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Graphical Abstract

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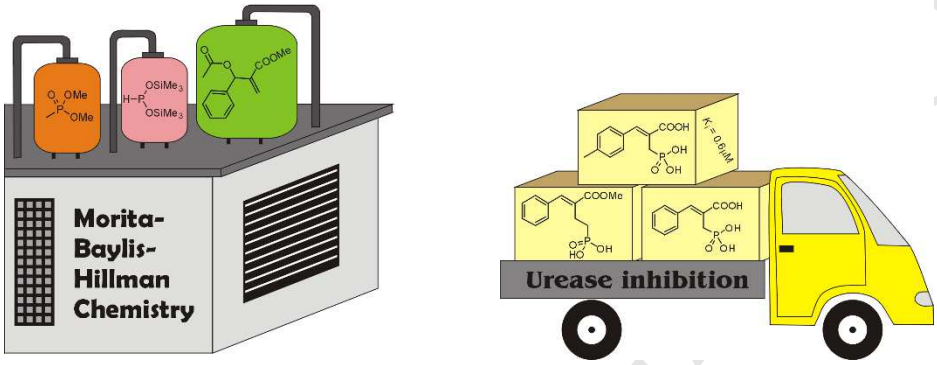

Novel organophosphorus scaffolds of urease inhibitors obtained by substitution of Morita-Baylis-Hillman adducts with phosphorus nucleophiles

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Keywords: phosphonic and phosphinic acids, substitution of allyl acetates, enzyme inhibitors, structure-activity relationship, binding mode.

Abstract

The reactivity of Morita-Baylis-Hillman allyl acetates was employed to introduce phosphorus-containing functionalities to the side chain of the cinnamic acid conjugated system by nucleophilic displacement. The proximity of two acidic groups, the carboxylate and phosphonate/phosphinate groups, was necessary to form interactions in the active site of urease by recently described inhibitor frameworks. Several organophosphorus scaffolds were obtained and screened for inhibition of the bacterial urease, an enzyme that is essential for survival of urinary and gastrointestinal tract pathogens. α-Substituted phosphonomethyl- and 2-phosphonoethyl-cinnamate appeared to be the most potent and were further optimized. As a result, one of the most potent organophosphorus inhibitors of urease, α-phosphonomethyl-*p*-methylcinnamic acid, was identified, with K_i = 0.6 μM for *Sporosarcina pasteurii* urease. High complementarity to the enzyme active site was achieved with this structure, as any further modifications significantly decreased its affinity. Finally, this work describes the challenges faced in developing ligands for urease.

Introduction

Urease (EC 3.5.1.5) is a heteropolymeric amidohydrolase involved in the degradation of urea to ammonia and carbamate; the latter compound is then spontaneously hydrolyzed to another ammonia and carbonic acid [1,2]. Urease is characterized by an exceptional hydrolysis rate enhancement (10^{14}-fold) and holds a special position among milestone biochemistry developments as the first ever enzyme crystallized and demonstrated as nickel-dependent [3-5]. Resolution of the crystal structures of urease from different sources allowed investigators to determine the role of the two Ni^{2+} ions in the active site and the subsequent mechanism of action [5-10]. Urease is involved in global nitrogen circulation and occurs in plants, fungi and bacteria, such as *Proteus mirabilis*, *Klebsiella aerogenes* and *Helicobacter pylori* [11,12]. This distribution determines the importance of urease to humans and the environment in two fundamental ways. In agriculture, the efficacy of nitrogen fertilizers can be decreased by soil macrobiotic ureases [13], which requires heavy use of urea and causes water eutrophication [14]. In the context of public health, bacterial urease activity is associated with colonization of humans with pathogenic microorganisms and causes persistent infections/diseases, in particular those of the urinary and gastrointestinal tracts. A *Proteus mirabilis*-induced increase in the environment's pH results in the crystallization of urinary stones [11,12,15], while *Helicobacter pylori* is the main etiological agent responsible for peptic ulcers. In the stomach, the alkaline local environment facilitates *H. pylori* colonization of the mucosal lining and ultimately leads to disruption of the gastric mucous membrane [11,12,16-18]. Accordingly, massive efforts have been devoted to the development of inhibitors of bacterial urease, especially those responsible for human disorders [19-21]. Although these inhibitors are not precisely antibacterial, they can eradicate the microorganism-induced conditions essential for the development of pathogens. Organophosphorus compounds are one of the most important classes of urease inhibitors. Originally, they comprised phosphoramides of varied structures that mimicked the substrate in the transition state of the enzymatic process [4,22-24]. Unfortunately, the P-N bond is not fully stable in water conditions, which limits further applications of phosphoramides. Recently, compounds containing a P-C linkage (phosphonates and phosphinates) emerged as an alternative to overcome the hydrolytic lability. Several active di- and tri-functional α-aminomethylphosphonic and α-aminomethylphosphinic acids were found after extensive screening and structural optimization (Figure 1) [25-28].

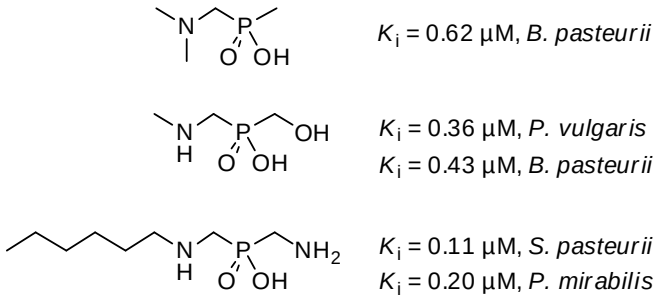


Figure 1. Exemplified *P*-methyl, *P*-hydroxymethyl and *P*-aminomethyl-substituted α-aminomethylphosphinic acids as inhibitors of bacterial ureases of submicromolar inhibition constants [26-28].

In this work, we suggest a novel framework for organophosphorus inhibitors of ureases. Our concept is partially based on the results presented in a study describing the crystal structure of *Sporosarcina pasteurii* urease complexed with citrate [29]. The polar groups of citrate, in particular two carboxylates, form a complex network of hydrogen bonds in the enzyme active site (Figure 2). Central 2-carboxylate is involved in bidendate nickel ion complexation and in an interaction with the imidazole nitrogen atom of Hiss222. Terminal 1-carboxylate forms two close hydrogen bonds with the oxygen atom of Gly280 and α-nitrogen atom of Arg339. The two remaining functionalities (3-carboxylate and hydroxyl) also interact with oxygen atoms of the neighboring amino acid residues, although these contacts seem to be less crucial for binding.

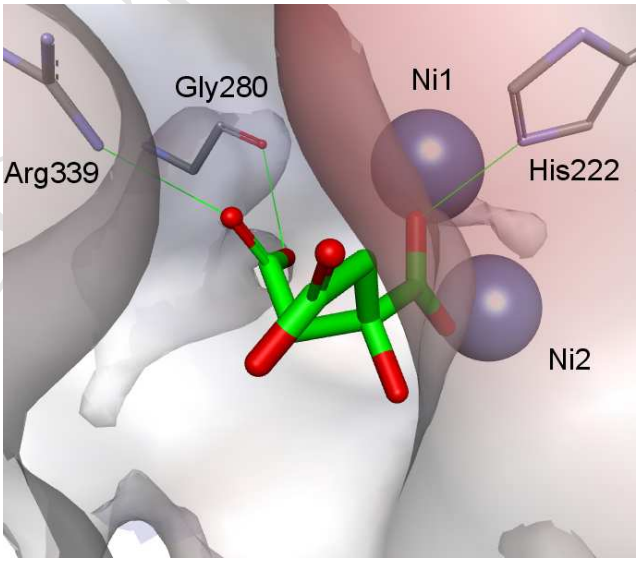
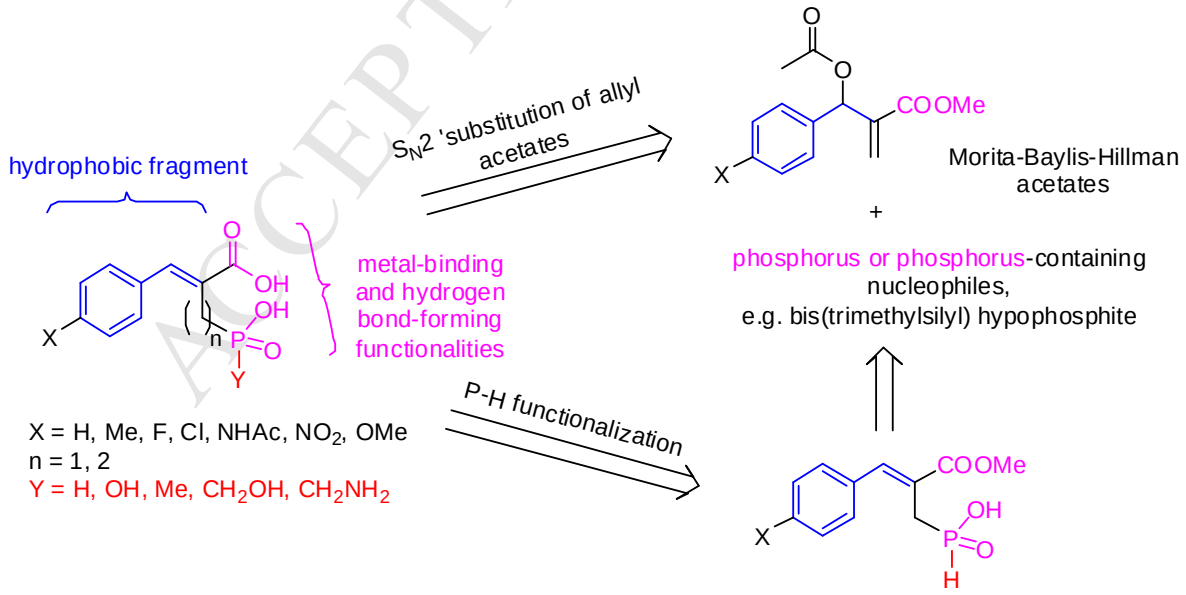


Figure 2. The mode of binding of citrate to *Sporosarcina pasteurii* urease (PDB id 4AC7) [29]. Inhibitor and enzyme amino acid residues are shown as sticks, while nickel ions are

shown as dark blue spheres. Hydrogen bonds are shown as thin green lines. Enzyme surface is colored according to the interpolated charge.

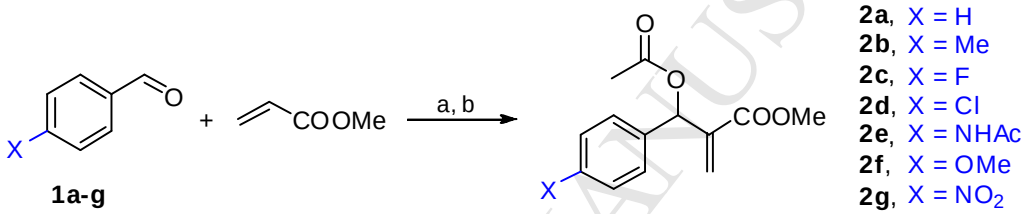
Inspired by these data, we propose construction of a phosphonate(phosphinate)/carboxylate system which can imitate the 1,2-dicarboxylate portion of citrate in its affinity to urease. Phosphonic acid should have improved complexation abilities with catalytic metal ions. Contrary to citrate, both acidic groups are planned to be fixed on a hydrophobic, partially rigid core. To examine the synthetic feasibility of such an arrangement, we turn our attention to appropriately modified cinnamic acids. These compounds can be readily obtained from activated allylic systems of Morita-Baylis-Hillman acetates under S_N2' nucleophilic displacement [30,31]. For phosphorus nucleophiles, such transformations were reported in the literature mostly with the use of tervalent phosphorus compounds [32-35]. Here, we intended to examine the reactivity of a broad range of P-nucleophiles with allyl acetates derived from aromatic aldehydes (Scheme 1). As an alternative to allylic substitution, certain derivatives could also be prepared by derivatization *via* intermediate *H*-phosphinic acids. We aimed to obtain various scaffolds containing phosphonic, *H*-, methyl-, hydroxymethyl- and aminomethyl-phosphinic functionalities and evaluate their affinity to *S. pasteurii* urease. Additionally, modification and optimization of the hydrophobic core structure was also planned.



Scheme 1. A general concept of the construction of novel inhibitors of urease and the retrosynthetic analysis.

Results and Discussion

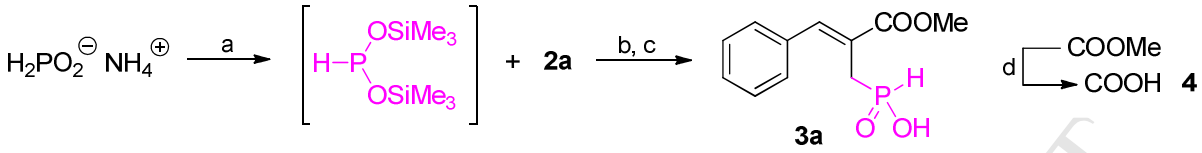
The Morita-Baylis-Hillman (MBH) acetates were synthesized in a typical two-step procedure (Scheme 2) [31,33]. First, benzaldehyde and its selected *p*-substituted analogues (**1a-g**) were reacted with methyl acrylate. As is standard, the reaction proceeded in the presence of DABCO as the catalyst for an extended time to form the corresponding MBH adducts, allyl alcohols, which were used in the subsequent step without purification. Acetylation of the hydroxyl group was readily performed with acetyl chloride and pyridine in CH_2Cl_2. Compounds **2a-g** were purified by column chromatography, and the yield was good to excellent (45-90%).



Scheme 2. The structure and preparation of the allylic substrates, the Morita-Baylis-Hillman acetates **2a-g**. Reagents and conditions: (a) DABCO, rt, 3 d (20 d for **1e**); (b) AcCl, pyridine, CH_2Cl_2, 0 °C, 1 h, rt, 16 h; 45-90%.

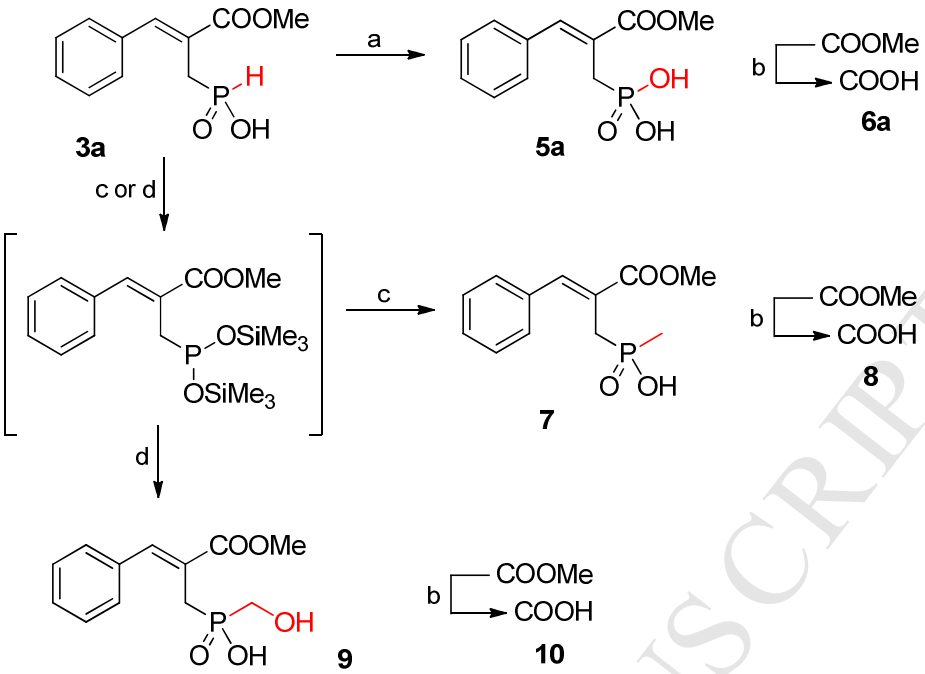
To establish the synthetic procedures of the allylic substitution with phosphorus and phosphorus-containing nucleophiles, benzaldehyde-derived compound **2a** was employed as the model substrate. The primary P-C bond formation was performed with bis(trimethylsilyl) hypophosphite (Scheme 3). The silylated starting material was obtained *in situ* by heating ammonium hypophosphite with hexamethyldisilazane (HMDS). After cooling to 0 °C, the esterification was followed by the addition of the MBH acetate **2a** and dissolved in CH_2Cl_2. Methanolysis of the produced silyl ester intermediate provided the final compound **3a** in 89% yield after column chromatography. The *Z* stereoisomer of phosphinic acid **3a** was exclusively obtained as determined by ^{1}H NMR. A single signal at 7.91 ppm appeared, and no resonance at a higher field (approximately 6.90-7.00 ppm) was observed. This assumption is in accordance with Georgiadis' data on the stereochemical preferences of MBH acetate substitution with phosphinic acid silyl esters [36]. Alkaline hydrolysis followed by

acidification provided the corresponding product **4a** in the form of free carboxylic acid in 96% yield.



Scheme 3. Substitution of the Morita-Baylis-Hillman acetate **2a** and with bis(trimethylsilyl) hypophosphite to obtain *H*-phosphinic acid **3a**, and hydrolysis of the latter to **4**. Reagents and conditions: (a) HMDS, 120 °C, 2 h; (b) **2a** in CH_2Cl_2, 0 °C → rt, 16 h; (c) MeOH, rt, 30 min; 89%, three steps; (d) 0.75 M NaOH, MeOH, rt, then H^+; 96%.

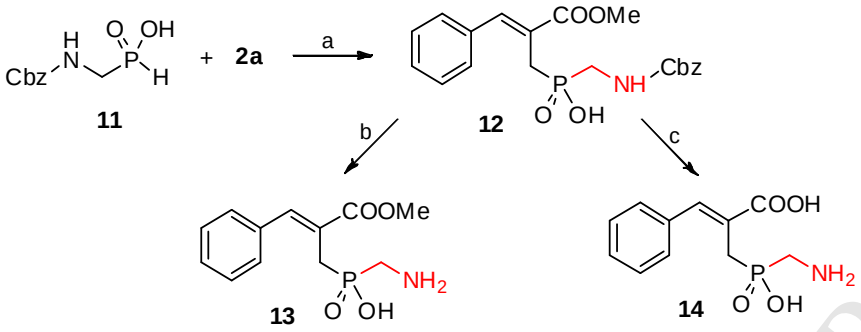
The *H*-phosphinic acids contain functionality that can be divergently modified. We envisaged three types of transformations: P-H oxidation, P-alkylation and P-H addition to a carbonyl compound (Scheme 4). The P-H oxidation of phosphinic acid **3a** with I_2/DMSO provided phosphonic acid **5a** in 81% yield after chromatographic purification. Apparently, the phosphorus group transformation does not alter the diastereometric composition, and the *Z* isomer was solely obtained. This result was again verified by the presence of an exclusive peak at 7.75 ppm in ^{1}H NMR. Acidic hydrolysis of **5a** in refluxing 6 M HCl resulted in free carboxylic acid **6a** in 74% yield after purification on reversed phase column chromatography. Typically, such α-(phosphonomethyl)cinnamic acids are obtained by substitution of compounds **2** with a trialkyl phosphite [33] followed by exhaustive hydrolysis. Here, we envisaged an alternative route that provides easy access to more diversified products including alkyl cinnamates **3** and **5** and cinnamic acids **4** and **6** containing a free phosphorus moiety.



Scheme 4. Divergent modifications of *H*-phosphinic acid **3a** leading to oxidized and P-alkylated products. Reagents and conditions: (a) DMSO, I_2, THF, reflux, 24 h; 81%; (b) 6 M HCl, reflux, 24 h, reversed phase column chromatography; 74% for **6a**, 83% for **8** and 69% for **10**; (c) CH_3I, CH_2Cl_2, BSA, 0 °C → rt, 24 h; 68%; (d) HCHO, CH_2Cl_2, BSA, 0 °C → rt, 24 h, 35%.

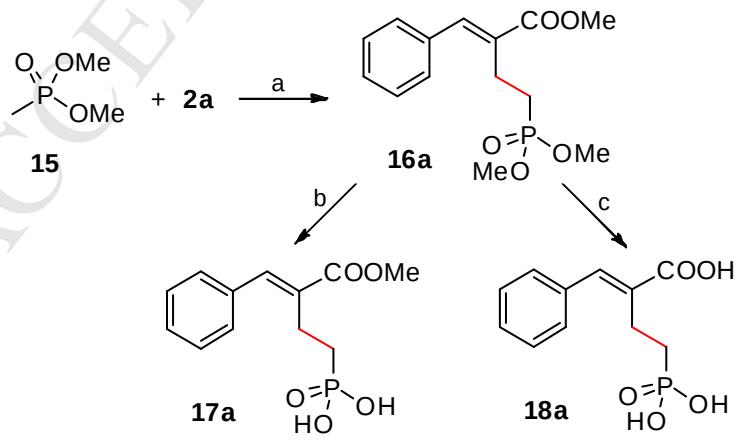
The subsequent P-C bonds were formed by reactions involving *in situ* generation of tervalent bis(trimethylsilylated) phosphorus species, in this case using BSA (*N*,*O*-bis(trimethylsilyl)acetamide). The activation was followed by either an Arbuzov-type reaction with iodomethane to obtain **7** or with the addition of formaldehyde to obtain **9**. The corresponding phosphinic acids were obtained after chromatographic purification in 68% and 35% yield, respectively. Both compounds were converted to the form of free carboxylates by acidic hydrolysis to give compounds **8** and **10** in 83% and 69% yield, respectively (Scheme 4).

The Cbz-protected *H*-phosphinic analog of glycine **11** [25] was selected as the appropriate nucleophile for the elaboration of aminomethylphosphinic acid **12** (Scheme 5). The conversion was achieved *via* BSA-mediated allylic substitution of MBH acetate **2a** with bis(trimethylsilyl) phosphinate intermediate (41% yield after purification on column chromatography). The Cbz-protecting group was successfully removed, in the presence of the double bond, with 33% HBr/AcOH giving phosphinic acid **13** in 94% yield. Simultaneous N- and C-deprotection were achieved in refluxing HCl to obtain **14** in 67% yield.



Scheme 5. Substitution of MBH acetate **2a** with *N*-benzyloxycarbonylaminomethyl-*H*-phosphinate **11**. Reagents and conditions: (a) CH_2Cl_2, BSA, 0 °C → rt, 24 h; 41%; (b) 33% HBr/AcOH, 30 min, rt, then reversed phase column chromatography; 94%; (c) 6 M HCl, reflux, 24 h, then reversed phase column chromatography; 67%.

Finally, allylic substitution with a dimethyl methylphosphonate anion was required to extend the inhibitor backbone (Scheme 6). The starting material **15** was deprotonated with LiHMDS (lithium bis(trimethylsilyl)amide) at -15 °C and reacted with acetate **2a**. Phosphonate **16a** of predominantly *E* stereochemistry (7.71 ppm in ^{1}H NMR) was obtained in 41% yield. Selective demethylation of the phosphonate group in the presence of the carboxylic methyl ester was achieved with TMSBr (bromotrimethylsilane) in CH_2Cl_2 giving **17a** in 79% yield. The fully deprotected compound **18a** was obtained by acidic hydrolysis in a manner similar to the cases mentioned above.

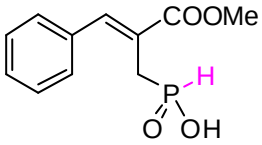
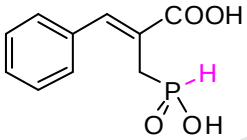
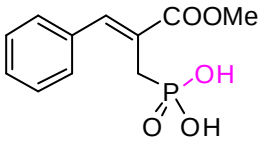
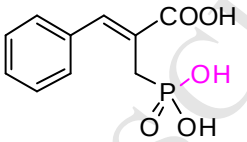
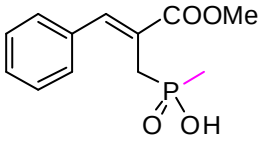
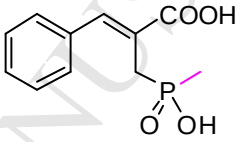
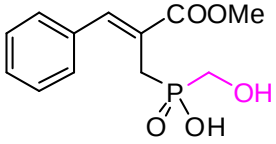
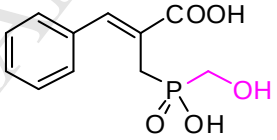
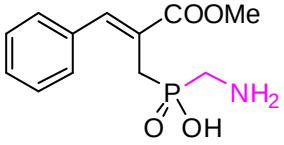
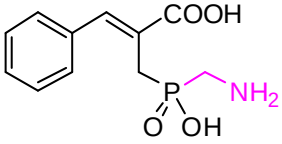
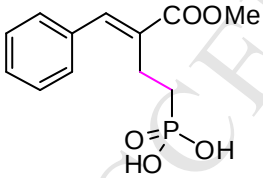
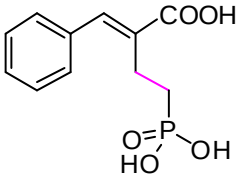


Scheme 6. Substitution of MBH acetate **2a** with dimethyl methylphosphonate **11**. Reagents and conditions: (a) THF, -15 °C, LiHMDS, 1 h; 41%; (b) TMSBr, CH_2Cl_2, 0 °C → rt, 24 h; 79%; (c) 6 M HCl, reflux, 24 h, reversed phase column chromatography; 67%.

All the synthesized structural variants, both esters and acids, together with the starting allyl acetates, were tested for inhibition of urease derived from *Sporosarcina pasteurii*. The MBH acetates **2** either did not inhibit or weakly inhibited the enzyme (Table S1), showing irreversible mode of binding to the target protein. The most likely mechanism of action involved formation of a covalent complex *via* nucleophilic displacement of the allylic system with thiolate of Cys322. As illustrated by molecular modeling such a modification does not produce any further specific ligand-protein contacts (Figure S1).

Several phosphorus compounds were found to be poor or completely inactive inhibitors, while the others appeared to range from moderate to good inhibitors (Table 1). Contrary to MBH acetates and in agreement with the designing concept they all bound to the enzyme in a competitive and reversible manner (see below for details). C-P(O)(OH)-C phosphinic derivatives, namely, *P*-methyl **7** and **8**, *P*-hydroxymethyl **9** and **10** and *P*-aminomethyl acids **13** and **14** belonged to the less potent structures. The majority of them indicated no effect on urease activity. P-H phosphinic compounds **3a** and **4** acted somewhat more efficiently. Their inhibition constants K_i were measured at approximately 100 μM and did not depend on the status of carboxylate function, methyl ester **3a** or free acid **4**. Phosphonic compounds **6a**, **17a** and **18a** (with the exception of **5a**) exhibited the most significant inhibitory influence on urease. Evidently, the presence of three oxygen atoms in the phosphonate group was favored for effective binding. α-(Phosphonomethyl)cinnamic acid **6a** displayed a low micromolar inhibition constant, while the corresponding carboxylate ester **5a** unexpectedly appeared inactive. Even more surprising was that their higher homologs behaved in a quite opposite manner. The ester **17a** was found to be the most active compound among all the tested scaffolds with K_i ~ 3 μM, approximately 20-fold more potent than the free acid **18a**. Considering the data set, phosphonic compounds **6**, **17** and **18** were selected for further structural optimization—phenyl ring modifications.

Table 1. Inhibition of *S. pasteurii* urease by methyl cinnamates and cinnamic acids α-substituted with phosphorus-containing functionalities. Scaffold validation and optimization of the structure of the phosphonate/phosphinate moiety. The most significant inhibition is highlighted in bold.

Entry	Ester	K_i [μM]	Entry	Acid	K_i [μM]
3a		97.5 ± 14	**4**		117 ± 8.9
5a		NI	**6a**		13.1 ± 2.3
7		NI	**8**		NI
9		293 ± 20	**10**		NI
13		NI	**14**		1 319 ± 126
17a		**3.05 ± 0.16**	**18a**		66.7 ± 6.6

To facilitate the structure development of the inhibitor, the binding mode of the two most potent compounds **6a** (data not shown) and **17a** (Figure 3), and a moderate one (**4,** Figure S2), was studied in some detail by molecular modeling. The optimized inhibitor-enzyme complexes had general similarities in their interactions at the enzyme active site. In all cases, the phosphonate/*H*-phosphinate group coordinates with two nickel ions, while the aromatic portion of the inhibitors is lipophilically stuck to the hydrophobic surface of the entrance cavity formed by Ala170 and Ala366. The carboxylate oxygen atom of methyl ester **17a**

forms a hydrogen bond with Arg339. However, the distance between the ester and phosphonic functions in **17a** seems to be too long to fully employ the potential of both oxygen-rich moieties as the ligand driving forces. For **6a**, the inclusion is somewhat deeper and conveniently involves charge-assisted hydrogen bonds with Arg339 (for details, see Figure 4 showing the binding mode of *p*-methyl derivative **6b** with improved inhibitory activity). In the case of *H*-phosphinic acid **4** the binding is not optimal. The lack of the third oxygen atom on phosphorus prevents formation of an interaction with His222. The less polar and spatial *H*-phosphinate group is moved closer to the nickel ions. This shift partially disables the potential of hydrogen bonding between the ligand carboxylate and the guanidine of Arg339. In conclusion, as the following optimization step, we suggested substitution of the phenyl ring with small groups to modify the properties and size of the lipophilic fragment of phosphonic acid-containing scaffolds.

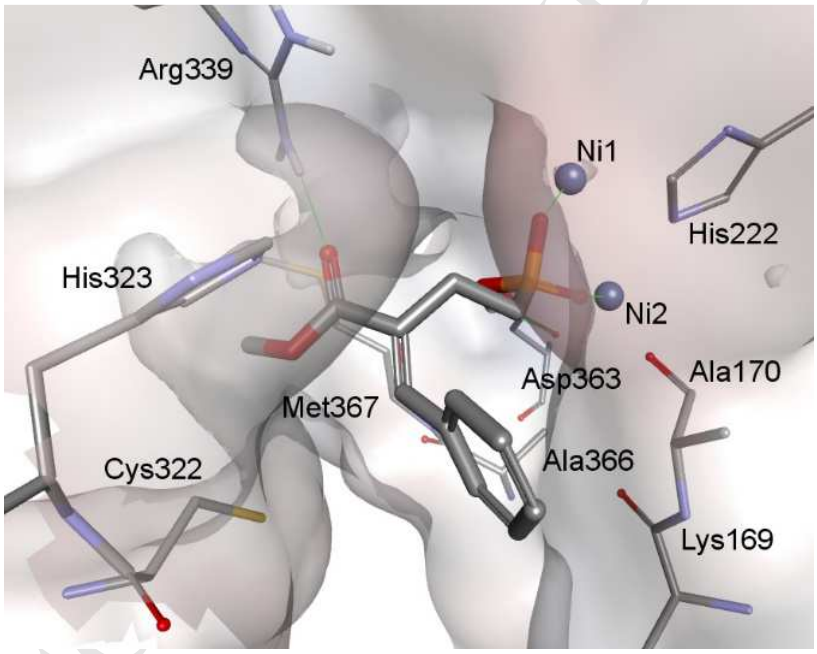
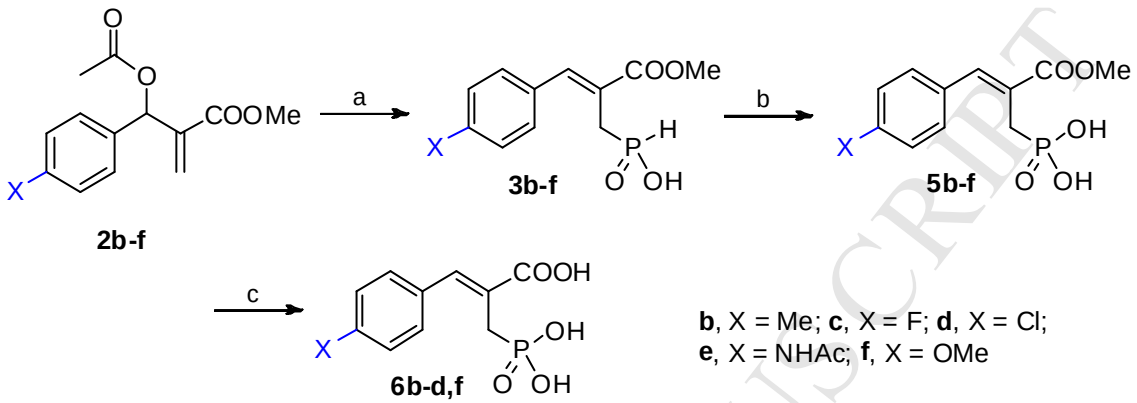


Figure 3. Modeled binding mode of compound **17a** to *S. pasteurii* urease. Inhibitor and enzyme amino acid residues are shown as sticks, while nickel ions as dark blue spheres. Hydrogen bonds and interactions with metal ions are shown as thin green lines. Enzyme surface is colored according to interpolated charge.

Substituted α-(phosphonomethyl)cinnamic acids **6b-f** were obtained *via* corresponding esters **5b-f** starting from the MBH acetates **2b-f** (Scheme 7), following the conditions of the allylic substitution procedure outlined in Scheme 3. Subsequently, oxidation of intermediate *H*-

phosphinic acids **3b-f** was conducted analogously to the reaction shown in Scheme 4. Similarly to the model derivatives, the substitution of **2b-f**, oxidation of **3b-f** and hydrolysis of **5d-b** and **5f** produced carboxylates **6d-b** and **6f** in good to excellent yield (**6e** was not obtained).

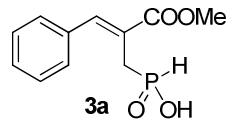
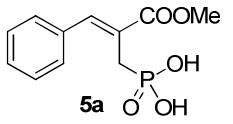
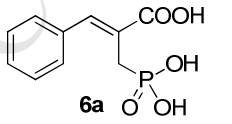
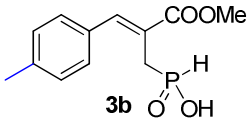
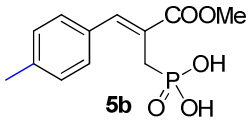
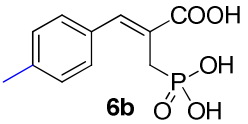
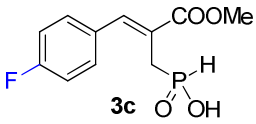
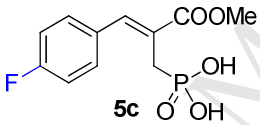
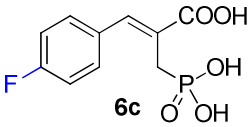
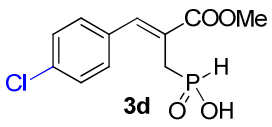
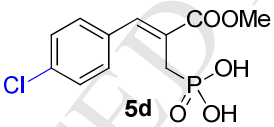
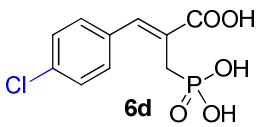
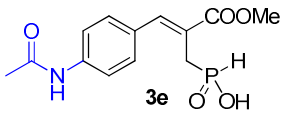
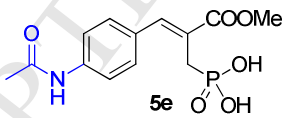
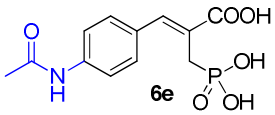
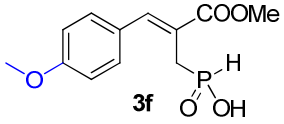
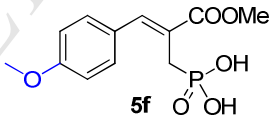
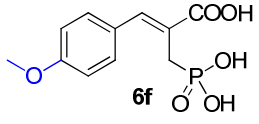


Scheme 7. Synthesis of methyl α-*H*-(phosphinomethyl)cinnamates, methyl α-(phosphonomethyl)cinnamates and α-(phosphonomethyl)cinnamic acids comprising a *p*-substituted phenyl ring. Reagents and conditions: (a) bis(trimethylsilyl) hypophosphite, CH_2Cl_2, 0 °C → rt, 16 h; then MeOH, rt, 30 min; 49-98%; (b) DMSO, I_2, THF, reflux, 24 h; 49-94%; (c) 6 M HCl, reflux, 24 h for **5b-d**; 0.75 M NaOH, MeOH, rt, 3 d for **5f**; then reversed phase column chromatography; 50-52% for **6b-d**, 89% for **6f**. **6e** was not obtained (hydrolysis of the amide bond).

Different small-size groups, including an alkyl—methyl group, halogens—fluoro and chloro groups, and heteroatom-derived—acetamido and methoxy groups, were used to modify the properties of the phenyl portion of the molecules. The anti-urease activity was evaluated for *H*-phosphinic intermediates and phosphonic products, and almost all substitutions gave rise to activity improvement (Table 2). For *H*-phosphinic carboxylate esters **3b-e** the potency reached a K_i range of 13.4-23.4 μM, which is 5-7-fold lower than that measured for reference compound **3a**. Only methoxy derivative **3f** did not follow the same tendency. The improvement was also visible for phosphonic carboxylate esters **5b-f** compared to the simplest analogue **5a** which did not inhibit urease. The most significant result was obtained for phosphonic carboxylate acids. The addition of *p*-methyl to the aromatic ring produced **6b**, the most potent inhibitor identified in this study and one of the most potent organophosphorus ligands of the enzyme reported so far (K_i ~ 0.6 μM). Substitution of phenyl with halogens

retained the activity of **6c** and **6d** at a low micromolar level. Interestingly, methoxy-substituted compound **6f** was only slightly less potent than the analogous esters of *H*-phosphinic **3f** and phosphonic acid **5f**.

Table 2. Inhibition of *S. pasteurii* urease by methyl α-*H*-(phosphinomethyl)cinnamates **3**, methyl α-(phosphonomethyl)cinnamates **5** and α-(phosphonomethyl)cinnamic acids **6**. Exploration of the structure of phenyl substituents. The most significant inhibition is highlighted in bold.

Entry	K_i [μM]	Entry	K_i [μM]	Entry	K_i [μM]
 **3a**	97.5 ± 14	 **5a**	NI	 **6a**	13.1 ± 2.3
 **3b**	23.4 ± 1.4	 **5b**	20.7 ± 1.3	 **6b**	**0.594 ± 0.028**
 **3c**	14.5 ± 1.2	 **5c**	62.9 ± 3.2	 **6c**	9.79 ± 0.65
 **3d**	16.7 ± 1.3	 **5d**	81.2 ± 4.9	 **6d**	16.2 ± 0.98
 **3e**	13.4 ± 0.85	 **5e**	386 ± 19	 **6e**	-
 **3f**	149 ± 8.2	 **5f**	229 ± 13	 **6f**	34.4 ± 2.1

The modeled structure of the optimized **6b**-urease complex confirmed a favorable binding mode of the inhibitor (Figure 4). In particular, the phosphonic acid group perfectly chelates the catalytic nickel cations. One of the phosphoryl oxygen atoms forms a bridge between both metallic centers and is additionally involved in a hydrogen bond with the imidazole nitrogen atom of neighboring His222. Each of the two remaining P-oxygen atoms coordinate monodendately counterpart Ni^{2+} ions. In contrast to **17a** (Figure 3) and **4** (Figure S2), the carboxylate group of **6b** is located opposite to guanidine of Arg339 and forms a classical two-hydrogen bond-assisted salt bridge. Thus, the polar heads of **6b** are in a suitable arrangement

to be involved in strong interactions in the enzyme active site. The *p*-methylphenyl group is conveniently accommodated in the hydrophobic cleft.

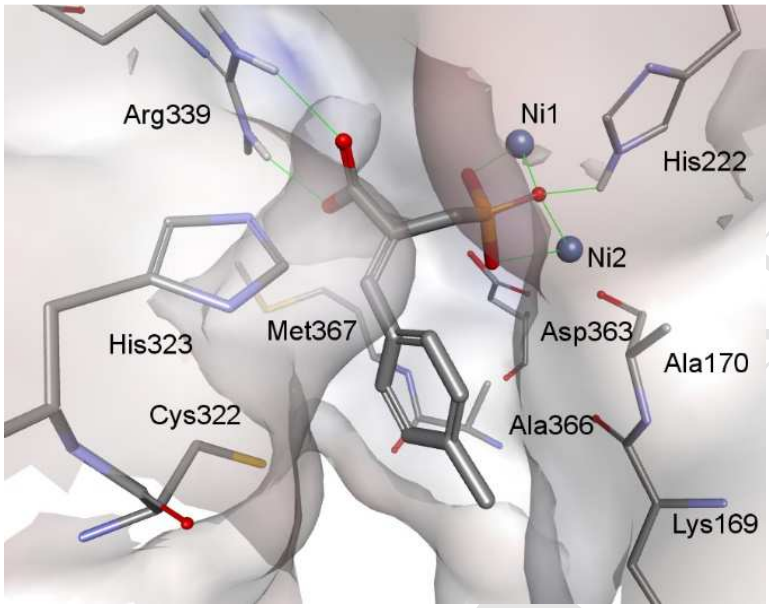
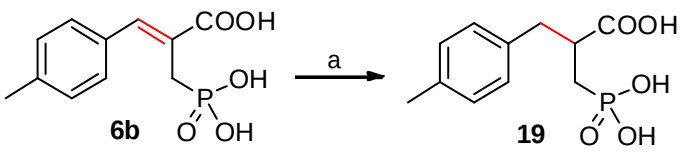


Figure 4. Modeled mode of binding of compounds **6b** to *Sporosarcina pasteurii* urease. Inhibitor and enzyme amino acid residues are shown as sticks, while nickel ions are shown as dark blue spheres. Hydrogen bonds and interactions with metal ions are shown as thin green lines. Enzyme surface is colored according to interpolated charge.

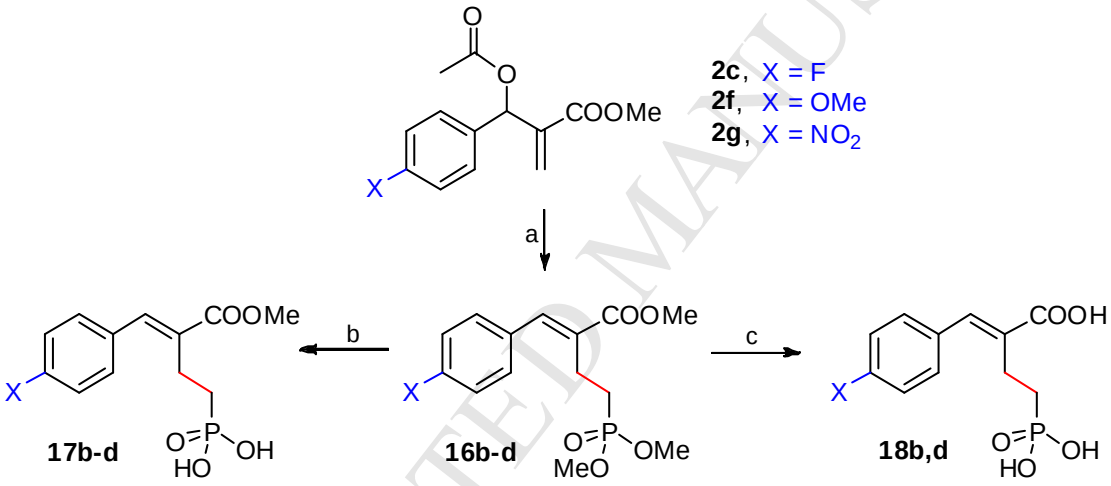
The α,β-unsaturated system of **6b**, conjugated with free carboxylate and an aromatic ring, of a certain size and rigidity appeared to be the most complementary compound to the sterical and electronic demands of the enzyme active site. High specificity towards the organophosphorus ligands is not an unusual observation for urease [26-28]. To further prove the significance of scaffold rigidity, the potency of **6b** was compared with a saturated counterpart. The latter (**19**) was obtained by catalytic hydrogenation over Pd/C. Indeed, the drop in activity reached 150-fold (Scheme 8).



K_i = 0.594 ± 0.028 μM K_i = 90.2 ± 5.3 μM

Scheme 8. Synthesis and activity of saturated phosphonic analog of the most potent inhibitor identified **6b**. Reagents and conditions: (a) H_2, 10% Pd/C, MeOH, 1 atm, rt, 2 d; 60%.

Phenyl substitution of extended phosphonic structures **17a** and **18a** was not as profitable as in the cases of methylene-shorter compounds. Selected phosphonates **17b-d** (carboxylate esters) and **18b,d** (carboxylic acids) were synthesized by the LiHMDS deprotonation procedure followed by either TMSBr-mediated phosphonate demethylation or acidic hydrolysis (Scheme 9), similar to that envisaged in Scheme 6. Any structural extension or modification of non-substituted ester **17a** dramatically altered the activity of the products, at least by an order of magnitude (Table 3). For acids (**18b** and **18d**), the K_i values were quite similar to esters and remained at a moderate, micromolar level.



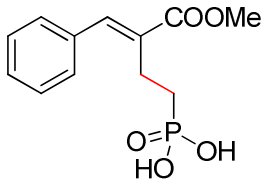
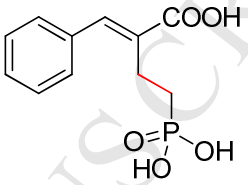
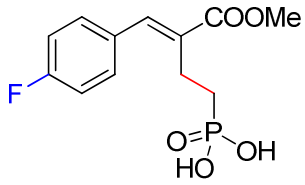
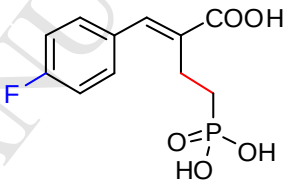
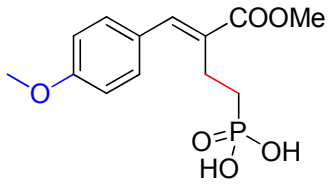
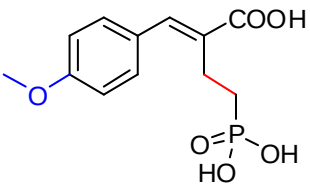
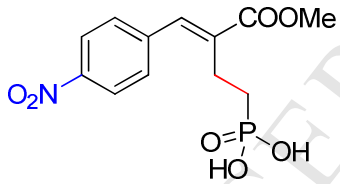
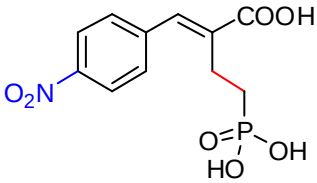
16-18b, X = F; **16-18c**, X = OMe; **16-18d,** X = NO_2

Scheme 9. Synthesis of methyl α-(2-phosphonoethyl)cinnamates and α-(2-phosphonoethyl)cinnamic acids comprising a *p*-substituted phenyl ring. Reagents and conditions: (a) $MeP(O)(OMe)_2$, THF, -15 °C, LiHMDS, 1 h; 11-44%; (b) TMSBr, CH_2Cl_2, 0 °C → rt, 24 h; 43-60% (c) 6 M HCl, reflux, 24 h, then reversed phase column chromatography; 37% (**18b**), 68% (**18d**).

The majority of studied organophosphorus inhibitors appeared irreversible, competitive and fast-binding. *p*-Nitro derivatives **17d** and **18d** showed a slow-binding mechanism of action, while a few more compounds exhibited a mixed mechanism. To achieve the stationary state for all the ligands presented in Tables 1-3, they were preincubated with urease before data

collection. To distinguish between the mechanisms, the inhibition was also measured without incubation. The initial and stationary state inhibition constants are compared in Supporting Information (Table S2).

Table 3. Inhibition of *S. pasteurii* urease by methyl α-(2-phosphonoethyl)cinnamates **17** and α-(2-phosphonoethyl)cinnamic acids **18**. Exploration of the structure of phenyl substituents. **18c** was not obtained.

Entry	Ester **17**	K_i [μM]	Entry	Acid **18**	K_i [μM]
17a		3.05 ± 0.16	**18a**		66.7 ± 6.6
17b		47.4 ± 1.3	**18b**		27.9 ± 1.3
17c		292 ± 18	**18c**		-
17d		54.1 ± 3.6*	**18d**		59.9 ± 3.2*

* stationary state (slow-binding mechanism)

Conclusions

Structure-based design and development of low molecular mass competitive inhibitors of urease is not a trivial task. The enzyme active site is adapted to accommodate and to perform hydrolysis of the small specific substrate [5-10,25]; thus, the potential ligands are limited to compounds that fulfill those sterical and functional restrictions. P-C organophosphorus compounds have been recently proven to satisfy the complementary to the enzyme demands and emerged as one of the most important classes of inhibitors of urease. Chemically stable

and resistant to hydrolysis, phosphonic and phosphinic acids offer a tetrahedral functionality which combines analogously to that of the *gem*-diolate substrate in the transition state of an enzymatic reaction with metal-binding properties. Nevertheless, to achieve a substantial level of inhibition these generally favorable features needed to be extensively optimized. The SAR studies performed for modifications of a lead structure typically appointed the optimization process to a specific compound of a particular size and functional group arrangement. For example, among collections of methyl-, hydroxymethyl- and aminomethyl(aminophosphinic) acids, only single structures of submicromolar activity were identified. They outscored even their closest homologs/analogs in affinity [26-28].

In this work, we found new organophosphorus ligand leads of a bacterial urease (*Sporosarcina pasteurii*). The two most potent compounds (**6b** and **17a**) were additionally tested for inhibition of urease from pathogenic *Proteus mirabilis* strain. According with high homology of these bacterial enzymes they exhibited quite a similar potency, with K_i = 0.485 ± 0.029 μM for *P. mirabilis* urease (0.594 ± 0.028, *S. pasteurii*) for **6b**, and K_i = 1.52 ± 0.11 μM for *P. mirabilis* urease (3.05 ± 0.16, *S. pasteurii*) for **17a**. They were selected among several structural variants obtained by nucleophilic displacement in the allylic system of the Morita-Baylis-Hillman acetates with phosphorus and phosphorus-containing reactants. The phosphonomethyl group installed as the α-substituent of the cinnamic acid conjugated system gave rise to the most significant results. Similar to the aforementioned literature cases, inhibition was spectacularly structure-specific (Figure 5). Only a single compound, α-phosphonomethyl-*p*-methylcinnamic acid (**6b**), exhibited the submicromolar K_i value. The lowest possible structural variations diminished this activity by an order of magnitude, at least 20-fold. An alteration of the aromatic portion bulkiness and hydrophobicity appeared to be relatively less influential on the potency. A transformation of one of the functional groups, either by esterification of carboxylate or through a phosphonic-to-phosphinic replacement (one oxygen atom loss), caused the K_i to increase to 20-25 μM (35-40-fold). The backbone restrictions were found to be the most important. Separation of the acidic groups from a three- to four-bond distance was manifested by a two-orders of magnitude decrease in activity (110-fold). Loss of compound rigidity appeared even more fatal and induced a 150-fold decrease in K_i. The enzyme preferences were quite uniquely satisfied by inhibitor **6b**, which was selected among a variety of fundamental structures and further optimized. Convenient tools to accomplish the syntheses, structure diversification and optimization were provided by the allylic system of the Morita-Baylis-Hillman adducts.

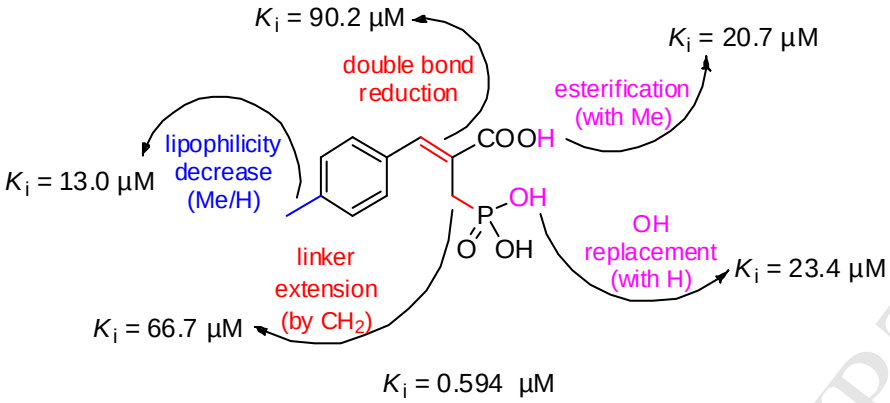


Figure 5. The specificity of inhibition of *Sporosarcina pasteurii* urease by compound **6b** and its close analogs/homologs.

Experimental

1. Chemistry – general

All used reagents were purchased at the highest quality from commercial suppliers, and were used without further purification. Reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm silica gel plates (silica gel 60F254) and components were visualized by UV light absorbance. Purification of compounds by column chromatography was carried out on silica gel (70-230 mesh) or reversed phase silica gel (Kieselgel 60, RP-18, 40-63 µm) with the indicated solvents. ^{1}H, ^{13}C and ^{31}P NMR spectra were recorded on a 200 MHz Mercury spectrometer. ^{1}H and ^{13}C spectra are referenced according to the residual peak of the solvent based on literature data. ^{31}P NMR chemical shifts are reported in ppm downfield from 85% H_3PO_4 (external standard). ^{13}C and ^{31}P NMR spectra are fully proton decoupled. ESI mass spectral analyses were performed on a mass spectrometer, using direct sample injection. Negative or positive ion ESI spectra were acquired by adjusting the needle and cone voltages accordingly. HRMS spectra were registered using a Bruker Maxis Impact QTOF mass spectrometer. All synthesized compounds gave satisfactory NMR spectra confirming >95% purity. All tested organophosphorus compounds **3a-f**, **4, 5a-f**, **6a-d**, **6f**, **7-10**, **13**, **14**, **17a-d**, **18a**, **18b** and **18d** gave satisfactory HRMS analysis (found value within 0.0014 *m/z* unit of the calculated value of the parent ion). The spectroscopy and analytical data were obtained in the Laboratory of Organic Chemistry of the University of Athens.

2. Allylic substitution of MBH acetates with bis(trimethylsilyl) hypophosphite, the general procedure for the synthesis of H-phosphinic acids 3a-f

In an oven-dried flask, $NH_4H_2PO_2$ (0.25 g, 3.00 mmol) and HMDS (0.74 mL, 3.60 mmol) are

placed and the mixture was heated at 110 °C for 2 h under Ar. Then, the reaction mixture was cooled at 0 °C and the appropriate MBH acetate **2** (1.20 mmol) dissolved in CH_2Cl_2 (5 mL) was added. The mixture was stirred at room temperature for 16 h, then, MeOH (5 mL) was added and stirring was continued for 30 min. The volatiles were evaporated under vacuum and the residue was acidified to pH = 1 with 6 M HCl, taken up with CH_2Cl_2, washed with 2 M HCl and H_2O. The organic phase was dried over Na_2SO_4, evaporated *in vacuo* and purified by column chromatography using $CHCl_3$/MeOH/AcOH, 7:1:0.5 (7:1.5:1 for **3e**) as eluent.

Methyl (*Z*)-2-{[hydrogen(hydroxy)phosphoryl]methyl}-3-phenylpropenoate, 3a

Colorless viscous oil, 0.64 g, yield 89%. R_f ($CHCl_3$/MeOH/AcOH, 7:1:0.5) = 0.27. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.91 (d, $^3J_{P\text{-}H}$ = 4.5 Hz, 1H), 7.50-7.33 (m, 5H), 7.30 (d, $^1J_{P\text{-}H}$ = 578.5 Hz, 1H), 3.81 (s, 3H), 3.10 (d, $^1J_{P\text{-}H}$ = 20.5 Hz, 2H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 167.5, 142.9 (d, $^3J_{C\text{-}P}$ = 10.7 Hz), 134.2, 129.1, 129.0, 128.5, 122.2 (d, $^2J_{C\text{-}P}$ = 9.7 Hz), 52.3, 29.9 (d, $^1J_{C\text{-}P}$ = 94.8 Hz). ^{31}P NMR (81 MHz, $CDCl_3$) δ 33.1. MS (ESI) m/z calculated for $C_{11}H_{12}O_4P^-$ $[M\text{-}H]^-$ 239.0, found 239.0. HRMS m/z calculated for $C_{11}H_{12}O_4P^-$ $[M\text{-}H]^-$ 239.0479, found 239.0479.

Methyl (*Z*)-2-{[hydrogen(hydroxy)phosphoryl]methyl}-3-(4-methylphenyl)propenoate, 3b

Yellowish foamy solid, 0.49 g, yield 98%. R_f ($CHCl_3$/MeOH/AcOH, 7:1:0.5) = 0.35. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.66 (s, 1H), 7.35-7.05 (m, 4H), 7.09 (d, $^1J_{P\text{-}H}$ = 527.5 Hz, 1H), 3.65 (s, 3H), 3.00 (m, 2H), 2.20 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 168.9, 140.9, 138.7, 131.9, 129.4, 129.1, 123.6, 52.3, 31.8 (d, $^1J_{P\text{-}C}$ = 82.4 Hz), 21.1. ^{31}P NMR (81 MHz, $CDCl_3$) δ 26.1. MS (ESI) m/z calculated for $C_{12}H_{14}O_4P^-$ $[M\text{-}H]^-$ 253.1, found 253.1. HRMS m/z calculated for $C_{12}H_{14}O_4P^-$ $[M\text{-}H]^-$ 253.0635, found 253.0642.

Methyl (*Z*)-3-(4-fluorophenyl)-2-{[hydrogen(hydroxy)phosphoryl]methyl}propenoate, 3c

White sticky solid, 0.35 g, yield 78%. R_f ($CHCl_3$/MeOH/AcOH, 7:1:0.5) = 0.32. ^{1}H NMR (200 MHz, CD_3OD) δ 7.56-6.77 (m, 5H), 7.09 (d, $^1J_{P\text{-}H}$ = 525.7 Hz, 1H), 3.74 (s, 3H), 3.06 (d, $^2J_{P\text{-}H}$ = 22.1 Hz, 2H). ^{13}C NMR (50 MHz, CD_3COOD) δ 170.4, 164.8 (d, $^1J_{F\text{-}C}$ = 250.0 Hz), 142.4, 134.6, 132.1, 125.8, 117.2, 53.8, 33.1 (d, $^1J_{P\text{-}C}$ = 75.6 Hz). ^{31}P NMR (81 MHz, CD_3OD) δ 23.6. MS (ESI) m/z calculated for $C_{11}H_{11}FO_4P^-$ $[M\text{-}H]^-$ 257.0, found 257.2. HRMS m/z calculated $C_{11}H_{11}FO_4P^-$ $[M\text{-}H]^-$ 257.0384, found 257.0384.

Methyl (*Z*)-3-(4-chlorophenyl)-2-{[hydrogen(hydroxy)phosphoryl]methyl}propenoate, 3d

Foamy solid, 0.47 g, yield 94%. R_f ($CHCl_3$/MeOH/AcOH, 7:1:0.5) = 0.31. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.68 (s, 1H), 7.42 (d, J = 7.9 Hz, 2H), 7.25 (d, J = 7.9 Hz, 2H), 7.12 (d, $^1J_{P\text{-}H}$ = 549.2 Hz, 1H), 3.71 (s, 3H), 2.97 (d, $^2J_{P\text{-}H}$ = 18.2 Hz, 2H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 168.3, 140.3 (d, $^3J_{C\text{-}P}$ = 9.7 Hz), 134.8, 133.0, 130.7, 128.7, 124.3 (d, $^2J_{C\text{-}P}$ = 9.0 Hz), 52.5, 31.3 (d, $^1J_{C\text{-}P}$ = 89.5 Hz). ^{31}P NMR (81 MHz, $CDCl_3$) δ 27.78. MS (ESI) m/z calculated for $C_{11}H_{11}ClO_4P^-$ [M-H]$^-$ 273.0, found 273.1. HRMS m/z calculated for $C_{11}H_{11}ClO_4P^-$ [M-H]$^-$ 273.0089, found 273.0087.

Methyl (*Z*)-3-(4-acetamidophenyl)-2-{[hydrogen(hydroxy)phosphoryl]methyl}propenoate, 3e

White solid, 0.39 g, yield 49%. R_f ($CHCl_3$/MeOH/AcOH, 7:1.5:1) = 0.35. ^{1}H NMR (200 MHz, CD_3COOD) δ 7.89 (d, $^4J_{P\text{-}H}$ = 4.5 Hz, 1H), 7.68 (d, J = 8.4 Hz, 2H), 7.54 (d, J = 8.4 Hz, 2H), 7.36 (d, $^1J_{P\text{-}H}$ = 587.7 Hz, 1H), 3.85 (s, 3H), 3.28 (d, $^2J_{P\text{-}H}$ = 20.0 Hz, 2H), 2.21 (s, 3H). ^{13}C NMR (50 MHz, CD_3COOD) δ 172.8, 169.3, 143.8, 140.2, 131.3, 122.3, 120.9, 113.1, 53.2, 30.9 (d, $^1J_{P\text{-}C}$ = 90.1 Hz), 23.9. ^{31}P NMR (81 MHz, CD_3OD) δ 44.6. MS (ESI) m/z calculated for $C_{13}H_{15}NO_5P^-$ [M-H]$^-$ 296.1, found 296.1. HRMS m/z calculated for $C_{13}H_{15}NO_5P^-$ [M-H]$^-$ 296.0693, found 296.0693.

Methyl (*Z*)-2-{[hydrogen(hydroxy)phosphoryl]methyl}-3-(4-methoxyphenyl)propenoate, 3f

White solid, 0.34 g, yield 68%. R_f ($CHCl_3$/MeOH/AcOH, 7:1:0.5) = 0.29. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.60-6.79 (m, 5H), 7.07 (d, $^1J_{P\text{-}H}$ = 535.2 Hz, 1H), 3.65 (br s, 6H) 3.18 (d, $^2J_{P\text{-}H}$ = 20.5 Hz, 2H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 169.5, 160.1, 140.8, 131.4, 127.3, 122.1, 113.9, 55.1, 52.4, 32.0 (d, $^1J_{C\text{-}P}$ = 84.5 Hz).^{31}P NMR (81 MHz, $CDCl_3$) δ 24.8. MS (ESI) m/z calculated for $C_{12}H_{14}O_5P^-$ [M-H]$^-$ 269.1, found 269.1. HRMS m/z calculated for $C_{12}H_{14}O_5P^-$ [M-H]$^-$ 269.0584, found 269.0586.

3. Alkaline hydrolysis of carboxylate methyl esters, the general procedure

To a stirred solution of a methyl ester (1 mmol) in MeOH (5.4 mL), 4 M NaOH (1.25 mL, 5 mmol) was added and the reaction mixture was stirred at room temperature till total consumption of the starting material (typically 4-5 days, the reaction progress was monitored by TLC). Then, the volatiles were evaporated *in vacuo* and the residue was acidified with 6 M

HCl to pH = 1. The aqueous phase was extracted with AcOEt, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by normal phase or reversed phase column chromatography using $CHCl_3$/MeOH/AcOH, 7:2:0.5 (**4**) or CH_3CN/H_2O, 1:9 to 9:1 (**6f**) as eluent.

(*Z*)-2-{[Hydrogen(hydroxy)phosphoryl]methyl}-4-phenylpropenoic acid, 4

Phosphinic acid **4** was obtained from **3a** (70.8 mg, 0.29 mmol) as colorless viscous oil after column chromatography using $CHCl_3$/MeOH/AcOH, 7:2:0.5 as eluent in 96% yield (64 mg). R_f ($CHCl_3$/MeOH/AcOH, 7:2:0.5) = 0.19. ^{1}H NMR (200 MHz, CD_3OD) δ 7.94 (d, $^3J_{P\text{-}H}$ = 3.8 Hz, 1H), 7.57-7.40 (m, 5H), 7.09 (d, $^1J_{P\text{-}H}$ = 643.0 Hz, 1H), 3.08 (d, $^1J_{P\text{-}H}$ = 20.2 Hz, 2H). ^{13}C NMR (50 MHz, DMSO) δ 168.8, 140.6 (d, $^3J_{C\text{-}P}$ = 9.8 Hz), 134.9, 129.5, 129.2, 128.7, 124.8 (d, $^2J_{C\text{-}P}$ = 9.4 Hz), 31.2 (d, $^1J_{C\text{-}P}$ = 88.5 Hz). ^{31}P NMR (81 MHz, DMSO) δ 26.5. MS (ESI) m/z calculated for $C_{10}H_{10}O_4P^-$ [M-H]$^-$ 225.0, found 225.0. HRMS m/z calculated for $C_{10}H_{10}O_4P^-$ [M-H]$^-$ 225.0322, found 225.0323.

4. Oxidation of H-phosphinic acids 3a-f to phosphonic acids 5a-f, the general procedure

The appropriate *H*-phosphinic acid **3** (1.00 mmol) was dissolved in THF (7 mL), then DMSO (148 μL, 2.00 mmol) and I_2 (2 mg) were added, and the reaction mixture was refluxed for 24 h. The volatiles were removed *in vacuo* and the residue was purified by column chromatography using $CHCl_3$/MeOH/AcOH, 7:1:0.5 (7:1.5:1 for **5e**) as eluent.

Methyl (*Z*)-3-phenyl-2-(phosphonomethyl)propenoate, 5a

Phosphonic acid **5a** was obtained from *H*-phosphinic acid **3a** (0.25 g, 1.04 mmol) as highly viscous oil in 81% yield (0.21 g). R_f ($CHCl_3$/MeOH/AcOH, 7:1:0.5) = 0.36. ^{1}H NMR (200 MHz, DMSO) δ 8.33 (br s, 2H), 7.76-7.38 (m, 5H), 3.71 (s, 3H), 2.96 (d, $^1J_{P\text{-}H}$ = 22.1 Hz, 2H). ^{13}C NMR (50 MHz, DMSO) δ 168.2, 139.1 (d, $^3J_{C\text{-}P}$ = 10.4 Hz), 134.9, 129.8, 129.1, 128.7, 126.2 (d, $^2J_{C\text{-}P}$ = 10.9 Hz), 52.3, 28.5 (d, $^1J_{C\text{-}P}$ = 134.6 Hz). ^{31}P NMR (81 MHz, DMSO) δ 21.0. MS (ESI) m/z calculated for $C_{11}H_{12}O_5P^-$ [M-H]$^-$ 255.0, found 255.1. HRMS m/z calculated for $C_{11}H_{12}O_5P^-$ [M-H]$^-$ 255.0428, found 255.0414.

Methyl (*Z*)-3-(4-methylphenyl)-2-(phosphonomethyl)propenoate, 5b

White solid, 0.36 g, yield 89%. R_f ($CHCl_3$/MeOH/AcOH, 7:1:1) = 0.35. ^{1}H NMR (200 MHz, CD_3COOD) δ 7.74 (s, 1H), 7.51 (d, *J* = 7.2 Hz, 2H), 7.19 (d, *J* = 7.2 Hz, 2H), 3.79 (s, 3H), 3.16 (d, $^2J_{P\text{-}H}$ = 22.2 Hz, 2H), 2.33 (s, 3H). ^{13}C NMR (50 MHz, CD_3COOD) δ 170.8, 142.3 (d,

$^{3}J_{P\text{-}C}$ = 12.1 Hz), 140.0, 132.9, 130.5, 129.9, 125.3 (d, $^{2}J_{P\text{-}C}$ = 11.1 Hz), 53.0, 29.4 (d, $^{1}J_{P\text{-}C}$ = 133.8 Hz), 20.7. ^{31}P NMR (81 MHz, CD_3COOD) δ 33.2. MS (ESI) m/z calculated for $C_{12}H_{14}O_5P^-$ $[M\text{-}H]^-$ 269.1, found 269.1. HRMS m/z calculated for $C_{12}H_{14}O_5P^-$ $[M\text{-}H]^-$ 269.0584, found 269.0584.

Methyl (*Z*)-3-(4-fluorophenyl)-2-(phosphonomethyl)propenoate, 5c

White solid, 0.14 g, yield 90%. R_f ($CHCl_3$/MeOH/AcOH, 7:1:1) = 0.39 ^{1}H NMR (200 MHz, CD_3COOD) δ 7.77-7.63 (m, 3H), 7.12 (t, J = 8.7 Hz, 2H), 3.81 (s, 3H), 3.17 (d, $^{2}J_{P\text{-}H}$ = 21.8 Hz, 2H). ^{13}C NMR (50 MHz, CD_3COOD) δ 170.5, 164.0 (d, $^{1}J_{F\text{-}C}$ = 248.2 Hz), 140.9, 132.3, 126.3 (d, $^{2}J_{P\text{-}C}$ = 10.4 Hz), 116.3 (d, $^{2}J_{F\text{-}C}$ = 21.9 Hz), 114.8, 53.1, 29.4 (d, $^{1}J_{P\text{-}C}$ = 134.0 Hz). ^{31}P NMR (81 MHz, CD_3COOD) δ 33.5. MS (ESI) m/z calculated for $C_{11}H_{11}FO_5P^-$ $[M\text{-}H]^-$ 273.0, found 273.1. HRMS m/z calculated for $C_{11}H_{11}FO_5P^-$ $[M\text{-}H]^-$ 273.0334, found 273.0331.

Methyl (*Z*)-3-(4-chlorophenyl)-2-(phosphonomethyl)propenoate, 5d

White solid, 0.28 mg, yield 94%. R_f ($CHCl_3$/MeOH/AcOH, 7:1:1) = 0.32. ^{1}H NMR (200 MHz, CD_3COOD) δ 7.71-7.38 (m, 5H), 3.81 (s, 3H), 3.12 (m, 2H). ^{13}C NMR (50 MHz, CD_3COOD) δ 168.7, 140.5, 135.0, 134.2, 131.7, 129.0, 126.9, 52.9, 30.4 (d, $^{1}J_{P\text{-}C}$ = 36.8 Hz). ^{31}P NMR (81 MHz, CD_3COOD) δ 31.8. MS (ESI) m/z calculated for $C_{11}H_{11}ClO_5P^-$ $[M\text{-}H]^-$ 289.0, found 289.1. HRMS m/z calculated for $C_{11}H_{11}ClO_5P^-$ $[M\text{-}H]^-$ 289.0038, found 289.0041.

Methyl (*Z*)-3-(4-acetamidophenyl)-2-(phosphonomethyl)propenoate, 5e

White solid, 0.11 g, yield 49%. R_f ($CHCl_3$/MeOH/AcOH, 7:1.5:1) = 0.23. ^{1}H NMR (200 MHz, CD_3COOD) δ 7.73-7.62 (m, 5H), 3.79 (s, 3H), 3.17 (d, $^{2}J_{P\text{-}H}$ = 21.6 Hz, 2H), 2.19 (s, 3H). ^{13}C NMR (50 MHz, CD_3COOD) δ 172.6, 170.8, 141.3, 139.8, 131.8, 131.4, 125.3, 120.5, 53.0, 29.4 (d, $^{1}J_{P\text{-}C}$ = 112.6 Hz), 23.9. ^{31}P NMR (81 MHz, CD_3COOD) δ 33.2. MS (ESI) m/z calculated for $C_{13}H_{15}NO_6P^-$ $[M\text{-}H]^-$ 312.1, found 312.1. HRMS m/z calculated for $C_{13}H_{15}NO_6P^-$ $[M\text{-}H]^-$ 312.0642, found 312.0636.

Methyl (*Z*)-3-(4-methoxyphenyl)-2-(phosphonomethyl)propenoate, 5f

White solid, 0.27 g, yield 91%. R_f ($CHCl_3$/MeOH/AcOH, 7:1:1) = 0.27. ^{1}H NMR (200 MHz, CD_3COOD) δ 7.73 (d, $^{4}J_{P\text{-}H}$ = 5.0 Hz, 1H), 7.60 (d, J = 8.6 Hz, 2H), 6.93 (d, J = 8.6 Hz, 2H), 3.80 (s, 3H), 3.79 (s, 3H), 3.17 (d, $^{2}J_{P\text{-}H}$ = 22.3 Hz, 2H). ^{13}C NMR (50 MHz, CD_3COOD) δ

171.2, 161.4, 142.2, 132.7 (d, $^{3}J_{P\text{-}C}$ = 18.4 Hz), 128.3, 123.7 (d, $^{2}J_{P\text{-}C}$ = 11.4 Hz), 114.7, 55.6, 53.1, 29.4 (d, $^{1}J_{P\text{-}C}$ = 133.8 Hz). ^{31}P NMR (81 MHz, CD_3COOD) δ 33.2. MS (ESI) m/z calculated for $C_{12}H_{16}O_6P^-$ [M-H]$^-$ 287.1, found 287.1. HRMS m/z calculated for $C_{12}H_{14}O_6P^-$ [M-H]$^-$ 285.0533, found 285.0534.

5. Acidic hydrolysis of carboxylate methyl esters, the general procedure

The corresponding methyl ester (2 mmol) was subjected to reflux in 6 M HCl (10 mL) for 24 h. The reaction mixture was concentrated under reduced pressure and the residue was purified using reversed phase column chromatography with CH_3CN/H_2O, 1:9 to 9:1 as eluent.

(*Z*)-3-Phenyl-2-(phosphonomethyl)propenoic acid, 6a

Phosphonic acid **6a** was obtained from **5a** (0.21 g, 0.80 mmol) as white solid in 74% yield (0.14 g). R_f (*n*-BuOH/AcOH/H_2O, 4:1:1) = 0.51. ^{1}H NMR (200 MHz, DMSO) δ 7.75-7.09 (m, 6H), 2.88 (d, $^{1}J_{P\text{-}H}$ = 22.0 Hz, 2H). ^{13}C NMR (50 MHz, DMSO) δ 169.5, 138.5 (d, $^{3}J_{C\text{-}P}$ = 10.2 Hz), 135.2, 129.8, 129.0, 128.8, 127.3 (d, $^{2}J_{C\text{-}P}$ = 10.6 Hz), 28.6 (d, $^{1}J_{C\text{-}P}$ = 135.1 Hz). ^{31}P NMR (81 MHz, DMSO) δ 20.8. MS (ESI) m/z calculated for $C_{10}H_{10}O_5P^-$ [M-H]$^-$ 241.0, found 241.1. HRMS m/z calculated for $C_{10}H_{10}O_5P^-$ [M-H]$^-$ 241.0271, found 241.0259.

(*Z*)-3-(4-Methylphenyl)-2-(phosphonomethyl)propenoic acid, 6b

White solid, 0.18 g, yield 52%. R_f (*n*-BuOH/AcOH/H_2O, 4:1:1) = 0.47. ^{1}H NMR (200 MHz, CD_3COOD) δ 7.90 (s, 1H), 7.56 (d, *J* = 7.2 Hz, 2H), 7.24 (d, *J* = 7.2 Hz, 2H), 3.30 (d, $^{2}J_{P\text{-}H}$ = 17.7 Hz, 2H), 2.36 (s, 3H). ^{13}C NMR (50 MHz, CD_3COOD) δ 170.7, 140.8, 132.8, 131.1, 130.8, 130.2, 123.6, 28.5 (d, $^{1}J_{P\text{-}C}$ = 132.8 Hz), 21.4. ^{31}P NMR (81 MHz, CD_3COOD) δ 37.9. MS (ESI) m/z calculated for $C_{11}H_{12}O_5P^-$ [M-H]$^-$ 255.0, found 255.0. HRMS m/z calculated for $C_{11}H_{12}O_5P^-$ [M-H]$^-$ 255.0428, found 255.0428.

(*Z*)-3-(4-Fluorophenyl)-2-(phosphonomethyl)propenoic acid, 6c

White solid 77 mg, yield 50%. R_f (*n*-BuOH/AcOH/H_2O, 4:1:1) = 0.50. ^{1}H NMR (200 MHz, CD_3COOD) δ 7.93 (d, $^{4}J_{P\text{-}H}$ = 5.1 Hz, 1H), 7.73-7.66 (m, 2H), 7.17 (t, *J* = 8.8 Hz, 2H), 3.31 (d, $^{2}J_{P\text{-}H}$ = 22.8 Hz, 2H). ^{13}C NMR (50 MHz, CD_3COOD) δ 173.3, 164.3 (d, $^{1}J_{F\text{-}C}$ = 249.0 Hz), 133.1, 131.9, 124.1 (d, $^{2}J_{P\text{-}C}$ = 11.2 Hz), 119.9, 116.6 (d, $^{2}J_{F\text{-}C}$ = 18.1 Hz), 27.9 (d, $^{1}J_{P\text{-}C}$ = 139.7 Hz). ^{31}P NMR (81 MHz, CD_3COOD) δ 39.4. MS (ESI) m/z calculated for $C_{10}H_9FO_5P^-$ [M-H]$^-$ 259.0, found 259.2. HRMS m/z calculated for $C_{10}H_9FO_5P^-$ [M-H]$^-$ 259.0177, found 259.0178.

(*Z*)-3-(4-Chlorophenyl)-2-(phosphonomethyl)propenoic acid, 6d

White solid, 76 mg, yield 52%. R_f (*n*-BuOH/AcOH/H_2O, 4:1:1) = 0.54. ^{1}H NMR (200 MHz, CD_3COOD) δ 7.90 (d, $^4J_{P\text{-}H}$ = 5.7 Hz, 1H), 7.63 (d, *J* = 8.5 Hz, 2H), 7.43 (d, *J* = 8.5 Hz, 2H), 3.30 (d, $^2J_{P\text{-}H}$ = 22.8 Hz, 2H). ^{13}C NMR (50 MHz, CD_3COOD) δ 173.0, 136.1, 134.2, 132.1 (d, $^3J_{P\text{-}C}$ = 14.9 Hz), 129.9, 129.6, 125.1 (d, $^2J_{P\text{-}C}$ = 11.4 Hz), 28.0 (d, $^1J_{P\text{-}C}$ = 137.3 Hz). ^{31}P NMR (81 MHz, CD_3COOD) δ 38.7. MS (ESI) m/z calculated for $C_{10}H_9ClO_5P^-$ [M-H]$^-$ 275.0, found 275.0. HRMS m/z calculated for $C_{10}H_9ClO_5P^-$ [M-H]$^-$ 274.9882, found 274.9883.

(*Z*)-3-(4-Methoxyphenyl)-2-(phosphonomethyl)propenoic acid, 6f

Following the general alkaline hydrolysis (chapter 3), phosphonic acid **6f** was obtained from **5f** as white solid in 89% yield (76 mg). R_f (*n*-BuOH/AcOH/H_2O, 4:1:1) = 0.50. ^{1}H NMR (200 MHz, CD_3COOD) δ 7.93 (d, $^4J_{P\text{-}H}$ = 5.1 Hz, 1H), 7.65 (d, *J* = 8.5 Hz, 2H), 6.99 (d, *J* = 8.5 Hz, 2H), 3.84 (s, 3H), 3.38 (d, $^2J_{P\text{-}H}$ = 23.0 Hz, 2H). ^{13}C NMR (50 MHz, CD_3COOD) δ 173.7, 162.0, 144.7, 132.9 (d, $^3J_{P\text{-}C}$ = 22.0 Hz), 127.9, 121.0 (d, $^2J_{P\text{-}C}$ = 11.5 Hz), 115.1, 55.7, 27.7 (d, $^1J_{P\text{-}C}$ = 140.0 Hz). ^{31}P NMR (81 MHz, CD_3COOD) δ 40.6. MS (ESI) m/z calculated for $C_{11}H_{12}O_6P^-$ [M-H]$^-$ 271.0, found 271.2. HRMS m/z calculated for $C_{11}H_{12}O_6P^-$ [M-H]$^-$ 271.0377, found 271.0377.

6. P-Methylphosphinic compounds 7 and 8

Methyl (*Z*)-2-{[methyl(hydroxy)phosphoryl]methyl}-3-phenylpropenoate, 7

Methyl iodide (0.23 mL, 3.66 mmol) was added to an oven-dried flask containing **3a** (0.24 g, 1.00 mmol) in dry CH_2Cl_2 (2.5 mL). After cooling to 0 ^{o}C, BSA (1.80 mL, 7.36 mmol) was added dropwise under argon, and the reaction mixture was stirred at room temperature for 24 h. Then, 1.5 M HCl (10 mL) was added and the organic phase was taken up with AcOEt (30 mL), washed with H_2O, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified with column chromatography using $CHCl_3$/MeOH/AcOH, 7:1:0.5 giving **7** as viscous oil in 68% yield (0.17 g). R_f ($CHCl_3$/MeOH/AcOH, 7:1:0.5) = 0.62. ^{1}H NMR (200 MHz, $CDCl_3$) δ 10.18 (br s, 1H), 7.82 (d, $^3J_{P\text{-}H}$ = 3.8 Hz, 1H), 7.52-7.20 (m, 5H), 3.78 (s, 3H), 3.16 (d, $^1J_{P\text{-}H}$ = 18.4 Hz, 2H), 1.42 (d, $^1J_{P\text{-}H}$ = 14.3 Hz, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 168.0, 141.9 (d, $^3J_{C\text{-}P}$ = 9.8 Hz), 134.6, 129.3, 128.9, 128.5, 123.9 (d, $^2J_{C\text{-}P}$ = 9.6 Hz), 52.3, 30.6 (d, $^1J_{C\text{-}P}$ = 92.7 Hz), 15.9 (d, $^1J_{C\text{-}P}$ = 94.7 Hz). ^{31}P NMR (81 MHz, $CDCl_3$) δ 52.2. MS (ESI) m/z calculated for $C_{12}H_{14}O_4P^-$ [M-H]$^-$ 253.06, found 253.16. HRMS m/z

calculated for $C_{12}H_{14}O_4P^-$ [M-H]$^-$ 253.0635, found 253.0637.

(*Z*)-2-{[Methyl(hydroxy)phosphoryl]methyl}-3-phenylpropenoic acid, 8

Following the general acidic hydrolysis procedure (chapter 5), phosphinic acid **8** was obtained from **7** (99.6 mg, 0.39 mmol) as white solid after chromatographic purification with $CHCl_3$/MeOH/AcOH, 7:2:0.5 in 83% yield (78 mg). R_f ($CHCl_3$/MeOH/AcOH, 7:2:1) = 0.32. ^{1}H NMR (200 MHz, DMSO) δ 7.70-7.39 (m, 6H), 3.00 (d, $^1J_{P-H}$ = 18.2 Hz, 2H), 1.32 (d, $^1J_{P-H}$ = 14.2 Hz, 3H). ^{13}C NMR (50 MHz, DMSO) δ 170.4, 141.6 (d, $^3J_{C-P}$ = 9.7 Hz), 135.5, 130.7, 130.4, 129.8, 125.9 (d, $^2J_{C-P}$ = 9.6 Hz), 29.7 (d, $^1J_{C-P}$ = 87.9 Hz), 16.9 (d, $^1J_{C-P}$ = 91.5 Hz). ^{31}P NMR (81 MHz, DMSO) δ 45.2. MS (ESI) m/z calculated for $C_{11}H_{12}O_4P^-$ [M-H]$^-$ 239.1, found 239.2. HRMS m/z calculated for $C_{11}H_{12}O_4P^-$ [M-H]$^-$ 239.0479, found 239.0480.

7. P-Hydroxymethylphosphinic compounds 9 and 10

Methyl (*Z*)-2-{[hydroxymethyl(hydroxy)phosphoryl]methyl}-3-phenylpropenoate, 9

Paraformaldehyde (0.11 g, 3.66 mmol) was added to an oven-dried flask containing **3a** (0.24 g, 1.00 mmol) in dry CH_2Cl_2 (2.5 mL). After cooling to 0 ^{o}C, BSA (1.80 mL, 7.36 mmol) was added dropwise under argon and the reaction mixture was stirred at room temperature for 24 h. Then, 1.5 M HCl (10 mL) was added and the organic phase was taken up with AcOEt (30 mL), washed with H_2O, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified with column chromatography using $CHCl_3$/MeOH/AcOH, 7:1:0.5 giving **9** as viscous oil in 35% yield (95 mg). R_f ($CHCl_3$/MeOH/AcOH, 7:1:0.5) = 0.40. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.82 (s, 1H), 7.51-7.25 (m, 5H), 3.83 (d, $^1J_{P-H}$ = 22.5 Hz, 2H), 3.63 (s, 3H), 3.16 (d, $^1J_{P-H}$ = 17.2 Hz, 2H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 168.4, 142.7 (d, $^3J_{C-P}$ = 8.9 Hz), 134.4, 129.3, 129.1, 128.6, 122.9 (d, $^2J_{C-P}$ = 8.8 Hz), 60.0 (d, $^1J_{C-P}$ = 105.5 Hz), 52.5, 28.7 (d, $^1J_{C-P}$ = 93.7 Hz). ^{31}P NMR (81 MHz, $CDCl_3$) δ 38.1. MS (ESI) m/z calculated for $C_{12}H_{14}O_5P^-$ [M-H]$^-$ 269.1, found 269.1. HRMS m/z calculated for $C_{12}H_{14}O_5P^-$ [M-H]$^-$ 269.0584, found 269.0585.

(*Z*)-2-{[Hydroxymethyl(hydroxy)phosphoryl]methyl}-3-phenylpropenoic acid, 10

Following the general acidic hydrolysis procedure (chapter 5), phosphinic acid **10** was obtained from **9** (0.14 g, 0.52 mmol) as white solid after reversed phase column chromatography in 69% yield (92 mg). R_f (*n*-BuOH/AcOH/H_2O, 4:1:1) = 0.42. ^{1}H NMR (200 MHz, DMSO) δ 7.75 (d, $^3J_{P-H}$ = 4.3 Hz, 1H), 7.32-7.28 (m, 5H), 3.41 (s, 2H), 2.72 (d, $^1J_{P-H}$ =

13.9 Hz, 2H). ^{13}C NMR (50 MHz, DMSO) δ 171.2, 136.6 (d, $^{3}J_{C\text{-}P}$ = 7.9 Hz), 135.6, 130.0, 129.4, 128.4, 128.1, 60.9 (d, $^{1}J_{C\text{-}P}$ = 112.2 Hz), 29.1 (d, $^{1}J_{C\text{-}P}$ = 74.6 Hz). ^{31}P NMR (81 MHz, DMSO) δ 34.1. MS (ESI) m/z calculated for $C_{11}H_{12}O_5P^-$ [M-H]$^-$ 255.0, found 255.1. HRMS m/z calculated for $C_{11}H_{12}O_5P^-$ [M-H]$^-$ 255.0428, found 255.0425.

8. P-Aminomethylphosphinic compounds 13 and 14

Methyl (*Z*)-2-{[*N*-benzyloxycarbonylaminomethyl(hydroxy)phosphoryl]methyl}-3-phenylpropenoate, 12

MBH acetate **2a** (1.52 g, 6.49 mmol) was added to an oven-dried flask containing *N*-benzyloxycarbonylaminomethyl-*H*-phosphinic acid **11** (0.40 g, 1.75 mmol) in dry CH_2Cl_2 (4.5 mL). After cooling to 0 ^{o}C, BSA (3.20 mL, 13.09 mmol) was added dropwise under argon and the reaction mixture was stirred at room temperature for 24 h. Then, 1.5 M HCl (10 mL) was added and the organic phase was taken up with AcOEt (30 mL), washed with H_2O, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified with column chromatography using $CHCl_3$/MeOH/AcOH, 7:0.5:0.5 giving **12** as sticky solid in 41% yield (0.29 g). R_f ($CHCl_3$/MeOH/AcOH, 7:0.5:0.5) = 0.56. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.77 (s, 1H), 7.54-7.02 (m, 10H), 6.13 (br s, 1H), 5.00 (s, 2H), 3.69 (d, $^{1}J_{P\text{-}H}$ = 12.8 Hz, 2H), 3.50 (s, 3H), 3.08 (d, $^{1}J_{P\text{-}H}$ = 17.2 Hz, 2H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 168.5, 156.6, 142.0 (d, $^{3}J_{C\text{-}P}$ = 9.4 Hz), 136.1, 134.4, 129.2, 128.8, 128.7, 128.2 (d, $^{2}J_{C\text{-}P}$ = 10.5 Hz), 128.0, 127.8, 127.7, 66.7, 52.3, 40.5 (d, $^{1}J_{C\text{-}P}$ = 107.8 Hz), 28.0 (d, $^{1}J_{C\text{-}P}$ = 95.7 Hz). ^{31}P NMR (81 MHz, $CDCl_3$) δ 43.3. MS (ESI) m/z calculated for $C_{20}H_{21}NO_6P^-$ [M-H]$^-$ 402.1, found 402.0. HRMS m/z calculated for $C_{20}H_{21}NO_6P^-$ [M-H]$^-$ 402.1112, found 402.1107.

Methyl (*Z*)-2-{[aminomethyl(hydroxy)phosphoryl]methyl}-3-phenylpropenoate, 13

33% HBr/CH_3COOH (2.0 mL) was added to **12** (0.27 g, 0.67 mmol) and the mixture was stirred at room temperature for 30 min. Then, toluene was added and the mixture was concentrated *in vacuo* (3 times). Phosphinic acid **13** was obtained as white solid after reversed phase column chromatography purification using CH_3CN/H_2O, 1:9 to 9:1 in 94% yield (0.17 g). R_f (*n*-BuOH/AcOH/H_2O, 4:1:1) = 0.50. ^{1}H NMR (200 MHz, CD_3COOD) δ 7.89 (d, $^{3}J_{P\text{-}H}$ = 4.7 Hz, 1H), 7.68-7.44 (m, 5H), 3.86 (s, 3H), 3.37 (d, $^{1}J_{P\text{-}H}$ = 8.8 Hz, 2H), 3.20 (d, $^{1}J_{P\text{-}H}$ = 18.6 Hz, 2H). ^{13}C NMR (50 MHz, CD_3COOD) δ 169.7, 135.3 (d, $^{3}J_{C\text{-}P}$ = 3.0 Hz), 131.3, 130.3, 129.3, 129.1, 125.2 (d, $^{2}J_{C\text{-}P}$ = 10.3 Hz), 52.9 (d, $^{1}J_{C\text{-}P}$ = 4.0 Hz), 40.7, 31.1 (d, $^{1}J_{C\text{-}P}$ = 93.4 Hz). ^{31}P NMR (81 MHz, CD_3COOD) δ 40.6. MS (ESI) m/z calculated for $C_{12}H_{15}NO_4P^-$ [M-

$H]^-$ 268.1, found 268.2. HRMS m/z calculated for $C_{12}H_{15}NO_4P^-$ $[M-H]^-$ 268.0744, found 268.0740.

(*Z*)-2-{[Aminomethyl(hydroxy)phosphoryl]methyl}-3-phenylpropenoic acid, 14

Following the general acidic hydrolysis procedure (chapter 5), phosphinic acid **14** was obtained from **12** (93.5 mg, 0.35 mmol), as white solid after reversed phase column chromatography using in 67% yield (60 mg). R_f (*n*-BuOH/AcOH/H_2O, 4:1:1) = 0.40. ^1H NMR (200 MHz, CD_3COOD) δ 7.96 (s, 1H), 7.66-7.17 (m, 5H), 3.42 (d, $^1J_{P-H}$ = 8.8 Hz, 2H), 3.21 (d, $^1J_{P-H}$ = 18.8 Hz, 2H). ^{13}C NMR (50 MHz, CD_3COOD) δ 172.9, 144.9 (d, $^3J_{C-P}$ = 9.0 Hz), 135.5, 131.6, 130.5, 129.4, 124.7 (d, $^2J_{C-P}$ = 10.4 Hz), 40.0 (d, $^1J_{C-P}$ = 91.6 Hz), 31.0 (d, $^1J_{C-P}$ = 94.6 Hz). ^{31}P NMR (81 MHz, CD_3COOD) δ 41.8. MS (ESI) m/z calculated for $C_{11}H_{13}NO_4P^-$ $[M-H]^-$ 254.1, found 254.0. HRMS m/z calculated for $C_{11}H_{13}NO_4P^-$ $[M-H]^-$ 254.0588, found 254.0579.

9. Allylic substitution of MBH acetates with dimethyl methylphosphonate, the general procedure for the synthesis of phoshonates 16a-d

Dry THF (2.9 mL) was added to an oven-dried flask containing the appropriate MBH acetate **2** (1.75 mmol) and dimethyl methylphosphonate **15** (0.20 mL, 1.88 mmol). After cooling to -15 ^oC, LiHMDS (1 M in THF, 1.88 mL, 1.88 mmol) was added dropwise under argon and the reaction mixture was stirred at -15 ^oC for 1h. Then, 1 M HCl was added and the organic phase was taken up in AcOEt, washed with H_2O, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified with column chromatography using AcOEt followed by $CHCl_3$/MeOH, 9.5:0.5 as eluent.

Trimethyl (*E*)-3-phenyl-2-(2-phosphonoethyl)propenoate, 16a

Phosphonate **16a** was obtained from **2a** (0.40 g, 1.75 mmol) as viscous oil in 41% yield (0.20 g). R_f ($CHCl_3$/MeOH, 9.5:0.5) = 0.73. ^1H NMR (200 MHz, $CDCl_3$) δ 7.71 (s, 1H), 7.43-7.25 (m, 5H), 3.81-3.69 (m, 9H), 2.85-2.72 (m, 2H), 2.10-1.93 (m, 2H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 167.9, 140.3, 134.8, 131.1 (d, $^3J_{C-P}$ = 18.3 Hz), 129.1, 128.7, 128.5, 52.3, 52.2, 52.0, 23.8 (d, $^1J_{C-P}$ = 138.5 Hz), 20.6 (d, $^2J_{C-P}$ = 4.0 Hz). ^{31}P NMR (81 MHz, $CDCl_3$) δ 34.2. MS (ESI) m/z calculated for $C_{14}H_{20}O_5P^+$ $[M+H]^+$ 299.1, found 299.0. HRMS m/z calculated for $C_{14}H_{20}O_5P^+$ $[M+H]^+$ 299.1043, found 299.1042.

Trimethyl (*E*)-3-(4-flourophenyl)-2-(2-phosphonoethyl)propenoate, 16b

Phosphonate **16b** was obtained from **2c** as yellowish thick oil in 25% yield (48 mg). R_f ($CHCl_3$/MeOH, 9:1) = 0.43. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.59 (s, 1H), 7.32-7.25 (m, 2H), 7.01 (t, J = 8.6 Hz, 2H), 3.81-3.59 (m, 9H), 2.76-2.63 (m, 2H), 2.03-1.85 (m, 2H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 167.6, 162.4 (d, $^1J_{F-C}$ = 249.9 Hz), 138.8, 130.9 (d, $^3J_{F-C}$ = 8.3 Hz), 130.7, 130.6, 115.5 (d, $^2J_{F-C}$ = 21.6 Hz), 52.1, 52.0, 51.9, 23.5 (d, $^1J_{P-C}$ = 138.7 Hz), 20.3 (d, $^2J_{P-C}$ = 3.6 Hz). ^{31}P NMR (81 MHz, $CDCl_3$) δ 34.0. MS (ESI) m/z calculated for $C_{14}H_{19}FO_5P^+$ $[M+H]^+$ 317.1, found 317.1.

Trimethyl (*E*)-3-(4-methoxyphenyl)-2-(2-phosphonoethyl)propenoate, 16c

Phosphonate **16c** was obtained from **2f** as yellowish thick oil in 44% yield (65 mg). R_f ($CHCl_3$/MeOH, 9:1) = 0.30. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.66 (s, 1H), 7.37 (d, J = 8.8 Hz, 2H), 6.93 (d, J = 8.8 Hz, 2H), 3.83-3.70 (m, 12H), 2.90-2.77 (m, 2H), 2.12-1.95 (m, 2H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 168.3, 160.0, 139.9, 131.1, 129.0, 127.3, 114.1, 55.3, 52.4, 52.3, 52.0, 23.8 (d, $^1J_{P-C}$ = 138.2 Hz), 20.7. ^{31}P NMR (81 MHz, $CDCl_3$) δ 34.3. MS (ESI) m/z calculated for $C_{15}H_{22}O_6P^+$ $[M+H]^+$ 329.1, found 329.2.

Trimethyl (*E*)-3-(4-nitrophenyl)-2-(2-phosphonoethyl)propenoate, 16d

Phosphonate **16d** was obtained from **2g** as yellowish glassy solid in 11% yield (24 mg). R_f ($CHCl_3$/MeOH, 9:1) = 0.83. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.26 (d, J = 8.8 Hz, 2H), 7.72 (s, 1H), 7.53 (d, J = 8.8 Hz, 2H), 3.85-3.69 (m, 9H), 2.83-2.70 (m, 2H), 2.11-1.94 (m, 2H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 167.2, 147.4, 141.4, 137.6, 134.6 (d, $^3J_{P-C}$ = 17.3 Hz), 129.9, 123.8, 52.5, 52.4, 52.4, 23.8 (d, $^1J_{P-C}$ = 139.8 Hz), 20.9 (d, $^2J_{P-C}$ = 3.4 Hz). ^{31}P NMR (81 MHz, $CDCl_3$) δ 33.6. MS (ESI) m/z calculated for $C_{14}H_{19}NO_7P^+$ $[M+H]^+$ 344.1, found 344.1.

10. Demethylation of phosphonate esters 16a-d with TMSBr, the general procedure for the synthesis of phosphonic acids 17a-d

The appropriate dimethyl phosphonate (0.34 mmol) was dissolved in dry CH_2Cl_2 (2.4 mL) and cooled to 0 ^{o}C. TMSBr (0.26 mL, 2.04 mmol) was added and the reaction mixture was kept at room temperature for 24 h. Then, MeOH (5 mL) was added and the mixture was stirred for 30 min. The volatiles were removed under reduced pressure and the residue was purified by reversed phase column chromatography using CH_3CN/H_2O, 1:9 to 9:1 as eluent.

Methyl (*E*)-3-phenyl-2-(2-phosphonoethyl)propenoate, 17a

Phosphonic acid **17a** was obtained from **16a** (0.10 g, 0.34 mmol) as white solid in 79% yield

(73 mg). R_f (*n*-BuOH/AcOH/H_2O, 4:1:1) = 0.62. ^{1}H NMR (200 MHz, CD_3COOD) δ 7.73 (s, 1H), 7.43-7.25 (m, 5H), 3.83 (s, 3H), 2.83 (m, 2H), 2.05 (m, 2H). ^{13}C NMR (50 MHz, CD_3COOD) δ 170.0, 141.5, 135.7, 132.4 (d, $^3J_{P\text{-}C}$ = 10.5 Hz), 130.7, 129.3, 129.2, 52.9, 27.1 (d, $^2J_{P\text{-}C}$ = 124.8 Hz), 21.7. ^{31}P NMR (81 MHz, CD_3COOD) δ 41.6. MS (ESI) m/z calculated for $C_{12}H_{14}O_5P^-$ [M-H]$^-$ 269.1, found 269.1. HRMS m/z calculated for $C_{12}H_{14}O_5P^-$ [M-H]$^-$ 269.0584, found 269.0584.

Methyl (*E*)-3-(4-flourophenyl)-2-(2-phosphonoethyl)propenoate, 17b

White solid, 34 mg, yield 60%. R_f (*n*-BuOH/AcOH/H_2O, 4:1:1) = 0.54. ^{1}H NMR (200 MHz, CD_3COOD) δ 7.69 (s, 1H), 7.50-7.43 (m, 2H), 7.15 (t, *J* = 8.6 Hz, 2H), 3.83 (s, 3H), 2.88-2.76 (m, 2H), 2.15 (m, 2H). ^{13}C NMR (50 MHz, CD_3COOD) δ 169.8, 163.7 (d, $^1J_{F\text{-}C}$ = 248.4 Hz), 140.3, 139.8, 132.1, 131.8, 116.4 (d, $^2J_{F\text{-}C}$ = 20.7 Hz), 52.9, 26.8 (d, $^1J_{P\text{-}C}$ = 135.8 Hz), 21.5. ^{31}P NMR (81 MHz, CD_3COOD) δ 41.5. MS (ESI) m/z calculated for $C_{12}H_{13}FO_5P^-$ [M-H]$^-$ 287.0, found 287.1. HRMS m/z calculated for $C_{12}H_{13}FO_5P^-$ [M-H]$^-$ 287.0490, found 287.0488.

Methyl (*E*)-3-(4-methoxyphenyl)-2-(2-phosphonoethyl)propenoate, 17c

White solid, 38 mg, yield 43%. R_f (*n*-BuOH/AcOH/H_2O, 4:1:1) = 0.53. ^{1}H NMR (200 MHz, CD_3COOD) δ 7.65 (s, 1H), 7.44 (d, *J* = 8.6 Hz, 2H), 6.98 (d, *J* = 8.6 Hz, 2H), 3.82 (s, 6H), 2.84 (m, 2H), 2.10 (m, 2H). ^{13}C NMR (50 MHz, CD_3COOD) δ 170.4, 161.1, 141.2, 133.5, 129.8 (d, $^3J_{P\text{-}C}$ = 19.5 Hz), 128.1, 114.8, 55.6, 52.8, 27.2 (d, $^1J_{P\text{-}C}$ = 137.0 Hz), 21.8. ^{31}P NMR (81 MHz, CD_3COOD) δ 40.2. MS (ESI) m/z calculated for $C_{13}H_{16}O_6P^-$ [M-H]$^-$ 299.1, found 299.1. HRMS m/z calculated for 299.0690, found 299.0677.

Methyl (*E*)-3-(4-nitrophenyl)-2-(2-phosphonoethyl)propenoate, 17d

White solid, 28 mg, yield 45%. R_f (*n*-BuOH/AcOH/H_2O, 4:1:1) = 0.47. ^{1}H NMR (200 MHz, CD_3COOD) δ 8.27 (d, *J* = 8.7 Hz, 2H), 7.76 (s, 1H), 7.64 (d, *J* = 8.7 Hz, 2H), 3.86 (s, 3H), 2.87-2.70 (m, 2H), 2.15 (m, 2H). ^{13}C NMR (50 MHz, CD_3COOD) δ 169.1, 148.3, 143.7, 142.3, 135.8 (d, $^3J_{P\text{-}C}$ = 18.8 Hz), 131.4, 124.6, 53.1, 27.0 (d, $^1J_{P\text{-}C}$ = 135.0 Hz), 21.9. ^{31}P NMR (81 MHz, CD_3COOD) δ 40.35. MS (ESI) m/z calculated for $C_{12}H_{13}NO_7P^-$ [M-H]$^-$ 314.0, found 314.0. HRMS m/z calculated for $C_{12}H_{13}NO_7P^-$ [M-H]$^-$ 314.0435, found 314.0435.

11. Acidic hydrolysis of esters 16a, 16b and 16d to phosphonic acids 18a, 18b and 18d

(*E*)-3-Phenyl-2-(2-phosphonoethyl)propenoic acid, 18a

Following the general acidic hydrolysis (chapter 5), phosphonic acid **18a** was obtained from **16a** (80 mg, 0.27 mmol) as white solid in 40% yield (28 mg). R_f (*n*-BuOH/AcOH/H_2O, 4:1:1) = 0.45. ^{1}H NMR (200 MHz, CD_3COOD) δ 7.80 (s, 1H), 7.59-7.24 (m, 5H), 2.83 (m, 2H), 2.05 (m, 2H). ^{13}C NMR (50 MHz, CD_3COOD) δ 172.9, 142.4, 135.6, 132.5, 132.1, 131.5, 130.6 (d, $^3J_{C\text{-}P}$ = 12.3 Hz), 27.9 (d, $^1J_{C\text{-}P}$ = 139.2 Hz), 21.9. ^{31}P NMR (81 MHz, CD_3COOD) δ 38.2. MS (ESI) m/z calculated for $C_{11}H_{12}O_5P^-$ [M-H]$^-$ 255.0, found 255.1. HRMS m/z calculated for $C_{11}H_{12}O_5P^-$ [M-H]$^-$ 255.0428, found 255.0416.

(*E*)-3-(4-Fluorophenyl)-2-(2-phosphonoethyl)propenoic acid, 18b

White solid, 28 mg, yield 37%. R_f (*n*-BuOH/AcOH/H_2O, 4:1:1) = 0.54. ^{1}H NMR (200 MHz, CD_3COOD) δ 7.75 (s, 1H), 7.54-7.38 (m, 2H), 7.17 (t, *J* = 8.5 Hz, 2H), 2.90-2.66 (m, 2H), 2.15 (m, 2H). ^{13}C NMR (50 MHz, CD_3COOD) δ 172.8, 163.7 (d, $^1J_{F\text{-}C}$ = 248.9 Hz), 141.0, 133.8, 132.5, 132.1 (d, $^3J_{F\text{-}C}$ = 7.4 Hz), 116.6 (d, $^2J_{F\text{-}C}$ = 17.8 Hz), 27.8 (d, $^1J_{P\text{-}C}$ = 135.6 Hz), 21.9. ^{31}P NMR (81 MHz, CD_3COOD) δ 37.7. MS (ESI) m/z calculated for $C_{11}H_{11}FO_5P^-$ [M-H]$^-$ 273.0, found 273.1. HRMS m/z calculated for $C_{11}H_{11}FO_5P^-$ [M-H]$^-$ 273.0334, found 273.0324.

(*E*)-3-(4-Nitrophenyl)-2-(2-phosphonoethyl)propenoic acid, 18d

White solid, 28 mg, yield 68%. R_f (*n*-BuOH/AcOH/H_2O, 4:1:1) = 0.53. ^{1}H NMR (200 MHz, CD_3COOD) δ 8.29 (d, *J* = 8.8 Hz, 2H), 7.89 (s, 1H), 7.66 (d, *J* = 8.8 Hz, 2H), 2.92-2.79 (m, 2H), 2.28-2.10 (m, 2H). ^{13}C NMR (50 MHz, CD_3COOD) δ 172.4, 148.8, 142.2, 140.5, 140.1, 135.2 (d, $^3J_{P\text{-}C}$ = 18.6 Hz), 131.6, 131.1, 124.7, 26.7 (d, $^1J_{P\text{-}C}$ = 137.9 Hz), 21.5. ^{31}P NMR (81 MHz, CD_3COOD) δ 44.6. MS (ESI) m/z calculated for $C_{11}H_{11}NO_7P^-$ [M-H]$^-$ 300.0, found 300.2. HRMS m/z calculated for $C_{11}H_{11}NO_7P^-$ [M-H]$^-$ 300.0279, found 300.0274.

12. 2-[(4-Methylphenyl)methyl]-3-phosphonopropanoic acid, 19, hydrogenation of 6b

A mixture of 10% Pd/C (5 mg) and **6b** (45 mg, 0.17 mmol) in methanol (5 mL) was stirred at room temperature under an atmosphere of hydrogen until the starting material was consumed as observed by TLC (3 d). The catalyst was filtered off through Celite, and the mixture was washed with methanol and water-methanol mixture (1:9). Solvents were combined and evaporated *in vacuo* to give a solid that was purified with reversed phase column chromatography using CH_3CN/H_2O, 1:9 to 9:1 as eluent to give **19** as white solid in 60%

yield (26 mg). R_f (*n*-BuOH/AcOH/H_2O, 4:1:1) = 0.49. ^{1}H NMR (200 MHz, CD_3COOD) δ 7.09 (m, 4H), 3.14-2.93 (m, 3H), 2.31-2.28 (m, 5H). ^{13}C NMR (50 MHz, CD_3COOD) δ 172.9, 137.0, 136.2, 130.0, 43.6, 39.8 (d, $^2J_{P\text{-}C}$ = 11.8 Hz), 30.4 (d, $^1J_{P\text{-}C}$ = 138.3 Hz), 21.8. ^{31}P NMR (81 MHz, CD_3COOD) δ 36.4. MS (ESI) m/z calculated for $C_{11}H_{14}O_5P^-$ $[M\text{-}H]^-$ 257.1, found 257.3. HRMS m/z calculated for $C_{11}H_{14}O_5P^-$ $[M\text{-}H]^-$ 257.0584, found 257.0581.

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Supporting Information

Details regarding preparation, purification and characterization of the MBH acetates **2a-g**, the enzyme preparation, the kinetic data with the methodology used to calculate the inhibition constants, and the molecular modeling methodologies, with the NMR spectra included are available free of charge via the Internet at http://.

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Highlights

Novel organophosphorus scaffolds of urease inhibitors obtained by substitution of Morita-Baylis-Hillman adducts with phosphorus nucleophiles

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MBH (allyl) acetates are readily substituted with diverse phosphorus nucleophiles.
Cinnamate frameworks with phosphonic/phosphinic-modified side-chains were obtained.
The structural arrangement appeared advantageous to construct urease inhibitors.