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Note

Synthesis of water-soluble phthalocyanines bearing four or eight D-galactose units

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ABSTRACT

The synthesis and structural characterization of two glycophthalocyanines with four or eight unprotected D-galactose units is reported. The sugar units are linked to the macrocycle via the hydroxyl group located at C-6. The water solubility promoted by the carbohydrate moieties provides a potential application of these phthalocyanine derivatives as photosensitizers in photodynamic therapy.

The conjugation of molecules with different properties is a very attractive area of research. The building of multifunctional systems, such as carbohydrates with non-natural organic compounds (porphyrins or phthalocyanines, for instance) has been a recent focus of interest. Phthalocyanines (Pcs), besides their use as industrial dyes and pigments, have found a great technological interest,[1] and a prominent potential application in medicine as photosensitizers (PS) for photodynamic therapy (PDT) of cancer diseases.[2,3]

Phthalocyanines show intense red shifted Q-band in their UV–vis spectra and some of them have a high quantum yield of singlet oxygen generation. These properties make them more advantageous over other PS which have been used in PDT (e.g., porphyrin-related compounds).[3] However, a limitation of most Pc is their insolubility in physiological fluids. The syntheses of ionic and neutral water-soluble phthalocyanine derivatives have been described.[4,5]

The combination of carbohydrate moieties with macrocycles has attracted interest, mainly due to the specific recognition of several carbohydrates for cancer cells.[6,7] Furthermore, these carbohydrate units can provide water solubility to the macrocycles.

Countless examples of carbohydrate-substituted porphyrins have been described.[8] However, the corresponding phthalocyanine-carbohydrate conjugates are quite uncommon. The first synthesis of a phthalocyanine substituted with four D-glucofuranose moieties was reported by Momenteau.[9] Hanack and co-workers[10] have prepared 'symmetrically' glycosylated zinc(II) phthalocyanines at the anomeric carbon of the glycoside units and we[11] have described the synthesis of asymmetrical glycophthalocyanines with four D-galactose units linked to the same isoindolyl moiety. Recently, Ng and co-workers reported the synthesis of zinc(II) phthalocyanines bearing four isopropylidene protected D-glucose or D-galactose units linked by the 3- or 6-positions, respectively.[12] The synthesis of silicon(IV) phthalocyanine complexes axially substituted with D-galactose units has also been described.[13] Herein, we report the synthesis of two glycophthalocyanines **5** and **9** with four or eight D-galactose units, respectively. In both cases, the carbohydrate units are linked to the macrocycle by the hydroxyl group located in carbon C-6.

The synthetic route to glycophthalocyanine **5** is shown in Scheme 1. It involved the preparation of the glycophthalonitrile **3**, which was obtained by nucleophilic substitution starting from the 4-nitrophthalonitrile (**2**) and 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**1**). Tetramerization of **3** in *N*,*N*-dimethylaminoethanol (DMAE) at 140 °C, in the presence of $ZnCl_2$, afforded phthalocyanine **4** in 43% yield as a mixture of positional isomers. Subsequent treatment of **4** with aqueous trifluoroacetic acid (TFA) afforded the unprotected Pc **5** in 84% yield. It was purified by reverse phase column chromatography using a gradient of water and THF as the eluent. During the preparation of this manuscript, syntheses for the intermediate phthalonitrile **3** and phthalocyanine **4** were published.[12] The authors attempted to remove the isopropylidene protecting groups of the phthalocyanine **4** by treatment with 9:1

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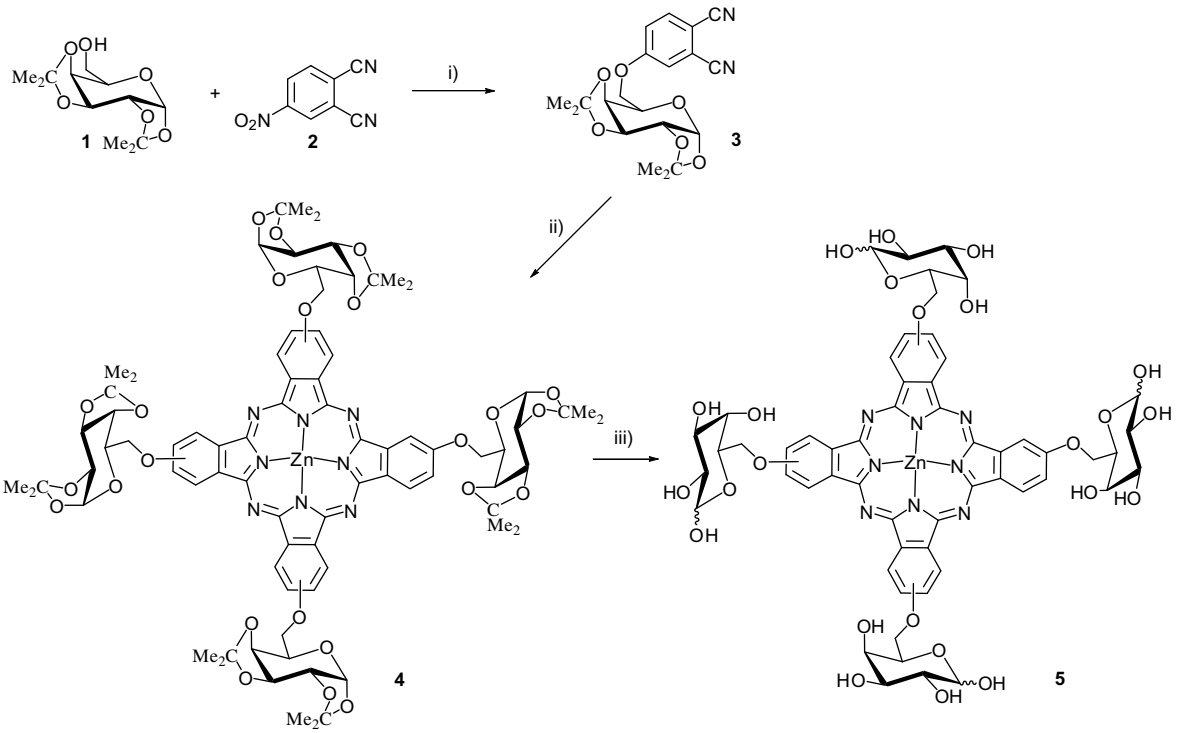


Scheme 1. (i) NaH, toluene, N_2, rt; (ii) $ZnCl_2$, DMAE, N_2, 140 °C; (iii) TFA/H_2O (9:1), rt.

TFA-water but were unable to purify the resulting phthalocyanine **5** either by chromatography or recrystallization. Therefore, the unprotected phthalocyanine **5**, the main target of this synthetic route, is reported here for the first time.

The glycophthalocyanine with eight D-galactose units **9** was prepared by a synthetic route similar to that described above. In this case, nucleophilic substitution of the bromine atoms of 4,5-bis(bromomethyl)phthalonitrile (**6**)[14] by D-galactopyranose units afforded glycophthalonitrile **7**. Phthalocyanine **8** was then obtained in 62% yield by the classical phthalocyanine template reaction. Finally, treatment of compound **8** with aqueous TFA gave the unprotected Pc **9** (Scheme 2).

The water-soluble phthalocyanines **5** and **9** were purified by reverse phase column chromatography using a gradient of H_2O/THF as the eluent.

The structures of all new compounds were confirmed by NMR and UV–vis spectroscopy and high resolution mass spectrometry (HRMS). The ^{1}H NMR spectra of **3** and **4** are identical to those already reported.[12] The ^{1}H NMR spectrum of phthalonitrile **7** shows three singlets between δ 1.4 and 1.6 ppm attributed to the resonances of the isopropylidene methyl groups. The signals due to the carbohydrate protons appear between δ 3.8 and 5.6 ppm. In the aromatic zone, only a singlet is observed at δ 8.0 ppm corresponding to the two equivalent aromatic protons.

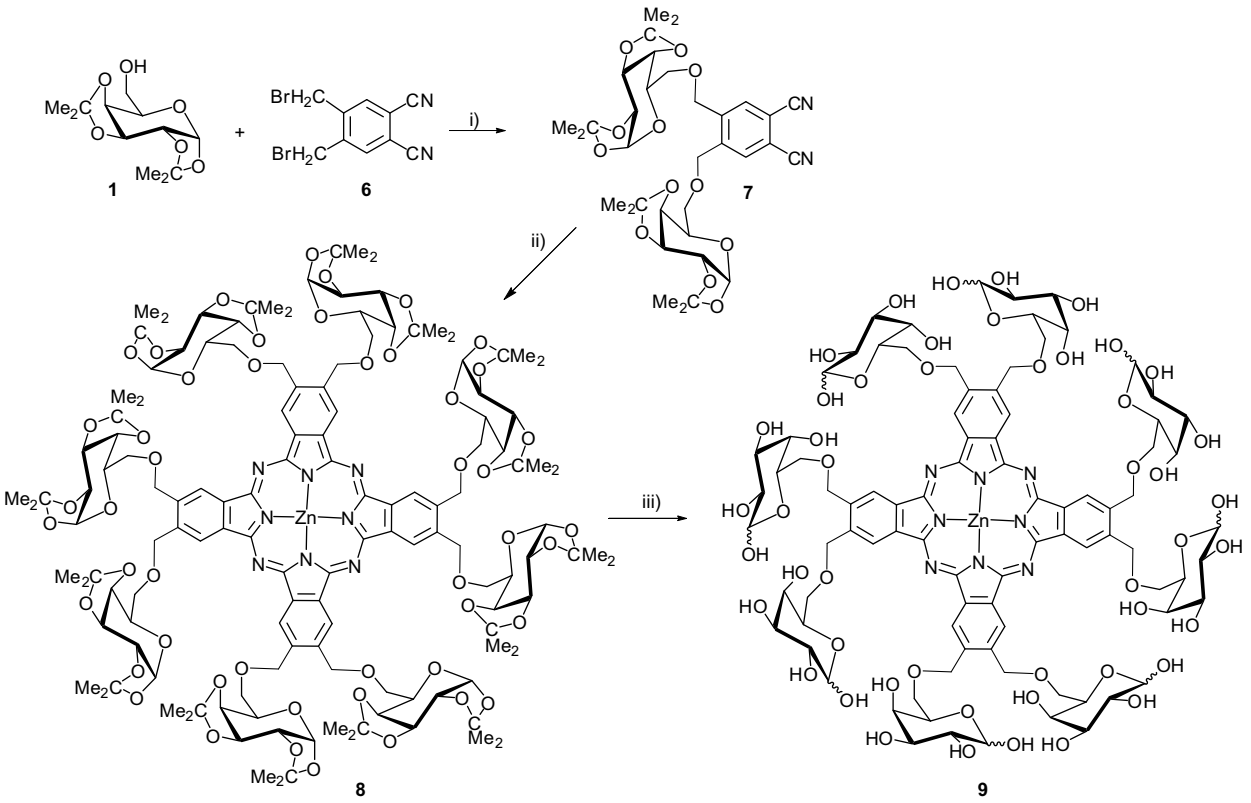
The ^{1}H NMR spectrum of Pc **5** in Me_2SO-d_6 is quite well defined although it is a mixture of isomers. The deprotection of the carbohydrate moieties is confirmed by the disappearance of the signals due to the isopropylidene protons. The resonances of the anomeric OH protons appear between δ 6.4 and 6.9 ppm as singlets (these signals disappear when D_2O is added). The multiplets at δ 3.4–5.4 ppm are due to the resonances of the carbohydrate units, while the phthalocyanine protons appear at lower field as a set of three multiplets, two multiplets between δ 8.5 and 9.1 ppm due to the resonances of the eight Pc-alpha protons and a multiplet at δ 7.6 and 7.8 ppm due to the four β-Pc protons.

In order to minimize the aggregation of the macrocycles, the ^{1}H NMR spectrum of Pc **8** was recorded in $CDCl_3$ in the presence of a trace amount of pyridine-d_5. The resonances due to the isopropylidene methyl groups appear as several singlets at δ 1.2–1.6 ppm. The signals between δ 4.0 and 5.6 ppm are due to the resonances of the carbohydrate and CH_2 protons, and the multiplet at lower field (between δ 9.2 and 9.7 ppm) is attributed to the resonances of the α-Pc protons.

The ^{1}H NMR spectrum of Pc **9** in Me_2SO-d_6 confirms the deprotection of the carbohydrate moieties showing no signals due to the isopropylidene protons. The signals corresponding to the carbohydrate units and CH_2 protons appear in the region δ 3.4–5.2 ppm. The resonances of the anomeric OH protons are also well distinguished from the other signals appearing between δ 6.3 and 6.7 ppm as singlets (confirmed by their disappearance when D_2O was added). The resonances of the eight α-Pc protons appear between δ 8.7 and 9.3 ppm.

The UV–vis spectra of the phthalocyanine derivatives **4**, **5**, **8** and **9** are characteristic for phthalocyanine zinc complexes, showing a Q-band maximum around 680 nm. The UV–vis spectra of Pc **5** and **9** in Me_2SO and in water are shown in Figure 1. In both cases, the intensity of the Q-band is much lower in water than in Me_2SO, thus suggesting intermolecular aggregation of these phthalocyanine derivatives. It is also shown that the number of the sugar moieties attached to the macrocycle affects significantly the solubility of these compounds in water. Pc **9** is much more soluble in water than Pc **5**.

In summary, we have prepared and characterized glycophthalocyanines with four or eight D-galactose units. The precursor glycophthalonitriles were prepared by nucleophilic substitution starting from 4-nitrophthalonitrile and 4,5-bis(bromomethyl)phthalonitrile, respectively. The incorporation of carbohydrate moieties at the periphery of the phthalocyanine macrocycle provides hydrophilicity to the new compounds, which is a useful parameter for drug administration. Furthermore, the specific affinity of carbohydrates for tumour tissues suggests good perspectives for the potential application of such compounds as photosensitizers in PDT.



Scheme 2. (i) NaH, toluene, N_2, 70 °C; (ii) $ZnCl_2$, DMAE, N_2, 140 °C; (iii) TFA/H_2O (9:1), rt.

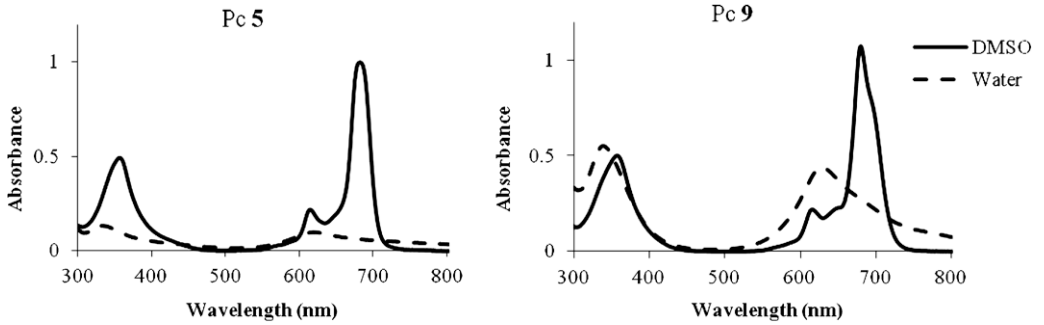


Figure 1. UV–vis spectra of Pc **5** and **9** in Me_2SO (–) and in water (- - -) at the same concentration (6 μM).

1. Experimental

1.1. General methods

^1H NMR spectra were recorded on a Bruker Avance 300 at 300.13 MHz using $CDCl_3$ or Me_2SO-d_6 as solvent and TMS as internal reference. HRMS were recorded on a Bruker REFLEX III instrument. The UV–vis spectra were recorded on a Uvikon spectrophotometer using $CHCl_3$ or Me_2SO as solvent. Reverse phase column chromatography was carried out using Sep-Pak® Vac 35 cc (10 g) columns. The starting reagents 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**1**) and 4-nitrophthalonitrile (**2**) were acquired from Sigma–Aldrich (Madrid).

1.2. [2,9(10),16(17),23(24)-Tetrakis(α/β-D-galactopyranos-6-*O*-yl)phthalocyaninato]zinc(II) (5)

A suspension of phthalocyanine **4**[12] (50 mg, 31.1×10^{-3} mmol) in 9:1 TFA–water (20 mL) was stirred at room temperature for 4 h. The mixture was neutralized with aqueous K_2CO_3 and the product was purified by a reverse phase column chromatography using a gradient of water and THF as the eluent. Compound **5** was precipitated with acetone, filtered, washed with acetone and dried under diminished pressure (34 mg, 84% yield). Mp > 300 °C; ^1H NMR (300.13 MHz; Me_2SO-d_6): δ 3.37–5.37 (m, 40 H, H-Gal), 6.41–6.85 (m, 4 H, Gal-OH α/β), 7.63–7.79 (m, 4 H, H_β-Pc), 8.49–8.70 (m, 4 H, H_α-Pc), 8.97-9.06 (m, 4 H, H_α-Pc); UV–vis (Me_2SO): λ_{max} (log ε) = 357 (4.9), 615 (4.5), 682 (5.2) nm; HRM-ALDI-TOFMS: *m/z* calcd for $C_{56}H_{56}N_8O_{24}Zn$ M^+: 1288.2693, found: 1288.2720.

1.3. 4,5-Bis(1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranos-6-*O*-ylmethyl)phthalonitrile (7)

D-Galactopyranose derivative **1** (1.7 g, 6.53 mmol) and NaH (0.15 g, 6.25 mmol) were stirred in dry toluene (16 mL) for 30 min, at 70 °C, under a N_2 atmosphere. Then, 4,5-bis(bromomethyl)phthalonitrile[14] (1.0 g, 3.18 mmol) was added and the mixture was stirred for 4 h. The reaction was cooled and neutralized with a saturated aqueous solution of citric acid. The organic layer

was diluted with CH_2Cl_2, washed with brine and water, dried (Na_2SO_4) and concentrated. The product was purified by column chromatography (silica gel) with a 3:1 mixture of hexane/ethyl acetate as the eluent. Phthalonitrile **7** was crystallized from CH_2Cl_2/MeOH affording a white solid (0.58 g, 27% yield). Mp 65–67 °C; ^{1}H NMR (300.13 MHz; $CDCl_3$): δ 1.35, 1.37, 1.45, 1.55 [4s, 24H, $C(CH_3)_2$], 3.75 (dd, *J* 6.2 and 10.0 Hz, 2H, H-6′a or H-6′b), 3.77 (dd, *J* 6.2 and 10.0 Hz, 2H, H-6′a or H-6′b), 4.04 (dt, *J* 1.4 and 6.2 Hz, 2H, H-5′), 4.28 (dd, *J* 1.4 and 7.9 Hz, 2H, H-4′), 4.34 (dd, *J* 2.3 and 5.0 Hz, 2H, H-2′), 4.57–4.71 (m, 6H, H-3′ and CH_2), 5.55 (d, *J* 5.0 Hz, 2H, H-1′), 7.97 (s, 2H, H-3); HRMALDI-TOF-MS: *m/z* calcd for $C_{34}H_{44}N_2O_{12}Na$ $(M+Na)^+$: 695.2787, found: 695.2772.

1.4. [2,3,9,10,16,17,23,24-Octakis(1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranos-6-*O*-ylmethyl) phthalocyaninato]zinc(II) (8)

Phthalonitrile **7** (489 mg, 0.72 mmol) and $ZnCl_2$ (50 mg, 0.37 mmol) in DMAE (3.0 mL) were stirred at 140 °C under argon atmosphere for 16 h. After being cooled to room temperature, the reaction mixture was precipitated with 1:3 MeOH–water, the solid was filtered, washed with water and MeOH and dried under diminished pressure. Compound **6** was purified by silica gel column chromatography using a 1:1 mixture of hexane/THF as the eluent. It was obtained as a dark blue solid (308 mg, 62% yield) after being washed with MeOH. Mp = 77–79 °C; ^{1}H NMR (300.13 MHz; $CDCl_3$ + Py-d_5): δ 1.18–1.64 (m, 96H, $C(CH_3)_2$), 4.01–4.66 (m, 48H, H-Gal), 5.17–5.29 (m, 16H, CH_2), 5.61–5.63 (m, 8H, H-1(α/β)-Gal); 9.20–9.70 (m, 8H, H_α-Pc); UV–vis ($CHCl_3$): λ_{max} ($\log\varepsilon$) = 351 (4.8), 613 (4.3), 680 (5.2) nm; HRMALDI-TOFMS: *m/z* calcd for $C_{136}H_{176}N_8O_{48}Zn$ M^+: 2753.0863, found: 2753.0813.

1.5. [2,3,9,10,16,17,23,24-Octakis(α/β-D-galactopyranos-6-*O*-ylmethyl)phthalocyaninato]zinc(II) (9)

Phthalocyanine **8** (50 mg, 18.3×10^{-3} mmol) in 9:1 TFA–water (12 mL) was stirred at room temperature for 4 h. The mixture was neutralized with aqueous K_2CO_3 and the product was purified by reverse phase column chromatography using a gradient of water and THF as the eluent. Compound **9** was precipitated with acetone, filtered, washed with acetone and dried under diminished pressure (34 mg, 87% yield). Mp > 300 °C; ^{1}H NMR (300.13 MHz; Me_2SO-d_6): δ 3.38–5.19 (m, 96H, H-Gal and CH_2), 6.28–6.70 (m, 8H, Gal-OH α/β), 8.70–9.29 (m, 8H, H_α-Pc); UV–vis (Me_2SO): λ_{max} ($\log\varepsilon$) = 357 (4.9), 616 (4.5), 680 (5.2) nm; MALDIMS: *m/z* calcd for $C_{88}H_{113}N_8O_{48}Zn$ $(M+H)^+$: 2112.6, found: 2112.4.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2008.12.009.

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