JOC*Note*

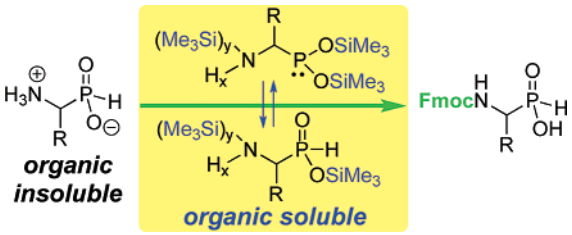
Application of in Situ Silylation for Improved, Convenient Preparation of Fluorenylmethoxycarbonyl (Fmoc)-Protected Phosphinate Amino Acids

Shunzi Li,† John K. Whitehead,† and Robert P. Hammer*

Department of Chemistry, Louisiana State University, 232 Choppin Hall, Baton Rouge, Louisiana 70803

rphammer@lsu.edu

Received February 7, 2007



A convenient and efficient method has been developed for the preparation of 9-fluorenylmethoxycarbonyl (Fmoc)-protected 1-aminoalkylphosphinic acids. Reproducible procedures for the synthesis and purification of free α-amino *H*-phosphinates are provided. Protection of free amino phosphinates as the *N*-Fmoc derivative was achieved by in situ trimethylsilylation of aminoalkylphosphinic acids, which then reacted with Fmoc-Cl to provide corresponding products in excellent yields and in high purity after simple extractive isolation. Mechanistic aspects of the silylation are discussed, and the application of the procedure to another class of amino phosphorus acids is presented.

A general approach to create inhibitors of proteolytic enzymes, amidases, and esterases, as well as inhibitors of transferases, ligases, and synthetases, involves replacing the trigonal planar, scissile amide or ester bond of the substrate with a tetrahedral analogue that mimics the intermediate formed during enzyme-catalyzed nucleophilic attack on the carbonyl. One of the most popular and effective classes of such "transition state" mimetics are phosphorus acid derivatives[1] in which the amide or ester bond is replaced by either a phosphonate ester (-PO_2-O-),[2] a phosphonamide (-PO_2-NH-),[3] or a phosphinate linkage (-PO_2-CH_2-).[4] The synthesis of each type of these phosphonopeptide mimetics depends on the ready availability of a protected form of a reduced phosphorus amino acid, typically the Boc or Fmoc amino *H*-phosphinic acid. In our hands, protocols for the synthesis of amino phosphinates and their protected derivatives have not led to reproducible results and often resulted in poor yields.

One difficulty we have invariably encountered is achieving high purity of the free phosphinate amino acids. A number of procedures in the literature report the precipitation of the free amino acids by neutralization of the HCl or HBr salts by addition of propylene oxide,[5,6] a non-ionic acid scavenger. This protocol generally does not work in our hands, giving either no precipitate or very impure products. Another difficulty that we have is synthesizing the carbamate-protected phosphinate amino acids under Schotten−Baumann conditions in the presence of $NaHCO_3$ or Na_2CO_3 in mixed aqueous organic solvents.[7,8] Particularly, the Fmoc amino acids are difficult to prepare as both the phosphinate amino acid and Fmoc-OSu are not very soluble under the previously reported solvent conditions, which leads to long reaction times and low yields. Also, homogeneous Fmoc amino acids can only be obtained after chromatography. Herein, we report reproducible procedures for preparation of *H*-phosphinate amino acids in analytically pure form by modification of literature protocols and careful ion-exchange chromatographic purification. Also, we have adapted a non-aqueous amino acid Fmoc-protection strategy, which has been utilized by our laboratory for preparation of natural, hydrophobic, and highly sterically hindered amino carboxylic acids [9] that involves trimethylsilylation for solubilization and protection of the H-phosphinate amino acid as a trimethylsilyl ester. The silylated amino phosphinate is then reacted in situ with Fmoc-Cl to give the Fmoc-amino phosphinate in pure form after simple extractive workup.

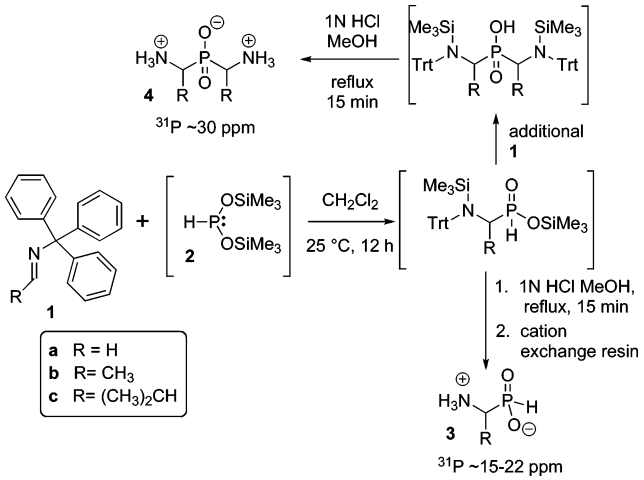
1-Aminoalkylphosphinic acids are generally prepared according to a procedure adapted from Baylis et al.,[5] which consists of the addition of hypophosphorous acid to *N*-(diphenylmethyl) imines followed by hydrolysis to cleave the benzhydryl group. The main drawback of this method is the need for drastic acidic conditions such as 49% hydrobromic acid to cleave the *N*-alkyl group. More recently, Jiao et al. proposed an easy and fast general one-pot method to prepare differently functionalized 1-aminoalkylphosphinic acids in high yield, using an addition of bis(trimethylsilyl) phosphonite to *N*-tritylalkanimines.[10] Trityl protection was used for the amino group and had the advantage of being rapidly removed by dilute acid, such as 1 N hydrochloric acid in methanol. Herein, the latter procedure was utilized to synthesize 1-aminoalkylphos-

† Contributed equally to this work.

(1) Collinsova, M.; Jiracek, J. *Curr. Med. Chem.* **2000**, *7*, 629−647.
(2) Bartlett, P. A.; Hanson, J. E.; Giannousis, P. P. *J. Org. Chem.* **1990**, *55*, 6268−6274. Kaplan, A. P.; Bartlett, P. A. *Biochemistry* **1991**, *30*, 8165−8170. Bartlett, P. A.; Giangiordano, M. A. *J. Org. Chem.* **1996**, *61*, 3433−3438.
(3) Jacobsen, N. E.; Bartlett, P. A. *J. Am. Chem. Soc.* **1981**, *103*, 654−657. Bartlett, P. A.; Marlowe, C. K. *Biochemistry* **1983**, *22*, 4618−4624.
(4) Bartlett, P. A.; Kezer, W. B. *J. Am. Chem. Soc.* **1984**, *106*, 4282−4283. Buchardt, J.; Ferreras, M.; Krog-Jensen, C.; Delaisse, J. M.; Foged, N. T.; Meldal, M. *Chem.−Eur. J.* **1999**, *5*, 2877−2884. Gall, A. L.; Ruff, M.; Kannan, R.; Cuniasse, P.; Yiotakis, A.; Dive, V.; Rio, M. C.; Basset, P.; Moras, D. *J. Mol. Biol.* **2001**, *307*, 577−586.
(5) Baylis, E. K.; Campbell, C. D.; Dingwall, J. G. *J. Chem. Soc., Perkin Trans. 1* **1984**, 2845−2853.
(6) Grobelny, D. *Synthesis* **1987**, 942−943.
(7) Dumy, P.; Escale, R.; Girard, J. P.; Parello, J.; Vidal, J. P. *Synthesis* **1992**, 1226−1228.
(8) Georgiadis, D.; Matziari, M.; Yiotakis, A. *Tetrahedron* **2001**, *57*, 3471−3478.
(9) Fu, Y. W.; Hammarstrom, L. G. J.; Miller, T. J.; Fronczek, F. R.; McLaughlin, M. L.; Hammer, R. P. *J. Org. Chem.* **2001**, *66*, 7118−7124. Fu, Y. W.; Etienne, M. A.; Hammer, R. P. *J. Org. Chem.* **2003**, *68*, 9854−9857.
(10) Jiao, X. Y.; Verbruggen, C.; Borloo, M.; Bollaert, W.; Degroot, A.; Dommisse, R.; Haemers, A. *Synthesis* **1994**, 23−24.

10.1021/jo070266p CCC: $37.00 © 2007 American Chemical Society
Published on Web 03/22/2007

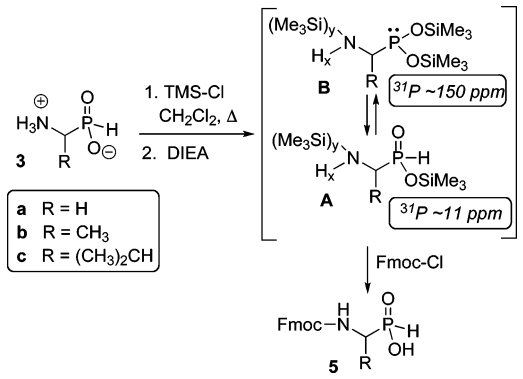
SCHEME 1. Preparation of 1-Aminoalkylphosphinic Acids



phinic acids **3a**−**c** with different R substitution groups that correspond to phosphorus analogues of natural amino acids Gly, Ala, and Val, respectively (Scheme 1). Jiao and co-workers reported that during the workup **3a**−**c** were precipitated from an ethanolic solution with excess of propylene oxide. The precipitated products were isolated by suction, washed with ethanol, and dried in a vacuum to obtain the desired pure compound. In contrast with their work, we generally do not observe a precipitate after treatment with propylene oxide. In general, we have found the only reliable and reproducible method for purifying amino acids is by ion exchange chromatography. In addition, we found that using equimolar *N*-tritylalkanimine **1** and bis(trimethylsilyl) phosphonite **2** as described by Jiao and co-workers produces a significant (20−50%) amount of undesired dialkylated phosphinic acids **4**. To overcome this problem, a 2-fold excess of phosphonite **2** over imine **1** was used in our procedures, which then produced pure amino acids **3** with only minor contamination with disubstituted phosphinic acid **4** (<5% as estimated by ^{31}P NMR of the crude product). The diamino phosphinate **4** side-product could be eliminated almost entirely by use of larger (5-fold) excesses of bis(trimethylsilyl) phosphonite (**2**), but workup of these reactions was impractical because of the large amount of hypophosphorous acid and its disproportionation products generated (data not shown). Crude amino acids were purified by cation exchange chromatography by elution with ammonium hydroxide to obtain **3a**−**c** in high purities as the small amount of diaminophospinate **4** present was more strongly retained on the cation exchange resin.

The synthesis of Fmoc-protected 1-aminoalkylphosphinic acids has been previously reported by Dumy et al.[7] and Georgiadis et al.,[8] who carried out the reaction using Schotten−Baumann-type methods as described (e.g., water−dioxane mixtures, pH 9−10). Our difficulties with these procedures led us to think about conditions that would not require the use of aqueous organic mixtures. For the synthesis of carbamate-protected amino carboxylic acids, Bolin et al. developed an efficient procedure involving in situ intermediate *N*,*O*-bis(trimethylsilyl) amino acids[1,1] that subsequently reacted with acylating agents such as Fmoc-Cl.[12] This strategy minimized

SCHEME 2. Preparation of Fmoc-Protected Amino Phosphinic Acids



the formation of dipeptides and tripeptides, which are difficult to remove by crystallization, and yielded products with purity superior to that achievable by traditional methods. This reaction also occurs in organic solvents such as methylene chloride or acetonitrile as the amino acids become soluble in organic solvents once they are silylated. Our lab has successfully employed this procedure to protect the amino group of very sterically hindered α,α-dialkylated amino carboxylic acids. [9]

Herein, we present the first preparation of Fmoc-protected 1-aminoalkylphosphinic acids using an in situ silylation procedure (Scheme 2). In this method, **3a**−**c** were refluxed in an aprotic solvent with TMS-Cl, and then diisopropylethylamine was added to give a clear solution. NMR-scale silylation reactions with **3a** (R = H) were analyzed by ^{31}P NMR, which revealed a mixture of major peaks at 11.8 and 150.4 ppm in a 3:1 ratio, along with a number of smaller peaks at 49.0, 45.6, 32.5, and 28.3 ppm. We assign the major peak at 11 ppm to the *O*-silyl *H*-phosphinate ester **A** and the smaller peak at 150 ppm to the *O*,*O*-bistrimethylsilyl phosphonite **B**.[13] ^{1}H NMR analysis still shows the distinctive doublet of the P−H bond (6.85 ppm, J_{PH} = 525 Hz), which is presumably *O*-silyl *H*-phosphinate ester **A**. Also, trimethylsilyl peaks at 0.25 ppm indicate some *N*-silylation, suggesting that some of the intermediates **A** and **B** may have been *N*-silylated (Scheme 2, x = 1, y = 1). The mixture of silylated intermediates **A** and **B** was treated in situ with a slightly less than 1 equiv (based on starting amino acid) of Fmoc-Cl.[14] After consumption of the Fmoc-Cl, the reaction mixture was subjected to a simple aqueous workup where the phosphinates are extracted into aqueous $NaHCO_3$, washed with Et_2O, and then precipitated from the aqueous phase by addition of HCl to pH ∼2, to afford the corresponding products **5a**−**c** as white powders in high yields (85−88%), which were of high purity as judged by NMR and elemental analysis. While we are unsure of what all of the components of the silylation reaction are, the high yields and purity of resultant Fmoc-protected amino phosphinates **5** suggest that the majority of the silylated products are converted to the desired material.

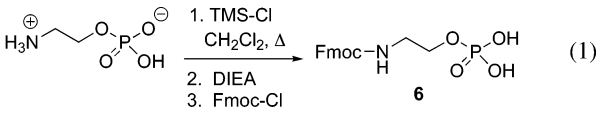
(11) Birkofer, L.; Ritter, A. *Angew. Chem., Int. Ed. Engl.* **1965**, *4*, 417−429. Kricheldorf, H. R. *Liebigs Ann. Chem.* **1972**, *763*, 17−38.

(12) Bolin, D. R.; Sytwu, I. I.; Humiec, F.; Meienhofer, J. *Int. J. Pept. Protein Res.* **1989**, *33*, 353−359.

(13) We assign the peak at 11 ppm as **A** in analogy to literature compounds Me_3SiO-P(O)(H)CH_2NR_2, ^{31}P NMR δ ∼17 ppm (Prishchenko, A. A.; Livantsov, M. V.; Lorents, K. L.; Grigor'ev, E. V.; Luzikov, Yu, N. *Zh. Obsh. Khim.* **1994**, *64*, 1577−1578) and the peak at 150 ppm as **B** in analogy to $(Me_3SiO)_2PCH_2NR_2$, ^{31}P NMR δ ∼152 ppm (Prishchenko, A. A.; Livantsov, M. V.; Pisarnitskii, D. A.; Shagi-Mukhametova, N. M.; Petrosyan, V. S. *Zh. Obsh. Khim.* **1990**, *60*, 699−701). Smaller peaks in the range of 28−50 ppm are consistent with the phosphinate anhydride species that may have formed under the silylation conditions. For examples of mixed phosphinate anhydride species, see: Plack, V.; Schmutzler, R. *Z. Anorg. Allg. Chem.* **1999**, *625*, 497−502.

(14) The purity of the Fmoc-Cl should be rigorously checked.

To test the potential generality of this method for other types of amino phosphorus acids, we utilized the phosphate ester of ethanolamine as a substrate under identical conditions as for the phosphinate amino acids (eq 1). While it is not clear what



(1)

the structure of the silylated intermediate is in this case, an *N*,*O*-bis(trimethylsilyl) or an *O*,*O*-bis(trimethylsilyl) phosphate derivative, as before the reaction became homogeneous after addition of the DIEA and Fmoc reaction proceeded readily to give a high yield of the Fmoc amino phosphate **6**. Presumably this silylation strategy would work for amino-protection of other amino acids including amino sulfonates, amino sulfates, and amino phosphonates. It is a practical advance for the synthesis of protected *H*-phosphinate amino acids both in terms of yields, ease of workup, and purity of final products. Also, since silylation can be carried out under very mild (nonacidic) conditions (e.g., bistrimethylsilylacetamide; see ref 12), this method may be applicable to the synthesis of protected trifunctional phosphinate amino acids such as side-chain-protected glutamic acid or lysine phosphinate analogues.

These detailed procedures for the synthesis of phosphinate amino acids and the use of the in situ silylation procedure for carbamate protection should provide ready access to Fmoc-protected amino phosphinates that are valuable in a variety of biological applications such as the synthesis of protease inhibitors.[15]

Experimental Section

1-Aminomethylphosphinic Acid (3a). *N*-Tritylmethanimine **1a** was prepared as described previously.[10] Ammonium phosphinate (2.6 g, 31.4 mmol) and hexamethyldisilazane (6.6 mL, 31.4 mmol) were heated together at 120 °C under argon for 2 h in a 100-mL round-bottom flask fitted with a heating mantle and condenser to prepare bis(trimethylsilyl) phosphonite **2** (31.4 mmol). After cooling to room temperature, the reaction was cooled in an ice bath, and CH_2Cl_2 (10 mL) was added to the in situ generated **2.** After an additional 5 min, a solution of **1a** (4.3 g, 15.7 mmol) in CH_2Cl_2 (25 mL) was then gradually injected at 0 °C, and stirring was continued at room temperature for 12 h. The solvent was removed under reduced pressure, and the residue was dissolved in 1 N HCl in MeOH (30 mL) and refluxed for 15 min. The solvent was evaporated under reduced pressure, H_2O (30 mL) was added, and the mixture was extracted with Et_2O (3 × 30 mL). The aqueous layer was retained for subsequent ion-exchange chromatography.

Dowex 50WX8-400 resin (~250 g) was placed in a Buchner funnel and activated by washing it with 2 N HCl until acidic (pH ~1). The resin was then washed with double distilled water until neutral (pH ~7) followed by 2 N NH_4OH until basic (pH ~14). The resin was then washed again with double distilled water until neutral (pH ~7). The whole process was repeated again. This washing procedure removes colored impurities and soluble polymer that are present in the matrix. This resin was loaded as a slurry into a large chromatography column (3 cm diameter). This column is suitable for purification of up to 50 mmol of crude amino phosphinate, but we use it routinely for the 15 mmol preparations as described here.

The neutral solution containing the crude amino phosphinate was then loaded onto the column packed with activated Dowex 50WX8-400. Eluent fractions were monitored by ^{31}P NMR. The column was then washed with double distilled water (300 mL), absolute ethanol (300 mL), and then again with water (300 mL). This wash removes unreacted hypophosphorus acid (^{31}P ~5 ppm; elutes in the aqueous phase) and any residual organics not removed by the extraction (ethanol fraction). Next, 2 N NH_4OH was added to the column, and 100 mL fractions were collected, which eluted first the desired phosphinate amino acid (^{31}P ~15−22 ppm) and subsequently the double addition product (^{31}P ~30 ppm). The aqueous eluate containing **3a** was evaporated and dried over P_2O_5 to provide a white powder. Yield: 43%. Mp: 274−275 °C (lit. mp 179−185 °C,[10] 254−256 °C[5]). ^1H NMR (250 MHz, D_2O): δ 7.1 (d, J_{P-H} = 540.0 Hz, 1H), 3.0 (d, J = 9.2 Hz, 2H). ^{31}P NMR (101 MHz, D_2O): δ 15.4. ESMS *m*/*z*: 96.0 (M + H, calcd 96.0), 118.1 (M + Na, calcd, 118.0). Anal. Calcd for CH_6NO_2P: C, 12.64; H, 6.36; N, 14.74. Found: C, 13.03; H, 6.14; N, 14.50.

1-(*N*-(9-Fluorenylmethoxycarbonyl)amino)-methylphosphinic Acid (5a). Finely ground amino phosphinate **3a** (47.5 mg, 5 mmol) was placed in a 100-mL round-bottom flask fitted with a heating mantle and condenser. The solid was suspended in anhydrous CH_2Cl_2 (20 mL) and stirred vigorously. TMS-Cl (1.27 mL, 10 mmol) was added in one portion and then refluxed for 2 h. The mixture was cooled in an ice bath, and DIEA (1.59 mL, 9.1 mmol) and Fmoc-Cl (1.20 g, 4.6 mmol) were added sequentially. The solution was stirred with cooling for 20 min and at room temperature for 6 h. The mixture was concentrated and then distributed between Et_2O (30 mL) and 2.5% $NaHCO_3$ (35 mL). The phases were separated, and the aqueous layer was extracted with Et_2O (5 × 30 mL). The aqueous layers were acidified to pH 2 with 6 N HCl. After standing for 24 h at 0 °C, the solid precipitate was filtered, washed with cold water (3 × 20 mL) and Et_2O (3 × 20 mL), and dried over P_2O_5 to provide **5a** as a white powder. Yield: 87%. Mp: 178−180 °C; ^1H NMR (250 MHz, CD_3SOCD_3): δ 7.9 (d, J = 7.5 Hz), 7.7 (d, J = 7.5 Hz), 7.4 (t, J = 7.4 Hz), 7.3 (t, J = 7.4 Hz), 6.9 (d, J_{P-H} = 539 Hz, 1H), 4.3 (m, 3H), 3.3 (m, J = 7.3 Hz, 2H). ^{13}C NMR (63 MHz, CD_3SOCD_3): δ 156.4, 143.8, 140.8, 127.7, 127.1, 125.2, 120.1, 65.9, 46.6, 40.7 (d, J_{P-C} = 103.3 Hz). ^{31}P NMR (101 MHz, CD_3SOCD_3): δ 25.1. ESMS *m*/*z*: 318.1 (M + H, calcd 318.1), 340.1 (M + Na, calcd 340.1). Anal. Calcd for $C_{16}H_{16}NO_4P$: C, 60.57; H, 5.08; N, 4.41. Found: C, 60.34; H, 5.13; N, 4.56.

Acknowledgment. We thank Dr. Dale Treleaven, Mr. Guangyu Li, Dr. Tracy McCarley, and Dr. Michelle Beeson for expert technical assistance. We are indebted to Prof. Gregg B. Fields of Florida Atlantic University for inspiring our work on phosphinate amino acids. This work was supported by a grant from the National Cancer Institute of the National Institutes of Health (CA98799) and an NSF IGERT (CHE-9987603) Fellowship (to JWK).

Supporting Information Available: General procedures and ^1H, ^{13}C, and ^{31}P NMR spectra for the compounds **3a**−**c**, **5a**−**c**, and **6**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO070266P

(15) Yiotakis, A.; Georgiadis, D.; Matziari, M.; Makaritis, A.; Dive, V. *Curr. Org. Chem.* **2004**, *8*, 1135−1158.