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Synthesis and bioactivity of β -substituted fosmidomycin analogues targeting 1-deoxy-D- xylulose-5-phosphate reductoisomerase

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ABSTRACT. Blocking the MEP pathway for isoprenoid biosynthesis offers interesting prospects for inhibiting *Plasmodia* or *Mycobacteria* growth. Fosmidomycin (**1**) and its homologue FR900098 (**2**) potently inhibit 1-deoxy-D-xylulose-5-phosphate reductoisomerase (Dxr), a key enzyme in this pathway. Here we introduced aryl or aralkyl substituents at the β -position of the hydroxamate analogue of **2**. While direct addition of a β -aryl moiety resulted in poor inhibition, longer linkers between the carbon backbone and the phenyl ring were generally associated with better binding to the enzymes. X-ray structures of the parasite Dxr-inhibitor complexes show that the “longer” compounds generate a substantially different flap structure, in which a key tryptophan residue is displaced, and the aromatic group of the ligand lies between the tryptophan and the hydroxamate’s methyl group. Although the most promising new Dxr inhibitors lack activity against *E. coli* and *M. smegmatis*, they proved to be highly potent inhibitors of *Plasmodium falciparum* *in vitro* growth.

Introduction

Malaria and tuberculosis feature on the World Health Organization's work plan as infectious diseases requiring urgent attention. *Plasmodium falciparum* is responsible for the most severe malaria cases,¹ while human tuberculosis (TB) results mainly from infection with *Mycobacterium tuberculosis* (Mtb). Global statistics reveal that between 2000 and 2012, scale-up of interventions helped to reduce malaria incidence rates by 29%. However, in 2012 an estimated 1300 children under the age of five years died from malaria every day, and the annual number of fatalities due to malaria was close to one million.² The same year, 8.6 million people developed TB and 1.3 million died from the disease, many of whom were HIV-positive.³ Even though malaria and tuberculosis are preventable and usually treatable, the high incidence of both diseases and evolving resistance to many drugs compel us to continue efforts toward the discovery of novel therapeutics.

A common denominator for both pathogens is their dependence on the 2-C-methyl-D-erythritol-4-phosphate (MEP) pathway for the biosynthesis of the isoprenoid precursors, isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP).^{4,5} Humans obtain these vital building blocks via an alternate route, known as the mevalonate pathway.⁶ Since the two pathways utilize non-homologous enzymes, the MEP pathway has several interesting targets for new therapeutics against malaria and/or tuberculosis.⁷ In this regard, 1-deoxy-D-xylulose-5-phosphate reductoisomerase (Dxr), the enzyme catalyzing the first committed step⁸ in the MEP pathway, has received most attention and is the subject of a recent review.⁹ Dxr catalyzes the conversion of 1-deoxy-D-xylulose-5-phosphate (DOXP) to MEP.¹⁰ Fosmidomycin (**1**, Figure 1), a natural phosphonate-type antibiotic extracted from *Streptomyces lavendulae*, is a potent inhibitor of Dxr.¹¹ FR900098 (**2**), the *N*-acetyl homologue of fosmidomycin, is twice as potent

against *P. falciparum* *in vitro* and against *P. vinckei* in a mouse model of infection.⁴ Protein prenylation, isoprene-containing quinone production, *N*-linked glycosylation as well as carotenoid and vitamin E biosynthesis are believed to be essential isoprenoid-dependent physiological processes in *Plasmodium*.¹² Interrupting any of these processes kills the parasite. In combination with clindamycin, fosmidomycin has undergone clinical evaluation for the treatment of uncomplicated *P. falciparum* malaria.^{13,14,15} However, a study showed that this combination had inadequate efficacy in clearing uncomplicated malaria in Mozambican children less than three years old.¹⁶

Although fosmidomycin is a remarkably safe antimalarial agent, low oral absorption, short serum half-life and malaria recrudescence preclude its use in monotherapy.^{17,18,19} Similarly, while fosmidomycin and **2** effectively inhibit purified recombinant MtbDxr, these highly hydrophilic antibiotics cannot penetrate the notoriously thick and lipophilic mycobacterial cell wall and lack significant activity against intact bacteria.⁵ Furthermore, Mtb lacks the glycerol-3-phosphate transporter (*glpT*), which is known to actively import fosmidomycin/**2** into a variety of bacterial pathogens such as *Escherichia coli* and some apicomplexan parasites like *Plasmodium*.^{5,20,21,22} Consequently, the development of more lipophilic Dxr inhibitors able to passively permeate into cells with improved pharmacokinetic properties could lead to more efficacious agents.

(Figure 1)

Both the phosphonate and the retro-hydroxamate groups of fosmidomycin/**2** are essential for Dxr inhibitory activity,^{23,24,25} however, modification of the three-carbon spacer linking these moieties has yielded analogues with exciting potency profiles. SAR studies reveal that a propyl linker is optimal.^{26,27} Previously, we discovered that analogue **3a** (Figure 2), featuring a 3,4-

dichlorophenyl substituent in the α -position of the phosphonate, surpasses fosmidomycin's potency in inhibiting *P. falciparum* growth.²⁸ Extensive crystallographic studies of various α -aryl derivatives in complex with EcDxr,²⁹ MtbDxr^{30,31,32} and PfDxr³³ showed that the active-site flap became disordered in these structures. This prevented the interaction of the indole ring of a highly conserved tryptophan residue (Trp211 in EcDxr, Trp203 in MtbDxr, Trp296 in PfDxr) with the fosmidomycin backbone, as had been observed in earlier antibiotic-NADPH-active-site metal containing ternary complexes.^{34,35,36} The changes to the flap are a direct consequence of the mode of binding of these analogues to the Dxr active site, since extensive clashes would otherwise result with the indole ring's placement in the ternary complexes. Recent complexes of various α -aryl derivatives with PfDxr,^{37,38} however, show quite well defined flaps (including the tryptophan); the substrate-binding site undergoes less drastic changes, but has opened up sufficiently to accommodate the inhibitors. The major conformational changes make it difficult to pinpoint the precise cause of the enhanced potency of **3a** and the derivatives described by Jansson et al.³¹ Analogues **3b** and **4a** showed comparable inhibitory activity, in line with the report by the Rohmer group that the hydroxamate counterparts of fosmidomycin or **2** (**4b**) have comparable inhibitory activity against EcDxr to the natural products.³⁹ Kurz *et al.* confirmed that adding electron-withdrawing substituents to the α -phenyl ring of the bispivaloyloxymethyl (bis-POM) ester prodrug **5a** slightly improved *P. falciparum* inhibition.⁴⁰ An aromatic substituent may also increase the compound's overall lipophilicity, thereby enhancing cellular uptake of these analogues.

(Figure 2)

Although substitution of the α -position of fosmidomycin has been widely explored, manipulation of the β -position has received much less attention. After we found that the β -oxa

derivative was almost equipotent to **2**,⁴¹ Brucher *et al.* demonstrated that β -oxa modifications combined with α -aryl substituents may afford potent PfDxr inhibitors (*e.g.*, **6a**), with promising *in vitro* antiparasmodial activity.⁴² The Kurz group showed that replacement of the β -methylene group with a sulfur atom in **6b** resulted in lower IC₅₀ values for *E. coli* and MtbDxr compared to the oxa ligand **6a**.⁴³ Furthermore, they demonstrated that the PfDxr inhibitory activity of the *S*-(+)-enantiomers was clearly superior to that of the *R*-(-) diastomers (*e.g.*, *S*-(+)-**6c** IC₅₀: 9.4 nM, *R*-(-)-**6c** IC₅₀: 12 μ M), in agreement with results from crystallographic studies on the Dxr binding of **3a** and related analogues carried out by Andaloussi *et al.*³⁰ and Jansson *et al.*³¹

The objective of this work was to assess the effect of introducing aromatic moieties in the β -position of the propyl backbone of **4b** on Dxr inhibition (Figure 3). First, we decided to introduce aryl groups (**7a–e**) based on Topliss' scheme,⁴⁴ an operational decision tree that suggests the optimum substitution pattern on a phenyl ring for attaining drug potency. Additionally, an analog of compound **4b** bearing a methyl group in the β -position (**7f**) was prepared. Although the latter compound has not been reported before, its retro-hydroxamate formyl and acetyl counterparts appear in a patent⁴⁵ and have been studied *in silico*.^{46,47} The known **4b39** was resynthesized to serve as a positive control for evaluation. Compounds **8a–d** were synthesized to assess the optimal linker length between the propyl backbone of fosmidomycin and the phenyl ring.

(Figure 3)

Results and discussion

Synthesis

(Scheme 1)

Scheme 1 shows the synthetic route followed to prepare compounds **7a–f**. The commercially available cinnamic acids **10a–e** were esterified by treatment with di-*tert*-butyl dicarbonate in *tert*-butanol.⁴⁸ The resulting *tert*-butyl cinnamates **11a–c** and the purchased *tert*-butyl crotonate **11f** served as Michael acceptors in a reaction with dibenzyl methylphosphonate to furnish predominantly the desired 1,4-addition adducts **12a–c,f** as described by Yamaguchi and co-workers.⁴⁹ Previously encountered complications during catalytic hydrogenation of related compounds bearing a chlorinated phenyl ring led to the use of diethyl methylphosphonate as a Michael donor for addition to **11d** and **11e** to yield **14d** and **14e**, respectively. Hydrolysis of the *tert*-butyl ester group of **12a–c,f** and **14d,e** with 20% TFA in CH₂Cl₂ and subsequent EDC-mediated coupling of the resulting carboxylic acids with *O*-benzyl-*N*-methyl-hydroxylamine yielded the protected *N*-methylhydroxamates **13a–c,f** and **15d,e**. Compounds **13a–c** and **13f** were deprotected by catalytic hydrogenolysis to access target phosphonates **7a–c** and **7f**. The hydroxamate group of **15d** and **15e** was unmasked with BCl₃. Bromotrimethyl silane (TMSBr) mediated deprotection of phosphonate esters **16d** and **16e** and basic workup yielded **7d–e** as bisammonium salts.

The synthesis of target compounds **8a–d** is outlined in Scheme 2. The preparation of the appropriate Michael acceptors **19a–d** commenced with a Dess-Martin oxidation of commercially available alcohols **17a–d** to afford aldehydes **18a–d**, which were swiftly transformed to the corresponding *tert*-butyl esters **19a–d** via Wittig olefination. Michael addition of dibenzyl methylphosphonate to **19a–d** predominantly afforded 1,4-addition adducts **20a–d** due to steric

hindrance of the *tert*-butyl group. Compounds **20a–d** were converted to the desired phosphonates **8a–d** as before. ³⁵P-NMR spectra of **7a–f** and **8a** indicate that these appear as rotameric mixtures, a known^{50,51} phenomenon that was further validated by variable-temperature ³¹P-NMR studies.

(Scheme 2)

Biological evaluation

Final compounds were tested for inhibition of recombinant enzymes using a spectrophotometric assay monitoring the substrate-dependent oxidation of NADPH associated with the Dxr-catalyzed reaction.¹¹ Initially, Dxr inhibition was studied at a compound concentration of 100 μ M (Figure 4). At this point, differences among the enzymes and compounds were already evident.

(Figure 4)

As anticipated, the known hydroxamate **4b39** was highly effective at inhibiting MtbDxr. A lipophilic prodrug of this compound has recently been shown to effectively inhibit *M. smegmatis* growth in Kirby-Bauer disk diffusion assays.⁵² In contrast, compounds **7a–e** had only modest activity on MtbDxr and EcDxr. Interestingly, compound **7f**, characterized by the presence of a β -methyl substituent rather than the bulkier aromatic group, retained good inhibitory activity. Other noteworthy trends were the fact that **8a–d** were more potent on EcDxr than the **7**-series compounds, and that PfDxr was more effectively inhibited than the other two enzymes.

IC₅₀ values of the most promising compounds (determined as described in the Supporting Information) are reported in Table 1. Again, differences between the various enzymes were apparent. PfDxr was most easily inhibited (followed by EcDxr), and for this enzyme, the **8**-series

compounds were more potent than **7a–c**. For the homologue series **8a–d**, the activity against EcDxr was most dependent on the linker length, although all enzymes were sensitive to this parameter to some degree. In general, a 3-carbon linker appeared to be best for EcDxr and MtbDxr, but both 3 and 4-carbon linkers showed very good activity on PfDxr.

Table 2 summarizes the antibacterial/antiparasitic activity of these compounds. Of the compounds evaluated against *E. coli*, only **4b** showed moderate activity (MIC₅₀ 15.6–31.25 µM, generally comparable to those of fosmidomycin and **2**). The most promising MtbDxr inhibitors, **4b** and **8c**, were also tested against intact *M. smegmatis* cells. *M. smegmatis* was used as a model for Mtb due to its non-pathogenicity and shorter doubling time.⁵³ However, as for the other compounds tested (Table 2), they showed no activity (MIC₅₀ > 250 µM). Overall, the lack of correlation between activity against purified enzymes and activity against whole bacteria suggests a lack of uptake and/or active efflux of these molecules.

All analogues were also evaluated *in vitro* for schizontocidal activity against the *P. falciparum* K1 strain. This activity correlated surprisingly well with the PfDxr inhibitory activity summarized in Table 1. Compounds **7a–e** were not active against blood stage *P. falciparum*. Compound **4b**, the congener of **2** with a hydroxamate group, showed potent antiplasmodial activity (IC₅₀ < 0.26 µM). Introduction of a β-methyl group (**7f**) was well tolerated (IC₅₀ = 0.74 µM). The activity trend in the homologue series **8b–d** was similar to that observed for PfDxr enzyme inhibition, and indicates that a linker of three or four carbons is optimal in this case. Comparison of the *P. falciparum* K1 growth inhibition assay with the enzyme activity results suggests that there is an improved interaction with the *Plasmodium* enzyme with longer linker lengths, but there could also be a positive influence on cell permeability.

X-ray structures of PfDxr in complex with four inhibitors

As described in more detail in the Supplementary Information, the structures of PfDxr in complex with four of the new β -substituted inhibitors (**7a**, **7b**, **8c** and **8d**) have been solved at resolutions of 1.9, 2.1, 1.6 and 1.9 Å, respectively. These structures were refined to crystallographic R-factors of 18.7, 21.7, 17.7 and 16.5%, and free R-factors of 21.8, 25.2, 20.6, and 19.6%. Each structure includes a dimer in the asymmetric unit, with a manganese ion and inhibitor in both active sites. The overall electron density is of high quality and complete models of the enzyme are deposited at the PDB for residues 77-486 in each chain. Although the compounds were synthesized as racemic mixtures, the high resolution of the study (Figures 5, 6 and S1) allowed us to identify the favored enantiomer for each ligand. Tests of both enantiomers in the refinement strongly suggested that all compounds were bound primarily as the *R*-enantiomer, although chemical rules of priority mean that the actual arrangement of the β -substituent at the chiral carbon is different for **7a** and **7b**, compared to **8c** and **8d**.

The protein in the complexes with **7a** and **7b** is for the most part identical, with an r.m.s. distance of only 0.2 Å when the subunits are compared. The largest difference is at Pro294 of the active-site flap, where the C α position differs by approximately 1 Å in the two structures; there is well-ordered electron density for all residues in the respective flaps. This movement is directly linked to the addition of the methyl group in **7b**, and correlates well with the observation that IC₅₀ increases as larger groups are added at this position. Interactions with the hydroxamate and phosphonate are essentially the same. The methyl group of the hydroxamic acid in each case makes close interactions with the indole ring of Trp296 (Figure 6B). Both of these protein structures are quite similar to the complexes with fosmidomycin and **2**, with the caveat that His293, Met298 and Ile302 must adjust somewhat to accommodate the new β -substituent.

More substantial differences are observed for the complexes with **8c** and **8d**. As can be seen

in Figure 6C, the β -substituents are placed in a very different way in the two series of compounds. Again, most of the protein is very similar, but the active-site flaps are pushed further away as the β -substituent of **8c** and **8d** displaces Trp296. In the **8c** complex, density is weak between 293-295 of both chains. Only the flap of the A molecule of the **8d** complex has continuous density at the RMS of the map; in the B-site there is a break between residues 292-296. In each complex, the methylene linker of the inhibitor is found in a depression described in numerous C α -aryl complexes, wedged between three ordered and one often-disordered loop (containing PfDxr residues 272, 338, 358 and 296, respectively). As shown in Figure 5C and 6C, the flexibility of the linker means it can take on a boomerang shape that allows the phenyl group of each inhibitor to interact with its methyl group (Figure 5B). The phenyl ring occupies the place normally assumed by the indole ring of the conserved tryptophan of the flap (Figure 5C), resulting in acyl-group-to-ring interactions similar to those seen in the **7a** and **7b** complexes (Figures 6B and S1), as well as **2** ternary complexes.³⁶ In three of the four active sites where we are able to observe Trp296, the face of the indole ring stacks on the edge of the phenyl ring of the inhibitor (Figure 5B). These favorable interactions are achieved in the **8c** complex with the same ligand backbone conformation normally observed in antibiotic/NADPH ternary complexes. In the **8d** complex, fitting in the extra methylene group, while conserving the position of the phenyl group, requires a rearrangement of the fosmidomycin backbone (Figures 5C and 6C). While the position of the hydroxamate remains unchanged, there are small but significant changes in the orientation of the phosphonate. These changes do not cause a ripple of differences in protein side chains involved in phosphonate binding, but instead there is a rearrangement of which phosphonate oxygen interacts with which hydrogen-bond donor. Figure 6C shows a superposition of the hydrogen-bond donors that interact with **7a**, **7b**, **8c** or **8d**. Clearly, they are

structurally highly conserved, while the phosphonate groups are not so tightly clustered. Table S3 highlights the various sets of interactions, and shows that only one phosphonate oxygen has the full set of three interacting groups in all complexes, a second oxygen has two interacting groups, and the third has either two or three interacting groups. Only oxygen 1 has the same interacting groups in all four complexes, and only five groups interact in the same site in all complexes, while one group (Lys312-NZ) interacts in all complexes, but with two different oxygens. The rearrangement of the fosmidomycin backbone in **8c** would produce a close contact with the usual conformation seen for Lys312, and so a small conformational change is needed to relieve this clash while maintaining an interaction with the phosphonate (Figure 6C). The side-chain of His293 does not contribute to phosphonate binding in any of the complexes, in contrast to observations in antibiotic/NADPH ternary complexes.

In all active sites, in all complexes, the hydroxamic acid group adopts a synperiplanar conformation ($\text{O}=\text{C}-\text{N}-\text{O}$ angle is 0°), in which both oxygen atoms coordinate to the manganese, as does a single carboxyl oxygen atom from each of the highly conserved acidic residues, Asp231, Asp233 and Glu315. The differences from the usual set of interactions observed in the antibiotic/NADPH ternary complexes are merely a consequence of the hydroxamate group in the new structures. Figures 5C and 6B show that the hydrogen bonding interactions are conserved, and the *N*-formyl oxygen now accepts a hydrogen bond from Asn311-ND2, while the *N*-hydroxyl oxygen interacts with Ser232-OG and a conserved water molecule.

At present, it is not clear why such large differences are observed for the inhibition of the different enzymes. Modelling experiments were therefore performed in an attempt to gain insights into how the new inhibitors might interact with MtbDxr. Specifically, we were interested in understanding how the phenylpropyl substituent of **8c** might be interacting with the Trp-containing loop of Dxr. In the X-ray structure of MtbDxr in complex with **2** (PDB code 4A03),⁵⁴ a loop containing Trp203 closes over the bound ligand, while this loop is disordered in the reported X-ray structures of Dxr co-complexes with α -phenyl-substituted analogues, which are all less potent inhibitors of the enzyme.

When comparing the predicted binding of **7f** and the aromatic analogues **8a–d** to the measured binding of fosmidomycin, one makes an interesting observation with respect to Ser213. In the co-crystal structure with fosmidomycin bound, Ser213 is hydrogen bonded to the phosphate of the ligand. This same orientation is predicted when **7f** is bound. For the aromatic analogues, however, Ser213 is reoriented and points instead towards His200. It appears that the hydrogen bonding between Ser213 and the phosphate is disrupted upon binding of the aromatic analogues. His203 appears to bind to Ser213 in order to compensate for the loss of interaction with the phosphate. This might explain the loss of activity of aromatic analogues **8a–d** relative to fosmidomycin.

The modeled structure of **8c** (white carbons) compared to the X-ray structure of the protein with fosmidomycin bound (green carbons) is shown in Figure 7.

(Figure 7)

In the minimized structure of **8c**, the aromatic ring occupies almost exactly the same position as the phenyl portion of Trp203 in the complex with fosmidomycin. Since the aromatic ring of Trp203 occupies what is presumably a stable position in the folded protein, it could be assumed

that this position would also be favourable for a ligand to occupy when the loop needs to be displaced for steric reasons. This could be described as an aromatic “hotspot”. Therefore, it could be that this results in favourable van der Waals and lipophilic interactions of the phenyl ring in this position which accounts for the better activity of **8c** compared to the shorter **8b**, which may not reach the hotspot, and **8d**, where the carbon chain is too long for the phenyl to occupy the same position. In addition, Trp203 in the loop of the minimized structure with **8c** makes an edge-to-face interaction with the phenyl ring of the ligand, potentially stabilising the loop *and* ligand in this position.

Conclusions

We present here the first systematic study on β -substituted analogues of fosmidomycin. A series of analogues with different aromatic moieties connected directly to the β -carbon (**7a–e**) failed to inhibit EcDxr and MtbDxr and proved moderately active against PfDxr. None of these compounds was capable of inhibiting the growth of *E. coli*, *M. smegmatis* or *P. falciparum* strain K-1. Exploration of alkyl linkers of different lengths between the β -carbon and a phenyl ring resulted in establishment of a 3-carbon linker (**8c**) as optimal for *E. coli* and *M. tuberculosis* Dxr inhibition, and a 4-carbon linker (**8d**) for inhibition of *P. falciparum* Dxr. While compounds **8a–d** also lacked activity against *E. coli* and *M. smegmatis*, **8c** and **8d** showed submicromolar schizontocidal activity against the *P. falciparum* K1 strain, where essentially the same SAR was observed as for PfDxr inhibition. Interestingly, the activity of **8c** and **8d** surpassed that of **7f**, indicating a favourable contribution of the phenylpropyl and phenylbutyl substituents to antiplasmodial growth inhibition.

Crystallographic studies on four of the compounds most active on PfDXR (**7a**, **7b**, **8c** and **8d**), show two different, novel modes of binding to the enzyme. The compounds showing the best enzyme inhibition, and best *in vitro* activity against the parasite, mimic the favourable interactions between the indole ring of the conserved tryptophan in the flap with the fosmidomycin backbone that have been seen in a number of antibiotic-bound ternary complexes. However, this mimicry is achieved by intramolecular interactions within each inhibitor (**8c**, **8d**), such that the phenyl ring common to this series spatially overlaps the usual position of the indole ring. Rearrangement of the flap results in favourable interactions between the phenyl ring of the inhibitors and the tryptophan. The improved activity of **8d** compared to **8c** is likely a consequence of this set of interactions.

Experimental Section

General

All reactions described were performed under an argon atmosphere and at ambient temperature unless stated otherwise. All reagents and solvents were purchased from Sigma-Aldrich (Diegem, Belgium), Acros Organics (Geel Belgium) or TCI Europe (Zwijndrecht, Belgium) and used as received (except THF). THF was dried over sodium/benzophenone. NMR solvents were purchased from Eurisotop (Saint-Aubin, France). Reactions were monitored by TLC analysis using TLC aluminium sheets (Macherey-Nagel, Alugram Sil G/UV₂₅₄). Detection was by spraying with a solution of (NH₄)₆Mo₇O₂₄·4H₂O (25 g/L) and (NH₄)₄Ce(SO₄)₄·2H₂O (10 g/L) in H₂SO₄ (10 %) followed by charring or immersion in an aqueous solution of KMnO₇ (20 g/L) and K₂CO₃ (10 g/L) or an ethanolic solution of ninhydrin (2 g/L) and acetic acid (1% v/v) followed by charring. Silica gel column chromatography was performed manually using Grace Davisil

60Å silica gel (40-63 μm) or automated using a Grace Reveleris X2 system and the corresponding flash cartridges. High-resolution spectra were recorded with a Waters LCT Premier XE Mass spectrometer. ¹H- and ¹³C-NMR spectra were recorded with a Varian Mercury-300BB (300/75 MHz) spectrometer. Chemical shifts are given in ppm (δ) relative to tetramethylsilane (¹H NMR) or the NMR solvent (¹³C NMR) as an internal standard. In ³¹P NMR, signals are referenced to the CDCl₃ or D₂O lock resonance frequency according to IUPAC referencing, with H₃PO₄ set to 0.00 ppm. Coupling constants are given in Hz. Preparative HPLC purifications were carried out using a Laprep preparative HPLC system equipped with an Xbridge Prep C18 column (19×250 mm, 5 micron) using a water/acetonitrile/formic acid gradient solvent system. All synthesized compounds were ≥95% pure as verified by LCMS. NMR analysis showed a rotameric mixture for some of the prepared compounds. At high temperature (80 °C), the phosphorus signals of the two rotameric forms of a representative compound (**7b**) merged into a single peak.

General Procedure I: Synthesis of *tert*-butyl cinnamates 11a–e

To a 0.1 M solution of the appropriate cinnamic acid (40-45 mmol, 1 equiv) in *tert*-butanol at 35 °C, was added di-*tert*-butyl dicarbonate (2.0 equiv) and 4-dimethylaminopyridine (0.3 equiv). The mixture was heated to 35 °C overnight and then poured into water and extracted three times with dichloromethane. The organic fractions were pooled, washed once with brine and dried over NaSO₄. Filtration, *in vacuo* concentration and subsequent silica gel column chromatography gave the respective *tert*-butyl cinnamates. Characterization was in agreement with reported data.^{55,56}

General Procedure II: Dess-Martin oxidation and concomitant Wittig olefination

A 0.05 M solution of the starting material in CH_2Cl_2 and a nitrogen atmosphere was cooled to 0 °C. Solid Dess-Martin periodinane (2.0 equiv) was added and the temperature was allowed to rise to RT. Upon completion of the reaction (TLC monitoring; typically 3 h), the mixture was washed with a mixture (5:1 v/v) of NaHCO_3 (sat. aq.) and $\text{Na}_2\text{S}_2\text{O}_3$ (aq. 2.0 M). The formed water layer was then extracted three times with diethyl ether. The organic fractions were combined, washed with HCl (0.1 M, once), brine (once) and dried over anhydrous Na_2SO_4 before *in vacuo* concentration. The resulting crude aldehyde was dissolved in toluene under a nitrogen atmosphere and 3.0 equiv of *tert*-butyl (triphenylphosphoranylidene)acetate was added. The mixture was refluxed at 120 °C overnight. It was allowed to cool to RT and concentrated *in vacuo*. The crude mixture was adsorbed onto celite and purified by silica gel chromatography.

General procedure III: Michael addition of methylphosphonatediesters to α,β -unsaturated *tert*-butyl esters.

To a 1.0 M solution of dibenzylmethyl- or diethylmethyl-phosphonate (2.0 equiv) in THF was added *n*-BuLi (1.6 M solution in hexanes, 2.0 equiv) at -78 °C under a N_2 atmosphere. After 30 minutes, a 0.5 M solution of the α,β -unsaturated ester (1.0 equiv) in THF was added dropwise. When the reaction was complete (typically 3 h), the reaction was quenched with sat. aq. NH_4Cl and transferred to a separatory funnel. The aqueous solution was extracted three times with EtOAc. The organic fractions were combined, washed once with brine, dried (Na_2SO_4), filtered and concentrated. Column chromatography of the residue yielded the Michael adduct.

General procedure IV: Acidic cleavage of the *tert*-butyl ester and protected hydroxamate formation.

A 0.1 M solution of the *tert*-butyl ester (1.0 equiv) in CH₂Cl₂/TFA (80:20) at 0 °C, was stirred for 2 h, after which an excess of toluene was added to the reaction mixture and concentrated *in vacuo*. The crude acid was redissolved in CH₂Cl₂ (0.1 M), followed by addition of EDC (1.2 equiv), DMAP (1.2 equiv) and DiPEA (2.0 equiv). *O*-Benzyl-*N*-methylhydroxylamine TFA salt (1.2 equiv) was added as a 0.2 M solution in CH₂Cl₂, and the ensemble was allowed to stir overnight at room temperature. The mixture was quenched with sat. aq. NaHCO₃, extracted three times with CH₂Cl₂, washed with brine and dried over Na₂SO₄. Column chromatography produced the protected hydroxamic acids.

General procedure V: Catalytic hydrogenolysis of benzyl protective groups.

The benzyl protected compound was dissolved in MeOH (10 mg/mL) under a nitrogen atmosphere and a catalytic amount of Pd/C (10 %) was added. The resulting mixture was then stirred under a hydrogen atmosphere for 10 minutes. Upon completion of the reaction (confirmed by MS), the mixture was filtered and neutralized with 1.0 equiv of NaOH (aq. 1.0M). It was then concentrated *in vacuo*, re-dissolved in a mixture of water and *tert*-butanol (1:1 v/v, 1.0 mL/10 mg starting material), frozen and lyophilized affording the desired phosphonic acid monosodium salts in quantitative yield.

General procedure VI: Boron trichloride mediated selective debenzylation of the hydroxamate.

A 0.1 M solution of the benzylated hydroxamate in dichloromethane was cooled to -75 °C. BCl₃ (1M solution in CH₂Cl₂, 3.0 equiv) was added dropwise and the mixture allowed to stir at this temperature for 45 min. Next, the reaction mixture was poured into aqueous NaHCO₃, and

extracted 4 times with CH₂Cl₂. The organic fractions were combined, washed with brine, dried (Na₂SO₄), filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography to obtain the free hydroxamate.

General procedure VII: Trimethylbromosilane mediated deprotection of diethyl phosphonates.

To a 0.1 M solution of starting material in dichloromethane, was added BSTFA (4.0 equiv). The mixture was allowed to stir at room temperature for 15 min before an ice bath was installed, and TMSBr (10.0 equiv) was added. The ice bath was removed after 10 min, and the reaction allowed to stir until phosphorus NMR confirmed complete deprotection. All volatiles were removed *in vacuo* and the resultant oil was re-dissolved in acetonitrile. Concentrated ammonia was added, and the mixture was allowed to stir at room temperature for 20 min. Evaporation of volatiles and subsequent lyophilization from a mixture of *tert*-butanol and water afforded the compound in quantitative yield.

Dibenzyl 2-phenyl-3-(*tert*-butoxycarbonyl)propylphosphonate (12a). Prepared from compound **11a** (715 mg, 3.50 mmol) according to general procedure III. Colorless oil; purification 1.5:1 Hex/EtOAc v/v; yield 72%. ¹H NMR (300 MHz, CDCl₃) δ_H ppm 1.25 (br, 9H, *t*-Bu), 2.17 (dd, *J* = 3.5 Hz, 18.6 Hz, 1H, P-CH₂-), 2.19 (dd, *J* = 2.8 Hz, 18.6 Hz, 1H, P-CH₂-), 2.52 (ddd, *J* = 1.3 Hz, *J* = 9.3 Hz, 15.2 Hz, 1H, -CH₂-CO), 2.78 (dd, *J* = 6.2 Hz, 15.2 Hz, 1H, -CH₂-CO), 3.44-3.62 (m, 1H, -CH-), 4.66-4.87 (m, 4H, -CH₂Ph), 7.17-7.36 (m, 15H, Ar-H). ¹³C-NMR (75 MHz, CDCl₃) δ_C ppm 27.8, 32.8 (d, ¹*J*_{C-P} = 139.2 Hz), 36.9 (d, ²*J*_{C-P} = 3.1 Hz), 43.1 (d, ³*J*_{C-P} = 12.9 Hz), 66.8 (d, ²*J*_{C-P} = 6.5 Hz), 67.1 (d, ²*J*_{C-P} = 6.4 Hz), 80.5, 126.9, 127.5, 127.8,

127.9, 128.3, 128.4, 136.2 (d, $^3J_{C-P}$ = 6.2 Hz), 136.3 (d, $^3J_{C-P}$ = 6.1 Hz), 142.9 (d, $^3J_{C-P}$ = 8.9 Hz), 170.5. ^{31}P -NMR (121.5 MHz, CDCl_3): δ_{P} ppm = 32.9. HRMS (ESI): calculated for $\text{C}_{28}\text{H}_{34}\text{O}_5\text{P}$ $[(\text{M}+\text{H})^+]$, 481.2138; found 481.2148.

Dibenzyl 2-(*p*-methylphenyl)-3-(*tert*-butoxycarbonyl)propylphosphonate (12b). Prepared from compound **11b** (680 mg, 3.11 mmol) according to general procedure III. Colorless oil; purification 2:1 Hex/EtOAc v/v; yield 73%. ^1H NMR (300 MHz, CDCl_3) δ_{H} ppm 1.27 (br, 9H, *t*-Bu), 2.11-2.23 (m, 2H, P-CH₂-), 2.28 (s, 3H, -Ph-CH₃), 2.50 (ddd, J = 1.5 Hz, 9.2 Hz, 15.2 Hz, 1H, -CH₂-CO), 2.75 (dd, J = 6.4 Hz, 15.2 Hz, 1H, -CH₂-CO), 3.40-3.57 (m, 1H, -CH-), 4.68-4.88 (m, 4H, -CH₂-Ph), 7.01-7.11 (m, 4H, Ar-H), 7.18-7.36 (m, 10H, Ar-H). ^{13}C -NMR (75 MHz, CDCl_3) δ_{C} ppm 21.0, 27.9, 32.8 (d, $^1J_{C-P}$ = 139.6 Hz), 36.5 (d, $^2J_{C-P}$ = 3.2 Hz), 43.3 (d, $^3J_{C-P}$ = 12.8 Hz), 66.8 (d, $^2J_{C-P}$ = 6.4 Hz), 67.0 (d, $^2J_{C-P}$ = 6.6 Hz), 80.4, 127.3, 127.8, 127.8, 128.2, 128.5, 129.1, 136.3 (d, $^2J_{C-P}$ = 6.1 Hz), 136.4 (d, $^2J_{C-P}$ = 6.0 Hz), 139.9 (d, $^3J_{C-P}$ = 8.8 Hz), 170.7. ^{31}P -NMR (121.5 MHz, CDCl_3): δ_{P} ppm = 31.4. HRMS (ESI): calculated for $\text{C}_{29}\text{H}_{36}\text{O}_5\text{P}$ $[(\text{M}+\text{H})^+]$, 495.2295; found 495.2315.

Dibenzyl 2-(*p*-methoxyphenyl)-3-(*tert*-butoxycarbonyl)propylphosphonate (12c). Prepared from compound **11c** (700 mg, 2.99 mmol) according to general procedure III. White crystal; purification 1:1 Hex/EtOAc v/v; yield 69%. ^1H NMR (300 MHz, CDCl_3) δ_{H} ppm 1.27 (br, 9H, *t*-Bu), 2.04-2.27 (m, 2H, P-CH₂-), 2.48 (ddd, J = 1.4 Hz, 9.3 Hz, 15.2 Hz, 1H, -CH₂-CO), 2.73 (dd, J = 6.3 Hz, 15.2 Hz, 1H, -CH₂-CO), 3.40-3.57 (m, 1H, -CH-), 3.74 (s, 3H, -Ph-O-CH₃), 4.68-4.91 (m, 4H, -CH₂Ph), 6.78 (d, J = 8.5 Hz, 2H, Ar-H), 7.11 (d, J = 8.5 Hz, 2H, Ar-H), 7.18-7.37 (m, 10H, Ar-H). ^{13}C -NMR (75 MHz, CDCl_3) δ_{C} ppm 27.8, 32.9 (d, $^1J_{C-P}$ = 137.7 Hz), 36.2 (d, $^2J_{C-P}$ = 3.5 Hz), 43.4 (d, $^3J_{C-P}$ = 12.2 Hz), 55.1, 66.8 (d, $^2J_{C-P}$ = 5.8 Hz), 67.0 (d, $^2J_{C-P}$ = 5.8 Hz), 80.4, 113.7, 127.8, 127.8, 128.2, 128.4, 134.9 (d, $^3J_{C-P}$ = 8.5 Hz), 136.2 (d, $^3J_{C-P}$ = 5.9 Hz), 136.3

(d, $^3J_{C-P}$ = 5.3 Hz), 158.4, 170.5. ^{31}P -NMR (121.5 MHz, CDCl_3): δ_{P} ppm = 30.25 HRMS (ESI): calculated for $\text{C}_{29}\text{H}_{36}\text{O}_6\text{P}$ $[(\text{M}+\text{H})^+]$, 511.2244; found 511.2260.

Dibenzyl 2-methyl-3-(*tert*-butoxycarbonyl)propylphosphonate (12f). Prepared from compound *tert*-butylcrotonate (890 mg, 6.26 mmol) according to general procedure III. Colorless oil; purification 1.5:1 Hex/EtOAc v/v; yield 62%. ^1H NMR (300 MHz, CDCl_3) δ_{H} ppm 1.08 (d, J = 6.5 Hz, 3H, -CH(CH₃)-), 1.42 (br, 9H, *t*-Bu), 1.61-1.79 (m, 2H, P-CH₂-), 1.83-2.00 (m, 1H, -CH₂-CO), 2.06-2.20 (m, 1H, -CH-), 2.28-2.47 (m, 2H, -CH₂-CO), 4.90-5.10 (m, 4H, -CH₂-Ph), 7.27-7.41 (m, 10H, Ar-H). ^{13}C -NMR (75 MHz, CDCl_3) δ_{C} ppm 21.7 ($^3J_{C-P}$ = 8.6 Hz), 25.1 ($^2J_{C-P}$ = 5.2 Hz), 27.8, 32.4 ($^1J_{C-P}$ = 139.5 Hz), 39.7 ($^3J_{C-P}$ = 12.0 Hz), 67.01 ($^2J_{C-P}$ = 6.5 Hz), 67.03 ($^2J_{C-P}$ = 6.5 Hz), 76.2, 127.9, 127.9, 129.2, 134.5, 136.17 ($^3J_{C-P}$ = 5.9 Hz), 173.3. ^{31}P -NMR (121.5 MHz, CDCl_3): δ_{P} ppm = 32.87. HRMS (ESI): calculated for $\text{C}_{23}\text{H}_{32}\text{O}_5\text{P}$ $[(\text{M}+\text{H})^+]$, 419.1982; found 419.1904.

Dibenzyl 3-(*N*-(benzyloxy)-*N*-methylecarbonyl)-2-phenylpropylphosphonate (13a). Prepared from compound **12a** (1.0 g, 2.08 mmol) according to general procedure IV. Colorless oil; purification 97:3 $\text{CH}_2\text{Cl}_2/\text{MeOH}$ v/v; yield 51%. ^1H NMR (300 MHz, CDCl_3) δ_{H} ppm 2.04-2.39 (m, 2H, P-CH₂-), 2.69-2.90 (m, 2H, -CH₂-CON-), 3.07 (s, 3H, N-CH₃), 3.56-3.75 (m, 1H, -CH(Ph)-), 4.63 (s, 2H, NOCH₂Ph) 4.67-4.91 (m, 4H, POCH₂Ph), 7.13-7.41 (m, 20H, Ar-H). ^{13}C -NMR (75 MHz, CDCl_3) δ_{C} ppm 32.2 (d, $^1J_{C-P}$ = 137.6 Hz), 36.1 (d, $^2J_{C-P}$ = 3.4 Hz), 39.8 (d, $^3J_{C-P}$ = 12.6 Hz), 66.8 (d, $^2J_{C-P}$ = 6.4 Hz), 67.1 (d, $^2J_{C-P}$ = 6.4 Hz), 76.1, 126.8, 127.5, 127.8, 127.9, 128.1, 128.2, 128.5, 128.7, 128.9, 129.2, 134.5, 136.3, (d, $^3J_{C-P}$ = 6.6 Hz), 136.4 (d, $^3J_{C-P}$ = 6.4 Hz), 143.6 (d, $^3J_{C-P}$ = 8.4 Hz), 172.9. ^{31}P -NMR (121.5 MHz, CDCl_3): δ_{P} ppm = 33.16. HRMS (ESI): calculated for $\text{C}_{32}\text{H}_{35}\text{NO}_5\text{P}$ $[(\text{M}+\text{H})^+]$, 544.2247; found 544.2318.

Dibenzyl 3-(*N*-(benzyloxy)-*N*-methylecarbamoyl)-2-*p*-tolylpropylphosphonate (13b).

Prepared from compound **12b** (980 mg, 1.98 mmol) according to general procedure IV. Colorless oil; purification 97:3 CH₂Cl₂/MeOH v/v; yield 56%. ¹H NMR (300 MHz, CDCl₃) δ_H ppm 2.04-2.37 (m, 5H, P-CH₂-, Ph-CH₃), 2.69-2.90 (m, 2H, -CH₂CO), 3.08 (s, 3H, N-CH₃), 3.54-3.71 (m, 1H, -CH-), 4.64 (s, 2H, -NOCH₂Ph), 4.70-4.89 (m, 4H, POCH₂Ph), 7.05 (m, 4H, Ar-H), 7.15-7.40 (m, 15H, Ar-H). ¹³C-NMR (75 MHz, CDCl₃) δ_C ppm 20.9, 32.2 (d, ¹J_{C-P} = 139.6 Hz), 35.5, 39.7 (d, ³J_{C-P} = 11.8 Hz), 66.6 (d, ²J_{C-P} = 5.7 Hz), 66.8 (d, ³J_{C-P} = 6.4 Hz), 66.4, 127.2, 127.6, 127.7, 128.0, 128.3, 128.5, 128.7, 130.0, 129.1, 134.4, 136.0, 136.2 (d, ³J_{C-P} = 6.9 Hz), 136.3 (d, ³J_{C-P} = 6.3 Hz), 140.4 (d, ³J_{C-P} = 8.3 Hz), 172.7. ³¹P-NMR (121.5 MHz, CDCl₃): δ_P ppm = 31.67. HRMS (ESI): calculated for C₃₃H₃₇NO₅P [(M+H)⁺], 558.2404; found 558.2408.

Dibenzyl 3-(*N*-(benzyloxy)-*N*-methylecarbamoyl)-2-(4-methoxyphenyl)propylphosphonate (13c).

Prepared from compound **12c** (850 mg, 1.67 mmol) according to general procedure IV. Colorless oil; purification gradient 0–5% MeOH in CH₂Cl₂; yield 47%. ¹H NMR (300 MHz, CDCl₃) δ_H ppm 2.01-2.37 (m, 2H, P-CH₂-), 2.66-2.88 (m, 2H, -CH₂-CO), 3.07 (s, 3H, N-CH₃), 3.53-3.70 (m, 1H, -CH-), 3.72 (s, 3H, Ph-O-CH₃), 4.64 (s, 2H, NOCH₂Ph), 4.70-4.90 (m, 4H, -CH₂-Ph), 6.76 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.08 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.14-7.39 (m, 15H, Ar-H). ¹³C-NMR (75 MHz, CDCl₃) δ_C ppm 32.3 (¹J_{C-P} = 138.72 Hz), 35.2 (³J_{C-P} = 3.99 Hz), 39.9 (³J_{C-P} = 13.1 Hz), 55.0, 66.6 (²J_{C-P} = 7.1 Hz), 66.8 (²J_{C-P} = 7.0 Hz), 75.9, 113.7, 127.6, 127.8, 128.1, 128.1, 128.3, 128.3, 128.6, 128.8, 129.1, 134.4, 135.5 (³J_{C-P} = 9.0 Hz), 136.2 (³J_{C-P} = 7.1 Hz), 136.33 (³J_{C-P} = 7.1 Hz), 158.2. ³¹P-NMR (121.5 MHz, CDCl₃): δ_P ppm = 30.59. HRMS (ESI): calculated for C₃₃H₃₇NO₆P [(M+H)⁺], 574.2353; found 574.2306.

Dibenzyl 3-(*N*-(benzyloxy)-*N*-methylecarbamoyl)-2-methylpropylphosphonate (13f).

Prepared from compound **12f** (1.0 g, 2.39 mmol) according to general procedure IV. Colorless

oil; purification gradient 0–5% MeOH in CH₂Cl₂; yield 62%. ¹H NMR (300 MHz, CDCl₃) δ_H ppm 1.06 (d, *J* = 6.5 Hz, 3H, -CH(CH₃)-CH₂-), 1.61-1.73 (m, 1H, P-CH₂-), 1.88-2.05 (m, 1H, P-CH₂-), 2.26-2.60 (m, 3H, -CH(CH₃)-, -CH₂-CO), 3.15 (s, 1H, N-CH₃), 4.75 (s, NOCH₂Ph), 4.89-5.09 (m, 4H, -CH₂-Ph), 7.27-7.42 (m, 15H, Ar-H). ¹³C-NMR (75 MHz, CDCl₃) δ_C ppm 21.4 (³*J*_{C-P} = 8.6 Hz), 25.2 (²*J*_{C-P} = 5.2 Hz), 32.3 (¹*J*_{C-P} = 138.5 Hz), 39.7 (³*J*_{C-P} = 12.9 Hz), 67.0 (²*J*_{C-P} = 6.5 Hz), 67.0 (²*J*_{C-P} = 6.5 Hz), 76.2, 127.9, 127.9, 128.3, 128.5, 128.7, 128.9, 129.3, 134.5, 136.5 (³*J*_{C-P} = 6.1 Hz), 173.7. ³¹P-NMR (121.5 MHz, CDCl₃): δ_P ppm = 33.16. HRMS (ESI): calculated for C₂₇H₃₃NO₅P [(M+H)⁺], 482.2091; found 482.2086.

Diethyl 2-(*p*-chlorophenyl)-3-(*tert*-butoxycarbonyl)propylphosphonate (14d). Prepared from compound **11d** (784 mg, 3.28 mmol) according to general procedure III. Pale yellow oil; purification 1.5:1 Hex/Me₂CO v/v; yield 71%. ¹H NMR (300 MHz, CDCl₃) δ_H ppm 1.17-1.34 (m, 6H, P-CH₂CH₃), 1.29 (br, 9H, *t*-Bu), 1.96-2.22 (m, 2H, -CH₂-), 2.52 (dd, *J* = 9.5 Hz, 15.8 Hz, 1H, -CH₂-CO), 2.80 (dd, *J* = 6.3 Hz, 15.8 Hz, 1H, -CH₂-CO), 3.40-3.58 (m, 1H, -CH-), 3.86-4.05 (m, 4H, P-CH₂CH₃), 7.18 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.27 (d, *J* = 8.4 Hz, 2H, Ar-H). ¹³C-NMR (75 MHz, CDCl₃) δ_C ppm 16.1 (d, ³*J*_{C-P} = 16.1 Hz), 16.1 (d, ³*J*_{C-P} = 6.3 Hz), 27.7, 32.2 (d, ¹*J*_{C-P} = 140.8 Hz), 36.3 (d, ²*J*_{C-P} = 3.5 Hz), 42.8 (d, ³*J*_{C-P} = 12.7 Hz), 61.2 (d, ²*J*_{C-P} = 6.9 Hz), 61.4 (d, ²*J*_{C-P} = 6.9 Hz), 80.5, 128.3, 128.8, 132.3, 141.5 (d, ³*J*_{C-P} = 9.6 Hz), 170.2. ³¹P-NMR (121.5 MHz, CDCl₃): δ_P ppm 28.47. HRMS (ESI): calculated for C₁₈H₂₉ClO₅P [(M+H)⁺], 391.1436; found 391.1610.

Diethyl 2-(3,4-dichlorophenyl)-3-(*tert*-butoxycarbonyl)propylphosphonate (14e). Prepared from compound **11e** (656 mg, 2.40 mmol) according to general procedure III. Pale yellow oil; purification 1:1 Hex/Me₂CO v/v; yield 73%. ¹H NMR (300 MHz, CDCl₃) δ_H ppm 1.22 (t, *J* = 7.1 Hz, 3H, -OCH₂CH₃), 1.23 (t, *J* = 7.2 Hz, 3H, -OCH₂CH₃), 1.32 (br, 9H, *t*-Bu), 1.95-2.21 (m, 2H,

P-CH₂-), 2.52 (ddd, $J = 1.2$ Hz, 9.3 Hz, 15.8 Hz, 1H, -CH₂-CO), 2.80 (dd, $J = 6.1$ Hz, 15.8 Hz, 1H, -CH₂-CO), 3.40-3.55 (m, 1H, -CH-), 3.88-4.07 (m, 4H, OCH₂CH₃), 7.10 (dd, $J = 2.1$ Hz, 8.4 Hz, 1H, Ar-H), 7.34 (d, $J = 2.1$ Hz, 1H, Ar-H), 7.37 (d, $J = 8.4$ Hz, 1H, Ar-H). ¹³C-NMR (75 MHz, CDCl₃) δ_C ppm 16.2 (d, $^3J_{C-P} = 2.9$ Hz), 16.3 (d, $^3J_{C-P} = 2.4$ Hz), 27.9, 32.1 (d, $^1J_{C-P} = 140.7$ Hz), 36.3 (d, $^2J_{C-P} = 3.9$ Hz), 42.7 (d, $^3J_{C-P} = 12.1$ Hz), 61.5 (d, $^2J_{C-P} = 7.8$ Hz), 61.6 (d, $^2J_{C-P} = 7.3$ Hz), 80.9, 126.9, 129.6, 130.2, 130.6, 132.2, 143.4 (d, $^3J_{C-P} = 9.0$ Hz), 170.1. ³¹P-NMR (121.5 MHz, CDCl₃): δ_P ppm = 27.96. HRMS (ESI): calculated for C₁₈H₂₈Cl₂O₅P [(M+H)⁺], 425.1046; found 425.1029.

Diethyl 3-(*N*-(benzyloxy)-*N*-methylecarbamoyl)-2-(4-chlorophenyl)propylphosphonate

(15d). Prepared from compound **14d** (870 mg, 2.23 mmol) according to general procedure IV.

Colorless oil; purification 97:3 CH₂Cl₂/MeOH v/v; yield 46%. ¹H NMR (300 MHz, CDCl₃) δ_H ppm 1.16 (app. t, $J = 7.1$ Hz, 3H, P-CH₂CH₃), 1.21 (app. t, $J = 7.1$ Hz, 3H, P-CH₂CH₃), 1.91-2.26 (m, 2H, P-CH₂-), 2.67-2.89 (m, 2H, -CH₂-CO-), 3.10 (s, 3H, N-CH₃), 3.50-3.66 (m, 1H, -CH-), 3.86-4.03 (m, 4H, POCH₂Ph), 4.72 (app. d., $J = 3.3$ Hz, 2H, NOCH₂Ph), 7.02-7.15 (m, 2H, Ar-H), 7.21-7.27 (m, 2H Ar-H), 7.30-7.42 (m, 5H, Ar-H). ¹³C-NMR (75 MHz, CDCl₃) δ_C ppm 16.11-6.39 (m), 31.88 (d, $^1J_{C-P} = 140.2$ Hz), 35.50 (d, $^2J_{C-P} = 3.2$ Hz), 39.60 (d, $^3J_{C-P} = 12.5$ Hz), 61.33 (d, $^2J_{C-P} = 6.4$ Hz), 61.55 (d, $^2J_{C-P} = 6.6$ Hz), 76.2, 128.4, 128.7, 128.9, 129.0, 129.3, 132.3, 134.4, 140.4, 142.3 (d, $^3J_{C-P} = 8.3$ Hz), 167.1. ³¹P-NMR (121.5 MHz, CDCl₃): δ_P ppm 29.87. HRMS (ESI): calculated for C₂₂H₃₀ClNO₅P [(M+H)⁺], 454.1545; found 454.0736.

Diethyl 3-(*N*-(benzyloxy)-*N*-methylecarbamoyl)-2-(3,4-dichlorophenyl)propylphosphonate

(15e). Prepared from compound **14e** (700 mg, 1.65 mmol) according to general procedure IV.

Colorless oil; purification 95:5 CH₂Cl₂/MeOH v/v; yield 49%. ¹H NMR (300 MHz, CDCl₃) δ_H ppm 1.18 (app. t, $J = 7.1$ Hz, 3H, P-CH₂CH₃), 1.22 (app. t, $J = 7.2$ Hz, 3H, P-CH₂CH₃), 1.86-

2.24 (m, 2H, P-CH₂-), 2.61-2.85 (m, 2H, -CH₂-CO-), 3.12 (s, 3H, N-CH₃), 3.44-3.62 (m, 1H, -CH-), 3.82-4.05 (m, 4H, POCH₂CH₃), 4.75 (s, 2H, NOCH₂Ph), 7.02 (dd, $J = 2.2$ Hz, 8.34 Hz, 1H, Ar-H), 7.23-7.44 (m, 7H, Ar-H). ¹³C-NMR (75 MHz, CDCl₃) δ_C ppm 16.2 (d, $^3J_{C-P} = 6.8$ Hz), 16.3 (d, $^3J_{C-P} = 6.6$ Hz), 31.7 (d, $^1J_{C-P} = 140.4$ Hz), 33.4, 35.3 (d, $^2J_{C-P} = 3.3$ Hz), 39.3 (d, $^3J_{C-P} = 13.1$ Hz), 61.4 (d, $^2J_{C-P} = 6.7$ Hz), 61.6 (d, $^2J_{C-P} = 6.6$ Hz), 76.1, 127.1, 128.7, 129.0, 129.3, 129.5, 130.2, 130.4, 132.1, 134.3, 144.1 (d, $^3J_{C-P} = 7.9$ Hz), 172.3. ³¹P-NMR (121.5 MHz, CDCl₃): δ_P ppm 28.22. HRMS (ESI): calculated for C₂₂H₂₉Cl₂NO₅P [(M+H)⁺], 488.1155; found 488.1144.

Diethyl 3-(*N*-hydroxy-*N*-methylecarbamoyl)-2-(4-chlorophenyl)propylphosphonate (16d).

Prepared from compound **15d** (400 mg, 0.88 mmol) according to general procedure VI. Colorless oil; purification gradient 0–10% MeOH in CH₂Cl₂, 1% triethylamine; yield 79%. ¹H NMR (300 MHz, CDCl₃) δ_H ppm 1.18 (app. t, $J = 7.1$ Hz, 3H, P-CH₂CH₃), 1.26 (app. t, $J = 7.0$ Hz, 3H, P-CH₂CH₃), 1.90-2.37 (m, 2H, P-CH₂-), 2.66-2.80 (m, 1H of -CH₂-CO-), 3.11-3.29 (m, 3H, N-CH₃, 1H of -CH₂-CO-), 3.51-3.74 (m, 1H, -CH-), 3.82-3.05 (m, 4H, POCH₂Ph), 7.15-7.33 (m, 4H, Ar-H), 9.46 (s, 1H, N-OH). ¹³C-NMR (75 MHz, CDCl₃) δ_C ppm 16.0-16.5 (m), 31.5 (d, $^1J_{C-P} = 139.2$ Hz), 33.2, 35.8 (d, $^3J_{C-P} = 8.7$ Hz), 37.7 (d, $^2J_{C-P} = 7.0$ Hz), 61.9 (d, $^2J_{C-P} = 7.2$ Hz), 62.2 (d, $^2J_{C-P} = 6.5$ Hz), 128.6, 128.7, 132.5, 142.7 (d, $^3J_{C-P} = 11.1$ Hz), 171.7. ³¹P-NMR (121.5 MHz, CDCl₃): rotamers at δ_P ppm 29.46, 30.85. HRMS (ESI): calculated for C₁₅H₂₄ClNO₅P [(M+H)⁺], 364.1075; found 364.0480.

Diethyl 3-(*N*-hydroxy-*N*-methylecarbamoyl)-2-(3,4-dichlorophenyl)propylphosphonate (16e).

Prepared from compound **15e** (350 mg, 0.72 mmol) according to general procedure VI. Colorless oil; purification gradient 0–10% MeOH in CH₂Cl₂, 1% triethylamine; yield 74%. ¹H NMR (300 MHz, CDCl₃) δ_H ppm 1.21 (app. t, $J = 7.2$ Hz, 3H, P-CH₂CH₃), 1.27 (app. t, $J = 7.1$

Hz, 3H, P-CH₂CH₃), 2.00-2.27 (m, 2H, P-CH₂-), 2.74 (dd, $J = 6.5$ Hz, 15.17 Hz, 1H, -CH₂CO), 3.19 (dd, $J = 8.1$ Hz, 15.2 Hz, 1H, -CH₂CO), 3.22 (s, 3H, N-CH₃), 3.52-3.72 (m, 1H, -CH-), 3.87-4.07 (m, 4H, -POCH₂CH₃), 7.14 (d, $J = 8.1$ Hz, 1H, Ar-H), 7.32-7.45 (m, 2H, Ar-H), 9.44 (s, 1H, N-OH). ¹³C-NMR (75 MHz, CDCl₃) δ_C ppm 16.2 (d, $^3J_{C-P} = 6.7$ Hz), 16.3 (d, $^3J_{C-P} = 6.4$ Hz), 31.3 (d, $^1J_{C-P} = 141.7$ Hz), 35.6 (d, $^2J_{C-P} = 3.3$ Hz), 35.9, 37.6 (d, $^3J_{C-P} = 7.5$ Hz), 62.0 (d, $^2J_{C-P} = 6.9$ Hz), 62.3 (d, $^2J_{C-P} = 7.8$ Hz), 126.9, 129.3, 130.4, 132.4, 130.7, 144.5 (d, $^3J_{C-P} = 12.2$ Hz), 171.4. ³¹P-NMR (121.5 MHz, CDCl₃): rotamers at δ_P ppm 29.01, 30.45. HRMS (ESI): calculated for C₁₅H₂₃Cl₂NO₅P [(M+H)⁺], 398.0685; found 398.0705.

(E)-tert-butyl 4-phenylbut-2-enoate (19a). Prepared from compound **17a** (1.0 g, 8.19 mmol) according to general procedure II. Yellow oil; purification 97:3 Hex/Et₂O v/v; yield 80% over two steps. ¹H NMR (300 MHz, CDCl₃) δ_H ppm 1.46 (br, 9H, *t*-Bu), 3.46 (dd, $J = 1.6$ Hz, 6.7 Hz, 2H -CH₂-), 5.73 (dt, $J = 1.7$ Hz, 15.6 Hz, 1H, -CH=CH-CO-), 6.99 (dt, $J = 6.7$ Hz, 15.5 Hz, 1H, -CH=CH-CO-), 7.14-7.34 (m, 5H, Ar-H). ¹³C-NMR (75 MHz, CDCl₃) δ_C ppm 28.1, 38.1, 80.2, 124.1, 126.5, 128.6, 128.8, 137.9, 145.9, 165.8.

(E)-tert-butyl 5-phenylpent-2-enoate (19b). Prepared from compound **17b** (1.0 g, 7.34 mmol) according to general procedure II. Colorless oil; purification 97:3 Hex/Et₂O v/v; yield 84% over two steps. ¹H NMR (300 MHz, CDCl₃) δ_H ppm 1.48 (br, 9H, *t*-Bu), 2.43-2.54 (m, 2H, -CH₂C=CH-), 2.78 (t, $J = 7.4$ Hz, 2H, Ph-CH₂-), 5.78 (dt, $J = 1.8$ Hz, 15.5 Hz, 1H, -CH=CH-CO-), 6.90 (dt, $J = 6.7$ Hz, 15.5 Hz, 1H, -CH=CH-CO-), 7.13-7.43 (m, 5H, Ar-H). ¹³C-NMR (75 MHz, CDCl₃) δ_C ppm 28.2, 33.8, 34.5, 80.1, 123.4, 126.1, 128.3, 128.5, 141.1, 147.0, 166.0.

(E)-tert-butyl 6-phenylhex-2-enoate (19c). Prepared from compound **17c** (1.5 g, 9.99 mmol) according to general procedure II. Colorless oil; purification 98:2 Hex/Et₂O v/v; yield 79% over two steps. ¹H NMR (300 MHz, CDCl₃) δ_H ppm 1.48 (br, 9H, *t*-Bu), 1.78 (app. quin, 2H, -CH₂-),

2.15-2.25 (m, 2H, -CH₂CH=CH-), 2.64 (t, J = 7.6 Hz, 2H, Ph-CH₂-), 5.75 (dt, J = 1.6 Hz, 15.6 Hz, 1H, CH=CH-CO-), 6.87 (dt, J = 6.8 Hz, 15.6 Hz, 1H, -CH=CH-CO-), 7.13-7.33 (m, 5H, Ar-H). ¹³C-NMR (75 MHz, CDCl₃) δ_C ppm 28.2, 29.8, 31.5, 35.3, 80.1, 123.3, 125.9, 128.4, 128.4, 141.8, 147.5, 166.1. HRMS (ESI): calculated for C₁₆H₂₃O₂ [(M+H)⁺], 247.1693; found 247.1630.

(E)-tert-butyl 7-phenylhept-2-enoate (19d). Prepared from compound **17d** (1.0 g, 6.09 mmol) according to general procedure II. Colorless oil; purification 98:2 Hex/Et₂O v/v; yield 87% over two steps. ¹H NMR (300 MHz, CDCl₃) δ_H ppm 1.44-1.55 (m, 9H, *t*-Bu, 2H, -CH₂-), 1.58-1.71 (m, 2H, -CH₂-), 2.14-2.24 (m, 2H, -CH₂-), 2.61 (t, J = 7.5 Hz, 2H, Ph-CH₂-), 5.7 (dt, J = 1.6 Hz, 15.6 Hz, 1H, -CH=CH-CO-), 6.84 (dt, J = 6.9 Hz, 15.6 Hz, 1H, -CH=CH-CO-), 7.13-7.32 (m, 5H, Ar-H). ¹³C-NMR (75 MHz, CDCl₃) δ_C ppm 27.7, 28.2, 30.9, 31.9, 35.7, 80.0, 123.1, 125.7, 123.3, 128.4, 142.3, 147.7, 166.1. HRMS (ESI): calculated for C₁₇H₂₅O₂ [(M+H)⁺], 261.1849; found 261.1865.

Dibenzyl 2-((tert-butoxycarbonyl)methyl)-3-phenylpropylphosphonate (20a). Prepared from compound **19a** (1.2 g, 5.50 mmol) according to general procedure III. Colorless oil; purification 5:1 Hex/Me₂CO v/v; yield 66%. ¹H NMR (300 MHz, CDCl₃) δ_H ppm 1.41 (br, 9H, *t*-Bu), 1.624-2.03 (m, 2H, P-CH₂-), 2.32-2.40 (m, 2H, -CH₂-CO), 2.38-2.56 (m, 3H, -CH(CH₂-Ph)-), 4.81-5.18 (m, 4H, O-CH₂-Ph), 7.01-7.41 (m, 15H, Ar-H). ¹³C-NMR (75 MHz, CDCl₃) δ_C ppm 28.0, 29.4 (d, ¹ J_{C-P} = 138.9 Hz), 30.5 (d, ² J_{C-P} = 4.5 Hz), 32.2, 37.1 (d, ³ J_{C-P} = 11.1 Hz), 41.2 (d, ³ J_{C-P} = 9.1 Hz), 67.12 (d, ² J_{C-P} = 6.1 Hz), 67.13 (d, ² J_{C-P} = 6.50 Hz), 80.40, 127.30, 127.32, 127.9, 128.4, 139.81 (d, ³ J_{C-P} = 6.06 Hz), 139.84 (d, ³ J_{C-P} = 6.1 Hz), 141.1, 173.3. ³¹P-NMR (121.5 MHz, CDCl₃): δ_P ppm = 33.0. HRMS (ESI): calculated for C₂₉H₃₆O₅P [(M+H)⁺], 495.2295; found 495.2321.

Dibenzyl 2-((*tert*-butoxycarbonyl)methyl)-4-phenylbutylphosphonate (20b). Prepared from compound **19b** (1.0 g, 4.30 mmol) according to general procedure III. Colorless oil; purification 5:1 Hex/Me₂CO v/v; yield 63%. ¹H NMR (300 MHz, CDCl₃) δ_H ppm 1.41 (br, 9H, *t*-Bu), 1.62-2.00 (m, 4H, -CH₂-), 2.22-2.39 (m, 2H, -CH₂-), 2.43-2.52 (m, 1H, -CH-), 2.56 (t, *J* = 8.4 Hz, 2H, -CH₂-), 4.89-5.09 (m, 4H, -CH₂-Ph), 7.07-7.26 (m, 5H, Ar-H), 7.30-7.37 (m, 10H, Ar-H). ¹³C-NMR (75 MHz, CDCl₃) δ_C ppm 28.1, 29.8 (d, ¹*J*_{C-P} = 138.8 Hz), 30.1 (d, ²*J*_{C-P} = 4.7 Hz), 32.7, 36.33 (d, ³*J*_{C-P} = 10.1 Hz), 40.1 (d, ³*J*_{C-P} = 9.4 Hz), 67.0 (d, ²*J*_{C-P} = 6.4 Hz), 67.1 (d, ²*J*_{C-P} = 6.5 Hz), 80.4, 125.7, 127.9, 128.3, 128.5, 136.3 (d, ³*J*_{C-P} = 6.1 Hz), 136.4 (d, ³*J*_{C-P} = 6.1 Hz), 141.7, 171.5. ³¹P-NMR (121.5 MHz, CDCl₃): δ_P ppm = 33.04. HRMS (ESI): calculated for C₃₀H₃₈O₅P [(M+H)⁺], 509.2451; found 509.2466.

Dibenzyl 2-((*tert*-butoxycarbonyl)methyl)-5-phenylpentylphosphonate (20c). Prepared from compound **19c** (1.35 g, 5.48 mmol) according to general procedure III. Colorless oil; purification 6:1 Hex/Me₂CO v/v; yield 71%. ¹H NMR (300 MHz, CDCl₃) δ_H ppm 1.41 (br, 9H, *t*-Bu), 1.42-1.63 (m, 4H, -CH₂-), 1.72-2.03 (m, 2H, -CH₂-), 2.16-2.56 (m, 4H, -CH₂-, 1H, -CH-), 4.86-5.09 (m, 4H, -CH₂-Ph), 7.03-7.47 (m, 15H, Ar-H). ¹³C-NMR (75 MHz, CDCl₃) δ_C ppm 28.0, 28.3, 29.8 (d, ¹*J*_{C-P} = 139.4 Hz), 30.3 (d, ²*J*_{C-P} = 4.1 Hz), 34.2 (d, ³*J*_{C-P} = 10.4 Hz), 35.8, 40.2 (d, ³*J*_{C-P} = 9.6 Hz), 66.9 (d, ²*J*_{C-P} = 6.2 Hz), 67.1 (d, ²*J*_{C-P} = 6.3 Hz), 80.3, 125.6, 127.9, 128.2, 128.3, 128.49, 129.4, 136.4 (d, ³*J*_{C-P} = 6.1 Hz), 136.4 (d, ³*J*_{C-P} = 6.1 Hz), 142.2, 171.6. ³¹P-NMR (121.5 MHz, CDCl₃): δ_P ppm 33.16. HRMS (ESI): calculated for C₃₁H₄₀O₅P [(M+H)⁺], 523.2608; found 523.2411.

Dibenzyl 2-((*tert*-butoxycarbonyl)methyl)-6-phenylhexylphosphonate (20d). Prepared from compound **19d** (1.0 g, 3.84 mmol) according to general procedure III. Colorless oil; purification 6:1 Hex/Me₂CO v/v; yield 68%. ¹H NMR (300 MHz, CDCl₃) δ_H ppm 1.20-1.35 (m, 2H, -CH₂-),

1.41 (br, 9H, *t*-Bu), 1.43-1.61 (m, 4H, -CH₂-), 1.77-1.92 (m, 2H, -CH₂-), 2.16-2.32 (m, 3H, -CH₂-, -CH-), 2.55 (t, *J* = 7.6 Hz, 2H, -CH₂-), 4.90-5.08 (m, 4H, -CH₂-Ph), 7.10-7.37 (m, 15H, Ar-H). ¹³C-NMR (75 MHz, CDCl₃) δ_C ppm 26.0, 28.1, 29.9 (d, ¹*J*_{C-P} = 138.4 Hz), 30.3 (d, ²*J*_{C-P} = 3.9 Hz), 31.3, 34.4 (d, ³*J*_{C-P} = 10.2 Hz), 35.7, 40.2 (d, ³*J*_{C-P} = 9.7 Hz), 67.0 (d, ²*J*_{C-P} = 6.6 Hz), 67.1 (d, ²*J*_{C-P} = 6.7.1 Hz), 80.3, 125.6, 127.9, 128.2, 128.3, 128.3, 128.5, 136.4 (d, ³*J*_{C-P} = 6.3 Hz), 136.4 (d, ³*J*_{C-P} = 6.3 Hz), 142.5, 171.7. ³¹P-NMR (121.5 MHz, CDCl₃): δ_P ppm = 33.32. HRMS (ESI): calculated for C₃₂H₄₂O₅P [(M+H)⁺], 537.2764; found 537.2784.

Dibenzyl 2-((*N*-(benzyloxy)-*N*-methylecarbamoyle)methyl)-3-phenylpropylphosphonate

(21a). Prepared from compound **20a** (1.0 g, 2.02 mmol) according to general procedure IV. Colorless oil; purification gradient 0–5% MeOH in CH₂Cl₂; yield 43%. ¹H NMR (300 MHz, CDCl₃) δ_H ppm 1.77-2.07 (m, 2H, P-CH₂-), 2.40-2.83 (m, 5H, -CH₂-CO-, -CH-CH₂-Ph), 3.11 (s, 3H, N-CH₃), 4.63 (s, 2H, NOCH₂Ph), 4.86-5.05 (m, 4H, POCH₂Ph), 7.07-7.42 (m, 20H, Ar-H). ¹³C-NMR (75 MHz, CDCl₃) δ_C ppm 29.0 (d, ¹*J*_{C-P} = 138.7 Hz), 31.6 (d, ²*J*_{C-P} = 3.1 Hz), 35.9 (d, ³*J*_{C-P} = 9.6 Hz), 40.9 (d, ³*J*_{C-P} = 11.4 Hz), 67.2 (d, ²*J*_{C-P} = 6.6 Hz), 67.3 (d, ²*J*_{C-P} = 6.7 Hz), 76.1, 126.2, 127.9, 127.9, 128.3, 128.3, 128.5, 128.6, 128.8, 129.2, 129.4, 134.5, 136.6 (d, ³*J*_{C-P} = 6.8 Hz), 136.7 (d, ³*J*_{C-P} = 6.4 Hz), 139.6, 165.7. ³¹P-NMR (121.5 MHz, CDCl₃): δ_P ppm 33.28. HRMS (ESI): calculated for C₃₃H₃₇NO₅P [(M+H)⁺], 558.2404; found 558.2431.

Dibenzyl 2-((*N*-(benzyloxy)-*N*-methylecarbamoyle)methyl)-4-phenylbutylphosphonate (21b).

Prepared from compound **20b** (1.0 g, 1.97 mmol) according to general procedure IV. Colorless oil; purification gradient 0–5% MeOH in CH₂Cl₂; yield 60%. ¹H NMR (300 MHz, CDCl₃) δ_H ppm 1.64-2.09 (m, 4H, -CH₂-), 2.31-2.58 (m, 3H, -CH₂-, 1H, -CH-), 2.65 (dd, *J* = 7.1 Hz, *J* = 16.5 Hz, 1H, -CH₂-), 3.14 (s, 3H, N-CH₃), 4.73 (br. s, 2H, NOCH₂Ph), 4.87-5.08 (m, 4H, -CH₂-Ph), 7.04-7.26 (m, 5H, Ar-H), 7.27-7.39 (m, 15H, Ar-H). ¹³C-NMR (75 MHz, CDCl₃) δ_C ppm

29.6 (d, $^2J_{\text{C-P}} = 4.0$ Hz), 29.6 (d, $^1J_{\text{P-C}} = 138.2$ Hz), 32.9, 33.5, 36.5 ($^3J_{\text{C-P}} = 9.6$ Hz), 36.6 ($^3J_{\text{C-P}} = 6.7$ Hz), 67.0 ($^2J_{\text{C-P}} = 6.5$ Hz), 76.1, 125.7, 127.9, 127.9, 128.3, 128.3, 128.5, 128.6, 128.9, 129.3, 134.6, 136.4 ($^3J_{\text{C-P}} = 6.3$ Hz), 173.7. ^{31}P -NMR (121.5 MHz, CDCl_3): δ_{P} ppm = 32.26. HRMS (ESI): calculated for $\text{C}_{34}\text{H}_{39}\text{NO}_5\text{P}$ $[(\text{M}+\text{H})^+]$, 572.2560; found 572.2585.

Dibenzyl 2-((*N*-(benzyloxy)-*N*-methylcarbamoyl)methyl)-5-phenylpentylphosphonate (21c).

Prepared from compound **20c** (1.5 g, 2.87 mmol) according to general procedure IV. Colorless oil; purification gradient 0–5% MeOH in CH_2Cl_2 ; yield 68%. ^1H NMR (300 MHz, CDCl_3) δ_{H} ppm 1.36–1.63 (m, 4H, $-\text{CH}_2-$), 1.72–2.03 (m, 2H, $-\text{CH}_2-$), 2.26–2.66 (m, 5H, $-\text{CH}_2-$, $-\text{CH}-$), 3.12 (s, 3H, N- CH_3), 4.72 (s, 2H, NOCH_2Ph), 4.82–5.06 (m, 4H, POCH_2Ph), 7.03–7.43 (m, 20H, Ar-H). ^{13}C -NMR (75 MHz, CDCl_3) δ_{C} ppm 28.2, 28.6, 29.7 (d, $^2J_{\text{C-P}} = 3.2$ Hz), 29.7 (d, $^1J_{\text{C-P}} = 138.6$ Hz), 34.6 (d, $^3J_{\text{C-P}} = 9.3$ Hz), 35.8, 36.7 (d, $^3J_{\text{C-P}} = 8.3$ Hz), 66.8–67.2 (m), 76.1, 125.7, 127.9, 128.2, 128.3, 128.4, 128.51, 128.53, 128.7, 128.9, 129.3, 136.5 (d, $^3J_{\text{C-P}} = 7.0$ Hz), 142.4, 172.1. ^{31}P -NMR (121.5 MHz, CDCl_3): δ_{P} ppm 33.40. HRMS (ESI): calculated for $\text{C}_{35}\text{H}_{41}\text{NO}_5\text{P}$ $[(\text{M}+\text{H})^+]$, 586.2717; found 586.2709.

Dibenzyl 2-((*N*-(benzyloxy)-*N*-methylcarbamoyl)methyl)-6-phenylhexylphosphonate (21d).

Prepared from compound **20d** (1.28 g, 2.39 mmol) according to general procedure IV. Colorless oil; purification gradient 0–15% MeOH in CH_2Cl_2 ; yield 74%. ^1H NMR (300 MHz, CDCl_3) δ_{H} ppm 1.12–1.31 (m, 2H, $-\text{CH}_2-$), 1.34–1.56 (m, 4H, $-\text{CH}_2-$), 1.71–2.02 (m, 2H, $-\text{CH}_2-$), 2.22–2.63 (m, 4H, $-\text{CH}_2-$, 1H, $-\text{CH}-$), 3.11 (s, 3H, N- CH_3), 4.69 (s, 2H, $-\text{NOCH}_2\text{Ph}$), 4.88–5.06 (m, 4H, POCH_2Ph), 7.07–7.42 (m, 20H, Ar-H). ^{13}C -NMR (75 MHz, CDCl_3) δ_{C} ppm 25.9, 27.9, 29.4 (d, $^2J_{\text{C-P}} = 3.9$ Hz), 29.5 (d, $^1J_{\text{C-P}} = 137.7$ Hz), 31.1, 34.4 (d, $^3J_{\text{C-P}} = 9.4$ Hz), 35.5, 36.5 (d, $^3J_{\text{C-P}} = 9.4$ Hz), 66.6–66.8 (m), 75.8, 125.4, 127.7, 128.0, 128.1, 128.2, 128.3, 128.4, 128.6, 129.1, 134.4,

136.2-136.3 (m), 142.3, 173.6. ^{31}P -NMR (121.5 MHz, CDCl_3): δ_{P} ppm = 32.52. HRMS (ESI): calculated for $\text{C}_{36}\text{H}_{43}\text{NO}_5\text{P}$ $[(\text{M}+\text{H})^+]$, 600.2873; found 600.2814.

Sodium hydrogen 3-(*N*-hydroxy-*N*-methylcarbamoyl)-2-phenylpropylphosphonate (7a).

Prepared from compound **13a** (150 mg, 0.28 mmol) according to general procedure V. White powder. ^1H NMR (300 MHz, D_2O) δ_{H} ppm 1.69-1.96 (m, 2H, P- CH_2 -), 2.89-3.14 (m, 5H, - CH_2 -, N- CH_3), 3.23-3.50 (m, 1H, -CH-) 7.16-7.41 (m, 5H, Ar-H). ^{13}C -NMR (75 MHz, D_2O) δ_{C} ppm 29.4, 36.1 (d, $^1J_{\text{C-P}} = 127.7$ Hz), 38.1 (d, $^2J_{\text{C-P}} = 2.8$ Hz), 38.5 (d, $^3J_{\text{C-P}} = 5.3$ Hz), 126.3, 127.3, 128.4, 146.1 (d, $^3J_{\text{C-P}} = 12.1$ Hz), 174.0. ^{31}P -NMR (121.5 MHz, D_2O): rotamers at δ_{P} ppm 19.64, 19.87. HRMS (ESI): calculated for $\text{C}_{11}\text{H}_{15}\text{NO}_5\text{P}$ $[(\text{M}-\text{H})^-]$, 272.0693; found 272.0622.

Sodium hydrogen 3-(*N*-hydroxy-*N*-methylcarbamoyl)-2-*p*-tolylpropylphosphonate (7b).

Prepared from compound **13b** (150 mg, 0.27 mmol) according to general procedure V. White powder. ^1H NMR (300 MHz, D_2O) δ_{H} ppm 1.73-1.97 (m, 2H, P- CH_2 -), 2.26 (s, 3H, Ph- CH_3), 2.86-3.09 (m, 5H, - CH_2 -CO, N- CH_3), 3.20-3.46 (m, 1H, -CH-), 7.10-7.25 (m, 4H, Ar-H). ^{13}C -NMR (75 MHz, D_2O) δ_{C} ppm 20.2, 35.9, 36.1 (d, $^1J_{\text{C-P}} = 130.5$ Hz), 37.7 (d, $^2J_{\text{C-P}} = 2.8$ Hz), 38.9 (d, $^3J_{\text{C-P}} = 6.7$ Hz), 127.5, 129.2, 136.5, 142.8 (d, $^3J_{\text{C-P}} = 13.1$ Hz), 174.4. ^{31}P -NMR (121.5 MHz, D_2O): rotamers at δ_{P} ppm 24.34, 24.65. HRMS (ESI): calculated for $\text{C}_{12}\text{H}_{17}\text{NO}_5\text{P}$ $[(\text{M}-\text{H})^-]$, 286.0849; found 286.0816.

Sodium hydrogen 3-(*N*-hydroxy-*N*-methylcarbamoyl)-2-(4-methoxyphenyl)propylphosphonate (7c).

Prepared from compound **13c** (200 mg, 0.35 mmol) according to general procedure V. White powder. ^1H NMR (300 MHz, D_2O) δ_{H} ppm 1.55-1.81 (m, 2H, P- CH_2 -), 2.72-2.98 (m, 5H, - CH_2 -CO, N- CH_3), 3.11-3.37 (m, 1H, -CH-), 3.71 (s, 3H, Ph-O- CH_3), 6.84 (d, $J = 7.4$ Hz, 2H, Ar-H), 7.18 (d, $J = 7.4$ Hz, 2H, Ar-H). ^{13}C -NMR (75 MHz, D_2O) δ_{C} ppm 36.8, 37.1 (d, $^1J_{\text{C-P}} = 132.9$ Hz), 40.3 (d, $^3J_{\text{C-P}} = 8.3$ Hz), 45.6 (d, $^2J_{\text{C-P}} = 5.8$ Hz),

55.4, 113.6, 128.6, 139.5 (d, $^3J_{C-P}$ = 11.7 Hz), 156.7, 167.8. ^{31}P -NMR (121.5 MHz, D_2O): δ_{P} ppm = 20.79, 20.83. HRMS (ESI): calculated for $\text{C}_{12}\text{H}_{17}\text{NO}_6\text{P}$ [(M-H) $^-$], 302.0799; found 302.0926.

3-(*N*-hydroxy-*N*-methylcarbamoyl)-2-(*p*-chlorophenyl)propylphosphonic acid, bisammonium salt (7d). Prepared from compound **16d** (200 mg, 0.55 mmol) according to general procedure VII. Brown powder. ^1H NMR (300 MHz, D_2O) δ_{H} ppm 1.87-2.09 (m, 2H, P-CH₂-), 2.81-2.97 (m, 2H, -CH₂-CO-), 3.04 (s, 5/6 of N-CH₃), 3.11 (s, 1/6 of N-CH₃), 3.24-3.51 (m, 1H, -CH-), 7.20-7.38 (m, 4H, Ar-H). ^{13}C -NMR (75 MHz, D_2O) δ_{C} ppm 34.8 (d, $^1J_{C-P}$ = 131.6 Hz), 35.6, 37.0 (d, $^2J_{C-P}$ = 2.5 Hz), 39.4 (d, $^3J_{C-P}$ = 10.8 Hz), 128.3, 128.8, 131.5, 142.7 (d, $^3J_{C-P}$ = 9.5 Hz), 173.6. ^{31}P -NMR (121.5 MHz, D_2O): rotamers at δ_{P} ppm 23.25, 23.52. HRMS (ESI): calculated for $\text{C}_{11}\text{H}_{14}\text{ClNO}_5\text{P}$ [(M-H) $^-$], 306.0304; found 306.0306.

3-(*N*-hydroxy-*N*-methylcarbamoyl)-2-(3,4-dichlorophenyl)propylphosphonic acid, bisammonium salt (7e). Prepared from compound **16e** (150 mg, 0.38 mmol) according to general procedure VII. Brown powder. ^1H NMR (300 MHz, D_2O) δ_{H} ppm 1.84-2.08 (m, 2H, P-CH₂-), 2.57-3.02 (m, 2H, -CH₂-CO), 3.06 (s, 5/6 of N-CH₃), 3.16 (s, 1/6 of N-CH₃), 3.24-3.50 (m, 1H, -CH-), 7.18 (dd, J = 2.18 Hz, 8.50 Hz, 1H, Ar-H), 7.40-7.50 (m, 2H, Ar-H). ^{13}C -NMR (75 MHz, D_2O) δ_{C} ppm 34.8 (d, $^1J_{C-P}$ = 131.5 Hz), 35.9, 37.2 (d, $^2J_{C-P}$ = 2.7 Hz), 39.5 (d, $^3J_{C-P}$ = 11.3 Hz), 127.51, 129.53, 129.8, 130.4, 131.6, 144.9 (d, $^3J_{C-P}$ = 9.4 Hz), 173.6. ^{31}P -NMR (121.5 MHz, D_2O): rotamers at δ_{P} ppm 22.47, 22.72. HRMS (ESI): calculated for $\text{C}_{11}\text{H}_{13}\text{Cl}_2\text{NO}_5\text{P}$ [(M-H) $^-$], 339.9914; found 340.0130.

Sodium hydrogen 3-(*N*-hydroxy-*N*-methylcarbamoyl)-2-methylpropylphosphonate (7f). Prepared from compound **13f** (125 mg, 0.26 mmol) according to general procedure V. Colorless oil. ^1H NMR (300 MHz, D_2O) δ_{H} ppm 1.01 (d, J = 8.40 Hz, 3H, -CH(CH₃)-), 1.41-1.72 (m, 2H, P-CH₂-), 2.06-2.34 (m, 1H, -CH-), 2.36-2.49 (m, 1H, -CH₂-CO), 2.59 (dd, J = 6.3 Hz, 14.02 Hz),

3.20 (s, 5/6 of N-CH₃), 3.38 (s, 1/6 of N-CH₃). ¹³C-NMR (75 MHz, D₂O) δ_C ppm 20.6 (d, ³J_{C-P} = 8.0 Hz), 28.6 (d, ²J_{C-P} = 3.4 Hz), 35.0 (d, ³J_{C-P} = 132.1 Hz), 36.1, 40.2 (d, ³J_{C-P} = 13.3 Hz), 175.2. ³¹P-NMR (121.5 MHz, D₂O): rotamers at δ_P ppm 25.03, 25.42. HRMS (ESI): calculated for C₆H₁₃NO₅P [(M-H)⁻], 210.0537; found 210.1632.

Sodium hydrogen2-((*N*-hydroxy-*N*-methylcarbamoyl)methyl)-3-phenylpropylphosphonate

(8a). Prepared from compound **21a** (200 mg, 0.36 mmol) according to general procedure V. White powder. ¹H NMR (300 MHz, D₂O) δ_H ppm 1.46-1.79 (m, 2H, P-CH₂-), 2.34-2.66 (m, 4H, -CH₂-), 2.80-2.9 (m, 1H, -CH-), 3.08 (s, 5/6 of N-CH₃), 3.18 (s, 1/6 of N-CH₃), 7.13-7.39 (m, 5H, Ar-H). ¹³C-NMR (75 MHz, D₂O) δ_C ppm 32.2 (d, ¹J_{C-P} = 132.3 Hz), 32.7 (d, ²J_{C-P} = 3.8 Hz), 35.8, 36.6, 41.0 (d, ³J_{C-P} = 9.2 Hz), 126.2, 128.4, 129.4, 140.4, 174.6. ³¹P-NMR (121.5 MHz, D₂O): rotamers at δ_P ppm 24.68, 25.01. HRMS (ESI): calculated for C₁₂H₁₇NO₅P [(M-H)⁻], 286.0850; found 286.0821.

Sodium hydrogen2-((*N*-hydroxy-*N*-methylcarbamoyl)methyl)-4-phenylbutylphosphonate
(8b)

White powder. Prepared from compound **21b** (130 mg, 0.23 mmol) according to general procedure V. ¹H NMR (300 MHz, D₂O) δ_H ppm 1.34-1.84 (m, 4H, -CH₂-), 2.08-2.27 (m, 1H, -CH-), 2.54-2.77 (m, 4H, -CH₂-), 3.20 (s, 5/6 of N-CH₃), 3.35 (s, 1/6 of N-CH₃), 7.17-7.40 (m, 5H, Ar-H). ¹³C-NMR (75 MHz, D₂O) δ_C ppm 32.0 (d, ¹J_{C-P} = 3.7 Hz), 32.5, 33.0 (d, ¹J_{C-P} = 129.9 Hz), 36.1, 36.3 (d, ³J_{C-P} = 6.4 Hz), 37.7 (d, ³J_{C-P} = 10.6 Hz), 126.0, 128.7, 128.8, 143.5, 175.0. ³¹P-NMR (121.5 MHz, D₂O): δ_P ppm 22.47. HRMS (ESI): calculated for C₁₃H₁₉NO₅P [(M-H)⁻], 300.1006; found 300.1204.

Sodium hydrogen 2-((*N*-hydroxy-*N*-methylcarbamoyl)methyl)-5-phenylpentylphosphonate

(8c). Prepared from compound **21c** (175 mg, 0.30 mmol) according to general procedure V.

White powder. ^1H NMR (300 MHz, D_2O) δ_{H} ppm 1.28-1.70 (m, 6H, $-\text{CH}_2-$), 2.05-2.26 (m, 1H, $-\text{CH}-$), 2.50-2.72 (m, 4H, $\text{P}-\text{CH}_2-$, $\text{CH}_2-\text{CON}-$), 3.18 (s, 5/6 of $\text{N}-\text{CH}_3$), 3.35 (s, 1/6 of $\text{N}-\text{CH}_3$), 7.18-7.36 (m, 5H, Ar-H). ^{13}C -NMR (75 MHz, D_2O) δ_{C} ppm 28.2, 31.8 (d, $^2J_{\text{C-P}} = 3.6$ Hz), 33.0 (d, $^1J_{\text{C-P}} = 130.1$ Hz), 35.0 (d, $^3J_{\text{C-P}} = 10.3$ Hz), 35.3, 36.1, 36.6 (d, $^3J_{\text{C-P}} = 6.2$ Hz), 126.0, 128.7, 128.8, 143.7, 175.2. ^{31}P -NMR (121.5 MHz, D_2O): δ_{P} ppm = 23.18. HRMS (ESI): calculated for $\text{C}_{14}\text{H}_{21}\text{NO}_5\text{P}$ $[(\text{M}-\text{H})^-]$, 314.1163; found 314.1101.

Sodium hydrogen 2-((*N*-hydroxy-*N*-methylcarbamoyl)methyl)-6-phenylhexylphosphonate

(8d). Prepared from compound **21d** (175 mg, 0.29 mmol) according to general procedure V.

White powder. ^1H NMR (300 MHz, D_2O) δ_{H} ppm 1.20-1.66 (m, 8H, $-\text{CH}_2-$), 2.11 (m, 1H, $-\text{CH}-$), 2.46-2.68 (m, 4H, $-\text{CH}_2-$), 3.18 (s, 5/6 of $\text{N}-\text{CH}_3$), 3.34 (s, 1/6 of $\text{N}-\text{CH}_3$), 7.15-7.38 (m, 5H, Ar-H). ^{13}C -NMR (75 MHz, D_2O) δ_{C} ppm 25.6, 31.2, 31.9 (d, $^2J_{\text{C-P}} = 3.8$ Hz), 33.1 (d, $^1J_{\text{C-P}} = 130.2$ Hz), 35.1, 35.3 (d, $^3J_{\text{C-P}} = 10.0$ Hz), 36.1, 36.6 (d, $^3J_{\text{C-P}} = 7.0$ Hz), 125.9, 128.7, 128.8, 143.7, 175.3. ^{31}P -NMR (121.5 MHz, D_2O): δ_{P} ppm = 23.20. HRMS (ESI): calculated for $\text{C}_{15}\text{H}_{23}\text{NO}_5\text{P}$ $[(\text{M}-\text{H})^-]$, 328.1319; found 328.1340.

FIGURES

Figure 1: Inhibition of isoprenoid biosynthesis via the MEP pathway by fosmidomycin and compound **2**.

Figure 2: Structures of relevant fosmidomycin analogues, previously reported.

Figure 3: β-substituted fosmidomycin analogues.

Figure 4. Residual Dxr activity upon treatment with 100 μ M of the indicated compounds. The enzyme assays for EcDxr and MtbDxr were performed at 37 $^{\circ}$ C with a saturating concentration of NADPH (150 μ M), a DOXP concentration fixed at its K_M (47 μ M for MtbDxr and 2 mM for EcDxr), and 100 μ M of the indicated inhibitor. For MtbDxr, the results were identical whether the enzyme was preincubated with the inhibitor for 10 min prior to the addition of NADPH or when the enzyme was preincubated with NADPH prior to the addition of the inhibitor. For EcDxr, the enzyme was preincubated with the inhibitor and the cofactor for 5 min at 37 $^{\circ}$ C. Enzyme activity was initiated by adding the substrate DOXP. Details specific to the assay for PfDxr are given in the Supporting Information. Enzyme activity was spectrophotometrically monitored immediately following the addition of DOXP at 340 nm. Residual enzyme activity is relative to an assay performed with vehicle alone (DMSO). All assays were performed in

duplicate for MtbDxr and in triplicate for EcDxr and PfDxr. Each value represents the average of three determinations per experiment and bars indicate standard deviation.

Figure 5. The active site of PfDxr bound to **8c** and **8d**. The background cartoon was created with a red-to-blue rainbow coloring for one chain. Water molecules are shown as small red spheres, and the Mn^{2+} ion is gold. (A) Electron density for the inhibitor **8c** and selected nearby residues is contoured at the r.m.s. value of the σ -weighted ($2m|F_o| - D|F_c|$) electron-density map⁵⁷ ($0.33 \text{ e}/\text{\AA}^3$) in light blue, as well as at $2.5 \text{ e}/\text{\AA}^3$ (gold) to show the higher electron density near the metal ion. (B) Magenta colored bubbles show hydrogen-bond interactions between **8c** and protein or solvent, while gold ones show metal coordination; blue and cyan bubbles indicate close contacts ($< 3.7 \text{ \AA}$) between the phenyl group of **8c** and the indole ring of Trp296 from the flap, or within the inhibitor, respectively. (C) The structures of bound **8c** and **8d** are superimposed. The flap in the complex with **7a** is shown in magenta for comparison.

Figure 6. The active site of PfDxr bound to **7a** and **7b**. The cartoon is presented as described for Figure 5. (A) Electron density for **7a** and selected nearby residues is contoured at the r.m.s. of the map ($0.28 \text{ e}/\text{\AA}^3$) in light blue, as well as at $2.5 \text{ e}/\text{\AA}^3$ (gold) to show the higher electron density near the metal ion. (B) Magenta-colored bubbles show hydrogen-bond interactions between **7a** and protein or solvent, while gold ones show metal coordination; blue bubbles indicate close contacts ($< 3.7 \text{ \AA}$) between the methyl group of **7a** and the indole ring of Trp296 from the flap. (C) The structures of bound **7a**, **7b**, **8c** and **8d** are superimposed. The flap in the complex with **7a** is shown in magenta for comparison with that of **8c**. Bubbles indicate the hydrogen bond interactions of **8c** with the enzyme and solvent.

Figure 7. Optimized geometry of **8c** in the minimized protein structure PDB entry 4A03 (MtbDxr). Only relevant residues close to the ligand are shown for clarity and labeled according to their residue numbers in the 4A03 structure. The position of Trp203 in 4A03 is shown with green carbon atoms. The carbon atoms of the optimized protein residues are shown in orange, while the ligand carbon atoms are shown in white. The position of the Trp203 indole ring overlaps with the position of the phenyl ring in **8c**. In the optimized structure, Trp203 forms a T-stacking interaction with the phenyl ring of **8c**.

SCHEMES

Scheme 1. Synthesis of β -aryl analogues of **4b**.^a

^a Reagents and conditions: i) Boc_2O , DMAP, *tert*-BuOH, rt, overnight; ii) $(\text{BnO})_2\text{OPMe}$ (for **12a-c, f**), $(\text{EtO})_2\text{OPMe}$ (for **14d** & **14e**), *n*-BuLi, THF, -78°C , 2.5 h; iii) (a) TFA, CH_2Cl_2 , 45 min, 0°C to rt; (b) $\text{MeNH}(\text{OBn})$, EDC, DMAP, CH_2Cl_2 , rt, overnight; iv) H_2 , Pd/C, MeOH, NaOH aq., 25°C , 10–15 min; v) BCl_3 , CH_2Cl_2 , -78°C , 1 h; vi) (a) TMSBr, BSTFA, CH_2Cl_2 , 0°C to rt, 22 h; (b) H_2O , NH_4OH aq.

Scheme 2. Synthesis of β -phenylalkyl homologues of 4b.^a

^a Reagents and conditions: i) Dess-Martin periodinane, CH₂Cl₂; ii) Ph₃P=CHCOO*tert*-Bu, toluene, 120 °C; iii) (BnO)₂OPMe, n-BuLi, THF, -78 °C, 2.5 h; iv) (a) TFA, CH₂Cl₂, 45 min, 0 °C to rt; (b) MeNH(OBn), EDC, DMAP, CH₂Cl₂, rt; v) H₂, Pd/C, MeOH, NaOH(aq.), 25 °C, 10–15 min.

TABLES.

Table 1. IC₅₀ ± sd values for recombinant Dxrs from *E. coli*, *M. tuberculosis* and *P. falciparum*.

Compound	EcDxr	MtbDxr	PfDxr
	IC ₅₀ (μM)	IC ₅₀ (μM)	IC ₅₀ (μM)
Fosmidomycin	0.030 ± 0.008 ²⁸	0.438 ± 0.09	0.036 ± 0.006
4b	0.159 ± 0.018	1.15 ± 0.21	nd
7a	nd	nd	3.3 ± 0.17
7b	nd	nd	9.3 ± 0.75
7c	nd	nd	18.8 ± 4.2
7f	0.205 ± 0.052	7.13 ± 1.2	nd
8a	nd	nd	nd
8b	31.39 ± 21.61	nd	1.36 ± 0.02
8c	0.843 ± 0.163	10.35 ± 1.3	0.117 ± 0.012
8d	6.67 ± 1.48	273.2 ± 54.6	0.069 ± 0.005

nd = not determined.

Table 2. MIC₅₀ values against *in vitro* growth of *E. coli*, *M. smegmatis* and IC₅₀ values against the *P. falciparum* K1 strain.

Compound	<i>E. coli</i> ATCC 8739 MIC ₅₀ (μM)	<i>E. coli</i> ATCC 25922 MIC ₅₀ (μM)	<i>E. coli</i> K-12 MIC ₅₀ (μM)	<i>M.</i> <i>smegmatis</i> ATCC 607 MIC ₅₀ (μM)	<i>M.</i> <i>smegmatis</i> ATCC 700084 MIC ₅₀ (μM)	<i>P.</i> <i>falciparum</i> -K1 MIC ₅₀ (μM)
Fosmidomyci						1.73 ±
n (1)	<u>0.98</u>	<u>250</u>	<u>7.8</u>	>250	>250	0.89 ⁵⁸
2	<u>7.8</u>	<u>62.5</u>	<u>15.6</u>	>250	>250	0.42 ± 0.17 ⁵⁸
4b	<u>31.25</u>	<u>15.6</u>	<u>31.25</u>	>250	>250	<u>0.26 ± 0.02</u>
7a	>250	>250	>250	>250	>250	>64
7b	>250	>250	>250	>250	>250	>64
7c	>250	>250	>250	>250	>250	>64
7d	>250	>250	>250	>250	>250	>64
7e	>250	>250	>250	>250	>250	>64
7f	nd	nd	nd	nd	nd	<u>0.74 ± 0.13</u>
8a	nd	nd	nd	nd	nd	≥56.8 ± 10.1
8b	>250	>250	>250	>250	>250	<u>35.4 ± 8.7</u>
8c	>250	>250	>250	>250	>250	<u>0.43 ± 0.09</u>
8d	>250	>250	>250	>250	>250	<u><0.25±0.00</u>

nd = not determined

ASSOCIATED CONTENT

Supporting Information Available. Additional information concerning cloning, expression, protein purification, enzyme assays, pathogen testing and crystallographic work, as well as ^1H , ^{13}C , and ^{31}P NMR spectra of compound **7a-f** and **8a-d**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Accession Codes

Coordinates and structure factor data have been deposited at the PDB with entry codes 4Y6R, 4Y6S, 4Y6T and 4Y6P, for complexes with **7a**, **7b**, **8c** and **8d**, respectively.

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ABBREVIATIONS USED

DOXP, 1-deoxy-D-xylulose-5-phosphate; Dxr, 1-deoxy-D-xylulose-5-phosphate reductoisomerase; Ec, *Escherichia coli*; MEP, 2-C-methyl-D-erythritol-3-phosphate; Mtb, *Mycobacterium tuberculosis*; Pf, *Plasmodium falciparum*; SAR, structure-activity relation; TB, tuberculosis; TMSBr, bromotrimethylsilane

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TABLE OF CONTENTS GRAPHIC

Fosmidomycin

$R_1 = \text{H or Me}$

5 examples

$n = 1, 2, 3, 4$