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

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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=diethylenetriaminepentacetic 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), O₂, 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 bleomycin,2 cholic acid,3 methidiumpropyl4 aminobenzyl,5 or ρ-iodoan obtobenzyl6 moiety 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-tetraakis(4-pyridyl)porphyrin and Gd-tetraakis(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_{5}TPPH_{2}) was synthesized since nucleophilic substitutions on some pentafluorophenyl residues is known to selectively occur with replacement of the p-F atom.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_{5}TPPH_{2} in 8.5% yield. As shown in Scheme 1, treatment of F_{5}TPPH_{2} (30 mg, 4.03x10^{-5} mol) with excess of ethylenediamine (0.27 ml, 4.04x10^{-3} mol) in DMF (15 ml) give the ethylenediamine-containing porphyrin derivative 5-[2,3,5,6-tetrafluoro-4-ethylenediaminophenyl]-10,15,20-triphenylporphyrin, F_{5}TPPH_{2}-EN, with a yield of 89%.13 A solution of ethylenediaminetetraacetic dihydride (EDTAD, 45.5 mg, 1.77x10^{-4} mol) and 3 drops of triethylamine in anhydrous DMSO (20 ml) was
placed into a round-bottomed flask with a nitrogen purge and additional funnel. A solution of \text{F.2TPPH}_2-\text{EN} (132 mg, 1.774×10^{-4} mol) 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 \text{F.2TPPH}_2-\text{EN}-\text{EDTA}\text{V} was purified by silica-gel chromatography (70-230 mesh) with methyl chloride/ methanol (v/v=9/1) as the eluant (yield=65%).

Evidence for the formation of \text{F.2TPPH}_2-\text{EN}-\text{EDTA} is found in spectroscopic analysis. The TOF-MALDI spectrum shows a M+H\textsuperscript{+} ion peak at 1020.3 (ms\textsuperscript{2}, calcd for C\textsubscript{42}H\textsubscript{42}F\textsubscript{3}N\textsubscript{6}O\textsubscript{7}H\textsubscript{2} 1020.02) and its IR spectrum shows broad overlap of \nu\textsubscript{max} and \nu\textsubscript{max} at 1590 cm\textsuperscript{-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 electronic spectrum of \text{F.2TPPH}_2-\text{EN}-\text{EDTA}.

Reaction of \text{F.2TPPH}_2-\text{EN}-\text{EDTA} (100 mg, 9.337×10^{-3} mol) with iron bromide (28 mg, 9.337×10^{-4} mol) in chloroform and methanol yielded a F.2TPPH\textsubscript{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 \text{F.2TPPH}_2-\text{EN}-\text{EDTA}-Fe (III) complex. A broad \textsuperscript{1}H NMR spectrum and Fe-O stretching peaks at 617, 474, 416 cm\textsuperscript{-1} in the FT-IR spectrum indicate the formation of paramagnetic \text{F.2TPPH}_2-\text{EN}-\text{EDTA-Fe} (III) complexes.

As shown in Scheme 1, same treatment of \text{F.2TPPH}_2-\text{EN} (128 mg, 1.72×10^{-4} mol) with diethylenetriaminepentaaetic dianhydride (DTPAD, 60 mg, 1.72×10^{-4} mol) in 3 drops of triethylamine and 30 ml of DMF, and a subsequent reaction with distilled water afforded the crude product: \text{F.2TPPH}_2-\text{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 \text{F.2TPPH}_2-\text{EN-DTPA} conjugate\textsuperscript{6} in 61% yield.

The TOF-MALDI spectrum shows a strong M+H\textsuperscript{+} ion peak at 1121.2 (ms\textsuperscript{2}, calcd for C\textsubscript{42}H\textsubscript{42}F\textsubscript{3}N\textsubscript{6}O\textsubscript{7}H\textsubscript{2} 1121.14) and its IR spectrum shows \nu\textsubscript{max} at 1651 cm\textsuperscript{-1} and (OH) at 1590 cm\textsuperscript{-1}. In the electronic spectrum of \text{F.2TPPH}_2-\text{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 porphyrin structure.

Further evidence for the formation of \text{F.2TPPH}_2-\text{EN-DTPA} conjugate and \text{F.2TPPH}_2-\text{EN-DTPA} conjugate comes from resonance peaks at 6.70 ppm and 6.77 ppm for the amide C(O)-NH resonance in the \textsuperscript{1}H NMR spectrum, respectively.

As mentioned earlier, upon complexation with transition metals such as Fe or Gd, \text{F.2TPPH}_2-\text{EN-EDTA} and
F,TPhH,-EN-DTPA are expected to have potential applicabilities in the field of a selective cleaving agent of DNA and a MRI enhancement agent, respectively. Metallation and reactivity studies of the F,TPhH,-EN-DTPA conjugate and the F,TPhH,-EN-DTPA conjugate are in progress.

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REFERENCES

13. UV-Vis (λmax, CHCl3): 220, 514, 548, 593 nm. 1H NMR (200 MHz, CDCl3), δ 8.98 (m, 8H, β-pyrrole), 8.16 (m, 6H, o-phenyl), 7.54 (m, 9H, m,p-phenyl), 4.97 (t, 2H, CH2), 2.49 (t, 2H, CH2), -1.12 (s, 2H, pyrrole-NH). TOF MALDI-M+H: m/z 745.4 (calcd for C40H22,N1,14,F1,114). 1H NMR (200 MHz, DMSO-d6), δ 9.16, 8.80 (m, 8H, β-pyrrole), 8.17 (b, 6H, o-phenyl), 7.78 (b, 9H, m,p-phenyl), 6.79 (s, 1H, CO-NH), 4.04 (s, EDTA ligand), -2.93 (s, 2H, pyrrole-NH).
15. 1H NMR (200 MHz, DMSO-d6), 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-NH), 3.9-3.3 (b, DTPA ligand), -2.96 (s, 2H, pyrrole-NH).