f = 0.53$ (hexane/CH₂Cl₂, 1:1); $[\alpha]_D^{20} = +8.6$ ($c = 2.1$, CH₂Cl₂); Ref.[18]: $[\alpha]_D^{20} =$

+ 8.4 ($c=3.0$, CH_2Cl_2) for the compound dideuterated at C-1; IR (NaCl): $\tilde{\nu}=2977, 2868, 1454, 1376, 1137, 1115, 1088\text{ cm}^{-1}$; $\text{de} > 98\%$ ($^1\text{H NMR}$); $^1\text{H NMR}$ (400 MHz, CDCl_3): $\delta=1.31$ (d, $J=6.1$ Hz, 3H, Me), 3.36 (brd, $J=5.9$ Hz, 1H, CHDBr), 3.73 (quint, $J=5.9$ Hz, 1H, CHMe), 4.57 (AB system, $J=12.0$ Hz, 2H, OCH_2), 7.30 (m, 5H, H_{arom}) ppm; $^{13}\text{C NMR}$ (100.6 MHz, CDCl_3): $\delta=18.86$ (Me), 36.15 (t, $J(\text{C,D})=22.9$ Hz, CHDBr), 71.10 (OCH_2), 74.14 (CHO), 127.68 ($2 \times \text{CH}_{\text{arom}}$), 127.71 (CH_{arom}), 128.41 ($2 \times \text{CH}_{\text{arom}}$), 138.13 (C_{arom}) ppm.

(1S,2S)-(+)-Diethyl (2-benzyloxy-[1-D₁]propyl)phosphonate [(1S,2S)-14]: Bromide (1R,2S)-13 (827 mg, 3.59 mmol) was transformed (reaction time: 3 h) into phosphonate (1S,2S)-14 by a literature procedure that used a round bottomed flask silylated on the inner surface.^[6] Excess phosphite was removed in vacuo and the residue was purified by flash chromatography ($\text{CH}_2\text{Cl}_2/\text{EtOAc}$, 10:1) to give phosphonate (1S,2S)-14 (838 mg, 81%) as an oil. $R_f=0.29$ ($\text{CH}_2\text{Cl}_2/\text{EtOAc}$, 10:1); $[\alpha]_{\text{D}}^{20}=+11.1$ ($c=1.1$, CH_2Cl_2); Ref.[18]: $[\alpha]_{\text{D}}^{20}=+12.32$ ($c=2.8$, CH_2Cl_2) for the compound dideuterated at C-1; $\text{de}=88\%$ (by $^1\text{H NMR}$); IR (NaCl): $\tilde{\nu}=2980, 1250, 1098, 1028, 966\text{ cm}^{-1}$; $^1\text{H NMR}$ (400 MHz, CDCl_3): $\delta=1.25$ and 1.28 (2t, $J=7.1$ Hz, 2×3 H, CH_2CH_3), 1.33 (d, $J=6.1$ Hz, 3H, $\text{H}_3\text{C}(3)$), 1.89 (brdd, $J=7.1, 17.9$ Hz, CHDP of (1R,2S)-18), 2.18 (brdd, $J=5.8, 19.2$ Hz, 1H, CHDP), 3.87 (m, 1H, CHO), 4.05 (m, 4H, $2 \times \text{CH}_2\text{OP}$), 4.51 (AB system, $J=11.6$ Hz, 2H, OCH_2Ph), 7.29 (m, 5H, H_{arom}) ppm; $^{13}\text{C NMR}$ (100.6 MHz, CDCl_3): $\delta=16.33$ and 16.35 2d, $J=6.1$ Hz, $2 \times \text{CH}_2\text{CH}_3$), 21.05 (d, $J=9.2$ Hz, $\text{CH}_3(3)$), 33.38 (dt, $J(\text{C,D})=19.1$ Hz, $J(\text{P,C})=137.7$ Hz, CHDP), 61.33 (d, $J=6.1$ Hz, CH_2OP), 61.56 (d, $J=6.9$ Hz, CH_2OP), 70.52 (CHO), 70.61 (OCH_2), 127.52 (CH_{arom}), 127.72 ($2 \times \text{CH}_{\text{arom}}$), 128.28 ($2 \times \text{CH}_{\text{arom}}$), 138.35 (C_{arom}) ppm.

Ammonium salt of (1S,2S)-2-hydroxy-[1-D₁]propylphosphonic acid [(1S,2S)-8·(NH₃)_x]: Phosphonate (1S,2S)-14 (810 mg, 2.82 mmol) was deblocked according to a literature procedure.^[18] The acid was dissolved in water (10 mL) and concentrated ammonia (1 mL) was added. The solution was concentrated in vacuo. The residue was dissolved in water (30 mL) and filtered. The solution was concentrated again in vacuo. Drying (0.5 mm, ambient temperature) furnished the salt (1S,2S)-8·(NH₃)_x (375 mg) as a crystalline solid of unknown structure. The salt was determined to be pure by NMR spectroscopy. The yield was assumed to be 90%. $^1\text{H NMR}$ (400 MHz, D_2O): $\delta=1.16$ (d, $J=6.1$ Hz, 3H, Me), 1.60 (dd, $J=6.9, 16.3$ Hz, 1H, CHDP), 4.01 (sext, $J=6.0$ Hz, CHO) ppm; $^{13}\text{C NMR}$ (100.6 MHz, D_2O): $\delta=23.51$ (d, $J=10.7$ Hz, Me), 37.92 (dt, $J(\text{C,D})=19.1$ Hz, $J=127.5$ Hz, CHDP), 65.24 (CHO) ppm.

(1R,2S)-(+)-2-Benzyloxy-[1-D₁]propyl benzoate [(1R,2S)-15]: Alcohol (1S,2S)-12 (821 mg, 4.91 mmol) was esterified by a literature procedure^[17] to give benzoate (1R,2S)-15 (1.24 g, 93%) as a colourless oil. $R_f=0.50$ (CH_2Cl_2); $[\alpha]_{\text{D}}^{20}=+15.3$ ($c=4.6$, CH_2Cl_2); IR (NaCl): $\tilde{\nu}=2976, 2869, 1721, 1452, 1315, 1272, 1115, 1071, 1027\text{ cm}^{-1}$; $^1\text{H NMR}$ (400 MHz, CDCl_3): $\delta=1.23$ (d, $J=6.3$ Hz, 3H, Me), 3.83 (dq, $J=4.3, 6.3$ Hz, 1H, CHMe), 4.27 (d, $J=4.0$ Hz, 1H, CHD), 4.57 (AB system, $J=12.0$ Hz, 2H, OCH_2), 7.24 (m, 5H, H_{arom}), 7.37 (m, 2H, H_{arom}), 7.49 (m, 1H, H_{arom}), 7.98 (m, 2H, H_{arom}) ppm; $^{13}\text{C NMR}$ (100.6 MHz, CDCl_3): $\delta=17.05$ (Me), 67.49 (t, $J(\text{C,D})=22.6$ Hz, CHD), 71.09 (OCH_2), 72.61 (CHO), 127.57 (CH_{arom}), 127.62 ($2 \times \text{CH}_{\text{arom}}$), 128.34 ($2 \times \text{CH}_{\text{arom}}$), 128.35 ($2 \times \text{CH}_{\text{arom}}$), 129.64 ($2 \times \text{CH}_{\text{arom}}$), 130.15 (C_{arom}), 132.95 (CH_{arom}), 138.47 (C_{arom}), 166.43 (CO) ppm; elemental analysis (%): calcd for $\text{C}_{17}\text{H}_{17}\text{DO}_3$ (271.34): C 75.25, H + D 7.05; found: C 75.25, H + D 6.69.

(1R,2S)-(+)-2-Benzyloxy-[1-D₁]propanol [(1R,2S)-16]: A solution of benzoate (1R,2S)-15 (1.143 g, 4.21 mmol) in MeOH/MeONa obtained by dissolving Na (26.4 mg, 1.15 mmol) in dry methanol (5 mL) was stirred for 16 h and then concentrated in vacuo.^[25] The residue was purified by flash chromatography (CH_2Cl_2 to elute methyl benzoate; hexane/ethyl acetate, 1:1, to elute alcohol) to elute alcohol (1R,2S)-16

(643 mg, 91%) as a colourless liquid. $R_f=0.58$ (hexane/ethyl acetate, 1:1); diastereomeric ($\geq 98\%$) and enantiomeric excess ($\geq 99\%$) were determined by $^1\text{H NMR}$ spectroscopy (400 MHz, CDCl_3) of the (R)-Mosher ester, diagnostic resonance at 4.32 (d, $J=3.8$ Hz, CHDO); $[\alpha]_{\text{D}}^{20}=+46.7$ ($c=0.9$, CH_2Cl_2); Ref.[24]: $\alpha_{\text{D}}^{25}=+28.6$ (neat), $[\alpha]_{\text{D}}^{20}=+44.8$ ($c=0.945$, CHCl_3); see (1S,2S)-12 for alcohol dideuterated at C-1; $^1\text{H NMR}$ (400 MHz, CDCl_3): $\delta=1.17$ (d, $J=6.3$ Hz, 3H, Me), 2.02 (d, $J=7.8$ Hz, 1H, OH), 3.58 (m, 1H, CHDO; no resonance for CHDO of (1S,2S)-12 at 3.47), 3.66 (dq, $J=3.4, 6.3$ Hz, 1H, CHO), 4.56 (AB system, $J=11.6$ Hz, 2H, OCH_2Ph), 7.31 (m, 5H, H_{arom}) ppm.

(1S,2S)-(+)-2-Benzyloxy-1-bromo-[1-D₁]propane [(1S,2S)-17]: Alcohol (1R,2S)-16 (585 mg, 3.5 mmol) was transformed into bromide (1S,2S)-17 (771 mg, 96%) by using the procedure for the preparation of [(1R,2S)-13]. $R_f=0.60$ (hexane/ CH_2Cl_2 , 1:1), $[\alpha]_{\text{D}}^{20}=+8.3$ ($c=0.9$, CH_2Cl_2); Ref.[18]: $[\alpha]_{\text{D}}^{20}=+8.4$ ($c=3.0$, CH_2Cl_2) for compound dideuterated at C-1; $\text{de} > 96\%$ ($^1\text{H NMR}$); IR (NaCl): $\tilde{\nu}=3030, 2868, 1454, 1376, 1325, 1144, 1093, 1028\text{ cm}^{-1}$; $^1\text{H NMR}$ (400 MHz, CDCl_3): $\delta=1.31$ (d, $J=6.3$ Hz, 3H, Me), 3.43 (dt, $J(\text{H,D})=1.4$ Hz, $J=4.9$ Hz, 1H, CHDBr), 3.36 (brd, $J=5.9$ Hz, CHDBr of (1R,2S)-13), 3.73 (quint, $J=5.9$ Hz, 1H, CHMe), 4.58 (AB system, $J=12.1$ Hz, 2H, OCH_2), 7.25–7.38 (m, 5H, H_{arom}) ppm; $^{13}\text{C NMR}$ (100.6 MHz, CDCl_3): $\delta=18.85$ (Me), 36.17 (t, $J(\text{C,D})=22.9$ Hz, CHDBr), 71.09 (OCH_2), 74.12 (CHO), 127.68 ($2 \times \text{CH}_{\text{arom}}$), 127.71 (CH_{arom}), 128.41 ($2 \times \text{CH}_{\text{arom}}$), 138.13 (C_{arom}) ppm.

(1R,2S)-(+)-Diethyl (2-benzyloxy-[1-D₁]propyl)phosphonate [(1R,2S)-18]: Bromide (1S,2S)-17 (755 mg, 3.28 mmol) was transformed into phosphonate (1R,2S)-18 (665 mg, 71%) by using the procedure for the preparation of (1S,2S)-14. The round-bottomed flask (10 mL) used for the Arbusov reaction was cleaned (fuming nitric acid, 70 °C, 30 min) and rinsed with water and acetone. A solution of dry pyridine (2 mL) and triisopropylsilylchloride (1 mL) was refluxed in this flask for 1 h. Triethylsilylchloride (1 mL) was added and refluxing was continued for 1 h. The flask was again rinsed with water and acetone and subsequently dried. $R_f=0.15$ (hexane/EtOAc, 1:2); $[\alpha]_{\text{D}}^{20}=+11.9$ ($c=1.1$, CH_2Cl_2); Ref.[18]: $[\alpha]_{\text{D}}^{20}=+12.32$ ($c=2.8$, CH_2Cl_2) for compound dideuterated at C-1; $\text{de}=95\%$ (by $^1\text{H NMR}$); IR (NaCl): $\tilde{\nu}=2980, 1249, 1028, 995\text{ cm}^{-1}$; $^1\text{H NMR}$ (400 MHz, CDCl_3): $\delta=1.24$ and 1.27 (2t, $J=7.0$ Hz, 2×3 H, CH_2CH_3), 1.32 (dd, $J=0.8, 6.1$ Hz, 3H, $\text{H}_3\text{C}(3)$), 1.88 (brdd, $J=7.1, 18.2$ Hz, 1H, CHDP), 2.18 (brdd, $J=5.8, 19.2$ Hz, CHDP of (1S,2S)-14), 3.91 (m, 1H, CHO), 4.04 (m, 4H, $2 \times \text{CH}_2\text{OP}$), 4.50 (AB system, $J=11.4$ Hz, 2H, OCH_2Ph), 7.24 (m, 1H, H_{arom}), 7.26–7.35 (m, 4H, H_{arom}) ppm; $^{13}\text{C NMR}$ (100.6 MHz, CDCl_3): $\delta=16.33$ and 16.34 (two d, $J=6.1$ Hz, $2 \times \text{CH}_3$), 21.05 (d, $J=8.4$ Hz, $\text{H}_3\text{C}(3)$), 33.37 (dt, $J(\text{C,D})=19.3$ Hz, $J(\text{P,C})=138.2$ Hz, CHDP), 61.33 (d, $J=6.9$ Hz, CH_2OP), 61.56 (d, $J=6.9$ Hz, CH_2OP), 70.52 (CHO), 70.60 (OCH_2), 127.52 (CH_{arom}), 127.72 ($2 \times \text{CH}_{\text{arom}}$), 128.28 ($2 \times \text{CH}_{\text{arom}}$), 138.34 (C_{arom}) ppm.

(1R,2S)-2-Hydroxy-[1-D₁]propylphosphonic acid [(1R,2S)-10·(NH₃)_x]: Phosphonate (1R,2S)-18 (659 mg, 2.29 mmol) was deblocked and transformed into ammonium salt (1R,2S)-10·(NH₃)_x (380 mg) by the procedure used for the preparation of (1S,2S)-8·(NH₃)_x. $^1\text{H NMR}$ (400 MHz, D_2O): $\delta=1.18$ (d, $J=6.1$ Hz, 3H, Me), 1.66 (dd, $J=6.1, 17.2$ Hz, 1H, CHDP), 4.02 (sext, $J=6.0$ Hz, OCH) ppm; $^{13}\text{C NMR}$ (100.6 MHz, D_2O): $\delta=23.48$ (d, $J=9.9$ Hz, Me), 37.92 (dt, $J(\text{C,D})=18.9, 128.0$ Hz, CHDP), 64.98 (CHO) ppm.

(1R,2R)-(+)-Dimethyl (1-benzyloxy-2-benzoylamino)propylphosphonate [(1R,2R)-23]: A mixture of aminophosphonic acid (1R,2R)-19 (50 mg, 0.32 mmol), TBDMSCl (0.54 g, 3.58 mmol), dry pyridine (0.3 mL) and dry acetonitrile (12 mL) was vigorously stirred and refluxed for 5 h.^[26] The mixture was allowed to cooled to room temperature and benzoyl chloride (0.4 mL, 3.45 mmol) was added and stirring was continued for 16 h. Volatile components were removed in vacuo (0.5 mm, warming to 50 °C). HCl (0.6 M, 25 mL) was

added to the residue. The mixture was refluxed for 10 min, cooled and extracted with diethyl ether (2 × 20 mL). The aqueous phase was concentrated on a rotary evaporator. The residue was dissolved in water (10 mL) and concentrated again. The residue was dissolved in water and applied (about 20 mL) to a column of Dowex 50 (H⁺) and eluted with water until neutral. The eluate was concentrated in vacuo. The residue was dissolved in dry methanol and esterified with a distilled solution of CH₂N₂ in diethyl ether. The solvents were evaporated and the residue was flash chromatographed to yield amide (1*R*,2*R*)-**23** (29 mg, 23%) as a crystalline solid. M.p.: 104–106 °C (toluene/hexane), *R*_f = 0.36 (EtOAc); [α]_D²⁰ = +26.5 (*c* = 1.0, CH₂Cl₂); IR (nujol): $\tilde{\nu}$ = 3271, 1727, 1659, 1316, 1241, 1038 cm⁻¹; ¹H NMR (250 MHz, CDCl₃): δ = 1.48 (d, *J* = 7.3 Hz, 3 H, CH₃), 3.83 (d, *J* = 11.0 Hz, 3 H, OCH₃), 3.87 (d, *J* = 11.0 Hz, 3 H, OCH₃), 4.82 (m, 1 H, CHN), 4.82 (d, *J* = 4.8, 9.4 Hz, 1 H, CHO), 7.14 (d, *J* = 9.5 Hz, NH), 7.48 (m, 5 H, H_{arom}), 7.61 (m, 1 H, H_{arom}), 7.84 (m, 2 H, H_{arom}), 8.10 (m, 2 H, H_{arom}) ppm; elemental analysis (%): calcd for C₁₄H₂₂NO₆P (391.36): C 58.31, H 5.67, N 3.58; found: C 58.49, H 5.64, N 3.49.

General procedure for derivatisation of (1*S*,2*R*)-21**, a mixture of (1*R*,2*R*)-**19** and (1*S*,2*R*)/(1*R*,2*S*)-**21**^[26] and samples of biological origin:**

Biological samples were used directly or after removal of the major portion of (1*R*,2*R*)-**19** by crystallisation from water (the solvent was evaporated slowly). A mixture of the sample (up to 14 mg), dry pyridine (3 mL) and TMSCl (0.05 mL, 0.39 mmol) was stirred vigorously and refluxed for 1.5 h (bath temperature 80 °C).^[27] Benzoyl chloride (0.25 mL, 2.15 mmol) was added and stirring was continued for 3 h at 50 °C. Volatile components were removed in vacuo (rotary evaporator, then 0.5 mm/warming to 50 °C). HCl (0.6 mL, 10 mL) was added to the residue. The mixture was heated for 10 min at 50 °C, cooled and extracted with diethyl ether (2 × 20 mL). Further purification and esterification with diazomethane was done as described for the preparation of amide (1*R*,2*R*)-**23**. The residue was purified by preparative TLC (hexane/ethyl acetate, 1:2). Yields were up to 36% for pure, and mixtures of pure, aminophosphonic acids and about 15% for samples of biological origin; Amides (1*R*,2*R*)-**23** and (1*S*,2*R*)-**24** have the same polarity (*R*_f value). ¹H NMR (400 MHz, DMSO-*D*₆) of (1*R*,2*R*)-**23**: δ = 1.39 (d, *J* = 7.1 Hz, 3 H, CH₃), 3.751 (d, *J* = 10.9 Hz, 3 H, OCH₃), 3.762 (d, *J* = 10.6 Hz, 3 H, OCH₃), 4.75 (m, 1 H, CHN), 5.52 (t, *J* = 8.5 Hz, 1 H, CHO), 7.41 (m, 2 H, H_{arom}), 7.49 (m, 1 H, H_{arom}), 7.54 (m, 2 H, H_{arom}), 7.69 (m, 3 H, H_{arom}), 7.99 (m, 2 H, H_{arom}), 8.46 (d, *J* = 8.1 Hz, NH) ppm; ¹H NMR (400 MHz, DMSO-*D*₆) of (1*S*,2*R*)-**24**: δ = 1.43 (d, *J* = 7.1 Hz, 3 H, CH₃), 3.748 (d, *J* = 10.6 Hz, 3 H, OCH₃), 3.781 (d, *J* = 10.6 Hz, 3 H, OCH₃), 4.60 (m, 1 H, CHN), 5.85 (dd, *J* = 4.0, 10.4 Hz, 1 H, CHO), 7.47 (m, 2 H, H_{arom}), 7.54 (m, 1 H, H_{arom}), 7.62 (m, 2 H, H_{arom}), 7.76 (m, 1 H, H_{arom}), 7.81 (m, 2 H, H_{arom}), 8.06 (m, 2 H, H_{arom}), 8.57 (d, *J* = 7.6 Hz, NH) ppm. The resonances at 7.99 and 8.06 were normally used for the calibration of the integration to determine the extents of deuteration at C-1 and the ratio of (1*R*,2*R*)-**23** to (1*S*,2*R*)-**24**, respectively.

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- [1] Review: H. Seto, T. Kuzuyama, *Nat. Prod. Rep.* **1999**, *16*, 589–596.
- [2] Review: F. M. Kahan, J. S. Kahan, P. J. Cassidy, H. Kropp, *Ann. N. Y. Acad. Sci.* **1974**, *235*, 364–386.
- [3] E. Bayer, K. H. Gugel, K. Hägele, H. Hagenmaier, S. Jessipow, W. A. König, H. Zähler, *Helv. Chim. Acta* **1972**, *55*, 224–239.
- [4] T. Hidaka, H. Iwakura, S. Imai, H. Seto, *J. Antibiot.* **1992**, *45*, 1008–1010; M. S. McQueney, S.-I. Lee, W. H. Swartz, H. L. Ammon, P. S. Mariano, D. Dunaway-Mariano, *J. Org. Chem.* **1991**, *56*, 7121–7130.
- [5] H. Nakashita, K. Watanabe, O. Hara, T. Hidaka, H. Seto, *J. Antibiot.* **1997**, *50*, 212–219.
- [6] F. Hammerschmidt, H. Kählig, *J. Org. Chem.* **1991**, *56*, 2364–2370.
- [7] T. Kuzuyama, T. Hidaka, K. Kamigiri, S. Imai, H. Seto, *J. Antibiot.* **1992**, *45*, 1812–1814.
- [8] F. Hammerschmidt, *Angew. Chem.* **1994**, *106*, 334–335; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 341–342.
- [9] F. Hammerschmidt, *J. Chem. Soc. Perkin Trans. 1* **1991**, 1993–1996.
- [10] F. Hammerschmidt, G. Bovermann, K. Bayer, *Liebigs Ann. Chem.* **1990**, 1055–1061.
- [11] P. Liu, K. Murakami, T. Seki, X. He, S.-M. Yeung, T. Kuzuyama, H. Seto, H.-w. Liu, *J. Am. Chem. Soc.* **2001**, *123*, 4619–4620.
- [12] T. Hashimoto, J. Kohno, Y. Yamada, *Plant. Physiol.* **1987**, *84*, 144–147.
- [13] A. Archelas, R. Furstoss, *Annu. Rev. Microbiol.* **1997**, *51*, 491–525.
- [14] B. Peric Simov, F. Wuggenig, M. Lämmerhofer, W. Lindner, E. Zarbl, F. Hammerschmidt, *Eur. J. Org. Chem.* **2002**, 1139–1142.
- [15] J. B. Jones, I. J. Jakovac, *Can. J. Chem.* **1982**, *60*, 19–28.
- [16] B. Zagalak, P. A. Frey, G. L. Karabatsos, R. H. Abeles, *J. Biol. Chem.* **1966**, *241*, 3028–3035; G. J. Karabatsos, J. S. Fleming, N. Hsi, R. H. Abeles, *J. Am. Chem. Soc.* **1966**, *88*, 849–850.
- [17] F. Hammerschmidt, *Liebigs Ann. Chem.* **1988**, 955–960, and references cited therein.
- [18] F. Hammerschmidt, *Monatsh. Chem.* **1991**, *122*, 389–398.
- [19] For a review on heme-containing oxygenases, see: M. Sono, M. Roach, E. D. Coulter, J. H. Dawson, *Chem. Rev.* **1996**, *96*, 2841–2887.
- [20] For a review on dioxygen activation by enzymes with mononuclear nonheme iron active sites, see: L. Que, Jr., R. Y. N. Ho, *Chem. Rev.* **1996**, *96*, 2607–2624.
- [21] M. H. Gelb, D. C. Heimbrook, P. Malkonen, S. G. Sligar, *Biochemistry* **1982**, *21*, 370–377.
- [22] J. E. Baldwin, M. Bradley, *Chem. Rev.* **1990**, *90*, 1079–1088; P. L. Roach, I. J. Clifton, C. M. H. Hensgens, N. Shibata, C. J. Schofield, J. Hajdu, J. E. Baldwin, *Nature* **1997**, *387*, 827–830.
- [23] F. Hammerschmidt, *Liebigs Ann. Chem.* **1991**, 469–475.
- [24] K. Mislow, R. E. O'Brien, H. Schaefer, *J. Am. Chem. Soc.* **1962**, *84*, 1940–1944.
- [25] F. Hammerschmidt, *Liebigs Ann. Chem.* **1988**, 961–964.
- [26] F. Hammerschmidt et al., unpublished results.
- [27] F. Hammerschmidt, *Liebigs Ann. Chem.* **1988**, 531–535.

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