Monatshefte für Chemie 133, 393–426 (2002)

Monatshefte für Chemie
Chemical Monthly

Invited Review

From Lianas to Glycobiology Tools: Twenty-Five Years of 2,5-Dideoxy-2,5-imino-*D*-mannitol

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Summary. 2,5-Dideoxy-2,5-imino-*D*-mannitol (*DMDP*) has been isolated from several natural sources. Many synthetic approaches are available, and many derivatives have been synthesized and their biological activities have been investigated. An overview on isolation, syntheses, and biological data of *DMDP* as well as some closely related compounds will be given in this review.

Keywords. 2,5-Dideoxy-2,5-imino-*D*-mannitol; Glycosidase inhibitors; Iminosugars; Iminoalditols; Glycosidase inhibitory activities.

Introduction

In 1976, *Welter* and coworkers isolated a new alkaloid from the leaves of *Derris eliptica* (Leguminosae) (Fig. 1) with the molecular formula of $C_6H_{13}NO$. Giving a positive nihydrine reaction, IR and NMR data as well as the obtained optical rotation led to the conclusion that the structure must be 2,5-dihydroxymethyl-3,4-dihydroxypyrrolidine (*DMDP*) having either a (2*R*,3*R*,4*R*,5*R*) or an all-*S* configuration [1]. Soon the crystal structure of this compound was determinated, and the all-*R* configuration was confirmed [2].

Ten years before the isolation of this alkaloid and its examination as a glycosidase inhibitor, important synthetic and structural studies on sugar analogues with nitrogen instead of oxygen in the ring had been conducted by *Paulsen* and coworkers [3]. Studies of glycosidases date back as early as the eighteen-forties when *Liebig* and *Wöhler* made their first contributions to this emerging area [4]. Recently, these important enzymes have been investigated comprehensively by

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Fig. 1. *Derris elliptica*

Legler [5], *Sinnot* [6], and the *Withers* group [7] and are believed to be well understood today.

Glycosidase inhibitors [8] are known as compounds which inhibit these carbohydrate processing enzymes. This class of compounds can mainly be divided into high and low molecular mass structures. The most important subgroup in the low molecular class is formed by polyhydroxylated derivatives of piperidines, pyrrolidines, indolizidines, pyrrolizidines, and nortropane alkaloids. Such compounds can be regarded as mimetics of the corresponding sugar with nitrogen instead of oxygen in the ring. Some of the most investigated compounds in this class are 2,5-dideoxy-2,5-imino-*D*-mannitol (**1**), 1-deoxynojirimycin (*DNJ*, **2**), castanospermine (**3**), and autraline (**4**), all being excellent reversible inhibitors of *D*-glucosidases (Fig. 2).

1-Deoxynojirimycin (**2**), which had been synthesized by *Paulsen* and his group in 1966 [9], was later found in the fermentation broths of a *Bacillus* species [10] as well as in that of *Streptomyces lavandulae* [11]. Castanospermine (**3**), which can be regarded as a bicyclic version of 1-deoxynojirimycin (**2**) also exhibiting a *D*-gluco

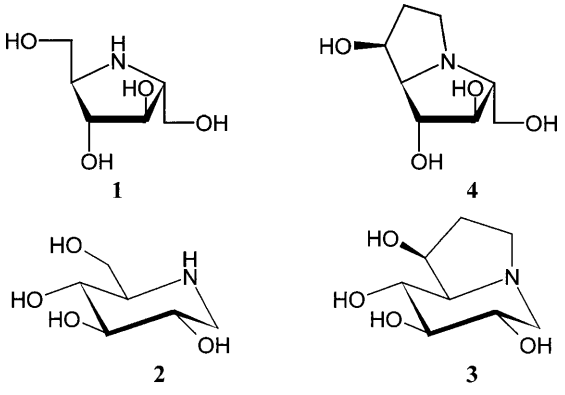


Fig. 2. *DMDP* (**1**), *DNJ* (**2**), castatonspermine (**3**), australine (**4**)

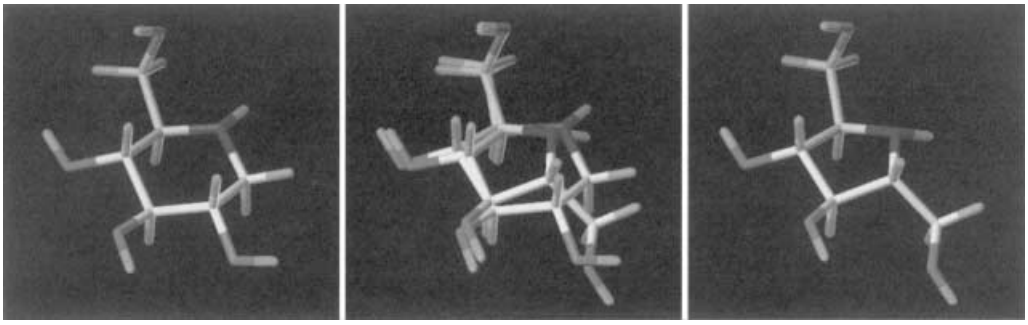


Fig. 3. Superposition (middle) of *DNM* (left) and *DMDP* (right)

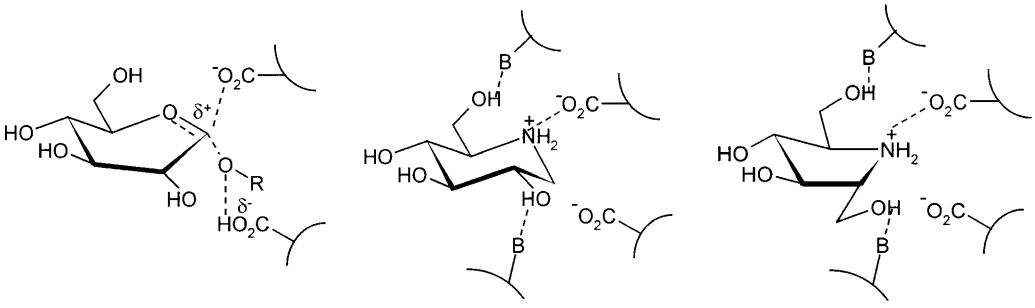


Fig. 4. Comparison of *DNJ* and *DMDP* with the carboxonium ion transition state

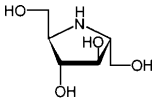
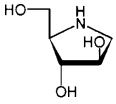
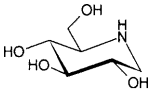
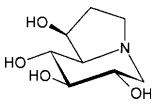
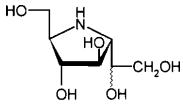
configuration pattern, was isolated from the Australian legume *Castanospermum australae* [12]. Australine (**4**) was also found in the fruit of this tree [13] and is related to *DMDP* in the same way as is **2** to **3**. Whereas in the case of **2** and **3** the *D*-gluco configuration is quite obvious, **1** and **4** are apparently rather α-*D*-fructofuranose analogues. Nevertheless, **1** is in some cases even more active against *D*-glucosidases than **2** or **3**. From the superposition of **2** and **1** (Fig. 3) it can be seen that all hydroxyl groups as well as the ring nitrogen match very well.

1 features a C_2 axis of symmetry in contrast to **4** in which one of the hydroxymethyl groups is locked in the bicyclic system which abolishes the symmetry and changes inhibitory properties relative to **1**. When compared to 1-deoxynoirimycin and castanospermine, the more flat five-membered ring in **1** is closer mimicking the half-chair conformation found in the oxocarbonium ion transition state of (enzymatic) glycoside hydrolysis [14] (Fig. 4).

The five-carbon-containing natural product 1,4-dideoxy-1,4-imino-*D*-arabinitol **5** (Fig. 5) is structurally closely related to *DMDP* and was isolated from two types of leguminose plants in 1985 [15]. Several synthetic approaches have been devised for this compound [16] which is an inhibitor of α-glucosidases, mammalian glycoprotein trimming glucosidases such as ER α-glucosidase II, plant α-mannosidase and *Golgi* located α-mannosidases I and II, and intestinal isomaltase and trehalase [22] (see Table 1).

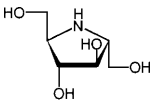
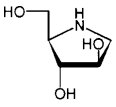
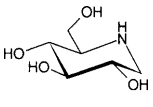
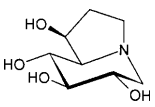
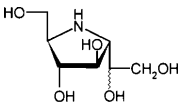
Another closely related natural product is 2-hydroxymethyl-3,4-dihydroxy-6-methylpyrrolidine (6-deoxy-*DMDP*, **6**; Fig. 5). This compound was isolated from

Table 1. Biological activities of **1**, **2**, **3**, **4**, and **6** (*D* and *L* are indicated at the values); K_i and IC_{50} values in μM; NI: no inhibition, ng: not given, nd: not determined

Enzyme	pH	 **1**	 **5**	 **2**	 **3**	 **7**	Ref.
α-Glucosidase	6.5	$K_i = 0.73$	ng	$K_i = 25$	$K_i \geqslant 1500$	ng	[37]
Yeast		$K_i = 7$	ng	ng	ng	$K_i = 54$	[24b]
	6.8	$IC_{50} = 3.3$	$IC_{50} = 0.18$	$IC_{50} = 190$	ng	ng	[16a], [20]
Brewer's yeast	opt	$IC_{50} = 0.0599$	ng	$IC_{50} = 9.57$	ng	ng	[27]
Baker's yeast		$IC_{50} = 15$	$IC_{50} = 7.0$	ng	ng	$IC_{50} = 85$ (*DL*)	[24a]
		$IC_{50} = 3.6$	ng	ng	ng	$IC_{50} = 270$ (*DL*)	[18]
α-Glucosidase	opt	$IC_{50} = 300$	ng		ng	$IC_{50} = 130$ (*DL*)	[18], [25]
Rice		NI	ng	$IC_{50} = 1.44$	ng	ng	[27]
		$IC_{50} = 200$	ng		ng	$IC_{50} = 130$ (*DL*)	[24b], [24c]
α-Glucosidase (*saccharomyces sp*)	7.0	$K_i = 330$	ng	ng	ng	$K_i = 28$ (*D*)	[48]
α-Glucosidase (rabbit gut)	opt	NI	$IC_{50} = 85$	ng	ng	NI	[24a]
α-Glucosidase	opt						
Rat intestinal	6.8	$IC_{50} = 290$	$IC_{50} = 55$	$IC_{50} = 0.36$	ng	$IC_{50} = 400$ (*DL*)	[18], [22], [24b], [24c]
maltase							
isomaltase	6.8	$IC_{50} = 91$	$IC_{50} = 5.8$	$IC_{50} = 0.3$	ng	NI	[24b], [22]
sucrase		NI	ng	ng	ng	$IC_{50} = 300$ (*DL*)	[24b]
α-Glucosidase	opt						
Rat liver	6.8	$IC_{50} = 92$	$IC_{50} = 100$	$IC_{50} = 0.4$	ng	NI	[24b], [22]
lysosome							
ER glucosidase II		NI	$IC_{50} = 20$	$IC_{50} = 4.6$	ng	ng	[22]
α-Glucosidase							[27]
Porcine small intestine	6.8						
Starch		$IC_{50} = 307$	ng	$IC_{50} = 0.479$	ng	ng	
Maltose		$IC_{50} = 9.57$	ng	$IC_{50} = 0.00957$	ng	ng	
Sucrose		$IC_{50} = 20.1$	ng	$IC_{50} = 0.144$	ng	ng	
α-Glucosidase							
Bovine liver lysosome	4.0	$IC_{50} = 170$	$IC_{50} = 89$	$IC_{50} = 0.4$	ng	ng	[22]
β-Glucosidase (*Asp. wentii*)	5.0	$K_i = 57$	ng	$K_i = 2.0$	$K_i = 0.9$	ng	[37]
β-Glucosidase (bovine kidney)	5.0	$K_i = 44$	ng	$K_i = 250$	$K_i = 25$	ng	[37]
β-Glucosidase (bitter almonds)	6.8	$IC_{50} = 7.8$	$IC_{50} = 200$	$IC_{50} = 81$	ng	ng	[16a], [20]
β-Glucosidase	opt	$IC_{50} = 11$	ng	ng	ng	$IC_{50} = 3.8$ (*DL*)	[24b]

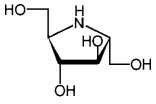
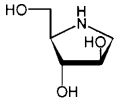
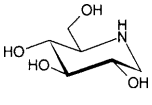
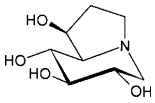
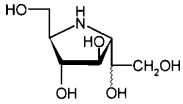
(*continued*)

Table 1 (*continued*)

Enzyme	pH	 **1**	 **5**	 **2**	 **3**	 **7**	Ref.
(*Caldocellum saccharolyticum*)						$IC_{50} = 3.8$ (*DL*)	[24c]
β-Glucosidase	5.0	$K_i = 1.7$	ng	$K_i = 300$	$K_i = 1.5$	ng	[37]
(almonds)		$IC_{50} = 13$	ng	ng	ng	$IC_{50} = 23$	[18]
		$IC_{50} = 2.40$	ng	$IC_{50} = 153$	ng	ng	[27]
		$K_i = 10$	ng	ng	ng	$K_i = 1.5$	[24b]
	5.0	ng	ng	ng	ng	$K_i = 24.5$ (*D*)	[47]
	opt	$K_i = 10$	$K_i = 280$	ng	ng	$K_i = 1.5$ (*DL*)	[24a]
	opt	$IC_{50} = 9$	$IC_{50} = 965$	ng	ng	$IC_{50} = 4$ (*DL*)	[24a]
	5.0	$K_i = 50$	ng	ng	ng	$K_i = 2.6$ (*D*)	[48]
	4.8	$K_i = 5.2$	ng	ng	ng	$IC_{50} = 23$ (*DL*)	[24c]
		$IC_{50} = 7.3$	ng	ng	ng	ng	[68]
β-Glucosidase (rat intestine cellobiase)	4.0	$IC_{50} = 34$	NI	$IC_{50} = 520$	ng	ng	[22]
β-Galactosidase	opt	$IC_{50} = 2.5$	ng	ng	ng	$IC_{50} = 4.4$ (*DL*)	[24b], [24c]
(bovine liver)	opt	$IC_{50} = 2.2$	ng	ng	ng	$IC_{50} = 4.4$ (*DL*)	[18], [25]
cytosolic	6.8	$IC_{50} = 3.3$	$IC_{50} = 1000$	NI	ng	$IC_{50} = 4.4$ (*DL*)	[22]
β-Galactosidase (rat intestinal lactase)	opt	$IC_{50} = 3.6$	$IC_{50} = 260$	$IC_{50} = 26$	ng	$IC_{50} = 4.0$ (*DL*)	[24b], [22], [24c]
α-Mannosidase (jack beans)	opt	NI	$IC_{50} = 750$			$IC_{50} = 695$ (*DL*)	[24a]
		NI	$IC_{50} = 100$				[16a]
β-Mannosidase (snail)	opt	NI	ng	ng	ng	$IC_{50} = 400$ (*DL*)	[24c]
α-Mannosidase					ng	ng	[22]
rat liver					ng	ng	
Golgi I	5.5	NI	$IC_{50} = 53$	NI	ng	ng	
Golgi II	5.5	NI	$IC_{50} = 46$	NI	ng	ng	
lysosomal	4.5	NI	$IC_{50} = 110$	$IC_{50} = 1000$	ng	ng	
soluble	6.5	$IC_{50} = 260$	$IC_{50} =$ NI	NI	ng	ng	
rat epididymis		NI	$IC_{50} = 84$	$IC_{50} = 320$	ng	ng	
α,α-Trehalase					ng		
rat instestine	opt	$IC_{50} = 360$	$IC_{50} = 25$	$IC_{50} = 42$	ng	$IC_{50} = 2.0$ (*DL*)	[22], [24b], [24b]
porcine kidney		$IC_{50} = 200$	$IC_{50} = 4.8$	$IC_{50} = 41$	ng	$IC_{50} = 5.0$ (*DL*)	[22], [24c], [18]
		$IC_{50} = 500$	ng	ng	ng	NI	[25], [24b]
		$K_i = 150$	ng	ng	ng	ng	[25]

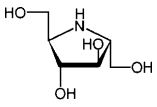
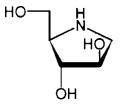
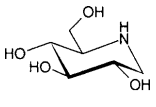
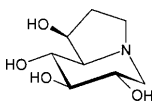
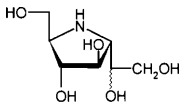
(*continued*)

Table 1 (*continued*)

Enzyme	pH	 **1**	 **5**	 **2**	 **3**	 **7**	Ref.
Trehalase (*Corynebacterium sp.*)	ng	$IC_{50} = 0.35$	ng	ng	ng	ng	[30]
Trehalase (*Plutella xylostella*)	ng	$IC_{50} = 10$	ng	ng	ng	ng	[30]
Sucrase (baker's yeast)	ng	$IC_{50} = 0.957$	ng	NI	ng	ng	[27]
β-Fructofuranosidase	4.6	$IC_{50} = 52.5$	ng	NI	nd	ng	[20]
Yeast	5.0	$K_i = 6.8$	ng	$K_i \geqslant 5000$	nd	ng	[37]
	6.0	$K_i = 3.5$	ng	nd	nd	ng	[37]
	7.0	$K_i = 1.1$	ng	nd	nd	ng	[37]
Invertase (*Pleum pratense*)	5.5	$K_i = 78$	$K_i = 1070$	ng	ng	$K_i = 77$ (*DL*)	[24a]
β-Xylosidase (*Asp. niger*)	5	$IC_{50} = 250$	NI	$IC_{50} = 400$	ng	ng	[16a], [20]
α-*L*-Fucosidase (bovine epidermis)	opt	$IC_{50} = 110$	ng	ng	ng	ng	[24c]
Amyloglucosidase (*Rhizophus*)	ng	$IC_{50} = 38.3$	ng	$IC_{50} = 613$	ng	ng	[27]
Amyloglucosidase (*A. niger*)	opt	$IC_{50} = 19$	ng	ng	ng	$IC_{50} = 180$	[18], [24c]
Thioglucosidase (mustard myrosinase):							[62]
singrin acetate	5	$IC_{50} = 9.3$	NI	NI	$IC_{50} = 10$	ng	
phosphate	7	$IC_{50} = 33$	NI	NI	$IC_{50} = 180$	ng	
progoitin acetate	5	$IC_{50} = 35$	NI	NI	$IC_{50} = 36$	ng	
citrate	5	$IC_{50} = 150$	NI	NI	$IC_{50} = 76$	ng	
phosphate	7	$IC_{50} = 51$	NI	NI	$IC_{50} = 340$	ng	
Thioglucosidase (*Brevicoryne brassicae*)							[62]
singrin acetate	5	$IC_{50} = 240$	NI	NI	$IC_{50} = 90$	ng	
phosphate	7	$IC_{50} = 100$	$IC_{50} = 280$	$IC_{50} = 970$	$IC_{50} = 43$	ng	
progoitin acetate	5	$IC_{50} = 140$	NI	NI	$IC_{50} = 58$	ng	
citrate	5	$IC_{50} = 400$	$IC_{50} = 570$	$IC_{50} = 230$	$IC_{50} = 6.0$	ng	
phosphate	7	$IC_{50} = 87$	$IC_{50} = 340$	$IC_{50} = 850$	$IC_{50} = 34$	ng	
Glucosidase I (mung bean seedlings)	6.5	$IC_{50} = 40$	ng	$IC_{50} = 5–7$	$IC_{50} = 2–3$		[68]

(*continued*)

Table 1 (*continued*)

Enzyme	pH	 **1**	 **5**	 **2**	 **3**	 **7**	Ref.
Activity of mouse small intestinal mucosal homogenates:							[61]
p-nitrophenyl-α-*D*-glucoside	6	$IC_{50} = 300$	$IC_{50} = 47$	$IC_{50} = 0.83$	$IC_{50} = 2.8$	ng	
maltose	6.4	$IC_{50} = 200$	$IC_{50} = 35$	$IC_{50} = 0.37$	$IC_{50} = 0.83$	ng	
trehalose	6	$IC_{50} = 320$	$IC_{50} = 22$	$IC_{50} = 67$	$IC_{50} = 9.8$	ng	
sucrose	6	$IC_{50} = 42$	$IC_{50} = 23$	$IC_{50} = 0.06$	$IC_{50} = 0.042$	ng	
isomaltose	6.4	$IC_{50} = 23$	$IC_{50} = 4.0$	$IC_{50} = 0.12$	$IC_{50} = 3.1$	ng	
turanose	6	$IC_{50} = 71$	$IC_{50} = 28$	$IC_{50} = 0.14$	$IC_{50} = 0.23$	ng	
palatinose	6	$IC_{50} = 56$	$IC_{50} = 13$	$IC_{50} = 0.25$	$IC_{50} = 43$	ng	
p-nitrophenyl-β-*D*-glucoside	6	$IC_{50} = 10$	NI	NI	$IC_{50} = 17$	ng	
cellobiose	6	$IC_{50} = 26$	NI	NI	$IC_{50} = 8.5$	ng	
gentiobiose	6	$IC_{50} = 2.2$	$IC_{50} = 100$	$IC_{50} = 44$	$IC_{50} = 0.73$	ng	
p-Nitrophenyl-β-*D*-galactoside	6	$IC_{50} = 2.0$	$IC_{50} = 210$	$IC_{50} = 220$	$IC_{50} = 5.5$	ng	
Lactose	6	$IC_{50} = 2.1$	NI	$IC_{50} = 57$	$IC_{50} = 0.58$	ng	

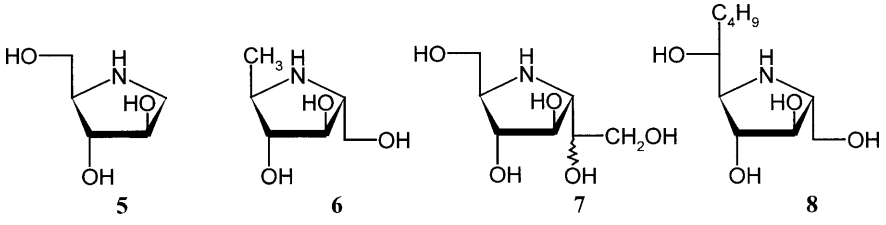


Fig. 5. Structures of *DMDP*-related natural compounds

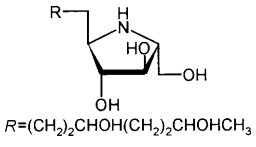
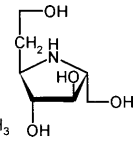
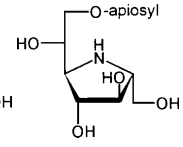
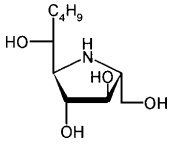
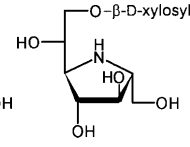
seeds of the African legume *Angylocalyx pynaerii*. Its configuration was proved by stereospecific synthesis, and it could be shown to be a selective inhibitor of β-mannosidase from *Aspergillus niger* with an IC_{50} of 380 μM without any effect on α-mannosidase from jack beans and several other glycosidases [17].

A homologue of *DMDP* containing seven carbons, 2,5-dideoxy-2,5-imino-*D*-glycero-*D*-manno-heptitol (**7**, Fig. 5), synthesized by *Wong* and coworkers [47] prior to its discovery in nature, was isolated from *Hyacinthoides nonscripta* together with **1** and **5**. The absolute configuration at C-6 in compound **7** could

not be determinated at this time, so all biological studies were referred to 2,5-dideoxy-2,5-imino-*DL*-glycero-*D*-manno-heptitol (**7**) [24a]. This inhibitor could be shown to be more potent against almond β-glucosidase (see Table 1) and to be more active against sucrase, maltase, and lactase than the parent compound. Recently, the 6-C-butyl derivative of *DMDP* (**8**, Fig. 5) was found in *Adenophora triphylla* var. *japonica* (Campanilacae) and showed interesting inhibitory activity against β-glucosidase and amyloglucosidase [18] (Table 2).

Broussonetines (Fig. 6) were discovered as a family of compounds having *DMDP*-type structures and could be shown to be β-galactosidase and β-mannosidase inhibitors. These compounds contain the same five-membered ring structure as *DMDP* with diversely modified lipophilic side chains at position C-1 such as in, for example, **9** and **10** [19].

Table 2. IC_{50} values of different derivatives (μM)

Enzyme	*pH*						Ref.
α-Glucosidase (rice)	opt	NI	$IC_{50} = 2.2$	NI	NI	NI	[24b]
α-Glucosidase rat intestinal	opt	NI	$IC_{50} = 2.5$	NI	NI	NI	[24b]
maltase		NI	$IC_{50} = 11$	NI	NI	NI	[24b]
α-Glucosidase rat liver lysosomal	opt	ng	$IC_{50} = 7.2$	ng	ng	ng	[24b]
β-Glucosidase (almond)	opt	NI	ng	NI	$IC_{50} = 68$	$IC_{50} = 4.6$	[18], [24c]
β-Glucosidase *C. saccharolyticum*	opt	NI	$IC_{50} = 380$	NI	ng	$IC_{50} = 0.34$	[24b], [24c]
β-Galactosidase (bovine liver)	opt	NI	$IC_{50} = 60$	$IC_{50} = 40$	$IC_{50} = 390$	$IC_{50} = 24$	[24c], [24b] [18]
β-Galactosidase (rat intestinal lactase)	opt	NI	$IC_{50} = 320$	$IC_{50} = 1.6$	ng	$IC_{50} = 0.18$	[24b], [24c]
β-Mannosiase (snail)	opt	NI	ng	NI	ng	$IC_{50} = 140$	[24c]
α,α-Trehalase (rat instestine)	opt	NI	$IC_{50} = 380$	NI	ng	NI	[24b]
α-*L*-Fucosidase (bovine epidermis)	opt	$IC_{50} = 110$	ng	NI	ng	NI	[24c]
Amyloglucosidase (*A. niger*)	opt	$IC_{50} = 40$	ng	$IC_{50} = 60$	$IC_{50} = 40$	$IC_{50} = 100$	[24c] [18]

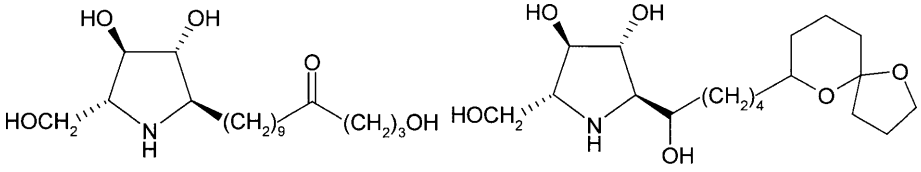


Fig. 6. Structures of two broussonetines

Natural Sources and Isolation

After the first isolation and recognition of the biological activities of *DMDP* and related compounds and the understanding of the potential biological importance of these structures, further screening of all kinds of sources became a research topic in the nineteen-eighties. The compound could be found in plants, animals, and microorganisms, demonstrating to be a fairly common metabolite.

Plant sources

Because of its first discovery in a leguminose plant, many others were screened for the presence of *DMDP*. The seeds of *Lonchocarpus sericeus* (Leguminosae) [20] and the leaves of the large liana *Omphalea diandra L.* [21] as well as the leaves of *Derris malaccensis* [22] were found to contain *DMDP* and other alkaloids such as deoxynojirimycin and derivatives. More recently, *DMDP* was found, besides other iminoalditols such as six-membered homologues with *D*-gluco, *D*-manno, or *D*-galacto configurations, in extracts of the plant *Aglaonema treubii* Engl. (Araceae) [23].

Since livestock have been poisoned by grazing the leaves of bluebells, *Hyacinthoides nonscripta* was screened for alkaloids, and *DMDP* as well as **5** and the homo-derivative **7** could be isolated from the British plant. Other species of Hyacinthaceae such as *Hyacinthus orientalis* and the bulbs of *Scilla campanulata*, were also investigated and found to be rich in alkaloids such as *DMDP* and related compounds, for example the 6-deoxy-homo-*DMDP* which had not been found in nature before [24]. Furthermore, *DMDP* and many other iminosugar derivatives were found in the plant *Lobelia sessilifolia* and the roots of *Adenophora spp.* (Campanulaceae) [25].

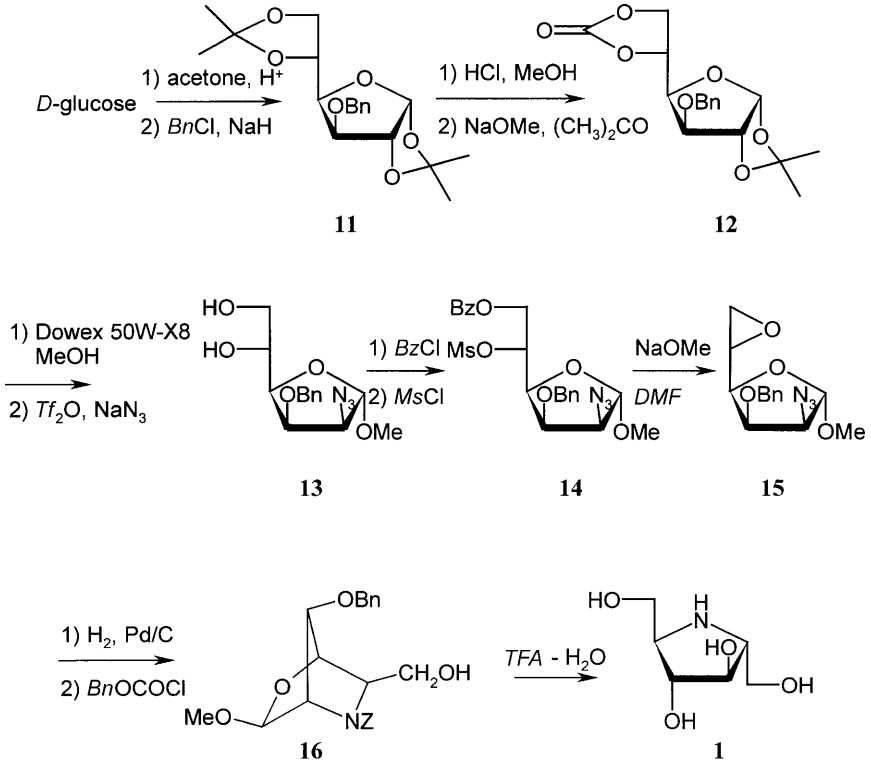
Kite and coworkers have screened many species of *Nephthytis* Schott, *Anchomanes* Schott, *Pseudohydrosme* Engl., *Aglaonema* Schott, and *Aglaodorum* Engl. where they found reasonable amounts of *DMDP* together with six-membered ring iminoalditols [26]. In their search for α-glucosidase inhibitors, *Kim* and his group became interested in *Commelina communis* L., the dried aerial parts of which have been used in traditional medicine as an antipyretic for noninfectious fever and to treat ascites, edema, and hordeolum. This herb was also very popular in Korea for the treatment of diabetes. Consequently, the extract was screened for glycosidase inhibitory activity, and *DMDP* together with four piperidine-related alkaloids were found [27].

Animal and microbial sources

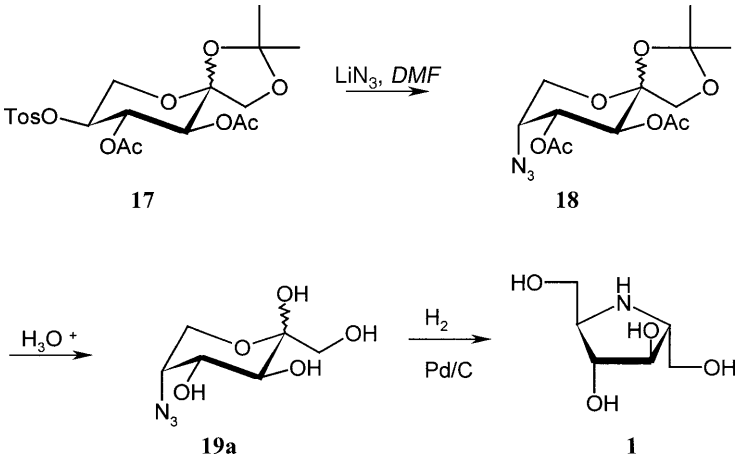
The larvae of the day-flying moth *Urania fulgens*, which was found to feed exclusively on the liana *O. diandra*, was consequently thought to accumulate *DMDP*, and it could be shown that the adult form contains *DMDP* and other alkaloids in all body parts including their wings [28]. This was the first example of the occurrence of such a compound in animals. Several other moths were found to contain this alkaloid, such as adults of *Alcides metaurus*, *Lyssa macleayi*, and *Urapteroides astheniata* [29]. *DMDP* was also isolated from the fermentation broth of *Steptomyces sp.* KSC-5791 when it was screened for trehalase inhibitor activity, showing the general presence of *DMDP* not only in plants and animals but also in microorganisms [30].

Syntheses

Because of the biological importance of *DMDP*, great interest in synthetic approaches arose. The first synthesis was reported by *Fleet* who employed D-glucose as the starting material in 1985 [31] (Scheme 1). Protection with acetone followed by O-3 benzylation gave the protected sugar **11**. The 5,6-O-isopropylidene group was exchanged by a carbonate residue to yield **12**. Acidic cleavage of the 1,2-O-isopropylidene acetal in methanol furnished the α,β-glucosides. The α-isomer with O-2 available for triflate formation and subsequent S_N2 substitution at C-2 with



Scheme 1



17 **18** **19a** **1**

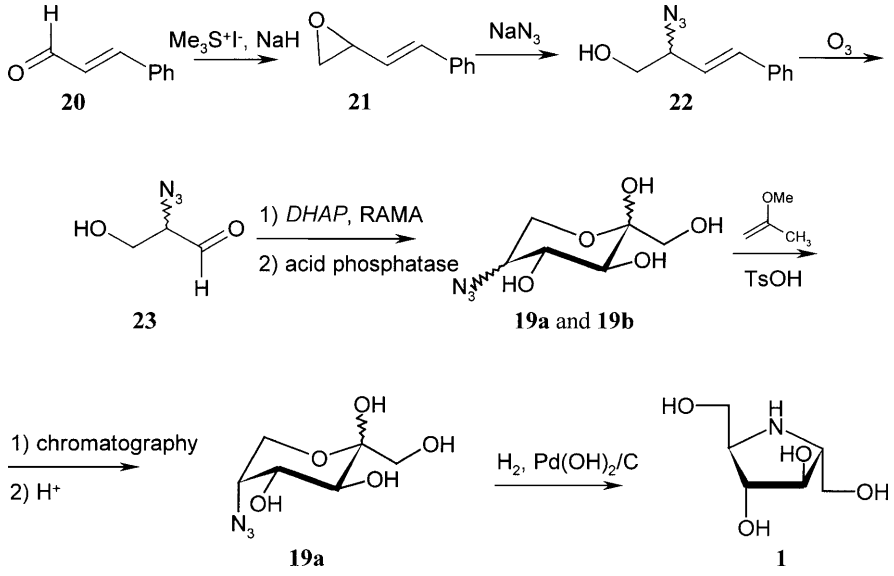
Scheme 2

sodium azide gave key intermediate **13**. The azidodeoxymannofuranoside can undergo subsequent cyclization *via* C-5 during an intramolecular nucleophilic attack with overall retention of configuration at this center by a nitrogen nucleophile generated from the azide yielding the bicyclic amine **16**. This approach was carried out with the epoxide **15** which subsequently suffered attack of the nitrogen to the desired bicyclic compound **16**. Subsequent deprotection converted **16** into *DMDP*. **13** can also undergo cyclization *via* C-6 leading to 1-deoxymannojirimycin.

At about the same time, *Card* and *Hitz* [32] who were interested in sucrose transport and inhibitors of invertase (*β*-*D*-fructofuranosidase) reported a different approach (Scheme 2). Starting from tosylate **17**, readily available from *L*-sorbose in three steps, they introduced the azide by substitution of the tosylate to obtain **18**. Deprotection under acidic conditions gave 5-azido-5-deoxy-*D*-fructose **19a** which underwent cyclization during hydrogenation to form *DMDP* in an overall yield of 68% for these three steps.

A double reductive amination of suitable dicarbonyl sugars, such as 5-keto-*D*-fructose, was introduced by *Reitz* and *Baxter*. With benzylamine as the amino compound the reaction gave *DMDP* only as a side product (6%), the corresponding *D*-gluco-epimer being the main product [33]. This unnatural isomer was also synthesized by an aldolase catalyzed carbon–carbon bond formation by *Wong* and coworkers [34].

The first chemoenzymatic synthesis had been conducted by *Whitesides* and coworkers in 1991 [35] who used RAMA (rabbit muscle aldolase, EC 4.1.2.13) to synthesize polyhydroxylated amines from C_3 fragments (Scheme 3). The synthesis of the key intermediate **23**, an azidoaldehyde, started with the methylenation of cinnamaldehyde **20** to epoxide **21**, followed by nucleophilic opening with sodium azide to **22**. Subsequent ozonolysis provided **23** for the RAMA-catalyzed reaction with dihydroxy acetone phosphate (*DHAP*) from which a 1:1-mixture of C-5 epimers **19a** and **19b** was obtained. Separation of the isopropylidene protected ketoses by silica gel chromatography and subsequent hydrogenation of the 5-azidodeoxy-*D*-fructose **19a** cleanly afforded the C_2-symmetric pyrrolidine **1** in approximately 10% overall yield.



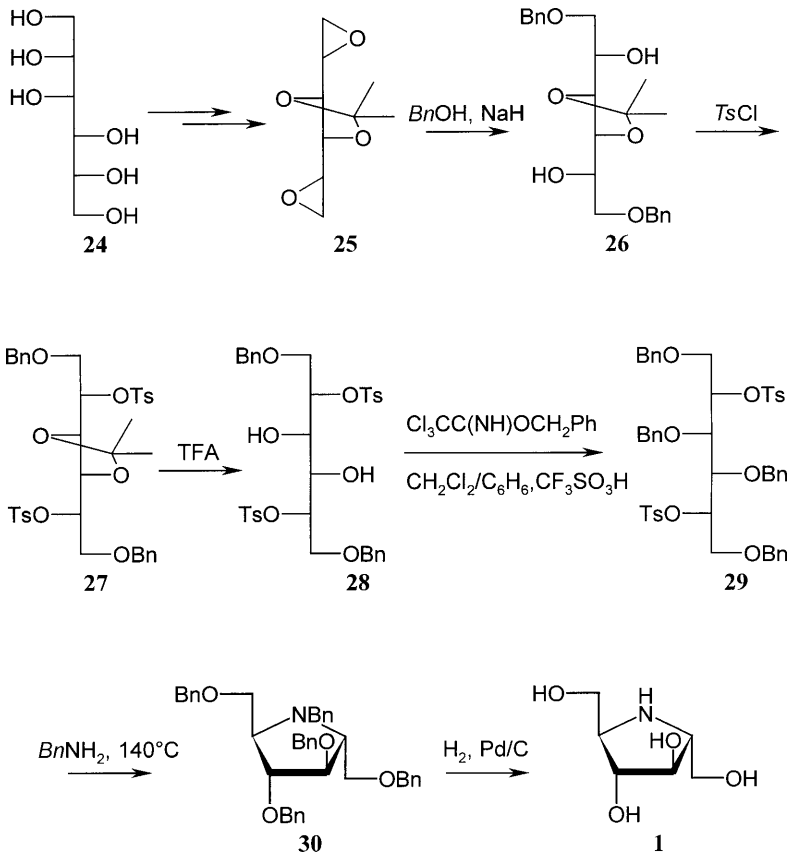
Scheme 3

Duréault and coworkers [36] employed *D*-mannitol (**24**) as the starting material (Scheme 4). Regioselective opening of the easily available *L*-ido-bisepoxide **25** with benzyl alcohol gave 1,6-di-O-benzyl-*L*-iditol derivative **26**. Ditosylation of the secondary alcohols to furnish **27** followed by cleavage of the 3,4-O-isopropylidene group to **28** and subsequent dibenzylation employing benzyltrichloroacetamidate gave the key indermediate **29**. The pyrrolidine ring **30** was obtained by heating of **29** in benzylamine for 12 h at 140°C. Hydrogenolysis of the benzyl groups gave **1** in an overall yield of 20%.

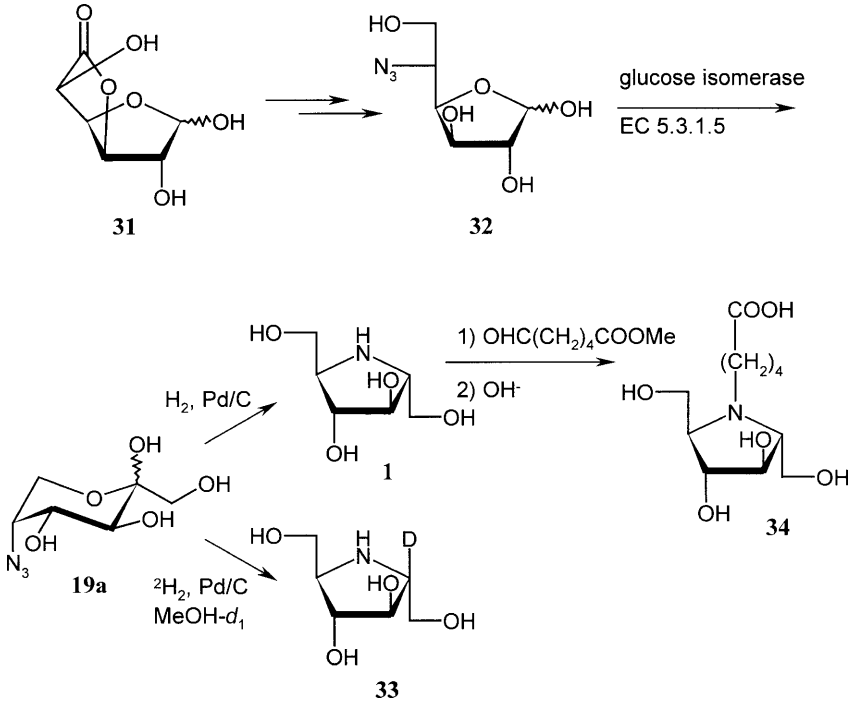
Another chemoenzymatic approach has been reported by *Stütz* and coworkers (Scheme 5) who exploited glucose isomerase (E.C. 5.3.1.5) in the key step [37]. 5-Azido-glucose **32**, easily available in six steps from *D*-glucurono-6,3-lactone **31**, was isomerized quantitatively to the 5-azidodeoxyfructose **19a** employing this enzyme. Conventional hydrogenation with concomitant intramolecular reductive amination furnished **1**. The N-(5-carboxypentyl) derivative **34** was prepared by reductive alkylation with adipic methyl ester hemialdehyde and subsequent saponification of the ester. Coupling to aminohexylsepharose with water-soluble carbodiimide and N-hydroxysuccinimide for activation led to an immobilized inhibitor which could be used as affinity ligand for the purification of glycosidases as previously demonstrated with the corresponding 1-deoxynojirimycin derivative attached to sepharose. The 2-^{2}H-derivative of *DMDP* **33** was prepared employing deuterium gas in methanol-d_1 as the solvent [38].

The N-methyl derivative **139** was made available by reductive amination of **1** with formaldehyde [39], and the iodide of the N,N-dimethyl derivative **140** by N-methylation with iodomethane [40] (Fig. 7).

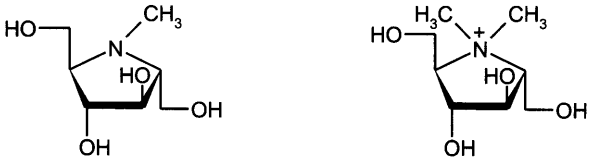
Liu and coworkers reported a synthesis starting from arabinofuranose derivative **35** [41] (Scheme 6). *Wittig* methenylation to **36** and subsequent *Swern* oxidation gave an unstable unsaturated ketone which was immediately converted to oxime **37**. Reduction and protection of the nitrogen with benzyloxycarbonyl chloride (*Cbz*)



Scheme 4

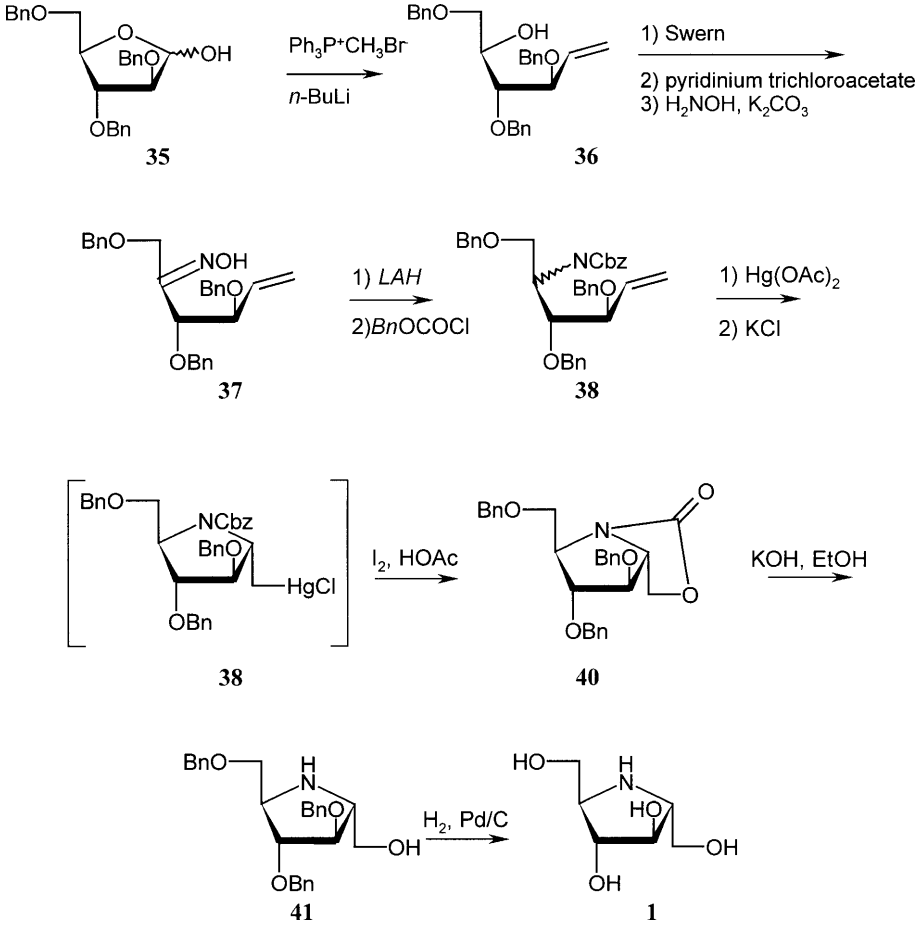


Scheme 5



139 **140**

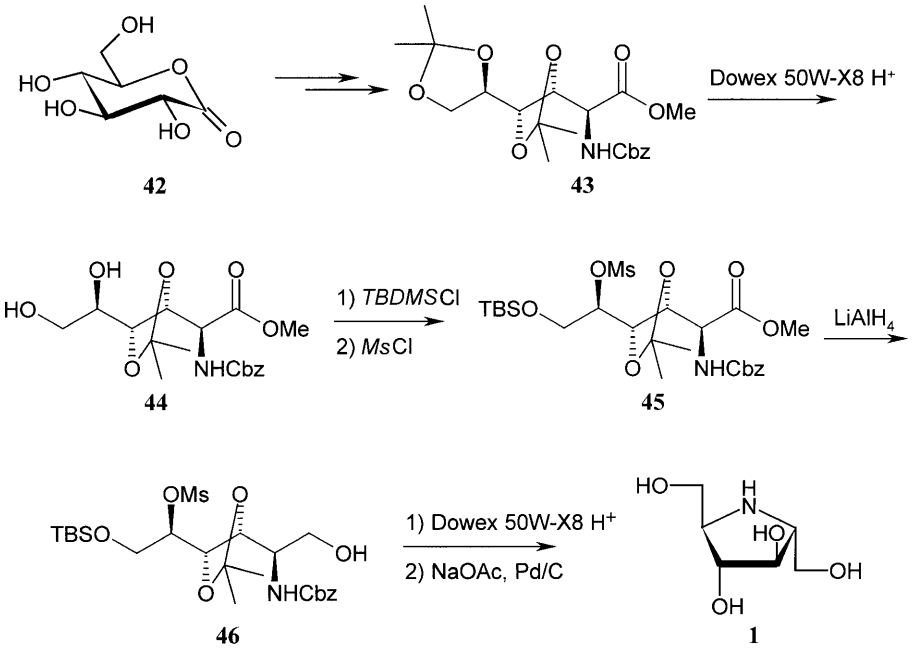
Fig. 7. Structures of compounds **139** and **140**



Scheme 6

gave a mixture of C-5 epimers **38** in a ratio of 7:1 for the desired *Cram* product which was separated by HPLC. Cyclization gave the correctly configured product **39** which was converted to **41** *via* **40**. Subsequent deprotection yielded **1**.

Lee and coworkers [42] developed a synthetic approach starting from D-glucono-δ-lactone **42** (Scheme 7) which was transformed to mannonate **43**. The deprotection of the terminal 5,6-O-isopropylidene group was carried out with Dowex 50W-X8 H^+ to yield 96% **44**. The primary hydroxyl group was silylated, followed by O-mesylation to afford **45**. Reduction of the methyl ester gave alcohol **46**. The remaining acid-labile protecting groups were cleaved with Dowex 50W-X8 H^+,



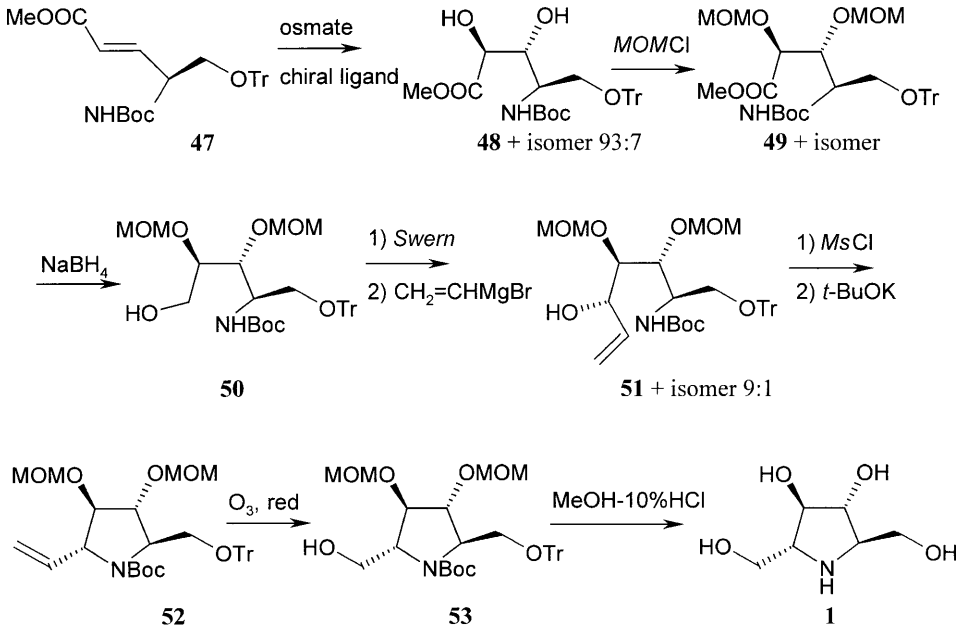
Scheme 7

and removal of the carbamate gave **1** in 67% yield from the di-O-isopropyl derivative **43**.

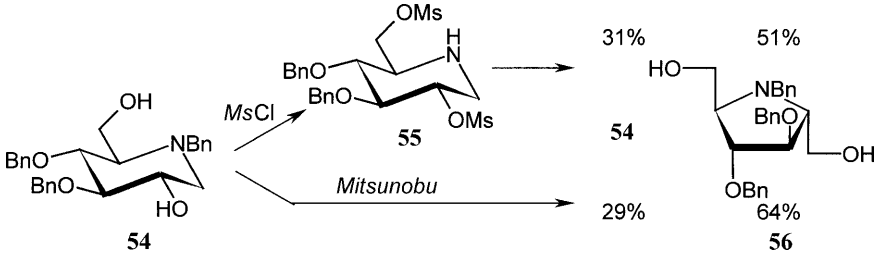
Ikota [43] used a dihydroxylation protocol for the functionalization of **47** (Scheme 8). The dihydroxylation was carried out with potassium osmate employing hydroquinidine-9-phenanthryl ether as a chiral ligand in the presence of $K_3Fe(CN)_6$ and K_2CO_3 in *tert*-BuOH-H_2O to give precursor **48** as the main product which could be separated from its isomer after O-methoxymethylation of the hydroxyl groups. Reduction to alcohol **50** followed by *Swern* oxidation and subsequent reaction of the resulting aldehyde with vinylmagnesium bromide gave a mixture of isomers in a ratio of 9:1 in favour of the desired intermediate **51**. This was, after separation by column chromatography, mesylated and cyclized with potassium *tert*-butoxide to furnish intermediate **52**. Ozonolysis and reduction to **53** followed by acidic removal of the protecting groups gave **1**.

Merrer and coworkers (Scheme 9) found that isomerization of partially protected polyhydroxypiperidines such as 1-deoxynojirimycin derivative **54** can be achieved by activating C-2 to give **55** which can undergo various transformations [44]. Activation of derivative **54** was carried out either by mesylation of the free hydroxyl groups or under *Mitsunobu* conditions (Ph_3P-*DEAD*-$PhCO_2H$, *THF*) at 0°C. In both cases a mixture of starting material and ring-contracted compound was obtained in favour of the pyrrolidine **56** in ratios of 31%:51% and 29%:64%, respectively. This has been explained by the ease of neighbouring nitrogen participation, since the leaving group is in equatorial position, and subsequent ring opening of the aziridinium ring occurs at the less substituted side.

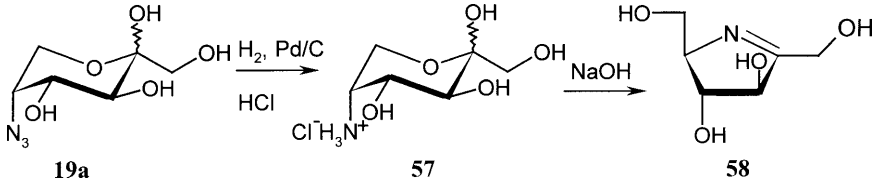
Efforts were also made to trap the cyclic imine resulting from the reduction and ring closure of the corresponding azidodeoxyfructose derivative **19a** (Scheme 10),



Scheme 8

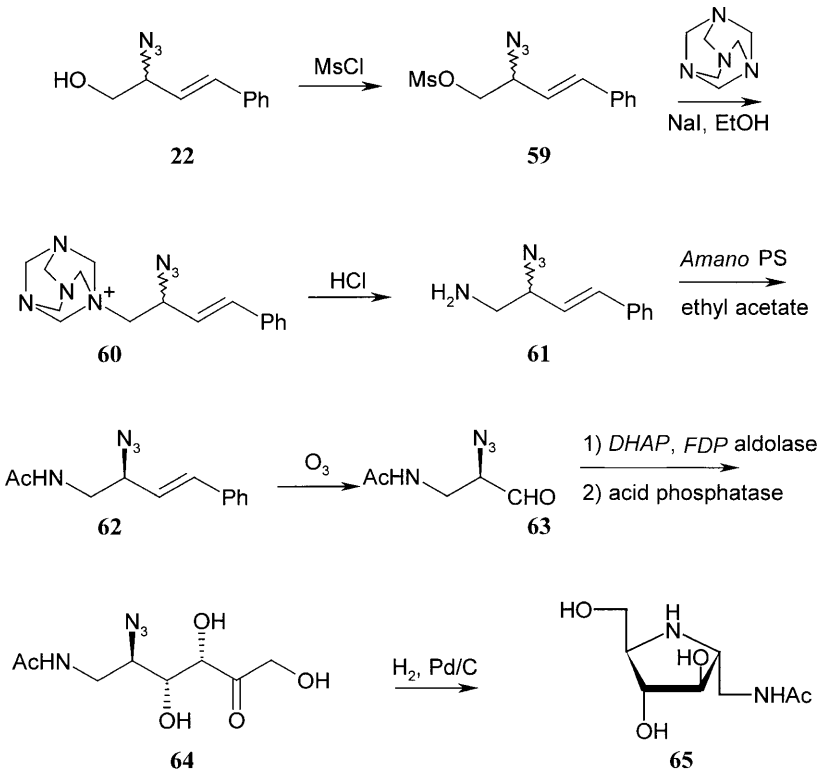


Scheme 9



Scheme 10

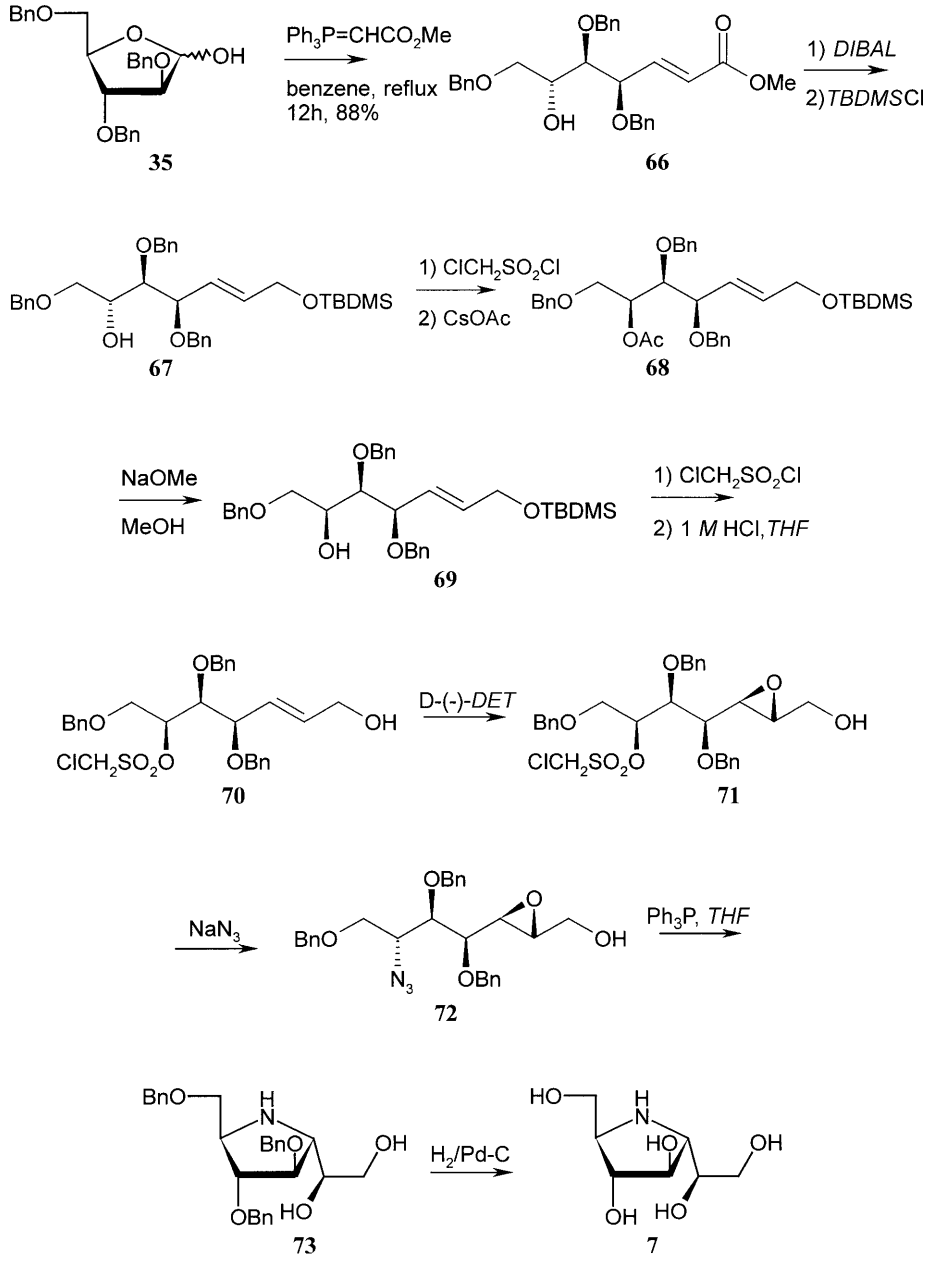
whose shape and charge were assumed to mimic the transition state stabilized by glycoprocessing enzymes [45]. Thus, the aminosugar intermediate was trapped as the corresponding hydrochloride **57**. When the salt was transferred into basic solution, the compound immediately formed an equilibrium in which the cyclic imine **58** was the major species present.



Scheme 11

Following the work of *Whitesides* and his group, the 1-acetamidodeoxy derivative of *DMDP* **65** was synthesized by *Wong* and coworkers employing a chemoenzymatic approach [46] (Scheme 11). The synthesis of the azidoaldehyde **63**, the key intermediate for the enzymatic step, started from azidoalcohol **22** whose mesylation gave **59** which by treatment with hexamethylenetetramine afforded ammonium salt **60**. The primary amine **61** was obtained by hydrolysis of **60** in concentrated hydrocloric acid. Enzymatic resolution exploiting *Pseudomonas* lipase in ethyl acetate gave the desired stereomer **62** in a yield of 34% based on a mixture of the primary amines **61**. Ozonolysis in methanol followed by reductive work-up employing dimethyl sulfide produced the azidoaldehyde derivative **63** required for the *FDP*-aldolase catalyzed condensation with *DHAP*. The phosphates were removed with acid phosphatase, and subsequent hydrogenation led to the desired 1-acetamido derivative **65**.

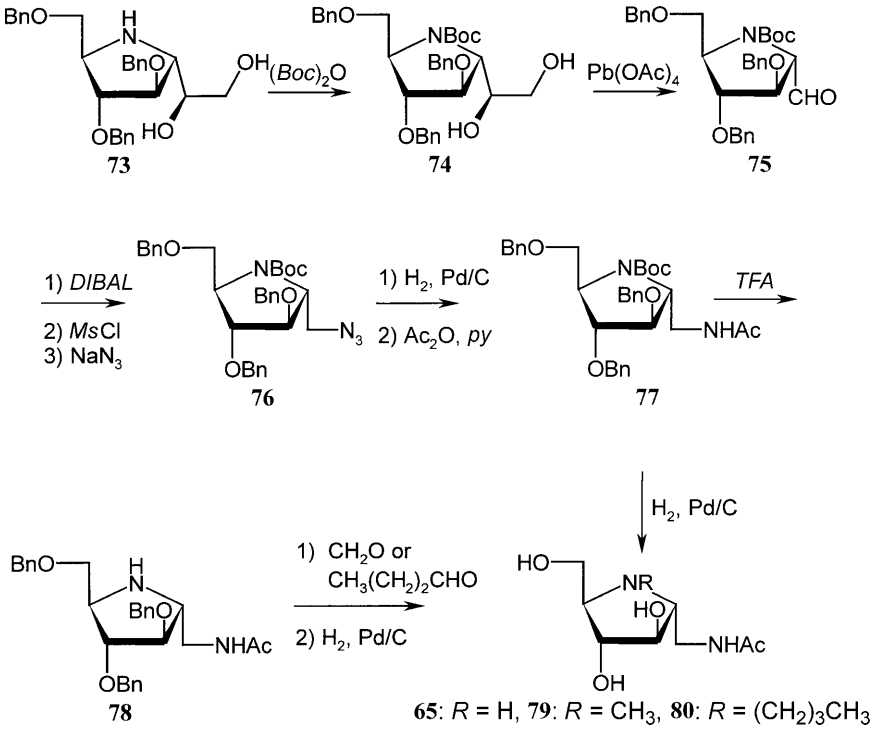
The *DMDP* homologue **7** was also synthesized by *Wong et al.* [47] who started from 2,3,5-tri-O-benzylarabinose **35** (Scheme 12). *Wittig* reaction gave the *E*-configured **66** in good yield. Reduction of the ester to the corresponding alcohol and subsequent protection of the latter as silylether furnished **67** bearing a free hydroxy group at position C-6. This was activated with chloromethylsulfonyl chloride, and a S_N2 reaction gave **68** in 60% yield. *Zemplen* deprotection gave **69** and was followed by chloromethylsulfonation and subsequent removal of the silyl protecting group to obtain **70**. Sharpless epoxidation employing *D*-(–)-diethyl tartrate led to the epoxide **71** in a highly stereoselective reaction. Replacement of the



Scheme 12

chloromethylsulfonate with azide allowed the desired introduction of the azido group at this carbon under formal overall retention of configuration at this center to yield **72**. Reduction of the azide to the amine, ring closure to **73**, and removal of the protecting groups furnished **7**.

The same group has reported the synthesis of several N-alkylated derivatives [48] of **7** as well as of the 1-acetamidodeoxy derivative **65** (Scheme 13). Starting from the precursor **73**, straightforward derivatization, N-*Boc* protection to **74**, and introduction of the acetamido group at C-1 led to derivative **77** which was either converted to the known compound **65** or, by deprotection of the nitrogen with *TFA*,



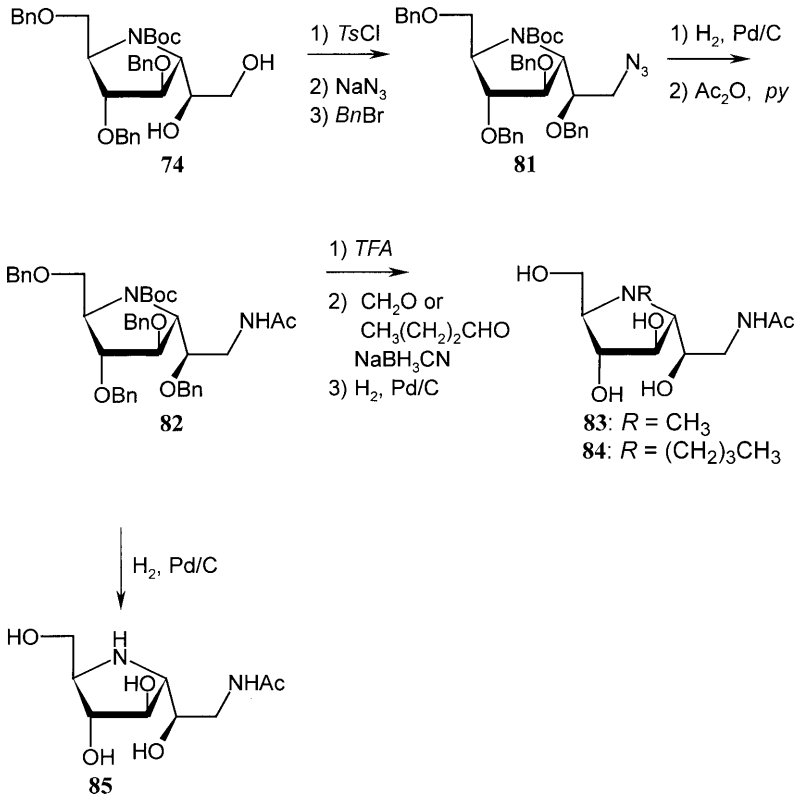
Scheme 13

to **78**. N-Alkylation employing two different aldehydes followed by deprotection of the hydroxyl groups led to N-alkylated derivatives **79** and **80**. These three products exhibited K_i values with β-N-acetylglucosaminidase in the low micromolar range.

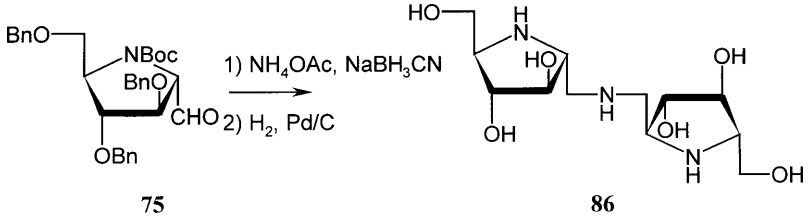
The acetamidodeoxy homologues were synthesized in a similar manner (Scheme 14). Tosylation of the primary hydroxyl group of **74**, followed by substitution employing sodium azide and protection with benzyl bromide, furnished **81**. Reduction of the azide and acetylation of the resulting amine gave acetamide **82** which underwent hydrogenolysis yielding derivative **85**. On the other hand, deprotection of the nitrogen, reductive alkylation employing aldehydes, and hydrogenolysis gave the two N-alkylated compounds **83** and **84**.

A dimeric form of *DMDP* featuring a nitrogen bridge between the to rings was prepared by coupling aldehyde **75** under reductive amination conditions employing ammonium acetate and sodium cyanoborhydride to yield **86**.(Scheme 15)

The synthesis of (difluoromethyl)-phosphonate iminosugars was investigated by *Guillerm* and coworkers [49] when they became interested in using such compounds as glycosyl transferase inhibitors (Scheme 16). 2,3,5-Tri-O-benzyl-*L*-xylose **87** was reacted with benzylamine to yield furanosylamine **88** which was exposed to (diethylphosphinoyl)-difluoromethyllithium furnishing a separable mixture of the two possible diastereomers **89a** and **89b** with moderate diastereoselectivity. For the final cyclization step, the hydroxyl group was activated as the methanesulfonate which underwent intramolecular nucleophilic displacement by the amino group leading to **90**.



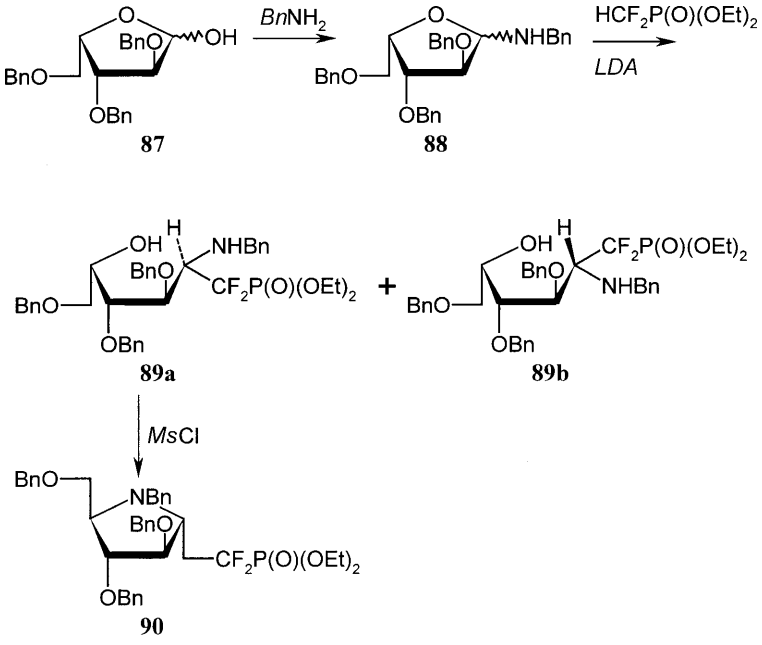
Scheme 14



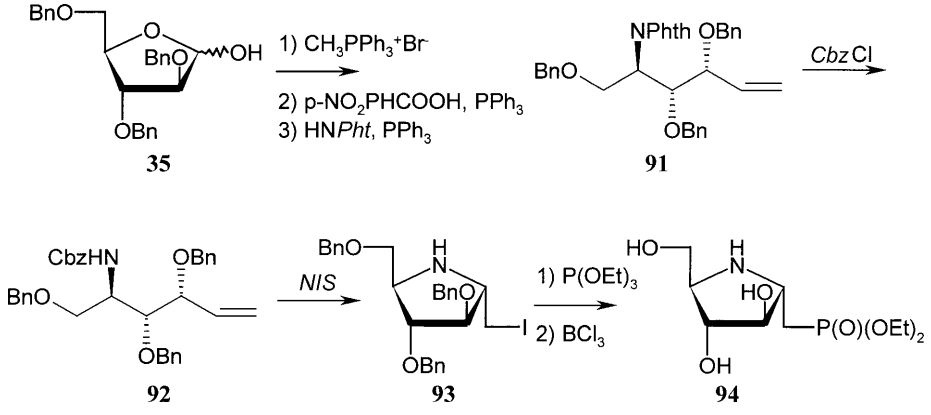
Scheme 15

Eustache and his group [50] were also interested in such 1-phosphonate derivatives in the context of glycosyltransferases. Starting from 2,3,5-tri-O-benzyl-*D*-arabinofuranose (**35**, Scheme 17) which underwent *Wittig* olefination and subsequent *Mitsunobu* reaction employing *p*-nitrobenzoic acid followed by phthalimide protection of the nitrogen, **91** was obtained. The nitrogen protecting group was then exchanged to N-*Cbz* protected **92**. Ring closure was achieved by treatment with *NBI*, and reaction of iododeoxy compound **93** with triethylphosphite followed by deprotection yielded the desired 1-phosphonate **94**.

A C-1 aryl derivative was synthesized recently by *Correia* and coworkers [51] during their synthetic studies of codonopsine and codonopsinine, two pyrrolidine alkaloids (Scheme 18). The key step was the *Heck* reaction on endocyclic enecarbamates **95** and **96**, which was carried out in the presence of a diazonium salt



Scheme 16

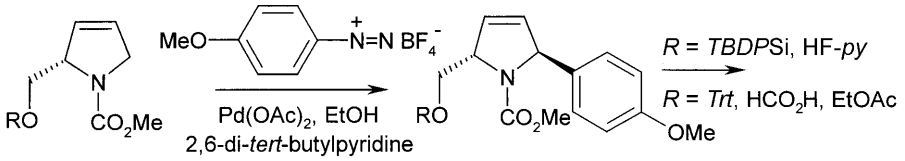


Scheme 17

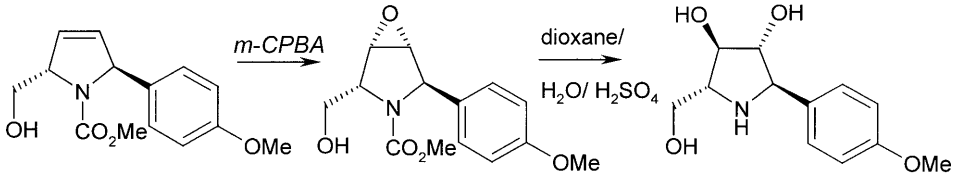
and 2,6-di-*t*-butylpyridine to yield the C-1-arylated compounds **97** and **98** in a highly regio- and stereoselective manner. The silyl and trityl groups in **97** and **98**, respectively, were removed to yield intermediate **99** which was converted to a single epoxide **100** employing *m-CPBA*. Subsequent acidic hydrolysis led to **101**.

Fleet and coworkers synthesized the achiral aliphatic version of *DMDP* starting from dimeric dihydroxyacetone **102** by reductive amination employing ammonium chloride. **103** showed no biological activities with the common glycosidases [52] (Scheme 19).

The first O-glycosylated derivative of *DMDP* was synthesized by *Stütz* and coworkers [53] (Scheme 20) by reacting intermediate 5-azidodeoxyfructose **19a** with

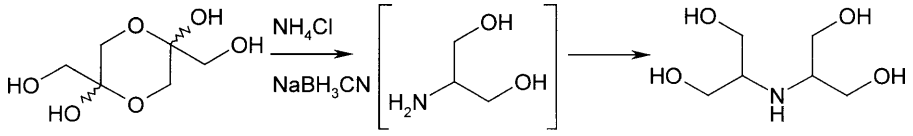


95: *R* = *TBDPS*
96: *R* = trityl
97: *R* = *TBDPS*
98: *R* = trityl



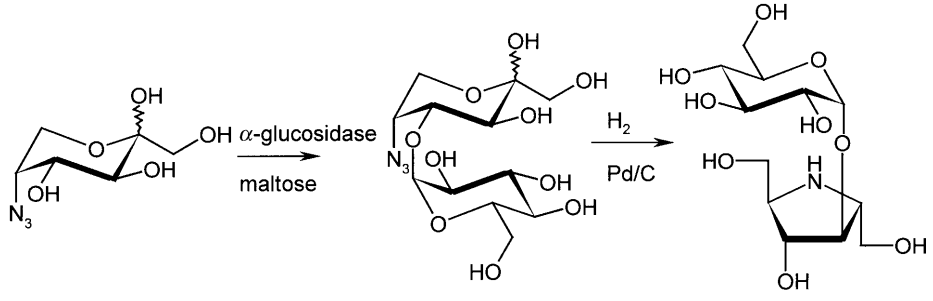
99 **100** **101**

Scheme 18



102 **103**

Scheme 19

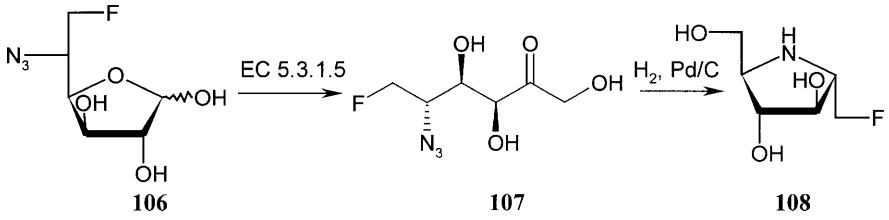


19a **104** **105**

Scheme 20

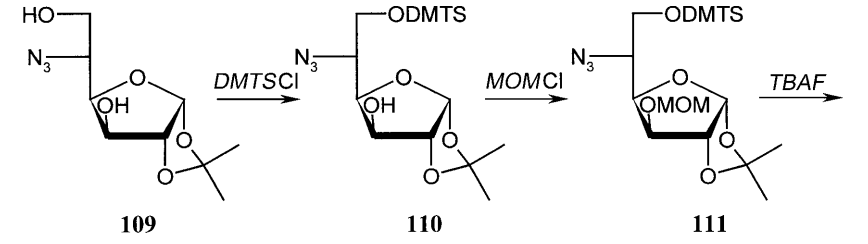
α-glucosidase from yeast in the presence of maltose as the donor. Glycosylation took place at positions O-1 and O-4; the isomers could be separated by chromatography on charcoal. Conventional hydrogenation of disaccharide **104** over Pd/C led to the O-4 glucosylated derivative **105**.

In the mid-nineteen-nineties, C-1 substituted derivatives of *DMDP* aroused interest. The first compounds prepared were the 1-deoxyfluoro-, 1-O-methyl, and 1-aminodeoxy derivatives of *DMDP* (**108**, **116**, and **118**). The 1-deoxyfluoro compound was easily accessible in two steps from known 5-azido-5,6-dideoxy-6-fluoro-D-glucofuranose (**106**, Scheme 21) by enzymatic isomerization to the

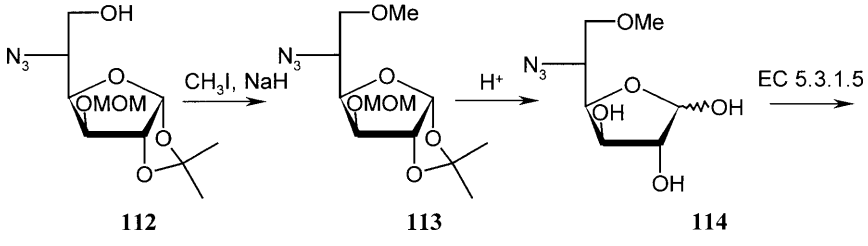


106 **107** **108**

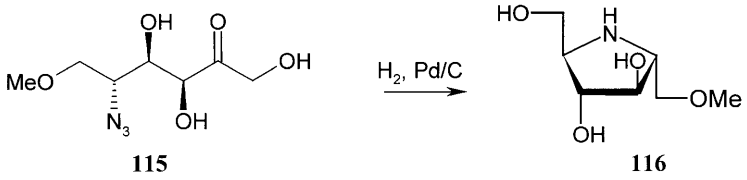
Scheme 21



109 **110** **111**



112 **113** **114**



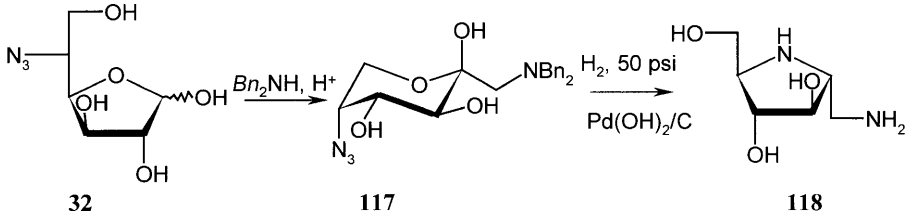
115 **116**

Scheme 22

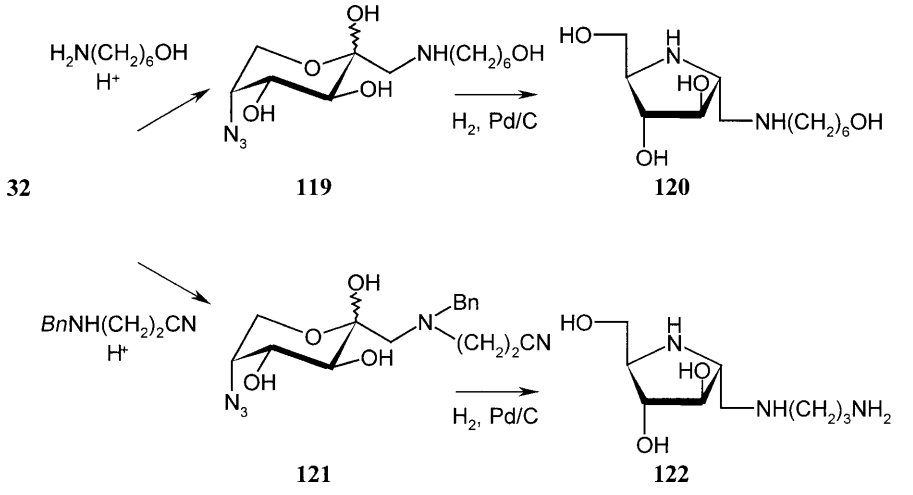
open-chain ketose **107** employing glucose isomerase, followed by conventional intramolecular reductive amination leading to the 1-fluoro derivative **108** [54].

The 1-O-methyl derivative **116** [54] was prepared from 5-azido-5-deoxy-1,2-O-isopropylidene-α-*D*-glucofuranose (**109**, Scheme 22). Reaction with chlorodimethyl-(1,1,2-trimethylpropyl)-silane gave O-6 silyl ether **110**. O-Methoxymethylation afforded the fully protected sugar **111**. Subsequent desilylation of O-6 using tetrabutylammonium fluoride to obtain **112** and O-methylation with iodomethane in the presence of sodium hydride gave intermediate **113**. Acidic deprotection to **114** and enzymatic isomerization to the corresponding open-chain *D*-fructose derivative **115** followed by hydrogenation yielded **116**.

The 1-aminodeoxy compound **118** was obtained following a different approach [55] (Scheme 23). Bearing in mind that the release of ring strain in the 5-membered



Scheme 23



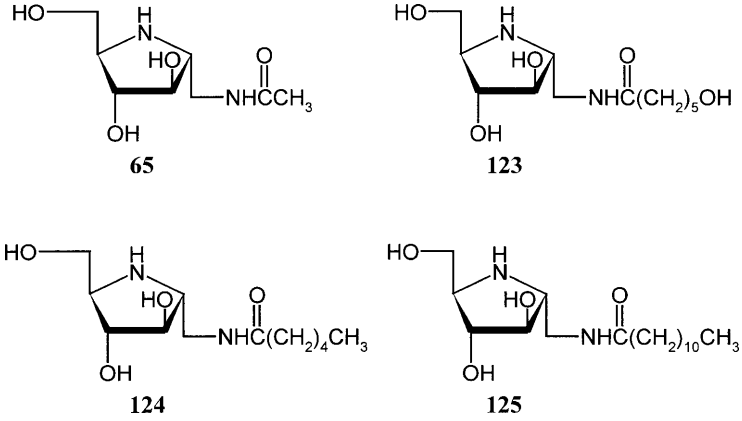
Scheme 24

ring **32** is a strong driving force for the quantitative isomerization into the corresponding D-fructopyranose isomer [56], it could be expected that an *Amadori* rearrangement reaction would introduce the desired amino group under concomitant formation of the D-fructopyranose derivative. Thus, employing dibenzylamine in an *Amadori* rearrangement [57] on 5-azidodeoxy-D-glucose **32** gave the rearrangement product **117** in almost quantitative yield. Hydrogenolysis and concomitant intramolecular reductive amination afforded **118**.

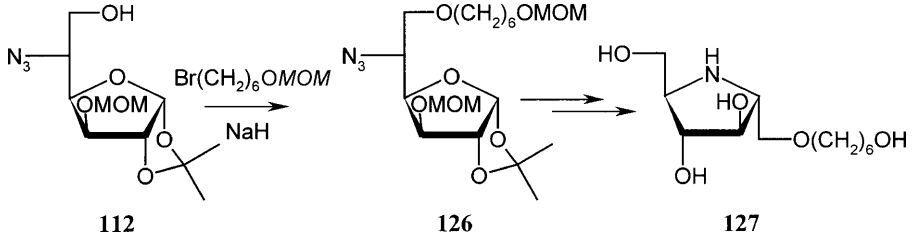
The *Amadori* rearrangement of 5-azidodeoxyglucose **32** was also performed employing 6-aminohexanol as well as 3-(N-phenyl)-amino propionitile to furnish the rearrangement products **119** and **121**, respectively (Scheme 24). Catalytic hydrogenation gave the 6-hydroxyhexyl derivative **120** and the triamine **122** [58].

118 was subsequently shown to react highly chemo- and regioselectively with acylating agents at the primary amine (Scheme 25); the 1-acetamido derivative **65** could easily be obtained by reaction of **118** with acetic anhydride in methanol, and the hydroxyhexanoyl, hexanoyl, and dodecanoyl derivatives **123**, **124**, and **125** resulted from the reaction with caprolactone, hexanoic anhydride, and dodecanoic anhydride, respectively [59].

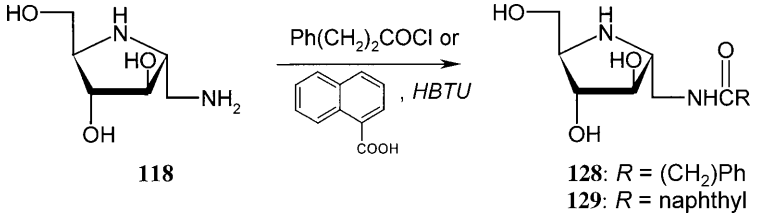
To extend the series of hydroxyhexyl derivatives, the corresponding 1-O-hydroxyhexyl ether **127** was synthesized taking advantage of the same synthetic approach as reported for the methyl ether **116**, employing methoxymethyl-protected 6-hydroxybromohexane for the ether formation to obtain precursor **126** (Scheme 26).



Scheme 25



Scheme 26

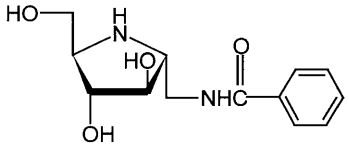


Scheme 27

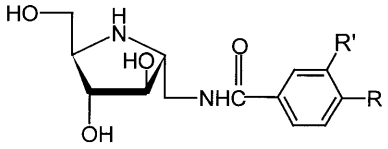
This was converted to the desired inhibitor **127** following the conventional route [58]. Introduction of aromatic substituents at position 1 of **118** gave, for example, the phenylpropanoyl and naphthoyl derivatives **128** and **129** [59] (Scheme 27).

Other examples in this series are the phenyl (**130**), the 4- and the 3-(dimethylamino)-phenyl (**131** and **132**, respectively), and the coumarin derivative **134** (Scheme 28). These compounds were synthesized by coupling the primary amino group in **118** with the corresponding carboxylic acid moieties employing *HBTU* as the coupling reagent. Reaction of **118** with naphthylsulfonic chloride gave the corresponding sulfonamide derivative **133** [59].

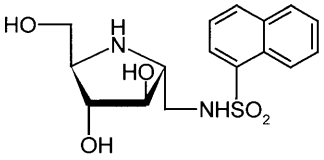
Interestingly, all compounds bearing an aromatic substituent exhibited very good to excellent inhibitory activities with the β-glucosidase from *Agrobacterium sp.*



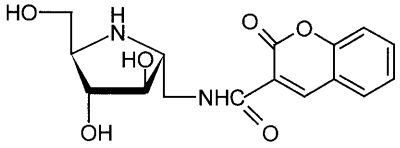
130



131: $R = NMe_2$, $R' = H$
132: $R = H$, $R' = NMe_2$

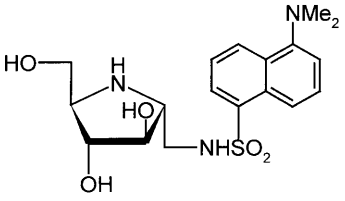


133

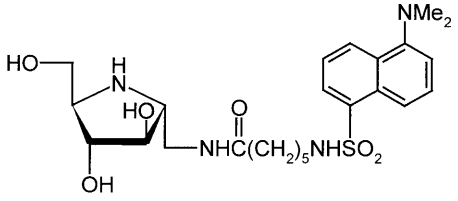


134

Scheme 28



135



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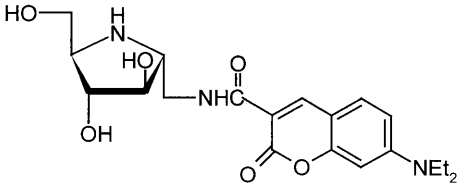
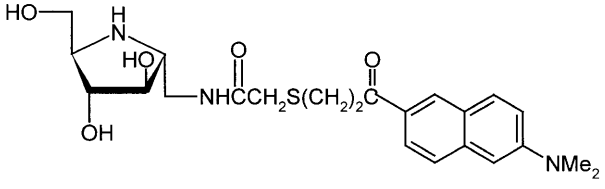


137



138

Scheme 29

with K_i values in the low micromolar to nanomolar range (Table 4). Thus, it was envisaged that derivatives of *DMDP* labelled with aromatic fluorescent tags might also be good inhibitors and, consequently, might be useful potential diagnostic compounds for monitoring enzyme–inhibitor interactions exploiting fluorescence

spectrometry methods. N-Dansyl derivative **135** as well as a related compound featuring a C_6 spacer arm between the dye and the inhibitor (**136**) were synthesized [60] (Scheme 29). Their excellent inhibitory activities in the single-figure nanomolar range prompted the subsequent preparation of the diethylaminocoumarin and acrylodan derivatives **137** and **138**.

Biological Activities and Applications

DMDP has been shown to be a very interesting compound concerning its biological activities such as interaction with glycoprotein processing glucosidase I, anti-retroviral activity (including HIV), antifeedant properties against important pest insects, and trehalase, invertase, and *D*-fructofuranosidase inhibition. It also exhibits plant growth regulatory activity as well as anti-cancer properties.

Activity of DMDP and related compounds against various glycosidases

DMDP has been screened against a wide variety of glycosidases from several different sources as shown in Table 1. Compared to closely related compounds such as **2**, **3**, **5**, and **7**, *DMDP* shows to be a universal inhibitor of such enzymes.

A very interesting study was performed by *Fleet* and coworkers who investigated the inhibitory properties of *DMDP*, **2**, **3**, and **5** against mouse gut dissaccharidases. Inhibitors of such enzymes bear potential for the treatment of diabetes, obesity, and related metabolic disorders. Although these compounds failed to inhibit α-amylase, which to date is only inhibited by the pseudotetrasaccharide acarbose, these compounds have potential as research tools and can help to understand many aspects of carbohydrate metabolism [61].

A structural basis of inhibition of mammalian glycosidases has been deduced by *Asano* and coworkers when testing **1**, **2**, and **5** amongst other iminoalditols against several of these enzymes. *DMDP* was shown not to be an inhibitor of ER α-glucosidase II [22], whereas it is known to inhibit α-glucosidase I in cell cultures causing the accumulation of glycoproteins with high-mannose oligosaccharide structures and, thus, is a specific inhibitor of this enzyme [66].

The ability of *DMDP* and related iminoalditols to inhibit the hydrolysis of the glucoinolates sinigrin and progitin, which are thioglucosides present in the cruciferae having a variety of biological properties such as insect antifeedant activity, by inhibition of thioglucosidase has been shown by *Scofield et al.* [62]. Additionally, the protein processing glucosidase I from mung bean seedings was also shown to be inhibited by *DMDP* ($IC_{50} = 40\ \mu M$), **2** ($IC_{50} = 5\text{–}7\ \mu M$) and **3** ($IC_{50} = 2\text{–}3\ \mu M$) exhibited stronger activities [63].

Early investigations have shown that *DMDP* is an inhibitor of invertase (β-*D*-fructofuranosidase), an enzyme which is known to act by a mechanism analogous to that of glucosidases and that hydrolyses sucrose. *DMDP* showed 50% inhibition at about $1.5\ \mu M$ for the invertase at a *pH* above 6.5 [32]. *DMDP* also inhibits *PFP* (pyrophosphate-*D*-fructose-6-phosphate-1-phosphotransferase), an enzyme in plants, which is activated by fructose 2,6-diphosphate and catalyzes a reversible reaction at a crucial point in the synthesis of sucrose in young tissues and the utilization of this sugar in meristems. Because *DMDP* inhibits *PFP* to an extent of

48% in the forward direction and 52% in the reverse direction, it was expected to be useful in the bio-rational design of herbicides [41].

Anti-viral activity

Anti-HIV activity of *DMDP* is caused by inhibition of α-glucosidase I, an enzyme involved in the processing of N-linked olidosaccharides on glycoproteins. The inhibitory effect is weak when compared to the value of **2** and interestingly, contrasting **2**, N-alkylation does not lead to any increase in its potency [64]. Tested on influenza-infected MDCK cells, *DMDP* inhibited the processing of the viral hemagglutin by inhibiting α-glucosidase I [65] and inhibited at a concentrations of 250 μM α-glucosidase I to accumulate glycopeptides exhibiting Glc_3-Man_9-($Glac$NAc)$_2$ as the major oligosaccharide [66].

Effects on insects

Very early, *DMDP* has been found to be toxic to insects. The potential role as a plant protective chemical against a range of phytophagous insects was investigated by examining the effects on development, feeding behaviour, and functioning of taste receptors, and it could be shown that *DMDP* is an effective anti-feedant [67]. Furthermore, *DMDP* was found to be active against digestive enzymes from larvae of *Callosobruchus maculatus*, making it an interesting lead substance in the search for new insecticidal compounds [68]. IC_{50}-values with these enzymes were 0.6 micromolar against α-glucosidase and 340 micromolar against β-glucosidase, showing its lethal activity to the larvae of the bruchid beetle. The antifeedant activities against other insects such as the larvae of the lepidopterans *Spodoptera littoralis*, *Spodoptera frugiperda*, *Heliothis virescens*, and *Helicoverpa armigera* turned out to be consistently effective [69].

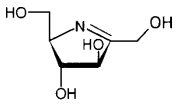
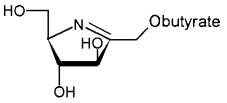
Several other insect species such as the orders *Orthoptera*, *Phasmida*, *Dictyoptera*, *Diptera*, and *Cleoptera* were screened for their inhibition of glycosidase activity by *DMDP* as well as other polyhydroxylated alkaloids. *DMDP* inhibited both α-glucosidase, including trehalase, and β-glucosidase activity strongly in these insects [70a]. Differentiation of glycosidase activity in some *Hemiptera* and *Lepidoptera* was also examined. The data obtained suggest that several separate enzymes or active sites are responsible for maltose and sucrose hydrolysis in aphids and *H. melpomone*, a species of larval *Lepidoptera* [70b]. Additionally, *DMDP* shows also potent antifeedant activity against important pest insects [71].

Activities of derivatives of DMDP

Related natural products bearing modifications at position 6 such as compounds **7** and **8** were found in various Hyacinthaceae and were tested against several glycosidases (Table 2). These studies were used for the explanation of the symptoms of poisoning live stock by bluebells [24].

Iminosugars **58** and its O-1-butyrate were believed to mimic the transition state stabilized by glycoprocessing enzymes and were tested against a few glycosidases

Table 3. Activities of **58** and **140**

Enzyme	*pH*	*DMDP*	 **58**		Ref.
β-Glucosidase (almonds)	6.8	$K_i = 7.8$	$K_i = 13 \pm 0.7$	NI	[45]
α-Glucosidase (brewer's yeast)	6.8	$K_i = 3.3$	$K_i = 2.6 \pm 0.2$	$K_i = 167 \pm 29$	[45]
β-Galactosidase (*E. coli*)	6.8	NI	$K_i = 276 \pm 51$	$K_i = 253 \pm 44$	[45]
α-Mannosidase (jack beans)	6.8	NI	$K_i = 17 \pm 1$	NI	[45]
α-Fucosidase (bovine epidimysis)	6.8	NI	$K_i = 381 \pm 71$	$K_i = 336 \pm 54$	[45]

(Table 3). Most notable was the inhibition of **58** against α-mannosidase, as *DMDP* itself does not show any activity against this enzyme at all [45].

Information about the active site of invertase (β-fructofuranosidase), the plant enzyme that splits sucrose into *D*-glucose and *D*-fructose, was obtained from the N-methyl and N,N-dimethyl derivatives **139** and **140** (Fig. 7) as well as with the N-(carboxypentyl) derivative **34** (K_i values of **139**, **140**, and **34** were 20, 130, and 50 μM, respectively, at $pH = 6$). The reduced biological activities of the N-alkyl derivatives as compared to parent compound **1** indicate steric clashes of the N-substituents with the active site. The *pH* dependence of the K_i values as well as the moderate impairment of inhibitory potency places yeast invertase among the small group of glycosidases which are strongly inhibited by cationic rather than basic glycon analogues [37]. Interestingly, *DMDP* had virtually no effect on the enzyme from the mammalian gut which catalyzes the same reaction [72].

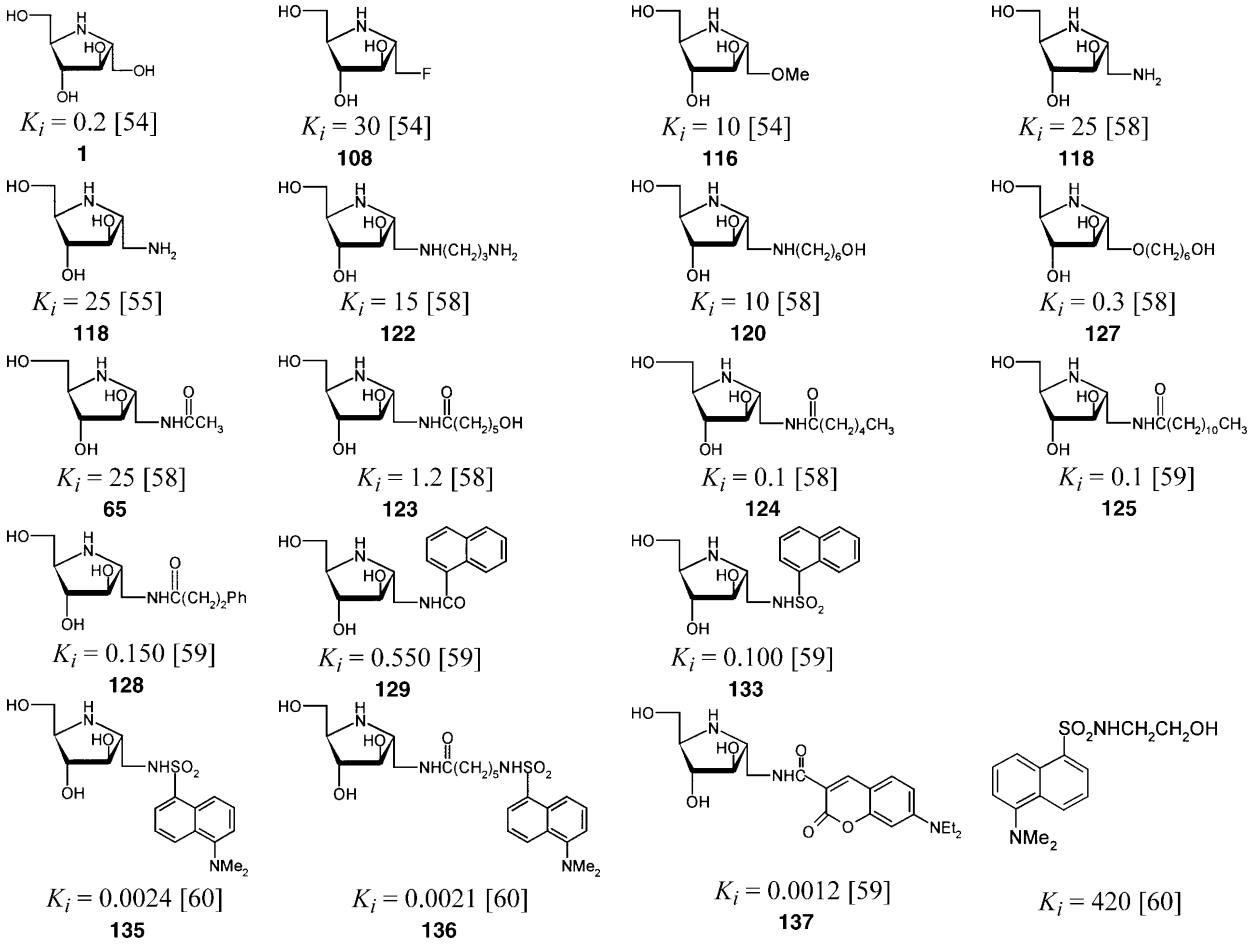
The 1-acetamido derivative **65** was found to be a potent inhibitor of N-acetyl-glucosaminidases with K_i values of 9.8 and 1.9 μM for β-N-acetylglucosaminidase from bovine kidney and jack beans, respectively, at *pH* 6.5 [46] but exhibited only weak activity against α-glucosidase from *Saccharomyces sp.* ($K_i = 380\ \mu M$ at *pH* 7) [48] as would have been predictable from its structure.

Recently, **65** and its N-methyl derivative **79** were suggested as new drug leads for the therapy of osteroarthritis. This disease is characterized by a decreased concentration of glycosaminoglycans in articular cartilage, the degradation being mainly caused by hexosaminidases. This findings might open new applications of *DMDP* and its derivatives as drugs with chondroprotective activity [73].

Interestingly, the homologous N-acetamido compound **85** as well as the corresponding N-alkylated derivatives **83** and **84** showed activities against β-glucosidase from sweet almonds (K_i values of 2.2, 45, and 120 μM, respectively, at *pH* 5) [48]. The dimeric form of *DMDP* (**86**) exhibited K_i values of 53 μM against α-glucosidase from *Saccaromyces sp.* (*pH* 7) and 37 μM against β-glucosidase from sweet almonds (*pH* 5) [48].

Good glucosidase inhibitory activities were found for C-1 modified analogues of *DMDP*. The 1-deoxyfluoro, 1-O-methyl, and 1-aminodeoxy derivatives **108**,

Table 4. Activities of C-1 derivatives of *DMDP* against β-glucosidase *Agrobacterium sp.*, $pH = 7.0$



116, and **118** exhibited moderate activities against β-glucosidase from *Argobacterium sp.* in the μM range (Table 4). Triamine **122** and the 1-N-hydroxyhexyl derivative **120** were similarly active. Surprisingly, all 1-N-acylamino derivatives such as **123**, **124**, and **125** showed K_i values comparable to that of **1**. Improved activities were detected with analogues bearing aromatic amides such as **128**, the corresponding sulfonamide **133**, and **129**. Fluorescently tagged compounds **135**, **136**, and **137** were found to be active in the low nanomolar range. This is noteworthy as only a few examples of related natural products and analogues, such as, for example, 1-deoxygalactonojirimycin (**141**, $K_i = 2\,\mathrm{n}M$ with α-D-galactosidase from human placenta), 2-acetamido-1,2-dideoxygalactonijirimycin (**142**, $K_i = 1.2\,\mathrm{n}M$ with N-Ac-β-D-glucosaminidase from jack beans), and 1-deoxyfuconojirimycin (**143** $K_i = 40\,\mathrm{p}M$ with canine α-L-fucosidase) have been reported to show similar activities [8] (Fig. 8).

Exploitation of the fluorescence properties of compound **135** has shown that this inhibitor is bound as a 1:1-complex with the enzyme. The experiment was conducted by quenching the intrinsic tryptophane fluorescence of a defined amount

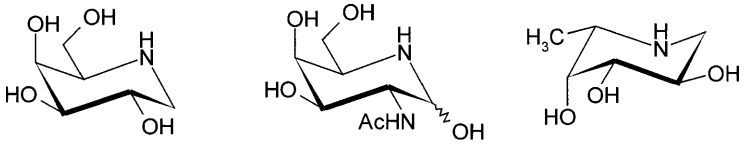


Fig. 8. Structures of compounds **141**–**143**

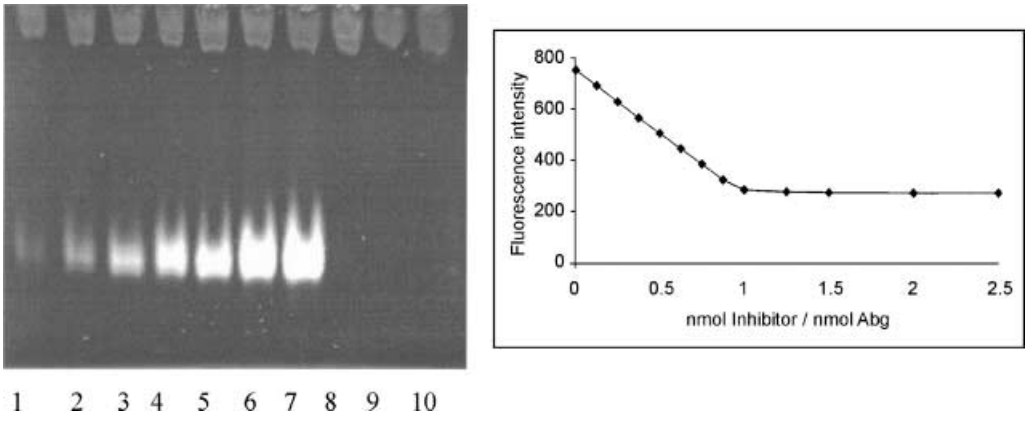


Fig. 9. Visualization of the fluoresent complex of **136** with *Agrobacterium sp.* β-glucosidase on native page and quenching of the intrinsic tryptophan fluorescence of *Agrobacterium sp.* β-glucosidase by titration with **136**

of *Agrobacterium sp.* β-glucosidase by titration with compound **135** (Fig. 9). The strong binding to the enzyme could be visualized very nicely on native PAGE gel with different amounts of enzyme incubated with **135** (Fig. 9, lanes 1–7), whereas other enzymes such as galactosidase, glucose isomerase, or albumin did not show binding to the inhibitor (lanes 8–10) [60].

From the studies mentioned above it can be concluded that the presence of aromatic substituents causes a dramatic increase of the inhibitory activities, resulting in K_i values in the nanomolar range. Consequently, it would be very interesting to test derivative **101** [51] as well as the corresponding allyl derivative [74].

Conclusions

Some well-known glycosidase inhibitors such as miglitol (**144**) and N-butyldeoxynojirimycin (**145**, Fig. 10) have been marketed for the treatment of non-insulin-

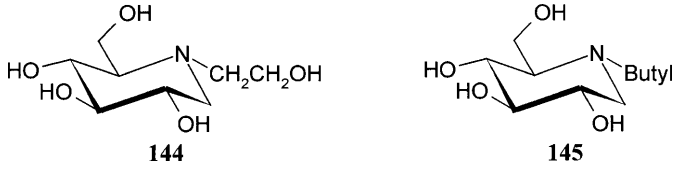


Fig. 10. Structures of compounds **144** and **145**

dependent and insulin-dependent diabetes mellitus, or, as in the case of **145**, undergo clinical trials due to promising anti-retroviral properties [8].

DMDP and quite a few of its derivatives were found to show very interesting biological activities in different glycosidase mediated processes, but, so far, despite many promising leads and fascinating biological properties, no commercial application has been reported. Nevertheless, compound **1** and some of its analogues have been shown to be powerful tools for glycobiology research. For example, purification of glycosidases by affinity chromatography employing immobilized **1** or fluorescent derivatives as reagents for high-throughput assays for enzyme or inhibitor discovery appear to be at hand.

Recent suggestions that *DMDP* derivatives might be new drug candidates for the therapy of osteroarthritis open venues towards pharmaceutical exploitation. A close relative of *DMDP* has recently been found to exhibit, albeit limited, antimycobacterial properties. Clearly, further investigations will show if the current gap from the established glycobiology tool to products of commercial value can be bridged.

Acknowledgements

The author thanks the Austrian *Fonds zur Förderung der wissenschaftlichen Forschung* (FWF), Vienna, for a *Hertha-Firnberg*-Fellowship (T-18 CHE) as well as an *Erwin Schrödinger* Research Grant (J 2092).

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Received November 14, 2001. Accepted November 19, 2001