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ScienceDirect
Tetrahedron Letters
Tetrahedron Letters 47 (2006) 9177–9180

[1,2,3,4-Tetrakis(α/β-D-galactopyranos-6-yl)-phthalocyaninato]zinc(II): a water-soluble phthalocyanine

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Received 6 September 2006; revised 26 October 2006; accepted 27 October 2006

Abstract—A novel water-soluble asymmetrical sugar-phthalocyanine was prepared via a statistical cross-condensation of tetrakis(1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranos-6-yl)phthalonitrile with phthalonitrile. The new compound, with amphiphilic character, can be useful as a selective photosensitizer in photodynamic therapy, as well as for constructing phthalocyanine-based supramolecular systems.

1. Introduction

A great scientific interest has been focused on the synthesis of phthalocyanines (Pcs) due to several technological applications already found for this type of compounds.[1–4] One of the most promising applications is their use as photosensitizers in photodynamic therapy (PDT), an emerging treatment for a large variety of tumours and infectious diseases.[5–8]

Pcs have adequate photophysical features to be used as photosensitizers in PDT, such as strong absorption bands in the 600–800 nm region and efficient singlet oxygen production.[9,10] However, a serious limitation of Pcs is their insolubility in physiological fluids, requiring usually the use of difficult formulations,[11] such as incorporation into liposomes, biopolymers or cyclodextrins.[12,13]

Water-soluble anionic sulfonated phthalocyanines have received great attention with regard to photodynamic efficacy,[14] but the purification of these compounds can be a problem and the final products are typically mixtures of several sulfonated derivatives. Furthermore, these compounds have been observed to aggregate at relatively low concentrations in aqueous media, which results in loss of the photosensitizing ability. Cationic phthalocyanine photosensitizers[15,16] have also not found a broader application in PDT.

The design of molecules that can target specific cells is an important goal in the development of new drugs for PDT. This goal can be reached by incorporating selected biological subunits on the photosensitizer. Studies with a range of porphyrin–carbohydrate conjugates have shown that this type of compounds are efficient photosensitizers in PDT.[7,17] Similarly, we expect that the conjugation of phthalocyanines with carbohydrate residues may increase their water solubility, avoiding the use of a delivery system to tumour cells, and also to provide better tumour specificity.[18,19]

Recently, we described the synthesis of covalently linked β-cyclodextrin-Pcs dyads, which corresponds to a new methodology to obtain stable water solutions of neutral Pcs.[20] Maillard et al.[21] and Hanack and co-workers[22] have described the preparation of water-soluble 'symmetrically' substituted phthalocyanines with four D-glucose units. In these two cases, each D-glucose unit is linked to a different isoindolyl group by the hydroxyl group located in the C-3 or C-1 carbons, respectively. Here, we describe the synthesis of asymmetrical

Keywords: Water-soluble phthalocyanines; Carbohydrates; Photosensitizers; PDT.
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doi:10.1016/j.tetlet.2006.10.155

glycophthalocyanines with four D-galactose units linked to the same isoindolyl group by the hydroxyl group located in carbon C-6. While in the two previous examples the glycophthalocyanines are obtained as mixtures of positional isomers, in our case a single isomer is formed.

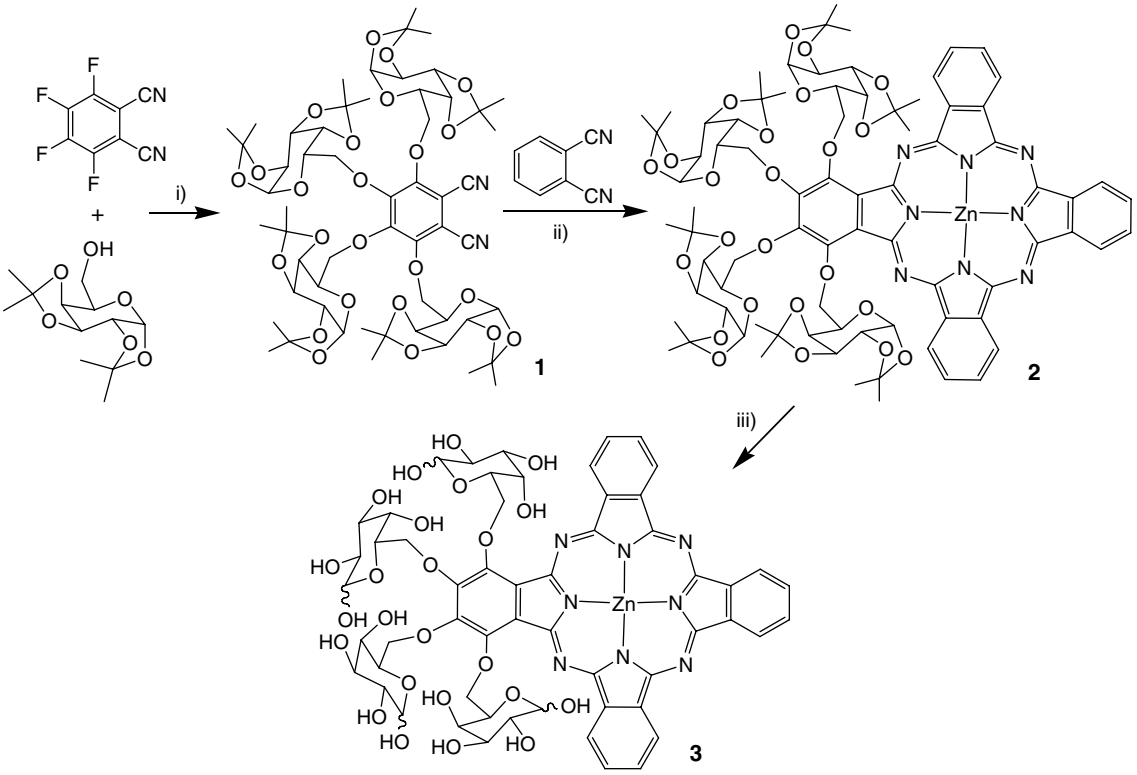
Our synthetic methodology is presented in Scheme 1. It involves, as the first step, the preparation of glycophthalonitrile **1**.[23] This phthalonitrile was obtained by nucleophilic substitution of the four fluorine atoms of tetrafluorophthalonitrile by four 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose units. Phthalocyanine **2** was then prepared by statistical cross-condensation of glycophthalonitrile **1** with an excess of 1,2-dicyanobenzene, in the presence of zinc chloride.[24] The reaction was carried out in refluxing *N*,*N*-dimethylaminoethanol (DMAE), affording the desired Pc **2** and the symmetric zinc Pc formed by self-condensation of phthalonitrile. The desired product was purified by silica gel column chromatography using a gradient of petroleum ether/THF as the eluent. The removal of the carbohydrate protection groups in compound **2** was performed with aqueous TFA at room temperature.[25] The water-soluble Pc **3** was purified by reverse-phase column chromatography using a gradient of H_2O/THF as the eluent.

The structures of products **1**–**3** were confirmed by NMR spectroscopy, UV–vis and HRMS-MALDI-TOF. The ^{1}H NMR spectrum of **1** shows a multiplet at 1.3–1.6 ppm due to the resonances of the isopropylidene methyl groups and five multiplets from 4.1 to 5.5 ppm attributed to the resonances of the protons of the carbohydrate units. No signals are observed in the ^{19}F NMR spectrum of **1**, confirming the substitution of all fluorine atoms by the monosaccharide units.

The ^{1}H NMR spectrum of compound **2** shows two distinct regions: the signals at lower field (between δ 7.8 and 9.0 ppm) are due to the protons of the Pc moiety, while the signals at higher field (between 1.2 and 5.6 ppm) are due to the protons of the carbohydrate units. The resonances due to the isopropylidene protons appear between 1.2 and 1.8 ppm and the ones due to the other protons of the carbohydrate units appear between 4.4 and 5.6 ppm.

The ^{1}H NMR spectrum of compound **3** in DMSO-d_6 is well-resolved and confirms the deprotection of the carbohydrate moieties (disappearance of the signals due to the isopropylidene protons). It shows two multiplets between 9.41 and 9.70 ppm due to the resonances of the six Pc-alpha protons and a broad singlet at 8.25 ppm due to the six Pc-beta protons. The signals corresponding to proton H-1 of the galactosyl moieties (in alpha and beta configurations) appear between 6.20 and 6.85 ppm, and the resonances of the remaining galactosyl protons appear between 3.51 and 5.45 ppm.

High resolution MS spectra (MALDI-TOF)[26] of compounds **1**–**3** provided a definitive proof for their characterization. In the case of the phthalonitrile derivative **1**, NaI was added for improving ionization results. In compounds **2** and **3**, a better ionization took place in the absence of NaI. Peaks corresponding to the molecular ions of compounds **2** and **3** were detected but, as expected,



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

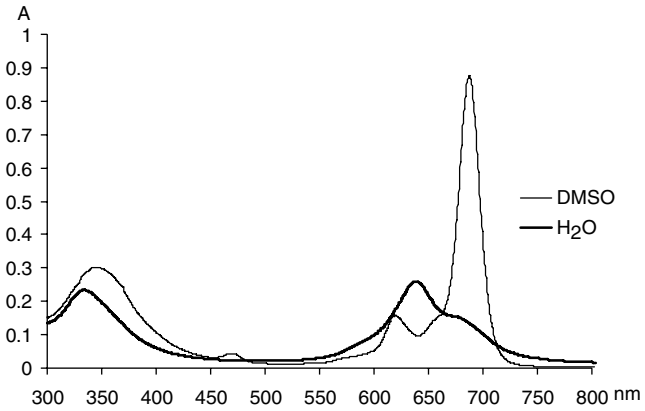


Figure 1. UV–vis spectra of Pc **3** in DMSO (—) and in H_2O (▬). Both solutions are 10 μM.

complex isotopic distributions were observed. The corresponding monoisotopic peak was selected for comparison with the standard.

Figure 1 shows the UV–vis spectra of dyad **3** in DMSO and in water. The spectrum in DMSO is well-defined, showing no intermolecular aggregation. The sharp Q-band at 687 nm indicates monomeric species in solution. However, the optical features of this compound in water differ remarkably from those in DMSO. In water, the intensity of the Q-band is much lower than in DMSO, indicating aggregation due to cofacial arrangement of the Pcs.[27a,b] The B-band is slightly shifted to shorter wavelength (332 nm), whereas the Q-band is also blue-shifted and split into two main absorptions at 638 and 670–680 nm. The solubility of compound **3** in water was determined as being 3.2 mg/mL.

2. Outlook

An easy methodology to access water-soluble phthalocyanines has been described. The asymmetric structure of the new water-soluble Pc **3** provides an amphiphilic character useful for drug administration (hydrophilicity) and transport through the organism (lipophilicity).[28] In addition, considering the specific affinity of carbohydrates for cancer cells and their strong influence on the bioavailability of the corresponding conjugates, good perspectives can be anticipated for this new generation of photosensitizers based on Pc-carbohydrate derivatives. Galactosyl-phthalonitrile **1** can also be used as *synthon* for preparing water-soluble nonlinear optically active subphthalocyanines,[29] as well as novel amphiphilic phthalocyanines with potential application in the construction of Pc-based nanostructures.[30]

Acknowledgements

Thanks are due to CAPES and CNPq—Brazil, to Fundação para a Ciência e a Tecnologia (Portugal) and FEDER for funding the Organic Chemistry Research Unit of the University of Aveiro, and Ministerio de Educación y Ciencia, Spain (Grant CTQ 2005-08933 BQU), for funding. Anderson O. Ribeiro thanks CAPES and CNPq and João P. C. Tomé thanks FCT for their postdoctoral grants.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2006.10.155.

References and notes

1. Kadish, K. M.; Smith, K. M.; Guilard, R. In *The Porphyrin Handbook*; Academic Press: San Diego, 2003; Vols. 15–20.
2. McKeown, N. B. *Phthalocyanine Materials: Synthesis, Structure and Function*; Cambridge University Press: Cambridge, 1998.
3. Leznoff, C. C.; Lever, A. B. P. *Phthalocyanines: Properties and Applications*; VCH: Weinheim, 1989, 1993, 1996; Vols. 1–4.
4. (a) de la Torre, G.; Vázquez, P.; Agulló-López, F.; Torres, T. *Chem. Rev.* **2004**, *104*, 3723; (b) de la Torre, G.; Vázquez, P.; Agulló-López, F.; Torres, T. *J. Mater. Chem.* **1998**, *8*, 1671.
5. Bonnett, R. *Chemical Aspects of Photodynamic Therapy*; Gordon and Breach Science: London, 2000.
6. DeRosa, M. C.; Crutchely, R. J. *Coord. Chem. Rev.* **2002**, *233*, 351.
7. Tomé, J. P. C.; Neves, M. G. P. M. S.; Tomé, A. C.; Cavaleiro, J. A. S.; Mendonça, A. F.; Pegado, I. N.; Duarte, R.; Valdeira, M. L. *Bioorg. Med. Chem.* **2005**, *13*, 3878.
8. Tomé, J. P. C.; Neves, M. G. P. M. S.; Tomé, A. C.; Cavaleiro, J. A. S.; Soncin, M.; Magaraggia, M.; Ferro, S.; Jori, G. *J. Med. Chem.* **2004**, *47*, 6649.
9. Bonnet, R.; Martinez, G. *Tetrahedron* **2001**, *57*, 9513.
10. Macdonald, I. J.; Dougherty, T. J. *J. Porphyrins Phthalocyanines* **2001**, *5*, 105.
11. Allen, C. M.; Sharman, W. M.; Van Lier, J. E. *J. Porphyrins Phthalocyanines* **2001**, *5*, 161.
12. Nyman, E. S.; Hynninen, P. H. *J. Photochem. Photobiol. B: Biol.* **2004**, *73*, 1.
13. Lang, K.; Mosinger, J.; Wagnerová, D. M. *Coord. Chem. Rev.* **2004**, *248*, 321.
14. Ogunsipe, A.; Nyokong, T. *J. Photochem. Photobiol. A: Chem.* **2005**, *173*, 211.
15. Filippis, M. P.; Dei, D.; Fantetti, L.; Roncucci, G. *Tetrahedron Lett.* **2000**, *41*, 9143.
16. Dei, D.; Chiti, G.; De Filippis, M. P.; Fantetti, L.; Giuliani, F.; Giuntini, F.; Soncin, M.; Jori, J.; Roncucci, G. *J. Porphyrins Phthalocyanines* **2006**, *10*, 147.
17. See, for instance: (a) Sylvain, I.; Zerrouki, R.; Granet, R.; Huang, Y. M.; Lagorce, J.-F.; Guilloton, M.; Blais, J.-C.; Krausz, P. *Bioorg. Med. Chem.* **2002**, *10*, 57; (b) Laville, I.; Figueiredo, T.; Loock, B.; Pigaglio, S.; Maillard, Ph.; Grierson, D. S.; Carrez, D.; Croisy, D.; Blais, J. *Bioorg. Med. Chem.* **2003**, *11*, 1643.
18. Sharon, N.; Lis, H. *Science* **1989**, *246*, 227.
19. Dwek, R. A. *Chem. Rev.* **1996**, *96*, 683.
20. Ribeiro, A. O.; Tomé, J. P. C.; Neves, M. G. P. M. S.; Tomé, A. C.; Cavaleiro, J. A. S.; Serra, O. A.; Torres, T. *Tetrahedron Lett.* **2006**, *47*, 6129.

21. Maillard, P.; Guerquin-Kern, J.-L.; Momenteau, M. S.; Gaspard, S. *J. Am. Chem. Soc.* **1989**, *111*, 9125.
22. Alvarez-Mico, X.; Calvete, M. J. F.; Hanack, M.; Ziegler, T. *Tetrahedron Lett.* **2006**, *47*, 3283.
23. Tetrakis(1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranos-6-yl)phthalonitrile, **1**. 1,2:3,4-Di-*O*-isopropylidene-α-D-galactopyranose (2.1 g, 8.0 mmol) and sodium hydride (0.2 g, 8.3 mmol) were stirred in dry toluene (3 mL) for 1 h, at 70 °C, under a N_2 atmosphere. Then, tetrafluorophthalonitrile (0.2 g, 1.0 mmol) was added and the mixture was stirred for 12 h. The reaction was cooled, a saturated aqueous solution of citric acid (3 mL) was added and the mixture was stirred for 5 min. The organic layer was diluted with CH_2Cl_2, washed with water, dried (Na_2SO_4) and concentrated. The product was purified by silica gel column chromatography starting with CH_2Cl_2 as the eluent to elute the sugar used in excess. Phthalonitrile **1** was then eluted with CH_2Cl_2/MeOH (90:10) and crystallized from CH_2Cl_2/petroleum ether (colourless powder, 0.45 g, 39% yield). Mp = 108–110 °C; ^{1}H NMR ($CDCl_3$) δ: 5.50 (m, 4H, H-1 anomeric), 4.65–4.55 (m, 4H, H-2), 4.40–4.50 (m, 4H, H-5), 4.25–4.35 (m, 8H, H-3 and 4), 4.10–4.20 (m, 8H, H-$6_{a,b}$), 1.3–1.6 (m, 48H, C(CH_3)$_2$). ^{13}C NMR ($CDCl_3$) δ: (sugar): 24.3, 24.9, 25.9, 66.5, 70.3, 70.6, 70.9, 73.5, 103.8 (C-1 anomeric), 108.6, 109.2 (*C*(CH_3)$_2$); (phthalonitrile): 96.2 (*C*–CN), 113.1 (CN), 149.9 and 152.2 (C-3,4,5,6); HRMS (MALDI-TOF, PEG+NaI): *m*/*z* $(M+Na)^+$ ($C_{56}H_{76}N_2O_{24}Na$). Calculated (monoisotopic peak): 1183.4680. Found: 1183.4692.
24. [1,2,3,4-Tetrakis(1,2:3,4-di-*O*-isopropylidene-α-D-galacto-pyranos-6-yl)phthalocyaninato]zinc(II), **2**. Phthalonitrile **1** (0.15 g, 0.13 mmol), 1,2-dicyanobenzene (0.18 g, 1.4 mmol) and $ZnCl_2$ (0.2 g, 1.4 mmol) in DMAE (3 mL) were stirred at 100 °C under a N_2 atmosphere for 12 h. The product was pre-purified by a Celite column and then it was purified by a silica gel column chromatography (THF/petroleum ether, 90:10). The eluent was evaporated to dryness under reduced pressure, the solid was dissolved in CH_2Cl_2 and precipitated out with petroleum ether affording 60 mg (30% yield). Mp >300 °C; ^{1}H NMR ($CDCl_3$) δ: 9.10–8.80 and 8.45–8.20 (2m, 2H and 4H, Pc-Hα), 7.75 (m, 6H, Pc-Hβ), 5.62 (d, $J = 4.5$ Hz, 4H, H-1 anomeric), 5.05–5.15 (m, 4H, H-2), 4.75–4.79 (m, 12H, H-3,4,5), 4.40–4.45 (m, 8H, H-6), 1.24–1.84 (m, 48H, C(CH_3)$_2$); ^{13}C NMR ($CDCl_3$) δ: (sugar): 24.5, 25.1, 26.8, 30.9 (C(CH_3)$_2$), 67.5, 70.6, 73.2, 76.5, 77.4, 96.0, 109.1; (Pc): 121.3, 122, 112.7, 125.0, 128.0, 128.5, 137.3, 137.8, 145.4, 147.7, 151.6, 152.1, 152.4, 152.8, 207.2; UV–vis ($CHCl_3$): λ_{max} ($\log\varepsilon$) = 687 (4.93), 630 (4.24), 343 (4.56) nm; HRMS (MALDI-TOF, PEG): *m*/*z* $(M+H)^+$ ($C_{80}H_{89}N_8O_{24}Zn$). Calculated (monoisotopic peak): 1609.5276. Found: 1609.5278.
25. [1,2,3,4-Tetrakis(α/β-D-galactopyranosyl)phthalocyaninato]-zinc(II), **3**. Pc **2** (10 mg, 6.2 μmol) in TFA and H_2O (9:1) (4 mL) was stirred in the dark at room temperature for 4 h. The mixture was neutralized with aqueous Na_2CO_3 and the product was purified by a reverse phase column chromatography using a gradient of H_2O/THF as eluent. The solvent was evaporated to dryness under reduced pressure and phthalocyanine **3** was washed with acetone (6.8 mg, 85% yield). Mp >300 °C; ^{1}H NMR (DMSO-d_6) δ: 9.70–9.45 and 9.43–9.41 (2m, 2H and 4H, Pc-Hα), 8.25 (s, 6H, Pc-Hβ), 6.85–6.20 (m, 4H, Gal-Hα and β), 5.45–3.51 (m, 40H, Gal-H and OH); UV–vis (DMSO): λ_{max} ($\log\varepsilon$) = 688 (4.94), 620 (4.20), 345 (4.48) nm; UV–vis (H_2O): λ_{max} ($\log\varepsilon$) = 631 (4.41), 333 (4.37) nm; HRMS (MALDI-TOF, PEG): *m*/*z* M^+ ($C_{56}H_{56}N_8O_{24}Zn$): Calculated (monoisotopic peak): 1288.2693. Found: 1288.2689.
26. High resolution MS-MALDI-TOF spectra were taken in a MALDI TOF-TOF instrument (Applied Biosystems 4700 Proteomic) using PEG (MW 1000 and 1500) as an internal standard.
27. (a) Tau, P.; Ogunsipe, A. O.; Maree, S.; Mearee, M. D.; Nyokong, T. *J. Porphyrins Phthalocyanines* **2003**, *7*, 439; (b) Snow, A. W. In *The Porphyrin Handbook*; Kadish, K. M., Smith, K. M., Guillard, R., Eds.; Academic Press: San Diego, 2003; Vol. 17, pp 129–176.
28. Shrman, W. M.; Allen, C. M.; van Lier, J. E. *Adv. Drug Deliv. Rev.* **2004**, *56*, 53.
29. (a) del Rey, B.; Keller, U.; Torres, T.; Rojo, G.; Agulló-López, F.; Nonell, S.; Marti, C.; Brasselet, S.; Ledoux, I.; Zyss, J. *J. Am. Chem. Soc.* **1998**, *120*, 12808; (b) Claessens, C. G.; González-Rodríguez, D.; Torres, T. *Chem. Rev.* **2002**, *102*, 835.
30. (a) de la Escosura, A.; Martínez-Díaz, M. V.; Thordarson, P.; Rowan, A. E.; Nolte, R. J. M.; Torres, T. *J. Am. Chem. Soc.* **2003**, *125*, 12300; (b) Guldi, D. M.; Gouloumis, A.; Vázquez, P.; Torres, T.; Georgakilas, V.; Prato, M. *J. Am. Chem. Soc.* **2005**, *127*, 5811.