Iminosugars

From synthesis to therapeutic applications

Editors

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CNRS, University of Orleans, France

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Wiley-VCH Verlag GmbH, Boschstr. 12, D-69469 Weinheim, Germany

John Wiley & Sons Australia Ltd, 33 Park Road, Milton, Queensland 4064, Australia

John Wiley & Sons (Asia) Pte Ltd, 2 Clementi Loop #02-01, Jin Xing Distripark, Singapore 129809

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Anniversary Logo Design: Richard J. Pacifico

Library of Congress Cataloging in Publication Data

Iminosugars : from synthesis to therapeutic applications / edited by
Philippe Compain and Olivier R. Martin.
p. ; cm.
Includes bibliographical references and index.
ISBN 978-0-470-03391-3 (cloth : alk. paper)
1. Imino sugars—Synthesis. 2. Imino sugars—Therapeutic use. I. Compain, Philippe. II. Martin, Olivier R. [DNLM: 1. Imino Sugars—chemical synthesis. 2. Imino Sugars—therapeutic use. 3. Glycoside Hydrolases—antagonists & inhibitors. QU 54 I32 2007]
QP702.I45I45 2007
612′.01578—dc22
2007023155

British Library Cataloguing in Publication Data

A catalogue record for this book is available from the British Library

ISBN 978-0-470-03391-3

Typeset in 10.5/12.5pt Minion by Integra Software Services Pvt. Ltd, Pondicherry, India
Printed and bound in Great Britain by Antony Rowe Ltd., Chippenham, Wiltshire
This book is printed on acid-free paper responsibly manufactured from sustainable forestry in which at least two trees are planted for each one used for paper production.

Contents

Foreword

The field of carbohydrate chemistry has a long and distinguished history in the annals of organic molecules that dates back to its founder Emil Fischer. Carbohydrates (sugars) are among the most abundant natural products. Their occurrence and importance transcends traditional boundaries separating them from other small molecule gifts provided by Nature such as amino acids, hydroxy acids, terpenes and related compounds. Carbohydrates are constituents of biologically important functional macromolecules such as polysaccharides, glycoproteins and lipopolysaccharides, not to mention RNA and DNA. The role of carbohydrates in molecular recognition at the cellular and subcellular level is the primary basis of immunity in living organisms including man.

Carbohydrates have also been associated with many therapeutically important medicines including antibiotics, antiviral agents and antitumour compounds. As monosaccharides, they have provided a playground for synthetic chemists to explore their exquisitely disposed functionality on conformationally distinct and stereochemically defined cyclic hemiacetals. No other class of natural products offers as much stereochemical diversity embodied in enantiopure chiral compounds as is found in the family of aldopyranose and aldofuranose sugars, as well as their aldulose (ketose) equivalents.

Stereoelectronic effects, exemplified by the anomeric effect, have been a fertile area of study for theoretical as well as experimental chemists with important consequences in the emerging field of glycobiology.

Perhaps the most revealing aspect of a natural carbohydrate analogue was the discovery in 1966 of nojirimycin, a D-glucose molecule containing nitrogen in the ring rather than the traditional oxygen. Its antibiotic properties instigated the synthesis of other congeners such as 1-deoxy-nojirimycin, formally a polyhydroxylated 2-hydroxymethyl piperidine of known 'D-*gluco*' configuration. The isolation of 1-deoxy-nojirimycin from natural sources, and the realization that it had inhibitory properties towards α-glucosidases, started a new class of carbohydrate analogues which have been named iminosugars. Remarkably, iminosugars have demonstrated a range of biological activities that span a wide cross section of diseases. The approval of Glyset^{TM} and Zavesca^{TM}, within the last decade, for the treatment of complications associated with type II diabetes and for Gaucher's disease respectively, is a testament to their importance as medicines for unmet medical needs. Clearly, the field of iminosugars has emerged as a fertile area for research on both chemical and biological fronts.

This book covers a wide cross section of iminosugar chemistry, biology and medicinal chemistry in 14 chapters. The Editors have done an excellent job of gathering some of the most active researchers in the field. The coverage of specific topics is extensive and clearly exposed.

It is remarkable that such a small, yet electronically distinct, difference in the replacement of oxygen by the more basic nitrogen, can have dramatically beneficial therapeutic effects. Certainly, the future augurs well to uncover many more fascinating aspects of iminosugars, especially with regard to their biological activities, mode of action and therapeutic potential in treating disease.

Stephen Hanessian
Montréal, Canada
March, 2007

Preface

> *"The productive scientist must be a traditionalist who enjoys playing intricate games by preestablished rules in order to be a successful innovator who discovers new rules and new pieces with which to play them."*
>
> Thomas S. Kuhn in *The Essential Tension*
> (University of Chicago Press, 1977).

The field of iminosugars is a very exciting area of research. These carbohydrate mimetics were first imagined and synthesized by chemists in the 1960s before being isolated from Nature a few years later. Since the discovery of their biological activity as potent glycosidase inhibitors in the 1970s, iminosugars have been the subject of intense studies at the interface between organic synthesis, glycobiology and medicinal science. In 1999, Arnold Stütz edited the first book devoted to iminosugars (*Iminosugars as Glycosidase Inhibitors: Nojirimycin and Beyond*, Wiley-VCH, New York; 1999). The title of this outstanding monograph reflected the main features of the field at that time and the strong interplay between isolation of natural products such as nojirimycin, synthesis and biochemical research. However, the most striking word of the title is undoubtedly the preposition 'beyond' which was premonitory. The pace of discovery in the area of iminosugars has indeed increased spectacularly over the past decade. The scope of their biological activity has been extended to the inhibition of numerous proteins including enzymes acting on sugars (glycosyltransferases, glycogen phosphorylases, nucleoside-processing enzymes, UDP-Gal*p* mutase) but also metalloproteinases. Glyset^{TM} and Zavesca^{TM}, the two first examples of iminosugar-based drugs, have been commercialized for complications associated with diabetes and for the treatment of Gaucher's disease, respectively. New biological and medicinal applications are being uncovered almost every month!

In view of these recent breakthroughs, it appeared to us very timely to publish a new book on the latest developments in the synthesis and the biological evaluation of iminosugars of therapeutic interest with some of the major experts in the area. During the genesis of this project, we have had the opportunity of physically gathering most of the authors at a symposium we organized in August 2005 during the 230th American Chemical Society meeting in Washington DC (*Iminosugars: synthesis and therapeutic potential*, Oral symposium # 7057). The success of this stimulating meeting further reinforced our wish to edit an interdisciplinary book devoted to iminosugars. We are very grateful to the authors, from no less than four continents, for having contributed to the high level of quality of this book. We would like to thank external reviewers for helpful comments, their time and their expertise. Special thanks are also due to

Fiona Woods, Joan Marsh, Andrea Baier and other members of the publishers' team for their faith in our project and for their constant help. Finally, we are most grateful to Stephen Hanessian, a pioneer in the field, for having accepted with enthusiasm our invitation to write the Foreword for this book.

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January, 2007

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1

Iminosugars: past, present and future

Philippe Compain and **Olivier R. Martin**

Iminosugars are sugars in which the endocyclic oxygen is replaced by a basic nitrogen atom. This apparently simple substitution raises many synthetic challenges and opens the way to remarkable biological properties. As such, iminosugars undoubtedly form the most attractive class of carbohydrate mimics reported so far. The origin of their therapeutic use goes back to ancient times and traditional Chinese phytomedicines. In Occident, Haarlem oil, the first medication produced on an industrial scale in the 17th century, was recommended for the treatment of diabetes and for whitening the skin. One of the major constituents of Haarlem oil was an extract from leaves of *Morus alba*, the white mulberry, an extremely rich source of iminosugars [1]. The scientific history of iminosugars began in the early 1960s with the almost simultaneous reports of the synthesis of sugar derivatives containing a nitrogen atom in the ring by the groups of Paulsen [2], Jones [3] and Hanessian [4]. At that time, the replacement of the endocyclic oxygen atom in sugars by heteroatoms (N, S, P) [5] to form 'heteroses' [6] was purely an academic exercise. In 1966, Paulsen published the first synthesis of 1-deoxynojirimycin (DNJ) [7] (Figure 1.1). The same year, Inouye *et al.* isolated nojirimycin from bacteria (*Streptomyces*) and identified its antibiotic properties [8]. The first renaissance of iminosugars came from the isolation of DNJ from natural sources and the finding of its biological activity as an α-glucosidase inhibitor by Bayer chemists in 1976. This discovery triggered an enormous amount of interest in imino analogues of carbohydrates [9].

We are currently witnessing a second renaissance in this field. Since the late 1990s, the rate of discoveries has increased dramatically. Original structures have been designed and synthesized such as seven- or eight-membered iminoalditols, conformationally constrained analogues of iminosugars and complex glycoconjugate mimetics (Figure 1.2). Innovative synthetic strategies have been developed, including combinatorial approaches to iminosugar libraries [10]. The asymmetric synthesis of a pyrrolidinol in only four steps in water has been reported [11] as well as concise *de novo* approaches

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Iminosugars: From synthesis to therapeutic applications Edited by Philippe Compain and Olivier R. Martin
ISBN 978-0-470-03391-3

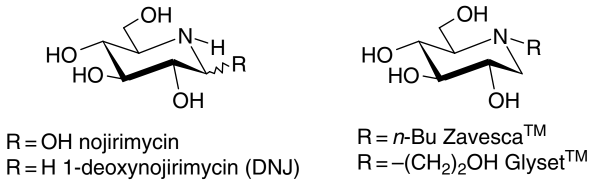


Figure 1.1 Some emblematic iminosugars

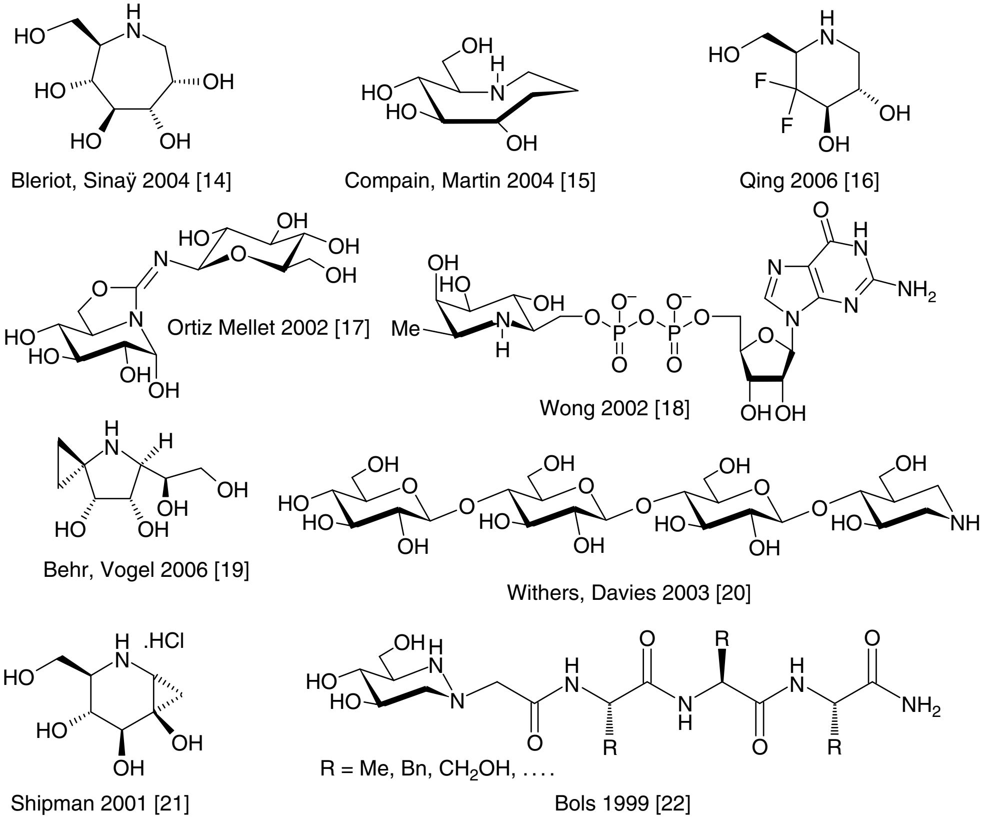


Figure 1.2 Recent examples of original iminosugar structures

by way of enantioselective proline-catalysed reactions [12]. The new concept of dynamic combinatorial chemistry has been applied to accelerate the discovery of iminoalditol-based glycosidase inhibitors [13].

The scope of the biological activity of iminosugars has been extended to the inhibition of a number of enzymes of medicinal interest such as glycosyltransferases [23], glycogen phosphorylases [24], nucleoside-processing enzymes [25], a sugar nucleotide mutase [26] and metalloproteinases [27]. Thanks to iminosugars, significant progress has thus been made in glycobiology in the past 10 years. One of the most spectacular breakthroughs is certainly the discovery that reversible competitive inhibitors could positively influence the folding state of abnormal glycosidases, thus preventing their destruction by quality control in the endoplasmic reticulum (ER) and ER-associated degradation (ERAD) [28]. This new concept, known as chemical chaperone therapy, is being evaluated clinically with a DNJ analogue (Phase II) for the treatment of Fabry disease, highlighting its strong potential as a new therapeutic option for lysosomal diseases. One may also consider the incredibly powerful inhibitors of nucleoside phosphorylases based on iminosugar *C*-nucleosides. These compounds designed as transition-state analogues display inhibition values in the femtomolar range and are among the most powerful inhibitors described for any enzyme to date [29]!

The amazing diversity of enzymes inhibited by iminosugars promises a new generation of medicines in a wide range of diseases such as diabetes, viral infections, lysosomal storage disorders or tumour metastasis (Table 1.1) [30]. Various structures are currently involved in clinical trials and the first successes are being recorded. Recently, two iminosugar-based drugs have been approved: Glyset$^{\mathrm{TM}}$ in 1996 for the treatment of complications associated with type II diabetes, and Zavesca$^{\mathrm{TM}}$ in 2003 as the first oral treatment for Gaucher disease, a severe lysosomal storage disorder (Figure 1.1).

The aim of this book is to present the multifaceted aspects of iminosugars from their structure to their biological activities, and from synthesis to therapeutic applications. Chapter 2 focuses on naturally occurring iminosugars. Chapter 3 is devoted to recent synthetic strategies and combinatorial approaches towards iminosugar libraries. Important classes of iminosugars and their biological activities are presented in Chapter 4 (imino-*C*-glycosides), Chapter 5 (imino-*C*-disaccharides), Chapter 6 (isoiminosugars, i.e. 1-aza carbasugars) and Chapter 8 (iminosugar *C*-nucleosides). Chapter 7 deals with recent developments in the field of glycosyltransferase inhibitors. Moving closer to therapeutic applications, Chapters 9 and 12 are devoted to iminosugars as antiviral and antitumour agents respectively. The two main strategies for the chemotherapeutic treatment of lysosomal diseases are reviewed in Chapter 10 (substrate reduction therapy) and Chapter 11 (chaperone therapy). Chapter 13 is an overview of the medicinal use of iminosugars, including key reflections on their therapeutic potential. Finally, Chapter 14 provides tables which correlate the structure of more than 600 iminosugars of therapeutic interest with their biological activities, where such data are available. Compounds have been selected for their potential as therapeutic agents. The goal of these at-a-glance tables is to facilitate and to stimulate further research in the area of iminosugar by relating structure to properties.

Table 1.1 Enzymatic and therapeutic targets of iminosugars

Enzymatic targets	Therapeutic targets
Glycosidases mid-1970s [9]	Diabetes mid-1970s
Glycosyltransferases 1992 [23]	Viral diseases 1980s
Nucleoside-processing enzymes 1993 [25]	Cancers 1980s
UDP Gal mutase 1997 [26]	Lysosomal diseases 1990s
Glycogen phosphorylases 1997 [24]	Psoriasis 2000s [31]
Metalloproteinases 2004 [27]	Cystic fibrosis 2006 [32]

'IMINOSUGARS'

4-amino-4-deoxypentofuranose

5-amino-5-deoxyhexopyranose

'IMINOALDITOLS'

1,4-dideoxy-1,4-iminopentitol

1,5-dideoxy-1,5-iminohexitol

2,6-dideoxy-2,6-iminoheptitol

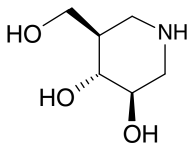
1,5-dideoxy-1-*C*-(2-imidazolyl)-1,5-iminohexitol or 2-(5-amino-5-deoxy-α/β-D/L-hexopyranosyl)imidazole

Figure 1.3

Before concluding, we would like to make some remarks concerning the nomenclature of iminosugars and derivatives. For several years, chemists have used the term 'azasugars' to refer to analogues of pyranoses and furanoses in which the ring oxygen atom is replaced by nitrogen. This nomenclature was adopted because it permitted a clear distinction between common amino sugars, such as glucosamine derivatives, and the new class of carbohydrate analogues. However, this nomenclature is evidently incorrect since, strictly, *aza* is to be used when carbon is replaced by nitrogen. Using the standard rules of carbohydrate nomenclature [33], it is possible to name the nitrogen analogues of sugars without resorting to special descriptors (Figure 1.3).

The use of azasugar should therefore be abandoned in preference to the above nomenclature. In addition, we have recently seen an increasing use of the term 'iminocyclitol'. This term should not be used as it relates to a totally different class of compounds based on a carbocyclic ring.

The new class of compounds, referred to as 1-*N*-iminosugars, is even more confusing. Strictly, 1-aza-analogues of sugars represent a little-studied class of compounds in which the C-1 carbon atom is replaced by nitrogen, thus giving a saturated 1,2-oxazine-type structure. The further replacement of the ring oxygen by a carbon atom leads to aza-carba analogues of sugars. Evidently, it is simpler to name such compounds as branched-chain alditols or as substituted pyrrolidines or piperidines. For example, the so-called 'isofagomine' is 1,2,5-trideoxy-2-hydroxymethyl-1,5-imino-D-xylitol (Figure 1.4) – a short name for this family of compounds could be isoiminosugars.



‘Isofagomine’

1,2,5-trideoxy-2-hydroxymethyl-
1,5-imino-D-xylitol

Figure 1.4

Regarding the impressive series of discoveries in the field over the past 10 years, we can confidently conclude that it is a ‘boom time’ for iminosugar chemistry and biology! These multitask molecules have now moved from the laboratory to the clinic and it is likely that so far we have only seen the tip of the iceberg.

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2

Naturally occurring iminosugars and related alkaloids: structure, activity and applications

Naoki Asano

2.1 Introduction

Great interest in iminosugars (also known as azasugars) has been aroused in recent years because it has been shown that such compounds and their derivatives have enormous therapeutic potential in many diseases such as diabetes, viral infection and lysosomal storage disorders [1–3]. Iminosugars are sugar mimics with a nitrogen atom in place of the ring oxygen and they inhibit glycosidases. The biological properties can be explained by their structural resemblance to the terminal sugar moiety in the natural substrates. Glycosidases are involved in a wide range of important biological processes, such as intestinal digestion, post-translational processing of the sugar chain of glycoproteins, quality-control systems in the endoplasmic reticulum (ER) and ER-associated degradation mechanisms and the lysosomal catabolism of glycoconjugates. Hence, inhibition of these glycosidases can have profound effects on carbohydrate catabolism in the intestine, maturation, transport and secretion of glycoproteins, and can alter cell–cell or cell–virus recognition processes.

Naturally occurring alkaloids inhibiting glycosidases are classified into five structural classes: polyhydroxylated pyrrolidines, piperidines, indolizidines, pyrrolizidines and nortropanes [1, 4]. Furthermore, they also occur in glycosylated forms. However, the structures of natural products are much more diverse. There is no doubt that there are more novel structures waiting to be discovered. This review describes the recent studies on natural inhibitors of α- and β-glucosidases, α- and β-galactosidases and α-mannosidase, as well as their biological applications.

Iminosugars: From synthesis to therapeutic applications Edited by Philippe Compain and Olivier R. Martin

ISBN 978-0-470-03391-3

2.2 α-Glucosidase inhibitors

2.2.1 Structures and *in vitro* inhibitory activity

Over 40 years have passed since nojirimycin (NJ) (**1**) was discovered as the first natural glucose mimic with a nitrogen atom in place of the ring oxygen [5]. NJ is stored as a bisulphite adduct because it bears a hydroxyl group at the anomeric position (C-1) and is fairly unstable. NJ is a good inhibitor of both α- and β-glucosidases, with IC_{50} values of 9 μM toward human lysosomal α-glucosidase and 19 μM toward human lysosomal β-glucosidase (unpublished data). 1-Deoxynojirimycin (DNJ) (**2**) was originally prepared by catalytic hydrogenation of NJ with a platinum catalyst or by chemical reduction with $NaBH_4$ [6]. DNJ was later isolated from the roots of mulberry trees and called molanoline [7]. Mulberry trees (*Morus* spp.) are cultivated in China, Korea and Japan, and their leaves are used to feed silkworms (*Bombyx mori*). Mulberry leaves have been used traditionally to cure and prevent 'Xiao-ke' (diabetes) in Chinese herbal medicine. The root bark of mulberry trees has been used as a Chinese herbal medicine called 'Sang-bai-pi' (Japanese name 'Sohakuhi') for anti-inflammatory, diuretic, antitussive and antipyretic purposes, while the fruits are used as a tonic and sedative. In 1994, the improvement of the purification procedures using a variety of ion-exchange resins led to the isolation of a number of water-soluble alkaloids from the genus *Morus* (Moraceae) [8, 9]. Fagomine (1,2-dideoxynojirimycin) (**3**) and *N*-methyl-DNJ (**4**) as polyhydroxylated piperidines are also isolated from all parts of mulberry trees [8–10]. Although DNJ is a potent inhibitor of all kinds of α-glucosidases, the deoxygenation of DNJ at C-2 to give fagomine markedly lowered its inhibition toward α-glucosidases [11]. The biosynthesis of oligosaccharide chains in *N*-linked glycoprotein involves the cotranslational transfer of a $Glc_3Man_9(GlcNAc)_2$ precursor from a dolichol carrier onto the asparagine residues in the proper sequon of the protein [12]. Initial processing of the oligosaccharides starts with the removal of the outermost α–1,2-linked glucose residue by α-glucosidase I, and α-glucosidase II successively removes the two remaining α–1,3-linked glucose residues in the endoplasmic reticulum (ER). *N*-Methyl-DNJ is a more potent inhibitor of α-glucosidase I than of α-glucosidase II [13, 14]. Mulberry trees additionally contain 1,4-dideoxy-1,4-imino-D-arabinitol (DAB) (**5**), which was originally isolated from fruits of *Angylocalyx boutiqueanus* (Leguminosae) [15] and found to be a potent inhibitor of yeast α-glucosidase [16]. We later reported that DAB is a good inhibitor with a broad inhibitory spectrum toward mammalian glycosidases, such as ER α-glucosidase II, Golgi α-mannosidases I and II and digestive α-glucosidases [11]. In particular, DAB is a more potent inhibitor of α-1,6-glucosidase (isomaltase) than α-1,4-glucosidase (maltase) [11].

In 1988, α-homonojirimycin (α-HNJ) (**6**) was isolated from the neotropical liana, *Omphalea diandra* (Euphorbiaceae), as the first example of a naturally occurring DNJ derivative with a carbon substituent at C-1 [17]. However, before the isolation of the natural product, the 7-*O*-β-D-glucopyranosyl-α-HNJ (Glc-HNJ) (**7**) had been designed and synthesized as a potential drug for the treatment of diabetes [18, 19]. α-HNJ has been detected in adults, pupae and eggs of the neotropical moth, *Urania fulgens*, whose larvae feed on *O. diandra*, and the level of α-HNJ in pupae was about 0.5 per cent

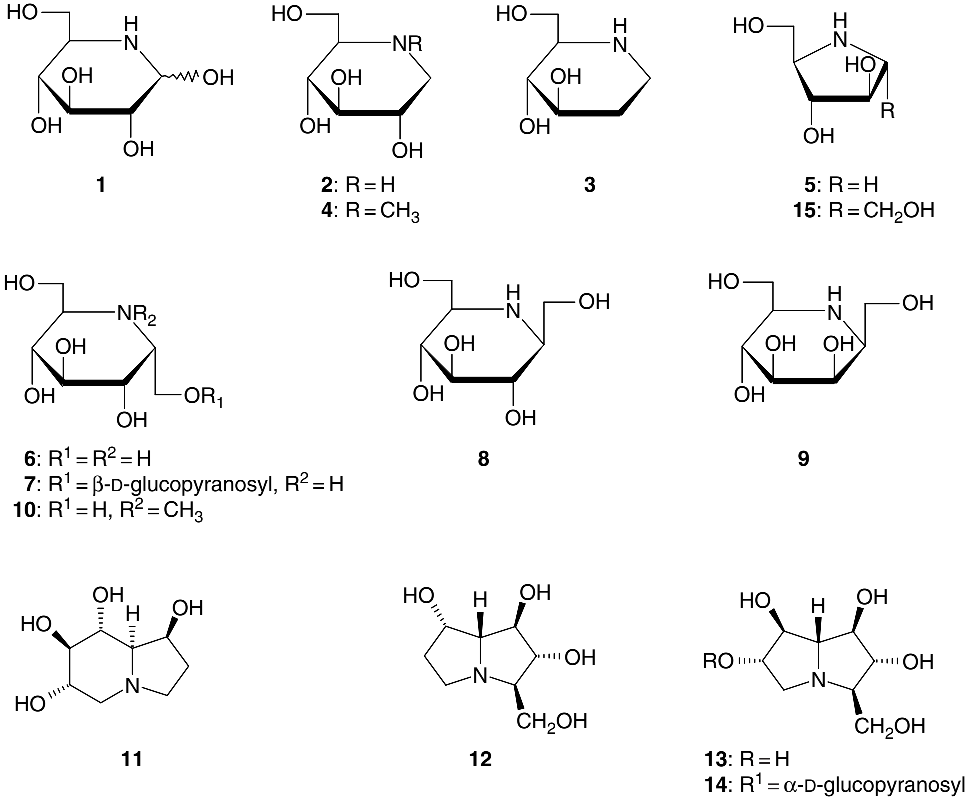


Figure 2.1 Structures of α-glucosidase inhibitors

dry weight [20]. Until 1990, the natural occurrence of α-HNJ had been strictly limited to the Euphorbiaceae family plants. *Aglaonema treubii* (Araceae) is a very common indoor foliage plant and a native to the tropical rainforests of South-East Asia. In 1997, a 50 per cent aqueous EtOH extract of *A. treubii* was found to inhibit α-glucosidase strongly and was subjected to various ion-exchange column chromatographic steps to give a number of polyhydroxylated pyrrolidine and piperidine alkaloids including **6**, **7**, β-homonojirimycin (β-HNJ) (**8**), and β-homomannojirimycin (β-HMJ) (**9**) [21]. α-HNJ is a more specific α-glucosidase inhibitor than DNJ, showing no significant inhibition of other glycosidases [22]. Interestingly, β-HNJ and β-HMJ are not inhibitors of β-glucosidase and β-mannosidase, respectively, but inhibitors of α-glucosidases [22]. α-HNJ and its synthetic *N*-methyl derivative (*N*-methyl-α-HNJ) (**10**) were evaluated for the inhibition of *N*-linked oligosaccharide processing of the viral envelope glycoproteins using an influenza virus-infected Madin–Darby canine kidney (MDCK) cell [23]. By treatment with 100 μg/ml of *N*-methyl-α-HNJ in the medium, essentially all of the *N*-linked oligosaccharide chains of the virus were of the high mannose type with the major structure being characterized as $Glc_3Man_9(GlcNAc)_2$. Similar results were obtained with α-HNJ although this compound was less effective both *in vitro* and *in vivo*.

The toxicity of the legume *Castanospermum australe* for livestock led to the isolation of the toxic principal castanospermine (**11**) [24] and this alkaloid gave rise to a great impetus in research on *N*-containing sugars and its application. In 1981, castanospermine was first isolated from the immature seeds, with the yield of 0.057 per cent [24]. Castanospermine is a potent inhibitor of lysosomal α-glucosidase [25] and disturbs the lysosomal catabolism of glycogen [26]. The syndrome resembles the genetic disorder Pompe disease. When administered to various types of animal cells in culture, castanospermine prevented glycoprotein processing and therefore caused the production of *N*-linked glycoproteins having oligosaccharides mostly of the $Glc_3Man_9(GlcNAc)_2$ type [27–29]. *C. australe* coproduces a pyrrolizidine alkaloid, australine (**12**), which can be regarded as a ring-contracted form of castanospermine [30] but is a much weaker inhibitor of α-glucosidases than castanospermine [31, 32]. A highly hydroxylated pyrrolizidine alkaloid casuarine (**13**) and its 6-*O*-α-D-glucoside (**14**) have been isolated from the bark of *Casuarina equisetifolia* (Casuarinaceae) [33, 34]. Casuarine is a potent inhibitor of rat digestive maltase and *Aspergillus niger* amyloglucosidase, with IC_{50} values in a submicromolar range, and its 6-*O*-α-D-glucoside is a potent competitive inhibitor of porcine kidney trehalase, with an IC_{50} value of 18 nM [32].

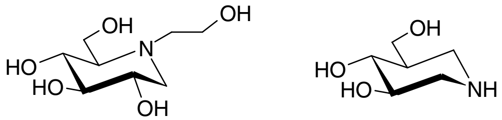
In 1976, 2,5-dideoxy-2,5-imino-D-mannitol (DMDP) (**15**), mimicking β-D-fructofuranose, was found in leaves of the legume *Derris elliptica* [35]. DMDP is a more potent inhibitor of yeast α-glucosidase than mammalian α-glucosidases [36, 37]. Recently, the L-enantiomer of DMDP was synthesized from D-gulonolactone and found to be a more powerful and more specific α-glucosidase inhibitor than the natural product DMDP [38]. More interestingly, the natural D-enantiomer is a competitive inhibitor of α-D-glucohydrolases but its synthetic L-enantiomer is a noncompetitive inhibitor [39]. Similarly, the L-enantiomer of DAB was found to be a more potent inhibitor of mammalian digestive α-glucosidases than the enantiomeric natural product DAB and also to be a noncompetitive inhibitor [39]. Recent studies suggest that D-iminosugars are competitive inhibitors of D-glycohydrolases but their L-enantiomers are noncompetitive inhibitors of the enzymes [39, 40].

2.2.2 Applications

The intestinal oligo- and disaccharidases are fixed components of the cell membrane of the brush border region of the wall of the small intestine. These enzymes digest dietary carbohydrate to monosaccharides which are absorbed through the intestinal wall. They include sucrase, maltase, isomaltase, lactase, trehalase and hetero-β-glucosidase. In the late 1970s, it was realized that inhibition of some, or all, of these activities by inhibitors could regulate the absorption of carbohydrate and these inhibitors could be used therapeutically in the oral treatment of the non-insulin-dependent diabetes mellitus (NIDDM or type 2 diabetes) [41].

The strong inhibition of digestive α-glucosidases by DNJ, which are produced by *Bacillus* and *Streptomyces* [41–43], and mulberry trees [8–10], attracted the interest of various research groups and a large number of *N*-substituted DNJ derivatives were prepared in the hope of increasing the *in vivo* activity. Miglitol (**16**) was identified as one of the most favourable candidates showing a desired glucosidase inhibitory profile [44]. Miglitol differs from acarbose in that it is almost completely absorbed from the intestinal tract, and may possess systemic effects in addition to the effects in the intestinal border [45, 46]. In 1996, Glyset (miglitol) tablets were granted market clearance by the US Food and Drug Administration (FDA) and introduced onto the market in 1999 as a more effective second-generation α-glucosidase inhibitor with fewer gastrointestinal side effects. In 2006, it was introduced onto the market in Japan under the brand name Seibule. α-Glucosidase inhibitors are especially suited for patients whose blood glucose levels are slightly above normal and can also benefit those who have high blood glucose immediately after a meal, a condition known as postprandial hyperglycemia. These drugs slow the rate at which carbohydrates are broken down into monosaccharides in the digestive tract and therefore lengthen the digestive process. Other antidiabetic agents such as sulphonylureas and biguanides sometimes are prescribed in combination with α-glucosidase inhibitors to help increase the effectiveness of this therapy. Protective effects of the α-glucosidase inhibitors have been reported for various diabetic complications. Interestingly, α-glucosidase inhibitors are also being studied as a possible treatment for heart disease, a common complication in diabetic patients. Although repetitive postprandial hyperglycemia increases ischemia/reperfusion injury, this effect can be prevented by treatment with α-glucosidase inhibitors [47].

In type 2 diabetes, hepatic glucose production is increased [48]. A possible way to suppress hepatic glucose production and lower blood glucose in type 2 diabetes patients may be through inhibition of hepatic glycogen phosphorylase [49]. In enzyme assays, Fosgerau *et al.* reported that DAB (**5**), which has a broad inhibitory spectrum toward mammalian glycosidases [11], is a potent inhibitor of hepatic glycogen phosphorylase [50]. Furthermore, in primary rat hepatocytes, DAB was shown to be the most potent inhibitor (IC_{50} 1 μM) of basal and glucagon-stimulated glycogenolysis ever reported [51]. Recently, Jakobsen *et al.* have reported that isofagomine (IFG) (**17**) synthesized chemically is a potent inhibitor of hepatic glycogen phosphorylase, with an IC_{50} value of 0.7 μM and, furthermore, is able to prevent basal and glucagon stimulated glycogen degradation in cultured hepatocytes with IC_{50} values of 2–3 μM [52]. However, its *N*-substitution always resulted in a loss of activity compared with the parent compound, and fagomine (**3**) was a weak inhibitor of this enzyme, with an IC_{50} value of 200 μM [52]. Glycogen phosphorylase inhibitors would be a beneficial target to attack in the development of new antihyperglycemic agents.

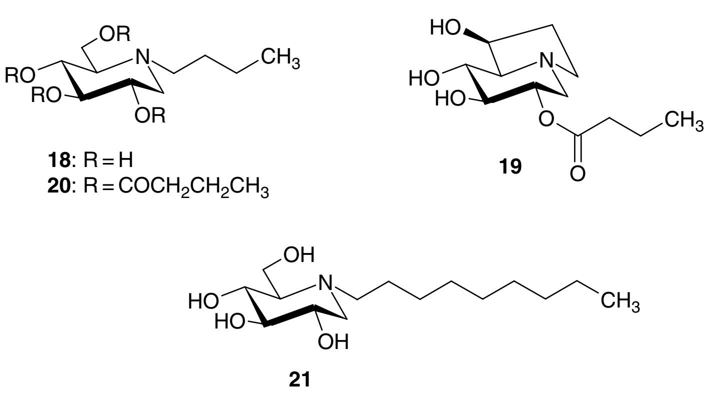


16 **17**

Figure 2.2 Structures of miglitol as a commercially available antidiabetic agent and isofagomine as a glycogen phosphorylase inhibitor

The viral envelope glycoproteins are often essential for virion assembly and secretion and/or infectivity. Compounds that interfere with the glycosylation processes of viral glycoproteins can be expected to be antiviral agents. In fact, α-glucosidase inhibitors such as DNJ, *N*-butyl-DNJ (**18**), castanospermine (**11**) and 6-*O*-butanoylcastanospermine (MDL 28574) (**19**) inhibit human immunodeficiency virus (HIV) replication and HIV-mediated syncytium formation *in vitro* [53–56]. The anti-HIV activity (EC_{50}), determined by syncytial counts, of DNJ, *N*-butyl-DNJ, castanospermine, 6-*O*-butanoylcastanospermine are 560, 56, 29 and 1.1 μM, respectively, but these compounds are not as potent as zidovudine ($EC_{50} = 0.1\ \mu M$) [56]. These sugar analogues showing anti-HIV activity have the common property that they are potent processing α-glucosidase inhibitors but not processing α-mannosidase inhibitors. The activities of these inhibitors toward processing α-glucosidase I correlate well with the antiviral effects [56, 57]. The *in vivo* data obtained to date do not promise practical use of processing α-glucosidase I inhibitors as anti-HIV agents. Problems exist in achieving therapeutic serum concentrations of inhibitors needed to inhibit α-glucosidase I sufficiently and side effects such as diarrhoea occur. With respect to diarrhoea, the prodrug of *N*-butyl-DNJ, glycovir (SC 49483) (**20**), was developed as a candidate anti-HIV agent. This prodrug is *N*-butyl-DNJ tetrabutanoate, which will be converted into active *N*-butyl-DNJ after it passes through the intestine, avoiding the diarrhoea [58]. However, it is believed that morphologic changes in various tissue cells were the result of nonspecific inhibition of host α-glucosidases by the prodrug, causing clinically silent perturbation in host cell glycoprotein processing and/or glycoprotein transport [59].

In contrast to the heavily glycosylated HIV envelope glycoproteins, the envelope glycoproteins of the hepatitis B virus (HBV) contain only two glycosylation sites [60]. However, the HBV glycoproteins are sensitive to inhibitors of the *N*-linked glycosylation pathway. In this virus, correct glycosylation appears to be necessary for processes involved in transport of the virus out of the host cell. *In vitro* treatment of HBV with *N*-butyl-DNJ results in a high proportion of virus particles being retained inside the cells [61]. Block *et al.* [62] reported that *N*-nonyl-DNJ (**21**) reduces the viremia in chronically infected woodchucks in a dose dependent manner. *N*-Nonyl-DNJ is 100–200



18: R = H

20: $R = COCH_2CH_2CH_3$

19

21

Figure 2.3 Structures of α-glucosidase inhibitors as antiviral agents times more potent than *N*-butyl-DNJ in inhibiting HBV in cell based assays [60]. Furthermore, *N*-nonyl-DNJ, compared with *N*-butyl-DNJ, exhibits a prolonged hepatic retention of bovine viral diarrhoea virus (BVDV), a tissue culture surrogate of the human hepatitis C virus (HCV) [63] (see Chapter 9 in this volume). A single drug against HBV and HCV may be of great therapeutic value. However, when processing α-glucosidase inhibitors are used as antiviral agents, it remains to be determined what effects occur on host cell glycoprotein processing and/or glycoprotein transport.

2.3 β-Glucosidase inhibitors

2.3.1 Structures and *in vitro* inhibitory activity

In mammals, three prominent β-glucosidases have been well characterized: lysosomal glucocerebrosidase (GCase), intestinal lactase-phlorizin hydrolase (LPH) and the cytosolic β-glucosidase present in the liver of mammalian species. LPH is enterocyte-specific disaccharidase crucial for the digestion of dietary lactose and β-glucosides, while GCase hydrolyses glucosylceramide derived from endogenous membrane glycolipids. However, a metabolic role for the cytosolic β-glucosidase has not yet been established.

Nojirimycin (NJ) is a moderate inhibitor of human GCase, with an IC_{50} value of 19 μM. The removal of the anomeric OH group to give DNJ markedly lowered its inhibitory potential toward GCase [64]. Interestingly, the DNJ derivatives with a longer chain than the butyl group at the imino group enhance the GCase activity with increasing chain length [64, 65]. In particular, *N*-5-(adamantane-1-yl-methoxy)pentyl-DNJ (**22**) is a very powerful inhibitor of the non-lysosomal ($IC_{50} = 1.7$ nM) and membrane ($IC_{50} = 48$ nM) glucosylceramidases [65]. Recently, Compain, Martin *et al.* synthesized a series of DNJ derivatives bearing an alkyl chain at the pseudo-anomeric position (C-1) in order to assess GCase inhibitory activity [64, 66–68]. Naturally occurring α-1-*C*-methyl-DNJ (**23**) showed weak inhibition toward GCase, with an IC_{50} value of 150 μM. Introduction of the butyl group at the C-1α position to give α-1-*C*-butyl-DNJ (**24**) slightly improved its inhibition toward GCase, whereas that to the C-1β position to give β-1-*C*-butyl-DNJ (**25**) abolished the inhibitory activity. Further elongation of an alkyl chain at C-1α, as seen in the *N*-alkyl-DNJ derivatives, remarkably enhanced the GCase inhibitory activity with increasing chain length [64]. In particular, α-1-*C*-octyl-DNJ (**26**) and α-1-*C*-nonyl-DNJ (**27**) showed 460-fold and 890-fold stronger inhibition toward GCase than DNJ, with IC_{50} values of 0.50 and 0.27 μM, respectively.

A number of polyhydroxylated nortropane alkaloids have been isolated from the families Solanaceae and Convolvulaceae [1, 4]. They were designated as calystegines. Calystegines possess three structural features in common: a nortropane ring system; two to four secondary hydroxyl groups varying in position and stereochemistry; and, a novel aminoketal functionality, which generates a tertiary hydroxyl group at the bicyclic ring bridgehead. The known members of calystegines have been subdivided into three groups on the basis of the number of the hydroxyl groups present, namely calystegines A with three OH groups, B with four OH groups and C with five OH groups. Among calystegines, calystegines A_3 (**28**), B_1 (**29**), B_2 (**30**) and C_1 (**31**) are inhibitors of almond

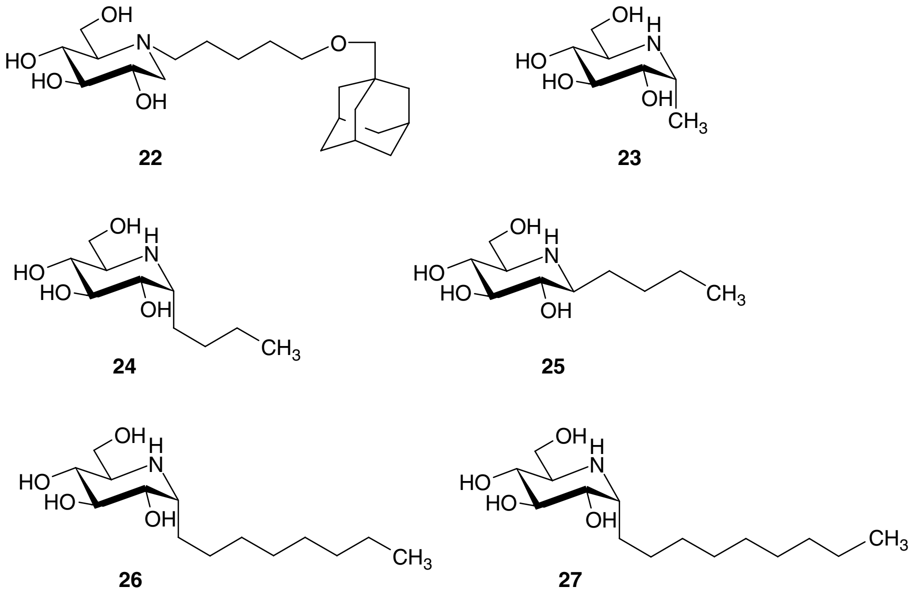


Figure 2.4 Structures of β-glucosidase-inhibiting *N*-alkyl- and *C*-alkyl-DNJ derivatives

β-glucosidase [69] and also potent inhibitors of GCase, with IC_{50} values of 3.1, 2.5, 1.0 and 2.5 μM, respectively [70]. Calystegines can be viewed as derivatives of 1,5-dideoxy-1,5-iminoxylitol (DIX) (**32**), with an ethano bridge across the 1,5-positions. DIX is a very specific inhibitor of GCase, with an IC_{50} value of 1.9 μM [70]. The fact that the inhibitory potency of α-1-*C*-alkyl-DNJ derivatives increases with the length of the alkyl chain suggests that introduction of an alkyl chain to the pseudo-anomeric position of DIX may lead to highly potent and selective inhibitors of GCase. Incorporation of a nonyl chain into DIX to give α-1-*C*-nonyl-DIX (**33**) dramatically improved its inhibitory potency, with an IC_{50} value of 6.8 nM [71]. In addition, α-1-*C*-nonyl-DIX inhibited GCase in a competitive manner, with a K_i value of 2.2 nM. Although *N*-nonyl-DIX (**34**) was prepared for comparison, this compound showed only slight improvement in inhibitory activity toward GCase. Very interestingly, the NMR data indicated that the introduction of a nonyl chain into the C-1 position of DIX caused a piperidine ring inversion from $^4C_1$ to $^1C_4$ conformation [71]. Replacement of the hydroxyl group at C-2 of DIX with a hydroxymethyl group gives isofagomine IFG (**17**), which enhanced its inhibitory potency by 60-fold, with an IC_{50} value of 40 nM [70]. The *N*-alkylation of IFG markedly lowered its activity, suggesting that an intact imino moiety at that position is essential for maintaining potent inhibition of GCase. On the other hand, incorporation of an alkyl chain longer than a butyl group to the C-6α position of IFG remarkably enhanced inhibitory activity toward GCase [72]. The most potent inhibitor α-6-*C*-nonyl-IFG (**35**) displayed a remarkable IC_{50} value of 0.6 nM, which is 93-fold more potent relative to IFG. Unlike **33**, compound **35** exists exclusively in the $^4C_1$ conformation with all substituents equatorial. Interestingly, the inhibition mode for IFG