Glucosidase Inhibitors: Structures of Deoxynojirimycin and Castanospermine

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High-resolution structures of the glucosidase inhibitors deoxynojirimycin (dNM) and castanospermine (CAST) have been determined by X-ray diffraction. The crystal parameters are a = 10.751(3) and 8.788(3) Å, b = 9.263(3) and 8.172(3) Å, c = 7.719(2) and 6.507(2) Å, and space group $P2_12_12_1$ and $P2_1$ for dNM and CAST, respectively. (β = 105.44(8)° for CAST.) The absolute configuration of CAST has also been established. Stereochemical comparisons with natural glucosidase substrates such as maltose and methyl glucoside show great similarities in the positioning of functional groups, and indicate the basis for enzyme inhibition. Conformational comparison between dNM and CAST suggests the greater activity of CAST may be due to the fixed axial positioning of the O6 atom; the results have implications for the design of analogues for potential anti-HIV and other antiviral therapies.

Infection of cells by human immunodeficiency virus (HIV) is initiated by binding of the viral surface glycoprotein gp120 to the cellular viral receptor CD4. This binding is dependent on the state of glycosylation of gp120; a nonglycosylated product of a recombinant gp120 gene or enzymatically deglycosylated gp120 was not able to bind to the receptor,[1,2] nor does the more highly glycosylated precursor protein gp160. Thus compounds that interfere with accurate carbohydrate processing of this viral glycoprotein may prevent viral binding to cellular receptors and hence may be useful anti-HIV agents.

The antibiotics nojirimycin and 1-deoxynojirimycin, produced by several strains of bacteria, are glucose analogues with an NH group substituting for the oxygen atom in the pyranose ring. Castanospermine (1,6,7,8-tetrahydroxyoctahydroindolizine) is a plant alkaloid isolated from *Castanosperum australe*. These compounds have been shown to be potent inhibitors of glucosidase enzymes, with varying specificities for glucosidases from different sources. Of particular medicinal interest is their ability to interfere with glycoprotein processing by inhibiting a trimming enzyme, α-glucosidase I, of the endoplasmic reticulum, resulting in inadequate cleavage of precursor oligosaccharide side chains during the biosynthesis of N-linked complex glycoproteins. Because this cellular enzyme is thought to be involved in viral glycoprotein processing, these agents may have possible anti-HIV properties.

Deoxynojirimycin (dNM) and castanospermine (CAST) have been tested and shown to prevent HIV-induced syncytium formation and inhibit virus binding *in vitro*[3-5] and *in vivo*.[6] CAST was found to be more effective against HIV-1 then dNM, but N-alkylation of dNM resulted in analogues with increased potency and specificity.[7,8] Currently there is considerable interest in developing further analogues of both these species which will be better anti-HIV drugs. We have determined the three-dimensional structures of dNM and CAST in order to investigate stereochemical correlations of activity of these enzyme inhibitors.

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Table I. Crystal Data

	deoxynojirimycin	castanospermine
formula	$C_6H_{13}O_4N$	$C_6H_{15}O_4N$
mol wt	163.1	189.1
a (Å)	10.751(3)	8.788(3)
b (Å)	9.263(3)	8.172(3)
c (Å)	7.719(2)	6.507(2)
β (deg)		105.44(8)
V (Å^3)	768.7	450.4
no. molec/cell	4	2
space group	$P2_12_12_1$	$P2_1$
total number of reflections	779	830
no. of data significantly above background ($I > 3\sigma(I)$)	749	818
crystal dimension (mm)	0.3 × 0.5 × 0.4	0.3 × 0.4 × 0.3

Experimental Section

Single crystals of dNM and CAST were obtained by slow evaporation of methanol–water solutions over a 1-week period. Crystal data are given in Table I. Precession-camera photographs were used to determine the space groups. X-ray diffraction data were collected on an automated four-circle diffractometer using Cu Kα radiation. The $\theta/2\theta$ scan technique was employed with $2\theta_{max}$ = 130°. Both crystal structures were solved by direct methods using the MULTAN80 program package.[9] Full-matrix least-squares was used to refine the structures, and all of the hydrogen atoms were located on difference Fourier maps. Atomic parameters were refined except for hydrogen thermal factors, which were fixed isotropically at B = 3.0 Å^2. Unitary weights were used. The final discrepancy factors are R = 0.0327 for dNM and 0.0307 for CAST, for data with $I > 3\sigma(I)$. The maximum atomic shift/esd ratios in the final cycle of refinement were 0.02 and 0.03. There were no significant features on final difference Fourier distributions: maximum and minimum peak heights were ±0.3 e Å^{-3} for both compounds. Anomalous dispersion of the oxygen atoms was utilized to investigate the absolute configuration of CAST; the Hamilton test[10] indicated that the difference between R_1 = 3.14 and R_2 = 3.07 was significant, thus allowing the absolute configuration to be specified, while for dNM the relationship to D-glucose establishes its configuration. The ORXFLS3[11] least-squares-refinement programs were used. Atomic scattering factors were as cited for non-hydrogen[12] and hydrogen.[13]

Results

(a) Molecular Structures. Figure 1 shows the structures of dNM and CAST. In both molecules the six-membered rings adopt chair conformations with all (non-

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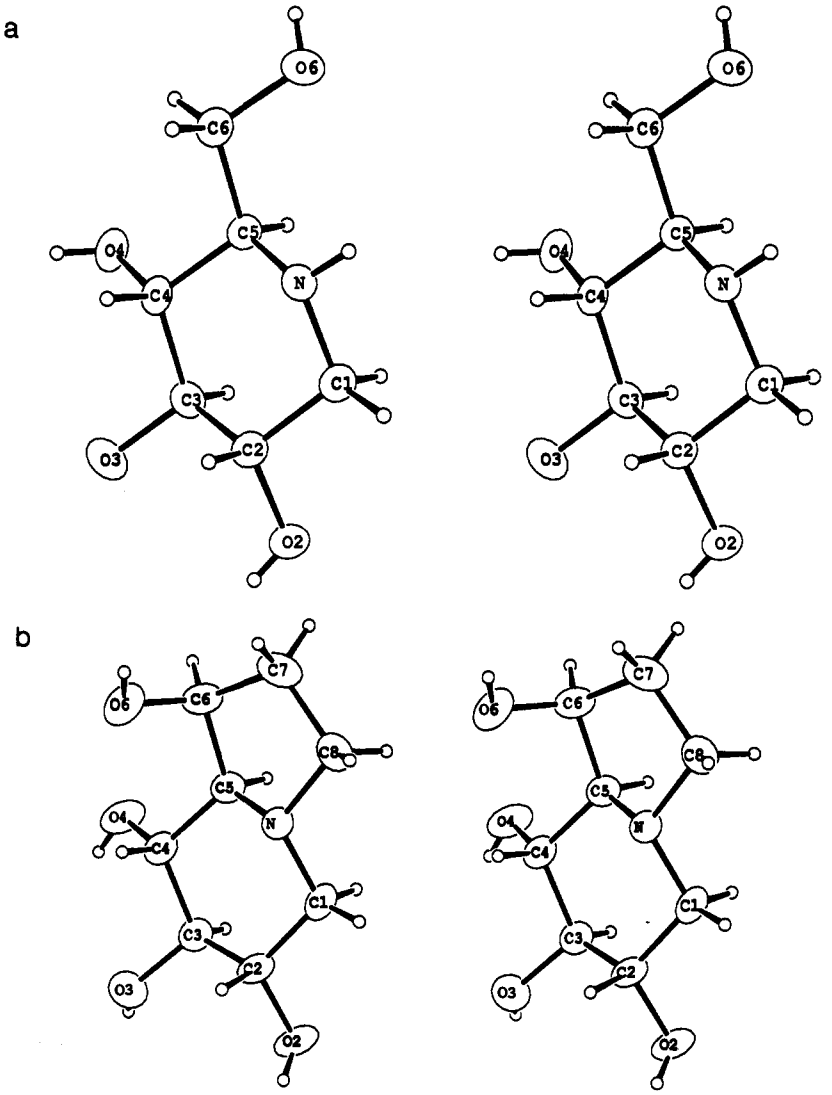

Figure 1. Stereoscopic drawings of the structures of (a) deoxynojirimycin and (b) castanospermine. Atomic ellipsoids are drawn at the 50% probability level.

hydrogen) substituents in equatorial positions. As shown, the absolute configuration indicated for CAST by the Hamilton test is 2(*S*),3(*R*),4(*R*),5(*R*),6(*S*). The pyrrolidine ring of CAST has an envelope conformation with the nitrogen atom lying 0.58 Å from the almost rigorously coplanar unit formed by the four carbon atoms. Bond lengths and angles in the two molecules are normal. Atomic coordinates for dNM and CAST are presented in Table II.

(b) Crystal Structures. The dNM structure forms a three-dimensional network in which the nitrogen and the four hydroxyl oxygens are all both donors and acceptors in intermolecular hydrogen bonding. The four hydroxyls in CAST are also donors in intermolecular H-bonds, the acceptors being the nitrogen atom and three of the hydroxyl oxygens. The hydrogen-bonding network is two-dimensional, forming molecular layers held together by van der Waals interactions.

After completion of this work we discovered a previous structure determination[14] of castanospermine. Although a slightly different monoclinic cell was chosen in that work, identical hydrogen-bonding parameters in the two structures prove that the crystals were the same. The present study measured 25% more data points than the previous one and appears to be of higher precision (*R* factor of 0.03 vs 0.05). As shown above, this higher precision allowed the establishment of the absolute configuration of CAST, which was not possible in the previous determination. The conformational structures appear to be identical within experimental error in the two cases, although the incorrect absolute configuration is depicted in the previous publication. The crystal structure of an analogue of CAST, 6-deoxy-6-fluorocastanospermine, has also recently been determined[15] (the atomic numbering is different from ours: C6 corresponds to C2 to in the present work). The overall conformation is closely similar to that of CAST. The absolute configuration was not established.

Discussion

The ability of dNM and CAST to interfere with glucosidase action suggests stereochemical similarities between these inhibitors and normal glucosidase substrates. Superpositions of the structures of dNM and CAST with the nonanomeric terminal glucose residue in the crystal structure-derived conformation of the disaccharide maltose[16] (Figure 2) clearly shows the coincidence of functionally similar features in three-dimensional structure, and indicates how the inhibitors could occupy the glucose-binding site of the enzyme. A similar superpostion of deoxymannojirimycin, the C2-epimer of dNM, with methyl α-D-glucopyranoside[17] (Figure 3) shows the axial positioning of the C2-hydroxy group; the fact that

Table II. Fractional Atomic Coordinates (×10⁴) and Equivalent Isotropic Temperature Factors for Deoxynojirimycin and Castanospermine $B_{eq} = 4/3(B(1,1)a^2+B(2,2)b^2+B(3,3)c^2+B(1,2)ab\cos\gamma+B(1,3)ac\cos\beta + B(2,3)bc\cos\alpha$

atom	X	Y	Z	B_{eq} (Å²)
		Deoxynojirimycin		
C1	6333(3)	3590(3)	5092(4)	2.4
C2	5175(2)	2833(3)	5749(4)	2.0
C3	5231(2)	1223(3)	5380(3)	1.9
C4	5480(2)	941(2)	3466(3)	1.8
C5	6630(2)	1754(3)	2850(3)	1.8
C6	6856(3)	1553(3)	925(4)	2.4
N	6449(2)	3302(2)	3222(3)	2.0
O2	5105(2)	3089(2)	7567(3)	2.9
O3	4092(2)	589(2)	5908(3)	2.5
O4	5677(2)	-574(2)	3182(3)	2.2
O6	7969(2)	2294(2)	445(3)	2.8
		Castanospermine		
C1	3904(3)	3159(5)	-2692(4)	2.7
C2	3687(3)	2374(4)	-676(4)	2.2
C3	2033(3)	2662(4)	-439(4)	2.3
C4	1518(2)	4445(4)	-778(4)	2.3
C5	1843(3)	5147(4)	-2765(4)	2.2
C6	1608(3)	6987(0)	-3174(4)	2.8
C7	2718(4)	7353(5)	-4592(5)	3.7
C8	3757(4)	5839(5)	-4495(4)	3.4
N	3510(2)	4898(4)	-2681(3)	2.2
O2	3978(2)	654(4)	-724(3)	2.9
O3	1951(2)	2209(4)	1640(3)	3.1
O4	-144(2)	4525(4)	-1000(3)	3.7
O6	2026(2)	7844(4)	-1195(3)	3.5

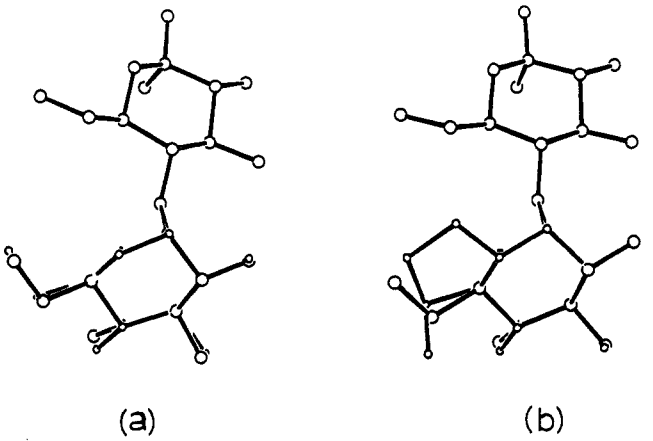

Figure 2. Superposition of (a) dNM and (b) CAST with maltose (large circles, unshaded bonds). (Two hydroxy oxygens are shown at the anomeric carbon atom of maltose because the structure determined was a mixture of α- and β-anomers.)

this compound is not a glucosidase inhibitor indicates the importance of the equatorial hydroxyl at C2.

Although both dNM and CAST have demonstrated anti-HIV activity, CAST appears somewhat more effective.[5] A comparison of the structures of the two molecules, Figure 4, allows structure–activity correlations to be made. The only difference in configuration of functional groups between the two is the positioning of the O6 hydroxyl; in CAST it is axial and fixed in position by the 5-membered ring closure, while in dNM it is equatorial to the sugar ring in the crystal structure. Thus it is likely that the O6 configuration in CAST is the one required for interaction with glucosidases.

Support for this conclusion may be found in a recent structure determination[18] of a complex of dNM and a closely functionally-related enzyme, glucoamylase. Deoxynojirimycin, which is an inhibitor of this enzyme as well, best fits the active site of the enzyme with the O6 hydroxyl

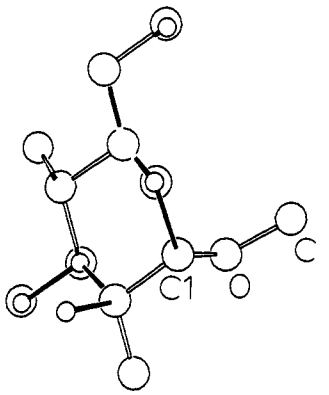

Figure 3. Superposition of deoxymannojirimycin (small circles, shaded bonds) with methyl α-D-glucopyranoside. The deoxymannojirimycin structure was derived by mathematical inversion of the dNM configuration at C2.

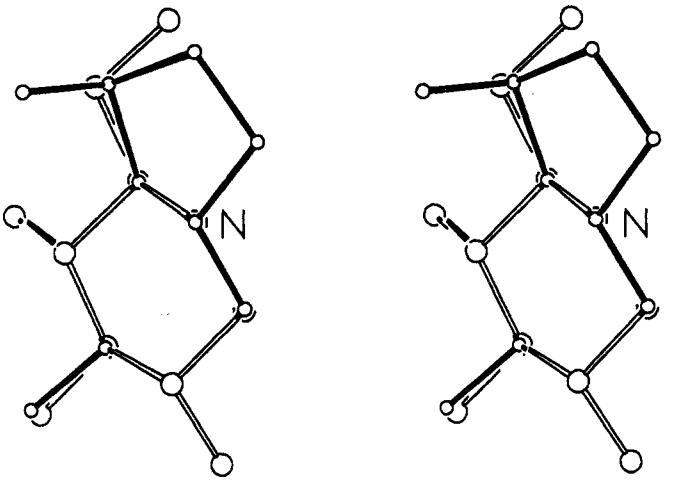

Figure 4. Stereoscopic drawing of the superposition of the structures of dNM (large circles, unshaded bonds) and CAST.

group oriented axially to the sugar ring; it is held in that configuration through hydrogen bonding to an aspartic acid side chain of the protein. This interaction appears to be vital; previous work[19] had shown that mutation of this particular aspartic acid to asparagine or tyrosine leads to loss of enzymatic activity. Because of the similar enzymatic functions of glucoamylases and glucosidases and the similar inhibition of both by dNM, these results provide corroborative evidence regarding the active O6 configuration we have postulated on the basis of the structural comparisons of dNM and CAST.

Although there is ostensibly free rotation about the C5–C6 bond in dNM, the observed crystal-structure conformation may be marginally favored because of a weak intramolecular attraction between the imino hydrogen and O6. This interaction is best characterized by the H(N)–N–C6–O6 torsion angle of 4°, indicating that the equatorial C6–O6 group approaches planarity with the equatorial NH group (the N···O distance is 2.85 Å and the NH···O angle is 104°). To investigate the stability of this conformation vs the O6 axial configuration, we have used both molecular mechanics[20] and *ab initio* [21] methods to calculate[22] the molecular energy as a result of rotation of O6 about the C5–C6 bond; the results are shown in Figure 5. Both methods give similar results: the O6 hydroxyl equatorial (crystal structure) conformation and the axial conformation (corresponding to that observed for CAST) are the lowest energy molecular configurations, with energy barriers separating them.

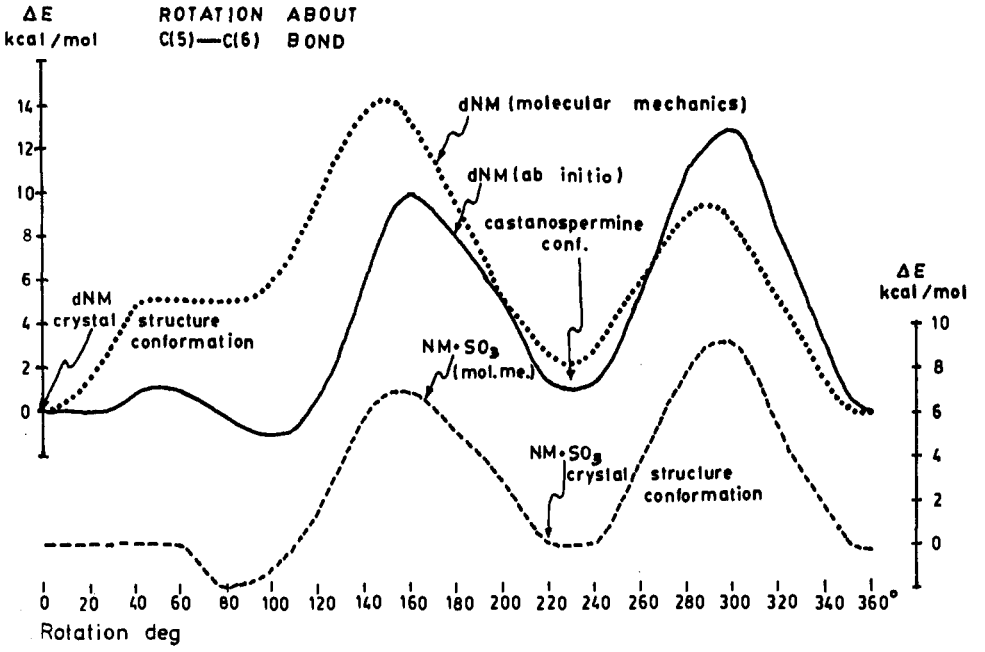

Figure 5. Plot of difference in molecular energy vs rotation of O6 about C5–C6 bond in dNM. For NM bisulfite (right ordinate) the curve has been aligned with that of dNM, i.e. 0° corresponds to C6–O6 being equatorial to the sugar ring. Energies were calculated every 10° for all curves.

Further evidence that the intramolecular O6···HN interaction plays a significant role in stabilizing the molecular conformation in this type of compound may be seen in the structure of a nojirimycin (NM) bisulfite adduct[23] which contains two independent NM molecules in the crystal cell. Presumably as a consequence of the bulky bisulfite group at C1, the ring adopts a chair conformation which has the hydrogen on the nitrogen atom axial to the ring in both molecules, rather than equatorial as seen in uncomplexed dNM. However, the intramolecular interaction between the NH and O6 and hence the parallel relationship between the NH and C6–O6 bonds is maintained: O6 is also axial to the ring in both NM molecules, with H(N)–N–C6–O6 torsion angles of 7° and 9°. Energy calculations for C5-C6 bond rotation in the NM bisulfite adduct are also shown in Figure 5; the overall features are similar to those of dNM.

The evidence is thus persuasive that the NH···O6 intramolecular interaction stabilizes the C6–O6 conformation in dNM. Thus substitution at the nitrogen atom in dNM, which would disrupt the N···O6 interaction, might be expected to increase the potency of dNM analogues, by favoring the C6-O6 axial conformation. Although one cannot rule out enhanced hydrophobic interactions, the loss of this intramolecular attraction may be the primary explanation for the increased enzyme inhibition of N-alkylated dNM.[8] Further, design of glucosidase inhibitors based on chemically similar structures may well benefit from attention to this stereochemistry.

We have calculated charge densities in the dNM and CAST structures, using the INDO procedure, to investigate electronic effects in the molecules accompanying the N···C6 ring formation in CAST. The charge on the nitrogen decreases marginally (from −0.26 to −0.20) in CAST; all other atomic charges do not change significantly.

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Supplementary Material Available: Tables of H-atom coordinates, hydrogen-bonding distances, anisotropic thermal parameters, bond lengths and angles, and figures showing crystal packing (6 pages); tables of observed and calculated structure factors (10 pages). Ordering information is given on any current masthead page.

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