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Synthesis of chlorin–carbohydrate conjugates by 'click chemistry'

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A synthesis of a conjugate of chlorin e_6 with β-D-lactose has been carried out by 1,3-dipolar cycloaddition of a sugar azide to a propargyl derivative of chlorin e_6.

Combinatory chemistry, which makes it possible to synthesise the libraries of new organic compounds for creating medicines with required therapeutic properties, has been rapidly developed. New approaches to the synthesis of biologically active compounds are being developed. It is mandatory for reactions of this kind that the starting compounds be readily accessible and easy to obtain, the reaction conditions be mild and the yields of target products be high. The [3 + 2] cycloaddition of azides to alkynes with a terminal triple bond suggested by Huisgen[1] is versatile and usable for constructing complex bioconjugates, immobilising biomolecules on various supports and attaching medicines to drug targeting systems.[2,3] The use of the above reaction in biological and pharmaceutical chemistry was encouraged by a considerable increase in the reaction rates owing to the use of Cu^{I}-compounds as catalysts.[4]

The insertion of mono- or disaccharides into a pigment molecule makes it possible to adjust the amphiphilicity of pharmaceuticals directly. Carbohydrate substituents not only increase the water solubility of porphyrins and chlorins but also ensure the vector delivery of pharmaceuticals into the cell by receptor-mediated endocytosis.[5] It is well known that cancer cells have high expression of galectins (*e.g.*, Galectin-1), that is, proteins having a carbohydrate-recognising domain with high affinity to β-galactosides.[6] The photocytotoxicity of purpurinimides conjugated with galactose and lactose is much higher than that of free pigments.[7] The photodynamic efficiency of porphyrin glycoconjugates with galactose and mannose residues due to enhanced expression of their surface receptors that have high affinity to the above sugars has been shown in *in vitro* experiments with a retinoblastoma cell line.[8]

In this work, the 'click chemistry' method described above was used to synthesise glycoconjugates based on chlorin e_6 in order to obtain new amphiphile photosensitisers for the photodynamic therapy of cancer.

Methylpheophorbide *a* **1** was used as a key compound in the synthesis; it was converted into a chlorin e_6 derivative with a terminal triple bond (Scheme 1). It is well known that the pentanone exocycle is opened on treatment with nucleophiles, including primary and secondary amines. In the latter case, corresponding amides are formed.[9] In this study, the reaction with propargylamine gave amide **2** in ~90% yield.

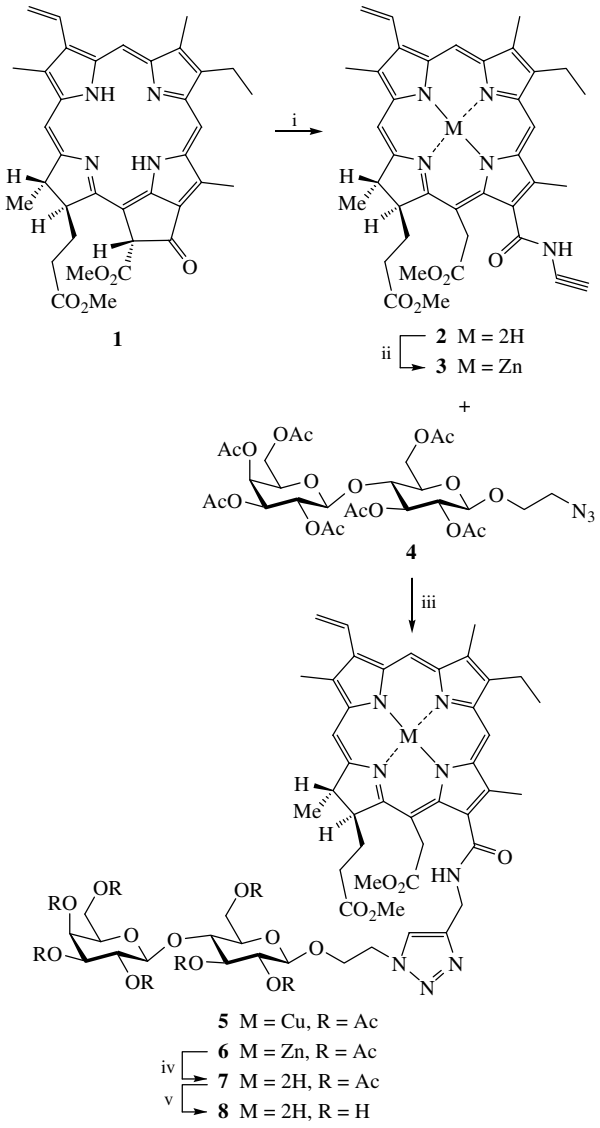
The structure of compound **2** was confirmed by NMR experiments.[†] The signal of the ethynyl proton is a triplet at δ 2.39, while the protons of the methylene group of the propargyl residue manifest themselves as a multiplet at δ 4.50, giving correlation in the COSY spectrum with the adjacent NH proton of the amide group and with the proton at the triple bond.

Ethynyl derivative **2** was condensed with 1-*O*-(2-azidoethyl)-β-D-lactose peracetate **4**, which was obtained by the treatment of a chloroethoxy derivative of acetylated lactose with sodium azide using a procedure described previously.[10]

The cycloaddition was carried out in the presence of a catalytic amount of copper(I) iodide. Within the first 10 min, the colour of the reaction mixture changed due to a hypso-

[†] The solvents were purified using standard procedures. Pheophorbide *a* was obtained using a published procedure.[12] All of the reactions were carried out away from direct light in an argon atmosphere. Electronic spectra were recorded in chloroform using a Jasco-UV 7800 spectrophotometer. NMR experiments were carried out at 303 K on a Bruker DRX spectrometer (500 MHz) for solutions in $CDCl_3$. Mass spectra were obtained by the MALDI method on a Bruker Ultraflex TOF/TOF mass spectrometer using dihydroxybenzene (DHB) as the substrate. High-resolution mass spectra were obtained with an LTQ FT hybrid mass spectrometer (Thermo, Germany). A Finnigan Ion Max Source in the positive ion mode was used as the ion source. The sample was electrosputtered at 3.6 kV across the capillary emitter and 1 μl min^{-1} sample solution feed rate to the emitter needle. The sample was dissolved in an acetonitrile–water mixture (50:50). Accurate measurements of molecular ion masses were performed using an FTICR mass spectrometer. Fragmentation of ions and measurement of the mass spectra of fragments were carried out in a linear quadrupole ion trap. Mass spectra were processed and analysed using the Qual Browser software (Thermo, Germany). IR spectra of compounds in KBr pellets were recorded on a Bruker EQUINOX 55 spectrometer. Column chromatography was carried out on 40/60 silica gel (Merk). Preparative TLC was performed on silica gel 60 (Merck) using 20×20 plates with a layer thickness of 1 mm. Analytical TLC was carried out on Kieselgel 60 F_{245} plates (Merck).

*13'-(*N*-Propargylamide)chlorin* e_6 *methyl ester* **2**: yield 86%. ^{1}H NMR, δ: 9.70 (s, H, 10-H), 9.63 (s, H, 10-H), 8.82 (s, H, 20-H), 8.07 (dd, H, 3^1-H), 6.71 (t, H, 13^2-NH), 6.35 (dd, H, *E*-3^2-H), 6.14 (dd, H, *Z*-3^2-H), 5.50 (d, H, 15-CH_2), 5.26 (d, H, 15-CH_2), 4.50 (m, 2H, 13^3-CH_2), 4.46 (m, H, 18-H), 4.39 (d, H, 17-H), 3.83 (s, 3H, 15^4-COOMe), 3.78 (q, 2H, 8^1-CH_2), 3.62 (s, 3H, 17^5-COOMe), 3.56 (s, 3H, 12^3-Me), 3.49 (s, 3H, 2-Me), 3.30 (s, 3H, 7-Me), 2.56 (m, H, 17^2-CH_2), 2.39 (t, H, 13^5-H), 2.25 (m, H, 17^1-CH_2), 2.15 (m, H, 17^2-CH_2), 1.80 (m, H, 17^1-CH_2), 1.73 (d, 3H, 18-Me), 1.71 (t, 3H, 8^2-Me), –1.60 (H, NH), –1.83 (H, NH). MS (MALDI), *m/z*: 662 (M^+). UV-VIS [CH_2Cl_2, λ_{max}/nm (ε/dm^3 mol^{-1} cm^{-1})]: 391 (116500), 500 (11550), 528 (4300), 557 (2100), 608 (4200), 663 (33500). IR (KBr, ν/cm^{-1}): 3294 (C–H alkyne), 2120 (C–C alkyne), 1734 (C=O ester), 1659 ('amide-I'), 1512 ('amide-II').



Scheme 1 Preparation of chlorin e_6 glycoconjugates and metal complexes. *Reagents and conditions*: i, propargylamine, CH_2Cl_2; ii, $(MeCOO)_2Zn$, $CHCl_3$–MeOH; iii, CuI, MeCN, Et_3N; iv, HCl; v, MeONa, MeOH.

chromic shift of the long-wave absorption band from 663 to 635 nm owing to the formation of a chlorin copper complex. The progress of the reaction was monitored chromatographically until the original ethynyl chlorin disappeared almost completely. Glycoconjugate **5** was purified using preparative TLC. The yield of the target product was 82%.

However, the drastic conditions (18% solution of sulfuric acid in trifluoroacetic acid[11]) required for copper removal from the macrocycle resulted in the decomposition of the glycosylated pigment.

Therefore, we developed another approach to the synthesis of glycosylated metal-free chlorin e_6 using a Zn complex of amide **3** to give corresponding glycoconjugate **6**. The zinc complex of chlorin e_6 is sufficiently stable under the reaction conditions and is not replaced by a copper ion. This 'protection' with a Zn^{2+} cation followed by demetallation in a weakly acidic medium allowed us to remove the metal, to purify glycoconjugate **7** by chromatography and then to hydrolyse the carbohydrate acetyl groups in the presence of sodium methoxide in a quantitative yield to give water-soluble glycoside **8**.

The structure of compound **7** was revealed by 1D and 2D NMR spectroscopy.‡ The ^{13}C and ^1H NMR spectra were completely assigned using 2D $\{^1H,^1H\}$ correlation spectroscopy (COSY), total correlation spectroscopy (TOCSY), rotating frame nuclear Overhauser effect spectroscopy (ROESY), $\{^1H,^{13}C\}$ heteronuclear single quantum coherence (HSQC) and heteronuclear multiple bond correlation (HMBC) experiments.

The proton spin system of the glucose residue (Glc) was separated by TOCSY and assigned from the data of COSY which showed all sequential correlations from H1 to H6. The line shapes of H2 and H3 identified this residue as having a *gluco*-confguration. β-Anomeric configuration followed from the observed $^3J_{H1–H2}$ of 9 Hz. The ring type was confirmed by *intra*-residue correlations in ROESY (H1–H3, H1–H5, H2–H4, H4–H6a,b, H5–H6a).

The proton spin system of the galactose residue (Gal) was separated by TOCSY, assigned by the data of COSY from H1 to H4 and distinguished using the line shape of H4 ($^3J_{H3–H4}$ of 4 Hz). H5 was found using H1–H5 and H4–H5 correlations in ROESY and H6 was tracked from H5–H6 correlation in COSY. β-Anomeric configuration followed from the observed $^3J_{H1–H2}$ of 9 Hz. Other *intra*-residue cross-peaks in ROESY (H1–H3, H4–H6a,b) confirmed the ring and anomeric configuration.

The ^{13}C NMR spectra of sugar residues were assigned using the HSQC data, which showed all sugar ring correlations. Abnormal low-field displacement of Gal H1, Glc H1 and Glc H4 indicated substitution at these positions. Gal(1-4)Glc glycosidic bond and its conformation (protons point to one side) were confirmed by Gal H1–Glc H4 *inter*-residue correlation in ROESY.

Signals due to the triazole residue and attached spacers (Trz) were assigned using HSQC, ROESY and HMBC data. Two $H1^1$ signals were found from the cross-peaks with characteristic chlorine e_6 $H13^2$ in COSY and ROESY. C1 was assigned from C1–$H1^1$ correlation in HMBC, and this signal led to the assignment of H2 *via* C1–H2 correlation in HMBC. $H3^1$ signals were distinguished from the ROESY correlation H2–$H3^1$, which confirmed (together with H2–$H1^1$ correlation) the chlorine e_6 attachment site in the triazole ring. $H3^2$ signals were attached

‡ Gglycoconjugate **7**: yield 82%. ^1H NMR, δ: 9.96 (s, H, 10-H_{Chl}), 9.64 (s, H, 5-H_{Chl}), 8.81 (s, H, 20-H_{Chl}), 8.07 (dd, H, 3^1-H_{Chl}), 7.89 (s, H, 2-H_{Trz}), 7.33 (t, H, 13^2-NH_{Chl}), 6.35 (dd, H, *E*-3^2-H_{Chl}), 6.14 (dd, H, *Z*-3^2-H_{Chl}), 5.49 (d, H, 15^{1a}-$CH_{2\,Chl}$), 5.30 (d, H, 4-H_{Gal}), 5.22 (d, H, 15^{1b}-$CH_{2\,Chl}$), 5.11 (m, 2H, 1^{1a}-H_{Trz}; t, 3-H_{Glc}), 5.07 (dd, H, 2-H_{Gal}), 4.93 (m, 2H, 1^{1b}-H_{Trz}; dd, 3-H_{Gal}), 4.83 (t, H, 2-H_{Glc}), 4.54 (m, H, 3^1-H_{Trz}), 4.48 (q, H, 18-H_{Chl}), 4.46 (d, H, 6^a-H_{Glc}), 4.40 (d, H, 1-H_{Gal}), 4.39 (m, H, 17-H_{Chl}), 4.38 (d, H, 1-H_{Glc}), 4.08 (dd, H, 6^b-H_{Glc}), 4.07 (m, H, 3^{2a}-H_{Trz}), 3.99 (m, 2H, $6^{a,b}$-H_{Gal}) 3.85 (m, H, 3^{2b}-H_{Trz}), 3.80 (q, 2H, 8^1-$CH_{2\,Chl}$), 3.72 (m, 2H, 5-H_{Gal}; t, 4-H_{Glc}), 3.69 (s, 3H, 15^4-$COOMe_{Chl}$), 3.62 (s, 3H, 17^5-$COOMe_{Chl}$), 3.56 (m, H, 5-H_{Glc}), 3.52 (s, 3H, 12^1-Me_{Chl}), 3.49 (s, 3H, 2^1-Me_{Chl}), 3.31 (s, 3H, 7^1-Me_{Chl}), 2.54 (dt, H, 17^{2a}-$CH_{2\,Chl}$), 2.54 (dt, H, 17^{2a}-H_{Chl}) 2.23 (dt, H, 17^{1a}-H_{Chl}), 2.15 (m, H, 17^{2b}-H_{Chl}), 2.14–1.84 (s, 21H, 4-OAc_{Gal}, 3-OAc_{Glc}), 1.80 (m, H, 17^1-CH_2), 1.72 (t, 3H, 8^2-Me_{Chl}), 1.71 (d, 3H, 18^1-Me_{Chl}), –1.59 (H, NH), –1.79 (H, NH). ^{13}C NMR, δ: 173.5 [15^2-C_{Chl}, 17^3-C_{Chl} (*C*OOMe)], 170.3–168.9 [19-C_{Chl}, 13^1-C_{Chl}, 7OAc (*C*O–$Me_{Gal,Glc}$)], 166.8 (16-C_{Chl}), 154.0 (6-C_{Chl}), 148.8 (9-C_{Chl}), 144.7 (8-C_{Chl}), 144.0 (1-C_{Trz}), 138.9 (2-C_{Chl}), 136.0 (7-C_{Chl}), 135.2 (14-C_{Chl}), 135.1 (12-C_{Chl}), 134.8 (4-C_{Chl}), 134.6 (3-C_{Chl}), 130.2 (1-C_{Chl}), 129.9 (11-C_{Chl}), 129.4 (3^1-C_{Chl}), 127.8 (13-C_{Chl}), 123.8 (2-C_{Trz}), 121.7 (3^2-C_{Chl}), 101.3 (15-C_{Chl}), 101.2 (10-C_{Chl}), 101.0 (1-C_{Gal}), 100.1 (1-C_{Glc}), 98.8 (5-C_{Chl}), 93.7 (20-C_{Chl}), 76.1 (4-C_{Glc}), 72.9 (3-C_{Glc}), 72.5 (5-C_{Glc}), 71.5 (2-C_{Glc}), 70.9 (3-C_{Gal}), 70.6 (5-C_{Gal}), 69.1 (2-C_{Gal}), 67.8 (3^2-C_{Trz}), 66.6 (4-C_{Gal}), 61.8 (6-C_{Glc}), 60.6 (6-C_{Gal}), 53.1 (17-C_{Chl}), 52.1 (15^4-C_{Chl}), 51.5 (17^5-C_{Chl}), 50.0 (3^1-C_{Trz}), 52.1 (15^4-C_{Chl}), 49.2 (18-C_{Chl}), 38.0 (15^1-C_{Chl}), 36.1 (1^1-C_{Trz}), 31.1 (17^2-C_{Chl}), 29.7 (17^1-C_{Chl}), 23.0 (18^1-C_{Chl}), 20.8–20.5 [7OAc (CO–$Me_{Gal,Glc}$)], 19.6 (8^1-C_{Chl}), 17.6 (8^2-C_{Chl}), 12.1 (2^1-C_{Chl}), 12.0 (12^1-C_{Chl}), 11.3 (7^1-C_{Chl}). MS (MALDI), *m*/*z*: 1367.63 (M^+). HRMS, calc.: 1366.5493; found: 1367.557 ($M + H^+$). UV-VIS [CH_2Cl_2, λ_{max}/nm (ε/dm^3 mol^{-1} cm^{-1})]: 390 (116000), 500 (11500), 528 (4300), 560 (2000), 606 (4200), 662 (33000). IR (KBr, ν/cm^{-1}): 3200 (C–H triazole), 1602 (C=C triazole), 1753 (C=O ester), 1654 ('amide-I'), 1515 ('amide-II').

Glycoconjugate **8**: yield 97%. MS (MALDI), *m*/*z*: 1073 (M^+).

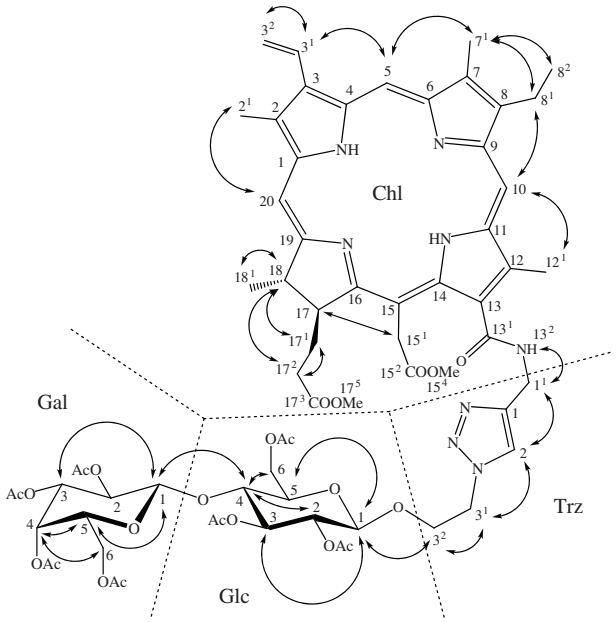


Figure 1 Primary, steric and tautomeric structure of compound **I**. Dashed lines separate residues: chlorine e_6 (Chl), Triazole and spacers (Trz), β-D-Glucopyranose (Glc), β-D-Galactopyranose (Gal). Observed 1H–1H NOEs are marked with biheaded arrows, including those not mentioned in the text.

from the cross-peaks H3^1-H3^{2a,b} in COSY and demonstrated an *inter*-residue correlation with Glc H1 in the HMBC spectrum, which confirmed the glycosidic linkage between C3^2 and Glc C1. The conformation of this region (protons point to one side) followed from Glc H1–Trz H3^2 *inter*-residue correlation in ROESY. The rest carbon signals of the residue were assigned using HSQC data.

The assignment of chlorine e_6 signals (Chl) started from H5, H10 and H20. H20 was distinguished by the characteristic chemical shift, and H5 was distinguished from H5–H3^1 correlation in ROESY, which also revealed the orientation of the vinyl group. Other observed spatial correlations (H20–H18, H20–H2^1, H5–H7^1, H7^1–H8^1, H8^1–H10, H10–H12^1) allowed assignment of H2^1, H7^1, H8^1, H8^1 (with COSY data), H12^1 to be accomplished. Together with signals of the meso-protons these signals were the basis for ^{13}C NMR spectrum assignment from far hetero-nuclear correlations in HMBC. The aliphatic chains at the 15-, 17- and 18-positions were separated using HMBC data from ring carbons and assigned from COSY (H17–H17^1–H17^2) and HMBC (from carboxymethyl groups) data. The ROESY spectrum showed the following correlations which confirmed the conformation of sp^3 nodes at 15^1-, 17- and 18-positions: H15^{1a}–H17, H17^{1a,b}–H18, H17^{2a}–H18, H18–H18^1.

Thus, the structure, tautomeric form and conformation shown in Figure 1 was proven for compound **I**. However, dihedral of 13^1–13^2 bridge could not be determined from the NMR data, and the position of the Trz–Glc–Gal chain against the chlorine e_6 residue remains unknown.

The photophysical properties of resulting conjugate **7** and the starting propargylamide of bacteriochlorin *e* **2** have been studied. The lactose residue does not affect the quantum yields of fluorescence ($\varphi \sim 0.25$) and singlet oxygen ($\varphi^1O_2 \sim 0.75$). The high generation of singlet oxygen in combination with the enhanced selectivity of accumulation in cancer cells due to the availability of a lactose residue in the molecule might make this glycoconjugate a promising pharmaceutical for the photodynamic therapy of cancer.

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References

1 R. Huisgen, in *1,3-Dipolar Cycloaddition Chemistry*, ed. A. Padwa, Wiley, New York, 1984.
2 H. C. Kolb and B. Sharpless, *Drug Discovery Today (DDT)*, 2003, **8**, 1128.
3 V. D. Bock, H. Hiemstra and J. H. van Maarseveen, *Eur. J. Chem.*, 2006, 51.
4 C. W. Tornoe, C. Christensen and M. Meldal, *J. Org. Chem.*, 2002, **67**, 3057.
5 P. Sears and C. H. Wong, *Angew. Chem., Int. Ed. Engl.*, 1999, **38**, 2301.
6 S. H. Barondes, V. Castronovo, D. N. W. Cooper, R. D. Cummings, K. Drickamer, T. Feizi, M. A. Gitt, J. Hirabayashi, C. Hughes, K. Kasai, H. Leffler, F. Liu, R. Lotan, A. M. Mercurio, M. Monsigny, S. Pillai, F. Poirer, A. Raz, P. W. J. Rigby, J. M. Rini and J. L. Wang, *Cell*, 1994, **76**, 597.
7 G. Zheng, A. Graham, M. Shibata, J. R. Missert, A. R. Oseroff, T. J. Dougherty and R. K. Pandey, *J. Org. Chem.*, 2001, **66**, 8709.
8 I. Laville, S. Pigaglio, J.-C. Blais, F. Doz, B. Loock, P. Maillard, D. S. Grierson and J. Blais, *J. Med. Chem.*, 2006, **49**, 2558.
9 G. Zheng, M. Aoudia, D. Lee, M. A. Rodgers, K. M. Smith, T. J. Dougherty and R. K. Pandey, *J. Chem. Soc., Perkin Trans. 1*, 2000, 3113.
10 P. E. Cheshev, E. A. Khatuntseva, Yu. E. Tsvetkov, A. S. Shashkov and N. E. Nifant'ev, *Bioorg. Khim.*, 2004, **30**, 68 (*Russ. J. Bioorg. Chem.*, 2004, **30**, 123).
11 M. G. H. Vicente and K. M. Smith, *J. Org. Chem.*, 1991, **56**, 4407.
12 A. S. Brandis, A. N. Kozyrev and A. F. Mironov, *Tetrahedron*, 1992, **48**, 6485.

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