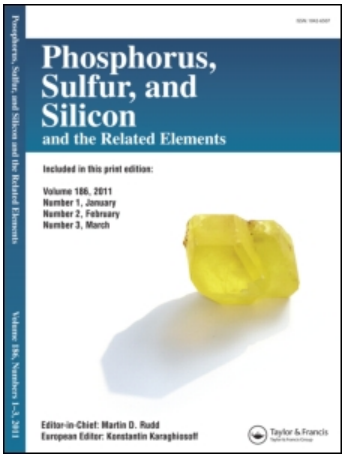

Phosphorus, Sulfur, and Silicon and the Related Elements
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713618290

THE CHEMICAL PROPERTIES OF N-(O,O-DIISOPROPYL)PHOSPHORYL-ARGININE

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To cite this Article Li, Yong-Fang , Wang, Qian , Zhao, Yu-Fen , Ji, Gai-Jiao and Li, Yuan-Chao(1995) 'THE CHEMICAL PROPERTIES OF N-(O,O-DIISOPROPYL)PHOSPHORYL-ARGININE', Phosphorus, Sulfur, and Silicon and the Related Elements, 106: 1, 131 — 136
To link to this Article: DOI: 10.1080/10426509508027898
URL: http://dx.doi.org/10.1080/10426509508027898

Phosphorus, Sulfur, and Silicon, 1995, Vol. 106, pp. 131–136
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THE CHEMICAL PROPERTIES OF N-(O,O-DIISOPROPYL)PHOSPHORYL-ARGININE

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(Received January 5, 1995; in final form May 23, 1995)

N-(O,O-diisopropyl)phosphoryl-arginine (DIPP—Arg) **3** and N-(O,O-diisopropyl)phosphoryl-arginine methyl ester (DIPP—Arg—OMe) **4a** were synthesized. In compound **3** the co-participation of the three functional groups of the phosphoryl, guanidine and carboxyl was essential for the ester exchange reaction at the phosphoryl group and esterification at carboxyl group. In contrast, when the carboxyl group was blocked, as in compound **4a**, no corresponding reaction occurred. A mixed anhydride penta-coordinate phosphorus transition state was proposed for the self-activation mechanism.

Key words: Phosphorylated amino acid, N-phosphorylated arginine, ester exchange on phosphate.

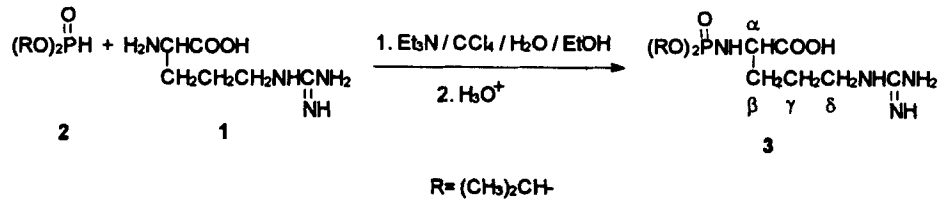
INTRODUCTION

A novel synthesis of N-phosphorylated amino acids was found by Zhao *et al.*,[1] and peptide formation, esterification and ester exchange reactions of these compounds were studied.[2-3] In terms of basic amino acids, such as arginine, histidine and lysine, there were some problems in obtaining the pure phosphoryl products. Therefore, compound **3** was synthesized through a modified method, and its properties were studied.

RESULTS AND DISCUSSIONS

Since there were two basic functional groups such as amino and guanidine groups present in arginine **1**, if diisopropylphosphite (DIPPH) **2** was in excess, both amino groups were phosphorylated. The ratio of DIPPH **2** and arginine **1** was maintained at 1:1, and the reaction temperature was kept below $-10°C$ for at least 30 minutes. Only the α-amino group was phosphorylated (Scheme 1).

Compounds **4a** and DIPP—Ala[1] **9** were also synthesized. The purity and structure of compounds **3** and **4a** were determined by fast atom bombardment mass spectrometry (FAB-MS), HRFAB-MS, ^{1}H-NMR, ^{31}P-NMR and ^{13}C-NMR.



SCHEME 1. Synthesis of DIPP—Arg 3

Ester Exchange and Esterification Reactions

A solution of compound **3** in 1-butanol was kept at 40°C for 20 hr. The FAB-MS of the mixture indicated that in addition to the molecular peak at $(M + 1)^+/z$ 339 for compound **3**, there were several new signals at $(M + 1)^+/z$ 395, 409, 423, 353, 367, which corresponded to the products from mono- and di-ester exchange at the phosphoryl group, i.e., N—(iPrO)(BuO)P(O)—Arg **5b** and N—$(BuO)_2P(O)$—Arg **6b**, together with the esterification products DIPP—Arg—OBu **4b**, N—(iPrO)(BuO)P(O)—Arg—OBu **7b**, N—$(BuO)_2P(O)$—Arg—OBu **8b**, respectively (Table I). These compounds showed overlapping ^{31}P-NMR signals at 6.27, 7.36, 8.46 ppm. According to the calculated ^{31}P-NMR shifts for N-phosphoryl amino acids,[7] the ^{31}P-NMR shifts for compounds **3**, **5b** and **6b** should be 6.27, 7.27 and 8.27 ppm, respectively. Since the ^{31}P-NMR shifts of N-phosphoryl amino acid and its ester were almost the same, they gave an overlapping signal. The relative amount of each type of compound were reported as the title ^{31}P-NMR peak intensity.

Similarly, after keeping freshly prepared compound **3** in methanol at 40°C for 20 hours, the compounds listed in Table II also showed overlapping ^{31}P-NMR shift values at 6.45, 8.80, 11.18 ppm, respectively. These deviated somewhat from the calculated values of 6.45, 8.25, 10.05 ppm. All these reactions are shown in Scheme 2.

Mechanistic Study

When DIPP—Arg—OMe **4a** was dissolved in 1-butanol or methanol and kept at 40°C for 20 hr, the ^{31}P-NMR spectrum indicated that no similar reaction occurred.

TABLE I

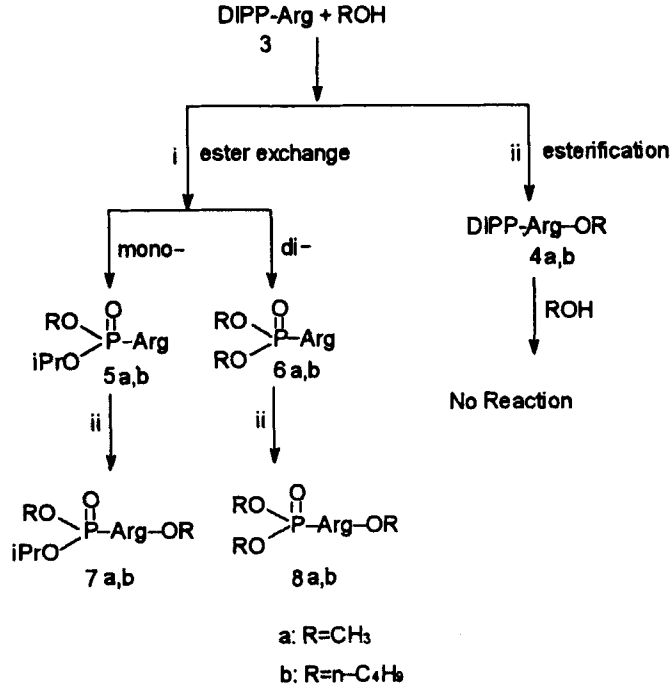
The FAB-MS and ^{31}P-NMR data of DIPP—Arg 3 after reacted with 1-butanol at 40°C for 20 hr

$(M+1)^+/z$	^{31}P-NMR (ppm) & relative amount	possible compd.	ester exchange	esterification
339	6.27 (28.2%)	DIPPArg		
395=339+56		DIPPArgOBu		ester
353=339+14	7.36 (44.0%)	(BuO)(iPrO)P(O)Arg	mono-	
409=353+56		(BuO)(iPrO)P(O)ArgOBu	mono-	ester
367=339+28	8.46 (27.8%)	$(BuO)_2P(O)$Arg	di-	
423=367+56		$(BuO)_2P(O)$ArgOBu	di-	ester

TABLE II

The FAB-MS and ^{31}P-NMR data of DIPP—Arg **3** after reacted with methanol at 40°C for 20 hr

$(M+1)^+/z$	^{31}P-NMR (ppm) & relative amount	possible compd.	ester exchange	esterification
339	6.45 (38.9%)	DIPPArg		
353=339+14		$DIPPArgOCH_3$		ester
311=339-28	8.80 (40.8%)	$(CH_3O)(iPrO)P(O)Arg$	mono-	
325=311+14		$(CH_3O)(iPrO)P(O)ArgOCH_3$	mono-	ester
283=339-56	11.18 (20.3%)	$(CH_3O)_2P(O)Arg$	di-	
297=283+14		$(CH_3O)_2P(O)ArgOCH_3$	di-	ester



SCHEME 2. The reaction of DIPP—Arg **3** with alcohol

It might be deduced that the co-participation of the phosphoryl and carboxyl groups in DIPP—Arg **3** was essential for the phosphoryl ester exchange and esterification reactions. Furthermore, the carboxyl group was an intramolecular catalytic group. If the carboxyl group were acting only as the acid, it would be expected that simple phosphoamidate DIPP—NH—Bu^t **12** should undergo the same reactions in the presence of acetic acid. However, with acetic acid addition into the solution of compound **12** in methanol, the ^{31}P-NMR spectra showed that no reaction occurred after 20 hr at 40°C. Therefore, it implied that the catalysis must be intramolecular, not a simple acidic catalysis reaction.

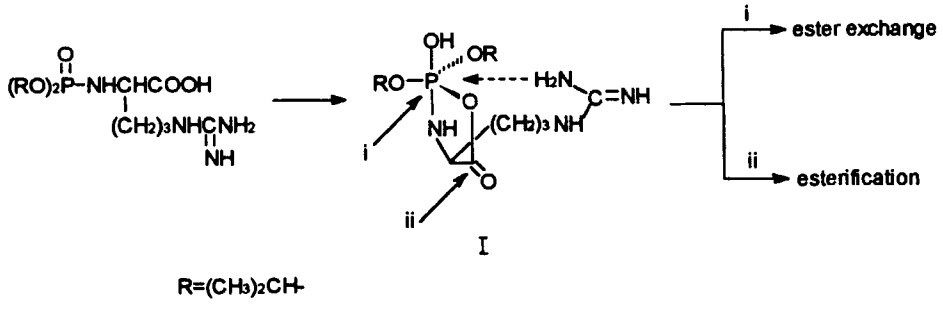
In addition, the guanidine group also participated as an intramolecular catalytic

group, because a simple phosphoryl amino acid without a side chain functional group, such as DIPP—Ala **9** had much lower reactivity. Furthermore, on addition of guanidine to the solution of DIPP-Ala **9** in methanol, there was no intermolecular base catalysis. On the contrary, the ester exchange and esterification reactions were completely inhibited. This implies that the guanidine group was not a general base catalysis, but an intramolecular catalytic group.

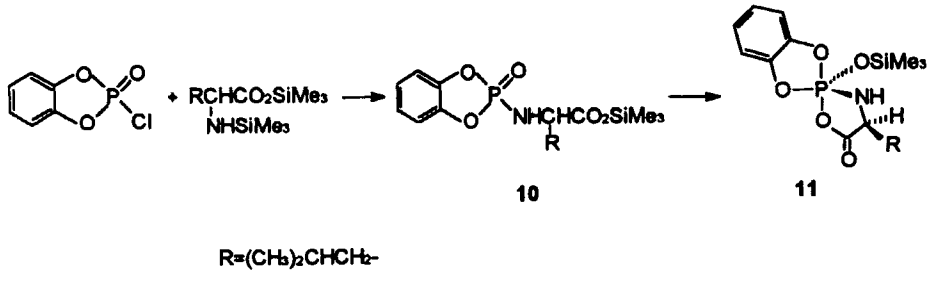
To account for these facts, the co-participation of all three groups in the DIPP—Arg **3** is proposed for the esterification and ester exchange reactions (Scheme 3). The transition state was a pentacoordinate phosphorus compound, and it could be subsequently activated by the intramolecular guanidine group.

In this reaction, the lifetime of the transition state was too short to be trapped by ^{31}P-NMR spectroscopy. In the following reaction, a model compound, leucine derivative **10**, with a stabilizing trimethylsilyl group $(Me)_3SiO$— was found to be transferred into the pentacoordinate product **11** completely (Scheme 4). Compound **11** was stable and its structure was identified. But for DIPP—Arg **3**, the derivative corresponding to compound **11** could not be trapped by ^{31}P-NMR yet. However, the identification of compound **11** might give some support to the proposed mechanism.

DIPP—Leu **13** could also undergo the same reactions as DIPP—Arg **3** at a much lower reactivity, because there was no side chain catalytic group in compound **13**.



SCHEME 3. Proposed transition state for the reaction of DIPP—Arg **3** with alcohol



SCHEME 4. Synthesis of transition state (I) analogue—compound **11**

CONCLUSION

As mentioned above, since the phosphoryl group in N—DIPP—Arg—OMe **4b** was inert, neither the esterification nor ester exchange occurred. However, in DIPP—Arg **3** with a free carboxyl to activate the phosphoryl group, together with an intramolecular guanidine catalytic effect, the phosphoryl ester exchange and esterification reactions were possible. Esterification and ester exchange at the phosphoryl group are fundamental biochemical reactions. Esterification of the amino acid is the first step in protein bio-synthesis. Ester exchange at the phosphoryl group is the key of the self-catalysis of RNA, and this has been postulated to relate to the foundation of biocatalysis.[4–6] In many cases enzymes are activated by a phosphorylation mechanism, and the position of phosphorylation in protein was found to be mostly in the arginine, histidine, serine, threonine and tyrosine residues. The results of this paper might provide a clue to arginine's side chain catalytic function in enzymes.

EXPERIMENTAL

Methods

The ^{1}H-NMR, ^{13}C-NMR and ^{31}P-NMR spectra were taken on a JEOL-JNM FX-100 and BRUKER AC200P spectrometer. The positive-ion FAB-MS data were obtained on a KYKY ZHP-5 double-focusing mass spectrometer, equipped with a standard KYKY fast atom gun. Infrared spectra were determined with a Carlzeiss, Jena Sepeord 751R instrument. EI and HR EI-MS data were taken on a ZAR-HS GC-MS spectrometer.

Preparation of N-(diisopropylphosphoryl)-arginine **3** (DIPP—Arg)

To an ice-salt cooled solution of arginine mono-hydrochloride (2.107 g, 10 mmol) in Et_3N (7 ml), H_2O (10 ml) and EtOH (5 ml) a mixture of diisopropyl phosphite **2** (1.66 g, 10 mmol) and CCl_4 (5 ml) was added dropwise. The mixture was stirred at −15°C for 2 hr and R.T. for 30 min. The mixture was evaporated in vacuum and the residue washed with ether (2 × 20 ml). The water layer was adjusted to pH 3–4 by 1.5 N HCl and extracted with 1:2 terbutyl alcohol-ethyl acetic ester (5 × 20 ml). The combined extracts were washed with saturated aqueous NaCl, dried ($MgSO_4$) and concentrated in vacuum. A colorless solid product **3** was obtained, yield 72%. ^{31}P-NMR: 6.11 ppm (BuOH—CH_3COOEt). ^{1}H-NMR: (d_6-DMSO) 1.28 (d, 12H, J = 6 Hz, 4 × CH_3), 1.60 (m, 4H, β,γ—CH_2), 3.17 (m, 2H, δ—CH_2), 3.55 (m, 1H, NH—P), 4.52 (m, 3H, CH—O and α—CH), 7.28 (m, 3H, H_2N—C=NH), 7.92 (m, 1H, —C—NH), 11.5 (s, 1H, —COOH). ^{13}C-NMR (d-DMSO): 23.6 (—CH_3), 37.8 (β—CH_2), 31.3 (γ—CH_2), 55.0 (δ—CH_2), 67.4 (α—CH), 69.8 (—CH—O), 157.5 (—NH—C=NH), 177.0 (—COOH). Positive FAB-MS: $(M + 1)^+/z$ = 339. IR: 1652, 1438, 1388, 1000, 3400, 3320, 3240, cm^{-1}. HRFAB-MS: molecular formula $C_{12}H_{27}N_4PO_5$, $(M + 1)^+/z$ = 339.1797 (calculated), 339.1794 (measurement).

Preparation of N—DIPP—Arg—OMe **4b**

To an ice-salt cooled solution of Arg—OMe.2HCl (1.3 g, 5 mmol) in methanol (10 ml) and Et_3N (2.8 ml) a mixture of diisopropyl phosphite **2** (1 ml, 6 mmol) and CCl_4 (10 ml) was added dropwise. The mixture was stirred for 10 hr, then evaporated. The residue was dissolved in saturated aqueous NaCl and washed with petroleum ether (3 × 20 ml). Then the water layer was extracted with ethyl acetate ester (3 × 20 ml), and the combined extracts were washed with saturated aqueous NaCl and dried ($MgSO_4$) and concentrated in vacuum. A colorless oil **4b** was obtained, yield 85%. ^{31}P-NMR: 5.36 ppm. ^{1}H-NMR: (d_6-DMSO) 1.32(d, 12H, J = 6 HZ, 4 × CH_3), 1.77 (m, 4H, β, γ—CH_2), 3.32 (m, 2H, δ—CH_2), 3.75 (s, 3H, —OCH_3), 3.8 (m, 2H, α—CH and NH—P), 4.54 (m, 2H, CH—O), 7.28 (m, 3H, H_2N—C=NH), 7.92 (m, 1H, —C—NH). ^{13}C-NMR (d-DMSO): 23.6(—CH_3), 40.7 β—CH_2), 33.5 (γ—CH_2), 52.3 (δ—CH_2), 53.5 (α—CH), 71.6 (—CH—O), 157.2 (—NH—C=NH), 173.3 (—COOH), 77.6 (—OCH_3). IR (film): 3156, 1740, 1665, 1220, 990 cm^{-1}. FAB-MS: $(M + 1)^+/z$ = 353. HRFAB-MS: for molecular formula $C_{13}H_{29}N_4PO_5$, $(M + 1)^+/z$ = 353.2001 (measurement), 353.1983 (calculated).

Preparation of compound **11**

All operation was under protection of nitrogen. 0.4 ml 0.5 M N,O,O-tri(methylsilyl) leucine[8] in CCl_4 and 0.2 ml 1M O,O-phenylene phosphochloridate[9] in CCl_4 were injected into a 5 mm NMR tube. The tube was sealed immediately and the reaction was traced by ^{31}P-NMR. First, the phosphoryl amino acid **10** was formed, (^{31}P-NMR: 21.3 ppm) and then it was transferred into the penta-coordinate product **11** completely as checked by ^{31}P-NMR. The solvent was removed in vacuum and the colorless oil product **11** was obtained, yield almost 99%. ^{31}P-NMR: −45.1, −45.6. ^{1}H-NMR: 0.43 (s, 9H), 0.91–1.02 (q, 6H), 1.71–1.82 (m, 1H), 3.83–3.91 (m, 1H), 4.56 (s, 1H), 6.75–7.04 (m, 4H). ^{13}C-NMR: 0.82, 21.71, 23.01, 25.18, 42.86, 53.35, 100.86, 110.38, 111.16, 111.95, 120.27, 123.30, 169.50. EI(m/z): 357 ($C_{15}H_{24}NO_5PSi$). HR EI-MS: 357.1165 (measurement), 357.116 (calculated).

Preparation of DIPP—Ala **9**, DIPP—NH—Bu **12**, DIPP—Leu **13** is referred to reference 1.

Acetic acid action on DIPP—NH—Bu **12**

60 mg (1 mmol) acetic acid was added into 1 ml 0.5 M compound **12** solution in methanol, then the solution was kept at 40°C for 20 hr. ^{31}P-NMR spectra showed that there was no reaction occurred.

Guanidine action on DIPP—Ala **9**

60 mg(1 mmol) guanidine was added into 1 ml 0.5 M compound **9** solution in methanol, then the solution was kept at 40°C for 20 hr. ^{31}P-NMR spectra showed that the ester exchange and esterification reactions were completely inhibited.

ACKNOWLEDGEMENTS

This work was supported by the Chinese National Science Foundation, the National Science and Technology Committee of China and Tsinghua University.

REFERENCES

1. G. J. Ji, C. B. Xue, J. N. Zeng, L. P. Li, W. G. Chai and Y. F. Zhao, *Synthesis*, **6**, 444 (1988).
2. Y. M. Li, D. Q. Zhang, H. W. Zhang, G. J. Ji and Y. F. Zhao, *Bioorganic Chemistry*, **20**, 285 (1992).
3. Y. M. Li and Y. F. Zhao, *Phosphorus, Sulfur and Silicon*, **7**, 15 (1993).
4. G. J. Zaug and T. R. Cech, *Science*, **231**, 470 (1986).
5. B. L. Bass and T. R. Cech, *Nature*, **308**, 820 (1986).
6. G. J. Zaug and T. R. Cech, *Cell.*, **19**, 331 (1986).
7. Y. Chen, Y. F. Zhao, Y. W. Yin and X. Q. Yang, *Phosphorus, Sulfur and Silicon*, **61**, 31 (1991).
8. T. A. Khwaja, C. B. Reese and J. C. M. Swewart, *J. Chem. Soc. (C)*, 2092 (1970).
9. E. D. Smith and H. Sheppaed, *Nature*, **208**, 878 (1965).