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SYNTHESIS AND ESTROGEN RECEPTOR BINDING AFFINITY OF A PORPHYRIN-ESTRADIOL CONJUGATE FOR TARGETED PHOTODYNAMIC THERAPY OF CANCER

David A. James,[a] Narasimha Swamy,[a] Nancy Paz,[a] Robert N. Hanson,[b] and Rahul Ray [a*]

[a]*Bioorganic Chemistry & Structural Biology Group, Department of Medicine, Boston University School of Medicine, Boston MA 02118, USA, and* [b]*Department of Pharmaceutical Sciences, Northeastern University, Boston, MA 02215, USA*

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Abstract: A tetraphenylporphyrin-C_{11}-β-estradiol conjugate has been synthesized. Competitive binding assay of the conjugate with estrogen receptor (ER)-ligand-binding domain showed that the conjugate binds specifically to the protein with high affinity. Potential use of this conjugate to selectively deliver cytotoxic porphyrins to ER-positive cells in various carcinomas is discussed.

Photodynamic therapy (PDT) is a relatively new method for the treatment of cancer that has received considerable attention in recent years.[1] PDT relies upon the selective accumulation of a photosensitizer into cancerous tissues followed by irradiation of the diseased area. Upon irradiation, the excited state of the photosensitizer generates singlet oxygen (or other reactive oxygen species) that damages cellular components and ultimately leads to cell death. The photosensitizer, Photofrin®, has been approved for the photodynamic therapy (PDT) of a variety of cancers (particularly bladder, esophageal, and lung) in numerous countries around the world.[1] However, there are several problems with the use of Photofrin® as a PDT agent. These problems include the fact that Photofrin is a complex mixture of compounds, and the exact composition can vary with each preparation. A major side effect is long-lasting skin photosensitivity due to relatively low selectivity of accumulation into cancerous tissues and slow rate of clearance.[1] As a result, more selective photosensitizers, which could accumulate into higher degree of selectivity are desired.

A certain degree of selectivity has been achieved by combining (covalent and non-covalent) porphyrins with antibodies,[2] microspheres,[3] liposomes[3] and lipoproteins[4] and targeting cancer cells. However limitations of these targeted-deliveries are well-documented.[4]

The goal of this present study is to employ certain steroid hormones as vehicles to selectively deliver photosensitizing porphyrins to cognate nuclear receptors of the steroid. Early-stage breast cancer cells are known to over-express estrogen receptor (ER).[5] Therefore, ER represents a potential site for directing photosensitizers, and increasing cellular uptake compared to normal cells via a receptor mediated process. Furthermore, by targeting the

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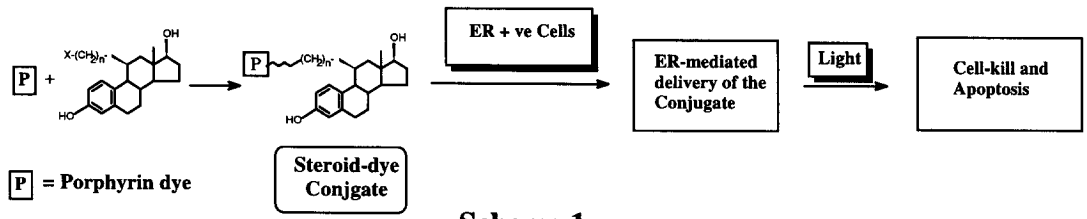
nuclear ER, light-induced damage may be directed at the nucleus, which has been acknowledged to be one of the more sensitive sites for photodynamic damage.[1] A sketch of the projected situation is depicted in Scheme 1.

The concept of ER-targeting has been previously investigated using phenylindole-aniline mustards.[6] Phenylindoles are known to bind effectively to ER and aniline mustards are efficient DNA cleaving agents. These

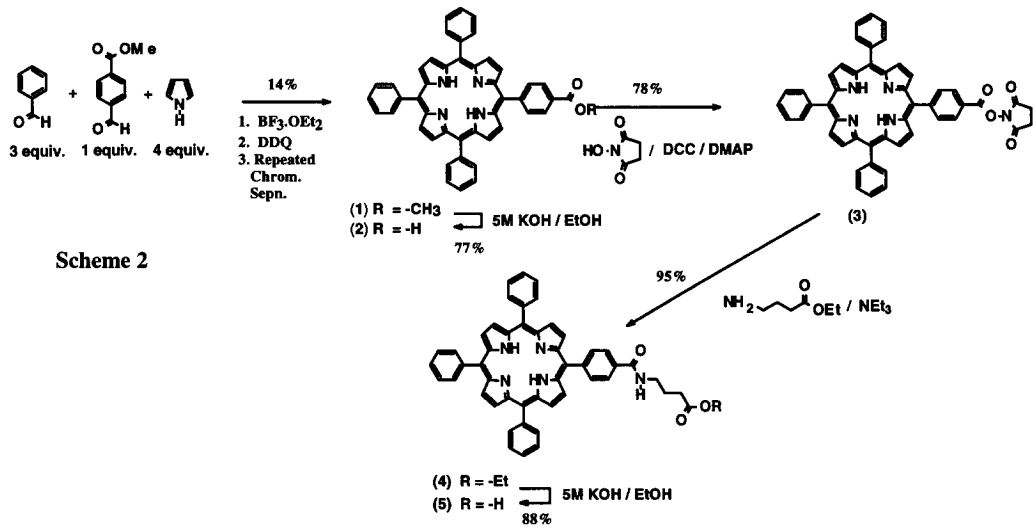


Scheme 1

conjugates showed affinity for calf-uterine ER and selective toxicity for the ER-positive cell line MCF-7, compared to the ER-negative cell line MDA-MB231. However, it was suggested that the selective toxicity was not due to nuclear ER delivery, as similar levels of interstrand crosslinks were observed in both cells lines. Jones and co-workers have studied various enediyne-estradiol conjugates and observed selective toxicity for MCF-7 cells compared to the enediyne alone.[7]

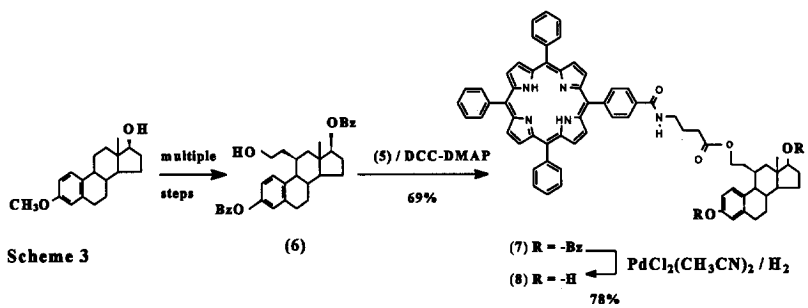
Extensive structure - function studies have been carried out with estrogens/antiestrogens and ER.[8] It has been amply demonstrated that modifications at the C-11β position of estradiol, particularly with hydrophobic groups, are well-tolerated towards ER-binding. In general, nonpolar groups are fairly well-tolerated, but presence of a polar group close to the main steroid structure is detrimental.[8] These observations have been substantiated by the recent determination of the three-dimensional structure of the ligand-binding domain of ER, which showed that C_{11}-β-position is largely devoid of steric interference from the protein backbone structure.[9,10] Based on this knowledge, we have synthesized a tetraphenylporphyrin-estradiol conjugate linked through the estradiol C_{11}–β-position via an eight (8) atom tether.

Huang and co-workers have previously reported the synthesis of the carboxymethyl-porphyrin (**1**).[11] We adopted a more convenient procedure based on the popular method of Lindsey and co-workers[12] for the synthesis of unsymmetrical tetraphenylporphyrins. This procedure involved the $BF_3.OEt_2$-catalyzed condensation of methyl-4-formylbenzoate, benzaldehyde and pyrrole followed by oxidation with DDQ. Formation of the mono-(carboxymethylphenyl)porphyrin was favored by adjusting the stoichiometry of the reaction as indicated in Scheme 2 and high dilution. A mixture of porphyrins resulted and **1** was obtained after repeated flash column chromatography. Following the hydrolysis of **1**, a DCC-mediated coupling of **2** with *N*-hydroxy succinimide produced the activated ester **3** which readily reacted with ethyl 4-aminobuyrate resulting in the protected porphyrin-amino acid **4**. Hydrolysis of the ester group in **4**, followed by DCC-coupling reaction with 3,17-dibenzyl,11β-(2')



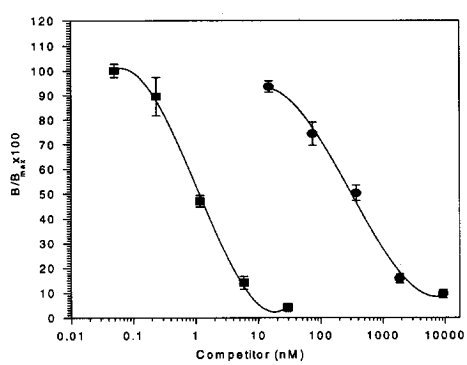
Scheme 2

hydroxy ethyl estradiol **6**[13,14] afforded the coupled product **7**, which, upon removal of the protecting groups, produced the porphyrin-estradiol conjugate **8** (Scheme 3).



Scheme 3

Figure 1. Competitive Binding Assay of (8) (EC_{50} = 274 nM) vs. estradiol (EC_{50} = 1 nM) to recombinant ER ligand binding domain.



A competitive radioligand binding assay was used to determine the ability of the porphyrin-estradiol conjugate **8** to bind to recombinant ER ligand binding domain compared to estradiol, the natural ligand for ER. The results of the binding assays, shown in Figure 1, demonstrated that the conjugate **8** displaced [^{3}H]-estradiol, bound to ER-ligand binding domain, in a dose-dependant manner similar to estradiol. However, the binding

affinity for the conjugate **8** was lower than that of the natural ligand, estradiol [EC_{50} for estradiol and conjugate **8** were one (1) and 274 nM, respectively). These results confirmed that the C_{11}-β-position of estradiol is tolerant to substitution with a large moiety such as a porphyrin.

Inherent fluorescence of porphyrins have been used for their detection in culture-grown cells as well as in tumors.[15] In addition, in several recent studies, metalloporphyrins have been used as tumor-localizing agents by magnetic resonance imaging (MRI).[16-18] It is well-known that fluorescence spectrum (wave lengths of absorption and emission) and quantum yields of a fluorescent molecule are highly dependent on its micro-environment[19] as well as any changes in its structure. For example, emission-quantum yield of fluorescein is significantly quenched by covalent attachment of avidin, apparently due to the interaction between fluorescein and amino acids in the protein.[20]

Fluorescence spectra (emission) of the conjugate **8** and the porphyrin **4** (on a molar basis, in methanol) are shown Figure 2 ($\lambda_{Excitation}$ = 413 nm, $\lambda_{Emission}$ = 650 nm). There was a 27% decrease in the quantum yield at 650 nm with the conjugate **8**, compared with the porphyrin **4**. Conversely, the peak at 604 nm increased by 1220% with the conjugate **8**. When we added different amounts of estradiol to a methanolic solution of the porphyrin **4**, there was no quenching at 650 nm, and the peak at 604 nm was unchanged. Collectively these results suggested that there could be some overlap of the electron-clouds of the porphyrin part and the aromatic ring of the conjugate **8**.

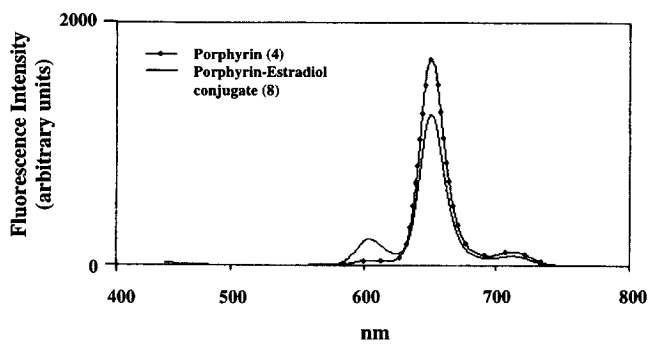


Figure 2 : Fluorescence emission spectra of (4) and (8).

In conclusion, we have synthesized, for the first-time, a conjugate of an estradiol-derivative and a porphyrin, in which specific and high-affinity ER-binding property of estradiol and fluroscence property of the porphyrin are largely retained. This compound provides us with a molecular probe and a vehicle for the nuclear- targeting of ER-positive cells such as MCF-7 (breast cancer), OVCAR (ovarian cancer) etc. Cellular uptake and photo-toxicity studies of the conjugate **8** in ER - positive MCF-7 and ER - negative Hs578T (breast cancer) cells are in progress; and the results will be reported in a future publication.

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