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Title: ${ }^{\alpha-A m i n o p h o s p h o n a t e ~ i n h i b i t o r s ~ o f ~ m e t a l l o-~} \beta$-lactamases NDM-1 and VIM-2

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# $\alpha$-Aminophosphonate inhibitors of metallo- $\beta$ lactamases NDM-1 and VIM-2† 

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#### Abstract

The upswing of antibiotic resistance is an escalating threat to human health. Resistance mediated by bacterial metallo- $\beta$-lactamases is of particular concern as these enzymes degrade $\beta$-lactams, our most frequently prescribed class of antibiotics. Inhibition of metallo- $\beta$-lactamases could allow the continued use of existing $\beta$-lactam antibiotics, such as penicillins, cephalosporins and carbapenems, whose applicability is becoming ever more limited. The design, synthesis, and NDM-1, VIM-2, and GIM-1 inhibitory activities (IC $\mathrm{C}_{50}$ 4.1-506 $\mu \mathrm{M}$ ) of a series of novel non-cytotoxic $\alpha$-aminophosphonate-based inhibitor candidates are presented herein. We disclose the solution NMR spectroscopic and computational investigation of their NDM-1 and VIM-2 binding sites and binding modes. Whereas the binding modes of the inhibitors are similar, VIM-2 showed a somewhat higher conformational flexibility, and complexed a larger number of inhibitor candidates in more varying binding modes than NDM-1. Phosphonate-type inhibitors may be potential candidates for development into therapeutics to combat metallo- $\beta$-lactamase resistant bacteria.


## Introduction

Antimicrobial resistance is a major threat to human health that already causes over a million deaths annually. ${ }^{1}$ It is expected to

[^0]become the leading cause of mortality by $2050 .{ }^{2}$ Out of the multiple resistance mechanisms that bacteria possess, ${ }^{3}$ metallo-$\beta$-lactamase mediated degradation pathways are currently the most worrisome ${ }^{4}$ as they render $\beta$-lactam antibiotics inactive (Fig. 1), ${ }^{5}$ including also carbapenems, a class of drugs of lastresort. ${ }^{6,7}$ Historically, $\beta$-lactams constitute the most widely used, cheapest to produce and most prescribed class of antibiotics. ${ }^{4,8}$ The New Delhi metallo- $\beta$-lactamase 1 (NDM-1) ${ }^{9}$ and the Verona integron-encoded metallo- $\beta$-lactamase $2(\mathrm{VIM}-2)^{10}$ are of particular concern as they are commonly found in clinical isolates and are spread worldwide. ${ }^{11}$ The transmutation of the plasmid-borne $\beta$-lactamase genes (bla) between Gram-negative bacteria $^{12}$ made $b l a_{\text {NDM-1 }}$ the predominant $\beta$-lactamase in Europe and endemic in the Indian subcontinent. ${ }^{4}$

The mutation rate of metallo- $\beta$-lactamases outnumbers that of antibiotic discovery by far, leading to a rapid increase in the number of bacteria insensitive to $\beta$-lactam antibiotics. Out of the $7279 \beta$-lactamases reported so far, 38 are NDM-1 and 74 are VIM- 2 variants, ${ }^{13}$ with further $\beta$-lactamases being continuously identified. ${ }^{14}$

Besides the design of novel $\beta$-lactams reluctant to hydrolysis by metallo- $\beta$-lactamases, the development of metallo- $\beta$-lactamase inhibitors is the most promising strategy to counteract metallo- $\beta$-lactamase-based antibiotic resistance. ${ }^{15}$ These inhibitors, when combined with antibiotics and formulated into a single product, may prolong the usefulness of existing $\beta$-lactam antibiotics. This strategy


Fig. 1 The schematic mechanism of $\beta$-lactam hydrolysis facilitated by metallo- $\beta$-lactamases. ${ }^{5}$
was demonstrated to be successful by the introduction of serine- $\beta$-lactamase inhibitors, such as tazobactam ${ }^{16}$ and clavulanic acid. ${ }^{17}$

Metallo- $\beta$-lactamases do not share an evolutionary relationship with serine- $\beta$-lactamases. They possess different structures, active sites and catalytic mechanisms. Consequently, serine- $\beta$-lactamase inhibitors are inactive against metallo- $\beta$-lactamases, except for the recently developed taniborbactam. ${ }^{18}$ Captopril derivatives, thiorphan, tiopronin, 2,3-dimercaprol, ML302F, L-CS319, 2-(4fluorobenzoyl)benzoic acid, 3-formylchromone, cyclic boronates and ANT-2681 are examples of compounds that act by ligand replacement, whereas SIT-Z5 and aspergillomarasmine $\mathrm{A}^{19}$ act by metal sequestration. These are both Zn -dependent strategies. ${ }^{20,21}$ Schofield's isoquinolines $\left(\mathrm{IC}_{50} \quad 10-1000 \mu \mathrm{M}\right)^{22}$ are the most successful Zn -independent inhibitors. Most inhibitors discovered so far have $\mathrm{IC}_{50} \mathrm{~S}$ in the $\mu \mathrm{M}$ range, although a few compounds with nM potency have also been reported. ${ }^{23}$ The field has been extensively reviewed. ${ }^{24-27}$ Importantly, no clinically applicable metallo- $\beta$-lactamase inhibitors are available to date, and merely two candidates have yet been reported to have reached clinical trials. ${ }^{8}$ Consequently, the development of new classes of metallo- $\beta$-lactamase inhibitors that provide protection to some of our clinically most precious antibiotics is expected to be one of the possibly most valuable contributions to combating antibiotic resistance. ${ }^{28}$

## Results and discussion

## Design

Mimetics of the tetrahedral transition state of $\beta$-lactam hydrolysis ${ }^{29}$ (Fig. 1) are expected to yield potential metallo- $\beta$ lactamase inhibitors. The active site of NDM-1 and of VIM-2 are structurally similar (Fig. 2, and section S3 of ESI $\dagger$ ), and involve the same amino acid residues to facilitate zinc coordination. In addition, both enzymes use flexible loops to allow for adjustment of the active site to enable the binding of a wide range of $\beta$-lactam antibiotics.

In our design of novel inhibitors, we introduced a phosphonic acid and a piperidine ring as key binding motives (Scheme 1). Phosphonic acid derivatives ${ }^{30}$ and
phosphonamidates ${ }^{31}$ have previously been studied in the context of metallo- $\beta$-lactamase inhibition. The phosphonate group was incorporated to mimic the amide carbonyl of $\beta$-lactams and is expected to coordinate the zinc ions of metallo- $\beta$-lactamases. ${ }^{32,33}$ The piperidine possibly interacts with the catalytic site of metallo- $\beta$-lactamases. Initially, phospholactams (I) showed low stability in aqueous solutions in preliminary experiments, ${ }^{34}$ so we turned to their chemically stable analogues II, as their tetrahedral phosphonate motif resembles the tetrahedral transition state of hydrolysed $\beta$-lactams (Scheme 1). The phosphonic acid core is thus expected to interact within the active site, by strongly coordinating the zinc ions with its oxygens, analogous to that reported for structurally related phosphonate-based metallo- $\beta$-lactamase inhibitors (see for example pdb: 5HH6). ${ }^{32,35}$

We further introduced thiophene moieties with variation in their regioisomeric pattern, steric bulk and flexibility (Scheme 2) as sulfur substitution is common in several classes of $\beta$-lactams (penams, penems, cephems, etc.). The


Fig. 2 Superimposed structures of NDM-1 (blue, pdb: 6NY7) and VIM2 (orange, pdb: 6O5T). ${ }^{29}$ The amino acid residues that are most important for binding of $\beta$-lactam antibiotics are highlighted to indicate the conserved part of the active site.


I


II

Scheme 1 Piperidine substituted phospholactams and $\alpha$-aminophosphonates are expected to bind metallo- $\beta$-lactamases as the geometry of their $\mathrm{P}=\mathrm{O}$ motif resembles the tetrahedral intermediate of $\beta$-lactam hydrolysis.


Scheme 2 Thiophenes with varying regioisomer patterns, steric bulk, polarity and flexibility have been used as the " $R$ "-substituents (Scheme 1) of the $\alpha$-aminophosphonate-type metallo- $\beta$-lactamase inhibitors developed herein.
(thiophene-2-yl)acet-amido and thiophene moieties, which are bioisosteres of benzyl and phenyl groups, respectively, ${ }^{36}$ were anticipated to interact with the hydrophobic and aromatic amino acid residues of loop 3 (Phe70/Tyr65). These sidechains vary in hydrophobicity, which we deemed worth exploring. In SAR studies of $\beta$-lactamase inhibitors, the properties of aromatic moieties were found important as they contribute to crucial $\pi-\pi$ interactions. ${ }^{37}$ The structural variation of the aromatic moiety in the present series is also expected to affect the solubility of the compounds.

## Synthesis

To synthesize 5a-l, we used a pathway (Scheme 3) that relies on the hafnium chloride catalyzed Kabachnik-Fields reaction, ${ }^{38}$ which simultaneously introduces the phosphonic acid and the amine functional groups, in its key first step. Next, the benzyl protecting group of 2 was hydrogenated under mild conditions using Pearlman's catalyst, ${ }^{39} \mathrm{Pd}(\mathrm{OH})_{2} / \mathrm{C}$ and $\mathrm{H}_{2}$ under atmospheric pressure, yielding 3. Subsequently, a variety of amides 4a-l were generated from this
intermediate with a range of carboxylic acids (Scheme 3). Out of the variety of coupling reagents and solvents examined, HATU in combination with dry EtOAc as solvent provided the highest yields. ${ }^{40}$ The diastereomers of $\mathbf{4 a - 1}$ were separated using preparative HPLC, and one set of enantiomeric pairs was crystalized to identify the relative configuration of the intermediates by single-crystal X-ray diffraction (section 5, ESI $\dagger$ ). The phosphonic ester was then hydrolyzed with McKenna's method using TMSBr, resulting in simultaneous Boc-deprotection of 4a-l. However, it was observed that ethyl bromide liberated as a side product led to unwanted $N$-alkylation of the target compounds $\mathbf{5 a - l}$. This could be avoided by the addition of $\mathrm{Et}_{3} \mathrm{~N}$ in combination with 3-mercaptopropyl functionalized silica gel to the reaction mixture. To enhance the aqueous solubility, the final products were converted to their hydrochloric salts (5a-1). In our hands, the compounds were chemically stable in solution for a prolonged time (weeks to months, Fig. S187-S192, ESI $\dagger$ ).

## Metallo- $\beta$-lactamase inhibition and cytotoxic activities

The 24 synthesized compounds 5a-l were tested for their inhibitory activities against NDM-1, VIM-2 (Table 1), and GIM-1 in an in vitro enzymatic assay (ESI $\dagger$ ). ${ }^{41}$ The inhibitory activity was measured using enzyme concentrations of 0.1 nM VIM-2, 10 nM NDM-1 or 1 nM GIM-1, with an excess Zn (II) ( $100 \mu \mathrm{M}$, see Experimental methods), with inhibitor compounds with concentrations from 0 to $800 \mu \mathrm{M}$. Each data point was measured in triplicates. Almost all compounds showed metallo- $\beta$-lactamase inhibitory activities with 5 g -2 being the most active ( $\mathrm{IC}_{50}=4.1 \mu \mathrm{M}$, VIM-2). A larger number of compounds inhibited VIM-2 than NDM-1 or GIM-1, and those with $R R / S S$ configuration typically inhibited VIM-2 at lower concentrations. Our observation that a larger number of compounds inhibited VIM-2 than NDM-1 may be unexpected as both enzymes belong to the B1-subclass of metallo- $\beta$-lactamases. This is in line with previous reports. ${ }^{42,43}$ In general, the more rigid compounds showed higher affinity (compare $\mathbf{5 b}$ to $\mathbf{5 j}$, for instance). Accordingly, upon introduction of a flexible methylene group, inhibitors with a 3-thiophene ring showed a 10 -fold reduction of activity, whilst 2 -thiophene substituted ones showed up to 45 -fold decreased activity. Importantly, none of the compounds showed cytotoxicity against human MCF-7 or HepG cells (section 2, ESI $\dagger$ ).

## Bacterial membrane permeability

To evaluate the compounds' ability to cross bacterial outer membranes we prepared outer membrane vesicles carrying VIM-2 inside their lumen. ${ }^{44}$ Outer membrane vesicles maintain the protein as well as the lipid composition of the bacterial membrane they originate from, and thus serve as an in situ model system for assessing transmembrane uptake. ${ }^{45}$ The activity of VIM-2 encapsulated inside the outer membrane vesicles was determined using the colorimetric



4(a-1)-2
5(a-l)-2

Scheme 3 Synthesis of the target compounds. Reagents and conditions: (a) $\mathrm{BnNH}_{2}, \mathrm{HP}(\mathrm{O})(\mathrm{OEt})_{2}, \mathrm{HfCl}_{4}$ (cat), $\mathrm{EtOH}, 60{ }^{\circ} \mathrm{C}, 12 \mathrm{~h}, 32 \%$; (b) $\mathrm{H}_{2}(1$ atm), $\mathrm{Pd}\left(\mathrm{OH}_{2}\right) / \mathrm{C}$ (cat), EtOH, r.t., $16 \mathrm{~h}, 99 \%$; (c) R-COOH, HATU, DIPEA, dry EtOAc, r.t., 12-24 h, 60-80\%; (d) TMSBr, 3-mercaptopropyl functionalized silica gel, $E t_{3} N, M e C N, ~ r . t ., 12-48 h, 19-94 \%$. For the full structures of $5(a-l)$, and hence of the R-groups, see Table 1 below.
$\beta$-lactamase substrate CENTA in presence and absence of the compounds. ${ }^{46}$ We observed a reduction in VIM-2 activity for most compounds (Fig. 3), in agreement with the in vitro assay, indicating that they effectively cross the Gram-negative outer membrane.

## Protein-inhibitor interaction studies

Solution NMR spectroscopy is a powerful tool to assess protein-ligand interactions under close to physiological conditions. ${ }^{47}$ We, therefore, performed NMR chemical shift perturbation (CSP, for details, see section S8, ESI $\dagger$ ) experiments to identify the amino acid residues of NDM-1 and VIM-2 that are involved in inhibitor binding, making use of the previously published protein assignments. ${ }^{48,49}$ Titrations were conducted using ${ }^{15} \mathrm{~N}$-labelled proteins with 0 , $0.5,1,2,4,8$, and 16 equiv. of 5 inhibitors for NDM-1 and of 7 inhibitors for VIM-2.

As performing NMR titrations for all inhibitors in combination with both proteins was deemed unrealistic, we selected compounds based on their inhibitory activity and aqueous solubility. Titrations with concentrated ligand samples were preferred to minimize dilution-induced chemical shift changes. Titrations were carried out with NDM-1 and VIM-2 due to their high clinical relevance.

Throughout the titrations of NDM-1 with selected inhibitors (Table 2), Phe70, Asp124, and Lys211 surrounding the suggested $\beta$-lactam binding site showed significant chemical shift perturbations (Fig. 4 and S164-S168, ESI $\dagger$ ). We also observed large chemical shift perturbations for Ser191, which has previously been reported to take part in substrate
binding (numbered as Ser198). ${ }^{4,50}$ The observed spectral changes are characteristic for binding whereas not compatible with zinc(II)-depletion of the enzyme. ${ }^{44,51}$ Some amino acid residues in the C-terminal increased in intensity with increasing concentration of the added inhibitor, suggesting binding-induced alteration corresponding to the rate of the conformational dynamics of this enzyme. ${ }^{52}$

We observed significant chemical shift perturbations for Tyr65, His177, Tyr199, Arg203, Ser205, and His238 of VIM-2, in the expected binding site (Fig. 5 and S169-S174, section S7, ESI $\dagger$ ), and for a few additional amino acid residues at the N terminal, the latter presumably due to binding induced conformational change of the enzyme. The line broadening was observed for Tyr65, Gly69, Thr83, His177, Tyr199, Ser202, Ser205, Ala206, His238, and Gly239 throughout at least one of the titrations, indicating the presence of a process with intermediate rate exchange, possibly related to the binding of the inhibitor and/or a binding induced conformational change.

Comparable chemical shift perturbations were observed for NDM-1 upon titrations with $\mathbf{5 i} \mathbf{- 1}, \mathbf{5 b} \mathbf{- 1}, \mathbf{5 c} \mathbf{- 1}$ and $\mathbf{5 j} \mathbf{- 1}$ to that observed for $5 \mathrm{i}-2$ ( $R^{2} \sim 0.81-0.84$, Fig. 6), a compound that inhibits both NDM-1 and VIM-2, and revealed that the $\alpha$-amino-phosphonate inhibitors bind to the same binding cleft and have comparable binding modes. Due to the structural differences leading to certain amino acid residues being involved in the binding of one but not of another inhibitor, the chemical shift perturbations are not expected to show perfect correlation. However, a reasonable correlation reveals the involvement of certain amino acid residues in the binding of all inhibitors studied herein. We also observed correlation of the chemical perturbations of

Table 1 The NDM-1 and VIM-2 inhibitory activities of compounds 5a-l

| No. | Compound | Stereochemistry ${ }^{a}$ | Enzyme $\mathrm{IC}_{50}{ }^{c}(\mu \mathrm{M})$ |  |
| :---: | :---: | :---: | :---: | :---: |
|  |  |  | NDM-1 | VIM-2 |
| $\begin{aligned} & 5 a-1 \\ & 5 a-2 \end{aligned}$ |  | $\begin{aligned} & S R / R S \\ & R R / S S \end{aligned}$ | $\begin{aligned} & \text { N.I. }{ }^{b} \\ & \text { N.I. } \end{aligned}$ | $\begin{aligned} & 353 \pm 62 \\ & \text { N.I. } \end{aligned}$ |
| $\begin{aligned} & 5 b-1 \\ & 5 b-2 \end{aligned}$ |  | $\begin{aligned} & S R / R S \\ & R R / S S \end{aligned}$ | $\begin{aligned} & 230 \pm 28 \\ & \text { N.I. } \end{aligned}$ | $\begin{aligned} & 118 \pm 60 \\ & 7.2 \pm 0.4 \end{aligned}$ |
| $\begin{aligned} & 5 c-1 \\ & 5 c-2 \end{aligned}$ |  | $\begin{aligned} & S R / R S \\ & R R / S S \end{aligned}$ | $\begin{aligned} & 219 \pm 14 \\ & \text { N.I. } \end{aligned}$ | $\begin{aligned} & 33 \pm 3 \\ & 14.6 \pm 2.3 \end{aligned}$ |
| 5d-1 <br> 5d-2 |  | $\begin{aligned} & S R / R S \\ & R R / S S \end{aligned}$ | $\begin{aligned} & \text { N.I. } \\ & \text { N.I. } \end{aligned}$ | $\begin{aligned} & 244 \pm 35 \\ & 4.5 \pm 0.8 \end{aligned}$ |
| $\begin{aligned} & 5 e-1 \\ & 5 e-2 \end{aligned}$ |  | $\begin{aligned} & S R / R S \\ & R R / S S \end{aligned}$ | $\begin{aligned} & 76 \pm 12 \\ & \text { N.I. } \end{aligned}$ | $\begin{aligned} & 69.7 \pm 26.2 \\ & 46.1 \pm 15.6 \end{aligned}$ |
| $\begin{aligned} & 5 f-1 \\ & 5 f-2 \end{aligned}$ |  | $\begin{aligned} & S R / R S \\ & R R / S S \end{aligned}$ | N.I. <br> N.I. | $\begin{aligned} & 37 \pm 3 \\ & 18.8 \pm 0.5 \end{aligned}$ |
| $\begin{aligned} & 5 g-1 \\ & 5 g-2 \end{aligned}$ |  | $\begin{aligned} & S R / R S \\ & R R / S S \end{aligned}$ | $\begin{aligned} & 46.7 \pm 9.7 \\ & 46.8 \pm 5.5 \end{aligned}$ | $\begin{aligned} & 45 \pm 5 \\ & 4.1 \pm 2.1 \end{aligned}$ |
| $\begin{aligned} & 5 h-1 \\ & 5 h-2 \end{aligned}$ |  | $\begin{aligned} & S R / R S \\ & R R / S S \end{aligned}$ | N.I. <br> N.I. | $\begin{aligned} & 40.6 \pm 3.9 \\ & 11.6 \pm 4.7 \end{aligned}$ |
| $\begin{aligned} & 5 \mathrm{i}-1 \\ & 5 \mathrm{i}-2 \end{aligned}$ |  | $\begin{aligned} & S R / R S \\ & R R / S S \end{aligned}$ | $\begin{aligned} & 296 \pm 87 \\ & 271 \pm 34 \end{aligned}$ | $\begin{aligned} & \text { N.I. } \\ & 328 \pm 116 \end{aligned}$ |
| $\begin{aligned} & 5 \mathbf{j}-1 \\ & 5 \mathbf{j}-2 \end{aligned}$ |  | $\begin{aligned} & R S / S R \\ & R R / S S \end{aligned}$ | $\begin{aligned} & 506 \pm 333 \\ & \text { N.I. } \end{aligned}$ | $\begin{aligned} & 319 \pm 69 \\ & \text { N.I. } \end{aligned}$ |
| 5k-1 <br> 5k-2 |  | $\begin{aligned} & S R / R S \\ & R R / S S \end{aligned}$ | $\begin{aligned} & \text { N.I. } \\ & 22.4 \pm 6.3 \end{aligned}$ | $\begin{aligned} & 56.6 \pm 1.0 \\ & 28.7 \pm 10.1 \end{aligned}$ |
| $\begin{aligned} & 51-1 \\ & 51-2 \end{aligned}$ |  | $\begin{aligned} & R R / S S \\ & S R / R S \end{aligned}$ | $\begin{aligned} & 7.9 \pm 1.6 \\ & 27.8 \pm 21.0 \end{aligned}$ | $\begin{aligned} & \text { N.I. } \\ & 94.4 \pm 5.8 \end{aligned}$ |

${ }^{a}$ The compounds were separated as diastereomeric pairs, which are shown here with the stereocenters being highlighted with *. ${ }^{b}$ N.I. means no enzyme inhibitory activity was observed. ${ }^{c} \mathrm{IC}_{50}$ values were calculated from 3 parallel measurements.
the ${ }^{1} \mathrm{H},{ }^{15} \mathrm{~N}$ resonances of the amino acid residues of VIM-2 upon titration with $\mathbf{5 b} \mathbf{- 1 / 5 b} \mathbf{- 2}, \mathbf{5 c}-\mathbf{1}, \mathbf{5 a} \mathbf{- 1}, \mathbf{5 c}-\mathbf{2}$ and $\mathbf{5 j} \mathbf{- 1}$ to that observed for 5i-2 (Fig. 7).

By superimposing the chemical shift perturbations observed for NDM-1 and VIM-2 upon titration with $\mathbf{5 b} \mathbf{- 1}$, the amino acid residues important for binding of these compounds to both
enzymes were identified (Fig. 8). Hence, we observed that His177 and His238 of VIM-2, and Asp124 of NDM-1, holding the zinc ions of the catalytic sites, showed large chemical shift perturbations. Moreover, Phe70/Tyr65 (NDM-1/VIM-2) of the lipophilic loop 3 and Lys214/Arg203 (NDM-1/VIM-2) of loop 10 are also pointed out as key residues for inhibitor binding.

Research Article


Fig. 3 Normalized relative activity of VIM-2 encapsulated in outer membrane vesicles in absence (control) and presence of compounds. Error bars represent S.D. of 3 measurements. * indicates ambiguous readout due to compound precipitation. Compound concentrations are specified in Table S189. $\dagger$

Table 2 NDM-1 and VIM-2 binding affinities as determined via NMR chemical shift perturbation experiments ${ }^{a}$

| $\frac{\text { Protein }}{}$ |  |  |
| :--- | :--- | :--- |
| Compound | NDM-1 $K_{\mathrm{d}}[\mathrm{mM}]$ | VIM-2 $K_{\mathrm{d}}[\mathrm{mM}]$ |
| $\mathbf{5 a - 1}$ | n.d. | $0.9 \pm 0.1$ |
| $\mathbf{5 b - 1}$ | $2.0 \pm 0.1$ | $0.4 \pm 0.04$ |
| $\mathbf{5 b - 2}$ | n.d. | $1.4 \pm 0.1$ |
| $\mathbf{5 c - 1}$ | $3.1 \pm 0.3$ | $0.4 \pm 0.03$ |
| $\mathbf{5 c - 2}$ | n.d. | n.d. |
| $\mathbf{5 i - 1}$ | $0.5 \pm 0.1$ | $1.5 \pm 0.2$ |
| $\mathbf{5 i} \mathbf{i} \mathbf{2}$ | $1.4 \pm 0.1$ | $1.1 \pm 0.1$ |
| $\mathbf{5 j - 1}$ | $2.2 \pm 0.2$ | $0.9 \pm 0.1$ |
| ${ }^{a}$ For details, see section S8 of the ESI; $\dagger$ n.d., not determined. |  |  |

Fig. 4 The chemical shift perturbation of the backbone amides (CSP) of ${ }^{15} \mathrm{~N}$-labelled NDM -1 (pdb: 6 O 3 R ) upon addition of 16 equivalents of
$5 \mathrm{~b}-1 / 2,5 \mathrm{c}-1 / 2,5 \mathrm{i}-2$ and $5 \mathrm{j}-2$ (grey). CSPs greater than the population of ${ }^{15} \mathrm{~N}$-labelled NDM-1 (pdb: 6 O 3 R ) upon addition of 16 equivalents of
$5 \mathrm{~b}-1 / 2,5 \mathrm{c}-1 / 2,5 \mathrm{i}-2$ and $5 \mathrm{j}-2$ (grey). CSPs greater than the population mean and one standard deviation $(\mu+1 \sigma)$ were considered significant and are colored. Amino acid residues highlighted in blue were and are colored. Amino acid residues highlighted in blue were
influenced by binding of each inhibitor and thus are essential for binding. Those highlighted in purple were affected in the majority of the titrations.
${ }^{a}$ For details, see section S8 of the ESI; $\dagger$ n.d., not determined.



Fig. 5 The chemical shift perturbation (CSP) of the backbone amides of ${ }^{15} \mathrm{~N}$-labelled VIM-2 (pdb: 6O5T) upon titration with ligands $5 \mathrm{~b}-1 / 2$, $5 c-1 / 2,5 i-1 / 2$ and $5 \mathrm{j}-1$ (grey). CSPs greater than the population mean plus one standard deviation $(\mu+1 \sigma)$ were considered significant and are colored as for Fig. 4.

The sequestration of $\mathrm{Zn}^{2+}$ ions from NDM-1 or VIM-2 were reported to cause extensive changes in their ${ }^{1} \mathrm{H},{ }^{15} \mathrm{~N}$ HSQC spectrum. ${ }^{44}$ Importantly, such large spectral alterations have not been observed at the titrations described above, which indicates that $\mathbf{5 a - j}$ do not act by metal sequestration, but rather by ligand replacement in the active site.

## Computational docking

In order to visualize the binding of the studied inhibitors, we performed flexible docking for the compounds that have been selected for NMR titration above, using the software Glide (Schrödinger Inc.). The calculations were started from the experimental protein structures pdb $603 R^{32}$ and pdb $6 \mathrm{O} 5 \mathrm{~T},{ }^{32}$ followed by MM-GBSA rescoring, which was selected as it is known as the quickest force-field based method for


Fig. 6 The chemical shift perturbation of NDM-1 upon titration with $5 \mathrm{~b}-1,5 \mathrm{c}-1,5 \mathrm{i}-1$, and $5 \mathrm{j}-1$ correlates to that of $5 \mathrm{i}-2$ with $R^{2} \sim 0.81-$ 0.84 , suggesting comparable binding modes of all ligands. For details, see Fig. S175-S178, ESI. $\dagger$


Fig. 7 The chemical shift perturbation of VIM-2 upon addition of 16 equivalents of $5 \mathrm{~b}-1 / 5 \mathrm{~b}-2,5 \mathrm{c}-1,5 \mathrm{a}-1,5 \mathrm{c}-2$ and $5 \mathrm{j}-1$ correlate to that observed upon titration with $5 \mathrm{i}-2\left(R^{2} \sim 0.61-0.82\right)$, suggesting comparable binding modes for the ligands. For details, see Fig. S179S184, ESI. $\dagger$


Fig. 8 Chemical shift perturbations observed for NDM-1 (blue, pdb: 6NY7) and VIM-2 (pink and orange, pdb: 6O5T), shown superimposed, upon titration with 5b-1 revealed the amino acid residues that are important for $\alpha$-aminophosphonate-type inhibitor binding for both metallo- $\beta$-lactamases. The zinc ions are shown as blue spheres.
calculation of the free energy of binding. ${ }^{53}$ Ligands were prepared with LigPrep (Schrödinger Inc.), generating all possible stereoisomers and reasonable protonation states at pH $7 \pm 2$. Factors such as tautomerisation, metal binding, and the presence or absence of salts were also taken into account. To avoid bias or forced positions, docking was performed up to 40 times for each generated state for each ligand without experimental restraints. The resulting docking modes were evaluated in comparison to the experimentally observed ${ }^{1} \mathrm{H},{ }^{15} \mathrm{~N}$ NMR chemical shift perturbations (see the $\mathrm{ESI} \dagger$ ) and those compatible with the experimental data were kept. Next, they were ranked according to their binding free energies $(\Delta G)$ obtained by MM-GBSA rescoring, and the most feasible binding pose was selected. The stronger binding of the $R S$ as compared to the $S R$, and of the $R R$ as compared to
the $S S$ diastereomers of the studied inhibitors to both VIM-2 and NDM-1 (Tables S8 and S9, ESI $\dagger$ ) was observed, as a general trend. This suggests that these stereoisomers ( $R S, R R$ ) are primarily responsible for the enzyme inhibitory activities are shown in Table 1. The superposition of the binding modes (Fig. 4, VIM-2, and Fig. 5, NDM-1, pdb coordinates are provided as ESI $\dagger$ ) indicates that the $\alpha$-aminophosphonatetype inhibitors coordinate Zn1 of both NDM-1 (Fig. 9) and VIM-2 (Fig. 10) via an oxygen of the phosphonic acid. This computational observation is corroborated by the line broadening of the ${ }^{1} \mathrm{H},{ }^{15} \mathrm{~N}$ HSQC signal of His177 that directly coordinates $\mathrm{Zn}-1$, and is in agreement with our design (inhibition by a tetrahedral phosphonate). Moreover, another oxygen of the phosphonic acid forms a hydrogen bond with Asn 220 of NDM-1 or a salt bridge to Arg203 of VIM-2. The piperidine nitrogen of the inhibitors is involved in a $\pi$-cation interaction ${ }^{53,54}$ with Phe70 of NDM-1 or Tyr65 in case of VIM2 , whereas the thiophene ring of the inhibitors is oriented towards loop 10 of the enzyme. The binding poses of the inhibitors are similar, as revealed by the average $\mathrm{RMSD}^{55}$ of $2.6 \pm 0.1 \AA$ for NDM-1, and $2.8 \pm 0.4 \AA$ for VIM-2 binding (Fig. 4 and 5). The latter similarity is expected for structurally related inhibitors, and is supported by the good correlation of the NMR chemical shift perturbations observed for the same protein upon titration with different $\alpha$-aminophosphonates (Fig. 6 and 7). Overall, the energetically preferred binding modes (Fig. 9 and 10) are in good agreement with the NMR chemical shift perturbations.

Pemberton et al. ${ }^{32}$ have shown that the binding pose of phosphonate-bearing coumarin inhibitors of NDM-1 may be pH dependent. At pH 7.5 , the active site hydroxide is retained, and the phosphonate group interacts with this hydroxide and Zn 2 , rather than with both Zn (II) ions as at lower pH . The NMR titrations reported herein were performed at pH 7 , however, based on the ${ }^{15} \mathrm{~N}$ NMR chemical shift perturbation data the possibility that $\mathbf{5 a - 1}$ could bind via a bridging hydroxide ion cannot be excluded. The binding poses of phosphonates presented herein and of the coumarin phosphonate derivatives of Pemberton, an example being depicted in Fig. 11, show noticeable similarities.

Comparing our data, we note that the ligand-induced chemical shift perturbation observed upon titration with 5a-1 showed a wider spread of chemical shift changes for VIM-2 (Fig. 6) as compared to NDM-1 (Fig. 7), and that the binding modes of the inhibitors to VIM-2 are being slightly more dissimilar (Fig. 4) as compared to those of NDM-1 (Fig. 5). This may indicate somewhat higher flexibility of VIM-2 as compared to NDM-1, which may allow it to offer a larger degree of substrate promiscuity. This is corroborated by the observation of a larger number of $\alpha$-aminophosphonate inhibitors showing activity against VIM-2 than NDM-1 in this study.

## Conclusions

We designed and synthesized a series of $\alpha$-aminophosphonate-type inhibitors for NDM-1 and VIM-2.


Fig. 9 The interactions of (RS)-5c-1 with the active site of NDM-1 (pdb: 6NY7), as determined by computational docking directed by NMR (ligand induced protein chemical shift perturbation). The inhibitor is shown in green, the amino acids involved in the interaction in blue whereas the zinc(॥) ions in cyan. The lowest $\Delta G_{\text {bind }}$ docked positions of all inhibitor candidates (pdb) are provided as ESI, uploaded to the open access repository Zenodo (DOI: https://doi.org/10.5281/zenodo.6971841).


Fig. 10 The interactions of (RS)-5c-1 with the active site of VIM-2 (pdb: 6O5T), as determined by computational docking directed by NMR (ligand induced protein chemical shift perturbation). The inhibitor is shown in green, the amino acids involved in the interaction in blue whereas the zinc(॥) ions in cyan. The lowest $\Delta G_{\text {bind }}$ docked positions of all inhibitor candidates (pdb) are provided as ESI, uploaded to the open access repository Zenodo (DOI: https://doi.org/10.5281/zenodo.6971841).

Their evaluation in enzyme assays revealed several compounds to possess $<10 \mu \mathrm{M}$ inhibitory activity ( $\mathrm{IC}_{50}$ ) against these metallo- $\beta$-lactamases, while showing no cytotoxicity, indicating their potential for further development. These compounds were designed to enhance the activity of $\beta$-lactam antibiotics, such as meropenem. Being the first members of a new type of inhibitors, they showed moderate to low inhibitory potency and are obviously not directly applicable in the clinical treatment of NDM-1 and VIM-2 expressing bacterial strains. As none of the


Fig. 11 Superimposition of the computationally predicted binding mode of (RS)-5c-1 (green) to NDM-1 (grey) with the X-ray structure of [(5-methoxy-7-methyl-2-oxo-2H-chromen-4-yl)methyl]-phosphonic acid (magenta) in complex with NDM-1 (blue, PDB code 6D1E). ${ }^{32}$
enantiomers of 5a-l can be expected to have nM activity, it was not worth to separate or selectively generate their isomers. The development of stereoselective synthetic routes to $\alpha$-aminophosphonates will therefore be the task for upcoming studies at the identification of more active members of this structural group. This may be achieved, for instance, by the structural modifications of the piperidine moiety of 5a-l. Hence, whereas these compounds aren't clinically applicable, their future structural modifications may yield compounds applicable for the treatment of NDM-1 and VIM-2 expressing bacterial strains. The inhibitor binding sites were indicated by ${ }^{1} \mathrm{H} /{ }^{15} \mathrm{~N}$ NMR chemical shift perturbation experiments, evaluating the data of selected compounds in the series. Comparable magnitudes of chemical shift perturbation on the same amino acid residues upon titration with differently substituted inhibitors indicated that the $\alpha$-aminophosphonic acid derivatives have a common binding site on both enzymes. Computational docking revealed that the $R S$ and $R R$ stereoisomers of the synthesized inhibitors are primarily responsible for NDM-1 and VIM-2 binding ( $K_{\mathrm{d}}=0.4-3.1 \mathrm{mM}$ ). A stronger correlation of the inhibitor induced chemical shift perturbations on NDM-1 as compared to that observed for VIM-2 may indicate a lower substrate specificity of VIM-2. This was corroborated by the observation of VIM-2 binding a larger number of phosphonates with more different binding poses, as
determined by NMR guided computational docking. The amino acid residues Phe70, Asp124, Lys211, Ser191 and Ser249 for NDM-1, and Tyr65, His177, Tyr199, Arg203, Ser205, and His238 for VIM-2 Phe70/Tyr65 (VIM-2/NDM-1) were suggested to be necessary for inhibitor binding. This structural knowledge is expected to be useful for the development of future wide spectrum metallo- $\beta$-lactamase inhibitors. Accordingly, the compounds presented herein may be useful in fragment-based drug discovery, in addition to possibly providing a starting point for the development clinically applicable metallo- $\beta$-lactamase inhibitors.

## Experimental methods

## General methods

Reagents and reactants for synthesis were purchased from commercial suppliers (Sigma-Aldrich, Merck, Fluorochem, VWR, Arcos, Fischer Scientific) and were used without further purification. Reactions were monitored by LCMS (Agilent 1100 Series) equipped with an ESI-MS detector (Waters Micromass ZQ 2000) with a UV range of 200-600 nm , and an analytical column (Phenomenex, Gemini C18 column, $5 \mu \mathrm{~m}, 110 \AA$ A , $\varnothing 3.00 \mathrm{~mm}$, L 50 mm ) or by TLC-MS (API, Advion Express). Purification of the compounds was performed on Merck silica gel ( $0.035-0.079 \mathrm{~mm}, 5 \AA$ ) by manual column chromatography or by using Biotage Isolera One flash column chromatography system with solvent gradients. Additionally, diastereomers were separated using preparative RP-HPLC (VWR LaPrep P110) with single wavelength detection, using a Kromasil C8 column ( $10 \mu \mathrm{~m}$, $100 \AA, \varnothing 39 \mathrm{~mm}, \mathrm{~L} 250 \mathrm{~mm}$ ), and chiral column Phenomenex Lux Amylose-1 ( $5 \mu \mathrm{~m}, 1000 \AA, \varnothing 21.2 \mathrm{~mm}, \mathrm{~L}$ 250 mm ) and gradients of $\mathrm{CH}_{3} \mathrm{CN} / \mathrm{H}_{2} \mathrm{O}$ with/without $0.1 \%$ formic acid as mobile phase at $10 / 15 \mathrm{~mL} \mathrm{~min}^{-1}$ flow rate. NMR spectra of the synthetic intermediates were recorded at room temperature on a Varian Agilent MR400-DD2 with OneNMR probe ( ${ }^{1} \mathrm{H}, 400 \mathrm{MHz}$ ), and a Bruker Avance Neo with TXO cryogenic probe $\left({ }^{1} \mathrm{H}, 500 \mathrm{MHz}\right)$. In order to assign diastereomers and rotamers, diastereomers were separated by preparative chiral HPLC. Protein-related spectra (series of ${ }^{1} \mathrm{H}-{ }^{15} \mathrm{~N}$ HSQC spectra was used for the CSP experiment) were recorded in $90 \% \mathrm{H}_{2} \mathrm{O} / 10 \% \mathrm{D}_{2} \mathrm{O}+2.5 \%$ DMSO- $d_{6}$ with a protein concentration of 0.25 mM at a Bruker Avance Neo $\left({ }^{1} \mathrm{H}, 600 \mathrm{MHz}\right)$ with TCI cryogenic probe, using 3 mm NMR tubes.

## Crystallography

Crystallization of $\mathbf{4 a - 1}$ was done by vapour diffusion or by layering. Layering dioxane over $\mathrm{H}_{2} \mathrm{O}$ at $12{ }^{\circ} \mathrm{C}$ provided single crystals suitable for X-ray diffraction analysis. Vapour diffusion was performed with THF/cyclohexane or methylformate $/ n$-pentane at $-20^{\circ} \mathrm{C}, 12{ }^{\circ} \mathrm{C}$, or at r.t. The crystallographic data were collected with either a Rigaku SuperNova single-source diffractometer equipped with an Eos CCD detector using mirror-monochromated Mo $\mathrm{K} \alpha$ ( $\lambda=$ $0.71073 \AA$ ) or a Rigaku SuperNova dual wave-length
diffractometer equipped with an Atlas CCD area detector with $\mathrm{Cu}-\mathrm{K} \alpha$ radiation $(\lambda=1.54184 \AA)$. Data collection, reduction, and Gaussian or analytical face-index based absorption correction were performed with CrysAlisPro. ${ }^{56}$ Structures were solved using SHELXT ${ }^{57}$ and refined by full-matrix leastsquares on $F^{2}$ using SHELXL. ${ }^{58}$ In all data, anisotropic displacement parameters were introduced for all atoms except hydrogens, which were calculated or refined into their ideal positions using isotropic displacement parameters of 1.2 or 1.5 times that of the host atom. The figures were made using the Mercury program. ${ }^{59}$ The structures have been deposited to CCDC with codes 2203659 (4a-2), 2203660 (4b-2), 2203661 (4c-2), 2203662 (4d-2), 2203663 and 2203670 (4e-2), 2203664 (4f-1), 2203665 ( $\mathbf{4 g}-\mathbf{1}$ ), 2203666 (4h-2), 2209101 (4i-1), 2203667 (4j-1), 2203668 ( $\mathbf{4 k} \mathbf{k}$ ), and 2203669 (41-1). For a detailed description of the crystallographic data, see section S5 of the ESI. $\dagger$

## Synthetic procedures

The synthetic route towards $\mathbf{5 a - 1} \mathbf{- 1 / 2}$ is summarized in Scheme 3, and the details are given below.

## tert-Butyl 2-((benzylamino)(diethoxyphosphoryl)methyl)-piperidine-1-carboxylate (2)

Compound 1 ( $3.55 \mathrm{~g}, 16.7 \mathrm{mmol}$ ), benzylamine ( 1.84 mL , $16.8 \mathrm{mmol}, 1.0$ equiv.) and diethyl phosphite ( $2.19 \mathrm{~mL}, 17.0$ mmol, 1.0 equiv.) were dissolved in absolute EtOH ( 17 mL ). $\mathrm{HfCl}_{4}(107 \mathrm{mg}, 2 \mathrm{~mol} \%)$ was added in one portion and the reaction was stirred at $60{ }^{\circ} \mathrm{C}$ for 12 h under an inert atmosphere. The solvent was removed under reduced pressure and the crude product was purified by $\mathrm{FCC}\left(\mathrm{SiO}_{2}\right.$, gradient EtOAc in hexane $80 \%$ to $100 \%$ ), $R_{\mathrm{f}}$ (EtOAc: Hex $4: 1$ ) $=0.47$. The product was obtained as a pale yellow solid (2.36 $\mathrm{g}, 5.35 \mathrm{mmol}, 32 \%)$. HRESIMS $\mathrm{m} / \mathrm{z} 441.2529[\mathrm{M}+\mathrm{H}]^{+}$ (441.2513 calcd for $\mathrm{C}_{22} \mathrm{H}_{38} \mathrm{~N}_{2} \mathrm{O}_{5} \mathrm{P}$ ).

## Compound 2, diastereomer 1 (rotamer ratio 1:1)

${ }^{1} \mathrm{H}$ NMR ( $500 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta 7.31-7.27(\mathrm{~m}, 4 \mathrm{H}, 11-\mathrm{H}, 12-\mathrm{H}$, $14-\mathrm{H}, 15-\mathrm{H}), 7.22$ (m, 1H, 13-H), 4.53 (br. s, 1H, 2-H), 4.26$4.16\left(\mathrm{~m}, 4 \mathrm{H}, 1-\mathrm{P}\left(\mathrm{OCH}_{2}\right)_{2}\right), 4.07\left(\mathrm{~d},{ }^{2} J=12.9 \mathrm{~Hz}, 1 \mathrm{H}\right.$, part A of AB, $9-\mathrm{H}$ ), 3.98 (br. d, $J=14.1 \mathrm{~Hz}, 1 \mathrm{H}, 4-\mathrm{H}$ ), 3.78 (d, ${ }^{2} J=12.9$ $\mathrm{Hz}, 1 \mathrm{H}$, part B of AB, $9-\mathrm{H}), 3.24\left(\mathrm{dd},{ }^{2} J_{H P}=10.6 \mathrm{~Hz}, J=10.6\right.$ $\mathrm{Hz}, 1 \mathrm{H}, 1-\mathrm{H}), 2.27$ (m, 1H, 4-H), 2.14 (br. d, $J=13.8 \mathrm{~Hz}, 1 \mathrm{H}$, $7-\mathrm{H}), 1.71-1.42\left(\mathrm{~m}, 5 \mathrm{H}, 5-\mathrm{H}_{2}, 6-\mathrm{H}_{2}, 7-\mathrm{H}\right), 1.38(\mathrm{~s}, 1 \mathrm{H}, 9 \mathrm{H}$, $\left.2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{OC}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), 1.37-1.32\left(\mathrm{~m}, 6 \mathrm{H}, 1-\mathrm{P}\left(\mathrm{OCH}_{2} \mathrm{CH}_{3}\right)_{2}\right) .{ }^{13} \mathrm{C}$ NMR ( $\left.126 \mathrm{MHz}, \mathrm{CDCl}_{3}\right) \delta 155.5\left(2-\mathrm{N}-\mathrm{C}_{\mathrm{q}}(\mathrm{O})-\mathrm{O}\right), 140.0\left(10-\mathrm{C}_{\mathrm{q}}\right)$, 128.7 ( $11-\mathrm{CH}, 15-\mathrm{CH}$ or $12-\mathrm{CH}, 14-\mathrm{CH}$ ), 128.5 ( $11-\mathrm{CH}, 15-\mathrm{CH}$ or $12-\mathrm{CH}, 14-\mathrm{CH})$, $127.3(13-\mathrm{CH})$, $79.9\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{O}-\mathrm{C}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right)$, $62.6\left(\mathrm{~d},{ }^{2} J_{C P}=7.4 \mathrm{~Hz}, 1-\mathrm{POCH}_{2}\right), 62.1$ (br. s., $\left.1-\mathrm{POCH}_{2}\right), 53.8$ $\left(9-\mathrm{CH}_{2}\right), 53.4\left(\mathrm{~d},{ }^{1} J_{C P}=152.4 \mathrm{~Hz}, 1-\mathrm{CH}\right), 51.0\left(\mathrm{~d},{ }^{2} J_{C P}=13.8\right.$ $\mathrm{Hz}, 2-\mathrm{CH}), 38.9\left(4-\mathrm{CH}_{2}\right), 28.6\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{OC}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), 27.1$ $\left(7-\mathrm{CH}_{2}\right), 25.2\left(5-\mathrm{CH}_{2}\right), 19.5\left(6-\mathrm{CH}_{2}\right), 16.8\left(\mathrm{~d},{ }^{3} J_{C P}=5.4 \mathrm{~Hz}\right.$, 1- $\mathrm{POCH}_{2} \mathrm{CH}_{3}$ ), $16.8\left(\mathrm{~d},{ }^{3} J_{C P}=4.8 \mathrm{~Hz}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right) .{ }^{31} \mathrm{P}$ NMR $\left(162 \mathrm{MHz}, \mathrm{CDCl}_{3}\right) \delta 27.02$.

## Compound 2, diastereomer 2

${ }^{1} \mathrm{H}$ NMR ( $500 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta 7.37-7.27(\mathrm{~m}, 4 \mathrm{H}, 11-\mathrm{H}, 12-\mathrm{H}$, $14-\mathrm{H}, 15-\mathrm{H}), 7.25(\mathrm{~m}, 1 \mathrm{H}, 13-\mathrm{H}), 4.33$ (ddd, $J=13.7,4.4,4.4$ $\mathrm{Hz}, 1 \mathrm{H}, 2-\mathrm{H}), 4.22-4.07\left(\mathrm{~m}, 4 \mathrm{H}, 1-\mathrm{P}\left(\mathrm{OCH}_{2}\right)_{2}\right), 4.06\left(\mathrm{dd},{ }^{2} J=\right.$ $12.7 \mathrm{~Hz}, J=1.7 \mathrm{~Hz}, 1 \mathrm{H}$, part A of AB, $9-\mathrm{H}), 3.98(\mathrm{~m}, 1 \mathrm{H}, 4-\mathrm{H})$, $3.86\left(\mathrm{dd},{ }^{2} J=12.7 \mathrm{~Hz}, J=2.2 \mathrm{~Hz}, 1 \mathrm{H}\right.$, part B of $\left.\mathrm{AB}, 9-\mathrm{H}\right), 3.33$ (dd, $J=14.0,8.7 \mathrm{~Hz}, 1 \mathrm{H}, 1-\mathrm{H}), 2.96(\mathrm{dd}, J=11.8,11.8 \mathrm{~Hz}, 1 \mathrm{H}$, $4-\mathrm{H}), 2.09(\mathrm{~m}, 1 \mathrm{H}, 7-\mathrm{H}), 1.59-1.37\left(\mathrm{~m}, 5 \mathrm{H}, 5-\mathrm{H}_{2}, 6-\mathrm{H}_{2}, 7-\mathrm{H}\right)$, $1.44\left(\mathrm{~s}, 9 \mathrm{H}, 2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{OC}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), 1.34(\mathrm{t}, J=7.1 \mathrm{~Hz}, 3 \mathrm{H}$, 1- $\mathrm{POCH}_{2} \mathrm{CH}_{3}$ ), $1.33\left(\mathrm{t}, J=7.0 \mathrm{~Hz}, 3 \mathrm{H}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right) .{ }^{13} \mathrm{C}$ NMR $\left(126 \mathrm{MHz}, \mathrm{CDCl}_{3}\right) \delta 154.8\left(2-\mathrm{N}-\mathrm{C}_{\mathrm{q}}(\mathrm{O})-\mathrm{O}\right), 140.2\left(10-\mathrm{C}_{\mathrm{q}}\right), 128.7$ ( $11-\mathrm{CH}, 15-\mathrm{CH}$ or $12-\mathrm{CH}, 14-\mathrm{CH}), 128.5(11-\mathrm{CH}, 15-\mathrm{CH}$ or $12-$ $\mathrm{CH}, 14-\mathrm{CH}), 127.28(13-\mathrm{CH}), 79.4\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{O}-\mathrm{C}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right)$, $61.9\left(\mathrm{~d},{ }^{2} J_{C P}=8.1 \mathrm{~Hz}, 1-\mathrm{POCH}_{2}\right), 61.8\left(\mathrm{~d},{ }^{2} J_{C P}=8.1 \mathrm{~Hz}\right.$, $\left.1-\mathrm{POCH}_{2}\right), 54.3\left(\mathrm{~d},{ }^{1} J_{C P}=139.6 \mathrm{~Hz}, 1-\mathrm{CH}\right), 53.3\left(9-\mathrm{CH}_{2}\right), 51.7$ (br. s, $2-\mathrm{CH}$ ), 40.2 (br. s, $\left.4-\mathrm{CH}_{2}\right), 28.6\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{OC}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right)$, $24.8\left(5-\mathrm{CH}_{2}\right), 24.7\left(\mathrm{~d},{ }^{3} J_{C P}=7.9 \mathrm{~Hz}, 7-\mathrm{CH}_{2}\right), 19.4\left(6-\mathrm{CH}_{2}\right), 16.7$ $\left(\mathrm{d},{ }^{3} J_{C P}=5.8 \mathrm{~Hz}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right), 16.7\left(\mathrm{~d},{ }^{3} J_{C P}=5.7 \mathrm{~Hz}\right.$, 1- $\mathrm{POCH}_{2} \mathrm{CH}_{3}$ ). ${ }^{31} \mathrm{P}$ NMR ( $162 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta 27.35$.

## tert-Butyl 2-(amino(diethoxyphosphoryl)methyl)piperi-dine-1carboxylate (3)

Compound $2(2.36 \mathrm{~g}, 5.35 \mathrm{mmol})$ and $\mathrm{Pd}(\mathrm{OH})_{2} / \mathrm{C}, 50 \%$ wet ( $10 \% \mathrm{w} / \mathrm{w}, 1.28 \mathrm{~g}, 0.91 \mathrm{mmol}, 0.17$ equiv.) were dissolved in EtOH ( 54 mL ). A strong argon flow was used to bubble argon through the reaction mixture for 5 min . Subsequently, $\mathrm{H}_{2}$ was bubbled through the solution for 30 s and the reaction vial was equipped with an $\mathrm{H}_{2}$-filled balloon. The reaction was stirred at r.t. for 16 h . The reaction mixture was filtered through a plug of Celite and a syringe filter to remove Pd traces. The solvent was removed under reduced pressure. The product was obtained as a colorless liquid ( 1.87 g , 5.33 mmol, 99\%). HRESIMS $m / z 351.2066[\mathrm{M}+\mathrm{H}]^{+}$(351.2043 calcd for $\mathrm{C}_{15} \mathrm{H}_{32} \mathrm{~N}_{2} \mathrm{O}_{5} \mathrm{P}$ ).

## Compound 3, diastereomer 1

${ }^{1} \mathrm{H} \operatorname{NMR}\left(400 \mathrm{MHz}, \mathrm{CDCl}_{3}\right) \delta 4.32(\mathrm{~m}, 1 \mathrm{H}, 2-\mathrm{H}), 4.13(\mathrm{q}, J=$ $7.1 \mathrm{~Hz}, 2 \mathrm{H}, 1-\mathrm{POCH}_{2}$ or $\left.1-\mathrm{P}(\mathrm{OCH})_{2}\right), 4.13(\mathrm{q}, J=7.1 \mathrm{~Hz}, 2 \mathrm{H}$, $1-\mathrm{POCH}_{2}$ or $\left.1-\mathrm{P}(\mathrm{OCH})_{2}\right), 4.00(\mathrm{~m}, 1 \mathrm{H}, 4-\mathrm{H}), 3.42\left(\mathrm{dd},{ }^{1} J_{H P}=\right.$ $10.4 \mathrm{~Hz}, J=10.4 \mathrm{~Hz}, 1 \mathrm{H}, 1-\mathrm{H}), 2.89(\mathrm{dd}, J=12.1,12.1 \mathrm{~Hz}, 1 \mathrm{H}$, $4-\mathrm{H}$ ), 2.09 (br. d, $J=11.7 \mathrm{~Hz}, 1 \mathrm{H}, 7-\mathrm{H}$ ), 1.67-1.37 (m, 5H, $\left.5-\mathrm{CH}_{2}, 6-\mathrm{H}_{2}, 7-\mathrm{H}\right), 1.45\left(\mathrm{~s}, 9 \mathrm{H}, 2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{OC}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), 1.31(\mathrm{t}, J$ $\left.=6.8 \mathrm{~Hz}, 6 \mathrm{H}, 1-\mathrm{P}\left(\mathrm{OCH}_{2} \mathrm{CH}_{3}\right)_{2}\right){ }^{13} \mathrm{C}$ NMR $\left(101 \mathrm{MHz}, \mathrm{CDCl}_{3}\right) \delta$ $154.7\left(2-\mathrm{N}-\mathrm{C}_{\mathrm{q}}(\mathrm{O})-\mathrm{O}\right), 79.6\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{O}-\mathrm{C}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), 62.3$ (d, $\left.{ }^{2} J_{C P}=7.3 \mathrm{~Hz}, 1-\mathrm{POCH}_{2}\right), 62.1\left(\mathrm{~d},{ }^{2} J_{C P}=7.3 \mathrm{~Hz}, 1-\mathrm{POCH}_{2}\right), 51.9$ $\left(\mathrm{d},{ }^{2} J_{C P}=2.5 \mathrm{~Hz}, 2-\mathrm{CH}\right), 48.2\left(\mathrm{~d},{ }^{1} J_{C P}=147.0 \mathrm{~Hz}, 1-\mathrm{CH}\right), 40.3$ (br. s, $4-\mathrm{CH}_{2}$ ), $28.6\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{OC}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), 25.0\left(5-\mathrm{CH}_{2}\right), 24.8$ $\left(\mathrm{d},{ }^{3} J_{C P}=9.4 \mathrm{~Hz}, 7-\mathrm{CH}_{2}\right), 19.3\left(6-\mathrm{CH}_{2}\right), 16.7\left(\mathrm{~d},{ }^{3} J_{C P}=3.8 \mathrm{~Hz}\right.$, 1- $\mathrm{POCH}_{2} \mathrm{CH}_{3}$ ), $16.6\left(\mathrm{~d},{ }^{3} J_{C P}=4.1 \mathrm{~Hz}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right) \cdot{ }^{31} \mathrm{P}$ NMR $\left(162 \mathrm{MHz}, \mathrm{CDCl}_{3}\right) \delta 27.42$.

## Compound 3, diastereomer 2 (rotamer ratio 1:1.3)

${ }^{1} \mathrm{H}$ NMR ( $400 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta 4.36$ (br. s, $1 \mathrm{H}, 2-\mathrm{H}$ ), 4.24-4.08 $\left(\mathrm{m}, 5 \mathrm{H}, 1-\mathrm{P}\left(\mathrm{OCH}_{2}\right)_{2}, 4-\mathrm{H}\right), 3.37\left(\mathrm{dd},{ }^{2} J_{H P}=11.6 \mathrm{~Hz}, J=11.6\right.$

Hz, 1H, 1-H), 2.59 (br. dd, $J=12.7,12.7 \mathrm{~Hz}, 1 \mathrm{H}, 4-\mathrm{H}), 2.16$ (br. d, $J=13.0 \mathrm{~Hz}, 1 \mathrm{H}, 7-\mathrm{H}), 1.73-1.50\left(\mathrm{~m}, 5 \mathrm{H}, 5-\mathrm{H}_{2}, 6-\mathrm{CH}_{2}\right.$, $7-\mathrm{H}), 1.45$ (s, 9H), 1.34 (dd, $J=7.1,7.1 \mathrm{~Hz}, 3 \mathrm{H}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}$ ), 1.33 (dd, $J=7.0,7.0 \mathrm{~Hz}, 3 \mathrm{H}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}$ ). ${ }^{13} \mathrm{C}$ NMR (126 $\left.\mathrm{MHz}, \mathrm{CDCl}_{3}\right) \delta 155.7\left(2-\mathrm{N}-\mathrm{C}_{\mathrm{q}}(\mathrm{O})-\mathrm{O}\right)$, $80.0\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{O}-\mathrm{C}_{\mathrm{q}}-\right.$ $\left.\left(\mathrm{CH}_{3}\right)_{3}\right), 62.5\left(\mathrm{~d},{ }^{2} J_{C P}=7.2 \mathrm{~Hz}, 1-\mathrm{POCH}_{2}\right), 62.3\left(\mathrm{~d},{ }^{2} J_{C P}=6.9\right.$ $\left.\mathrm{Hz}, 1-\mathrm{POCH}_{2}\right), 52.4\left(\mathrm{~d},{ }^{2} J_{C P}=10.5 \mathrm{~Hz}, 2-\mathrm{H}\right), 47.4\left(\mathrm{~d},{ }^{1} J_{C P}=\right.$ $149.5 \mathrm{~Hz}, 1-\mathrm{H}), 38.8(4-\mathrm{CH}), 28.6\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{OC}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), 26.9$ $\left(7-\mathrm{CH}_{2}\right), 25.2\left(5-\mathrm{CH}_{2}\right), 19.4\left(6-\mathrm{CH}_{2}\right), 16.7\left(\mathrm{~d},{ }^{3} J_{C P}=5.5 \mathrm{~Hz}\right.$, 1-P $\left.\left(\mathrm{OCH}_{2} \mathrm{CH}_{3}\right)_{2}\right) .{ }^{31} \mathrm{P}$ NMR ( $\left.162 \mathrm{MHz}, \mathrm{CDCl}_{3}\right) \delta(27.83), 27.62$.

## General procedure A for 4a-l

The carboxylic acid (2.0 equiv.) and HATU (2.1 equiv.) were dissolved in ethyl acetate. DIPEA (4.0 equiv.) was added dropwise and the reaction mixture was stirred at r.t. for at least 10 min . The reaction mixture was added to a solution of 3 in EtOAc. The reaction was stirred at r.t. for $12-24 \mathrm{~h}$. The reaction mixture was then diluted with EtOAc and washed $(3 \times)$ with a sat. solution of $\mathrm{NaHCO}_{3}$ (or alternatively, with a mixture of $\mathrm{NaHCO}_{3}$ and brine). The aqueous layer was reextracted with EtOAc ( $2 \times$ ) and the combined organic layers were washed with brine and dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$. The solvent was removed under reduced pressure. The crude product was purified using preparative HPLC ( C 8 column, MeCN: $\mathrm{H}_{2} \mathrm{O}$ ). The product was obtained as separated diastereomers (in a total yield of $60-80 \%$ ).
tert-Butyl 2-((diethoxyphosphoryl)(2-(4-methyl-2-thioxo-2,3-dihydrothiazol-5-yl)acetamido)methyl)piperidine-1-carboxylate (4a). The synthesis was performed according to general procedure A, 3 ( $316 \mathrm{mg}, 0.902 \mathrm{mmol}$ ) and 2-mercapto-4-methyl-5-thiazoleacetic acid ( $341 \mathrm{mg}, 1.80 \mathrm{mmol}, 2.0$ equiv.) gave the total product ( $330 \mathrm{mg}, 0.632 \mathrm{mmol}, 70 \%$ ). 4a-1 was obtained as a light orange solid ( $152 \mathrm{mg}, 0.291 \mathrm{mmol}, 32 \%$ ). 4a-2 was obtained as a light orange solid ( $178 \mathrm{mg}, 0.341$ mmol, 38\%).

## Compound 4a-1, diastereomer 1

${ }^{1} \mathrm{H}$ NMR ( 500 MHz, DMSO- $d_{6}$ ) $\delta 12.93$ (s, 1H, 13-NH), 8.67 (d, $J=9.9 \mathrm{~Hz}, 1 \mathrm{H}, 1-\mathrm{NH}), 4.71\left(\mathrm{ddd},{ }^{2} J_{H P}=15.7 \mathrm{~Hz}, J=10.2,10.2\right.$ $\mathrm{Hz}, 1 \mathrm{H}, 1-\mathrm{H}), 4.42(\mathrm{~m}, 1 \mathrm{H}, 2-\mathrm{H}), 4.09-3.69(\mathrm{~m}, 5 \mathrm{H}, 4-\mathrm{H}$, $\left.1-\mathrm{P}\left(\mathrm{OCH}_{2}\right)_{2}\right), 3.46\left(\mathrm{~s}, 2 \mathrm{H}, 10-\mathrm{H}_{2}\right), 2.74(\mathrm{~m}, 1 \mathrm{H}, 4-\mathrm{H}), 2.06(\mathrm{~s}$, $3 \mathrm{H}, 15-\mathrm{C}_{\mathrm{q}} \mathrm{CH}_{3}$ ), 1.61 (br. d, $J=13.4 \mathrm{~Hz}, 1 \mathrm{H}$ ), $1.53-1.43$ (m, 3 H , $\left.5-\mathrm{H}, 6-\mathrm{H}_{2}\right), 1.40\left(\mathrm{~s}, 9 \mathrm{H}, 2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{OC}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), 1.36-1.19(\mathrm{~m}$, $2 \mathrm{H}, 5-\mathrm{H}, 7-\mathrm{H}$ ), 1.17 (dd, $J=7.1 \mathrm{~Hz}, 3 \mathrm{H}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}$ ), 1.15 (dd, $J=7.1 \mathrm{~Hz}, 3 \mathrm{H}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}$ ). ${ }^{13} \mathrm{C}$ NMR ( 126 MHz , DMSO- $d_{6}$ ) $\delta 186.9(13-\mathrm{CH}), 168.2\left(\mathrm{~d},{ }^{3} J_{C P}=4.8 \mathrm{~Hz}\right), 153.5$ $\left(2-\mathrm{N}-\mathrm{C}_{\mathrm{q}}(\mathrm{O})-\mathrm{O}\right), 135.0\left(15-\mathrm{C}_{\mathrm{q}}\right), 117.0\left(11-\mathrm{C}_{\mathrm{q}}\right), 78.7\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{O}-\right.$ $\left.\mathrm{C}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), 62.1$ (br. s, 1- $\mathrm{POCH}_{2}$ ), 61.4 (br. s, 1- $\mathrm{POCH}_{2}$ ), 50.2 (br. s, 2-CH), 43.6 (d, ${ }^{1} J_{C P}=156.7 \mathrm{~Hz}, 1-\mathrm{CH}$ ), 38.5 (br. s, $4-\mathrm{CH}), 32.3\left(10-\mathrm{CH}_{2}\right), 28.1\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{OC}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), 24.7$ (br. s, $7-\mathrm{CH}_{2}$ ), 24.5 (br. s, $5-\mathrm{CH}_{2}$ ), $18.8\left(6-\mathrm{CH}_{2}\right), 16.3\left(\mathrm{~d},{ }^{3} J_{C P}=5.5 \mathrm{~Hz}\right.$, $\left.1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right), 16.1\left(\mathrm{~d},{ }^{3} J_{C P}=6.0 \mathrm{~Hz}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right), 11.3(15-$ $\mathrm{C}_{\mathrm{q}} \mathrm{CH}_{3}$ ). ${ }^{31} \mathrm{P}$ NMR ( 162 MHz , DMSO- $d_{6}$ ) $\delta$ 22.48. HRESIMS $\mathrm{m} / \mathrm{z}$ $522.2179[\mathrm{M}+\mathrm{H}]^{+}$(calcd 522.1856 for $\mathrm{C}_{21} \mathrm{H}_{37} \mathrm{~N}_{3} \mathrm{O}_{6} \mathrm{PS}_{2}$ ).

## Compound 4a-2, diastereomer 2 (rotamer ratio 2.3:1)

${ }^{1} \mathrm{H}$ NMR ( 500 MHz, DMSO- $d_{6}$ ) $\delta 12.89$ ( $\mathrm{s}, 1 \mathrm{H}, 13-\mathrm{NH}$ ), 8.45 (d, $J=10.2 \mathrm{~Hz}, 1 \mathrm{H}, 1-\mathrm{NH}), 4.71\left(\mathrm{ddd},{ }^{2} J_{H P}=17.4 \mathrm{~Hz}, J=10.9\right.$, $10.9 \mathrm{~Hz}, 1 \mathrm{H}, 1-\mathrm{H}), 4.46(\mathrm{~m}, 1 \mathrm{H}, 2-\mathrm{H}), 4.10-3.95(\mathrm{~m}, 4 \mathrm{H}$, 1-P( $\left.\mathrm{OCH}_{2}\right)_{2}$ ), $3.74-3.65(\mathrm{~m}, 1 \mathrm{H}, 4-\mathrm{CH}), 3.47\left(\mathrm{~d},{ }^{2} J=15.9 \mathrm{~Hz}\right.$, 1 H , part A of AB, $10-\mathrm{H}$ ), $3.33\left(\mathrm{~d},{ }^{2} J=15.9 \mathrm{~Hz}\right.$, part B of $\mathrm{AB}, 10-$ H), 2.71 (dd, $J=13.7,13.7 \mathrm{~Hz}, 1 \mathrm{H}, 4-\mathrm{CH}), 2.05(\mathrm{~m}, 1 \mathrm{H}, 7-\mathrm{CH})$, $2.03\left(\mathrm{~s}, 3 \mathrm{H}, 15-\mathrm{C}_{\mathrm{q}} \mathrm{CH}_{3}\right), 1.58-1.47\left(\mathrm{~m}, 4 \mathrm{H}, 5-\mathrm{CH}, 6-\mathrm{CH}_{2}, 7-\mathrm{CH}\right)$, $1.36\left(\mathrm{~s}, 9 \mathrm{H}, 2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{OC}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), 1.24(\mathrm{~m}, 1 \mathrm{H}, 5-\mathrm{CH}), 1.19$ $\left(\mathrm{t}, J=7.1 \mathrm{~Hz}, 6 \mathrm{H}, 1-\left(\mathrm{POCH}_{2} \mathrm{CH}_{3}\right)_{2}\right) .{ }^{13} \mathrm{C}$ NMR ( 101 MHz , DMSO- $\left.d_{6}\right) \delta 187.4\left(13-\mathrm{C}_{\mathrm{q}}\right), 168.8\left(\mathrm{~d},{ }^{3} J_{C P}=4.9 \mathrm{~Hz}, 9-\mathrm{C}_{\mathrm{q}}\right)$, $154.6 \quad\left(2-\mathrm{N}-\mathrm{C}_{\mathrm{q}}(\mathrm{O})-\mathrm{O}\right), \quad 135.1 \quad\left(15-\mathrm{C}_{\mathrm{q}}\right), 117.5 \quad\left(11-\mathrm{C}_{\mathrm{q}}\right), \quad 79.1$ $\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{O}-\mathrm{C}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), 62.9\left(\mathrm{~d},{ }^{2} J_{C P}=7.0 \mathrm{~Hz}, 1-\mathrm{POCH}_{2}\right)$, $62.1\left(\mathrm{~d},{ }^{2} J_{C P}=6.7 \mathrm{~Hz}, 1-\mathrm{POCH}_{2}\right), 50.1\left(\mathrm{~d},{ }^{2} J_{C P}=13.6 \mathrm{~Hz}\right.$, $2-\mathrm{CH}), 44.1\left(\mathrm{~d},{ }^{1} J_{C P}=151.3 \mathrm{~Hz}, 1-\mathrm{CH}\right)$, $39.8\left(4-\mathrm{CH}_{2}\right), 32.5$ $\left(10-\mathrm{CH}_{2}\right), \quad 28.5\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{OC}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), \quad 26.4\left(7-\mathrm{CH}_{2}\right), \quad 25.3$ $\left(5-\mathrm{CH}_{2}\right), 19.2\left(6-\mathrm{CH}_{2}\right), 16.7\left(\mathrm{~d},{ }^{3} J_{C P}=5.5 \mathrm{~Hz}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right), 16.5$ (d, $\left.{ }^{3} J_{C P}=6.0 \mathrm{~Hz}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right), 11.7\left(15-\mathrm{C}_{\mathrm{q}} \mathrm{CH}_{3}\right) .{ }^{31} \mathrm{P}$ NMR (162 MHz, DMSO- $d_{6}$ ) $\delta 22.92$ (22.71). HRESIMS $m / z 522.2255$ [ $\mathrm{M}+$ $\mathrm{H}]^{+}$(calcd for $\mathrm{C}_{21} \mathrm{H}_{37} \mathrm{~N}_{3} \mathrm{O}_{6} \mathrm{PS}_{2}$ 522.18559). The solution tautomer was confirmed by ${ }^{1} \mathrm{H}^{15} \mathrm{~N}$ HSQC, and by X-ray diffraction.

## tert-Butyl 2-((diethoxyphosphoryl)(thiophene-2-carbox-amido) methyl)piperidine-1-carboxylate (4b)

The synthesis was performed according to general procedure A, 3 ( $211 \mathrm{mg}, 0.603 \mathrm{mmol}$ ) and thiophene-2-carboxylic acid ( $154 \mathrm{mg}, 1.19 \mathrm{mmol}, 2.0$ equiv.) gave the total product ( 212 $\mathrm{mg}, 0.460 \mathrm{mmol}, 76 \%$ ). $\mathbf{4 b} \mathbf{- 1}$ was obtained as a colorless solid ( $100 \mathrm{mg}, 0.217 \mathrm{mmol}, 36 \%$ ). 4b-2 was obtained as a colorless solid ( $112 \mathrm{mg}, 0.243 \mathrm{mmol}, 40 \%$ ).

## Compound 4b-1, diastereomer 1

${ }^{1} \mathrm{H}$ NMR ( $500 \mathrm{MHz}, \mathrm{CD}_{3} \mathrm{CN}$ ) $\delta 7.71$ (d, $\left.J=3.8 \mathrm{~Hz}, 1 \mathrm{H}, 14-\mathrm{H}\right)$, 7.64 (d, $J=5.0 \mathrm{~Hz}, 1 \mathrm{H}, 12-\mathrm{H}$ ), 7.35 (br. m, $1 \mathrm{H}, 1-\mathrm{NH}$ ), 7.12 (dd, $J=5.0,3.8 \mathrm{~Hz}, 1 \mathrm{H}, 13-\mathrm{H}), 5.04\left(\mathrm{ddd},{ }^{2} J_{H P}=15.7 \mathrm{~Hz}, J=\right.$ $10.5,10.5 \mathrm{~Hz}, 1 \mathrm{H}), 4.74(\mathrm{~m}, 1 \mathrm{H}, 2-\mathrm{H}), 4.12-3.89(\mathrm{~m}, 5 \mathrm{H}$, $\left.1-\mathrm{P}\left(\mathrm{OCH}_{2}\right)_{2}, 4-\mathrm{H}\right), 2.94(\mathrm{~m}, 1 \mathrm{H}, 4-\mathrm{H}), 1.74$ (br. d, $J=11.5 \mathrm{~Hz}$, $1 \mathrm{H}, 7-\mathrm{H}), 1.69-1.49\left(\mathrm{~m}, 4 \mathrm{H}, 5-\mathrm{H}, 6-\mathrm{H}_{2}, 7-\mathrm{H}\right), 1.44(\mathrm{~s}, 9 \mathrm{H}$, $\left.2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{OC}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), 1.34(\mathrm{~m}, 1 \mathrm{H}, 5-\mathrm{H}), 1.24(\mathrm{t}, J=7.1 \mathrm{~Hz}$, $\left.3 \mathrm{H}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right), 1.19\left(\mathrm{t}, J=7.1 \mathrm{~Hz}, 3 \mathrm{H}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right) .{ }^{13} \mathrm{C}$ NMR ( $101 \mathrm{MHz}, \mathrm{CD}_{3} \mathrm{CN}$ ) $\delta 162.3$ (d, $J=4.8 \mathrm{~Hz}, 9-\mathrm{C}_{\mathrm{q}}$ ), 155.2 $\left(2-\mathrm{N}-\mathrm{C}_{\mathrm{q}}(\mathrm{O})-\mathrm{O}\right), 139.9\left(10-\mathrm{C}_{\mathrm{q}}\right), 132.1(12-\mathrm{CH}), 129.5(14-\mathrm{CH})$, $129.0(13-\mathrm{CH}), 79.9\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{O}-\mathrm{C}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), 63.5\left(\mathrm{~d},{ }^{2} J_{C P}=8.1\right.$ $\left.\mathrm{Hz}, 1-\mathrm{POCH}_{2}\right), 63.0\left(\mathrm{~d},{ }^{2} J_{C P}=6.7 \mathrm{~Hz}, 1-\mathrm{POCH}_{2}\right), 51.1(2-\mathrm{CH})$, $45.4\left(\mathrm{~d},{ }^{1} J_{C P}=157.2 \mathrm{~Hz}, 1-\mathrm{CH}\right), 41.4\left(4-\mathrm{CH}_{2}\right), 28.6\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O})\right.$ $\left.\mathrm{OC}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), 26.0\left(\mathrm{~d},{ }^{3} J_{C P}=10.0 \mathrm{~Hz}, 7-\mathrm{CH}_{2}\right), 25.8\left(5-\mathrm{CH}_{2}\right)$, $20.1\left(6-\mathrm{CH}_{2}\right), 16.8\left(\mathrm{~d},{ }^{3} J_{C P}=5.7 \mathrm{~Hz}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right), 16.7(\mathrm{~d}$, $\left.{ }^{3} J_{C P}=6.1 \mathrm{~Hz}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right) .{ }^{31} \mathrm{P}$ NMR ( $162 \mathrm{MHz}, \mathrm{CDC}_{3} \mathrm{~N}$ ) $\delta$ (21.77), 21.67. HRESIMS $m / z 461.1854[\mathrm{M}+\mathrm{H}]^{+}$(calcd for $\mathrm{C}_{20} \mathrm{H}_{34} \mathrm{~N}_{2} \mathrm{O}_{6} \mathrm{PS} 461.18697$ ).

## Compound 4b-2, diastereomer 2 (rotamer ratio 2.3:1)

${ }^{1} \mathrm{H}$ NMR ( $500 \mathrm{MHz}, \mathrm{CD}_{3} \mathrm{CN}$ ) $\delta 7.61(\mathrm{~d}, J=4.8 \mathrm{~Hz}, 1 \mathrm{H}, 14-\mathrm{H})$, $7.55(\mathrm{~d}, J=3.8 \mathrm{~Hz}, 1 \mathrm{H}, 12-\mathrm{H}), 7.08$ (dd, $J=4.8,3.8 \mathrm{~Hz}, 1 \mathrm{H}$,
$13-$ ), 6.88 (d, $J=9.6 \mathrm{~Hz}, 1 \mathrm{H}, 1-\mathrm{NH}), 4.95$ (ddd, ${ }^{2} J_{H P}=16.0$ $\mathrm{Hz}, J=10.7,10.7 \mathrm{~Hz}, 1 \mathrm{H}, 1-\mathrm{H}), 4.60(\mathrm{~m}, 1 \mathrm{H}, 2-\mathrm{H}), 4.18-4.02$ $\left(\mathrm{m}, 4 \mathrm{H}, 1-\mathrm{P}\left(\mathrm{OCH}_{2}\right)_{2}\right), 3.84(\mathrm{dd}, J=13.5,3.4 \mathrm{~Hz}, 1 \mathrm{H}, 4-\mathrm{H})$, 2.77 (ddd, $J=13.2,13.2,1.9 \mathrm{~Hz}, 1 \mathrm{H}, 4-\mathrm{H}$ ), 2.26 (br. d, $J=$ $11.9 \mathrm{~Hz}, 1 \mathrm{H}, 7-\mathrm{H}), 1.68-1.58\left(\mathrm{~m}, 4 \mathrm{H}, 5-\mathrm{H}, 6-\mathrm{H}_{2}, 7-\mathrm{H}\right), 1.34$ $(\mathrm{m}, 1 \mathrm{H}, 5-\mathrm{H}), 1.25\left(\mathrm{t}, J=7.0 \mathrm{~Hz}, 3 \mathrm{H}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right), 1.25(\mathrm{t}, J$ $\left.=7.1 \mathrm{~Hz}, 3 \mathrm{H}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right), 1.20\left(\mathrm{~s}, 9 \mathrm{H}, 2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{OC}_{\mathrm{q}}{ }^{-}\right.$ $\left.\left(\mathrm{CH}_{3}\right)_{3}\right) .{ }^{13} \mathrm{C}$ NMR ( $101 \mathrm{MHz}, \mathrm{CD}_{3} \mathrm{CN}$ ) $\delta 162.0\left(9-\mathrm{C}_{\mathrm{q}}\right), 157.0$ $\left(2-\mathrm{N}-\mathrm{C}_{\mathrm{q}}(\mathrm{O})-\mathrm{O}\right), 139.4\left(10-\mathrm{C}_{\mathrm{q}}\right), 132.1$ (12-CH), 129.5 ( $14-\mathrm{CH}$ ), $128.9(13-\mathrm{CH}), 80.5\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{O}-\mathrm{C}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), 63.8\left(\mathrm{~d},{ }^{2} J_{C P}=\right.$ $\left.7.0 \mathrm{~Hz}, 1-\mathrm{POCH}_{2}\right), 63.3\left(\mathrm{~d},{ }^{2} J_{C P}=6.5 \mathrm{~Hz}, 1-\mathrm{POCH}_{2}\right), 51.0(\mathrm{~d}$, $\left.{ }^{3} J_{C P}=12.4 \mathrm{~Hz}, 2-\mathrm{CH}\right), 46.5\left(\mathrm{~d},{ }^{1} J_{C P}=153.3 \mathrm{~Hz}, 1-\mathrm{CH}\right), 40.8$ $\left(4-\mathrm{CH}_{2}\right), \quad 28.3 \quad\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{OC}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), \quad 26.9 \quad\left(7-\mathrm{CH}_{2}\right), \quad 25.87$ $\left(5-\mathrm{CH}_{2}\right), 19.8\left(6-\mathrm{CH}_{2}\right), 16.8\left(\mathrm{~d},{ }^{3} J_{C P}=5.7 \mathrm{~Hz}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right)$, $16.7\left(\mathrm{~d},{ }^{3} J_{C P}=6.0 \mathrm{~Hz}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right) .{ }^{31} \mathrm{P}$ NMR $(162 \mathrm{MHz}$, $\left.\mathrm{CD}_{3} \mathrm{CN}\right) \delta(21.78)$, 21.74. HRESIMS $\mathrm{m} / \mathrm{z} 461.2128[\mathrm{M}+\mathrm{H}]^{+}$ (461.18697 calcd for $\mathrm{C}_{20} \mathrm{H}_{34} \mathrm{~N}_{2} \mathrm{O}_{6} \mathrm{PS}$ ).
tert-Butyl 2-((diethoxyphosphoryl)(thiophene-3-carbox-amido) methyl)piperidine-1-carboxylate (4c)
The synthesis was performed according to general procedure A, 3 ( $211 \mathrm{mg}, 0.601 \mathrm{mmol}$ ) and thiophene-3-carboxylic acid ( $153 \mathrm{mg}, 1.20 \mathrm{mmol}, 2.0$ equiv.) gave the total product (219 $\mathrm{mg}, 0.476 \mathrm{mmol}, 79 \%$ ). $\mathbf{4 c} \mathbf{c} 1$ was obtained as a colorless solid ( $101 \mathrm{mg}, 0.219 \mathrm{mmol}, 37 \%$ ); 4c-2 was obtained as a colorless solid ( $118 \mathrm{mg}, 0.256 \mathrm{mmol}, 43 \%$ ).

## Compound 4c-1, diastereomer 1

${ }^{1} \mathrm{H} \operatorname{NMR}\left(400 \mathrm{MHz}, \mathrm{CDCl}_{3}\right) \delta 7.98(\mathrm{~d}, J=2.2 \mathrm{~Hz}, 1 \mathrm{H}, 11-\mathrm{H})$, $7.46(\mathrm{dd}, J=5.1,1.3 \mathrm{~Hz}, 1 \mathrm{H}, 13-\mathrm{H}), 7.34(\mathrm{dd}, J=5.1,3.0 \mathrm{~Hz}$, $1 \mathrm{H}, 14-\mathrm{H}), 6.96(\mathrm{~m}, 1 \mathrm{H}, 1-\mathrm{NH}), 5.08(\mathrm{~m}, 1 \mathrm{H}, 1-\mathrm{H}), 4.60(\mathrm{~m}$, $1 \mathrm{H}, 2-\mathrm{H}), 4.17-4.04\left(\mathrm{~m}, 5 \mathrm{H}, 1-\mathrm{P}\left(\mathrm{OCH}_{2}\right)_{2}, 4-\mathrm{H}\right), 3.01(\mathrm{~m}, 1 \mathrm{H}$, $4-\mathrm{H}), 1.85-1.68$ (m, 2H, $6-\mathrm{H}, 7-\mathrm{H}), 1.63-1.53(\mathrm{~m}, 3 \mathrm{H}, 5-\mathrm{H}, 6-\mathrm{H}$, $7-\mathrm{H}), 1.48\left(\mathrm{~s}, 9 \mathrm{H}, 2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{OC}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), 1.40(\mathrm{~m}, 1 \mathrm{H}, 5-\mathrm{H})$, $1.29\left(\mathrm{t}, J=7.1 \mathrm{~Hz}, 3 \mathrm{H}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right), 1.23(\mathrm{t}, J=7.1 \mathrm{~Hz}, 3 \mathrm{H}$, 1- $\mathrm{POCH}_{2} \mathrm{CH}_{3}$ ). ${ }^{13} \mathrm{C}$ NMR ( $101 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta 162.4\left(\mathrm{~d},{ }^{3} \mathrm{~J}_{C P}=\right.$ $\left.4.8 \mathrm{~Hz}, 9-\mathrm{C}_{\mathrm{q}}\right), 154.8\left(2-\mathrm{N}-\mathrm{C}_{\mathrm{q}}(\mathrm{O})-\mathrm{O}\right), 136.8\left(10-\mathrm{C}_{\mathrm{q}}\right), 129.1$ (11$\mathrm{CH}), 126.7$ ( $13-\mathrm{CH}$ ), 126.4 ( $14-\mathrm{CH}), 79.9\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{O}-\mathrm{C}_{\mathrm{q}}-\right.$ $\left.\left(\mathrm{CH}_{3}\right)_{3}\right), 63.1\left(\mathrm{~d},{ }^{2} J_{C P}=5.5 \mathrm{~Hz}, 1-\mathrm{POCH}_{2}\right), 62.5\left(\mathrm{~d},{ }^{2} J_{C P}=6.9\right.$ $\left.\mathrm{Hz}, 1-\mathrm{POCH}_{2}\right), 51.4(2-\mathrm{CH}), 45.1\left(\mathrm{~d},{ }^{1} J_{C P}=156.7 \mathrm{~Hz}, 1-\mathrm{CH}\right)$, $40.4\left(4-\mathrm{CH}_{2}\right), 28.6\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{OC}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), 25.4\left(\mathrm{~d},{ }^{3} J_{C P}=9.7\right.$ $\left.\mathrm{Hz}, 7-\mathrm{CH}_{2}\right), 24.8\left(5-\mathrm{CH}_{2}\right), 19.5\left(6-\mathrm{CH}_{2}\right), 16.6\left(\mathrm{~d},{ }^{3} J_{C P}=5.6 \mathrm{~Hz}\right.$, $\left.1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right), 16.5\left(\mathrm{~d},{ }^{3}{ }_{C P}=6.2 \mathrm{~Hz}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right) .{ }^{31} \mathrm{P}$ NMR ( $162 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta$ (23.06), 22.83. HRESIMS $\mathrm{m} / \mathrm{z} 461.1781$ [ M $+\mathrm{H}]^{+}\left(461.1870\right.$ calcd for $\left.\mathrm{C}_{20} \mathrm{H}_{34} \mathrm{~N}_{2} \mathrm{O}_{6} \mathrm{PS}\right)$.

## Compound 4c-2, diastereomer 2 (rotamer ratio 6.6:1)

${ }^{1} \mathrm{H} \operatorname{NMR}\left(400 \mathrm{MHz}, \mathrm{CDCl}_{3}\right) \delta 7.89(\mathrm{~d}, J=2.8 \mathrm{~Hz}, 1 \mathrm{H}, 11-\mathrm{H})$, $7.40(\mathrm{~m}, 1 \mathrm{H}, 13-\mathrm{H}), 7.28$ (dd, $J=4.2,3.0 \mathrm{~Hz}, 1 \mathrm{H}, 14-\mathrm{H}), 6.72$ $(\mathrm{d}, J=9.6 \mathrm{~Hz}, 1 \mathrm{H}, 1-\mathrm{NH}), 5.03\left(\mathrm{ddd},{ }^{2} J_{H P}=15.4, J=10.7,10.7\right.$ $\mathrm{Hz}, \quad 1 \mathrm{H}, 1-\mathrm{H}), 4.70(\mathrm{~m}, 1 \mathrm{H}, 2-\mathrm{H}), 4.19-4.09(\mathrm{~m}, ~ 4 \mathrm{H}$, $\left.1-\mathrm{P}\left(\mathrm{OCH}_{2}\right)_{2}\right), 3.85(\mathrm{~m}, 1 \mathrm{H}, 4-\mathrm{H}), 2.80(\mathrm{ddd}, J=13.4,13.4,2.9$ $\mathrm{Hz}, 1 \mathrm{H}, 4-\mathrm{H}), 2.44-2.30(\mathrm{~m}, 1 \mathrm{H}, 7-\mathrm{H}), 1.73-1.55(\mathrm{~m}, 4 \mathrm{H}, 5-\mathrm{H}$, $\left.6-\mathrm{H}_{2}, 7-\mathrm{H}\right), 1.37(\mathrm{~m}, 1 \mathrm{H}, 5-\mathrm{H}), 1.30\left(\mathrm{t}, J=6.9 \mathrm{~Hz}, 1 \mathrm{H}, 1-\mathrm{POCH}_{2}-\right.$ $\left.\mathrm{CH}_{3}\right), 1.28\left(\mathrm{t}, J=6.8 \mathrm{~Hz}, 1 \mathrm{H}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right), 1.23(\mathrm{~s}, 9 \mathrm{H}$,
$\left.2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{OC}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right) .{ }^{13} \mathrm{C}$ NMR ( $\left.101 \mathrm{MHz}, \mathrm{CDCl}_{3}\right) \delta 162.3(\mathrm{~d}$, $\left.{ }^{3} J_{C P}=3.8 \mathrm{~Hz}, 9-\mathrm{C}_{\mathrm{q}}\right), 156.7\left(2-\mathrm{N}-\mathrm{C}_{\mathrm{q}}(\mathrm{O})-\mathrm{O}\right), 136.8\left(10-\mathrm{C}_{\mathrm{q}}\right), 129.0$ $(11-\mathrm{CH}), 126.5(13-\mathrm{CH}), 126.4(14-\mathrm{CH}), 80.1\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{O}-\mathrm{C}_{\mathrm{q}}-\right.$ $\left.\left(\mathrm{CH}_{3}\right)_{3}\right), 62.9\left(\mathrm{~d},{ }^{2} J_{C P}=5.6 \mathrm{~Hz}, 1-\mathrm{POCH}_{2}\right), 62.9(\mathrm{~d}, J=5.5 \mathrm{~Hz}$, $\left.1-\mathrm{POCH}_{2}\right), 50.1\left(\mathrm{~d},{ }^{2} J_{C P}=12.0 \mathrm{~Hz}, 2-\mathrm{CH}\right), 45.6\left(\mathrm{~d},{ }^{1} J_{C P}=152.8\right.$ $\mathrm{Hz}, 1-\mathrm{CH}), 40.2\left(4-\mathrm{CH}_{2}\right), 28.3\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{OC}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), 26.3$ $\left(7-\mathrm{CH}_{2}\right), 25.2\left(5-\mathrm{CH}_{2}\right), 19.2\left(6-\mathrm{CH}_{2}\right), 16.6\left(\mathrm{~d},{ }^{3} J_{C P}=5.4 \mathrm{~Hz}\right.$, 1- $\mathrm{POCH}_{2} \mathrm{CH}_{3}$ ), $16.4\left(\mathrm{~d},{ }^{3} J_{C P}=6.2 \mathrm{~Hz}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right) .{ }^{31} \mathrm{P}$ NMR ( $162 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta$ (23.43), 23.10. HRESIMS $m / z 461.1836$ [M $+\mathrm{H}]^{+}\left(461.1870\right.$ calcd for $\left.\mathrm{C}_{20} \mathrm{H}_{34} \mathrm{~N}_{2} \mathrm{O}_{6} \mathrm{PS}\right)$.
tert-Butyl 2-((diethoxyphosphoryl)(4,5,6,7-tetrahydro-benzo-[b] thiophene-2-carboxamido)methyl)piperidine-1-carboxylate (4d)
The synthesis was performed according to general procedure A, $3(241 \mathrm{mg}, 0.689 \mathrm{mmol})$ and $4,5,6,7-$ tetrahydrobenzo[b]thiophene-2-carboxylic acid ( 251 mg , 1.38 mmol, 2.0 equiv.) gave the total product ( $269 \mathrm{mg}, 0.523$ mmol, 76\%). 4d-1 was obtained as a colorless solid (127 $\mathrm{mg}, 0.247 \mathrm{mmol}, 36 \%) .4 d-2$ was obtained as a colorless solid ( $142 \mathrm{mg}, 0.256 \mathrm{mmol}, 43 \%$ ).

## Compound 4d-1, diastereomer 1

${ }^{1} \mathrm{H}$ NMR ( $400 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta 7.26$ (s, 1H, 18-H), 6.23 (br. s, $1 \mathrm{H}, 1-\mathrm{NH}), 5.06\left(\mathrm{ddd},{ }^{2} J_{H P}=16.7 \mathrm{~Hz}, J=9.9,9.9 \mathrm{~Hz}, 1 \mathrm{H}, 1-\mathrm{H}\right)$, 4.57 (br. s, 1H, 2-H), 4.19-3.98 (m, 5H, 1-P( $\left.\left.\mathrm{OCH}_{2}\right)_{2}, 4-\mathrm{H}\right), 2.94$ (br. s, 1H, 4-H), 2.78 (dd, $J=6.1,6.1 \mathrm{~Hz}, 1 \mathrm{H}, 16-\mathrm{H}_{2}$ ), 2.60 (dd, $\left.J=6.1,6.1 \mathrm{~Hz}, 1 \mathrm{H}, 13-\mathrm{H}_{2}\right), 1.91-1.66\left(\mathrm{~m}, 8 \mathrm{H}, 6-\mathrm{H}, 7-\mathrm{H}, 14-\mathrm{H}_{2}\right.$, $15-\mathrm{H}_{2}$ ), $1.64-1.53(\mathrm{~m}, 3 \mathrm{H}, 5-\mathrm{H}, 6-\mathrm{H}, 7-\mathrm{H}), 1.49$ (s, 9 H , $\left.2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{OC}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), 1.40(\mathrm{~m}, 1 \mathrm{H}, 5-\mathrm{H}), 1.29\left(\mathrm{t},{ }^{3} J_{H P}=6.9 \mathrm{~Hz}\right.$, $\left.3 \mathrm{H}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right), 1.26\left(\mathrm{t},{ }^{3} J_{H P}=7.2 \mathrm{~Hz}, 3 \mathrm{H}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right)$. ${ }^{13} \mathrm{C}$ NMR ( $126 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta 161.6\left(\mathrm{~d},{ }^{3} J_{C P}=4.8 \mathrm{~Hz}, 9-\mathrm{C}_{\mathrm{q}}\right)$, $154.7\left(2-\mathrm{N}-\mathrm{C}_{\mathrm{q}}(\mathrm{O})-\mathrm{O}\right), 142.4\left(17-\mathrm{C}_{\mathrm{q}}\right), 136.6\left(12-\mathrm{C}_{\mathrm{q}}\right), 133.5$ ( $10-$ $\mathrm{C}_{\mathrm{q}}$ ), $129.8(18-\mathrm{CH}), 79.9\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{O}-\mathrm{C}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), 63.1$ (br. s, $\left.1-\mathrm{POCH}_{2}\right), 62.5\left(\mathrm{~d},{ }^{2} J_{C P}=6.9 \mathrm{~Hz}, 1-\mathrm{POCH}_{2}\right), 51.2(2-\mathrm{CH})$, $45.0\left(\mathrm{~d},{ }^{1} J_{C P}=159.9 \mathrm{~Hz}, 1-\mathrm{CH}\right), 40.7\left(4-\mathrm{CH}_{2}\right), 28.6$ $\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{OC}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), 25.6\left(13-\mathrm{CH}_{2}\right), 25.5\left(16-\mathrm{CH}_{2}\right), 25.4$ $\left(\mathrm{d},{ }^{3} J_{C P}=8.5 \mathrm{~Hz}, 7-\mathrm{CH}_{2}\right), 24.9\left(5-\mathrm{CH}_{2}\right), 23.4\left(15-\mathrm{CH}_{2}\right), 22.7$ $\left(14-\mathrm{CH}_{2}\right), 19.5\left(6-\mathrm{CH}_{2}\right), 16.6\left(\mathrm{~d},{ }^{3} J_{C P}=5.5 \mathrm{~Hz}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right)$, $16.4\left(\mathrm{~d},{ }^{3} J_{C P}=6.3 \mathrm{~Hz}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right) .{ }^{31} \mathrm{P}$ NMR $(162 \mathrm{MHz}$, $\left.\mathrm{CDCl}_{3}\right) \delta$ 22.72. HRESIMS $m / z 515.2324[\mathrm{M}+\mathrm{H}]^{+}(515.2339$ calcd for $\mathrm{C}_{22} \mathrm{H}_{38} \mathrm{~N}_{2} \mathrm{O}_{5} \mathrm{P}$ ).

## Compound 4d-2, diastereomer 2 (rotamer ratio 4.9:1)

${ }^{1} \mathrm{H}$ NMR ( $400 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta 7.12$ ( $\mathrm{s}, 1 \mathrm{H}, 18-\mathrm{H}$ ), 6.55 ( $\mathrm{d}, J=$ $9.6 \mathrm{~Hz}, 1 \mathrm{H}, 1-\mathrm{NH}), 4.99\left(\mathrm{ddd},{ }^{2} J_{H P}=15.0 \mathrm{~Hz}, J=11.6,9.6 \mathrm{~Hz}\right.$, $1 \mathrm{H}, 1-\mathrm{H}), 4.68(\mathrm{~m}, 1 \mathrm{H}, 2-\mathrm{H}), 4.19-4.10\left(\mathrm{~m}, 4 \mathrm{H}, 1-\mathrm{P}\left(\mathrm{OCH}_{2}\right)_{2}\right)$, $3.84(\mathrm{~m}, 1 \mathrm{H}, 4-\mathrm{H}), 2.78$ (ddd, $J=13.3,13.3,2.9 \mathrm{~Hz}, 1 \mathrm{H}, 4-\mathrm{H})$, $2.76-2.72\left(\mathrm{~m}, 2 \mathrm{H}, 13-\mathrm{H}_{2}\right.$ or $\left.16-\mathrm{H}_{2}\right), 2.64-2.44\left(\mathrm{~m}, 2 \mathrm{H}, 16-\mathrm{H}_{2}\right.$ or $13-\mathrm{H}_{2}$ ), 2.37 (br. d, $J=11.5 \mathrm{~Hz}, 1 \mathrm{H}, 7-\mathrm{H}$ ), $1.87-1.72(\mathrm{~m}, 4 \mathrm{H}$, $\left.14-\mathrm{H}_{2}, 15-\mathrm{H}_{2}\right), 1.69-1.56\left(\mathrm{~m}, 4 \mathrm{H}, 5-\mathrm{H}, 6-\mathrm{H}_{2}, 7-\mathrm{H}\right), 1.40(\mathrm{~m}, 1 \mathrm{H}$, $5-\mathrm{H}), 1.31\left(\mathrm{t},{ }^{3} J_{C P}=7.1 \mathrm{~Hz}, 3 \mathrm{H}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right), 1.28(\mathrm{~s}, 9 \mathrm{H}$, $\left.2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{OC}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), 1.28\left(\mathrm{t}, J=7.3 \mathrm{~Hz}, 3 \mathrm{H}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right)$. ${ }^{13} \mathrm{C}$ NMR ( $101 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta 161.7\left(\mathrm{~d}, J=3.8 \mathrm{~Hz}, 9-\mathrm{C}_{\mathrm{q}}\right), 156.7$ $\left(2-\mathrm{N}-\mathrm{C}_{\mathrm{q}}(\mathrm{O})-\mathrm{O}\right), 142.1\left(17-\mathrm{C}_{\mathrm{q}}\right), 136.2\left(12-\mathrm{C}_{\mathrm{q}}\right), 133.9\left(10-\mathrm{C}_{\mathrm{q}}\right)$, $129.1(18-\mathrm{CH}), 80.1\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{O}-\mathrm{C}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), 62.9(\mathrm{~d}, J=5.5$
$\left.\mathrm{Hz}, 1-\mathrm{POCH}_{2}\right), 62.8\left(\mathrm{~d},{ }^{2} J_{C P}=6.8 \mathrm{~Hz}, 1-\mathrm{POCH}_{2}\right), 50.0\left(\mathrm{~d},{ }^{2} J_{C P}=\right.$ $12.2 \mathrm{~Hz}, 2-\mathrm{CH}), 45.7\left(\mathrm{~d},{ }^{1} J_{C P}=152.7 \mathrm{~Hz}, 1-\mathrm{CH}\right), 40.2\left(4-\mathrm{CH}_{2}\right)$, $28.3\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{OC}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), 26.3\left(7-\mathrm{CH}_{2}\right), 25.5\left(13-\mathrm{CH}_{2}\right.$ or $16-$ $\left.\mathrm{CH}_{2}\right), 25.4\left(13-\mathrm{CH}_{2}\right.$ or $\left.16-\mathrm{CH}_{2}\right), 25.2\left(5-\mathrm{CH}_{2}\right), 23.4\left(15-\mathrm{CH}_{2}\right)$, $22.8\left(14-\mathrm{CH}_{2}\right), 19.2\left(6-\mathrm{CH}_{2}\right), 16.7\left(\mathrm{~d},{ }^{3} J_{C P}=5.3 \mathrm{~Hz}, 1-\mathrm{POCH}_{2}-\right.$ $\left.\mathrm{CH}_{3}\right), 16.5\left(\mathrm{~d},{ }^{3} J_{C P}=6.3 \mathrm{~Hz}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right) .{ }^{31} \mathrm{P}$ NMR (162 $\left.\mathrm{MHz}, \mathrm{CDCl}_{3}\right) \delta(23.42)$, 23.05. HRESIMS $\mathrm{m} / \mathrm{z} 515.2316[\mathrm{M}+$ $\mathrm{H}]^{+}\left(515.2339\right.$ calcd for $\left.\mathrm{C}_{22} \mathrm{H}_{38} \mathrm{~N}_{2} \mathrm{O}_{5} \mathrm{P}\right)$.

## tert-Butyl 2-((diethoxyphosphoryl)(4,5,6,7-tetrahydro-benzo-[b] thiophene-3-carboxamido)methyl)piperidine-1-carboxylate (4e)

Synthesis was performed according to the general procedure A, 3 ( $322 \mathrm{mg}, 0.919 \mathrm{mmol}$ ) and 4,5,6,7-tetrahydrobenzo[b] thiophene-3-carboxylic acid ( $336 \mathrm{mg}, 1.84 \mathrm{mmol}$ ) gave the total product ( $317 \mathrm{mg}, 0.616 \mathrm{mmol}, 67 \%$ ). 4e-1 was obtained as a colorless solid ( $117 \mathrm{mg}, 0.227 \mathrm{mmol}, 25 \%$ ). $4 \mathrm{e}-2$ was obtained as a colorless solid ( $201 \mathrm{mg}, 0.391 \mathrm{mmol}, 43 \%$ ).

## Compound 4e-1, diastereomer 1

${ }^{1} \mathrm{H}$ NMR ( $400 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta 7.50$ (s, 1H, 11-H), 6.35 (br. s, $1 \mathrm{H}, 1-\mathrm{NH}), 5.07\left(\mathrm{ddd},{ }^{2} J_{H P}=16.8 \mathrm{~Hz}, J=9.9,9.9 \mathrm{~Hz}, 1 \mathrm{H}, 1-\mathrm{H}\right)$, $4.55(\mathrm{~m}, 1 \mathrm{H}, 2-\mathrm{H}), 4.18-4.03\left(\mathrm{~m}, 4 \mathrm{H}, 1-\mathrm{P}\left(\mathrm{OCH}_{2}\right)_{2}\right), 3.97(\mathrm{~m}$, $1 \mathrm{H}, 4-\mathrm{H}), 3.00(\mathrm{~m}, 1 \mathrm{H}, 4-\mathrm{H}), 2.88-2.70\left(\mathrm{~m}, 4 \mathrm{H}, 14-\mathrm{CH}_{2}, 17-\right.$ $\mathrm{CH}_{2}$ ), 1.90-1.72 (m, 6H, 6-H, 7-H, $15-\mathrm{CH}_{2}, 16-\mathrm{CH}_{2}$ ), $1.65-1.53$ $(\mathrm{m}, 4 \mathrm{H}, 5-\mathrm{H}, 6-\mathrm{H}, 7-\mathrm{H}), 1.48\left(\mathrm{~s}, 9 \mathrm{H}, 2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{OC}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right)$, $1.40(\mathrm{~m}, 1 \mathrm{H}, 5-\mathrm{H}), 1.29\left(\mathrm{~m}, 6 \mathrm{H}, 1-\mathrm{P}\left(\mathrm{OCH}_{2} \mathrm{CH}_{3}\right)_{2}\right) .{ }^{13} \mathrm{C}$ NMR $\left(126 \mathrm{MHz}, \mathrm{CDCl}_{3}\right) \delta 163.9\left(\mathrm{~d}, J=4.7 \mathrm{~Hz}, 9-\mathrm{C}_{\mathrm{q}}\right), 154.8(2-\mathrm{N}-$ $\left.\mathrm{C}_{\mathrm{q}}(\mathrm{O})-\mathrm{O}\right), 138.3\left(13-\mathrm{C}_{\mathrm{q}}\right.$ or $\left.18-\mathrm{C}_{\mathrm{q}}\right), 135.6\left(10-\mathrm{C}_{\mathrm{q}}\right), 134.7\left(13-\mathrm{C}_{\mathrm{q}}\right.$ or $\left.18-\mathrm{C}_{\mathrm{q}}\right), 124.8(11-\mathrm{CH}), 79.8\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{O}-\mathrm{C}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), 62.8$ (br. s, 1-POCH ${ }_{2}$ ), $62.5\left(\mathrm{~d},{ }^{2} J_{C P}=6.6 \mathrm{~Hz}, 1-\mathrm{POCH}_{2}\right), 51.2(2-\mathrm{CH})$, $44.4\left(\mathrm{~d},{ }^{1} J_{C P}=156.2 \mathrm{~Hz}, 1-\mathrm{CH}\right), 40.6\left(4-\mathrm{CH}_{2}\right), 28.5\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O})\right.$ $\left.\mathrm{OC}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), 25.7\left(14-\mathrm{CH}_{2}\right.$ or $\left.17-\mathrm{CH}_{2}\right), 25.5\left(14-\mathrm{CH}_{2}\right.$ or $17-$ $\left.\mathrm{CH}_{2}\right), 25.5\left(\mathrm{~d},{ }^{3} J_{C P}=8.1 \mathrm{~Hz}, 7-\mathrm{CH}_{2}\right), 24.9\left(5-\mathrm{CH}_{2}\right), 23.2(15-$ $\mathrm{CH}_{2}$ or $\left.16-\mathrm{CH}_{2}\right), 22.7\left(15-\mathrm{CH}_{2}\right.$ or $\left.16-\mathrm{CH}_{2}\right)$, $19.5\left(6-\mathrm{CH}_{2}\right), 16.6$ $\left(\mathrm{d},{ }^{3} J_{C P}=5.7 \mathrm{~Hz}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right), 16.5\left(\mathrm{~d},{ }^{3} J_{C P}=6.1 \mathrm{~Hz}\right.$, 1- $\mathrm{POCH}_{2} \mathrm{CH}_{3}$ ). ${ }^{31} \mathrm{P}$ NMR ( $162 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta$ (22.91), 22.54. HRESIMS $\mathrm{m} / \mathrm{z} 515.2349[\mathrm{M}+\mathrm{H}]^{+}$(515.2339 calcd for $\mathrm{C}_{22} \mathrm{H}_{38} \mathrm{~N}_{2} \mathrm{O}_{5} \mathrm{P}$ ).

## Compound 4e-2, diastereomer 2 (rotamer ratio 3.6:1)

${ }^{1} \mathrm{H}$ NMR ( $400 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta 7.48$ (s, 1H, 11-H), 6.39 (d, $J=$ $9.9 \mathrm{~Hz}, 1 \mathrm{H}, 1-\mathrm{NH}), 5.02$ (ddd, ${ }^{2} J_{H P}=15.4 \mathrm{~Hz}, J=11.6,9.9 \mathrm{~Hz}$, $1 \mathrm{H}, 1-\mathrm{H}), 4.67(\mathrm{~m}, 1 \mathrm{H}, 2-\mathrm{H}), 4.27-4.08\left(\mathrm{~m}, 4 \mathrm{H}, 1-\mathrm{P}\left(\mathrm{OCH}_{2}\right)_{2}\right)$, 3.85 (br. dd, $J=13.52,3.7 \mathrm{~Hz}, 1 \mathrm{H}, 4-\mathrm{H}$ ), $2.88-2.59$ (m, 5 H , $4-\mathrm{H}, 14-\mathrm{H}_{2}, 17-\mathrm{H}_{2}$ ), 2.39 (br. d, $J=10.1 \mathrm{~Hz} 1 \mathrm{H}, 7-\mathrm{H}$ ), 1.92-1.72 (m, $4 \mathrm{H}, 15-\mathrm{H}_{2}, 16-\mathrm{H}_{2}$ ), 1.70-1.56 (m, 4H, $\left.5-\mathrm{H}, 6-\mathrm{H}_{2}, 7-\mathrm{H}\right), 1.40$ $(\mathrm{m}, 1 \mathrm{H}, 5-\mathrm{H}), 1.34\left(\mathrm{t}, J=7.2 \mathrm{~Hz}, 3 \mathrm{H}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right), 1.29(\mathrm{t}, J=$ $\left.7.1 \mathrm{~Hz}, 3 \mathrm{H}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right), 1.24\left(\mathrm{~s}, 9 \mathrm{H}, 2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{OC}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right)$. ${ }^{13} \mathrm{C}$ NMR $\left(101 \mathrm{MHz}, \mathrm{CDCl}_{3}\right) \delta 163.7\left(\mathrm{~d}, J=4.0 \mathrm{~Hz}, 9-\mathrm{C}_{\mathrm{q}}\right), 156.4$ $\left(2-\mathrm{N}-\mathrm{C}_{\mathrm{q}}(\mathrm{O})-\mathrm{O}\right), 137.8\left(13-\mathrm{C}_{\mathrm{q}}\right.$ or $\left.18-\mathrm{C}_{\mathrm{q}}\right)$, $135.3\left(10-\mathrm{C}_{\mathrm{q}}\right), 134.3$ $\left(13-\mathrm{C}_{\mathrm{q}}\right.$ or $\left.18-\mathrm{C}_{\mathrm{q}}\right), 124.9(11-\mathrm{CH}), 79.8\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{O}-\mathrm{C}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right)$, $62.7\left(\mathrm{~d},{ }^{2} J_{C P}=6.0 \mathrm{~Hz}, 1-\mathrm{POCH}_{2}\right), 62.5\left(\mathrm{~d},{ }^{2} J_{C P}=7.2 \mathrm{~Hz}\right.$, $\left.1-\mathrm{POCH}_{2}\right), 49.8\left(\mathrm{~d},{ }^{2} J_{C P}=12.4 \mathrm{~Hz}, 2-\mathrm{H}\right), 44.8\left(\mathrm{~d},{ }^{1} J_{C P}=152.2\right.$ $\mathrm{Hz}, \quad 1-\mathrm{H}), 40.0\left(4-\mathrm{CH}_{2}\right), 28.1 \quad\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{OC}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), 26.1$ $\left(7-\mathrm{CH}_{2}\right), 25.5\left(14-\mathrm{CH}_{2}\right.$ or $\left.17-\mathrm{CH}_{2}\right), 25.3\left(14-\mathrm{CH}_{2}\right.$ or $\left.17-\mathrm{CH}_{2}\right)$,
$25.1\left(5-\mathrm{CH}_{2}\right), 23.0\left(15-\mathrm{CH}_{2}\right.$ or $\left.16-\mathrm{CH}_{2}\right), 22.6\left(15-\mathrm{CH}_{2}\right.$ or $16-$ $\left.\mathrm{CH}_{2}\right), 19.00\left(6-\mathrm{CH}_{2}\right), 16.5\left(\mathrm{~d},{ }^{3} J_{C P}=5.4 \mathrm{~Hz}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right), 16.3$ $\left(\mathrm{d},{ }^{3} J_{C P}=6.2 \mathrm{~Hz}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right) .{ }^{31} \mathrm{P}$ NMR ( $162 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta$ (23.57), 23.27. HRESIMS $m / z 515.2302[\mathrm{M}+\mathrm{H}]^{+}$(515.2339 calcd for $\mathrm{C}_{22} \mathrm{H}_{38} \mathrm{~N}_{2} \mathrm{O}_{5} \mathrm{P}$ ).
tert-Butyl 2-((diethoxyphosphoryl)(4,5,6,7-tetrahydro-benzo[c] thiophene-1-carboxamido)methyl)piperidine-1-carboxylate (4f)
The synthesis was performed according to general procedure A, $3(303 \mathrm{mg}, 0.865 \mathrm{mg}$ ) and 4,5,6,7-tetrahydrobenzo[c] thiophene-1-carboxylic acid ( $336 \mathrm{mg}, 1.84 \mathrm{mg}, 2.1$ equiv.) gave the total product ( $275 \mathrm{mg}, 0.534 \mathrm{mmol}, 62 \%$ ). $4 \mathrm{f}-1$ was obtained as a colorless solid ( $121 \mathrm{mg}, 0.235 \mathrm{mmol}, 27 \%$ ). 4f-2 was obtained as a colorless solid ( $201 \mathrm{mg}, 0.391 \mathrm{mmol}, 45 \%$ ).

## Compound 4f-1, diastereomer 1

${ }^{1} \mathrm{H}$ NMR ( $400 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta 6.99$ (s, 1H, 12-H), 6.18 (s, 1H, $1-\mathrm{NH}$ ), 5.08 (ddd, $\left.{ }^{2} J_{H P}=16.4 \mathrm{~Hz}, J=9.5,9.5 \mathrm{~Hz}, 1 \mathrm{H}, 1-\mathrm{H}\right)$, $4.55(\mathrm{~m}, 1 \mathrm{H}, 2-\mathrm{H}), 4.27-3.86\left(\mathrm{~m}, 5 \mathrm{H}, 1-\mathrm{P}\left(\mathrm{OCH}_{2}\right)_{2}\right), 3.10-2.86$ (m, 3H, $4-\mathrm{H}, 17-\mathrm{H}_{2}$ ), 2.71 (br. dd, $J=6.0,6.0 \mathrm{~Hz}, 2 \mathrm{H}, 14-\mathrm{H}_{2}$ ), $1.85-1.70\left(\mathrm{~m}, 6 \mathrm{H}, 6-\mathrm{H}, 7-\mathrm{H}, 15-\mathrm{H}_{2}, 16-\mathrm{H}_{2}\right), 1.58(\mathrm{~m}, 3 \mathrm{H}, 5-\mathrm{H}$, $6-\mathrm{H}, 7-\mathrm{H}), 1.48\left(\mathrm{~s}, 9 \mathrm{H}, 2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{OC}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), 1.40(\mathrm{~m}, 1 \mathrm{H}$, $5-\mathrm{H}), 1.29\left(\mathrm{t}, J=7.0 \mathrm{~Hz}, 6 \mathrm{H}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right) .{ }^{13} \mathrm{C}$ NMR (126 $\left.\mathrm{MHz}, \mathrm{CDCl}_{3}\right) \delta 162.4\left(\mathrm{~d}, J=4.6 \mathrm{~Hz}, 9-\mathrm{C}_{\mathrm{q}}\right), 154.9\left(2-\mathrm{N}-\mathrm{C}_{\mathrm{q}}(\mathrm{O})-\right.$ O), $141.9\left(13-\mathrm{C}_{\mathrm{q}}\right), 140.7\left(18-\mathrm{C}_{\mathrm{q}}\right), 129.0\left(10-\mathrm{C}_{\mathrm{q}}\right), 122.7(12-\mathrm{CH})$, $79.8\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{O}-\mathrm{C}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), 62.8$ (br. s, 1- $\left.\mathrm{POCH}_{2}\right), 62.6(\mathrm{~d}, J=$ $\left.6.6 \mathrm{~Hz}, 1-\mathrm{POCH}_{2}\right), 51.3\left(\mathrm{~d},{ }^{2} J_{C P}=2.9 \mathrm{~Hz}, 2-\mathrm{CH}\right), 44.8\left(\mathrm{~d},{ }^{1} J_{C P}=\right.$ $155.3 \mathrm{~Hz}, 1-\mathrm{CH})$, $40.6\left(4-\mathrm{CH}_{2}\right), 28.6\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{OC}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right)$, $27.0\left(17-\mathrm{CH}_{2}\right), 26.6\left(14-\mathrm{CH}_{2}\right), 25.5\left(\mathrm{~d},{ }^{3} J_{C P}=9.5 \mathrm{~Hz}, 7-\mathrm{CH}_{2}\right)$, $24.8\left(5-\mathrm{CH}_{2}\right), 23.1\left(16-\mathrm{CH}_{2}\right), 22.9\left(15-\mathrm{CH}_{2}\right), 19.5\left(6-\mathrm{CH}_{2}\right), 16.6$ $\left(\mathrm{d},{ }^{3} J_{C P}=5.7 \mathrm{~Hz}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right), 16.5\left(\mathrm{~d},{ }^{3} J_{C P}=6.0 \mathrm{~Hz}\right.$, 1- $\mathrm{POCH}_{2} \mathrm{CH}_{3}$ ). ${ }^{31} \mathrm{P}$ NMR ( $162 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta$ 22.81, 22.42. HRESIMS $\mathrm{m} / \mathrm{z} 515.2312[\mathrm{M}+\mathrm{H}]^{+}$(515.2339 calcd for $\mathrm{C}_{22} \mathrm{H}_{38} \mathrm{~N}_{2} \mathrm{O}_{5} \mathrm{P}$ ).

## Compound 4f-2, diastereomer 2 (rotamer ratio 3.5 :1)

${ }^{1} \mathrm{H}$ NMR ( $400 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta 6.96$ ( $\mathrm{s}, 1 \mathrm{H}, 12-\mathrm{H}$ ), 6.34 (d, $J=$ $9.8 \mathrm{~Hz}, 1 \mathrm{H}, 1-\mathrm{NH}), 5.06$ (ddd, ${ }^{2} J_{H P}=15.3 \mathrm{~Hz}, J=11.6,9.8 \mathrm{~Hz}$, $1 \mathrm{H}, 1-\mathrm{H}), 4.68(\mathrm{~m}, 1 \mathrm{H}, 2-\mathrm{H}), 4.25-4.09\left(\mathrm{~m}, 4 \mathrm{H}, 1-\mathrm{P}\left(\mathrm{OCH}_{2}\right)_{2}\right)$, 3.85 (br. dd, $J=13.6,2.6 \mathrm{~Hz}, 1 \mathrm{H}, 4-\mathrm{H}), 3.08-2.91$ (m, 2H, 14$\mathrm{CH}_{2}$ or $17-\mathrm{CH}_{2}$ ), 2.87 (ddd, $J=13.4,13.4,2.8 \mathrm{~Hz}, 1 \mathrm{H}, 4-\mathrm{H}$ ), $2.75-2.57\left(\mathrm{~m}, 2 \mathrm{H}, 14-\mathrm{CH}_{2}\right.$ or $\left.17-\mathrm{CH}_{2}\right), 2.41(\mathrm{~m}, J=9.0 \mathrm{~Hz} 1 \mathrm{H}$, $7-\mathrm{H}), 1.91-1.56\left(\mathrm{~m}, 8 \mathrm{H}, 5-\mathrm{H}, 6-\mathrm{H}_{2}, 7-\mathrm{H}, 15-\mathrm{CH}_{2}, 16-\mathrm{CH}_{2}\right), 1.39$ $(\mathrm{m}, 1 \mathrm{H}, 5-\mathrm{H}), 1.34\left(\mathrm{t}, J=7.1 \mathrm{~Hz}, 3 \mathrm{H}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right), 1.28(\mathrm{t}, J=$ $\left.7.1 \mathrm{~Hz}, 3 \mathrm{H}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right), 1.20\left(\mathrm{~s}, 9 \mathrm{H}, 2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{OC}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right)$. ${ }^{13} \mathrm{C}$ NMR $\left(101 \mathrm{MHz}, \mathrm{CDCl}_{3}\right) \delta 162.6\left(\mathrm{~d}, J=4.4 \mathrm{~Hz}, 9-\mathrm{C}_{\mathrm{q}}\right), 156.6$ $\left(2-\mathrm{N}-\mathrm{C}_{\mathrm{q}}(\mathrm{O})-\mathrm{O}\right), 140.8\left(13-\mathrm{C}_{\mathrm{q}}\right.$ or $\left.18-\mathrm{C}_{\mathrm{q}}\right), 140.2\left(13-\mathrm{C}_{\mathrm{q}}\right.$ or $\left.18-\mathrm{C}_{\mathrm{q}}\right)$, $130.9\left(10-\mathrm{C}_{\mathrm{q}}\right), 123.1(12-\mathrm{CH}), 79.9\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{O}-\mathrm{C}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right)$, $62.9\left(\mathrm{~d},{ }^{2} J_{C P}=6.1 \mathrm{~Hz}, 1-\mathrm{POCH}_{2}\right), 62.7\left(\mathrm{~d},{ }^{2} J_{C P}=7.2 \mathrm{~Hz}\right.$, $\left.1-\mathrm{POCH}_{2}\right), 49.9\left(\mathrm{~d},{ }^{2} J_{C P}=12.3 \mathrm{~Hz}, 2-\mathrm{CH}\right), 45.3\left(\mathrm{~d},{ }^{1} J_{C P}=151.9\right.$ $\mathrm{Hz}, 1-\mathrm{CH}), 40.2\left(4-\mathrm{CH}_{2}\right), 28.1\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{OC}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), 26.7(14-$ $\mathrm{CH}_{2}$ or $\left.17-\mathrm{CH}_{2}\right), 26.6\left(14-\mathrm{CH}_{2}\right.$ or $\left.17-\mathrm{CH}_{2}\right), 26.2\left(7-\mathrm{CH}_{2}\right), 25.2$ $\left(5-\mathrm{CH}_{2}\right), 23.2\left(15-\mathrm{CH}_{2}\right.$ or $\left.16-\mathrm{CH}_{2}\right), 22.9\left(15-\mathrm{CH}_{2}\right.$ or $\left.16-\mathrm{CH}_{2}\right)$, $19.2\left(6-\mathrm{CH}_{2}\right), 16.7\left(\mathrm{~d},{ }^{3} J_{C P}=5.5 \mathrm{~Hz}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right), 16.5(\mathrm{~d}$, $\left.{ }^{3} J_{C P}=6.0 \mathrm{~Hz}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right) .{ }^{31} \mathrm{P}$ NMR ( $162 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta$
(23.42), 23.15. HRESIMS $\mathrm{m} / \mathrm{z} 515.2329[\mathrm{M}+\mathrm{H}]^{+}$(515.2339 calcd for $\mathrm{C}_{22} \mathrm{H}_{38} \mathrm{~N}_{2} \mathrm{O}_{5} \mathrm{P}$ ).

## tert-Butyl 2-((benzo[b]thiophene-2-carboxamido)(di-ethoxyphosphoryl)methyl)piperidine-1-carboxylate (4g)

The synthesis was performed according to the general procedure A, 3 ( $236 \mathrm{mg}, 0.673 \mathrm{mmol}$ ) and benzo[b]thiophene-2-carboxylic acid ( $238 \mathrm{mg}, 1.34 \mathrm{mmol}, 2.0$ equiv.) gave the total product ( $270 \mathrm{mg}, 0.529 \mathrm{mmol}, 79 \%$ ). $\mathbf{4 g} \mathbf{g} \mathbf{1}$ was obtained as a colorless solid ( $110 \mathrm{mg}, 0.215 \mathrm{mmol}, 32 \%$ ). $\mathbf{4 g}-2$ was obtained as a colorless solid ( $153 \mathrm{mg}, 0.300 \mathrm{mmol}, 44 \%$ ).

## Compound 4g-1, diastereomer 1

${ }^{1} \mathrm{H}$ NMR ( $400 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta 7.90$ (s, 1H, 18-H), 7.86 (dd, $J=$ $8.1,1.1 \mathrm{~Hz}, 1 \mathrm{H}, 13-\mathrm{H}$ ), 7.84 (dd, $J=7.7,1.7 \mathrm{~Hz}, 1 \mathrm{H}, 16-\mathrm{H}$ ), 7.43 (ddd, $J=7.1,7.1,1.7 \mathrm{~Hz}, 1 \mathrm{H}$, part C of ABCD, $14-\mathrm{CH}$ ), 7.40 (ddd, $J=7.1,7.1,1.6 \mathrm{~Hz}, 1 \mathrm{H}$, part D of ABCD, $15-\mathrm{CH}$ ), 7.00 (s, 1H, 1-NH), 5.14 (m, 1H, 1-H), 4.65 (m, 1H, 2-H), 4.22$3.91\left(\mathrm{~m}, 5 \mathrm{H}, 1-\mathrm{P}\left(\mathrm{OCH}_{2}\right)_{2}, 4-\mathrm{H}\right), 2.92(\mathrm{~m}, 1 \mathrm{H}, 4-\mathrm{H}), 1.85(\mathrm{~m}$, $1 \mathrm{H}, 7-\mathrm{H}), 1.75$ (m, 1H, 6-H), 1.66-1.54 (m, 3H, 5-H, 6-H, 7-H), $1.49\left(\mathrm{~s}, 9 \mathrm{H}, 2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{OC}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), 1.42(\mathrm{~m}, 1 \mathrm{H}, 5-\mathrm{H}), 1.31(\mathrm{t}, J$ $\left.=7.1 \mathrm{~Hz}, 3 \mathrm{H}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right), 1.24\left(\mathrm{t}, J=7.2 \mathrm{~Hz}, 3 \mathrm{H}, 1-\mathrm{POCH}_{2}-\right.$ $\left.\mathrm{CH}_{3}\right) \cdot{ }^{13} \mathrm{C}$ NMR ( $126 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta 160.8\left(\mathrm{~d}, J=5.0 \mathrm{~Hz}, 9-\mathrm{C}_{\mathrm{q}}\right)$, $153.9\left(2-\mathrm{N}-\mathrm{C}_{\mathrm{q}}(\mathrm{O})-\mathrm{O}\right), 140.2\left(12-\mathrm{C}_{\mathrm{q}}\right), 138.1\left(17-\mathrm{C}_{\mathrm{q}}\right), 136.8$ ( $10-$ $\mathrm{C}_{\mathrm{q}}$ ), $125.5(14-\mathrm{CH}), 124.7(18-\mathrm{CH}), 124.2(16-\mathrm{CH}), 123.9$ ( $15-$ $\mathrm{CH}), 121.7(13-\mathrm{CH}), 78.8\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{O}-\mathrm{C}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), 62.2$ (br s, 1- $\mathrm{POCH}_{2}$ ), $61.5\left(\mathrm{br} \mathrm{s}, 1-\mathrm{POCH}_{2}\right), 50.1(2-\mathrm{CH}), 44.7\left(\mathrm{~d},{ }^{1} J_{C P}=\right.$ $148.9 \mathrm{~Hz}, 1-\mathrm{CH})$, $39.4\left(4-\mathrm{CH}_{2}\right), 27.4\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{OC}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right)$, $24.3\left(\mathrm{~d},{ }^{3} J_{C P}=9.8 \mathrm{~Hz}, 7-\mathrm{CH}_{2}\right), 23.7\left(5-\mathrm{CH}_{2}\right), 18.3\left(6-\mathrm{CH}_{2}\right), 15.5$ $\left(\mathrm{d},{ }^{3} J_{C P}=5.6 \mathrm{~Hz}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right), 15.3\left(\mathrm{~d},{ }^{3} J_{C P}=6.3 \mathrm{~Hz}\right.$, 1- $\mathrm{POCH}_{2} \mathrm{CH}_{3}$ ). ${ }^{31} \mathrm{P}$ NMR ( $162 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta$ 22.79, 22.43. HRESIMS $\mathrm{m} / \mathrm{z} 511.2017[\mathrm{M}+\mathrm{H}]^{+}$(511.2026 calcd for $\left.\mathrm{C}_{24} \mathrm{H}_{36} \mathrm{~N}_{2} \mathrm{O}_{6} \mathrm{PS}\right)$.

## Compound 4g-2, diastereomer 2 (rotamer ratio 6.6:1)

${ }^{1} \mathrm{H}$ NMR ( $400 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta 7.84(\mathrm{~d}, J=7.9,1.2 \mathrm{~Hz}, 1 \mathrm{H}, 13-$ CH), 7.78 (dd, $J=7.1,1.6 \mathrm{~Hz}, 1 \mathrm{H}, 16-\mathrm{H}), 7.73$ (s, 1H, 18-H), 7.41 (ddd, $J=7.4,7.4,1.4 \mathrm{~Hz}, 1 \mathrm{H}$, part C of ABCD $14-\mathrm{CH}$ ), 7.37 (ddd, $J=7.4,7.4,1.4 \mathrm{~Hz}, 1 \mathrm{H}$, part D of ABCD, $15-\mathrm{CH}$ ), $6.90(\mathrm{~d}, J=9.5 \mathrm{~Hz}, 1 \mathrm{H}, 1-\mathrm{NH}), 5.05\left(\mathrm{ddd},{ }^{2} J_{H P}=15.0 \mathrm{~Hz}, J=\right.$ $11.6,9.5 \mathrm{~Hz}, 1 \mathrm{H}, 1-\mathrm{H}), 4.75(\mathrm{~m}, 1 \mathrm{H}, 2-\mathrm{H}), 4.32-4.14(\mathrm{~m}, 4 \mathrm{H}$, $\left.1-\mathrm{P}\left(\mathrm{OCH}_{2}\right)_{2}\right), 3.89(\mathrm{dd}, J=14.0,4.0 \mathrm{~Hz}, 1 \mathrm{H}, 4-\mathrm{H}), 2.82(\mathrm{ddd}, J$ $=13.3,13.3,2.7 \mathrm{~Hz}, 1 \mathrm{H}, 4-\mathrm{H}$ ), 2.38 (br. d, $J=12.4 \mathrm{~Hz}, 1 \mathrm{H}$, 7-H), 1.80-1.58 (m, 4H, 5-H, 5-H2, 7-H), 1.41 (m, 1H, $5-\mathrm{H})$, $1.33\left(\mathrm{t}, J=7.1 \mathrm{~Hz}, 3 \mathrm{H}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right), 1.30(\mathrm{t}, J=7.1 \mathrm{~Hz}, 3 \mathrm{H}$, 1- $\mathrm{POCH}_{2} \mathrm{CH}_{3}$ ), $1.23\left(\mathrm{~s}, 9 \mathrm{H}, 2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{OC}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right) .{ }^{13} \mathrm{C}$ NMR $\left(126 \mathrm{MHz}, \mathrm{CDCl}_{3}\right) \delta 161.8\left(\mathrm{~d}, J=3.6 \mathrm{~Hz}, 9-\mathrm{C}_{\mathrm{q}}\right), 156.9(2-\mathrm{N}-$ $\left.\mathrm{C}_{\mathrm{q}}(\mathrm{O})-\mathrm{O}\right), 141.4\left(12-\mathrm{C}_{\mathrm{q}}\right), 139.2\left(17-\mathrm{C}_{\mathrm{q}}\right), 138.2\left(10-\mathrm{C}_{\mathrm{q}}\right), 126.5$ ( $14-\mathrm{CH}$ ), 125.3 ( $18-\mathrm{CH}$ ), 125.1 ( $16-\mathrm{CH}$ ), 125.0 ( $15-\mathrm{CH}$ ), 122.8 $(13-\mathrm{CH}), 80.3\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{O}-\mathrm{C}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), 63.1\left(\mathrm{~d},{ }^{2} J_{C P}=6.3 \mathrm{~Hz}\right.$, $\left.1-\mathrm{POCH}_{2}\right), 63.0\left(\mathrm{~d},{ }^{2} J_{C P}=7.5 \mathrm{~Hz}, 1-\mathrm{POCH}_{2}\right), 50.0\left(\mathrm{~d},{ }^{2} J_{C P}=\right.$ $11.7 \mathrm{~Hz}, 2-\mathrm{H}), 46.2\left(\mathrm{~d},{ }^{1} J_{C P}=152.7 \mathrm{~Hz}, 1-\mathrm{H}\right), 40.2\left(4-\mathrm{CH}_{2}\right), 28.3$ $\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{OC}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), \quad 26.3 \quad\left(7-\mathrm{CH}_{2}\right), \quad 25.2 \quad\left(5-\mathrm{CH}_{2}\right), \quad 19.2$ $\left(7-\mathrm{CH}_{2}\right), 16.7\left(\mathrm{~d},{ }^{3} J_{C P}=5.4 \mathrm{~Hz}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right), 16.5\left(\mathrm{~d},{ }^{3} J_{C P}=\right.$ 6.3 Hz, 1-POCH $\mathrm{CH}_{3}$ ). ${ }^{31} \mathrm{P}$ NMR ( $162 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta(22.97)$,
22.53. HRESIMS $m / z 511.2029[\mathrm{M}+\mathrm{H}]^{+}$(511.2026 calcd for $\mathrm{C}_{24} \mathrm{H}_{36} \mathrm{~N}_{2} \mathrm{O}_{6} \mathrm{PS}$ ).

## tert-Butyl 2-((benzo[b]thiophene-3-carboxamido)(di-

 ethoxyphosphoryl)methyl)piperidine-1-carboxylate (4h)Synthesis was performed according to the general procedure A, 3 ( $241 \mathrm{mg}, 0.689 \mathrm{mmol}$ ) and benzo[b]thiophene-3carboxylic acid ( $245 \mathrm{mg}, 1.38 \mathrm{mmol}, 2.0$ equiv.) gave the total product ( $257 \mathrm{mg}, 0.503 \mathrm{mmol}, 73 \%$ ). 4h-1 was obtained as a colorless solid ( $104 \mathrm{mg}, 0.204 \mathrm{mmol}, 30 \%$ ). 4h-2 was obtained as a colorless solid ( $153 \mathrm{mg}, 0.300 \mathrm{mmol}, 44 \%$ ).

## Compound 4h-1, diastereomer 1

${ }^{1} \mathrm{H}$ NMR ( $400 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta 8.43$ (d, $J=7.8 \mathrm{~Hz}, 1 \mathrm{H}, 17-\mathrm{H}$ ), 8.07 (br. s, $1 \mathrm{H}, 11-\mathrm{H}$ ), 7.87 (d, $J=7.8 \mathrm{~Hz}, 1 \mathrm{H}, 14-\mathrm{H}$ ), 7.45 (ddd, $J=7.6,7.6,1.3 \mathrm{~Hz}, 1 \mathrm{H}, 16-\mathrm{CH}), 7.40$ (ddd, $J=7.6,7.2$, $1.4 \mathrm{~Hz}, 1 \mathrm{H}, 15-\mathrm{CH}), 7.11(\mathrm{~m}, 1 \mathrm{H}, 1-\mathrm{NH}), 5.20\left(\mathrm{ddd},{ }^{2} J_{H P}=17.7\right.$ $\mathrm{Hz}, J=9.9,9.9 \mathrm{~Hz}, 1 \mathrm{H}, 1-\mathrm{H}), 4.66(\mathrm{~m}, 1 \mathrm{H}, 2-\mathrm{H}), 4.25-3.92(\mathrm{~m}$, $\left.5 \mathrm{H}, 1-\mathrm{P}\left(\mathrm{OCH}_{2}\right)_{2}, 4-\mathrm{H}\right), 3.05(\mathrm{~m}, 1 \mathrm{H}, 4-\mathrm{H}), 2.13-1.72(\mathrm{~m}, 2 \mathrm{H}$, $7-\mathrm{H}, 6-\mathrm{H}), 1.67-1.55(\mathrm{~m}, 3 \mathrm{H}, 5-\mathrm{H}, 6-\mathrm{H}, 7-\mathrm{H}), 1.48(\mathrm{~s}, 9 \mathrm{H}$, $\left.2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{OC}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), 1.40(\mathrm{~m}, 1 \mathrm{H}, 5-\mathrm{H}), 1.31(\mathrm{t}, J=7.1 \mathrm{~Hz}$, $\left.3 \mathrm{H}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right), 1.24\left(\mathrm{t}, J=6.9 \mathrm{~Hz}, 3 \mathrm{H}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right) \cdot{ }^{13} \mathrm{C}$ NMR (126 MHz, $\mathrm{CDCl}_{3}$ ) $\delta 163.4\left(\mathrm{~d}, J=4.7 \mathrm{~Hz}, 9-\mathrm{C}_{\mathrm{q}}\right), 155.0$ $\left(2-\mathrm{N}-\mathrm{C}_{\mathrm{q}}(\mathrm{O})-\mathrm{O}\right), 140.3\left(13-\mathrm{C}_{\mathrm{q}}\right), 137.1\left(18-\mathrm{C}_{\mathrm{q}}\right), 131.1\left(10-\mathrm{C}_{\mathrm{q}}\right)$, 130.6 (br. s, $11-\mathrm{C}_{\mathrm{q}}$ ), $125.4(16-\mathrm{CH}), 125.3(15-\mathrm{CH}), 124.5$ $(17-\mathrm{CH}), 122.6(14-\mathrm{CH}), 79.9\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{O}-\mathrm{C}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), 63.1$ $\left(\mathrm{d},{ }^{2} J_{C P}=4.4 \mathrm{~Hz}, 1-\mathrm{POCH}_{2}\right), 62.6\left(\mathrm{~d},{ }^{2} J_{C P}=5.5 \mathrm{~Hz}\right.$, 1- $\mathrm{POCH}_{2}$ ), $51.2(2-\mathrm{CH}), 44.9\left(\mathrm{~d},{ }^{1} J_{C P}=155.7 \mathrm{~Hz}, 1-\mathrm{CH}\right)$, $40.6\left(4-\mathrm{CH}_{2}\right), 28.6\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{OC}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), 25.5\left(\mathrm{~d},{ }^{3} J_{C P}=\right.$ $\left.10.1 \mathrm{~Hz}, 7-\mathrm{CH}_{2}\right), 24.9\left(5-\mathrm{CH}_{2}\right), 19.5\left(6-\mathrm{CH}_{2}\right), 16.6\left(\mathrm{~d},{ }^{3} J_{C P}=5.6\right.$ $\mathrm{Hz}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}$ ), $16.5\left(\mathrm{~d},{ }^{3} J_{C P}=6.2 \mathrm{~Hz}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right) .{ }^{31} \mathrm{P}$ NMR ( $162 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta 22.71$. HRESIMS $m / z 511.2016[\mathrm{M}+$ $\mathrm{H}]^{+}$(511.2026 calcd for $\mathrm{C}_{24} \mathrm{H}_{36} \mathrm{~N}_{2} \mathrm{O}_{6} \mathrm{PS}$ ).

## Compound 4h-2, diastereomer 2 (rotamer ratio 4.8:1)

${ }^{1} \mathrm{H}$ NMR ( $400 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta 8.46$ (d, $\left.J=7.9 \mathrm{~Hz}, 1 \mathrm{H}, 17-\mathrm{H}\right)$, 7.92 (s, 1H, 11-H), 8.46 (d, $J=7.9 \mathrm{~Hz}, 1 \mathrm{H}, 14-\mathrm{H}), 7.45$ (ddd, $J$ $=8.2,7.1,1.2 \mathrm{~Hz}, 1 \mathrm{H}, 16-\mathrm{H}), 7.39$ (ddd, $J=8.0,6.9,1.2 \mathrm{~Hz}$, $1 \mathrm{H}, 15-\mathrm{H}), 6.66(\mathrm{~d}, J=9.9 \mathrm{~Hz}, 1 \mathrm{H}, 1-\mathrm{NH}), 5.13\left(\mathrm{ddd},{ }^{2} J_{H P}=\right.$ $15.4 \mathrm{~Hz}, J=11.6,9.8 \mathrm{~Hz}, 1 \mathrm{H}, 1-\mathrm{H}), 4.74(\mathrm{~m}, 1 \mathrm{H}, 2-\mathrm{H}), 4.27-$ $4.15\left(\mathrm{~m}, 4 \mathrm{H}, 1-\mathrm{P}\left(\mathrm{OCH}_{2}\right)_{2}\right), 3.89(\mathrm{ddd}, J=13.6,4.7,2.1 \mathrm{~Hz}, 1 \mathrm{H}$, $4-\mathrm{H}), 2.90$ (ddd, $J=13.4,13.4,2.8 \mathrm{~Hz}, 1 \mathrm{H}, 4-\mathrm{H}), 2.42$ (m, 1H, $7-\mathrm{H}), 1.77-1.58\left(\mathrm{~m}, 4 \mathrm{H}, 5-\mathrm{H}, 6-\mathrm{H}_{2}, 7-\mathrm{H}\right), 1.43(\mathrm{~m}, 1 \mathrm{H}, 5-\mathrm{H})$, $1.35\left(\mathrm{t}, J=7.1 \mathrm{~Hz}, 3 \mathrm{H}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right), 1.31(\mathrm{t}, J=7.2 \mathrm{~Hz}, 3 \mathrm{H}$, 1- $\mathrm{POCH}_{2} \mathrm{CH}_{3}$ ), $1.16\left(\mathrm{~s}, 9 \mathrm{H}, 2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{OC}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right) \mathrm{ppm} .{ }^{31} \mathrm{P}$ NMR ( $\left.162 \mathrm{MHz}, \mathrm{CDCl}_{3}\right) \delta(23.40), 22.99 \mathrm{ppm}$. HRESIMS $\mathrm{m} / \mathrm{z}$ $511.2008[\mathrm{M}+\mathrm{H}]^{+}$(511.2026 calcd for $\mathrm{C}_{24} \mathrm{H}_{36} \mathrm{~N}_{2} \mathrm{O}_{6} \mathrm{PS}$ ).

## tert-Butyl 2-((diethoxyphosphoryl)(2-(thiophen-2-yl)-acetamido)methyl)piperidine-1-carboxylate (4i)

The synthesis was performed according to general procedure A, 3 ( $255 \mathrm{mg}, 0.897 \mathrm{mmol}$ ) and 2-(thiophen-2-yl)acetic acid ( $255 \mathrm{mg}, 1.79 \mathrm{mmol}, 2.0$ equiv.) gave the total product ( 256 $\mathrm{mg}, 0.539 \mathrm{mmol}, 60 \%$ ). 4i-1 was obtained as a colorless solid
( $125 \mathrm{mg}, 0.238 \mathrm{mmol}, 27 \%$ ). 4i-2 was obtained as a colorless solid ( $139 \mathrm{mg}, 0.293 \mathrm{mmol}, 33 \%$ ).

## Compound 4i-1, diastereomer 1

${ }^{1} \mathrm{H}$ NMR $\left(400 \mathrm{MHz}, \mathrm{CDCl}_{3}\right) \delta 7.23(\mathrm{dd}, J=4.7,1.7 \mathrm{~Hz}, 1 \mathrm{H}, 13-$ CH), 6.98 (dd, $J=3.5,3.5 \mathrm{~Hz}, 1 \mathrm{H}, 14-\mathrm{H}), 6.97(\mathrm{~m}, 1 \mathrm{H}, 15-\mathrm{H})$, $6.46(\mathrm{~m}, 1 \mathrm{H}, 1-\mathrm{NH}), 4.90\left(\mathrm{ddd},{ }^{2} J_{H P}=16.0 \mathrm{~Hz}, J=10.2,10.2\right.$ $\mathrm{Hz}, 1 \mathrm{H}, 1-\mathrm{H}), 4.41(\mathrm{~m}, 1 \mathrm{H}, 2-\mathrm{H}), 4.16-3.88\left(\mathrm{~m}, 5 \mathrm{H}, 1-\mathrm{P}\left(\mathrm{OCH}_{2}\right)_{2}\right.$, $4-\mathrm{H}), 3.83$ (s, 2H, 10- $\mathrm{H}_{2}$ ), 2.85 (m, 1H, 4-H), 1.69-1.42 (m, 5H, $\left.5-\mathrm{H}, 6-\mathrm{H}_{2}, 7-\mathrm{H}_{2}\right), 1.44\left(\mathrm{~s}, 9 \mathrm{H}, 2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{OC}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), 1.37(\mathrm{~m}$, $1 \mathrm{H}, 5-\mathrm{H}), 1.27\left(\mathrm{t}, J=7.1 \mathrm{~Hz}, 3 \mathrm{H}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right), 1.22(\mathrm{t}, J=7.1$ $\left.\mathrm{Hz}, 3 \mathrm{H}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right) .{ }^{13} \mathrm{C}$ NMR ( $101 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta 169.5(\mathrm{~d}$, $\left.J=3.7 \mathrm{~Hz}, 9-\mathrm{C}_{\mathrm{q}}\right), 154.6\left(2-\mathrm{N}-\mathrm{C}_{\mathrm{q}}(\mathrm{O})-\mathrm{O}\right), 135.9\left(11-\mathrm{C}_{\mathrm{q}}\right), 127.4(14-$ CH or $15-\mathrm{CH}), 127.4(14-\mathrm{CH}$ or $15-\mathrm{CH}), 125.6(13-\mathrm{CH}), 79.8$ $\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{O}-\mathrm{C}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), 62.9\left(\mathrm{~d},{ }^{2} J_{C P}=7.2 \mathrm{~Hz}, 1-\mathrm{POCH}_{2}\right), 62.4$ $\left(\mathrm{d},{ }^{2} J_{C P}=6.5 \mathrm{~Hz}, 1-\mathrm{POCH}_{2}\right), 50.7(2-\mathrm{CH}), 44.8\left(\mathrm{~d},{ }^{1} J_{C P}=155.2\right.$ $\mathrm{Hz}, 1-\mathrm{CH}), 40.6\left(4-\mathrm{CH}_{2}\right), 37.5\left(10-\mathrm{CH}_{2}\right), 28.5\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O})\right.$ $\left.\mathrm{OC}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), 25.2\left(\mathrm{~d},{ }^{3} J_{C P}=9.8 \mathrm{~Hz}, 7-\mathrm{CH}_{2}\right), 24.9\left(5-\mathrm{CH}_{2}\right)$, $19.4\left(6-\mathrm{CH}_{2}\right), 16.5\left(\mathrm{~d},{ }^{3} J_{C P}=6.0 \mathrm{~Hz}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right), 16.5$ $\left(\mathrm{d},{ }^{3} J_{C P}=6.5 \mathrm{~Hz}, \quad 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right) .{ }^{31} \mathrm{P}$ NMR ( 162 MHz , $\left.\mathrm{CDCl}_{3}\right) \quad \delta$ 22.15. HRESIMS m/z $475.1967 \quad[\mathrm{M}+\mathrm{H}]^{+}$ (475.2026 calcd for $\mathrm{C}_{21} \mathrm{H}_{36} \mathrm{~N}_{2} \mathrm{O}_{6}$ PS).

## Compound 4i-2, diastereomer 2 (rotamer ratio 3.2:1)

${ }^{1} \mathrm{H}$ NMR ( $400 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta 7.21$ (d, $J=5.0 \mathrm{~Hz}, 1 \mathrm{H}, 13-\mathrm{H}$ ), 6.96 (dd, $J=5.0,3.5 \mathrm{~Hz}, 1 \mathrm{H}, 14-\mathrm{H}), 6.93(\mathrm{~m}, 1 \mathrm{H}, 15-\mathrm{H}), 6.10$ $(\mathrm{m}, 1 \mathrm{H}, 1-\mathrm{NH}), 4.84\left(\mathrm{ddd},{ }^{2} J_{H P}=15.4 \mathrm{~Hz}, J=11.5,10.1 \mathrm{~Hz}\right.$, $1 \mathrm{H}, 1-\mathrm{H}), 4.55(\mathrm{~m}, 1 \mathrm{H}, 2-\mathrm{H}), 4.19-3.98\left(\mathrm{~m}, 4 \mathrm{H}, 1-\mathrm{P}\left(\mathrm{OCH}_{2}\right)_{2}\right)$, $3.88(\mathrm{~m}, 1 \mathrm{H}, 4-\mathrm{H}), 3.69\left(\mathrm{~s}, 2 \mathrm{H}, 10-\mathrm{H}_{2}\right), 2.76(\mathrm{~m}, 1 \mathrm{H}, 4-\mathrm{H}), 2.30$ (m, 1H, 7-H), 1.70-1.53 (m, 4H, 5-H, 6-H2, 7-H), 1.45 (s, 9H, $\left.2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{OC}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), 1.35(\mathrm{~m}, 1 \mathrm{H}, 5-\mathrm{H}), 1.25(\mathrm{t}, J=7.1 \mathrm{~Hz}$, $\left.6 \mathrm{H}, 1-\mathrm{P}\left(\mathrm{OCH}_{2} \mathrm{CH}_{3}\right)_{2}\right) .{ }^{13} \mathrm{C}$ NMR ( $101 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta 169.5(\mathrm{~d}, J$ $\left.=3.2 \mathrm{~Hz}, 9-\mathrm{C}_{\mathrm{q}}\right), 156.3\left(2-\mathrm{N}-\mathrm{C}_{\mathrm{q}}(\mathrm{O})-\mathrm{O}\right), 135.8\left(11-\mathrm{C}_{\mathrm{q}}\right), 127.2(14-$ $\mathrm{CH}), 127.1(15-\mathrm{CH}), 125.4(13-\mathrm{CH}), 80.0 \quad\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{O}-\mathrm{C}_{\mathrm{q}}-\right.$ $\left.\left(\mathrm{CH}_{3}\right)_{3}\right), 62.9$ (br. s, 1- $\mathrm{POCH}_{2}$ ), 62.6 (br. s, 1- $\mathrm{POCH}_{2}$ ), 49.9 $(2-\mathrm{CH}), 45.5\left(\mathrm{~d},{ }^{1} J_{C P}=163.1 \mathrm{~Hz}, 1-\mathrm{CH}\right), 40.1\left(4-\mathrm{CH}_{2}\right), 37.7(10-$ $\left.\mathrm{CH}_{2}\right), 28.6\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{OC}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), 26.3\left(7-\mathrm{CH}_{2}\right), 25.2\left(5-\mathrm{CH}_{2}\right)$, $19.1\left(6-\mathrm{CH}_{2}\right), 16.5\left(\mathrm{~d},{ }^{3} J_{C P}=5.6 \mathrm{~Hz}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right), 16.4(\mathrm{~d}$, $\left.{ }^{3} J_{C P}=6.2 \mathrm{~Hz}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right) .{ }^{31} \mathrm{P}$ NMR ( $162 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta$ (22.97), 22.44. HRESIMS $m / z 475.1997[\mathrm{M}+\mathrm{H}]^{+}$(475.2026 calcd for $\mathrm{C}_{21} \mathrm{H}_{36} \mathrm{~N}_{2} \mathrm{O}_{6} \mathrm{PS}$ ).

## tert-Butyl 2-((diethoxyphosphoryl)(2-(thiophen-3-yl)-acetamido)methyl)piperidine-1-carboxylate (4j)

The synthesis was performed according to general procedure A, 3 ( $235 \mathrm{mg}, 0.670 \mathrm{mmol}$ ) and 2-(thiophene-3-yl)acetic acid ( $191 \mathrm{mg}, 1.34 \mathrm{mmol}, 2.0$ equiv.) gave the total product ( 254 $\mathrm{mg}, 0.535 \mathrm{mmol}, 80 \%$ ). $\mathbf{4 j} \mathbf{- 1}$ was obtained as s slightly yellow solid ( $109 \mathrm{mg}, 0.230 \mathrm{mmol}, 34 \%$ ). $4 \mathrm{j}-2$ was obtained as a slightly yellow solid ( $145 \mathrm{mg}, 0.306 \mathrm{mmol}, 46 \%$ ).

## Compound 4j-1, diastereomer 1

${ }^{1} \mathrm{H}$ NMR ( $400 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta 7.32$ (dd, $J=5.0,2.9 \mathrm{~Hz}, 1 \mathrm{H}, 14-$ H), $7.18(\mathrm{~d}, J=2.2 \mathrm{~Hz}, 1 \mathrm{H}, 12-\mathrm{H}), 7.01(\mathrm{~d}, J=5.0 \mathrm{~Hz}, 1 \mathrm{H}, 15-$
H), 6.09 (br. s, 1H, 1-NH), 4.90 (ddd, ${ }^{2} J_{C P}=17.2 \mathrm{~Hz}, J=10.2$, $10.2 \mathrm{~Hz}, 1 \mathrm{H}, 1-\mathrm{H}), 4.37(\mathrm{~m}, 1 \mathrm{H}, 2-\mathrm{H}), 4.14-3.89(\mathrm{~m}, 5 \mathrm{H}$, $\left.1-\mathrm{P}\left(\mathrm{OCH}_{2}\right)_{2}, 4-\mathrm{H}\right), 3.63\left(\mathrm{~s}, 2 \mathrm{H}, 10-\mathrm{H}_{2}\right), 2.85(\mathrm{~m}, 1 \mathrm{H}, 4-\mathrm{H}), 1.63-$ $1.48\left(\mathrm{~m}, 5 \mathrm{H}, 5-\mathrm{H}, 6-\mathrm{H}_{2}, 7-\mathrm{H}_{2}\right), 1.45\left(\mathrm{~s}, 9 \mathrm{H}, 2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{OC}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right)$, $1.35(\mathrm{~m}, 1 \mathrm{H}, 5-\mathrm{H}), 1.27\left(\mathrm{t}, J=7.1 \mathrm{~Hz}, 3 \mathrm{H}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right), 1.22(\mathrm{t}$, $\left.J=7.1 \mathrm{~Hz}, 3 \mathrm{H}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right) .{ }^{13} \mathrm{C}$ NMR ( $126 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta$ $170.0\left(9-\mathrm{C}_{\mathrm{q}}\right), 154.6\left(2-\mathrm{N}-\mathrm{C}_{\mathrm{q}}(\mathrm{O})-\mathrm{O}\right), 134.5\left(11-\mathrm{C}_{\mathrm{q}}\right), 128.5(15-\mathrm{CH})$, $126.8(14-\mathrm{CH}), 123.5(12-\mathrm{CH}), 79.8\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{O}-\mathrm{C}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), 62.7$ (br. s, 1-POCH 2 ), $62.5\left(\mathrm{~d},{ }^{2} J_{C P}=6.6 \mathrm{~Hz}, 1-\mathrm{POCH}_{2}\right), 50.8(2-\mathrm{CH})$, $44.7\left(\mathrm{~d},{ }^{1} J_{C P}=154.7 \mathrm{~Hz}, 1-\mathrm{CH}\right), 40.5\left(4-\mathrm{CH}_{2}\right), 38.2\left(10-\mathrm{CH}_{2}\right), 28.5$ $\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{OC}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), 25.2\left(\mathrm{~d},{ }^{3} J_{C P}=9.7 \mathrm{~Hz}, 7-\mathrm{CH}_{2}\right), 24.8$ $\left(5-\mathrm{CH}_{2}\right), 19.4\left(6-\mathrm{CH}_{2}\right), 16.5\left(\mathrm{~d},{ }^{3} J_{C P}=5.9 \mathrm{~Hz}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right), 16.5$ $\left(\mathrm{d},{ }^{3} J_{C P}=6.6 \mathrm{~Hz}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right) .{ }^{31} \mathrm{P}$ NMR ( $162 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta$ 22.37. HRESIMS $m / z 475.2032[\mathrm{M}+\mathrm{H}]^{+}(475.2026$ calcd for $\left.\mathrm{C}_{21} \mathrm{H}_{36} \mathrm{~N}_{2} \mathrm{O}_{6} \mathrm{PS}\right)$.

## Compound 4j-2, diastereomer 2 (rotamer ratio 2.8:1)

${ }^{1} \mathrm{H}$ NMR ( $400 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta 7.27(\mathrm{dd}, J=5.0,3.1 \mathrm{~Hz}, 1 \mathrm{H}, 14-$ H), $7.15-7.12(\mathrm{~m}, 1 \mathrm{H}, 12-\mathrm{H}), 7.00(\mathrm{dd}, J=5.0,1.3 \mathrm{~Hz}, 1 \mathrm{H}, 15-$ H), $6.12(\mathrm{~d}, J=9.9 \mathrm{~Hz}, 1 \mathrm{H}, 1-\mathrm{NH}), 4.84\left(\mathrm{ddd},{ }^{2} J_{C P}=15.5 \mathrm{~Hz}, J\right.$ $=11.6,10.0 \mathrm{~Hz}, 1 \mathrm{H}, 1-\mathrm{H}), 4.56(\mathrm{~m}, 1 \mathrm{H}, 2-\mathrm{H}), 4.19-3.95(\mathrm{~m}$, $\left.4 \mathrm{H}, 1-\mathrm{P}\left(\mathrm{OCH}_{2}\right)_{2}\right), 3.88(\mathrm{~m}, 1 \mathrm{H}, 4-\mathrm{H}), 3.50(\mathrm{~s}, 2 \mathrm{H}, 10-\mathrm{H}), 2.78(\mathrm{dd}$, $J=13.4,13.4,2.6 \mathrm{~Hz}, 1 \mathrm{H}, 4-\mathrm{H}), 2.32(\mathrm{~m}, 1 \mathrm{H}, 7-\mathrm{H}), 1.67-1.55(\mathrm{~m}$, $\left.4 \mathrm{H}, 5-\mathrm{H}, 6-\mathrm{H}_{2}, 7-\mathrm{H}\right), 1.44\left(\mathrm{~s}, 9 \mathrm{H}, 2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{OC}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), 1.36(\mathrm{~m}$, $1 \mathrm{H}, 5-\mathrm{H}), 1.23\left(\mathrm{t}, J=6.9 \mathrm{~Hz}, 3 \mathrm{H}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right), 1.22(\mathrm{t}, J=6.7$ $\left.\mathrm{Hz}, 3 \mathrm{H}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right) .{ }^{13} \mathrm{C}$ NMR ( $126 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta 169.9(\mathrm{~d}$, $\left.{ }^{3} J_{C P}=3.6 \mathrm{~Hz}, 9-\mathrm{C}_{\mathrm{q}}\right), 156.4\left(2-\mathrm{N}-\mathrm{C}_{\mathrm{q}}(\mathrm{O})-\mathrm{O}\right), 134.5\left(11-\mathrm{C}_{\mathrm{q}}\right), 128.5$ $(15-\mathrm{CH}), 126.2(14-\mathrm{CH}), 123.0(12-\mathrm{CH}), 80.0\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{O}-\mathrm{C}_{\mathrm{q}}-\right.$ $\left.\left(\mathrm{CH}_{3}\right)_{3}\right), 62.8\left(\mathrm{~d},{ }^{2} J_{C P}=6.0 \mathrm{~Hz}, 1-\mathrm{POCH}_{2}\right), 62.6\left(\mathrm{~d},{ }^{2} J_{C P}=7.2 \mathrm{~Hz}\right.$, $\left.1-\mathrm{POCH}_{2}\right), 49.8\left(\mathrm{~d},{ }^{2} J_{C P}=12.0 \mathrm{~Hz}, 2-\mathrm{CH}\right), 45.3\left(\mathrm{~d},{ }^{1} J_{C P}=151.5\right.$ $\mathrm{Hz}, 1-\mathrm{CH}), 40.1\left(4-\mathrm{CH}_{2}\right), 38.3\left(10-\mathrm{CH}_{2}\right), 28.6\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{OC}_{\mathrm{q}}{ }^{-}\right.$ $\left.\left(\mathrm{CH}_{3}\right)_{3}\right), 26.2\left(7-\mathrm{CH}_{2}\right), 25.2\left(5-\mathrm{CH}_{2}\right), 19.2\left(6-\mathrm{CH}_{2}\right), 16.5\left(\mathrm{~d},{ }^{3} J_{C P}=\right.$ $5.4 \mathrm{~Hz}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}$ ), $16.4\left(\mathrm{~d},{ }^{3} J_{C P}=6.2 \mathrm{~Hz}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right) .{ }^{31} \mathrm{P}$ NMR ( $162 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta$ (23.29), 22.69. HRESIMS $\mathrm{m} / \mathrm{z}$ $475.2012[\mathrm{M}+\mathrm{H}]^{+}$(475.2026 calcd for $\left.\mathrm{C}_{21} \mathrm{H}_{36} \mathrm{~N}_{2} \mathrm{O}_{6} \mathrm{PS}\right)$.

## tert-Butyl 2-((2-(benzo[b]thiophen-2-yl)acetamido)(di-ethoxyphosphoryl)methyl)piperidine-1-carboxylate (4k)

The synthesis was performed according to general procedure A, 3 ( $308 \mathrm{mg}, 0.879 \mathrm{mmol}$ ) and 2-(benzo[b]thiophen-2-yl) acetic acid ( $330 \mathrm{mg}, 1.72 \mathrm{mmol}, 2.0$ equiv.) gave the total product ( $280 \mathrm{mg}, 0.534 \mathrm{mmol}, 61 \%$ ). 4k-1 was obtained as a slightly orange solid ( $125 \mathrm{mg}, 0.238 \mathrm{mmol}, 27 \%$ ). $\mathbf{4 k}-2$ was obtained as a slightly orange solid ( $154 \mathrm{mg}, 0.294 \mathrm{mg}, 33 \%$ ).

## Compound $\mathbf{4 k} \mathbf{k} \mathbf{1}$, diastereomer 1

${ }^{1} \mathrm{H}$ NMR ( $400 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta 7.74(\mathrm{dd}, J=7.8,1.0 \mathrm{~Hz}, 1 \mathrm{H}, 14-$ H), 7.67 (dd, $J=6.9,1.1 \mathrm{~Hz}, 1 \mathrm{H}, 17-\mathrm{H}), 7.30(\mathrm{ddd}, J=7.3,7.3$, $1.3 \mathrm{~Hz}, 1 \mathrm{H}$, part C of ABCD, 16-H), 7.24 (ddd, $J=7.3,7.3,1.3$ $\mathrm{Hz}, 1 \mathrm{H}$, part D of ABCD, $15-\mathrm{H}), 7.18$ (d, $J=1.1 \mathrm{~Hz}, 1 \mathrm{H}, 19-\mathrm{H})$, 7.00 (br. s, 1H, 1-NH), 4.89 (m, 1H, 1-H), 4.44 (m, 1H, 2-H), 4.16-3.90 (m, 5H, 1-P( $\left.\left.\mathrm{OCH}_{2}\right)_{2}, 4-\mathrm{H}\right), 3.88\left(\mathrm{~s}, 2 \mathrm{H}, 10-\mathrm{H}_{2}\right), 2.99-$ $2.69(\mathrm{~m}, 1 \mathrm{H}, 4-\mathrm{H}), 1.73-1.41\left(\mathrm{~m}, 5 \mathrm{H}, 5-\mathrm{H}, 6-\mathrm{H}_{2}, 7-\mathrm{H}_{2}\right), 1.40(\mathrm{~s}$, $\left.9 \mathrm{H}, 2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{OC}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), 1.34(\mathrm{~m}, 1 \mathrm{H}, 5-\mathrm{H}), 1.24(\mathrm{t}, J=7.1$ $\left.\mathrm{Hz}, 3 \mathrm{H}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right), 1.12\left(\mathrm{t}, J=7.1 \mathrm{~Hz}, 3 \mathrm{H}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right)$.
${ }^{13} \mathrm{C}$ NMR ( $\left.126 \mathrm{MHz}, \mathrm{CDCl}_{3}\right) \delta 168.9\left(9-\mathrm{C}_{\mathrm{q}}\right), 154.5\left(2-\mathrm{N}-\mathrm{C}_{\mathrm{q}}(\mathrm{O})-\right.$ O), $140.1\left(13-\mathrm{C}_{\mathrm{q}}\right), 139.8\left(18-\mathrm{C}_{\mathrm{q}}\right), 137.2\left(11-\mathrm{C}_{\mathrm{q}}\right), 124.4(16-\mathrm{CH})$, $124.2(14-\mathrm{CH}), 123.8(19-\mathrm{CH}), 123.3(17-\mathrm{CH}), 122.2(14-\mathrm{CH})$, $79.6\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{O}-\mathrm{C}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), 62.8\left(\mathrm{~d},{ }^{2} J_{C P}=7.1 \mathrm{~Hz}, 1-\mathrm{POCH}_{2}\right)$, $62.3\left(\mathrm{~d},{ }^{2} J_{C P}=6.7 \mathrm{~Hz}, 1-\mathrm{POCH}_{2}\right), 50.7(2-\mathrm{CH}), 44.7\left(\mathrm{~d},{ }^{1} J_{C P}=\right.$ $156.0 \mathrm{~Hz}, 1-\mathrm{CH}), 40.4\left(4-\mathrm{CH}_{2}\right), 38.28\left(10-\mathrm{CH}_{2}\right), 28.4\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O})\right.$ $\left.\mathrm{OC}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), 25.2\left(\mathrm{~d},{ }^{3} J_{C P}=10.0 \mathrm{~Hz}, 7-\mathrm{CH}_{2}\right), 24.8\left(5-\mathrm{CH}_{2}\right)$, $19.3\left(6-\mathrm{CH}_{2}\right), 16.4\left(\mathrm{~d},{ }^{3} J_{C P}=5.9 \mathrm{~Hz}, 1-\mathrm{P}\left(\mathrm{OCH}_{2} \mathrm{CH}_{3}\right)_{2}\right) .{ }^{31} \mathrm{P}$ NMR $\left(162 \mathrm{MHz}, \mathrm{CDCl}_{3}\right) \delta$ 22.30. HRESIMS $m / z 525.2177[\mathrm{M}+\mathrm{H}]^{+}$ (525.2183 calcd for $\mathrm{C}_{25} \mathrm{H}_{38} \mathrm{~N}_{2} \mathrm{O}_{6} \mathrm{PS}$ ).

## Compound 4k-2, diastereomer 2 (rotamer ratio 2.7:1)

${ }^{1} \mathrm{H}$ NMR ( $500 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta 7.69$ (d, $J=7.8 \mathrm{~Hz}, 1 \mathrm{H}, 14-\mathrm{H}$ ), $7.62(\mathrm{~d}, J=7.5 \mathrm{~Hz}, 1 \mathrm{H}, 17-\mathrm{H}), 7.24(\mathrm{~m}, 1 \mathrm{H}, 16-\mathrm{H}), 7.21(\mathrm{~m}$, $1 \mathrm{H}, 15-\mathrm{H}), 7.09(\mathrm{~s}, 1 \mathrm{H}, 19-\mathrm{H}), 6.43(\mathrm{~m}, 1 \mathrm{H}, 1-\mathrm{NH}), 4.80$ (ddd, $\left.{ }^{2} J_{H P}=15.5 \mathrm{~Hz}, J=10.7,10.7 \mathrm{~Hz}, 1 \mathrm{H}, 1-\mathrm{H}\right), 4.51(\mathrm{~m}, 1 \mathrm{H}, 2-\mathrm{H})$, 4.10-3.89 (m, 4H, 1-P( $\left.\left.\mathrm{OCH}_{2}\right)_{2}\right), 3.83(\mathrm{~m}, 1 \mathrm{H}, 4-\mathrm{H}), 3.70\left(\mathrm{~d},{ }^{2} J=\right.$ $15.9 \mathrm{~Hz}, 1 \mathrm{H}$, part A of $\mathrm{AB}, 10-\mathrm{H}), 3.68\left(\mathrm{~d},{ }^{2} J=15.9 \mathrm{~Hz}, 1 \mathrm{H}\right.$, part B of AB, 10-H), 2.74 (ddd, $J=13.4,13.4,2.6 \mathrm{~Hz}, 1 \mathrm{H}, 4-\mathrm{H}$ ), 2.25 (br. d, $J=10.5 \mathrm{~Hz}, 1 \mathrm{H}, 7-\mathrm{H}$ ), 1.60-1.49 (m, 4H, $5-\mathrm{H}, 6-\mathrm{H}_{2}$, $7-\mathrm{H}), 1.37\left(\mathrm{~s}, 9 \mathrm{H}, 2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{OC}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), 1.32(\mathrm{~m}, 1 \mathrm{H}, 5-\mathrm{H})$, $1.15\left(\mathrm{t}, J=7.1 \mathrm{~Hz}, 3 \mathrm{H}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right), 1.08(\mathrm{t}, J=7.0 \mathrm{~Hz}, 3 \mathrm{H}$, 1- $\mathrm{POCH}_{2} \mathrm{CH}_{3}$ ). ${ }^{13} \mathrm{C}$ NMR ( $126 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta 168.5(\mathrm{~d}, J=3.3$ $\left.\mathrm{Hz}, 9-\mathrm{C}_{\mathrm{q}}\right), 156.2\left(2-\mathrm{N}-\mathrm{C}_{\mathrm{q}}(\mathrm{O})-\mathrm{O}\right), 140.0\left(13-\mathrm{C}_{\mathrm{q}}\right), 139.7\left(18-\mathrm{C}_{\mathrm{q}}\right)$, 137.1 ( $11-\mathrm{C}_{\mathrm{q}}$ ), 124.3 ( $16-\mathrm{CH}$ ), 124.1 ( $\left.15-\mathrm{CH}\right), 123.3$ ( $19-\mathrm{CH}$ ), $123.2(17-\mathrm{CH}), 122.0(14-\mathrm{CH}), 79.9\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{O}-\mathrm{C}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right)$, $62.7\left(\mathrm{~d},{ }^{2} J_{C P}=5.2 \mathrm{~Hz}, 1-\mathrm{POCH}_{2}\right), 62.6\left(\mathrm{~d},{ }^{2} J_{C P}=7.3 \mathrm{~Hz}\right.$, 1- $\mathrm{POCH}_{2}$ ), $49.7\left(\mathrm{~d},{ }^{2} J_{C P}=11.9 \mathrm{~Hz}, 2-\mathrm{CH}\right), 45.4\left(\mathrm{~d},{ }^{1} J_{C P}=151.6\right.$ $\mathrm{Hz}, 1-\mathrm{CH}), 40.0\left(4-\mathrm{CH}_{2}\right), 38.3\left(10-\mathrm{CH}_{2}\right), 28.4\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{OC}_{\mathrm{q}}-\right.$ $\left.\left(\mathrm{CH}_{3}\right)_{3}\right), 26.1\left(7-\mathrm{CH}_{2}\right), 25.1\left(5-\mathrm{CH}_{2}\right), 19.0\left(6-\mathrm{CH}_{2}\right), 16.3\left(\mathrm{~d},{ }^{3} J_{C P}\right.$ $\left.=3.9 \mathrm{~Hz}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right), 16.2\left(\mathrm{~d},{ }^{3} J_{C P}=4.9 \mathrm{~Hz}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right)$. ${ }^{31} \mathrm{P}$ NMR ( $162 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta(22.87)$, 22.42. HRESIMS $\mathrm{m} / \mathrm{z}$ $525.2191[\mathrm{M}+\mathrm{H}]^{+}\left(525.2183\right.$ calcd for $\left.\mathrm{C}_{25} \mathrm{H}_{38} \mathrm{~N}_{2} \mathrm{O}_{6} \mathrm{PS}\right)$.

## tert-Butyl 2-((2-(benzo[b]thiophen-3-yl)acetamido)(di-ethoxyphosphoryl)methyl)piperidine-1-carboxylate (41)

The synthesis was performed according to general procedure A, 3 ( $301 \mathrm{mg}, 0.860 \mathrm{mmol}$ ) and 2-(benzo[b]thiophen-3-yl) acetic acid ( $330 \mathrm{mg}, 1.72 \mathrm{mmol}, 2.0$ equiv.) gave the total product ( $304 \mathrm{mg}, 0.579 \mathrm{mg}, 68 \%$ ). 4l-1 was obtained as a colorless solid ( $141 \mathrm{mg}, 0.269 \mathrm{mmol}, 31 \%$ ). 41-2 was obtained as a colorless solid ( $163 \mathrm{mg}, 0.311 \mathrm{mmol}, 36 \%$ ).

## Compound 4l-1, diastereomer 1

${ }^{1} \mathrm{H}$ NMR ( $400 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta 7.86(\mathrm{~m}, 1 \mathrm{H}, 15-\mathrm{H}), 7.78(\mathrm{~m}$, $1 \mathrm{H}, 18-\mathrm{H}), 7.44-7.32$ (m, 3H, $16-\mathrm{H}, 17-\mathrm{H}, 12-\mathrm{H}$ ), 6.30 (br. $\mathrm{s}, 1 \mathrm{H}, 1-\mathrm{NH}), 4.88\left(\mathrm{ddd},{ }^{2} J_{P H}=18.8 \mathrm{~Hz}, J=10.5,10.5 \mathrm{~Hz}\right.$, $1 \mathrm{H}, \quad 1-\mathrm{H}), \quad 4.32(\mathrm{~m}, ~ 1 \mathrm{H}, 2-\mathrm{H}), 4.07-3.88(\mathrm{~m}, ~ 4 \mathrm{H}$, $\left.1-\mathrm{P}\left(\mathrm{OCH}_{2}\right)_{2}\right), 3.86\left(\mathrm{~s}, 2 \mathrm{H}, 10-\mathrm{H}_{2}\right), 3.83(\mathrm{~m}, 1 \mathrm{H}, 4-\mathrm{H}), 2.82$ (m, 1H, 4-H), 1.61-1.47 (m, 4H, 5-H, 6-H2, 7-H), 1.41 (s, $\left.9 \mathrm{H}, \quad 2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{OC}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), \quad 1.38-1.27(\mathrm{~m}, \quad 2 \mathrm{H}, \quad 5-\mathrm{H}, \quad 7-\mathrm{H})$, $1.23\left(\mathrm{t}, J=7.1 \mathrm{~Hz}, 3 \mathrm{H}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right), 1.09(\mathrm{t}, J=7.1 \mathrm{~Hz}$, $\left.3 \mathrm{H}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right) .{ }^{13} \mathrm{C}$ NMR ( $126 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta 169.5$ $\left(9-\mathrm{C}_{\mathrm{q}}\right), 154.7\left(2-\mathrm{N}-\mathrm{C}_{\mathrm{q}}(\mathrm{O})-\mathrm{O}\right), 140.6\left(14-\mathrm{C}_{\mathrm{q}}\right), 138.4\left(19-\mathrm{C}_{\mathrm{q}}\right)$, $129.2\left(11-\mathrm{C}_{\mathrm{q}}\right), 125.3(12-\mathrm{CH}), 124.9(16-\mathrm{CH}), 124.5(17-\mathrm{CH})$,
$123.0(15-\mathrm{CH}), 122.1(18-\mathrm{CH}), 79.7\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{O}-\mathrm{C}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right)$, $62.6\left(\mathrm{~d},{ }^{2} J_{C P}=4.3 \mathrm{~Hz}, 1-\mathrm{POCH}_{2}\right), 62.4\left(\mathrm{~d},{ }^{2} J_{C P}=6.5 \mathrm{~Hz}\right.$, $\left.1-\mathrm{POCH}_{2}\right), 50.7(2-\mathrm{CH}), 44.8\left(\mathrm{~d},{ }^{1} J_{C P}=154.6 \mathrm{~Hz}, 1-\mathrm{CH}\right)$, $40.4\left(4-\mathrm{CH}_{2}\right), 36.89\left(10-\mathrm{CH}_{2}\right), 28.5\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{OC}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right)$, $25.2\left(\mathrm{~d},{ }^{3} J_{C P}=9.4 \mathrm{~Hz}, 7-\mathrm{CH}_{2}\right), 24.7\left(5-\mathrm{CH}_{2}\right), 19.27\left(6-\mathrm{CH}_{2}\right)$, $16.4\left(\mathrm{~d},{ }^{3} J_{C P}=6.1 \mathrm{~Hz}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right), 16.4\left(\mathrm{~d},{ }^{3} J_{C P}=5.9\right.$ $\left.\mathrm{Hz}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right) .{ }^{31} \mathrm{P}$ NMR ( $162 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta 22.05$. HRESIMS $\mathrm{m} / \mathrm{z} 525.2181[\mathrm{M}+\mathrm{H}]^{+}(525.2183$ calcd for $\left.\mathrm{C}_{25} \mathrm{H}_{38} \mathrm{~N}_{2} \mathrm{O}_{6} \mathrm{PS}\right)$.

## Compound 4l-2, diastereomer 2 (rotamer ratio 2.5:1)

${ }^{1} \mathrm{H}$ NMR ( $400 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta 7.84(\mathrm{~d}, J=7.0 \mathrm{~Hz}, 1 \mathrm{H}, 15-\mathrm{H})$, $7.74(\mathrm{~d}, J=7.5 \mathrm{~Hz}, 1 \mathrm{H}, 18-\mathrm{H}), 7.38$ (dd, $J=7.0,7.0 \mathrm{~Hz}, 1 \mathrm{H}$, $17-\mathrm{H}), 7.35$ (m, 1H, 12-H), 7.33 (m, 1H, 16-H), 6.17 (d, $J=$ $9.9 \mathrm{~Hz}, 1 \mathrm{H}, 1-\mathrm{NH}), 4.84\left(\mathrm{ddd},{ }^{2} J_{H P}=15.4 \mathrm{~Hz}, J=11.7\right.$, 9.9 $\mathrm{Hz}, 1 \mathrm{H}, 1-\mathrm{H}), 4.55(\mathrm{~m}, 1 \mathrm{H}, 2-\mathrm{H}), 4.14-3.80(\mathrm{~m}, 5 \mathrm{H}$, $\left.1-\mathrm{P}\left(\mathrm{OCH}_{2}\right)_{2}, 4-\mathrm{H}\right), 3.71\left(\mathrm{~s}, 2 \mathrm{H}, 10-\mathrm{H}_{2}\right), 2.82$ (ddd, $J=13.5$, $13.5,2.6 \mathrm{~Hz}, 1 \mathrm{H}, 4-\mathrm{H}), 2.34(\mathrm{~m}, 1 \mathrm{H}, 7-\mathrm{H}), 1.67-1.55(\mathrm{~m}, 4 \mathrm{H}$, $\left.5-\mathrm{H}, 6-\mathrm{H}_{2}, 7-\mathrm{H}\right), 1.46\left(\mathrm{~s}, 9 \mathrm{H}, 2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{OC}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), 1.43(\mathrm{~m}$, $1 \mathrm{H}, 5-\mathrm{H}), 1.14\left(\mathrm{t}, J=7.1 \mathrm{~Hz}, 3 \mathrm{H}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right), 1.08(\mathrm{t}, J=$ $\left.7.1 \mathrm{~Hz}, 3 \mathrm{H}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right) \cdot{ }^{13} \mathrm{C}$ NMR ( $126 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta$ $169.2\left(\mathrm{~d}, J=3.6 \mathrm{~Hz}, 9-\mathrm{C}_{\mathrm{q}}\right), 156.3$ (2-N-C $\left.\mathrm{C}_{\mathrm{q}}(\mathrm{O})-\mathrm{O}\right), 140.3$ $\left(14-\mathrm{C}_{\mathrm{q}}\right), 138.4\left(19-\mathrm{C}_{\mathrm{q}}\right), 129.1\left(11-\mathrm{C}_{\mathrm{q}}\right), 124.5(16-\mathrm{CH}$ or $12-\mathrm{CH})$, $124.5(16-\mathrm{CH}$ or $12-\mathrm{CH}), 124.2(17-\mathrm{CH})$, $122.8(15-\mathrm{CH}), 121.9$ $(18-\mathrm{CH}), 79.9\left(2-\mathrm{NC}_{\mathrm{q}}(\mathrm{O}) \mathrm{O}-\mathrm{C}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), 62.7\left(\mathrm{~d},{ }^{2} J_{C P}=6.0 \mathrm{~Hz}\right.$, $\left.1-\mathrm{POCH}_{2}\right), 62.4\left(\mathrm{~d},{ }^{2} J_{C P}=7.3 \mathrm{~Hz}, 1-\mathrm{POCH}_{2}\right), 49.7\left(\mathrm{~d},{ }^{2} J_{C P}=\right.$ $11.9 \mathrm{~Hz}, 2-\mathrm{CH}), 45.3\left(\mathrm{~d},{ }^{1} J_{C P}=151.5 \mathrm{~Hz}, 1-\mathrm{CH}\right), 40.1\left(4-\mathrm{CH}_{2}\right)$, $36.8\left(10-\mathrm{CH}_{2}\right), 28.5\left(\mathrm{OC}_{\mathrm{q}}-\left(\mathrm{CH}_{3}\right)_{3}\right), 26.1\left(7-\mathrm{CH}_{2}\right), 25.1\left(5-\mathrm{CH}_{2}\right)$, $19.1\left(6-\mathrm{CH}_{2}\right), 16.2\left(\mathrm{~d},{ }^{3} J_{C P}=1.6 \mathrm{~Hz}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right), 16.2(\mathrm{~d}$, $\left.{ }^{3} J_{C P}=2.2 \mathrm{~Hz}, 1-\mathrm{POCH}_{2} \mathrm{CH}_{3}\right) .{ }^{31} \mathrm{P}$ NMR ( $162 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta$ (22.93), 22.32. HRESIMS $m / z 525.2153[\mathrm{M}+\mathrm{H}]^{+}$(525.2183 calcd for $\mathrm{C}_{25} \mathrm{H}_{38} \mathrm{~N}_{2} \mathrm{O}_{6} \mathrm{PS}$ ).

## General procedure B for 5a-l

Corresponding compounds $\mathbf{4}$ and 3-mercaptopropylfunctionalized silica gel ( 3.5 equiv., $1.2 \mathrm{mmol} \mathrm{g}{ }^{-1}$ loading) were dried under high vacuum for at least 30 min. Dry MeCN and $\mathrm{Et}_{3} \mathrm{~N}$ ( 6.0 equiv.) were added, and the mixture was stirred at r.t. for 5 min . The reaction mixture was subsequently cooled to $0{ }^{\circ} \mathrm{C}$. TMSBr ( 5.0 equiv.) in MeCN was added dropwise. The reaction was stirred for $12-48 \mathrm{~h}$. All the compounds were converted to HCl salts by adding 0.1 M HCl solution (aq) and the solvent was removed by freezedrying, due to poor solubility of either the free base or formate salts.

## ((2-(4-Methyl-2-thioxo-2,3-dihydrothiazol-5-yl)acet-amido) (piperidin-2-yl)methyl)phosphonic acid hydro-chloride (5a)

The synthesis was performed according to general procedure B. Compound $\mathbf{4 a - 1}(50.8 \mathrm{mg}, 0.0974 \mathrm{mmol})$ gave $\mathbf{5 a - 1}$ as a slightly orange solid ( $18.8 \mathrm{mg}, 0.0515 \mathrm{mmol}, 48 \%$ ). Compound 4a-2 ( $50.6 \mathrm{mg}, 0.0970 \mathrm{mmol}$ ) gave $\mathbf{5 a - 2}$ as a slightly orange solid ( $8.6 \mathrm{mg}, 0.0235 \mathrm{mmol}, 22 \%$ ).

## Compound 5a-1, diastereomer 1

${ }^{1} \mathrm{H}$ NMR ( 500 MHz, DMSO- $d_{6}$ ) $\delta 12.94(\mathrm{~s}, 1 \mathrm{H}, 15-\mathrm{NH}), 8.32(\mathrm{~s}$, $1 \mathrm{H}, 1-\mathrm{NH}), 3.96\left(\mathrm{dd},{ }^{2} J_{H P}=15.9 \mathrm{~Hz}, J=8.5,8.5 \mathrm{~Hz}, 1 \mathrm{H}, 1-\mathrm{H}\right)$, $3.55\left(\mathrm{~d},{ }^{2} J=17.3 \mathrm{~Hz}, 1 \mathrm{H}\right.$, part A of AB, $\left.10-\mathrm{H}\right), 3.54\left(\mathrm{~d},{ }^{2} J=17.3\right.$ $\mathrm{Hz}, 1 \mathrm{H}$, part B of $\mathrm{AB}, 10-\mathrm{H}$ ), 3.23 (br. d, $12.3 \mathrm{~Hz}, 1 \mathrm{H}, 4-\mathrm{H}$ ), 3.09 (ddd, $J=8.8,8.8,8.8 \mathrm{~Hz}, 1 \mathrm{H}, 2-\mathrm{H}$ ), 2.84 (ddd, $J=12.0$, $12.0,2.3 \mathrm{~Hz}, 1 \mathrm{H}, 4-\mathrm{H}$ ), $2.06\left(\mathrm{~s}, 3 \mathrm{H}, 15-\mathrm{CH}_{3}\right), 1.81$ (br. d, $J=$ $10.5 \mathrm{~Hz}, 1 \mathrm{H}, 7-\mathrm{H}), 1.76-1.59(\mathrm{~m}, 2 \mathrm{H}, 5-\mathrm{H}, 6-\mathrm{H}), 1.52(\mathrm{~m}, 1 \mathrm{H}$, $6-\mathrm{H}), 1.45-1.30(\mathrm{~m}, 2 \mathrm{H}, 5-\mathrm{H}, 7-\mathrm{H}) .{ }^{13} \mathrm{C}$ NMR ( 126 MHz , DMSO$\left.d_{6}\right) \delta 186.9\left(13-\mathrm{C}_{\mathrm{q}}\right), 168.9\left(\mathrm{~d},{ }^{3} J_{C P}=6.2 \mathrm{~Hz}, 9-\mathrm{C}_{\mathrm{q}}\right), 134.7$ (15$\left.\mathrm{C}_{\mathrm{q}}\right), 117.5\left(11-\mathrm{C}_{\mathrm{q}}\right), 57.3(2-\mathrm{CH}), 48.6\left(\mathrm{~d},{ }^{1} J_{C P}=134.7 \mathrm{~Hz}, 1-\mathrm{CH}\right)$, $43.7\left(4-\mathrm{CH}_{2}\right), 32.2\left(10-\mathrm{CH}_{2}\right), 25.0\left(\mathrm{~d},{ }^{3} J_{C P}=7.6 \mathrm{~Hz}, 7-\mathrm{CH}_{2}\right)$, $22.0\left(5-\mathrm{CH}_{2}\right), 21.6\left(6-\mathrm{CH}_{2}\right), 11.4\left(15-\mathrm{CH}_{3}\right) .{ }^{31} \mathrm{P}$ NMR $(162 \mathrm{MHz}$, DMSO- $d_{6}$ ) $\delta$ 11.71. HRESIMS $m / z 366.0718[\mathrm{M}+\mathrm{H}-\mathrm{Cl}]^{+}$ ( 366.0706 calcd for $\mathrm{C}_{12} \mathrm{H}_{21} \mathrm{~N}_{3} \mathrm{O}_{4} \mathrm{PS}_{2}$ ).

## Compound 5a-2, diastereomer 2

${ }^{1} \mathrm{H}$ NMR ( 500 MHz, DMSO- $d_{6}$ ) $\delta 12.92$ (s, 1H, 15-NH), 8.69 ( s , $1 \mathrm{H}, 1-\mathrm{NH}$ ), 4.01 (ddd, $\left.{ }^{2} J_{H P}=17.1 \mathrm{~Hz}, J=8.8,3.5 \mathrm{~Hz}, 1 \mathrm{H}, 1-\mathrm{H}\right)$, $3.61\left(\mathrm{~d},{ }^{2} J=16.7 \mathrm{~Hz}, 1 \mathrm{H}\right.$, part A of AB, $\left.10-\mathrm{H}\right), 3.56\left(\mathrm{~d},{ }^{2} J=16.7\right.$ $\mathrm{Hz}, 1 \mathrm{H}$, part B of $\mathrm{AB}, 10-\mathrm{H}$ ), 3.34-3.13 (m, 2H, 2-H, $4-\mathrm{H}), 2.82$ (dd, $J=12.4,12.4 \mathrm{~Hz}, 1 \mathrm{H}, 4-\mathrm{H}), 2.07\left(\mathrm{~s}, 3 \mathrm{H}, 15-\mathrm{CH}_{3}\right), 1.82-1.70$ (m, 2H, 6-H, 7-H), 1.69-1.47 (m, 3H, 5-H2, 7-H), 1.41 (m, 1H, $6-\mathrm{H}) .{ }^{13} \mathrm{C}$ NMR ( 126 MHz , DMSO- $d_{6}$ ) $\delta 186.9\left(13-\mathrm{C}_{\mathrm{q}}\right), 168.8(\mathrm{~d}$, $\left.{ }^{3} J_{C P}=6.7 \mathrm{~Hz}, 9-\mathrm{C}_{\mathrm{q}}\right), 134.7(15-\mathrm{CH}), 117.5(11-\mathrm{CH}), 57.1(2-\mathrm{CH})$, 48.9 (d, $\left.{ }^{1} J_{C P}=134.7 \mathrm{~Hz}, 1-\mathrm{CH}\right), 44.3\left(4-\mathrm{CH}_{2}\right), 32.3\left(10-\mathrm{CH}_{2}\right)$, $25.6\left(\mathrm{~d},{ }^{3} J_{C P}=5.2 \mathrm{~Hz}, 7-\mathrm{CH}_{2}\right), 22.1\left(5-\mathrm{CH}_{2}\right), 21.8\left(6-\mathrm{CH}_{2}\right), 11.4$ $\left(15-\mathrm{CH}_{3}\right) \cdot{ }^{31} \mathrm{P}$ NMR ( 162 MHz , DMSO- $d_{6}$ ) $\delta$ 11.79. HRESIMS $\mathrm{m} /$ $z 366.0699[\mathrm{M}+\mathrm{H}-\mathrm{Cl}]^{+}$(calcd for $\mathrm{C}_{12} \mathrm{H}_{21} \mathrm{~N}_{3} \mathrm{O}_{4} \mathrm{PS}_{2}$ ).

## (Piperidin-2-yl(thiophene-2-carboxamido)methyl)phos-phonic acid hydrochloride (5b)

The synthesis was performed according to general procedure B. Compound $\mathbf{4 b} \mathbf{- 1}(31.5 \mathrm{mg}, 0.0684 \mathrm{mmol})$ gave $\mathbf{5 b}-\mathbf{1}$ as a colorless solid ( $15.8 \mathrm{mg}, 0.0464 \mathrm{mmol}, 68 \%$ ). Compound $\mathbf{4 b - 2}$ ( $40.1 \mathrm{mg}, 0.0871 \mathrm{mmol}$ ) gave $\mathbf{5 b} \mathbf{b}-2$ as a colorless solid ( 22.5 $\mathrm{mg}, 0.0660 \mathrm{mmol}, 76 \%)$.

## Compound 5b-1, diastereomer 1

${ }^{1} \mathrm{H}$ NMR ( 400 MHz, DMSO- $d_{6}$ ) $\delta 8.53$ (m, 1H, 1-NH), 8.10 (m, $1 \mathrm{H}, 14-\mathrm{H}), 7.80(\mathrm{~d}, J=4.9 \mathrm{~Hz}, 1 \mathrm{H}, 12-\mathrm{H}), 7.16$ (dd, $J=5.0,3.7$ $\mathrm{Hz}, 1 \mathrm{H}, 13-\mathrm{H}), 4.57$ (m, 1H, 1-H), $3.42(\mathrm{~m}, 1 \mathrm{H}, 2-\mathrm{H}), 3.24(\mathrm{~m}$, $1 \mathrm{H}, 4-\mathrm{H}$ ), $2.94(\mathrm{~m}, 1 \mathrm{H}, 4-\mathrm{H}), 2.06$ (br. d, $J=13.5 \mathrm{~Hz}, 1 \mathrm{H}, 7-\mathrm{H}$ ), 1.78-1.38 (m, 1H, 5- $\mathrm{H}_{2}, 6-\mathrm{H}_{2}$ ), 1.57 (m, 1H, 7-H). ${ }^{13} \mathrm{C}$ NMR $\left(126 \mathrm{MHz}\right.$, DMSO- $\left.d_{6}\right) \delta 161.7$ (d, ${ }^{3} J_{C P}=5.6 \mathrm{~Hz}, 9-\mathrm{C}_{\mathrm{q}}$ ), 139.2 ( $10-\mathrm{C}_{\mathrm{q}}$ ), 131.3 ( $12-\mathrm{CH}$ ), 129.4 ( $14-\mathrm{CH}$ ), 127.9 ( $13-\mathrm{CH}$ ), 57.1 (d, $\left.{ }^{2} J_{C P}=8.1 \mathrm{~Hz}, 2-\mathrm{CH}\right), 48.6\left(\mathrm{~d},{ }^{1} J_{C P}=145.5 \mathrm{~Hz}, 1-\mathrm{CH}\right), 44.8$ $\left(4-\mathrm{CH}_{2}\right), 24.6\left(7-\mathrm{CH}_{2}\right), 21.5\left(5-\mathrm{CH}_{2}, 6-\mathrm{CH}_{2}\right) .{ }^{31} \mathrm{P}$ NMR (162 MHz , DMSO- $d_{6}$ ) $\delta$ 14.26. HRESIMS $m / z 305.0751[\mathrm{M}+\mathrm{H}-\mathrm{Cl}]^{+}$ (305.0719 calcd for $\mathrm{C}_{11} \mathrm{H}_{18} \mathrm{~N}_{2} \mathrm{O}_{4} \mathrm{PS}$ ).

## Compound 5b-2, diastereomer 2

${ }^{1} \mathrm{H}$ NMR ( 400 MHz, DMSO- $d_{6}$ ) $\delta 8.68$ (d, $J=9.7 \mathrm{~Hz}, 1 \mathrm{H}$, $1-\mathrm{NH}), 8.14(\mathrm{~d}, J=3.7,1.1 \mathrm{~Hz}, 1 \mathrm{H}, 14-\mathrm{H}), 7.79(\mathrm{dd}, J=5.0,1.1$
$\mathrm{Hz}, 1 \mathrm{H}, 12-\mathrm{H}), 7.17$ (dd, $J=5.0,3.7 \mathrm{~Hz}, 1 \mathrm{H}, 13-\mathrm{H}), 4.44$ (ddd, $\left.{ }^{2} J_{H P}=17.7 \mathrm{~Hz}, J=9.7,7.2 \mathrm{~Hz}, 1 \mathrm{H}, 1-\mathrm{H}\right), 3.45(\mathrm{~m}, 1 \mathrm{H}, 2-\mathrm{H})$, 3.28 (br. d, $J=12.2 \mathrm{~Hz}, 1 \mathrm{H}, 4-\mathrm{H}$ ), 2.86 (dd, $J=11.3,11.3 \mathrm{~Hz}$, $1 \mathrm{H}, 4-\mathrm{H}$ ), 2.07 (br. d, $J=12.1 \mathrm{~Hz} 1 \mathrm{H}, 7-\mathrm{H}), 1.81-1.72(\mathrm{~m}, 1 \mathrm{H}$, $6-\mathrm{H}$ or $5-\mathrm{H}), 1.68(\mathrm{~m}, 1 \mathrm{H}, 5-\mathrm{H}$ or $6-\mathrm{H}), 1.65-1.55(\mathrm{~m}, 2 \mathrm{H}, 7-\mathrm{H}$, $5-\mathrm{H}$ or $6-\mathrm{H}$ ), $1.47(\mathrm{~m}, 1 \mathrm{H}, 5-\mathrm{H}$ or $6-\mathrm{H}) .{ }^{13} \mathrm{C}$ NMR ( 126 MHz , DMSO- $d_{6}$ ) $\delta 161.3\left(\mathrm{~d}, J=4.0 \mathrm{~Hz}, 9-\mathrm{C}_{\mathrm{q}}\right), 139.2(10-\mathrm{CH}), 131.2$ (12-CH), 129.5 ( $14-\mathrm{CH}$ ), 127.9 ( $13-\mathrm{CH}$ ), 56.3 (d, ${ }^{2} J_{C P}=7.1 \mathrm{~Hz}$, $2-\mathrm{CH}), 49.1\left(\mathrm{~d},{ }^{1} J_{C P}=147.1 \mathrm{~Hz}, 1-\mathrm{CH}\right), 44.9\left(4-\mathrm{CH}_{2}\right), 26.1(\mathrm{~d}$, $\left.{ }^{3} J_{C P}=4.5 \mathrm{~Hz}, 7-\mathrm{CH}_{2}\right)$, $21.7\left(5-\mathrm{CH}_{2}\right.$ or $\left.6-\mathrm{CH}_{2}\right), 21.5\left(6-\mathrm{CH}_{2}\right.$ or $5-\mathrm{CH}_{2}$ ). ${ }^{31} \mathrm{P}$ NMR ( 162 MHz , DMSO- $d_{6}$ ) $\delta$ 14.67. HRESIMS $m / z$ $305.0761[\mathrm{M}+\mathrm{H}-\mathrm{Cl}]^{+}$( 305.0719 calcd for $\mathrm{C}_{11} \mathrm{H}_{18} \mathrm{~N}_{2} \mathrm{O}_{4} \mathrm{PS}$ ).

## (Piperidin-2-yl(thiophene-3-carboxamido)methyl)phos-phonic acid hydrochloride (5c)

The synthesis was performed according to general procedure B. Compound $\mathbf{4 c - 1}(32.6 \mathrm{mg}, 0.0708 \mathrm{mmol})$ gave $5 \mathrm{c}-1$ as a colorless solid ( $22.6 \mathrm{mg}, 0.0663 \mathrm{mmol}, 94 \%$ ). Compound $4 \mathrm{c}-1$ ( $32.6 \mathrm{mg}, 0.0974 \mathrm{mmol}$ ) gave $5 \mathrm{c}-2$ as a colorless solid (21.8 $\mathrm{mg}, 0.0640 \mathrm{mmol}, 90 \%)$.

## Compound $5 \mathbf{c - 1}$, diastereomer 1

${ }^{1} \mathrm{H}$ NMR ( 400 MHz, DMSO- $d_{6}$ ) $\delta 8.37(\mathrm{dd}, J=2.9,1.4 \mathrm{~Hz}, 1 \mathrm{H}$, $11-\mathrm{H}), 8.23(\mathrm{~d}, J=8.1 \mathrm{~Hz}, 1 \mathrm{H}, 1-\mathrm{NH}), 7.58(\mathrm{~m}, 1 \mathrm{H}, 14-\mathrm{H}), 7.56$ $(\mathrm{m}, 1 \mathrm{H}, 13-\mathrm{H}), 4.48\left(\mathrm{ddd},{ }^{2} J_{H P}=16.7 \mathrm{~Hz}, J=9.0,6.5 \mathrm{~Hz}, 1 \mathrm{H}\right.$, $1-\mathrm{H}), 3.35(\mathrm{~m}, 1 \mathrm{H}, 2-\mathrm{H}), 2.89$ (ddd, $J=12.3,12.3,3.5 \mathrm{~Hz}, 1 \mathrm{H}$, $4-\mathrm{H}), 2.01$ (br. d, $J=12.9 \mathrm{~Hz}, 1 \mathrm{H}, 7-\mathrm{H}), 1.78-1.61(\mathrm{~m}, 2 \mathrm{H}, 5-\mathrm{H}$, $6-\mathrm{H}), 1.61-1.47(\mathrm{~m}, 1 \mathrm{H}, 5-\mathrm{H}$ or $6-\mathrm{H}, 7-\mathrm{H}), 1.40(\mathrm{~m}, 1 \mathrm{H}, 5-\mathrm{H}$ or $6-\mathrm{H}) .{ }^{13} \mathrm{C}$ NMR ( 126 MHz, DMSO- $d_{6}$ ) $\delta 162.4\left(\mathrm{~d},{ }^{3} J_{C P}=5.7 \mathrm{~Hz}\right.$, $9-\mathrm{C}_{\mathrm{q}}$ ), 137.1 ( $10-\mathrm{C}_{\mathrm{q}}$ ), 129.5 ( $11-\mathrm{CH}$ ), 127.4 ( $14-\mathrm{CH}$ ), 126.4 ( $13-$ CH), $57.3\left(\mathrm{~d},{ }^{2} J_{C P}=6.2 \mathrm{~Hz}, 2-\mathrm{CH}\right), 48.4\left(\mathrm{~d},{ }^{1} J_{C P}=141.9 \mathrm{~Hz}\right.$, 1-CH), $44.5\left(4-\mathrm{CH}_{2}\right), 24.8\left(\mathrm{~d},{ }^{3} J_{C P}=5.2 \mathrm{~Hz}, 7-\mathrm{CH}_{2}\right), 21.7\left(5-\mathrm{CH}_{2}\right.$ or $6-\mathrm{CH}_{2}$ ), $21.6\left(5-\mathrm{CH}_{2}\right.$ or $\left.6-\mathrm{CH}_{2}\right) .{ }^{31} \mathrm{P}$ NMR ( 162 MHz , DMSO$\left.d_{6}\right) \delta$ 13.77. HRESIMS $m / z 305.0737[\mathrm{M}+\mathrm{H}-\mathrm{Cl}]^{+}(305.0719$ calcd for $\mathrm{C}_{11} \mathrm{H}_{18} \mathrm{~N}_{2} \mathrm{O}_{4} \mathrm{PS}$ ).

## Compound 5c-2, diastereomer 2

${ }^{1} \mathrm{H}$ NMR ( 500 MHz, DMSO- $d_{6}$ ) $\delta 8.44$ (m, 1H, 11-H), 7.65 (d, $J$ $=5.0 \mathrm{~Hz}, 1 \mathrm{H}, 14-\mathrm{H}), 7.58(\mathrm{dd}, J=5.1,2.9 \mathrm{~Hz}, 1 \mathrm{H}, 13-\mathrm{H}), 4.47$ (ddd, $\left.{ }^{2} J_{H P}=17.7 \mathrm{~Hz}, J=9.7,6.9 \mathrm{~Hz}, 1 \mathrm{H}, 1-\mathrm{H}\right), 3.44(\mathrm{~m}, 1 \mathrm{H}$, $2-\mathrm{H}), 3.29$ (br. d, $J=12.9 \mathrm{~Hz}, 1 \mathrm{H}, 4-\mathrm{H}), 2.86$ (dd, $J=12.6,12.6$ $\mathrm{Hz}, 1 \mathrm{H}, 4-\mathrm{H}), 2.06$ (br. d, $J=11.9 \mathrm{~Hz}, 1 \mathrm{H}, 7-\mathrm{H}), 1.81-1.39(\mathrm{~m}$, $4 \mathrm{H}, 5-\mathrm{H}_{2}, 6-\mathrm{H}_{2}$ ), 1.61 (m, 1H, 7-H). ${ }^{13} \mathrm{C}$ NMR ( 126 MHz , DMSO- $d_{6}$ ) $\delta 162.2\left(\mathrm{~d},{ }^{3} J_{C P}=4.0 \mathrm{~Hz}, 9-\mathrm{C}_{\mathrm{q}}\right), 137.1\left(10-\mathrm{C}_{\mathrm{q}}\right), 129.8$ (11-CH), 127.5 (14-CH), 126.3 (13-CH), 56.4 (d, ${ }^{2} J_{C P}=6.9 \mathrm{~Hz}$, $2-\mathrm{CH}), 48.8\left(\mathrm{~d},{ }^{1} J_{C P}=147.0 \mathrm{~Hz}, 1-\mathrm{CH}\right), 44.9\left(4-\mathrm{CH}_{2}\right), 26.2(\mathrm{~d}$, $\left.{ }^{3} J_{C P}=4.8 \mathrm{~Hz}, 7-\mathrm{CH}_{2}\right), 21.7\left(5-\mathrm{CH}_{2}\right.$ or $\left.6-\mathrm{CH}_{2}\right), 21.5\left(5-\mathrm{CH}_{2}\right.$ or $6-\mathrm{CH}_{2}$ ). ${ }^{31} \mathrm{P}$ NMR ( 162 MHz, DMSO- $d_{6}$ ) $\delta$ 12.03. HRESIMS $m / z$ $305.0745[\mathrm{M}+\mathrm{H}-\mathrm{Cl}]^{+}\left(305.07194\right.$ calcd for $\left.\mathrm{C}_{11} \mathrm{H}_{18} \mathrm{~N}_{2} \mathrm{O}_{4} \mathrm{PS}\right)$.

## (Piperidin-2-yl(4,5,6,7-tetrahydrobenzo[b]thiophene-2carboxamido)methyl)phosphonic acid hydrochloride (5d)

The synthesis was performed according to general procedure B. Compound $\mathbf{4 d - 1}(52.1 \mathrm{mg}, 0.101 \mathrm{mmol})$ gave $\mathbf{5 d} \mathbf{- 1}$ as a
slightly yellow solid (26.8 mg, 0.0679 mmol , 67\%). Compound 4d-2 ( $45.8 \mathrm{mg}, 0.0890 \mathrm{mmol}$ ) gave $\mathbf{5 d - 2}$ as a colorless solid ( $21.4 \mathrm{mg}, 0.0542 \mathrm{mmol}, 61 \%$ ).

## Compound 5d-1, diastereomer 1

${ }^{1} \mathrm{H}$ NMR ( $500 \mathrm{MHz}, \mathrm{DMSO}-d_{6}$ ) $\delta 8.30(\mathrm{~d}, J=9.7 \mathrm{~Hz}, 1 \mathrm{H}$, $1-\mathrm{NH}$ ), $7.74(\mathrm{~s}, 1 \mathrm{H}, 18-\mathrm{H}), 4.53$ (ddd, ${ }^{2} J_{H P}=19.4 \mathrm{~Hz}, J=9.5$, $5.3 \mathrm{~Hz}, 1 \mathrm{H}, 1-\mathrm{H}), 3.40(\mathrm{~m}, 1 \mathrm{H}, 2-\mathrm{H}), 3.26$ (br. d, $J=12.4 \mathrm{~Hz}$, $1 \mathrm{H}, 4-\mathrm{H}), 2.97-2.88(\mathrm{~m}, 1 \mathrm{H}, 4-\mathrm{H}), 2.76-2.69\left(\mathrm{~m}, 2 \mathrm{H}, 13-\mathrm{H}_{2}\right)$, 2.62-2.53 (m, 2H, 16-H2), 2.03 (br. d, $J=12.3 \mathrm{~Hz}, 1 \mathrm{H}, 7-\mathrm{H}$ ), $1.84-1.63\left(\mathrm{~m}, 5 \mathrm{H}, 14-\mathrm{H}_{2}, 15-\mathrm{H}_{2}, 5-\mathrm{H}\right.$ or $\left.6-\mathrm{H}\right), 1.61-1.49(\mathrm{~m}$, $2 \mathrm{H}, 7-\mathrm{H}, 5-\mathrm{H}$ or $6-\mathrm{H}), 1.42(\mathrm{~m}, 1 \mathrm{H}, 5-\mathrm{H}$ or $6-\mathrm{H}) .{ }^{13} \mathrm{C}$ NMR (126 MHz, DMSO- $d_{6}$ ) $\delta 161.9\left(\mathrm{~d},{ }^{3} J_{C P}=5.6 \mathrm{~Hz}, 9-\mathrm{C}_{\mathrm{q}}\right), 140.9\left(12-\mathrm{C}_{\mathrm{q}}\right)$, $135.8\left(17-\mathrm{C}_{\mathrm{q}}\right), 134.7\left(10-\mathrm{C}_{\mathrm{q}}\right), 130.1(18-\mathrm{CH}), 57.1\left(\mathrm{~d},{ }^{2} J_{C P}=8.3\right.$ $\mathrm{Hz}, 2-\mathrm{CH}), 48.5\left(\mathrm{~d},{ }^{1} J_{C P}=145.5 \mathrm{~Hz}, 1-\mathrm{CH}\right), 44.8\left(4-\mathrm{CH}_{2}\right), 25.0$ $\left(16-\mathrm{CH}_{2}\right), 24.7\left(13-\mathrm{CH}_{2}\right), 24.5\left(\mathrm{~d},{ }^{3} J_{C P}=4.3 \mathrm{~Hz}, 7-\mathrm{CH}_{2}\right), 22.9$ $\left(14-\mathrm{CH}_{2}\right), 22.2\left(15-\mathrm{CH}_{2}\right), 21.5\left(6-\mathrm{CH}_{2}, 5-\mathrm{CH}_{2}\right) .{ }^{31} \mathrm{P}$ NMR (162 MHz, DMSO- $d_{6}$ ) $\delta$ 14.58. HRESIMS $m / z 359.1207[\mathrm{M}+\mathrm{H}-\mathrm{Cl}]^{+}$ (359.1189 calcd for $\mathrm{C}_{15} \mathrm{H}_{24} \mathrm{~N}_{2} \mathrm{O}_{4} \mathrm{PS}$ ).

## Compound 5d-2, diastereomer 2

${ }^{1} \mathrm{H}$ NMR ( $500 \mathrm{MHz}, \mathrm{DMSO}_{6}$ ) $\delta 8.42(\mathrm{~d}, J=9.5 \mathrm{~Hz}, 1 \mathrm{H}$, $1-\mathrm{NH}), 7.75(\mathrm{~s}, 1 \mathrm{H}, 18-\mathrm{H}), 4.40\left(\mathrm{ddd},{ }^{2} J_{H P}=17.7 \mathrm{~Hz}, J=9.7\right.$, $7.4 \mathrm{~Hz}, 1 \mathrm{H}, 1-\mathrm{H}), 3.41(\mathrm{~m}, 1 \mathrm{H}, 2-\mathrm{H}), 3.27$ (br d, $J=10.6 \mathrm{~Hz}$, $1 \mathrm{H}, 4-\mathrm{H}), 2.84(\mathrm{dd}, J=12.6,12.6 \mathrm{~Hz}, 1 \mathrm{H}, 4-\mathrm{H}), 2.76-2.68(\mathrm{~m}$, $\left.2 \mathrm{H}, 13-\mathrm{H}_{2}\right), 2.66-2.53\left(\mathrm{~m}, 2 \mathrm{H}, 16-\mathrm{H}_{2}\right), 2.08(\mathrm{~m}, 1 \mathrm{H}, 7-\mathrm{H}), 1.85-$ $1.65\left(\mathrm{~m}, 5 \mathrm{H}, 14-\mathrm{H}_{2}, 15-\mathrm{H}_{2}, 5-\mathrm{H}\right.$ or $\left.6-\mathrm{H}\right), 1.63-1.53(\mathrm{~m}, 2 \mathrm{H}, 7-\mathrm{H}$, $5-\mathrm{H}$ or $6-\mathrm{H}$ ), $1.44(\mathrm{~m}, 1 \mathrm{H}, 5-\mathrm{H}$ or $6-\mathrm{H}) .{ }^{13} \mathrm{C}$ NMR ( 126 MHz , DMSO- $d_{6}$ ) $\delta 160.9\left(\mathrm{~d},{ }^{3} J_{C P}=3.9 \mathrm{~Hz}, 9-\mathrm{C}_{\mathrm{q}}\right), 140.3\left(12-\mathrm{C}_{\mathrm{q}}\right), 135.2$ $\left(17-\mathrm{C}_{\mathrm{q}}\right), 134.2\left(10-\mathrm{C}_{\mathrm{q}}\right), 129.5(18-\mathrm{CH}), 55.7\left(\mathrm{~d},{ }^{2} J_{C P}=7.2 \mathrm{~Hz}\right.$, $2-\mathrm{CH}), 48.4\left(\mathrm{~d},{ }^{1} J_{C P}=147.1 \mathrm{~Hz}, 1-\mathrm{CH}\right), 44.3\left(4-\mathrm{CH}_{2}\right), 25.6(\mathrm{~d}$, $\left.{ }^{3} J_{C P}=4.2 \mathrm{~Hz}, 7-\mathrm{CH}_{2}\right), 24.5\left(16-\mathrm{CH}_{2}\right), 24.1\left(13-\mathrm{CH}_{2}\right), 22.3$ (14$\left.\mathrm{CH}_{2}\right), 21.7\left(15-\mathrm{CH}_{2}\right), 21.1\left(5-\mathrm{CH}_{2}\right.$ or $\left.6-\mathrm{CH}_{2}\right), 20.9\left(5-\mathrm{CH}_{2}\right.$ or $6-\mathrm{CH}_{2}$ ). ${ }^{31} \mathrm{P}$ NMR ( 162 MHz , DMSO- $d_{6}$ ) $\delta$ 15.36. HRESIMS $m / z$ $359.1219[\mathrm{M}+\mathrm{H}-\mathrm{Cl}]^{+}$(359.1189 calcd for $\mathrm{C}_{15} \mathrm{H}_{24} \mathrm{~N}_{2} \mathrm{O}_{4} \mathrm{PS}$ ).

## (Piperidin-2-yl(4,5,6,7-tetrahydrobenzo[b]thiophene-3-

 carboxamido)methyl)phosphonic acid hydrochloride (5e)The synthesis was performed according to the general procedure B. Compound $\mathbf{4 e} \mathbf{e} \mathbf{1}(59.4 \mathrm{mg}, 0.115 \mathrm{mmol})$ gave $\mathbf{5 e}$ 1 as a colorless solid ( $17.6 \mathrm{mg}, 0.0446 \mathrm{mmol}, 39 \%$ ). Compound $4 \mathrm{e}-2(68.5 \mathrm{mg}, 0.133 \mathrm{mmol})$ gave $5 \mathrm{e}-2$ as a colorless solid ( $11.5 \mathrm{mg}, 0.0291 \mathrm{mmol}, 22 \%$ ).

## Compound 5e-1, diastereomer 1

${ }^{1} \mathrm{H}$ NMR ( 400 MHz, DMSO- $d_{6}$ ) $\delta 8.07$ ( $\mathrm{s}, 1 \mathrm{H}, 11-\mathrm{H}$ ), 7.95 (dd, $J$ $=9.7,3.5 \mathrm{~Hz}, 1 \mathrm{H}, 1-\mathrm{NH}$ ), 4.54 (ddd, ${ }^{2} J_{H P}=19.2 \mathrm{~Hz}, J=9.5,5.5$ $\mathrm{Hz}, 1 \mathrm{H}, 1-\mathrm{H}), 3.39(\mathrm{~m}, 1 \mathrm{H}, 2-\mathrm{H}), 3.26$ (br. d, $J=11.7 \mathrm{~Hz}, 1 \mathrm{H}$, $4-\mathrm{H}), 2.93$ (ddd, $J=11.7,11.7,3.6 \mathrm{~Hz}, 1 \mathrm{H}, 1-\mathrm{H}), 2.84-2.65(\mathrm{~m}$, $4 \mathrm{H}, 14-\mathrm{H}_{2}, 17-\mathrm{H}_{2}$ ), 2.01 (br. d, $J=12.2 \mathrm{~Hz}, 1 \mathrm{H}, 7-\mathrm{H}$ ), $1.83-1.64$ $\left(\mathrm{m}, 5 \mathrm{H}, 15-\mathrm{H}_{2}, 16-\mathrm{H}_{2}, 5-\mathrm{H}\right.$ or $\left.6-\mathrm{H}\right), 1.63-1.51(\mathrm{~m}, 2 \mathrm{H}, 7-\mathrm{H}, 5-\mathrm{H}$ or $6-\mathrm{H}), 1.44(\mathrm{~m}, 1 \mathrm{H}, 5-\mathrm{H}$ or $6-\mathrm{H}) .{ }^{13} \mathrm{C}$ NMR ( 101 MHz , DMSO$\left.d_{6}\right) \delta 163.8\left(9-\mathrm{C}_{\mathrm{q}}\right), 136.0\left(10-\mathrm{C}_{\mathrm{q}}\right.$ or $13-\mathrm{C}_{\mathrm{q}}$ or $\left.18-\mathrm{C}_{\mathrm{q}}\right), 134.9(10-$ $\mathrm{C}_{\mathrm{q}}$ or $13-\mathrm{C}_{\mathrm{q}}$ or $\left.18-\mathrm{C}_{\mathrm{q}}\right), 134.8\left(10-\mathrm{C}_{\mathrm{q}}\right.$ or $13-\mathrm{C}_{\mathrm{q}}$ or $\left.18-\mathrm{C}_{\mathrm{q}}\right), 125.9$
(11-CH), 57.1 (d, $\left.{ }^{2} J_{C P}=7.3 \mathrm{~Hz}, 2-\mathrm{CH}\right), 47.9\left(\mathrm{~d},{ }^{1} J_{C P}=145.4 \mathrm{~Hz}\right.$, $1-\mathrm{CH}), 44.7\left(4-\mathrm{CH}_{2}\right), 25.4\left(14-\mathrm{CH}_{2}\right.$ or $\left.17-\mathrm{CH}_{2}\right), 24.7\left(14-\mathrm{CH}_{2}\right.$ or $\left.17-\mathrm{CH}_{2}\right), 24.5\left(\mathrm{~d},{ }^{3} J_{C P}=3.8 \mathrm{~Hz}, 7-\mathrm{CH}_{2}\right), 22.7\left(14-\mathrm{CH}_{2}\right.$ or $17-$ $\left.\mathrm{CH}_{2}\right), 22.1\left(14-\mathrm{CH}_{2}\right.$ or $\left.17-\mathrm{CH}_{2}\right), 21.5\left(5-\mathrm{CH}_{2}, 6-\mathrm{CH}_{2}\right) .{ }^{31} \mathrm{P}$ NMR ( 162 MHz, DMSO- $d_{6}$ ) $\delta$ 14.73. HRESIMS $m / z 359.1161$ [M + H$\mathrm{Cl}]^{+}$(359.1189 calcd for $\mathrm{C}_{15} \mathrm{H}_{24} \mathrm{~N}_{2} \mathrm{O}_{4} \mathrm{PS}$ ).

## Compound 5e-2, diastereomer 2

${ }^{1} \mathrm{H}$ NMR ( 500 MHz, DMSO- $\mathrm{d}_{6}$ ) $\delta 8.22(\mathrm{~s}, 1 \mathrm{H}, 11-\mathrm{H}), 8.17$ (d, $J=$ $9.9 \mathrm{~Hz}, 1 \mathrm{H}, 1-\mathrm{NH}), 4.45$ (ddd, ${ }^{2} J_{H P}=17.9 \mathrm{~Hz}, J=8.8,8.8 \mathrm{~Hz}$, $1 \mathrm{H}, 1-\mathrm{H}), 3.45(\mathrm{~m}, 1 \mathrm{H}, 2-\mathrm{H}), 3.26$ (br. d, $J=12.4 \mathrm{~Hz}, 1 \mathrm{H}, 4-\mathrm{H}$ ), 2.85 (m, 1H, 4-H), 2.81-2.67 (m, 4H, 14- $\mathrm{H}_{2}, 17-\mathrm{H}_{2}$ ), 2.10 (br. $\mathrm{d}, J=13.9 \mathrm{~Hz}, 1 \mathrm{H}, 7-\mathrm{H}), 1.79-1.36\left(\mathrm{~m}, 9 \mathrm{H}, 5-\mathrm{H}_{2}, 6-\mathrm{H}_{2}, 7-\mathrm{H}\right.$, $\left.15-\mathrm{H}_{2}, 16-\mathrm{H}_{2}\right) .{ }^{13} \mathrm{C}$ NMR ( 126 MHz, DMSO- $d_{6}$ ) $\delta 163.5\left(\mathrm{~d},{ }^{3} J_{C P}\right.$ $\left.=3.4 \mathrm{~Hz}, 9-\mathrm{C}_{\mathrm{q}}\right), 135.9\left(10-\mathrm{C}_{\mathrm{q}}\right.$ or $13-\mathrm{C}_{\mathrm{q}}$ or $\left.18-\mathrm{C}_{\mathrm{q}}\right), 135.2\left(10-\mathrm{C}_{\mathrm{q}}\right.$ or $13-\mathrm{C}_{\mathrm{q}}$ or $\left.18-\mathrm{C}_{\mathrm{q}}\right), 135.0\left(10-\mathrm{C}_{\mathrm{q}}\right.$ or $13-\mathrm{C}_{\mathrm{q}}$ or $\left.18-\mathrm{C}_{\mathrm{q}}\right)$, $126.0(11-$ $\mathrm{CH}), 55.9\left(\mathrm{~d},{ }^{2} J_{C P}=8.5 \mathrm{~Hz}, 2-\mathrm{CH}\right), 48.4\left(\mathrm{~d},{ }^{1} J_{C P}=147.7 \mathrm{~Hz}\right.$, $1-\mathrm{CH}), 44.7\left(4-\mathrm{CH}_{2}\right), 26.0\left(7-\mathrm{CH}_{2}\right), 25.4\left(14-\mathrm{CH}_{2}\right.$ or $\left.17-\mathrm{CH}_{2}\right)$, $24.7\left(14-\mathrm{CH}_{2}\right.$ or $\left.17-\mathrm{CH}_{2}\right), 22.8\left(15-\mathrm{CH}_{2}\right.$ or $\left.16-\mathrm{CH}_{2}\right), 22.2(15-$ $\mathrm{CH}_{2}$ or $\left.16-\mathrm{CH}_{2}\right)$, $21.6\left(5-\mathrm{CH}_{2}\right.$ or $\left.6-\mathrm{CH}_{2}\right)$, $21.5\left(5-\mathrm{CH}_{2}\right.$ or $\left.6-\mathrm{CH}_{2}\right)$. ${ }^{31} \mathrm{P}$ NMR ( 162 MHz, DMSO- $d_{6}$ ) $\delta$ 14.34. HRESIMS $m / z 3591$ $170[\mathrm{M}+\mathrm{H}-\mathrm{Cl}]^{+}$(359.1189 calcd for $\mathrm{C}_{15} \mathrm{H}_{24} \mathrm{~N}_{2} \mathrm{O}_{4} \mathrm{PS}$ ).

## (Piperidin-2-yl(4,5,6,7-tetrahydrobenzo $[c]$ thiophene-1carboxamido)methyl)phosphonic acid hydrochloride (5f)

The synthesis was performed according to general procedure B. Compound $\mathbf{4 f} \mathbf{- 1}(45.3 \mathrm{mg}, 0.0880 \mathrm{mmol})$ gave $\mathbf{5 f} \mathbf{- 1}$ as a colorless solid ( $28.9 \mathrm{mg}, 0.0732 \mathrm{mmol}, 83 \%$ ). Compound $\mathbf{4 f - 2}$ ( $56.1 \mathrm{mg}, 0.109 \mathrm{mmol}$ ) gave $\mathbf{5 f}-2$ as a colorless solid ( 32.2 mg , $0.0815 \mathrm{mmol}, 75 \%)$.

## Compound 5f-1, diastereomer 1

${ }^{1} \mathrm{H}$ NMR ( 500 MHz, DMSO- $d_{6}$ ) $\delta 7.32$ (s, 1H, 12-H), 7.02 (m, $1 \mathrm{H}, 1-\mathrm{NH}), 4.33(\mathrm{~m}, 1 \mathrm{H}, 1-\mathrm{H}), 3.32(\mathrm{~m}, 1 \mathrm{H}, 2-\mathrm{H}), 3.28(\mathrm{~m}, 1 \mathrm{H}$, $4-\mathrm{H}), 3.00-2.92\left(\mathrm{~m}, 2 \mathrm{H}, 17-\mathrm{CH}_{2}\right), 2.88(\mathrm{~m}, 1 \mathrm{H}, 4-\mathrm{H}), 2.72-2.62$ $\left(\mathrm{m}, 2 \mathrm{H}, 14-\mathrm{H}_{2}\right), 1.95$ (br. d, $\left.J=13.2 \mathrm{~Hz}, 1 \mathrm{H}, 7-\mathrm{H}\right), 1.81-1.63$ (m, $\left.6 \mathrm{H}, 15-\mathrm{H}_{2}, 16-\mathrm{H}_{2}, 5-\mathrm{H}, 6-\mathrm{H}\right), 1.61-1.48(\mathrm{~m}, 2 \mathrm{H}, 7-\mathrm{H}, 5-\mathrm{H}$ or $6-\mathrm{H}), 1.43(\mathrm{~m}, 1 \mathrm{H}, 5-\mathrm{H}$ or $6-\mathrm{H}) .{ }^{13} \mathrm{C}$ NMR ( 126 MHz , DMSO- $d_{6}$ ) $\delta 162.5\left(\mathrm{~d},{ }^{3} J_{C P}=6.2 \mathrm{~Hz}, 9-\mathrm{C}_{\mathrm{q}}\right), 140.2\left(13-\mathrm{C}_{\mathrm{q}}\right), 139.5\left(18-\mathrm{C}_{\mathrm{q}}\right)$, $131.0\left(10-\mathrm{C}_{\mathrm{q}}\right), 123.5(12-\mathrm{CH}), 57.4\left(\mathrm{~d},{ }^{2} J_{C P}=5.7 \mathrm{~Hz}, 2-\mathrm{CH}\right)$, $48.5\left(\mathrm{~d},{ }^{1} J_{C P}=139.5 \mathrm{~Hz}, 1-\mathrm{CH}\right), 44.4\left(4-\mathrm{CH}_{2}\right), 26.2\left(17-\mathrm{CH}_{2}\right)$, $26.0\left(14-\mathrm{CH}_{2}\right), 24.8\left(\mathrm{~d},{ }^{3} J_{C P}=5.5 \mathrm{~Hz}, 7-\mathrm{CH}_{2}\right), 22.7\left(15-\mathrm{CH}_{2}\right.$ or $\left.16-\mathrm{CH}_{2}\right), 22.4\left(15-\mathrm{CH}_{2}\right.$ or $\left.16-\mathrm{CH}_{2}\right), 21.7\left(5-\mathrm{CH}_{2}\right.$ or $\left.6-\mathrm{CH}_{2}\right), 21.6$ $\left(5-\mathrm{CH}_{2}\right.$ or $\left.6-\mathrm{CH}_{2}\right) .{ }^{31} \mathrm{P}$ NMR ( 162 MHz, DMSO- $d_{6}$ ) $\delta 13.89$. HRESIMS $\mathrm{m} / \mathrm{z} 359.1149[\mathrm{M}+\mathrm{H}-\mathrm{Cl}]^{+}$(359.1189 calcd for $\mathrm{C}_{15} \mathrm{H}_{24} \mathrm{~N}_{2} \mathrm{O}_{4} \mathrm{PS}$ ).

## Compound 5f-2, diastereomer 2

${ }^{1} \mathrm{H}$ NMR ( 500 MHz, DMSO- $d_{6}$ ) $\delta 7.30(\mathrm{~s}, 1 \mathrm{H}, 12-\mathrm{H}$ ), 4.33 (m, $1 \mathrm{H}, 1-\mathrm{H}), 3.42(\mathrm{~m}, 1 \mathrm{H}, 2-\mathrm{H}), 3.28$ (br. d, $J=12.1 \mathrm{~Hz}, 1 \mathrm{H}, 4-\mathrm{H})$, 2.98 (m, 2H, $17-\mathrm{H}_{2}$ ), 2.83 (dd, $\left.J=12.7,12.7,3.0 \mathrm{~Hz}, 1 \mathrm{H}, 4-\mathrm{H}\right)$, $2.66\left(\mathrm{~m}, 2 \mathrm{H}, 14-\mathrm{CH}_{2}\right), 1.99(\mathrm{~m}, 1 \mathrm{H}, 7-\mathrm{H}), 1.81-1.49(\mathrm{~m}, 6 \mathrm{H}$, $15-\mathrm{CH}_{2}, 16-\mathrm{CH}_{2}, 7-\mathrm{H}, 5-\mathrm{H}^{\prime}, 6 \mathrm{H}^{\prime}, 5-\mathrm{H}^{\prime \prime}$ or $6-\mathrm{H}^{\prime \prime}$ ), 1.42 (m, 1 H , $5-\mathrm{H}^{\prime \prime}$ or $6-\mathrm{H}^{\prime \prime}$ ). ${ }^{13} \mathrm{C}$ NMR ( 126 MHz, DMSO- $d_{6}$ ) $\delta 162.3\left(\mathrm{~d},{ }^{3} J_{C P}\right.$
$\left.=5.4 \mathrm{~Hz}, 9-\mathrm{C}_{\mathrm{q}}\right), 140.6\left(13-\mathrm{C}_{\mathrm{q}}\right), 139.3\left(18-\mathrm{C}_{\mathrm{q}}\right), 130.7\left(10-\mathrm{C}_{\mathrm{q}}\right)$, $123.3\left(12-\mathrm{C}_{\mathrm{q}}\right), 56.3\left(\mathrm{~d},{ }^{2} J_{C P}=4.8 \mathrm{~Hz}, 2-\mathrm{CH}\right), 49.0\left(\mathrm{~d},{ }^{1} J_{C P}=\right.$ $142.7 \mathrm{~Hz}, 1-\mathrm{CH}), 44.7\left(4-\mathrm{CH}_{2}\right), 26.2\left(17-\mathrm{CH}_{2}\right), 26.0\left(14-\mathrm{CH}_{2}\right)$, $25.9\left(\mathrm{~d},{ }^{3} J_{C P}=4.8 \mathrm{~Hz}, 7-\mathrm{CH}_{2}\right), 22.7\left(15-\mathrm{CH}_{2}\right.$ or $\left.16-\mathrm{CH}_{2}\right), 22.4$ $\left(15-\mathrm{CH}_{2}\right.$ or $\left.16-\mathrm{CH}_{2}\right), 21.9\left(5-\mathrm{CH}_{2}\right.$ or $\left.6-\mathrm{CH}_{2}\right)$, $21.6\left(5-\mathrm{CH}_{2}\right.$ or $6-\mathrm{CH}_{2}$ ). ${ }^{31} \mathrm{P}$ NMR ( 162 MHz, DMSO- $d_{6}$ ) $\delta$ 13.37. HRESIMS $m / z$ $359.1155[\mathrm{M}+\mathrm{H}-\mathrm{Cl}]^{+}$( 359.1189 calcd for $\mathrm{C}_{15} \mathrm{H}_{24} \mathrm{~N}_{2} \mathrm{O}_{4} \mathrm{PS}$ ).

## ((Benzo[b]thiophene-2-carboxamido)(piperidin-2-yl)methyl) phosphonic acid hydrochloride (5g)

The synthesis was performed according to general procedure B. Compound $\mathbf{4 g}$ - $\mathbf{1}(49.7 \mathrm{mg}, 0.0973 \mathrm{mmol})$ gave $\mathbf{5 g}-\mathbf{1}$ as a colorless solid ( $19.7 \mathrm{mg}, 0.0504 \mathrm{mmol}, 52 \%$ ). Compound $\mathbf{4 g - 2}$ ( $51.5 \mathrm{mg}, 0.101 \mathrm{mmol}$ ) gave $5 \mathrm{~g}-2$ as a colorless solid ( 20.8 mg , $0.0532 \mathrm{mmol}, 53 \%)$.

## Compound 5g-1, diastereomer 1

${ }^{1} \mathrm{H}$ NMR ( 400 MHz, DMSO- $d_{6}$ ) $\delta 8.81$ (m, 1H, 1-NH), 8.47 ( s , $1 \mathrm{H}, 18-\mathrm{H}), 8.02(\mathrm{~d}, J=7.7 \mathrm{~Hz}, 1 \mathrm{H}, 13-\mathrm{H}), 7.95(\mathrm{~d}, J=7.8 \mathrm{~Hz}$, $1 \mathrm{H}, 18-\mathrm{H}$ ), 7.47 (ddd, $J=8.1,8.1,1.5 \mathrm{~Hz}, 1 \mathrm{H}$, part C of ABCD, $14-\mathrm{H}), 7.44$ (ddd, $J=8.1,8.1,1.6 \mathrm{~Hz}, 1 \mathrm{H}$, part D of ABCD, 15H), $4.66\left(\mathrm{ddd},{ }^{2} J_{H P}=19.7 \mathrm{~Hz}, J=9.9,5.0 \mathrm{~Hz}, 1 \mathrm{H}, 1-\mathrm{H}\right), 3.49$ (m, 1H, 2-H), 3.28 (br. d, $J=12.7 \mathrm{~Hz}, 1 \mathrm{H}, 4-\mathrm{H}$ ), 2.95 (br. dd, $J$ $=11.9,11.9 \mathrm{~Hz}, 1 \mathrm{H}, 4-\mathrm{H}), 2.11$ (br. d, $J=13.6 \mathrm{~Hz}, 1 \mathrm{H}, 7-\mathrm{H}$ ), $1.76(\mathrm{~m}, 1 \mathrm{H}, 5-\mathrm{H}), 1.71-1.53\left(\mathrm{~m}, 4 \mathrm{H}, 6-\mathrm{H}_{2}, 7-\mathrm{H}\right), 1.45(\mathrm{~m}, 1 \mathrm{H}$, $5-\mathrm{H}) .{ }^{13} \mathrm{C}$ NMR ( 126 MHz, DMSO- $d_{6}$ ) $\delta 161.7$ (d, ${ }^{3} J_{C P}=5.5 \mathrm{~Hz}$, $10-\mathrm{C}_{\mathrm{q}}$ ), $139.8\left(12-\mathrm{C}_{\mathrm{q}}\right), 138.6\left(10-\mathrm{C}_{\mathrm{q}}\right), 138.5\left(17-\mathrm{C}_{\mathrm{q}}\right), 125.8$ ( $18-$ $\mathrm{CH}), 125.8$ ( $14-\mathrm{CH}$ ), 124.8 ( $16-\mathrm{CH}$ ), 124.4 ( $15-\mathrm{CH}$ ), 122.2 ( $13-$ CH), $56.5\left(\mathrm{~d},{ }^{2} J_{C P}=8.5 \mathrm{~Hz}, 2-\mathrm{H}\right), 48.3\left(\mathrm{~d},{ }^{1} J_{C P}=146.0 \mathrm{~Hz}, 1-\mathrm{H}\right)$, $44.3\left(4-\mathrm{CH}_{2}\right), 23.9\left(7-\mathrm{CH}_{2}\right), 21.0\left(5-\mathrm{CH}_{2}\right.$ or $\left.6-\mathrm{CH}_{2}\right), 21.0\left(5-\mathrm{CH}_{2}\right.$ or $6-\mathrm{CH}_{2}$ ). ${ }^{31} \mathrm{P}$ NMR ( 162 MHz , DMSO- $d_{6}$ ) $\delta$ 14.19. HRESIMS $m / z 355.0803[\mathrm{M}+\mathrm{H}-\mathrm{Cl}]^{+}\left(355.0876\right.$ calcd for $\left.\mathrm{C}_{15} \mathrm{H}_{20} \mathrm{~N}_{2} \mathrm{O}_{4} \mathrm{PS}\right)$.

## Compound 5g-2, diastereomer 2

${ }^{1} \mathrm{H}$ NMR ( $400 \mathrm{MHz}, \mathrm{DMSO}-d_{6}$ ) $\delta 8.93$ (d, $J=9.8 \mathrm{~Hz}, 1 \mathrm{H}, 1-\mathrm{NH}$ ), 8.42 (s, 1H, 18-CH), 8.03 (dd, $J=6.7,1.7 \mathrm{~Hz}, 1 \mathrm{H}, 13-\mathrm{H}), 7.96$ (dd, $J=7.6,1.7 \mathrm{~Hz}, 1 \mathrm{H}, 6-\mathrm{H}), 7.48$ (ddd, $J=5.8,5.8,1.7 \mathrm{~Hz}, 1 \mathrm{H}$, part C of ABCD, 14-H), 7.45 (ddd, $J=5.8,5.8,1.7 \mathrm{~Hz}, 1 \mathrm{H}$, part D of ABCD, $15-\mathrm{H}$ ), 4.48 (ddd, $\left.{ }^{3} J_{C P}=17.8 \mathrm{~Hz}, J=9.6,8.0 \mathrm{~Hz}, 1 \mathrm{H}, 1-\mathrm{H}\right)$, 3.48 (m, 1H, 2-H), 3.31 (m, 1H, 4-H), 2.87 (br. dd, $J=10.1,10.1$ Hz, 1H, 4-H), 2.23-2.13 (br. d, $J=12.0 \mathrm{~Hz}, 1 \mathrm{H}, 7-\mathrm{H}$ ), $1.86-1.55$ ( $\mathrm{m}, 4 \mathrm{H}, 7-\mathrm{H}, 5^{\prime}-\mathrm{H}, 6^{\prime}-\mathrm{H}, 5^{\prime \prime}-\mathrm{H}$ or $6^{\prime \prime}-\mathrm{H}$ ), 1.48 ( $\mathrm{m}, 1 \mathrm{H}, 5^{\prime \prime}-\mathrm{H}$ or $6^{\prime \prime}-\mathrm{H}$ ). ${ }^{13} \mathrm{C}$ NMR ( $\left.126 \mathrm{MHz}, \mathrm{DMSO}-d_{6}\right) \delta 161.8\left(\mathrm{~d},{ }^{3} J_{C P}=3.8 \mathrm{~Hz}, 9-\mathrm{C}_{\mathrm{q}}\right)$, $140.3\left(12-\mathrm{C}_{\mathrm{q}}\right), 139.2\left(10-\mathrm{C}_{\mathrm{q}}\right), 139.1\left(17-\mathrm{C}_{\mathrm{q}}\right), 126.3(14-\mathrm{H}), 126.2$ $(18-\mathrm{H}), 125.2(16-\mathrm{H}), 125.0(15-\mathrm{H}), 122.8(13-\mathrm{H}), 56.3\left(\mathrm{~d},{ }^{2} J_{C P}=7.7\right.$ $\mathrm{Hz}, 2-\mathrm{CH}), 49.4\left(\mathrm{~d},{ }^{1} J_{C P}=147.4 \mathrm{~Hz}, 1-\mathrm{CH}\right), 44.8\left(4-\mathrm{CH}_{2}\right), 26.2(\mathrm{~d}$, $\left.{ }^{3} J_{C P}=3.5 \mathrm{~Hz}, 7-\mathrm{CH}_{2}\right), 21.7\left(5-\mathrm{CH}_{2}\right.$ or $\left.6-\mathrm{CH}_{2}\right), 21.5\left(5-\mathrm{CH}_{2}\right.$ or $6-\mathrm{CH}_{2}$ ). ${ }^{31} \mathrm{P}$ NMR ( 162 MHz, DMSO- $d_{6}$ ) $\delta$ 14.41. HRESIMS $m / z$ $355.0806[\mathrm{M}+\mathrm{H}-\mathrm{Cl}]^{+}\left(355.0876\right.$ calcd for $\left.\mathrm{C}_{15} \mathrm{H}_{20} \mathrm{~N}_{2} \mathrm{O}_{4} \mathrm{PS}\right)$.

## ((Benzo[b]thiophene-3-carboxamido)(piperidin-2-yl)met-hyl) phosphonic acid hydrochloride (5h)

The synthesis was performed according to general procedure B. Compound $\mathbf{4 h} \mathbf{- 1}(49.4 \mathrm{mg}, 0.0973 \mathrm{mmol})$ gave $\mathbf{5 h} \mathbf{- 1}$ as a
colorless solid ( $25.8 \mathrm{mg}, 0.0660 \mathrm{mmol}, 68 \%$ ). Compound $\mathbf{4 h - 2}$ ( $39.3 \mathrm{mg}, 0.0770 \mathrm{mmol}$ ) gave $\mathbf{5 h} \mathbf{- 2}$ as a colorless solid ( 15.2 $\mathrm{mg}, 0.0389 \mathrm{mmol}, 51 \%)$.

## Compound 5h-1, diastereomer 1

${ }^{1} \mathrm{H}$ NMR ( 400 MHz, DMSO- $d_{6}$ ) $\delta 8.70(\mathrm{~s}, 1 \mathrm{H}, 11-\mathrm{H}), 8.48(\mathrm{~d}, J=$ $7.5 \mathrm{~Hz}, 1 \mathrm{H}, 17-\mathrm{H}), 8.04$ (dd, $J=7.5,1.6 \mathrm{~Hz}, 1 \mathrm{H}, 13-\mathrm{H}), 7.45$ (ddd, $J=7.5,7.5,1.3 \mathrm{~Hz}, 1 \mathrm{H}$, part C of ABCD, $16-\mathrm{H}$ ), 7.42 (ddd, $J=7.5,7.5,1.6 \mathrm{~Hz}, 1 \mathrm{H}$, part D of $\mathrm{ABCD}, 15-\mathrm{H}$ ), 4.71 (ddd, $\left.{ }^{2} J_{\mathrm{HP}}=19.5 \mathrm{~Hz}, J=9.5 \mathrm{~Hz}, 4.8 \mathrm{~Hz}, 1 \mathrm{H}, 1-\mathrm{H}\right), 3.46(\mathrm{~m}$, $1 \mathrm{H}, 2-\mathrm{H}$ ), 3.29 (br. d, $J=12.5 \mathrm{~Hz}, 1 \mathrm{H}, 4-\mathrm{H}$ ), 2.96 (ddd, $J=12.5$, $12.5,2.08 \mathrm{~Hz}, 1 \mathrm{H}, 4-\mathrm{H}$ ), 2.09 (br. d, $J=12.1 \mathrm{~Hz}, 1 \mathrm{H}$ ), $1.81-$ $1.38\left(\mathrm{~m}, 5 \mathrm{H}, 5-\mathrm{H}_{2}, 6-\mathrm{H}_{2}, 7-\mathrm{H}\right) .{ }^{13} \mathrm{C}$ NMR ( 126 MHz , DMSO- $d_{6}$ ) $\delta 163.5$ (d, $\left.{ }^{3} J_{\mathrm{CP}}=5.4 \mathrm{~Hz}, 9-\mathrm{Cq}\right), 139.2$ (13-Cq), 137.3 (18-Cq), 132.4 ( $11-\mathrm{CH}$ ), 130.0 ( $10-\mathrm{Cq}$ ), 124.9 ( $15-\mathrm{CH}$ ), 124.8 ( $16-\mathrm{CH}$ ), $124.6(17-\mathrm{CH}), 122.8(14-\mathrm{CH}), 57.2\left(\mathrm{~d},{ }^{2} J_{\mathrm{CP}}=8.7 \mathrm{~Hz}, 2-\mathrm{CH}\right)$, $48.2\left(\mathrm{~d},{ }^{1} J_{\mathrm{CP}}=146.3 \mathrm{~Hz}, 1-\mathrm{CH}\right), 44.8\left(4-\mathrm{CH}_{2}\right), 24.5\left(\mathrm{~d},{ }^{3} J_{\mathrm{CP}}=\right.$ $\left.3.8 \mathrm{~Hz}, 7-\mathrm{CH}_{2}\right), 21.6\left(5-\mathrm{CH}_{2}, 6-\mathrm{CH}_{2}\right) .{ }^{31} \mathrm{P}$ NMR ( 162 MHz , DMSO- $d_{6}$ ) $\delta$ 14.68. HRESIMS $m / z 355.0807[\mathrm{M}+\mathrm{H}-\mathrm{Cl}]^{+}$ (355.0876 calcd for $\mathrm{C}_{15} \mathrm{H}_{19} \mathrm{~N}_{2} \mathrm{O}_{4} \mathrm{PS}$ ).

## Compound 5h-2, diastereomer 2

${ }^{1} \mathrm{H}$ NMR ( 500 MHz, DMSO- $d_{6}$ ) $\delta 8.75(\mathrm{~s}, 1 \mathrm{H}, 11-\mathrm{H}), 8.54(\mathrm{~d}, J=$ $9.8 \mathrm{~Hz}, 1 \mathrm{H}, 1-\mathrm{NH}), 8.47(\mathrm{~d}, J=7.2 \mathrm{~Hz}, 1 \mathrm{H}, 17-\mathrm{H}), 8.06(\mathrm{~d}, J=$ $7.0 \mathrm{~Hz}, 1 \mathrm{H}, 14-\mathrm{H}$ ), 7.46 (ddd, $J=7.3,7.3,1.2 \mathrm{~Hz}, 1 \mathrm{H}$, part C of ABCD, 16-H), 7.45 (ddd, $J=7.3,7.3,1.2 \mathrm{~Hz}, 1 \mathrm{H}$, part D of ABCD, 15-H), 4.56 (ddd, $\left.{ }^{2} J_{H P}=18.0 \mathrm{~Hz}, J=9.1 \mathrm{~Hz}, 1 \mathrm{H}\right), 3.47$ (m, 1H, 2-H), 3.30 (br. d, $J=12.0 \mathrm{~Hz}, 1 \mathrm{H}, 4-\mathrm{H}$ ), 2.87 (ddd, $J=$ $11.9,11.9,11.9 \mathrm{~Hz}, 1 \mathrm{H}, 4-\mathrm{H}), 2.23$ (d, $J=13.3 \mathrm{~Hz}, 1 \mathrm{H}, 7-\mathrm{H})$, 1.85-1.42 (m, 5H, 5-H2, 6-H2, 7-H). ${ }^{13} \mathrm{C}$ NMR ( 126 MHz , DMSO- $d_{6}$ ) $\delta 163.0\left(\mathrm{~d},{ }^{3} J_{C P}=3.3 \mathrm{~Hz}\right), 139.3\left(13-\mathrm{C}_{\mathrm{q}}\right), 137.3$ (18$\mathrm{C}_{\mathrm{q}}$ ), $132.2(11-\mathrm{CH}), 130.2\left(10-\mathrm{C}_{\mathrm{q}}\right), 124.9(15-\mathrm{CH}$ or $15-\mathrm{CH})$, 124.8 ( $15-\mathrm{CH}$ or $15-\mathrm{CH}$ ), 124.6 ( $17-\mathrm{CH}$ ), 122.7 ( $14-\mathrm{CH}$ ), 56.2 (d, $\left.{ }^{2} J_{C P}=8.8 \mathrm{~Hz}, 2-\mathrm{H}\right), 48.7\left(\mathrm{~d},{ }^{1} J_{C P}=148.0 \mathrm{~Hz}, 1-\mathrm{H}\right), 44.7\left(4-\mathrm{H}_{2}\right)$, $26.1\left(7-\mathrm{CH}_{2}\right), 21.7\left(5-\mathrm{CH}_{2}\right.$ or $\left.6-\mathrm{CH}_{2}\right), 21.5\left(5-\mathrm{CH}_{2}\right.$ or $\left.6-\mathrm{CH}_{2}\right)$. ${ }^{31} \mathrm{P}$ NMR ( $162 \mathrm{MHz}, \mathrm{DMSO}-d_{6}$ ) $\delta$ 14.94. HRESIMS $\mathrm{m} / \mathrm{z}$ $355.0849[\mathrm{M}+\mathrm{H}-\mathrm{Cl}]^{+}$( 355.0876 calcd for $\mathrm{C}_{15} \mathrm{H}_{20} \mathrm{~N}_{2} \mathrm{O}_{4} \mathrm{PS}$ ).

## (Piperidin-2-yl(2-(thiophen-2-yl)acetamido)methyl)phosphonic acid hydrochloride (5i)

The synthesis was performed according to general procedure B. Compound $\mathbf{4 i} \mathbf{i} \mathbf{1}(60.8 \mathrm{mg}, 0.128 \mathrm{mmol})$ gave $\mathbf{5 i - 1}$ as a colorless solid ( $21.3 \mathrm{mg}, 0.0600 \mathrm{mmol}, 47 \%$ ). Compound 4i-2 ( $61.1 \mathrm{mg}, 0.129 \mathrm{mmol}$ ) gave $5 \mathrm{i}-2$ as a colorless solid $(31.0 \mathrm{mg}$, $0.0874 \mathrm{mmol}, 68 \%)$.

## Compound 5i-1, diastereomer 1

${ }^{1} \mathrm{H}$ NMR ( $400 \mathrm{MHz}, \mathrm{DMSO}-d_{6}$ ) $\delta 8.36(\mathrm{~d}, J=9.8 \mathrm{~Hz}, 1 \mathrm{H}$, $1-\mathrm{NH}), 7.36$ (dd, $J=4.9,1.6 \mathrm{~Hz}, 1 \mathrm{H}, 13-\mathrm{H}), 7.04-6.89(\mathrm{~m}, 2 \mathrm{H}$, $14-\mathrm{H}, 15-\mathrm{H}), 4.43$ (ddd, ${ }^{3} J_{H P}=20.1 \mathrm{~Hz}, J=9.7,4.5 \mathrm{~Hz}, 1 \mathrm{H}$, $1-\mathrm{H}), 3.90\left(\mathrm{~d},{ }^{2} J=16.1 \mathrm{~Hz}, 1 \mathrm{H}\right.$, part A of B, $\left.10-\mathrm{H}\right), 3.79\left(\mathrm{~d},{ }^{2} \mathrm{~J}=\right.$ $16.1 \mathrm{~Hz}, 1 \mathrm{H}$, part B of $\mathrm{AB}, 10-\mathrm{H}), 3.37(\mathrm{~m}, 1 \mathrm{H}, 2-\mathrm{H}), 3.24$ (br. $\mathrm{d}, J=12.4 \mathrm{~Hz}, 1 \mathrm{H}, 4-\mathrm{H}), 2.93(\mathrm{dd}, J=11.5,11.5 \mathrm{~Hz}, 1 \mathrm{H}, 4-\mathrm{H})$, 2.01 (br. d, $J=12.6 \mathrm{~Hz}, 1 \mathrm{H}, 7-\mathrm{H}), 1.81-1.37\left(\mathrm{~m}, 5 \mathrm{H}, 5-\mathrm{H}_{2}\right.$,
$\left.6-\mathrm{H}_{2}, 7-\mathrm{H}\right) .{ }^{13} \mathrm{C}$ NMR ( 126 MHz, DMSO- $d_{6}$ ) $\delta 170.1\left(\mathrm{~d},{ }^{3} J_{C P}=\right.$ $5.6 \mathrm{~Hz}, 9-\mathrm{C}_{\mathrm{q}}$ ), 137.2 ( $11-\mathrm{C}_{\mathrm{q}}$ ), 126.4 ( $14-\mathrm{CH}$ ), 126.2 ( $15-\mathrm{CH}$ ), $124.9(13-\mathrm{CH}), 56.9\left(\mathrm{~d},{ }^{2} J_{C P}=10.0 \mathrm{~Hz}, 2-\mathrm{CH}\right), 48.1\left(\mathrm{~d},{ }^{1} J_{C P}=\right.$ $148.5 \mathrm{~Hz}, 1-\mathrm{CH}), 44.8\left(4-\mathrm{CH}_{2}\right), 36.00\left(10-\mathrm{CH}_{2}\right), 24.0\left(\mathrm{~d},{ }^{3} J_{C P}=\right.$ $\left.3.4 \mathrm{~Hz}, 7-\mathrm{CH}_{2}\right), 21.5\left(5-\mathrm{CH}_{2}, 6-\mathrm{CH}_{2}\right) .{ }^{31} \mathrm{P}$ NMR ( 162 MHz , DMSO- $d_{6}$ ) $\delta$ 14.76. HRESIMS $m / z 319.0848[\mathrm{M}+\mathrm{H}-\mathrm{Cl}]^{+}$ (319.0876 calcd for $\mathrm{C}_{12} \mathrm{H}_{20} \mathrm{~N}_{2} \mathrm{O}_{4} \mathrm{PS}$ ).

## Compound 5i-2, diastereomer 2

${ }^{1} \mathrm{H}$ NMR ( 400 MHz, DMSO- $d_{6}$ ) $\delta 8.49$ (m, 1H, 1-NH), 7.35 (dd, $J=4.6,1.7 \mathrm{~Hz}, 1 \mathrm{H}, 13-\mathrm{H}), 6.97-6.92(\mathrm{~m}, 2 \mathrm{H}, 14-\mathrm{H}, 15-\mathrm{H}), 4.22$ (ddd, $\left.{ }^{2} J_{H P}=17.8 \mathrm{~Hz}, J=9.6,6.4 \mathrm{~Hz}, 1 \mathrm{H}, 1-\mathrm{H}\right), 3.83(\mathrm{~s}, 2 \mathrm{H}, 10-$ $\mathrm{CH}_{2}$ ), 3.35-3.21 (m, 2H, 2-H, 4-H), 2.87 (br. dd, $J=11.4,11.4$ $\mathrm{Hz}, 1 \mathrm{H}, 4-\mathrm{H}$ ), 2.00 (br. d, $J=13.2 \mathrm{~Hz}, 1 \mathrm{H}, 7-\mathrm{H}$ ), 1.79-1.39 (m, $5 \mathrm{H}, 5-\mathrm{H}_{2}, 6-\mathrm{H}_{2}, 7-\mathrm{H}$ ). ${ }^{13} \mathrm{C}$ NMR ( 126 MHz, DMSO- $d_{6}$ ) $\delta 169.5$ $\left(\mathrm{d},{ }^{3} J_{C P}=5.1 \mathrm{~Hz}, 9-\mathrm{C}_{\mathrm{q}}\right), 137.2\left(11-\mathrm{C}_{\mathrm{q}}\right), 126.4(14-\mathrm{CH}), 126.3$ (15-CH), 124.9 (13-CH), 56.8 (d, $\left.{ }^{2} J_{C P}=5.7 \mathrm{~Hz}, 2-\mathrm{CH}\right), 48.4$ (d, $\left.{ }^{1} J_{C P}=146.4 \mathrm{~Hz}, 1-\mathrm{CH}\right), 44.9\left(4-\mathrm{CH}_{2}\right), 36.1\left(10-\mathrm{CH}_{2}\right), 25.8(\mathrm{~d}$, $\left.{ }^{3} J_{C P}=4.9 \mathrm{~Hz}, 7-\mathrm{CH}_{2}\right), 21.8\left(5-\mathrm{CH}_{2}\right.$ or $\left.6-\mathrm{CH}_{2}\right), 21.5\left(5-\mathrm{CH}_{2}\right.$ or $6-\mathrm{CH}_{2}$ ). ${ }^{31} \mathrm{P}$ NMR ( 162 MHz, DMSO- $d_{6}$ ) $\delta$ 15.26. HRESIMS $\mathrm{m} / \mathrm{z}$ $319.0833[\mathrm{M}+\mathrm{H}-\mathrm{Cl}]^{+}$(319.0876 calcd for $\mathrm{C}_{12} \mathrm{H}_{20} \mathrm{~N}_{2} \mathrm{O}_{4} \mathrm{PS}$ ).

## (Piperidin-2-yl(2-(thiophen-3-yl)acetamido)methyl)phos-phonic acid hydrochloride (5j)

The synthesis was performed according to general procedure B. Compound $\mathbf{4 j - 1}(48.0 \mathrm{mg}, 0.101 \mathrm{mmol})$ gave $\mathbf{5 j} \mathbf{- 1}$ as a colorless solid ( $24.4 \mathrm{mg}, 0.0688 \mathrm{mmol}, 68 \%$ ). Compound $\mathbf{4 j - 2}$ ( $58.2 \mathrm{mg}, 0.122 \mathrm{mmol}$ ) gave $\mathbf{5 j}$-2 as a slightly yellow solid ( $26.8 \mathrm{mg}, 0.0755,62 \%$ ).

## Compound 5j-1, diastereomer 1

${ }^{1} \mathrm{H}$ NMR ( 400 MHz, DMSO- $d_{6}$ ) $\delta 8.30(\mathrm{dd}, J=9.9,3.2 \mathrm{~Hz}, 1 \mathrm{H}$, $1-\mathrm{NH}), 7.44$ (dd, $J=4.9,2.9 \mathrm{~Hz}, 1 \mathrm{H}, 14-\mathrm{H}$ ), 7.27 (dd, $J=2.9$, $1.2 \mathrm{~Hz}, 1 \mathrm{H}, 12-\mathrm{H}$ ), 7.07 (dd, $J=4.9,1.2 \mathrm{~Hz}, 1 \mathrm{H}, 15-\mathrm{H}), 4.46$ (ddd, $\left.{ }^{3} J_{H P}=20.4 \mathrm{~Hz}, J=9.9,4.3 \mathrm{~Hz}, 1 \mathrm{H}, 1-\mathrm{H}\right), 3.69\left(\mathrm{~d},{ }^{2} J=\right.$ $15.2 \mathrm{~Hz}, 1 \mathrm{H}$, part A of AB, 10-H), $3.56\left(\mathrm{~d},{ }^{2} J=15.2 \mathrm{~Hz}, 1 \mathrm{H}\right.$, part B of AB, $10-\mathrm{H}$ ), $3.39(\mathrm{~m}, 1 \mathrm{H}, 2-\mathrm{H}), 3.24$ (br. d, $J=12.3 \mathrm{~Hz}$, $1 \mathrm{H}, 4-\mathrm{H}), 2.93(\mathrm{~m}, 1 \mathrm{H}, 4-\mathrm{H}), 2.00$ (br. d, $J=13.5 \mathrm{~Hz}, 1 \mathrm{H}, 7-\mathrm{H})$, 1.81-1.35 (m, 4H, 5-H2, 6-H2, 7-H). ${ }^{13} \mathrm{C}$ NMR ( 126 MHz , DMSO- $d_{6}$ ) $\delta 170.8\left(\mathrm{~d},{ }^{3} J_{C P}=5.5 \mathrm{~Hz}, 9-\mathrm{C}_{\mathrm{q}}\right), 136.0\left(11-\mathrm{C}_{\mathrm{q}}\right), 129.0$ (15-CH), 125.4 (14-CH), 122.3 (12-CH), 56.9 (d, ${ }^{2} J_{C P}=10.3 \mathrm{~Hz}$, $2-\mathrm{CH}), 48.0\left(\mathrm{~d},{ }^{1} J_{C P}=148.9 \mathrm{~Hz}, 1-\mathrm{CH}\right), 44.8\left(4-\mathrm{CH}_{2}\right), 36.7(10-$ $\left.\mathrm{CH}_{2}\right), 24.0\left(\mathrm{~d},{ }^{3} J_{C P}=3.3 \mathrm{~Hz}, 7-\mathrm{CH}_{2}\right), 21.5\left(5-\mathrm{CH}_{2}\right.$ or $\left.6-\mathrm{CH}_{2}\right)$, $21.4\left(5-\mathrm{CH}_{2}\right.$ or $\left.6-\mathrm{CH}_{2}\right) .{ }^{31} \mathrm{P}$ NMR ( 162 MHz , DMSO- $d_{6}$ ) $\delta 15.07$. ${ }^{15} \mathrm{~N}$ NMR ( 51 MHz, DMSO- $d_{6}$ ) $\delta-272.06$ (1-NH), -331.80 ( $2-\mathrm{NH}_{2}$ ). HRESIMS $m / z 319.0801[\mathrm{M}+\mathrm{H}-\mathrm{Cl}]^{+}$(319.0876 calcd for $\mathrm{C}_{12} \mathrm{H}_{20} \mathrm{~N}_{2} \mathrm{O}_{4} \mathrm{PS}$ ).

## Compound 5j-2, diastereomer 2

${ }^{1} \mathrm{H}$ NMR ( $400 \mathrm{MHz}, \mathrm{DMSO}-d_{6}$ ) $\delta 8.40(\mathrm{~d}, J=7.1 \mathrm{~Hz}, 1 \mathrm{H}$, $1-\mathrm{NH}), 7.44(\mathrm{dd}, J=4.9,3.0 \mathrm{~Hz}, 1 \mathrm{H}, 14-\mathrm{H}), 7.30(\mathrm{~d}, J=3.0 \mathrm{~Hz}$, $1 \mathrm{H}, 12-\mathrm{H}), 7.08(\mathrm{~d}, J=4.9 \mathrm{~Hz}, 1 \mathrm{H}, 15-\mathrm{H}), 4.27\left(\mathrm{ddd},{ }^{2} J_{H P}=\right.$ $17.3 \mathrm{~Hz}, J=9.7,7.1 \mathrm{~Hz}, 1 \mathrm{H}), 3.60\left(\mathrm{~s}, 2 \mathrm{H}, 10-\mathrm{H}_{2}\right), 3.38-3.22$ (m, 2H, 2-H, 4-H), $2.89(\mathrm{dd}, J=12.1,12.1 \mathrm{~Hz}, 1 \mathrm{H}, 4-\mathrm{H}), 2.06$
(br. d, $J=13.8 \mathrm{~Hz}, 1 \mathrm{H}, 7-\mathrm{H}$ ), 1.78-1.36 (m, $5 \mathrm{H}, 5-\mathrm{H}_{2}, 6-\mathrm{H}_{2}$, 7-H). ${ }^{13} \mathrm{C}$ NMR ( 126 MHz, DMSO- $d_{6}$ ) $\delta 169.5$ (d, $J=4.4 \mathrm{~Hz}$, $9-\mathrm{C}_{\mathrm{q}}$ ), 135.3 ( $11-\mathrm{C}_{\mathrm{q}}$ ), 128.5 ( $15-\mathrm{CH}$ ), 124.8 ( $14-\mathrm{CH}$ ), 121.8 ( $12-$ CH), $55.9\left(\mathrm{~d},{ }^{2} J_{C P}=6.8 \mathrm{~Hz}, 2-\mathrm{CH}\right), 47.7\left(\mathrm{~d},{ }^{1} J_{C P}=148.3 \mathrm{~Hz}\right.$, 1-CH), $44.2\left(4-\mathrm{CH}_{2}\right), 36.3\left(10-\mathrm{CH}_{2}\right), 25.3\left(\mathrm{~d},{ }^{3} J_{C P}=4.1 \mathrm{~Hz}\right.$, $\left.7-\mathrm{CH}_{2}\right), 21.2\left(5-\mathrm{CH}_{2}\right.$ or $\left.6-\mathrm{CH}_{2}\right), 20.9\left(5-\mathrm{CH}_{2}\right.$ or $\left.6-\mathrm{CH}_{2}\right) .{ }^{31} \mathrm{P}$ NMR ( 162 MHz, DMSO- $d_{6}$ ) $\delta$ 15.26. HRESIMS $m / z 319.0885$ $[\mathrm{M}+\mathrm{H}-\mathrm{Cl}]^{+}$(319.0876 calcd for $\left.\mathrm{C}_{12} \mathrm{H}_{20} \mathrm{~N}_{2} \mathrm{O}_{4} \mathrm{PS}\right)$.

## ((2-(Benzo[b]thiophen-2-yl)acetamido)(piperidin-2-yl)methyl) phosphonic acid hydrochloride ( 5 k )

The synthesis was performed according to general procedure B. Compound $\mathbf{4 k} \mathbf{k} \mathbf{1}(52.2 \mathrm{mg}, 0.100 \mathrm{mmol})$ gave $\mathbf{5 k} \mathbf{- 1}$ as a slightly orange solid ( $17.5 \mathrm{mg}, 0.0432 \mathrm{mmol}, 43 \%$ ). Compound $4 \mathbf{k}-2(53.7 \mathrm{mg}, 0.102 \mathrm{mmol})$ gave $5 \mathbf{k}-2$ as a colorless solid ( $13.4 \mathrm{mg}, 0.0331 \mathrm{mmol}, 32 \%$ ).

## Compound 5k-1, diastereomer 1

${ }^{1} \mathrm{H}$ NMR ( 400 MHz, DMSO- $d_{6}$ ) $\delta 8.45(\mathrm{~d}, J=9.3 \mathrm{~Hz}, 1 \mathrm{H}$, $1-\mathrm{NH}), 7.87(\mathrm{~d}, J=7.7 \mathrm{~Hz}, 1 \mathrm{H}, 14-\mathrm{H})$, $7.80-7.69$ (dd, $J=$ $7.8,1.0 \mathrm{~Hz}, 1 \mathrm{H}, 17-\mathrm{H}), 7.32$ (ddd, $J=7.4,7.4,1.3 \mathrm{~Hz}, 1 \mathrm{H}$, part C of ABCD, 16-H), 7.28 (ddd, $J=7.4,7.4,1.3 \mathrm{~Hz}$, 1 H, part D of $\mathrm{ABCD}, 15-\mathrm{H}$ ), 7.25 (s, $1 \mathrm{H}, 19-\mathrm{H}$ ), 4.39 (ddd, $\left.{ }^{2} J_{H P}=19.5 \mathrm{~Hz}, J=9.7,4.9 \mathrm{~Hz}, 1 \mathrm{H}, 1-\mathrm{H}\right), 4.01\left(\mathrm{~d},{ }^{2} J=\right.$ $16.0 \mathrm{~Hz}, 1 \mathrm{H}$, part A of $\mathrm{AB}, 10-\mathrm{H}), 3.90\left(\mathrm{~d},{ }^{2} J=16.0 \mathrm{~Hz}\right.$, 1 H, part B of AB, 10-H), 3.35 (m, 1H, $2-\mathrm{H}$ ), 3.25 (br. d, $J=$ $12.4 \mathrm{~Hz}, 1 \mathrm{H}, 4-\mathrm{H}$ ), 2.92 (br. dd, $J=11.7,11.7 \mathrm{~Hz}, 1 \mathrm{H}$, $4-\mathrm{H}$ ), 2.02 (br. d, $J=13.1 \mathrm{~Hz}, 1 \mathrm{H}, 7-\mathrm{H}$ ), 1.80-1.35 (m, 5H, $5-\mathrm{H}_{2}, 6-\mathrm{H}_{2}, 7-\mathrm{H}$ ). ${ }^{13} \mathrm{C}$ NMR ( 126 MHz, DMSO- $d_{6}$ ) $\delta 169.6$ $\left(\mathrm{d},{ }^{3} J_{C P}=5.8 \mathrm{~Hz}, 9-\mathrm{C}_{\mathrm{q}}\right), 139.4\left(13-\mathrm{C}_{\mathrm{q}}\right), 139.3\left(18-\mathrm{C}_{\mathrm{q}}\right), 138.9$ ( $11-\mathrm{C}_{\mathrm{q}}$ ), 124.1 ( $16-\mathrm{CH}$ ), 123.7 ( $15-\mathrm{CH}$ ), 122.9 ( $17-\mathrm{CH}$ ), 122.7 (19-CH), 122.1 (14-CH), 57.0 (d, $\left.{ }^{2} J_{C P}=8.7 \mathrm{~Hz}, 2-\mathrm{H}\right), 48.2$ $\left(\mathrm{d},{ }^{1} J_{C P}=146.6 \mathrm{~Hz}, 1-\mathrm{CH}\right), 44.7\left(4-\mathrm{CH}_{2}\right), 36.9\left(10-\mathrm{CH}_{2}\right)$, $24.2\left(\mathrm{~d},{ }^{3} J_{C P}=3.8 \mathrm{~Hz}, 7-\mathrm{CH}_{2}\right), 21.6\left(5-\mathrm{CH}_{2}\right.$ or $\left.6-\mathrm{CH}_{2}\right), 21.5$ $\left(5-\mathrm{CH}_{2}\right.$ or $\left.6-\mathrm{CH}_{2}\right) .{ }^{31} \mathrm{P}$ NMR ( $162 \mathrm{MHz}, \mathrm{DMSO}-d_{6}$ ) $\delta$ 14.27. HRESIMS $\mathrm{m} / \mathrm{z} 369.1008[\mathrm{M}+\mathrm{H}-\mathrm{Cl}]^{+}$(369.1032 calcd for $\mathrm{C}_{16} \mathrm{H}_{22} \mathrm{~N}_{2} \mathrm{O}_{4} \mathrm{PS}$ ).

## Compound 5k-2, diastereomer 2

${ }^{1} \mathrm{H}$ NMR ( $500 \mathrm{MHz}, \mathrm{DMSO}-d_{6}$ ) $\delta 8.57(\mathrm{~d}, J=10.0 \mathrm{~Hz}, 1 \mathrm{H}$, $1-\mathrm{NH}), 7.88(\mathrm{~d}, J=7.8 \mathrm{~Hz}, 1 \mathrm{H}, 14-\mathrm{H}), 7.75(\mathrm{~d}, J=7.7 \mathrm{~Hz}, 1 \mathrm{H}$, $17-\mathrm{H}), 7.33$ (dd, $J=7.4,7.4 \mathrm{~Hz}, 1 \mathrm{H}, 16-\mathrm{H}), 7.29$ (dd, $J=7.4$, $7.4 \mathrm{~Hz}, 1 \mathrm{H}, 15-\mathrm{H}), 7.27(\mathrm{~s}, 1 \mathrm{H}, 19-\mathrm{H}), 4.29\left(\mathrm{ddd},{ }^{2} J_{H P}=17.3\right.$ $\mathrm{Hz}, J=9.6,7.4 \mathrm{~Hz}, 1 \mathrm{H}, 1-\mathrm{H}), 3.97\left(\mathrm{~d},{ }^{2} J=16.2 \mathrm{~Hz}, 1 \mathrm{H}\right.$, part A of AB, $10-\mathrm{H}), 3.93\left(\mathrm{~d},{ }^{2} J=16.2 \mathrm{~Hz}, 1 \mathrm{H}\right.$, part A of $\left.\mathrm{AB}, 10-\mathrm{H}\right)$, $3.34-3.25$ (m, 2H, 2-H, 4-H), 2.88 (br. dd, $J=12.2,11.5 \mathrm{~Hz}$, $1 \mathrm{H}, 4-\mathrm{H}$ ), 2.11 (br. d, $J=13.9 \mathrm{~Hz}, 1 \mathrm{H}, 7-\mathrm{H}$ ), 1.85-1.40 (m, 5H, $5-\mathrm{H}_{2}, 6-\mathrm{H}_{2}, 7-\mathrm{H}$ ). ${ }^{13} \mathrm{C}$ NMR ( 126 MHz, DMSO- $d_{6}$ ) $\delta 168.9$ (d, $\left.{ }^{3} J_{C P}=4.2 \mathrm{~Hz}, 9-\mathrm{C}_{\mathrm{q}}\right), 139.5\left(13-\mathrm{C}_{\mathrm{q}}\right.$ or $\left.18-\mathrm{C}_{\mathrm{q}}\right), 139.4\left(18-\mathrm{C}_{\mathrm{q}}\right.$ or $13-$ $\mathrm{C}_{\mathrm{q}}$ ), $138.8\left(11-\mathrm{C}_{\mathrm{q}}\right), 124.2(16-\mathrm{CH}), 123.8(15-\mathrm{CH}), 123.0$ ( $17-$ $\mathrm{CH}), 122.9(19-\mathrm{CH})$, $122.2(14-\mathrm{CH}), 56.2$ (d, ${ }^{2} J_{C P}=7.6 \mathrm{~Hz}$, $2-\mathrm{CH}), 48.5\left(\mathrm{~d},{ }^{1} J_{C P}=148.5 \mathrm{~Hz}, 1-\mathrm{CH}\right), 44.7\left(4-\mathrm{CH}_{2}\right), 37.2(10-$ $\left.\mathrm{CH}_{2}\right), 25.9\left(7-\mathrm{CH}_{2}\right), 21.7\left(5-\mathrm{CH}_{2}\right.$ or $\left.6-\mathrm{CH}_{2}\right), 21.5\left(5-\mathrm{CH}_{2}\right.$ or $6-\mathrm{CH}_{2}$ ). ${ }^{31} \mathrm{P}$ NMR ( 162 MHz , DMSO- $d_{6}$ ) $\delta$ 13.32. HRESIMS $\mathrm{m} / \mathrm{z}$ $369.1052[\mathrm{M}+\mathrm{H}-\mathrm{Cl}]^{+}$(369.1032 calcd for $\mathrm{C}_{16} \mathrm{H}_{22} \mathrm{~N}_{2} \mathrm{O}_{4} \mathrm{PS}$ ).

## ((2-(Benzo[b]thiophen-3-yl)acetamido)(piperidin-2-yl)methyl) phosphonic acid hydrochloride (51)

The synthesis was performed according to general procedure B. Compound $\mathbf{4 l} \mathbf{- 1}(53.0 \mathrm{mg}, 0.101 \mathrm{mmol})$ gave $\mathbf{5 l}-1$ as a colorless solid ( $7.9 \mathrm{mg}, 0.0195 \mathrm{mmol}, 19 \%$ ). Compound $\mathbf{4 1}-2$ $(50.7 \mathrm{mg}, 0.0966 \mathrm{mmol})$ gave $\mathbf{5 l - 2}$ as a colorless solid (15.9 $\mathrm{mg}, 0.0393 \mathrm{mmol}, 41 \%)$.

## Compound 51-1, diastereomer 1

${ }^{1} \mathrm{H}$ NMR ( $400 \mathrm{MHz}, \mathrm{DMSO}-d_{6}$ ) $\delta 8.49(\mathrm{~d}, J=9.5 \mathrm{~Hz}, 1 \mathrm{H}$, $1-\mathrm{NH}), 7.97(\mathrm{~d}, J=6.7 \mathrm{~Hz}, 1 \mathrm{H}, 15-\mathrm{H}), 7.88(\mathrm{~d}, J=6.9 \mathrm{~Hz}, 1 \mathrm{H}$, $18-\mathrm{H}), 7.54(\mathrm{~s}, 1 \mathrm{H}, 12-\mathrm{H}), 7.39(\mathrm{~m}, 1 \mathrm{H}, 17-\mathrm{H}), 7.36(\mathrm{~m}, 1 \mathrm{H}, 16-$ H), $4.46(\mathrm{~m}, 1 \mathrm{H}, 1-\mathrm{H}), 3.93\left(\mathrm{~d},{ }^{2} \mathrm{~J}=15.6 \mathrm{~Hz}, 1 \mathrm{H}\right.$, part A of AB , $10-\mathrm{H}), 3.83\left(\mathrm{~d},{ }^{2} J=15.6 \mathrm{~Hz}, 1 \mathrm{H}\right.$, part B of AB, $\left.10-\mathrm{H}\right), 3.39(\mathrm{~m}$, $1 \mathrm{H}, 2-\mathrm{H}$ ), 3.25 (br. d, $J=12.1 \mathrm{~Hz}, 1 \mathrm{H}, 4-\mathrm{H}$ ), 2.93 (br. dd, $J=$ $12.6,12.6 \mathrm{~Hz}, 1 \mathrm{H}, 4-\mathrm{H}$ ), 2.03 (br. d, $J=13.0 \mathrm{~Hz}, 1 \mathrm{H}, 7-\mathrm{H}$ ), 1.86-1.35 (m, 5H, 5-H2, 6-H2, 7-H). ${ }^{13} \mathrm{C}$ NMR ( 126 MHz , DMSO- $d_{6}$ ) $\delta 170.4\left(\mathrm{~d},{ }^{3} J_{C P}=5.5 \mathrm{~Hz}, 9-\mathrm{C}_{\mathrm{q}}\right), 139.3\left(14-\mathrm{C}_{\mathrm{q}}\right), 138.9$ $\left(19-\mathrm{C}_{\mathrm{q}}\right), 130.6\left(11-\mathrm{C}_{\mathrm{q}}\right), 124.3$ ( $16-\mathrm{CH}$ ), 124.1 ( $12-\mathrm{CH}$ ), 124.0 ( $17-$ $\mathrm{CH})$, 122.7 ( $15-\mathrm{CH}$ ), 122.4 ( $18-\mathrm{CH}$ ), 56.9 ( $\mathrm{d},{ }^{2} J_{C P}=9.9 \mathrm{~Hz}$, $2-\mathrm{CH}), 48.1\left(\mathrm{~d},{ }^{1} J_{C P}=148.4 \mathrm{~Hz}, 1-\mathrm{CH}\right), 44.8\left(4-\mathrm{CH}_{2}\right), 35.1(10-$ $\left.\mathrm{CH}_{2}\right), 24.1\left(\mathrm{~d},{ }^{3} J_{C P}=2.5 \mathrm{~Hz}, 7-\mathrm{CH}_{2}\right), 21.5\left(5-\mathrm{CH}_{2}\right.$ or $\left.6-\mathrm{CH}_{2}\right)$, $21.5\left(5-\mathrm{CH}_{2}\right.$ or $\left.6-\mathrm{CH}_{2}\right) .{ }^{31} \mathrm{P}$ NMR ( 162 MHz , DMSO- $d_{6}$ ) $\delta 14.97$. HRESIMS m/z $3690859[\mathrm{M}+\mathrm{H}-\mathrm{Cl}]^{+}$(369.1032 calcd for $\mathrm{C}_{16} \mathrm{H}_{22} \mathrm{~N}_{2} \mathrm{O}_{4} \mathrm{PS}$ ).

## Compound 51-2, diastereomer 2

${ }^{1} \mathrm{H}$ NMR ( 400 MHz, DMSO- $d_{6}$ ) $\delta 8.56(\mathrm{~d}, J=9.3 \mathrm{~Hz}, 1 \mathrm{H}$, 1-NH), 7.96 (m, 1H, 15-H), 7.90 (m, 1H, 18-H), 7.57 (s, 1H, 12$\mathrm{H}), 7.38(\mathrm{~m}, 1 \mathrm{H}, 17-\mathrm{H}), 7.36(\mathrm{~m}, 1 \mathrm{H}, 16-\mathrm{H}), 4.28\left(\mathrm{ddd},{ }^{3} \mathrm{H}_{H P}=\right.$ $17.1 \mathrm{~Hz}, J=9.7,6.7 \mathrm{~Hz}, 1 \mathrm{H}, 1-\mathrm{H}), 3.88\left(\mathrm{~d},{ }^{2} J=16.3 \mathrm{~Hz}, 1 \mathrm{H}\right.$, part A of AB, $10-\mathrm{H}$ ), $3.86(\mathrm{dd}, J=16.3 \mathrm{~Hz}, 1 \mathrm{H}$, part B of AB , $10-\mathrm{H}), 3.36-3.22(\mathrm{~m}, 2 \mathrm{H}, 2-\mathrm{H}, 4-\mathrm{H}), 2.88$ (dd, $J=11.8,11.8 \mathrm{~Hz}$, $1 \mathrm{H}, 4-\mathrm{H}), 2.04(\mathrm{~d}, J=13.8 \mathrm{~Hz}, 1 \mathrm{H}, 7-\mathrm{H}), 1.82-1.38(\mathrm{~m}, 5 \mathrm{H}$, $\left.5-\mathrm{H}_{2}, 6-\mathrm{H}_{2}, 7-\mathrm{H}\right) .{ }^{13} \mathrm{C}$ NMR ( 126 MHz, DMSO- $\mathrm{d}_{6}$ ) $\delta 169.8$ (d, $\left.{ }^{3} J_{C P}=4.7 \mathrm{~Hz}, 9-\mathrm{C}_{\mathrm{q}}\right), 139.3\left(14-\mathrm{C}_{\mathrm{q}}\right), 138.9\left(19-\mathrm{C}_{\mathrm{q}}\right), 130.5\left(11-\mathrm{C}_{\mathrm{q}}\right)$, 124.3 ( $16-\mathrm{CH}$ ), 124.3 ( $12-\mathrm{CH}$ ), 123.9 ( $17-\mathrm{CH}$ ), 122.7 ( $15-\mathrm{CH}$ ), $122.4(18-\mathrm{CH}), 56.7\left(\mathrm{~d},{ }^{2} J_{C P}=6.4 \mathrm{~Hz}, 2-\mathrm{CH}\right), 48.4\left(\mathrm{~d},{ }^{1} J_{C P}=\right.$ $147.5 \mathrm{~Hz}, 1-\mathrm{CH})$, $44.9\left(4-\mathrm{CH}_{2}\right), 35.2\left(10-\mathrm{CH}_{2}\right), 25.8\left(\mathrm{~d},{ }^{3} J_{C P}=\right.$ $\left.4.3 \mathrm{~Hz}, 7-\mathrm{CH}_{2}\right), 21.8\left(5-\mathrm{CH}_{2}\right.$ or $\left.6-\mathrm{CH}_{2}\right), 21.5\left(5-\mathrm{CH}_{2}\right.$ or $\left.6-\mathrm{CH}_{2}\right)$. ${ }^{31} \mathrm{P}$ NMR ( 162 MHz, DMSO- $d_{6}$ ) $\delta$ 15.13. HRESIMS $\mathrm{m} / \mathrm{z}$ $369.0975[\mathrm{M}+\mathrm{H}-\mathrm{Cl}]^{+}$(369.10324 calcd for $\left.\mathrm{C}_{16} \mathrm{H}_{22} \mathrm{~N}_{2} \mathrm{O}_{4} \mathrm{PS}\right)$.

## ${ }^{1} \mathrm{H},{ }^{15} \mathrm{~N}$ HSQC titration experiments

For the titrations, ${ }^{15} \mathrm{~N}$-labelled NDM-1 and ${ }^{15} \mathrm{~N}$-labelled VIM-2 $(250 \mu \mathrm{M})$, and titrated ligands ( 0.5 equiv. in $1 \mu \mathrm{~L}$ ) were prepared in the same buffer as the protein $(20 \mathrm{mM} \mathrm{KPO}$ $\mathrm{mM} \mathrm{ZnCl} 2, \mathrm{pH} 7.0$ with $10 \% \mathrm{D}_{2} \mathrm{O}$ and $2.5 \%$ DMSO- $d_{6}$ ). ${ }^{1} \mathrm{H},{ }^{15} \mathrm{~N}$ HSQC spectra were acquired with $128 \times 1024$ complex points (F1 $\times$ F2) and spectral width of $9090 \times 2740 \mathrm{~Hz}$ on ${ }^{15} \mathrm{~N}$-labeled NDM-1/VIM-2 and with every titration step up to $1: 16$ ratio between protein:ligand $(0,0.5,1,2,4,8$, and 16 equiv. of ligand). All experiments were recorded on a Bruker 600 MHz spectrometer at 310 K equipped with a 5 mm TCI cryogenic
probe. The NMR data were processed on the MestReNova software with the Mnova Binding plugin. The weighted average chemical shift perturbations (CSP) for the backbone amides were calculated from the observed chemical shift differences in the proton and nitrogen dimensions using the equation (chemical shift scaling factors: $F_{\mathrm{H}}=1, F_{\mathrm{N}}=0.156$ ) $\mathrm{CSP}=\Delta \delta_{1 \mathrm{H}, 15 \mathrm{~N}}=\sqrt{\left(\left(1 / F_{\mathrm{H}} \times \Delta \delta\left({ }^{1} \mathrm{H}\right)\right)^{2}+\left(1 / F_{\mathrm{N}} \times \Delta \delta\left({ }^{15} \mathrm{~N}\right)\right)^{2}\right)}$.

## Molecular docking

Schrödinger software (Schrödinger, LLC, New York, NY, 2019) was used and unless otherwise stated, default settings were used in the computations. The crystal structures of VIM-2 (pdb 6O5T) and NDM-1 (pdb 6NY7) were prepared using the Protein Preparation Wizard; ${ }^{60}$ involving Prime ${ }^{52}$ to fill in missing side chains and loops, Epik ${ }^{61,62}$ to generate heteroatom states (pH $7.0 \pm 2.0$ ), PropKa ( pH 7 ) to optimize the hydrogen bond network (sampling of water orientations when appropriate), and the OPLS3e force field ${ }^{63}$ in the restrained minimization (hydrogens only). The Receptor Grid Generation module in Glide ${ }^{64-66}$ was used to define the active site by creating a rectangular box centered on the native ligand (crystal structure) and extending in all directions to encompass ligands of similar size (i.e. $16.5 \AA$ ) or ligands $<22 \AA$. Grids with no water molecules in the active site were generated and validated. The ligands were converted to 3D all atom structures using LigPrep. Epik was used to generate possible ionization states at target $\mathrm{pH} 7 \pm 2$, including metal binding states. Tautomers were selected to be generated. The molecular docking was performed using Glide. The inhibitors were docked using the flexible docking mode, with sampling of nitrogen inversions and ring conformations, and with extra precision (XP) rescoring. Biased sampling of torsions was selected for amides by penalizing non-planar conformations. Epik state penalties was selected to be added to the docking score. A maximum of 40 poses were selected to be generated for each ligand. Post-docking minimization was used, and per-residue interaction scores were written for residues within $10 \AA$ from the grid center. Prime MM-GBSA rescoring of docking poses (VSGB 2.0 solvation model and OPLS3e force field) were performed providing poses ranked based on free energy of binding ( $\Delta G_{\text {bind }}$ in kcal $\left.\mathrm{mol}^{-1}\right)$. For these calculations, flexibility was allowed for residues within $10 \AA$ from the ligands, and minimization was selected as sampling method. Structural interaction fingerprints (SIFts) in Canvas was used for representation and analysis of the docking poses. Side chain, zinc ion, and hydrogen bonding interactions were used to generate the SIFts. The similarity between the SIFts were evaluated using the Tanimoto similarity metric, and clustering was performed using the Flexible Beta linkage method. Epik (Schrödinger, LLC, New York, NY, 2020) was used for empirical predictions of aqueous $\mathrm{p} K_{\mathrm{a}}$ values.

## Dose rate inhibition studies for $\mathbf{I C}_{50}$ determination

The half-maximal inhibitory concentration $\left(\mathrm{IC}_{50}\right)$ against the NDM-1, VIM-2 and GIM-1 enzymes were determined by using
twelve different concentration of inhibitor compounds ranging from $0 \mu \mathrm{M}$ to $800 \mu \mathrm{M}$. A $100 \mu \mathrm{l}$ solution with buffer ( 50 mM HEPES ( pH 7.2 ), $100 \mu \mathrm{M} \mathrm{ZnCl}_{2}, 2.5 \%$ DMSO), purified enzyme ( 0.1 nM VIM-2, 10 nM NDM-1 or 1 nM GIM1) and $800-0 \mu \mathrm{M}$ inhibitor was incubated in a 96 well plate at $25{ }^{\circ} \mathrm{C}$ for 5 min . In addition, the enzyme buffer contained $400 \mu \mathrm{~g} \mathrm{ml}^{-1}$ bovine serum albumin (BSA) to prevent protein unfolding and loss of activity due to low concentrations. ${ }^{67,68}$ $100 \mu \mathrm{M}$ of the reporter substrate nitrocefin (VIM-2, GIM-1) or imipenem (NDM-1) was added to the enzyme-inhibitor solution and the increase in absorbance at 482 nm (nitrocefin) or 300 nm (imipenem) was recorded on a Spectramax M4 spectrophotometer (Molecular Devices). Each data point was performed in triplicates and the initial velocity for each inhibitor concentration was analyzed by a $\log$ [inhibitor] vs. response curve fitting to calculate $\mathrm{IC}_{50}$ in GraphPad Prism 9.2 software.

## Cytotoxicity assay

MCF-7 and HepG2 cells were cultured in Dulbecco's modified Eagle's medium supplemented with $10 \%$ fetal bovine serum and $1 \%$ of penicillin-streptomycin antibiotics (SigmaAldrich). Cells were cultured in a permanent exponential growth at $37{ }^{\circ} \mathrm{C}, 5 \% \mathrm{CO}_{2} .{ }^{69}$ Briefly, cells were seeded in 96well plates (TPP® tissue culture plates 92097) at a density that allowed continued exponential growth. Stock solutions of the compounds dissolved in DMSO were added to a final DMSO concentration of $0.3 \% \mathrm{v} / \mathrm{v}$ in the cell culture medium. Two-fold serial dilutions were made to estimate $\mathrm{EC}_{50}$. After 24 h of incubation in the presence of the compounds, cell viability was determined using PrestoBlue cell viability reagent (ThermoFisher). POLARstar Omega plate reader (BMG Lab Tech) was used to measure resorufin fluorescence ( $544 \mathrm{~nm} / 590 \mathrm{~nm}$ ). Cells with no treatment, cells treated only with DMSO $0.3 \% \mathrm{v} / \mathrm{v}$, and cells treated with SDS $1 \% \mathrm{w} / \mathrm{v}$ were used as controls. Three independent replicate experiments were used to calculate the $\mathrm{EC}_{50}$. The statistical analysis and plots were performed using R version 3.6.3 ( R core team). ${ }^{70}$ The viability of the cells was plotted using ggplot $2,{ }^{71}$ as the fluorescence of treated cells/fluorescence of the lowest concentration of the compound.

## Protein expression

The expression and purification of uniformly [ ${ }^{15} \mathrm{~N}$ ]-labelled NDM-1 and VIM-2 were performed according to the procedure reported previously. ${ }^{48,49}$ For enzymatic assays, NDM-1, VIM-2 and GIM-1 were expressed and purified as published earlier. ${ }^{41}$

## Molecular cloning

The full-length $b l a_{\mathrm{VIM}-2}$ gene was amplified using primers ATA CAT ATG TTC AAA CTT TTG AGT AAG TTA TTG and TCA CTC GAG CTA CTC AAC GAC TGA GCG ATT TG and subcloned into a pET30a vector between the NdeI and XhoI restriction sites resulting in plasmid pY339.

## Preparation of outer membrane vesicles (OMVs) and in situ activity testing

OMVs carrying active VIM-2 inside their lumen were prepared as described. ${ }^{72}$ Briefly, E. coli strain BL21(DE3) $\Delta$ ompA carrying plasmid pY339 was grown at $37^{\circ} \mathrm{C}$ in LB media supplemented with $10 \mu \mathrm{M} \mathrm{ZnCl} 2$ and $50 \mu \mathrm{~g} \mathrm{~mL}$ - Kanamycin. When cultures reached an optical density of 0.4 protein expression was induced by addition of $50 \mu \mathrm{M}$ IPTG and growth was maintained for 2 h . Cells were removed by centrifugation at $10000 \times g$ for 10 min and cleared supernatants were filtered using $0.45 \mu \mathrm{~m}$ bottle top filters. OMVs were collected by centrifugation at $38400 \times g$ for 2 h , washed in OMV buffer (DPBSS, SigmaAldrich D8662, supplemented with $10 \mu \mathrm{M}_{\mathrm{ZnCl}}^{2}$ ), filtered using $0.45 \mu \mathrm{~m}$ syringe filters, recollected by centrifugation at $74500 \times$ $g$ for 30 min and resuspended in OMV buffer.

Activity of the encapsulated VIM-2 in presence of the compounds was determined in clear flat bottom half area 96 well microplates (Corning 3696) in a total volume of $50 \mu \mathrm{~L}$ containing $400 \mu \mathrm{~g} \mathrm{ml}{ }^{-1}$ OMVs, $250 \mu \mathrm{~g} \mathrm{ml}{ }^{-1}$ CENTA $\beta$-lactamase substrate (Sigma-Aldrich 219475), and $2.5 \%$ DMSO in OMV buffer using a Fluostar Omega plate reader (BMG Lab Tech) by measuring the increase in absorbance at 405 nm 10 minutes after addition of the substrate.

## Author contributions

KP and FD contributed equally. FD and KP synthesized the molecules, KP performed the NMR titrations and computational docking, SS and HKSL determined $\mathrm{IC}_{50} \mathrm{~S}$, GPDSM and PS performed the cytotoxicity assay, JT determined membrane permeability, AAR expressed and purified proteins, and AV determined X-ray structures. HA, KP and ME designed the structures. The synthesis was developed by HA, KP and FD. PS, HKSL, KP, HA and ME analysed and discussed the data. The manuscript was written with the contributions of all authors, and all authors have approved the final version of the manuscript.

## Conflicts of interest

There are no conflicts to declare.

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