On the Synthesis of Phosphonamidate Peptides

Hans-Jürgen Musiol, Frank Grams, Sabine Rudolph-Böhner, and Luis Moroder*

Max-Planck-Institut für Biochemie, 82152 Martinsried, Germany

Received July 11, 1994®

Summary: Oxalyl chloride-mediated preparation of phosphonochloridates allowed the improvement of the synthesis of phosphonamidate peptides, particularly if AgCN was used as catalyst or upon conversion of the chloridate into the 7-aza-1-hydroxybenzotriazole ester.

The concept of mimicking tetrahedral transition states of enzyme-mediated peptide bond hydrolysis[1] led in the past years to the successful design and synthesis of phosphonamide containing peptides as a promising new class of proteinase inhibitors.[2]

So far, phosphonamidate peptides were synthesized by reacting monoalkyl or monoaryl N-protected (aminoalkyl)phosphonochloridates with suitably protected amino acids or peptides.[2] For this purpose the chloridates are obtained from phosphonate diesters with PCl_5[2c,d] or by selective base hydrolysis of phosphonate diesters to the monoesters followed by reaction with thionyl chloride.[2b] Alternatively, phosphonic acids are converted with oxalyl chloride to the dichloridate[3] and subsequently with alcohol to the monoester chloridate.[2g] By the use of this activated species unsymmetrical target diesters are prepared in high and reproducible yields, whereas related phosphonamidates are formed in moderate, if not low yields. Besides the facile hydrolysis of phosphonamidates in acidic media,[2b,4] their unsatisfactory synthetic accessibility is severely limiting the use of this interesting class of compounds as tools in enzyme chemistry.

In the present study we have analyzed possible improvements of the phosphonamide bond formation more systematically using the reaction shown in Figure 1 as a model. For this purpose, Z-Phe[PO(OMe)]-OH (**1**) was synthesized in a straightforward manner from the corresponding diphenyl phosphonate[5] via transesterification in the presence of KF/crown ether[6] followed by selective base hydrolysis of the resulting dimethyl ester.[2b,7] Compound **1** was used in the following study as a diastereomeric mixture. The two peptides H-Leu-Phe-NH_2 (**2**) and H-Ala-Pro-Phe-OMe (**3**) were prepared by classical methods of peptide synthesis in solution. By applying the standard thionyl chloride procedure[2b] (compound **1**/$SOCl_2$, 1:2; in CH_2Cl_2; rt; 2 or 4 h) the model phosphonamidate peptides **4** and **5** were obtained as diastereomeric mixtures in 50% and 65% yield, respectively. As observed throughout this study the yields of the tripeptide **4** were lower than those of the tetrapeptide **5** indicating significant steric effects (leucine against alanine) in the aminolysis of the phosphonochloridate. Addition of pyridine or DCHA/pyridine at optimized ratios to improve the rates and yields of the chloridate formation as in the case of acid chlorides[8,9] was found to decrease the yields of compound **5** to 50% and 10%, respectively. Conversely, addition of 0.1 equiv of DMF allows for more reproducible yields.

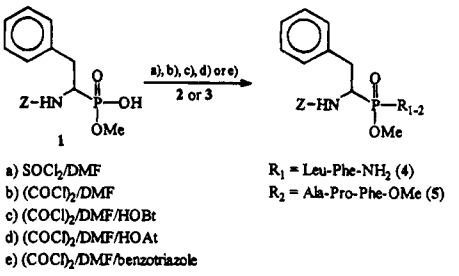

Figure 1.

Because of the known disadvantages of thionyl chloride, e.g., fast decomposition, difficult handling, and purity of the reagent, alternative chloridating reagents were examined. With triphosgene,[10] even in the presence of DMF as catalyst, only trace amounts of the desired phosphonopeptide **4** were obtained in addition to a main product of unknown nature. The identical result was obtained by activating the phosphonic acid **1** via the imidoyl chloride procedure,[11] i.e., with oxalyl chloride/DMF at a 1:3 molar ratio, as recommended for the production of acid chlorides. However, when the monomethyl phosphonate **1** was reacted with 2 equiv of oxalyl chloride in the presence of 0.1 equiv of DMF as proposed for the formation of acid chlorides[12] and phosphonodichloridates,[3] the phosphonopeptides **4** and **5** were isolated in yields significantly superior to those resulting from the standard thionyl chloride procedure (see Table 1). Decomposition of the phosphonochloridates has recently been assessed by ^{31}P-NMR, however, without identification of the products formed and of the rates of

* To whom correspondence should be addressed. Tel.: (089)-8578-3905. Fax: (089)-8578-3777.

® Abstract published in *Advance ACS Abstracts,* October 1, 1994.

(1) (a) Lienhard, G. E. *Ann. Rep. Med. Chem.* **1972**, *23*, 249. (b) Lienhard, G. E. *Science* **1973**, *180*, 149. (c) Wolfenden, R. *Ann. Rev. Biophys. Bioeng.* **1976**, *5*, 271.

(2) (a) Kam, C.-M.; Nishino, N.; Powers, J. C. *Biochemistry* **1979**, *18*, 3032. (b) Jacobsen, N. E.; Bartlett, P. A. *J. Am. Chem. Soc.* **1981**, *103*, 654. (c) Thorsett, E. D.; Harris, E. E.; Peterson, E. R.; Greenlee, W. J.; Patchett, A. A.; Ulm, E. H.; Varsil, T. C. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 2176. (d) Elliott, R. L., Marks, N., Berg, M. J., Portoghese, P. S. *J. Med. Chem.* **1985**, *28*, 1208. (e) Bartlett, P. A.; Marlowe, C. K. *Biochemistry* **1983**, *22*, 4618. (f) Bartlett, P. A.; Marlowe, C. K. *Science* **1987**, *235*, 569. (g) Bertenshaw, S. R.; Rogers, R. S.; Stern, M. K.; Norman, B. H.; Moore, W. M., Jerome, G. M., Branson, L. M.; McDonald, J. F.; McMahon, E. G.; Palomo, M. A. *J. Med. Chem.* **1993**, *36*, 173.

(3) Rogers, R. S. *Tetrahedron Lett.* **1992**, *33*, 7473.

(4) The stability of Z-Phe[PO(OH)]Ala-Pro-Phe-OH has been analyzed in 100 mM sodium phosphate buffer at pH 6.0, 7.0, and 8.0, respectively (12 °C) by HPLC. The $t_{1/2}$ values were 70 h at pH 6.0 and ca. 2 weeks at pH 7.0, and there was nearly no decay at pH 8.0 in 2 weeks. Jacobsen and Bartlett[2b] found $t_{1/2}$ values of some min at pH 2.3.

(5) Oleksyszyn, J.; Subokowska, L.; Mastalerz, P. *Synthesis* **1979**, 985.

(6) Lejczak, B., Kafarski, P.; Szewczyk, J. *Synthesis* **1982**, 412.

(7) (a) Camp, N. P.; Hawkins, P. C. D.; Hitchcock, P. E.; Gani, D. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 1047. (b) Galardy, R. E.; Kontoyiannidou-Ostrem, V.; Kortylewicz, Z. P. *Biochemistry* **1983**, *22*, 1990.

(8) Matsuda, F.; Yanagiya, M.; Matsumoto, T. *Tetrahedron Lett.* **1982**, *23*, 4043.

(9) Matsuda, F.; Itoh, S.; Hattori, N.; Yanagiya, M.; Matsumoto, T. *Tetrahedron* **1985**, *41*, 3625.

(10) Eckert, H.; Forster, B. *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 894.

(11) Stadler, P. A. *Helv. Chim. Acta* **1978**, *61*, 1675.

(12) Perlow, D. S.; Erb, J. M.; Gould, N. P.; Tung, R. D.; Freidinger, R. M.; Williams, P. D.; Veber, D. F. *J. Org. Chem.* **1992**, *57*, 4394.

0022-3263/94/1959-6144$04.50/0

Table 1. Synthesis of Phosphonamidate Peptides via Oxalyl Chloride Using the Following Activation Procedure: Compound 1/$(COCl)_2$/DMF; 1:2:0.1; rt; 2 h in CH_2Cl_2

amino component (CH_2Cl_2)	**1/2** (or **3**) molar ratios	yield (%)
2·HCl/TEA; 1:2.5; rt; 12 h	1	65, **4**
3·HCl/TEA; 1:2.5; rt; 12 h	1	75, **5**
2; rt; 12 h	2.5	75, **4**
3; rt; 12 h	2.65	85, **5**
2/TEA; 2.1:1; rt; 12 h	2.1	80, **4**
3/AgCN; 1:1.6; rt; 12 h; in toluene	1	90, **5**

decay.[13] This decomposition may reasonably be attributed to the formation of phosphonic acid anhydride via elimination of alkyl chloride as previously reported for monoalkyl alkylphosphonic acid chloridate.[14] This reaction apparently occurs at rates competitive with those of aminolysis. The significantly higher yields obtained by operating with excesses of amino components, i.e., by increasing the rate of consumption of the phosphonochloridate, confirm this assumption (Table 1). A similar effect was therefore expected from suitable catalysts; in fact, catalysis by AgCN[12] as recommended for the acid chloride procedure was found to increase the yields up to nearly 90% (Table 1). Moreover, it seems interesting to note that using the amino component to capture nascent HCl leads to higher yields than by neutralization with TEA.

In the search of alternative methods for the phosphonamide bond formation we have examined the Mukaiyama procedure[15] which failed completely in the present case. The mixed anhydrides of alkylphosphonic acid monoesters, e.g., with pivaloyl chloride, are known to generate unsymmetrical diesters in good yields.[16] The nucleophilic attack by amino groups, however, was found to occur prevalently at the carbonyl carbon, thus leading almost exclusively to the *N*-pivaloylpeptide derivatives, despite the steric bulk of the *tert*-butyl group. Since esterification of alkylphosphonate monoesters is insensitive to the bulkiness of the phosphonic acid side chain,[17] steric effects should not be responsible for the lack of phosphonamide bond formation. Phosphoric and phosphonic acid active esters of the HOBt and HONp type have been used, particularly for the preparation of unsymmetrical diesters.[18-20] By reaction of the phosphonic acid **1** with DCC/HOBt no active ester was formed; similarly unsuccessful were attempts to prepare the phosphonamidate peptides **4** and **5** with PyBOP, whereas related esters were obtained in good yields with this reagent.[20] This observation fully agrees with the findings that even phosphinic acid amides are not accessible with PyBOP.[21] Conversely, treatment of the chloridate of **1** with 1 equiv of KOBt (5 min; rt), followed by reaction with the model dipeptide **2**, led to the phosphonopeptide **4** in yields comparable to those obtained directly with the phosphonochloridate (Table 2). Therefore, DBU was used instead of TEA as this strong base has been reported to catalyze efficiently the aminolysis of at least one ester group of bis(4-nitrophenyl) phosphonates.[19] Although *in situ* formation of the HOBt ester is confirmed by precipitation of KCl, storage of the active species in solution or its isolation led to significant decomposition as monitored by the yields of **4** (Table 2). This observation fully confirms previous reports which unfortunately did not disclose the method of preparation of the HOBt ester.[13] In the case of carboxylic acids the HOBt esters exist in equilibrium with the less active benzotriazolyl 1-oxide form;[22-24] in solvents which favor the O → N shift a reduced reactivity was observed.[24,25] A similar O → N shift is apparently occurring for the phosphonic acid HOBt ester, too, as ^{31}P resonances of the NMR spectrum of the isolated inactivated species in $CDCl_3$ (δ 22.8, 23.3, 24.2, 24.8) are very similar to those of phosphonic acid benzotriazolide. Formation of the *N*-oxide form could not be confirmed by IR because of the overlap of the N–O and P–O stretching band. It has recently been reported that the HOAt esters of carboxylic acids are more reactive than the corresponding HOBt esters, possibly because of stabilization of the ester form by intramolecular hydrogen bonding.[26] In order to examine this possible advantage the chloridate of **1** was reacted with KOAt and the resulting HOAt ester allowed for production of the model peptide **4** in significantly higher yields than the HOBt derivative (Table 2). Conversion of the reactive species into unreactive ones is occurring at lower rates than in the case of the HOBt ester. However, the ^1H-NMR spectrum taken after 24 h in DMSO revealed a set of signals in the region of the heterocyclic protons (7.1–8.6 ppm) which supports an O → N shift with concomitant formation of an isomeric mixture of *N*-oxide compounds;[24] this was further confirmed with the spray reagent for *N*-oxides.[27] Of significant interest is the fact that upon removal of CH_2Cl_2, the HOAt derivative is sufficiently stable in DMF to be used for the phosphonamide bond formation in solvents suitable for higher mass peptides or for solid phase synthesis. The lower yields obtained in DMF may well be attributed to a different equilibrium between the ester and amide form of the HOAt derivative as recently suggested for the differentiated reactivities of carboxylic acid HOAt esters in CH_2Cl_2 and DMF.[24,26] Since DBU is too basic to use in solid phase synthesis of phosphonamidate peptides with the Fmoc-strategy, it has been replaced by DIEA. No effect was observed in DMF (Table 2), while in CH_2Cl_2 the results obtained with DIEA confirm the usefulness of the strong base DBU.[19] Further attempts to produce stable reactive intermediates of the phosphonic acid **1** led us to the benzotriazolide. Although this derivative is apparently more stable than the HOAt ester, it was found to be less reactive as monitored by the yields of the phosphonopeptide **4** (Table 2).

To conclude, this comparative study allowed for significant improvements in the synthesis of phosphonamidate peptides by optimizing the preparation of the phosphonochloridates with oxalyl chloride in presence of

(13) Dumy, P.; Maffre, D.; Escale, R.; Girard, J. P.; Parello, J.; Vidal, J. P. In *Peptides 1992*; Schneider, C. H., Eberle, A. N., Eds.; ESCOM: Leiden, 1993; pp 340–341.
(14) *Houben Weyl, Methoden der Organischen Chemie*; Georg Thieme: Verlag, Stuttgart, 1963; Vol. 12/1, p 612.
(15) Mukaiyama, T. *Angew. Chem., Int. Ed. Engl.* **1976**, *15*, 94.
(16) Froehler, B. C.; Matteucci, M. C. *Tetrahedron Lett.* **1986**, *27*, 469.
(17) Campbell, D. A. *J. Org. Chem.* **1992**, 57, 6331.
(18) van der Marel, G.; van Boeckel, C. A. A.; Wille, G.; van Boom, J. H. *Tetrahedron Lett.* **1981**, *39*, 3887.
(19) Tawfik, D. S.; Eshhar; Z.; Bentolila, A.; Green, B. S. *Synthesis* **1993**, *968*.
(20) Campagne, J.-M.; Coste J.; Jouin; P. *Tetrahedron Lett.* **1993**, *34*, 6743.
(21) Campagne, J.-M.; Coste, J.; Guillou, L.; Heitz, A.; Jouin, P. *Tetrahedron Lett.* **1993**, *34*, 4181.
(22) Horiki, K. *Tetrahedron Lett.* **1977**, *22*, 1897.
(23) Barlos, K.; Papaioannou, D.; Voliotis, S.; Prewo, R.; Bieri, J. H. *J. Org. Chem.* **1985**, *50*, 696.
(24) Coste, J., Frerot, E. Jouin, P. *J. Org. Chem.* **1994**, 2437.
(25) Carpino, L. A.; El-Faham, A. *J. Org. Chem.* **1994**, *59*, 695.
(26) Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397.
(27) Drabowicz, J.; Kotynski, A.; Kudzin, Z. H.; Skowronski, R. *J. Chromatogr.* **1989**, *473*, 287.

Table 2. Synthesis of Phosphonamidates via Phosphonic Acid Esters and Benzotriazolide Using the Compounds 1 and 2 at a 1:1 Molar Ratio

activation procedure	amino component	yield of 4 (%)
procedure 1: 1/$(COCl)_2$/DMF; 1:2:0.1; rt; 2 h; KOBt; 1; 5 min; in CH_2Cl_2	2·HCl/DBU; 1:2; rt; in CH_2Cl_2; 12 h	65
procedure 1 and 3 h storage in solution, rt; in CH_2Cl_2	2·HCl/DBU; 1:2; rt; in CH_2Cl_2; 12 h	25
procedure 1 and 12 h storage in CH_2Cl_2; filtering of KCl; isolation from hexane; storage for 6 d in the cold	2·HCl/DBU; 1:2; rt; in CH_2Cl_2; 12 h	0
procedure 2: 1/$(COCl)_2$/DMF; 1:2:0.1; rt; 2 h; benzotriazole/TEA; 1:1; 5 min; in THF	2·HCl/TEA; 1:1.5; rt; in THF; 12 h	45
procedure 3: 1/$(COCl)_2$/DMF; 1:2:0.1; rt; 2 h; KOAt; 1; 5 min; in CH_2Cl_2	2·HCl/DBU; 1:2; rt; in CH_2Cl_2; 12 h	75
procedure 3 and storage in solution for 3 h in CH_2Cl_2	2·HCl/DBU; 1:2; rt; in CH_2Cl_2; 12 h	75
procedure 4: 1/$(COCl)_2$/DMF; 1:2:0.1; rt; 2 h; KOAt; 1; 5 min; in CH_2Cl_2	2·HCl/DIEA; 1:2; rt; in CH_2Cl_2; 12 h	54
procedure 4 and evaporation and dissolution in DMF	2·HCl/DBU; 1:2; rt; in DMF; 12 h	49
procedure 4 and evaporation and dissolution in DMF	2·HCl/DIEA; 1:2; rt; in DMF; 12 h	49

catalytic amounts of DMF which are then employed under AgCN catalysis for the phosphonamide bond formation. Moreover, conversion of the phosphonochloridate into the HOAt derivative leads to a sufficiently stable compound to be used in the synthesis of higher molecular weight peptides both in solution and on solid supports.

Abbreviations: DCHA, dicyclohexylamine; DIEA, diisopropylethylamine; TEA, triethylamine; HONp, 4-nitrophenol; HOBt, 1-hydroxybenzotriazole; HOAt, 7-aza-1-hydroxybenzotriazole; AcOEt, ethyl acetate; MeOH, methanol; Phe[PO(OH)], (1-amino-(1*R*,*S*)-2-phenylethyl)-phosphonic acid; Phe[PO(OMe)], methyl (1-amino-(1*R*,*S*)-phenylethyl)phosphonate; Z, benzyloxycarbonyl; Fmoc, fluorenylmethyloxycarbonyl; PyBOP, (benzotriazol-1-yloxy)trispyrrolidinophosphonium hexafluorophosphate.

Supplementary Material Available: Experimental procedures and compound characterization data (5 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.