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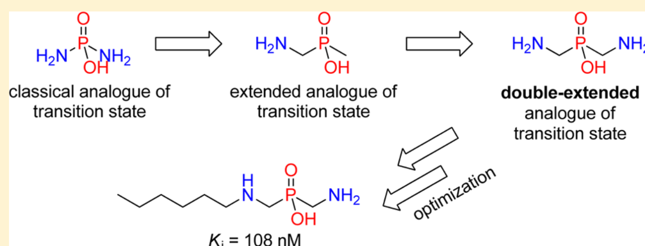
Bis(aminomethyl)phosphinic Acid, a Highly Promising Scaffold for the Development of Bacterial Urease Inhibitors

Katarzyna Macegoniuk, Anna Dziełak, Artur Mucha, and Łukasz Berlicki*

Department of Bioorganic Chemistry, Faculty of Chemistry, Wrocław University of Technology, Wybrzeże Wyspiańskiego 27, 50-370 Wrocław, Poland

Supporting Information

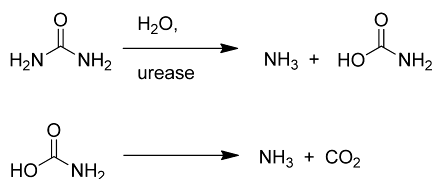
ABSTRACT: Inhibitors of bacterial ureases are considered to be promising compounds in the treatment of infections caused by *Helicobacter pylori* in the gastric tract and/or by urealytic bacteria (e.g., *Proteus* species) in the urinary tract. A new, extended transition state scaffold, bis(aminomethyl)phosphinic acid, was successfully explored for the construction of effective enzyme inhibitors. A reliable methodology for the synthesis of phosphinate analogues in a three-component Mannich-type reaction was elaborated. The obtained molecules were assayed against ureases purified from *Sporosarcina pasteurii* and *Proteus mirabilis*, and aminomethyl(*N*-*n*-hexylaminomethyl)phosphinic acid was found to be the most potent inhibitor, with a K_i = 108 nM against the *S. pasteurii* enzyme.



KEYWORDS: *ureases, inhibitor, urealytic bacteria, Mannich-type reaction*

Rapid microbial decomposition of urea, catalyzed by urease, causes a substantial local increase in pH due to the release of ammonia (Scheme 1).[1−3] This process is a key factor in serious medical complications.[4,5]

Scheme 1



First, the stomach and duodenum form a suitable microenvironment for survival of colonizing *Helicobacter pylori* in the highly acidic conditions in these regions of the gastrointestinal tract.[6,7] In addition, a high concentration of ammonia causes damage of the stomach mucosa and increases the negative impact of the pathogen on human health. Second, rapid decomposition of urea by bacteria infecting the urinary tract, such as *Proteus mirabilis* or *Proteus vulgaris*, considerably elevates the pH of urine, leading to the formation of urinary stones.[8−12] Thus, inhibition of urease is of considerable interest for the treatment of both infections. Although urease inhibitors do not show direct antimicrobial activity, their application can help in the eradication of *Helicobacter pylori* infections or the suppression of troublesome symptoms from ureolytic bacterial infections of the urinary tract.

These important, medicinally relevant applications have stimulated intensive studies on urease inhibitors.[4,5,13] Several classes of compounds have been found to alter the activity of these enzymes, with phosphoramidates being the most active.[14−17] The high activity of phosphoric acid amides is apparently related to their close similarity to the transition state of the enzymatic reaction. Other classes of inhibitors are hydroxamic acids,[18−20] quinones,[21,22] polyphenols,[23,24] heavy metal ions,[25,26] and organosulfur compounds.[27] Disappointingly, attempts at the application of the most active phosphoramidates failed because of the low stability of the P−N bonds in aqueous solutions.[28] Alternatively, the use of hydroxamic acids is restricted by their side effects, with teratogenicity being the most serious.[29,30] Nevertheless, acetohydroxamic acid was introduced to the market for the treatment of chronic infections of the urinary tract. We have focused on exploration of the N−C−P structural motif for the development of urease inhibitors (Figure 1).[31−35]

The major drawback of the classical transition state analogue (**1**), the low stability of the P−N bond, was eliminated by introduction of an inert P−C linkage; therefore, the N−C−P scaffold (**2**) was proposed as a so-called "extended transition state analogue".[36] Computer-aided optimization of such a core has led to many structural variations, namely, phosphonates, *P*-methylphosphinates, *P*-hydroxymethylphosphinates, and thiophosphinates (examples **3**−**6**, Figure 1b), which exhibited considerable antiureolytic activity.

In this study, we explore a potentially promising modification of the previous scaffolds, bis(aminomethyl)phosphinic acid. This lead structure shows a greater opportunity for substitution as it contains two amino groups that can be feasibly modified.

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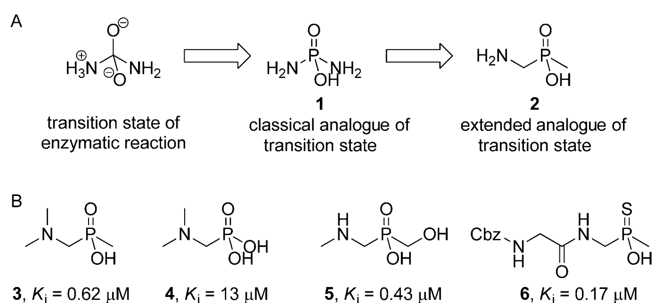
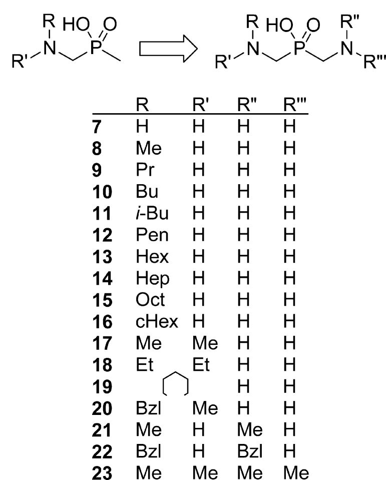


Figure 1. Design principle for the N−C−P scaffold (a), and selected examples of inhibitors constructed according to this approach with their inhibition constants against *S. pasteurii* urease (b).

In comparison to the formerly developed "one-handed" motifs, the new core provides more options to optimize the ligand−enzyme interactions and modulate the inhibitory potencies. The design, synthesis and evaluation of bis(aminomethyl)-phosphinic acids against ureases purified from *Sporosarcina pasteurii* and *Proteus mirabilis* are presented.

Aminomethylphosphinic inhibitors of ureases, presented in a series of earlier publications, showed highly interesting properties that include high potency and water stability.[33,34] In the previous studies, we successfully explored aminomethyl-(*P*-methyl)phosphinic acid (scaffold **2**),[32] followed by aminomethyl(*P*-hydroxymethyl)phosphinic acids.[33] These studies resulted in a highly potent compound (**5**) with a K_i = 0.36 μM against urease from *Proteus vulgaris*. Interestingly, various other modifications, such as elongation of the amino group substituents or substitution of the hydroxymethyl moiety, led to a substantial decrease in activity.[35] Thus, we decided to apply a new backbone that can be modified in multiple positions. The incorporation of the second nitrogen atom in the extended transition state analogue **2**, resulting in bis(aminomethyl)-phosphinic acid scaffold **7**, provides various possibilities (Figure 2). First, the amino moiety can be substituted with alkyl/aryl groups without losing the hydrogen bond donating abilities. Second, it is positively charged under physiological conditions and can form charge-assisted interactions with the enzyme residues. To test all of the possible scaffold substitution patterns, various symmetrical and nonsymmetrical modifications with the methyl groups were envisaged (**8**, **17**, **21**, and **23**) (Figure 2). Additionally, several longer chains (**9−16**, **18**, and **19**) or aromatic rings (**20** and **22**) were incorporated to test the possibility of structural extension of the inhibitors.

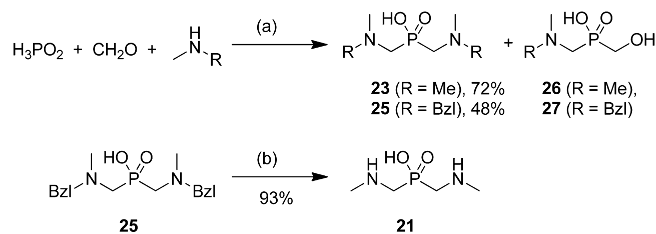


	R	R'	R''	R'''
7	H	H	H	H
8	Me	H	H	H
9	Pr	H	H	H
10	Bu	H	H	H
11	*i*-Bu	H	H	H
12	Pen	H	H	H
13	Hex	H	H	H
14	Hep	H	H	H
15	Oct	H	H	H
16	cHex	H	H	H
17	Me	Me	H	H
18	Et	Et	H	H
19	[ring]		H	H
20	Bzl	Me	H	H
21	Me	H	Me	H
22	Bzl	H	Bzl	H
23	Me	Me	Me	Me

Figure 2. Structures of the designed bis(aminomethyl)phosphinic acid inhibitors.

The unsubstituted core compound, namely, bis-(aminomethyl)phosphinic acid (**7**), was obtained according to the method reported by Meier, starting from bis-(chloromethyl)phosphinic acid (**24**) by reaction with benzylamine to yield **22**, which was subsequently subjected to catalytic hydrogenation.[37] The symmetrically substituted bis-(aminomethyl)phosphinic acids were synthesized in a three-component reaction of hypophosphorous acid, formaldehyde, and an appropriate secondary amine in 6 M hydrochloric acid (Scheme 2). The desired compounds (**23** and **25**) were

Scheme 2[a]



[a]Reagents and conditions: (a) 6 M HCl, reflux; (b) H_2, Pd/C, methanol.

typically accompanied by hydroxymethylated byproducts (**26** and **27**). High yields of the target molecules were obtained when a 1:2:5 molar ratio of substrates (H_3PO_2/CH_2O/amine) was applied. Bis(methylaminomethyl)phosphinic acid (**21**) was obtained by hydrogenolysis of the benzyl groups from **25**.

A convenient synthetic strategy was applied to the synthesis of nonsymmetrical analogues, both mono- (**8−16**) and disubstituted (**17−20**). A three-component phospha-Mannich type reaction of an N-protected aminomethyl-*H*-phosphinic acid, formaldehyde, and a secondary amine was used to build the additional (substituted) aminomethylene fragment of the target molecules **29−40** (Scheme 3). The phosphorus component of the condensation and the precursor of the unsubstituted N−C−P portion, *N*-benzyloxycarbonylamino-methyl-*H*-phosphinic acid (**28**), was obtained by addition of sodium hypophosphite to a mixture of hydroxylamine hydrochloride and formaldehyde, followed by hydrolysis and N-protection as described elsewhere.[38,39]

As proven in our previous study, the phospha-Mannich condensation was not efficient for primary amines and produced a complex mixture of the desired product and byproducts.[40] Therefore, obtaining monosubstituted bis-(aminomethyl)phosphinic acid analogues demanded the use of the appropriate *N*-benzyl-*N*-alkylamines as secondary precursors. Accordingly, primary amines were subjected to *N*-benzylation by a reductive amination and then used in the three-component condensation to give **32−40** in reasonable yields and purity.

The products of condensation were purified chromatographically to obtain the target protected compounds **29−40** in satisfactory yields (55−86%). The strategy for deprotection depended on the structure of the desired target inhibitors. To obtain disubstituted compounds **17−20**, removal of the Cbz group from **29−32** was needed, and this was achieved by the use of hydrobromic acid in acetic acid. When removal of both Cbz and *N*-benzyl protecting groups in **32−40** was needed, to

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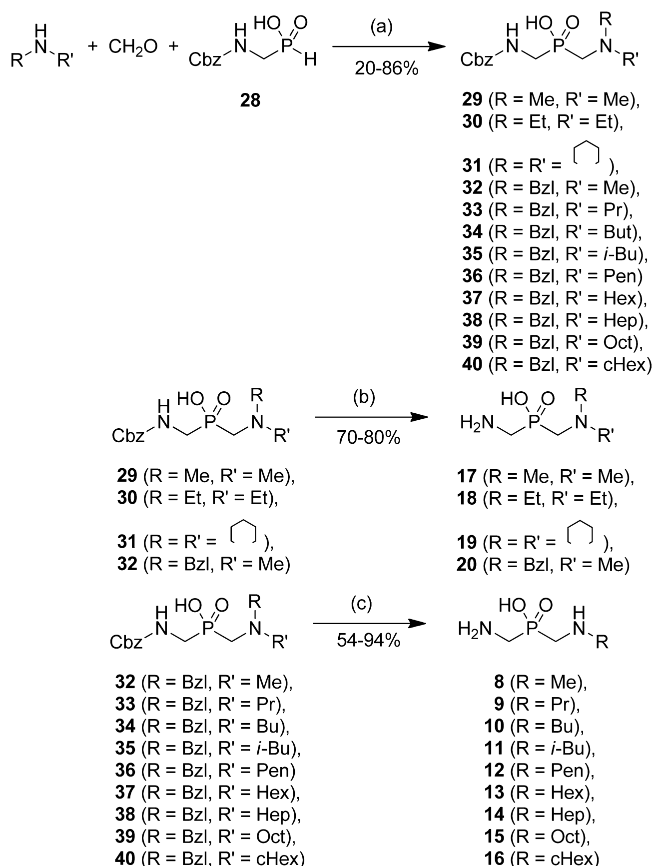
Scheme 3[a]



[a]Reagents and conditions: (a) HCl_{conc}, ethanol, reflux; (b) HBr/AcOH; (c) H_2, Pd/C, MeOH.

obtain monosubstituted bis(aminomethyl)phosphinic acids **8**–**16**, catalytic hydrogenation on a palladium catalyst was performed.

Bis(aminomethyl)phosphinic acid **7** and all of its synthesized substituted analogues (**8**–**23**) were tested for their inhibitory activities against two bacterial ureases, namely, those purified from *Sporosarcina pasteurii* and *Proteus mirabilis* (Table 1). Both native enzymes were prepared from bacterial cultures using a five-step chromatographic protocol (see details in Supporting Information). The majority of the studied compounds showed significant inhibitory potency in a low micromolar range, similarly to the positive control, a well-known urease inhibitor acetohydroxamic acid. Phosphinates **7**–**19** and **21**–**23** exhibited a competitive mode of action. The most suitable pattern for scaffold substitution can be deduced from comparison of the compounds containing variously distributed methyl substituents (**8**, **17**, **21**, and **23**). In the case of the *S. pasteurii* urease, the compound with a single methyl substituent (**8**) was the most active, while symmetrically and unsymmetrically substituted dimethyl analogues **17** and **21** exhibited slightly lower potency. The activities of **8**, **17**, and **21** against the *P. mirabilis* enzyme were similar. Tetramethylated compound **23** was found to have no activity (*S. pasteurii*) or be very weak in potency (*P. mirabilis*). Thus, our main efforts were directed toward the analysis of monosubstituted analogues. A series of compounds with linear substituents showed high activity up to the *n*-hexyl derivative **13**, which exhibited the highest activity among all of the phosphinic

Table 1. Inhibitory Activities (K_i Values) of Bis(aminomethyl)phosphinic Acid and Its Derivatives against Ureases from *S. pasteurii* and *P. mirabilis*[a]

no	compound	K_i [μM] *Sporosarcina pasteurii*	K_i [μM] *Proteus mirabilis*
7		80.0 ± 7.6	82 ± 12
8		1.59 ± 0.32	34 ± 8
9		11.2 ± 1.9	16.0 ± 1.1
10		9.6 ± 1.9	25.9 ± 3.0
11		270 ±37	416 ± 50
12		13.3±0.9	19.0 ± 1.1
13		0.108 ± 0.016	0.202 ± 0.057
14		543 ±75	778 ± 127
15		NI	NI
16		NI	NI
17		4.66 ± 0.71	24.4 ± 4.1
18		NI	NI
19		NI	NI
20		14.2 ± 2.2	58.3 ± 8.0
21		4.3 ± 0.9	17.2 ± 2.5
22		30.1 ± 6.6	24.5 ± 4.5
23		NI	243 ± 62
AHA		3.3 ± 0.4	5.7 ± 0.4

[a]NI, no inhibition.

compounds (K_i = 108 nM against the *S. pasteurii* enzyme). Further extension of the length of the substituent chain led to a dramatic drop in activity (**14** and **15**). Weak inhibition was also observed for compounds with branched or cyclic substituents (**11** and **16**). An extension of the substituent structure showed steric preferences for the active site. The increase in size, from methyl substituents in **21** to benzyl substituents in **22**, did not improve the affinity toward ureases. Interestingly, a small change in the size of the geminal substituents in **17** (e.g., from methyl to ethyl) resulted in a dramatic loss in potency: both phosphinates **18** and **19** displayed no activity. Interestingly, **20** exhibited a different mode of action compared to all other studied inhibitors. Kinetic studies indicated that its mode of binding was uncompetitive.

The mode of binding of the most active inhibitor to *S. pasteurii* urease was studied by molecular modeling (Figure 3). The optimized inhibitor–enzyme complex structure indicates

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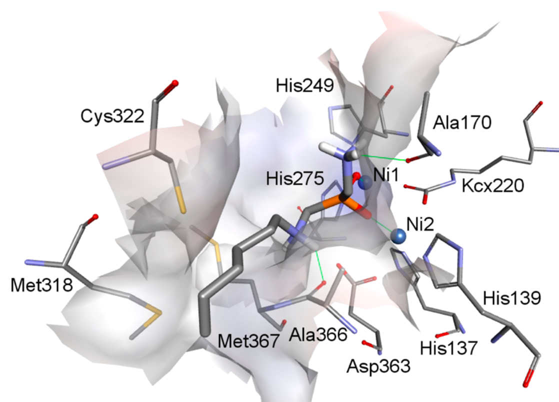

Figure 3. Modeled structure of the inhibitor **13**–urease (*S. pasteurii*) complex. Inhibitor–enzyme hydrogen bonds and inhibitor–nickel ion interactions are marked by solid green lines. The surface is colored according to interpolated charge.

several interactions including binding of the phosphinate group to both nickel ions and hydrogen bonds of the inhibitor amine groups with carbonyls of Ala170 and Ala366. The long aliphatic chain is well docked to neutral cleft formed by Met318, Met367, and Cys322.

The efforts to optimize the structure of phosphinate inhibitors of bacterial ureases led to validation of a new scaffold: bis(aminomethyl)phosphinic acid. The main advantage of this core in comparison to the previously studied phosphinates is the variety of feasible modifications. Moreover, compounds constructed on this framework exhibit high stability and solubility (up to 50 mM) in aqueous solutions, as shown by ^{1}H and ^{31}P NMR studies on compound **13**, which remained unchanged within 7 days at pH 2. In particular, the efficient, elaborated synthesis of such derivatives provides the possibility to assay several structurally different molecules. The availability of inhibitors with various patterns of substitution led us to identify the most potent phosphinate inhibitor of bacterial ureases: aminomethyl(*N*-*n*-hexylaminomethyl)phosphinic acid, with a K_i = 108 nM.

■ ASSOCIATED CONTENT

Supporting Information

Detailed synthetic procedures, NMR spectra, high-resolution ESI mass spectra, and elemental analyses for all target compounds; enzyme purification methods and assays for inhibitory activity and molecular modeling algorithms. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +48 71 320 3344. Fax: +48 71 320 2427. E-mail: lukasz.berlicki@pwr.edu.pl.

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

Bu, butyl; Bzl, benzyl; Cbz, benzyloxycarbonyl; cHex, cyclohexyl; Et, ethyl; Hep, heptyl; Hex, hexyl; Me, methyl; Oct, octyl; Pen, pentyl; Pr, propyl

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