Journal of the Korean Chemical Society
1999, *Vol. 43, No. 6*
Printed in the Republic of Korea

속 보

포르피린-EDTA 및 포르피린-DTPA의 합성

申 株* · 兪大植
세종대학교 화학과
(1999. 9. 1 접수)

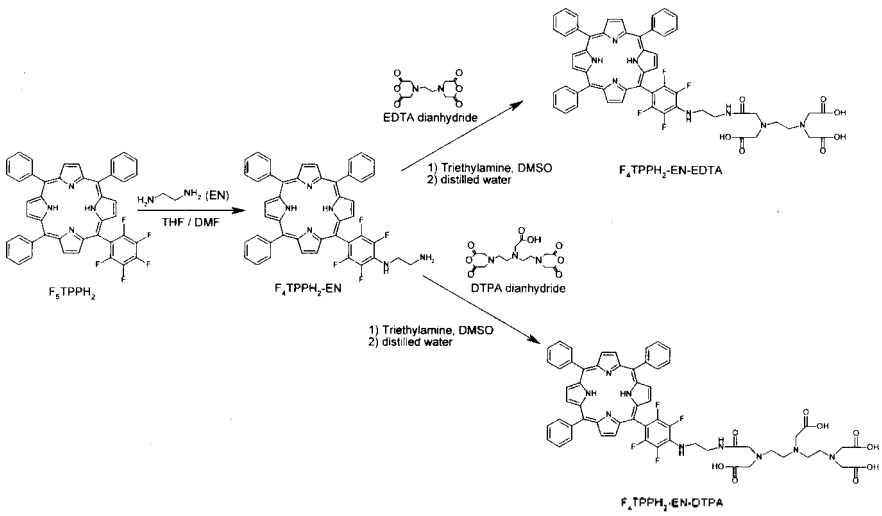
Facile Synthesis of Porphyrin-EDTA Conjugate and Porphyrin-DTPA Conjugate

Koo Shin* and Dae-Hawn Yeu
Department of Chemistry, Sejong University, Seoul 143-747, Korea
(Received September 1, 1999)

There has been considerable recent research directed towards the design and synthesis of EDTA (EDTA=ethylenediaminetetraacetic acid) conjugate of protein- and DNA-binding molecule or DTPA (DTPA=diethylenetriaminepentaacetic acid) conjugate of protein- and DNA-binding molecule. Studies of EDTA conjugates of protein- and DNA-binding molecule have shown that these species, in the presence of iron(II), O_2, and a suitable reducing agent, can effect the cleavage of the protein and DNA backbone.[1] Many conjugates of the protein- and DNA-binding molecule such as bleomycine,[2] cholic acid,[3] methidiumpropyl,[4] aminobenzyl,[5] or *p*-isothiocyanatobenzyl[6] moeity have been synthesized. Recently EDTA conjugates of sapphyrin, a class of expanded porphyrin, have been synthesized but the synthesis is somewhat tedious.[7]

Magnetic resonance imaging (MRI) frequently involves the use of gadolinium(Gd)-DTPA as an enhancement agent. Gd-DTPA accumulates in areas where the blood-brain barrier is damaged, but may not be specific to the tumor and rapidly washed out from the kidney.[8] Porphyrin derivatives have been used for MRI due to the selective uptake by the tumor tissue. Gd porphyrin derivatives such as Gd-tetrakis(N-methyl-4-pyridyl)porphyrin and Gd-tetrakis(4-sulfonatophenyl)porphyrin have been developed as MRI enhancement agents, but these are thought to be unstable *in vivo*.[9] Gd is known to be unstable if placed directly into the porphyrin cage. Therefore, porphyrin derivatives incorporating a strong chelating agent outside the porphyrin has been developed.[10] Herein, we report facile synthesis of a porphyrin-EDTA conjugate and a porphyrin-DTPA conjugate.

For the synthesis of the porphyrin-EDTA conjugate and the porphyrin-DTPA conjugate, it was necessary to consider a porphyrin derivative that bears a peripheral functionality for attachment to an EDTA (or DTPA) subunit. In order to obtain monofunctional porphyrin derivative, 5,10,15-triphenyl-20-pentafluorophenylporphyrin (F_5TPPH_2) was synthesized since nucleophilic substitutions on some pentafluoroarenes was known to selectively occur with replacement of the *p*-F atoms.[11] The condensation of pentafluorobenzaldehyde with 3 mol of benzaldehyde and 4 mol of pyrrole according to the method reported by Lindsey using *p*-chloranil as the oxidant produces mixture of products.[12] The concentrating solution of the mixture was purified from silica gel chromatography with chloroform/hexane (v/v=1/4). The 3rd fraction (Rf=0.2) was evaporated and the product recrystallized from chloroform/methanol to give a pure product of F_5TPPH_2 in 8.5% yield. As shown in *Scheme* 1, treatment of F_5TPPH_2 (30 mg, 4.03×10^{-5} mol) with excess of ethylenediamine (0.27 ml, 4.04×10^{-3} mol) in DMF (15 ml) give the ethylenediamine-containging porphyrin derivative 5-[2',3',5',6'-tetrafluoro-(4'-ethylenediamino)phenyl]-10,15,20-triphenylporphyrin, F_5TPPH_2-EN, with a yield of 89%.[13] A solution of ethylenediaminetetraacetic dianhydride (EDTAD, 45.5 mg, 1.774×10^{-4} mol) and 3 drops of triethylamine in anhydrous DMSO (20 ml) was



Scheme 1.

placed into a round-bottomed flask with a nitrogen purge and additional funnel. A solution of F_4TPPH_2-EN (132 mg, 1.774×10^{-4}) in anhydrous DMSO (10 ml) was added dropwise with stirring over 10 min. The solution was stirred for an additional 30 min, after which distilled water (50 ml) was added. Stirring was continued for 30 min. The crude F_4TPPH_2-EN-EDTA[14] was purified by silica-gel chromatography (70-230 mesh) with methylene chloride/ methyl alcohol (v/v=9/1) as the eluant (yield=65%).

Evidence for the formation of F_4TPPH_2-EN-EDTA is found in spectroscopic analysis. The TOF-MALDI spectrum showsa M+H$^+$ ion peak at 1020.3 (*m/z*, calcd for $C_{50}H_{40}F_4N_8O_7$+H$^+$ 1020.02) and its IR spectrum shows broad overlap of $\nu_{C=O}$ and ν_{N-H} at 1590 cm^{-1}. Absorption bands at 223 nm due to the EDTA ligand and 416(Soret), 513, 547, 589, 644 nm due to the porphyrin structure are appeared in the eletronic spectrum of F_4TPPH_2-EN-EDTA.

Reaction of F_4TPPH_2-EN-EDTA (100 mg, 9.337×10^{-5} mol) with iron bromide (28 mg, 9.337×10^{-5} mol) in chloroform and methanol yielded a F_4TPPH_2-EN-EDTA-Fe (III) complex. The concentrating solution was subjected to chromatography on silica gel (70~230 mesh) with chloroform/methanol (v/v=5/1). The 2nd fraction was evaporated, and the product recrystallized from dichloromethane/acetone to give the F_4TPPH_2-EN-EDTA-Fe (III) complex. A broad ^{1}H NMR spectrum and Fe-O stretching peaks at 617, 474, 416 cm^{-1} in the FT-IR spectrum indicate the formation of paramagnetic F_4TPPH_2-EN-EDTA-Fe(III) complexes.

As shown in *Scheme* 1, same treatment of F_4TPPH_2-EN (128 mg, 1.72×10^{-4} mol) with diethylenetriaminepentaacetic dianhydride (DTPAD, 60 mg, 1.72×10^{-4} mol) in 3 drops of triethylamine and 30 ml of DMF, and a subsquent reaction with distilled water afforded the crude product, F_4TPPH_2-EN-DTPA conjugate. The crude product was dissolved in chloroform and subjected to chromatography on silica gel (70-230 mesh). The second fraction was evaporated, and the product recrystallized from dichloromethane/acetone to give the pure F_4TPPH_2-EN-DTPA conjugate[15] in 61% yield.

The TOF-MALDI spectrum shows a strong M+H$^+$ ion peak at 1121.2 (*m/z*, calcd for $C_{60}H_{53}F_4N_9O_{10}$+H$^+$ 1121.14) and its IR spectrum shows $\nu_{C=O}$ at 1651 cm^{-1} and ν_{N-H} at 1590 cm^{-1}. In the electronic spectrum of F_4TPPH_2-EN-DTPA, similar absorption bands are observed at 236 nm due to the DTPA ligand and 421(Soret), 513, 547, 587, 649 nm due to the porphyin structure.

Further evidence for the formation of F_4TPPH_2-EN-EDTA conjugate and F_4TPPH_2-EN-DTPA conjugate comes from resonance peaks at 6.70 ppm and 6.77 ppm for the amide C(O)-N*H* resonance in the ^{1}H NMR spectrum, respectively.

As mentioned earlier, upon complexation with transition metals such as Fe or Gd, F_4TPPH_2-EN-EDTA and

F_4TPPH_2-EN-DTPA are expected to have potential applicabilities in the field of a selective cleavaging agent of DNA and a MRI enhancement agent, respectively. Metallation and reactivity studies of the F_4TPPH_2-EN-EDTA conjugate and the F_4TPPH_2-EN-DTPA conjugate are in progress.

Acknowledgement. We are indebted to the Korean Cancer Research Hospital for the financial support of this work and the Korea Basic Science Institute for obtaining mass spectra.

REFERENCES

1. a) Meares, C. F.; Wensel, T. G. *Acc. Chem. Res.* **1984**, *17*, 202. b) Bailly, C.; Henichart, J.-P. *Bioconjugate Chem.* **1991**, *2*, 379.
2. Michael, W.; Dyke, V.; Dervan, P. B. *Nucleic Acids Res.* **1983**, *11*, 5555.
3. Betebenner, D. A.; Carney, P. L.; Zimmer, A. M.; Kazikiewicz, J. M.; Brucher, E.; Sherry, A. D.; Johnson, D. K. *Bioconjugate Chem.* **1991**, *2*, 117.
4. Van Dyke, M. W.; Hertzberg, R. P.; Dervan, P. B. *Proc. Natl. Acad. Sci. USA* **1982**, *79*, 5470.
5. Studer, M.; Meares, C. F. *Bioconjugate Chem.* **1992**, *3*, 420.
6. Brechbiel, M. W.; Gansow, O. A.; Atcher, R. W.; Schlom, J.; Esteban, J.; Simpson, D. E.; Colcher, D. *Inorg. Chem.* **1986**, *25*, 2772.
7. Iverson, B. L.; Shreder, K.; Morishima, T.; Rosingana, M.; Sessler, J. L. *J. Org. Chem.* **1995**, *60*, 6616.
8. Lauffer, R. B. *Chem. Rev.* **1987**, *87*, 901.
9. Lyon, R. C.; Faustino, P. J.; Cohen, J. S.; Katz, A.; Momex, F.; Colcher, D.; Baglin, C.; Koening, S. H.; Hambrigt, P. *Magn. Reson. Med.* **1987**, *4*, 24.
10. Matsumura, A.; Shibata, Y.; Yamamoto, T.; Nakagawa, K.; Yasuda, S.; Nakajima, S.; Sakata, I.; Yoshizawa, T.; Nose, T. *Neurol. Med. Chir.* **1997**, *37*, 327.
11. Battioni, P.; Brigand, O.; Desyaux, H.; Mansuy, D.; Trayler, T. G. *Tetrahedron Lett.* **1991**, *32*, 2893.
12. Lindsey, J. S.; Schreiman, I. C.; Hsu, H. C.; Kearney, P. C.; Marguerettaz, A. M. *J. Org. Chem.* **1989**, *54*, 827.
13. UV-Vis (λ_{max}, $CHCl_3$): 420, 514, 548, 593 nm. ^1H NMR (200 MHz, $CDCl_3$): δ 8.98 (m, 8H, β-pyrrole), 8.17 (m, 6H, *o*-phenyl), 7.54 (m, 9H, *m,p*-phenyl), 4.97 (b, 1H, phenyl-N*H*), 3.25(t, 2H, CH_2), 2.49(t, 2H, CH_2), -2.12(s, 2H, pyrrole-N*H*). TOF MALDI(M+H)$^+$: *m/z* 745.4 (calcd for $C_{48}H_{32}F_4N_8$+H$^+$ 745.80).
14. ^1H NMR (200 MHz, DMSO-d_6): δ 9.12, 8.80(m, 8H, β-pyrrole), 8.17(b, 6H, *o*-phenyl), 7.78(b, 9H, *m,p*-phenyl), 6.70(s, 1H, CO-N*H*), 4.0-3.4(b, EDTA ligand), -2.93 (s, 2H, pyrrole-N*H*).
15. ^1H NMR (200 MHz, DMSO-d_6): δ 9.16, 8.82(m, 8H, β-pyrrole), 8.21(b, 6H, *o*-phenyl), 7.82(b, 9H, *m,p*-phenyl), 6.77(s, 1H, CO-N*H*), 3.9-3.3(b, DTPA ligand), -2.96 (s, 2H, pyrrole-N*H*).