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A three-component Mannich-type condensation leading to phosphinic dipeptides—extended transition state analogue inhibitors of aminopeptidases

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ABSTRACT

N-Protected α-aminoalkylphosphinic acids bearing a P–H function were found to be novel practical building blocks in three-component condensations with formaldehyde and secondary amines (amino acids). Such Mannich-type *N*-phosphonomethylation is a common approach for phosphorus acid derived substrates and leads to multifunctional (phosphonic/amino/carboxylic) compounds of diverse relevance. The utility of this reaction was examined for construction, in a single synthetic step, of advanced phosphinic pseudodipeptides designed to act as extended transition state analogue inhibitors of selected aminopeptidases. Phosphinomethylation of primary amino acids was less efficient and yielded mixtures of products which were separated into individual components, and their structures identified.

Phosphinic dipeptide analogues have been reported to be potent competitive inhibitors of the M17 cytosolic leucine aminopeptidase (E.C.3.4.11.1, LAP) and the M1 microsomal alanyl aminopeptidase (E.C.3.4.11.2, APN/CD13), the most recognized representatives of metallo-containing exopeptidases of biomedical importance.[1] These phosphorus compounds are believed to act as high energy transition state (TS) analogues in amide hydrolysis.[2] Accordingly, the pseudodipeptides **1** containing hydrophobic P1 and P1′ residues, which were linked via a –P(O)(OH)–CH_2– moiety mimicking the peptide bond, exhibited inhibition constants for LAP and APN in the nanomolar range (Scheme 1).[3] The same ligands **1** were also found to be very effective towards *Plasmodium falciparum* M1 and M17 recombinant enzymes.[4] Interestingly, phosphonamidate dipeptides **2**, containing a direct nitrogen-to-phosphorus bond [–P(O)(OH)–NH–], were predicted to be more favourable TS analogues. As calculated theoretically, they should be an order of magnitude more potent inhibitors of LAP than phosphinates owing to the energy gain from a very specific hydrogen bond NH···O=C that followed interaction of the substrate with the enzyme Leu360 upon cleavage.[3] Unfortunately, the P–N moiety adjacent to a free amino group was discovered to be extremely labile in water (pH <11) which excluded application of phosphonamidate compounds such as **2** in kinetic assays.[5]

In the present work, we report the synthesis and validation of extended TS analogues [–P(O)(OH)–CH_2–NH–, general structure **3**, Scheme 1] as inhibitors of LAP and APN aminopeptidases. The novel pseudodipeptidic backbone should combine the advantages of hydrolytically stable phosphinates with the enhanced active site interactions characteristic for phosphonamidates. Optimal hydrophobic residues were selected as side-chain substituents of the modified compounds: 2-phenylethyl for the P1 position and benzyl, *p*-hydroxybenzyl or isobutyl for P1′. A simple and versatile synthetic strategy, the three-component Mannich-type condensation of α-aminoalkylphosphinate **4**, formaldehyde and an amino acid, was examined as a method to achieve the target compounds (Scheme 1).

The traditional synthesis of phosphinic dipeptide analogues involves a multistep process. Two building blocks bearing an appropriate P1 (frequently an N-protected α-aminoalkyl-*H*-phosphinic acid[6]) and P1′ fragment (e.g., an acrylate[7]) are synthesized individually. In the final step, the nucleophilic phosphinate, upon activation (most commonly via silylation), adds to the double bond in a Michael reaction.[8] As this methodology does not allow simple variation of the substituents, many efforts have been dedicated to the development of alternative synthetic approaches.[9] To achieve the structures designed here, we intended to investigate a convenient, Mannich-type, three-component condensation of a P–H compound, formaldehyde and an amine. Presently, there is only one paper describing a corresponding structure, which was prepared via alkylation of an amino ester with a chloromethylphosphinic acid.[10] The Mannich reaction is frequently applied for partial and exhaustive phosphonomethylation[11] of primary and secondary amines/amino acids (glyphosate herbicide, antiscale agents, water softeners, etc.), under acidic or basic catalysis.[12] Typically, phosphorus

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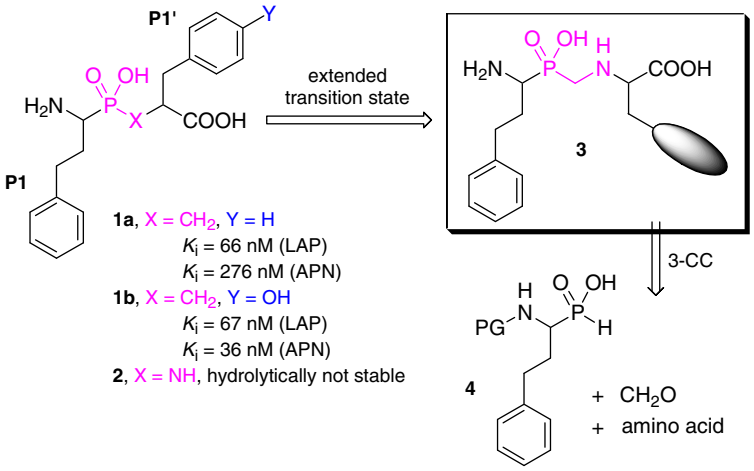


Scheme 1. The structures of the phosphinic (**1**) and phosphonamidate (**2**) lead compounds, and the target phosphinic dipeptides with a modified backbone (**3**). The planned synthetic strategy to achieve the designed compounds via a three-component condensation starting from phosphinic acid **4**.

acid or its esters (dialkyl phosphites), but also hypophosphorus acid, P–H phosphines and phosphinoxides, are used. Herein, according to the best of our knowledge, N-protected α-aminoalkyl-*H*-phosphinic acids were subjected to these conditions for the first time. Unprotected, enantiomerically pure amino acids were selected as the amino components. This makes the P1′ building blocks easily accessible and stereochemically defined. It also increases the availability and structural diversity of the target compounds, which in turn allows extensive optimization of the P1′–S1′ ligand–protein interactions.

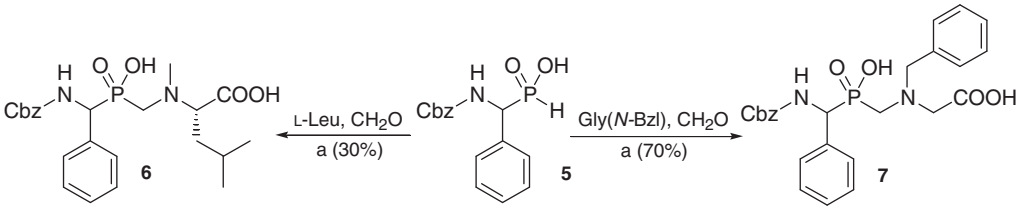
The analogue of phenylglycine **5**,[13] easily obtained on multigram scale, was selected as a model phosphinate for preliminary optimization of the reaction conditions. The substrate was reacted in water with excess formaldehyde and an amino acid (L-leucine or *N*-benzylglycine) possessing a primary or secondary amino group, respectively (Scheme 2). It was necessary to use an organic solvent to achieve satisfactory solubility of the phosphorus component. Thus, acetic acid was added for this purpose. However, its acidity was not sufficient to ensure effective catalysis and addition of a few drops of conc. HCl was also required. Basic catalysis was excluded to avoid racemization of the amino acid. Additionally, esterification of the phosphorus component would be required to maintain its reactivity under alkaline conditions.

Initially, the reaction was performed with a 10-fold molar excess of formaldehyde and was heated under reflux for 5–6 h. For the amino acid containing a primary amino moiety, unexpected N-methylated pseudodipeptide **6** (Scheme 2) crystallized preferentially from the complex mixture of organophosphorus products (see below for discussion), and was isolated in 30% yield. In contrast, phosphinomethylation of secondary *N*-benzylglycine was not problematic and led to the desired product. The above-mentioned conditions afforded the pure target product **7** that crystallized after cooling, without any work-up, in a 70% yield. Interestingly, compound **7** existed as two isomers (trans and cis forms of the carbamate[14]) as was evident in the ^{31}P NMR spectrum (1:0.4 ratio). This is somewhat surprising since usually the cis isomer content is negligible. It seems that for this compound, the presence of the *N*-benzyl group stabilizes the cis carbamate arrangement. Indeed, after removal of the Cbz group, both forms coalesced into a single ^{31}P NMR signal.

To optimize the conditions for the condensation of **5** with L-leucine (to avoid N-methylation), the reaction time was fixed for 1 h at reflux. The condensation proceeded slowly at lower temperature, whereas prolongation of refluxing increased the complexity of the product mixture. Similar complications were observed when a high excess of formaldehyde was used, and only two equivalents of CH_2O proved to be the optimum amount. Typically, a water solution of formaldehyde was added in two portions to a refluxing water/AcOH/HCl_{concd} mixture (1:1:0.05, v/v) containing both amino acid components.[15] The separated product still consisted of several compounds. After removal of the benzyloxycarbonyl protection, these components were purified by HPLC, characterized and identified. Their structures (**8–14**) are presented in Scheme 3.

The phosphonic analogue of phenylglycine **8** predominated in the HPLC amino acid fraction. This oxidized form of the phosphorus substrate represented 25–30% of the total content. As various N-methylated products (e.g., **10**, **12** and **14**, Scheme 3) were also present, the reduction of formimines (or formiminium cations) to the corresponding *N*-methyl derivatives must have accompanied the oxidation reaction (Scheme 4). Such an observation was reported earlier in the literature.[16]

The N-methylation involved the amino group of both substrates and was combined with other side-reactions. Cleavage of the Cbz protection under acidic conditions was then evident as the first reaction. Condensation of only two components, namely (deprotected) phosphinic phenylglycine with formaldehyde was also apparent. These reactions led to P-hydroxymethylated



Scheme 2. The products of condensation of 1-*N*-benzyloxycarbonylamino(phenyl)methane-*H*-phosphinic acid (**5**) with L-leucine or *N*-benzylglycine and formaldehyde. Reagents and conditions: (a) amino acid (2 equiv), CH_2O (36–38% aqueous solution, 10 equiv), H_2O/AcOH/HCl_{concd} (1:1:0.05, v/v), reflux, 5 h.