velocities were linear for at least 30 min and were proportional to the amount of enzyme added at the levels of enzyme activity employed.

Inhibition studies were made with six to eight levels of MgATP or L-methionine in the range 0.5–4.0 × K_M for each of two inhibitor levels that were in the range 1–5 × K_M and for control mixtures lacking inhibitor. Inhibitors were tested as their 1:1 Mg complexes formed by admixture of stock solutions with equimolar amounts of $MgCl_2$. Inhibition constants (K_i values) were obtained to within ±15% from replots of inhibitor concentrations vs. slopes or intercepts on the vertical axis of double-reciprocal plots of velocity vs. substrate level. All of the latter plots were linear, as were the replots.

Acknowledgment. This work was supported by Public Health Service Research Grant CA-11196 from the National Cancer Institute, by grants to the Institute for Cancer Research (USPHS Grants CA-06927 and RR-05539 and an appropriation from the Commonwealth of Pennsylvania), and by a grant, PCM 82-09954, from the National Science Foundation. Mass spectral determinations were carried out at the Middle Atlantic Mass Spectrometry Laboratory, a National Science Foundation Shared Instrumentation Facility.

Registry No. 1, 89301-78-0; **2a**, 101249-43-8; **2b**, 101249-44-9; **3a**, 101249-45-0; **3b**, 101249-46-1; **4a** · 2Na, 101249-47-2; **4a** monophenyl ester, 101249-48-3; **4a** · Et_3N, 101249-50-7; **4b** · 2Na, 101249-51-8; **4b** monophenyl ester, 101249-52-9; **4b** · Et_3N, 101249-54-1; **5a** · 4Na, 101249-55-2; **5b** · 4Na, 101249-56-3; **6a** · XNa, 101249-57-4; **6b** · XNa, 101249-58-5; **7a**, 101249-59-6; **7b**, 101249-60-9; **8a**, 101249-61-0; **8b**, 101399-22-8; **9a**, 101249-62-1; **9b**, 101249-63-2; **10a**, 101249-64-3; **10b**, 101249-65-4; **11a**, 101249-66-5; **11a** · Bu_3N, 101249-67-6; **11b**, 101249-68-7; **12a**, 101249-69-8; **13a**, 101249-70-1; **14a**, 101249-71-2; **14a** · 4Na, 101314-63-0; **14b**, 101249-72-3; **14b** · 4Na, 101249-73-4; **15a**, 101249-74-5; **15a** · 4Na, 101249-75-6; **16a** (isomer 1), 101249-76-7; **16a** (isomer 2), 101399-23-9; **17a**, 101249-77-8; **18a** · Bu_3N, 101249-79-0; **19a**, 101249-80-3; *tert*-butyl mercaptan, 75-66-1; bis(tri-*n*-butylammonium) pyrophosphate, 5975-18-8; L-homocysteine sodium salt, 73292-23-6; di-*tert*-butyl pyrocarbonate, 24424-99-5; imidodiphosphate tri-*n*-butylammonium salt, 101249-81-4; methionine adenosyltransferase, 9012-52-6.

Synthesis and α-D-Glucosidase Inhibitory Activity of N-Substituted Valiolamine Derivatives as Potential Oral Antidiabetic Agents[1]

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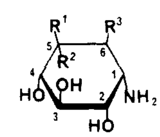
Various kinds of N-substituted valiolamine derivatives, including compounds **23a**, **24a**, and **34a**, which are structurally analogous to the key pseudodisaccharides (**25a** and **26a**) of naturally occurring oligosaccharide α-D-glucosidase inhibitors, have been synthesized and estimated by the measure of inhibitory activity against porcine sucrase and maltase. The N-substituted valiolamine derivatives evaluated in this study have been found to be more potent than the corresponding N-substituted valienamine derivatives as well as the parent valiolamine. It is noteworthy that even simple N-substituted valiolamine derivatives such as *N*-[2-hydroxy-1-(hydroxymethyl)ethyl]-, *N*-[(1*R*,2*R*)-2-hydroxycyclohexyl]-, and *N*-[(*R*)-(−)-β-hydroxyphenethyl]valiolamine (**6**, **8a**, and **9a**) have the stronger α-D-glucosidase inhibitory activity against porcine intestinal maltase and sucrase than naturally occurring oligosaccharide α-D-glucosidase inhibitors.

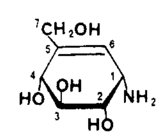
Since the middle 1970s, quite a few pseudooligosaccharides of microbial origin that exhibit a very pronounced inhibitory effect on intestinal α-D-glucosidase have been reported,[2–5] and some of them have aroused medical interest in the treatment of metabolic disease such as diabetes. In general, these microbial α-D-glucosidase inhibitors have valienamine (**2**)[6] as their key constituent, which was first found in validamycins. As previously reported, **2** itself is an inhibitor for α-D-glucosidase, and some *N*-alkyl- and *N*-aralkylvalienamine derivatives have stronger inhibitory activity against porcine sucrase and maltase than the parent valienamine.[7] These results suggest that the 4,6-dideoxy- and the 4-deoxy-D-glucopyranose units of **25a** and **26a**, found in the naturally occurring pseudooligosaccharide α-D-glucosidase inhibitors, such as the acarboses,[2] trestatins,[3] amylostatins,[4] and adiposins,[5] are not essential to sucrase and maltase inhibitory activity and are substitutable by some other structural unit.

We also found that the valiolamine (**1**),[8,9] (1*S*)-(1-(OH),2,4,5/1,3)-5-amino-1-*C*-(hydroxymethyl)-1,2,3,4-cyclohexanetetrol, has more potent α-D-glucosidase inhibitory activity than the other pseudo amino sugars such as **2**, validamine (**3**),[10] hydroxyvalidamine (**4**),[10] and epi-

Chart I



	R^1	R^2	R^3
1	$-{}^7CH_2OH$	–OH	–H
3	$-{}^7CH_2OH$	–H	–H
4	$-{}^7CH_2OH$	–H	–OH
5	–OH	$-{}^7CH_2OH$	–H



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(1) Part of this work and related experimental results are disclosed in the following patent applications: Horii, S.; Kameda, Y.; Fukase, H. (Takeda Chemical Industries, Ltd.), Eur. Pat. Appl. EP 56194 (1982); *Chem. Abstr.* **1982**, *97*, 198515r; and EP 89812 (1983); *Chem. Abstr.* **1984**, *101*, 38779c.

0022-2623/86/1829-1038$01.50/0

Scheme I

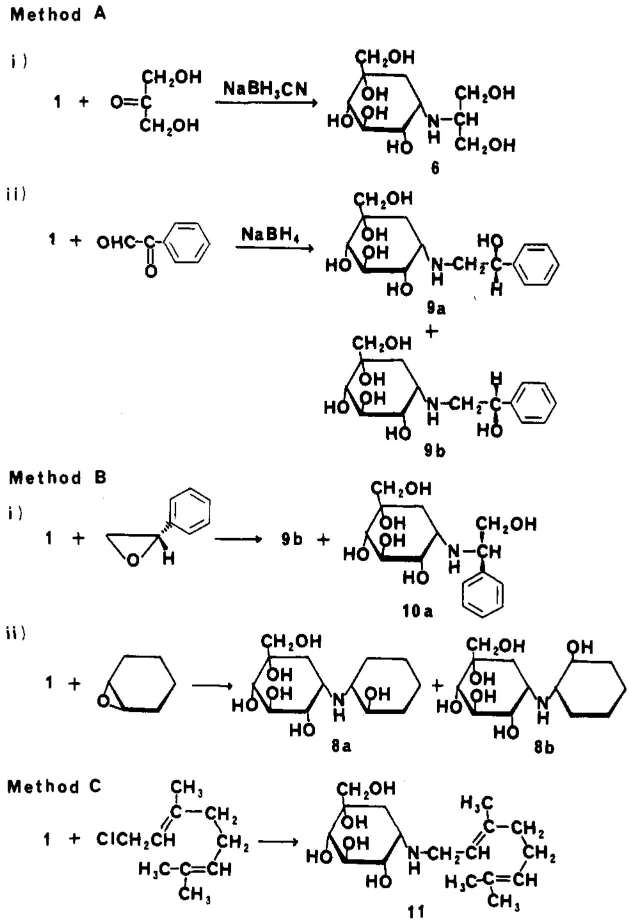
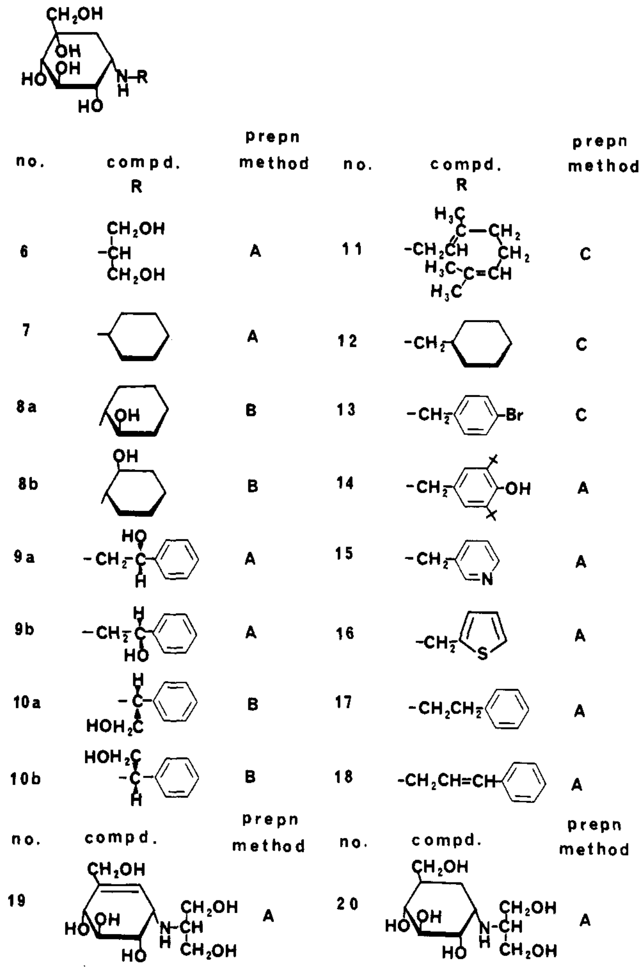


Chart II



valiolamine (5)[9] against porcine intestinal sucrase and maltase.[8,9] On the basis of the above results, we have embarked on a program of synthesis of N-substituted valiolamine derivatives in order to find more potent α-D-glucosidase inhibitor than naturally occurring oligosaccharide α-D-glucosidase inhibitors.

In this paper we described the preparation of a series of N-substituted valiolamine derivatives and discuss the α-D-glucosidase inhibitory activity of these compounds in comparison with corresponding N-substituted valienamine derivatives.

Chemistry. Compounds **1**,[9] **2**,[11] and **3**[11] were prepared by the method described in the previous papers. N-Substituted derivatives of **1** and **3** were synthesized by methods similar to those described for the preparation of N-substituted valienamine,[7] and the methods are summarized as follows. (a) Condensation of **1** (or **3**) with an appropriate ketone or aldehyde, and reduction of the resulting Schiff base (reductive alkylation of pseudo amino sugars; method A). (b) Reaction of **1** with epoxide (method B). (c) Reaction of **1** with an alkyl halide, cyclohexyl halide, or aralkyl halide (method C). If necessary, the resulting diastereoisomers were resolved chromatographically. Representative examples of these three methods are illustrated in Scheme I and are described below in detail.

N-[2-Hydroxy-1-(hydroxymethyl)ethyl]valiolamine (**6**) was prepared from **1** and 1,3-dihydroxyacetone by a reductive alkylation method (method A). Synthetically, especially for large-scale preparation, **6** is more attractive than derivatives that have an asymmetric carbon in their N-substituted moieties and require a stereoresolution.

N-(β-Hydroxyphenethyl)valiolamines (**9a** and **9b**) were first synthesized from **1** and phenylglyoxal by method A and resolved into the *R*-(−) isomer **9a** (the faster moving component) and the *S*-(+) isomer **9b** (the slower moving

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(10) Horii, S.; Iwasa, T.; Mizuta, E.; Kameda, Y. *J. Antibiot.* **1971**, *24*, 59–63.

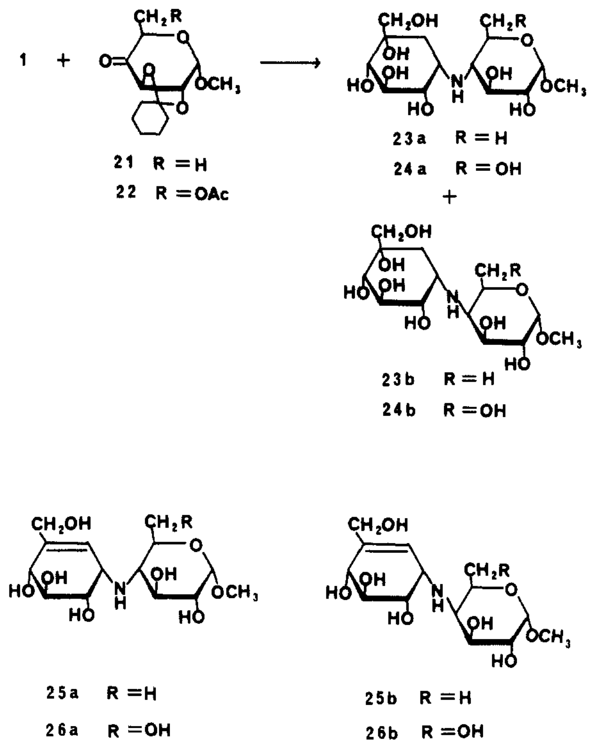
(11) Kameda, Y.; Asano, N.; Teranishi, M.; Matsui, K. *J. Antibiot.* **1980**, *33*, 1573–1574.

component) by Amberlite CG-50 (NH_4^+) chromatography with water. Compounds **9a** and **9b** were also synthesized from **1** and racemic β-styrene oxide by method B. The latter method was accompanied by the formation of *N*-[α-(hydroxymethyl)benzyl]valiolamines (**10a** and **10b**); the structural isomers **10a** and **10b** (the faster moving components) were separated from **9a** and **9b** (the slower moving components) by Amberlite CG-50 (NH_4^+) chromatography with water. The *R*-(−) isomer **10a** was crystallized out of aqueous solution of **10a** and **10b**, while the *S*-(+) isomer remained in the mother liquor. The *S*-(+) isomer **10b** was purified by Dowex 1 × 2 (OH^-) chromatography with water and freed from the *R*-(−) isomer **10a** (the slower moving component). The stereochemistry of the aralkyl units of the isomer **9a**, **9b**, **10a**, and **10b** was determined by comparing their optical rotations with those of *N*-[(*S*)-β-hydroxyphenethyl]valiolamine (**9b**, $[\alpha]^{24}_D$ +17.3° (*c* 1, H_2O)) and *N*-[(*R*)-α-(hydroxymethyl)-benzyl]valiolamine (**10a**, $[\alpha]^{24}_D$ −10.6° (*c* 1, H_2O)), which were synthesized from optically active (*S*)-β-styrene oxide and **1** by method B. *N*-(*trans*-2-Hydroxycyclohexyl)valiolamine was synthesized from **1** and 1,2-epoxycyclohexane (method B) and resolved into the 1*R*,2*R* isomer (**8a**, the faster moving component, $[\alpha]^{24}_D$ −41.9° (*c* 1, H_2O), −21.7° (*c* 1, 0.1 N HCl); ^{1}H NMR (D_2O) δ 2.57 (1 H, dt, J = 3, 10, 10 Hz, NCH)) and the 1*S*,2*S* isomer (**8b**, the slower moving component, $[\alpha]^{24}_D$ +43.4° (*c* 1, H_2O), +59.8° (*c* 1, 0.1 N HCl); ^{1}H NMR (D_2O) δ 2.57 (1 H, dt, J = 3, 10, 10 Hz, NCH)) by Amberlite CG-50 (NH_4^+) chromatography with water. The stereochemistry of the two isomers was presumed by comparison of optical rotation.[12] *N*-Geranylvaliolamine (**11**) is illustrated as a typical derivative that was prepared by method C.

In this paper, the numberings of pseudo amino sugars with trivial names, such as valienamine, validamine, and valiolamine, are assigned as those illustrated in Chart I, which are analogous to carbohydrate numbering system, because it is convenient to discuss the assignment of NMR spectra and the structure–activity relationships of pseudosaccharides in analogy with saccharides, while the positional numbers of substituents of valiolamine [(1*S*)-(1(OH),2,4,5/1,3)-5-amino-1-*C*-(hydroxymethyl)-1,2,3,4-cyclohexanetetrol] are different from those of the corresponding substituents of valienamine [(1*R*)-(1,3,4/2)-4-amino-6-*C*-(hydroxymethyl)-5-cyclohexene-1,2,3-triol] and validamine [(1*R*)-(1,3,4/2,6)-4-amino-6-*C*-(hydroxymethyl)-1,2,3-cyclohexanetriol] as well as sugars in IUPAC–IUB numbering rules.

The N-substituted valioamine derivative **23a**, of which the structure differs from the α-anomer (**25a**) of methylacarviosins (the methyl glycoside of the common and essential building block of acarbose homologous series),[2b] in that the valienamine portion of **25a** is replaced by valiolamine unit, was prepared as illustrated in Scheme II. The starting material, methyl 2,3-*O*-cyclohexylidene-6-deoxy-α-D-*xylo*-hexopyranosid-4-ulose (**21**) was prepared from methyl 4-*O*-benzoyl-6-bromo-6-deoxy-α-D-glucopyranoside via methyl 2,3-*O*-cyclohexylidene-6-deoxy-α-D-glucopyranoside by a method analogous to that described for benzyl 2,3-di-*O*-benzyl-6-*O*-trityl-α-D-*xylo*-hexopyranosid-4-ulose.[13] The coupling of **1** and the 4-ulose **21** by reductive alkylation with $NaBH_3CN$ (method A) and subsequent removal of the protecting group gave a mixture of **23a** and its epimer at C-4′ (**23b**). The mixture was purified by Dowex 50W × 8 (H^+) chromatography with 0.5 N NH_4OH and Amberlite CG-50 (NH_4^+) chromatography with water and then separated by Dowex 1 × 2 (OH^-) chromatography with water into the *gluco* isomer **23a** (the slower moving component, $[\alpha]^{22}_D$ +105.6° (*c* 1, H_2O) and the *galacto* isomer **23b** (the faster moving component, $[\alpha]^{22}_D$ +130.7° (*c* 1, H_2O). The stereochemistry at the C-4′ position of **23a** and **23b** was determined by ^{1}H NMR spectra ((400 MHz, D_2O) δ 2.47 (t, J = 9.7 Hz, 4′-CH) for **23a** and δ 2.96 (br d, $J_{3',4'}$ = 4.2 Hz, 4′-CH) for **23b**).

Scheme II



Similarly, the N-substituted valiolamine derivative **24a**, which is structurally corresponding to the methyl glycoside (**26a**) of the key pseudodisaccharide (6′-hydroxy analogues of acarviosin) of adiposins[4] and its C-4′ epimer (**24b**), were also prepared by coupling the 4-ulose **22** with **1** in a procedure similar to that of **23a** and **23b** as follows. The 4-ulose **22** was prepared by oxidation ($Me_2SO/(CF_3CO)_2O$) of methyl 6-*O*-acetyl-2,3-*O*-cyclohexylidene-α-D-glucopyranoside. Reductive alkylation of **1** with **22** and $NaBH_3CN$, successive removal of the protecting groups, and chromatographic separation of the resulting two epimer led to the isolation of the *gluco* isomer **24a** and the *galacto* isomer **24b** (^{1}H NMR (400 MHz, D_2O) δ 2.72 (apparent t, $J_{3',4'}$ = 9.5 Hz, $J_{4',5'}$ = 10.2 Hz, 4′-CH) for **24a** and δ 3.10 (br d, $J_{3',4'}$ = 4.0 Hz, 4′-CH) for **24b**).

To our knowledge, the key pseudodisaccharide (acarviosin) of the acarbose homologous series has been isolated only in the form of the methyl glycosides (**25a** and its β-anomer), because free acarviosin easily undergoes a rearrangement to less active tricyclic pyrrolo[2,1-*b*]benzoxazole derivative (component 1 of acarbose series) under the conditions of hydrolysis of glycoside linkage.[2] Therefore, the preparation of the chemically stable pseudodisaccharides **34a,b** in which the ring oxygen of the 4,6-dideoxy-D-glucopyranose moiety of **23a** is replaced by a methylene unit was designed. Further, **34a,b** would be analogues of the unstable free sugars derivable from **23a**.

The preparation of **34a,b** was carried out by way of **33a** with use of **1** and 2,3-*O*-cyclohexylidene-(2*R*)-(2,6/3,4)-

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Table I. Inhibitory Effects of N-Substituted Valiolamine, Valienamine, and Validamine Derivatives on Porcine Maltase and Sucrase

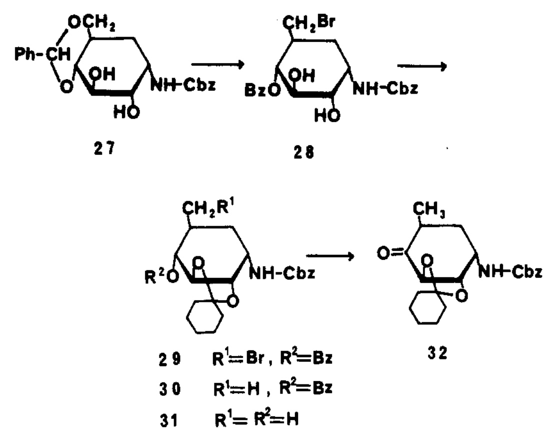
compd	IC_{50}, M maltase	IC_{50}, M sucrase	compd	IC_{50}, M maltase	IC_{50}, M sucrase
1	2.2×10^{-6}	4.9×10^{-8}	**13**	2.3×10^{-7}	1.5×10^{-8}
6	1.5×10^{-8}	4.6×10^{-9}	**14**	4.3×10^{-8}	6.8×10^{-9}
7	4.1×10^{-7}	1.5×10^{-8}	**15**	3.3×10^{-7}	1.5×10^{-8}
8a	6.1×10^{-9}	5.2×10^{-9}	**16**	2.0×10^{-7}	3.3×10^{-9}
8b	1.6×10^{-6}	1.6×10^{-7}	**17**[a]	1.0×10^{-7}	2.3×10^{-9}
9a	5.8×10^{-9}	2.9×10^{-9}	**18**[a]	2.7×10^{-7}	1.4×10^{-8}
9b	5.0×10^{-8}	1.9×10^{-8}			
10a	1.3×10^{-8}	6.6×10^{-9}	**2**	3.4×10^{-4}	5.3×10^{-5}
10b	3.4×10^{-5}	3.0×10^{-7}	**19**	5.9×10^{-6}	1.8×10^{-7}
11[a]	5.2×10^{-7}	1.1×10^{-8}	**3**	1.1×10^{-4}	7.5×10^{-6}
12	2.4×10^{-7}	9.3×10^{-9}	**20**	5.5×10^{-7}	1.8×10^{-7}

[a] Hydrochloride.

Table II. Inhibitory Effects of Pseudodisaccharides on Porcine Maltase and Sucrase

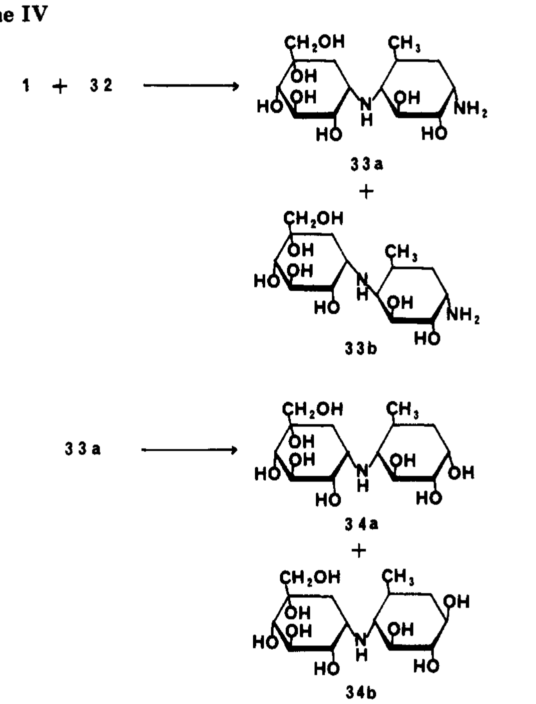
compd	IC_{50}, M maltase	IC_{50}, M sucrase	compd	IC_{50}, M maltase	IC_{50}, M sucrase
23a	4.9×10^{-9}	1.0×10^{-8}	**26a**	7.2×10^{-6}	3.2×10^{-7}
23b	6.5×10^{-7}	2.5×10^{-7}	**26b**	8.0×10^{-5}	4.0×10^{-6}
24a	7.2×10^{-8}	8.0×10^{-8}	**33a**	2.8×10^{-8}	7.5×10^{-9}
24b	1.0×10^{-7}	2.3×10^{-7}	**33b**	1.5×10^{-6}	5.3×10^{-8}
25a	3.2×10^{-6}	1.6×10^{-7}	**34a**	6.8×10^{-8}	3.6×10^{-8}
25b	1.0×10^{-4}	6.6×10^{-5}	**34b**	7.0×10^{-8}	3.5×10^{-8}

Scheme III



4-[(benzyloxycarbonyl)amino]-2,3-dihydroxy-6-methylcyclohexanone (**32**) as the starting material as shown in Scheme IV. To begin with, **32**, one of the two synthons, was prepared as shown in Scheme III. Bromination of the primary hydroxyl group of (1*R*)-(1,3,4/2,6)-1,7-*O*-benzylidene-4-[(benzyloxycarbonyl)amino]-6-*C*-(hydroxymethyl)-1,2,3-cyclohexanetriol (**27**) with *N*-bromosuccinimide gave the 6-*C*-(bromomethyl) derivative **28**, which was converted into the 2,3-*O*-cyclohexylidene derivative **29** by treatment with 1,1-dimethoxycyclohexane and *p*-toluenesulfonic acid. Hydrogenolysis of the bromomethyl group of **29** with tri-*n*-butyltin hydride and α,α′-azobis(isobutyronitrile) in toluene and subsequent de-*O*-benzoylation gave the 4-hydroxy derivative **31**. Finally, the oxidation of **31** with Me_2SO and $(CF_3CO)_2O$ gave the 4-oxo derivative **32**. The coupling of **1** and **32** to yield **33a** and its C-4′ epimer **33b** was carried out in a procedure similar to the preparation of **23a** and **23b**. After removal of the protecting groups, the resulting two stereoismers were separated by Amberlite CG-50 (NH_4^+) chromatography with 0.1 N NH_4OH into **33a** (the faster moving component; $[\alpha]^{26}_D$ +42.6° (*c* 1, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 2.25 (t, J = 9.7 Hz, 4′-CH)) and **33b** (the slower moving component; $[\alpha]^{26}_D$ +2.0° (*c* 1, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 2.99 (dd, $J_{3',4'}$ = 3.4 Hz, $J_{4',5'}$ = 4.6 Hz, 4′-CH)). The conversion of the primary amino group of **33a** into the hydroxyl group was carried out by oxidation with 3,5-di-*tert*-butyl-1,2-benzoquinone and subsequent reduction with $NaBH_4$. The resulting two isomers were separated into **34a** (the slower moving component; $[\alpha]^{26}_D$ +31.1° (*c* 1, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 4.02 (apparent q, $J_{1',2'}$ = 3.3 Hz, $J_{1',6'ax}$ = 2.4 Hz, $J_{1',6'eq}$ = 3.7 Hz, 1′-CH)) and **34b** (the faster moving component; $[\alpha]^{26}_D$ +18.9° (*c* 1, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 3.50 (ddd, $J_{1',2'}$ = 9.5 Hz, $J_{1',6'ax}$ = 12.2 Hz, $J_{1',6'eq}$ = 4.5 Hz, 1′-CH)) by Amberlite CG-50 (NH_4^+) chromatography with 0.02 N NH_4OH.

Scheme IV



Biological Results and Discussion

Compounds were tested for α-D-glucosidase inhibitory activity, and the enzyme inhibition data in vitro (molar concentrations required for a 50% inhibition: IC_{50} (M)) against porcine maltase and sucrase are shown in Tables I and II.

As reported in the previous papers,[8,9] the porcine intestinal disaccharidase inhibitory activity of 1 is much more active than that of related pseudo amino sugars such as 2–5. Additionally, the chemical conversion of the hydroxymethyl group of 1 into methyl caused a great decrease in the potency [IC_{50} (M) of 7-deoxyvaliolamine: 7.5 $\times 10^{-4}$ (maltase), 2.4 $\times 10^{-5}$ (sucrase)].[14] These results show that the presence and configuration of hydroxymethyl and tertiary hydroxyl groups of 1 play a very important role for potency.

A typical simple N-substituted valiolamine derivative **6** has been compared to the corresponding N-substituted valienamine derivative **19** and validamine derivative **20**. As shown in Table I, the valiolamine derivative was more potent than the corresponding valienamine and validamine derivatives as well as the parent valiolamine, and the IC_{50} values of some other representative simple N-substituted valiolamine derivatives were also generally more potent than the corresponding N-substituted valienamine derivatives.[7] Furthermore, the inhibitory activity tends to increase especially against porcine maltase with introduction of a hydroxyl group into a proper position on the alkyl, cyclohexyl, or aralkyl moiety of N-substituted group, which is supposed to interact with the aglycon binding subsite of the enzyme.

Stereochemistry of the hydroxyl group on the cyclohexyl unit of *N*-(hydroxycyclohexyl)valiolamine exerts an influence on activity in either a positive or negative sense. The hydroxyl group of the *N*-[(1*R*,2*R*)-2-hydroxycyclohexyl] isomer **8a** exerts a positive effect on activity, while the hydroxyl group of the *N*-[(1*S*,2*S*)-2-hydroxycyclohexyl] isomer **8b** exerts a negative effect on activity in comparison with nonsubstituted cyclohexyl derivative **7**.

The presence and configuration of hydroxyl group of aralkyl unit of *N*-[(*R*)-β-hydroxyphenethyl]valiolamine (**9a**) also markedly affect the inhibitory activity, as observed in comparison with the (*S*)-β-hydroxyphenethyl derivative **9b** and the phenethyl derivative **17**. For this results, it is presumed that the *R* configuration of the β-hydroxyl function is the more preferred configuration to fit into the active site of enzyme protein, as compared with the *S* configuration.

Replacement of the valienamine unit of **25a** and **26a**, the key pseudodisaccharides of naturally occurring oligosaccharide α-D-glucosidase inhibitors, with valiolamine unit has led to a remarkable increase in porcine maltase and sucrase inhibitory activity, especially enhancement in maltase inhibitory activity as shown in Table II. By the way, the 6′-deoxy analogue **23a** showed stronger inhibitory activity than **24a**. The C-4′ epimers **23b** and **24b** exhibit a decrease in inhibitory activity as compared with **23a** and **24a** but still showed slightly stronger activity than or almost the same activity as the valienamine derivatives **25a** and **26a**.

Pseudodisaccharide **34a** and **34b**, which were formed by coupling of two pseudosugar units, valiolamine and 7-deoxypseudo-D-glucopyranose, through an –NH– bond, showed undiminished potency as compared to **23a**. The enzyme inhibitory activity was not so much affected by the functional group (hydroxyl or amino group, and stereochemistry (α or β)) on the C-1′ carbon atom of **33a**, **34a**, and **34b**, which corresponds to the anomeric carbon atom of reducing end group of acarviosin. However, the C-4′ epimer **33b** again exhibits a recognizable decrease in maltase inhibitory activity compared to **33a**.

(14) Unpublished results (for a preparation of 7-deoxyvaliolamine, see: EP 89 812, 1983).

The high activity of these N-substituted valiolamine derivatives suggests that 4,6-dideoxy-D-glucopyranose unit, which is one of the key components of acarbose homologous series, is not indispensable for α-D-glucosidase inhibitory activity and reveals that even simple substitution can lead to more effective substances than naturally occurring pseudooligosaccharide α-D-glucosidase inhibitors.

However, practically none of these N-substituted valiolamine derivatives showed α-amylase inhibitory activity ($IC_{50} > 1 \times 10^{-3}$ M).

***N*-[2-Hydroxy-1-(hydroxymethyl)ethyl]valiolamine (6).** Compound **6** (code number AO-128), one of simple N-substituted valiolamine derivatives, is a potent α-D-glucosidase inhibitor as shown in Table I. The K_i values of **6** for maltase and sucrase (competitive inhibition) were 3.8×10^{-9} and 2.0×10^{-9} M (Lineweaver–Burk plot), respectively, which are 10^{-6} times smaller than the K_m values (2.9×10^{-3} M for maltase and 3.0×10^{-3} M for sucrase).

Although detailed experimental results of animal tests will be reported elsewhere, the ED_{50} values (the doses that suppressed the postprandial blood sugar increase by 50%) were ca. 0.1 mg/kg in sucrose loading (2.5 g/kg) and 0.5 mg/kg in starch loading (1.0 g/kg) in rats given **6** together with the corresponding carbohydrate.

This strong inhibitory activity of **6** is explicable by assuming that the hydroxyl group of the N-substituent unit ($-CH(CH_2OH)_2$) can take the three-dimensional position that is very similar to that of the C-3′ hydroxyl group of maltose and the C-1′ and C-3′ hydroxyls of sucrose by rotation around the pseudoglycosidic linkage bond between the valiolamine unit and the $-CH(CH_2OH)_2$ unit. Compound **6** was thus selected for further biological evaluation for reducing postprandial hyperglycemia and is presently undergoing clinical trial as an adjunct to the dietary management of carbohydrate-dependent metabolic disorders such as diabetes, obesity, hyperglycemia, and hyperlipemia.

Experimental Section

Chemistry. Melting points were determined with a Yamato MP-21 apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer Model 141 polarimeter or a JASCO DIP-181 polarimeter. ^1H NMR spectra were recorded, with tetramethylsilane (Me_4Si) as the external standard in D_2O and as the internal standard in $CDCl_3$, with a Varian EM-390 spectrometer (90 MHz), unless noted that they were recorded with a JEOL JNM-GX400 spectrometer at 400 MHz, or with a Varian XL-100A spectrometer at 100 MHz for decoupling experiments. ^{13}C NMR spectra were recorded with a Varian XL-100A spectrometer at 25.2 MHz. IR spectra were recorded with a Hitachi 270-30 infrared spectrometer. Thin-layer chromatography (TLC) was performed on precoated Kieselgel F_{254} plates (Merck) with *n*-PrOH–AcOH–H_2O (4:1:1), unless otherwise specified. Chromatography columns of silica gel were prepared with Kieselgel (70–230 mesh; Merck). Column chromatography was monitored by refractive index (with a Waters differential refractometer, R-403) and/or ultraviolet (254 nm) detection (with a Uvicord II instrument). Ratios for mixtures of solvents are expressed by volume (v/v).

***N*-[2-Hydroxy-1-(hydroxymethyl)ethyl]valiolamine (6).** HCl (2 N, 7.5 mL) and $NaBH_3CN$ (13 g, 0.2 mol) were added to a solution of **1** (10 g, 0.05 mol) and 1,3-dihydroxyacetone (17 g, 0.2 mol) in DMF (250 mL), and the solution was stirred for 15 h at 50–60 °C. The mixture was concentrated and the remaining solvent was removed by azeotropic distillation with toluene. The residue was dissolved in H_2O (500 mL), and Dowex 50W × 8 (H^+, 1 L) was added to the solution. The mixture was stirred for 10 min at room temperature and then poured onto a column packed with Dowex 50W × 8 (H^+, 500 mL). The column was washed with H_2O and eluted with 0.5 N NH_4OH. The eluate was concentrated and chromatographed on a column of Amberlite CG-50 (NH_4^+, 1.8 L) with H_2O. The eluate was evaporated and EtOH (500 mL) was added to the residue. The mixture was boiled under reflux for 30 min and then refrigerated to give **6** (9.3 g, 73%) as colorless crystals: mp 162–163 °C; $[\alpha]^{25}_D$ + 26.2° (*c* 1, H_2O); TLC, R_f 0.29; ^{1}H NMR (400 MHz, D_2O) δ 2.86 (1 H, m, $CH(CH_2OH)_2$), 3.59 (dd, *J* = 6.6, 11.5 Hz), and 3.66 (dd, *J* = 4.9, 11.5 Hz) (1 H each, CH_2O), 3.64 (dd, *J* = 3.8, 11.7 Hz) and 3.71 (dd, *J* = 5.1, 11.7 Hz) (1 H each, CH_2O); ^{13}C NMR (D_2O) δ 30.3 (t), 55.2 (d), 57.4 (d), 59.4 (t), 62.9 (t), 65.9 (t), 72.8 (d), 73.8 (d), 74.7 (d), 76.8 (s). Anal. ($C_{10}H_{21}NO_7$) C, H, N.

***N*-[2-Hydroxy-1-(hydroxymethyl)ethyl]valienamine (19).** Compound **19** (17 g, 66%) was prepared from **2** (20 g, 0.1 mol) by a procedure similar to that described for **6**: colorless crystals, mp 94–95 °C; $[\alpha]^{25}_D$ +121.9° (*c* 1, H_2O); TLC, R_f 0.42; ^{13}C NMR (D_2O) δ 54.4 (d), 60.3 (d), 62.2 (t), 62.5 (t), 62.5 (t), 70.5 (d), 72.2 (d), 74.0 (d), 124.5 (d), 139.7 (s). Anal. ($C_{10}H_{19}NO_6$) C, H, N.

***N*-[2-Hydroxy-1-(hydroxymethyl)ethyl]validamine (20).** Compound **20** (7.8 g, 55%) was prepared from **3** (10 g, 0.056 mol) by a procedure similar to that described for **6**: a white solid; $[\alpha]^{25}_D$ +74.0° (*c* 1, H_2O); TLC, R_f 0.35; ^{13}C NMR (D_2O) δ 28.6 (t), 39.0 (d), 55.6 (d), 59.4 (d), 61.4 (t), 62.8 (t), 63.4 (t), 74.1 (d), 74.4 (d), 75.6 (d). Anal. ($C_{10}H_{21}NO_6$) C, H, N.

***N*-[(*R*)-β-Hydroxyphenethyl]valiolamine (9a) and *N*-[(*S*)-β-Hydroxyphenethyl]valiolamine (9b).** To a solution of **1** (1.0 g, 0.005 mol) and phenylglyoxal monohydrate (1.5 g, 0.01 mol) in MeOH (20 mL) was added $MgSO_4$ (2.3 g). The mixture was stirred at room temperature for 20 h. The filtrate of the mixture was evaporated and Et_2O was added to the residue. The resulting precipitates were collected by filtration, dried in vacuo, and then dissolved in MeOH (20 mL). $NaBH_4$ (400 mg, 0.01 mol) was added to the solution with ice cooling and the solution was stirring for 3 h. H_2O and Me_2CO were added to the mixture, and the mixture was concentrated with *n*-BuOH. The resulting aqueous solution was adjusted to pH 1 with 2 N HCl and washed with EtOAc. The aqueous layer was evaporated and then chromatographed on a column of MCI Gel CHP20P resin (180 mL) that was eluted with H_2O. The eluate was adjusted to pH 10 with 1 N NaOH and concentrated. The concentrate was chromatographed on a column of Amberlite CG-50 (NH_4^+, 250 mL). The column was eluted with H_2O to resolve the two stereoisomers (**9a** and **9b**). The earlier eluted fraction was concentrated and lyophilized to give the β-*R* isomer **9a** (320 mg, 21%) as a white solid, and the later eluted fraction was concentrated and lyophilized to give the β-*S* isomer **9b** (310 mg, 21%) as a white solid. **9a**: $[\alpha]^{24}_D$ -11.0° (*c* 1, H_2O), -8.3° (*c* 1, 0.1 N HCl); TLC, R_f 0.63. Anal. ($C_{15}H_{23}NO_6 \cdot {}^1/_4H_2O$) C, H, N. **9b**: $[\alpha]^{24}_D$ 17.3° (*c* 1, H_2O), +62.1° (*c* 1, 0.1 N HCl); TLC, R_f 0.62. Anal. ($C_{15}H_{23}NO_6 \cdot {}^1/_4H_2O$) C, H, N.

***N*-[(*R*)-α-(Hydroxymethyl)benzyl]valiolamine (10a) and *N*-[(*S*)-β-Hydroxyphenethyl]valiolamine (9b).** To a solution of (*S*)-styrene oxide (2.0 g, 0.016 mol) in MeOH (50 mL) was added **1** (2.0 g, 0.01 mol). The solution was boiled under reflux for 18 h and evaporated. The residue was dissolved in H_2O (200 mL) and the solution was washed with Et_2O. The aqueous solution was concentrated and chromatographed on a column of Amberlite CG-50 (NH_4^+, 400 mL) with H_2O. The earlier fractions were concentrated and then refrigerated to give **10a** as colorless crystals (825 mg, 27%). The latter fractions were concentrated and lyophilized to give **9b** (2.0 g, 66%) as a white solid. **10a**: mp 157–158 °C; $[\alpha]^{24}_D$ -10.6° (*c* 1, H_2O), -6.5° (*c* 1, 0.1 N HCl); TLC, R_f 0.59. Anal. ($C_{15}H_{23}NO_6 \cdot {}^1/_4H_2O$) C, H, N.

***N*-[(*S*)-α-(Hydroxymethyl)benzyl]valiolamine (10b).** Compound **10b** was prepared by chromatographic separation [**10a** and **10b** were faster moving components than **9a** and **9b** by Amberlite CG-50 (NH_4^+) chromatography with H_2O, and **10b** was a faster moving component than **10a** on Dowex 1 × 2 (OH^-) chromatography with H_2O] and fractional crystallization (**10b** was more soluble in H_2O than **10a** and remained in mother liquor) of the mixture of **9a**, **9b**, **10a**, and **10b**, which was synthesized in the same manner as **10a**, by using racemic styrene oxide in place of (*S*)-styrene oxide. **10b**: $[\alpha]^{24}_D$ +43.2° (*c* 1, H_2O), +55.7° (*c* 1, 0.1 N HCl); TLC, R_f 0.61. Anal. ($C_{15}H_{23}NO_6 \cdot {}^1/_2H_2O$) C, H, N.

***N*-Cyclohexylvaliolamine (7).** Compound **7** (1.4 g, 52%) was prepared by reductive N-alkylation of **1** (2.0 g, 0.01 mol) with cyclohexanone (3.5 mL, 0.034 mol) and $NaBH_3CN$ (2.6 g, 0.04 mol) in DMF (50 mL). Purification was achieved by chromatography on a column of Dowex 50W × 8 (H^+) with 0.5 N NH_4OH and Amberlite CG-50 (NH_4^+) with H_2O: a white solid; $[\alpha]^{24}_D$ +10.8° (*c* 1, H_2O); TLC, R_f 0.56. Anal. ($C_{13}H_{25}NO_5 \cdot {}^1/_2H_2O$) C, H, N.

***N*-[(1*R*,2*R*)-2-Hydroxycyclohexyl]valiolamine (8a) and *N*-[(1*S*,2*S*)-2-Hydroxycyclohexyl]valiolamine (8b).** A solution of **1** (2.0 g, 0.01 mol) and cyclohexene oxide (2 mL, 0.02 mol) in MeOH (100 mL) was refluxed with stirring for 5 h. Additional cyclohexene oxide (4 mL, 0.04 mol) was added to the solution and the solution continued to reflux with stirring for additional 10 h. After evaporation of the solution, Et_2O was added to the residue to give a precipitate. The precipitate was chromatographed on a column of Amberlite CG-50 (NH_4^+, 400 mL) with H_2O. The 1*R*,2*R* isomer **8a** was eluted prior to the 1*S*,2*S* isomer **8b**. Each fraction was concentrated and lyophilized to give **8a** (1.1 g, 39%) and **8b** (0.9 g, 32%) as white solids. **8a**: $[\alpha]^{24}_D$ -41.9° (*c* 1, H_2O), -21.7° (*c* 1, 0.1 N HCl); TLC, R_f 0.51; ^{1}H NMR (D_2O) δ 1.1–2.33 (8 H, m, CH_2 × 4), 2.57 (1 H, dt, *J* = 3, 10, 10 Hz, CHN). Anal. ($C_{13}H_{25}NO_6 \cdot {}^1/_4H_2O$) C, H, N. **8b**: $[\alpha]^{24}_D$ +43.4° (*c* 1, H_2O), +59.8° (*c* 1, 0.1 N HCl); TLC, R_f 0.48; ^{1}H NMR (D_2O) δ 0.8–2.45 (8 H, m, CH_2 × 4), 2.57 (1 H, dt, *J* = 3, 10, 10 Hz, CHN). Anal. $C_{13}H_{25}NO_6 \cdot {}^1/_2H_2O$) C, H, N.

***N*-Geranylvaliolamine Hydrochloride (11).** Geranyl chloride (5.5 mL, 0.35 mol) and $NaHCO_3$ (3.4 g) were added to a solution of **1** (2.0 g, 0.01 mol) in DMF (55 mL). The mixture was stirred at room temperature for 48 h. The filtrate of reaction mixture was concentrated and then followed by repeated azeotropic distillation with toluene. H_2O was added to the residue. The mixture was adjusted to pH 2 and then washed with EtOAc. The aqueous layer was concentrated and chromatographed on a column of MCI Gel CHP20P resin (180 mL). The column was washed with H_2O and then eluted with a gradient of H_2O–MeOH. The eluate was concentrated and then lyophilized to give **11** (2.3 g, 63%) as a light yellow solid: $[\alpha]^{26}_D$ +17.4° (*c* 1, H_2O); TLC, R_f 0.73. Anal. ($C_{17}H_{31}NO_5 \cdot HCl \cdot H_2O$) C, H, N, Cl.

***N*-(Cyclohexylmethyl)valiolamine (12).** Compound **12** (1.6 g, 58%) was prepared from **1** (2.0 g, 0.01 mol) and cyclohexylmethyl bromide (3.5 g, 0.02 mol) by a method similar to that described for **11**, except that **12** was isolated as free base: a white solid; $[\alpha]^{26}_D$ +3.3° (*c* 1, H_2O); TLC, R_f 0.61. Anal. ($C_{14}H_{27}NO_5$) C, H, N.

***N*-(4-Bromobenzyl)valiolamine (13).** Compound **13** (750 mg, 49%) was prepared as colorless crystals from **1** (900 mg, 0.004 mol) and *p*-bromobenzyl bromide (3.0 g, 0.012 mol) by a method similar to that described for **11**, except that MeOH–dioxane (5:4) was used as reaction solvent and **13** was isolated as free base. Recrystallization from EtOH: mp 206–208 °C dec; $[\alpha]^{24}_D$ +3.9° (*c* 1, MeOH); TLC, R_f 0.63. Anal. ($C_{14}H_{20}BrNO_5$) C, H, Br, N.

***N*-(3,5-Di-*tert*-butyl-4-hydroxybenzyl)valiolamine (14).** Compound **14** (3.0 g, 47%) was prepared by reductive N-alkylation of **1** (3.0 g, 0.015 mol) with 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde (7.0 g, 0.03 mol) and $NaBH_4$ (1.0 g, 0.026 mol) in MeOH: a light yellow solid; $[\alpha]^{24}_D$ -2.3° (*c* 1, MeOH); TLC, R_f 0.80. Anal. ($C_{22}H_{37}NO_6$) C, H, N.

***N*-(3-Pyridylmethyl)valiolamine (15).** Compound **15** (0.6 g, 42%) was prepared by reductive N-alkylation of **1** (1.0 g, 0.005 mol) with 3-pyridinecarboxaldehyde (0.6 mL, 0.006 mol) and $NaBH_4$ (270 mg, 0.007 mol) in MeOH. Purification was achieved by chromatography on a column of MCI Gel CHP20P with a gradient of H_2O–60% aqueous MeOH and Dowex 1 × 2 (OH^-) with H_2O: a white solid; $[\alpha]^{26}_D$ +9.2° (*c* 1, H_2O); TLC, R_f 0.13. Anal. ($C_{13}H_{20}N_2O_5 \cdot H_2O$) C, H, N.

***N*-Thenylvaliolamine (16).** Compound **16** (500 mg, 36%) was prepared by reductive N-alkylation of **1** (1.0 g, 0.005 mol) with 2-thiophenecarboxaldehyde (1.0 mL, 0.01 mol) and $NaBH_4$ (210 mg, 0.0056 mol) in MeOH. Purification was achieved by chromatography on a column of MCI Gel CHP20P with a gradient of H_2O–MeOH: colorless crystals; mp 144–145 °C; $[\alpha]^{26}_D$ +25.6° (*c* 1, H_2O); TLC, R_f 0.54. Anal. ($C_{12}H_{19}NO_5S$) C, H, N, S.

***N*-Phenethylvaliolamine Hydrochloride (17).** Compound **17** (1.0 g, 62%) was prepared by reductive N-alkylation of **1** (1.0 g, 0.005 mol) with phenylacetaldehyde (5 mL of 50% solution in diethyl phthalate, 0.02 mol) and $NaBH_4$ (400 mg, 0.01 mol) in MeOH. Purification was achieved by chromatography on a column of MCI Gel CHP20P with H_2O: a white solid; $[\alpha]^{26}_D$ +35.2° (*c* 1, H_2O); TLC, R_f 0.61. Anal. ($C_{15}H_{23}NO_5 \cdot HCl \cdot {}^1/_2H_2O$) C, H, N, Cl.

N-(3-Phenylallyl)valiolamine Hydrochloride (18). Compound 18 (1.3 g, 77%) was prepared by reductive N-alkylation of 1 (1.0 g, 0.005 mol) with cinnamaldehyde (1.3 mL, 0.01 mol) and $NaBH_4$ (210 mg, 0.0056 mol) in MeOH. Purification was achieved by chromatography on MCI Gel CHP20P with a gradient of H_2O–MeOH: a white solid; $[\alpha]^{26}_D$ +36.0° (c 1, H_2O); TLC, R_f 0.66. Anal. ($C_{16}H_{23}NO_5{\cdot}HCl{\cdot}{}^1/_2H_2O$) C, H, N, Cl.

Methyl 2,3-O-Cyclohexylidene-6-deoxy-α-D-xylo-hexopyranosid-4-ulose (21). A solution of $(CF_3CO)_2O$ (11.1 mL, 0.08 mol) in CH_2Cl_2 (20 mL) was added to a solution of Me_2SO (7.5 ml, 0.1 mol) in CH_2Cl_2 (20 mL) with the temperature <−65 °C (dry ice–acetone bath), and the solution was stirred at the same temperature for 10 min. To this solution was added dropwise a solution of methyl 2,3-O-cyclohexylidene-6-deoxy-α-D-glucopyranoside (6.8 g, 0.026 mol) in CH_2Cl_2 (30 mL). The solution was stirred for 1 h and then Et_3N (22.2 mL) was added to the mixture. The reaction temperature was maintained below −65 °C during the above processes. The mixture was stirred to warm to room temperature and partitioned between CH_2Cl_2 (200 mL) and ice–water (200 mL). The organic layer was separated and washed with 2 N HCl and aqueous $NaHCO_3$. After evaporation of the solvent, the residue was chromatographed on a column of silica gel (400 mL) with toluene–EtOAc (17:3). The eluate was evaporated and dried in vacuo to give 21 (5.3 g, 79%) as a colorless syrup: $[\alpha]^{24}_D$ +143.3° (c 1, MeOH); IR (Nujol) 1760 cm^{-1} (C=O). Anal. ($C_{13}H_{20}O_5$) C, H.

Methyl 6-O-Acetyl-2,3-O-cyclohexylidene-α-D-xylo-hexopyranosid-4-ulose (22). Compound 22 (10.2 g, 87%) was prepared from methyl 6-O-acetyl-2,3-O-cyclohexylidene-α-D-glucopyranoside (11.8 g, 0.037 mol) by a procedure similar to that described for 21: colorless syrup; $[\alpha]^{24}_D$ +120.4° (c 1, MeOH); IR (Nujol) cm^{-1} 1760, 1750 (C=O). Anal. ($C_{15}H_{22}O_7$) C, H.

Methyl 4-[[(1S,2S)-(2,4,5(OH)/3,5)-2,3,4,5-Tetrahydroxy-5-(hydroxymethyl)cyclohexyl]amino]-4,6-dideoxy-α-D-glucopyranoside (23a) and Methyl 4-[[(1S,2S)-(2,4,5-(OH)/3,5)-2,3,4,5-Tetrahydroxy-5-(hydroxymethyl)cyclohexyl]amino]-4,6-dideoxy-α-D-galactopyranoside (23b). To a solution of 1 (2.0 g, 0.01 mol) and 21 (5.3 g, 0.02 mol) in DMF (50 mL) were added $NaBH_3CN$ (2.6 g, 0.04 mol) and 2 N HCl (1.5 mL). The solution was stirred for 15 h at 50–65 °C and then concentrated by azeotropic distillation with toluene. H_2O (150 mL) and Dowex 50W × 8 (H^+, 150 mL) were added to the residue. The mixture was stirred for 2 h at room temperature and poured onto a column packed with Dowex 50W × 8 (H^+, 30 mL). The column was washed with H_2O and eluted with 0.5 N NH_4OH. The eluate was evaporated and the residue was chromatographed on a column of Amberlite CG-50 (NH_4^+, 250 mL) with H_2O. The eluate was evaporated to give a mixture of the *gluco* isomer **23a** and the *galacto* isomer **23b**. The mixture was chromatographed on a column of Dowex 1 × 2 (OH^-, 1.5 L) with H_2O. The earlier fractions were concentrated and lyophilized to give **23b** (0.25 g, 7%) as a white solid. Concentration and lyophilization of the latter fractions afforded **23a** (1.47 g, 43%) as a white solid. **23a**: $[\alpha]^{22}_D$ +105.6° (c 1, H_2O); TLC, R_f 0.44; ^1H NMR (400 MHz, D_2O) δ 1.34 (3 H, d, J = 6.1 Hz, 6′-CH_3), 2.47 (1 H, t, J = 9.7 Hz, 4′-CH), 3.71 (1 H, t, J = 9.7 Hz, 3′-CH), 3.72 (1 H, dq, J = 9.7, 6.1 Hz, 5′-CH). Anal. ($C_{14}H_{27}NO_9{\cdot}{}^1/_2H_2O$) C, H, N. **23b**: $[\alpha]^{22}_D$ +130.7° (c 1, H_2O); TLC R_f 0.32; ^1H NMR (400 MHz, D_2O) δ 1.33 (3 H, d, J = 6.8 Hz, 6′-CH_3), 2.96 (1 H, br d, J = 4.2 Hz, 4′-CH), 3.82 (1 H, dd, J = 4.2, 10.3 Hz, 3′-CH), 4.17 (1H, br q, J = 6.8 Hz, 5′-CH). Anal. ($C_{14}H_{27}NO_9{\cdot}{}^1/_2H_2O$) C, H, N.

Methyl 4-[[(1S,2S)-(2,4,5(OH)/3,5)-2,3,4,5-Tetrahydroxy-5-(hydroxymethyl)cyclohexyl]amino]-4-deoxy-α-D-glucopyranoside (24a) and Methyl 4-[[(1S,2S)-(2,4,5(OH)/3,5)-2,3,4,5-Tetrahydroxy-5-(hydroxymethyl)cyclohexyl]amino]-4-deoxy-α-D-galactopyranoside (24b). To a solution of 1 (2.0 g, 0.01 mol) and 22 (5.5 g, 0.017 mol) in DMF (35 mL) were added $NaBH_3CN$ (2.6 g, 0.04 mol) and 2 N HCl (1.5 mL). The solution was stirred for 18 h at 60–70 °C and then azeotropically concentrated with toluene. The residue was dissolved in 50% aqueous MeOH (150 mL), and Dowex 50W × 8 (H^+, 150 mL) was added to the solution. The mixture was stirred for 1.5 h at room temperature and poured onto a column packed with Dowex 50W × 8 (H^+, 30 mL). The column was washed with H_2O and eluted with 0.5 N NH_4OH. The eluate was evaporated, and the residue was dissolved in 2 N NH_4OH (200 mL). The solution was kept for 15 h at room temperature. The mixture was evaporated and the residue was chromatographed on a column of Amberlite CG-50 (NH_4^+, 450 mL) with H_2O. The eluate was concentrated and chromatographed on a column of Dowex 1 × 2 (OH^-, 850 mL) with H_2O. The eluate was divided into three fractions in order of elution. The third fraction was concentrated and lyophilized to give the glucopyranoside isomer **24a** (435 mg, 12%) as a white solid. The second fraction was concentrated and chromatographed on a column of Dowex 1 × 2 (OH^-, 270 mL) with H_2O. The appropriate fraction in this chromatography and the first fraction in the preceding chromatography were combined, concentrated, and chromatographed on a column of Amberlite CG-50 (NH_4^+, 250 mL) with H_2O. The eluate was concentrated and lyophilized to give **24b** (160 mg, 4%) as a white solid. **24a**: $[\alpha]^{23}_D$ +102.1° (c 1, H_2O); TLC, R_f 0.35; ^1H NMR (400 MHz, D_2O) δ 2.72 (1 H, apparent t, J = 9.5, 10.2 Hz, 4′-CH), 3.63 (1 H, ddd, J = 2.2, 4.6, 10.2 Hz, 5′-CH), 3.76 (1 H, t, J = 9.5 Hz, 3′-CH). Anal. ($C_{14}H_{27}NO_{10}{\cdot}{}^1/_2H_2O$), C, H, N. **24b**: $[\alpha]^{23}_D$ +105.4° (c 1, H_2O); TLC, R_f 0.33; ^1H NMR (400 MHz, D_2O) δ 3.10 (1 H, br d, J = 4.0 Hz, 4′-CH), 3.84 (1 H, dd, J = 4.0, 10.5 Hz, 3′-CH), 4.02 (1 H, br dd, J = 4.6, 8.3 Hz, 5′-CH). Anal. ($C_{14}H_{27}NO_{10}{\cdot}H_2O$) C, H, N.

Methyl 4-[[(1S,2S)-(2,4/3)-2,3,4-Trihydroxy-5-(hydroxymethyl)-5-cyclohexen-1-yl]amino]-4,6-dideoxy-α-D-glucopyranoside (25a) and Methyl 4-[[(1S,2S)-(2,4/3)-2,3,4-Trihydroxy-5-(hydroxymethyl)-5-cyclohexen-1-yl]amino]-4,6-dideoxy-α-D-galactopyrnaoside (25b). Compound **25a** (1.2 g, 34%) and **25b** (370 mg, 10%) were prepared from **2** (2.0 g, 0.01 mol) and **21** (5.5 g, 0.02 mol) by a procedure similar to that described for the valiolamine derivatives **23a** and **23b**. **25a** [the later eluted isomer by Dowex 1 × 2 (OH^-) chromatography with H_2O]: colorless crystals (crystallized from H_2O–EtOH); mp 153–154 °C; $[\alpha]^{22}_D$ +131.5° (c 1, H_2O); TLC, R_f 0.54; ^1H NMR (D_2O) δ 1.68 (3 H, d, J = 6.5 Hz, 6′-CH_3), 2.88 (1 H, m, 4′-CH), 3.74 (3 H, s, OCH_3), ∼5.05 (1 H, 1′-CH), 6.25 (1 H, dd, J = 1.5, 5 Hz, 6-CH). Anal. ($C_{14}H_{25}NO_8{\cdot}{}^1/_2H_2O$) C, H, N. **25b** [the earlier eluted isomer by Dowex 1 × 2 (OH^-) chromatography with H_2O]: a white solid; $[\alpha]^{22}_D$ +133.6° (c 1, H_2O); TLC, R_f 0.45; ^1H NMR (D_2O) δ 1.64 (3 H, d, J = 6.5 Hz, 6′-CH_3), 3.37 (1 H, dd, J = 1.5, 4 Hz, 4′-CH), 3.75 (3 H, s, OCH_3), ∼5.1 (1 H, 1′-CH), 6.32 (1 H, dd, J = 1.5, 4 Hz, 6-CH). Anal. ($C_{14}H_{25}NO_8{\cdot}{}^1/_2H_2O$) C, H, N.

Methyl 4-[[(1S,2S)-(2,4/3)-2,3,4-Trihydroxy-5-(hydroxymethyl)-5-cyclohexen-1-yl]amino]-4-deoxy-α-D-glucopyranoside (26a) and Methyl 4-[[(1S,2S)-(2,4/3)-2,3,4-Trihydroxy-5-(hydroxymethyl)-5-cyclohexen-1-yl]amino]-4-deoxy-α-D-galactopyranoside (26b). Compound **26a** (430 mg, 15%) and **26b** (230 mg, 7%) were prepared from **2** (1.5 g, 0.008 mol) and **22** (3.8 g, 0.012 mol) by a procedure similar to that described for **24a** and **24b**. **26a** [the later eluted isomer by Dowex 1 × 2 (OH^-) chromatography with H_2O]: a white solid; $[\alpha]^{22}_D$ +174.7° (c 1, H_2O); TLC, R_f 0.49; ^1H NMR (D_2O) δ 2.77–3.03 (1 H, m, 4′-CH), 3.62 (3 H, s, OCH_3), 5.02 (1 H, d, J = 3 Hz, 1′-CH), 6.11 (1 H, d, J = 4.5 Hz, 6-CH). Anal. ($C_{14}H_{25}NO_9{\cdot}{}^1/_2H_2O$) C, H, N. **26b** [the earlier eluted isomer by Dowex 1 × 2 (OH^-) chromatography with H_2O]: a white solid, $[\alpha]^{22}_D$ +192.4° (c 1, H_2O); TLC, R_f 0.40; ^1H NMR (D_2O) δ 3.42 (1 H, br d, J = 4 Hz, 4′-CH), 3.62 (3 H, s, OCH_3), 5.04 (1 H, d, J = 3.6 Hz, 1′-CH), 6.23 (1 H, d, J = 4.5 Hz, 6-CH). Anal. ($C_{14}H_{25}NO_9$) C, H, N.

(1R)-(1,3,4/2,6)-1,7-O-Benzylidene-4-[(benzyloxycarbonyl)amino]-6-C-(hydroxymethyl)-1,2,3-cyclohexanetriol (27). A mixture of N-(benzyloxycarbonyl)validamine (55.3 g, 0.18 mol), benzaldehyde dimethyl acetal (27.2 g, 0.18 mol), and p-toluenesulfonic acid (0.2 g, 0.001 mol) in DMF (190 mL) was stirred at 60–65 °C for 1 h under 60 mmHg and then evacuated at the same temperature under 18–20 mmHg. The residue was partitioned between H_2O and EtOAc. The organic layer was washed with aqueous $NaHCO_3$. After removal of the solvent, Et_2O (1.5 L) was added to the residue and then the mixture was refrigerated to give **27** (67.7 g, 95%) as a white solid: $[\alpha]^{26}_D$ +56.2° (c 1, MeOH). Anal. ($C_{22}H_{25}NO_6$) C, H, N.

(1R)-(1,3,4/2,6)-1-O-Benzoyl-4-[(benzyloxycarbonyl)amino]-6-C-(bromomethyl)-1,2,3-cyclohexanetriol (28). A mixture of **27** (42.5 g, 0.106 mol), N-bromosuccinimide (21.5 g, 0.12 mol), and $BaCO_3$ (35 g, 0.177 mol) in CCl_4 (500 mL) and $CHCl_2CHCl_2$ (100 mL) was boiled under reflux with stirring for 1 h. The precipitate was filtered off while the mixture was hot and washed with CCl_4. The filtrate and washings were combined and evaporated. The residue was dissolved in EtOAc (500 mL) and washed with 2 N HCl and aqueous $NaHCO_3$. After evaporation of the solvent, the residue was chromatographed on a column of silica gel (600 mL). The column was washed with toluene–EtOAc (4:1) and eluted with toluene–EtOAc (1:1). The eluate was concentrated, and Et_2O–petroleum ether (1:5, 800 mL) was added to the residue. The mixture was refrigerated to give **28** (29.8 g, 59%) as a white solid: $[\alpha]^{25}_D$ +47.0° (*c* 1, MeOH). Anal. ($C_{22}H_{24}BrNO_6$) C, H, Br, N.

(1*R*)-(1,3,4/2,6)-1-*O*-Benzoyl-2,3-*O*-cyclohexylidene-4-[(benzyloxycarbonyl)amino]-6-*C*-(bromomethyl)-1,2,3-cyclohexanetriol (29). A mixture of **28** (23 g, 0.048 mol), 1,1-dimethoxycyclohexane (20 mL, 0.14 mol), and *p*-toluenesulfonic acid (0.5 g, 0.0026 mol) in DMF (50 mL) was stirred for 2 h at 55 °C under weakly diminished pressure (40 mmHg). The mixture was concentrated and partitioned between EtOAc and H_2O. The organic layer was washed with aqueous $NaHCO_3$ and evaporated. The residue was chromatographed on a column of silica gel (550 mL) with toluene–EtOAc (19:1). The eluate was evaporated and dried overnight in vacuo to give **29** (25.5 g, 95%) as a colorless syrup: $[\alpha]^{25}_D$ +69.0° (*c* 1, MeOH). Anal. ($C_{28}H_{32}BrNO_6$) C, H, Br, N.

(1*R*)-(1,3,4/2,6)-1-*O*-Benzoyl-2,3-*O*-cyclohexylidene-4-[(benzyloxycarbonyl)amino]-6-*C*-methyl-1,2,3-cyclohexanetriol (30). A mixture of **29** (25 g, 0.045 mol), tri-*n*-butyltin hydride (13.5 mL, 0.05 mol), and α,α'-azobis(isobutyronitrile) (0.1 g, 0.6 mmol) in toluene (300 mL) was boiled under reflux for 1 h. The cooled mixture was washed with 1 N HCl and aqueous $NaHCO_3$ and evaporated. The residue was chromatographed on a column of silica gel (600 mL). The column was washed with toluene and eluted with toluene–EtOAc (9:1). The eluate was evaporated and the residue was dried in vacuo to give **30** (21 g, 98%) as a colorless syrup: $[\alpha]^{25}_D$ +4.0° (*c* 1, MeOH). Anal. ($C_{28}H_{33}NO_6$) C, H. N.

(1*R*)-(1,3,4/2,6)-2,3-*O*-Cyclohexylidene-4-[(benzyloxycarbonyl)amino]-6-*C*-methyl-1,2,3-cyclohexanetriol (31). To a solution of **30** (20 g, 0.04 mol) in acetone–EtOH (3:2, 500 mL) was added 1 N NaOH (100 mL). The solution was stirred for 1 h at room temperature. The mixture was adjusted to pH 4.5 with 2 N HCl and then to pH 7.5 with 28% NH_4OH with cooling (ice–water bath). After addition of H_2O (500 mL), the mixture was concentrated to evaporate the organic solvent and extracted with EtOAc. The extract was washed with aqueous $NaHCO_3$ and evaporated. The residue was chromatographed on a column of silica gel (500 mL) with toluene–EtOAc (3:1). The eluate was evaporated and Et_2O–petroleum ether (1:4, 500 mL) was added to the residue. The mixture was refrigerated to give **31** (13.9 g, 89%) as colorless crystals: mp 110–111 °C; $[\alpha]^{25}_D$ +17.0° (*c* 1, MeOH). Anal. ($C_{21}H_{29}NO_5$) C, H, N.

(2*R*)-(2,6/3,4)-2,3-*O*-Cyclohexylidene-4-[(benzyloxycarbonyl)amino]-2,3-dihydroxy-6-methylcyclohexanone (32). Compound **32** (9.3 g, 78%) was prepared from **31** (12 g, 0.03 mol) by a procedure similar to that described for **21**: a colorless syrup; $[\alpha]^{25}_D$ +75.4° (*c* 1, MeOH); IR (KBr) cm^{-1} 1727, 1710 (C=O). Anal. ($C_{21}H_{27}NO_5$) C, H, N.

***N*-[(1*R*,2*S*)-(2,6/3,4)-4-Amino-2,3-dihydroxy-6-methylcyclohexyl]valiolamine (33a) and *N*-[(1*S*,2*S*)-(2,6/3,4)-4-Amino-2,3-dihydroxy-6-methylcyclohexyl]valiolamine (33b).** To a solution of **1** (4.0 g, 0.019 mol) and **32** (9.2 g, 0.025 mol) in DMF (120 mL) were added 2 N HCl (3 mL) and $NaBH_3CN$ (5.6 g, 0.09 mol). The solution was stirred for 18 h at 60–65 °C. The mixture was azeotropically concentrated with toluene. The residue was partitioned between EtOAc and H_2O. The organic layer was separated and washed with H_2O. After evaporation of the solvent, Et_2O (500 mL) was added to the residue. The mixture was refrigerated to give a white solid (3.0 g). A solution of the solid in 80% aqueous AcOH (100 mL) was stirred for 1 h at 50–60 °C and then evaporated. Pd black (600 mg) was added to a solution of the residue in MeOH–H_2O–AcOH (3:5:2, 100 mL), and the mixture was stirred in a stream of hydrogen for 4 h at room temperature. The catalysts were filtered off and washed with H_2O. The combined filtrate and washings were evaporated. The residue was chromatographed on a column of Amberlite CG-50 (NH_4^+, 250 mL). The column was washed with H_2O and eluted with 0.1 N NH_4OH. The eluate was divided into two fractions in order of elution. The earlier fractions were concentrated and rechromatographed on a column of amberlite CG-50 (NH_4^+, 400 mL) with 0.1 N NH_4OH. The eluate was concentrated and lyophilized to give **33a** (505 mg, 8%) as a white solid. The latter fractions were concentrated and rechromatographed on a column of Dowex 1 × 2 (OH^-, 150 mL) with H_2O. The eluate was concentrated and lyophilized to give **33b** (490 mg, 7%) as a white solid. **33a**: $[\alpha]^{26}_D$ +42.6° (*c* 1, H_2O); ^1H NMR (400 MHz, D_2O) δ 2.25 (1 H, t, *J* = 9.7 Hz, 4'-CH), 3.63 (1 H, t, *J* = 9.7 Hz, 3'-CH). Anal. ($C_{14}H_{28}N_2O_7 \cdot H_2O$) C, H, N. **33b**: $[\alpha]^{26}_D$ +2.0° (*c* 1, H_2O); ^1H NMR (400 MHz, D_2O) δ 2.99 (1 H, dd, *J* = 3.4, 4.6 Hz, 4'-CH), 3.80 (1 H, dd, *J* = 3.4, 5.0 Hz, 3'-CH). Anal. ($C_{14}H_{28}N_2O_7 \cdot H_2O$) C, H, N.

***N*-[(1*R*,2*S*)-(2,6/3,4)-2,3,4-Trihydroxy-6-methylcyclohexyl]valiolamine (34a) and *N*-[(1*R*,2*S*)-(2,4,6/3)-2,3,4-Trihydroxy-6-methylcyclohexyl]valiolamine (34b).** A solution of **33a** (200 mg, 0.56 mmol) and 3,5-di-*tert*-butyl-1,2-benzoquinone (180 mg, 0.8 mmol) in MeOH (5 mL) was stirred for 15 h at room temperature and then adjusted to pH 1 with 1 N H_2SO_4. After stirring for 3 h at room temperature, the mixture was partitioned between H_2O (100 mL) and $CHCl_3$ (50 mL). The aqueous layer was separated, washed with $CHCl_3$, and concentrated to 50 mL. $NaBH_4$ (200 mg) was added to the concentrate with cooling (ice–water bath). The solution was stirred for 2 h with cooling and then for 1 h at room temperature. The mixture was adjusted to pH 5 with AcOH and applied to a column of Dowex 50W × 8 (H^+, 160 mL). The column was washed with H_2O and eluted with 0.5 N NH_4OH. The eluate was evaporated to give a mixture of **34a** and **34b**. The mixture was chromatographed on a column of Amberlite CG-50 (NH_4^+, 180 mL) with 0.02 N NH_4OH. Compound **34b** was eluted prior to **34a**. Each fraction was concentrated and lyophilized to give **34a** (32 mg, 16%) and **34b** (57 mg, 29%) as white solids. **34a**: $[\alpha]^{26}_D$ +31.1° (*c* 1, H_2O); TLC, R_f 0.30; ^1H NMR (400 MHz, D_2O) δ 1.40 (1 H, ddd, *J* = 2.4, 12.5, 14.5 Hz, 6'-CHax), 1.84 (1 H, dt, *J* = 3.7, 3.7, 14.5 Hz, 6'-CHeq), 3.47 (1 H, dd, *J* = 3.3, 9.5 Hz, 2'-CH), 4.02 (1 H, apparent q, *J* = 2.4, 3.3, 3.7 Hz, 1'-H). Anal. ($C_{14}H_{27}NO_8 \cdot {}^1/_2H_2O$) C, H, N. **34b**: $[\alpha]^{26}_D$ +18.9° (*c* 1, H_2O); TLC, R_f 0.29; ^1H NMR (400 MHz, D_2O) δ 1.22 (1 H, q, *J* = 12.2 Hz, 6'-CHax), 1.93 (1 H, apparent dt, *J* = 3.6, 4.5, 12.2 Hz, 6'-CHeq), 3.24 (1 H, t, *J* = 9.5 Hz, 2'-CH), 3.50 (1 H, ddd, *J* = 4.5, 9.5, 12.2 Hz, 1'-CH). Anal. ($C_{14}H_{27}NO_8 \cdot {}^1/_2H_2O$) C, H, N.

Biology. In Vitro Assays of α-D-Glucosidase Inhibition Activity. Sucrase and maltase were prepared from porcine small intestine mucosa according to the method of Borgström and Dahlqvist.[15] The inhibitory activity was determined by incubating a solution (0.25 mL) of α-D-glucosidase with a 0.2 M substrate solution (0.25 mL) and a solution (0.5 mL) of inhibitor (at several different concentrations) in 0.02 M phosphate buffer (pH 6.8, in a final volume of 1.0 mL) at 37 °C for 10 min and the by measuring the amount of released D-glucose by glucose oxidase method using the commercially available Glucose B-Test Wako kit (Wako Pure Chem. Ind., Osaka). The concentration producing 50% inhibition (IC_{50}) was determined from a plot of percent vs. the concentration.

Sucrose and Starch Tolerance Test of 6 in Rats. Seven- to 8-week-old, male Sprague–Dawley rats (Jcl:SD, Clea Japan Inc., Osaka) were used. After being fasted for 20 h, they were orally given 5 mL of 50% (w/v) sucrose solution or 5 mL of 20% (w/v) soluble starch solution per kg with or without **6** at doses of 0.025, 0.1, 0.2, and 0.4 mg/kg for the sucrose tolerance test and of 0.1, 0.3, and 0.5 mg/kg for the starch tolerance test. Each group consisted of five or six rats. Blood was collected from tail vein before and 30, 60, and 120 min after the test solution was given, and its glucose concentration was determined by glucose oxidase method with use of the Glucose B-Test Wako kit. ED_{50} values (doses that suppress the postprandial hyperglycemia by 50%) were determined from a plot of δ-glucose area vs. dose.

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Supplementary Material Available: ^{1}H NMR data of compounds **6**, **8a,b–10a,b**, **19–22**, **23a,b**, **24a,b**, **32**, **33a,b**, and **34a,b**, and ^{13}C NMR data of compounds **23a**, **24a**, **25a,b**, and **26a** (8 pages). Ordering information is given on any current masthead page.

Structural Studies on Bioactive Compounds. 4.[1] A Structure–Antitumor Activity Study on Analogues of *N*-Methylformamide

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A series of derivatives of *N*-methylformamide (NMF), an experimental antitumor agent, has been prepared, having the general formula $R^3C(X)NR^1R^2$ where R^1 = H, CH_3, CD_3, CH_2CF_3, CH_2CH_2Cl, cyclopropyl, C_2H_5, CH_2OH, CH_2OR, $CH_2N(CH_3)_2$; R^2 = H, CH_3; R^3 = H, CF_3, CCl_3, CH_3, Ph, $NHCH_3$, $N(CH_3)_2$; and X = O, S, NH. A further short series of "push-pull" olefins of the general formula $R^1R^2C{=}CHNR^3R^4$ has been synthesized where R^1 = H, CH_3 and R^2 = H, NO_2, CN, CHO, CH_3 and R^3 = H and R^4 = H, CH_3, morpholino. These compounds have been tested for activity against the M5076 ovarian sarcoma and the TLX5 lymphoma in mice. NMF was by far the most potent agent of both series with activity against both tumors. Some other compounds showed weak activity, but there is a rigorous structural requirement for activity and most analogues were inactive. Certain members of the series exist as equilibrium mixtures of rotamers about the amide or pro-amide bonds as shown by NMR.

The antitumor activity of *N*-methylformamide (NMF; NSC 3051: 1) in experimental use was first described[2] in 1953. A subsequent clinical trial[3] in five patients was terminated when indications of hepatotoxicity intervened. We have shown that the hepatotoxicity of NMF toward mice can be minimized if the drug is scheduled in divided doses;[4] moreover, optimum antitumor activity is elicited if the drug is administered in a chronic schedule.[4] On the basis of these preclinical studies, a new phase 1 trial was conducted, and the dose-limiting toxicities were hyperbilirubinemia, nausea, and malaise. Remarkably the agent has no myelosuppressive activity in rodents or in man.[5] Beneficial effects of 1 in combination with conventional (myelosuppressive) antitumor agents have been demonstrated against rodent experimental tumors.[6] The drug is now in phase 2 trial particularly against lung and colon tumors since the compound is very active against the NCI lung (LX-1), colon (CX-1), and mammary (MX-1) human tumor xenografts implanted in mice.[7]

Earlier studies on analogues of NMF tested against the Ehrlich ascites[8] and sarcoma 180 tumors[2] revealed that only the simplest amides, NMF, and formamide **2** had antitumor activity. We have screened a range of formamides, thioformamides, acetamides, benzamides, ureas, thioureas, guanidines, enamines, and vinylogous amides **3–35** and some related compounds **36–39** against either the TLX5 lymphoma or the M5076 reticulum cell sarcoma (or both). These tumors are sensitive to a range of agents that have an *N*-alkyl group bearing an electron-withdrawing substituent. The TLX5 lymphoma is especially sensitive to nitrosoureas,[9] triazenes,[10] and the recently discovered imidazotetrazines[11] whereas the M5076 tumor is additionally responsive to the 1,3,5-triazine series based on hexamethylmelamine.[12] Structure–activity studies in the aforementioned agents have confirmed a requirement for either an *N*-methyl or *N*-(2-haloethyl) fragment for optimum antitumor activity. It was of interest, therefore, to investigate whether or not there are similar structural

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