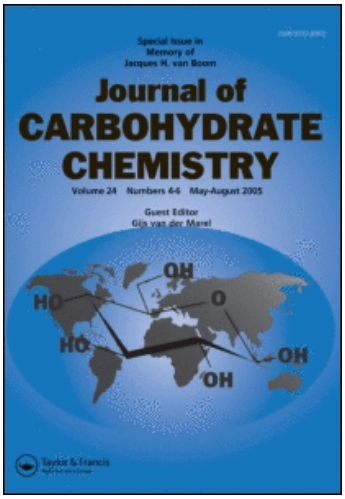

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REVIEW

MODIFICATION OF GLYCOSYLATION AS A THERAPEUTIC STRATEGY[1]

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1. INTRODUCTION

Glycoconjugates play important roles in many cellular and physiological processes, including development, differentiation, cell to cell contact, formation of the intracellular matrix, recognition of microorganisms, macromolecules and small molecules and intracellular transport of proteins.[2,3]

Therefore, it is essential that each cell type can synthesise and maintain the appropriate range of glycoconjugates. Failure to do this as a result of a genetic defect or malignant or viral transformation will lead to abnormal function and disease. Many enzymic steps are involved in the biosynthesis and catabolism of glycoconjugates and the regulation of these processes. Modulation of the activity of these carbohydrate-recognising enzymes using analogues of the relevant carbohydrate has enormous therapeutic potential. Sugar analogues have been used to modify protein glycosylation by altering the supply of precursors, inhibiting processing or catabolism. They have also been used to decrease the rate of synthesis of glycolipids in the glycosphingolipidoses (lysosomal storage diseases). Recombinant therapeutic enzymes can be targeted to specific cell receptors by enzymic modification of their glycosylation.

2. OVERVIEW OF GLYCOSYLATION

The repertoire of glycans that can be synthesised by a cell, the glycotype, varies from one cell type to another, but the overall scheme for the biosynthesis and catabolism of glycoconjugates is remarkably similar in all mammalian cells (Fig.1). The different glycotypes result from the expression of different combinations of glycosidases and glycosyltransferases. The complement of enzymes expressed is determined genetically but may be modulated within a cell type by physiological signals. The fidelity of biosynthesis of individual glycans is maintained by regulation of the expression of these enzymes and not by synthesis on a template as is the case for proteins and nucleic acids.

The immediate donors of glycosyl residues for the biosynthesis of most glycoconjugates, e.g., glycolipids, glycosaminoglycans and the *O*-linked glycans of glycoproteins, are nucleoside phosphate sugars, which are formed from monosaccharide-1-phosphates. Intracellular monosaccharides, which may be of exogenous (dietary) or endogenous origin, can be interconverted to provide an appropriate supply of these monosaccharide-1-phosphates. In contrast, the asparagine *N*-linked glycans of glycoproteins are formed by the transfer *en bloc* of a common oligosaccharide precursor from a lipid carrier to the nascent protein in the lumen of the endoplasmic reticulum. The oligosaccharide precursor is assembled on dolichol pyrophosphate by the stepwise addition of single glycosyl residues from nucleoside phophate sugars and dolichol-phosphate monosaccharides (Fig.2). After being subjected to a quality control test in the endoplasmic reticulum, the glycoproteins are transported to their sites of action via the endoplasmic reticulum and Golgi apparatus. During this intracellular journey the *N*-linked glycans are processed to the vast array of glycans found in mature glycoproteins by glycosidases and glycosyltransferases located in the endoplasmic reticulum and Golgi apparatus. Over 30 enzymes can be involved in the synthesis of a particular *N*-linked glycan from simple monosaccharides.

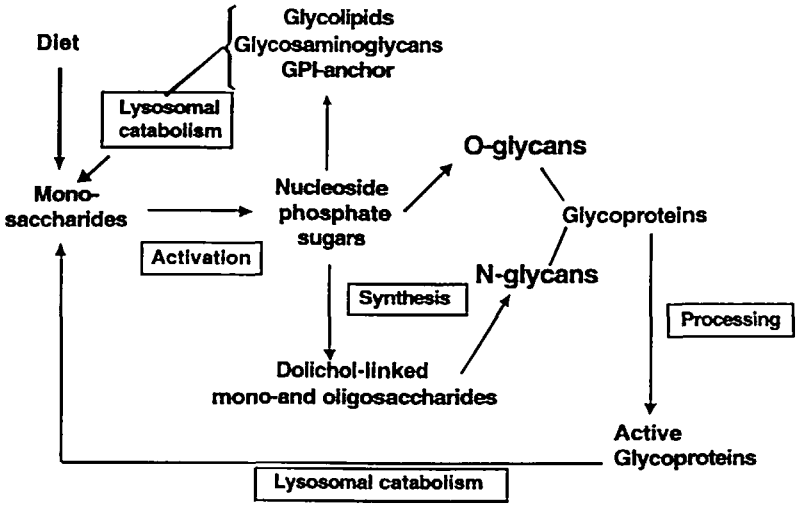


Figure 1. Overview of glycosylation

Glycoconjugates are continually being turned over to maintain the appropriate glycosylation for a cell or tissue. Catabolism of glyconconjugates takes place predominantly in lysosomes, which contain a cocktail of enzymes capable of degrading all naturally occurring glycoconjugates to their monomeric constituent components.[4] The resultant monosaccharides derived from glycoconjugates can pass through the lysosomal membrane for reutilisation by the cell for biosynthetic or energetic purposes.

Cellular glycosylation is a dynamic process with the rate of synthesis and breakdown of the different classes of glycoconjugates being regulated by complex mechanisms, which are not very well understood. A genetic defect in an enzymic step in the glycosylation pathway will lead to abnormal or lack of glycosylation of a group of glycoconjugates, with consequent change or loss of function. These changes may be incompatible with life or lead to specific clinical symptoms.

3. THERAPEUTIC STRATEGIES

Modification of glycosylation may be exploited therapeutically in two general ways.

3.1 Modification of abnormal endogenous glycosylation

It may be possible to bypass a defective step in glycosylation by providing alternative precursor molecules or by supplying a molecule that stimulates an alternative

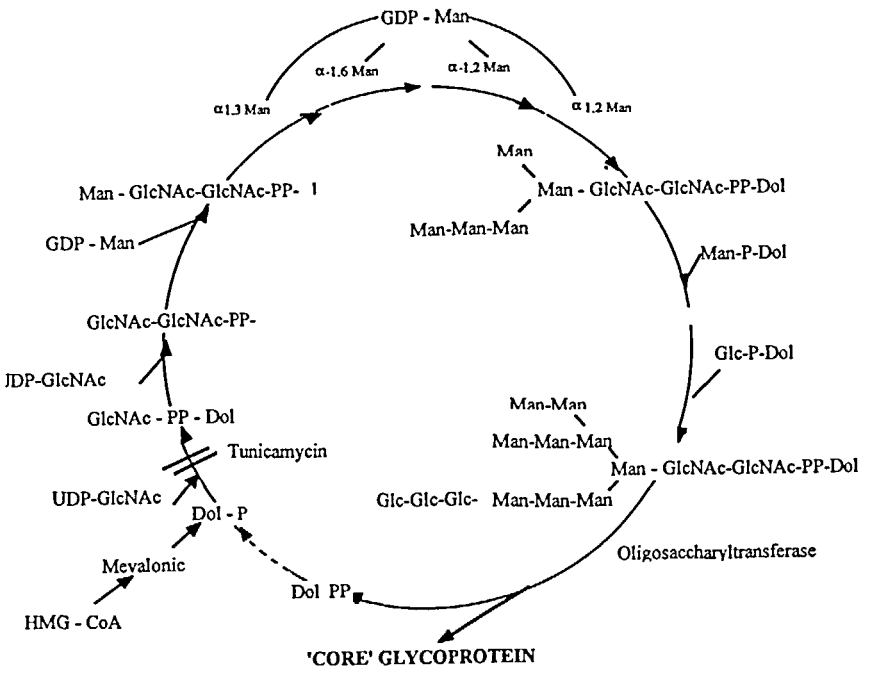


Figure 2. Assembly of common oligosaccharide precursor

pathway. In cancer the regulation of the expression of glycosyltransferases is often disrupted leading to abnormal glycosylation of many proteins,[5] which may contribute to the disease but also be used as a diagnostic marker. It may be possible to prevent or decrease this abnormal glycosylation by inhibiting the overexpressed glycosyltransferase directly or inhibiting an earlier enzyme in the pathway thereby depriving it of its substrate.

3.2 Modification of normal glycosylation

It may be beneficial to modify the normal glycosylation pattern of cells for therapeutic purposes. Transfection of cells with viruses results in viral proteins with the host cell pattern of glycosylation. Changing the host cell glycosylation may prevent the replication or infectivity of the virus. Inducing changes in the normal cell surface glycosylation may decrease binding of infectious organisms or affect the immunological properties of the cell or its constituent proteins and glycolipids.

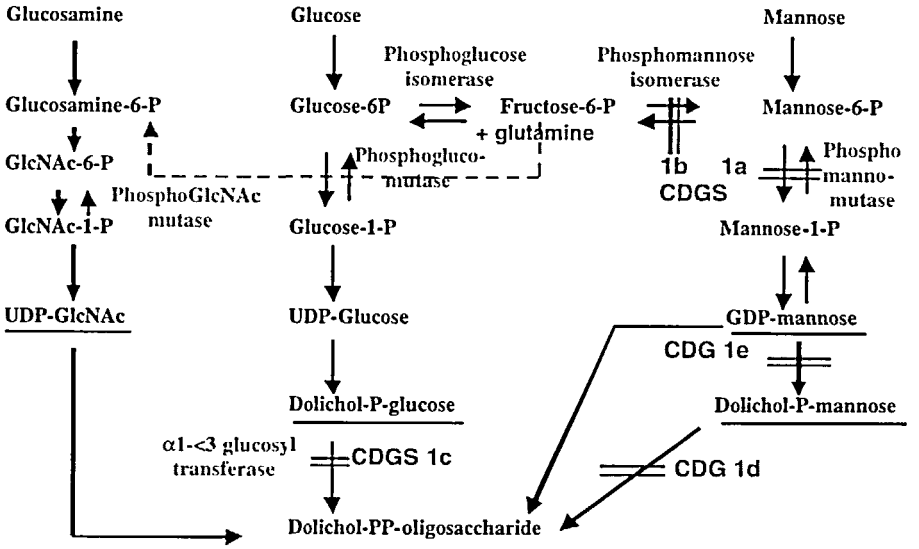


Figure 3. Assembly of glycosylation precursors

4. MODIFICATION OF PROTEIN GLYCOSYLATION

It is convenient and logical from a metabolic point of view to separate the *N*-glycosylation process into three parts when considering strategies for its modification: (1) assembly of glycosylation precursors, (2) processing of *N*-linked glycans and (3) catabolism.

4.1 Assembly of glycosylation precursors

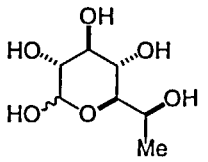
Several genetic metabolic diseases have been shown to result from defects in the assembly of glycosylation precursors (Fig 3). The carbohydrate-deficient glycoprotein syndromes (CDG) are characterised biochemically by the undersialylation of serum proteins.[6] This aberrant glycosylation leads to a multisystem disease, which is often fatal in childhood. In CDG type I the undersialylation is due to non-occupancy of *N*-glycosylation sites resulting from an inadequate supply of the common lipid-linked oligosaccharide

precursor. Five different enzymic defects have been discovered in these patients: phosphomannomutase (CDG-1a),[7] phosphomannose isomerase (CD-1b),[8] α1>3 glucosyltransferase (CD-1c),[9] α1->3 manno syltransferase (CDG-1d) and dolichol-P-mannose synthetase (CDG-1e).

Currently there is no treatment for CDG 1a,1c, 1d and 1e but some cases of CDG1b have responded to increased dietary mannose, which can be converted directly to GDP-mannose and dolichol-P-mannose, thereby bypassing the block in the formation of mannose-6-phosphate from glucose.[8] It had been assumed that the mannose in glycoconjugates had been derived predominantly from the most abundant monosaccharide, glucose. The fact that only certain cell types are affected in CDGS 1b shows that some cells normally use mannose directly. Furthermore, the simple therapeutic strategy shows that even those cells that normally derive most of their mannose from glucose can utilise an alternative pathway if the concentration of mannose is increased. This illustrates an important aspect of therapy for glycosylation defects, the use of alternative pathways. Understanding the origin of the glycosyl residues in different glycoconjugates in different cells will be essential for developing therapies for these disorders.

An obvious analogous therapy for CDGS1a would be to supply dietary mannose-1-phosphate or dolichol-P-mannose. However charged sugar phosphates are not taken up by cells.

We have discovered that *6S*- and *6R*-methylglucose **1** and **2** and *6S*- and *6R*-methylmannose **3** and **4** are specific inhibitors of the phosphohexose mutases in the pathway for the interconversion of monosaccharide phosphates.[10,11] As they are neutral molecules they should permeate the plasma membrane. They are being used to measure the flux of precursors in cells grown in the presence of different monosaccharides to try to identify alternative pathways which might be exploited therapeutically.



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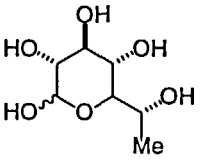

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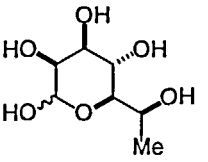

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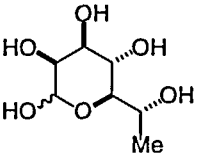

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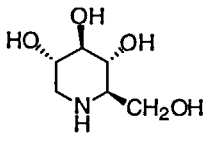
4.2 Processing

The preliminary steps in the processing of *N*-linked glycans are catalysed by α-glucosidases and α-mannosidases located in the endoplasmic reticulum and Golgi apparatus. These glycosidases are susceptible to selective inhibition by aminosugars **5-10**, which bear a strong structural resemblance to the glycosyl moieties of the bonds hydrolysed by the glycosidases.[12,13] This inhibition can be used to block the processing

at a particular stage, thereby altering the structures and properties of cellular glycoproteins. These inhibitors have been used extensively to investigate the function of the glycosylation in whole cells, on individual proteins and at specific glycosylation sites and to alter glycosylation for therapeutic purposes.[12,14]



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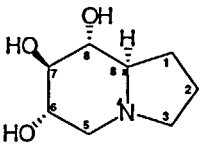

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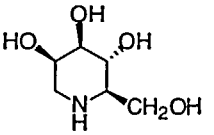

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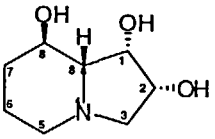

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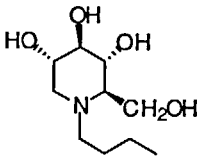

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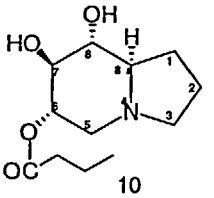

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Anti-viral activity

The α-glucosidase inhibitors, deoxynojirimycin (DNJ) **5** and castanospermine **6** and their derivatives **9,10**, but not the α-mannosidase inhibitors deoxymannonojirimycin and swainsonine **7** and **8**, have been found to inhibit replication of the human immunodeficiency virus-1 (HIV-1) in cultures of CD4+ T cells and to prevent syncitium formation at concentrations non-cytopathic to lymphocytes.[15,16] This was presumed to be due to disruption of processes mediated by processed viral glycoproteins. These encouraging results *in vitro* led to human clinical trials. The first compound to be tested was the *N*-butyl derivative of DNJ **9**[17] which was taken up into cells more readily than DNJ **5**. The efficacy of zidovudine (AZT, 3`-azido-3`-deoxythymidine) alone was compared with it in combination with *N*-butylDNJ in a phase II clinical trial.[18] Diarrhea due to inhibition of intestinal glucosidases by the *N*-butylDNJ complicated interpretation of the results but the decrease in antibodies against some viral glycoproteins suggested that *N*-butylDNJ did have anti-HIV activity *in vivo*. A pro-drug, the perbutyrylated ester of *N*-butylDNJ, which is hydrolysed to *N*-butylDNJ *in situ,* was developed to overcome this problem. [15]

A comparison of the anti-HIV activity of a series of castanospermine analogues revealed that the pro-drug 6-*O*-butanoylcastanospermine **10** (BuCast, MDL 28574) was 20-30 times more active than castanospermine despite having an IC_{50} 10 times greater

than castanospermine for inhibition of α-D-glucosidase.[19,20] The expression of the integrin LFA-1 (CD 18/CD 11a), which may decrease cell adhesion of uninfected mononuclear leukocytes[21] is also decreased by BuCast and this may play a role in the prevention of transfer of HIV-1 from cell to cell.[22] BuCast, may have several other important applications. Oral treatment of mice infected with herpes simplex virus 1 with BuCast decreased infection in the brain and delayed development of lesions.[23] It also blocked growth of herpes simplex virus-2.[24] The basis of the activity of castanospermine against herpes simplex virus is probably the blocking of the association of herpes simplex viral glycoproteins with calnexin.[25]

The precise mechanism by which α-D-glucosidase inhibitors decrease HIV-infectivity is not known but experiments with *N*-butyldeoxynojirimycin **9** show that it impairs viral entry at a post-CD4 binding step.[26]

Anti-cancer

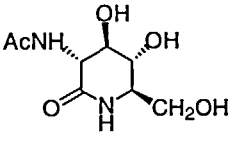
Many tumour cells display aberrant glycosylation due to altered expression of glycosyltransfeases.[5,27] Tumour progression is often associated with increased β1-6 branching of the trimannosyl core of *N*-linked glycans, which is a key step in the formation of polylactosamine and extended chain Lewis antigens.[28] Prevention of this branching by mutations or inhibition of processing with castanospermine **6**[29] or swainsonine **8**[30,31] decreases tumour growth and metastasis. The antitumour activity of swainsonine, which inhibits Golgi α-mannosidase II, has been extensively investigated in murine experimental tumour systems.[32] On the basis of these results, clinical trials were carried out using intravenous infusion[32] and oral administration[33] of swainsonine. Although some patients showed symptomatic improvement there were considerable side effects attributable to the drug, including edema, liver dysfunction and in addition fatigue and neurological effects with oral administration. Further investigation of the pharmacokinetics of swainsonine and the effects of derivatives of swainsonine are in progress.

Swainsonine has also been shown to modulate the immune system.[34,35] The proliferation of natural killer cells is increased at sub-optimal concentrations of interleukin2 and murine bone marrow is protected from radiation and chemotherapy when swainsonine is present. It has been suggested that lymphocytes are more responsive to cytokines in the presence of swainsonine.[36] The alteration in the expression of cell surface glycoproteins induced by castanospermine may also be useful in modulating the immune response. The reduction in the expression of adhesion molecules can prolong heart allograft survival in rats[37] and pancreaticoduodenal allograft survival is prolonged in an experimental form of treatment for diabetes in rats.[38] Castanospermine can also act synergistically with other drugs in prolonging allografts.[39,40]

4.3 Catabolism

The intracellular breakdown of glycoconjugates in lysosomes is catalysed predominantly by hydrolases, including glycosidases, that have acidic pH-optima. Many aminosugars are also potent and specific inhibitors of the lysosomal glycosidases. Paradoxically, ingestion of the potent α–mannosidase inhibitor, swainsonine **8**, by grazing animals leads to induction of a phenocopy of the lysosomal storage disease, α–mannosidosis.[41] As swainsonine is a weak base (pKa, 7.4), it accumulates in lysosomes and inhibits the lysosomal α–mannosidase *in situ*, mimicking the genetic deficiency of α–mannosidase which is the cause of α–mannosidosis.[42] Although the inhibition of lysosomal glycosidases by aminosugars has been a very powerful tool for studying the catabolism of glycoconjugates and enzyme replacement therapy for lysosomal storage diseases, the complication of induced lysosmal storage must be taken into account when using these compounds for therapeutic purposes.

The levels of secreted lysosomal glycosidases, particularly β-hexosaminidase, are elevated in serum in many malignant diseases.[43] It is believed that these glycosidases and other hydrolases such as proteases are secreted by the transformed cells to digest basement membranes to facilitate tumour cell invasion during metastasis.[44] A correlation between secretion of hydrolytic enzymes and degradaton of the extracellular matrix has been shown for human ovarian carcinoma (HOC) cells.[45] The *N*-acetylglucosamine analogues, 2-acetamido-2-deoxy-1,5-gluconolactone **11** and 2-acetamido-2-deoxy-1,5 imino-1,2,5-trideoxy-D-glucitol **12** competitively inhibited the β-hexosaminidase isoenzymes secreted by HOC cells and the degradation of extracellular basement membranes,[46] suggesting that β-hexosaminidase inhibitors might be useful antiinvasive agents.



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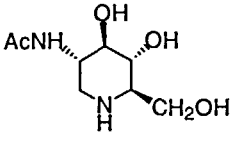

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The bacterium, *Streptococcus oralis,* is a major cause of infective endocarditis in immunocompromised patients and those with malignancies.[47] It has been shown in model systems that *S. oralis* produces a novel range of exoglycosidases that can degrade intact host glycoproteins to provide monosaccharides for its growth and survival.[47,48] Specific

inhibition of these glycosidases by sugar analogues may be a very effective way of controlling or preventing such infections.

5. MODIFCATION OF RECOMBINANT GLYCOPROTEINS

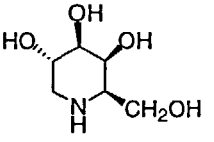
Most lysosomal storage diseases result from a deficiency of a lysosomal hydrolase. Replacement of the missing enzyme is a logical and attractive therapeutic strategy, especially as receptor-mediated endocytosis offers a natural mechanism for the efficient delivery of exogenous proteins to lysosomes. Several of these cell surface receptors recognise the carbohydrate structures on glycoproteins. Some receptors are widely distributed whereas others have a more restricted distribution. Therefore it should be possible to target exogenous replacement lysosomal enzymes to different cells by modifying their glycosylation. Gaucher disease, which is the most common lysosomal storage disease, results from a deficiency of the enzyme β-glucocerebrosidase, leading to the accumulation of its substrate, glucocerebroside within lysosomes. Storage of glucocerebroside is particularly marked in macrophages because they are the site of turnover of the glycolipids of red blood cells. The non-neuronopathic form of Gaucher disease can be treated successfully by targeting replacement natural or recombinant β glucocerebrosidase to macrophages. This is achieved by sequential enzymic deglycosylation of the glycan chains of β -glucocerebrosidase from the non-reducing end to expose α-linked mannose residues, which are recognised by the abundant mannose-binding receptor on the surface of macrophages.[49]

6. INHIBITION OF GLYCOLIPID BIOSYNTHESIS

An alternative therapeutic strategy for lysosomal storage diseases is to try to decrease the rate of accumulation of indigestible material by decreasing the load of substrate being delivered to lysosomes for catabolism – substrate deprivation.[50] The aminosugar analogue, *N*-butyldeoxynojirimycin **9**, which was developed as an anti-HIV agent is also a partial inhibitor of the ceramide-specific β-glucosyltransferase that catalyses the first step in the biosynthesis of the glycan chain of glycosphingolipids. It was shown first in cells in culture and subsequently in mice that the rate of accumulation of GM2-ganglioside in Tay-Sachs and Sandhoff diseases is decreased in the presence of *N*-butyldeoxynojirimycin.[51,52] The decrease in rate of accumulation in the brains of the mice delayed the onset of symptoms and prolonged the life of the Sandhoff mice. The galactose analogue, *N*-butyldeoxygalactonojirimycin, is a more selective inhibitor of glycosphingolipid biosynthesis and may be more suitable for long term human therapy. Substrate deprivation using these inhibitors of glycosphingolipid biosynthesis has

enormous potential for treating the glycosphingolipidoses as it can affect the pathology of the central nervous system and could be used in conjunction with various ways of replacing the missing enzyme.

Paradoxically, competitive inhibitors of lysosomal enzymes may also be therapeutic for lysosomal enzyme deficiencies. 1-Deoxygalactonojirimycin (DGJ, **13**) the galactose analogue of DNJ, stabilised and accelerated the transport and maturation of a mutant α-galactosidase at sub-inhibitory levels in lymphoblast cells from a patient with Fabry disease.[52] It has been proposed that competitive inhibitors may act as 'chemical chaperons' at sub-inhibitory intracellular concentrations.



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7. ACKNOWLEDGEMENTS

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