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Targeting an aromatic hotspot in *Plasmodium falciparum* 1-deoxy-
d-xyulose-5-phosphate reductoisomerase with β-arylpropyl-
alogues of fosmidomycin

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Structural Biology
Structure-activity relationships

Abstract

Blocking the 2-C-methyl-D-erythritol-4-phosphate pathway for isoprenoid synthesis offers new ways to inhibit Plasmodium spp. growth. Fosmidomycin (\(\text{I}\): \(3-(N\)-hydroxyformamido)propylphosphonic acid) and its acetyl homologue FR-900098 (\(\text{II}\): \(3-(N\)-hydroxyacetamido)propylphosphonic acid) potently inhibit 1-deoxy-D-xylulose-5-phosphate reductoisomerase, a key enzyme in this pathway. Arylpropyl substituents were introduced at the \(\beta\)-position of the hydroxamate analogue of \(\text{II}\) to study changes in lipophilicity, as well as electronic and steric properties. The potency of several new compounds on the \(P. falciparum\) enzyme approaches that of \(\text{I}\) and \(\text{II}\). Activities against the enzyme and parasite correlate well, supporting the mode of action. Seven X-ray structures show that all of the new arylpropyl substituents displace a key tryptophan residue of the active-site flap, which had made favorable interactions with \(\text{I}\) and \(\text{II}\). Plasticity of the flap allows substituents to be accommodated in many ways; in most cases, the flap is largely disordered. Compounds can be separated into two classes based on whether the substituent on the aromatic ring is \textit{meta} or \textit{para}. Generally, \textit{meta}-compounds are better inhibitors, and in both classes, smaller size is linked to better potency.
Introduction

Despite major efforts to reduce its incidence in the last decade, malaria remains one of the leading causes of death from a single infectious agent. The disease, caused mostly by *Plasmodium falciparum*, was responsible for an estimated 438,000 deaths in 2015.\(^{11}\) Significant gains in recent years are being undermined by mounting resistance of the parasite to currently available drugs, so there is an urgent need for new chemical entities acting on new targets.

After Jomaa and coworkers demonstrated that *Plasmodia* synthesize isoprenoids via the nonmevalonate or 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway,\(^{2}\) while humans obtain these essential molecules via the orthogonal mevalonate pathway,\(^{3,4}\) blocking the MEP pathway became an attractive strategy to stem the proliferation of this and other pathogens.\(^{5}\)

1-Deoxy-D-xylulose-5-phosphate reductoisomerase (Dxr; EC 1.1.1.267) catalyzes the first committed step in the MEP pathway, *i.e.* the transformation of 1-deoxy-D-xylulose 5-phosphate (DOXP) to MEP.\(^{6,7}\) The natural antibiotic fosmidomycin (I, Figure 1), a potent inhibitor of Dxr,\(^{8,9}\) has been clinically evaluated for the treatment of malaria, alone and in combination therapy, but unfavorable pharmacokinetic properties and low intestinal absorption have prevented it from reaching the market.\(^{10,11,12}\) The acetyl homologue of fosmidomycin, FR-900098 (2), has been reported to be twice as potent against *P. falciparum in vitro*, and against *P. vinckei* in a mouse malaria model.\(^{2}\) Extensive medicinal chemistry efforts seeking new antimalarial agents have yielded various analogues of fosmidomycin, the subject of multiple reviews.\(^{13,14,15}\) The phosphonate group and the metal-chelating reverse hydroxamate moiety are both required for Dxr inhibitory activity. Aryl substitutions at the α-position (with respect to the phosphonate), when attached to the inhibitor backbone via a linker of 3-4 carbon units, have yielded some of the most promising analogues to date.

![Figure 1](image-url)  
*Figure 1:* Conversion catalyzed by Dxr, and two inhibitors of this enzyme.
Dxr enzymes contain a strictly conserved tryptophan residue within a flexible loop (flap) that undergoes an induced-fit conformational change upon fosmidomycin binding, closing over and interacting with the bound inhibitor. This flap is considered essential for Dxr’s catalytic activity.\cite{16,17,18,19,20} Murkin and coworkers demonstrated a change in the rate-limiting step of the \textit{Mycobacterium tuberculosis} Dxr-catalyzed reaction upon alteration of Trp203 in the flap, thereby establishing a functional link between this amino acid and chemical barrier crossing.\cite{21} Inhibition and binding studies with fosmidomycin confirmed the importance of the flap, and the conserved tryptophan in particular, for ligand binding. Structural evaluation of a series of Dxr-bound compounds including 3 and 4 (Figure 2) showed that the indole group of Trp211 in the \textit{Escherichia coli} enzyme (EcDxr) is displaced in order to accommodate the inhibitors’ pyridine/quinoline rings, which form π-π stacking or charge-transfer interactions with the indole of the tryptophan side chain.\cite{22}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{dxr_enzymes.png}
\caption{Relevant Dxr inhibitors (3-5) and target β-substituted (6a-j) fosmidomycin analogues.}
\end{figure}

Recently, we assessed the ability of Dxr to accommodate substituents in the β-position of fosmidomycin analogues bearing a hydroxamate rather than the original reverse hydroxamate group.\cite{23} We observed that direct introduction of aromatic rings at the β-carbon (as in 5a) afforded moderate Dxr inhibitors. Exploration of different linkers between the β-carbon and a phenyl ring (5b-e) suggested that a 3-carbon linker (5d) was optimal for inhibition of EcDxr.
and *M. tuberculosis* Dxr, while both phenylpropyl (5d) and phenylbutyl (5e) substituents afforded potent *P. falciparum* Dxr (PfDxr) inhibitors. These results were rationalized by crystallographic studies of PfDxr in complex with 5d and 5e, which showed that the phenyl rings of these compounds displace the indole ring of the conserved Trp296 residue, and occupy its 'usual' position in both active-site metal-containing\textsuperscript{16,18, 24} and metal-free structures.\textsuperscript{17} This allows an intra-molecular interaction between the phenyl ring and the methyl group on the hydroxamic acid that is equivalent to the inter-molecular interactions observed in ternary complexes with 2.\textsuperscript{18,24} Rearrangement of the residues of the flap results in favorable interactions between these phenyl rings and the tryptophan residue. Importantly, both analogues showed submicromolar schizontocidal activity against the *P. falciparum* K1 strain, and essentially the same SAR was observed as for PfDxr inhibition.

This follow-up study explored the influence of lipophilicity, electronic and steric properties in variants of the phenylpropyl side chain of 5d. We anticipated that analogues 6a-j would retain the capacity to occupy the aromatic 'hotspot', while their phenyl substituents might reinforce intra- or inter-molecular interactions.

**Results and discussion**

**Synthesis**

Scheme 1. Reagents and conditions: i) but-3-yn-1-ol, PdCl\(_2\)(PPh\(_3\)), Cul, Et\(_3\)N, 117 °C, 50%–98%; ii) H\(_2\), Pd/C, MeOH, 69–94%; iii) Dess-Martin periodinane, CH\(_2\)Cl\(_2\); iv) Ph\(_3\)P=CHCOO\(_{\text{tert}}\)-Bu, toluene, 120 °C, 49%–67%; v) (BnO\(_2\))OPMe, n-BuLi, THF, -78 °C.
The synthesis of 6a-j (Scheme 1) was achieved starting from commercially available aryl iodides 7a-j. Sonogashira coupling with but-3-yn-1-ol afforded the corresponding alkynols 8a-j, which were readily converted to 9a-j upon catalytic hydrogenation. Dess-Martin oxidation to the corresponding aldehydes 10a-j and subsequent Wittig olefination afforded the α,β-unsaturated esters 11a-j, which served as electrophiles in a Michael reaction with dibenzyl methylphosphonate to yield the 1,4-addition adducts 12a-j. Hydrolysis of the tert-butyl ester and EDC mediated coupling with O-benzyl-N-methyl-hydroxylamine gave 13a-j. Finally, removal of all benzyl protecting groups by catalytic hydrogenolysis afforded the desired analogues 6a-j.

Evaluation of function

The final compounds were tested for inhibition of recombinant EcDxr and PfDxr using a spectrophotometric assay monitoring the substrate-dependent oxidation of NADPH associated with the Dxr-catalyzed reaction (Table 1).[16,23, 25]

Table 1. In vitro inhibition of recombinant Dxr enzymes, and as well as IC₅₀ values against in vitro growth of the P. falciparum K1 strain. The IC₅₀ values reported for EcDxr were calculated from a single curve, while those for PfDxr are based on triplicates (the confidence interval is shown in parentheses). IC₅₀ values on Plasmodium growth are the mean and standard deviation from three separate experiments.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>EcDxr</th>
<th>PfDxr</th>
<th>P. falciparum K1 IC₅₀ (μM)[b]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image" alt="Structure" /></td>
<td>nd[c]</td>
<td>0.036 (0.032 – 0.040)</td>
<td>1.7 ± 0.9[26]</td>
</tr>
<tr>
<td>2</td>
<td><img src="image" alt="Structure" /></td>
<td>0.03 (0.02-0.05)[d]</td>
<td>0.045 (0.041-0.049)[d]</td>
<td>0.4 ± 0.2[26]</td>
</tr>
<tr>
<td>5d</td>
<td><img src="image" alt="Molecule 5d" /></td>
<td>0.84^{23} \ (0.51-1.40)</td>
<td>0.079 (0.066-0.093)</td>
<td>2.7 ± 0.14</td>
</tr>
<tr>
<td>6a</td>
<td><img src="image" alt="Molecule 6a" /></td>
<td>1.04 (0.6-1.8)</td>
<td>0.175 (0.129-0.237)</td>
<td>1.4 ± 1.2</td>
</tr>
<tr>
<td>6b</td>
<td><img src="image" alt="Molecule 6b" /></td>
<td>10.40 (2.8-388.3)</td>
<td>0.05 (0.043-0.049)</td>
<td>10.9 ± 4.5</td>
</tr>
<tr>
<td>6c</td>
<td><img src="image" alt="Molecule 6c" /></td>
<td>1.18 (0.4-3.3)</td>
<td>0.12 (0.096-0.142)</td>
<td>3.8 ± 2.5</td>
</tr>
<tr>
<td>6d</td>
<td><img src="image" alt="Molecule 6d" /></td>
<td>0.97 (0.62-1.5)</td>
<td>0.15 (0.137-0.154)</td>
<td>7.8 ± 3.7</td>
</tr>
<tr>
<td>6e</td>
<td><img src="image" alt="Molecule 6e" /></td>
<td>10.53 (2.4-46.2)</td>
<td>3.2 (2.83-3.63)</td>
<td>51.4 ± 21.8</td>
</tr>
<tr>
<td>6f</td>
<td><img src="image" alt="chemical structure" /></td>
<td>2.00 (1.1-3.7)</td>
<td>0.067 (0.057-0.078)</td>
<td>5.7 ± 4.4</td>
</tr>
<tr>
<td>6g</td>
<td><img src="image" alt="chemical structure" /></td>
<td>7.33 (0.9-59.6)</td>
<td>0.27 (0.248-0.295)</td>
<td>45.7 ± 21.3</td>
</tr>
<tr>
<td>6h</td>
<td><img src="image" alt="chemical structure" /></td>
<td>13.91 (4.3-45.5)</td>
<td>0.28 (0.272-0.298)</td>
<td>49.3 ± 18.9</td>
</tr>
<tr>
<td>6i</td>
<td><img src="image" alt="chemical structure" /></td>
<td>4.07 (1.5-11.2)</td>
<td>0.87 (0.780-0.977)</td>
<td>46.6 ± 15.4</td>
</tr>
<tr>
<td>6j</td>
<td><img src="image" alt="chemical structure" /></td>
<td>nd[^a]</td>
<td>1.6 (1.16-2.158)</td>
<td>&gt; 64</td>
</tr>
</tbody>
</table>

[^a]: The IC₅₀ values reported for EcDxr were calculated from a single curve, while those for PfDxr are based on triplicates three experiments; the confidence interval for each value is shown in parentheses.

[^b]: IC₅₀ values on *Plasmodium* growth are the mean and standard deviation from three separate experiments performed on different dates.

[^c]: nd = not determined; a value of 0.030 ± 0.008 was reported earlier.\[^{27}\]

[^d]: Values of 0.051 and 0.018 were reported earlier for EcDxr and PfDxr, respectively.\[^{28}\]

[^e]: A value of 0.117 ± 0.012 was reported earlier.\[^{25}\]

[^f]: A value of 0.43 ± 0.09 was reported earlier.\[^{23}\]

[^g]: nd = not determined; 86% activity remained after the addition of 100 μM 6j.
None of the changes introduced in 6a-6j relative to 5d improve inhibition of EcDxr relative to 5d, although 6a, 6c, 6d and 6f are essentially equipotent to 5d. Two changes improve inhibition of PfDxr relative to 5d (6b and 6f), while three others give similar IC₅₀ values (6a, 6c and 6d). The remaining changes are associated with weaker inhibition of PfDxr (6e, 6g-6j). Surprisingly, methyl-substitution of the aromatic ring at the meta-position (6b) increases PfDxr inhibition, while it unfavorably influences EcDxr inhibition (Figure S1). The same may be true for the meta-fluoro analogue 6f.

None of the changes introduced in 6a-6j relative to 5d significantly improve the IC₅₀ values regarding in vitro growth of the multidrug-resistant P. falciparum K1 strain (Table 1). However, improvement of IC₅₀ for PfDxr is well correlated with improvements in the IC₅₀ against the parasite (Figure 3), indicating that Dxr is in fact the primary target of this series of compounds. Only inhibitors with IC₅₀s better than approximately 0.3 μM (pIC₅₀ greater than 6.5) had significant effects against the parasite at the highest concentration tested (64 μM).

**Figure 3.** Plot of IC₅₀ against in vitro growth of the P. falciparum K1 strain versus pIC₅₀ against PfDxr activity, for the new compounds; 1 and 2 are shown for reference. Compounds marked with blue boxes represent those for which X-ray structures of complexes with PfDxr are reported in the present paper, while red/orange boxes represent structures reported elsewhere (ref. 23 and 18, respectively).

Results against Trypanosoma brucei brucei Squib 427, T. cruzi Tulahuen LacZ (clone C4) and Leishmania infantum MHOM/MA(BE)/67 strains were also negative at the highest
concentrations tested (64 µM). Since these parasites do not have Dxr, these were effectively control experiments. Cytotoxicity, as assessed against MRC-5SV2 (human lung fibroblast) cells, was also negligible for all compounds at this concentration.

**X-ray structures of PfDxr in complex with seven inhibitors**

The structures of PfDxr in complex with seven of the new β-substituted inhibitors (6a-d, 6f, 6g, 6h) are described in more detail in the Supporting Information. Although all of the new compounds were entered into crystallization trials, only inhibitors with IC50s better than ~0.5 µM (pIC50 greater than ~6.2) produced structures (Figures 3-5). The structures have been solved at resolutions in the range of 1.4-1.8 Å, and refined to crystallographic R-factors of ~18% and free R-factors of 20-21%. Complete data collection and refinement statistics are given in Table S2. Each complex has been crystallized in space group P1, with similar unit cell constants. A dimer is found in the asymmetric unit, and a manganese ion and an inhibitor molecule are very clear in each active site (Figure 4A4a). The overall electron density is of good quality, and complete models of the enzyme are deposited at the PDB for residues 77-486 in each chain. However, the electron density for even the main chain in some of the flap (residues 292-298) is sometimes poorly defined, see Table S3. Only in the complex with 6h, and in one molecule of 6d, is the main-chain electron density of each flap continuous at the r.m.s. value of the relevant map. The indole ring of Trp296 is poorly defined in six of the 14 views of the active site. Although each compound was synthesized as a racemic mixture, the high resolution of the structures allowed us to identify the favored R-enantiomer in each complex, as observed in our earlier work. The protein structures are highly conserved; a structural superposition of each chain onto the A chain of the 5d complex (PDB code 4Y67) produces r.m.s. values of 0.16-0.36 Å for 398-410 pairs of Cα atoms, when using a 1-Å Cα-pair cut-off. The overlapping Cα-traces show separation into A and B chain clusters at the start of two helices in the cofactor-binding domain, probably as a result of differences in the crystal environment. Because the electron density in the flap is often poorly defined, the chains are not tightly clustered, but short regions before and after the flap show separate tight A- and B-chain clusters, again separated by ~0.5-1.0 Å, probably as a result of different crystal contacts.
Figure 4. X-ray structures of PfDxr in complex with inhibitors. The cartoon representation of the protein backbone is color-coded according to position in the sequence, going through the rainbow from red to blue. (A) Electron density for the inhibitor 6h and selected nearby atoms, contoured at the r.m.s. value of the σA-weighted (2m|F_o| − D|F_c|) electron-density map (0.43 e/Å^3) in light blue, as well as at 2.5 e/Å^3 (gold) to show the higher electron density near the metal ion. (B) Superimposed structures of the meta-class compounds, 6b (light brown), 6d (orange), 6f (dark brown) and 6h (yellow), on 5d (silver gray). The well-defined flap residue, Trp296, of 6h and 5d is seen to undergo a conformational change. (C) Superimposed structures of the para-class compounds, 6a (dark green), 6c (light green) and 6g (cyan), on 5d (silver gray). Fluorine atoms are shown in magenta. (D) All the structures superimposed, using the same coloring scheme defined in panels B and C.

There is no clear correlation between strength of inhibition and temperature factors or fit to electron density (Figure S2), which is perhaps to be expected given the variable conformations observed for the flap.

Numerous crystallographic studies have shown that fosmidomycin analogues bind in the substrate/product-binding site of Dxr. The phosphonate group at one end is held firmly in place by multiple hydrogen-bonding interactions with protein and solvent, and the hydroxamic acid group at the other end is coordinated to the active-site metal ion. In ternary complexes with 1 and 2, a well-defined flap has been observed, with a number of highly conserved amino-acid side chains contributing to the binding site.[16,17,18,24] In particular, the
indole ring of Trp296 (PfDxr numbering) packs against the backbone of the two compounds, and interacts with the methyl group of the acetyl derivative 2. In the numerous structures with \( \alpha \)-aryl analogues, the flap is either disordered or has moved to allow the substituents to bind in a depression located between three ordered loops and a usually disordered flap. This depression is large enough to accommodate analogues with formyl, acetyl or phenyl substituents at the hydroxamate group, for example. For the \( \beta \)-aryl substituents, however, show at least two modes of binding. For the \( 5a \) complex that lacks a linker, the phenyl group is positioned ‘under’ a well-defined flap, and the methyl group on the hydroxamic acid interacts with the indole ring of Trp296. For the \( 5d \) and \( 5e \) complexes, however, the linkers adopt boomerang-like conformations that, together with small changes in the fosmidomycin backbone, allow their respective phenyl groups to interact with the methyl group of their hydroxamic acid. In this strikingly different way of dealing with the substitution, the linker occupies the volume normally occupied by \( \alpha \)-aryl substitutions, while the phenyl ring is co-spatial with the indole ring of the flap tryptophan, as seen in ternary complexes. The edge of the phenyl ring, in turn, interacts with the indole ring of Trp296 in the flap. All seven of the new \( \beta \)-substituted complexes take on the same general structure seen in the \( 5d \) complex, but can be grouped into two sets depending on whether they represent meta- or para-substitutions to the phenyl ring. We have structures for 3 meta- (6b, 6d, 6f) and 3 para- (6a, 6c, 6g) substitutions, as well as 6h, which we consider as a member of the meta-class.

The members of the meta-class form a tight cluster of eight independent structures (including both subunits of the dimers), where the phenyl rings of the new compounds closely overlap that observed in the \( 5d \) complex (Figure 4B4b). Interactions of the phosphonate and hydroxamic acid groups in all complexes are essentially identical to those of the \( 5d \) complex, as is the overall conformation of the fosmidomycin backbone. All members adopt the same pose, where the substituent is directed towards the indole ring of Trp296 in the \( 5d \) complex; none points in the other direction, towards His341. However, all inhibitors have an effect on the positioning of the indole ring, and on the quality of the electron density of the flap (Table S3). Unsurprisingly, the introduction of a naphthalene ring in 6h causes a large change in the conformation of Trp296, which is needed to prevent clashes (Figure 4B4b); the flap in this structure is well defined. Although there are no close contacts to the indole ring, only a few atoms of one edge of the naphthalene ring are solvent-exposed. The slightly smaller methoxyphenyl substituent in 6d causes a different movement of the tryptophan
residue, needed to prevent close contacts between the indole ring and the methyl group. The flap is rather well defined in both chains, as is the density for the indole group, but the change in conformation results in a loss of the close contacts to the indole seen in the 5d complex. The methoxyphenyl group is approximately planar, and so occupies the same place as the corresponding portion of the naphthalene ring of 6h (Figure 4B4b). The methoxy group does not form any hydrogen-bonding interactions with the protein, and is shielded from the solvent by residues near 360. The introduction of the methyl and fluorine substituents in 6b and 6f, respectively, would result in close contacts to one edge of the indole, if the conformation seen in the 5d complex were maintained (three contacts are predicted, of 2.5 Å and ~3.1Å, in 6b and 6f, respectively). Instead, the flap moves, and the electron density in a three-residue segment of the flap (residues 294-296) becomes poorly defined (Table S3); the indole ring is moderately clear in only one of the four active sites (that of the 6f A-chain). Compounds 6b and 6f show equal or better IC50s than 5d, however, while the other two meta-compounds have higher values (Figure 3 and Table 1). The lack of well-defined interactions between the best inhibitors and the indole ring of Trp296 suggests that the interactions with the indole that are observed in the 5d complex are not the most important determinants of the observed IC50s. However, it is striking that the larger the substituent, the higher the IC50 observed (Figure 5A5a). We suggest that the most energetically favorable intra-molecular phenyl/methyl interactions are harder for inhibitors with larger substituents to attain in the complexes, because they occur in the context of enzyme-inhibitor interactions.

![Figure 5](image_url)

**Figure 5.** Relationship between size of substituents and pIC50. The inhibitors and the residues of their respective flaps are colored on a common scale, going through the rainbow from the best pIC50 (red, using 1 as the endpoint) to the poorest (blue). (Aa) Complexes of the meta-cluster are shown. It should be remembered that the 6b complex, in particular, has poor electron density for the indole of Trp296. (Bb) Complexes of the para-cluster are shown.
Note that the 6g complex has no significant electron density in the flap, as well as weaker electron density in the 3-carbon linker of the inhibitor.

The members of the para-group (6a, 6c, 6g) form a cluster of six similar structures that are distinct from those of the meta-group (Figure 4C). The interactions at the phosphonate and hydroxamic acid moieties are essentially identical in all complexes, as is the conformation of the fosmidomycin backbone. While their phenyl rings are closely co-planar with that of the 5d complex, the rings are not so tightly clustered and each is shifted to some degree within the binding site by virtue of the flexible 3-carbon linker. The smallest shift from 5d is associated with the smallest substitution, and so on: ~0.5 Å for 6a, ~0.7Å for 6c and ~1.0 Å for 6g. This results from steric constraints near Met360/Pro363 that “push” the larger substituents away. Small differences in the torsion angles give the effect of splaying the linker, while sliding the ring in the plane of the phenyl ring seen in 5d (Figure 4C). The important intra-ligand ring-methyl group interactions are, therefore, maintained in all complexes, but with variations (Figure 4D). Again, the electron density in each complex is poorly defined in regions of the flap; the indole ring of Trp296 is well defined in only three active sites, see Table S3. In these three complexes, Trp296 is similar to the 5d complex, and the indole ring helps to shield the edge of the phenyl ring of the inhibitor. However, the tryptophan is not forced out of the active site, as was observed for the largest meta-group compounds. Two of the fluorine atoms in 6g interact with a water molecule that is highly conserved in the various structures (although slightly displaced in the 6a complex), while the oxygen atom in the methoxy-substituent of 6c accepts a hydrogen bond from the main-chain amide nitrogen of residue 359. In general, the larger the para-group substituent, the poorer the IC₅₀ (Figure 5B). This is probably due to the translation of the relevant phenyl group from its energetically-preferred position in the 5d complex, although this is compensated for in part by interactions of 6g and 6c with structurally-conserved polar atoms. Overall, the para-substituted ligands are poorer inhibitors than their meta-equivalents (placing 6i in the para-group, as the para-equivalent of 6h).

Conclusions

In the present work, we continued our systematic study of β̅β̅-substituted hydroxamate
analouges of fosmidomycin. Specifically, we explored the effects of changes in lipophilicity, electronic and steric properties versus the phenylpropyl side chain of the earlier compound 5d. Several of the new compounds exhibit potency on PfDxr that approaches that of 1 and 2. There is a good correlation between activity against the enzyme, and activity against the parasite, indicating that their primary mode of biological action is in fact via PfDxr. Seven new X-ray structures show that all of the new arylpropyl substituents displace the key tryptophan residue of the active-site flap, which had made favorable interactions with the reverse hydroxamate group of 1 and its acetyl homologue 2. The plasticity of the flap allows the various compounds to be accommodated in many ways, and indeed in most cases, the flap is largely disordered. However, the structures results can be separated into two classes groups, based on whether the substituent on the aromatic ring is meta or para. Generally, meta-compounds are better inhibitors, and in both classes smaller substituents are associated with better potency. The large lipophilic biphenyl and naphthyl substituents provided poor inhibitors of Dxr, which was not compensated for by any advantages such compounds might provide regarding entry into the parasite cell and apicoplast, or other factors. Future directions should include tests of additional small substituents, particularly ones that could make good interactions with one of the favored conformations of the enzyme. However, it remains to be seen whether intestinal absorption, pharmacokinetic properties or other properties are improved for any of the β-arylpropyl analogues. The strength of the present study is that it provides multiple viable compounds for additional biological work, increasing the chances of ultimate success in the effort to develop useful drugs of the fosmidomycin class.

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). Tetrahydrofuran 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 observed by spraying with a solution of
(NH₄)₆Mo₇O₂₄·4H₂O (25 g/L) and (NH₄)₄Ce(SO₄)₂·2H₂O (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 Revelelis 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.

**General Procedure I: Sonogashira coupling towards aralkynols 8a–j**

To a solution of the aryl iodides (7a–j) in degassed triethylamine, was added PdCl₂(PPh₃)₂, CuI and but-3-yn-1-ol. The reaction mixture was refluxed at 117 °C for 3 h after which, it was cooled and concentrated in vacuo. Column chromatography using a Hex/EtOAc solvent system afforded compounds 8a–j.

**General Procedure II: Triple bond reduction**

To a solution of the alkynes 8a–j in MeOH, was added 10 % of Pd/C under a nitrogen atmosphere. Molecular hydrogen (H₂) was bubbled through the mixture for 30 minutes followed by filtration through a Whatman filter paper path. In vacuo concentration yielded compounds 9a–j which were used for the next step without further purification.

**General Procedure III: Dess-Martin oxidation and concomitant Wittig olefination**

A solution of the starting materials (9a–j) in CH₂Cl₂ and a nitrogen atmosphere was cooled to 0 °C. Dess-Martin periodinane (2.0 equiv) was added and the mixture allowed to attain RT. After stirring for 3 h, TLC analysis showed a completed reaction. The reaction mixture was washed once with a 5:1 mixture of NaHCO₃ (sat. aq.) and Na₂S₂O₃ (aq. 2.0 M), and the water layer extracted three times with diethyl ether. The combined organic layer was washed successively with a 0.1 M solution of HCl and brine, dried over anhydrous Na₂SO₄ and concentrated in vacuo to obtain the corresponding aldehyde (10a–j) which was used without...
further purification. The aldehyde was dissolved in toluene under nitrogen atmosphere and tert-butyl (triphenylphosphoranylidene)acetate (3 equiv) was added. An overnight reflux at 120 °C, was followed by cooling and in vacuo concentration. Sorption of the crude on celite and silica gel chromatography gave access to the tert-butyl esters 11a–j.

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

To a solution of dibenzylmethyl phosphonate (2 eq.) in THF and under a nitrogen atmosphere was added n-BuLi (2 eq.) at -78 °C. After 30 minutes, a solution of the ester was added to the reaction mixture dropwise. Three hours later, the reaction showed to be complete by TLC and was quenched with NH₄Cl (sat. aq.). The water layer was extracted three times with EtOAc. Organic fractions were pooled, washed once with brine and dried over anhydrous Na₂SO₄. Column chromatography (EtOAc/Hex system) afforded the adducts 12a–j.

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

A 0.1 M solution of the starting materials 12a–j in CH₂Cl₂/TFA (80:20), at 0 °C, was stirred for two hours, 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 triethylamine (2.0 equiv). O-Benzyl-N-methylhydroxylamine TFA salt (1.2 equiv) was added as a 0.2 M solution in CH₂Cl₂, and the mixture stirred overnight at RT. The reaction was subsequently quenched with sat. aq. NaHCO₃, extracted three times with CH₂Cl₂, washed with brine and dried over Na₂SO₄. Column chromatography (CH₂Cl₂/MeOH system) produced the protected hydroxamic acids yielded compounds 13a–j.

**General procedure VI: Catalytic hydrogenolysis of benzyl protective groups**

The benzyl protected compounds 13a–j (100-130 mg) was dissolved in MeOH (10 ml) under inert atmosphere and 10 % of Pd/C was added. The resulting mixture was then stirred under hydrogen atmosphere while monitoring the progress by mass spectroscopy. At completion (about 10 minutes), the reaction mixture was filtered and neutralized with NaOH (1 equiv). The reaction mixture was then concentrated in vacuo, re-dissolved in a 1:1 (v/v) mixture of water and tert-butanol, frozen and lyophilized to afford the desired targets compounds 6a–j as monosodium phosphonic acid salts in quantitative yield.
**Tert-butyl (E)-6-(p-tolyl)hex-2-enoate (11a):** Prepared according to general procedure III. Purification 1:1 toluene/hexane v/v; yield 59%. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$H ppm 1.47 (br. s, 9H, t-Bu), 1.75 (app. quint. $J$ = 8.0 Hz, 2H, -CH$_2$-), 2.12-2.23 (m, 2H, -CH$_2$-), 2.30 (s, 3H, Ph-CH$_3$), 2.58 (t, $J$ = 7.5 Hz, 2H, -CH$_2$-), 5.74 (dt, $J$ = 1.5 Hz, 15.6 Hz, 1H, -CH=CHCO), 6.87 (dt $J$ = 7.1 Hz, 15.6 Hz, 1H, -CH=CHCO), 7.00-7.16 (m, 4H, Ar-H). $^{13}$C-NMR (75 MHz, CDCl$_3$) $\delta$C ppm 20.9, 28.1, 29.7, 31.4, 34.7, 79.9, 123.2, 128.2, 128.9, 135.2, 138.6, 147.5, 170.0. HRMS (ESI): calculated for C$_{17}$H$_{25}$O$_2$ [(M+H)$^+$], 261.1849; found 261.1856.

**Tert-butyl (E)-6-(m-tolyl)hex-2-enoate (11b):** Prepared according to general procedure III. Purification 1:1 toluene/hexane v/v; yield 51%. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$H ppm 1.44 (br. s, 9H, t-Bu), 1.76 (app. quint. $J$ = 7.7 Hz, 2H, -CH$_2$-), 2.14-2.24 (m, 2H, -CH$_2$-), 2.32 (s, 3H, Ph-CH$_3$), 2.59 (t, $J$ = 7.7 Hz, 2H, -CH$_2$-), 5.75 (dt, $J$ = 1.6 Hz, 15.6 Hz, 1H, -CH=CHCO), 6.87 (dt $J$ = 6.9 Hz, 15.5 Hz, 1H, -CH=CHCO), 6.93-7.02 (m, 3H Ar-H), 7.12-7.20 (m, 1H, Ar-H). $^{13}$C-NMR (75 MHz, CDCl$_3$) $\delta$C ppm 21.3, 28.1, 29.7, 31.5, 35.2, 80.0, 123.3, 125.4, 126.6, 128.2, 129.2, 137.8, 141.7, 147.5, 166.0. HRMS (ESI): calculated for C$_{17}$H$_{25}$O$_2$ [(M+H)$^+$], 261.1849; found 261.1852.

**Tert-butyl (E)-6-(4-methoxyphenyl)hex-2-enoate (11c):** Prepared according to general procedure III. Purification 1:1 toluene/hexane v/v; yield 64%. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$H ppm 1.47 (br. s, 9H, t-Bu), 1.73 (app. quint. $J$ = 7.7 Hz, 2H, -CH$_2$-), 2.14-2.24 (m, 2H, -CH$_2$-), 2.32 (s, 3H, Ph-CH$_3$), 2.57 (t, $J$ = 7.4 Hz, 2H, -CH$_2$-), 3.76 (s, 3H, PhOCH$_3$), 5.74 (dt, $J$ = 1.7 Hz, 15.7 Hz, 1H, -CH=CHCO), 6.82 (dt $J$ = 6.4 Hz, 15.7 Hz, 1H, -CH=CHCO), 7.26-7.42 (m, 4H, Ar-H). $^{13}$C-NMR (75 MHz, CDCl$_3$) $\delta$C ppm 28.1, 29.8, 31.3, 34.2, 55.1, 78.3, 113.7, 123.2, 129.2, 133.7, 147.5, 157.7, 166.0. HRMS (ESI): calculated for C$_{17}$H$_{25}$O$_3$ [(M+H)$^+$], 277.1798; found 277.1790.

**Tert-butyl (E)-6-(3-methoxyphenyl)hex-2-enoate (11d):** Prepared according to general procedure III. Purification 1:1 toluene/hexane v/v; yield 65%. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$H ppm 1.46 (br. s, 9H, t-Bu), 1.76 (app. quint. $J$ = 7.6 Hz, 2H, -CH$_2$-), 2.16-2.24 (m, 2H, -CH$_2$-), 2.57 (t, $J$ = 7.6 Hz, 2H, -CH$_2$-), 3.72 (s, 3H, PhOCH$_3$), 5.73 (dt, $J$ = 1.6 Hz, 15.7 Hz, 1H, -CH=CHCO), 6.87 (dt $J$ = 6.9 Hz, 15.7 Hz, 1H, -CH=CHCO), 6.92-7.04 (m, 3H Ar-H), 7.12-7.21 (m, 1H, Ar-H). $^{13}$C-NMR (75 MHz, CDCl$_3$) $\delta$C ppm 27.9, 28.1, 29.4, 31.5, 35.8, 80.0, 123.3, 125.4, 126.6, 128.2, 129.2, 137.8, 141.7, 146.8, 165.4. HRMS (ESI): calculated for C$_{17}$H$_{25}$O$_3$ [(M+H)$^+$], 277.1798; found 277.1790.

**Tert-butyl (E)-6-(4-fluorophenyl)hex-2-enoate (11e):** Prepared according to general procedure III. Purification 1:1 toluene/hexane v/v; yield 53%. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$H ppm 1.47 (br. s, 9H, t-Bu), 1.66-1.92 (app. quint. $J$ = 6.3 Hz, 2H, -CH$_2$-), 2.19 (m, 2H, -CH$_2$-), 2.35 (t, $J$ = 7.5 Hz, 2H, -CH$_2$-), 5.74 (dt, $J$ = 1.7 Hz, 15.7 Hz, 1H, -CH=CHCO), 6.85 (dt $J$ = 6.5 Hz, 15.7 Hz, 1H, -CH=CHCO), 6.92-7.04 (m, 3H Ar-H), 7.12-7.21 (m, 1H, Ar-H). $^{13}$C-NMR (75 MHz, CDCl$_3$) $\delta$C ppm 28.1, 29.8, 31.3, 34.2, 55.1, 78.3, 113.7, 123.2, 129.2, 133.7, 147.5, 157.7, 166.0. HRMS (ESI): calculated for C$_{17}$H$_{25}$O$_3$ [(M+H)$^+$], 277.1798; found 277.1790.
2.60 (t, J = 7.4 Hz, 2H, -CH₂-), 5.75 (dt, J = 1.7 Hz, 15.80 Hz, 1H, -CH=CHCO), 6.80-7.00 (m, 3H, -CH=CHCO, Ar-H), 7.06-7.15 (m, 2H, Ar-H). ¹³C-NMR (75 MHz, CDCl₃) δC ppm 28.1, 29.8, 31.3, 34.4, 80.0, 115.0 (d, ¹JC-F = 20.9 Hz), 123.4, 129.7 (d, ¹JC-F = 8.8 Hz), 137.3 (d, ¹JC-F = 3.6 Hz), 147.2, 161.3 (d, ¹JC-F = 242.9 Hz), 166.0. HRMS (ESI): calculated for C₁₆H₂₂FO₂ [(M+H)+], 265.1598; found 265.1597.

Tert-butyl (E)-6-(3-fluorophenyl)hex-2-enoate (11f): Prepared according to general procedure III. Purification 1:1 toluene/hexane v/v; yield 49%. ¹H NMR (300 MHz, CDCl₃) δH ppm 1.48 (br. s, 9H, t-Bu), 1.78 (app. quint. J = 8.0 Hz, 2H, -CH₂-), 2.15-2.25 (m, 2H, -CH₂-), 2.63 (t, J = 7.7 Hz, 2H, -CH₂-), 5.75 (dt, J = 1.7 Hz, 15.6 Hz, 1H, -CH=CHCO), 6.79-6.97 (m, 4H, -CH=CHCO, Ar-H), 7.18-7.27 (m, 1H, Ar-H). ¹³C-NMR (75 MHz, CDCl₃) δC ppm 28.1, 29.4, 31.3, 34.9 (d, ¹JC-F = 1.8 Hz), 80.1, 112.7, 115.2 (d, ¹JC-F = 21.1 Hz), 123.5, 124.0 (d, ¹JC-F = 2.8 Hz), 129.7 (d, ¹JC-F = 8.2 Hz), 144.3 (d, ¹JC-F = 7.1 Hz), 147.1, 162.8 (d, ¹JC-F = 245.5 Hz), 166.0. HRMS (ESI): calculated for C₁₆H₂₂FO₂ [(M+H)+], 265.1598; found 265.1599.

Tert-butyl (E)-6-(4-(trifluoromethyl)phenyl)hex-2-enoate (11g): Prepared according to general procedure III. Purification 1:1 toluene/hexane v/v; yield 60%. ¹H NMR (300 MHz, CDCl₃) δH ppm 1.48 (br. s, 9H, t-Bu), 1.80 (app. quint. J = 7.5 Hz, 2H, -CH₂-), 2.11 (m, 2H, -CH₂-), 2.69 (t, J = 7.8 Hz, -CH₂-), 5.76 (dt, J = 1.8 Hz, 15.6 Hz, 1H, -CH=CHCO), 6.86 (dt, J = 6.8 Hz, 15.6 Hz, 1H, -CH=CHCO), 7.28 (d, J = 9.0 Hz, 2H, Ar-H), 7.54 (d, J = 8.5 Hz, 2H, Ar-H). ¹³C-NMR (75 MHz, CDCl₃) δC ppm 28.1, 29.4, 31.3, 34.9 (d, ¹JC-F = 1.8 Hz), 80.1, 112.7, 115.2 (d, ¹JC-F = 21.1 Hz), 123.5, 124.0 (d, ¹JC-F = 2.8 Hz), 129.7 (d, ¹JC-F = 8.2 Hz), 144.3 (d, ¹JC-F = 7.1 Hz), 147.1, 162.8 (d, ¹JC-F = 245.5 Hz), 166.0. HRMS (ESI): calculated for C₁₇H₂₂F₃O₂ [(M+H)+], 315.1566; found 315.1570.

Tert-butyl (E)-6-(naphthalen-1-yl)hex-2-enoate (11h): Prepared according to general procedure III. Purification 1:1 toluene/hexane v/v; yield 67%. ¹H NMR (300 MHz, CDCl₃) δH ppm 1.47 (br. s, 9H, t-Bu), 1.90 (app. quint. J = 7.6 Hz, 2H, -CH₂-), 2.21 (m, 2H, -CH₂-), 3.08 (t, J = 7.6 Hz, -CH₂-), 5.78 (dt, J = 1.5 Hz, 15.6 Hz, 1H, -CH=CHCO), 6.91 (dt, J = 6.9 Hz, 15.6 Hz, 1H, -CH=CHCO), 7.26-7.53 (m, 4H, Ar-H), 7.70 (d, J = 8.4 Hz, 1H, Ar-H), 7.80-8.00 (m, 2H, Ar-H). ¹³C-NMR (75 MHz, CDCl₃) δC ppm 28.1, 28.9, 31.8, 32.4, 80.0, 123.4, 123.6, 125.4, 125.5, 125.7, 126.0, 126.7, 128.3 (quart., ¹JC-F = 72.9 Hz), 128.4 (quart., ¹JC-F = 56.7 Hz), 128.7, 145.9, 146.9, 165.9. HRMS (ESI): calculated for C₂₀H₂₅O₂ [(M+H)+], 297.1849, found 297.1854.

Tert-butyl (E)-6-(naphthalen-2-yl)hex-2-enoate (11i): Prepared according to general procedure III. Purification 1:1 toluene/hexane v/v; yield 67%. ¹H NMR (300 MHz, CDCl₃) δH ppm 1.48 (br. s, 9H, t-Bu), 1.84 (app. quint. J = 8.1 Hz, 2H, -CH₂-), 2.21 (m, 2H, -CH₂-), 2.77 (t, J = 7.7 Hz, -CH₂-), 5.76 (dt, J = 1.6 Hz, 15.2 Hz, 1H, -CH=CHCO), 6.89 (dt, J = 7.3 Hz, 15.8 Hz, 1H, -CH=CHCO), 7.29 (dd, J = 1.8 Hz, 8.4 Hz, 1H, Ar-H), 7.36-7.47 (m, 2H, Ar-H), 7.54-7.64 (m, 2H, Ar-H), 7.70-7.76 (m, 2H, Ar-H), 7.82-7.89 (m, 1H, Ar-H). ¹³C-NMR (75 MHz, CDCl₃) δC ppm 28.1, 28.9, 31.8, 32.4, 80.0, 123.4, 123.6, 125.4, 125.5, 125.7, 126.0, 126.7, 128.3 (quart., ¹JC-F = 72.9 Hz), 128.4 (quart., ¹JC-F = 56.7 Hz), 128.7, 145.9, 146.9, 165.9. HRMS (ESI): calculated for C₂₀H₂₅O₂ [(M+H)+], 297.1849, found 297.1854.
7.58 (s, 1H, Ar-H), 7.71-7.81 (m, 2H, Ar-H). 13C-NMR (75 MHz, CDCl3) δ ppm 28.1, 29.5, 31.4, 35.3, 80.0, 123.3, 125.1, 125.8, 126.4, 127.1, 127.3, 127.5, 127.9, 132.0, 133.5, 139.2, 147.4, 166.0. HRMS (ESI): calculated for C20H25O2 [(M+H)+], 297.1849; found 297.1852.

Tert-butyl (E)-6-([1,1'-biphenyl]-4-yl)hex-2-enoate (11j): Prepared according to general procedure III. Purification 1:1 toluene/hexane v/v; yield 61%. 1H NMR (300 MHz, CDCl3) δ ppm 1.48 (br. s, 9H, t-Bu), 1.84 (app. quint. J = 7.5 Hz, 2H, -CH2-), 2.21-2.35 (m, 2H, -CH2-), 2.65 (t, J = 7.8 Hz, -CH2-), 5.75 (dt, J = 1.7 Hz, 15.7 Hz, 1H, -CH=CHCO), 6.90 (dt, J = 6.8 Hz, 15.7 Hz, 1H, -CH=CHCO), 7.22 (d, J = 8.5 Hz, 2H, Ar-H), 7.27-7.67 (m, 7H, Ar-H). 13C-NMR (75 MHz, CDCl3) δ ppm 28.1, 29.6, 31.4, 34.8, 80.0, 123.4, 126.9, 127.0, 127.1, 128.7, 128.8, 138.8, 140.8, 141.0, 147.4, 166.0. HRMS (ESI): calculated for C22H27O2 [(M+H)+], 323.2006, mass not found.

Tert-butyl 3-((bis(benzyloxy)phosphoryl)methyl)-6-(p-tolyl)hexanoate (12a): Prepared according to general procedure IV. Purification 2:1 hexane/ethyl acetate v/v; yield 54%. 1H NMR (300 MHz, CDCl3) δ ppm 1.38 (br. s, 9H, t-Bu), 1.42-1.62 (m, 4H, -CH2-), 1.76-1.95 (m, 2H, -CH2-), 2.19-2.32 (m, 5H, Ph-CH3, -CH2-), 2.35-2.54 (m, 3H, -CH2-, -CH-), 4.87-5.08 (m, 4H, -CH2-Ph), 6.97-7.08 (m, 4H, Ar-H), 7.28-7.35 (m, 10H, Ar-H). 13C-NMR (75 MHz, CDCl3) δ ppm 21.2, 28.3, 28.7, 30.1 (d, J_C-P = 138.6 Hz), 30.6 (d, J_C-P = 4.1 Hz), 31.1, 34.5 (d, J_C-P = 10.1 Hz), 35.6, 40.5 (d, J_C-P = 9.3 Hz), 66.9 (d, J_C-P = 6.6 Hz), 67.0 (d, J_C-P = 6.6 Hz), 80.2, 127.9, 127.9, 128.2, 128.3, 128.5, 128.9, 135.0, 136.4 (d, J_C-P = 6.2 Hz), 136.4 (d, J_C-P = 6.0 Hz), 139.1, 171.6. 31P-NMR (121.5 MHz, CDCl3): δ ppm = 33.29. HRMS (ESI): calculated for C32H42O5P [(M+H)+], 537.2764; found 537.2778.

Tert-butyl 3-((bis(benzyloxy)phosphoryl)methyl)-6-(m-tolyl)hexanoate (12b): Prepared according to general procedure IV. Purification 2:1 hexane/ethyl acetate v/v; yield 45%. 1H NMR (300 MHz, CDCl3) δ ppm 1.39 (br. s, 9H, t-Bu), 1.42-1.63 (m, 4H, -CH2-), 1.78-1.98 (m, 2H, -CH2-), 2.17-2.34 (m, 5H, Ph-CH3, -CH2-), 2.35-2.54 (m, 3H, -CH-, -CH2-), 4.88-5.08 (m, 4H, -CH2-Ph), 6.88-7.00 (m, 3H, Ar-H), 7.14 (t, J = 7.7 Hz, 1H, Ar-H), 7.28-7.37 (m, 10H, Ar-H). 13C-NMR (75 MHz, CDCl3) δ ppm 21.6, 28.3, 28.6, 30.1 (d, J_C-P = 138.6 Hz), 30.6 (d, J_C-P = 9.3 Hz), 66.9 (d, J_C-P = 6.6 Hz), 67.0 (d, J_C-P = 6.6 Hz), 80.6, 125.6, 126.7, 128.2, 128.4, 128.8, 128.9, 135.0, 136.4 (d, J_C-P = 6.2 Hz), 136.4 (d, J_C-P = 6.0 Hz), 139.1, 171.6. 31P-NMR (121.5 MHz, CDCl3): δ ppm = 33.29. HRMS (ESI): calculated for C32H42O5P [(M+H)+], 537.2764; found 537.2786.

Tert-butyl 3-((bis(benzyloxy)phosphoryl)methyl)-6-(4-methoxyphenyl)hexanoate (12c): Prepared according to general procedure IV. Purification 2:1 hexane/ethyl acetate v/v; yield 53%. 1H NMR (300 MHz, CDCl3) δ ppm 1.39 (br. s, 9H, t-Bu), 1.43-1.97 (m, 6H, -CH2-),...
2.17-2.52 (m, 5H, -CH2-, -CH-), 3.77 (s, 3H, PhOCH3), 4.88-5.10 (m, 4H, -CH2-Ph), 6.79 (d, J = 9.1 Hz, 2H, Ar-H), 7.02 (d, J = 9.1 Hz, 2H, Ar-H), 7.26-7.44 (m, 10H, Ar-H). 13C-NMR (75 MHz, CDCl3) δ ppm 28.0, 28.5, 29.8 (d, 1JCP = 138.5 Hz), 30.3 (d, 2JCP = 3.9 Hz), 34.2 (d, 3JCP = 10.7 Hz), 34.9, 40.2 (d, 2JCP = 6.1 Hz), 67.0 (d, 2JCP = 6.7 Hz), 80.3, 113.7, 127.9, 128.3, 128.5, 129.2, 134.3, 136.4 (d, 3JCP = 5.3 Hz), 136.4 (d, 3JCP = 5.9 Hz), 157.7, 171.6. 31P-NMR (121.5 MHz, CDCl3): δ ppm = 32.18. HRMS (ESI): calculated for C32H42O6P [(M+H)+], 553.2714; found 553.2717.

Tert-butyl 3-((bis(benzyloxy)phosphoryl)methyl)-6-(3-methoxyphenyl)hexanoate (12d): Prepared according to general procedure IV. Purification 2:1 hexane/ethyl acetate v/v; yield 47%. 1H NMR (300 MHz, CDCl3) δ ppm 1.39 (br. s, 9H, t-Bu), 1.42-1.63 (m, 4H, -CH2-), 1.76-1.93 (m, 2H, -CH2-), 2.19-2.54 (m, 5H, -CH2-, -CH-), 2.65-2.74 (m, 3H, Ar-H), 7.16 (t, J = 8.1 Hz, 1H, Ar-H), 7.28-7.36 (m, 10H, Ar-H). 13C-NMR (75 MHz, CDCl3) δ ppm 28.3, 28.5, 30.2 (d, 1JCP = 139.8 Hz), 30.6 (d, 2JCP = 4.1 Hz), 34.5 (d, 3JCP = 11.0 Hz), 36.1, 40.4 (d, 3JCP = 9.4 Hz), 55.3, 67.2 (d, 3JCP = 4.1 Hz), 67.3 (d, 2JCP = 4.3 Hz), 80.6, 111.2, 114.4, 121.0, 128.2, 128.6, 128.8, 129.5, 136.6 (d, 3JCP = 1.5 Hz), 136.7 (d, 3JCP = 6.0 Hz), 144.1, 159.8, 171.9. 31P-NMR (121.5 MHz, CDCl3): δ ppm = 33.24. HRMS (ESI): calculated for C32H42O6P [(M+H)+], 553.2714; found 553.2717.

Tert-butyl 3-((bis(benzyloxy)phosphoryl)methyl)-6-(4-fluorophenyl)hexanoate (12e): Prepared according to general procedure IV. Purification 2:1 hexane/ethyl acetate v/v; yield 43%. 1H NMR (300 MHz, CDCl3) δ ppm 1.38 (br. s, 9H, t-Bu), 1.42-1.61 (m, 4H, -CH2-), 1.76-1.96 (m, 2H, -CH2-), 2.16-2.34 (m, 2H, -CH2-), 2.18-2.52 (m, 3H, -CH2-, -CH-), 4.89-5.08 (m, 4H, -CH2-Ph), 6.78-6.96 (m, 2H, Ar-H), 7.03 (m, 2H, Ar-H), 7.28-7.37 (m, 10H, Ar-H). 13C-NMR (75 MHz, CDCl3) δ ppm 28.0, 28.4, 29.9 (d, 1JCP = 138.9 Hz), 30.3 (d, 2JCP = 3.9 Hz), 34.1 (d, 3JCP = 10.2 Hz), 34.9, 40.2 (d, 3JCP = 10.2 Hz), 67.0 (m), 80.3, 114.9 (d, 3JCP = 20.4 Hz), 127.9, 128.3, 128.5, 129.6 (d, 3JCP = 7.7 Hz), 136.4 (d, 3JCP = 6.3 Hz), 137.8 (d, 3JCP = 3.3 Hz), 161.1 (d, 1JCP = 243.0 Hz), 171.6. 31P-NMR (121.5 MHz, CDCl3): δ ppm = 33.00. HRMS (ESI): calculated for C31H39FO5P [(M+H)+], 541.2514; found 541.2519.

Tert-butyl 3-((bis(benzyloxy)phosphoryl)methyl)-6-(3-fluorophenyl)hexanoate (12f): Prepared according to general procedure IV. Purification 2:1 hexane/ethyl acetate v/v; yield 47%. 1H NMR (300 MHz, CDCl3) δ ppm 1.33-1.40 (br. s, 9H, t-Bu), 1.41-1.61 (m, 4H, -CH2-), 1.72-1.97 (m, 2H, -CH2-), 2.18-2.33 (2H, m, -CH2-), 2.36-2.55 (m, 3H, -CH2-, -CH-), 4.89-5.08 (m, 4H, -CH2-Ph), 6.76-6.90 (m, 3H, Ar-H), 7.19 (td, 1H, J = 6.08 Hz, 13.96 Hz, Ar-H), 7.29-7.39 (m, 10H, Ar-H). 13C-NMR (75 MHz, CDCl3) δ ppm 28.2, 28.3, 30.2 (d, 1JCP =
139.9 Hz), 30.5 (d, $\gamma_{C,P} = 4.9$ Hz), 34.4 (d, $\gamma_{C,P} = 10.8$ Hz), 35.7, 40.5 (d, $\gamma_{C,P} = 9.8$ Hz), 66.9 (d, $\gamma_{C,P} = 6.6$ Hz), 67.0 (d, $\gamma_{C,P} = 6.4$ Hz), 80.6, 112.8 (d, $\gamma_{C,P} = 21.0$ Hz), 115.4 (d, $\gamma_{C,P} = 20.7$ Hz), 124.2 (d, $\gamma_{C,P} = 3.1$ Hz), 128.2, 128.6, 128.8, 129.9 (d, $\gamma_{C,P} = 8.3$ Hz), 136.3 (d, $\gamma_{C,P} = 6.3$ Hz), 136.4 (d, $\gamma_{C,P} = 6.4$ Hz), 145.1 (d, $\gamma_{C,P} = 7.3$ Hz), 162.8 (d, $\gamma_{C,P} = 246.5$ Hz), 171.8 31P-NMR (121.5 MHz, CDCl3): $\delta_p$ ppm = 33.16. HRMS (ESI): calculated for C31H39FO5P [(M+H)$^+$], 541.2514; found 541.2515.

Tert-butyl 3-((bis(benzyloxy)phosphoryl)methyl)-6-(4-(trifluoromethyl)phenyl)hexanoate (12g): Prepared according to general procedure IV. Purification 2:1 hexane/ethyl acetate v/v; yield 62%. 1H NMR (300 MHz, CDCl3) $\delta_H$ ppm 1.37 (br. s, 9H, t-Bu), 1.41-1.64 (m, 4H, -CH2-), 1.72-1.96 (m, 2H, -CH2-), 2.18-2.61 (m, 5H, -CH2-, -CH-), 4.90-5.09 (m, 4H, -CH2-Ph), 7.19 (d, $J = 8.0$ Hz, 2H, Ar-H), 7.28-7.36 (m, 10H, Ar-H), 7.49 (d, $J = 8.0$ Hz, 2H, Ar-H). 13C-NMR (75 MHz, CDCl3) $\delta_C$ ppm 28.3, 28.4, 30.2 (d, $\gamma_{C,P} = 138.2$ Hz), 30.6 (d, $\gamma_{C,P} = 5.4$ Hz), 34.4 (d, $\gamma_{C,P} = 9.2$ Hz), 67.4 (d, $\gamma_{C,P} = 6.7$ Hz), 67.5 (d, $\gamma_{C,P} = 6.6$ Hz), 80.8, 124.6 (quart., $\gamma_{C,F} = 271.5$ Hz), 125.5 (quart., $\gamma_{C,F} = 3.8$ Hz), 128.3, 128.5 (quart., $\gamma_{C,F} = 27.1$ Hz), 128.7, 128.9, 129.0, 136.7 (d, $\gamma_{C,F} = 6.0$ Hz), 146.7, 171.9. 31P-NMR (121.5 MHz, CDCl3): $\delta_p$ ppm = 31.86 HRMS (ESI): calculated for C32H39F3O5P [(M+H)$^+$], 591.2482; found 591.2487.

Tert-butyl 3-((bis(benzyloxy)phosphoryl)methyl)-6-(naphthalen-1-yl)hexanoate (12h): Prepared according to general procedure IV. Purification 2:1 hexane/ethyl acetate v/v; yield 55%. 1H NMR (300 MHz, CDCl3) $\delta_H$ ppm 1.37 (br. s, 9H, t-Bu), 1.45-1.75 (m, 4H, -CH2-), 1.77-1.94 (m, 2H, -CH2-), 2.20-2.49 (m, 3H, -CH2-, -CH-), 2.98 (t, $J = 7.1$ Hz, 2H, -CH2-), 4.86-5.09 (m, 4H, -CH2-Ph), 7.21-7.39 (m, 12H, Ar-H), 7.41-7.51 (m, 2H, Ar-H), 7.68 (d, $J = 8.4$ Hz, 1H, Ar-H), 7.80-7.86 (m, 1H, Ar-H), 7.93-7.99 (m, 1H, Ar-H). 13C-NMR (75 MHz, CDCl3) $\delta_C$ ppm 27.8, 28.2, 30.1 (d, $\gamma_{C,P} = 138.8$ Hz), 30.5 (d, $\gamma_{C,P} = 4.5$ Hz), 33.1, 34.8 (d, $\gamma_{C,P} = 10.9$ Hz), 40.3 (d, $\gamma_{C,P} = 10.0$ Hz), 66.9 (d, $\gamma_{C,F} = 6.7$ Hz), 7.2 (d, $\gamma_{C,F} = 6.4$ Hz), 80.5, 123.9, 125.5, 125.6, 125.84, 126.0, 126.7, 128.1, 128.5, 128.7, 128.9, 131.9, 134.0, 135.3 (d, $\gamma_{C,F} = 6.4$ Hz), 135.7 (d, $\gamma_{C,P} = 6.1$ Hz), 138.5, 171.7. 31P-NMR (121.5 MHz, CDCl3): $\delta_p$ ppm = 33.23. HRMS (ESI): calculated for C35H42O5P [(M+H)$^+$], 573.2764; found 573.2761.

Tert-butyl 3-((bis(benzyloxy)phosphoryl)methyl)-6-(naphthalen-2-yl)hexanoate (12i): Prepared according to general procedure IV. Purification 2:1 hexane/ethyl acetate v/v; yield 49%. 1H NMR (300 MHz, CDCl3) $\delta_H$ ppm 1.39 (br. s, 9H, t-Bu), 1.44-1.96 (m, 6H, -CH2-), 2.21-2.53 (m, 3H, -CH2-, -CH-), 2.71 (t, $J = 7.2$ Hz, 2H, -CH2-), 4.91-5.10 (m, 4H, -CH2-Ph), 7.25-7.49 (m, 13H, Ar-H), 7.56 (s, 1H, Ar-H), 7.73-7.83 (m, 3H, Ar-H). 13C-NMR (75 MHz, CDCl3) $\delta_C$ ppm 28.0, 28.1, 29.9 (d, $\gamma_{C,P} = 139.1$ Hz), 30.3 (d, $\gamma_{C,P} = 4.5$ Hz), 34.2 (d, $\gamma_{C,P} = ...
10.5 Hz), 35.9, 40.2 (d, $J_{C-P} = 9.8$ Hz), 66.9 (d, $J_{C-P} = 5.8$ Hz), 67.00 (d, $J_{C-P} = 6.9$ Hz), 80.3, 125.0, 125.8, 126.3, 127.2, 127.3, 127.5, 127.8, 127.9, 128.3, 128.5, 131.9, 133.5, 136.4 (d, $J_{C-P} = 6.2$ Hz), 139.7, 171.6. 31P-NMR (121.5 MHz, CDCl3): $\delta_P$ ppm = 32.13. HRMS (ESI): calculated for C$_{35}$H$_{42}$O$_5$P [(M+H)+], 573.2764; found 573.2772.

**Tert-butyl 6-[(1,1'-biphenyl)-4-yl]-3-((bis(benzyloxy)phosphoryl)methyl)hexanoate (12j):**

Prepared according to general procedure IV. Purification 2:1 hexane/ethyl acetate v/v; yield 71%. 1H NMR (300 MHz, CDCl3) $\delta_H$ ppm = 1.38 (br. s, 9H, $t$-Bu), 1.42-1.99 (m, 6H, -CH$_2$-), 2.20-2.63 (m, 5H, -CH$_2$-, -CH-), 4.88-5.09 (m, 4H, -CH$_2$-Ph), 7.17 (d, $J = 8.2$ Hz, 2H, Ar-H), 7.25-7.60 (m, 17H, Ar-H). 13C-NMR (75 MHz, CDCl3) $\delta_C$ ppm 28.0, 28.2, 29.9 (d, $J_{C-P} = 138.5$ Hz), 30.3 (d, 2$J_{C-P} = 3.9$ Hz), 34.2 (d, 3$J_{C-P} = 10.9$ Hz), 35.4, 36.6 (d, 3$J_{C-P} = 9.2$ Hz), 67.0 (d, 2$J_{C-P} = 6.6$ Hz), 67.1 (d, 2$J_{C-P} = 6.4$ Hz), 80.3, 126.9, 127.0, 127.9, 128.0, 128.3, 128.5, 128.6, 136.3 (d, $J_{C-P} = 6.1$ Hz), 136.4 (d, $J_{C-P} = 6.1$ Hz), 138.6, 141.0, 141.3, 171.6. 31P-NMR (121.5 MHz, CDCl3): $\delta_P$ ppm = 31.83. HRMS (ESI): calculated for C$_{37}$H$_{44}$O$_5$P [(M+H)+], 599.2921; found 599.2928.

**Dibenzyl (2-(2-((benzyloxy)(methyl)amino)-2-oxoethyl)-5-(p-tolyl)pentyl)phosphonate (13a):**

Prepared according to general procedure V. Purification 3:1 hexane/acetone v/v; yield 71%. 1H NMR (300 MHz, CDCl3) $\delta_H$ ppm 1.34-1.58 (m, 4H, -CH$_2$-), 1.72-2.05 (m, 3H, -CH$_2$-, -CH-), 2.30 (s, 3H, Ph-CH$_3$), 2.37-2.65 (m, 4H, -CH$_2$-), 3.13 (s, 3H, N-CH$_3$), 4.72 (s, 2H, NOCH$_2$Ph), 4.86-5.06 (m, 4H, -POCH$_2$Ph), 6.98 (d, $J = 8.1$ Hz, 2H, Ar-H), 7.05 (d, $J = 8.1$ Hz, 2H, Ar-H), 7.28-7.36 (m, 15H, Ar-H). 13C-NMR (75 MHz, CDCl3) $\delta_C$ ppm 19.2, 21.0, 28.7, 29.6 (d, $J_{C-P} = 139.3$ Hz), 29.6 (d, 2$J_{C-P} = 5.1$ Hz), 33.5, 34.6 (d, 3$J_{C-P} = 10.2$ Hz), 35.4, 36.6 (d, 2$J_{C-P} = 9.2$ Hz), 67.1 (d, 2$J_{C-P} = 6.3$ Hz), 67.5 (d, 2$J_{C-P} = 6.1$ Hz), 76.1, 127.9, 127.9, 128.2, 128.3, 128.5, 128.6, 128.9, 129.3, 134.5, 135.0, 136.5 (m), 139.3, 173.8. 31P-NMR (121.5 MHz, CDCl3): $\delta_P$ ppm = 33.55. HRMS (ESI): calculated for C$_{36}$H$_{43}$NO$_5$P [(M+H)+], 600.2873; found 600.2903.

**Dibenzyl (2-(2-((benzyloxy)(methyl)amino)-2-oxoethyl)-5-(m-tolyl)pentyl)phosphonate (13b):**

Prepared according to general procedure V. Purification 98:2 dichloromethane/methanol v/v; yield 72%. 1H NMR (300 MHz, CDCl3) $\delta_H$ ppm 1.37-1.59 (m, 4H, -CH$_2$-, -CH-), 2.30 (s, 3H, Ph-CH$_3$), 2.37-2.65 (m, 5H, -CH$_2$-, -CH-), 3.12 (s, 3H, N-CH$_3$), 4.72 (s, 2H, NOCH$_2$Ph), 4.89-5.05 (m, 4H, -POCH$_2$Ph), 6.86-7.00 (m, 3H, Ar-H), 7.13 (t, $J = 7.4$ Hz, 1H, Ar-H), 7.27-7.36 (m, 15H, Ar-H). 13C-NMR (75 MHz, CDCl3) $\delta_C$ ppm 21.3, 28.5, 28.6, 28.6 (d, 2$J_{C-P} = 8.8$ Hz), 29.5 (d, 1$J_{C-P} = 138.8$ Hz), 29.6 (d, 2$J_{C-P} = 4.9$ Hz), 34.6 (d, 1$J_{C-P} = 10.8$ Hz), 35.7, 66.9 (m), 76.0, 125.3, 126.3, 127.8, 128.1, 128.2, 128.5, 128.6, 128.8, 129.1, 129.2, 136.4 (m), 137.6, 142.3, 171.9. C 31P-NMR (121.5 MHz, CDCl3): $\delta_P$ ppm = 33.55. HRMS (ESI): calculated for C$_{36}$H$_{43}$NO$_5$P [(M+H)+], 600.2873; found 600.2903.
MHz, CDCl<sub>3</sub>): δ<sub>p</sub> ppm = 33.54. HRMS (ESI): calculated for C<sub>36</sub>H<sub>43</sub>NO<sub>5</sub>P [(M+H)<sup>+</sup>], 600.2873; found 600.2883.

**Dibenzy (2-(2-((benzyloxy)(methyl)amino)-2-oxoethyl)-5-(4-methoxyphenyl)pentyl)phosphonate (13c):** Prepared according to general procedure V. Purification 3:1 hexane/acetone v/v; yield 57%. 1H NMR (300 MHz, CDCl<sub>3</sub>) δ<sub>H</sub> ppm 1.34-1.59 (m, 4H, -CH<sub>2</sub>), 1.75-2.04 (m, 2H, -CH<sub>2</sub>), 2.26-2.65 (m, 5H, -CH<sub>2</sub>, -CH), 3.13 (s, 3H, N-CH<sub>3</sub>), 3.75 (s, 3H, PhOCH<sub>3</sub>), 4.72 (s, 2H, -NOCH<sub>2</sub>Ph), 4.88-5.07 (m, 4H, -POCH<sub>2</sub>Ph), 6.78 (d, J = 9.6 Hz, 2H, Ar-H), 7.00 (d, J = 9.6 Hz, 2H, Ar-H), 7.27-7.39 (m, 15H, Ar-H). 13C-NMR (75 MHz, CDCl<sub>3</sub>) δ<sub>C</sub> ppm 29.1, 29.9 (d, J<sub>C-P</sub> = 4.3 Hz), 30.0, 30.1 (d, J<sub>C-P</sub> = 136.9 Hz), 34.8 (d, J<sub>C-P</sub> = 9.8 Hz), 35.1, 36.9 (d, J<sub>C-P</sub> = 9.2 Hz), 55.5, 67.2 (d, J<sub>C-P</sub> = 6.7 Hz), 67.3 (d, J<sub>C-P</sub> = 6.1 Hz), 76.3, 113.9, 128.2, 128.5, 128.8, 128.9, 129.1, 129.9, 129.6, 134.7, 134.8 (d, J<sub>C-P</sub> = 5.5 Hz), 136.7, 157.9, 173.5. 31P-NMR (121.5 MHz, CDCl<sub>3</sub>): δ<sub>p</sub> ppm = 32.35. HRMS (ESI): calculated for C<sub>36</sub>H<sub>43</sub>NO<sub>6</sub>P [(M+H)<sup>+</sup>], 616.2823; found 616.2830.

**Dibenzy (2-(2-((benzyloxy)(methyl)amino)-2-oxoethyl)-5-(3-methoxyphenyl)pentyl)phosphonate (13d):** Prepared according to general procedure V. Purification 5:1 dichloromethane/ethyl acetate v/v; yield 51%. 1H NMR (300 MHz, CDCl<sub>3</sub>) δ<sub>H</sub> ppm 1.35-1.61 (m, 4H, -CH<sub>2</sub>), 1.72-2.07 (m, 2H, -CH<sub>2</sub>), 2.27-2.67 (m, 5H, -CH<sub>2</sub>, -CH), 3.12 (s, 3H, N-CH<sub>3</sub>), 3.75 (s, 3H, PhOCH<sub>3</sub>), 4.72 (s, 2H, -NOCH<sub>2</sub>Ph), 4.87-5.07 (m, 4H, -POCH<sub>2</sub>Ph), 6.64-6.74 (m, 3H, Ar-H), 7.16 (t, J = 7.9 Hz, 1H, Ar-H), 7.26-7.37 (m, 15H, Ar-H). 13C-NMR (75 MHz, CDCl<sub>3</sub>) δ<sub>C</sub> ppm 28.4, 29.5 (d, J<sub>C-P</sub> = 4.5 Hz), 29.6 (d, J<sub>C-P</sub> = 138.4 Hz), 34.4 (d, J<sub>C-P</sub> = 10.0 Hz), 35.8, 36.6 (d, J<sub>C-P</sub> = 9.0 Hz), 55.0, 66.8 (d, J<sub>C-P</sub> = 6.7 Hz), 66.9 (d, J<sub>C-P</sub> = 6.6 Hz), 76.0, 110.9, 114.0, 120.7, 127.8, 128.2, 128.4, 128.6, 128.8, 129.1, 129.2, 134.5, 136.4 (d, J<sub>C-P</sub> = 6.1 Hz), 136.4 (d, J<sub>C-P</sub> = 6.1 Hz), 144.0, 159.5, 173.7. 31P-NMR (121.5 MHz, CDCl<sub>3</sub>): δ<sub>p</sub> ppm = 32.31. HRMS (ESI): calculated for C<sub>36</sub>H<sub>43</sub>NO<sub>6</sub>P [(M+H)<sup>+</sup>], 616.2823; found 616.2831.

**Dibenzy (2-(2-((benzyloxy)(methyl)amino)-2-oxoethyl)-5-(4-fluorophenyl)pentyl)phosphonate (13e):** Prepared according to general procedure V. Purification 3:1 hexane/acetone v/v; yield 71%. 1H NMR (300 MHz, CDCl<sub>3</sub>) δ<sub>H</sub> ppm 1.36-1.56 (m, 4H, -CH<sub>2</sub>), 1.71-2.02 (m, 2H, -CH<sub>2</sub>), 2.23-2.50 (m, 5H, -CH<sub>2</sub>, -CH), 3.13 (s, 3H, N-CH<sub>3</sub>), 4.73 (s, 2H, -NOCH<sub>2</sub>Ph), 4.87-5.09 (m, 4H, -POCH<sub>2</sub>Ph), 6.85-7.06 (m, 4H, Ar-H), 7.27-7.39 (m, 15H, Ar-H). 13C-NMR (75 MHz, CDCl<sub>3</sub>) δ<sub>C</sub> ppm 28.6, 29.6 (d, J<sub>C-P</sub> = 4.3 Hz), 29.7 (d, J<sub>C-P</sub> = 10.0 Hz), 34.4 (d, J<sub>C-P</sub> = 10.0 Hz), 34.9, 36.7 (d, J<sub>C-P</sub> = 10.0 Hz), 37.0, 66.9 (d, J<sub>C-P</sub> = 6.6 Hz), 67.0 (d, J<sub>C-P</sub> = 6.3 Hz), 76.1, 114.9 (d, J<sub>C-P</sub> = 21.8 Hz), 127.8 (d, J<sub>C-P</sub> = 9.8 Hz), 127.9, 128.3, 128.6, 128.9, 129.2, 130.9, 134.5, 136.4 (d, J<sub>C-P</sub> = 6.7 Hz), 137.9 (d,
\[ J_{C-P} = 4.2 \text{ Hz}, \] 161.2 (d, \[ J_{C-P} = 242.42 \text{ Hz} \]), 173.4. \[ \delta_p \text{ ppm} = 32.11. \] HRMS (ESI): calculated for C\text{35}H\text{40}F\text{NO5}P [(M+H)+], 604.2623; found 604.2657.

**Dibenzyl (2-(2-((benzyloxy)(methyl)amino)-2-oxoethyl)-5-(3-fluorophenyl)pentyl)phosphonate (13f):** Prepared according to general procedure V. Purification 3:1 hexane/acetonene v/v; yield 69%. \[ \delta_h \text{ ppm} 1.32-1.58 \] (m, 4H, -CH\text{2}-), 1.69-2.03 (m, 2H, -CH\text{2}-), 2.25-2.53 (m, 5H, -CH\text{2}-, -CH-), 3.13 (s, 3H, N-CH\text{3}), 4.73 (s, 2H, -NOCH\text{2}Ph), 4.86-5.09 (m, 4H, -POCH\text{2}Ph), 6.73-7.92 (m, 3H, Ar-H), 7.14-7.38 (m, 16H, Ar-H). \[ \delta_c \text{ ppm} 28.2, 29.6 \] (d, \[ J_{C-P} = 4.1 \text{ Hz} \]), 29.7 (d, \[ J_{C-P} = 137.6 \text{ Hz} \]), 31.6, 34.4 (d, \[ J_{C-P} = 9.83 \text{ Hz} \]), 35.5, 36.7 (d, \[ J_{C-P} = 9.1 \text{ Hz} \]), 66.9 (d, \[ J_{C-P} = 6.7 \text{ Hz} \]), 67.0 (d, \[ J_{C-P} = 6.0 \text{ Hz} \]), 76.1, 112.5 (d, \[ J_{C-P} = 22.0 \text{ Hz} \]), 115.1 (d, \[ J_{C-P} = 22.0 \text{ Hz} \]), 124.0 (d, \[ J_{C-P} = 3.1 \text{ Hz} \]), 127.9, 128.3, 128.5, 128.6, 128.7, 129.9, 129.5 (d, \[ J_{C-P} = 8.6 \text{ Hz} \]), 134.5, 136.4 (d, \[ J_{C-P} = 6.1 \text{ Hz} \]), 144.9 (d, \[ J_{C-P} = 7.7 \text{ Hz} \]), 162.8 (d, \[ J_{C-P} = 244.7 \text{ Hz} \]), 173.25. \[ \delta_p \text{ ppm} = 33.43. \] HRMS (ESI): calculated for C\text{35}H\text{40}F\text{NO5}P [(M+H)+], 604.2623; found 604.2656.

**Dibenzyl (2-(2-((benzyloxy)(methyl)amino)-2-oxoethyl)-5-(4-(trifluoromethyl)phenyl)pentyl)phosphonate (13g):** Prepared according to general procedure V. Purification 3:1 hexane/acetonene v/v; yield 55%. \[ \delta_h \text{ ppm} 1.32-1.59 \] (m, 4H, -CH\text{2}-), 1.70-2.05 (m, 2H, -CH\text{2}-), 2.25-2.64 (m, 5H, -CH\text{2}-, -CH-), 3.12 (s, 3H, N-CH\text{3}), 4.72 (s, 2H, -NOCH\text{2}Ph), 4.87-5.08 (m, 4H, -POCH\text{2}Ph), 7.17 (d, \[ J = 8.2 \text{ Hz} \]), 2H, Ar-H), 7.32 (m, 15H, Ar-H), 7.48 (d, \[ J = 8.2 \text{ Hz} \]), 2H, Ar-H). \[ \delta_c \text{ ppm} 28.2, 29.5 \] (d, \[ J_{C-P} = 3.6 \text{ Hz} \]), 29.7 (d, \[ J_{C-P} = 138.2 \text{ Hz} \]), 32.8, 34.4 (d, \[ J_{C-P} = 10.1 \text{ Hz} \]), 35.5, 36.7 (d, \[ J_{C-P} = 9.5 \text{ Hz} \]), 66.9 (d, \[ J_{C-P} = 6.4 \text{ Hz} \]), 67.0 (d, \[ J_{C-P} = 6.6 \text{ Hz} \]), 76.1, 124.5 (quart., \[ J_{C-P} = 272.7 \text{ Hz} \]), 125.1 (quart., \[ J_{C-P} = 3.8 \text{ Hz} \]), 127.9, 128.2 (quart., \[ J_{C-P} = 22.1 \text{ Hz} \]), 128.3, 128.5, 128.6, 128.9, 129.2, 134.5, 136.4 (d, \[ J_{C-P} = 6.3 \text{ Hz} \]), 146.4, 173.8. \[ \delta_p \text{ ppm} = 33.43. \] HRMS (ESI): calculated for C\text{36}H\text{40}F\text{3}NO5P [(M+H)+], 654.2591; found 654.2601.

**Dibenzyl (2-(2-((benzyloxy)(methyl)amino)-2-oxoethyl)-5-(naphthalen-1-yl)pentyl)phosphonate (13h):** Prepared according to general procedure V. Purification 98:2 dichloromethane/methanol v/v; yield 44%. \[ \delta_h \text{ ppm} 1.50-2.04 \] (m, 6H, -CH\text{2}-), 2.29-2.64 (m, 3H, -CH\text{2}-, -CH-), 2.90-3.00 (m, 2H, -CH\text{2}-), 3.12 (s, 3H, N-CH\text{3}), 4.68 (s, 2H, -NOCH\text{2}Ph), 4.88-5.05 (m, 4H, -POCH\text{2}Ph), 7.19-7.40 (m, 17H, Ar-H), 7.43-7.50 (m, 2H, Ar-H), 7.66-7.72 (m, 1H, Ar-H), 7.80-7.86 (m, 1H, Ar-H), 7.93-7.99 (m, 1H, Ar-H). \[ \delta_c \text{ ppm} 28.1, 29.9 \] (d, \[ J_{C-P} = 138.4 \text{ Hz} \]), 29.9 (d, \[ J_{C-P} = 4.9 \text{ Hz} \]), 33.2, 35.2 (d, \[ J_{C-P} = 9.8 \text{ Hz} \]), 36.9 (d, \[ J_{C-P} = 8.5 \text{ Hz} \]), 67.2 (d, \[ J_{C-P} = 6.7 \text{ Hz} \]), 67.3 (d, \[ J_{C-P} =
Dibenzyl (2-(2-(benzyloxy)(methyl)amino)-2-oxoethyl)-5-(naphthalen-2-yl)pentylphosphonate (13i): Prepared according to general procedure V. Purification 3:1 hexane/acetone v/v; yield 78%. \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta_H\) ppm 1.37-2.02 (m, 6H, -CH\(_2\)-), 2.26-2.75 (m, 5H, -CH-, -CH\(_2\)-), 3.11 (s, 1H, N-CH\(_3\)), 4.71 (s, 2H, -NOCH\(_2\)-Ph), 4.85-5.08 (m, 4H, -POCH\(_2\)-Ph), 7.21-7.34 (m, 16H, Ar-H), 7.36-7.47 (m, 2H, Ar-H), 7.53 (s, 1H, Ar-H), 7.70-7.81 (m, 3H, Ar-H). \(^13\)C-NMR (75 MHz, CDCl\(_3\)) \(\delta_C\) ppm 28.7, 29.9 (d, \(^2J_{C,P} = 3.9\) Hz), 30.0 (d, \(^2J_{C,P} = 138.8\) Hz), 32.9, 34.8 (d, \(^2J_{C,P} = 10.4\) Hz), 36.2, 36.9 (d, \(^2J_{C,P} = 9.1\) Hz), 47.1 (d, \(^2J_{C,P} = 6.6\) Hz), 67.2 (d, \(^2J_{C,P} = 6.6\) Hz), 67.3 (d, \(^2J_{C,P} = 6.0\) Hz), 76.3, 125.3, 126.0, 126.6, 127.5, 127.6, 127.8, 128.0, 128.8, 128.9, 129.1, 129.5, 132.2, 133.8, 134.8, 136.7 (d, \(^2J_{C,P} = 6.6\) Hz), 136.8 (d, \(^2J_{C,P} = 6.2\) Hz), 140.1, 170.4. \(^{31}\)P-NMR (121.5 MHz, CDCl\(_3\)) \(\delta_P\) ppm = 33.49. HRMS (ESI): calculated for C\(_{39}\)H\(_{43}\)NO\(_5\)P [(M+H)+], 636.2873; found 636.2880.

Dibenzyl (5-([1,1'-biphenyl]-4-yl)-2-(2-(benzyloxy)(methyl)amino)-2-oxoethyl)pentylphosphonate (13j): Prepared according to general procedure V. Purification 97:3 dichloromethane/ethyl acetate v/v; yield 68%. \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta_H\) ppm 1.37-1.64 (m, 4H, -CH\(_2\)-), 1.75-2.04 (m, 2H, -CH\(_2\)-), 2.28-2.66 (m, 5H, -CH-, -CH\(_2\)-), 3.13 (s, 3H, N-CH\(_3\)), 4.72 (s, 2H, NOCH\(_2\)-Ph), 4.89-5.07 (m, 4H, -POCH\(_2\)-Ph), 7.11-7.19 (m, 3H, Ar-H), 7.25-7.60 (m, 21H, Ar-H). \(^13\)C-NMR (75 MHz, CDCl\(_3\)) \(\delta_C\) ppm 28.5, 29.6 (d, \(^2J_{C,P} = 4.7\) Hz), 30.3, 29.6 (d, \(^2J_{C,P} = 10.2\) Hz), 35.4, 36.6 (d, \(^2J_{C,P} = 10.2\) Hz), 66.9 (d, \(^2J_{C,P} = 6.1\) Hz), 70.0 (d, \(^2J_{C,P} = 6.6\) Hz), 76.0, 126.9, 127.9, 128.2, 128.5, 128.6, 128.7, 128.8, 129.1, 132.2, 133.8, 134.8, 136.4 (d, \(^2J_{C,P} = 6.4\) Hz), 138.5, 140.1, 141.5, 172.6. \(^{31}\)P-NMR (121.5 MHz, CDCl\(_3\)) \(\delta_P\) ppm = 32.33. HRMS (ESI): calculated for C\(_{41}\)H\(_{45}\)NO\(_5\)P [(M+H)+], 662.3039; found 662.3039.

Sodium hydrogen (2-(2-(hydroxy(methyl)amino)-2-oxoethyl)-5-(p-tolyl)pentyl)phosphonate (6a): White powder. Prepared from compound 13a (150 mg, 0.25 mmol) according to general procedure VI. \(^1\)H NMR (300 MHz, D\(_2\)O) \(\delta_H\) ppm 1.29-1.78 (m, 6H, -CH\(_2\)-), 1.94-2.41 (m, 4H, -CH\(_2\)-Ph, CH\(_3\)-), 2.45-2.70 (m, 4H, -CH\(_2\)-), 3.01 (s, 5/6 of N-CH\(_3\)), 3.23 (s, 1/6 of N-CH\(_3\)). \(^13\)C-NMR (75 MHz, D\(_2\)O) \(\delta_C\) ppm 20.0, 27.6, 30.5 (d, \(^2J_{C,P} = 4.3\) Hz), 31.6 (d, \(^2J_{C,P} = 130.7\) Hz), 34.1 (d, \(^2J_{C,P} = 12.7\) Hz), 34.5, 37.0, 39.2 (d, \(^2J_{C,P} = 7.1\) Hz), 128.4, 128.9, 135.4, 139.8, 177.8. \(^{31}\)P-NMR (121.5 MHz, D\(_2\)O): \(\delta_P\) ppm 25.97. HRMS (ESI): calculated for C\(_{11}\)H\(_{17}\)NO\(_3\)P [(M-H)-], 328.1319; found 328.1320.
Sodium hydrogen (2-(2-(hydroxy(methyl)amino)-2-oxoethyl)-5-(m-tolyl)pentyl)phosphonate (6b): White powder. Prepared from compound 13b (150 mg, 0.25 mmol) according to general procedure VI. \(^1^H\) NMR (300 MHz, \(D_2O\)) \(\delta_H\) ppm 1.22-1.69 (m, 6H, -CH\(_2\)), 1.99-2.23 (m, 1H, -CH=), 2.28 (s, 3H, Ph-CH\(_3\)), 2.49-2.68 (m, 4H, -CH\(_2\)), 3.18 (s, 5/6 of N-CH\(_3\)), 3.35 (s, 1/6 of N-CH\(_3\)), 7.02-7.15 (m, 3H, Ar-H), 7.23 (app. t, \(J = 7.4\) Hz, 1H, Ar-H). \(^{13}C\)-NMR (75 MHz, \(D_2O\)) \(\delta_C\) ppm 20.5, 28.4, 32.2 (d, \(J_{C-P} = 4.1\) Hz), 33.4 (d, \(J_{C-P} = 130.7\) Hz), 35.3, 35.4 (d, \(J_{C-P} = 9.8\) Hz), 36.2, 36.3 (d, \(J_{C-P} = 5.9\) Hz), 125.8, 126.5, 128.7, 129.4, 138.7, 143.8, 174.4. \(^{31}P\)-NMR (121.5 MHz, \(D_2O\)): rotamers at \(\delta_P\) ppm 22.14 and 22.25. HRMS (ESI): calculated for C\(_{15}\)H\(_{23}\)NO\(_5\)P \([\text{M-H}]^-\), 328.1319; found 328.1318.

Sodium hydrogen (2-(2-(hydroxy(methyl)amino)-2-oxoethyl)-5-(4-methoxyphenyl)pentyl)phosphonate (6c): White powder. Prepared from compound 13c (125 mg, 0.20 mmol) according to general procedure VI. \(^1^H\) NMR (300 MHz, \(D_2O\)) \(\delta_H\) ppm 1.23-1.66 (m, 6H, -CH\(_2\)), 2.01-2.26 (m, 1H, -CH=), 2.47-2.67 (m, 4H, -CH\(_2\)), 3.17 (s, 5/6 of N-CH\(_3\)), 3.34 (s, 1/6 of N-CH\(_3\)), 3.78 (s, 3H, PhOCH\(_3\)), 6.91 (m, 2H, Ar-H), 7.21 (m, 2H, Ar-H). \(^{13}C\)-NMR (75 MHz, \(D_2O\)) \(\delta_C\) ppm 28.3, 32.0 (d, \(J_{C-P} = 4.0\) Hz), 33.3 (d, \(J_{C-P} = 129.9\) Hz), 34.4, 35.1 (d, \(J_{C-P} = 10.7\) Hz), 36.1, 36.4 (d, \(J_{C-P} = 6.2\) Hz), 55.6, 114.1, 129.9, 136.2, 156.9, 175.0. \(^{31}P\)-NMR (121.5 MHz, \(D_2O\)): \(\delta_P\) ppm = 22.48. HRMS (ESI): calculated for C\(_{15}\)H\(_{23}\)NO\(_6\)P \([\text{M-H}]^-\), 344.1268; found 344.1269.

Sodium hydrogen (2-(2-(hydroxy(methyl)amino)-2-oxoethyl)-5-(3-methoxyphenyl)pentyl)phosphonate (6d): White powder. Prepared from compound 13d (150 mg, 0.24 mmol) according to general procedure VI. \(^1^H\) NMR (300 MHz, \(D_2O\)) \(\delta_H\) ppm 1.24-1.69 (m, 6H, -CH\(_2\)), 2.02-2.26 (m, 1H, -CH=), 2.51-2.66 (m, 4H, -CH\(_2\)), 3.17 (s, 5/6 of N-CH\(_3\)), 3.34 (s, 1/6 of N-CH\(_3\)), 3.79 (s, 3H, PhOCH\(_3\)), 6.77-6.93 (m, 3H, Ar-H), 7.26 (app. t, \(J = 7.9\) Hz, 1H, Ar-H). \(^{13}C\)-NMR (75 MHz, \(D_2O\)) \(\delta_C\) ppm 28.0, 31.8 (d, \(J_{C-P} = 4.1\) Hz), 33.1 (d, \(J_{C-P} = 130.2\) Hz), 35.1 (d, \(J_{C-P} = 10.6\) Hz), 35.4, 36.1, 36.5 (d, \(J_{C-P} = 7.3\) Hz), 55.4, 111.5, 114.2, 121.7, 129.8, 145.5, 159.0, 175.2. \(^{31}P\)-NMR (121.5 MHz, \(D_2O\)): \(\delta_P\) ppm = 21.72. HRMS (ESI): calculated for C\(_{15}\)H\(_{23}\)NO\(_6\)P \([\text{M-H}]^-\), 344.1268; found 344.1269.

Sodium hydrogen (2-(2-(hydroxy(methyl)amino)-2-oxoethyl)-5-(4-fluorophenyl)pentyl)phosphonate (6e): White powder. Prepared from compound 13e (125 mg, 0.20 mmol) according to general procedure VI. \(^1^H\) NMR (300 MHz, \(D_2O\)) \(\delta_H\) ppm 1.14-1.68 (m, 6H, -CH\(_2\)), 1.92-2.20 (m, 1H, -CH=), 2.42-2.66 (m, 4H, -CH\(_2\)), 3.14 (s, 5/6 of N-CH\(_3\)), 3.27 (s, 1/6 of N-CH\(_3\)), 6.97-7.09 (m, 2H, Ar-H), 7.20-7.33 (m, 2H, Ar-H). \(^{13}C\)-NMR (75 MHz, \(D_2O\)) \(\delta_C\) ppm 28.0, 31.4 (d, \(J_{C-P} = 4.5\) Hz), 33.9 (d, \(J_{C-P} = 139.3\) Hz), 34.6 (d, \(J_{C-P} = 8.1\) Hz), 34.7, 36.3 (d, \(J_{C-P} = 8.3\) Hz), 37.1, 114.7 (d, \(J_{C-P} = 21.2\) Hz), 129.9 (d, \(J_{C-P}\).
$f = 8.3 \text{ Hz}$), 139.2 (d, $J_{C-F} = 3.7 \text{ Hz}$), 160.7 (d, $J_{C-F} = 239.1 \text{ Hz}$), 169.0. $^{31}$P-NMR (121.5 MHz, D$_2$O): rotamers at $\delta$ ppm 21.40, 21.50. HRMS (ESI): calculated for C$_{14}$H$_{21}$FNO$_5$P [(M-H)$^-$], 332.1069; found 332.1088.

**Sodium hydrogen (5-(3-fluorophenyl)-2-(2-(hydroxy(methyl)amino)-2-oxoethyl)pentyl)phosphonate (6f):** White powder. Prepared from compound 13f (130 mg, 0.22 mmol) according to general procedure VI. $^1$H NMR (300 MHz, D$_2$O) $\delta$H ppm 1.23-1.68 (m, 6H, -CH$_2$-), 2.02-2.23 (m, 1H, -CH-), 2.50-2.69 (m, 4H, -CH$_2$-), 3.17 (s, 5/6 of N-CH$_3$), 3.35 (s, 1/6 of N-CH$_3$), 6.88-7.10 (m, 3H, Ar-H), 7.25-7.34 (m, 1H, Ar-H). $^{13}$C-NMR (75 MHz, D$_2$O) $\delta$C ppm 27.9, 31.9 (d, $J_{C-P} = 4.1 \text{ Hz}$), 33.1 (d, $J_{C-P} = 129.1 \text{ Hz}$), 35.0 (d, $J_{C-F} = 8.2 \text{ Hz}$), 35.1, 36.1, 36.4 (d, $J_{C-P} = 7.0 \text{ Hz}$), 112.5 (d, $J_{C-F} = 23.32 \text{ Hz}$), 115.3 (d, $J_{C-F} = 21.9 \text{ Hz}$), 124.6 (d, $J_{C-F} = 2.7 \text{ Hz}$), 130.1 (d, $J_{C-F} = 8.7 \text{ Hz}$), 146.2 (d, $J_{C-F} = 8.7 \text{ Hz}$), 162.9 (d, $J_{C-F} = 247.1 \text{ Hz}$), 175.0. $^{31}$P-NMR (121.5 MHz, D$_2$O): $\delta$P ppm = 22.75. HRMS (ESI): calculated for C$_{14}$H$_{21}$FNO$_5$P [(M-H)$^-$], 332.1069; found 332.1067.

**Sodium hydrogen (2-(2-(hydroxy(methyl)amino)-2-oxoethyl)-5-(4-(trifluoromethyl)phenyl)pentyl)phosphonate (6g):** White powder. Prepared from compound 13g (150 mg, 0.25 mmol) according to general procedure VI. $^1$H NMR (300 MHz, D$_2$O) $\delta$H ppm 1.27-1.71 (m, 6H, -CH$_2$-), 2.00-2.26 (m, 1H, -CH-), 2.50-2.61 (m, 2H, -CH$_2$-), 2.66 (t, $J = 7.4 \text{ Hz}$, 2H, -CH$_2$-), 3.16 (s, 5/6 of N-CH$_3$), 3.34 (s, 1/6 of N-CH$_3$), 3.79 7.40 (d, $J = 7.9 \text{ Hz}$, 2H, Ar-H), 7.61 (d, $J = 7.9 \text{ Hz}$, 2H, Ar-H). $^{13}$C-NMR (75 MHz, D$_2$O) $\delta$c ppm 27.9, 31.9 (d, $J_{C-P} = 4.1 \text{ Hz}$), 32.8 (d, $J_{C-P} = 130.7 \text{ Hz}$), 34.5 (d, $J_{C-F} = 11.0 \text{ Hz}$), 34.9, 35.8, 36.3 (d, $J_{C-P} = 6.8 \text{ Hz}$), 124.4 (quart., $J_{C-P} = 270.5 \text{ Hz}$), 125.0 (quart., $J_{C-P} = 4.1 \text{ Hz}$), 128.9 (app. s), 147.6, 174.9. $^{31}$P-NMR (121.5 MHz, D$_2$O): $\delta$P ppm = 21.49. HRMS (ESI): calculated for C$_{15}$H$_{20}$F$_3$NO$_5$P [(M-H)$^-$], 382.1037; found 382.1039.

**Sodium hydrogen (2-(2-(hydroxy(methyl)amino)-2-oxoethyl)-5-(naphthalen-1-yl)pentyl)phosphonate (6h):** White powder. Prepared from compound 13h (150 mg, 0.24 mmol) according to general procedure VI. $^1$H NMR (300 MHz, D$_2$O) $\delta$H ppm 1.38-1.84 (m, 6H, -CH$_2$-), 2.02-2.27 (m, 1H, -CH-), 2.46-2.65 (m, 2H, -CH$_2$-), 3.07 (t, $J = 7.6 \text{ Hz}$, 2H, -CH$_2$-), 3.13 (s, 5/6 of N-CH$_3$), 3.24 (s, 1/6 of N-CH$_3$), 7.39-7.62 (m, 4H, Ar-H), 7.78 (dd, $J = 2.4 \text{ Hz}$, 7.4 Hz, 1H, Ar-H), 7.92 (dd, $J = 2.4 \text{ Hz}$, 8.1 Hz, 1H, Ar-H), 8.18 (d, $J = 8.1 \text{ Hz}$, 1H, Ar-H). $^{13}$C-NMR (75 MHz, D$_2$O) $\delta$c ppm 27.8, 32.1 (d, $J_{C-P} = 4.5 \text{ Hz}$), 32.7, 33.8 (d, $J_{C-F} = 129.3 \text{ Hz}$), 34.5, 35.7 (d, $J_{C-F} = 10.8 \text{ Hz}$), 36.4 (d, $J_{C-F} = 7.6 \text{ Hz}$), 36.6, 124.4, 126.1, 126.2, 126.3, 126.4, 128.8, 131.6, 133.7, 139.7, 172.7. $^{31}$P-NMR (121.5 MHz, D$_2$O): rotamers at $\delta$ ppm 21.94, 22.18. HRMS (ESI): calculated for C$_{18}$H$_{20}$NO$_5$P [(M-H)$^-$], 364.1319; found 364.1315.
Sodium hydrogen (2-(2-(hydroxy(methyl)amino)-2-oxoethyl)-5-(naphthalen-2-yl)pentyl)phosphonate (6i): White powder. Prepared from compound 13i (150 mg, 0.24 mmol) according to general procedure VI. $^1$H NMR (300 MHz, D$_2$O) $\delta$H ppm 1.23-1.80 (m, 6H, -CH$_2$-), 2.01-2.29 (m, 1H, -CH-), 2.46-2.65 (m, 2H, -CH$_2$-), 2.78 (t, $J$ = 7.8 Hz, 2H, -CH$_2$-), 3.12 (s, 5/6 of N-CH$_3$), 3.30 (s, 1/6 of N-CH$_3$), 7.44-7.55 (m, 3H, Ar-H), 7.77 (s, 1H, Ar-H), 7.84-7.92 (m, 3H, Ar-H). $^{13}$C-NMR (75 MHz, D$_2$O) $\delta$C ppm 29.8, 31.8 (d, $^3$J$_{C-P}$ = 3.7 Hz), 34.1 (d, $^3$J$_{C-P}$ = 130.8 Hz), 35.0 (d, $^3$J$_{C-P}$ = 10.9 Hz), 35.8, 36.5 (d, $^3$J$_{C-P}$ = 8.0 Hz), 37.3, 125.5, 126.3, 126.4, 127.5, 127.7, 127.9, 128.1, 131.7, 133.5, 141.6, 169.7. $^{31}$P-NMR (121.5 MHz, D$_2$O): $\delta$P ppm = 22.48. HRMS (ESI): calculated for C$_{18}$H$_{23}$NO$_5$P [(M-H)$^-$], 364.1319; found 364.1315.

Sodium hydrogen (5-(1,1'-biphenyl)-4-yl)-2-(2-(hydroxy(methyl)amino)-2-oxoethyl)pentyl)phosphonate (6j): White powder. Prepared from compound 13j (200 mg, 0.30 mmol) according to general procedure VI. $^1$H NMR (300 MHz, D$_2$O) $\delta$H ppm 1.23-1.73 (m, 6H, -CH$_2$-), 1.98-2.24 (m, 1H, -CH-), 2.42-2.70 (m, 4H, -CH$_2$-), 3.14 (s, 5/6 of N-CH$_3$), 3.30 (s, 1/6 of N-CH$_3$), 7.35-7.53 (m, 5H, Ar-H), 7.58-7.71 (m, 4H, Ar-H). $^{13}$C-NMR (75 MHz, D$_2$O) $\delta$C ppm 27.9, 31.5 (d, $^3$J$_{C-P}$ = 4.1 Hz), 33.0 (d, $^3$J$_{C-P}$ = 130.1 Hz), 34.5 (d, $^3$J$_{C-P}$ = 10.1 Hz), 35.0, 36.3 (d, $^3$J$_{C-P}$ = 8.3 Hz), 37.1, 126.7, 126.8, 127.4, 129.0, 129.3, 137.8, 140.4, 143.1, 169.2. $^{31}$P-NMR (121.5 MHz, D$_2$O): rotamers at $\delta$P ppm 21.37, 21.54. HRMS (ESI): calculated for C$_{20}$H$_{25}$NO$_5$P [(M-H)$^-$], 390.1476; found 390.1479.

X-ray crystallography

Protein was produced and assayed as described earlier.[23] The water-soluble ligands (6a, 6b, 6c, 6d, 6f, 6g, 6h) were incubated (final concentration, 1 mM) with the protein solution (0.3 mM in 20 mM Tris-HCl, pH 7.8, 200-300 mM NaCl, 5% (v/v) glycerol, 2 mM dithiothreitol and 1 mM MnCl$_2$) for 10-15 min at 20 °C before the co-crystallization experiments were set up in 2-well MRC plates (Molecular Dimensions, UK) with a Mosquito robot (TTP Labtech, UK). Reservoir solutions consisted of 40 µL, and the sitting droplets contained equal volumes (100 nL each) of the protein–ligand mixture and reservoir solution. Previous screening[23] had shown the Morphues screen[24] was highly effective for this protein, and crystals (0.1 x 0.1 x 0.1 mm) appeared in 1-3 days at 20 °C in multiple conditions (Table S1). Crystals were harvested without further cryoprotection, and plunged into liquid nitrogen for transport to the relevant synchrotron beamline.
Diffraction data were collected at 100 K at the European Synchrotron Radiation Facility (ESRF, Grenoble, France) or at Diamond, Oxford, England (see Table S2). All crystals had the symmetry of the triclinic space group P1, and could be classified into the same one of the two related groups of P1 cells discussed earlier. Diffraction images were processed and scaled with XDSC and SCALA respectively, using the CCP4 package. Rigid-body refinement was used for initial placement of the structures, to maintain a similar position relative to that seen earlier. Structures were then subjected to alternating rounds of reciprocal space refinement with REFMAC and manual rebuilding with O. Solvent was added using the water tools in O. The ligands and respective stereochemical restraints were built and generated with the qds tools in O. Refinement restraints were then generated from the fitted models by REFMAC5, and manually edited as needed. Hydroxamate groups were restrained to planarity, and metal-coordination target distances were taken from Harding (2006). Complete data collection and refinement statistics are included in Table S2.

Briefly, the structures of the seven new β-substituted enzyme-inhibitor complexes (6a, 6b, 6c, 6d, 6f, 6g, 6h) were solved at resolutions of 1.55, 1.8, 1.7, 1.7, 1.6 and 1.4 Å, respectively, and refined to crystallographic R-factors of 18.6, 17.7, 18.2, 16.9, 18.2, 18.0 and 18.7% (R-frees are 20.5, 20.4, 21.1, 20.3, 20.8 and 20.7%, respectively). Each complex has a dimer in the asymmetric unit, with a manganese ion and ligand in each active site. Although the new compounds were synthesized as racemic mixtures, the high resolution of the study (e.g. Figure 4A4a) allowed us to define the respective enantiomer of each ligand. The overall electron density is of high quality, and complete models of the enzyme are deposited for residues 77-486 in each chain. Density for residues in the active-site flap is discussed in the main text, and described further in Table S3. Structural comparisons were made with the lsq commands in O with close-pair Ca cut-offs (1.0 Å Ca - Ca separations). Figures were created in O, using secondary structure assignments from the yasspa algorithm, and rendered in Molray. Structures of the various complexes were deposited at the Protein Data Bank, as follows: 6a (5JMW), 6b (5JO0), 6c (5JBI), 6d (5JC1), 6f (5JMP), 6g (5JNL) and 6h (5JAZ). Electron density for each entry is available at the Uppsala Electron Density Server.

**Supporting Information**
Additional experimental details are presented, including comparisons of pIC50 for EcDxr and PfDxr, comparisons of pIC50 with crystallographic temperature factors and fit to electron density, crystallization conditions used in the reported X-ray structures, statistics for data collection and refinement, and summary of electron density in residues of the flap.

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Abbreviations used

Bn, benzyl; DOXP, 1-deoxy-D-xylulose 5-phosphate; DMAP, 4-(dimethylamino)-pyridine; Dxr, 1-deoxy-D-xylulose-5-phosphate reductoisomerase; EcDxr, Escherichia coli Dxr; EDC, N-(3-Dimethylaminopropyl)-N’-ethylcarbodiimide; h, hour(s); MEP, 2-C-methyl-d-erythritol-3-phosphate; min, minutes; PfDxr, Plasmodium falciparum Dxr; r.m.s., root-mean-square; rt, room temperature; tert-Bu, tertiary butyl; TFA, trifluoroacetic acid; THF, tetrahydrofuran.

References


