Biosynthesis of Natural Products with a P–C Bond. 7. Synthesis of [1,1-$^2H_2$]-, [2,2-$^2H_2$]-, (*R*)- and (*S*)-[1-$^2H_1$](2-Hydroxyethyl)phosphonic Acid and (*R*,*S*)-[1-$^2H_1$](1,2-Dihydroxyethyl)phosphonic Acid and Incorporation Studies into Fosfomycin in *Streptomyces fradiae*[1]

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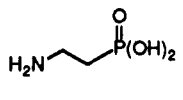
Nondeuteriated and deuteriated 2-(benzyloxy)ethanols (**8a–c**) were transformed into (2-hydroxyethyl)phosphonic acids (**12a–c**). **8c** was prepared from dimethyl 2,3-*O*-isopropylidene-L-tartrate. **12b,c** were fed to *Streptomyces fradiae* and were incorporated into fosfomycin (**2**), which was converted to amino phosphonic acid (–)-**13**. (–)-**13** derived from **12b,c** contained 42% and 34% deuterium, respectively. (1,2-Dihydroxyethyl)phosphonic acid ((±)-**29**) was not incorporated into fosfomycin. (*S*)- and (*R*)-(2-hydroxy[1-$^2H_1$]ethyl)phosphonic acids ((*S*)- and (*R*)-**34**) were prepared from (*S*)-2-(benzyloxy)[1-$^2H_1$]ethanol ((*S*)-**30**) and fed to *S. fradiae*. The deuterium of (*R*)-**34** was lost, and the deuterium of (*S*)-**34** was retained on incorporation into fosfomycin. The optical purity of (*S*)- and (*R*)-**34** (84% and 86%, respectively) was determined by transformation to dimethyl ester and esterification with (+)-MTPA-Cl to afford **39b,c**. **41** obtained from (*S*)-**32** was identical with an authentic sample prepared from (*S*)-(2-amino[1-$^2H_1$]ethyl)phosphonic acid.

Introduction

The number of known natural products containing a P–C bond has been steadily growing since the report of isolation of (2-aminoethyl)phosphonic acid (AEP) (**1**) from protozoa in 1959.[2a] The most important members of this



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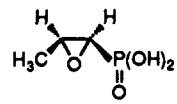

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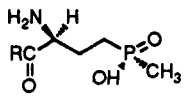
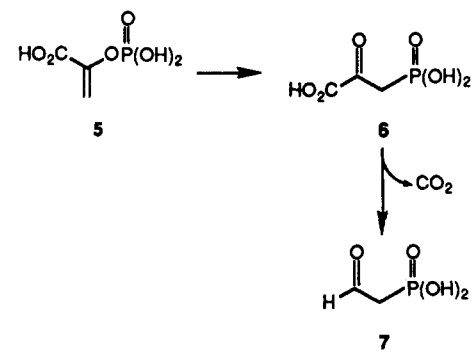


3 R = OH
4 R = L-ala-L-ala

small class[2b] of compounds are fosfomycin (**2**) and phosphinothricin (**3**). Fosfomycin is an antibiotic produced[3] as a secondary metabolite by various strains of *Streptomyces* and is used clinically.[4] It inhibits cell wall biosynthesis of bacteria by alkylating an SH group in the active site of phosphoenolpyruvate:UDP-GlcNAc-enolpyruvyl transferase.[4b] Phosphinothricin is produced by *Streptomyces viridochromogenes*[5a] and *hygroscopicus*[5b] as alanylalanine derivative **4** called bialaphos. It is an inhibitor of glutamine synthetase[5a] essential for nitrogen metabolism in plants and bacteria, which is the basis for the application of **3** and **4** as a herbicide.[5c]

The biosynthesis of compounds with a P–C bond diverges from primary metabolism with the intramolecular rearrangement[6] of phosphoenol pyruvate (**5**) to phosphonopyruvic acid (**6**), which is decarboxylated to phosphonoacetaldehyde (**7**). The enzyme catalyzing the rearrangement has been isolated recently,[7] but it could not be detected in a cell-free extract of *Streptomyces wedmorensis* producing fosfomycin.[7b] A reaction sequence for the biosynthesis of **4** has been proposed on the basis of compounds isolated from the broths of blocked mutants of *S. hygroscopicus*.[8]



5 **6** **7**

The building blocks for fosfomycin are a P–C_2 unit, possibly phosphonoacetaldehyde, and methyl from methionine on the basis of experiments with ^{14}C-labeled compounds.[9] A mutant strain of *S. wedmorensis* incorporated AEP and (2-hydroxyethyl)phosphonic acid via the putative intermediate **7** into fosfomycin.[10] The oxirane oxygen atom was not derived from di[^{18}O]oxygen, thus excluding (*Z*)-1-propenylphosphonic acid as precursor.[11]

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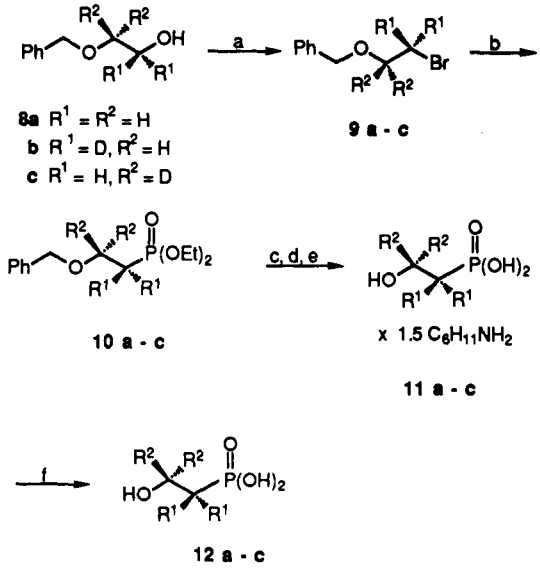
Scheme I[a]



[a] Key: (a) Ph_3P/NBS, CH_2Cl_2, −78 → +35 °C; (b) $(EtO)_3P$, reflux; (c) Me_3SiBr/(allyl)$SiMe_3$ in CCl_4, 50 °C, EtOH; (d) H_2, Pd–C, EtOH; (e) cyclohexylamine; (f) Dowex 50 (H^+).

AEP was only taken up by *Streptomyces fradiae* after attaching L-Ala or L-Ala-L-Ala to the amino group to produce peptide mimetics transported by peptide permeases.[1]

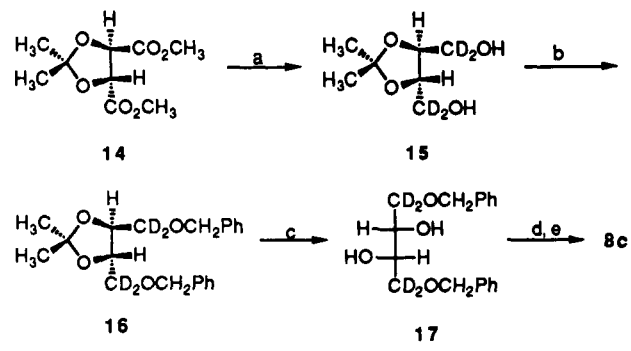
L-Methionine is transformed to *S*-adenosyl-L-methionine (SAM) acting as a donor of "CH_3^+" for alkylation of nucleophilic centres (C, N, O, S). A direct reaction with the electrophilic carbon of the carbonyl function of phosphonoacetaldehyde (**7**) is precluded unless its polarity at carbon or the polarity of methyl is reversed by umpolung.

To get deeper insight into the construction of the oxirane ring of **2** and to determine the stereochemistry at C-1, whether the pro-*R* or pro-*S* hydrogen is replaced by oxygen, various deuteriated (2-hydroxyethyl)phosphonic acids were synthesized and fed to *S. fradiae*. (2-Hydroxyethyl)phosphonic acid is also a compound excreted into the culture broth by a mutant strain of *S. hygroscopicus*, producing the tripeptide bialaphos.[12]

Results and Discussion

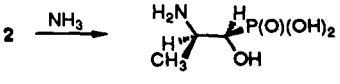
Preparation and Incorporation of Doubly Labeled (2-Hydroxyethyl)phosphonic Acids. It was intended to develop a simple entry into labeled (2-hydroxyethyl)-phosphonic acids. At first, the synthesis was tested for unlabeled material, a known compound prepared by addition of dimethyl phosphite to vinyl acetate followed by hydrolysis.[13] 2-(Benzyloxy)ethanol (**8a**) obtained by reduction[14] of methyl *O*-benzylglycolate with $LiAlH_4$ was treated with triphenylphosphine/NBS[15] under mild conditions in dichloromethane from −78 to +35 °C to yield bromide **9a** (86% yield) (Scheme I). Phosphorus tribromide in pyridine used to convert various 2-alkoxyethanols, but not 2-(benzyloxy)ethanol, to the corresponding bromides seemed to be less suited.[16] Bromide **9a** was refluxed with distilled triethyl phosphite (Arbuzov reaction) to afford phosphonate **10a** in 76% yield. Removal of ethyl ester groups was effected by dealkylation with bromotrimethylsilane[17] in carbon tetrachloride at 50 °C in the presence of allyltrimethylsilane as scavenger for traces of hydrogen bromide. Volatile material was removed in vacuo, and the silyl ester formed was hydrolyzed in ethanol. The benzyl group was hydrogenolyzed over palladium (10%)–carbon, and the free (hydroxyethyl)-phosphonic acid was converted to crystalline cyclohexylammonium salt **11a** in 61% yield with a molar ratio of amine to acid of 1.5:1 as determined by ^1H NMR spectroscopy and elemental analysis. Free acid **12** can be prepared by passing an aqueous solution of salt **11** through Dowex 50 (H^+).

Scheme II[a]



[a] Key: (a) $LiAlD_4$ (99% D), THF; (b) NaH, DMF/THF, $PhCH_2Br$; (c) EtOH/H_2O/HCl, 50 °C; (d) $Pb(OAc)_4$, C_6H_6, 0 °C; (e) $NaBH_4$, EtOH.

The first deuteriated (2-hydroxyethyl)phosphonic acid synthesized was **12b**. One of its deuterium atoms should be retained during transformation to fosfomycin, if it is taken up and dehydrogenated to **7** by an enzyme, because one deuterium of D-[6,6-$^2H_2$]glucose is incorporated into fosfomycin at C-1.[11] The requisite 2-(benzyloxy)[1,1-$^2H_2$]ethanol (**8b**) was obtained by reduction[14] of methyl *O*-benzylglycolate with $LiAlD_4$ (99% D) and transformation to **11b** as before. Scrambling of deuterium between C-1 and C-2 via neighboring group participation of the benzyloxy substituent to displace bromide with formation of a symmetrical cyclic oxonium species, which is attacked by phosphite at C-1 or C-2, occurs to the extent no more than 1% (^1H NMR of **10b**), if at all. Free acid **12b** was added to the growth medium before sterilization at a concentration of 400 μg/mL. Six 1-L Erlenmeyer flasks with 220 mL of cornstarch medium were inoculated with 7 mL of a 24-h culture of *S. fradiae* in the same medium, and shake cultured at 28 °C for 64 h.[11] The cells were removed by centrifugation. The titer of fosfomycin in the supernatant was determined by microbiological assay to be 8–10 μg/mL in each flask. The cells were discarded, and the supernatant was saturated with gaseous ammonia and kept for 3 days at 60 °C to open the oxirane ring to give as major component (1*R*,2*R*)-(2-amino-1-hydroxypropyl)phosphonic acid ((−)-**13**) isolated by ion-exchange chromatography.[11] A 3.5-mg portion of crystalline (−)-**13**



was obtained on feeding **12b**. The ^1H NMR spectrum (400 MHz) of (−)-**13** showed indirectly the presence of 42%

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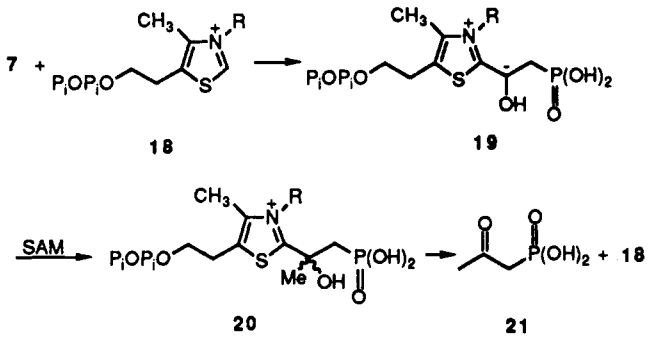
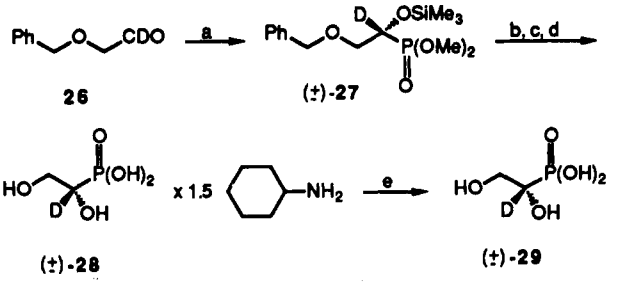
deuterium at C-1 because of lower signal area for H-1 compared to H-2. The $^{13}C\{^1H\}$ NMR spectrum (100.6 MHz) shows three doublets (73.13, 53.64, and 20.07 ppm) for the three carbon atoms. C-2 and C-3 had satellite doublets shifted to higher field, which are diagnostic for molecules bearing deuterium at C-1. The ^2H-induced β-shift (for C-2) is 0.06 ppm, and the γ-shift (for C-3) is 0.034 ppm in agreement with reported values.[18] This experiment demonstrates that **12b** is passing into the cells of *S. fradiae* and that it is efficiently incorporated into fosfomycin, probably via phosphonoacetaldehyde enzymatically formed by dehydrogenation. The preparation of (2-hydroxy[2,2-$^2H_2$]ethyl)phosphonic acid (**12c**) involved more steps than the preparation of **12b**. The synthesis started with L-tartaric acid as a four-carbon unit that was cleaved at the end into *O*-benzyl[2,2-$^2H_2$]glycolaldehyde (Scheme II). Dimethyl 2,3-*O*-isopropylidene-L-tartrate (**14**) was reduced[19] with $LiAlD_4$ (99% D) to tetradeuteriated, partially protected tetrol **15** obtained in 85% yield after bulb to bulb distillation Benzylation[20] with sodium hydride/benzyl bromide produced protected L-threitol **16** in 90% yield. Removal of the isopropylidene group was effected at 50 °C in a mixture of ethanol and water, being 0.5 N in HCl. Crystalline **17** was cleaved by lead tetraacetate in benzene to aldehyde, which was immediately reduced by $NaBH_4$ in ethanol to 2-(benzyloxy)[2,2-$^2H_2$]ethanol (**8c**). The transformation **8c** to **11c** was exactly as for **8a** to **11a**. Free phosphonic acid (**12c**) was fed (500 μg/mL) to *S. fradiae*. The amino phosphonic acid (−)-**13** contained 34% deuterium at C-2 (^1H NMR, 400 MHz). The $^{13}C\{^1H\}$ NMR spectrum (100.6 MHz) showed ^2H-induced β-shifted satellite doublets for C-1 and C-3 (0.07 and 0.113 ppm, respectively). This experiment proves that one deuterium at C-2 of **12c** is retained on transformation to fosfomycin. A possible involvement of a thiamin pyrophosphate adduct **19** for umpolung as used for glycolaldehyde[21] in carbohydrate metabolism is therefore excluded. Formation of adduct **19** with concomitant loss of both deuteriums from C-2 of **12c** furnishes a nucleophilic carbon that could be methylated by SAM. **20** is cleaved to (2-oxopropyl)phosphonate (**21**) and thiamin pyrophosphate.



Floss et al.[22] reported that the methyl group of the hydroxyethyl side chain of thienamycin is biosynthesized by addition of intact methyl from a methylated corrin to the β-carbon of an α,β-unsaturated carbonyl intermediate by using L-[*methyl*-^{13}C,$^2H_3$]methionine. A chirally labeled

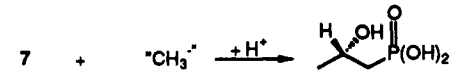
Scheme III[a]



[a] Key: (a) $(MeO)_2POSiMe_3$, CH_2Cl_2; (b) Me_3SiBr/(allyl)$SiMe_3$, CCl_4, 20 °C, EtOH; (c) H_2, Pd–C, EtOH; (d) cyclohexylamine; (e) Dowex 50 (H^+).

methyl group is transfered from methionine to an acceptor carbon with net retention of configuration, in agreement with two inversions.

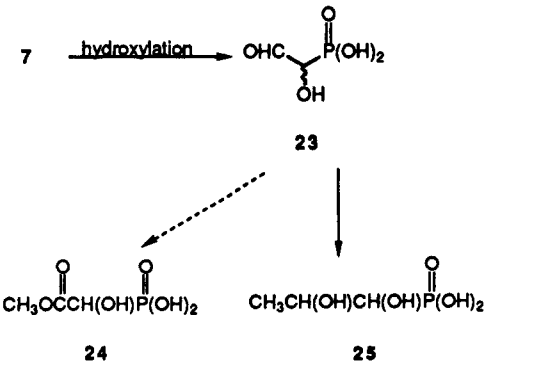
The most plausible mechanism for biosynthesis of the P–C_3 unit of fosfomycin is addition of CH_3^- from a methylated corrin, possibly methylcobalamin,[23] to phosphonoacetaldehyde to give (2-hydroxypropyl)phosphonic acid (**22**). Further elaboration to fosfomycin could involve



22

either an oxidative ring closure with removal of two hydrogens, one being from C-1, or hydroxylation at C-1 with dioxygen, activation of one hydroxyl, and displacement by the other. Both processes have no precedence in epoxide biosynthesis. As the oxirane oxygen atom is not derived from $^{18}O_2$, it should be the hydroxy group at C-1 that is activated and replaced, if a diol is an intermediate. In this case, the configuration at C-2 of **22** should be the same as at C-2 of fosfomycin, that is *S*.

Preparation and Feeding of (*R*,*S*)-(1,2-Dihydroxy-[1-$^2H_1$]ethyl)phosphonic Acid. The hydroxylation could also occur already at the stage of the P–C_2 unit. The arguments for direct methylation of phosphonoacetaldehyde apply to the hydroxylated aldehyde **23**, too. The phosphonic acid component **24** of fosfazinomycins A and B[24] isolated from *Streptomyces lavendofoliae* Nr. 630 is a P–C_2 unit with a hydroxy group at C-1, biogenetically easily accessible from **23** by oxidation and methylation.



(1,2-Dihydroxyethyl)phosphonic acid ((±)-**29**) might be a stable precursor for aldehyde **23** generated by a de-

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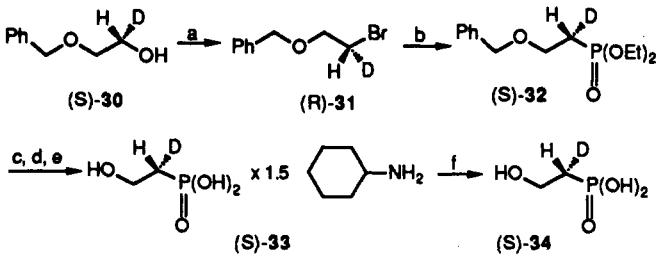
Scheme IV[a]



[a] (a)–(f) as in Scheme I.

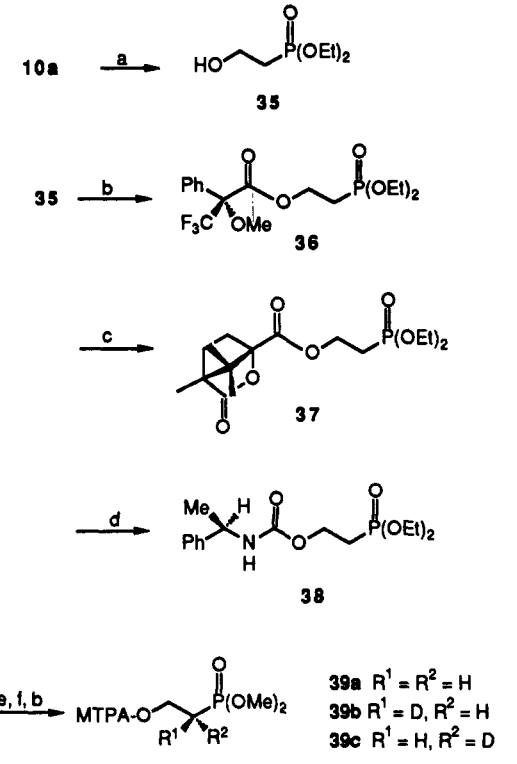
hydrogenase, if it gets into the cells. Protected (dihydroxyethyl)phosphonate (±)-27 was prepared in 84% yield by addition of dimethyl trimethylsilyl phosphite[25] to deuteriated *O*-benzylglycolaldehyde (26)[26] (Scheme III). Removal of protecting groups, purification as cyclohexylammonium salt 28 (69% yield), and passage through Dowex 50 (H^+) afforded free acid (±)-29, which was fed to *S. fradiae* (100 μg/mL). The amino phosphonic acid (−)-13 isolated from this experiment did not contain deuterium. This result can be explained by impermeability of the cell membrane for phosphonic acid (±)-29, by absence of a dehydrogenase accepting it as an analogue for a natural substrate to produce deuteriated 7, or by not being involved in fosfomycin biosynthesis.

Whatever the actual structure of the intermediate might be, a three-carbon phosphorus unit with OH at C-2 with *S* configuration seems to be plausible. The *R* configuration of (*R*)-[3-(*N*-acetyl-*N*-hydroxyamino)-2-hydroxypropyl]-phosphonic acid,[27] another natural product with a P–C bond, at C-2 indicates that it is formed by a mechanism different from that via 22.

Preparation and Incorporation of (*R*)- and (*S*)-(2-Hydroxy[1-$^2H_1$]ethyl)phosphonic Acid. Chirally labeled (2-hydroxy[1-$^2H_1$]ethyl)phosphonic acids were prepared to determine the overall stereochemistry of epoxide ring formation. One hydrogen at C-1 is replaced by oxygen. Stereospecifically deuteriated (*S*)-(benzyloxy)[1-$^2H_1$]-ethanol (30) was easily prepared by HLAD-catalyzed reduction of the corresponding aldehyde.[26] (*R*)-30 was obtained from (*S*)-30 by using triphenylphosphine/diethyl azodicarboxylate/benzoic acid (Mitsunobu reaction) and Zemplen saponification of the ester formed. The two enantiomeric alcohols were subjected to the same reactions as used for unlabeled compound 8a (Scheme IV, structures only given for transformation of (*S*)-30). The hydroxy group is displaced by bromide with inversion[15] of configuration, which in turn is substituted again with inversion of configuration by triethyl phosphite. The configuration of phosphonate 32 should be identical with configuration of starting material.

Determination of optical purity of 34 was necessary, as partial racemization, especially during Arbuzov reaction at 170 °C could not be excluded. In a preliminary experiment, the benzyl group of nondeuteriated phosphonate 10a was hydrogenolyzed to 35 and the free hydroxy group was derivatized with chiral reagents such as (*S*)-(+)-MTPA-Cl (Mosher's reagent),[28] (−)-camphanoyl chloride,[29] and (*R*)-(+)-1-phenylethyl isocyanate to form esters 36 and

Scheme V[a]



[a] Key: (a) H_2, Pd–C, EtOH; (b) (+)-MTPA-Cl, C_5H_5N; (c) (−)-camphanoyl chloride, C_5H_5N; (d) (*R*)-(+)-1-phenylethyl isocyanate, CH_2Cl_2; (e) Dowex 50 (H^+); (f) CH_2N_2, MeOH.

37 and urethane 38, respectively (Scheme V). ^1H NMR spectra (400 MHz) were recorded in $CDCl_3$, C_6D_6, and DMSO-d_6. The spectrum of 36 recorded in $CDCl_3$ showed for hydrogens at C-1 and the spectrum recorded in C_6H_6 for hydrogens at C-2 a well resolved AB system coupling with phosphorus and hydrogens at C-2 or C-1, respectively. Only ester 36 with Mosher's chloride proved suitable for determination of optical purity. The signal pattern for dimethyl ester 39a prepared by esterification of free acid 12a, prepared from salt 11a, with diazomethane, followed by (+)-MTPA-Cl, was similar (Figure 1).

(*S*)- and (*R*)-(2-hydroxy[1-$^2H_1$]ethyl)phosphonic acid (34) were derivatized analogously (Scheme V). The $^{13}C\{^1H\}$ (100.6 MHz) and $^{31}P\{^1H\}$ NMR (161.98 MHz) spectra of a mixture of 39b and 39c (2:1) were recorded in $CDCl_3$ as well. The two diastereomeric compounds exhibited no detectable difference in both spectra.

(*R*)-34 was a mixture of 93% *R* and 7% *S*, and (*S*)-34, a mixture of 92% *S* and 8% *R* as determined by cutting and weighing the signal areas of H-1 on irradiation at H-2 of 39c and 39b (Figure 1). This corresponds to an enantiomeric excess of 86% for (*R*)-34 and 84% for (*S*)-34 (see Figure 1). Furthermore, (*S*)-32 was converted to 41 without characterization of reaction products (Scheme VI). The hydroxy group was deprotected by hydrogenolysis, converted to triflate with trifluoromethanesulfonic acid anhydride/pyridine,[30] reacted in situ with aqueous ammonia, and deprotected with refluxing in 6 N hydrochloric acid. [1-$^2H_1$]AEP (41) obtained by purification with ion-exchange chromatography[31] was transformed to amide with (−)-camphanoyl chloride and esterified with diazomethane according to reference[26] to afford 41. Its ^1H NMR (400-MHz) spectrum recorded in C_6D_6 was identical with the

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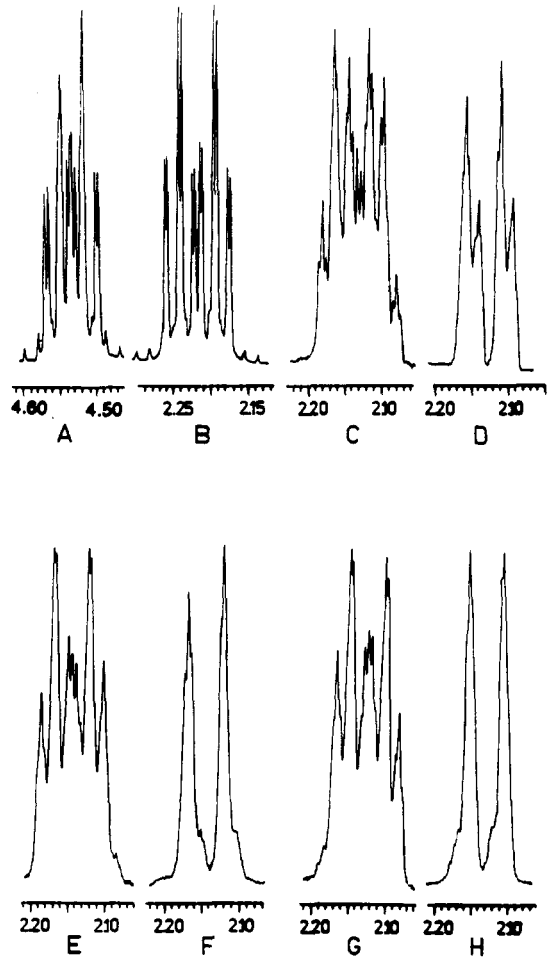
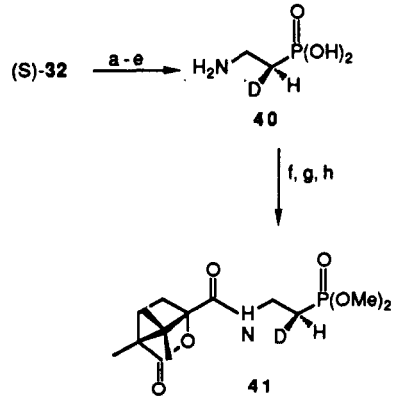


Figure 1. Sections [protons at C-1 (H-1) or C-2 (H-2) of (2-hydroxyethyl)phosphonate part] of ^1H NMR spectra (400 MHz) of compounds **39**, recorded in $CDCl_3$: A, **39a**, H-2; B, **39a**, H-1; C, mixture of **39b** and **39c** (2:1), H-1; D, as C, with irradiation at H-2; E, **39b**, H-1; F, **39b**, H-1, with irradiation at H-2; G, **39c**, H-1; H, **39c**, H-1, with irradiation at H-2.

Scheme VI[a]



[a] Key: (a) H_2, Pd–C, EtOH; (b) $(CF_3SO_2)_2O$, C_5H_5N, CH_2Cl_2; (c) NH_3/H_2O, EtOH; (d) 6 N HCl, reflux; (e) Dowex 50 (H^+), Dowex 1 (OH^-); (f) Me_3SiCl/C_5H_5N, (–)-camphanoyl chloride, H_2O; (g) Dowex 50 (H^+); (h) CH_2N_2, MeOH, PTLC, overall yield 17%.

same derivative of (*S*)-[1-$^2H_1$]AEP prepared by Arbuzov reaction of (*R*)-*N*-(2-bromo[2-$^2H_1$]ethyl))phthalimide with triethyl phosphite and deprotection.

Free phosphonic acids (*R*)- and (*S*)-**34**, each at a concentration of 150 μg/mL, were fed to *S. fradiae*. The amino phosphonic acid (–)-**13** isolated was investigated by ^1H and $^{13}C\{^1H\}$ NMR spectroscopy. The deuterium of (*R*)-**34** was lost (the few percent possibly present is derived from the *S* enantiomer present in (*R*)-**34**), and the deuterium of (*S*)-**34** was retained (30% deuterium in (–)-**13**) on incorporation into fosfomycin. Thus the pro-*R* hydrogen at C-1 of (2-hydroxypropyl)phosphonic acid (**22**) is replaced with inversion of configuration by the C–O bond. This result is in agreement with hydroxylation at C-1 (with retention of configuration), activation, and displacement by OH at C-2 with inversion of configuration. Introduction of a still unknown functionality instead of OH at C-1 and radical processes for the removal of two hydrogens (an oxidative process) from **22** remain a possibility. Oxidative four- and five-membered ring closures such as in clavaminic acid[32] and penicillin[33] biosynthesis occur with retention of configuration at carbon. Experiments are under way to test the incorporation of labeled (2-hydroxypropyl)- and (1,2-dihydroxypropyl)phosphonic acids into fosfomycin.

Experimental Section

General Methods. TLC was carried out on glass plates precoated with 0.25-mm Kieselgel 60 F_{254} (E. Merck). Spots were visualized by UV and/or spraying with 2% cerium(IV) sulfate solution in 2 N sulfuric acid and charring on a hot plate. Flash chromatography[34] was carried out with use of silica gel (E. Merck; Kieselgel 60, 230–400 mesh). Chemical shifts of ^1H NMR spectra recorded in D_2O are relative to HDO (δ 4.80); ^{13}C NMR spectra recorded in D_2O are relative to DSS (δ 0.0). Optical rotation values given for compounds with $[\alpha]_D < 1°$ are mean values of five measurements. Melting points are uncorrected.

Materials. Solvents were distilled before use. Petroleum ether had bp 65–85 °C. Dry solvents were prepared as follows: THF was distilled over potassium with benzophenone ketyl as indicator, CH_2Cl_2 over P_2O_5, DMF and pyridine over CaH_2, and benzene over Na. (*S*)-(+)-MTPA-Cl (JPS-Chimie) had $[\alpha]^{20}_D = +135°$ (*c* 5.2, CCl_4) and ee ≥ 99%.

Reactions were carried out under an atmosphere of argon. Boiling points given for bulb-to-bulb distillations refer to the temperature of the air bath.

2-Bromoethyl Phenylmethyl Ether (9a). A solution of triphenylphosphine (6.29 g, 24 mmol) in dry CH_2Cl_2 (50 mL) was added dropwise within 10 min to a stirred mixture of NBS (4.27 g, 24 mmol) in dry CH_2Cl_2 (70 mL) at –78 °C in the dark. Stirring was continued until all NBS had dissolved (10 min). A solution of 2-(benzyloxy)ethanol (**8a**) (3.04 g, 20 mmol) in dry CH_2Cl_2 (30 mL) was added dropwise. The cooling bath was removed, and stirring was continued for 1 h at room temperature and 30 min in a water bath (35 °C). Dry methanol (2 mL) and toluene (30 mL) were added, and the solvents were removed in vacuo. The residue was purified by flash chromatography with CH_2Cl_2/hexane (1:1, R_f = 0.64) as the eluting solvent and bulb-to-bulb distillation (bp 80 °C/0.001 mmHg) (lit.[35] bp 95–95.5 °C/3 mmHg) to give **9a** (3.71 g, 86%) as a colorless liquid: IR (CH_2Cl_2) 3030, 2960, 1455, 1360, 1110 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 3.46 (t, 2 H, *J* = 6 Hz, CH_2Br), 3.76 (t, 2 H, *J* = 6 Hz, OCH_2), 4.59 (s, 2 H, $PhCH_2$), 7.33 (m, 5 H, Ar H); EIMS *m/z* (relative intensity) 216 and 214 (13 and 9, M^+), 91 (100).

2-Bromo[2,2-$^2H_2$]ethyl Phenylmethyl Ether (9b). **8b**[14] was transformed to **9b** according to the procedure given for **9a** in 97% yield: bp 98 °C/3 mmHg; IR (CH_2Cl_2) 3060, 2815, 1455, 1134, 1103 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 3.77 (s, 2 H, OCH_2), 4.59 (s, 2 H, $PhCH_2$), 7.33 (m, 5 H, Ar H); EIMS *m/z* (relative intensity) 218 and 216 (6 and 6, M^+), 91 (100).

2-Bromo[1,1-$^2H_2$]ethyl Phenylmethyl Ether (9c). **8c** was transformed to **9c** according to the procedure given for **9a** in 47% yield: bp 80 °C/0.001 mmHg; IR (CH_2Cl_2) 3030, 2860, 2180, 2073, 1452, 1205, 1100 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 3.48 (s, 2 H, CH_2Br), 4.59 (s, 2 H, $PhCH_2$), 7.33 (m, 5 H, Ar H); EIMS *m/z* (relative intensity) 218 and 216 (11 and 13, M^+), 91 (100).

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2,3-*O*-Isopropylidene-L-[1,1,4,4-$^2H_4$]threitol (15). Dimethyl 2,3-*O*-isopropylidene-L-tartrate (17.31 g, 79 mmol) was reduced with $LiAlD_4$ (5 g, 119 mmol, 99% D) in dry THF instead of ether.[19] The crude material was purified by bulb-to-bulb distillation to give **15** (11.1 g, 85%) as a colorless oil: bp 110 °C/0.5 mmHg, $[\alpha]^{20}_D$ = +5.3° (*c* 4.6, $CHCl_3$) (lit.[19] for unlabeled compound, bp 96–96.5 °C/0.5 mmHg, $[\alpha]^{20}_D$ = +4.1° (*c* 5, $CHCl_3$)); IR (CH_2Cl_2) 3600, 3460, 2985, 2880, 2210, 2100, 1380, 1371, 1240, 1220, 1168, 1092, 1070 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 1.45 (s, 6 H, 2 Me), 3.45 (br s, 2 H, 2 OH), 3.98 (s, 2 H, 2 CHO). Anal. Calcd for $C_7H_{10}D_4O_4$: C, 50.59; H, 6.09; D, 4.85. Found: C, 50.58; H, 6.20; D, 4.96.

1,4-Di-*O*-benzyl-2,3-*O*-isopropylidene-L-[1,1,4,4-$^2H_4$]threitol (16). NaH (7.0 g, dispersion in mineral oil, 55–60%) was washed three times with petroleum ether and dried in vacuo. Dry DMF (270 mL) was added, followed by **15** (5.9 g, 35.5 mmol) in dry THF (90 mL) with stirring at room temperature. The mixture was then heated for 1.5 h in an oil bath (40 °C). Benzyl bromide (7.5 mL) was added to the viscous reaction mixture, and stirring was continued for 18 h at room temperature. Three hours after the addition of concentrated ammonia (5 mL), ether and water were added. The organic layer was washed twice with water, 0.5 N HCl (300 mL), water, a saturated aqueous solution of $NaHCO_3$ and dried ($MgSO_4$). Concentration in vacuo gave an oily residue that was purified by flash chromatography with CH_2Cl_2/hexane (1:2, R_f = 0.15) as the eluting solvent to give **16** as a colorless oil (2.3 g containing a trace of an impurity and 8.8 g of pure compound, total yield 90%): $[\alpha]^{20}_D$ = −9.3° (*c* 4.1, CH_2Cl_2) (lit.[20] for unlabeled compound, $[\alpha]^{25}_D$ = −7.5° (*c* 2.6, $CHCl_3$)); IR (CH_2Cl_2) 3050, 2985, 2860, 2180, 2078, 1455, 1380, 1370, 1210, 1105, 1072 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 1.42 (s, 6 H, 2 Me), 4.03 (s, 2 H, 2 CHO), 4.56 (s, 4 H, 2 CH_2O), 7.32 (m, 10 H, Ar H); d_2 at each site >98%. Anal. Calcd for $C_{21}H_{22}D_4O_4$: C, 72.80; H, 6.40; D, 2.33. Found: C, 72.35; H, 6.46; D, 2.35.

1,4-Di-*O*-benzyl-L-[1,1,4,4-$^2H_2$]threitol (17). A solution of **16** (8.8 g, 25.4 mmol) in a mixture of ethanol (60 mL), water (25 mL), and concentrated HCl (4 mL) was kept at 50 °C for 3 h. After concentration (to 50 mL) in vacuo, water and CH_2Cl_2 were added. The organic layer was washed with water and a saturated aqueous solution of $NaHCO_3$, dried (Na_2SO_4), and concentrated in vacuo to afford **17** (7.4 g, 95%) as a solid, R_f = 0.2 (CH_2Cl_2/ethyl acetate, 10:1); the analytical sample was recrystallized from diisopropyl ether: mp 58–59 °C, $[\alpha]^{20}_D$ = −7.3° (*c* 1.15, CH_2Cl_2) (lit.[20] for unlabeled compound, mp 58–59 °C, $[\alpha]^{25}_D$ = −5.0° (*c* 5.0, $CHCl_3$)); IR (CH_2Cl_2) 3565, 3030, 2860, 2184, 2180, 1455, 1385, 1113, 1065 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 2.71 (br s, 2 H, 2 OH), 3.86 (s, 2 H, 2 CHO), 4.54 (AB system, 4 H, *J* = 11.6 Hz, 2 CH_2O), 7.32 (m, 10 H, Ar H). Anal. Calcd for $C_{18}H_{18}D_4O_4$: C, 70.56; H, 5.92; D, 2.63. Found: C, 70.75; H, 5.99; D, 2.66.

2-(Benzyloxy)[2,2-$^2H_2$]ethanol (8c). Powdered lead tetraacetate (4.38 g, 9.9 mmol) was added under exclusion of moisture in one portion to a stirred solution of **17** (3.18 g, 10.4 mmol) in dry benzene (80 mL) at 0 °C. After 15 min, glycerol (10 drops) was added, and stirring was continued for another 15 min. The mixture was filtered through Celite. The filtrate was washed twice with ice-cold water and diluted with 96% ethanol (100 mL). Five equal portions of a solution of $NaBH_4$ (0.5 g, 13.2 mmol) in water (5 mL) were added within 5 min to the stirred solution at room temperature. Twenty minutes later, the solution was concentrated (50 mL) in vacuo and water and CH_2Cl_2 were added. The organic layer was washed twice with water, dried (Na_2SO_4), and evaporated. The residue was bulb-to-bulb distilled to furnish **8c** (2.2 g, 69%) as a colorless liquid: R_f = 0.21 (CH_2Cl_2); bp 70–80 °C/0.005 mmHg (lit.[35] for unlabeled compound, bp 90–92 °C/0.2 mmHg); IR (CH_2Cl_2) 3600, 3470, 3050, 2926, 2875, 2186, 2085, 1454, 1389, 1183, 1103, 1067, 1050 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 2.26 (t, 1 H, *J* = 5.6 Hz, OH), 3.74 (d, 2 H, *J* = 5.6 Hz, CH_2O), 4.54 (s, 2 H, $ArCH_2$), 7.32 (m, 5 H, Ar H); EIMS *m/z* (relative intensity) 154 (17, M^+), 107 (29), 91 (100); d_2 = 97%.

Diethyl [2-(Benzyloxy)ethyl]phosphonate (10a). A solution of **9a** (1.88 g, 8.75 mmol) in distilled triethyl phosphite (3.5 mL) was refluxed (oil bath temperature 165–170 °C) with stirring in a round-bottomed flask fitted with an air condenser for 3 h. The air condenser was removed every 30 min for a few seconds to allow the bromoethane formed to escape. After the mixture was cooled to room temperature, excess phosphite was removed in vacuo (up to 70 °C/0.05 mmHg). The oily residue was purified either by bulb-to-bulb distillation (bp 120 °C/0.05 mmHg) or by flash chromatography (CH_2Cl_2/acetone, 10:1; R_f = 0.27) and bulb-to-bulb distillation to afford **10a** (1.8 g, 76%) as colorless oil: IR (CH_2Cl_2) 3043, 2980, 1240, 1095, 1050, 1027, 960 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 1.30 (t, 6 H, *J* = 7 Hz, 2 CH_3), 2.18 (dt, 2 H, *J* = 19, 7.5 Hz, PCH_2), 3.75 (dt, 2 H, *J* = 12, 7.5 Hz, OCH_2), 4.11 (pseudoquint, 4 H, *J* = 7 Hz, 2 $POCH_2$), 4.54 ((s, 2 H, $ArCH_2$), 7.32 (m, 5 H, Ar H); EIMS *m/z* (relative intensity) 166 (100, M^+ − C_7H_6O), 138 (75), 111 (60), 91 (86). Anal. Calcd for $C_{13}H_{21}O_4P$: P, 11.38. Found: P, 11.15.

Diethyl [2-(Benzyloxy)[1,1-$^2H_2$]ethyl]phosphonate (10b). **9b** was transformed according to the procedure given for **10a** to **10b** (yield 88%) with only bulb-to-bulb distillation: bp 110–120 °C/0.001 mmHg; IR (CH_2Cl_2) 3035, 2970, 1385, 1360, 1232, 1115, 1092, 1045, 1025, 965 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 1.30 (t, 6 H, *J* = 7 Hz, 2 CH_3), 3.74 (d, 2 H, *J* = 12.1 Hz, OCH_2), 4.09 (pseudoquint, 4 H, *J* = 7 Hz, 2 $POCH_2$), 4.52 (s, 2 H, $ArCH_2$), 7.34 (m, 5 H, Ar H); EIMS *m/z* (relative intensity) 168 (100, M^+ − C_7H_6O), 138 (60), 111 (56), 91 (96); $d_2 \geq$ 98%.

Diethyl [2-(Benzyloxy)[2,2-$^2H_2$]ethyl]phosphonate (10c). **10c** was prepared from **9c** according to the procedure given for **10a** in 86% yield, with bulb-to-bulb distillation: bp 120 °C/0.005 mmHg; IR (CH_2Cl_2) 3050, 2985, 2190 and 2077 (very weak), 1390, 1238, 1055, 1028, 966 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 1.30 (t, 6 H, *J* = 7.2 Hz, 2 CH_3), 2.15 (d, 2 H, *J* = 18 Hz, PCH_2), 4.1 (pseudoquint, 4 H, *J* = 7.2 Hz, 2 $POCH_2$), 4.52 (s, 2 H, $ArCH_2$), 7.32 (m, 5 H, Ar H); EIMS *m/z* (relative intensity) 168 (100, M^+ − C_7H_6O), 138 (49), 111 (63), 91 (90); d_2 = 97%.

(2-Hydroxyethyl)phosphonic Acid–1.5 Cyclohexylamine (11a). Bromotrimethylsilane (3.89 g, 25.41 mmol, 3.3 mL) was added to a stirred solution of **10a** (1.73 g, 6.35 mmol) and allyltrimethylsilane (0.5 mL) in dry CCl_4 (10 mL) at room temperature with exclusion of moisture. The solution was heated in an oil bath (50 °C) for 2.5 h and then cooled. Volatile material was removed in vacuo (0.5 mmHg/up to 40 °C). The oily residue was dissolved in ethanol and concentrated, again dissolved in water/ethanol (1:1), and evaporated. The residue was hydrogenolyzed over palladium (10%)–carbon (300 mg) in dry ethanol (50 mL) in a Parr apparatus for 2 h at 3.7 bar. The catalyst was filtered off, and the filtrate was concentrated in vacuo. The residue was dissolved in a small volume of ethanol, and cyclohexylamine (1.89 g, 19.06 mmol, 2.2 mL) was added. The solvent was evaporated, and the crystalline residue was dried (20 °C/0.1 mmHg) for 1 h. The residue was dissolved in hot ethanol. After the solution was cooled to below 30 °C, the salt was precipitated by adding ether. The crystals were collected and dried (45 °C/0.005 mmHg, 2 h) to give **11a**: 1.06 g, 61%; mp 128–133 °C; IR (Nujol) broad band from 3500 to 2000, 1640, 1555, 1140, 1127, 1057, 1020 cm^{-1}; ^1H NMR (250 MHz, D_2O) δ 1.0–2.0 (m, 17 H, PCH_2, 15 cyclohexyl H), 3.09 (m, 1.5 H, 1.5 CHN), 3.74 (q, 2 H, *J* = 7.6 Hz, CH_2O). Anal. Calcd for $C_{11}H_{26.5}N_{1.5}O_4P$: C, 48.08; H, 9.72; N, 7.65. Found: C, 47.67; H, 9.69; N, 7.39. Small deviations from the molar ratio of amine to acid of 1.5:1 for cyclohexylammonium salts prepared by this procedure are possible.

(2-Hydroxy[1,1-$^2H_2$]ethyl)phosphonic Acid–1.5 Cyclohexylamine (11b). **11b** was prepared from **10b** according to the procedure given for **11a**: 77% yield; mp 143–145 °C; IR (Nujol) broad band from 3600 to 2000, 1645, 1235, 1105, 1070, 1050, 1000 cm^{-1}; ^1H NMR (250 MHz, D_2O) δ 1.0–2.0 (m, 15 H, 15 cyclohexyl H), 3.1 (m, 1.5 H, 1.5 CHN), 3.74 (d, 2 H, *J* = 7.7 Hz, CH_2O).

(2-Hydroxy[2,2-$^2H_2$]ethyl)phosphonic Acid–1.5 Cyclohexylamine (11c). **11c** was prepared from **10c** according to the procedure given for **11a**: 96% yield (contained 2.6% ethanol, ^1H NMR); mp 118–121 °C; IR (Nujol) broad band from 3600 to 2000, 1630, 1560, 1140, 1040, 1020 cm^{-1}; ^1H NMR (250 MHz, D_2O) δ 1.0–2.05 (m, 17 H, PCH_2, 15 cyclohexyl H), 3.09 (m, 1.5 H, 1.5 CHN).

(*R*,*S*)-Dimethyl [2-(Benzyloxy)-1-[(trimethylsilyl)oxy][1-$^2H_1$]ethyl]phosphonate ((±)-27). Dimethyl trimethylsilyl phosphite[25] (4 mL) was added to a solution of *O*-benzyl[1-$^2H_1$]-glycolaldehyde[26] (2.37 g, 15.67 mmol) in dry CH_2Cl_2 (50 mL) at room temperature. The warm solution (exothermic reaction) was allowed to cool to room temperature and was then concentrated in vacuo. The oily residue was purified by flash chromatography (ethyl acetate/petroleum ether, 3:1; R_f = 0.42) to give (±)-**27** (4.4

g, 84%) as colorless oil: IR (CH_2Cl_2) 3020, 2950, 2850, 1450, 1237, 1160, 1102, 1033 cm^{-1}; ^1H NMR (250 MHz, C_6D_6) δ 0.2 (s, 9 H, $SiMe_3$), 3.47 and 3.48 (two d, 6 H, J = 10.5 Hz, 2 OCH_3), 3.82 (AB system, $J_{A,B}$ = 10.2 Hz, $J_{A,P}$ = 5.5 Hz, $J_{B,P}$ = 3.9 Hz, $PCCH_2$), 4.32 (s, 2 H, $PhCH_2$), 7.05–7.32 (m, 5 H, Ar H); EIMS m/z (relative intensity) 333 (1, M^+), 318 (12), 227 (41), 212 (36), 167 (30), 91 (100). Anal. Calcd for $C_{14}H_{24}DO_5PSi$: C, 50.43; H, 7.26; D, 0.60; P, 9.29. Found: C, 50.39; H, 7.19; D, 0.60; P 9.28.

(*R*,*S*)-(1,2-Dihydroxy[1-$^2H_1$]ethyl)phosphonic Acid–1.5 Cyclohexylamine ((±)-28). (±)-28 was prepared from (±)-27 according to the procedure given for **11a** except that the dealkylation was carried out at 25 °C for 2 h. The crude cyclohexylammonium salt was dried in vacuo (45 °C/0.1 mmHg), and after recrystallization it was dried for 2 h (45 °C/0.005 mmHg) to give (±)-28: 69% yield; mp 133–137 °C; IR (Nujol) broad band from 3600 to 2000, 1640, 1585, 1123, 1070, 1045, 1035, 990; ^1H NMR (250 MHz, D_2O) 1.10–2.07 (m, 15 H, 15 cyclohexyl H), 3.19 (m, 1.5 H, 1.5 CHN), 3.82 (AB system, coupling with P, $J_{A,B}$ = 12 Hz, $J_{A,P}$ = 4 Hz, $J_{B,P}$ = 4.8 Hz, CH_2O). Anal. Calcd for $C_{11}H_{25.5}DN_{1.5}O_5P$: N, 7.20. Found: N, 7.30.

(*R*)-(−)-2-Bromo[2-$^2H_1$]ethyl Phenylmethyl Ether ((*R*)-31). (*S*)-30[26] (1.2 g, 7.8 mmol) was transformed according to the procedure given for **9a** to (*R*)-**31** (1.6 g, 95%) and purified by flash chromatography and bulb-to-bulb distillation: bp 70 °C/0.007 mmHg; $[\alpha]^{20}_D$ = −0.17° (*c* 11.45, CH_2Cl_2), $[\alpha]^{20}_{365}$ = −6.64°. (*S*)-**31** was prepared similarly from (*R*)-30[26] in 86% yield: bp 60 °C/0.001 mmHg; $[\alpha]^{20}_D$ = +0.15° (*c* 12.35, CH_2Cl_2), $[\alpha]^{20}_{365}$ = +6.40°. The IR, ^1H NMR, and EIMS spectra of (*R*)- and (*S*)-**31** were identical: IR (CH_2Cl_2) 3037, 2863, 1495, 1453, 1360, 1151, 1089, 1075 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 3.46 (tt, 1 H, J = 6.2, 1.5 Hz, CHD), 3.77 (d, 2 H, J = 6.2 Hz, OCH_2), 4.57 (s, 2 H, $PhCH_2$), 7.33 (m, 5 H, Ar H); EIMS m/z (relative intensity) 217 and 215 (3 and 4, M^+), 91 (100). Anal. Calcd for $C_9H_{10}DBrO$: C, 50.02; H, 4.66; D, 0.93; Br, 36.98. Found: C, 49.92; H, 4.66; D, 0.93; Br, 37.54.

(*S*)-(+)-Diethyl [2-(Benzyloxy)[1-$^2H_1$]ethyl]phosphonate ((*S*)-32). (*R*)-**30** (1.6 g, 7.4 mmol) was reacted (4 h) with triethyl phosphite according to the procedure given for **10a**. The flask and the air condenser were cleaned with hot, fuming HNO_3, thoroughly rinsed with water and acetone, and air-dried. Dry pyridine (5 mL) and bromotrimethylsilane (1 mL) were refluxed in the flask fitted with the air condenser and a normal condenser for 30 min under exclusion of moisture. After cooling, the apparatus was rinsed and dried as before.[26] Flash chromatography and bulb-to-bulb distillation afforded (*S*)-**32**: 1.8 g, 89%; bp 120 °C/0.002 mmHg; $[\alpha]^{20}_D$ = +0.016° (*c* 12.47, CH_2Cl_2), $[\alpha]^{20}_{365}$ = +0.096°. (*R*)-**32** was prepared from (*S*)-**31** similarly in 97% yield: bp 120 °C/0.001 mmHg; $[\alpha]^{20}_D$ = +0.014° (*c* 13.25, CH_2Cl_2), $[\alpha]^{20}_{365}$ = −0.11°. The IR, ^1H NMR, and EIMS spectra of (*S*)- and (*R*)-**32** were identical: IR (CH_2Cl_2) 3044, 2980, 2905, 2865, 1453, 1391, 1363, 1237, 1103, 1053, 1027, 965 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 1.27 (t, 6 H, J = 7 Hz, 2 CH_3), 2.10 (dtt, 1 H, J = 18.7, 7.3, 1.5 Hz, CHD), 3.71 (dd, 2 H, J = 11.9, 6.5 Hz, CH_2O), 4.06 (m, 4 H, 2 $POCH_2$), 4.49 (s, 2 H, $PhCH_2$), 7.30 (m, 5 H, Ar H); EIMS m/z (relative intensity) 167 (100, $M^+ - C_7H_6O$), 138 (66), 111 (55), 91 (86); d_1 = 99% for (*S*)- and (*R*)-**32**. Anal. Calcd for $C_{13}H_{20}DO_4P$: P, 11.33. Found: P, 11.28.

(*S*)-(−)-(2-Hydroxy[1-$^2H_1$]ethyl)phosphonic Acid–1.5 Cyclohexylamine ((*S*)-(33). (*S*)-**32** (1.49 g, 5.45 mmol) was transformed according to the procedure given for **11a** to (*S*)-**33**: 1.39 g, 92%; mp 136–140 °C; $[\alpha]^{20}_D$ = −0.023° (*c* 8.65, H_2O), $[\alpha]^{20}_{365}$ = −0.13°. (*R*)-**33** was prepared from (*R*)-**32** similarly in 91% yield: mp 135–140 °C; $[\alpha]^{20}_D$ = +0.024° (*c* 8.4, H_2O), $[\alpha]^{20}_{365}$ = +0.11°. The IR, ^1H NMR, and EIMS spectra of (*S*)- and (*R*)-**33** were identical: IR (Nujol) broad band from 3600 to 2000, 1635, 1555, 1140, 1050, 1020 cm^{-1}; ^1H NMR (250 MHz, D_2O) δ 1.0–2.1 (m, 16 H, CHD, 15 cyclohexyl H), 3.12 (m, 1.5 H, 1.5 CHN), 3.75 (t, 2 H, J = 8 Hz, CH_2O). Anal. Calcd for $C_{11}H_{25.5}DN_{1.5}O_4P$: N, 7.62. Found: N, 7.48.

Preparation of 36–38. **10a** was hydrogenolyzed in dry ethanol containing 1 drop of concentrated HCl on Pd (10%)–carbon in a Parr hydrogenation apparatus at 3.7 bar for 2.5 h. **35** (0.125 g, dried by azeotropic distillation with toluene and then drying at 0.005 mm/20 °C) and a solution (2 mL) of (+)-MTPA-Cl (1.0 g of (+)-MTPA-Cl and dry CH_2Cl_2 to give a final volume of 5 mL) were reacted in dry pyridine (3 mL) for 18 h. Workup and flash chromatography (CH_2Cl_2/ethyl acetate, 5:1, R_f = 0.25) gave **36** as an oil. **35** (0.125 g) and 0.3 g of (−)-camphanoyl chloride were reacted in dry pyridine (3 mL) for 18 h. Workup and flash chromatography (CH_2Cl_2/ethyl acetate, 3:1; R_f = 0.2) gave **37** as an oil. A solution of **35** (100 mg) in dry CH_2Cl_2 (5 mL) was reacted with (*R*)-(+)-1-phenylethyl isocyanate (0.3 mL) for 18 h at room temperature. **38** was isolated by flash chromatography (CH_2Cl_2/ethyl acetate, 1:1; R_f = 0.25) as an oil.

Preparation of 39a–c. **11a** (or (*S*)- or (*R*)-**33**) (100 mg) was dissolved in water and the resultant solution passed through Dowex 50 (H^+). The solution was concentrated, and the free acid was dissolved in dry methanol and esterified with a distilled ethereal solution of diazomethane. The carefully dried ester was esterified in dry pyridine (2 mL) with a solution (1 mL, see preparation of **36**) of (+)-MTPA-Cl in CH_2Cl_2 for 18 h. Workup and flash chromatography (CH_2Cl_2/ethyl acetate, 2:1, R_f = 0.32) afforded **39a** (or **39b** or **39c**) as an oil.

Preparation of 41 from (*S*)-32. (*S*)-**32** (117 mg, 0.43 mmol) was hydrogenolyzed (see preparation of **36**). The carefully dried reaction product was dissolved in dry CH_2Cl_2 (3 mL) containing dry pyridine (0.3 mL). After the mixture was cooled to −20 °C (bath temperature), triflic anhydride (0.536 g, 1.9 mmol) was added and stirring was continued for 20 min. The flask was cooled to −40 °C, ammonia (25% aqueous solution, 5 mL) and dry ethanol (5 mL) were added, the cooling bath was removed, and stirring was continued for 1 h at room temperature. The solution was concentrated, and the residue was refluxed with 6 N HCl (50 mL) for 18 h. **40**[31] was isolated by ion-exchange chromatography and derivatized to give **41**;[26] 39 mg was obtained after flash chromatography, and further purification by PTLC (ethyl acetate) gave 24 mg (17%).

Feeding Experiments[11] with *S. fradiae*. Six 1-L Erlenmayer flasks each containing cornstarch medium (220 mL) with the free phosphonic acid (see general part, prepared by passing a solution of the corresponding salt through Dowex 50 (H^+)). Fosfomycin was transformed into (−)-**13**, which was isolated by ion-exchange chromatography.

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