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Synthesis of α-aryl substituted and conformationally restricted fosmidomycin analogues as promising antimalarials

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Abstract

Fosmidomycin represents a new antimalarial drug, acting by inhibition of 1-deoxy-D-xylulose 5-phosphate reductoisomerase, an essential enzyme of the mevalonate independent pathway of isoprenoid biosynthesis. This work describes the synthesis of a series of α-aryl substituted fosmidomycin analogues exhibiting improved antimalarial activity. A linear synthetic route, involving a 3-aryl-3-phosphoryl propanal intermediate, proved practical to prepare these derivatives. A phospha-Michael addition to cyclopent-2-enone gave access to conformationally restricted analogues.

1. Introduction

In the 1970s Kuemmerle and co-workers reported the first isolation of fosmidomycin as a structurally simple antibiotic from *Streptomyces lavendulae*. In recent years, fosmidomycin received considerable attention due to its promising antimalarial activity and recent clinical trials conducted in Gabon and Thailand confirmed the potential of fosmidomycin as antimalarial drug.[1,2] In 1998 the molecular target of fosmidomycin was discovered to be 1-deoxy-D-xylulose 5-phosphate (DOXP) reductoisomerase.[3] This enzyme plays an essential role in the mevalonate-independent pathway for the synthesis of isoprenoids and is absent in humans.[4] Fosmidomycin was found to be a potent inhibitor for DOXP reductoisomerase (DXR) of *P. falciparum*.[5,6] After this important discovery much attention has been focused on the chemical synthesis of fosmidomycin analogues. FR900098, the acetyl analogue of fosmidomycin, was shown to be approximately twice as active against *P. falciparum in vitro* as well as in a *P. vinckei* mouse model.[3]

Chemical variations of fosmidomycin were mainly directed to increase the inhibitory activity against DXR or to achieve inhibitors with improved physicochemical properties. To study the structure-activity relationships, hydroxamic moiety modifications, including benzoxazolone and oxazolopyridinone functionalities, have been reported.[7] Also, the phosphonate moiety has been altered to produce prodrugs with improved oral bioavailability.[8,9,10]

Surprisingly, modifications addressing the three carbon spacer are scarce. Recently, we reported the discovery of a series of α-aryl substituted fosmidomycin or FR900098 derivatives **1** and **2**, which generally proved superior to fosmidomycin in inhibiting *P. falciparum* growth.[11] To sort out the influence of the lipophilicity and electronic properties of this phenyl moiety, substituents were introduced according to Topliss' methodology.[12]

Briefly, in this methodology an operational scheme is used to quickly identify the optimum substitution on a benzene ring for maximizing drug potency by virtue of resulting changes in hydrophobic, electronic and steric effects.

Here, we describe the detailed procedure used to synthesize these α-substituted analogues. Although strategies to synthesise products with a C-P bond are well documented,[13] introducing aryl substituents in α-position of a phosphonate (resulting in a P-CH(Ar)-C motive) is quite challenging. Fosmidomycin was first synthesised in the early eighties by Hemmi et al. using a Michaelis-Becker reaction.[14] This approach cannot be easily adapted to allow the synthesis of α-substituted derivatives.

In this study 3-aryl substituted 3-phosphoryl propanals were anticipated to be appropriate intermediates for the synthesis of a small series of α-aryl substituted fosmidomycin analogues. Depending on the availability of the starting material, a lithiation-allylation-alkene oxidation sequence or a Michael addition will be considered for the synthesis of these intermediates (Scheme 1). A drawback of this strategy is that every derivative has to be synthesized *de novo*, which does not permit to prepare an extended series of the envisaged analogues. However, when the proposed routes allow to obtain the desired analogues in good overall yields, they might be valuable for scale-up purposes, e.g., to prepare a selected inhibitor for *in vivo* studies. Interestingly, when applied to cyclopent-2-enone the Michael addition should be a useful approach to design unprecedented fosmidomycin analogues **3** and **4**, in which the 3C spacer is part of a cyclopentane ring. Indeed, by incorporating the α- and β-carbon in a cyclopropane ring, we recently demonstrated that rigidification of fosmidomycin might result in potent DXR inhibitors.[15]

2. Result and discussion

2.1. Synthesis of α-aryl substituted fosmidomycin and FR900098 analogues

Retrosynthetic analysis toward the synthesis of the desired α-substituted fosmidomycin analogues is depicted in Scheme 1.

[Scheme 1]

Two synthetic pathways toward the aldehyde synthons were followed (Scheme 2). The first one started from the appropriate diethyl benzylphosphonate, which upon treatment with n-BuLi in the presence of allyl bromide, afforded **6a**,**b** in 97 and 33 % yield.[16] Oxidation of **6a**,**b** to the vicinal *cis*-diol with osmium tetraoxide in the presence of 4-methylmorpholine *N*-oxide followed by sodium periodate cleavage gave aldehydes **9a**,**b**, which could be used in the next step without further purification.

[Scheme 2]

When the desired benzylphosphonate was not commercially available, an alternative strategy to prepare the desired aldehydes was followed. A 1,4-addition of triethyl phosphite to the appropriately substituted cinnamaldehyde in the presence of phenol gave the acetals **8c**-**e** in 70-85 % yield.[17] Subsequent deprotection of the diphenyl acetal afforded in 76-83 % yield the corresponding aldehydes, which appeared stable enough to be purified by flash chromatography. If necessary, substituted cinnamaldehydes were synthesized. Several procedures are described in the literature. In our hands a palladium-catalyzed synthesis from acrolein diethyl acetal and the corresponding aryl iodide was very efficient.[18] Only the (*E*)-isomer was obtained as deduced from the large coupling (16 Hz) between the vinylic hydrogens.

[Scheme 3]

Conversion of the appropriate aldehydes to the desired analogues **1** and **2** is depicted in Scheme 3. Treatment of **9a**-**e** with *O*-benzylhydroxylamine yielded (67-92 %) oximes **10a**-**e**. ^{13}C-NMR revealed the presence of two geometric isomers, which were reduced with sodium

cyanoborohydride to produce the benzyloxyamines **11a-e** in 91-96 % yield. Subsequent acetylation of 11a-e with acetyl chloride afforded **13a-e** in good yield. For the formylation of **11** different methods were investigated. Since the mixed anhydride method was unsuccessful, compound **11a** was formylated with 2-thioxothiazolidine-3-carbaldehyde, prepared by reaction between 2-mercaptothiazoline and formic acid using DCC as coupling agent. A drawback of this approach was the long reaction time (more than 3 days at room temperature). Consequently, **11c**,**d**,**e** were formylated using formic acid and 1,1'-carbonyl-diimidazole in dichloromethane. This method reduced the reaction time considerably.

Benzyl deprotection by catalytic hydrogenation proved tricky, especially in the formyl series, where this reaction generally led to the formation of two reaction products. After their separation, MS was useful to assign these compounds as the desired product and the corresponding deoxygenated derivative, i.e. the amide. Further structural evidence for this deoxygenation was furnished by a ^{1}H COSY NMR experiment of the side product, showing a strong coupling between the NCH_2 protons and a heteroatom bound proton at 7.04 ppm, which is normally absent in the desired products as may be expected for such long range ^{4}J (CH_2NOH) coupling. Also the characteristic ^{13}C NMR upfield shifts of the N-CH_2 carbon are in agreement with the absence of the OH-group on nitrogen (β-substituent effect). Indeed, for the deoxygenated product the N-CH_2 signal appeared at 35.8 ppm, while for product **14c** two signals at 47 and 44 ppm were found. This indicates that **14c** (and also **14e**) exists as a mixture of *syn* and *anti* NOH rotamers in a 2:1 ratio.

Compounds **14c**,**e** and **15a-e** were finally deprotected with 4 eq of TMSBr in CH_2Cl_2 at ambient temperature to afford pure **1c**,**e** and **2a-e** after purification by reversed phase HPLC. Although this reaction was almost quantitative, minor amounts of deacylated products were detected, probably due to small amounts of HBr in the TMSBr reagent.

2.2. Synthesis of conformationally restricted fosmidomycin and FR900098 analogues

The approach used to convert the cinnamaldehydes **7c**-**e** to the corresponding α-substituted fosmidomycin derivatives, was also successfully applied to prepare four 5-membered cyclic fosmidomycin analogues from cyclopent-2-enone (Scheme 4). Michael addition of triethyl phosphite to this cyclic α,β-unsaturated ketone gave direct access to the diethyl 3-oxocyclopentylphosphonate.[17] The remaining part of the synthesis involved the same transformations as used for the α-aryl phosphonates. Separation of the diastereomeric pairs was realized after the hydrogenolysis. The *cis*- and *trans*-isomers were assigned by ^{1}H NOEDIF NMR experiments: an interaction between the NOH and the methyls of the phosphonate ester was observed for *cis*-**22** and cis-**23**, as opposed to the *trans* isomer where such a contact was missing. The ^{13}C NMR spectra of compounds **22** further point to the presence of a major and a minor form, most probably as a result of restricted rotation in the hydroxamic group with preferential formation of the syn isomer due to a likely hydrogen bond between NOH and the carbonyl.

By using the described procedures, we have synthesized eleven analogues, allowing to perform initial SAR studies for the α-aryl series.[11] Although these studies revealed that the α-aryl analogues were generally weaker *E. coli* DXR inhibitors than fosmidomycin, these analogues unambiguously surpassed the activity of fosmidomycin to inhibit *P. falciparum* growth. Remarkably, the formyl analogues **1c** and **1e** consistently outperformed the acetyl derivatives **2c** and **2e**, both in the enzyme and the parasite growth inhibition assay.

With an IC_{50} value of 0.036 μM compound **1e** emerged as the most promising analogue. Amongst the fosmidomycin analogues in which the C-C-C spacer is part of a cyclopentane ring, the *trans* analogues proved notably more active than the *cis* isomers (Table 1). This is in agreement with recent results obtained with cyclopropane fosmidomycin analogues, where a *trans* orientation of the phosphonate group and the hydroxyamide moiety also yielded the

most potent inhibitor.[15] Remarkably, in the cyclopropane series, the inhibitory activity of the formyl analogues surpassed that of the acetyl derivatives, while an opposite trend was observed in the cyclopropane series.

3. Conclusion

In conclusion, a synthetic procedure for the preparation of α-aryl substituted fosmidomycin analogues was developed starting from (a ring substituted) benzylphosphonate. Alternatively, these analogues were also accessible via a Michael addition of triethyl phosphite to an appropriate cinnamaldehyde. The latter method was also successfully used to prepare a series of cyclopentyl analogues of fosmidomycin.

4. Experimental

General

IUPAC names were generated with Chemdraw Ultra 8.0 (Chemoffice 2004, Cambridge Soft, Cambridge, USA). Most reactions were carried out under inert (N_2) atmosphere. Precoated Merck silica gel F_{254} plates and precoated Macherey-Nagel (Düren, Germany) silica gel F_{254} plates were used for TLC and spots were examined under UV light at 254 nm and revealed by a phosphomolybdic-cerium sulphate solution, iodine vapour or a dinitrophenol solution. Column chromatography was performed on ICN silica gel (63-200 μM). NMR spectra were obtained with a Varian Mercury 300 spectrometer. Chemical shifts are given in parts per million (ppm) (δ relative to residual solvent peak, in the case of DMSO-d_6 2.54 ppm for ^{1}H and 40.5 ppm for ^{13}C, in the case of $CDCl_3$ 7.26 ppm for ^{1}H and 77.4 ppm for ^{13}C and in the case of acetone 2.05 ppm for ^{1}H and 29.84 and 206.26 ppm for ^{13}C. Coupling constants are expressed in Hz. Abbreviations used are: s = singlet, d = doublet, t = triplet, q = quartet, m =

multiplet, br = broad. All signals assigned to hydroxyl and to amino groups were exchangeable with D_2O. Structural assignment was confirmed with COSY, DEPT, HMQC and/or NOEDIF/NOESY if necessary. Mass spectra and exact mass measurements were performed on a quadrupole/orthogonal-acceleration time-of-flight (Q/oaTOF) tandem mass spectrometer (qTof 2, Micromass, Manchester, U.K.) equipped with a standard electrospray ionization (ESI) interface. Samples were infused in a acetonitrile/water (1:1) mixture at 3μL/min. Most chemicals were obtained from Sigma-Aldrich or Acros Organics and were used without further purification.

Diethyl 1-phenylbut-3-enylphosphonate (6a)

To a stirred solution of **5a** (12 mL, 57.4 mmol) in dry THF (100 mL), cooled at -50 to -70 °C, was added under N_2 atmosphere a 1.6 M solution of *n*BuLi (39 mL, 63.2 mmol) in hexane. After stirring for 15 minutes at the same temperature allyl bromide (5 mL, 57.4 mmol) was added. One hour after this addition the reaction mixture was refluxed for 2 h. After cooling to room temperature the reaction mixture was evaporated *in vacuo*, and the resulting oil was diluted with toluene (200 mL), washed with 10% NH_4Cl (200 mL) and water (200 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Purification of the residue by flash chromatography (*n*-hexane/ethyl acetate 8:2→7:3→6:4) yielded compound **6a** as a transparent oil (14.96 g, 97%).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.06 (3H, t, *J* = 7.0 Hz, OCH_2CH_3); 1.25 (3H, t, *J* = 7.0 Hz, OCH_2CH_3); 2.61 – 2.74 (1H, m, allyl CH_2); 2.76 – 2.88 (1H, m, allyl CH_2); 3.05 (1H, ddd, $J_{H,P}$ = 22.0 Hz, *J* = 4.4 Hz en *J* = 11.1 Hz, CHP); 3.62 – 3.75 (1H, m, OCH_2CH_3); 3.80 – 3.93 (1H, m, OCH_2CH_3); 3.95 – 4.09 (2H, m, OCH_2CH3); 4.85 – 4.89 (1 H, m, $CH=CH_{2,\ cis}$); 4.93 – 5.00 (1 H, m, $CH=CH_{2,\ trans}$); 5.51 – 5.65 (1 H, m, $CH=CH_2$), 7.17 – 7.30 (5 H, m, arom. H) ppm.

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 16.46 (d, $^3J_{C,P}$ = 5.7 Hz, OCH_2CH_3); 16.63 (d, $^3J_{C,P}$ = 6.0 Hz, OCH_2CH_3); 34.26 (d, $^2J_{C,P}$ = 2.9, CH_2CHP); 44.81 (d, $^1J_{C,P}$ = 137.1 Hz, CHP); 62.00 (d, $^2J_{C,P}$ = 7.2 Hz, OCH_2CH_3); 62.77 (d, $^2J_{C,P}$ = 7.2 Hz, OCH_2CH_3); 117.03; 127.34; 128.62; 129.55; 135.56; 135.82 ppm.

Exact mass (ESI-MS): calculated for $C_{14}H_{22}O_3P$ $[M+H]^+$: 269.1306; found: 269.1292

General method for synthesis of **8c-e**

A mixture of the appropriate acryl aldehyde (6.17 mmol), triethylphosphite (1.34 mL, 7.71 mmol) and phenol (1.54 g, 16 mmol) was heated to 100 °C. After 24 h TLC analysis (hexane/ethyl acetate 6:4) indicated that the reaction was finished and the reaction mixture was subsequently evaporated. The crude product was purified by flash chromatography hexane/ethyl acetate 6:4. After evaporation of the pure fractions, the desired acetals **8c-e** were obtained as slightly yellow oils.

Diethyl 1-(3,4-dichlorophenyl)-3,3-diphenoxypropylphosphonate (8e). Yield: 70% (2.29 g).

^{1}H-NMR (300 MHz, $CDCl_3$): δ= 1.13 (3H, t, *J* = 7.0 Hz, OCH_2CH_3); 1.25 (3H, t, *J* = 7.0 Hz, OCH_2CH_3); 2.43 – 2.57 (1H, m, $PCHCH_2$); 2.70 – 2.82 (1H, m, $PCHCH_2$); 3.38 (1H, ddd, $J_{H,P}$ = 22.7 Hz, *J* = 4.7 Hz and *J* = 10.3 Hz, CHP); 3.74 – 3.87 (1H, m, OCH_2CH_3); 3.89 – 3.99 (1H, m, OCH_2CH_3); 3.99 – 4.12 (2H, m, OCH_2CH_3); 5.66 (1H, dd, *J* = 6.8 Hz and *J* = 4.4 Hz, $CH(OPh)_2$); 6.84 – 6.92 (3H, m, arom. H); 6.97 – 7.03 (2H, m, arom. H); 7.18 – 7.27 (6H, m, arom. H); 7.37 – 7.45 (2H, m, arom. H) ppm.

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 16.51 (app t, $^3J_{C,P}$ = 5.8 Hz, OCH_2CH_3); 34.61 (d, $^2J_{C,P}$ = too small for detection, $PCHCH_2$); 39.70 (d, $^1J_{C,P}$ = 139.9 Hz, CHP); 62.57 (d, $^2J_{C,P}$ = 7.2 Hz, OCH_2CH_3); 63.16 (d, $^2J_{C,P}$ = 6.6 Hz, OCH_2CH_3); 99.34 (d, $^3J_{C,P}$ = 15.8 Hz, $CH(OPh)_2$);

117.66 (=CH); 117.68 (=CH); 122.99 (=CH); 123.01 (=CH); 128.75 (d, =CH); 129.83 (=CH); 129.85 (=CH); 130.81 (d, =CH); 131.29 (d, =CH); 131.87 (d, =C); 133.01 (d, =C); 136.27 (d, =C); 155.95 (=C); 156.04 (=C) ppm.

Exact mass (ESI-MS): calculated for $C_{25}H_{27}Cl_2O_5PNa$ $[M+Na]^+$: 531.0871; found: 531.0872

General method for synthesis of **9a,b**

To a mixture of alkene **6a** or **6b** (6.56 mmol) and 4-methylmorpholine *N*-oxide (0.92 g, 7.87 mmol) in dioxane (40 mL) was added an aqueous 1 % solution of OsO_4 (99.1 mg, 0.39 mmol). After stirring overnight at room temperature and protected from light, the starting material was completely converted according to TLC. Then sodium periodate (2.24 g, 10.5 mmol) was added in small portions. After completion of the reaction (2 h), the mixture was diluted with ethyl acetate (100 mL), filtered through celite, and solids were washed with ethyl acetate. The combined filtrates were washed with saturated aqueous NaCl (100 mL), dried over $MgSO_4$, and evaporated under vacuum to yield crude **9a** or **9b**, which were used in the next step whitout further purification.

General method for synthesis of **9c-e**

Acetals **8c-e** (5.0 mmol) were hydrolyzed by treatment with a mixture of water (7 mL), acetone (35 mL) and 2 N HCl (8 mL). After heating to 60-70 °C for 3-4 h TLC analysis (ethyl acetate) confirmed that the reaction was finished. The solvents were evaportated under *vacuo* and the residue was dissolved in ethyl acetate (200 mL) and transferred to a separatory funnel were it was washed twice with water (200 mL). The organic layer was dried with $MgSO_4$ and evaporated. The residue was purified by flash chromatography using ethyl acetate as eluens yielding **9c-e** as transparent oils.

Remark: NMR revealed by disappearance of CH=O, that **9a-e** are prone to oxidation upon storage when dissolved in $CDCl_3$.

Diethyl 1-(3,4-dichlorophenyl)-2-formylethylphosphonate (9e). Yield: 76% (1.28 g).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.11 (3H, t, *J* = 7.0 Hz, OCH_2CH_3); 1.23 (3H, t, *J* = 7.0 Hz, OCH_2CH_3); 2.95 – 3.20 (2H, m, $PCHCH_2$); 3.62 (1H, ddd, $J_{H,P}$ = 22.7 Hz, *J* = 4.7 Hz and *J* = 9.7 Hz, CHP); 3.74 – 3.83 (1H, m, OCH_2CH_3); 3.84– 3.95 (1H, m, OCH_2CH_3); 3.96– 4.07 (2H, m, OCH_2CH3); 7.12 – 7.40 (4H, m, arom. H); 9.61 – 9.62 (3H, m, HC=O) ppm.

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 16.54 (app t, $^3J_{C,P}$ = 6.2 Hz, OCH_2CH_3); 37.20 (d, $^1J_{C,P}$ = 141.9 Hz, CHP); 44.09 (d, $^2J_{C,P}$ = 2.3 Hz, $PCHCH_2$); 62.75 (d, $^2J_{C,P}$ = 7.2 Hz, OCH_2CH_3); 63.34 (d, $^2J_{C,P}$ = 6.9 Hz, OCH_2CH_3); 128.70 (d, $^3J_{C,P}$ = 6.3 Hz, $=C_oH$); 130.75 (d, $^4J_{C,P}$ = 2.6 Hz, $=C_mH$); 131.15 (d, $^3J_{C,P}$ = 6.9 Hz, $=C_oH$); 131.95 (d, $^5J_{C,P}$ = 3.7 Hz,. $=C_p$); 132.92 (d, $^4J_{C,P}$ = 2.9 Hz, $=C_m$); 136.03 (d, $^2J_{C,P}$ = 7.2 Hz, $=C_i$); 198.13 (d, $^3J_{C,P}$ = 15.0 Hz, HC=O) ppm.

Exact mass (ESI-MS): calculated for $C_{13}H_{18}Cl_2O_4P$ $[M+H]^+$: 339.03204; found: 339.0325

General method for synthesis of **10a-e** and **18**

A mixture of aldehydes **9a-e** (3.86 mmol) and *O*-benzylhydroxylamine hydrochloride (0.61 g, 3.86 mmol) in pyridine/ethanol, 1:1 (14 mL) was stirred for 1.5 to 6 h at room temperature under nitrogen. After the solvent was removed by evaporation, the residue was coevaporated three times with toluene and subsequently chromatographed on a silica gel column (*n*-hexane/ethyl acetate 6:4 or 6:4→1:1) to give a mixture of benzyloxyimines **10a-e** as transparent oils.

(*E*) and **(*Z*)-Diethyl 3-(benzyloxy)imino-1-(3,4-dichlorophenyl)propylphosphonate (10e).** Yield: 92% (1.57 g)

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.17 (3H, dt, *J* = 7.0 Hz, $J_{H,P}$ = 1.47 Hz, OCH_2CH_3); 1.28 (3H, dt, *J* = 7.0 Hz, $J_{H,P}$ = 3.2 Hz, OCH_2CH_3); 2.72 – 3.05 (2H, m, $CHPCH_2$); 3.17 – 3.32 (1H, m, PCH); 3.81 – 3.90 (1H, m, OCH_2CH_3); 3.93 – 3.98 (1H, m, OCH_2CH_3); 3.99 – 4.10 (2H, m, OCH_2CH3); 4.97 (1H, s, OCH_2Ph); 5.08 (1H, s, OCH_2Ph); 6.55 (1H, t, *J* = 5.3 Hz, HC=N); 7.11 – 7.39 (8H, m, arom. H) ppm.

Exact mass (ESI-MS): calculated for $C_{20}H_{25}Cl_2NO_4P$ $[M+H]^+$: 444.090; found: 444.091

Diethyl 3-(benzyloxy)iminocyclopentylphosphonate (18). Yield: 90% (3.07 g).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.30 (3H, t, *J* = 7.0 Hz, OCH_2CH_3); 1.31 (3H, t, *J* = 7.0 Hz, OCH_2CH_3); 1.80 – 2.89 (7H, m, C_5H_7P); 4.04 – 4.15 (4H, m, OCH_2CH_3); 5.06 (2H, s, CH_2Ph); 7.26 – 7.34 (5H, m, arom. H) ppm.

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 16.74 (d, $^{3}J_{C,P}$ = 5.8 Hz, OCH_2CH_3); 25.68 (d, $J_{C,P}$ = 2.6 Hz, CH_2); 26.03 (d, $J_{C,P}$ = 3.2 Hz, CH_2); 27.92 (d, $J_{C,P}$ = 12.1 Hz, CH_2); 29.19 (d, $J_{C,P}$ = 1.7 Hz, CH_2); 30.91 (d, $J_{C,P}$ = 11.5 Hz, CH_2); 32.07 (d, CH_2); 34.77 (d, $J_{C,P}$ = 151.4 Hz, CHP); 34.99 (d, $J_{C,P}$ = 151.7 Hz, CHP); 62.06 (m, OCH_2CH_3); 75.92 (OCH_2Ph); 75.95 (OCH_2Ph); 127.93 (=CH); 127.95 (=CH); 128.16 (=CH); 128.18 (=CH); 128.56 (=CH); 128.55 (=CH); 138.26 (=C); 138.33 (=C); 164.12 (d, $^{3}J_{C,P}$ = 13.8 Hz, C=N); 164.33 (d, $^{3}J_{C,P}$ = 15.0 Hz, C=N) ppm.

^{31}P-NMR (120 MHz, $CDCl_3$): δ = 32.12 and 32.36 ppm.

Exact mass (ESI-MS): calculated for $C_{16}H_{25}NO_4P$ $[M+H]^+$: 326.1521; found: 326.1523

General procedure for the reduction of the *O*-benzyloximes **10a-e** to **11a-e** and **18** to **19**

Sodium cyanoborohydride (12.95 mmol, 0.81 g) was added to a solution of *O*-benzyloximes **10a-e** (2.59 mmol) in methanol (15 mL). Two drops of methyl orange indicator were added followed by dropwise addition of concentrated hydrochloric acid, until the solution remained pink and milky for at least half an hour. The reaction mixture was stirred for 3 to16 h at room

temperature. The solvent was removed under *vacuo*. The residue was taken up in CH_2Cl_2 (100 mL) and washed until alkaline with 1 M potassium hydroxide solution and extracted thrice with CH_2Cl_2 (3 × 100 mL). The combined organic extracts were dried $MgSO_4$, filtered and the solvent was removed. The residue was brought on silica column and eluted with CH_2Cl_2/MeOH 95:5 or *n*-hexane/ethyl acetate 4:6. After evaporation of the appropriate fractions *O*-benzyloxyamines **11a-e** were obtained as clear oils.

Diethyl 3-(benzyloxyamino)-1-(3,4-dichlorophenyl)propylphosphonate (11e). Yield: 91% (1.05 g).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.16 (3H, t, *J* = 7.0 Hz, OCH_2CH_3); 1.28 (3H, t, *J* = 7.0 Hz, OCH_2CH_3); 1.97 – 2.13 (1H, m, CH_2CHP); 2.28 – 2.42 (1H, m, CH_2CHP); 2.64 – 2.74 (1H, m, CH_2N); 2.85 – 2.93 (1H, m, CH_2N); 3.20 (1H, ddd, $J_{H,P}$ = 22.6 Hz, *J* = 4.1 Hz and *J* = 11.1 Hz, CHP); 3.77 – 3.89 (1H, m, OCH_2CH_3); 3.91 – 3.99 (1H, m, OCH_2CH_3); 3.99 – 4.13 (2H, m, OCH_2CH_3); 4.61 – 4.70 (2H, m, $PhCH_2O$); 7.13 – 7.17 (1H, m, arom. H); 7.26 – 7.40 (7H, m, arom. H) ppm.

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 16.56 (d, $^3J_{C,P}$ = 7.8 Hz, OCH_2CH_3); 16.64 (d, $^3J_{C,P}$ = 8.1 Hz, OCH_2CH_3); 27.73 (d, $^2J_{C,P}$ = 2.9 Hz, CH_2CHP); 41.35 (d, $^1J_{C,P}$ = 139.6 Hz, CHP); 49.48 (d, $^3J_{C,P}$ = 15.3 Hz, NCH_2); 62.36 (d, $^2J_{C,P}$ = 6.9 Hz, OCH_2CH_3); 62.88 (d, $^2J_{C,P}$ = 6.9 Hz, OCH_2CH_3); 77.88 (OCH_2Ph); 128.13 (=CH); 128.61 (=CH); 128.63 (=CH); 128.87 (d, $J_{C,P}$ = 6.6 Hz, =CH); 130.65 (d, $J_{C,P}$ = 2.6 Hz, =CH); 131.42 (d, $J_{C,P}$ = 6.9 Hz, =CH); 131.53 (=C); 132.74 (d, $J_{C,P}$ = 2.9 Hz, =C); 136.73 (d, $J_{C,P}$ = 6.9 Hz, =C); 137.88 (=C) ppm.

Exact mass (ESI-MS): calculated for $C_{20}H_{27}Cl_2NO_4P$ $[M+H]^+$: 446.1055; found: 446.1060

Diethyl 3-(benzyloxyamino)cyclopentylphosphonate (19). Yield: 80% (1.83 g, mixture of *cis* and *trans*).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.30 (3H, t, *J* = 7.0 Hz, OCH_2CH_3); 1.30 (3H, t, *J* = 7.0 Hz, OCH_2CH_3); 1.42 – 2.40 (8H, m, C_5H_8P); 3.58 – 3.69 (1H, m, NH); 4.03 – 4.15 (4H, m, OCH_2CH_3); 4.69 (1H, s, CH_2Ph); 4.75 (1H, s, CH_2Ph); 7.26 – 7.56 (5H, m, arom. H) ppm.

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 16.76 (d, $^3J_{C,P}$ = 5.8 Hz, OCH_2CH_3); 25.27 (d, $J_{C,P}$ = 2.9 Hz, CH_2); 25.72 (d, $J_{C,P}$ = 2.6 Hz, CH_2); 30.18 (s, CH_2); 30.34 (s, CH_2); 31.54 (d, $J_{C,P}$ = 2.3 Hz, CH_2); 31.67 (d, CH_2); 33.73 (d, $J_{C,P}$ = 147.9 Hz, CHP); 34.55 (d, $J_{C,P}$ = 147.4 Hz, CHP); 61.65 – 62.13 (d, OCH_2CH_3 and 2 x d, NCH); 77.59 (OCH_2Ph); 76.95 (OCH_2Ph); 128.11 (=CH);128.61 (=CH); 128.67 (=CH); 129.57 (=CH); 130.42; 137.86 (=C) ppm.

Exact mass (ESI-MS): calculated for $C_{16}H_{27}NO_4P$ $[M+H]^+$: 328.1678; found: 328.1660

Two methods have been used for the formylation of compounds **11** and **19**.

Method A involves the use of 2-thioxothiazolidine-3-carbaldehyde, which was obtained as follows. Formic acid (1 eq.) and 2-mercaptothiazoline (1 eq.) were dissolved in CH_2Cl_2 (0.5 M), cooled to 0 °C and DCC (1 eq.) was added in one portion. After the reaction mixture was filtered and evaporated, the residue was chromatographed (CH_2Cl_2) to afford 2-thioxothiazolidine-3-carbaldehyde as a yellow solid.

Diethyl 3-(N-(benzyloxy)formamido)-1-(phenyl)propylphosphonate (12a).

2-Thioxothiazolidine-3-carbaldehyde (1 eq.) was dissolved in CH_2Cl_2 and added to a solution of **11a** (1 eq.) in CH_2Cl_2 (0.1 M). The reaction mixture was stirred for 3 days. The reaction mixture was extracted with water, dried over $MgSO_4$ and evaporated *in vacuo*. The residue was purified by flash chromatography (CH_2Cl_2/MeOH 95:5) to yield **12a** in a 89% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.07 (3H, t, *J* = 7.0 Hz, OCH_2CH_3); 1.26 (3H, t, *J* = 7.0 Hz, OCH_2CH_3); 2.17 (1H, m, CH_2CHP); 2.45 – 2.48 (1H, m, CH_2CHP); 3.06 (1H, ddd, $J_{H,P}$ = 23.0 Hz, *J* = 4.1 Hz and *J* = 11.1 Hz, CHP); 3.39 (1H, m, CH_2N); 3.50 (1H, m, CH_2N); 3.62 – 3.75 (1H, m, OCH_2CH_3); 3.81 – 3.91 (1H, m, OCH_2CH_3); 3.97 – 4.10 (2H, m, OCH_2CH_3);

4.74 and 4.94 (2H, 2 x br s, PhC*H*$_2$O); 7.26 – 7.35 (10H, m, arom. H); 8.16 (1H, br s, HC=O) ppm.

^{13}C-NMR (75 MHz, CDCl$_3$): δ = 16.40 (d, $^3J_{C,P}$ = 5.7 Hz, OCH$_2$*C*H$_3$); 16.58 (d, $^3J_{C,P}$ = 6.0 Hz, OCH$_2$*C*H$_3$); 27.42 (m, *C*H$_2$CHP); 40 (d, $^1J_{C,P}$ = 140 Hz, CHP); 43.11 (m, NCH$_2$); 62.19 (d, $^2J_{C,P}$ = 7.2 Hz, O*C*H$_2$CH$_3$); 63.96 (d, $^2J_{C,P}$ = 7.2 Hz, O*C*H$_2$CH$_3$); 77.42 (O*C*H$_2$Ph); 127.77 (arom. C); 128.91 (arom. C); 129.26 (arom. C); 129.47 (arom. C); 129.56 (arom. C); 129.63 (arom. C); 131.10 (arom. C); 135.09 (arom. C); 163.32 (m, HC=O) ppm.

Mass (ESI-MS): calculated for $C_{21}H_{29}NO_5P$ $[M+H]^+$: 406.1783; found: 406.1

Method B for synthesis of **12c,e** and **20**

In a three-neck flask containing a solution of formic acid (0.61 mmol, 30 µl) in 0.6 mL CH_2Cl_2 was added 1,1'-carbonyl-diimidazol (0.64 mmol, 0.10 g). After 20 minutes benzyloxyamines **11c,e** (0.61 mmol) were dissolved in 1 mL CH_2Cl_2 and were transferred to the three-neck flask. After 5 h the mixture was partioned between water (70 mL) and CH_2Cl_2 (70 mL). The water layer was extracted twice with CH_2Cl_2 (70 mL). The combined organic layers were dried with $MgSO_4$ and evaporated *in vacuo* and the residue was purified by flash chromatography (*n*-pentane/acetone 6:4) to give **12c,e** as transparent oils.

Diethyl 3-(*N*-(benzyloxy)formamido)-1-(3,4-dichlorophenyl)propylphosphonate (12e).

Yield: 85% (245 mg).

^{1}H-NMR (300 MHz, CDCl$_3$): δ = 1.14 (3H, t, *J* = 7.0 Hz, OCH$_2$C*H*$_3$); 1.27 (3H, t, *J* = 7.0 Hz, OCH$_2$C*H*$_3$); 2.07 – 2.21 (1H, m, C*H*$_2$CHP); 2.37 – 2.51 (1H, m, C*H*$_2$CHP); 3.00 (1H, ddd, $J_{H,P}$ = 23.0 Hz, *J* = 4.1 Hz and *J* = 11.4 Hz, CHP); 3.23 – 3.38 (1H, m, CH$_2$N); 3.44 – 3.46 (1H, m, CH$_2$N); 3.78 – 3.88 (1H, m, OC*H*$_2$CH$_3$); 3.89 – 3.99 (1H, m, OC*H*$_2$CH$_3$); 3.99 – 4.11 (2H,

m, OC*H*$_2$CH$_3$); 4.75 and 4.91 (2H, 2 x br s, PhC*H*$_2$O); 7.12 – 7.40 (8H, m, arom. H); 8.16 (1H, br s, HC=O) ppm.

^{13}C-NMR (75 MHz, CDCl$_3$): δ = 16.50 (d, $^3J_{C,P}$ = 6.1 Hz, OCH$_2$*C*H$_3$); 16.61 (d, $^3J_{C,P}$ = 6.1 Hz, OCH$_2$*C*H$_3$); 27.34 (m, *C*H$_2$CHP); 41.46 (d, $^1J_{C,P}$ = 138.48 Hz, CHP); 42.50 (m, NCH$_2$); 62.59 (d, $^2J_{C,P}$ = 7.2 Hz, O*C*H$_2$CH$_3$); 63.09 (d, $^2J_{C,P}$ = 6.9 Hz, O*C*H$_2$CH$_3$); 78.29 (O*C*H$_2$Ph); 128.86 (arom. C); 128.95 (arom. C); 129.94 (arom. C); 129.69 (arom. C); 130.81 (arom. C); 131.24 (arom. C); 131.92 (arom. C); 132.93 (arom. C); 134.27 (arom. C); 135.87 (arom. C); 163.32 (m, HC=O) ppm.

Exact mass (ESI-MS): calculated for $C_{21}H_{27}Cl_2NO_5P$ $[M+H]^+$: 474.1004; found: 474.1000

Diethyl 3-(*N*-(benzyloxy)formamido)cyclopentylphosphonate (20). Yield: 97% (1.3 g, mixture of *cis* and *trans*).

^{1}H-NMR (300 MHz, CDCl$_3$): δ = 1.23 (6H, m, OCH$_2$C*H*$_3$); 1.64 – 2.39 (7H, m, C$_5$H$_7$P); 3.98 – 4.09 (4H, m, OC*H*$_2$CH$_3$); 4.4 (1H, m, CHN); 4.90 (2H, br s, OC*H*$_2$Ph); 7.30 – 7.32 (4H, m, arom. H); 8.12 (1H, br d, HC=O) ppm.

^{13}C-NMR (75 MHz, CDCl$_3$): δ = 16.76 (2C, d, $^3J_{C,P}$ = 5.8 Hz, OCH$_2$*C*H$_3$); 24.40 (d, CH$_2$); 25.60 (s, CH$_2$); 29.98 (d, CH$_2$); 33.31 (d, $^1J_{C,P}$ = 150.6 Hz, CHP); 33.66 (d, $^1J_{C,P}$ = 149.7 Hz, CHP); 57.73 (m, CHN), 59.03 (m, CHN); 62.01 (m, O*C*H$_2$CH$_3$); 79.50 (m, O*C*H$_2$Ph); 128.95 – 129.63 (three =CH); 134.72 (=C); 165.10 (m, HC=O) ppm.

Exact mass (ESI-MS): calculated for $C_{17}H_{27}NO_5P$ $[M+H]^+$: 356.1627; found: 356.1629

General method for the benzyl deprotection of **12**,**13, 20** and **21**.

A solution of compounds **12** and **13** or **20** and **21** (0.9 mmol) in MeOH (8 mL) was hydrogenated at atmospheric pressure in the presence of Pd 10 wt. % on activated carbon (40 mg). After 5 h stirring the reaction mixture was filtered over a celite pad. The solvent was

removed under *vacuo* and the crude mixture was purified by column chromatography on silica gel (CH_2Cl_2/MeOH 95:5).

Diethyl 3-(*N*-hydroxyformamido)-1-(3,4-dichlorophenyl)propylphosphonate (14e). Yield: 57% (157 mg).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.09 – 1.24 (6H, m, OCH_2CH_3); 2.11 (1H, m, CH_2CHP); 2.46 (1H, m, CH_2CHP); 3.01 – 3.17 (1H, m, CHP); 3.22 – 3.35 (1H, m, CH_2N); 3.45 – 3.56 (1H, m, CH_2N); 3.77 – 4.04 (4H, m, OCH_2CH_3); 7.08 and 7.11 (1H, arom. H); 7.32 – 7.37 (2H, m, arom. H); 7.55 (1H, br s, HC=O); 8.24 (1H, s, NOH) ppm.

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 16.48 (app t, $^3J_{C,P}$ = 5.8 Hz, OCH_2CH_3); 26.90 (CH_2CHP, major); 27.08 (CH_2CHP, minor); 40.09 (d, $^1J_{C,P}$ = 139.3 Hz, CHP, major); 40.96 (d, $^1J_{C,P}$ = 139.3 Hz, CHP, minor); 44.60 (d, $^3J_{C,P}$ = 15.8 Hz, NCH_2, minor); 47.45 (d, $^3J_{C,P}$ = 15.0 Hz, NCH_2, major); 62.84 (d, $^2J_{C,P}$ = 6.9 Hz, OCH_2CH_3, major); 63.01 (d, $^2J_{C,P}$ = 7.2 Hz, OCH_2CH_3, minor); 63.24 (d, $^2J_{C,P}$ = 6.9 Hz, OCH_2CH_3, major); 63.32 (d, $^2J_{C,P}$ = 6.1 Hz, OCH_2CH_3, minor); 128.81 (d, $J_{C,P}$ = 6.3 Hz, =CH, major); 128.95 (d, $J_{C,P}$ = 6.9 Hz, =CH, minor); 130.69 (=CH, minor); 130.91 (=CH, major); 131.14 (d, $J_{C,P}$ = 6.9 Hz, =CH, major); 131.24 (d, $J_{C,P}$ = 9.2 Hz, =CH, minor); 131.74 (d, $J_{C,P}$ = 3.8, =C, minor); 131.98 (d, $J_{C,P}$ = 3.8, =C, major); 132.70 (d, $J_{C,P}$ = 2.6, =C, minor); 133.01 (d, $J_{C,P}$ = 2.6, =C, major); 135.60 (d, $J_{C,P}$ = 7.2, =C, major); 136.00 (d, $J_{C,P}$ = 7.5, =C, minor); 157.37 (C=O, major); 163.03 (C=O, minor) ppm.

Exact mass (ESI-MS): calculated for $C_{14}H_{21}Cl_2NO_5P$ $[M+H]^+$: 384.0535; found: 384.0530

Diethyl 3-(*N*-hydroxyformamido)cyclopentylphosphonate (22cis). Yield: 19 % (90 mg).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.26 (6H, m, OCH_2CH_3); 1.75 – 2.42 (7H, m, C_5H_7P); 4.03 – 4.22 (4H, m, OCH_2CH_3); 4.83 (1H, s, CHNOH), 7.88 (1H, s, HC=O); 8.25 (1H, s, HC=O); 9.70 (1H, s, NOH) ppm.

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 16.65 (d, $^{3}J_{C,P}$ = 5.8 Hz, OCH_2CH_3); 26.15 (major, CH_2); 26.66 (minor, CH_2); 29.21 (minor d, $J_{C,P}$ = 12.7 Hz, CH_2); 29.81 (major d, $J_{C,P}$ = 11.5 Hz, CH_2); 29.81 (minor, CH_2); 30.44 (major, CH_2); 33,54 (major d, $^{1}J_{C,P}$ = 148.3 Hz, CHP); 35,51 (minor d, $^{1}J_{C,P}$ = 148.9 Hz, CHP); 55.08 (major d, $^{3}J_{C,P}$ = 12.7 Hz, CHN); 60.19 (minor d, $^{3}J_{C,P}$ = 11.5 Hz, CHN); 62.05 (OCH_2CH_3); 62.15 (OCH_2CH_3); 156.49 (major, C=O); 162.37 (minor, C=O) ppm.

Exact mass (ESI-MS): calculated for $C_{10}H_{21}NO_5P$ $[M+H]^+$: 266.1158; found: 266.1131

Diethyl 3-(*N*-hydroxyformamido)cyclopentylphosphonate (22trans). Yield: 35 % (170 mg).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.18 (3H, t, *J* = 7.0 Hz, OCH_2CH_3); 1.18 (3H, t, *J* = 6.8 Hz, OCH_2CH_3); 1.75 – 2.08 (7H, m, C_5H_7P); 3.95 (4H, m, OCH_2CH_3); 4.68 (1H, s, CHNOH), 7.80 (1H, minor s, HC=O); 8.17 (1H, major s, HC=O); 9.78 (1H, br s, NOH) ppm.

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 16.60 (d, $^{3}J_{C,P}$ = 5.5 Hz, OCH_2CH_3); 25.25 (CH_2); 28.08 (major d, $J_{C,P}$ = 10.4 Hz, CH_2); 28.83 (minor d, $J_{C,P}$ = 11.2 Hz, CH_2); 29.4 (major s, CH_2); 30.07 (minor, CH_2); 33.41 (major d, $^{1}J_{C,P}$ = 148.3 Hz, CHP); 34.22 (minor d, $^{1}J_{C,P}$ = 150.0 Hz, CHP); 55.44 (major d, $^{3}J_{C,P}$ = 15.8 Hz, CHN); 60.50 (minor d, $^{3}J_{C,P}$ = 18.1 Hz, CHN); 62.10 – 62.47 (2C, m, OCH_2CH_3); 156.43 (minor, C=O); 162.48 (major, C=O) ppm.

Exact mass (ESI-MS): calculated for $C_{10}H_{21}NO_5P$ $[M+H]^+$: 266.1158; found: 266.1143

General method for the phosphonate deprotection

Esters **14**, **15**, **22** and **23** (0.84 mmol) were dissolved in CH_2Cl_2 (10 mL) and treated dropwise with TMSBr (3.36 mmol, 0.50 g) under N_2. The reaction mixture was stirred for 2 h at room temperature. After completion of the reaction the volatile compounds were removed *in vacuo* to give the corresponding phosphonic acids in almost quantitative yield. All final compounds were purified using a preparative HPLC system on a C18 column (5µm; Phenomenex; Luna; 250 x 21.2 mm) with a linear gradient of acetonitrile in 5 mM NH_4OAc solution over 20 min at a flow rate of 17.5 mL/min. The purity of all target compounds was assessed by analytical HPLC (5µm; Phenomenex; C18(2); 250 x 4.6 mm) using the same gradient at a flow rate of 1 mL/min. All final compounds were obtained as hygroscopic powders after lyophilisation. All powders were white, except the 5-membered cyclic analogues which were obtained as orange powders.

3-(*N*-hydroxyformamido)-1-(3,4-dichlorophenyl)propylphosphonic acid (1e).

^{1}H NMR (300 MHz; D_2O): δ = 1.93 – 2.15 (1H, m, β-CH); 2.24 – 2.38 (1H, m, β-CH); 2.73 – 2.87 (1H, m, α-CH); 3.17 – 3.47 (2H, m, γ-CH_2); 7.07 – 7.12 (1H, m, arom. H); 7.33 – 7.39 (2H, m, arom. H); 7.44 and 8.07 (1H, 2 x s, major and minor HC=O) ppm.

^{13}C NMR (75 MHz; D_2O): δ = 26.49 (s, β-CH_2); 42.82 (d, α-CH, $^1J_{C,P}$ = 129.6 Hz); 48.86 (d, γ-CH_2, $^3J_{C,P}$ = 17.0 Hz); 128.92 (d, $J_{C,P}$ = 5.8 Hz, =CH); 130.04 (d, $J_{C,P}$ = 3.8 Hz, =C) ; 130.55 (d, $J_{C,P}$ = 2.6 Hz, =CH); 130.73 (d, $J_{C,P}$ = 6.0 Hz, =CH); 131.88 (d, $J_{C,P}$ = 3.2 Hz, =C); 138.80 (d, $J_{C,P}$ = 7.2 Hz, =C); 159.70 and 163.76 (2 x s, major and minor C=O) ppm.

^{31}P NMR (121 MHz; D_2O): δ = 21.46 and 21.78 ppm. (major and minor isomer)

Exact mass (ESI-MS): calculated for $C_{10}H_{11}Cl_2NO_5P$ $[M-H]^-$: 325.9751; found: 325.9745

(1*R*,3*R*)-3-(*N*-hydroxyformamido)cyclopentylphosphonic acid and (1*S*,3*S*)-3-(*N*-hydroxyformamido)cyclopentylphosphonic acid (*trans*-3).

^{1}H NMR (300 MHz; D_2O): δ = 1.73 – 2.04 (7H, m, α-CH and CH_2); 4.13 (1H, br s, NCH); 7.84 and 8.07 (1H, 2 x s, major and minor HC=O) ppm.

^{13}C NMR (75 MHz; D_2O): δ = 25.55 (d, CH_2); 28.56 (d, CH_2, $J_{C,P}$ = 10.4 Hz); 31.10 (d, CH_2); 35.94 (d, $^{1}J_{C,P}$ = 141.7 Hz, α-CH); 61.46 (d, $J_{C,P}$ = 17.3 Hz, NCH) ; 159.23 (s, C=O) ppm.

^{31}P NMR (121 MHz; D_2O): δ = 27.71 and 27.91 ppm. (major and minor isomer)

Exact mass (ESI-MS): calculated for $C_6H_{11}NO_5P$ $[M-H]^-$: 208.0374; found: 208.0366

(1*R*,3*S*)-3-(*N*-hydroxyformamido)cyclopentylphosphonic acid and (1*S*,3*R*)-3-(*N*-hydroxyformamido)cyclopentylphosphonic acid (*cis*-3).

^{1}H NMR (300 MHz; D_2O): δ = 1.49 – 2.17 (7H, m, α-CH and CH_2); 4.21 (1H, br m, NCH); 7.88 and 8.09 (1H, 2 x s, major and minor HC=O) ppm.

^{13}C NMR (75 MHz; D_2O): δ = 25.73 (d, CH_2); 29.89 (d, CH_2, $J_{C,P}$ = 11.2 Hz); 31.76 (s, CH_2); 36.47 (d, $^{1}J_{C,P}$ = 141.5 Hz, α-CH); 61.36 (d, $J_{C,P}$ = 10.9 Hz, NCH) ; 159.11 (s, C=O) ppm.

^{31}P NMR (121 MHz; D_2O): δ = 27.74 ppm. (major and minor isomer)

Exact mass (ESI-MS): calculated for $C_6H_{11}NO_5P$ $[M-H]^-$: 208.0374; found: 208.0378

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Supporting Information Available: Experimental details (^{1}H, ^{13}C, ^{31}P NMR, MS) for intermediates (**6b**, **7d-e**, **8c-d**, **9c-d**, **10a-d**, **11a-d**, **12c**, **13a-e**, **14c**, **15a-e**, **17**, **21**, trans-**23**,

cis-**23**) and final products (**1c**, **2a**-**e**, trans-**4**, cis-**4**). This material is available free of charge on the WWW under http://www.eurjoc.org or from the author.

Tables

Table 1. Inhibitory activity on *E. coli* DXR enzyme

Compounds	**IC_{50} (μM)**
fosmidomycin	0.029
FR900098	0.035
trans-**3**	0.20
cis-**3**	2.3
trans-**4**	2.3
cis-**4**	12

References and notes

[1] B. Lell, R. Ruangweerayut, J. Wiesner, M.A. Missinou, A. Schindler, T. Baranek, M. Hintz, D. Hutchinson, H. Jomaa and P.G. Kremsner *Antimicrob. Agents Chemother.* **2003**, *47*, 735-738.

[2] M.A. Missinou, S. Borrmann, A. Schindler, S. Issifou, A.A. Adegnika, P.B. Matsiegui, R. Binder, B. Lell, J. Wiesner, T. Baranek, H. Jomaa, P.G. Kremsner, *Lancet* **2002**, *360*, 1941-1942.

[3] H. Jomaa, J. Wiesner, S. Sanderbrand, B. Altincicek, C. Weidemeyer, M. Hintz, I. Turbachova, M. Eberl, J. Zeidler, H.K. Lichtenthaler, D. Soldati and E. Beck *Science* **1999**, *285*, 1573-1576.

[4] M. Rohmer, *Nat. Prod. Rep.* **1999**, 16, 565 – 574.

[5] J. Zeidler, J. Schwender, C. Muller, J. Wiesner, C. Weidemeyer, E. Beck, H. Jomaa *Z. Naturforsch. C* **1998**, *53*, 980-986.

[6] T. Kuzuyama, T. Shimizu, S. Takahashi, H. Seto *Tetrahedron Lett.* **1998**, *39*, 7913-7916.

[7] M. Courtois, Z. Mincheva, F. Andreu, M. Rideau, M. C. Viaud-Massuard *J. Enzyme Inhib. Med. Chem.* **2004**, *19*, 559-565.

[8] R. Ortmann, J. Wiesner, A. Reichenberg, D. Henschker, E. Beck, H. Jomaa, M. Schlitzer *Arch. Pharm.* **2005**, *338*, 305-314.

[9] R. Ortmann, J. Wiesner, A. Reichenberg, D. Henschker, E. Beck, H. Jomaa, M. Schlitzer *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2163-2166.

[10] A. Reichenberg, J. Wiesner, C. Weidemeyer, E. Dreiseidler, S. Sanderbrand, B. Altincicek, E. Beck, M. Schlitzer, H. Jomaa *Bioorg. Med. Chem. Lett.* **2001**, *11*, 833-835.

[11] T. Haemers, J. Wiesner, S. Van Poecke, J. L. Goeman, H. Jomaa, S. Van Calenbergh *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1888-1891.

[12] a) J. G. Topliss *J. Med. Chem.* **1972**, *15*, 1006-1011; b) J. G. Topliss *J. Med. Chem.* **1977**, *20*, 463-469.

[13] a) S. C. Fields, *Tetrahedron* **1999**, 55, 12237-12273. b) D. Enders, A. Saint-Dizier, M.-I. Lannou, A. Lenzen *Eur. J. Org. Chem.* **2006**, 29-49.

[14] K. Hemmi, H. Takeno, M. Hashimoto, T. Kamiya *Chem. Pharm. Bull.* **1982**, 30, 111.

[15] V. Devreux, J. Wiesner, J. L. Goeman, J. Van der Eycken, H. Jomaa, S. Van Calenbergh *J. Med. Chem.* **2006**, accepted for publication.

[16] W. Flitsch, W. Lubisch, J. Rosche *Liebigs Ann. Chem.* **1987**, *8*, 661-664.

[17] R. G. Harvey *Tetrahedron* **1966**, *22*, 2561-2573.

[18] G. Battistuzzi, S. Cacchi, G. Fabrizi *Org. Lett.* **2003**, *5*, 777-780.

Legends

Figure 1. Structures of fosmidomycin, FR900098 and analogues under study.

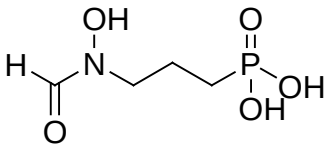
Scheme 1. Retrosynthetic route toward analogues **1** and **2**.

Scheme 2. Reagents and conditions: (a) (i) n-BuLi, THF, -50 to -70 °C, (ii) allyl bromide, -70 °C; (b) (i) OsO_4, 4-methyl-morpholine N-oxide, dioxane (ii) $NaIO_4$; (c) triethyl phosphite, phenol, 100 °C; (d) 2N HCl, rt.

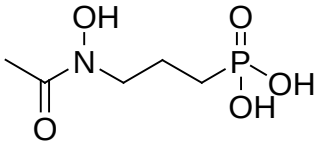
Scheme 3. Reagents and conditions: (a) *O*-benzylhydroxylamine, pyridine, EtOH, rt; (b) $NaCNBH_3$, MeOH, HCl, rt; (c) Acetyl chloride, CH_2Cl_2, EtN_3, 0 °C or carbonyldiimidazole, HCOOH, CH_2Cl_2, rt (or 2-thioxothiazolidine-3-carbaldehyde for **12a**) ; (d) H_2, Pd/C, MeOH, rt; (e) TMSBr, CH_2Cl_2, rt.

Scheme 4. Reagents and conditions: (a) triethyl phosphite, phenol, 100 °C; (b) *O*-benzylhydroxylamine, pyridine, EtOH, rt; (c) $NaCNBH_3$, MeOH, HCl, rt; (d) Acetyl chloride, CH_2Cl_2, EtN_3, 0 °C or carbonyldiimidazole, HCOOH, CH_2Cl_2, rt; (e) H_2, Pd/C, MeOH, rt; (f) TMSBr, CH_2Cl_2, rt.

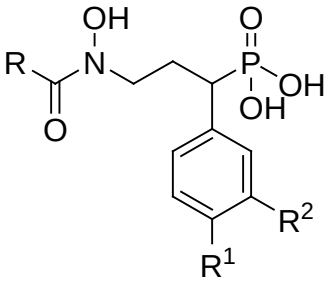
Figure 1



fosmidomycin

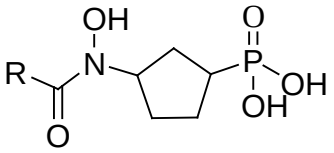


FR900098



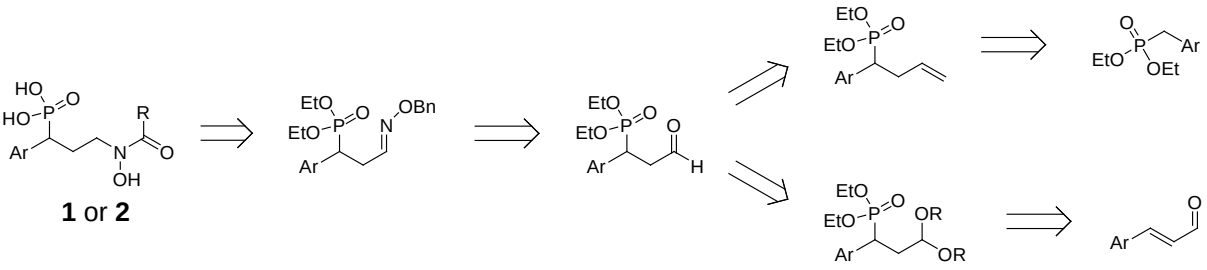
1a-e (R = H)
2a-e (R = CH_3)

a: R^1 = H; R^2 = H
b: R^1 = Me; R^2 = H
c: R^1 = OMe; R^2 = H
d: R^1 = Cl; R^2 = H
e: R^1 = Cl; R^2 = Cl



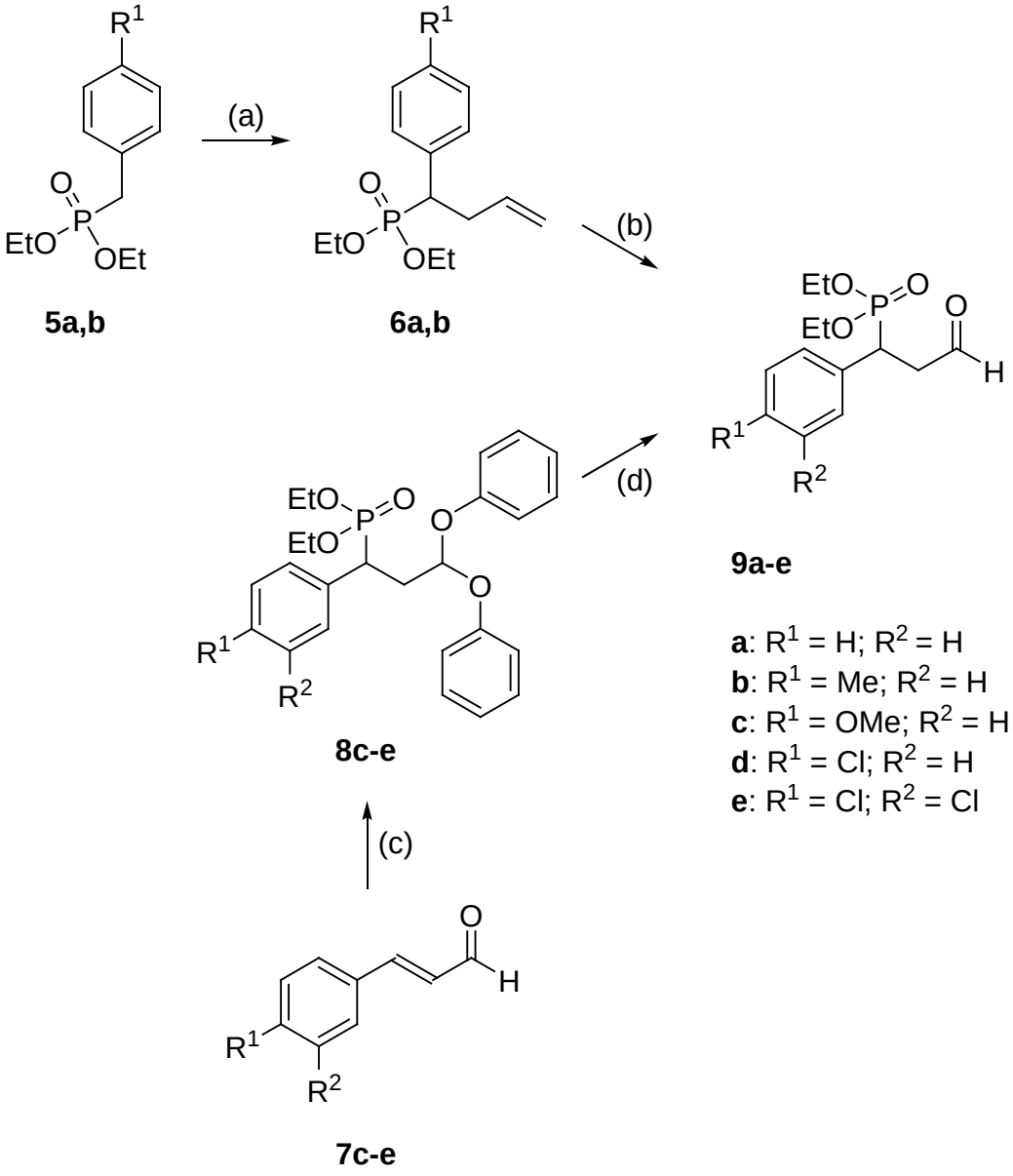
trans-**3** (R = H)
cis-**3** (R = H)
trans-**4** (R = CH_3)
cis-**4** (R = CH_3)

Scheme 1



1 or **2**

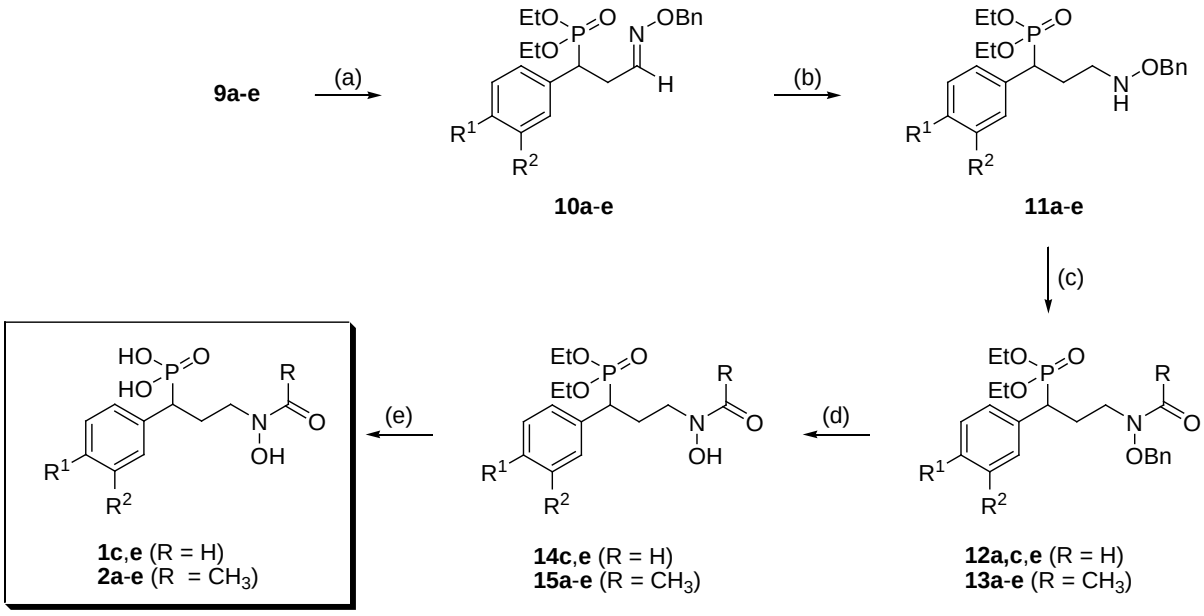
Scheme 2



9a-e

a: R^1 = H; R^2 = H
b: R^1 = Me; R^2 = H
c: R^1 = OMe; R^2 = H
d: R^1 = Cl; R^2 = H
e: R^1 = Cl; R^2 = Cl

Scheme 3



Scheme 4