(C=O)]. To a solution of the acid chloride in a mixture of 1.5 mL of acetonitrile and 1.5 mL of dry THF was added, at 0 °C, 371 mg (3.25 mmol) of (trimethylsilyl)diazomethane[23] in 2 mL of the same mixture of solvents. Stirring at room temperature for 15 h gave a solution of a silicon-containing diazo ketone [IR (THF-CH_3CN) 2100 (CHN_2), 1625 (C=O), 1110 (C-O) 1250, 845 cm^{-1} (SiC); the two latter peaks of the trimethylsilyl groups disappeared after further stirring of the mixture for 24 h]. Evaporation of the solvents under reduced pressure yielded **41** as a yellow oil [300-MHz ^1H NMR ($CDCl_3$) δ 1.114 (s, 12, CH_3), 3.403 (s, 4, OCH_2CO), 3.592 (m, 12, CH_2O)].

The yellow solution of 144 mg (0.36 mmol) of the diazo ketone **41** in 75 mL of degassed dioxane and 75 mL of degassed MeOH was irradiated under argon and cooling with ice water in a Pyrex well with a high-pressure 450-W mercury lamp. When the solution was decolorized, the solvent was removed under reduced pressure. The residue was dissolved in ether and purified by column chromatography on silica gel (ether-hexane mixtures as eluent) to give 104 mg (71%) of dimethyl 3,3,16,16-tetramethyl-5,8,11,14-tetraoxaoctadecanedioate (**42**) as a pale yellow oil: IR ($CHCl_3$) 1725 (C=O), 1115 cm^{-1} (C-O); 200-MHz ^1H NMR ($CDCl_3$) δ 0.972 (s, 12, CCH_3), 2.265 (s, 4, CCH_2CO), 3.206 (s, 4, OCH_2C), 3.616 (m, 18, OCH_3, OCH_2). Anal. ($C_{20}H_{38}O_8$) C, H.

Hydrolysis of **42** with boiling aqueous KOH gave 59% of the free dicarboxylic acid **43** as a pale yellow oil: IR ($CHCl_3$) 3000 (OH), 1700 (C=O), 1100 cm^{-1} (C-O); 200-MHz ^1H NMR ($CDCl_3$) δ 1.010 (s, 12, CCH_3), 2.303 (s, 4, CCH_2CO), 3.265 (s, 4, OCH_2C), 3.623 (m, 12, OCH_2), 7.822 (br s, 2, COOH); EI MS (70 eV, 75 °C) m/z (rel intensity) 319 ($C_{16}H_{31}O_6^+$, 24), 318 ($C_{16}H_{30}O_6^{\bullet+}$, 22) 263 ($C_{12}H_{23}O_6^+$, 6), 247 ($C_{12}H_{23}O_5^+$, 100), 219 ($C_{10}H_{19}O_5^+$, 51), 203 ($C_{10}H_{19}O_4^+$, 26), 202 ($C_{10}H_{13}O_4^+$, 40), 200 ($C_{10}H_{16}O_4^{\bullet+}$, 32). Anal. ($C_{18}H_{34}O_8$) C, H.

Biochemistry. Incorporation of ^3H_2O into Liver Total Lipids, Saponified Fatty Acids, and 3-β-Hydroxysterols; Induction of Liver Peroxisomal Activities. Rats of the Hebrew University strain weighing 140-160 g were starved for 48 h and pair-refed for 3 consecutive nights a 15-g meal of a high-carbohydrate fat-free powdered diet (Fat-free Test diet, ICN, Cleveland, OH) in the absence and in the presence of MEDICA analogues added to the diet as stated. In the morning following the last meal, the rats were injected intraperitoneally with 10 mCi of ^3H_2O in saline and were sacrificed by cervical dislocation 1 h later. The liver was quickly excised, rinsed in saline, and cooled in ice. The incorporation of ^3H_2O into liver total lipids, saponified fatty acids, and 3-β-hydroxysterols was evaluated as previously described.[3] The induction of liver peroxisomal proliferation was determined by evaluating the CN-insensitive palmitoyl-CoA oxidation or the peroxisomal enoyl-CoA hydratase activities in weighed samples of the liver as previously described.[11]

Incorporation of ^3H_2O into Total Lipids, Saponified Fatty Acids, and 3-β-Hydroxysterols in Cultured Rat Hepatocytes. This was determined as previously described.[4]

Induction of Liver Peroxisomal Activities in Cultured Rat Hepatocytes. This was determined as previously described.[11]

Liver ATP-Citrate Lyase Activity. This was determined as previously described.[4]

The Hypotriglyceridemic-Hypocholesterolemic Effect. Male Lewis rats weighing 150-180 g (SAND-Iwanovas, Kissleg, GFR) were fed ad libitum with standard chow. The test compounds were administered orally in 1% hydrous tylose suspension for the time periods as specified. Control animals were treated with the same amount of vehicle. Blood was drawn by puncture of the retrobulbar venous plexus 3 h following the application of the compounds. Plama cholesterol and triacylglycerols were determined spectrophotometrically by using the respective enzymatic kits (Boehringer Mannheim, GmBH).[29,30]

Blood Glucose and Glucose Tolerance in ob/ob Mice. Female diabetic ob/ob mice (C57BL/6J-ob obtained from Jackson Laboratory, Bar Harbor, ME 04609), aged 10-12 weeks and weighing ca. 40 g, were kept at room temperature of 23 ± 1 °C and relative humidity of 55 ± 5%. The animals had free access to water and standard chow (Sniff-R powder, Sniff Versuchsdiaten GmBH, Soest, FRG). The test compounds were mixed into the powder. Blood samples (5 μL) were taken from the tail tip. Blood glucose concentrations were measured in the hemolysate using the hexokinase/glucose-6-phosphate dehydrogenase method (Boehringer Mannheim GmBH).[31] The oral glucose tolerance was evaluated following the application by gavage of 1 g of glucose/kg of body weight (10% hydrous solution).

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Design of Potential Anti-HIV Agents. 1. Mannosidase Inhibitors

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A molecular orbital and molecular graphics study of 12 substrates, inhibitors, reaction intermediates, and substrate analogues of α-mannosidase was undertaken. The results indicated that potent inhibitors must be good topographical analogues of the mannopyranosyl cation, an intermediate in the reaction catalyzed by the enzyme. Enzyme recognition and strong binding by the inhibitors requires that they contain, as part of their structures, electronegative atoms which are the topographical equivalent of the mannosyl cation C_2 and C_3 hydroxyl groups and ring heteroatom. The absence of a topographical analogue of the C_4 hydroxyl group of the cation appeared to have little effect on the binding and activity of inhibitors. These results have been utilized in the design of potential anti-HIV drugs whose synthesis is now under consideration.

Inhibitors of glycosidases have the potential to produce a number of kinds of beneficial therapeutic effects. They have been used or suggested, as antihyperglycemic compounds,[1] inhibitors of tumour metastasis,[2] antiobesity drugs,[3,4] fungistatic compounds,[5] insect antifeedants,[6-8] and antivirals.[9-12]

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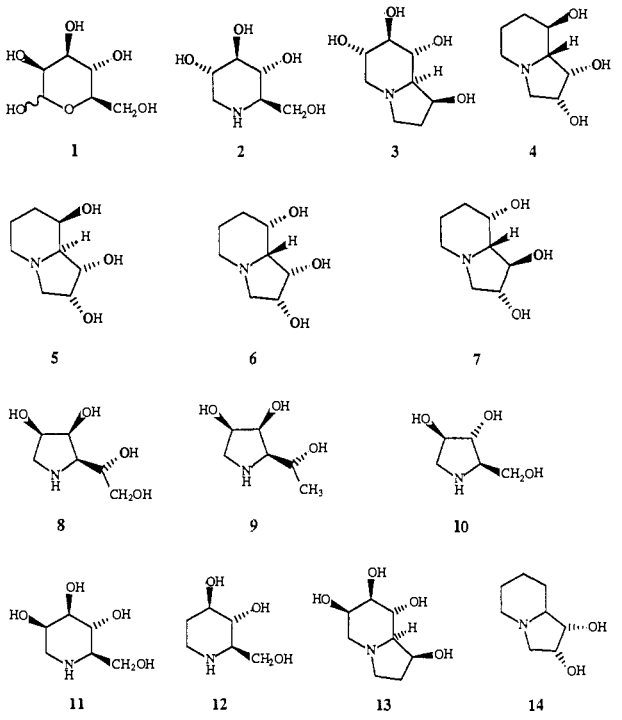
0022-2623/89/1832-2084$01.50/0

Chart I



Scheme I

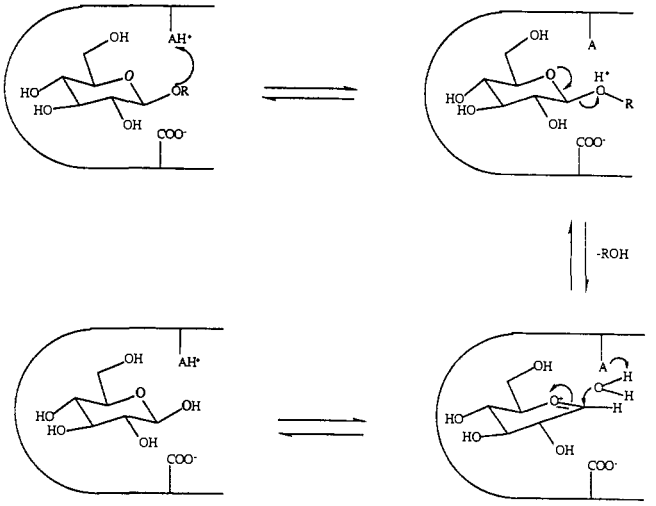


Table I. Biological Activity of Mannosidase Inhibitors

compd	IC_{50}, μM	mannosidase
1	20000[35]	jack bean (*Canavalia ensiformis*)
4	1.75,[21] 2,[36] 8[32]	jack bean
5	~10[29]	rat liver[a]
6	~50[30]	rat liver[a]
7	~50[30]	rat liver[a]
8	0.5,[31] 0.5[32]	jack bean
9	0.6[33]	jack bean
10	100[31]	jack bean
11	150,[20] 400[35 b]	jack bean
12	>1000[28]	jack bean
13	>1000[23]	jack bean
14	7500[34]	rat liver

[a] Values estimated from inhibition relative to swainsonine. [b] pH dependent.

The observation that many glycosidase inhibitors show antiviral activity[10] was of particular relevance to our efforts to rationally design compounds exhibiting activity against HIV, the causative agent in AIDS. As the envelope glycoprotein of HIV is heavily glycosylated, it seems plausible that inhibitors of one of the enzymes required for processing glycoproteins may prevent envelope formation and affect virus infectivity. Recent work has shown that a number of trimming glycosidase inhibitors (e.g., castanospermine (3)) exhibit activity against this virus.[10,12,13]

Glycosidase inhibitors appear to work by preventing the processing of N-linked complex oligosaccharides[14] (consisting of high mannose, complex, and hybrid structures, all of which arise from processing of the common precursor, $Glc_3Man_9GlcNAc_2$[12-15]). This results in the disruption of synthesis of viral coat glycoproteins, such as glycoprotein 120 (gp120) and is believed to be the specific, primary mode of action[10-12,16] of trimming glycosidase inhibitors in HIV. The disruption of viral coat gp120 formation results in loss of recognition by the CD-4 receptor of the target cell, resulting in a reduction in syncytia formation[10] with consequential reduction in virus infectivity and inhibition of viral replication.

In general there is a high degree of substrate specificity in glucosidases and mannosidases.[17,18] Reversible inhibitors of glucosidases (excluding transition-state analogues) often have structures closely resembling glucose.[19] Inspection of inhibitors of α-mannosidases (EC 3.2.1.24), however, shows that they generally do not have structures similar to mannose (1), with the exception of deoxymannojirimycin (11) (Chart I). Swainsonine (4) and its epimers (5–7), for example, exhibit potent α-D-mannosidase activity,[20-22] but any structural similarity they

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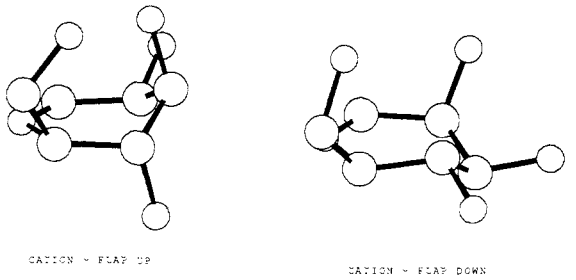

Figure 1. Geometries of MO-optimized half-chair forms of the mannopyranosyl cation. Hydrogen atoms have been omitted for clarity.

may have to α-D-mannose (1) is not immediately obvious. The lack of α-mannosidase activity of 6-*epi*-castanospermine (**13**),[23] which superficially appears to be an excellent α-D-mannose (1) analogue, is equally puzzling. This suggests that the mode of binding of inhibitors and possibly substrates and products to α-mannosidases may not be as straightforward as in the analogous case in glucosidases.

It has been speculated that inhibitors of glycosidases have structures which resemble those of the respective glycopyranosyl cations.[21,22] These cations are intermediates in the reactions catalyzed by glycosylases,[4,24-26] as Scheme I shows.

Most work on glycosylation inhibitors has focused on glucosidase inhibition. It was of considerable interest to undertake studies of mannosidase inhibitors to see whether they hold promise as antiviral (especially anti-HIV) agents, particularly in the light of recent results on the chemical modification of glucosidase inhibitors[27] which have lead to large increases in anti-HIV activity of some glucosidase inhibitors.

In this paper, we report results of molecular orbital calculations of the geometries and relative stabilities of the two half-chair forms of the mannopyranosyl cation. The results of these calculations have been used in a computer-graphics-based structure-activity study of the activities of α-D-mannose analogues and mannosidase inhibitors (1, and **4**–**14**) in α-D-mannosidase.

Results and Discussion

The activities of the 12 compounds as mannosidase inhibitors are summarized in Table I.

Table II. Atom Charges (u) for Mannopyranosyl Cation

	"flap up"		"flap down"	
atom	semiempirical	ab initio	semiempirical	ab initio
O	−0.08	−0.13	−0.06	−0.13
C1	0.25	0.28	0.29	0.29
C2	−0.01	0.07	−0.02	0.05
C3	−0.02	0.05	−0.01	0.06
C4	−0.03	0.05	−0.04	0.05
C5	−0.03	0.08	−0.01	0.09
C6	−0.03	0.00	−0.03	0.00

Molecular Orbital Calculations. The optimized geometries of two half-chair forms of the mannosyl cation, obtained from the semiempirical and ab initio MO calculations, are given in Figure 1.

The two types of MO calculation yielded essentially identical geometries for the two ring conformations. The semiempirical AMPAC calculation found that the "flap up" form was 21.1 kJ/mol (5.0 kcal/mol) lower in energy than the "flap down" form, consistent with the crystal structure of glycan and gluconolactone. the ab initio calculation gave the energy difference as a much smaller value of 1.2 kcal/mol, and care must be taken in interpreting such a small difference in energy as being significant, particularly when minimal basis set calculations are being used.

In both MO calculations and for both ring conformations of the mannopyranosyl cation, the carbon atom (C_1) double bonded to the ring oxygen atom retained a significant positive charge (approximately 0.2–0.3 u). Although the pyranose ring oxygen atom is represented as bearing a formal positive charge in the reaction mechanism schemes, the molecular orbital calculations indicate that this atom actually acquires a small negative charge, as Table II shows. This may explain why most inhibitors, in which the ring heteroatom is an electron-rich nitrogen instead of oxygen, appear to bind to the active site so strongly.

Molecular Modeling. In order to determine whether the structures of inhibitors of mannosidases more closely resemble that of mannose (as glucosidase inhibitors resemble glucose) or the mannopyranosyl cation (as suggested from the mechanism of the reaction catalyzed by the enzyme[4,24-26]), attempts were made to superimpose the good inhibitors, poor inhibitors, and inactive mannose analogues onto α-D-mannose and each of the two mannopyranosyl cation structures in turn.

Topographical Binding Models Based on the Structure of α-D-Mannose. Initial molecular modeling studies attempted to find a common binding topography for the active mannosidase inhibitors which would also be inaccessible to the inactive mannose analogues, such as 6-*epi*-castanospermine (**13**). It was not possible to superimpose the active mannosidase inhibitors onto the chair form of α-D-mannose and obtain a close fit of the respective binding groups (primarily the hydroxyl oxygen atoms and ring heteroatoms) of all of them. Inhibitors such as deoxymannojirimycin (**11**) superimposed well on mannose but other, potent inhibitors, such as swainsonine, did not.

Moreover, the crude binding model, obtained from the best fit of all inhibitors onto α-D-mannose, incorrectly predicted that inactive compounds such as 6-*epi*-castanospermine (**13**) would fit the receptor very well and thus should be excellent inhibitors of the mannosidase. Clearly, the resulting "receptor model" was not a good representation of the binding of the inhibitors and could not discriminate between the active and inactive compounds at all.

Topographical Binding Model Based on the Structure of the Mannosyl Cation. Further modelling

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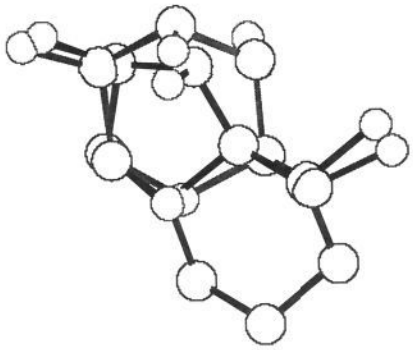


Figure 2. Superimposition of the mannopyranosyl cation on swainsonine (**4**). Hydrogen atoms have been omitted for clarity.

studies indicated that the apparently inconsistent activities of inhibitors of α-D-mannosidase (vide ultra) may be reconciled if the assumption is made that it is necessary for their structures to mimic one of the lowest energy half-chair forms of the mannosyl cation, rather than the chair form of mannose. Both energy-minimized structures for the mannosyl cation were used as templates for the superimposition of mannosidase inhibitors. The cation geometry which the molecular orbital calculations predicted to be of lowest energy was found to be a better template for superimposition of the mannosidase inhibitors. Consequently, in the discussion which follows, this lowest energy mannosyl cation geometry only was used as a template.

A common assumption of receptor modelling studies is that equivalent atoms in each of the various ligands which bind at a given receptor should all occupy approximately the same points in space. However, there is more likely considerable flexibility in the way active-site groups interact with the appropriate electronegative binding groups of the inhibitors.[46] In the case of mannosidase inhibitors, if these groups interact with the enzyme via hydrogen bonds, then these bonds can exist for a range of donor···H···receptor orientations and very close correspondence of topographical binding groups in different mannosidase inhibitors is not critical for activity.

The mannosidase inhibitors superimpose well on the mannosyl cation structure obtained from the molecular orbital calculations. Figure 2 shows the superimposition of the mannosidase inhibitor swainsonine (**4**) on the mannosyl cation structure. The electronegative atoms, such as the ring hydroxyl oxygen atoms and ring heteroatoms, superimpose well, with an average mismatch of less than 0.5 Å. The vicinal C_1 and C_2 hydroxyl groups of swainsonine superimpose on the C_2 and C_3 hydroxyl groups of the mannosyl cation, respectively. The C_8 hydroxyl of swainsonine also superimposes on the C_6 primary hydroxyl group of the cation.

The three epimers of swainsonine (**5**–**7**) are also able to be superimposed onto the mannosyl cation, in a similar manner to swainsonine, but the fit is not as good in this case. For the 8-epi compound (**6**), it is now necessary for its five-membered ring to twist slightly to allow the 8-OH to lie in the same region of space as the 6-OH of the mannosyl cation. In the 8a-epi swainsonine (**5**), it is not possible for its 8-OH to superimpose with the 6-OH of the cation. Instead, it lies in a similar region of space as the 4-OH of the cation. The 1,8-diepi compound (**7**) does not superimpose as well as the other epimers and would be expected to exhibit the lowest activity.

1,4-Dideoxy-1,4-imino-D-mannitol (**8**) and 1,4,6-trideoxy-1,4-imino-D-mannitol (**9**) superimpose very well on the mannosyl cation. Compound 8 can superimpose in one

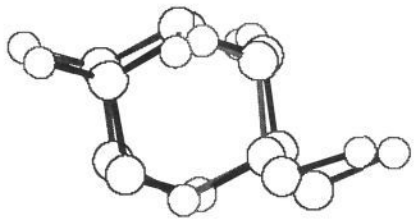


Figure 3. Superimposition of the mannopyranosyl cation on deoxymannojirimycin (**11**). Hydrogen atoms have been omitted for clarity.

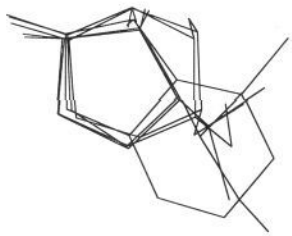


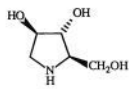
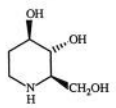
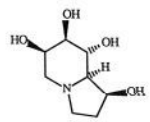
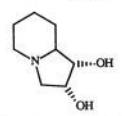
Figure 4. Superimposition of most potent mannosidase inhibitors **4**, **5**, **8**, and **9** on α-D-mannose. Hydrogen atoms have been omitted for clarity.

of two ways. In both cases the two ring secondary hydroxyl groups can superimpose on the C_2 and C_3 hydroxyl groups of the cation and the ring heteroatoms also coincide. The difference between the two possible binding modes lies in the alignment of the mannosyl cation C_6 hydroxyl group with either the primary or secondary hydroxyl group on the alkyl substituent of the inhibitor. Both superimpositions produce good correspondence between the important binding groups on the respective molecules although, when the primary hydoxyl group of the inhibitor is superimposed on the primary hydroxyl group of the cation, the secondary hydroxyl group of the inhibitor then lies close to the binding region for the ring heteroatom. This may allow an additional binding interaction to occur with the active site of the enzyme. The trideoxy compound **9** can only superimpose on the cation if its secondary hydroxyl group on C_5 superimposes on the primary C_6 hydroxyl group of the cation.

The superimposition of deoxymannojirimycin (**11**), which is a less potent inhibitor of mannosidases than swainsonine (see Table I), is illustrated in Figure 3. In order for deoxymannojirimycin to superimpose well it was necessary to assume it binds in the 1C ring conformation (see Reeves[47] for pyranose ring convention), which allows the C_3 region of the ring to adopt a similar "flap up" configuration to that of the mannosyl cation. The hydroxyl groups on the cation and deoxymannojirimycin (**11**) superimpose very well, with an average mismatch of less than 0.4 Å. However, when the hydroxyl oxygen atoms are superimposed in this way, the ring heteroatoms do not lie in exactly the same region of space, as the ring nitrogen atom of deoxymannojirimycin lies approximately 1.0 Å below the mannosyl cation ring oxygen atom. This is due to deoxymannojirimycin (**11**) adopting a chair conformation while the cation adopts a half-chair conformation. This mismatch of the ring heteroatoms may be responsible for the lower activity of deoxymannojirimycin (**11**) compared with swainsonine (**4**).

Figure 4 shows the superimposition of the four most potent mannosidase inhibitors (**4**, **5**, **8**, and **9**) on the lowest energy half-chair form of the mannopyranosyl cation. It can be seen that there is good overlap of the rings and that the topographically equivalent ring hydroxyls and ring

Table III. Summary of Topographical Properties of Inactive Compounds

compound	reason for inactivity
	lack of OH group equivalent to cation C_2-OH
	lack of OH group equivalent to cation C_2-OH
	incorrect ring conformation for good superimposition
	lack of OH group equivalent to cation C_6-OH

heteroatoms cluster in relatively small, common regions of space. These regions delineate the topographical properties which inhibitors must possess to in order to bind most efficiently to the active site.

Discrimination of the Model against Inactive Inhibitor or Substrate Analogues. The binding model was able to discriminate among the active compounds, those less active, and the inactive compounds. The rationale for the poor activity of compounds **10**, and **12–14** is summarized in Table III.

Pyrrolidine **10** is a weak inhibitor of mannosidases. It superimposes well on the binding model based on the mannosyl cation geometry and has its two ring hydroxyl groups lying in the same region of space as C_3 and C_4 of the cation, with the CH_2OH moieties also being topographically equivalent. However, the C_2 hydroxyl group of the cation has no equivalent in the inhibitor. It appears to be vital for recognition and binding of an inhibitor to the enzyme active site that there be an electronegative functional group within the inhibitor which is a topographical equivalent of the C_2 hydroxyl of the cation. Any compounds which lack this important group are inactive.

Fagomine (**12**), which is inactive, also lacks this important binding group equivalent to the C_2 hydroxyl of the sugar. The loss of one strong hydroxyl group interaction in the enzyme active site can also have an effect on the strength of binding to the site, even if recognition of the inhibitor by the enzyme occurs. Andrews et al.[48] have deduced an average binding energy of 2.5 kcal/mol for the hydroxyl group, loss of which could result in an approximately 70-fold decrease in binding constant at 298 K. This factor is a likely explanation for the apparent inactivity of the *cis*-indolizidine diol **14**, which has only two hydroxyl groups and the ring heteroatom to bind to the active site.

Thus, the lack of a third hydroxyl group appears to be sufficient to prevent binding and/or recognition of the compound at the active site of the enzyme. However, possession of the requisite number of hydroxyl groups does not ensure activity if the hydroxyl groups are not correctly orientated to bind to the active site of the mannosidase. For example, the inactivity of excellent mannose analogues, such as 6-*epi*-castanospermine (**13**), is easily explained when this compound is superimposed onto the mannosyl cation template. The superimposition is very poor as the conformation of the the six-membered ring of the inhibitor does not allow optimum overlap of the ring hydroxyl groups with those of the template. The hydroxyl groups of the inhibitor which are topographically equivalent to

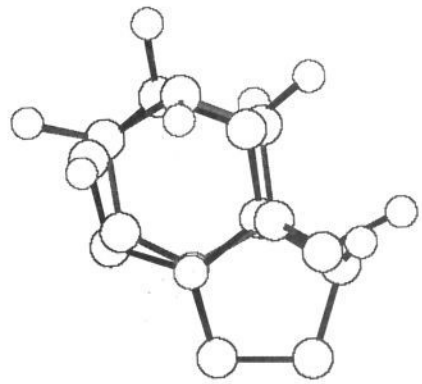


Figure 5. Superimposition of the mannopyranosyl cation onto 6-*epi*-castanospermine (**13**). Hydrogen atoms have been omitted for clarity.

axial cation hydroxyl groups adopt an equatorial configuration. The equatorial hydroxyl groups of the mannosyl cation are similarly equivalent to axial hydroxyl groups in the inhibitor, as Figure 5 shows.

In deoxymannojirimycin (**11**), sufficient flexibility is inherent in the ring to allow the ring to flip (vide ultra) and provide optimum alignment of the topographically equivalent hydroxyl groups of inhibitor and cation. In 6-*epi*-castanospermine (**13**), the five-membered ring fused to the six-membered ring prevents the ring flip from occurring. This poor topographical equivalence between hydroxyl groups on the cation and inhibitor prevents the inhibitor from binding effectively to the enzyme active site.

The hydroxyl groups on the inhibitors which are topographically equivalent to the C_4 hydroxyl group of the cation appear to be relatively unimportant in effective recognition and binding of inhibitors to the enzyme. Thus, the swainsonine epimers (**4–7**) and pyrrolidines (**8** and **9**) are very active inhibitors of mannosidase, in spite of lacking an hydroxyl group which is the topographically equivalent of the mannosyl cation C_4 hydroxyl group.

In summary, inhibitors of mannosidases require the correct spatial disposition of binding groups in order to interact with the active site of the enzyme efficiently. The role of the ring heteroatom has not been clearly defined, but it is likely to be essential for correct recognition of the inhibitor by the enzyme and for tight binding into the active site. It is relatively unimportant for inhibitors to possess a binding group topographically equivalent to the 4-OH in mannose. The equivalent of the 6-OH appears to assist in binding of inhibitors into the active site, but it is not essential for activity. The topographical equivalents of the 2- and 3-hydroxyl groups in the sugar appear to have the greatest influence on potency and specificity of the inhibitors. The group at an equivalent position to that of the 2-OH in mannose is the main determinant of specificity of the inhibitor, while the 3-OH equivalent is essential for potency. A ring heteroatom and at least three ring hydroxyl groups appear to be the minimum requirements for activity.

The active site model based on the geometry of the mannopyranosyl cation provides useful insight into the topographical requirements for mannosidase inhibition. It may be a useful tool for the design of new classes of inhibitors and can direct modifications to existing mannosidase inhibitors which will increase their activity. Synthesis of several new inhibitors which were designed in this manner is currently being undertaken. These compounds will be screened for activity against HIV.

Experimental Section

Biological Activity. The biological activities of the inhibitors of mannosidase were taken from the literature. Where several values were cited for activity of some compounds, the range of values was taken. The activities of the compounds studied are summarized in Table I. Several compounds had been tested for their inhibitory activity in rat liver mannosidase instead of the jack bean enzyme. Howard et al.[36] reported the activity of swainsonine (4) in mannosidases from several biological sources, including rat liver and jack bean, and found relatively small differences in IC_{50}.

Molecular Orbital Calculations. Molecular orbital calculations were performed on a MicroVAX II computer, using the AMPAC[37] semiempirical molecular orbital and the GAUSSIAN 80 ab initio molecular orbital packages. The AMPAC molecular orbital method appears to provide a better description of hydrogen bonding and to yield more realistic geometries than MINDO/3.[38,39] It was used to optimize the geometry of each half-chair form of the mannopyranosyl cation. The AMPAC starting geometries for the two possible half-chair forms of the mannosyl cation were adapted from the crystal structure of α-D-mannose.[40] The starting geometry of the second half-chair form was derived from the geometry of the first half-chair form with the appropriate ring flip applied via the modelling capabilities of CHEM-X.[41] This optimum geometry obtained from the semiempirical MO calculation was used as the starting geometry for a fully geometry-optimized ab initio MO calculation to obtain the relative energies of the two half-chair forms. These calculations utilized the GAUSSIAN 80[42] package and employed the minimal STO-3G basis set.

Molecular Modelling. The molecular modelling was carried out on a MicroVAX II computer using the CHEM-X[41] software. Crystal structures were available for swainsonine (3)[43] and nojirimycin,[44] α-D-mannose (1),[40] and castanospermine (3).[45] The geometries of the other inhibitors were obtained by modification of castanospermine, swainsonine, and nojirimycin and by the use of the molecular modification and building capabilities of CHEM-X. The derived geometries were optimized by means of a molecular mechanics calculation (in CHEM-X). Errors in the molecular structures derived by these means are usually small and do not significantly affect the results of the molecular superimposition studies.

The superimpositions were accomplished by choosing several pairs of topographically equivalent atoms on the two molecules being studied and by using a least-squares procedure to move one of the structures to ensure optimum overlap of the nominated atoms. The choice of topographically equivalent atoms for the superimpositions was usually clear, with hydroxyl groups of inhibitors and cation being superimposed, as were the pyranose ring oxygen atom and the heterocyclic nitrogen atoms of the inhibitors. In most cases the inhibitors studied were relatively rigid, cyclic compounds and superimpositions were performed with rigid geometries. Relaxation of geometries during the superimpositions would be expected to improve the quality of the fit, but the extra computational effort was not justified.

Where the molecules being superimposed exhibited conformational flexibility in a side chain, an energy-weighted superimposition was carried out allowing relaxation of the flexible torsion angles in the side chain. In this procedure the molecular geometry used for superimposition was a compromise between the lowest energy conformations and those most consistent with good overlap between equivalent functional groups on the two molecules. In all cases this resulted in a geometry which overlapped well with the template but which still adopted an acceptably low energy (<40 kJ/mol above the global minimum) conformation. In effect, the superimposition was weighted to account for the conformational energy of the flexible groups.

Note Added in Proof. One of the referees noted that there was no published work showing that mannosidase inhibitors exhibited activity against HIV. However, Montefiori et al. have recently shown that deoxymannojirimycin (11) dramatically attenuates the infectivity of HIV-1 in micromolar concentrations, although bromoconduritol (a glucosidase inhibitor) and swainsonine were inactive in vivo. Montefiori, D. C.; Robinson, W. E.; Mitchell, W. M. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 9248-52.

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New Anticancer Agents: Chiral Isomers of Ethyl 5-Amino-1,2-dihydro-2-methyl-3-phenylpyrido[3,4-*b*]pyrazine-7-carbamate

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Racemic ethyl 5-amino-1,2-dihydro-2-methyl-3-phenylpyrido[3,4-*b*]pyrazine-7-carbamate (1a) has shown antitumor activity in a variety of in vivo experiments. The preparation of the *R* (9) and *S* (10) isomers gave compounds with significant differences in potency in several biological tests.

A series of 1,2-dihydropyrido[3,4-*b*]pyrazine-7-carbamates (e.g., **1a,b**)[1] have shown activity against P388 leukemia in mice.[2,3] In addition, **1a** has demonstrated activity against L1210 leukemia, M5076 ovarian sarcoma, mouse

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