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SYNTHESIS AND INVESTIGATION OF A GALACTOPYRANOSYL-CHOLESTERYLOXY SUBSTITUTED PORPHYRIN

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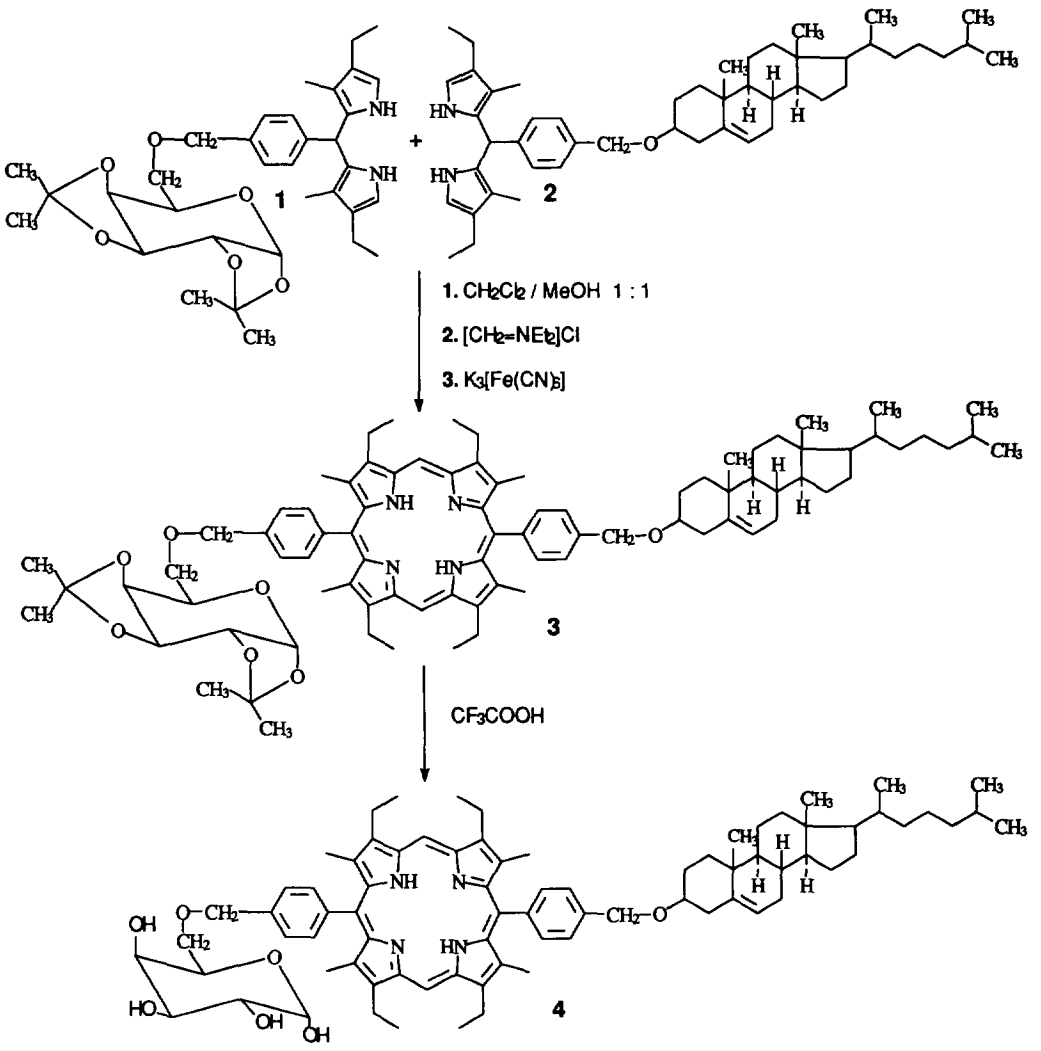
Abstract: The synthesis of a new carbohydrate cholesterol substituted porphyrin is described. Due to its amphiphilic character this compound is easily built into model membranes. Furthermore, the vesicle forming properties of compound **4** were investigated by light scattering experiments and electron microscopy.

One of the most interesting applications of porphyrins is photodynamic therapy (PDT) of tumours.[1] This treatment is based on the high selectivity of porphyrinic compounds to tumour tissue and on the production of singlet oxygen by irradiation of the porphyrin photosensitizer with visible light. Thus, formation of singlet oxygen in tumour cells causes cell death and tumour necrosis. Besides long wavelength absorption solubility in water is one of the requirements that a photosensitizer must fulfill. Therefore a number of carbohydrate substituted porphyrins were synthesized.[2] It was shown recently that some carbohydrate substituted porphyrins exhibit enhanced selectivity to cancer cells.[3] Although the mechanism of sensitizer uptake is not yet clarified, there is evidence that hydrophobic and amphiphilic porphyrins associate strongly to LDL and are introduced into the tumour cell by receptor mediated endocytosis.[4] Amphiphilic porphyrins may be also incorporated into plasma membranes of tumour cells. Incorporation of a photosensitizer into a plasma membrane leads to a high quantum yield of cell deactivation as shown recently by Shulok et al.[5]

We herein describe the synthesis and some preliminary investigations of a new amphiphilic porphyrin derivative that exhibits very interesting chemical and physical properties. The synthesis was performed by coupling of a carbohydrate substituted dipyrrylmethane (**1**) with a cholesteryloxy substituted dipyrrylmethane (**2**) by an aminomethylation procedure [6] and oxidation with $K_3[Fe(CN_6)]$.[7] Porphyrin **3** was isolated in 5% yield after chromatographic work up. Deprotection of the carbohydrate moiety was achieved by reaction with CF_3COOH/ H_2O in nearly quantitative yield (Scheme 1). Spectroscopic and analytical data were in accordance with the assumed structure of compounds **3** and **4**.[8]



Scheme 1

Due to its amphiphilic character compound **4** could be easily introduced into phosphatidyl ethanolamine vesicles. Phosphatidyl ethanolamine vesicles have been often used as a model system for cell membranes. We were able to incorporate up to 5% of porphyrin **4** into these vesicles without significant aggregation. The absorption spectrum of a water solution of porphyrin containing vesicles exhibited a normal Soret absorption at 407 nm and four Q-bands in the region of 505 to 630 nm. Thus, no significant aggregation of porphyrins in the membrane could be detected by

absorption spectroscopy. Formation of vesicles was proved by light scattering experiments (vesicle diameter 250 nm) and measurement of the K^+-diffusion potential.

Injection of a DMSO solution of **4** into water (dilution 1:1000) led to the formation of vesicle-like structures or aggregates. In the optical spectra of this solution the Soret band is split into a blue-shifted (375 nm) and a red-shifted (480 nm) absorption. The red-shifted absorption is dominant (E_{375} / E_{480} = 1:1.6). It is well known that a blue-shifted absorption could be attributed to a face-to-face orientation and a red-shifted absorption is due to an edge-to-edge orientation. The appearance of both a red-shifted absorption and a blue-shifted absorption leads to the conclusion that no defined structure in the aggregates is formed. Nevertheless, face-to-face orientation seems to be of minor importance. Furthermore, we were able to obtain defined vesicles formed by compound **4** using a different method. Dilution of 5 mg of **4** in 10 ml of a SDS (sodium dodecylsulfate) solution at 60°C and filtration (1 µm Microfilter) led to a clear solution. Vesicles were formed after dialysis and ultrasound treatment (Branson Sonifier, 30% pulse, 50W) of this solution. Light scattering experiments and electron microscopy (Jeol JSM-840 Scanning Microscope) revealed that spherical vesicles of 500 nm diameter were formed (Figure 1). Interestingly, no splitting of the Soret absorption (407 nm) was observed. The lack of Soret splitting might be due to incorporation of several SDS molecules acting as spacer groups between the porphyrin chromophores. In marked contrast to this, the Soret absorption band is split into a blue-shifted (386 nm) and a red-shifted (441 nm) absorption (E_{386} / E_{441} = 1:1.9) if CHAPS (3[(3-cholamidopropyl)dimethylammonio]-1-propane-sulfonate) is used instead of SDS. Formation of vesicles under these conditions was proved by measurement of the K^+-diffusion potential. Nevertheless, more detailed investigations are necessary to clarify the molecular structure of the obtained vesicles.

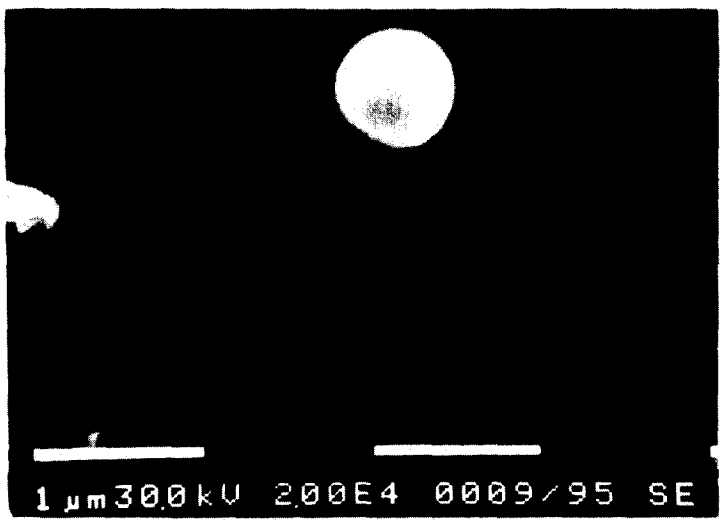


Figure 1: Electron micrograph of vesicles formed by **4**. Magnification: 20000

In summary, we have synthesized a new type of amphiphilic porphyrin that exhibits very interesting properties. These properties are of considerable interest for the design of new potential agents for photodynamic cancer treatment. Work is underway in our laboratories to elucidate the structures of the vesicles and to obtain information upon the interaction between the vesicles formed by **4** and different serum proteins.

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8. **3**: FAB-MS: m/e = 1300 (M^+ + 1).- UV-vis (CH_2Cl_2): λ (log ε) = 407 (5.329), 506 (4.217), 539 (3.690), 573 (3.820), 625 (2.954) nm.- ^{1}H NMR (500 MHz, $CDCl_3$): δ = -2.4 (br s, 2 H, NH), 0.60-2.40 (m, 28 H, Chol), overlapped by: 0.64 (s, 3 H, Chol-19-H), 0.81 (d, *J* = 7.3 Hz, 6 H, Chol-26-H and Chol-27-H), 0.86 (d, *J* = 6.4, 3 H, Chol-21-H), 1.03 (s, 3 H, Chol-18-H), 1.32 (s, 3 H, Gal-CH_3), 1.36 (s, 3 H, Gal-CH_3), 1.48 (s, 3 H, Gal-CH_3), 1.58 (s, 3 H, Gal-CH_3), 1.69 (t, *J* = 7.6 Hz, 12 H, Por-CH_2-CH_3), 2.40 (s, 12 H, Por-CH_3), 3.45 (m, 1 H, Chol-3-H), 3.80-4.02 (m, 10 H, Por-CH_2-CH_3, 2 H, Gal-6-H), 4.15 (dt, *J* = 6.4 Hz, *J* = 2.0 Hz,1 H, Gal-5-H), 4.33 (dd, *J* = 4.9 Hz, *J* = 2.4 Hz, 1 H, Gal-2-H), 4.38 (dd, *J* = 7.8 Hz, *J* = 2.0 Hz, Gal-4-H), 4.63 (dd, *J* = 7.8 Hz, *J* = 2.4 Hz, 1 H, Gal-3-H), 4.85 (d, *J* = 12.7 Hz, 1 H, CH_2-O-Gal), 4.88 (s, 2 H, -CH_2-O-Chol), 4.95 (d, *J* = 12.7 Hz, 1 H, -CH_2-O-Gal), 5.37-5.40 (m, 1 H, Chol-6-H), 5.59 (d, *J* = 4.9 Hz, 1 H, Gal-1-H), 7.65 (d, *J* = 7.8 Hz, 4 H, H_m, $H_{m'}$), 7.97 (d, *J* = 7.8 Hz, 4 H, H_o, $H_{o'}$), 10.15, (s, 2 H, Por-10-H, Por-20-H).- ^{13}C NMR (125 MHz, $CDCl_3$): δ = 11.88 (q, Chol-C-18), 14.53 (q, Por-CH_3), 14.58 (q, Por-CH_3), 17.59 (q, Por-CH_2-CH_3), 18.72 (q, Chol-C-21), 19.48 (q, Chol-C-19), 19.92 (t, Por-CH_2-CH_3), 21.12 (t, Chol-C-11), 22.57 (q, Chol-C-26), 22.82 (q, Chol-C-27), 23.83 (t, Chol-C-23), 24.31 (t, Chol-C-15), 24.56 (q, C(CH_3)$_2$), 25.00 (q, C(CH_3)$_2$), 26.07 (q, C(CH_3)$_2$), 26.20 (q, C(CH_3)$_2$), 28.03 (d, Chol-C-25), 28.25 (t, Chol-C-12), 28.72 (t, Chol-C-2), 31.94 (d, Chol-C-8), 32.01 (t, Chol-C-7), 35.79 (d, Chol-C-20), 36.17 (t, Chol-C-22), 36.99 (s, Chol-C-10), 37.43 (t, Chol-C-1), 39.42 (t, Chol-C-4), 39.52 (t, Chol-C-24), 39.80 (t, Chol-C-16), 42.36 (s, Chol-C-13), 50.24 (d, Chol-C-9), 56.14 (d, Chol-C-17), 56.80 (d, Chol-C-14), 67.09 (d, Gal-C-5), 68.99 (t, Gal-C-6), 70.19 (t, CH_2-O-Chol), 70.63 and 70.75 (d, Gal-C-3, C-4), 71.38 (d, Gal-C-2), 73.28 (t, CH_2-O-Gal), 79.02 (d, Chol-C-3), 96.37 (d, Por-C-10, C-20), 96.47 (d, Gal-C-1), 108.05 (s, Por-C-5, C-15), 108.65 (s, C(CH_3)$_2$), 109.34 (s, C(CH_3)$_2$), 117.77 (s, Por-C-10), 117.83 (s, Por-C-20), 121.68 (d, Chol-6), 126.54 (d, $C_{m'}$), 126.70 (d, C_m), 132.79 (d, C_o, $C_{o'}$), 135.88 (s, Por-C_α), 138.40 (s, C_p), 139.38 (s, $C_{p'}$), 140.96 (s, Por-C_β), 141.05 (s, C_i), 141.24 (s, Chol-C-5), 141.43 (s, Por-C_β), 144.52 (s, $C_{i'}$), 145.15 (s, Por-C_α).- Anal. Calc. for $C_{85}H_{112}N_4O_7 \cdot H_2O$ (1319.9): C 77.35, H 8.71, N 4.24. Found: C 76.99, H 8.79, N 4.06. **4**: FAB-MS: m/e = 1221 (M^+ + 1).- UV-vis (CH_2Cl_2): λ (log ε) = 409 (5.325), 507 (4.218), 541 (3.730), 573 (3.848), 624 (3.136) nm.- Anal. calc. for $C_{79}H_{104}N_4O_7 \cdot 3H_2O$ (1275.7): C 74.38, H 8.69, N 4.39. Found: C 74.20, H 8.32, N 4.40. Due to aggregation in organic solvents no well resolved NMR spectra could be obtained.

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