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Synthesis of an octasubstituted galactose zinc(II) phthalocyanine

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ABSTRACT

4,5-Bis(1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranos-6-yl)phthalonitrile (**3**) was prepared by S_NAr reaction of diacetone galactose **1** and 4,5-difluorophthalonitrile (**2**) in 96% yield. Cyclotetramerization of **3** was achieved via its isoindoline derivative **4**, affording the peripherally octasubstituted galactose zinc(II) phthalocyanine **5** in 29% yield. Deprotection of **5** gave the highly water soluble octasubstituted galactose zinc(II) phthalocyanine **6** in 81% yield which will be applied as a photosensitizer in photodynamic therapy.

Porphyrins and phthalocyanines (Pc's) have been widely used in photodynamic therapy (PDT)[1–6] due to their ability to generate singlet oxygen via transfer of energy from the triplet excited state of the porphyrinoid to the triplet ground state of oxygen.[7] However, the low solubility of Pc's in physiological fluids significantly limits their applications in PDT. Likewise, Pc's show a low and rather unspecific uptake by tumour cells due to their poor solubility in water.[8–10] Water soluble anionic sulfonated Pc's, on the other hand, tend to aggregate in water resulting in a loss of their photosensitizing ability and making them less suitable for PDT.[11] Similarly, water soluble cationic Pc's also did not find a broader application in PDT so far.[12,13]

Conjugates of carbohydrates and porphyrins or Pc's are considered to be suitable for solving these persistent problems regarding poor cellular uptake, low tumour selectivity and reduced photosensitizing ability. Therefore, various porphyrin–carbohydrate conjugates have been synthesized by several research groups, assuming that the presence of the carbohydrate moiety might improve the membrane interaction of such conjugates, and thus, also increase their tumour selectivity when applied in PDT.[14–18] In contrast, phthalocyanine–carbohydrate conjugates are less common, despite their great potential in PDT.[19–23]

Recently, we have described for the first time a zinc(II) phthalocyanine peripherally tetra-glucosylated.[24] This glucosylated Pc exhibited a high solubility in water, the primary prerequisite to potential biological applications. A crucial step in the synthesis of such peripherally glycolsylated Pc's was the efficient accessibility of their precursors, that is, glycosylated phthalonitriles, for which we developed a new glycosidation protocol via S_NAr reaction of nitro-phthalonitriles with anomerically unprotected glycopyranoses and 1-thio-glycopyranoses.[25] Thus, a series of Pc's peripherally tetra-glycosylated with D-glucopyranose, 1-thio-β-D-glucopyranose, β-D-galactopyranose, 1-thio-β-D-cello- and lactobiose could be synthesized (Fig. 1).[26]

Preliminary cell uptake studies performed with WISH cells, a transformed human amniotic epithelial cancer cell line, point towards a therapeutic potential of the respective phthalocyanine derivative as possible lead compounds for PDT. A major drawback of the glycosylated Pc's we have prepared so far, however, is the fact that these Pc's were obtained as mixtures of constitutional isomers which are difficult to separate.

In order to circumvent the formation of constitutional isomers upon tetramerization of mono-glycosylated phthalonitriles, we now prepared symmetrically 4,5-di-glycosylated phthalonitriles which afford constitutional uniform octa-glycosylated Pc's.

Here, we describe as a first example the synthesis of a Pc symmetrically octasubstituted with D-galactose residues as outlined in Figure 2.

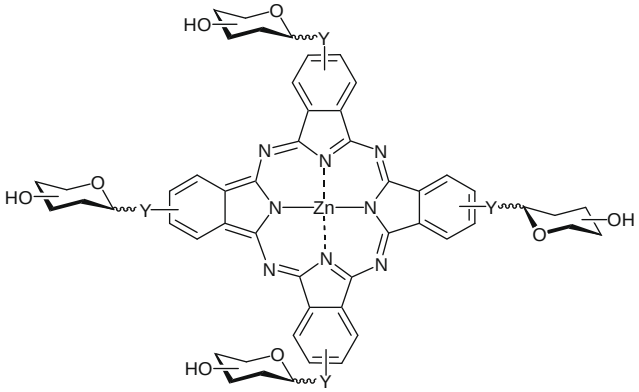

Figure 1. Peripherally tetra-glycosylated zinc(II) phthalocyanines. The glycosyl moieties represent glucose, galactose, cellobiose and lactose residues. Y = O or S.

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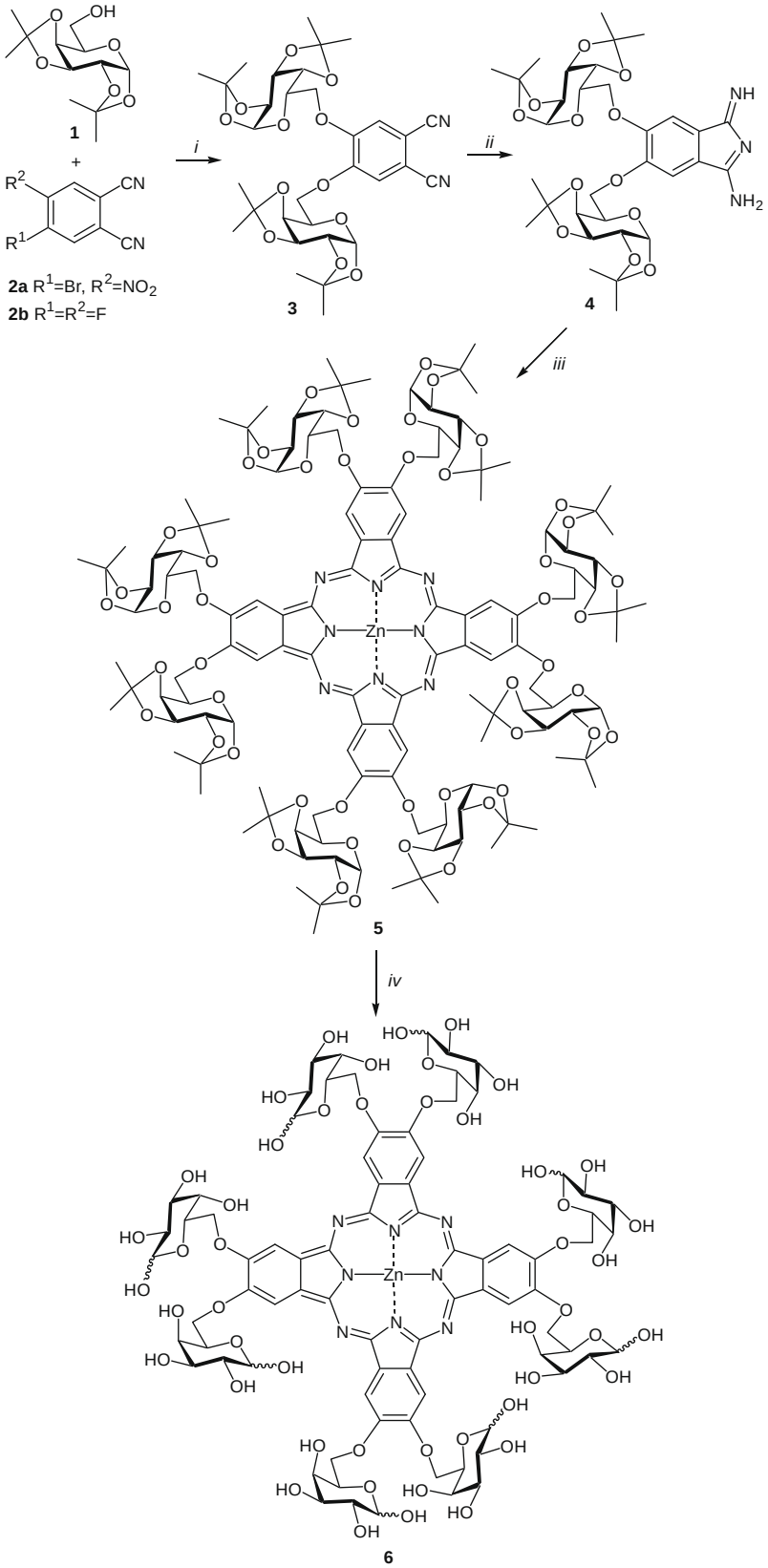

Figure 2. Reagents and conditions: (i) **1** + **2b**, DMF or DMAC, NaH, 35–40 °C, 48 h, 96% **3**; (ii) **3**, THF/MeOH, NH_3, $NaOCH_3$, 0 °C to rt, 1 h, reflux, 1 h, 95% **4**; (iii) **4**, DMAE, $Zn(AcO)_2{\cdot}2H_2O$, 24 h, reflux, 29% **5**; (iv) **5**, TFA/H_2O, rt, 4 h, 81% **6**.

As sugar moiety, we chose 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**1**) which is easily available from galactose,[27] and which was planned to be attached to the macrocycle via its position 6. In our earlier reported tetraglycosylated Pc's, the sugar moieties were anomerically attached to the Pc macrocycle (see Fig. 1). Here, however, we changed the attachment mode of the galactose residue from the anomeric position to position 6 because we anticipated that a better recognition by the cancer cells in PDT

can be achieved this way. Previously, glycosylated phthalonitriles were prepared from 4-nitrophthalonitrile by nucleophilic displacement of nitrite with a partially protected glycopyranose.[24–26] However, attempts to synthesize 4,5-dinitrophthalonitrile starting from 1,2-dibromo-4,5-dinitrobenzene under Rosenmund–von-Braun reaction conditions were unsuccessful. 4-Bromo-5-nitrophthalonitrile (**2a**) which was previously used for the preparation of 4,5-disubstituted phthalonitriles also could not be efficiently disubstituted with **1**.[28] Reaction of **1** with **2a** using K_2CO_3 or NaH in DMF predominately resulted in the substitution of bromo group. The disubstituted product **3** was only formed in a small amount. Wherefore, diacetone galactose **1** was instead reacted with 4,5-difluorophthalonitrile (**2b**).[29] Under optimized conditions (dimethyl acetamide or DMF, NaH, 35–40 °C, 48 h), the S_NAr reaction proceeded smoothly and gave the bis-galactosylated phthalonitrile **3** in a virtually quantitative yield.[30]

Next, attempts to cyclize compound **3** under various conditions which had been previously found[24–26] suitable failed. Heating **3** in various solvents in the presence of $Zn(OAc)_2$ or $ZnCl_2$ did not alter the starting material. Therefore, phthalonitrile **3** was first converted into the isoindoline **4** by bubbling gaseous NH_3 through a refluxing solution of **3** in THF/MeOH (1:1). Thus, obtained crude isoindoline **4** (95%)[31] was pure enough for the subsequent cyclization step. Refluxing a solution of **4** and $Zn(AcO)_2{\cdot}2H_2O$ in DMAE for 24 h and purification of the obtained product by chromatography afforded Pc **5** in 29% yield as a green amorphous solid (method A)[32] Alternatively, heating **4** and $Zn(AcO)_2{\cdot}2H_2O$ without a solvent in a sealed glass tube for 15 h also yielded **5** in 30% yield (method B). Since compound **5** is sensitive to light, all experimental manipulations were performed in the dark. The NMR spectra of **5** showed only one doublet for H-1 of the 8 galactose moieties. This indicates that the galactoses in **5** are not sterically hindered and can freely rotate at room temperature. Deprotection of the 16 isopropylidene groups was achieved by treating **5** with aqueous trifluoroacetic acid (TFA). Final purification by reverse phase HPLC afforded the fully deprotected octasubstituted Pc **6** in 81% yield.[33]

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30. 4,5-Bis(1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranos-6-yl)phthalonitrile (**3**). NaH (800 mg, 20 mmol) was added to a mixture of 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**1**) (5.2 g, 20 mmol) and 3,4-difluorophthalonitrile (**2b**) (1.64 g, 10 mmol) in 50 ml DMAC or DMF, and the reaction mixture was heated at 35–40 °C for two days. The mixture was cooled to room temperature, poured into water, and the precipitate was collected by filtration and dried in vacuo. Purification of the crude product was carried out by column chromatography on silica gel using toluene/acetone (5:1) as eluent to afford **3** (6.2 g, 96%) as a crystalline colourless solid. Mp: 198 °C. $[\alpha]_D^{20}$ −138.5 (*c*, 0.8, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.17 (s, 2H, H–Ar), 5.51 (d, $J_{1,2}$ = 5.0 Hz, 2H, H-1, 1′), 4.63 (dd, $J_{2,3}$ = 2.5 Hz, $J_{3,4}$ = 7.9 Hz 2H, H-3,3′), 4.37 (br d, $J_{4,5}$ < 1.0 Hz 2H, H-4,4′), 4.32 (dd, 2H, H-2,2′), 4.20–4.13 (m, 6H, H-5,5′, H-6a,b,6a′,b′), 1.49 (s, 6H, CH_3), 1.42 (s, 6H, CH_3), 1.32 (s, 12H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 152.0 (2C, C^{Ar}–O), 116.7 (2C, C^{Ar}–H), 115.5 (2C, CN), 109.4 (2C, C^{Ar}–CN), 108.8 (2C, $C(CH_3)_2$), 108.6 (2C, $C(CH_3)_2$), 96.1 (2C, C-1,1′), 70.4, 70.3, 68.1, 66.1 (4C, 2C, 2C, 2C, C-2,2′,3,3′,4,4′,5,5′,6,6′), 25.8, 25.7, 24.8, 24.1 (2C, 2C, 2C, 2C, CH_3); IR (KBr): 2987, 2933, 2231, 1590, 1519, 1370, 1212, 1115, 1003, 920, 536 cm^{-1}; FTICR MS *m/z* 644 $[M]^+$; Anal. Calcd for $C_{32}H_{40}N_2O_{12}$: C, 59.62; H, 6.25; N, 4.35. Found: C, 59.78; H, 6.32; N, 4.10.
31. 4,5-Bis(1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranos-6-yl)isoindoline (**4**). NaOMe (502 mg, 9.3 mmol) was added to 40 ml of a 1:1 mixture of dry THF and MeOH. The mixture was cooled to 0 °C, and gaseous NH_3 was passed through the mixture. Phthalonitrile **3** (6.0 g, 9.3 mmol) was added and the mixture was kept at 0 °C under a stream of NH_3 for 0.5 h, followed by stirring at 20 °C for 1 h and finally refluxing for 1 h. After the reaction mixture was allowed to cool to room temperature, the flow of NH_3 was stopped and the reaction mixture was poured into water. The precipitate was filtered off, washed with water, and small portions of MeOH and dichloromethane. Dried in vacuo to afford crude **4** (5.9 g, 95%), which was sufficiently pure for the next step. ^{1}H NMR (400 MHz, $CDCl_3$, significant signals): δ 7.49 (br s, 2H, H–Ar), 5.51 (d, $J_{1,2}$ = 5.0 Hz, 2H, H-1, 1′), 4.62 (br dd, $J_{3,4}$ = 8.0 Hz 2H, H-3,3′), 4.43 (br d, $J_{4,5}$ < 1.0 Hz 2H, H-4,4′), 4.30 (dd, 2H, H-2,2′), 4.20–4.16 (m, 6H, H-5,5′, H-6a,b,6a′,b′), 3.75–3.59 (m, 3H, NH); 1.45, 1.42, 1.31, 1.30 (4s, 24H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, significant signals): δ 152.7 (2C, C^{Ar}–O), 109.8 (2C, $C(CH_3)_2$), 109.1 (2C, $C(CH_3)_2$), 96.7 (2C, C-1,1′), 26.3, 25.4, 24.7, 23.5 (2C, 2C, 2C, 2C, CH_3); LC–MS: *m/z* 684 $[M+Na]^+$.
32. [2,3,9,10,16,17,24,25-Octakis(1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranos-6-yl)phthalocyaninato]zinc(II) (**5**). Method A: Isoindoline **4** (661 mg, 1 mmol) and $Zn(AcO)_2{\cdot}2H_2O$ (110 mg, 0.5 mmol) were refluxed in 1 ml DMAE for 24 h. The reaction mixture was cooled to room temperature and poured into 50% aqueous MeOH. The precipitate was filtered off and dried in vacuo. Chromatography on silica gel with *n*-hexane/ethyl acetate (1:1) mixture to remove side products, followed by ethyl acetate to elute the product afforded **5** (196 mg, 29%). Method B: **4** (661 mg, 1 mmol) and $Zn(AcO)_2{\cdot}2H_2O$ (220 mg, 1 mmol) were mixed in a mortar and filled into sealed glass tube. The tube was heated at 140–145 °C overnight. Chromatography of the solid material from the tube as described in method A afforded **5** (198 mg, 30%). $[\alpha]_D^{20}$ −160 (*c*, 0.05, $CHCl_3$). ^{1}H NMR (400 MHz, DMSO-d_6, significant signals): δ 7.16 (br s, 8H, H–Ar), 5.66 (d, $J_{1,2}$ = 4.8 Hz, 8H, H-1), 4.76–4.43 (m, 48 H, H-2,3,4,5,6), 1.53–1.36 (m, 96 H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6, significant signals): δ 109.8 (8C, $C(CH_3)_2$), 109.1 (8C, $C(CH_3)_2$), 96.9, 96.7 (8C, C-1,1′), 26.5, 26.3, 25.5, 25.3, 24.8, 24.7 (32C, CH_3); IR (KBr): 2987, 2936, 1609, 1492, 1456, 1383, 1281, 1211, 1070, 1005, 891, 748, 511 cm^{-1}; UV/vis (DMSO): λ_{max} (log ε) = 669 (4.95), 604 (4.48), 352 (4.91), 289 nm (4.67); MALDI–TOF MS (2,5-dihydroxybenzoic acid): *m/z* 2644 $[M]^+$. Anal. Calcd for $C_{128}H_{160}N_8O_{48}Zn$: C, 58.14; H, 6.10; N, 4.24. Found: C, 58.35; H, 6.10; N, 4.12.
33. [2,3,9,10,16,17,24,25-Octakis(α/ß-D-galactopyranos-6-yl)phthalocyaninato]zinc(II) (**6**). Phthalocyanine **5** (100 mg, 39 μmol) was dissolved in a 9:1 mixture of trifluoroacetic acid and water (10 ml) for 4 h at room temperature. The acid was neutralized by slow addition of aqueous Na_2CO_3 solution and concentrated in vacuo. Chromatography of the residue by reverse phase HPLC using acetonitrile/H_2O as eluent afforded **6** (60 mg, 81%). ^{1}H NMR (400 MHz, DMSO-d_6, significant signals): δ 8.99 (br s, 8 H, H–Ar), 5.16–3.71 (m, 56 H, H–Gal); IR (KBr): 3428, 2925, 1716, 1637, 1495, 1399, 1256, 1074, 744, 616, 580 cm^{-1}; UV/vis (DMSO): λ_{max} (log ε) = 679 (4.67), 613 (3.90), 360 (4.34), 291 nm (4.12); MALDI–TOF MS (2,5-dihydroxybenzoic acid): *m/z* 2000.9 $[M]^+$.