

# Synthesis of glycoconjugated chlorin $p_6$ cycloimide

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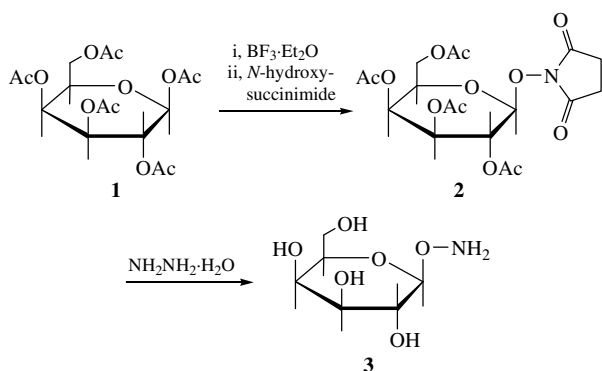
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The synthesis of N-substituted cycloimide chlorin  $p_6$  with a D-galactose residue by simultaneous glycosylation of the main macrocycle followed by replacement of oxygen in the additional ring of purpurin 18 by nitrogen has been carried out to give a rigid spacer section in the form of a six-membered cycloimide.

The introduction of an extra six-membered imide ring conjugated with the main macrocycle considerably changes the spectral properties of natural chlorins.<sup>1</sup> The bathochromic shift of long-wave band Q in the electronic spectrum is about 40–50 nm. This is extremely important in the development of photosensitizers for the photodynamic therapy (PDT) of cancer since light with wavelengths above 700 nm weakly dissipates in tissues and hence the PDT efficiency increases considerably.<sup>2</sup> We have previously suggested the synthesis of a new group of derivatives of natural chlorophyll *a* by the reaction of purpurin 18 with hydroxylamine. A distinctive feature of these compounds is that the cycloimide ring contains a hydroxyl group at the nitrogen atom.<sup>3</sup> It was shown that this group was reactive and could be used to obtain various alkyl and acyl derivatives.<sup>4</sup> However, attempts to introduce a carbohydrate residue at the nitrogen atom using this method failed.

Since carbohydrate-containing chlorins are of considerable interest for PDT due to the higher accumulation of similar photosensitizers in tumor<sup>5–8</sup> and due to better solubility in polar solvents, we elaborated a new method to obtain such compounds using not hydroxylamine but its carbohydrate derivative in condensation with purpurin 18. We were solving two tasks simultaneously: replacement of oxygen in the anhydride ring of purpurin 18 by nitrogen to give a cycloimide and addition of a sugar molecule to chlorin.

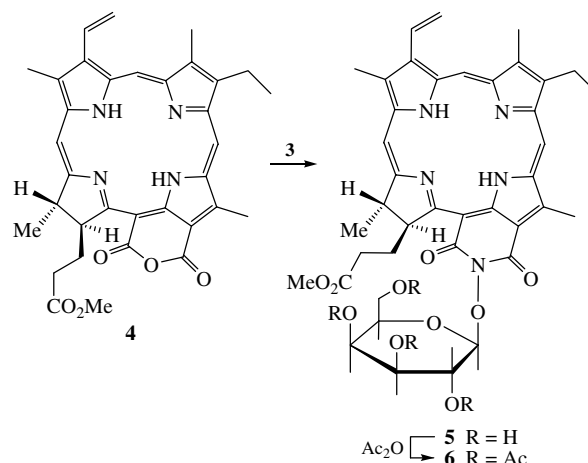
The starting *O*-β-D-galactopyranosylhydroxylamine was synthesised according to Scheme 1. The reaction of D-galactose pentaacetate **1** with *N*-hydroxysuccinimide under acidic catalysis conditions gave *N*-hydroxysuccinimide ether **2**, which was subsequently treated with hydrazine hydrate to give target compound **3**. The target and intermediate products were characterised by <sup>1</sup>H NMR and IR spectroscopic data.<sup>†</sup>



Scheme 1

In comparison with the previously reported synthesis of similar glycosides *via* bromo derivatives,<sup>9</sup> this approach simplifies the synthesis of reactive hydroxyamines considerably and opens up a way for their subsequent use in syntheses of neoglycoconjugates.

Methyl ester of purpurin 18 **4** reacts with *O*-β-D-galactopyranosylhydroxylamine **3** in pyridine at room temperature to give *N*-galactosyloxycycloimide **5** (Scheme 2). Note that the synthesis of cycloimide **5** is carried out without preliminary protection of hydroxy groups in the carbohydrate. The yield of the target product was 30%.



Scheme 2

The electronic spectrum of compound **5**<sup>‡</sup> shows a bathochromic shift of the Q band from 700 to 714 nm. The mass spectrum of conjugate **5** contains a molecular ion peak with *m/z* 756.2. The incorporation of the hydrophilic carbohydrate

<sup>†</sup> The <sup>1</sup>H NMR spectra of compounds **2** and **3** were recorded using a Bruker WM-200 spectrometer. The IR spectra were recorded in Vaseline oil using a Shimadzu-IR-435 spectrophotometer. The optical rotation angles were measured with a Digytar Vasco DIP 360 photoelectric polarimeter.

**2**: [ $\alpha$ ]<sub>D</sub> –50° (*c* 1.0, CHCl<sub>3</sub>). <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$ : 5.40–5.31 (m, 2H, 2-H, 4-H), 5.03 (dd, 1H, 3-H), 4.84 (d, 1H, 1-H, *J*<sub>1,2</sub> 8.2 Hz), 4.22–4.03 (m, 2H, 6-H), 3.84 (m, 1H, 5-H), 2.70 (s, 4H, CH<sub>2</sub>CH<sub>2</sub>), 2.14–1.92 (4s, 12H, COMe). Found (%): C, 48.43; H, 5.25; N, 3.24. Calc. for C<sub>18</sub>H<sub>23</sub>NO<sub>12</sub> (%): C, 48.54; H, 5.21; N, 3.14.

**3**: <sup>1</sup>H NMR (D<sub>2</sub>O)  $\delta$ : 4.49 (d, 1H, 1-H, *J*<sub>1,2</sub> 8 Hz), 3.93 (dd, 1H, 3-H), 3.80–3.51 (m, 5H, 2-H, 4-H, 5-H, 6-H). IR (Vaseline oil,  $\nu_{\max}$ /cm<sup>–1</sup>): 3320 (O–H), 3320 (N–H), 2900 (C–H), 1630 (N–H), 1430, 1360 (C–H), 1225 (C–O), 1110–1130 (C–O, 4 bands, carbohydrate frame).

fragment into the structure of the hydrophobic chlorin macrocycle enhances the solubility of the sensitizer in polar solvents. The  $^1\text{H}$  NMR spectrum of compound **5** is insufficiently informative; therefore, the compound was treated with acetic anhydride in pyridine to additionally confirm its structure.

The structure of compound **6** was confirmed by  $^1\text{H}$  NMR spectroscopy, mass spectrometry and electronic absorption spectroscopy. The  $^1\text{H}$  NMR spectrum of conjugate **6** contained, along with the signals of protons in the chlorin macrocycle, signals of carbohydrate ring protons in the regions of  $\delta$  5.50–5.30 and 4.44–4.03, as well as protons of acetoxy groups in the region of 2.42–1.90 (Figure 1). The anomeric centre was determined to have a  $\beta$ -anomer configuration ( $\delta$  4.44). No signals corresponding to the  $\alpha$ -anomer were found in the spectrum. The mass spectrum of compound **6** contains a molecular ion peak with  $m/z$  924.5 ( $\text{M}^+ + \text{H}$ ).

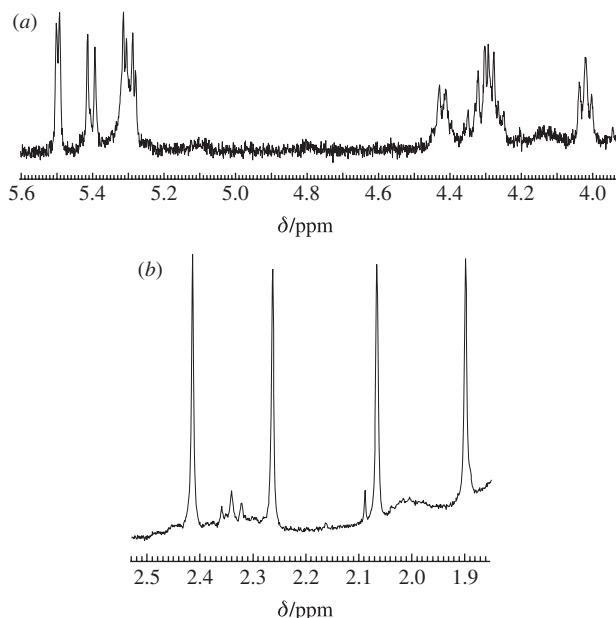
Thus, the synthesis of glycosylated chlorin  $p_6$  described opens up a new promising method for the creation of photosensitizers for the PDT of cancer.

‡ The electronic spectra of compounds **5** and **6** were recorded with a Jasco 7800 spectrophotometer in  $\text{CHCl}_3$  at 400–800 nm. The  $^1\text{H}$  NMR spectra were recorded in  $\text{CDCl}_3$  on a Bruker AMX 500 spectrometer. The mass spectra were recorded on a Vision 2000 instrument using the MALDI method combined with a time-of-flight analyzer.

**5**: *O*- $\beta$ -D-galactopyranosylhydroxylamine **3** (30.0 mg, 0.15 mmol) was added to a solution of purpurin 18 methyl ester **4** (10.0 mg, 0.017 mmol) in pyridine (5 ml), and the mixture was stirred for 48 h at 25 °C. The reaction mixture was diluted with chloroform (5 ml) and repeatedly washed with water. The organic layer was separated and dried with sodium sulfate; the solvent was concentrated *in vacuo*. The residue was chromatographed on a plate with silica gel Kieselgel 60 H (Merck, Germany) in the chloroform–ethanol (70:13) system. The yield of target product **5** was 3.9 mg (30%).

Electronic spectrum [ $\lambda_{\text{max}}$ /nm (relative intensities)]: 420, 485, 514, 555, 658, 714 (1:0.06:0.05:0.20:0.06:0.36). MS,  $m/z$  (%): 756.2 ( $\text{M}^+$ , 100), 779.2 ( $\text{M}^+ + \text{Na}$ , 22), 795.2 ( $\text{M}^+ + \text{K}$ , 18).

**6**:  $^1\text{H}$  NMR,  $\delta$ : 9.85 (s, 1H, 10-H), 9.57 (s, 1H, 5-H), 8.78 (s, 1H, 20-H), 7.90 (dd, 1H, 3<sup>1</sup>-CH,  $J$  19 and 12 Hz), 6.32 (dd, 1H, 3<sup>2</sup>-CH<sub>2</sub>,  $J$  19 Hz), 6.25 (dd, 1H, 3<sup>2</sup>-CH<sub>2</sub>,  $J$  12 Hz), 5.77 (d, 1H, 17-H,  $J$  7 Hz), 5.50 (m, 1H, Gal-H), 5.41 (m, 1H, Gal-H), 5.30 (m, 1H, Gal-H), 4.44 (m, 1H, Gal-H), 4.30 (m, 3H, Gal-CH<sub>2</sub> and 18-H), 4.03 (m, 1H, Gal-H), 3.87 (s, 3H, 17<sup>5</sup>-Me), 3.74 (quint, 2H, 8<sup>1</sup>-CH<sub>2</sub>,  $J$  7 Hz), 3.56 (s, 3H, 12-Me), 3.34 (s, 3H, 2-Me), 3.25 (s, 3H, 7-Me), 2.68 (m, 2H, 17<sup>1</sup>-CH<sub>2</sub>), 2.42 (s, 3H, MeCO), 2.35 (m, 2H, 17<sup>2</sup>-CH<sub>2</sub>), 2.26, 2.06, 1.90 (3s, 3 $\times$ 3H, MeCO), 1.80 (d, 3H, 18-Me,  $J$  7 Hz), 1.39 (t, 3H, 8<sup>2</sup>-Me,  $J$  7 Hz), 0.01 (s, 2H, NH). Electronic spectrum [ $\lambda_{\text{max}}$ /nm (relative intensities)]: 417, 481, 510, 549, 598, 650, 708 (1:0.06:0.07:0.22:0.03:0.08:0.38). MS,  $m/z$  (%): 579.7 (34), 924.5 ( $\text{M}^+ + \text{H}$ , 100), 946.5 ( $\text{M}^+ + \text{Na}$ , 54), 962.5 ( $\text{M}^+ + \text{K}$ , 24).



**Figure 1** Signals of (a) carbohydrate ring protons and (b) protons of acetoxy groups of compound **6**.

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