Competitive Binding Assay. A modification[36] of the previously described method of Sharkey and Blumberg[17] was used to assess analogue affinity for PDBu-receptor binding. Briefly, rat brain cytosol was prepared from whole Sprague–Dawley rat brains by homogenization (Brinkman polytron at a setting of 60, two times for 10 s each) in an equal volume of 50 mM Tris-HCl buffer (pH 7.4) with 1 mM magnesium acetate, 1 mM phenylmethanesulfonyl fluoride, 10 mM ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N*-tetraacetic acid, 2 mM ethylenediaminetetraacetic acid, and 1 mM dithiothreitol. The homogenate was centrifuged at 100000*g* for 60 min at 4 °C. The supernatent was divided into aliquots and stored at −70 °C. Protein concentration was determined by the method of Bradford.[37]

Diacylglycerols were stored in chloroform at a concentration of 10^{-3} M. Aliquots were added to test tubes along the 15 μL of 10 mg/mL PS in chloroform, and the solvent was removed with a stream of nitrogen. To each tube, an aqueous solution of Tris-HCl, calcium chloride, and bovine γ-globulin (with concentrations such that the final concentrations in the reactions were as listed below) was added. Each tube was sonicated twice for 15 s at a setting of 3–4. A solution of [^{3}H]PDBu was added to each tube and vortexed.

Reaction mixtures with a total volume of 250 μL contained 0.02 μM [^{3}H]PDBu, rat brain cytosol (200 μg/mL protein), 0.05 M Tris-HCl (pH 7.4), 0.1–4 mM calcium chloride, 100 μg/mL PS, and 2 mg/mL bovine γ-globulin. Nonspecific binding was determined by measuring binding in parallel reactions containing 12 μM PDBu. The reactions were carried out in polyethylene microfuge tubes, which were incubated at 37 °C for 30 min and then put on ice for 5 min. Polyethylene glycol (PEG, 187 μL of a 35% solution in water) was added, and the tubes were vortexed and left on ice for 15 min. They were spun in a microfuge for 5–10 min. The supernate was removed, and the pellets were washed with 400 μL of 15% PEG, cut from the centrifuge tubes, dried, and dissolved in scintillation fluid. Radioactivity was determined by liquid-scintillation spectroscopy with a Beckman L58100 counter. All experiments were conducted in duplicate or triplicate.

Protein Kinase C Activation Assay. The enzyme was prepared as described by Leach and Blumberg.[20] Activity was determined by measuring the transfer of ^{32}P from [^{32}P]ATP to histone IIIs. The reaction mixture had a total volume of 50 μL and contained 40 mM Tris-HCl (pH 7.4), 15 mM magnesium acetate, 1 μg/mL PS, 50 μM [^{32}P]ATP (225-325 dpm/pmol), 75 μg of histone IIIs, and 11 μg of enzyme. Diacylglycerols were stored in stock $CHCl_3$ solutions. PS and an appropriate amount of diacylglycerol for the highest concentration to be assayed were measured into a tube and the solvent was removed by a stream of nitrogen. Tris-HCl buffer (0.5 mL) was added to the tube and the mixture was sonicated three times for 20 s each. Lower concentrations of diacylglycerol were obtained by diluting the sonicated mixture with a solution of Tris-HCl and PS and vortexing. The reactions were carried out in 1.5 mL tubes for 7 mins at 30 °C. The tubes were placed on ice immediately after incubation, and 25 μL from each was spotted on a 2 × 2 cm square of phosphocellulose paper. The paper was washed five times with water and once with acetone, dried, and counted by liquid-scintillation spectroscopy. All experiments were carried out in triplicate.

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Registry No. 1, 121269-62-3; **2**, 121269-63-4; **3**, 121269-64-5; 4, 555-68-0; **5**, 5396-71-4; **6**, 10039-64-2; **7**, 121269-65-6; **8**, 68034-75-3; **9**, 1664-57-9; **10**, 63307-44-8; 11, 1823-16-1; **12**, 121269-66-7; **13**, 71655-36-2; 14, 2009-59-8; **15**, 121269-67-8; **16**, 121269-68-9; **17**, 121269-69-0; **18**, 121269-70-3; **19**, 22323-83-7; **20a**, 115228-29-0; **20b**, 121348-86-5; **21a**, 19670-49-6; **21b**, 93713-40-7; **22a**, 121269-71-4; **22b**, 121269-77-0; **23a**, 121269-72-5; **23b**, 121269-78-1; **24**, 96-26-4; **25**, 121269-73-6; **26**, 121269-74-7; **27**, 121269-75-8; **28**, 121269-76-9; Pk-C, 9026-43-1; $CH_3(CH_2)_6COCl$, 111-64-8; cyclododecanone, 830-13-7.

Supplementary Material Available: The IR and NMR data not included in the text (5 pages). Ordering information is given on any current masthead page.

(36) Martell, R. E.; Simpson, R. U.; Taylor, J. M. *J. Biol. Chem.* **1987**, *262*, 5570.
(37) Bradford, M. M. *Anal. Biochem.* **1976**, *72*, 248.

Carbohydrate Biguanides as Potential Hypoglycemic Agents

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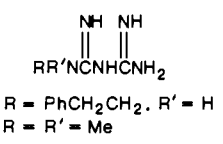
A series of monosaccharides containing a biguanide functionality was prepared and evaluated for hypoglycemic activity. Among the analogues prepared were those involving D-glucose substituted on the 6- or 1-position (**19** and **24**), D-galactose substituted on the 6-position (**7**), and D-arabinose (**31**). The target compounds were evaluated in a modified rat glucose-tolerance test (oral glucose load/oral drug, 100 mg/kg). Compounds **8** [6-biguanidino-1,2:3,5-bis-*O*-(1-methylethylidene)-6-deoxy-α-D-glucofuranose] and **23** [methyl 6-biguanidino-6-deoxy-2,3,4-*O*-tribenzyl-α-D-glucopyranoside] were the most active, exhibiting nearly equivalent hypoglycemic activity to that of phenformin (**1**) and metformin (**2**), as measured by the inhibition of the rise of blood glucose. Compound **31** was somewhat less active with 26% inhibition, as compared to 64% inhibition with **1** and 41% inhibition with **2**.

There are several approaches to the treatment of non-insulin-dependent diabetes mellitus (NIDDM). One fruitful avenue of investigation has centered on the use of biguanide-containing drugs, such as phenformin (**1**) and metformin (**2**), as oral hypoglycemic agents.[1] Unfortunately, since biguanide drugs can also cause fatal lactic



1: R = $PhCH_2CH_2$. R' = H
2: R = R' = Me

[†] Department of Chemical Research.
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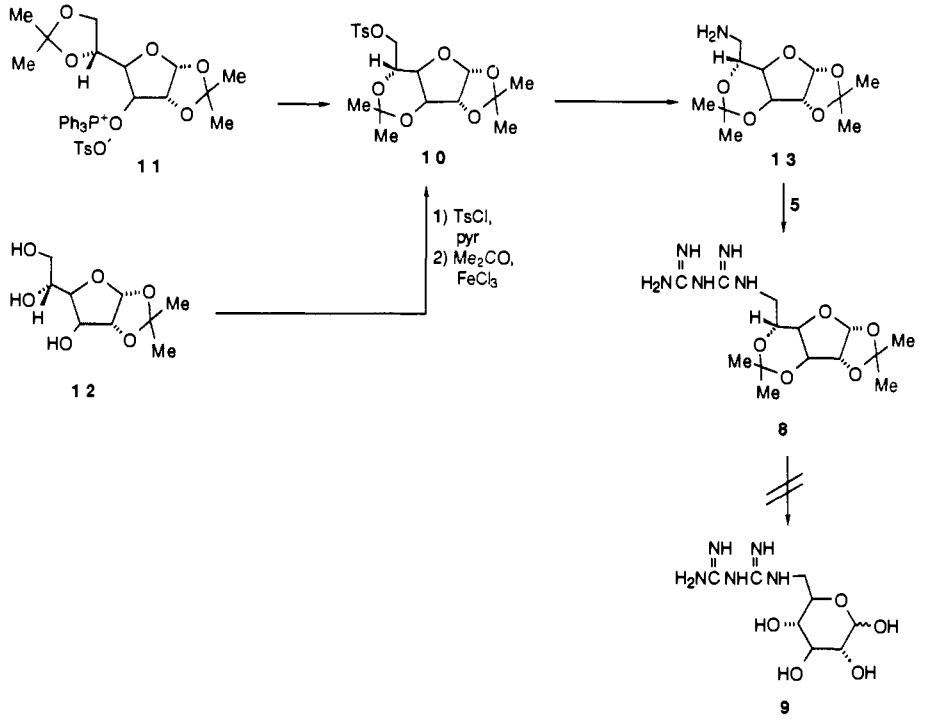
Scheme I



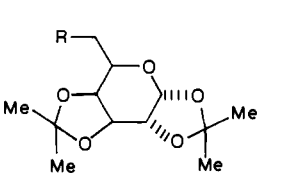
acidosis, their usefulness has been severely restricted.[2]

Although the mechanism of action of the biguanides is not fully understood, several studies have indicated that a large part of their activity is due to nonselective inhibition of the uptake of monosaccharides, such as glucose, from the intestinal tract.[3] Monosaccharides are transported across the intestinal membrane by an energy-requiring active transport system.[4] In support of the proposal that biguanides inhibit or delay this transport, compound 1 was found to be much more effective when glucose is administered orally than when it is administered parenterally.[5] The biguanide drugs also increase peripheral glucose utilization and inhibit hepatic gluconeogenesis, both of which contribute to the observed hypoglycemic effect via nonspecific disruption of membrane structure and function.[3] Indeed, the binding of biguanides to membranes has been shown to alter membrane potential and transport of metabolites and increase membrane rigidity.[3] Recently, Nicholls and Leese proposed that phenformin (1) delays carbohydrate uptake in the small intestines primarily by disrupting mitochondrial function in the epithelial cells and only partially by a direct effect on the brush border.[6]

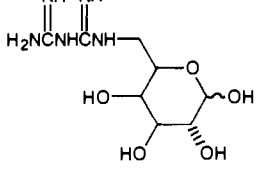
Since relatively high doses of biguanides (ca. 50–100 mg/kg) are required to achieve a hypoglycemic effect, we hypothesized that attaching the biguanide moiety to appropriate monosaccharides could result in enhanced specificity by targeting the biguanide directly to the site of the transport system. With this line of reasoning, the biguanide functionality should optimally be attached to monosaccharides with structural elements likely to effect good binding to the hexose transporter. The structural specificity of this membrane-bound transport system has been investigated in detail.[7] The configuration and presence of the hydroxyl group at C-2 is very crucial for binding and transport. The presence of the other hydroxyl groups is required to varying degrees; for example, the hydroxyl group at C-6 can be replaced by fluorine, but not hydrogen.[7d] Larger halogens (e.g. iodine) result in considerably poorer transport. The configuration of the hydroxyl at C-4 is relatively unimportant (e.g., gluco vs galacto configurations).[7] We focused on either glucose or galactose derivatives and a novel furanose congener. In the pyranose analogues, the biguanide was incorporated at C-1 or C-6, due to the factors mentioned above as well as the feasibility of synthesis.

Chemical Synthesis

Protected D-galactose **3** was converted into amine **4** by formation of the tosylate, treatment with sodium azide, and reduction of the azide group with hydrogen and palladium.[8] Compound **4** (as an HCl salt) was heated with



3: R = OH
4: R = NH_2
6: R = NHC(=NH)NHC(=NH)NH_2



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(1) (a) Wolff, M. E. *Burger's Medicinal Chemistry*; 4th ed., Part II; John Wiley: New York, 1979; p 1059. (b) Shapiro, S. L.; Parrino, V. A.; Greiger, K.; Kobrin, S.; Freedman, L. *J. Am. Chem. Soc.* **1957**, *46*, 689. (c) Ungar, G.; Freedman, L.; Shapiro, S. L. *Proc. Soc. Exp. Biol. Med.* **1957**, *95*, 190. (d) El-Kerdamy, M. M.; Selim, H. A. *Pharmazie*, **1975**, *30*, 768. (e) Hermann, L. S. *Diabete Metab.* **1979**, *5*, 233.

(2) (a) Sarges, R. *Prog. Med. Chem.* **1981**, *18*, 191. (b) Krall, L. P. In *Joslin's Diabetes Mellitus*, 12th ed.; Lea and Febiger: Philadelphia, 1985; pp 412–452.

(3) (a) Schafer, G. *Biochem. Pharmacol.* **1976**, *25*, 2005. (b) Schafer, G. *Biochem. Pharmacol.* **1976**, *25*, 2015.

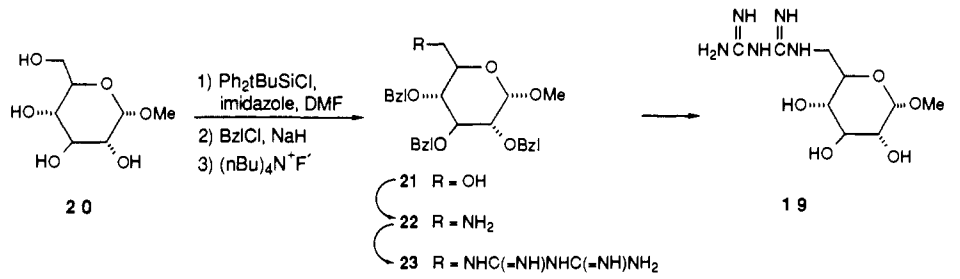
(4) Crane, R. K.; Code, C. F.; Heidel, W. *Handbook of Physiology*; American Physiology Society: Washington, D.C., 1968; Vol. 3, Section 6, p 1323.

(5) (a) Larch, E. *Diabetologia* **1971**, *7*, 195. (b) Hollobaugh, S. L.; Rao, M. B.; Kruger, F. A. *Diabetes* **1970**, *19*, 45.

(6) Nicholls, T. J.; Leese, H. J. *Biochem. Pharmacol.* **1984**, *33*, 771.

(7) (a) Barnett, J. E. G.; Jarvis, W. T. S.; Munday, K. A. *Biochem. J.* **1968**, *109*, 61. (b) Barnett, J. E. G.; Ralph, A.; Munday, K. A. *Biochem. J.* **1969**, *114*, 569. (c) Bihler, I. *Biochim. Biophys. Acta* **1969**, *183*, 169. (d) Berlin, R. D. *Science* **1970**, *168*, 1539.

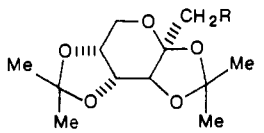
Scheme II



dicyandiamide [5; $H_2NC(=NH)NHCN$], until the two substances fused together in a melt,[9] to give **6** in 45% yield. Removal of the isopropylidene groups of **6** was effected by heating a 0.1 N HCl solution of **6** to afford **7** in 51% yield (35:65 mixture of α/β anomers in D_2O).

Biguanide **8** was prepared as well in an attempt to acquire **9** (see Scheme I). Tosylate **10** was synthesized by Kunz and co-workers by thermal rearrangement of tosylate salt **11** (67% yield).[10] We prepared **10** by the Kunz procedure and also by the following sequence. Tosylation of **12**, followed by insertion of the second isopropylidene group with acetone and ferric chloride,[11] resulted in **10**. Amine **13** was secured from **10** by the procedure used for the synthesis of **4**. Amine **13** proved to be more heat sensitive than **4** and decomposed when subjected to the same conditions used to convert **4** into **6**. We found that heating **13** with **5** in DMSO, with careful control of the reaction conditions, allowed for the preparation of **8**. Deprotection of **8**, attempted under a variety of different conditions (Dowex 50X8 resin, HCl, TsOH, HOAc), was unsuccessful. Decomposition of the molecule competed with removal of the final isopropylidene group, unlike the analogous reaction with **6**.

Since sugar biguanides such as **6** and **8** might be expected to exhibit hypoglycemic activity, they were also evaluated in our screening program. Additionally, we prepared biguanide **15**, a protected D-fructose derivative which is structurally related to topiramate (**14**), an anti-

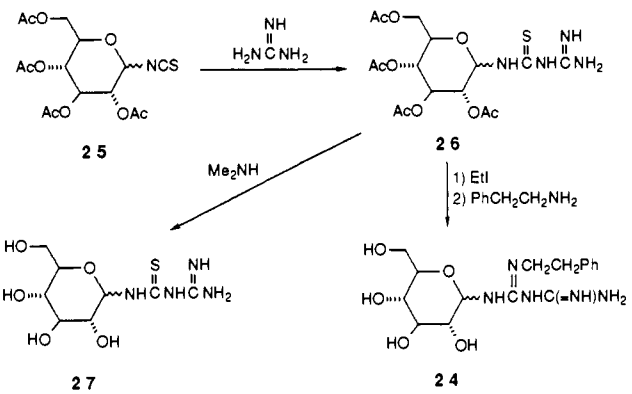


14: R = OSO_2NH_2
15: R = $NHC(=NH)NHC(=NH)NH_2$
16: R = N_3
17: R = OH
18: R = NH_2

convulsant developed in our laboratories and currently undergoing phase II clinical trials.[12] Azide **16** was obtained from alcohol **17** by treatment with tosyl chloride and sodium azide. Reduction of **16** gave amine **18**, which was converted to biguanide **15** by reaction with **5** in DMSO.

Since **9** had eluded us, we decided to prepare α-methyl glycoside **19**, reasoning that it would be more stable than

Scheme III



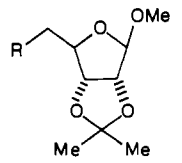
9 by virtue of a less reactive anomeric center. Furthermore, methyl α-D-glucopyranoside (**20**) is recognized as well as glucose itself at the hexose transporter.[7] Compound **20** was converted to **21** by the sequence of steps indicated in Scheme II. Amine **22** was then obtained by conversion of **21** into the corresponding phthalimide derivative by using the Mitsunobo reaction, followed by hydrazinolysis. Amine **22** and **5** were combined in DMSO under carefully controlled conditions to furnish **23**. Hydrogenolysis of **23** was best achieved with palladium on carbon and hydrogen in 1:1 methanol/water, after the addition of a catalytic quantity of HCl, to give target **19**.

After having inserted the biguanide functionality into the 6-position of glucose structure **19**, we also prepared **24**, in which the biguanide unit is directly attached to the anomeric carbon of glucose (see Scheme III). To this end, isothiocyanate **25** was treated with guanidine to afford **26**. Guanidines are capable of promoting deacetylation,[13] so we had to be careful to avoid this side reaction. Substituted guanidines, such as *N,N*-dimethylguanidine, produced multiple products upon reaction with **25**, presumably due their enhanced capacity to promote deacetylation, relative to guanidine itself. Compound **26** was treated with dimethylamine and mercury oxide in an attempt to simultaneously displace the sulfur and deacetylate.[9] Only the product of deacetylation (**27**) was isolated. Alternatively, **26** was treated with ethyl iodide, followed by an excess of phenethylamine to give **24** (a 7:3 mixture of β/α anomers). Structure **24** is a direct analogue of **1**, in which a glycosyl residue is attached to one of the biguanide nitrogens.

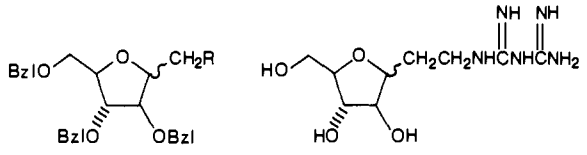
We also prepared a furanose-derived analogue to examine the effect of the sugar ring size on the biological activity. The furanoses are more reactive than the corresponding pyranoses at the anomeric center, so we were concerned that this enhanced reactivity would translate into greater instability during the condensation reaction

(8) Szarek, W. A.; Jones, J. K. N. *Can. J. Chem.* **1965**, *43*, 2345.
(9) Ray, P. *Chem. Rev.* **1961**, *61*, 313.
(10) Kunz, H.; Schmidt, P. *Liebigs Ann. Chem.* **1982**, 1245. We could only achieve 10–20% yields of **10** when repeating this procedure on 10-g quantities of **11**.
(11) Singh, P. P.; Gharia, M. M.; Dasgupta, F.; Srivastava, H. C. *Tetrahedron Lett.* **1977**, *18*, 439.
(12) Maryanoff, B. E.; Nortey, S. O.; Gardocki, J. F.; Shank, R. P.; Dodgson, S. P. *J. Med. Chem.* **1987**, *30*, 880. Maryanoff, B. E.; Margul, B. L. *Drugs Future* **1989**, *14*, 342.
(13) Kunesch, N.; Miet, C.; Poisson, J. *Tetrahedron Lett.* **1987**, *28*, 3569.

with **5**. Indeed, amine **28**, obtained from **29** by the two-step



28: R = NH_2
29: R = OH
30: R = NHC(=NH)NHC(=NH)NH_2



32: R = CN
33: R = CH_2NH_2
34: R = CH_2NHC(=NH)NHC(=NH)NH_2

31

Mitsunobo reaction/hydrazinolysis sequence, decomposed under all our attempts to convert it into biguanide **30**, including conditions with or without added solvent. We thus prepared **31**, which as a *C*-glycoside lacks a reactive anomeric acetal functionality. Nitrile **32** (α/β anomeric composition of 3:2)[14] was converted into amine **33** by reduction with diborane. Compound **33** was then condensed with **5** to give biguanide **34**, which was deprotected with trimethylsilyl iodide[15] and hydrogenolysis, affording **31**.

Although biguanides generally do not provide distinguishing molecular ions by chemical-ionization mass spectrometry, they are readily detected by fast-atom-bombardment (FAB) mass spectrometry, with extremely high sensitivity.[16] For example, the molecular ion for compound **15** could be detected at a concentration of 10^{-9} M. The ability to see biguanides by FAB mass spectrometry at the nanogram level allowed us to conveniently monitor the biguanide-forming reactions as they proceeded.

Biological Testing

The hypoglycemic activity of the target carbohydrate biguanide compounds is compared to that of reference biguanide glucose-absorption inhibitors phenformin (**1**) and metformin (**2**) in Table I. Tolerance to oral glucose was significantly improved by orally administered phenformin (50–300 mg/kg) and metformin (100 mg/kg), with the degree of inhibition being dose-dependent for phenformin.[5] The tolerance to parenteral glucose administration was not improved by phenformin or metformin (data not shown).

As indicated in Table I, three of the nine compounds displayed significant hypoglycemic activity. Compound **8**, a protected glucose biguanide deriative, produced non-dose-dependent activity (48% and 45% inhibition at 100 and 200 mg/kg, respectively), which was comparable to that produced by 100 mg/kg of metformin (47% inhibition). Similarly, compound **23** (protected glucose biguanide) at 100 mg/kg significantly inhibited (47%) the rise of blood glucose. Compound **31** (*C*-arabinosylbiguanide) also significantly improved oral glucose tolerance, however, the effect was approximately one-half of that produced by metformin at an identical dose. As expected for glucose-absorption inhibitors, these compounds did not lower fasting blood glucose. The other compounds (**6**, **7**, **15**, **19**, **24**, and **27**) were inactive at the doses tested.

Conclusion

The reference biguanides **1** and **2** appear to exert their hypoglycemic effect by disrupting the transport of monosaccharides across the intestinal wall.[3] Attaching biguanide groups onto monosaccharides resulted in complete loss of activity (cf. **7**, **19**, and **24**), except in the case of **31**, which has a pendant arabinose-derived tetrahydrofuran ring. Compounds **8** and **23** produced hypoglycemic activity which was nearly equivalent to that of **1** and **2**.[17]

Experimental Section

General Procedures. ^{1}H NMR spectra were recorded on either a Varian EM-390 (90 MHz), Bruker AM-360WB (360 MHz), or Bruker AM-400 (400 MHz) spectrometer. ^{13}C NMR spectra were recorded on a JEOL FX-60Q (15.0 MHz) or Bruker AM-360WB (100 MHz) spectrometer. For NMR work, $CDCl_3$ was used as a solvent unless otherwise noted, and tetramethylsilane was used as an internal standard. Elemental analyses were performed primarily by Galbraith Laboratories (Knoxville, TN). Melting points were determined in open capillary tubes with a Thomas-Hoover apparatus and are corrected. Chemical-ionization mass spectra (CI-MS) were recorded on a Finnigan 3300-6100 system with methane as the reagent gas. Fast-atom-bombardment mass spectra (FAB-MS) were obtained on a VG 7070E spectrometer. An Ion Tech saddlefield gun, which generated a primary beam of argon atoms at 8 keV and 2 mA, was used for the FAB analysis. Where analyses are reported by symbols of elements, the results were within 0.4% of the calculated values.

6-Amino-1,2:3,4-bis-*O*-(1-methylethylidene)-6-deoxy-α-D-galactopyranose Hydrochloride (4). Alcohol **3** (25 g, 96.0 mmol) and *p*-toluenesulfonyl chloride (21 g, 109.3 mmol) were dissolved in 300 mL of a 2:1 mixture of pyridine and acetonitrile. After 6 h, the solution was added to ether, washed three times with water and once with 0.2 N HCl, concentrated, and triturated with EtOAc/hexane 1:10 to give 31.96 g of tosylate product as a white solid (80% yield). The tosylate (42.3 g, 0.102 mol) was dissolved in 50 mL of DMSO and treated with sodium azide (13.3 g, 0.202 mol). The solution was heated at 115 °C for 24 h, during which time the sodium azide dissolved in the DMSO. After cooling, the mixture was added to water and the product was extracted into ether, washed three times with water, dried ($MgSO_4$), filtered and concentrated. This procedure resulted in 28.4 g of an oil, which was pure by TLC (98%). This oil was dissolved in EtOH and treated with 10% Pd/C (2 g). After shaking with hydrogen for 20 h (50 psig), the solution was filtered through Celite. The filtrate was concentrated, giving 23.3 g of the amine, pure by TLC. A portion of this amine (8 g) was converted into the HCl salt by treatment with HCl/ether (8.8 g): mp 210–228 °C dec; ^{1}H NMR (D_2O, 90 MHz) δ 1.48 (s, 6 h), 1.52 (s, 3 H), 1.58 (s, 3 H), 3.3 (d, 2 H, J = 5 Hz), 4.05–4.9 (m, 4 H), 5.8 (d, 1 H, J = 5 Hz); ^{13}C NMR (D_2O, 15.0 MHz) δ 23.8, 23.2, 24.8 (2 C), 39.7, 64.4, 69.7, 70.1, 71.0, 95.8, 109.9, 110.4. Anal. ($C_{12}H_{22}ClNO_5$) C, H, N.

6-[[[(Aminoiminomethyl)amino]iminomethyl]amino]-1,2:3,4-bis-*O*-(1-methylethylidene)-6-deoxy-α-D-galactopyranose Hydrochloride (6). Amine **4** and dicyandiamide (**5**) were carefully mixed together and heated under nitrogen. At 170–180 °C, the bulk of the material melted into a viscous oil that became brown upon continued heating. After 1.3 h at 180 °C, the oil was cooled, dissolved into EtOH, decolorized with charcoal, and triturated with ether, producing **6** as white needles, pure by TLC (3.62 g, 45%): mp 180–183 °C; $[\alpha]^{23}_D$ 38.5° (*c* 0.12, D_2O); IR (KBr) ν_{max} 3280, 3180, 2992, 2940, 1634, 1548, 1385, 1260, 1214, 1168, 1066 cm^{-1}; ^{1}H NMR (D_2O, 90 MHz) δ 1.32 (s, 6 H), 1.44 (s, 3 H), 1.50 (s, 3 H), 3.3–3.5 (m, 2 H), 3.72–4.7 (m, 4 H), 5.57 (d, 1 H, J = 5 Hz); ^{13}C NMR (D_2O, 15.0 MHz) δ 23.3, 24.0, 24.9 (2

(14) Maryanoff, B. E.; Nortey, S. O.; Inners, R. R.; Campbell, S. A.; Reitz, A. B.; Liotta, D. *Carbohydr. Res.* **1987**, *171*, 259.
(15) Olah, G. A.; Narang, S. C. *Tetrahedron* **1982**, *38*, 2225.
(16) Caldwell, G. W.; Reitz, A. B.; Masucci, J. A. *Org. Mass. Spectrom.* In press.

(17) We do not know whether **8** or **23** have decreased liability to cause lactic acidosis relative to **1** and **2**. The fact that the potency of **8** and **23** is not increased significantly relative to **1** and **2**, which already require doses of 50–100 mg/kg, lessened our interest in **8** and **23**.

Table I. Effect of Oral Administration of Compounds on Oral Glucose Tolerance in Fasted Nondiabetic Rats[a]

compd	dose, mg/kg	N	blood glucose at min after glucose load: 0	blood glucose at min after glucose load: 30	glucose rise	% inhibn of glucose rise
vehicle (no glucose)		28	72 ± 1	82 ± 1	10 ± 1	
vehicle (with glucose)		64	69 ± 1	137 ± 2	68 ± 2[b]	
phenformin (1)	10	3	61 ± 2	129 ± 5	68 ± 6	0
	50	9	63 ± 1	98 ± 2	35 ± 2[c]	57
	100	8	70 ± 2	101 ± 3	31 ± 3[c]	64
	150	9	58 ± 2	84 ± 3	26 ± 3[c]	71
	300	6	70 ± 3	91 ± 5	21 ± 4[c]	81
metformin (2)	100	15	68 ± 2	113 ± 3	45 ± 3[c]	41
6	100	3	67 ± 1	141 ± 4	74 ± 4	−12
7	100	4	68 ± 3	145 ± 4	77 ± 6	−14
8	100	3	78 ± 5	118 ± 6	40 ± 6[c]	48
	200	3	78 ± 1	121 ± 8	43 ± 8[c]	45
15	100	4	67 ± 2	152 ± 6	85 ± 4	−29
19	100	4	72 ± 3	133 ± 3	61 ± 5	12
23	100	3	82 ± 1[c]	123 ± 6	41 ± 6[c]	47
24	100	4	74 ± 2	137 ± 1	63 ± 3	9
27	100	4	76 ± 4	139 ± 6	63 ± 1	9
31	100	3	74 ± 3	127 ± 13	53 ± 14[c]	26

[a] Values are mean ± SEM (mg/dL) of n rats per treatment group. Percent inhibition of glucose rise was calculated as described in the Experimental Section. Oral glucose load was 1.0 g/kg. [b] $p < 0.001$ vs vehicle group without oral glucose load. [c] $p < 0.05$ vs vehicle group with oral glucose load.

C), 41.7, 66.6, 70.1, 70.2, 70.9, 95.9, 109.9 (2 C), 160.0, 169.9. Anal. ($C_{14}H_{26}ClN_5O_5 \cdot 0.3H_2O$) C, H, N, H_2O.

6-[[[(Aminoiminomethyl)amino]iminomethyl]amino]-6-deoxy-β-D-galactopyranose Hydrochloride Hydrate (7). Compound **6** (1.9 g, 4.94 mmol) was dissolved in 60 mL of 0.1 N HCl and stirred at 70 °C. After 16 h, the water was neutralized by addition of Amberlite IR-45 resin (until pH ~3), and the solution was filtered and the water was removed by evaporation, giving a lightly yellow powder. This material was recrystallized from MeOH/ether to give a white powder, which was dried under vacuum at ambient temperature (0.88 g, 51%): mp 73–107 °C dec; IR (KBr) ν_{max} 3400, 1740, 1640, 1615, 1540, 1080 cm^{-1}; ^1H NMR (D_2O, 360 MHz) δ 3.15–4.0 (m, 6 H), 4.32 (d, J = 7.5 Hz, β-pyranose anomeric proton, 0.65 H), 5.02 (d, J = 4.0 Hz, α-pyranose anomeric proton, 0.35 H); the presence of a trace amount of MeOH was detected; ^{13}C NMR (D_2O, 15.0 MHz) δ 42.8, 68.2, 69.1, 71.7, 72.6, 92.4 (α isomer, 0.35 C), 95.5 (β isomer, 0.65 C), 157, 158. Anal. ($C_8H_{17}N_5O_5 \cdot 1.5HCl \cdot 1.4H_2O \cdot 0.2MeOH$) C, H, N, Cl, H_2O.

1,2:3,5-Bis-O-(1-methylethylidene)-6-(p-tolylsulfonyl)-α-D-glucofuranose (10). A solution of 1,2-isopropylidene-α-D-glucofuranose (100 g, 0.45 mol) and 50 mg of 4-pyrrolidinopyridine in a 5:3:2 mixture of ether/pyridine/chloroform was cooled to −10 °C and treated with 104 g of tosyl chloride (0.55 mol). The solution was allowed to warm to ambient temperature and stir for 4 days. The majority of the solvent was removed, and the residual pyridine was azeotropically evaporated with toluene. The resulting substance was dissolved in acetone (500 mL), and $FeCl_3$ (140 g), $MgSO_4$ (40 g), and dried, 3-Å molecular sieves (50 g) were added. The solution was refluxed for 4 days, cooled, and treated with 100 mL of 10% Na_2CO_3. The solution was filtered, and the filtrate was treated with ether, washed eight times with water, dried ($MgSO_4$), filtered, and concentrated. This material was purified on two Waters Prep-500 columns (EtOAc/hexane, 1:3) to give 20.2 g of **10**, pure by TLC (11%): ^{13}C NMR (15.0 MHz) δ 23.9, 21.7, 23.9, 26.6, 27.3, 69.9 (C 6), 70.3, 74.5, 78.8, 84.0, 101.2, 106.6, 112.5, 129.9 (2 C), 128.2 (2 C), 133.4, 144.8.

6-[[[(Aminoiminomethyl)amino]iminomethyl]amino]-1,2:3,5-bis-O-(1-methylethylidene)-6-deoxy-α-D-glucofuranose Hydrochloride Hydrate (8). A solution of **10** (20 g, 0.05 mol) in DMSO (60 mL) was treated with 8.0 g of sodium azide. The slightly yellow solution was heated at 110–115 °C overnight. After heating for 20 h, the heat was removed and the solution was treated with water. The product was extracted into ether. The ether layer was washed twice with water, dried ($MgSO_4$), filtered, and concentrated to give 15.3 g of yellow oil, nearly pure by TLC. This material was dissolved into 50 mL of EtOH, treated with 1.3 g of 10% Pd/C, and set shaking on a Parr apparatus under 50 psig of hydrogen. After 2 days, the solution was filtered, the catalyst was washed with EtOH, and the solvent was removed to give **13** as an oil (13.7 g). This material was treated with HCl in ether to precipitate 10.9 g of the HCl salt (74% from **10**): FAB-MS m/e 260 (M + 1), 282 (M + Na); ^1H NMR (DMSO-d_6, 400 MHz) δ 1.23 (s, 3 H), 1.30 (s, 3 H), 1.32 (s, 3 H), 1.42 (s, 3 H), 3.0 (m, 2 H), 3.82 (m, 1 H), 4.20 (d, 1 H), 4.28 (m, 1 H), 4.59 (m, 1 H), 5.99 (d, 1 H), 8.3 (br s, 3 H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 23.6, 24.2, 26.5, 27.0, 68.7, 74.5, 79.4, 83.2, 100.8, 105.9, 111.5. This salt (10.36 g, 35 mmol) and **5** (10.08 g, 120 mmol) were suspended in 10 mL of DMSO, and the mixture was heated at 90–100 °C. After heating for 2 days, an additional 3.0 g (36 mmol) of **5** was added, and after another day, 1 g more of **5** (12 mmol) was added. After a total of 4 days, the solution was cooled and treated with ether, and the yellow solid that resulted was dissolved in water and lyophilized. The resulting powder was chromatographed on 500 g of dry silica gel ($CHCl_3$/MeOH/HOAc, 88:11:1), which produced 5.8 g of **8**, pure by TLC (40%). A portion (ca. 1 g) was recrystallized from $CHCl_3$/ether for the analytical sample. The product, as was true with most of the biguanides prepared, was very hygroscopic: mp 108–128 °C (bubbling); IR (KBr) ν_{max} 3320, 3220, 1640, 1633, 1564, 1370, 1250, 1180, 1090, 1070 cm^{-1}; ^1H NMR (D_2O, 360 MHz) δ 1.10 (s, 3 H), 1.13 (s, 3 H), 1.16 (s, 3 H), 1.23 (s, 3 H), 3.2 (m, 2 H), 3.54 (d, 1 H, J = 4.3 Hz), 4.15 (m, 2 H), 4.49 (d, J = 3.3 Hz), 5.86 (d, J = 3.3 Hz); ^{13}C NMR (D_2O, 15.0 MHz) δ 15.7 (2 C), 17.9, 18.4, 35.9, 62.9, 66.9, 72.5, 75.6, 94.4, 98.3, 105.7, 152.4 (br; other biguanide carbon broadened into the base line). Anal. ($C_{14}H_{25}N_5O_5 \cdot 1.5HCl \cdot 0.8H_2O$) C, H, N, Cl; H_2O; calcd, 3.49; found, 2.19.

1-Amino-1-deoxy-2,3:4,5-bis-O-(methylethylidene)-β-D-fructopyranose Hydrochloride (18). A solution of **17** (40 g, 0.154 mol) and p-toluenesulfonyl chloride (33.8 g, 0.178 mol) in 50 mL of pyridine was stirred for 2 days. The solution was treated with water and stirred an additional 0.5 h. The product was extracted into ether, washed with water, dried ($MgSO_4$), filtered, and concentrated to a yellow oil (64.6 g). The majority of this material (ca. 64 g) was dissolved in 80 mL of DMSO and treated with sodium azide (20 g) and heated at 110 °C overnight. The temperature was increased to 150–160 °C, and the reaction was allowed to proceed another day. The solution was cooled; saturated aqueous NaCl and ether were added, and the product was extracted into the ether layer. After drying ($MgSO_4$), the solvent was removed to give 35.4 g of an oil, which was immediately dissolved in 250 mL of EtOH and shaken with hydrogen (50 psig) and 4 g of 10% Pd/C. After 1 day, the solution was filtered and treated with saturated HCl in ether, and the precipitate was collected and dried to give 31.2 g of yellow powder (59% from **17**). Some of this powder (11.5 g) was recrystallized from methylene chloride/ether to give 10.0 g of white powder, which was dried overnight under vacuum at 40 °C: mp 159–173 °C dec; IR (KBr) ν_{max} 3420, 2990, 2970, 1600, 1510, 1470, 1400, 1260, 1240, 1040 cm^{-1}; CI-MS m/e 261 (M + 1), 289 (M + 29);

^{1}H NMR (D_2O, 90 MHz) δ 1.40 (s, 3 H), 1.43 (s, 3 H), 1.50 (s, 3 H), 1.58 (s, 3 H), 3.3 (s, 2 H), 3.8 (m, 2 H), 4.3–4.8 (m, 4 H). Anal. ($C_{12}H_{22}ClNO_5$) C, H, N.

1-[[[(Aminoiminomethyl)amino]iminomethyl]amino]-1-deoxy-2,3:4,5-bis-*O*-(1-methylethylidene)-β-D-fructopyranose Hydrochloride Hydrate (15). Compounds 18 (13.0 g, 44.07 mmol) and 5 (3.44 g, 40.9 mmol) were dissolved in 6.5 mL of DMSO and heated under a drying tube at 105 °C for 30 h. The brown solution was cooled, dissolved in EtOH, and dried to give a tacky, buff solid. This substance was recrystallized from methylene chloride/ether, with charcoal to remove most of the color, to yield 13 g of tan powder (75%). In order to remove the minor amount of 18 (ca. 2%) still visible by TLC, the material was further recrystallized from 2-propanol/ether to give 3.04 g of 15: mp 115–122 °C (bubbling); FAB-MS *m/e* 344 (M + 1); IR (KBr) ν_{max} 3340, 3240, 1640, 1550, 1380, 1260, 1250, 1080 cm^{-1}; ^{1}H NMR (D_2O, 90 MHz) δ 1.38 (s, 6 H), 1.47 (s, 3 H), 1.51 (s, 3 H), 3.52 (s, 2 H), 3.7–4.6 (m, 5 H). Anal. ($C_{14}H_{25}N_5O_5$·1.1HCl·0.5H_2O) C, H, Cl; N: calcd, 17.84; found, 18.79.

Methyl 6-Amino-6-deoxy-2,3,4-*O*-tris(phenylmethyl)-α-D-glucopyranoside Hydrochloride (22). A solution of methyl α-D-glucopyranoside (62.48 g, 0.321 mol), chloro-*tert*-butyldiphenylsilane (92.8 g, 0.337 mol), and imidazole (21.9 g, 0.337 mol) in 50 mL of DMF was stirred under a drying tube at 0 °C and then at room temperature.[18] After 2.5 days, water was added, and the product was extracted into methylene chloride, washed five times with water, dried ($MgSO_4$), filtered, and treated with 1.2 volumes of hexane, whereupon a voluminous precipitate emerged. This was collected and dried 3 days under vacuum to yield 91 g of white solid. A considerable portion of this material (75 g, 0.174 mol) was dissolved in 130 mL of DMF and treated with 60% NaH in an oil dispersion (24.4 g, 0.61 mol). After ca. 5 min of bubbling, benzyl bromide (64.2 mL, 0.54 mol) was added at ca. 5 °C, and the solution was allowed to stir at room temperature overnight. Water was then carefully added, and the product was extracted into ether, washed twice with water, dried ($MgSO_4$), filtered, and concentrated. The resulting solid was dissolved in 150 mL of THF and treated with 1 M tetrabutylammonium fluoride/THF (180 mL). After stirring of the reaction mixture overnight, TLC showed disappearance of the silylated intermediate and formation of a more polar product. The solution was treated with water, and the product was extracted into EtOAc, washed with water, 1 N HCl, and H_2O, dried ($MgSO_4$), filtered, and concentrated. The product was purified on two Waters Prep-500 HPLC columns (EtOAc/hexane, 15:85) to give 68.3 g (0.148 mol) of 21 (56% from 20), pure by TLC. This was dissolved in 100 mL of THF and treated with phthalimide (22.7 g, 0.15 mol) and triphenylphosphine (40.3 g, 0.15 mol) and cooled to 5 °C. Diethyl azodicarboxylate (25.4 mL, 0.16 mol) was added dropwise for 40 min. After stirring overnight at room temperature, the material was treated with ether, washed with water, 1 N NaOH, and saturated aqueous NaCl, dried ($MgSO_4$), filtered, and concentrated. The residue was dissolved into 100 mL of EtOH and treated with hydrazine hydrate (9 mL, 0.15 mol). After refluxing for 4 h, the solution was treated with 1:1 ether/EtOAc, washed with water and saturated aqueous $NaHCO_3$, dried ($MgSO_4$), filtered, and concentrated. The hydrochloride salt was prepared by treating a solution of the free base in 2:1 ether/hexane with saturated HCl in ether, giving 60 g of 22 (81% from 21): ^{1}H NMR (400 MHz) δ 3.34 (s, 3 H), 3.52 (s, 2 H), 3.63 (m, 1 H), 3.70 (dd, 1 H), 4.01 (t, 1 H), 4.52 (d, 1 H), 4.62 (dd, 2 H), 4.8 (m, 3 H), 5.00 (d, 1 H), 7.35 (m, 15 H).

Methyl 6-[[[(Aminoiminomethyl)amino]iminomethyl]amino]-6-deoxy-2,3,4-*O*-tris(phenylmethyl)-α-D-glucopyranoside Hydrochloride Hydrate (23). Compounds 22 (37.85 g, 76 mmol) and 5 (6.05 g, 72.2 mmol) were mixed together and heated to 155 °C. After 3 h, TLC of the brown liquid revealed that the reaction was over. The solution was cooled, dissolved in EtOH, treated with decolorizing carbon, and filtered. Trituration with ether and then $CHCl_3$/ether produced a brown powder, which was dried overnight under vacuum. This material was purified on 750 g of dry silica gel ($CHCl_3$/MeOH/HOAc, 80:15:5) to give a tacky solid, which was treated with toluene and concentrated (azeotropic removal of HOAc) and recrystallized from $CHCl_3$/ether to give 12.2 g of 23 (26%): mp 70–90 °C (softening), 90–95 °C (bubbling, melting); IR (KBr) ν_{max} 3320, 3240, 2900, 1620, 1540, 1090 cm^{-1}; FAB-MS *m/e* 548 (M + 1); ^{1}H NMR (D_2O, 360 MHz) δ 3.2–4.0 (m, 9 H), 4.3–5.0 (m, 10 H), 7.0–7.3 (m, 15 H); ^{13}C NMR (D_2O, 15.0 MHz) δ 42.3, 55.5, 69.5, 73.3, 80.4, 81.9, 98.0, 127–129 (15 C), 138.3, 138.5, 138.8, 157, 161. Anal. ($C_{30}H_{37}N_5O_5$·1.1HCl·1.2H_2O) C, H, N, Cl.

Methyl 6-[[[(Aminoiminomethyl)amino]iminomethyl]amino]-6-deoxy-α-D-glucopyranoside Hydrochloride Hydrate (19). Compound 23 (89.6 g, 0.014 mol) was dissolved in 80 mL of MeOH and treated with 5 g of 10% Pd/C and 2.2 mL of concentrated HCl. After shaking on a Parr apparatus for 1 day under 50 psig of hydrogen, the flask was removed, and the solution was filtered. The filtrate was triturated with ether, whereupon there emerged a white powder, which was dried at 40 °C under vacuum (4.35 g) and then recrystallized from EtOH/EtOAc to give 2.3 g of material pure by TLC (45%): IR (KBr) ν_{max} 3320, 1695, 1632, 1537, 1150, 1050 cm^{-1}; ^{1}H NMR (D_2O, 90 MHz) δ 3.3 (s, 3 H), 3.2–3.6 (m, 6 H), 4.7 (d, 1 H, *J* = 3 Hz); ^{13}C NMR (D_2O, 15.0 MHz) δ 43.5, 55.3, 69.5, 70.7, 71.0, 72.7, 99.3, 154.2, 155.0; FAB-MS *m/e* 278 (M + 1). Anal. ($C_9H_{19}ClN_5O_5$·2.1HCl·0.8H_2O) C, H, Cl; N: calcd, 19.02; found, 17.14.

1-[[[(Aminoiminomethyl)amino]thiocarbonyl]amino]-1-deoxy-2,3,4,6-tetra-*O*-acetyl-D-glucopyranose (26). A mixture of glycosyl isothiocyanate 25 (prepared by a literature procedure,[19] undetermined anomeric configuration; 21.55 g, 55.0 mmol), guanidine nitrate (79.42 g, 60.9 mmol), and triethylamine (8.5 mL, 60.8 mmol) in 700 mL of acetonitrile was stirred for 21 h at ambient temperature, and at 77 °C for 3 h. The solvent was then concentrated, and the brown residue was dissolved in $CHCl_3$, washed with water (5 × 200 mL), dried ($MgSO_4$), filtered, and concentrated to give a tacky solid, which was triturated with $CHCl_3$ and hexane to give 20.6 g of brown solid. This material was purified on a Waters Prep-500 HPLC using 1:9 MeOH/$CHCl_3$ to afford 12.7 g of tan solid (51%): FAB-MS *m/e* 449 (M + 1); ^{1}H NMR (400 MHz) δ 2.01 (s, 3 H), 2.03 (s, 3 H), 2.11 (s, 3 H), 2.13 (s, 3 H), 3.8–4.0 (m, 1 H), 4.2 (m, 2 H), 4.9–5.1 (m, 2 H), 5.2–5.4 (m, 1 H), 5.57 (t, 0.24 H, anomeric H of α isomer), 5.86 (d, 0.76 H, anomeric H of β isomer), 6.8–7.1 (br s, 5 H); ^{13}C NMR (100 MHz) δ 20.52 (2 C), 20.56, 20.81, 61.86, 62.28, 70.47, 73.27, 79.62 (0.21 C, anomeric C of α isomer), 81.64 (0.79 C, anomeric C of β isomer), 161.43 (ca. 0.8 C), 161.94 (ca. 0.2 C), 169.66, 169.91, 170.90, 171.55, 187.19 (ca. 0.3 C), 189.74 (ca. 0.7 C).

1-[[[(Aminoiminomethyl)amino](phenethylimino)methyl]amino]-1-deoxy-D-glucopyranose Hydriodide Hydrate (24). Compound 26 (7.1 g, 0.016 mol) was dissolved in 80 mL of MeOH and treated with 6 mL of ethyl iodide. The solution was refluxed for 3 h. The solvent was then removed, leaving a foam, which was dissolved in 100 mL of MeOH, and treated with 20 mL of phenethylamine. After 2.8 days, the solution was triturated with 300 mL of ether and 50 mL of hexane. A yellow oil emerged, the solvent was decanted, and ca. 1.9 g of yellow powder was formed upon drying. This was recrystallized from EtOH/ether to give a buff powder (1.0 g) pure by TLC ($CHCl_3$/MeOH/HOAc, 5:4:1; 13% yield): mp 63–80 °C (softening), 81–129 °C (foaming), 130–142 dec °C; IR (KBr) ν_{max} 3400, 1610, 1090, 1040 cm^{-1}. ^{1}H NMR (D_2O, 90 MHz) δ 2.76 (t, 2 H), 3.2–3.8 (m, 8 or 9 H), 4.6 (anomeric H masked by HOD peak), 7.1–7.3 (s, 5 H); ^{13}C NMR (D_2O, 15.0 MHz) δ 34.4 (ca. 0.7 C), 34.8 (ca. 0.3 C), 42.9, 43.2 (CH_2N), 60.6, 63.6 (CH_2OH), 68.2, 69.5, 72, 73 (1 C), 76–78 (2 C), 82.2 (ca. 0.3 C, anomeric C of α isomer), 86.2 (ca. 0.7 C, anomeric C of β isomer), 126.4, 126.8, 128–129 (4 C), 133.3, 138.6. Anal. ($C_{16}H_{25}N_5O_5$·0.6HI·1.5H_2O) C, H, N, I.

1-[[[(Aminoiminomethyl)amino]thiocarbonyl]amino]-1-deoxy-D-glucopyranose Hemifumarate Hydrate (27). To a stirred solution of compound 26 (9.89 g, 22.0 mmol) and mercury oxide (5.73 g, 27.0 mmol) in 100 mL of MeOH at 0 °C was bubbled dimethylamine gas for 30 min. The reaction mixture was stirred at 0 °C for 2 h and at ambient temperature for 18 h. After 18 h, TLC (9:1, $CHCl_3$/MeOH) showed complete disappearance of 26 with the formation of an origin spot. The reaction mixture

(18) Hanessian, S.; Lavallee, P. *Can. J. Chem.* **1975**, *53*, 2975.

(19) Johnson, T. R.; Bergmann, W. *J. Am. Chem. Soc.* **1932**, *54*, 3360.

was filtered to remove mercury salts, concentrated under vacuum, dissolved in water, and washed several times with $CHCl_3$. The aqueous solution was lyophilized to give 5.5 g of crude yellow solid. Most (5.0 g) of the crude product was reacted with fumaric acid in MeOH, and the resultant salt was precipitated from solution with ether. The salt was recrystallized from water to give 1.57 g of white crystalline solid (18%): mp 149–151 °C; IR (KBr) ν_{max} 3314, 2906, 1710, 1658, 1548, 1376, 1281, 1186, 1076 cm^{-1}; FAB-MS m/e 281 (M + 1); ^1H NMR (D_2O, 90 MHz) δ 3.4–3.9 (m, 6 H), 5.4–5.6 (m, 1 H), 6.6 (s, 1.2 H, 0.6 molar equiv of fumaric acid); ^{13}C NMR (DMSO-d_6, 15.0 MHz) δ 60.9, 70.0, 72.7, 77.6, 78.0, 80.9 (ca. 0.6 C, α anomer), 83.2 (ca. 0.4 C, β anomer), 134.1 ($CHCO_2H$), 160.5 (0.4 C), 161.8 (0.6 C), 166.4 (CO_2H), 186.1 (0.6 C), 187.7 (0.4 C). Anal. ($C_8H_{16}N_4O_5S \cdot 0.6C_4H_4O_4 \cdot 2.0H_2O$) C, H, N.

1-[2-[[[(Aminoiminomethyl)amino]iminomethyl]amino]-ethyl]-1-deoxy-D-arabinose Hydrochloride Hydrate (31). To a solution of nitrile **32**[13] (18.20 g, 0.041 mol) in THF at 0 °C under nitrogen was added 41.0 mL (0.041 mol) of 1 M BH_3/THF. The ice bath was then removed and the reaction was refluxed for 5.5 h. An additional 28.0 mL of 1 M BH_3/THF (0.028 mol) was then added, and the solution was refluxed for an additional 18 h. The excess BH_3 was carefully destroyed by adding ice. The THF was removed to give a white oil suspended in water. The solution was acidified with 100 mL of 0.1 N HCl and heated for 1.5 h. The resulting HCl salt was neutralized with 3.0 N NaOH and extracted into ether. The ether extracts were dried ($MgSO_4$), filtered, and concentrated to yield 15.9 g of **33**. This free base was redissolved in ether and then acidified with ether saturated with HCl to afford 17.0 g of viscous oil (86%): CI-MS m/e 448 (M + 1), 476 (M + 29). To 21.96 g (45.3 mmol) of this amine hydochloride in DMSO (15 mL) under nitrogen was added **5** (19.07 g), and the slurry was heated at 115 °C for 7 days. The reaction mixture was dissolved in $CHCl_3$, filtered, washed with saturated aqueous NaCl, dried ($MgSO_4$), filtered, and concentrated to a brown oil. This material was triturated with ether, to facilitate removal of residual DMSO. The crude product (25.8 g) was chromatographed on dry silica gel ($CHCl_3$/MeOH/HOAc, 89:7:4) to give 9.30 g (37%) of **34**, as a brown syrup: CI-MS m/e 538 (M + 1). To 8.55 g (15.0 mmol) of **34** in methylene chloride (50 mL) at 0 °C under nitrogen was added iodotrimethylsilane (17.5 mL, 0.123 mol) via a syringe. The reaction was allowed to stir at 0 °C. After 1.5 h, the reaction was quenched with water and washed with additional methylene chloride. The aqueous phase was treated with Amberlite CG-400 anion-exchange resin (chloride anion form), filtered, treated with decolorizing carbon, filtered, and lyophilized to give 3.56 g of slightly yellow solid. Since analysis of the FAB-MS and 90-MHz ^1H NMR spectrum revealed that ca. 25% of the product still retained a benzyl group on it, the reaction was completed by catalytic hydrogenolysis of the remaining benzyl group. Thus, 1.26 g of this material was dissolved in water (50 mL), treated with 2 g of 10% Pd/C, and shaken for 2 days under 50 psig hydrogen. The catalyst was filtered and the aqueous mixture was lyophilized to yield 650 mg of tacky, white solid, pure by TLC (33%): mp 36–111 °C (softening), 111–116 °C (foaming); IR (KBr) ν_{max} 3872, 3388, 2926, 2182, 2162, 1641, 1554, 1036 cm^{-1}; FAB-MS m/e 262 (M + 1), 284 (M + Na); ^1H NMR (D_2O, 90 MHz) δ 1.7 (m, 2 H), 3.2 (t, 2 H), 3.5 (m, 2 H), 3.8 (m, 3 H). Anal. ($C_9H_{19}N_5O_4 \cdot 1.9HCl \cdot 2.0H_2O$) C, H, Cl; N: calcd, 19.10; found, 17.40.

Glucose-Tolerance Test. Male Sprague–Dawley rats (200–275 g), obtained from Charles River Breeding Laboratories (Wilmington, MA), were maintained on a regular 12 h light/dark cycle with standard rodent chow and water provided ad libitum. Rats were deprived of food for 18 h prior to glucose tolerance testing. Groups of rats were orally dosed at approximately 9 a.m. with either vehicle or test drugs 60 min prior to an oral glucose load (1.0 g/kg). Separate groups of rats received vehicle without glucose challenge. Blood samples were obtained from the tail vein (tail cut method) just before (0 min) and 30 min following glucose administration. Blood glucose was determined by the glucose oxidase method (Autoflo, Boehringer-Mannheim) on whole blood immediately deproteinized with $Ba(OH)_2$ and $ZnSO_4$. Test compounds were prepared either as a 0.5% (w/v) methylcellulose (Fisher Scientific) suspension or as an aqueous solution with control rats receiving the same vehicle as drug-treated rats. The rats were dosed based on the mean body weight per treatment group and all doses were calculated on a salt-free basis. Data are presented as the mean ± SEM for n rats per treatment group. The incremental change in glucose (mg/dL) from the t = 0 min value, in response to the glucose load, was calculated for each rat and corrected by subtracting the glucose change in vehicle-treated rats which did not receive glucose. The mean change in glucose for each drug-treated group (ΔG_D) was statistically compared to that of vehicle-treated controls (ΔG_V) with ANOVA and Dunnett's t test with a p value of 0.05 or less taken as significant. Percent inhibition of the glucose increase by each drug was calculated as $100(\Delta G_V - \Delta G_D)/(\Delta G_V)$.

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Evaluation and Synthesis of Aminohydroxyisoxazoles and Pyrazoles as Potential Glycine Agonists

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Except for structurally similar small amino acids, such as alanine, β-alanine, and serine, compounds acting as glycine-receptor agonists are an unknown class of pharmacological agents. To investigate the potential of small, substituted heterocycles to act as glycine agonists, we have evaluated the similarities between glycine and a series of hydroxy- and amino-substituted pyrazoles and isoxazoles through complementary molecular modeling techniques. Using a "scorecard approach" to determine the overall similarity of projected agonist structures to glycine, we prioritized synthesis and subsequently prepared several novel derivatives. The biological activity of these compounds was compared to that of glycine by using a [^3H]strychnine-mediated glycine receptor binding assay. Despite the close similarity in the calculated parameters when compared to glycine, no significant receptor-binding activity was observed for the targeted analogues. These results illustrate the structurally exacting nature of the glycine receptor.

Glycine, the structurally least complex of the naturally occurring amino acids, is a major inhibitory neurotransmitter located primarily in the spinal cord.[2] Activation of the glycine receptor complex by its endogenous ligand

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(2) Aprison, M. H.; Daly, E. C. *Adv. Neurochem.* **1978**, *3*, 205.

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