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Synthesis, Characterization and Photocytotoxicity of a Glycoconjugated *meso*-Monoarylbenzochlorin

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Abstract: Amphiphilic glycoconjugated benzochlorin was prepared efficiently from meso monoaryl porphyrin and 3-(dimethylamino)acrolein by regiospecific Vilsmeier's reaction followed by cyclisation under acidic conditions and glycosylation. This compound displays a good in vitro photocytotoxicity on tumor cell lines after irradiation with light > 590 nm. © 1997 Elsevier Science Ltd.

In the active field of photodynamic therapy, the design of new photosensitizers having well-defined structure with amphiphilic properties, high selectivity for tumor cells, quick elimination from healthy cells and strong absorption in the red region of visible spectrum is an important challenge for chemists[1]. Syntheses of many tetrapyrrolic compounds such as purpurins[2], chlorins[3], phthalocyanins[4] and benzochlorins[5] have been developed. Smith *et al.*[6], Gunter *et al.*[7] and more recently Dolphin *et al.*[8] reported the syntheses of a series of 5,15-diaryl substituted benzochlorins by electrophilic Vilsmeier formylation[9] of symmetrical nickel 5,15-diphenylporphyrins, followed by cyclisation under acidic conditions to the single possible benzochlorin. Furthermore, Kohli *et al.*[10] have described the preparation of functionalized benzochlorins from *meso*-unsubstituted porphyrins. However the possibility to prepare *meso*-monosubstituted benzochlorins from *meso*-monosubstituted porphyrins following the same strategy has so far never been explored.

In this paper, we wish to report the efficient regioselective preparation of an amphiphilic glycoconjugated *meso*-monoaryl-benzochlorins. Nickel (II) porphyrin **1** (scheme) was obtained by cyclo-condensation of 2, 3, 7, 8, 12, 13, 17, 18-octaethyl-1'-8'-dideoxy-a-c-biladiene hydrobromide[11] on *para*-methoxy benzaldehyde according to the method of Harris *et al.*[12] and then metallation with nickel acetate in methanol. Electrophilic substitution with 3-(dimethylamino)acrolein under Vilsmeier's conditions, led to the two isomeric nickel(II) complexes **2** and **3** (total yield 85%, ratio **2/3**, 85.5/14.5)[13], in which the 2"-formylvinyl group is linked either at the adjacent *meso*-carbon (C_{10}) or at the opposite (C_{15}) to the *meso*-aryl position. The structure of each *meso*-(2"-formylvinyl)porphyrin was determined by ^{1}H NMR studies[14]. Treatment of porphyrin **2**, by trifluoroacetic acid under argon atmosphere at room temperature, afforded nickel(II) benzochlorin **4** in 58% yield. HPLC analysis and ^{1}H NMR studies showed the presence of a single compound **4** corresponding exclusively to one of the two possible nickel monoarylbenzochlorins[15]. Dealkylation of the methoxy group by boron tribromide[16] in dry methylene chloride afforded complex **5** in 73 % yield[17]. Demetallation in concentrated sulfuric acid of **5** gave the metal-free benzochlorin **6**[18] in 70% yield. Glycosylation of **6** was performed, in dimethylformamide in the presence of potassium carbonate, by 1-bromoethoxy-per-acetyl-maltose **9** available from condensation of *per*-acetylated maltose with 1-bromo ethanol using boron trifluoride diethyl etherate in dry methylene chloride[19]. This afforded glycosylated benzochlorin **7**[20] in 95% yield. Glycoconjugated derivative **8**[21] was obtained from **7** by deacetylation of maltose moieties by the method of Zemplén *et al.*[22] in quantitative yield.

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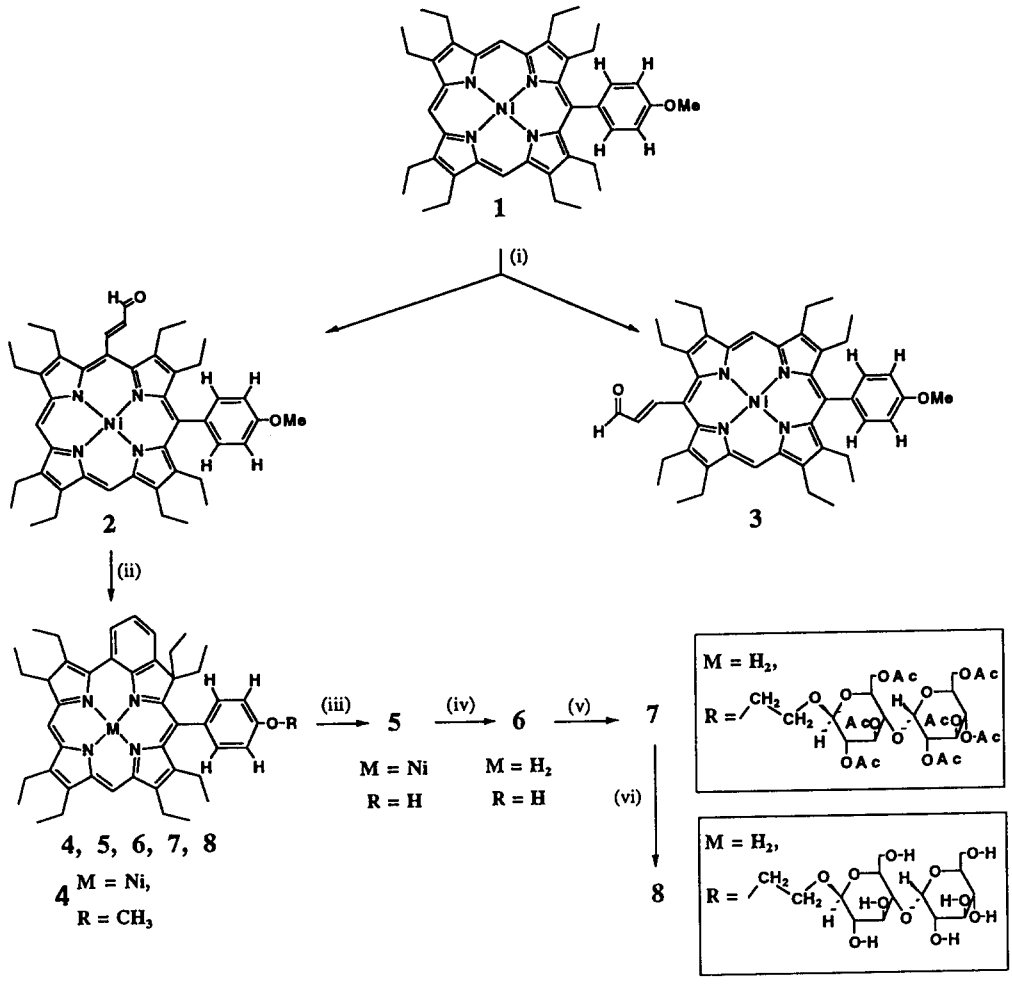
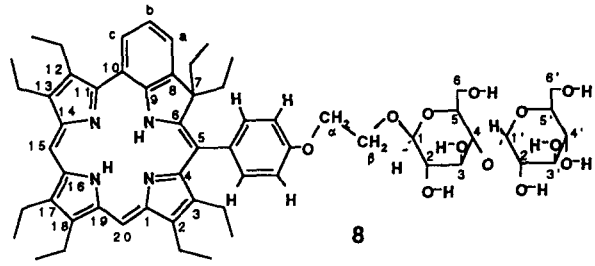


Reagents : (i) 3-(dimethylamino)acrolein /$POCl_3$, (ii) CF_3CO_2H/Ar, (iii) BBr_3/dry CH_2Cl_2, (iv) H_2SO_4, (v) 9 and K_2CO_3 in DMF/60°, (vi) MeONa/MeOH .

Scheme : Synthesis of glycoconjugated benzochlorin

The UV characteristics of **6-8** have absorptions similar to those of Gunter[7]. Our monophenyl compound has not lost the shift and the increased absorbance in the red region which were seen with compounds bearing two phenyl groups.

1D and homonuclear 2D ^1H NMR studies enabled us to confirm the structures. NOESY cross correlation peak were seen between ethyl groups carried by carbon 7 and the two *ortho* protons of the *meso*-phenyl group (figure). Moreover ^1H NMR 2D spectra of benzochlorin **7** showed NOE interactions between the ten protons (1.96 ppm CH_2 ethyl and -0.02 ppm CH_3) of the C_7 ethyl and $H_{2'}$ and $H_{6'}$ *ortho* protons of the *meso*-phenyl group (7.79 ppm). Such a behaviour corresponds to a cyclisation of the C_5 *meso* carbon atom on the nearest pyrrole.



8

Structure and numbering of glycoconjugated benzochlorin **8**

To evaluate the influence of a sugar substitution on the photobiological activity of benzochlorin **8**, its photocytotoxicity was determined and compared with that obtained with sugar-free benzochlorin **6** (Table). **6** did not show any cytotoxicity and photocytotoxicity while **8**, irradiated either with white light (IC_{50} = 8 μM) or above 590 nm light (IC_{50} = 5.7 μM) showed significant photocytotoxicity. Although the fluence was lower in the last case and may affect the results, it is interesting to note that a red light irradiation appears to be more efficient than a full spectrum one.

Table

Survival Fraction of HT 29 Tumor Cells.

Compound	Dose μg/ML	Survival fraction % of controls without light [a]	Survival fraction % of controls, white light [a] [b]	Survival fraction % of controls, light (λ>590 nm)[a] [c]
6	10	94 (2.3)	110 (6.3)	83 (6.8)
	5	88 (7)	116 (2.3)	85 (3.0)
	2	92 (1.0)	113 (13.5)	88 (7.5)
	1	86 (3.7)	115 (10.2)	85 (1.9)
8	10	96 (5.9)	37 (19.2)	15 (1.5)
	5	86 (3.3)	75 (14.1)	55 (9.4)
	2	104 (0.6)	109 (1.2)	85 (10.4)
	1	101 (7.2)	115 (2)	95 (3.2)

[a] Standard deviation, [b] Total dose 2.3 J / cm^2, fluence 3.8 mW / cm^2, [c] 520 nm 0% T, 590 nm 80% T, dose 2.5 J / cm^2, fluence 2 mW / cm^2. HT29 cells were grown in DMEM supplemented with 10% FCS. Surviving fraction was estimated using the MTT assay

In summary, an amphiphilic glycoconjugated benzochlorin with spectroscopic properties suitable for use in photodynamic therapy can be prepared in good yield from a-c biladiene. This compound displays a good photocytotoxicity *in vitro* on HT 29 tumor cells after irradiation with red light > 590 nm.

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13 Ratio determined by HPLC analysis using a Gilson apparatus with a dynamic mixer module Gilson 811, a manometric module Gilson 802, a pump Gilson 303 and a holochrom module Gilson (detection at 450 nm). Column: Hibar Lichrosorb SI 60, 7-µm Merck eluted by a mixture heptane / methylene chloride (1.5 ml/mn). HPLC gradient (time mn: % heptane): t = 0: 80%, t = 15: 50%, t = 49: 80%.

14 **2** ^{1}H NMR ($CDCl_3$) δ: 9.71 (d, *J* = 8 Hz, 1H, CHO), 9.24 (d, *J* = 15 Hz, 1H, H_α vinyl), 9.14 (s, 1H, H *meso*), 9.08 (s, 1H, H *meso*), 7.76 (d, *J* = 8 Hz, 2H, phenyl), 7.09 (d, *J* = 8 Hz, 2H, phenyl), 5.63 (dd, *J* = 8 and 15 Hz, 1H, Hβ vinyl), 3.64 (m, 12H, CH_2), 2.46 (m, 4H, CH_2), 1.65 (m, 18H, CH_3), 1.02 (t, *J* = 7.3 Hz, 3H, CH_3), 0.44 (t, *J* = 7.3 Hz, 3H, CH_3). UV-vis. spectrum in CH_2Cl_2: λ_{max}, nm (ε, L mmol^{-1} cm^{-1}): 454 (101.9), 548 (7,6), 618.5 (11.6). **3** ^{1}H NMR ($CDCl_3$) δ: 9.77 (d, *J* = 8 Hz, 1H, CHO), 9.29 (d, *J* = 15 Hz, 1H, H_α vinyl), 9.16 (s, 2H, H *meso*), 7.75 (d, *J* = 8 Hz, 2H, phenyl), 7.07 (d, *J* = 8 Hz, 2H, phenyl), 5.56 (dd, *J* = 8 and 15 Hz, 1H, Hβ vinyl), 4.0 5(s, 3H, OMe), 3.67 (m, 12H, CH_2), 2.60 (q, 4H, CH_2), 1.65 (m, 18H, CH_3), 0.91 (t, *J* = 7.3 Hz, 6H, CH_3). UV-vis. spectrum in CH_2Cl_2: λ_{max}, nm (ε, L mmol^{-1} cm^{-1}): 420 (65.5), 448.5 (77.1), 548 (6.7), 581 (8), 608 (8.2).

15 **4** ^{1}H NMR ($CDCl_3$) δ: 8.75 (m, 2H, H *meso*, and H_c benzo), 8.40 (s,1H, H *meso*), 7.62 (d, *J* = 8 Hz, 2H, phenyl), 7.64 (m, 2H, H_a and H_b benzo), 6.96 (d, 2H, *J* = 8 Hz, phenyl), 3.96 (s, 3H, OMe), 3.40 (m, 8H, CH_2), 2.11 (q, 2H, CH_2), 1.88 (q, 2H, CH_2), 1.55 (m, 12H, CH_3), 0.89 (t, 3H, CH_3), 0.63 (t, 3H, CH_3), 0.06 (t, 6H, CH_3). UV-vis. spectrum in CH_2Cl_2: λ_{max}, nm (ε, L mmol^{-1} cm^{-1}): 429.5 (70), 523.5 (shoulder), 590 (shoulder), 642 (shoulder), 693.5 (31.1).

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17 **5** UV-vis. spectrum in CH_2Cl_2: λ_{max}, nm (ε, L mmol^{-1} cm^{-1}): 429.5 (92.7), 523.5 (shoulder), 647 (shoulder), 693 (43.2).

18 **6** ^{1}H NMR ($CDCl_3$) δ: 9.22 (d, *J* = 8 Hz, 1H, H_c benzo), 8.89 (s, 1H, H *meso*), 8.35 (s, 1H, H *meso*), 7.89 (t, *J* = 7.8 Hz, 1H, H_b benzo), 7.74 (d, *J* = 8 Hz, 2H, H *ortho* phenyl), 7.72 (d, *J* = 8 Hz, 1H, H_a benzo), 6.96 (d, *J* = 8 Hz, 2H, H *meta* phenyl), 3.69 (q, 2H, CH_2), 3.63 (q, 2H, CH_2), 3.43 (q, *J* = 7.6 Hz, 6H, CH_2), 2.70 (broad, 1H, OH), 2.30 (s, 1H, NH), 2.25 (q, 2H, CH_2), 2.18 (q, 2H, CH_2), 1.99 (m, 2H, CH_2), 1.73 (t, *J* = 7.4 Hz, 3H, CH_3), 1.55 (m, 12H, CH_3), 0.92 (t, *J* = 7.2 Hz, 3H, CH_3), -0.02 (t, *J* = 7.2 Hz, 6H, CH_3). UV-vis. spectrum in CH_2Cl_2: λ_{max}, nm (ε, L mmol^{-1} cm^{-1}): 418 (95.4), 548.5 (7.4), 581.5 (9.6), 618 (1.1), 673 (26.1).

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20 **7** ^{1}H NMR ($CDCl_3$) δ: 9.22 (d, *J* = 8.5 Hz, 1H, H_c benzo), 8.88 (s, 1H, H *meso*), 8.36 (s, 1H, H *meso*), 7.90 (t, *J* = 8 Hz, 1H, H_b benzo), 7.79 (d, *J* = 8.25 Hz, 2H, *ortho* phenyl), 7.69 (d, *J* = 8 Hz, 1H, H_a benzo), 7.01 (d, *J* = 8.25 Hz, 2H, *meta* phenyl), 5.45 (d, *J* = 4 Hz, 1H, $H_{1'}$ Malt), 5.39 (dd, *J* = 10 Hz, 1H, $H_{3'}$ Malt), 5.34 (dd, *J* = 9 Hz, 1H, H_3 Malt), 5.07 (t, *J* = 10 Hz, 1H, $H_{4'}$ Malt), 4.95 (d, *J* = 8 Hz, 1H, H_2 Malt), 4.88 (dd, *J* 2'-1'= 4 Hz, *J* 2'-3'= 11 Hz, 1H, $H_{2'}$ Malt), 4.80 (d, *J* = 8 Hz, 1H, H_1 Malt), 4.57 (dd, 1H, H_6 Malt), 4.29 (m, 2H, $CH_2\alpha$), 4.27 (dd, 2H, $H_{6'}$ and H_6 Malt), 4.07 (m, 2H, H_4 and $H_{6'}$ Malt), 3.99 (dd, *J* 5'-6'= 2.5 Hz, *J* 5'-4'= 10 Hz, 1H, $H_{5'}$ Malt), 3.99 (m, 2H, $CH_2\beta$), 3.78 (dd, 1H, H_5 Malt), 3.69 (q, *J* = 7.5 Hz, 2H, CH_2 C_{12}), 3.62 (q, *J* = 7.5 Hz, 2H, CH_2 C_2), 3.45 (q, *J* = 7.5 Hz, 2H, CH_2 C_{18}), 3.42 (q, *J* = 7.5 Hz, 4H, CH_2 C_{13} and C_{17}), 2.19 (q, *J* = 7.5 Hz, 2H, CH_2 C_3), 2.15 (m, 2H, CH_2 C_7), 1.96 (q, *J* = 7.5 Hz, 2H, CH_2 C_7), 1.73 (t, *J* = 7.5 Hz, 3H, CH_3 C_{12}), 1.58 (t, *J* = 7.5 Hz, 6H, CH_3 C_{17} and C_{18}), 1.52 (t, *J* = 7.5 Hz, 6H, CH_3 C_2 and C_{13}), 0.90 (t, *J* = 7.5 Hz, 3H, CH_3 C_3), -0.02 (t, *J* = 7.5 Hz, 6H, CH_3 C_7), UV-vis. spectrum in CH_2Cl_2: λ_{max}, nm (ε, L mmol^{-1} cm^{-1}): 418 (74.5), 548.5 (shoulder), 581.5 (7.9), 618.5 (9), 673 (22.7).

21 **8** ^{1}H NMR (pyridine d_5) δ: 9.50 (d, *J* = 8 Hz, 1H, H_c benzo), 9.26 (s, 1H, H *meso*), 8.68 (s, 1H, H *meso*), 8.12 (t, *J* = 7.5 Hz, 1H, H_b benzo), 7.96 (d, *J* = 8.5 Hz, 2H, *ortho* phenyl), 7.89 (d, *J* = 7 Hz, 1H, H_a benzo), 7.49 (m, 2H, OH C_2 and C_3), 7.48 (t, 1H, OH $C_{2'}$), 7.24 (d, *J* = 9 Hz, 2H, *meta* phenyl), 7.10 (m, 2H, OH $C_{3'}$ and $C_{4'}$), 6.38 (t, 1H, OH C_6), 6.32 (t, 1H, OH $C_{6'}$), 5.95 (d, *J* = 4 Hz, 1H, $H_{1'}$ Malt), 4.95 (d, *J* = 8 Hz, 1H, H_1 Malt), 4.61 (dd, *J* = 10 Hz, 1H, $H_{3'}$ Malt), 4.54 (t, 1H, H_5 Malt), 4.51 (dd, *J* = 5 Hz, 2H, H_6 Malt), 4.47 (t, *J* = 2.5 Hz, 2H, $CH_2\alpha$), 4.40 (m, 2H, H_3 and H_4 Malt), 4.39 (t, 2H, $CH_2\beta$), 4.36 (m, 2H, $H_{6'}$ Malt), 4.21 (t, 1H, $H_{4'}$ Malt), 4.19 (dd, 1H, $H_{2'}$ Malt), 4.09 (m, 1H, H_2 Malt), 3.87 (m, 1H, $H_{5'}$ Malt), 3.67 (t, *J* = 7.5 Hz, 2H, CH_2 C_{12}), 3.63 (t, *J* = 7.5 Hz, 2H, CH_2 C_{12}), 3.49 (t, *J* = 7.5 Hz, 2H, CH_2), 3.45 (q, *J* = 7.5 Hz, 2H, CH_2), 2.38 (q, 4H, CH_2 C_7 and C_3), 2.14 (m, 2H, CH_2 C_7), 1.72 (t, *J* = 7.5 Hz, 3H, CH_3 C_{12}), 1.66 (t, *J* = 7.5 Hz, 3H, CH_3), 1.65 (t, *J* = 7.5 Hz, 3H, CH_3), 1.58 (t, *J* = 7.5 Hz, 3H, CH_3), 1.57 (t, *J* = 7.5 Hz, 3H, CH_3), 1.03 (t, *J* = 7.5 Hz, 3H, CH_3 C_3), 1.20 and 0.82 (m, 3H, CH_3), 0.15 (t, *J* = 7.5 Hz, 3H, CH_3 C_7), UV-vis. spectrum in MeOH: λ_{max}, nm (ε, L mmol^{-1} cm^{-1}): 415 (68.9), 546 (shoulder), 582 (10.2), 618 (11.3), 672 (24.3).

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