Synthesis and Biological Activity of Aminoguanidine and Diaminoguanidine Analogues of the Antidiabetic/Antiobesity Agent 3-Guanidinopropionic Acid

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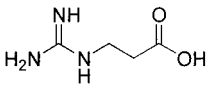
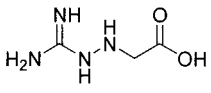
3-Guanidinopropionic acid (**1**) has been demonstrated both to improve insulin sensitivity and to promote weight loss selectively from adipose tissue in animal models of non-insulin-dependent diabetes mellitus (NIDDM). However, **1** has also been shown to be a substrate for both the creatine transporter and creatine kinase, leading to marked accumulation in muscle tissue as the corresponding *N*-phosphate. The corresponding aminoguanidine analogue **2** was recently discovered to retain the antidiabetic activity of **1** while being markedly less susceptible to creatine-like metabolism, suggesting that it should have less potential to accumulate in muscle. Further structural modification of **2** was undertaken to investigate whether the antidiabetic potency could be augmented while maintaining resistance to creatine-like metabolism. Modifications such as α-alkylation, homologation, and bioisosteric replacement of the aminoguanidine all were detrimental to antidiabetic activity. However, the simple regioisomeric aminoguanidinoacetic acid **9** and diaminoguanidinoacetic acid analogue **7** were found to be equipotent to **2**, leading eventually to the discovery of the significantly more potent diaminoguanidinoacetic acid regioisomers **52** and **53**. Further attempts to modify the more active template represented by **52** led only to reductions in antidiabetic activity. Each of the new active analogues displayed the same resistance to creatine-like metabolism as **2**. Further testing of **7**, **9**, and **53** in obese diabetic *ob/ob* mice confirmed that weight loss is induced selectively from adipose tissue, similar to the lead **1**. Administration of **53** to insulin-resistant rhesus monkeys led to reductions in both fasting and post-prandial plasma glucose levels with concomitant reductions in plasma insulin levels, suggesting that the compound improved the action of endogenous insulin. Compounds **7** and **53** were selected for further preclinical development.

Introduction

3-Guanidinopropionic acid (**1**) has been demonstrated both to improve insulin sensitivity and to promote weight loss selectively from adipose tissue in animal models of non-insulin-dependent diabetes mellitus (NIDDM).[1,2] However, **1** has also been shown to be a substrate for both the creatine transporter and creatine kinase, leading to marked accumulation in muscle tissue as the corresponding *N*-phosphate with concomitant depletion of intracellular creatine phosphate.[3] In the preceding paper, we described an analogue program aimed at identifying novel, more potent compounds retaining the antidiabetic activity of **1** but lacking its ability to be incorporated into the creatine transport/kinase cycle. Although **1** proved to be highly intolerant of structural modification, the simple aminoguanidinoacetic acid analogue **2** was eventually discovered to be an equipotent antidiabetic agent that was significantly less susceptible to creatine-like metabolism, suggesting that it should be less prone to accumulate in muscle. A second phase of analogue work was thus launched with the goal of discovering more potent analogues of **2** which maintained this resistance to creatine-like metabolism.



1, PNU-10483



2, PNU-99022

Chemistry

Tables 1−8 depict the analogues that were prepared and evaluated for this study. Compounds **8**,[4] **9**,[5] and **18**[6] were prepared according to literature procedures. Compound **13** is an item of commerce.

Table 1 shows a variety of substituted analogues of **2**. Esterification of **2** with methanol and HCl provides **3**. α-Substituted analogues of **2** could be prepared from the α-bromo acids **83** (eq 1). Treatment with hydrazine produced the hydrazino acids[7] which could be guanylated by reaction with 2-methyl-2-thiopseudourea sulfate to give the α-substituted aminoguanidinoacetic acids **4**−**6**. Preparation of the amino-substituted analogue **7** could be accomplished by reaction of methyl

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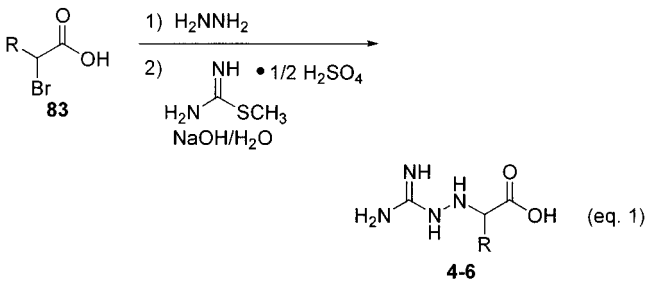
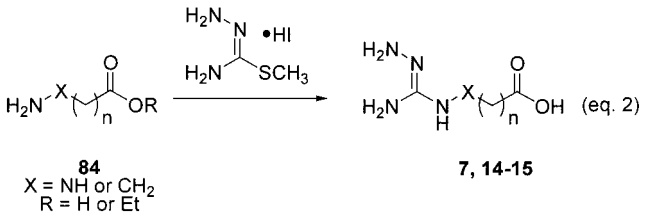
hydrazonothiocarbamate hydroiodide[8] with hydrazinoacetic acid (prepared in situ by saponification of ethyl-

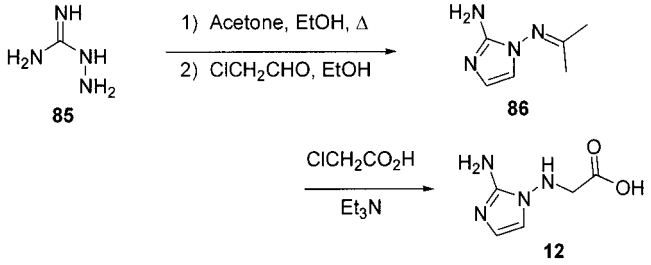


hydrazinoacetate hydrochloride (**84**, X = NH, R = OEt) (eq 2). Two regioisomeric analogues of **2** were also



prepared. Alkylation of aminoguanidine with 2-bromoacetic acid gave **10.** Saturation of the known triazinone **31**[6] by catalytic hydrogenation in aqueous HCl gave the cyclic analogue **11**. A heteroaromatic replacement of the aminoguanidine moiety was also investigated. The heterocycle was prepared by first protecting the terminal amino group of aminoguanidine as the corresponding isopropylidene imine, followed by reaction with 2-chloroacetaldehyde to give **86** (Scheme 1). The heterocycle was then simultaneously deprotected and alkylated with 2-chloroacetic acid to provide the desired analogue **12**.

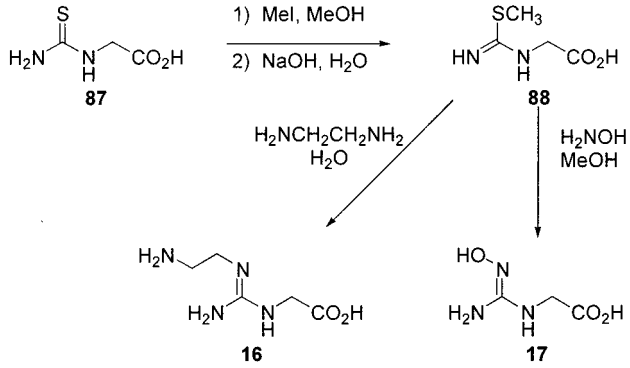
Scheme 1



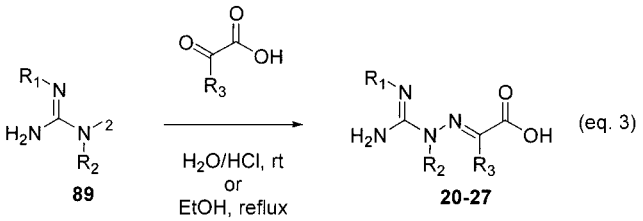
Analogues of the regioisomeric aminoguanidine **9** are shown in Table 2. Chain-extended analogues **14** and **15** were prepared by the procedure shown in eq 2 from the amino acids **84** (X = CH_2, R = H). Other analogues that varied the terminal amino-substituent on the guanidine of **9** could be prepared as shown in Scheme 2. The thiourea **87**[9] was alkylated with methyl iodide. Neutralization of the resulting salt with aqueous sodium hydroxide gave the desired isothiourea **88**. Reactions with 1,2-diaminoethane or hydroxylamine afforded the aminoethyl **(16)** and hydroxy **(17)** analogues, respectively.

Table 3 depicts a variety of imine analogues that were prepared. Esterification of **18** with methanol provided **19**. α-Substituted analogues **20**−**29** were also prepared according to literature procedures (eq 3).[10,11] Analogues substituted on one of the nitrogens of the guanidine

Scheme 2

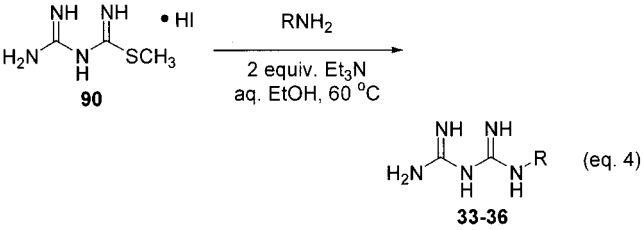


(25−**27)** could be prepared by condensation of the appropriately substituted aminoguanidine[12,13] with glyoxylic acid (R_3 = H) (eq 3). Condensation of 2-hydrazino-



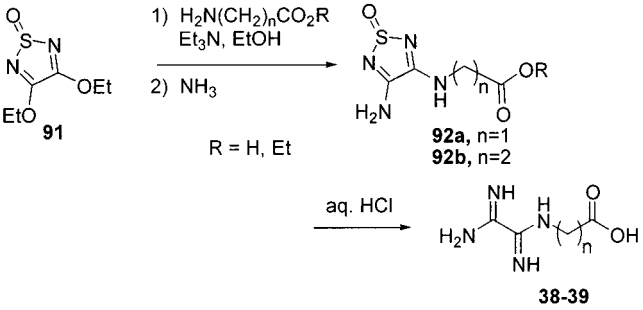
4,5-dihydro-1*H*-imidazole with chloral hydrate provided **29**. Condensation of diaminoguanidine with glyoxylic acid in water at room temperature provided the dimer **30**.

Biguanidine and oxamidine analogues related to **1** and **2** were prepared and are shown in Table 4. The biguanide transfer reagent **90** (eq 4) was prepared by



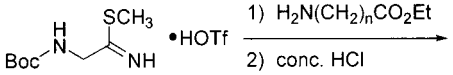
alkylation of iminothiobiuret with methyl iodide.[14] This compound was then condensed with glycine, *β*-alanine, 3-aminopropionic acid, and taurine to give the desired biguanide analogues **33**−**36**. Esterification of **34** provided **37**. The synthesis of the oxamidine analogues **38**−**39** is shown in Scheme 3. The starting material **91**, a thiadiazole 1-oxide, was prepared according to a literature procedure.[15] Addition of the requisite amino acid (or ethyl ester) to **91** followed by direct ammonolysis of

Scheme 3

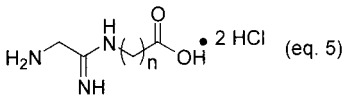


the intermediate afforded **92**. Hydrolysis of **92** with concentrated aqueous HCl afforded the desired amidines **38**−**39**. Esterification of **39** provided **40**.

Analogues in which the guanidine is replaced by an aminomethylamidine are shown in Table 5. Both possible isomers of these analogues were investigated. Analogues with a terminal amino group were prepared as shown in eq 5. The thioimidate **93** was prepared



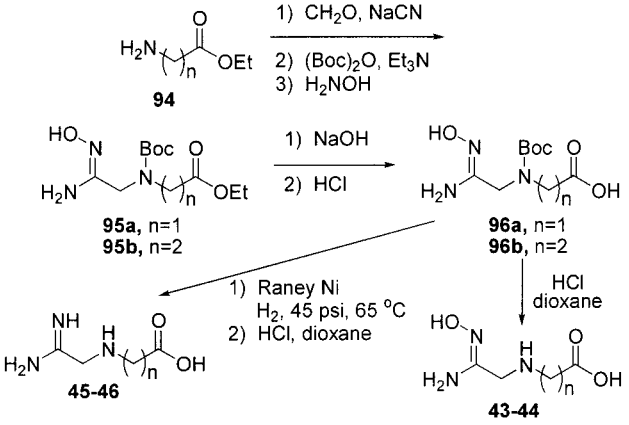
93



41-42

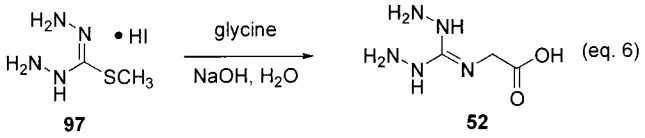
according to a literature procedure from glycinamide.[16] Treatment of **93** with glycine or β-alanine followed by deprotection with concentrated HCl gave the desired analogues **41**−**42** as bis-hydrochloride salts. The synthesis of the isomer in which the internal guanidine nitrogen of **2** was replaced by an aminomethyl group is summarized in Scheme 4. Glycine or β-alanine ethyl

Scheme 4



esters were formylated in the presence of sodium cyanide to prepare the corresponding nitriles[17] that were Boc protected and treated with hydroxylamine to give the hydroxyamidines **95**. Saponification of the esters provided the acids **96**. At this point, deprotection with HCl in dioxane furnished the α-aminohydroxyamidines **43**−**44**. Alternatively, hydrogenolysis of the N−O bond[18] and deprotection with HCl in dioxane gave the aminomethylamidine analogues **45**−**46**.

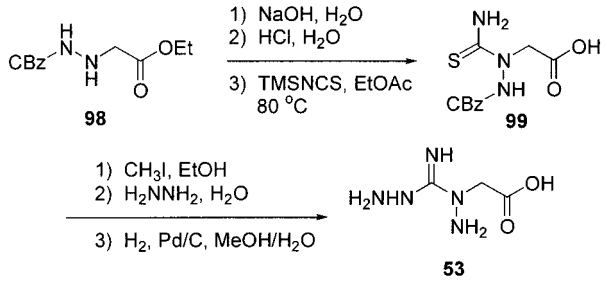
Additional diaminoguanidine analogues such as substituted analogues, a cyclic analogue, an analogue with a heterocyclic replacement of the diaminoguanidine, and regioisomeric analogues are depicted in Table 6. Condensation of the diaminoguanidine **7** with acetone or benzaldehyde provided **47** and **48**, respectively. Reduction of **47** with sodium cyanoborohydride provided **49**.[19] A cyclic analogue of **7** was prepared by hydrogenation of the previously described cyclic imine **32**.[20] Alkylation of 4*H*-1,3,4-triazole-3,4-diamine with 2-chloroacetic acid afforded **51**. The regioisomeric diamoguanidinoacetic acid **52** was prepared by the reaction of glycine with the diaminoisothiourea **97** (prepared by methylation of thiocarbohydrazide, eq 6). The synthesis of the other



97 **52**

regioisomeric diamoguanidinoacetic acid **53** is depicted in Scheme 5. Starting from CBz-protected ethylhydrazinoacetate **98**,[21] saponification of the ester followed by

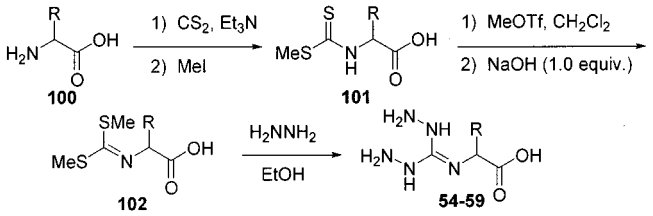
Scheme 5



treatment with trimethylsilyl isothiocyanate afforded the thiourea **99**. Alkylation with methyl iodide followed by displacement of methanethiol with hydrazine and deprotection of the benzyloxycarbonyl group afforded **53**.

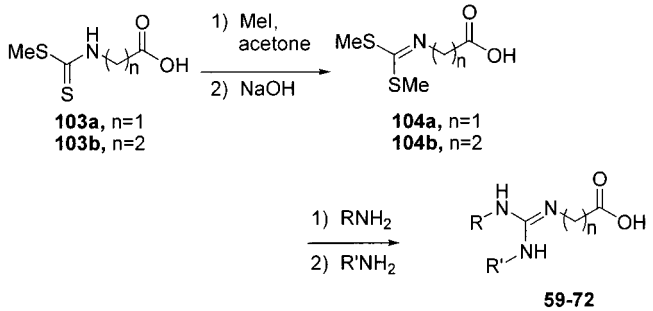
Analogues of the regioisomeric diaminoguanidinoacetic acid **52** are shown in Tables 7−8. α-Substituted analogues **54**−**58** were synthesized from the corresponding α-substituted amino acids **100** (Scheme 6).

Scheme 6



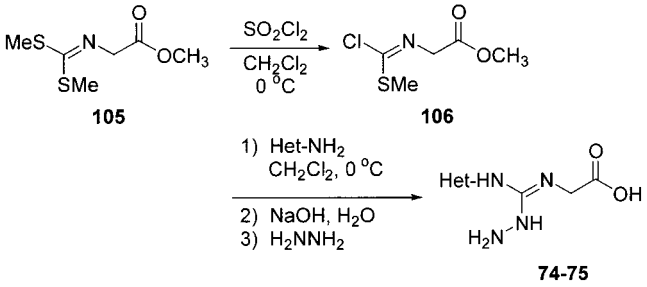
Treatment with carbon disulfide followed by alkylation with methyl iodide gave the dithiocarbamates **101**. Further alkylation with methyl triflate and neutralization of the resulting salt led to the bis(methylthio)imines **102**. Displacement of both methanethiol groups with excess hydrazine gave the desired α-substituted analogues **54**−**58**. Analogues with variable chain lengths and substituted analogues could be prepared as shown in Scheme 7. Methylation of the known dithiocarbamates **103**[22] with methyl iodide in acetone followed by

Scheme 7



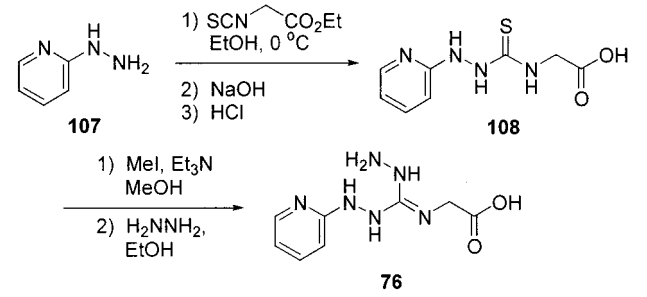
neutralization of the resulting salts gave the bis(methylthio)imines **104**. Exposure of **104** to excess hydrazine gave the chain-extended analogues **59**−**60**. Alternatively, sequential displacement of the methanethiol groups of the bis(methylthio)imine **104** (n = 1)[23] with an amine and hydrazine provided the monosubstituted analogues **61**−**64**. Treatment of the bis(methylthio)imine **104** (n = 1) with an excess of the appropriate amine provided the disubstituted analogues **65**−**72**. Deprotection of the bis-Boc-protected amino analogue **67** with HCl in THF gave the bis(aminoethyl) analogue **73**. For heterocycle-substituted analogues **74**−**75**, addition of the nucleophile to **104** was too sluggish to be useful. To facilitate these reactions, one of the methanethiol groups of **105** was converted to a chloride by treatment with sulfuryl chloride to give **106** (Scheme 8).[24] The chloride could then be selectively displaced by

Scheme 8



the less reactive heterocyclic amine. Saponification of the ester followed by treatment with excess hydrazine gave **74**−**75**. The third approach to the synthesis of substituted analogues is shown for the synthesis of **76** (Scheme 9). 2-Hydrazinopyridine was added to ethyl

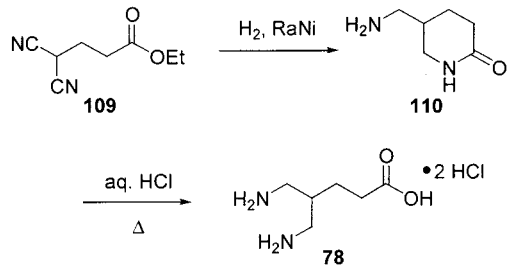
Scheme 9



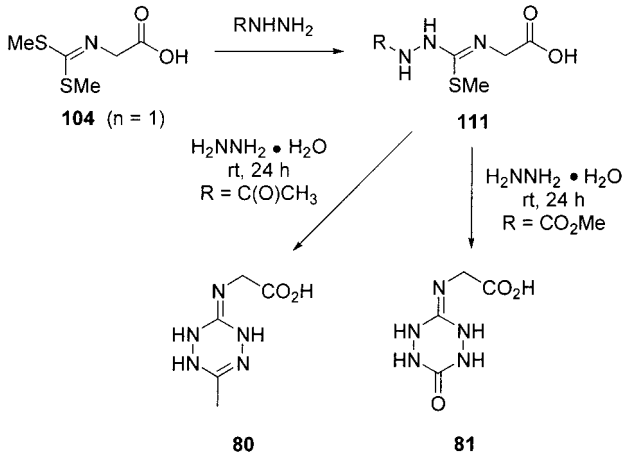
isothiocyanatoacetate. Saponification of the ester resulted in the thiosemicarbazide **108**. Alkylation with methyl iodide followed by treatment with excess hydrazine gave **76**. Condensation of **52** with excess 2,4-pentanedione[25] resulted in the formation of the bis(dimethylpyrazolyl) analogue **77**. The final analogue depicted in Table 8 is one which retains only the terminal amino groups, replacing the remainder of the diaminoguanidine moiety with carbon atoms. The synthesis of this compound is outlined in Scheme 10. Raney nickel reduction of the bis-nitrile **109**[26] afforded the lactam **110** which was opened by heating in aqueous HCl to afford the desired diamine **78**.

Conformationally restricted, cyclic diaminoguanidine analogues are shown in Table 9. Compound **79** was prepared as shown (eq 7) by the reaction of the bis(methylthio)imine **104a** with ethylene bis(hydrazine).[27]

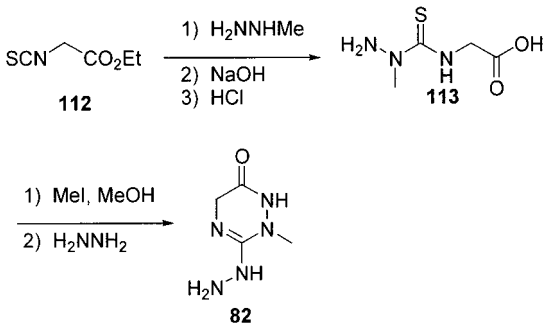
Scheme 10



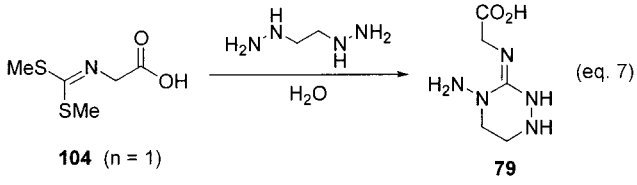
Scheme 11



Scheme 12

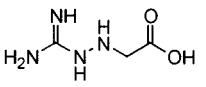
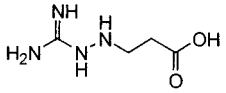
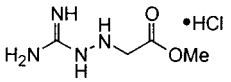
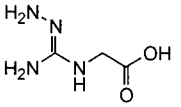
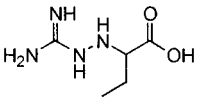
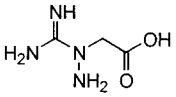
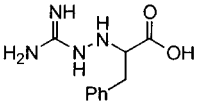
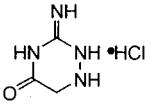
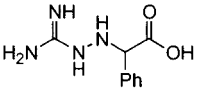
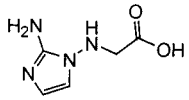
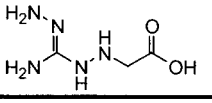


Through a two-step condensation, tetrazino analogues **80** and **81** could also be prepared from the bis(thiomethyl)imine **104a** (Scheme 11). Reaction of **104a** with



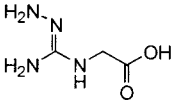
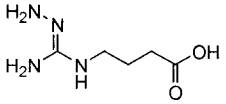
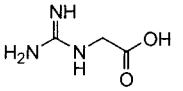
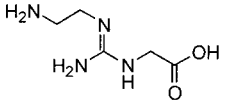
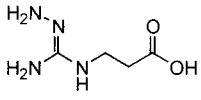
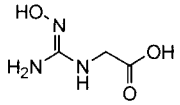
N-acetylhydrazine or methyl hydrazinocarboxylate gave the isothiosemicarbazide **111** (Scheme 11). Further reaction with hydrazine gave the cyclic analogues **80** and **81**. Finally, an additional cyclic analogue was prepared as shown in Scheme 12. Ethyl isothiocyanatoacetate was treated with methylhydrazine to give the corresponding thiosemicarbazide.[28] Saponification of the ester afforded **113**. Treatment with methyl iodide followed by treatment with excess hydrazine gave the cyclic analogue **82**.

Table 1. Substituted Aminoguanidine Analogues

Cpd	Structure	MISS T/C[a]	Cpd	Structure	MISS T/C[a]
2		0.61 (±0.29)**	**8**		1.11
3		1.12	**9**		0.51 (±0.24)***
4		1.08	**10**		1.04
5		0.87	**11**		1.08
6		1.11	**12**		0.99
7		0.69 (±0.22)***			

[a] Mouse insulin sensitizing screen nonfasting blood glucose levels (test/control); see Experimental Section: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Table 2. Isomeric Aminoguanidine Analogues

Cpd	Structure	MISS T/C[a]	Cpd	Structure	MISS T/C[a]
9		0.51 (±0.24)***	**15**		1.14
13		0.62 (±0.23)**	**16**		0.99
14		0.83	**17**		1.06

[a] Mouse insulin sensitizing screen nonfasting blood glucose levels (test/control); see Experimental Section: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

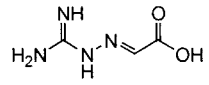
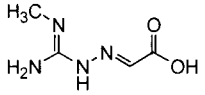
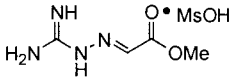
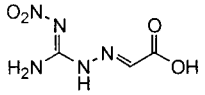
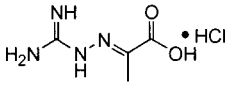
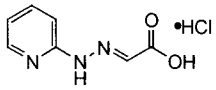
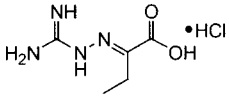
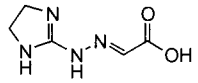
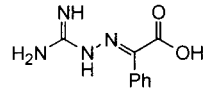
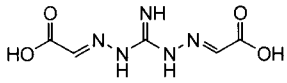
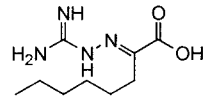
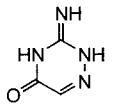
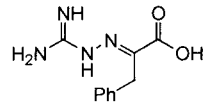
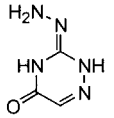
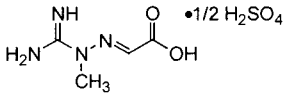
Biology

Antihyperglycemic Activity in KKA^y Mice. New analogues were evaluated in obese hyperglycemic, hyperinsulinemic, insulin-resistant KKA^y mice as previously described.[29] Animals were grouped into treatment and control groups ($n = 6$) following pretest blood glucose measurements with an Alpkem glucose autoanalyzer. Treatment groups had the selected compound administered as a food mixture at 500 mg/kg for 4 days. The glucose level for the treated group (T) versus the control group (C) was utilized to determine the antihyperglycemic activity of the test compounds. The results are summarized in Tables 1−9 as test/control (T/C) values. For those compounds with T/C < 0.80, the control values were statistically compared to the treatment values using the nonparametric Wilcoxon rank sum test. Those compounds determined to effect statistically significant reductions in nonfasting blood glucose are indicated with asterisks as defined in the tables.

The substituted analogues in Table 1 show that, as previously observed with **1**, even simple modifications of **2** were detrimental to activity. The methyl ester **3** was inactive as were the α-substituted analogues **4**−**6** and the one-carbon homologue **8**. Cyclization to the corresponding lactam **11** also led to a loss of activity, and incorporating the aminoguanidine into a cyclic structure **(12)** was unfavorable as well. In this series of substituted analogues, the only compounds that were found to retain antidiabetic activity were the isomeric aminoguanidine **9** and the diaminoguanidine **7.** It was intriguing to note that each of these analogues possessed a terminal amino group. It was around these compounds that we focused further SAR studies.

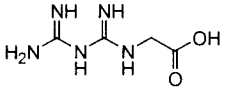
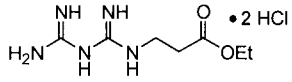
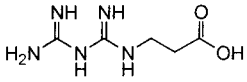
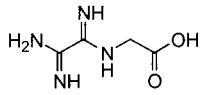
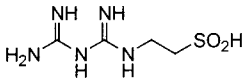
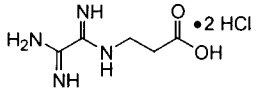
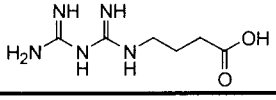
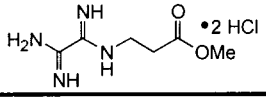
Table 2 shows analogues of the isomeric aminoguanidine **9**. Once again, most changes rendered the molecule inactive. The one- and two-carbon homologues **14** and **15** were inactive as was the compound in which the terminal amino group of **9** was removed by two carbons **(16).** Replacement of the terminal amino group with hydroxyl also resulted in an inactive analogue **(17)**. For comparison, guanidinoacetic acid **13** has been included in the table, a compound we previously found to have

Table 3. Imine Analogues

Cpd	Structure	MISS T/C[a]	Cpd	Structure	MISS T/C[a]
18		0.52 (±0.24)***	**26**		0.94
19		1.16	**27**		1.23
20		0.90	**28**		0.73 (±0.21)*
21		0.77 (±0.31)	**29**		1.01
22		1.04	**30**		0.88
23		0.73 (±0.27)	**31**		0.98
24		0.68 (±0.34)	**32**		0.84
25		0.95			

[a] Mouse insulin sensitizing screen nonfasting blood glucose levels (test/control); see Experimental Section: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Table 4. Biguanides and Amidines

Cpd	Structure	MISS T/C[a]	Cpd	Structure	MISS T/C[a]
33		1.01	**37**		1.06
34		0.88	**38**		1.08
35		0.92	**39**		1.10
36		1.05	**40**		1.00

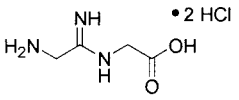
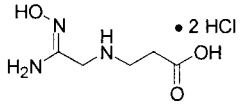
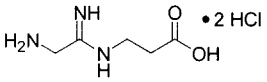
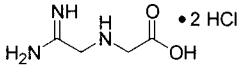
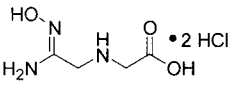
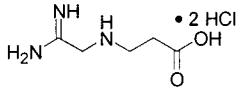
[a] Mouse insulin sensitizing screen nonfasting blood glucose levels (test/control);. see Experimental Section: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

reduced significance versus control due to poor reproducibility. On the basis of the decreased activity of **13** and the inactivity of **16** and **17**, we speculated that the terminal amino group on the guanidine functionality was a key component of the antidiabetic activity of **9**.

The analogues in Table 3 represent a series of imine analogues that were prepared. Although the unsubstituted analogue **18** and the heterocyclic analogue **28** did display statistically significant activity in the assay, these analogues were found to increase bilirubin levels in a nondiabetic C57 mouse and were not investigated further. Other modifications of the imine template, including α-substitution, substitution on the guanidine functionality and cyclization to the corresponding lactams led to a loss of activity.

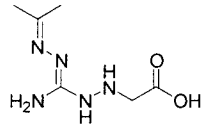
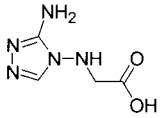
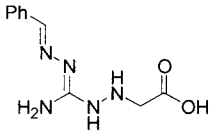
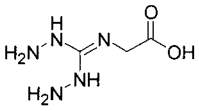
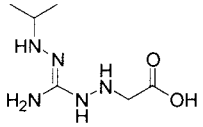
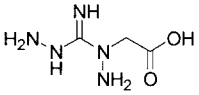
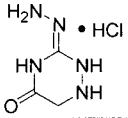
The structural similarity of the compounds reported in this manuscript to Metformin was noted. With this in mind, biguanide and amidine analogues were prepared and are shown in Table 4. As can be seen from the table, biguanide and amidine analogues were universally inactive within this series of compounds. This may be the result of either the increased size of the molecules or the change in the basicity of the aminoguanidine functionality.

Table 5. Carbon Insertions

Cpd	Structure	MISS T/C[a]	Cpd	Structure	MISS T/C[a]
41		0.95	**44**		1.00
42		0.97	**45**		1.18
43		0.88	**46**		0.96

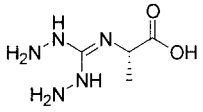
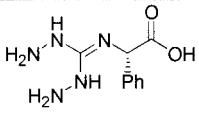
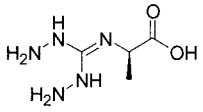
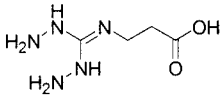
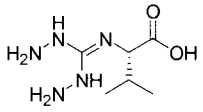
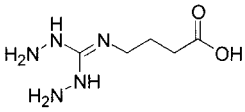
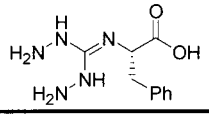
[a] Mouse insulin sensitizing screen nonfasting blood glucose levels (test/control); see Experimental Section: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

Table 6. Diaminoguanidine Analogues

Cpd	Structure	MISS T/C[a]	Cpd	Structure	MISS T/C[a]
47		0.98	**51**		0.96
48		0.89	**52**		0.23 (±0.05)**
49		0.90	**53**		0.43 (±0.2)***
50		1.00			

[a] Mouse insulin sensitizing screen nonfasting blood glucose levels (test/control); see Experimental Section: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

Table 7. Analogues of **52**: Variations in the Carbon Chain

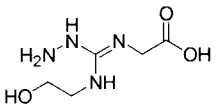
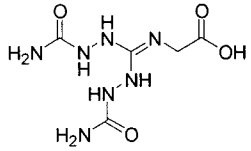
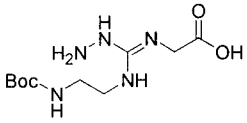
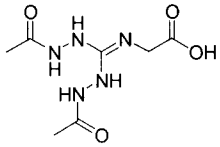
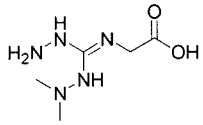
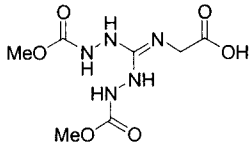
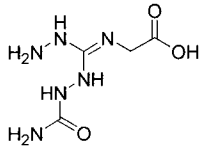
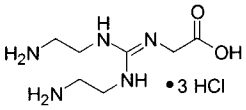
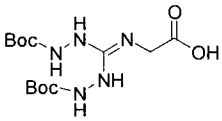
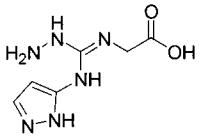
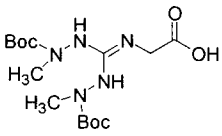
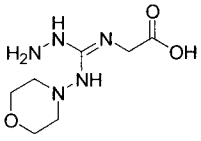
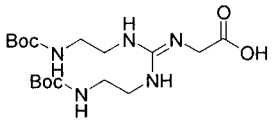
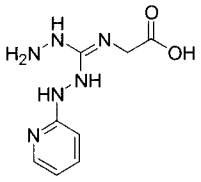
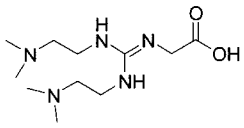
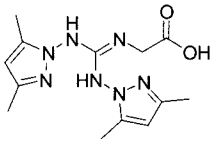
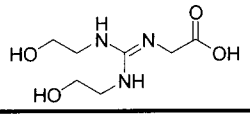
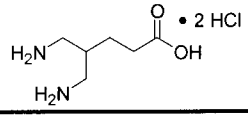
Cpd	Structure	MISS T/C[a]	Cpd	Structure	MISS T/C[a]
54		0.57 (±0.28)**	**58**		1.06
55		0.66 (±0.29)**	**59**		0.78 (±0.14)*
56		0.56 (±0.21)***	**60**		0.93
57		0.88			

[a] Mouse insulin sensitizing screen nonfasting blood glucose levels (test/control); see Experimental Section: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

Analogues in which one of the amino groups of the aminoguanidine moieties of **2** or **9** is extended out by one methylene are shown in Table 5. These analogues were targeted to try to avoid the potentially labile N−N bonds of the leads. Unfortunately, these alterations were uniformly detrimental to activity. The *N*-hydroxy analogues **43** and **44** that were intermediates in the syntheses of these analogues were also inactive.

Table 6 depicts analogues of the diaminoguanidinoacetic acid **7**. As can be seen from the biological data, simple substitutions, including imines (**47** and **48**) and N-alkylation (**49**), resulted in a loss of activity. Cycliza-

Table 8. Analogues of **52**: Variation in the Aminoguanidine Region

Cpd	Structure	MISS T/C[a]	Cpd	Structure	MISS T/C[a]
61		1.01	**70**		0.86
62		0.81	**71**		0.84
63		0.80	**72**		0.92
64		0.86	**73**		0.86
65		0.86	**74**		0.89
66		0.85	**75**		1.00
67		0.90	**76**		0.91
68		0.95	**77**		1.00
69		1.07	**78**		0.84

[a] Mouse insulin sensitizing screen nonfasting blood glucose levels (test/control); see Experimental Section: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

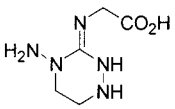
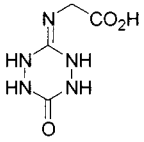
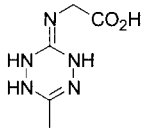
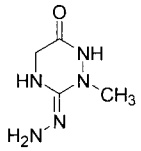
tion to the corresponding lactam (**50**) and incorporating the diaminoguanidine into a cyclic structure (**51**) also led to inactive analogues. The isomeric diaminoguanidinoacetic acids **52** and **53**, however, displayed improved antidiabetic activity with T/C values of 0.23 and 0.43, respectively. Dose−response testing of these compounds indicated potencies 5−10 times that of **7** (Figures 2, 3). Further SAR studies thus focused on these two diaminoguanidine analogues.

The analogues shown in Table 7 are versions of **52** in which variations have been made to the carbon chain of the molecule. As in the other series that were previous described, α-substitution (e.g. compounds **54**−**58**) results in a decrease in activity. Unlike other series, however, some of these compounds retain significant activity. The one- and two-carbon homologues **59** and **60** were inactive.

Table 8 depicts compounds in which the guanidine moiety of **49** has been altered. Replacement of one or both of the hydrazine fragments of **52** with various amines and heterocycles is the basis of analogues **61**−**77**. All of these examples proved to be inactive. Replacement of all of the nitrogens except for the terminal amino group with carbon resulted in **78** which was also inactive.

Conformationally restricted analogues of the diaminoguanidines are shown in Table 9. Analogues **79**−**81** were attempts to restrict the diaminoguanidine func-

Table 9. Cyclic Diaminoguanidine Analogues

Cpd	Structure	MISS T/C[a]	Cpd	Structure	MISS T/C[a]
79		0.77 (±0.38)	**81**		1.04
80		0.97	**82**		1.16

[a] Mouse insulin sensitizing screen nonfasting blood glucose levels (test/control); see Experimental Section: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

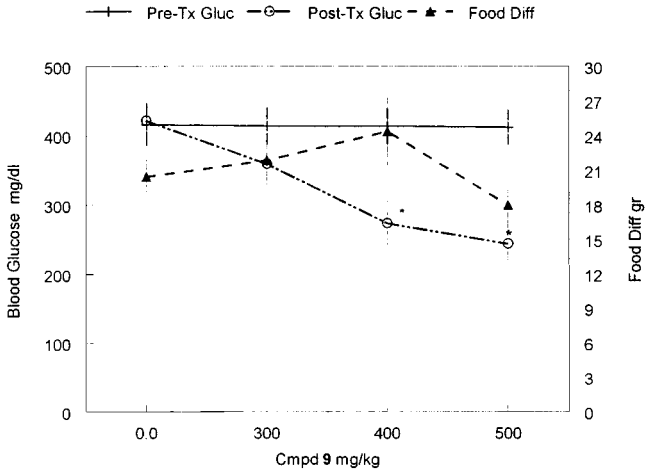


Figure 1. Compound **9** given as admixture to food. Whole blood glucose measured prior to treatment and day 3 using the Alpkem 2700 Analyzer; $*p < 0.05$.

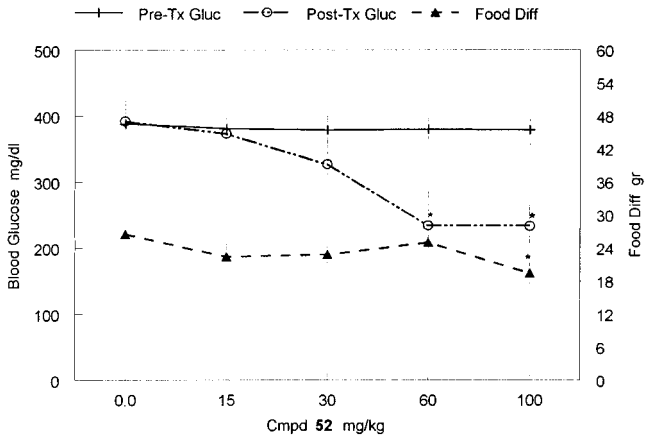


Figure 2. Compound **52** given as admixture to food. Whole blood glucose measured prior to treatment and day 3 using the Alpkem 2700 Analyzer; $*p < 0.05$.

tionality into a heterocycle. These analogues were also inactive, as was the lactam **82**.

Dose–Response Studies in KKA^y Mice. Compounds **52**, **53**, and **9** were evaluated at multiple doses in KKA^y mice to determine their potency relative to the lead compounds **1** and **2**. At the same time, food intake was measured to determine the extent, if any, to which nonfasting blood glucose (NFBG) lowering might be correlated with reductions in food intake. Experiments were performed under the identical protocol to that described above, except that animals were divided into

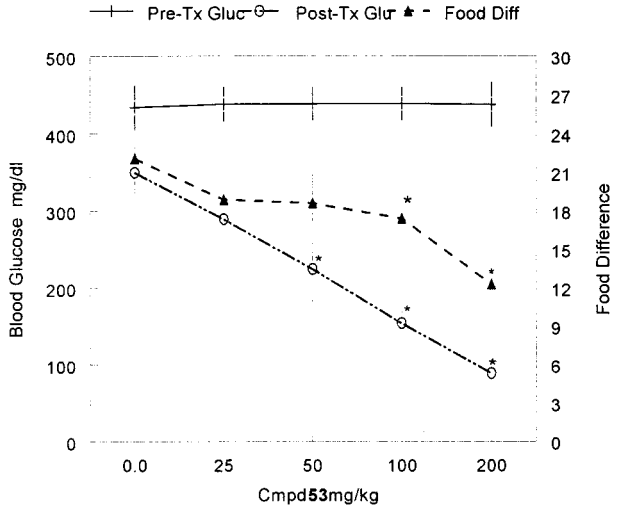


Figure 3. Compound **53** given as admixture to food. Whole blood glucose measured prior to treatment and day 3 using the Alpkem 2700 Analyzer; $*p < 0.05$.

multiple groups for dosing at different levels. Based on the high degree of efficacy displayed by **52** and **53** at 500 mg/kg, dosing was undertaken at substantially reduced levels relative to analogue **9**. The results are summarized graphically in Figures 1–3 (numerical tabular data can be found in Supporting Information). The least potent analogue **9** did not achieve a significant reduction in NFBG under 400 mg/kg, indicating that its potency is not markedly improved over that of **1** and **2**. At the 400 and 500 mg/kg doses, statistically significant reductions in NFBG were apparent *without* concomitant reductions in food intake. Both **52** and **53**, on the other hand, proved to be considerably more potent, inducing significant reductions in NFBG at 60 and 50 mg/kg, respectively. Although food intake was clearly suppressed at the highest doses administered, neither compound induced statistically significant reductions in food intake at the lowest effective doses for glucose lowering. These results indicate that the onset of NFBG lowering is not directly correlated to significant effects on food intake and that the observed antidiabetic effects are more likely to be caused by differences in energy expenditure. These results are similar to those observed in the *ob/ob* mice studies (vide infra).

Creatine Transport and Creatine Kinase Activity. 3-Guanidinopropionic acid **(1)** was found to accumulate in muscle tissue. A likely mechanism for this accumulation is that **1** is known to be a substrate for

Table 10. Inhibition of Creatine Transport

test substance (1 mM)	inhib of [^{14}C]creatine uptake (%)
creatine	92
1	97
2	36
7	26
52	88

Table 11. Creatine Kinase Activity

substrate	reaction rate (relative to creatine)
creatine (1 mM)	100
1 (100 mM)	2.4
2 (50 mM)	0.24
7	0
52	0.1
53	0

the plasma membrane creatine transporter[30] and to reduce cellular creatine concentration by antagonizing cellular creatine uptake.[31] In addition, **1** is also known to be a substrate for creatine kinase[32] and to reduce cellular ATP concentrations under conditions where the forward flux rate (β-GPA + ATP $\rightarrow$ β-GPAP + ADP) is high.[33] The active analogues were therefore tested as substrates for these two enzymes. Reduction in activity with either of these enzymes should lead to decreased accumulation in muscle tissue. To determine the relative antagonism of creatine transport by analogues of **1**, the effect of these compounds on the uptake of [^{14}C]-creatine by cultured rat smooth muscle cells was determined. The results are summarized in Table 10. These data indicate that the creatine transporter discriminates between the analogues of **1**. Inhibition of A10 cell creatine uptake by **7** is low relative to that of **1**.

To explore the relative substrate discrimination among the analogues of **1** by creatine kinase, creatine kinase activity was assayed in the forward direction by measuring the conversion of ATP to ADP by enzyme extracted from rabbit muscle (specific activity 350 U/mg; Boehringer Mannheim, Indianapolis, IN). The results are shown in Table 11. These data indicate that creatine kinase discriminates between the analogues of **1**. Phosphorylation of **7** and **53** was not detectable in this assay. Compound **52** had a negligible rate of phosphorylation. These results suggest that the likelihood of these compounds to accumulate in muscle was very low.

Pharmacology in *ob/ob* Mice. Pharmacology studies with 3-guanidinopropionic acid (**1**) demonstrated a reduction in hyperglycemia in obese, diabetic mice as well as weight loss in the animals. To determine if these activities were retained in the active compounds from the current series, exemplary compounds from each of the active series were tested with *ob/ob* mice. These mice are hyperglycemic and obese compared to normal, wild-type mice due to a mutation in the leptin signal sequence.[34] The mice received test substances as an admixture in the diet. Three of the test substances were add to the diet at similar concentrations and produced nearly similar levels of drug intake (Table 12). One of the test substances, **53**, was known to be more potent based on earlier studies and was added to the diet at a lower concentration. This resulted in an averaged daily drug intake for **53** of approximately 6% of the dose of the other test substances.

The pretreatment blood glucose concentration was similar in experimental groups (Table 12). After treatment for 14 days, compounds **7** and **9** decreased the blood glucose level of *ob/ob* mice ($p < 0.05$). The effects of these compounds were statistically different from control but not significantly different from the initial lead **1**. By comparison, **53** also decreased the blood glucose level of *ob/ob* mice, but its effect was significantly greater than that of the initial lead, **1** ($p < 0.05$).

The mean initial body weights for four of the experimental groups were approximately the same (52−55 g; Table 12). In one group (**53**) the mean initial body weight was greater (59 g) due to a randomization error. This represents a negative bias in the data for weight loss. On the basis of comparison of the terminal body weights in the mice, **1** and **9** produced a similar, statistically significant loss of body weight by the mice ($p < 0.05$). Compounds **7** and **53** produced a significant loss of body weight compared to the controls ($p < 0.05$) as well as compared to the mice treated with **1** or **9** ($p < 0.05$). The average daily food consumption was significantly reduced by **1** and **53** ($p < 0.05$), but there was no significant effect of **7** and **9** on this parameter. This indicates that the relationship of reduced food intake and weight loss is a weak one and that differences in energy expenditure by the experimental groups are likely to have occurred.

Body composition analysis demonstrated that the lean body mass of mice that received the initial lead compound, **1**, was nearly similar to that in controls (Table 12). By comparison, the fat body mass of the mice was significantly reduced by **1** compared to the control mice ($p < 0.05$). Compounds **7**, **9**, and **53** produced small but statistically significant reductions in lean body mass compared to controls ($p < 0.05$). These compound also produced statistically significant reductions in the fat body mass of the mice ($p < 0.05$). For each of these compounds, the reduction in fat body mass was 2 times the lean tissue mass that was lost. This indicates that the reduction of body weight in response to these compounds may involve a relatively selective mobilization of stored fat and subsequent clearance of this material from the system.

Pharmacology in Insulin-Resistant Monkeys. A previous study of the initial lead compound, **1**, demonstrated that the compound improved the disposal rate for glucose in monkeys that were insulin-resistant. To determine whether **53** retained this important property of the initial lead, three insulin-resistant rhesus monkeys were given **53** or a placebo once daily for 4 days every other week. The treatment sequence was placebo, 10 mg/kg/day **53**, placebo, and 3 mg/kg/day **53**. Plasma glucose and insulin levels were determined after an overnight fast and for 3 h after a liquid meal. The first and second placebo phases produced similar results; therefore, the placebo data were averaged for the purpose of statistical analysis. The results are shown in Table 13. Compound **53** produced a statistically significant reduction in the fasting plasma glucose level of rhesus monkeys ($p = 0.02$ treatment effect in repeated measures ANOVA). Post hoc analysis of the results indicated that the effect was statistically significant at a dose of 10 mg/kg/day. The fasting plasma insulin concentration, post-prandial glucose response, and post-prandial plasma insulin AUC all tended to be decreased by drug treatment ($p = 0.10$, 0.23, and 0.07, respectively,

Table 12. Effect of 2 Weeks of Oral Administration of Test Compounds on *ob/ob* Mice

test substance	control	**1**	**7**	**9**	**53**
sample size (*n*)	15	15	8	8	8
average daily dose (mg/kg)	0	377 ± 20	406 ± 12	436 ± 20	26 ± 1
initial blood glucose (mg/dL)	228 ± 19	229 ± 18	226 ± 20	227 ± 20	228 ± 34
terminal blood glucose (mg/dL)	226 ± 29	147 ± 18*	85 ± 13*	97 ± 11*	57 ± 3*,‡
initial body weight (g)	55 ± 1	54 ± 1	52 ± 1	52 ± 1	59 ± 2*
terminal body weight (g)	53 ± 1	48 ± 1*	43 ± 1*,‡	46 ± 1*	44 ± 1*,‡
average daily food consumption (g/kg)	90 ± 3	75 ± 4*	81 ± 2	87 ± 5	52 ± 2*,‡
terminal lean body weight (g)	25 ± 1	24 ± 0.4	22 ± 0.4*,‡	23 ± 0.4*	22 ± 0.7*
terminal fat body weight (g)	28 ± 0.9	24 ± 1*	20 ± 0.7*,‡	23 ± 0.8*	22 ± 0.9*

*$p < 0.05$ vs control. ‡ $p < 0.05$ vs **1**.

Table 13. Effect of **53** in Insulin-Resistant Rhesus Monkeys

	placebo[a]	**53** (3 mg/kg/d)	**53** (10 mg/kg/d)
fasting plasma glucose (mg/dL)	83 ± 11	81 ± 8	64 ± 9*
post-prandial plasma glucose AUC (g·min/mL)	185 ± 36	171 ± 12	146 ± 19
fasting plasma insulin (μU/mL)	111 ± 43	41 ± 11	35 ± 7
post-prandial plasma insulin AUC (mU·min/dL)	94 ± 44	38 ± 24	30 ± 20

[a] Mean of first and second placebo phases. *$p < 0.05$ vs control.

for the treatment effect in repeated measures ANOVA analyses). The finding that the fasting and post-prandial plasma glucose levels are reduced by **53** in the presence of a reduced level of circulating insulin suggests that the test compound improved the action of endogenous insulin. These findings indicate that this compound retains the activity of the initial lead in nonhuman primates.

Conclusion

3-Guanidinopropionic acid (**1**) was previously found to possess antidiabetic activity and to induce weight loss selectively from adipose tissue in obese animals. An initial phase of analogue work led to the discovery of an aminoguanidinoacetic acid analogue (**2**) that retained equipotent antidiabetic activity but was less susceptible to processing by the creatine transport/kinase system, thereby possessing a reduced potential to accumulate in muscle. A second phase of analogue work was launched to try to identify compounds with greater antidiabetic potency that retained the resistance to creatine-like metabolism exhibited by **2**. The SAR about **2** proved to be just as narrow as that established for **1**, with simple substituted, homologated, and bioisosteric analogues losing all activity. Eventually it was discovered that the simple regioisomeric aminoguanidinoacetic acid **9** retained activity, as did three closely related diaminoguandinoacetic acid analogues, one of which **(7)** was equipotent to **2** and two of which (**52** and **53**) were substantially more potent. After establishing that all three diaminoguanidinoacetic acid regioisomers were less susceptible to creatine-like metabolism than **1** and therefore less likely to accumulation in muscle, testing in additional animal models of obesity and diabetes was undertaken. All three induced weight loss in obese diabetic *ob/ob* mice. The decrease in fat body mass was twice that of lean body mass, suggesting that the weight loss occurred selectively from adipose tissue in a manner similar to **1**. Furthermore, it was demonstrated that **52** and **53** induce reductions in NFBG in diabetic KKA^y mice that are independent of statistically significant reductions in food consumption, suggesting that they are altering the overall rate of metabolism. Finally, when **53** was administered to insulin-resistant rhesus monkeys, it induced reductions in fasting and post-prandial serum glucose with concomitant reductions in plasma insulin levels, suggesting that it is improving the actions of endogenous insulin. Both **7** and **53** were selected for further preclinical development.

Experimental Section

Chemistry. All melting points (mp) were obtained on a capillary melting point apparatus and are uncorrected. Proton magnetic resonance spectra were recorded on a Bruker AM-300 spectrometer in the deuterated solvents indicated. Chemical shifts were recorded in parts per million (δ scale) and are reported relative to internal tetramethylsilane or 3-(trimethylsilyl)-1-propanesulfonic acid, sodium salt. ^{13}C NMR spectra were recorded on a Bruker AM-300 spectrometer at 75.4 MHz. Flash column chromatography separations were carried out using EM Science silica (mesh 230−400). Electron impact (EI) mass spectra were obtained with an ionization voltage of 70 eV. Alternatively, ionization was achieved by fast atom bombardment (FAB). Reagents and solvents were purchased from common suppliers and were used as received. All non-aqueous reactions were run under a nitrogen atmosphere. All starting materials were commercially available unless otherwise noted. Compound **13** is available from commercial sources. Compounds **8**,[4] **9**,[5] **18**,[6] **28**,[35] **31**,[7] and **32**[21] were prepared according to literature procedures.

General Procedure for the Synthesis of 4−6. A solution of the hydrazino acid (2.36 g, 20 mmol) and 2-methyl-2-thiopsuedourea sulfate (2.78 g, 20 mmol) in 20 mL 1 N NaOH was heated to reflux for 2 h. The solution was concentrated to one-half volume and cooled. The resulting product was filtered and washed with water and dried in vacuo.

2-[2-(Aminoiminomethyl)hydrazino]butanoic Acid (4). Compound **4** was prepared from 2-hydrazinobutyric acid[7] [mp 187−189 °C] by the general method above: yield 63%; mp 229−232 °C dec; ^{1}H NMR (D_2O) δ 3.25 (t, J = 6.5 Hz, 1 H), 1.64−1.54 (m, 2 H), 0.89 (t, J = 7.5 Hz, 3 H); ^{13}C NMR (DMSO) δ 179.1, 160.4, 67.9, 25.3, 13.0; MS (FAB) *m/z* (relative intensity) 161 (M + H, base), 115 (8), 74 (2), 60 (3). Anal. ($C_5H_{12}N_4O_2$) C, H, N.

[2-(Hydrazinoiminomethyl)hydrazino]acetic Acid (7). Ethylhydrazinoacetate hydrochloride (9.28 g, 60 mmol) was saponified by refluxing in 120 mL of 1 N NaOH for 2 h. To the hot solution was then added *N*-amino-*S*-methylisothiourea hydroiodide[8] (13.98 g, 60 mmol) and the solution was refluxed for an additional 2 h. The solvent was removed. The crude product was dissolved in methanol and filtered to remove the NaCl. The filtrate was condensed and dried by high vacuum. The residue was then stirred with 150 mL MeOH overnight. The resulting white solid was filtered. This solid was then refluxed in 100 mL MeOH for 2 h to remove any impurities. The mixture was then cooled and filtered. The resulting solid was dried in vacuo to yield 2.14 g (24%) of **7** as an off-white solid: mp 201−203 °C dec; ^{1}H NMR (D_2O) δ 3.39 (s, 2 H); ^{13}C NMR (D_2O) δ 178.6, 159.5, 54.0; MS (FAB) *m/z* (relative intensity) 148 (M + H, base). Anal. ($C_3H_9N_5O_2$) C, H, N.

2-{1-[Amino(imino)methyl]hydrazino}acetic Acid (10). To a stirring suspension of aminoguanidine bicarbonate (100 g, 734 mmol) in water (200 mL) was added bromoacetic acid (100 g, 720 mmol). After initial effervescence the homogeneous solution was refluxed overnight, cooled to ambient temperature, and solvent evaporated to dryness. The residue was suspended in EtOH (200 mL) and sonicated. The solid was filtered to afford 13.6 g (9%) of the title compound as a white solid: mp 163−165 °C; ^{1}H NMR (D_2O) δ 4.25 (s, 2 H); ^{13}C NMR (D_2O) δ 60.6, 158.0, 175; MS (FAB+) *m*/*z* 133 (M + H). Anal. ($C_3H_8N_4O_2$·HBr) C, H, N, Br. To a stirring solution of LiOH·H_2O (0.98 g, 23.4 mmol) in MeOH (20 mL) was added the salt from above (5.0 g, 23.4 mmol) in MeOH (50 mL). After a solid begins to form (∼15 min) stirring was continued an additional 30 min. The resulting solid was filtered and dried in vacuo at 25 °C to afford 2.08 g (66%) of the title compound as a white solid: mp 118−119 °C; ^{1}H NMR (D_2O) δ 4.10 (s, 2 H); ^{13}C NMR (D_2O) δ 56.19, 158.4, 171; MS (FAB+) *m*/*z* 133 (M + H). Anal. ($C_3H_8N_4O_2$·0.17H_2O) C, H, N.

3-Amino-1,6-dihydro-1,2,4-triazin-5(2*H*)-one Monohydrochloride (11). A solution of **31**[6] (1.65 g, 14.7 mmol) in H_2O (40 mL) and concentrated HCl (8 mL) was hydrogenated over 10% Pd/C (165 mg) at 40 psi for 1.5 h. The reaction mixture was filtered and the filtrate concentrated in vacuo. The residue was recrystallized from MeOH to yield **11** (1.48 g, 68%) as a white solid: mp 204−205 °C dec; ^{1}H NMR (CD_3OD) δ 3.60 (s, 2 H); ^{13}C NMR (CD_3OD) δ 175.2, 139.6, 51.7; MS (EI) *m*/*z* (relative intensity) 114 (M^+, 39), 85 (50), 42 (base). Anal. ($C_3H_6N_4O$·HCl) C, H, N, Cl.

***N*-(2-Amino-1*H*-imidazol-1-yl)-*N*-(1-methylethylidene)-amine (86).** To a mechanically stirring solution of aminoguanidine carbonate (100 g, 734 mmol) in EtOH (1.2 L) and concentrated aqueous HCl (1 mL) was added acetone (116.8 g, 2 mol) and the resulting solution heated at reflux overnight. The resulting solution was cooled to ambient temperature and solvent evaporated to ∼800 mL. To the resulting solution was added chloroacetaldehyde (28.8 g, 367 mmol) and the mixture heated to reflux for 4 h. The solvent was removed under reduced pressure and the residue suspended between H_2O and CH_2Cl_2. The layers were shaken, the organics separated, dried over Na_2SO_4 and the solvent removed in vacuo. The aqueous layer was continuously extracted with CH_2Cl_2 overnight and the organic layer evaporated. The extracted organic residues were combined and purified via SiO_2 flash column chomatography (eluant 7% MeOH/CH_2Cl_2). The chromatographed residue was recrystallized from EtOAc/hexane to afford 15.26 g (15%) of the title compound as a highly crystalline white solid: mp 123−125 °C; ^{1}H NMR ($CDCl_3$) δ 2.07 (s, 3 H), 2.19 (s, 3 H), 4.13 (br s, 2 H), 6.59 (d, J = 2.0 Hz 1 H), 6.64 (d, J = 2.0 Hz, 1 H); MS (EI) *m*/*z* 138 (M^+), 138, 82, 69, 56, 55, 53, 42, 41, 40, 25. Anal. ($C_6H_{10}N_4$) C, H, N.

2-[(2-Amino-1*H*-imidazol-1-yl)amino]acetic Acid (12). To a stirring solution of **86** (5.0 g, 36.18 mmol) in H_2O (40 mL) in a pressure tube at ambient temperature was added chloroacetic acid (3.42 g, 36.18 mmol) and triethylamine (3.65 g, 36.18 mmol). The tube was closed and heated at 100 °C overnight and cooled to ambient temperature. The aqueous solution was washed with EtOAc (50 mL) and the aqueous layer evaporated to dryness. The residue was taken up in EtOH (50 mL) and heated on a steam bath to remove byproducts and unreacted starting material. The solids were filtered and dried in vacuo to afford 2.16 g (38%) of the title compound as a tan solid: mp 265−267 °C; ^{1}H NMR (D_2O) δ 4.52 (s, 2 H), 6.73 (d, J = 2.0 Hz, 1 H), 6.91 (d, J = 2.0 Hz, 1 H); ^{13}C NMR (D_2O) δ 49.4, 114.6, 117.8, 146.9, 177.4; MS (FAB) *m*/*z* 157 (M + H), 469, 314, 313, 189, 158, 157, 142, 141, 112, 99. Anal. ($C_5H_8N_4O_2$) H; C: calcd, 38.46; found, 36.39. N: calcd, 35.88; found, 33.98.

2-{[Imino(methylsulfanyl)methyl]amino}acetic Acid (88). A solution of **87** (9.5 g, 71 mmol) and iodomethane (8.8 mL, 142 mmol) in methanol (140 mL) was stirred at room temperature for 18 h. Attempted trituration of a small sample yielded the hydroiodide salt of **88** as a gummy oil, so the entire reaction mixture was concentrated in vacuo to a viscous oil. This material was dissolved in water (70 mL), and the solution was chilled in ice before neutralization to pH 7 with 1 N NaOH (required about 55 mL). Acetone was added to the resulting solution until no more precipitate appeared (ca. 300 mL). The mixture was stirred in the ice bath for 10 min before filtering, giving **88** as a fine white solid (8.97 g, ca. 85% yield): mp 184 °C dec; ^{1}H NMR (D_2O) δ 3.98 (br s, 2 H), 2.63 (s, 3 H); ^{13}C NMR (D_2O) δ 174.0, 169.5, 46.9, 13.2; FAB MS *m*/*z* (rel intensity) 149 (M + H, 100). Anal. ($C_4H_8N_2O_2S$) C, H, N.

2-{[[(2-Aminoethyl)amino](imino)methyl]amino}-acetic Acid (16). To a solution of isothiourea **88** (4.0 g, 27.0 mmol) in water (54 mL), cooled in a cold water bath, was added in one portion ethylenediamine (9.02 mL, 135 mmol). The solution was stirred at room temperature for 7 h. The solution was then diluted with isopropyl alcohol to a volume of 250 mL. Chilling at 0 °C overnight afforded **16** as a fine white solid (2.83 g, 65%): mp 157−158 °C dec; ^{1}H NMR (D_2O) δ 3.81 (s, 2 H), 3.29 (t, J = 6 Hz, 2 H), 2.82 (t, J = 6 Hz, 2 H); ^{13}C NMR (D_2O) δ (mult) 175.3 (s), 156.3 (s), 44.7 (t), 43.6 (t), 39.4 (t); FAB MS *m*/*z* (rel intensity) 161 (M + H, 100). Anal. ($C_5H_{12}N_4O_2$·0.86H_2O) C, H, N.

General Procedure for the Synthesis of 20−22. Concentrated (37%) hydrochloric acid (10 mL, 120 mmol) was added slowly and cautiously to a magnetically stirred suspension of aminoguanidine bicarbonate (15.0 g, 110 mmol) in 20 mL of distilled water. Gas evolved and a clear solution of aminoguanidine hydrochloride was formed. To this solution was added 125 mmol of the keto acid all at once. The mixture was diluted with 15 mL of distilled water to facilitate stirring, and it was stirred 24 h at 25 °C. After an additional 24 h at 5 °C, it was filtered and the filtrant was washed with two successive 20 mL portions of distilled water. The solid was dried several hours at 25 °C in a stream of air, then at 20 Torr/45 °C/4 days.

2-[(Aminoiminomethyl)hydrazono]propanoic Acid Hydrochloride (20). Compound **20** was prepared from pyruvic acid according to the general procedure above; yield 66% of **20** as a white powder: mp 238−239 °C dec (lit. 130°, 230 °C); ^{1}H NMR (D_2O) δ 1.97 (s, major (87%) stereoisomer, 3 H), 1.95 (s, minor (13%) stereoisomer, 3 H); ^{13}C NMR (D_2O) δ 166.9 (major stereoisomer), 155.9 (major stereoisomer), 145.8 (major stereoisomer), 20.0 (minor stereoisomer), 12.1 (major stereoisomer); MS(FAB) *m*/*z* (relative intensity) 145 (M + H^+, base). Anal. ($C_4H_8N_4O_2$·HCl) C, H, N, Cl.

General Procedure for the Synthesis of 33−36. To the amino acid (4.33 g, 58.2 mmol) suspended in absolute ethanol (0.3 L), in a 60 °C oil bath, was added in order, triethylamine (11.67 g, 0.115 mol) and water (30 mL). The mixture was stirred until the majority of the amino acid had dissolved (30 min), then **90**[14] (30 g, 0.115 mol) was added in 6 approximately equal portions over 6 h. The mixture was allowed to stir for 12 h at 60 °C, then was cooled to room temperature. The solid was isolated by filtration, washed with absolute ethanol and dried in vacuo.

Imidocarbonimidic Diamide *N*-(Acetic acid) (33). Compound **33** was prepared from glycine according to the general procedure above; yield 28% of **33** as a fine, powdery, pale yellow solid: mp 226−228 °C dec; ^{1}H NMR (D_2O) δ 3.79 (s, 2 H); ^{13}C NMR (D_2O) δ 172.5, 160.3, 45.3, 41.6; IR (Nujol) 3396, 3316, 3119, 1635, 1601, 1557, 1506, 1405, 1363, 1275, 733 cm^{-1}; FAB/MS 319 (19.6), 160 (M^+ + 1, base); HRMS *m*/*z* calcd for $C_6H_{13}N_5O_3$ 160.0834, found 160.0831.

General Procedure for the Synthesis of 92a,b. To a stirring solution of **91**[15] (10.0 g, 52.5 mmol) in EtOH (350 mL) was added the amino acid (52.5 mmol) and triethylamine (5.35 g, 52.57 mmol). Water was added incrementally until a solution was realized (∼50 mL) and the mixture stirred for 1.5 h. The solvent was evaporated to dryness to afford a hygroscopic solid. Dry ammonia was slowly bubbled into a stirring solution of this compound (45.8 mmol) in EtOH (125 mL). The resulting solution was stirred for 15 min in which time a solid began to form. The suspension was stirred for 1 h, and the solid filtered.

92a was prepared from glycine according to the general procedure above: yield 99% as an off-white solid; ^{1}H NMR (D_2O) δ 4.03 (s, 2H); ^{13}C NMR (D_2O) δ 47.5, 159.0, 159.9, 175.3; MS (FAB) *m*/*z* 191 (M + H). Anal. ($C_4H_6N_4O_3S{\cdot}NH_3{\cdot}0.4H_2O$) H, N; C: calcd, 22.40; found, 22.83.

2-[(2-Amino-2-iminoethanimidoyl)amino]acetic Acid (38). 92a (8.6 g, 41.3 mmol) was dissolved in concentrated aqueous HCl (25 mL) and allowed to stir for 2 h. The solution was evaporated to dryness and dried under vacuo at 25 °C overnight. The resulting solid (10.0 g, 37 mmol) was taken up in minimal water and neutralized by dropwise addition of 1 M aqueous NaOH (74 mL, 74 mmol). The resulting solid was filtered and washed with water (2 × 50 mL). The solid was dried overnight in vacuo at 25 °C to afford 4.82 g (81%) of **38** as a tan solid: mp >300 °C; ^{1}H NMR (D_2O/DCl) δ 4.31 (s, 2 H); ^{13}C NMR (D_2O/DCl) δ 44.31, 154.0, 155.0, 168.9; MS (FAB+) *m*/*z* 145 (M + H). Anal. ($C_4H_8N_4O_2{\cdot}1.1H_2O$) C, H, N.

General Procedure for the Synthesis of 41−42. To a solution of **93**[16] (20.0 g, 56.8 mmol) in CH_3CN (250 mL) at 0 °C was added a solution of the neutralized amino acid ethyl ester (56.8 mmol) in CH_3CN (200 mL) over 10 min. After warming to room temperature, the mixture was stirred for 2 h. The mixture was concentrated under reduced pressure to provide the triflate salt as a waxy solid which was used directly in the next reaction. The triflate salt was dissolved in 12 M HCl (100 mL), and the mixture was heated at 95 °C for 1 h. The reaction mixture was cooled to −15 °C in a freezer and seeded. The mixture was allowed to stand in the freezer for 2.5 days. The solid was collected by filtration and washed with THF.

2-[(2-Aminoethanimidoyl)amino]acetic Acid Dihydrochloride (41). Compound **41** was prepared from glycine ethyl ester hydrochloride according to the general procedure above: yield 84% as a white solid; mp 191−192 °C; ^{1}H NMR (D_2O) δ 4.25 (s, 2 H), 4.18 (s, 2 H); MS (FAB) *m*/*z* 263, 133, 132 (base), 45, 30. Anal. ($C_4H_9N_3O_2{\cdot}2HCl$) C, H, N, Cl.

General Procedure for the Synthesis of 95. To a solution of the amino acid ethyl ester hydrochloride (**94**) (36 mmol) in H_2O (10 mL) at 0 °C was added 37% aqueous formaldehyde (2.7 mL) and a solution of sodium cyanide (1.75 g) in H_2O (3.6 mL). The resulting mixture was stirred at 0−5 °C for 4 h and extracted with Et_2O (6 × 30 mL). The combined organic phase was dried (Na_2SO_4) and filtered. Anhydrous HCl (g) was bubbled through the ethereal extract until a milky white mixture formed. After cooling to 0 °C, the white solid which formed was collected by filtration to provide the desired nitrile as an off-white solid. To a suspension of the nitrile (22.0 mmol) and triethylamine (3.1 mL, 22.0 mmol) in CH_3CN (25 mL) was added di-*tert*-butyl dicarbonate (9.6 g, 44.0 mmol). After stirring the mixture for several minutes, 4-(dimethylamino)pyridine (0.67 g, 5.5 mmol) was added, and the mixture was stirred at room temperature for 24 h. The reaction mixture was concentrated to near dryness in vacuo and partitioned between EtOAc (50 mL) and H_2O. After the aqueous phase was extracted with EtOAc (1 × 50 mL), the combined organic phase was washed with 1 N HCl (1 × 25 mL) and dried ($MgSO_4$). The solvent was removed under reduced pressure, and the residue was purified by chromatography (20% EtOAc: hexane) to provide the Boc-protected aminonitrile as a colorless oil. To a solution of $NaHCO_3$ (3.5 g, 42.1 mmol) in H_2O (30 mL) was added slowly a solution of hydroxylamine hydrochloride (2.9 g, 42.1 mmol) in H_2O (5 mL). The aqueous solution of hydroxylamine was added to a solution of the Boc-protected aminonitrile (42.1 mmol) in 95% EtOH (400 mL). The mixture was heated at reflux overnight. The EtOH was removed under reduced pressure, and the residue was partitioned between EtOAc (100 mL) and H_2O (50 mL). The aqueous phase was extracted with EtOAc (1 × 50 mL), and the combined organic phase was dried ($MgSO_4$). The solvent was removed in vacuo.

Compound **95a** was prepared from glycine ethyl ester hydrochloride according to the general procedure above; yield 87% of a solid that was approximately a 9:1 mixture of the desired **95a** and the cyclized lactam. The crude product could be separated by chromatography (70% EtOAc:hexane): mp 101−102 °C; ^{1}H NMR ($CDCl_3$) δ 5.04 (br d, 2 H, NH_2), 4.19 (q, J = 7 Hz, 2 H), 3.97, 3.90 (two s, 4 H), 1.49, 1.44 (two s, 9 H), 1.28 (t, J = 7 Hz, 3 H), mixture of rotamers and/or E, Z isomers; ^{13}C NMR ($CDCl_3$) δ 170.3 (minor), 167.7 (major), 156.3 (major), 155.2 (minor), 151.7 (major), 151.3 (minor), 81.6 (minor), 81.3 (major), 61.4 (minor), 61.2 (major), 49.2 (major), 49.0 (minor), 48.4, 28.3 (minor), 28.2 (major), 14.2; MS *m*/*z* 276 (M + H), 260, 220 (base), 204, 176, 116, 57. Anal. ($C_{11}H_{21}N_3O_5$) C, H, N.

2-[[2-Amino-2-(hydroxyimino)ethyl](*tert*-butoxycarbonyl)amino]acetic Acid (96a). To a suspension of **95a** (1.95 g, 7.1 mmol) in 95% EtOH (20 mL) was added 1.0 M NaOH (28.5 mL). After the mixture was stirred at room temperature for 2.5 h, 1.0 M HCl (28.5 mL) was added, and the mixture was cooled at 0 °C overnight. The white solid formed was filtered and washed with cold H_2O to provide 1.48 g (85%) of **96a** as a white solid: mp 219−220 °C; ^{1}H NMR (DMSO) δ 9.33 (1 H), 5.81 (br s, 2 H), 3.87 (s, 2 H), 3.82, 3.79 (two s, 2 H), 1.38, 1.35 (two s, 9 H); ^{13}C NMR (DMSO) δ 171.3, 154.5, 150.9, 79.6, 50.4, 50.0, 27.8; MS (FAB) *m*/*z* 249, 248 (M + H), 247, 232, 176, 192, 148, 57. Anal. ($C_9H_{17}N_3O_5$) C, H, N.

2-{[2-Amino-2-(hydroxyimino)ethyl]amino}acetic Acid (43). 96a (2.0 g, 8.1 mmol) was dissolved in a solution of anhydrous HCl in dioxane (2.8 M, 15 mL). A solid precipitated very rapidly, and the mixture was stirred for 5 h. The mixture was concentrated under reduced pressure, and the solid which was obtained was washed with Et_2O to provide 1.6 g (89%) of **43** as a hygroscopic white solid: mp 78−80 °C; ^{1}H NMR (D_2O) δ 3.97 (s, 2 H), 3.88 (s, 2 H); ^{13}C NMR (D_2O) δ 168.8, 153.3, 48.0, 43.8; MS (FAB) *m*/*z* 204, 148 (base), 40, 18; exact mass calcd for $C_4H_9N_3O_3$ 148.0722, found 148.0726. Anal. ($C_4H_9N_3O_3{\cdot}2HCl{\cdot}0.44H_2O$) C, H, N, Cl.

2-[(2-Amino-2-iminoethyl)amino]acetic Acid Dihydrochloride (45). To a suspension of **96a** (3.0 g) in MeOH (200 mL) in a Parr reaction vessel was added Raney nickel (50% slurry in H_2O). The reaction vessel was pressurized with H_2 (45 psi) and heated to 65 °C using a YSI Thermistemp temperature controller for 2 h. The mixture was cooled to room temperature and carefully filtered through Celite under N_2. The solvent was removed in vacuo to provide 2.5 g (89%) of a white solid: mp 250 °C dec; ^{1}H NMR (DMSO) δ 11.0 (br s), 10.9 (br s), 10.2 (br s), 4.10 (s, 2 H), 3.67 (s, 2 H), 1.35 (s, 9 H); ^{13}C NMR (DMSO) δ 175.5, 170.3, 169.7, 154.7, 153.8, 79.7, 79.6, 54.1, 53.3, 50.3, 50.0, 27.8, some signals doubled due to rotamers; MS (FAB) *m*/*z* 232 (M + H, base), 176, 58, 57, 41. Anal. ($C_9H_{17}N_3O_4{\cdot}0.22H_2O$) C, H, N.

The solid from the above reaction (5.0 g, 21.6 mmol) was suspended in a solution of anhydrous HCl in dioxane (2.8 M, 60 mL). The suspension was stirred at room temperature for 40 h. An ^{1}H NMR of an aliquot showed that some starting material remained. Anhydrous HCl in dioxane (3.3 M, 50 mL) was added, and the suspension was stirred at room temperature for another 24 h. The reaction was judged complete by ^{1}H NMR. The suspension was filtered, and the filter cake was washed with Et_2O to obtain 3.97 g (90%) of **45** as a white solid: mp 172−174 °C; ^{1}H NMR (D_2O) δ 4.19 (s, 2 H), 3.96 (s, 2 H); ^{13}C NMR (D_2O) δ 169.8, 162.5, 48.8, 46.4; MS (FAB) *m*/*z* 132 (base), 88, 58, 45. Anal. ($C_4H_9N_3O_2{\cdot}2HCl$) C, H, N, Cl.

Methyl 1-Hydrazinecarbohydrazonothioate Hydroiodide (97). A mixture of thiocarbohydrazide (25.76 g, 242.7 mmol) and methyl iodide (18.2 mL, 291.9 mmol) in methanol (240 mL) was stirred under nitrogen at reflux for 1 h 45 min. The resulting pale yellow solution was cooled to room temperature until crystallization commenced, and then it was diluted with ether (200 mL) to facilitate complete crystallization. After chilling in ice for 2 h, the mixture was filtered and the collected solid was washed with ether. Drying in vacuo gave pale yellow prisms (48.27 g, 80%). The solid discolors in air, so it is stored in a dark bottle in the refrigerator: ^{1}H NMR (D_2O) δ 2.49 (s); ^{13}C NMR (DMSO-d_6) δ 170.8, 12.7.

2-[(Dihydrazinomethylene)amino]Acetic Acid (52). A solution of **97** (25.0 g, 101 mmol) and glycine (6.314 g, 83.98 mmol) in water (50 mL) and 12.5 N NaOH (8.89 mL, 111 mmol) was stirred under nitrogen at 75−80 °C for 3 h. The

solution was chilled in ice while still under nitrogen before the portionwise addition of absolute ethanol (550 mL in 50 mL portions), stirring between each addition until precipitation was complete. Seeding with authentic product greatly facilitates this initial trituration. The mixture was then stirred for 15 min at 0 °C before filtering. The collected solid was washed thoroughly with absolute ethanol. Drying gave a light pink powder (8.04 g). The crude solid was dissolved in water (30 mL), filtered to remove some fine insoluble material, and then diluted to a volume of 250 mL with absolute ethanol. Precipitation began almost immediately and was accelerated by sonication for a few seconds. After standing at room temperature for 10 min, the mixture was filtered, giving a pale rose powder (5.25 g, 42%): mp 200 °C dec. An analytical sample could be prepared as follows: 2.04 g was dissolved in 6 mL water, and the pink solution was stirred with activated charcoal for 10 min at room temperature. The mixture was filtered through a fine glass frit, and the nearly colorless filtrate was diluted to 100 mL with absolute ethanol. After standing 1 h at room temperature with occasional sonication, 1.61 g of white powder was obtained. This was dissolved again in water, treated with charcoal again, filtered, and diluted to 100 mL with absolute ethanol. After 2 days at room temperature, 1.35 g of white prisms (mp 202 °C, dec) were obtained. The mp could not be raised any further by additional crystallizations: ^{1}H NMR (D_2O) δ 3.78 (s); ^{13}C NMR (D_2O) δ 175.3, 158.1, 43.7; FAB MS *m*/*z* (rel intens.) 148 (M + H, 100), 133 (3), 116 (1). Anal. ($C_3H_9N_5O_2$) C, H, N.

Benzyl 2-(2-Ethoxy-2-oxoethyl)-1-hydrazinecarboxylate (98). To a stirring suspension of ethyl hydrazinoacetate hydrochloride (5.0 g, 32.34 mmol) and *N*-methylmorpholine (3.26 g, 32.34 mmol) at 0 °C was added solid *N*-(benzyloxycarbonyloxy)succinimide (8.06 g, 32.34 mmol). The mixture was allowed to warm to ambient temperature overnight and the solvent removed in vacuo. The residue was suspended between EtOAc/H_2O, the layers shaken, the organics separated and dried over Na_2SO_4. The solvent was removed and the residue chomatographed via SiO_2 flash chromatography (eluant 4:1 hexane/EtOAc) to afford 5.7 g (70%) of **98** as a white solid: mp 95−97 °C; ^{1}H NMR ($CDCl_3$) δ 1.27 (t, J = 7 Hz, 3 H), 3.66 (s, 2 H), 4.19 (q, J = 7 Hz, 2 H), 5.13 (s, 2 H), 6.77 (brs, 1 H), 7.33 (m, 5 H); MS (EI) *m*/*z* (rel intensity) 252 (M^+, 5), 208 (16), 135 (21), 117 (33), 92 (36), 91 (99), 89 (11), 77 (13), 65 (32), 61 (15), 51 (10). Anal. ($C_{12}H_{16}N_2O_4$) C, H, N.

2-{1-(Aminocarbothioyl)-2-[(benzyloxy)carbonyl]hydrazino}acetic Acid (99). To a stirring suspension of **98** (3.0 g, 11.89 mmol) in EtOH (30 mL) at ambient temperature was added aqueous NaOH (1 N, 11.89 mL). To the mixture was added additional H_2O (10 mL) and stirring was continued for 1 h. (The mixture became a homogeneous solution and then a solid precipitated.) Aqueous HCl (1 N, 11.89 mL) was then added, the ethanol removed in vacuo and the aqueous extracted with EtOAc (2 × 100 mL). The organic layers were combined, dried over Na_2SO_4, and the solvent removed to afford 2.31 g (87%) of the CBz protected acid as white solid: mp 131−133 °C; ^{1}H NMR (CD_3OD) δ 3.59 (s, 2 H), 5.15 (s, 2 H), 7.37 (m, 5 H); MS (FAB) *m*/*z* (rel intensity) 225 (M + H, 55), 449 (7), 359 (14), 247 (19), 237 (7), 226 (7), 225 (55), 224 (12), 181 (8), 92 (9), 91 (99). Anal. ($C_{10}H_{12}N_2O_4 \cdot 0.12H_2O$) C, H, N.

To a stirring suspension of the acid (25.44 g, 112.7 mmol) in EtOAc (500 mL) was added trimethylsilyl isothiocyanate (14.79 g, 112.7 mmol) and the mixture was heated at gentle reflux (80 °C) overnight. The resulting solution was cooled to ambient temperature and washed with H_2O (2 × 100 mL). The organic layer was separated, dried over Na_2SO_4, and the solvent evaporated to dryness. The oily residue was dissolved in CH_2Cl_2 and allowed to stand at ambient temperature for 3 min in which time a solid formed. The solid was filtered, washed with CH_2Cl_2 (100 mL) and dried in vacuo. The solid was slurried in hot EtOAc (300 mL) to dissolve any sulfur related byproducts and triturated with hexane (200 mL) to afford 17.1 g of **99** (53%) as a white solid: mp 148−149 °C; ^{1}H NMR (CD_3OD) δ 5.20 (s, 2 H), 7.30 (m, 5 H) remaining CH_2 not observable; ^{13}C NMR (CD_3OD) δ 54.8, 68.7, 129.1, 129.2, 129.4, 137.1, 171.8, 185.3, 198.6; MS (FAB) *m*/*z* (rel intensity) 284 (M + H, 99), 567 (9), 566 (8), 392 (8), 362 (7), 361 (10), 360 (57), 285 (14), 284 (99), 92 (6), 91 (72). Anal. ($C_{11}H_{13}N_3O_4S$) C, H, N.

2-{1-[Hydrazino(imino)methyl]hydrazino}acetic Acid (53). To a stirring solution of **99** (5.0 g, 17.64 mmol) in EtOH (150 mL) at ambient temperature was added methyl iodide (2.73 g, 19.41 mmol) and the resulting solution stirred overnight. The solvent was removed in vacuo to afford 7.50 g (quant) of the *S*-methyl isothiosemicarbazide as a yellow foam: ^{1}H NMR (CD_3OD) δ 2.69 (brs, 0.6 H), 2.84 (brs, 0.4H), 4.40−4.70 (m, 2H), 5.31 (brs, 2H), 7.46 (m, 5H); MS (FAB) *m*/*z* (rel intensity) 298 (M + H, 99), 452 (4), 374 (4), 327 (4), 326 (21), 300 (6), 299 (15), 298 (99), 208 (3), 164 (12), 91 (24). Anal. ($C_{12}H_{15}N_3O_4S$) C, H, N.

To a vigorously stirring solution of this compound (25.5 g, 60 mmol) in H_2O (100 mL) at ambient temperature was added hydrazine hydrate (6.06 g, 120 mmol) slowly until 1/2 had been added. H_2O (10 mL) was added to the solid mass which had formed and the solids broken up mechanically with a spatula. The remaining hydrazine was then added and the solution vigorously stirred for 1 h. The heterogeneous mixture was sonicated and stirring continued until a thick mass had formed. EtOH (50 mL) was added, the solid filtered, washed with EtOH and dried in vacuo to afford 9.24 g (55%) of a white solid: mp 168−170 °C; ^{1}H NMR (D_2O) δ 3.86 (brs, 1 H), 4.21 (brs, 1 H), 5.17 (s, 2 H), 7.39 (s, 5 H); ^{13}C NMR (D_2O) δ 54.4, 68.7, 128.1, 128.3, 128.9, 135.3, 162.0, 169.1, 173.1; MS (FAB) *m*/*z* (rel intensity) 282 (M + H, 99), 358 (11), 283 (14), 282 (99), 281 (12), 264 (10), 238 (4), 192 (4), 148 (4), 91 (27), 87 (11). Anal. ($C_{11}H_{15}N_5O_4$) C, H, N.

To a solution of this solid (9.20 g, 32.71 mmol) in MeOH/H_2O (400 mL, ∼2:1 v/v) was added 10% Pd−C (1.0 g) and the mixture hydrogenated at 30 psi for 4 h. The catalyst was filtered through diatomaceous earth and 10% Pd−C (1.0 g) was again added. The mixture was hydrogenated at 30 psi for 2.5 h and determined to be complete by TLC (eluant 85:14:1 CH_2Cl_2/MeOH/HCO_2H). The mixture was filtered through diatomaceous earth and solvent concentrated to ∼50 mL at which time a solid precipitated. The solid was filtered, washed with a minimum amount of H_2O and dried in vacuo to afford 3.60 g (75%) of **53** as an off white solid: mp 196−198 °C. A second crop was obtained by concentrating the filtrate until a solid formed. Filtration afforded 0.90 g (19%, total yield: 94%) additional material having identical melting point: ^{1}H NMR (D_2O) δ 4.06 (s, 2 H); ^{13}C NMR (D_2O) δ 55.7, 160.1, 174.3; MS (FAB) *m*/*z* (rel intensity) 148 (M + H, 99), 302 (8), 300 (3), 295 (8), 256 (3), 224 (16), 180 (3), 149 (5), 148 (99), 147 (8), 87 (5). Anal. ($C_3H_9N_5O_2 \cdot 0.89H_2O$) C, H, N.

General Procedure for the Synthesis of 101. To a suspension of the amino acid (10.0 g, 0.11 mol) and triethylamine (33.5 mL, 0.24 mol) in EtOH (90 mL) and H_2O (6 mL) was added carbon disulfide (7.2 mL, 0.12 mol). After stirring overnight, methyl iodide (7.5 mL, 0.12 mol) was added to the yellow solution. The mixture was stirred for 1 h and concentrated to a slurry. The residue was dissolved in H_2O (25 mL), and concentrated HCl was added until acidic. The mixture was extracted with Et_2O (3 × 100 mL), and the organic phase was dried ($MgSO_4$) and concentrated to provide **101**.

Compound **101** (R = (*S*)-Me) was prepared from L-alanine according to the general procedure above; yield 93% of **101** (R = (*S*)-Me). A analytically pure sample was obtained by recrystallization from Et_2O/hexane: mp 90−92 °C; ^{1}H NMR (D_2O) δ 4.89 (q, J = 7 Hz, 1 H), 2.59 (s, 3 H), 1.52 (d, J = 7 Hz, 3 H); ^{13}C NMR (D_2O) δ 201.5, 176.0, 55.7, 17.5, 16.9; MS (EI) *m*/*z* (rel intensity) 179 (M^+, 93), 132 (26), 131 (14), 91 (30), 88 (16), 86 (99), 60 (20), 59 (10), 48 (10), 46 (13). Anal. ($C_5H_9NO_2S_2$) C, H, N, S.

General Procedure for the Synthesis of 102. To a solution of the dithocarbamate **101** (28 mmol) in methylene chloride (50 mL) at 0 °C was added methyl trifluoromethanesulfonate (3.5 mL, 31 mmol). The mixture was warmed to room temperature and stirred for 20 h. The mixture was concen-

trated under reduced pressure to a colorless oil. The resulting oil was dissolved in H_2O (5 mL), and 1.0 M NaOH (28 mmol) was added. The mixture was extracted with EtOAc (3 × 100 mL), and the organic phase was dried ($MgSO_4$). After filtration, the solvent was removed in vacuo to provide **102**.

Compound **102** (R = (*S*)-Me) was prepared from **101** (R = (S)-Me) according to the general procedure above: ^{1}H NMR (MeOH) δ 4.46 (q, J = 7.0 Hz, 1 H), 2.59 (s, 3 H), 2.42 (s, 3 H), 1.40 (d, J = 7.0 Hz, 3 H).

General Procedure for the Synthesis of 54−58. 102 was dissolved in absolute EtOH (25 mL), and anhydrous hydrazine (0.14 mol) was added. The mixture was stirred for 1.5 h, and the solid which formed was collected by filtration. The white powder was further purified by crystallization from H_2O/IPA to give **54−58.**

(2*S*)-2-[(Dihydrazinomethylene)amino]propanoic Acid (54). Following the general procedure, **54** was prepared from **102** (R = (*S*)-Me): yield 49% as a white powder; mp 174−176 °C dec; ^{1}H NMR (D_2O) δ 3.69 (q, J = 7 Hz, 1 H), 1.20 (d, J = 7 Hz, 3 H); ^{13}C NMR (D_2O) δ 178.7, 157.6, 51.6, 18.3; MS (FAB) *m*/*z* (rel intensity) 162 (M + H, 99), 323 (14), 296 (4), 163 (7), 162 (99), 161 (5), 147 (8), 144 (11), 116 (4), 44 (8), 18 (3); HRMS (FAB) calcd for $C_4H_{11}N_5O_2$ + H 162.0991, found 162.0994. Anal. ($C_4H_{11}N_5O_2$) C, H, N.

***N*-[Bis(methylsulfanyl)methylene]-β-alanine (104a).** A solution of **103a**[22] (12.12 g, 67.62 mmol) and methyl iodide (9.7 mL, 156 mmol) in acetone (100 mL) was stirred overnight at room temperature. NMR analysis of the mixture indicated it was only about 60% complete. The mixture was then stirred at reflux for 5 h. After cooling to room temperature, the mixture was filtered, giving **104a** as a fine white solid (19.57 g, 90%) which was analytically pure: mp 138 °C dec; ^{1}H NMR (D_2O) δ 3.99 (t, J = 7 Hz, 2 H), 2.90 (t, J = 7 Hz, 2 H), 2.84 (s, 3 H), 2.79 (s, 3 H); ^{13}C NMR (D_2O) δ 193.7, 174.9, 44.3, 31.8, 16.0, 15.7; MS (FAB) *m*/*z* 194 (M + H), 348, 224, 196, 195, 194, 180, 148, 146, 134, 73; HRMS (FAB) calcd for $C_6H_{11}NO_2S_2$ + H 194.0309, found 194.0320. Anal. ($C_6H_{12}INO_2S_2$) C, H, N, S.

3-[(Dihydrazinomethylene)amino]propanoic Acid (59). To a 0 °C solution of **104a** (10.00 g, 31.15 mmol) in 1.00 M aq NaOH (31 mL) was added hydrazine monohydrate (7.57 mL, 156 mmol). The solution was stirred 5 min before removing the ice bath. Upon warming to room temperature, gas evolution became apparent. After stirring for 3.5 h at room temperature, the solution was chilled in an ice bath and diluted with 2-propanol in 50 mL portions (with seeding) to a total volume of 600 mL. The mixture was sonicated and stirred 15 min at 0 °C before collecting the resulting light pink solid by filtration (4.21 g). The crude solid was dissolved in water (50 mL), treated with Norite, and filtered through a fine glass frit, giving an almost colorless solution. The solution was diluted with 2-propanol to a volume of 1 L, seeded, and left at room temperature overnight. The mixture was briefly sonicated to losen crystals on the side, resulting in precipitation of additional product. The mixture was chilled 4 h in a refrigerator before filtering, affording **59** (3.16 g, 63%) as fine white crystals: mp 192 °C dec; ^{1}H NMR (D_2O) δ 3.39 (t, J = 7 Hz, 2 H), 2.48 (t, J = 7 Hz, 2 H); ^{13}C NMR (D_2O) δ 180.1, 158.2, 37.8, 36.9; MS (FAB) *m*/*z* 162 (M + H), 323, 316, 296, 238, 163, 162, 161, 147, 118, 89; HRMS (FAB) calcd for $C_4H_{11}N_5O_2$ + H 162.0991, found 162.0992. Anal. ($C_4H_{11}N_5O_2 \cdot 0.43H_2O$) C, H, N.

2-({(*Z*)-Hydrazino[(2-hydroxyethyl)amino]methylidene}amino)acetic Acid (61). A solution of **104a** (5.00 g, 27.9 mmol) and ethanolamine (1.68 mL, 27.9 mmol) in water (13 mL) was stirred at room temperature for 24 h. The solution was diluted with THF to a volume of 250 mL. An oil separated which solidified with stirring. The mixture was stirred and sonicated until the solid was finely divided. Filtration gave a white solid (3.85 g), sufficiently pure to carry into the next step. Structure analysis showed that both methanethiol groups has been displaced by ethanolamine to give the cyclic oxazolidine. An analytical sample was obtained by recrystallization as follows: 0.85 g crude was dissolved in water (20 mL). The solution was diluted with 2-propanol (100 mL), seeded, and left at 0 °C for several hours, affording a fine white solid (542 mg): mp 198 °C dec; MS (FAB) *m*/*z* (rel intensity) 145 (M + H, 99), 299 (5), 290 (2), 289 (15), 221 (2), 146 (6), 145 (99), 143 (2), 100 (2), 99 (4), 87 (2); HRMS (FAB) calcd for $C_5H_8N_2O_3$ + H 145.0613, found 145.0611. Anal. ($C_5H_8N_2O_3$) H, N; C: calcd, 41.67; found, 41.06.

A solution of the solid from above (3.00 g, 20.8 mmol) in water (20 mL) was cooled to 0 °C before the addition of hydrazine monohydrate (3.03 mL, 62.4 mmol). The solution was stirred at 0 °C for 10 min and then at room temperature for 5 h. The solution was diluted with 2-propanol to a volume of 250 mL and seeded with authentic product. Precipitation of product commenced immediately. After 30 min of occasional stirring and sonication, the mixture was filtered, providing the product as a white solid (2.87 g, 78%): mp 182 °C dec; ^{1}H NMR (D_2O) δ 3.82 (s, 2 H), 3.74 (t, J = 5 Hz, 2 H), 3.39 (bs, 2 H); ^{13}C NMR (D_2O) δ 175.4, 157.2, 60.2, 44.3, 43.2; MS (FAB) *m*/*z* (rel intensity) 177 (M + H, 99), 353 (10), 178 (7), 177 (99), 176 (4), 175 (2), 162 (2), 159 (3), 102 (2), 62 (2), 44 (2). Anal. ($C_5H_{12}N_4O_3$) C, H, N.

2-({Bis[2-(*tert*-butoxycarbonyl)hydrazino]methylene}amino)acetic Acid (65). To a suspension of **104a** (0.18 g, 1 mmol) in THF (1 mL) was added *tert*-butyl carbazate (0.66 g, 5 mmol). The resulting homogeneous solution was stirred at room temperature overnight. The solid which formed was collected by filtration and washed with cold THF to provide 0.23 g (66%) of **65** as a white solid: mp 179−180 °C dec; ^{1}H NMR (D_2O) δ 3.85 (s, 1 H), 1.46 (s, 1 H); ^{13}C NMR (D_2O) δ 174.0, 158.8, 157.5, 83.4, 44.5, 27.5; MS (FAB) *m*/*z* (rel intensity) 348 (M + H, 99), 695 (11), 349 (17), 348 (99), 292 (17), 237 (7), 236 (81), 192 (6), 57 (23), 41 (6), 29 (6). Anal. ($C_{13}H_{25}N_5O_6$) C, H; N: calcd, 20.16; found, 19.71.

Methyl 2-{[Chloro(methylsulfanyl)methylidene]amino}acetate (106). To a stirring solution of bis(methylthio)methyleneglycine ethyl ester **(105)** (5.0 g, 25.9 mmol) in CH_2Cl_2 (45 mL) at 0 °C was added SO_2Cl_2 (2.28 mL, 28.84 mmol) in CH_2Cl_2 (5 mL) dropwise over 20 min. After addition, the mixture was stirred at 0 °C for 10 min then allowed to warm to ambient temperature over 1h. The solvent was removed in vacuo to afford 4.7 g (quant) of the desired product as a slightly yellow liquid. This material was suitable for use without further purification: ^{1}H NMR ($CDCl_3$) δ 2.5 (s, 3 H), 3.78 (s, 3 H), 4.37 (s, 2 H); ^{13}C NMR ($CDCl_3$) δ 16.4, 51.8, 54.0, 144.8, 168.8.

2-{[(*E*)-Hydrazino(1*H*-pyrazol-5-ylamino)methylidene]amino}acetic Acid (74). To a stirring solution of 3-aminopyrazole (4.30 g, 51.7 mmol) in CH_2Cl_2 (60 mL) at 0 °C was added **106** (9.40 g, 51.7 mmol) in CH_2Cl_2 (20 mL) dropwise over 15 min. The resulting green suspension was stirred at 0 °C for 30 min, then allowed to warm to ambient temperature and stirred 1 h. H_2O (100 mL) and CH_2Cl_2 (50 mL) were added, the layers shaken, and the aqueous layer separated. The aqueous layer was neutralized with solid $NaHCO_3$ to pH 7 and extracted with CH_2Cl_2 (3 × 75 mL). The organic layers were combined, dried over Na_2SO_4 and solvent removed under reduced pressure to afford 5.40 g (45%) of the desired compound as a tan solid: mp 95−97 °C; ^{1}H NMR ($CDCl_3$) δ 3.74 (s, 3 H), 4.17 (s, 2 H), 6.06 (brs, 1 H), 7.41 (d, J = 3 Hz, 1 H) (NH's absent); MS (EI) *m*/*z* (rel intensity) 228 (M^+, 64), 228 (64), 181 (49), 169 (12), 153 (39), 146 (16), 140 (27), 125 (19), 124 (40), 121 (99), 114 (38). To a stirring suspension of this solid (5.0 g, 21.9 mmol) in H_2O (5 mL) at 0 °C was added aqueous NaOH (1 M, 21.9 mL) and EtOH (2 mL). The resulting green solution was treated with hydrazine monohydrate (5.47 g, 109 mmol) and the resulting solution was warmed to room temperature and stirred overnight. Aqueous HCl (1 M, 21.9 mL) was added and the resulting precipitate cooled to 0 °C and filtered. The solid was dried in vacuo at 25 °C to afford 1.62 g of **74**. Additional material which appeared in the filtrate (2.0 g) was filtered and found to be identical to the first crop (total yield 83%): mp 205−207 °C dec; ^{1}H NMR (D_2O) δ 3.97 (s, 2 H), 6.00 (d, J = 3 Hz, 1 H), 7.49 (d, J = 3 Hz, 1 H); MS (FAB) *m*/*z* (rel intensity) 199 (M + H, 99), 397 (2), 353 (4),

289 (2), 275 (3), 245 (2), 200 (9), 199 (99), 198 (7), 177 (2), 84 (2). Anal. ($C_6H_{10}N_6O_2 \cdot H_2O$) C, H, N.

2-[{[2-(2-Pyridinyl)hydrazino]carbothioyl}amino]-acetic Acid (108). To a mixture of 2-hydrazinopyridine (4.6 g, 24.2 mmol) in absolute EtOH (25 mL) at 0 °C was added ethyl isothiocyanatoacetate (3.0 mL, 23.0 mmol), and the mixture was stirred at 0 °C for approximately 20 min. A thick suspension had formed which made stirring difficult. The ice bath was removed, and absolute EtOH was added until stirring resumed. The mixture was then stirred at room temperature for 30 min, cooled to 0 °C, and filtered. The solid was washed with ice cold absolute EtOH and dried (house vacuum, 50 °C) to yield 4.5 g (77%) the desired thiosemicarbazide as a white solid: mp 149−151 °C dec; ^{1}H NMR (DMSO) δ 9.63 (s, 1 H), 8.50 (s, 1 H), 8.40 (m, 1 H), 8.12 (d, J = 4 Hz, 1 H), 7.62 (t, 1 H), 6.81 (t, 1 H), 6.57 (d, J = 8 Hz, 1 H), 4.18 (d, J = 6 Hz, 2 H), 4.08 (q, J = 7 Hz, 2 H), 1.18 (t, J = 7 Hz, 3 H); ^{13}C NMR (DMSO) δ 185, 172, 161, 148.5, 148, 140.0, 139, 117.7, 117.6, 109, 108.7, 62.3, 57, 46.4, 18, 14.5; MS (EI) m/z (rel intensity) 254 (M^+, 26), 254 (26), 221 (99), 152 (20), 151 (93), 147 (29), 109 (36), 94 (50), 93 (17), 79 (41), 67 (21); HRMS (EI) calcd for $C_{10}H_{14}N_4O_2S$ 254.0837, found 254.0548. Anal. ($C_{10}H_{14}N_4O_2S \cdot 0.21H_2O$) C, H, N, S.

A mixture of this solid (4.5 g, 17.7 mmol) in 1.0 M NaOH (35 mL) was stirred at room temperature for 1.5 h. 1.0 M HCl (35 mL) was added, and a solid immediately precipitated. The solid was collected by filtration, and dried (house vacuum 50 °C, 18 h) to provide 3.9 g (98%) of **108** as a tan solid: mp 198−199 °C dec; ^{1}H NMR (DMSO) δ 9.55 (s, 1 H), 8.43 (s, 1 H), 8.10 (m, 1 H), 8.10 (d, 1 H), 7.59 (t, 1 H), 6.78 (t, 1 H), 6.56 (d, J = 6 Hz, 1 H), 4.11 (d, J = 4 Hz, 2 H); ^{13}C NMR (DMSO) δ 183.2, 171.4, 159.8, 148.1, 138.2, 116.1, 107.4, 45.5; MS (EI) m/z (rel intensity) 226 (M^+, 13), 208 (81), 193 (72), 175 (59), 151 (99), 136 (79), 109 (33), 94 (58), 93 (70), 79 (69), 78 (35); HRMS (FAB) calcd for $C_8H_{10}N_4O_2S$ + H 227.0603, found 227.0600. Anal. ($C_8H_{10}N_4O_2S \cdot 0.21H_2O$) C, H, N, S.

2-({(*E*)-Hydrazono[2-(2-pyridinyl)hydrazino]methyl}-amino)acetic Acid (76). To a mixture of **108** (3.65 g, 16.1 mmol) and triethylamine (4.5 mL, 32.2 mmol) in MeOH (15 mL) at room temperature was added methyl iodide (3.0 mL, 48.3 mmol). After approximately 3 min, an exothermic reaction was observed, and a yellow solid precipitated. The mixture was stirred at room temperature for 3 h, and then cooled to 0 °C. The solid was collected by filtration, washed with cold MeOH, and dried (house vacuum, 50 °C, 18 h) to give 2.8 g (72%) of the desired product (2:1 mixture of rotational isomers) as a bright yellow solid: mp 149−151 °C; ^{1}H NMR (DMSO + DCl) δ 8.00 (m, 2 H), 7.25 (m, 1 H), 7.00 (m, 1 H), 4.28, 4.15 (s, 2 H), 2.72, 2.55 (s, 3 H); ^{13}C NMR (DMSO + DCl) δ 170.9, 151.3, 144.4, 140.3, 136.8, 115.1, 112.9, 112.4, 45.6, 45.2, 14.5, 14.1; MS (FAB) m/z (rel intensity) 241 (M + H, 99), 317 (3), 243 (5), 242 (13), 241 (99), 240 (7), 193 (8), 166 (6), 116 (4), 95 (6), 94 (4); HRMS (FAB) calcd for $C_9H_{12}N_4O_2S$ + H 241.0759, found 241.0766.

To a suspension of this solid (2.6 g, 10.9 mmol) in absolute EtOH (11 mL) at room temperature was added hydrazine monohydrate (1.1 mL, 21.7 mmol). After stirring for approximately 2 h, a homogeneous solution formed. After stirring for approximately an additional 1 h (3 h total), a solid precipitated, and the mixture was cooled to 0 °C. The solid was isolated by filtration, and washed with cold absolute EtOH to yield 2.0 g (82%) of **76** as a white solid: mp 187−188 °C; ^{1}H NMR (D_2O) δ 8.06 (d, 1 H), 7.70 (3, 1 H), 6.95 (3, 1 H), 6.85 (d, 1 H), 3.70 (br s, 2 H); MS (EI) m/z (rel intensity) 206 (39), 148 (12), 147 (99), 94 (44), 93 (10), 79 (22), 78 (38), 67 (19), 66 (9), 51 (10); MS (FAB) m/z (rel intensity) 225 (M + H, 99), 449 (7), 377 (3), 301 (15), 226 (11), 225 (99), 224 (27), 180 (4), 108 (4), 95 (11), 94 (6); HRMS (FAB) calcd for $C_8H_{12}N_6O_2$ + H 225.1100, found 225.1098. Anal. ($C_8H_{12}N_6O_2 \cdot 0.4H_2O$) C, H, N.

5-(Aminomethyl)-2-piperidinone (110). In a hydrogenation vessel was placed bisnitrile **109**[26] (11 g, 66 mmol) in ethanol (absolute, 600 mL) and Raney nickel (50% aqueous emulsion, 50 g). The heterogeneous solution was hydrogenated at 40 psi H_2 while being shaken vigorously over a period of 6 h at which time the hydrogen pressure was reduced to atmospheric pressure. The reaction vessel was recharged with H_2 (40 psi) and shaken over a period of 17 h. The reaction mixture was filtered over a plug of Celite (rinsing with wet ethanol) and concentrated to give an amorphous solid. Purification via column chromatography (silica gel, 550 g, 15% MeOH/CH_2Cl_2 doped with 3% concentrated ammonium hydroxide) afforded 5.1 g (61%) of **110** as an amorphous solid. Analytical material could be obtained via recrystallization with Et_2O/$CHCl_3$ (1:1, 2% methanol) at −20 °C: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53 (br s, 1 H), 3.46−3.37 (m, 1 H), 2.99 (dd, J = 10, 10 Hz, 1 H), 2.71−2.65 (m, 2 H), 2.46−2.25 (m, 2 H), 1.98−1.86 (m, 1 H), 1.86−1.74 (m, 3 H), 1.55−1.44 (m, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.6, 45.1, 44.5, 36.1, 30.1, 24.4; HRMS calcd for $C_6H_{12}N_2O$ m/z 128.0905, found m/z 128.0960.

5-Amino-4-(aminomethyl)pentanoic Acid Dihydrochloride (78). To a stirring solution of **110** (1.57 g, 12.3 mmol) and water (24 mL) was added concentrated hydrochloric acid (12 M, HCl, 4.0 mL, 50 mmol), and the solution was heated to reflux over a period of 18 h and concentated to give an amorphous solid. The crude solid was recrystallized by dissovling in hot water/ethanol (1:4, 25 mL) and cooling to −20 °C over a period of 48 h. **78** was collected by filtration to give 1.43 g (53%) as a white solid: mp 183−184 °C; ^{1}H NMR (300 MHz, D_2O) δ 3.19−2.99 (m, 4 H), 2.54 (t, J = 7 Hz, 2 H), 2.21 (heptet, J = 6 Hz, 1 H), 1.82 (q, J = 6 Hz, 2 H); ^{13}C NMR (75 MHz, D_2O) δ 177.2, 40.1, 34.4, 29.9, 22.9; MS (FAB) m/z (rel intensity) 147 (M + H, 99), 247 (3), 245 (7), 148 (8), 147 (99), 130 (3), 129 (6), 118 (5), 116 (3), 45 (3), 30 (5). Anal. ($C_6H_{14}N_2O_2 \cdot 2HCl$) C, H, N.

2-[(4-Amino-1,2,4-triazinan-3-ylidene)amino]acetic Acid Monohydrate (79). To a stirring solution of **104a** (11.4 g, 37.0 mmol) in water (300 mL) was added a solution of ethylene bis(hydrazine)[27] (3.3 g, 37.0 mmol) and aqueous sodium hydroxide (1 M NaOH, 37 mL) via pipet. The reaction mixture was allowed to stir at ambient temperature over a period of 22 h and concentrated to give a tan solid. The crude solid was dissolved in hot ethanol/water (120 mL, 10 mL) and filtered to remove insoluble material and concentrated to give 3 g of a viscous oil. Purification via column chromatography (silica gel, 500 g, 7:2:1 MeOH/CH_2Cl_2/NH_4OH(conc)) gave 2.0 g (31%) of **79** ~95% purity judged by ^{1}H NMR. Analytically pure material could be obtained by dissolving in hot ethanol and triturating with diethyl ether at 0 °C: white solid; mp 130−132 °C (sublimed); ^{1}H NMR (300 MHz, D_2O) δ 3.74 (s, 2 H), 3.57 (t, J = 6 Hz, 2 H), 3.21 (t, J = 6 Hz, 2 H); ^{13}C NMR (75 MHz, D_2O) δ 175.7, 155.4, 50.6, 44.4, 42.4. Anal. ($C_5H_{11}N_5O \cdot H_2O$) C, H, N.

2-{[(2-Acetylhydrazino)(methylsulfanyl)methylidene]-amino}acetic Acid (111 (R = C(O)CH_3)). A solution of **104a** (1.00 g, 5.58 mmol) and acetic hydrazide (0.62 g, 8.4 mmol) in 2-propanol (5.6 mL) was stirred at room temperature for 1 h. Cold water bath cooling was used for the first 5 min. The precipitate was collected by filtration, affording **111** (R = C(O)-CH_3) as a white solid (1.04 g, 91%): mp 118 °C dec; ^{1}H NMR (D_2O) δ 4.00 (bs, 2 H), 2.68, 2.48 (2 bs, 3 H), 2.11 (bs, 3 H); ^{13}C NMR (D_2O) δ 173.9, 171.5, 47.3, 20.3 (broad), 13.3 (broad), one peak missing; MS (FAB) m/z (rel intensity) 206 (M + H, 99), 296 (6), 260 (6), 216 (6), 207 (9), 206 (99), 205 (7), 149 (7), 132 (6), 109 (6), 45 (7); HRMS (FAB) calcd for $C_6H_{11}N_3O_3S$ + H 206.0599, found 206.0605. Anal. ($C_6H_{11}N_3O_3S$) C, H, N.

2-{[6-Methyl-1,4-dihydro-1,2,4,5-tetraazin-3(2*H*)-ylidene]-amino}acetic Acid (80). To a mixture of **111** (R = C(O)CH_3) (7.53 g, 36.7 mmol) in water (37 mL), cooled in a water bath, was added hydrazine monohydrate (3.56 mL, 73.5 mmol). The resulting solution was stirred at room temperature for 24 h. The solution was diluted with 2-propanol to a volume of 500 mL. An oil separated which was induced to solidify by sonication and seeding with authentic product. After standing at 0 °C overnight, filtration afforded **80** as a white solid (1.74 g, 28%): mp >280 °C; ^{1}H NMR (D_2O) δ 3.92 (s, 2 H), 2.39 (s, 3 H); ^{13}C NMR (D_2O) 175.7, 152.3, 151.1, 45.7, 9.1; MS (FAB) m/z (rel intensity) 172 (M + H, 99), 344 (2), 343 (8), 326 (4), 280 (1), 248 (4), 173 (9), 172 (99), 127 (2), 126 (5), 111 (1);

HRMS (FAB) calcd for $C_5H_9N_5O_2$ + H 172.0835, found 172.0832. Anal. ($C_5H_9N_5O_2$) C, H, N.

2-{[(1-Methylhydrazino)carbothioyl]amino}acetic Acid (113). To a mixture of methyl hydrazine (0.86 mL, 16 mmol) in absolute EtOH (15 mL) at 0 °C was added ethyl isothiocyanatoacetate **(112)** (2 mL, 16 mmol) dropwise over 5 min. The mixture was stirred at 0 °C for an additional 10 min, warmed to room temperature, and stirred for 0.5 h. After the mixture was cooled back to 0 °C, the white solid was isolated by filtration, washed with cold EtOH, and dried (house vacuum, 50 °C, 18 h) to yield 2.1 g (68%) of the desired thiosemicarbazide as a white solid: mp 121−123 °C; ^{1}H NMR (CD_3OD) δ 4.28 (s, 2 H), 4.18 (q, J = 7 Hz, 2 H), 3.55 (s, 3 H), 1.27 (t, J = 7 Hz, 3 H); ^{13}C NMR (CD_3OD) δ 183.2, 172.0, 62.0, 47.2, 43.3, 14.3; MS (FAB) m/z (rel intensity) 192 (M + H, 99), 383 (5), 300 (5), 268 (25), 194 (5), 193 (8), 192 (99), 191 (11), 160 (6), 89 (11), 46 (11); HRMS (FAB) calcd for $C_6H_{13}N_3O_2S$ + H 192.0807, found 192.0805. Anal. ($C_6H_{13}N_3O_2S$) C, H, N.

To a portion of this solid (1.7 g, 8.8 mmol) was added 1.0 M NaOH (18 mL) and the mixture was stirred for 1 h. The solid dissolved after 10−15 min of stirring to produce a homogeneous solution. 1.0 M HCl (18 mL) was added, and the volume was reduced in vacuo until a solid formed. The mixture was cooled to 0 °C and filtered to yield 1.06 g (74%) of **113** as a white solid: mp 151−153 °C; ^{1}H NMR (CD_3OD) δ 4.27 (s, 2 H), 3.55 (s, 3 H); ^{13}C NMR (CD_3OD) δ 183.2, 173.8, 47.2, 43.5; MS (FAB) m/z (rel intensity) 164 (M + H, 99), 374 (8), 316 (6), 298 (21), 272 (11), 240 (39), 174 (6), 165 (6), 164 (99), 163 (18), 46 (6); HRMS (FAB) calcd for $C_4H_9N_3O_2S$ + H 164.0494, found 164.0504. Anal. ($C_4H_9N_3O_2S$) C, H, N, S.

3-Hydrazino-2-methyl-2,5-dihydro-1,2,4-triazin-6(1*H*)-one (82). To a suspension of **113** (0.40 g, 2.45 mmol) in MeOH (2.5 mL) was added methyl iodide (0.46 mL, 7.40 mmol). The mixture was heated at reflux for 1 h, then cooled to 0 °C. The solid was isolated by filtration and washed with cold MeOH to give 0.23 g of a white solid. To the filtrate was added Et_2O (30 mL), and the mixture was cooled to 0 °C. Filtration provided a second crop 0.38 g of a white solid. The two crops were combined to yield 0.61 g (86%) of a white solid: mp 188−189 °C dec; ^{1}H NMR (D_2O) δ 3.89 (s, 2 H), 3.37 (s, 3 H), 2.47 (s, 3 H); MS (FAB) m/z (rel intensity) 160 (M + H, 99), 447 (3), 319 (7), 236 (2), 162 (7), 161 (7), 160 (99), 159 (4), 146 (1), 114 (1), 88 (4); HRMS (FAB) calcd for $C_5H_9N_3OS$ + H 160.0545, found 160.0547. Anal. ($C_5H_9N_3OS\cdot HI$) C, H, N, S.

To a suspension of the solid from above (7.42 g, 25.8 mmol) in absolute EtOH (25 mL) was added 12.5 M NaOH (1.94 mL). Hydrazine monohydrate (2.4 mL, 48.6 mmol) was added to the mixture, and the mixture was stirred at room temperature for 1 h. A solid precipitated producing a thick suspension, and absolute EtOH (30 mL) was added. The mixture was stirred for an additional 1 h and filtered. The isolated solid was washed with cold absolute EtOH and dried to give 3.1 g of a white solid. A second crop (1.3 g) was realized by allowing the filtrate to stand at room-temperature overnight. The two crops were combined (4.4 g), and recrystallized ($EtOH/H_2O$) to provide 2.2 g (52%) of **82** (monohydrate) as a white solid: mp 189−191 °C; ^{1}H NMR (D_2O) δ 3.52 (s, 2 H), 2.92 (s, 3 H); ^{13}C NMR (D_2O) δ 165.3, 151.8, 40.8, 36.4; MS (EI) m/z (rel intensity) 143 (M^+, 99), 100 (24), 99 (20), 70 (26), 69 (20), 58 (21), 57 (33), 55 (38), 46 (62), 45 (30); HRMS (EI) calcd for $C_4H_9N_5O$ 143.0807, found 143.0813. Anal. ($C_4H_9N_5O\cdot H_2O$) C, H, N.

Biological Procedures. In Vivo Antihyperglycemic Activity in KKA^y Mice. New analogues were evaluated in obese hyperglycemic, hyperinsulinemic, insulin-resistant KKA^y mice as previously described.[29] Animals were grouped into treatment and control groups (n = 6) following pretest blood glucose measurements with an Alpkem glucose autoanalyzer. Treatment groups had the selected compound administered as a food mixture at 500 mg/kg for 4 days. For those compounds with T/C < 0.80, the control values were statistically compared to the treatment values using the nonparametric Wilcoxon rank sum test. Those compounds determined to effect statistically significant reductions in nonfasting blood glucose are indicated with asterisks as defined in the tables. For comparison, administration of troglitazone (Rezulin) in this assay at a dose of 200 mg/kg gives a T/C of 0.78, and administration of pioglitazone[43] at a dose of 100 mg/kg gives a T/C of 0.49. Metformin (Glucophage) is not active in the assay when administered at 500 mg/kg.

Dose−Response Studies in KKA^y Mice. Experiments were run as described above. The difference between the pre- and post-treatment blood glucose values was computed for individual animals. These delta values were used to compare treatment groups using a one-way analysis of variance. Treatment groups were compared to the control group using a t-test where the estimate of the standard error comes from a pooled estimate using all the treatment groups. The food difference data for each treatment group was compared in the same fashion. All calculations were done using the SAS computer package.[36]

Body Composition Analysis. Diabetic, obese KKA^y mice of either sex, aged 12−16 weeks, were obtained from Clea Nippon (Osaka, Japan[37]). Diabetic, obese female C57BL6J *ob/ob* mice, aged 10−14 weeks, were obtained from Jackson Laboratories (Bar Harbor, ME[38]). Mice were housed in the Pharmacia Kalamazoo animal care facility at 21 ± 1 °C and kept on a 12 h light−dark cycle. Fresh water and rodent chow were available at all times. Diabetic mice received a diet of Purina 5015 chow (26% of calories from fat; 4.35 kcal/g) and normal mice and rats received Purina 5002 rodent chow (11.6% of calories from fat; 4.06 kcal/g). Test compounds were administered as an admixture in milled Purina chow for 14 days. Drug intake was determined by measuring the difference in the weight of the food bowl before and after feeding. Blood samples were obtained by puncture of the retro-orbital sinus. Heparinized blood was stored on ice and promptly analyzed or centrifuged for preparation of plasma. Plasma samples were stored at −80 °C until assayed. Body composition was assessed by injecting ^{3}HOH intraperitoneally using the method of Pace et al.[39] The lean and fat body mass was determined based on the difference in the ^{3}HOH space of the two types of tissue. The ^{3}HOH space was converted to lean body mass according to eq 1. Fat body mass was calculated according to eq 2:

$$\text{LBM} = (^3\text{HOH space})/0.729 \quad (1)$$

where LBM = lean body mass and

$$\text{FBM} = \text{BW} - \text{LBM} \quad (2)$$

where FBM = fat body mass and BW = body weight.

Studies in Nonhuman Primates. Adult rhesus macaques (Macaca mulatta) were maintained on a nutritionally adequate diet of PMI Certified Primate bisquits (St. Louis, MO) and a piece of fruit daily. The animals were all surgically ovariectomized females and varied in age from 15−30 years. These monkeys were selected because their fasting plasma insulin and glucose concentrations were elevated relative to the mean value for normal rhesus monkeys in our colony (20 μU/mL and 59 mg/dL, respectively; n = 14). Oral dosing was performed by dissolving the test substance in diet orange soda and allowing the animals to voluntarily drink the solution from the tip of a syringe. Dosing was performed once daily at approximately 8:00 a.m. Meal tolerance tests were performed in conscious monkeys that had been fasted overnight. A mixed liquid meal was administered via an orogastric tube to start the test. The composition of the meal was carbohydrate, 5.21 kcal/kg body weight; protein, 1.12 kcal/kg; fat, 2.34 kcal/kg. Blood samples were obtained by percutaneous puncture of the femoral vein at t = 0, 30, 60, 90, and 120 min.

Glucose was assayed using either a YSI Biochemical Analyzer or an Alpkem Autoanalyzer. Insulin concentration was determined with a double-antibody radioimmunoassay technique using guinea pig antihuman insulin serum and ^{125}I-porcine insulin as the tracer (DuPont-NEN, Wilmington, DE). For assays of monkey samples, human insulin was used as the standard (Linco, St. Louis, MO).

Statistical analysis was performed using SigmaStat Software (Jandel, San Rafael, CA). In studies that used mice, two-way ANOVA was used. Post hoc comparison of sample means was performed using the Student−Newman−Keuls procedure. In the studies that used monkeys, the sample sizes were small and animals were often heterogeneous with respect to the measured parameters. To compensate for the high inter-subject variability, the results were analyzed using a multiway ANOVA design. Three-way ANOVA was used in order to partition the sources of variation according to subject, treatment, and time. With this approach, the variation due to inter-subject differences was partitioned to allow concentration on the effects attributable to the treatment and time. Post hoc comparison of sample means was performed using the Tukey Test.

In Vitro Evaluation of Creatine Uptake/Kinase. These assays were run as described in the previous manuscript. Results are reported in Tables 10 and 11.

Supporting Information Available: Numerical tabular data to support Figures 1−3 and experimental procedures for analogues **3, 5, 6, 8, 14, 15, 17, 19, 21**−**27, 29, 30, 34**−**37, 39, 40, 42, 44, 46**−**51, 55**−**58, 60, 62**−**64, 66**−**73, 75, 77**, and **81**. This material is available free of charge via the Internet at http://pubs.acs.org.

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