Synthesis and Biological Activity of Analogues of the Antidiabetic/Antiobesity Agent 3-Guanidinopropionic Acid: Discovery of a Novel Aminoguanidinoacetic Acid Antidiabetic Agent

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3-Guanidinopropionic acid (**1**, PNU-10483) has been demonstrated to both 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 **4**. In an effort to identify novel entities that maintain antidiabetic potency without susceptibility to creatine-like metabolism, an analogue program was undertaken to explore the effects of various structural modifications, including homologation, simple substitution, single atom mutations, and bioisosteric replacements for the guanidine and carboxylic acid. Overall, the scope of activity encompassed by the set of new analogues proved to be exceedingly narrow. Notable exceptions demonstrating equivalent or improved antidiabetic activity included the α-amino derivative **29**, aminopyridine **47,** isothiourea **67**, and aminoguanidine **69**. On the basis of its superior therapeutic ratio, aminoguanidine **69** was selected for preclinical development and became the foundation for a second phase of analogue work. Furthermore, in vitro studies demonstrated that **69** is markedly less susceptible to phosphorylation by creatine kinase than the lead **1,** suggesting that it should have less potential for accumulation in muscle tissue than **1**.

Introduction

Non-insulin-dependent diabetes mellitus (NIDDM) is a metabolic disorder characterized by a resistance of the peripheral target tissues to fully respond to the binding of insulin and insufficient insulin secretion by the pancreas to overcome this reduced response.[1,2] The result of these two pathologies is impaired glucose uptake and metabolism, leading to fasting hyperglycemia. The etiology of NIDDM is complex but is now generally accepted to entail the initial development of insulin resistance in the prediabetic state that leads to compensatory hyperinsulinemia. Eventually the *β*-cells of the pancreas can no longer maintain the hyperinsulinemic state, and the ensuing insulin deficiency leads to chronic hyperglycemia. Thus insulin resistance appears to be the trigger for the development of the final disease, and there is reason to believe that improvement in the insulin sensitivity of target tissues may not only impede the development of NIDDM but may actually prevent its onset altogether.[3,4]

Meglasson et al. recently reported that 3-guanidinopropionic acid (**1**) possesses both antihyperglycemic and antiobesity activity in the KK^{y} mouse, a rodent model of NIDDM.[5,6] Although the biochemical mechanism for these effects remains obscure, it was shown that glucose disposal was augmented without effects on gluconeogenesis, hepatic glycogen content, or intestinal glucose absorption. Furthermore, insulin suppression tests indicated an improvement in insulin sensitivity. In *ob/ob* mice, **1** reduces both hyperglycemia and hyperinsulinemia, and in insulin-resistant rhesus monkeys it accelerates the disappearance of i.v. glucose.[6] Although the antidiabetic potential of lipophilic guanidine derivatives bearing alkyl, aryl, and arylalkyl side chains has been recognized for decades,[7] this was the first report that suggested that polar zwitterionic guanidine analogues might be useful in the treatment of NIDDM.[8] The extreme hydrophilicity of **1** in fact may offer an advantage over more lipophilic guanidine antidiabetic agents, which have historically been associated with lactic acidosis, a potentially fatal overproduction of lactic acid resulting from inhibition of mitochondrial oxidative phosphorylation.[9,10] It has been demonstrated that lipophilicity, binding to mitochondrial membranes, and inhibition of oxygen consumption are closely correlated among mono-, di-, and biguanidine compounds. The higher observed incidence of lactic acidosis in patients receiving phenformin (**2**) relative to that observed in patients treated with the closely related but markedly less lipophilic drug, metformin (Glucophage, **3**), lends strong clinical support to the hypothesis that lipophi-

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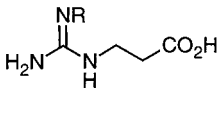
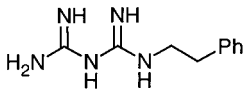
licity and toxicity are positively correlated.[10,11]



1: R = H
4: R = PO_3H



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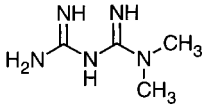

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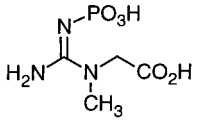


5

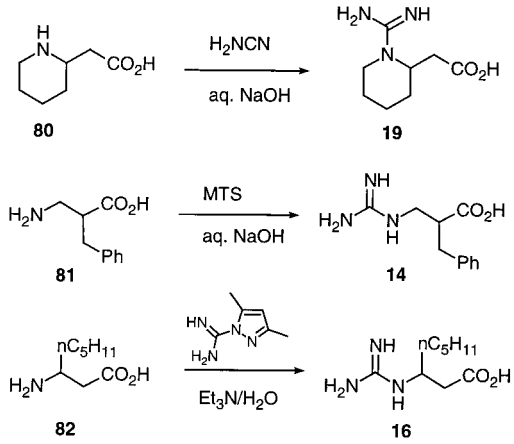
Two significant liabilities associated with **1** that sparked a search for improved analogues are its relatively low potency and its propensity to accumulate in muscle as the corresponding *N*-phosphate **4** when administered chronically at high doses. **1** is known to be a substrate for the creatine transporter and to be susceptible, albeit much less so than creatine, to N-phosphorylation by creatine kinase, both of which result in a gradual accumulation of **4** in muscle tissue and concomitant depletion of cellular creatine phosphate (**5**).[12] Chronic administration of **1** to rats eventually results in the observation of cardiac hypertrophy in association with mitochondrial paracrystalline inclusions of proliferated creatine kinase.[13] Although these pathologies are fully reversible over a period of months following withdrawal of treatment, muscle accumulation was clearly not a desirable attribute for a drug that could be anticipated to be administered over a period of many years. For these reasons, we elected to undertake an analogue program to identify novel, more potent entities retaining the antidiabetic activity of **1** but lacking its ability to be incorporated into the creatine transport/kinase cycle.

Chemistry

Homologues and simple alkyl- and aryl-substituted derivatives of **1** (Table 1) were prepared by standard amidination of the corresponding amino acids, using either 2-methyl-2-thiopseudourea sulfate (MTS),[14] cyanamide,[15] or 1-aminomethylimino-3,5-dimethylpyrazole[16] under basic conditions (examples depicted in Scheme 1). Yields were typically modest, often reflecting significant losses of the highly water-soluble products during isolation and crystallization. Compounds **11**, **12**, and **17** were prepared similarly by adding β-alanine to the appropriately N-substituted and activated isothioureas (exemplified by **11** and **17** in Scheme 2). Analogues **20** and **21** in Table 1 were prepared as described in the literature,[14] and compound **24** was purchased from a commercial source.

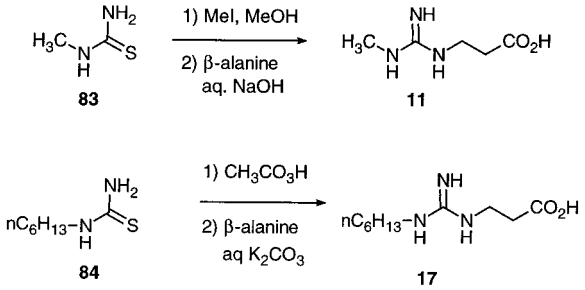
Analogues bearing electron-withdrawing groups on the guanidine are presented in Table 2. Compounds **25**

Scheme 1



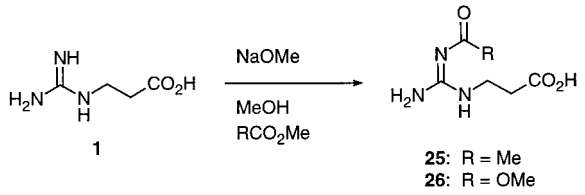
80 **19** **81** **14** **82** **16**

Scheme 2



83 **11** **84** **17**

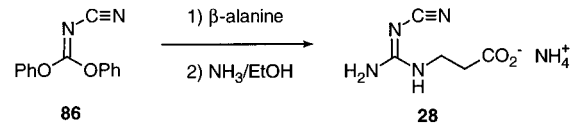
Scheme 3



1

25: R = Me
26: R = OMe

Scheme 4

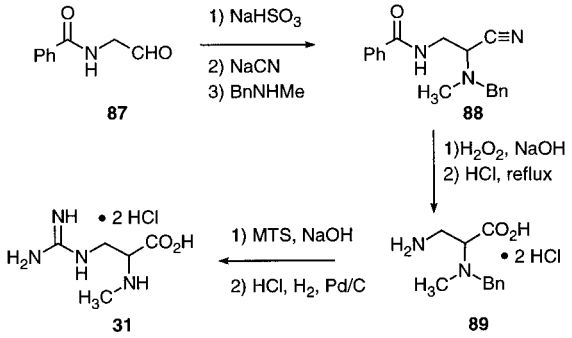


86 **28**

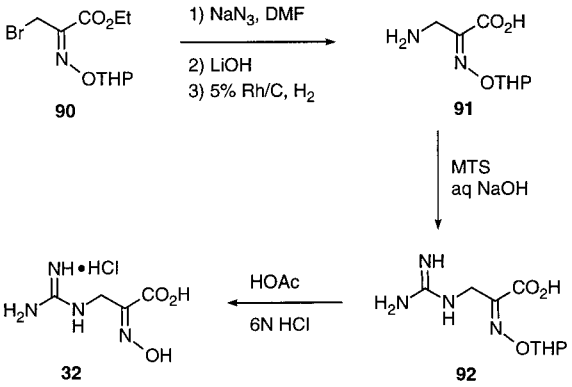
and **26** were prepared by acylating **1** with methyl acetate or dimethyl carbonate, respectively, under basic conditions (Scheme 3). Cyanoguanidine **28** was synthesized from diphenylcarbonimidate **86**[17] by sequential addition of β-alanine and ammonium hydroxide (Scheme 4). Nitroguanidine **27** was prepared as described in the literature.[18]

Table 3 contains analogues incorporating heteroatom substitution α to the carboxyl group. Compounds **30**,[19] **33**,[20] and **34**[21] were all prepared as described in the literature. Methylamino analogue **31** was synthesized as depicted in Scheme 5. Conversion of the known aldehyde **87**[22] to amino nitrile **88** was accomplished in three steps. The nitrile was hydrolyzed to carboxylic acid **89** via the corresponding carboxamide, and the amino acid **89** was amidinated with MTS. Debenzylation by catalytic hydrogenation then afforded the desired analogue **31**. The preparation of oxime analogue **32** is outlined in Scheme 6. Displacement of the bromide of **90**[23] with sodium azide followed by ester saponification

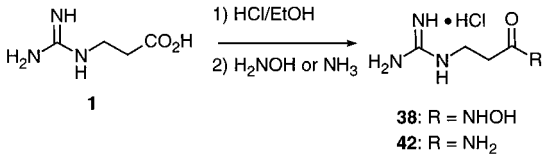
Scheme 5



Scheme 6



Scheme 7

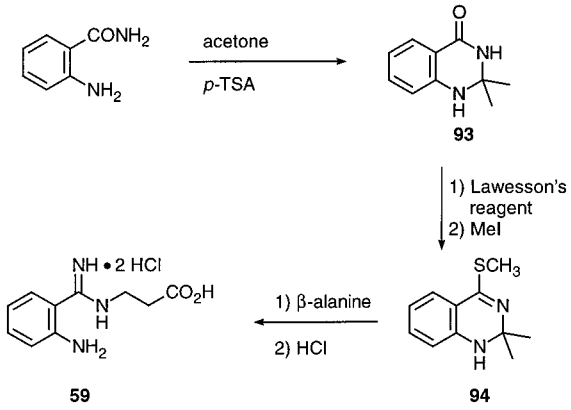


and catalytic hydrogenation afforded amino acid **91**. The primary amine was amidinated with MTS to afford guanidino acid **92**. Deprotection of the oxime via THP hydrolysis then afforded the oxime **32**.

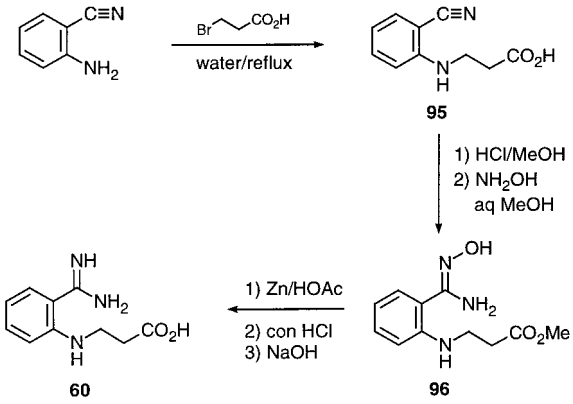
Analogues of **1** wherein the carboxyl group has been replaced with potentially bioisosteric functionality are compiled in Table 4. Compounds **36**[24] and **37**[25] were prepared as described in the literature. **1** could be esterified with ethanolic HCl, affording **41**. Subsequent addition of basic ammonia or hydroxylamine provided amide **42** or hydroxamic acid **38**, respectively (Scheme 7). Heterocyclic bioisostere analogues **39** and **40** could be obtained by direct amidination of the known corresponding primary amines[26,27] with cyanamide and MTS, respectively.

Analogues incorporating replacements for the guanidine moiety are presented in Table 5. Literature procedures were employed to produce compounds **43**,[28] **44**,[29] **47**,[30] **49**,[31] **50**,[32] **53**,[33] **54**,[34] and **55**.[35] Novel analogues **48**, **52**, **56**, and **57** were prepared via Michael additions of the appropriate heterocycle with either ethyl propiolate or ethyl acrylate, followed by hydrogenation and/or saponification (see Experimental Section). Pyridinium salt **51** was prepared by directly alkylating 3-aminopyridine with chloroacetic acid, and pyrimidinone analogue **58** could be synthesized in a single step from **1** by condensation with ethyl 3-oxovalerate.

Scheme 8

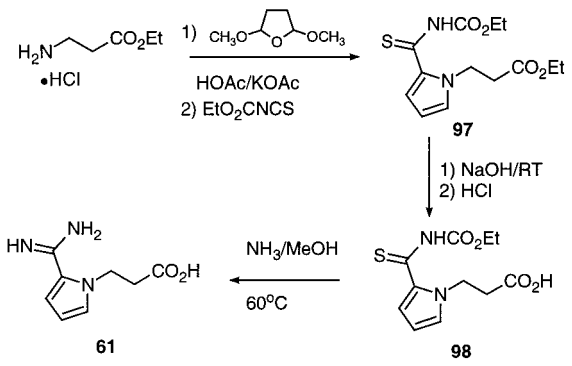


Scheme 9

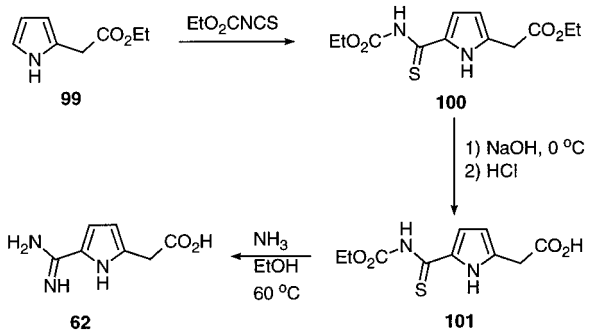


The last four guanidine surrogate analogues in Table 5 required multistep syntheses. **59** was prepared as outlined in Scheme 8. Both nitrogens of anthranilamide were protected by conversion to cyclic acetonide **93**. Transformation to the corresponding thioamide with Lawesson's reagent was followed by methylation with iodomethane, affording thioimidate **94**. Displacement of methanethiol by β-alanine and subsequent hydrolysis of the acetonide afforded the desired amidine **59**. The other arylamidine regioisomer **60** was synthesized as in Scheme 9. Anthranilonitrile was alkylated with 3-bromopropionic acid to afford **95**. The nitrile was converted to the corresponding methyl imidate with methanolic HCl prior to addition of hydroxylamine, affording hydroxyamidine **96**. Reductive cleavage of the *N*-hydroxyl group and hydrolysis of the methyl ester then provided **60**. The synthesis of pyrrole amidine analogue **61** is summarized in Scheme 10. The primary amine of β-alanine ethyl ester was first converted to a pyrrole ring with 2,5-dimethoxytetrahydrofuran. Regioselective acylation at C-2 was then effected with ethoxycarbonyl isothiocyanate to provide the acyl thioamide **97**, which was saponified to the corresponding acid **98**. Heating with methanolic ammonia then provided the target pyrroloamidine **61**. Scheme 11 presents the synthesis of pyrroloamidine **62**. The known pyrrole acetic acid ester **99**[36] was regioselectively acylated with ethoxycarbonyl isothiocyanate, affording acylated thioamide **100**. Alkaline hydrolysis under mild conditions provided carboxylic acid **101**, which could be converted directly to the desired amidine **62** with ethanolic ammonia.

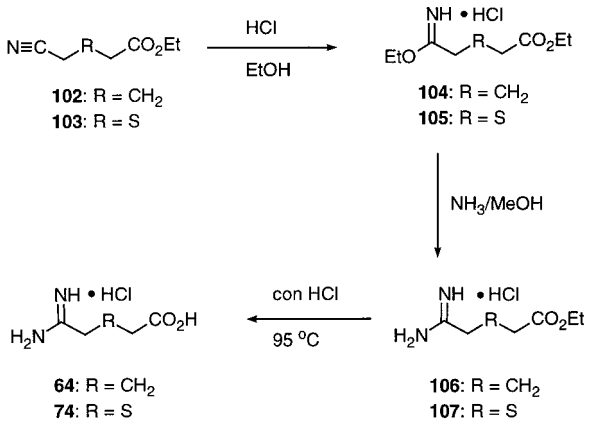
Scheme 10



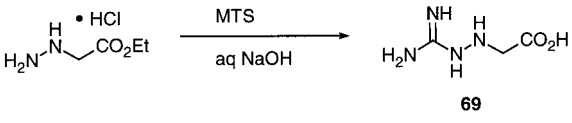
Scheme 11



Scheme 12



Scheme 13



Analogues wherein a single atom of the lead **1** has been changed are presented in Table 6. **63**,[37] **66**,[38] and **68**[39] were prepared as described in the literature. Amidine **64** was obtained by a modification of a literature route,[40] presented in Scheme 12. Ethyl 4-cyanobutyrate was converted into the corresponding imidate **104** with ethanolic HCl, followed by addition of ammonia, affording amidine **106**. Hydrolysis with aqueous HCl then provided amidine acid **64** as the HCl salt. Aminoguanidine **69** was prepared in a single step by direct amidination of hydrazinoacetic acid, obtained in situ from the saponification of commercially available ethyl hydrazinoacetate (Scheme 13). Confirmation that amidination had taken place on the terminal nitrogen of the hydrazinoacetic acid was obtained via a single-crystal X-ray structure of **69**.[41]

Table 7 presents analogues of isothiourea **67**, which were targeted following the observation of antidiabetic activity with this compound (vide infra). Homologues and analogues substituted on the terminal nitrogens were synthesized by S-alkylation of the appropriately substituted urea with a chloroalkanoic acid. Unsaturated analogues were prepared isomerically pure by a modification of the reported route.[42] Addition of thiourea to propiolic acid in aqueous HCl afforded with remarkable stereoselectivity (40:1) the *Z*-isomer **73**. On the other hand, the *E*-isomer **72** was readily obtained by simply using *trans*-3-chloropropenoic acid as the starting material and recrystallizing the crude mixture. Isomeric isothiourea **75** was prepared by S-methylation of the known thiourea **66**[38] (Table 6). Synthesis of amidine analogue **74** was effected via the classic Pinner protocol used to prepare **64**, starting with commercially available nitrile **103** (Scheme 12). The synthesis of **79** was accomplished via Michael addition of 2-amino-2-thiazoline to acrylic acid.

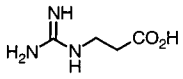
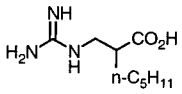
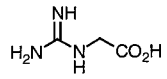
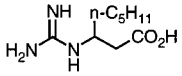
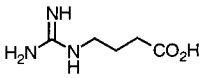
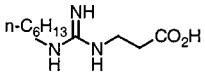
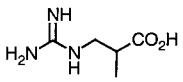
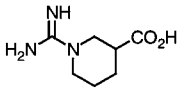
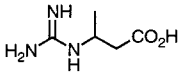
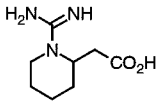
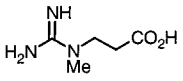
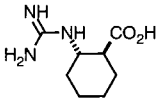
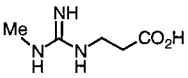
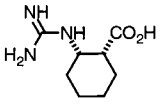
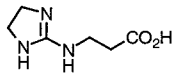
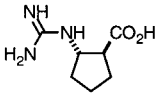
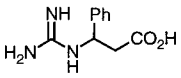
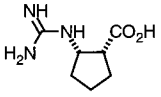
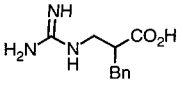
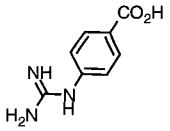
Biology

Antihyperglycemic Activity in KKA^y Mice. All compounds were evaluated in obese hyperglycemic, hyperinsulinemic, insulin-resistant KKA^y mice as previously described.[43] These mice are a cross between glucose-intolerant black KK female mice and obese yellow male A^y mice. Animals were separated into treatment and control groups ($n = 6$) following pretest blood glucose measurements. Samples were obtained by bleeding from the retroorbital sinus, and glucose levels were measured with an Alpkem glucose autoanalyzer. Treatment groups had the selected compound administered as a food mixture equivalent to a daily dose of 500 mg/kg for 4 days. The glucose level for the treated group (T) over the control group (C) was utilized to determine the antihyperglycemic activity of the test compounds. 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.

It is immediately evident from the analogues compiled in Table 1 that alkyl or aryl substitution at *any* position of the lead **1** effectively eliminated antidiabetic activity. Homologating the alkyl chain of **1** by a single methylene unit also was not tolerated (**7**). Guanidinoacetic acid **6** exhibited significant activity but in repeated testing has proven to be slightly less effective than **1**. Thus the optimum chain length was found to be two methylenes. Unfortunately, this intolerance for substitution likely precluded a meaningful evaluation of the effect of restricting the conformation of the parent molecule (e.g. compounds **20**−**23**, Table 1), information that might have proven valuable in elucidating a mechanism of action. Substitution of the guanidine group by electron-withdrawing functionality (Table 2) was no less detrimental, perhaps indicating that the basicity of this group is a key element of the activity.

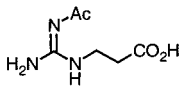
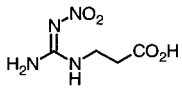
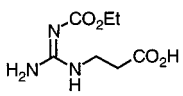
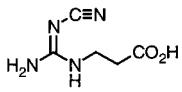
Heteroatomic substitutions were investigated next (Table 3). α-Hydroxy, mercapto, and oxime groups all

Table 1. Alkyl- and Aryl-Substituted Guanidinoacetic Acids

Cpd	Structure	MISS T/C[a]	Cpd	Structure	MISS T/C[a]
1		0.52 (±0.24)**	**15**		0.79
6		0.62 (±0.23)**	**16**		0.88
7		0.80	**17**		0.85
8		0.86	**18**		1.00
9		0.77	**19**		0.91
10		1.02	**20**		0.93
11		1.02	**21**		0.92
12		0.97	**22**		0.90
13		0.95	**23**		0.97
14		0.93	**24**		0.80

[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. 3-Guanidinopropionic Acids N-Substituted with Electron-Withdrawing Groups

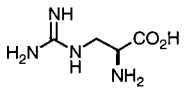
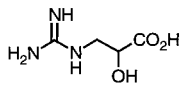
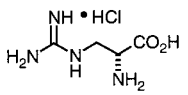
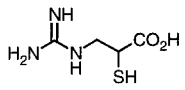
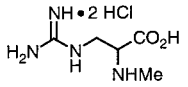
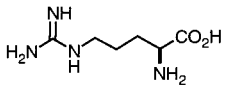
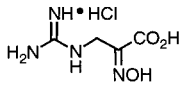
Cpd	Structure	MISS T/C[a]	Cpd	Structure	MISS T/C[a]
25		0.88	**27**		0.84
26		0.88	**28**		0.92

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

proved to be inactive. Only the *R*-amino analogue **29** maintained significant antidiabetic potency with a T/C actually less than that of the lead **1**. Remarkably, the opposite enantiomer **30** was completely devoid of activity. Further substitution of the α-amino group of **29** with a simple methyl group (**31**) obliterated the activity, even when the racemic mixture was subsequently tested at double the standard dose. The longer analogue L-arginine (**35**) did not maintain the antidiabetic activity of **29**, consistent with the optimum chain length of two indicated by the results in Table 1.

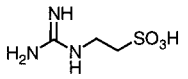
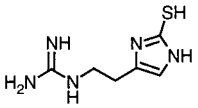
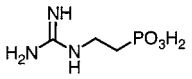
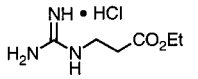
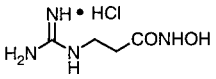
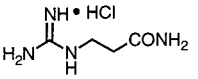
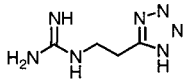
Our attention then turned to examining the possibility of replacing either the carboxyl or the guanidine group with potential bioisosteres (Tables 4 and 5). Standard surrogates for the carboxyl group, including sulfonic, phosphonic, and hydroxamic acids, as well as tetrazole, all proved to be inactive. It is noteworthy that simple carboxamide **42** lacked antidiabetic activity in

Table 3. α-Heteroatom-Substituted Guanidinoalkanoic Acids

Cpd	Structure	MISS T/C[a]	Cpd	Structure	MISS T/C[a]
29		0.35 (±0.20)***	**33**		1.10
30		0.92	**34**		0.83
31		0.96	**35**		1.02
32		0.97			

[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. Carboxyl Bioisostere Analogues

Cpd	Structure	MISS T/C[a]	Cpd	Structure	MISS T/C[a]
36		0.91	**40**		0.80
37		0.81	**41**		0.87
38		1.05	**42**		0.96
39		0.94			

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

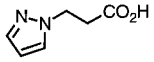
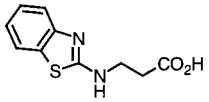
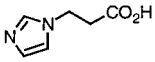
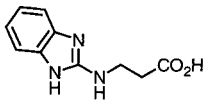
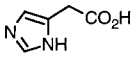
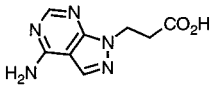
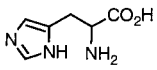
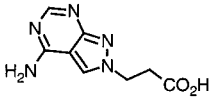
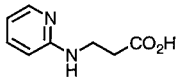
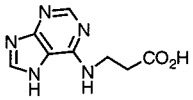
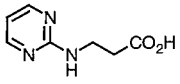
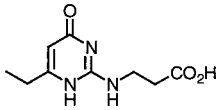
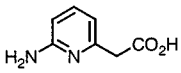
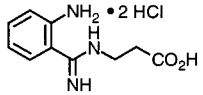
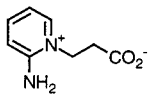
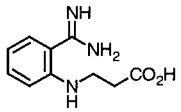
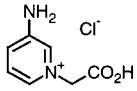
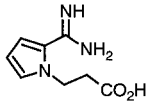
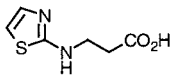
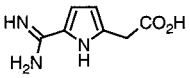
our model, in light of the report in the literature that the homologous 4-guanidinobutyramide is an antidiabetic.[8a] Potential prodrug ester **41** failed to elicit activity, indicating either that hydrolysis in vivo is not occurring or that the ester is not orally bioavailable.

Attempts to replace the guanidine group of **1** proved no less frustrating. Emphasis was placed on basic heterocycles to add complexity to the molecule and to preclude phosphorylation by creatine kinase. As Table 5 indicates, every example investigated failed to exhibit significant antidiabetic activity except for imidazole acetic acid **45** and aminopyridine **47**. The former was less effective than the lead **1** and ultimately proved to lower blood glucose in normal mice,[47] diminishing our interest in it. Unfortunately the strong antihyperglycemic activity of **47** was also associated with an unacceptable level of toxicity,[47] so it was dropped from further consideration. Remarkably, the closely related pyrimidine analogue **48** was devoid of activity. In an effort to retreat to analogues more closely resembling the lead **1**, the arylamidines **59**−**62** were prepared by multistep routes. It was anticipated that the combination of increased basicity and availability of N−H bonds would more closely mimic the guanidine of **1**, and it has been reported in the literature that arylamidines can function successfully as guanidine bioisosteres.[44] Unfortunately, none of the permutations depicted in the final four entries of Table 5 retained antidiabetic activity.

The results to this point suggested that only the most minimal of alterations were going to be tolerated in this series. Consequently, single atom mutations were evaluated via the analogues depicted in Table 6. Urea analogues **65** and **66** lacked activity, which was not altogether unexpected since the basicity of the guanidine was completely lacking. However, even the strongly basic amidines **63** and **64** were still inactive. Only when the internal nitrogen of the guanidine was replaced with sulfur (**67**) was antidiabetic activity finally maintained. Replacement of guanidines by isothioureas with some maintenance of biological activity has been reported previously.[45] Only one of the chain methylenes of **1** was amenable to alteration, and it was replaced with oxygen (**68**) and nitrogen (**69**). The former lost all activity, while the latter maintained significant antihyperglycemic activity.

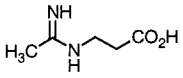
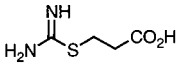
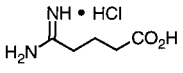
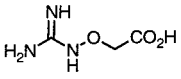
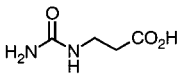
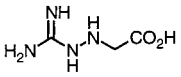
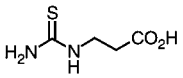
The equipotency of isothiourea **67** with **1** prompted a further investigation of isothiourea analogues (Table 7). The detrimental effect of homologation encountered with **1** was also apparently operative with **67**, as indicated by the lack of activity of simple analogues **70** and **71**. Substitution of the isothiourea was similarly

Table 5. Guanidine Bioisostere Analogues

Cpd	Structure	MISS T/C[a]	Cpd	Structure	MISS T/C[a]
43		1.11	**53**		0.87
44		0.81	**54**		0.95
45		0.68 (±0.35)*	**55**		1.27
46		0.80	**56**		1.08
47		0.20 (±0.17)**	**57**		0.85
48		1.01	**58**		0.96
49		1.08	**59**		0.92
50		1.07	**60**		0.73
51		0.94	**61**		0.93
52		0.99	**62**		1.10

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

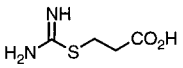
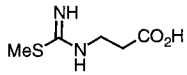
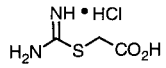
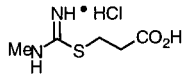
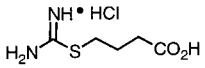
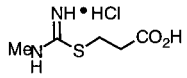
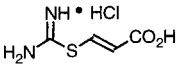
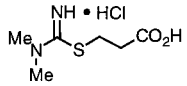
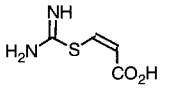
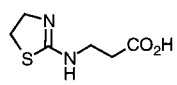
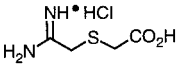
Table 6. Single Atom Replacement Analogues

Cpd	Structure	MISS T/C[a]	Cpd	Structure	MISS T/C[a]
63		0.77	**67**		0.53 (±0.34)*
64		0.93	**68**		0.83
65		1.06	**69**		0.61 (±0.29)***
66		0.99			

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

detrimental, with only the monomethyl analogue **76** achieving a statistically significant reduction in blood glucose. This was a bit unexpected, in light of the total loss of activity experienced by **1** following an analogous terminal N-methylation (compound **11**, Table 1). Regioisomeric isothiourea **75** and amidine **74** also lacked activity. The ready accessibility of unsaturated analogues **72** and **73** provided an interesting opportunity to evaluate restricting the molecular conformation. Interestingly, only the *Z*-isomer **73** retained significant

Table 7. Analogues of Isothiourea **67**

Cpd	Structure	MISS T/C[a]	Cpd	Structure	MISS T/C[a]
67		0.53 (±0.34)*	**75**		1.10
70		0.86	**76**		0.59 (±0.33)*
71		0.87	**77**		1.06
72		0.95	**78**		1.08
73		0.68 (±0.33)**	**79**		0.85
74		0.92			

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

Table 8. Affinity of **1** and **69** for Creatine Transporter and Creatine Kinase

substrate	% inhib of [^{14}C]creatine uptake (1 mM) by substrate (1 mM)	relative rates of phosphorylation of substrate by creatine kinase
creatine (1 mM)	92	100
1[a]	97	5
69[a]	36	0.5

[a] 50 mM concentration in creatine kinase assay. See Experimental Section.

activity, perhaps indicating that the preferred conformation of **1** in solution has the oppositely charged guanidine and carboxyl functionalities in close proximity, a result that is both intuitive and supported by molecular mechanics calculations.[46] Unfortunately, **67** ultimately exhibited an unacceptable therapeutic ratio[47] that precluded its further development and discouraged us from pursuing any additional isothiourea analogues.

Creatine Uptake/Kinase Activity. Among the few active analogues identified in this work, only aminoguanidine **69** was determined to have a therapeutic ratio[47] that warranted further development. To assess its potential to accumulate in muscle tissue via the same mechanisms responsible for the accumulation of **1,** two assays were performed to determine susceptibility to the creatine uptake/kinase system (Table 8).

1 is known to be a substrate for creatine kinase[47] and to reduce cellular [ATP] under condition where the forward flux rate (10483 + ATP → 10483-phosphate + ADP) is high.[48] Creatine kinase activity was assayed in the forward direction by measuring conversion of ATP to ADP by enzyme extracted from rabbit muscle. **69** was assayed as the corresponding HCl salt, due to its superior aqueous solubility. The rates of the ATP to ADP reaction relative to that measured in the presence of 1mM creatine are reported in Table 8 for **69** and **1**. It is evident from the results in the table that **69** is phosphorylated by creatine kinase at a rate of only about one-tenth that of **1**, thereby suggesting a reduced propensity to accumulate in muscle as the corresponding phosphate.

1 is also known to be a substrate for the plasma membrane creatine transporter[49,50] and to reduce cellular creatine concentrations by antagonizing cellular creatine uptake.[51] To determine the relative antagonism of creatine transport, the effect of **69** on the uptake of [^{14}C]creatine by cultured rat smooth muscle cells was determined. The results in Table 8 indicate that **69** is significantly less effective at competing with creatine for cellular uptake by the creatine transporter than is **1**, also suggesting an attenuated potential to accumulate in muscle tissue.

Conclusion

β-Guanidinopropionic acid (**1**) was previously shown to be an effective antihyperglycemic in the KKA^y mouse model of NIDDM. The propensity of **1** to accumulate in muscle tissue via uptake and phosphorylation by creatine-processing enzymes prompted a search for novel antidiabetic analogues lacking the potential for muscle accumulation. The SAR around this lead was determined to be remarkably narrow, with simple manipulations such as minor substitutions and homologations rendering the lead inactive. Carboxyl and guanidine replacements were all ineffective with the exception of 2-aminopyridine (**47**), but the gross toxicity of this analogue suggests that it may be acting by a different mechanism than **1**. The only modifications determined to be tolerated were substitution by a primary amine group (**29**) and single atom mutations (**67** and **69**). Of these three analogues, **69** was found to exhibit the best therapeutic ratio,[47] so it was selected for further preclinical development. In vitro assays established that **69** is a poorer substrate than **1** for both creatine kinase and the creatine transporter, suggesting that it should be less susceptible to accumulation in muscle upon chronic administration. The promising spectrum of in vivo and in vitro activities associated with aminoguanidine **69** encouraged us to pursue a new analogue program exploring further aminoguanidine derivatives and analogues, an effort that eventually led to the

identification of compounds with substantially improved activity, and that is reported in the following article.

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 nonaqueous reactions were run under a nitrogen atmosphere. All starting materials were commercially available unless otherwise noted. Analogues **6**, **7**, **10**, **12**, **24**, **29**, **35**, **45**, **46**, **65**, and **67** were all purchased from commercial suppliers.

***N*-[Imino(methylamino)methyl]-β-alanine (11).** To a suspension of *N*-methylthiourea (12.0 g, 133 mmol) in absolute methanol (60 mL) was added methyl iodide (21.3 g, 150 mmol). The mixture became a solution within 5 min and was slightly exothermic. After 1 h, the product was triturated with ether and collected by filtration, affording 31.5 g (ca. 100%) of 1-methyl-2-methyl-2-thiopseudourea hydrogen iodide as white crystals. A solution of β-alanine (5.75 g, 64.7 mmol) and 1-methyl-2-methyl-2-thiopseudourea hydrogen iodide (10.0 g, 43.1 mmol) and 12.5 M aqueous NaOH (3.45 mL) in water (10 mL) was stirred at 65−70 °C for 3 h. After cooling, the solution was concentrated in vacuo to a colorless oil. Flash chromatography (chloroform/methanol/concentrated aqueous ammonia) gave an oil that solidified. Recrystallization from methanol/acetone provided the title product as white crystals (4.49 g, 72%): ^{1}H NMR (D_2O) δ 3.38 (t, 2H, J = 7 Hz), 2.79 (s, 3H), 2.43 (t, 2H, J = 7 Hz); MS (FAB) m/z 146 (M + H). Anal. ($C_5H_{11}N_3O_2 \cdot 0.1H_2O$) C, H, N.

***N*-[Amino(imino)methyl]-2-benzyl-β-alanine (14).** To a suspension of 3-amino-2-(phenylmethyl)propanoic acid hydrochloride **81** (5.0 g, 23.2 mmol) MTS (3.88 g, 13.9 mmol) in methanol (23 mL) was added a 3.0 N aqueous solution of sodium hydroxide (17.0 mL). The mixture was stirred for 5 days at room temperature under a nitrogen atmosphere, then it was cooled to 0 °C and filtered through a medium porosity glass frit. The collected solid was washed with water and dried, affording 3.4 g (67%) of the title compound as a white powder: mp 232−233 °C (decomposes with gas evolution); ^{1}H NMR (D_2O/trace DCl) δ 7.30−7.00 (m, 5H), 3.35−3.12 (m, 2H), 2.88 (tt, J = 6.1, 7.2 Hz, 1H), 2.76 (d, J = 7.2 Hz, 2H); MS (FAB) m/z 222 (M + H$^+$). Anal. ($C_{11}H_{15}N_3O_2 \cdot 0.1H_2O$) C, H, N.

3-{[Amino(imino)methyl]amino}octanoic Acid (16). The free base of 1-carboxamidino-3,5-dimethylpyrazole was prepared in the following manner. The commercially available nitric acid salt (52.3 g, 260 mmol) was partitioned between excess aqueous 3 N sodium hydroxide solution and ethyl acetate. After washing the aqueous phase with several portions of ethyl acetate, the combined organic extracts were washed with brine and dried ($MgSO_4$). The solvent was then evaporated at reduced pressure to give 40.0 g of a colorless oil. To this oil was added 3-aminooctanoic acid hydrochloride **82** (10.0 g, 51.7 mmol), 70.0 mL of distilled water, 3.0 N aqueous sodium hydroxide (17.2 mL, 51.7 mmol), and triethylamine (36.4 mL, 260 mmol). The mixture was stirred under a nitrogen atmosphere at reflux for 2 h, then at room temerature for 1 h. The resulting suspension was diluted with 400 mL of ether and filtered through a medium porosity glass frit. The filtrant was suspended in distilled water (25 mL) and was stirred for 5 h. It was collected by filtration and then dried to give 6.9 g (62%): mp 255−256 °C dec; ^{1}H NMR (dilute DCl in D_2O) δ 3.70−3.55 (sym. m, 1H), 2.45 (dd, J = 16.4, 4.6 Hz, 1H), 2.29 (dd, J = 8.4, 16.4 Hz, 1H), 1.45−1.20 (sym. m, 2H), 1.20−0.95 (m, 6H), 0.58 (t, J = 6.6 Hz, 3H); MS (FAB) m/z 202 (M + H$^+$). Anal. ($C_9H_{19}N_3O_2 \cdot 0.1H_2O$) C, H, N.

***N*-[(Hexylamino)(imino)methyl]-β-alanine (17).** To a solution of 32% peracetic acid in dilute aqueous acetic acid (41.5 mL, 197 mmol) at 0 °C was added a solution of hexylthiourea **84** (10.05 g, 62.7 mmol) in methanol (50 mL), dropwise at a rate such that the temperature of the reaction did not rise above 20 °C (total addition time 1.5 h). After stirring at 25 °C for an additional 20 h, the mixture was evaporated almost to dryness at reduced pressure (high vacuum, 25 °C). *Explosion hazard! This operation should be performed only on a limited scale and should be performed behind a blast shield! Avoid distilling completely to dryness!* The product was dissolved in a mixture of glacial acetic acid (25 mL) and distilled water (15 mL). The solution was evaporated almost to dryness at reduced pressure (high vacuum, 25 °C), and the product was precipitated by adding 20 mL of 0 °C water. The white solid was collected by filtration and washed with ice water (2 × 10 mL). It was dried in a stream of air at 25 °C and then at 0.1 Torr/25 °C/45 min. This procedure gave 8.9 g (68%) of hexylamidinosulfonic acid: mp 191.5−192.5 °C.

A mixture of hexylamidinoaminosulfonic acid (4.0 g, 19.2 mmol), β-alanine (1.71 g, 19.2 mmol), and potassium carbonate (2.65 g, 19.2 mmol) in 15:4 distilled water/methanol (19 mL) was stirred for 4 days at room temperature. The reaction mixture was cooled to 0 °C and filtered. The filtrant was washed with ice water (3 × 7 mL). It was dried in a stream of air at 25 °C and then at 20 Torr/50 °C/24 h. It was suspended in ethyl acetate (20 mL) and stirred for 4 h. The mixture was filtered. The filtrate was diluted with an equal volume of ether and filtered. The two filtrants were combined and dried at 45 °C/20 Torr/18 h to give 2.2 g (53%) of a white solid: mp 217−218 °C dec; ^{1}H NMR (D_2O/trace DCl) δ 3.27 (t, J = 6.3 Hz, 2H), 2.96 (t, J = 6.9 Hz, 2H), 2.46 (t, J = 6.3 Hz, 2H), 1.42−1.25 (m, 2H), 1.20−0.95 (m, 6H), 0.64 (t, J = 6.6 Hz, 3H); MS (FAB) m/z 216 (M + H$^+$). Anal. ($C_{10}H_{21}N_3O_2 \cdot 0.25H_2O$) C, H, N.

2-{1-[Amino(imino)methyl]-2-piperidinyl}acetic Acid (19). To a mixture of **80** (7.44 g, 41.4 mmol) in water (5 mL) was added 12.5 N NaOH (3.3 mL, 41 mmol). Water was then added slowly until a solution was achieved (required 6 mL). To this solution was added cyanamide (2.09 g, 49.7 mmol). The solution was stirred at room temperature for 5 days. Precipitate began to form at 2 days. The reaction was chilled in an ice bath for 1 h before suction filtration. The collected solid was washed with cold water and air-dried. The resulting white powder (3.27 g) was recrystallized from water (40 mL) to afford a white solid (1.69 g, 22%): mp 276−278 °C dec; ^{1}H NMR (D_2O) δ 4.35 (m, 1H), 3.56 (d, 1H, J = 14 Hz), 3.14 (td, 1H, J = 3, 14 Hz), 2.76 (dd, 1H, J = 10, 15 Hz), 2.41 (dd, 1H, J = 5, 15 Hz), 1.4−1.8 (m, 6H); MS (EI) m/z (rel intensity) no M$^+$, 167 (99), 166 (47), 112 (99), 84 (44), 82 (50), 55 (64), 42 (61), 41 (49), 40 (63), 27 (49). Anal. ($C_8H_{15}N_3O_2$) C, H, N.

3-{[(Acetylamino)(imino)methyl]amino}propanoic Acid (25). To a suspension of **1** (7.0 g, 55.4 mmol) in MeOH (5 mL) was added a solution of sodium methoxide in MeOH (25 wt%, 12.6 mL, 55.4 mmol). Ethyl acetate (27.0 mL, 0.28 mol) was added, and the resulting mixture was heated at 55 °C for 48 h. After cooling to room temperature, the solvent was removed under reduced pressure, producing a white solid. The solid was dissolved in H_2O (40 mL), and concentrated HCl (4.6 mL, 55.4 mmol) was added which resulted in the formation of a white solid. Filtration gave 5.65 g (59%) of **25** as a white powder: mp 212−214 °C; ^{1}H NMR (D_2O + DCl) δ 2.69 (d, J = 5.4 Hz, 2H), 1.87 (d, J = 5.4 Hz, 2H), 1.31 (s, 3H); MS (EI) m/z 173 (M$^+$). Anal. ($C_6H_{11}N_3O_3 \cdot 0.2H_2O$) C, H, N.

3-({Imino[(methoxycarbonyl)amino]methyl}amino)-propanoic Acid (26). To a suspension of **1** (7.0 g, 55.4 mmol) in MeOH (5 mL) was added a solution of sodium methoxide in MeOH (25 wt%, 12.6 mL, 55.4 mmol). Dimethyl carbonate (23.3 mL, 0.28 mol) was added, and the mixture was heated at 50 °C for 48 h. After cooling to room temperature, the solvent was removed under reduced pressure, providing a

white solid. The solid was dissolved in H_2O (50 mL), and concentrated HCl (4.6 mL, 55.4 mmol) was added which caused the product to precipitate from solution. More H_2O (20 mL) was added and stirring was continued for several minutes. Filtration yielded 10.4 g (99%) of **26** as a white powder: mp 194−197 °C; ^{1}H NMR (D_2O + DCl) δ 3.45 (s, 3H), 3.24 (t, J = 6.2 Hz, 2H), 2.40 (t, J = 6.2 Hz, 2H); ^{13}C NMR (D_2O + DCl) δ 175.3, 175.2, 154.1, 153.3, 54.2, 37.2, 32.5; MS (EI) *m*/*z* 189 (M^+). Anal. ($C_6H_{11}N_3O_4 \cdot 0.15H_2O$) C, H, N.

Ammonium *N*-[Amino(cyanoimino)methyl]-*β*-alanine (28). A slurry of *β*-alanine (3.65 g, 41 mmol), 1 M sodium hydroxide (41.1 mL) and diphenylcyanocarbonimidate **86**[17] (9.79 g, 41 mmol) in 100 mL EtOH was stirred at room temperature for 2.75 h. The reaction was concentrated in vacuo to a yellow oil, which was taken up in 75 mL H_2O and extracted with 3 × 40 mL portions of Et_2O. The aqueous phase was acidified to pH 3 with 1 M HCl, and the resulting white precipitate was collected and dried to afford 8.00 g (83%) of *N*-[(cyanoamino)phenoxymethylene]-*β*-alanine**,** sufficiently pure to use directly in the next step. A solution of *N*-[(cyanoamino)-phenoxymethylene]-*β*-alanine (3.75 g, 16.1 mmol) in 50 mL EtOH/NH_3 was heated at 60 °C in a sealed tube overnight. The reaction was cooled and opened and after 1 h a precipitate formed which was collected and dried in vacuo. This provided 1.92 g (69%) of **28** as a white solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.54 (bs, 1H), 6.99 (bs, 2H), 6.03 (bs, 4H), 3.18 (d, J = 4.9 Hz, 2H), 2.12 (t, J = 5.9 Hz, 2H); MS (EI) *m*/*z* 172 ($M - 1)^+$. Anal. ($C_5H_{11}N_5O_2$) C, H, N.

3-{[Amino(imino)methyl]amino}-*N*-hydroxypropanamide Hydrochloride (38). A fresh solution of hydroxylamine (prepared from hydroxylamine hydrochloride (55.59 g, 0.800 mol) in absolute methanol (80 mL) with sodium methoxide in methanol (25 wt % sodium methoxide, 187 mL, 0.82 mol)) was poured into a large Buchner funnel (350 mL, coarse frit) and filtered with nitrogen pressure into a flask containing ester **41** (76.3 mmol) which was cooled in an ice bath. The flask was then put under positive nitrogen pressure and the ice bath removed. After reaching room temperature, the flask was stoppered tightly and stirred for 48 h. Most solvent was then removed in vacuo, leaving a foamy oil. This was redissolved in absolute methanol (120 mL), filtered to removed insolubles, and *slowly* triturated with ether. After the first 30 mL were added, the precipitate was collected (646 mg). Ether addition to the filtrate was continued in 5 mL portions, adding small amounts of methanol whenever the precipitate began to become pasty. After another 50 mL of ether and 20 mL of methanol had been added, the crude product was still very sticky. The mixture was left to stir vigorously at room temperature for several hrs., turning to a finely divided solid. More ether (25 mL) was added and the mixture was stirred overnight. The precipitate was collected (9.57 g), and the filtrate was further triturated with ether (to a total volume of ca. 500 mL), giving an additional 3.47 g. Examination of the three crops by NMR indicated that the third was the purest. This was redissolved in methanol (170 mL) at room temperature, and ether was added slowly until precipitate just began to form (120 mL). This mixture was allowed to stand at room temperature for 1 h and at 0 °C overnight. Filtration and washing with cold 1/1 ether/methanol provided a fine white solid (2.11 g): mp 165 °C dec; ^{1}H NMR (D_2O) δ 3.50 (t, 2H, J = 7 Hz), 2.46 (t, 2H, J = 7 Hz); ^{13}C NMR (D_2O) δ 170.0, 156.8, 37.4, 31.9; MS (FAB) *m*/*z* 147 (M + H). Anal. ($C_4H_{11}ClN_4O_2 \cdot 0.2H_2O$) C, H, N.

3-{[Amino(imino)methyl]amino}propanamide (42). Ethyl ester **41** (38.2 mmol) was diluted with methanol (150 mL) that had been saturated with ammonia gas. The vessel was sealed and kept at room temperature for 24 h. The mixture was filtered to remove ammonium chloride, and the filtrate was concentrated in vacuo. The residue was taken up in a minimum amount of absolute ethanol (15−20 mL) and filtered. This process was repeated until no further solid formed. The gummy material was taken up in ether and gassed with HCl, yielding a white solid: mp 122−125 °C. Anal. ($C_4H_{10}N_4O \cdot HCl$) C, H; N: calcd, 27.59; found, 28.20. Cl: calcd, 34.92; found, 33.62.

***N*-[(2-Aminophenyl)(imino)methyl]-*β*-alanine (59).** To a stirring solution of anthranilamide (15 g, 110 mmol) in acetone (150 mL) was added *p*-toluenesulfonic acid monohydrate (0.125 g, 0.65 mmol), and the resulting homogeneous mixture was refluxed for 1 h. The solvent was subsequently cooled to ambient temperature and removed under reduced pressure. The resulting solid was partitioned between EtOAc (250 mL) and saturated aqueous $NaHCO_3$ (250 mL). The layers were shaken, and the organics were separated and washed additionally with H_2O (2 × 100 mL). The organic layer was separated, dried over Na_2SO_4 and solvent evaporated to approximately one-half its volume. At this point, solid began to crystallize from the organic layer. The organic layer was filtered and found to be analytically pure **93** (18.6 g, 96%) as white prisms: mp 183−184 °C; ^{1}H NMR (DMSO-d_6) δ 1.52 (s, 6H), 6.77 (m, 3H), 7.34 (t, 1H, J = 7 Hz), 7.73 (d, 1H, J = 6 Hz), 8.11 (s, 1H).

To a stirring solution of **93** (50 g, 283 mmol) in THF (600 mL) at 40 °C was added Lawesson's reagent (57.38 g, 142 mmol). The resulting mixture was heated to 80 °C for 3 h, cooled to ambient temperature and solvent removed in vacuo. The resulting foamy residue was triturated with CH_2Cl_2 (200 mL), and the solid was filtered to yield 56 g solid. The filtered solid was recrystallized from EtOAc/hexanes (three crops) and subsequently air-dried in a Buchner funnel overnight to afford 46.2 g (85%) of the corresponding thioamide as a highly crystalline yellow solid: mp 161−163 °C; ^{1}H NMR (DMSO-d_6) δ 1.20 (s, 6H), 6.44 (m, 2H), 6.78 (s, 1H), 7.05 (t, 1H, J = 8 Hz), 7.85, (d, 1H, J = 8 Hz), 9.95 (s, 1H).

To a stirring solution of the thioamide (30 g, 156 mmol) in MeOH (300 mL) was added CH_3I (29.1 mL, 467 mmol). The resulting dark yellow solution was stirred for 2 h, and the solvent was removed under reduced pressure. The residue was partitioned between EtOAc (150 mL) and saturated aqueous $NaHCO_3$ (150 mL). The layers were shaken, the organic layer separated, dried over Na_2SO_4 and solvent removed in vacuo. The residue was recrystallized from hexanes to yield **94** (31.6 g, 99%) as a yellow solid: mp 65−66 °C; ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 6H), 2.39 (s, 3H), 3.91 (brs, 1H), 6.49 (d, 1H, J = 8 Hz), 6.65 (t, 1H, J = 8 Hz), 7.18 (t, 1H, J = 8 Hz), 7.43 (d, 1H, J = 7 Hz).

To a stirring solution of **94** (10 g, 48.72 mmol) in MeOH (150 mL) was added *β*-alanine (4.34 g, 48.72 mmol). The resulting homogeneous solution was heated to reflux overnight, cooled to ambient temperature, and the mixture was filtered of unreacted starting material and concentrated in vacuo. SiO_2 flash column chromatography of the resulting residue (eluant 85:13:2 CH_2Cl_2/MeOH/NH_4OH) afforded 8.62 g (71%) of the adduct as a yellow amorphous solid: ^{1}H NMR (D_2O) δ 1.47 (s, 6H), 2.52 (t, 2H, J = 12 Hz), 3.55 (t, 2H, J = 12 Hz), 6.73 (d, 1H, J = 8 Hz), 6.80 (t, 1H, J = 8 Hz), 7.38 (t, 1H, J = 8 Hz), 7.45 (d, 1H, J = 8 Hz).

The above acetonide (4.1 g, 16.6 mmol) was heated at reflux in 6 N HCl (100 mL) overnight and cooled to ambient temperature. The residue was heated in a vacuum oven overnight at 50 °C in order to remove residual HCl. The residue was taken up in isopropyl alcohol (150 mL) and sonicated. The solid was filtered and dried in vacuo to afford 1.62 g of **59** as a white solid: mp 198−201 °C; ^{1}H NMR (D_2O) δ 2.89 (t, 2H, J = 8 Hz), 3.75 (t, 2H, J = 8 Hz), 7.38 (m, 2H), 7.54 (d, 1H, J = 8 Hz), 7.66 (t, 1H, J = 8 Hz); MS (EI) *m*/*z* 207 (M^+); ^{13}C NMR (D_2O) δ 31.5, 38.6 119.8, 121.6, 124.8, 129.8, 134.0, 135.0, 162.3, 175.0. Anal. ($C_{10}H_{13}N_3O_2 \cdot 2HCl$) C, H, N.

3-{2-[Amino(imino)methyl]anilino}propanoic Acid (60). To a stirring solution of anthranilonitrile (25 g, 211 mmol) in water (460 mL) was added 3-bromopropionic acid (32.37 g, 211 mmol). The resulting suspension was refluxed overnight. The resulting homogeneous solution was cooled to ambient temperature and extracted with EtOAc (3 × 100 mL). The organic layers were combined, dried over Na_2SO_4 and the solvent evaporated to dryness. The resulting residue was partitioned

between EtOAc (100 mL) and saturated aqueous $NaHCO_3$ (100 mL), the layers were shaken and the aqueous phase was separated, washed with additional EtOAc (100 mL) and acidified to pH 6 with 1 N HCl. The aqueous layer was extracted with EtOAc (3 × 100 mL), the organic layers were combined, dried over Na_2SO_4 and solvent removed under reduced pressure. The residue was recrystallized from EtOAc/hexane to afford 9.87 g (25%) of **95** as a yellow solid: mp 113−115 °C; ^{1}H NMR ($CDCl_3$) δ 2.72 (t, 2H, J = 8 Hz), 3.57 (t, 2H, J = 8 Hz), 6.70 (m, 2H), 7.40 (m, 2H).

Dry HCl gas was slowly bubbled into a stirring solution of **95** (8.54 g, 44.93 mmol) in MeOH (200 mL) at ambient temperature until saturated. The resulting homogeneous solution was stirred overnight at ambient temperature and solvent evaporated to dryness. The residue was partitioned between EtOAc (100 mL) and saturated aqueous $NaHCO_3$ (100 mL). The layers were shaken, the organics separated, dried over Na_2SO_4 and evaporated to dryness to afford 8.44 g (92%) of the corresponding imidate as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 2.66 (t, 2H, J = 8 Hz), 3.57 (q, 2H, J = 8,14 Hz), 3.97 (s, 3H), 4.90 (brt, 1H), 6.69 (m, 2H), 7.39 (m, 2H).

To a stirring solution of hydroxylamine hydrochloride (3.40 g, 48.96 mmol) in water (50 mL) was added solid $NaHCO_3$ (4.11 g, 48.96 mmol) at ambient temperature. The above imidate (10.0 g, 48.96 mmol) in EtOH (150 mL) was added, and the solution was refluxed overnight. The solution was cooled, EtOH evaporated, and the aqueous solution acidified to pH 3−4 with 1 N HCl. The aqueous layer was washed with EtOAc (3 × 50 mL), neutralized with solid $NaHCO_3$ and extracted with EtOAc (3 × 100 mL). The organic layers were combined, dried over Na_2SO_4 and solvent removed in vacuo to afford 9.70 g (83%) of **96** as a brown oil: ^{1}H NMR ($CDCl_3$) δ 2.64 (t, 2H, J = 8 Hz), 3.49 (t, 2H, J = 8 Hz), 3.68 (s, 3H), 4.89 (brs, 2H), 6.64−6.70 (brs, 2H), (6.69 (d, 2H, J = 8 Hz), 7.24 (m, 2H), 7.33 (m, 2H).

To a mechanically stirred solution of **96** (9.70 g, 40.88 mmol) in acetic acid (300 mL) was added activated zinc metal (30 g). The mixture was stirred overnight at 110 °C filtered hot through Celite and concentrated in vacuo. The residue was taken up in concentrated HCl (75 mL) and heated at 95 °C for 3 h. The resulting solution was cooled to ambient temperature and evaporated to dryness. The resulting solid was taken up in 10−15 mL H_2O and neutralized by dropwise addition of 1 N NaOH. The resulting solid was collected by filtration and dried in vacuo at 20 °C to afford 4.26 g crude product. The solid (2.5 g, 12 mmol) was taken up in 1 N NaOH (12 mL, 12 mmol), filtered through Celite and the resulting solution neutralized with vigorous stirring with 1 N HCl (12 mL, 12 mmol). The solid which precipitated was filtered and dried in vacuo at 20 °C to afford 2.23 g of **60** as a white solid: mp 266−267 °C dec; ^{1}H NMR (DMSO-d_6) δ 2.50 (t, 2H, J = 8 Hz), 3.29 (t, 2H, J = 8 Hz), 6.73 (t, 1H, J = 8 Hz), 6.80 (d, 1H, J = 8 Hz), 7.19 (d, 1H, J = 8 Hz), 7.35 (t, 1H, J = 8 Hz), 9.12 (brs, 1H), 9.20 (brs, 1H); MS (FAB) m/z 208 (M + H). Anal. ($C_{10}H_{13}N_3O_2 \cdot 0.11H_2O$) C, H, N.

3-{2-[Amino(imino)methyl]-1*H*-pyrrol-1-yl}propanoic Acid (61). β-Alanine ethyl ester (23.66 g, 0.154 mol) and potassium acetate (24.86 g, 0.253 mol) were dissolved in water (60 mL) before the addition of acetic acid (154 mL, 2.69 mol) and 2,5-dimethoxytetrahydrofuran (20.0 mL, 0.154 mol). The reaction mixture was stirred at reflux for 4 h 15 min. After cooling of the reaction mixture in ice, it was diluted with water (220 mL) before the addition of solid sodium hydroxide (113.7 g, 2.84 mol) in portions over about 45 min. Neutral pH was reached before the addition of the last few grams, at which time addition was stopped. The mixture was extracted with ethyl acetate (2 × 250 mL, 1 × 100 mL), and the extracts were dried over sodium sulfate. Concentration in vacuo left an oil (30.5 g). Kugelrohr distillation (130−140 °C, 15−20 mmHg) provided ethyl 3-(1-pyrrolo)propanoate as a colorless oil (22.94 g, 89%): ^{1}H NMR ($CDCl_3$) δ 6.65 (t, 2H, J = 2 Hz), 6.13 (t, 2H, J = 2 Hz), 4.19 (t, 2H, J = 7 Hz), 4.13 (q, 2H, J = 7 Hz), 2.75 (t, 2H, J = 7 Hz), 1.24 (t, 3H, J = 7 Hz).

Ethyl 3-(1-pyrrolo)propanoate (10.00 g, 59.84 mmol) and ethoxycarbonyl isothiocyanate (7.06 mL, 59.8 mmol) were combined neat while cooling in a room temperature water bath. The solution was stirred under nitrogen for 2.5 days. NMR analysis of the resulting thick red oil indicated it was nearly pure **97**, but it could not be induced to crystallize. The crude oil was taken directly into the next step: ^{1}H NMR ($CDCl_3$) δ 8.60 (bs, 1H), 7.06 (m, 1H), 6.71 (m, 1H), 6.13 (m, 1H), 4.75 (t, 2H, J = 7 Hz), 4.23 (q, 2H, J = 7 Hz), 4.12 (q, 2H, J = 7 Hz), 2.86 (t, 2H, J = 7 Hz), 1.32 (t, 3H, J = 7 Hz), 1.22 (t, 3H, J = 7 Hz).

The crude **97** from above (ca. 17.5 g, ca. 59 mmol) was combined with ice cold aqueous NaOH (7.03 g, 176 mmol, in 63 mL of degassed water). The mixture was swirled in an ice bath until all of the oil had dissolved (ca. 10 min) and then stirred at room temperature for 15 min. The solution was chilled again in ice before acidifying with con HCl. The resulting gummy mixture was seeded with authentic product and sonicated until the gum had become a finely divided solid. The mixture was chilled in ice before suction filtration. Drying in vacuo left an orange solid (12.9 g). The crude solid was dissolved in methylene chloride (250 mL) at reflux. Hexane was added in portions until the solution became cloudy (required about 250 mL). Cooling to room temperature with seeding, then at 0 °C for 2 days, gave **98** (11.42 g, 72% overall) as orange crystals: mp 117−119 °C; ^{1}H NMR ($CDCl_3$) δ 8.62 (bs, 1H), 7.07 (m, 1H), 6.71 (m, 1H), 6.14 (m, 1H), 4.75 (t, 2H, J = 7 Hz), 4.26 (q, 2H, J = 7 Hz), 2.95 (t, 2H, J = 7 Hz), 1.32 (t, 3H, J = 7 Hz).

98 (11.08 g, 41.0 mmol) was divided evenly into two pressure tubes equipped with Teflon screw caps and magnetic stir bars, each containing absolute ethanol (90 mL) which had been saturated with ammonia gas at 0 °C. After capping the reaction tubes tightly, the solutions were stirred at 60 °C for 45 h. The resulting mixtures were chilled in ice before opening and filtration. The collected solid was washed thoroughly with absolute ethanol and dried in vacuo, leaving **61** as a shimmery solid (4.88 g, 66%): mp 248 °C dec; ^{1}H NMR (D_2O) δ 7.24 (t, 1H, J = 2 Hz), 6.88 (m, 1H), 6.33 (m, 1H), 4.32 (t, 2H, J = 6 Hz), 2.74 (t, 2H, J = 6 Hz); MS (EI) m/z 181 (M^+). Anal. ($C_8H_{11}N_3O_2$) C, H, N.

2-{5-[Amino(imino)methyl]-1*H*-pyrrol-2-yl}acetic Acid (62). Ethoxycarbonyl isothiocyanate (1.53 mL, 13.1 mmol) and pyrrole ester **99**[36] (2.00 g, 13.1 mmol) were combined at 0 °C. The solution was stirred for 5 min before removal of the ice bath and replacement with a room temperature water bath. The solution solidified after about 1 h, and it was left standing overnight at room temperature. The crude solid was taken up in boiling 2-propanol (50 mL). Cooling to room temperature gave **100** as golden needles (2.81 g, 75%): mp 114−115 °C; ^{1}H NMR ($CDCl_3$) δ 10.27 (bs, 1H), 8.64 (bs, 1H), 6.79 (m, 1H), 6.15 (m, 1H), 4.29 (q, 2H, J = 7 Hz), 4.22 (q, 2H, J = 7 Hz), 3.68 (s, 2H), 1.34 (t, 3H, J = 7 Hz), 1.31 (t, 3H, J = 7 Hz).

A solution of 1.0 M aqueous NaOH (110 mL) and water (30 mL) was chilled in ice before the addition of **100** (13.52 g, 47.55 mmol). The solution was stirred in the ice bath for 15 min, at which time TLC indicated the reaction was complete. The solution was acidified with con HCl. After stirring in the ice bath for 20 min, the precipitate was collected, washed with water, and dried in vacuo, leaving a bright yellow powder (12.70 g, ca. 100%), sufficiently pure to carry into the next step. An analytical sample could be prepared by recrystallization from methylene chloride/hexane, giving **101** as fine orange-yellow needles (360 mg): mp 133 °C dec; ^{1}H NMR ($CDCl_3$) δ 10.44 (bs, 1H), 9.24 (bs, 1H), 6.86 (m, 1H), 6.15 (m, 1H), 4.29 (q, 2H, J = 7 Hz), 3.67 (s, 2H), 1.35 (t, 3H, J = 7 Hz).

Crude **101** (ca. 46 mmol) was dissolved in saturated ethanolic ammonia as described for the preparation of **61**. After capping tightly, the solutions were stirred at 60 °C for 48 h and at 65 °C for 24 h. The reaction tubes were chilled in ice and sonicated to break up the solid which had formed before opening carefully. The solid was collected by filtration and washed thoroughly with absolute ethanol. Drying in vacuo left a cream powder (4.36 g). The crude solid was recrystallized

from methanol/water, affording **62** as colorless needles (4.06 g, 53%): mp 310 °C dec; ^{1}H NMR (D_2O/DCl) δ 7.01 (d, 1H, J = 4 Hz), 6.25 (d, 1H, J = 4 Hz), 3.80 (s, 2H); ^{13}C NMR (D_2O/DCl)(mult) δ 174.7 (s), 156.6 (s), 133.6 (s), 118.4 (s), 117.6 (d), 111.6 (d), 33.1 (t); MS (EI) m/z 167 (M^+). Anal. ($C_7H_9N_3O_2$) C, H, N.

5-Amino-5-iminopentanoic Acid (64). A solution of ethyl 4-cyanobutyrate **102** (9.84 g, 69.3 mmol) and absolute ethanol (8.5 mL, 145 mmol) in dry ether (100 mL) was cooled in an ice bath before gently saturating with HCl gas. The solution was then allowed to come to room temperature before capping the flask tightly and stirring at room temperature for 18 h. The solution was concentrated in vacuo to a viscous oil which rapidly crystallized on standing. No attempt was made to purify the sensitive imidate salt **104**; the crude material was sufficiently pure to carry into the next step.

To the crude **104** from above under nitrogen was added a solution of ammonia in methanol (2.0 M, 40 mL, 80 mmol) with ice bath cooling. The mixture was stirred vigorously for 5 min. before removing the ice bath. Stirring was continued for 1 h. The solvent was evaporated in vacuo, leaving a yellow oil and solid. Absolute ethanol (100 mL) was added, and the mixture was stirred until all of the oil had dissolved. The mixture was chilled overnight at −20 °C before filtering. Concentration of the filtrate in vacuo left an oil which still contained some solid. The mixture was taken up in chloroform (100 mL) and again filtered. Concentration of the filtrate left the crude amidine ester **106** as a viscous yellow oil (ca. 15 g).

The crude **106** from above was dissolved in concentrated aqueous HCl (150 mL) and stirred at 95 °C (bath temperature) for 1 h. The solution was concentrated in vacuo (aspirator first, then 0.5 mm), leaving a pale yellow crystalline solid (10.8 g). 9.84 g of the crude solid was recrystallized from absolute ethanol (130 mL) and pyridine (ca. 0.5 mL), affording pure **64** as white flakes (7.34 g, 70% overall): mp 175−176 °C; ^{1}H NMR (DMSO) δ 12.27 (bs, 1H), 9.14 (bs, 2H), 8.83 (bs, 2H), 2.43 (t, 2H, J = 7 Hz), 2.27 (t, 2H, J = 7 Hz), 1.84 (quintet, 2H, J = 7 Hz); MS (FAB) m/z 131 (M + H). Anal. ($C_5H_{11}ClN_2O_2$) C, H, N, Cl.

[2-(Aminoiminomethyl)hydrazino]acetic Acid (69). Ethylhydrazinoacetate hydrochloride (7.73 g, 50 mmol) was saponified by refluxing in 100 mL of 1 N NaOH for 2 h. To the hot solution was then added MTS (6.95 g, 50 mmol) and the solution was refluxed for an additional 2 h. The mixture was concentrated to ∼1/2 volume at which time a white solid precipitated. The solution was cooled and filtered to yield 3.34 g of a white solid. Recrystallization from water afforded 2.41 g (36%) of **69** as a highly crystalline white solid: mp 247−248 °C dec; ^{1}H NMR (D_2O) δ 3.40 (s, 2H); ^{13}C NMR (D_2O) δ 181.7, 162.0, 57.6; MS (FAB) m/z 133 (M + H). Anal. ($C_3H_8N_4O_2$) C, H, N.

***(Z)*-3-{[Amino(imino)methyl]sulfanyl}-2-propenoic Acid (73).** To a 0 °C solution of thiourea (7.61 g, 100 mmol) in 1.0 M aqueous HCl (100 mL) was added propiolic acid (6.16 mL, 100 mmol). A precipitate appeared within 15 min. The ice bath was removed, and stirring was continued for 2 h. The mixture was cooled again in ice before the addition of 1.0 M aqueous NaOH (100 mL). The resulting thick slurry was stirred for 15 min before suction filtration. The collected solid was washed with cold water and dried in vacuo, leaving a white powder (10.66 g, 73%, mp 164 °C dec; lit.[42] mp 168 °C). Attempted recrystallization of a small sample from hot water resulted in decomposition: ^{1}H NMR (D_2O) δ 7.53 (d, 2H, J = 9.8 Hz), 6.31 (d, 2H, J = 9.8 Hz); ^{13}C NMR (D_2O)(mult) δ 169.3 (s), 169.1 (s), 137.5 (d), 118.8 (d); MS (FAB) m/z 147 (M + H). Anal. ($C_4H_6N_2O_2S$) C, H, N, S.

3-{[Imino(methylamino)methyl]sulfanyl}propanoic Acid, Hydrochloride (76). Prepared from *N*-methylthiourea and chloropropionic acid as described for **77**. Recrystallization from ^{i}PrOH afforded a white solid: ^{1}H NMR (D_2O) δ 3.33 (t, J = 7 Hz, 2H), 2.98 (s, 3H), 2.82 (t, J = 7 Hz, 2H); MS (FAB) m/z 163 (M + H). Anal. ($C_5H_9N_2O_2S$·HCl) C, H, N, S, Cl.

3-{[(Methylamino)(methylimino)methyl]sulfanyl}-propanoic Acid (77). A mixture of 1,3-dimethyl-2-thiourea (10.42 g, 100 mmol) and 3-chloropropionic acid (10.85 g, 100 mmol) in acetone (22 mL) was stirred at reflux for 48 h. Upon cooling, a large amount of oil separated. More acetone (ca. 150 mL) was added, and the mixture was stirred vigorously until the oil solidified. The solid was broken up and stirred until fine before filtering (required about 30 min). The solid was collected by filtration under a nitrogen atmosphere and washed with acetone. Drying in vacuo left a hygroscopic white solid (14.70 g, 69%) which was analytically pure but retained an odor of chloropropionic acid. The crude solid was dissolved in warm 2-propanol (120 mL) and diluted with ether until cloudy (80 mL). After standing at room temperature for several hours and 0 °C for several days, a crusty solid was obtained (9.61 g): mp 83−86 °C; ^{1}H NMR (D_2O) δ 3.36 (t, 2H, J = 7 Hz), 3.06 (s, 3H), 2.98 (s, 3H), 2.85 (t, 2H, J = 7 Hz); MS (FAB) m/z 177 (M + H). Anal. ($C_6H_{13}N_2O_2SCl$) C, H, N, Cl, S.

Biological Procedures. In Vivo Antihyperglycemic Activity. Compounds were evaluated in obese, hyperglycemic, insulin-resistant KKA^y mice as previously described.[43] Compounds were administered as a food admixture at 500 mg/kg for 4 days. Results are reported in Tables 1−7 as the ratio of glucose levels for the treated group (T) over the control group (C). 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.

In Vitro Evaluation of Creatine Uptake/Kinase. Creatine kinase activity was assayed by measuring the conversion of ATP to ADP by enzyme extracted from rabbit muscle (specific activity 350 U/mg; Boehringer Mannhein). The reaction was performed at 30 °C using a buffer composed of 50 mM Hepes (pH 7.5), 2.5 mM EDTA, 10 mM $MgCl_2$ and 5 mM ATP. Creatine or test compounds were added as substrate. The reaction was started by adding 20 units of creatine kinase. At times of 0, 30, 60, 120, and 180 min, an aliquot of the reaction medium was removed and added to 1 volume of cold 2 M $HClO_4$ and centrifuged at 11600*g* for 10 min at 4 °C in an Eppendorf model 5402 centrifuge. The supernatant was removed, the pH neutralized with 2.5 M potassium carbonate, and the sample recentrifuged. The supernatant was immediately used for nucleotide analysis using a Waters 600E HPLC and an Adsorbosphere HS C18 column (100 × 4.6 mm, 3 μm particle size; Alltech Associates). The mobile phase contained 30 mM ammonium dihydrogen sulfate and 5 mM tetrabutylammonium hydrogen sulfate, pH 5.1. Elution was accomplished at room temperature with a 5−36% methanol linear gradient for 30 min at a flow rate of 1 mL/min. Nucleotides were quantitated by area-to-response factors calculated from standards at 260 nm. The activity of creatine kinase with creatine or test compounds as substrate was determined by linear regression of the sample ADP concentration vs time over the initial linear phase of the reaction.

The effect of test compounds on cellular creatine transport was determined using A10 smooth muscle cells (American Tissue Culture Collection). A10 cells were grown in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum (Gibco) in 35 mm tissue culture dishes. The cells were studied while in the logarithmic growth phase. A10 cells were washed with Hanks buffer (pH 7.4) containing 10 mM Hepes, 20 mM glucose and 0.2% fatty-acid-free bovine serum albumin. The cells were then incubated at 37 °C in the same buffer supplemented with [^{14}C]creatine (Sigma). After 30, 60, 90, or 120 min, the medium was aspirated from the culture dish, the cells were washed twice with 2 mL of ice cold Hanks-Hepes buffer, and 0.2 N NaOH was added to lyse the cells. The dishes were scraped with a Teflon policeman. Aliquots of cell extract were transferred to scintillation vials. Insta-Gel XF scintillant was added and liquid scintillation counting was performed

using a Beckman 5801 counter with automatic quench correction. Creatine uptake was determined by dividing the ^{14}C per tissue culture dish by the specific activity of the incubation medium and the protein concentration of the cell extract. Preliminary studies indicated that under these conditions cell accumulation of [^{14}C]creatine increased as a linear function of incubation time. The affinity for creatine uptake (K_M) was 43 ± 3 μM and the maximum velocity was 8.7 ± 1.3 nmol/h/mg protein ($n = 6$ experiments). Analysis of the kinetics for cell uptake using a double-reciprocal plot (Lineweaver−Burk) plot, indicated that compound **1** was a simple competitive inhibitor of creatine uptake with a K_i value of 19 ± 1 μM ($n = 3$).

Supporting Information Available: Crystallographic data for analogue **69**; experimental procedures for analogues **8**, **9**, **13, 15, 18, 22, 23, 31, 32, 39−41, 48, 51, 52, 56−58, 70−72, 74, 75, 78,** and **79**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(46) The neutral molecule **1** was minimized via a Monte Carlo multiple minimum run (with solvation) which assigned random dihedral angles to the four nonterminal rotatable bonds. Each new conformation was automatically minimized and duplicates were eliminated. The six lowest energy unique conformations were then assigned charges and subjected to AM1 geometry optimization. The lowest energy conformation from this run possessed a stacked arrangement of the carboxyl and guanidine groups. We are grateful to Jeff Howe and Jim Petke of Computer Aided Drug Discovery for this analysis.
(47) Active analogues were administered at multiple doses to normal C57 mice as a preliminary gauge of gross toxicity. A panel of liver enzymes, as well as fasting blood glucose, was measured (data not shown). An "unacceptable therapeutic ratio" is indicative of significant alterations in one or more liver enzymes or reductions in blood glucose (not desireable in nondiabetic animals) at doses less than 5 times the active dose in KKA^y mice.
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