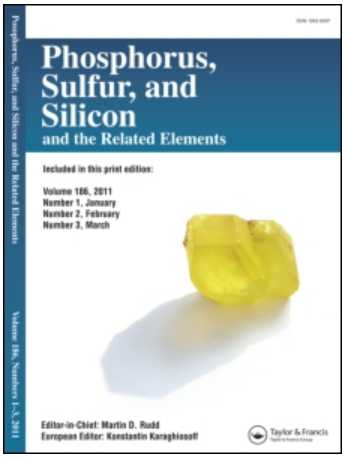

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PHOSPHORYLATION OF TYROSINE AND THE REACTIVITY OF DIALKYL PHOSPHITES WITH TYROSINE

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New methods of synthesizing N-phosphoryltyrosine and N,O-bisphosphoryl tyrosine ester were studied. The reactivity of dialkyl phosphites with tyrosine was also studied.

Key words: Phosphorylation, tyrosine, dialkyl phosphite.

INTRODUCTION

The pharmacological properties of derivatives of N-phosphorylamino acids and low-molecular weight peptides have stimulated investigation of the synthesis of these compounds. Several methods for their synthesis are known. A series of phosphorylated amino acids and low-molecular weight peptides were synthesized in one step using various dialkyl phosphites as the phosphorylating reagent in mixed aqueous media.[1–3] The phosphoryl tyrosine is of great pharmaceutical and biological interest. It is important to synthesize the phosphoryl tyrosine in order to study its chemical, biological and pharmacological properties.

RESULTS AND DISCUSSION

Synthesis of N-phosphoryltyrosine 2

Tyrosine was phosphorylated in a water and triethylamine medium and then extracted by ethyl acetate, as described in the literature[1–3] (Scheme I). The reactant tyrosine could not be dissolved in the reaction system completely and there was none or little expected product, N-phosphoryl tyrosine 2 (Table I (1)). When the amount of triethylamine was increased to five millilitres, tyrosine still could not be dissolved completely (Table I, (2)). When the amount of triethylamine reached 10 ml, the reactant can be dissolved well. After reaction, there was still none or little expected product (Table I (3)). When N-phosphorylaspartic acid 3 was synthesized, it was found that the phase transfer catalyst, such as *n*-tetrabutylammonium bromide was essential.[4] After comparing the solubility of aspartic acid and tyrosine in

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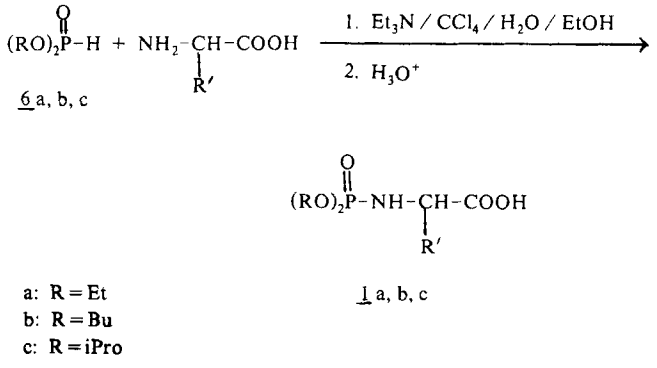


SCHEME I Previous method to synthesize the N-phosphoryl amino acids.

water, the same phase transfer catalyst was used in the N-phosphorylation of tyrosine. From Table I (4), it was shown that the function of *n*-tetrabutylammonium bromide was significant. The yield of N-diisopropylphosphoryl tyrosine (DIPP-Tyr 2c) reached 57.7%.

In our previous study, ethanol was used to make water and the other organic compounds (e.g. triethylamine, carbon tetrachloride, and dialkyl phosphite) miscible. When ethanol was omitted, the yield of the expected product 2 was low and the purity was poor (Table I, (5)). Ethanol not only can make the reaction system miscible, but also has some other function. Further work was needed to clarify this.

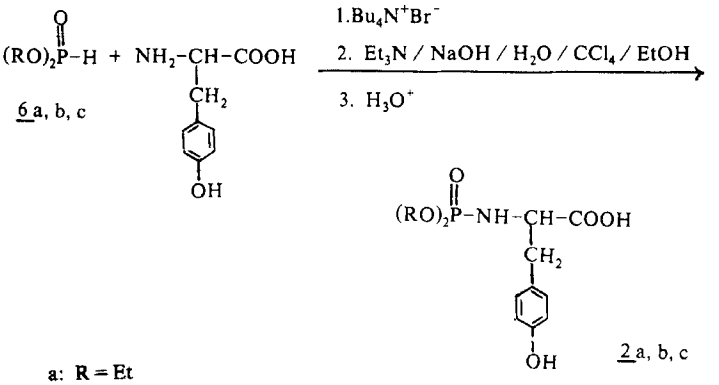
Method (4) in Table I needed a large amount of triethylamine, if a stronger base (e.g. NaOH) was chosen, a smaller amount of base is expected. NaOH gave poor results (Table I, (6)). When a mixture of Et_3N and NaOH was used, the pure N-phosphoryltyrosine 2 was easily obtained with higher yield (Table I, (7)). The new synthetic method for N-phosphoryltyrosine 2 is shown in Scheme II and is described in detail in the experimental section.

TABLE I

Methods to synthesis N-phosphoryl tyrosine 2c

Method	Entity							Solubility	Yield (%)
	L-Tyr (mmol)	DIPPH* (mmol)	H_2O (ml)	EtOH (ml)	Et_3N (ml)	4N NaOH (ml)	$Bu_4N^+Br^-$ (g)		
(1)	3	3.6	5	5	1.7	0	0	poor	~0
(2)	3	3.6	5	5	5	0	0	poor	~0
(3)	3	3.6	5	5	10	0	0	good	~0
(4)	10	10	10	10	35	0	0.32	good	57.7
(5)	10	10	10	0	35	0	0.32	good	impure
(6)	10	10	10	10	0	10	0.32	good	20.0
(7)	10	10	5	5	10	1	0.32	good	63.8

Notes: DIPPH 6c: diisopropyl phosphite



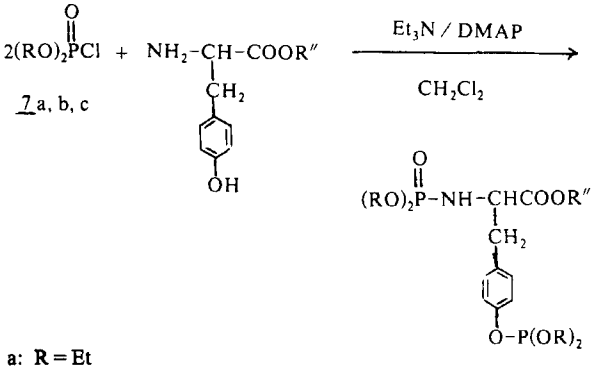
a: R = Et
b: R = Bu
c: R = iPro

SCHEME II New method to synthesize N-phosphoryl tyrosine.

Synthesis of N,O-bisphosphoryl Tyrosine Ester 4

When one mol of tyrosine was dissolved in the $Et_3N/NaOH/H_2O/CCl_4/EtOH$ system and 2 mol diisopropyl phosphite (DIPPH, 6c) was added as described above, N,O-bisphosphoryl tyrosine 5 is synthesized with $(MH)^+/z = 510$ and ^{31}P-NMR: 5.01 ppm, −8.67 ppm. The yield of the expected product 5 was low and N-phosphoryl tyrosine 2 was the main product. The ratio of N-phosphorylated and N,O-bisphosphorylated product in the mixture was generally about 6:1. Increasing the amount of dialkyl phosphite 6 always leads to the formation of some other by-product, e.g. polyphosphate.

When tyrosine was esterified, higher N,O-product ratio was obtained using the same procedure. There still exists some percentage of the N-phosphoryl product. After studying the properties of dialkyl phosphite 6, it was found that the dialkylphosphite/CCl_4/Et_3N system has higher N-selective ability.[5] Since dialkylphos-



a: R = Et
b: R = Bu
c: R = iPro

SCHEME III Synthesis of N,O-bisphosphoryl tyrosine.

phoryl chloride 7 is not so selective, it was chosen as the phosphorylation reagent. DMAP (dimethylaminopyridine) is a good O-acetylating catalyst which is widely used in organic synthesis. It has not been used in the phosphorylation reaction to date.[6-8] In order to synthesize the N,O-bisphosphoryl product, DMAP was used in the reaction system. The result showed that not only the expected product 4 was obtained in high yield, but also the reaction time was decreased. The reaction proceeded as shown in Scheme III.

Reactivity of Dialkyl Phosphites 6 with Tyrosine

In our study, we found that different dialkyl phosphites have different reactivity with tyrosine. When DEPH 6a (diethyl phosphite, ^{31}P-NMR: 6.78 ppm) was added into the reaction system, it changed to DEPCl 7a (diethyl phosphoryl chloride, ^{31}P-NMR: 3.88 ppm) immediately. After 5 min, there was only 14.3% DEPCl 7a left and the yield of N-DEP-Tyr 2a (N-diethylphosphoryl tyrosine, ^{31}P-NMR: 8.40 ppm) is 14.3%. After 85 min, none of the DEPCl 7a could be detected and the yield of N-DEP-Tyr 2a is only about 26.5% (Figure 1).

The reaction between DBPH 6b (dibutyl phosphite, ^{31}P-NMR: 6.84 ppm) and tyrosine was not as rapid as 6a. First, DBPH 6b changed into DBPCl 7b (dibutyl phosphoryl chloride, ^{31}P-NMR: 4.25 ppm) quickly. After 15 min, there was still 62.5% DBPCl 7b left and the yield of N-DBP-Tyr 2b (N-dibutylphosphoryl tyrosine, ^{31}P-NMR: 8.40 ppm) was only 6.2%. After 102 min, there was 8.8% DBPCl 7b left and the yield of N-DBP-Tyr 2b was 56.1%. DBPCl 7b did not decay completely until after 225 min. At that time, the yield of N-DBP-Tyr 2b was 74.2% (Figure 2).

The reaction between DIPPH 6c (diisopropyl phosphite, ^{31}P-NMR: 4.95 ppm) and tyrosine was the slowest one. First, DIPPH 6c changed into DIPPCl 7c (diisopropylphosphoryl chloride, ^{31}P-NMR: 1.67 ppm) immediately. There was still 85.7% DIPPCl 7c and the yield of N-DIPP-Tyr 2c (N-diisopropylphosphoryl tyrosine, ^{31}P-NMR: 6.40 ppm) was only 7.1% after 20 min. After 245 min, there was

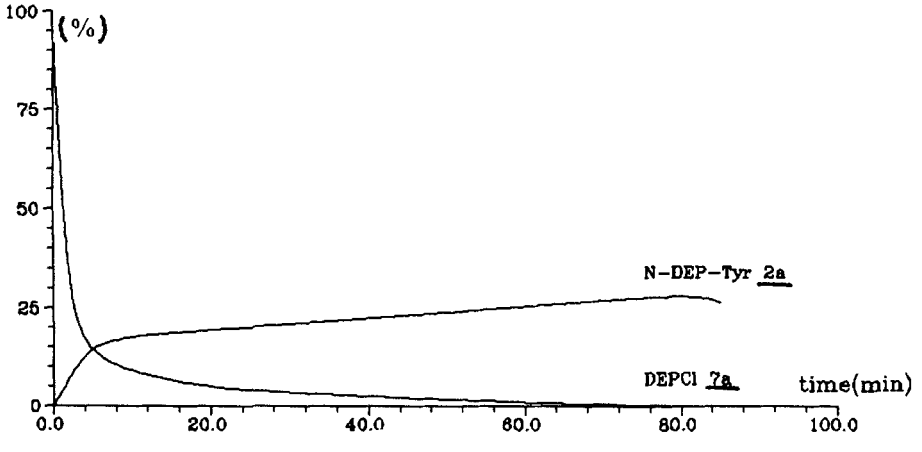


FIGURE 1 Reaction of diethyl phosphite 6a with Tyr.

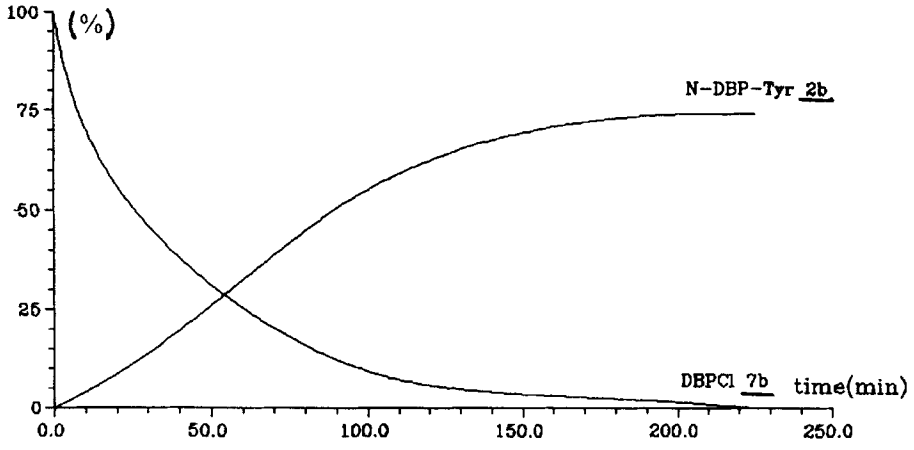


FIGURE 2 Reaction of dibutylphosphite 6b with Tyr.

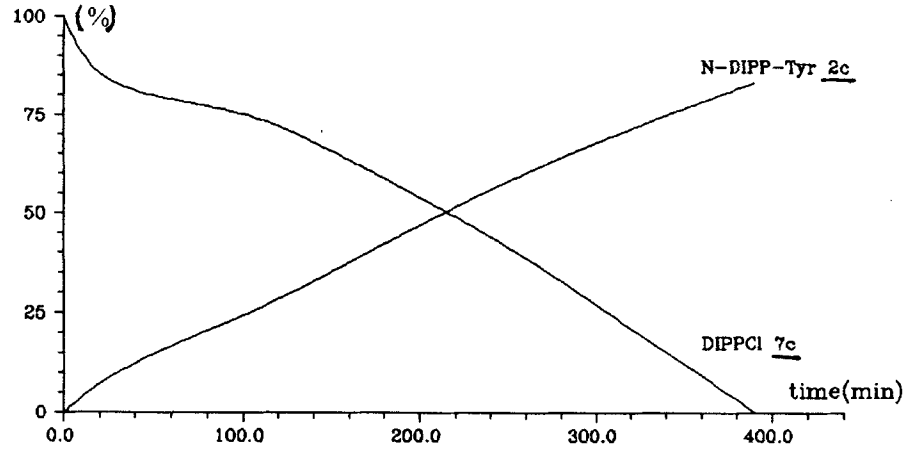


FIGURE 3 Reaction of disisopropyl phosphite 6c with Tyr.

42.8% DIPPCl 7c left and the yield of N-DIPP-Tyr 2c was 57.1%. DIPPCl 7c did not disappear completely until 390 min. At that time, the yield of N-DIPP-Tyr 2c was 83.8% (Figure 3).

From the results given above, it can be seen that the reactivity of DEPH 6a, DBPH 6b and DIPPH 6c with tyrosine is:

$$\text{DEPH} > \text{DBPH} > \text{DIPPH}$$

In order to compare the competition of these three dialkyl phosphites, a mixture of DEPH 6a, DBPH 6b and DIPPH 6c (1:1:1 mol/mol/mol) was added into the tyrosine reaction system. The result is listed in Figure 4. Because the ^{31}P-NMR shifts of N-DEP-Tyr 2a and N-DBP-Tyr 2b are almost the same, the two peaks overlapped at about 8.45 ppm. Figure 4 gives the total amount of N-DEP-Tyr 2a and N-DBP-Tyr 2b. From Figure 4, it can be seen that DEPCl 7a and DBPCl 7b decayed faster than DIPPCl 7c, and the total amount of N-DEP-Tyr 2a and N-

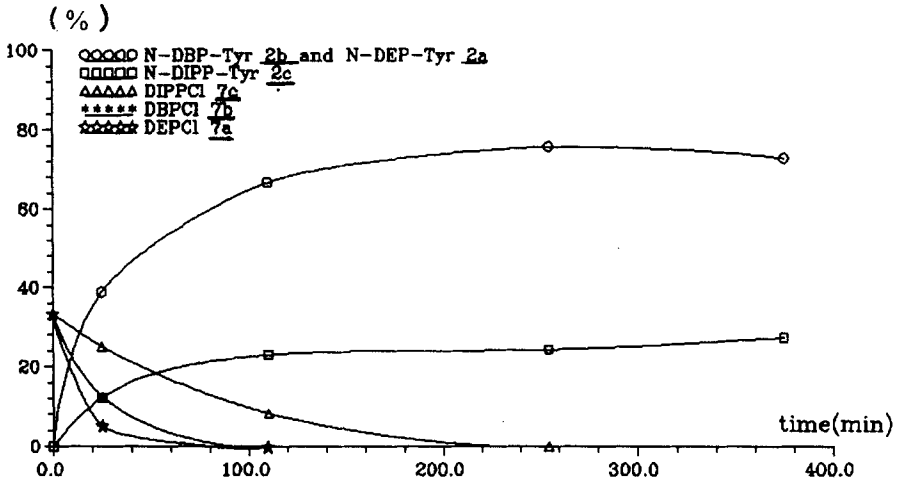


FIGURE 4 Reaction of mixed dialkylphosphites with tyrosine.

DBP-Tyr 2b was larger than the double value of N-DIPP-Tyr, 2c, e.g. after 25 min, the total amount of N-DEP-Tyr 2a and N-DBP-Tyr 2b was 38.8% and the amount of N-DIPP-Tyr 2c is only 12.2% (38.8% > 12.2% × 2). So we may come to the conclusion that DIPPH 6c has the lowest reactivity with tyrosine. The reactivity of DEPH 6a and DBPH 6b is hard to compare from this competition result. The comparison of DBPCl 7b and DEPCl 7a's decay may give some side evidence, i.e. DEPH 6a is more reactive than DBPH 6b.

CONCLUSION

Synthesis of N-phosphoryl tyrosine 2 and N,O-bisphosphoryl tyrosine ester 4 needed different reaction mediums and phase transfer catalyst or DMAP as catalyst. Different dialkyl phosphites have different reactivity with tyrosine. DEPH 6a has the highest reactivity, DBPH 6b is second one while DIPPH 6c reacts slowest with tyrosine.

EXPERIMENTAL

The ^{31}P-NMR, ^{1}H-NMR and ^{13}C-NMR spectra were taken on JEOL FX-90Q, JEOL FX-100 and Bruker AC-200 spectrometer. The ^{31}P-NMR shifts used 85% phosphoric acid as the external reference. The ^{13}C-NMR spectra used chloroform-d as the internal reference at 76.9 ppm. TMS was used as the internal standard for the ^{1}H-NMR spectra. Positive ion FAB-MS data were obtained on a KYKY zhp-5 double-focusing mass spectrometer from the Scientific Instrument Factory (Beijing, China) equipped with a standard KYKY fast atom gun. Infrared spectra were determined with Shimadzu 408 and PE-2000 system FTIR.

Preparation

Synthesis of compounds 6a, 6b, 6c, 7a, 7b, 7c were carried out according to the literature.[9] Synthesis of compound 1 was carried out according to the literature.[1] Synthesis of compound 3 was carried out according to the literature.[4] The preparation of tyrosine ester hydrochloride was carried out according to the literature.[10] Before use, the tyrosine ester hydrochloride was neutralized by triethylamine. All

physical constants and spectroscopic data of the products synthesized above agreed with the literature values.

Synthesis of N-phosphoryl Tyrosine 2

Take the synthesis of N-diisopropyl phosphoryl tyrosine 2c for example. A solution of 1.81 g L-tyrosine (10 mmol) and 0.32 g $(n\text{-}Bu)_4N^+Br^-$ (1 mmol) in Et_3N (10 ml), H_2O (5 ml), EtOH (5 ml), 4 N NaOH (1 ml) was cooled to 0°C. A mixture of 2.0 g diisopropyl phosphite (12 mmol) and CCl_4 (10 ml) was added dropwise at 0°C and the mixture was stirred for 10 hours at room temperature. Then 20 ml H_2O was added and the mixture was concentrated by water aspiration at 40°C. The residue was extracted with petroleum ether (20 ml × 2), ether (20 ml × 2) and ethyl acetate (20 ml × 2) successively. The water layer was acidified to pH = 4 with 1 M HCl. The mixture was then extracted with a mixed solvent of *t*-BuOH and EtOAc (1:1.5 vol/vol, 20 ml × 3). The combined extract was dried ($MgSO_4$) and evaporated under reduced pressure at a temperature lower than 40°C. A white powder (2.2 g) was obtained with the yield 63.8%. ^{31}P-NMR: 5.17 ppm. FAB-MS: $[MH]^+/z$ = 346. ^{13}C-NMR (ppm): 175.1 (d, J = 4.4 Hz, COOH), 55.5 (C_α), 39.3 (C_β), 155.4, 130.5, 126.7, 115.3 (C_γ), 72.9 (=CHO—), 23.2 (CH_3—). ^{1}H-NMR (ppm): 1.00–1.50 (d, 12H), 3.10–3.50 (br, 2H), 4.00–5.30 (m, 5H), 7.00–7.50 (m, 4H), 9.80–10.00 (br, 1H). IR (cm^{-1}): 3400, 3100, 3500–2350, 1730, 1600, 1380, 1200, 1100, 1000. 2a and 2b were synthesized by the same method 2a: yield 64.0%, ^{31}P-NMR: 7.24 ppm, FAB-MS: $(MH)^+/z$ = 318. 2b: yield: 64.2%, ^{31}P-NMR: 7.02 ppm, FAB-MS: $(MH)^+/z$ = 374.

Synthesis of N,O-bisphosphoryl Tyrosine Ester 4

Take the synthesis of N,O-bis(diisopropylphosphoryl) tyrosine methyl ester 4c for example. 1.16 g L-tyrosine methyl ester (5 mmol) and 20 mg DMAP was dispersed in Et_3N (10 ml) and CH_2Cl_2 (5 ml). A mixture of 2.2 g diisopropylphosphoryl choride (11 mmol) and 5 ml CH_2Cl_2 was added dropwise at room temperature. The mixture was stirred for another hour. The mixture was concentrated and then acidified to PH = 3 with 1 M HCl. The reaction mixture was extracted with EtOAc (10 ml × 3). The combined extract was washed with saturated NaCl solution and then dried ($MgSO_4$). After evaporation under reduced pressure at room temperature, a colorless oily product was obtained (2.2 g, yield: 84%). ^{31}P-NMR (ppm): 5.51, −7.57, FAB-MS: $(MH)^+/z$ = 524. ^{13}C-NMR (ppm): 172.5 (d, J = 4.4 Hz, COOH), 55.2 (C_α), 39.2 (C_β), 119.5, 130.2, 132.3, 149.5 (C_γ), 51.5 (—OCH_3), 70.5 (=CHO), 23.2 (CH_3), ^{1}H-NMR (ppm): 1.20–1.43 (m, 24H), 3.01 (d, 2H), 3.66 (s, 3H), 4.03–4.19 (m, 1H), 4.44–4.50 (m, 1H), 4.71–4.80 (m, 4H), 7.13–7.17 (m, 4H). IR (cm^{-1}): 3200, 2980, 1750, 1500, 1380, 1250, 1000. Elemental analysis: $C_{22}H_{39}NP_2O_9$, measure value: C 49.84, H 7.63, N 2.35, calculated value: C 50.48, H 7.46, N 2.68.

Reactivity of Dialkyl Phosphites 6 with Tyrosine

A solution of 0.36 g L-tyrosine (2 mmol) and 0.06 g $(n\text{-}Bu)_4N^+Br^-$ (0.16 mmol) in Et_3N (2 ml), H_2O (1 ml), EtOH (1 ml), 4 N NaOH (0.2 ml) was cooled to 0°C. A mixture of 2.4 mmol dialkyl phosphite 6 or the mixture of 0.8 mmol DEPH 6a, 0.8 mmol DBPH 6b and 0.8 mmol DIPPH 6c and CCl_4 (2 ml) was added immediately at 0°C. 0.6 ml of the reaction mixture was removed to NMR tube. The reaction was traced by ^{31}P-NMR.

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