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Prospects of Metal Complexes Peripherally Substituted with Sugars in Biomedicinal Applications

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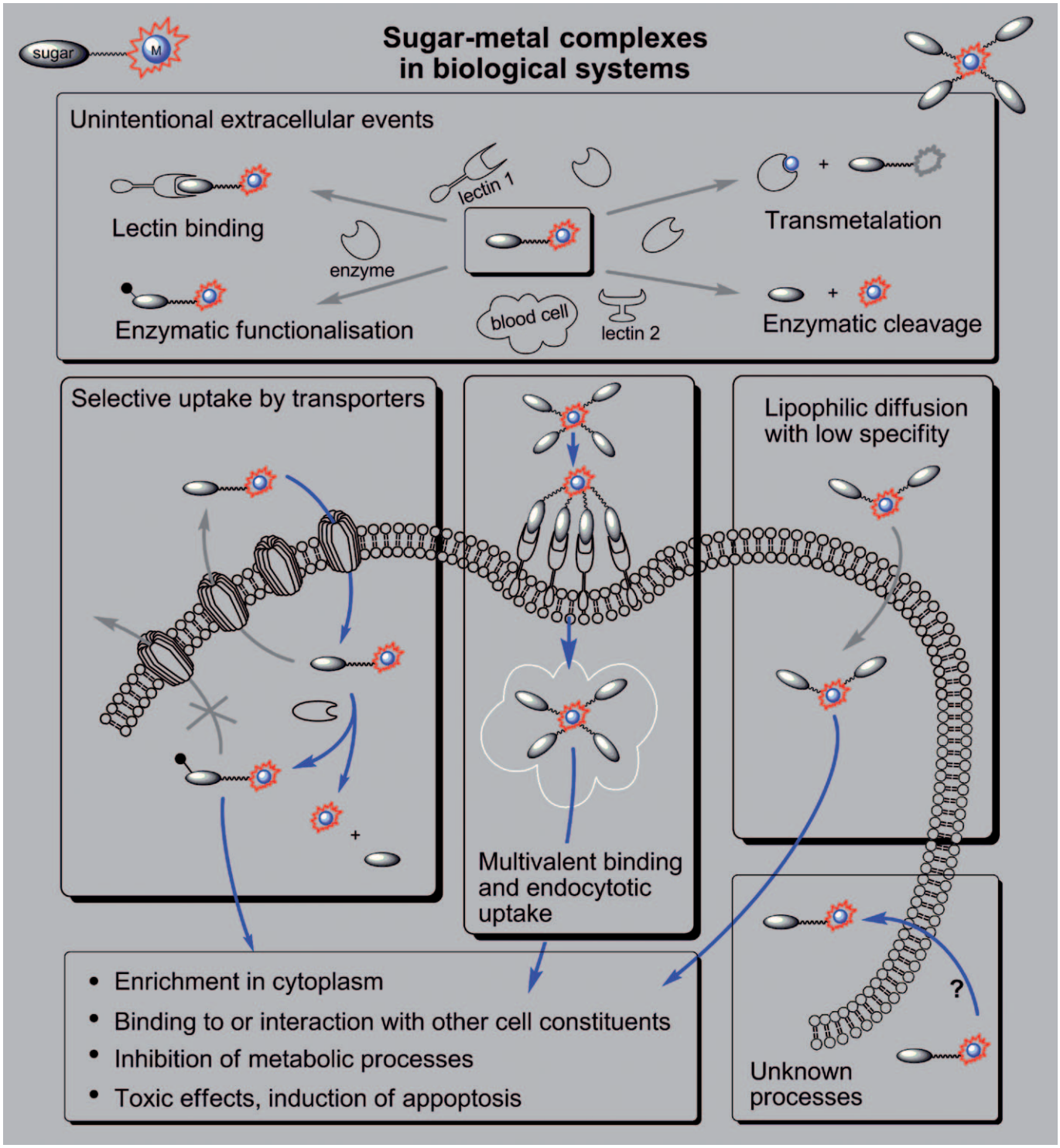

Abstract: Metal complexes possess unique tunable properties, such as radioactivity, cytotoxicity or photophysical features, enabling them to act as diagnostic tracers or therapeutic agents. In applying them in biological systems, it is often necessary to enhance their solubility and biocompatibility. To achieve such goals, like the targeting of binding domains, transport systems and enzyme activities, the attachment of carbohydrate moieties appears to be suitable. Sugar-substitution in the periphery of metal complexes has therefore become a strongly growing field of research. Outlined herein is a selection of recent examples.

Keywords: antitumor agents · bioinorganic chemistry · carbohydrates · imaging agents · lanthanides · transition metals

Introduction

Metal ions and their complexes offer an unmatched structural and functional diversity. Introduced into biological systems they are able to act as diagnostic tracers or therapeutic agents. Their specific effects in terms of biodistribution and mode of action can be influenced in a wide range by the design of the coordinating ligand. One general principle used in nature is the attachment of biomolecules to active species to obtain highly efficient targeting conjugates. In many cases glycosidically bound sugars fulfil the function to mask, direct or activate naturally occurring ingredients. Thus, the combination of sugars and metal ions represents a highly suitable approach to creating new materials with combined properties of both building blocks: saccharides and metal complexes.

Synthetically the coordination of metal ions to sugars can be achieved in two different ways. With the main focus on utilising carbohydrates as chiral ligands for the development of enantioselective catalysts, a number of compounds has been described binding the metal ion directly at the sugar skeleton after introduction of diverse donor atoms (e.g. nitrogen or phosphorous) and additional chelating units (Figure 1, left). These attempts led to a variety of new coordination patterns and reactivity profiles of metal complexes and have been reviewed elsewhere.[1–4] As a consequence,

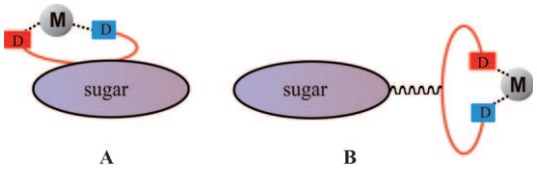

Figure 1. Principles for the design of carbohydrate metal complexes A: the metal ion is directly bound to the saccharide skeleton; B: entire metal complex attached through a spacer to a peripherally sugar residue.

complexes with unusual coordination geometries and supramolecular architectures, which are combined with often unpredictable reactivity behaviour, result from this approach.[5–12] The use of a predefined metal chelating core for the design of the ligands allows the maintenance of the designated properties, as well as others, such as fluorescence and photoactivity of the resulting complexes (Figure 1, right). In such cases, the sugar residues are bound in the periphery, and influence principally only the solubility of the compounds.[13] Since saccharides are involved in many intra- and extracellular events primarily as energy sources and also contributing to various recognition or specific transformation processes,[14] the idea arose not only to modify the solubility of the metal complexes by suitable substituents, but also to influence their biocompatibility, activity and targeting properties by a substitution with sugar moieties in the periphery of the metal centre. This article outlines the progress in the field, with a specific focus on low-molecular-weight compounds.[15] The Concept article will at first discuss the principles for uptake and binding of unfunctionalised sugars in biological systems, necessary to develop consequences for the ligand design synthetic strategies, as well as the designated properties of the obtained complexes in terms of stability and biological or medicinal application.

Binding and Uptake of Sugars and Their Derivatives

Different possibilities and mechanisms for the binding and/or uptake of saccharides and their derivatives exist and were discussed in the literature.[16] While unprotected monosaccharides are supposed to be unable to easily pass through a cell membrane by lipohilic diffusion (due to their hydrophilicity), derivatives carrying lipophilic substituents can take this rather nonselective path into a cell. However, simple, unsubstituted monosaccharides, which mainly serve as energy sources and building blocks for more complex structures, require certain specific transporters located in the membrane which enable them to enter a cell. The uptake as

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well as the post translational metabolic processing is cell specific and varies for example, for breast cancer cells.[17,18] For larger aggregates bearing more than one sugar residue, multiple steps are involved until they reach the cytoplasm. It is generally agreed that, in a first move, they bind to sugar-specific receptors at the cell surface, followed by a mainly endocytotic uptake and encapsulation into vesicles. Afterwards, they remain in these vesicles or are released into the cytoplasm in different ways.[19] Additionally, one has to consider that there are always free enzymes accessible which are able to bind and process the saccharides and their derivatives.

Transporters for selective sugar uptake: Keeping in mind that there are life-form-specific proteins responsible for realising the transport of monosaccharides and related substrates through membranes, two classes of transporters are known for humans. One is the family of facilitative glucose transporters (GLUT). These proteins mediate the sugar transport in most tissues and cells as a bidirectional and energy-independent process. For enrichment within the cells, the sugars have to be metabolised subsequently by internal enzymes. Only within the small intestine and in the kidneys are sugars absorbed or reabsorbed against their electrochemical gradient by a second active transport mechanism, which uses the sodium concentration gradient established by Na^+/K^+/ATP pumps. This process is realised by the second family of proteins, the so-called solute carriers (SGLT).[20] From a genome-wide search thirteen members of the GLUT-family (GLUT1–12 and HMIT), and six different SGLT-proteins (SGLT1–6) have been identified.[21] The identification of additional proteins and isoforms, the investigation of their structure and function, as well as the determination of their substrate specificity have advanced constantly.[22] As related to the targeted delivery of compounds, it is most interesting to note that the currently identified transporters are already expressed differently in certain cell types and tissues, and possess unequal substrate specificity. Furthermore, investigations on the uptake of substituted sugar derivatives suggest that for certain sugars with dedicated substitution patterns, the transport process and the post translational modification are not effected, as shown for D-fructose-based photoaffinity labels for the GLUT5 transporter and fluorescent-labelled fructoses selectively taken up by breast cancer cells.[23,24]

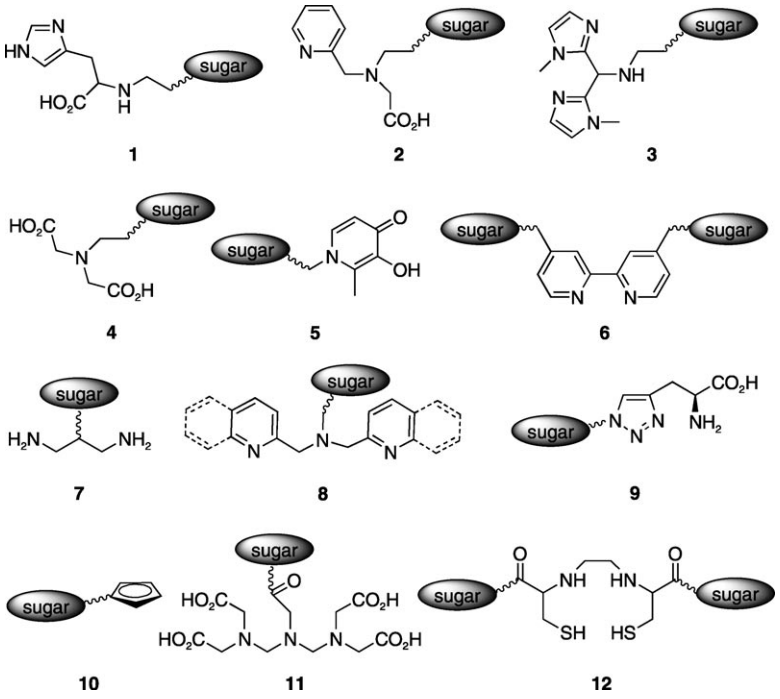
Binding to receptors and proteins: Receptor proteins for the binding of glycans are divided into intra- and extracellular lectins. They are located in luminal compartments of the secretory pathway and are secreted into the extra cellular matrix or body fluids, or localised to the plasma membrane. These proteins operate in the trafficking, sorting and targeting of maturing glycoproteins[25] and mediate a range of functions including cell adhesion, cell signalling, glycoprotein clearance and pathogen recognition.[26] The number of identified pairs of specific lectins and certain carbohydrate moieties is constantly increasing, and the knowledge is collected in extended databases.[27] These proteins are able to recognise various different structures like heparin sulfate and hyaluronic acid proteoglycans as polysaccharide-like molecules, and may also specifically bind to the oligosaccharide moieties of glycoproteins and glycolipids.[28] Due to the "glycoside cluster effect"—which describes the affinity enhancement (typically an increase of orders of magnitude) involved in binding several carbohydrate residues at the same time in a multivalent fashion[29]—numerous synthetic glycopolymers and dendrimers have been developed to selectively bind, influence and/or circumvent these processes.[30,31]

Interaction with processing enzymes: In addition to binding proteins, many sugar-processing enzymes are described. Hexokinases for phosphorylation, glycosidases for hydrolytic cleavage of glycosidic bonds, oxidases for dehydrogenation of certain substrates and transferases for glycoside formation are ubiquitous.[32] An updated list of known enzymes correlated to their various functions is available online.[33]

Development of Tracers

To monitor the aforementioned sugar-based biological and biochemical events, a number of labelled compounds have been prepared and were tested to some extent for their applicability as tracers in vitro and in vivo. In the following section, selected examples will be discussed.

Radioactive-labelled sugar-containing ligands: Due to the rapid development of imaging techniques like single-photon emission computed tomography (SPECT) or positron emission tomography (PET) and coupled procedures like PET/computed tomography (CT), radioactive-labelled biomolecules are increasingly used for the visualisation of certain cell types and tissues. Since the detected types of radiation easily permeate tissue and the applied concentrations are very low, these are excellent methods for in vivo imaging. Additionally, progress in radiochemistry generated a large variety of accessible radionuclides and procedures for processing them.[34] The most abundant sugar transporter is GLUT1. It is over-expressed in tissue with an accelerated energy requirement, like fast-growing cancer cells.[35] In the majority of cases to-date 2-^{18}F-2-deoxy-D-glucose (FDG) is used as a sugar-based tracer to monitor GLUT1 activity by PET/CT, and to image tumour cell metabolism.[36] The need of a cyclotron for the production of ^{18}F, the suboptimal uptake (FDG is not only into malignant tumours, but often also into inflammatory tissue)[37,38] and the missing uptake into certain tumours (e.g. highly differentiated prostate carcinoma)[39] are major drawbacks for the use of FDG, and have resulted in analogue studies with the intention of finding substitutes. Different chelating units were examined to complex the most frequently used radionuclide for SPECT-imaging, ^{99m}Tc and its therapeutic meaningful analogue ^{186}Re (a selection of sugar-based ligands (**1**–**12**) used for the



coordination of radioactive nuclides is shown here, see also Table 1).[40–56] For comparison and structural elucidation, the "cold" rhenium analogues have also been prepared. To expand the techniques towards PET/CT-imaging (positron emitters as nuclides) $^{55}Co^{II}$ complexes and first examples of non-radioactive Ga^{III} complexes of **5** could be obtained.[45,46] The stability of the complexes was determined by ligand exchange experiments against naturally occurring substrates with known high affinities towards the respective metal ion (for $^{99m}Tc^{I}$ and Re^{I} typically histidine, cysteine or blood plasma). It is crucial to enable the tracer to reach its destination without being captured by competing ligands (e.g. blood proteins). Complexes with Tc^{I} and Re^{I} of tridentate (or higher) chelators were found to show a high stability in these tests over a period of 24 h. Only the bidentate derivatives **5**, **6** and **7** gave lower stabilities under the mentioned conditions.

Most of the synthesised complexes are glucose-based aiming to take advantage of the over expression of GLUT1 (Table 1). Schibli and co-workers tested the cellular uptake of the complexes bound to the C-1, C-2, C-3 or C-6 position of glucose by oxygen (into colon carcinoma cells HT29), their ability to act as inhibitors for hexokinase (by kinetic experiments and molecular docking calculations) and pseudo-substrates for the phosphorylation at position C-6.[41] The results show that the glucose C-2 position is the most favoured one for substitution in terms of post-processing of the derivatives by internal enzymes. However, no internalisation of these complexes by GLUT1 was observed (only increased unspecific uptake of the most lipophilic derivative **3** bound to 3-*O*-Glc). Therefore, GLUT1 no longer appears to be the most suitable target for the design of sugar-based metal complexes.

The synthetic approach of using "click" chemistry to obtain derivatives of the basic structure **9** is particularly promising to study the dependence of uptake and the position of sugar functionalisation as well as to screen for other selective transporters, since it provides fast and easy access to various substitution patterns.[52] So far, the most promising candidates to act as functional imaging agents are the ^{99m}Tc-/^{188}Re-DTPA (diethylenetriamine pentaacetic acid) complexes (**11**), and the appropriately labelled ECDG (ethylenedicysteine deoxyglucose) ligand (**12**), both of which are derived from 2-*N*-2-deoxy-D-glucoseamine. These derivatives were phosphorylated by hexokinase, exhibit cellular uptake (probably by a multifunctional glucose transport system) and reveal higher tumor-to-tissue ratios than observed for FDG in in vivo experiments (small animal tumour models).[54–56]

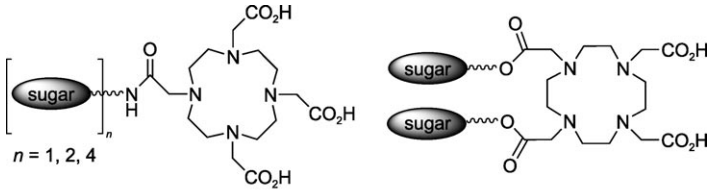
Sugar-containing ligands for MRI applications: Magnetic resonance imaging (MRI) has become one of the most important techniques in diagnostic clinical medicine and biomedical research. Complexes of lanthanide metal ions are

Table 1. Overview of known combinations of chelating units with differently substituted sugars and coordinated metal ions.

Chelating unit[a]	Bound sugars[b]	Complexed metal ions	Ref.
1, **2**, **3**	3-*O*-Glc	$^{99m}Tc^{I}$	[40,41]
4	1-*O*-Glc, 2-*O*-Glc, 3-*O*-Glc, 6-*O*-Glc, 5-*N*-Thy[c]	$^{99m}Tc^{I}$, Re^{I}	[40–43]
5	2-*N*-Glc, 1-*O*-Glc, 6-*N*-Glc	$^{99m}Tc^{I}$, $^{186}Re^{I}$, $^{55}Co^{II}$, In^{III}, Ga^{III}	[44–46]
6	1-*S*-Glc, 1-*S*-Gal, 1-*S*-Man	$^{99m}Tc^{I}$, Re^{I}	[47]
7	1-*O*-Glc, 1-*O*-Xyl, 1-*O*-Man, 1-*O*-Gal, 1-*O*-Mal	$^{99m}Tc^{I}$, Re^{I}	[48]
8	1-*O*-Glc, 2-*O*-Glc, 2-*N*-Glc, 1-*O*-Xyl, 1-*O*-Man	$^{99m}Tc^{I}$, $^{186}Re^{I}$	[49–51]
9	1-*N*-Gal, 3-*N*-Thy[c]	$^{99m}Tc^{I}$, Re^{I}	[52]
10	2-*N*-Glc	$^{99m}Tc^{I}$, Re^{I}	[53]
11	2-*N*-Glc	$^{99m}Tc^{I}$, $^{188}Re^{I}$	[54,55]
12	2-*N*-Glc	$^{99m}Tc^{I}$	[56]

[a] For the chelating units **1**–**12** additional spacers are not considered. [b] With position and mode of substitution: Glc = D-glucose, Thy = thymidine, Xyl = D-xylose, Man = D-mannose, Gal = D-galactose, Mal = D-maltose. [c] Since the chelating unit is bound to the ribose part of the molecules, the thymidine derivatives have been included.

widely used to enhance the local water-proton relaxation rate, which is a precondition for this imaging technique.[57] Among them, Gd^{III} complexes are the most popular class of tracers.[58] To obtain suitable coordination compounds with a high stability (especially in light of the toxicity associated with free lanthanide cations), which are also able to interact with water molecules, the most favoured motifs involve the use of hexa-, hepta- or octadentate ligands, often-based on macrocylic structures. To take advantage of selective binding and uptake phenomena of peripherally sugar-substituted ligands, derivatives carrying one or more saccharide residues have been prepared (a selection of sugar-substituted chelating units (**13**–**17**) for use in MRI is shown here, see also Table 2). To determine their binding to the galactose specific



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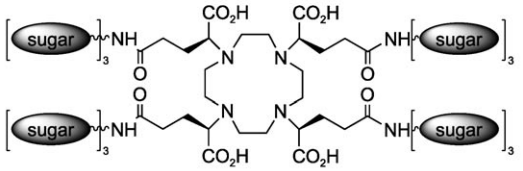

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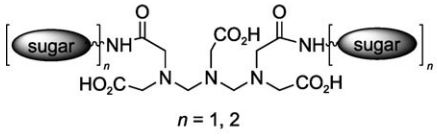

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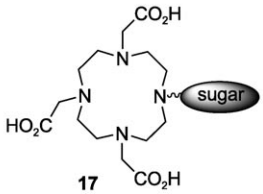

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hepatic asyaloglycoprotein receptor in a multivalent fashion (ASGP-R, mainly found on hepatic cells but also in liver cancer and, to some extent, in its metastases), DOTA (1,4,7,10-tetrakis(carboxymethyl)-1,4,7,10-tetraazacyclododecane) derivatives **13** and **14** have been synthesised carrying up to four sugar residues.[59] All sugar units were bound thioglycosidically, in order to prevent them from being cleaved off by enzymes. To be able to compare the results for MRI with γ-imaging studies of the corresponding Gd^{III} complexes, the ligands have been complexed to γ-emitting ^{153}Sm nuclides.[60] The results of this work are prime examples for the aforementioned "glycoside cluster effect". After 24 h post injection in Wistar rats, the compounds bearing four galactose residues clearly show a much better enrichment than the mono- or di-substituted systems in the liver, binding to the ASGP-R in a multivalent fashion. The affinity decreases also if lactose is attached and has been shown to be the lowest for the glucose-derived compounds. In this context the potential of the recently patented DTPA–Gd^{III} complexes **16**, containing various sugar residues connected by different spacers and substitution pattern, as well as of the truly dendrimeric systems **15** remains to be seen.[61,62] A contrary but very exciting approach was introduced and advanced by Maede and co-workers. Using a locally concentrated cleaving enzyme, the bound sugar is cleaved off. This process initially "switches on" the MRI activity of the tracer. A galactose derivative of type **17** was used to monitor β-galactosidase activity. The enzyme is a commonly used marker to follow the regulation of individual genes, for example, in transgenic animals. This combination of a sugar and a Gd^{III} complex could be shown to be useful for in vitro and in vivo (*X. laevis* embryos) visualisation and localisation of gene expression by MRI.[63,64] However, results from the biodistribution of the analogue $^{111}In^{III}$-radiolabelled compound in mice revealed that most of the galactose-substituted compound was located in the liver (taken up by ASGP-R) already two hours post injection.[65] Inspired by other β-glucuronide prodrugs, Meade et al. expanded their approach towards derivatives of type **17** substituted ligands with glucuronic acid, using a nitrodihydroxybenzyl spacer, aiming to take advantage of the enhanced concentration of endogenous extracellular β-glucuronidase around tumours.[66]

Contrast-staining sugar-based metal complexes: For the in vitro detection of distribution or monitoring of binding events, sugar-based metal complexes possessing suitable MLCT absorption characteristics and/or luminescent properties are promising target structures. In particular, due to their detectability at even relatively low concentrations, fluorescently labelled compounds are advantageous. For this purpose adequate complexes designed for MRI, as models for radioactive tracers or therapeutics, would already be suitable candidates to examine the distribution of the contrast agents in vitro, for example, the Re^{I} complexes of the bipyridyl derivatives **6** as well as the bis(quinolyl) structures of **8** with an emission maximum at 610 or 550 nm, respectively.[47,49] The luminescent Pt^{II} complexes (for example, see complex **25** later) with emissions up to 730 nm were used to monitor the interaction with ctDNA due to the threefold en-

Table 2. Overview on chelating units with differently substituted sugars and coordinated metal ions as potential MRI contrast agents.

Chelating unit[a]	Bound sugars[b]	Complexed metal ions	Ref.
13, **14**	1-*S*-Glc, 1-*S*-Gal, 1-*S*-Lac	Gd^{III}, $^{153}Sm^{III}$	[59,60]
15	1-*O*-Glc	Gd^{III}	[61]
16	various	Gd^{III}	[62]
17	1-*O*-Gal, 1-*O*-Glu	Gd^{III}, Eu^{III}, $^{111}In^{III}$	[63–67]

[a] For the chelating units **13**–**17** additional spacers are not considered. [b] With position and mode of substitution: Glc = D-glucose, Lac = D-lactose, Gal = D-galactose, Glu = D-glucuronic acid.

hancement of intensity during binding.[81] Additionally, the fluorescent properties of Gd^{III} and Eu^{III} complexes of type **17** were used to gain mechanistic insights into their water binding behaviour.[67] Polypyridine-based ligands (for example, **18**–**23** shown here, see aslo Table 3) are useful building blocks to obtain self-assembled glycoclusters or even dendrimers, bearing a compact saccharide shell around the metal centre. Depending on the nature of the metal ion and the coordinating ligand, complexes of the general formula [ML], [ML_2] or [ML_3] could be obtained. In this way, the copper(II) complexes of type **18** carrying four or eight α-D-*N*-acetyl-galactosamine residues ([ML_2] with the so-called "Tn-antigen cancer marker" α-D-NAcGal) were synthesised.[68] Due to the fact that these complexes exhibit no fluorescence, their glycolide-dependent lectin-binding properties, and the effect of the length of the spacer in between the metal ion and carbohydrate residue compared to allyl-α-D-*N*-acetylgalactoside as a substrate were studied by using a solid-phase competition assay with asialoglycophorin and horseradish peroxidase-labelled lectin (*Vicia villosa*). The enhancement of binding could be shown to be proportional to the number of sugar residues and the length of the spacer (down to IC_{50}-values of 0.63 μM for the eight sugar residues carrying complex with the longest spacer) due to the glycoside cluster effect and the enhanced flexibility. Metal ions with a preferred octahedral coordination sphere, that is, $iron^{II}$ and $ruthenium^{II}$, lead to complexes of the general formula [ML_3] when complexed to the bipyridyl derivatives **18**. In contrast to the Fe^{II} complexes, the ruthenium compounds were found to be stable even at low concentrations, possessing amplified strong luminescence accompanied by a 40% change in intensity when binding to an appropriate lectin.[69] A similar dependency of the affinity towards ConA with respect to bound sugar and spacer length was observed down to IC_{min}-values of 10^{-8} M (the highest affinity was associated with the Ru^{II} complex, with six α-D-mannose residues and the longest spacer). Supported by molecular dynamics calculations, it could be shown that different spacers can have a dramatic influence on the flexibility of the sugar residues (expressed as a fluctuation in the distance of the anomeric carbon and the metal ion). The enhanced luminescence before binding was attributed to the almost complete shielding of the metal centre by the compact saccharide shell which disappears during lectin binding or even solvent exchange from water towards organic solvents. Interestingly, different values were measured for the resulting Λ- and Δ-diastereomers.

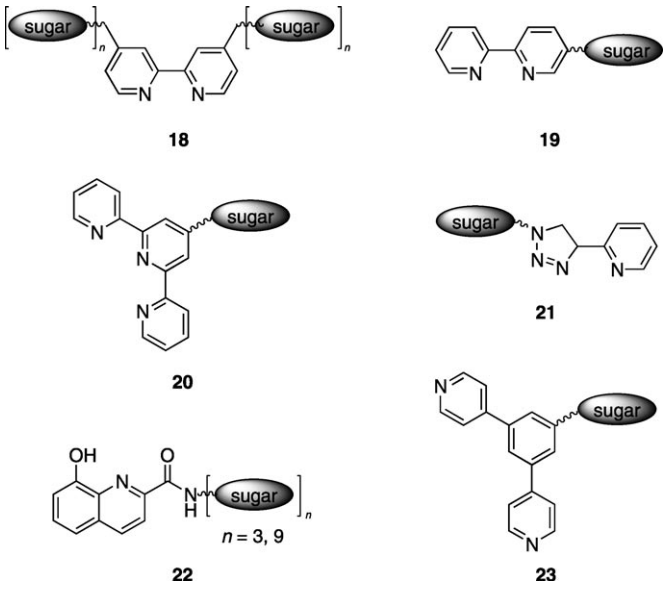


Table 3. Overview on chelating units with differently substituted sugars and coordinated metal ions as staining probes.

Chelating unit[a]	Bound sugars[b]	Complexed metal ions	Ref.
18	1-*O*-NAcGal, 1-*N*-Glc, 1-*N*-Gal, 1-*N*-Mal, 1-*N*-Lac, 1-*O*-Glc, 1-*O*-Man, 1-*O*-Gal, 1-*O*-Glc-YDS	Cu^{II}, Ru^{II}, Fe^{II}	[68–70]
19	1-*O*-NAcGal	Fe^{II}	[71]
20	1-*O*-Glc, 6-*O*-Gal	Fe^{II}, Ru^{II}	[72]
21	1-*O*-Glc	Re(I)	[73]
22	1-*O*-Glc, 1-*O*-Gal, 1-*O*-Man	Zn^{II}, Al^{III}, Gd^{III}	[74]
23	1-*O*-Glc, 1-*O*-Gal, 1-*O*-Man, 1-*O*-Lac, 1-*O*-Mal, 1-*O*-Mat	Pd^{II}	[75]

[a] For the chelating units **18**–**23** additional spacers are not considered. [b] With position and mode of substitution: NAcGal = *N*-acetyl-D-galactoseamine, Glc = D-glucose, Lac = D-lactose, Gal = D-galactose, Man = D-mannose, Mal = D-maltose, Glc-YDS = yolk disialo complex-type oligosaccharide, Mat = maltotriose.

By attaching the specific oligosaccharide YDS (branched yolk disialo complex-type nonasaccharide) to the outer sphere of the ruthenium complexes by means of the transglycosidation reaction with *endo*-glycosidase, excellent affinities for binding to type-A influenza viruses (IC_{50} of 8.4 μM) were found. Due to the strong depression of the luminescence intensity during virus-binding such aggregates may be further used to monitor such specific binding events.[70] The lower stability of the [ML_3] monosubstituted bipyridine-based Fe^{II} complexes **19** results in a dynamic equilibrium between the four possible isomeric forms even at room temperature. It enables the compounds to change the spatial arrangement of the three sugar substituents in order to fit into the multivalent carbohydrate binding site of a target lectin, as shown for the 1-*O*-NAcGal-substituted derivatives during their binding to *Vicia villosa* B_4 lectin.[71]

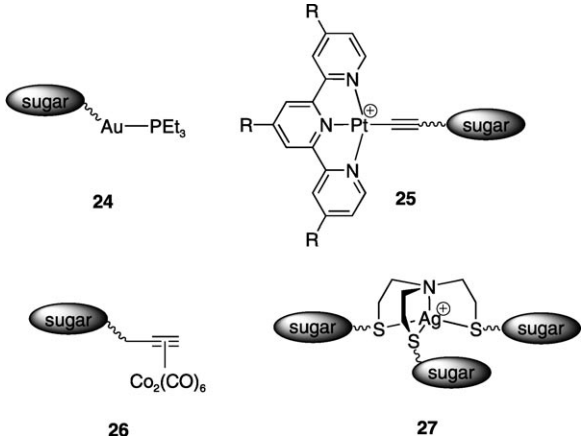
Constable and co-workers synthesised the first iron(II) and ruthenium(II) complexes of 2,2′:6′,2″-terpyridines (**20**), functionalised with D-glucose and D-galactose at the 1- or 6-position of the sugar, bound directly or coupled through eth-

ylene glycol spacers.[72] Due to the generation of $[ML_2]$ complexes, the formation of isomers is excluded, but only the complexes containing a spacer were found to be chemically stable. Exposed to β-glucosidase, the D-glucose-substituted ligands were hydrolysed, whereas the respective Fe^{II}- and Ru^{II} complexes were not, offering possibilities for the design of metal-activated drug delivery approaches. When compared to the aforementioned bipyridine-based complexes, the drawback of the terpyridine derivatives is their missing luminescence at room temperature. By means of copper(I)-catalysed 1,3-dipolar cycloaddition, bipyridine-like ligands of type **21** have recently been obtained.[73] Besides the vast potential of this synthetic approach, these ligands act as bipyridine mimics with strongly electron-donating substituents. The resulting Re^{I} complexes possess enhanced luminescence intensities and superior life times, making them suitable candidates as luminescent probes in time-gated imaging. Recently, the first fluorescent glycodendrimers based on self-assembled metal complexes were obtained by Seeberger and co-workers.[74] Substituted 8-hydroxyquinilines containing branched spacers bearing three or nine sugar residues were complexed to Zn^{II}, Al^{III} or Gd^{III} ions to yield $[ML_2]$ or $[ML_3]$ complexes with up to eighteen saccharide units. Binding studies of the complexes towards Concavalin A (ConA) lectin by turbidity measurements revealed the formation of aggregates (multivalent binding), best for the larger D-mannose containing dendron with Zn^{II} metal centre. Additionally, a slight quenching of the fluorescence signal during agglutination is observed for this cluster, which opens up the possibility to use such aggregates as sensors for lectin binding. Although not coloured or fluorescent, the self-assembled clusters resulting from the coordination of Pd^{II} ions by ligands of type **23** obtained by Fujita and co-workers should be mentioned in this context.[75] The square-planar coordination of the palladium results in the formation of complexes of the general formula $[M_{12}L_{24}]$ and, thus, to aggregates with a high metal content. Expanding this approach towards suitable metal ions (e.g., radionuclides) may lead to applications where a high concentration of bound metals is desired.

Development of Therapeutics

Organometallic compounds offer a variety of useful therapeutic properties.[76,77] Generally, the efforts of developing effective sugar-based therapeutics are constrained by the fact that substitution of an active metal complex by a hydrophilic carbohydrate (in most cases, D-glucose) results in a reduction of its biological activity. On the other hand, glycoconjugates not based on metal complexes are already well studied as therapeutics,[78] for example, as anti-cancer agents.[79] Thus, combining the useful effects of metal complexes an glycoconjugates seems to be promising.

Hydrophilicity versus cytotoxicity: Considering that the mode of action of a sugar–metal complex is not determined by a selective uptake through sugar transporters (which is probably the case for most of the known derivatives, due to the high specificity of the transporters) or endocytotic uptake, the nonselective lipophilic diffusion into the cytoplasm is the assumed alternative. Post-uptake events subsequently define the selectivity of the drugs. Probably the most well-known sugar-containing drug is Auranofin (**24**),



an antirheumatic agent which is structurally an organogold compound derived from 2,3,4,6-*O*-tetraacetyl-β-D-glucopyranosyl-1-thiol, which carries a peracetylated (lipophilised) sugar (Table 4). Its exact mode of action is still under investigation.[80] Ma et al. synthesised a series of Pt^{II} complexes (**25**) containing various peracetylated sugar residues.[81] The highly lipophilic derivative, which carries *tert*-butyl groups as residues at the terpyridine connected to peracetylated glucose by a phenyl bridge, was found to be the most effective compound (100 times higher cytotoxicities against human cancer cell lines than cisplatin). According to gel-mobility-shift assays, the interaction with ctDNA by a non-intercalating binding mode can be assumed to be the reason for its induction of high levels of apoptosis compared to the other derivatives. Ott et al. showed that the cytotoxicity of

Table 4. Overview of selected sugar residues of biologically active complexes.

Metal complex[a]	Bound sugars[b]	Ref.
24	1-*S*-Glc(Ac)$_4$	[80]
25	1-*O*-Glc(Ac)$_4$, 1-*O*-Gal(Ac)$_4$, 1-*O*-Mal(Ac)$_7$, 1-*O*-NAcGlc(Ac)$_3$	[81]
26	3-*O*-Fru(*i*Pr)$_2$, 3-*O*-Fru(*i*Pr), 3-*O*-Fru	[82]
27	1-Glc(Ac)$_4$, 1-Gal(Ac)$_4$, 1-Man(Ac)$_4$, 1-Glc, 1-Gal 1-Man	[83]

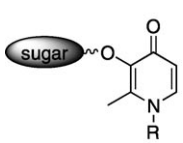
[a] For complexes shown **24**–**27**, additional spacers are not considered. [b] With position and mode of substitution: Glc(Ac)$_4$ = 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose, Gal(Ac)$_4$ = 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranose, Mal(Ac)$_7$ = peracetylated maltose, NAcGlc(Ac)$_3$ = peracetylated *N*-acetyl-glucosamine, Fru(*i*Pr)$_2$ = 1,2:4,5-*O*-diisopropylidene-fructopyranose, Fru(*i*Pr) = 1,2-*O*-isopropylidene-fructopyranose, Man(Ac)$_4$ = 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranose, Glc = D-glucose, Gal = D-galactose, Man = D-mannose.

fructose substituted cobalt–carbonyl complexes strongly depends on the lipophilicity of the compounds. The diisopropylidene derivative of **26** possesses the highest activity compared to the mono-isopropylidene and the unprotected complex, with the lowest cytotoxic action against MCF-7 cells.[82]

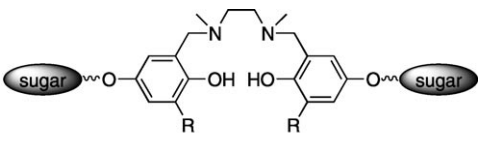
The hydrophilic unprotected silver(I) complexes of type **27** were found to be less toxic against mammalian cells than free silver salts, but exhibit doubled anti-microbial activity towards numerous bacteria and fungi. Since a sugar-depending activity could not be determined, the mode of action is revealed to be solely depending on the release of the silver ions to the organisms.[83] In general, detoxifying effects of sugar substitution were observed for hydrophilic cisplatin analogues of ligands **7** against cancer cell lines and for in vivo testing in CDF1 mice.[84,85] However, in these cases the activity was found to be sugar-dependent. Different sugars, anomers or spacers resulted in varying activities, the analogue Pd^{II} complexes were found to be almost inactive and cisplatin-resistant cells were less effected.[86] Therefore, a pre-uptake processing of the complexes seems to be plausible, which may include selective hydrolysis or binding to blood proteins prior to membrane permeation of the then activated metal core.

Initially metal-free systems: Taking advantage of three of the described effects—detoxification of metal ions, possibility of targeting by sugar substitution and the permanent ability of *O*-glycosides to be cleaved off by ubiquitous enzymes—an alternative strategy arises to develop useful therapeutics by designing and administering solely sugar-containing ligands. Orvig et al. suggested a strategy for the design of such multifunctional molecules to combat Alzheimer's disease (AD). In the etiology of AD, Fe, Zn and Cu ions were found to be jointly responsible for neurodegeneration by oxidative stress and the formation of plaques by Aβ-peptide aggregation. Complexation and passivation of these metals by chelation therapy may contribute to the treatment of AD in the future.

The glucose-substituted pyridinone conjugates **28** are designed as pro-ligands to pass the blood-brain-barrier based on the sugar substitution. Cleavage of the glucose units by enzymes liberates the hydroxypyridone ligand, which subsequently acts as tissue-depending metal binder and ROS (reactive oxygen species) scavenger. It could be shown that the ^{125}I-labelled pro-ligands **28** are able to permeate the blood-brain-barrier (by rat brain perfusion technique), β-glucosidase can cleave off the sugar moiety and the resulting hydroxypyridones possess efficient binding capacity towards Zn^{II} and Cu^{II}, trap radicals and dissolve Aβ plaque.[87] The ligands **29** were found to have antioxidant activity, complex Zn^{II} and Cu^{II} ions with high affinities at physiological pH and reduce the Aβ aggregation in vitro.[88]



28



29

Conclusion

Functionalisation of metal complexes by saccharides in their periphery leads to hybrid molecules the properties of which can be adjusted in a wide range. Crucial factors for the design of the compounds and their designated application in biological systems are the number and type of connected sugars, the position of substitution on the saccharide, as well as the chemical and biological stability of the connection. The length, branching and flexibility of the spacer control the ligand affinity, particularly for multivalent targets. Finally, the metal centre needs to possess the right properties and stability for its desired task. The application of the compounds for monitoring binding events and studying protein-carbohydrate interactions in vitro has made large progress.

During the development of low-molecular-weight tracers and therapeutics targeting highly specific transport proteins in cell membranes it showed up that the tolerance of a metal-containing appendix is limited to a very small number of structures up to now. Additionally, they have to be functionalised post-uptake by enzymes to achieve enrichment within the cell. The most promising candidates so far feature a non-glycosidic linkage of the sugar, preferably without substitution of the 6-position, to enable subsequent phosphorylation by an internal hexokinease. This causes more complicated synthesis pathways (protection/deprotection steps of the glycosidic and primary hydroxyl groups) and difficult purification and characterisation of the resulting ligands (only varying mixtures of ring and open-chain forms can be obtained). Additionally, the variability in terms of applicable metal ions is restricted to oxidation states which are not reduced by the sugar carbonyl groups. These prerequisites probably limited the number of attempts for the elucidation of these molecules. Nevertheless, the discovery of a sugar substitution pattern that selectively targets a certain transporter expressed only in the intended tissue will remain the ultimate goal for the delivery of these active metal complexes. Binding to receptors at cell surfaces and lectines by multivalent molecules has the advantage that the sugars can be glycosidically bound to the metal complex, like their naturally occurring counterparts. However, for a highly selective binding in vivo the distances of the residue as well as the flexibility of the spacers have to be carefully adjusted and the aggregates have to be stable against degradation.

Despite the drawbacks described, the synthesis and characterisation of peripherally sugar-substituted metal complexes offer great possibilities for the discovery of better targeted drugs, and will help to gain insights into the processing of sugars and their derivatives in biological systems. The growing number of pending patents provides an indication of the fast growth within the field and the assumed prospects of impact (see, for example, references [62,89,90]).

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