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A phthalocyanine–mestranol conjugate for photodynamic therapy prepared via click chemistry

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ABSTRACT

Introduction of an azide group onto the periphery of a photodynamically effective phthalocyanine (Pc) derivative gives a useful building block for the preparation of Pc conjugates via Cu(I)-catalyzed azide-alkyne 1,3-dipolar cycloaddition ('click chemistry'). In this way, Pc–mestranol and Pc–hept-1-yne conjugates (for comparison purposes) are synthesized and their absorption, photochemical, and photophysical properties are studied. The conjugate retains the advantageous photophysical and photochemical properties of Pc. Conjugation with mestranol may lead to improved localization in tumors whereas the Pc unit is responsible for the photodynamic effect.

Phthalocyanines (Pc) are well-known organic dyes with applications in many areas. Medical applications include mainly photodynamic therapy (PDT) which combines a photosensitizer (PS), light, and oxygen to produce a cytotoxic effect.[1] However, the low targeting of the PS can often limit their use. This problem is usually solved by the synthesis of PS conjugates with biomolecules or other targeting moieties.[2,3] Among them, alkynylestradiol–porphyrin conjugates have shown good promise for hormone-sensitive tumors where the estrogen receptor (ER) is overexpressed.[4] In another study, Pc–estradiol conjugates showed good receptor binding affinities for the ER, particularly when the Pc was lipophilic.[5] Nevertheless, the published syntheses of PS conjugates are difficult and often suffer from low yields. The highly efficient, selective and mild Cu(I)-catalyzed azide-alkyne 1,3-dipolar cycloaddition ('click' chemistry) is an attractive strategy for the preparation of bioconjugates.[6] Although 'click' chemistry has been thoroughly explored in recent years, only a few examples of Pc postmodification by this interesting reaction can be found,[7,8] most likely due to the limited solubility of some Pcs.[9]

Recently, we described the synthesis of photosensitizer **1** (Scheme 1) which displayed advantageous photophysical and photochemical properties for application in PDT.[10] It is highly soluble in common organic solvents and absorbs light at wavelengths that allow deeper penetration of activating light into human tissues (726 nm). It possesses very good photosensitizing properties ($\Phi_\Delta = 0.80$) and emits fluorescence for detection of PS localization ($\Phi_F = 0.06$).[10] Herein, we describe a reliable synthesis of its derivative **2**, which is a suitable building block for 'click' chemistry with any molecule bearing a terminal alkyne. For example, conjugation with mestranol and hept-1-yne is described.

First, 3-azidopropylamine (**3**) was prepared from the corresponding bromide (Scheme 1). Amidation of **1** with **3** using HBTU as the activator gave **2**. However, the published synthesis of **1** is relatively low-yielding (11%) due to problems with silica binding during isolation.[10] As compound **2** seemed to lack such problems, we decided to synthesize it directly using a modified precursor to obtain better overall yields (Scheme 1). Thus, amidation of 2,3-dicyanoquinoxaline-6-carboxylic acid with **3** using $SOCl_2$ gave **4** in a reasonable 65% yield. Its mixed cyclotetramerization with 4,5-bis(*tert*-butylsulfanyl)phthalonitrile using magnesium butoxide as the initiator gave a statistical mixture composed of at least six different Pcs (AAAA, AAAB, AABB, etc.). A 3:1 ratio of starting material shifted the reaction toward the desired AAAB type. The magnesium(II) complex of **2** was isolated in 33% yield and its demetalation using *p*-toluenesulfonic acid gave metal-free **2** which was subsequently chelated with zinc(II) to give Zn–**2** in an overall 26% yield (based on starting dicarbonitriles). The higher overall yield may be attributed to the significantly decreased silica binding which seemed to be the limiting factor during purification of **1**.

Compound **2** is a suitable active part of PS conjugates either with improved physicochemical properties (e.g., water solubility) or with targeting properties. Due to the above-mentioned reasons we focused on PS–estrogen conjugates, but any other biomolecule (e.g., saccharides, oligonucleotides) modified with a terminal alkyne can be used for conjugation. The 17α-ethynyl group of mestranol represents a suitable reactive center for 'click' chemistry. Various conditions were tested for the 'click' reaction. Refluxing **2** with mestranol in THF with CuI and DIPEA was revealed as the most suitable procedure giving very high yields of conjugate **5**.[11] For example, DMF as the solvent and DBU as the base led to only

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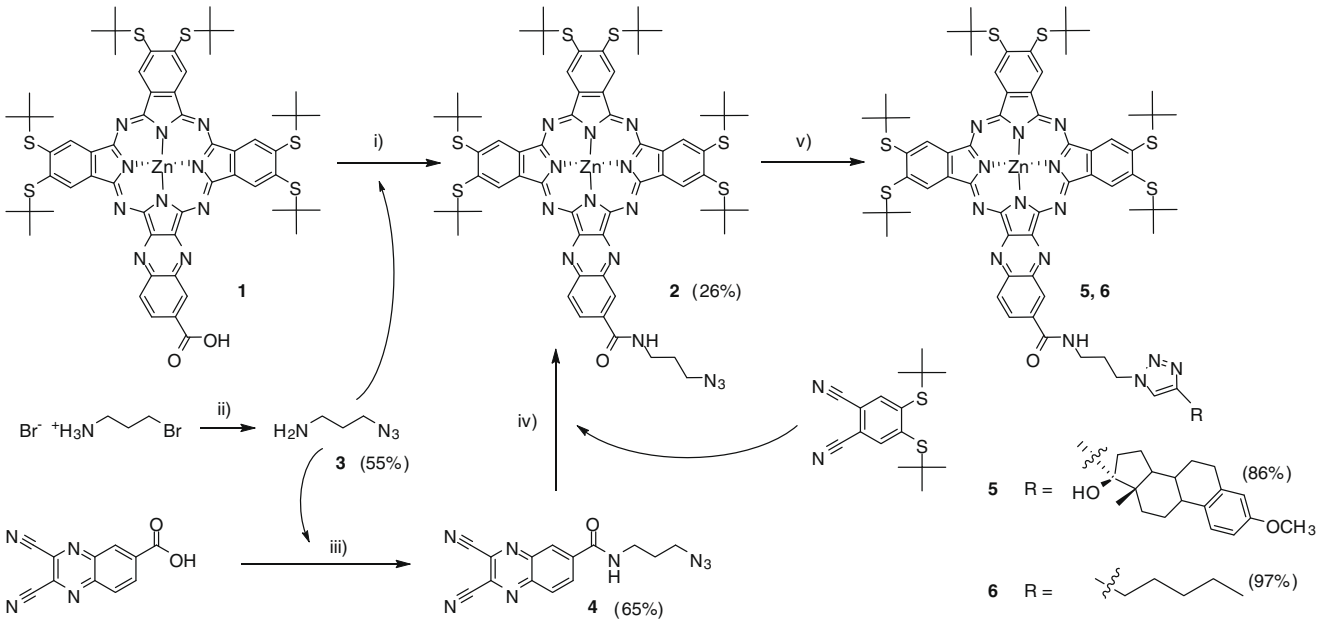

Scheme 1. Reaction conditions: (i) HBTU, anhyd. DMF, Et_3N, rt, 18 h; (ii) NaN_3, H_2O, 90 °C, 23 h; (iii) $SOCl_2$, anhyd toluene/THF, reflux, 6 h; (iv) Mg, anhyd butanol, reflux, 19 h→*p*TSA, THF/$CHCl_3$, rt, 4 h→$Zn(COOCH_3)_2$, pyridine and DMF, 120 °C, 5 h; (v) **5**-C≡CH or **6**-C≡CH, CuI, THF, DIPEA, reflux, 19 h.

a 29% yield and significant amounts of side products were detected on TLC. For comparative purposes, **2** was also reacted with hept-1-yne to give **6** almost quantitatively. All the synthesized compounds were fully characterized by NMR, IR, and UV–vis spectral methods and the purity was confirmed by elemental analysis. MALDI-TOF spectra showed clusters at expected *m/z* values with isotope patterns corresponding with calculated distributions.

An important question is whether the promising photophysical and photochemical properties of **1** are retained in conjugates **5** and **6**. No significant changes were observed in the absorption or fluorescence emission spectra of **1**, **5**, and **6** (Fig. 1). The Q-band maxima were found at 727 nm allowing photosensitization using light that penetrates deeper into human tissues. The broad Q-band caused by splitting due to the unsymmetrical composition of the macrocyclic system may be considered as another advantage. More photons are expected to be absorbed during irradiation by conventional lamps emitting full-spectrum light. The singlet oxygen quantum yield (Φ_Δ), the most important photochemical parameter in PDT, was determined by a comparative method based on the decomposition of 1,3-diphenylisobenzofuran. The results summarized in Table 1 showed that neither introduction of an azide group as in **2** nor conjugation with mestranol (**5**) or hept-1-yne (**6**) led to significant reduction of Φ_Δ values. All the tested compounds can

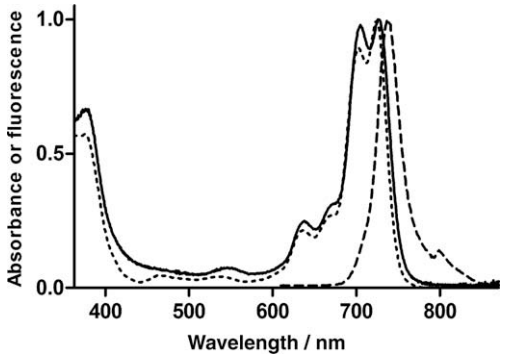

Figure 1. Normalized absorption spectra of **1** (dotted line) and **5** (full line) in pyridine. Normalized emission spectrum of **5** in pyridine (dashed line).

Table 1
Absorption maxima of the Q-bands (λ_{max}), singlet oxygen quantum yields (Φ_Δ), and fluorescence quantum yields (Φ_F) of **1**, **2**, **5**, and **6** in pyridine

Compd	λ_{max} (nm)/ε ($\times 10^5$ M^{-1} cm^{-1})	Φ_Δ	Φ_F/λ_{em} (nm)
1[a]	726/1.390	0.80	0.060/734
2	727/1.503	0.78	0.040/738
5	727/1.045	0.84	0.042/738
6	727/1.335	0.69	0.040/738

[a] Data from Ref. 10.

therefore be considered as very efficient photosensitizers. The fluorescence emission of **1**, which can be a useful tool for tumor visualization or photosensitizer distribution monitoring, is also retained for **2** as well as for conjugates **5** and **6**.

In conclusion, we have developed an efficient synthesis of a photosensitizer substituted with an azide group **2** which is a versatile substrate for 'click' chemistry with various molecules bearing a terminal alkyne for both improved targeting (e.g., steroids, proteins, oligonucleotides) and/or physicochemical properties (e.g., increase of water-solubility after conjugation to oligosaccharides). Formation of the stable linkage after Cu(I)-catalyzed azide-alkyne 1,3-dipolar cycloaddition does not affect the good photosensitizing properties of the starting PS and no quenching of the excited states occurs at the azide or final 1,2,3-triazolyl-modified Pc. Thus click chemistry can be used for the preparation of targeting PS with no decrease of the photosensitizing properties of the active part. Conjugate **5** bearing mestranol should have improved targeting properties for hormone-sensitive tumors where the ER is overexpressed and biological testing is under investigation.

Acknowledgments

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Supplementary data

Supplementary material includes full synthetic details and characterization of all compounds including ^1H NMR spectra of final

compounds. Absorption, emission, and excitation spectra are also presented. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.12.075.

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11. Preparation of conjugate **5**: Compound **2** (20 mg, 0.016 mmol), CuI (3.0 mg, 0.016 mmol) and mestranol (7.3 mg, 0.023 mmol) under argon were dissolved in THF (10 mL). *N,N*-Diisopropylethylamine (DIPEA) (60.5 mg, 0.468 mmol) was added dropwise. The solution was heated at reflux under argon for 7 h after which furthur portions of CuI (3.0 mg, 0.016 mmol) and mestranol (7.3 mg, 0.023 mmol) were added and reflux was continued for 10 h. The crude product was purified by column chromatography on silica with toluene/pyridine 10:2 as eluent and washed with methanol. Yield: 21.3 mg (86%), cyan-blue solid. Anal. Calcd $C_{83}H_{96}N_{14}O_3S_6Zn+4H_2O$: C, 59.78; H, 6.29; N, 11.76. Found: C, 59.50; H, 6.06; N, 11.34. *m/z* (MALDI-TOF) 1593 $[M+H]^+$, 1615 $[M+Na]^+$, 1631 $[M+K]^+$; δ_H(300 MHz, pyridine-d_5) 1.35 (3H, s, CH_3Mestr.), 1.53 (9H, s, CCH_3), 1.57 (9H, s, CCH_3), 1.67 (18H, s, CCH_3), 1.69 (18H, s, CCH_3), 1.45–1.75 (4H, m, aliphatic Mestr), 1.88–1.95 (4H, m, aliphatic Mestr), 2.14–2.29 (4H, m, aliphatic Mestr), 2.43–2.60 (3H, m, aliphatic Mestr and $CH_2CH_2CH_2$), 2.70–2.83 (1H, m, aliphatic Mestr), 2.91–3.04 (1H, m, aliphatic Mestr), 3.56 (3H, s, OCH_3), 3.89 (2H, q, J = 7.1 Hz, $NHCH_2$), 4.81 (2H, t, J = 6.8 Hz, NCH_2), 6.64 (1H, d, J = 2.5 Hz, Ar-Mestr.), 6.79 (1H, dd, J_1 = 2.6 Hz, J_2 = 8.8 Hz, Ar-Mestr.), 6.82 (1H, s, Ar-Mestr), 8.41 (1H, s, CH triazole), 8.89 (1H, d, J = 9.1 Hz, Ar-H), 9.01 (1H, d, J = 8.8 Hz), 9.81–9.86 (2H, m, Ar-H), 10.14 (2H, s, Ar-H), 10.16 ppm (3H, s, Ar-H); δ_C(75 MHz, pyridine-d_5) 15.07, 24.47, 26.97, 27.85, 30.15, 29.98, 31.38, 33.73, 37.98, 38.85, 39.98, 43.85, 47.84, 48.29, 48.74, 48.76, 48.90, 48.97, 54.94, 82.28, 111.94, 114.01, 126.72, 130.11, 130.73, 131.17, 131.42, 132.43, 132.59, 133.08, 134.83, 137.58, 138.07, 138.17, 138.28, 138.32, 141.67, 141.72, 142.63, 142.66, 143.16, 143.39, 143.44, 144.68, 154.56, 155.39, 155.66, 155.68, 155.93, 157.87 and 167.16 ppm. Additional analytical data can be found in the Supplementary data.