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Convenient Synthesis and Diversification of Dehydroalaninyl Phosphinic Peptide Analogues

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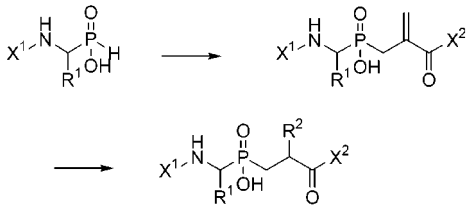
ABSTRACT



Dehydroalaninyl phosphinic dipeptide analogues were synthesized, via an efficient tandem Arbuzov addition/allylic rearrangement, in high yields. The susceptibility of the conjugate system to 1,4 nucleophilic additions was investigated. C-Elongation of the dipeptides was performed, and the efficiency of 1,4 addition to the resulting acrylamidic moiety was evaluated. Derivatization of such phosphinic templates is a powerful approach for rapid access to large number of phosphinic pseudopeptides bearing various side chains in the P_1' position.

Several studies have demonstrated that the synthesis of phosphinic peptides is a very effective approach for the development of highly potent and selective inhibitors of various Zn metalloproteases.[1,2] In many zinc metalloproteases, one of the key side chain for the control of the inhibitor selectivity is the so-called P_1' position,[3] (Figure 1), which in the case of phosphinic peptide inhibitors corresponds to the side chain situated at the right of the phosphinic moiety (standard representation of peptides).

The classical approach to prepare phosphinic peptides relies on the synthesis of phosphinic pseudodipeptidic units, which are obtained by an Arbuzov addition[4] of N-protected silyl aminoalkylphosphonites to suitably substituted acrylates.[5]

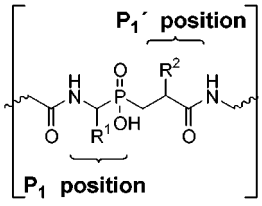


Figure 1.

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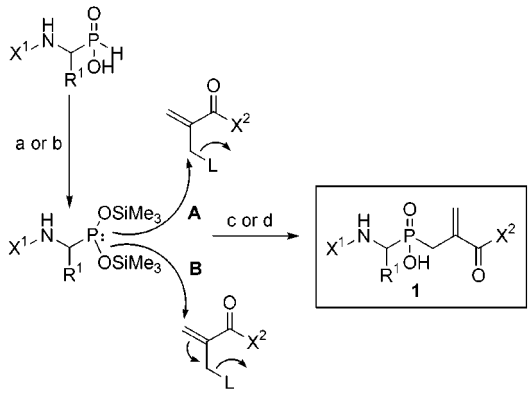
Such an approach, which implies several steps and depends on the availability of acrylates, is not convenient to prepare rapidly large series of P_1' substituted phosphinic peptides. Herein, we report a strategy allowing diversification of the P_1' position of phosphinic peptides via the functionalization, at the final step of the synthesis, of phosphinic precursors.

Esters of 2-(acetoxymethyl) and 2-(bromomethyl) acrylic acid have been widely used as substrates for nucleophilic additions, mainly of organometallic species and in some cases of other nucleophiles such as phenoxides, thiolates, and hydrides.[6,15] In all cases, the reaction leads efficiently to β-substituted acrylates. We speculated that a similar rearrangement would occur during an Arbuzov reaction of silyl aminoalkylphosphonites with this kind of acrylates. As it is shown in Scheme 1, aminoalkylphosphonous acids were activated in the form of their silyl esters, and their subsequent reaction with 1.3 equiv of any of these acrylates led smoothly to the formation of compound **1**. Actually, dehydroalanine derivative **1** can be formed either by nucleophilic substitution (pathway **A**) or by allylic rearrangement (pathway **B**) since the allylic cation is symmetrical. According to literature references, the reaction should follow pathway **B** when L = OAc and mainly pathway **A** when L = Br. Indeed, the structure of the main products obtained by similar additions of carbanions and thiolates to unsymmetrical allylic cations strongly supports these mechanistic pathways.[7]

Scheme 1



[a] TMSCl (4.5 equiv), DIPEA (4.5 equiv) in CH_2Cl_2, 0 °C to rt, 3 h. [b]HMDS (3 equiv), 110 °C, 1 h. [c](for TMSCl activation) $H_2C{=}C(CH_2L)COX^3$ L = Br or OAc (1.3 equiv), 0 °C to rt, 24 h, then EtOH. [d](for HMDS activation) $H_2C{=}C(CH_2Br)COX^3$ (1.3 equiv), 110 °C, 3 h, then EtOH. All steps are performed under an inert atmosphere.

As illustrated in Table 1, compounds type **1**, harboring different protecting groups, can be easily obtained using the experimental procedure described in Scheme 1. The reaction is mild and versatile, proceeds with excellent yields, and involves no byproducts. Moreover, there is no need for the prior protection of the hydroxyphosphinyl function. In addition, the reactants are easily accessible since β-aminoalkyl phosphonous acids are prepared by the method described by Baylis et al.[9] and 2-(bromomethyl) acrylic acid is commercially available. As compared to the method of Schoen et al., which consists of three synthetic steps and prior protection of the hydroxyphosphinyl function, the present procedure is more convenient.[10]

Table 1. List of Compounds of Type **1**

entry	X^1	R^1	X^2	L	yields (%)
1a[8]	Cbz	$PhCH_2$	OEt	Br	92
1a	Cbz	$PhCH_2$	OEt	OAc	89
1b	Cbz	$PhCH_2$	OBu^t	Br	94
1c	Fmoc	$PhCH_2$	OBu^t	Br	85

Phosphinic peptides type **1** undergo a variety of transformations that increase their value as synthetic intermediates.

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(8) **Experimental Procedure for the Synthesis of Compound 1a.** To an ice-cold suspension of (*R,S*)-((1-*N*-(benzyloxycarbonyl)amino)-2-phenylethyl)phosphinic acid (1 mmol) in CH_2Cl_2 (7 mL) were added *N,N*-diisopropylethylamine (4.5 mmol, 0.58 g, 0.78 mL) and chlorotrimethylsilane (4.5 mmol, 0.49 g, 0.57 mL) under an argon atmosphere. This solution was stirred for 3 h at room temperature. Then, the mixture was cooled to 0 °C, and ethyl 2-(bromomethyl) acrylate (1.3 mmol) was added dropwise. The solution was stirred for 24 h at room temperature. Then, absolute ethanol (0.8 mL) was added dropwise, and the mixture was stirred for 20 min. The solvents were evaporated. The residue was dissolved in 5% $NaHCO_3$ (10 mL), and the resulting suspension was extracted with diethyl ether (2 × 3 mL). The crude product was precipitated by acidification with 1 N HCl to pH 1. Purification by column chromatography using chloroform/methanol/acetic acid, (7:0.4:0.4) as eluent afforded the product as a white solid, mp 90−93 °C. **NMR and typical analysis data for compound 1a**: ^{1}H NMR (200 MHz, $CDCl_3/d_1$-TFA 99.5/0.5) δ 1.27 (t, $^3J_{HH}$ = 7.3 Hz, 3H, CH_2CH_3), 2.78−3.20 (m, 3H, PCH_2, PhC*H*H), 3.20−3.35 (m, 1H, PhCH*H*), 4.15−4.46 (m, 3H, CH_2CH_3, PC*H*), 4.82−5.07 (br s, 2H, OCH_2Ph), 5.70−5.92 (m, 2H, N*H*, C=CH*H*), 6.35 (s, 1H, C=C*H*H), 7.05−7.34 (m, 10H, aryl); ^{13}C NMR (50 MHz, $CDCl_3/d_1$-TFA 99.5/0.5) δ 13.9 (CH_2CH_3), 29.7 (d, $^1J_{PC}$ = 86.9 Hz, PCH_2), 33.7 (CH_2Ph), 50.5 (d, $^1J_{PC}$ = 104.8 Hz, PCH), 61.4 (CH_2CH_3), 66.7 (OCH_2Ph), 126.5, 126.9, 127.6, 127.8, 128.2, 128.3, 129.1, 129.5, 130.6, 130.7, 136.3, 136.6, 136.8 (aryl, vinyl), 156.1 (s, OCONH), 166.5 (s, COOEt); ^{31}P NMR (81 MHz, $CDCl_3/d_1$-TFA 99.5/0.5) δ 48.66; ESMS *m/z* calcd for $C_{22}H_{25}NO_6P$ $(M - H)^-$ 430.4, found 430.2. Anal. Calcd for $C_{22}H_{26}NO_6P$ (431.4): C, 61.25; H, 6.07; N, 3.25. Found: C, 61.59; H, 5.89; N, 3.30.

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(14) To reduce the number of diastereoisomers, the pure diastereoisomeric form (*R,S*) of compound **3c** was used for the reactions described in Scheme 4. (*R,S*)-**3c** was prepared by the procedures described in this letter starting from optically pure (*R*)-$ZPhePO_2H_2$. The choice of the (*R*) configuration was based exclusively on data concerning enzymatic preferences.

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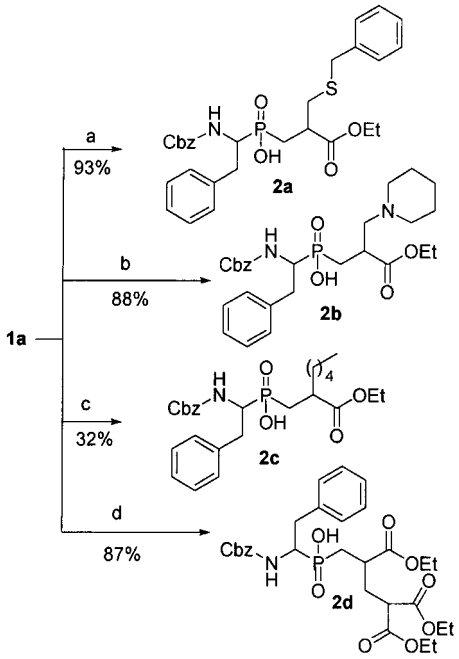
Sulfur and nitrogen nucleophiles, as well as carbanions, can be added to the acrylic unit of compounds type **1**, as shown in Scheme 2.

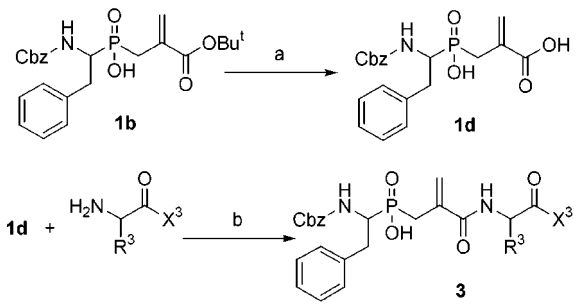
Scheme 2



[a] BnSH, Et_3N in CH_2Cl_2, 8 h, rt. [b]Piperidine in CH_2Cl_2, 48 h, rt. [c]*n*-BuLi, CuJ, TMSCl in Et_2O/THF, 4 h, −20 °C. [d]Sodium diethylmalonate, in EtOH, 12 h, rt.

To explore the utility of our strategy, we checked the possibility to functionalize directly phosphinic peptides type **3**. Scheme 3 outlines the general method used for the synthesis of compounds type **3**.

Scheme 3



[a] TFA/CH_2Cl_2 50%, 1 h, rt. [b]EDC·HCl (4 equiv), HOBt (1 equiv), DIPEA (1.9 equiv) in CH_2Cl_2/THF, 1 h, rt.

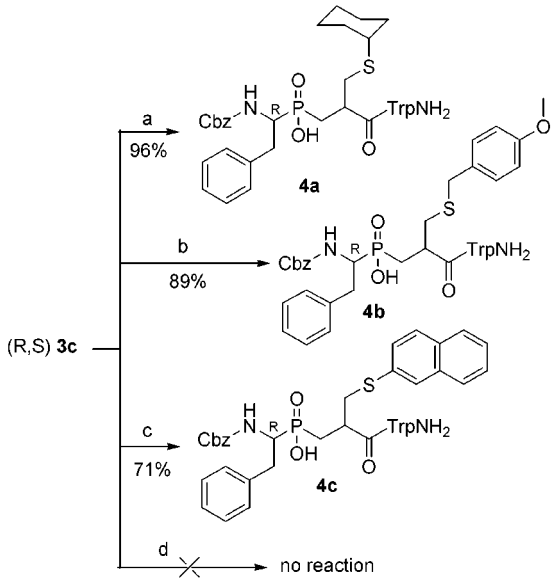
Phosphinic dipeptide **1b** was chosen as starting material. This compound was deprotected under acidic conditions to afford quantitatively pseudodipeptide **1d**. Coupling of such units to aminopeptidyl moieties, without protection of the hydroxyphosphinyl group, may imply low yields and/or byproduct formation.[11] These drawbacks are overcome by short reaction times and the use of a large excess of the carbodiimide coupling reagent in slightly acidic solutions.[12] Under these conditions, phosphinic tripeptides of type **3** were produced in satisfactory yields, as illustrated in Table 2.

Table 2. List of Compounds of Type **3**

entry	R^3	X^3	yields (%)
3a	$PhCH_2$	OBu^t	72
3b	$PhCH_2$	OMe	65
3c	3-indolylmethyl	NH_2	69

Pseudotripeptides **3** can also serve as Michael-type substrates undergoing nucleophilic additions. Alkyl, benzyl, and aryl thiols react readily with the phoshinopeptidic precursor **3c**, leading to cysteine analogues in high yields. However, secondary amines such as piperidine do not add to **3c**. This can be attributed to the reduced reactivity of the acrylamidic system as a result of inductive and mesomeric effects, which cause an increase on the β carbon electron density.[13] Thereby, nucleophilic additions occur only when the nucleophile is strong enough to attack the relatively inactivated conjugate amide system. Reaction conditions are outlined in Scheme 4.[14]

Scheme 4



[a] Cyclohexyl mercaptan (4 equiv), EtONa/EtOH (2 equiv) in THF, 1 h, rt. [b]4-Methoxy-α-toluenethiol (4 equiv), EtONa/EtOH (2 equiv) in THF, 8 h, rt. [c]2-Naphthalenethiol (4 equiv), EtONa/EtOH (2 equiv) in THF, 40 h, rt. [d]Piperidine in THF, CH_2Cl_2, MeOH, heat, various bases.

Two of the compounds described in this communication (**4b** and **4c**, Scheme 4) were tested toward different MMPs, and biological results are shown in Table 3.

Table 3. K_i Values of Compounds **4b** and **4c** toward MMP-8 (HNC), MMP-2 (Gel-A), and MMP-9 (Gel-B)

entry	MMP-8	MMP-2	MMP-9
4b	0.7 nM	6 nM	3 nM
4c	3 nM	100 nM	500 nM

As already reported, compound **4b** is a potent but mixed inhibitor of MMP-8 (human neutrophil collagenase, HNC), MMP-2 (gelatinase A, Gel-A), and MMP-9 (gelatinase B, Gel-B).[15] Compound **4c** represents an example of the key role played by the P_1' side chain in governing the selectivity of the inhibitors toward MMPs. Given the wide and easy diversification of the P_1' position of the phosphinopeptidic precursors reported in this letter, this new chemical strategy may lead to the development of phosphinic inhibitors with specificity for small sets of MMPs. More generally, given the key function of the P_1' position in inhibitors of many zinc metalloproteases, this novel approach to prepare diversified phosphinic peptides will find a broad application.

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Supporting Information Available: Experimental procedures and spectroscopic data for compounds **1a**, **1b**, **1d**, **2a**, **3a**, **3c**, and **4a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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