New strategy for targeting of photosensitizers. Synthesis of glycodendrimeric phenylporphyrins, incorporation into a liposome membrane and interaction with a specific lectin†

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Received (in Cambridge, UK) 15th September 2008, Accepted 27th October 2008
First published as an Advance Article on the web 19th November 2008
DOI: 10.1039/b816128c

Two glycodendrimeric phenylporphyrins were synthesized and their interaction with phospholipids was studied at the air–water interface and in liposome bilayers; such liposomes bearing glycodendrimeric porphyrin could constitute an efficient carrier for drug targeting in photodynamic therapy.

The incorporation of carbohydrates on tetrapyrrolic macrocyclic cores usable in photodynamic therapy (PDT) continues to be pursued vigorously by a number of research teams.[1] In our laboratories, efforts have been focused on the preparation and *in vitro* evaluation of the phototoxicity of a series of neutral glycoconjugated tetrapyrrolic macrocycles as potential photosensitizing agents for photodynamic therapy.[2] However, these compounds are usually poorly water-soluble molecules, and tend to form aggregates in the aqueous solution. This affects unfavourably both their formulation and bioavailability, and limits pharmacologic studies. Glycoconjugation modifies the amphiphilicity of macrocycles, and can favor their interaction with the tumor cell surface membrane.[3] Concerning this latter property, indications are that glycosylation provides the possibility for specific interaction of the resulting conjugate with lectin type receptors overexpressed in certain malignant cells.[4] Glycoconjugation can thus be a potentially effective strategy for targeting photosensitizers toward tumor cells.[5] The identification of the transport mechanisms through the biological membranes was a crux. However, it would be advantageous to use these mechanisms for the optimization of photosensitizer targeting towards tumor cells. In this context, the use of glycodendrimers as recognition motifs was very exciting. It has been widely accepted that carbohydrate–protein interactions play a crucial role in a large number of biological processes.[6] Since most proteins possess multiple carbohydrate-recognition domains and typically exist as oligomeric structures, this limitation is often overcome through multivalency.[7,8] Lectin receptors are multisubunit and multivalent proteins with many important biological functions. Due to the weak nature, in the millimolar range, of interactions between a single specific carbohydrate and a receptor protein subunit, nature uses cluster carbohydrates in order to obtain biologically meaningful affinities for the receptors. The cluster effect appears when the multivalent carbohydrates interact with more than one receptor binding site simultaneously and cooperatively, resulting in better cellular recognition. Several methods of carbohydrate clustering have been described, including the attachment of carbohydrates to natural scaffolds, synthetic polymers, synthetic glycopeptides or simple oligomerization through organic linkers.[9] Among these various ways, dendritic structures (glycodendrimers) are emerging as ligands for carbohydrate-binding proteins.[10] Due to rapid advances in this area, promising potential medicinal applications have appeared in the last ten years, including treatment of cancers.[11–13]

In a continuation of our work to prepare and study neutral targeting glycoconjugated photosensitizers, we report the synthesis, characterization and behavior in liposomes of glycodendrimers linked to *meso*-tetraaryl-porphyrins shown in Fig. 1.

Recently, Ballardini *et al.* described the synthesis of two symmetric dendrimers, incorporating tetrasubstituted porphyrin units as the core and, in one case, four benzyloylated or deprotected and in the other case, twelve acetylated-β-D-glucopyranosyl- or β-D-glucopyranosyl residues at the peripheries of the tetrapyrrolic macrocycle.[14] These unprotected tetrasubstituted molecules are very water-soluble. We have shown that an amphiphilic structure of the glycoconjugated photosensitizers induces a better photocytotoxicity *in vitro*.[5] With the aim of increasing this photoefficiency, we designed a new family of glycoconjugated photosensitizers bearing only one glycodendrimer moiety, with variable length for the spacer linking the carbohydrate to the porphyrin, on the *para* position of one *meso*-phenyl group.

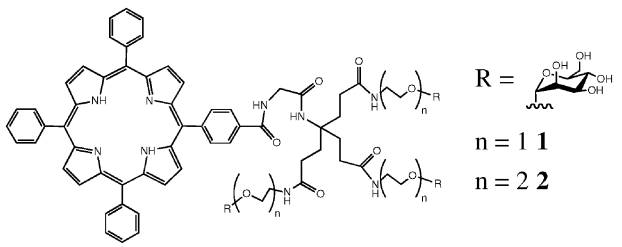

Fig. 1 Structures of glycodendrimeric porphyrins.

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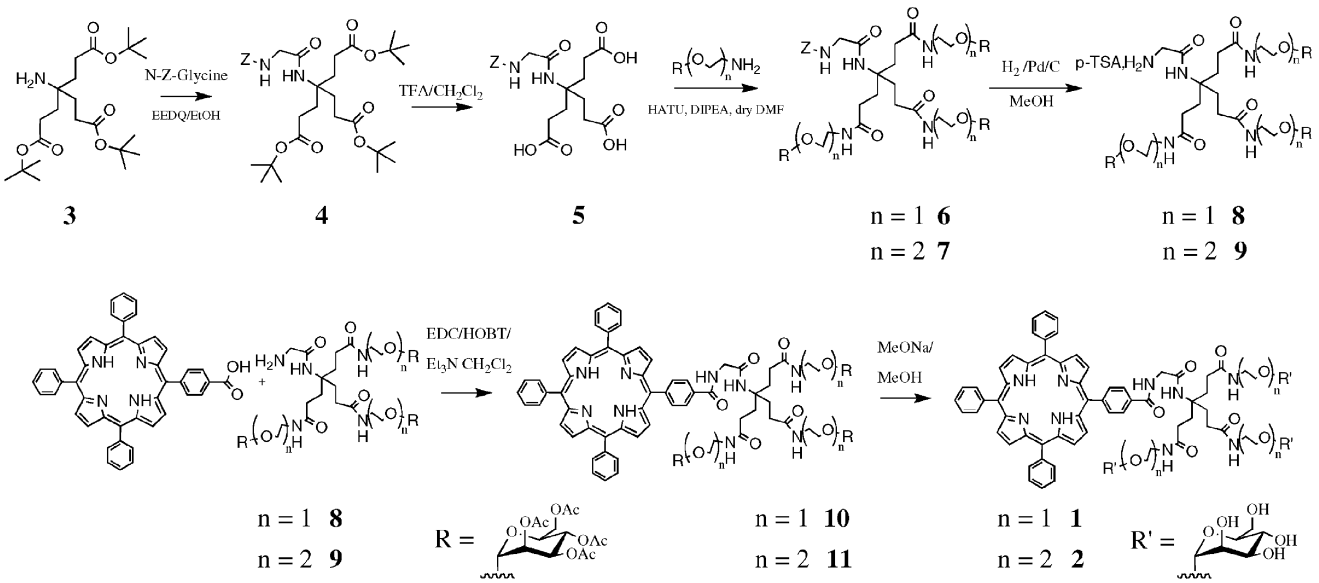
† Electronic supplementary information (ESI) available: Further experimental details. See DOI: 10.1039/b816128c



Scheme 1 Synthesis of glycodendrimeric porphyrins.

Synthesis of the glycodendrimeric porphyrins is shown in Scheme 1. *N*-Z-glycine was linked to **3** by EEDQ in ethanol (yield 86%) to give **4** then the *tertio*-butyl protection of carboxylic acid was removed by trifluoroacetic acid in methylene chloride to give compound **5** (92%). 2-Aminoethoxy-*O*-α-peracetyl-D-mannose, and 2-aminoethoxy-ethoxy-*O*-α-peracetyl-D-mannose prepared by the protocol described by Dahmen *et al.* and Sasaki *et al.*[15] were selected for peptide coupling with triacid **5**. HATU[16] promoted peptide coupling allowed the preparation of branched glycopeptides with good yields (**6** 70%, **7** 72%).[17] Catalytic hydrogenation was used for selective cleavage of benzyloxycarbonyl group into amine, in the presence of *p*-toluensulfonic acid (H_2–Pd/C 10%, MeOH) to afford the key building blocks with good yields (**8** 96%, **9** 82%). Dendrimeric moieties **8** and **9** were linked to 5-*para*-benzoic acid-10,15,20-triphenyl porphyrin using a mixture of HOBT, EDC and Et_3N with good or acceptable yields to give protected glycodendrimeric porphyrins **10**, and **11** (yield 86% and 57%, respectively) then were quantitatively O-deacetylated under Zemplén's conditions to afford glycodendrimeric porphyrins **1** and **2**.[18]

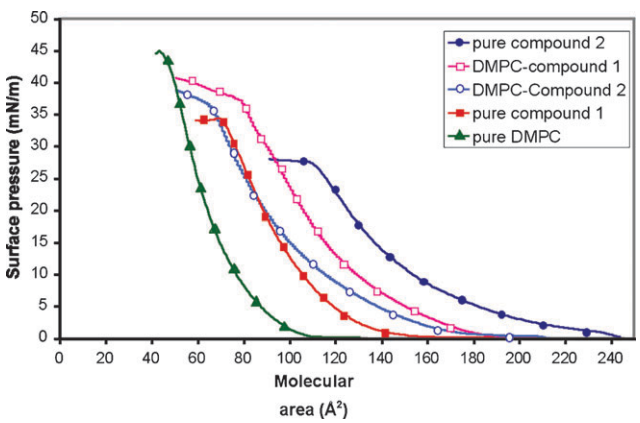


Fig. 2 Interfacial behaviour of mixed monolayers (1 : 1) of dimyristoylphosphatidylcholine and compounds **1** and **2**.

To evaluate the conditions of incorporation of compounds **1** and **2** into a liposome membrane, the two derivatives were mixed with dimyristoylphosphatidylcholine (DMPC) in a (1 : 1) ratio and the mixtures were spread at the air–water interface.[19,20] Fig. 2 shows that, whereas the isotherm of the DMPC-compound **2** mixed monolayer lies between those of the pure components, that of the mixed DMPC-compound **1** one is located at higher molecular areas and surface pressures. Obviously in both cases, the phospholipid and porphyrin derivatives interacted. However, if for compound **2**, this interaction was apparently attractive, for compound **1**, it was most probably repulsive. Thus, DMPC would mix better with compound **2** than with compound **1**.

The incorporation of compound **2** in liposome membranes led to the formation of larger vesicles than DMPC ones (Table 1). This is consistent with the π–A isotherms in Fig. 2, which show an expansion of the phospholipid monolayer in the presence of compound **2**. For DMPC liposomes bearing compound **1**, vesicles appeared smaller and less stable with time than those prepared from DMPC or mixtures of DMPC and compound **2**. This is also in agreement with the results in Fig. 2 that show an unfavourable interaction between DMPC and compound **1**, probably due to the

Table 1 Mean diameter of liposomes before and after incubation with Concanavalin A (Con A) at room temperature for 1 hour

Liposome composition	Mean diameter before Con A addition ± σ (nm)	Polydispersity index	Mean diameter after Con A addition ± σ (nm)	Polydispersity index
Pure DMPC	185.0 ± 0.08	0.103 ± 0.029	187.0 ± 1.3	0.142 ± 0.036
DMPC–compound **1**	178.0 ± 2.2	0.096 ± 0.013	210.0 ± 4.15	0.229 ± 0.007
DMPC–Compound **2**	218.0 ± 1.2	0.179 ± 0.034	2510 ± 821	0.617 ± 0.229

presence of the sugar moieties in the vicinity of phospholipid headgroups. This repulsive interaction between DMPC and compound **1** could hinder the formation and stabilisation of vesicles and lead to the separation of DMPC vesicles on one side and self-aggregated compound **1** molecules on another.

Fresh vesicles batches of DMPC and its mixtures with the glycodendrimeric phenylporphyrin derivatives were left in contact for 1 hour with Concanavalin A (Con A), a mannose-specific lectin (0.5 mg/ml). Their diameters were measured before and after addition of Con A. The results in Table 1 show that the size of vesicles of pure DMPC and DMPC–compound **1** was not affected (or only slightly) by addition of the lectin. Conversely, for liposomes of DMPC–compound **2**, a dramatic increase in the vesicle diameter and polydispersity index was observed.

These striking results could originate from (i) the poor mixing properties of compound **1** with DMPC that would lead to the low incorporation rate of this porphyrin into phospholipid bilayers, and thus to a limited interaction of those liposomes with Con A, (ii) the longer spacer in compound **2** compared to that in compound **1**, which would increase the mobility of mannose moieties and facilitate their interaction with Con A, and (iii) the existence of Con A dimers and tetramers at the studied pH, allowing lectin interaction with more than one porphyrin molecule possibly borne by different liposomes. Such a multiple interaction would lead to the formation of a network of vesicles bridged by Con A molecules, resulting in a dramatic increase in their apparent size.

In this work, two glycodendrimeric phenylporphyrins (compounds **1** and **2**) were synthesized and their interaction with phospholipids was studied at the air–water interface and in liposome bilayers. The expansion of the DMPC-compound **1** mixed monolayer compared to monolayers of the pure components accounts for an unfavourable interaction that affected the formation and stabilisation of liposomes. Conversely, compound **2** favourably interacted with phospholipid molecules and formed mixed liposomes, which aggregated in the presence of α-mannose specific concanavalin A. These results show that the tetrapyrrolic macrocycle **2** was indeed embedded into the phospholipid bilayer and that its sugar moieties protruded into the surrounding aqueous phase. Such liposomes bearing glycodendrimeric phenylporphyrin could constitute an efficient carrier for drug targeting in photodynamic therapy.

The authors acknowledge financial support from the "Programme Incitatif et Coopératif Rétinoblastome" of Institut Curie, and for A. Makky's PhD grant, from ARC (Association pour la Recherche contre le Cancer).

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