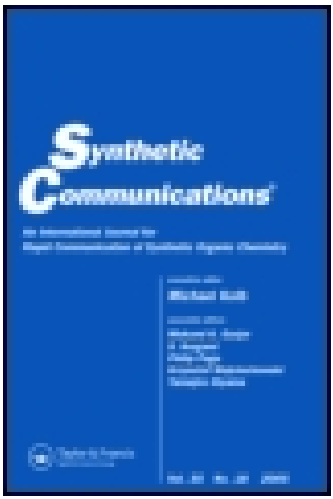


Synthetic Communications: An International Journal for Rapid Communication of Synthetic Organic Chemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lsyc20

Preparation of N-Acylated Phosphonopeptides with Free Phosphonic Group

Vladimir Solodenko [a], Tamara Kasheva [a] & Valeri Kukhar [a]
[a] Institute of Bioorganic Chemistry, Ukrainian Academy of Sciences , Murmanskaya Str., 5, Kiev, 252660, USSR
Published online: 23 Sep 2006.

SYNTHETIC COMMUNICATIONS, 21(15&16), 1631-1641 (1991)

PREPARATION OF N-ACYLATED PHOSPHONOPEPTIDES WITH FREE PHOSPHONIC GROUP

Vladimir Solodenko*, Tamara Kasheva, Valeri Kukhar

Institute of Bioorganic Chemistry, Ukrainian Academy of Sciences, Murmanskaya Str., 5, Kiev 252660, USSR

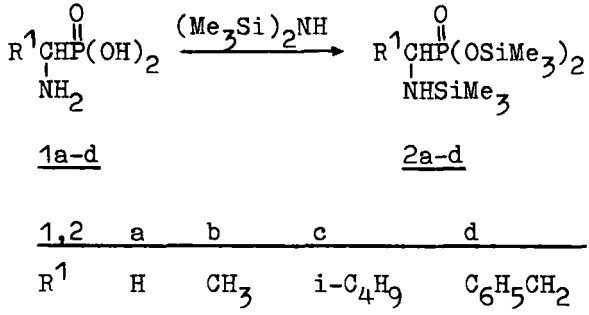
Abstract: Aminoalkylphosphonic acids on heating with hexamethyldisilazane yield tris(trimethylsilyl) derivatives, which are used as amino components for the synthesis of N-acylated phosphonopeptides with free phosphonic group. These transformations proceed with complete retention of the stereoconfiguration of aminophosphonate moiety.

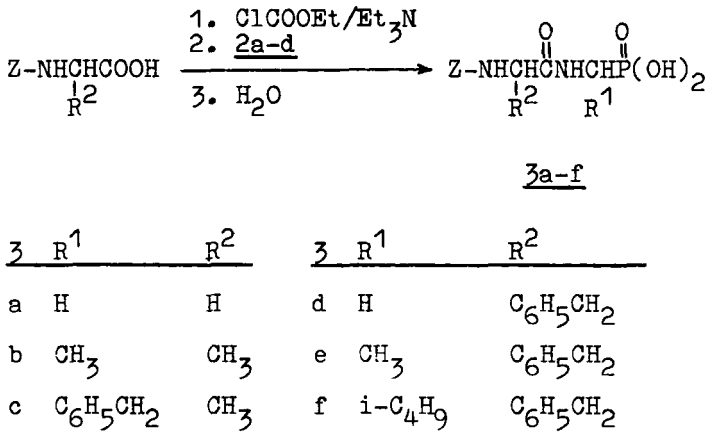
The incorporation of aminoalkylphosphonic acids into peptide chain leads to the phosphonopeptides. Phosphonopeptides with P-terminal aminophosphonate residues draw increasing attention because of their promising biological activities - antimicrobial[1] (alafosfalin 10 seems to be the most interesting example), analgesic[2], plant growth regulating[3], etc. Our interest in the area of potentially bioactive phosphonopeptides led us to synthesize N-acylated phosphonopeptides with free phosphonic group. These compounds are of interest as the analogues of bioactive N-acylated common pepti-

* To whom correspondence should be addressed.

des such as chemotactic peptide For-Met-Leu-Phe[4], schizophrenia related peptide Moz-Thr-Val-Leu[5], virus replication inhibiting peptide Z-D-Phe-Phe-Gly[6], morphine tolerance peptide Boc-Pro-D-Leu[7], and so on. In contrast to well described totally protected or fully unblocked phosphonopeptides[8], relatively few reports have involved the synthesis of N-acylated phosphonopeptides with free phosphonic group. Earlier these compounds were prepared by coupling of N-protected amino acids or their active esters with free aminophosphonic acids in aqueous-organic media[9-11] or by P-deprotection of totally protected phosphonopeptides with chlorotrimethylsilane[11]. However, these methods have some drawbacks (e.g., complicated multi-step procedure of product isolation[9,10] or prolonged time of reaction[11]) and, in our hands, often failed to give the desired products in reasonable yields, thus prompting us to search for other approaches.

We describe here the facile preparation of N-acylated phosphonopeptides with free phosphonic group, which is based on the usage of trimethylsilyl derivatives of aminophosphonic acids as amino components of phosphonopeptide synthesis. This approach was chosen because the hydrolysis of trimethylsilyl esters of phosphonic acids is known to proceed very easily under mild conditions[12]. 1-Aminoalkylphosphonic acids 1a-d on heating with the excess of hexamethyldisilazane[13] are converted in good yields into tris(trimethylsilyl) aminophosphonates 2a-d, which can be isolated by vacuum distillation in individual state. These compounds react with acylating agents similarly to trimethylsilyl derivatives of aminocarboxylic acids[14] resulting in amide bond formation. This fact was used for synthesis of phosphonopeptides with P-terminal aminophosphonate residues by the standard mixed carboxylic-car-

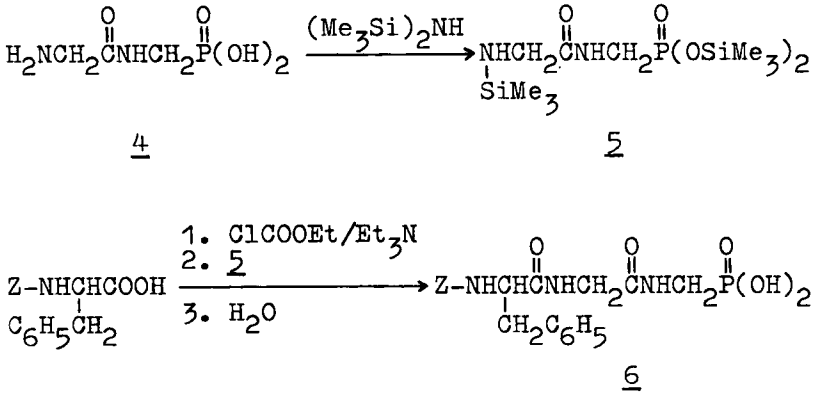




Scheme 1

bonic anhydride method (Scheme 1). The treatment of reaction mixture with aqueous solutions causes the easy removal of trimethylsilyl groups resulting in N-acylated phosphonopeptides 3a-f with free phosphonic function[15].

These compounds can be converted in totally unblocked phosphonopeptides (e.g. by treatment with



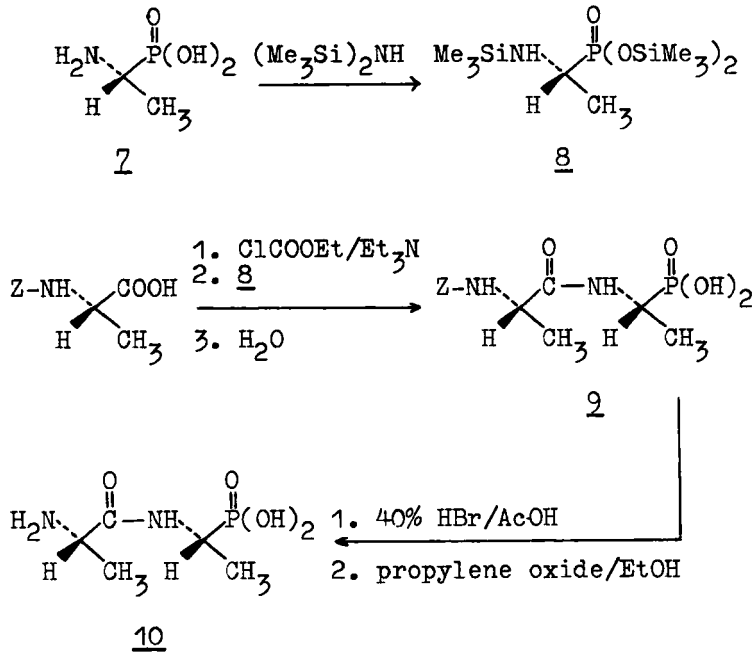
Scheme 2

40% hydrogen bromide solution in glacial acetic acid, r.t., 2 h) as was demonstrated by the transformation of compounds 3a and 9 into compounds 4 and 10, respectively.

Similarly, phosphonopeptide 4 was converted into tris(trimethylsilyl) derivative 5, which was used further for the synthesis of phosphonotripeptide 6 (Scheme 2).

Starting with enantiomeric (L)-1-aminoethylphosphonic acid 7, N-protected phosphonopeptide 9 of L,L configuration was obtained. Removal of N-protecting group from 9 yielded the pure alafosfalin 10[1,10] (Scheme 3). Thus, the reactions outlined in Scheme 3 proceed with the complete retention of aminoalkylphosphonate moiety stereoconfiguration.

In summary, the described preparative procedure has some advantages in simplicity, number of steps, time consumption, as well as in the yields of the products. Moreover, enantiomeric aminoalkylphosphonic acids can be easily incorporated into peptide chain.



Scheme 3

This finding is of great importance because the bioactivity of phosphonopeptides was shown to depend essentially on the stereochemistry of incorporated aminophosphonic acids which, as previously reported, must correspond to L-configuration of natural amino acids[10].

The bioactivity of certain N-acylated phosphonopeptides prepared by this method is under investigation and will be reported elsewhere.

Experimental

Melting points were determined in open capillary tubes and are uncorrected. NMR spectra were obtained

on a Varian Gemini-200 and Bruker WP-200 spectrometers and chemical shifts are presented in ppm from TMS (NMR-^{1}H) and 85% H_3PO_4 (NMR-^{31}P) as standards in CD_2Cl_2 for compounds 2a-d and 5 and CD_3OD for compounds 3a-f and 6. Optical rotations were measured on a Polamat A and Spectropol 1 polarimeters in methanol (c = 1). N-Benzyloxycarbonyl-L-amino acids were purchased from Reanal. 1-Aminoalkylphosphonic acids were prepared as previously described[5,16,17]. All reactions were carried out under unhydrous conditions in dry solvents.

(((N-Glycyl)amino)methyl)phosphonic acid 4 was obtained by N-deprotection of 3a with 40% HBr solution in glacial acetic acid, as described below for alafosfalin 10; yield 67%, mp 254-256°C (dec)(water/ethanol). Lit[18]. mp 221-223°C (dec).

N,O,O'-Tris(trimethylsilyl) 1-aminoalkylphosphonates 2a-d; General Procedure:

A mixture of racemic 1-aminoalkylphosphonic acid (1a-d; 20 mmol) and hexamethyldisilazane (16.7 mL, 80 mmol) is heated on an oil bath at 150-160°C until all solid is dissolved (2-5 h). Excess of hexamethyldisilazane is removed in vacuo and the residue is distilled under reduced pressure to give the following compounds[19]:

2a, yield 91%, bp 140-141°C/15 mbar. NMR-^{1}H, δ: 0.03 (s, 9H), 0.24 (s, 18H), 0.56 (br, 1H), 2.88 (dd, J = 7.8 Hz, 11.4 Hz, 2H). δ_P 11.6.

2b, yield 80%, bp 138-140°C/15 mbar. NMR-^{1}H, δ: 0.04 (s, 9H), 0.26 (s, 18H), 0.57 (br, 1H), 1.20 (dd, 3H, J = 6.9 Hz, 17.6 Hz), 2.90 (m, 1H). δ_P 13.4.

2c, yield 87%, bp 148-150°C/15 mbar. NMR-^{1}H, δ: 0.04 (s, 9H), 0.26 (s, 18H), 0.86, 0.92 (2d, 3H each, J = 6.7 Hz), 1.00-1.50 (m, 4H), 2.80 (m, 1H). δ_P 14.6.

2d, yield 76%, bp 118-120°C/0.08 mbar. NMR - ^{1}H, δ:

0.08 (s, 9H), 0.31 (s, 18H), 1.17 (m, 1H), 2.40-3.00 (m, 2H), 3.11 (m, 1H), 7.26 (m, 5H). δ_P 12.5.

((L,D)-(1-((N-Benzyloxycarbonyl)-L-aminoacyl)amino)alkyl)phosphonic acids 3a-f; General Procedure:

A mixture of N-benzyloxycarbonyl-L-amino acid (10 mmol) and Et_3N (1.39 mL, 10 mmol) in ethyl acetate (10 mL; in the case of N-benzyloxycarbonylglycine dioxane (5 mL) is added additionally to obtain a clear solution) is cooled to -5°C. Ethyl chloroformate (0.95 mL, 10 mmol) is added, and the mixture is stirred at -5°C for 0.5 h. A solution of tris(trimethylsilyl) aminophosphonate (2a-d, 11 mmol) in ethyl acetate (7 mL) is added dropwise, and the mixture is stirred at -5°C for 2 h, then at r.t. for 2 h, and heated at 80°C for 1 h. All volatiles are removed in vacuo, the residue is dissolved in sat. aq. $NaHCO_3$ solution (15 mL), and the solution is acidified to pH 2 with 10% HCl. This solution is extracted with EtOAc (3x40 mL), the combined organic extracts are dried (Na_2SO_4) and evaporated in vacuo to give crude 3, which is crystallized from the appropriate solvent. The following phosphonodipeptides are prepared[20]:

3a, yield 72%, mp 180-182°C (MeOH/EtOAc). NMR-^{1}H, δ: 3.41 (d, 2H, J = 12.8 Hz), 3.76 (s, 2H), 5.04 (s, 2H), 7.27 (s, 5H). δ_p 18.2.

3b, yield 70%, mp 160-162°C (EtOAc). $[\alpha]_D^{20}$ -27.6°. NMR-^{1}H, δ: 1.26 (dd, 3H, J = 7.0 Hz, 15.9 Hz), 1.28 (d, 3H, J = 7.0 Hz), 4.02 (m, 2H), 5.0 (s, 2H), 7.29 (m, 5H). NMR-$^{31}P\{^1H\}$, δ: 23.35, 23.65 (two diastereomeric phosphonopeptides).

3c, yield 78%, mp 173-176°C (EtOAc). $[\alpha]_D^{20}$ -28.6°. NMR-^{1}H, δ: 0.87, 1.10 (2d, 3H, J = 7.0 Hz), 2.60-3.30 (m, 2H), 4.02 (m, 1H), 4.39 (m, 1H), 5.0 (s, 2H), 7.13-7.28 (m, 10H). δ_P 22.0.

3d, yield 68%, mp 174-176°C (MeOH/EtOAc). $[\alpha]_D^{20}$ -11.5°.

NMR-^{1}H, δ : 2.65-3.14 (m, 2H), 3.47 (d, 2H, J = 12.5 Hz), 4.32 (m, 1H), 4.89 (s, 2H), 7.12-7.17 (m, 10H). δ_P 20.6. Lit.[11] mp 183-184°C, $[\alpha]_D^{20}$ -10.9° (c = 1, MeOH).

3e, yield 78%, mp 188-189°C (EtOAc). $[\alpha]_D^{20}$ -15.9°. NMR-^{1}H, δ : 1.06, 1.24 (2dd, 3H, J = 7.0 Hz, 16.0 Hz), 2.66-3.17 (m, 2H), 4.03 (m, 1H), 4.33 (m, 1H), 4.91 (s, 2H), 7.17-7.21 (m, 10H). δ_P 23.7.

3f, yield 56%, mp 175-177°C (EtOAc/hexane). $[\alpha]_D^{20}$ -8.6°. NMR-^{1}H, δ : 0.70, 0.74 (2d, 3H each, J = 6.5 Hz), 1.20-1.75 (m, 3H), 2.60-3.14 (m, 2H), 4.17 (m, 1H), 4.37 (m, 1H), 4.94 (s, 2H), 7.18-7.23 (m, 10H). δ_P 23.6.

((((N-Benzyloxycarbonyl)-L-phenylalanyl)glycyl)amino)-methyl)phosphonic acid (6).

A mixture of phosphonopeptide 4 (0.23 g, 1.37 mmol) and hexamethyldisilazane (5 mL, 24 mmol) is heated on an oil bath at 150-160°C for 5 h, the remaining solid is filtered off, and the clear filtrate is evaporated in vacuo (15 mbar) on an oil bath at 80°C. The colorless mobile residue is sufficiently pure 5[19], yield 0.37 g (70%). NMR-^{1}H, δ : 0.07 (s, 9H), 0.27 (s, 18H), 1.20 (m, 1H), 3.32 (d, 2H, J = 9.0 Hz), 3.33 (dd, 2H, J = 6.0 Hz, 13.0 Hz), 7.30 (br, 1H). δ_P 5.8. This product is used in the next step without further purification. A mixture of N-benzyloxycarbonyl-L-phenylalanine (0.27 g, 0.9 mmol) and Et_3N (0.125 mL, 0.9 mmol) in ethyl acetate (7 mL) is cooled to -5°C. Ethyl chloroformate (0.085 mL, 0.9 mmol) is added, and the mixture is stirred at -5°C for 0.5 h. A solution of 5 (0.37 g, 0.96 mmol) in ethyl acetate (5 mL) is added dropwise, and the mixture is stirred at -5°C for 2 h, then at r.t. for 2 h, and heated at 80°C for 1 h. The volatile components are removed in vacuo, the residue is dissolved in sat. aq. $NaHCO_3$ solution (10 mL), and the resulting solution is adjusted to pH 2 with 10% HCl. The precipitated crude 6 is filtered off. The

filtrate is extracted with EtOAc (3x30 mL), the combined organic phase is dried (Na_2SO_4) and evaporated in vacuo to give further portion of the product. The combined crude 6 is crystallized from MeOH/EtOAc; yield 0.25 g (62%)[20], mp 195-196°C. $[\alpha]_D^{20}$ -5.6°. NMR-^{1}H, δ : 2.77-3.15 (m, 2H), 3.53 (d, 2H, J = 12.0 Hz), 3.79 (m, 2H), 4.30 (m, 1H), 4.98 (s, 2H), 7.17-7.21 (m, 10H). δ_P 20.6.

((L)-(1-((N-L-Alanyl)amino)ethyl))phosphonic acid (10, Alafosfalin):

(L)-1-Aminoethylphosphonic acid 7 is converted into tris(trimethylsilyl) 1-aminoethylphosphonate 8 (yield after distillation 79%, bp 138-140°C/15 mbar), which is used for coupling with N-benzyloxycarbonyl-L-alanine as described above for 3a-f, resulting in phosphonopeptide 9; yield 67%, mp 172-173°C (EtOAc). $[\alpha]_D^{20}$ -40.8°(c = 1, MeOH). NMR-^{1}H is as for 3b. NMR-^{31}P-{^{1}H}, δ : 23.35. Phosphonopeptide 9 (0.2 g, 0.6 mmol) is dissolved in 40% HBr solution in glacial acetic acid (3 mL) and the mixture is stirred at r.t. for 2 h. Anhydrous Et_2O (10 mL) is added, and the mixture is stirred for 10 min and the upper phase is decanted. The residue is evaporated, the remaining gum is dissolved in absolute ethanol (20 mL) and propylene oxide (3 mL) is added. The solid precipitate is filtered off and crystallized from water/ethanol to give pure alafosfalin 10; yield 0.11 g (93%), mp 274-276°C (dec). $[\alpha]_D^{20}$ -46.0° (c = 0.5, H_2O). NMR-^{1}H (D_2O/TMS), δ: 1.04 (dd, 3H, J = 6.8 Hz, 15.0 Hz), 1.28 (d, 3H, J = 6.8 Hz), 3.78 (m, 2H). NMR-^{31}P-{^{1}H}(D_2O/H_3PO_4), δ: 20.15[21]. Lit.[10] mp 294-295.5°C (dec), $[\alpha]_D^{20}$ -46.3° (c = 1, H_2O).

References and Notes

1. Allen, J.G., Atherton, F.R., Hall, M.J., Hassall, C.H., Holmes, S.W., Lambert, R.W., Nisbet, L.J. and Ringrose, P.S., Nature, 1978, 272, 56.
2. Mastalerz, P., Kupczyk-Subotkowska, L., Herman, Z.S. and Laskawiec, G., Naturwissenschaften, 1982, 69, 46.
3. Gancarz, R., Kafarski, P., Lejczak, B., Mastalerz, P., Wieczorek, J.S., Przybylka, E. and Czerwinski, W., Phosphorus and Sulfur, 1983, 18, 373.
4. Bloom, R.S., "Peptides, Synthesis-Function", Proceedings of the Seventh American Peptide Symposium, Rich, D.H. and Gross, E. (Eds), 1981, p. 577.
5. Frohman, C.E., German Patent 2 712 781; C.A., 1978, 88, 170493x.
6. Richardson, S.D., Virology, 1980, 105, 205.
7. Walter, R., Proc. Natl. Acad. Sci. USA, 1979, 76, 518.
8. Kafarski, P., Lejczak, B. and Mastalerz, P., Beitr. Wirkstofforsch., 1985, No. 25.
9. Kametani, T., Kigasawa, K., Hiiragi, M., Wakisaka, K., Haga, S., Sugi, H., Tanigawa, K., Suzuki, Y., Fukawa, K., Irino, O., Saita, O. and Yamabe, S., Heterocycles, 1981, 16, 1205.
10. Atherton, F.R., Hassall, C.H. and Lambert, R.W., J. Med. Chem., 1986, 29, 29.
11. Atherton, F.R., Hall, M.J., Hassall, C.H., Lambert, R.W. and Ringrose, P.S., German Patent 2 602 193 (1976); C.A., 1976, 85, 143525.
12. Rabinowitz, R., J. Org. Chem., 1963, 28, 2975.
13. Maier, L., J. Organomet. Chem., 1979, 178, 157.
14. Birkofer, L., Ritter, A. and Neuhausen, P., Liebigs Ann. Chem., 1962, 190.

15. When this work was in progress, the communication of Polish authors (Kafarski, P., Soroka, M. and Lejczak, B., Peptide Chemistry 1987. Shiba, T. and Sakakibara, S. (Eds), Protein Research Foundation, Osaka, 1988, p. 307; C.A., 1988, 109, 170892d) was published, where silylated aminophosphonic acids were reported to be used for the synthesis of totally unblocked phosphonodipeptides. However, N-acylated phosphonopeptides with free phosphonic group were not described in this communication.
16. Kudzin, Z.H.and Stec, W.J., Synthesis, 1978, 469.
17. Pulwer, M.J. and Balthazor, T.M., Synth. Commun., 1986, 16, 733.
18. Kafarski, P. and Lejczak, B., Synthesis, 1988, 307.
19. Satisfactory microanalyses were obtained: P ± 0.10, Si ± 0.29.
20. Satisfactory microanalyses were obtained: C ± 0.48, H ± 0.30, N ± 0.38, P ± 0.29. Compounds 3b,c,e,f are the mixtures of two diastereomers.
21. N-Deprotection of 3b affords the mixture of two diastereomeric phosphonopeptides, which are distinctly distinguishable in NMR-^{31}P-$\{^{1}H\}$ spectrum ($\Delta\delta_p$ 0.12 ppm).

(Received in UK 23 April, 1991)