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The first example of anomeric glycoconjugation to phthalocyanines

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Abstract—Preparation and characterization of peripherally glucose substituted zinc(II) phthalocyanine **6**, linked via the anomeric carbon through a novel glycosidation method is reported for the first time, for which classical Pc template chemistry with the unprotected phthalonitrile **4** could be used. Phthalocyanine **6** was formed in high yield and is displaying a high solubility in water as a primary condition for a potential biological application.
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The combination of saccharides with non-natural organic compounds is an increasingly important area of research. For instance, the conjugation of carbohydrates with macrocycles, such as porphyrins, has been widely considered, namely for use in photodynamic therapy (PDT). This therapeutic method uses porphyrin photosensitizers as a source of singlet oxygen in cancer therapy. Porphyrin-type compounds (e.g., porphyrins and/or phthalocyanines) photosensitize the formation of highly reactive singlet oxygen via transfer of energy from the triplet excited state of the porphyrinoid to the triplet ground state of oxygen. Singlet oxygen is a potent oxidant that reacts with numerous functional groups of biomolecules such as double bonds in lipids, bases of nucleic acids, aromatic amino acids, and both phosphate backbones and subcellular organelles.[1] However, the drugs used in PDT still display a low chemical selectivity toward the intended targets and uptake by cells mostly arises from passive or diffusion processes.[2]

Because the balance between hydrophobicity and hydrophilicity is acknowledged as a significant aspect for the design of new photosensitizers,[3,4] diverse research groups have synthesized a variety of new porphyrinoid–carbohydrate conjugates[5] assuming that the presence of the carbohydrate moiety could improve the membrane interaction, therefore increasing their tumor

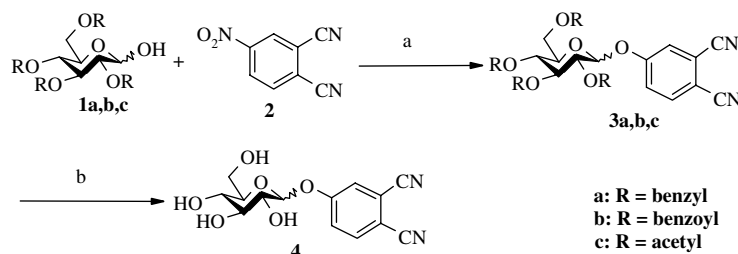selectivity. Moreover, various types of glucose transporters are specific for different monosaccharides in cancer cells.[6]

Phthalocyanine–carbohydrate conjugates are quite uncommon. To our knowledge, only the synthesis and characterization of a phthalocyaninato zinc(II) complex peripherally substituted with four glucose moieties[7] and a phthalocyaninato silicone(IV) complex axially substituted with two galactose moieties have been reported so far.[8] However, in none of the cases the phthalocyanine is linked to the carbohydrate through the anomeric carbon, like in most of the porphyrinoid–carbohydrate conjugates.[9] Of all the possible carbohydrate and peptide derivatives available attached to phthalocyanine, glucose might be a priority to start with, because the specific recognition of glycosidated photosensitizers by cell membrane receptors is dependent on cell type, but cancer cells in general have a high need for glucose due to their elevated metabolic rate. So, glucose-attached complexes indeed may have an enhanced incorporation due to specific interactions.[10]

Taking into account, the significance of the premises referred above we report here for the first time the synthesis of peripherally glucose substituted zinc(II) phthalocyanine **5** linked via the anomeric carbon (see Scheme 3).[11]

For the synthesis of **5a,b** and **6**, the precursors **3a–c** were needed. For this purpose, we used a novel glycosidation method (Scheme 1). Glycosidation of aromatic

Scheme 1. Glycosidation and deprotection of the correspondent protected carbohydrate with 4-nitropthalonitrile. Reagents and conditions—(a) for **1a**: NaH (1 equiv), dry DMF, rt, 1 h; for **1b**, **1c**: K$_2$CO$_3$ (6 equiv), DMF, rt, 12 h; (b) for **3c**: cat. NaOMe, dry MeOH, rt, 1 h.
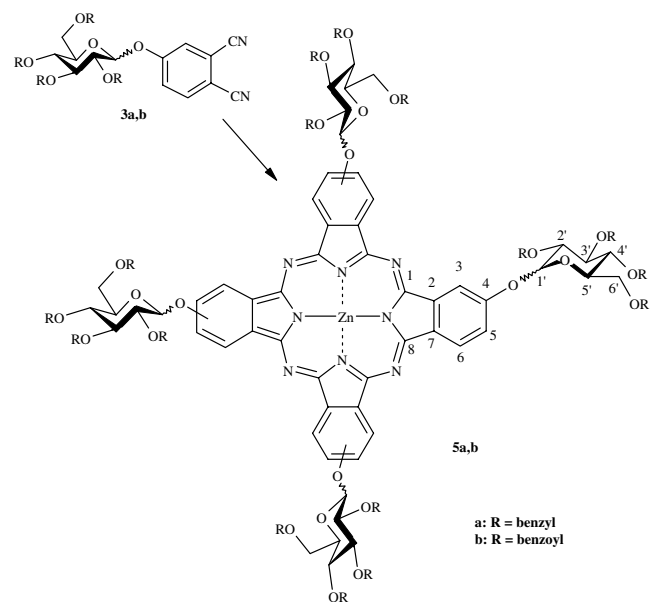
compounds with carbohydrates is a devoted area of carbohydrate research. However, application of known glycosylation methodologies to the preparation of the desired glucosyloxy-phthalonitriles **3a**–**c** starting from 4-hydroxyphthalonitrile was unsuccessful. Therefore, we turned our attention to a method which is routinely used to prepare precursors for Pcs, through nucleophilic reaction of alcohols with 4-nitrophthalonitrile (**2**) (Scheme 1). Nucleophilic aromatic substitution turned out to be extremely efficient and allowed to obtain quantitative yields of **3a**, **3b** and **3c**, respectively. Furthermore, we have been able to carry out a direct anomeric O-arylation of glucose, starting from a nitro substituted phenyl (nitrophthalonitrile), under very mild conditions, at room temperature with high yield for the first time.

The glycosidation reaction between 2,3,4,6-tetra-O-benzyl-, benzoyl- and acetyl-protected glucopyranoses[12] **1a**–**c** and 4-nitrophthalonitrile **2** was carried out in the case of **1a** with sodium hydride (NaH), while in the case of **1b** and **1c** potassium carbonate (K$_2$CO$_3$) was employed as the base. The main nucleophilic aromatic substitution products were their respective α-anomers (α/β ratio: **3a**-9/1; **3b**-10/1 and **3c**-10/1) independently of the base.[12] Glycoside **3c** was subsequently deprotected under Zemplén's conditions[13] to afford **4**.

Initially, we tried to accomplish the synthesis of tetra-glucose substituted zinc phthalocyanine **6** by applying the classical phthalocyanine template reaction on protected dinitriles **3a** and **3b** in order to obtain the Pcs **5a** and **5b**, respectively (Scheme 2) and subsequent deprotection of these compounds to afford **6**.

Tetra-O-benzyl-glucose PcZn **5a** was prepared in 58% yield starting from **3a** and showed to be stable up to 150 °C.[14] However, deprotection of the benzyl groups by catalytic hydrogenation with Pd/C turned out to be difficult and did not result in the desired phthalocyanine **6**. On the other hand, benzoyl protecting groups in **3b** revealed to be unstable under the harsh template conditions (160 °C) resulting in a significantly lower yield of **5b**. Similarly, deprotection of the carbohydrate moieties of **5b** under Zemplén's conditions failed. Only partial debenzoylation could be accomplished. MALDI-TOF spectra revealed that at least two benzoyl groups could not be removed.
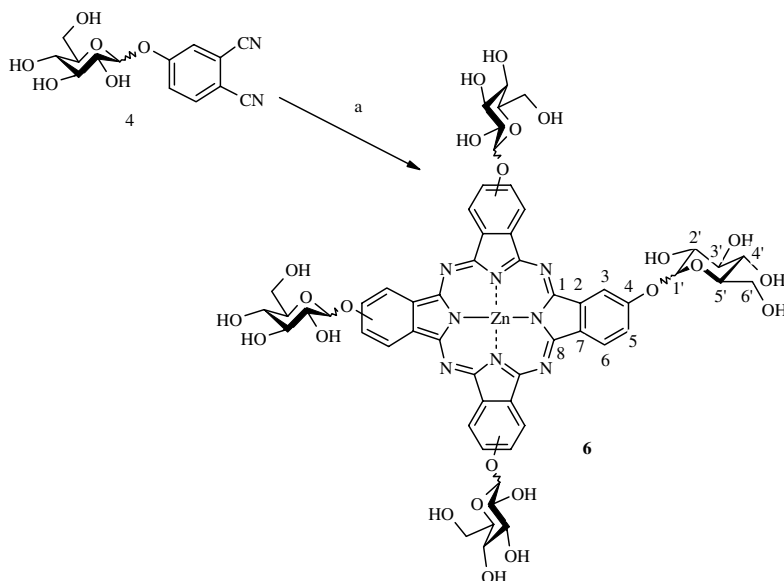
In contrast, the synthesis of Pc **6** could be easily carried out through template reaction as shown in Scheme 3.

Scheme 2. Protected glucose PcZn **5**. Reagents and conditions: (a) *n*-pentanol, zinc acetate (Zn(ac)$_2$·2H$_2$O), 150 °C, 5 h; (b) DMAE, zinc acetate (Zn(ac)$_2$·2H$_2$O), 160 °C, 5 h.

Reacting **4** with zinc acetate in a mixture of solvents (DMAE/*n*-butanol 2:1) (see Scheme 3),[15] whereby the chosen mixture of solvents plays an important role, afforded **6** in 51% yield. When the reaction was carried out either in DMAE or in *n*-butanol alone yields were low. This is due to the fact that DMAE dissolves both the starting material and the formed macrocycle well. If the formed Pc was kept in solution during the course of the template reaction it decomposed partially leading to a decrease in yield. The addition of butanol reduced this effect since it allowed the formed Pc to precipitate from the solution. This revealed to be the most adequate method for the preparation of **6**, since it requires only one step to prepare Pc **6** in a yield comparable to the synthesis of common zinc phthalocyanines. To our knowledge, a direct synthesis of Pcs peripherally substituted with alkyl chains bearing alcohol groups via template reaction has not been described in the literature so far.

Spectroscopic data of compound **6** (as well as **5a**) are in full agreement with common substituted phthalocyanines. Due to the fact that compound **6** is a mixture of isomers,[16] its ^{1}H- and ^{13}C NMR spectra (recorded in DMSO-*d*$_6$) show typical chemical shifts for phthalocya-

Scheme 3. Synthesis of phthalocyanine–carbohydrate conjugate. Reagents and conditions: (a) DMAE/n-butanol (2:1), dry zinc acetate (Zn(ac)$_2$), 100 °C, 24 h.

nines. However, the phthalocyanine protons appear at lower fields (7.95, 9.02 and 9.27 ppm for **5** and 7.98, 9.25 and 9.39 ppm for **5a**), when compared to other oxo-substituted Pcs.[16] This might be due to the de-shielding effect of the carbohydrate moieties. The chemical shift of the proton from the anomeric carbon is also affected by the electron-rich core of the Pc and therefore appearing at 6.05 ppm. The UV/vis spectrum of **6** is also characteristic for zinc-substituted phthalocyanines, showing a Q-band maximum at 680 nm. MALDI-TOF measurements confirmed unambiguously the molecular mass of compounds **5a** ($m/z = 2730$) and **6** ($m/z = 1289$).

In summary, we have prepared and characterized peripherally glucose-substituted zinc(II) phthalocyanine **6** linked via the anomeric carbon through a novel glycosidation method. Classical Pc template reactions with unprotected phthalonitrile **4** were applied for the first time. Pc **6** displays very high solubility in water, a primary condition for further biological testing.

Acknowledgements

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14. Synthesis of tetrakis-2(3),9(10),16(17),23(24)-(2,3,4,6-tetra-benzyl-α/β-D-glucopyranosyl) phthalocyaninato zinc: a mixture of 3,4-dicyanophenyl-2,3,4,6-tetra-O-benzyl-α/β-D-glucopyranoside **3a** (3.5 g, 5.2 mmol) and zinc acetate dihydrate (0.57 g, 2.6 mmol) in pentanol (10 ml) was stirred under argon 5 h at 150 °C. After cooling, the mixture was poured into MeOH (100 ml) and the solid was filtered. The crude product was purified by chromatography over silica gel [eluent: CH$_2$Cl$_2$] to give **5a**. Yield 1.9 g (58%); UV–vis (DMSO): λ (Rel. Int. %) = 354 (42, B-band), 612 (19, sh), 680 nm (100, Q-band); ^{1}H NMR (250 MHz, DMSO-d_6): δ = 3.75–5.37 (m, 24H, H-2′, H-3′, H-4′, H-5′, H-6′), 6.29 (br, 4H, H-1′), 7.03–7.55 (br, 80H, H-Bn), 7.98 (br, 4H, H-5), 9.25 (s, 4H, H-3), 9.39 (br d,

4H, H-6); ^{13}C NMR (62.9 MHz, DMSO-d_6): $\delta = 69.9$ (C-6'α), 73.1 (C-5'α), 73.7, 74.0, 74.2, 75.7, 76.3, 76.5 [C–CH$_2$Bnα/β], 78.8 (C-4'α), 81.4 (C-2'α), 83.2 (C-3'α), 83.5 (C-4'β), 85.9 (C-3'β), 96.9, 97.2 [C-1'α], 102.7 (C-1'β), 110.5 (C-3), 119.0 (C-5), 124.7 (C-6), 127–129 [C–Bn], 134.0 (C-7), 139–140 [C–Bn], 141.5 (C-2), 154.0 (C-1, C-8), 160 (C-4); MS MALDI-TOF: $m/z = 2730$ [M$^+$+H]; Anal. Calcd for C$_{168}$H$_{152}$N$_8$O$_{24}$Zn: C, 73.85; H, 5.61; N, 4.10. Found: C, 73.09; H, 5.65; N, 4.10.

15. Synthesis of tetrakis-2(3),9(10),16(17),23(24)-α/β-D-gluco-pyranosyl phthalocyaninato zinc: a mixture of 3,4-dicyano-phenyl-2,3,4,6-tetra-*O*-acetyl-α/β-D-glucopyranoside **2c** (1 g, 2 mmol) was suspended in dry MeOH (25 ml). NaOMe (100 μl) was added and the solution was stirred for 1 h. Dowex 50WX8-400 ion exchanger was added to neutralize the solution. The ion exchanger was then filtered off and the solvent evaporated to obtain **4**. Without further purification, to the deprotected dinitrile **4** dissolved in a mixture of DMAE (1 ml) and butanol (0.5 ml), zinc acetate (183 mg, 1 mmol) was added. The reaction mixture was stirred under argon for 24 h at 100 °C. After cooling, it was dissolved in a minimal amount of water and acetone was added. The solid was filtered, dissolved again in a minimal amount of water, reprecipitated adding acetone and collected after filtration. The crude product was purified by reverse phase HPLC (C18) chromatography [eluent: H$_2$O/CH$_3$CN] to give **6**. Yield 320 mg (51%); UV–vis (DMSO) λ (Rel. Int. %): 354 (41, B-band), 613 (17, sh), 681 nm (100, Q-band); ^{1}H NMR (250 MHz, DMSO-d_6): $\delta = 6.05$ (br, 4H, H-1'), 7.95 (br, 4H, H-6), 9.08 (br, 4H, H-3), 9.35 (s, 4H, H-5); ^{13}C NMR (62.9 MHz, DMSO-d_6): $\delta = 61.3$ (C-6'), 70.5 (C-5'), 72.3 (C-4'), 73.8 (C-2'), 74.8 (C-3'), 98.9 (C-1'), 109.8 (C-3), 120.2 (C-5), 124.2 (C-6), 132.5 (C-7), 140.1 (C-2), 153 (C-1, C-8), 159 (C-4); MS MALDI-TOF (high resolution): $m/z = 1289.197$ [M$^+$].

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