Practical Synthesis of Phosphinic Dipeptides by Tandem Esterification of Aminophosphinic and Acrylic Acids under Silylating Conditions

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Abstract: In this report, a synthetic protocol for the preparation of phosphinic dipeptides of type 5 is presented. These compounds serve as valuable building blocks for the development of highly potent phosphinopeptidic inhibitors of medicinally relevant Zn-metalloproteases and aspartyl proteases. The proposed method is based on the tandem esterification of α-aminophosphinic and acrylic acids under silylating conditions in order to subsequently participate in a P-Michael reaction. The scope of the transformation was investigated by using a diverse set of readily available acrylic acids and (R)-α-aminophosphinic acids, and high yields were achieved in all cases. In most examples reported herein, the isolation of biologically relevant (R,S)-diastereoisomers became possible by simple crystallization from the crude products, thus enhancing the operational simplicity of the proposed method. Finally, functional groups corresponding to acidic or basic natural amino acids are also compatible with the reaction conditions. Based on the above, we expect that the practicality of the proposed protocol will facilitate the discovery of pharmacologically useful bioactive phosphinic peptides.

Keywords: phosphinic; pseudopeptides; P-Michael; silylation; hexamethyldisilazane; inhibitors; Zn-metalloproteases; acrylic acids; aminophosphinic; organophosphorus

1. Introduction

Among the plethora of bioactive scaffolds with numerous applications in drug discovery, phosphinic peptides undoubtedly hold a prominent position [1–9]. By acting as transition state analogues of natural peptides during proteolysis, phosphinic peptides have provided a large number of potent inhibitors of zinc and aspartyl proteases over previous decades [2,5,6]. In addition, their propensity to bind tightly to their target has fueled efforts towards the development of enzymatic probes for imaging applications [10–14]. Their synthesis is typically based on building block strategies where side chains of the phosphinopeptidic scaffold are either preinstalled or introduced at a later stage of the synthetic plan by post-modification protocols [3,4]. Therefore, it becomes evident that synthetic methodologies aiming to the fast and reliable production of phosphinic dipeptide building blocks lie at the heart of every medicinal project involving this privileged class of bioactive compounds.

There are two possible synthetic approaches towards a phosphinodipeptidic building block: the “NP + C approach”, which relies on P-C bond-forming reactions between aminophosphinic derivatives and carbon electrophiles, and the less-commonly
used “N + PC approach” which involves Mannich-type or amidoalkylation reactions with 3-phosphinoyl propionates of type I (Scheme 1) [3,15,16]. The preference of synthetic chemists for the NP+C approach mainly stems from the fact that optically pure α-aminophosphinic acids can be accessed by well-established methods [17–19], thus allowing stereochemical efficiency and scalability. In particular, diacids of type 5 (Scheme 2) have been successfully employed as starting materials in the discovery of potent and selective inhibitors of a wide range of proteases, such as ER aminopeptidases [20], neutral aminopeptidase (APN) [21,22], matrix metalloproteinases (MMPs) [23], neprilysin [22], angiotensin-converting enzymes (ACE) 1 and 2 [24,25], endothelin-converting enzyme (ECE) [24] and many more [22,26–28]. In most cases, such compounds are produced by the initial conversion of α-aminophosphinic acids of type 1 to the respective bis(trimethylsilyl)phosphonites 1′, followed by the addition of α-substituted acrylates (2) to the resulting P-nucleophiles (Scheme 2) [3,29,30]. Since the reaction affords carboxylic esters (4), an additional cleavage step is required to furnish the final compounds of type 5 [20,23,31]. Interestingly, a more direct, protecting-group-free approach which involves the use of acrylic acids of type 3 as electrophiles (Scheme 2) has to our knowledge never been reported in the literature, probably because acrylic acids are intuitively excluded when different electrophile alternatives are considered.

Since P-Michael additions using phosphinic acids are mainly performed under silylating conditions, we assumed that acrylic acids could also be silylated towards the corresponding esters and react with the P-nucleophiles generated in situ (Scheme 2). By extending our literature search to different types of phosphinic acids, only scarce examples of relevant reactions were identified, either leading to moderate yields [32] or employing highly reactive electrophiles [33]. Given this striking gap in the literature, we decided to investigate the feasibility of such a transformation, aiming not only to a shorter synthetic route for the preparation of phosphinic building blocks of type 5, but also to increased simplicity and scalability. Moreover, there are several examples in the literature where the biologically relevant, less soluble (R,S)-diastereoisomer of Cbz-protected diacids 5 can be easily separated from the (R,R)-isomer by crystallization [20,23,25,31,34], which implies that the proposed protocol may have the potential to lead directly to optically pure (R,S)-phosphinic dipeptides without the need of chromatographic separation. To this respect, in the present study we explore the synthesis of phosphinopeptidic building blocks of type 5 by tandem activation of α-aminophosphinic acids (I) and temporary esterification of acrylic acids (3) in a P-Michael reaction under silylating conditions, aiming to a protocol that will be characterized by high overall practicality and synthetic convenience.
Scheme 2. Schematic representation of previously described protocol towards the synthesis of building blocks of type 5 and proposed protocol in this study.

2. Results and Discussion
2.1. Preparation of Acrylic Acids of Type 3

Minimization of chromatographic purification steps in a synthetic plan is a crucial parameter that needs to be taken into account for assessing the overall cost efficiency of a preparation, especially when upscaling is required. In this regard, as it was mentioned in the introduction part, simple α-aminophosphinic acids of type 1 can be accessed in multigram quantities and optically pure form by following the classical method of Baylis et al. [19]. On the other hand, α-substituted acrylic acids of type 3 can be accessed by a 3-step procedure starting from diethyl malonate and simple alkyl halides, as described in Scheme 3.

Scheme 3. Synthesis of acrylic acids 3a and 3c-k used in this study (see Supplementary Material).  
\(^{a}\) Isolated yields are given.  \(^{b}\) NaH was used for the alkylation step [35].  \(^{c}\) Prepared according to ref. [20].
In the first step, the alkylation of diethyl malonate leads to malonic derivatives of type 6, generally contaminated with small amounts of dialkylated by-products (6′) and unreacted materials. The removal of dialkylated derivatives 6′ becomes possible at the next step by taking advantage of their low reactivity towards hydrolysis due to increased steric hindrance. This allows the facile isolation of the target malonic acids of type 7 by simple aqueous workup. At the final step of the procedure, a Doebner–Knoevenagel condensation with formaldehyde takes place, leading to adequately pure target acrylic acids of type 3 without the need for chromatographic purification and overall yields ranging from 24 to 84% for 3 steps. The structure of acrylic acids of type 3 prepared by the above method is shown in Scheme 3.

2.2. Optimization

Next, we proceeded to the evaluation of our proposed method by employing the Cbz-protected aminophosphinic acid 1a and acrylic acid 3a as starting materials in our initial experiments (Table 1).

Table 1. Optimization of reaction conditions.

| Entry | Acrylic Acid (Equiv) | Reaction Conditions | Conversion of 1a, % (yield to 5a, %) |
|-------|----------------------|---------------------|-------------------------------------|
| 1     | 3a (1.3)             | BSA (4 equiv), CH₂Cl₂, rt, 48 h | 53 (41)                             |
| 2     | 3a’ (1.3)            | BSA (4 equiv), CH₂Cl₂, rt, 48 h  | 90 (89) 3                           |
| 3     | 3a (1.3)             | TMSCl (7 equiv), Pr₂EtN (7 equiv), CH₂Cl₂, rt, 48 h | 86 (81) |
| 4     | 3a (1.2)             | HMDS (6 equiv), 100 °C, 2 h     | 100 (99)                            |
| 5     | 3a (1.2)             | HMDS (6 equiv), 70 °C, 2 h      | 72 (70)                             |

1 All reactions were performed in a 0.3 mmol scale of 1a under inert conditions. 2 Conversions and yields were determined by integration of the 31P-NMR spectra of crude products. 3 Conversion to 5a’.

Based on previous reports [32,33], we first tested the efficiency of BSA [N,O-bis(trimethylsilyl)acetamide] in promoting the P-Michael reaction between 1a and 3a. The reaction of a mixture of 1a (1.0 equiv), 3a (1.2 equiv) and BSA (4 equiv) for 48 h led to just a 53% conversion of the starting material and 41% formation of target compound 5a, as it was judged by the 31P-NMR spectrum of the crude mixture. This was in accordance with the low efficiency observed in the literature for a similar reaction [32]. When unsubstituted acrylic acid (3a’) was used, the conversion to product 5a’ increased to 90% under the same conditions, which implies that sterics due to the presence of a substituent in 2-position of acrylic acids are partially responsible for the low reactivity of studied electrophiles. TMSCl as silylating agent in the presence of Hüning’s base proved more efficient for the formation of product 5a (82%), based on 31P-NMR, leaving only 13% of unreacted 1a. Despite the improved reaction profile, long reaction times associated with these conditions prompted us to explore hexamethyldisilazane (HMDS) as an alternative, which is known to cause silylation of acidic functionalities upon heating [29]. Indeed, heating of a mixture of 1a, 3a and 6 equiv of HMDS to 100 °C during 2 h under inert conditions led to quantitative formation of the desired product 5a. Lowering the temperature to 70 °C had a negative effect to the conversion of phosphinic acid 1a, presumably due to incomplete activation of the reactants.
2.3. Substrate Scope

After identifying the optimal conditions for the P-Michael addition of α-aminophosphinic acids to acrylic acids through their tandem esterification by silylation, we proceeded to the application of our protocol in a wide range of substrates, as shown in Scheme 4.

Scheme 4. Substrate scope for the proposed P-Michael reaction of aminophosphinic (1) and acrylic (3) acids by tandem esterification under silylating conditions. Isolated yields are provided in all examples. "outside parentheses: isolated yields to 5 and d.r. values after separation of (R,S)-isomer by crystallization; inside parentheses: conversions to 5 and d.r. values from crude products, as estimated by integration of their $^{31}$P-NMR spectra; isolated yield after column purification; d.r. was estimated by integration of their $^{31}$P-NMR spectrum in CD$_3$OD; recrystallized by CHCl$_3$; isolated yield after treatment with Et$_2$O; d.r. was estimated by integration of the $^{31}$C-NMR spectrum of isolated product 5q.
Since (R)-α-aminophosphinic acids were used, a mixture of two diastereoisomers [(R,S) and (R,R)] was obtained in all cases. However, as mentioned above, the (R,S)-diastereoisomer of Cbz-protected diacids of type 5 has a higher tendency to crystallize compared to (R,R) isomers. This was observed in 12 out of 16 examples of Cbz-protected diacids prepared in this study, allowing the isolation of target compounds of type 5 with high diastereoisomeric purity, as determined by 31P-NMR, without the need of chromatographic purification. Considering that starting materials were also obtained without column chromatography, it becomes evident that the proposed protocol is characterized by high practicality and scalability. Boc-protected α-aminophosphinic acids are also compatible with this protocol leading to compounds 5o and 5r in high yields. However, in these cases the presence of Boc group hampered diastereoisomeric separation. Furthermore, acrylic acids with functional groups corresponding to the side chains of acidic (5l) or basic (5k) aminoacids were also compatible with the proposed method.

3. Materials and Methods

3.1. General Information

All P-Michael reactions were carried out under argon atmosphere. All solvents were used without further purification. Reagents were purchased at the highest commercial quality from Aldrich (St. Louis, MI, USA), Acros (Belgium), Fluka and Fluorochem and were used without further purification. Reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm silica gel plates (E. Merck silica gel 60F254) and components were visualized by the following methods: UV light absorbance, and/or charring after spraying with a solution of NH4HSO4 or an aqueous solution of cerium molybdate/H2SO4 ("Blue Stain"), or an aqueous solution of KMnO4 and heating. Purification of compounds by column chromatography was carried out on silica gel (Merck (Darmstadt, Germany), 70–230 mesh) and the indicated solvents. Melting points (measured on an Electrothermal apparatus) are uncorrected. 1H, 13C and 31P NMR spectra were recorded on a Varian 200 MHz Mercury spectrometer and on a Bruker Avance Neo 400 MHz at 25 °C. 1H and 13C spectra are referenced according to the residual peak of the solvent based on literature data [36]. 31P NMR chemical shifts are reported in ppm downfield from 85% H3PO4 (external standard). 13C and 31P NMR spectra are fully proton-decoupled. Phosphinic diacids of type 5 tend to aggregate, causing peak broadening in the NMR spectra. In all products of type 5, small signals observed upfield from the main peaks corresponded to rotamers observed in this type of compound, as it has been reported in the literature [37]. Signals marked with an asterisk (*) in the NMR assignment correspond to minor isomers. Optical rotation data were obtained in an Optical Activity instrument. High-resolution mass spectra were obtained on a Bruker Maxis Impact QTOF spectrometer or an AB Sciex 4600 Triple TOF mass spectrometer.

3.2. General Procedure for the HMDS-Mediated P-C Bond-Forming Reaction between Phosphinic and Acrylic Acids

Phosphinic acid of type 1 (1.0 equiv) and acrylic acid of type 3 (1.2 equiv) were added to a round-bottomed flask. Then, HMDS (6.0 equiv) was slowly added and the flask was firmly closed with a septum and thoroughly purged with argon. Then, the mixture was slowly heated to 100 °C, ensuring that gas NH3 was released (by periodically piercing the septum with a needle, taking care to always maintain a positive pressure inside the flask). Production of gas NH3 starts at ~60 °C and was completed at 100 °C. The mixture was heated at 100 °C during 2 h and then cooled at 70 °C. At this temperature, EtOH (0.2 mL/mmol of HMDS) was slowly added under a gentle flow of argon, and stirring was continued for 15 min. The resulting mixture was diluted with HCl 2M (15 mL/mmol of 1) and AcOEt (20 mL/mmol of 1). Isolation of the final product of type 5 is described separately for each example given below.

(2S)-2-Benzyl-3-{[(R)-1-(benzyloxycarbonylamino)-2-phenylethyl](hydroxy)phosphoryl}propanoic acid (5a): Phosphinic acid 1a (100 mg, 0.31 mmol) and acrylic acid 3a (61 mg, 0.37 mmol) were subjected to
the general HMDS-mediated P-C bond-forming reaction protocol. After the addition of HCl 2 M and AcOEt, a solid was precipitated that was collected by filtration. The solid product was recrystallized by AcOEt to afford compound 5a (d.r. >99:1) as a white solid in 48% yield (72 mg, 0.15 mmol). m.p. 190–195 °C; TLC Rf (CHCl3/Methanol/ AcOEt: 7:2:1) = 0.58. 1H NMR (500 MHz, d6-DMSO + 4%TFA) δ 7.68 (d, J = 9.5 Hz, 1H), 7.37–6.94 (m, 15H), 4.95 (d, J = 13 Hz, 1H), 4.93 (d, J = 13 Hz, 1H), 3.14–2.84 (m, 4H), 2.78–2.68 (m, 1H), 2.10–1.92 (m, 1H), 1.84–1.74 (m, 1H); 13C NMR (50 MHz, d6-DMSO) δ 176.5 (d, JPC = 7.6 Hz), 156.1 (d, JPC = 3.8 Hz), 138.9, 138.6, 138.4, 137.3, 129.2, 128.4, 127.7, 127.1, 126.4, 65.3, 52.7 (d, JPC = 104 Hz), 40.35, 38.3, 27.6 (d, JPC = 89 Hz); 31P NMR (81 MHz, d6-DMSO + 4%TFA) δ 46.4; HRMS (m/z): [M + Na]+ calcd. for C18H25NNaO6P+, 504.1547 found, 504.1565.

(2S)-2-Benzyl-3-[[[(R)-1-(benzyloxycarbonylamino)-2-phenylethyl](hydroxy)phosphoryl]propanoic acid (5b): Phosphinic acid 1b (100 mg, 0.41 mmol) and acrylic acid 3a (80 mg, 0.49 mmol) were subjected to the general HMDS-mediated P-C bond-forming reaction protocol. After the addition of HCl 2M and AcOEt, a solid was precipitated that was collected by filtration. The solid product was recrystallized by AcOEt to afford compound 5b (d.r. >97:3) as a white solid in 54% yield (90 mg, 0.22 mmol). m.p. 180–185 °C; TLC Rf (CHCl3/Methanol/ AcOEt: 7:2:1) = 0.33. 1H NMR (200 MHz, d6-DMSO + 4.5%TFA) δ 7.52 (d, J = 9.0 Hz, 1H), 7.39–7.13 (m, 10H), 5.11–4.96 (m, 9H), 3.85–3.72 (m, 1H), 3.00–2.79 (m, 3H), 2.04–1.94 (m, 1H), 1.91–1.61 (m, 1H), 1.20 (dd, J = 14.2, 7.3 Hz, 3H); 13C NMR (50 MHz, d6-DMSO + 4.5%TFA) δ 175.4 (d, JPC = 9.4 Hz), 155.9 (d, JPC = 4.1 Hz), 138.9, 137.1, 129.1, 128.5, 128.3, 127.9, 127.8, 126.4, 65.7, 46.2 (d, JPC = 105 Hz), 40.53, 40.45, 38.5, 27.3 (d, JPC = 88 Hz), 13.9; 31P NMR (81 MHz, d6-DMSO + 4.5%TFA) δ 47.6; HRMS (m/z): [M + H]+ calcd. for C20H25NO6P+, 404.1614 found, 404.1624.

3-[[[(R)-1-(Benzyloxycarbonylamino)-2-phenylethyl](hydroxy)phosphoryl]-2-methylpropanoic acid (5c): Phosphinic acid 1a (250 mg, 0.78 mmol) and methacrylic acid (3b) (81 mg, 0.94 mmol) were subjected to the general HMDS-mediated P-C bond-forming reaction protocol. After the addition of HCl 2M and AcOEt, the two phases were separated and the organic layer was dried with Na2SO4 and evaporated in vacuo. The crude product was purified by silica gel column chromatography using CHCl3/Methanol/ AcOEt 70:0.1:0.01 → 70:4:0.4 as eluent solvent system. Compound 5c (d.r. 2:1) was isolated as a white solid in 84% yield (266 mg, 0.65 mmol). TLC Rf (CHCl3/Methanol/ AcOEt: 7:2:1) = 0.39. 1H NMR (200 MHz, d6-DMSO) δ 7.72 & 7.71 (2 × d, J = 9.5 Hz, 1H), 7.36–7.07 (m, 10H), 4.92 (s, 2H), 3.97–3.73 (m, 1H), 3.18–2.94 (m, 1H), 2.84–2.57 (m, 2H), 2.19–1.93 (m, 1H), 1.73–1.45 (m, 1H), 1.19 & 1.17 (2 × d, J = 7.0 Hz, 3H); 13C NMR (50 MHz, d6-DMSO) δ 176.90, 176.86, 176.7, 156.1, 156.0, 138.8, 138.7, 138.5, 138.44, 137.37, 137.3, 129.1, 128.4, 128.2, 127.6, 127.1, 127.0, 126.2, 65.1, 56.6 (d, JPC = 104 Hz), 52.2* (d, JPC = 105 Hz), 33.4, 33.35, 33.28, 33.2, 32.7, 30.6, 30.4, 28.88, 28.68, 19.0 (d, JPC = 7.4 Hz), 18.7 (d, JPC = 6.0 Hz); 31P NMR (81 MHz, d6-DMSO + 5%TFA) δ 46.5; HRMS (m/z): [M − H]− calcd. for C20H23NO6P−, 404.1268 found, 404.1264.

(2S)-2-[[[(R)-1-(Benzyloxycarbonylamino)-2-phenylethyl](hydroxy)phosphoryl)methyl]-4-methylpropanoic acid (5d): Phosphinic acid 1a (100 mg, 0.31 mmol) and acrylic acid 3c (48 mg, 0.37 mmol) were subjected to the general HMDS-mediated P-C bond-forming reaction protocol. After the addition of HCl 2M and AcOEt, the two phases were separated and the organic layer was dried with Na2SO4 and evaporated in vacuo. The residue was recrystallized twice with AcOEt to afford compound 5d (d.r. 95:5) as a white solid in 43% yield (60 mg, 0.13 mmol). m.p. 153–156 °C; [α]D25 −32 (c = 0.31, DMSO); TLC Rf (CHCl3/Methanol/ AcOEt: 7:2:1) = 0.6. 1H NMR (200 MHz, d6-DMSO) δ 7.66 (d, J = 9.5 Hz, 1H), 7.39–6.88 (m, 10H), 5.02–4.78 (m, 2H), 4.02–3.73 (m, 1H), 3.17–2.97 (m, 1H), 2.82–2.57 (m, 2H), 2.11–1.86 (m, 1H), 1.78–1.26 (m, 4H), 0.86 (app t, J = 6.3 Hz, 6H); 13C NMR (50 MHz, d6-DMSO) δ 176.5 (d, JPC = 7.6 Hz), 156.1 (d, JPC = 3.8 Hz), 138.6, 138.4, 137.3, 129.1, 128.4, 128.2, 127.6, 127.1, 126.3, 65.1, 52.8 (d, JPC = 104 Hz), 42.7 (d, JPC = 9.0 Hz), 36.8 (d, JPC = 4.0 Hz), 32.8, 28.9 (d, JPC = 88 Hz), 25.60, 23.01, 21.87; 31P NMR (81 MHz, d6-DMSO) δ 45.7*, 45.4; HRMS (m/z): [M − H]− calcd. for C23H28NO6P−, 446.1738 found, 446.1739.
(2S)-2-\{[[R]-1-(Benzyloxycarbonylamino)-2-phenylethyl](hydroxy)phosphoryl]methyl\}-4-phenylbutanoic acid (5e): Phosphinic acid 1a (200 mg, 0.63 mmol) and acrylic acid 3d (133 mg, 0.76 mmol) were subjected to the general HMDS-mediated P-C bond-forming reaction protocol. After the addition of HCl 2M and AcOEt, a solid was precipitated that was collected by filtration. The solid product was recrystallized with AcOEt and the hot suspension was filtered. The procedure is repeated to afford compound 5f (d.r. 98:2) as a white solid in 47% yield (147 mg, 0.30 mmol). m.p. 196–199 °C; [α]D25 = −31.2 (c = 0.9, DMSO); TLC Rf (CHCl3/MeOH/AcOH: 7/2/1) = 0.62. 1H NMR (200 MHz, d6-DMSO) δ 7.71 (d, J = 9.7 Hz, 1H), 7.40–6.90 (m, 15H), 4.92–3.74 (m, 1H), 3.98–3.73 (m, 1H), 3.20–2.95 (m, 4H, overlapped by dPC = 4.2 Hz), 34.8 (d, JPC = 8.0 Hz), 32.8, 32.5, 28.1 (d, JPC = 89 Hz); 31P NMR (81 MHz, d6-DMSO + 2% TFA) δ 46.2; HRMS (m/z): [M – H]− calcld. for C24H29NO6P−, 494.1738 found, 494.1744.

(2S)-2-\{[R]-1-(Benzyloxycarbonylamino)-2-phenylethyl\}(hydroxy)phosphoryl]methyl\}-5-phenylpentanoic acid (5f): Phosphinic acid 1a (200 mg, 0.63 mmol) and acrylic acid 3e (143 mg, 0.75 mmol) were subjected to the general HMDS-mediated P-C bond-forming reaction protocol. After the addition of HCl 2M and AcOEt, the two phases were separated and the organic layer was dried with Na2SO4 and evaporated in vacuo. The crude product was purified by gel column chromatography using CHCl3/MeOH/AcOH 7:0.01:0.01 → 7:0.4:0.4 as eluent solvent system. Compound 5g was isolated as an off-white solid in 94% yield (255 mg, 0.59 mmol). Isolation of diastereoisomer (R,S)-5g (d.r. 99:1) was achieved after 3 recrystallizations with CHCl3 in 23% yield (62 mg, 0.14 mmol). m.p. 131–133 °C; [α]D25 = −32.8 (c = 1, MeOH); TLC Rf (CHCl3/MeOH/AcOH: 7/2/1) = 0.44. 1H NMR (200 MHz, d6-DMSO) δ 7.70 (d, J = 9.2 Hz, 1H), 7.39–7.03 (m, 10H), 5.81–5.55 (m, 1H), 4.01–3.84 (m, 1H), 3.17–3.02 (m, 2H), 2.92–2.51 (m, 4H, overlapped by dPC = 3.7 Hz), 141.5, 138.6, 138.4, 137.3, 129.1, 128.4, 128.3, 128.2, 127.6, 127.1, 126.3, 125.9, 52.7 (d, JPC = 104 Hz), 38.4 (d, JPC = 4.2 Hz), 34.8 (d, JPC = 8.0 Hz), 32.8, 32.5, 28.1 (d, JPC = 89 Hz); 31P NMR (81 MHz, d6-DMSO) δ 45.0; HRMS (m/z): [M – H]− calcld. for C24H29NO6P−, 508.1894 found, 508.1889.

(2S)-2-\{[R]-1-(Benzyloxycarbonylamino)-2-phenylethyl\}(hydroxy)phosphoryl]methyl\}-pent-4-enioic acid (5g): Phosphinic acid 1a (200 mg, 0.63 mmol) and acrylic acid 3f (84 mg, 0.75 mmol) were subjected to the general HMDS-mediated P-C bond-forming reaction protocol. The addition of HCl 2M and AcOEt, the two phases were separated and the organic layer was dried with Na2SO4 and evaporated in vacuo. The crude product was purified by gel column chromatography using CHCl3/MeOH/AcOH 7:0.01:0.01 → 7:0.4:0.4 as eluent solvent system. Compound 5g was isolated as an off-white solid in 94% yield (255 mg, 0.59 mmol). Isolation of diastereoisomer (R,S)-5g (d.r. 99:1) was achieved after 3 recrystallizations with CHCl3 in 23% yield (62 mg, 0.14 mmol). m.p. 131–133 °C; [α]D25 = −32.8 (c = 1, MeOH); TLC Rf (CHCl3/MeOH/AcOH: 7/2/1) = 0.44. 1H NMR (200 MHz, d6-DMSO) δ 7.70 (d, J = 9.2 Hz, 1H), 7.39–7.03 (m, 10H), 5.81–5.55 (m, 1H), 4.01–3.84 (m, 1H), 3.17–3.02 (m, 2H), 2.92–2.51 (m, 4H, overlapped by dPC = 3.7 Hz), 141.5, 138.6, 138.4, 137.3, 129.1, 128.4, 128.3, 128.2, 127.6, 127.1, 126.9, 126.2, 125.7, 65.0, 52.8 (d, JPC = 104 Hz), 35.0, 33.0, 32.8, 28.6, 28.5 (d, JPC = 104 Hz); 31P NMR (81 MHz, d6-DMSO) δ 45.0; HRMS (m/z): [M – H]− calcld. for C24H29NO6P−, 508.1894 found, 508.1889.

(2S)-2-\{[R]-1-(Benzyloxycarbonylamino)-2-phenylethyl\}(hydroxy)phosphoryl]methyl\}-pent-4-enioic acid (5h): Phosphinic acid 1a (410 g, 1.3 mmol) and acrylic acid 3g (170 mg, 1.54 mmol) were subjected to the general HMDS-mediated P-C bond-forming reaction protocol. After the addition of HCl 2M and AcOEt, the two phases were separated and the organic layer was dried with Na2SO4 and evaporated in vacuo. The solid product was recrystallized with AcOEt and the hot suspension is filtrated. The procedure is repeated to afford compound 5h (d.r. 96:4) as a white solid in 51% yield (284 mg, 0.66 mmol). m.p. 171–174 °C; [α]D25 = −43 (c = 1, DMSO); TLC Rf (CHCl3/MeOH/AcOH: 7/2/1) = 0.59. 1H NMR (400 MHz, d6-DMSO) δ 7.70 (d, J = 9.4 Hz, 1H), 7.36–6.94 (m, 10H), 4.95 (d, J = 13 Hz, 1H), 4.90 (d, J = 13 Hz, 1H), 4.01–3.84 (m, 1H), 3.17–3.02 (m, 2H), 2.92–2.51
(2S)-3-[[1,1′-Biphenyl]-4-yl]-2-[[[(R)-1-(benzyloxycarbonylamino)-2-phenylethyl](hydroxy)phosphoryl]methyl]propanoic acid (5i): Phosphinic acid 1a (200 mg, 0.63 mmol) and acrylic acid 3i (180 mg, 0.76 mmol) were subjected to the general HMDS-mediated P-C bond-forming reaction protocol. After the addition of HCl 2M and AcOEt, a solid was precipitated that was collected by filtration. The solid product was refluxed with AcOEt and the hot suspension was collected by filtration. The solid product was refluxed with AcOEt and the hot suspension was filtered. The procedure was repeated to afford compound 5i (d.r. >99:1) as a white solid in 54% yield (167 mg, 0.34 mmol). m.p. 143–146°C; [α]D 25° = 31.0 (c = 0.9, MeOH); TLC Rf (CHCl3/MeOH/AcOH: 7/2/1) = 0.34. 1H NMR (200 MHz, d6-DMSO) δ 7.69 (d, J = 9.4 Hz, 1H), 7.39–6.92 (m, 10H), 4.92 (s, 2H), 4.79–4.70 (m, 1H), 3.96–3.67 (m, 5H), 3.14–2.97 (m, 1H), 2.81–2.56 (m, 2H), 2.12–1.88 (m, 1H), 1.81–1.43 (m, 5H); 13C NMR (50 MHz, d6-DMSO) δ 156.0 (d, JPC = 9.6 Hz), 156.0 (d, JPC = 3.8 Hz), 140.0, 138.6, 138.3, 138.2, 137.2, 129.7, 129.0, 129.6, 128.3, 128.2, 127.6, 127.3, 127.0, 126.6, 126.2, 65.1, 52.6, (d, J1PC = 105 Hz), 32.71, 27.7 (d, J1PC = 89 Hz); 31P NMR (81 MHz, d6-DMSO + 4%TFA) δ 46.6; HRMS (m/z): [M + Na]+ calcd. for C32H30N2O7P+, 556.1894 found, 556.1896.

(2S)-2-[[[(R)-1-(benzyloxycarbonylamino)-2-phenylethyl](hydroxy)phosphoryl]methyl]-4-(1,3-dioxolan-2-yl)butanoic acid (5j): Phosphinic acid 1a (200 mg, 0.63 mmol) and acrylic acid 3j (130 mg, 0.76 mmol) were subjected to the general HMDS-mediated P-C bond-forming reaction protocol. After the addition of HCl 2M and AcOEt, a solid was precipitated that was collected by filtration. The solid product was recrystallized with AcOEt to afford compound 5j (d.r. 3.5:1) as a white foam in 87% yield (239 g, 0.43 mmol). m.p. 208–210°C; [α]D 25° = –23.8 (c = 0.95, DMSO); 1H NMR (200 MHz, d6-DMSO + 4%TFA) δ 7.77–6.95 (m, 20H), 5.02–4.74 (m, 2H), 4.03–3.77 (m, 1H), 3.16–2.82 (m, 4H), 2.81–2.59 (m, 1H), 2.19–1.93 (m, 1H), 1.92–1.67 (m, 1H); 13C NMR (50 MHz, d6-DMSO) δ 138.2, 137.2, 129.7, 129.0, 128.3, 128.2, 127.6, 127.1, 126.3, 103.3, 65.1, 52.6, (d, J1PC = 105 Hz), 32.17, 27.7 (d, J1PC = 89 Hz); 31P NMR (81 MHz, d6-DMSO + 4%TFA) δ 46.6; HRMS (m/z): [M + H]+ calcd. for C23H23NO7P+, 428.1268 found, 428.1262.

2-[[[(R)-1-(benzyloxycarbonylamino)ethyl](hydroxy)phosphoryl]methyl]-4-(tert-butoxy)-4-oxo-2-yl)butanoic acid (5k): Phosphinic acid 1b (500 mg, 1.57 mmol) and acrylic acid 3k (48 mg, 0.37 mmol) were subjected to the general HMDS-mediated P-C bond-forming reaction protocol. After the addition of HCl 2M and AcOEt, the two phases were separated and the organic layer was dried with Na2SO4 and evaporated in vacuo. The residue was treated with Et2O, filtrated and washed with Et2O. Compound 5l (d.r. 2:5:1) was isolated as a white
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solid in 93% yield (129 mg, 0.29 mmol). TLC Rf (CHCl3/MeOH/AcOH: 7/2/1) = 0.28. 1H NMR (200 MHz, d6-DMSO) δ [7.58 (d, J = 9.0 Hz) & 7.56* (d, J = 9.0 Hz)], 1H), 7.41–7.25 (m, 5H), 5.11–4.95 (m, 2H), 3.87–3.62 (m, 1H), 3.04–2.81 (m, 1H), 2.80–2.63 (m, 1H), 2.58–2.38 (m, 1H, overlapped by d6-DMSO), 2.13–1.92 (m, 1H), 1.83–1.60 (m, 1H, 1.37 (s, 9H), 1.20 (dd, J = 14.4, 7.4 Hz, 3H); 13C NMR (50 MHz, d6-DMSO) δ 175.3 (d, 3JPC = 13.5 Hz), 170.7, 156.0 (d, 3JPC = 4.9 Hz), 128.5, 128.0, 127.84, 127.79, 80.0, 65.8, 46.3 (d, 1JPC = 105 Hz)*, 46.1 (d, 1JPC = 107 Hz), 37.18* (s, 3.16*), 36.93, 36.87, 35.0*, 34.9*, 35.2, 35.1, 27.8, 26.0, 13.9; 31P NMR (81 MHz, d6-DMSO) δ 47.3, 46.9; HRMS (m/z): [M – H–] calcld. for C19H22NO9P2−, 428.1480 found, 428.1467.

(2S)-2-(((R)-1-(((Benzyloxy)carbonyl)amino)-3-phenylpropyl)(hydroxy)phosphoryl)methyl)-4-meth ylpentanoic acid (5m): Phosphinic acid 1c (1.0 g, 3.0 mmol) and acrylic acid 3c (461 mg, 3.6 mmol) were subjected to the general HMDS-mediated P-C bond-forming reaction protocol. After the addition of HCl 2M and AcOEt, a solid was precipitated that was collected by filtration. The solid product was refluxed with AcOEt and the hot suspension was filtrated to afford compound 5m (d.r. 96:4 as a white solid in 53% yield (733 mg, 1.59 mmol). m.p. 184–186 °C; [a]D 25 = −19 (c = 1, DMSO); TLC Rf (CHCl3/MeOH/AcOH: 7/2/1) = 0.7. 1H NMR (400 MHz, d6-DMSO + 20%CD3OD) δ 7.57 (d, J = 9.2 Hz, 0.2H partially exchanged), 7.41–7.08 (m, 10H), 5.08 (s, 2H), 3.68–3.54 (m, 1H), 2.77–2.57 (m, 2H), 2.55–2.42 (m, 1H, overlapped by d6-DMSO), 2.03–1.86 (m, 2H), 1.85–1.72 (m, 1H), 1.67–1.56 (m, 1H), 1.55–1.28 (m, 3H), 0.85 & 0.82 (2 × d, J = 6.4 Hz, 6H); 13C NMR (101 MHz, d6-DMSO + 20%CD3OD) δ 176.4 (d, 3JPC = 7.8 Hz), 156.4 (d, 3JPC = 3.8 Hz), 141.3, 137.3, 128.5, 128.4, 128.3, 127.9, 127.7, 125.9, 65.6, 50.3 (d, 1JPC = 105 Hz), 42.6 (d, 1JPC = 8.5 Hz), 36.7 (d, 1JPC = 3.9 Hz), 31.7 (d, 1JPC = 12 Hz), 29.3, 28.8 (d, 1JPC = 89 Hz), 25.6, 22.9, 21.8; 31P NMR (162 MHz, d6-DMSO + 20%CD3OD) δ 45.0; HRMS (m/z): [M + H+]* calcd. for C24H32N2O6P2+, 462.2040 found, 462.2046.

(2S)-2-(((R)-1-((Benzyloxy)carbonyl)amino)-3-phenylpropyl)(hydroxy)phosphoryl)methyl)4-meth ylpentanoic acid (5n): Phosphinic acid 1c (1.5 g, 4.5 mmol) and acrylic acid 3g (594 mg, 5.4 mmol) were subjected to the general HMDS-mediated P-C bond-forming reaction protocol. After the addition of HCl 2M and AcOEt, a solid was precipitated that was collected by filtration. The solid product was refluxed with AcOEt and the hot suspension was filtrated to afford compound 5n (d.r. 95:5) as a white solid in 56% yield (1.1 g, 2.5 mmol). m.p. 162–164 °C; [a]D 25 = −22 (c = 1, DMSO); TLC Rf (CHCl3/MeOH/AcOH: 7/0.5/0.5) = 0.16. 1H NMR (400 MHz, d6-DMSO + 5% TFA) δ 7.63 (d, J = 9.3 Hz, 1H), 7.42–7.08 (m, 10H), 5.06 (s, 2H), 3.73–3.57 (m, 1H), 2.84–2.61 (m, 2H), 2.62–2.43 (m, 2H, overlapped by d6-DMSO), 2.10–1.92 (m, 2H), 1.87–1.72 (m, 2H); 13C NMR (50 MHz, d6-DMSO) δ 174.6 (d, 3JPC = 11.8 Hz), 156.7 (d, 3JPC = 3.7 Hz), 141.5, 137.4, 128.7, 128.7, 128.2, 128.0, 126.2, 81.6, 73.1, 66.0, 50.3 (d, 1JPC = 106 Hz), 37.9, 31.9 (d, 1JPC = 12 Hz), 29.4, 26.7 (d, 1JPC = 87 Hz), 21.8; 31P NMR (162 MHz, d6-DMSO + 5% TFA) δ 45.7; HRMS (m/z): [M + Na]+ calcd. for C24H32N2NaO6P2+, 466.1390 found, 456.1368.

2-(((R)-1-((tert-Butoxycarbonyl)amino)-3-phenylpropyl)(hydroxy)phosphoryl)methyl)pent-4-ynoic acid (5o): Phosphinic acid 1d (450 mg, 1.50 mmol) and acrylic acid 3g (198 mg, 1.80 mmol) were subjected to the general HMDS-mediated P-C bond-forming reaction protocol. After the addition of HCl 2M and AcOEt, the two phases were separated and the organic layer was dried with Na2SO4 and evaporated in vacuo. The crude product was purified by silica gel column chromatography using CHCl3/MeOH/AcOH: 7:0.1:0.01 → 7:0:4:0 as eluent solvent system. Compound 5o (d.r. 1:3.1) was isolated as an off-white solid in 89% yield (547 mg, 1.34 mmol). TLC Rf (CHCl3/MeOH/AcOH: 7/0.5/0.5) = 0.16. 1H NMR (400 MHz, d6-DMSO) δ 7.35–7.07 (m, 6H), 3.62–3.32 (m, 1H), 2.80–2.65 (m, 3H), 2.61–2.50 (m, 2H, overlapped by d6-DMSO), 2.06–1.89 (m, 2H), 1.84–1.68 (m, 2H), 1.41 & 1.37 (2 × s, 9H); 13C NMR (101 MHz, d6-DMSO) δ 174.2 (d, 3JPC = 12 Hz), 155.7, 155.64, 155.61, 141.39, 141.36, 128.5, 128.3, 125.8, 81.4, 78.3*, 78.2, 72.7, 48.2 (d, 1JPC = 107 Hz), 37.8* (d, 1JPC = 2.7 Hz), 37.6 (d, 1JPC = 2.6 Hz), 31.7 (d, 1JPC = 12 Hz), 31.74, 31.62, 29.0, 28.2, 27.9, 26.4* (d, 1JPC = 89 Hz), 26.5 (d, 1JPC = 89 Hz), 21.8* (d, 1JPC = 6.3 Hz), 21.4 (d, 1JPC = 5.7 Hz); 31P NMR (162
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(2S)-2-({[(R)-1-(Benzyloxycarbonylamino)-3-(phenylthio)propyl](hydroxy)phosphoryl}methyl)-4-methylpentanoic acid (5p): Phosphinic acid 1e (2.0 g, 5.5 mmol) and acrylic acid 3c (840 mg, 6.6 mmol) were subjected to the general HMDS-mediated P-C bond-forming reaction protocol. After the addition of HCl 2M and AcOEt, a solid was precipitated that was collected by filtration. The solid product was refluxed with AcOEt and the hot suspension was filtered. The procedure was repeated to afford compound 5p (d.r. 97:3) as a white solid in 59% yield (1.6 g, 2.5 mmol). m.p. 130–135 °C; [α]25 = −14.2 (c = 1, DMSO); TLC Rf (CHCl3/MeOH/AcOH: 7/2/1) = 0.65. 1H NMR (500 MHz, d6-DMSO) δ 7.50 & 7.49 (2 × d, J = 9.6 Hz, 1H), 7.26 & 7.21–7.13 (m, 10H), 5.07 (d, J = 13 Hz, 1H), 5.02 (dd, J = 13, 2 Hz, 1H), 3.70–3.57 (m, 1H), 3.20–2.92 (m, 3H), 2.80–2.58 (m, 1H), 2.01–1.74 (m, 3H), 1.76–1.57 (m, 4H), 0.83 [app t, J = 6.3 Hz, 6H]. 13C NMR (50 MHz, d6-DMSO) δ 176.4 (d, JPC = 7.8 Hz, 1H), 156.4 (d, JPC = 1 H Bez), 137.2, 135.9, 129.2, 128.4, 128.1, 127.9, 127.6, 125.7, 125.6, 65.6, 49.9 (d, JPC = 104 Hz), 42.6 (d, JPC = 8.9 Hz), 36.8 (d, JPC = 4.0 Hz), 29.0 (d, JPC = 12 Hz), 28.9 (d, JPC = 89 Hz), 25.6, 23.0 (d, 3H), 18.5 (d, 3H), 17.6 (d, JPC = 5.8 Hz). 31P NMR (162 MHz, d6-DMSO) δ 45.1; HRMS (m/z): [M + H]+ calcd. for C26H30NO8P5S3, 494.1761 found, 494.1760.

3-{{[(R)-1-(Benzyloxycarbonylamino)-4-(phenylthio)butyl](hydroxy)phosphoryl}-2-methylpropanoic acid (5q): Phosphinic acid 1f (937 mg, 2.47 mmol) and methylacrylic acid (3b) (255 mg, 2.97 mmol) were subjected to the general HMDS-mediated P-C bond-forming reaction protocol. After the addition of HCl 2M and AcOEt, the two phases were separated and the organic layer was dried with Na2SO4 and evaporated in vacuo. The residue was solidified on standing, treated with Et2O and filtrated. Compound 5q (d.r. 1:1) was isolated as a white solid in 92% yield (1.06 g, 2.27 mmol). TLC Rf (CHCl3/MeOH/AcOH: 7/2/1) = 0.61. 1H NMR (400 MHz, d6-DMSO) δ 7.50 & 7.49 (2 × d, J = 9.6 Hz, 1H), 7.26 & 7.21–7.13 (m, 10H), 5.07 (d, J = 13 Hz, 1H), 5.02 (dd, J = 13, 2 Hz, 1H), 3.70–3.57 (m, 1H), 3.20–2.92 (m, 3H), 2.80–2.58 (m, 1H), 2.01–1.74 (m, 3H), 1.76–1.57 (m, 4H), 0.83 [app t, J = 6.3 Hz, 6H]. 13C NMR (300 MHz, d6-DMSO) δ 176.4 (d, JPC = 7.8 Hz, 1H), 156.4 (d, JPC = 1 Hz), 137.2, 135.9, 129.2, 128.4, 128.1, 127.9, 127.6, 125.7, 125.6, 65.6, 49.9 (d, JPC = 104 Hz), 42.6 (d, JPC = 8.9 Hz), 36.8 (d, JPC = 4.0 Hz), 29.0 (d, JPC = 12 Hz), 28.9 (d, JPC = 89 Hz), 25.6, 23.0, 21.8. 31P NMR (81 MHz, d6-DMSO) δ 46.0; HRMS (m/z): [M + H]+ calcd. for C24H30NO6P5S3, 476.1416 found, 476.1413.

3-{{[(R)-1-(Benzyloxycarbonylamino)-5′-(phenylthio)butyl](hydroxy)phosphoryl)-2-methylpropanoic acid (5r): Phosphinic acid 1g (310 mg, 0.77 mmol) and acrylic acid 3b (100 mg, 0.33 mmol) were subjected to the general HMDS-mediated P-C bond-forming reaction protocol. After the addition of HCl 2M and AcOEt, the two phases were separated and the organic layer was dried with Na2SO4 and evaporated in vacuo. The crude product was purified by silica gel column chromatography using CHCl3/MeOH/AcOH 7/0.1:0.1:0.1 → 7/0.4:0.4 as eluent solvent system. Compound 5r (d.r. 2:1) was isolated as a white foam in 92% yield (193 mg, 2.27 mmol). TLC Rf (CHCl3/MeOH/AcOH: 7/0.5/0.5) = 0.43. 1H NMR (200 MHz, d6-DMSO) δ 7.83–7.06 (m, 18H), 3.73–3.47 (m, 1H), 3.20–2.92 (m, 3H), 2.80–2.58 (m, 1H), 2.58–2.37 (m, 1H, overlapped by d3-DMSO), 2.08–1.58 (m, 4H), 1.34 & 1.41 (2 × s, 9H). 13C NMR (50 MHz, d6-DMSO) δ 175.2, 175.1, 174.9, 129.0, 128.9, 128.5, 127.7, 127.1, 126.9, 125.9, 123.5, 78.4, 78.3, 49.5 (d, JPC = 107 Hz), 49.1 (d, JPC = 106 Hz), 31.9, 31.7, 29.2, 29.0, 28.3, 28.0, 26.5. 31P NMR (81 MHz, d6-DMSO) δ 47.0, 46.7; HRMS (m/z): [M + H]+ calcd. for C24H31NO6P5S3, 464.2666 found, 464.2666.

4. Conclusions

In this work, a useful method for the synthesis of phosphinic dipeptides of type 5 is presented. These compounds are valuable synthetic intermediates in the field of phosphinic peptides and protocols that facilitate their preparation are of great interest, especially when...
large quantities are required for medicinal chemistry purposes. Based on the observation that acrylic acids are neglected electrophile alternatives for the key $\text{P-Michael addition}$ between $\alpha$-aminophosphinic acids and acrylic acceptors, we propose that an unreactive mixture of an aminophosphinic and an acrylic acid can be activated under silylating conditions by acting simultaneously on both reactants, to afford in a single step the target compounds of type 5. A great advantage of the presented method is that in several cases, bioactive ($R,S$)-isomers of type 5 can be directly isolated from the crude products by means of selective crystallization. This tendency is well-described in the literature and has been used in the past for the synthesis of several dipeptides of type 5; for example, 5h and 5m, which are also described in this report and were found spectroscopically identical to previously reported compounds [20,23]. In particular, compounds 5h [20] and 5m [23] have been converted to tripeptides that were stereochemically characterized either by NMR [23] or by X-ray crystallographic analysis of their complexes with Zn-metalloproteases [38,39]. Taking together the availability of starting acids and the practicality of the proposed P-C bond-forming reaction, we believe that the reported protocol will greatly contribute to the simplification of synthetic procedures targeting this important class of compounds.

**Supplementary Materials:** Synthetic protocol and characterization for compounds 3a and 3c–k. Copies of $^1\text{H}$, $^{13}\text{C}$ and $^{31}\text{P}$ NMR spectra for compounds 3a, 3c–k and 5a–r.

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**Sample Availability:** Samples of the compounds are not available from the authors.

**Abbreviations**

| Abbreviation | Definition |
|--------------|------------|
| HMDS | 1,1,1,3,3,3-hexamethyldisilazane |
| BSA | $N,O$-bis(trimethylsilyl)acetamide |
| TMSCl | chlorotrimethylsilane |

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