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# Chemoselective Protection of Solid-Phase Compatible Fmoc-Phosphinic Building Blocks

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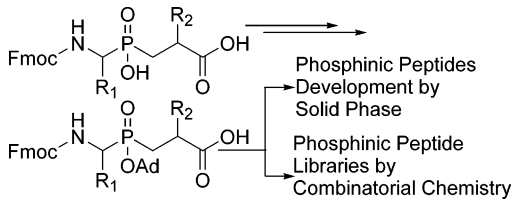


An efficient four-step synthetic strategy able to fully discriminate hydroxyphosphinyl and carboxylic groups of Fmoc-phosphinic building blocks and related analogues has been developed. The proposed method applies selective acidic removal of the phenacyl (Pac) group from the hydroxyphosphinyl functionality and protection by the 1-adamantyl (Ad) group. Reductive removal of the Pac group from the carboxylic functionality leads to Fmoc-protected phosphinic pseudodipeptidic units suitable for phosphinic peptide and library development using solid-phase peptide synthesis (SPPS).

The intensive use of combinatorial chemistry in the discovery of novel biologically active compounds has provided large amounts of information concerning structure−activity relationships. Solid-phase synthesis has been the major tool of combinatorial chemistry with apparent advantages over solution-phase chemistry.[1]

Synthesis of phosphinic peptides has been successfully accomplished on solid phase, using either parallel[2] or combinatorial strategies.[3] Such approaches have led to the rapid access and identification of not only highly potent but also selective inhibitors of various zinc metalloproteases, such as angiotensin-converting enzyme (ACE)[4] and matrix metalloproteinases (MMPs),[5] among others.

The most popular strategy to synthesize natural peptides on solid support applies the Fmoc protocol. Application of this strategy to phosphinic peptides requires the use of an orthogonal protection scheme, compatible with Fmoc solid-phase chemistry. Although two alternative synthetic strategies provide such blocks,[6] both require careful planning in terms of protection and deprotection steps of the numerous functional groups of the pseudodipeptidic unit. The chemoselective deprotection of either the hydroxyphosphinyl by TFA,[7] TMSBr,[8] LiI,[9] LiBr,[10] and phosphodiesterase I[11] or the carboxylate group by Carlsberg subtilisin,[12] pig liver esterase,[13] or $(Bu_3Sn)_2O$[14] of phosphinic peptide blocks has been achieved for methyl and ethyl esters, which are not compatible with the Fmoc solid-phase peptide synthesis (SPPS) protocol.

We report herein a new chemoselective strategy, able to discriminate between the two acidic groups of the phosphinic units, namely, the hydroxyphosphinyl and the carboxylic acid functionalities. Although phosphinic building blocks of type **1** (Scheme 1) which are unprotected at the hydroxyphosphinyl group have been successfully used in SPPS,[2,15,16] the use of protected blocks of type **5** remains the method of choice for the development of phosphinic peptide libraries devoid of byproducts and incomplete reactions.

Fmoc-phosphinic building blocks of type **1** have been recently reported to be easily synthesized by a three-component condensation reaction.[16] Any attempt to discriminate between the two acidic functionalities, using a variety of protecting groups, (e.g., *tert*-butyl, benzyl, diphenylmethyl, trityl, etc.) has failed.[17] In practice, a phenacyl (Pac) protecting group offered the expedient method for the efficient discrimination between the two acidic functionalities. The simultaneous protection of both hydroxyphosphinyl and carboxylic functional groups by Pac was easily achieved using PacBr in the presence of $Et_3N$, in very good yields.

Completely chemoselective deprotection of the hydroxyphosphinyl functional group of building blocks **2** (Scheme 1) was accomplished under acidic conditions ($TFA/CH_2Cl_2$), where the carboxylate Pac-ester remains perfectly intact. Hydroxyphos-

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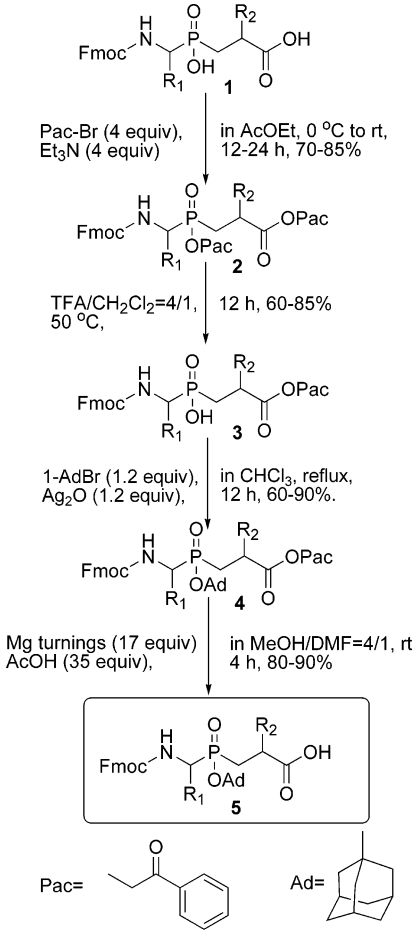
**TABLE 1. Yields and Side Chains of Compounds 2−5**

| entry | $R_1$ | $R_2$ | yields of **2** (%) | yields of **3** (%) | yields of **4** (%) | yields of **5** (%) |
|---|---|---|---|---|---|---|
| a | H− | $PhCH_2$− | 70 | 62 | 61 | 89 |
| b | $CH_3$− | $PhCH_2$− | 78 | 82 | 90 | 81 |
| c | $(CH_3)_2CH$− | $PhCH_2$− | 72 | 65 | 60 | 85 |
| d | $CH_3OOCCH_2CH_2$− | $PhCH_2$− | 80 | 75 | 85 | 83 |
| e | Ph− | $PhCH_2$− | 82 | 78 | 74 | 91 |
| f | $PhCH_2OCH_2$− | $PhCH_2$− | 81 | 60 | 84 | 87 |
| g | $CH_3$− | $(CH_3)_2CHCH_2$− | 81 | 71 | 73 | 80 |
| h | $(CH_3)_2CHCH_2$− | H | 82 | 70 | 62 | 86 |

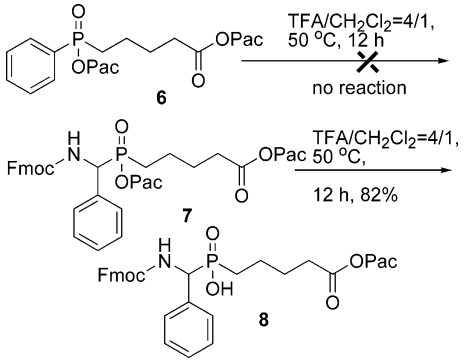
**SCHEME 1. Chemoselective Protection of Fmoc Pseudodipeptidic Building Blocks 5**




phinyl reprotection by the 1-Ad group[6] (**3**) and final removal of the Pac-protecting group from the carboxylic functionality of **4** by metal reduction[18] afforded the target compounds **5** in good yields (Table 1). As it has been previously shown, phosphinic blocks of type **1** are formed as two pairs of enantiomers, namely, four diastereoisomers, in nearly the same amount, which can be easily separated by RP-HPLC, once incorporated into a peptide sequence.[16] Although protection of the hydroxyphosphinyl group, as present in compounds **5**, creates a new stereogenic center, removal of the Ad protecting group simultaneously with peptide cleavage from the resin reconstitutes the number and ratio of the aforementioned diastereoisomers.

To examine the applicability of the proposed method to nonpeptidic phosphinic compounds, compound **6** (Scheme 2)

**SCHEME 2. Phosphinic Ester Acidic Cleavage via *β*-Carbamido-Induced Assistance**




was synthesized[19] and subjected to the same acidic conditions as compounds **2** (Scheme 1). In this case, Pac-cleavage did not take place from the hydroxyphosphinyl or from the carboxylic functional groups.

On the contrary, acidic treatment of compound **7** (Scheme 2) resulted in the selective Pac-phosphinyl deprotection, leading to compound **8** (Scheme 2). These results are consistent with pertinent reports of the literature, where assisted acidic cleavage of methyl *β*-carboxyphosphinates,[20] or *β*-carboxamides,[7] was observed. According to those results, the cleavage of methyl phosphinate is promoted by the *β*-carboxamide or the *β*-carboxylic groups due to the formation of a five-membered reactive intermediate. In the present case, a similar mechanistic pathway is proposed, involving an α-fluorenylmethyl carbamide-induced cleavage via a five-membered cyclic intermediate formation (Scheme 3). Alternatively, electrophilic activation of the P═O group has also been proposed to proceed through hydrogen bonding with the carbamate N−H.[21] However, compound **6** cannot form such a reactive intermediate, and therefore, the Pac-cleavage is not favorable in this case.

In conclusion, we have described here an efficient method for the chemoselective protection of the hydroxyphosphinyl group of Fmoc-phosphinopeptidic building blocks, suitable for SPPS and combinatorial library development. To the best of our knowledge, the proposed strategy constitutes the first successful method to the vexing problem of discriminating between the two acidic functionalities of the Fmoc-phosphinic units. The *β*-carbamido-induced selective Pac-phosphinic ester cleavage can thus be applied to any Fmoc-protected α-aminophosphinic analogues. Such blocks are of great importance in the development of potent and selective Zn-metalloprotease phosphinic inhibitors.

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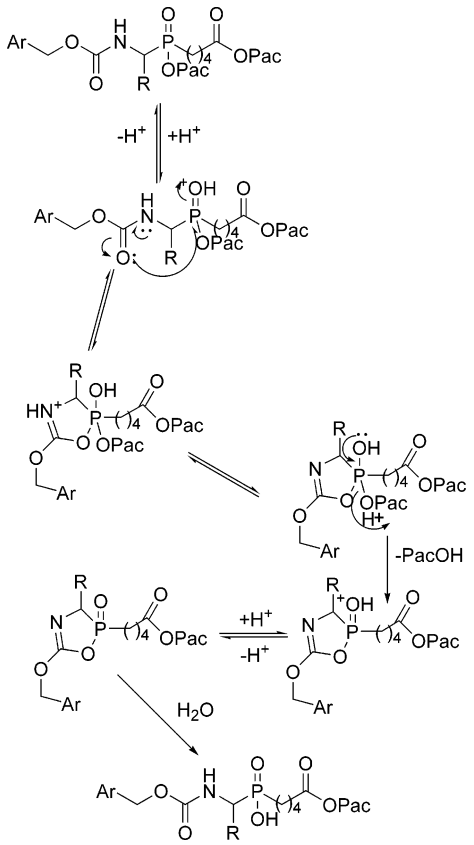
**SCHEME 3. Proposed Mechanism for the Acid-Catalyzed Cleavage of the Phosphinic Ester via *β*-Carbamido-Induced Assistance**



## Experimental Section

**General Method for Diphenacyl Protection for Compounds of Type 2.** Phosphinic acid of type **1** (1 mmol) is dissolved in AcOEt (1 mL) then cooled at 0 °C, and PacBr (4 mmol) is added, followed by $Et_3N$ (4 mmol). After stirring at rt overnight, the reaction mixture is diluted with AcOEt, washed with HCl (1 N), and brine and dried over $Na_2SO_4$. Evaporation of the solvent and purification by column chromatography, using $CHCl_3$/MeOH = 9.8:0.2 as the eluent system, afford the pure products as white solids, after trituration with petroleum ether 40−60 °C.

**2-Benzyl-3-[[1-(9*H*-fluoren-9-ylmethoxycarbonylamino)-ethyl]-(2-oxo-2-phenyl-ethoxy)-phosphinoyl]-propionic Acid 2-Oxo-2-phenyl-ethyl Ester (2b).** White solid. Yield 78%, 569 mg. TLC $R_f$ ($CHCl_3$/MeOH = 95:5) 0.63. $^1$H NMR (200 MHz, $CDCl_3$) $\delta$ 1.24−1.46 (m, 3H), 2.06−2.19 (m, 2H), 2.28−2.60 (m, 1H), 2.88−3.07 (m, 1H), 3.27−3.40 (m, 2H), 4.09−4.41 (m, 3H), 5.14−5.60 (m, 4H), 5.71−6.34 (m, 1H), 7.12−7.90 (m, 23H). $^{13}$C NMR (50 MHz, $CDCl_3$) $\delta$ 14.6, 14.8, 27.6, 39.3, 39.5, 40.8, 40.9, 45.2, 47.0, 66.1, 66.3, 67.2, 119.9, 125.1, 126.7, 127.0, 127.7, 128.5, 128.8, 128.9, 129.1, 129.2, 133.8, 133.9, 134.2, 137.6, 141.1, 143.7, 155.8, 173.6, 191.7. $^{31}$P NMR (81 MHz, $CDCl_3$) $\delta$ 55.28, 55.45, 56.14. ESMS *m*/*z* calcd for $C_{43}H_{41}NO_8P$ $(M + H)^+$ 730.3, found 730.3.

**General Method for Selective Cleavage of Phosphinate Phenacyl Ester for Compounds of Type 3.** A phosphinic diester of type **2** (1 mmol) is dissolved in $CH_2Cl_2$ (1.6 mL), and TFA (6.4 mL) is added. The reaction mixture is allowed to stir at 50 °C overnight. Evaporation of the solvent and purification by column chromatography, using $CHCl_3$/MeOH/AcOH = 7:0.2:0.2 as the eluent system, afford the pure products as white solids, after trituration with petroleum ether 40−60 °C.

**2-Benzyl-3-{[1-(9*H*-fluoren-9-ylmethoxycarbonylamino)-ethyl]-hydroxy-phosphinoyl}-propionic Acid 2-Oxo-2-phenyl-ethyl Ester (3b).** White solid. Yield 82%, 502 mg. TLC $R_f$ ($CHCl_3$/MeOH = 4:1) 0.30. HPLC $t_R$ 48.42, 48.69. $^1$H NMR (200 MHz, $CDCl_3$) $\delta$ 1.27−1.43 (m, 3H), 1.94−2.06 (m, 1H), 2.24−2.36 (m, 1H), 2.89−3.00 (m, 1H), 3.22−3.28 (m, 2H), 4.08−4.35 (m, 4H), 5.18−5.36 (m, 2H), 5.77−5.84 (m, 1H), 7.18−7.83 (m, 18H). $^{13}$C NMR (50 MHz, $CDCl_3$) $\delta$ 13.9, 14.1, 26.3, 26.8, 28.2, 28.6, 29.6, 39.2, 40.7, 44.2, 44.7, 46.4, 47.0, 66.2, 67.2, 119.9, 125.1, 126.7, 127.0, 127.7, 127.8, 128.5, 128.7, 129.1, 133.9, 137.6, 141.2, 143.7, 143.8, 155.9, 173.4, 192.4. $^{31}$P NMR (81 MHz, $CDCl_3$) $\delta$ 53.94, 54.42. ESMS *m*/*z* calcd for $C_{35}H_{35}NO_7P$ $(M + H)^+$ 612.2, found 612.3.

**General Method for Phosphinate Adamantyl Protection for Compounds of Type 4.** Phosphinic ester of type **3** (1 mmol) and 1-AdBr (1.2 mmol) are dissolved in $CHCl_3$ (20 mL), and the reaction mixture is refluxed. To this refluxing mixture, silver oxide (1.2 mmol) is added in five equal portions over 50 min. After the solution is refluxed overnight, the solvent is removed in vacuo and the residue is diluted in $Et_2O$ and filtered through a pad of celite. Evaporation of the solvent and purification by column chromatography, using P.E./AcOEt = 1:2 as the eluent system, afford the pure products as white solids, after trituration with petroleum ether 40−60 °C.

**3-{(Adamantan-1-yloxy)-[1-(9*H*-fluoren-9-ylmethoxycarbonylamino)-ethyl]-phosphinoyl}-2-benzyl-propionic Acid 2-Oxo-2-phenyl-ethyl Ester (4b).** White solid. Yield 90%, 671 mg. TLC $R_f$ ($CHCl_3$/MeOH =95:5) 0.62. $^1$H NMR (200 MHz, $CDCl_3$) $\delta$ 1.25−1.42 (m, 3H), 1.56−1.58 (m, 7H), 1.91−2.08 (m, 9H), 2.27−2.55 (m, 1H), 2.81−2.90 (m, 1H), 3.22−3.30 (m, 2H), 4.06−4.23 (m, 1H), 4.31−4.37 (m, 3H), 5.15−5.94 (m, 3H), 7.18−7.86 (m, 18H). $^{13}$C NMR (50 MHz, $CDCl_3$) $\delta$ 14.6, 14.8, 30.3, 30.8, 31.1, 31.2, 35.6, 39.7, 39.9, 41.7, 42.1, 44.1, 44.2, 45.0, 47.1, 66.1, 66.3, 67.2, 83.6, 119.9, 125.2, 125.5, 126.8, 127.0, 127.7, 128.5, 128.6, 128.8, 129.3, 133.8, 134.1, 138.0, 138.1, 141.2, 143.8, 143.9, 155.9, 173.8, 192.1. $^{31}$P NMR (81 MHz, $CDCl_3$) $\delta$ 47.81, 48.56. ESMS *m*/*z* calcd for $C_{45}H_{49}NO_7P$ $(M + H)^+$ 746.3, found 746.2.

**General Method for Selective Deprotection of Carboxylate Esters for Compounds of Type 5.** To a solution of phosphinic diester of type **4** (1 mmol) in MeOH/DMF (4:1) (17 mL) were added AcOH (35 mmol) and Mg turnings (17 mmol). After stirring for 4 h at rt, the reaction mixture was filtered. The filtrate is concentrated in vacuo, and the residue is diluted with DCM, washed with HCl (1 N), and brine and dried over $Na_2SO_4$. Evaporation of the solvent and purification by column chromatography, using $CHCl_3$/MeOH = 9.8:0.2 as the eluent system, afford the pure products as white solids, after trituration with petroleum ether 40−60 °C.

**3-{(Adamantan-1-yloxy)-[1-(9*H*-fluoren-9-ylmethoxycarbonylamino)-ethyl]-phosphinoyl}-2-benzyl-propionic Acid (5b).** White solid. Yield 81%, 508 mg. TLC $R_f$ ($CHCl_3$/MeOH = 95:5) 0.31. $^1$H NMR (200 MHz, $CDCl_3$) $\delta$ 1.25−2.40 (m, 20H), 2.42−2.79 (m, 1H), 2.98−3.41 (m, 2H), 3.85−4.42 (m, 4H), 6.62−6.57 (m, 1H), 7.17−7.89 (m, 13H). $^{13}$C NMR (50 MHz, $CDCl_3$) $\delta$ 13.6, 14.1, 14.9, 29.3, 29.7, 30.8, 31.1, 35.2, 35.4, 40.2, 40.4, 41.8, 42.6, 44.0, 44.1, 44.2, 44.3, 47.0, 67.5, 83.1, 84.6, 119.8, 125.4, 125.7, 126.0, 126.6, 127.1, 127.6, 128.5, 129.0, 129.4, 138.0, 138.5, 140.0, 141.2, 143.7, 144.0, 144.3, 156.4, 157.1, 176.1, 176.4. $^{31}$P NMR (81 MHz, $CDCl_3$) $\delta$ 48.99, 52.09. ESMS *m*/*z* calcd for $C_{37}H_{43}NO_6P$ $(M + H)^+$ 628.3, found 628.1.

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**Supporting Information Available:** Detailed experimental procedures, spectroscopic and analytical data for all compounds, copies of $^1$H, $^{13}$C, and $^{31}$P NMR spectra for compounds **3a**−**h**, **5a**−**h**, **2b**, **3b**, and **4b**, and HPLC chromatograms for compounds **3a**−**h**. This material is available free of charge via the Internet at http://pubs.acs.org.

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