Synthesis of Glycoporphyrins Using Trichloroacetimidates as Glycosyl Donors

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Abstract: The trichloroacetimidate method has been utilized for the glycosylation of porphyrins. The corresponding glycoconjugates were obtained rapidly, in high yields, and excellent purity. A three-step sequence using well-matched (Lewis) acids was found to be highly effective and reliable.

Key words: porphyrins, glycosylations, glycoconjugates, antitumor agents, photodynamic therapy

Porphyrin-based compounds play a key role as antitumor agents in photodynamic therapy (PDT). PDT is a cancer therapy where a photosensitizer produces cytotoxic singlet oxygen in malignant cells after treatment with laser light.[1] However, selective accumulation of the sensitizer in tumor cells is still one of the major challenges. As yet, the mechanism of tumor uptake is not fully understood but there is evidence that an amphiphilic substitution pattern is beneficial.[2]

Promising approaches for improved tumor selectivity utilize conjugates of porphyrins or chlorins with carbohydrate moieties. The glyco substituent increases the hydrophilicity and could improve the targeting process by molecular-recognition features.[3] Some carbohydrate species actually possess a specific affinity for cancer cells.[4] In fact, several groups have proven the photodynamic activity of such glycoporphyrins[5] and in vivo studies on the pharmacokinetics of these hybrid compounds[6] have already been carried out.[7]

Different kinds of porphyrin–carbohydrate conjugates are described in the literature,[8] including N-,[9] S-,[10] C-,[11] and O-glycosides[5,7,12] as well as spacer-linked glycosides[13] (Figure 1). The most common synthesis of O-connected glycoporphyrins is based on glycosylation of hydroxyl-substituted benzaldehydes which act as the starting material for subsequent porphyrin condensations.[14]

While glycosylation of *para*-hydroxybenzaldehydes proceeds in fair yields the preparation of the corresponding *meta* derivatives is much less effective.[5] Therefore, other approaches prepared porphyrin–carbohydrate conjugates after formation of the tetrapyrrole system via a Koenigs–Knorr reaction. These protocols require long reaction times, large excess of glycosyl bromide, and produce a mixture of free-base porphyrin and the corresponding silver complex.[12a,d] Recently, the glycosylation of β-vinyl-substituted porphyrins using a cross-metathesis as the connecting step was reported.[15]

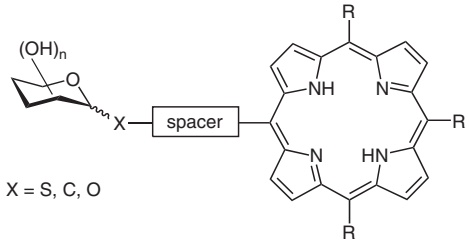

Figure 1 Schematic representation of a porphyrin–carbohydrate conjugate

Herein we present a procedure for the highly efficient preparation of glycoporphyrins using trichloroacetimidates as glycosyl donors.[16] Our initial studies were carried out using 5-(3-hydroxyphenyl)-10,15,20-triphenylporphyrin (**1a**) as a model compound (Scheme 1). Due to their increased biological activity *meta*-hydroxyphenyl-substituted porphyrins were used instead of the *ortho* or *para* derivatives.[17] Porphyrin **1a** was synthesized by condensation of pyrrole, benzaldehyde, and 3-hydroxybenzaldehyde under equilibrium conditions.[18] For glycosylation 1.5–2.0 equivalents of 2,3,4,6-tetra-*O*-acetyl-α-D-gluco-pyranosyl trichloroacetimidate[16,19] and $BF_3{\cdot}OEt_2$ or TMSOTf as promoters in dichloromethane were used. Initial experiments were performed at 0 °C but only little conversion was observed. A large excess of glycosyl donor or Lewis acid, higher temperatures, and longer reaction times did not affect the outcome significantly. We assumed that the acid catalyst formed a complex involving the inner nitrogen atoms of the porphyrin and that this Lewis acid/base complex is unreactive as a glycosyl acceptor. We therefore considered the use of a suitable metal to mask the free-base porphyrin without deactivating it. Copper and nickel are commonly used for porphyrin protection but their demetalation requires treatment with very strong acids, which was not compatible with our substrates, and metal-free glycoporphyrins are needed with respect to an application in PDT. Zinc seemed to be a better suited candidate: Porphyrins can easily be converted to their Zn(II) derivatives and subsequent demetalation usually occurs efficiently under relatively mild conditions.[20] Accordingly, Zn(II)–porphyrin **2a** was pre-

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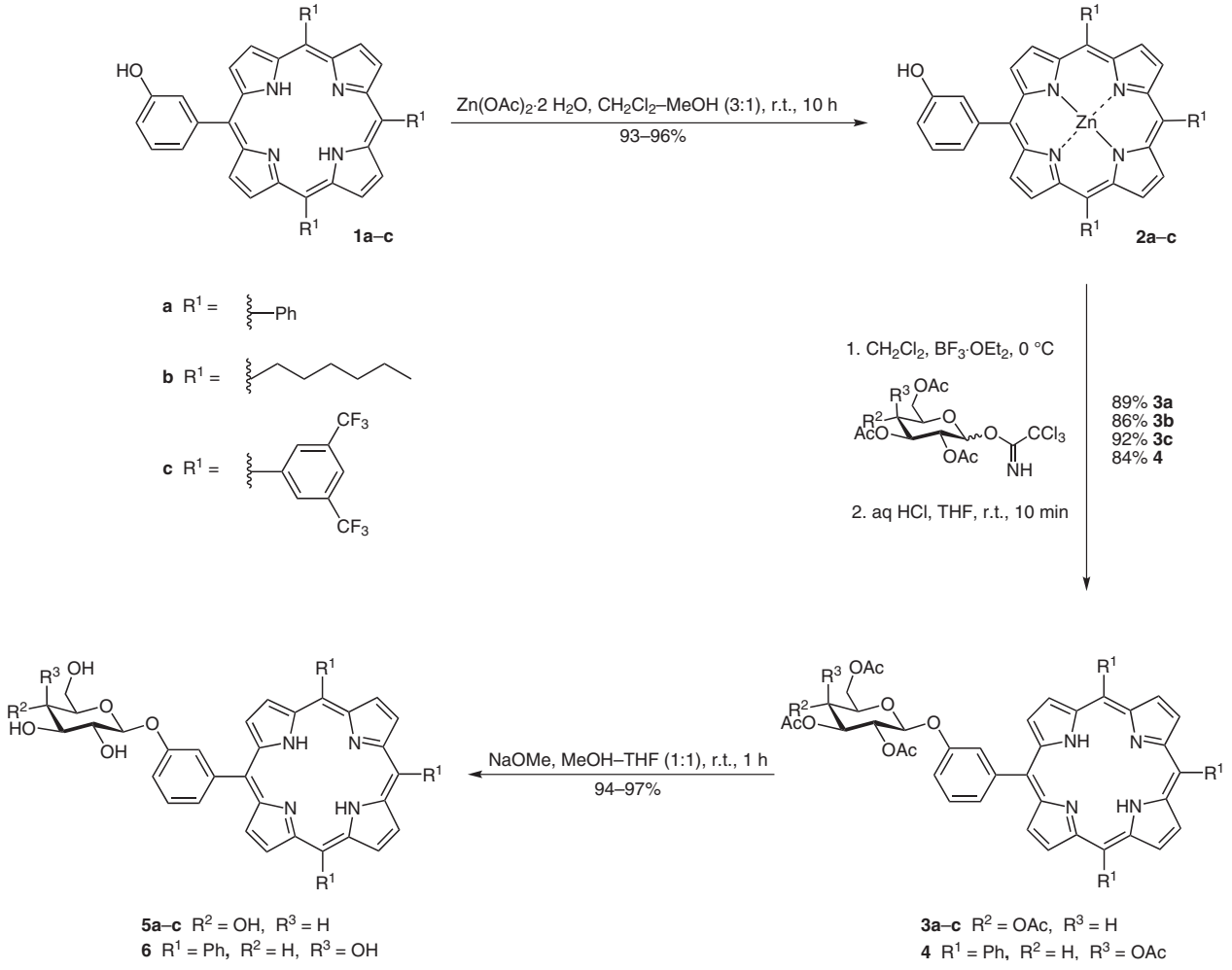
pared by treatment of 5-(3-hydroxyphenyl)-10,15,20-triphenyl-porphyrin (**1a**) with $Zn(OAc)_2$ in a mixture of dichloromethane–methanol in almost quantitative yield. Pleasingly, conversion of this metalated porphyrin **2a** to the corresponding glycoconjugate proceeded within 10 minutes when promoted by catalytic amounts of $BF_3{\cdot}OEt_2$ in presence of 1.85 equivalents of the glycosyl donor. Subsequent demetalation using hydrochloric acid in tetrahydrofuran yielded glycoporphyrin **3a** in 89% yield (Scheme 1). No hydrolysis of the glycosidic bond was observed. As expected, both the α- and β-trichloroacetimidate led exclusively to formation of the β-glycosylated porphyrin due to neighboring-group participation of the acetyl protecting group. According to these results an efficient synthesis of glycoporphyrins requires i) appropriate protection of the porphyrin core, ii) glycosylation of the peripheral hydroxyl group(s), and finally iii) deprotection of the tetrapyrrole. Therefore, three subsequent (Lewis) acid mediated transformations are required and a fine adjustment of the three acids is crucial to the success and generality of this protocol.

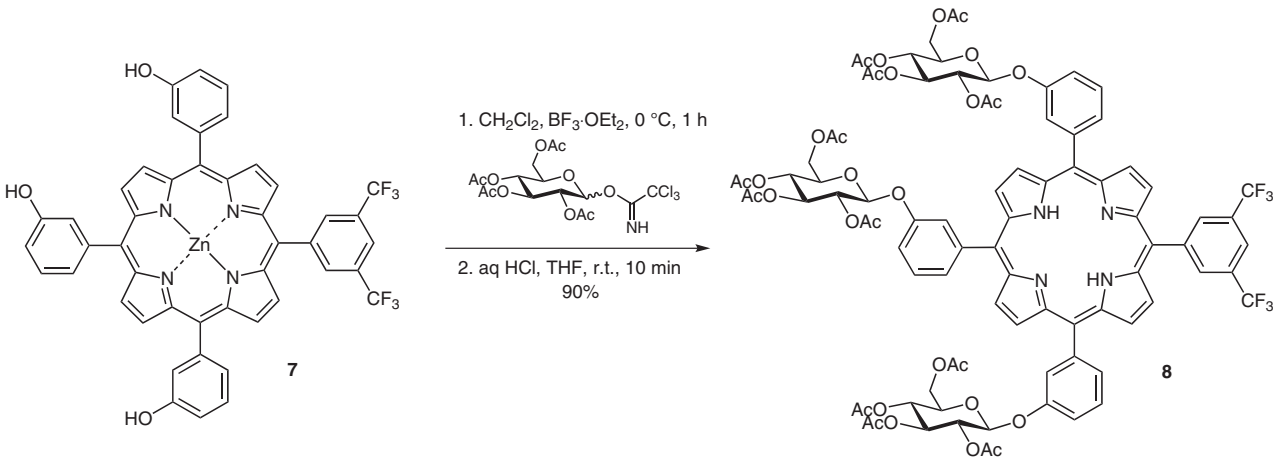
In order to investigate the scope of this reaction sequence it was applied to different *meso*-substituted porphyrins. Both aryl- and alkyl-substituted tetrapyrroles were used as substrates. The hexyl-substituted porphyrin **1b** and aryl-substituted porphyrin **1c** were synthesized under equilibrium conditions.[18] Glycosylation of their corresponding Zn(II) derivatives **2b** and **2c** yielded the glycoconjugates **3b** and **3c** in 86% and 92% yield, respectively. Moreover, this method is not limited to glucosylation as shown by the investigation of galactosyl trichloroacetimidate[21] as sugar donor. In this case, the sugar donor seemed to be less reactive and therefore the galactosylation required larger amounts of donor and longer reaction times. Nevertheless, no demetalation was observed during glycoconjugate formation and the galactosylated porphyrin **4** was obtained in 84% yield after acidic removal of the zinc ion (Scheme 1). Glycosylations with other trichloroacetimidates are currently under investigation.

The deacetylation of the carbohydrate protecting groups succeeded through treatment with catalytic amounts of sodium methanolate. As the porphyrins **3a–c** and **4** are nearly insoluble in methanol the reaction was carried out in a mixture of tetrahydrofuran–methanol (1:1). After 2 hours reaction time complete conversion was observed and the deprotected glycoconjugates **5a–c** and **6** were obtained in yields between 94–97% (Scheme 1).

With respect to biological activity, triglycosylated tetrapyrrolic compounds were found to exhibit a particularly



Scheme 1 Glucosylation and galactosylation of different hydroxyphenylporphyrin derivatives using trichloroacetimidates.



Scheme 2 Polyglycosylation of a porphyrin

high phototoxicity.[22] As summarized in Scheme 2, the procedure presented here is also highly efficient for multiple glycosylations and therefore superior to existing protocols.[5,7,12] Thus, porphyrin **7** which is substituted with three phenolic hydroxyl groups was converted into the corresponding glycosylated derivative **8** in 90% yield within short reaction time (Scheme 2). Due to its high hydrophilicity and amphiphilicity this photosensitizer is a promising candidate for further investigations.

In conclusion, a simple and highly efficient protocol for the preparation of glycoporphyrins was developed using trichloroacetimidates as glycosyl donors. Zinc protection of the porphyrin core and a sequence of well-matched (Lewis) acids is essential for the success of this procedure. Thus, short reaction times, high yields, and excellent purities of amphiphilic glycoconjugates can be accomplished.[23,24] We believe that this protocol will find application in the synthesis of other monoglycosylated as well as polyglycosylated tetrapyrrolic compounds.

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(23) **Typical Glycosylation Procedure**
Zn(II) 5-(3-hydroxyphenyl)-10,15,20-triphenylporphyrin (**2a**, 100 mg, 0.14 mmol) was dissolved in dry CH_2Cl_2 (20 mL) under an argon atmosphere. Then 2,3,4,6-tetra-*O*-acetyl-β-D-gluco-pyranosyl trichloroacetimidate (130 mg, 0.26 mmol, 1.85 equiv) or 2,3,4,6-tetra-*O*-acetyl-α-D-galacto-pyranosyl trichloroacetimidate (350 mg, 0.70 mmol, 5.0 equiv) was added in three portions followed by $BF_3{\cdot}OEt_2$ (5.0 μL, 0.04 mmol). After stirring for 15 min for glucosylation or 120 min for galactosylation the mixture was transferred to a separatory funnel. The organic layer was washed with H_2O (2 × 50 mL), and the solvent was evaporated under reduced pressure. The residue was dissolved in THF (20 mL) and HCl (25%, 0.5 mL) were added. After stirring for 10 min H_2O (50 mL) and CH_2Cl_2 (75 mL) were added. The organic layer was separated and washed with H_2O (2 × 50 mL). After drying with Na_2SO_4 the solvent was evaporated under reduced pressure. Further purification was achieved by flash chromatography using CH_2Cl_2–EtOAc (95:5) as the eluent. The analytically pure product was obtained as a violet crystalline solid after recrystallization from CH_2Cl_2–MeOH.

5-[3-(2,3,4,6-Tetraacetyl-β-D-glucosyl)phenyl]-10,15,20-triphenylporphyrin (3a)
Yield 123 mg, 89%; mp 205 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ = –2.70 (m, 2 H, NH), 1.37 (s, 3 H, OAc), 2.02 (s, 3 H, OAc), 2.07 (s, 3 H, OAc), 2.12 (s, 3 H, OAc), 3.83 (ddd, *J* = 2.4, 5.8, 10.0 Hz, 1 H, H-5'ose'), 4.08 (dd, *J* = 2.4, 12.2 Hz, 1 H, H-6_A'ose'), 4.20 (dd, *J* = 5.8, 12.2 Hz, 1 H, H-6_B'ose'), 5.20 (dd, *J* = 9.1, 10.0 Hz, 1 H, H-4'ose'), 5.32 (dd, *J* = 9.1, 9.1 Hz, 1 H, H-3'ose'), 5.35 (d, *J* = 7.8 Hz, 1 H, H-1'ose'), 5.40 (dd, *J* = 7.8, 9.1 Hz, 1 H, H-2'ose'), 7.42–7.45 (m, 1 H, Ar), 7.66–7.69 (m, 1 H, Ar), 7.74–7.81 (m, 9 H, Ph), 7.88–7.89 (m, 1 H, Ar), 7.95–7.97 (m, 1 H, Ar), 8.20–8.26 (m, 6 H, Ph), 8.85–8.89 (m, 8 H, β-pyrrole-H). ^{13}C NMR (126 MHz, $CDCl_3$): δ = 19.83, 20.45, 20.53, 20.63, 61.98, 68.44, 71.42, 72.29, 72.86, 99.41, 116.75, 118.97, 120.28, 120.30, 120.44, 122.95, 126.69, 127.68, 127.78, 129.94, 131.16, 134.54, 142.15, 143.88, 155.41, 169.28, 170.11, 170.27. ESI-HRMS: *m/z* calcd for $C_{58}H_{49}N_4O_{10}^+$ $[M + H]^+$: 961.3443; found: 961.3481. UV/vis (CH_2Cl_2): λ_{max} (ε) = 417 (298600), 515 (18400), 549 (10700), 591 (8700), 646 (6400) nm.

5-[3-(2,3,4,6-Tetraacetyl-β-D-galactosyl)phenyl]-10,15,20-triphenylporphyrin (4)
Yield 116 mg, 84%; mp 169 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ = –2.71 (s, 2 H, NH), 1.20 (s, 3 H, OAc), 2.00 (s, 3 H, OAc), 2.10 (s, 3 H, OAc), 2.16 (s, 3 H, OAc), 4.02 (ddd, *J* = 1.1, 6.4, 6.4 Hz, 1 H, H-5'ose'), 4.11–4.14 (m, 2 H, H-6'ose'), 5.17 (dd, *J* = 3.4, 10.4 Hz, 1 H, H-3'ose'), 5.35 (d, *J* = 8.0 Hz, 1 H, H-1'ose'), 5.44 (dd, *J* = 1.1, 3.4 Hz, 1 H, H-4'ose'), 5.64 (dd, *J* = 8.0, 10.4 Hz, 1 H, H-2'ose'), 7.47–7.49 (m, 1 H, Ar), 7.68–7.71 (m, 1 H, Ar), 7.77–7.83 (m, 9 H, Ph), 7.94–7.95 (m, 1 H, Ar), 7.97–8.00 (m, 1 H, Ar), 8.23–8.27 (m, 6 H, Ph), 8.87–8.90 (m, 8 H, β-pyrrole-H). ^{13}C NMR (126 MHz, $CDCl_3$): δ = 19.72, 20.48, 20.52, 20.71, 61.45, 67.07, 68.86, 70.92, 71.33, 99.98, 116.70, 118.99, 120.24, 120.28, 120.42, 123.07, 126.68, 127.65, 127.76, 129.91, 131.09, 134.54, 142.14, 143.85, 155.42, 169.32, 169.96, 170.02, 170.09. ESI-HRMS: *m/z* calcd for $C_{58}H_{49}N_4O_{10}^+$ $[M + H]^+$: 961.3443; found: 961.3411. UV/vis (CH_2Cl_2): λ_{max} (ε) = 417 (308600), 515 (20900), 549 (16300), 591 (13600), 646 (10800) nm.

(24) **Typical Procedure for Deacetylation**
To a stirred solution of 5-[3-(2,3,4,6-tetraacetyl-β-D-glucosyl)phenyl]-10,15,20-triphenylporphyrin (**3a**, 50 mg, 0.05 mmol) in dry THF–MeOH (1:1, 10 mL) under an argon atmosphere a solution of sodium methanolate in dry MeOH (1.5 mL, 0.02 N) was added. After 2 h the solvent was evaporated under reduced pressure, and the crude product was purified by flash chromatography using CH_2Cl_2–MeOH (9:1) as the eluent. The pure product was obtained as a violet crystalline solid after recrystallization from CH_2Cl_2–MeOH aq.

5 (3-β-D-Glucosylphenyl)-10,15,20-triphenylporphyrin (5a)
Yield 40 mg, 97%, mp 160 °C. ^{1}H NMR [700 MHz, $(CD_3)_2SO$]: δ = –2.90 (s, 2 H, NH), 3.22–3.26 (m, 1 H, H'ose'), 3.31–3.38 (m, 3 H, H'ose'), 3.47–3.51 (m, 1 H, H-6_A'ose'), 3.68–3.71 (m, 1 H, H-6_B'ose'), 4.57 (dd, *J* = 5.9, 5.9 Hz, 1 H, OH-6'ose'), 5.01 (d, *J* = 5.3 Hz, 1 H, OH'ose'), 5.11 (d, *J* = 5.1 Hz, 1 H, OH'ose'), 5.22 (d, *J* = 7.6 Hz, 1 H, H-1'ose'), 5.44 (d, *J* = 4.9 Hz, 1 H, OH'ose'), 7.53–7.55 (m, 1 H, Ar), 7.72–7.75 (m, 1 H, Ar), 7.80–7.87 (m, 1 H, Ar, 9 H, Ph), 7.91–7.92 (m, 1 H, Ar), 8.20–8.24 (m, 6 H, Ph), 8.80–8.96 (m, 8 H, β-pyrrole-H). ^{13}C NMR [176 MHz, $(CD_3)_2SO$]: δ = 61.13, 70.17, 73.89, 77.06, 77.47, 100.85, 116.29, 120.01, 120.51, 120.58, 122.87, 127.47, 128.59, 129.00, 134.72, 141.66, 142.87, 156.39. ESI-HRMS: *m/z* calcd for $C_{50}H_{41}N_4O_6^+$ $[M + H]^+$: 793.3021; found: 793.2900. UV/vis (CH_2Cl_2): λ_{max} (ε) = 417 (333600), 515 (22800), 549 (16800), 591 (13900), 646 (10700) nm.

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