Chinese Chemical Letters Vol. 15, No. 12, pp 1427-1429, 2004 1427
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Synthesis and Photocytotoxicity of Mono-functionalised Porphyrin with Valine Moiety

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Abstract: A mono-funtionalised tetraphenylporphyrin (TPP) bearing valine moiety at the phenyl ring was synthesized for photocytotoxicity examination in four steps, starting from regiospecific mono-nitration of TPP at the phenyl ring. The in vitro photocytotoxicitic effect against SPC-A1 adenocarcinona cell line was tested.

Keywords: Mono-functionalised, porphyrin, tetraphenylporphyrin, photocytotoxicity.

In the past decades, much interest has been focused on the synthesis of well-defined porphyrins for potential application as photosensitizer in photodynamic therapy (PDT) of cancer[1]. Though there are many aspects to attain to this purpose, one important issue to porphyrin derivatives is their availability[2]. Up to now, the most porphyrins investigated are obtained from naturally occurring porphyrins like hematoporphyrin[3] and proto-porphyrin[4], involving complicated modification reaction and tedious separation. Due to the fact that meso-tetraphenylporphyrin[5] (TPP, **1**, **Scheme 1**) was particularly readily prepared, it should be a direct way to get a variety of substituted porphyrins by introducing suitable group to TPP, especially at the phenyl rings. Previously Kruper *et al*.[6] reported mono-nitration at phenyl ring of TPP with excess of fuming nitric acid. Now we report our exploitation of this method to synthesize a mono-funtionalised TPP bearing valine moiety at the phenyl ring (**5**, **Scheme 1**). Its *in vitro* photocytotoxicity against SPC-A1 adenocarcinona cell line was also tested.

The synthesis procedure was shown in **Scheme 1**. The starting porphyrin TPP was subjected to regiospecific mono-nitration to give 5-(4-nitrophenyl)-10, 15, 20-triphenyl porphyrin **2**. Usual reduction[7] of the nitro group with $SnCl_2$/HCl was applied to give 5-(4-aminophehyl)-10,15,20-triphenylporphyrin **3**. Followed by the condensation reaction with Boc protected valine in the presence of dicyclohexylcarbodiimide (DCC) in CH_2Cl_2, the amino acid was introduced into the porphyrin to give compound **4**, which was treated with TFA to remove Boc group to afford porphyrin **5**

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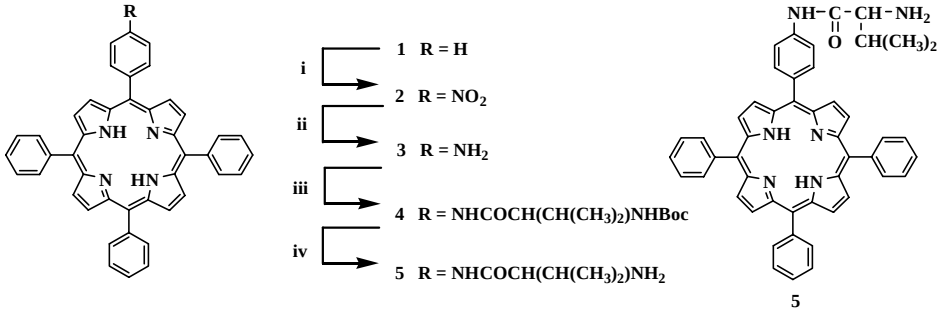
Scheme 1



i) nitric acid (65%, w/w), 64%; ii) $SnCl_2$, HCl, 83%; iii) Boc-valine, DCC, CH_2Cl_2, 90%; iv) TFA, CH_2Cl_2, 92%.

Table 1 Effect of nitration reagent on nitration of TPP[a]

Nitric acid	Stoichiometry of nitric acid	Yield[b] of mononitro（%）
Fuming nitric acid (95%)	19	54
Nitric acid (65%)	19	64
Acetic acid / Fuming nitric acid (95%)	19	38
Urea / Fuming nitric acid (95%)	19	44

[a] In CH_2Cl_2 for 2 hr. [b] Isolated yield

Enhancing the yield of porphyrin **2** is essential for the total synthesis. Four different nitration reagents were screened, as shown in **Table 1**. Instead of fuming nitric acid (95%, w/w), which was used in the Kruper's method, the less-concentrated nitric acid (65%, w/w) was found to be much preferable for this reaction.

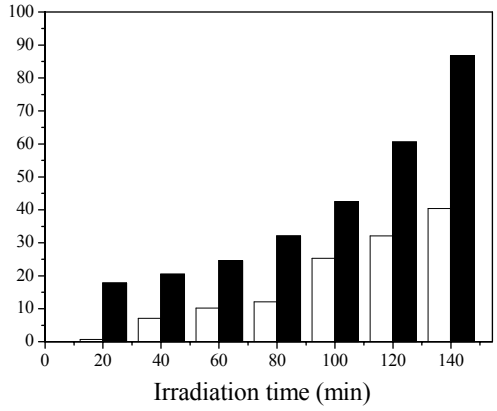
The photocytotoxicity of porphyrin **5** was tested against SPC-A1 adenocarcinona cell line. A normal cell line, L929 mouse Fibroblast cell line, was also applied to the test for comparison. Cells were suspended in a RPMI (cell culture medium for *in vitro* diagnostic use, received from GIBCO Co., Ltd.) medium containing 10^{-4} mol/L porphyrin. The suspension was irradiated with fluorescent light (fluence = 60 watt / m^2) for a certain period of time. After further 24 hour incubation in dark at 37°C, the dead cells were identified as propidium iodide (PI) permeable ones, and the counts were measured by flow cytometry.

Figure 1 displays dead cells counts in function of irradiation time with porphyrins **5** against SPC-A1 cells and L929 mouse Fibroblast cells, respectively. For both of the cells, the dead cell percentage increased with augmentation of irradiation time, and attained a maximum at 140 min. The dead SPC-A1 cell counts were always higher than L929 cells in the same irradiation time. The maximum of dead SPC-A1 cell percentage (80%, 140 min) was nearly twice of that of dead L929 cell, indicating that the photocytotoxicity against tumor cell of **5** was much higher than that against normal cell.

Experimental

Synthesis of porphyrins: 5-(4-Nitrophenyl)-10, 15, 20-triphenylporphyrin **2** was prepared according to the reference[6]. The synthetic procedures for porphyrins **3**, **4** and **5** were similar to those in our previous report[8]. Porphyrin **5**: ^{1}H NMR δ($CDCl_3$, ppm), 8.89 (d, 2 H, J = 4.3, β-pyrrole), 8.84 (s, 6 H, β-pyrrole), 8.20 (m, 2 H, 4-aminophenyl; 6 H, ortho triphenyl), 8.12 (d, 4 H, J = 8.3, 4-aminophenyl), 7.76 (m, 9 H, meta/para triphenyl), 4.10 (d, 1H, J = 6.4, CH-N), 2.08 (m, 1H, CH-C), 0.99 (d, 6 H, J = 3.5, Valine-CH_3). UV: λ_{max} ($CHCl_3$) 423, 521, 557, 595, 651 nm. Anal. Calcd. For $C_{49}H_{40}N_6O$: C, 80.74; H, 5.53; N, 11.53. Found: C, 80.80; H, 5.65; N, 11.34.

Figure 1 Percentage of dead SPC-A1 cells and L929 cells *vs* irradiation time in the presence of porphyrin **5**



void bars: L929 mouse Fibroblast cell line

solid bars: SPC-A1 adenocarcinona cell line

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 59983002).

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Received 3 November, 2003