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8

## **Glycoconjugates: Advantages of conjugation analyzed fragment to fragment and determination of physico-chemical properties useful for wide applications**

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### **Introduction**

From now on, the brand new chemical products to be applied in health, bioremediation, industrial as well as technological fields necessarily are going to be related to sustainability, bioavailability, biodegradation and reduced toxicity, among other green

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characteristics coupled within the normal requirements for high performance substances. Hence, nowadays these are the worldwide and up to date optimization variables in molecular design. As has been often employed, a great number of industrial products and processes were obtained due to a mimetic approach to Mother Nature providing novelties. In this line the molecular design of compounds comprising natural building blocks such as *amino acids*, *carbohydrates*, *lipids* and *nucleic acids* is a key role in order to reach the desired sustainability of the new-line chemical products as well as to maintain the optimized usual process variables. Moreover, conjugated molecules formed by moieties from these four naturally occurring building blocks (*amino acids*, *carbohydrates*, *lipids* and *nucleic acids*) should result in advantageous physicochemical properties since by nature the conjunctions among these molecular bricks have been proven to work well in a broad diversity of experimental conditions, e.g. extreme variations of pH, salinity, sour, ionic strength, etc.

Particularly, these conjoin between *amino acids*, *carbohydrates*, *lipids* and *nucleic acids* with themselves and other chemical functionalities have been coined as **bioconjugates** [1-3]. Its IUPAC definition is as follows: “*bioconjugate* - A molecular species produced by living systems when it is composed of two parts of different origins, e.g. a conjugate of a **xenobiotic**<sup>1</sup> with some groups, e.g. glutathione, sulfate or glucuronic acid, to make it soluble in water or compartmentalized within the cell” [4]. In this sense, the study of the whole combinatory implying biochemical substance conjugation is enormous and in our competence we will focus this review to the link between glycosides with other functionalities. The justification to center within sugar type building blocks is pointed out as follows, i) the first step is centered in the synthetic approaches to achieve the **glycoconjugation**;<sup>2</sup> ii) the second is centered in the study of physicochemical properties of such glycoconjugates; and iii) the last step is centered in the possibility of applications due to low toxicity and synergic properties demonstrated for this type of chemical substances.

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<sup>1</sup>“A **xenobiotic** (Greek, xenos “foreign”, bios “life”) is a compound that is foreign to a living organism. Principal xenobiotics include: drugs, carcinogens, and various compounds that have been introduced into the environment by artificial means” [4].

<sup>2</sup>Please note that **Glycoconjugation**, **Glycosylation**, **Glyco-substances** and their linguistic and chemical derivatives comprises all the combinations of **saccharides** or **sugars** (glycosides) with other molecules or groups; meanwhile when specific naming is applied such as **Glucoconjugation**, **Gluco-substances** and so on, this is only related to the combinations of **glucose** with other molecules or groups, or **Xyloconjugation**, **Xylo-substances** and so on is only related to the combinations of **xylose** with other molecules or groups. Please be aware of these differences since these naming rules will be also applied for the other types of sugars discussed herein.

Glyco-substances are of great potential value because they comprise a source of naturally-occurring building blocks (glycosides) that are modifiable through bio- or chemical synthesis, are of low cost, they have defined stereochemistry and as previously cited are environmentally friendly. One important emerging application of glyco-substances is in the obtaining and formulation of non-fossil fuels [5, 6], hence another point to center this contribution in saccharides. The versatility itself is dependant of the particular conjugation. The conjugation can be carried on with naturally and none naturally occurring substances, the latter family of molecules is better known as xenobiotics, hence the nature of the non glycoside counterpart would provide its own physicochemical and biological properties to the resulting glycoconjugate.

Particularly, for simplicity purposes we are going to center the discussion in simpler glycoconjugate systems since even they are by now not fully understood, e.g. pretty few theoretical, structural and reactivity studies have been found before and during the development of this effort. To achieve such simplicity we have focused the central building block to a glycoside such as natural hexoses (e.g. D-glucose, D-mannose, D-galactose) or pentoses (e.g. D-ribose, D-arabinose, D-xylose). This selection is related to ring stability since these glycosides are able to easily provide six and five membered rings, which are among the most stable rings in chemistry that can be attached to other functionalities also in order, for example, to acquire better solubility in water or to trespass biological barriers or to carry/remove malignant molecules from a sick organism, etc.

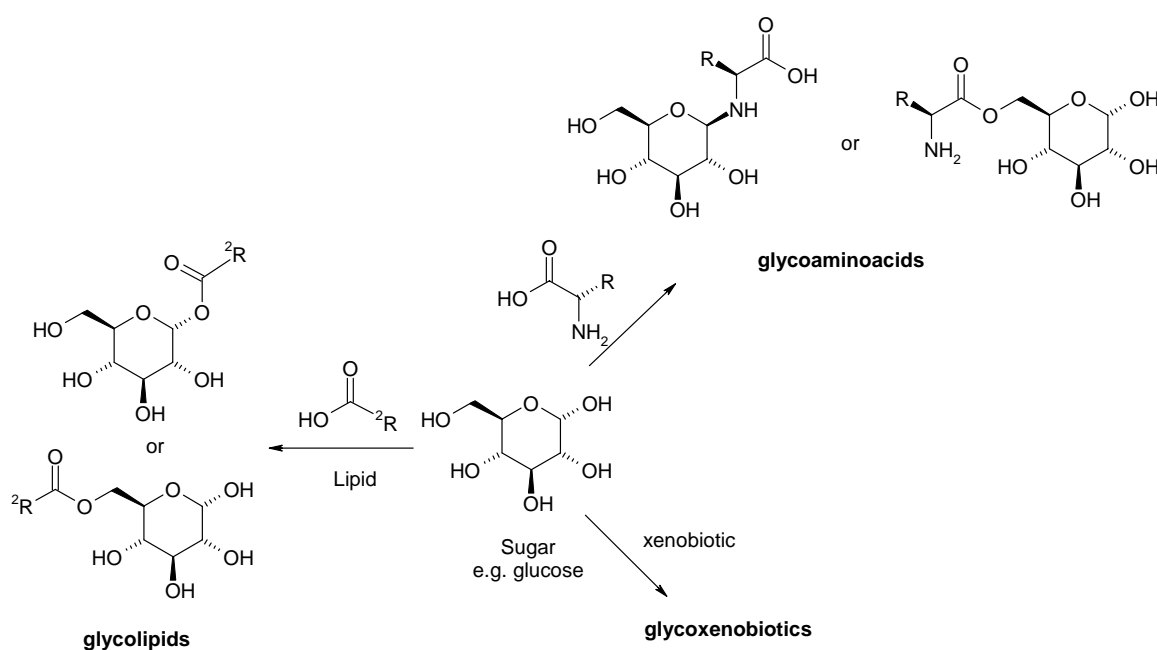
The conjoint is also going to be tracked taking into account the characteristics of the building blocks. One set of glycoconjugates is related to linkages with other natural-occurring substances such as essential amino-acids, fatty acids and amines, etc. which by conjugation led, for example, to **glyco-amino-acids**, **glyco-amides**, **glyco-esters**, **glyco-ethers** and **glyco-amines**. If the length of employed tails in each of these unions is big enough then it should generate **glyco-lipids** (see figure 1).

Another set of glycoconjugates belongs to those conjunctions comprising none biologically occurring substances, as cited above known as xenobiotics, to deliver a new family denoted **glyco-xenobiotics** (see figure 1). The most xenobiotic the compound is the higher its toxicity; we cite this as a toxicological precision for further developments comprising glycoconjugates.

Depending on the chosen conjoint it is possible to prepare water soluble, amphiphile (from the Greek *αμφίς*, *amphis*: both and *φιλία*, *philia*: love, friendship, term used to describe molecules with both a hydrophobic and a hydrophilic region), or hydrophobic compounds. This solubility schemes are not only industrially but pharmacologically as well as biologically

transcendent and also important to reach the desired sustainability. According to the already mentioned conjugations, as well as depending on the applicability in pharmacy and industry, the resulting surfactant molecules can be molecularly designed and engineered to be multifunctional, e.g. self-carrier drugs and pro-drugs, self-carrier chemical products, bio-degradable, bio-metabolic, and so on. In order to achieve these goals the survey of important physicochemical characteristics is also of applicable meaning to understand the structural key roles to achieve a certain molecular behavior. Being some of them the solubility in water, ethanol, ionic liquids and other media; as well as their capability to trespass biologic barriers like the cell membrane or the cell wall, depending on the target organism, not affecting their function or just in the desired way to do it.

Several uses have been underlined for this type of green molecules. As mentioned, many molecules of pro-drug or pro-chemical product types endure the difficulties of solubility in water and lyophilic solubility; therefore, one of the major uses of sugar surfactants is as solubilizers in biological as well as in industrial formulations. In a personal point of view, the surfactant employment has a lot of potential, which indeed has been the common practice instead of the modification of the lead molecule to achieve multifunctionality [7] that nowadays has been turned into the main goal in research and development., hence the raising question is e.g.: why not to become the lead molecule its own surfactant and turn it to be multifunctional?



**Figure 1.** General scheme of glycoconjugates.

Actually, non ionic surfactants are the most common in this type of applications due to its lower toxicity compared against the ionic ones. At these moments, polyethylene oxide based surfactants are products frequently used in the carriage of active molecules. Even though some of them possess low hemolytic activity, they are not chemically stable in solution, being hydrolyzed and self-oxidized to yield toxic products e.g. formaldehyde [8]. Consequently, there is the need of new surfactants having a well defined composition, non toxic, biodegradables and preferentially coming from renewable sources to be applied in any type of industry. Fulfilling all these prerequisites, surfactants based in saccharides [9] and amino-acids [10] seemed to be two prominent options. Additionally is worthwhile to mention that glycoconjugates connected through heteroatoms are the goal of the present study but several examples of C-glycosylation are present in the literature mainly in the search of natural products for drug development and chemical studies, the C-glycoside flavonoid, xanthonoid and C-glycated (in the indole fragment) tryptophan and aromatic amino acid families are clear examples of this particular topic comprising other types of Golgi derived glycans or synthetic examples. For further reviews and recent efforts please see references [11-31].

## 1. Glycoconjugates with amines and amino acids

There is a traditional preparative scheme among bioconjugates, which is known to mainly occur in hexoses (but can occur in pentoses as well) and a compound comprising an amino functionality, including e.g. *amino* acids, *amino* phosphonates, fatty *amines*, etc. This type of conjugation produces glyco-amine derivatives. In the first step the Schiff base or glycosil-imine is formed as the central intermediate (Figure 1.1). This compound is unstable in the presence of water, small amounts of acid or atmospheric conditions and tends to transform quickly through domino or tandem reactions known as *Amadori rearrangement*. By taking the example of glucose it can direct the reaction coordinates to lead i) to glucose-amine passing by the ***Imino-cetal route***, or ii) to amino-fructopyranose, passing by the ***Imino-cetose route***, depending on the reaction conditions. These reaction pathways are described above and depicted in Figure 1.1.

***Imino-cetose route***: immediately after the creation of the imine intermediate, an enaminal species is generated, which due to dehydration, forms an open chain cetose, and then the cyclic form of *1-amino-fructopyranose* is achieved by cetalization.

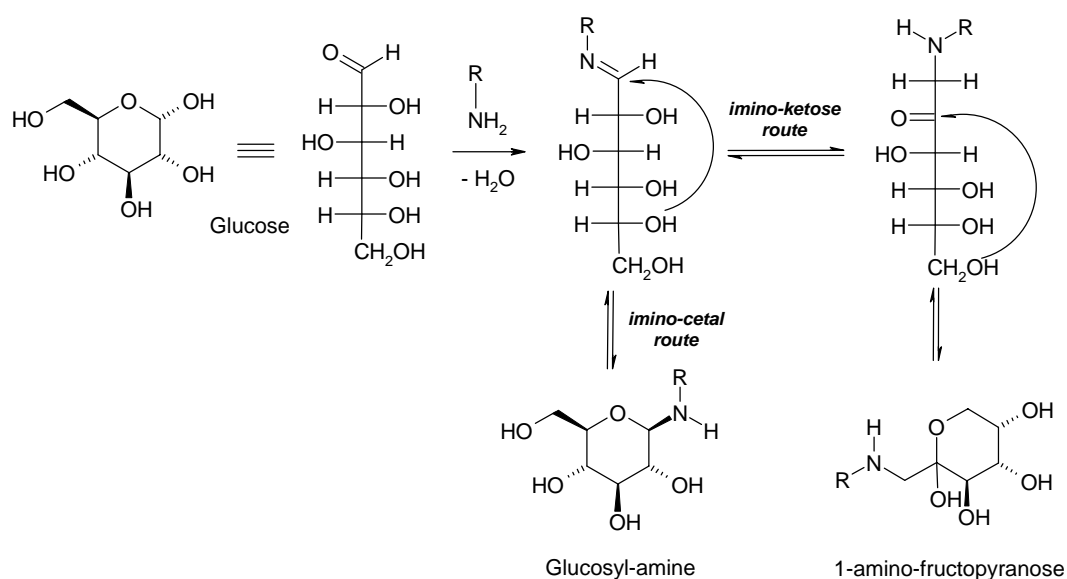
***Imino-cetal route***: another transformation route, also following the formation of the imine, implies that the cetalization step is faster than the

construction of the enaminal species. Hence the reaction course to the formation of a *glucosyl-amine* is achieved.

Biologically, these conjugations occur even specifically, but they are synthetically harsh, producing diverse types of dehydration products, hence difficult to characterize since they form a part of the equilibrium of the system [32, 33]. Amadori developed methodical studies for these reactions at the early 1900s giving his name to the general tandem set of transformations. This researcher was pretty clever to achieve the isolation of his products even publishing that the general reaction proceeds without major setbacks between a primary amine (being aromatic, aliphatic or taking part of more complex molecules as stated) and saccharides of aldose type, such as glucose, galactose, mannose, etc.

In Nature there have been found many bioconjugates that have passed through this type of transformation promoted by light, enzymatically or by coupled mechanisms, where additionally it has been determined that the final products resemble pretty low acute toxicity and hence low general toxicity [32, 33].

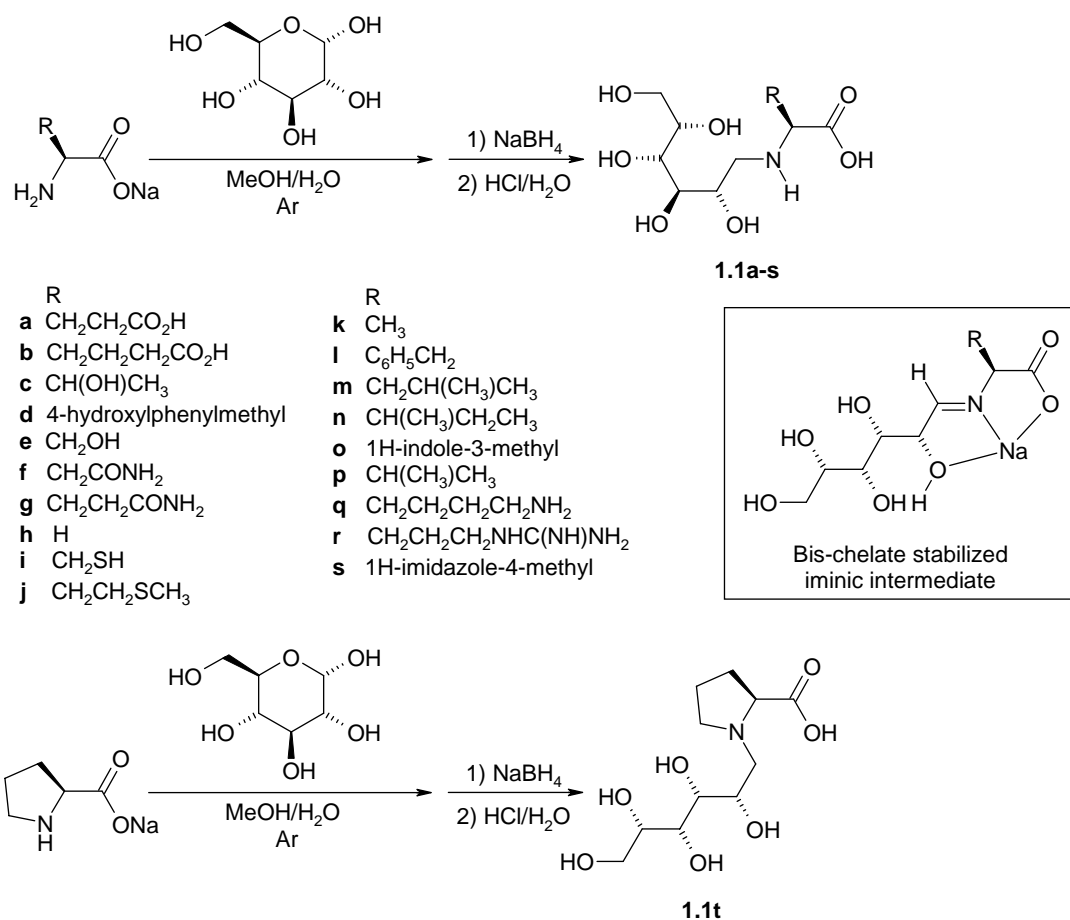
In the inner part of a proteic system the Amadori rearrangement takes place in the terminal amino functional group but also into the side chains of amino acids comprising other amino groups such as lysine, arginine, histidine and ornithine, or in the amidic part of glutamine and asparagine. Due to the fact that the Amadori rearrangement holds a central role in the biochemistry of carbohydrates we will go deep in the survey of the process to molecular design new derivatives with enhanced properties. Basically it comprises the



**Figure 1.1.** Amadori rearrangement depicted for a hexose (e.g. glucose) and a primary amine.

smooth equilibrium stated in Fig. 1.1 implying the conversion of saccharides among aldoses and ketoses [32, 33].

Unfortunately, the straightforward Amadori rearrangement to obtain one of the most important families of bioconjugates, employing amino acids as the amine bearing moiety, the one has not received the deserved attention due to the synthetic difficulties to obtain pure products when the alpha substitution is the goal [32-35]. Nevertheless, elaborated examples of not so simple molecules have been prepared and reviewed going beyond the scope of the present contribution, this selection should be referred and consulted [36-38]. Only a few simple examples have arisen giving proof that this transformation can be carried out without organic protections into the backbone of the central carbohydrate. One of these examples is successful since the  $\alpha$ -amino-acid moiety is previously transformed (and certainly inorganically protected) into the sodium salt of the carboxylate (Figure 1.2) and also the important iminic intermediate is reduced *in situ* avoiding further transformations through both the *imino-cetal* or *imino-ketose* routes (see Fig.



**Figure 1.2.** Protection of  $\alpha$ -aminoacids for their successful reaction with glucose to obtain polyhydroxylated  $\alpha$ -amino acids (**1.1a-t**).

1.1) banning the second step dehydration reactions since the electrophile carbonyl group has been already reduced at this stage [39]. This specific transformation gives place to pure gluco-amino-acid derivatives **1.1a-t**. Wang, et al. stated that within the present reaction conditions the Amadori rearrangement is banned giving cleanliness single products. Additionally, the selectivity in the formation of the imine is directed due to the presence of the sodium ion through the formation of a coordination compound as the sodium salt of each amino-acid. Hence, it is preferred the formation of the  $\alpha$ -imine, instead of the other iminic possibilities e.g L-lysine, due to a bis-chelating effect, therefore the indifference of the nature of the side chain in the yield of the final product (see right side of Fig. 1.2).

As previously stated, in the inner part of a polypeptidic (proteic) system the Amadori rearrangement can take place both in the terminal amino functional group and into the side chains of amino acids [40-43]. It is well known [44] that in protein molecules the *Asn-Xaa-Ser/Thr* sequence is a consensus potential site for the carbohydrate binding to the amido group of an asparagine (Asn) side chain. This type of amino acid modification is known as N-glycosylation, where the N stands for the nitrogen atom of Asn covalently bound to the glycosilic moiety. Also it has been observed that proline between Asn and Ser/Thr will inhibit N-glycosylation [45]; therefore the presence of this consensus sequence is not sufficient to conclude that an asparagine residue is always glycosylated, due to the fact that the folding of the protein plays an important role in the regulation of N-glycosylation [46]. Other consensus sequences have been found to undergo the possibility of N-glycosylation, an example of this is the *Asn-Xaa-Cys* triad. A recent extension of the consensus sequence includes a cysteine residue: *Asn-Xaa-Cys* where experimental evidences stated that this non-standard bioconjugation site has been found in the plasma protein C [47]. Nevertheless, the presence of these consensus sequences in a protein is not sufficient to conclude that the asparagine residue would be glycosylated, due to different reasons: first the folding of the protein must locate the Asn side chain in the molecular surface to permit an enzyme called oligosaccharyltransferase to catalyze the binding of the carbohydrate [46]; there exists some metabolic regulation on glycosylation, and finally, some species like most bacteria lack this kind of enzymatic apparatus for glycosylation.

A different type of protein glycosylation is carried out on the oxygen atom of threonine or serine side chains, and so it is called O-glycosylation. Unlike the previous type, there no exists a known consensus sequence for potential O-glycosylation, being determined mainly by geometrical constraints due to folding of the polypeptidic chain and the presence of



condensable functional groups, as the hydroxyl groups of carboxylic side chains, as in aspartic and glutamic acids, and other simple hydroxyl groups, present in threonine and serine, and included in the latter the thiol group of cysteine, providing the S-glycosylation. Several differences are known between N- and O-glycosylation. For example, while Asn glycosylation occurs in the endoplasmic reticulum, Ser or Thr glycosylation happens at a later stage during posttranslational processing of a protein, in the Golgi apparatus. The N- type always starts (at least in eukaryotes) with the binding of the same branched oligosaccharide formed by 14 sugar units containing 3 glucose, 9 mannose and 2 N-acetylglucosamine residues. This initial core is then modified by the removal first of all glucose and then some mannose residues, followed by the addition of few to many units of other saccharides yielding a vast variety of glycosylation types. On the contrary, several different mechanisms of O-glycosylation are known being each protein family a particular case. In both glycosylation cases, the process of binding a carbohydrate to a protein involves several steps controlled by a set of different enzymes. Such biological investment conserved through evolution must yield strong benefits to living organisms. One of them is control and diversity, since and a single gene which generates a particular polypeptide might result in a great variety of products due to the variety in number and kind of sugar chains than can be attached. The selection of the precise combination of sugars is determined by metabolic regulation and produces different biological functions. Due to the broad biological diversity of monosaccharides and their property of forming different types of covalent bonds between them, a huge number of distinct carbohydrate chains can be constructed, more than with amino acids. For this reason sugar chains attached to proteins frequently function to a “zip code” for protein deliver to the appropriate cellular compartment or tissues. Also they often are involved in the self-recognition of the own cells of an organism and hence in the identification of exogenous cells, so they have been considered as a “bar code” for an organism. The biological properties of these molecules can be adapted for technological or medical functions, and for this reason the terms glycome, glycobiology and glycomics are becoming the emerging fields of study that conjoin sugar-biology and -chemistry in a broader sense.

**A sightseeing of Glycome.** The glycome means the whole set of carbohydrates present in a certain species, and it can be considered as more complicated than the two previous -ome terms, *genome* and *proteome*. While the genome can be determined by sequencing the DNA of an organism (a complicated task by itself), the proteome is the collection of the regulated products of the genome. So the set of proteins in each stage of the organism is not the same, existing some of these biomolecules only during child

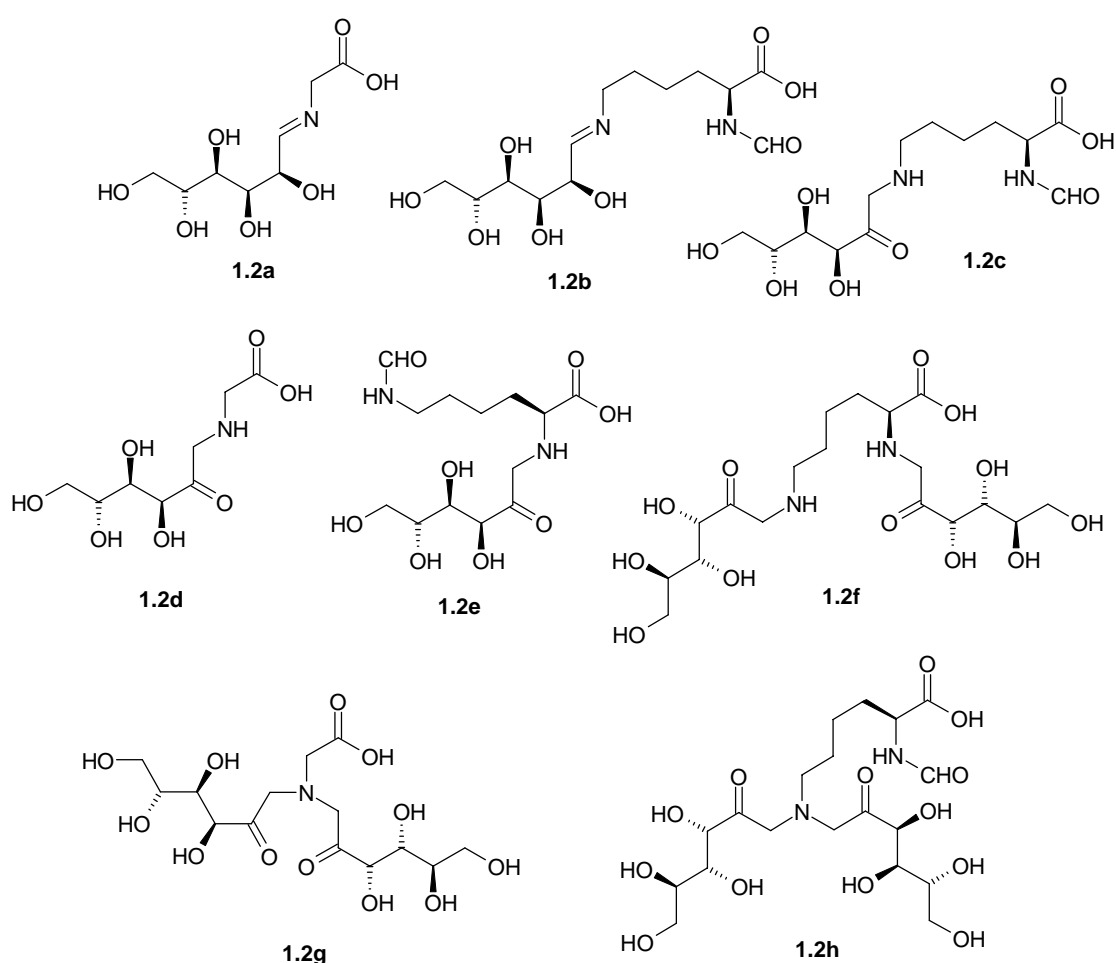
growth, disease or stress. Moreover, one single gene from the genome can yield different products by posttranslational processes or alternative splicing. These facts show the proteome more complicated than the genome. But evolutionary pressures have impelled the glycome to be much more complex [48], since it is not possible even the one-gene one-protein first approximation, and also because their metabolic regulation, the diversity of building blocks (monosaccharides) and the variability in the form they can covalently combine among them and to non saccharide building blocks is much higher than in proteins [49]. An important part of the glycome is present in the form of glycoconjugates with peptide and proteins, lipids, and other biomolecules; and these compounds perform structural, regulation, targeting, recognition and catalytic functions, among others. A huge range of potential and “already in use” applications of this knowledge emerge. For example, glycoconjugates present in cell surface control cellular differentiation, targeting of cells to specific tissues, cell adhesion, and recognition of several types (cell, virus or antibody) and their study stimulates the adaptation of new methodologies (such as mass spectrometry, microarrays and bioinformatics) for the investigation of the role that each glycoconjugate plays [50] and their use in diagnostics, therapy or industry. A new high-throughput methodology uses the precise recognition between proteins and carbohydrates observed in lectins to put them in a microarray and hence following the dynamic changes in glycome due to tumor cell metastasis [51] and cell differentiation [52].

**Molecular models to ascertain and understand the importance of glycomics.** In order to aboard the complexity and coherence of glycomic systems, small models among glycosides and peptides would approach important roles of both types of molecular superfamilies. E.g. when small peptidic models are the subject of study, the directed monosaccharide coupling takes place into the terminal amino groups leading to both types of Amadori rearrangement products depending on experimental conditions [53] or through novel supported reactions to yield site specific Amadori products [54, 55], which have been the recent developed methodologies to obtain site specific glycated-peptides.

Within this context in mind, the smaller systems of study have been performed between amino acids and hexoses, as in the natural occurring N- and O-glycosylation. Particularly speaking, in the case of the N-glycosylation it has been evidenced that this reaction provides rearrangement products via imino-cetal route through alkali metal stabilization of the imine as in **1.1** intermediates, giving sodium N-glucosyl-glycinate (**1.2a**) or sodium N<sup>ε</sup>-glucosyl-N<sup>α</sup>-formyl-L-lysinate (**1.2b**) [56, 57]. Another analogous reaction, in this case via the imino-ketose route (without the formation of alkaline salt)

catalyzed with mild acidic media, provided as the resulting compounds (the most populated species) the 1-amino-fructopyranose derivatives as follows: N<sup>ε</sup>-fructose-N<sup>α</sup>-formyl-L-lysine (**1.2c**), N-fructose-glycine (**1.2d**) and N,N-difructose-glycine (**1.2g**) were prepared as in their corresponding former descriptions [56, 58]. N<sup>α</sup>-fructose-N<sup>ε</sup>-formyl-L-lysine (**1.2e**) was prepared in a similar pathway [58-60] to **1.2c**. Syntheses of N<sup>ε</sup>,N<sup>α</sup>-di-fructose-L-lysine hydrochloride (**1.2f**), and N<sup>ε</sup>,N<sup>ε</sup>-di-fructose-N<sup>α</sup>-formyl-L-lysine (**1.2h**) are carried out with similar procedures employed to obtain **1.2d** and **1.2g** respectively (Fig. 1.3).

Additionally, due to the generation of oxygen free radicals by glycated proteins, or by the generation of glycated proteins due to enhanced oxidative stress; are widely believed to be among the major setbacks in diabetes and aging [61-66]. As an interesting behavior of the di-glycated amino acids **1.2f-h**, the rates of superoxide radical formation in their aqueous solutions were significantly higher than in the mono-glycated ones (**1.2a-e**), therefore modulators of the glycosylation in proteins, through mechanisms comprising

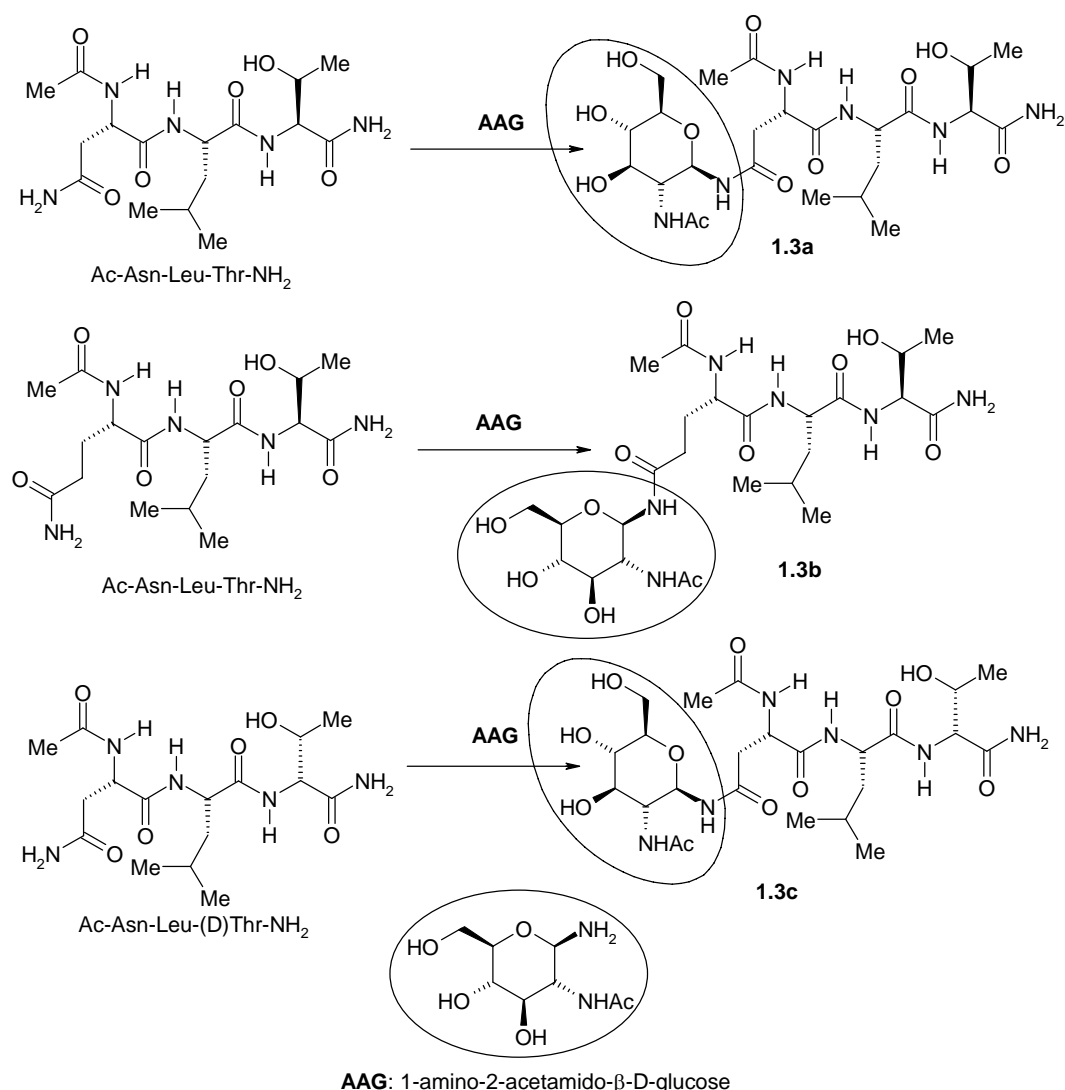


**Figure 1.3.** Mono-glycated (**1.2a-e**) and di-glycated (**1.2f-h**) amino acids.

molecular design of Amadori derivatives, would serve as a therapeutic basis to overcome these sicknesses since the number and type of attached glycoside units seem to have very important biological roles.

The conjugation among glycans with amino acids is of such biological importance that signaling pathways in cells employ this communication scheme and hence the coherent study of glycomics can serve as well as prognosis and diagnosis of sicknesses. It has been stated that the union of these two superfamilies of biomolecules produce important changes in the structural and physicochemical characteristics of the bioconjugate molecule. As previously cited, the consensus sequences indicate possible glycosylation sites, but not all serve for these means dependant on the positioning, availability and other already unknown structural, physicochemical as well as coded factors related to their previous and posterior neighboring amino acids. Nevertheless, due to its basic importance in the field of glycomics a thorough model involving changes affecting chemical specificity, stereochemistry and other slight variations in the consensus sequence has been reported by the group of Davies [67, 68]. They report the preparation of three N-linked glycopeptides (**1.3a-c**) from their corresponding amido-N-acetyl peptides, Ac-Asn-Leu-Thr-NH<sub>2</sub>, Ac-Gln-Leu-Thr-NH<sub>2</sub> and Ac-Asn-Leu-(D)Thr-NH<sub>2</sub> (the diastereomer of Ac-Asn-Leu-Thr-NH<sub>2</sub>) through their transamidation reaction with 1-amino-2-acetamido- $\beta$ -D-glucose (**AAG**) in mild reaction conditions at room temperature in the presence of dimethylsulfoxide/dichloromethane avoiding the presence of water (see Fig. 1.4). This study comprises the analysis of small <sup>1</sup>H chemical shift differences depending on structure variations while changing the length of the connecting group from Asn to Gln as well as by the change in stereochemistry at the third amino acid of the peptide from L-Thr to D-Thr. The results evidenced that only **1.3a** is able to efficiently intramolecularly interact among the glycoside and the peptidic sequence. Important evidence arises when the glucosamine moiety attached to the asparagine side chain is able to sense the stereochemistry at the remote Thr residue by comparison of **1.3a** and **1.3c**. They have stated through spectroscopic methods that there exist important glycoside-peptide intramolecular interactions highly dependent on the peptidic sequence within the change in secondary structure at the peptidic moiety by the measurement of significant changes in the equilibrium populations of GlcNAc C5–C6 rotamers. This small model of N-linked glycoproteins serves as platform in further studies where local peptide-sugar interactions are present in glycosylated-proteins and this also resembles the importance of an intrinsic interaction/stabilization/recognition property of the well known *Asn-Xaa-Thr/Ser* glycosylation site.

Moreover, when other types of amino comprising fragments are employed, the obtaining of the glycosylamines or their intermediate Schiff

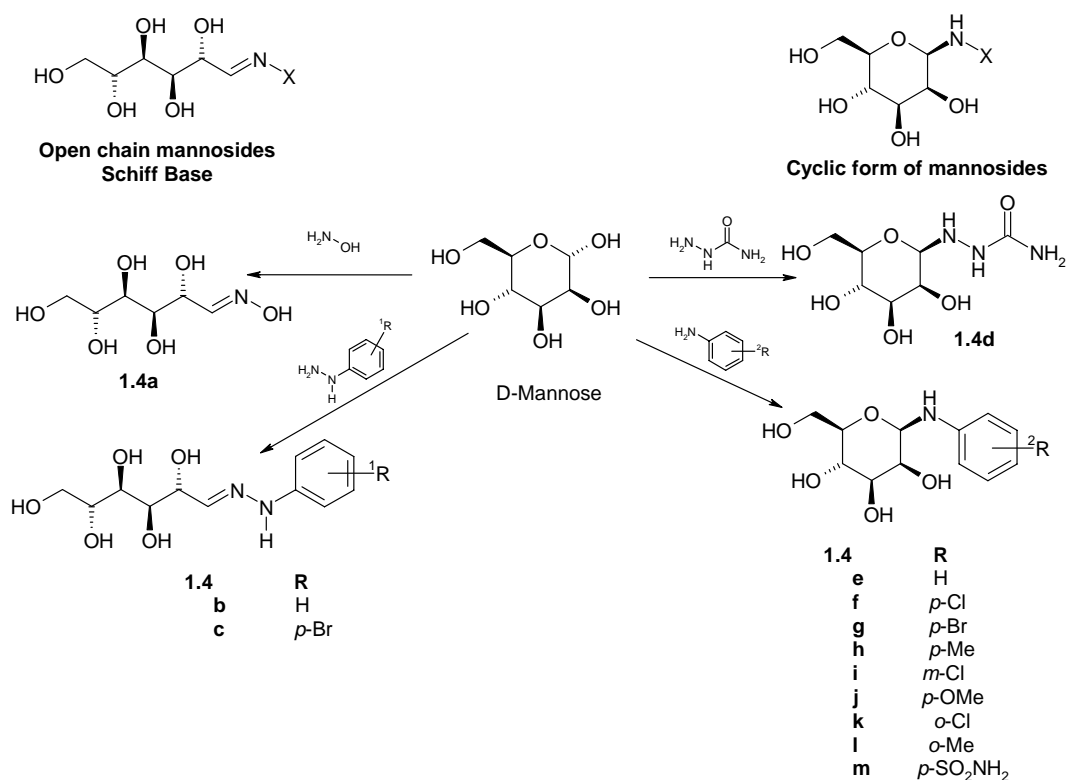


**Figure 1.4.** Intramolecular interaction of N-glycated peptides as model of natural occurring proteic glycans and their structural modifications due to conjugation.

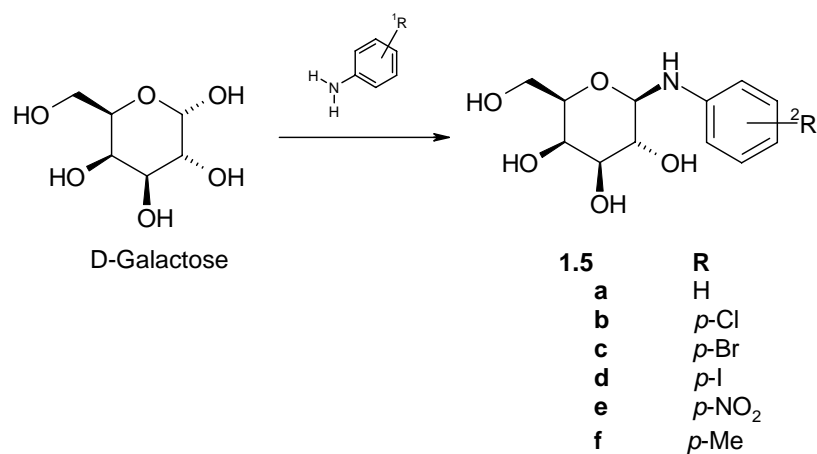
bases is achieved in good yields [69-72]. An example of this situation is from the reaction of D-mannose with hydroxylamine, phenylhydrazine or *para*-bromo-phenylhydrazine to yield an open chain Schiff base β-imino-pyranose (**1.4a-d**), and with semicarbazide, and *para*-chloro-, *para*-bromo-, *para*-methyl-, *meta*-chloro-, *para*-methoxy-, *ortho*-chloro-, *ortho*-methyl- and *para*-sulfamoyl- anilines, and aniline itself to yield the reduced (cyclic) form of the β-amino-pyranoses (**1.4e-m**) see figure 1.5 [70-72].

In a similar fashion, the preparation of parent reduced form of the β-amino-pyranoses (**1.5a-f**) was carried on with D-galactose and the corresponding aromatic amines (see fig. 1.6).

Even though when lower family aldoses, in this case pentoses, D-ribose and D-arabinose, were employed for these means the reduced cyclic form of



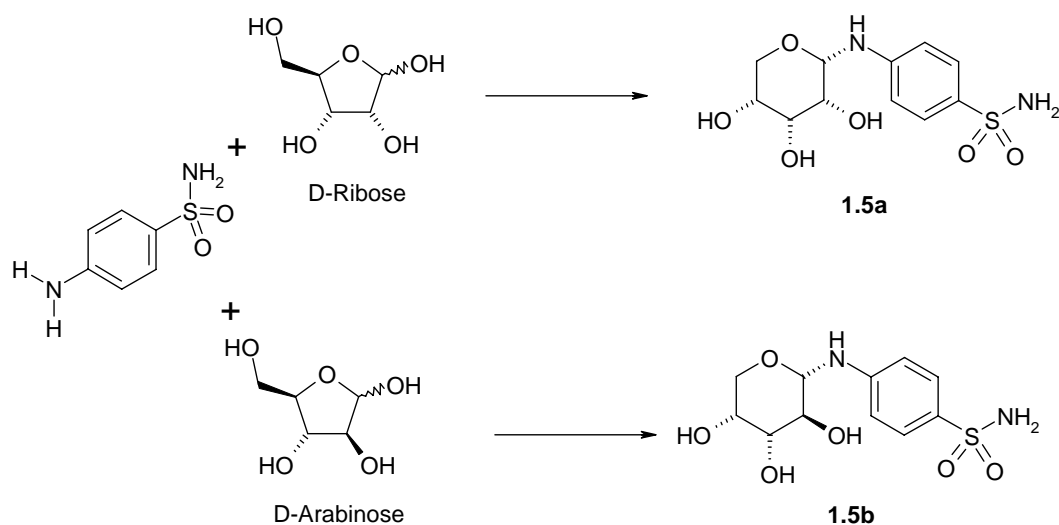
**Figure 1.5.** Preparation of  $\beta$ -amino-pyranoses (**1.4a-m**) starting from amines and D-mannose.



**Figure 1.6.** Preparation of  $\beta$ -amino-pyranoses (**1.5a-f**) starting from anilines and D-galactose.

the compounds was preferred [70] forming the N-(*p*-sulfamoylphenyl)-  $\alpha$ -D-ribofuranosylamine (**1.6a**) and N-(*p*-sulfamoylphenyl)-  $\alpha$ -D-arabinofuranosylamine (**1.6b**) see Figure 1.7.

It has been encountered that when acidic conditions are employed in the transformation of glycosides the preference of the imino ketose route is evident



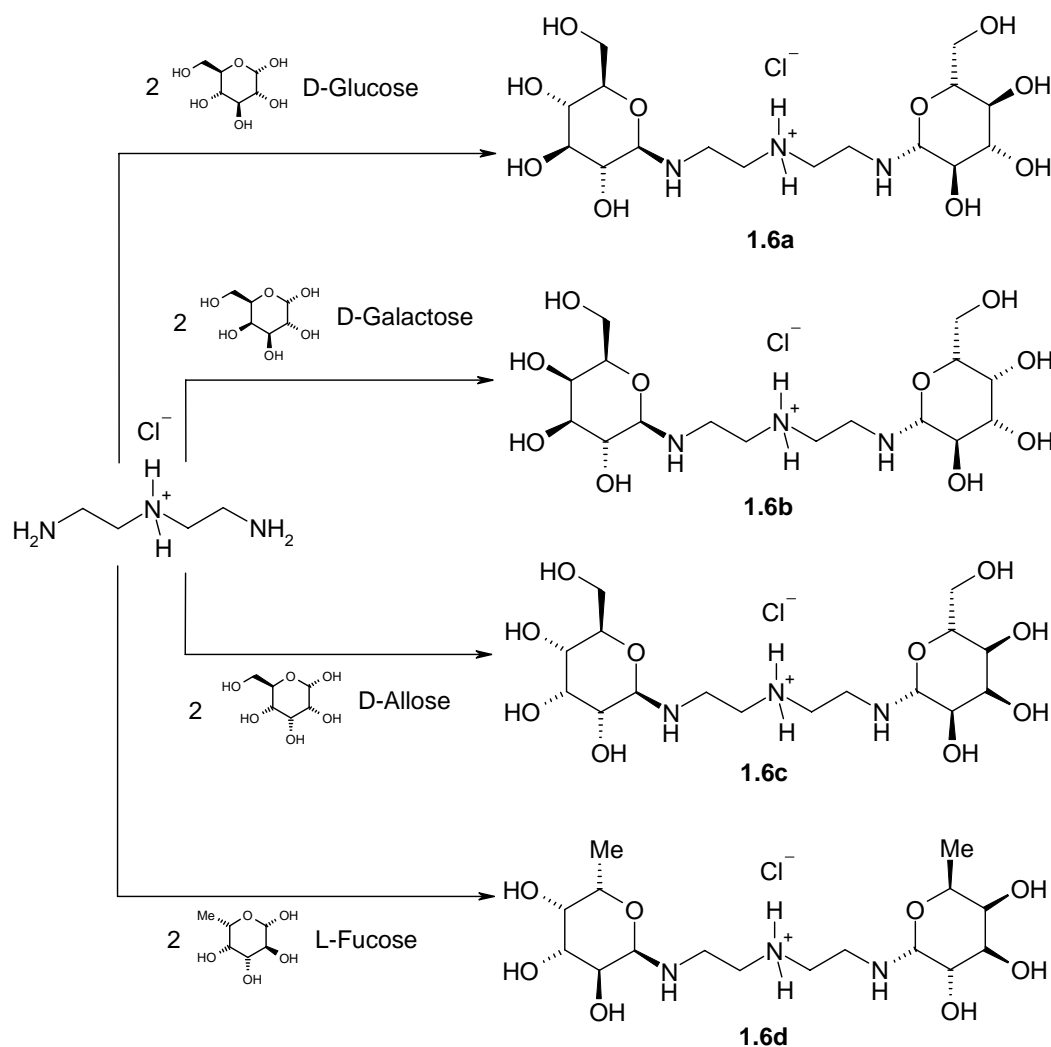
**Figure 1.7.** Preparation of  $\alpha$ -amino-pyranoses (**1.5a-b**) starting from amines and pentoses, e.g. D-ribose or D-arabinose.

within the formation of varying amounts of products and in many cases the major isomer preserves the 1-amino-fructopyranose structure. One exception of this behavior is in the formation of bis-glycosides through DETA mono hydrochloride employment [73]. In this particular case the mono hydrochloride form of DETA acts as a protected species into the secondary amine fragment as the ammonium salt, providing solely the terminal bis-glycosylation with D-glucose, to yield **1.6a**; also with D-galactose, to yield **1.6b**; with D-allose, to yield **1.6c**; and with L-fucose, providing the **1.6d**. Independently of the configuration at the employed hexose, and including the change in hydroxyl groups from the first three hexoses compared with L-fucose, they were not significant chemical variations to ban the formation of the bis- $\beta$ -amino-pyranoses **1.6a-d**, instead being the exclusive product (Fig. 1.8).

All these derivatives are clear examples of the preference of the imino-cetal route in the Amadori rearrangement independently of the iminic or reduced (cyclic) forms of the resulting species. Particularly, compounds **1.4a-c** are present as the *E* (*trans*) stereoisomer as a fully extended carbon chain denoting a high preference of polar substituents to rely in the open chain state, very similar to their reduced **1.1a-t** analogues, which presumably rely in this form due to maximization of hydrogen bond interactions within the employed solvent. Typically, when acidic media is present the imino-ketose route is preferred, and it becomes as a platform for other complex reaction products, starting from the major one observed in mild reaction conditions, the 1-amino-fructopyranose. Other chemical variations, including xenobiotics, of the reactions presented in this section will be seen in section

3. Products with kinetically and thermodynamically enhanced stability can be generated if the glycoside moiety is protected in strategic positions as will be seen in section 4. Additionally, as we will see forthcoming in section 2, this type of compounds are useful building blocks of Gemini type surfactants being examples of glycolipids with the corresponding chemical functionalizations.

Notwithstanding, the obtaining of other types of glycoaminoacids is feasible when enzymatic synthesis is carried on [74]. These examples are the glyco-esters of N-acyl protected amino acids in positions 2, 3 and 6, as well as the 2, 6- and 3-6-di-esters as shown in figure 1.9. The preparation of these derivatives was carried out in special experimental conditions employing not only four different types of lipases or another four proteases but lipase-surfactant-ensembles (LSE) or protease-surfactant-ensembles (PSE), thus the

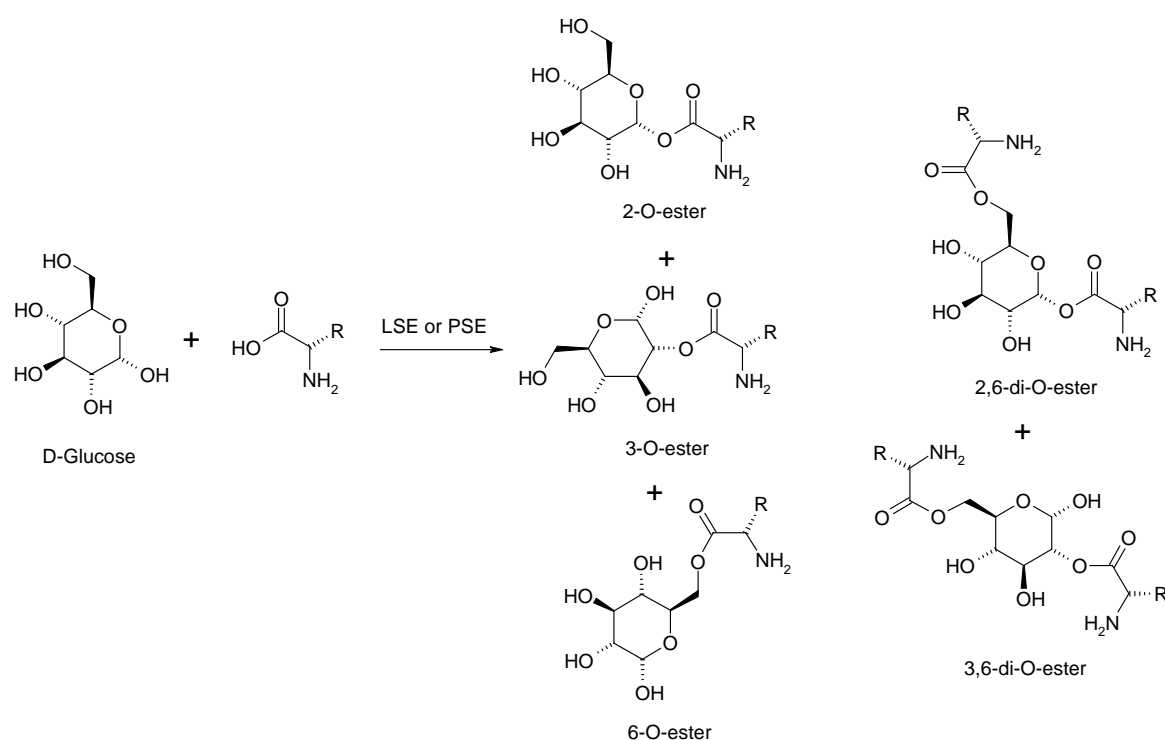


**Figure 1.8.** Preparation of bis-β-amino-pyranoses (**1.6a-d**) starting from mono hydrochloride DETA and hexoses.



enzymatic function can be maintained with high activity even if substantial amounts of organic solvents were employed [74] (fig. 1.9). The authors have carried out a thorough systematic study regarding the change of organic solvents as well as the change in the glyco moiety with the aim of optimize the efficiency of the conversion, which indeed resulted better with *tert*-butanol and 10% of dimethylsulphoxide, also with better conversion in the case of fructose in the latter media, and finally the selectivity, promoting as major product the formation of the 6-O-ester among the other possibilities.

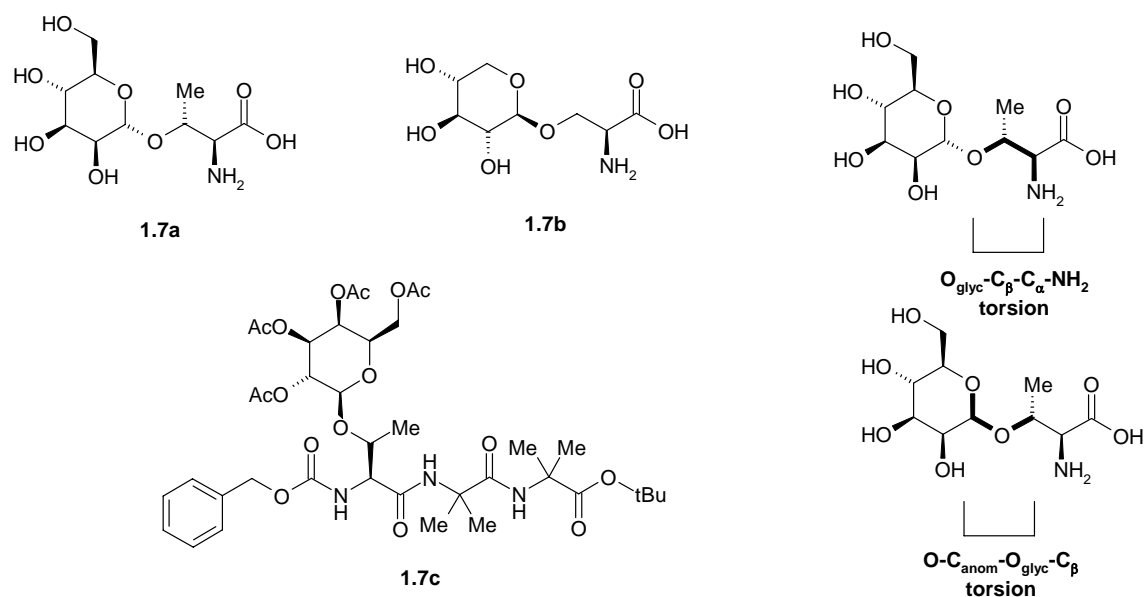
Indeed, the O-glyco-amino esters of amino acids are examples of O-glycosylation products. Nevertheless they are not the only examples of O-glycosylation of amino acids. Other possibilities arise when hydroxyl bearing side chains of the protein building blocks are taken into account [75-77]. Few examples of this new family have been reported and found in the literature when small molecules are the subject of study [78-80]. The first one comprises the linkage of a mannose sugar at its position 1 within the hydroxyl side chain of threonine. Thus, the prepared compound is the O- $\alpha$ -D-mannopyranosyl (1 $\rightarrow$ 3)-L-threonine (**1.7a**), and the observed stereochemistry is for the alpha anomer determined by X-ray and NMR data, as well as the observation of a particular conformation of the threonine molecule in which H $_{\alpha}$  and H $_{\beta}$  are nearly *gauche* [78]. The X-ray structure of the free threonine, in which H $_{\alpha}$  and H $_{\beta}$  are in an *anti* disposition, is different



**Figure 1.9.** Preparation of glyco-esters of N-acyl-amino acids.

from that reported for **1.7a**, being important the analysis of this variation due to the glycosidic union and some other crystallographic contacts (that are not necessarily conserved in solution but the conformation of the backbone does due to the data obtained by NMR) in order to track if there is a common behavior in this union that could result useful for glycomic bioinformatics. The second example of a single amino acid linkage is the O- $\beta$ -D-xylopyranosyl (1 $\rightarrow$ 3)-L-serine (**1.7b**) [79] where also its copper(II) coordination compound is reported underlining the perspective of this family as chelators for metals. The final example of this family is a tripeptide, the N-benzyloxycarbonyl-O-(2, 3, 4, 6-tetra-O-acetyl- $\beta$ -D-galactopyranosyl)-L-threonyl- $\alpha$ -aminoisobutyryl- $\alpha$ -aminoisobutyric acid *tert*-butyl ester (**1.7c**, or in its condensed reported form [Z-( $\beta$ -D-GalAc4)-L-Thr-Aib-Aib-OtBu] [80]. As the authors state, in **1.7c**, the peptide backbone is fully extended at Thr(1), left-handed helical at Aib(2), while it is right-handed helical at Aib(3). Taking into account the glycoconjugate nature of **1.7c** and the possibility to analyze the intramolecular interactions present, there has been shown that owing to the peptide–sugar H-bonds, the peptide backbone is forced to adopt a conformation dramatically different from the  $\beta$ -bend/ $3_{10}$ -helical conformation, usually observed for Aib-rich peptides.

Both compounds **1.7a-b** are present in their crystallographic state as the zwitterions, and the protected nature of **1.7c** impede the comparison with their smaller analogues **1.7a-b**. The torsion angles for the fragment  $\text{NH}_2\text{-C}_\alpha\text{-C}_\beta\text{-O}_{\text{glyc}}$  are  $79.9^\circ$ , for **1.7a**, and  $-58.2^\circ$ , for **1.7b**; they are placed *gauche* but in enantiotopic conformations, and of  $-177.2^\circ$  in the case of **1.7c**, showing an



**Figure 1.10.** O-glycosylation of amino acids in their side chains.

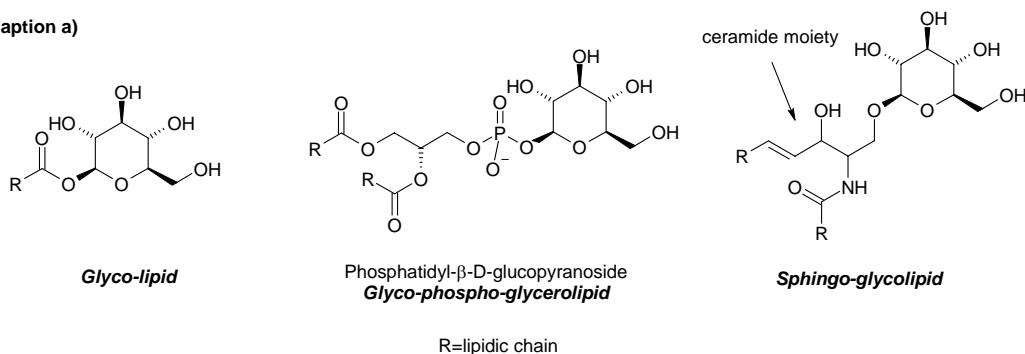
*anti* disposition. Other important geometrical parameter to compare are the  $O-C_{\text{anom}}-O_{\text{glyc}}-C_{\beta}$  angles, of  $79.8^{\circ}$  for **1.7a**, of  $-84.6^{\circ}$  for **1.7b**, and of  $-81.2^{\circ}$  for **1.7c**, placing the amino acid in a *gauche* disposition taking as reference the glycoside, please see fig. 1.10 for torsion angle references. These structural variations maybe due to the differences of the  $\alpha$  and  $\beta$  anomer type, or the variation of hexose and pentose sugar, or the presence of an additional methyl group for **1.7a** not present in **1.7b**, due to the formation of the peptidic bond when compare among **1.7a-b** versus **1.7c**, also due to the presence of bulkier substituents as in **1.7c**, etc. Anyway they are the only examples present for small molecules of this type and further studies are necessary in this topic to understand behaviors.

## 2. Glycolipids

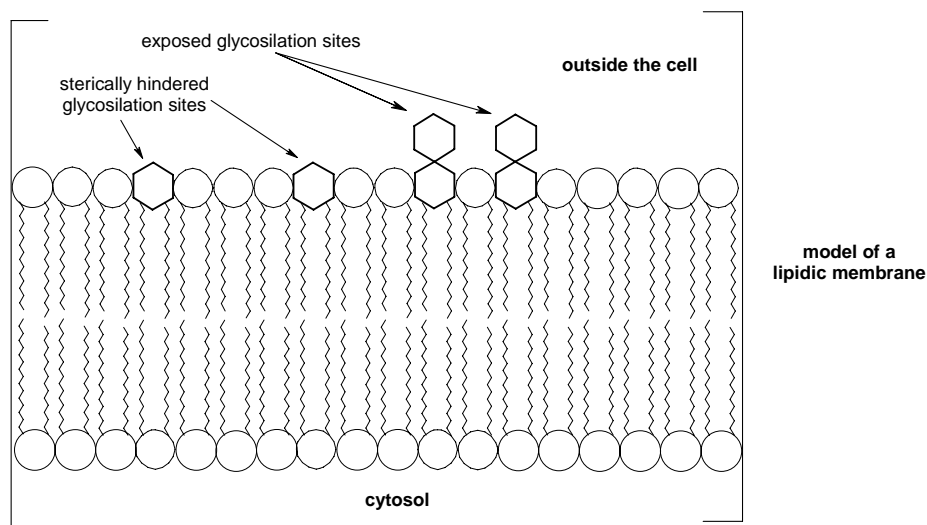
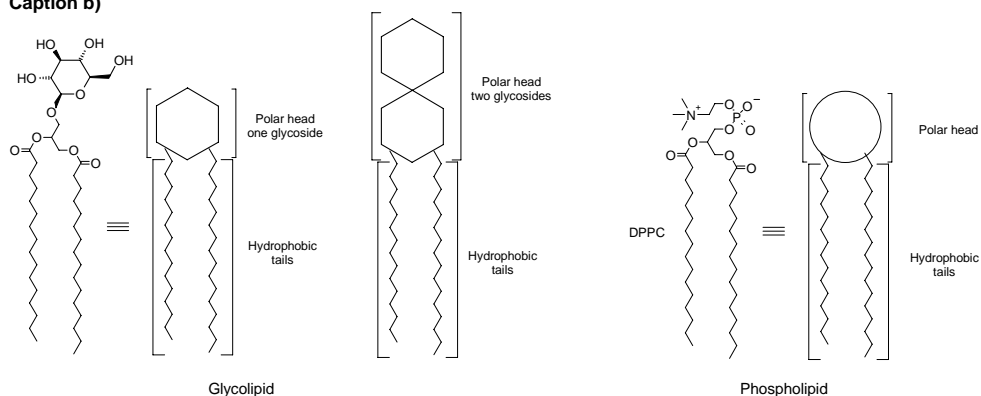
Natural lipidic molecules containing glyco head groups are very important in many biological processes e.g. in the cell-cell recognition, migration and adhesion, in coagulation of blood, in immunological responses, scarification, wound and affected tissue healing, being these some of the most important roles among others [81]. In many of the latter cases the functional glycoside moiety suffers bioconjugations when it covalently binds to a protein or lipid that finally becomes the bioactive molecule. Interestingly, there are lipidic molecules which specificity and coherent function have been consistently asymmetrically distributed in cell membranes due to their biological roles. These specific conjugates are the sugar-containing lipid molecules called **glycolipids**. Their specificity is of biologic activity means due to the experimental evidences indicating that they are found exclusively in the non-cytoplasmic half of the lipid bilayer, suggesting inter-cell interactions and other extra-cell processes [82, 83]. Herein we will cite three important types of glycolipids found in biological systems; they comprise simple **glycolipids**, when the lipidic substitution is directly bound to glyco-moiety; the **glycero-** and **phospho- glycolipids** [84], when in between the aforementioned moieties relays another connecting group such as glycerol or phosphate fragments, additionally the inter-position of the groups can generate another type of conjugation namely **glyco-phospholipids** and **glyco-glycerolipids**; and finally the **sphingo-glycolipids**, when ceramides and their derivatives are connected with the glyco head group (fig. 2.1, caption a) [82, 83]. Although there are a wide diversity of glycolipids there are specific reviews comprising this selection, which goes beyond the scope of the present contribution [84, 85].

Resembling the important biological characteristics and specific positioning of glycolipids, their asymmetric distribution in the bilayer results

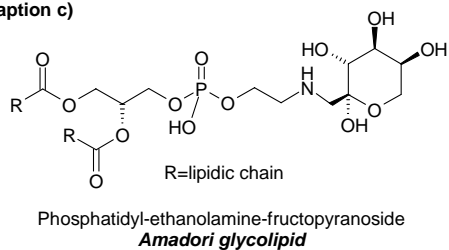
Caption a)



Caption b)



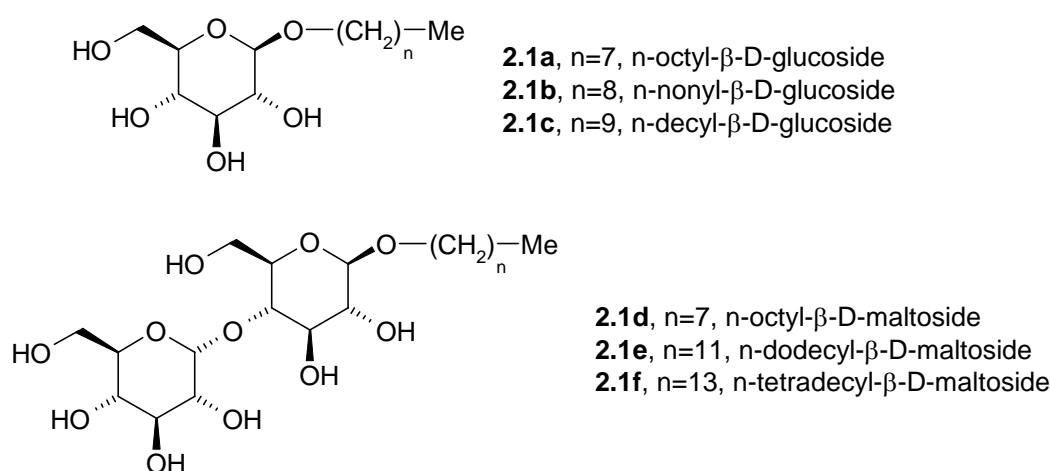
Caption c)



**Figure 2.1.** a) Typical glycolipids found in biological systems; b) comparison of glycolipids with other lipids (DPPC) and their specific positioning into the lipidic bilayer; c) Amadori glycolipid.

from the addition of sugar groups to the lipid molecules in the lumen of the Golgi apparatus, which is topologically equivalent to the exterior of the cell thus acting as specific recognition sites for glycosylated or free proteins, there relies their biologic importance [81]. As well, the glyco substitution of lipidic molecules results in a particular site for interaction and recognition due to the semi-rigid (many times bigger) size of the glyco-conjugations, see figure 2.1 (caption b) for a schematic view. The proportion of glycolipids has been chosen to be in a 3-28% of the total amount of lipids, among phospholipids (such as DPPC), cholesterol, etc. in biologic systems [82, 83] denoting a wide variety of concentration dependent activities and functions. As we have reviewed in the last section, it is well known that amino bearing compounds interact with sugars. Similarly, it has been shown that phosphatidylethanolamine also reacts with glucose, through the imino ketose route to yield the Amadori derivative covalently attached to the phosphatidylethanolamine moiety [86] (fig. 2.1, caption c).

Simple models of glycolipids (**2.1a-f**) have been prepared mainly by recent enzymatic substitution reactions [87] towards the anomeric center of sugar molecules, glucose or maltose in the present case, providing the  $\beta$ -D-glucosides or  $\beta$ -D-maltosides of varying chain lengths (Fig. 2.2). Instead of the enzymatic efforts, the chemical synthesis of these derivatives has not been further modified nor improved since the works of Fischer itself [88-90] and crew of his group [91]. Besides that and a sort of small variations reported by Noller and Rockwell in the latter thirties [92] there were no other reported efforts to achieve. They employed the per-acylated-bromo-glucose as starting material which by substitution with the specific alkyl-alcohol and removal of bromhydric acid and posterior saponification of the per-acylated derivative [91, 92] obtained the corresponding alkyl-glucoside. Other similar

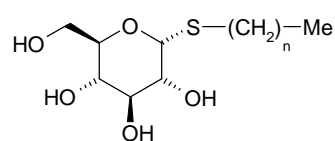


**Figure 2.2.** Simple glycolipids **2.1a-f**.

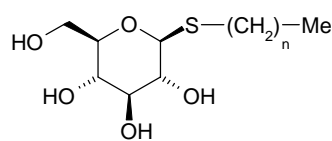
or more complex alkyl-glycosides can be obtained as well by enzymatic or chemical transformations of this type. These derivatives resemble the simplest glycolipids ever found and therefore they have been further employed and thoroughly physicochemically characterized so far [93, 94], see section 6 for physicochemical details and further applications in section 7.

Other examples of simple glycolipids should contain not only the union of glycosides and alkyl chains but the incorporation of other linking groups such as heteroatoms as well as other groups such as aromatics. Hence, the thio and phenoxy glycolipids in their  $\alpha$  (**2.2a-f**) and  $\beta$  (**2.2g-l**) forms have been prepared [95], compared [96] and studied [85] (Figure 2.3) in order to optimize their physicochemical characteristics. Due to experimental evidences [85] these molecular designed compounds have liquid crystalline phases initiating at a four carbon chain for the thio derivatives and a six carbon chain for the phenoxy family, see figure 2.3 to identify molecular species. Indeed, as a general trend, the presence of aryl groups increases the melting and clearing temperature of the liquid crystalline phase due to stabilizing  $\pi$ - $\pi$  interactions, which reinforces the supramolecular assemblies. As the authors have underlined, the temperature range between melting and clearing (liquid crystalline state) phases increases with the length of the hydrocarbon chain and it reaches what is called a maximum of the property. Additionally, the  $\alpha$  form stabilizes the liquid crystal behavior in slightly higher temperatures than does the  $\beta$  stereoisomer. Specifically, the sulfur bearing molecules for their  $\alpha$  form have a narrower temperature range than their corresponding  $\beta$  anomers.

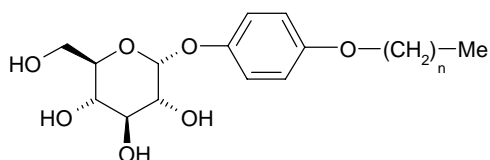
It has been hypothesized that the incorporation of not only one but two or more alkyl chains into a single molecular backbone could enhance the resulting



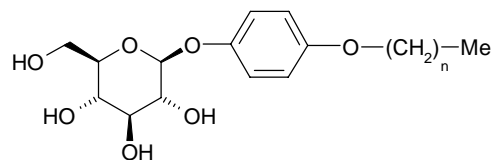
**2.2a**, n=4, n-pentyl- $\alpha$ -D-thioglucoside  
**2.2b**, n=9, n-decyl- $\alpha$ -D-thioglucoside  
**2.2c**, n=11, n-dodecyl- $\alpha$ -D-thioglucoside



**2.2g**, n=4, n-pentyl- $\beta$ -D-thioglucoside  
**2.2h**, n=9, n-decyl- $\beta$ -D-thioglucoside  
**2.2i**, n=11, n-dodecyl- $\beta$ -D-thioglucoside



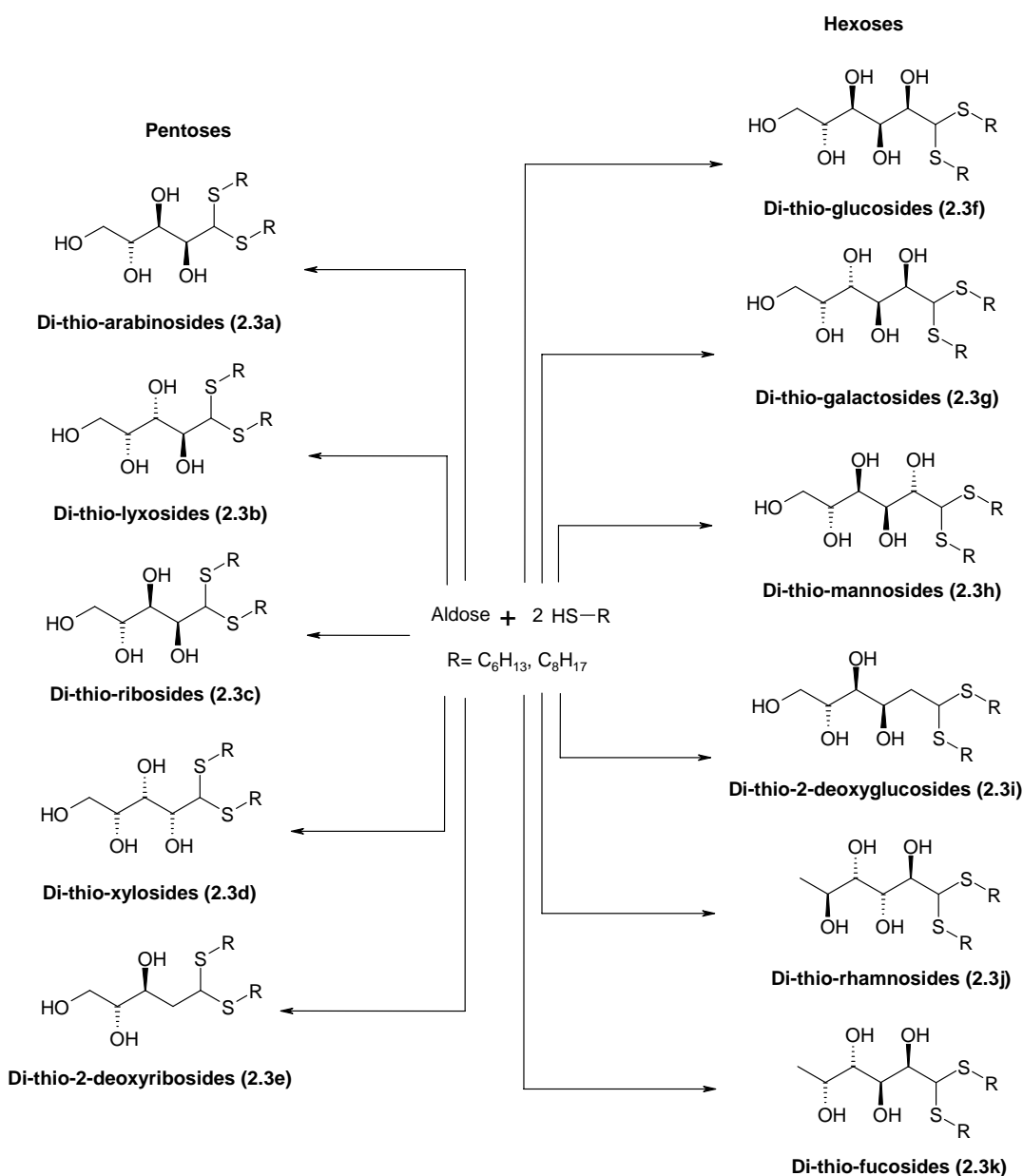
**2.2d**, n=0, 4-methoxy-phenoxy- $\alpha$ -D-maltoside  
**2.2e**, n=9, 4-n-decyloxy-phenoxy- $\alpha$ -D-maltoside  
**2.2f**, n=11, 4-n-dodecyloxy-phenoxy- $\alpha$ -D-maltoside



**2.2j**, n=0, 4-methoxy-phenoxy- $\beta$ -D-maltoside  
**2.2k**, n=9, 4-n-decyloxy-phenoxy- $\beta$ -D-maltoside  
**2.2l**, n=11, 4-n-dodecyloxy-phenoxy- $\beta$ -D-maltoside

**Figure 2.3.** Thio and phenoxy glycolipids in  $\alpha$  (**2.2a-f**) and  $\beta$  (**2.2g-l**) forms.

surfactant properties as well as others of biological means such as toxicity and biodegradability. Some interesting examples of open chain glycosides can be prepared through almost any aldose by its dissolution in concentrated HCl and the addition of two equivalents of alkanethiol, forming the glycodithioacetals (**2.3a-k**) (Figure 2.3), another form of glycolipids that surprisingly if the employed alkyl chains are above six carbon atoms can behave as mesophase surfactants; this happens with the formation of small amounts of alkyl-thioglycoside [85, 97-99] just as the sulfur bearing **2.2** compounds. The goal to prepare these open chain dithioacetals was due to the

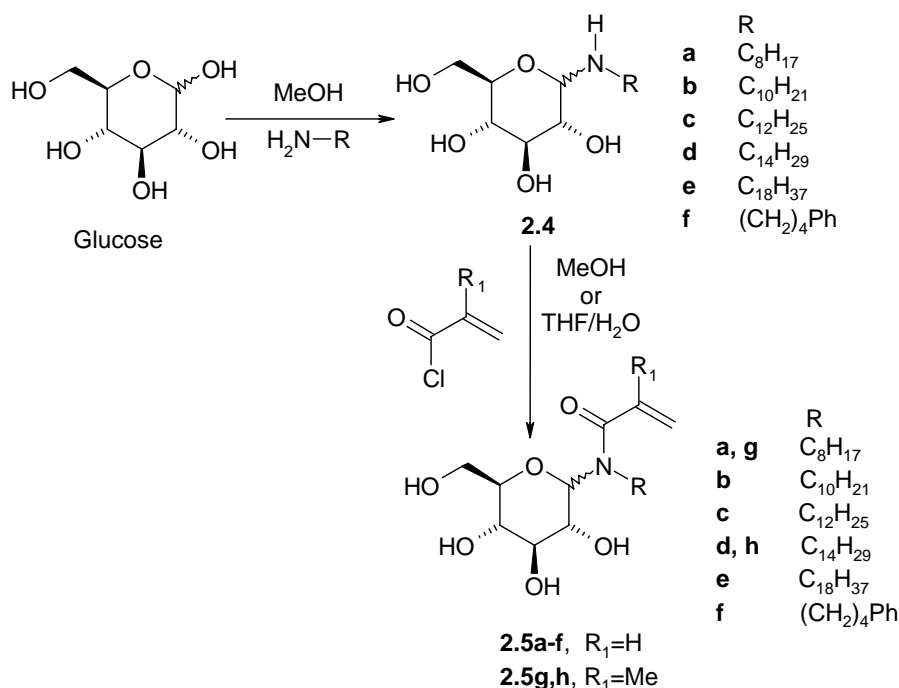


**Figure 2.4.** Preparation of open chain glyco-di-thio-acetals (**2.3a-k**) starting from fatty thiols and aldoses.

belief that the double thio-alkyl chain and the presence of thioether moieties itself will produce surfactant species harder to metabolize by bacteria, but this thinking lacks of funded evidences yet [85]. Even if the variation of the aldose is thoroughly examined the formation of the **2.3** type compound is always observed.

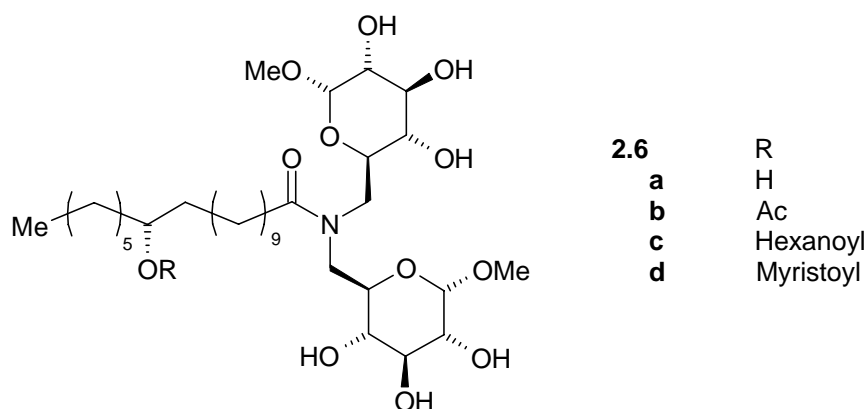
Other example of glycolipids is formed by the conjoint of aldoses with fatty amines instead of fatty alcohols or thiols (as in compounds **2.3**). Synthetically, the union among e.g fatty amines with D-glucose can be carried out in methanol, in the absence of water, giving place to glucolipids (**2.4a-f**) (Fig. 2.5) [100]. These interesting molecules were functionalized with  $\alpha,\beta$ -unsaturated fragments, through the formation of the corresponding amides leading to other variation of glucolipids (**2.5a-f** ( $R_1=H$ ) and **2.5g,h** ( $R_1=Me$ ), Fig. 2.5) [100] in order to provide useful building blocks (or synthons as known in organic synthesis) for the development of more complex surfactants as will be seen in the next section. These reactive species can serve also in other applications through coupling or addition reactions, e.g polymerization, or Michael addition. The critical micelle concentration (CMC) of the title compounds were reported as follows: 12.0 (**2.5a**), 1.2 (**2.5b**), 0.14 (**2.5c**), 0.03 (**2.5d**), 8.0 (**2.5g**), 0.017 (**2.5h**) mM.

Other type of glycolipids, in this case bearing a double glycoside moiety as polar head group (**2.6a-d**)[100] were prepared. The synthetic route comprised the starting from the 1-methyl, 2,3,4-tribenzyl-glucose and its self

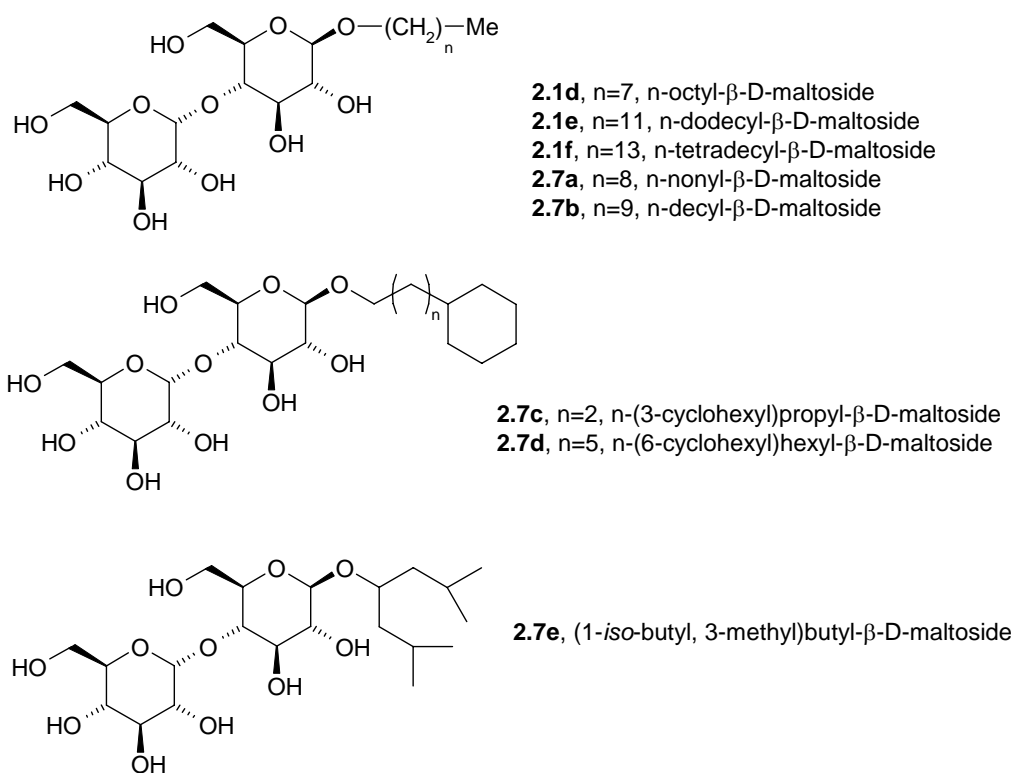


**Figure 2.5.** Preparation of glycolipids (**2.5a-h**) united by amide moieties.





**Figure 2.6.** Preparation of glycolipids **2.6a-d**.

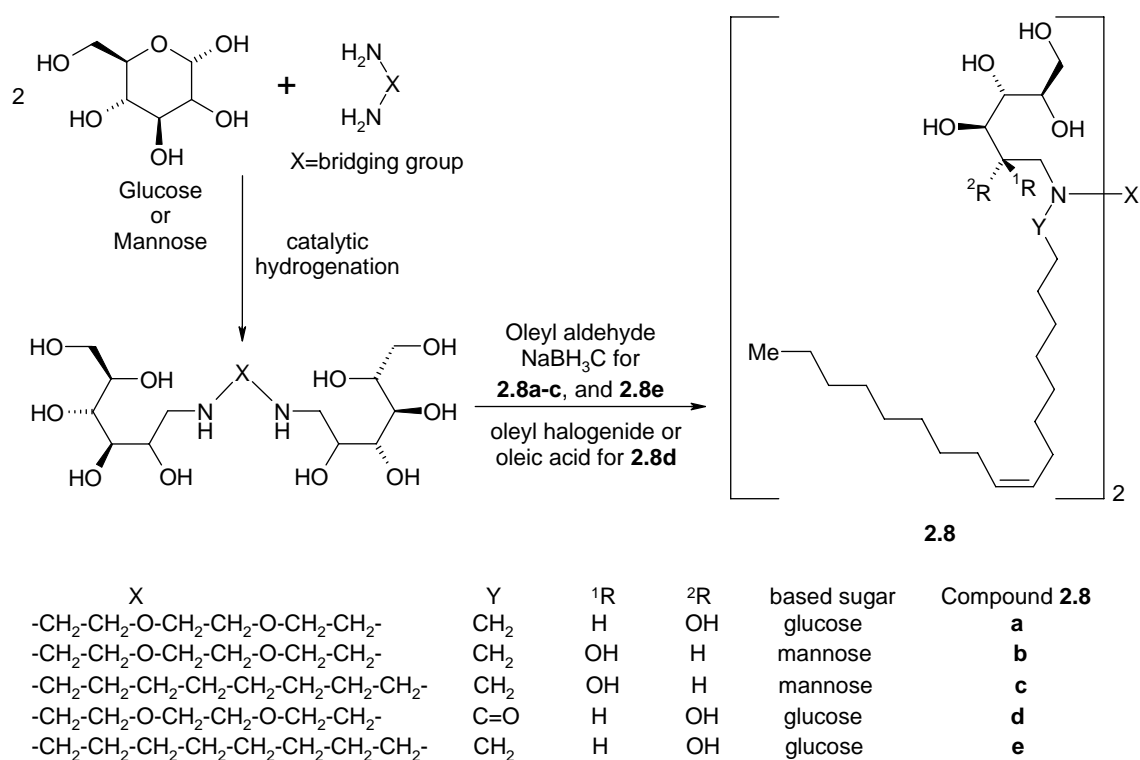


**Figure 2.7.** Preparation of glycolipids **2.7a-e**.

coupling to yield the amine bridging double glycosidic polar head group which by further condensation with the 12-hydroxy stearic acid led the goal glycolipids set together through the amidic functionality. The search for enhanced polar head groups [101] is herein foreseen in order to determine the new physicochemical properties within this type of modifications. The first three surfactants (**2.6a-c**) are water soluble and the inclusion of a myristoyl side chain at the stearic moiety was enough to ban this property due to liophilic balance. Further physicochemical properties are discussed in section 6.

There have been prepared and studied other derivatives of **2.1** type glycolipids, they were **2.7a-e** (see their structures in figure 2.7) [7] preserving the disaccharide maltopyranoside moiety and varying the alcohol moiety with linear chains, in order to complete series **2.1**, as well with cyclic and branched substitutions, as in **2.7c-e**, to determine the modification of physicochemical properties in comparison with their linear, **2.1d-f** and **2.7a-b**, homologues. Their hemolytic activities were systematically determined in order to correlate their structural changes regarding CMCs within specific changes in the *in vitro* tests, and further discussion of this is placed in section 6.

The Gemini type surfactants, classified as *bolaamphiphiles*<sup>3</sup>, have received attention due to their designable modifications that also found plenty potential applications. The name Gemini has been initially employed due to



**Figure 2.8.** Preparation of Gemini glycolipids **2.8a-e**.

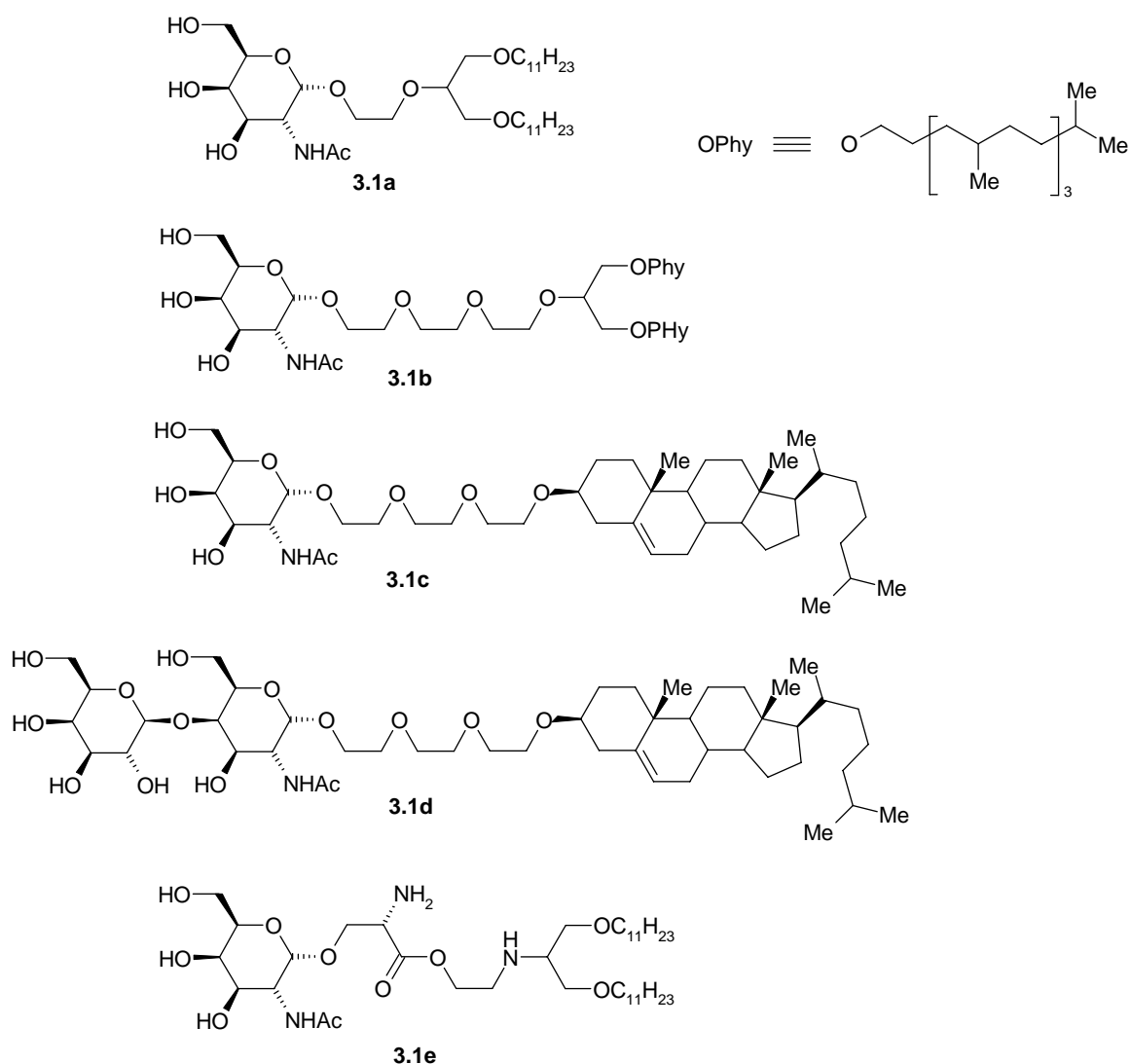
<sup>3</sup>The term *bolaamphiphile* is as a result of the acronym among *bolaform* and *amphiphile*. This particular type of amphiphiles tends to ensemble as spherical or elliptical micelles due to a highly sensitive ratio among two or more hydrophilic groups (heads) and at least one long alkyl chain. See page 168 of reference [102] for further details in this particular theme. The **2.8** type Gemini molecules are examples of two polar heads with two non polar hydrocarbon chains, lying in the definition of *bolaamphiphile* not only due to their structural but to their physicochemical properties.

the presence of two amphiphiles connected through a modifiable bridging group hence they comprise twin groups; in figure 2.8 the symmetry of the Gemini molecule is employed for drawing simplicity. A particular case of geminis are prepared through a two step reaction pathway in which three molecules through the catalytic hydrogenation of two synthetic equivalents of glucose or mannose and the corresponding bridged N,N'-diamine ensemble the hydrophilic head. The second step consisted in the employment of this extended polar head to perform the *in situ* condensation and reduction with the oleyl aldehyde and sodium cyanoborohydride, as achieved to obtain **2.8a-c** and **2.8e**, or through the same extended polar head and N-acylation giving place to **2.8d**, see figure 2.8 for structural details [103-105]. Physicochemical characteristics of they are discussed in section 6.

### 3. Other examples of glycoconjugates, mainly glyco-xenobiotics

Some other examples of a diversity of glycoconjugates can be found in the literature. The non glycosidic building block can take almost any chemical shape and according to this they can be classified as lipidic, hydrophilic or amphiphilic. One important synthetic effort to obtain glycolipids with interesting functionalities yielded N-acetyl- $\alpha$ -D-galactosamines (**3.1a-e**) [106], please refer to fig. 3.1 in order to revise the chemical structure. Compounds **3.1c** and **3.1d** bear cholesterol anchoring groups, the former with a single sugar hydrophilic group and the latter with a double sugar counterpart. Compound **3.1a** is functionalized with a double condensed glycerol O-allylic groups, meanwhile **3.1b** is functionalized with two phytol (from phytol alcohol) groups. And finally, another example of O-glycosylation of an  $\alpha$ -aminoacid, the **3.1e** derivative, in this case the L-serine central part is modified as the amino-amide form with the same bis-O-alkylated group as in **3.1a**, while in the O-glycosylated position is attached an  $\alpha$ -D-galactosamine. In the original reference is stated that these derivatives are going to be evaluated according to their variations in the hydrophobic anchors in the adsorption process in some particular cancer cell surfaces to give further insights into their biochemical roles.

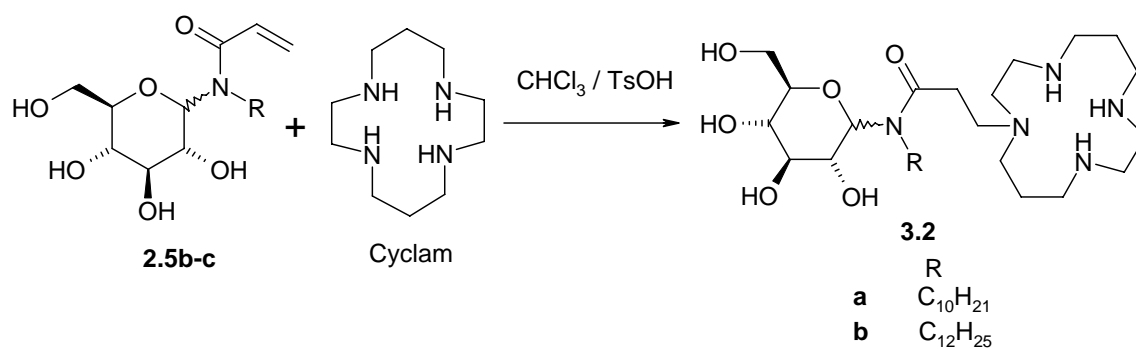
In this line, an important piece of work states that through the formation of a  $\beta$ -alanine bridge from the condensation of cyclam and the corresponding N-alkylglucosylacrylamide (**2.5b-c**, which preparation is pointed out in section 2 [107]) in chloroform, the preparation of N-cyclam-N-alkylglucopyranosides (**3.2a-b**) [108] was taken. Is important to notice that the amino-acid moiety is constructed within the reaction pathway and it remains in a protected or less reactive form, the amino-amide state (Fig. 3.2). Additionally,



**Figure 3.1.** Preparation of N-acetyl- $\alpha$ -D-galactosamines (**3.1a-e**) with different bridging and hydrophobic groups.

the formation of an inverse Amadori product, where the connection of the glycoconjugate is through the carbonyl bearing fragment instead of the amino terminal group, is a good example of stability when compared with the normal Amadori product that is almost unreachable when it is tried to be prepared without protection of the sugar. Regrettably no more physicochemical studies were developed for these compounds to compare among the parent glycolipids.

Fortunately, the latter effort has led to a superlative step in the preparation and molecular design of compounds of the **3.2** type. Larpent *et al.* [109] systematically developed azamacrocycles bearing the same N-alkyl-glucopyranoside functionalities as chiral scaffolds [107], and demonstrated the enhanced and tunable surfactant properties of the newly designed compounds

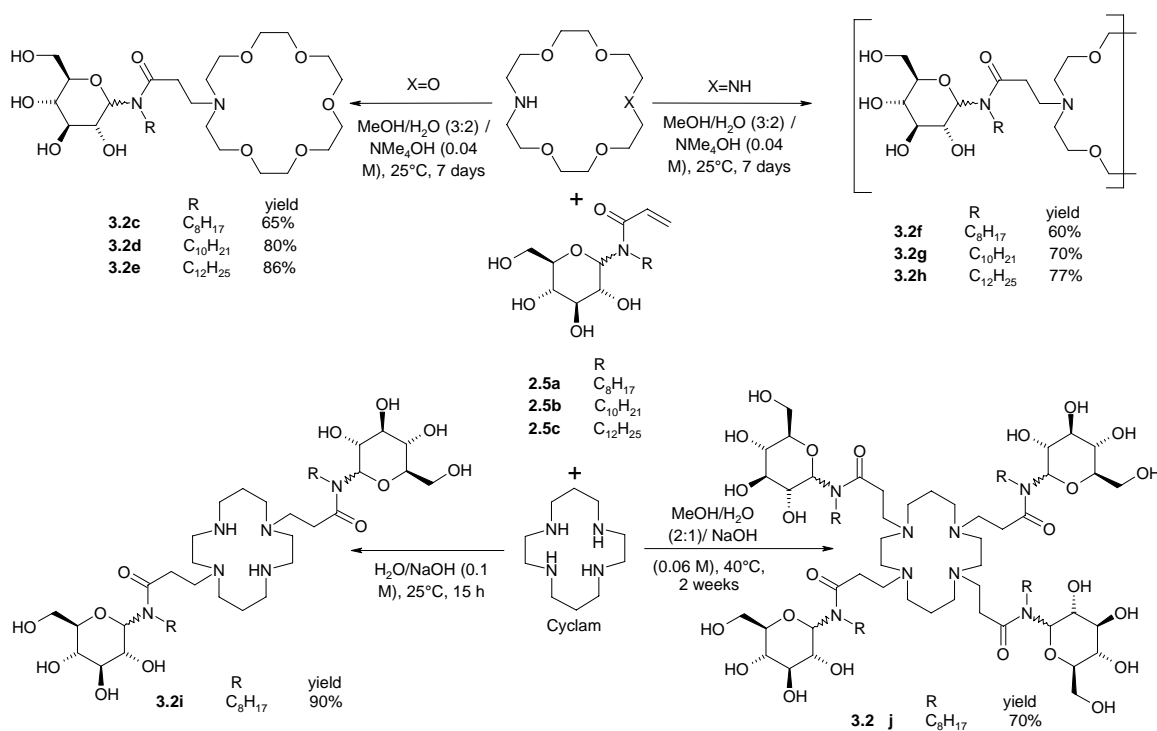


**Figure 3.2.** Preparation of inverse Amadori derivatives with  $\beta$ -alanine bridges (**3.2a-b**).

**3.2c-j** [109] (Fig. 3.3). As stated by the authors, the characteristic properties of any surfactant are i) a narrow range of CMC and ii) self-assembly towards the formation of stable micellar ensembles. Molecules of type **3.2** bearing two or four surfactant moieties behave like the well known gemini surfactants [110], slightly seen in section 2. These compounds have lower CMC values compared with their starting glycoconjugates. The comparisons among compounds bearing octylic side chains are 0.24 (**3.2c**), 1.3 (**3.2f**), 0.14 (**3.2i**), 0.6 (**3.2j**) with 12 mM for **2.4a**; meanwhile with decyl side chains are 0.05 (**3.2d**) and 0.03 (**3.2g**) versus 1.2 mM for **2.4b**; and finally, with dodecyl side chains are 0.16 (**3.2e**) and 0.01 (**3.2h**) that can be compared with 0.14 mM for **2.4c**. These comparisons clearly evidenced the physicochemical differences related to number of substitutions and length of alkyl chain. Those compounds with just one glycoconjugation resemble much similarity to their respective reagents, like **3.2e**, and thus present pretty similar CMC values, in this case 0.16 mM, a value nearby that of **2.4c**. The solubilities of **3.2c-j** in water were reported to rely in the range 0.01–0.05 M. All of the macrocyclic containing surfactants self-assemble in water as small micelles with a prolate shape with the lipophilic zone being the hydrocarbon chains and a hydrophilic counterpart that contains the macrocycles and the glycopyranosides.

Simpler couplings have been tested among glycosides and other molecules of pharmacological interest. An example of this is the set of glycosides (**3.3a-d**) derived from hexoses and pentoses reacted with 2-(*ortho*-aminophenyl)-benzimidazole [111] (see figure 3.4).

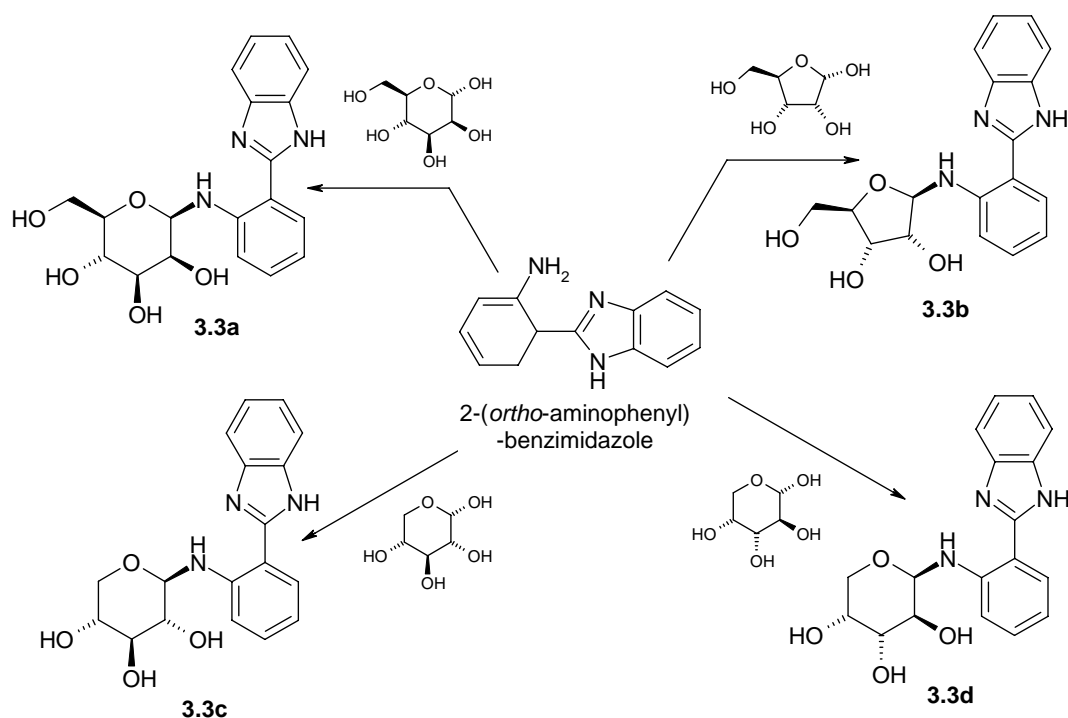
It is well known the importance of conjugation among sugars with phosphate units and nucleic bases, to obtain e.g. ATP, being a triple bioconjugate molecule. Due to its inherent nature, nucleotides, such as ATP, have three selective coordinating moieties, i) planar pi-pi recognition bases, as nitrogen containing fragments, ii) puckered sugar, mainly as diol-coordinating systems, and iii) phosphate side chain, which depending on the number of connected phosphate units, serves as the mono-, bi-, tri- and multi-



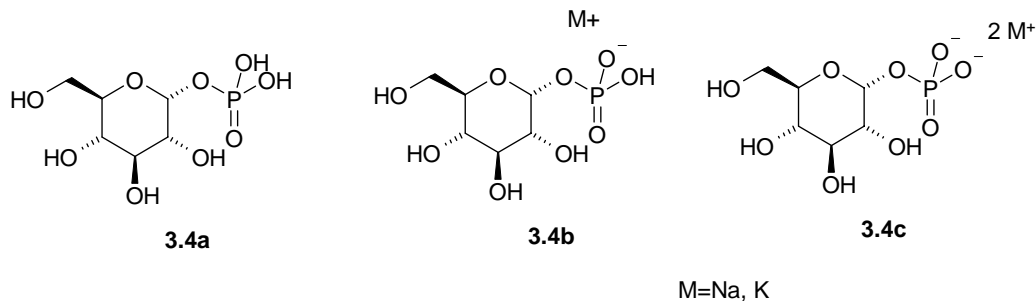
**Figure 3.3.** Azamacrocycles (**3.2c-j**) united by  $\beta$ -alanine moieties with N-alkylglucopyranosides.

dentate ionic binding motif [112-116]. The biological roles that play nucleotides make them subjects of study nevertheless that their multicharged nature, such as ATP<sup>4-</sup>, have turn them as hard crystallizing molecules. In spite of this, some examples of ATP-M(II) systems have been reported so far. It has been observed that metal transition cations (Co<sup>2+</sup>, Zn<sup>2+</sup>, Cd<sup>2+</sup>), with N/O ratio placed in the middle selectivity preference, tend to occupy specific sites for the metal to ligand interaction. Thus being a needed counterion, the outer sphere [M(H<sub>2</sub>O)<sub>6</sub>]<sup>2+</sup> hydrated cation, principally responsible to electroneutralize the ATP<sup>4-</sup> tetra-anion.

Other simpler possibilities, than the ATP molecule, are present in the case of a combination of just two of the three fragments, in this case a given sugar with a phosphate unit. One of them is the  $\alpha$ -D-glucose-1-phosphate (**3.4a**). This type of bioconjugate, due to the characteristics of the phosphate group is that it can be employed as mono-, di- or tri-dentate ligands just by taking into account the phosphate ligation group, which sometimes indeed bans the utilization of the glycosidic coordination atoms towards the metallic center [113, 114]. The versatility of these starting materials is that they can be isolated as its mono-salt (**3.4b**) [117], or di-salt (**3.4c**) [118, 119] coordination compounds (see figure 3.5). When  $\alpha$ -D-glucose-1-phosphate [120] as its disodium salt (**3.4c**) [118] (see figure 3.5) was employed for the generation of



**Figure 3.4.** Preparation of glycoconjugates of 2-(*ortho*-aminophenyl)-imidazole (**3.3a-d**).

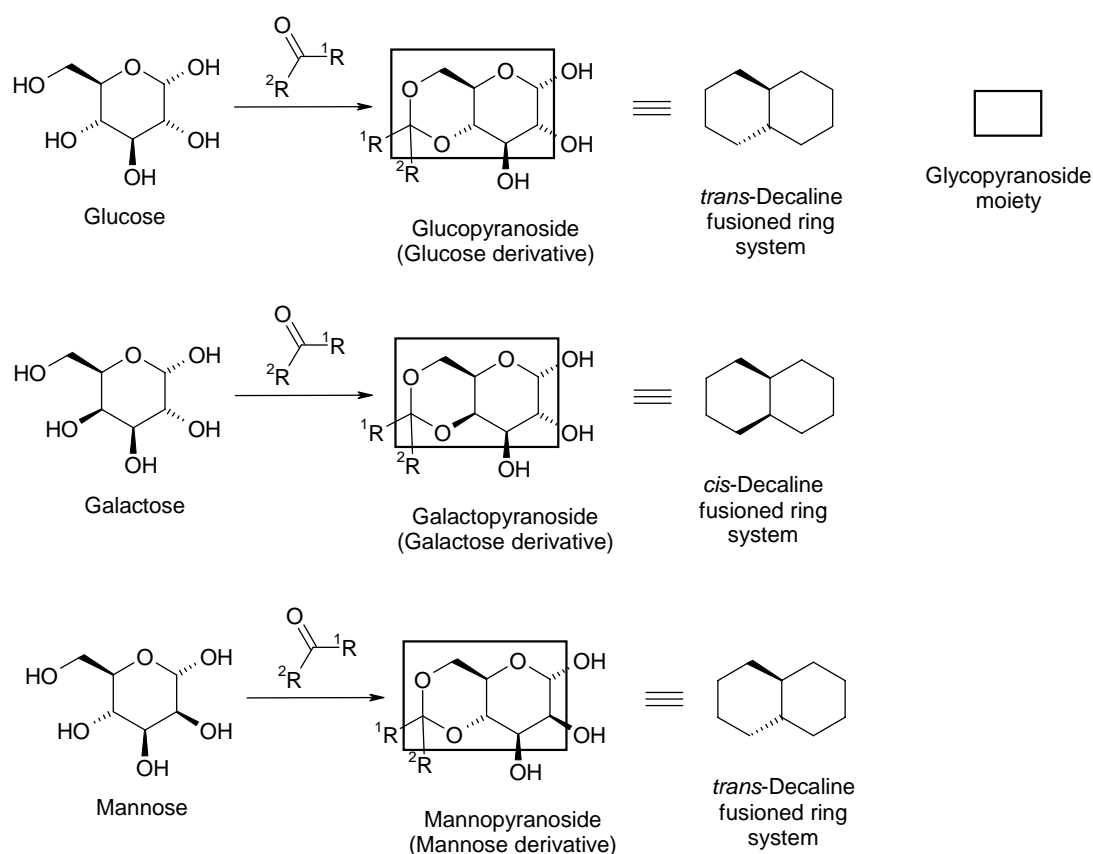


**Figure 3.5.** Preparation of glycoconjugates with phosphate metal linking groups (**3.4a-c**).

coordination compounds [120] and within the formation of metallic clusters with copper as the metallic building moiety and other ligands such as 2,2'-pipyridine, other mono- and bi-dentate ligands as well as with sugar acids, the preparation of coordination polymers and the characteristic reinforced metallic clusters as building motifs for 2D and 3D architectures was achieved [120]. Other effort to obtain coordination compounds using this bioconjugate **3.4a** building block was with the employment of aza-macrocyclic ligands [121]. Some of the compounds and materials obtained resemble metal-organic frameworks or their building blocks opening this line of state of the art research.

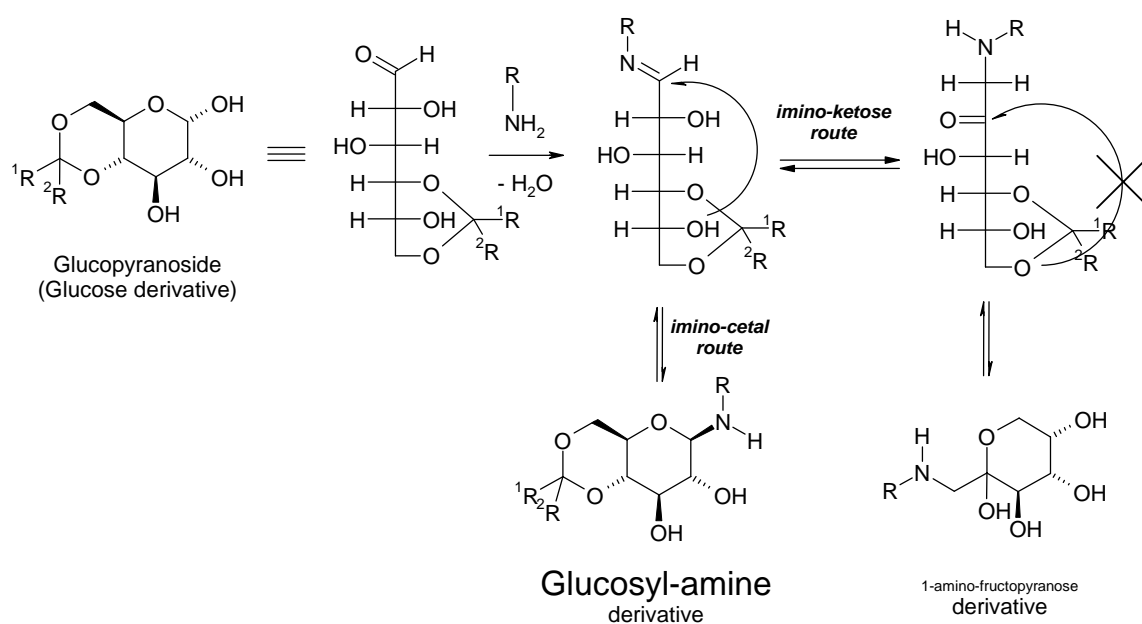
## 4. Glycoconjugates with protection of the glycopyranoside moiety

Anyway, if we intend to perform a wider range of preparation conditions, the harsh synthetic workup and isolation of the native Amadori products as well as other difficulties observed for the isolation of pure glycolipids has been reduced by the blockade of one of the Amadori pathways, the *imino-ketose route* (see Fig. 1.1) that also serve as protecting group for other syntheses. The strategy consisted, as mentioned in the last paragraph, in the protection of the hexoses at 4, 6 positions via the production of 4,6-<sup>1</sup>R-<sup>2</sup>R-glycopyranosides (e.g. for glucose, mannose, galactose, etc., even for pentoses) employing aldehydes or ketones for this means (<sup>1</sup>RC<sup>2</sup>RO) [111, 122]. The protected carbohydrates are reasonably more stable due to the formation of fused rings analogues of *cis*- and *trans*-decalines [85] depending on the stereochemistry of the starting material (see Fig. 4.1). Due to this protection just leaves place to the *imino-cetal route* (see Fig. 1.2 vs. Fig. 4.2) [111, 122] when nucleophilic attack is the synthetic strategy. Other



**Figure 4.1.** Protection of carbohydrates (e.g. hexoses) to obtain a glycopyranoside skeleton. More stable moiety than the original hexose due to the formation of *cis*- and *trans*-decaline analogues and fused rings.





**Figure 4.2.** Specificity for the reaction of amines and 4,6-<sup>1</sup>R-<sup>2</sup>R-glycopyranosides (e.g. with carbohydrate = glucose).

advantage is that, depending on the aldehyde or ketose conjoint to achieve protection, the solubility can be compared with the starting carbohydrate or even improved [32, 33]. Another important point to obtain stereochemical controlled scaffolds is that this condensation reaction, due to various aspects such as rigidity of the *cis*- and *trans*-decaline backbones, when aldehydes are the protecting groups, its substituent will end up in the preferred equatorial position, consistent with the indicated R absolute configuration for this newly formed stereogenic center as stated in reference [85] and therein citations.

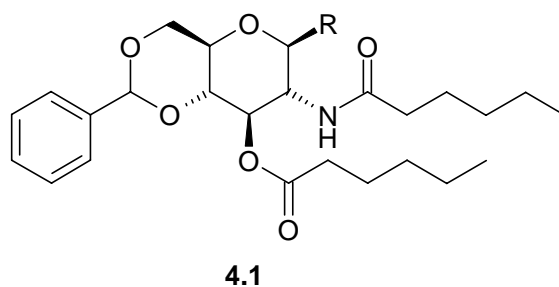
Some studies related to the applicability of substances derived from the Amadori rearrangement clearly mention their antioxidant character and also include them into the family of Maillard reactions. This latter set of transformations are mainly dehydrations of Amadori products coupled with isomerizations that led to nitrogen containing polymers and furfural based molecules [32, 33]. Maillard reactions are mainly carried out between aminoacids and saccharides occurring at low both temperature and concentration. To differentiate among Amadori products and Maillard continuing reactions is important to underline that the second dehydration step is the stated separator for these coupled transformations. This second step led the caramel stage products known as reductones and dehydroreductones that in the reduced chemical form are employed in the conservation (antioxidant activity) of food such as bread [32, 33]. One coarse example of this is in the employment of the so called browning reactions occurring when egg and sucrose are mixed and this resulting mixture is

applied as painting over the surface of a piece of mass and hence baked, thus propitiating the shining surface in bread as a natural conservator [32, 33]. In further dehydration stages the variety of products is wide but mainly via the formation of furfurals or  $\alpha$ ,  $\beta$  unsaturated imines, to reach the final step reactions where multiple couplings occur yielding melanoidins (nitrogenated polymeric mixtures with antioxidant properties) [32, 33].

The powerful strategy of the conjoint of 4, 6-<sup>1</sup>R-<sup>2</sup>R-glycopyranosides with amino acids has not been already exploited synthetically and it is a central strategy for the generation of entire families of new pure products between carbohydrates and amino acids [123, 124]. It is therefore an opportunity for basic and applied research and development of technology. Another wide open possibility of glycoconjugates arises when stated the prove that the 4,6-<sup>1</sup>R-<sup>2</sup>R-glycopyranosides have been coupled with simple aromatic and aliphatic amines [111, 122] since the employment of e.g. fatty, bis-, amino acid, xenobiotic, etc. amines would led to a complete family of glycoconjugates, not yet fully synthesized neither applied, and as a pulse opportunity of research and development, as will be seen forthcoming.

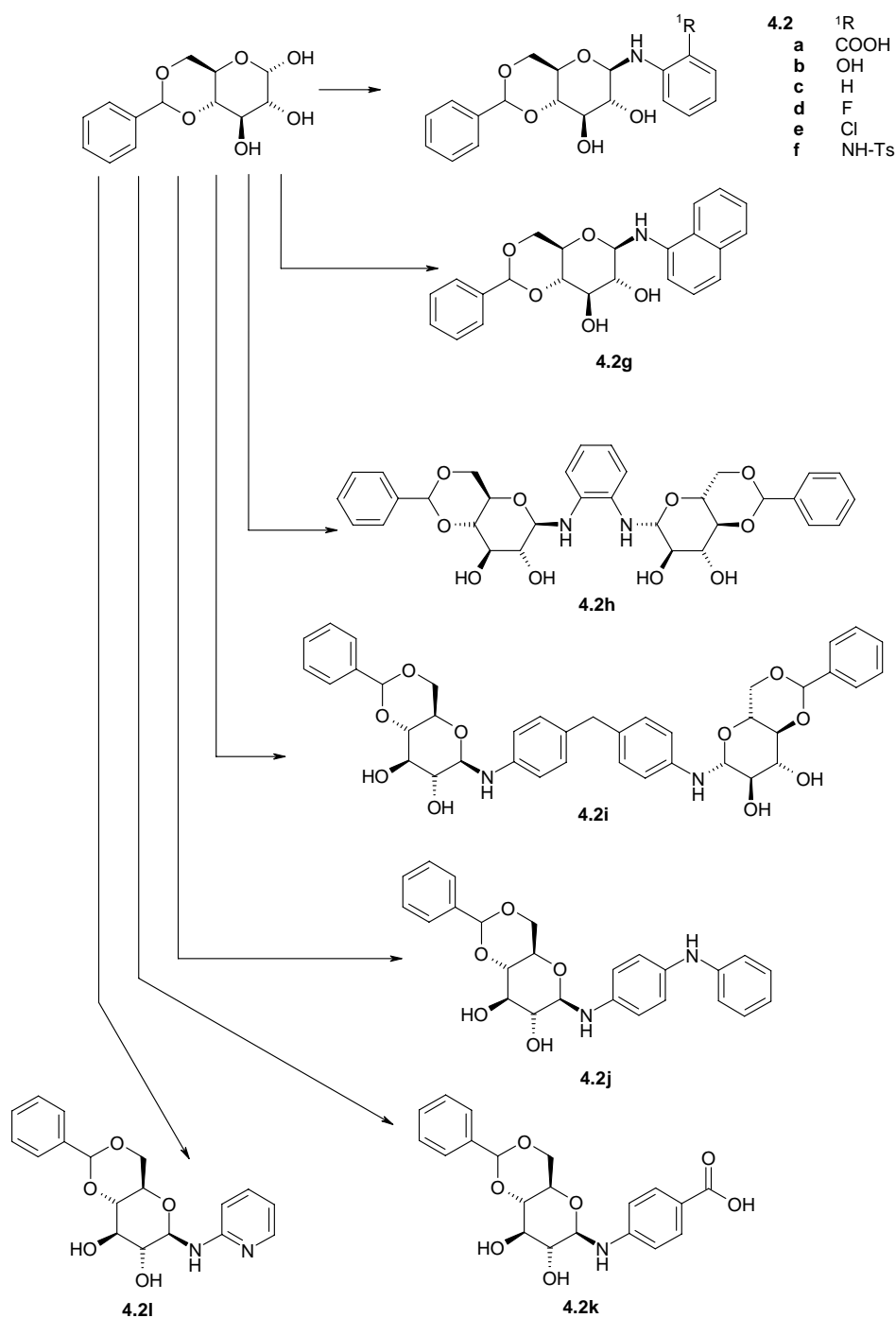
Some lead compounds of possible pharmaceuticals, for further molecular design and evaluation, are cited as follows, they are conformed of two fatty acid chains attached in positions 2- and 3- as their amide and ester functionalities into the 2-glucosamine backbone, which was previously protected as its phenyl glucopyranoside. Besides, specific groups were substituted in position 1 of the hexose in order to modulate physicochemical properties impacting the biological responses through the knowledge of specific variation of the chemical structure in **4.1** (Figure 4.3). Indeed, type **4.1** compounds have shown potential as prototypes of antitumor agents [125, 126].

Almost the same precursor as for **4.1** is herein applied for the obtaining of glucopyranosides bearing amine functionalities in position 1 (see compounds **4.2a-1** in figure 4.4). In all cases, the  $\beta$  anomer was observed, either from NMR or X-ray diffraction analyses [111]. Compounds **4.2a** and



R=several grups to achieve structure-activity relationships

**Figure 4.3.** Glucopyranoside protected glucolipids (**4.1**) as potential antitumor agents.



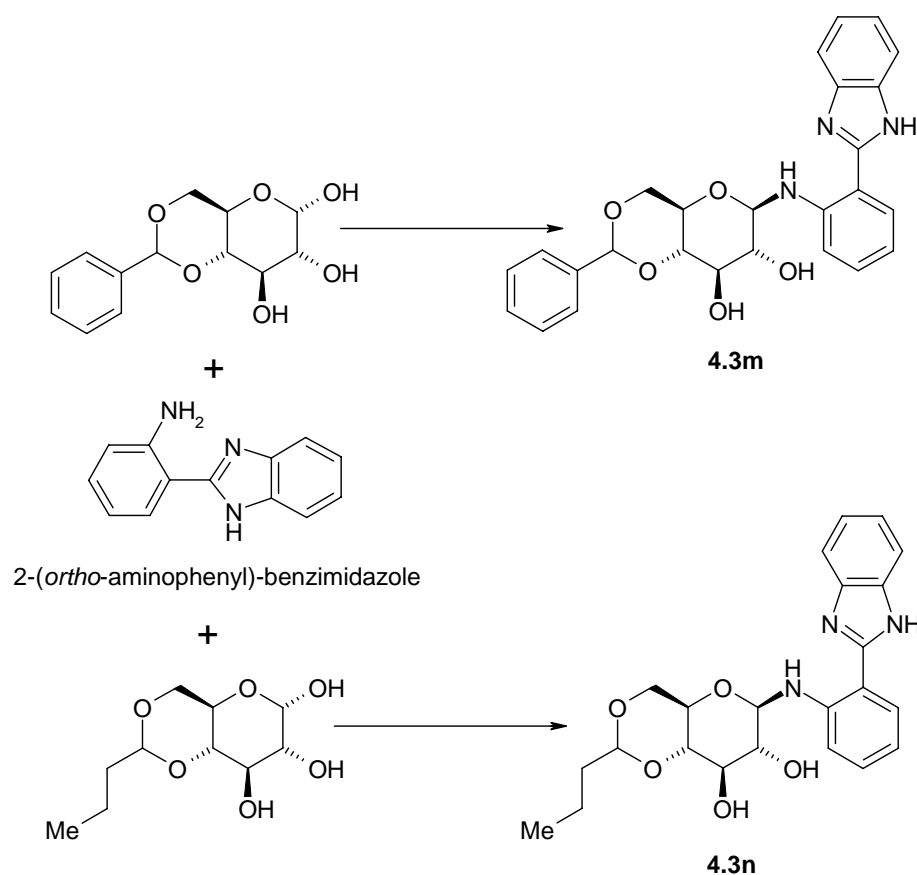
**Figure 4.4.** Glucopyranosides (**4.2a-l**) bearing amines at position 1 of the glyco moiety.

**4.2k** are clear examples of the preference of the imino-cetal route when aromatic amino acids are employed. No bibliographic information was found for examples bearing proteinogenic aminoacids within the glycopiranoside moiety, being another source of lead compounds of molecular designable pharmaceuticals. The authors state that due to hydrogen bonds present in

structures **4.2a** and **4.2b**, and the fingerprint of a tridentate ligand due to the presence of the ONO should be useful for the chelation of metals. Besides, other interesting fragments found in chelating ligands are present in **4.2i** where the presence of a pyridine nitrogen atom is the replacement of the donor atoms, carboxyl and hydroxyl groups, found in **4.2a** and **4.2b**. Finally, a supramolecular building block, due to the position of the carboxylate moiety, is herein represented in **4.2k**.

Also as in **3.4** derivatives, the coupling of molecules of pharmacological interest such as 2-(*ortho*-aminophenyl)-benzimidazole, in this case with the glucopyranosides protected in 4 and 6 position due to the formation of the acetal, provided the **4.3m** and **4.3n** derivatives [111], see figure 4.5. In these two cases the varying from glucopyranoside moiety was in the protected fragment including both 4,6-O-benzylidene, in **4.3m**, and 4,6-O-butylidene, in **4.3n**.

Through the development of **4.2a-n** compounds, the preparation of their simpler analogues derivatives of primary amines were also synthesized, giving place to **4.2o-p** by variation of the protecting acetalic moiety. These two compounds were employed as starting materials for the development of

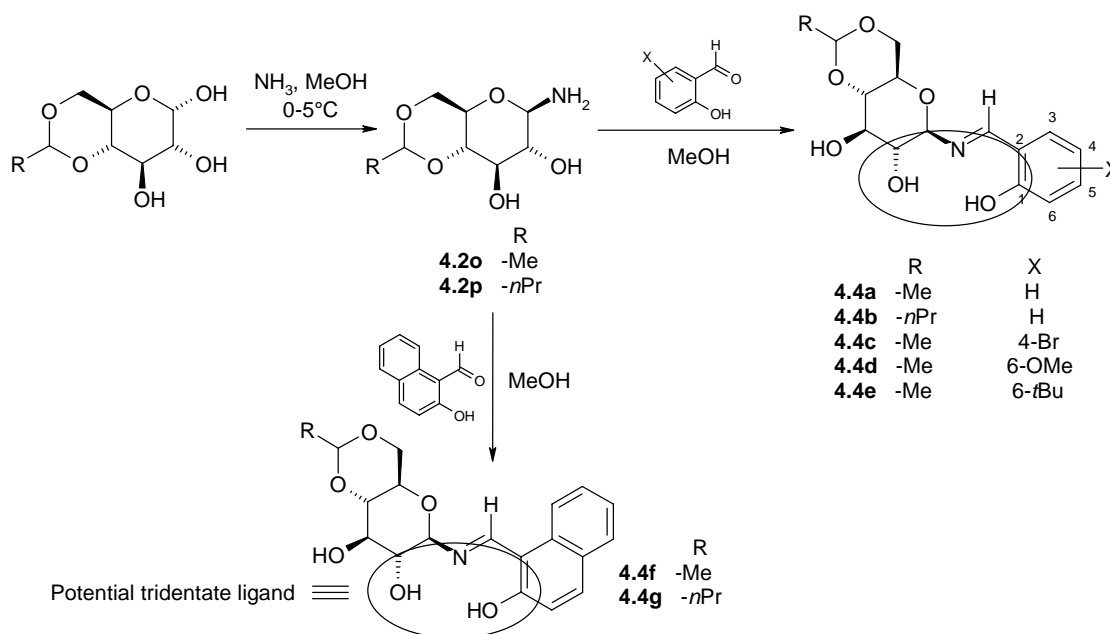


**Figure 4.5.** Glucopyranosides (**4.2m-n**) bearing 2-(*ortho*-aminophenyl)-benzimidazole at position 1 of the glyco moiety.

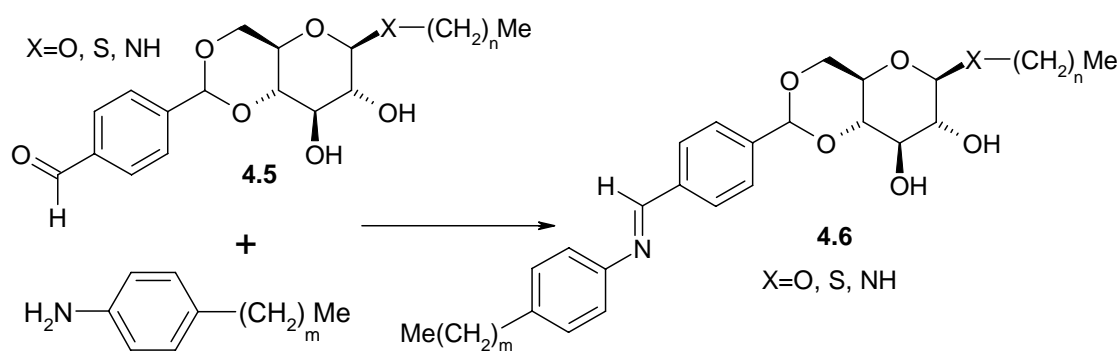
their respective glycosides bearing in this case the salicylideneimino functionality (**4.4a-g**) by the condensation reaction of **4.2o-p** with salicylaldehyde or 6-methoxy-, 4-bromo-, 6-*tert*-butyl- salicylaldehydes or 2-hydroxy-naphthaldehyde in methanol [122, 127-129]. For the coordination chemist, compounds **4.4** have the ideal prerequisites of a normal O-N-O tridentate ligand. Indeed, their employment as chelating agents for copper [128-133], zinc [134, 135], nickel [136], molybdenum [137], vanadium [137] and uranium [137] has already been reported.

More elaborated glucopyranosyl-derivatives can be afforded when reactive groups are attached into the condensable aldehyde fragment. An example of this versatility is reached when the terephthalaldehyde is mono condensed with *S*-decyl- $\beta$ -D-glucopyranose to yield compound **4.5**. The latter was employed as starting material for the preparation of another condensation product with fatty-aromatic-amines yielding compounds of type **4.6** with extended chromophoric groups (Fig. 4.7) [85]. Although only compound **4.6** with X=S, m=11 and n=9 has been prepared and evaluated, and resulted to have interesting mesogenic properties. Nevertheless all the other possibilities resemble new opportunities of molecular design, to e.g. obtain liquid crystals with non linear optic behavior or as fluorescent probes of active pockets in macroreceptors, etc.

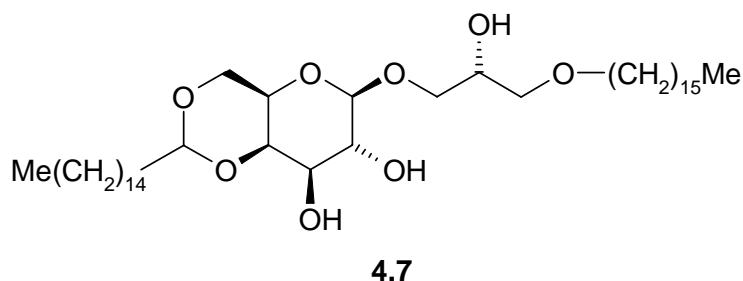
Due to its importance in synthetic chemistry, the protection of glycosides has its own examples in biological systems as well. A novel glycolipid, the



**Figure 4.6.** Simple glucopyranosylamines (**4.2o-p**) and their reaction with salicylaldehydes or 2-hydroxy-naphthaldehyde to led glucopyranosylimines (**4.4a-g**).



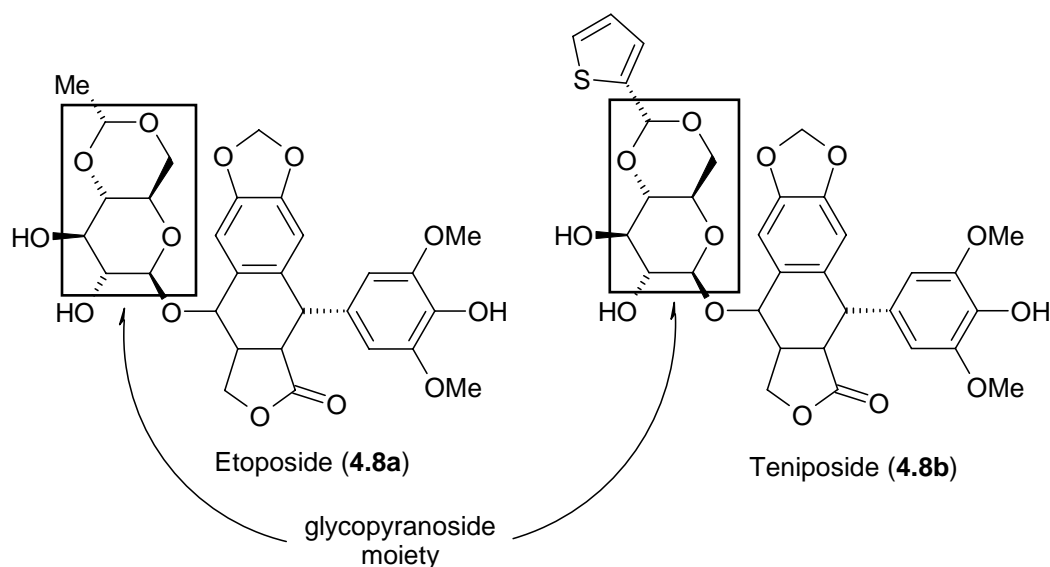
**Figure 4.7.** Condensable glucopyranosyl-derivatives (**4.5**) and their coupling with fatty-aromatic amines to yield mesogenic glucopyranosylimines (**4.6**).



**Figure 4.7.** Natural occurring galactosylalkylglycerol (**4.7**) protected at the glycoside moiety.

galactosylalkylglycerol (**4.7**), modified with a long-chain cyclic acetal attached at the 4- and 6- positions of the sugar moiety was isolated from equine brain and named plasmalogalactosylalkylglycerol [138] see figure 4.7. The chain lengths of alkyl and acetal groups were C14 and C16 for the former and C16 and C18 for the latter. The acetal group appears thus similar to that found in plasmalogalactosyl ceramide previously isolated from equine brain. According to the results of this research and references therein the whole equine brain contains about 5 mg of this new acyl protected glycolipid.

Moreover, the success of the strategy of glyco-protection, within the observed beneficial antioxidant properties and some other biological activities, has been taken as advantage in the generation of branded pharmaceuticals. Former 80s international patents protect the use of glycoconjugates comprising the reinforced glycopyranoside (*decaline analogue or fused rings systems*) molecular skeleton and their potential as wide spectra antitumor agents, tested *in vivo* in humans and with diminished toxicity [139, 140]. The application studies of these 4,6-<sup>1</sup>R-<sup>2</sup>R-glycopyranosides have resulted in the generation of important chemical structures such as etoposide (**4.8a**) and its derivatives (e.g. teniposide (**4.8b**),



**Figure 4.8.** Molecular designed bioconjugates with 4,6-<sup>1</sup>R-glycopyranosides (4.8).

see fig. 4.8). The applicability of this family of compounds as first line chemotherapy is well founded [141-145].

## 5. Theoretical and computational studies of glycoconjugates

As has been described on the previous pages, research on glycoconjugates (glycoaminoacids, glycoamines, glycopeptides, glycolipids, etc.), and their biological roles, has made impressive progress in recent years. This has left footprints in virtually all fields of biology and medicine, not only in immunology (e.g. lymphocyte homing), but also in general cell biology, developmental and reproductive biology, neurobiology, and pharmacology. The role of glycoconjugates in biological events has been recognized and glycobiology has emerged as a new and challenging research area at the interface of biology and chemistry [146].

Glycoconjugates have intrigued biologists for decades as mediators of complex cellular events. The glycolipids, glycoproteins, and glycosaminoglycans present at the cell surface, display diversity in glycosylation pattern between species which appear to be driven by evolutionary selection pressures [147] but which also can occur between cell types in the same organism. Modifications of cell glycosylation also occur during cell activation, inflammation, and cancer [148].

With respect to structural diversity, the glycoconjugates have the capacity to far exceed proteins and nucleic acids. This structural variance allows them to encode information for specific molecular recognition and to

serve as determinants of protein folding, stability, and pharmacokinetics. Elucidation of the three-dimensional structures and the dynamical properties of glycoconjugates is a prerequisite for a better understanding of the biochemistry of recognition processes and for the rational design of carbohydrate-derived drugs. Therefore, an understanding of the three-dimensional structure of these compounds is of considerable importance. The three-dimensional structure of these compounds is primarily determined by the conformations about the glycosidic linkage. The conformational behavior of the glycosidic linkage is controlled by a balance of many different interaction energies, of intra- and inter-molecular origin. In solution, the flexibility of certain glycosidic linkages produces multiple conformations which coexist in equilibrium. The use of spectroscopic methods, with appropriate time resolution, is necessary for analysis of the conformational behavior of such molecules [149, 150]. Hence, procedures for molecular modeling of glycoconjugates have been devised as an important tool for structural studies of these compounds. Since the pioneering work of Prof. Lemieux and co-workers [151], various molecular modeling methods have been developed [152] and widely used for the determination of the conformations present in glycoconjugates. The progress made in algorithms and computational power now allows for the simulation of glycoconjugates in their natural environment, i.e., solvated in water or in organic solvent, in concentrated solution, or in the binding site of a protein receptor.

The level of theory employed is a compromise between the known reliability of the method and the computational cost. The latter is strongly dependent on the size of the system and the number of possible structural permutations. In most cases, since many structures will be possible, a typical strategy begins with the generation of a large set of feasible conformers or cluster structures using a molecular dynamics or a Monte Carlo search procedure implemented from a molecular modeling suite, and continues with the optimization of each one using a Quantum Chemistry method, e. g. Semi-empirical approximation, Hartree-Fock method (HF), Møller–Plesset perturbation theory (MP2, MP4), or Density Functional Theory (DFT) [153–158], and standard basis sets of moderate size, e.g. 6–31+G(d). Stationary points, characterized by calculation of harmonic frequencies using analytical second derivatives, yield the vibrational frequencies used to determine zero-point energy (ZPE) corrections and also frequencies and intensities for comparison with experimental IR spectra. Depending upon the level of theory used and the relevant IR spectral region, appropriate scaling factors can be introduced to compare computed frequencies with those actually observed. DFT/B3LYP calculations do not fully take dispersion interactions into account and the relative energies they provide can be unreliable; better



estimates are usually obtained through single-point MP2 calculations using a bigger basis set, typically 6–311++G(d,p). For the larger, more complicated molecules, a second round of conformational searching may be performed using either the results from experiments or the MP2 level relative energies as a guide. Calculations of glycoconjugate conformations in solution are constrained by the additional complexity introduced by interactions with the solvent. When taken together, these limitations represent a very significant challenge to experiment and to reliable molecular modeling.

### 5.1. Molecular dynamic simulations of glycoconjugates

Molecular mechanics potential-energy functions have been developed to describe a variety of systems, such as various small molecules, including the important case of water, simple organic compounds, proteins, and nucleic acids. Unfortunately, because the characteristics of a particular functional group may depend on the chemical environment, it is usually not possible to transfer molecular mechanics potential-energy parameters developed for a specific case to the description of the same group in a different environment. For this reason, potential energy functions and parameter sets developed for proteins or for general organic molecules may not be appropriate for glycoconjugates systems, particularly for the carbohydrates. Several carbohydrate potential-energy functions and/or parameter sets are available in the literature, and these have been used extensively in the past. The following force fields are widely used or some of them have been designed especially for glycoconjugates and carbohydrates.

The MM2 and MM3 force fields are molecular mechanics force fields initially meant for hydrocarbons but now applicable to a wide range of compounds [159-162]. Tvaroska and Pérez published a modified version especially for oligosaccharides called MM2CARB [163]. The GROMOS force field was developed for molecular dynamics simulations of proteins, nucleotides, or sugars in aqueous or apolar solutions or in crystalline form [164] and has been modified to include the *exoanomeric* effect [165]. The CHARMM force field is designed for the modeling of macromolecular systems [166]. Several revisions for carbohydrates have been proposed [167, 168]. Kouwijzer and Grootenhuis redeveloped the CHEAT force field: a CHARMM-based force field for carbohydrates in which a compound in aqueous solution is mimicked by a simulation of the isolated molecule [169, 170]. The AMBER force field was developed for simulations of proteins and nucleic acids [171]. A modification of this, for conformational analysis of oligosaccharides, was made by Homans [172]. Glennon et al. [173] and more recently Momany and Willet [174, 175] presented an AMBER-based force field especially modified for  $\alpha$ -(1→4) linkages. Woods et al. developed the

GLYCAM parameter set for molecular dynamics simulations of glycoproteins and glycol-amino acids that is consistent with AMBER [152]. The AMBER\* version used in the MacroModel package [176] has been expanded with carbohydrate parameters and validated by free-energy calculations on various simple sugars and disaccharides [177]. The consistent force field (CFF), originally a molecular mechanics force field for cycloalkane and *n*-alkane molecules optimized on structural and vibrational data [178], has been developed, in later versions, for other classes of compounds including carbohydrates (PEF95SAC) [179, 180]. The SPACIBA program has been developed as a vibrational force field with particular emphasis on monosaccharides and oligosaccharides [181]. The TRIPOS molecular mechanics force field is designed to simulate both biomolecules (peptides) and small organic molecules [182]. Additional parameters for conformational analysis of oligosaccharides, including sulfated glycosaminoglycan fragments and glycopeptides, were derived by Imberty *et al.* [183, 184]. The CVFF and CFF force fields available from Biosym Technologies have been evaluated for modeling carbohydrates [185]. Recently, methods for deriving class II force fields [186] have been applied to carbohydrates and the parameters incorporated into the CFF force field. The DREIDING force field, developed for the simulation of organic, biological, and main-group inorganic molecules, is one of the newer force fields in this list [187]. The OPLS force field in its first version [188] has been later expanded to include carbohydrates [189]. The Merck Molecular Force Field (MMFF94) has been published [190], it seeks to achieve MM3-like accuracy for small molecules in a combined “organic/protein” force field equally applicable to proteins and other systems of biological significance. A comparison and chemometric analysis of 20 of these molecular mechanics force fields and parameter sets applied to carbohydrates and glycoconjugates has been performed [191].

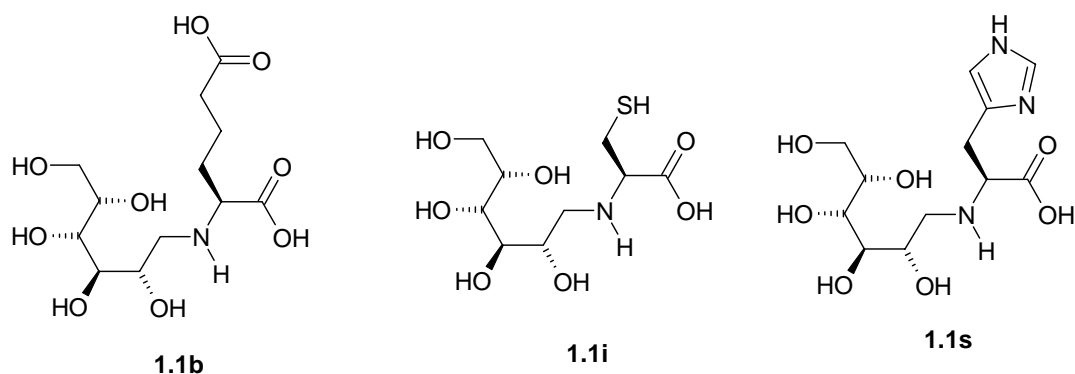
## 6. Physicochemical characteristics of glycoconjugates

In depth understanding of glycoconjugates physicochemical properties is of obvious direct relevance for all the different applications that they have, as well as to identify or molecularly design new ones. In particular, the influence of salt and temperature on self-aggregation and general behavior has a direct influence on dispersing and solubilizing capacity of a given surfactant in various applications. For the case of ionic and nonionic surfactants the correlation between physical factors and the details of self assembling is well-known. However, this knowledge and understanding for the case of glycoconjugates are much less extensive. In this section, we will

focus our review specifically on how does the molecular design of glycoconjugates affects the physicochemical properties and applications and to do so we will rely on some of the relatively new synthesized and studied glycoconjugates reported herein and elsewhere.

Glycoaminoacids have been found to be useful as chelating agents. Thus the potential of these compounds as heavy metal scavengers has been proved for lead intoxication [39] both *in vitro* and *in vivo*. A series of synthesized enantiopure pentahydroxylhexylamino acids, **1.1a-t** [39], have proved their potential as antagonist for lead intoxication. The assays performed *in vivo* confirmed the lowering of the heavy metal contents in treated mice in the liver, kidney, bone, and brain, increasing them in feces and urine, even with equal or better levels as shown for DL-penicillamine, setting this family of glycoconjugates as a model for further molecular design within this type of applications. It seems that the therapeutic efficiency of the lead chelating agents depends on factors that affect the stability constants of the formed lead compounds. The membrane permeability of representative compounds was also evaluated. A critical determining factor for complex stability is the hardness/softness characteristics of electron donors and acceptors. The thiol side chain of the Cys residue in **1.1i** acts as a soft donor ligand binding to soft and moderately soft metal acids, that is  $\text{Cd}^{2+}$  and  $\text{Pb}^{2+}$ , rather than the hard acid metals, that could be  $\text{Fe}^{2+}$  and  $\text{Cu}^{2+}$ . This further confirmed that the sulfhydryl group was very important for the formation of complexes of chelating agents with heavy metals. On the contrary, the carboxylic acid side chain in the Asp residue acts as a hard donor ligand, preferentially binding with hard acid metals, that is  $\text{Cu}^{2+}$  and  $\text{La}^{3+}$  but not  $\text{Pb}^{2+}$ . When taking into account the effect of the length of an acyl chain it was found that the longer more flexible chain of **1.1b** rendered the molecule with a favorable conformation for metal complexation [39]. In general, it was found that the presence of the N-terminal amino and nearby carbonyl group on these new chelating agents is necessary for lead binding. Also, the stability of the complex is enhanced with the increase in the number of donor groups, as is the case of **1.1s** which has two extra nitrogen atoms from the histidyl residue that serve as an additional ligand for metal ion binding. Indeed the side chain of the amino acid moiety plays various important roles, another among the already listed is the formation of supramolecular ensembles within this side chains giving extra stabilization in the glycoconjugate while the scavenging has happened forming more polar substances, thus the excretion is plausible at this stage.

An ideal lead chelating agent should possess greater affinity for the toxic metal, low toxicity and rapid elimination of the metal, high water solubility, and capacity to penetrate the cell membrane, oral administration, and minimal



**Figure 6.1.** Glycoconjugates as heavy metal scavengers.

toxic metabolites. For successful lead chelation, the scavenging agent must be capable of entering the cell and mobilizing the metal and eliminating it without accumulation in the kidney or distribution to other sensitive organs. The membrane permeability of the chelating agents not only affects the absorption and transportation of the chelating agents but also impacts the metal decorporation potency of chelating agents. Hydrophilic chelators most effectively promote renal metal excretion, but they inefficiently form complexes with intracellular deposits. Lipophilic chelators can decrease intracellular stores but may redistribute toxic metals to other organs, for example, the brain. It seems that the optimum structure for balancing the lead-mobilizing activity, stability, and bioavailability require some compromise of lead mobilizing activity. In that study, compounds **1.1b**, **1.1j**, **1.1m** and **1.1s** possess a balanced combination of stability, lipophilic and hydrophilic (the glucose stereochemistry in these cases) groups, and a potent cell permeability and were found to be highly effective in mobilizing lead from the liver and kidney with preferential elimination through bile without carrying it to the brain [39].

Alkyl-glucosides and -maltosides are one of the most studied glycoconjugates so far. An extensive review of related derivatives can be found elsewhere [9]. Recently, Ericsson *et al.*, [94] studied the influence of salt, temperature, and deuterium oxide on the self-aggregation of n-nonyl- $\beta$ -D-glucoside (**2.1b**) in dilute solution by static and dynamic light scattering, neutron scattering, and tensiometry. Deuterium effect was used since it has been observed that it has unexpected and dramatic effect on micelles of some alkylglucosides. The use of **2.1b** was supported on the fact that it represents a border line between discrete and infinite micellar systems formed in dilute solution by **2.1a** and **2.1c**, respectively. Consequently, it was reasonable to assume that this surfactant was a key molecular entity for the understanding of the micellization of the alkylglucosides as a class.

The scattering data obtained show that the micelles can be described as relatively stiff, elongated structures with a circular cross section. With a decrease of temperature, the micelles grow in one dimension, which makes it surprising that the CMC shows a concomitant increase. On the other hand, substitution of D<sub>2</sub>O for H<sub>2</sub>O causes a large increase in micelle size at low temperatures, without any appreciable effect on CMC. With increasing temperature, the deuterium effect on the micelle size diminishes. The effects of salt on the micelle size and CMC were found to follow the Hofmeister series. Thus, at constant salt concentration, the micelle size decreased according to the sequence SO<sub>4</sub><sup>2-</sup> > Cl<sup>-</sup> > Br<sup>-</sup> > NO<sub>3</sub><sup>-</sup> > I<sup>-</sup> > SCN<sup>-</sup>, whereas the effect on CMC displays the opposite trend. Here, I<sup>-</sup> and SCN<sup>-</sup> are salting-in anions. Similarly, the effects of cations decrease with increasing polarizability in the sequence Li<sup>+</sup> > Na<sup>+</sup> > K<sup>+</sup> > Cs<sup>+</sup>. At high ionic strength, the systems separate into two micellar phases. With these results, the authors concluded that the particular size of **2.2b** micelles is extremely sensitive to changes in the headgroup size. More specifically, temperature and salt effects on effective headgroup size, including intermolecular interactions and hydration water, are suggested to be more decisive for the micelle morphology than the corresponding effects on unimer<sup>4</sup> solubility [94].

According to this line of reasoning, temperature effects on headgroup hydration and hydrogen bonding increase the headgroup size with increasing temperature, which tends to favor smaller aggregates. This effect counteracts and overruns the effects of decreasing unimer solubility, which would tend to favor larger aggregates at higher temperatures. Data from systems in which deuterium oxide was substituted for water support the idea that even moderate changes in headgroup size give rise to substantial effects on micelle size. The O-D bond is somewhat shorter than the O-H bond and in average, the headgroup is therefore expected to be smaller in D<sub>2</sub>O than in H<sub>2</sub>O. This effect provides light to explain the observation that the micelles are considerably larger in the former solvent. Tensiometric data show that it is less attractive to explain this pronounced deuterium effect in terms of unimer solubility, since the CMC is the same in the two solvents [94].

In order to test to what extent these conclusions are applicable to alkylglycosides as a class Ericson, et al. [93], did a similar study on n-tetradecyl-β-D-maltoside (**2.1f**). The results showed that micelles ensemble with maltosides are as sensitive to external factors as glucoside ones. Just as

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<sup>4</sup> The term unimer must not be confused with monomer, e.g. “The free or unassociated surfactant is referred to in the literature either as ‘monomer’ or ‘unimer’. In this text we will use ‘unimer’ and the term ‘monomer’ will be restricted to the polymer building block.” taken from page 3 of reference [192].

for **2.1b**, **2.1f** shows that a relatively minor increase of hydrophobicity within a given homologous surfactant series (i.e. going from **2.1e** to **2.1f** or from **2.1a** to **2.1b**) may lead to a dramatic amplification of the effects of temperature, salt, and deuterium oxide. Compound **2.1f** forms flexible polymer-like micelles with an elliptical cross section. Exchange of D<sub>2</sub>O for H<sub>2</sub>O and addition of salting-out salts lead to increasing size of the micelles, whereas salting-in salts give the opposite effect. In qualitative terms, these effects exactly parallel those previously observed for **2.1b** micelles and can be attributed to effects on micelle size, on the other hand the difference between **2.1f** and **2.1b** is striking. Here, they observed that **2.1f** micelles increase in size with temperature, whereas **2.1b** micelles show the opposite behavior. The difference cannot be rationalized as effects of temperature on unimer solubility (CMC). Rather, it can be understood as a larger relative importance of temperature effects on the hydrocarbon chain for the longer-chain surfactant. Consequently, results from the study of **2.1f** substantiate the conclusion that effects on unimer geometry are much more important for the micelle size of alkylglucosides and alkylmaltosides than are those on unimer solubility [93].

It is worth emphasizing that an increase of the alkyl chain length by two carbons (i.e. going from **2.1e** to **2.1f**) leads to transitions from near spherical micelles with little temperature dependence to wormlike micelles that grow dramatically with temperature. This delicate dependence of physical properties on molecular structure is equally evident for alkylglucosides. For instance, the **2.1b**/water system has a wide micellar domain, whereas the corresponding part of the **2.1c**/water phase diagram is dominated by phase separation into two micellar phases. Therefore, the results of the present work highlight the pronounced sensitivity of alkylglycoside micelle morphology and phase behavior on surfactant molecular structure [93].

Another property that has been found for many simple sugar derivatives is that in general they can possess mesogenic, liquid crystal, phases. This is achieved since most of the compounds are amphiphilic, i.e. with a hydrophilic sugar head group and a hydrophobic tail. The types of liquid crystalline phases formed can differ greatly depending on the molecular structure of the sugar counterpart. The effects of stereochemistry of the sugar moiety and of the alkyl chain length on liquid crystalline behavior were examined on thiol sugar derivatives by van Doren *et al.*, [85, 193]. The richness of stereochemical variation obtained by these authors using simple monosaccharides is illustrated with compounds **2.3a-k**, see Figure 2.4. On these compounds, at chain lengths of R of 6-8 carbon atoms, dependent on the sugar, smectic A liquid crystalline phase was systematically observed except for compounds **2.3j** and **2.3k** that presented discotic phases. In a

smectic phase the individual molecules are arranged parallel to each other to form layers and in the case of amphiphilic sugar derivatives the layers are arranged so that the sugar moieties can contact each other to form intermolecular hydrogen bonds. Subcategories of smectic arrangements are in essence the consequence of various tilting or additional ordering within the layers. In the discotic phase, columns are formed in which the head groups form the cores. The hydrophobic tails extend out in a rather disorganized fashion so that a disk-like arrangement is formed. In the case of compounds **2.3j** and **2.3k**, the observed average distance between the columns suggest that the tails of different disks are locked into each other [85, 193].

From these investigations and the study of various carbohydrate derivatives, with even more variation in the size and number of alkyl chains, it became clear that their mesogenic behavior can be predicted on the basis of the relative sizes of the hydrophilic and hydrophobic parts present in the molecule, e.g. as their ratio. Derivatives with only one alkyl chain (nearly) always present smectic A phases, analogous to the lyotropic lamellar phases, whereas most derivatives with two alkyl chains will form columnar hexagonal phases (compared to the inverted hexagonal-HII-phase). Also, the authors were able to show that increasing the size of hydrophilic head groups may eventually again lead to columnar hexagonal phases, similar to the “normal” hexagonal-HI-phase [85, 193].

Van Doren and colleagues also studied a series of sugar surfactants to evaluate their molecular structure on the CMC. Glucose, lactose, glucitol and lactitol sugar molecules were studied with different chain lengths. The CMC obtained have the same order of magnitude ( $10^{-2}$  to  $10^{-4}$  M) as generally shown by nonionic surfactants. The CMCs decrease regularly by roughly a factor of ten on increase of the length of the alkyl chain by two methylene groups, similarly to what it is shown by polyethoxylated surfactants and other nonionic surfactants. The length of the alkyl chain is thus the major determining factor for the order of magnitude of the CMCs. Other factors such as the head group size (monosaccharide vs. disaccharide), shape (cyclic, acyclic or combination thereof), length of the chain have a smaller influence on the CMC [85, 193]. Moreover, the configuration of the stereogenic centers and number of hydroxyl groups, e.g. glucose-derived surfactants have lower CMCs than the lactose derived ones, owing to the smaller hydrophilic head group and, as a consequence, relatively larger hydrophobic unit. Surfactants with reduced saccharide head group (going from hexoses to pentoses) have smaller CMCs.

One of the most innovative applications of sugar surfactants is as solubilizers in pharmaceutical formulations. To assess this task different sugar based surfactants (**2.6a-d**) derived from glucose and (R)-12-hydroxy-stearic

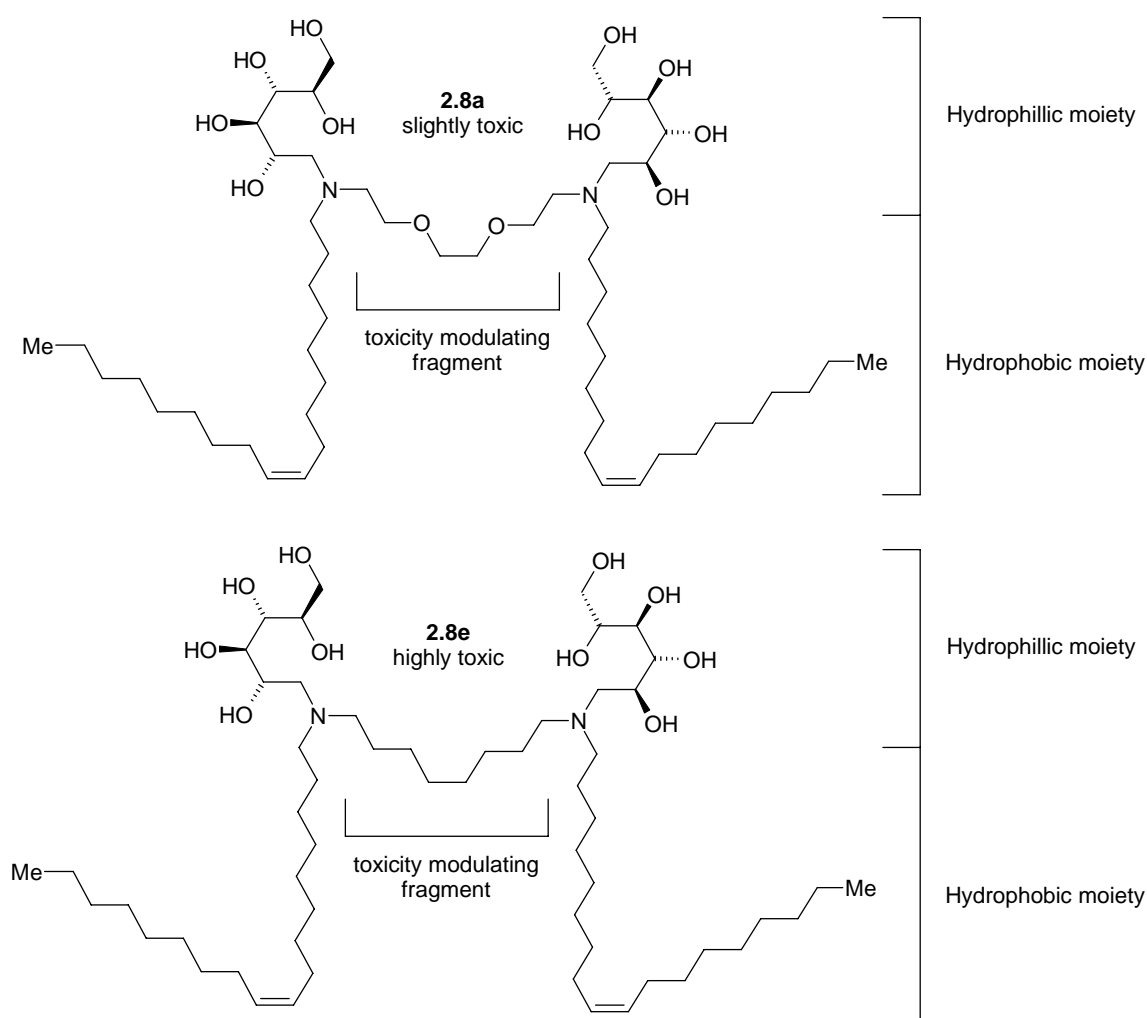
acid were synthesized [100]. The surfactants have an oxygen bearing group in the hydrophobic part, which is either free or acylated using acetyl chloride, hexanoyl chloride, or myristoyl chloride, see figure 2.6. In this case, the authors considered that in order to increase the water solubility of glucamide type surfactants the tendency of these surfactants to crystallize should be decreased and also that the large hydrophobic group should be balanced with a sufficiently large water-soluble head group. To meet both of these requirements, dicephalic surfactants with sugar based head groups were synthesized and studied. Although the CMCs obtained for surfactants 1-3 are in the same range as other sugar-based dicephalic surfactants, they are higher in comparison to the CMCs for poly-ethylene oxide based surfactants with the same hydrophobic part (approximately  $1^{-5} \times 10^{-6}$  M). Also, it was found that surfactants 1-3 are all hemolytic close to their respective CMC indicating that their use as parenteral formulations may be limited. Nevertheless, because of their large hydrophobic group they are expected to have a higher solubilization capacity compared to other previously studied sugar surfactants [100].

In this context, the hemolytic activity of a number of maltopyranoside surfactants have been studied, see figure 2.7 [7]. It was found that alkyl maltopyranosides become more hemolytic the longer the alkyl chain becomes. Branching or presence of cyclic groups clearly decreases hemolytic activity, but it also increases the CMC. As a result, the cyclic or branched surfactants do not become better solubilizing excipients than the straight-chain surfactants. The most useful surfactant for pharmaceutical applications appears to be tetradecyl maltopyranoside, which is the least hemolytic surfactant relative to its CMC [7].

Recently, the group of Engberts has investigated the use of a novel class of pH sensitive sugar-based gemini surfactants as DNA transfection vectors [103]. Five different surfactants were employed, **2.8a-e**, and their structural characteristics are shown in figures 2.8 and 6.2. All compounds contain (unsaturated) oleoyl hydrocarbon tails that are well known fluidizing hydrocarbon chains. The extended head group is constructed via a bis-ethylene oxide bridging or spacer moiety (**2.8a**, **2.8b** and **2.8d**) or a C6 aliphatic ones (**2.8c** and **2.8e**) comprising either double ended reduced mannose or glucose carbohydrates thus present as their open chain forms. The two amino moieties in the head group of **2.8a-c** and **2.8e** are weak bases and are fully protonated at mild acidic pH, whereas the non-titrable amido linkage in **2.8d** gives rise to a net neutral charge. The surfactants with amino groups were studied and it was shown that at physiological pH, these compounds form bilayer vesicles, but they undergo a lamellar-to-micellar phase transition in the endosomal pH range as a consequence of an increased



protonation state. In the same way, lipoplexes made with these amphiphiles exhibit a lamellar morphology at physiological pH and a non lamellar phase at acidic pH. This characteristic was used to investigate the mechanism of Gemini-mediated transfection, considering that this property would convey colloidal stability to Gemini lipoplexes before cellular uptake, while exerting destabilizing properties necessary for gene delivery only after internalization within mild acidic endosomal compartments. The results showed that surfactants **2.8a-c** and **2.8e** can efficiently form complexes with plasmid DNA and mediate transfection *in vitro*. The effect of head group changes among glucose and mannose, or the effect of the spacer, C6-alkyl versus bis-ethylene oxide does not appear to modulate the level of transfection to a significant extent. Surfactants **2.8a**, **2.8b** and **2.8c** all show a transfection efficiency of approximately 70%. However, surfactants with aliphatic C6 spacer showed a relatively high toxicity [103]. One important aspect in the molecular



**Figure 6.2.** Molecular design of gemini glycolipids to optimize two properties/ activities.

design is that the molecular shape of the monoprotonated Gemini surfactant is such that it conveys a higher colloidal stability than similar complexes formed by different cationic amphiphiles. The preferred morphology of the Gemini lipoplex at neutral pH is lamellar. In contrast, cationic amphiphiles are often characterized by a packing parameter (relatively small head group versus relative large hydrocarbon tail area) that leads to the formation of lipoplexes organized in inverted phases. As lipoplexes with a lamellar organization are less prone to aggregation than lipoplexes that have adopted an inverted hexagonal phase, such a property would preclude them from accumulating in the lungs, as commonly seen for cationic delivery vehicles [103].

## **7. Present applications, future outlook and www resources**

In this final section we intend to underline and make clear the advantages of glycoconjugates besides typical chemical products nowadays yet employed in many applications, hence the importance of the study of this sugar based promissory molecules and the present applications and future outlook of them. As well we cite some of the new www resources available at this moment that could be useful to the reader, graduate and undergraduate researchers. This citing of electronic resources is as a result of self experience in research directed to glycoconjugates. Also they were selected in order to direct the sight of novel students in this interesting field whose new efforts and design through electronic/experimental tools in the employment of these green substances will provide the new generation of glycoconjugates. Please go deep within this final gathering of information.

The great importance of sugar-related compounds and the rapidly increasing number of glycoproteins with structure known at atomic detail has made necessary the existence of an annotated data bank called Glycoconjugate Data Bank (build from a subset of the Protein Data Bank, PDB), where crystallographic structures can be freely retrieved or analyzed ([www.glycostructures.jp](http://www.glycostructures.jp)) [194]. Also, the Kyoto Encyclopedia of Genes and Genomes (KEGG), has a special section for glycome informatics ([www.genome.jp/kegg/glycan](http://www.genome.jp/kegg/glycan)) [195] including a set of different tools to integrate genomics and chemistry and a Data Bank for carbohydrate structures equivalent to the PDB for proteins. Another web site with useful tools for bioinformatics with glycoconjugates is [www.glycosciences.de](http://www.glycosciences.de), where one of them is GlycoMapsDB to explore the accessible conformational space of glycosidic linkages [196]. An additional effort, in this case a thoroughly funded and composed set of not only electronic but also experimental research utilities and contacting info was shuttled by the Nature

group (<http://www.nature.com/>). It is the Center of Functional Glycomics (CFG) that is reachable in <http://www.functionalglycomics.org/>. CFG is a large research initiative composed of more than 300 Participating Investigators and seven scientific Core laboratories. This electronic set of tools was funded by the National Institute of General Medical Sciences (NIGMS, <http://www.nigms.nih.gov/>) in 2001 in order to determine the roles of glycoconjugates of carbohydrate-protein type and their most important interactions. Specialty databases for glycan-binding proteins, glycan structures, and glycosyltransferases are also available at this site in order e.g. to determine the structure (branching, number of attached monomers, stereochemistry, etc.) of the N- or O-linked or free glycosides and some other important characteristics. The available resources at this stage are present in <http://www.functionalglycomics.org/static/consortium/resources.shtml>, and they comprise i) Carbohydrate Compounds, Glycan-binding Proteins and Antibodies; ii) Glyco-gene Chip Analysis; iii) Glycan Analysis; among others.

Simple glycolipids such as **2** type derivatives have resulted useful for the obtaining of high-quality crystals for X-ray diffraction of membrane proteins that until now has proven to be a very difficult and serendipitous task to achieve its optimization [197]. Persson, et al. recently presented a methodology comprising the employment of 1-monooleoyl-rac-glycerol for the successful crystallization, besides, the careful taking of the proteins from their native environment requires the use of surfactants, in this particular case, the employment of **2.1a** was reported as a superlative example of a phase transition reagent in order to obtain suitable crystals for the diffraction studies.

Regarding new therapeutic treatments involving glycoconjugates, a recent study demonstrated that oral administration of a glucosylceramide in mice can be delivered to skin to overcome chronic or acute perturbations on skin barrier functions [198], opening the possibility of novel oral therapies based on glycoconjugates to deal with other sicknesses. Also, liposomes coated by chitosan can protect oral drugs from the gastric fluid [199-201]. Glycolipids also are implicated in infectious processes and can modulate protein conformation being also involved in HIV infection, prion propagation, and amyloid aggregation in Alzheimer's and Creutzfeldt-Jakob's diseases [202], leading a promising future of this group of glycoconjugates as therapeutic targets or drug leads for treatment of human diseases [203-205] and a wide range of applications in medicine [206, 207].

Biosurfactants on the other hand, are emulsifiers or detergents synthesized by living cells, and a special type of those is glycolipids. Due to their diversity, non-toxicity and biodegradability they are involved in an increasing number of applications: from emulsification of hydrocarbons [208, 209] and complexation of heavy metals in bioremediation of contaminated

sites [210]; passing through pesticide and herbicide solubilization and formulations [211]; to a vast variety of uses in cosmetic, food, textile, paper and ceramic industries [212, 213]. Research on the biological production in high scale of specific glycolipids is a very active biotechnological field [214]; these studies have allowed the discovery of specific strains of microorganisms with efficient production of such compounds, for example for different types of mannosylerythritol lipids [215]. Another group of glycolipids produced by bacterium *Pseudomonas aeruginosa* known as rhamnolipids possesses good detergent properties as well as a high foaming rate, used in oil recovery in petroleum industry [216] and currently under investigation as additives in lung surfactants for medical applications [217].

Other important application has been developed in the field of corrosion inhibition means as glyco based corrosion inhibitor formulations [218-220] (instead of the thoroughly molecular designed imidazoline based prototypes [221]). There have been reported glycoconjugates with potential in the corrosion inhibition of dental pieces as well [222]. Additionally into the formulation of high added value chemical products, the preparation of biodiesel starting from simple glycosides through coupling, condensation, microwave assisted [223] among other chemical synthesis has been also pointed out [224, 225].

The versatility of the glycoconjugation at this stage is astonishing, hence the future outlook of this platform of new green (bio)chemicals as useful scaffolds can be aimed in the obtaining of new renewable fuels, pharmaceuticals with diminished side effects and very low dosages even at homeopathic level, new conjugations to improve physicochemical properties such as the liquid crystal behavior, as templates for the preparation of functionalized catalysts, as green corrosion inhibitors and metal scavengers, as simpler glycomimetics resembling peptidomimetic molecules and thus as high throughput prototypes of cheaper drugs, in the roots of the generation of knowledge/patterns/memory related to the complex and coherent signaling pathways of organisms, in the molecular recognition, selectivity and specificity level of molecular interactions, in the prognosis and diagnosis of sicknesses at molecular level such as HIV, cancer, diabetes, aging, etc., in the reconstitution of damaged DNA and thus enhancement of longevity and effective cloning of partial and complete organisms, as well as other promising technological/biological advances not sight seen at this stage yet.

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