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Synthesis of a carotenobenzoporphyrin from a *meso*-diphenylporphyrin†

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Abstract

A carotenobenzoporphyrin has been prepared by covalently joining a benzoporphyrin derivative and an anilinocarotenoid by an amide linkage. A synthetic *meso*-diphenylporphyrin is the precursor of the benzoporphyrin moiety. The carotenobenzoporphyrin is highly fluorescent and does not sensitize measurable amounts of singlet oxygen. © 2000 Elsevier Science Ltd. All rights reserved.

Carotenoporphyrins have been synthesized as prototypes of imaging agents for the detection of malignancy.[1–3] The carotenoid moiety functions to suppress the phototoxicity associated with porphyrin-based compounds by eliminating the formation of singlet oxygen, the principal agent responsible for the phototoxic effects of porphyrins. Singlet oxygen is produced by energy transfer from the porphyrin triplet species to ground state oxygen in a bimolecular process. The role of the carotenoid is to interdict this bimolecular process by rapidly quenching the porphyrin triplet species. Rapid quenching requires close proximity of the carotenoid moiety to the porphyrin and effective electronic coupling between the chromophores to promote extremely rapid energy transfer from the porphyrin triplet state to the carotenoid.[4] Quenching the porphyrin triplet state to <200 ns renders it kinetically incapable of singlet oxygen sensitization at physiological concentrations of O_2 ($\sim 10^{-3}$ M). On the other hand, the electronic coupling provided by the linkage between the carotenoid and the porphyrin necessary for singlet oxygen suppression often results in quenching of the porphyrin fluorescence. This is an undesirable property for a fluorescence-based imaging agent. Fortunately, in carotenobenzoporphyrin **1** the carotenoid does not excessively quench the fluorescence of the porphyrin because the linkage that joins both chromophores limits the magnitude of their electronic interaction.

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† This paper is dedicated to Professor Harry H. Wasserman on the occasion of his 80th birthday.

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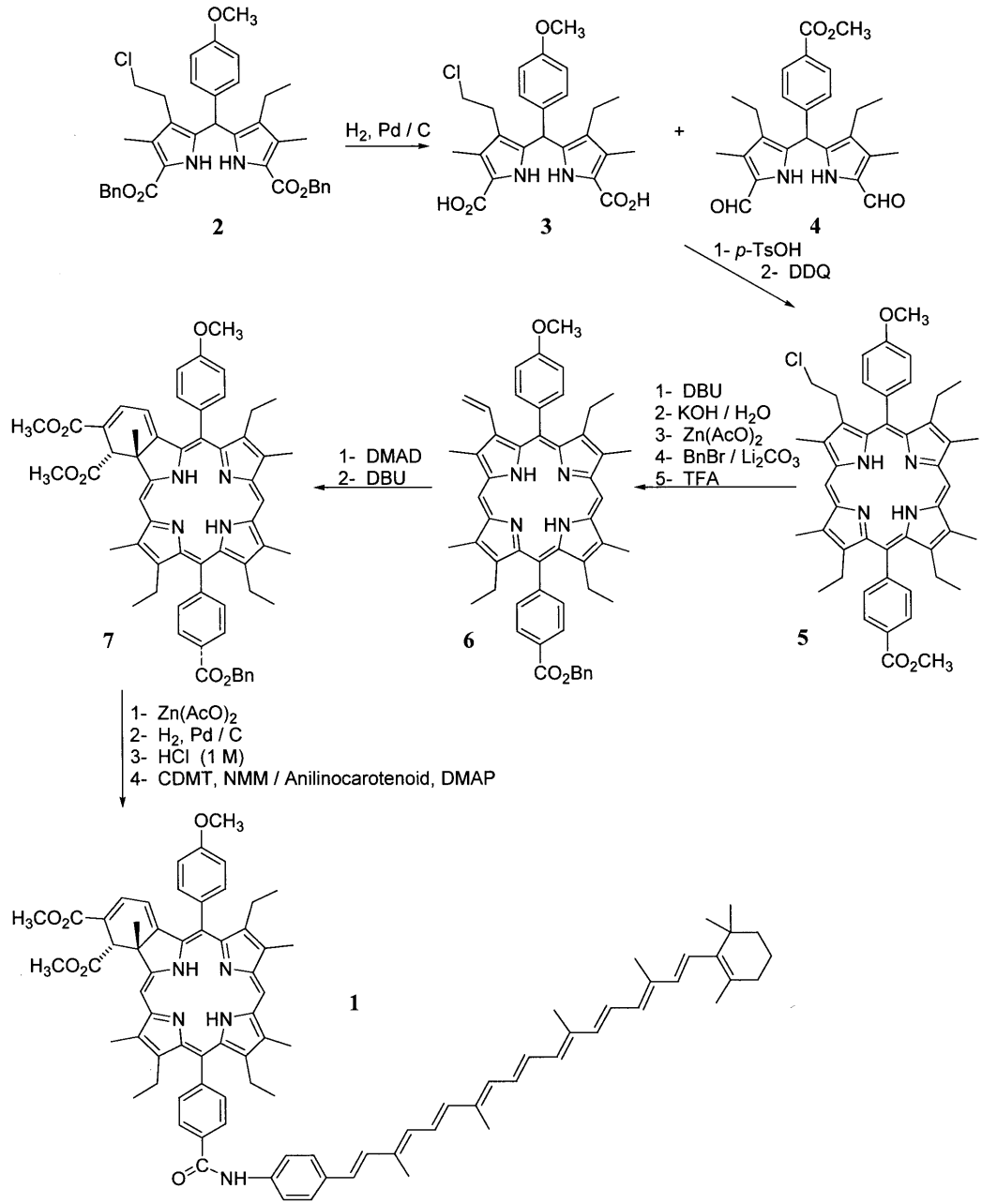
Carotenobenzoporphyrin **1** is highly fluorescent, approximately 1.3 times more fluorescent than tetrakis-5,10,15,20-(4-methylphenyl)porphyrin (TTP), does not sensitize measurable amounts of singlet oxygen at typical physiological O_2 concentrations and because of the extended chromophore of the benzoporphyrin moiety, its absorption is red shifted compared to simple octaalkyl porphyrins.[5]

Diels–Alder reactions have been frequently employed to extend the chromophore of porphyrins. The vinyl group of compounds such as protoporphyrin IX in conjunction with the ring A β,β-double bond mimics a diene unit which undergoes the cycloaddition reaction with a number of dienophiles. Benzoporphyrin derivatives (BPDs) are examples of compounds obtained by this [4+2] cycloaddition reaction which are presently being used in a number of biomedical applications including the treatment of cancer by photodynamic therapy (PDT).[6] A synthetic problem associated with BPDs prepared from protoporphyrin IX and acetylenedicarboxylate is the isolation of the desired compound from a complex mixture. To avoid these problems several laboratories have used other naturally occurring porphyrins instead of protoporphyrin IX.[7–9] In the present report we describe the synthesis of a BPD from a synthetic *meso*-diphenylporphyrin (**6**).

The synthesis of porphyrin **6**, requisite for the preparation of **1**, started with the coupling of two dipyrromethanes **3**, obtained by catalytic hydrogenation of **2**, and **4** (Scheme 1). The coupling reaction to form porphyrin **5** was effected in a solvent mixture of methanol and dichloromethane (CH_2Cl_2) with an excess of *p*-toluenesulfonic acid, at room temperature. The reaction mixture was treated with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ), and after column chromatography porphyrin **5** was isolated in 54% yield. Dehydrohalogenation to introduce the vinyl group at the β-position of the macrocycle was carried out by treatment of **5** with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in dimethylformamide at 120°C (81% yield). Four other steps were followed to complete the synthesis of **6**. They consisted of base hydrolysis of the methyl ester, metallation of the porphyrin with zinc acetate, esterification of the free acid with benzyl bromide and lithium carbonate, and removal of the central metal by acid treatment. Quantitative yields were obtained in each of these steps.

The fused six-membered ring of porphyrin **7** was built by the standard Diels–Alder reaction between **6** and dimethyl acetylenedicarboxylate (DMAD).[6] The reaction was carried out in a sealed tube, in toluene at 110°C for 4 days. At this point, TLC indicated that although starting material was still present, the major component was the more polar Diels–Alder adduct. The solvent and unreacted dimethyl acetylenedicarboxylate were removed under vacuum and the crude residue was redissolved in CH_2Cl_2. The solution was treated with an excess of DBU to isomerize the initial adduct of the cycloaddition reaction to the chlorin structure of **7**. After stirring for 2 h, TLC showed that only a single major product had formed. After column chromatography on silica gel (CH_2Cl_2 containing 2–2.5% acetone) pure benzoporphyrin **7** was obtained in 63% yield.

For the preparation of **1** the carboxylic acid masked by the benzylic ester of **7** had to be deprotected. The deprotection was effected by catalytic hydrogenation of zinc benzoporphyrin **7** dissolved in tetrahydrofuran with 10% palladium on carbon at a hydrogen pressure of $\sim$1.1 atm. After 2 h TLC showed that all the starting material was converted to a much more polar compound. The catalyst was removed by filtration (Celite) and the CH_2Cl_2 solution of the monoacid zinc-benzoporphyrin was shaken vigorously with 1 M hydrochloric acid to remove the zinc metal from the macrocycle.



Scheme 1.

The coupling reaction of the anilinocarotenoid (7′-apo-7′-(4-aminophenyl)-β-carotene)[10] with the benzoporphyrin monoacid acid was carried out with 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT) and *N*-methylmorpholine (NMM) in CH_2Cl_2. The solution of the benzoporphyrin monoacid was cooled to 0°C and a slight excess of the triazine derivative was added. The reaction was allowed to proceed at 0°C under a nitrogen atmosphere for 15 min and then at room temperature for 4 h. TLC of the reaction mixture indicated that most of the benzopor-

phyrin acid had been converted to the reactive intermediate. To the reaction mixture were added an equimolar amount of anilinocarotenoid and two equivalents of 4-dimethylaminopyridine (DMAP). After stirring the reaction mixture for 3 days, TLC indicated that the only major product was the desired carotenobenzoporphyrin. The reaction mixture was applied to a silica gel column and eluted with CH_2Cl_2 containing 2–3.5% acetone. A final recrystallization from CH_2Cl_2/methanol gave pure carotenobenzoporphyrin **1** in 70% yield.[11]

Briefly summarizing the photophysics of compounds **1** and the trimethyl ester of **7** (**8**), their fluorescence quantum yields have been determined in toluene using steady state measurements by the comparative method with TTP as the standard. The fluorescence quantum yield of **1** is 0.14 while that of model benzoporphyrin **8** is 0.18. In agreement with the steady-state fluorescence data, the fluorescence lifetime of **1** is 4.6 ns in toluene and that of **8** is 6 ns in the same solvent. In **1**, the rate constant for triplet energy transfer from the porphyrin to the carotenoid measured by the rise time of the carotenoid triplet is 5.6×10^6 s^{-1}.

The amide linkage used to connect the benzoporphyrin and the carotenoid chromophores allows partial conjugation between the two. However in **1**, the attachment of the carotenoid is through an amide linkage at the *meso*-aromatic position of the macrocycle. For steric reasons, the presence of the β-pyrrolic ethyl groups of the rings flanking the *meso*-aromatic substituent limit the π–π overlap between the benzoporphyrin (fluorophore) and the carotenoid. This structural arrangement serves to isolate to some extent the chromophores and consequently reduces the fluorescence quenching by the nearby carotenoid. As a result, carotenobenzoporphyrin **1** is approximately three times more fluorescent than a related carotenopurpurin bearing the same amide linkage but lacking the conformationally restricted *meso*-aromatic spacer.[12]

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11. Selected spectroscopic data for **1**. NMR (500 MHz, $CDCl_3$) δ: −1.54 (1H, s, –NH), −0.64 (1H, brs, –NH), 1.03 (6H, s, Car 16-CH_3, Car 17-CH_3), 1.04 (3H, t, $J=8$ Hz, –CH_3), 1.11 (3H, t, $J=8$ Hz, –CH_3), 1.16 (3H, t, $J=8$ Hz, –CH_3), 1.46–1.48 (2H, m, Car 2-CH_2–), 1.61–1.63 (2H, m, Car 3-CH_2–), 1.73 (3H, s, Car 18-CH_3), 1.78 (3H, s, –CH_3), 1.98 (3H, s, Car 19-CH_3), 1.99 (3H, s, Car 20-CH_3), 2.01 (3H, s, 20′-CH_3), 2.01–2.03 (2H, m, Car 4-CH_2–), 2.09 (3H, s, Car 19′-CH_3), 2.46–2.50 (2H, m, –CH_2–), 2.66–2.70 (2H, m, –CH_2–), 2.81 (1H, m, –CH_2–), 2.94–2.98 (1H, m, –CH_2–), 2.97 (3H, s, –CO_2CH_3), 3.32 (3H, s, –CH_3), 3.38 (3H, s, –CH_3), 3.47 (3H, s, –CH_3), 3.90 (3H, s, –CO_2CH_3), 4.07 (3H, s, O–CH_3), 5.03 (1H, s, –CH–CO_2CH_3), 5.38 (1H, d, $J=6$ Hz, vinyl-H), 6.12–6.94 (14H, m, Car vinyl-H), 7.18 (1H, dd, $J=9$ and 3 Hz, Ar-H), 7.29 (1H, dd, $J=9$ and 3 Hz, Ar-H), 7.41 (1H, d, $J=6$ Hz, vinyl-H), 7.52 (2H, d, $J=8$ Hz, Car Ar-H), 7.71 (1H, dd, $J=8$ and 2 Hz, Ar-H), 7.79 (2H, d, $J=8$ Hz, Car Ar-H), 8.01 (1H, dd, $J=8$ and 2 Hz, Ar-H), 8.06 (1H, d, $J=8$ Hz, Ar-H), 8.09 (1H, d, $J=8$ Hz, Ar-H), 8.19 (2H, m, Ar-H and –NH), 8.29 (1H, d, $J=8$ Hz, Ar-H), 9.00 (1H, s, *meso*-H), 9.88 (1H, s, *meso*-H); HRFAB-MS m/z calcd for $C_{89}H_{97}N_5O_6$: 1331.7438; found: $(M+H)^+$ 1332.7461; UV–vis (CH_2Cl_2) λ_{max} nm (ε M^{-1} cm^{-1}): 362 (96,598), 470 (203,487), 509 (125,618), 585 (19,026), 625 (11,978), 684 (35,066).
12. Unpublished data.