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Chemical modification of azasugars, inhibitors of N-glycoprotein-processing glycosidases and of HIV-I infection.

Review and structure–activity relationships

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Abstract. The synthesis of a series of analogues of the α-glucosidase inhibitor 1-deoxynojirimycin (dNM, **1**) and of the α-mannosidase inhibitor 1-deoxymannojirimycin (dMM, **3**) is described. The ability of dNM, dMM and a series of *N*-alkylated dNM and dMM derivatives to interfere with biosynthesis, transport and maturation of the glycoprotein α_1-antitrypsin in human hepatoma HepG2 cells and with the syncytium-inducing capacity of HIV-infected cells was investigated. A strong correlation was observed between α-glucosidase inhibition found in HepG2 cells and antiviral activity in HIV-I-infected cells. *N*-Butyl- (**35**), *N*-pentyl- (**38**), *N*-benzyl- (**41**) and *N*-decyl-dNM (**47**) showed high activity in both assays. *N*-Decyl-dNM was active at 0.01 mM but showed drug-related cell toxicity at concentrations exceeding 0.1 mM. Branching of the *N*-alkyl side chain reduced the activity of dNM derivatives considerably. *N*-Benzyl-6-*O*-butyryl-dNM (**42**) and *N*-decyl-6-*O*-benzoyl-dNM (**48**) showed activity comparable to that of *N*-benzyl-dNM (**41**) and *N*-decyl-dNM (**47**), respectively. None of the dMM analogues prevented HIV-induced syncytia formation. The 3-hydroxyl group in dNM and in dMM plays a crucial role in the interaction of these drugs with the corresponding processing glycosidases: 3-*O*-methyl-dNM (**49**) and 3-*O*-methyl-dMM (**56**) were inactive in both assays. The plasma membrane constitutes a permeability barrier for castanospermine (CAS, **2**), but not for dNM, since the activity of CAS in streptolysin-*O*-permeabilized HepG2 cells was significantly higher than that in intact HepG2 cells. Finally, an overview is given of structures, other than the many *N*-substituted derivatives that were described, related to dNM and dMM that have appeared in the literature in recent years.

Introduction [b]

Many pyranoses and furanoses with the ring oxygen replaced by an amino group are natural products and are found to inhibit specific enzymes, *e.g.* glycosidases involved in the biosynthesis of *N*-glycoproteins, lysosomal hydrolases and intestinal glucosidases like sucrase and maltase[1]. This discovery has stimulated interest in the development of specific glycosidase inhibitors for studying and treating metabolic disorders such as diabetes[2] and lysosomal storage diseases[3], or as antiviral[4], antibacterial[5] and anticancer agents[6] or as immunomodulators[6,7]. For example, analogues of the α-glucosidase inhibitors 1-de-

[a] Present address: MIT Center for Cancer Research, 77 Massachusetts Ave, E17-322, Cambridge, MA 02139-4307, USA.
[b] **Nomenclature and abbreviations:** AIDS = Acquired immune-deficiency syndrome, Bn = benzyl, CAS = castanospermine (**2**), Cl_2-TMB = chlorine-3,3′,5,5′-tetramethylbenzidine, dNM = 1-deoxynojirimycin = 1,5-dideoxy-1,5-imino-D-glucitol (**1**), dMM = 1-deoxymannojirimycin = 1,5-dideoxy-1,5-imino-D-mannitol (**3**), HIV = human immunodeficiency virus, HM = high mannose, 2,6-lutidine = 2,6-dimethylpyridine, SDS-PAGE = Sodium-dodecyl-sulphate polyacrylamide-gel-electrophoresis, SW = swainsonine (**4**), triflate = trifluoromethane sulphonate.

oxynojirimycin (dNM, **1**)[4a,b,e] and castanospermine (CAS, **2**)[4h] were shown to be promising antiviral drugs, while the α-mannosidase inhibitors 1-deoxymannojirimycin (dMM, **3**) and swainsonine (SW, **4**) (see Figure 1) were shown to block human B cell development[7].

The enzymes involved in the maturation of *N*-linked glycans are found in the endoplasmic reticulum (ER) and Golgi complex where they act upon newly synthesized glycoproteins to generate an array of different structures from a common oligosaccharide precursor[1a-d]. *N*-linked glycoproteins receive $Glc_3Man_9GlcNAc_2$ oligosaccharides cotranslationally on asparagine residues in the proper sequon. Initial processing of the oligosaccharides occurs in the ER and starts with removal, *i.e.* trimming, of the outermost α1,2-linked glucose residue by glucosidase I. Subsequently, glucosidase II removes the two remaining α1,3-linked glucose residues to produce a glycoprotein with high mannose-type $Man_9GlcNAc_2$ oligosaccharides. $Glc_{3-1}Man$ can also be cleaved directly from glucose-containing high-mannose-type oligosaccharides by a Golgi endomannosidase to produce $Man_8GlcNAc_2$ oligosaccharides, thereby making the action of glucosidase I and II redundant[8]. The products of glucosidase I and II may be further processed to complex-type oligosaccharides by sequential action of trimming α-mannosidases and glycosyltransferases. The isolation and purification of a number of processing α-glucosidases and α-mannosidases from different origins have been reported[1f], but unfortunately, only few analogues of dNM and dMM have been tested for *in-vitro* enzyme inhibition using these enzymes (see Figures 1, 2 and Table I, upper part).

The action of glycosidase inhibitors, which block glycan modification at different stages, results in the biosynthesis of aberrant *N*-glycans and it is assumed that thus modified *N*-glycoproteins cause some of the biological effects described above. However, other mechanisms of action may exist[1g] and the following remarks have to be considered. Although *N*-butyl-dNM (**35**), an inhibitor of α-glucosidase I, shows anti-HIV activity[4a,b,e], it was never established unambiguously that modified, glucose-retaining, *N*-glycans on the virus envelope gp120 were responsible for the effect[1g,4g]. In many studies glycosidase inhibition was tested using commercially available enzymes (*e.g.* sweet almond β-glucosidase and yeast α-glucosidase) instead of the mammalian processing glycosidases. Finally, in several studies, the biological activity was suggested to be related to inhibition of *N*-glycoprotein trimming enzymes, although a clear correlation was not shown[4a,b]. On the other hand, *Sunkara* and collaborators did report a correlation between glucosidase-I inhibition in mouse cells and syncytia formation by HIV-I[4h].

Structure–activity relationship (SAR) studies on azasugar glycosidase inhibitors have mainly focussed on the synthesis of simple *N*-substituted derivatives of the natural products. For instance, the synthesis of over one hundred different *N*-substituted dNM analogues was reported[4a-b,9]. On the other hand, only a few derivatives were investigated for which it was found to be detrimental to the biological activity to *N*-alkylate, *e.g.* dMM, or in cases that quaternary ammonium derivatives would be

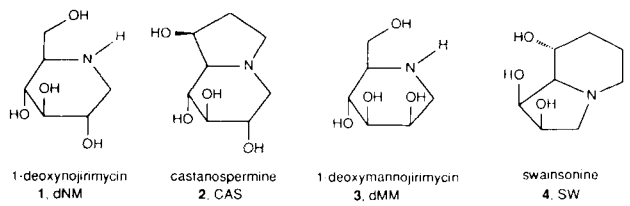


Figure 1. Structures of dNM, CAS, dMM and SW. See Tables I and II for biological activity data on these compounds.

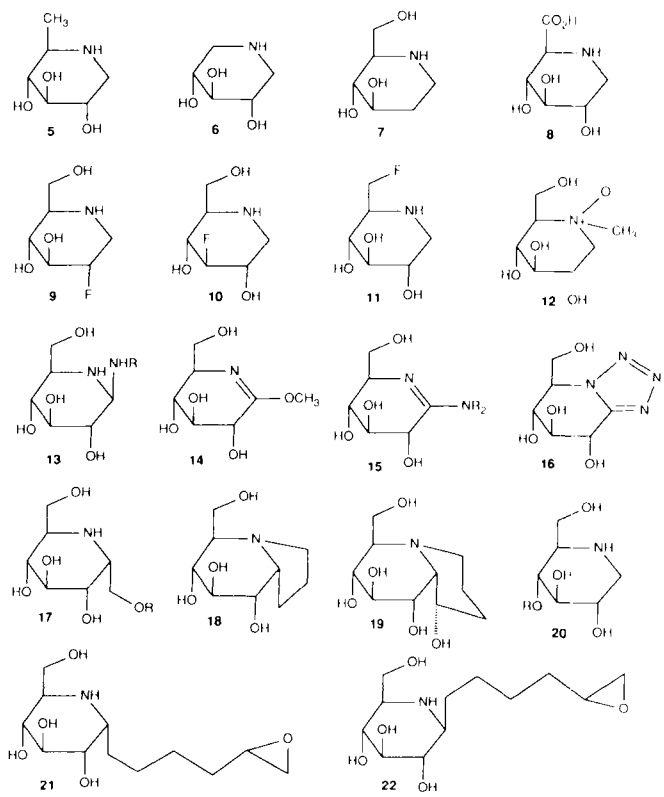


Figure 2. Azapyranose derivatives related to dNM (1). Compounds 5–12 and 20 are 1-deoxy analogues, 13–16 are substituted at C-1 with a hetero atom, and 17–19, 21 and 22 are substituted with a carbon atom at C-1. See Table I for biological activity data on these compounds and for the identity of group R in compounds 13, 15, 17 and 20. See also Ref. 37b for (6S)-6-C-ethyl-dNM (structure not shown).

formed, *e.g.* the tertiary amines CAS and SW. This paper will present an overview of several azapyranose derivatives closely resembling dNM and dMM of which 25 structures are related to dNM, *i.e.* **5–22** (Figure 2), and of which 11 correspond to dMM, *i.e.* **23–33** (Figure 3)[10-37]. Some selected enzyme inhibition data and the biological effect(s) of these compounds, when known, are presented in Table I[10-37].

We describe here also our results on the chemical synthesis and biological activity of a series of related compounds, 20 (*i.e* **34–53**) derived from dNM (**1**) and 3 (*i.e.* **54–56**) derived from dMM (**2**) (see Figure 4 and Tables II and III). The biological tests included: (i) inhibition of human hepatoma-processing α-glycosidases in intact HepG2 cells as well as in (ii) permeabilized HepG2 cells and (iii) inhibition of syncytia formation of HIV-I infected U937 cells. This is the first study of this class of compounds in which a therapeutically relevant activity is presented in relation to the ability to inhibit a particular human *N*-glycoprotein-processing glycosidase. In this study, we show that the effects of the inhibitors on processing glycosidases in HepG2 cells correlates closely with their ability to inhibit syncytia formation of HIV-infected cells and that impaired transport of azasugar inhibitors across the plasma membrane decreases their biological activity.

Results and discussion

Review of enzyme-inhibition data and biological effect(s) of a series of azapyranose derivatives closely resembling dNM and dMM[37b-d]

In the last few years the synthesis of a number of new compounds closely related to dNM (*i.e.* **5–22**, see Figure

2) and to dMM (*i.e.* **23–33**, see Figure 3) has been reported[10–37]. In Table I (middle and lower part) some biological data on these analogues are shown. Much of the work on chemical modification of dNM, and to a much lesser extent of dMM, has been focussed on *N*-substitution. Soon after the isolation of dNM and dMM it was shown that the α-glucosidase inhibitory activity of dNM could be improved by alkylating the amine. Com-

Table I Review of selected glycosidase inhibitor enzyme data and biological effects.

For structures of **1–4** see Figure 1; for structures of the other compounds see Figures 2 and 3. See also Refs. 37b–d for enzyme activity data on (6S)-6-ethyl-dNM (**31**, R = H) and α-homomannojirimycin. Abbreviations used: K_i, inhibition constant; IC_{50}, 50% inhibitory concentration; α-Glc I, processing α-glucosidase I; α-Glc, α-D-glucose or α-glucosidase; β-Glc, β-D-glucose or β-glucosidase; β-GlcA, β-glucuronidase; α-Man I, processing α-mannosidase I; α-Man II, processing α-mannosidase II; α-Man, α-Mannosidase; β-Gal, β-D-galactose; sweet-almond β-glucosidase, emulsin; Bn, benzyl.

No.	Inhibition	Enzyme	Activity
1 dNM	K_i 18-370 μM	sweet almond β-Glc[10,18]	anti-HIV
	K_i 0.05 μM	rat intestine sucrase[11]	anti-diabetic
	K_i 1.0 μM	calf liver α-Glc I[12]	
34	K_i 0.07 μM	calf liver α-Glc I[12]	anti-HIV
35	K_i 0.09 μM	calf liver α-Glc I[12]	anti-HIV
47	K_i 0.4 μM	calf liver α-Glc I[12]	anti-HIV
51	K_i 37 μM	sweet almond β-Glc[10]	no data
	K_i 40 nM	bovine liver β-GlcA[13]	
2 CAS	IC_{50} 0.10 μM	pig kidney α-Glc I[14]	anti-HIV, anti-diabetic
	K_i 0.5 nM	rat intestine sucrase[11]	immunomodulator
3 dMM	K_i 7 μM	calf liver α-Man I[15]	immunomodulator
	K_i 5 μM	pig liver α-Man I[15]	
4 SW	K_i 0.2 μM	α-Man II[16]	immunomodulator
	0% at 0.1 mM	α-Man I[16]	
	IC_{50} 0.1 μM	jack bean α-Man[17]	
5	K_i 1.56 mM	yeast α-Glc[18]	no data
6	K_i 0.43 mM	sweet almond β-Glc[19]	no data
7	IC_{50} 0.3 mM	mouse gut α-Glc[20]	no anti-diabetic
8	K_i 80 μM	human liver β-GlcA[13]	no data
9	24% at 1 mM		
	64% at 5 mM	yeast α-Glc[21]	no data
10	11% at 1 mM		
	18% at 5 mM	yeast α-Glc[21]	no data
11	no data	[22]	no data
12	K_i > 10 mM	yeast α-Glc[18]	no data
	K_i 80 μM	sweet almond β-Glc[18]	
13 R = H	K_i 40 μM	sweet almond β-Glc[23]	no data
R = Bn	K_i 20 μM	sweet almond β-Glc[23]	no data
14	K_i 0.8 mM	sweet almond β-Glc[18]	no data
15 R = H	K_i 9 μM	jack bean α-Man[24]	no data
	K_i 8 μM	sweet almond β-Glc[24]	
R = Me	K_i 83 μM	sweet almond β-Glc[24]	no data
R = NH_2, H	K_i 8.4 μM	sweet almond β-Glc[25]	no data
R = OH, H	K_i 13.8 μM	sweet almond β-Glc[25]	no data
16	IC_{50} 80 μM	sweet almond β-Glc[26]	no data
	IC_{50} 30 mM,		
	K_i 18 mM	pig liver α-Glc I[26]	
17 R = H	IC_{50} 81 nM	mouse intestine sucrase[27]	anti-diabetic
R = β-Glc	K_i 5 μM	rat intestine sucrase	no data
	K_i 2 μM	rat intestine sucrase[2a]	anti-hyperglycemic
18	IC_{50} 0.30 μM	pig kidney α-Glc I[14]	no data
19	IC_{50} 0.15 μM	pig kidney α-Glc I[14]	no data
20 R = α-Glc	no data	mammalian intestine α-Glc[28]	no data
R = β-Glc	no data	bacterial endocellulase[29]	no data
R = β-Gal	no data	[30]	no data
21	K_i 27 μM	α-amyloglucosidase[31]	no data
22	K_i 0.4 mM	sweet almond β-Glc[31]	no data
23	no data	[18]	no data
24	60% at 1 mM	jack bean α-Man[19]	no data
25	no data	[32]	no anti-HIV
26	no data	[4a]	no anti-HIV
27	no data	[18]	no data
28	K_i > 1 mM	jack bean α-Man[18]	no data
	K_i > 1 mM	sweet almond β-Glc[18]	
29	no data	[33]	no data
30	K_i 0.92 μM	apricot β-Glc[34]	no data
	K_i 95 μM	rat epididymal α-Man[34]	
31 R = NH_2	K_i 166 nM	jack bean α-Man[25]	no data
	IC_{50} 4 μM	Golgi α-Man I[25]	
	IC_{50} 90–100 nM	α-Man II[25]	
	IC_{50} 1 μM	ER α-Man[25]	
R = OH	no data	[25]	no data
32	no data	[35]	no anti-HIV
33	IC_{50} 20–50 nM	mung bean α-Man I[36]	immunomodulator
	IC_{50} 0.1 μM	rat liver Golgi α-Man I[36]	
	IC_{50} 20 nM	MDCK α-Man I[36]	

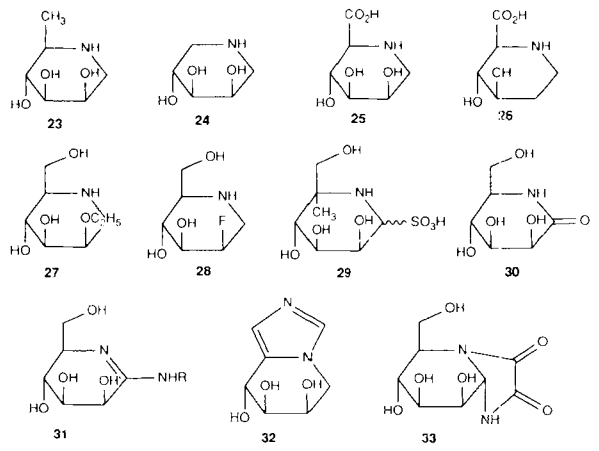


Figure 3. Azapyranose analogues related to dMM (3). Compounds ***23–28*** *and* ***32*** *are 1-deoxy derivatives,* ***29–31*** *and* ***33*** *are substituted at C-1. Compound* ***26*** *is also related to dNM. See Table I for biological activity data on these compounds and for the identity of group R in compound* ***31****. See also Ref. 37c for compound* ***31*** *(R = H) and Ref. 37d for α-homomannojirimycin (structures not shown).*

pounds **34** (*N*-methyl-dNM) and **35** (*N*-butyl-dNM) show K_i values at a 10-fold lower concentration than dNM (see Table I, upper part)[12]. However, longer alkyl chains do not show further increases in activity, *e.g.* compound **47** (*N*-decyl-dNM). So far no data have been published of analogues of dNM having K_i values in the nM range. CAS shows a K_i value close to that of *N*-butyl-dNM. DMM also shows a K_i value in the μM range against the processing α-mannosidase I. However, *N*-substitution of dMM decreases enzyme inhibitory activity by a factor 10 for *N*-methyl-dMM and by a factor 20 for *N*-(carboxypentyl)-dMM[15]. DNM and CAS were also reported to be potent inhibitors of rat intestine sucrase[11]. Therefore, much effort was put in developing these agents as potential anti-diabetic drugs[2]. Strangely enough, as far as we know, no data have been reported on inhibition of intestinal α-glucosidases by most of the analogues of dNM and CAS.

The following general conclusions can be drawn with regard to the data described in Table I. In most studies, enzyme kinetic data have been obtained using commercially available enzymes from plants and yeast, instead of using the processing glycosidases. Many of the azasugars show a broad spectrum of enzyme inhibitory activities. From most studies it remains unclear what the full spectrum of enzyme inhibition of a particular compound looks like. More attention should be paid in our opinion to the ability of a compound to inhibit selectively one of the human processing glycosidases, which could be of more interest for the development of therapeutic compounds.

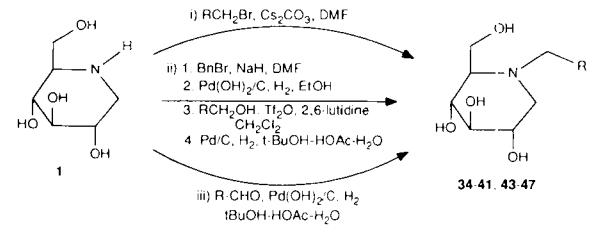


Figure 4. Three routes to N-alkylated dNM derivatives. For example (i) $R = C_6H_5$ *(**41**), (ii)* $R = p\text{-}F\text{-}C_6H_4\text{-}OCH_2$ *(**46**) and (iii)* $R = C_9H_{19}$- *(**47**). Compounds **37**, **40**, **43**, **44**, **45** and **35**, **36**, **38**, **39** were also prepared via route (i) and (iii), respectively. See also Table III for structures.*

Table II Estimated glucose retention on α_1-antitrypsin in HepG2 cells and inhibition of HIV-induced syncytia formation.

For structures of **1–4** see Figure 1; the structures of the dNM and dMM derivatives are shown in Table III.

No.	Compound	Glc_n [a]	HIV-I [b]
1	dNM	$Glc_{2\ 1}$	+/−
34	*N*-methyl	$Glc_{3\ 2}$	+
35	*N*-butyl	$Glc_{3\ 2}$	+ +
36	*N*-isobutyl	$Glc_{2\ 1}$	+/−
37	*N*-cyclopropylmethyl	$Glc_{1\ 0}$ [c]	+/−
38	*N*-pentyl	$Glc_{3\ 2}$	+ + [k]
39	*N*-isopentyl	$Glc_{3\ 2}$	+/−
40	*N*-cyclobutylmethyl	$Glc_{1\ 0}$ [c]	+/−
41	*N*-benzyl	$Glc_{3\ 2}$	+ + [k]
42	*N*-benzyl-6-*O*-butyryl	$Glc_{3\ 2}$	+
43	*N*-*p*-CF_3-benzyl	$Glc_{3\ 0}$ [d]	−
44	*N*-*m*-CF_3-benzyl	$Glc_{3\ 0}$ [d]	−
45	*N*-phenylpropyl	$Glc_{2\ 1}$	+
46	*N*-*p*-*F*-phenoxyethyl	$Glc_{2\ 1}$	+/−
47	*N*-decyl	$Glc_{2\ 1}$ [e]	+ + [l]
48	*N*-decyl-6-*O*-benzoyl	$Glc_{2\ 1}$ [f]	+ + [l]
49	3-*O*-methyl	Glc_0	−
50	*N*-decyl-3-*O*-methyl	Glc_0 [g]	+ + [l]
51	1-oxo	Glc_0	−
52	1-β-sulphonyl	Glc_0	+ + [l]
53	1-α-cyano	Glc_0	−
2	CAS	$Glc_{3\ 2}$	+ +
3	dMM	[h]	−
54	*N*-benzyl	[i]	−
55	*N*-2-hydroxyethyl	[j][l]	−
56	3-*O*-methyl	[l]	−
4	SW	[m]	−

[a] Estimated number of glucose residues (Glc_n) retained on α_1-antitrypsin at 3 mM. [b] Syncytia formation after incubation with inhibitor at 1 mM. + +: no syncytia, +: clear inhibition, +/−: inhibition noticeable, −: no inhibition. [c] Active from 1 mM. [d] Active from 1 mM, partially lytic at 3 mM. [e] $Glc_{2\ 1}$ at 0.1–0.5 mM, lytic at 0.1–0.5 mM. [f] $Glc_{2\ 1}$ at 1 mM, lytic at 3 mM. [g] Lytic at 0.5 mM. [h] 100% inhibition of α-mannosidase I at 1 mM. [i] 0% inhibition of α-mannosidase I at 0.01–3.0 mM. [j] intermediate complex-type glycans at 3 mM. [k] TCID reduction 100 fold. [l] p24 levels decreased compared to control. [m] 100% inhibition of α-mannosidase II at 3 mM.

Chemistry

Synthesis of N-alkylated derivatives. The synthesis of compounds **34–39**, **41**, **45**, **47** and **51–53** has been reported[9a,b,38]. Compound **52**, *i.e.* nojirimycin bisulfite, was converted to nojirimycin before use[39]. The synthesis of dNM was carried out as described earlier.[48] The synthesis of the remaining compounds listed in Table II is described below (structures are shown in Table III). The preparation of *N*-substituted dNM or dMM derivatives can be achieved in several ways. In Figure 4, three routes to *N*-alkylated dNM analogues are outlined. The most commonly used route to *N*-alkylation is reaction of the secondary amine **1** with an alkyl halide, frequently an alkyl bromide, in DMF using potassium carbonate or cesium carbonate as a base. For reactive (ar)alkyl bromides, *e.g.* benzyl bromide, a smooth reaction after several hours at room temperature takes place giving the alkylated amine, *e.g.* **41** (*N*-benzyl-dNM), in high yields.

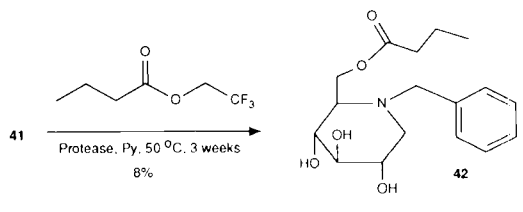
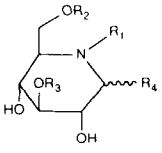
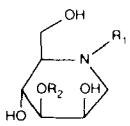


*Figure 5. Enzymatic synthesis of N-benzyl-6-O-butyryl-dNM (**42**).*

Table III Structures of dNM derivatives ***34–53*** *and dMM derivatives* ***54–56.***



No.	R_1	R_2	R_3	R_4
34	CH_3	H	H	H
35	$(CH_2)_3CH_3$	H	H	H
36	$CH_2CH(CH_3)_2$	H	H	H
37	$CH_2CH(-(CH_2)_2-)$	H	H	H
38	$(CH_2)_4CH_3$	H	H	H
39	$CH_2CH(CH_3)CH_2CH_3$	H	H	H
40	$CH_2CH(-(CH_2)_3-)$	H	H	H
41	$CH_2C_6H_5$	H	H	H
42	$CH_2C_6H_5$	$C(O)(CH_2)_2CH_3$	H	H
43	$CH_2C_6H_5$-p-CF_3	H	H	H
44	$CH_2C_6H_5$-m-CF_3	H	H	H
45	$(CH_2)_3C_6H_5$	H	H	H
46	$(CH_2)_2O$-C_6H_4-p-F	H	H	H
47	$(CH_2)_9CH_3$	H	H	H
48	$(CH_2)_9CH_3$	$C(O)C_6H_5$	H	H
49	H	H	CH_3	H
50	$(CH_2)_9CH_3$	H	CH_3	H
51	H	H	H	=O
52	H	H	H	β-SO_3H
53	H	H	H	α-CN



No.	R_1	R_2
54	$CH_2C_6H_5$	H
55	$(CH_2)_2OH$	H
56	H	CH_3

However, less reactive alkyl bromides, *e.g.* cyclobutylmethyl bromide, react slowly and even at higher temperatures poor conversion is observed resulting in low (isolated) yields, e.g. 16% yield of **40** [*N*-(cyclobutylmethyl)-dNM] (*vide infra*). A much more reactive nucleophilic substitution involves reaction of the secondary amine **1** with an alkyl triflate. In case of azasugars like **1**, protection of the free hydroxyl groups, as for instance with benzyl ethers, is required. The triflate is generated *in situ* and allowed to react with the amine in dichloromethane. This method provides alkylated dNM derivatives in good yields. Compound **46** [*N*-[2-(*p*-fluorophenoxy)ethyl]-dNM] was synthesized via this route (*vide infra*, Figure 6). The obvious disadvantage of this method concerns the hydroxyl-group-protection/deprotection steps. The third route involves a reductive amination of the secondary amine **1** with the required aldehyde, *e.g.* decanal for the synthesis of compound **47** (*N*-decyl-dNM). Usually Pearlman's catalyst (*i.e.* 20% $Pd(OH)_2/C$) is used and the hydrogenation is carried out (in a Parr apparatus) in tBuOH + HOAc + H_2O. Generally, the *N*-alkylated azasugar is obtained in high yields. The only disadvantage of this method is the need to remove the (slight) excess of aldehyde. Usually, column chromatography on silica gel or on a strongly acidic ion-exchange resin (H^+ form) is performed to separate the product from the aldehyde. In our hands, the reductive amination is preferred to the nucleophilic substitution of a bromide or a triflate, unless more stringent conditions are necessary.

Compound **40** (*N*-(cyclobutylmethyl)-dNM) was prepared in 16% yield in a sluggish reaction of dNM with cyclobutylmethyl bromide in DMF with cesium carbonate as base at 70°C for 12 days (*vide supra*). Compounds **43** and **44** were synthesized in a straightforward way. Reaction at

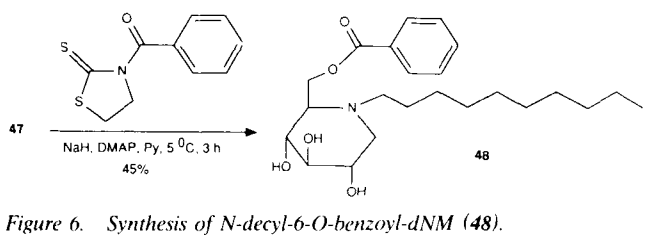


*Figure 6. Synthesis of N-decyl-6-O-benzoyl-dNM (**48**).*

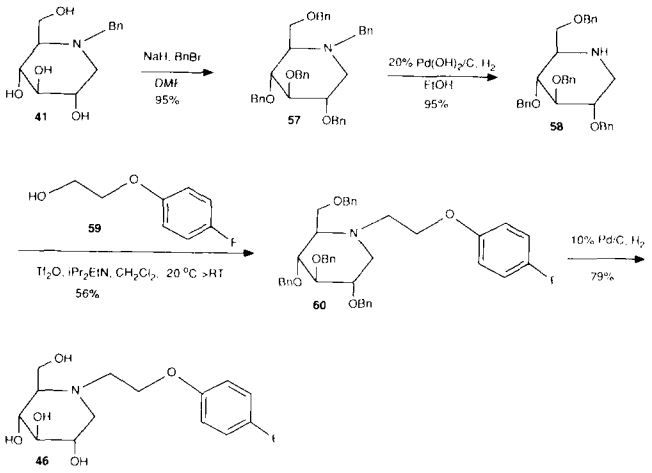


*Figure 7. Synthesis of N-[2-(p-fluorophenoxy)ethyl]-dNM (**46**).*

room temperature for 2 h of dNM with *p*-(trifluoromethyl)benzyl bromide or *m*-(trifluoromethyl)benzyl bromide in DMF with cesium carbonate as base afforded **43** [*N*-[*p*-(trifluoromethyl)benzyl]-dNM] (56% yield) and **44** [*N*-[*m*-(trifluoromethyl)benzyl]-dNM] (51% yield), respectively.

Compound **46** [*N*-[2-(*p*-fluorophenoxy)ethyl]-dNM] was synthesized from tetra-*O*-benzyl-dNM (**58**)[26] (Figure 7). Compound **58** was prepared in two steps from **41**. *O*-Benzylation of **41** with benzyl bromide and sodium hydride in DMF yielded the fully benzylated azasugar **57** in 95% yield. Selective *N*-debenzylation (95% yield) was achieved by hydrogenation using 20% $Pd(OH)_2/C$ according to the procedure described recently[40]. Reaction of **58** in dichloromethane from −20°C to room temperature with *N*,*N*-diisopropylethylamine as base with 2-(p-fluorophenoxy)ethyl triflate, generated *in situ* from **59**, afforded in 56% yield the coupled product *N*-[2-(*p*-fluorophenoxy)ethyl]-tetra-*O*-benzyl-dNM (**60**). Subsequently, hydrogenolysis of the benzyl ether protecting groups generated the desired end-product **46** in 79% yield.

Synthesis of ester derivatives. Several reports describe the chemical or enzymatic synthesis of lipophilic ester analogues of glycosidase inhibitors in order to improve the biological activity of the parent compound[41–44]. Thus, 6-*O*-butyryl-CAS was shown to be 20 times more active than CAS itself in inhibiting HIV replication *in vitro*[4h]. The synthesis of two 6-*O*-acyl-dNM derivatives, *i.e.* *N*-benzyl-6-*O*-butyryl-dNM (**42**) and *N*-decyl-6-*O*-benzoyl-dNM (**48**) was undertaken. Compound **42** was synthesized enzymatically as follows (Figure 5). *N*-Benzyl-dNM (**41**) was reacted with 2,2,2-trifluoroethyl butyrate in dry pyridine, using the protease subtilisin[43]. After 3 weeks at 50°C a mixture (1.6 : 1) of the desired mono-butyrylated derivative **42** (8%) and of *N*-benzyl-2,6-di-*O*-butyryl-dNM (5%) (structure not shown) were obtained. The mono- and di-ester were separated chromatographically on silica gel. In addition to the starting material, two other products were formed but could not be isolated. It is noteworthy that the desired butyrate **42** was not formed using lipase as the catalyst. This is in contrast to the lipase-catalyzed 6-*O*-acylation of methyl α-D-glucose which showed a high selectivity (6 : 1) and a moderate yield (49%) was obtained[45,46]. This phenomenon should be attributed to the lower reactivity of the C6-OH group in *N*-alkylated dNM derivatives compared to that of methyl α-D-glucose (*vide infra*). This is supported by the fact that subtilisin-catalyzed 6-*O*-benzoylation of dNM, using vinyl benzoate, occurred in only 24% yield with concomitant formation of

2,6-di-*O*-benzoyl-dNM (36% yield)[41]. Furthermore, a mixture of products was formed when **41** was treated with 1-hydroxybenzotriazole butyrate in dichloromethane/pyridine/triethylamine. In the synthesis of compound **48** (*N*-decyl-6-*O*-benzoyl-dNM) the chemical approach to 6-*O*-benzoylation of **47** proved to be the method of choice (Figure 6). Reaction of **47** with 2-mercaptothiazoline benzoate in dry pyridine with sodium hydride and a catalytic amount of 4-(dimethylamino)pyridine for 3 h at 5°C afforded solely the ester **48** in 45% yield[47]. Enzyme-catalyzed, *e.g.* subtilisin, esterification of **47** using 2,2,2-trifluoroethyl benzoate in dry pyridine only gave less than 10% conversion after 2 weeks. In conclusion, selective chemical or enzymatic 6-*O*-acylation of basic *N*-alkylated azapyranose derivatives related to dNM proved to be a difficult reaction contrary to what has been reported[41–44]. It resulted in poor regioselectivity, low yields and long reaction periods and also depended on the structure of the ester group to be introduced. Enzyme-catalyzed acylation always took long reaction times. Appropriate selection of the activating group in chemical acylation is our preferred method (*vide infra*).

Synthesis of 3-O-methyl derivatives. The 3-hydroxyl group is the only position in the pyranose ring that is stereochemically conserved in a series of important glycopyranoses: glucose, mannose, galactose, iduronic acid, glucuronic acid, *N*-acetylglucosamine and *N*-acetylgalactosamine (see Figure 8). For each of the latter sugars, corresponding glycosidases (*i.e.* hydrolases) exist and for most of these enzymes the corresponding azasugar is found to be an inhibitor. We reasoned that either this hydroxyl group could easily be replaced with a methoxy group or, in contrast, that it would be involved in a specific interaction with the enzyme and should therefore be left untouched. The synthesis of compounds **49** (3-*O*-methyl-dNM) and **56** (3-*O*-methyl-dMM) was carried out as follows (Figure 9, see also Ref. 48). Removal of the benzyl ether protecting group in the idose building block **61**[49] with 10% palladium on carbon/hydrogen in DMF was followed by reaction of **62** with methyl iodide and sodium hydride in dry DMF to give the 3-*O*-methyl idofuranose epoxide **63** in 65% overall yield. Two by-products were isolated when the hydrogenation was carried out in methanol: 1,2-*O*-isopropylidene-6-deoxy-β-L-idofuranose and 1,2-isopropylidene-6-*O*-methyl-β-L-idofuranose (structures not shown). Opening of the epoxide function in **63** with benzyl alcohol and sodium hydride in dry DMF (**64**, 43% yield), activation of the secondary hydroxyl group with triflic anhydride and 2,6-lutidine in dry DMF (**65**) and subsequent nucleophilic substitution of the triflate with sodium azide in dry DMF resulted in the formation

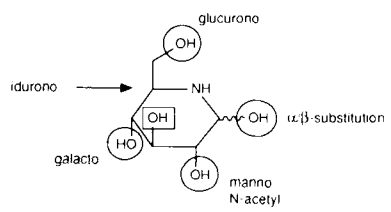


Figure 8. Structure of nojirimycin, an azaglucopyranose analogue. The 3-hydroxyl group (square box) in azapyranose derivatives, i.e. in the corresponding glucose, mannose, galactose, iduronic acid, glucuronic acid, N-acetyl glucosamine and N-acetyl-galactosamine analogues (circles), is the only stereochemically conserved position. For each of these sugars corresponding glycosidases (i.e. hydrolases) exist and for most of these enzymes the corresponding azasugar is found to be an inhibitor. The effect of substituting this hydroxyl group for a methoxy group was investigated in case of 3-O-methyl-dNM (49) and 3-O-methyl-dMM (56).

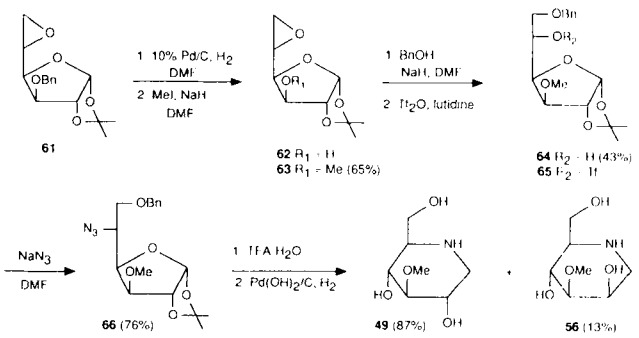


Figure 9. Synthesis of 3-O-methyl-dNM (49) and 3-O-methyl-dMM (56).

of the azide **66** (76% overall yield). Removal of the 1,2-*O*-isopropylidene protecting group in **66** with 80% trifluoroacetic acid in water and subsequent intramolecular reductive amination using hydrogen and 20% $Pd(OH)_2/C$ gave a mixture of the expected 3-*O*-methyl-dNM (**49**) and 3-*O*-methyl-dMM (**56**). The formation of the corresponding manno derivative is due to acid-catalyzed epimerization at C2 during removal of the 1,2-*O*-isopropylidene function in **66**[48]. A by-product was formed due to β-elimination of the triflate **65**. The structure was identified as the (Z) (C5–C6-enol benzyl ether (see also Ref. 50). Compounds **49** (87% yield) and **56** (13% yield) were separated by column chromatography on silica gel. Compound **50** (*N*-decyl-3-*O*-methyl-dNM) was prepared in 50% yield from **49** by reaction with decanal in a reductive amination using hydrogen and 20% $Pd(OH)_2/C$ according to reaction route iii depicted in Figure 4. Compound **54** (*N*-benzyl-dMM) was synthesized by reaction of **3** with benzyl bromide and cesium carbonate in DMF in 95% yield (reaction route i in Figure 4). Compound **55** [*N*-(2-hydroxy-ethyl)-dMM] was prepared in 95% yield by reaction of **3** with ethylene oxide in DMF in a pressure reaction vessel (reaction scheme not shown).

Conformation

During ^{1}H-NMR analysis of the *N*-alkylated dNM structures a remarkable difference in conformation between the parent compound and all *N*-substituted derivatives was observed. The C6-OH group in dNM is in a *gauche–trans* conformation (H6, dd, ^3J 3 Hz; H6′, dd, ^3J 6 Hz), placing the hydroxyl group in the plane of the azapyranose ring (see Figure 10). In all *N*-alkylated dNM analogues described in Table II, a *gauche–gauche* conformation is preferred, positioning the C6-OH group perpendicular to the plane of the azapyranose ring, *i.e.* a pseudo-axial position: H6 and H6′, dd, ^3J ±3 Hz. This difference in conformation was found to be independent of the solvents used for dNM and the various analogues, *i.e.* D_2O, CD_3OD and $CDCl_3$. For 3-*O*-methyl-dNM (**49**) and *N*-decyl-3-*O*-methyl-dNM (**50**) exactly the same difference in conformation was found. The C6-OH group of compound **53** (1-α-cyano-dNM) is also in a *gauche–trans* conformation as judged from ^{1}H-NMR coupling constants. This finding was recently corroborated by molecular modelling studies on dNM and *N*-methyl-dNM by *Wong* and collaborators[18]. The dihedral angle N–C5–C6–O6 in dNM of 77° changed to −71° for *N*-methyl-dNM. We postulated that this conformational difference may explain the fact that only dNM and not any of its *N*-substituted analogues reduced the synthesis of lipid-linked oligosaccharides resulting in the synthesis of proteins containing a reduced number of *N*-linked glycans[39]. The C1-OH group in CAS and the C6-OH group in valiolamine occupy a position that is almost perpendicular to

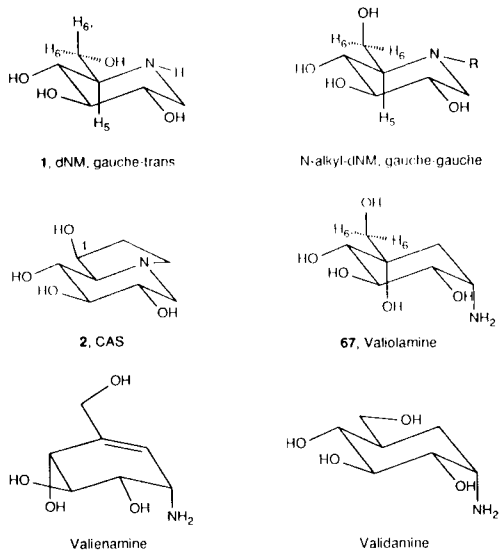


Figure 10. Preferred conformation of dNM, N-alkylated dNM derivatives, CAS and valiolamine. N-alkylation of dNM changes the preferred gauche-trans *conformation for dNM into a* gauche-gauche *conformation placing the C6-OH group in a position perpendicular to the plane of the azapyranose ring. A similar position is taken by the C1-OH group of CAS and the C6-OH group of valiolamine. The structures of valienamine and validamine are also shown.*

the plane of the ring(s) (Figure 10). We have proposed that, in the case of CAS, this preferred conformation is responsible for its strong antiviral and α-glucosidase inhibitory activity[39]. In the case of valiolamine, the additional α-C5-OH group will force the C6-OH group in a pseudo-axial position and again this may be the basis for valiolamine's strong α-glucosidase inhibitory activity. Valiolamine shows significantly higher activity than two related pseudo-amino sugars, *i.e.* valienamine and validamine[51]. The pseudo-axial position of the C6-OH group in *N*-substituted dNM analogues is probably the reason for our difficulties in synthesizing the C6 esters **42** (*N*-benzyl-6-*O*-butyryl-dNM) and **48** (*N*-decyl-6-*O*-benzoyl-dNM) due to a reduced reactivity of the primary hydroxyl group (*vide supra*).

Biological activity

Assays. The effects of the series of glycosidase inhibitors (see Table II, structures not shown) on the biosynthesis, maturation, oligosaccharide structure and secretion of α_1-antitrypsin were investigated in HepG2 cells[39] (Figures 11–13 and 15). In Table II, the estimated number of glucose residues on α_1-antitrypsin after incubation with inhibitor at 3 mM is given. The data for the α-glucosidase inhibitor CAS (**2**) and the α-mannosidase inhibitor SW (**4**) are depicted in Table II for comparison. Streptolysin *O*-permeabilized HepG2 cells[52] were used to investigate the role of transport of inhibitors across the plasma membrane on their glucosidase inhibitory activity (Figure 14). Antiviral activity was investigated by HIV^{IIIb}-induced syncytia formation[4d,53].

α-Glucosidase inhibitory activity in intact HepG2 cells[39]. (i) N-alkylation. The following conclusions can be drawn on the α-glucosidase inhibitory activity as measured in HepG2 cells (Table II)[39]. Inhibition of α-glucosidases is increased by *N*-substitution of dNM with a straight (ar)alkyl chain: *N*-methyl- (**34**), *N*-butyl- (**35**), *N*-pentyl- (**38**) and *N*-benzyl-dNM (**41**) (see also Figure 11) showed significantly higher activity than the parent drug dNM. *N*-Benzyl-dNM (**41**) (see also Figure 12) showed a maximal inhibitory

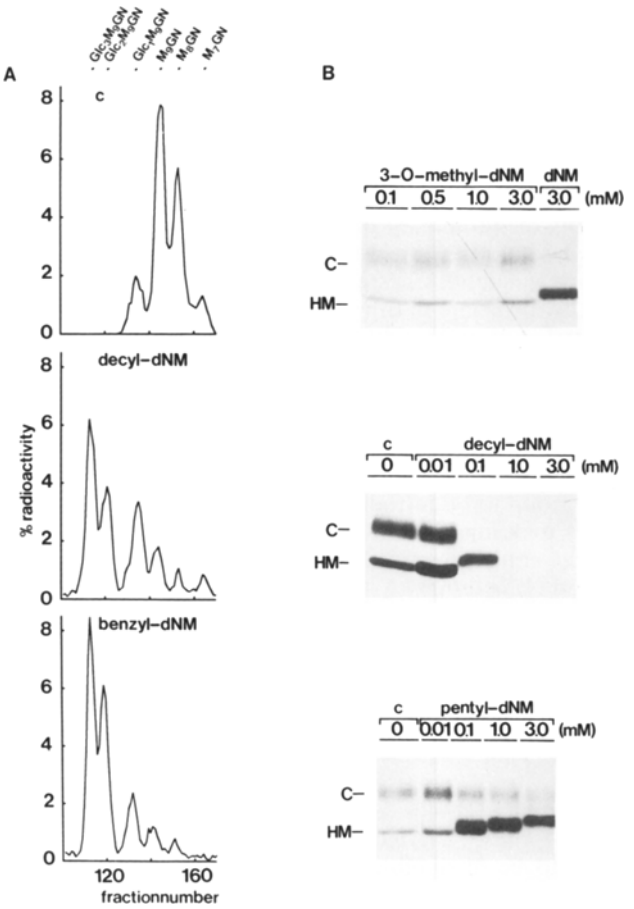


*Figure 11. Titration (B) and analysis of Endo-H-released α_1-antitrypsin oligosaccharides (A) from inhibitor-treated HepG2 cells. HepG2 cells were pretreated for 2 h with indicated concentrations of inhibitor and labeled for 1 h with 100 μCi of D-[2-^{3}H]mannose (A) and 20 μCi of [^{35}S]methionine-cysteine (B) in continuous presence of inhibitors. α_1-Antitrypsin was isolated by immunoprecipitation from cell lysates for analysis by SDS-PAGE (10% AA) (B) or its oligosaccharides were released by digestion with Endo H for analysis by gel filtration (Bio-Gel P-4 column). A: Gel filtration profiles of oligosaccharides derived from control cells (c), cells treated with 0.5mM N-decyl-dNM (**47**) and 3.0mM benzyl-dNM (**41**) are shown. The elution positions of the marker oligosaccharide structures $Glc_{3-1}Man_9GlcNAc$ and $Man_{9-7}GlcNAc$ are indicated. B: Effects of 3-O-methyl-dNM (**49**), N-decyl-dNM (**47**) and N-pentyl-dNM (**38**) were examined on the biosynthesis, maturation and transport of α_1-antitrypsin and analysed on SDS-PAGE. The position of α_1-antitrypsin with the high mannose (HM) and mature complex (C) type forms as determined for untreated cells (c, control) are indicated. α_1-Antitrypsin-carrying glucose-containing HM-type oligosaccharides that accumulate in the inhibitor-treated cells, migrate between the HM and C bands.*

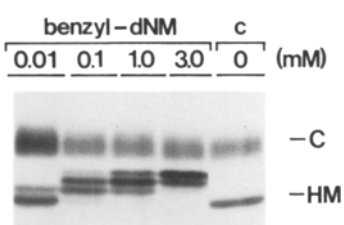


*Figure 12. Titration of N-benzyl-dNM (**41**) in HepG2 cells. HepG2 cells were pretreated for 2 h with indicated concentrations of N-benzyl-dNM and labelled for 1 h with 20 μCi of [^{35}S]methionine-cysteine in continuous presence of inhibitor. α_1-Antitrypsin was isolated by immunoprecipitation from cell lysates and analysed by SDS-PAGE (10% AA). The positions of α_1-antitrypsin with high mannose (HM) and complex (C) type oligosaccharides are indicated as determined for untreated cells (c). α_1-Antitrypsin-carrying glucose-containing HM type oligosaccharides that accumulates in the inhibitor-treated cells, migrates between the HM and C bands.*

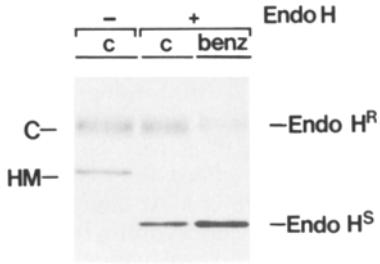


*Figure 13. Analysis of Endo-H sensitivity of α_1-antitrypsin derived from N-benzyl-dNM-(**41**)-exposed HepG2 cells. α_1-Antitrypsin was isolated by immunoprecipitation from untreated (c) and N-benzyl-dNM treated cells as described in Figure 12, digested with Endo H and analysed by SDS-PAGE (10% AA). The positions of Endo-H-resistant (Endo H^R) and Endo-H-sensitive (Endo H^S) α_1-antitrypsin are indicated.*

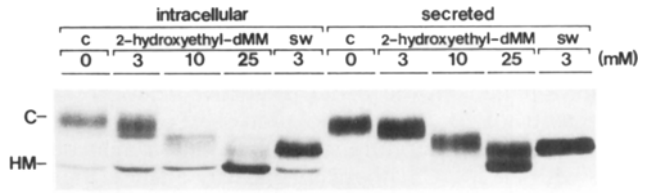


*Figure 15. Effect of swainsonine and N-(2-hydroxyethyl)-dMM (**55**) in HepG2 cells. HepG2 cells were pretreated for 2 h with swainsonine (SW) and N-(2-hydroxyethyl)-dMM at the concentrations indicated and labelled for 1 h with 20 μCi [^{35}S]methionine-cysteine in the continuous presence of inhibitors. α_1-Antitrypsin was isolated by immunoprecipitation from the cell lysates (intracellular) and medium (secreted) and analysed by SDS-PAGE (10% AA). The positions of α_1-antitrypsin with high-mannose (HM) and complex (C) type oligosaccharides are indicated.*

effect at 1.0 mM, whereas for the other dNM derivatives inhibition was maximal at 3.0 mM. Figure 12 illustrates the stepwise dose-dependent formation of Glc_1- (0.01 mM), Glc_2- (0.1 mM) and $Glc_3Man_9GlcNAc_2$ (1.0 mM) α_1-antitrypsin oligosaccharides due to the action of a glucosidase inhibitor. Inhibition of α-glucosidase I is still incomplete at 3.0 mM. Glucosidase-inhibitor-treated cells synthesize glucosylated α_1-antitrypsin oligosaccharides which are sensitive to Endo H treatment; this is illustrated in Figure 13 for *N*-benzyl-dNM. The length of the *N*-alkyl chain cannot be extended beyond a certain limit due to detergent-like properties as observed with *N*-decyl-dNM (**47**) (see also in Figure 11 the lack of α_1-antitrypsin in the lanes of 1.0 and 3.0 mM). However, clear inhibition of α-glucosidase at concentrations a factor 5–10 lower than for some of the other *N*-alkyl-dNM analogues, *e.g.* **34**, **35** and **38**, was observed. It would be of great interest to modify the chemical structure of **47** in such a way that glucosidase inhibitory activity is retained and the drug-related cell toxicity is eliminated. Studies directed at this goal are underway and will be reported in due time.

(ii) Branching of the N-alkyl side chain. Branching of the *N*-alkyl side chain leads to a remarkable reduction in the ability of the drug to alterate glucose trimming. The *N*-isobutyl-dNM (**36**) isomer is less active than *N*-butyl-dNM, while the *N*-(cyclopropylmethyl)-dNM (**37**) isomer is almost inactive. In case of the *N*-pentyl-dNM (**38**) analogues, *N*-isopentyl-dNM (**39**) shows activity comparable to that of **38**, while *N*-(cyclobutylmethyl)-dNM (**40**) is almost inactive. Also, the position of the phenyl group in the alkyl side chain as well as substitution of the phenyl group in *N*-benzyl-dNM (**41**) is of importance. *N*-(3-Phenylpropyl)-dNM (**45**) as well as *N*-[*p*-(trifluoromethyl)benzyl]-dNM (**43**) and *N*-[*m*-(trifluoromethyl)benzyl]-dNM (**44**) show decreased activity as compared to compound **41**. *N*-[2(*p*-Fluorophenoxy)ethyl]-dNM (**46**), a derivative of **45**, is comparably active to **45** but less active than **41**.

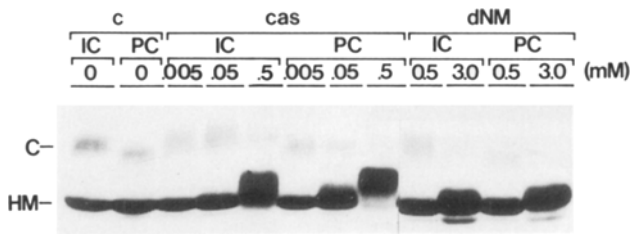


Figure 14. Effect of CAS and dNM in intact and streptolysin-O-permeabilized HepG2 cells. HepG2 cells were pretreated for 1½ h with CAS and dNM, transferred to 0°C and incubated without (intact cells, IC) or with 0.5 U/ml streptolysin O (permeabilized cells, PC). Unbound streptolysin O was removed by washing, followed by transfer of the cells to 37°C for 1½ h in the presence of inhibitors and 20 μCi [^{35}S]methionine-cysteine. The positions of α_1-antitrypsin with high-mannose (HM) and complex (C) type oligosaccharides are indicated.

(iii) Ester derivatives. Of two structures, *N*-benzyl- (**41**) and *N*-decyl-dNM (**47**), ester prodrugs were synthesized, *i.e.* compounds **42** and **48**, respectively (*vide supra*). *N*-Benzyl-6-*O*-butyryl-dNM (**42**) and *N*-decyl-6-*O*-benzoyl-dNM (**48**) showed activity comparable to that of their respective parent compounds. These results are contrary to what has been reported for 6-*O*-butyryl-CAS. Surprisingly, this ester derivative was found to be 20 times more active in vitro than CAS itself[4h]. The role of the position of the ester group in *N*-substituted dNM derivatives on biological activity has not yet been studied and we are currently investigating this.

(iv) 3-O-Methyl derivatives. Subsequently, the effect of replacing the 3-hydroxyl group in dNM and dMM with a methoxy group, *i.e.* compounds **49** and **56**, respectively, was investigated. As shown in Table II, 3-*O*-methyl-dNM (**49**) (see also Figure 11) and 3-*O*-methyl-dMM (**56**) displayed no activity. Apparently, the 3-hydroxyl group is important for binding of dNM and dMM to the enzymes, presumably through an interaction with a hydrogen bond acceptor. A study in the literature reported that 3-deoxy-3-fluoro-dNM (**10**) showed 26% inhibition at 1 mM and 63% inhibition at 5 mM against yeast α-glucosidase[21]. The corresponding *N*-butyl-3-deoxy-3-fluoro-dNM derivative (structure not given) showed, however, much less activity: 11% at 1mM and 18% at 5mM concentrations[21]. Our data indicate that caution should be taken in interpreting biological data on enzymes: α-glucosidases obtained from yeast and from mammalian cells are distinct in their sensitivity to azasugar inhibitors. The unfavourable interaction of the 3-*O*-methylated azapyranose ring in **49** with trimming α-glucosidase could not be compensated for by introduction of an *N*-alkyl side chain as in **50**. *N*-Decyl-3-*O*-methyl-dNM (**50**) still lacked biological activity; it showed drug-related cell toxicity at concentrations comparable to those for *N*-decyl-dNM (**47**). A point relating to what has been described above is the following. In a previous paper we reported the lack of anti-α-glucosidase inhibitory activity of two tetra-acetate derivatives: *N*-benzyl-tetra-*O*-acetyl-dNM and *N*-butyl-tetra-*O*-acetyl-dNM (structures not shown)[39]. Given the high activity of the 6-mono-esterified analogue(s) **42** (and **48**) it can be concluded that presumably no intracellular hydrolysis of ester prodrugs occurs when no free hydroxyl group is present.

(v) C1 derivatives. Compounds **51**, **52** and **53** are derivatives of dNM modified at C1. None of these drugs showed

α-glucosidase inhibitory activity in HepG2 cells. The lactam 1-oxo-dNM (**51**) was earlier reported to have a K_i of 37 μM against sweet almond β-glucosidase[10]. However, this information proved of no predictive relevance as to the activity of **51** against the processing α-glucosidase. From data in the literature it is known that azaglucopyranoses with an sp^2 C1 atom show activity against β-glucosidases rather than against α-glucosidases[4h-j]. The lack of activity of 1-α-cyano-dNM (**53**) is surprising since several 1-α-substituted dNM derivatives, *e.g.* **17**, **18**, **19** and **20**, have been reported to possess α-glucosidase inhibitory activity (see Table I).

α-Glucosidase inhibitory activity in permeabilized HepG2 cells[52]

CAS is the most powerful α-glucosidase inhibitor that we have tested in HepG2 cells thus far. CAS, but not dNM, exhibited a stronger inhibitory activity in streptolysin *O*-permeabilized[52] than in intact HepG2 cells (Figure 14). It has been reported that dNM rapidly enters the cell, in unprotonated form, through non-facilitated diffusion[54-55]. The plasma membrane apparently constitutes a permeability barrier for CAS[1g], but not for dNM to enter the cytosol in order to gain access to the target enzymes in the ER. This unexpected finding calls for further investigation on the membrane permeability properties of glycosidase inhibitors. Also, concentrations required for significant inhibition of α-glucosidase in HepG2 cells and syncytia formation of HIV-infected cells (IC_{50} in mM range) are much higher than the concentrations required to inhibit isolated α-glucosidases (IC_{50} in μM range). This discrepancy is likely due to differences in accessibility of enzymes *in vitro* and *in vivo*.

α-Mannosidase inhibitory activity in intact HepG2 cells[39]

Analogues of the α-mannosidase inhibitor dMM, *i.e.* **54–56**, did not show activity in the HepG2 assay. This observation confirms earlier data that *N*-alkylation of dMM, unlike *N*-alkylation of dNM, resulted in loss of α-mannosidase inhibitory activity (*vide supra*)[15]. Incorporation of a phenyl group (*N*-benzyl-dMM, **54**) or a hydroxyl group [*N*-2(hydroxyethyl)-dMM, **55**] in the side chain of dMM resulted in complete loss of activity. However, compound **55** showed an inhibitory effect different from the effects of dMM and SW (Figure 15). At high concentration (25 mM), the effects of *N*-(2-hydroxyethyl)-dMM, **55** resemble that of SW. We have no explanation for these observations.

Antiviral activity[4d,53]

The following conclusions can be drawn regarding the antiviral activity of compounds **34–56** (Table II). For selected compounds (*i.e.*, **1**, **2**, **35**, **36**, **38**, **41**, **47** and **49**) the dose dependency of inhibition is shown in Figure 16. A remarkable parallel in activity in the HepG2 and the HIV-I assays was observed[see also ref. 4h]. However, for compounds **43** and **44** a poor correlation was found, underscoring the need for parallel testing. The most potent α-glucosidase inhibitors, *i.e.* *N*-butyl- (**35**), *N*-pentyl- (**38**), *N*-benzyl- (**41**) and *N*-decyl-dNM (**47**) and CAS (**2**), also showed the strongest inhibition of HIV-induced cytopathogenicity. In agreement with observations of others, *N*-butyl-dNM (**35**) exhibited potent antiviral activity[4a,b,e]. In our hands compounds **35**, **38** and **41** were at least as effective as CAS (**2**). Compounds **38** and **41** showed a 100-fold lower $TCID_{50}$ (50% tissue culture infectious dose), similar to CAS[4d]. Neither dMM[4a,d] nor one of its analogues **54–56** showed antiviral activity. The results described above indicate a strong correlation between glucosidase inhibition observed in human hepatoma HepG2 cells and antiviral activity observed in HIV-infected cells. The use of both these assays should enable us to select α-glucosidase inhibitors which show promise for antiviral chemotherapy. Special attention will be paid to the membrane permeability properties of the inhibitors using permeabilized HepG2 cells.

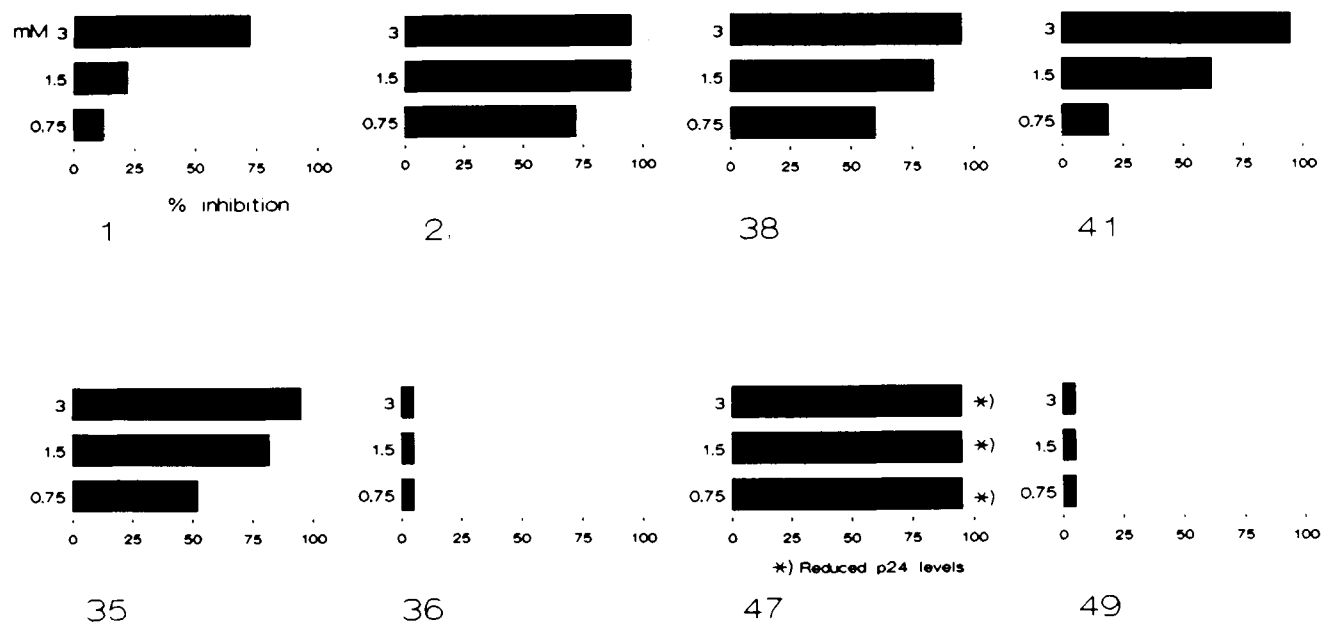


Figure 16. Improved effectiveness of N-alkylated dNM compounds in inhibiting HIV-induced syncytium formation. The HIV-I-infected promonocytic cell line U937 was grown in the presence of the indicated amounts of inhibitors for at least 3 days in Iscove's modified Dulbecco's medium containing 10% fetal calf serum in 1 ml cultures. Compounds shown are dNM (1), CAS (2), N-butyl- (35), N-isobutyl- (36), N-pentyl- (38), N-benzyl- (41), N-decyl- (47) and 3-O-methyl-dNM (49). Cells were harvested, counted and mixed with uninfected supT1 cells in 96-well Nunc plates. After 6 h and 24 h cultures were scored for syncytia with a microscope and compared to controls in a semi-quantitative manner (< 5%, 25%, 75% and > 95%, see also Table II: ++, +, +/− and −, respectively). Mean inhibition percentages of three experiments are shown.

Experimental

Chemistry

^{1}H-NMR (200 or 360 MHz), ^{13}C-NMR (50 MHz) and ^{19}F-NMR (188 MHz) spectra were measured using a Bruker WM-200 spectrometer; chemical shifts are given in ppm (δ) relative to TMS for spectra run in $CDCl_3$ and CD_3OD or relative to D_2O. Optical rotations were recorded at ambient temperature on a Perkin–Elmer 241 polarimeter. TLC analysis was performed on Merck DC-Fertigplatten (Kieselgel 60 F_{254}). Compounds were visualized by spraying with H_2SO_4/EtOH, 1:9 or Cl_2-TMB. For column chromatography Merck Kieselgel 60 (230–400 mesh) was used. The synthesis of dNM (**1**) was carried out as described earlier[48].

*N(Cyclobutylmethyl)-dNM (**40**)*

Cesium carbonate (2.1 mmol, 684 mg) was added to a stirred suspension of dNM (**1**, 2 mmol, 326 mg) and cyclobutylmethyl bromide (4 mmol, 0.42 ml) in 10 ml of dry DMF. The suspension was stirred for 12 days at 70°C. After completion of the reaction, the suspension was filtered and the filtrate concentrated. Column chromatography on silica gel (eluent iPrOH/H_2O/NH_4OH, 190:10:1) afforded the pure compound **40** in 16% yield. TLC: R_f 0.63 (eluent iPrOH/H_2O/NH_4OH, 160:40:1). ^{1}H NMR (CD_3OD): 1.65–2.15 (m, 8H, $CH_2CH_2CH_2$, H1ax, H_5), 2.52 (dd, 1H, NCH_2CH, ^3J 6 Hz, ^2J 13 Hz), 2.56–2.67 (m, 1H, NCH_2CH), 2.89 (dd, 1H, NCH_2CH, ^3J 7 Hz, ^2J 13 Hz), 2.99 (dd, H1eq, ^3J 5 Hz, ^2J 11 Hz), 3.12 (t, H3, J 9 Hz), 3.34 (t, H_4, J 9 Hz), 3.44 (ddd, H2, $J_{2,4}$ 5 Hz, $J_{2,3}$ 9 Hz), 3.82 (dd, H6, ^3J 3 Hz, ^2J 12 Hz), 3.86 (dd, H6, ^3J 3 Hz, ^2J 12 Hz). ^{13}C NMR (CD_3OD): 19.60 ($CH_2CH_2CH_2$), 29.12 and 29.25 ($CH_2CH_2CH_2$), 34.09 (NCH_2CH), 57.91, 59.25 and 59.96 (C1, C6, NCH_2CH), 67.90, 70.53, 71.76 and 80.34 (C2, C3, C4, C5). $[\alpha]_D^{20}$ −30.0° (*c* 1.0, CH_3OH).

*N-Benzyl-6-O-butyryl-dNM (**42**)*

2,2,2-Trifluoroethyl butyrate (5 mmol, 850 mg) and subtilisin (100 mg)[43] were added to a stirred solution of *N*-benzyl-dNM (**41**, 0.5 mmol, 126.5 mg) in 10 ml of dry pyridine. The reaction mixture was stirred for 3 weeks at 50°C. The mixture was filtered and the filtrate concentrated. According to TLC a mixture of reaction products was obtained. Column chromatography on silica gel (eluent toluene/ethanol, 95:5) afforded the desired monobutyrate (**42**, 8% yield) as well as *N*-benzyl-2,6-di-*O*-butyryl-dNM (structure not shown, 5% yield). TLC: R_f 0.25 (**42**) (eluent toluene/ethanol, 9:1). The hydrochloride of **42** was prepared from 0.25N HCl in MeOH. ^{1}H NMR (D_2O): 0.76 (t, 3H, CH_2CH_3, J 7 Hz), 1.47 (sextet, 2H, CH_2CH_3, J 7 Hz), 2.29 (t, $C(O)CH_2$, J 7 Hz), 2.74 (dd, H1ax, ^3J 11 Hz, ^2J 12 Hz), 3.15 (dd, H1eq, ^3J 5 Hz, ^2J 12 Hz), 3.28–3.58 (m, 4H, H2, H3, H4, NCH_2Ph), 4.00 (d, 1H, NCH_2Ph, J 13 Hz), 4.53 (dd, H6, ^3J 2 Hz, ^2J 12 Hz), 4.62 (dd, H6′, ^3J 2 Hz, ^2J 12 Hz), 7.25–7.38 (m, 5H, Ph). ^{13}C NMR (D_2O): 15.56 (CH_2CH_3), 20.52 (CH_2CH_3), 38.02 [$C(O)CH_2$], 55.90 and 60.21 (C1, C_6, NCH_2Ph), 67.42, 68.69 and 70.08 (C2, C4, C5), 78.58 (C3), 131.24–133.99 (Ph), 178.48 [C(O)].

*N-[p-(Trifluoromethyl)benzyl]-dNM (**43**)*

This compound was prepared in 56% yield from dNM (**1**, 2 mmol, 326 mg) and *p*-(trifluoromethyl)benzyl bromide (4 mmol, 0.61 ml) according to the procedure used to prepare **40** (*vide supra*). TLC: R_f 0.93 (eluent iPrOH/H_2O/NH_4OH, 160:40:1). ^{1}H NMR (CD_3OD): 1.88 (dd, H1ax, ^3J ^2J 11 Hz), 2.14 (ddd, H5, $J_{5,6}$ 3 Hz, $J_{5,4}$ 9 Hz), 2.74 (dd, H1eq, ^3J 5 Hz, ^2J = 11 Hz), 3.12 (t, H3, J 9 Hz), 3.22–3.43 (m, 3H, H2, H4, NCH_2Ph), 3.87 (dd, H6, ^3J 3 Hz, ^2J 12 Hz), 4.05 (dd, H6′, ^3J 3 Hz, ^2J 12 Hz), 4.32 (d, 1H, NCH_2Ph), 7.55 (ABq, 4H, Ph, J 9 Hz). ^{13}C NMR ($CDCl_3$): 55.96, 56.26 and 58.89 (C1, C6, NCH_2Ph), 66.18, 69.01, 70.94 and 78.80 (C2, C3, C4, C5). 124.08 (d, CF_3, J 270 Hz), 125.15 (Ph), 129.17 (Ph), 129.46 (d, CCF_3, J 29 Hz), 142.17 (Ph). $[\alpha]_D^{20}$ −35.6° (*c* 1.0, CH_3OH).

*N-[m-(Trifluoromethyl)benzyl]-dNM (**44**)*

This compound was prepared in 51% yield from dNM (**1**, 2 mmol, 326 mg) and *m*-(trifluoromethyl)benzyl bromide (4 mmol, 0.61 ml) according to the procedure used to prepare **40** (*vide supra*). TLC: *R*f 0.92 (eluent iPrOH/H_2O/NH_4OH, 160:40:1). ^{1}H NMR (CD_3OD): 1.94 (dd, H1ax, ^3J ^2J 11 Hz), 2.20 (br d, H5, J 9 Hz), 2.78 (dd, H1eq, ^3J 5 Hz, ^2J 11 Hz), 3.15 (t, H3, J 9 Hz), 3.30–3.47 (m, 3H, H2, H4, NCH_2Ph), 3.93 (dd, H6, ^3J 3 Hz, ^2J 12 Hz), 4.08 (dd, H6′, ^3J 3 Hz, ^2J 12 Hz), 4.37 (d, 1H, NCH_2Ph), 7.45–7.70 (m, 4H, Ph). ^{13}C NMR ($CDCl_3$): 59.73, 56.27 and 58.90 (C1, C6, NCH_2Ph), 66.10, 68.88, 70.97 and 78.74 (C2, C3, C4, C5), 124.00 (d, CF_3, J 270 Hz), 124.08, 125.51 and 128.88 (Ph), 130.62 (d, CCF_3, J 32 Hz), 132.44 (Ph), 138.88 (Ph). $[\alpha]_D^{20}$ −30.8° (*c* 1.0, CH_3OH).

*N-[p-(Fluorophenoxy)ethyl]-dNM (**46**)*

A 10% solution of Pd/C (330 mg) was added to a solution of **60** (0.5 mmol, 330 mg) in tBuOH/H_2O/HOAc (3:3:1) and hydrogen was passed through the solution. After 24 h the catalyst was filtered off and the filtrate was treated with Amberlite OH$^-$ and concentrated. Yield 119 mg (79%). TLC: *R*f 0.78 (eluent CH_2Cl_2/MeOH, 8:2). ^{1}H NMR (D_2O): 2.40–2.53 (m, 2H, H1ax, H5), 2.98–3.27 (m, 3H, H1eq, NCH_2CH_2), 3.30 (t, H4, J 9 Hz), 3.44 (t, H3, J 9 Hz), 3.59 (ddd, H2, J 5 Hz, $J_{2,3}$ 9 Hz), 3.89 (dd, *H6*, ^3J 3 Hz, ^2J 13 Hz), 4.02 (dd, H6′, ^3J 2.5 Hz, ^2J 13 Hz), 4.20 (t, NCH_2CH_2, J 5 Hz), 6.93–7.19 (m, 4H, Ph). ^{13}C NMR (D_2O) 53.05, 58.77, 60.34 and 67.98 (C1, C6, NCH_2CH_2), 68.22, 71.42 and 72.64 (C2, C4, C5), 80.99 (C3), 118.50–118.96 (Ph), 156.77 (Ph), 160.13 (d, CF, J 236 Hz). ^{19}F NMR (D_2O) −127.68 (CF). $[\alpha]_D^{20}$ −2.4° (*c* 1.0, H_2O).

*N-Decyl-6-O-benzoyl-dNM (**48**)*

NaH (0.14 mmol, 6 mg)[47] was added to a solution of *N*-decyl-dNM (**47**, 0.07 mmol, 21.2 mg), 2-mercaptothiazoline benzoate (0.07 mmol, 15.6 mg) and a catalytic amount of 4-(dimethylamino)pyridine under nitrogen at 0°C. The reaction mixture was stirred for 3 h, Dowex 50Wx4 (H^+ form) was added and stirring was continued for 30 min. After filtration the filtrate was concentrated and the product was purified by column chromatography on silica gel (eluent toluene/ethanol, 9:1) to give 13 mg (45%) of the desired ester **48**. TLC: R_f 0.68 (eluent toluene/ethanol, 9:1). The hydrochloride of **48** was prepared from 0.25N HCl in MeOH. ^{1}H NMR (CD_3OD) 0.73 (t, 3H, CH_2CH_3), 1.00–1.25 [m, 14H, $(CH_2)_7CH_3$], 1.50–1.70 (m, 2H, NCH_2CH_2), 2.90 (dd, H1ax, ^3J, ^2J 11 Hz), 3.20–3.75 (m, 7H, H1eq, H2, H3, H4, H5, NCH_2CH_2), 4.63 (dd, H6, ^3J 3.5 Hz, ^2J 12 Hz), 4.73 (dd, H6′, ^3J 2.5 Hz, ^2J 12 Hz), 7.30–7.54 and 7.94–8.00 (m, 5H, Ph). ^{13}C NMR (CD_3OD) 14.33 (CH_3), 23.59, 27.53, 30.07, 30.26, 30.45 and 32.99 [$(CH_2)_8CH_3$], 54.92 (NCH_2CH_2, C1), 60.10 (C6), 65.60, 67.79 and 69.43 (C2, C3, C4, C5), 129.61, 130.37, 130.85 and 134.71 (Ph), 167.19 (C(O)). $[\alpha]_D^{20}$ −0.5° (*c* 0.5, CH_3OH).

*3-O-Methyl-dNM (**49**) and 3-O-Methyl-dMM (**56**)*

A solution of **66** (3.58 mmol, 1.25 g) in 15 ml of trifluoroacetic-acid/water, 9:1 was stirred at room temperature for approximately 30 min until no starting material could be detected by TLC analysis (eluent EtOAc/hexane, 3:7)[48]. An excess of toluene was added and the solution was concentrated and dried (1.197 g). The residue was used without purification for the subsequent reaction.

To a solution of the isopropylidene-deprotected compound (1.154 g) in 15 ml of tBuOH/H_2O/HOAc, 4:1:1, was added 20% $Pd(OH)_2$/C (1.15 g). The reaction mixture was hydrogenated overnight in a Parr apparatus at 55 psi H_2[48]. The catalyst was filtered off, the filtrate concentrated and the remaining oil was purified by column chromatography on silica gel (eluent iPrOH/H_2O/NH_4OH, 185:15:1) to give 549 mg (87% yield) of **49** and 81 mg (13% yield) of **56** TLC: *R*f 0.42 (**49**) and 0.20 (**56**) (eluent iPrOH/H_2O/NH_4OH, 160:40:1).

Compound **49**. ^{1}H NMR (D_2O): 2.60 (dd, H1ax, ^3J 11 Hz, ^2J 12 Hz), 2.73 (ddd, H5, $J_{5,6}$ 3 Hz, $J_{5,6'}$ 6 Hz, $J_{5,4}$ 9 Hz), 3.17 (t, H3, J 9 Hz), 3.23 (dd, H1eq, ^3J 5 Hz, ^2J 12 Hz), 3.43 (t, H4, J 9 Hz), 3.58–3.70 (m, H2), 3.66 (s, OCH_3), 3.65 (dd, H6′, ^3J 6 Hz, ^2J 12 Hz), 3.87 (dd, H6, ^3J 3 Hz, ^2J 12 Hz). ^{13}C NMR (D_2O): 50.80 (C1), 62.83 (OCH_3), 63.05 (C6), 63.17 (C5), 72.18 and 72.81 (C2, C4), 90.40 (C3).

Compound **56**. ^{1}H NMR (D_2O): 2.63 (ddd, H5, $J_{5,6'}$ 4 Hz, $J_{5,4}$ 10 Hz), 2.84 (dd, H1eq, ^3J 1.5 Hz, ^2J 14 Hz), 3.14 (dd, H1ax, ^3J 3 Hz, ^2J 14 Hz), 3.29 (dd, H3, $J_{3,2}$ 3 Hz, $J_{3,4}$ 9 Hz), 3.47 (s, OCH_3), 3.70 (t, H4, J 9 Hz), 3.82 (d, H6, H6′, J 4 Hz), 4.30–4.35 (m, H2). ^{13}C NMR (D_2O): 50.57 (C1), 58.85 (OCH_3), 62.90 (C6), 63.25, 66.79 and 69.57 (C2, C4, C5), 86.13 (C3).

*N-Decyl-3-O-methyl-dNM (**50**)*

To a solution of **49** (0.45 mmol, 80 mg) in 15 ml of tBuOH/H_2O/NH_4OH, 18:9:3 under N_2 was added 80 mg 20% $Pd(OH)_2$/C and decanal (4.5 mmol, 847 µl). The mixture was hydrogenated in a Parr apparatus at 55 psi H_2 for 2 days. The catalyst was removed by filtration and the filtrate was extracted with

CH_2Cl_2 repeatedly to remove decanal. The product was obtained in 50% yield. TLC: *Rf* 0.80 (eluent iPrOH/H_2O/NH_4OH, 160:40:1). The hydrochloride of **50** was prepared from 0.25N HCl in MeOH. ^1H NMR (CD_3OD) 0.89 (br t, $CH_2C\underline{H}_3$), 1.15–1.40 [br s, 14H, $(CH_2)_7$], 1.50–1.70 [br s, 2H, $C\underline{H}_2(CH_2)_7$], 2.64 (br t, 2H, H1ax, H5, *J* 10 Hz), 2.83–3.30 (m, 4H, H2, H1eq, $NC\underline{H}_2CH_2$), 3.00 (t, H3, *J* 9 Hz), 3.55 (t, H4, *J* 9 Hz), 3.64 (s, OCH_3), 3.85 (dd, H6, *J* 12 Hz), 3.97 (dd, H6′, *J* 12 Hz). ^{13}C NMR (CD_3OD): 14.36 ($CH_2\underline{C}H_3$), 23.62, 24.59, 27.98, 30.32, 30.54 and 32.94 [$(CH_2)_8$], 53.89 ($N\underline{C}H_2CH_2$), 55.98 (C1), 56.84 (C6), 61.15 (OCH_3), 67.36, 68.81 and 69.70 (C2, C4, C5), 88.84 (C3).

N-Benzyl-dMM (**54**)

This compound was prepared in 95% yield from dMM (**2**, 2.5 mmol, 400 mg) and benzyl bromide (2.6 mmol, 0.31 ml) according to the procedure used to prepare **40** (*vide supra*). TLC: R_f 0.85 (eluent iPrOH/H_2O/NH_4OH, 160:40:1). ^1H NMR (D_2O): 2.25–2.40 (m, H1ax, H5), 2.94 (dd, H1eq, ^3J 3 Hz, ^2J 13 Hz), 3.47 (dd, H3, J_{3-2} 3 Hz, J_{3-4} 9 Hz), 3.45 (d, 1H, $C\underline{H}_2Ph$, *J* 13 Hz), 3.78 (t, H4, *J* 9 Hz), 3.90 (ddd, H2, *J* 1.5 Hz *J* 3 Hz), 4.02 (dd, H6, ^3J 3 Hz, ^2J 3 Hz, ^2J 13 Hz), 4.19 (dd, H6′, ^3J 3 Hz, ^2J 13 Hz), 4.20 (d, 1H, $C\underline{H}_2Ph$, *J* 13 Hz), 7.35–7.45 (m, 5H, Ph). ^{13}C NMR (D_2O): 57.20, 59.27 and 60.47 (C1, $N\underline{C}H_2Ph$, C6), 69.25, 70.27, 70.45 and 77.06 (C2, C3, C4, C5), 130.44, 131.30 and 132.71 (Ph). $[\alpha]_D^{20}$ −45.8° (*c* 0.5, H_2O).

N-(2-Hydroxyethyl)-dMM (**55**)

An excess of ethylene oxide was added to a solution of dMM (**2**, 0.61 mmol, 100 mg) in 4 ml of dry DMF in a pressure reaction vessel. The sealed container was stirred for 1 day at 80°C. Concentration of the solvent *in vacuo* and purification over silica gel (eluent EtOH/H_2O/NH_4OH, 16:1:1) afforded 121.3 mg (95% yield) of the desired product. TLC: R_f 0.25 (eluent iPrOH/H_2O/NH_4OH, 160:40:1). ^1H NMR (D_2O): 2.25 (ddd, H5, J_{5-6}, $J_{5-6'}$ 3 Hz, J_{5-4} 9 Hz), 2.52–2.67 (m, 2H, $NC\underline{H}_2CH_2$, H1ax), 2.91–3.08 (m, 2H, $NCHC\underline{H}_2$, H1eq), 3.46 (dd, H3, J_{3-2} 3 Hz, J_{3-4} 9 Hz), 3.58–3.75 (m, 3H, $NCH_2C\underline{H}_2$, H4), 3.82 (dd, H6, ^3J 3 Hz, ^2J 13 Hz), 3.93 (dd, H6′, ^3J 3 Hz, ^2J 13 Hz), 3.90–3.94 (m, H2). ^{13}C NMR (D_2O): 56.06, 58.03, 60.71 and 60.83 (C1, C6, NCH_2CH_2), 68.84, 70.52, 70.58 and 77.09 (C2, C3, C4, C5). $[\alpha]_D^{20}$ −64.8° (*c* 1.0, H_2O).

N-Benzyl-2,3,4,6-tetra-O-benzyl-dNM (**57**)

NaH (oil suspension, 4.5 mmol, 166 mg) was added to a solution of *N*-benzyl-dNM (**41**, 1 mmol, 253 mg) in 10 ml of dry DMF at 0°C under nitrogen. To this suspension benzyl bromide (4.25 mmol, 0.5 ml) was added slowly. The reaction mixture was stirred overnight and allowed to come to room temperature. Methanol was added to the reaction mixture and the solvents were removed *in vacuo*. The residue was taken up in CH_2Cl_2 and extracted with a saturated $NaHCO_3$ solution. The organic layer was separated, dried and concentrated. Column chromatography on silica gel (eluent EtOAc/hexane, 1:9) yielded 581 mg (95%) of the desired product. TLC: R_f 0.48 (eluent EtOAc/hexane, 1:9). ^1H NMR ($CDCl_3$): 2.01 (dd, H1ax, ^3J 10 Hz, ^2J 11 Hz), 2.40 (ddd, H5, J_{5-6} 3 Hz, $J_{5-6'}$ 2 Hz, J_{5-4} 9 Hz), 3.03 (dd, H1eq, ^3J 5 Hz, ^2J 11 Hz), 3.37–3.68 (m, 4H, H2, H3, H4, $NC\underline{H}_2Ph$), 3.72 (dd, H6, ^3J 3 Hz, ^2J 11 Hz), 3.81 (dd, H6′, ^3J 2 Hz, ^2J 11 Hz), 4.07 (d, 1H, $NC\underline{H}_2Ph$), 4.44–4.99 (4 ABq, 8H, $OC\underline{H}_2Ph$), 7.12–7.36 (m, 25H, Ph). ^{13}C NMR ($CDCl_3$): 54.24 and 56.61 (C1, $N\underline{C}H_2Ph$), 65.07 (C5), 66.63 (C6), 72.58, 73.33 and 75.30 ($O\underline{C}H_2Ph$), 78.28 and 78.75 (C2, C4), 87.35 (C3), 127.02–129.06 (Ph), 138.04–139.08 (Ph).

2,3,4,6-Tetra-O-benzyl-dNM (**58**)[26]

$Pd(OH)_2$/C (20% w/w, 215 mg) was added to a solution of **57** (2.59 mmol, 1.59 g) in 30 ml of dry ethanol (0.1 M). Hydrogenation at 55 psi H_2 in a Parr apparatus overnight resulted in selective *N*-debenzylation in accordance with a recent report[40]. The product was purified by column chromatography on silica gel (eluent toluene/acetone, 95:5 → 9:1) to yield 1.28 g (91%) of **58**. TLC: R_f 0.66 (eluent MeOH/CH_2Cl_2, 95:5). ^1H NMR ($CDCl_3$): 2.05 (br s, NH), 2.50 (dd, H1ax, ^3J 10 Hz, ^2J 12 Hz), 2.72 (ddd, H5, J_{5-6} 3 Hz, $J_{5-6'}$ 6 Hz, J_{5-4} 9 Hz), 3.24 (dd, H1eq, ^3J 5 Hz, ^2J 12 Hz), 3.34 (t, H3, *J* 9 Hz), 3.43–3.57 (m, 3H, H2, H_4, $H_{6'}$), 3.67 (dd, H6, ^3J 3 Hz, ^2J 9 Hz), 4.38–5.01 (4 ABq, 8H, $C\underline{H}_2Ph$), 7.17–7.38 (m, 20H, Ph). ^{13}C NMR ($CDCl_3$) 48.12 (C1), 59.74 (C5), 70.25, 72.83, 73.43, 75.24 and 76.45 (C6, $\underline{C}H_2Ph$), 80.08 and 80.64 (C2, C3), 87.35 (C4), 127.59–128.45 (Ph), 137.99, 138.41, 138.51 and 138.93 (Ph).

N-[(p-Fluorophenoxy)ethyl]-2,3,4,6-tetra-O-benzyl-dNM (**60**)

Triflic anhydride (1.44 mmol, 0.242 ml) was slowly added to a solution of 2-(*p*-fluorophenoxy)ethanol (1.6 mmol, 250 mg) and *N,N*-diisopropylethylamine (6.4 mmol, 1.12 ml) in 10 ml of dry CH_2Cl_2 at −20°C. After stirring for 30 min, a solution of **58** (1 mmol, 523 mg) in 5 ml of dry CH_2Cl_2 was added slowly. The reaction mixture was allowed to warm to room temperature and was stirred overnight. To the reaction mixture 0.5N NaOH and water was added and the products were extracted with CH_2Cl_2. The organic layer was separated, dried and concentrated. Column chromatography on silica gel (toluene/acetone, 97.5:2.5) afforded 369 mg (56% yield) of **60**. TLC: R_f 0.72 (eluent toluene/acetone, 9:1). ^1H NMR ($CDCl_3$): 2.44 (dd, H1ax, ^3J 10 Hz, ^2J 11 Hz), 2.52 (ddd, H5, J_{5-6}, $J_{5-6'}$ 2 Hz, J_{5-4} 9 Hz), 3.11 (dt, 2H, $NC\underline{H}_2CH_2$, ^3J 6 Hz, ^2J 1 Hz), 3.18 (dd, H1eq, ^3J 5 Hz, ^2J 11 Hz), 3.43–3.70 (m, 3H, H2, H3, H4), 3.72 (d, H6, H6′, *J* 2 Hz), 3.98 (t, 2H, $NCH_2C\underline{H}_2$, ^3J 6 Hz), 4.38–4.99 (4 ABq, 8H, $C\underline{H}_2Ph$), 6.72–7.35 (m, 24H, Ph). ^{13}C NMR ($CDCl_3$): 50.97 and 55.38 (C1, $N\underline{C}H_2CH_2$), 64.18 (C5), 65.63 and 66.04 (C6, $NCH_2\underline{C}H_2$), 72.86, 73.52, 75.28 and 75.44 ($\underline{C}H_2Ph$), 78.38 and 78.40 (C2, C4), 87.25 (C3), 115.40, 115.63 and 116.09 (Ph), 127.66, 127.75, 127.95 and 128.41 (Ph), 137.76, 138.50 and 138.98 (Ph).

1,2-O-Isopropylidene-3-O-methyl-5,6-anhydro-β-L-idofuranose (**63**)

Pd/C (10%, 0.6 g) was added by a stirred solution of **61**[48,49] (10.2 mmol, 3 g) in 12 ml of dry DMF under N_2. Hydrogen was passed through overnight until complete conversion as judged from TLC analysis. The catalyst was removed by filtration over hyflo and the filtrate was used without purification for subsequent methylation. TLC: R_f 0.47 (**62**) and 0.87 (**61**) (eluent EtOAc/hexane, 3:2).
Two by-products were isolated when the hydrogenation was carried out in MeOH after purification by column chromatography: 1,2-*O*-isopropylidene-6-deoxy-β-L-idofuranose and 1,2-*O*-isopropylidene-6-*O*-methyl-β-L-idofuranose (structures not shown).
The stirred solution was put under a N_2 atmosphere and an extra 23 ml of dry DMF was added. To this solution, MeI (3 eq. 1.92 ml) was added and the mixture was cooled to 10–15°C. NaH (oil suspension, 12 mmol, 483 mg) was added in portions and after complete addition the reaction mixture was allowed to warm to room temperature and stirred for 1 h. MeOH and subsequently water were added and the mixture was extracted with EtOAc. The organic layer was separated and extracted with a $NaHSO_3$ solution, separated, dried and concentrated *in vacuo*. The product was purified by column chromatography on silica gel (eluent EtOAc/toluene, 1:9) to give 1.44 g (65% yield) of **63**. TLC: R_f 0.23 (eluent EtOAc/toluene, 2:8). ^1H NMR ($CDCl_3$) 1.33 and 1.47 [2 s, $C(CH_3)_2$], 2.68 (dd, H6, ^3J 3 Hz, ^2J 5 Hz), 2.85 (dd, H6′, ^3J 4 Hz, ^2J 5 Hz), 3.23 (ddd, H5, J_{5-6} 3 Hz, $J_{5-6'}$ 4 Hz, J_{5-4} 6 Hz), 3.45 (s, OCH_3), 3.79 (d, H3, *J* 3 Hz), 3.85 (dd, H4, J_{4-3} 3 Hz, J_{4-5} 6 Hz), 4.62 (d, H_2, *J* 3 Hz), 5.79 (d, H1, *J* 3 Hz).

1,2-O-Isopropylidene-3-O-methyl-6-O-benzyl-β-L-idofuranose (**64**)

NaH (oil suspension, 1.1 eq, 565 mg) was added to a stirred solution of **63** (13.88 mmol, 3.01 g) in 10 ml of dry DMF under N_2 at 0°C. Subsequently, benzyl alcohol (1.05 eq, 1.5 ml) was added dropwise[48]. The reaction mixture was allowed to warm to room temperature and was stirred overnight at 55°C. Diluted HOAc was added to the reaction mixture until neutral pH and the solvents were removed *in vacuo*. The residue was dissolved in Et_2O and extracted with a saturated NH_4Cl solution and a saturated NaCl solution. The organic layer was separated, dried and concentrated. The resulting oil was purified by column chromatography on silica gel (eluent Et_2O/hexane, 1:2 → 2:3) to give 1.953 g (43% yield) of **64**. TLC: *Rf* 0.21 (eluent Et_2O/hexane, 7:3). ^1H NMR ($CDCl_3$): 1.33 and 1.50 [2 s, $C(CH_3)_2$], 2.94 (d, OH), 3.33 (s, OCH_3), 3.57 (d, H6, ^3J 1.5 Hz), 3.59 (d, H6′, ^3J 1 Hz), 3.70 (d, H3, *J* 3 Hz), 4.14 (ddd, H5, *J* 3 Hz, J_{5-4} 5 Hz, *J* 10 Hz), 4.27 (dd, H4, J_{4-3} 3 Hz, J_{4-5} 5 Hz), 4.57 (d, H2, *J* 4 Hz), 4.58 (ABq, $C\underline{H}_2Ph$, *J* 12 Hz), 5.96 (d, H1, J_{1-2} 4 Hz), 7.28–7.38 (m, 5H, Ph). ^{13}C NMR ($CDCl_3$): 26.34 and 26.81 [$C(CH_3)_2$], 57.56 (OCH_3), 69.42 (C5), 70.70 (C6), 73.42 ($\underline{C}H_2Ph$), 79.78, 81.69 and 85.48 (C2, C3, C4), 104.81 (C1), 111.79 [$\underline{C}(CH_3)_2$], 127.69, 127.84 and 128.38 (Ph), 138.04 (Ph).

1,2-O-Isopropylidene-3-O-methyl-5-deoxy-5-azido-6-O-benzyl-α-D-glucofuranose (**66**)

To a solution of **64** (5.91 mmol, 1.916 g) and 0.5 g of 4 Å molecular sieves in 25 ml of dry CH_2Cl_2 under N_2 at −20°C was added 2,6-di-*tert*-butylpyridine (1.4 eq, 1.86 ml) and then triflic anhydride

(1.3 eq, 1.30 ml)[48]. The reaction was complete after 1 h stirring and the mixture was poured into a cold saturated $NaHCO_3$ solution. The organic layer was subsequently extracted with a saturated NaCl solution, separated, dried and concentrated. The resulting oil was used without further purification.

The product was dissolved in 10 ml of dry DMF and sodium azide (2 eq, 768 mg) was added[48]. The reaction mixture was stirred overnight at 50°C. The solvent was removed *in vacuo*, the residue taken up in CH_2Cl_2 and the organic layer was extracted with a saturated $NaHCO_3$ solution, a saturated NaCl solution, separated, dried and concentrated. The resulting oil was purified by column chromatography on silica gel (eluent Et_2O/hexane, 15:85) to give 1.569 g (76% yield) of **66** and 161 mg (9% yield) of a by-product which was identified as the triflate-eliminated C5–C6-(*Z*)-alkene (structure not shown, $J_{5,6}$ 7 Hz). TLC: *Rf* 0.35 (**66**) and 0.15 (alkene) (eluent Et_2O/hexane, 1:4). ^1H NMR ($CDCl_3$): 1.33 and 1.48 [2 s, $C(CH_3)_2$], 3.47 (s, OCH_3), 3.56–3.65 (m, H6), 3.80 (d, H3, $J_{3,4}$ 3 Hz), 3.88–3.99 (m, 2H, H5, H6'), 4.07 (dd, H4, $J_{4,3}$ 3 Hz, $J_{4,5}$ 9 Hz) 4.58 (d, H2, $J_{2,1}$ 4 Hz), 4.60 (s, 2H, CH_2Ph), 5.87 (d, H1, $J_{1,2}$ 4 Hz), 7.27–7.37 (m, 5H, Ph). ^{13}C NMR ($CDCl_3$): 26.24 and 26.76 [$C(CH_3)_2$], 57.62 (OCH_3), 59.29 (C5), 70.75 (C6), 73.32 (CH_2Ph), 78.52, 81.09 and 83.50 (C2, C3, C4), 105.28 (C1), 111.93 [$C(CH_3)_2$], 127.53 and 128.37 (Ph), 137.98 (Ph).

(Z)-1,2-O-Isopropylidene-3-O-methyl-5-deoxy-5,6-dehydro-6-O-benzyl-α-D-glucofuranose. ^1H NMR ($CDCl_3$): 1.33 and 1.53 [2 s, $C(CH_3)_2$], 3.38 (s, OCH_3), 3.64 (d, H3, *J* 3 Hz), 4.59 (d, H2, *J* 4 Hz), 4.67 (dd, H5, $J_{5,6}$ 7 Hz, $J_{5,4}$ 9 Hz), 4.85 (AB*q*, CH_2Ph, *J* 12 Hz), 5.22 (ddd, H4, $J_{4,6}$ 1 Hz, $J_{4,3}$ 3 Hz, $J_{4,5}$ 9 Hz), 5.90 (d, H1, *J* 4 Hz), 6.24 (dd, H6, $J_{6,5}$ 7 Hz, $J_{6,4}$ 1 Hz), 7.30–7.42 (m, 5H, Ph). ^{13}C NMR ($CDCl_3$): 26.28 and 26.79 [$C(CH_3)_2$], 58.11 (OCH_3), 74.10 (CH_2Ph), 74.22 (C2), 82.20 (C3), 85.34 (C4), 100.74 (C1), 104.37 (C5), 111.32 [$C(CH_3)_2$], 127.36, 128.01 and 128.52 (Ph), 137.11 (Ph), 147.75 (C6).

Biochemistry

The effects of inhibitors on the biosynthesis, maturation, oligosaccharide structure and secretion of α_1-antitrypsin were investigated in HepG2 cells[39]. The effect on retention of glucose residues on α_1-antitrypsin was tested for each compound in the range of 0.01–0.1–0.5–1.0–3.0 mM. We have reported earlier a full description of the methods that were used, *i.e. Titration of Inhibitors, Biochemical Analysis, Digestion with Endoglycosidase H, Oligosaccharide Analysis* and *Preparation of Permeabilized Cells* in order to perform the biochemical assays described in this study[39,52].

Virology

Antiviral activity was investigated after incubating an HIV-I ($HTLV^{IIIb}$)-infected cell line, *i.e.* U937[c], for three days with increasing amounts of inhibitors, after which the infected cells were co-incubated with an uninfected T-cell line. Syncytium formation between infected and uninfected cells was scored visually with a microscope, allowing semiquantitative analysis (see Table II). Samples were taken after the three-day incubation period and measured for p24 levels in a p24 capture Elisa[53]. Of selected compounds, infectious titres ($TCID_{50}$) were quantitated and compared to untreated samples[4d]. The *Cell Fusion Assay* and *p24 Elisa* that were used to assess HIV-induced syncytia formation and the determination of infectious titres have been described previously.

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