Russian Chemical Reviews **83** (6) 523 – 554 (2014) © 2014 Russian Academy of Sciences and Turpion Ltd

DOI 10.1070/RC2014v083n06ABEH004426

Glycoconjugates of porphyrins with carbohydrates: methods of synthesis and biological activity †

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Data on the main approaches to preparation of mono- and oligodentate glycoconjugates based on porphyrin scaffolds are surveyed. The prospects for using these compounds as sensitizers for photodynamic therapy of cancer and for suppression of bacterial and viral pathogens are considered. Data on the synthesis of oligodentate blocking agents for carbohydrate-binding proteins (lectins) based on porphyrin scaffolds are discussed.
The bibliography includes 161 references.

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I. Introduction

Porphyrins — derivatives of the tetrapyrrole macrocycle porphin — are involved in vital processes of the living nature such as respiration and photosynthesis. In recent years, the chemistry of porphyrins and related compounds (chlorins, bacteriochlorins, phthalocyanines) has been vigorously developed and many of their derivatives have already found use in related fields of science: medicine, catalysis,[1] materials science,[2] nanotechnology[3] and so on. Medical applications are mainly related to the photosensitizing properties of porphyrins, which determine the prospects for using them in the photodynamic therapy of cancer and cardiovascular, ophthalmic, skin and infectious diseases.[4–6]

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Current research interests of the authors: carbohydrate chemistry.

Received 28 August 2013
Uspekhi Khimii **83** (6) 523 – 554 (2014); translated by Z P Svitanko

The essence of the photodynamic therapy (PDT)[7] is that special photosensitizer (PS) molecules are accumulated in the target cells (*e.g.*, cancer cells), which is followed by irradiation of tissues or organs with light having desired characteristics. The photosensitizer absorbs light energy and passes to the triplet excited state. Upon interaction with the environment, the excited PS returns to the starting non-excited state to give reactive oxygen species (ROS). The interaction with biological substrates by the electron transfer mechanism leads to generation of radicals and radical ions (type I reactions) and reactions with oxygen molecules by the energy transfer mechanism lead to generation of highly reactive singlet oxygen (type II reactions). All these ROS are cytotoxic and destroy cell organelles: membrane, mitochondria, lysosomes and so on, and this kills the cell.

Currently, relatively few porphyrin photosensitizer are allowed for use for the PDT of cancer,[7] which is due to some drawbacks of these compounds, first of all, low specificity of accumulation in tumour tissues. This stimulates the search for new sensitizers and ways of modification of known tetrapyrrole derivatives by attachment of biovectors for better delivery. As such modification of porphyrins, binding of mono- and oligosaccharide fragments to them has been studied in the last two decades. The conjugates in which one or several carbohydrate substituents are attached to porphyrin through glycosidic or another bond and also

† Dedicated to Academician B A Trofimov on the occasion of his 75th birthday.

through spacer groups are often called glycoporphyrins. Carbohydrates are among the most naturally abundant classes of organic compounds. They are involved in biological processes such as energy exchange, intercellular recognition, inflammation, tumour growth and metastasis, *etc.*[8–11]

What new useful properties can be imparted to the porphyrin macrocycle by carbohydrate ligands conjugated with it? First, this is solubility in water necessary for medical application. Second, it is the possibility of fine tuning of amphiphilic properties of glycoporphyrins, which is an equally important aspect of biodistribution of potential photosensitizers in the body. The third feature is the capability of molecular recognition by specific receptors (lectins) on the cell membrane surface of, for example, a bacterium, which may be favourable for selective accumulation of photosensitizers on the surface of the target cells.

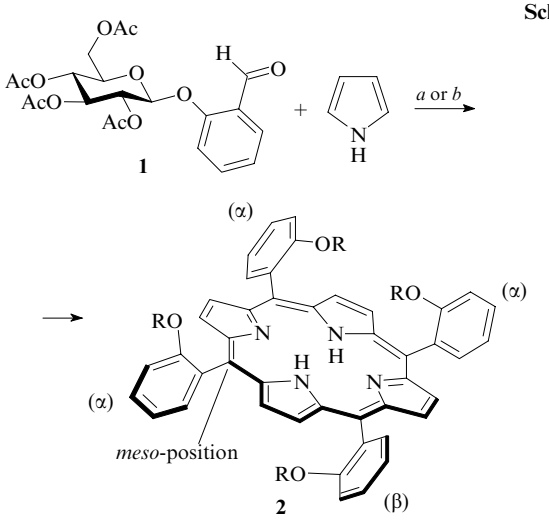
Some aspects of this subject matter were covered in reviews.[12–15] The review by Mironov *et al.*[12] deals with the main approaches to the synthesis of porphyrin and chlorin conjugates with carbohydrates and presents the analysis of the structure – biological activity relationship. A paper by Cavaleiro *et al.*[13] is devoted only to preparation methods of glycoporphyrins, which are considered in sufficient detail. In addition, the review covers the methods for preparation of glycochlorins, glycodendrimer derivatives of porphyrins and more intricate structures with several tetrapyrrole macrocycles. The next review of the same group of authors [14] is focused on the synthesis and biological activity assays of O- and S-glycoconjugates of porphyrins and chlorins.

The present review considers all currently known procedures for the synthesis of glycoporphyrins. In particular, data about compounds obtained by [3 + 2]-cycloaddition according to Sharpless (click chemistry) are integrated and analyzed for the first time.[16] Methods for the synthesis and properties of glycoconjugates based on other macrocyclic scaffolds related to porphyrin such as chlorins and phthalocyanines are beyond the scope of this review due to the large amount of available published information about these compounds. The primary attention is given to publications that present the results of biological experiments with the glycoporphyrins in question. The present review continues our earlier reviews devoted to the synthesis and studies of glycoconjugates based on low-molecular-mass oligodentate scaffolds — calixarenes [17] and cyclooligosaccharides.[18]

II. Glycoporphyrins obtained by condensation of pyrroles with aldehydes

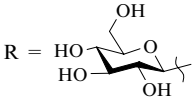
Binding of the already formed scaffolds to carbohydrate components by appropriate reactions is the most widely used strategy for the synthesis of glycoconjugates based on low-molecular-mass carriers. This approach is used, for example, to prepare glycocalixarenes [17] and cyclooligosaccharide conjugates with carbohydrates.[18] In the case of glycoporphyrins, the most popular method is the condensation of pyrroles with aldehydes. The proper porphin and its derivatives are prepared in laboratory by acid-catalyzed condensation of pyrrole (or dipyrrolylmethane) with the desired aldehyde. The condensation stage yields partially reduced porphyrin (porphyrinogen), which is oxidized *in situ* to the target product. If the initial aldehyde or dipyrrolylmethane contains a glycosyl substituent, then glycosylated porphyrin is formed as the product (Scheme 1). Drawbacks of this method are low regioselectivity of condensation (which, however, can be increased by optimizing the stoichiometry of the reactants) and low yields of glycoporphyrins (often $\leqslant$10%). On the other hand, the condensation may yield a series of glycoconjugates of different denticity, which presents some interest for the subsequent biological assays of the products. Owing to the ready availability of the starting reactants, large amounts can be used in the reactions and the desired glycoporphyrins can be isolated in reasonable amounts for further investigations.

Scheme 1



(13%, method *a*; 42%, method *b*)

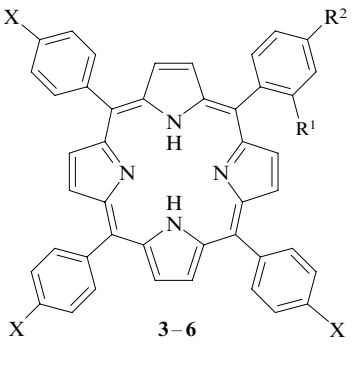
(*a*) 1) AcOH; 2) Py, Δ (Py is pyridine); 3) MeONa, MeOH;
(*b*) 1) $BF_3 \cdot Et_2O$, CH_2Cl_2; 2) chloranil, Δ; 3) MeONa, MeOH



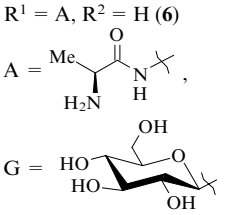
An early paper [19] on the chemical synthesis of glycoporphyrins describes the preparation of water-soluble glycoconjugates in which the glucopyranosyl residues are attached to the *ortho*-positions of the *meso*-aryl groups (see Scheme 1). The condensation of aldehyde **1** with pyrrole under the action of acetic acid (conditions *a*) resulted in the target product **2** in a relatively low yield. The same compound was obtained in a somewhat higher yield by the condensation according to the method proposed by Lindsey *et al.*[20, 21] (Lindsey method, conditions *b* in Scheme 1). It is noteworthy that both procedures afford glycoporphyrin **2** as three atropoisomers, αβαβ, ααββ and αααβ, in a 1 : 2 : 4 ratio (Scheme 1 shows the αααβ-isomer). The procedure for the preparation of the αααα-atropoisomer by isomerization of compound **2** was developed and optimized by Maillard *et al.*[22] The isomerization is carried out in a toluene – acetonitrile mixture in the presence of silica gel at 80 °C; the yield of the target product is 22%.

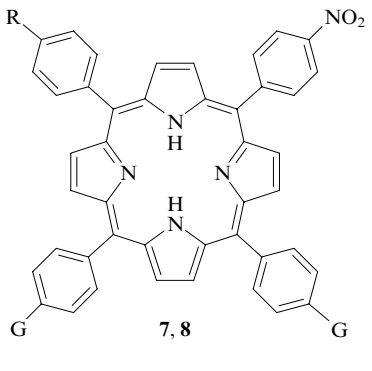
Subsequently a glycoporphyrin isomeric to compound **2** was prepared in which the glucosyl residues are in the *para*- or *meta*-positions of the *meso*-aryl group.[23] The molecule of this compound has no single bonds with hindered rotation around them; therefore, it exists as a single isomer (no atropoisomers are formed).

Structures 3 – 8

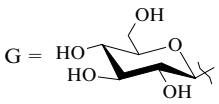


X = A: R^1 = H, R^2 = GO (**3**);
R^1 = GO, R^2 = H (**4**);
X = GO: R^1 = H, R^2 = A (**5**);
R^1 = A, R^2 = H (**6**)





R = GO (**7**), NO_2 (**8**);



The preparation of glycosylated porphyrin derivatives of different denticity by the Lindsey method [condensation induced by $BF_3 \cdot Et_2O$ and subsequent oxidation of porphyrinogen with chloranil or 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ)] has been considered in several publications;[24 – 37] the Adler – Longo synthesis[38] (refluxing in propionic acid), the Little synthesis[39] (refluxing in propionic acid with acetic anhydride)[37, 40, 41] and the condensation induced by other acids (trifluoroacetic and *p*-toluenesulfonic acid) have also been reported.[35, 36, 42 – 44] Below we consider the results of biological testing of some of the synthesized glycotetrapyrroles.

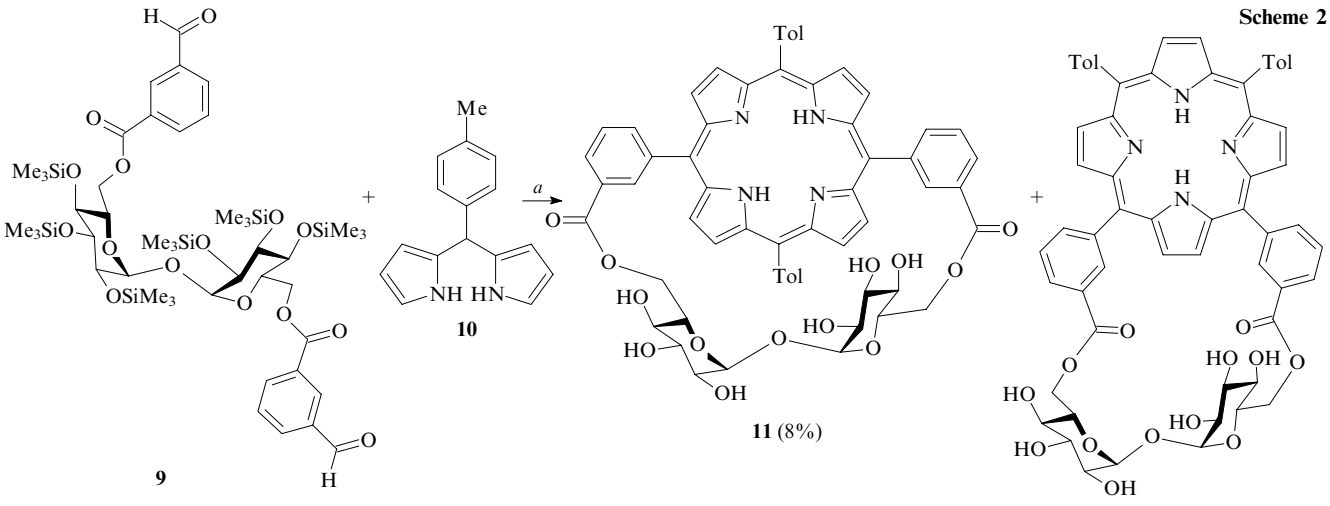
In order to search for potential photosensitizers for the photodynamic therapy of cancer, mono- and trisubstituted *meso*-glucosyloxyphenylporphyrins **3** – **6** were synthesized by the Lindsey method.[45] The authors investigated the photocytotoxicity of glycoporphyrins with respect to the K562 cells of human chronic myelogenous leukemia. These cells were placed into a medium containing glycoporphyrins **3** – **6** in a concentration of 2×10^{-6} mol litre^{-1} and irradiated for 100 min. Immediately after the irradiation, the percentage of dead cells was measured by flow cytometry; however, in all cases, it was rather low (< 10%). Then the cells were incubated in the dark for 24 h. In the case of porphyrin **6**, this killed 90% of the cells. Other conjugates proved to be less active (not more than 50% – 60% of the cancer cells were killed). The photocytotoxicity of the bidentate conjugates synthesized later[46] with respect to the K562 cells was not higher than that for hematoporphyrin.

The same authors[47] synthesized porphyrins **7** and **8** functionalized at the *meso*-positions by the *p*-glucosyloxyphenyl and *p*-nitrophenyl groups.[47] The glycoconjugates thus obtained were studied as agents suppressing the growth of gram-positive *Staphylococcus aureus* and gram-negative *Escherichia coli* bacteria. *E. coli* was insensitive even to high doses of porphyrins and long-term irradiation but compounds **7** and **8** exhibited bactericidal effect *in vitro* with respect to *S. aureus* under the experimental conditions used. Note that this is in line with the known higher stability of gram-negative bacteria against phototoxic impact, which is likely to be related to the specific cell wall structure.

The condensation of dialdehyde trehalose derivative **9** with dipyrrolylmethane **10** affords bridged porphyrins **11** and **12** (Scheme 2).[48] These compounds exhibit photocytotoxicity, although to a lesser extent than hematoporphyrin, with respect to the K562 tumour cells. Even at longer irradiation times, the activities of conjugate **11** and hematoporphyrin only become equal.

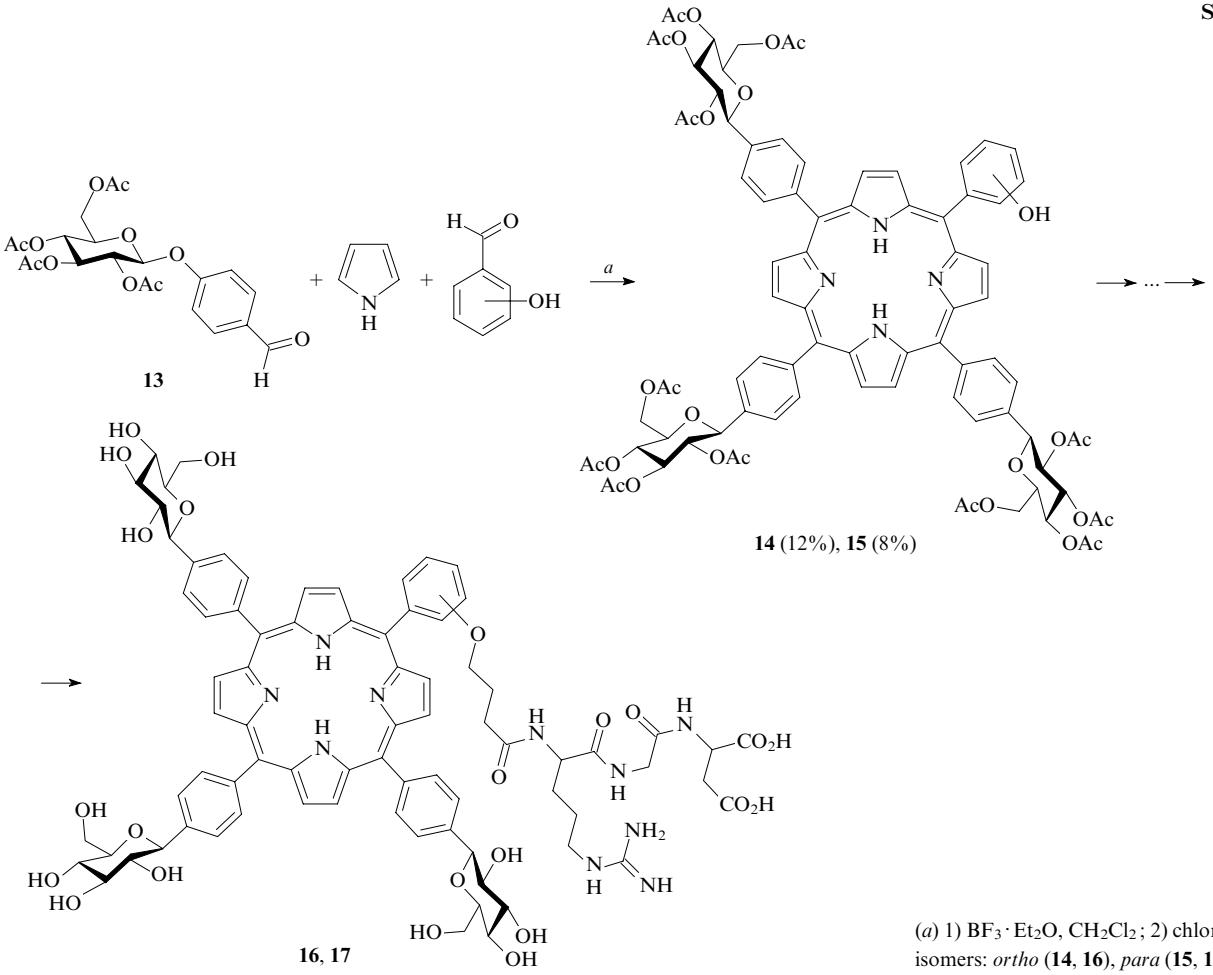
The activities of two other potential photosensitizers were studied by Krausz and co-workers[49] against the K562 cells. Using various procedures for the condensation of pyrrole with aldehydes (Little and Lindsey methods), they converted aldehyde **13** to glycoporphyrins **14** and **15** (Scheme 3).[49] It is noteworthy that the Lindsey method afforded higher product yields. These conjugates differ by the position (*ortho* or *para*) of the hydroxy groups in the phenyl substituent. These compounds were used to prepare the desired glycoconjugates **16** and **17** modified by a RGD tripeptide sequence attached through a spacer. It is assumed

Scheme 2



(*a*) 1) $BF_3 \cdot Et_2O$, CH_2Cl_2; 2) chloranil, CH_2Cl_2; 3) Bu^n_4NF; Tol is *p*-tolyl

Scheme 3

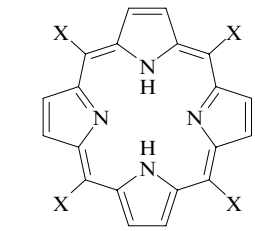


that the RGD motif in the porphyrin structure may increase the therapeutic efficacy of potential photosensitizers. The results of biological experiments showed that porphyrins **16** and **17** kill only 10% of the cancer cells immediately after the irradiation, whereas the first-generation photosensitizer Photofrin, authorized for use, kills about 40% of the cancer cells. If after irradiation, the cells are incubated in a solution of compound **16** or **17** in the dark, the percentage of killed cells increases to 70% – 80%. The authors deem that apoptosis is activated in the presence of compounds **16** and **17**.

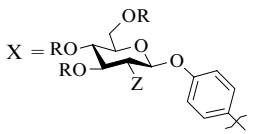
The uterine cervix tumour HeLa cells, which are widely used in scientific research, express receptors that bind to various carbohydrate ligands. A series of glycoporphyrins **18** – **25** obtained earlier[27,33] were used in experimental investigation of the photocytotoxic activity against these cells.[50] The lowest amount of cancer cells surviving after incubation with glycoconjugates **18** – **25** followed by activation by irradiation was found for acetylated derivative **18**. This compound is effective even at concentration of 1.4×10^{-6} mol litre^{-1} (>90% dead cell count). It is noteworthy that the nature of the beneficial effect of the fully acetylated glucosyl substituent in compound **18** remains obscure as this substituent is not recognized by receptors on the tumour cell surface.

Template synthesis and a modified Lindsey procedure were used to prepare[51 – 53] 16 tetradentate glycoporphyrins **19**, **23** and **26** – **39**. In these conjugates, the porphyrin scaffold is linked to glucose, galactose, xylose and arabinose residues, the conjugates being obtained both as complexes with the Zn^{2+} ion and in the free form. The effect of

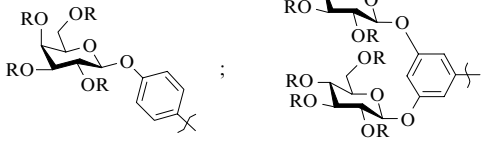
Structures 18 – 25



18 – **25**



[Z = OR: R = Ac (**18**), H (**19**);
Z = NHAc: R = Ac (**20**), H (**21**)];



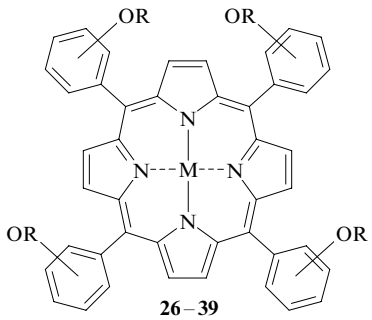
[R = Ac (**22**), H (**23**)]

[R = Ac (**24**), H (**25**)]

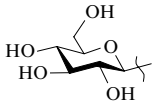
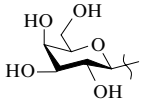
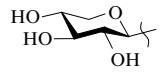
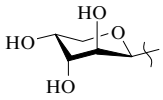
structural factors (type of the carbohydrate residue, type of the scaffold, position of the O-glycosyl groups in the phenyl substituents) on the glycoporphyrin photocytotoxicity was studied in relation to the HeLa cells. After incubation for 24 h with the cells, none of the photosensitizers showed cytotoxicity in the dark but most showed clear-cut photocytotoxicity (except for compounds **19**, **26** and **30**). The measured cellular uptake of the glycoconjugates was little correlated with the photocytotoxicity, which is in line with the results of other studies.[54, 55] The experiment in which the cells were first incubated with photosensitizers and sodium azide serving as a singlet oxygen trap demonstrated a substantial increase in the survival rate of the cells after irradiation. This proves that for all glycoporphyrins tested (**19**, **26** – **28**, **30**, **32**), irrespective of the structure, it is singlet oxygen rather than, for example, hydroxyl radical, that is the major species that destroys cell organelles. Analysis of the structural factors revealed that glycoconjugates with xylose residues had higher photocytotoxicity, while those with arabinose residues had lower photocytotoxicity; however, no unambiguous correlation was found. This indicates that no receptor-mediated mechanism of phototoxicity apparently exists, because xylose-binding lectins on HeLa tumour cell surface are unknown. The effect of the scaffold type (either with or without a Zn^{2+} ion) was more obvious: zinc porphyrin derivatives were less effective than porphyrin conjugates. According to the authors, a possible explanation to this fact is the difference between the molar absorption coefficients of the scaffolds. The most clear-cut dependence of the photocytotoxicity on the photosensitizer structure is observed for isomers differing in the position of O-glycosyl groups in phenyl substituents: conjugates with *meta*-glycosyl groups are almost in all cases much more active than the *para*-derivatives (even despite other factors). The authors suggested that this may be due to the globular structure of *meta*-photosensitizers, which reduces the number of undesirable collisions of reactive oxygen species with endogenous quenching traps.

As noted above, the photocytotoxicity of conjugates **19**, **23**, **29** and **31** is not directly related to the degree they are internalized by the HeLa tumour cells. Using these four compounds, an attempt was made to explain the difference in the exhibited photocytotoxicity for different types of carbohydrate substituents.[56] It was found that electronic absorption spectra of solutions of these glycoporphyrins are identical in DMSO but are markedly different in a physiological salt solution (phosphate buffer) containing 10 mass % of bovine serum albumin (BSA). This additive was not chosen by chance: the interactions between human serum albumin and pharmaceutical agents have a pronounced effect on the pharmaceutical action.[57, 58] The calculation of the oscillator strength (the area below the absorption spectral curve) demonstrated a good agreement with the order of variation of the photocytotoxicity of the conjugates. By means of titration assisted by fluorescence spectroscopy and circular dichroism spectroscopy, the binding constants and the binding stoichiometry of glycosylated porphyrins with BSA were calculated. However, these data were little correlated with the photocytotoxicity data and provided no explanation for the role played by different carbohydrate ligands in the glycoporphyrin – protein and glycoporphyrin – cell interactions on exposure to radiation.

Structures 26 – 39



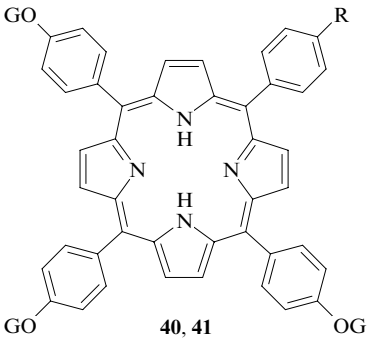
26 – 39

R	Isomer	M	Compound
	*para*	Zn	**26**
	meta	Zn	**32**
	meta	2 H	**33**
	*para*	Zn	**27**
	meta	Zn	**34**
	meta	2 H	**35**
	*para*	Zn	**28**
	para	2 H	**29**
	meta	Zn	**36**
	meta	2 H	**37**
	*para*	Zn	**30**
	para	2 H	**31**
	meta	Zn	**38**
	meta	2 H	**39**

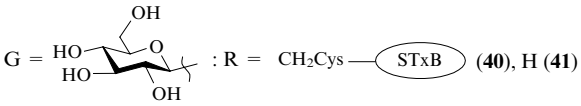
A key problem limiting the use of PDT in medicine is insufficiently selective accumulation of photosensitizers in the tumour. As noted above, attempts are made to solve this problem by attaching biovectors to the photosensitizer molecule. However, the cytotoxicity of these conjugates may decrease when they get inside the cells due to the non-optimal intracellular acid – base balance or degradation in late endosomes or lysosomes. The glycosphingolipid globotriaosylceramide (Gb3), which specifically binds to the bacterial Shiga toxin, was expressed on a multitude of human tumour cells.[59 – 61] This toxin consists of two subunits, one of them, subunit B (STxB) being responsible for the specific binding to Gb3. In the Shiga toxin-sensitive cells, the subunit STxB participates in the toxin delivery from the plasmatic membrane to the endoplasmic reticulum through early endosomes and Golgi complex. This transport route is called the retrograde transport.[62] The traffic by the retrograde route allows STxB to prevent degradation in lysosomes and to be stably associated with the cells. In order to utilize these vector properties of STxB in the photodynamic therapy, Amessou *et al.*[63] synthesized its conjugate with glycoporphyrin obtained by the Lindsey method. In conjugate **40**, the glycoporphyrin moiety is linked to the protein component *via* a cysteine spacer. A fluorescence immunoassay showed that conjugate **40** is effectively accumulated in the Golgi complex of the HeLa tumour cells (which also express the receptor Gb3), which is indicative of retention of the functional properties of STxB after conjugation and purification stages. The quantum

yield of the formation of singlet oxygen for compound **40** was somewhat lower than for reference compound **41**.[27] Nevertheless, conjugate **40** was 5 times more photocytotoxic with respect to the HeLa cells than compound **41**. Pretreatment of the HeLa cells with 2-hexadecanoylamino-3-morpholino-1-phenylpropan-1-ol inhibits the expression of the receptor Gb3. It was found that glycoporphyrin **41** equally induces cell death for Gb3-expressing and Gb3-deficient cells. Conversely, owing to the vector STxB, conjugate **40** exhibited high selectivity and had no toxic action on Gb3-deficient cells. Treatment of the HeLa cells with brefeldin A causes accumulation of STxB in early endosomes.[64] These cells are less susceptible to the phototoxic action of compound **40** (by 20% compared with untreated cells), which confirms once again the efficiency of the retrograde transport resulting in accumulation of the photosensitizer in the Golgi complex. The authors do not comment on the role of glucose residues used in the work. Probably, they are needed to ensure the porphyrin solubility during conjugation with the STxB protein.

Structures 40, 41



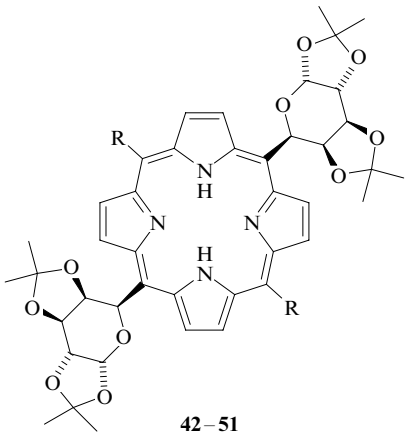
40, **41**



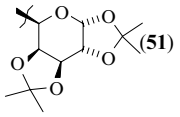
G = HO, HO, OH, O, OH : R = CH_2Cys—STxB (**40**), H (**41**)

Using the Lindsey method, Vedachalam *et al.*[65] prepared a series of glycoporphyrins **42**–**51**, which are distinguished by rather unusual positions of the carbohydrate moieties — they are attached to the *meso*-positions *via* C–C bonds. In the target conjugates, the galactose residues are protected by isopropylidene groups, which makes these molecules metabolically stable and facilitates their penetration inside the tumour cells, according to the author' assumption, owing to the enhanced hydrophobicity. The photocytotoxic effect against the HeLa and HCT116 cancer cells was found only for bidentate glycoconjugates **44**, **45** and **50** and tetradentate conjugate **51**, whereas without radiation these compounds were inactive. It is noteworthy that compounds with electron-withdrawing substituents (**46**–**49**) proved to be non-toxic in the light or in the dark. It was shown by fluorescence microscopy that the cellular uptake of glycoporphyrin **51** by HeLa cells is 3–8 times higher than that of glycoporphyrins **44**, **45** and **50**. The authors suggested that the isopropylidene groups present in these compounds are cleaved in the acid medium within the tumour cells, and the hydroxyl radicals thus formed additionally facilitate the binding. The experiments carried out to elucidate the intracellular location of compound **51** demonstrated that the conjugate is mainly accumulated in the lysosomes of the HeLa and HCT116 cells, while the subsequent photoinduced apoptosis follows the caspase activation mechanism.

Structures 42 – 51

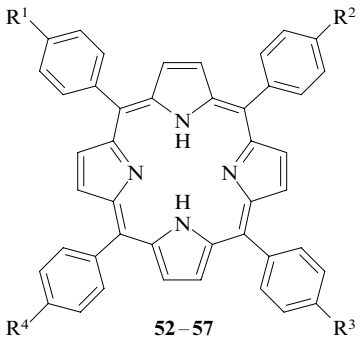


42–**51**

R = Ph (**42**), 2,6-$(MeO)_2C_6H_3$ (**43**), 4-$MeOC_6H_4$ (**44**), 4-$MeSC_6H_4$ (**45**) 4-$CF_3OC_6H_4$ (**46**), C_6F_5 (**47**), 4-ClC_6H_4 (**48**), 4-$O_2NC_6H_4$ (**49**), 3-Th (**50**) (Th is thienyl),  (**51**)

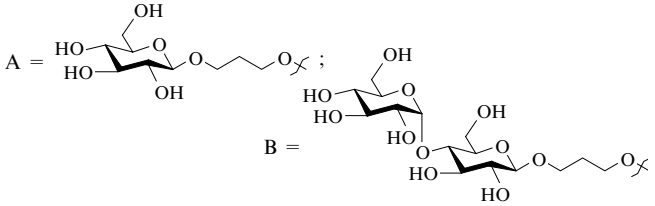
The yeast *Saccharomyces cerevisiae* is one of the best known model organisms used to study eukaryotic cells. It is easily cultured and is not pathogenic to humans. This culture was used[30] to study the properties of a series of glycoporphyrins **52**–**57**, which demonstrated their photodynamic activity.[66] All compounds killed the yeast upon photoactivation at different rates, which was detected based on fluorescence of the propidium iodide — a dye that stains only dead cells. However, in experiments this staining always occurred after the cell population lost the ability to form colonies. This implies that yeast was killed by intracellular destruction of organelles (first of all, DNA) rather

Structures 52 – 57



52–**57**

R^1 = A, R^2 = R^3 = R^4 = Me (**52**); R^1 = R^2 = A, R^3 = R^4 = Me (**53**); R^1 = R^3 = A, R^2 = R^4 = Me (**54**); R^1 = R^2 = R^3 = A, R^4 = Me (**55**); R^1 = R^2 = R^3 = R^4 = A (**56**), B (**57**);



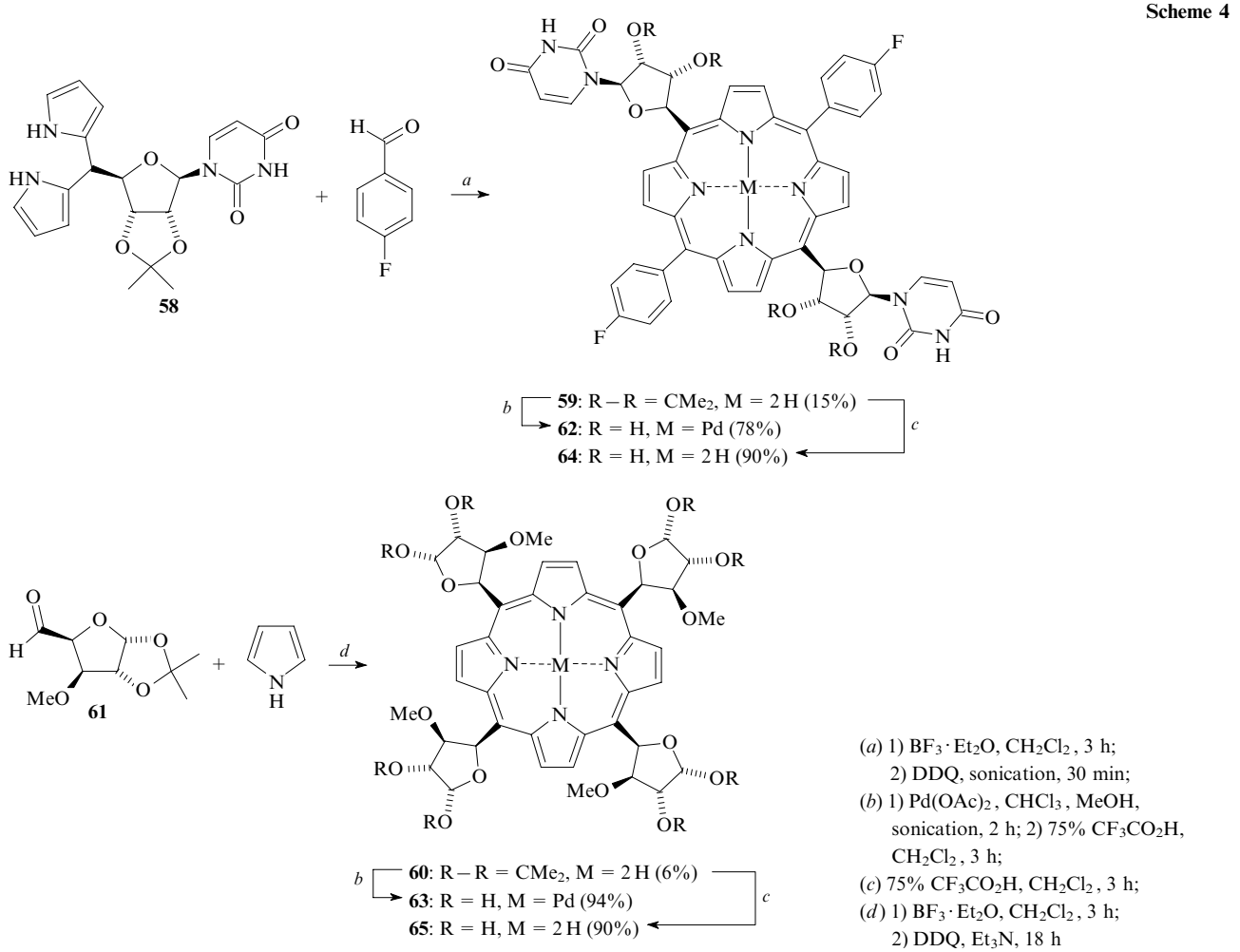
than by mere destruction of the membrane. Special experiments with the most active (compound **52**) and least active (compound **55**) photosensitizers detected DNA fragmentation. The short lifetime of the singlet oxygen $[(1-3)\times 10^{-6}$ s] confirms additionally the penetration of glycoporphyrins inside the cells. Hydrophobic conjugates **52**–**54** penetrated into the cells of *Saccharomyces cerevisiae* much better than hydrophilic conjugates **55**–**57**. *cis*-Glycoporphyrin **53** showed better cellular uptake than *trans*-isomer **54**, as its structure has separate hydrophilic and hydrophobic areas.

A series of di- and tetradentate C-*meso*-glycoporphyrins bearing modified glucofuranose residues as peripheral components have been obtained.[67] The condensation of compound **58** with *p*-fluorobenzaldehyde by the MacDonald method[68] gave glycoconjugate **59** with alternating *meso*-substituents — the *p*-fluorophenyl group and the uridine residue — in a low yield (Scheme 4). Conjugate **60** was obtained in a 6% yield by the Lindsey method from compound **61**. Compounds **59** and **60** were converted to glycometalloporphyrins **62** and **63**, respectively, by metallation with palladium(II) acetate and deprotection. Free glycoconjugates **64** and **65** were also obtained. Compounds that are able to cleave DNA molecules on exposure to light are of interest as regards the search for new anticancer and antiviral drugs.[69,70] The authors demonstrated that palladium complexes **62** and **63** can cleave supercoiled pUC18 DNA (plasmid from *E. coli*) only on exposure to visible light to give circular single-stranded DNA. Irradiation without the addition of glycoporphyrins did not result in cleavage of the DNA molecule, while the efficiency of conjugates **64** and **65** containing no palladium atom was 4 times lower than that of complexes **62** and **63**.

The synthesis of C-glycosylated porphyrins substituted at the *meso*-position and study of DNA photocleavage by these compounds was also considered by Pasetto *et al.*[71]

As noted above, studies are in progress to elucidate the possibility of using photodynamic therapy to suppress bacterial and viral infectious agents, in particular, for *ex vivo* disinfection of blood and protein products from blood. It is especially important to find effective agents for photodynamic suppression of viruses.[72] The mechanism of virus–photosensitizer interaction has not been finally elucidated as yet. Bacteriophage T7 is a frequently used model of a virus for various investigations. Its interaction with glucoporphyrins **19** and **41** prepared previously[27] has been studied.[73] In the absence of irradiation (dark toxicity), the asymmetric conjugate **41** decreases substantially the phage activity, while symmetric conjugate **19** is less effective. However, on irradiation the situation is reversed. The addition of special compounds — a singlet oxygen quencher and a trap for hydroxyl radicals — decreases, although does not eliminate, the photoinactivating action of compounds **19** and **41**. This indicates that each of the conjugates forms both reactive oxygen species that inactivate viruses. The attempts to identify the primary receptor for the action of

Scheme 4



Scheme 5

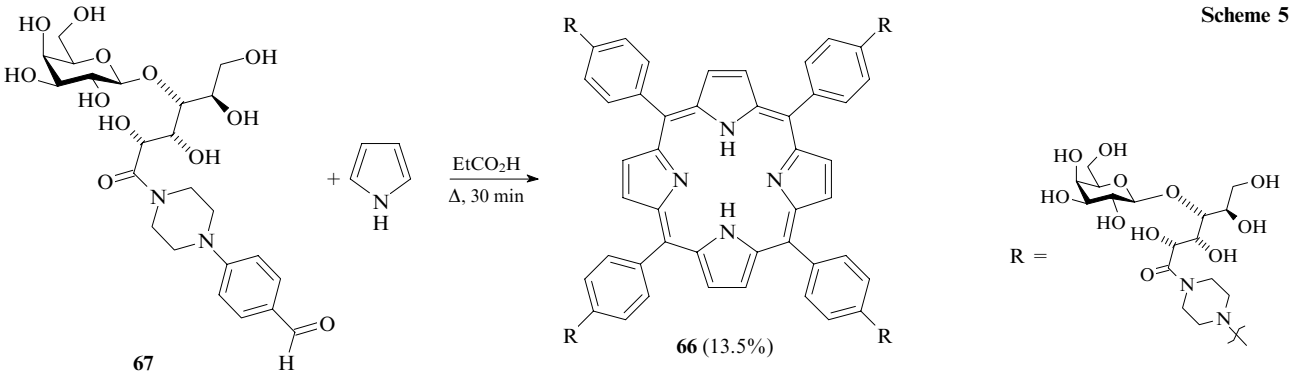


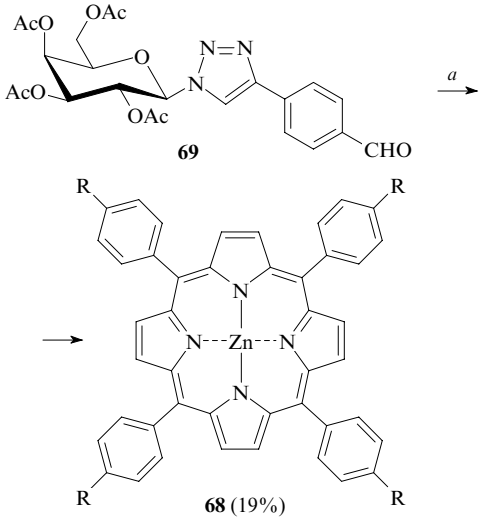
photo-produced toxic agents failed: the experiments on thermal DNA denaturation in the presence of photosensitizers and on exposure to radiation demonstrated that phage inactivation was not caused by DNA damage. Quantitative PCR analysis (PCR is polymerase chain reaction) also supported this result. Although symmetric product **19** exhibits higher phototoxicity, for drawing conclusion about the possibility of using it as an antiviral photosensitizer, it is also necessary to prove that it is harmless with respect to blood cells and protein components (first of all, red blood cells and platelets).

Compounds incorporating a piperazine residue are widely used in the pharmaceutical industry. Some of them exhibited antitumour activity.[74] The lactobionic acid residue containing a terminal β-D-galactopyranosyl moiety was tested as a biovector for targeted drug delivery to liver cells (hepatocytes).[75] Both above-mentioned structural elements are present in molecule **66** prepared by condensation of pyrrole with aldehyde **67** under Adler's conditions (Scheme 5).[76] The cytotoxic effect of compound **66** on the cells Bel-7404 (hepatic cancer), MCG (gastric cancer) and HNE1 (nasopharyngeal cancer) was studied in comparison with the known anticancer drugs Cisplatin and Fluorouracil. The dead cell counts observed without irradiation were lower for compound **66** than for the drugs; however, on exposure to laser radiation, the toxic effect of glycoporphyrin **66** was twice higher. It was also shown that toxicity is related particularly to the porphyrin moiety rather than to galactosylated piperazine residues.

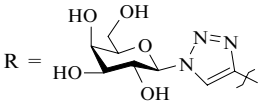
β-D-galactopyranosyl substituents were studied as possible vectors for the delivery of sensitizers to the tumours with a view to recognition of these vectors with tumour-associated lectins (galectins), which are expressed by cancer cells. Hao *et al.*[77] described the synthesis of tetradentate galactoporphyrin **68** as a complex with zinc ion (Scheme 6). The Lindsey condensation of glycosylaldehyde **69** with pyrrole afforded the target conjugate in a relatively high yield (19%). The accumulation of glycoporphyrin **68** upon incubation with HEp2 human carcinoma cells occurs in 2 h. It was shown by fluorescence microscopy that conjugate **68** is mainly located within lysosomes.[78, 79] The authors do not report the ability of the synthesized compounds to function as photosensitizers upon laser irradiation.

Yet another galactoso-containing glycoporphyrin prepared using the above-described approaches is compound **70**.[27] It is structurally related to non-glycosylated chlorin **71** (mTHPC), the active component of the second-generation photosensitizer Foscan®, which found use in the therapy of

Scheme 6

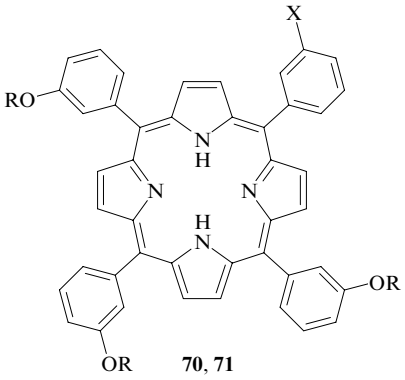


(*a*) 1) pyrrole, $BF_3 \cdot Et_2O$, CH_2Cl_2, 1 h; 2) DDQ, 1 h; 3) MeONa, MeOH; 4) $Zn(OAc)_2$, MeOH, 2 h;

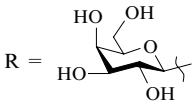


dermoid head and neck cancer and some other types of cancer (see Refs 80 – 82 and references cited therein). The initial preclinical tests of glycoporphyrin **70**[83] demonstrated its higher photocytotoxicity compared to the reference agent **71**. Conjugate **70** was excreted from the rat body faster than chlorin **71**, which is an obvious advantage, because this reduces the residual photosensitivity side effect. One more interesting finding is that the initial plasma concentration of compound **70** depends linearly on the administered dose in the range of doses studied. This fact enables pharmacodynamic extrapolation and dose correction, which is important for minimization of the residual (*e.g.*, skin) photosensitivity. Analysis of biodistribution of compound **70** showed that the largest amounts are accumulated in rat lungs, liver and spleen. Meanwhile, glycoporphyrin **70** was completely absent in the healthy brain, which ruled out the possible neurological side effects. It was also noted that active metabolites of compound **70** were not detected in the blood plasma or tissues of test animals. Although the above data attest to favourable properties of compound **70**, it has not been further investigated.

Structures 70, 71



70, **71**



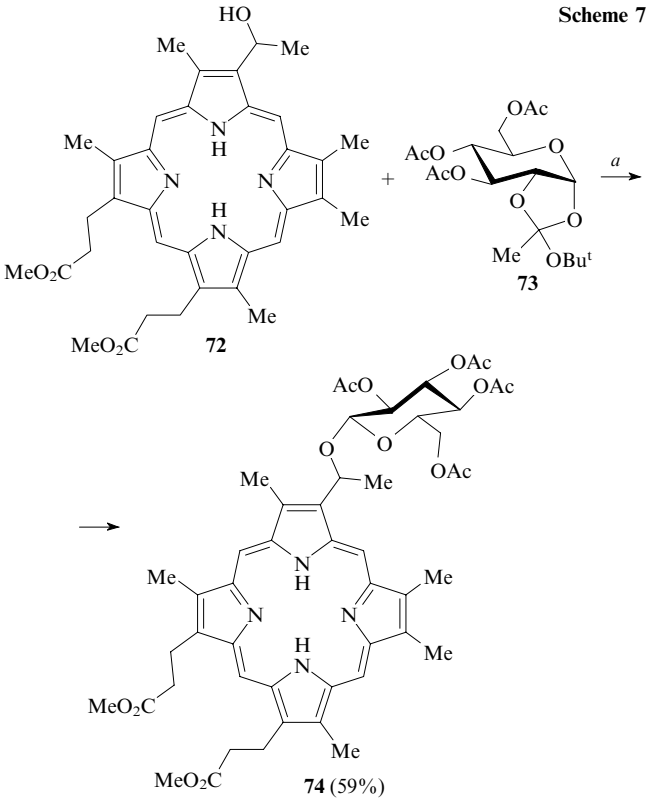
R = , X = H (**70**); R = H, X = OH (**71**)

III. Glycoporphyrins obtained by glycosylation

Glycosylation is not widely used to attach carbohydrate residues to the porphyrin scaffolds. This is apparently due to the fact that the reaction procedure is fairly cumbersome and the yields are relatively low. Moreover, low yields of monoglycosylation make the application of this reaction in the synthesis of oligodentate glycoporphyrins inexpedient.

Chronologically, the first work on the preparation of glycoporphyrin was that published in 1978 by Mironov *et al.*[84] describing glycosylation of porphyrin derivative **72** with orthoester **73** under the action of lutidinium perchlorate (Scheme 7); product **74** was isolated in a 59% yield.

Scheme 7

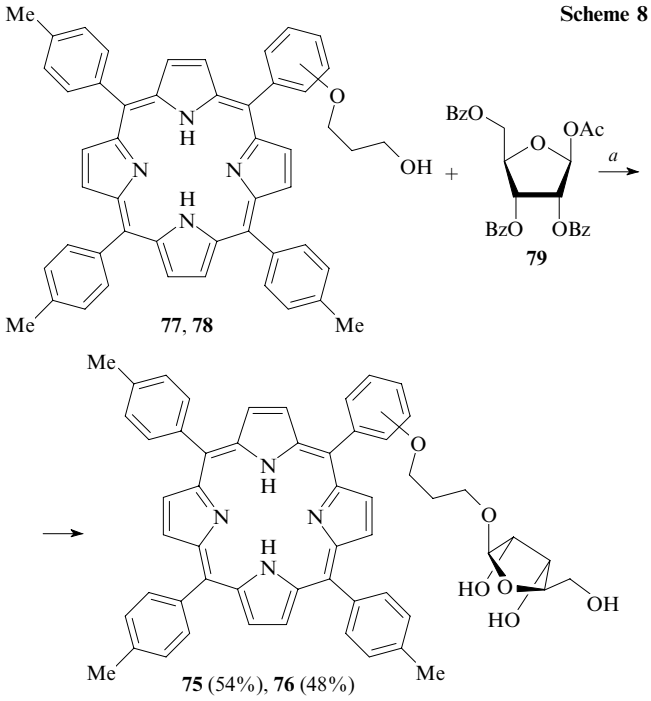


(*a*) 2,6-lutidinium perchlorate, $ClCH_2CH_2Cl$, Δ, 2 h

Further development of the synthetic strategy and systematic research into the biological properties of glycoporphyrins were continued only in the late 1980s. For instance, the synthesis of mono- and bidentate glycoporphyrins containing glucose, galactose, ribose, maltose and lactose residues was reported.[30, 85, 86] Linking of the porphyrin core to carbohydrates was performed by Helferich glycosylation induced by silver(I) carbonate or tin(IV) chloride.

Glycoporphyrins **75** and **76** (Scheme 8) in which the ribose residues are attached through spacer groups have been prepared.[87] The key stage of the synthesis of compounds **75** and **76** is tin(IV) chloride-promoted glycosylation of porphyrins **77** and **78** by fully acylated β-ribofuranose derivative **79**. It is noteworthy that the initial attempts at Helferich glycosylation of the same acceptors by ribofuranosyl bromide were unsuccessful. The paper also mentions the synthesis of glucoporphyrins similar to derivatives **75** and **76** but containing glucose residues. These compounds were effectively prepared by the Helferich reaction.

Scheme 8

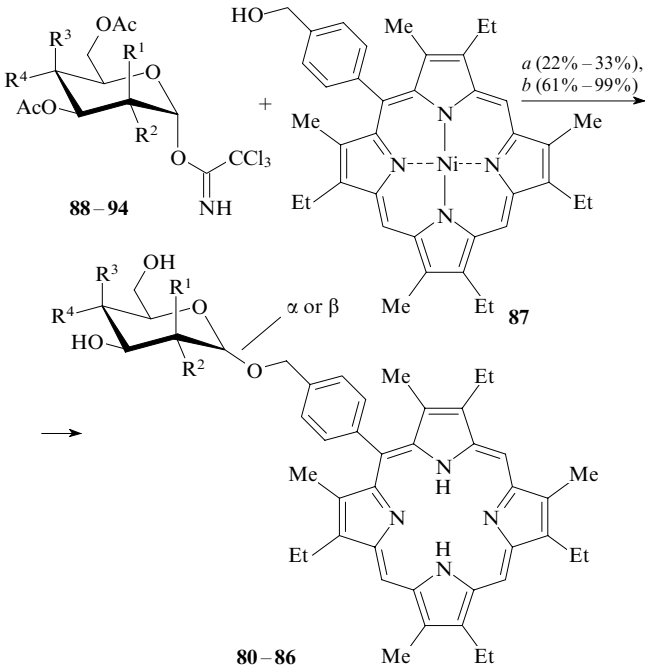


(*a*) 1) $SnCl_4$, CH_2Cl_2; 2) NaOH, MeOH, CH_2Cl_2, 0 °C;
isomers: *ortho* (**75**, **77**), *para* (**76**, **78**)

The accumulation of photosensitizers in cells depends on numerous physicochemical and biological factors. One of them is interaction of porphyrin-based compounds with cell membranes. Schell and Hombrecher[88] studied the interaction of porphyrin glycoconjugates **80** – **86** (Scheme 9) with phosphatidylethanolamine (PE) liposomes and dimyristoylphosphatidylcholine (DMPC) liposomes as cell membrane models. The conjugates were prepared by glycosylation of metal porphyrin **87** by various trichloroacetimidates **88** – **94**. The formation of a α-glycosidic bond was observed only for mannoside derivative **80**, which is consistent with the anchimeric assistance by the acetyl protecting groups at O(2). Note that glycosylation with the use of metal-free porphyrin analogue **87** affords a complex mixture (> 10 components) of non-porphyrin destruction products.

The same outcome of glycosylation by trichloroacetimidates of both metal and metal-free porphyrins was observed in another work.[89] The interaction of conjugates **80** – **86** with liposomes was studied by fluorescence spectroscopy. The binding constants obtained for DMPC liposomes were lower than those for PE liposomes. However, all of the constants were higher than those measured for binding of hematoporphyrin to PE liposomes. The highest binding constant with PE (187 ± 20 litre g^{-1}) was found for conjugate **86**.

Scheme 9



(*a*) $ZnCl_2 \cdot Et_2O$, CH_2Cl_2, MS 4 Å, 24 h;
(*b*) 1) MeONa, MeOH, CH_2Cl_2; 2) CF_3CO_2H, $HS(CH_2)_3SH$

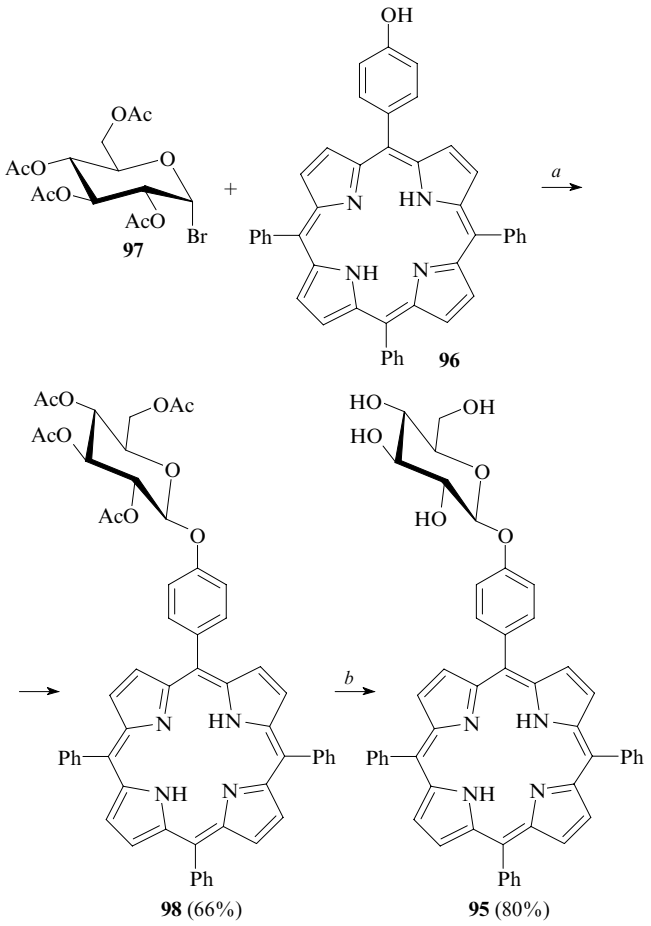
Compound	R^1	R^2	R^3	R^4
80, **88**	OX	H	H	OX
81, **89**	H	OX	OX	H
82, **90**	H	OX	H	OX
83, **91**	H	NHAc	H	OX
84, **92**	H	OX	H	α(1,4)Glc(OX)$_4$
85, **93**	H	OX	H	β(1,4)Gal(OX)$_4$
86, **94**	H	OX	H	β(1,4)Glc(OX)$_4$

X = H (**80** – **86**), Ac (**88** – **94**); Glc and Gal are glucose and galactose residues, respectively.

Glucoporphyrin **95** was synthesized to search for new photoactivated antiviral agents (Scheme 10).[90] Glycosylation of acceptor **96** with bromide **97** promoted by freshly prepared silver(I) carbonate afforded glycoside **98** and the same product complexed with a silver ion. The complex was destroyed (to give glycoside **98**) by treatment with trifluoroacetic acid. The subsequent deprotection yielded the target conjugate **95**.

The cytotoxic effect of glycoporphyrins **95**, **96** and **98** was studied on the Vero cells infected with the herpes simplex virus of two types (HSV-1 and HSV-2). The drugs Acyclovir and Foscarnet were used as the references.

Scheme 10



(*a*) 1) Ag_2CO_3, Na_2SO_4, CH_2Cl_2, 24 h; 2) CF_3CO_2H;
(*b*) MeONa, MeOH, CH_2Cl_2

The maximum non-cytotoxic concentrations with respect to non-infected Vero cells were $(1.5-3) \times 10^{-5}$ g ml^{-1} for compounds **95**, **96** and **98**, 10^{-4} g ml^{-1} for Acyclovir and 10^{-3} g ml^{-1} for Foscarnet. At these concentrations, the suppression of the virus replication cycle was the same (100%) for conjugates **95** and **96** and for the reference drugs. Meanwhile, for acetylated derivative **98** this value was only 40%. Conjugate **95** showed activity even when the working concentration was decreased to 2.5×10^{-6} g ml^{-1} (*i.e.*, 7-fold). When the concentration was 10^{-8} g ml^{-1}, the activity still exceeded 40%. It is noteworthy that in the absence of irradiation, conjugate **95** suppresses the virus replication twice less efficiently than upon light activation.

IV. Glycoporphyrins obtained by N- or O-acylation reaction

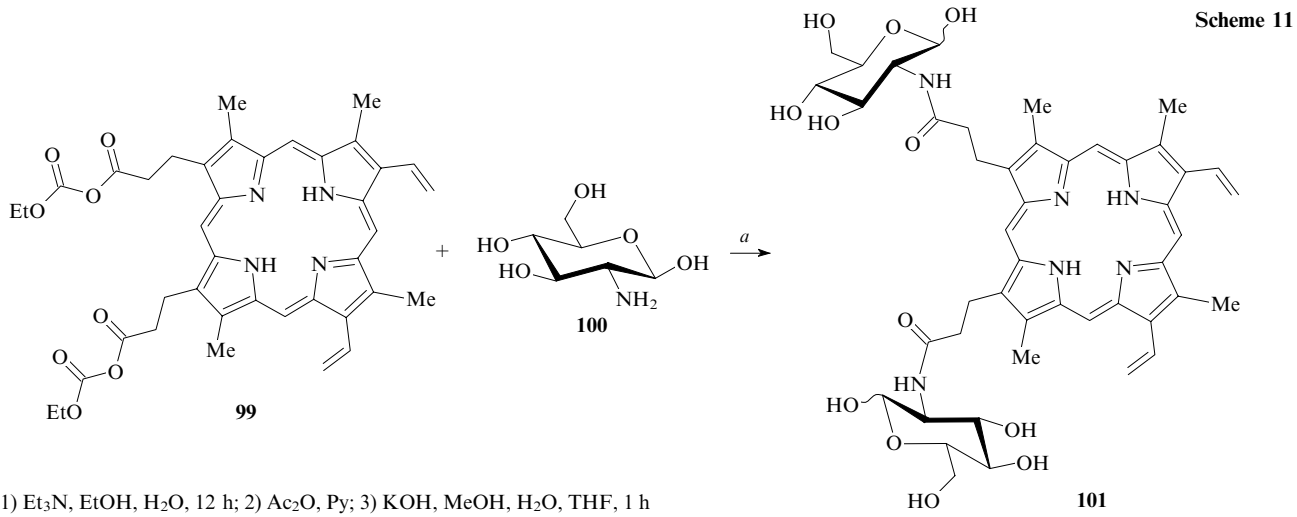
Acylation of amino groups (more rarely hydroxy groups) by carboxylic acid derivatives is a fairly facile and reliable method for the carbohydrate conjugation with porphyrin scaffolds. The acylating agents used for this purpose include acid anhydrides, lactones and activated esters prepared *in situ* from the acid and various condensing reagents [dicyclohexylcarbodiimide (DCC), 3-ethyl-1-(3-dimethyl-

aminopropyl)carbodiimide (EDAC)]. The key advantage of this method of conjugation is the formation (in the case of amines) of the amide bond stable over a broad range of conditions. For example, mixed anhydride **99** acylated glucosamine (**100**) to give the target conjugate **101** as a mixture of anomers in a 70% total yield (Scheme 11).[91] Similarly, glycoporphyrins were synthesized from anhydride **99**, D- or L-mannosamine and D-galactosamine.

The search for photodynamic agents active against the K562 cells of human chronic myelogenous leukemia was also carried out among glycoporphyrins obtained by acylation. The acylation of amines **102** and **103** with DCC-activated acid **104** furnished monodentate conjugates **105** and **106** in which the carbohydrate residue is linked to porphyrin *via* the alanine spacer (Scheme 12).[46] Hematoporphyrin was used as the reference compound in the biological assays. The immediate dead cell counts for K562 cells were always lower in the case of compounds **105** and **106** than in the control runs. However, the K562 dead cell counts increased (4 – 5-fold as compared with the early necrosis rate) during the subsequent incubation (24 h) in the dark. The delayed cell death may be caused by apoptosis induced by the photoradiation followed by secondary necrosis. Generally, compounds **105** and **106** are 2 – 2.5 times inferior to hematoporphyrin in the photocytotoxicity.

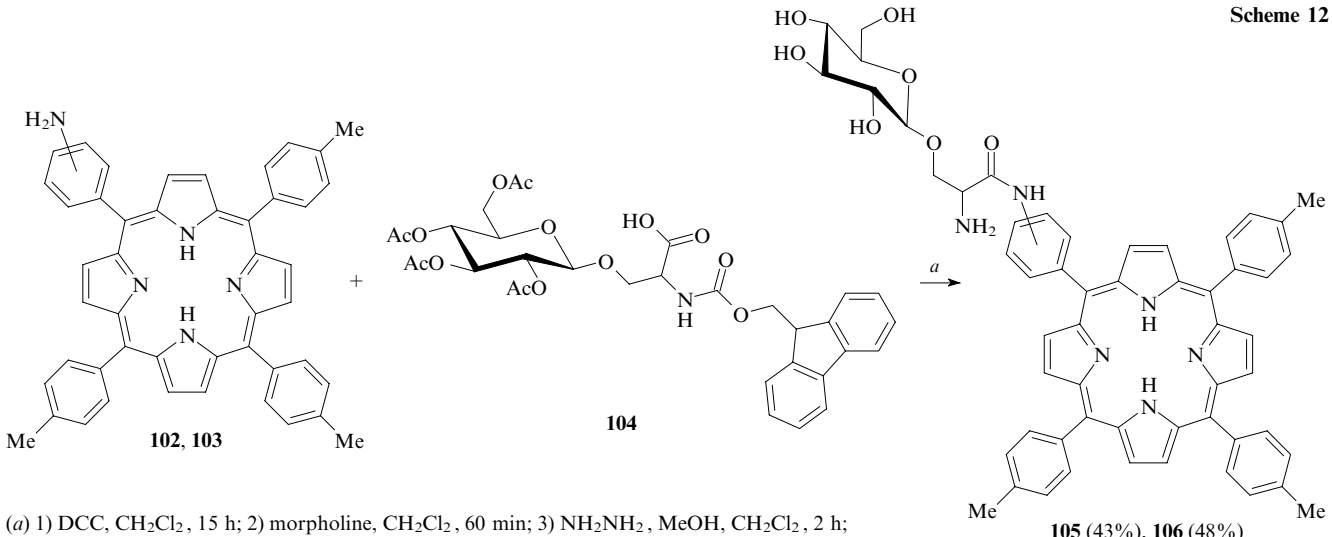
The conjugation stage in this method was successfully accomplished using lactone derivatives. Amines **107**, **108** and lactone **109** were converted to two monodentate glycoporphyrins **110** and **111** in high yields (Scheme 13).[92] The K562 tumour cells, which are used traditionally for biological assays by the Krausz group, served to assess the *in vitro* photocytotoxic activity of conjugates **110** and **111**. The drug Photofrin and non-glycosylated porphyrin **112** were used as the reference agents. The immediate dead cell counts for K562 cells just after irradiation were much higher for *ortho*-isomer **110** than for *para*-isomer **111** but still lower than for Photofrin. The subsequent cell incubation in the dark for 24 h did not induce a significant increase in the dead cell count in the case of glycoporphyrins **110** and **111** unlike Photofrin. Presumably, the phototoxic action of conjugates **110** and **111** induces only the early cell necrosis and does not induce apoptosis. The higher photoactivity of the *ortho*-derivative compared with the *para*-derivative was not explained by the authors. The low photocytotoxicity exhibited by non-glycosylated porphyrin **112** attests to the

Scheme 11



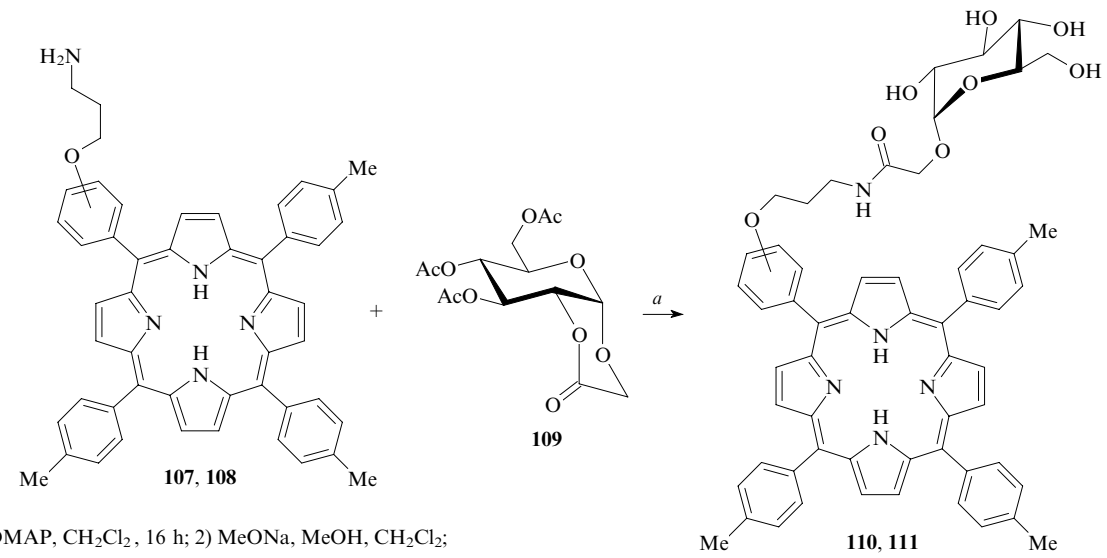
(*a*) 1) Et_3N, EtOH, H_2O, 12 h; 2) Ac_2O, Py; 3) KOH, MeOH, H_2O, THF, 1 h

Scheme 12



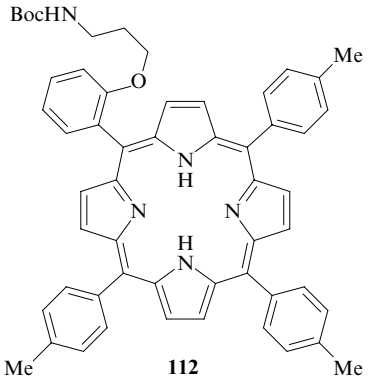
(*a*) 1) DCC, CH_2Cl_2, 15 h; 2) morpholine, CH_2Cl_2, 60 min; 3) NH_2NH_2, MeOH, CH_2Cl_2, 2 h; isomers: *ortho* (**102**, **105**), *para* (**103**, **106**)

Scheme 13



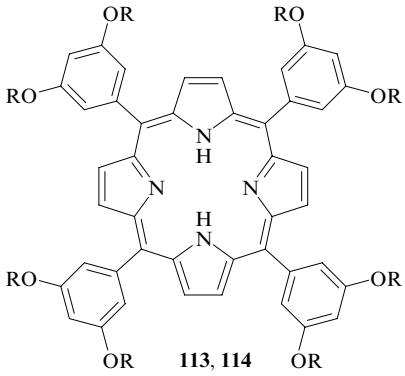
(*a*) 1) DMAP, CH_2Cl_2, 16 h; 2) MeONa, MeOH, CH_2Cl_2;
isomers: *ortho* (**107**, **110**), *para* (**108**, **111**); DMAP is dimethylaminopyridine

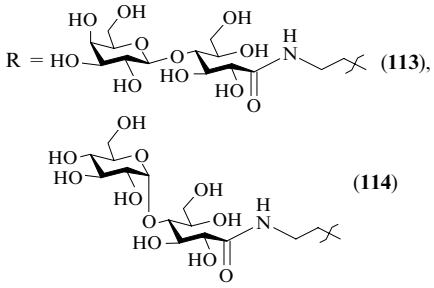
Structure 112



Boc = Bu^{t}OC(O)

Structures 113, 114



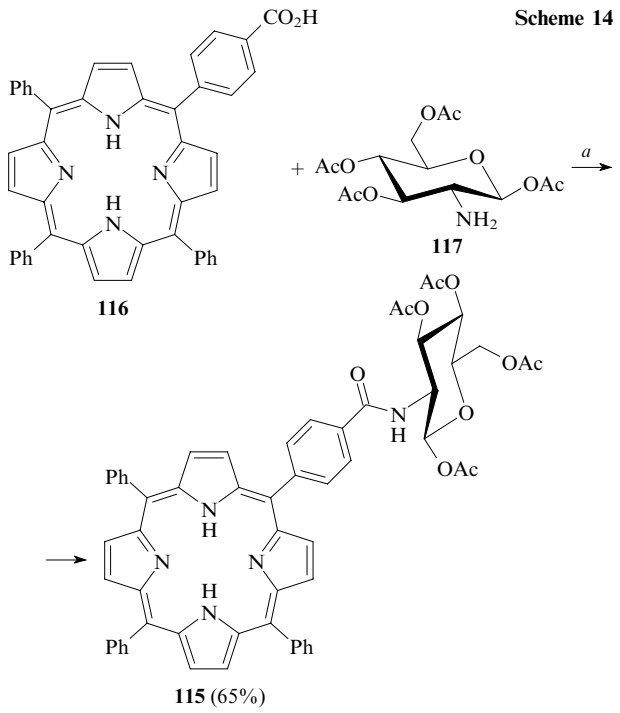


beneficial influence of the peripheral carbohydrate residues on the photoactivity of porphyrin derivatives.

Fujimoto *et al.*[93] obtained octadentate glycoclusters **113** and **114** by treating the corresponding octaamine with maltonolactone or lactonolactone. The interaction of compound **113** and **114** with RLC-16 hepatocytes was studied by fluorescence microscopy. Hepatocytes have receptors that bind to the terminal galactose residues. It was shown that glyco-cluster **113** was attached to the hepatic cells, whereas its analogue **114** showed no affinity for these cells. Cancer cells are known to bind to various molecules (*e.g.*, porphyrins) owing to non-specific hydrophobic interactions. However, in the case of compound **114**, this did not take place, probably, because the hydrophobicity of the porphyrin core of this molecule is completely hidden by the shell of hydrophilic glucose residues.

D-Glucosamine can be conveniently conjugated with non-carbohydrate compounds containing acidic moieties by acylation of the primary amino group. The anomeric position in glucosamine should be permanently protected even in the target conjugates in order to avoid the formation of difficult-to-separate mixtures of stereoisomers (anomers). These principles were used in the synthesis of glycoporphyrin **115** (65% yield) by the reaction of EDAC-activated acid **116** with glucosamine derivative **117** (Scheme 14).[94] Related bidentate glycoporphyrin **118** with symmetrical arrangement of the carbohydrate moieties was prepared by condensation of benzaldehyde with glycosylated dipyrrolylmethane. The use of protected carbohydrate derivatives such as compounds **115** and **118** not only provides stereoisomerically pure compounds but, in the authors' opinion, it also increases their accumulation in lipophilic cell membranes and decreases the toxic effect on healthy tissues.[50, 95] The HT29 human adenocarcinoma cells accumulate conjugates **115** and **118** better than tetraphenylporphyrin by factors of 11.5 and 5, respectively. These compounds do not exhibit cytotoxicity without light activation up to concentration of 10^{-5} (**115**) and 4×10^{-6} mol litre^{-1} (**118**). The photocytotoxicity of conjugates **115** and **118** was 5.8 and 2.7 times, respectively, higher than that of tetraphenylporphyrin, which is correlated with the data on accumulation of these compounds in the cells. This finding confirms the observation[96] that compounds with symmetrically arranged

Scheme 14

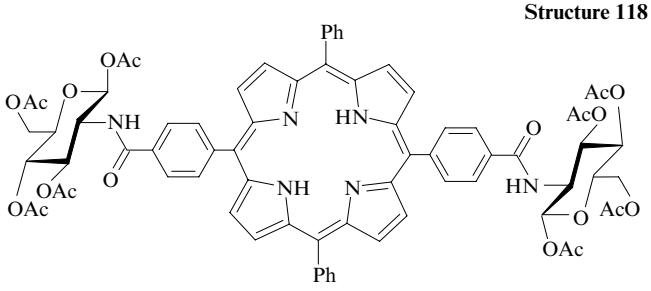


(*a*) EDAC, DMAP, CH_2Cl_2, 0 → 25 °C, 27 h

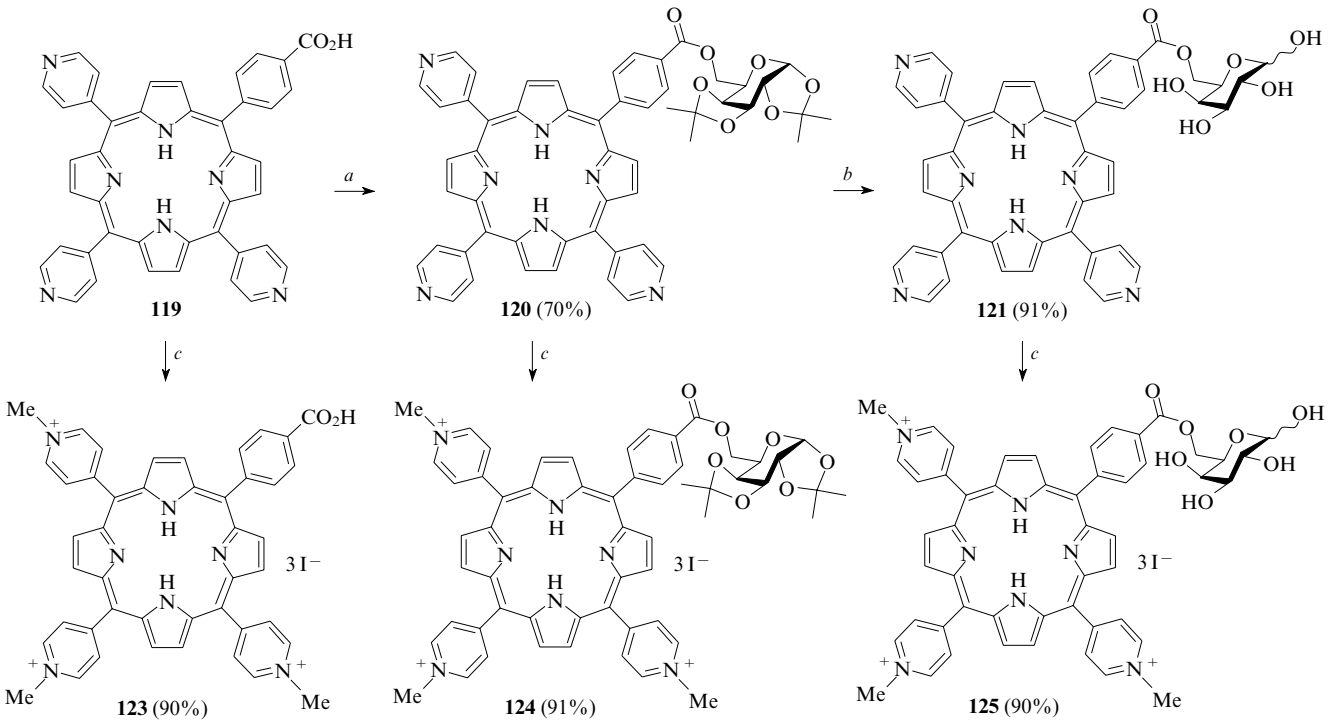
ligands (as in diagonal bidentate or tetradentate glycoporphyrins) are generally less phototoxic than asymmetric molecules. It was shown by fluorescence microscopy that conjugate **115** is mainly accumulated in the endoplastic reticulum of the HT29 cells.

Cavaleiro's group[97] performed the search for potential antiviral agents based on porphyrin (see also Section III), in particular, with the use of acylation for the conjugation of porphyrin and carbohydrate compounds. The results of these studies, which used porphyrin **119** with a free carboxyl group as the starting compound, are summarized in Scheme 15. The obtained compounds included neutral (**120**, **121**) and cationic derivatives **123** – **125**. All of the synthesized conjugates were stable on exposure to white light for 15 min; they were capable of generating singlet oxygen and had no photocytotoxicity against the Vero cells up to a concentration of 10^{-8} mol litre^{-1}. These glycoporphyrins showed a weak virucidal effect with respect to HSV-1 virions (human herpes simplex virus) without photoactivation. On irradiation, the antiviral activity substantially increased (in particular, upon increase in the

Structure 118

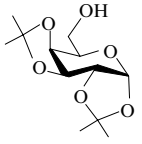


Scheme 15



(*a*) 1) $SOCl_2$, Py, 50 °C, 30 min; 2) *N*-hydroxysuccinimide, Py, 50 °C, 3 h; 3) **122**, NaH, PhMe, 90 min; (*b*) CF_3CO_2H, H_2O, 30 min; (*c*) MeI, DMF, 40 °C, 3 h

122 =

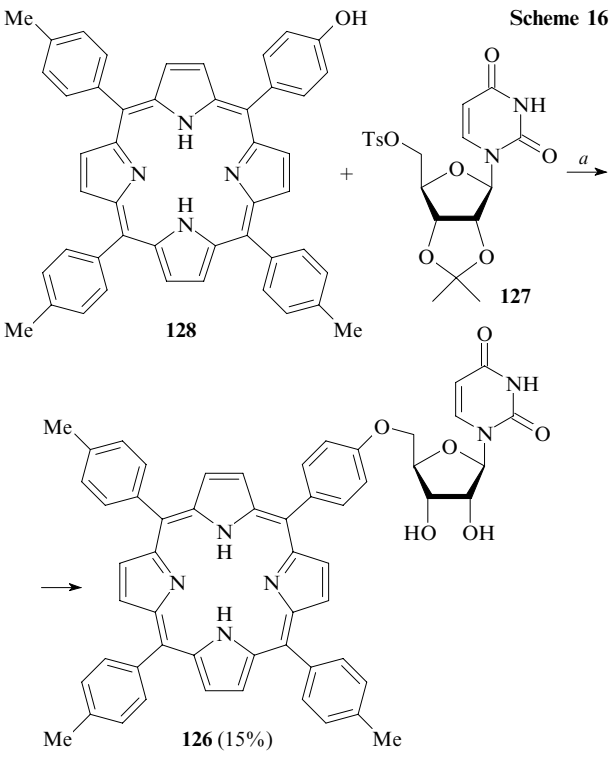


photoactivation time). The starting porphyrin **119** and neutral derivative **121** were most effective, while cationic derivatives were generally less active. The ability to inhibit the replication of HSV-1 in the Vero cells was studied for galactoporphyrins **121** and **125**. It was found that photoactivation substantially decreases the amount of the virus, although cationic porphyrin **125** was also active without irradiation. Kinetic experiments performed with and without irradiation ascertained that neutral conjugate **121** is more effective at later stages of the virus replication cycle but virtually does not exhibit inhibitory activity if the cells are treated with this agent immediately after being infected. Meanwhile, conjugate **125** is effective irrespective of how much time has elapsed from the cellular uptake of the virus to glycoporphyrin treatment (0, 2, 4 or 16 h). The authors deem that compounds **121** and **125** affect the replication of HSV-1 by different mechanisms. Compound **121** is most active after 16-h post-uptake maintenance, *i.e.*, when new viral particles have already formed. The authors attributed this activity to high virucidal effect of porphyrin **121**.

V. Glycoporphyrins obtained by nucleophilic substitution

The attachment of carbohydrate residues to porphyrins *via* nucleophilic substitution reaction is quite widely used to synthesize glycoporphyrins. Most often, S-nucleophiles are employed, more rarely, O- and N-nucleophiles. Glycoconjugates synthesized in this way usually contain a linear alkyl spacer. This is due to the fact that the nucleophilic substitution occurs most effectively at the primary carbon atom by the S_N2 mechanism. Halides serve most often as leaving groups. In some papers, the fluorine atom in the *para*-position of perfluorinated phenyl substituents acts as the nucleofuge.

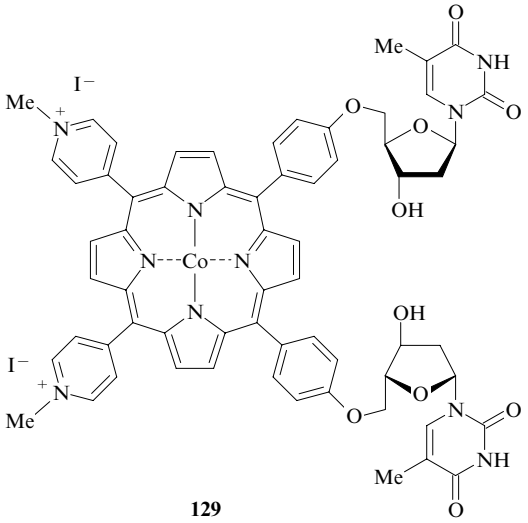
Scheme 16



(*a*) 1) NaH, DMF; 2) CF_3CO_2H, MeOH; Ts is tosyl

The synthesis of porphyrinyl nucleoside **126** is the first example of using nucleophilic substitution to link the porphyrin scaffold to a carbohydrate derivative (Scheme 16).[98] In this compound, the residue of nitrogenous base (uracil) is linked to ribose, which in turn is connected to the porphyrin core. The yield of substituton of porphyrin moiety **128** for tosylate in substrate **127** was 42%. Similarly, porphyrinyl nucleoside with the adenosine residue and also conjugates of *meso*-substituted porphyrin with uridine, adenosine and thymidine[99, 100] and some other porphyrinyl nucleosides were synthesized.[101] When present in a concentration of 2.5×10^{-5} mol litre^{-1} (without irradiation), metal porphyrin **129** with two thymidine residues obtained similarly to conjugate **126** suppressed the growth of M21-HPB human melanoma cells by 95% as compared with the reference sample (cells without addition of the conjugates).[102] The authors assume that the cytotoxic effect of glycoporphyrin **129** is due to the ability of the porphyrin moiety and the thymidine residues it contains to interact with the polynucleotide chains of the cell.[103]

Structure 129

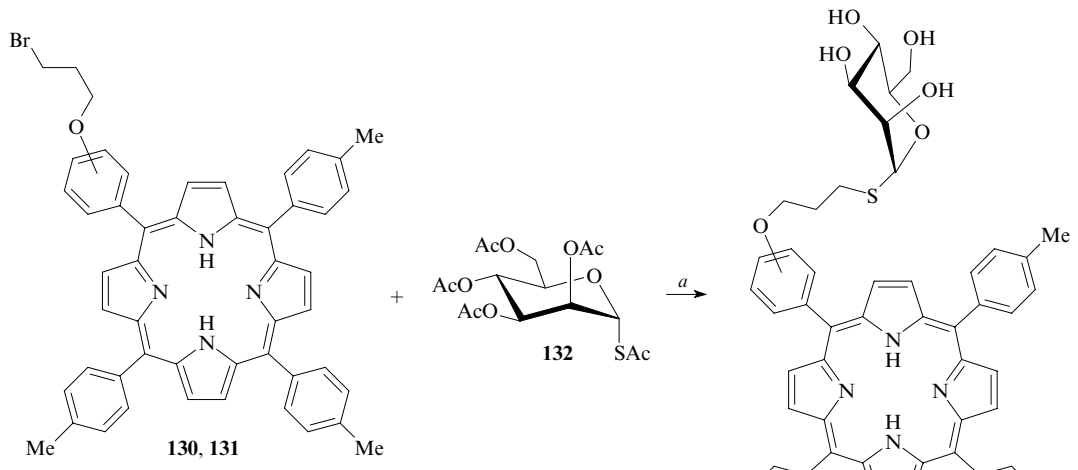


129

A study[104] is devoted to the synthesis of glycoporphyrins with one thioglycoside residue. The advantage of the S-glycosidic bond over the O-glycosidic bond is its higher stability with respect to intracellular glycosidases, which increases the chances of the delivery of potential agents based on such glycoporphyrins to tumour cells. The reaction of tris(*p*-tolyl)porphyrin monobromo derivatives **130** and **131** with *S*-acetyl-1-thio-α-D-mannopyranoside (**132**) followed by deprotection provided the synthesis of target mannoporphyrins **133** and **134** (Scheme 17). Glucoporphyrins **135** and **136** and galactoporphyrins **137** and **138** were synthesized exactly in the same way. *S*-Glucosylporphyrins **135**, **136** together with their *O*-glucosylated analogues prepared previously were tested for the stability against enzymatic hydrolysis of the glycosidic bond by β-glucosidase.[105] No cleavage of *S*-glucosides was observed, while in solutions of *O*-glucosides, glucose was found. This fact confirms once again the stability of *S*-glycosides against the action of glycosidases under physiological conditions.

The photocytotoxicity of compounds **133** – **138** was tested in relation to the cells of the yeast *Saccharomyces cerevisiae* strain FL100 (ATCC 29383). Hematoporphyrin was chosen as the reference compound. The dead cell counts

Scheme 17



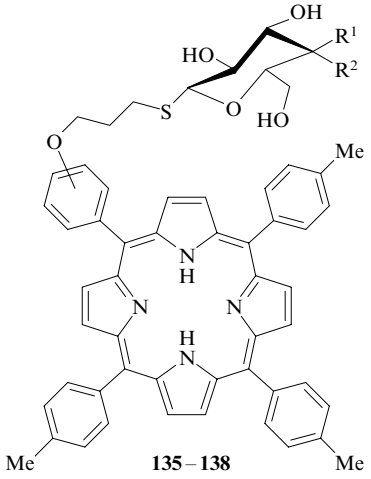
(*a*) 1) Et_2NH, DMF, 6 h; 2) MeONa, MeOH, CH_2Cl_2, 1 h;
isomers: *ortho* (**130**, **133**), *para* (**131**, **134**)

depending on the duration of photoactivation were measured by flow cytometry. For attaining the dead cell counts obtained in the control run in the presence of hematoporphyrin, longer irradiation time was generally required for the tested glycoconjugates **133** – **138**. The specificity of the cytotoxic action was not directly correlated with the nature of the carbohydrate residue. The authors note that *ortho*-isomers were more effective than the corresponding *para*-isomers. A similar observation was also made in another work [93] (see Section IV).

Krausz and co-workers [105] synthesized a series of bidentate *S*-glycosylporphyrins **139** – **144** based on protoporphyrin IX. The synthesis was conducted by analogy with the synthesis of compounds **133** – **138** (see Scheme 17), which were also used, along with **139** – **144**, in biological assays. The photocytotoxicity of conjugates **133** – **138** and **139** – **144** against human chronic myelogenous leukemia cells (K562) was measured based on the dead cell count immediately after photoactivation and after 24 h of incubation when kept in the dark. Photofrin was used as the reference agent. In all cases, the cell death rate increased after a 24-h incubation. Among compounds **133** – **138**, all *ortho*-isomers (**133**, **135** and **137**) and one *para*-isomer (**136**) were photoactive. The number of dead cells immediately after irradiation was in all cases lower than in the Photofrin experiment; however, the efficiency of action became comparable with that of Photofrin after a 24 h incubation. Among conjugates **139** – **144**, compounds **139** – **141** were photoactive and more effective than Photofrin. In the authors' opinion, the photocytotoxicity of esters **139** – **141** is due to the absence of easily ionizable groups, which facilitates the diffusion of non-polar glycoporphyrins into the cell. If this is really so, the photocytotoxic activity of the tested compounds depends to a larger extent on the hydrophobic – hydrophilic balance than on the nature of the carbohydrate residue.

Amphiphilic glycoporphyrins are very promising as regards the use in photodynamic therapy. On the one hand, the functionalization of porphyrins by oligosaccharide fragments enables specific interaction with cell membranes and selective accumulation of the compounds in the target tissues. On the other hand, lipophilic functional

Structures 135–138

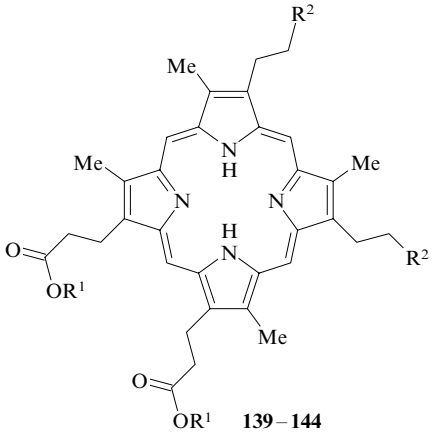


R^1 = OH, R^2 = H (**135**, **136**); R^1 = H, R^2 = OH (**137**, **138**);
isomers: *ortho* (**135**, **137**), *para* (**136**, **138**)

groups also increase the affinity to cell membranes. Using the Lindsey method, porphyrin **145** was synthesized and then it was modified by attachment of the maltohexaosyl residue from compound **146** (Scheme 18).[106] The yield of conjugation was 55%. The photocytotoxicity of amphiphilic glycoconjugate **147** against the HeLa cells was assessed in comparison with 5,10,15,20-tetrakis(*m*-hydroxyphenyl)porphyrin (*m*-THPP). Neither of these compounds showed toxicity in the dark, while upon photoactivation the number of surviving cells at the porphyrin concentration of 5×10^{-6} mol litre^{-1} was 43% (for compound **147**) and 4% (for *m*-THPP). However, at a concentration of 10^{-5} mol litre^{-1}, the efficiency of both photosensitizers was already virtually equal.

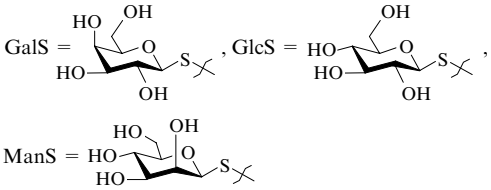
Subsequently, Mikata *et al.*[107] synthesized a series of amphiphilic glycoporphyrins containing each three maltohexaose residues and one linear aliphatic substituent. The carbohydrate substituent was attached to the scaffold by a method similar to that presented in Scheme 18. In investigations of the obtained compounds, the authors focused

Structures 139 – 144



139 – 144

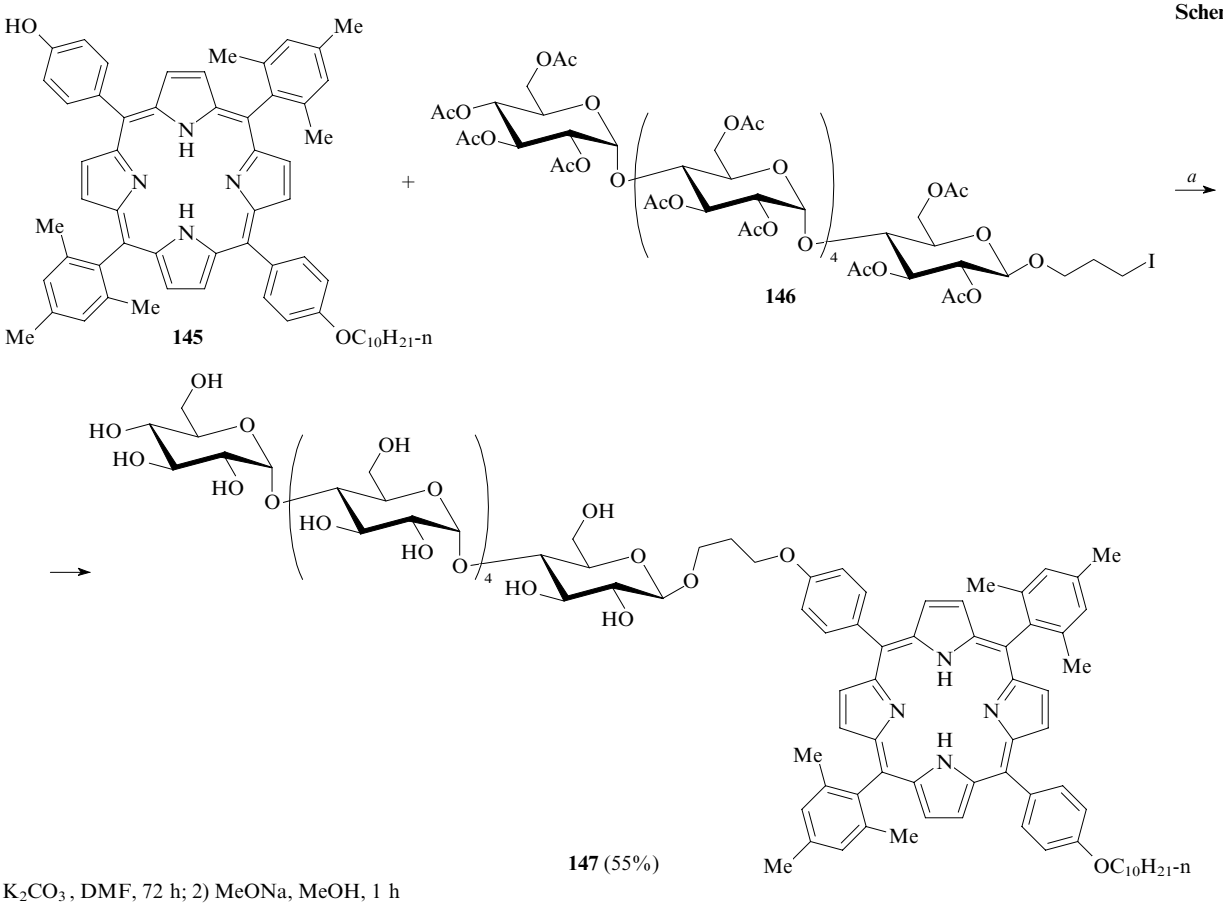
R^1 = Me: R^2 = GalS (**139**), GlcS (**140**), ManS (**141**);
R^1 = H: R^2 = GalS (**142**), GlcS (**143**), ManS (**144**);



on their capability for aggregation rather than on the role of photosensitizers during laser irradiation.

As a continuation of the previous work, a series of zinc glycoporphyrins **148 – 157** comprising different numbers of maltohexaose residues have been prepared.[108] Glycoporphyrins **148 – 152** were obtained similarly to compound **147** (see Scheme 18) and formed as mixtures, which were separated into components by gel filtration and preparative thin-layer chromatography. The series of zinc-containing conjugates **153 – 157** were prepared in the same way. All of the listed compounds proved to be readily soluble in water, especially the zinc complexes (the solubility is $> 10^{-7}$ mol litre^{-1}). The cytotoxicity and phototoxicity were assessed in relation to the human uterine cervix cancer HeLa cells. None of compounds **148 – 157** was toxic in the dark. On exposure to radiation, most photocytotoxic were monodentate conjugates **148** and **153**, which probably have the optimal hydrophilic – hydrophobic balance. They proved to be more active than not only other conjugates synthesized in this work but also than compounds **19** and **23** (see Section II). Generally, the phototoxicity of sensitizers decreased upon increase in the number of carbohydrate residues. The authors demonstrated that this has nothing to do with generation of the singlet oxygen. To explain the differences in the photocytotoxicity, it was suggested that glycoconjugates **148 – 157** are uptaken by the HeLa cells to different extents. It was shown by fluorescence microscopy that compounds with one or two maltohexaosyl moieties penetrate rather easily through the cell membrane, while those containing more carbohydrate ligands, for example, compound **152** get inside the cells with difficulty. These observations suggest that the hydrophobic porphyrin scaffold in conjugate **152** is fully shielded by the hydrophilic ligand environment, which resembles the interaction of octadentate glycoporphyrin **114** (see Section IV) with the

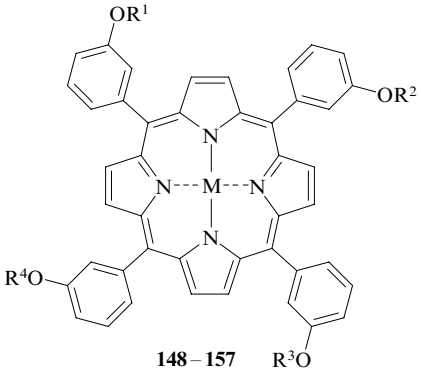
Scheme 18



(*a*) 1) K_2CO_3, DMF, 72 h; 2) MeONa, MeOH, 1 h

RLC-16 hepatocytes.[94] It is also of interest that *cis*-conjugate **149** was more photocytotoxic than *trans*-derivative **150**. This may also be directly related to the different degrees of cellular uptake, which was observed, for example, for compounds **53** and **54** (see Section II).[27] However, for metal porphyrins **154** and **155**, the photocytotoxicity difference was minor (effect of the heavy atom on the biological activity is considered below in this Section).

Structures 148 – 157

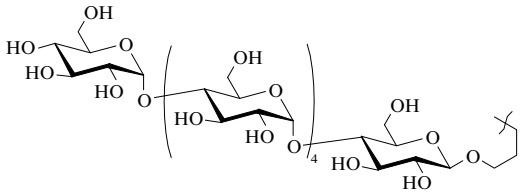


148 – 157

M = 2 H (**148 – 152**), Zn (**153 – 157**)

Compounds	R^1	R^2	R^3	R^4
148, **153**	Sug	H	H	H
149, **154**	Sug	Sug	H	H
150, **155**	Sug	H	Sug	H
151, **156**	Sug	Sug	Sug	H
152, **157**	Sug	Sug	Sug	Sug

Sug =



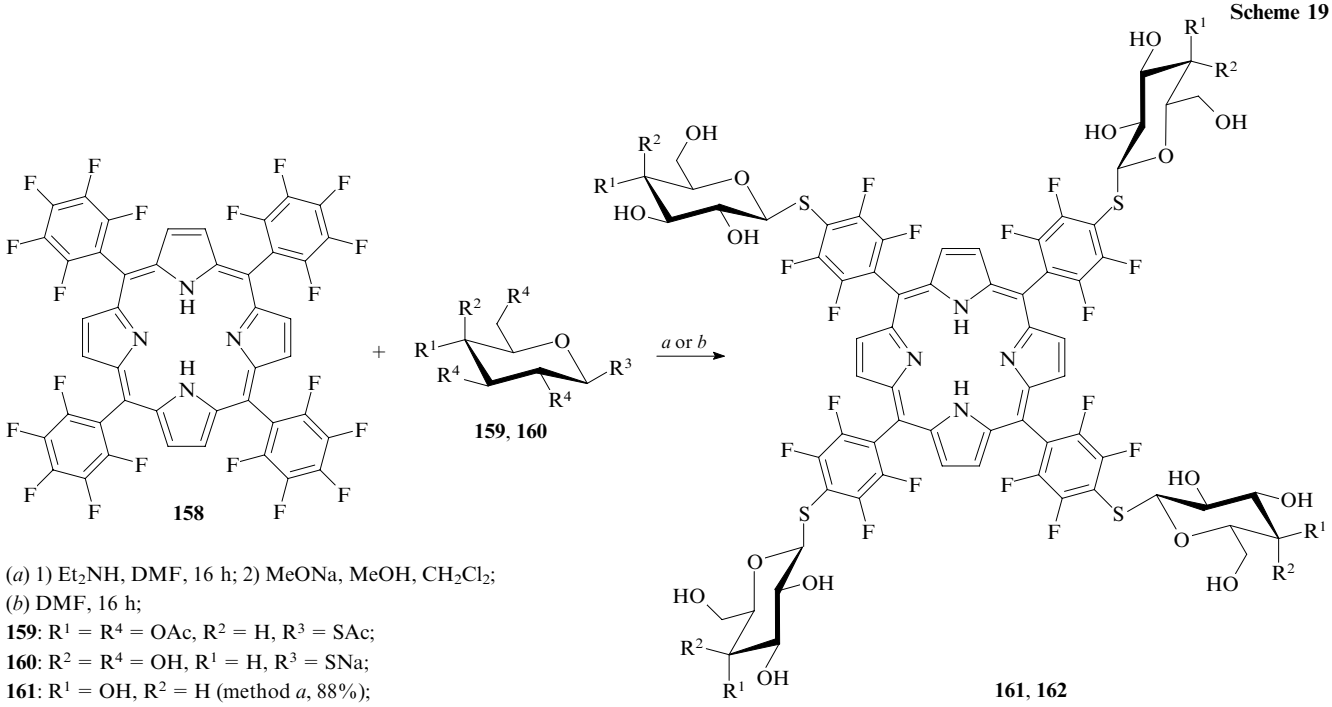
The replacement of the fluorine atom in the *para*-position of the pentafluorophenyl group was used to modify porphyrins by a variety of nucleophilic substitution reactions.[109, 110] This approach to the synthesis of glycoporphyrins was first proposed by Lambrecht *et al.*;[72] however, the authors did not report synthetic schemes or experimental data. Two glycoporphyrins **161** and **162** have been prepared by nucleophilic substitution of thiosugar residues from compounds **159** and **160** for the fluorine atoms in the *para*-position of *meso*-pentafluorophenyl groups in compound **158** (Scheme 19).[111] Note that fluorinated porphyrin derivatives are characterized by higher quantum yields of the triplet state than non-halogenated porphyrins and, hence, they are more promising photosensitizers.[112]

The problem of selectivity of photosensitizers based on porphyrin derivatives with respect to tumour or healthy tissues has not been ultimately solved. Due to accelerated metabolism as compared with healthy cells, most tumour cells require a larger amount of glucose as an energy source. Therefore, porphyrins functionalized by glucose residues can have enhanced affinity for tumour cells. It was shown by fluorescence microscopy that the MDA-MB-231 breast cancer cells uptake glucosylated conjugate **161** twice more actively than galactosylated analogue **162**. Compound **158** devoid of carbohydrate residues is not uptaken by the MDA-MB-231 cells at all, which is indicative of importance of the lipophilic – hydrophilic balance. The authors studied the photocytotoxicity of conjugate **161** and demonstrated that its cytotoxic effect correlates with the conjugate concentration and with the irradiation duration and intensity. An increase in the concentration of compound **161** or irradiation intensity results in cell necrosis, while a decrease in the irradiation intensity induces apoptosis. Milder irradiation conditions decrease the cell mobility and, hence, reduce the tumour invasion. Additional experiments on rat fibroblasts with different degrees of malignancy (3Y1, $3Y1^{c\text{-}Src}$, $3Y1^{v\text{-}Src}$) demonstrated that the affinity of glucoporphyrin **161** for healthy 3Y1 cells is minimum, while that for tumour $3Y1^{v\text{-}Src}$ cells is high (according to fluorescence microscopy data). The photocytotoxicity of conjugate **161** correlates with the affinity data and decreases in the series $3Y1^{v\text{-}Src} > 3Y1^{c\text{-}Src} > 3Y1$. Thus, the obtained results attest to selective affinity of compound **161** for tumour cells unlike healthy tissues.

As has already been noted, low doses of photosensitizer **161** and lower irradiation intensity promote apoptotic death of the MDA-MB-231 cancer cells. Thompson *et al.*[113] made an attempt to study the mechanism of apoptosis of these cells by various methods. The data of confocal fluorescence microscopy demonstrated that 90% of glycoporphyrin **161** absorbed by the MDA-MB-231 cells is located in the endoplasmic reticulum. The subsequent photoactivation triggers the release of Ca^{2+} ions from the endoplasmic reticulum to the cytosol. Then cytochrome *c*, which participates in the caspase 3 activation, is released from mitochrondria. The caspase 3 cleaves and, hence, inactivates poly(ADP-ribose) polymerase, which results in chromatin condensation and cell death.

The search for optimal photodynamic agents affecting the MDA-MB-231 tumour cells was performed by the same group. For this purpose, a library of 21 glycoporphyrins based on *meso*-tetrakis(pentafluorophenyl)porphyrin was designed.[114] All compounds were simultaneously [each in the concentration of $(1-5)\times10^{-6}$ mol litre^{-1}] incubated with the MDA-MB-231 cells for 24 h, and after then the cells were separated and the porphyrins that had attached to the cells were extracted as a mixture and analyzed by MALDI mass spectrometry. Conjugates **163** and **164** were the major identified glycoporphyrins. Relying on the earlier finding that porphyrin *cis*-isomers are better attached to membranes and other tumour cell components than the corresponding *trans*-isomers,[115] conjugate **163** was excluded from further consideration, while isomer **164** was deliberately synthesized and isolated in a pure state. In addition, compound **164** was converted to cationic glycoporphyrin **165**. The affinity of compounds **164** and **165** for the MDA-MB-231 cells was assessed in comparison with conjugate **161** (see Scheme 19) by fluorescence microscopy. An increase in the time of cell incubation with glycoporphyrins from one day to one week was found to markedly increase the cellular uptake of these compounds. The authors attributed this difference to the fact that compounds **161**, **164** and **165** occur initially in the aggregated state (according to dynamic light-scattering data), which prevents their interaction with cells, and, hence, some time is required for their disaggregation, which occurs rather slowly. The cellular

Scheme 19

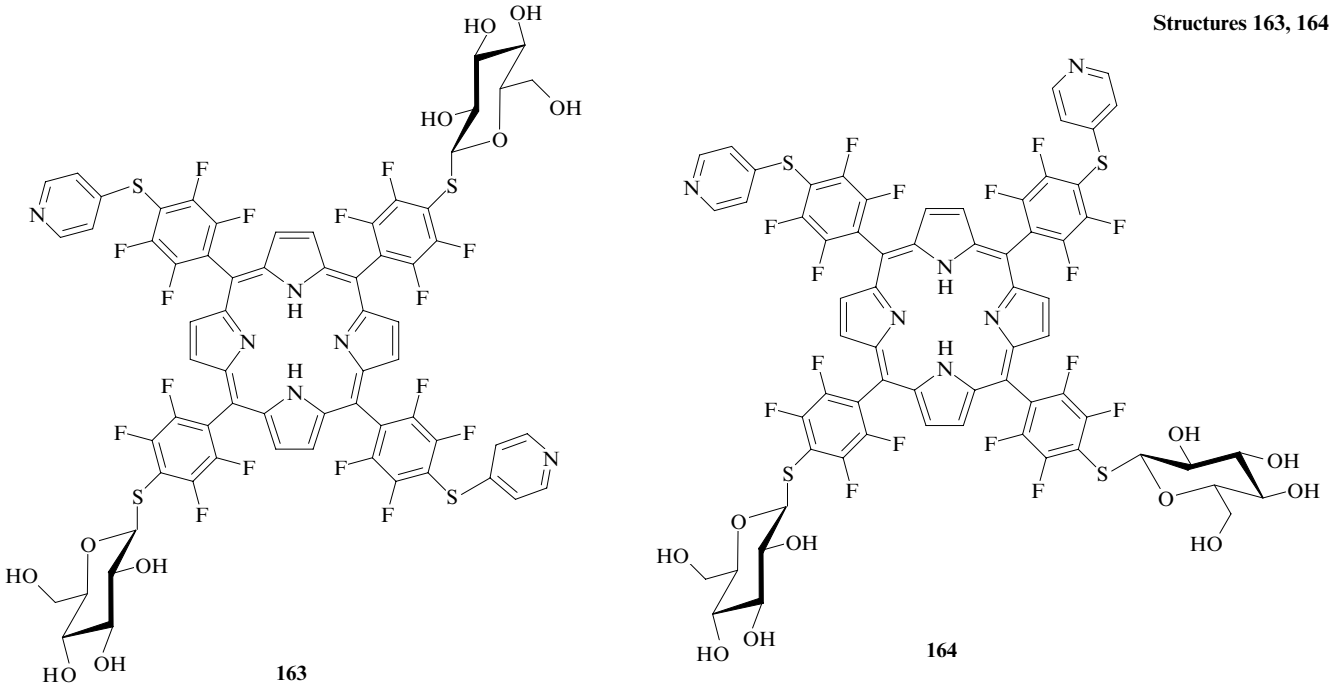


(*a*) 1) Et_2NH, DMF, 16 h; 2) MeONa, MeOH, CH_2Cl_2;
(*b*) DMF, 16 h;
159: $R^1 = R^4 = OAc$, $R^2 = H$, $R^3 = SAc$;
160: $R^2 = R^4 = OH$, $R^1 = H$, $R^3 = SNa$;
161: $R^1 = OH$, $R^2 = H$ (method *a*, 88%);
162: $R^1 = H$, $R^2 = OH$ (method *b*, 92%);

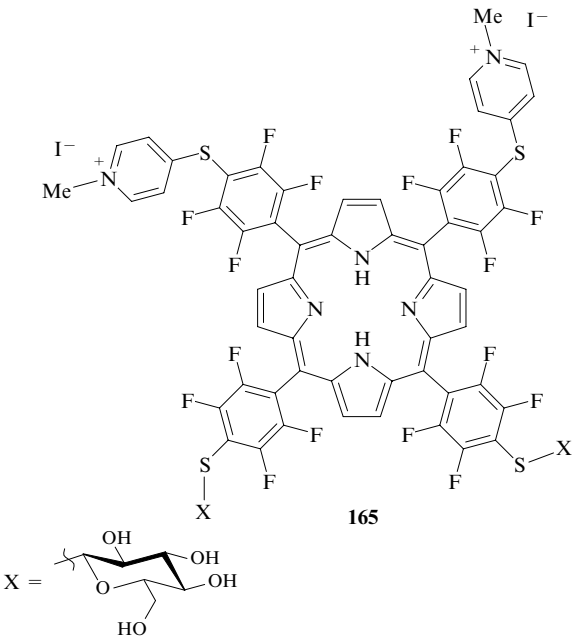
uptake of glycoconjugates **161** and **165** is ~1.4 times higher than in the case of compound **164**. The photocytotoxic effect of compounds **161**, **164** and **165** was assessed for concentrations of 2.5×10^{-6} and 10^{-5} mol litre^{-1}. At concentrations of 10^{-5} mol litre^{-1} and immediately after irradiation for 20 min, all three compounds showed a photocytotoxic effect of ~25%. After 24 h of incubation, the dead cell count increased to 80% for compounds **161**, **165** and to 60% for compound **164**. The following result is noteworthy: the number of necrotic cells for compound **161** immediately after irradiation increased from 28% to 43% as the concentration decreased 4-fold (down to 2.5×10^{-6} mol litre^{-1}). The authors attributed this finding to a lower initial degree of aggregation of glycoporphyrin, which facilitates the formation of triplet excited states of the dye, resulting in enhancement of the sensitizer action.

Ahmed *et al.*[116] synthesized a series of cationic glycoporphyrins **166** – **169** and studied their photodynamic activity *in vitro*. Apart from the photodynamic effect, the cationic water-soluble porphyrins exhibit considerable cytotoxicity in the dark. Conjugates **166** – **169** were tested for photodynamic inactivation of the HT-29 human colon adenocarcinoma cells. The highest activity was found for compound **168** ($LD_{90} = 2.5 \times 10^{-5}$ mol litre^{-1}) and the

Structures 163, 164



Structure 165

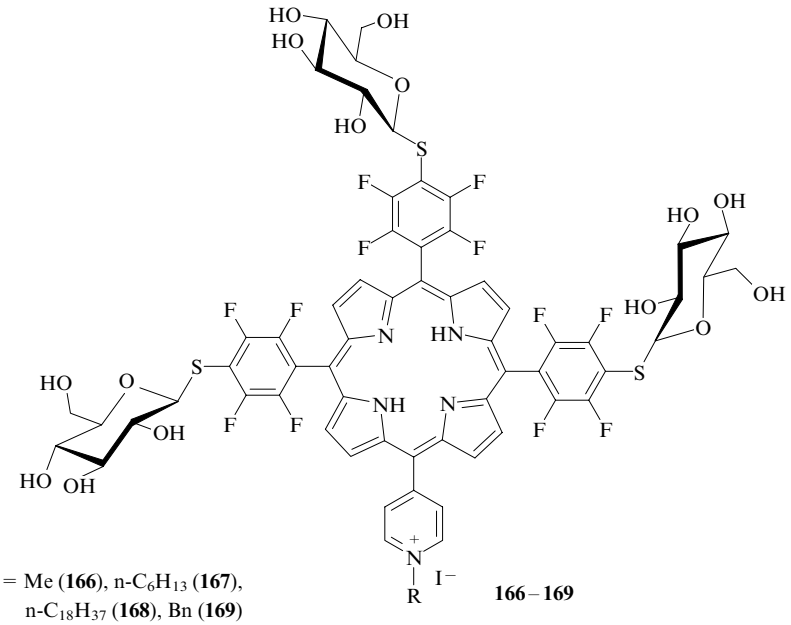


lowest activity was found for conjugate **169** ($LD_{90} = 5 \times 10^{-5}$ mol litre^{-1}) (LD_{90} is the concentration needed to kill 90% of the cells). The authors proposed no explanation to the observed dependence of cytotoxicity on the type of hydrocarbon group. None of the glucoporphyrins exhibited toxicity to the cells without light when present in the maximum concentration of 5×10^{-5} mol litre^{-1}. It is of interest that derivative **170** having no carbohydrate residue was most active ($LD_{90} = 5 \times 10^{-6}$ mol litre^{-1}) and showed also considerable toxicity in the dark (80% at 5×10^{-5} mol litre^{-1}).

Apart from conjugate **95** obtained by glycosylation of porphyrin derivative **96** (see Scheme 10), the synthesis of one more series of porphyrin conjugates has been reported.[91] The nucleophilic substitution reaction involving the carboxylate ion formed from acid **116** and iodide **171** yielded glycoporphyrins **172** – **174** (Scheme 20). Conjugates **175** and **176** were prepared in a similar way (in this case, the fluorine atom in the *meso*-aryl substituent served as the nucleofuge and the alkoxide ion acted as the nucleophile; the values in parentheses are the yields for two steps of the synthesis). The antiviral photocytotoxic activity of conjugates **172** – **176** was tested in relation to the Vero cells infected by two types of herpes simplex virus (HSV-1 and HSV-2). Acyclovir and Foscarnet were used as reference compounds. The maximum non-cytotoxic concentrations of compounds **172** – **176** with respect to non-infected Vero cells were $(5-50) \times 10^{-6}$ g ml^{-1}. At these concentrations, the inhibition of the virus replication cycle was approximately the same (90% – 100%) for porphyrins **172** – **176** and for reference agents. Conjugates **172** and **176** retained the antiviral activity at the level of >40% as the working concentration decreased to 10^{-8} g ml^{-1}, which is comparable in efficiency with glycoporphyrin **95**. Methylglycosides **173** and **174** inhibited the viral replication with the same efficiency as compound **172** with an unprotected semiacetal OH group.

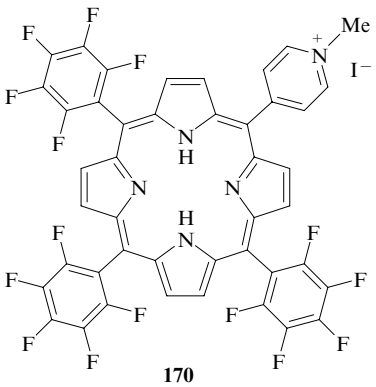
Retinoblastoma is the most frequently encountered intraocular tumour in children. The conservative treatment is based on Carboplatin (platinum complex) chemotherapy and heat treatment of the tumour. This type of chemotherapy may cause short- and long-term side effects, which stimulates the development of alternative therapeutic approaches. One of these is a photodynamic therapy protocol with the use of the photosensitizer Photofrin. However, its activity is rather low, which requires increasing the photosensitizer dose and laser irradiation intensity. This, in turn, also induces side effects. To overcome this restriction, Laville *et al.*[117] prepared a series of tridentate galacto- and mannoporphyrins **177** – **181**, in which the carbohydrate residues are attached through spacers, and performed biological assays for these products. The Y79 human retinoblastoma cells were used as the biological target. Previously, it was shown that tridentate glycoconjugates as sensitizers are more phototoxic than the initial tetrapyrrole derivatives or symmetric tetraglycoconjugates based on them.[97] Porphyrin **182** containing no carbohydrate residues and con-

Structures 166 – 170



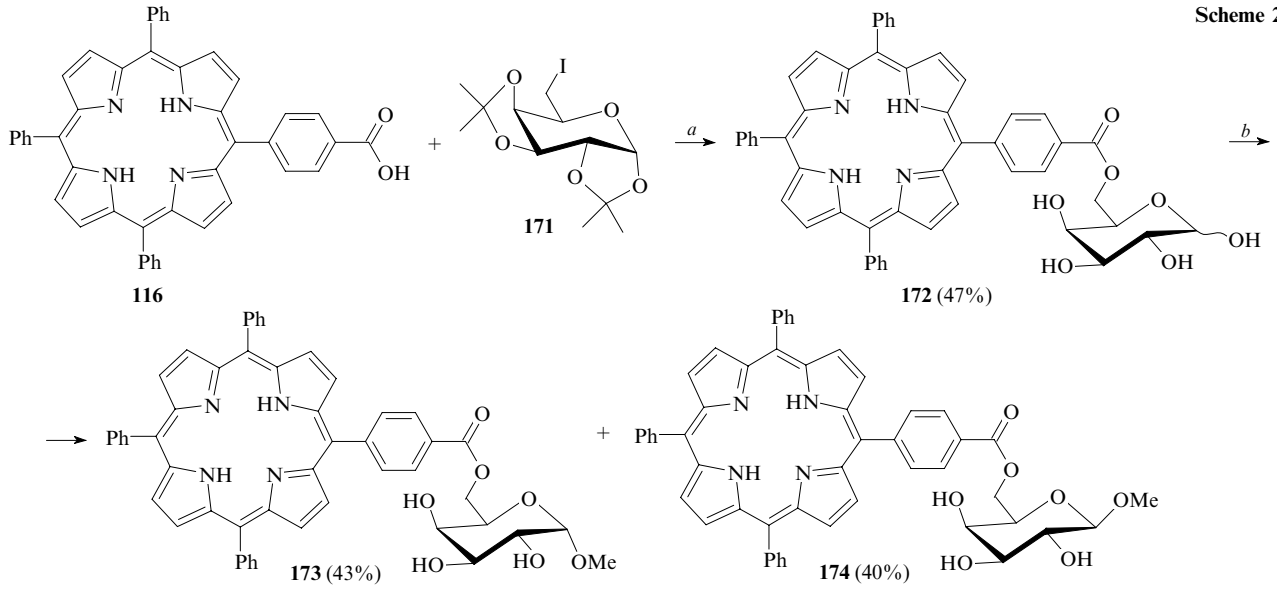
R = Me (**166**), n-C_6H_{13} (**167**), n-$C_{18}H_{37}$ (**168**), Bn (**169**)

166 – 169



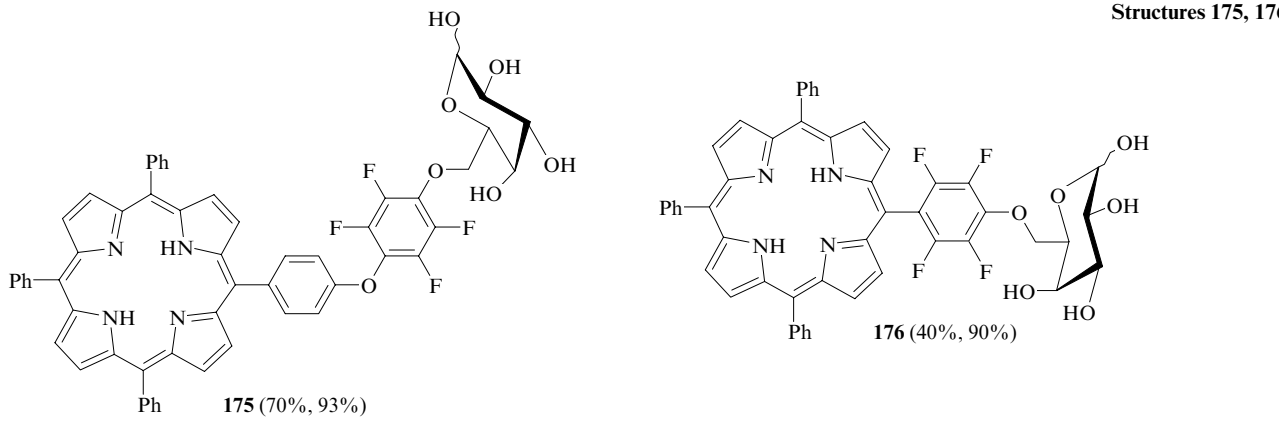
170

Scheme 20



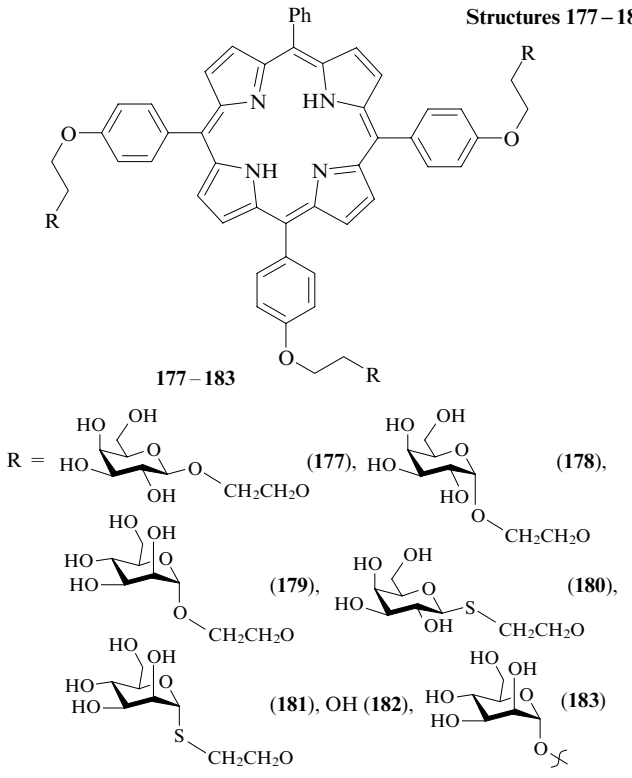
(*a*) 1) K_2CO_3, DMF, 100 °C, 30 h; 2) CF_3CO_2H, H_2O, 30 min; (*b*) MeOH, HCl (gas), $CHCl_3$, 4 h

Structures 175, 176



Structures 177 – 183

jugate **183**[97] with a monoethylene glycol spacer were used as reference agents. The cellular uptake of the photosensitizers for the Y79 cells was measured by flow cytometry. Compound **182** was poorly accumulated in the cells as compared with glycoconjugates, and the cellular uptake of glycoporphyrins **178**, **181** and **183** was only 1.4 – 2 times higher. Higher values were observed for β-galactosides **177** and **180**, which apparently indicates that this anomeric configuration is preferred in the galactose series. The cells absorbed conjugate **180** best of all (a 5-fold difference as compared with **182** and **183**). Upon incubation of the Y79 cells with compounds **178** and **179** in a glucose-containing medium, the cellular uptake of glycoporphyrins was inhibited by 50%, whereas that of porphyrin **182** did not depend on the presence of glucose. The authors believe that this attests to the involvement of glucose transporters present on the cell surface into the uptake. None of the obtained conjugates exhibited cytotoxicity in the dark, while their activity upon irradiation was generally correlated with the efficiency of their accumulation in the cells. However, α-galactosylated porphyrin **178** was most phototoxic ($LD_{50} = 5 \times 10^{-8}$ mol litre^{-1}), while for conjugate **179** having the highest cell affinity, the LD_{50} value was 3.5×10^{-7} mol litre^{-1}. Glycoporphyrins **177** – **179** also exhibited clear-cut photocytotoxic activity against HT29 human colon adenocarcinoma cells.[118]



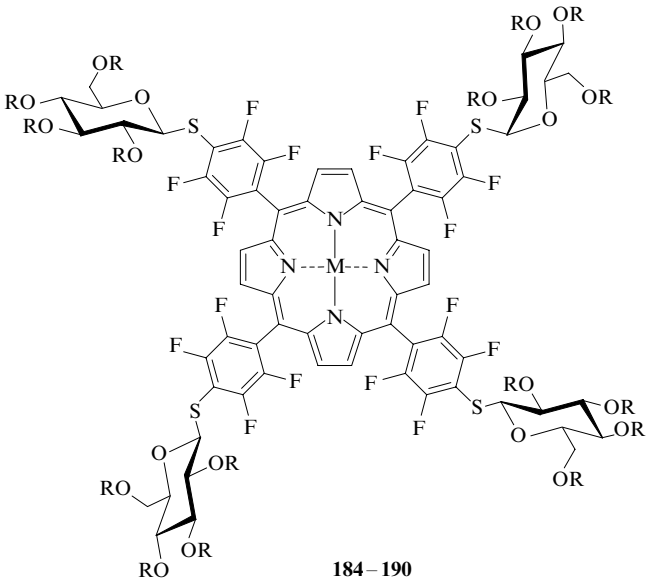
Compound **19**, **33**, **179** and some other neutral porphyrin derivatives were assessed for their ability to interact with dimyristoylphosphatidylcholine liposomes mimicking cell membranes and with human serum albumin.[119] An increase in the number of carbohydrate residues attached to the tetraphenylporphyrin scaffold increases the water solubility of glycoporphyrins, decreases self-aggregation and causes fast association with liposomes and albumin. A decrease in the lipophilicity of structures due to conjugation with sugars leads to lower affinity for the phospholipid bilayer for all of the studied compounds. On the other hand, the binding constant with albumin increases. It was noted that α-mannoside conjugate **179** with the diethylene glycol linker is more prone to self-aggregation in water than other studied compounds and has low affinity for albumin and DMPC. It is of interest that *para*-derivative **19** is less efficiently attached to liposomes than *meta*-derivative **33**. Some other works are also devoted to the interaction of glycoporphyrins with liposomes [120 – 122] and protein components of blood plasma.[123]

The effect of a heavy atom complexed with glycoporphyrin on the photocytotoxic properties against HeLa tumour cells was studied.[124] The authors synthesized Zn^{II}, Pd^{II} and Pt^{II} complexes with the porphyrin ligand containing four glucose residues (compounds **161** and **184** – **190**). The obtained results corresponded to the known data that heavy metal atoms increase the quantum yield of the singlet oxygen generation.[125] Production of singlet oxygen by zinc **185**, **188** and palladium **186**, **189** complexes was indeed higher than for free glycoporphyrins **161** and **184**, whereas platinum complexes **187** and **190** unexpectedly poorly generated $^1O_2$. None of the conjugates destroyed the HeLa cells in the dark at concentrations of 5×10^{-7} mol litre^{-1}. When present in the same concentration but upon photoactivation, compounds **161**, **188** and **189** destroyed the cell culture almost completely but other studied conjugates were less active. A decrease in the concentration of photosensitizers **161**, **188** and **189** to 2×10^{-7} mol litre^{-1} did not reduce their photocytotoxic effect. The incubation of the HeLa cells simultaneously with glycoporphyrins **161**, **188** – **190** and sodium azide (singlet oxygen inhibitor) considerably decreased the photocytotoxic effect. Therefore, the authors concluded that it is singlet oxygen rather than other active species (hydroxyl radical, superoxide anion) formed during photochemical transformations that is the major mediator of tumour cell death.

The first example of systematic research into the photocytotoxic properties of mono-, di-, tri- and tetradentate glycosylated porphyrins is the work by Hirohara *et al.*[126] Conjugates **161** and **191** – **194** were obtained by nucleophilic displacement of fluorine atoms in 5,10,15,20-tetrakis(pentafluorophenyl)porphyrin on treatment with 2,3,4,6-tetra-*O*-acetyl-*S*-acetyl-1-thio-β-D-glucopyranose. Under these conditions, a mixture of all compounds was formed, and it was separated into components by HPLC and then subjected to O-deacetylation. The electronic absorption spectra of solutions of glycoporphyrins **161** and **191** – **194** in DMSO are identical but the spectra of solutions in the phosphate buffer are considerably different. Especially distinguished is the spectrum of bidentate compound **193** with *trans*-arrangement of the carbohydrate ligands: the oscillator strength is almost 1.5 higher than the corresponding values for other S-glycosylated porphyrins. The generation of reactive oxygen species under photoirradiation, which takes place upon energy transfer giving singlet oxygen, for compounds **161** and **191** – **194** does not depend on the number and arrangement of glucose residues. The formation of superoxide anions, hydroxyl radicals and other reactive species — products of electron transfer — is most intense for mono- and *trans*-bidentate derivatives **191** and **193**. The incubation of glycoporphyrins for 24 h ($c = 8 \times 10^{-8}$ mol litre^{-1}) with HeLa tumour cells demonstrated that most active is compound **193**, which is accumulated in the cells in almost three times higher amount than other photosensitizers. This is a noteworthy result, because earlier it has been repeatedly emphasized that *cis*-glycoporphyrins have higher affinity for cells. Possibly, in this case, perfluorinated substituents have the crucial effect on the hydrophobic – hydrophilic balance.

Study of the photocytotoxicity of compounds **161** and **191** – **194** demonstrated that *trans*-glycoporphyrin **193** is

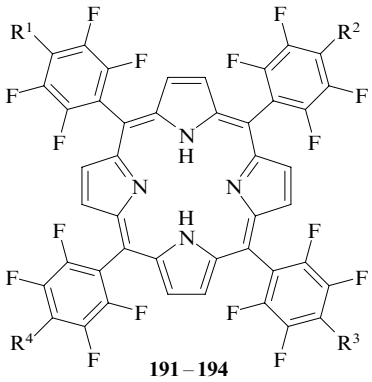
Structures 184 – 190



184 – 190

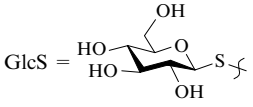
R = Ac: M = 2 H (**184**), Zn (**185**), Pd (**186**), Pt (**187**);
R = H: M = Zn (**188**), Pd (**189**), Pt (**190**)

Structures 191 – 194



191 – 194

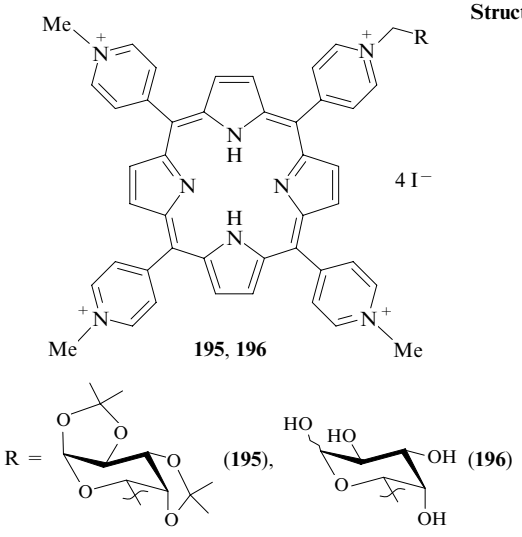
191: R^1 = GlcS, $R^2 = R^3 = R^4$ = F;
192: $R^1 = R^2$ = GlcS, $R^3 = R^4$ = F;
193: $R^1 = R^3$ = GlcS, $R^2 = R^4$ = F;
194: $R^1 = R^2 = R^3$ = GlcS, R^4 = F;



more than 20 times as efficient as other compounds ($LD_{50} < 5 \times 10^{-9}$ mol litre^{-1}). The morphological changes of the HeLa cells according to the confocal laser scanning microscopy data attested to cell death as a result of apoptosis upon photodynamic treatment in the presence of compound **193**. The authors concluded that hydrophobic photosensitizers similar to compound **193** with the *trans*-arrangement of carbohydrate substituents are quite promising for the possible use as photosensitizers.

Unlike the PDT of tumours, the main targets of the antimicrobial photodynamic inactivation are cell membrane elements.[127] Therefore, potential photosensitizers should not necessarily penetrate into the cellular space to achieve the desired effect.[128] Lipids, proteins and carbohydrate compounds that are present on the cell surface undergo molecular destruction under the action of reactive oxygen species.[129] Singlet oxygen oxidizes these cell membrane components, which results in their destruction and the subsequent cell death [130, 131] or disrupts their functioning.[132] A study [133] was devoted to the synthesis of new cationic galactoporphyrins **195** and **196** and estimation of their inactivating potential with respect to gram-positive *Micrococcus* and gram-negative *Pseudomonas* bacterial strains stable [134] against ultraviolet radiation. In this work, apart from compounds **195** and **196**, glycoporphyrins **124** and **125** (see Scheme 15), able to generate singlet oxygen upon irradiation, were also used. In reference experiments, bacteria were not killed when being treated with conjugates without photoactivation or on exposure to radiation alone without a photosensitizer. The *Micrococcus* cells were killed most rapidly under the action of photosensitizers **124**, **125** and **196**, whereas the rate of killing the *Pseudomonas* bacteria was much lower even when 25 times more concentrated solutions of photosensitizers were used (compounds **124** and **196** were the leaders). As noted above, the higher stability of gram-negative bacteria is probably related to a thicker cell wall.[135] It is noteworthy that the *Micrococcus* cells adsorbed all of the studied compounds to almost equal extents; therefore, their photodynamic activity was determined only by the output of singlet oxygen. Conversely, the *Pseudomonas* cells adsorbed most actively conjugates **124** and **196**, which proved to be most active bactericidal agents in this case. The amount of malondialdehyde formed upon the oxidation of lipids was higher in experiments with *Micrococcus*, which is related to lower content of polyunsaturated fatty acids in gram-negative bacteria. These lipids are located in the cytoplasmic membrane, which appears to be destroyed most of all. Moreover, the lifetime of the singlet oxygen is very short; therefore, its diffusion inside the cell is unlikely. By special experiments for both types of bacteria, oxidation of the surface proteins under the action of photosensitizers was also detected. In the authors' opinion, in the case of *Pseudomonas* and compound **196**, it is the oxidation of proteins (rather than lipids) that was the major cause of cell death.

Structures 195, 196

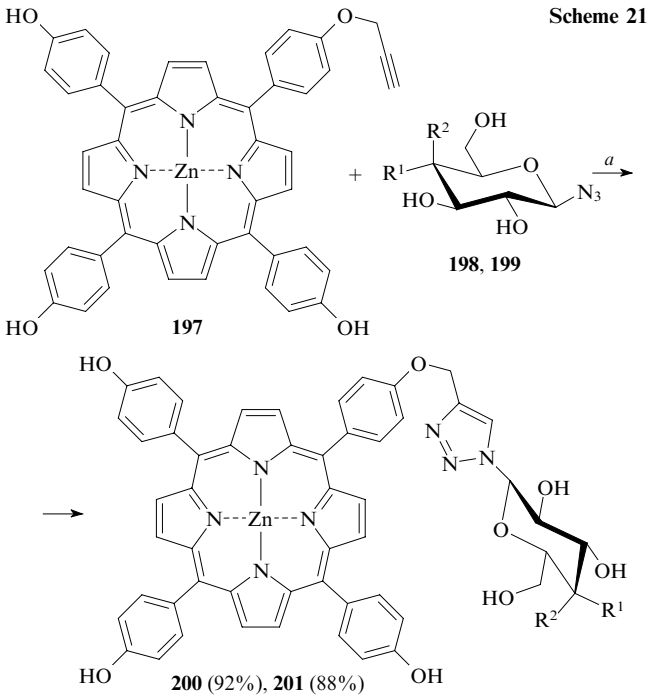


VI. Glycoporphyrins obtained by 1,3-dipolar cycloaddition reaction

An effective modern method for cross-linking diverse molecular fragments is the so-called click-reaction proposed by Sharpless and co-workers [16] and based on 1,3-dipolar cycloaddition of azides to alkynes to give 1,2,3-triazoles.[136] The key reasons for popularity of this reaction among synthetic chemists are as follows: (1) experimental ease; (2) high (often quantitative) yields; (3) regiospecificity of addition. The catalytic version of the reaction in the presence of copper(I) salts is used especially frequently. A number of recent studies deal with conjugated photosensitizers obtained by click reactions. It is known from published data that the formed triazole derivatives are relatively metabolically stable and have no clear-cut toxicity.[137] This is important for photosensitizers, which should be sufficiently stable in physiological media.

One of the first studies of glycoporphyrins synthesized by the 1,3-dipolar cycloaddition reaction was reported by Hao *et al*.[77] Monopropargyl derivative of porphyrin **197** and galactosyl (**198**) and lactosyl azides (**199**) were converted to conjugates **200** and **201** in high yields (Scheme 21).

Scheme 21



(*a*) $CuSO_4 \cdot 5H_2O$, sodium ascorbate, Bu^{t}OH, H_2O, 70 °C, 24 h;
R^1 = H, R^2 = OH (**198**, **200**); R^1 = β-D-Gal, R^2 = H (**199**, **201**)

It is of interest that a similar reaction of azides **198** and **199** with porphyrin devoid of a metal atom did not bring about the desired result, which was attributed to the complex formation between porphyrin and the copper ion, the latter being thus removed from the catalytic cycle.

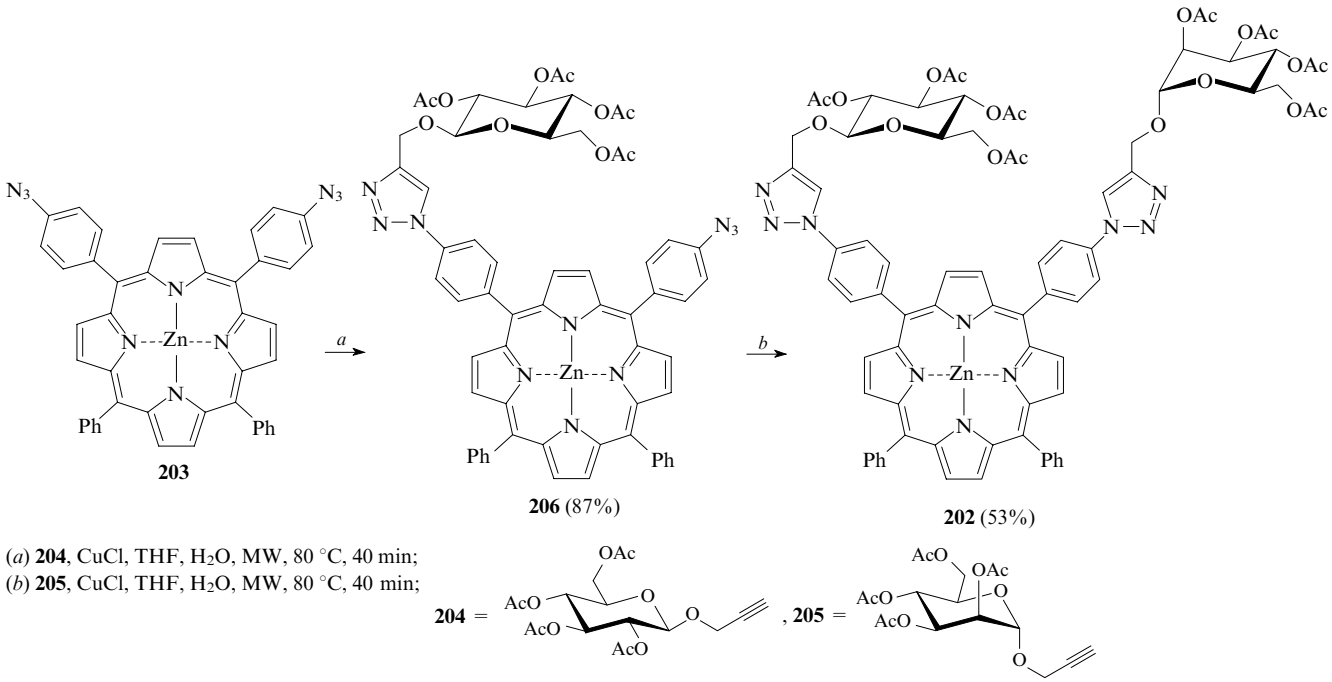
The accumulation of glycoporphyrins **200** and **201** in the HEp2 human carcinoma cells took place for 2 h and, after that, their content in the cell no longer increased. The difference in the accumulation between sensitizers **200** and **201** was insignificant. It was shown by fluorescence microscopy that the synthesized conjugates are mainly localized inside the endosomes and endoplasmic reticulum. It is of interest that tetragalactosylporphyrin **68** (see Scheme 6) obtained in the same work was mainly accumulated in lysosomes.

The optimization of click chemistry techniques for the preparation of various glycoporphyrins has been considered.[138] Thus microwave (MW) radiation was found to considerably reduce the reaction time (for some substrates, from 3 days to 20 min). In addition, bidentate glycoporphyrin **202** (Scheme 22) substituted simultaneously by glucose and mannose residues was synthesized for the first time. The residues were introduced to the initial azidoporphyrin **203** stepwise using propargyl glycosides **204** and **205**; porphyrin **206** was formed as the intermediate.

As a continuation of the previous study,[138] the same research group prepared a library of glycoporphyrins including compounds **207**–**218** containing one to three carbohydrate substituents.[139] The 1,3-dipolar cycloaddition was carried out for unprotected propargylglycosides of mono- and oligosaccharides, including the trisaccharide Lewis^X, which is a part of some biologically significant glycolipids and glycoproteins. Under the elucidated optimal conditions of conjugation, the target compounds were obtained in fairly high yields (56%–93%). It was noted in the study cited that glycoporphyrins known to date contain mainly mono- and disaccharide residues, the latter being mainly represented by lactose. However, many tumour cells express lectins, which are bound to more complex oligosaccharides.[140–142] The biological part of the study concerned the photocytotoxic activity of glycoporphyrins **207**–**218** against the human esophageal squamous carcinoma cell line OE21. The photosensitizer Foscan (compound **71**) was used as the reference. None of the conjugates was found to be toxic in the dark or upon irradiation, whereas the reference agent **71** markedly reduced cell proliferation upon irradiation. The absence of any bioactivity of the tested conjugates brought about the question of whether they were absorbed by the OE21 cells. Experiments demonstrated that only compounds **207**–**212** exhibited intracellular fluorescence, which, however, was inferior in intensity to that of agent **71**. This attests to slower penetration of porphyrin derivatives **207**–**212** into the OE21 cells compared to **71**. Among compounds **207**–**212**, *cis*-conjugates **207**–**210** are absorbed by the cells most actively; the advantage of this arrangement of carbohydrate ligands for penetration into cells was repeatedly noted above. It was also demonstrated that on exposure to radiation, lactosamine-containing conjugate **210** produces singlet oxygen but less intensely than compound **71**. The authors give no explanation to the absence of phototoxic activity of the conjugates against the OE21 cells.

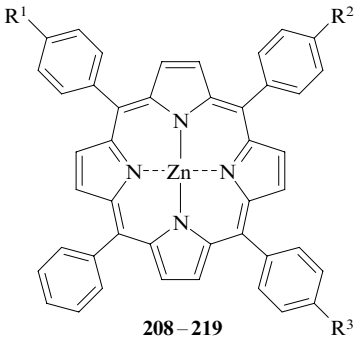
Yet another series of tridentate glycoporphyrins **219**–**224** in which monosaccharide residues are attached through a triazole-containing spacer were prepared by 1,3-diploar cycloaddition assisted by microwave radiation.[143] The cytotoxicity and photocytotoxicity of compounds **219**–**224** were determined in the HT29 human colon adenocarcinoma cells and Y79 retinoblastoma cells. None of the glycoporphyrins of this series showed a cytotoxic activity in the dark. The HT29 cells express membrane lectins specific to β-glucose while the Y79 cells express α-mannose- and β-galactose-specific lectins. However, in this study, the photocytotoxicity of compounds **219**–**224** against the Y79 cells was 10–20 times higher than that for the HT29 cells irrespective of the carbohydrate residue. The authors failed to elucidate a relationship between the structure and the activity. Mannoporphyrin

Scheme 22



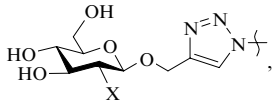
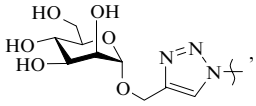
222 proved to be the most effective photosensitizer. It is of interest that it is similar in structure to compound **183**, which, however, killed the HT29 cells 25 times more actively.[118] Thus, the introduction of a triazole moiety decreases the photocytotoxicity in *in vitro* experiments irrespective of its position in the spacer (closer either to the porphyrin part or to the carbohydrate ligand).

Structures 207 – 218

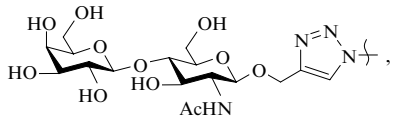


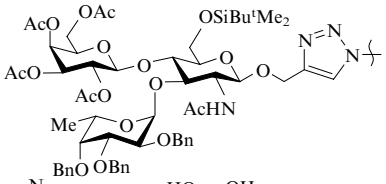
208 – 219

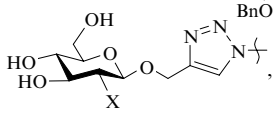
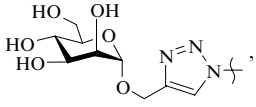
Compound	R^1	R^2	R^3	Compound	R^1	R^2	R^3
207	**A**	**A**	H	**213**	**A**	**A**	**A**
208	**B**	**B**	H	**214**	**B**	**B**	**B**
209	**C**	**C**	H	**215**	**C**	**C**	**C**
210	**D**	**D**	H	**216**	**D**	**D**	**D**
211	**B**	H	**B**	**217**	**B**	**C**	H
212	**D**	H	**D**	**218**	**E**	H	H

A, **C** = , **B** = ,

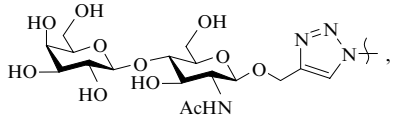
X = OH (**A**), NHAc (**C**),

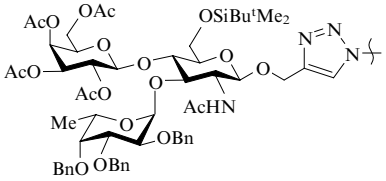
D = ,

E = 

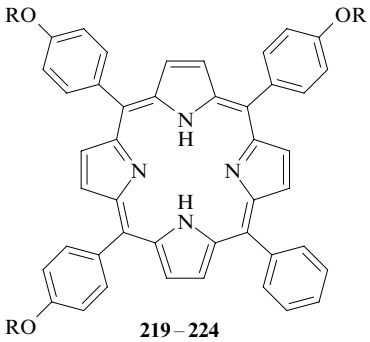
A, **C** = , **B** = ,

X = OH (**A**), NHAc (**C**),

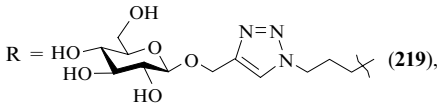
D = ,

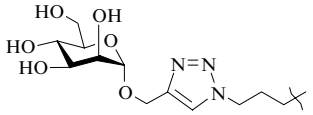
E = 

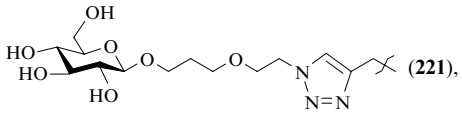
Structures 219 – 224

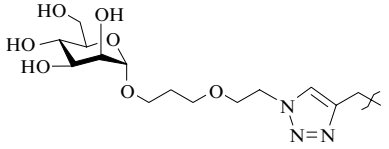


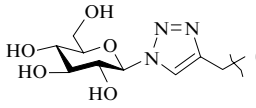
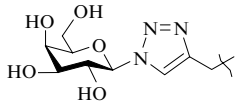
219 – 224

R =  (**219**),

 (**220**),

 (**221**),

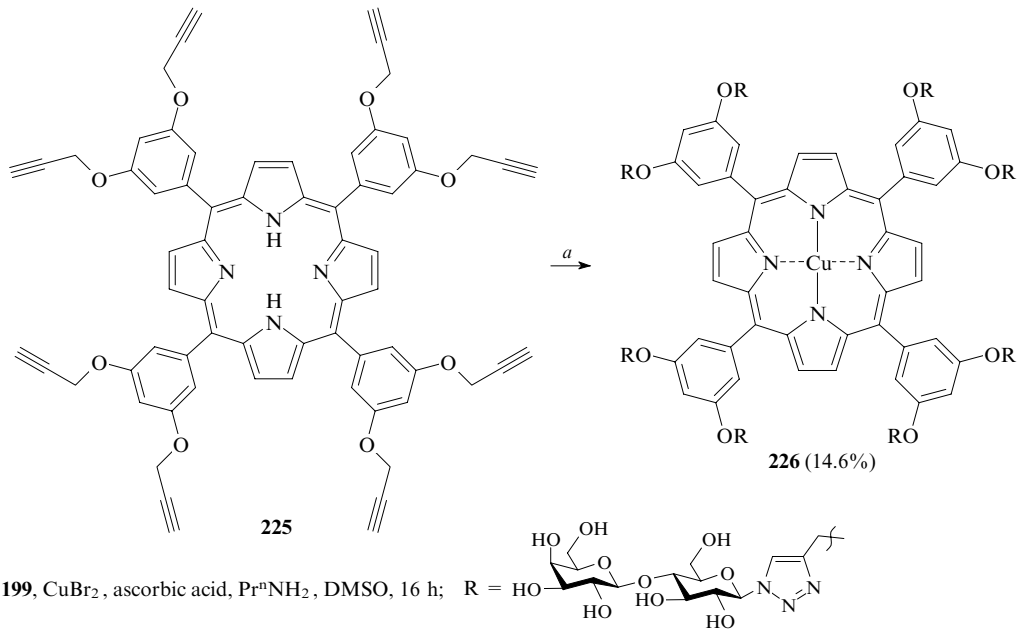
 (**222**),

 (**223**),  (**224**)

Hasegawa and co-workers[144] used propargylated porphyrin **225** and lactosyl azide **199** to prepare easily water-soluble octadentate glycoporphyrin **226** (Scheme 23). The authors took a more than twofold excess of copper(II) bromide with respect to porphyrin **225** to prevent complete catalyst deactivation due to complexation. The yield of product **226** (15%) can be considered to be rather high, as this transformation actually comprises eight separate reactions of addition of carbohydrate ligands to the porphyrin core. The affinity of product **226** for fluorescein isothiocyanate (FITC)-labelled lactose-specific lectin RCA 120 was measured by fluorescence titration. The fluorescence intensity of FITC-RCA 120 decreased following the addition of glycoporphyrin. The dissociation constant of the ligand – lectin complex was 3.2×10^{-5} mol litre^{-1}. On the other hand, no fluorescence changes were detected during titration of the labelled lectin ConA (specific to mannose and glucose) with conjugate **226**, which attests to highly specific interaction of compound **226** and lectin RCA 120. Unfortunately, in this or in subsequent works, the authors did not report on the photodynamic properties of glycoporphyrin **226**.

The biological assays of almost all glycoporphyrins considered above were carried out to elucidate their applicability as photosensitizers for photodynamic therapy. However, porphyrin and its derivatives having a rigid planar structure can also be of interest as low-molecular-mass scaffolds for the design of cluster glycoconjugates with a definite topology, which may find a whole range of possible

Scheme 23



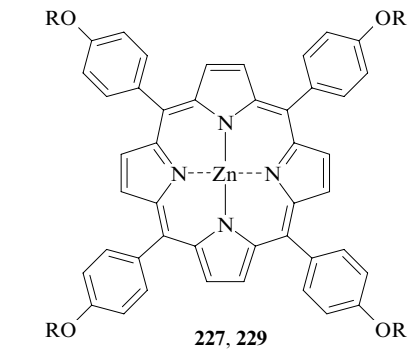
(*a*) **199**, $CuBr_2$, ascorbic acid, Pr^nNH_2, DMSO, 16 h; R =

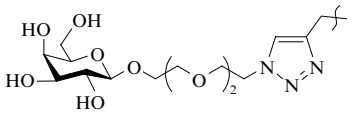
applications, for example, for the fabrication of biochips or blocking agents of bacterial adhesins (see below). From this standpoint, glycoporphyrins were studied by S Vidal's and A Imberty's research groups. For example, galactoporphyrin **227** was synthesized and its interaction with two galactose-specific lectins — adhesin of *P. aeruginosa* PA-IL and lectin ECA from the plant *Erythrina cristagalli* — was studied by a variety of procedures.[145] Galactoside **228** served as a monodentate reference compound. In the hemagglutination inhibition assay (HIA) with rabbit erythrocytes, cluster **227** proved to be 159 times more efficient than compound **228** or almost 40 times more efficient per glycoside substituent, which attests to a considerable cluster effect. This outcome indicates that by optimizing the structure of conjugate **227**, one can obtain even more active blocking agents for the lectin PA-IL needed for medical applications.

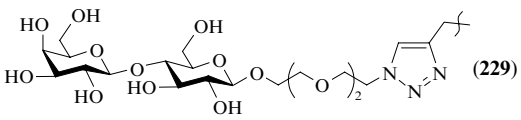
Unlike the interaction with PA-IL, compounds **227** and **228** bind to the lectin ECA almost equally poorly, which has been demonstrated, in particular, by surface plasmon resonance (SPR). This conclusion was also supported by the results of inhibition by glycoporphyrin **227** and galactoside **228** of rabbit erythrocyte hemagglutination induced by both lectins. Using isothermal titration microcalorimetry (ITC) of lectin PA-IL by glycoporphyrin **227**, the dissociation constant (K_D) of the ligand – lectin complex was determined to be 3.3×10^{-7} mol litre^{-1} (for compound **228**, $K_D = 1.5 \times 10^{-4}$ mol litre^{-1}). It is noteworthy that tetragalactoside based on the cyclo-oligoglucosamine scaffold synthesized by our research group is currently the most high-affinity ligand for the lectin PA-IL ($K_D = 8 \times 10^{-8}$ mol litre^{-1}).[146]

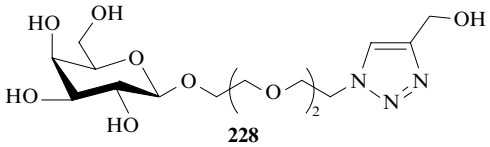
Vidal's and Imberty's studies dealing with the synthesis of lectin blocking agents based on the porphyrin scaffold were continued by Cecioni *et al.*,[147] who described the preparation of tetralactosylporphyrin **229**. The interaction of this compound with lectins ECA and galectin-1 (also specific to galactose) was studied by HIA, SPR and enzyme-linked lectin assay (ELLA). Lactoside **230** was used as the reference compound. Glycoconjugate **229** was found to bind to this lectin only 8 – 11 times (depending on the method) more effectively than compound **230**. The conditions of the ELLA experiment, particularly, the use of peroxidase, are inapplicable to galectin-1 due to disturbance of its tertiary structure under the action of the oxidant. Therefore, the authors studied the interaction of porphyrin **229** with galectin-1 by HIA and SPR. Compound **229** inhibited

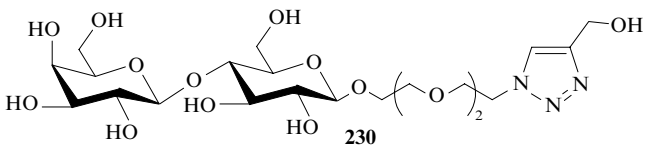
Structures 227 – 230



R =  (**227**),

 (**229**)



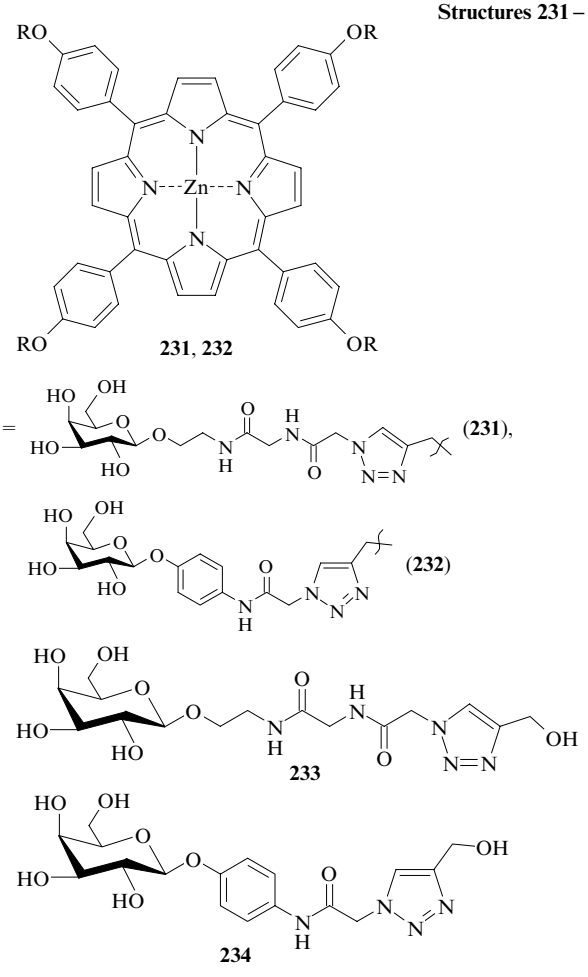


hemagglutination of erythrocytes induced by galectin-1 when present in a 250 times lower concentration than derivative **230**. However, the SPR test showed that adhesion is inhibited by porphyrin **229** only 6.4 times more efficiently than by compound **230**, which means the absence of the cluster effect. This difference between the results obtained by HIA and SPR methods was attributed to the fact that these methods generally study different processes: biomolecular interactions on the erythrocyte surface (HIA) or on the surface of a gold chip coated by lactosylated polyacrylamide polymer (SPR).

A series of glycoclusters **227**, **231** and **232** and the corresponding reference compounds **228**, **233** and **234** were prepared to study the influence of the nature of spacers (linkers) between the porphyrin scaffolds and carbohydrate ligands on the interaction with lectin PA-IL.[148] Generally, an increase in the linker rigidity reduces the entropy contribution to the binding energy, resulting in higher affinity. However, molecular modelling demonstrated that the linkers in compounds **227** and **231** have almost equal rigidity. On the other hand, the more hydrophobic the linker, the stronger the binding of the ligand to lectin owing to non-specific hydrophobic interactions. For example, derivative **234** binds to lectin PA-IL rather effectively according to various data (HIA, ELLA, SPR). In the HIA test, glycoporphyrins **227** and **231** exhibited activity in micromolar concentrations and proved to be 159 and 2500 times, respectively, more effective than monodentate analogues (glycocluster **232** was not studied under these conditions as it can induce erythrocyte hemolysis). High affinity of the glycoporphyrins with respect to lectins was also confirmed by the ELLA method, because glycoporphyrins **231** and **232** are not sufficiently soluble in water, which is necessary for isothermal titration calorimetry. This is why the authors specially noted that good solubility is necessary for lectin blocking agents and emphasized the complexity of predicting the influence of the rigidity – flexibility of the ligand structure on the thermodynamics of binding.

Glycoclusters with porphyrin scaffolds similar to glycoporphyrin **227** [149] but containing α-D-mannose or α-L-fucose residues instead of galactose residues have found use for the design of biosensors based on single-walled carbon nanotubes or chemically modified graphene;[150] this sensors can be used to detect various lectins including PA-IL, PA-IIL and ConA. Owing to the presence of a planar aromatic skeleton, glycoporphyrins are non-covalently bound to the nanotube or graphene surface, thus providing spatial accessibility of carbohydrate residues for biorecognition. Note that the sensitivity and selectivity were higher for biosensors based on nanotubes. The authors attributed this to the fact that nanotubes have one-dimensional structure, unlike two-dimensional graphene, and, therefore, glycoporphyrins attached to them are more accessible for binding to lectins. The dissociation constants of multidentate ligand – lectin complexes measured by biosensors differed only slightly from the constants measured by ITC. The authors believe that the nanoelectron platform they fabricated will be suitable, after some modification, for fast assessment of the bacteria *P. aeruginosa* and other pathogens in water and soil samples, which is needed to prevent outbreaks of diseases and to preserve human health.

Structures 231 – 234



231, **232**

(**231**),

(**232**)

233

234

VII. Miscellaneous methods for the preparation of glycoporphyrins

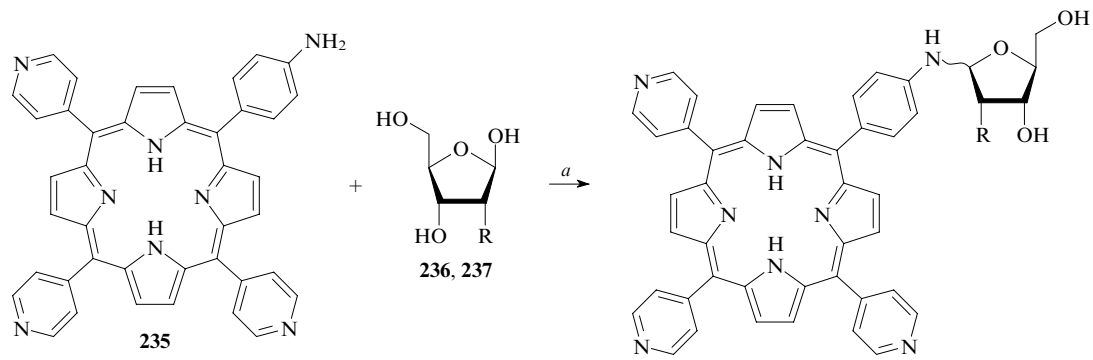
Some known methods for the synthesis of glycoconjugates based on porphyrins have been illustrated so far by single examples and have not been further systematically developed. Nevertheless, they deserve mentioning in the review, although no data on the biological activities of products are available. Treatment of monoamine **235** with monosaccharide derivatives **236** and **237** in the presence of ammonium chloride in anhydrous ethanol afforded N-substituted glycoporphyrins **238** and **239** (Scheme 24).[151] The conjugates were isolated as mixtures of anomers with isomer ratios $\alpha:\beta = 1:1.7$ (for compound **238**) and $1:1.9$ (for compound **239**).

The palladium-catalyzed Heck reaction [152] was also used for the synthesis of porphyrinyl nucleosides (Scheme 25).[153] Treatment of vinylporphyrin **240** with chloromercurouridine **241** yielded a mixture of compounds **242** and **243** in a total yield of 36%; the mixture was separated into components after removal of the zinc ion from the complexes.

Transesterification was applied in the synthesis of a series of oligodentate galactoporphyrins.[32] As an example, Scheme 26 presents the reaction of ester **244** and galactose derivative **122** to give conjugate **245** readily soluble in water. The yield of the conjugation step was 5%, while the removal of isopropylidene protections occurred quantitatively.

By aminomethylation followed by oxidation and deprotection, compounds **246** and **247** were converted in a low yield to porphyrin derivative **248** (Scheme 27) containing galactose and cholesterol moieties as substituents.[154, 155]

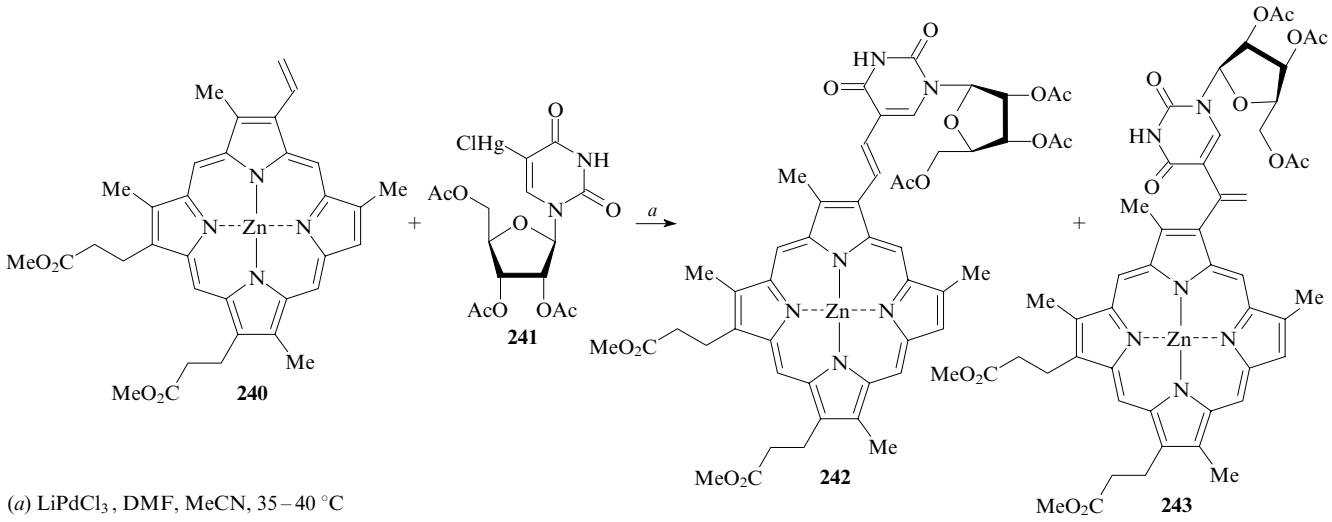
Scheme 24



235 **236**, **237** **238** (64%), **239** (54%)

R = OH (**236**, **238**), H (**237**, **239**); (*a*) NH_4Cl, EtOH, 36 h

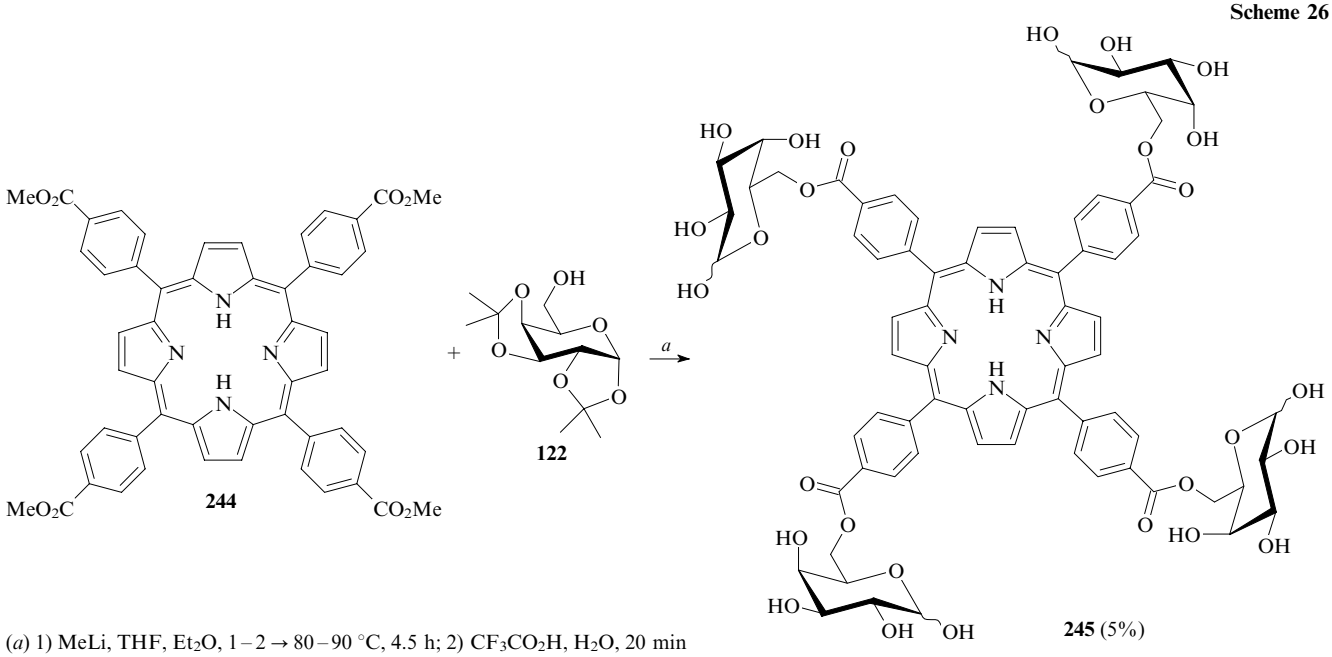
Scheme 25



240 **241** **242** **243**

(*a*) $LiPdCl_3$, DMF, MeCN, 35 – 40 °C

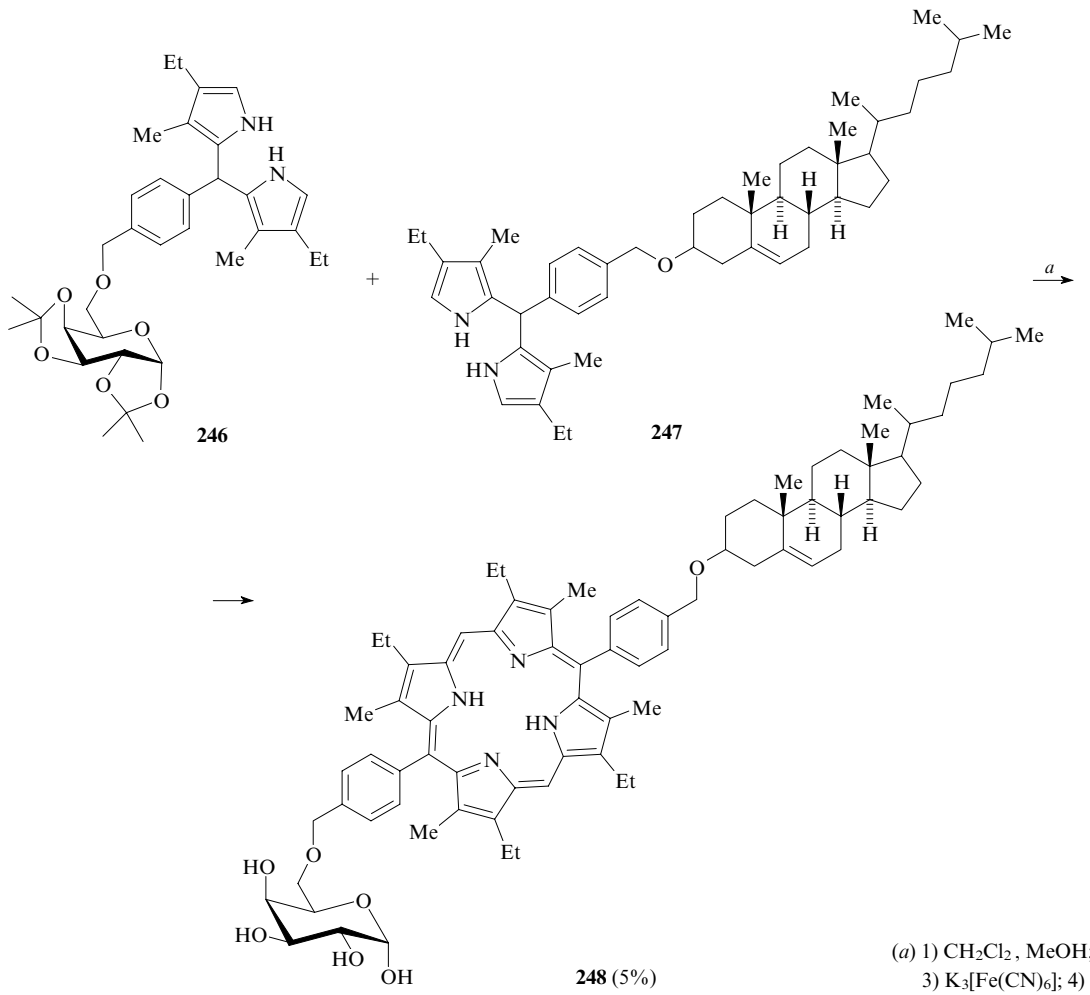
Scheme 26



244 **122** **245** (5%)

(*a*) 1) MeLi, THF, Et_2O, 1 – 2 → 80 – 90 °C, 4.5 h; 2) CF_3CO_2H, H_2O, 20 min

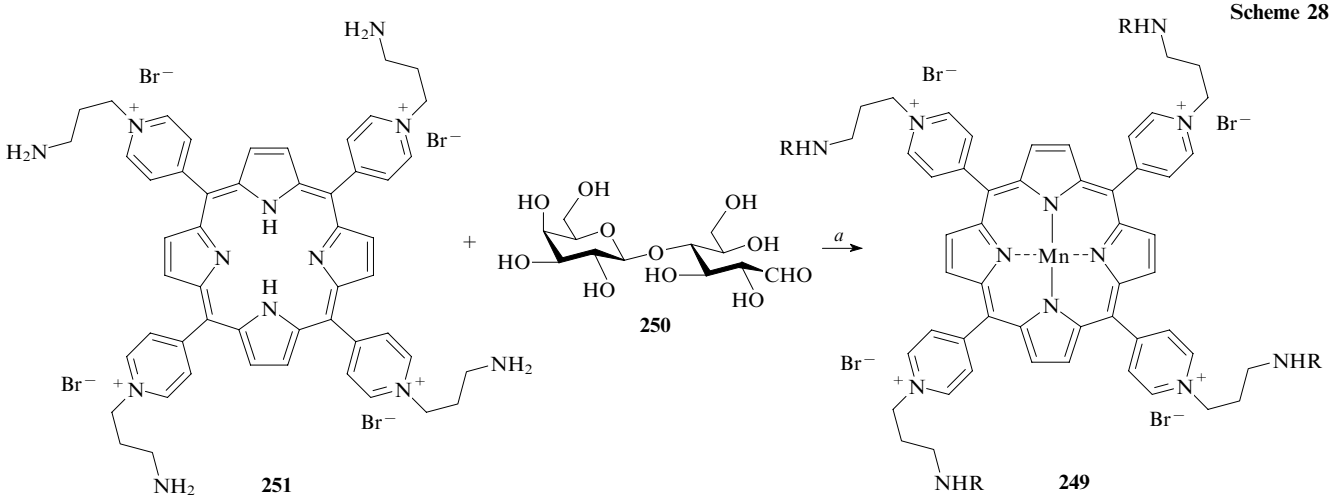
Scheme 27



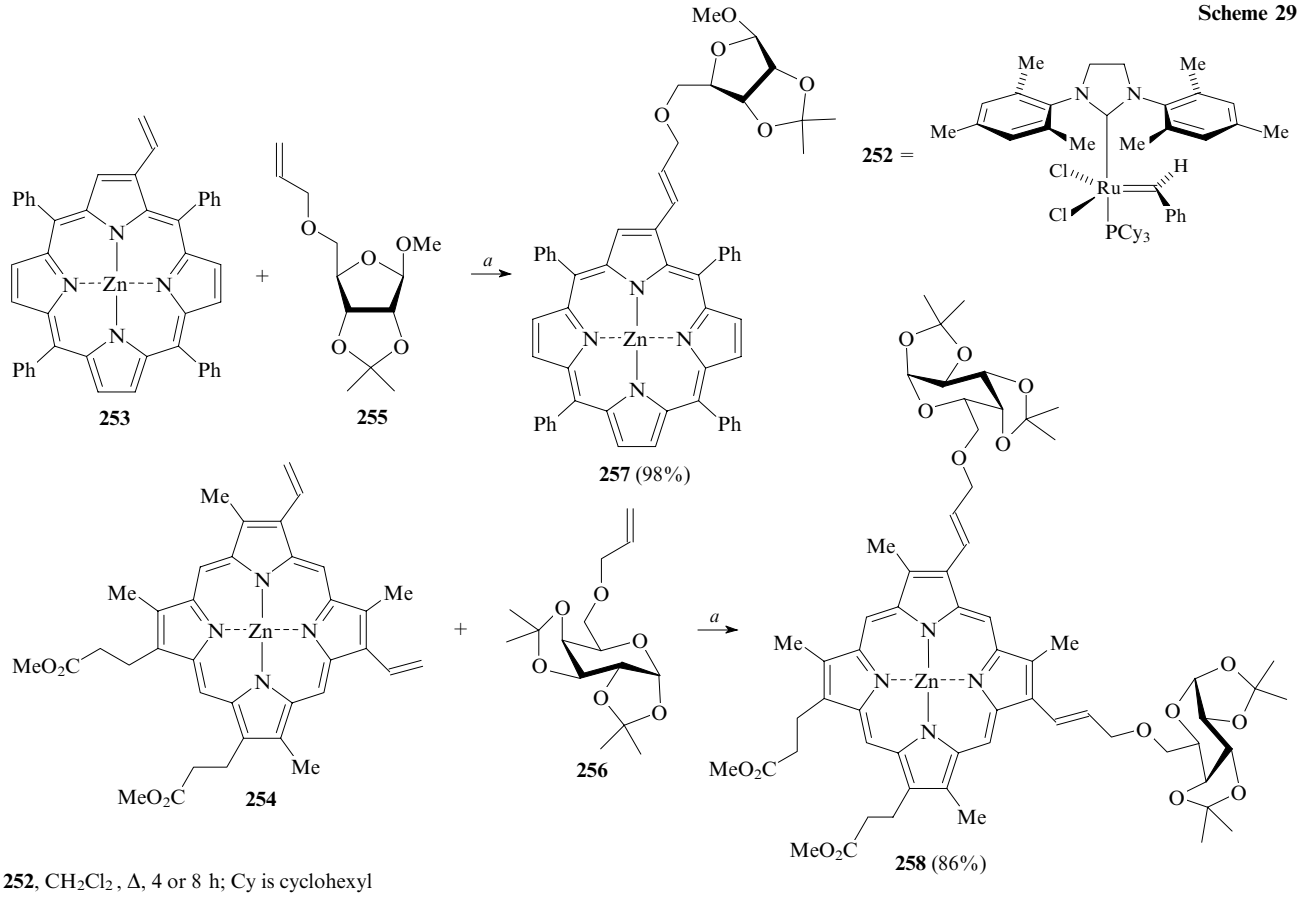
The authors demonstrated that, despite the amphiphilic nature, conjugate **248** is virtually unable to be incorporated into phosphatidylethanolamine liposomes, which are often used as cell membrane models. This might be related to aggregation of the molecules of **248** in water to give stable vesicles.

Asayama *et al.*[156] described the synthesis of tetradentate glycoporphyrin in which the carbohydrate residues are bound to the porphyrin carrier by three-carbon spacers. Compound **249** was prepared by reductive amination of aldehyde **250** involving amino groups of derivative **251** in the presence of the reducing agent $NaBH_3CN$ followed by

Scheme 28



Scheme 29



(*a*) **252**, CH_2Cl_2, Δ, 4 or 8 h; Cy is cyclohexyl

metallation (Scheme 28). Unfortunately, the authors do not present the yields of the chemical transformations. This work was performed within the framework of the search for potential mimicking agents for superoxide dismutase (SOD), which is the key enzyme protecting the body from constantly forming aggressive oxygen radicals. It was shown that conjugate **249** has a considerable SOD activity. Moreover, owing to the presence of pseudolactosyl residues, the HepG2 human hepatoma cells effectively recognize compound **249**. The addition of lactose or galactosylated glycoprotein asialofetuin inhibits the process of recognition. In authors' opinion, this implies that glycoconjugate **249** specifically interacts with asialoglycoprotein receptors on the surface of HepG2 cells.

Alkene cross-metathesis is fairly promising in the synthesis of various glycoporphyrins. Using the second-generation Grubbs catalyst **252**, vinyl porphyrin derivatives **253** and **254** and allyl D-ribose (**255**) and D-galactose (**256**) derivatives were converted to conjugates **257** and **258** in high yields (Scheme 29).[157] Glycoporphyrins with glucose and fructose residues were equally efficiently prepared from compounds **253** and **254**. A somewhat lower yield observed in the case of bidentate conjugate **258** was attributed to lower catalyst : alkene ratio compared to that in the synthesis of compound **257**. All reactions resulted only in *E*-isomers.

VIII. Conclusion

The present review devoted to methods for the synthesis and biological activity assays of glycoporphyrins completes the series of reviews dealing with mono- and oligodentate conjugates based on low-molecular-mass scaffolds [the previous reviews covered glycoclusters based on calixarenes (resorcinarenes)[17] and cyclooligosaccharides].[18] The methods of conjugation of carbohydrate ligands with all types of scaffolds are largely similar and have been studied rather extensively. However, the application areas being developed for cluster glycoconjugates of the listed types, especially possible biological applications, are substantially different. For example, glycocalixarenes and glycoconjugates based on cyclooligosaccharide scaffolds have been synthesized mainly for the search for effective blocking agents of microbial lectins the operation of which is based on glycoside cluster effect.[158–160] Conversely, the proportion of original works dealing with interactions of oligodentate glycoporphyrins with lectins is very low, as the principal incentive for the studies of glycoporphyrins is the search for effective photosensitizers for photodynamic therapy. However, many authors note that it is often wrong to analyze and compare the results of biological assays of glycoporphyrins, because there are no generally accepted protocols of these assays. Various works use different cell cultures, concentrations of active components, parameters and irradiation times. In this respect, it should be noted that most of papers that claim a high potential of obtained compounds as sensitizers for photodynamic therapy relying on the results of *in vitro* experiments usually have no data on the activity of compounds in the presence of saliva, protein components of blood or native blood, which often suppress the photodynamic activity of porphyrin derivatives.

As has already been noted, the introduction of carbohydrate moieties into porphyrin conjugates was intended to solve several topical problems. One problem was successfully solved, namely, increase in the solubility of porphyrins in water, which is a necessary condition for both biological experiments and possible therapeutic applications. The other problem is to achieve the optimal hydrophilic – hydrophobic balance by varying the number and topology of conjugated carbohydrate ligands. Indeed, the highest affinity for various test cells was repeatedly observed for glycoporphyrins with two or three carbohydrate residues located in the *cis*-positions (for bidentate conjugates) relative to each other.

The third problem is the introduction of the carbohydrate vector that would allow glycoporphyrins to interact with specific lectins on the cell membrane surface, thus providing targeted delivery of glycoporphyrins to the cells. In our opinion, the practicability of this approach is doubtful, because the human and animal carbohydrate antigens used as vectors cannot be active in the presence of large amounts of analogous natural oligosaccharides and their derivatives that are commonly present in blood plasma. It is not surprising that in none of the papers was the efficiency of oligosaccharides as vectors for the delivery of porphyrins to biological targets ever proved. It is also characteristic that in *in vitro* experiments, the variation of the type of the carbohydrate residue had almost no influence on the interaction of conjugates with living subjects.

Apparently, it is most rational and workable to study and develop exactly multidentate ligand (with respect to lectins) properties of glycoporphyrins. The works carried out along this line by research groups of S Vidal and A Imberty seem promising as regards the practical application of the cluster conjugates of porphyrins with carbohydrates. This is true, in particular, for their research on the use of glycoporphyrins for the design of biosensors.[161]

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