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Looking glass inhibitors: L-DMDP, a more potent and specific inhibitor of α-glucosidases than the enantiomeric natural product DMDP†‡

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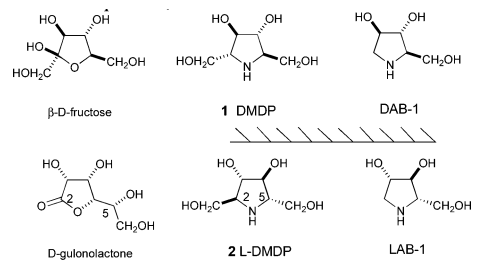
L-DMDP, prepared from D-gulonolactone, is a highly specific inhibitor of a number of plant and mammalian α-glucosidases [between 2 and 4 orders of magnitude more potent than the enantiomeric natural product DMDP] but is not an inhibitor of bacterial and yeast α-glucosidases. Additionally *N*-butyl-DMDP is a potent inhibitor of ceramide-specific glucosyltransferase but *N*-butyl-L-DMDP shows no inhibition.

Over 100 polyhydroxylated alkaloids have been isolated from plants and micro-organisms.[1] Such natural products and synthetic analogues have potential as mechanistic probes and chemotherapeutic agents for an ever widening number of diseases.[2] Many can be viewed as mimics of individual sugars in which the ring oxygen has been replaced by nitrogen.[3] Although glycosidase inhibition has been the driving force for the initial investigations of this class of compounds,[4] it is probable that many of their biological properties[5] are not related to their ability to inhibit glycosidases[6]—even apparent mimics of sugars in practice act as mimics of other compounds, such as ceramide.[7]

Among iminosugar pyrrolidine analogues, DMDP **1** [Scheme 1] is one of the most widespread of secondary metabolite sugar mimics.[8] DMDP can be viewed as a nitrogen analogue of β-fructose and is related to another natural product DAB-1[9] by the removal of either of its hydroxymethyl groups. *Both* the natural product DAB-1 and the synthetic enantiomer LAB-1[10] inhibited glucosidases, although their relative abilities to inhibit a particular glucosidase varied;[11] antiviral properties of derivatives of both DAB-1[12] and LAB-1[13] have been reported. DMDP has long been reported as a good glucosidase inhibitor with mild inhibition of some other glycosidases; the inhibitory properties of the enantiomer L-DMDP **2** have not been studied. This paper reports that L-DMDP **2**, synthesised from D-gulonolactone, is a more powerful and more specific α-glucosidase inhibitor than the enantiomeric natural product DMDP **1**. Also the fact that *N*-butyl DMDP inhibits ceramide-specific glucosyltransferase[14] may make L-DMDP more attractive in the inhibition of gut disaccharidases than the less specific natural product.

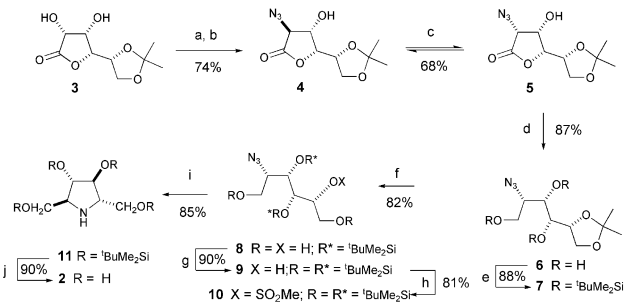


Scheme 1

Although the first syntheses of DMDP **1** appeared in 1985,[15,16] new approaches are still regularly reported from carbohydrate[17] and other starting materials.[18,19] In contrast, the only synthesis of L-DMDP **2** hitherto described involves the diastereoselective reduction of a ketosulfoxide with an overall yield from a protected D-tartrate of 10% in 11 steps.[20] The formation of L-DMDP from D-gulonolactone requires the introduction of a nitrogen bridge between C-2 [with retention of configuration] and C-5 [with inversion of configuration] and adjustment of the oxidation level at C-1 [Scheme 2].

Treatment of D-gulonolactone with 2-methoxypropene in DMF in the presence of *p*-toluenesulfonic acid (*p*-TSA) afforded the kinetic acetonide of D-gulonolactone **3**[21] which underwent preferential esterification of the C-2 hydroxyl group by reaction with triflic anhydride in acetonitrile; treatment of the resulting triflate with sodium azide in DMF gave the azide **4** [yields for each step of the synthesis are given in Scheme 2].

In order to introduce the nitrogen with overall retention of configuration at C-2 it was necessary to equilibrate the *trans*-2-azido-lactone **4** to the thermodynamically more stable all *cis*-2-azido-lactone **5**.[22] The remote stereochemistry at C-5 plays an important role in the epimerisation; the *gluco trans*-azide (5-*epi*-**4**) was easily epimerised to the *manno cis*-azide (5-*epi*-**5**) by stirring with sodium azide at room temperature in DMF. In contrast, the *idono*-azide **4** was stable under the same conditions, with negligible epimerisation taking place. However, the *idono*-azide **4** may be equilibrated by pyridinium *p*-toluenesulfonate (PPTS), to the more stable *gulono*-azide **5** isolated in 68% yield together with 27% of **4**;



Scheme 2 (a) $(CF_3SO_2)_2O$, MeCN, pyridine, N_2, −30 °C, 3 h. (b) NaN_3, DMF, r.t., 26 h. (c) pyridinium *p*-toluenesulfonate, DMF, N_2, r.t., 7 days. (d) $LiBH_4$, THF, N_2, −30 °C, 3 h. (e) $^tBuMe_2SiOTf$, DCM, pyridine, N_2, r.t., 14 h. (f) AcOH–H_2O (4 : 1), 70–74 °C, 1 h. (g) $^tBuMe_2SiCl$, pyridine, N_2, r.t., 18 h. (h) MsCl, DCM, DMAP, pyridine, N_2, r.t., 1 h. (i) H_2, Pd–C, AcOEt, AcONa, r.t., 18 h. (j) HCl–MeOH, r.t., 17 h.

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there are no prior examples of the use of such azide epimerisations in the synthesis of imino sugars.

Reduction of **5** by lithium borohydride in THF gave the triol **6** with the carbohydrate at the correct oxidation level to form the final product **2**; all that remained to be done was to introduce a leaving group at C-5 prior to reduction of the azide to permit subsequent cyclisation by the amine. Accordingly the triol **6** was treated with *tert*-butyldimethylsilyl (TBDMS) triflate to afford the fully protected gulitol **7** in 88% yield. Treatment of **7** with aqueous acetic acid caused removal of the acetonide and of the primary TBDMS ether [but without affecting the secondary silyl ethers] resulting in the formation of the triol **8**. Subsequent protection of the two primary hydroxyl groups in **8** with TBDMS chloride in pyridine gave **9** with only the C-5 OH group exposed. Esterification of **9** with mesyl chloride gave the corresponding mesylate **10** which on subsequent hydrogenation in the presence of palladium on carbon in the presence of sodium acetate led to the formation of the *O*-silyl-protected DMDP **11** in 85% yield. All the protecting groups in **11** were removed with methanolic hydrogen chloride; subsequent purification by ion-exchanger resin furnished L-DMDP 2 in 90% yield. The overall yield of L-DMDP **2** from **3** was approximately 11%.

The synthetic L-DMDP **2** was carefully compared with the naturally occurring enantiomer DMDP **1**. GCMS analysis of permethylated trimethylsilyl ethers is an exquisitively sensitive method of detecting any diastereomeric impurities; the trimethylsilyl ether of the synthetic L-isomer was precisely identical in retention time to that of the naturally occurring D-enantiomer. Additionally the ^{1}H- and ^{13}C-NMR spectra of the synthetic sample of L-DMDP were identical to those of an authentic sample of the natural product. The specific rotation of the synthetic L-DMDP: $\{[\alpha]_D^{23} = -52.7$ (c = 0.28, H_2O)$\}$ was consistent with that of the prior synthesis[20] $\{[\alpha]_D^{23} = -54.3$ (c = 0.3, H_2O)$\}$ and opposite to that of the authentic natural product $\{[\alpha]_D^{20} = +53.8$ (c = 0.32, H_2O)$\}$.

The natural product DMDP was compared as a glycosidase inhibitor with the synthetic enantiomer L-DMDP [Table 1]. First L-DMDP **2** is a highly specific [and potent] competitive inhibitor of a number of α-glucosidases – it is between 2 and 4 orders of magnitude more potent an inhibitor than DMDP **1** of the plant and mammalian α-glucosidases tested, but not an inhibitor of bacterial and yeast α-glucosidases. DMDP also shows some inhibitory activity against some β-glucosidases as well as against a β-galactosidase; L-DMDP was a more specific inhibitor of α-glucosidase and had showed no inhibition of any other glycosidases. Additionally there was no significant inhibition by either enantiomer against α-galactosidases (human lysosome, coffee bean), α-mannosidase (Jack bean) or α-fucosidase (human placenta). The differential inhibition of glucosidases implies considerable subtlety in the recognition of these pyrrolidine inhibitors by the enzymes.

Table 1 Effects of D-DMDP **1** and L-DMDP **2** on glycosidases

	IC_{50} (μM)	
Enzyme	D-DMDP	L-DMDP
α-Glucosidases		
rice	370	1.5 (K_i 2.02)
Bacillus stearothermophilus	(K_i 0.13)	NI[a]
sucrase (rat intestine)	81	0.1
sucrase (porcine)	55	1.5
maltase (rat intestine)	NI	1.4
isomaltase (rat intestine)	75	0.05
isomaltase (bakers yeast)	1.1	NI
Other glycosidases		
β-glucosidase (almond)	17	NI
β-glucocerebrosidase (human placenta)	340	NI
β-galactosidase (bovine liver)	4.6	NI
Amyloglucosidase (*Aspergillus niger*)	85	NI

[a] NI = No inhibition (less than 50% inhibition at 1000 μM).

A number of *N*-butyl imino sugars inhibit ceramide-specific glucosyltransferase and may have promise as agents for the treatment of Gauchers disease;[23] *N*-butyl DMDP was a potent inhibitor giving 86% inhibition at 200 μM. The enantiomer *N*-butyl-L-DMDP showed no inhibition at 1 mM. Full details of the enzyme assays are provided in the supplementary material.

In summary, the ability of synthetic imino sugars L-DMDP and LAB-1 to be as potent inhibitors of glucosidases as their naturally occurring enantiomers is remarkable. No substantive explanation of the ability of the enantiomers of DAB has been advanced even though the experimental results were published nearly 20 years ago. This further example of enantiomeric inhibition by DMDP and L-DMDP shows this may be a more general feature of pyrrolidine sugar mimics. It may be that in general *both* enantiomers of pyrrolidine analogues with partial or complete *gluco*-stereochemistry will inhibit related enzymes; there are chemotherapeutic arguments for the development of a range of specific inhibitors which differentiate *N*-acetylglucosaminidases and *N*-acetylgalactosaminidases. The evaluation, synthesis and rationalization of specificities of inhibitors of individual hexosaminidases are likely to present a significant challenge.

The specificity between different glucosidases of the two enantiomers may be exploitable; the lack of inhibition of ceramide-specific glucosyltransferase may make L-DMDP a much better agent for the inhibition of glucosidases.

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