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Synthesis of 5-(4′-carboxyphenyl)-10,15,20-tris-(4 pyridyl)-porphyrin and its peptidyl phosphonate derivatives

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The synthesis and characterization of three new 4-pyridyl porphyrin-peptidyl-phosphonate compounds, containing a diphenyl 3-pyridylmethyl-phosphonate moiety, is described in this article. Nitrogen atoms in the pyridine rings of the obtained compounds were alkylated using methyl iodide, to give additional three, water soluble derivatives of these peptidyl-porphyrin conjugates. All the synthesized compounds could serve as potential photosensitizers for the photodynamic therapy (PDT) method of tumor therapy and displayed activity as inhibitors of aminopeptidase N.

Keywords: 4-pyridyl-porphyrins; diphenyl phosphonates; 4-pyridyl-porphyrin peptidyl phosphonates; aminopeptidase N inhibition

Introduction

Since 1931, when Hans Fischer was awarded the Nobel Prize for his work on haemin synthesis, the interest in synthesis and application of porphyrin derivatives in various areas of science has grown significantly [1]. One of their most important applications in medicine is application of them as photosensitizers. When porphyrin compounds are irradiated with visible light, the formation of cytotoxic singlet oxygen in tumor cells occurs. This initiates a tumor necrosis, which is a base of photodynamic therapy (PDT) method. This method has been developed about three decades ago [2], but up to now it has not been applied widely. Photosensitization can be a useful approach for destroying microbial cells since it has been shown that certain porphyrin derivatives display phototoxicity against bacteria and yeast; therefore, it is called *photodynamic antimicrobial chemotherapy* (PACT) method [3.] Investigation has also been carried out on the destruction of various types of viruses including the human immunodeficiency virus (HIV); which is in turn a base of the Photodynamic Destruction of Viruses (PDV) method) [4].

Our earlier investigations concerning the preparation of some potential photosensitizers have shown that certain derivatives of porphyrins containing an amide bond in the molecule showed good photobiological properties, based on singlet oxygen quantum yield [5–7]. Such porphyrin–peptide conjugates possessed a strictly defined structure in which the porphyrin structure was covalently linked with an amino acid or dipeptidyl moiety. It was an improvement, in comparison with the 'Photofrin II' [4] that is widely applied for PDT therapy, which, in reality, is a mixture of various haematoporphyrins.

The aminophosphonic acids are phosphorus analogs of regular amino acids. As it is known, these acids and their esters possess a great biological activity [8–10]. Therefore, combining the porphyrins and aminophosphonate moieties may lead to new compounds with interesting biological properties, as for example enzyme inhibitors [11,12]. Hence is our interest in the porphyrin-aminophosphonate derivatives, which might prove to be new substances with a great biological activity.

Material and Methods

Chemical Part

General procedures

NMR spectra were recorded on a Bruker Avance TM DRX 300 MHz in $CDCl_3$ using 300 MHz for ^{1}H NMR and 121.51 MHz for ^{31}P NMR spectra in the Department of Organic Chemistry at the Wroclaw University of Technology. IR spectra were measured on a Perkin Elmer 1600 FTIR spectrophotometer in the Department of Organic Chemistry at the Wroclaw University of Technology and UV-Vis spectra were measured on Genesis 6 Thermo Spectronic Instrument in the Institute of Chemistry at the University of Silesia, Katowice. MS analyses were performed on a Finnigan TSQ 700 Instrument (electrospray ionization, mode ESI + Q1MS) in the Department of Chemistry at the University of Wroclaw.

All reagents were purchased from the Sigma Aldrich Company.

Working on the synthesis of porphyrin derivatives, we have obtained the 5-(4′-carboxyphenyl)-10,15,20-tri-(4-pyridyl)-porphyrin (**1**), starting from 4-pyridinecarboxaldehyde, pyrrole and 4-carboxybenzaldehyde (Scheme 1), following the method described for synthesis of some other porphyrins [13–15].

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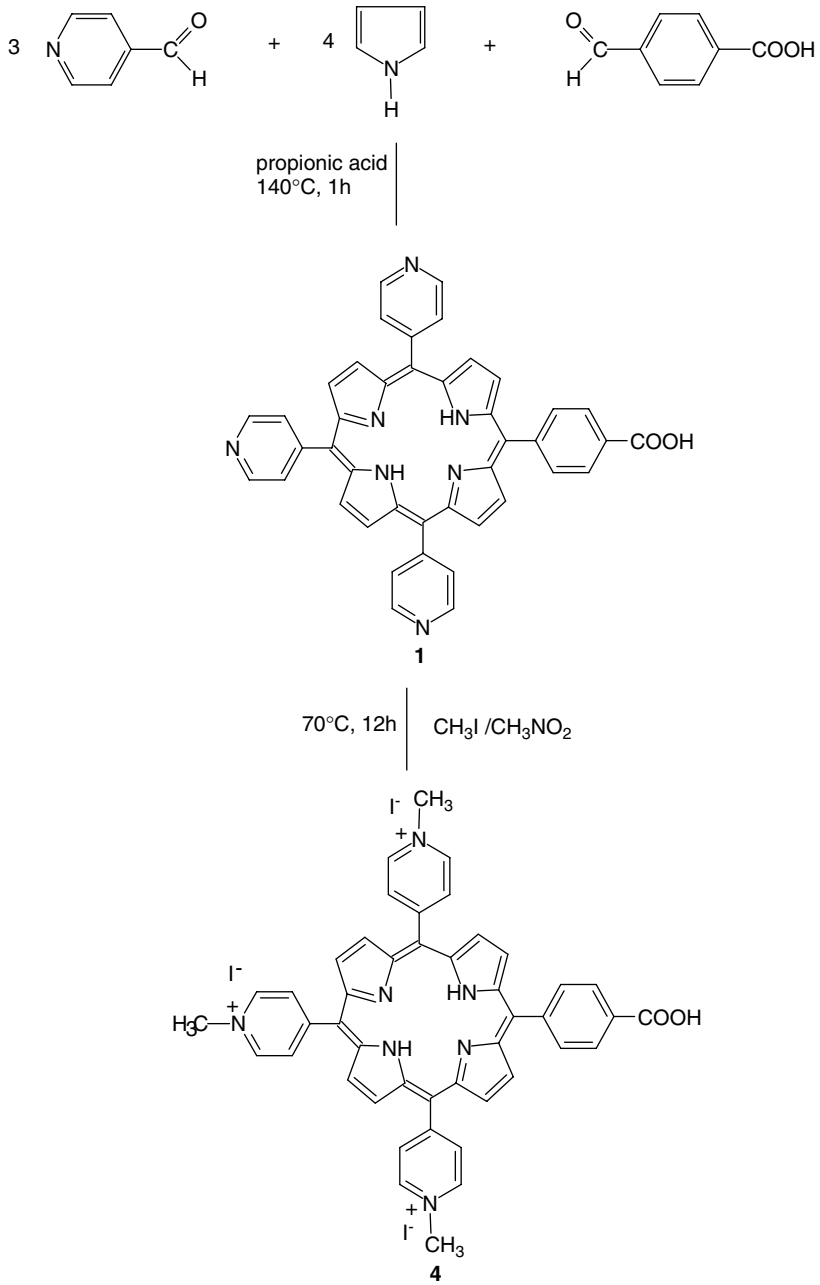

Scheme 1. Preparation of 4-pyridylporphyrin **1** and its methyl iodide **4**.

The 4-pyridylporphyrin **1** was then alkylated by using methyl iodide to form the corresponding *N*-methyl derivative **4,** in a typical procedure (Scheme 1).

Having the porphyrin **1**, we have synthesized three new 4-pyridylporphyrin-peptidyl-phosphonate conjugates **3a-c**, possessing the pyridine-3-yl-methylphosphonate diphenyl moiety. A course of the reaction is shown in Scheme 2.

The starting pyridine-3-yl Cbz-phosphonodipeptides [16] used for reaction with the pyridylporphyrin **1**, were synthesized from chosen protected *L*-aminoacids, which were then converted to the corresponding hydrobromides **2a′ – c′** (Scheme 2), suitable for this peptide-coupling reactions [13,14]. For stability and long storage, the amine groups in the phosphonodipeptides [16] were protected by Cbz groups, which could be easily removed by means of the 30% HBr solution in anhydrous acetic acid, in a one-pot procedure [13,14].

The side peptidyl chains in the products **3a-c** were composed with an aminoacid and pyridine-3-yl-methyl diphenyl phosphonate moiety, 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**).

Coupling of the peptidyl-phosphonates **2a′–c′** with the 4-pyridylporphyrin **1** enabled the combination of the 4-pyridylporphyrin moiety with the known inhibitory activity of the peptidyl diphenyl phosphonates [9,10], which are well recognized as effective inhibitors of the serine proteases. Also, an introduction

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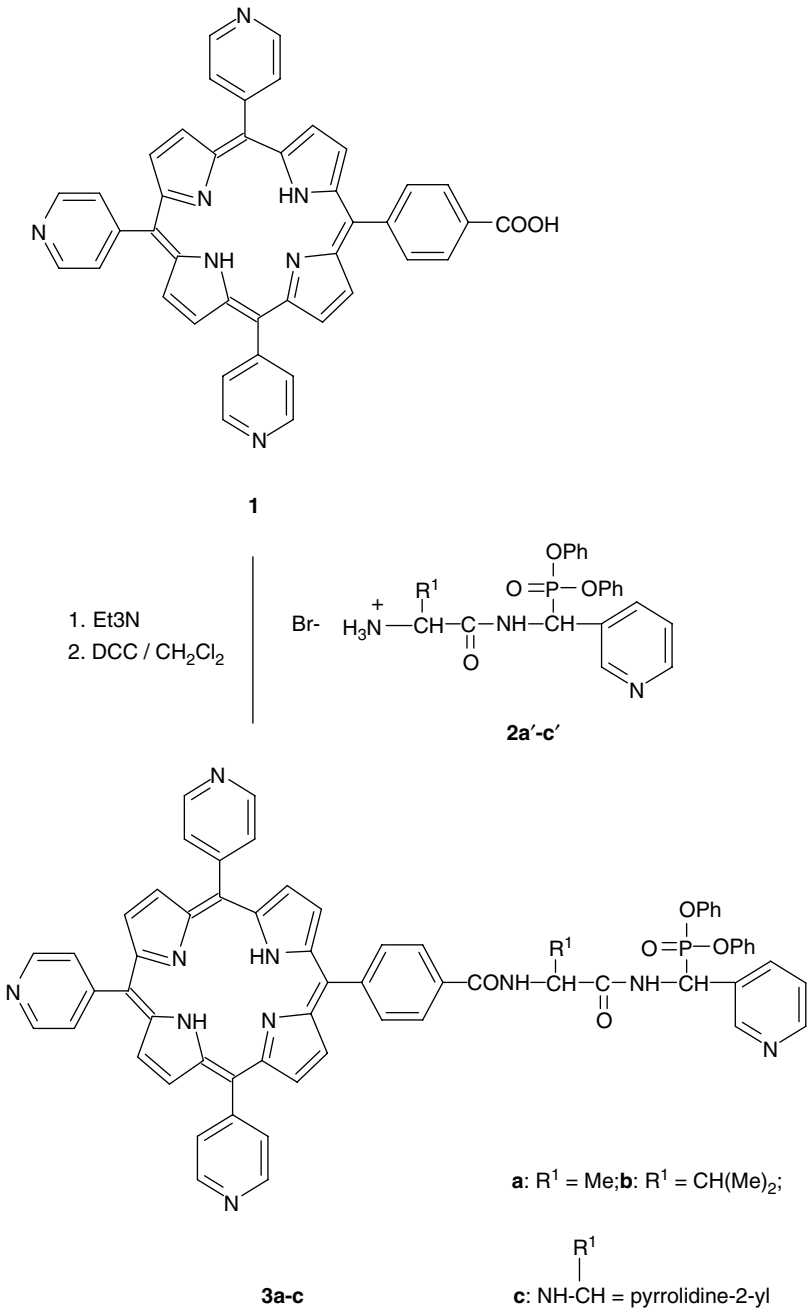


Scheme 2. Synthesis of the 4-pyridylporphyrin-peptidyl-diphenyl phosphonates **3a-c**.

of the pyridine ring into the porphyrin moiety should increase the solubility of such compounds in aqueous systems, which is an important factor in searching of biological active compounds.

In summary, the title compounds were synthesized in a good yield, using a simple dicyclohexylcarbodiimide (DCC) coupling method, frequently applied in a peptide synthesis. The isolation of the products **3a–c** was done in a regular way, which is used in preparation of short peptides.

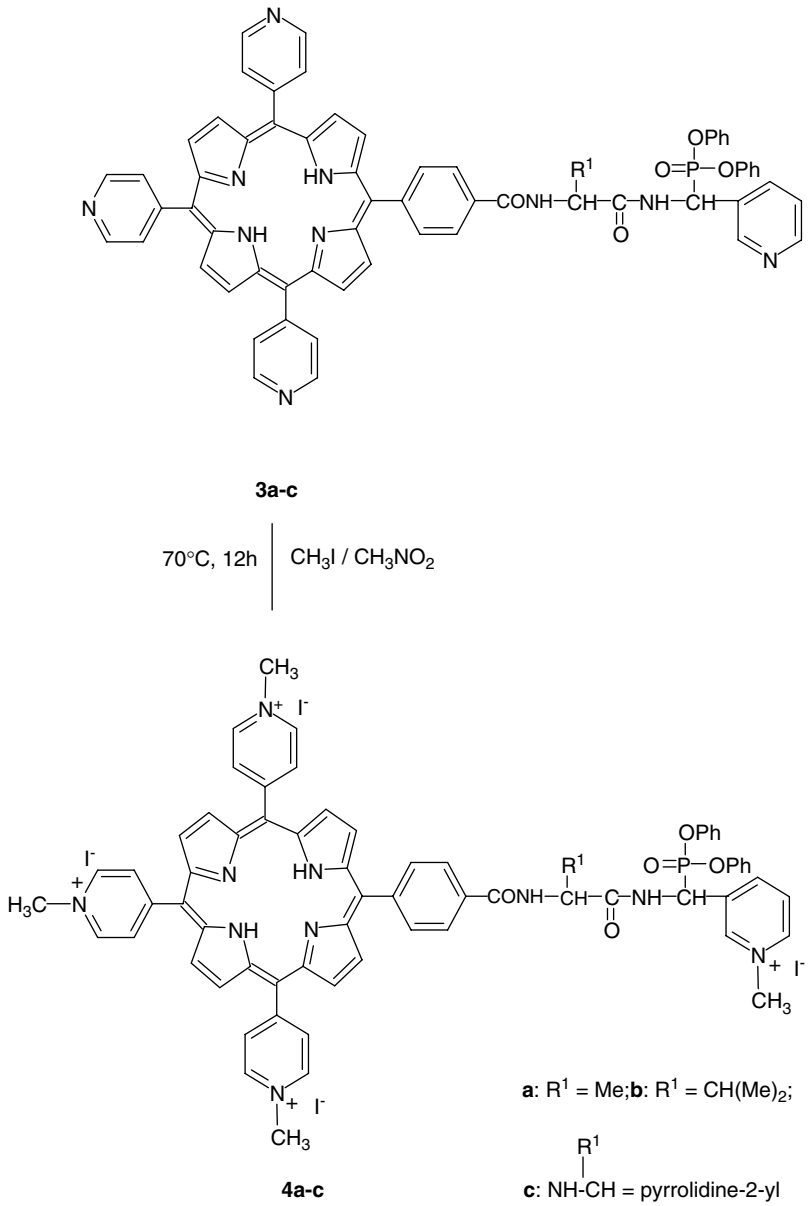
The formed products were additionally purified by means of column chromatography.

The methodology applied here for synthesis of porphyrin-peptidyl phosphonates was already elaborated by us for synthesis of some other phosphono peptides [16] and tolylporphyrin-peptidyl phosphonates [13,14].

The pyridylporphyrin peptidyl conjugates **3a–c** were easily transformed to corresponding methyl iodide derivatives **4a–c**, (Scheme 3) due to an existence of nitrogen atoms in the pyridyl-porphyrins. The formed quaternary ammonium iodides (**4a–c**) have some solubility in water, suitable for the corresponding inhibitory tests. Conversions of the **3a–c** into iodides **4** and **4a–c** were achieved using methyl iodide in nitromethane solution.

Procedure for preparation of 5-(4-carboxyphenyl)-10,15,20-tris-(4-pyridyl)-porphyrin (1)

In a 1l round-bottom flask, equipped with an efficient mechanical stirrer, were placed consecutively: propionic acid (600 ml), 4-pyridinecarboxaldehyde (3.2 g, 30 mmol) and 4-



Scheme 3. Conversion of 4-pyridylporphyrin peptidyl phosphonates **3a–c** into its methyl iodide derivatives **4a–c**.

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 additional 30 min. and then the mixture cooled. The mixture was left for 24 h and a separated product was filtered off, washed several times with water and with a mixture of water and methanol (v/v: 4 : 1). Washing was continued until the filtrate became colorless and odorless. The product was dried to give a dark violet solid (m = 1.7 g, 20%), which was a mixture of various porphyrins). The product was separated by column chromatography (silica gel: 60–230 mesh; eluant : chloroform-methanol; v/v 9 : 1), collecting the eluate with a second purple band containing the 4-pyridylporphyrin **1.** After the evaporation of the solvent, a dark-violet product (**1**) was obtained [15,17].

1: Yield: 300 mg, 4%. ^{1}H NMR (DMSO), δ, ppm: 8.98(d, 6H, J = 5.76 Hz, 2,6-Py.), 8.83(d, 8H, J = 4.08 Hz, pyrroles), 8.30(m, 4H, Ph's.), 8.20(d, 6H, J = 5.78 Hz, 3,5-Py), −3.10 (s, 2H, NH-pyrrole). IR (film) cm^{-1}: 3316 (NH), 3130, 2922, 3034, 3091, 3130 (CH), 1711 (C=O), 1595 (C=C), (MS: ESI + Q1MS; 661 (M. + 1).

Hydrobromides of the diphenyl phosphonates **2a′–c′** were prepared as described in [14,16].

General procedure for preparation of the tris-(4-pyridyl)-porphyrin-peptidyl-phosphonates 3a–c

DCC (1,3- dicyclohexylcarbodiimide, 21 mg, 0.1 mmol) (a coupling agent) was added with stirring to the solution of 5-(4-carboxyphenyl)-10,15,20-tris-(4-pyridyl)-porphyrin (**1,** 66 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 DMAP [4-(dimethylamino)pyridine, 12 mg], (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. Then, the reaction mixture was evaporated to dryness, the residue treated with ethyl

acetate (50 ml), stirred and filtered. The filtrate was subsequently washed with 1.0 M aqueous solution of $NaHCO_3$, (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 tris-(4-pyridyl)-porphyrin-peptidyl-phosphonate. After the evaporation of the eluate, the desired product (**3a–c**) was obtained as a dark-violet solid.

3a: Yield: 65%, ^{1}H NMR($CDCl_3$), δ, ppm: 8.99 (d, 6H, J = 5.69 Hz, 2,6-Py), 8.85–8.70 (m, 12H, pyrroles, plus aromatic protons), 8.10 (d, 6H, J = 5.72 Hz, 3,5-Py), 7.30–7.08 (m, 14H, Ph's),6.80 (d, 1H, J = 6.12 Hz, CH-P), 4.10–1.18 (m, 7H, NH, CH, CH_3), −2.96 (s, 2H, NH-pyrrole). ^{31}P NMR ($CDCl_3$), δ, ppm: 13.28 (s). IR (KBr), cm^{-1}: 3325 (NH); 2928; 2850 (CH) 1626, (C═O); 1574; 1537; 1489; 1261; 1243 (P═O); 1087; 941; 892; 800; 689; 641. (MS: ESI + Q1MS; 1056 (M + 1).

3b: Yield: 70%, ^{1}H NMR($CDCl_3$), δ, ppm: 8.98 (d, 6H, J = 5.61 Hz, 2,6-Py), 8.79–8.75 (m, 8H, pyrroles), 8.51–8.16 (m, 4H, arom.), 8.11 (d, 6H, J = 5.70 Hz, 3,5-Py), 7.23–7.08 (m, 14H, Ph's), 6.86 (d, 1H, J = 9.94 Hz, CH-P), 4.16–0.79 (m, 11H, NH, CH, CH_3), −2.96 (s, 2H, NH-pyrrole). ^{31}P NMR ($CDCl_3$), δ, ppm: 13.42 (s). IR (KBr), cm^{-1}: 3323 (NH); 3034; 2927; 2850; 1626 (C═O); 1591; 1489; 1311; 1244 (P═O); 1227; 1210; 1088; 1024; 941; 892; 799; 762; 688; 641. (MS: ESI + Q1MS; 1084 (M + 1).

3c: Yield 75%, ^{1}H NMR($CDCl_3$), δ, ppm: 9.01 (d, 6H, J = 4.56 Hz, 2,6-Py), 8.86–8.77 (m, 12H, pyrroles, plus aromatic protons), 8.10 (d, 6H, J = 4.87 Hz, 3,5-Py), 7.48–7.03 (m, 14H, Ph's),6.85 (d, 1H, J = 6.98 Hz, CH-P), 4.10–0.85 (m, 9H, NH, CH, CH_2), −2.96 (s, 2H, NH-pyrrole). ^{31}P NMR ($CDCl_3$), δ, ppm: 13.34 (s). IR (KBr), cm^{-1}: 3325 (NH); 3034; 2929; 2851 (CH); 1687; 1626 (C═O); 1589; 1487; 1403; 1272; 1244; 1212 (P═O); 1084; 940; 891; 798; 729; 660; 643. (MS: ESI + Q1MS; 1082 (M + 1).

Procedure for Preparation of Methyl Iodides 4 and 4a-c

The conversion of **1** and **3a–c** to methyl iodide derivatives was conducted by a modified procedure, using CH_3I as a methylating agent: To 25-ml conical flask equipped with magnetic stirrer 0.01 mM of **1** (7 mg) or 0.01 mmol of **3a-c** and a solution of methyl iodide (2 ml) in nitrometane (3 ml) were added, respectively. The mixture was refluxed for 2 h and cooled. The reaction was monitored by TLC chromatography. Following the evaporation of the solvent, a dark-purple product was obtained.

4: Yield 75%, ^{1}H NMR (DMSO), δ, ppm: 9.45(d, 6H, J = 5.64 Hz, 2,6-Py.), 9.12(m, 8H, pyrroles), 9.00(d, 6H, J = 5.62 Hz, 3,5-Py.), 8.80–8.30(m, 4H, Ph's.), 4.68(s, 9H, CH_3), −3.10 (s, 2H, NH-pyrrole).

IR (film) cm^{-1}: 3326 (NH), 2928, 2917, 2850 (CH), 1626 (C═O), 1591 (C═C), 1577 (C═C).

4a: Yield 80%, ^{1}H NMR (DMSO), δ, ppm: 9.43 (d, 6H, J = 5.52 Hz, 2,6-Py), 9.28–9.02 (m, 12H, pyrroles, plus aromatic protons), 8.96 (d, 6H, J = 5.72 Hz, 3,5-Py), 7.50–7.02 (m, 14H, Ph's), 6.71 (d, 1H, J = 8.79 Hz, CH-P), 4.67(s, 9H, CH_3), 4.2–1.1 (m, 7H, NH, CH, CH_3), −3.07 (s, 2H, NH-pyrrole). ^{31}P NMR (DMSO), δ, ppm: 11.74 (s).

IR (film) cm^{-1}: 3328, 3252 (NH), 2928, 2850 (CH), 1627 (C═O), 1575, 1536 (C═C), 1089 1024 (P–O).

4b: Yield 70%, ^{1}H NMR (DMSO), δ, ppm: 9.47 (d, 6H, J = 3.12, 2,6 Py), 9.16–9.07 (m, 12H pyrroles, plus aromatic protons), 8.99 (d, 6H, J = 5.60 Hz, 3,5 Py), 7.40–7.18 (m, 14H, Ph's), 5.56 (d, 1H, J = 7.11 Hz, CH-P), 4.71 (s, 9H, CH_3), 4.47–1.14 (m, 7H, NH, CH, CH_3), −3.02 (s, 2H, NH-pyrrole}. ^{31}P NMR (DMSO), δ, ppm: 11.97 (d). J = 22.69 Hz

IR (film) cm^{-1}: 3310, 3228 (NH), 2925, 2848 (CH), 1624 (C═O), 1582, 1537 (C═C), 1085, 1028 (P–O)

4c: Yield 70%, ^{1}H NMR (DMSO), δ, ppm: 9.47(d, 6H, J = 5.64 Hz, 2,6 Py), 9.15–9.00 (m, 12H, pyrroles, plus aromatic protons), 8.92 (d, 6H, J = 6.72 Hz, 3,5-Py), 7.40–7.02 (m, 14H, Ph's), 6.96 (d, 1H, J = 8.79 Hz, CH-P), 4.71(s, 9H, CH_3), 4.2–1.1 (m, 7H, NH, CH, CH_3), ^{31}P NMR (DMSO), δ, ppm: 12.29 (d), J = 23.13 Hz, 11.96(d) J = 23.55 Hz

IR (film) cm^{-1}: 3399, 3325, 3234 (NH), 2925, 2848 (CH), 1628 (C═O), 1583, 1536 (C═C), 1087, 1071 (P–O).

UV-vis spectra of porphyrinpeptidyl phosphonate derivatives 3a-c and 4a-c

Electronic spectroscopy is an important tool in investigation of the porphyrins due to unique characteristic spectra of them in a UV and visible region (Figure 1).

The UV-vis spectrum within range 400–700 nm, and especially the values of molar absorption coefficients (ε), (Table 1) are the source of information concerning their photochemical properties, as for example, the potential photosensitizers in cancer therapy (PDT method). Also, the UV part of spectrum gives information about the possible aggregations at applied concentrations, what is unwanted process, limiting their applications as photosensitizers. This process is often observed among some cationic porphyrins, e.g. the methylated pyridylporphyrins, during a long storage in the solutions.

Spectra of **3a-c** and **4a-c** were obtained in chloroform and water solution, respectively. Electronic spectra of the synthesized porphyrinpeptidyl derivatives are characteristic for free base porphyrins with a highly intensive band in UV region (Soret band) and four up to ten times less intensive bands in visible region (Q bands). (Figure 1) The ratio between four Q bands in all these derivatives placed the synthesized compounds as etio type porphyrins. No splitting of the Soret absorption band were observed even after several months, what suggested the lack of aggregations in these solvents in used concentrations of the porphyrins (ca 0.1mM) and therefore the stability of their photochemical properties. Also, relatively high values of molar absorption coefficients {ε} for Q_1 band (red region) equal to ≅1 × 10^4 M^{-1} cm^{-1} were observed (see Table 1). In summary, there are essential properties of the porphyrins for photochemical investigation in the aspect of using them as potential photosensitizers.

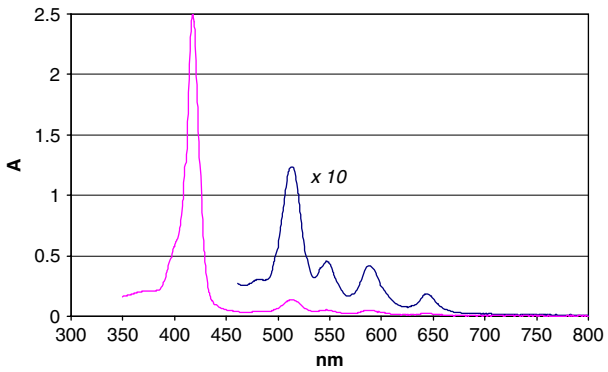


Figure 1. UV-vis spectra of **3b**. This figure is available in colour online at www.interscience.wiley.com/journal/jpepsci.

Table 1. UV-vis spectral data of 3a-c and 4a-c

	Soret		Q_4		Q_3		Q_2		Q_1	
	λ [nm]	ε [$\times 10^6$ M^{-1} cm^{-1}]	λ [nm]	ε [$\times 10^4$ M^{-1} cm^{-1}]	λ [nm]	ε [$\times 10^4$ M^{-1} cm^{-1}]	λ [nm]	ε [$\times 10^4$ M^{-1} cm^{-1}]	λ [nm]	ε
3	418	1.12	513	17.0	547	6.16	588	6,05	645	1.32
3a	418	1.35	513	7.99	547	2.90	588	2.75	644	1.32
3b	418	1.43	513	7.04	547	2.58	588	2.38	645	1.06
3c	418	1.39	513	6.84	547	2.41	588	2.35	644	1.05
4	423	0.68	520	4.68	558	2.20	584	2.28	641	1.09
4a	421	1.17	519	8.23	556	4.25	583	4.12	641	1.69
4b	422	1.15	519	7.50	556	3.70	584	3.73	641	1.47
4c	420	1.04	519	6.94	557	3.40	585	3.40	640	1.34

Biological part

Aminopeptidase N (APN/CD13 EC 3.4.11.2) is a transmembrane protease present in a wide variety of human tissues and cells [18,19]. This enzyme is usually overfilled in tumor cells and plays a critical role in angiogenesis, which is a complex cascade process playing a central role in tumor growth [19]. Therefore, inhibition of APN/CD13 may lead to the development of anti-cancer and anti-inflammatory drugs [18]. There are many known natural and synthetic APN inhibitors [18]. Known synthetic inhibitors are analogs of amino acids including aminohydroxamates, β-aminothiols, aminoaldehydes and aminophosphonic acids [18]. Some of them have potent inhibitory activities with Ki values in the nanomolar range [11,18].

Lately, some phosphonate and phosphinate analogs of amino acids and short peptides have been developed as new inhibitors of APN [19]. For example, phosphinic analogs of alanine and phenylalanine and other acids are good, potent inhibitors of APN with Ki $\cong 10^{-7}$ M [19]. In turn, some optically pure phosphinate analogs of short peptides have even better inhibitory potencies, with the range of Ki $\sim 10^{-8}$ M.

Because of the existence of phosphono-dipeptide fragments in the porphyrin-like products, we have decided to execute some preliminary tests with the aminopeptidase N (APN/CD13) in order to check their inhibitory activity.

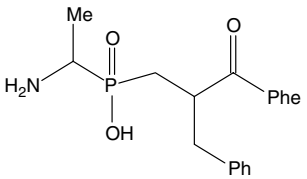
The results of the inhibitory activity of the diphenyl porphyrin-peptidyl phosphonates **3a-c** and **4a-c** toward APN are given in the Table 2. For comparison, the best phosphinate derivative inhibitor [19] of APN is included in the Table 2. The methodology used for these inhibitory tests was described earlier in the literature [12].

The products **3a–c** and **4a–c** showed a moderate inhibition of aminopeptidase N. Among them, the best inhibitor of APN/CD 13 was the **4c** [MeI-TPP-Pro-3PyP$(OPh)_2$] (IC_{50} = 9.48 μM). Surprisingly, the methyl iodide derivative of the 5-(4′-carboxyphenyl)-10,15,20-tris-(4-pyridyl)-porphyrin (**4**) was quite good inhibitor itself, with IC_{50} equal to 12.40 μM.

According to the literature data [18,19] it seems, that replacement of phosphonate group [-P(O)$(OPh)_2$] in the products **3a-c** by free phosphonic acid moiety [-PO_3H_2] should improve the inhibitory potency.

The preliminary tests indicated that the peptidyl derivatives of 4-pyridylporphyrin showed to be promising inhibitors of

Table 2. Inhibition of aminopeptidase N [CD 13][a] by **3a–c** and **4a–c**

Compound no.	Compound; abbreviation	Calc. molar weight	IC_{50} [μM][b,c]
3	TPyP-COOH	661	Not determined
3a	TPyP-Ala-3PyP$(OPh)_2$	1055	60.16
3b	TPyP-Val-3PyP$(OPh)_2$	1083	49.94
3c	TPyP-Pro-3PyP$(OPh)_2$	1081	53.36
4a	MeI-TPyP-Ala-3PyP$(OPh)_2$	1623	45.27
4b	MeI-TPyP-Val-3PyP$(OPh)_2$	1651	24.36
4c	MeI-TPyP-Pro-3PyP$(OPh)_2$	1649	9.48
4	MeI-TPyP-COOH	1087	12.40
– [d]		418	Ki = 2.2 nM Lit. [19]

[a] Concentration of enzyme; 0.2 μg/ml.
[b] Concentration of substrate; (L-leucylo-7-amido-4-methyl-coumarin): 12.5 μM/ml.
[c] Buffer: KH_2PO_4-Na_2HPO_4 with pH = 7.2 was used.
[d] Phosphinate inhibitor of APN [19].

aminopeptidase N and therefore may serve as biologically active agents for further investigation in this field.

Conclusions

The synthesis of the three new tris-(4-pyridyl)porphyrin-peptidyl-phosphonates **3a–c** and their water soluble derivatives **4a–c** is described. These compounds were obtained in a satisfactory yield from the 5-(4-carboxyphenyl)-10,15,20-tris-(4-pyridyl)-porphyrin **1** and phosphonodipeptides **2a′–c′**, 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, the obtained compounds could serve as a new class of inhibitors of aminopeptidase N, after further structural modifications.

Acknowledgements

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