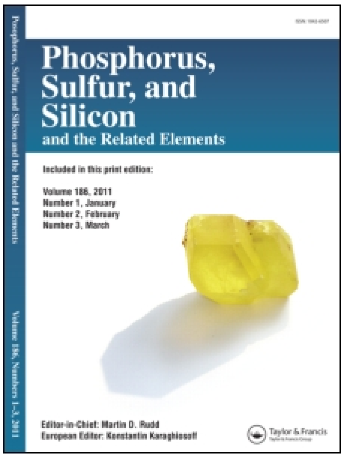

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The Phosphorus-Mediated Oligomerization of Glycinesters

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The Phosphorus-Mediated Oligomerization of Glycinesters

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The catalytic polymerization of α-aminoacids or stable α-aminoesters in the presence of a phosphorus template is proposed as an alternative to that of sensitive N-carboxyanhydrides for the synthesis of polyaminoacids. The synthesis of methane thiophosphonyl derivatives of polyglycine (an average of ten residues) was actually carried out in polar solvents (dimethylformamide, acetonitrile) in the presence of diazabicycloundecene and trimethylsilyldiethylamine.

Keywords Cyclization; peptides; phosphorus; polycondensation; silicon

INTRODUCTION

Polyaminoacids constitute a class of polymers with interesting applications. As a simplified protein model, they have, firstly, contributed to elucidating the secondary structure of proteins.[1] More recently they have been used as antigens,[2] as biodegradable substrates releasing active components,[3] or, when formed with amino acids possessing a functional side-chain (particularly serine or lysine), as anchors for various chemical groups.[4] Typically, their synthesis involves *N*-carboxyanhydride polymerization.[5,6] However, these heterocycles are very fragile, and alternative methods using more stable compounds are therefore attractive. Indeed a different approach using α-aminoesters, the salts of which are stable and easy to prepare, has recently been published. Their polymerization is affected intramolecularly within a metal complex.[7] In fact, the principle of this method is identical to what we published previously[8] with a phosphorus template instead of a metal template, and even more advantageously, with *free* (unprotected)

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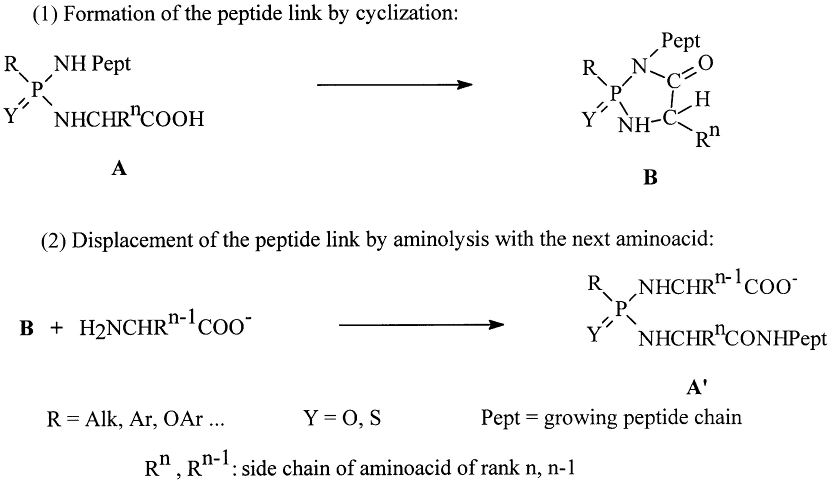
α-aminoacids in place of α-aminoesters. As success was limited to a low degree of polymerization,[7] it is of interest to examine also whether with the phosphorus template[8] the size of the oligomers could be enhanced.

RESULTS AND DISCUSSION

Our aforementioned peptide synthesis method[8] requires the repetition of two reactions (Scheme 1), with only one, the second, for the steps comprising the displacement of the peptide link and the attachment of the next α-amino acid, which are otherwise separated:[7]



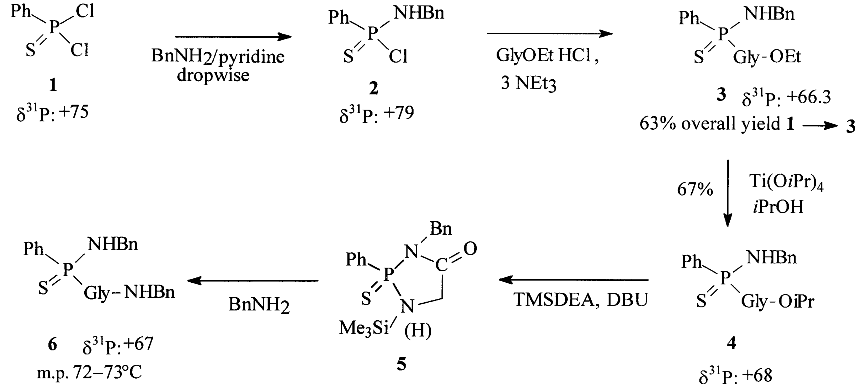
SCHEME 1

In **A**′ the arrangement is the same as in **A**, with permutation of the respective positions of the growing peptide chain and of the amino acid: The two reactions can be repeated alternately, ad. libitum. Moreover, implicitly, the synthesis is regulated by the use of different media for each reaction: for example, cyclization (1) in acid, and aminolysis (2) in a basic medium. Conversely, if each reaction can be carried out under the same conditions, the synthesis is unregulated: Polyaminoacids should then be obtained.[8b]

Many years ago, in the search for such a medium, we first considered operating with amino acids in carboxylic acids, since in those they are sufficiently soluble, while their amino function is still reactive toward such strong acylating reagents as trifluoraocetic anhydride[9] or mixed formic-acetic anhydride.[10] As for the aminolysis (2), cyclization (1) may also be a priori carried out in them in light of the known acid catalyzed conversion[11] of thiocarbamoyl amino acids into thiohydantoins (analogous to **B** by replacing RP(Y) with CS). Nevertheless, in practice, this was not feasible: In the presence of amines and acetic acid, heterocycles **B** react exclusively with the acetic acid.[12] They are also extremely easily hydrolyzed (water is produced by the assumed cyclization reaction). Furthermore, phosphonic acid diamides **A** are not very stable in an acidic medium.[8,13]

At this stage we considered two alternatives:[8] (1) the intramolecular catalysis of the aminolysis of **B**, making possible the use of free amino acids in a carboxylic acid medium, as previously mentioned. This study was started recently and is in progress.[14–18] (2) The second alternative was the use of aminoesters: each reaction (Scheme 1) may then be affected in the same medium, i.e., an aprotic organic solvent. The problem is that in reaction (1) an alcohol will be released, which is known to react much more easily with **B** than amines.[12] In order to suppress this alcoholysis, the use of alcohol trapping reagents may be considered. Of course, they could also react with the aminoesters. We therefore selected aminosilanes as it is known that they react with amines in a displaced equilibrium[19] (i.e., leaving, as required, some free, reactive amino groups), unlike the way they react with alcohols; hexamethyldisilazane was discarded because free ammonia would react easier with phosphorus than aminoesters. Trimethylsilyldiethylamine (TMSDEA) was chosen because the more bulky secondary amine should not interfere in the course of the reaction. We operated in three steps.

First, we examined (Scheme 2) the feasibility of the synthesis of **B**. As it had already been prepared in another way,[20] we chose heterocycle **5**, starting from phenyl phosphonic diamide **4**. This isopropylester, pure and easily crystallized, was prepared using Seebach's method[21]

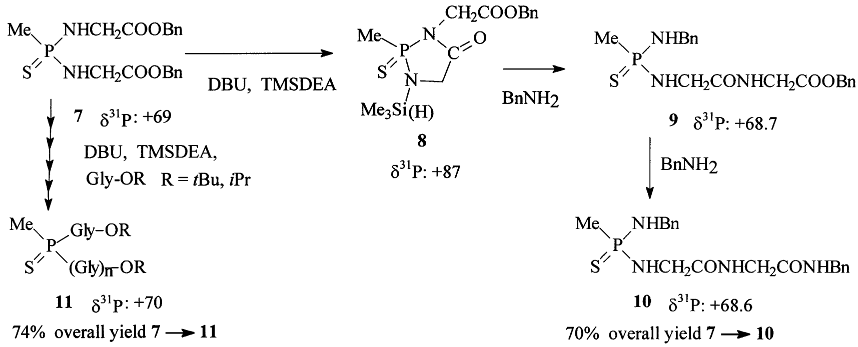


SCHEME 2

from ethylester **3** in its crude state. Silylated heterocycle **5** was actually obtained under the following conditions (which are similar to those already described for the synthesis of phosphorus heterocycles derived from β-amino amides[15] or sarcosamide[22]): (1) catalytic amounts of the strong base Diazabicycloundecene (DBU) (triethylamine proved to be inefficient); (2) a polar solvent such as DMF (in which the nucleophilicity of anions is known to be enhanced) or acetonitrile; and (3) the TMSDEA trapping reagent.

After an addition of a large excess (8 equivalents) of benzylamine, which is a good model for a glycinester, and heating to 65°C, aminolysis leading to **6** by the attack on phosphorus[12] was completed in only a week. Clearly, these conditions preclude the use of α-aminoesters, particularly of glycine derivatives, because of their well-known easier self-condensation in dioxopiperazine.[23]

Second, in order to obtain the required free phosphorus access accelerating aminolysis, we turned to the less crowded methyl phosphonamide **7** (Scheme 3), previously prepared in the laboratory.[24] Indeed this proved to be considerable: Reaction at phosphorus of heterocycle **8** is completed in only a few hours at r.t. A second, slower, aminolysis (performed overnight) of transient benzylester **8** leading eventually to phosphondiamide **10** was also observed. The cyclization of **7** was also faster (2 h at 60°C) than that of 4, while being slower than the aminolysis of **8**.



SCHEME 3

Third, instead of benzylamine we used esters of glycine (Scheme 3, left). Since their self-condensation reaction must be slower than reactions (1) and (2) (Scheme 1), we did not employ the ethylester used by Hoffmüller et al.[7] (leading, by transesterification [MeOH, NEt_3], to the methylester, even more prone to self-condensation[25]), which is known to lead very easily to dioxopiperazine,[25] and even to polyglycine in the absence of template.[26] Rather, we used isopropylester (easily prepared from hydrochloride using Hillman's excellent method[27]) and tert-butylester.[28] They are known to be, respectively, rather[25] and very[28] resistant to self-condensation, while the cyclization (1) remains possible.[8] Moreover, we checked that in the most favorable case for this reaction (i.e., with isopropylester), no dioxopiperazine was produced, as the stable[29] *N*-silylated aminoester was formed. Finally, NMR analysis of the insoluble material produced showed that it was a mixture of polymers with an average number of ten glycine residues (nearly eight in the experiment of Hoffmüller et al.[7]).

In conclusion, the relatively low degree of polymerization obtained confirms the explanation given by Hoffmüller et al.:[7] "notoriously insoluble polyglycine peptides in organic solvents." (p. 730) Work is in progress to improve the solubility by using more lipophilic aminoesters derived from other α-aminoacids, provided the increase of steric bulk does not drastically reduce the aminolysis reaction,[30]. and phosphonyl templates, which are more electrophilic than MEPS (e.g., CF_3PO). These are expected to improve both reactions in Scheme 1, particularly cyclization, thus avoiding the use of DBU prone to racemizing the synthesized polymers.

EXPERIMENTAL[31]

The general conditions are the same as indicated elsewhere.[18]

Phosphondiamide Ethylester 3

To a pyridine (20 mL) solution of **1** (0.58 g, 2.8 mmol), under vigorous magnetic stirring and ice cooling, a pyridine (20 mL) solution of benzylamine (0.3.3 g, 1.1 equiv.) was added dropwise during 20 min. The resulting solution was added dropwise at r.t. under stirring during 35 min to a pyridine (6 mL) and triethylamine (0.85 g, 3.35 equiv.) suspension of Gly-OEt.HCl (0.55 g, 1.4 equiv.). After stirring overnight and concentration, an addition of CCl_4 (50 mL), extraction with water, 10% citric acid, and 5% $NaHCO_3$ solutions (30 mL each), the CCl_4 was evaporated, and the residue dissolved in a mixture of pyridine (35 mL) and water (2.5 mL). After 4 days, the solution was concentrated to dryness; the residue was dissolved in ether (60 mL) and extracted with a 5% $NaHCO_3$ solution (3 × 20 mL). After removal of the water (Na_2SO_4) and concentration to dryness, the raw product (83% of the phosphorus content) was used for the following transesterification reaction. Crystals (m.p. 42–44°C) were obtained from diisopropylether after 2 months.

ν_{max} 3337, 1737 cm^{-1}; δ_H ($CDCl_3$): 8 (m, 2H, 2 H ortho C_6H_5PS), 7.4 (m, 3H, 2 H meta + H para C_6H_5PS), 7.27 (s, 5H, $C_6H_5CH_2$),4.3 (m, 4H, ethyl and benzyl CH_2), 3.78 (dd, J_{HH} 6.2, J_{PH} 11.4, 2H, CH_2Gly), 3 (broad s, 2H, 2NH), 1.22 (t, J_{HH} 7.1, 3H, CH_3); δ_C ($CDCl_3$): 171.5 (d, J_{PC} 8.1, CO), 138.5 (d, J_{PC} 8.7, quart. C benzyl), 134.5 (d, J_{PC} 123.1, C ipso), 131.9 (d, J_{PC} 2.8, CH para PhPS), 131.0 (d, J_{PC} 11.2, 2CH meta PhPS), 128.6 (d, J_{PC} 13.4, 2CH ortho PhPS), 127.7 and 128.7 (2 × 2CH ortho/meta benzyl), 127.4 (CH para benzyl), 61.5 (CH_2 ethyl), 45.4 (CH_2 benzyl), 42.8 (CH_2 Gly), 14.2 (CH_3); δ_P ($CDCl_3$): +66.3.

Phosphondiamide Isopropylester 4

Isopropanol (90 mL), raw **3** (14 g; 40 mmol), and $Ti(OiPr)_4$ (3.46 g, 0.3 equiv.) were heated for 24 h at 70°C. After work-up as described,[21] the product crystallized rapidly from diisopropylether: colorless crystals, mp 70–71°C. (Found: C, 58.73; H, 6.58; N, 7.70; S, 8.63. $C_{18}H_{23}N_2O_2PS$ requires C, 58.65; H, 6.40; N, 7.73; S, 8.85); ν_{max} 3364, 3326, 1724 cm^{-1}; δ_H ($CDCl_3$): 7.85 (m, 2 H, 2H ortho PhPS), 7.40 (m, 3H, 2H meta +H para PhPS), 7.27 (s, 5H, $C_6H_5CH_2$),5.00 (sept., J_{HH} 6.3, 1H, CH isopropyl), 4.12 (dd, J_{HH} 6.6, J_{PH} 8.6, 2H, CH_2 benzyl), 3.75 (dd, J_{HH} 6.1, J_{PH} 11.3, 2H, CH_2 Gly), 1.2 (d, J_{HH} 6.3, 6H, 2CH_3); δ_C ($CDCl_3$): 171.0 (d, J_{PC} 8.1, CO), 138.5 (d, J_{PC} 8.7, quart. C benzyl), 134.5 (d, J_{PC} 122.9, C ipso), 131.8 (d, J_{PC} 2.9, CH para), 131.0 (d, J_{PC} 11.2, 2 CH meta PhPS), 128.6 (d, J_{PC} 13.7, 2CH ortho PhPS), 128.6 and 127.7 (2 × CH ortho/meta benzyl), 127.4 (CH para benzyl), 68.3 (CH isopropyl), 45.4 (CH_2 benzyl), 43.0 (CH_2 Gly), 21.8 (2 CH_3); δ_P ($CDCl_3$): +66.7.

Dibenzylamide 6

In an NMR tube filled with argon we added a capillary of deuteriated benzene, DMF (0.4 mL), DBU (25 mg, 1.4 equiv.), and TMSDEA (85 mg, 5.5 equiv.), and after 20 h (time allowed for dehydration of the medium), **4** (44 mg, 0.12 mmol). After 2 h at r.t., ^{31}P NMR showed two peaks δ 78.2 (silylated **5**) and 67.2 (**4**) in a 38:61 ratio. A septum connected with a syringe filled with $CaCl_2$ was then adapted, the tube was heated at 65°C. Ratio **5/4**: 61:38 after 1 h 30 min; 81:8 after 6 h 30 min; 88:2 after 20 h. A mixture (118 mg) of benzylamine (80%: 7.8 equiv.) and bis(silyl)acetamide (10%) was then added at r.t. ^{31}P NMR showed practically two peaks. δ 78.1 (**5**), 68.1 (**6**) in the ratio 81:13, 63:31 after 6 h, 11:85 after 2 days, and 5:81 after 3 days. After 1 week, CH_2Cl_2 (40 mL) was added. After extractions with 50% then 10% citric acid, 5% $NaHCO_3$ solutions (20 × 30 mL each), removal of water (Na_2SO_4), and concentration to dryness, **6** was obtained as an oil in an 83% yield. After a year at −15°C in a saturated EtOAc/diisopropylether solution the product crystallized: white powder, mp 72–73°C. (Found: C, 64.28; H, 5.88; N, 8.83. $C_{22}H_{24}N_3OPS$ requires C, 64.54; H, 5.81; N, 10.26). ν_{max} 3376, 1662 cm^{-1}; δ_H ($CDCl_3$): 8.00 (m, 2H, 2H ortho PhPS), 7.45 (m, 3H, 2H meta + H para PhPS), 7.25 (s, 10H, 2 C_6H_5 benzyl), 6.62 (broad s, 1H, NH carboxamide), 4.34 (d, J_{HH} 5.8, 2H, CH_2 benzylcarboxamide), 4.08 (dd J_{HH} 6.2, J_{PH} 10,4, 2H, CH_2 benzylphosphonamide), 3.68 (nonsymmetric dd, J_{HH} 7.3, J_{PH} 12.3, 2H, CH_2 Gly), 3.34 (m, 1H, NH), 3.02 (m, 1H, NH); δ_C ($CDCl_3$): 170.2 (d, J_{PC} 6.1, CO), 138.2 (d, J_{PC} 7.8, quart. C benzylphosphonamide). 137.8 (quart. C benzylcarboxamide), 134.1 (d, J_{PC} 121.8, C ipso), 132.2–127.6 (m, 15 C, 15 aromatic CH), 45.4 and 43.5 (2s, $2CH_2$); δ_P ($CDCl_3$): +66.7.

Dibenzylamide 10

In an NMR tube, as previously described, an acetonitrile (0.6 mL) solution of **7** (85 mg, 0.24 mmol), DBU (33 mg, 0.8 equiv.) and TMSDEA (130 mg, 3.7 equiv.) was heated for 3 h to 70°C (^{31}P NMR: $\delta + 87$, **8**). Benzylamine (168 mg, 6.5 equiv.) was then added. After 20 h at r.t. (^{31}P NMR showed practically one signal at δ +68.3), the reaction mixture was concentrated and diluted with CH_2Cl_2 (25 mL). The solution was extracted with 10% citric acid and 5% $NaHCO_3$ solutions (3 × 20 mL each), dried (Na_2SO_4), and concentrated to dryness leaving an oil as a mixture of **10** (72% yield) and trimethylsilylated benzylalcohol. After a year at −15°C in a saturated EtOAc/ether solution, the product crystallized: small white balls. ν_{max} 3280, 1647 cm^{-1}; δ_H ($CDCl_3$): 7.54 (t, J_{HH} 5.5, 1H, NH carboxamide), 7.30 (m, 11H, $2C_6H_5$ + 1 NH carboxamide), 4.31 (d, J_{HH} 5–8, s with D_2O, 2H, CH_2 benzylcarboxamide), 4.04 (dd, J_{HH} 5.8, J_{PH} 11.6, 2H, CH_2 benzylphosphonamide), 3.84 (d, J_{HH} 5.7, s with D_2O, 2H, CH_2 carboxamide), 3.5 (m, 3H, 2H with D_2O, CH_2 Gly phosphonamide + NH phosphonamide), 3.30 (m, 1H, disappeared with D_2O, NH phosphonamide), 1.72 (d, J_{PH} 14.2, 3H, CH_3); δ_C ($CDCl_3$): 171.8 (d, J_{PC} 5.1, CO Gly linked to phosphorus), 168.3 (CO other Gly), 138.5 and 138.0 (2 quart. C), 128.7–126.6 (m, aromatic CH, two phenyl groups), 45.1, 43.8, 43.3, 43.0 (4 s, 4 CH_2), 21.8 (d, J_{PC} 88.8, CH_3); δ_P ($CDCl_3$): +68.6.

When the reaction was stopped after 2 h 30 min, a mixture of trimethylsilylated benzylalcohol, **10** (40%), and **8** (60%) was obtained. The latter product was identified by IR (additional νCO 1745 cm^{-1}), ^1H NMR (a characteristic low-field signal of the methylene protons of a benzylic ester at δ 5.13; methyl protons at δ 1.87 J_{PH} 14.2), and ^{31}P NMR (δ 68.7, slighty upfield compared to **8**).

Polycondensation

An NMR tube, as previously described, was loaded with acetonitrile (0.4 mL), 7 (71 mg, 0.17 mmol), TMSDEA (132 mg, 0.81 mmol, 5.34 eq.), and DBU (25 mg, 0.17 mmol). The addition of Glycine-*tert*-butylester (Lancaster) was effected in successive sections of 75 mg (0.57 mmol, 3.3 eq.). In this way, ^{31}P NMR enabled the control of both aminolysis ($\delta \sim +70$, several hours at r.t.) and the cyclization ($\delta \sim 87$, 3 h at 60°C: slightly slower than with benzylester **7**). After three cycles of this treatment, a gel-like substance appeared; at the same time ^{31}P signals became less intense. Subsequently, this phenomenon became more and more pronounced, and in the end no clear signals were observed. Finally, after an addition of 12 equivalents of aminoester, the mixture was concentrated to dryness, and the residue was taken up in ether, in particular in order to remove DBU and silylated alcohols. The IR spectrum of the insoluble material (74% yield in **11**) showed an intense carbonyl absorption at 1630–1650 cm^{-1} (amide) and a weak one at 1737 cm^{-1} (ester). The ^{31}P NMR spectrum in DMSO-d6 indicated the presence not of cyclic products ($\delta \sim 87$) but of diamides ($\delta \sim 70$). The ^{1}H NMR spectrum proved, as for **10**, the lack of a signal at 5.2 ppm characteristic for benzylester: This confirmed that there were two *tert*-butylesters present. The ratio of integration of glycine CH_2 (at $\delta \sim 3.2$ ppm) and of *tert*-butylesters CH_3 confirmed polycondensation, with an average number of 10 glycine residues.

The same result was also obtained with isopropylester or with an addition of 25 *tert*-butylester equivalents. It is important to note that by means both of IR and NMR no dioxopiperazine, which has a very characteristic spectrum, was observed.

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[30] In that case, the aminolysis will still be easier with the participation of the sulfonamido group[18] by intramolecular nucleophilic catalysis.[14]

[31] More details are available in ref. 4b (chapter 3).