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Synthesis of porphyrin glycoconjugates bearing thiourea, thiocarbamate and carbamate connecting groups: Influence of the linker on chemical and photophysical properties

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ABSTRACT

In the present work, we have synthesized new porphyrin conjugates bearing thiourea, thiocarbamate and carbamate linkers using conventional and ultrasound-assisted methods. These molecules were synthesized by addition reactions using isothiocyanate derivates and amino- or hydroxyl-functionalized *meso*-tetra(aryl)porphyrins to give porphyrin analogs linked to glycosyl or benzyl moieties. Glycoporphyrin derivates could be obtained with better yields than their respective benzyl analogs. Thiourea and carbamate glycoporphyrins were also successfully synthesized through an ultrasound protocol with a remarkable decreasing of the reaction time. By NMR experiments we were able to evaluated the linker conformations which showed a preferred *Z,Z* conformation for the thiourea and carbamate derivatives. In addition, porphyrins were evaluated in terms of their UV–Vis spectra, singlet oxygen production, photostability and aggregation properties. Correlations between the linker type and the corresponding photophysical properties were observed. Carbamate porphyrins seem to be the most promising photosensitizers among the studied molecules due to their high singlet oxygen production, photostability and absence of self-assembling behavior in aqueous media.

1. Introduction

Since porphyrins have become part of the therapeutic arsenal for cancer treatment through photodynamic therapy (PDT), numerous studies have been dedicated to the synthesis of new and more efficient macrocyclic photosensitizers (PS). The first generation of PS is represented by hematoporphyrin derivates (e.g. Photofrin®), which gave place to a second generation that presented improved chemical homogeneity and radiation absorptivity at higher wavelengths of the visible spectrum [1–3]. However, both first and second generations of PS typically act as unspecific cytotoxic drugs. In this case, PDT selectivity towards the tumor tissue depends on the spatial focusing of the light source, as well as on the more active metabolism presented by malignant cells, which amplifies the photodamage promoted by the PS [4,5]. On the other hand, the lack of PS intrinsic selectivity can compromise the surrounding healthy tissue during PDT course. In this way, conjugation of PS to molecular moieties that can be selectively recognized by tumor cells have become of interest. Conjugated PS are then referred as the third generation of PS and their study currently represents an active research area [3,6].

A number of structural motifs based on biomolecules, such as proteins [7], immunoglobulins [8], cholesterol [9] and carbohydrates [10,11], have been chemically associated to macrocyclic rings in order to enhance the selectivity of PS uptake by the target cell. In this field, PS conceived through the attachment of glycosyl moieties to the porphyrin ring have proved to be promising candidates when targeted systems are desired [12]. This is related to the fact that cancer cells present higher amounts of lectins (carbohydrate binding proteins) on their surfaces than do regular cells [13,14]. Many porphyrin glycoconjugates have already been synthesized with ether [15,16], ester [17] or triazole [18,19] groups as linkers for binding the porphyrin to carbohydrate moieties. Nevertheless, these linkers could not be considered ideal because the deglycosylation process [20] as well as decreasing of biological activity [21] have already been reported as result of *in vitro* assays employing

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those PS glycoconjugates. Thus, it is considered important to synthesize PS conjugates having different kinds of linkers in order to evaluate their chemical stability and their influence on photodynamic properties.

The formation of thiourea bridges is a well-known approach for binding different types of molecules through convergent synthesis. This kind of linkage was first utilized for the attachment of fluorescent probes to sensitive substrates, which could be performed under very mild conditions in the absence of catalysts [22]. Because of the versatility and robustness of this functional group, thiourea bridges became a common linker for binding carriers to biologically active molecules. However, there are only a few examples of the synthesis of porphyrins bearing thiourea linkers. Clark and Boyle (1999) [23] synthesized thiourea-containing porphyrins by using isothiocyanate-functionalized porphyrins and a series of amino compounds, including amino acids, aliphatic and aromatic amines as starting materials. Other related studies made use of reactions employing amino porphyrins and isothiocyanate compounds [24]. Thiourea-related linkers, such as thiocarbamate and carbamate groups, have also facile synthesis methodologies; nevertheless they are poorly explored for the synthesis of conjugated porphyrins. Although the thiocarbamate linker can be promptly obtained from reactions of hydroxyl compounds with isothiocyanate derivates, to the best of our knowledge there is no example of this kind of linkage involving porphyrin synthesis. This fact could be related to the previously noted instability of the thiocarbamate bridge [25,26]. There are two reported examples of carbamate-linked porphyrins where hydroxy-porphyrins and isocyanate-functionalized compounds were utilized as starting materials [27,28]. The above cited linkers have never been previously used for functionalizing porphyrins with carbohydrate moieties. Also, a comparative evaluation of the influence of such linkers on porphyrin photophysical properties is yet to be performed. PS photophysical evaluation is considered key in order to understand, or even predict, porphyrin photodynamic potentialities [29].

Here, we have investigated the synthesis of porphyrin conjugates bearing thiourea, thiocarbamate and carbamate linkers. For this purpose, we utilized amino- or hydroxy-functionalized *meso*-tetra(aryl)porphyrins and per-*O*-acetyl-β-D-glucopyranosyl isothiocyanate as starting materials. We have also employed benzyl isothiocyanate in order to obtain non-glycosyl porphyrin conjugate analogs for intercomparison of their properties. The linker conformation of the porphyrin conjugates was evaluated by NMR spectroscopy. Due to the potential interest in this class of compounds in PDT, photophysical and physicochemical properties of those conjugates were studied, including determination of singlet oxygen production and photostability, evaluation of the electronic spectra and aggregation studies.

2. Material and methods

2.1. General procedures

The ^{1}H NMR, ^{13}C NMR and 2D experiments (HMBC-^{1}H/^{13}, NOESY ^{1}H/^{1}H) were obtained with a Bruker AVANCE III 400 spectrometer with the indicated solvents operating at 400.15 MHz and 100.62 MHz respectively. Chemical shifts (δ) are expressed in parts per million (ppm) and coupling constants (J) in Hertz (Hz) using residual solvent peaks as internal standards. Low resolution mass spectra were obtained with a Bruker MALDI-TOF spectrometer with HCCA matrix. The high resolution mass spectra (HRMS) were recorded with two different spectrometers: (a) a Bruker MicroTOF-Q II XL operating in the positive-ion mode with porphyrins at 100 μg mL^{-1} in methanol using sodium formate solution as reference or (b) a Bruker LTQ Orbitrap operating in the positive-ion mode with porphyrins at 10 μg mL^{-1} in methanol using Ultramark 1621 as reference. Optical rotation values were obtained with a Jasco P-200 polarimeter equipped with a sodium light source from 1% (g 100 mL^{-1}) porphyrin solutions in DMSO. UV–Vis spectra were recorded with a Shimadzu UV-1800 spectrometer using porphyrin concentration at 12 μmol L^{-1} in chloroform. Fluorescence emission spectra were recorded with an RF-5301PC Shimadzu spectrofluorometer using porphyrin concentrations as indicated in Fig. 8 and chloroform as solvent. Infrared (IR) experiments were performed with KBr in an FTIR Bomen/MB analyzer. The light source used for the photophysical assays was a Lumacare LC 122A with a halogen/quartz 250 W lamp. All compounds were stored at 5 °C in a 1 mmol L^{-1} DMSO solution (stock solutions) before the photophysical assays. These experiments were conducted at room temperature in a dark room to avoid any impact of ambient light over the main light source. Compounds **9** and **11** were analyzed just after purification with less than 24 h storing.

2.2. Synthesis and purification

Reagents and solvents were of reagent grade. For anhydrous reactions, the solvents were dried according to stated methods [30]. Acetonitrile was dried by soaking it with silica gel for 24 h followed by distillation. DMF was dried by soaking it for 24 h with anhydrous $MgSO_4$. The dried solvents were then stored in flasks containing molecular sieves 4 Å under nitrogen atmosphere. Column chromatography was performed with the indicated solvents using Sigma silica gel 60 (particle size 220–440 mesh). Yields refer to chromatographically and spectroscopically pure compounds. Compounds **1** [31], **2** [32], **3** [31], **4** [33], **5** [34] and **6** [35] are known compounds and their spectroscopic data coincided to those reported previously (for spectroscopy data see Supplementary Information).

2.2.1. Synthesis of 5-[4-((2′,3′,4′,6′-tetra-O-acetyl-β-D-glucopyranosyl)thioureido)-phenyl]-10,15,20-triphenylporphyrin (**7**)

Method A: 5-(4-aminophenyl)-10,15,20-triphenylporphyrin **3** (63 mg 0.1 mmol) and per-*O*-acetyl-β-D-glucosyl isothiocyanate **5** (78 mg, 0.2 mmol) were dissolved in chloroform (4 mL) and stirred under reflux for 24 h. The resulting mixture was concentrated under vacuum and the residue was purified by silica gel column using chloroform/methanol (99:1) as mobile phase to give **7** as a purple solid (73 mg, 72% yield). Method B: **3** (63 mg 0.1 mmol) and **5** (78 mg, 0.2 mmol) were dissolved in chloroform (4 mL) and placed in an ultrasonic bath (42 MHz) for 1 h at room temperature. The resulting mixture was concentrated under reduced pressure and the residue was chromatographed as outlined for Method A, giving **7** with 69% yield (70 mg).

$[a]_D^{25}$ + 6.25 (*c* 1.0, DMSO); **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm) 10.35 (s, 1H, N–H linker); 8.92 (d, J = 4.3 Hz, 2H, β-pyrrole), 8.83 (m, 6H, β-pyrrole); 8.50 (d, $J_{HN\text{-}H1'}$ = 8.5 Hz, 1H, N′-H linker), 8.21 (d, J = 5.6 Hz, 6H, *o*-phenyl); 8.18 (d, J = 8.4 Hz; 2H, *o*-phenyl linker); 7.96 (d, J = 8.4 Hz; 2H, *m*-phenyl linker); 7.84 (m, 9H, *m*- and *p*-phenyl); 6.04 (t, $J_{H1'\text{-}HN}$ = 8.5 Hz, 1H, H-1′); 5.45 (t, 1H, $J_{H2'\text{-}H3'}$ = 9.5 Hz, 1H, H-3′); 5.15 (t, $J_{H2'\text{-}H3'}$ = 9.5 Hz, 1H, H-2′); 5.03 (t, $J_{H4'\text{-}H5'}$ = 9.7 Hz, 1H, H-4′); 4.27–4.13 (m, 2H, H-5′ e H-6a′); 4.07 (m, $J_{6a\text{-}6b}$ = 12.0 Hz, H-6b′); 2.09–1.99 (4s, 12H, CH_3); −2.89 (s, NH-pyrrole). **^{13}C NMR** (101 MHz, DMSO-d_6) δ (ppm)181.9 (C=S); 170.1; 169.9; 169.6; 169.4 (4C, C=O); 141.2; 138.7; 137.5; 135.2; 134.5; 131.4; 128.1; 127.0; 121.5; 120.0; 119.6 (44C, tetraarylporphyrin); 81.4; 72.9; 72.3, 70.6; 68.0; 61.8 (6C, sugar moiety); 20.6; 20.5; 20.4; 20.3 (4C, CH_3).UV–Vis ($CHCl_3$) λ_{max} (log ε) 419 (5.38), 516 (4.04), 551 (3.70), 590 (3.52), 646 (3.38) nm. IR (KBr):