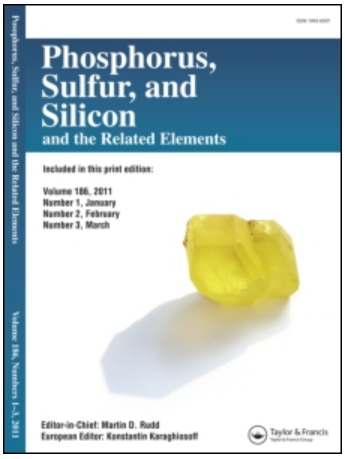

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Qing-Jin Yan[a]; Qian Wang[a]; W-Fen Zhao[a]
[a] Bioorganic Phosphorus Chemistry Laboratory, Department of Chemistry, Tsinghua University, Beijing, P.R. China

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KINETICS OF PHOSPHORYL TRANSFER REACTIONS OF PHOSPHOAMINO ACIDS AND ESTERS IN THE PRESENCE OF IMIDAZOLE

QING-JIN YAN, QIAN WANG and YU-FEN ZHAO*

Bioorganic Phosphorus Chemistry Laboratory, Department of Chemistry, Tsinghua University, Beijing, 100084, P.R. China

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In the presence of imidazole N-phosphoamino acids were prompted to undergo ester exchanges on the phosphorus as well as N to O migration 10–38 times faster than in its absence. Only phosphoserine and threonine methyl esters, were activated by imidazole to give similar reactions. For the diastereomeric N-phosphonoserine methyl ester containing a phosphorus-carbon bond, a stereoselective reaction occurred to provide an O-phosphonoserine derivative with an isomeric ratio of 65 to 35. The mechanism may involve the formation of pentacoordinate intermediates promoted by imidazole.

Key words: Phosphoryl transfer reaction, imidazole, stereoselectively, N-phosphoamino acids.

INTRODUCTION

It is known that many enzymes' activities are regulated through phosphorylation and dephosphorylation mechanisms. In most cases the active sites of enzymes consist of amino acid residues, such as imidazolyl on histidine, hydroxyl on serine or threonine, and a carboxyl function on aspartic acid or glutamic acid.[1,2] For example, acetylchiolinesterase (AChE) was inhibited by organophosphorus compounds through the phosphorylation of the hydroxyl group on serine by the assistance of an imidazolyl group on histidine.[3] It would be useful to understand the intrinsic relationships between the imidazole, hydroxyl, carboxyl functional groups and phosphorus.

It has been shown that imidazole can catalyze many biological and chemical reactions involving organophosphorus compounds, such as, cleavage and isomerization of a dinucleotide,[4] syntheses of polypeptides,[5] retardation of the aging rate of phosphorylated enzymes,[6] as well as enantioselective hydrolysis of esters of amino acids.[7] This paper presents the kinetics of ester exchange and phosphoryl transfer reactions of N-phosphoryl amino acids and the related esters in the presence of imidazole.

RESULTS AND DISCUSSION

Imidazole Activation of Phosphoamino Acids

It was found that when N-phosphoamino acids were incubated in alcohol at 40°C, two types of phosphoryl transfer reactions took place: intermolecular ester exchange and intramolecular N to O migration.[8,9] Our kinetic results showed that the extent of the reactions changed as the amino acid side chains of the phosphoamino acids **2**–**11** was varied. The rate constants for the disappearance of compound **2**–**11** ranged

TABLE I

Rate of disappearance of N-phosphoryl amino acids and methyl esters incubated in 1-butanol at 40°C without or with the presence of imidazole[a]

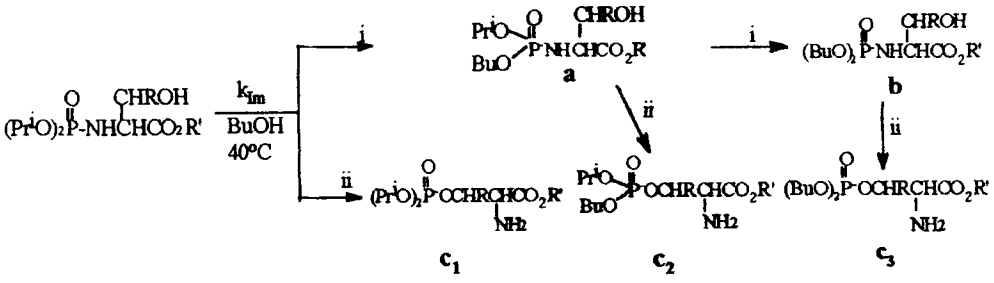
Compd.	without imidazole $k(10^{-6}, s^{-1})$	$t_{1/2}$(h)	with imidazole $k_{Im}(10^{-6}, s^{-1})$	$t_{1/2}$(h)	ratio k_{Im}/k
1 DIPP-β-Ala[b]	no reaction		no reaction		
2 DIPP-Pro	0.4	481	0.5	385	1.2
3 DIPP-Gly	0.1	1925	2.1	92	21
4 DIPP-Leu	0.7	275	26	7.3	38
5 DIPP-Tyr	1.2	160	44	4.4	36
6 DIPP-Phe	1.8	107	30	6.4	17
7 DIPP-Ala	1.9	100	19	10	10
8 DIPP-Ser	2.8	69	31	6.2	11
9 DIPP-Asp	3.1	62	63	3.0	20
10 DIPP-Thr	4.1	47	67	2.9	16
11 DIPP-His	11.9	16	124	1.5	10
12 DIPP-AlaOMe	no reaction		no reaction		
13 DIPP-TyrOMe	no reaction		no reaction		
14 DIPP-CysOMe	no reaction		no reaction		
15 DIPP-$NHCH_2CH_2OH$	no reaction		no reaction		
16 DIPP-SerOMe	no reaction		3.0	64	
17 DIPP-ThrOMe	no reaction		34	6.0	
18 MIPP-SerOMe[c]	no reaction		35	5.5	
19 MIPP-AlaOMe	no reaction		no reaction		
20 MIPP-TyrOMe	no reaction		no reaction		

Note: a. The original concentration C_0=1.0M, [reactant]/[imidazole] =1:2, k and k_{im} represent rate of disappearance of sample without and with the presence of imidazole, respectively, deviation = 6-10%;

b. DIPP=O,O-diisopropyl phosphoryl; c. MIPP=O-isopropyl methylphosphonyl.

from $0.7-11.9 \times 10^{-6}$ s^{-1}, with half lives of 16–1925 hours. Compounds **8–10**, whose side chains contained a hydroxyl and carboxyl, were significantly faster than the others. DIPP-His **11** was the most reactive compound with a reaction rate one hundred fold faster than the glycine derivative, due to the presence of an intramolecular imidazolyl group. What is the origin of the intermolecular imidazole effect?

Our experiments indicated that phosphoryl transfer reactions of most phosphoamino acids **3–11** were accelerated when imidazole was added to the solution under the same conditions. The rates of disappearance k_{Im} were about 10–38 times faster than their self activated reactions k (Table I). If imidazole were replaced by other organic bases such as triethylamine, the reactions were completely inhibited. Therefore, a general base catalysis mechanism was not responsible for the enhanced reactivity of DIPP-His **11**. To rationalize this phenomena, imidazole participation was



i: Intermolecular ester exchange; ii: Intramolecular N to O migration

SCHEME I The phosphoryl transfer reaction products of N-phosphoserine and threonine and related esters as incubated in 1-butanol at 40°C in the presence of imidazole.

TABLE II

^{31}P-NMR chemical shifts (ppm) of some N-phosphoamino acids and methyl esters as incubated in 1-butanol at 40°C in the presence of two equivalent of imidazole

Compd.	Time(h)	Reactant(%)	Ester exchange(%)		Migration(%)	Other
			a	b	c	product(%)*
8 DIPP-Ser	5	6.6(55.6)	7.7(21.0)	8.8(3.2)	-0.5(4.0)	5.7(3.0)
					-1.6(1.5)	-9.7(7.0)
					-2.6(2.8)	-8.8(1.9)
10 DIPP-Thr	2.5	7.7(55.0)	8.8(21.7)	9.9(5.0)	-0.7(12)	/
					-2.7(6.3)	
16 DIPP-SerOMe	16	6.6(86.0)	7.7(14.0)	/	/	/
17 DIPP-ThrOMe	16	7.2(15.7)	8.2(16.7)	9.4(5.6)	-2.4(42.0)	/
					-0.5(20.0)	
18 MIPP-SerOMe	16	33.6(7.8)	/	/	23.2(21.9)	15.9(7.6)
		34.3(7.8)			24.3(43.7)	1.1(13.2)

*5.7ppm(phosphopeptide), -9.7 and -8.8ppm(pyrophosphate), 15.9ppm(methylphosphonic acid), 1.1ppm(pyrophosphonate).

postulated. The structures of the phosphoamino acids products catalyzed by imidazole were confirmed by ^{31}P-NMR and FAB-MS spectra. These were the same as the self-activated phosphoamino acid products (Scheme I and Table II).

The reaction rates k_{Im} were influenced by the relative concentrations of reactants and imidazole. It was also noticeable that changing the concentration of imidazole caused k_{Im} to vary (Figure 1). Without imidazole the three N-phosphoamino acids **7–9** had similar reaction rates k. However, when two molar equivalents of imidazole were added, the k_{Im}'s differences were enhanced. For example, the k_{Im} values for phosphoalanine **7** increased slowly from 1.1×10^{-5} to 2.1×10^{-5} s^{-1} as the relative concentration of imidazole changed from 0.5 to 4 molar equivalent, while for phosphoserine **8**, 0.5 molar equivalent of imidazole was the maximum effective amount

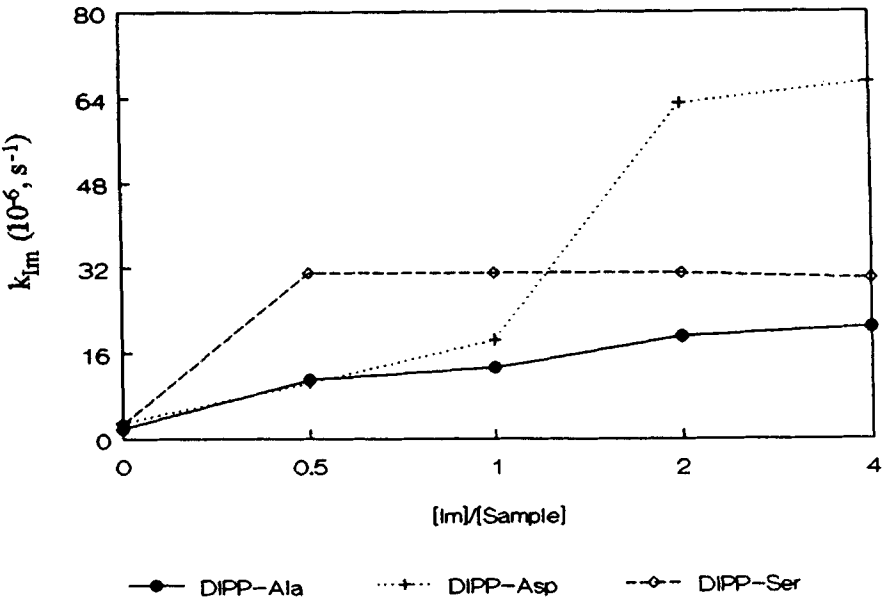


FIGURE 1 The relationship between the concentration of imidazole and rate k_{Im}.

for the promotion. However, for phosphoaspartic acid **9**, it took four molar equivalents of imidazole to reach the maximum acceleration effect, and the change from one to two molar equivalent imidazole caused a three fold enhancement in the rate, k_{Im}. These diverse phenomena indicate much more complicated chemical interactions between the imidazole and various amino acids side chains. The detailed mechanism is under further investigation.

Imidazole Activation on Phosphoserine and Threonine Methyl Esters

The more complicated cases were the hydroxyl amino acids serine and threonine ester derivatives **16**–**17**, which were promoted by the imidazole to undergo inter and intra phosphoryl transfer reactions (Table II). The non-hydroxyl amino acid analogues phosphoalanine ester **12** and phosphocysteine ester **14** were not influenced by the presence of imidazole. Phosphotyrosine ester **13**, in which the hydroxyl group is on the side chain phenyl group, was not affected by imidazole (Table I). Perhaps only the neighboring hydroxyl group is able to co-participate in the reaction. To differentiate this structural effect, a model compound **15** without the carboxyl group but with a hydroxyl group on the same carbon as the phosphoserine ester **16** was tested. It was found that there was no imidazole promotional effect. So, not only a proper distance of hydroxyl group but also a carboxyl group were essential for this promotion.

Similarly, most diastereomeric N-phosphonoamino acid methyl esters containing a phosphorus carbon bond were also stable.[10] When they were incubated in 1-butanol at 40°C with imidazole, only the diastereomeric N-(O-isopropyl methylphosphonyl)serine methyl ester **18** was converted into O-phosphonyl serine derivative without any ester exchange product (Table II). Unexpectedly the two diastereomers

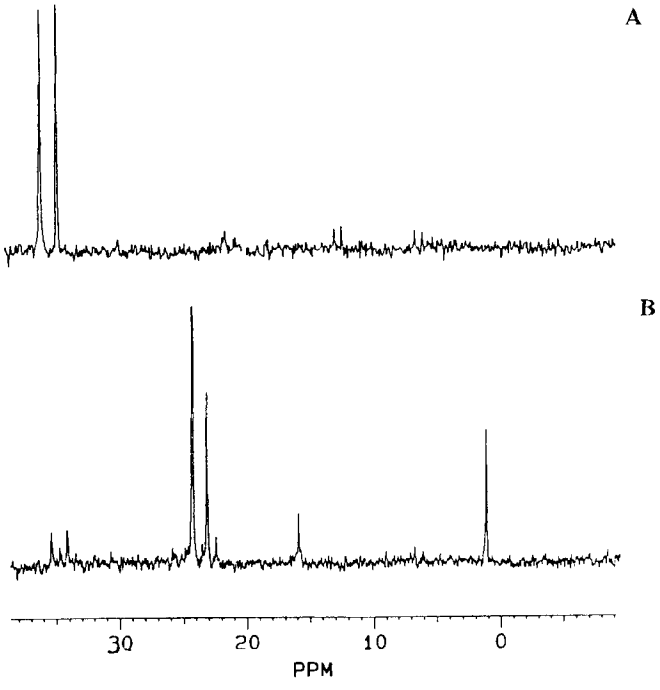
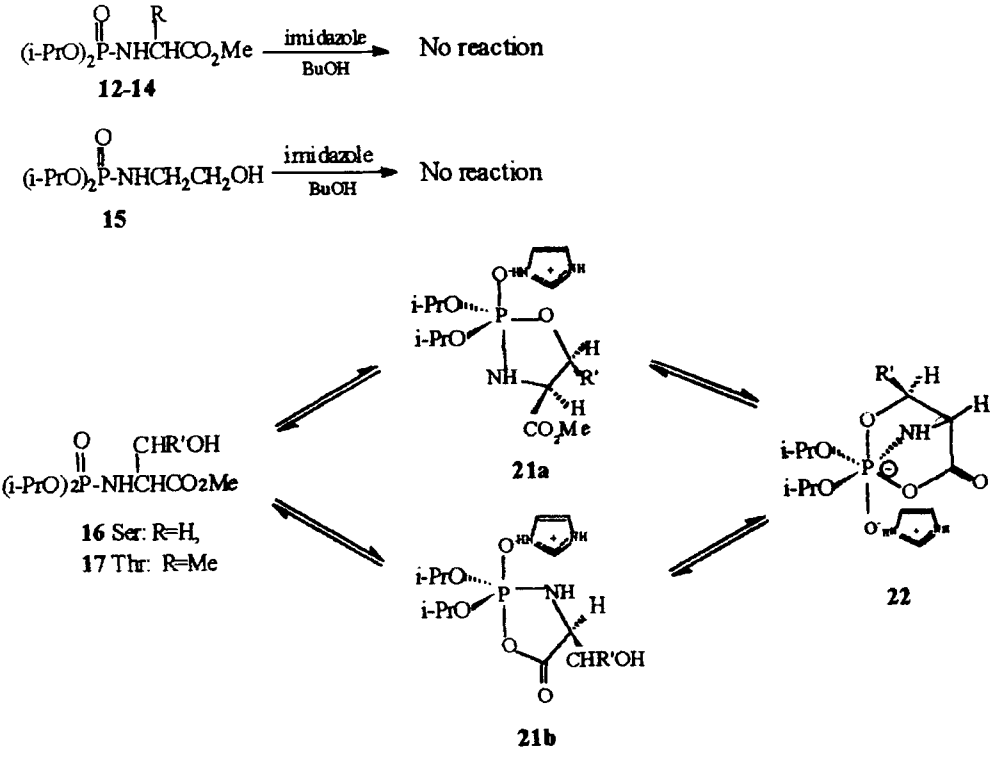


FIGURE 2 ^{31}P-NMR spectra of intramolecular phosphoryl transfer of N-(O-isopropyl methylphosphonyl) serine methyl ester **18**. (A) represents diastereomeric compound **18** (δ 33.6, 34.3 ppm); (B) **18** is incubated in 1-butanol at 40°C for 16 hours. New signals indicate O-(O-isopropyl methylphosphonyl) serine ester (δ 23.2, 24.3 ppm), methylphosphonic acid (δ 15.9 ppm) and pyrophosphonate (δ 1.1 ppm).

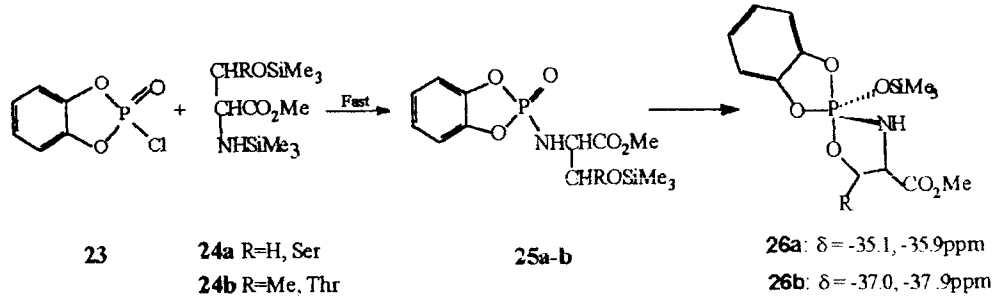
of the O-substituted product were unequal, with a ratio of 65 to 35 (Figure 2). This indicated that there was a stereoselective phosphonyl transfer reaction of diastereomeric phosphonoserine ester in the presence of imidazole. The analogue of the threonine ester was not capable of synthesis due to instability.

Activation Mechanism

Considering these observations, the imidazole activation on the phosphoserine and threonine esters required the co-participation of the carboxyl and hydroxyl group simultaneously. To account for the facts, the imidazole action on phosphoserine and threonine estersis outlined in Scheme II, in which two pentacoordinate phosphorus intermediates **21a** and **21b**, formed by nucleophilic attacks of activated carboxyl group and side chain hydroxyl group onto the phosphorus atom, respectively, are proposed. Further a hexacoordinate phosphorus intermediate **22** is postulated. To support intermediate **21a**, some model pentacoordinate phosphorus compounds were synthesized as shown in Scheme III. Model compounds with a structure similar to **21b** had been reported.[11]



SCHEME II Proposed pentacoordinate phosphorus intermediate of phosphoserine and threonine methyl esters in the presence of imidazole.



SCHEME III Formation of **26a** and **26b** with a structure similar to **21a**.

The tetracoordinate phosphoamino acid ester **25a**, which formed immediately by the reaction of N,O-disilylated serine methyl ester **24a** with O,O-phenylene phosphochloride **23**, was completely transformed into the spiro pentacoordinate phosphorus compound **26a** in about half an hour. Compound **26a** appeared as a doublet at −35.1 and −35.9 ppm in the ^{31}P NMR spectrum due to formation of a pair of diastereoisomers.

A similar roped reaction were observed when the N,O-disilylated threonine methyl ester **24b** was used as starting compound, which reacted more rapidly. But for other amino acids, such as cysteine and tyrosine methyl ester, no product formed as evi-

dences by the observe and signal at −35 ppm. This indicated that a β-hydroxyl group on serine or threonine might participate in the formation of the pentacoordinate phosphorus intermediate **21**.

CONCLUSION

Imidazole activation of phosphoryl transfer reactions of N-phosphoamino acids and esters might go through nucleophilic attack of hydroxyl group on phosphorus to give a pentacoordinate intermediate. This was supported by the syntheses of spiro pentacoordinate phosphoranes which possess similar structures. The phosphonoserine ester containing P—C bond was more reactive than DIPP-SerOMe in the presence of imidazole, which could undergo intramolecular N → O phosphonyl migration stereoselectively.

EXPERIMENTAL

^{31}P-, ^{13}C- and ^{1}H-NMR spectra were recorded on a Brucker ACP-200 spectrometer. Chemical shifts of ^{31}P-NMR are referenced to external 85% H_3PO_4. ^{1}H-NMR and ^{13}C-NMR are referenced to internal tetramethylsilane (TMS) and $CDCl_3$, respectively. Positive and negative ion FAB-MS data were obtained on a KYKY Zhp-5 double focusing mass spectrometer from the Scientific Instrument Factory, Beijing, China. EI HR-MS data were taken from a ZAR-HS GC-MS spectrometer.

Syntheses of Phosphoamino Acids **1–11** *and Their Methyl Esters* **12–20**

According to the methods reported by Y. F. Zhao *et al.*[9,10,12]

Tracing the Ester Exchange Reactions of N-phosphoamino Acids and Esters in the Presence of Imidazole

Samples (10 mmol), imidazole (20 mmol) were dissolved in 10 ml 1-butanol, and then incubated at 40°C. The relative concentrations of each sample and products were determined by integration of each ^{31}P-NMR spectra. The disappearance rates k and k_{im} of the starting compounds were calculated using the equation; $\ln C_0/C = k \cdot t$ by the least square method.

Identification of the Products

All ester exchange and N to O migration products were checked by ^{31}P NMR, FAB-MS data. ^{31}P NMR shifts of the mono- and di-ester exchanged products in 1-butanol shifted about δ 1.1 ppm to low magnetic field with positive FAB-MS data at M + 1 + 14 and M + 1 + 28, respectively,[8,9] and the N to O migration products were at the chemical shifts of −0.5 to −2.5 ppm.[8] The above products were then confirmed through the syntheses of authentic samples as follows.

N-(O-isopropyl, O-butyl phosphoryl) Serine **8a**

A solution of serine (10 mmol) in triethylamine (25 mmol), water (5 ml) and ethanol (5 ml) was cooled to 0°C. A mixture of O-isopropyl O-butyl phosphochloride (1 mmol) and CCl_4 (5 ml) was added dropwise with stirring at 0°C for 2 hours. The reaction was quenched by acidifying the mixture to pH 3 with 1N HCl and extracted with ethyl acetate/*t*-butanol (3:2). The extract was dried by $MgSO_4$, then evaporated to afford an oily product (yield 60%).

^{31}P-NMR (BuOH) 7.7 ppm, ^{13}C NMR ($CDCl_3$, ppm, JHz), 13.5 (Me), 18.6 (CH_2), 23.5 (CH_2, 7.4 Hz), 32.1 (Me, 7.3 Hz), 65.8 (OCH_2, 5.9 Hz), 71.9 (OCH, 4.4 Hz), 56.0 (NCH), 64.1 (OCH_2, 2.9 Hz), 173.8 (C=O, 8.8 Hz). FAB HR-MS for $C_{10}H_{22}NPO_6$ m/z: 282.1086 (M-1, requires 282.1105).

N-(O,O-dibutyl phosphoryl) Serine **8b**

According to the methods reported by Y. F. Zhao *et al.*[12]

O-(O,O-diispropyl phosphoryl) Serine **8c**

N-(O,O-diisopropyl phosphoryl) serine **8** (10 mmol) was heated in EtOAc at 40°C until a solid formed. The solid portion was separated carefully and purified by crystallization in DMSO/petroleum ether, to give a 50% yield of product.

^{31}P-NMR (buoy) −2.5 ppm, ^{13}C NMR ($CDCl_3$, PPM, JHz) 31.8 (Me), 71.5 (OCH, 5.9 Hz), 61.8 (NCH), 55.0 (OCH_2, 2.9 Hz), 174.7 (C=O). FAB-MS for $C_{10}H_{22}NPO_6$ m/z: 284 (M + 1, 60%), 242 (M + 1 − C_3H_6, 40%), 200 (M + 1 − $2C_3H_6$ 100%).

Formation of Pentacoordinate Phosphorus Compounds **26a**

To a 0.4 ml 0.5 M solution of N,O-dimethylsilyl serine methyl ester **24a**[13] in dry $CHCl_3$ protected under a dry nitrogen atmosphere in a 5 mm NMR tube was added a 0.2 ml 1 M solution of O,O-phenylene phosphochloride **23** in dry CCl_4, and traced by ^{31}P NMR. The pentacoordinate phosphorus product **26a** was formed completely as confirmed by ^{31}P NMR. The crude product was then sent for the EI HR-MS determined by ZAR-HS GC-MS spectrometer.

^{31}P NMR ($CDCl_3$) −35.1, −35.9 ppm;
^{1}H NMR ($CDCl_3$) 0.01 (s, 9H, $SiMe_3$), 3.57–3.62 (m, 1H, α-CH), 3.66 (s, 3H, OMe), 3.86–4.21 (t, 2H, β-CH_2), 6.57–6.89 (m, 4H, C_6H_4).
^{13}C NMR ($CDCl_3$) 0.74 (SiMe), 51.8 (CH_2), 60.4 (CH), 54.9 (OMe), 109.2 (C_6H_4), 109.8 (C_6H_4), 122.4 (C_6H_4), 123.4 (C_6H_4), 142.3 (C_6H_4), 145.6 (C_6H_4), 175.0 (COOH).
EI HR-MS: $C_{13}H_{20}NO_6PSi$ 345.0784 (requires 345.0799).

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