Heteroatom Chemistry
Volume 19, Number 1, 2008

Synthesis of Peptidyl Phosphonates Containing 5-(4′-Carboxyphenyl)-10,15,20-Tritolylporphyrin

Jan Habdas[1] and Bogdan Boduszek[2]

[1]*Institute of Chemistry, University of Silesia, 40-006 Katowice, Poland*

[2]*Department of Organic Chemistry, Faculty of Chemistry, Wroclaw University of Technology, 50-370 Wroclaw, Poland*

Received 26 February 2007; revised 18 June 2007

ABSTRACT: *The synthesis and characterization of three new porphyrin-peptidyl-phosphonate derivatives, containing a diphenyl 3-pyridylmethyl-phosphonate moiety, is described. These compounds could serve as potential photosensitizers for the PDT method of tumor therapy and displayed activity as inhibitors of aminopeptidase N.* © 2008 Wiley Periodicals, Inc. Heteroatom Chem 19:107–111, 2008; Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/hc.20407

INTRODUCTION

Our earlier investigations concerning the preparation of some photosensitizers, used for the PDT method for the diagnosis and therapy of tumors, have shown that certain derivatives of porphyrins containing an amide bond in the molecule demonstrated the properties of the photosensitizers [1–3]. These porphyrin–peptide conjugates possessed a strictly defined porphyrin structure in which the porphyrin ring was covalently linked with an amino acid or dipeptidyl moiety. It was an improvement, in comparison with the widely applied "Photofrin II" [4] for the PDT therapy, which is a mixture of various hematoporphyrins.

As it is known, aminophosphonic acids are phosphorus analogs of regular amino acids with great biological activity [5,6]. Hence, we are interested in the porphyrin-aminophosphonate derivatives, which might turn out to be compounds with definite biological activity, such as enzyme inhibitors.

The aim of this work is the synthesis of new porphyrin-peptidyl-phosphonates, the substances with potential biological activity.

RESULTS AND DISCUSSION

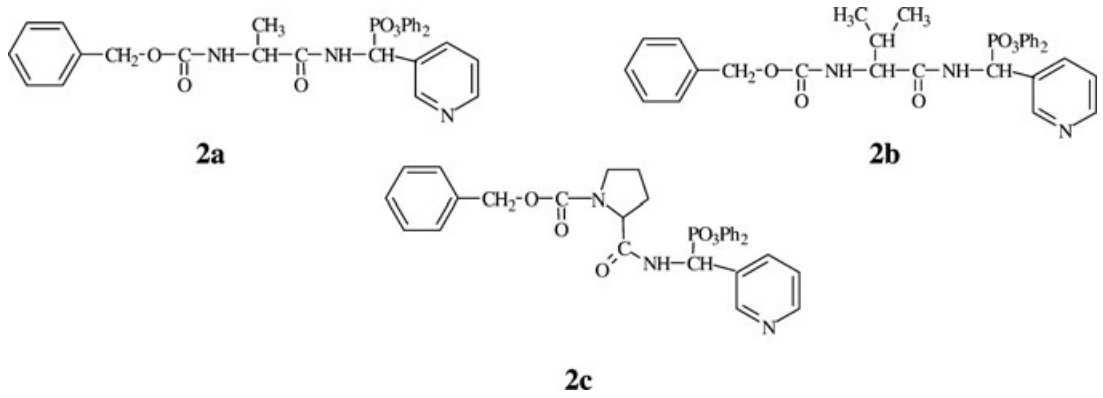
We have synthesized three new tolylporphyrin derivatives containing pyridine peptidyl-phosphonate fragments in a side chain of the porphyrin ring. The compounds were obtained in the reaction of 5-(4′-carboxyphenyl)-10,15,20-tritolylporphyrin (**1**) with pyridine derivatives of the diphenyl esters of selected phosphonodipeptides. The choice of the diphenyl phosphono-esters for synthesis was made because such esters of aminophosphonic acids showed great biological activity [5].

The pyridine-3-yl Cbz-phosphonodipeptides **2a–c** as single diastereomers, were synthesized

Correspondence to: Bogdan Boduszek; e-mail: bogdan.boduszek@pwr.wroc.pl

Contract grant sponsor: Institute of Chemistry, University of Silesia, Katowice, Poland.

Contract grant sponsor: Faculty of Chemistry, Wroclaw University of Technology, Wroclaw, Poland.



SCHEME 1 Structures of the Cbz-phosphono-peptides.

earlier from protected L-amino acids [7] and were transformed into the corresponding hydrobromides **2a′–c′** with nonsubstituted amino groups, suitable for this work.

Coupling the phosphonates **2a′–c′** with the tolylporphyrin **1** enabled the combination of the biological properties of the porphyrins with the known inhibitory activity of the peptidyl diphenyl phosphonates, which are well recognized as inhibitors of the serine proteases [6,8]. Also, an introduction of the pyridine derivatives of phosphonopeptides to such structures should increase the solubility in aqueous systems.

The formulas of the **2a–c** (Cbz-phosphono-peptides) used for the preparation of the corresponding hydrobromides **2a′–c′** are shown in Scheme 1.

The side peptidyl chains in the synthesized compounds were composed with chosen amino acids and pyridine-3-yl-methyl diphenyl phosphonate ester: namely, alanine-[(1-amino)-1-(pyridine-3-yl)-methylphosphonate diphenyl ester] (in the case of **3a**), valine-[(1-amino)-1-(pyridine-3-yl)-methylphosphonate diphenyl ester] (in the case of **3b**), and proline-[(1-amino)-1-(pyridine-3-yl)-methylphosphonate diphenyl ester] (in the case of **3c**).

The method applied for synthesis of similar peptidyl-phosphonate derivatives of the porphyrin is already elaborated by us in 2005 [9].

The considered products, that is, the tritolylporphyrin-pyridine-phospho-dipeptides **3a–c** were obtained from the tritolylporphyrin **1** [10], by the use of the DCC coupling method. A course of the reaction is shown in Scheme 2.

The isolation of the **3a–c** was done in a classical way, which was applied in the preparation of short peptides, and the products were additionally purified by means of column chromatography.

The obtained products were porphyrin-like substances, unstable at elevated temperatures.

Because of the existence of phosphono-dipeptide moieties in the final products, we have decided to perform preliminary inhibitory tests by using the selected enzyme, aminopeptidase N (APN/CD13). As it is known, the aminopeptidase N [11] is a cell surface peptidase, which occurs in a wide variety of tissues and cells. This enzyme is usually overfilled in tumor cells and plays a critical role in angiogenesis, which is, in turn, a complex cascade process playing a central role in tumor growth.

The results of the inhibitory activity of the diphenyl porphyrin-peptidyl-phosphonates **3a–c** toward APN/CD13 are given in Table 1. The method used for the inhibitory tests was previously described in the literature [12].

The products **3a–c** showed a moderate inhibition of aminopeptidase N. Among them, the best inhibitor of APN/CD 13 was the **3b** [TTP-Val-3PyP(OPh)] ($IC_{50} = 19.3\ \mu M$). Surprisingly, the starting material, that is, 5-(4′-carboxyphenyl)-10,15,20-tritolylporphyrin (**1**), with IC_{50} equal to 18.5 μM, was the best inhibitor.

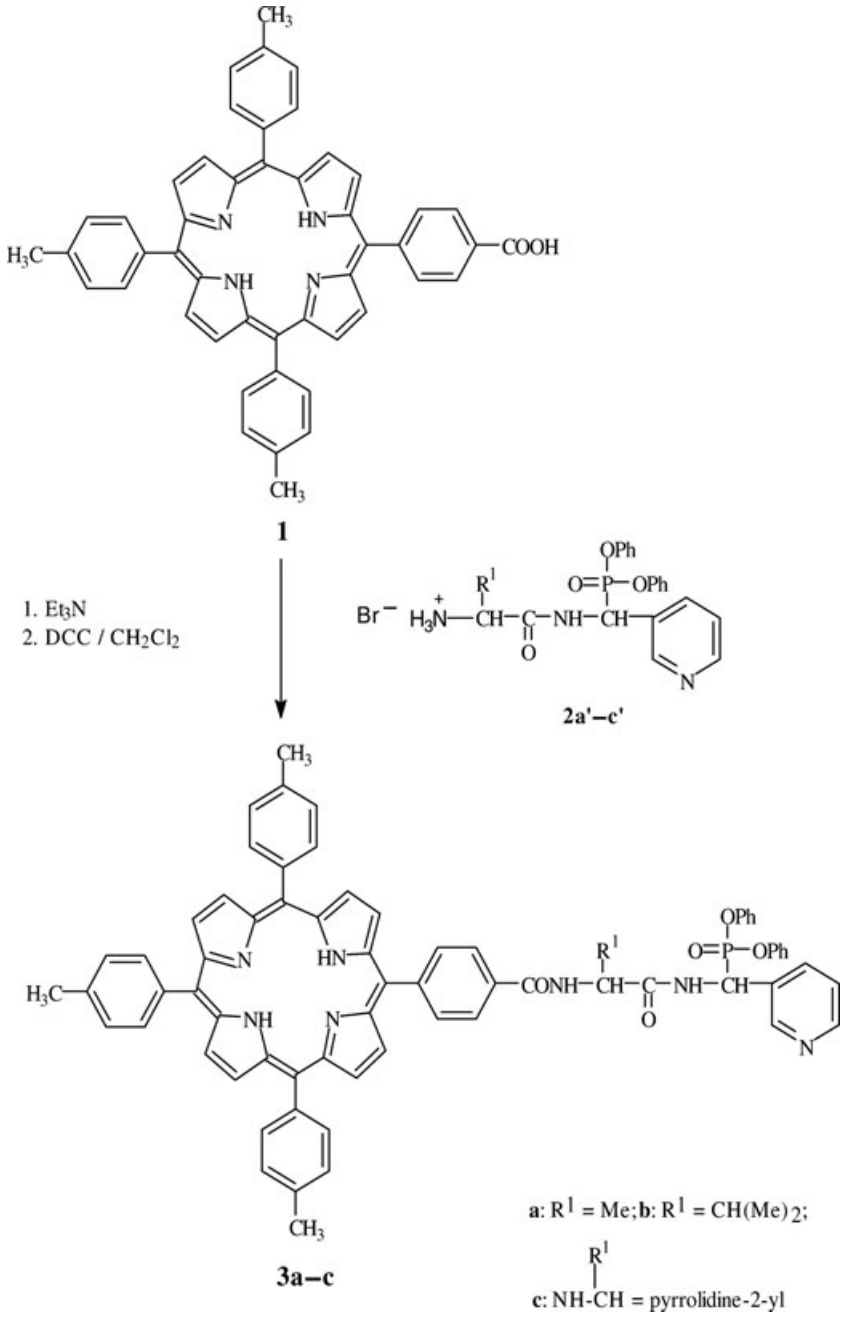
The preliminary tests showed that the peptidyl derivatives of tolylporphyrin and the carboxytolylporphyrin itself were significant inhibitors of aminopeptidase N and may be promising biologically active agents for further investigation in this field, which are already in progress.

In the near future, we are planning to obtain the free phosphonic acid derivatives of the porphyrin, without the diphenyl ester functions, which, as it is expected, should show higher inhibitory activity toward aminopeptidase N.

EXPERIMENTAL

General

NMR spectra were recorded on a Bruker Avance TM DRX 300 MHz in $CDCl_3$using 300 MHz for ^1H



SCHEME 2 Synthesis of the tolylporphyrin pyridine peptidyl diphenyl phosphonates **3a–c**.

TABLE 1 Inhibition of Aminopeptidase N [CD 13][a]

Compound No.	*Compound Abbreviation*	*Molar Weight*	*IC_{50} (μM)[b,c]*
3a	TTP-Ala-3PyP(OPh)	1094	87.3
3b	TTP-Val-3PyP(OPh)	1122	19.3
3c	TTP-Pro-3PyP(OPh)	1120	32.4
1	TTP-COOH	701	18.5

[a]Concentration of enzyme: 5 μg/mL.
[b]Concentration of substrate, L-leucylo-(4-nitroanilide): 250 μM/mL.
[c]Buffer: KH_2PO_4-Na_2HPO_4 with pH 7.2 was used.

NMR and 121.51 MHz for ^{31}P NMR spectra in the Department of Organic Chemistry at the Wroclaw University of Technology. IR and UV spectra were measured on a Perkin Elmer 1600 FTIR spectrophotometer in the Department of Organic Chemistry at the Wroclaw University of Technology and in the Institute of Chemistry at the University of Silesia, Katowice. MS analyses were performed by electrospray ionization with a Finnigan TSQ 700 instrument on mode ESI + Q1MS at the Department of Chemistry at the University of Wroclaw.

All reagents were purchased from the Sigma Aldrich Co.

Diphenyl pyridine-3-methyl-(Cbz-amino) phosphonate alanine derivative (**2a**), diphenyl pyridine-3-methyl-(Cbz-amino)phosphonate valine derivative (**2b**), and diphenyl pyridine-3-methyl-(Cbz-amino)phosphonate proline derivative **(2c**) were obtained as described in [7]. For stability and long storage, the amine groups in the **2a–c** were protected by the Cbz groups, which could be easily removed by means of the 30% HBr solution in anhydrous acetic acid, in a one-pot procedure.

5-(4-Carboxyphenyl)-10,15,20-tritolylporphyrin (**1**) was generally obtained by the Adler's method [10], with some modifications. In our case, 5-(4-carboxyphenyl)-10,15,20-tritolylporphyrin (**1**) was prepared by the condensation of 4-methylbenzaldehyde and 4-carboxybenzaldehyde with pyrrole in boiling propionic acid. The applied molar ratio of the reactants (3:1:4) was optimized and gave the highest yield of the **1** in the conditions used.

*Procedure for Preparation of 5-(4-Carboxyphenyl)-10,15,20-tris-p-tolylporphyrin (**1**)*

In a 1-L round-bottom flask, equipped with an efficient mechanical stirrer was placed consecutively: propionic acid (600 mL), 4-tolylaldehyde (3.6 g, 30 mmol), and 4-carboxybenzaldehyde (1.5 g, 10 mmol). The mixture was heated to 140°C and pyrrole (6.7 g, 40 mmol) was added dropwise for 30 min with stirring. Heating was continued for the next 30 min and then the mixture cooled. The mixture was left for 24 h and then a separated product was filtered off, washed several times with water and a mixture of water–methanol (v/v: 4:1). Washing was continued until the filtrate become colorless and odorless. The product was dried to give a dark violet solid (m = 2.1 g, 30%, which was a mixture of various porphyrins). The desired product was separated by column chromatography (silica gel 60–230 mesh, eluant: chloroform–methanol; v/v 9:1), collecting the eluate with the second purple band containing the tolylporphyrin **1**. After the evaporation of the solvent, a dark-violet product (**1**) was obtained.

1: Yield: 400 mg (5%). The spectroscopic data (UV, IR) of the **1** was in agreement with the literature [10].

^1H NMR ($CDCl_3$), δ, ppm: 8.86 (d, 2H, J = 4.8 Hz, arom.), 8.76 (d, 2H, J = 4.8 Hz, arom.), 8.28 (d, 2H, J = 8.19 Hz, arom.), 8.10 (d, 8H, J = 7.8 Hz, Phs), 7.96 (d, 2H, J = 8.19 Hz, arom.), 7.56 (d, 8H, J = 7.8 Hz, Phs), 2.71 (s, 6H, CH_3), 2.21 (s, 3H, CH_3), −2.75 (bs, 2H, NH-pyrrole). MS: ESI + Q1MS, 701 (M + 1).

*Preparation of Hydrobromides **2a′–c′***

The diphenyl peptidyl-pyridine phosphonate **2a–c** [7] (0.5 mmol) was mixed with 30% HBr solution in acetic acid (1.0 mL). The mixture was kept for 1–2 h, protected against moisture and then treated with anhydrous diethyl ether (20 mL) with stirring. The supernatant ethereal layer was removed by decantation, and a fresh portion of diethyl ether was added. The procedure was repeated three times, and the product was filtered to give a whitish solid, being the hydrobromide of the **2a′, 2b′** or**2c′**, which was used directly in the next step.

General Procedure for Preparation of the Tolylporphyrin-pyridine-peptidyl-phosphonates **3a–c**

1,3-Dicyclohexylcarbodiimide (21 mg, 0.1 mmol) (DCC, a coupling agent) was added with stirring to the solution of 5-(4-carboxyphenyl)-10,15,20-*p*-tritolylporphyrin (**1**, 70 mg, 0.1 mmol) in dry methylene chloride (10 mL). Then, a solution of the hydrobromide of the corresponding aminophosphonate **2a′–c′** (0.1 mmol) with triethylamine (11 mg) and 4-(dimethylamino)pyridine (12 mg, DMAP, a catalyst) in methylene chloride (10 mL) was added and the mixture was stirred for 2 h at 0°C and left for 24 h at room temperature. After this, the reaction mixture was evaporated to dryness, treated with ethyl acetate (50 mL), stirred, and filtered. The filtrate was subsequently washed with 0.1 M aqueous solution of citric acid (20 mL), 1.0 M aq. $NaHCO_3$, (2 × 20 mL), and water (3 × 20 mL), dried (anh. Na_2SO_4), filtered, and evaporated to give the crude product as a dark-violet solid. The product was purified by column chromatography (silica gel: 60–230 mesh, eluant: $CHCl_3$–MeOH, v/v 20:1), collecting the band containing the tritolylporphyrin-peptidyl-phosphonate **3a–c**. After the evaporation of the eluate, the particular product (**3a–c**) was obtained as a dark-violet solid.

3a: Yield, 55 mg (50%), ^{1}H NMR($CDCl_3$), δ (ppm): 8.88–6.90 (m, 38H, aromatic protons), 6.42 (d, 1H, CH—P, $J = 7.67$ Hz), 3.70–3.50 (m, 3H, NH, CH protons), 3.06 (s, 3H, CH_3 protons), 2.71 (s, 6H, CH_3 protons), 1.25 (m, CH_3), −2.78 (bs, 2H, NH-pyrrole). ^{31}P NMR ($CDCl_3$), δ (ppm): 12.84 (s). IR (KBr) (cm^{-1}): 3326; 2963; 2927; 2850; 1627 (C=O); 1573; 1262 (P=O); 891; 801; 692; 640. MS: ESI + Q1MS, 1094 (M).

3b: Yield: 60 mg (54%), ^{1}H NMR ($CDCl_3$), δ (ppm): 8.88–6.50 (m, 40H, aromatic, NH and CH—P protons), 4.4–3.8 (m, 5H, NH, CH, CH_3 protons), 3.03 (s, 3H, CH_3), 2.71 (s, 6H, CH_3), 1.1 (m., 3H, CH_3), −2.77 (bs, 2H, NH-pyrrole). ^{31}P NMR ($CDCl_3$), δ (ppm): 13.29 (s). IR (KBr) (cm^{-1}): 3326; 2928; 2850; 1626 (C=O); 1575; 1311; 1244 (P=O); 1227; 1088; 892; 801; 641. MS: ESI + Q1MS, 1121 (M – 1).

3c: Yield: 59 mg (53%), ^{1}H NMR ($CDCl_3$), δ (ppm): 8.89–7.17 (m, 38H, aromatic protons), 7.04 (d, 1H, CH—P, $J = 7.72$ Hz), 4.2–3.7 (m, 9H, NH, CH_2, CH protons), 2.94 (s 3H, CH_3), 2.71 (s, 6H, CH_3), –2.77 (bs, 2H, NH-pyrrole). ^{31}P NMR ($CDCl_3$), δ (ppm): 14.29 (s). IR (KBr) (cm^{-1}): 3323; 2927; 2850; 1646; 1626 (C=O); 1473; 1222 (P=O); 1069; 966; 891; 800; 693; 641. MS: ESI + Q1MS, 1120 (M).

CONCLUSIONS

The synthesis of the three new *p*-tritolylporphyrin-peptidyl-pyridine-3-yl phosphonates is described. These compounds were obtained in a satisfactory yield from the carboxy-*p*-tritolylporphyrin **1** and phosphonodipeptides **2a′–c′** with protected phosphonic acid functions by a typical peptide-synthesis method. The obtained products showed a moderate inhibitory activity toward aminopeptidase N, an enzyme responsible for tumor cell growth. However, these compounds and their derivatives could serve as a new class of inhibitors of some proteases.

ACKNOWLEDGMENTS

We thank Ms. Renata Grzywa and Dr. Marcin Sieńczyk from the Wroclaw University of Technology for performing the inhibitory tests.

REFERENCES

[1] Drzewiecka, A. K.; Urbańska, K.; Matuszak, Z.; Pinerio, M.; Arnaut, L. G.; Habdas, J.; Ratuszna, A.; Stochel, G. Acta Biochim Pol 2001, 48, 277–282.
[2] Szurko, A.; Kramer-Marek, G.; Widel, M.; Ratuszna, A.; Habdas, J.; Kuś, P. Acta Biochim Pol 2003, 50, 1165–1174.
[3] (a) Kramer-Marek, G.; Szurko, A.; Serpa, C.; Arnaut, L. G.; Kuś, P.; Habdas, J.; Ratuszna, A. Phys Med 2004, 20(Suppl. 1), 46–48; (b) Geier, G. R., III; Sasaki, T. Tetrahedron Lett 1997, 38, 3821–3824.
[4] Saczko, J.; Kulbacka, J.; Chwiłkowska, A.; Drag-Zalesinska, M.; Wysocka, T.; Ługowski, M.; Banaś T. Folia Histochem Cytobiol 2005, 43(3), 129–132.
[5] Kukhar, V. P.; Hudson, H. R. (Eds.). Aminophosphonic and Aminophosphinic Acids; Wiley: Chichester, UK, 2000; Vol. 443–482, pp. 559–621.
[6] Kafarski, P.; Lejczak, B. Phosphorus, Sulfur Silicon 1991, 63, 193–215.
[7] Boduszek, B. Phosphorus, Sulfur Silicon 2001, 176, 119–124.
[8] Boduszek, B.; Oleksyszyn, J.; Kam, Ch-M.; Selzler, J.; Smith, R. E.; Powers, J. C. J Med Chem 1994, 34, 3969–3976.
[9] Habdas, J., Boduszek, B. Phosphorus, Sulfur Silicon 2005, 180, 2039–2045.
[10] Adler, A. D.; Longo, F. R.; Finarelli, J. D.; Goldmacher, J.; Assour, J.; Korsakoff, L. J Org Chem 1967, 32, 476–481.
[11] Bauvois, B.; Pulffe, M. L.; Bongul, J. B.; Paillat, S.; Monneret, C.; Dauzonne, D. J Med Chem 2003, 46, 3900–3913.
[12] Sieńczyk, M.; Oleksyszyn, J. Bioorg Med Chem Lett 2006, 16, 2886–2890.