Synthesis of Water-Soluble C_{60}-Porphyrin Hybrid Compounds

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Water-soluble C_{60}-porphyrin hybrid molecules were first synthesized toward their pharmaceutical applications.

Key words fullerene; porphyrin; antioxidant; superoxide; water-solubility

Much interest has been generated in fullerene and porphyrin because of their unique properties, especially their photochemical activity; thus, many researchers are studying fullerene and porphyrin interactions in order to develop useful applications, *e.g.*, a photosynthetic system.[1] These fullerene and porphyrin hybrid compounds are all hydrophobic, and little is known about their water-soluble derivatives and their application.

Here we describe the synthesis of water-soluble C_{60}-porphyrin hybrid compounds in which one *meso*-position is occupied by the *p*-phenylene C_{60} derivative, while the remaining positions contain the *N*-methylpyridinium-4-yl substituents. We also show a preliminary example of their pharmaceutical application.

Our synthetic pathway is shown in Chart 1. 5-(4-Methoxycarbonylphenyl)-10,15,20-tris(4-pyridyl)porphine **1**[2] was obtained according to standard Adler's procedure with a 3% yield. The ester group of **1** was converted to aldehyde **3** in two steps. Then **3** was introduced to the C_{60} core by 1,3-dipolar cycloaddition with sarcosine to give **4**. The C_{60}-porphyrin hybrid compound **4** was methylated with excess methyl tosylate (30 eq) in chloroform to afford the tosylate salt of **5**, which was converted to its chloride salt by being passed through an anion-exchange resin (Amberlyte IRA 400). Finally, **6**, a Mn complex of **5**, was obtained from the tosylate salt of **5**. Mn was inserted into **5** using excess $Mn(OAc)_2$ (10 eq), purified as perchlorate salt, and then converted again to the chloride salt of **6** by an anion-exchange resin. The structures of these compounds were confirmed by standard instrumental analyses. **5** and **6** had sufficient water-solubility, as we had anticipated.

Next, we tested the pharmaceutical evaluation of **5** and **6**. We have previously studied fullerene derivatives against active oxygen toxicity and have already shown that anionic water-soluble C_{60} derivatives have moderate superoxide-quenching activity.[3] They actually decreased the active oxygen toxicity in *E. coli*.[4] Meanwhile, cationic water-soluble C_{60} derivatives had toxicity in *E. coli*[5] in spite of their potent activity on superoxide quenching.[6] Therefore, cationic C_{60} derivatives that have no toxicity against biological systems are expected for the application of fullerene as an antioxidant. On the other hand, manganese *meso*-tetrakis(*N*-methylpyridinium-4-yl)porphine (Mn-TMPyP) is well known for its potent superoxide-quenching activity,[7] which is why we became interested in the activity of the hybrid molecules.

We evaluated **5** and **6** for superoxide-quenching activity by the standard cytochrome c method.[8] Their IC_{50} was 23 μM for **5** and 0.76 μM for **6**, while their porphyrin control compounds TMPyP and Mn-TMPyP showed similar values (7.2 μM for TMPyP and 0.70 μM for Mn-TMPyP[7]). As we had anticipated, the activity of **5** and **6** was much higher than that of a water-soluble C_{60} molecule with a negative charge (IC_{50} *ca.* 100 μM).[6] Obviously, the difference in C_{60}-porphyrin hybrid compounds and porphyrin control on activity was not regarded as significant.

We expect that these derivatives reduce superoxide toxicity in a more hydrophobic environment, and we are now investigating their effect in *E. coli* systems.

Experimental

General Methods The ^{1}H-NMR spectra were recorded on a Varian VXR-200 or 500 spectrometer with tetramethylsilane as an internal standard. IR absorption spectra were recorded on a JASCO FT/IR-200 spectrometer. FAB-MS spectra were measured on a VG-70 spectrometer using *m*-nitrobenzyl alcohol as a matrix. Elemental analyses were performed on a Yanagimoto MT-5 CHN Corder elemental analyzer. Silica gel TLC was performed with Merck Kieselgel F_{254} precoated plates, and the silica gel used for column chromatography was Daisogel.

5-(4-Hydroxyphenyl)-10,15,20-tris(4-pyridyl)porphine (2) To a solution of 5-(4-methoxycarbonylphenyl)-10,15,20-tris(4-pyridyl)porphine (**1**, 710 mg, 1.05 mmol) in anhydrous THF (100 ml) was added $LiAlH_4$ (319 mg, 8.40 mmol) at room temperature. After being stirred for 30 min, AcOEt (10 ml) was added and then poured into H_2O (100 ml). The aqueous suspension was extracted with $CHCl_3$ (300 ml), washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure to yield **2** (360 mg, 53%). **2** was used for the next reaction without further purification. An aliquot was purified by silica gel column chromatography with $CHCl_3$–EtOH (97 : 3) for analysis. ^{1}H-NMR ($CDCl_3$) δ: −2.88 (2H, s, internal pyrrole), 5.10 (2H, s, –C<u>H</u>$_2$OH), 7.80 (2H, d, 3,5-phenyl, J=7.6 Hz), 8.19 (8H, m, 2,6-phenyl and 3,5-pyridyl), 8.86 (8H, m, pyrrole β), 9.06 (6H, d, 2,6-pyridyl, J=4.4 Hz). FAB-MS m/z: 648 ($[M+H]^+$).

5-(4-Formylphenyl)-10,15,20-tris(4-pyridyl)porphine (3) To a solution of $(COCl)_2$ (0.5 ml, 5.85 mmol) in anhydrous CH_2Cl_2 (5 ml) was dropped DMSO (0.5 ml, 7.05 mmol) in CH_2Cl_2 for 2 min at −60°. After being stirred for 15 min at the same temperature, **2** (99.4 mg, 0.153 mmol) in CH_2Cl_2 (50 ml) was dropped into the above mixture for 10 min. After being further stirred for 20 min at the same temperature, the reaction was completed by the addition of triethylamine (2.5 ml, 17.9 mmol) and was stirred for 30 min longer at the same temperature. After being warmed to room temperature, the organic layer was washed with water, 0.5 M $NaHCO_3$, and then brine. After being dried over $MgSO_4$ and evaporated under reduced pressure, the crude **3** was purified by silica gel column chromatography with $CHCl_3$–EtOH (97 : 3) to afford **3** (65.3 mg, 66%). ^{1}H-NMR ($CDCl_3$) δ: −2.89 (2H, s, internal pyrrole), 8.18 (6H, d, 3,5-pyridyl, J=4.8 Hz), 8.32 (2H, d, 3,5-phenyl, J=8.2 Hz), 8.41 (2H, d, 2,6-phenyl, J=8.2 Hz), 8.87 (8H, d, pyrrole β, J=2.6 Hz), 9.07 (6H, br, 2,6-pyridyl), 10.41 (1H, s, –C<u>H</u>O). FAB-MS m/z: 646 ($[M+H]^+$). IR (KBr) cm^{-1}: 1700 (C=O).

Trispyridylporphyrin-C_{60} Hybrid (4) To a solution of C_{60} (159 mg, 221 μmol) in bromobenzene (120 ml) was added **3** (95.0 mg, 147 μmol) and sarcosine (19.7 mg, 221 μmol). The reaction mixture was refluxed in an Ar atmosphere for 1 h and then evaporated under reduced pressure. The crude

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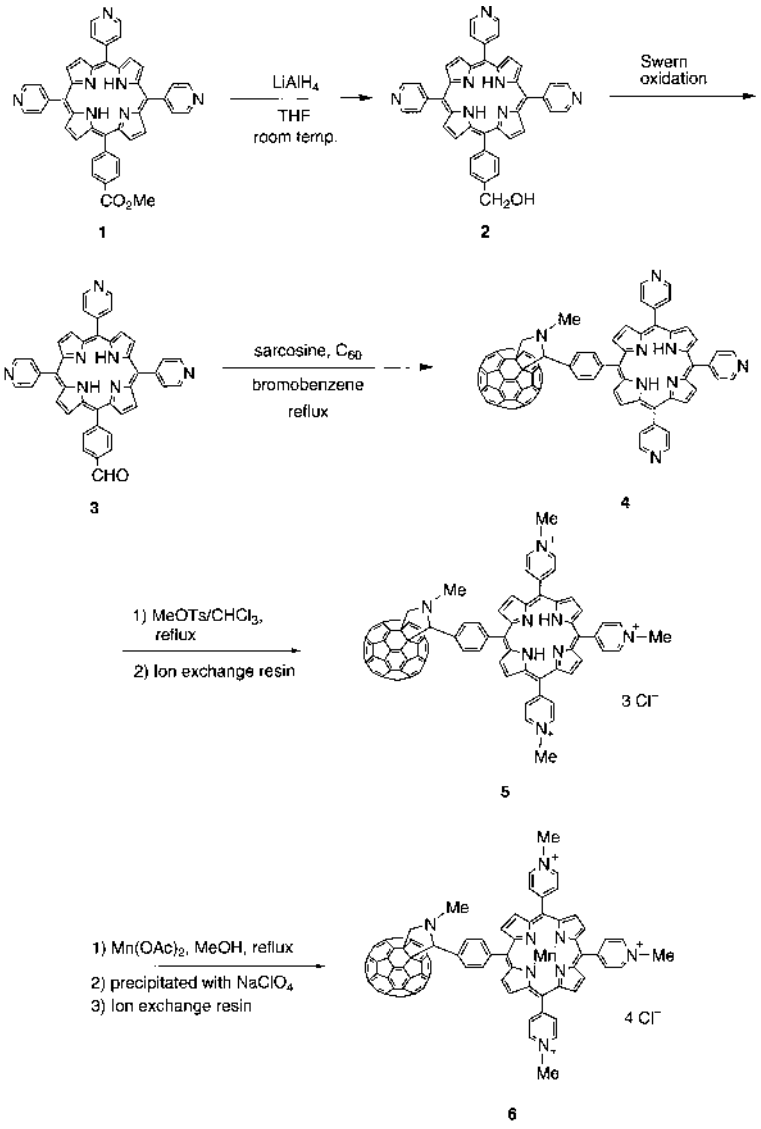

Chart 1. Preparation of **5** and **6**

mixture was subjected to silica gel column chromatography. After unreacted C_{60} was eluted with toluene, the elution with $CHCl_3$–EtOH (95 : 5) afforded **4** (135 mg, 63%). ^{1}H-NMR ($CDCl_3$) δ: −2.93 (2H, s, internal pyrrole), 2.97 (3H, s, N-Me), 4.00 (1H, d, J=9.2 Hz), 4.80 (1H, d, J=9.2 Hz), 4.81 (1H, s), 8.12 (10H, m, phenyl and 3,5-pyridyl), 8.83 (8H, m, pyrrole β), 9.04 (6H, br, 2,6-pyridyl). FAB-MS m/z: 1392 (M^+), 721 ($[C_{60}+H]^+$). UV λ_{max} (CH_2Cl_2) nm (ε): 417 (2.6×10^5), 513 (1.9×10^4), 547 (1.1×10^4), 588 (1.0×10^4), 645 (7.4×10^3).

Trismethylpyridylporphyrin-C_{60} Hybrid (5) To a solution of **4** (124 mg, 89.1 μmol) in bromobenzene (25 ml) was added methyl tosylate (0.4 ml, 2.65 mmol). The reaction mixture was refluxed in an Ar atmosphere for 1 h and then evaporated under reduced pressure. The crude tosylate salt of **5** was passed through an anion-exchange resin (Amberlyte IRA 400) repeatedly to yield **5** as a chloride salt (63.0 mg, 39%). ^{1}H-NMR (DMSO): −3.06 (2H, s, internal pyrrole), 3.11 (3H, s, N-Me), 4.51 (1H, d, J=9.2 Hz), 4.72 (9H, s, quaternary ammonium methyl), 5.24 (1H, d, J=8.6 Hz), 5.53 (1H, s), 8.32 (8H, m, pyrrole β), 8.96 (6H, d, 3,5-pyridyl, J=6.7 Hz), 9.12 (4H, m, phenyl), 9.49 (6H, d, 2,6-pyridyl, J=6.7 Hz). UV λ_{max} (H_2O) nm (ε): 426 (1.3×10^5), 524 (1.2×10^4), 560 (shoulder), 590 (shoulder), 652 (2.4×10^3). *Anal.* Calcd for $C_{107}H_{41}N_8Cl_3\cdot16H_2O$: C, 70.11; H, 4.01; N, 6.11. Found: C, 70.25; H, 3.85; N, 6.32.

Mn-Trismethylpyridylporphyrin-C_{60} Hybrid (6) To a solution of **5** (tosylate salt, 36.7 mg, 188 μmol) in methanol (20 ml) was added manganese (II) acetate tetrahydrate (46.2 mg, 0.189 mmol). The completion of the reaction was determined by a shift in the Soret band. After being refluxed for 1 h, the reaction mixture was evaporated under reduced pressure. The crude product was dissolved in distilled water (10 ml), and then the precipitate was obtained by the addition of sodium perchlorate (42.6 mg, 0.303 mmol). The precipitate was collected by centrifugation and then washed 5 times with 1.2% perchloric acid and 1 time with distilled water to yield **6** as a perchlorate salt. This material was converted by being passed through an anion-exchange resin (Amberlyte IRA 400) to yield **6** as a chloride salt (31.4 mg, 84%). UV λ_{max} (H_2O) nm (ε): 464 (1.1×10^5), 562 (1.2×10^4). *Anal.* Calcd for $C_{107}H_{39}N_8Cl_4Mn\cdot19H_2O$: C, 65.05; H, 3.93; N, 5.67. Found: C, 65.32; H, 3.64; N, 5.70.

Measurement of Superoxide-Quenching Activity This assay was performed according to the xanthine-xanthine oxidase/cytochrome c method.[8] Conditions were as follows: 0.05 M potassium phosphate buffer, 0.10 mM EDTA, pH 7.8, 50 μM xanthine, 10 μM cytochrome c, 700 units/ml catalase. The reproducibility of IC_{50} was within 10%. We checked that neither sample inhibited xanthine oxidase activity.

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