Full Paper

Synthesis of (1*R*,2*R*)- and (1*S*,2*R*)-1,2-Epoxy-3-hydroxypropylphosphonates as Analogues of Fosfomycin

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Cyclohexylammonium (1*R*,2*R*)-1,2-epoxy-3-hydroxypropylphosphonate was conveniently synthesized from dibenzyl (1*S*,2*R*)-2,3-*O*-cyclohexylidene-1,2,3-trihydroxypropylphosphonate by a reaction sequence including mesylation, hydrolysis of acetal, intramolecular Williamson reaction, and hydrogenation in the presence of cyclohexylamine. For dibenzyl (1*S*,2*R*)-2,3-*O*-cyclohexylidene-1,2,3-trihydroxypropylphosphonates the same approach was not successful, since prior the epoxide-ring closure tritylation of HO–C3 in dibenzyl (1*R*,2*R*)-2,3-dihydroxy-1-mesyloxypropylphosphonate was necessary and the hydrogenolysis of dibenzyl (1*S*,2*R*)-1,2-epoxy-3-trityloxypropylphosphonate yielded a complex reaction mixture.

Keywords: Antibacterial / Epoxides / Fosfomycin / Synthesis

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Introduction

A vast number of natural compounds possessing an oxirane ring displays antifungal, antibacterial [1–7], and anticancer activity [8–16] and also acts as enzyme inhibitors [17–23]. Fosfomycin ((1*R*,2*S*)-1,2-epoxypropylphosphonic acid **1**; Fig. 1) which was isolated in 1969 from strains of *Streptomyces fradiae* is among the best recognized natural epoxides pharmacologically useful in treatment of urinary tract infections caused by Gram-negative and Gram-positive bacteria [24, 25]. According to the generally accepted mode of action, fosfomycin inhibits the biosynthesis of the bacterial cell wall by inactivation of pyruvyl transferase [26].

We anticipated that replacement of the methyl group in fosfomycin for the hydroxymethyl group would significantly increase the polarity of the analogue and could introduce additional, hopefully binding, interactions within the enzyme active site. Synthesis of all enantiomers of diethyl 1,2-epoxy-3-hydroxypropylphosphonates has already been described [27, 28]. Since hydrolytic cleavage of phosphonate esters would destroy the epoxide ring, another approach was necessary and we decided to study the feasibility of the benzyl esters hoping to cleanly remove benzyl groups in the presence of the oxirane ring by the catalytic hydrogenolysis. The synthetic strategy is outlined in Scheme 1 and follows the methodology described earlier [27] except for the preparation of the starting materials and final products.

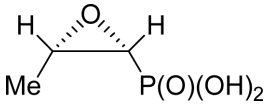

Figure 1. Structure of (1*R*,2*S*)-**1** (Fosfomycin).

Results and discussion

The triethylamine-catalyzed addition of dibenzyl phosphite to 2,3-*O*-cyclohexylidene-D-glyceraldehyde led to a 0.95 : 1 mixture of dibenzyl (1*R*,2*R*)- and (1*S*,2*R*)-2,3-*O*-cyclohexylidene-1,2,3-trihydroxypropylphosphonates **3** in almost quantitative yield. Diastereoisomeric phosphonates **3** were separated chromatographically on silica gel

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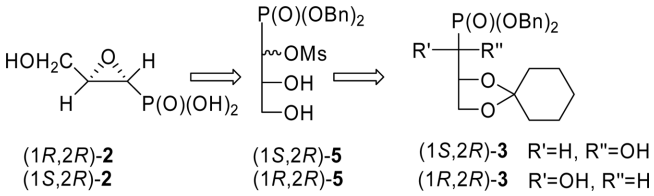
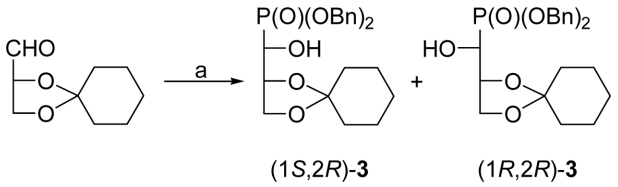


Scheme 1. Retrosynthesis of enantiomers of 1,2-epoxy-3-hydroxypropylphosphonate.

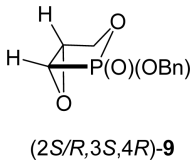


Reagents and conditions: a) $(BnO)_2P(O)H$, Et_3N, r.t., 24 h, 98%.

Scheme 2. Synthesis of dibenzyl (1*R*,2*R*)- and (1*S*,2*R*)-2,3-*O*-cyclohexylidene-1,2,3-trihydroxypropylphosphonates **3**.

to give (1*R*,2*R*)-**3** (δ ^{31}P: 22.23 ppm) and (1*S*,2*R*)-**3** (δ ^{31}P: 22.62 ppm) in 20 and 52% yield, respectively (Scheme 2). The absolute configurations at C-1 in (1*R*,2*R*)- and (1*S*,2*R*)-**3** were established by comparison of their ^{1}H and ^{31}P spectral data with those of the corresponding ethyl esters [29].

Mesylation of the HO – C-1 groups in the enantiomerically pure phosphonates (1*R*,2*R*)- and (1*S*,2*R*)-**3** was performed under standard conditions [27]. Pure 1-*O*-mesyl derivatives (1*R*,2*R*)- and (1*S*,2*R*)-**4** were obtained after chromatographic purification and crystallization from a diethyl ether / hexane mixture. Removal of the cyclohexylidene groups from (1*R*,2*R*)- and (1*S*,2*R*)-**4** was accomplished with aqueous trifluoroacetic acid without formation of any side products to give after two hours the diols (1*R*,2*R*)- and (1*S*,2*R*)-**5**, in 73 and 70% yield, respectively (Schemes 3 and 4) [30]. When hydrochloric acid was used to hydrolyse the acetals [27], the expected diols **5** were produced in low yield. Furthermore, formation of several unidentified phosphonates was observed by ^{31}P-NMR spectroscopy when the reaction time was extended.

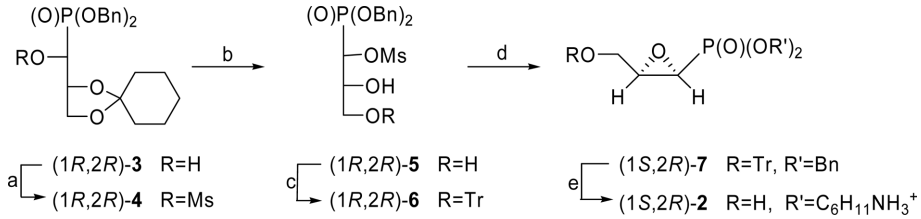


(2*S*/*R*,3*S*,4*R*)-**9**

Figure 2. Structure of compound (2*S*/*R*,3*S*,4*R*)-**9**.

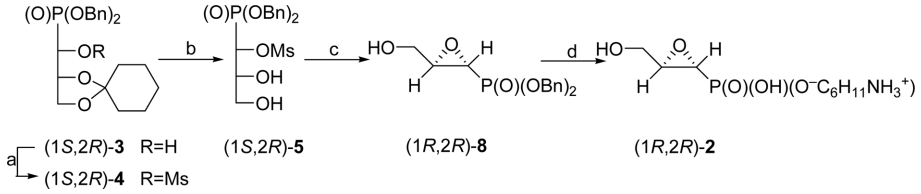
According to our earlier experience, the primary hydroxyl group had to be selectively protected by the trityl group [27] before the epoxide ring closure in phosphonate (1*R*,2*R*)-**5**. The respective 3-*O*-trityl derivative (1*R*,2*R*)-**6** was obtained in 60% yield only, because under the basic conditions employed in the tritylation the formation of by-products was noticed. Among them, based on the analyses of the ^{31}P-NMR spectra of the crude reaction mixture, P-epimeric 3,4-epoxy-1,2-oxaphospholanes **9** (δ ^{31}P: 31.09 and 29.95 ppm) were identified [27].

When the 3-*O*-trityl phosphonate (1*R*,2*R*)-**6** was treated with potassium carbonate suspended in THF at room



Reagents and conditions: a) MsCl, Et_3N, DMAP, 0°C to r.t., 5 h, 83%; b) 70% CF_3COOH, r.t., 2 h, 73%; c) TrCl, Et_3N, DMAP, 0°C to r.t., 20 h, 60%; d) K_2CO_3, THF, r.t., 20 h, 73%; e) H_2, Pd / C, $C_6H_{11}NH_2$, r.t., THF, 24 h.

Scheme 3. Synthesis of compound (1*S*,2*R*)-**2**.



Reagents and conditions: a) MsCl, Et_3N, DMAP, 0°C to r.t., 5 h, 79%; b) 70% CF_3COOH, r.t., 2 h, 70%; c) K_2CO_3, THF, r.t., 24 h, 70%; d) H_2, Pd / C, $C_6H_{11}NH_2$, r.t., THF, 24 h, 92%.

Scheme 4. Synthesis of compound (1*R*,2*R*)-**2**.

Table 1. Minimum inhibitory concentration (MIC in μg/mL) values of compounds, which presented any antibacterial activity.

Compound	S. aureus **strains:** **ATCC 25923** **ATCC 6538P**	B. subtilis **ATCC 6633**	E. faecalis **ATCC 29212**	E. coli **strains:** **ATCC 8739** **ATCC 35218** **ATCC 25922**	P. aeruginosa **ATCC 27853**
(1*R*,2*S*)-**10**	N	N	N	N	500
(1*S*,2*S*)-**10**	N	N	N	N	500
(1*R*,2*S*)-**11**	N	N	N	N	500
(1*S*,2*S*)-**11**	N	N	N	N	500
Reference compound (fosfomycin)	500 125	N	N	15.2 62.5 31.2	31.25

N – growth not inhibited.

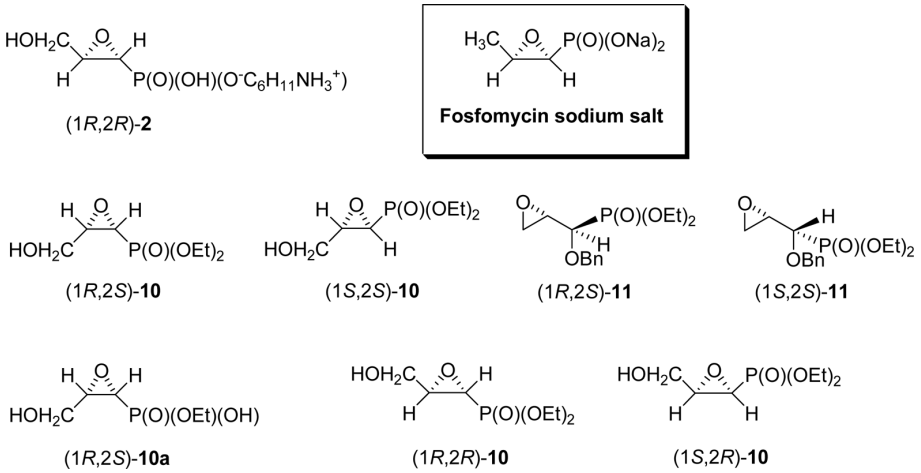


Figure 3. Epoxyphosphonates tested for antibacterial activity.

temperature for 20 h to execute the epoxide ring closure by the intramolecular Williamson reaction, the epoxyphosphonate (1*S*,2*R*)-**7** was obtained as a single product in 73% yield (Scheme 3). On the other hand, the transformation of the 1-*O*-mesyl phosphonate (1*S*,2*R*)-**5** into the epoxyphosphonate (1*R*,2*R*)-**8** was accomplished in the usual manner (potassium carbonate suspended in THF) without involvement of protective groups (Scheme 4). To avoid the formation of any transesterification products, tetrahydrofuran was applied as a solvent instead of low-boiling alcohols.

Several attempts to hydrogenolytically remove the trityl and benzyl protective groups from phosphonates (1*S*,2*R*)-**7** and (1*R*,2*R*)-**8** failed to give the epoxides (1*S*,2*R*)- and (1*R*,2*R*)-**2** in satisfactory yield and purity. Following a literature report [31], the epoxyphosphonate (1*R*,2*R*)-**8** was subjected to catalytic hydrogenolysis in the presence of cyclohexylamine in THF to provide pure (1*R*,2*R*)-**2** as a cyclohexylammonium salt in 92% yield (Scheme 4). However, under the same conditions the pure cyclohexylammonium salt (1*S*,2*R*)-**2** could not be isolated from the 3-*O*-trityl epoxyphosphonate (1*S*,2*R*)-**7** (Scheme 3).

The cyclohexylammonium salt (1*R*,2*R*)-**2** and several previously synthesized, structurally related epoxyhydroxypropylphosphonates (Fig. 1) were tested to find their presumable antibacterial activity against Gram-negative (*Escherichia coli, Pseudomonas aeruginosa*) and Gram-positive (*Staphylococcus aureus, Enterococcus faecalis, Bacillus subtilis*) bacteria. Minimum inhibitory concentration (MIC) values of all compounds were estimated in a decreasing concentrations mode starting from 500 μg/mL. Weak activity (MIC = 500 μg/mL) against *Pseudomonas aeruginosa* ATCC 27853 was detected for (1*R*,2*S*)- and (1*S*,2*S*)-1,2-epoxy-3-hydroxypropylphosphonates **10** and (1*R*,2*S*)- and (1*S*,2*S*)-1-benzyloxy-2,3-epoxypropylphosphonates **11**, while the reference fosfomycin sodium salt inhibited growth of this strain in a much lower concentration (Table 1).

Conclusions

Enantiomerically pure dibenzyl (1*R*,2*R*)- and (1*S*,2*R*)-2,3-*O*-cyclohexylidene-1,2,3-trihydroxypropylphosphonates

were obtained from 2,3-*O*-cyclohexylidene-D-glyceraldehyde and dibenzyl phosphite. After mesylation and acetal cleavage dibenzyl (1*R*,2*R*)- and (1*S*,2*R*)-2,3-dihydroxy-1-mesyloxypropylphosphonates were cleanly prepared to study the intramolecular Williamson reaction. Dibenzyl (1*R*,2*R*)-1,2-epoxy-3-hydroxypropylphosphonate (the *trans*-configured isomer) was obtained directly from dibenzyl (1*S*,2*R*)-2,3-dihydroxy-1-mesyloxypropylphosphonate. However, for the synthesis of dibenzyl (1*S*,2*R*)-1,2-epoxy-3-hydroxypropylphosphonate (isomer *cis*) tritylation of the primary hydroxy group in dibenzyl (1*R*,2*R*)-2,3-dihydroxy-1-mesyloxypropylphosphonate was necessary because under basic conditions intramolecular transesterification followed by hydrolysis of the already formed 1,2-oxaphospholane ring led to complex reaction mixtures. Catalytic hydrogenolysis of dibenzyl (1*R*,2*R*)-1,2-epoxy-3-hydroxypropylphosphonate was successfully accomplished in the presence of cyclohexylamine to only give the respective cyclohexylammonium salt. Several 1,2-epoxy-3-hydroxypropylphosphonates (Table 1) exhibited negligible activity against *Pseudomonas aeruginosa* ATCC 27853, while cyclohexylammonium (1*R*,2*R*)-1,2-epoxy-3-hydroxypropylphosphonate was found inactive against this strain.

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The authors have declared no conflict of interest.

Experimental

Chemistry

Melting points were determined on a Boetius apparatus and are uncorrected. Polarimetric measurements were conducted on a Perkin Elmer 241 MC apparatus (Perkin Elmer Beaconsfield, UK). IR spectral data were measured on an Infinity MI-60 FT-IR spectrometer (Mattson, Madison, WI, USA). ^{1}H-NMR spectra were recorded with a Varian Mercury-300 spectrometer (Varian Inc., Palo Alto, CA, USA) and Bruker DPX-250 (Bruker Bioscience, USA); chemical shifts δ in ppm with respect to TMS; coupling constants *J* in Hz. ^{13}C- and ^{31}P-NMR spectra were recorded on a Varian Mercury-300 machine at 75.5 and 121.5 MHz and Bruker DPX-250 machine at 62.9 and 101.5 MHz, respectively. Elemental analyses were performed by the Microanalytical Laboratory of this Faculty on a Perkin Elmer PE 2400 CHNS analyzer. The following absorbents were used: column chromatography, Merck silica gel 60 (70–230 mesh); analytical TLC, Merck TLC plastic sheets silica gel 60 F_{254} (Merck, Germany).

General procedure for the preparation of the starting materials **3**

A mixture of 2,3-*O*-cyclohexylidene-D-glyceraldehyde (5.00 g, 29.4 mmol), dibenzyl phosphite (3.59 mL, 27.9 mmol) and triethylamine (0.374 mL, 2.94 mmol) was left at room temperature for 24 h. After removal of all volatiles *in vacuo*, the residue was chromatographed on a silica gel column with chloroform / methanol (100 : 1, v / v) to give (1*R*,2*R*)-**3** (2.081 g, 23%) and (1*S*,2*R*)-**3** (4.80 g, 53%) both as fluffy white needles after crystallization from hexane.

Dibenzyl (1R,2R)-2,3-O-cyclohexylidene-1,2,3-trihydroxypropylphosphonate **3**

M.p.: 65.5–68°C; $[\alpha]_D^{20}$ = −3.5 (*c* = 1.2, $CHCl_3$); IR (KBr) ν [cm^{-1}]: 3303, 2934, 2859, 1251, 1103, 1037, 1009, 738, 697; ^{1}H-NMR (300 MHz, $CDCl_3$) δ [ppm]: 1.61–1.37 (m, 10H, $H_{cyclohex}$), 3.0–1.8 (brs, 1H, OH), 3.86 (dd, *J* = 9.9 Hz, *J* = 4.5 Hz, 1H, H-1), 3.90 (dd, *J* = 8.7 Hz, *J* = 6.6 Hz, 1H, H-3a), 4.03 (dd, *J* = 8.7 Hz, *J* = 6.6 Hz, 1H, H-3b), 4.48 (ddt, *J* = 6.6 Hz, *J* = 6.6 Hz, *J* = 4.5 Hz, *J* = 3.9 Hz, 1H, H-2), 5.10 (dd, J_{AB} = 7.5 Hz, 4H, H_2COP), 7.38–7.31 (m, 10H, Ar-H); ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ [ppm]: 24.0, 24.1, 24.2, 35.3, 36.3, 65.9 (d, *J* = 9.8 Hz, C-3), 68.3 and 68.6 (2d, *J* = 6.8 Hz, CH_2OP), 68.6 (d, *J* = 160.7 Hz, C-1), 74.4 (d, *J* = 3.8 Hz, C-2), 110.7, 127.0, 128.0, 128.1, 128.5, 128.6, 128.7, 136.0 and 136.2 (2d, *J* = 6.0 Hz, C_{ipso}); ^{31}P-NMR (121.5 MHz, $CDCl_3$) δ [ppm]: 22.24. Anal. calcd. for $C_{23}H_{29}O_6P$: C, 63.88; H, 6.76. Found: C, 63.82; H, 6.74.

Dibenzyl (1S,2R)-2,3-O-cyclohexylidene-1,2,3-trihydroxypropylphosphonate **3**

M.p.: 88.5–90°C; $[\alpha]_D^{20}$ = + 4.6 (*c* = 2.3, $CHCl_3$); IR (KBr) ν [cm^{-1}]: 3248, 2933, 2890, 1455, 1224, 1097, 1052, 997, 742, 696; ^{1}H-NMR (300 MHz, $CDCl_3$) δ [ppm]: 1.62–1.58 (m, 10$H_{cyclohex}$), 2.69 (brs, 1H, OH), 4.00 (dd, *J* = 8.7 Hz, *J* = 6.6 Hz, 1H, H-3a), 4.08 (dd, *J* = 8.7 Hz, *J* = 6.3 Hz, 1H, H-3b), 4.17 (dd, *J* = 8.4 Hz, *J* = 4.2 Hz, 1H, H-1), 4.37 (ddt, *J* = 6.6 Hz, *J* = 6.3 Hz, *J* = 4.2 Hz, *J* = 3.0 Hz, 1H, H-2), 5.08 (dd, *J* = 8.4 Hz, 4H, H_2COP), 7.39–7.29 (m, 10H, Ar-H); ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ [ppm]: 24.0, 24.2, 25.4, 35.0, 36.2, 65.2 (d, *J* = 5.7 Hz, C-3), 68.4 and 68.5 (2d, *J* = 6.8 Hz, CH_2OP), 68.5 (d, *J* = 161.2 Hz, C-1), 74.8 (d, *J* = 7.2 Hz, C-2), 110.0, 128.1, 128.1, 128.1, 128.6, 128.7, 128.7, 136.0 and 136.1 (2d, *J* = 6.0 Hz, C_{ipso}); ^{31}P-NMR (121.5 MHz, $CDCl_3$) δ [ppm]: 23.33. Anal. calcd. for $C_{23}H_{29}O_6P$: C, 63.88; H, 6.76. Found: C, 63.69; H, 7.10.

General procedure for the preparation of the mesylate **4**

Solutions of phosphonates in CH_2Cl_2 containing NEt_3 (three equiv.) were cooled to 0°C and mesyl chloride (two equiv.) was added dropwise. After 5 h at room temperature, the reaction mixtures were washed with water (3 × 25 mL), the organic phases were dried over $MgSO_4$, concentrated, and the crude products were chromatographed on a silica gel column with chloroform / methanol (100 : 1).

Dibenzyl (1R,2R)-2,3-O-cyclohexylidene-1-mesyloxy-2,3-dihydroxypropylphosphonate **4**

According to the general procedure, the reaction of phosphonate (1*R*,2*R*)-**3** (2.18 g, 5.04 mmol) with mesyl chloride (0.79 mL, 10.1 mmol) in the presence of NEt_3 (2.29 mL, 15.1 mmol) in CH_2Cl_2 (40 mL) afforded the mesylate (1*R*,2*R*)-**4** (2.14 g, 83%) as a white needles after crystallization from diethyl ether / hexane; M.p.: 79–80°C; $[\alpha]_D^{20}$ = −8.7 (*c* = 1.3, $CHCl_3$); IR (KBr) ν [cm^{-1}]: 700,

747, 1012, 1181, 1256, 1366, 2853, 2993, 2933; ^{1}H-NMR (300 MHz, $CDCl_3$) δ [ppm]: 1.65 – 1.38 (m, $10H_{cyclohex}$), 3.13 (s, 3H, CH_3SO_2), 3.96 (dd, *J* = 9.3 Hz, *J* = 6.6 Hz, 1H, H-3a), 4.06 (dd, *J* = 9.3 Hz, *J* = 6.0 Hz, 1H, H-3b), 4.43 (dddd, *J* = 7.5 Hz, *J* = 6.6 Hz, *J* = 6.0 Hz, *J* = 3.0 Hz, 1H, H-2), 4.88 (dd, *J* = 10.5 Hz, *J* = 7.5 Hz, 1H, H-1), 5.19-5.01 (m, 4H, CH_2OP), 7.39 – 7.33 (m, 10H, Ar-H); ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ [ppm]: 24.1, 24.2, 25.3, 35.2, 36.1, 39.5, 65.6 (d, *J* = 3.0 Hz,C-3), 69.0 and 69.7 (2d, *J* = 6.8 Hz, CH_2OP), 73.9 (d, *J* = 9.0 Hz, C-2), 77.5 (d, *J* = 162.2 Hz, C-1), 111.1, 128.4, 128.5, 128.8, 128.9, 128.9, 135.3 and 135.6 (2d, *J* = 6.0 Hz, C_{ipso}); ^{31}P-NMR (121.5 MHz, $CDCl_3$) δ [ppm]: 16.30. Anal. calcd. for $C_{24}H_{31}O_8PS \times 0.5\ H_2O$: C, 55.48; H, 6.21. Found: C, 55.77; H, 6.01.

*Dibenzyl (1S,2R)-2,3-O-cyclohexylidene-1-mesyloxy-2,3-dihydroxypropylphosphonate **4***

As described in the general procedure, from phosphonate (1*S*,2*R*)-**3** (2.01 g, 4.65 mmol) and mesyl chloride (0.73 mL, 9.3 mmol) in the presence of NEt_3 (1.94 mL, 13.9 mmol), the mesylate (1*S*,2*R*)-**4** (1.87 g, 79%) was obtained as white needles after crystallization from diethyl ether / hexane; M.p.: 81 – 82°C; $[\alpha]_D^{20}$ = +17.5 (*c* = 1.3, $CHCl_3$); IR (KBr) ν [cm^{-1}]: 699, 743, 1019, 1181, 1262, 1363, 2853, 2939; ^{1}H-NMR (300 MHz, $CDCl_3$) δ [ppm]: 1.61 – 1.38 (m, $10H_{cyclohex.}$), 3.08 (s, 3H, CH_3SO_2), 3.93 (dd, *J* = 8.7 Hz, *J* = 7.5 Hz, 1H, H-3a), 4.01 (dd, *J* = 8.7 Hz, *J* = 6.6 Hz, 1H, H-3b), 4.44 (dddd, *J* = 7.5 Hz, *J* = 6.6 Hz, *J* = 3.3 Hz, *J* = 1.2 Hz, 1H, H-2), 5.11 – 5.01 (m, 4H, CH_2OP), 5.16 (dd, *J* = 11.4 Hz, *J* = 3.3 Hz, 1H, H-1), 7.38 – 7.32 (m, 10H, Ar-H); ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ [ppm]: 23.9, 24.1, 25.3, 34.9, 35.9, 39.5, 64.5 (d, *J* = 2.3 Hz, C-3), 69.0 and 69.3 (2d, *J* = 7.5 Hz, CH_2OP), 73.3 (d, *J* = 9.8 Hz, C-2), 74.7 (d, *J* = 165.2 Hz, C-1), 110.5, 128.4, 128.5, 128.8, 129.0, 129.2, 135.3 and 135.4 (2d, *J* = 6.0 Hz, C_{ipso}); ^{31}P-NMR (121.5 MHz, $CDCl_3$) δ [ppm]: 17.15. Anal. calcd. for $C_{24}H_{31}O_8PS$: C, 56.46; H, 6.12. Found: C, 56.62; H, 6.25.

*General procedures for the preparation of 2,3-dihydroxy-1-mesyloxypropylphosphonate **5***

Solutions of phosphonates in 70% aqueous trifluoroacetic acid were vigorously stirred at room temperature for 2 h. The volatiles were evaporated and the residues were dissolved in CH_2Cl_2, neutralized with solid $NaHCO_3$ and finally dried over $MgSO_4$. After removal of the solids, the solutions was concentrated and the crude products were chromatographed on a silica gel column with chloroform / methanol (100 : 1, v / v) to give the corresponding diols.

*Dibenzyl (1R,2R)-2,3-dihydroxy-1-mesyloxypropylphosphonate **5***

According to the general procedure, phosphonate (1*R*,2*R*)-**4** (0.668 g, 1.31 mmol) in 70% aqueous trifluoroacetic acid (4.56 mL) was vigorously stirred at room temperature for 2 h and the diol (1*S*,2*R*)-**5** (0.383 g, 73%) was obtained as white needles after crystallization from chloroform / hexane; M.p.: 93 – 94.5°C; $[\alpha]_D^{20}$ = −10.0 (*c* = 1.0, $CHCl_3$); IR (KBr) ν [cm^{-1}]: 695, 730, 1000, 1105, 1178, 1224, 1361, 2887, 2932, 3322, 3395, 3462; ^{1}H-NMR (300 MHz, $CDCl_3$) δ [ppm]: 3.01 (s, 3H, CH_3SO_2), 3.71 (dd, *J* = 12.0 Hz, *J* = 6.0 Hz, 1H, H-3a), 3.83 (ddd, *J* = 12.0 Hz, *J* = 5.1 Hz, *J* = 1.2 Hz, 1H, H-3b), 4.10 (dddd, *J* = 6.0 Hz, *J* = 5.4 Hz, *J* = 5.1 Hz, *J* = 4.2 Hz, 1H, H-2), 5.03 (dd, *J* = 11.7 Hz, *J* = 4.2 Hz, 1H, H-1), 5.18 – 5.05 (m, 4H, CH_2OP), 7.38 – 7.32 (m, 10H, Ar-H); ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ [ppm]: 39.0, 62.4 (d, *J* = 8.3 Hz, C-3), 69.1 and 69.6 (2d, *J* = 6.8 Hz, CH_2OP), 70.7 (d, *J* = 3.0 Hz, C-2), 76.1 (d, *J* = 166.0 Hz, C-1), 128.5, 128.8, 128.8, 129.0, 129.8, 135.2 and 135.5 (2d, *J* = 7.5 Hz, C_{ipso}); ^{31}P-NMR (121.5 MHz, $CDCl_3$) δ [ppm]: 18.83. Anal. calcd. for $C_{18}H_{23}O_8PS \times 0.5\ H_2O$: C, 53.07; H, 5.94. Found: C, 53.11; H, 5.94.

*Dibenzyl (1S,2R)-2,3-dihydroxy-1-mesyloxypropylphosphonate **5***

As described in general procedure, from phosphonate (1*S*,2*R*)-**4** (0.891 g, 1.75 mmol) and aqueous trifluoroacetic acid (4.60 mL), the diol (1*S*,2*R*)-**5** (0.488 g, 70%) was obtained as a white amorphous powder after crystallization from chloroform / hexane; M.p.: 52 – 53°C; $[\alpha]_D^{20}$ = + 10.9 (*c* = 1.2, $CHCl_3$); IR (KBr) ν [cm^{-1}]: 695, 730, 979, 1129, 1176, 1226, 1349, 2927, 3337, 3397; ^{1}H-NMR (300 MHz, $CDCl_3$) δ [ppm]: 2.85 – 2.65 (brs, 2H, OH), 2.99 (s, 3H, CH_3SO_2), 3.75 (dd, *J* = 12.1 Hz, *J* = 4.4 Hz, 1H, H-3a), 3.85 (dd, *J* = 12.1 Hz, *J* = 2.6 Hz, 1H, H-3b), 4.09 (dddd, *J* = 10.7 Hz, *J* = 6.6 Hz, *J* = 4.4 Hz, *J* = 2.6 Hz, 1H, H-2), 5.00 (dd, *J* = 9.6 Hz, *J* = 6.6 Hz, 1H, H-1), 5.13 – 5.03 (m, 4H, CH_2OP), 7.40 – 7.27 (m, 10H, Ar-H); ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ [ppm]: 39.0, 62.1 (d, *J* = 6.6 Hz, C-3), 69.2 and 69.7 (2d, *J* = 6.8 Hz, CH_2OP), 70.8 (d, *J* = 3.7 Hz, C-2), 76.4 (d, *J* = 164.6 Hz, C-1), 128.4, 128.5, 128.8, 128.9, 129.0, 135.3 and 135.5 (2d, *J* = 6.0 Hz, C_{ipso}); ^{31}P-NMR (121.5 MHz, $CDCl_3$): δ [ppm] 19.28. Anal. calcd. for $C_{18}H_{23}O_8PS \times 1.5\ H_2O$: C, 50.83; H, 6.16. Found: C, 50.60; H, 5.86.

*Dibenzyl (1R,2R)-2-hydroxy-1-mesyloxy-3-trityloxypropylphosphonate **6***

To a solution of the diol (1*R*,2*R*)-**5** (0.35 g, 0.89 mmol) in CH_2Cl_2 (20 mL) cooled to 0°C, trityl chloride (0.298 g, 1.07 mmol) was added, followed by NEt_3 (0.20 mL, 1.4 mmol) and DMAP (0.003 g, 0.03 mmol). Then, the solution was stirred at room temperature for 20 h and later treated with cooled saturated NH_4Cl (20 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 15 mL), organic phases were combined and dried over $MgSO_4$. After concentration, the crude product was chromatographed on a silica gel column with chloroform / methanol / triethylamine (100 : 1 : 0.05, v / v) to give the trityl derivative (1*R*,2*R*)-**6** (0.360 g, 60%) as a colourless oil; $[\alpha]_D^{20}$ = − 8.0 (*c* = 1.6, $CHCl_3$); IR (film) ν [cm^{-1}]: 699, 745, 1008, 1156, 1217, 1363, 2889, 2936, 3033, 3061,3354; ^{1}H-NMR (300 MHz, $CDCl_3$) δ [ppm] 2.86 (s, 3H, CH_3SO_2), 3.05-2.90 (brs, 1H, HO), 3.31 (dd, *J* = 9.6 Hz, *J* = 6.0 Hz, 1H, H-3a), 3.41 (dd, *J* = 9.6 Hz, *J* = 6.0 Hz, 1H, H-3b), 4.20 – 4.05 (m, 1H, H-2), 5.13 – 4.94 (m, 4H, CH_2OP), 5.14 (dd, *J* = 10.8 Hz, *J* = 3.6 Hz, 1H, H-1), 7.40 – 7.20 (m, 25H, Ar-H); ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ [ppm]: 39.1, 63.4 (d, *J* = 9.2 Hz, C-3), 69.6 and 68.7 (2d, *J* = 7.0 Hz, CH_2OP), 69.5 (s, C-2), 75.7 (d, *J* = 167.5 Hz,C-1), 87.6, 127.1, 127.4, 127.7, 128.0, 128.3, 128.4, 128.6, 128.7, 128.8, 128.8, 128.9, 135.6 and 135.9 (2d, *J* = 5.9 Hz, C_{ipso}), 143.4; ^{31}P-NMR (121.5 MHz, $CDCl_3$) δ [ppm]: 18.85. Anal. calcd. for $C_{37}H_{37}O_8PS \times H_2O$: C, 64.34; H, 5.69. Found: C, 64.64; H, 5.56.

*Dibenzyl (1S,2R)-1,2-epoxy-3-trityloxypropylphosphonate **7***

To a solution of the trityl derivative (1*R*,2*R*)-**6** (0.43 g, 0.64 mmol) in THF (4 mL) anhydrous K_2CO_3 (0.13 g, 0.96 mmol) was added and the suspension was vigorously stirred at room temperature for 20 h. After removal of potassium carbonate by filtration through a layer of Celite, the solution was concentrated and the crude product was chromatographed on a silica gel column

with chloroform / methanol / triethylamine (100: 1 : 0.05, v / v) to give the epoxyphosphonate (1*S*,2*R*)-**7** (0.270 g, 73%) as a colourless oil; $[\alpha]_D^{20} = -10.7$ (*c* = 1.9, $CHCl_3$); IR (film) ν [cm^{-1}]: 704 745, 1019, 1073, 1216, 1264, 1449, 1492, 2854, 2926, 3032, 3059; ^{1}H-NMR (300 MHz, $CDCl_3$) δ [ppm]: 3.01 (dd, *J* = 27.6 Hz, *J* = 4.5 Hz, 1H, H-1), 3.34 (dddd, *J* = 6.9 Hz, *J* = 6.3 Hz, *J* = 4.5 Hz, *J* = 3.0 Hz, 1H, H-2), 3.51 (dd, *J* = 11.4 Hz, *J* = 3.0 Hz, 1H, H-3a), 3.59 (dd, *J* = 11.4 Hz, *J* = 6.9 Hz, 1H, H-3b), 4.97–4.85 (m, 4H, C*H*$_2$OP), 7.61–7.17 (m, 25H, Ar-H); ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ [ppm]: 49.2 (d, *J* = 203.8 Hz, C-1), 56.8 (s, C-3) 62.9 (s, C-2), 68.0 and 68.3 (2d, *J* = 6.0 Hz, Ph*C*H$_2$OP), 87.3, 128.0, 128.0, 128.1, 128.1, 128.7, 128.7, 128.8, 136.9, 137.0, 143.8; ^{31}P-NMR (121.5 MHz, $CDCl_3$) δ [ppm]: 20.01. Anal. calcd. for $C_{36}H_{33}O_5P$: C, 74.99; H, 5.77. Found: C, 75.29; H, 5.58.

Dibenzyl (1R,2R)-1,2-epoxy-3-hydroxypropylphosphonate ***8***

As described above, from the diol (1*S*,2*R*)-**5** (0.420 g, 1.05 mmol) dissolved in THF (6 mL) and K_2CO_3 (0.22 g. 1.56 mmol), the epoxyphosphonate (1*R*,2*R*)-**8** (0.280 g, 80%) was obtained after chromatography on a silica gel column with chloroform / methanol (100 : 1, v / v) as a colourless oil; $[\alpha]_D^{20} = +14.9$ (*c* = 0.8, $CHCl_3$); IR (film) ν [cm^{-1}]: 698, 740, 866, 1009, 1216, 1248, 1456, 2898, 2954, 3034, 3065, 3392; ^{1}H-NMR (300 MHz, $CDCl_3$) δ [ppm]: 3.09 (dd, *J* = 30.9 Hz, *J* = 2.4 Hz, 1H, H-1), 3.36 (dddd, *J* = 4.8 Hz, *J* = 3.3 Hz, *J* = 2.7 Hz, *J* = 2.4 Hz, 1H, H-2), 3.56 (dd, *J* = 12.9 Hz, *J* = 3.3 Hz, 1H, H-3a), 3.98 (ddd, *J* = 12.9 Hz, *J* = 2.7 Hz, *J* = 0.9 Hz, 1H, H-3b), 5.16–5.02 (m, 4H, CH_2OP), 7.38–7.26 (m, 10H, Ar-H); ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ [ppm]: 47.5 (d, *J* = 203.0 Hz, C-1), 56.8 (s, C-2), 60.2 (d, *J* = 0.7 Hz, C-3), 68.6 and 68.7 (2d, *J* = 6.0 Hz, Ph*C*H$_2$OP), 128.2, 128.3, 128.7, 128.8, 128.8, 135.8 (d, *J* = 4.8 Hz, C_{ipso}), 135.8 (d, *J* = 6.0 Hz, C_{ipso}); ^{31}P-NMR (121.5 MHz, $CDCl_3$) δ [ppm]: 20.32. Anal. calcd. for $C_{17}H_{19}O_5P \times 0.75\ H_2O$: C, 58.70; H, 5.94. C, 58.67; H, 6.12.

Cyclohexylammonium salt of (1R,2R)-1,2-epoxy-3-hydroxypropylphosphonic acid ***2***

A solution of the epoxyphosphonate (1*R*,2*R*)-**8** (0.11 g, 0.47 mmol) in THF (7 mL) was hydrogenated over 10% Pd / C (6 mg) in the presence of cyclohexylamine (0.11 mL, 0.94 mmol) at room temperature for 24 h. After removal of a catalyst on a layer of Celite, the residue was chromatographed on silica gel with water / methanol (1: 1, v / v) to give cyclohexylammonium salt of the epoxyphosphonic acid (1*R*,2*R*)-**2** (0.11 g, 92%) as grey needles which decomposed after heating above 200°C; $[\alpha]_D^{20} = +7.2$ (*c* = 1.1, H_2O); IR (KBr) ν [cm^{-1}]: 879, 976, 1061, 1085, 1228, 1323, 1395, 1535, 1633, 2548, 2599, 2642, 2701, 2857, 2943, 3428; ^{1}H-NMR (250 MHz, $CDCl_3$) δ [ppm]: 1.75–1.60 (m, 2H,), 1.80–1.76 (m, 4H), 2.10–1.90 (m, 4H), 2.75 (dd, *J* = 21.6 Hz, *J* = 3.0 Hz, 1H, H-1), 3.12–3.07 (m, 1H), 3.26 (dddd, *J* = 5.8 Hz, *J* = 3.0 Hz, *J* = 2.5 Hz, *J* = 2.2 Hz, 1H, H-2), 3.49 (dd, *J* = 12.9 Hz, *J* = 5.8 Hz, 1H, H-3a), 3.92 (dd, *J* = 12.9 Hz, *J* = 2.2 Hz, 1H, H-3b); ^{13}C-NMR (62.9 MHz, $CDCl_3$) δ [ppm]: 21.9, 22.4, 28.5, 48.4, 50.3 (d, *J* = 175.8 Hz, C-1), 60.1, 65.5; ^{31}P-NMR (101.3 MHz, $CDCl_3$) δ [ppm]: 11.16. Anal. calcd. for $C_9H_{20}NO_5P \times ½\ H_2O$: C, 41.22; H, 8.07; N, 5.34. Found: C, 40.95; H, 8.27; N, 5.59.

Antimicrobial activity tests

The method used for the bacterial susceptibility tests followed the instruction M7-A7 of the Clinical and Laboratory Standards Institute (CLSI) [32]. Stock solutions of phosphonates (Fig. 1) in dimethylsulfoxide (DMSO – analytical grade) were prepared at a concentration of 5000 μg/mL and sterilized by filtration. Series of twofold dilutions were prepared in Mueller–Hinton broth in a microtiter tray. Test strains (*Staphylococcus aureus* (ATCC 25923, ATCC 6538P), *Escherichia coli* (ATCC 8739, ATCC 35218, ATCC 25922), *Pseudomonas aeruginosa* ATCC 27853, *Bacillus subtilis* ATCC 6633, *Enterococcus faecalis* ATCC 29212) were then inoculated to each well to achieve a final concentration of 10^6 organism per 1 mL. After 24 h of incubation at 37°C, the increase of turbidity was measured. The MIC values represent the lowest concentrations of the investigated compounds with no measurable bacterial growth increase.

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