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Source: BIOORGANIC & MEDICINAL CHEMISTRY (2008), 16 (6), 3361-3371,

DOI: 10.1016/j.bmc.2007.12.001
Synthesis of β- and γ-oxa Isosteres of Fosmidomycin and FR900098 as Antimalarial Candidates

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Abstract

To expand the structure-activity relationships of fosmidomycin and FR900098, two potent antimalarials interfering with the MEP-pathway, we decided to replace a methylene group in β-position of the phosphonate moiety of these leads by an oxygen atom. β-oxa-FR900098 (11) proved equally active as the parent compound.

When applied to 4-[hydroxyl(methyl)amino]-4-oxobutyl phosphonic acid, featuring a hydroxamate instead of the retrohydroxamate moiety, a β-oxa modification yielded a derivative (13) with superior activity against a 3D7 *P. falciparum* strain than fosmidomycin, while a γ-oxa modification resulted in less active derivatives.

A bis(pivaloyloxymethyl)ester of phosphonate 13 proved twice as active in inhibiting cultured parasites than a similar prodrug of FR900098.

*Keywords*: Isoprenoid biosynthesis; Fosmidomycin; DOXP Reductoisomerase; Methylerthritol phosphate pathway
1. Introduction

1-Deoxy-D-xylulose 5-phosphate reductoisomerase (DXR) catalyzes the conversion of 1-deoxy-D-xylulose 5-phosphate (DOXP, 1) to 2-C-methyl-D-erythritol 4-phosphate (MEP, 3). This conversion involves two consecutive steps (Figure 1). The enzyme first catalyzes an intramolecular rearrangement of DOXP into the intermediate 2-C-methyl-D-erythrose 4-phosphate (2). This step requires a divalent metal ion to induce partial positive charge on the C-2 carbon, allowing the C-4 atom to attack it. Several studies have been reported on the mechanism of this isomerisation step.\(^1\) A second step involves an NADPH-dependent reduction of the aldehyde intermediate.

DXR is one of the enzymes involved in the MEP pathway for the biosynthesis of isoprenoids. As it is present in most eubacteria, green algae, and the plastids of higher plants, but not in humans,\(^2\) this pathway has attracted considerable interest for the development of new and safe antibiotics as well as herbicides. Furthermore, the MEP pathway has been found in *Plasmodium* spp. that harbour this pathway in their apicoplast compartments.\(^3\) The discovery that fosmidomycin (4), a metabolite isolated from *Streptomyces lavendulae* in the late 1970s,\(^4\) efficiently inhibits *P. falciparum* growth by targeting DXR,\(^3\) catalyzed research on the synthesis of analogues of this phosphonate and its acetyl congener FR900098 (5). Most reported efforts toward the synthesis of fosmidomycin analogues involve modifications of the retrohydroxamate\(^5\) and phosphonate moieties.\(^6\) Modifications of the phosphonate group are generally directed towards improving the relatively poor oral availability of fosmidomycin. By masking the phosphonic acid moiety of FR900098 as acyloxyalkyl esters, we were able to improve the activity in mice infected with *P. vinckei*.

Only recently, some studies were reported on analogues modified at the three carbon spacer that connects the retrohydroxamate and phosphonate functionalities.\(^7\) The X-ray structural data that are available today should aid in the defining of the structural requirements for the
design of potent DXR inhibitors. In a recent ternary complex of DXR with fosmidomycin and NADPH with a tight-binding conformation, hydrophobic contacts were observed between the three carbon spacer and the rigid part of the binding site or with the partially ordered catalytic loop. Recently, Proteau and coworkers described the synthesis of the phosphate analogues of fosmidomycin (6) and FR900098 (7). The former had previously been isolated from Pseudomonas fluorescens PK-52 and is known as fosfoxacin. The latter proved to be a very potent inhibitor of Synechocystis DXR. These results prompted us to report our investigations on related series of β- and γ-oxa analogues 10-15. Compounds 10 and 11 are analogues of fosmidomycin and FR900098, respectively, in which the β-methylene function was replaced by an oxygen atom. Since a phosphonomethoxy group has been successfully employed as a stable iso-electronic alternative of a methyl phosphate moiety to produce antiviral nucleosides, compounds 10 and 11 were selected as possible bioisosteres of 6 and 7.

On the one hand, the bridging oxygen of the phosphate group of DOXP may be involved in interactions with amino acids in the binding site. Since only hydrophobic contacts with the methylene linker were reported, the replacement of the β- or γ-methylene groups by an oxygen atom in search for hydrogen bonding seems less compelling. On the other hand, the introduction of the electronegative oxygen atom could cause a perturbation of the electronic properties, in casu the deprotonation of the phosphonate group or hydroxamate group, which becomes slightly more acidic. As a consequence of the logical decrease of the second pKa, the phosphonate could appear rather in its doubly ionised form, which is more favourable for an ideal binding. However, it can be concluded that differences in ionisation may contribute to a better binding, if the introduction of an oxygen in β-position gives better activity instead of a methylene moiety. Analogues 12 and 13 combine the β-oxa modification
with a hydroxamic acid moiety, as generally found as metal-binding group in matrix metalloproteinase inhibitors. Rohmer and coworkers reported that such metal chelating group, when replacing the N-hydroxyformamido moiety of fosmidomycin, as in compound 8 or its N-methyl analogue 9, yielded potent inhibitors of DXR which were also capable to inhibit *E. coli* growth.\textsuperscript{5b} Poulter and Walker reported an analogue of DOXP in which the acetyl was replaced by a hydroxamic acid, but it did not inhibit the enzyme,\textsuperscript{13} probably because the molecule no longer fit in the confined volume of the active site, as a consequence of its one-atom longer chain. This is consistent with the recently reported X-ray structure.\textsuperscript{8}

Analogues 14 and 15, which are constitutional isomers of 12 and 13, were also synthesized. They combine a hydroxamic acid moiety with an oxygen in γ-position that could possibly mimic the C4 hydroxyl group present in the substrate, known to be involved in hydrogen bonding with Glu152, Asn227 and Lys228.\textsuperscript{8}

Prompted by the instability of phosphonic acid 13, we set out on the synthesis of its bis(pivaloyloxymethyl)ester analogue 17.

### 2. Results and discussion

Scheme 1 depicts the synthesis of analogues 10 and 11. Retrohydroxamic acids 21 and 22, obtained by acylation of *O*-benzylhydroxylamine 20, were treated with paraformaldehyde and potassium tert-butoxide to give the hydroxymethylated products 23 and 24, which were subsequently converted to the chlorides 25 and 26 as reported by Zhu et al.\textsuperscript{14} Since attempts to crystallize the chlorides led to hydrolysis to alcohols 23 and 24, crude 25 and 26 were directly used in the Williamson synthesis with 19. Addition of one equivalent of NaH did not prove effective to increase the moderate yield. Compound 19 was prepared by a neat reaction with dibenzyl phosphite, paraformaldehyde and triethylamine. The perbenzylated compounds
27 and 28 were finally deprotected by catalytic hydrogenation to yield the phosphonic acids 10 and 11, which were purified by reversed phase HPLC. Since the N,O-acetal in 27 and 28 is acid sensitive, it was very important to use neutral Pd/C catalyst to prevent cleavage. Sajiki et al. reported a remarkable supplier dependent disparity in properties of Pd/C catalysts. Pd/C catalysts of some suppliers appeared to be acidic enough to cleave triethylsilyl ethers without H2.\textsuperscript{15} β-Oxa analogue 11 proved to be stable in H2O for more than two weeks, as revealed by \textsuperscript{1}H-NMR.

The synthesis of the hydroxamate 12 and its N-methyl analogue 13 is shown in Scheme 2. Diethylhydroxymethyl phosphonate (29) was reacted with ethylbromoacetate in the presence of two equivalents NaH in THF to give 30 in 80% yield. Hydrolysis of 30 with one equivalent of sodium hydroxide followed by neutralisation gave acid 31 in 94% yield. An attempt to react diethylhydroxymethyl phosphonate directly with chloroacetic acid in the presence of two equivalents of NaH resulted in a poor yield. Treatment of carboxylic acid 31 with carbonyldiimidazole and O-benzylhydroxylamine afforded the N-benzyloxyamide 32. A possible simplification consists of the direct conversion of the ester 30 to the N-benzyloxyamide 32 by a recently published procedure.\textsuperscript{16} A methyl group was introduced by deprotonation of 32 with NaH in THF followed by addition of methyl iodide to give 34. Finally, hydroxamic acid 12 and its N-methylated analogue 13 were obtained after catalytic debenzylation (Pd/C) and deprotection of the phosphonate moieties with two equivalents of bromotrimethylsilane in dichloromethane.

The synthesis of the N-hydroxy carbamates 14 and 15 is depicted in Scheme 3. Treatment of dimethylhydroxyethyl phosphonate (34) with carbonyldiimidazole and benzylhydroxyl amine afforded 37. N-methylation, as described for 34, yielded 39. The benzyl protecting groups of 37 and 39 were removed by catalytic hydrogenolysis (Pd/C) to give 38 and 40. Finally, the phosphonate esters were deprotected as outlined above.
The synthesis of the bis(pivaloyloxymethyl)ester of 13 from its diethyl ester 35 was realized under standard conditions.

All target compounds were tested for inhibition of recombinant *E. coli* DXR (Table 1). The assay was based on the photometric measurement of the NADPH turnover associated with the DXR catalysed reaction. Fosmidomycin and FR900098 were included as reference compounds.

In the series with the inversed hydroxamates and carbamates the *N*-methyl substituted analogues proved superior to their non-methylated analogues. Compound 9, made by Rohmer et al., was marginally less active than fosmidomycin and FR900098 in the *E. coli* DXR inhibition assay. The results of 13 and 15 showed that introduction of an oxygen atom in the propyl chain is more favourable in the β-position of the phosphonate moiety than in γ-position of the phosphonate moiety. Introduction of an oxygen atom in the γ-position of the phosphonate moiety generates carbamates 14 and 15 which were weak in inhibiting *E. coli* DXR, as shown by the relatively high *IC*₅₀ values. In contrast, compound 13 proved to be almost equipotent to FR900098 and at least as active as compound 9. Comparison of analogues 11 and 13 demonstrated that, when combined with a β-oxa modification, the hydroxamate seems favourable to the *N*-hydroxyacetamido group as metal chelating moiety. Inhibitor 13, when assayed as a fresh solution, is slightly more potent than 11. The introduction of an oxygen atom in the linker does not contribute favourably to the hydrophobic interactions around the linker which are present according to the crystal structure of DXR from Sweeney *et al.*, although on this *E. coli* DXR inhibition assay analogue 13 was comparably potent to analogue 9 with the methylene linker. This points out that an oxygen atom in β-position slightly influences the ionisation and as such the binding, even if hydrophobic interactions are playing a part. Likewise, it is also possible that the ether linkage is still hydrophobic enough.
to ensure a tight bonding of the inhibitor. In order to confirm that there is no hydrogen bonding involved, docking experiments would be useful.

Because of the varying IC_{50} values obtained with 13, we assessed its stability and inferred that in aqueous solution, slow but significant degradation of this compound took place. Hence, we evaluated its bis(pivaoxyloxymethyl)ester 17, which was compared to the same ester prodrug of FR900098. While both compounds were unable to inhibit *E. coli* DXR, 17 proved approximately twice more potent in inhibiting the growth of a 3D7 *P. falciparum* strain, further warranting in vivo evaluation of this compound.

Furthermore, compound 11 outperformed its formyl analogue 10. An opposite trend was observed in the fosmidomycin/FR900098 couple, where fosmidomycin is superior to FR900098. Nevertheless, in this series of compounds fosmidomycin stays the best inhibitor on this *E. coli* DXR inhibition assay.

In summary, we established an efficient and reliable synthesis for β- and γ-oxa fosmidomycin analogues. analogue 13, which combines a β-oxa modification with a hydroxamate moiety was almost as potent in inhibiting *E. coli* DXR as FR900098.

3. Experimental
3.1. Chemicals and solvents

Most chemicals were obtained from Sigma-Aldrich or Acros Organics and were used without further purification. Compound 16 was obtained as described earlier.\(^{5b}\) All reactions were carried out under inert (N\(_2\)) atmosphere.

3.2. Chromatography

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 sulfate solution, iodine vapour or bromocresol green solution. Column chromatography was performed on ICN silica gel (63-200 μM).

3.3. Instruments and analyses

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 CDCl₃ 7.26 ppm for ¹H and 77.4 ppm for ¹³C. Coupling constants are expressed in Hz. Abbreviations used are: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br. s = broad signal. All signals assigned to hydroxyl and to amino groups were exchangeable with D₂O. Structural assignment was confirmed with COSY, DEPT and/or HMQC 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 an acetonitrile/water (1:1) mixture at 3μL/min.

3.4. Dibenzyl hydroxymethylphosphonate (19)

Dibenzyl phosphite (20 mL, 76.27 mmol), triethylamine (10.60 mL, 76.27 mmol), and paraformaldehyde (2.45 g, 130 mmol) were mixed in a reaction vessel. The vessel was tightly sealed and the mixture was mechanically stirred for 12 h at 90 °C. All volatile components were subsequently removed under reduced pressure, and the remaining semisolid was dissolved in 25 mL of CH₂Cl₂. The solution was successively washed with saturated aqueous solutions of NH₄Cl, K₂CO₃ and again NH₄Cl, dried with MgSO₄ and the solvent removed under reduced pressure. The residue, a pale-yellow oil, was purified by column chromatography (CH₂Cl₂/ethyl acetate, 2:1 to CH₂Cl₂/acetone 1:1, after all benzyl alcohol had eluted from the column) to yield compound 19 (12.48 g, 42.7 mmol, 56%).
1\textsuperscript{H}-NMR (300 MHz, CDCl\textsubscript{3}): $\delta = 3.92$ (d, $J = 5.6$ Hz, 2 H, CH\textsubscript{2}P), 5.06 (m, 4 H, CH\textsubscript{2}Ph), 7.34 (s, 10 H, arom. H) ppm.

1\textsuperscript{3}C-NMR (75 MHz, CDCl\textsubscript{3}): $\delta = 57.66$ (d, $J = 160.7$ Hz, CH\textsubscript{2}P), 68.27 (d, $J = 6.6$ Hz OCH\textsubscript{2}Ar), 128.26 (=CH), 128.78 (=CH), 128.86 (=CH), 136.29 (=C) ppm.

Exact mass (ESI-MS): calculated for C\textsubscript{15}H\textsubscript{16}O\textsubscript{4}P [M-H]\textsuperscript{−}: 291.0785; found: 291.0772.

3.5. \textit{N-}(benzyloxy)formamide (21)

Formic acid (1.18 mL, 31.3 mmol) was dissolved in dry CH\textsubscript{2}Cl\textsubscript{2} (60 mL). To this solution carbonyldiimidazole (5.08 g, 31.3 mmol) was added. After stirring for 30 min a suspension of benzylhydroxylamine hydrochloride (5 g, 31.3 mmol) in 31 mL of CH\textsubscript{2}Cl\textsubscript{2} containing Et\textsubscript{3}N (4.35 ml, 31.3 mmol) was added. When TLC revealed disappearance of all the starting material, the reaction mixture was diluted with 150 mL CH\textsubscript{2}Cl\textsubscript{2} and washed six times with 1N HCl (100 mL). The organic layer was dried over MgSO\textsubscript{4}, concentrated under reduced pressure and the residue purified by chromatography (CH\textsubscript{2}Cl\textsubscript{2}/MeOH 95:5) to yield 4.13 g of \textit{N-}(benzyloxy)formamide 21 (yield 87%) as a pale yellow oil.

\textsuperscript{1}H-NMR (300 MHz, CDCl\textsubscript{3}): mixture of amide rotamers $\delta = 4.74$ and 4.80 (2 \times s, 2 H, CH\textsubscript{2}Ph, minor and major), 7.30 (m, 5 H, arom. H), 7.67 and 8.01 (2 \times s, 1 H, C(O)H, major and minor), 9.60 and 10.32 (2 \times s, 1 H, NH, minor and major) ppm.

\textsuperscript{13}C-NMR (75 MHz, CDCl\textsubscript{3}): mixture of amide rotamers $\delta = 78.56$ and 81.05 (2 \times s, OCH\textsubscript{2}Ar, major and minor), 128.79 (=CH), 129.07 (=CH), 129.41 (=CH), 135.09 (=C), 158.35 and 165.08 (2 \times s, C(O)H, major and minor) ppm.

Exact mass (ESI-MS): calculated for C\textsubscript{8}H\textsubscript{10}NO\textsubscript{2} [M+H]\textsuperscript{+}: 152.0712; found: 152.0426.

3.6. \textit{N-}(benzyloxy)acetamide (22)
O-benzylhydroxylamine hydrochloride (1g, 6.2 mmol) was dissolved in CH₂Cl₂ (20 mL) and triethylamine (1 mL, 6.8 mmol) and stirred for 1 h. This suspension was treated with acetyl chloride (0.5 mL, 6.8 mmol) dropwise and stirred for 1.5 h at room temperature. The reaction mixture was diluted with 60 mL of CH₂Cl₂ and washed with 100 mL of water. The organic solvent was removed under reduced pressure and the residue was purified (CH₂Cl₂/MeOH 95:5) to yield 0.43 g of N-(benzyloxy)acetamide 22 (yield 86%) as white crystals.

¹H-NMR (300 MHz, CDCl₃): mixture of amide rotamers δ = 1.80 and 1.96 (2 × s, 3 H, C(O)CH₃, major and minor), 4.77 and 4.84 (2 × s, 2 H, CH₂Ph, minor and major), 7.30 (m, 5 H, arom. H), 8.57 and 9.59 (2 × s, 1 H, NH, minor and major) ppm.

¹³C-NMR (75 MHz, CDCl₃): mixture of amide rotamers δ = 19.95 and 19.96 (2 × s, CH₃, minor and major); 78.24 and 79.53 (2 × s, OCH₂Ar, major and minor), 128.74 (=CH), 128.82 (=CH), 129.32 (=CH), 135.67 (=C), 168.45 and 175.23 (2 × s, C(O)CH₃, major and minor) ppm.

Exact mass (ESI-MS): calculated for C₉H₁₂NO₂ [M+H]⁺: 166.0868; found: 166.0875.


A mixture of the N-benzyloxyamide (5.71 mmol), paraformaldehyde (0.26 g, 8.56 mmol) and tBuOK (0.064 g, 0.57 mmol) was heated, in the absence of solvent, at 60 °C during 4 h. The reaction mixture was cooled and diluted with water (80 mL). After extraction with CH₂Cl₂ (3 x 80 mL) the organic layer was dried over anhydrous Na₂SO₄ and evaporated in vacuo. The residue was purified by chromatography (hexane/ethyl acetate, 1:1 for 24 or pentane/acetone/Et₃N, 6:4:0.1 for 23) to give 23 and 24 in 79 and 48% yield as white solids.

3.7.1. N-(benzyloxy)-N-(hydroxymethyl)formamide (23)
1H-NMR (300 MHz, CDCl₃): δ = 3.91 (br. s, 1 H, OH), 4.76 (s, 2 H, CH₂Ph), 4.98 (br. s, 2 H, HOCH₂), 7.37 (m, 5 H, arom. H), 8.08 (s, 1H, O=CH) ppm.

13C-NMR (75 MHz, CDCl₃): δ = 69.55 (s, HOCH₂, major), 73.10 (s, HOCH₂, minor), 77.71 (s, CH₂Ph, minor), 79.81 (s, CH₂Ph, major), 128.96 (=CH), 129.37 (=CH), 129.87 (=CH), 134.54 (=C), 159.48 (s, O=CH, minor), 164.27 (s, C(O)H, major) ppm.

Exact mass (ESI-MS): calculated for C₉H₁₂NO₃ [M+H]+: 182.0817; found: 182.0832.

3.7.2. N-(benzyloxy)-N-(hydroxymethyl)acetamide (24)

1H-NMR (300 MHz, CDCl₃): δ = 2.06 (s, 3 H, C(O)CH₃), 3.96 (br. s, 1 H, OH), 4.93 (s, 2 H, CH₂Ph), 5.03 (br. s, 2 H, HOCH₂), 7.38 (m, 5 H, arom. H) ppm.

13C-NMR (75 MHz, CDCl₃): δ = 20.86 (s, CH₃), 71.17 (s, HOCH₂), 78.45 (s, OCH₂Ar), 128.94 (=CH), 128.94 (=CH), 128.94 (=CH), 134.65 (=C), 174.26 (s, C(O)CH₃) ppm.

Exact mass (ESI-MS): calculated for C₁₀H₁₄NO₃ [M+H]+: 196.0974; found:196.0981.

3.8. General method for synthesis of N-(benzyloxy)-N-(chloromethyl)formamide (25) and N-(benzyloxy)-N-(chloromethyl)acetamide (26)

To a cooled solution (10 °C) of 23 or 24 (2.7 mmol) in 5.5 mL of CH₂Cl₂ was added thionyl chloride (0.55 mL, 7.5 mmol). After stirring for 30 min, the mixture was evaporated under vacuum to yield an oily residue that could be solidified by adding a drop of hexane and connecting the flask to high vacuum. In our hands it was not possible to recrystallize 26 from ethyl acetate due to hydrolysis to the alcohol 24. Therefore, 26 was directly used in the next step without further purification. The same holds for 25.

3.9. Dibenzyl(N-(N-benzyloxyformamido)methoxy)methylphosphonic acid (27)
To a solution of crude 25 (~ 0.37 mmol) in 5 mL of CH₂Cl₂ was added a solution 19 (0.16 g, 0.54 mmol) in 5 mL of CH₂Cl₂. After stirring for 16 h at room temperature the mixture was evaporated to dryness and purified by column chromatography (CH₂Cl₂/ethyl acetate, 2:1), affording 27 (35 mg, 21%) as a clear oil.

¹H-NMR (300 MHz, CDCl₃): δ = 3.75 – 3.87 (m, 2H, CH₂P), 4.74 – 5.15 (m, 8H, NCH₂O and PhCH₂O), 7.28 – 7.35 (m, 15H, arom. H), 8.07 and 8.15 (br. s and br. s, 1H, C(O)H) ppm.

¹³C-NMR (75 MHz, CDCl₃): δ = 63.60 (d, ¹J_C,P = 171.0 Hz, CH₂P), 68.24 and 68.32 (2 × s, PhCH₂), 80.04 (NCH₂O), 128.33 (=CH), 128.86 (=CH), 129.37 (=CH), 129.80 (=CH), 134.44 (=C), 136.18 (=C), 164.25 (C=O) ppm.


3.10. Dibenzyl(N-(N-benzyloxyacetamido)methoxy)methylphosphonic acid (28)

To a solution of crude 26 (~ 2.5 mmol) in 2 mL of CH₂Cl₂ was added a solution 19 (1.46 g, 5 mmol) in 3 mL of CH₂Cl₂. After stirring for 16 h at room temperature the mixture was evaporated to dryness and purified by column chromatography (CH₂Cl₂/ethyl acetate, 2:1) to give 28 (0.44 g, 38%) as a clear oil.

¹H-NMR (300 MHz, DMSO-d₆): δ = 2.06 (s, 3H, C(O)CH₃), 3.97 (d, ¹J = 8.8 Hz, 2H, CH₂P), 4.92 (s, 2H, NCH₂O), 5.02 (s, 2H, PhCH₂), 5.05 (d, ¹J = 1.5 Hz, 2H, PhCH₂), 5.08 (d, ¹J = 2.0 Hz, 2H, PhCH₂), 7.30 – 7.40 (m, 15H, arom. H) ppm.

¹³C-NMR (75 MHz, DMSO-d₆): δ = 21.16 (CH₃), 62.76 (d, ¹J_C,P = 219.3 Hz, CH₂P), 67.70 and 67.78 (2 × s, PhCH₂), 77.41 (NCH₂O), 128.48 (=CH), 128.93 (=CH), 129.13 (=CH), 129.15 (=CH), 129.40 (=CH), 130.10 (=CH), 135.35 (=C), 137.03 (d, ¹J_C,P = 6.1 Hz, =C), 173.18 (C=O) ppm.

Exact mass (ESI-MS): calculated for C₂₅H₂₉NO₆P [M+H]⁺: 470.1733; found: 470.1728.
3.11. General procedure for the benzyl deprotection of 27 and 28

A solution of compound 27 or 28 (140 mg) in MeOH (3 mL) was hydrogenated at atmospheric pressure in the presence of Pd 10 wt. % on activated carbon (70 mg). After 3 h stirring the reaction mixture was filtered over a celite pad. The solvent was removed under vacuum and the crude mixture was purified by column chromatography on silica gel (CH₂Cl₂/MeOH 90:10). Phosphonic acids 10 and 11 were purified according to the purification of compounds 12-15 (see further).

3.11.1. (N-(N-hydroxyformamido)methoxy)methylphosphonic acid (10): Yield: 34 % according to HPLC analysis.

\(^1\)H-NMR (300 MHz, D₂O): \(\delta = 3.68\) (d, \(J_{\text{H,P}} = 9.4\) Hz, 2H, CH₂P), 5.01 (s, 2H, OCH₂), 7.94 (s, 1H, C(O)H, minor), 8.21 (s, 1H, C(O)H, major) ppm.

\(^13\)C-NMR (75 MHz, D₂O): \(\delta = 58.11\) (d, \(^1\)J\text{C,P} = 153.88 Hz, CH₂P, major), 63.98 (d, \(^1\)J\text{C,P} = 153.88 Hz, CH₂P, minor), 81.26 (s, OCH₂, major), 81.40 (s, OCH₂, minor), 160.19 (s, C=O, minor), 161.032 (s, C=O, major) ppm.

\(^31\)P-NMR (121 MHz, D₂O): \(\delta = 15.05\) ppm.

Exact mass (ESI-MS): calculated for C₃H₇NO₆P [M-H]⁻: 184.0010; found: 184.0025.

3.11.2. (N-(N-hydroxyacetamido)methoxy)methylphosphonic acid (11): Yield: 43 % according to HPLC analysis.

\(^1\)H-NMR (300 MHz, D₂O): \(\delta = 2.07\) (s, 3H, CH₃, major), 2.08 (s, 3H, CH₃, minor), 3.43 (d, \(J_{\text{H,P}} = 8.9\) Hz, 2H, CH₂P, major), 3.49 (d, \(J_{\text{H,P}} = 8.9\) Hz, 2H, CH₂P, minor), 4.87 (s, 2H, OCH₂, major), 4.95 (s, 2H, OCH₂, minor) ppm.
\(^{13}\text{C-NMR}\ (75\ \text{MHz, D}_2\text{O})\): \(\delta = 19.79\) (CH\(_3\)), 66.38 (d, \(J_{C,P} = 137.0\ \text{Hz, CH}_2\text{P, major}\)), 70.87 (d, \(J_{C,P} = 137.0\ \text{Hz, CH}_2\text{P, minor}\)), 77.78 (s, OCH\(_2\), major), 77.93 (s, OCH\(_2\), minor), 175.74 (C=O) ppm.

\(^{31}\text{P-NMR}\ (121\ \text{MHz, D}_2\text{O})\): \(\delta = 15.16\) ppm.

Exact mass (ESI-MS): calculated for C\(_4\)H\(_{11}\)NO\(_6\)P \([\text{M+H}]^+\): 200.0324; found: 200.0632.

3.12. Diethyl ((ethoxycarbonyl)methoxy)methylphosphonate (30)
Sodium hydride (400 mg, 11.9 mmol) was added to a solution of diethylhydroxymethyl phosphonate (1 g, 5.9 mmol) in THF (26 mL) and stirred for 1 h. Subsequently, ethyl bromoacetate (1.29 g, 7.7 mmol) was added and, after 3 h, the reaction was quenched with sat. NH\(_4\)Cl (aq.). EtOAc was added, the two phases separated and the aqueous layer further extracted with three portions of EtOAc (100 mL). The combined organic layers were dried over MgSO\(_4\) and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (CH\(_2\)Cl\(_2\)/MeOH, 95:5) to give 30 (1.21 g, 80%) as an oil.

\(^1\text{H-NMR}\ (300\ \text{MHz, CDCl}_3\): \(\delta = 1.25\) (t, \(J = 7.0\ \text{Hz, 6H, C(O)OCH}_2\text{CH}_3\)), 1.32 (t, \(J = 7.0\ \text{Hz, 6H, POCH}_2\text{CH}_3\)), 3.91 (d, \(J_{H,P} = 8.5\ \text{Hz, 2H, CH}_2\text{P}\)), 4.12 – 4.22 (m, 8H, C(O)CH\(_2\)O and POCH\(_2\)CH\(_3\)) ppm.

\(^{13}\text{C-NMR}\ (75\ \text{MHz, CDCl}_3\): \(\delta = 14.37\) (C(O)OCH\(_2\)CH\(_3\)), 16.64 (d, \(J_{C,P} = 5.8\ \text{Hz, POCH}_2\text{CH}_3\)), 61.19 (C(O)OCH\(_2\)CH\(_3\)), 62.79 (d, \(J_{C,P} = 6.3\ \text{Hz, POCH}_2\text{CH}_3\)), 65.30 (d, \(J_{C,P} = 165.5\ \text{Hz, CH}_2\text{P}\)), 69.66 (d, \(J_{C,P} = 11.2\ \text{Hz, C(O)CH}_2\text{O}\)), 169.79 (C=O) ppm.

Exact mass (ESI-MS): calculated for C\(_9\)H\(_{20}\)O\(_6\)P \([\text{M+H}]^+\): 255.0998; found: 255.0987.

3.13. 2-((Ethoxyphosphono)methoxy)acetic acid (31)
A solution of 28 (1.21 g, 4.8 mmol) in 4.8 mL 1N aq. NaOH was stirred for 2 h at 30 °C.

When the hydrolysis was finished, as indicated by TLC, the reaction mixture was neutralized
with 1N HCl until pH ≈ 6. The desired carboxylic acid was extracted with CH₂Cl₂ (5 × 50 mL) and EtOAc (3 × 50 mL). The combined organic layers were dried over MgSO₄ and concentrated in vacuo. Purification by flash chromatography (CH₂Cl₂/MeOH/ HCOOH, 95:5:0.1) afforded 31 (1.0 g, 94%). The carboxylic acid was visualized on TLC with bromocresol green.

¹H-NMR (300 MHz, CDCl₃): δ = 1.36 (t, J = 7.0 Hz, 6H, OCH₂CH₃), 3.98 (d, J₄,₅ = 8.2 Hz, 2H, CH₂P), 4.16 – 4.26 (m, 6H, C(O)CH₂O and OCH₂CH₃), 6.79 (br. s, 1H, OH) ppm.

¹³C-NMR (75 MHz, CDCl₃): δ = 16.61 (d, J₃,₅ = 5.8 Hz, OCH₂CH₃), 63.34 (d, J₂,₃ = 6.3 Hz, OCH₂CH₃), 65.40 (d, J₃,₅ = 165.8 Hz, CH₂P), 69.74 (d, J₃,₅ = 10.1 Hz, C(O)CH₂O), 171.91 (C=O) ppm.

Exact mass (ESI-MS): calculated for C₇H₁₆O₆P [M+H]⁺: 227.0685; found: 227.0683.

3.14. Diethyl (benzyloxycarbamoyl)methoxy)methylphosphonate (32)

Compound 31 (0.80 g, 3.5 mmol) was dissolved in CH₂Cl₂ (4 mL). To this solution 1.1 eq. carbonyldiimidazole (0.60 g, 3.7 mmol) was added. After stirring for 0.5 h, a suspension of benzylhydroxylamine hydrochloride (0.59 g, 3.7 mmol) in 4 mL of CH₂Cl₂ containing Et₃N (0.38 g, 3.7 mmol) was added. After disappearance of all starting material as indicated by TLC, the reaction mixture was diluted with 50 mL CH₂Cl₂ and washed with 1N HCl (60 mL). The organic layer was dried over MgSO₄ and concentrated in vacuo. Purification by flash chromatography (CH₂Cl₂/MeOH, 95:5) afforded 32 as an oil in 83% yield (970 mg).

¹H-NMR (300 MHz, CDCl₃): δ = 1.31 (t, J = 7.0 Hz, 6H, OCH₂CH₃), 3.76 (d, J₂,₃ = 8.8 Hz, 2H, CH₂P), 4.07 – 4.17 (m, 6H, C(O)CH₂O and OCH₂CH₃), 4.96 (s, 2H, PhCH₂O), 7.34 – 7.44 (m, 5H, arom. H), 9.46 (br. s, 1H, NH) ppm.
$^{13}$C-NMR (75 MHz, CDCl$_3$): $\delta = 16.65$ (d, $^3J_{C,P} = 5.8$ Hz, OCH$_2$CH$_3$), 63.01 (d, $^2J_{C,P} = 6.6$ Hz, OCH$_2$CH$_3$), 66.19 (d, $^1J_{C,P} = 168.4$ Hz, CH$_2$P), 72.33 (d, $^3J_{C,P} = 10.1$ Hz, CH$_2$), 78.59 (PhCH$_2$O), 128.78 (=CH), 128.95 (=CH), 129.39 (=CH), 135.0 (=C), 168.91 (C=O) ppm.

Exact mass (ESI-MS): calculated for C$_{14}$H$_{23}$NO$_6$P [M+H]$^+$: 332.1263; found: 332.1272.

3.15. Diethyl (hydroxycarbamoyl)methoxy)methylphosphonate (33)

Benzyl deprotection of 32 was essentially performed as described for 10 and 11, yielding the title compound in 80% yield (92 mg).

$^1$H-NMR (300 MHz, CDCl$_3$): $\delta = 1.30$ (m, 6H, OCH$_2$C$_6$H$_5$), 3.85 (m, 2H, CH$_2$P), 4.14 (m, 6H, C(O)CH$_2$O and OCH$_2$CH$_3$), 10.02 (br. s, 1H, NH) ppm.

$^{13}$C-NMR (75 MHz, CDCl$_3$): $\delta = 16.59$ (d, $^3J_{C,P} = 5.5$ Hz, OCH$_2$CH$_3$), 63.23 (d, $^2J_{C,P} = 6.3$ Hz, OCH$_2$CH$_3$), 65.91 (d, $^1J_{C,P} = 166.7$ Hz, CH$_2$P), 71.57 (d, $^3J_{C,P} = 10.0$ Hz, C(O)CH$_2$O), 166.24 (C=O) ppm.

Exact mass (ESI-MS): calculated for C$_{8}$H$_{17}$NO$_6$P [M+H]$^+$: 254.0794; found: 254.0806.

3.16. Diethyl (N-(benzyloxy)-N-methylcarbamoyl)methoxy)methylphosphonate (34)

To solution of 32 (0.51 g, 1.5 mmol) in 16 mL of THF was added sodium hydride (67 mg, 1.7 mmol) and methyl iodide (0.24 g, 1.7 mmol). The mixture was stirred overnight at room temperature, quenched with a saturated solution of NH$_4$Cl (40 mL) and extracted with ether (3 $\times$ 40 mL). The organic layer was dried over MgSO$_4$ and concentrated in vacuo. Purification by flash chromatography (CH$_2$Cl$_2$/MeOH, 95:5) afforded 34 (300 mg, 56%).

$^1$H-NMR (300 MHz, CDCl$_3$): $\delta = 1.30$ (t, $J = 7.0$ Hz, 6H, OCH$_2$CH$_3$), 3.17 (s, 3H, NCH$_3$), 3.83 (d, $J_{H,P} = 8.2$ Hz, 2H, CH$_2$P), 4.09 – 4.19 (m, 4H, OCH$_2$CH$_3$), 4.25 (s, 2H, C(O)CH$_2$O), 4.77 (s, 2H, PhCH$_2$O), 7.26 – 7.37 (m, 5H, arom. H) ppm.
$^{13}$C-NMR (75 MHz, CDCl$_3$): $\delta = 16.63$ (d, $^3J_{C,P} = 5.8$ Hz, OCH$_2$CH$_3$), 33.73 (NCH$_3$), 62.71 (d, $^2J_{C,P} = 6.6$ Hz, OCH$_2$CH$_3$), 65.11 (d, $^1J_{C,P} = 164.7$ Hz, CH$_2$P), 69.91 (d, $^3J_{C,P} = 11.5$ Hz, C(O)CH$_2$O), 76.53 (PhCH$_2$O), 129.00 (=CH), 129.42 (=CH), 129.65 (=CH), 134.28 (=C), 171.13 (C=O) ppm.

Exact mass (ESI-MS): calculated for C$_{15}$H$_{25}$NO$_6$P [M+H]$^+$: 346.1420; found: 346.1408.

3.17. **Diethyl ((N-hydroxy-N-methylcarbamoylmethoxy)methoxy)methylphosphonate (35)**

Benzyl deprotection of 34 was essentially performed as described for 10 and 11, yielding the title compound in 89% yield (140 mg).

$^1$H-NMR (300 MHz, CDCl$_3$): $\delta = 1.31$ (t, $J = 7.0$ Hz, 6H, OCH$_2$CH$_3$), 3.19 (s, 3H, NCH$_3$), 3.91 (d, $^1J_{H,P} = 8.2$ Hz, 2H, CH$_2$P), 4.09 – 4.19 (m, 4H, OCH$_2$CH$_3$), 4.41 (s, 2H, C(O)CH$_2$O), 9.38 (br. s, 1H, OH) ppm.

$^{13}$C-NMR (75 MHz, CDCl$_3$): $\delta = 16.57$ (d, $^3J_{C,P} = 5.8$ Hz, OCH$_2$CH$_3$), 36.03 (NCH$_3$), 63.22 (d, $^2J_{C,P} = 6.6$ Hz, OCH$_2$CH$_3$), 65.02 (d, $^1J_{C,P} = 166.1$ Hz, CH$_2$P), 70.01 (d, $^3J_{C,P} = 11.8$ Hz, C(O)CH$_2$O), 169.83 (C=O) ppm.

Exact mass (ESI-MS): calculated for C$_8$H$_{19}$NO$_6$P [M+H]$^+$: 256.0950; found: 256.1052.

3.18. **Dimethyl 2-(N-benzyloxycarbonyloxy)ethylphosphonate (37)**

The title compound was prepared in 73% yield from 36 using a similar procedure as described for 32.

$^1$H-NMR (300 MHz, CDCl$_3$): $\delta = 2.13$ (dt, $J_{H,H} = 7.3$ Hz and $J_{H,P} = 18.8$ Hz, 2H, CH$_2$P), 3.68 (d, $J = 11.1$ Hz, 6H, OCH$_2$), 4.30 (dt, $J_{H,H} = 7.6$ Hz and $J_{H,P} = 12.9$ Hz, 2H, CH$_2$CH$_2$P), 4.81 (s, 2H, PhCH$_2$O), 7.29 – 7.34 (m, 5H, arom. H), 8.15 (br. s, 1H, NH) ppm.
$^{13}$C-NMR (75 MHz, CDCl$_3$): $\delta = 25.42$ (d, $^1J_{C,P} = 140.5$ Hz, CH$_2$P), 52.73 (d, $^2J_{C,P} = 6.6$ Hz, OCH$_3$), 59.79 (CH$_2$CH$_2$P), 78.78 (PhCH$_2$O), 128.69 (=CH), 128.78 (=CH), 129.32 (=CH), 135.68 (=C), 157.16 (C=O) ppm.

Exact mass (ESI-MS): calculated for C$_{12}$H$_{19}$NO$_6$P [M+H]$^+$: 304.0950; found: 304.0947.

3.19. Dimethyl 2-(N-hydroxycarbonyloxy)ethylphosphonate (38)

Benzyl deprotection of 37 was essentially performed as described for 10 and 11, yielding the title compound in 98% yield (350 mg).

$^1$H-NMR (300 MHz, CDCl$_3$): $\delta = 2.21$ (dt, $J_{H,H} = 7.0$ Hz and $J_{H,P} = 18.8$ Hz, 2H, CH$_2$P), 3.77 (d, $J = 11.1$ Hz, 6H, OCH$_3$), 4.40 (dt, $J_{H,H} = 7.0$ Hz and $J_{H,P} = 15.3$ Hz , 2H, CH$_2$CH$_2$P), 7.40 (br. s, 1H, NH) ppm.

$^{13}$C-NMR (75 MHz, CDCl$_3$): $\delta = 25.45$ (d, $^1J_{C,P} = 141.4$ Hz, CH$_2$P), 52.96 (d, $^2J_{C,P} = 6.6$ Hz, OCH$_3$), 59.82 (CH$_2$CH$_2$P), 158.38 (C=O) ppm.

Exact mass (ESI-MS): calculated for C$_5$H$_{13}$NO$_6$P [M+H]$^+$: 214.0481; found: 214.0479.

3.20. Dimethyl 2-(N-benzyloxy-N-methylcarbonyloxy)ethylphosphonate (39)

The title compound was prepared in 68% yield from 37 using a similar procedure as described for 34.

$^1$H-NMR (300 MHz, CDCl$_3$): $\delta = 2.03$ (dt, $J_{H,H} = 7.0$ Hz and $J_{H,P} = 18.8$ Hz, 2H, CH$_2$P), 2.91 (s, 3H, NCH$_3$), 3.56 (d, $J = 10.8$ Hz, 6H, OCH$_3$), 4.20 (dt, $J_{H,H} = 7.3$ Hz and $J_{H,P} = 13.2$ Hz , 2H, CH$_2$CH$_2$P), 4.69 (s, 2H, PhCH$_2$O), 7.15 – 7.25 (m, 5H, arom. H) ppm.

$^{13}$C-NMR (75 MHz, CDCl$_3$): $\delta = 25.33$ (d, $^1J_{C,P} = 140.5$ Hz, CH$_2$P), 36.89 (NCH$_3$), 52.52 (d, $^2J_{C,P} = 6.3$ Hz, OCH$_3$), 60.08 (CH$_2$CH$_2$P), 76.65 (PhCH$_2$O), 128.52 (=CH), 128.70 (=CH), 129.48 (=CH), 135.47 (=C), 157.06 (C=O) ppm.

Exact mass (ESI-MS): calculated for C$_{13}$H$_{21}$NO$_6$P [M+H]$^+$: 318.1107; found: 318.1111.
3.21. Dimethyl-2-(N-hydroxy-N-methylcarbonyloxy)ethylphosphonate (40)

Benzyl deprotection of 39 was essentially performed as described for 10 and 11, yielding the title compound in 97% yield (300 mg).

$^1$H-NMR (300 MHz, CDCl$_3$): $\delta = 2.09$ (dt, $J_{H,H} = 6.2$ Hz and $J_{H,P} = 18.8$ Hz, 2H, CH$_2$P), 3.08 (s, 3H, NCH$_3$), 3.64 (d, $J = 10.8$ Hz, 6H, OCH$_3$), 4.40 (dt, $J_{H,H} = 6.5$ Hz and $J_{H,P} = 13.8$ Hz, 2H, CH$_2$CH$_2$P) ppm.

$^{13}$C-NMR (75 MHz, CDCl$_3$): $\delta = 25.27$ (d, $^1J_{C,P} = 140.5$ Hz, CH$_2$P), 38.34 (NCH$_3$), 52.82 (d, $^2J_{C,P} = 6.3$ Hz, OCH$_3$), 59.76 (CH$_2$CH$_2$P), 157.22 (C=O) ppm.

Exact mass (ESI-MS): calculated for C$_6$H$_{15}$NO$_6$P [M+H]$^+$: 228.0637; found: 228.0624.

3.22. General method for the phosphonate deprotection towards compounds 12, 13, 14 and 15.

Esters 33, 35, 38 or 40 (0.39 mmol) were dissolved in CH$_2$Cl$_2$ (1.1 mL) and treated dropwise with 2 eq. of TMSBr (0.77 mmol, 83 mg) 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. 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$_4$OAc 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 foams or slight yellow oils after lyophilisation.

For compounds 12 and 13, this procedure was not very efficient since the target compounds showed poor retention (elution time: 3.6 min). Nevertheless it permitted to produce a pure fraction.
3.22.1. ((Hydroxycarbamoyl)methoxy)methylphosphonic acid (12): Yield: 52% according to HPLC analysis.

$^1$H-NMR (300 MHz, D$_2$O): $\delta = 3.49$ (d, $J_{H,P} = 9.1$ Hz, 2H, CH$_2$P), 3.92 (s, 2H, C(O)CH$_2$O, minor), 4.07 (s, 2H, C(O)CH$_2$O, major) ppm.

$^{13}$C-NMR (75 MHz, D$_2$O): $\delta = 69.47$ (d, $^1J_{C,P} = 152.3$ Hz, CH$_2$P), 70.76 (d, $^3J_{C,P} = 13.3$ Hz, C(O)CH$_2$OO), 169.31 (C=O) ppm.

$^{31}$P-NMR (121 MHz, D$_2$O): $\delta = 15.58$ ppm.

Exact mass (ESI-MS): calculated for C$_3$H$_7$NO$_6$P $[\text{M-H}]^-$: 184.0010; found: 184.0456.

3.22.2. (\(N\)-hydroxy-\(N\)-methylcarbamoyl)methoxy)methylphosphonic acid (13): Yield: 45% according to HPLC analysis.

$^1$H-NMR (300 MHz, D$_2$O): $\delta = 3.16$ (s, 3H, NCH$_3$, major), 3.22 (s, 3H, NCH$_3$, minor), 3.51 (d, $J_{H,P} = 8.5$ Hz, 2H, CH$_2$P), 4.25 (s, 2H, C(O)CH$_2$O, minor), 4.38 (s, 2H, C(O)CH$_2$O, major) ppm.

$^{13}$C-NMR (75 MHz, D$_2$O): $\delta = 36.09$ (NCH$_3$), 68.40 (d, $^1J_{C,P} = 157.5$ Hz, CH$_2$P), 69.37 (d, $^3J_{C,P} = 11.5$ Hz, C(O)CH$_2$O), 171.73 (C=O) ppm.

$^{31}$P-NMR (121 MHz, D$_2$O): $\delta = 15.42$ ppm.

Exact mass (ESI-MS): calculated for C$_4$H$_9$NO$_6$P $[\text{M-H}]^-$: 198.0166; found: 198.0160.

3.22.3. 2-(\(N\)-hydroxycarbonyloxy)ethyolphosphonic acid (14): Yield: 71 mg (quantitative).

$^1$H-NMR (300 MHz, MeOD): $\delta = 2.14$ (dt, $J_{H,H} = 7.0$ Hz and $J_{H,P} = 19.1$ Hz, 2H, CH$_2$P), 4.31 (dt, $J_{H,H} = 7.6$ Hz and $J_{H,P} = 10.6$ Hz , 2H, CH$_2$CH$_2$P) ppm.

$^{13}$C-NMR (75 MHz, MeOD): $\delta = 27.51$ (d, $^1J_{C,P} = 137.0$ Hz, CH$_2$P), 59.97 (CH$_3$CH$_2$P), 159.15 (C=O) ppm.
$^{31}$P-NMR (121 MHz, MeOD): $\delta = 25.63$ ppm.

Exact mass (ESI-MS): calculated for C$_3$H$_8$NO$_6$P [M-H]: 184.0010; found: 184.0007.

### 3.22.4. 2-(N-hydroxy-N-methylcarbonyloxy)ethylphosphonic acid (15): Yield: 153 mg (quantitative).

$^1$H-NMR (300 MHz, MeOD): $\delta = 2.16$ (m, 2H, CH$_2$P), 3.16 (s, 3H, NCH$_3$), 4.32 (m, 2H, CH$_2$CH$_2$P) ppm.

$^{13}$C-NMR (75 MHz, MeOD): $\delta = 27.43$ (d, $^1$J$_{C,P} = 138.2$ Hz, CH$_2$P), 37.68 (NCH$_3$), 60.68 (CH$_2$CH$_2$P), 157.74 (C=O) ppm.

$^{31}$P-NMR (121 MHz, MeOD): $\delta = 25.92$ ppm.

Exact mass (ESI-MS): calculated for C$_4$H$_9$NO$_6$P [M-H]: 198.0166; found: 198.0173.

### 3.23. Bis(pivaloyloxymethyl)ester of 13 (17)

To a solution of 34 in CH$_2$Cl$_2$ was added TMSBr (4eq.) dropwise at 0 °C. After one hour the icebath was removed and the mixture was stirred at rt for another five hours. All volatiles were removed under vacuum, the residual oil was redissolved in THF and treated with water. After five minutes the solvent was evaporated and the residue was dried in vacuo overnight. For the alkylation step, the resulting oil was dissolved in anhydrous DMF, treated with triethylamine and stirred for ten minutes. Chloromethyl pivalate was added and the mixture was stirred at 70 °C for 6.5 h, then at room temperature during the weekend. DMF was removed under reduced pressure and the residue was taken up in diethyl ether, filtered and extracted with water and saturated NaHCO$_3$-solution. The organic layer was dried over anhydrous MgSO$_4$ and the solvent was removed under reduced pressure. The resulting oil was purified by flash chromatography on silica gel with diethyl ether as an eluent. Benzyl deprotection was performed in ethyl acetate with H$_2$ at atmospheric pressure and Pd/C 10% as
a catalyst. After hydrogenating for four hours at room temperature the mixture was filtered over a celite pad, the solvent was evaporated and the resulting oil was purified by column chromatography on silica gel (Et₂O to Et₂O:EtOAc 1:1) to give 17 as a slightly purple oil in 56% yield.

\[ ^1H\text{-NMR} \text{(300 MHz, CDCl}_3 \text{)}: \delta = 1.23 \text{ (s, 18H, (CH}_3)_3\text{C(O)O), 3.26 (br.s, 3H, NCH}_3\text{), 4.00 (d, J}_{CP} = 6.6 \text{ Hz, 2H, CH}_2\text{P), 4.41 (s, 2H, C(O)CH}_2\text{O), 5.70 (m, 4H, (CH}_2\text{O})_2\text{P), 8.23 (br.s, 1H, NOH) ppm.} \]

\[ ^{13}\text{C-NMR} \text{(75 MHz, CDCl}_3 \text{)}: \delta = 27.02 ((\text{CH}_3)_3\text{), 36.11 (NCH}_3\text{), 38.95 (C(\text{CH}_3)_3\text{), 65.20 (d, J}_{CP} = 165.59 \text{ Hz, CH}_2\text{P), 70.07 (d, J}_{CP} =9.96 \text{ Hz, C(O)CH}_2\text{O), 82.08 (d, J}_{CP} =6.64 \text{ Hz, OCH}_2\text{O), 169.66 (C(O)N), 177.13 (C(O)O) ppm.} \]

\[ ^{31}\text{P-NMR} \text{(121 MHz, CDCl}_3 \text{): } \delta = 22.91 \text{ (s) ppm.} \]

Exact mass (ESI-MS): calculated for C\text{_{16}H}_{29}\text{NO}_{10}\text{P [M-H]}^-: 428.16799; found: 428.16729.

### 3.24. Biological assays

#### 3.24.1. DOXP reductoisomerase inhibition assay

The assay was performed in a reaction mixture containing 100 mM Tris HCl (pH 7.5), 0.2 % bovine serum albumin, 1 mM MnCl₂, 1 mM DOXP, 0.3 mM NADPH, and 1 µg/ml recombinant DOXP reductoisomerase of *E. coli*. The mixture without substrate was pre-incubated with a serial dilution of the test compounds on a 96 well plate at 30 °C for 5 min. The compounds had been dissolved in 100 mM Tris HCl (pH 7.5) and pre-diluted to the 10-fold final concentrations on a separate plate. The reaction was started by the addition of DOXP. The decrease of absorption was monitored at 340 nm using a SpectraMax 340PC microplate reader (Molecular Devices).
3.24.2. In vitro culture of *P. falciparum*

*P. falciparum* parasites were cultured in RPMI 1640 medium supplemented with 10% human serum and 25 mM HEPES buffer. Human erythrocytes served as host cells. For maintenance of the culture, Petri dishes with 10 cm diameter were used. Each Petri dish contained 10 ml medium with a haematocrit of 5%. The cultures were incubated at 37 °C under an atmosphere of 5% O₂, 3% CO₂ and 92% N₂. The medium was changed daily, and the cultures diluted at a maximal parasitaemia of 5%. The parasitaemia was checked microscopically using Giemsa-stained smears.

3.24.3. In vitro determination of antimalarial activity

For the in vitro determination of antimalarial activity a 96-well plate assay was used (Desjardins et al. 1979; Ancelin et al. 1998). To each well 0.2 ml of a suspension of *P. falciparum* infected erythrocytes with 2% haematocrit and 0.4% parasitaemia were added. Then, a serial dilution of the test compounds was prepared on the plate. After incubation for 48 h, 0.8μ Ci [³H]hypoxanthine in 50 μl medium were added to each well. The plates were further incubated for 24 h. The parasites were collected on glass fibre filters using a cell harvester (Micromate 196, Packard). The incorporated radioactivity was measured with a β-counter (Matrix 9600, Packard).
Acknowledgment

This study was supported by grants from the European Commission (QLK2-CT-2002-00887) and INTAS (03-51-4077). Technical assistance was provided by Dajana Henschker.
**Figure 1.** The conversion of 1-deoxy-d-xylulose 5-phosphate (DOXP, 1) to 2-C-methyl-d-erythritol 4-phosphate (MEP, 3) by deoxyxylulose 5-phosphate reductoisomerase (DXR).

**Chart 1.** Structures of fosmidomycin, FR900098, reported and synthesized analogues.

**Scheme 1.** Reagents and conditions: (a) acetyl chloride, CH$_2$Cl$_2$, Et$_3$N, 0 °C or carbonyldiimidazole, HCOOH, rt; (b) paraformaldehyde, tBuOK, 60 °C; (c) SOCl$_2$, CH$_2$Cl$_2$, 10 °C; (d) paraformaldehyde, Et$_3$N, 90 °C; (e) CH$_2$Cl$_2$, rt; (f) H$_2$, Pd/C, MeOH, rt.

**Scheme 2.** Reagents and conditions: (a) ethylbromoacetate, NaH, DMF, 60 °C; (b) NaOH, H$_2$O, rt; (c) carbonyldiimidazole, BnONH$_2$·HCl, Et$_3$N, rt; (d) MeI, NaH, rt; (e) H$_2$, Pd/C, MeOH, rt; (f) TMSBr, CH$_2$Cl$_2$, rt.

**Scheme 3.** Reagents and conditions: (a) carbonyldiimidazole, NH$_2$OBn·HCl, Et$_3$N, rt; (b) MeI, NaH, rt; (c) H$_2$, Pd/C, MeOH, rt; (d) TMSBr, CH$_2$Cl$_2$, rt.

**Scheme 4.** Reagents and conditions: (a) (i) TMSBr in CH$_2$Cl$_2$ 6h, 0 °C to rt, then H$_2$O in THF, rt; (ii) (CH$_3$)$_3$CC(O)OCH$_2$Cl, Et$_3$N, DMF, 70 °C to rt, 2d; (iii) H$_2$, Pd/C, EtOAc, rt.

**Table 1.** IC$_{50}$ values against recombinant *E. coli* DXR and in vitro growth inhibition of a 3D7 *P. falciparum* strain.
Figure 1

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\[
\begin{align*}
\text{DOXP (1)} & \xrightarrow{\text{Mg}^{2+}} \begin{array}{c}
\text{O} \\
\text{O} \\
\text{H}
\end{array} & \begin{array}{c}
\text{OH} \\
\text{O} \\
\text{H}
\end{array} & \begin{array}{c}
\text{O} \\
\text{O} \\
\text{P}
\end{array} \\
\text{NADPH} & \xrightarrow{\text{NADPH}} & \begin{array}{c}
\text{OH} \\
\text{O} \\
\text{H}
\end{array} & \begin{array}{c}
\text{OH} \\
\text{O} \\
\text{P}
\end{array} \\
\text{MEP (3)} & & \begin{array}{c}
\text{OH} \\
\text{O} \\
\text{H}
\end{array} & \begin{array}{c}
\text{OH} \\
\text{O} \\
\text{P}
\end{array}
\end{align*}
\]
```

Chart 1

```
\[
\begin{align*}
\text{Fosmidomycin (4)} & \quad \text{FR900098 (5)} \\
\text{Fosfoxacin (6)} & \quad \text{7} \\
\text{8} & \quad \text{9} \\
\text{10} & \quad \text{11} \\
\text{12} & \quad \text{13} \\
\text{14} & \quad \text{15} \\
\text{16} & \quad \text{17}
\end{align*}
\]
```
<table>
<thead>
<tr>
<th>compound</th>
<th>IC₅₀ (μM) <em>E. coli</em> DXRa</th>
<th>IC₅₀ (μM) 3D7a</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>0.097 ± 0.012</td>
<td>0.98 ± 0.06</td>
</tr>
<tr>
<td>10</td>
<td>1.11 ± 0.14</td>
<td>4.9 ± 0.4</td>
</tr>
<tr>
<td>11</td>
<td>0.087 ± 0.015</td>
<td>0.36 ± 0.02</td>
</tr>
<tr>
<td>12</td>
<td>&gt;3</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>0.072 ± 0.012</td>
<td>0.24 ± 0.09</td>
</tr>
<tr>
<td>14</td>
<td>&gt;3</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>&gt;3</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>&gt;30</td>
<td>0.099 ± 0.012</td>
</tr>
<tr>
<td>17</td>
<td>&gt;30</td>
<td>0.051 ± 0.010</td>
</tr>
<tr>
<td><strong>fosmidomycin</strong></td>
<td>0.054 ± 0.012</td>
<td>1.20 ± 0.18</td>
</tr>
<tr>
<td><strong>FR900098</strong></td>
<td>0.062 ± 0.012</td>
<td>0.43 ± 0.06</td>
</tr>
</tbody>
</table>

*Mean values ± standard deviation of 3 to 5 independent measurements*

Table 1
Scheme 1

20 → (a) → 21: R = H, 22: R = Me
(b) → 23: R = H, 24: R = Me
(c) → 25: R = H, 26: R = Me
(d) → 18
(e) → 19
(f) → 10: R = H, 11: R = Me
Scheme 2
Scheme 3

Scheme 4
References


18 Trager, W.; Jensen, J.B. Science 1976, 193, 673-5.