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A Practical Preparation of Diisopropyl Phosphoryl Protected Amino Acids.

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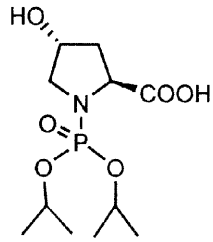
Abstract: *N*-Diisopropyl phosphoryl protected amino acids can be prepared in high yield and without racemization by slow addition of sodium hypochlorite to a solution of the amino acid and diisopropylphosphite in water while carefully maintaining a constant pH via the addition of sodium hydroxide.

Keywords: Amino acids and derivatives, Phosphoramidates, Protecting groups

As part of an ongoing drug development program we recently had a need for *N*-(*O,O*-diisopropyl phosphoryl)-*trans*-4-hydroxy-L-proline (**1**; DIPP-HyPro). The dialkyl phosphoryl group has been used only occasionally as an amine protecting group.[1] We were attracted to this protecting group since it had been reported that deprotection can be achieved under acidic conditions similar to those used to deprotect *tert*-butyl carbamates (BOC groups).[2,3] The DIPP protecting group offers particular advantages over the BOC group. Most importantly, diisopropylphosphite is a significantly cheaper reagent than di-*tert*-butyl dicarbonate in the protection step.



1

Several methods have been used to prepare dialkyl phosphoryl protected amino acids. *N,O*-Bistrimethylsilylation of amino acids followed by reaction with dialkylphosphorochloridates was recently reported.[4] Alternatively, several modifications of the classical Atherton and Todd[5] procedure have been reported. Most notably, Zhao's group has described the reaction of amino acids with diisopropylphosphite in a mixture of water, ethanol (or DMF), carbon tetrachloride and triethyl amine.[6] While this procedure provided **1** in good yield in the racemic series,[7] we considered this process impractical for scale-up since carbon tetrachloride is a known carcinogen. It is accepted that under the Atherton/Todd conditions dialkylphosphite **2** is chlorinated to give dialkyl phosphorochloridate **3** (Scheme 1).[5b] Reaction of **3** with the amino acid **4** produces the product **5**. A total of two equivalents of base are needed to accomplish these transformations with one equivalent presumably deprotonating the phosphite in the first step[8] and the other neutralizing the hydrogen chloride in the second step. It was our idea that the reaction of **2** with **4** to yield **5** could potentially be achieved under more practical conditions by the use of sodium hypochlorite which formally is a combination of a chlorinating agent and a base.

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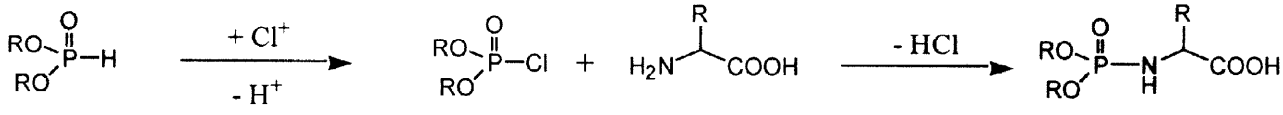
Scheme 1



2 **3** **4** **5**

In this letter we would like to report our discovery of exceedingly practical and general conditions for the high yielding preparation of diisopropyl phosphoryl protected amino acids under aqueous conditions via the reaction of diisopropylphosphite[9] with sodium hypochlorite in the presence of the amino acid. Optimal reaction conditions for protection of *trans*-4-hydroxy-L-proline were defined. Solutions of sodium hypochlorite and sodium hydroxide are added simultaneously to an ice-cold solution of the amino acid and diisopropylphosphite in water. While "household bleach" (a 5.25 wt% solution of sodium hypochlorite in water) is very effective under these conditions, the use of more concentrated sodium hypochlorite solutions (10-20 wt% NaOCl) was preferred in order to avoid undesirable dilution of the reaction.

Reactions were run maintaining the pH within different ranges. It was found that at pH<9.0 and pH>9.5 yields were lower than in the pH 9.0-9.5 range. It is possible that at pH<9.0 phosphoramidate formation is considerably slower due to significant conversion of sodium hypochlorite to hypochlorous acid (pKa 7.54) which has a different oxidation potential.[10] On the other hand, at pH>9.5 hydrolysis of the intermediate diisopropyl phosphorochloridate is presumably becoming competitive to phosphoramidate formation. The same trend was also observed for several other amino acids (*vide infra*). The pH during the reaction can be accurately controlled via the addition rates of the bleach and sodium hydroxide. For optimum reaction control we preferred to use a pump for addition of sodium hypochlorite at constant rate and a pH stat to control the addition of sodium hydroxide and maintain a constant pH.

The following observations are of interest to obtain optimal reaction performance. Sodium hypochlorite solutions, particularly at higher concentrations, are not stable indefinitely. Therefore, these solutions were titrated iodometrically shortly before use. Aged sodium hypochlorite solutions with decreased titers performed equally well in the reaction as long as the charge was based on iodometric titration. When the pH is kept constant in the optimal range (9.0-9.5) identical reaction yields are obtained regardless of the absolute addition rates of sodium hypochlorite and sodium hydroxide provided that the temperature is maintained in the 0-5 °C range. The reaction is quite exothermic and efficient cooling is important particularly when the more concentrated sodium hypochlorite is used. At higher temperatures reaction yields decrease, presumably because diisopropyl phosphorochloridate hydrolysis becomes competitive. On 5 g scale we were able to add the sodium hypochlorite over 1 h while maintaining the temperature at 0-2°C and the pH at 9.0-9.2, without any problem. The end of the reaction is conveniently determined. When all diisopropylphosphite has been consumed, the addition of more sodium hypochlorite leads to upward drift of the pH and a change of the color of the reaction mixture from colorless to faintly yellow. Upon scaling of a reaction using 20 wt% sodium hypochlorite it was found that efficient mixing of the reaction is critical. This is indeed expected from a reaction wherein a fast reacting reagent is added in high concentration.

Under the optimal conditions *N*-(*O,O*-diisopropyl phosphoryl)-*trans*-4-hydroxy-L-proline (**1**) is produced in 80-90% yield according to an HPLC assay of the crude reaction mixture. Diisopropylphosphite, sodium hypochlorite and sodium hydroxide are consumed in near stoichiometric quantities (1.1, 1.1 and 1.0 mol equivalents relative to the amino acid, respectively) under these conditions. Innocuous sodium chloride and water are formed as the only side-products. We were able to secure multikilogram quantities of **1** using this procedure.

We decided to probe the generality of this novel synthetic method by preparing several other diisopropyl phosphoryl (DIPP) protected amino acids under essentially identical conditions as used for **1**. Table 1 shows the results. For most entries the optimum pH window for the reaction was reexamined. In all of those cases it was found that optimum yields are achieved in the same pH 9.0-9.5 window. In most cases the products could be crystallized after an extractive workup (DIPP-Trp was precipitated as its DCHA salt from diethyl ether; entry 6). It was found that high concentration solutions of DIPP protected amino acids are generally rather unstable when held at RT and therefore the crystallizations were carried out in the cold. The rather low isolated yields in a few cases are more a reflection of poor (unoptimized) crystallization efficiency than of poor reaction performance. The reaction assay yields were uniformly good with the exception of cysteine (entry 5). As expected for this amino acid, sulfide oxidation competes with DIPP protection and as a result DIPP protected cystine was isolated in mediocre yield (even when 2 molequivalents of diisopropylphosphite are used in the reaction). However, tryptophan which is also oxidation sensitive yielded the expected product in good yield (entry 6).

Table 1: Preparation of DIPP protected amino acids using the optimum general procedure.

entry	amino acid [a]	product [a,b]	assay yield in % [c]	isolated yield in % [d]	m.p. in °C [e]	$[a]_D$ [f]
1	L-Ala	DIPP-Ala	80	35	118-120 (110-112)[6]	-5 (-7)[6]
2	L-Glu	DIPP-Glu	72	61	65-68 (73-75)[6]	+2 (-4)[6]
3	L-Phe	DIPP-Phe	86	36	64-66 (50-52)[6]	+7 (+6)[6]
4	L-PhGly	DIPP-PhGly	86	61	130-132	+103
5	L-Cys	DIPP-Cystine	57	44	166-171 (166-168)[11]	-68[g]
6	L-Trp	DIPP-Tryp•DCHA		82	172-175	-4
7	L-Pro	DIPP-Pro	93	57	58-60 (62-64)[6]	-49 (-63)[6]
8	L-Hyp	DIPP-Hyp	89	80	94-95	-45

a. Abbreviations: Ala = alanine; Glu = glutamic acid; Phe = phenylalanine; PhGly = phenylglycine; Cys = cysteine; Trp = tryptophan; Pro = proline; Hyp = 4-hydroxyproline; DCHA = dicyclohexylamine.
b. All products showed satisfactory ^{1}H, ^{13}C and ^{31}P spectra.
c. As determined before workup by reversed phase HPLC.
d. Yield of pure product after workup and crystallization.
e. Melting points are not corrected. Literature values, if available, are given in parenthesis.
f. All rotations were measured at ambient temperature in EtOH (c=1) unless indicated otherwise. Literature values under similar conditions, if available, are given in parenthesis.
g. Rotation measured at ambient temperature in MeOH/H_2O 1/1 (c=1).

It was determined that DIPP protection can be achieved without any racemization under the described conditions by close examination of the product of entry 4. Phenylglycine is considered to be the most racemization prone amino acid of those examined. SFC analysis of the methyl ester corresponding to the isolated (S)-(+)-DIPP-phenylglycine using a chiral stationary phase showed that the enantiomeric purity of this product was >99.5% e.e.[12]

In conclusion, we have developed an exceedingly practical method for the racemization-free preparation of diisopropyl phosphoryl protected amino acids.

Procedure for the preparation of *N*-(*O,O*-diisopropyl phosphoryl)-*trans*-4-hydroxy-L-proline:

trans-4-Hydroxy-L-proline (200 g) was dissolved in 500 mL of water at ambient temperature. The solution was cooled to 0-5 °C and the pH was adjusted from 5-6 to 9 by adding a 25% sodium hydroxide solution (18 mL). Diisopropylphosphite (280 g) was added in one portion. Sodium hypochlorite (20 wt%; 640 mL; concentration determined at 2.6 mol/L via iodometric titration) was added over 2.5 h to the resulting mixture while maintaining the pH at 9 using a 25% sodium hydroxide solution. The temperature was controlled at 0-5°C throughout. Any excess bleach was then quenched via addition of sodium bisulfite (30 g). The pH of the resulting solution was adjusted to 2 by slow addition at 0-5 °C of conc. hydrochloric acid (230 mL). Sodium chloride (170 g) was added and the resulting solution was extracted with cold *iso*-propyl acetate (2 x 2 L). The organic extracts were combined (DIPP-HyPro was assayed at 89% yield using HPLC) and partially concentrated *in vacuo*. The crystal slurry was flushed with fresh *iso*-propyl acetate. The crystals were filtered, washed and dried *in vacuo* at RT to yield 359 g of the product (80% isolated yield).

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