Journal of Medicinal Chemistry

Article

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J. Med. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jmedchem.8b01466 • Publication Date (Web): 20 Dec 2018

ACS Publications

is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

α-Amino diphenyl phosphonates as novel inhibitors of *Escherichia coli* ClpP protease

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ABSTRACT

Increased Gram-negative bacteria resistance to antibiotics is becoming a global problem and new classes of antibiotics with novel mechanisms of action are required. The caseinolytic protease subunit P (ClpP) is a serine protease conserved among bacteria that is considered as an interesting drug target.

ClpP function is involved in protein turnover and homeostasis, stress-response and virulence among other processes. The focus of this study was to identify new inhibitors of *Escherichia coli* ClpP and to understand their mode of action. A focused library of serine protease inhibitors based on diaryl phosphonate warheads was tested for ClpP inhibition and a chemical exploration around the hit compounds was conducted. Altogether 14 new potent inhibitors of *E. coli* ClpP were identified. Compounds **85** and **92** emerged as most interesting compounds from this study due to their potency and, respectively, to its moderate but consistent antibacterial properties as well as the favorable cytotoxicity profile.

INTRODUCTION

Antibiotic resistance is a major global problem in both developed and developing countries.[1] The selection pressures on microorganisms when in contact with antibacterial agents underlies the emergence of resistance,[2] and the efficacy of first and second line antibiotics is decreasing at an alarming rate.[3] The importance of antimicrobial drug discovery was underlined by the World Health Organization's (WHO) first global report on antibiotic resistance which attributed 25,000 deaths in Europe and 2 million worldwide per year to bacterial infections.[4] Of particular concern are Gram-negative multidrug-resistant bacteria (MDR) which are becoming more prevalent.[5] Among the antibiotic drugs launched since the year 2000, only five new classes were introduced and only one was directed against Gram-negative bacteria in combination with β-lactams.[6] To avoid key resistance mechanisms to pre-existing antibiotics, drug discovery research has focussed on addressing alternative targets with novel mechanisms of antibacterial action.[7]

The antibacterial drug target caseinolytic protease proteolytic subunit (ClpP) is a widely conserved protein which is present in bacteria, in many eukaryotes (including humans, localised in mitochondria), but is absent in archaea and mollicutes.[8-9] ClpP, a chymotrypsin-like serine protease,[10] is thought to play an important role in determining virulence and stress response by modulating virulence factor expression in several bacteria including *Staphylococcus aureus*, *Listeria monocytogenes* and *Streptococcus pneumoniae*.[11-14] ClpP degrades mistranslated, misfolded or aggregated proteins, arising as a result of stress factors (*e.g.* heat stress and antibiotics).[8] In *Listeria monocytogenes* ClpP was found

to be essential for bacterial survival in macrophages.[12] In *Streptococcus pneumoniae* the levels of ClpP were demonstrated to be correlated with nitric oxide stress.[14-15]

ClpP proteases in Gram-positive bacteria have been more thoroughly studied as drug targets, but also advances in Gram-negative ClpPs were recently reported. Robinson *et al.*[16] identified ClpP as potential target for antivirulence therapies by showing differences between growth curves of wild-type and *clpP*-defective *E. coli* under nitric oxide stress conditions. It has also been demonstrated that in *E. coli* is responsible for the cleavage of proteins involved in metabolism, transcription factors, as well as in oxidative stress response and starvation.[17] Furthermore, *clpP*-deficient *Legionella pneumophila* showed impaired virulence and reduced translocation of effector proteins in the studies from Zhao *et al*.[18] and ClpX and ClpP2 were identified by Qiu *et al.*[19] as part of the proteolytic network of the exopolysaccharide alginate biosynthesis in *Pseudomonas aeruginosa*, a marker for the onset of chronic lung infection in cystic fibrosis.

ClpP is a tetradecamer with a cylindrical shape. The 14 subunits are arranged in two heptameric rings and a central chamber which contains the active sites of each subunit.[8] Each active site comprises the canonical Ser-His-Asp catalytic triad (for most bacteria).[20] The peptidase activity, a characteristic of a chymotrypsin-like serine protease,[21] typically results in peptides of 7-8 residues length,[22] with cuts occurring after non-polar residues.[23] ClpP proteolytic activity requires the presence of specific ATPases (ClpX and ClpA in the case of *E. coli*),[23] of the AAA+ enzyme superfamily, whose function is to recognize, unfold and then transfer the substrates into the chamber, thus forming the Clp complex together with ClpP.[24] The interface between ClpP and the AAA+ partners has been investigated as a drug targeting site and several antibacterial peptides were identified, which activate and deregulate ClpP.[24-25] These acyldepsipeptides (ADEPs) prevent ATPases binding to the heptameric rings of ClpP, resulting in uncontrolled proteolysis of essential bacterial proteins and eventually in bacterial cell death.[8, 26]

A promising approach to target the virulence-related functions of ClpP is to develop enzyme inhibitors used in combination with existing antibiotics. The pioneering efforts of Böttcher and Sieber to target ClpP led to the development of a series of β-lactone inhibitors (among them D3, **Figure 1**) for *S. aureus* ClpP.[27] These inhibitors bind covalently to the catalytic serine, leading to irreversible inhibition of

proteolytic activity. Further characterization proved their ability to reduce bacterial virulence expression not only in *S. aureus* but also in *L. monocytogenes*.[28-29] The potency of this inhibitor was improved 3- to 5-fold with the optimized β-lactone U1 (**Figure 1**).[30] However, the reduced plasma stability of these compounds, due to the fast hydrolysis of the cyclic ester, impeded further clinical development.[31]

Thereafter, a new class of potent ClpP inhibitors with better plasma stability was discovered by the Sieber group.[31] The phenyl esters (AV170, **Figure 1**) irreversibly inhibited *S. aureus* ClpP, and triggered deoligomerization of the ClpP tetradecamer into inactive heptamers. Their higher potency, inhibition kinetics and plasma lifetime, compared to the β-lactone series, were countered by their lower anti-virulence activity. Furthermore, attempts to further improve their acyl-enzyme complex stability unfortunately let to a loss of ClpP reactivity.[31] A non-covalent inhibitor against *S. aureus* ClpP has been also identified in a high-throughput screening (HTS) campaign.[32] The inhibitor (AV145, **Figure 1**) bound to the handle region near the active site, locking *S. aureus* ClpP in a novel and inactive conformation. However, binding of ClpX to ClpP revoked the inhibitory effect of AV145 and its analogues in bacteria.[32]

Boron derived compounds have also shown evidence of successfully inhibiting ClpP in *Mycobacterium tuberculosis* as demonstrated for bortezomib by Moreira *et al.* or the substrate-based peptide boronate inhibitors by Akopian *et al.* (**Figure 1**).[33,34] Nevertheless, proteasome inhibition, short half-life, poor pharmacokinetics and its high cost limited the direct use of bortezomib, the most potent *in cellulo* of previously described compounds at *M. tuberculosis* treatment.[33]

Recently, also pyrimidines have been shown to inhibit ClpP.[35] Compounds (P33, **Figure 1**) targeting *Plasmodium falciparum* ClpP achieved inhibition of growth and segregation of the apicoplast during the cell cycle, leading to parasite death.

Although ClpPs have been investigated in several organisms, inhibition of Gram-negative bacteria ClpP remains untapped and the chloromethyl ketone (Z-LY-CMK, **Figure 1**) co-crystallized by Szyk and Maurizi was the only reported inhibitor for *E. coli* ClpP reported so far.[36]

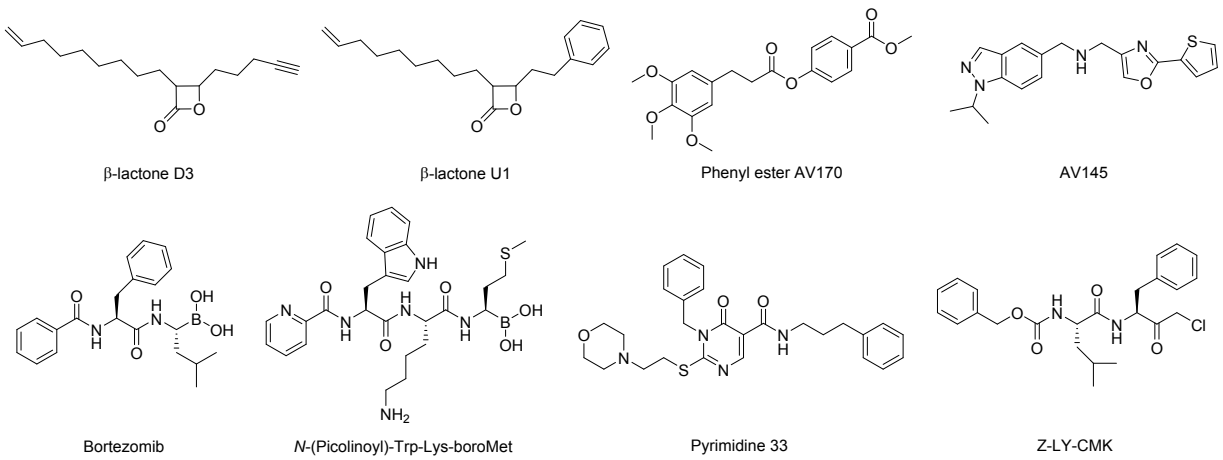


Figure 1. Examples of ClpP reported inhibitors.

Despite the demonstration of the potential of irreversible inhibitors on different ClpPs, inhibitors with a classical α-amino diaryl phosphonate warhead remained unexplored.[37] Thus far, several diaryl phosphonate compounds have been identified as potent, irreversible serine protease inhibitors. Some illustrative examples are a urokinase plasminogen activator (uPA) inhibitor reported by Joossens *et al.*,[38-39] a dipeptidyl peptidase 8 (DPP8) inhibitor by Van der Veken *et al.*,[40] an elastase inhibitor by Winiarski *et al.*,[41] a subtilisin inhibitor by Pietrusewicz *et al.*,[42] and the GluC and SplA inhibitors by Burchacka *et al.*[43-44]

The mode of action for this class of inhibitors (**Figure 2**) involves a nucleophilic attack by the hydroxyl of the active site serine on the electrophilic phosphorus atom, leading to the formation of a phosphonate ester. The initial enzyme-inhibitor complex is unstable. Therefore, hydrolysis of the aryl ester (with a half-life ranging from few hours to few days) leads to the formation of the "aged complex".

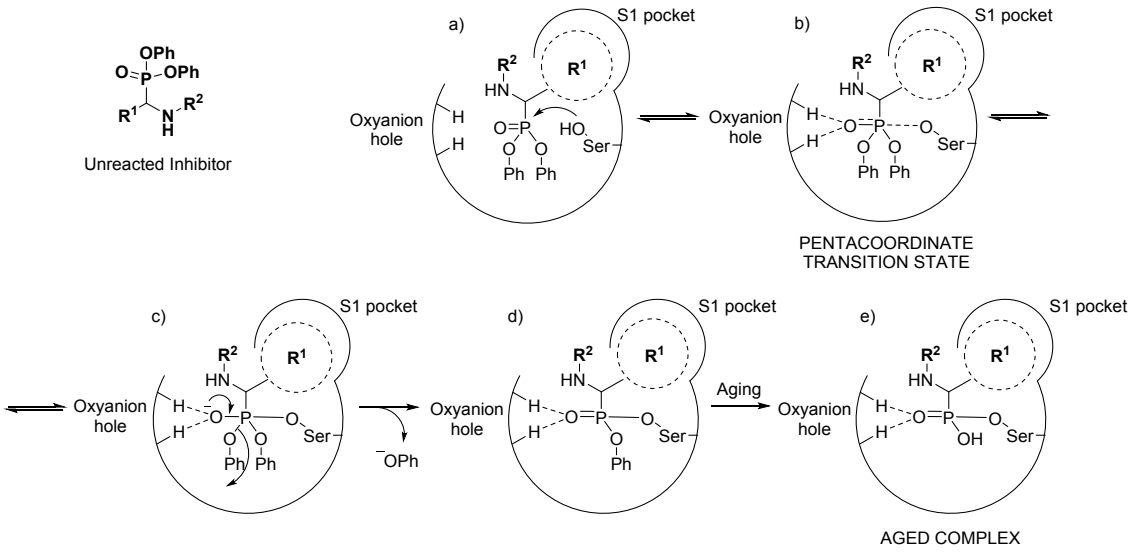


Figure 2. Binding mechanism of diphenyl phosphonates with serine proteases. a) The unreacted inhibitor enters the active site, with the R^1 moiety filling the S1 pocket while the phosphonate sits at a reachable distance from the oxyanion hole and the catalytic serine. b) Nucleophilic attack of the serine to the phosphonate facilitated by the hydrogen bonds of this group with the oxyanion hole residues to form the pentacoordinate transition state. c) Formalised bonds between the serine oxygen and phosphorus of the phosphonate lead to a negative charge on the oxygen that, when recovering the tetrahedral geometry, leads to the release of the phenol group. d) Stabilised configuration after covalent bonding between ligand and serine protease. e) Slow hydrolysis of the remaining phenolate leads to formation of the aged complex.

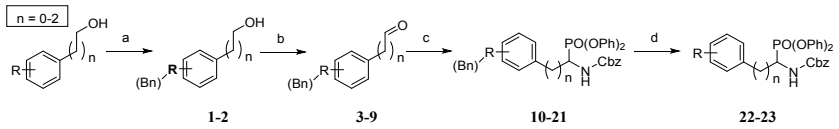
The aim of this work was to identify new classes of compounds as inhibitors of ClpP activity and investigate their mechanisms of action. We describe a series of α-amino diphenyl phosphonate esters as the first potent inhibitors of *E. coli* ClpP, using this species as a model organism for Gram-negative bacteria, encouraged by the availability of a crystal structure and by the previous studies where a clpP-defective strain showed a decreased growth under nitric oxide stress conditions.[16, 36]

RESULTS

Chemical explorations and enzymatic activity screening. The existing diarylphosphonate library of the Medicinal Chemistry group of the University of Antwerp (UAMC) was highly enriched in hydrophilic and polar residues in R^1 position, since it was mainly focused on targeting trypsin–like

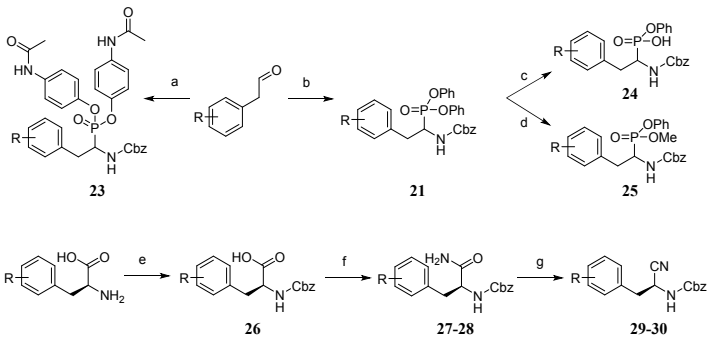
serine proteases. Based on the specificity of chymotrypsin-like serine proteases for lipophilic residues in the S1 pocket, a library of hydrophobic moieties in R^1 was designed. Some analogues of the previously described inhibitors (Z-LY-CMK and Lys-boroMet in **Figure 1**) were also included, together with variations on the warhead (diversity of arylphosphonates and nitriles).[34, 36]

Synthesis of the analogues with -Cbz in R^2 position and a diphenyl phosphonate as warhead (**10-23**) was carried out following the general synthesis described in **Scheme 1**, where protection of the hydroxyl groups on some of the aromatic rings was carried out in order to improve the yield of the following steps: Dess-Martin oxidation[45] and a modified alternative of the Birum-Oleksyszyn reaction previously reported by Van der Veken *et al*.[46] Those protected compounds, were finally debenzylated following the conditions of Okano *et al.*[47]



Scheme 1. Reagents and conditions. a) K_2CO_3, BnBr, DMF, rt, 4 h. b) Dess-Martin periodinane, DCM, 0-25 °C, 2 h. c) $CbzNH_2$, $P(OPh)_3$, $Cu(OTf)_2$, DCM, rt, 16 h. d) Pentamethylbenzene, BCl_3, DCM, -78 °C, 15 min.

Synthesis of the compounds with modifications on the phosphonate warhead or its substitution by a nitrile (**23-30**) were undertaken as described in **Scheme 2**, while dipeptidic diphenyl phosphonates (**32-41**) were obtained by prior cleavage of -Cbz and subsequent peptidic coupling. These protocols can be found in the experimental section.



Scheme 2. Reagents and conditions. a) $CbzNH_2$, tris(4-acetamidophenyl) phosphite, $Cu(OTf)_2$, DCM, rt, 16 h. b) $CbzNH_2$, $P(OPh)_3$, $Cu(OTf)_2$, DCM, rt, 16 h. c) KOH, H_2O:dioxane (1:1), rt, 16 h. d) NH_3,

NH_4Cl, MeOH, rt, 72 h. e) CbzCl, NaOH, H_2O, 0-25 °C, 2 h. f) Isobutylchloroformate, *N*-methylmorpholine, NH_3, DCM, 0-25 °C, 16 h. g) Burgess reagent, DCM, rt, 16 h.

The compounds **10-41** were evaluated for ClpP inhibition together with a subset of diaryl phosphonates from the UAMC library (**42-74**), selected in order to expand the variety of R^1 and R^2 residues (**Table 1**). ClpP inhibition was assessed by a high-throughput screen in 384-well format using a fluorescence assay with Suc-LY-AMC as fluorogenic substrate. The compounds were screened at 200 μM concentration. Compounds were considered as active if the percentage of inhibition (compared to a control without compounds) was higher or equal to 75 % (or ≤ 25% remaining activity).

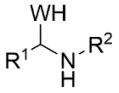
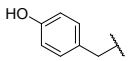
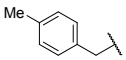
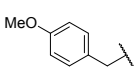
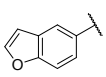
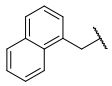
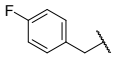
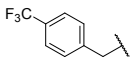
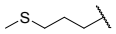
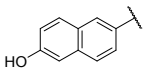
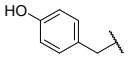
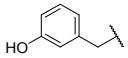
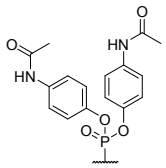
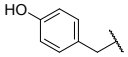
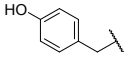
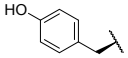
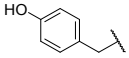
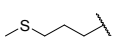
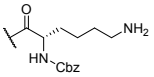
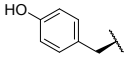
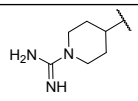
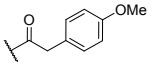
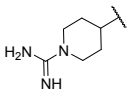
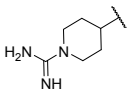
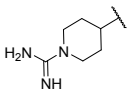
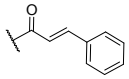
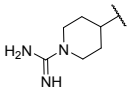
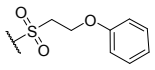
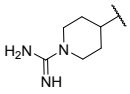
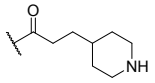
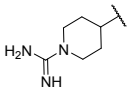
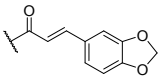
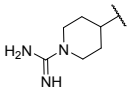
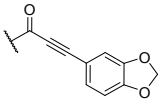
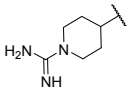
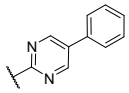
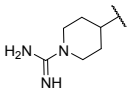
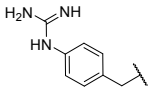
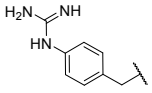
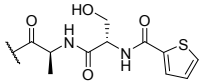
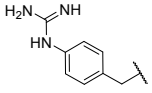
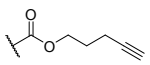
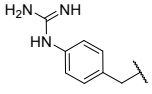
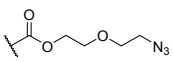
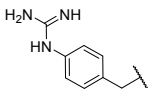
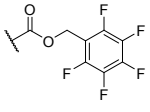


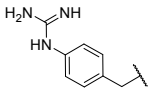
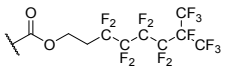
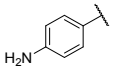
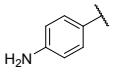
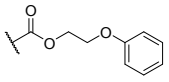
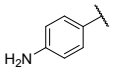
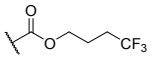
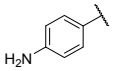
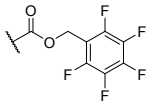
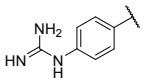
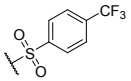
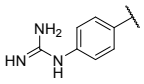
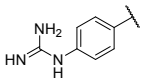
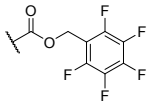
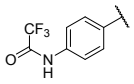
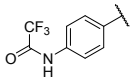
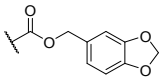
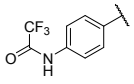
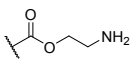
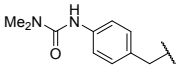
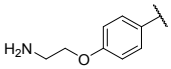
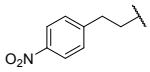
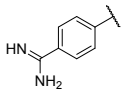
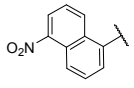
Figure 3. Generic compound structure.

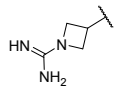
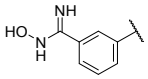
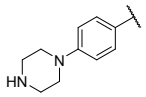
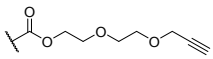
Table 1. Enzymatic inhibition of the apolar exploration and first library screening.

Compd.	R^1	R^2	Warhead	*E. coli* ClpP inhibition	
				%I (200 μM)	IC_{50} (μM)
Z-LY-CMK				100	14.4
10		-Cbz	$-PO(OPh)_2$	7	ND
11		-Cbz	$-PO(OPh)_2$	7	ND
12		-Cbz	$-PO(OPh)_2$	6	ND
13		-Cbz	$-PO(OPh)_2$	6	ND

14		-Cbz	-PO(OPh)$_2$	<1	ND
15		-Cbz	-PO(OPh)$_2$	13	ND
16		-Cbz	-PO(OPh)$_2$	**100**	**14.2 ± 1.3**
17		-Cbz	PO(OPh)$_2$	9	ND
18		-Cbz	-PO(OPh)$_2$	<1	ND
21		-Cbz	-PO(OPh)$_2$	<1	ND
22		-Cbz	-PO(OPh)$_2$	3	ND
23		-Cbz		9	ND
24		-Cbz		11	ND
25		-Cbz		14	ND
29		-Cbz	-CN	<1	ND
30		-Cbz	-CN	<1	ND
32			-PO(OPh)$_2$	<1	ND
36			-PO(OPh)$_2$	4	ND
41			-CN	13	ND
42			-PO(OPh)$_2$	13	ND

43			-PO(OPh)$_2$	13	ND
44			-PO(OPh)$_2$	24	ND
45			-PO(OPh)$_2$	18	ND
46			-PO(OPh)$_2$	27	ND
47			-PO(OPh)$_2$	6	ND
48			-PO(OPh)$_2$	4	ND
49			-PO(OPh)$_2$	24	ND
50			-PO(OPh)$_2$	12	ND
51			-PO(OPh)$_2$	11	ND
52			-PO(OPh)$_2$	27	ND
53			-PO(OPh)$_2$	**90**	**49.5 ± 0.5**
54			-PO(OPh)$_2$	23	ND
55			-PO(OPh)$_2$	68	ND
56			-PO(OPh)$_2$	25	ND

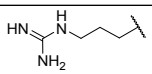
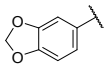
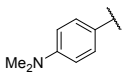
57			-PO(OPh)$_2$	55	ND
58		-Cbz	-PO(OPh)$_2$	**96**	**39.8 ± 2. 9**
59			-PO(OPh)$_2$	1	ND
60			-PO(OPh)$_2$	<1	ND
61			-PO(OPh)$_2$	28	ND
62			-PO(OPh)$_2$	27	ND
63			-PO(OPh)$_2$	29	ND
64			-PO(OPh)$_2$	48	ND
65			-PO(OPh)$_2$	64	ND
66			-PO(OPh)$_2$	**93**	**8.2 ± 0.8**
67			-PO(OPh)$_2$	73	ND
68		-Cbz	-PO(OPh)$_2$	9	ND
69		-Cbz	-PO(OPh)$_2$	15	ND
70		-Cbz	-PO(OPh)$_2$	**100**	**13.1 ± 1.2**
71			-PO(OPh)$_2$	**93**	**48.1 ± 1.7**
72			-PO(OPh)$_2$	17	ND

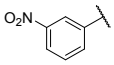
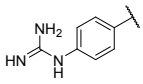
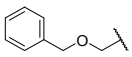
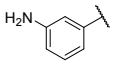
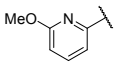
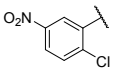
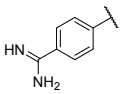
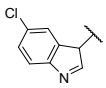
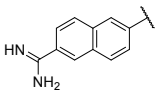
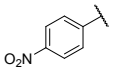
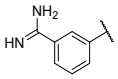
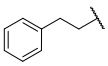
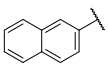
73			-PO(OPh)$_2$	12	ND
74			-PO(OPh)$_2$	23	ND
75			-PO(OPh)$_2$	25	ND

Six compounds emerged as active in the primary screen (**16**, **53**, **58**, **66**, **70** and **71**). Dose-response experiments confirmed all initial hits and the IC_{50} values ranged between 8.2 and 49.5 μM (**Table 1**). The biochemical tests revealed that the S1 pocket showed a preference for hydrophilic moieties, while **16** represents the only active compound with a lipophilic R^1 moiety. Regarding the R^2 substitution, a variety of simple carbamates were tolerated, -Cbz being the most common and chemically accessible. However, methyl carbamates and benzodioxol carbamates were also taken into account for future investigations.

After learning that the S1 pocket accepted a wider range of side chains, every remaining compound from the library with -Cbz in R^2 position (**76-95**) was submitted to a second round of experimental testing (**Table 2**).

Table 2. Enzymatic inhibition of second library screening.

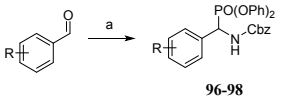
Compd.	**R^1**	**R^2**	**Warhead**	***E. coli* ClpP inhibition**	
				%I (200 μM)	**IC_{50} (μM)**
76		-Cbz	-PO(OPh)$_2$	15	ND
77		-Cbz	-PO(OPh)$_2$	6	ND
78		**-Cbz**	**-PO(OPh)$_2$**	**100**	**0.6 ± 0.1**
79		-Cbz	-PO(OPh)$_2$	27	ND

80		-Cbz	-PO(OPh)$_2$	6	ND
81		-Cbz	-PO(OPh)$_2$	3	ND
82		-Cbz	-PO(OPh)$_2$	4	ND
83		-Cbz	PO(OPh)$_2$	29	ND
84		-Cbz	-PO(OPh)$_2$	4	ND
85		**-Cbz**	**-PO(OPh)$_2$**	**100**	**0.5 ± 0.0**
86		**-Cbz**	**-PO(OPh)$_2$**	**100**	**38.0 ± 2.4**
87		-Cbz	-PO(OPh)$_2$	6	ND
88		-Cbz	-PO(OPh)$_2$	30	ND
89		**-Cbz**	**-PO(OPh)$_2$**	**88**	**100.5 ± 8.0**
90		**-Cbz**	**-PO(OPh)$_2$**	**81**	**79.7 ± 7.2**
91		-Cbz	-PO(OPh)$_2$	71	ND
92		**-Cbz**	**-PO(OPh)$_2$**	**100**	**0.5 ± 0.0**
93		-Cbz	PO(OPh)$_2$	<1	ND
94		-Cbz	-PO(OPh)$_2$	6	ND
95		-Cbz	-PO(OPh)$_2$	51	ND

1

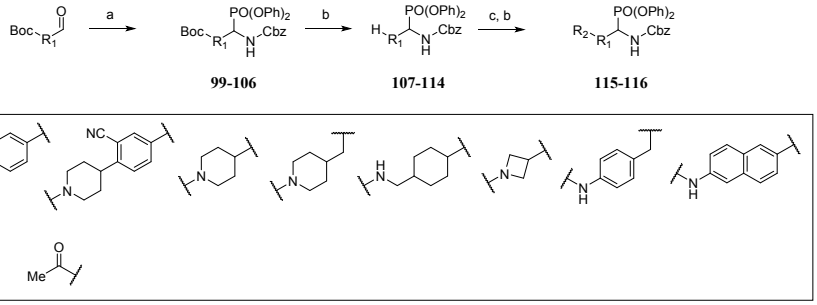
The biochemical tests resulted in the identification of six additional ClpP inhibitors (**78**, **85**, **86**, **89**, **90** and **92**), of which three inhibited the enzyme with a sub-micromolar IC_{50} value. From this and the previous screening, we concluded that hydrophilicity in the S1 pocket is preferred, and we therefore continued with a chemical exploration of polar groups in R^1 together with further modifications around three selected R^1 residues.

First, based on the polarity of the most active compounds identified so far, the scope of hydrophilic moieties for R^1 was enlarged, leaving the rest of the structure unchanged (**96-127**). Some of these compounds (**96-98**) were directly obtained from the commercial aldehydes after a Birum-Oleksyszyn reaction as stated in **Scheme 3**.



Scheme 3. Reagents and conditions. a) $CbzNH_2$, $P(OPh)_3$, $Cu(OTf)_2$, DCM, rt, 16 h.

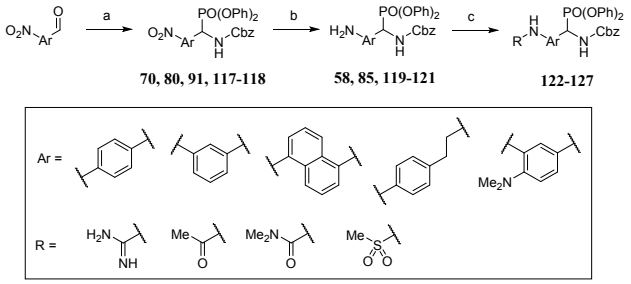
Still, most of them required a higher synthetic effort. The remaining compounds can be summarized in two synthetic schemes. For the first group (**107-116**), the Birum-Oleksyszyn reaction was conducted on the selected commercial aldehydes with Boc-protected amine, with the subsequent deprotection and guanylation for **115** and **116**. This group comprises a variety of aniline and piperidine related moieties in the R^1 position (**Scheme 4**).



Scheme 4. Reagents and conditions. a) $CbzNH_2$, $P(OPh)_3$, $Cu(OTf)_2$, DCM, rt, 16 h. b) TFA, DCM, rt, 1 h. c) *N,N′*-bis-Boc-1-guanylpyrazole, Et_3N, DCM, rt, 48 h.

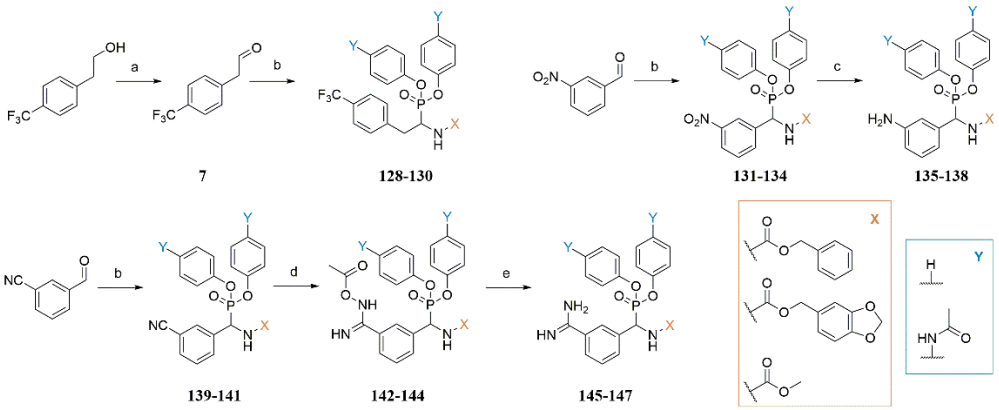
For a second group of aniline-related compounds and aromatic guanidines (**117-127**), the starting materials were a variety of commercial nitroaryl aldehydes that, after a Birum-Oleksyszyn reaction,

were reduced and subsequently substituted in some cases to generate methyl sulphoxyamines, guanydines, dimethylarylureas and methyl amides (**Scheme 5**). The biochemical tests for ClpP inhibition of these compounds revealed two inhibitors with IC_{50} values of 0.6 and 71.3, respectively (**Table 3**).

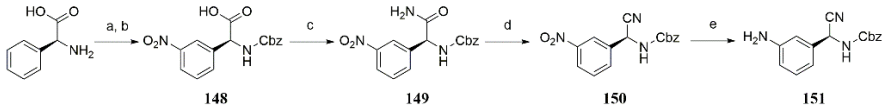


Scheme 5. Reagents and conditions. a) $CbzNH_2$, $P(OPh)_3$, $Cu(OTf)_2$, DCM, rt, 16 h. b) Zn, THF:NH_4Cl (sat. sol.) (1:1), 0 ˚C, 1 h. c) **124-125**: *N,N′*-bis-Boc-1-guanylpyrazole, Et_3N, DCM, rt, 48 h, then TFA, DCM, rt, 1 h; **122-123, 126-127**: RCl, DIPEA, DCM, rt, 2 h.

Finally, further investigation around the two most potent R^1 moieties (aniline **85** and amidine **92**) and the only lipophilic structure with activity (4-(trifluoromethyl)benzyl **16**) was undertaken, with substitution of the -Cbz by other active substituents from the first screening, together with some warhead alternatives (paracetamol-like phosphonates and nitriles). The chemistry regarding this exploration can be found in **Scheme 6** and **Scheme 7**. None of the 10 tested compounds revealed any pronounced ClpP inhibition (**Table 4**). A summary of all the compounds initial screening is reported in **Figure S1** in the supporting information.

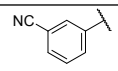
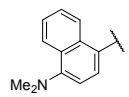


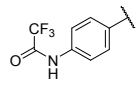
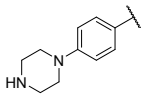
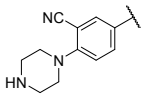
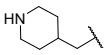
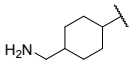
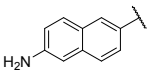
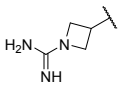
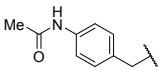
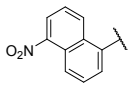
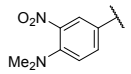
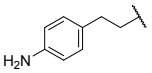
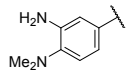
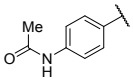
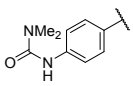
Scheme 6. Reagents and conditions. a) Dess-Martin periodinane, DCM, 0-25 °C, 2 h. b) $CbzNH_2$ (**129, 133, 140**)/methyl carbamate (**128, 130-132, 134, 139, 141**)/benzo[*d*][1,3]dioxol-5-ylmethyl carbamate (**132**), $P(OPh)_3$ (**128, 131-132, 139**)/ tris(4-acetamidophenyl) phosphite (**129-130, 133-134, 140-141**), $Cu(OTf)_2$, DCM, rt, 16 h. c) Zn, THF:NH_4Cl (aq. sat. sol.) (2:1), 0-25 °C, 16 h. d) NH_2OH□HCl, DIPEA, EtOH, 95 °C, 30-72 h, then acetic anhydride, MeCN, rt, 1 h; e) Pd(II)/C 10%, H_2 gas, AcOH, rt, 30 h.



Scheme 7. Reagents and conditions. a) HNO_3, H_2SO_4, rt, 2 h. b) CbzCl, NaOH, H_2O, 0-25 °C, 2 h. c) Isobutylchloroformate, *N*-methylmorpholine, NH_3, DCM, 0-25 °C, 16 h. d) Burgess reagent, DCM, rt, 16 h. e) Zn, THF:NH_4Cl (aq. sat. sol.) (2:1), 0-25 °C, 16 h.

Table 3. Enzymatic inhibition of the hydrophilic exploration.

Compd.	R^1	R^2	Warhead	*E. coli* ClpP inhibition	
				%I (200 μM)	IC_{50} (μM)
96		-Cbz	-$PO(OPh)_2$	15	ND
97		-Cbz	-$PO(OPh)_2$	<1	ND

98		-Cbz	-PO(OPh)$_2$	15	ND
107		-Cbz	-PO(OPh)$_2$	**100**	**0.6 ± 0.0**
108		-Cbz	-PO(OPh)$_2$	10	ND
109		-Cbz	-PO(OPh)$_2$	30	ND
110		-Cbz	PO(OPh)$_2$	16	ND
111		-Cbz	-PO(OPh)$_2$	15	ND
114		-Cbz	-PO(OPh)$_2$	<1	ND
115		-Cbz	-PO(OPh)$_2$	**100**	**71.3 ± 2.4**
116		-Cbz	-PO(OPh)$_2$	18	ND
117		-Cbz	-PO(OPh)$_2$	49	ND
118		-Cbz	-PO(OPh)$_2$	39	ND
120		-Cbz	-PO(OPh)$_2$	13	ND
121		-Cbz	PO(OPh)$_2$	22	ND
122		-Cbz	-PO(OPh)$_2$	8	ND
123		-Cbz	-PO(OPh)$_2$	22	ND

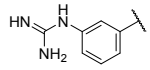
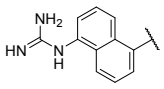
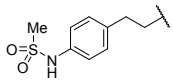
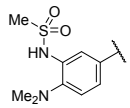
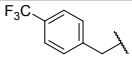
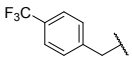
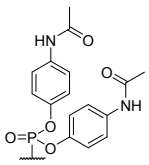
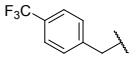
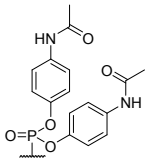
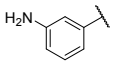
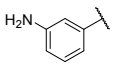
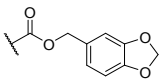
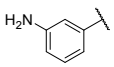
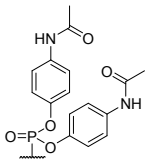
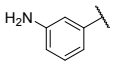
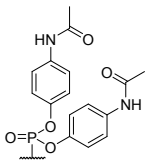
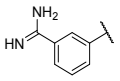
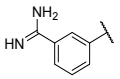
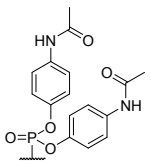
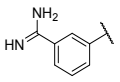
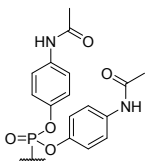
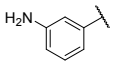
124		-Cbz	-PO(OPh)$_2$	15	ND
125		-Cbz	-PO(OPh)$_2$	38	ND
126		-Cbz	-PO(OPh)$_2$	10	ND
127		-Cbz	-PO(OPh)$_2$	46	ND

Table 4. Enzymatic inhibition of the exploration around **16**, **85** and **92**.

Compd.	**R^1**	**R^2**	**Warhead**	***E. coli* ClpP inhibition**	
				%I (200 μM)	**IC$_{50}$ (μM)**
128			-PO(OPh)$_2$	4	ND
129		-Cbz		1	ND
130				20	ND
135			-PO(OPh)$_2$	7	ND
136			-PO(OPh)$_2$	20	ND
137		-Cbz		21	ND

138				12	ND
145			-PO(OPh)$_2$	30	ND
146		Cbz		29	ND
147				12	ND
151		-Cbz	-CN	8	ND

The hydrophilic exploration resulted in two additional (**107** and **115**), with **107** having an IC_{50} in the sub-micromolar range. Unfortunately, every alteration on the structure of our reference compounds (**16**, **85** and **92**) led to loss of activity.

Biological evaluation of hits. The 14 hits identified after the different stages were submitted to further *in vitro* profiling. The selectivity properties *versus* chymotrypsin-like serine proteases were evaluated by screening the 14 compounds against α-chymotrypsin (bovine) at 200 μM concentration. Most compounds showed no significant inhibition of chymotrypsin with the exception of **85**, which resulted in a residual enzyme activity of 18 % (**Table 5**).

Cytotoxicity was tested against the human cell lines A549 (lung), HepG2 (liver) and HeLa (cervical cancer) in dose response (**Table 5**). Compounds **89**, **90** and **107** were toxic for all cell lines while compounds **66** and **58** exerted high cytotoxicity (<10-fold of compound IC_{50}) against the lung cell line A549. Compounds **78** and **85** showed moderate toxicity against A549 and HepG2 cell lines compared with their *in vitro* potency against the target (IC_{50}). The cytotoxicity effects reported here against human

cell lines could in principle be caused by the interaction with ClpP present in the human mitochondria as well by ClpP unrelated mechanisms.

Table 5. Enzymatic activity, cytotoxicity and activity against chymotrypsin of the selected hits.

Compd.	*E. coli* ClpP	Cytotoxicity EC_{50} (μM)			Chymotrypsin
	IC_{50} (μM)	HeLa	HepG2	A549	% of remaining activity (200 μM)
16	14.2 ± 1.3	≥100	≥100	≥100	93.9 ± 0.8
53	49.5 ± 0.5	≥100	≥100	≥100	≥ 100
58	39.8 ± 2. 9	≥100	≥100	57.8 ± 6.7	≥ 100
66	8.2 ± 0.8	≥100	≥100	41.5 ± 3.8	46.0 ± 2.9
70	13.1 ± 1.2	≥100	≥100	≥100	≥ 100
71	48.1 ± 1.7	≥100	≥100	≥100	≥ 100
78	0.6 ± 0.1	≥100	28.4 ± 3.5	65.6 ± 8.3	≥ 100
85	0.5 ± 0.0	≥100	23.8 ± 2.8	27.5 ± 2.3	17.9 ± 1.6
86	38.0 ± 2.4	≥100	≥100	≥100	≥ 100
89	100.5 ± 8.0	19.9 ± 1.8	10.5 ± 1.4	5.9 ± 8.5	≥ 100
90	79.7 ± 7.2	19.9 ± 2.3	28.3 ± 2.5	25.6 ± 2.7	≥ 100
92	0.5 ± 0.0	≥100	≥100	≥100	≥ 100
107	0.6 ± 0.0	8.6 ± 1.2	1.1 ± 0.1	0.4 ± 0.0	≥ 100
115	71.3 ± 2.4	≥100	≥100	≥100	≥ 100

In order to investigate the mode of interaction between ClpP and selected compounds, surface plasmon resonance measurements were conducted. The known covalently binding compound chloromethyl ketone (Z-LY-CMK)[36] was used as positive control for irreversible binding.

Compounds with IC_{50} values <10 μM were tested in a range of concentrations. In addition, the known covalent inhibitor Z-LY-CMK was tested as positive control. Z-LY-CMK clearly showed irreversible binding to ClpP, since the compound signal in the sensogram did not return to the baseline (0 RU), even

after stop of the compound injection (at ~350 seconds in all experiments) (**Figure 4A**). In contrast, all compounds from this study (**Figure 4B-F**) bound reversibly to the protein, as shown by the signal drop to the baseline after stopping injection. Moreover, the sensorgrams revealed rapid on- and off-rates for all newly identified ClpP inhibitors.

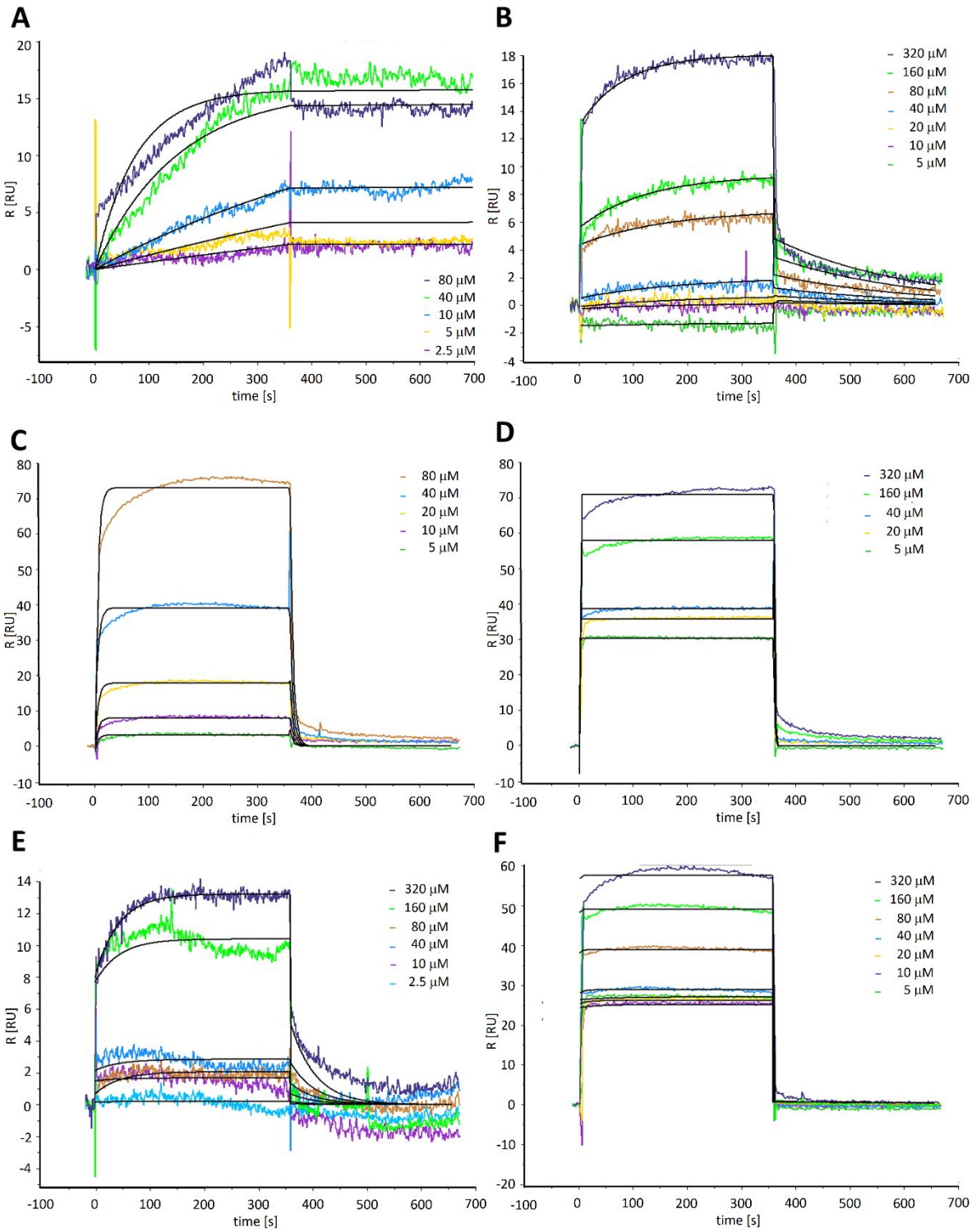


Figure 4. Surface plasmon resonance sensorgrams. A) Known covalent inhibitor Z-LY-CMK as control for irreversible binding.[36] B-F) Selected screening hits, B) **78**, C) **107**, D) **85**, E) **66**, F) **92**.

Antibacterial assays. Since ClpP is not an essential protease in *E. coli*, an assay was required to investigate the influence of the ClpP inhibitors on bacterial growth rates. We utilized a method reported

by Robinson *et al.*,[16] who observed that a ClpP deletion mutant recovered more slowly from nitric oxide stress than the corresponding wild type, and adapted this assay to a HTS format. Nitric oxide stress was induced by addition of DPTA NONOate (2 mM) to the *E. coli* WT and the isogenic *E. coli* ClpP deletion strain (Δ*clpP*). Although Δ*clpP* strain grew less well under our assay conditions (M9 minimal medium, 96-well format) compared to the wild type, we observed a small but significant difference in time to growth recovery after nitric oxide stress for the Δ*clpP* strain compared to the WT strain (**Figure 5**). Statistical analysis indicated that the ClpP deletion strain required approximately one hour longer than the wild type to for growth recovery (see **Figure S2** in the supporting information).

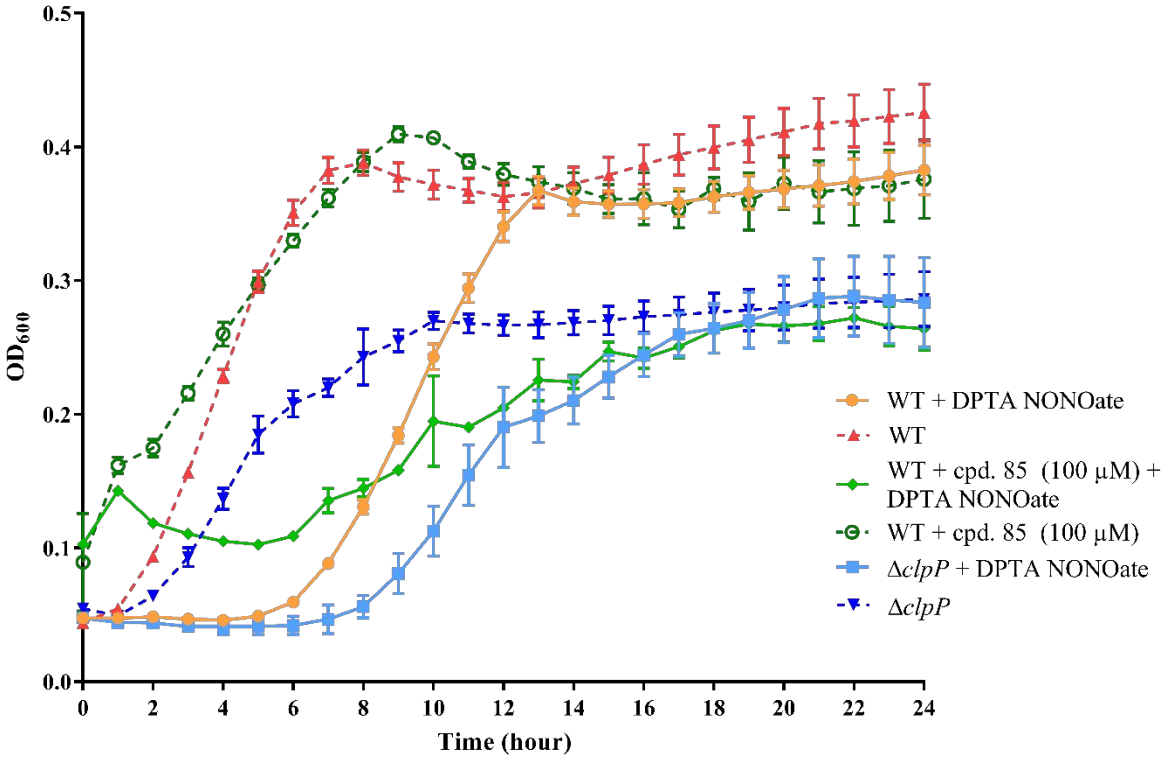


Figure 5. Comparison of the the growth curves, depicted by OD_{600}, of *E. coli* WT and the isogenic mutant *E. coli* JW0427-1 (*clpP*-defective mutant), both in absence and presence of the •NO chemical donor DPTA NONOate (2 mM) in minimal M9 medium supplemented with 10 mM of glucose. Effect of compound **85** on bacterial growth is quantified by OD_{600} of *E. coli* BW25-113 (WT) in presence and in absence of DPTA NONOate (2 mM). Each value represents the mean of three independent experiment ± standard deviation.

The fourteen hits identified in this study were tested in WT strain in presence of nitric oxide stress. Only compound **85** showed a remarkable effect in the WT growth (**Figure 5**), opposite to all the other compounds which were also tested but without showing any effect. Therefore, while not every hit achieved the desired inhibition, the effects of compound **85** on the stressed WT strain showed a comparable growth delay observed to the Δ*clpP* strain exposed to nitric oxide stress conditions, consistent with a potential ClpP protease inhibition mediated effect of compound **85**.

Figure 5 shows that in the presence of nitric oxide stress the growth rate of the WT strain is reduced, and requires additional 4 h to reach maximum absorbance (orange circle versus red triangle). The growth rate of the Δ*clpP* strain compared to the WT strain is reduced, and is further reduced in the presence of nitric oxide stress, taking an additional 6 h to reach maximum absorbance (light blue square versus dark blue inverted triangle). The addition of compound **85** at 100 μM does not affect the growth rate of the WT strain (green ring versus red triangle). However, under nitric oxide stress conditions, the WT strain growth is reduced in presence of compound **85** compared to nitric oxide stress only (green diamond versus orange circle) and, interestingly, the growth of Δ*clpP* is similar to that of the WT strain in the presence of compound **85** (blue square versus green diamond). Moreover, the effect of compound **85** in Δ*clpP* growth was tested in presence and absence of nitric oxide stress to ensure that the compound-mediated effect on the WT bacteria growth was due to ClpP inhibition and not due to off-target effect. As shown in **Figure S3** (in the supporting information), the compound did not significantly affect the growth of the Δ*clpP* bacteria either in absence or presence of nitric oxide stress conditions. This confirms that the effect of **85** on WT bacteria under nitric oxide stress conditions is most likely mediated through its inhibition of ClpP.

Selected compounds were also screened against the wild-type strains of *S. aureus, Acinetobacter baumannii, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *E. coli* in a standard bacterial growth assay. Two *E. coli* mutants with either *lpxC* defect (impaired in lipidA synthesis) or *tolC* defect (efflux pump defect) were also included. At 100 μM compound concentration, only **115** inhibited the growth of *S. aureus* WT (% inhibition of growth 99.9 ± 0.04), while **90** (98.9 ± 0.05), **107** (69.9 ± 5.6) and **115** (99.0 ± 0.5) inhibited the efflux pump deficient *E. coli* strain. The mode of action underlying this growth

inhibition remains elusive and could be caused by mechanisms unrelated to *E. coli* ClpP. In order to investigate whether the compounds are efflux pump substrates, the growth of *E. coli* wild type was examined in presence of test compounds (concentration 50 μM) and 25 μg/ml of phenylalanine-arginine beta-naphthylamide (PAβN), a known efflux pump substrate. At 24 h compound **90** inhibited bacterial growth (98.5 ± 1.2). In order to verify whether the observed effect of compound **90** was due to ClpP inhibition, the assay was repeated using the *E.coli* Δ*clpP* strain. The same output of the assay with the wild type strain was obtained (growth inhibition 99.4 ± 0.7), we can therefore assest that compound **90** addresses a different target that is influencing bacterial growth in presence of the efflux pump substrate. A summary of the compounds active in bacteria can be found in the supporting information (**Table S1**).

Molecular docking. Potential binding modes of the most potent inhibitors (**92** and **85)** within the active site of ClpP were investigated by molecular docking of the compounds into the X-ray crystal structure of *E. coli* ClpP (PDB ID 2FZS) using GOLD.

Clustering of the docking poses of **92** revealed two preferred binding modes (**Figure 6A-D**). The top-ranked pose of the first cluster (**Figure 6A&B**) shows the benzamidine group to be positioned deeply within the S1 pocket, while a hydrogen bond network between the phosphonate and residues Gly68 (constituent of the oxanion hole) and Leu125 is well established. However, the distance between the side chain oxygen atom of Ser97 and the phosphorus atom of the ligand is larger than required for the expected nucleophilic attack (3.35 Å). The second predominant binding mode revealed the benzamidine group to be solvent-exposed and the docked ligand shares several hydrogen bonds with the protein (**Figure 6C&D**). The interaction energy between docked ligand and ClpP was calculated using the Amber10:EHT force field. The top-ranked docking pose of cluster 1 (R^1 moiety placed in the S1 pocket) revealed a more favorable interaction energy (-62.5 kcal mol^{-1}) compared to the top-ranked pose of cluster 2 (-54.6 kcal mol^{-1}).

The predicted binding mode of **85** is shown in **Figure 6E&F**. Only one cluster was identified and the binding mode revealed the phenyl groups of the diarylphosphonate to be solvent-exposed, whilethe aniline moiety is positioned inside the S1 pocket. Again, several hydrogen bonds are formed between

the phosphonate group and the protein, but Ser97 did not display a favorable position for the nuclephilic attack.

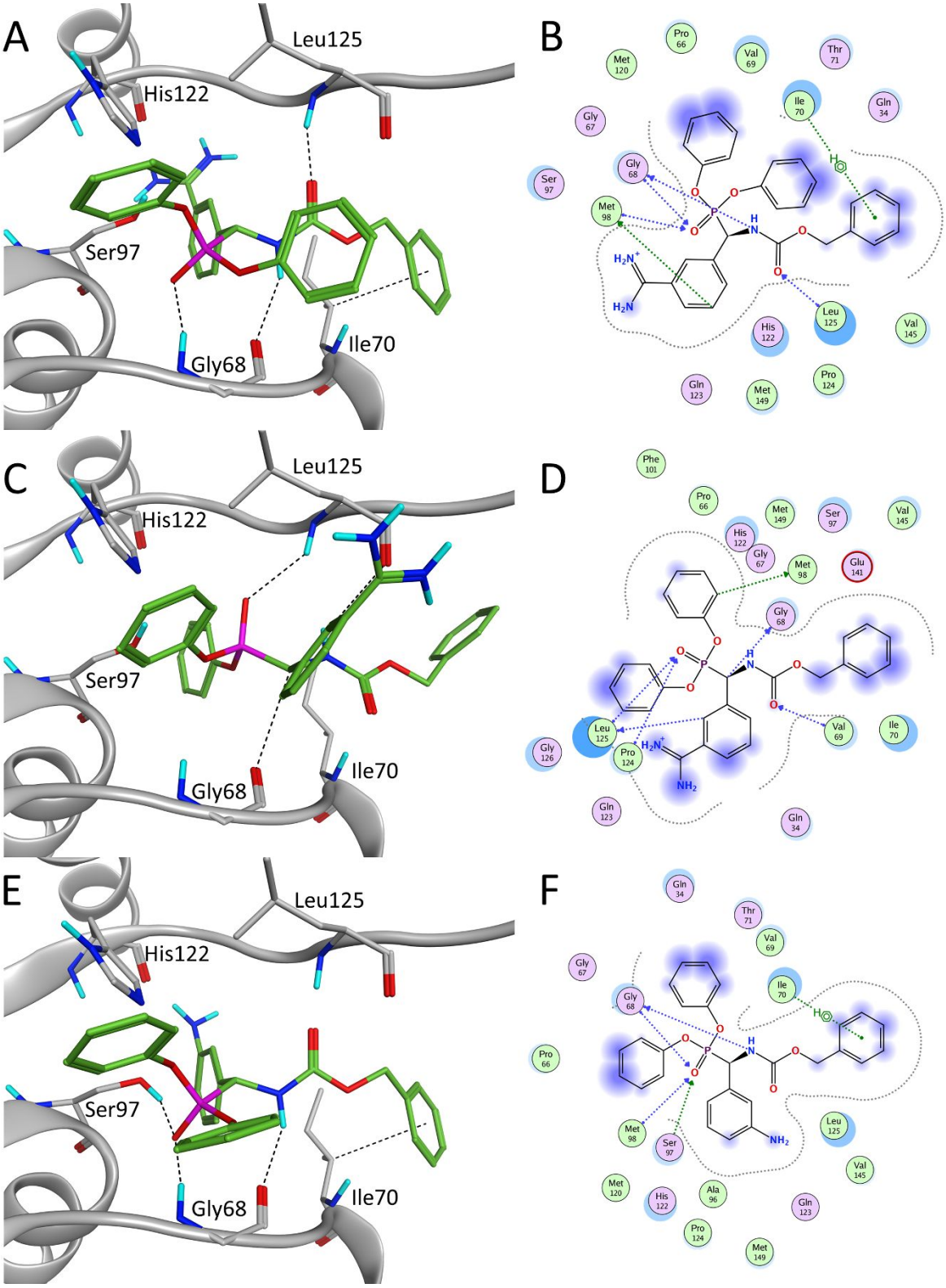


Figure 6. Computational prediction of potential binding modes for **92** and **85** within the *E. coli* ClpP crystal structure (PDB ID: 2FZS). A and C) Docking poses of the two main docking clusters of

compound **92**. E) Pose of compound **85**. Black dotted lines indicate hydrogen bonds between the ligands and the protein. B, D and F) 2D interaction plots between protein and **92** of cluster 1 (B), cluster 2 (D) and **85** (F) where the polar residues are indicated in purple, the non-polar or charged residues in green, and the solvent exposure by blue shadow. The black dotted line designates the proximity contour. The green dotted arrows indicate hydrogen bonds involving amino acid side chain atoms (donors and acceptors) while blue dotted arrows indicate hydrogen bonds accepted or donated by protein backbone atoms. Moreover, arene-H interactions are shown as green dotted line.

DISCUSSION AND CONCLUSIONS

An extensive chemical exploration and enzymatic screening identified 14 compounds inhibiting *E. coli* ClpP *in vitro* with sub-micromolar IC_{50} values for **78**, **85**, **92** and **107**. Despite the expected hydrophobicity of the protease recognition pocket, compounds containing polar residues in R^1 position displayed the highest inhibitory activity. Molecular docking analysis of compound **85** and **92** revealed that the most favorable poses had the aniline or benzamidine group deeply positioned within the recognition pocket. The diphenyl phosphonate warhead was crucial, with none of the replacements or small modifications attempted maintaining the inhibitory activity.

Surface plasmon resonance demonstrated a reversible binding for all tested compounds. With help of the docking studies of **85** and **92,** it can be hypothesized that the inhibitory poses do not allocate the phosphonate esters of the ligands in a favorable position to form the pentacoordinate transition state (**Figure 2**) after the attack by Ser97. Albeit unexpected, this reversible binding of the chemical family has a precedent in the KLK4 inhibitors reported by Van Soom *et al*.[48]

Benzamidine **92** emerges as the safest option for further optimization due to its potent enzymatic inhibition, absence of activity against chymotrypsin and lack of toxicity against the tested eukaryotic cell lines. Even though the latter could be related to a compound's incapability to enter eukaryotic cells or to the possibility of being substrate of an efflux pump and would also explain the lack of activity in the nitric oxide stress assay. At the same time, aniline **85** showed reduction of growth in *E. coli* WT under nitric oxide stress conditions, consistent with a ClpP-mediated effect. However, it requires further improvement in terms of limiting its toxicity against human cell lines and decreasing activity against

chymotrypsin**.** Both compounds, as well as **16**, **66**, **70**, **78** and **107** are significantly more potent ClpP inhibitors compared to the so far only known inhibitor Z-LY-CMK. In order to enlarge the scope and to understand the lack of effect of some of our inhibitors in the nitric oxide stress assay, further exploration of chemical alternatives is needed. Given the already known potential of covalent binding compounds as antimicrobial agents, for example the huge success of β-lactam antibiotics (*e.g.* penems, cephalosporins, carbapenems, monobactams), the development of a covalent binder for *E. coli* ClpP should focus on the replacement of the diaryl phosphonate by a different covalent warhead for serine proteases. The comprehensive R^1 moiety library developed in this study may guide future work in the field combining them with warheads such as boronates, based on the success of bortezomib with *M. tuberculosis* ClpP and its inhibition of human 26S proteasome.[33]

EXPERIMENTAL SECTION

CHEMISTRY. Reagents were obtained from commercial sources and were used without further purification. Characterization of all compounds was done with ^{1}H and ^{13}C NMR and mass spectrometry. ^{1}H and ^{13}C NMR spectra were recorded on a 400 MHz Bruker Avance III Nanobay spectrometer with Ultrashield working at 400 MHz and 100 MHz respectively; and analyzed by use of MestReNova analytical chemistry software. Chemical shifts are in ppm, and coupling constants are in hertz (Hz). The UPLC (Ultra Performance liquid chromatography), used to quantify the purity of the products was an ACQUITY UPLC H-Class System with a TUV detector Waters coupled to a MS detector Waters QDa. An Acquity UPLC BEH C18 1.7 μm (2.1 x 50 mm) column was used and as eluent a mixture of 0.1% FA in H_2O, 0.1% FA in MeCN, H_2O and MeCN. The wavelengths for UV detection were 254 nm and 214 nm. Key target compounds for the activity were analysed by High Resolution Mass: 10 μL of each sample (conc. = 10^{-5} M) was injected using the CapLC system (Waters, Manchester, UK) and electrosprayed using a standard electrospray source. Samples were injected with an interval of 5 min. Positive ion mode accurate mass spectra were acquired using a Q-TOF II instrument (Waters, Manchester, UK). The MS was calibrated prior to use with a 0.2% H_3PO_4 solution. The spectra were lock mass corrected using the known mass of the nearest H_3PO_4 cluster. Where necessary, flash column chromatography was performed on a Biotage ISOLERA One flash system equipped with an internal variable dual wavelength diode array detector (200−400 nm). For normal phase purifications SNAP cartridges (4 − 100 g, flow rate of 10 − 100 mL/min) were used, and reverse phase purifications were done making use of KP-C18 cartridges (4 - 30 g, flow rate of 10 − 50 mL/min). Dry sample loading was done by self-packing samplet cartridges using Celite 545. Gradients used varied for each purification.

The following sections comprise the synthetic procedures and analytical data for all compounds reported in this manuscript. Every reaction was performed under N_2 atmosphere if not stated otherwise. Several synthetic procedures that were used in the preparation of intermediates and final products are summarized here as “General Procedures”. Target compounds were obtained with a purity >95% and as amorphous solids, unless stated otherwise.

General Procedure A. K_2CO_3 (3 eq) was added to a solution of the selected **aromatic alcohol** (1 eq) in anhydrous DMF (1.5 M) and the reaction mixture was stirred at rt for 30 min. Benzyl bromide (1.05 eq) was added dropwise to the reaction mixture, that was left stirring for 4 h at rt. The reaction mixture was quenched with H_2O and extracted with EtOAc. The combined EtOAc were washed with H_2O, brine, dried over $MgSO_4$, filtered and the solvent was evaporated in vacuo to yield the corresponding **protected alcohol**.

General Procedure B. Dess-Martin periodinane (1.2 eq) was added portionwise to a stirred solution of the selected **primary alcohol** (1 eq) in anhydrous DCM (0.2 M) at 0 ˚C. The mixture was stirred at rt for 4 h and then the solvent was evaporated in vacuo. The crude was purified by flash column chromatography (SiO_2, EtOAc in heptane, 0/100 to 100/0). The desired fractions were collected and concentrated to yield the corresponding **aldehyde**.

General Procedure C. Selected **aldehyde** (1 eq), benzyl carbamate (if not stated otherwise) (1 eq) and triphenyl phosphite (if not stated otherwise) (1.1 eq) were dissolved in anhydrous DCM (0.3 M). Then, copper(II) triflate (0.1 eq) was added and the mixture was stirred at rt for 16 h. Then, solvent was evaporated and the residue dissolved in the minimum amount of MeOH. The solution was kept at - 20 °C for 48 h and then filtrated. When precipitation did not succeed, the crude was purified by flash column chromatography (SiO_2, EtOAc in heptane, 0/100 to 100/0) and if still not pure, by reverse phase column chromatography (C18, MeOH in H_2O, 0/100 to 100/0). The desired fractions were collected and concentrated to yield the corresponding **α-amino diarylphosphonate** as a racemic mixture.

General Procedure D. To a stirred solution of the selected **protected alcohol** (1 eq) and pentamethylbenzene (3 eq) in anhydrous DCM (0.3 M) was added boron trichloride (1 M in hexanes) (2 eq) dropwise at - 78 °C. After 15 min, the reaction was quenched with $CHCl_3$:MeOH (10:1, 1 mL) at - 78 °C, and the resulting mixture was allowed to reach rt. The organic solvents were evaporated in vacuo. The residue was purified by flash column chromatography (SiO_2, EtOAc in heptane 0/100 to 100/0) and then by reverse column chromatography (C18, MeOH in H_2O 0/100 to 100/0). The desired fractions were then collected and evaporated to yield the corresponding **deprotected alcohol**.

General Procedure E. To a solution of the selected **acid** (1 eq) in anhydrous DCM (0.3 M) at 0 °C was added 4-methylmorpholine (1.2 eq). This was followed by dropwise addition of isobutyl chloroformate

(1.2 eq) over 20 min. After 30 min of stirring at 0 °C, NH_3 (25%, aq. sol.) (6 eq) was added portionwise over 5 min. The reaction was stirred for 16 h at rt and then the DCM was evaporated in vacuo. The remaining solution was extracted with EtOAc, washed with citric acid citric acid (5% aq. sol.), $NaHCO_3$ (sat. sol.) and brine, dried over Na_2SO_4, filtered and the solvents were evaporated in vacuo to yield the corresponding **amide**.

General Procedure F. A solution of Burgess reagent (2.1 eq) in anhydrous DCM (0.3 M) was added over a suspension of the corresponding **amide** (1 eq) in anhydrous DCM (0.3 M) and the reaction mixture was stirred for 24 h. The reaction mixture was washed with AcOH (1% aq. sol.), brine, dried over Na_2SO_4, filtered and the solvents were evaporated in vacuo. The residue was purified by flash column chromatography (SiO_2, EtOAc in heptane 0/100 to 100/0). The desired fractions were collected and concentrated to yield the corresponding **nitrile**.

General Procedure G. Hydrochloric acid (4 M in dioxane) (20 eq) was added dropwise to a solution of the selected **protected amine** (1 eq) in anhydrous MeOH (0.1 M) at 0 °C. The reaction mixture was stirred at rt for 16 h. The mixture was concentrated. The solid was then dissolved in a mixture of Na_2CO_3 (10% aq. sol.). The free salt was extracted with EtOAc and the combined organic layers were then acidified with HCl (2 M) until pH = 1 to get the hydrochloric salt again. The organic layer was further extracted with HCl and the combined aqueous layers evaporated. The excess of HCl was removed by coevaporation with toluene. In case of final compounds, the crude was purified by reverse column chromatography (18C, MeOH in H_2O, 0/100 to 100/0). The desired fractions were then collected and evaporated to yield the corresponding **deprotected amine** as a hydrochloride salt.

General Procedure H. Selected **Boc-protected compound** (1 eq) was dissolved in anhydrous DCM (0.02 M) and TFA (100 eq) was added and the solution was stirred for 1 h at rt. The solvents were evaporated in vacuo and the mixture was co-evaporated with heptane to yield corresponding **deprotected amine** compound as a TFA salt.

General Procedure I. To a solution of the selected **amine** (1 eq) in anhydrous DCM (0.04 M) was added Et_3N (3 eq) followed by *N*,*N*′-bis-Boc-1-guanylpyrazole (2 eq). The reaction was stirred at rt for 48 h. After this time, the solvent was evaporated in vacuo and the crude was purified by flash column

chromatography (SiO_2, EtOAc in heptane 0/100 to 100/0) to yield the corresponding protected guanidine.

General Procedure J. Selected **acid chloride** (1.2 eq) was added dropwise to a solution of the selected **aniline** (1 eq) and DIPEA (1.5 eq) in anhydrous DCM (0.02 M) and the reaction mixture was stirred for 2 h at rt. Then, the reaction was quenched with HCl (1 M). This mixture was extracted with DCM, combined organic layers were washed with brine, dried over Na_2SO_4, filtered and the solvent was evaporated in vacuo. The crude was then purified by flash column chromatography (SiO_2, EtOAc in heptane: 20/80 to 80/20). The desired fractions were collected and concentrated to yield the corresponding **carbamate**.

General Procedure K. Zinc was first purified by stirring commercial Zn dust with HCl (2% aq. sol.) for 1 min. The acid was removed by filtration, and the Zn was washed with HCl (2% aq. sol.), distilled H_2O, EtOH, and finally with Et_2O. Then, selected **nitrobenzyl compound** (1 eq) was dissolved in mixture of THF (0.03 M) and NH_4Cl (sat. aq. sol.) (0.03 M) and cooled to 0 °C. The mixture was treated with the pre-treated Zn (5 eq) at vigorous stirring. The reaction mixture was stirred at rt for 1 h. The reaction mixture was filtered through celite while rinsing with THF. The mixture was extracted with EtOAc, washed with brine, dried over Na_2SO_4 and concentrated. When conversion was not complete, the crude was purified by reverse phase column chromatography (C18, MeOH in H_2O, 0/100 to 100/0). The desired fractions were collected and concentrated to afford the corresponding **aniline**.

General Procedure L. A mixture of the selected **cyanophenyl compound** (1 eq), hydroxylammonium chloride (2 eq) and DIPEA (2 eq) in EtOH (0.05 M) was heated to 80 ˚C for 48 h. The crude was filtrated, the filtrate was evaporated and the crude was dissolved in MeCN (0.1 M). Acetyl ether (3 eq) was added and the reaction was stirred at rt for 1 h. Then, the crude was concentrated, dissolved in MeOH and kept at - 20 °C for 16 h. The solid was filtered and rinsed with cold MeOH, the filtrate was concentrated to yield the corresponding ***N*-acetoxycarbamimidoyl)phenyl compound**.

General Procedure M. The selected ***N*-acetoxycarbamimidoyl)phenyl compound** (1 eq) was dissolved in AcOH (0.03 M) and wet Pd(II)/C 10 wt. % (0.1 eq) was added. The reaction mixture was stirred at rt under H_2 atmosphere (1 atm) for 24 h. Then, the palladium was filtrated off through a pad of celite from the mixture and the solvent was evaporated in vacuo. The crude was dissolved in MeOH

and kept at - 20 ˚C for 16 h. The solid was filtered and washed with cold MeOH. Then, the solid was purified by reverse phase column chromatography (C18, MeOH in H_2O, 0/100 to 100/0). The desired fractions were collected and concentrated to yield the corresponding **aromatic amidine**.

2-(4-(Benzyloxy)phenyl)ethan-1-ol (1). General procedure **A** with 2-(4-hydroxyphenyl) ethanol (2.00 g, 14.5 mmol) to yield 2-(4-(benzyloxy)phenyl)ethanol (2.75 g, 12.03 mmol, 83% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.46 - 7.28 (m, 5H), 7.16 - 7.11 (m, 2H), 6.96 - 6.89 (m, 2H), 5.04 (s, 2H), 3.81 (t, J = 6.5 Hz, 2H), 2.80 (t, J = 6.5 Hz, 2H). MS (ESI) *m/z* 211.0 $[M\text{-}OH]^+$.

2-(3-(Benzyloxy)phenyl)ethan-1-ol (2). General procedure **A** with 2-(3-hydroxyphenyl)-ethanol (800 mg, 5.79 mmol) to yield 2-(3-(benzyloxy)phenyl)ethanol (1.25 g, 5.47 mmol, 94% yield) as a white solid. MS (ESI) *m/z* 211.0 $[M\text{-}OH]^+$.

2-(*p*-Tolyl)acetaldehyde (3). General procedure **B** with 2-(4-methylphenyl) ethanol (800 mg, 5.87 mmol) to yield 2-(*p*-tolyl)acetaldehyde (580 mg, 4.32 mmol, 74% yield) as a colourless oil. No ionization found. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.73 (t, J = 2.5 Hz, 1H), 7.18 (d, J = 8.0 Hz, 2H), 7.12 - 7.10 (m, 2H), 3.65 (d, J = 2.5 Hz, 2H), 2.35 (s, 3H).

2-(4-Methoxyphenyl)acetaldehyde (4). General procedure **B** with 4-methoxybenzeneethanol (600 mg, 3.94 mmol) to yield 2-(4-methoxyphenyl)acetaldehyde (292 mg, 1.94 mmol, 49% yield) as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.72 (t, J = 2.5 Hz, 1H), 7.17 - 7.10 (m, 2H), 6.94 - 6.88 (m, 2H), 3.81 (s, 3H), 3.63 (d, J = 2.5 Hz, 2H). No ionization found.

2-(Naphthalen-2-yl)acetaldehyde (5). General procedure **B** with 2-(naphthalen-1-yl)ethanol (100 mg, 0,58 mmol) to yield 2-(naphthalen-2-yl)acetaldehyde (56 mg, 0.33 mmol, 57% yield) as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.80 (t, J = 2.5 Hz, 1H), 7.94 - 7.83 (m, 3H), 7.60 - 7.40 (m, 4H), 4.12 (d, J = 2.5 Hz, 2H). No ionization found.

2-(4-Fluorophenyl)acetaldehyde (6). General procedure **B** with 2-(4-fluorophenyl)-ethanol (0.89 mL, 7.31 mmol) to yield 2-(4-fluorophenyl)acetaldehyde (545 mg, 3.95 mmol, 55% yield) as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.75 (t, J = 2.0 Hz, 1H), 7.18 (m, 2H), 7.06 (m, 2H), 3.68 (d, J = 2.0 Hz, 2H). No ionization found.

2-(4-(Trifluoromethyl)phenyl)acetaldehyde (7). General procedure **B** with 2-(4-fluorophenyl)-ethanol (200 mg, 1.26 mmol) to yield 2-(4-(trifluoromethyl)phenyl)acetaldehyde (111 mg, 0.59 mmol, 56% yield) as a colourless oil. No ionization found. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.79 (t, J = 2.0 Hz, 1H), 7.63 (d, J = 8.0 Hz, 2H), 7.34 (d, J = 8.0 Hz, 2H), 3.79 (d, J = 2.0 Hz, 2H).

2-(4-(Benzyloxy)phenyl)acetaldehyde (8). General procedure **B** with 2-(4-(benzyloxy)phenyl)ethanol (**1**) (2.75 g, 12.0 mmol) to yield 2-(4-(benzyloxy)phenyl)acetaldehyde (2.03 g, 8.99 mmol, 75% yield) as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.73 (t, J = 2.5 Hz, 1H), 7.51 - 7.34 (m, 5H), 7.22 - 7.10 (m, 2H), 7.07 - 6.98 (m, 2H), 5.09 (s, 2H), 3.63 (d, J = 2.5 Hz, 2H). No ionization found.

2-(3-(Benzyloxy)phenyl)acetaldehyde (9). General procedure **B** with 2-(3-(benzyloxy)phenyl)ethanol (**2**) (1.25 g, 5.78 mmol) to yield 2-(3-(benzyloxy)phenyl)acetaldehyde (789 mg, 3.49 mmol, 64% yield) as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.73 (t, J = 2.5 Hz, 1H), 7.41 (tdd, J = 7.5, 7.0, 1.5 Hz, 4H), 7.36 - 7.27 (m, 1H), 6.95 - 6.90 (m, 2H), 6.86 - 6.80 (m, 2H), 5.07 (s, 2H), 3.66 (d, J = 2.5 Hz, 2H). No ionization found.

Benzyl (1-(diphenoxyphosphoryl)-2-phenylethyl)carbamate (10). Procedure and characterization consistent with previously reported data.[49]

Benzyl (1-(diphenoxyphosphoryl)-2-(*p*-tolyl)ethyl)carbamate (11). General procedure **C** with 2-(*p*-tolyl)acetaldehyde (**3**) (580 mg, 4.32 mmol), to give benzyl (1-(diphenoxyphosphoryl)-2-(*p*-tolyl)ethyl)carbamate (1.18 g, 2.36 mmol, 55% yield) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.36 - 7.26 (m, 5H), 7.26 - 7.20 (m, 7H), 7.15 (dd, J = 16.0, 8.0 Hz, 4H), 7.08 (d, J = 8.0 Hz, 5H), 5.32 - 5.10 (m, 5H), 5.09 - 4.87 (m, 1H), 4.87 - 4.73 (m, 2H), 3.38 (ddd, J = 14.5, 10.0, 4.5 Hz, 1H), 3.09 - 2.86 (m, 1H), 2.32 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 155.7, 150.3, 150.1, 136.7, 136.3, 132.8, 130.0, 129.8, 129.4, 129.3, 128.6, 128.2, 128.1, 125.6, 125.4, 120.8, 120.6, 7.2, 49.5 (d, J_{CP} = 158.0 Hz), 35.7, 21.25. MS (ESI) *m/z* 502.1 $[M+H]^+$. MP = 114-116 °C

Benzyl (1-(diphenoxyphosphoryl)-2-(4-methoxyphenyl)ethyl)carbamate (12). General procedure **C** with 2-(4-methoxyphenyl)acetaldehyde (**4**) (269 mg, 1.19 mmol) to yield benzyl (1-(diphenoxyphosphoryl)-2-(4-methoxyphenyl) ethyl)carbamate (354 mg, 0.68 mmol, 34% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.43 - 6.94 (m, 17H), 6.81 (d, J = 8.5 Hz, 2H), 5.18 (d, J = 10.5 Hz, 1H), 5.11 - 4.86 (m, 2H), 4.76 (dtd, J = 15.0, 10.5, 4.5 Hz, 1H), 3.78 (s, 3H), 3.35 (ddd, J = 14.5, 10.0, 4.5

Hz, 1H), 2.98 (dt, J = 14.5, 10.0 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 158.7, 155.7, 150.3, 150.1, 136.2, 130.5, 130.0, 129.8, 128.6, 128.3, 128.1, 127.9, 127.8, 125.6, 125.4, 120.8, 120.8, 120.6, 120.5, 114.1, 67.3, 55.3, 49.5 (d, J_{CP} = 157.5 Hz), 35.3. MS (ESI) *m/z* 518.2 $[M+H]^+$.

Benzyl (benzofuran-5-yl(diphenoxyphosphoryl)methyl)carbamate (13). General procedure **C** with 1-benzofuran-5-carbaldehyde (500 mg, 3.42 mmol) to yield benzyl (benzofuran-5-yl(diphenoxyphosphoryl)methyl)carbamate (100 mg, 0.95 mmol, 6% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.30-6.73 (m, 20H), 5.88 (br s, 1H), 5.62 (m, 1H), 5.10 (m, 2H). MS (ESI) *m/z* 536.0 $[M+Na]^+$.

Benzyl (1-(diphenoxyphosphoryl)-2-(naphthalen-2-yl)ethyl)carbamate (14). General procedure **C** with 2-(naphthalen-1-yl)acetaldehyde (**5**) (56 mg, 0.33 mmol) to give benzyl (1-(diphenoxyphosphoryl)-2-(naphthalen-2-yl)ethyl)carbamate (51 mg, 0.10 mmol, 29% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.10 - 8.03 (m, 1H), 7.88 (dd, J = 6.5, 3.0 Hz, 1H), 7.78 (d, J = 8.0 Hz, 1H), 7.56 - 7.47 (m, 2H), 7.42 - 6.99 (m, 17H), 5.75 (d, J = 10.5 Hz, 1H), 5.08 - 4.87 (m, 3H), 3.95 (ddd, J = 14.5, 8.0, 4.0 Hz, 1H), 3.44 (dt, J = 14.5, 10.5 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 155.8, 150.4, 150.1, 136.2, 134.0, 132.0, 130.0, 129.8, 129.1, 128.5, 128.1, 128.0, 127.9, 127.8, 126.6, 125.8, 125.6, 125.3, 123.2, 120.7, 120.5, 67.0, 49.1 (d, J_{CP} = 158.5 Hz), 33.12. MS (ESI) *m/z* 538.1 $[M+H]^+$.

Benzyl (1-(diphenoxyphosphoryl)-2-(4-fluorophenyl)ethyl)carbamate (15). General procedure **C** with 2-(4-fluorophenyl)acetaldehyde (**6**) (545 mg, 3.95 mmol), to give benzyl (1-(diphenoxyphosphoryl)-2-(4-fluorophenyl)ethyl)carbamate (1.39 g, 2.75 mmol, 70% yield) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.51 - 7.01 (m, 17H), 6.87 (dt, J = 17.0, 8.0 Hz, 2H), 5.30 (d, J = 10.5 Hz, 1H), 5.17 - 4.85 (m, 2H), 4.75 (dtd, J = 15.0, 10.5, 4.5 Hz, 1H), 3.37 (ddd, J = 14.0, 9.0, 4.5 Hz, 1H), 3.00 (dt, J = 14.5, 10.0 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 162.1, 155.8, 150.3, 150.0, 136.2, 131.0, 130.9, 130.0, 129.9, 128.6, 128.4, 128.1, 125.7, 125.5, 120.8, 120.7, 120.5, 120.5, 115.7, 115.5, 67.4, 49.4 (d, J_{CP} = 158.5 Hz), 35.4. MS (ESI) *m/z* 506.2 $[M+H]^+$. MP = 133-135 °C

Benzyl (1-(diphenoxyphosphoryl)-2-(4-(trifluoromethyl)phenyl)ethyl) carbamate (16). General procedure **C** with 2-(4-(trifluoromethyl)phenyl)acetaldehyde (**7**) (111 mg, 0.59 mmol) to give benzyl (1-(diphenoxyphosphoryl)-2-(4-(trifluoromethyl) phenyl)ethyl)carbamate (91 mg, 0,16 mmol, 28% yield) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.24 (d, J = 9.5 Hz, 1H), 7.66 (d,

J = 8.0 Hz, 2H), 7.56 (d, J = 8.0 Hz, 2H), 7.45 – 7.34 (m, 4H), 7.30 – 7.09 (m, 11H), 4.95 (m, 2H), 4.66 – 4.51 (m, 1H), 3.43 – 3.36 (m, 1H), 3.10 (m, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 155.9, 150.1, 149.8, 142.1, 137.0, 130.1, 130.0, 129.9, 128.3, 127.8, 127.3, 127.1 (q, J_{CF} = 31.5 Hz), 125.5, 125.3, 125.1 (q, J_{CF} = 3.5 Hz), 124.5 (q, J_{CF} = 272.0 Hz), 120.7, 120.7, 120.5, 120.4, 65.6, 49.6 (d, J_{CP} = 159.5 Hz), 34.0. MS (ESI) *m/z* 556.0 $[M+Na]^+$, (95%). HRMS: Calc: 556.15 Found: 556.1481 $[M+H]^+$.

Benzyl (1-(diphenoxyphosphoryl)-3-(methylthio)propyl)carbamate (17). Procedure and characterization consistent with previously reported data.[50]

Benzyl ((diphenoxyphosphoryl)(6-hydroxynaphthalen-2-yl)methyl)carbamate (18). General procedure **C** with 6-hydroxy-2-naphthaldehyde (289 mg, 1.68 mmol), to give benzyl ((diphenoxyphosphoryl)(6-hydroxynaphthalen-2-yl)methyl)carbamate (208 mg, 0.39 mmol, 23% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.85 (s, 1H), 8.98 (d, J = 10.0 Hz, 1H), 8.00 (s, 1H), 7.78 - 7.63 (m, 3H), 7.42 - 7.26 (m, 9H), 7.21 - 7.05 (m, 6H), 6.98 (d, J = 8.4 Hz, 2H), 5.78 - 5.59 (m, 1H), 5.10 (dd, J = 35.0, 12.5 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 156.9, 150.9, 137.6, 135.2, 130.8, 130.4, 129.3, 128.9, 128.5, 128.2, 127.4, 127.2, 126.2, 121.3, 120.1, 109.5, 67.1, 53.9 (d, J_{CP} = 157.5 Hz). MS (ESI) *m/z* 540.1 $[M+H]^+$. HRMS: Calc: 540.16 Found: 540.1584 $[M+H]^+$. MP = 166-168 ^{o}C.

Benzyl (2-(4-(benzyloxy)phenyl)-1-(diphenoxyphosphoryl)ethyl)carbamate (19) General procedure **C** with 2-(4-(benzyloxy)phenyl)acetaldehyde (**8**) (1.79 g, 7.89 mmol), to give benzyl (2-(4-(benzyloxy)phenyl)-1-(diphenoxyphosphoryl)ethyl) carbamate (3.55 g, 5.99 mmol, 76% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.46 - 7.27 (m, 12H), 7.25 - 7.02 (m, 10H), 6.89 (d, J = 8.5 Hz, 2H), 5.22 (d, J = 10.5 Hz, 1H), 5.03 (s, 2H), 5.02 (s, 2H), 4.83 - 4.70 (m, 1H), 3.35 (ddd, J = 14.5, 10.0, 4.5 Hz, 1H), 2.99 (dt, J = 14.5, 10.0 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 158.0, 155.8, 150.3, 150.1, 137.1, 136.2, 130.5, 130.0, 129.7, 128.7, 128.6, 128.3, 128.1, 127.6, 125.6, 125.4, 120.8, 120.5, 115.1, 70.1, 67.3, 49.6 (d, J_{CP} = 157.5 Hz), 35.3. MS (ESI) *m/z* 594.2 $[M+H]^+$.

Benzyl (2-(3-(benzyloxy)phenyl)-1-(diphenoxyphosphoryl)ethyl)carbamate (20). General procedure **C** with 2-(3-(benzyloxy)phenyl)acetaldehyde (**9**) (789 mg, 3.49 mmol), to give benzyl (2-(3-(benzyloxy)phenyl)-1-(diphenoxyphosphoryl)ethyl) carbamate (1.37 g, 2.31 mmol, 66% yield). ^{1}H

NMR (400 MHz, $CDCl_3$) δ: 7.48 - 7.06 (m, 21H), 6.96 - 6.85 (m, 3H), 5.33 (d, J = 10.5 Hz, 1H), 5.06 (s, 2H), 5.00 (s, 2H), 4.89 - 4.74 (m, 1H), 3.46 - 3.36 (m, 1H), 3.13 - 3.01 (m, 1H). MS (ESI) *m/z* 594.2 $[M+H]^+$.

Benzyl (1-(diphenoxyphosphoryl)-2-(4-hydroxyphenyl)ethyl)carbamate (21). General procedure **D** with benzyl (2-(4-(benzyloxy)phenyl)-1-(diphenoxyphosphoryl)ethyl)carbamate (**19**) (200 mg, 0.34 mmol) to yield benzyl (1-(diphenoxyphosphoryl)-2-(4-hydroxyphenyl)ethyl)carbamate (52 mg, 0.10 mmol, 31% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.33 (s, 1H), 8.17 (d, J = 9.5 Hz, 1H), 7.48 - 7.09 (m, 18H), 6.73 (t, J = 5.5 Hz, 2H), 5.08 - 4.94 (m, 2H), 4.53 - 4.38 (m, 1H), 3.19 (dt, J = 14.0, 3.5 Hz, 1H), 2.98 - 2.85 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 157.0, 151.1, 150.7, 137.9, 131.0, 130.8, 129.2, 128.6, 128.1, 126.3, 126.2, 121.6, 121.3, 116.0, 66.3, 51.2 (d, J_{CP} = 156.0 Hz), 34.1. MS (ESI) m/z 504.2 $[M+H]^+$. MP = 172-174 °C

Benzyl (1-(diphenoxyphosphoryl)-2-(3-hydroxyphenyl)ethyl)carbamate (22). General procedure **D** with benzyl (2-(3-(benzyloxy)phenyl)-1-(diphenoxyphosphoryl) ethyl)carbamate (**20**) (500 mg, 0.84 mmol), to yield benzyl (1-(diphenoxyphosphoryl)-2-(3-hydroxyphenyl)ethyl)carbamate (196 mg, 0.39 mmol, 46% yield) as a white solid. ^{1}H NMR (400 MHz, Acetone-d_6) δ: 8.33 (s, 1H), 7.50 - 7.20 (m, 14H), 7.16 (t, J = 8.0 Hz, 1H), 7.09 (d, J = 10.0 Hz, 1H), 6.96 - 6.84 (m, 2H), 6.78 (dd, J = 8.0, 2.0 Hz, 1H), 5.05 (s, 2H), 4.76 (dddd, J = 13.5, 12.0, 10.0, 3.5 Hz, 1H), 3.40 (ddd, J = 14.0, 5.0, 3.5 Hz, 1H), 3.09 (ddd, J = 14.0, 12.0, 8.5 Hz, 1H). ^{13}C NMR (100 MHz, Acetone-d_6) δ: 158.0, 156.6, 151.4, 151.14, 139.2, 137.8, 130.2, 129.9, 128.9, 128.2, 128.0, 125.8, 125.6, 121.4, 121.1, 120.9, 116.7, 114.3, 66.6, 50.8 (d, J_{CP} = 158.5 Hz), 35.6. MS (ESI) *m/z* 504.2 $[M+H]^+$. MP = 140-142 °C

Benzyl (1-((4-acetamidobenzyl)(4-acetamidophenoxy)phosphoryl)-2-phenylethyl) carbamate (23). General procedure **C** with phenylethanal (0.31 mL, 2.65 mmol) and tris(4-acetamidophenyl) phosphite (1.40 g, 2.91 mmol, 1.1 eq) to give benzyl (1-(bis(4-acetamidophenoxy)phosphoryl)-2-phenylethyl)carbamate (368 mg, 0.61 mmol, 23% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.00 (d, J = 3.0 Hz, 2H), 8.15 (d, J = 9.5 Hz, 1H), 7.61 - 7.50 (m, 4H), 7.39 - 7.20 (m, 8H), 7.19 - 7.05 (m, 6H), 5.01 - 4.77 (m, 2H), 4.47 (tdd, J = 14.5, 9.5, 3.0 Hz, 1H), 3.25 (dt, J = 7.5, 3.5 Hz, 1H), 2.98 (ddd, J = 13.5, 12.5, 8.0 Hz, 1H), 2.03 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 168.9, 155.9, 145.2, 145.0,

137.2, 136.5, 129.1, 128.2, 127.6, 127.2, 126.6, 120.8, 120.5, 120.1, 65.9, 49.8 (d, J_{CP} = 157.5 Hz), 34.1, 23.9. MS (ESI) *m/z* 602.2 $[M+H]^+$. HRMS: Calc: 602.21 Found: 602.2054 $[M+H]^+$.

Benzyl (1-(hydroxy(phenoxy)phosphoryl)-2-(4-hydroxyphenyl)ethyl)carbamate (24). KOH (58 mg, 0.99 mmol, 3 eq) was added to a solution of benzyl (1-(diphenoxyphosphoryl)-2-(4-hydroxyphenyl)ethyl)carbamate (**21**) (250 mg, 0.50 mmol) in H_2O (5 mL) and 1,4-dioxane (5 mL) and the resulting mixture was stirred at rt over 16 h. The crude reaction was evaporated and HCl (1N aq. sol.) was added to form the HCl salt. The residue was purified by reverse column chromatography (C18, MeOH in H_2O 0/100 to 100/0). The desired fractions were then collected and evaporated to yield benzyl (1-(hydroxy(phenoxy)phosphoryl)-2-(4-hydroxyphenyl)ethyl)carbamate hydrochloride (47 mg, 0.10 mmol, 20% yield). ^{1}H NMR (400 MHz, Methanol-d_4) δ: 7.38 - 7.27 (m, 5H), 7.25 - 7.15 (m, 5H), 7.10 (d, *J* = 8.5 Hz, 2H), 6.76 - 6.66 (m, 2H), 5.10 - 4.91 (m, 2H), 4.32 (dd, *J* = 20.0, 7.5 Hz, 1H), 3.26 - 3.16 (m, 1H), 2.89 - 2.77 (m, 1H). ^{13}C NMR (100 MHz, Methanol-d_4) δ: 157.2, 155.8, 150.9, 136.9, 129.9, 129.3, 128.0, 127.4, 127.0, 124.4, 120.4, 114.8, 66.1, 50.4 (d, J_{CP} = 155.0 Hz), 34.2. MS (ESI) *m/z* 428.2 $[M+H]^+$.

Benzyl (2-(4-hydroxyphenyl)-1-(methoxy(phenoxy)phosphoryl)ethyl)carbamate (25). NH_3 (7N in MeOH) (0.09 mL, 0.60 mmol) was added to the stirred solution of benzyl (1-(diphenoxyphosphoryl)-2-(4-hydroxyphenyl)ethyl)carbamate (**21**) (200 mg, 0.40 mmol) and NH_4Cl (32 mg, 0.60 mmol) in MeOH (4 mL). The reaction mixture was stirred at rt and for 3 days. The reaction mixture was concentrated and purified by reverse column chromatography (C18, MeOH in H_2O, 0/100 to 100/0). The desired fractions were then collected and evaporated to yield benzyl (2-(4-hydroxyphenyl)-1-(methoxy(phenoxy)phosphoryl)ethyl)carbamate (26 mg, 0.06 mmol, 15% yield) as a colourless oil. ^{1}H NMR (400 MHz, Methanol-d_4) δ: 7.73 (dd, *J* = 9.5, 6.0 Hz, 1H), 7.41 - 7.32 (m, 2H), 7.32 - 7.24 (m, 3H), 7.24 - 7.13 (m, 5H), 7.12 - 6.96 (m, 2H), 6.71 (dd, *J* = 8.5, 3.5 Hz, 2H), 4.99 (ddd, *J* = 33.0, 12.5, 7.5 Hz, 2H), 4.52 - 4.31 (m, 1H), 3.91 - 3.72 (m, 3H), 3.23 - 3.09 (m, 1H), 2.91 - 2.71 (m, 1H). ^{13}C NMR (100 MHz, Methanol-d_4) δ: 158.3, 157.4, 151.7, 138.2, 131.1, 130.9, 129.4, 128.8, 128.4, 126.4, 121.57, 116.3, 67.5, 54.7, 51.1 (d, J_{CP} = 158.0), 35.3. MS (ESI) *m/z* 442.1 $[M+H]^+$.

(*S*)-2-(((Benzyloxy)carbonyl)amino)-3-phenylpropanoic acid (26). Procedure and characterization consistent with previously reported data.[51]

(*S*)-Benzyl (1-amino-3-(4-hydroxyphenyl)-1-oxopropan-2-yl)carbamate (27). General procedure **E** with ((benzyloxy)carbonyl)tyrosine (382 mg, 1.23 mmol) to yield (*S*)-benzyl (1-amino-3-(4-hydroxyphenyl)-1-oxopropan-2-yl)carbamate (140 mg, 0.45 mmol, 37% yield). MS (ESI) *m/z* 315.1 $[M+H]^+$.

(*S*)-Benzyl (1-amino-1-oxo-3-phenylpropan-2-yl)carbamate (28). General procedure **E** with (*S*)-2-(((benzyloxy)carbonyl)amino)-3-phenylpropanoic acid (**26**) (100 mg, 0.33 mmol) to yield (*S*)-benzyl (1-amino-1-oxo-3-phenylpropan-2-yl)carbamate (98 mg, 0.33 mmol, 98% yield). MS (ESI) *m/z* 299.1 $[M+H]^+$.

Benzyl (*S*)-(1-cyano-2-(4-hydroxyphenyl)ethyl)carbamate (29). General procedure **F** with (*S*)-benzyl (1-amino-3-(4-hydroxyphenyl)-1-oxopropan-2-yl)carbamate (**27**) (140 mg, 0.45 mmol) to yield (*S*)-benzyl (1-cyano-2-(4-hydroxyphenyl)ethyl)carbamate (113 mg, 0.38 mmol, 86% yield). ^{1}H NMR (400 MHz, Acetone-d_6) δ: 8.33 (s, 1H), 7.48 - 7.32 (m, 5H), 7.25 - 7.18 (m, 2H), 6.89 - 6.78 (m, 2H), 5.13 (m, 2H), 4.88 - 4.74 (m, 1H), 3.15 (d, J = 7.5 Hz, 2H). ^{13}C NMR (100 MHz, Acetone-d_6) δ: 158.3, 157.0, 138.4, 132.2, 130.0, 129.6, 129.5, 127.8, 120.5, 117.0, 116.9, 68.0, 46.2, 39.3. MS (ESI) *m/z* 297.1 $[M+H]^+$.

Benzyl (*S*)-(1-cyano-2-phenylethyl)carbamate (30). General procedure **F** with (*S*)-benzyl (1-amino-1-oxo-3-phenylpropan-2-yl)carbamate (**28**) (460 mg, 1.54 mmol) to yield (*S*)- benzyl (1-cyano-2-phenylethyl)carbamate (323 mg, 1.15 mmol, 75% yield). ^{1}H NMR (400 MHz, Acetone-d_6) δ: 7.43 - 7.25 (m, 10H), 5.13 (s, 2H), 4.90 (dt, J = 8.0, 5.5 Hz, 1H), 3.30 - 3.20 (m, 2H). ^{13}C NMR (100 MHz, Acetone-d_6) δ: 157.0, 138.4, 137.2, 131.1, 130.2, 130.0, 129.6, 129.5, 128.9, 120.4, 68.1, 45.9, 40.0. MS (ESI) *m/z* 281.1 $[M+H]^+$. MP = 132-134 °C. Characterization consistent with previously reported data.[52]

Diphenyl (1-amino-2-(4-hydroxyphenyl)ethyl)phosphonate hydrobromide (31). Benzyl (2-(4-(benzyloxy)phenyl)-1-(diphenoxyphosphoryl)ethyl)carbamate (**19**) (1.00 g, 1.69 mmol)) was dissolved in AcOH (2 mL) and then, 33% HBr/AcOH solution (1.22 mL, 6.74 mmol, 4 eq). The reaction was performed at rt for 6 h. Then, the reaction mixture was concentrated in vacuo. The crude was purified by reverse phase column chromatography (C18, MeOH in H_2O 0/100 to 60/40). The desired fractions were collected and concentrated to yield diphenyl (1-amino-2-(4-hydroxyphenyl)ethyl)phosphonate

hydrobromide (374 mg, 49% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.41 - 7.35 (m, 4H), 7.26 - 7.16 (m, 6H), 7.11 (d, J = 8.5 Hz, 2H), 6.70 (d, J = 8.5 Hz, 2H), 3.49 (td, J = 10.0, 3.5 Hz, 1H), 3.16 - 3.07 (m, 1H), 2.70 (dt, J = 14.0, 10.5 Hz, 1H). MS (ESI) *m/z* 369.2 $[M+H]^+$.

Benzyl ((2S)-1-((1-(diphenoxyphosphoryl)-2-(4-hydroxyphenyl)ethyl)amino)-4-methyl-1-oxopentan-2-yl)carbamate (32). To a stirred solution of *N*-carbobenzyloxy-L-leucine (71 mg, 0.27 mmol, 1.2 eq) in MeCN (3 mL) and DMF (1 mL), 1-hydroxybenzotriazolehydrate (37 mg, 0.24 mmol, 1.1 eq) and *N*,*N*′-dicyclohexylcarbodiimide (92 mg, 0.44 mmol, 2 eq) were added and the solution was stirred for 10 min at rt. Then, a solution of diphenyl (1-amino-2-(4-hydroxyphenyl)ethyl)phosphonate hydrobromide (**31**) (100 mg, 0.22 mmol) and Et_3N (0.03 mL, 0.22 mmol, 1 eq) in DCM (2 mL) at 0 ºC and the mixture was left stirring at rt for 16 h. Then, the precipitate was filtrated off. The solvent was evaporated in vacuo from the filtrate and the crude was purified by flash column chromatography (SiO_2, EtOAc in heptane 0/100 to 100/0) to yield benzyl ((2*S*)-1-((1-(diphenoxyphosphoryl)-2-(4-hydroxyphenyl)ethyl)amino)-4-methyl-1-oxopentan-2-yl)carbamate (**32**) (24 mg, 18% yield) as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.39 - 7.28 (m, 12H), 7.23 - 7.12 (m, 7H), 7.06 (d, J = 8.0 Hz, 2H), 5.28 (d, J = 8.5 Hz, 1H), 5.14 (s, 2H), 4.62 (td, J = 9.0, 4.5 Hz, 1H), 3.62 (td, J = 10.5, 3.0 Hz, 1H), 3.47 - 3.37 (m, 1H), 2.93 (dt, J = 14.0, 10.5 Hz, 1H), 1.87 - 1.77 (m, 2H), 1.73 - 1.65 (m, 1H), 1.03 - 1.00 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 172.0, 156.1, 150.4, 149.5, 136.2, 135.3, 130.5, 129.9, 128.7, 128.4, 128.3, 125.4, 121.7, 120.7, 67.3, 52.8, 50.7 (d, J_{CP} = 157.5 Hz), 41.7, 37.2, 25.0, 23.0, 22.0. MS (ESI) *m/z* 617.3 $[M+H]^+$.

***Tert*-butyl (1-(diphenoxyphosphoryl)-3-(methylthio)propyl)carbamate (33).** General procedure **C** with 4-thiapentanal (4.40 g, 42.20 mmol) and *O*-*tert*-butylcarbamate (4.95 g, 42.20 mmol) to give *tert*-butyl (1-(diphenoxyphosphoryl)-3-(methylthio)propyl)carbamate (5.480 g, 12.53 mmol, 30% yield). MS (ESI) *m/z* 438.2 $[M+H]^+$.

Diphenyl (1-amino-3-(methylthio)propyl)phosphonate hydrochloride (34). General procedure **G** with *tert*-butyl (1-(diphenoxyphosphoryl)-3-(methylthio)propyl)carbamate (**33**) (500 mg, 1.14 mmol) to yield diphenyl (1-amino-3-(methylthio)propyl)phosphonate hydrochloride (425 mg, 1.14 mmol, 99% yield). MS (ESI) *m/z* 338.2 $[M+H]^+$.

Benzyl *tert*-butyl ((5S)-6-((1-(diphenoxyphosphoryl)-3-(methylthio)propyl)amino)-6-oxohexane-1,5-diyl)dicarbamate (35). To a stirred solution of (*R*)-2-(((benzyloxy)carbonyl)amino)-6-((*tert*-butoxycarbonyl)amino)hexanoic acid (122 mg, 0.32 mmol) in MeCN (3 mL) and DMF (1 mL), 1-hydroxybenzotriazolehydrate (53 mg, 0.35 mmol) and *N*-Ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride (62 mg, 0.32 mmol) were added and the solution was stirred for 10 min at rt. Then, a solution of diphenyl (1-amino-3-(methylthio)propyl)phosphonate hydrochloride (**34**) (100 mg, 0.27 mmol) and Et_3N (0.08 mL, 0.59 mmol) in MeCN (3 mL) was added at 0 ºC and the mixture was left stirring at rt for 16 h. Then, the precipitate was filtrated off. The solvent was evaporated in vacuo from the filtrate and the crude was purified by flash column chromatography (SiO_2, EtOAc in heptane 0/100 to 100/0). Desired fractions were collected and concentrated to yield benzyl *tert*-butyl ((5S)-6-((1-(diphenoxyphosphoryl)-3-(methylthio)propyl)amino)-6-oxohexane-1,5-diyl)dicarbamate (**35**) (220 mg, 0.252 mmol, 94% yield). MS (ESI) *m/z* 700.4 $[M+H]^+$.

Benzyl ((2*S*)-6-amino-1-((1-(diphenoxyphosphoryl)-3-(methylthio)propyl)amino)-1-oxohexan-2-yl)carbamate hydrochloride (36). General procedure **G** with benzyl *tert*-butyl ((5*S*)-6-((1-(diphenoxyphosphoryl)-3-(methylthio)propyl)amino)-6-oxohexane-1,5-diyl)dicarbamate (**35**) (250 mg, 0.36 mmol) to yield benzyl ((2*S*)-6-amino-1-((1-(diphenoxyphosphoryl)-3-(methylthio)propyl)amino)-1-oxohexan-2-yl)carbamate hydrochloride (**36**) (123 mg, 0.19 mmol, 54% yield) as a colourless oil. ^{1}H NMR (400 MHz, Methanol-d_4) δ: 7.52 - 7.05 (m, 15H), 5.20 - 4.93 (m, 3H), 4.32 - 4.08 (m, 1H), 2.95 - 2.74 (m, 2H), 2.74 - 2.38 (m, 2H), 2.37 - 2.12 (m, 2H), 2.11 - 1.98 (m, 3H), 1.92 - 1.54 (m, 4H), 1.54 - 1.33 (m, 2H). ^{13}C NMR (100 MHz, Methanol-d_4) δ: 175.0, 158.3, 151.4, 138.1, 131.1, 130.9, 129.5, 129.1, 128.9, 126.8, 121.8, 121.6, 67.7, 56.3, 46.3 (d, J_{CP} = 160.0 Hz), 40.4, 32.6, 31.1, 29.5, 28.1, 23.7, 15.3. MS (ESI) *m/z* 600.3 $[M+H]^+$.

(*S*)-2-((*Tert*-butoxycarbonyl)amino)-3-(4-hydroxyphenyl)propanoic acid (37). Di-*tert*-butyldicarbonate (1.21 g, 5.52 mmol) was added to a solution of (*S*)-(-)-Tyrosine (1.00 g, 5.52 mmol) in a mixture of dioxane (5 mL), H_2O (2.5 mL) and NaOH (1 M, 5 mL) at 0 °C and the above mixture and stirred for 6 h at rt. Then the solution was concentrated in vacuum, cooled in an ice water bath, covered with a layer of EtOAc and acidified with a dilute solution of $KHSO_4$ such that the solution pH 2-3. The aqueous phase was extracted with EtOAc, dried (Na_2SO_4), filtered and solvents and evaporated

in vacuo to yield (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-hydroxyphenyl)propanoic acid (1.55 g, 5.16 mmol, 94% yield). MS (ESI) *m/z* 304.2 $[M+Na]^+$.

***Tert*-butyl (*S*)-(1-amino-3-(4-hydroxyphenyl)-1-oxopropan-2-yl)carbamate (38).** General procedure **E** with (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-hydroxyphenyl)propanoic acid (**37**) (1.00 g, 3.55 mmol) to yield (*S*)-*tert*-butyl (1-amino-3-(4-hydroxyphenyl)-1-oxopropan-2-yl)carbamate (1.09 g, 3.51 mmol, 99% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.13 (s, 1H), 7.02 (d, J = 8.5 Hz, 2H), 6.64 (d, J = 8.5 Hz, 2H), 3.98 (dd, J = 9.5, 5.0 Hz, 1H), 2.82 (dd, J = 14.0, 4.5 Hz, 1H), 2.61 (dd, J = 14.0, 10.0 Hz, 1H), 1.36 - 1.26 (m, 9H). MS (ESI) m/z 303.2 $[M+Na]^+$.

(*S*)-2-Amino-3-(4-hydroxyphenyl)propanamide hydrochloride (39). General procedure **G** with (*S*)-*tert*-butyl (1-amino-3-(4-hydroxyphenyl)-1-oxopropan-2-yl)carbamate (**38**) (1.09 g, 3.90 mmol) to yield (*S*)-2-amino-3-(4-hydroxyphenyl)propanamide hydrochloride (804 mg, 3.71 mmol, 95% yield). ^{1}H NMR (400 MHz, Methanol-d_4) δ: 7.18 - 7.11 (m, 2H), 6.82 - 6.74 (m, 2H), 4.06 (dd, J = 8.0, 6.0 Hz, 1H), 3.16 (dd, J = 14.0, 6.0 Hz, 1H), 2.98 (dd, J = 14.0, 8.0 Hz, 1H). MS (ESI) *m/z* 181.1 $[M+H]^+$.

Benzyl ((*S*)-1-(((*S*)-1-amino-3-(4-hydroxyphenyl)-1-oxopropan-2-yl)amino)-4-methyl-1-oxopentan-2-yl)carbamate (40). A solution of (*S*)-2-amino-3-(4-hydroxyphenyl)propanamide hydrochloride (**39**) (804 mg, 3.71 mmol) and *N,N*-diisopropylethylamine (0.65 mL, 3.71 mmol) in DCM (1 mL) was added dropwise to a solution of Z-Leu-OSu (1.61 g, 4.45 mmol) in DCM (10 mL) at 0 °C. The reaction mixture was stirred at rt for 16 h. The mixture was concentrated, dissolved in EtOAc, washed with $NaHCO_3$ sat. and HCl (1 M) , dried (Na_2SO_4), filtered and solvents concentrated in vacuo to yield benzyl ((*S*)-1-(((*S*)-1-amino-3-(4-hydroxyphenyl)-1-oxopropan-2-yl)amino)-4-methyl-1-oxopentan-2-yl)carbamate (608 mg, 1.34 mmol, 36% yield). MS (ESI) *m/z* 428.3 $[M+H]^+$.

Benzyl ((*S*)-1-(((*S*)-1-cyano-2-(4-hydroxyphenyl)ethyl)amino)-4-methyl-1-oxopentan-2-yl)carbamate (41). General procedure **F** with benzyl ((*S*)-1-(((*S*)-1-amino-3-(4-hydroxyphenyl)-1-oxopropan-2-yl)amino)-4-methyl-1-oxopentan-2-yl)carbamate (**40**) (508 mg, 1.19 mmol) to yield benzyl ((*S*)-1-(((*S*)-1-cyano-2-(4-hydroxyphenyl)ethyl)amino)-4-methyl-1-oxopentan-2-yl)carbamate (137 mg, 0.34 mmol, 28% yield). ^{1}H NMR (400 MHz, Acetone-d_6) δ: 8.32 (s, 1H), 8.04 (d, J = 8.0 Hz, 1H), 7.60 - 7.26 (m, 5H), 7.17 (d, J = 8.5 Hz, 2H), 6.79 (d, J = 8.5 Hz, 2H), 6.43 (m, 1H), 5.08 (q, J = 12.5 Hz, 2H), 4.99 (dt, J = 7.5, 5.5 Hz, 1H), 4.22 (dd, J = 14.5, 8.5 Hz, 1H), 3.05 (d, J = 7.5 Hz,

2H), 1.78 - 1.63 (m, 1H), 1.63 - 1.40 (m, 2H), 0.89 (dd, J = 9.0, 6.5 Hz, 6H). ^{13}C NMR (100 MHz, Acetone-d_6) δ: 173.0, 157.6, 157.1, 138.1, 131.5, 129.2, 128.7, 126.9, 119.4, 116.2, 66.9, 54.3, 43.0, 41.7, 38.26, 25.3, 23.3, 21.8. MS (ESI) *m/z* 410.1 $[M+H]^+$.

Diphenyl ((1-carbamimidoylpiperidin-4-yl)(2-(4-methoxyphenyl)acetamido)methyl)phosphonate 2,2,2-trifluoroacetate (42). ^{1}H NMR (400 MHz, Methanol-d_4) δ: 7.33 (dt, J = 16.0, 8.0 Hz, 4H), 7.27-7.18 (m, 4H), 7.14 - 7.07 (m, 2H), 7.02 (dd, J = 7.5, 1.0 Hz, 2H), 6.87 - 6.79 (m, 2H), 4.77 (dd, J = 18.5, 6.5 Hz, 1H), 3.90 (t, J = 14.5 Hz, 2H), 3.74 (s, 3H), 3.54 (s, 2H), 3.19 - 3.02 (m, 2H), 2.39 (ddd, J = 18.5, 9.5, 5.5 Hz, 1H), 2.04 (d, J = 13.0 Hz, 2H), 1.49 (qd, J = 13.0, 4.0 Hz, 2H). MS (ESI) *m/z* 537.0 $[M+H]^+$. Synthetic procedures in the supporting information.

Diphenyl ((1-carbamimidoylpiperidin-4-yl)(nicotinamido)methyl)phosphonate 2,2,2-trifluoroacetate (43). ^{1}H NMR (400 MHz, Methanol-d_4) δ: 8.6-5.73 (m, 2H), 8.14 (d, J = 8.0 Hz, 1H), 7.56 (d, J = 4.5 Hz, 1H), 7.42 - 7.28 (m, 4H), 7.25 - 7.15 (m, 6H), 5.02 (dd, J = 17.5, 8.0 Hz, 1H), 3.96 (d, J = 13.5 Hz, 2H), 3.17 (ddd, J = 21.5, 14.0, 2.5 Hz, 2H), 2.54 (ddd, J = 11.5, 8.0, 3.5 Hz, 2H), 2.27 (d, J = 13.0 Hz, 1H), 2.09 (d, J = 13.5 Hz, 1H), 1.76 - 1.46 (m, 2H). MS (ESI) *m/z* 494.0 $[M+H]^+$. Synthetic procedures in the supporting information.

Diphenyl ((1-carbamimidoylpiperidin-4-yl)(furan-2-carboxamido)methyl)phosphonate 2,2,2-trifluoroacetate (44). ^{1}H NMR (400 MHz, Methanol-d_4) δ: 7.72 (dd, J = 1.5, 0.5 Hz, 1H), 7.45 - 7.27 (m, 4H), 7.26 - 7.07 (m, 7H), 6.62 (dd, J = 3.5, 1.5 Hz, 1H), 3.94 (d, J = 13.5 Hz, 2H), 3.15 (td, J = 15.5, 2.5 Hz, 2H), 2.52 (ddd, J = 16.0, 9.5, 6.0 Hz, 1H), 2.26 (d, J = 13.0 Hz, 1H), 2.08 (d, J = 13.5 Hz, 1H), 1.72 - 1.39 (m, 2H). MS (ESI) *m/z* 483.0 $[M+H]^+$. Synthetic procedures in the supporting information.

Diphenyl ((1-carbamimidoylpiperidin-4-yl)(cinnamamido)methyl)phosphonate bis(2,2,2-trifluoroacetate) (45). ^{1}H NMR (400 MHz, Methanol-d_4) δ: 7.62 (d, J = 15.5 Hz, 1H), 7.60 - 7.55 (m, 2H), 7.46 - 7.31 (m, 7H), 7.28 - 7.14 (m, 6H), 6.74 (d, J = 15.5 Hz, 1H), 4.96 (dd, J = 18.5, 6.5 Hz, 1H), 4.07 - 3.80 (m, 2H), 3.16 (td, J = 15.5, 2.5 Hz, 2H), 2.45 (ddd, J = 18.5, 9.5, 5.5 Hz, 1H), 2.12 (dd, J = 9.5, 4.0 Hz, 2H), 1.70 - 1.47 (m, 2H). MS (ESI) *m/z* 519.3 $[M+H]^+$. Synthetic procedures in the supporting information.

Diphenyl ((1-carbamimidoylpiperidin-4-yl)(2-phenoxyethylsulfonamido)methyl)phosphonate 2,2,2-trifluoroacetate (46). ^{1}H NMR (400 MHz, Methanol-d_4) δ: 7.35-7.30 (m, 4H), 7.25-7.17 (m, 6H),

7.13-7.07 (m, 2H), 6.97-6.91 (m, 1H), 6.90-6.84 (m, 2H), 4.42 (t, *J* = 6.5 Hz, 2H), 4.35 (dd, *J* = 19.0, 5.4 Hz, 1H), 3.98 (dd, *J* = 10.5, 3.5 Hz, 2H), 3.68 (t, *J* = 6.5 Hz, 2H), 3.14 (td, *J* = 15.0, 2.5 Hz, 2H), 2.52 - 2.34 (m, 1H), 2.21-1.98 (m, 2H), 1.87-1.56 (m, 2H). MS (ESI) *m/z* 573.2 $[M+H]^+$. Synthetic procedures in the supporting information.

Diphenyl ((1-carbamimidoylpiperidin-4-yl)(3-(piperidin-4-yl)propanamido)methyl)phosphonate bis(2,2,2-trifluoroacetate) (47). ^{1}H NMR (400 MHz, Methanol-d_4) δ: 7.44 - 7.33 (m, 4H), 7.29 - 7.19 (m, 4H), 7.15 - 7.09 (m, 2H), 4.82 (dd, *J* = 18.0, 7.0 Hz, 1H), 4.01 - 3.87 (m, 2H), 3.28 (d, *J* = 2.5 Hz, 2H), 3.14 (td, *J* = 13.0, 2.0 Hz, 2H), 2.84 - 2.70 (m, 2H), 2.48 - 2.29 (m, 3H), 2.16 - 2.02 (m, 2H), 1.94 - 1.82 (m, 2H), 1.68 - 1.45 (m, 5H), 1.41 - 1.25 (m, 2H). MS (ESI) *m/z* 528.3 $[M+H]^+$. Synthetic procedures in the supporting information.

Diphenyl (*E*)-diphenyl ((3-(benzo[d][1,3]dioxol-5-yl)acrylamido)(1-carbamimidoylpiperidin-4-yl)methyl)phosphonate 2,2,2-trifluoroacetate (48). ^{1}H NMR (400 MHz, Methanol-d_4) δ: 7.53 (d, *J* = 15.5 Hz, 1H), 7.36 (dd, *J* = 17.0, 8.5 Hz, 4H), 7.28 - 7.10 (m, 7H), 7.06 (dd, *J* = 8.0, 1.5 Hz, 1H), 6.86 (d, *J* = 8.0 Hz, 1H), 6.55 (d, *J* = 15.5 Hz, 1H), 6.01 (s, 2H), 5.04 - 4.90 (m, 1H), 3.94 (t, *J* = 11.0 Hz, 2H), 3.16 (dd, *J* = 24.0, 13.0 Hz, 2H), 2.56 - 2.32 (m, 1H), 2.20 - 1.99 (m, 2H), 1.70 - 1.48 (m, 2H). MS (ESI) *m/z* 563.2 $[M+H]^+$. Synthetic procedures in the supporting information.

Diphenyl ((3-(benzo[d][1,3]dioxol-5-yl)propiolamido)(1-carbamimidoylpiperidin-4-yl)methyl)phosphonate (49). ^{1}H NMR (400 MHz, Methanol-d_4) δ: 7.38 (td, *J* = 8.0, 3.0 Hz, 4H), 7.28 - 7.12 (m, 7H), 7.04 (d, *J* = 1.5 Hz, 1H), 6.89 (d, *J* = 8.0 Hz, 1H), 6.04 (s, 2H), 4.01 - 3.88 (m, 2H), 3.22 - 3.06 (m, 2H), 2.53 - 2.38 (m, 1H), 2.12 (t, *J* = 13.5 Hz, 2H), 1.69 - 1.48 (m, 2H). MS (ESI) *m/z* 561.2 $[M+H]^+$. Synthetic procedures in the supporting information.

Diphenyl ((1-carbamimidoylpiperidin-4-yl)((5-phenylpyrimidin-2-yl)amino)methyl)phosphonate 2,2,2-trifluoroacetate (50). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.79 – 8.59 (m, 2H), 8.07 (d, *J* = 10.0 Hz, 1H), 7.73 – 7.57 (m, 2H), 7.53 – 7.43 (m, 2H), 7.26 – 7.06 (m, 5H), 5.11 (ddd, *J* = 17.0, 10.5, 7.0 Hz, 1H), 7.36 (dt, *J* = 12.5, 4.0 Hz, 7H), 3.90 (t, *J* = 15.0 Hz, 2H), 3.19–2.95 (m, 2H), 2.49 – 2.42 (m, 1H), 2.00 (t, *J* = 10.5 Hz, 2H), 1.65–1.39 (m, 2H). MS (ESI) *m/z* 543.2 $[M+H]^+$. Synthetic procedures in the supporting information.

(*Z*)-Diphenyl ((1-carbamimidoylpiperidin-4-yl)(3-phenylacrylamido)methyl)phosphonate 2,2,2-trifluoroacetate (51). ^{1}H NMR (400 MHz, Methanol-d_4) δ: 7.52 (dd, J = 7.5, 1.5 Hz, 2H), 7.36 (t, J = 8.0 Hz, 4H), 7.27 – 7.18 (m, 5H), 7.17 – 7.11 (m, 4H), 6.87 (d, J = 12.5 Hz, 1H), 6.10 (dd, J = 12.5, 1.0 Hz, 1H), 4.92 – 4.88 (m, 1H), 3.91 (d, J = 14.0 Hz, 2H), 3.20 – 3.00 (m, 2H), 2.48 – 2.27 (m, 1H), 2.05 (dd, J = 25.5, 14.5 Hz, 2H), 1.65 – 1.36 (m, 2H). MS (ESI) *m/z* 519.3 $[M+H]^+$. Synthetic procedures in the supporting information.

Methyl (1-(diphenoxyphosphoryl)-2-(4-guanidinophenyl)ethyl)carbamate (52). Procedure and characterization consistent with previously reported data.[53]

Diphenyl (2-(4-guanidinophenyl)-1-((*S*)-2-((*S*)-3-hydroxy-2-(thiophene-2-carboxamido)propanamido)propanamido)ethyl)phosphonate (53). ^{1}H NMR ($CDCl_3$) δ: 7.8 - 7.1 (m, 17H), 5.1 (m, 1H), 4.2 - 4.3 (m, 2H), 3.9 (m, 2H), 3.4 (m, 2H), 1.3 (m, 3H). MS (ESI) m/z 679.3 $[M+H]^+$, (100%). Procedure and characterization consistent with previously reported data.[38]

Pent-4-yn-1-yl (1-(diphenoxyphosphoryl)-2-(4-guanidinophenyl)ethyl)carbamate (54). Procedure and characterization consistent with previously reported data.[54]

2-(2-Azidoethoxy)ethyl (1-(diphenoxyphosphoryl)-2-(4-guanidinophenyl)ethyl)carbamate (55). Procedure and characterization consistent with previously reported data.[54]

(Perfluorophenyl)methyl (1-(diphenoxyphosphoryl)-2-(4-guanidinophenyl)ethyl)carbamate 2,2,2-trifluoroacetate (56). ^{1}H NMR (400 MHz, Methanol-d_4) δ: 7.5-7.0 (m, 14H), 5.1 (m, 2H), 4.60 (m, 1H), 4.57 (m, 1H), 3.4 (m, 1H), 3.08 (m, 1H). MS (ESI) *m/z* 635.1 $[M+H]^+$. Synthetic procedures in the supporting information.

3,3,4,4,5,5,6,6,7,8,8,8-dodecafluoro-7-(trifluoromethyl)octyl 1-(diphenoxyphosphoryl)-2-(4-guanidinophenyl)ethylcarbamate 2,2,2-trifluoroacetate (57). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.02 (s, 1H)**,** 7.41-7.12 (m, 14H), 5.43 (m, 1H), 4.69 (m, 1H), 4.26 (m, 2H), 3.38 (m, 1H), 3.08 (m, 1H), 2.35 (m, 2H). MS (ESI) *m/z* 851.1 $[M+H]^+$. Synthetic procedures in the supporting information.

Benzyl ((4-aminophenyl)(diphenoxyphosphoryl)methyl)carbamate (58). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.82 (d, J = 10.0 Hz, 1H), 7.48 (dd, J = 16.0, 9.0 Hz, 2H), 7.42 – 7.26 (m, 8H), 7.18 (dd, J = 15.5, 8.0 Hz, 2H), 7.09 – 7.02 (m, 2H), 6.96 (t, J = 8.0 Hz, 3H), 5.50 (dd, J = 22.0, 10.0 Hz, 1H), 5.09 (dd, J = 33.5, 12.5 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 156.0, 155.9, 150.1, 149.8, 136.6,

131.0, 129.83, 129.78, 129.6, 129.5, 128.3, 127.9, 125.3, 125.2, 120.4, 120.35, 120.29, 120.2, 118.3, 66.1, 52.4 (d, J_{CP} = 159.0 Hz). HRMS: Calc: 489.16 Found: 489.1588 $[M+H]^+$. Procedure and characterization consistent with previously reported data.[55]

2-Phenoxyethyl ((4-aminophenyl)(diphenoxyphosphoryl)methyl)carbamate (59). Procedure and characterization consistent with previously reported data.[55]

4,4,4-Trifluorobutyl ((4-aminophenyl)(diphenoxyphosphoryl)methyl)carbamate (60). Procedure and characterization consistent with previously reported data.[55]

(Perfluorophenyl)methyl ((4-aminophenyl)(diphenoxyphosphoryl)methyl)carbamate (61). Procedure and characterization consistent with previously reported data.[55]

Diphenyl ((4-guanidinophenyl)((4-(trifluoromethyl)phenyl)sulfonamido)methyl)phosphonate (62). Procedure and characterization consistent with previously reported data.[55]

Diphenyl ((4-guanidinophenyl)(phenylsulfonamido)methyl)phosphonate (63). Procedure and characterization consistent with previously reported data.[55]

(Perfluorophenyl)methyl ((diphenoxyphosphoryl)(4-guanidinophenyl)methyl)carbamate (64). Procedure and characterization consistent with previously reported data.[55]

Methyl ((diphenoxyphosphoryl)(4-(2,2,2-trifluoroacetamido)phenyl)methyl)carbamate (65). ^{1}H NMR (400 MHz, Methanol-d_4) δ: 7.70 (m, 1H), 7.55 (m, 2H), 7.41 -7.07 (m, 10H), 5.6 (d, 1H, J = 20.0 Hz), 3,75 (s, 3H). MS (ESI) m/z 531.1 $[M+Na]^+$. Synthetic procedures in the supporting information.

Benzo[d][1,3]dioxol-5-ylmethyl ((diphenoxyphosphoryl)(4-(2,2,2-trifluoroacetamido) phenyl)methyl)carbamate (66). ^{1}H NMR (400 MHz, Methanol-d_4) δ: 7.70-7.50 (m, 4H), 7.40-6.80 (m, 13H), 5.80 (m, 2H), 5.60 (d, J = 28.0 Hz, 1H), 4.90 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 156.6, 156.1, 150.0, 149.7, 147.3, 146.8, 136.2, 131.5, 131.1, 130.0, 129.9, 125.5, 125.4, 122.1, 121.7, 121.1, 120.4, 120.4, 120.4, 120.0, 119.2 – 113.6 (m, CF_3), 108.5, 108.1, 101.4, 100.8, 66.1, 52.4 (d, J_{CP} = 158.0 Hz). MS (ESI) m/z 629.2 $[M+H]^+$, (96%). HRMS: Calc: 629.13 Found: 629.1301 $[M+H]^+$. Synthetic procedures in the supporting information.

2-Aminoethyl ((diphenoxyphosphoryl)(4-(2,2,2-trifluoroacetamido)phenyl)methyl)carbamate 2,2,2-trifluoroacetate (67). ^{1}H NMR (400 MHz, Methanol-d_4) δ: 7.75-7.5 (m, 4H), 7.40-6.93 (m,10H),

5.72 (m, 2H), 4.43 (m, 2H), 3.44 (m, 2H). MS (ESI) m/z 538.2 $[M-H]^-$. Synthetic procedures in the supporting information.

Benzyl 2-(4-(3,3-dimethylureido)phenyl)-1-(diphenoxyphosphoryl)ethylcarbamate (68). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.05 – 7.38 (m, 19H), 6.39 (s, 1H), 5.34 (d, J = 10.5 Hz, 1H), 4.95 – 5.10 (m, 2H), 4.69 – 4.84 (m, 1H), 3.36 (ddd, J = 4.5, 10.0, 14.5 Hz, 1H), 3.02 (s, 6H), 1.28 (s, 1H). MS (ESI) *m/z* 574.7 $[M+H]^+$. Synthetic procedures in the supporting information.

Benzyl ((4-(2-aminoethoxy)phenyl)(diphenoxyphosphoryl)methyl)carbamate 2,2,2-trifluoroacetate (69). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.60-6.80 (m, 19H), 5.58 (d, *J* = 22.5 Hz, 1H), 5.15 (m, 2H), 4.25 (t, *J* = 5.0 Hz, 2H), 3.37 (t, *J* = 5.0 Hz, 2H). MS (ESI) *m/z* 533.1 $[M+H]^+$. Synthetic procedures in the supporting information.

Benzyl (1-(diphenoxyphosphoryl)-3-(4-nitrophenyl)propyl)carbamate (70). General procedure **C** with 3-(4-nitrophenyl)propanal (420 mg, 2.34 mmol) to yield benzyl (1-(diphenoxyphosphoryl)-3-(4-nitrophenyl)propyl)carbamate (700 mg, 1.28 mmol, 55% yield). ^{1}H NMR (400 MHz, Acetone-d_6) δ: 8.24 - 8.05 (m, 2H), 7.57 - 7.44 (m, 2H), 7.44 - 7.27 (m, 9H), 7.26 - 7.05 (m, 6H), 5.22 - 5.08 (m, 2H), 4.58 - 4.25 (m, 1H), 3.06 (ddd, *J* = 14.0, 9.0, 5.0 Hz, 1H), 2.92 (ddd, *J* = 24.0, 15.0, 11.0 Hz, 1H), 2.46 - 2.17 (m, 2H). ^{13}C NMR (100 MHz, Acetone-d_6) δ: 157.9, 152.3, 152.0, 150.8, 148.2, 138.7, 131.4, 131.3, 130.0, 129.9, 129.6, 129.3, 126.8, 126.7, 125.1, 122.3, 122.0, 68.1, 49.7 (d, J_{CP} = 159.0 Hz), 33.2, 32.4. MS (ESI) m/z 547.1 $[M+H]^+$, (100%). HRMS: Calc: 547.16 Found: 547.1646 $[M+H]^+$.

Methyl ((4-carbamimidoylphenyl)(diphenoxyphosphoryl)methyl)carbamate (71). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.45 (s, 2H), 9.22 (s, 1H), 8.80 (d, *J* = 20.0 Hz, 1H), 7.90 – 7.85 (m, 4H), 7.40 – 7.36 (m, 4H), 7.24 – 7.20 (m, 2H), 7.10 – 7.00 (m, 4H) 5.78 – 5.72 (m, 1H), 3.61 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 165.4, 156.7, 149.9, 149.6, 140.5, 130.1, 128.9, 127.9, 125.4, 120.4, 120.38, 120.32, 120.27, 120.23, 64.9, 52.3 (d, J_{CP} = 157.5 Hz). MS (ESI) *m/z* 440.2 $[M+H]^+$, (100%). HRMS: Calc: 440.14 Found: 440.1369 $[M+H]^+$. Procedure and characterization consistent with previously reported data.[53]

Methyl ((diphenoxyphosphoryl)(5-nitronaphthalen-1-yl)methyl)carbamate (72). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.62-8.51 (m, 1H), 8.46-8.31 (m, 1H), 8.24-8.17 (m, 1H), 8.03-7.91 (m, 1H),

7.74-7.58 (m, 2H), 7.35-7.01 (m, 10H), 6.5-6.35 (m, 1H), 6.11-6.01 (m, 1H). MS (ESI) *m/z* 493.1 $[M+H]^+$. Synthetic procedures in the supporting information.

Diphenyl ((1-carbamimidoylazetidin-3-yl)(pyrimidin-2-ylamino)methyl)phosphonate 2,2,2-trifluoroacetate (73). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.47–8.24 (m, 2H), 8.08 (d, J = 9.5 Hz, 1H), 7.37 (td, J = 8.0, 3.0 Hz, 4H), 7.29 (s, 3H), 7.21 (td, J = 7.5, 3.5 Hz, 2H), 7.11 (d, J = 8.0 Hz, 3H), 6.73 (t, J = 5.0 Hz, 1H), 5.37 (dt, J = 15.5, 9.5 Hz, 1H), 4.13 – 4.26 (m, 2H), 4.07 (ddd, J = 9.5, 6.0, 3.5 Hz, 2H), 3.62-3.44 (m, 1H). MS (ESI) *m/z* 439.2 $[M+H]^+$. Synthetic procedures in the supporting information.

Diphenyl ((3-(*N*-hydroxycarbamimidoyl)phenyl)(pyrimidin-2-ylamino)methyl)phosphonate (74). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.67 (s, 1H), 8.47 (dd, J = 10.5, 2.0 Hz, 1H), 8.37 (d, J = 4.5 Hz, 2H), 8.05 (q, J = 2.0 Hz, 1H), 7.72 – 7.81 (m, 1H), 7.66 (dq, J = 8.0, 1.5 Hz, 1H), 7.41 (t, J = 8.0 Hz, 1H), 7.29 – 7.36 (m, 4H), 7.14 – 7.21 (m, 2H), 7.04 (dq, J = 7.8, 1.2 Hz, 2H), 6.98 (dq, J = 8.0, 1.0 Hz, 2H), 6.71 (t, J = 5.0 Hz, 1H), 6.26 (dd, J = 22.5, 10.5 Hz, 1H), 5.82 (s, 2H). No ionization found. Synthetic procedures in the supporting information.

2-(2-(Prop-2-yn-1-yloxy)ethoxy)ethyl (diphenoxyphosphoryl)(4-(piperazin-1-yl)phenyl)methylcarbamate 2,2,2-trifluoroacetate (75). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.42 (dd, J = 8.5, 1.5 Hz, 2H), 7.36 – 7.28 (m, 2H), 7.26 – 7.16 (m, 3H), 7.16 – 7.05 (m, 3H), 6.96 – 6.78 (m, 4H), 6.25 (d, J = 9.5 Hz, 1H), 5.53 (dd, J = 22.5, 9.5 Hz, 1H), 4.22 (d, J = 2.5 Hz, 2H), 4.17 (d, J = 2.5 Hz, 2H), 3.76 - 3.73 (m, 2H), 3.71 – 3.60 (m, 4H), 3.40 – 3.25 (m, 4H), 3.03 (s, 2H), 2.96 – 2.92 (m, 2H), 2.42 (s, 1H). MS (ESI) *m/z* 594.8 $[M+H]^+$. Synthetic procedures in the supporting information.

Benzyl (1-(diphenoxyphosphoryl)-4-guanidinobutyl)carbamate (76). Procedure and characterization consistent with previously reported data.[56]

Benzyl (benzo[d][1,3]dioxol-5-yl(diphenoxyphosphoryl)methyl)carbamate (77). Procedure and characterization consistent with previously reported data.[46]

Benzyl ((4-(dimethylamino)phenyl)(diphenoxyphosphoryl)methyl)carbamate (78). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.37 – 7.06 (m, 15H), 6.88 (d, J = 9.0 Hz, 2H), 6.69 (d, J = 8.5 Hz, 2H), 5.72 (d, J = 8.0 Hz, 1H), 5.47 (m, 1H), 5.09 (m, 2 H), 2.94 (s, 6 H, 2 CH3). MS (ESI) *m/z* 517.3 $[M+H]^+$, (95%).

HRMS: Calc: 517.19 Found: 517.1894 $[M+H]^+$. Procedure and characterization consistent with previously reported data.[46]

Benzyl ((diphenoxyphosphoryl)(pyridin-3-yl)methyl)carbamate (79). Procedure and characterization consistent with previously reported data.[57]

Benzyl ((diphenoxyphosphoryl)(3-nitrophenyl)methyl)carbamate (80). Procedure and characterization consistent with previously reported data.[58]

Benzyl (1-(diphenoxyphosphoryl)ethyl)carbamate (81). Procedure and characterization consistent with previously reported data.[49]

Benzyl (1-(diphenoxyphosphoryl)-3-methylbutyl)carbamate (82). Procedure and characterization consistent with previously reported data.[49]

Benzyl ((diphenoxyphosphoryl)(4-guanidinophenyl)methyl)carbamate (83). Procedure and characterization consistent with previously reported data.[55]

Benzyl (2-(benzyloxy)-1-(diphenoxyphosphoryl)ethyl)carbamate (84). Procedure and characterization consistent with previously reported data.[59]

Benzyl ((3-aminophenyl)(diphenoxyphosphoryl)methyl)carbamate (85). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.29 - 6.75 (m, 19H), 6.54 (s (b), 1H), 5.59 (dd, J = 22.5, 10.0 Hz, 1H), 5.05 (dd, J = 53.0, 12.2 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ: 155.6, 150.1, 146.5, 136.0, 135.2, 129.8, 129.7, 128.6, 128.3, 128.2, 125.4, 120.5, 118.6, 115.7, 115.0, 67.5, 52.9 (d, J_{CP} = 159.5 Hz). MS (ESI) *m/z* 489.2 $[M+H]^+$, (95%). HRMS: Calc: 489.16 Found: 489.1582 $[M+H]^+$. Procedure and characterization consistent with previously reported data.[58]

Benzyl ((diphenoxyphosphoryl)(6-methoxypyridin-2-yl)methyl)carbamate (86). General procedure **C** with 6-methoxypicolinaldehyde (200 mg, 1.46 mmol) to give benzyl (diphenoxyphosphoryl)(6-methoxypyridin-2-yl)methylcarbamate (298 mg, 0.59 mmol, 41% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.57 (dd, J = 25.0, 18.0 Hz, 1H), 7.51 – 7.21 (m, 8H), 7.19 – 6.92 (m, 6H), 6.75 (br s, 1H), 6.36 (m, 1H), 5.72 (d, J = 13.0 Hz, 2H), 5.19 (dd, J = 37.0, 12.0 Hz, 2H), 3.88 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 163.6, 155.7, 150.5, 150.4, 150.3, 150.2, 149.6, 139.4, 136.1, 129.7, 129.6, 128.7, 128.6, 128.3, 125.3, 125.2, 120.5, 120.3, 116.4, 111.0, 67.5, 56.3 (d, J = 156.5 Hz), 53.6. MS (ESI) *m/z* 505.6 $[M+H]^+$, (100%). HRMS: Calc: 505.15 Found: 505.1506 $[M+H]^+$.

Benzyl ((2-chloro-5-nitrophenyl)(diphenoxyphosphoryl)methyl)carbamate (87). Procedure and characterization consistent with previously reported data.[60]

Benzyl ((4-carbamimidoylphenyl)(diphenoxyphosphoryl)methyl)carbamate (88). Procedure and characterization consistent with previously reported data.[53]

Benzyl ((5-chloro-1H-indol-3-yl)(diphenoxyphosphoryl)methyl)carbamate (89). General procedure **C** with *tert*-butyl 5-chloro-3-formyl-1H-indole-1-carboxylate (500 mg, 1.78 mmol) to yield benzyl ((5-chloro-1H-indol-3-yl) (diphenoxyphosphoryl)methyl) carbamate (830 mg, 1.52 mmol, 85% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.43 (s, 1H), 8.70 (d, J = 9.5 Hz, 1H), 7.77 (s, 1H), 7.68 (t, J = 2.5 Hz, 1H), 7.46 – 7.25 (m, 10H), 7.24 – 7.05 (m, 6H), 6.97 (d, J = 8.0 Hz, 2H), 5.82 (dd, J = 21.0, 10.0 Hz, 1H), 5.22 – 4.97 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 156.1, 150.3, 150.0, 136.8, 134.4, 129.8, 128.4, 127.9, 127.4, 127.0, 127.0, 125.2, 123.9, 121.57, 120.4, 118.4, 113.3, 107.5, 66.1, 45.3 (d, J_{CP} = 165.5 Hz). MS (ESI) m/z 547.1 $[M+H]^+$, (95%).

Benzyl ((6-carbamimidoylnaphthalen-2-yl)(diphenoxyphosphoryl)methyl)carbamate (90). ^{1}H NMR (400 MHz, Methanol-d_4) δ: 8.80 – 8.60 (m, 1H), 8.46 (s, 1H), 8.22 (s, 1H), 8.15 – 8.08 (m, 2H), 8.00 - 7.65 (m, 4H), 7.64 – 7.58 (m, 1H), 7.45 – 7.12 (m, 10H), 7.08 – 6.95 (m, 5H), 6.00 – 5.78 (m, 1H), 5.25 – 5.11 (m, 2H). MS (ESI) *m/z* 566.2 $[M+H]^+$, (100%). Procedure and characterization consistent with previously reported data.[61]

Benzyl ((diphenoxyphosphoryl)(4-nitrophenyl)methyl)carbamate (91). Procedure and characterization consistent with previously reported data.[58]

Benzyl ((3-carbamimidoylphenyl)(diphenoxyphosphoryl)methyl)carbamate (92). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.63 (s, 2H), 9.34 (s, 2H), 8.96 (d, J = 10.0 Hz, 1H), 8.04 (d, J = 7.5 Hz, 2H), 7.80 (d, J = 7.5 Hz, 1H), 7.67 (t, J = 8.0 Hz, 1H), 7.43 – 7.28 (m, 9H), 7.20 (t, J = 7.0 Hz, 2H), 7.03 (dd, J = 18.0, 8.0 Hz, 4H), 5.72 (dd, J = 22.5, 10.0 Hz, 1H), 5.11 (dd, J = 39.0, 12.5 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 165.6, 158.4, 150.0, 149.6, 136.5, 135.4 133.2, 129.9, 129.9, 129.5, 128.6, 128.4, 128.1, 128.0, 125.5, 125.4, 120.3, 120.3, 120.2, 120.1, 66.4, 50.7 (d, J_{CP} = 159.5 Hz). MS (ESI) *m/z* 516.4 $[M+H]^+$, (100%). HRMS: Calc: 516.17 Found: 516.1703 $[M+H]^+$. Procedure and characterization consistent with previously reported data.[50]

Benzyl (1-(diphenoxyphosphoryl)-3-phenylpropyl)carbamate (93). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.26 (s, 20H), 5.26 - 5.07 (m, 3H), 4.59 - 4.46 (m, 1H), 2.92 - 2.81 (m, 1H), 2.80 - 2.67 (m, 1H), 2.44 - 2.29 (m, 1H), 2.15 - 1.98 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 156.0, 150.3, 150.1, 140.6, 136.1, 130.0, 129.9, 128.7, 128.7, 128.6, 128.5, 128.3, 126.4, 125.5, 120.7, 120.5, 67.6, 48.3 (d, J_{CP} = 158.0 Hz), 32.1, 32.0. MS (ESI) *m/z* 502.3 $[M+H]^+$. Synthetic procedures in the supporting information.

Benzyl ((diphenoxyphosphoryl)(naphthalen-1-yl)methyl)carbamate (94). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.24 (d, *J* = 8.5 Hz, 1H), 7.81 (dd, *J* = 14.0, 8.0 Hz, 3H), 7.55 - 6.89 (m, 14H), 6.60 (d, *J* = 8.0 Hz, 2H), 6.49 - 6.38 (m, 1H), 6.04 - 5.88 (m, 1H), 5.04 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 155.7, 150.1, 150.0, 136.0, 134.0, 131.4, 130.8, 129.9, 129.6, 129.0, 128.7, 127.2, 126.6, 126.2, 125.3, 123.3, 120.7, 120.2, 67.7, 48.4 (d, J_{CP} = 161.0 Hz). MS (ESI) *m/z* 524.2 $[M+H]^+$. Synthetic procedures in the supporting information.

Benzyl ((diphenoxyphosphoryl)(naphthalen-2-yl)methyl)carbamate (95). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.89 - 7.67 (m, 4H), 7.56 - 6.93 (m, 16H), 6.80 (m, 2H), 5.96 (m, 1H), 5.68 (m, 1H), 5.02 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 156.0, 150.2, 136.0, 133.3, 131.7, 129.9, 129.8, 128.9, 128.7, 128.5, 128.3, 127.8, 126.7, 125.6, 125.5, 120.6, 120.5, 67.8, 53.1 (d, J_{CP} = 157.0 Hz). MS (ESI) *m/z* 524.2 $[M+H]^+$. Synthetic procedures in the supporting information.

Benzyl ((3-cyanophenyl)(diphenoxyphosphoryl)methyl)carbamate (96). General procedure **C** with 3-cyanobenzaldehyde (447 mg, 3.31 mmol) to yield benzyl ((3-cyanophenyl)(diphenoxyphosphoryl)methyl)carbamate (998 mg, 2.00 mmol, 61% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.81-6.89 (m, 19H), 6.15-6.04 (m, 1H), 5.14 (dd, *J* = 23.0, 10.5 Hz, 1H). MS (ESI) *m/z* 499.5 $[M+H]^+$. HRMS: Calc: 499.14 Found: 499.1431 $[M+H]^+$.

Benzyl ((4-(dimethylamino)naphthalen-1-yl)(diphenoxyphosphoryl)methyl)carbamate (97). General procedure **C** with 4-dimethylamino-1-naphthaldehyde (200 mg, 1.004 mmol) to yield benzyl ((4-(dimethylamino)naphthalen-1-yl)(diphenoxyphosphoryl)methyl) carbamate (21 mg, 0.04 mmol, 4% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.18 (t, *J* = 8.0 Hz, 1H), 7.69 (dd, *J* = 8.0, 2.5 Hz, 1H), 7.51 - 7.38 (m, 1H), 7.28 - 6.85 (m, 7H), 6.56 (d, J = 8.1 Hz, 1H), 6.34 (dd, J = 22.5, 9.9 Hz, 1H), 5.98 - 5.93 (m, 1H), 5.00 (dt, *J* = 28.0, 12.0 Hz, 1H), 2.78 (s, 3H), 1.18 - 1.13 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$)

δ: 155.7, 152.0, 150.6, 150.2, 136.1, 132.6, 129.8, 129.1, 129.1, 128.6, 128.3, 126.9, 125.4, 125.1, 124.8, 123.6, 120.7, 120.2, 113.5, 67.0, 48.2 (d, J_{CP} = 162.0 Hz), 44.9. MS (ESI) *m/z* 567.2 $[M+H]^+$.

Benzyl ((diphenoxyphosphoryl)(4-(2,2,2-trifluoroacetamido)phenyl)methyl)carbamate (98). General procedure **C** with 2,2,2-trifluoro-*N*-(4-formylphenyl)acetamide (70 mg, 0.32 mmol) to yield benzyl ((diphenoxyphosphoryl)(4-(2,2,2-trifluoroacetamido)phenyl)methyl)carbamate (17 mg, 0.03 mmol, 9% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.00 (s, 1H), 7.55, 7.50 -7.00 (m, 14 H), 5.75 (m, 1H), 5.50 (m, 1H), 5.10 (m, 2H). MS (ESI) m/z 585.2 $[M+H]^+$.

***Tert*-butyl 4-(4-((((benzyloxy)carbonyl)amino)(diphenoxyphosphoryl) methyl)phenyl)piperazine-1-carboxylate (99).** General procedure **C** with *tert*-butyl 4-(4-formylphenyl)piperazine-1-carboxylate (200 mg, 0.69 mmol) to yield 4-(4-((benzyloxycarbonylamino)(diphenoxyphosphoryl) methyl)phenyl)piperazine-1-carboxylate (80 mg, 0.12 mmol, 17% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.85-7.40 (m, 19H), 5.95 (s, 1H), 5.49 (m, 1H), 5.09 (m, 2H), 3.57 (t, *J* = 5.0 Hz, 4H), 3.12 (t, *J* = 5.0 Hz, 4 H), 1.48 (s, 9H). MS (ESI) *m/z* 680.2 $[M+Na]^+$.

***Tert*-butyl 4-(4-((((benzyloxy)carbonyl)amino)(diphenoxyphosphoryl)methyl)-2-cyanophenyl)piperazine-1-carboxylate (100).** General procedure **C** with *tert*-butyl 4-(2-cyano-4-formylphenyl)piperazine-1-carboxylate (500 mg, 1.59 mmol), to yield *tert*-butyl 4-(4-((((benzyloxy)carbonyl)amino)(diphenoxyphosphoryl)methyl)-2-cyanophenyl)piperazine-1-carboxylate (457 mg, 0.67 mmol, 42% yield). MS (ESI) *m/z* 705.3 $[M+Na]^+$.

***Tert*-butyl 4-((benzyloxycarbonylamino)(diphenoxyphosphoryl)methyl)piperidine-1-carboxylate (101).** General procedure **C** with *tert*-butyl 4-formylpiperidine-1-carboxylate (120 mg, 0.56 mmol) to yield *tert*-butyl 4-((benzyloxycarbonylamino)(diphenoxyphosphoryl)methyl)piperidine-1-carboxylate (100 mg, 0.17 mmol, 30% yield). MS (ESI) *m/z* 603.1 $[M+Na]^+$.

***Tert*-butyl 4-(2-(benzyloxycarbonylamino)-2-(diphenoxyphosphoryl)ethyl)piperidine-1-carboxylate (102).** General procedure **C** with *tert*-butyl 4-(2-oxoethyl)piperidine-1-carboxylate (600 mg, 2.64 mmol) to give *tert*-butyl 4-(2-(benzyloxycarbonylamino)-2-(diphenoxyphosphoryl)ethyl)piperidine-1-carboxylate (250 mg, 0.42 mmol, 16% yield). MS (ESI) *m/z* 595.9 $[M+H]^+$.

Benzyl ((4-(((*tert*-butoxycarbonyl)amino)methyl)cyclohexyl)(diphenoxyphosphoryl)methyl)carbamate (103). General procedure **C** with *tert*-butyl ((1r,4r)-4-formylcyclohexyl)methylcarbamate (400 mg, 1.66 mmol) to yield benzyl ((4-(((*tert*-butoxycarbonyl)amino)methyl)cyclohexyl)(diphenoxyphosphoryl)methyl)carbamate (252 mg, 0.42 mmol, 25% yield). MS (ESI) *m/z* 609.6 $[M+H]^+$.

***Tert*-butyl 3-((((benzyloxy)carbonyl)amino)(diphenoxyphosphoryl)methyl)azetidine-1-carboxylate (104).** General procedure **C** with *tert*-butyl 3-formylazetidine-1-carboxylate (613 mg, 3.31 mmol) to yield *tert*-butyl 3-((((benzyloxy)carbonyl)amino)(diphenoxyphosphoryl) methyl)azetidine-1-carboxylate as a white solid (1.084 g, 1.96 mmol, 59% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 6.38 (dd, J = 23.5, 10.5 Hz, 1H), 6.74 (t, J = 5.0 Hz, 1H), 7.02 (ddq, J = 16.0, 8.0, 1.0 Hz, 4H), 7.15 - 7.24 (m, 2H), 7.28 - 7.40 (m, 4H), 7.63 (t, J = 8.0 Hz, 1H), 7.83 (dq, J = 8.0, 1.5 Hz, 1H), 8.04 - 8.15 (m, 1H), 8.27 (q, J = 2.0 Hz, 1H), 8.38 (d, J = 5.0 Hz, 2H), 8.65 (dd, J = 10.5, 2.5 Hz, 1H).

Benzyl (2-(4-((tert-butoxycarbonyl)amino)phenyl)-1-(diphenoxyphosphoryl)ethyl)carbamate (105). Procedure and characterization consistent with previously reported data.[53]

Benzyl ((6-((*tert*-butoxycarbonyl)amino)naphthalen-2-yl)(diphenoxyphosphoryl)methyl)carbamate (106). General procedure **C** with *tert*-butyl 6-formylnaphthalen-2-ylcarbamate (300 mg, 1,106 mmol) to yield benzyl ((6-((*tert*-butoxycarbonyl)amino)naphthalen-2-yl)(diphenoxyphosphoryl)methyl)carbamate (350 mg, 0.55 mmol, 50% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.57 (s, 1H), 8.94 (d, J = 10.0 Hz, 1H), 8.07 (br s, 1H), 7.98 (br s, 1H), 7.65 (d, J = 8.5 Hz, 1H), 7.28 (m, 9H), 7.47 (dd, J = 2.0, 8.5 Hz, 1H), 7.14 (t, J = 6.0 Hz, 2H), 7.01 (d, J = 8.5 Hz, 2H), 6.92 (d, J = 8.5 Hz, 2H), 5.65 (m, 1H), 5.09 (d, J = 12.5 Hz, 1H), 5.01 (d, J = 12.5 Hz, 1H), 1.47 (s, 9H). MS (ESI) *m/z* 639.1 $[M+H]^+$

Benzyl ((diphenoxyphosphoryl)(4-(piperazin-1-yl)phenyl)methyl)carbamate 2,2,2-trifluoroacetate (107). General procedure **H** with *tert*-butyl 4-(4-((benzyloxycarbonylamino)(diphenoxyphosphoryl)methyl)phenyl)piperazine-1-carboxylate (**98**) (800 mg, 1.22 mmol) to afford benzyl (diphenoxyphosphoryl)(4-(piperazin-1-yl)phenyl)methylcarbamate 2,2,2-trifluoroacetate (75 mg, 0.11 mmol, 9% yield). ^{1}H NMR (400 MHz,

Methanol-d_4) δ: 6.90-7.50 (m, 19H), 5.53 (d, J = 20.0 Hz, 1H), 5.12 (m, 2H), 3.48 (m, 4H), 3.37 (m, 4H). ^{13}C NMR (100 MHz, Methanol-d_4) δ: 158.3, 158.2, 151.7, 151.4, 138.0, 130.8, 130.7, 129.5, 129.1, 129.0, 126.6, 121.8, 121.7, 121.6, 121.5, 117.9, 117.8, 68.2, 53.7 (d, J_{CP} = 159.5 Hz), 47.5, 44.7. MS (ESI) *m/z* 558.2 $[M+H]^+$, (95%). HRMS: Calc: 558.22 Found: 558.2163 $[M+H]^+$.

Benzyl ((3-cyano-4-(piperazin-1-yl)phenyl)(diphenoxyphosphoryl)methyl)carbamate 2,2,2-trifluoroacetate (108). General procedure **H** with *tert*-butyl 4-(4-((((benzyloxy)carbonyl)amino)(diphenoxyphosphoryl)methyl)-2-cyanophenyl)piperazine-1-carboxylate (**100**) (120 mg, 0.18 mmol) to yield benzyl ((3-cyano-4-(piperazin-1-yl)phenyl)(diphenoxyphosphoryl)methyl)carbamate 2,2,2-trifluoroacetate (9 mg, 0.15 mmol, 9%) as a colourless oil. ^{1}H NMR (400 MHz, CDCl3) δ: 7.59 (dd, J = 28.5, 19.5 Hz, 2H), 7.44 - 7.27 (m, 6H), 7.24 - 6.74 (m, 11H), 5.99 (s, 1H), 5.48 (dd, J = 22.5, 9.5 Hz, 1H), 5.24 - 4.95 (m, 2H), 3.31 - 3.11 (m, 4H), 3.06 (s, 4H). ^{13}C NMR (100 MHz, CDCl3) δ: 156.0, 155.1, 150.0, 135.6, 133.9, 133.9, 130.0, 129.7, 128.8, 128.6, 128.4, 127.7, 125.7, 120.5, 120.4, 120.4, 120.3, 119.2, 117.9, 115.5, 105.7, 67.9, 52.6, 51.0 (d, J_{CP} = 151.5 Hz), 45.61. MS (ESI) *m/z* 583.2 $[M+H]^+$.

Benzyl ((diphenoxyphosphoryl)(piperidin-4-yl)methyl)carbamate 2,2,2-trifluoroacetate (109). General procedure **H** with tert-butyl 4-((((benzyloxy)carbonyl)amino)(diphenoxyphosphoryl)methyl)piperidine-1-carboxylate (**101**) (900 mg, 1.55 mmol) to give benzyl ((diphenoxyphosphoryl)(piperidin-4-yl)methyl)carbamate 2,2,2-trifluoroacetate (520 mg, 0.87 mmol, 56% yield). ^{1}H NMR (400 MHz, Methanol-d_4) δ: 8.05 (d, J = 10.5 Hz, 1H), 7.34 (m, 9H), 7.23 (m, 2H), 7.14 (m, 4H), 5.14 (m, 2H), 4.46 (m, 1H), 3.45 (m, 2H), 2.40 (m, 1H), 2.31 (m, 2H), 2.21 (m, 2H), 1.71 (m, 2H). MS (ESI) *m/z* 481.7 $[M+H]^+$.

Benzyl 1-(diphenoxyphosphoryl)-2-(piperidin-4-yl)ethylcarbamate 2,2,2-trifluoroacetate (110). General Procedure H with *tert*-butyl 4-(2-(benzyloxycarbonylamino)-2-(diphenoxyphosphoryl)ethyl)piperidine-1-carboxylate (**102**) (250 mg, 0.42 mmol) to give benzyl 1-(diphenoxyphosphoryl)-2-(piperidin-4-yl)ethylcarbamate 2,2,2-trifluoroacetate (200 mg, 0.33 mmol, 78% yield). ^{1}H NMR (400 MHz, Methanol-d_4) δ: 7.36 (m, 9H), 7.22 (m, 2H), 7.14 (m, 4H), 5.14 (q, J = 12.5 Hz, 2H), 4.53 (m, 1H), 3.60 (d, J = 12.5 Hz, 2H), 2.95 (dt, J = 12.5, 2.5 Hz, 1H), 2.85 (dt,

J = 12.5, 2.5 Hz, 1H), 2.05 (m, 1H), 1.90 (m, 3H), 1.81 (m, 1H), 1.52 (m, 1H), 1.35 (m, 1H). MS (ESI) *m/z* 495.2 $[M+H]^+$

Benzyl ((4-(aminomethyl)cyclohexyl)(diphenoxyphosphoryl)methyl)carbamate 2,2,2-trifluoroacetate (111). General procedure **H** with benzyl ((4-(((*tert*-butoxycarbonyl)amino)methyl)cyclohexyl)(diphenoxyphosphoryl)methyl)carbamate (**103**) (252 mg, 0.42 mmol) to yield benzyl ((4-(aminomethyl)cyclohexyl)(diphenoxyphosphoryl)methyl)carbamate 2,2,2-trifluoroacetate (180 mg, 0.35 mmol, 83% yield) as a colourless oil. ^{1}H NMR (400 MHz, Methanol-d_4) δ: 7.32 (m, 9H), 7.21 (m, 2H), 7.11 (m, 3H), 5.18 (d, *J* = 12.5 Hz, 1h), 5.10 (d, *J* = 12.5 Hz, 1H), 4.37 (m, 1H), 2.79 (d, *J* = 7.0 Hz, 2H), 2.10 (m, 3H), 1.90 (m, 2H), 1.60 (m, 2H), 1.30 (m, 2H), 1.11 (m, 2H). MS (ESI) *m/z* 509.1 $[M+H]^+$.

Benzyl (azetidin-3-yl(diphenoxyphosphoryl)methyl)carbamate 2,2,2-trifluoroacetate (112). General procedure **H** with *tert*-butyl 3-((((benzyloxy)carbonyl)amino)(diphenoxyphosphoryl) methyl)azetidine-1-carboxylate (**104**) (1.084 g, 1.96 mmol) to yield benzyl (azetidin-3-yl(diphenoxyphosphoryl)methyl)carbamate 2,2,2-trifluoroacetate (884 mg, 1.61 mmol, 82% yield). MS (ESI) *m/z* 453.4 $[M+H]^+$.

Benzyl (2-(4-aminophenyl)-1-(diphenoxyphosphoryl)ethyl)carbamate 2,2,2-trifluoroacetate (113). Procedure and characterization consistent with previously reported data.[53]

Benzyl ((6-aminonaphthalen-2-yl)(diphenoxyphosphoryl)methyl)carbamate (114)

General procedure **H** with benzyl ((6-((tert-butoxycarbonyl)amino)naphthalen-2-yl)(diphenoxyphosphoryl)methyl)carbamate (**106**) (350 mg, 0.55 mmol) to yield benzyl (6-aminonaphthalen-2-yl)(diphenoxyphosphoryl)methylcarbamate 2,2,2-trifluoroacetate (210 mg, 0.32 mmol, 59% yield) as a colourless oil. ^{1}H NMR (400 MHz, Methanol-d_4) δ: 9.76 (d, *J* = 10.0 Hz, 1H), 8.77 (s, 1H), 8.50 (m, 3H), 8.15 (m, 9H), 8.01 (m, 2H), 7.89 (m, 3H), 7.80 (d, *J* = 8.0 Hz, 2H), 6.49 (m, 1H), 5.96 (d, *J* = 12.5 Hz, 1H), 5.87 (d, *J* = 12.5 Hz, 1H). MS (ESI) *m/z* 539.9 $[M+H]^+$.

Benzyl ((1-carbamimidoylazetidin-3-yl)(diphenoxyphosphoryl)methyl)carbamate 2,2,2-trifluoroacetate (115). General procedure **I** followed by general procedure **H** with benzyl (azetidin-3-yl(diphenoxyphosphoryl)methyl)carbamate 2,2,2-trifluoroacetate (**112**) (884 mg, 1.61 mmol) to yield benzyl ((1-carbamimidoylazetidin-3-yl)(diphenoxyphosphoryl)methyl) carbamate 2,2,2-

trifluoroacetate (357 mg, 0.72 mmol, 45% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.35 (dd, J = 15.0, 10.0 Hz, 1H), 7.47 – 7.27 (m, 9H), 7.23 (dt, J = 11.5, 6.0 Hz, 2H), 7.15 (dd, J = 8.5, 7.0 Hz, 4H), 5.21 – 4.97 (m, 2H), 4.86 – 4.68 (m, 1H), 4.28 – 4.09 (m, 2H), 4.03 (dt, J = 17.5, 7.0 Hz, 2H), 3.42 – 3.27 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 156.3, 151.7, 149.6, 136.6, 130.0, 128.5, 128.0, 127.8, 125.5, 125.4, 120.6, 120.4, 66.2, 52.8, 49.7 (d, J_{CP} = 157.5 Hz), 28.0. MS (ESI) m/z 495.4 $[M+H]^+$, (100%). HRMS: Calc: 495.18 Found: 495.1785 $[M+H]^+$.

Benzyl 2-(4-acetamidophenyl)-1-(diphenoxyphosphoryl)ethylcarbamate (116). General procedure **J** with acetyl chloride (27 μL, 0.38 mmol, 1.2 eq) and benzyl 2-(4-aminophenyl)-1-(diphenoxyphosphoryl)ethylcarbamate (**113**) (160 mg, 0.32 mmol) to yield benzyl 2-(4-acetamidophenyl)-1-(diphenoxyphosphoryl)ethylcarbamate (104 mg, 0.19 mmol, 60% yield). ^{1}H NMR (400 MHz, Methanol-d_4) δ: 7.55 – 7.45 (m, 2H), 7.40 – 7.30 (m, 4H), 7.30 – 7.17 (m, 9H), 7.15 (dd, J = 5.5, 4.5 Hz, 4H), 5.06 – 4.93 (m, 2H), 4.63 (qd, J = 11.5, 5.5 Hz, 1H), 3.39 – 3.32 (m, 1H), 3.01 (ddd, J = 14.0, 12.0, 9.0 Hz, 1H), 2.12 (s, 3H). ^{13}C NMR (100 MHz, Methanol-d_4) δ: 171.6, 159.0, 151.8, 151.4, 138.9, 138.1, 133.6, 131.00, 130.9, 130.7, 129.4, 128.9, 128.6, 126.8, 126.7, 121.8, 121.8, 121.7, 121.6, 121.1, 67.7, 51.4 (d, J_{CP} = 158.5 Hz), 35.6, 23.8. MS (ESI) *m/z* 545.7 $[M+H]^+$

Benzyl ((diphenoxyphosphoryl)(5-nitronaphthalen-1-yl)methyl)carbamate (117). General procedure **C** with 5-nitro-1-naphthaldehyde (200 mg, 0.99 mmol), to give benzyl ((diphenoxyphosphoryl)(5-nitronaphthalen-1-yl)methyl)carbamate (568 mg, 0.35 mmol, 35% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.62 (d, J = 9.0 Hz, 1H), 8.48 (d, J = 9.0 Hz, 1H), 8.20 (d, J = 8.0 Hz, 1H), 7.99 (s, 1H), 7.76 – 7.62 (m, 2H), 7.42 – 7.28 (m, 8H), 7.13 (m, 7H), 6.73 (d, J = 8.5 Hz, 2H), 6.44 (d, J = 23.5 Hz, 1H), 6.03 (s, 1H), 5.12 (dd, J = 43.5, 12.0 Hz, 2H). MS (ESI) *m/z* 569.3 $[M+H]^+$.

Benzyl ((4-(dimethylamino)-3-nitrophenyl)(diphenoxyphosphoryl)methyl)carbamate (118). General procedure **C** with 5-nitro-1-naphthaldehyde with 4-(dimethylamino)-3-nitrobenzaldehyde (500 mg, 2.57 mmol), to yield benzyl ((4-(dimethylamino)-3-nitrophenyl)(diphenoxyphosphoryl)methyl)carbamate (793 mg, 1.41 mmol, 55% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.91 (s, 1H), 7.54 (d, J = 8.5 Hz, 1H), 7.35 (s, 5H), 7.30 - 7.20 (m, 5H), 7.20 - 7.05 (m, 4H), 7.00 (t, J = 9.0 Hz, 3H), 6.00 (dd, J = 9.0, 4.5 Hz, 1H), 5.51 (dd, J = 22.5, 9.5 Hz, 1H), 5.21 - 4.99 (m, 2H), 2.91 (s, 6H). ^{13}C NMR (100 MHz, CDCl3) δ: 155.7, 150.0, 145.9, 138.4, 135.8,

133.4, 129.8, 128.6, 128.4, 128.3, 126.5, 125.6, 123.8, 120.4, 120.3, 118.7, 67.7, 51.5 (d, J_{CP} = 159.0 Hz), 42.5. MS (ESI) *m/z* 562.2 $[M+H]^+$.

Benzyl ((5-aminonaphthalen-1-yl)(diphenoxyphosphoryl)methyl)carbamate (119). General procedure **K** with benzyl ((diphenoxyphosphoryl)(5-nitronaphthalen-1-yl)methyl)carbamate (**117**) (500 mg, 0.88 mmol) to give benzyl ((5-aminonaphthalen-1-yl)(diphenoxyphosphoryl)methyl)carbamate (440 mg, 0.82 mmol, 93% yield). MS (ESI) *m/z* 539.3 $[M + Na]^+$

Benzyl (3-(4-aminophenyl)-1-(diphenoxyphosphoryl)propyl)carbamate (120). General procedure **K** with benzyl (1-(diphenoxyphosphoryl)-3-(4-nitrophenyl)propyl)carbamate (**70**) (8.03 g, 14.69 mmol) to yield benzyl (3-(4-aminophenyl)-1-(diphenoxyphosphoryl)propyl)carbamate (4.24 g, 8.21 mmol, 56% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.12 (d, *J* = 9.5 Hz, 1H), 7.40 - 7.27 (m, 9H), 7.20 (q, *J* = 7.0 Hz, 2H), 7.08 (dd, *J* = 12.0, 8.5 Hz, 4H), 6.84 (d, *J* = 8.5 Hz, 2H), 6.51 (d, *J* = 8.5 Hz, 2H), 5.15 - 5.07 (m, 2H), 5.07 - 4.95 (m, 2H), 4.33 - 4.13 (m, 1H), 2.72 - 2.58 (m, 1H), 2.49 - 2.37 (m, 1H), 2.13 - 1.96 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 156.1, 150.0, 149.8, 146.5, 137.0, 130.3, 129.8, 129.0, 128.4, 127.9, 127.8, 127.5, 125.2, 120.6, 120.4, 114.2, 65.8, 47.5 (d, J_{CP} = 158.0 Hz), 30.9, 30.3. MS (ESI) m/z 517.2 $[M+H]^+$. HRMS: Calc: 517.19 Found: 517.1871 $[M+H]^+$.

Benzyl ((3-amino-4-(dimethylamino)phenyl)(diphenoxyphosphoryl)methyl)carbamate (121). General procedure **K** with benzyl ((4-(dimethylamino)-3-nitrophenyl)(diphenoxyphosphoryl)methyl)carbamate (**118**) (793 mg, 1.41 mmol) to give benzyl ((3-amino-4-(dimethylamino)phenyl)(diphenoxyphosphoryl)methyl)carbamate (310 mg, 0.58 mmol, 41% yield) . ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.40 - 7.28 (m, 4H), 7.25 - 7.04 (m, 9H), 6.95 (d, *J* = 8.0 Hz, 1H), 6.84 (d, *J* = 8.5 Hz, 4H), 5.88 (dd, *J* = 10.0, 3.0 Hz, 1H), 5.45 (dd, *J* = 22.0, 10.0 Hz, 1H), 5.09 (dd, *J* = 40.0, 12.0 Hz, 2H), 4.42 - 3.52 (m, 2H), 2.67 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 155.6, 150.2, 141.6, 136.0, 129.9, 129.7, 129.6, 128.6, 128.3, 125.4, 125.3, 120.6, 120.5, 120.1, 119.6, 118.4, 115.0, 67.5, 52.6 (d, J_{CP} = 159.0 Hz), 43.6. MS (ESI) *m/z* 532.2 $[M+H]^+$.

Benzyl ((4-acetamidophenyl)(diphenoxyphosphoryl)methyl)carbamate (122). General procedure **J** with acetyl chloride (28 μL, 0.39 mmol, 1.2 eq) and benzyl (4-aminophenyl)(diphenoxyphosphoryl)methylcarbamate (**58**) (160 mg, 0.33 mmol) to yield benzyl ((4-acetamidophenyl)(diphenoxyphosphoryl)methyl)carbamate (110 mg, 0.20 mmol, 63% yield) as a white

solid. ^{1}H NMR (400 MHz, Methanol-d_4) δ: 7.52 – 7.43 (m, 2H), 7.38 – 7.31 (m, 4H), 7.30 – 7.15 (m, 9H), 7.14 – 7.10 (m,4H), 5.65 - 5.51 (m, 1H), 5.02 – 4.97 (m, 2H), 2.15 (s, 3H). MS (ESI) *m/z* 531.7 $[M+H]^+$.

Benzyl ((4-(3,3-dimethylureido)phenyl)(diphenoxyphosphoryl)methyl)carbamate (123). General procedure **J** with dimethylcarbamoylchloride (87 mg, 0.81 mmol, 2.2 eq) and benzyl (4-aminophenyl)(diphenoxyphosphoryl)methylcarbamate (**58**) (180 mg, 0.37 mmol) to yield benzyl ((4-(3,3-dimethylureido)phenyl)(diphenoxyphosphoryl)methyl)carbamate (30 mg, 0.05 mmol, 15% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.42 (s, 3H), 7.06 – 7.41 (m, 15H), 6.87 – 6.96 (m, 2H), 6.43 (s, 1H), 5.87 (d, *J* = 9.5 Hz, 1H), 5.55 (dd, *J* = 9.5, 22.0 Hz, 1H), 5.17 (d, *J* = 12.0 Hz, 1H), 5.08 (d, *J* = 12.0 Hz, 1H), 3.04 (s, 6H). MS (ESI) *m/z* 560.7 $[M+H]^+$.

Benzyl ((diphenoxyphosphoryl)(3-guanidinophenyl)methyl)carbamate 2,2,2-trifluoroacetate (124). Procedure and characterization consistent with previously reported data.[62]

Benzyl ((diphenoxyphosphoryl)(5-guanidinonaphthalen-1-yl)methyl)carbamate 2,2,2-trifluoroacetate (125). General procedure **I** followed by general procedure **H** with benzyl ((5-aminonaphthalen-1-yl)(diphenoxyphosphoryl)methyl)carbamate (**119**) (440 mg, 0.82 mmol)) to yield benzyl ((diphenoxyphosphoryl)(5-guanidinonaphthalen-1-yl)methyl)carbamate 2,2,2-trifluoroacetate2,2,2-trifluoroacetate (69 mg, 0.10 mmol, 12% yield). ^{1}H NMR (400 MHz, Methanol-d_4) δ: 8.39 (d, *J* = 8.5 Hz, 1H), 8.13 – 8.01 (m, 2H), 7.72 – 7.65 (m, 2H), 7.58 (d, *J* = 7.0 Hz, 1H), 7.41 – 7.12 (m, 11H), 7.08 – 7.02 (m, 2H), 6.93 – 6.87 (m, 2H), 6.58 (d, *J* = 23.0 Hz, 1H), 5.14 (dd, *J* = 48.0, 12.5 Hz, 2H). MS (ESI) *m/z* 581.2 $[M+H]^+$. HRMS: Calc: 581.20 Found: 581.1940 $[M+H]^+$.

Benzyl (1-(diphenoxyphosphoryl)-3-(4-(methylsulfonamido)phenyl)propyl)carbamate (126). General procedure **J** with methanesulfonylchloride (0.42 mL, 5.43 mmol, 1.2 eq) and benzyl (3-(4-aminophenyl)-1-(diphenoxyphosphoryl)propyl)carbamate (**120**) (2.55 g, 4.94 mmol) to yield benzyl (1-(diphenoxyphosphoryl)-3-(4-(methylsulfonamido)phenyl)propyl)carbamate (1.62 g, 2.72 mmol, 55% yield) as an colourless foam. ^{1}H NMR (400 MHz, Acetone-d_6) δ: 8.49 (s, 1H), 7.58 - 7.13 (m, 19H), 7.08 (d, *J* = 10.0 Hz, 1H), 5.23 - 4.91 (m, 2H), 4.44 (dtt, *J* = 28.0, 25.0, 12.5 Hz, 1H), 3.01 - 2.80 (m, 4H), 2.79 - 2.65 (m, 1H), 2.42 - 2.09 (m, 2H). ^{13}C NMR (100 MHz, Acetone-d_6) δ: 157.1, 151.6, 151.3, 138.1, 138.0, 137.5, 130.5, 130.4, 129.2, 128.8, 126.0, 125.9, 121.6, 121.3, 67.2, 49.0 (d,

J_{CP} = 159.0 Hz), 39.2, 32.2, 31.9. MS (ESI) *m/z* 595.1 $[M+H]^+$. HRMS: Calc: 595.17 Found: 595.1647 $[M+H]^+$.

Benzyl ((4-(dimethylamino)-3-(methylsulfonamido)phenyl)(diphenoxyphosphoryl)methyl)carbamate (127). General procedure **J** with methanesulfonyl chloride (0.05 mL, 0.64 mmol, 1.2 eq) and benzyl ((3-amino-4-(dimethylamino)phenyl)(diphenoxyphosphoryl)methyl)carbamate (**121**) (310 mg, 0.58 mmol) to yield benzyl ((4-(dimethylamino)-3-(methylsulfonamido)phenyl)(diphenoxyphosphoryl)methyl)carbamate as a colourless foam. ^{1}H NMR (400 MHz, CDCl3) δ: 7.75 (s, 1H), 7.59 (s, 1H), 7.22 - 6.95 (m, 15H), 6.91 - 6.77 (m, 2H), 6.06 (dd, *J* = 10.0, 4.5 Hz, 1H), 5.47 (dt, *J* = 15.0, 7.5 Hz, 1H), 5.13 - 4.91 (m, 2H), 2.79 (s, 3H), 2.52 (s, 6H). ^{13}C NMR (100 MHz, CDCl3) δ: 155.6, 150.2, 142.85, 136.0, 133.6, 131.6, 129.9, 129.7, 128.6, 128.4, 128.3, 125.6, 125.4, 123.6, 121.7, 120.5, 120.4, 115.8, 67.6, 52.7 (d, J_{CP} = 158.0 Hz), 44.9, 39.3. MS (ESI) *m/z* 610.3 $[M+H]^+$.

Methyl ((diphenoxyphosphoryl)(3-(trifluoromethyl)phenyl)methyl)carbamate (128). General procedure **C** with 2-(4-(trifluoromethyl)phenyl)acetaldehyde (**7**) (290 mg, 1.54 mmol) and methyl carbamate (116 mg, 1.54 mmol, 1 eq) to give methyl (1-(diphenoxyphosphoryl)-2-(4-(trifluoromethyl)phenyl)ethyl)carbamate (150 mg, 0.31 mmol, 20% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.09 (d, *J* = 9.5 Hz, 1H), 7.68 (d, *J* = 8.0 Hz, 2H), 7.57 (t, *J* = 9.5 Hz, 2H), 7.44 - 7.36 (m, 5H), 7.22 (ddd, *J* = 17.0, 8.0, 2.0 Hz, 7H), 4.63 - 4.47 (m, 1H), 3.43 (s, 3H), 3.41 - 3.37 (m, 1H), 3.31 (s, 1H), 3.16 - 3.00 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 157.4, 150.9, 143.0, 131.0, 130.9, 128.3, 126.4, 126.0, 124.0, 121.4, 52.8, 50.6 (d, J_{CP} = 159.0 Hz), 35.0. MS (ESI) *m/z* 480.1 $[M+H]^+$. HRMS: Calc: 480.12 Found: 480.1179 $[M+H]^+$.

Benzyl (1-(bis(4-acetamidophenoxy)phosphoryl)-2-(4-(trifluoromethyl)phenyl)ethyl) carbamate (129). General procedure **C** with 2-(4-(trifluoromethyl)phenyl)acetaldehyde (**7**) (642 mg, 3.41 mmol), and tris(4-acetamidophenyl) phosphite (1.81 g, 3.75 mmol, 1.1 eq) to give benzyl (1-(bis(4-acetamidophenoxy)phosphoryl)-2-(4-(trifluoromethyl)phenyl)ethyl) carbamate (51 mg, 0.08 mmol, 2% yield). ^{1}H NMR (400 MHz, Acetone-d_6) δ: 9.22 (s, 2H), 7.63 (dt, *J* = 12.5, 5.0 Hz, 8H), 7.34 - 7.26 (m, 3H), 7.21 (dt, *J* = 5.0, 4.0 Hz, 2H), 7.18 - 7.10 (m, 4H), 5.07 - 4.88 (m, 2H), 4.82 - 4.64 (m, 1H), 3.58 - 3.44 (m, 1H), 3.21 (ddd, *J* = 14.0, 12.0, 8.5 Hz, 1H), 2.05 (d, *J* = 2.0 Hz, 6H). ^{13}C NMR (100 MHz,

Acetone-d_6) δ: 168.7, 156.8, 146.7, 146.5, 142.9, 137.9, 137.84, 137.7, 130.9, 129.1, 128.6, 128.4, 126.1, 126.0, 124.2, 121.7, 121.4, 120.9, 66.9, 50.6 (d, J_{CP} = 159.0 Hz), 35.7, 24.6. MS (ESI) *m/z* 670.2 $[M+H]^+$. HRMS: Calc: 670.19 Found: 670.1912 $[M+H]^+$.

Methyl (1-(bis(4-acetamidophenoxy)phosphoryl)-2-(4-(trifluoromethyl)phenyl)ethyl)carbamate (130). General procedure **C** with 2-(4-(trifluoromethyl)phenyl)acetaldehyde (**7**) (376 mg, 2.00 mmol), methyl carbamate (150 mg, 2.00 mmol, 1 eq) and tris(4-acetamidophenyl) phosphite (1.05 g, 2.20 mmol, 1.1 eq) to give methyl (1-(bis(4-acetamidophenoxy)phosphoryl)-2-(4-(trifluoromethyl)phenyl)ethyl)carbamate (8 mg, 0.01 mmol, 1% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.04 (s, 2H), 7.62-7.49 (m, 6H), 7.42-7.12 (m, 4H), 7.11 – 6.94 (m, 2H), 6.73 (m, 1H), 4.73 - 4.59 (m, 1H), 3.43 (s, 3H), 3.52 - 3.40 (m, 1H), 3.28 (ddd, *J* = 13.5, 12.5, 8.0 Hz, 1H), 2.03 (s, 6H). MS (ESI) *m/z* 594.1 $[M+H]^+$.

Methyl ((diphenoxyphosphoryl)(3-nitrophenyl)methyl)carbamate (131). General procedure **C** with 3-nitrobenzaldehyde (500 mg, 3.31 mmol) and methyl carbamate (248 mg, 3.31 mmol) to give methyl ((diphenoxyphosphoryl)(3-nitrophenyl)methyl)carbamate (1.04 g, 2.13 mmol, 64% yield). ^{1}H NMR (400 MHz, Acetone-d_6) δ: 8.61 (dd, *J* = 4.0, 2.2 Hz, 1H), 8.24 (dt, *J* = 8.0, 2.5 Hz, 1H), 8.13 (d, *J* = 7.5 Hz, 1H), 7.95 (d, *J* = 8.5 Hz, 1H), 7.73 (t, *J* = 8.0 Hz, 1H), 7.34 (ddd, *J* = 8.0, 4.0, 1.5 Hz, 4H), 7.23 - 7.14 (m, 4H), 7.14 - 7.09 (m, 2H), 5.87 (dd, *J* = 23.5, 10.0 Hz, 1H), 3.66 (s, 3H). MS (ESI) *m/z* 443.2 $[M+H]^+$.

Benzo[d][1,3]dioxol-5-ylmethyl ((diphenoxyphosphoryl)(3-nitrophenyl)methyl)carbamate (132). General procedure **C** with 3-nitrobenzaldehyde (200 mg, 1.32 mmol) and benzo[d][1,3]dioxol-5-ylmethyl carbamate (258 mg, 1.32 mmol) to yield benzo[d][1,3]dioxol-5-ylmethyl ((diphenoxyphosphoryl)(3-nitrophenyl)methyl)carbamate (250 mg, 0.39 mmol, 30% yield). MS (ESI) *m/z* 585.2 $[M+Na]^+$.

Benzyl ((bis(4-acetamidophenoxy)phosphoryl)(3-nitrophenyl)methyl)carbamate (133). General procedure **C** with 3-nitrobenzaldehyde (1.00 g, 6.62 mmol) and tris(4-acetamidophenyl) phosphite (7.46 g, 7.28 mmol) to yield benzyl ((bis(4-acetamidophenoxy)phosphoryl)(3-nitrophenyl)methyl)carbamate (134 mg, 0.21 mmol, 3% yield). MS (ESI) *m/z* 633.2 = $[M+H]^+$.

Methyl ((bis(4-acetamidophenoxy)phosphoryl)(3-nitrophenyl)methyl)carbamate (134). General procedure **C** with 3-nitrobenzaldehyde (1.00 g, 6.62 mmol), methyl carbamate (497 mg, 6.62 mmol) and tris(4-acetamidophenyl) phosphite (3.50 g, 7.28 mmol) to give methyl ((bis(4-acetamidophenoxy)phosphoryl)(3-nitrophenyl)methyl)carbamate (480 mg, 0.73 mmol, 11% yield). MS (ESI) *m/z* 443.2 $[M+H]^+$.

Methyl ((3-aminophenyl)(diphenoxyphosphoryl)methyl)carbamate (135). General procedure **K** with pent-4-yn-1-yl ((diphenoxyphosphoryl)(4-nitrophenyl)methyl)carbamate (**131**) (2.24 g, 4.54 mmol) to yield pent-4-yn-1-yl ((4-aminophenyl)(diphenoxyphosphoryl)methyl)carbamate (2.04 g, 4.40 mmol, 97% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.09 (d, J = 9.5 Hz, 1H), 7.68 (d, J = 8.0 Hz, 2H), 7.57 (t, J = 9.5 Hz, 2H), 7.44 - 7.36 (m, 5H), 7.22 (ddd, J = 17.0, 8.0, 2.0 Hz, 7H), 4.63 - 4.47 (m, 1H), 3.43 (s, 3H), 3.41 - 3.37 (m, 1H), 3.31 (s, 1H), 3.16 - 3.00 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 157.4, 150.9, 143.0, 131.0, 130.9, 128.3, 126.4, 126.0, 124.0, 121.4, 52.8, 50.6 (d, J_{CP} = 159.0 Hz), 35.0. MS (ESI) *m/z* 480.1 $[M+H]^+$. HRMS: Calc: 413.13 Found: 413.1256 $[M+H]^+$.

Benzo[d][1,3]dioxol-5-ylmethyl ((3-aminophenyl)(diphenoxyphosphoryl)methyl)carbamate (136). General procedure **K** with benzo[d][1,3]dioxol-5-ylmethyl ((diphenoxyphosphoryl)(3-nitrophenyl)methyl)carbamate (**132**) (250 mg, 0.44 mmol) to yield benzo[d][1,3]dioxol-5-ylmethyl ((3-aminophenyl)(diphenoxyphosphoryl)methyl)carbamate (40 mg, 0.08 mmol, 17% yield. ^{1}H NMR (400 MHz, Acetone-d_6) δ: 7.59 (dd, J = 58.0, 10.0 Hz, 1H), 7.37 - 7.23 (m, 4H), 7.22 - 6.97 (m, 7H), 6.96 - 6.84 (m, 4H), 6.84 - 6.76 (m, 1H), 6.67 - 6.60 (m, 1H), 5.99 (d, J = 1.0 Hz, 2H), 5.56 (ddd, J = 58.0, 22.0, 10.0 Hz, 1H), 5.11 - 4.90 (m, 2H), 4.71 (s, 2H). ^{13}C NMR (100 MHz, Acetone-d_6) δ: 156.8, 153.0, 151.4, 149.5, 148.6, 148.4, 136.2, 131.6, 130.4, 130.0, 125.9, 122.8, 121.3, 120.2, 117.4, 115.1, 109.6, 108.7, 102.0, 67.3, 54.2 (d, J_{CP} = 158.0 Hz). MS (ESI) m/z 533.1 $[M+H]^+$.

Benzyl ((3-aminophenyl)(bis(4-acetamidophenoxy)phosphoryl)methyl)carbamate (137). General procedure **K** with benzyl ((bis(4-acetamidophenoxy) phosphoryl)(3-nitrophenyl)methyl)carbamate (**133**) (130 mg, 0.21 mmol) to yield benzyl ((3-aminophenyl)(bis(4-acetamidophenoxy)phosphoryl)methyl)carbamate (40 mg, 0,07 mmol, 32% yield). ^{1}H NMR (400 MHz, Acetone-d_6) δ: 9.22 (d, J = 4.0 Hz, 2H), 7.78-7.66 (m, 1H), 7.54 (dd, J = 12.5, 6.0 Hz, 4H), 7.39 - 7.28 (m, 6H), 7.04 (dt, J = 17.0, 8.0 Hz, 3H), 6.94 - 6.87 (m, 3H), 6.67 (d, J = 2.0 Hz, 1H), 6.65 (d, J = 7.5

Hz, 1H), 5.63-5.49 (m, 1H), 5.11 (ddd, J = 34.5, 12.4, 3.0 Hz, 2H), 4.72 (s, 1H), 2.02 (d, J = 2.9 Hz, 6H). ^{13}C NMR (100 MHz, Acetone-d_6) δ: 168.9, 156.8, 153.0, 149.5, 146.7, 137.7, 136.3, 130.0, 129.9, 129.2, 128.8, 123.7, 121.5, 120.9, 120.4, 120.3, 117.5, 115.3, 115.1, 67.4, 54.8 (d, J_{CP} = 156.5 Hz), 53.4, 24.2. MS (ESI) *m/z* 603.2 $[M+H]^+$.

Methyl ((3-aminophenyl)(bis(4-acetamidophenoxy)phosphoryl)methyl)carbamate (138). General procedure **K** with methyl ((bis(4-acetamidophenoxy)phosphoryl)(3-nitrophenyl)methyl)carbamate (**134**) (480 mg, 0.86 mmol) *to* yield methyl ((3-aminophenyl)(bis(4-acetamidophenoxy)phosphoryl)methyl)carbamate (135 mg, 0.26 mmol, 30% yield). ^{1}H NMR (400 MHz, CD_3CN) δ: 8.38 (d, J = 6.5 Hz, 1H), 7.52 - 7.42 (m, 4H), 7.10 (td, J = 8.0, 1.0 Hz, 1H), 7.02 - 6.97 (m, 2H), 6.92 - 6.86 (m, 2H), 6.79 (s, 1H), 6.78 - 6.76 (m, 2H), 6.73 (m, 1H), 6.63 - 6.59 (m, 1H), 5.38 (dd, J = 22.5, 10.0 Hz, 1H), 3.63 (s, 3H), 2.01 (t, J = 3.5 Hz, 6H). ^{13}C NMR (100 MHz, CD_3CN) δ: 169.5, 157.2, 149.3, 146.6, 137.5, 136.3, 130.40, 121.7, 121.35, 117.75, 115.4, 115.0, 53.9 (d, J_{CP} = 156.5 Hz), 53.2, 24.2. MS (ESI) *m/z* 527.2 $[M+H]^+$.

Methyl ((3-cyanophenyl)(diphenoxyphosphoryl)methyl)carbamate (139). General procedure **C** with 3-cyanobenzaldehyde (800 mg, 6.10 mmol) and methyl carbamate (458 mg, 6.10 mmol), to yield methyl ((3-cyanophenyl) (diphenoxyphosphoryl)methyl)carbamate (2.58 g, 6.11 mmol, 99% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.84 (d, J = 10.0 Hz, 1H), 8.14 (d, J = 1.5 Hz, 1H), 8.00 (d, J = 8.0 Hz, 1H), 7.87 - 7.82 (m, 1H), 7.63 (t, J = 8.0 Hz, 1H), 7.37 (dd, J = 16.0, 7.5 Hz, 4H), 7.21 (td, J = 7.5, 3.5 Hz, 2H), 7.08 (d, J = 8.5 Hz, 2H), 7.03 - 6.98 (m, 2H), 5.77 (dd, J = 23.0, 10.5 Hz, 1H), 3.61 (s, 3H). MS (ESI) *m/z* 423.2 $[M+H]^+$.

Benzyl ((bis(4-acetamidophenoxy)phosphoryl)(3-cyanophenyl)methyl)carbamate (140). General procedure **C** with 3-cyanobenzaldehyde (3.00 g, 22.9 mmol) and tris(4-acetamidophenyl) phosphite (28.7 g, 25.2 mmol) to give benzyl ((bis(4-acetamidophenoxy)phosphoryl)(3-cyanophenyl)methyl)carbamate (4.13 g, 5.12 mmol, 22% yield). MS (ESI) *m/z* 613.3 = $[M+H]^+$.

Methyl ((bis(4-acetamidophenoxy)phosphoryl)(3-cyanophenyl)methyl)carbamate (141). General procedure **C** with 3-cyanobenzaldehyde (3.00 g, 22.8 mmol), methyl carbamate (1.72 g, 22.8 mmol) and tris(4-acetamidophenyl) phosphite (30.1 g, 25.2 mmol) to yield methyl ((bis(4-

acetamidophenoxy)phosphoryl)(3-cyanophenyl)methyl) carbamate (2.13 g, 3.96 mmol, 17% yield). MS (ESI) *m/z* 537.2 = $[M+H]^+$.

Methyl ((3-(*N*-acetoxycarbamimidoyl)phenyl)(diphenoxyphosphoryl)methyl)carbamate (142). General procedure **C** with methyl ((3-cyanophenyl)(diphenoxyphosphoryl)methyl)carbamate (**139**) (1.00 g, 2.37 mmol) to yield methyl ((3-(*N*-acetoxycarbamimidoyl)phenyl)(diphenoxyphosphoryl)methyl)carbamate (2.34 g, 2.82 mmol). MS (ESI) *m/z* 498.2 $[M+H]^+$

Benzyl ((3-(*N*-acetoxycarbamimidoyl)phenyl)(bis(4-acetamidophenoxy)phosphoryl) methyl)carbamate (143). General procedure **L** with benzyl ((bis(4-acetamidophenoxy)phosphoryl)(3-cyanophenyl)methyl) carbamate (**140**) (4.13 g, 5.12 mmol) to yield benzyl ((3-(*N*-acetoxycarbamimidoyl)phenyl)(bis(4-acetamidophenoxy)phosphoryl)methyl)carbamate (950 mg, 1.38 mmol, 27% yield). MS (ESI) *m/z* 688.4 $[M+H]^+$.

Methyl ((3-(*N*-acetoxycarbamimidoyl)phenyl)(bis(4-acetamidophenoxy)phosphoryl) methyl)carbamate (144). General procedure **L** with methyl ((bis(4-acetamidophenoxy)phosphoryl)(3-cyanophenyl)methyl) carbamate (**141**) (2.13 g, 3.96 mmol) to yield methyl ((3-(*N*-acetoxycarbamimidoyl)phenyl)(bis(4-acetamidophenoxy)phosphoryl)methyl)carbamate (3.70 g, 3.75 mmol, 95% yield). MS (ESI) *m/z* 612.3 = $[M+H]^+$.

Methyl ((3-carbamimidoylphenyl)(diphenoxyphosphoryl)methyl)carbamate (145). General procedure **M** with methyl ((3-(*N*-acetoxycarbamimidoyl)phenyl)(diphenoxyphosphoryl)methyl)carbamate (**142**) (2.24 g, 2.79 mmol) to yield methyl ((3-carbamimidoylphenyl)(diphenoxyphosphoryl)methyl)carbamate (88 mg, 0.20 mmol, 7% yield). ^{1}H NMR (400 MHz, Methanol-d_4) δ: 7.99 (d, J = 7.5 Hz, 1H), 7.95 (s, 1H), 7.80 (d, J = 24.5, 12.5 Hz, 1H), 7.69 (t, J = 8.0 Hz, 1H), 7.34 (td, J = 8.0, 3.5 Hz, 4H), 7.22 (t, J = 7.5 Hz, 2H), 7.05 (dd, J = 22.5, 8.5 Hz, 4H), 5.79 (d, J = 23.5 Hz, 1H), 3.71 (s, 3H). ^{13}C NMR (100 MHz, Methanol-d_4) δ: 168.2, 158.8, 151.4, 137.3, 134.7, 131.0, 130.4, 129.2, 129.0, 127.0, 121.5, 54.6 (d, J_{CP} = 158.0 Hz), 53.4. MS (ESI) *m/z* 440.4 $[M+H]^+$.

Benzyl ((bis(4-acetamidophenoxy)phosphoryl)(3-carbamimidoylphenyl)methyl) carbamate (146). General procedure **M** with benzyl ((3-(*N*-acetoxycarbamimidoyl)phenyl)(bis(4-

acetamidophenoxy)phosphoryl) methyl)carbamate (**143**) (950 mg, 1.38 mmol) to yield benzyl ((bis(4-acetamidophenoxy)phosphoryl)(3-carbamimidoylphenyl)methyl)carbamate (172 mg, 0.273 mmol, 20% yield). ^{1}H NMR (400 MHz, Methanol-d_4) δ: 7.96 (d, J = 7.5 Hz, 1H), 7.93 (s, 1H), 7.79 (d, J = 7.5 Hz, 1H), 7.66 (t, J = 8.0 Hz, 1H), 7.51 - 7.46 (m, 4H), 7.39 - 7.30 (m, 5H), 6.95 (dd, J = 22.0, 8.5 Hz, 4H), 5.78 (d, J = 23.0 Hz, 1H), 5.14 (dd, J = 49.5, 12.5 Hz, 2H), 2.10 (s, 6H). ^{13}C NMR (100 MHz, Methanol-d_4) δ: 171.6, 168.4, 158.2, 147.0), 137.8, 137.2, 134.6, 131.0, 130.6, 129.5, 129.3, 129.1, 129.0, 122.3, 121.7, 68.4, 53.7 (d, J_{CP} = 158.5 Hz), 23.7. MS (ESI) *m/z* 630.3 $[M+H]^+$.

Methyl ((bis(4-acetamidophenoxy)phosphoryl)(3-carbamimidoylphenyl)methyl) carbamate (147). General procedure **M** with methyl ((3-(*N*-acetylcarbamimidoyl)phenyl)(bis(4-acetamidophenoxy)phosphoryl)methyl) carbamate (**144**) (3.70 g, 3.85 mmol) to yield methyl ((bis(4-acetamidophenoxy)phosphoryl)(3-carbamimidoylphenyl)methyl)carbamate (190 mg, 0.34 mmol, 9% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.17 (s, 3H), 9.44 (s, 1H), 9.29 (s, 1H), 8.80 (d, J = 10.0 Hz, 1H), 8.06 (s, 1H), 8.02 (d, J = 7.5 Hz, 1H), 7.83 (d, J = 7.0 Hz, 1H), 7.68 (t, J = 8.0 Hz, 1H), 7.60 - 7.46 (m, 4H), 6.98 (dd, J = 26.5, 8.5 Hz, 4H), 5.66 (dd, J = 22.5, 10.0 Hz, 1H), 3.61 (d, J = 10.5 Hz, 3H), 2.03 (d, J = 0.5 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 168.3, 165.5, 156.5, 144.9, 136.7, 135.7, 133.4, 129.3, 128.4, 128.1, 128.0, 120.5, 120.2, 52.2 (d, J_{CP} = 157.0 Hz), 52.3, 23.9. MS (ESI) *m/z* 554.3 = $[M+H]^+$.

(*S*)-2-(((Benzyloxy)carbonyl)amino)-2-(3-nitrophenyl)acetic acid (148). Procedure and characterization consistent with previously reported data.[63]

(*S*)-Benzyl (2-amino-1-(3-nitrophenyl)-2-oxoethyl)carbamate (149). Procedure and characterization consistent with previously reported data.[64]

(*S*)-Benzyl (cyano(3-nitrophenyl)methyl)carbamate (150). General procedure **F** with (*S*)-benzyl (2-amino-1-(3-nitrophenyl)-2-oxoethyl)carbamate (**149**) (1.92 g, 5.83 mmol) to yield (*S*)-benzyl (cyano(3-nitrophenyl)methyl)carbamate (245 mg, 0.60 mmol, 13% yield). MS (ESI) *m/z* 312.2 $[M+H]^+$.

(*S*)-Benzyl ((3-aminophenyl)(cyano)methyl)carbamate (151). General procedure **K** with (*S*)-benzyl (cyano(3-nitrophenyl)methyl)carbamate (**150**) (245 mg, 0.79 mmol) to afford (*S*)-benzyl ((3-aminophenyl)(cyano)methyl)carbamate (215 mg, 0.78 mmol, 99% yield). ^{1}H NMR (400 MHz, CDCl3) δ: 7.44 - 7.31 (m, 5H), 7.23 - 7.11 (m, 1H), 6.83 (d, J = 7.5 Hz, 1H), 6.74 (d, J = 11.0 Hz, 1H), 6.69

(ddd, J = 8.5, 5.0, 3.5 Hz, 1H), 5.74 (d, J = 8.5 Hz, 1H), 5.41 - 5.28 (m, 1H), 5.16 (d, J = 17.5 Hz, 2H), 4.04 - 3.68 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 155.1, 147.5, 135.6, 134.2, 130.5, 128.8, 128.7, 128.5, 117.7, 116.7, 116.2, 113.1, 68.0, 46.7. MS (ESI) *m/z* 282.1 $[M+H]^+$.

BIOLOGICAL EVALUATION.

Protein production. *E. coli* ClpP protein carrying a C-termial His_6 affinity tag was produced starting from pETDclpPec (ORF ECK0431).[65] pETDclpPec was transformed into *E. coli* SG1146a strain (Δ*clpP*) for overexpression. Overnight cultures were grown at 37 °C on an orbital shaker at 130 rpm, then diluted in fresh LB medium including 100 µg/mL ampicillin, and grown until an OD_{600} of 0.6 was reached. Induction was carried out in 5 L flasks by 1 mM isopropyl β-D-1-thiogalactopyranoside at 30 °C at 180 rpm over 5 h. Cell lysis of cooled samples was conducted using Precellys Evolution (Bertin Technologies, France). Supernatant was applied to Ni-NTA (Sigma Aldrich, USA) followed by batchwise washing and elution steps, sequentially applying buffer A (pH 7.6, 50 mM Tris-HCl buffer, 150 mM NaCl, 10 mM imidazole), buffer B (pH 7.6, 50 mM Tris-HCl buffer, 150 mM NaCl, 20 mM imidazole), buffer C (pH 7.6, 50 mM Tris-HCl buffer, 150 mM NaCl, 500 mM Imidazole) and buffer D (pH 7.6, 20 mM Tris-HCl buffer, 100 mM NaCl, 5 mM MgCl, 10% (v/v) glycerol). Purified ClpP protein was frozen in liquid nitrogen and stored at -80 °C. Protein purity and concentration were determined by SDS-PAGE and the Bradford assay, respectively.

Assay Development and Screening. A microplate screening assay with a fluorescence based readout was developed to measure ClpP proteolytic activity. The ClpP activity assay was performed with the fluorogenic substrate Suc-LY-AMC (Enzo Life Sciences, Germany) at 75 µM and *E. coli* ClpP at 625 nM in 100 mM NaCl and 100 mM Hepes pH 7.5, 0.05% Brij® 35 (#P1254, Sigma Aldrich, USA). Compound selectivity for ClpP was assessed by measuring the level of activity of the compounds in the presence of 40 nM of alpha-chymotrypsin (#C4129, Sigma-Aldrich, USA) and 100 µM of Suc-LY-AMC substrate, in a buffer containing 150 mM NaCl, 10 mM $CaCl_2$, 50 mM Tris-HCl and 0.05% Brij® 35. Assays were performed in 384-well black, flat-bottom microtiter plates (#3820, Corning Inc., Corning, USA). All compounds were dissolved in 99.8% DMSO (ROTIPURAN® CAS No.[67-68-5], Carl Roth GmbH, Germany), at a stock concentration of 10 mM. Compound serial dilutions were carried out in 1:3 or 1:2 dilutions and stored at -20 °C. Screening of compounds was conducted at a

final compound concentration of 200 μM in triplicates, with compound transfer carried out using acoustic liquid handling (Echo 550, Labcyte, USA). ClpP protein and compounds were incubated for 10 min at 30 °C, followed by addition of the fluorescent substrate Suc-LY-AMC. The reaction was monitored by following the increase of fluorescence (excitation 350 nm, emission 435 nm) at 30 °C over 1h. Vehicle controls contained the same DMSO concentration (2% v/v) without compound and chloromethyl ketone (Z-LY-CMK) (#4016342, Bachem, Switzerland) was used as a positive control (200 μM). Assays were performed under automated conditions (Fluent® 780, Tecan, Switzerland) equipped with a microplate reader (Infinite M1000 Pro, Tecan, Switzerland). Calculation of Z prime (Z') for validation was performed according to Zhang *et al.*[66] and plates were considered valid for further analyses where Z' was >0.6. Data analysis was conducted using Prism 7.02 (GraphPad Software, USA).

Surface Plasmon Resonance Spectroscopy (SPR). Measurements were conducted on a flow based SPR instrument (Sierra spr-16, Bruker Daltonics, USA). *E. coli* ClpP was immobilized to an amino coupling chip at a concentration of 80 μg/mL, in 10 mM sodium acetate pH 4, according to the manufacturer's protocols, with a buffer containing 150 mM NaCl, 10 mM Hepes, 3 mM EDTA and 0.05% (v/v) Tween-20. The flow rate for protein immobilization was 10 μL/min. The binding assay was performed in immobilization buffer with added DMSO (3.2% (v/v) final) at a flow rate of 20 μL/min, 6 min injection and up to 300 s of dissociation time. The compounds were tested in a range of concentration between 2.5 and 320 μM. To compensate for non-specific interactions and solvent effects, signals were subjected to reference channel subtraction, DMSO and bulk-shift correction and further analyzed with Analyzer 3 (Bruker Daltonics, USA).

Cytotoxicity and cell-viability assays. Potential toxicity of the tested compounds was evaluated by ATP quantification using the CellTiter-Glo® viability assay kit (Promega, USA) and human cell lines A549, HepG2, HeLa. Cells were cultured in 95% air incubator at 5% CO_2 at 37 °C (Heracell™ 240, Thermo Fisher Scirntific, USA). The assays were performed in 96 white, flat bottom, sterile plates (# 781073, Greiner Bio-One, Germany), with an assay volume of 20 μL. Cells were seeded at day zero at concentration of 2000 cells/well, except for A549 (500 cells/well), and placed in 95% air incubator, 5% CO_2 at 37 °C. After 24 h, 200 nL of test compounds were transferred into the plate using an Echo® 550

liquid handler (Labcyte, USA), resulting in a final DMSO concentration of 1%, and were further incubated for 48 h. Luminescence was quantified by EnVision plate reader (PerkinElmer, Germany) and compared to DMSO-treated cells. The compounds were tested in dose-response at 1:3 dilutions starting from 100 μM. DMSO (1%) and valinomycin (10 μM) were used as negative and positive controls respectively. Data analysis was conduced using Prism 7.02 (GraphPad Software, USA). Plates with Z' > 0.5 were accepted.

Antibacterial assays. Compounds were tested for antimicrobial activity using seven different strains (**Table S1**, supporting information). All assays were conducted in 96-well, flat bottom, sterile plates (#167008, Nunc, VWR, USA). 5 mL of fresh sterile saline solution was inoculated with a single colony from a Mueller Hinton Agar (MHA) plate of the bacteria strain (not older than 24 h). Bacterial supension was adjusted to contain $1x10^6$ CFU/mL in fresh sterile Mueller Hilton Broth (MHB) media. Compounds to be tested were transferred into the assay plate in triplicate for screening (at 100 μM), along with controls of 2% DMSO or ciprofloxacin (the latter employed at MIC concentration for each strain) and 100 μL of the bacterial solution was added (final incolum $5x10^5$ CFU/mL). The final volume employed for the assay was 200 μL.

The absorbance at 600 nm was measured with the Multiskan GO plate reader (Thermo Fisher Scientific, Finland) or Varioskan LUX plate reader (Thermo Fisher Scientific, Finland) at time 0 and different time points and used for quantifying bacterial growth. Plates were incubated in a plate shaker (500 rpm) at 37 °C between the measurements.

Selected studies were conducted in the presence of an efflux pump substrate (25 μM of Phe-Arg β-naphthylamide dihydrochloride (#P4157, Sigma-Aldrich, USA) with *Escherichia coli* BW25-113 (wild type) and isogenic *E.coli* JW0427-1 (Δ*clpP*),[67] (derived from *E. coli* BW25-113).

Nitric oxide stress was induced by adding 2 mM of DPTA NONOate ((Z)-1-[N-(3-aminopropyl)-N-(3-ammoniopropyl)amino]diazen-1-ium-1,2-diolate, Cayman Chemical), dissolved in NaOH (0.14 mM stock solution) in a *E. coli* BW25-113 culture at OD_{600} = 0.1 in M9 media supplemented with 10 mM glucose. The bacterial culture was previously grown in MHB overnight, shaking at 250 rpm at 37 °C. On the experimental day, fresh M9 medium supplemented with 10 mM glucose was inoculated at 1:100 ratio with the overnight culture and grown until approximately OD_{600} = 0.3. The assay was conducted

at 37 °C while shaking (500 rpm), and the bacterial growth was monitored measuring the OD_{600} every hour for 15 or 24 h. The isogenic mutant *E. coli* JW0427-1 was used as control, an internal control or as strain study in a control experiment. The mutant was treated in the same way of the WT.

Molecular Docking. Molecular docking was performed using GOLD version 5.4.1 (Cambridge Crystallographic Data Centre, Cambridge, UK). Selection of the optimal scoring function was carried out by redocking the only co-crystallized non-covalently bound ClpP inhibitor AV145 (PDB ID 5DL1) into *S. aureus* ClpP. For newly identified inhibitors, the *E. coli* ClpP X-ray crystal structure 2FZS was selected. All protein structures were prepared with the molecular modeling software suite Molecular Operating Environment (MOE, Chemical Computing Group Inc., Montreal, Canada) version 2016.0802 and energy minimized using an Amber10:EHT force field with implicit solvation model (R-Field). Three-dimensional coordinates of ligands to be docked were generated within MOE. Redocking of co-crystallized AV145 into the ClpP structure 5DL1 (all 14 chains) revealed the scoring function GoldScore to be best suited for docking non-covalent compounds into ClpP, resulting in top-ranked docking poses with heavy atom root-mean-square deviation (RMSD) values 0.68 Å for all 14 monomers. The search space for compounds to be docked into *E. coli* ClpP was defined by a sphere of 15 Å radius centered on atom C10 of the ligand. For each compound, 50 docking runs were conducted. The early termination option was switched off. The Asn150 amide side was allowed to flip by 180 degrees.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website:

-Detailed protocols, extended data series, additional charts and graphs for the *in vitro* HTS enzymatic assay and bacterial growth assays can be found in the associated content (PDF).

-Molecular formula strings (CSV).

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§Author Contributions. C.M.-C.[1] and E.S.[2] contributed equally to this study. The manuscript was written with contributions of all authors. All authors gave approval to the final version of the manuscript.

Funding. C.M.-C.[1] and E.S.[2] were financed by the MSCA-ITN-2014-ETN project INTEGRATE (grant number 642620). C.D.C and P.T. were supported by the Academy of Finland Grants 277001 and 304697. H. B.-O. and L.R. received DFG funding (SFB 766 and GRK 1708).

ACKNOWLEDGMENTS

This research was supported by the MSCA-ITN-2014-ETN project INTEGRATE (grant number 642620). The Laboratory of Medicinal Chemistry is a partner of the Antwerp Drug Discovery Network (www.addn.be). C.D.C and P.T. thank the Academy of Finland (Grants no. 277001 and 304697) for financial support. We thank Dr. Peter Sass for providing the pETDclpPec construct.

ABBREVIATIONS USED

ADEPs	Acyldepsipeptides
ClpA	Caseinolytic protease subunit A
ClpP	Caseinolytic protease proteolytic subunit
ClpX	Caseinolytic protease subunit X
DIPEA	*N*,*N*-diisopropylethylamine
DPP8	Dipeptidyl peptidase 8
DPTA NONOate	(*Z*)-1-[*N*-(3-Aminopropyl)-N-(3-ammoniopropyl)amino]diazen-1-ium-1,2-diolate
E. coli	*Escherichia coli*
KLK4	Kallikrein-related peptidase 4
L. monocytogenes	*Listeria monocytogenes*
MOE	Molecular Operating Environment
S. aureus	*Staphylococcus aureus*
SDS-PAGE	Sodium Dodecyl Sulphate - PolyAcrylamide Gel Electrophoresis
SPR	Surface Plasmon Resonance Spectroscopy
Suc-LY-AMC	4-(((*S*)-1-(((*S*)-2-(4-Hydroxyphenyl)-1-(4-methyl-2-oxo-2H-chromen-7-yl)ethyl)amino)-4-methyl-1-oxopentan-2-yl)amino)-4-oxobutanoic acid
UAMC	University of Antwerp Medicinal Chemistry group
uPA	Urokinase plasminogen activator
WT	Wild-type
Z’	Z prime
Z-LY-CMK	Benzyl ((*S*)-1-(((*S*)-4-chloro-1-(4-hydroxyphenyl)-3-oxobutan-2-yl)amino)-4-methyl-1-oxopentan-2-yl)carbamate
Δ*clpP*	Mutant defective in clpP
Δ*lpxC*	Mutant impaired in lipidA synthesis
Δ*tolC*	Mutant defective in tolC
•NO	Nitric oxide

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