Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 363–365

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis and Photodynamic Activity of a Cationic Zinc Monoazaporphyrin Bearing a Nitrogen Atom at the Peripheral Position

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Received 28 September 2000; accepted 16 November 2000

Abstract—A new cationic monoazaporphyrin, zinc 2-aza-8,12,13,17-tetraethyl-2,3,7,18-tetramethylporphyrinium iodide **3** was synthesized. Photodynamic activity of **3** in degradation of 2′,3′-isopropylideneguanosine **4** was compared with 2-aza-8,12,13,17-tetraethyl-3,7,18-trimethylporphyrin **1**, zinc 2-aza-8,12,13,17-tetraethyl-3,7,18-trimethylporphyrin **2**, and hematoporphyrin **5**. The quarternary ammonium **3** showed a remarkable increase of photodynamic activity compared with **5**, although no appreciable difference in the activity was observed between **1** and **5**.

Recently, in view of development of agents for photodynamic therapy (PDT)[1] and magnetic resonance imaging (MRI),[2] structurally modified and simplified porphyrins are of particular interests.

PDT is one of the therapeutic treatments of cancer which involves the photodynamic reaction of the nucleic acids of tumor cells with singlet oxygen generated by light, oxygen, and dye as a photosensitizer. Treatment protocol of PDT generally involves intravenous administration of photosensitizer, followed by localization in malignant tissue and subsequent irradiation of visible light. Therefore, photosensitizers are preferable to possess amphiphilic property, and various water-soluble porphyrins such as sulfonated porphyrins, pyridinium porphyrins, and glycosylated porphyrins have been synthesized and examined for photochemical property.[3] Almost all of these porphyrins possessed water-soluble groups on the side chain at the *meso* position or the peripheral position. Except for a cationic naphthalocyanine, there have been no reports on the synthesis of porphyrins possessing the hydrophilic moiety in the porphyrin skeleton whilst keeping aromaticity.[4]

So we were interested in the synthesis of the compounds with the hydrophilic porphyrin skeleton and in the investigation on the photodynamic activity of the porphyrin analogues.

Aiming at a search for new candidates for PDT agents, we have already reported the synthesis of novel porphyrin analogues having an extra nitrogen atom at the peripheral position, i.e. the β position of pyrrole ring in porphyrin skeleton, by use of '3 + 1' condensation of a tripyrrane derivative and an imidazole derivative.[5] It is expected that the azaporphyrin analogues are easily alkylated at the peripheral nitrogen atom to give porphyrin skeletons with hydrophilic property without losing aromaticity.

In this paper, we report the synthesis of a novel cationic monoazaporphyrin and evaluation of its photodynamic activity in the photosensitized oxidation of a guanosine derivative.

For the purpose of preparation of cationic monoazaporphyrin, we tried to quarternize a peripheral nitrogen atom of 2-aza-8,12,13,17-tetraethyl-3,7,18-trimethylporphyrin **1**, prepared by the recently reported method.[5] Thus, the compound **1** was treated with methyl iodide in acetone or dimethylformamide at room temperature for 4 days. Reaction, however, afforded no desired quarternized compound except for highly polar complex mixtures. The failure was suspected to be caused by over-alkylation not only at the peripheral nitrogen atom but also the inner ones.

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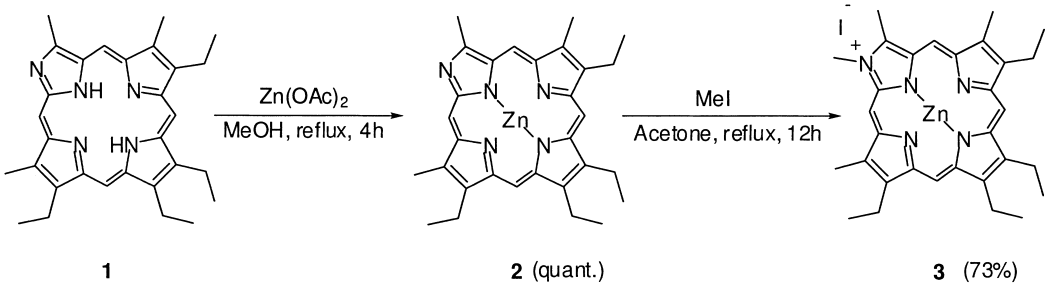
0960-894X/01/$ - see front matter
PII: S0960-894X(00)00663-6



Scheme 1.

Therefore, metalation of compound **1** in order to block alkylation at the inner nitrogen atoms, followed by quarternization of the nitrogen atom at the peripheral position was performed. We selected zinc as a coordination metal. A zinc complex **2** was quantitatively obtained by the reaction of compound **1** with zinc acetate in methanol under reflux for 4 h. Compound **2** was characterized by its proton NMR spectrum and high resolution mass spectrum.[6] Reaction of compound **2** with methyl iodide in acetone under reflux for 12 h gave the desired compound **3** in a 73% yield after silica gel column chromatography ($CHCl_3$:acetone = 3:2). The structure of compound **3** was confirmed by spectroscopic analysis (Scheme 1).[7]

It is known that dye-sensitized photooxidation of DNA occurs usually at guanine residue, and it has been investigated that the photooxidation of guanosine derivatives, 2′-deoxyguanosine and guanosine-5′-monophosphate, etc., with various photosensitizers for the explanation of the mechanism of DNA photodegradation.[8] Therefore, photodegradation of 2′,3′-*O*-isopropylidene guanosine **4** was investigated to evaluate photodynamic activity of the porphyrins **1**, **2** and **3**, now synthesized as PDT agents, and hematoporphyrin **5** as a standard photosensitizer which is the monomer of Photofrin®, a clinically used PDT agent (Fig. 1).[9] Comparison protocol of photodegradation of the guanosine derivative **4** was as follows. A mixture of 0.12 mM of the porphyrin derivative, (**1**, **2**, **3**, or **5**) and 0.62 mM of the guanosine derivative **4** in methanol (5 mL) saturated with oxygen was irradiated with a 100 W high pressure mercury lump for 1 or 2 h at room temperature. Photodegradation ratio of the guanosine deriviative **4** was calculated from the decay of absorbance at 254 nm ascribed to **4** in HPLC analysis (Table 1).

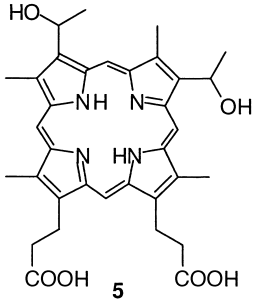
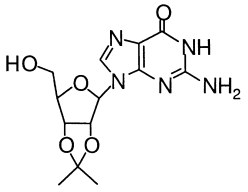


Figure 1.

The guanosine derivative **4** was intact under the conditions without photosensitizer. Compared with the results of monoazaporphyrin derivative **1** and hematoporphyrin **5**, no appreciable difference of the photodynamic activity was observed. This fact showed that introducing a nitrogen atom at the peripheral position of the porphyrin skeleton had little influence on the photodynamic activity. On the other hand, cationic monoazaporphyrin derivative **3** showed a remarkable increase of photodynamic activity.

As for UV–vis spectrum of the compound **3**, no hyperchromic shift was observed, although all absorption bands shifted a little bathochromically compared with **2**. This result showed that the high activity of compound **3** in degradation of **4** may not be ascribed to the difference of electronic property associated with π orbital conjugation of the porphyrin skeleton between **3** and others. Contribution of $^1O_2$ was presented to be crucial for the photodegradation of **4** by the fact that the photodynamic acitivity of **3** decreased by addition of sodium azide, a known quencher of $^1O_2$, to the reaction system (Table 1). Recently, DNA binding and photocleavage properties of cationic porphyrins, pyridinium porphyrins, and ammonium porphyrins have been reported.[10]

Table 1. Photodegradation ratio of 2′,3′-isopropylidene guanosine **4**



Photosensitizer	Irradiation time (h)	Degradation ratio of **4** (%)
None	2	0.0
1	1	20.3
1	2	39.8
2	1	5.5
2	2	18.2
3	1	34.7
3	2	55.9
3[a]	2	14.0
5	1	16.5
5	2	35.4
5[a]	2	15.7

[a]Sodium azide was added in a concentration of 6 mM/L.

Kubát et al. investigated the photodegradation of guanosine-5′-monophosphate (GMP) in the presence of cationic and anionic porphyrins as sensitizers, and found the high photosensitizing ability of cationic porphyrins.[10b] They suggested a mechanism that cationic porphyrins bind to GMP and generate singlet oxygen, which readily decomposes GMP.[10b] Therefore the enhancement of photodynamic acitivity of compound **3** may be attributed to its binding property with the guanosine derivative **4**.

In conclusion we synthesized a new cationic monoazaporphyrin **3** bearing a hydrophilic moiety at the porphyrin skeleton. Compound **3** showed a remarkable increase of photodynamic activity in degradation of a guanosine derivative **4** compared with a standard photosensitizer **5**, the monoazaporphyrin **1**, or **2**. It suggested that the association of the cationic monoazaporphyrin **3** with the guanosine derivative **4** is responsible for the increase of the degradation of guanosine derivative **4** with singlet oxygen. Thus, quarternization of azaporphyrins appears to have potential for development of an excellent photosensitizer for PDT.

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6. **2**: ^{1}H NMR (δ $CDCl_3$): 1.84 (br, 12H), 3.23 (br, 3H), 3.48 (br, 3H), 3.73 (br, 3H), 3.90–3.96 (m, 8H), 9.31 (s, 1H), 9.39 (s, 1H), 9.58 (s, 1H), 9.75 (br, 1H); λ_{max} nm (logε/M^{-1} cm^{-1}): 582 (4.18), 541 (4.05), 407 (5.35); HRMS (EI^+) calculated for $C_{30}H_{33}N_5Zn$ 527.2027, found 527.2045.

7. **2**: ^{1}H NMR (δ CD_3OD): 1.84 (m, 6H), 1.98 (t, 7.3 Hz, 6H), 3.25 (s, 3H), 3.42 (s, 6H), 3.92–4.03 (m, 8H), 4.52 (s, 3H), 8.41 (s, 1H), 8.83 (s, 1H), 9.26 (s, 1H), 9.35 (s, 1H); HRMS (FAB^+/*m*-Nitrobenzylalcohol) calculated for $C_{31}H_{36}N_5Zn^+$ 542.2261, found 542.2269; UV–vis ($CHCl_3$) λ_{max} nm (logε/M^{-1} cm^{-1}): 633 (3.85), 602 (4.33), 558 (3.77), 523 (3.44), 420 (4.93). When the methanol solution of **3** was irradiated without the substrate **4** under the same conditions, the decay of absorbance at 312 nm ascribed to **3** in HPLC analysis was not observed at all after the irradiation.

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