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Convenient solid-phase synthesis of oligopeptides using pentacoordinated phosphoranes with amino acid residue as building blocks

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Abstract

The reactive intermediates of pentacoordinated phosphoranes with amino acids (P(5)-AA) as building blocks, which were obtained by the reaction of *O*-phenylene phosphorochloridate with *N*,*O*-bis(trimethylsilyl)amino acids, were linked to a solid-phase support containing a hydroxymethyl polystyrene functional group. The first amino acid residue was coupled to the solid-phase support after washing the resin with organic solvent. Repeating the procedure led to oligopeptides linked on the resin. A series of free oligopeptides including tetra-Gly, di-Val, tri-Val, di-Leu, di-Phe, and Phe–Leu were obtained after cleavage from solid-phase support. The structure of these oligopeptides were determined by IR, ^{1}H NMR, FAB-MS, and HPLC.

Keywords: Pentacoordinated phosphoranes; Amino acid; Oligopeptides; Solid-phase synthesis

1. Introduction

Peptide chemistry is of great importance in pharmaceutical research having a major impact on immunology such as in the preparation of synthetic vaccines and in the study of biologically active molecules like enzymes and hormones [1]. The chemical

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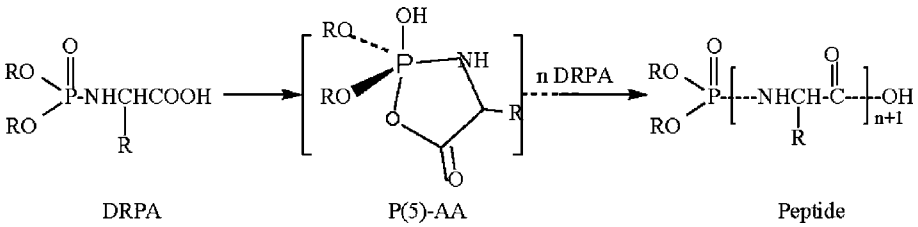


Scheme 1. Self-activation of *N*-phosphorylamino acids into peptides.

synthesis of large peptides has been accomplished by two distinct approaches. Efficient synthetic protocols to obtain pure peptides were first developed in solution (liquid-phase peptide synthesis LPPS) [2]. However, the stepwise addition of single amino acids was revolutionized by the Merrifield solid-phase technique (solid-phase peptide synthesis SPPS). Within the last thirty years this technique has assumed a prominent place in peptide synthesis [3,4].

In our previous work, we have shown that a series of *N*-phosphoryl amino acids formed oligopeptides in the absence of any kind of activator or coupling agent [5,6]. It has been reported that such organic phosphorous pentacoordinated phosphoranes are efficient intermediates which react with nucleophiles [7]. Some models have been proposed to explain the condensation process for peptide formation in aqueous media. An intermolecular mixed carboxylic phosphoric anhydride intermediate [pentacoordinated phosphorane with amino acid P(5)-AA] can exist in the conversion of *N*-dialkyloxy-phosphorylamino acid(DRPA) into oligopeptides (Scheme 1).

However, this intermediate could not be observed by any method in aqueous media because of its rapid hydrolysis [8]. In order to shed light on the reaction mechanism of peptide formation by *N*-phosphorylamino acid, we found that the *N*,*O*-bis(trimethylsilyl) amino acid (*N*,*O*-Bis-AA) could self-assemble into peptides by treatment with *O*-phenylene phosphochloridate (O-PPC) in the absence of any catalyst. The results showed that the pentacoordinated phosphoranes containing amino acid (P(5)-AA) were key reactive intermediates in the process of peptide formation [9–11]. In fact, under these conditions, it is not easy to control the length of oligopeptide. Usually, a series of mass numbers was found, which corresponded to oligopeptides ranging from monomers to different sized oligomers. For example, when reacting O-PPC with *N*,*O*-Bis-Phe under aqueous conditions, different length oligomers of Phe ranging from the monomer to the octamer were observed. We report here that pentacoordinated phosphoranes can be used as building blocks in the solid-phase synthesis of oligopeptides.

2. Materials and methods

2.1. Chemicals

4-Hydroxymethyl polystyrene resin ($HOCH_2$-R) was purchased from Sigma–Aldrich. Several amino acids, catechol, hexamethyldisilyldiamine, and other chemicals

were commercially available from Beijing Chemical Company. All solvents were purified and dried before use.

2.2. Synthesis of oligopeptide

Solid-phase synthesis was performed according to the following procedures. Traces of oxygen were removed in a home-built reactor at room temperature. In the first step, $HOCH_2(R)$ (0.25 g) was swelled in dry benzene (5 ml) for 2 h. Elongation of the peptide was carried out by coupling pentacoordinated phosphorane (P(5)-AA) (1.7 mmol) in benzene (5 ml) with solid support resin for 4 h. The resulting resin was washed with CH_2Cl_2 five times (5 ml each time). The elongation and washing steps were repeated. Subsequently, the peptide was cleaved from resin in 10% TFA containing the appropriate solvent. The crude peptide was obtained after removal of solvent. Purification of oligopeptide is accomplished by aqueous workup and/or reverse phase chromatography. The total yield of peptide varied between 50 and 70%.

A typical procedure for the synthesis of Phe–Leu was as follows. P(5)-Leu (0.1 mmol), dissolved in dry benzene (5 ml), was added to $HOCH_2(R)$ (0.25 g). The resulting solution was stirred for 4 h at room temperature, and washed with 5 ml CH_2Cl_2 ($\times 5$). After filtration, the resin was swelled in dry benzene (5 ml) and P(5)-Phe (0.1 mmol) in benzene (5 ml) was added to the reactor. The resulting solution was stirred for 4 h at room temperature, washed and filtered as described above. A solution of dioxane and acetonitrile containing 10% TFA was used to cleave the oligopeptide from the solid-phase support. The solvent was removed in vacua and the residue was dissolved in ethyl acetate. The solution was extracted with water and the final product was obtained in 56% yield after lyophilization of aqueous phase.

^{1}H NMR (200 MHz in $CDCl_3$): δ 7.1–7.3 (5H, m, aromatic H), 7.1 (1H NH of Leu), 2.9–3.1 (2H m, $C^{\alpha}H$ of Leu and $C^{\alpha}H$ of Phe), 2.3–2.5 (5H, m $C^{\beta}H$ of Phe, $C^{\beta}H$, $C^{\gamma}H$ of Leu), 0.5–0.6 (6H, d, $2 \times CH_3$ of Leu). IR(cm^{-1}, KBr) 3350/3225 (NH), 3125/3300 (Ph–H and $–CH_2–$), 1670 (CONH), 1550/1500 (NH), 1530 and 670 (benzene ring monosubstituted). FAB-MS $[M + H]^+$ m/z 279. HPLC R_t 2.690 min.

2.3. Analytical methods

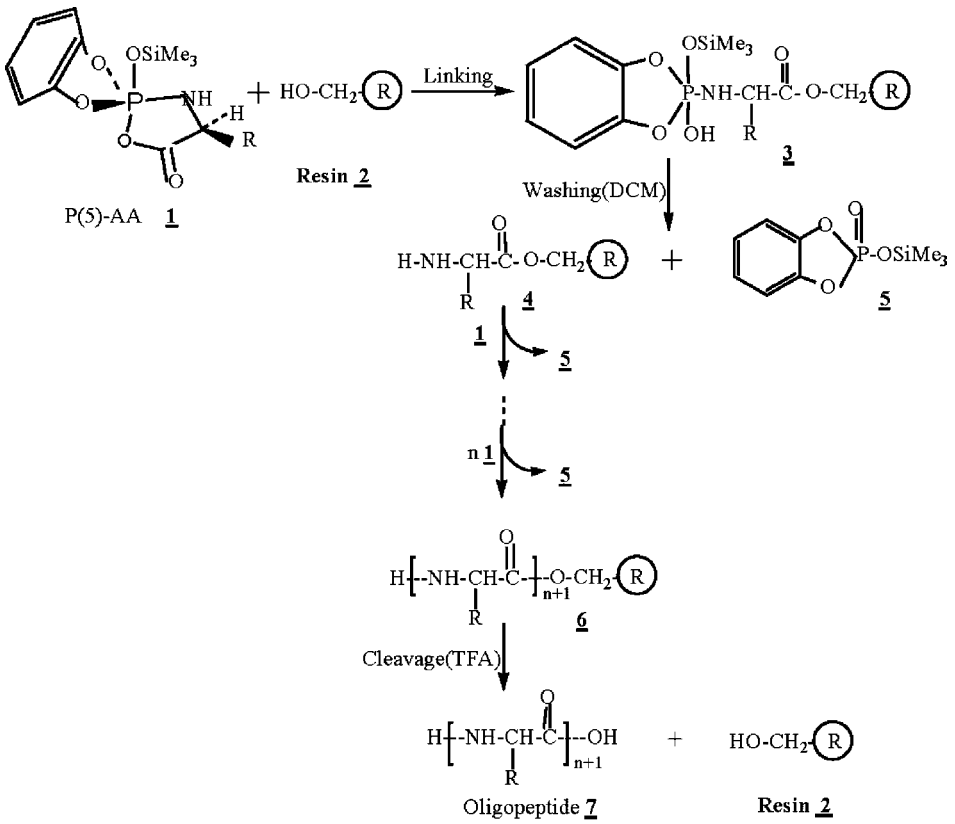
The IR spectra were measured as a KBr disc using a Nicolet 5DX FT-IR spectrometer. Positive ion FAB-MS data were obtained on a Finnigan MAT 90MS double-focusing magnetic mass spectrometer. The ^{1}H NMR spectra were recorded on a Bruker AM 200 spectrometer. The analysis of oligopeptides was carried out on SHIMADZU model LC-9A (RPC ZORBOX ODS C18 column 4.6 × 150 mm). An aqueous solution containing 0.02 M of KH_2PO_4 (adjusted to pH 2.5 using H_3PO_4) buffer was used to elute the oligopeptide formed in the solid-phase synthesis. Chromatography was performed at 25 °C, and the elution profile was monitored at 210 nm using a SHIMADZU SPD-6AV UV detector.

3. Results and discussion

3.1. Procedures of solid-phase synthesis

In general, the typical solid-phase peptide synthesis involves multiple steps, including the protection of the amino acid, activation, coupling, deprotection of the amino acid, and the final cleavage of the peptide from the solid-phase support. In our recent work, we found that (P(5)-AA) are reactive intermediates which react rapidly with (*N*,*O*-Bis-AA) to form a series of different length oligopeptides in aqueous solution. On the other hand, P(5)-(AA) can also be attacked by nucleophiles, such as ROH and RNH_2 [11]. In order to control the random elongation of oligopeptide, we selected a solid-phase support (4-hydroxymethyl polystyrene resin, resin **2** in Scheme 2) as an efficient nucleophile. The solid-phase synthesis of oligopeptide by this procedure is shown in Scheme 2.

As shown in Scheme 2, $HOCH_2(R)$ (**2**) is a nucleophile that rapidly attacks P(5)-AA (1) to form product **3**. The experimental results show that the P–N bond



Scheme 2. Solid-phase synthetic process of oligopeptide.

is unstable in compound **3**. Phosphate **5** could be removed to give **4** by washing with CH_2Cl_2. Product **4** was analyzed by IR spectra showing amide bond formation (Fig. 1). Compound **5** was a phosphate triester corresponding to the ^{31}P NMR signal at 3.5 ppm, which was determined by comparison with a control sample and identified by high resolution electron ionization mass spectrometry (EI-MS) [11]. Similar nucleophilic attack by the amino group in **4** to the carbonyl group in **1** led to dipeptide linking with resin as the leaving compound 5. Repetition of the process above yielded oligopeptide linked with resin **6**. The final oligopeptide was obtained after cleavage from the resin by trifluoracetic acid (TFA).

3.2. Infrared spectra of oligopeptides

We have made a series of oligopeptides using our novel solid-phase synthesis method, including Phe–Leu and Leu–Leu. Since IR spectroscopy is a very powerful technique for determination of functional groups linked to a solid support, we determined the IR spectra of $HOCH_2(R)$ (I), oligopeptide (Phe–Leu) (II) and (Leu–Leu) (III) linked to the resin, respectively. It can be seen in Fig. 1 that the polystyrene resin (I) showed a series of typical absorption peaks at 3125–3000 cm^{-1} (Ph–H and $–CH_2–$stretch region); 1500, 1050, 740, and 690 cm^{-1} (1,4-disubstituted benzene). The new peaks in their IR spectra appeared at 1640 and 1670 cm^{-1} for (II) and (III) respectively, which are different from those observed in (I). This result shows amide bond absorption consistent with peptide bond formation. It is interesting that the IR spectra of (II) showed an obviously monosubstituted benzene ring with characteristic absorption peaks at 1530 and 670 cm^{-1}, which is due to Phe and is not seen in the resin or in the Leu–Leu oligopeptide spectra.

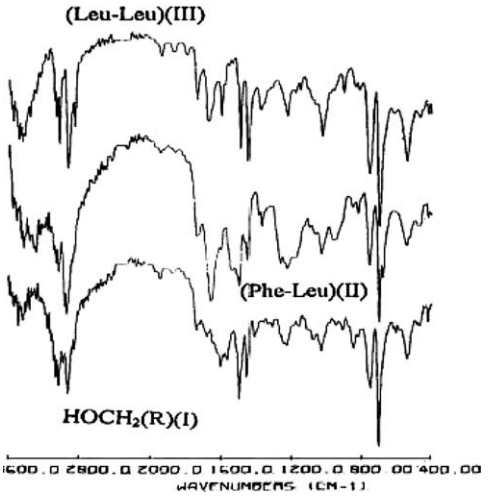


Fig. 1. The IR spectra of $HOCH_2(R)(I)$, oligopeptide (Phe–Leu)(II) and (Leu–Leu)(III) linked the resin, respectively.

Table 1
FAB mass spectral data of oligopeptides

Oligopeptides	tetra-Gly	di-Val	tri-Val	di-Leu	di-Phe	Phe–Leu
$[MH]^+$ *m/z*	247	217	316	245	313	279

3.3. FAB mass spectrometric measurements

The oligopeptides were identified by FAB mass spectrometry, and comparing results with the corresponding authentic samples. The positive ion FAB mass spectral data of oligopeptides formed in the solid-phase synthesis are listed in Table 1.

It is seen from Table 1 that the FAB mass spectral data of corresponding oligopeptides obtained in SPPS are all agreement with those of the authentic oligopeptides. It is found that the peak $[M + H]^+$ at *m/z* 247 (65%) in tetra-Gly was protonated tetra-Gly. There are still di-Gly $[M + H]^+$ at *m/z* 133 and a few hexa-Gly $[M + H]^+$ at *m/z* 361 in the synthesis of oligo-Gly in addition to the tetra-Gly. This is due to the rapid interaction of the produced higher peptide and neighboring group effects in the presence of polymer solid-phase support. However, except for Gly, our results show that the formation of the target molecular is the major product in these reactions.

3.4. HPLC analysis of oligopeptides

The reverse-phase HPLC analysis confirmed that the correct oligopeptides were formed in the solid-phase synthesis (Fig. 2). The samples synthesized by SPPS had retention times of 2.65 min (di-Phe) and 2.69 min (Phe–Leu) (Fig. 2C and D). The retention time of the synthesized di-Phe is compared to that of an authentic sample (2.67 min) (Fig. 2B). Phe has a retention time at 1.6 min (Fig. 2A).

Fig. 3 shows the HPLC profiles of products obtained using our solid-phase peptide synthesis (SPPS) and products obtained using liquid-phase peptide synthesis (LPPS). Fig. 3A corresponds to di-Phe obtained by SPPS (- - -) compared to an authentic sample (—).

There is very good agreement between the retention times of di-Phe synthesized by our SPSS with that of LPPS (Fig. 3B). These results indicated that the self-catalytic formation of peptide was better controlled in SPPS than the LPPS method. It appears to be a more efficient method for oligopeptide formation.

4. Conclusion

The solid-phase synthesis of oligopeptides using reactive pentacoordinated phosphoranes P(5)-AA as building blocks has been described. This strategy represents a modified solid-phase peptide synthesis method, which is different from the conventional approach, involving the protection of amino acid, activation, coupling and deprotection.

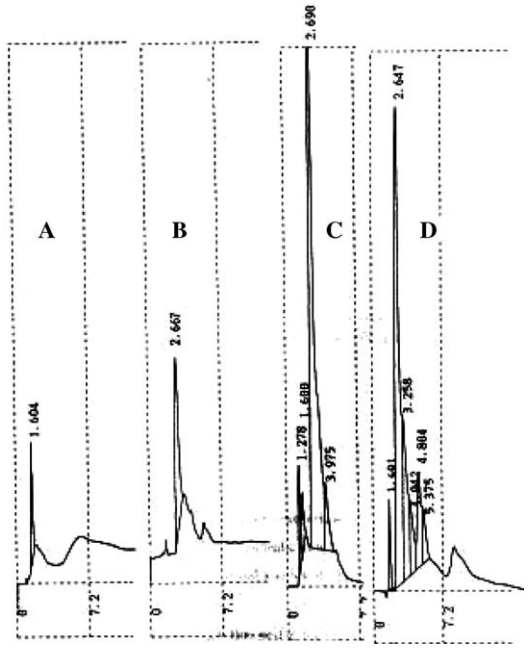


Fig. 2. HPLC profiles of oligopeptides and authentic samples: (A) phenylalanine; (B) di-Phe; (C) di-Phe(SPPS) and (D) Phe–Leu(SPPS).

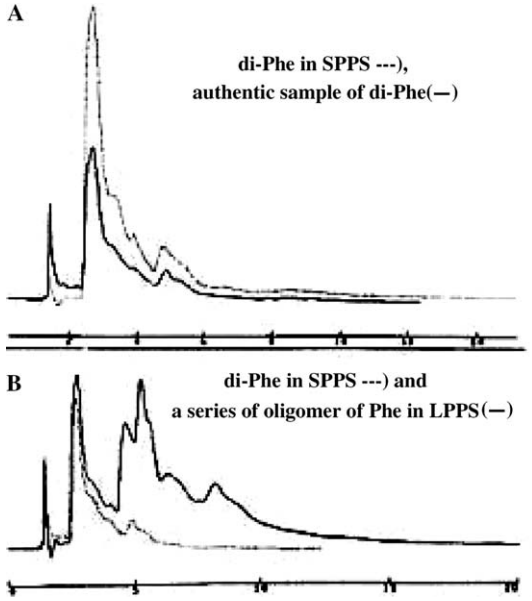


Fig. 3. HPLC profiles of oligopeptide synthesized by SPPS and LPPS method.

Acknowledgments

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