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Synthesis of α-Glucosidase I Inhibitors Showing Antiviral (HIV-1) and Immunosuppressive Activity

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Abstract

The synthesis of a series of analogues of the monosaccharide α-glucosidase I inhibitor *N*-decyl-1-deoxynojirimycin (**1**) is described. With the incorporation of a single oxygen atom particularly at position seven in the *N*-decyl side chain, i.e. to give *N*-7-oxadecyl-dNM (**4**), the therapeutic ratio (α-glucosidase I inhibitory activity over toxicity in HepG2 cells) increases considerably. *N*-7-Oxadecyl-dNM inhibits purified porcine liver α-glucosidase I with an IC50 value of 0.28 μM. The position of the oxygen atom in the *N*-decyl side chain is of importance since *N*-3-oxadecyl-dNM is less active and, moreover, is toxic to HepG2 cells at 3 mM. Subsequently, the synthesis of a disaccharide inhibitor of α-glucosidase I is described. The aminodisaccharide $ManNH_2\alpha 1,2Glc$ (**12**) inhibits α-glucosidase I with an IC50 value of 15·7 μM. Two closely related monosaccharide derivatives of **12** did not inhibit the enzyme at low μM concentrations (no inhibition at 5 μM), showing the additional effect of binding of the aglycon fragment of the molecule to the active site of α-glucosidase I. Next, the *N*-alkyl-dNM derivatives were analysed for antiviral and immunomodulatory activity in-vitro. It is found that the most potent α-glucosidase I inhibitor from this study, *N*-7-oxadecyl-dNM (**4**) inhibits HIV-1 induced syncytia formation and lymphocyte proliferation in-vitro. Finally, compound **4** was also investigated in-vivo. *N*-7-Oxadecyl-dNM (**4**) reduced adjuvant-induced arthritis in rats making this compound a potential candidate for treating autoimmune diseases like rheumatoid arthritis.

N-Alkyl derivatives of the glucose analogue 1-deoxynojirimycin (dNM) are powerful inhibitors of α-glucosidase I, an enzyme involved in *N*-glycoprotein processing and responsible for the hydrolysis of the terminal α1,2-linked glucose residue from the oligosaccharide precursor $Glc_3Man_9GlcNAc^2$-Asn (Winchester & Fleet 1992). Considerable effort has been made to develop therapeutic agents on the basis of the α-glucosidase I inhibition properties of these molecules. *N*-Butyl-dNM is effective against human immunodeficiency virus (HIV) infection in *Rhesus* primates (Aidsdrug Citations 1993). Preliminary positive results from a Phase I/II clinical trial in HIV-infected patients with *N*-butyl-dNM in combination with zidovudine (AZT) have been reported recently (Fischl et al 1993). Besides antiviral activity anti-inflammatory (i.e. immunosuppressive) activity in animal models has also been described for the α-glucosidase I inhibitor castanospermine (Willenborg et al 1992).

DNM is thought to mimic the terminal glucose residue of the oligosaccharide precursor $Glc_3Man_9GlcNAc_2$-Asn. It is thought that in α-glucosidases a beta-oriented carboxylate binds the cation (see Fig. 1) (Sinnott 1987; Legler 1990). In its protonated form, (*N*-alkylated-) dNM mimics the oxocarbenium ion intermediate of the hydrolase and inhibits the enzyme competitively by binding to the carboxylate in the enzyme active site as exemplified for *N*-butyl-dNM in Fig. 2. The same active site also interacts with the aglycon portion of the natural substrate, as evidenced by the linkage specificity shown by many glycosidases, e.g. α1,2 for α-glucosidase I versus α1,3 for α-glucosidase II. Recently, two crystal structures have appeared which show the binding of the pseudotetrasaccharide α-amylase inhibitor acarbose in the active sites of two completely different α-amylases (Aleshin et al 1994; Quian et al 1994). These show quite graphically that enzyme-aglycon interactions contribute to substrate binding in the active site. Clearly, some of these interactions will also be present in the transition state for glucose hydrolysis. In principal, effective and selective inhibition of a particular glycosidase could be achieved by designing mimics (e.g. disaccharides) of both portions of the natural substrate. Recently, a 1,4- "trehazoloid" glucosidase inhibitor was described that showed aglycon selectivity for α- and β-glucosidase (Knapp et al 1994).

Here, we describe the synthesis of a series of *N*-substituted dNM derivatives, i.e. **1**--**7** and their effective α-glucosidase I inhibitory, antiviral and immunosuppressive activity. Compound **4** shows activity in an animal model for rheumatoid arthritis. Further, we report the synthesis of the aminodisaccharide **12** and its effective inhibition of α-glucosidase I.

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Materials and Methods

Chemistry

^1H- and ^{13}C-NMR spectra were measured using a Bruker WM-200 or Bruker AM-360 spectrometer; chemical shifts are given in ppm (δ) relative to TMS for spectra run in $CDCl_3$ or relative to D_2O. Fast-atom bombardment (FAB)

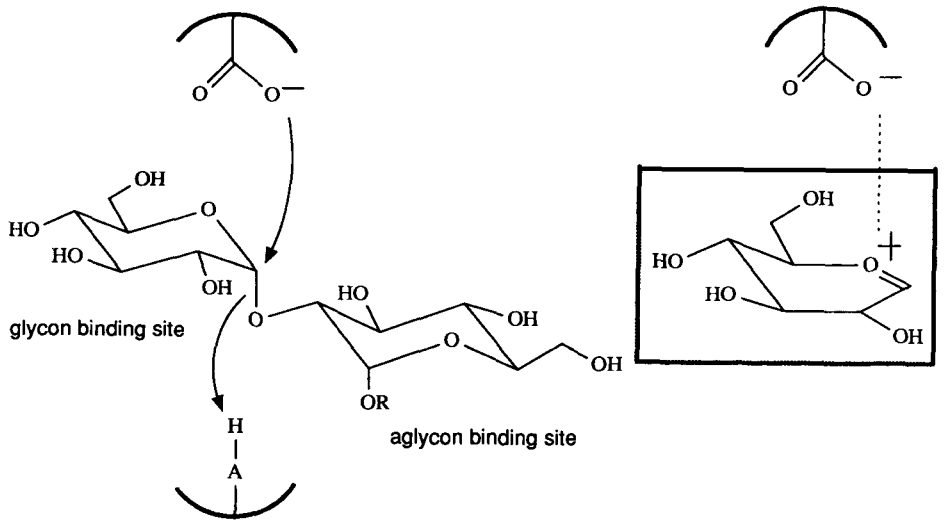


FIG. 1. Mechanism of hydrolysis of the terminal glucose residue of the substrate Glc(α1,2)Glc(α1,*R*) by α-glucosidase I with formation of an oxocarbenium ion intermediate (Sinnott 1987). The glycon and aglycon binding sites of the enzyme are shown.

mass spectra were recorded on a Finnigan MAT 90 mass spectrometer using glycerol as the matrix. Optical rotation was recorded at ambient temperature on a Perkin-Elmer 241 polarimeter. TLC analysis was performed on Merck DC-Fertigplatten (silica gel 60 F_{254}). Compounds were visualized by spraying with H_2SO_4-EtOH, 1:9. Merck silica gel 60 (230–400 mesh) was used for column chromatography.

Methyl 3-O-benzyl-4,6-O-benzylidene-2-O-(6-O-acetyl-2-azido-13,4-di-O-benzyl-2-deoxy-a-mannopyranosyl)-a-D-glucopyranoside (**11**)

To a stirred solution of **10** (Patroni et al 1988)† (200 mg, 0·53 mmol) and the imidate **9** (Paulsen & Stenzel 1978)‡ (307 mg, 0·53 mmol) in 10 mL of dry ether at −40°C under N_2, containing powdered 4 Å molecular sieves, was added trimethylsilyl trifluoromethanesulphonate (TMSOTf) (380 μl of 0·2 mM solution) in dry ether. After completion of the reaction (60 min), $NaHCO_3$ was added to the reaction mixture and stirring was continued for 30 min at −40°C. The mixture was filtered and the filtrate was washed with aqueous $NaHCO_3$ solution and brine, dried and concentrated. The residue was purified on silica gel (eluent CH_2Cl_2-acetone, 98:2) to give **11** (α-anomer) (108 mg, 42% yield) and β-anomer (68 mg, 26% yield). TLC R_F 0.60 (**11**) and 0·33 (β-anomer), respectively (eluent CH_2Cl_2-acetone, 97:3).

11: ^{1}H NMR (360 MHz, $CDCl_3$) 2·05 (s, $C(O)CH_3$), 3·44 (s, OCH_3), 3·64 (t, H-4, J = 6 Hz), 3·75 (t, H-6, J = 7 Hz), 3·79–3·88 (m, H-5, H-2, H-5′), 3·91 (t, H-3, J = 6 Hz), 4·04 (dd, H-2′, J2′-1′ = 1 Hz, J2′-3′ = 2·5 Hz), 4·09 (t, H-6′, J = 2 Hz), 4·11–4·19 (m, H-3′, H-4, H-6a′), 4·31 (dd, H-6a′, 3J = 3 Hz, 2J = 7 Hz), 4·55–4·90 (3 × ABq, CH_2Ph), 4·88 (d, H-1, J = 2·5 Hz), 4·91 (d, H-1′, J = 1 Hz), 5·58 (s, CHPh), 7·05–7·50 (m, Ph). ^{13}C NMR (90 MHz, $CDCl_3$ proton coupled) 95·0 (d, JC-1,H-1 = 171·4 Hz), 96·9 (d, JC-1′,H-1′ = 171·7 Hz). ^{13}C NMR (90 MHz, $CDCl_3$) 21·0 ($C(O)CH_3$), 55·5 (OCH_3), 61·0, 62·5, 63·0, 69·0, 70·0, 72·5, 73·5, 74·0, 75·5, 76·0, 77·0, 80·0, 82·5 (C-2, C-3, C-4, C-5, C-6, C-2′, C-3′, C-4′, C-5′, C-6′, 3 × CH_2), 95·0, 97·0, 101·5 (C-1, C-1′, CHPh), 126·0–129·0 (Ph), 137·5–138·5 (4x Ph), 171·0 (C(O)).

Characteristic signals for the β-anomer: ^{1}H NMR ($CDCl_3$), 4·70 (d, H-1′, J = 1 Hz), 4·84 (d, H-1, J = 2 Hz).

Methyl 2-O-(2-Amino-2-deoxy-α-D-mannopyranosyl)-α-D-glucopyranoside monoacetate (**12**)

Methyl 3-O-benzyl-2-O-(6-O-acetyl-2-azido-3,4-di-O-benzyl-2-deoxy-α-D-mannopyranosyl)-α-D-glucopyranoside

A stirred solution of **11** (142 mg, 0·18 mmol) in 2·5 mL of 70% HOAc was heated at 65°C for 3 h. The reaction

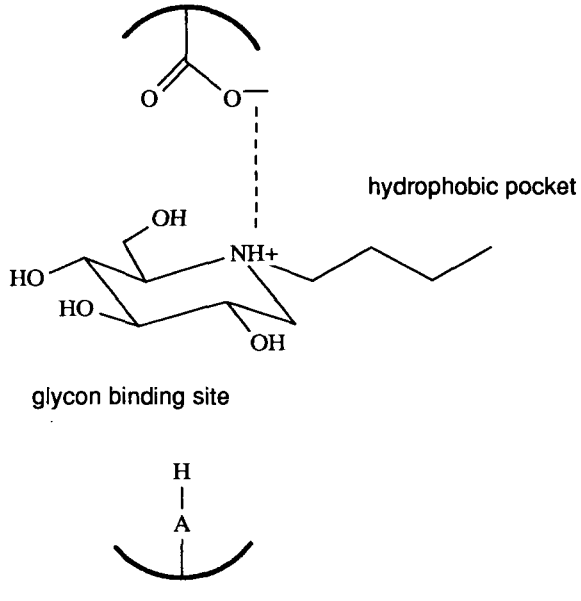


FIG. 2. Binding of *N*-butyl-dNM (**8**) to α-glucosidase I. The azasugar ring binds to the glycon binding site, while the *N*-alkyl side chain interacts with a hydrophobic site in the enzyme.

† Compound **10** was obtained by benzylation of methyl 4,6-O-benzylidene-α-D-glucoside and subsequent separation on silica gel.

‡ Compound **9** with mannose configuration was obtained as a by-product during the synthesis of the imidate from 6-O-acetyl-2-azido-3,4-di-O-benzyl-2-deoxy-D-glucopyranose.

Table 1. HepG2 and HIV-1 activity data of compounds **1-8** (for structures see Fig. 1).

dNM derivatives		Glc_n	Toxicity $HepG_2$	HIV-1
1	*N*-Decyl	$Glc_{2\text{-}1}$	0.1 mM	++ (toxic)
2	*N*-*p*-F-fenoxyethoxyethyl	$Glc_{2\text{-}1}$	none	+
3	*N*-3-Oxadecyl	Glc_2	3 mM	++
4	*N*-7-Oxadecyl	Glc_3	none	++
5	*N*-7-Oxadecyl-3-*O*-methyl	inactive	none	−
6	*N*-3,6,9-Trioxadecyl	inactive	none	+/−
7	*N*-7,10,13-Trioxatetradecyl	$Glc_{2\text{-}1}$	none	+/−
8	*N*-Butyl	$Glc_{2\text{-}3}$	none	++
12	Aminodisaccharide	inactive		not tested

The estimated number of glucose residues (Glcn) retained on α1-antitrypsin synthesized in HepG2 cells at 3 mM drug concentration is taken as a measure of α-glucosidase inhibitory activity (Tan et al 1991, 1994; Van den Broek et al 1993); the toxicity, as shown by reduced glycoprotein amounts detected on SDS-PAGE gels, observed in the HepG2 assay is also shown at the lowest concentration found. Syncytia formation at 1 mM drug concentration is taken as a measure for antiviral (HIV-1) activity: ++: no syncytia (= total inhibition), +: clear inhibition, ±: inhibition noticeable, −: no inhibition (Van den Broek et al 1993).

mixture was concentrated, dissolved in CH_2Cl_2 and extracted with aqueous $NaHCO_3$ solution and concentrated to give 103 mg (82%) of benzylidene deprotected material. The product was not further purified.

Methyl 3-O-benzyl-2-O-(2-azido-2-deoxy-3,4-di-O-benzyl-α-D-mannopyranosyl)-a-D-glucopyranoside

To a stirred solution of the crude product (81.7 mg, 0.11 mmol) in 3 mL of dioxane and 3 mL of methanol was added at 0°C under N_2, K_2CO_3 (45 mg). According to TLC (eluent CH_2Cl_2-acetone, 7:3 R_F 0·23) the reaction was complete after 6 h stirring. The K_2CO_3 was filtered off, the filtrate treated with Dowex H^+, filtered and concentrated to give 41·2 mg (54% yield) of the product. The reaction product was used without further purification.

To a stirred solution of the crude product (41 mg, 0·06 mmol) in 7 mL of *t*-BuOH, 10 mL of H_2O and 1·5 mL of HOAC was added 10% Pd/C (40 mg) and H_2 was passed through overnight. The catalyst was filtered off and the filtrate concentrated in-vacuo. The residue was freeze-dried from H_2O to give 19·3 mg (74% yield) of **12**. ^1H NMR (360 MHz, D_2O) 1·95 (s, $C(O)CH_3$), 3·43–3·49 (m, H-3), 3·45 (s, OCH_3), 3·55–3·59 (m, H-2′), 3·62–3·72 (m, H-4, H-4′), 3·72–3·75 (H-2), 3·75–3·80 (H-5), 3·81–3·91 (H-6, H-6a, H-6′, H-6a′), 3·95–4·00 (m, H-5′), 4·15 (dd, H-3′, J2′-3′ = 5 Hz, J3′-4′ = 10 Hz), 5·09 (d, H-1, J1-2 = 3 Hz), 5·19 (br s, H-1′). FAB^+ MS 448 [M + H + glycerol], 356 [M + H]. FAB-MS 354 [M-H]. $[\alpha]_{20}D + 107·9°$ (c 0·63, H_2O).

HepG2 assay

The effects of inhibitors on the biosynthesis, maturation, oligosaccharide structure and secretion of α-1-antitrypsin were investigated in human hepatoma HepG2 cells by the method of Tan et al. (1991; 1994). 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 and analysed by SDS-PAGE gel electrophoresis. The estimated number of glucose residues (Glcn) retained on α-1-antitrypsin synthesized in HepG2 cells at 3 mM drug concentration is taken as a measure of α-glucosidase inhibitory activity (Van den Broek et al 1993). The results of a single experiment are shown in Table 1. The toxicity, as shown by reduced glycoprotein amounts (of approximately 25%) detected on SDS-PAGE gels, is also shown at the lowest concentration found.

HIV-1 assay

Antiviral activity was investigated after incubating an HIV-1 (HTLVIIIb)-infected cell line, i.e. U937+, for 3 d with increasing amounts of inhibitors, after which the infected cells were co-incubated with an uninfected T-cell line (Van den Broek et al 1993). Syncytium formation between infected and uninfected cells was scored visually with a microscope, enabling semiquantitative analysis. Samples were taken after the three-day incubation period and measured for p24 levels in a p24 capture Elisa. Syncytia formation at 1 mM drug concentration is taken as a measure for antiviral (HIV-1) activity: ++: no syncytia (= total inhibition), +: clear inhibition, ±: inhibition noticeable, −: no inhibition. The average results of three separate experiments are shown in Table 1.

α-Glucosidase I activity

The glucosidase assay uses affinity-purified α-glucosidase I from porcine liver and metabolically radiolabelled $Glc_3Man_9GlcNAc_2$ (Karlsson et al 1993). To measure IC50 values, compound and enzyme were pre-incubated for 10 min at 37°C, substrate added and the reaction allowed to continue for 3·5 h at 37°C. The reaction products were separated by concavalin A chromatography and the amount of released radiolabelled glucose determined by scintillation counting. The results are shown in Table 2. Values given are the average of two experiments.

In-vitro immunomodulatory activity

Human peripheral blood mononuclear cells (PBMC) were isolated from normal donors as described by van Kemenade et al (1994). For proliferation, 40×10^3 PBMC were incubated with either antigen (tetanus toxoid, TT) or mitogen (anti-CD3 monoclonal antibody). PBMC were pulsed after 72 h (or 140 h in case of TT) for 24 h with 0·74 kBq

Table 2. Inhibitory activity against purified α-glucosidase I (Karlsson et al 1993) IC50 (= 50% inhibitory concentration) values for compounds **4**, **5**, **6**, **8** and **12** (for structures see Fig. 1 and Scheme 1) against porcine liver α-glucosidase I.

dNM derivatives		IC50
4	*N*-7-Oxadecyl	0.28 μM
5	*N*-7-Oxadecyl-3-*O*-methyl	3.93 mM
6	*N*-3,6,9-Trioxadecyl	21.41 μM
8	*N*-Butyl	0.57 μM
12	Aminodisaccharide	15.7 μM

^{3}H-Thymidine and incorporation was measured with liquid scintillation counting. For immunoglobulin (Ig) production PBMC were incubated with pokeweed mitogen (PWM) and the Ig content, determined in an enzyme-linked immunosorbent assay, was measured on day 8 in the supernatant. Inhibitors were added at the start of cultures. The average results of three separate experiments are shown in Table 3.

Adjuvant arthritis in rats

Mycobacterium butyricum suspended in mineral oil (10 mg mL^{-1}) was injected subplantar, at a dose of 0·05 mL per rat, in the right hind paw of male Lewis rats (Billingham & Davies 1979; Newbould 1963). This induces a polyarthritis with most adjuvant injected animals subsequently developing irreversible ankylosing of the joints. The development of the arthritis can be divided in two phases: the acute inflammatory phase (the local inflammatory reaction (paw swelling) immediately after the subplantar injection) and the secondary autoimmune response (from day 10 on when the inflammation spreads out over the total body; the non-injected hind paw increases in volume and general signs develop. Twenty one days after injection these signs are maximal).

Compound **4** was administered subcutaneously to male Lewis rats for 21 d at a dose of 50 and 25 mg kg^{-1} three times a day, respectively (10 rats per group). No major toxicity was observed in these experiments. *N*-7-Oxadecyl-dNM (**4**) at a dose of 50 mg kg^{-1} three times a day inhibited the acute inflammatory phase (unpublished observations) as well as the secondary autoimmune response. This effect on the secondary phase of adjuvant induced arthritis is shown in Fig. 6 by the strong reduction in arthrogram score. Compound **4** at a dose of 25 mg kg^{-1} three times a day only inhibited the acute inflammatory phase (unpublished observations), but had no effect on the autoimmune response as shown in Fig. 6 by the absence of an effect on the arthrogram score.

The animals were scored (arthrogram score, see Fig. 6) in a blind manner several times a week throughout the entire experiment. Score: ears (redness and/or nodular lesions), fore paws (redness and swelling), uninjected hind paw (redness and ankylosis) and tail (ankylosis). Range: 0 = none, 1 = slight, 2 = moderate and 3 = severe.

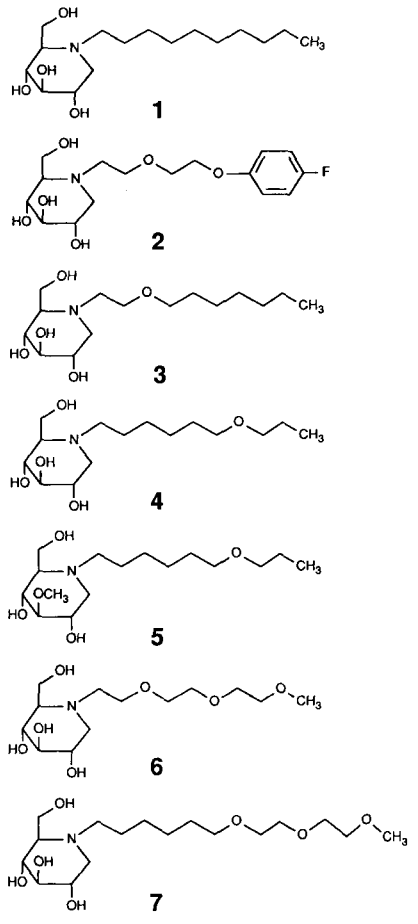


FIG. 3. The structures of *N*-alkyl 1-deoxynojirimycin (dNM) derivatives **1**–**7**.

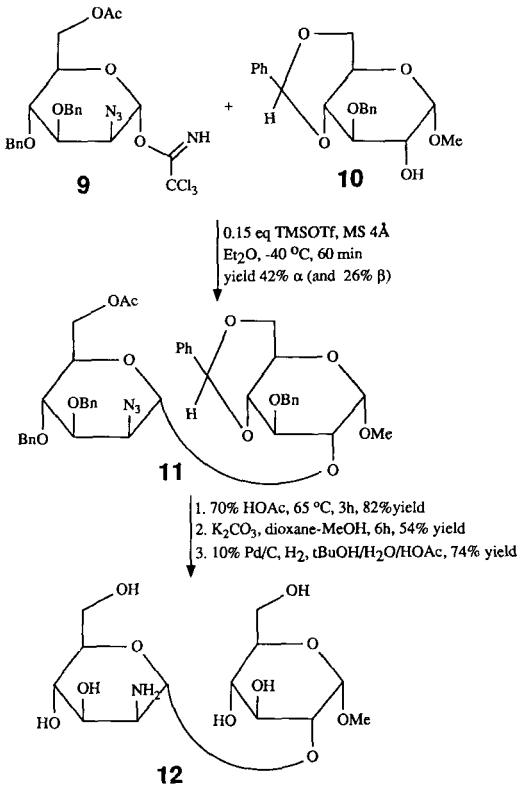
Results and Discussion

Chemistry

The synthesis of the monosaccharide dNM (= 1,5-didexoy-1,5-imino-D-glucitol) derivatives **1**-**7** (see Fig. 3) was carried out following two reaction routes which are described elsewhere (Van den Broek et al 1993; Van den Broek et al 1994). Briefly, one route involves the *N*-alkylation of 2,3,4,6-tetra-*O*-benzyl-dNM by nucleophilic substitution with the

Table 3. In-vitro immunomodulatory activity (Van Kemenade et al 1994). IC50 (= 50% inhibitory concentration) values for dNM and *N*-7-oxadecyl-dNM (**4**) in three assays: lymphocyte (peripheral blood mononuclear cells) proliferation induced by antigen (tetanus toxoid) or anti-CD3 monoclonal antibody and pokeweed mitogen-induced immunoglobulin production.

Compound	Tetanus toxoid	IC50	Pokeweed mitogen
dNM	>2 mM	>2 mM	1 mM
4 *N*-7-Oxadecyl dNM	0·5 mM	0.5 mM	0.01 mM



SCHEME 1. The synthesis of aminodisaccharide 12.

appropriate alkyl triflate; the other route proceeds via reductive alkylation of dNM using the appropriate aldehyde. The synthesis of the aminodisaccharide ManNH_2α1,2Glc (**12**) is outlined in Scheme 1 and involves a TMSOTf-catalysed coupling of imidate **9** with glycosyl acceptor **10** giving the desired α-coupled disaccharide **11** in 42% yield after chromatographic separation. Subsequent deprotection of **11** gave the desired aminodisaccharide **12** in 33% overall yield.

Biological activity

Compounds **1-7** and **8** (used as reference material) were tested for their α-glucosidase-inhibitory activity in human hepatoma HepG2 cells and antiviral activity (i.e. HIV-1 induced syncytia formation). The results are depicted in Table 1. Compounds **4**, **6**, **8** and **12** were also tested for their inhibitory activity against purified porcine liver α-glucosidase I. The results are shown in Table 2. From the data in Tables 1 and 2 the following conclusions can be drawn. *N*-7-Oxadecyl-dNM (**4**) is the most potent *N*-alkyl-dNM derivative tested. This is the only compound which shows complete inhibition of α-glucosidase I in intact HepG2 cells (i.e. formation of Glc_3-containing oligosaccharides on α-1-antitrypsin synthesized in HepG2 cells). It has an IC50 of 0·28 μM against the purified enzyme; being twice as active as the reference compound *N*-butyl-dNM (**8**) showing an IC50 of 0·57 μM. There is a good correlation between the data of the HepG2, HIV-1 and purified α-glucosidase I assays as exemplified by *N*-7-oxadecyl-dNM (**4**). Compound **5** is inactive in all three assays having an IC50 against the purified processing enzyme in the millimolar range. The inactivity of compound **6** in the HepG2 assay may be explained by its 100-fold lower affinity for α-glucosidase I than compound **4**. Some activity is detected in the HIV-1 assay.

The aminodisaccharide **12** shows a remarkably high affinity for α-glucosidase I with an IC50 of 15·7 μM slightly better than that of compound **6**. This is even more so considering that the molecule contains two hydrolysable glycosidic linkages (see below). No activity was observed for this compound in intact HepG2 cells, which is not surprising since disaccharides cannot pass the cell membrane unaided. Recently, we were able to show activity in intact cells of disaccharides which were chemically modified (unpublished observations). By incorporation of *N*-alkyl- and (hydrolysable) ester groups in disaccharide molecules the lipophilicity of the molecule can be increased to an extent enabling transport across the cell membrane (see below).

Binding of inhibitors to α-glucosidase I

The mechanism of hydrolysis of α-glucosidase I is depicted in Fig. 1 (Sinnott 1987). The terminal glucose residue binds to the glycon binding site of the active site. Nucleophilic attack of a beta-positioned carboxylate group at the anomeric centre cleaves the interglycosidic bond. An oxocarbenium ion intermediate is thought to be formed during the hydrolysis. It is believed that the azasugar inhibitors like *N*-butyl-dNM (**8**) (see Fig. 2) and *N*-7-oxadecyl-dNM (**4**) bind to the active site carboxylate of the enzyme in their protonated form (or are protonated by the enzyme itself, since these compounds are only weakly basic). The *N*-alkyl side chain interacts with a hydrophobic pocket in the same active site of the enzyme. *N*-Substitution also results in a change in conformation of the C-6 hydroxyl group from gauche-*trans* in dNM to gauche-gauche in *N*-substituted dNM derivatives (Van den Broek et al 1993).

We believe that the aminodisaccharide **12** binds with the mannosamine fragment bound to the glycon binding site, where the beta-amino group binds to the beta-positioned carboxylate in the enzyme active site (see Fig. 4). In doing so, hydrolysis of the interglycosidic and of the reducing glycosidic linkage is minimised or even prevented. The α1,2-linked glucose residue of **12** binds to the aglycon binding site

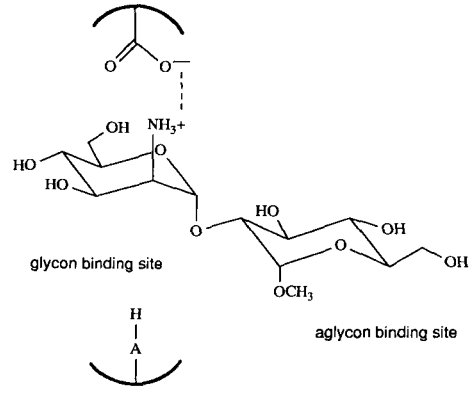


FIG. 4. Binding of the aminodisaccharide **12** (for structure see Scheme 1) to α-glucosidase I. The mannosamine residue of **12** is bound by the glycon binding site, whereas the glucose residue of **12** is bound by the aglycon binding site in the enzyme.

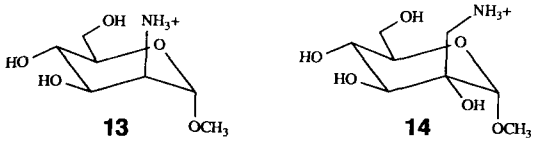


FIG. 5. Structures of two aminomonosaccharides, i.e. **13** and **14** with mannose and glucose configuration, respectively.

of the same active site of the enzyme thereby fulfilling the α1,2-linkage specificity of α-glucosidase I. Remarkably, the amino monosaccharide **13** (see Fig. 5) has no enzyme inhibitory activity (unpublished observations). The enzyme, in keeping with its substrate specificity, is therefore recognizing and deriving significant affinity from the presence and mode of linkage of the glucose moiety of **12**. Interestingly, the synthesis of a pseudodisaccharide "trehazoloid" inhibitor of yeast α-glucosidase (an α1,4-hydrolase) was reported recently (Knapp et al 1994). The design of this inhibitor was based upon the α,α-trehalase inhibitor trehazolin, a natural product. This 1,4-trehazoloid glucosidase inhibitor exhibited aglycon selectivity with α- but also β-glucosidase.

Even better, linkage-spanning, glycosidase inhibitors with effective selectivity of the aglycon portion could be synthesized by eliminating the labile glycosidic linkages (i.e. via pseudodisaccharides or C-disaccharides or via aza-C-glycosides containing *N*-alkyl-dNM), incorporating the missing C-2 hydroxyl group in **12** to obtain the gluco configuration (see below) and incorporating *N*-alkyl groups for favourable hydrophobic interaction with the enzyme. The synthesis of *N*-alkyl-dNM-containing aza-C-glucosides would have the additional benefit of the favourable C-6 hydroxyl conformation and interaction with the hydrophobic pocket. So far, disaccharides with dNM at the non-reducing end and with the correct α1,2-linkage have not been reported. An interesting recent report describes the synthesis of azapyranosyl thioglycosides which may have potential as glycosidase inhibitors with aglycon selectivity Suzuki & Hashimoto 1994). The amino monosaccharide **14**, maintaining the gluco configuration (see Fig. 5), showed no enzyme activity (unpublished observations). Obviously, it would be worthwhile to synthesise and test the α1,2-glucose derivative of **14** to evaluate any significant additional contribution to the activity of **12** due to the C-2 hydroxyl group.

Immunomodulatory activity

DNM and *N*-7-oxadecyl-dNM (**4**) were analysed for their effect in three assays that measure immune reactivity in-vitro (see Table 3). *N*-7-Oxadecyl-dNM (**4**) was more potent than its parent compound showing an IC50 of 10 μM in the pokeweed mitogen (PWM) driven immunoglobulin (Ig) production. This warranted further investigation of this compound in-vivo.

Adjuvant arthritis in rats

N-7-Oxadecyl-dNM (**4**) was studied, subsequently, on adjuvant-induced arthritis in rats. In a dose of 150 mg kg^{-1} d^{-1} compound **4** reduces the arthrogram score considerably; a dose of 75 mg kg^{-1} d^{-1} was ineffective (see Fig. 6). The compound inhibited the acute inflammatory

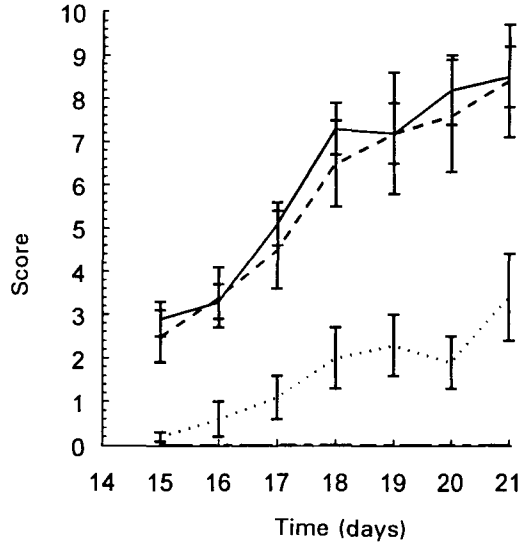


FIG. 6. Arthrogram score from days 15-21 of adjuvant-induced arthritis in rats for *N*-7-oxadecyl-dNM (**4**) (Billingham & Davies 1979; Newbould 1963). The compound was administered subcutaneously in three daily injections of 25 (- - -) or 50 (· · ·) mg kg^{-1} (total dose 75 or 150 mg kg^{-1} d^{-1}) for 21 d. Placebo (——) and control (– -) are also shown.

phase as well as the secondary autoimmune response. Thus, *N*-7-oxadecyl-dNM (**4**) is effective in arthritic rats at a dose of 150 mg kg^{-1} d^{-1}. This makes compound **4** a potential candidate for treating autoimmune diseases like rheumatoid arthritis.

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