Design and Synthesis of Benzoic Acid Derivatives as Influenza Neuraminidase Inhibitors Using Structure-Based Drug Design[1]

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A series of 94 benzoic acid derivatives was synthesized and tested for its ability to inhibit influenza neuraminidase. The enzyme−inhibitor complex structure was determined by X-ray crystallographic analysis for compounds which inhibited the enzyme. The most potent compound tested in vitro, **5** (4-(acetylamino)-3-guanidinobenzoic acid), had an $IC_{50} = 2.5 \times 10^{-6}$ M against N9 neuraminidase. Compound **5** was oriented in the active site of the neuraminidase in a manner that was not predicted from the reported active site binding of GANA (**4**) with neuraminidase. In a mouse model of influenza, **5** did not protect the mice from weight loss due to the influenza virus when dosed intranasally.

Introduction

Influenza is an acute respiratory disease that causes annual epidemics in humans afflicting between 20 and 40 million people in the United States each year. It is particularly dangerous to the very young, the elderly and/or debilitated patients, and those who have suppressed immune systems. Approximately 300 000 people are hospitalized, and about 15 000−20 000 persons die. This mortality figure can be as high as 40 000−70 000 during a more severe outbreak. Four times this century, a particularly virulent new strain of flu virus has emerged resulting in far more disease and deaths than usual. The pandemics of 1957 and 1968 together cost some $32 billion (in 1995 dollars) in lost productivity and medical expenses.[2]

Neuraminidase (EC 3.2.1.18) is one of two major surface glycoproteins of both type A and B influenza viruses and is essential for viral replication in vitro.[3] The enzyme active site of viral neuraminidase is conserved despite up to 75% sequence variation in amino acid sequence between the neuraminidases of influenza A and B, which makes it an attractive target for structure-based drug design (SBDD). Inhibition of neuraminidase by complexation with the active site could lead to drugs which have a broad spectrum of activity against various flu strains.

Sialic acid (**1**) has relatively weak ($IC_{50} \sim 10^{-3}$ M) in vitro viral neuraminidase inhibitory activity, but recent reports[4,5] have shown that deoxysialic acid derivatives **2**−**4** (Chart 1) are potent neuraminidase inhibitors. Compound **4**, which has been described as a transition-state inhibitor,[6] has shown activity against influenza in the clinic both prophylactically and therapeutically in experimentally infected humans.[7] The ring system in **2**−**4** assumes a more planar conformation than the ring system in **1**, and this observation has led to other reports[8,9] which have indicated that aromatic derivatives related to **5** (Chart 2) where the ring system is completely planar also bond to the neuraminidase active site and possess in vitro activity. However, one of the same reports[9] also indicated that **7** (Chart 2), an aromatic derivative closely related to **4**, was inactive in vitro in the H3N2 strain of influenza.

Chart 1. Sialic Acid Derivatives

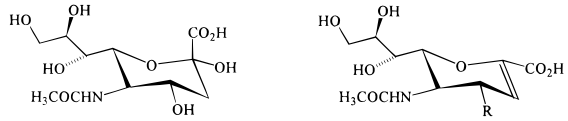
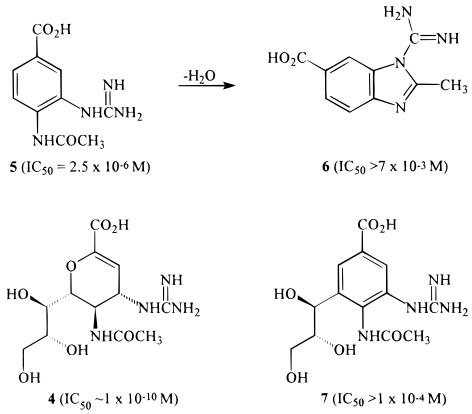


Chart 2. Inhibitors of Influenza Neuraminidase



Aromatic derivatives related to **5** are chemically more readily accessible than the more difficult-to-prepare and costly analogues of **4**. Our goals for this project were to prepare orally active, potent, and selective viral neuraminidase inhibitors which could be easily and economically prepared. Our concurrent studies with **5** in the H1N9 viral strain of influenza gave us similar results to those previously reported[8,9] for the B/Lee/40 and H3N2 strains; in addition, we observed cyclization in **5** (Chart 2) which resulted in loss of neuraminidase inhibitory potency. By studying the interactions of **5** and other derivatives with the active site of neuraminidase, we believed we could design analogues which contained substituents which would maximize the interactions with the active site and also impart chemical

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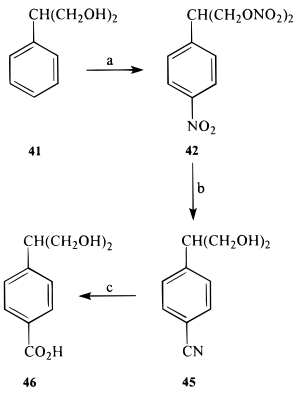
Scheme 1[a]



[a] Reagents: (a) fuming HNO_3; (b) 1. H_2, 10% Pd/C, MeOH, 2. HCl, $NaNO_2$, 3. CuCl, NaCN; (c) 1. 10% NaOH, EtOH, Δ, 4 h, 2. 1 N HCl.

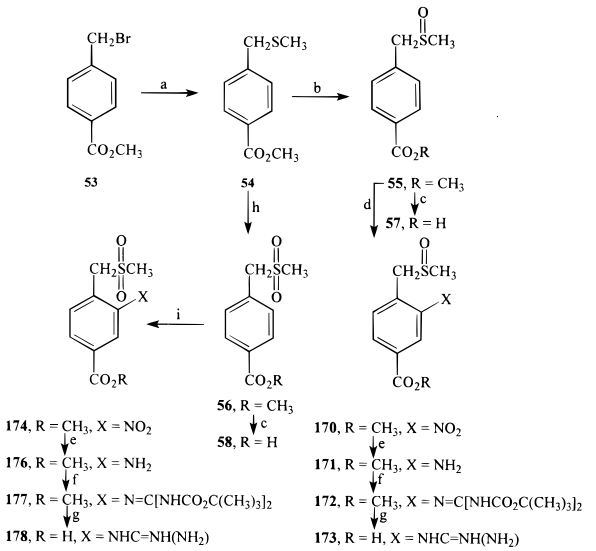
stability to these planar molecules. Hopefully, we could also explain any anomalous results such as the inactivity of **7** in vitro. This report describes our initial work to determine suitable replacements for each of the substituents on **5**. Our approach was to initially test the synthesized compound at a concentration of 7 mM in an in vitro assay[10] for the inhibition of N9 neuraminidase. If the compound showed good activity, an IC_{50} was determined. A solution of certain interesting compounds was also soaked (see Experimental Section) into crystals[11] of neuraminidase. X-ray diffraction data were collected for the enzyme−inhibitor complex and compared with the refined N9 native structure. The difference Fourier maps were calculated to identify the inhibitor in the active site of the enzyme. The inhibitory activity of the compound coupled with its mode of bonding in the active site was used to design other derivatives for synthesis.

Chemistry

Table 1 lists monosubstituted benzoic acid derivatives and isosteres that were tested for in vitro neuraminidase inhibitory activity. Most of these compounds could be purchased or prepared by literature methods. The synthesis of diol **46** is outlined in Scheme 1. Nitration of 2-phenyl-1,3-propanediol (**41**)[29] with fuming nitric acid gives predominantly the para isomer **42** which is relatively stable and can be characterized. Hydrogenation of **42** over palladium gives the amine **44** which was then diazotized and converted to the nitrile **45**. Hydrolysis of **45** gave **46**. Scheme 2 depicts the synthesis of sulfoxide **57** and sulfone **58**. Methyl 4-(bromomethyl)-benzoate (**53**) was converted to the key intermediate **54** with sodium thiomethoxide in DMF. The methylthio derivative was reacted with *m*-chloroperbenzoic acid to give sulfoxide **55** or reacted with H_2O_2 in acetic acid to give sulfone **56**. Hydrolysis of these esters gave **57** and **58**, respectively.

The three-dimensional structure of the active site of N9 neuraminidase and the structure of **2** bonded to the active site have been determined.[39] The active site has 12 amino acids in contact with **2**, while another 6 amino acids stabilize this site. The bonding environment is a very polar and charged environment. There is only one small hydrophobic pocket where the methyl substituent of the $-NHCOCH_3$ group resides. This polar requirement makes the synthesis and purification of com-

Scheme 2[a]



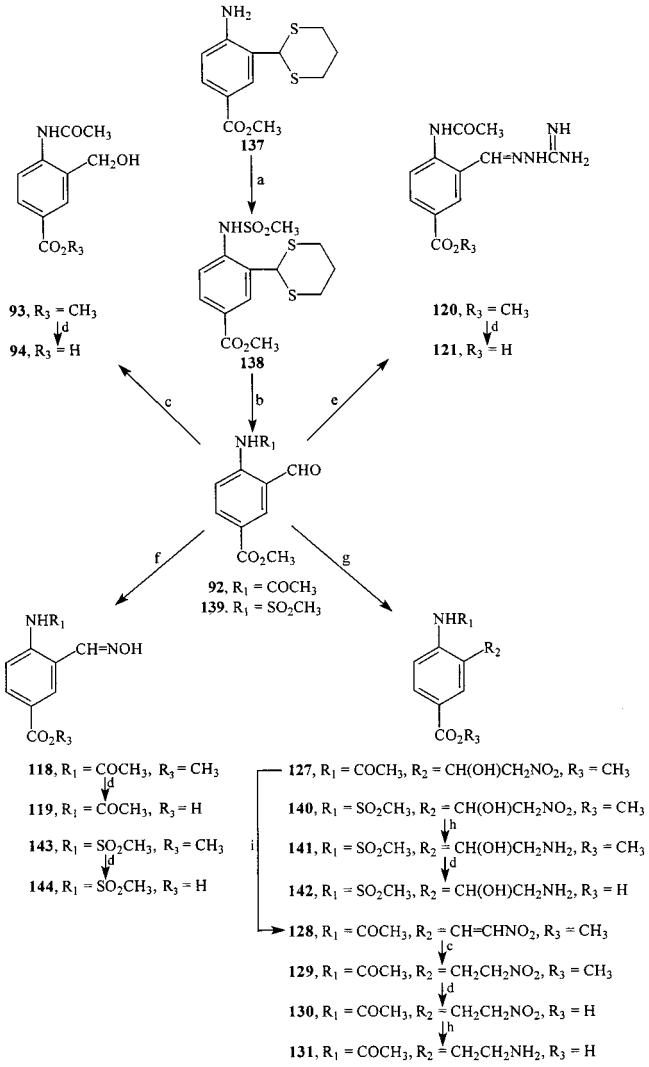
[a] Reagents: (a) $NaSCH_3$, DMF; (b) mClPBA, CH_2Cl_2; (c) 1. 1 N NaOH, 2. HCl; (d) KNO_3, H_2SO_4; (e) H_2, PtO_2, MeOH; (f) $S{=}C[NHCO_2(CH_3)_3]_2$, Et_3N, DMF, $HgCl_2$; (g) 1. TFA, 2. 1 N NaOH, 3. HCl; (h) H_2O_2, HOAc; (i) 90% fuming HNO_3.

pounds which contain several complimentary polar groups contiguously placed around a planar benzene ring a daunting chemical challenge. Replacement of the ring hydroxyl in **2** with the guanidine substituent in **4** increases the in vitro potency of this series of molecules by approximately 5 orders of magnitude, so it was desirable to use the guanidino group as a key substituent for our series of benzoic acid inhibitors. The chemistry associated with the synthesis of guanidine derivatives has been reviewed recently.[40] In most instances, our guanidino substituents were synthesized by three general methods: reaction of an amine with cyanamide; reaction of an amine with aminoiminomethanesulfonic acid;[34] and reaction of an amine with *N*,*N*′-bis(*tert*-butoxycarbonyl)thiourea[41] facilitated by $HgCl_2$.[42] The latter method allowed for protected guanidine derivatives to be prepared which could readily be purified by column chromatography before the charged groups such as carboxyl and/or guanidino were unmasked. Purification of the highly polar molecules after the final synthetic step proved troublesome. The *tert*-butoxy protecting groups could be readily removed by TFA,[43] or both *tert*-butoxy protecting group removal and hydrolysis of an ester could be accomplished in one step using 6 N HCl.

Table 2 lists the disubstituted benzoic acid derivatives which were tested for in vitro neuraminidase inhibitory activity. Most of the compounds listed in Table 2 are novel, and their preparations are described in the Experimental Section. Scheme 3 illustrates the synthesis for several targets from the key intermediates **92** and **139**. These intermediates were prepared using Gassman's method[45] for aldehyde synthesis. Aldehyde **92** was converted to the alcohol **94**, the iminoguanidine derivative **121**, and the aminoethyl analogue **131** by standard methods. Both aldehydes **92** and **139** were converted to their respective oximes **119** and **144**, while **139** was converted to the aminohydroxyethyl derivative **142**.

Scheme 4 depicts the preparation of various derivatives containing a two-carbon substituent ortho to the

Scheme 3[a]

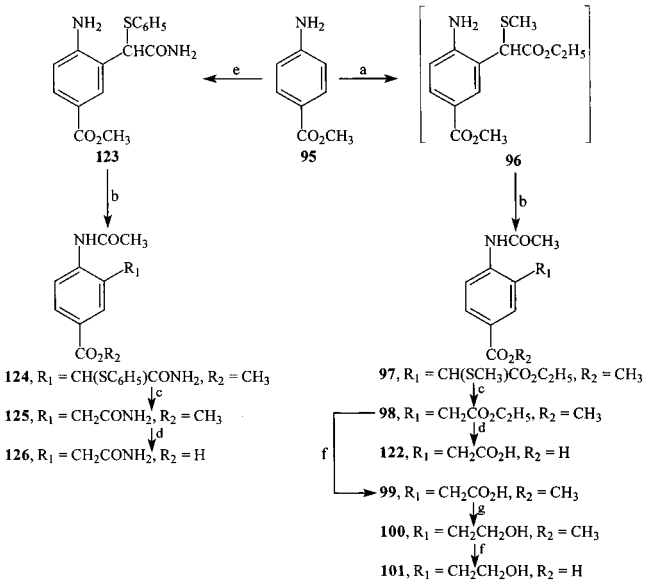


[a] Reagents: (a) MsCl, pyridine, CH_2Cl_2; (b) CuO, $CuCl_2$, acetone, DMF; (c) $NaBH_4$, MeOH; (d) 1. 1 N NaOH, 2. 1 N HCl; (e) aminoguanidine bicarbonate, HCl, EtOH; (f) hydroxylamine hydrochloride, EtOH; (g) NaH, $MeNO_2$, DMF; (h) H_2, PtO_2, EtOH; (i) DMAP, acetic anhydride, CH_2Cl_2.

amino function. These were again prepared utilizing a Gassman[52] procedure. Starting with ester **95**, the diester **98** was prepared by standard methods. The diester could be hydrolyzed to the diacid **122**, or the aliphatic ester could be selectively hydrolyzed to the monoacid **99** in the presence of an aromatic ester by using shorter reaction times. The selective reduction of an acid in the presence of an ester to give **100** was accomplished by preparing the mixed anhydride of the acid and reduction with $NaBH_4$. The amide **126** could be prepared from **95** in a similar manner.

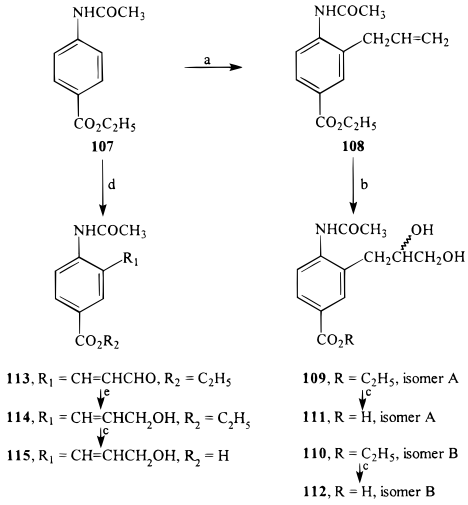
The syntheses of compounds which have substituents designed to mimic the glycerol substituent in **2** are outlined in Scheme 5. The reaction of **107** with palladium acetate and an appropriate alkene gave adducts **108** and **113**. Intermediate **108** was converted to the dihydroxy esters **109** and **110** using commercially available AD mix-α or -*β*. In this instance, the acids obtained after hydrolysis, **111** and **112**, had no optical rotation at the sodium D-line and were chemically, physically, and spectroscopically indistinguishable from each other. Aldehyde **113** was reduced with $NaBH_4$ to yield alcohol **115** after hydrolysis.

Scheme 4[a]



[a] Reagents: (a) *t*-BuOCl, $CH_3SCH_2CO_2C_2H_5$, Et_3N, CH_2Cl_2, −70 °C; (b) CH_3COCl, Et_3N; (c) Raney Ni, THF; (d) 1 N NaOH, 16 h; (e) *t*-BuOCl, $C_6H_5SCH_2CONH_2$, Et_3N, CH_2Cl_2, −70 °C; (f) 1 N NaOH, 1 h; (g) 1. $ClCO_2C_2H_5$, Et_3N, THF, 2. $NaBH_4$, MeOH, 3. HCl.

Scheme 5[a]

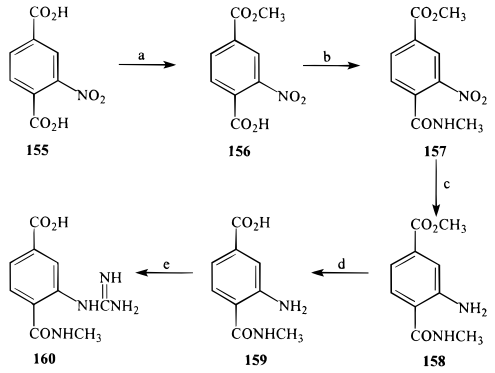


[a] Reagents: (a) 1. $Pd(OAc)_2$, toluene, 2. CH_2=$CHCH_2I$, HOAc; (b) AD mix-α for isomer A, AD mix-*β* for isomer B, *t*-BuOH, H_2O; (c) 1. 1 N NaOH, 2. HCl; (d) 1. $Pd(OAc)_2$, toluene, 2. CH_2=$CHCH(OC_2H_5)_2$, Et_3N; (e) $NaBH_4$, MeOH.

A terephthalic acid derivative (**160**) was prepared by the procedure given in Scheme 6. Selective esterification of one carboxyl group gave **156** which in turn was converted through standard methods to target **160**.

Scheme 7 depicts the synthesis of several 3,5-disubstituted benzoic acid derivatives. The acid group in **185**[51] could be selectively reduced in the presence of an ester using lithium tri-*tert*-butoxyaluminohydride to give both the aldehydo- (**186**) and the hydroxymethyl- (**187**) substituted compounds. The aldehyde was converted to the oxime (**188**), and both the nitro and hydroxyimino groups were reduced catalytically over palladium to give the diamine **189**. The aliphatic amino group in **189** could be preferentially substituted in the presence of the aromatic amino group to yield **191** via

Scheme 6[a]



[a] Reagents: (a) MeOH, H_2SO_4, Δ, 1 h; (b) 1. $SOCl_2$, DMF, 2. $MeNH_2$, H_2O, CH_2Cl_2; (c) $SnCl_2$, EtOH; (d) 1. 1 N NaOH, 2. HCl; (e) cyanamide, HCl, EtOAc, 37−38 °C, 16 h.

intermediate **190**, or both amino groups could be substituted simultaneously to give the disubstituted **193** via **192**.

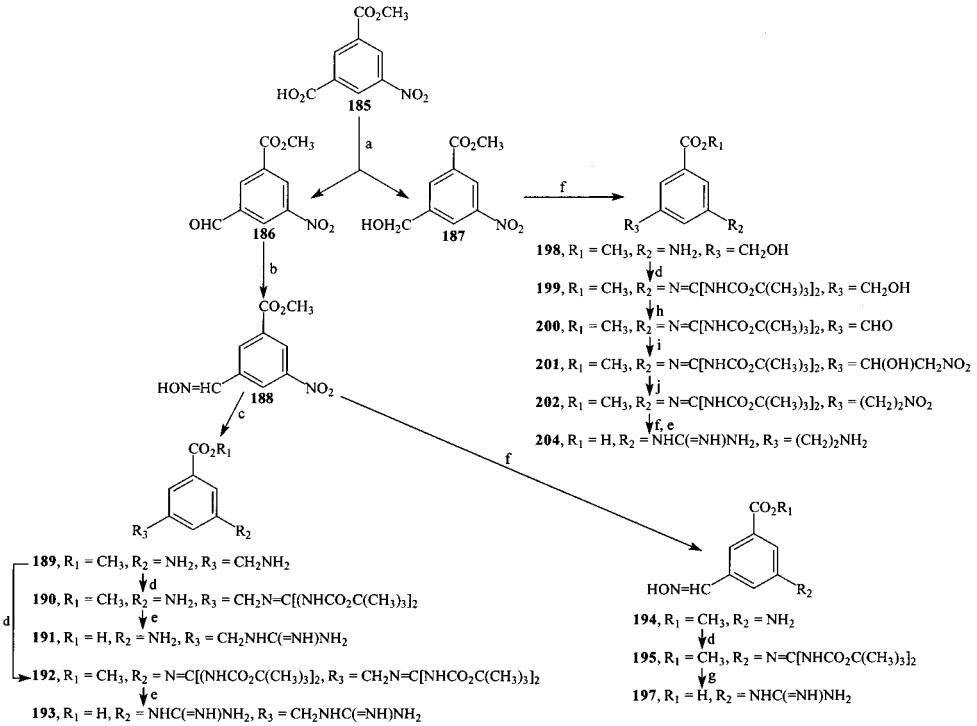
The nitro substituent in **188** could be reduced over platinum oxide preferentially in the presence of the hydroxyimino group to give the monoamine **194**, which is then converted to **197**. The hydroxymethyl-substituted **187** was converted to the aminoethyl-substituted **204** through a series of standard reactions.

Results and Discussion

Initial results from the testing of **11** (Table 1) were encouraging since this relatively simple monosubstituted benzoic acid derivative showed some in vitro inhibitory activity against the H1N9 strain of influenza neuraminidase. Furthermore, X-ray data showed that this weak inhibitor soaked into the N9 crystal and that the carboxyl and acetylamino substituents bonded to the active site in a similar manner and position as did the same substituents in **2**.[39] Compounds **9**−**58** listed in Table 1 were tested to determine suitable replacements for the acetylamino substituent located in a para position to the carboxyl group in the benzoic acid derivative. Derivatives **11**, **12**, **14**−**17**, **31**−**34**, **39**, **46**, **49**, and **57** showed a measurable degree of inhibitory activity at a concentration of 7 mM with **14**, **16**, and **31** being the most potent. Interestingly, **14** (C_6H_5CONH) had an IC_{50} = 1 mM, but no electon density corresponding to this inhibitor was present in the active site of the enzyme. One explanation for the results with **14** is that it interacts with the neuraminidase at a position other than the catalytic site. Derivatives with one (**48**) or two (**51**) $-CH_2-$ spacer groups between the acetylamino substituent and the aromatic ring were inactive at 7 mM in vitro.

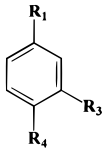
Compounds **59**−**82** contain substituents in a meta position to the carboxyl group in the benzoic acid derivatives. These substituents may substitute for the glycerol side chain in **2**[39] or for the guanidino substituent in **4**.[4] Of these derivatives tested, **60**, **64**, and **67** had the highest degree of activity at 7 mM; however, **67** could not be located in the active site. The −NHCN substituent in **60** and the guanidino substituent in **64** were positioned in the same site as the glycerol substituent in **2** and **4** and not in the same site as the guanidino substituent of **4**. This observation is in agreement with the results reported by Singh et al.[8] for **5** (Table 2) in B/Lee neuraminidase. The activity for the derivative **72**, where one $-CH_2-$ spacer is placed between the guanidino substituent and the aromatic ring, or **76**, where the guanidino group is replaced by an amidino substituent, is reduced and for the deriva-

Scheme 7[a]



[a] Reagents: (a) 1. $(ClCO)_2$, DMF, CH_2Cl_2, 2. CuI, $LiAlH[OC(CH_3)_3]_3$, THF; (b) $NH_2OH\cdot HCl$, EtOH; (c) H_2, 10% Pd/C, EtOH; (d) $S{=}C[NHCO_2C(CH_3)_3]_2$, Et_3N, DMF, $HgCl_2$; (e) 6 N HCl; (f) H_2, PtO_2, EtOH; (g) 1. TFA, 2. 1 N NaOH, 3. 1 N HCl; (h) pyridinium chlorochromate, CH_2Cl_2; (i) NaH, CH_3NO_2, DMF; (j) 1. $NaBH_4$, EtOH, 2. HOAc.

Table 1. In Vitro Inhibitory Activity and Soak Study Results in Influenza Neuraminidase (H1N9) for Monosubstituted Benzoic Acids



compd	R_1	R_3	R_4	formula[a]	mp, °C[b]	source[c]	in vitro activity: % inhibtn at 7 mM	in vitro activity: IC_{50} (mM)	density in active site (H1N9)[d]
9	CO_2H	H	H	$C_7H_6O_2$	122−123	purchased[e]	0		−
10	CO_2H	H	NH_2	$C_7H_7NO_2$	188−189	purchased[e]	0		−
11	CO_2H	H	$NHCOCH_3$	$C_9H_9NO_3$	258	purchased[e]	39		Y
12	CO_2H	H	$NHCOCF_3$	$C_9H_6F_3NO_3$	277−278	ref 13	56[f]		N
13	CO_2H	H	$NHCOCH(CH_3)_2$	$C_{11}H_{13}NO_3$	238−239	ref 14	0		N
14	CO_2H	H	$NHCOC_6H_5$	$C_{14}H_{11}NO_3$	278−280	ref 15		1.5	N
15	CO_2H	H	$NHCOCO_2H$	$C_9H_7NO_5$	235−237	ref 16	15[g]		N
16	CO_2H	H	$NHCSCH_3$	$C_9H_9NO_2S$	220	ref 17	1		Y
17	CO_2H	H	$NHSO_2CH_3$	$C_8H_9NO_4S$	248	ref 18	34		Y
18	CO_2H	H	$NHSO_2CF_3$	$C_8H_6F_3NO_4S$	228−229	ref 19	0		−
19	CO_2H	H	$NHSO_2C_6H_5$	$C_{13}H_{11}NO_4S$	214−215	ref 20	0[h]		−
22	CO_2H	H	$NHCO_2CH_3$	$C_{10}H_9NO_5 \cdot 0.25H_2O$	195−196	Exptl Section	0		−
23	CO_2H	H	$NHCONH_2$	$C_8H_8N_2O_3$	>400	ref 21	0		−
24	CO_2H	H	$NHCONHCH_3$	$C_9H_{10}N_2O_3$	>400	ref 22	0		−
25	CO_2H	H	$NHCON(CH_3)_2$	$C_{10}H_{12}N_2O_3$	237−239	ref 23	0		−
26	CO_2H	H	$NHC(=NH)NH_2$	$C_8H_9N_3O_2 \cdot HCl$	285 dec	purchased[e]	0		−
27	CO_2H	H	OH	$C_7H_6O_3$	214−216	purchased[e]	0		−
28	CO_2H	H	O_2CCH_3	$C_9H_8O_4$	186.5−188	purchased[i]	0		−
30	CO_2H	H	O_2CNHCH_3	$C_9H_9NO_4$	223−225	Exptl Section	0		−
31	CO_2H	H	SO_2NH_2	$C_7H_7NO_4S$	290−292	purchased[e]		2.5	Y
32	CO_2H	H	SO_2NHCH_3	$C_8H_9NO_4S$	239−241	ref 24	48		−
33	CO_2H	H	$CONH_2$	$C_8H_7NO_3$	>300	ref 25	14		−
34	CO_2H	H	$CONHCH_3$	$C_9H_9NO_3$	270	ref 17	27		Y
35	CO_2H	H	$C(=NOH)CH_3$	$C_9H_9NO_3$	275−277	ref 26	0[h]		−
37	CO_2H	H	$C[=NNHC(=NH)NH_2]CH_3$	$C_{10}H_{12}N_4O_2 \cdot 0.25H_2O$	328 dec	Exptl Section	0[i]		N
38	CO_2H	H	$COCH_3$	$C_9H_8O_3$	208−210	purchased[e]	0		−
39	CO_2H	H	$CH(OH)CH_3$	$C_9H_{10}O_3$	127−130	ref 27	11		−
40	CO_2H	H	CH_2CH_2OH	$C_9H_{10}O_3$	125	ref 28	0		−
46	CO_2H	H	$CH(CH_2OH)_2$	$C_{10}H_{12}O_4$	128−131	Exptl Section	20		−
48	CO_2H	H	$CH_2NHCOCH_3$	$C_{10}H_{11}NO_3$	195	Exptl Section	0		N
49	CO_2H	H	$CH_2NHC(=NH)NH_2$	$C_9H_{11}N_3O_2 \cdot H_2O$	322−324	ref 30	14[k]		Y
50	CO_2H	H	$CH_2CH_2NH_2$	$C_9H_{11}NO_2 \cdot HCl$	297−299	ref 30	0		N
51	CO_2H	H	$CH_2CH_2NHCOCH_3$	$C_{11}H_{13}NO_3$	180−184	ref 31	0		−
52	CO_2H	H	$CH_2CH_2NHC(=NH)NH_2$	$C_{10}H_{13}N_3O_2$	>300	ref 30	0		−
57	CO_2H	H	CH_2SOCH_3	$C_9H_{10}O_3S \cdot 0.25H_2O$	177−178	Exptl Section	6.5		−
58	CO_2H	H	$CH_2SO_2CH_3$	$C_9H_{10}O_4S$	249−253	Exptl Section	0		−
59	CO_2H	NH_2	H	$C_7H_7NO_2$	178−180	purchased[e]	0		−
60	CO_2H	NHCN	H	$C_8H_6N_2O_2 \cdot 0.2H_2O$	238−240	Exptl Section		4	Y
61	CO_2H	$NHCOCH_3$	H	$C_9H_9NO_3$	247.5−248.5	purchased[l]	0		−
62	CO_2H	$NHCONH_2$	H	$C_8H_8N_2O_3$	276−280	ref 21	0		−
63	CO_2H	$NHCSNH_2$	H	$C_8H_8N_2O_3S$	183−185	purchased[m]	0		−
64	CO_2H	$NHC(=NH)NH_2$	H	$C_8H_9N_3O_2 \cdot 0.5HCl$	278	ref 32		5	Y
67	CO_2H	$NHC(=NCN)NH_2$	H	$C_9H_8N_4O_2 \cdot 0.25H_2O$	>300	Exptl Section	68		N
68	CO_2H	OH	H	$C_7H_6O_3$	200−202	purchased[e]	0		−

69	CO_2H	$CH_2NHCOCH_3$	H	$C_{10}H_{11}NO_3$	165−166	Exptl Section	0	–
72	CO_2H	$CH_2NHC(=NH)NH_2$	H	$C_9H_{11}N_3O_2$	308−311	Exptl Section	18	N
76	CO_2H	$CH_2C(=NH)NH_2$	H	$C_9H_{10}N_2O_2$	219−221	Exptl Section	22[h]	N
77	CO_2H	$CH_2CH_2NH_2$	H	$C_9H_{11}NO_2 \cdot HCl$	256−258	ref 35	0	–
80	CO_2H	$CH_2CH_2NHCOCH_3$	H	$C_{11}H_{13}NO_3$	146−148	Exptl Section	0	–
81	CO_2H	$CH_2CH_2NHC(=NH)NH_2$	H	$C_{10}H_{13}N_3O_2$	>310	Exptl Section	0[h]	–
82	CO_2H	NH(5-tetrazolyl)	H	$C_8H_7N_5O_2$	243−245	Exptl Section	0[h]	–
83	$CONH_2$	H	$NHCOCH_3$	$C_9H_{10}N_2O_2$	262−263	ref 36	insol[n]	–
84	SO_2H	H	$NHCOCH_3$	$C_8H_8NO_3S \cdot Na \cdot 2H_2O$	313−316	purchased[o]	0	–
85	SO_2NH_2	H	$NHCOCH_3$	$C_8H_{10}N_2O_3S$	212.5−215	purchased[o]	0	–
86	SO_3H	H	$NHCOCH_3$	$C_8H_9NO_4S \cdot 0.25H_2O$	235−238	ref 37	30	–
87	PO_3H_2	H	$NHCOCH_3$	$C_8H_{10}NO_4P \cdot H_2O$	228	ref 38	32[p]	–
88	NO_2	H	$NHCOCH_3$	$C_8H_8N_2O_3$	216−218	purchased[e]	0	–
90	5-tetrazolyl	H	$NHCOCH_3$	$C_9H_9N_5O$	287	Exptl Section	0[h]	–
2						purchased[q]	0.013	Y

[a] All compounds were analyzed for C, H, and N, and results agreed to ±0.4% of theoretical values. [b] Melting points are uncorrected. [c] Compounds tested were purchased, prepared by literature procedures (see specific references), or synthesized as described in the Experimental Section. [d] Soak study results: Y = density found in active site; N = no density found in active site; – = not tested. [e] Purchased from Aldrich Chemical Co. [f] Concentration tested was 3.3 mM. [g] Concentration tested was 0.7 mM. [h] Concentration tested was 3.5 mM. [i] Purchased from Lancaster Synthesis. [j] Concentration tested was 0.26 mM. [k] Concentration tested was l.75 mM. [l] Purchased from Schweizerhall, Inc. [m] Purchased from TransWorld Chemicals. [n] Too insoluble to test. [o] Purchased from TCI America. [p] Concentration tested was 14 mM. [q] Purchased from Sigma.

Figure 1. Comparison of the binding of **5** (green) and **119** (white) in the active site of H1N9 neuraminidase.

tive **81**, where two $-CH_2-$ spacer groups are between the guanidino substituent and the aromatic ring, is abolished.

In compounds **83**−**90**, the *p*-acetylamino substituent was held constant while various isosteres were substituted for the carboxyl group. Of these, the derivatives containing the $-SO_3H$ (**86**) and $-PO_3H_2$ (**87**) groups retained activity.

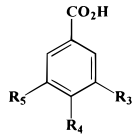
On the basis of the results obtained for the compounds listed in Table 1, the derivatives in Table 2 were prepared to determine the effect on potency when a second substituent for interaction with the active site was added to the benzoic acid derivatives. The propensity for substituents located in the meta and para positions of the benzoic acid derivatives to undergo chemical reactions with each other was also assessed. In compounds **91**−**115**, the *p*-acetylamino substituent was held constant while various hydroxylated substituents were placed in the meta position to mimic the glycerol side chain of **2**. The in vitro activity of these compounds was disappointing since none of the compounds had increased activity over **11** which has a hydrogen in the meta position.

Compounds **116**−**131** in Table 2 were tested to determine if other substituents would bond in the pocket where the glycerol substituent of **2** bonds. Clearly, **5** was the most potent compound tested in vitro. Although **122** and **126** possessed some in vitro activity, they could not be located in the difference electron density maps. Of the compounds which did bond to the active site (**5**, **117**, **119**, **130**, and **131**), only the hydroxyimino substituent of **119** did not point to the glycerol pocket. The hydroxyimino of **119** was positioned in the pocket in which the guanidino substituent of **4** bonds, and the acetylamino group had different interactions with the active site than in **5** (Figure 1).

Replacement of the acetylamino substituent was studied with compounds **136**−**178**. Of these, the (methylsulfonyl)amino (**136**), (methylamino)carbonyl (**160**), aminosulfonyl (**164**), and (methylsulfoxyl)methyl (**173**) all bonded in the same site as did the acetylamino group of **5** (Figures 2 and 3) albeit with different interactions.

Only the *o*-(methylamino)carbonyl substituent of **160** appeared to undergo cyclization with the guanidino

Table 2. In Vitro Inhibitory Activity and Soak Study Results in Influenza Neuraminidase (H1N9) for Disubstituted Benzoic Acids



compd	R_3	R_4	R_5	formula[a]	mp, °C[b]	in vitro activity: % inhibtn at 7 mM	in vitro activity: IC_{50} (mM)	density in active site (H1N9)[c]
5[d]	$NHC(=NH)NH_2$	$NHCOCH_3$	H	$C_{10}H_{12}N_4O_3 \cdot H_2O$	260		0.0025	Y
11[e]	H	$NHCOCH_3$	H	$C_9H_9NO_3$	258	39		Y
91[f]	OH	$NHCOCH_3$	H	$C_9H_9NO_4$	245−248	33		−
94	CH_2OH	$NHCOCH_3$	H	$C_{10}H_{11}NO_4$	180−181	31		−
101	CH_2CH_2OH	$NHCOCH_3$	H	$C_{11}H_{13}NO_4$	206−208 dec	42		Y
106	OCH_2CH_2OH	$NHCOCH_3$	H	$C_{11}H_{13}NO_5$	207.5−208.5	0		N
111	$CH_2CH(OH)CH_2OH$ (A)	$NHCOCH_3$	H	$C_{12}H_{15}NO_5 \cdot 0.2H_2O$	181−183	25		Y
112	$CH_2CH(OH)CH_2OH$ (B)	$NHCOCH_3$	H	$C_{12}H_{15}NO_5 \cdot 0.75H_2O$	180−182	35		Y
115	$CH=CHCH_2OH$	$NHCOCH_3$	H	$C_{12}H_{13}NO_4$	214−215	13		Y
116[g]	NO_2	$NHCOCH_3$	H	$C_9H_8N_2O_5$	215−230	15[h]		−
117[g]	NH_2	$NHCOCH_3$	H	$C_9H_{10}N_2O_3 \cdot 0.5H_2O$	272 dec	30[h]		Y
119	CH=NOH	$NHCOCH_3$	H	$C_{10}H_{10}N_2O_4 \cdot 0.25H_2O$	215−218 dec		5.5	Y
121	$CH=NNHC(=NH)NH_2$	$NHCOCH_3$	H	$C_{11}H_{13}N_5O_3 \cdot 2H_2O$	326−327	0[i]		N
122	CH_2CO_2H	$NHCOCH_3$	H	$C_{11}H_{11}NO_5$	240−241	49		N
126	CH_2CONH_2	$NHCOCH_3$	H	$C_{11}H_{12}N_2O_4 \cdot 0.15H_2O$	324−326	51		N
130	$CH_2CH_2NO_2$	$NHCOCH_3$	H	$C_{11}H_{12}N_2O_5$	220 dec		2.5	Y
131	$CH_2CH_2NH_2$	$NHCOCH_3$	H	$C_{11}H_{14}N_2O_3 \cdot 0.25H_2O$	212−213 dec	41		Y
136	$NHC(=NH)NH_2$	$NHSO_2CH_3$	H	$C_9H_{12}N_4O_4S \cdot 0.5H_2O$	200		0.1	Y
142	$CH(OH)CH_2NH_2$	$NHSO_2CH_3$	H	$C_{10}H_{14}N_2O_5S \cdot 0.2H_2O$	274−276	0[k]		N
144	CH=NOH	$NHSO_2CH_3$	H	$C_{10}H_{10}N_2O_5S$	250		2	Y
149	$NHC(=NH)NH_2$	$NHCOCH(CH_3)_2$	H	$C_{12}H_{16}N_4O_3 \cdot 0.5H_2O$	238−239.5		1	Y
154	$NHC(=NH)NH_2$	NHCO(2-furanyl)	H	$C_{13}H_{12}N_4O_4$	227−228	0[l]		N
160	$NHC(=NH)NH_2$	$CONHCH_3$	H	$C_{10}H_{12}N_4O_3 \cdot HCl \cdot H_2O \cdot 0.25CH_3OH$	>300		0.005	Y
164	$NHC(=NH)NH_2$	SO_2NH_2	H	$C_8H_{10}N_4O_4S \cdot 0.25H_2O$[m]	>320		0.009	Y
169	$NHC(=NH)NH_2$	CH_2CH_2OH	H	$C_{10}H_{13}N_3O_3 \cdot 0.25H_2O$	270 dec	42[n]		Y
173	$NHC(=NH)NH_2$	CH_2SOCH_3	H	$C_{10}H_{13}N_3O_3S \cdot H_2O$	211−213		0.14	Y
178	$NHC(=NH)NH_2$	$CH_2SO_2CH_3$	H	$C_{10}H_{13}N_3O_4S \cdot H_2O$	261−264		0.3	Y
179[e]	NH_2	H	NH_2	$C_7H_8N_2O_2 \cdot 2HCl \cdot 0.5H_2O$	>300	0		−
180	NHCN	H	NHCN	$C_9H_6N_4O_2$	300 dec	44[o]		−
183	NH_2	H	$NHC(=NH)NH_2$	$C_8H_9N_4NaO_2 \cdot H_2O$	198−203		2	Y
184	$NHC(=NH)NH_2$	H	$NHC(=NH)NH_2$	$C_9H_{12}N_6O_2 \cdot 1.5HCl \cdot 0.2C_2H_5OH$	280 dec		1.5	Y
191	NH_2	H	$CH_2NHC(=NH)NH_2$	$C_9H_{12}N_4O_2 \cdot 2HCl$	245−247	0		−
193	$NHC(=NH)NH_2$	H	$CH_2NHC(=NH)NH_2$	$C_{10}H_{14}N_6O_2 \cdot HCl \cdot 0.75H_2O$	>300	0[p]		N
196	$NHC(=NH)NH_2$	H	CH=NOH	$C_9H_{10}N_4O_3 \cdot 0.75H_2O$	248−253 dec		0.5	Y
204	$NHC(=NH)NH_2$	H	$CH_2CH_2NH_2$	$C_{10}H_{14}N_2O_2 \cdot 2HCl \cdot H_2O \cdot 0.25EtOAc$	hygroscopic	11		−
2[q]							0.013	Y

[a] All compounds were analyzed for C, H, and N, and results agreed to ±0.4% of theoretical values. [b] Melting points are uncorrected. [c] Soak study results: Y = density found in active site; N = no density found in active site; − = not tested. [d] Reference 8. [e] Purchased from Aldrich Chemical Co. [f] Reference 44. [g] Reference l2. [h] Dose tested was 3.5 mM. [i] Dose tested was 0.05 mM. [j] N: calcd, 11.72; found, 11.27. [k] Dose tested was 2.3 mM. [l] Dose tested was 0.9 mM. [m] N: calcd, 21.33; found, 21.88. [n] Dose tested was 2.6 mM. [o] Dose tested was 0.5 mM. [p] Dose tested was 0.35 mM. [q] Purchased from Sigma.

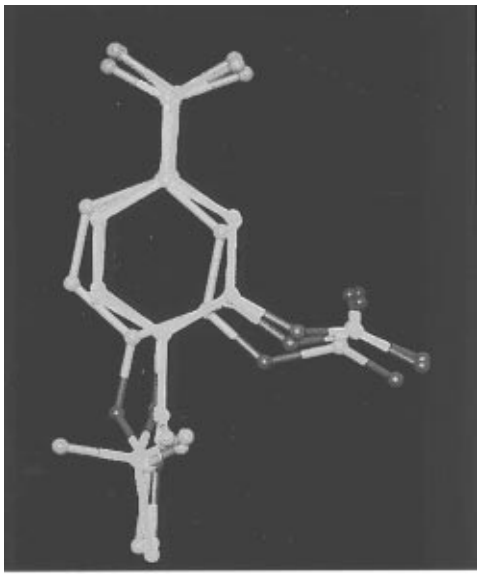


Figure 2. Comparison of the binding of **5** (green, $HNCOCH_3$), **136** (yellow, $NHSO_2CH_3$), and **160** (white, $CONHCH_3$) of the active site of H1N9 neuraminidase.

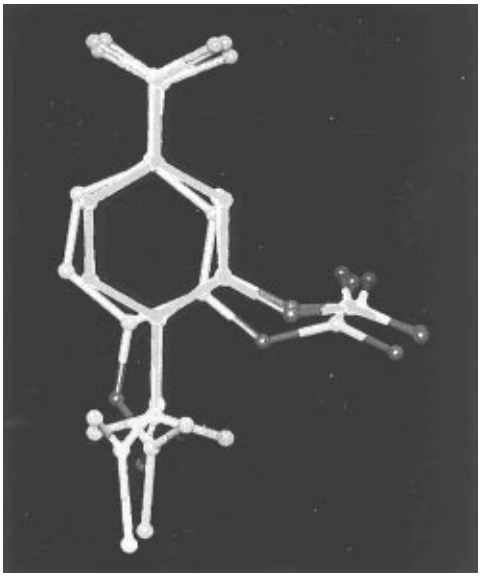


Figure 3. Comparison of the binding of **5** (green, $NHCOCH_3$), **164** (white, SO_2NH_2), and **173** (blue, CH_2SOCH_3) in the active site of H1N9 neuraminidase.

group at higher temperatures. Compounds **136**, **164**, and **173** appeared to be stable up to their melting points. Again, the hydroxyimino substituent of **144** bonded to the same pocket where the guanidino substituent in **4** bonded.

Examples of meta-substituted benzoic acids are compounds **179**−**204**. These compounds without the *p*-acetylamino group were tested to determine a combination of substituents which would bond in each of the guanidino and glycerol bonding sites of **4**. Figure 4 depicts the comparison of **5**, **184**, and **196** in the active site of the neuraminidase enzyme. The loss of the interactions of the acetylamino−substituent interaction with the active site reduced the in vitro potency compared with **5** for all of these (**179**−**204**) derivatives.

The most potent compound in this report against neuraminidase activity, **5**, was tested in vivo in an influenza infection weight loss model in the mouse according to the method of Johansson et al.[53] The

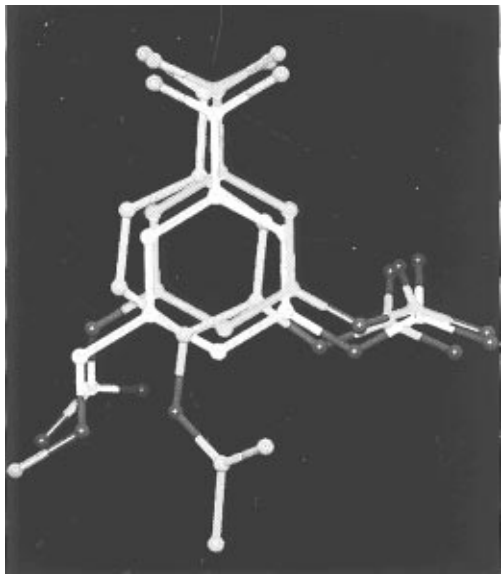


Figure 4. Comparison of the binding of **5** (green, 4-NH-$COCH_3$), **184** (yellow, 5-NHC(=NH)NH_2), and **196** (blue, 5-CH=NOH).

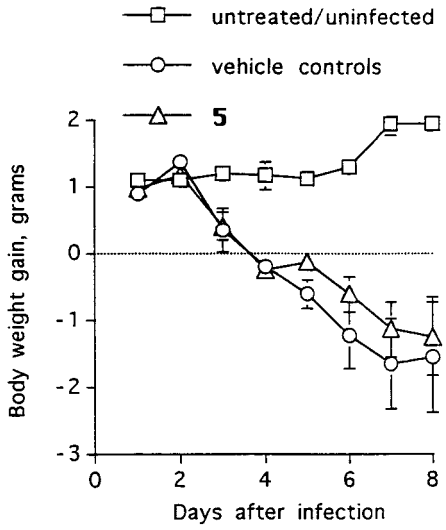


Figure 5. Effects of daily intranasal administration of **5**, 10 mg/kg, twice per day, on the time course of body weight loss in mice after infection with influenza virus. Values are means ± SE, $n = 5$/group.

effects of **5** intranasal treatment in influenza-infected mice are shown in Figure 5. Compound **5** failed to prevent weight loss and was not different from the vehicle controls. The effects of **2** (DANA) intranasal treatment are shown in Figure 6. Compound **2** produced a significant attenuation of weight loss compared to the vehicle control but did not completely prevent weight loss in response to infection compared to the uninfected, untreated control. The results in Figure 7 show that active immunization by infection with the influenza virus is completely effective in preventing weight loss in response to a second infection.

Conclusions

The planar benzene ring provides a scaffold to support substituents which will interact with the active site of influenza neuraminidase. Based upon the data presented herein, a direct correlation between the interactions observed for the substituents of DANA (**2**) and GANA (**4**) and the active site cannot be extended to the

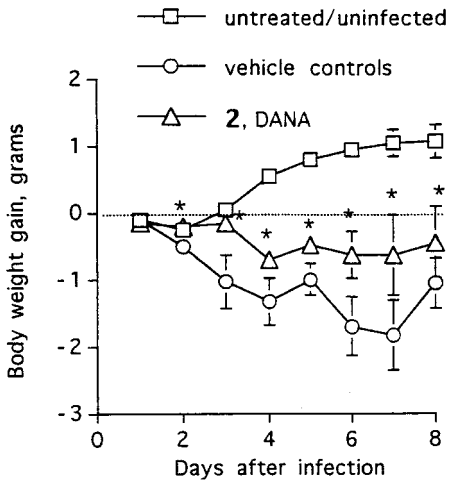


Figure 6. Effects of daily intranasal administration of **2** (DANA), 10 mg/kg, twice per day, on the time course of body weight loss in mice after infection with influenza virus. *Significantly different from vehicle-treated mice, $p < 0.05$. Values are means ± SE, $n = 5$/group.

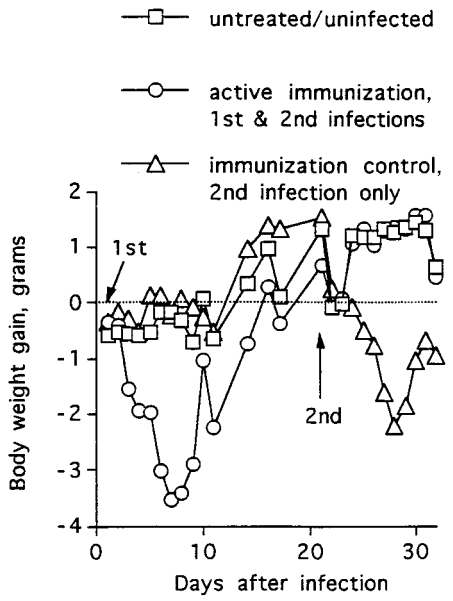


Figure 7. Effects of active immunization on weight loss in mice after infection with influenza. Values are means ± SE, $n = 10$/group.

benzene ring. From the enzyme−inhibitor complex of **5**, it can be seen that the interaction of the guanidino substituent is more favorable with the active site pocket where the glycerol substituent of **4** resides, rather than in the pocket where the guanidino substituent of **4** bonds. Considering this observation, it is not surprising that Williams et al.[9] did not see any in vitro inhibitory activity for **7**.

Interactions for individual substituents on the benzene ring with the active site cannot be considered to be additive. Each combination of substituents has unique steric and electronic interactions with each other which influences the overall interaction of the compound with the active site. This makes it difficult to predict a substituent arrangement for this series that will give an enhanced bonding to the active site.

Moieties which can be substituted for the $-NHCOCH_3$ group are $-NHSO_2CH_3$, $-CONHCH_3$, $-SO_2NH_2$, and $-CH_2SOCH_3$. These groups all bond to the same pocket as does the $-NHCOCH_3$ group albeit each in a different manner. The $-NHSO_2CH_3$, $-SO_2NH_2$, and $-CH_2SOCH_3$ groups appear to be stable at elevated temperatures when located contiguous to a guanidino group.

Although **2** (DANA), a relatively weak inhibitor of influenza neuraminidase, was moderately, but significantly, effective in preventing weight loss in influenza-infected mice, the most potent inhibitor, **5**, was not. It is not clear why **5** was not active in vivo, but it is possible that it may be cleared rapidly from the lung by metabolism and/or absorption. Active immunization demonstrated that weight loss can be prevented by this stimulus for antibody production against influenza, in agreement with the findings of Johansson et al.,[53] who further demonstrated that immunization with purified influenza neuraminidase was also completely effective in preventing weight loss in the influenza-infected mouse. Thus, it appears that **5** may not have properties suitable for in vivo efficacy. Similar results to those seen for **5** were obtained with **160**.

This present study lays the foundation to select appropriate substituents for arrangement around a benzene ring that would lead to compounds possessing inhibitory potency in the same range as **4**. Future reports will discuss compounds with more than three substituents about a benzene ring and the exploitation of a lipophilic pocket in the active site of neuraminidase recently reported.[54,55]

Experimental Section

Biochemistry. The in vitro assay is based on the method reported by von Itzstein et al.[10] The neuraminidase from the H1N9 strain of influenza was obtained by the method described by Laver et al.[11] Values for the IC_{50} were measured via a spectrofluorometric technique that uses 2′-(4-methylumbelliferyl)-α-D-acetylneuraminic acid as substrate. This substrate was cleaved by neuraminidase to yield a fluorescent product which can be quantified. The assay mixture contained inhibitors at various concentrations (4−6 points) and enzyme in 32.5 mM MES ((2-(*N*-morpholino)ethanesulfonic acid) buffer, 4 mM $CaCl_2$ at pH 6.5 (total volume = 80 μL). The reaction was started by the addition of 20 μL of the substrate to a final concentration of 75 μM. After 10 min at 37 °C, 2.4 mL of 0.1 M glycine/NaOH (pH 10.2) was added to 0.1 mL of the reaction mixture to terminate the reaction. A blank was run with the same substrate solution with no enzyme. Fluorescence was read using an Aminco-Bowman fluorescence spectrophotometer (excitation, 360 nm; emission, 450 nm), and substrate blanks were subtracted from the sample readings. The IC_{50} was calculated by plotting percent inhibition versus the inhibitor concentration, and determination of each point was performed in duplicate.

Biology. Mice (Balb/c females, 31−53 days of age) were infected intranasally under light ether anesthesia with A/Turkey/Mass/76A/Beijing/32/92[R] (H6N2). The IC_{50} of **5** against this strain was 2.25 μM ($n = 2$). The infection was at 200 times the MID_{50} (mouse infectious dose, 50%). Compound **5** was dissolved in phosphate-buffered saline and administered intranasally 5−10 min before infection at 10 mg/kg. The mice were treated thereafter at 10 mg/kg/day intranasally, twice a day. Body weight was measured daily. DANA (**2**) was tested for comparison under similar conditions at 10 mg/kg/day intranasally, twice a day. The IC_{50} of DANA against this strain was 6 μM ($n = 3$). A vehicle-treated group and an untreated/uninfected group were also included. There were 5 mice/group. In a second experiment mice were actively immunized with virus and then infected again to demonstrate that active immunity prevents weight loss in the mouse in response to intranasal influenza infection. Differences among groups were analyzed by analysis of variance with a least significant difference test.

Crystallography. Influenza neuraminidase has an N-terminal hydrophobic sequence which anchors the glycoprotein to the viral membrane. This sequence is followed by a highly variable "stalk" which raises the globular neuraminidase "heads" away from the membrane. Neuraminidase heads released with protease contain all the enzymatic and antigenic activity and are used for crystallization. Purified N9 neuraminidase[11] was crystallized using the hanging drop technique in which droplets of protein solution on siliconized cover slips are inverted on a linbro plate. The droplet consisted of equal volumes of protein solution (10−15 mg/mL in water) and potassium phosphate buffer (1.7 M, pH 6.6). This mixture was equilibrated through vapor phase with a reservoir of 1.9 M potassium phosphate buffer at pH 6.8. Large rhombic dodecahedral crystals grew in a few days. The space group has been identified as cubic, *I*432 with cell dimension $a = 183.8$ Å. The crystals are stable at room temperature indefinitely in the artificial mother liquor, and they diffract strongly to at least 2-Å resolution on a rotating anode source.

Inhibitor complexes with neuraminidase were prepared by diffusing the compound into N9 crystals that were transferred into a 2-mL solution of the stabilization buffer which was the same as the reservoir solution used in crystallization. The final concentration of the inhibitor in the solution was 1−2 mM. The N9 crystals were allowed to equilibrate with the inhibitor compound for at least 24 h before data collection.

All X-ray intensity measurements were recorded with a Nicolet/Siemens X-100 multiwire area detector. The detector is mounted on a Rigaku RU-300 rotating anode X-ray generator operating at 100 mA and 50 kV with a 0.3 × 0.3 focus and a Cu anode. The data collection parameters were crystal to detector distance of 16 cm, swing angle of 28°, frame width of 0.1°, and exposure time of 240−300 s. Each crystal provided about 800−900 frames of data before radiation damage deteriorated the diffraction quality.

The X-ray intensity data were processed using the XENGEN suite of programs. The integrated intensities were then scaled and merged to produce a final data set containing only unique reflections. The completeness of data to 2.1 Å is generally about 90%. The refinement of the inhibitor complexes was performed using X-PLOR simulated annealing protocol. Diffraction data in 10−2.1-Å resolution range were used in the refinement with a 2σ cutoff on F_o's. The starting model for the refinement of the inhibitor complexes is the 1.9-Å refined uncomplexed neuraminidase native structure. The water molecules in the active site of the native structure were removed prior to the refinement of the complex structure. A full cycle of X-PLOR simulated annealing protocol was carried out on this model. $F_o - F_c$ and $2(F_o - F_c)$ difference Fourier maps were calculated, and a model of the inhibitor molecule was fitted into the electron density in the active site. Water molecules were also placed in the active site based on the difference electron density maps, and these positions were compared to the active site water structure in the native neuraminidase model. At the end of the simulated annealing protocols and positional and temperature factor refinement, the crystallographic *R*-factor converged to 17−19% at 2-Å resolution for the inhibitor−complex structures.

Chemistry. General Procedures. Melting points were determined in open capillary tubes in a Melt-Temp II melting point apparatus and are uncorrected; ^{1}H NMR spectra were obtained in $CDCl_3$, Me_2SO-d_6, or TFA-*d* with Me_4Si as internal standard or in D_2O or ND_4OD with DSS as internal standard on a Bruker AM400 or Bruker AM360 spectrometer; IR spectra were run as KBr pellets on a BioRad FTS-7 FTIR spectrometer; mass spectra were determined on a Fisons Trio 2000 quadrupole mass spectrometer. Elemental analyses were obtained from Atlanta Microlab, Inc. (Norcross, GA) and are within 0.4% of the calculated values unless otherwise noted.

1-(Aminoiminomethyl)-2-methylbenzimidazole-6-carboxylic Acid (6). To a mixture of 0.97 g (0.005 mol) of 4-(acetylamino)-3-aminobenzoic acid (**8**)[12] in 40 mL of EtOH were added 0.44 mL (0.005 mol) of concentrated HCl and 1.5 g (0.036 mol) of cyanamide. The reaction mixture was stirred at ambient temperature for 48 h. Compound **5** precipitated and was removed by filtration. The filtrate was concentrated, and the residue was purified by column chromatography on silica gel (6:1:0.5 EtOAc−MeOH−H_2O). The appropriate fractions ($R_f = 0.45$) were combined and concentrated to give 0.14 g (12%) of **6** as a brown powder: mp 195−198 °C dec; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.52 (s, 3H), 5.41 (br s, 1H), 6.21 (br s, 1H), 6.57 (br s, 1H), 7.50 (d, $J = 7.8$ Hz, 1H), 7.75 (dd, $J = 7.8$, 1.4 Hz, 1H), 8.05 (s, 1H), 12.50 (s, 1H); IR (KBr) 3336, 1635, 1558, 1384, 1363 cm^{-1}; MS (ES$^+$) 177 (M + 1). Anal. ($C_{10}H_{10}N_4O_2 \cdot 0.6H_2O$) C, H; N: calcd, 24.46; found, 23.96.

4-[(Methoxycarbonyl)amino]benzoic Acid (22). A solution of 5.0 g (0.033 mol) of methyl *p*-aminobenzoate (**20**; Aldrich) in 100 mL of CH_2Cl_2 was cooled to 0−5 °C, and 3.2 mL (0.04 mol) of pyridine, 2.8 mL (0.04 mol) of methyl chloroformate, and 0.5 g of DMAP were added. The reaction mixture was stirred at ambient temperature for 16 h and then diluted with 100 mL of H_2O and 50 mL of CH_2Cl_2. The mixture was vigorously shaken, and the layers were separated. The organic layer was dried (Na_2SO_4) and concentrated to give 5 g (72%) of methyl 4-[(methoxycarbonyl)amino]benzoate (**21**): mp 177−178 °C.

A mixture of 1.5 g (0.006 mol) of **21**, 10 mL of deionized H_2O, and 7 mL of 1 N NaOH was heated at 60−70 °C for 8 h. The reaction mixture was filtered, and the filtrate was made acidic with 6 N HCl. The precipitate was collected by filtration, washed with H_2O, and dried to yield 0.75 g (54%) of **22** as a white solid: mp 195−196 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.69 (s, 3H), 7.56 (d, $J = 8.7$ Hz, 2H), 7.87 (d, $J = 8.7$ Hz, 2H), 10.04 (s, 1H), 12.62 (s, 1H); IR (KBr) 3330, 1716, 1687, 1610, 1552, 1420 cm^{-1}; MS (ES$^-$) 194 (M − 1). Anal. ($C_{10}H_9NO_5 \cdot 0.25H_2O$) C, H, N.

4-[[(Methylamino)carbonyl]oxy]benzoic Acid (30). To 0.24 g (0.0018 mol) of 4-hydroxybenzoic acid (**29**; Aldrich) in 10 mL of THF was added 460 μL (0.0035 mol) of Et_3N, and the mixture stirred for 15 min. A 230-μL (0.0018-mol) portion of trimethylsilyl bromide was added, and stirring continued for another 30 min followed by the addition of 114 μL (0.0019 mL) of methyl isocyanate. The reaction was quenched by the addition of 1 mL of H_2O, and the solvent was evaporated. To the residue were added 15 mL of H_2O and 2 mL of 6 N NaOH, and the suspended impurities were removed through filtration. The filtrate was acidified using 6 N HCl, and the precipitate was collected by filtration. The solid was dried to give 0.097 g (28%) of **30** as a white solid: mp 223−225 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.67 (d, $J = 4.6$ Hz, 3H), 7.21 (d, $J = 8.7$ Hz, 2H), 7.77 (m, 1H), 7.95 (d, $J = 8.7$ Hz, 2H), 12.90 (br s, 1H); IR (KBr) 3361, 1709, 1685, 1533, 1293, 1219 cm^{-1}; MS (CI$^+$) 196 (M + 1). Anal. ($C_9H_9NO_4$) C, H, N.

4-[2-[[(Aminoiminomethyl)amino]imino]ethyl]benzoic Acid (37). A mixture of 0.82 g (0.005 mol) of 4-acetylbenzoic acid (**36**; Aldrich), 0.68 g (0.005 mol) of aminoguanidine bicarbonate, and 0.084 g (0.01 mol) of concentrated HCl in 30 mL of EtOH was heated at reflux for 16 h. Upon cooling, a light brown precipitate formed, which was collected by filtration, washed with cold EtOH, and dried to give 0.9 g (79%) of **37** as a light brown powder: mp 328 °C dec; ^{1}H NMR (400 MHz, CF_3CO_2D) δ 2.45 (m, 3H), 7.94 (dd, $J = 6.9$, 1.7 Hz, 2H), 8.25 (dd, $J = 6.9$, 1.7 Hz, 2H); IR (KBr) 3180, 2382, 2350, 1679, 1635, 1594, 1384 cm^{-1}; MS (ES$^+$) 221 (M + 1). Anal. ($C_{10}H_{12}N_4O_2 \cdot 0.25H_2O$) C, H, N.

2-(4-Nitrophenyl)-1,3-*O*-dinitropropanediol (42). To 10 mL of cooled 90% fuming HNO_3 at 0−5 °C was added portionwise 1.0 g (0.0066 mol) of 2-phenyl-1,3-propanediol (**41**)[29] over a 10-min period. The reaction mixture was stirred for 30 min at 0−5 °C and for 30 min at ambient temperature and then poured into 100 mL of cold H_2O. The mixture was extracted thrice with 25 mL of EtOAc. The combined organic layers were washed with 20 mL of a saturated Na_2CO_3 solution and 20 mL of brine, dried ($MgSO_4$), and concentrated to give a yellow oil. This oil was purified by flash chromatography on silica gel (10−50% Et_2O in C_6H_{14}). The appropriate fractions were combined and concentrated to give 1.3 g (67%) of **42** as a light yellow solid: mp 53−55 °C. The 2-nitro isomer **43** was also obtained in a 20% yield as a light yellow oil. On a large scale, **42** can be obtained by crystallization of the crude oil from Et_2O: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.63 (m, 1H), 4.77 (dd, $J = 6.4$, 4.5 Hz, 4H), 7.48 (m, 2H), 8.26 (m, 2H); IR (KBr)

3076, 2883, 1593, 1522, 1359, 1285 cm^{-1}; MS (ES^-) 286.5 (M − 1). Anal. ($C_9H_9N_3O_8$) C, H, N.

2-(4-Cyanophenyl)-1,3-propanediol (45). To 3.0 g (0.01 mol) of **42** in 25 mL of MeOH was added 0.3 g of 10% Pd/C under N_2. The resulting slurry was hydrogenated for 30 min at 50 psi, evacuated to remove ammonia, and then further hydrogenated for an additional 30 min at 50 psi. The catalyst was removed by filtration through Celite, and the solvent was removed in vacuo to furnish 1.8 g of 2-(4-aminophenyl)-1,3-propanediol (**44**).

To 1.8 g (0.01 mol) of **44** in 5 mL of ice−concentrated HCl (2:3) cooled to 0 °C was added dropwise with stirring 0.7 g (0.01 mol) of sodium nitrite in 5 mL of H_2O. The resulting diazonium solution was neutralized carefully with a saturated sodium carbonate solution to neutral pH; 10 mL of toluene was added, and the reaction mixture was cooled to 0 °C. To this cold solution was added slowly a solution containing 0.6 g (0.003 mol) of CuCl and 0.37 g (0.007 mol) of NaCN in 2 mL of H_2O; the mixture stirred sequentially in the cold for 10 min, at room temperature for 1 h, and at 50 °C for 1 h. The reaction mixture was cooled, the organic layer was separated, and the aqueous layer was extracted with four 20-mL portions of EtOAc. The combined organic layers were washed with 20 mL of brine, dried (Na_2SO_4), and concentrated to furnish 1.6 g of crude product. The crude was purified by flash column chromatography (EtOAc) to furnish 1.0 g (57%) of **45** which was recrystallized from Et_2O to furnish **45** as a tan solid: mp 77−79 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 2.29 (s, 2H), 3.13 (m, 1H), 3.98 (m, 4H), 7.39 (d, J = 8.2 Hz, 2H), 7.62 (d, J = 8.2 Hz, 2H); IR (KBr) 3474, 3291, 2239, 1609, 1507, 1472, 1418; MS (ES^-) 176.1 (M − 1). Anal. ($C_{10}H_{11}NO_2$) C, H, N.

2-(4-Carboxyphenyl)-1,3-propanediol (46). To a solution of 0.3 g (0.0017 mol) of **45** in 2.5 mL of EtOH was added 7.5 mL of 10% NaOH solution. The solution was heated at reflux for 4 h and then cooled. The reaction mixture was acidified with 1 N HCl to pH 2 and extracted thrice with 20 mL of EtOAc. The combined organic layers were dried (Na_2SO_4) and concentrated to give 0.2 g (60%) of brown residue. The residue was recrystallized from EtOAc−Et_2O to obtain 0.08 g of **46** as a brown solid: mp 128−131 °C; ^1H NMR (400 MHz, DMSO-d_6) δ 2.88 (m, 1H), 3.68 (m, 4H), 4.59 (s, 2H), 7.40 (m, 2H), 7.81 (m, 2H), 12.79 (s, 1H); IR (KBr) 3339, 2875, 1690, 1320, 1296, 1037 cm^{-1}; MS (ES^-) 194.9 (M − 1). Anal. ($C_{10}H_{12}O_4$) C, H.

4-[(Acetylamino)methyl]benzoic Acid (48). A mixture of 1.51 g (0.01 mol) of 4-(aminomethyl)benzoic acid (**47**; Aldrich) and 1.5 g (0.018 mol) of anhydrous NaOAc in 5 mL of glacial HOAc was heated at reflux for 18 h. Upon cooling, the mixture was poured into 50 mL of cold H_2O. The precipitate was separated by filtration and washed several times with cold H_2O. The cake was recrystallized from H_2O to give 1.4 g (73%) of **48** as a white powder: mp 195 °C; ^1H NMR (400 MHz, DMSO-d_6) δ 1.89 (s, 3H), 4.31 (d, J = 5.9 Hz, 2H), 7.35 (d, J = 8.3 Hz, 2H), 7.89 (d, J = 8.3 Hz, 2H), 8.42 (t, J = 5.9 Hz, 1H), 12.87 (br s, 1H); IR (KBr) 3393, 1690, 1606, 1543, 1264, 1178 cm^{-1}; MS (ES^+) 194 (M + 1). Anal. ($C_{10}H_{11}NO_3$) C, H, N.

Methyl 4-[(Methylthio)methyl]benzoate (54). To 1.8 g (0.025 mol) of $NaSCH_3$ in 25 mL of DMF cooled to 0 °C was added 5.5 g (0.024 mol) of methyl 4-(bromomethyl)benzoate (**53**; Aldrich), and the mixture stirred under N_2 at ambient temperature for 16 h. The reaction mixture was poured into 150 mL of H_2O and extracted thrice with 50-mL portions of EtOAc. The combined organic layers were washed with 50 mL of brine, dried ($MgSO_4$), and concentrated to give 5.3 g of colorless liquid. The oil was vacuum-distilled to furnish 3.9 g (85%) of **54** as a colorless liquid, bp 122−124 °C/0.6 mmHg; ^1H NMR (600 MHz, $CDCl_3$) δ 2.0 (s, 3H), 3.7 (s, 2H), 3.9 (s, 3H), 7.4 (d, J = 8.2 Hz, 2H), 8.0 (d, J = 8.2 Hz, 2H); IR (KBr) 2951, 2916, 1720, 1610, 1435, 1280; MS (ES^+) 197 (M + 1). Anal. ($C_{10}H_{12}O_2S$) C, H.

Methyl 4-[(Methylsulfoxy)methyl]benzoate (55). To 1.0 g (0.003 mol) of *m*-chloroperbenzoic acid (50% in 10 mL of CH_2Cl_2) at 0 °C was added 0.5 g (0.0025 mol) of **54**. The mixture was stirred at ambient temperature overnight and then poured into 10 mL of a saturated Na_2CO_3 solution. The organic layer was separated, washed successively with 10 mL of a saturated $NaHCO_3$ solution and 10 mL of brine, dried (Na_2SO_4), and concentrated to give 0.68 g of oily residue. The crude was purified by flash column chromatography on silica gel (40−100% EtOAc in C_6H_{14} followed by 20% MeOH in EtOAc) to give 0.17 g (30%) ofthe sulfone **56** and 0.35 g (66%) of **55** as a white solid: mp 69−72 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 2.48 (s, 3H), 3.93 (s, 3H), 4.02 (d, J = 7.1 Hz, 2H), 7.37 (d, J = 8.3 Hz, 2H), 8.06 (d, J = 8.3 Hz, 2H); IR (KBr) 3417, 3002, 2950, 1719, 1610, 1435, 1280, 1101, 1032 cm^{-1}; MS (ES^+) 213.3 (M + 1). Anal. ($C_{10}H_{12}O_3S$) C, H.

4-[(Methylsulfoxy)methyl]benzoic Acid (57). A mixture of 1.2 g (0.0055 mol) of **55** and 17 mL (0.017 mol) of 1 N NaOH was stirred at ambient temperature for 1 h. The reaction mixture was filtered and the filtrate pH adjusted to 5 with concentrated HCl. The precipitate was collected by filtration and washed with H_2O and Et_2O to give 0.7 g (64%) of **57** as a white, crystalline solid: mp 177−178 °C; ^1H NMR (400 MHz, DMSO-d_6) δ 2.49 (s, 3H), 4.02 (d, J = 12.65 Hz, 1H), 4.23 (d, J = 12.65 Hz, 1H), 7.45 (d, J = 8.2 Hz, 2H), 7.94 (d, J = 8.2 Hz, 2H), 12.99 (br s, 1H); IR (KBr) 2919, 2778, 2618, 2496, 1701, 1262, 1005 cm^{-1}; MS (ES^+) 1992 (M + 1). Anal. ($C_9H_{10}O_3S \cdot 0.25H_2O$) C, H.

Methyl 4-[(Methylsulfonyl)methyl]benzoate (56). To a solution of 1.8 g (0.009 mol) of **54** in 10 mL of glacial HOAc was added 5.2 mL (0.046 mol) of 30% H_2O_2. The mixture was heated at reflux for 1 h and cooled, and the resulting precipitate was collected by filtration and dried to give 1.6 g (89%) of **56** as a white solid. An analytical sample, mp 162−164 °C, was recrystallized from EtOAc: ^1H NMR (400 MHz, DMSO-d_6) δ 2.94 (s, 3H), 3.87 (s, 3H), 4.62 (s, 2H), 7.56 (d, J = 8.4 Hz, 2H), 7.99 (d,J = 8.4 Hz, 2H); IR (KBr) 3005, 2982, 1720, 1614, 1440, 1300, 1277, 1125, 1101 cm^{-1}; MS (ES^+) 229.8 (M + 1). Anal. ($C_{10}H_{12}O_4S$) C, H.

4-[(Methylsulfonyl)methyl]benzoic Acid (58). A mixture of 0.26 g (0.001 mol) of **56** and 4 mL (0.004 mol) of 1 N NaOH was stirred at ambient temperature for 2 h. The mixture was filtered, and the filtrate pH was adjusted to 4 using 1 N HCl. The precipitate was collected by filtration, washed with H_2O, and recrystallized from EtOH to give 0.16 g (65%) of **58** as a white, crystalline solid: mp 249−253 °C; ^1H NMR (400 MHz, DMSO-d_6) δ 2.93 (s, 3H), 4.60 (s, 2H), 7.53 (d, J = 8.2 Hz, 2H), 7.96 (d, J = 8.2 Hz, 2H), 13.05 (br s, 1H); IR (KBr) 3017, 2945, 2829, 2682, 2551, 1686, 1286, 1131 cm^{-1}; MS (ES^-) 212.9 (M − 1). Anal. ($C_9H_{10}O_4S$) C, H.

3-(Cyanoamino)benzoic Acid (60). To 9.1 g (0.066 mol) of 3-aminobenzoic acid (**59**; Aldrich) in 70 mL of HOAc−H_2O (1:1) was added 8.1 g (0.099 mol) of NaOAc, and the mixture was cooled in an ice−water bath. A total of 8.4 g (0.079 mol) of cyanogen bromide was added in two batches over a 10-min period. The mixture was stirred at ambient temperature for 18 h and then poured into 350 g of an ice−water mixture with vigorous stirring. The precipitate was collected by filtration, washed with 70 mL of cold H_2O, air-dried, and recrystallized from MeOH−H_2O to give 10.4 g (95%) of **60** as a white solid: mp 238−240 °C; ^1H NMR (400 MHz, DMSO-d_6) δ 7.19−7.22 (m, 1H), 7.46−7.53 (m, 2H), 7.59−7.62 (m, 1H); IR (KBr) 3177, 2228, 1683, 1595, 1448, 1316 cm^{-1}; MS (ES^-) 161 (M − 1). Anal. ($C_8H_6N_2O_2 \cdot 0.2H_2O$) C, H, N.

Methyl 3-[[Amino(cyanoimino)methyl]amino]benzoate (66). A solution of 0.8 g (0.0086 mol) of sodium dicyanamide in 5 mL of H_2O was treated dropwise with a solution of 1.3 g (0.0086 mol) of methyl 3-aminobenzoate (**65**; Aldrich) in 15 mL of 1 N HCl. The resulting clear solution was heated at 80−90 °C for 2 h, and a precipitate formed upon cooling. The solid was collected by filtration and recrystallized from $CHCl_3$−MeOH to give 1.8 g (quantitative) of **66** as a white solid: mp 203−204 °C; ^1H NMR (400 MHz, DMSO-d_6) δ 3.17 (d, J = 5.1 Hz, 0.6H), 3.34 (s, 3H), 4.10 (q, J = 5.1 Hz, 0.2H), 7.14 (s, 2H), 7.43−7.47 (m, 1H), 7.60−7.66 (m, 2H), 8.00−8.01 (m, 1H), 9.32 (s, 1H); IR (KBr) 3420, 3329, 2179, 1720, 1562 cm^{-1}; MS (ES^+) 219.0 (M + 1). Anal. ($C_{10}H_{10}N_4O_2 \cdot 0.2MeOH$) C, H, N.

3-[[Amino(cyanoimino)methyl]amino]benzoic Acid (67). A mixture of 7.3 g (0.033 mol) of **66** in 20 mL of H_2O and 34 mL of 1 N NaOH was heated at reflux for 2 h. The cooled mixture was filtered, and the filtrate pH was adjusted to 4.0−

4.5 with 6 N HCl. The precipitate was collected by filtration, washed with 30 mL of H_2O, and dried to give 1.3 g (18%) of **67** as a white solid: mp >300 °C. Further acidification of the filtrate gave an additional 4 g of slightly impure **67**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.12 (s, 2H), 7.42 (m, 1H), 7.6 (m, 2H), 7.98 (m, 1H), 9.31 (s, 1H), 12.95 (br s, 1H); IR (KBr) 3434, 3361, 2196, 1702, 1658, 1565 cm^{-1}; MS (ES$^-$) 203 (M − 1). Anal. ($C_9H_8N_4O_2$·0.25H_2O) C, H, N.

3-[(Acetylamino)methyl]benzoic Acid (71). To a stirred mixture of 0.23 g (0.0012 mol) of **70**,[33] 0.037 g (0.003 mol) of DMAP, and 0.86 mL (0.006 mol) of Et_3N in 12 mL of CH_2Cl_2 was added dropwise 0.23 mL (0.0023 mol) of acetic anhydride. The mixture was stirred at ambient temperature for 4 h and then concentrated. The white residue was treated with 2 mL of H_2O, and the pH was adjusted to 8 with 1 N NaOH. The mixture was extracted thrice with 2 mL of EtOAc, and the combined extracts were dried ($MgSO_4$) and concentrated. The solid residue was recrystallized from EtOAc to yield 0.14 g (59%) of **71** as a white solid: mp 165−166 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.88 (s, 3H), 4.29 (d, J = 5.9 Hz, 2H), 7.45 (m, 2H), 7.82 (m, 2H), 8.42 (s, 1H), 12.95 (br s, 1H); IR (KBr) 3306, 2835, 1691, 1642, 1352, 1292 cm^{-1}; MS (ES$^-$) 192.2 (M − 1). Anal. ($C_{10}H_{11}NO_3$) C, H, N.

3-[[(Aminoiminomethyl)amino]methyl]benzoic Acid (72). A mixture of 0.1 g (0.0064 mol) of **70** in 7 mL of a saturated $NaHCO_3$ solution was treated dropwise with 6 N NaOH until all solids dissolved (2 drops). To this solution was added 0.08 g (0.0064 mol) of aminoiminomethanesulfonic acid;[34] the mixture was stirred at ambient temperature for 16 h and concentrated. The residue was recrystallized from H_2O to give 0.072 g (58%) of **72** as a white solid: mp 308−311 °C; ^{1}H NMR (400 MHz, CF_3CO_2D) δ 4.53 (s, 2H), 7.55−7.61 (m, 2H), 8.09 (s, 1H), 8.14 (d, J = 7.7 Hz, 1H); IR (KBr) 3399, 3248, 3079, 2363, 2337, 1670, 1628, 1550, 1396 cm^{-1}; MS (ES$^+$) 194.5 (M + 1). Anal. ($C_9H_{11}N_3O_2$) C, H, N.

Methyl 3-(Cyanomethyl)benzoate (74). A mixture of 5.0 g (0.022 mol) of methyl 3-(bromomethyl)benzoate (**73**; Maybridge), 2.9 g (0.044 mol) of KCN, and 0.5 g (0.0018 mol) of 18-crown-6 in 50 mL of CH_3CN was vigorously stirred for 24 h at ambient temperature. The mixture was filtered, the filtrate was concentrated to ∼30 mL, and the volume was adjusted to 100 mL with H_2O. The yellow mixture was extracted with portions of CH_2Cl_2 until the aqueous layer was almost colorless. The combined organic layers were dried ($MgSO_4$) and concentrated to give an oil. This oil was purified by column chromatography on silica gel eluted with Et_2O. The appropriate fractions were combined and concentrated. The residue was vacuum-distilled to give 3.5 g (92%) of **74** as a light yellow oil: bp 101.5 °C/0.05 mmHg; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.82 (s, 2H), 3.89 (s, 3H), 7.42 (m, 1H), 7.51 (m, 1H), 7.96 (m, 2H); IR (KBr) 2955, 2249, 1718, 1436, 1286, 1200 cm^{-1}; MS (no ionization). Anal. ($C_{10}H_9NO_2$) C, H, N

Methyl 3-(2-Amino-2-iminoethyl)benzoate (75). A solution of 1.9 g (0.011 mol) of **74** in 100 mL of dry CH_2Cl_2 and 15 mL of absolute EtOH was cooled to 0 °C and treated with a stream of HCl gas for 10 min. The mixture was refrigerated for 6 days and then concentrated in vacuo with the bath temperature at 0 °C. The white solid residue was dissolved in 200 mL of anhydrous MeOH, and the solution was treated with a stream of NH_3 gas for 20 min. The mixture was then warmed at 50 °C for 18 h. The mixture was cooled and filtered, and the filtrate was concentrated to give 2.5 g (quantitative) of white foam as a residue. The foam was pulverized and dried in vacuo at toluene reflux temperature to give **75** as a white solid: mp 139−140 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.82 (s, 2H), 3.87 (s, 3H), 7.55 (m, 1H), 7.78 (m, 1H), 7.91 (m, 1H), 8.08 (m, 1H), 8.89−9.34 (m, 4H); IR (KBr) 3362, 3030, 1718, 1688, 1313, 1285 cm^{-1}; MS (ES$^+$) 193 (M + 1). Anal. ($C_{10}H_{12}N_2O_2$·HCl) C, H, N.

3-(2-Amino-2-iminoethyl)benzoic Acid (76). A mixture of 0.2 g (0.0009 mol) of **75** and 5 mL of NH_4OH was stirred at ambient temperature for 24 h. The mixture was concentrated, the residue diluted with 5 mL of H_2O, and the resulting solid collected by filtration. The cake was washed with H_2O and dried in vacuo at acetone reflux temperature to give 0.14 g (88%) of **76** as a white powder: mp 219−221 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.42 (s, 2H), 6.92 (s, 1H), 7.33−7.94 (m, 8H); IR (KBr) 3354, 3161, 1670, 1636, 1402 cm^{-1}; MS (ES$^+$) 179 (M + 1). Anal. ($C_9H_{10}N_2O_2$·0.2H_2O) C, H, N.

Methyl 3-(2-Aminoethyl)benzoate (78). To a suspension of 0.095 g (0.0025 mol) of $NaBH_4$ in 2.5 mL of THF was added dropwise over a 10-min period at ambient temperature a solution of 0.19 mL (0.0025 mol) of TFA in 2.5 mL of THF. A solution of 0.18 g (0.001 mol) of **74** in 2.5 mL of THF was then added, and the mixture was stirred at ambient temperature for 4 h. The mixture was cooled, and H_2O was added dropwise while keeping the temperature below 10 °C. The mixture was concentrated, and 5 mL of H_2O was added to the residue. The mixture was made basic with 1 N NaOH and extracted thrice with 10-mL portions of CH_2Cl_2. The combined extracts were dried ($MgSO_4$) and concentrated to give an oil as a residue. The oil was converted to the hydrochloride and the resulting solid recrystallized from EtOH to furnish 0.12 g (52%) of **78** as a white solid: mp 120−122 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.00−3.05 (m, 4H), 3.85 (s, 3H), 7.47−7.51 (m, 1H), 7.57 (d, J = 7.7 Hz, 1H), 7.83−7.85 (m, 2H), 8.23 (br s, 2H); IR (KBr) 3423, 3003, 1724, 1591, 1285 cm^{-1}; MS (ES$^+$) 180.4 (M + 1). Anal. ($C_{10}H_{13}NO_2$·HCl·0.75H_2O) C, H, N.

Methyl 3-[2-(Acetylamino)ethyl]benzoate (79). To a mixture of 2.2 g (0.01 mol) of **78**, 7 mL (0.05 mol) of Et_3N, and 0.25 g (0.002 mol) of DMAP in 25 mL of CH_2Cl_2 was added dropwise with stirring at 0 °C 1.9 mL (0.02 mol) of acetic anhydride. The mixture was stirred at ambient temperature overnight and then poured into 20 mL of H_2O. The organic layer was separated, and the aqueous layer was extracted twice with 10-mL portions of CH_2Cl_2. The combined organic layers were dried ($MgSO_4$) and concentrated. The residue was purified by flash column chromatography on silica gel (75−80% EtOAc in C_6H_{14}) to yield 0.96 g (44%) of **79** as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.95 (s, 3H), 2.88 (t, J = 7.0 Hz, 2H), 3.53 (m, 2H), 3.92 (s, 3H), 5.62 (br s, 1H), 7.39 (m, 2H), 7.91 (m, 2H); IR (KBr) 3297, 3082, 2951, 1722, 1655, 1554, 1286, 1203 cm^{-1}; MS (ES$^+$) 222.4 (M + 1). Anal. ($C_{12}H_{15}NO_3$·0.25H_2O) C, H, N.

3-[2-(Acetylamino)ethyl]benzoic Acid (80). A mixture of 0.35 g (0.0016 mol) of **79** and 3.2 mL (0.0032 mol) of 1 N NaOH was stirred at ambient temperature for 2 h. The mixture was filtered, and the filtrate pH was adjusted to 6 using concentrated HCl. The mixture was concentrated, 2 mL of H_2O was added, and the precipitate was collected by filtration to yield 0.1 g (31%) of **80** as a white solid: mp 146−148 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.77 (s, 3H), 2.76 (t, J = 7.2 Hz, 2H), 3.26 (m, 2H), 7.42 (m, 2H), 7.79 (s, 2H), 7.91 (s, 1H), 12.89 (s, 1H); IR (KBr) 3333, 3130, 1688, 1628, 1591, 1567, 1313, 1267, 1199 cm^{-1}; MS (ES$^-$) 206.2 (M − 1). Anal. ($C_{13}H_{13}NO_3$) C, H, N.

3-[2-[(Aminoiminomethyl)amino]ethyl]benzoic Acid (81). A solution of 0.23 g (0.0011 mol) of **77** and 0.32 g (0.0023 mol) of Na_2CO_3 in 2.2 mL of H_2O was treated portionwise with 0.15 g (0.0011 mol) of aminoiminomethanesulfonic acid[34] over a 10-min period. The reaction mixture was stirred at ambient temperature for 1 h, and the solid which precipitated was collected by filtration and dried in vacuo at toluene reflux temperature to yield 0.15 g (64%) of **81** as a white solid: mp >310 °C; ^{1}H NMR (400 MHz, CF_3CO_2D) δ 3.1 (t, 2H), 3.7 (t, 2H), 7.6 (m, 2H), 8.1 (s, 1H), 8.2 (d, 1H); IR (KBr) 3354, 3149, 1658, 1519, 1386 cm^{-1}; MS (ES$^+$) 208.4 (M + 1). Anal. ($C_{10}H_{13}N_3O_2$) C, H, N.

5-[(3-Carboxyphenyl)amino]-1*H*-tetrazole (82). A mixture of 5.0 g (0.031 mol) of **60**, 2.4 g (0.037 mol) of NaN_3, and 2.0 g (0.037 mol) of NH_4Cl in 20 mL of dry DMF was heated at 160 °C for 16 h. The mixture was concentrated, and the residue was diluted with 150 mL of H_2O. The mixture was made basic with dilute NaOH and filtered, and the filtrate was acidified with 6 N HCl. The precipitate was collected by filtration, air-dried, and recrystallized from MeOH−EtOAc to give 2.3 g (36%) of **82** as a cream-colored solid: mp 243−245 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.44 (dd, J = 7.86, 8.0 Hz, 1H), 7.54 (ddd, J = 7.65, 1.17, 1.15 Hz, 1H), 7.77 (ddd, J = 8.16, 2.12, 1.12 Hz, 1H), 8.21 (m, 1H), 10.05 (s, 1H); IR (KBr) 3277, 3080, 1688, 1629, 1600, 1047, 1463, 1311, 1242, 1056 cm^{-1}; MS (ES$^+$) 206.3 (M + 1). Anal. ($C_8H_7N_5O_2$) C, H, N.

5-[4-(Acetylamino)phenyl]-1*H*-tetrazole (90). A stirred mixture of 10 g (0.062 mol) of 4′-cyanoacetanilide (**89**; Aldrich), 4.6 g (0.07 mol) of NaN_3, and 3.75 g (0.07 mol) of NH_4Cl in 100 mL of DMF was heated at 120 °C for 20 h and then poured into 800 mL of ice−water with vigorous stirring. The resulting precipitate was collected by filtration and slurried with 200 mL of H_2O with vigorous stirring. The solid was collected by filtration and dried to yield 3.4 g (27%) of **90** as a white solid: mp 287 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.07 (s, 3H), 7.78 (d, J = 8.8 Hz, 2H), 7.95 (d, J = 8.8 Hz, 2H), 10.24 (s, 1H); IR (KBr) 3312, 3267, 1678, 1603, 1549, 1502, 1321 cm^{-1}; MS (ES$^-$) 202 (M − 1). Anal. ($C_9H_9N_5O$) C, H, N.

Methyl 4-(Acetylamino)-3-(hydroxymethyl)benzoate (93). To a mixture of 3.3 g (0.015 mol) of methyl 4-(acetylamino)-3-formylbenzoate (**92**)[45] in 30 mL of MeOH was added 0.3 g (0.0078 mol) of $NaBH_4$ at 0 °C in three portions at 10-min intervals. The mixture was stirred at 0 °C for 0.75 h after the final addition was complete. The mixture was neutralized with H^+ resin and filtered, and the filtrate was concentrated. The residue was recrystallized from EtOAc−hexane to give 2.6 g (78%) of **93** as yellow needles: mp 131−132 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.10 (s, 3H), 3.84 (s, 3H), 4.56 (d, J = 5.5 Hz, 2H), 5.49 (t, J = 5.5 Hz, 1H), 7.82 (d, J = 1.2 Hz, 2H), 8.04 (s, 1H), 9.44 (s, 1H); IR (KBr) 3197, 1721, 1667, 1590, 1540, 1436, 1287, 1123 cm^{-1}; MS (ES$^+$) 206 (M − H_2O). Anal. ($C_{11}H_{13}NO_4$) C, H, N.

4-(Acetylamino)-3-(hydroxymethyl)benzoic Acid (94). A mixture of 0.84 g (0.0038 mol) of **93** in 4.5 mL (0.0045 mol) of 1 N NaOH was stirred at ambient temperature for 8 h. The mixture was filtered, the filtrate was made acidic with concentrated HCl, the resulting precipitate was collected by filtration, and the cake was recrystallized from H_2O to give 0.48 (61%) of **94** as a white powder: mp 180−181 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.09 (s, 3H), 4.55 (s, 2H), 5.46 (br s, 1H), 7.76−7.81 (m, 2H), 8.00 (s, 1H), 9.42 (s, 1H), 12.75 (br s, 1H); IR (KBr) 3261, 1696, 1662, 1527, 1286, 1241, 1015 cm^{-1}; MS (ES$^-$) 208 (M − 1). Anal. ($C_{10}H_{11}NO_4$) C, H, N.

Ethyl α-[2-(Acetylamino)-5-(methoxycarbonyl)phenyl]-α-(methylthio)acetate (97). To a mixture of 15.1 g (0.1 mol) of methyl 4-aminobenzoate (**95**; Aldrich) in 400 mL of CH_2Cl_2 at −70 °C was added a solution of 12.0 g of *tert*-butyl hypochlorite in 50 mL of CH_2Cl_2 dropwise over a 10-min period. The mixture was stirred for 10 min and then treated dropwise with a solution of 13.4 g (0.1 mol) of ethyl (methylthio)acetate in 50 mL of CH_2Cl_2 over a 10-min period. The stirring was continued for 1 h, and then the solution was treated dropwise with a solution of 11.2 g (0.11 mol) of triethylamine in 40 mL of CH_2Cl_2 over a 10-min period. The reaction mixture was allowed to warm to ambient temperature and then diluted with 120 mL of H_2O. The organic layer was separated, dried (Na_2SO_4), and concentrated to give crude **96** as an oil.

A mixture of the above **96**, 40 mL of triethylamine, and 200 mL of Et_2O was cooled to 0−5 °C in an ice bath. A solution of 8.6 g (0.11 mol) of acetyl chloride in 50 mL of Et_2O was added dropwise over a 15-min period. The reaction mixture was stirred for 1 h and then diluted with 200 mL of H_2O. The organic layer was separated, washed with H_2O, dried (Na_2SO_4), and concentrated to give a syrup as a residue. This syrup was purified by column chromatography on silica gel (EtOAc−hexane, 1:2). The appropriate fractions were combined and concentrated to give a solid residue. The solid was recrystallized from EtOAc−hexane to give 9.5 g (29%) of **97** as a white solid: mp 95−96 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.17 (t, J = 7.0 Hz, 3H), 2.08 (s, 3H), 2.10 (s, 3H), 3.85 (s, 3H), 4.12−4.17 (m, 2H), 5.10 (s, 1H), 7.67 (d, J = 8.5 Hz, 1H), 7.88 (dd, J = 8.5, 2.0 Hz, 1H), 8.14 (d, J = 2.0 Hz, 1H), 9.66 (s, 1H); IR (KBr) 3224, 1717, 1645, 1534, 1299 cm^{-1}; MS (ES$^+$) 326.4 (M + 1). Anal. ($C_{15}H_{19}NO_5S$) C, H, N.

Ethyl α-[2-(Acetylamino)-5-(methoxycarbonyl)phenyl]-acetate (98). A slurry of Raney nickel in THF was prepared by washing a 20-g sample of a commercial preparation with several portions of H_2O and then washing with three portions of THF. A stirred mixture of 6.5 g (0.02 mol) of **97** in 150 mL of THF was treated portionwise with the washed Raney nickel slurry over a 30-min period. The mixture was cautiously filtered through Celite, and the filtrate was concentrated. The residue was recrystallized from EtOAc−hexane to give 5.2 g (93%) of **98** as a white solid: mp 119 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.17 (t, J = 7.0 Hz, 3H), 2.06 (s, 3H), 3.83 (s, 2H), 3.84 (s, 3H), 4.07 (q, J = 7.0 Hz, 2H), 7.67 (d, J = 8.4 Hz, 1H), 7.85 (dd, J = 8.4, 2.0 Hz, 1H), 7.87 (d, J = 2.0 Hz, 1H), 9.56 (s, 1H); IR (KBr) 3350, 2993, 1712, 1691, 1589, 1521, 1279, 1213 cm^{-1}; MS (ES$^+$) 280.6 (M + 1). Anal. ($C_{14}H_{17}NO_5$) C, H, N.

α-[2-(Acetylamino)-5-(methoxycarbonyl)phenyl]acetic Acid (99). To a mixture of 4.18 g (0.015 mol) of **98** in 60 mL of MeOH was added 15 mL of 1 N NaOH over a 10-min period. The mixture was stirred at ambient temperature for 1 h and diluted with 50 mL of H_2O. The mixture was filtered, and the filtrate was neutralized with concentrated HCl. The precipitate was collected by filtration and washed with H_2O, and the cake was recrystallized from EtOAc to give 2.3 g (61%) of **99** as a white solid: mp 194 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.07 (s, 3H), 3.74 (s, 2H), 3.84 (s, 3H), 7.72 (d, J = 8.3 Hz, 1H), 7.82 (dd, J = 8.4, 2.0 Hz, 1H), 7.84 (d, J = 2 Hz, 1H), 9.55 (s, 1H), 12.40 (s, 1H); IR (KBr) 3476, 3327, 3008, 2958, 1723, 1709, 1693, 1676, 1590, 1531, 1439, 1289, 1205 cm^{-1}; MS (ES$^+$) 252.5 (M + 1). Anal. ($C_{12}H_{13}NO_5$) C, H, N.

Methyl 4-(Acetylamino)-3-(2-hydroxyethyl)benzoate (100). To a mixture of 1 g (0.004 mol) of **99**, 0.56 mL (0.004 mol) of Et_3N, and 10 mL of THF at 0 °C was added 0.38 mL of ethyl chloroformate over a 5-min period. The mixture was stirred at 0 °C for 20 min and then filtered. The filtrate containing the mixed anhydride was treated with 0.48 g (0.013 mol) of $NaBH_4$ followed by the dropwise addition of 2.6 mL of MeOH over a 1-h period at 10 °C. After addition was complete, the mixture was stirred for 30 min and then carefully quenched with 1 N HCl (pH ∼7). The mixture was treated with 25 mL of H_2O and 25 mL of Et_2O. The aqueous layer was extracted thrice with 10 mL of CH_2Cl_2. The organic layers were combined, dried (Na_2SO_4), and concentrated. The residue was purified by flash column chromatography in silica gel (75−100% EtOAc in hexane) to give 0.55 g (58%) of **100** as a white solid: mp 134−136 °C. The ^{1}H NMR indicated that this sample was contaminated with ∼15% of the ethyl ester: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.38 (t, J = 7.1 Hz, 3 × 0.15H), 2.16 (s, 3H), 2.52 (s, 1H), 2.89 (t, J = 5.3 Hz, 2H), 3.89 (s, 3 × 0.85H), 4.00 (m, 2H), 4.36 (q, J = 14.3, 7.1 Hz, 2 × 0.15H), 7.85 (d, J = 1.8 Hz, 1H), 7.90 (dd, J = 8.5, 2.0 Hz, 1H), 8.04 (d, J = 8.5 Hz, 1H), 9.33 (br s, 1H); IR (KBr) 3251, 1726, 1678, 1542, 1286 cm^{-1}; MS (ES$^-$) 236.1 (M − 1). Anal. ($C_{12}H_{15}NO_5$) C, H, N.

4-(Acetylamino)-3-(2-hydroxyethyl)benzoic Acid (101). A mixture of 0.71 g (0.003 mol) of **100** and 6 mL of 1 N NaOH was stirred at ambient temperature for 1 h. The mixture was filtered and the filtrate pH adjusted to 2−3 with 1 N HCl. The mixture was allowed to stand for 1 h, and the precipitate was collected by filtration, washed with H_2O and then Et_2O, and dried to give 0.58 g (87%) of **101** as a light brown solid: mp 206−208 °C dec; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.09 (s, 3H), 2.82 (t, J = 6.3 Hz, 2H), 3.64 (t, J = 6.3 Hz, 2H), 5.10 (br s, 1H), 7.75 (s, 2H), 7.82 (s, 1H), 9.63 (s, 1H), 12.75 (s, 1H); IR (KBr) 3346, 1683, 1590, 1532, 1431 cm^{-1}; MS (ES$^-$) 222 (M − 1). Anal. ($C_{11}H_{13}NO_4$) C, H, N.

Methyl 3-(2-Hydroxyethoxy)-4-nitrobenzoate (103). A mixture of 5.3 g (0.027 mol) of methyl 3-hydroxy-4-nitrobenzoate (**102**; Lancaster), 9.15 g (0.065 mol) of K_2CO_3, 0.8 g (0.0053 mol) of NaI, and 7.3 g (0.059 mol) of 2-bromoethanol in 50 mL of acetone was heated at reflux for 48 h. A TLC analysis (silica gel, 3:2 hexane−EtOAc) indicated that the reaction was not complete. An additional 11 g (0.089 mol) of 2-bromoethanol and 100 mL of acetone were added, and the mixture was heated at reflux for 60 h. The mixture was poured into 400 mL of H_2O and extracted thrice with 200-mL portions of EtOAc. The combined organic layers were concentrated, and the residue was recrystallized from EtOAc−hexane to give 3.3 g (50%) of **103** as an orange solid: mp 97−98 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.70−3.74 (m, 2H), 3.90 (s, 3H), 4.26 (t, J = 4.8 Hz, 2H), 4.94 (t, J = 5.8 Hz, 1H), 7.66 (dd, J = 8.4, 1.5 Hz, 1H), 7.81 (d, J = 1.5 Hz, 1H), 8.3 (d,

J = 8.4 Hz, 1H); IR (KBr) 3556, 3452, 1723, 1612, 1527, 1295 cm^{-1}; MS (did not ionize). Anal. ($C_{10}H_{11}NO_6$) C, H, N.

4-(Acetylamino)-3-(2-hydroxyethexy)benzoic Acid (106). A mixture of 3.3 g (0.0136 mol) of **103** and 0.05 g of PtO_2 in 50 mL of EtOH was hydrogenated for 40 min at 35 psi. The mixture was filtered and concentrated to give 2.9 g (100%) of methyl 4-amino-3-(2-hydroxyethoxy)benzoate (**104**) as an off-white solid.

To 3.0 g (0.014 mol) of **104** dissolved in 200 mL of CH_2Cl_2 were added 1.5 mL (0.016 mol) of acetic anhydride and 1.34 mL (0.0156 mol) of pyridine. After stirring for 3 h at room temperature, the reaction mixure was poured into 70 mL of H_2O and the organic layer washed thrice with 50 mL of H_2O. The organic layer was concentrated, treated with 50 mL of MeOH and a catalytic amount of $NaOCH_3$, and stirred for 1 h. The solution was neutralized with H^+ resin, filtered, and then concentrated. The residue was recrystallized from EtOAc to give 1.9 g (53%) of **105** as an off-white solid.

A mixture of 1.5 g (0.006 mol) of **105** and 12 mL (0.012 mol) of 1 N NaOH was stirred at ambient temperature for 1 h. The mixture was filtered and the filtrate neutralized with concentrated HCl. The resulting precipitate was collected by filtration, washed with H_2O, and dried to give 0.47 g (34%) of **106** as a tan solid: mp 207.5−208.5 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.16 (s, 3H), 3.77 (t, J = 4.5 Hz, 2H), 4.08 (t, J = 4.5 Hz, 2H), 5.13 (br s, 1H), 7.51 (d, J = 1.7 Hz, 1H), 7.54 (dd, J = 1.7, 8.4 Hz, 1H), 8.26 (d, J = 8.4 Hz, 1H), 9.20 (s, 1H), 12.79 (br s, 1H); IR (KBr) 3351, 1710, 1660, 1604, 1531, 1270 cm^{-1}; MS (ES^+) 240 (M + 1). Anal. ($C_{11}H_{13}NO_5$) C, H, N.

Ethyl 4-(Acetylamino)-3-(2-propenyl)benzoate (108). A solution of 2.1 g (0.01 mol) of ethyl 4-(acetylamino)benzoate (**107**)[46] and 1.1 g (0.005 mol) of $Pd(OAc)_2$ in 17 mL of dry toluene was heated at reflux for 0.75 h under a N_2 atmosphere. The solution was cooled to 60 °C, and the toluene was decanted from the residue. The residue was washed with fresh toluene, and the combined toluene layers were heated at reflux for 3 h. The solution was cooled, and the resulting precipitate was collected by filtration. The filter cake was combined with the residue, and the mixture was dried under vacuum for 3 h. A mixture of this palladium adduct, 8.4 g (0.05 mol) of allyl iodide, and 40 mL of glacial HOAc was stirred at ambient temperature for 16 h. The mixture was filtered through Celite, the filtrate was concentrated, and the residue was purified by column chromatography on silica gel (1:1 EtOAc−hexane) to give 0.85 g (69%) of **108** as a white powder: mp 127−128 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.39 (t, J = 7.1 Hz, 3H), 2.17 (s, 3H), 3.44 (d, J = 6.0 Hz, 2H), 4.36 (q, J = 7.1 Hz, 2H), 5.14 (d, J = 16.8 Hz, 1H), 5.24 (d, J = 10.0 Hz, 1H), 5.98 (m, 1H), 7.43 (br s, 1H), 7.87 (s, 1H), 7.95 (dd, J = 4.0, 2.0 Hz, 1H), 8.14 (br s, 1H); IR (KBr) 3298, 1716, 1658, 1525, 1280; MS (ES^+) 248 (M + 1). Anal. ($C_{14}H_{17}NO_3$) C, H, N.

Ethyl 4-(Acetylamino)-3-(2,3-dihydroxypropyl)benzoate, Isomer A (109). A mixture of 5 mL of *t*-BuOH, 5 mL of H_2O, and 1.4 g of AD mix-α (Aldrich) was stirred for 5 min. To this mixture was added 0.1 g (0.0004 mol) of **108**, and the mixture stirred at ambient temperature for 48 h. The mixture was cooled to 0 °C, 1.5 g (0.012 mol) of Na_2SO_3 was added, and the reaction mixture stirred at 0 °C for 20 min and then at ambient temperature for 30 min. The mixture was extracted thrice with 15-mL portions of EtOAc. The combined extracts were dried (Na_2SO_4) and concentrated, and the residue was purified by column chromatography on silica gel (50−100% EtOAc in hexane) to give 0.075 g (66%) of **109** as a white powder: mp 114−116 °C; ^{1}H NMR (400 MHz, CD_3COCD_3) δ 1.35 (t, J = 7.0 Hz, 3H), 2.10 (s, 3H), 2.83−3.00 (m, 2H), 3.47−3.60 (m, 2H), 3.91−3.97 (m, 1H), 4.21 (t, J = 5.0 Hz, 1H), 4.31 (q, J = 7.0 Hz, 2H), 4.85 (d, J = 3.5 Hz, 1H), 7.83−7.88 (m, 2H), 8.15 (m, 1H), 9.81 (br s, 1H); IR 3506, 3260, 1684, 1593, 1541, 1290 cm^{-1}; MS (ES^-) 280 (M − 1). Anal. ($C_{14}H_{19}NO_5$) C, H, N.

Ethyl 4-(Acetylamino)-3-(2,3-dihydroxypropyl)benzoate, Isomer B (110). Isomer B was prepared by the above procedure using 0.09 g of **108** and 1.4 g of AD mix-β (Aldrich). The method gave 0.068 g (75%) of **110** as a white powder: mp 116−117 °C; ^{1}H NMR (400 MHz, CD_3COCD_3) δ 1.35 (t, J = 7.1 Hz, 3H), 2.10 (s, 3H), 2.84−3.00 (m, 2H), 2.47−3.60 (m, 2H), 3.91−3.98 (m, 1H), 4.21 (t, J = 4.7 Hz, 1H), 4.31 (q, J = 7.1 Hz, 2H), 4.85 (d, J = 3.8 Hz, 1H), 7.83−7.88 (m, 2H), 8.15 (m, 1H), 9.81 (br s, 1H); IR 3508, 3260, 1684, 1591, 1541, 1290 cm^{-1}; MS (ES^+) 282 (M + 1). Anal. ($C_{14}H_{19}NO_5$) C, H, N.

4-(Acetylamino)-3-(2,3-dihydroxypropyl)benzoic Acid, Isomer A (111). A mixture of 0.12 g (0.0004 mol) of **109** and 2 mL of 1 N NaOH was stirred at ambient temperature for 15 h. The mixture was filtered and the filtrate made acidic with concentrated HCl. The mixture was concentrated; the residue was dissolved in 5 mL of MeOH and filtered. The filtrate was concentrated, and the residue was purified by column chromatography on silica gel (85:15:1 EtOAc−MeOH−HOAc) to give 0.06 g (54%) of **111** as an off-white powder: mp 181−183 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 2.17 (s, 3H), 2.82 (dd, J = 7.1, 7.7 Hz, 1H), 2.92 (dd, J = 7.0, 4.0 Hz, 1H), 3.48 (m, 2H), 3.84 (m, 1H), 7.82−7.88 (m, 2H), 7.93 (d, J = 1.5 Hz, 1H); IR (KBr) 3369, 3289, 1699, 1514, 1286 cm^{-1}; MS (ES^+) 254 (M + 1). Anal. ($C_{12}H_{15}NO_5 \cdot 0.2H_2O$) C, H, N.

4-(Acetylamino)-3-(2,3-dihydroxypropyl)benzoic Acid, Isomer B (112). Isomer B was prepared by the above procedure using 0.09 g (0.0003 mol) of **110** and 2 mL of 1 N NaOH. This method gave 0.055 g (67%) of **112** as an off-white powder: mp 180−182 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 2.17 (s, 3H), 2.82 (dd, J = 7.1, 7.7 Hz, 1H), 2.92 (dd, J = 9.3, 4.0 Hz, 1H), 3.48 (m, 2H), 3.84 (m, 1H), 7.82−7.88 (m, 2H), 7.93 (d, J = 1.3 Hz, 1H); IR (KBr) 3389, 3256, 1699, 1651, 1518, 1284 cm^{-1}; MS (ES^-) 252 (M − 1). Anal. ($C_{12}H_{15}NO_5 \cdot 0.75H_2O$) C, H, N.

Ethyl 4-(Acetylamino)-3-(3-oxo-1-propenyl)benzoate (113). Compound **113** was synthesized according to the procedure used to prepare **108**. A mixture of 8.3 g (0.04 mol) of **107**, 4.5 g (0.02 mol) of $Pd(OAc)_2$, 17.5 g (0.135 mol) of acrolein diethyl acetal, and 11.2 g (0.11 mol) of Et_3N gave 1.1 g (21%) of **113** as an off-white, flocculent solid: mp 171 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.34 (t, J = 7.0 Hz, 3H), 2.16 (s, 3H), 4.34 (q, J = 7.0 Hz, 2H), 6.82 (dd, J = 7.7, 15.7 Hz, 1H), 7.80−7.87 (m, 1H), 7.91 (d, J = 1 5.7 Hz, 1H), 7.98 (dd, J = 8.3, 2.0 Hz, 1H), 8.28 (d, J = 2.0 Hz, 1H), 9.73 (d, J = 7.7 Hz, 1H), 10.05 (s, 1H); IR (KBr) 3293, 1718, 1672, 1522, 1285, 1234 cm^{-1}; MS (ES^-) 260 (M − 1). Anal. ($C_{14}H_{15}NO_4 \cdot 0.2H_2O$) C, H, N.

Ethyl 4-(Acetylamino)-3-(3-hydroxy-1-propenyl)benzoate (114). A mixture of 0.52 g (0.002 mol) of **113** in 20 mL of MeOH at 0 °C was treated with a mixture of 0.09 g (0.0024 mol) of $NaBH_4$ in 2 mL of MeOH and then stirred for 15 min. The mixture was neutralized with H^+ resin (Dowex-50W) and filtered. The filtrate was concentrated, and the residue was purified by column chromatography on silica gel (19:1 $CHCl_3$−MeOH) to give 0.41 g (76%) of **114** as a pale yellow powder: mp 118−119 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.32 (t, J = 7.0 Hz, 3H), 2.10 (s, 3H), 4.17 (ddd, J = 5.3, 5.1, 1.8 Hz, 2H), 4.31 (q, J = 7.0 Hz, 2H), 4.93 (t, J = 5.3 Hz, 1H), 6.34 (dt, J = 15.8, 5.1 Hz, 1H), 6.81 (d, J = 15.8 Hz, 1H), 7.70 (d, J = 8.4 Hz, 1H), 7.78 (dd, J = 8.4, 2.0 Hz, 1H), 8.06 (d, J = 2.0 Hz, 1H), 9.65 (s, 1H); IR (KBr) 3273, 1720, 1662, 1527, 1287, 1257 cm^{-1}; MS (ES^-) 262 (M − 1). Anal. ($C_{14}H_{17}NO_4 \cdot 0.5H_2O$) C, H, N.

4-(Acetylamino)-3-(3-hydroxy-1-propenyl)benzoic Acid (115). A mixture of 0.27 g (0.001 mol) of **114** and 5 mL of 0.5 N NaOH was stirred at ambient temperature for 8 h. The mixture was neutralized with H^+ resin and quickly filtered. Upon cooling of the filtrate, a pale yellow solid precipitated. The solid was collected by filtration, washed with cold H_2O, and dried to give 0.065 g (28%) of **115**: mp 214−215 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.08 and 2.09 (2 s, 3H), 4.16 (d, J = 3.7 Hz, 2H), 4.92 (br s, 1H), 6.32 (dt, J = 15.8, 5.0 Hz, 1H), 6.80 (dt, J = 15.8, 1.6 Hz, 1H), 7.65 (d, J = 8.4 Hz, 1H), 7.77 (dd, J = 8.4, 2.0 Hz, 1H), 8.06 (d, J = 2.0 Hz, 1H) 9.63 (s, 1H), 12.86 (br s, 1H); IR (KBr) 3314, 2886, 1675, 1524, 1229 cm^{-1}; MS (ES^+) 218 (M + 1). Anal. ($C_{12}H_{13}NO_4$) C, H, N.

Methyl 4-(Acetylamino)-3-[(hydroxyimino)methyl]benzoate (118). A mixture of 0.44 g (0.002 mol) of **92**[45] and 0.17 g (0.0025 mol) of $HONH_2 \cdot HCl$ in 10 mL of EtOAc was heated at reflux for 16 h. The mixture was filtered, and the filtrate was concentrated. The residue was recrystallized from EtOAc−hexane to give 0.25 g (53%) of **118** as a pale yellow powder:

mp 200−203 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.15 (s, 3H), 3.85 (s, 3H), 7.92 (dd, J = 8.67, 2.0 Hz, 1H), 8.17 (d, J = 2.0 Hz, 1H), 8.33 (d, J = 8.6 Hz, 1H), 8.43 (s, 1H), 10.76 (s, 1H), 11.69 (s, 1H); IR (KBr) 3214, 3100, 3010, 1720, 1598, 1550, 1282 cm^{-1}; MS (ES$^+$) 237.1 (M + 1). Anal. ($C_{11}H_{12}N_2O_4$) C, H, N.

4-(Acetylamino)-3-[(hydroxyimino)methyl]benzoic Acid (119). A mixture of 0.14 g (0.0006 mol) of **118** and 1 mL of 1 N NaOH was stirred at ambient temperature for 4 h. The mixture was filtered, and the filtrate was neutralized with 1 N HCl. The resulting precipitate was collected by filtration, washed with H_2O, and dried to give 0.095 g (71%) of **119** as a white powder: mp 215−218 °C dec; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.15 (s, 3H), 7.90 (dd, J = 8.6, 2.0 Hz, 1H), 8.15 (d, J = 2.0 Hz, 1H), 8.31 (d, J = 8.6 Hz, 1H), 8.42 (s, 1H), 10.74 (s, 1H), 11.66 (s, 1H), 12.93 (s, 1H); IR (KBr) 3597, 3288, 3087, 2995, 2916, 1693, 1589, 1543, 1195 cm^{-1}; MS (ES$^-$) 220.4 (M − 1). Anal. ($C_{10}H_{10}N_2O_4 \cdot 0.25H_2O$) C, H, N.

Methyl 4-(Acetylamino)-3-[[(aminoiminomethyl)hydrazino]methyl]benzoate (120). A mixture of 2.2 g (0.01 mol) of **92**,[45] 1.4 g (0.01 mol) of aminoguanidine bicarbonate, 1.7 mL (0.02 mol) of concentrated HCl, and 50 mL of EtOH was heated at reflux for 4 h. The reaction mixture was concentrated to ∼0.5 times the original volume and cooled. The precipitate was collected by filtration and dried to give 2.1 g (69%) of **120** as a fluffy white solid: mp 278 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.15 (s, 3H), 3.87 (s, 3H), 7.90 (m, 6H), 8.33 (s, 1H), 8.45 (d, J = 2.0 Hz, 1H), 10.47 (s, 1H); IR (KBr) 3449, 3271, 1724, 1670, 1631, 1291 cm^{-1}; MS (ES$^+$) 278 (M + 1). Anal. ($C_{12}H_{15}N_5O_3 \cdot$HCl) C, H, N.

4-(Acetylamino)-3-[[(aminoiminomethyl)hydrazino]methyl]benzoic Acid (121). A mixture of 0.54 g (0.0017 mol) of **120** and 5 mL of 1 N NaOH was stirred at ambient temperature for 24 h. The mixture was filtered and the filtrate neutralized with concentrated HCl. The precipitate was collected by filtration, washed with H_2O, and dried to give 0.41 g (80%) of **121** as a white powder: mp 326−327 °C; ^{1}H NMR (400 MHz, CF_3COOD) δ 2.50 (s, 3H), 8.03 (d, J = 8.5 Hz, 1H), 8.33 (m, 2H), 8.64 (br s, 1H); IR (KBr) 3379, 3129, 1668, 1637, 1386, 1294 cm^{-1}; MS (ES$^+$) 264 (M + 1). Anal. ($C_{11}H_{13}N_5O_3 \cdot 2H_2O$) C, H, N.

2-[2-(Acetylamino)-5-carboxyphenyl]acetic Acid (122). A mixture of 0.3 g (0.001 mol) of **98** and 3 mL of 1 N NaOH was stirred at ambient temperature for 16 h. The mixture was filtered, and the filtrate was neutralized with 1 N HCl. The precipitate was collected by filtration, washed with H_2O, and recrystallized from MeOH−H_2O to give 0.14 g (59%) of **122** as cream-colored needles: mp 240−241 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.07 (s, 3H), 3.72 (s, 2H), 7.66 (d, J = 8.3 Hz, 1H), 7.79 (dd, J = 8.4, 2 Hz, 1H), 7.84 (d, J = 2.0 Hz, 1H), 9.53 (s, 1H), 12.61 (br s, 1H); IR (KBr) 3289, 3013, 1693, 1620, 1388, 1286 cm^{-1}; MS (ES$^-$) 236.5 (M − 1). Anal. ($C_{11}H_{11}NO_5$) C, H, N.

2-[2-Amino-5-(methoxycarbonyl)phenyl]-2-(phenylthio)acetamide (123). A solution of 10 g (0.066 mol) of methyl 4-aminobenzoate (**95**; Aldrich) in 400 mL of CH_2Cl_2 at −70 °C under a N_2 atmosphere was treated with a solution of 8.6 g (0.079 mol) of *tert*-butyl hypochlorite in 20 mL of CH_2Cl_2 over a 5-min period. The reaction mixture was stirred for 10 min and then treated in one portion with a solution of 11 g (0.066 mol) of 1-(phenylthio)acetamide (Parish) in 300 mL of CH_2Cl_2. The solution was stirred at −70 °C for 5 h, at −40 °C for 1 h, and at −10 °C for 1 h and then cooled again to −70 °C. The mixture was treated in one portion with 11 mL (0.079 mol) of Et_3N, stirred for 30 min at −70 °C, and then allowed to warm to room temperature. A suspended solid was collected by filtration, and the filter cake was suspended in ∼500 mL of hexane to remove color. The solid was collected by filtration and dried to give 16.9 g (81%) of **123**. An analytical sample, mp 181−182 °C, was recrystallized from EtOAc−hexene to yield a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.74 (s, 3H), 5.16 (s, 1H), 6.25 (s, 2H), 6.67 (d, J = 8.5 Hz, 1H), 7.22 (m, 1H), 7.32 (m, 5H), 7.57 (dd, J = 8.5, 2.1 Hz, 1H), 7.67 (s, 1H), 7.93 (d, J = 2.1 Hz, 1H); IR (KBr) 3369, 3259, 3183, 1687, 1661, 1602, 1509, 1290, 1236 cm^{-1}; MS (ES$^+$) 317.2 (M + 1, 40), 207.2 (M − C_6H_5SH, 10). Anal. ($C_{16}H_{16}N_2O_3S$) C, H, N.

2-[2-(Acetylamino)-5-(methoxycarbonyl)phenyl]-2-(phenylthio)acetamide (124). A mixture of 13 g (0.041 mol) of **123** and 16.4 mL (0.12 mol) of Et_3N in 400 mL of THF at 0 °C under a N_2 atmosphere was treated with 3.2 mL (0.045 mol) of acetyl chloride, and the mixture stirred at ambient temperature for 1 h. The reaction progress was monitored by TLC (silica gel, 3:1 EtOAc−hexene). An additional 1.2 mL (0.016 mol) of acetyl chloride was added, and the mixture stirred at ambient temperature overnight. The reaction was not complete so an additional 1.2 mL (0.010 mol) of acetyl chloride was added and the mixture stirred for 4 h. The mixture was diluted with 300 mL of EtOAc and 200 mL of H_2O. The organic layer was separated, washed twice with 100-mL portions of H_2O, and concentrated. The residue was chromatographed twice on silica gel (50−67% EtOAc in hexene). The appropriate fractions were combined and concentrated, and the residue was recrystallized twice from $CHCl_3$−hexane to give 1.5 g (10%) of **124** as a white solid: mp 173−174 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.13 (s, 3H), 3.92 (s, 3H), 5.34 (s, 1H), 7.29 (m, 5H), 7.63 (s, 1H), 7.84 (dd, J = 8.5, 2.0 Hz, 1H), 7.94 (m, 3H), 10.47 (s, 1H); IR (KBr) 3382, 3273, 3185, 1727, 1681, 1670, 1527, 1291, 1278, 1224 cm^{-1}; MS (ES$^+$) 359.2 (M + 1). Anal. ($C_{18}H_{18}N_2O_4S \cdot 0.25H_2O$) C, H, N.

2-[2-(Acetylamino)-5-(methoxycarbonyl)phenyl]acetamide (125). An activated sample of Raney Ni was prepared by washing 20 g of a commercial preparation thrice with H_2O and thrice with THF. This THF slurry was added portionwise over a 1-h period to a stirred solution of 1.25 g (0.0035 mol) of **124** in 70 mL of THF. The mixture was filtered through Celite, the filtrate concentrated, and the residue dried to give 0.68 (78%) of **125** as a white solid. An analytical sample, mp 235 °C, was recrystallized from MeOH: ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.11 (s, 3H), 3.55 (s, 2H), 3.84 (s, 3H), 7.28 (s, 1H), 7.82 (dd, J = 8.5, 2.0 Hz, 1H), 7.85 (s, 1H), 7.89 (d, J = 2.0 Hz, 1H), 7.96 (dd, J = 8.2, 5 Hz, 1H), 10.39 (s, 1H); IR (KBr) 3408, 3276, 3211, 1712, 1665, 1522, 1273 cm^{-1}; MS (ES$^+$) 251.3 (M + 1). Anal. ($C_{12}H_{14}N_2O_4$) C, H, N.

2-[2-(Acetylamino)-5-carboxyphenyl]acetamide (126). A mixture of 0.48 g (0.0019 mL) of **125**, 2.1 mL of 1 N NaOH, and 3 mL of H_2O was stirred at ambient temperature for 3 h followed by 1 h at 35 °C. The mixture was filtered, the filtrate pH was adjusted to 4.4 with 0.5 N HCl, and the volume of the filtrate was reduced by ∼50%. The precipitate was collected by filtration and then recrystallized twice from H_2O to give 0.1 g (22%) of **126** as a cream-colored solid: mp 324−326 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.10 (s, 3H), 3.53 (s, 2H), 7.26 (s, 1H), 7.79 (dd, J = 8.4, 2.0 Hz, 1H), 7.85 (s, 1H), 7.87 (d, J = 2.0 Hz, 1H), 7.91 (dd, J = 8.2, 5.2 Hz, 1H), 10.35 (s, 1H), 12.77 (s, 1H); IR (KBr) 3360, 3180, 1705, 1685, 1668, 1596, 1525, 1323, 1280 cm^{-1}; MS (ES$^+$) 237.3 (M + 1). Anal. ($C_{11}H_{12}N_2O_4 \cdot 0.15H_2O$) C, H; N: calcd, 11.72; found, 11.27.

Methyl 4-(Acetylamino)-3-(1-hydroxy-2-nitroethyl)benzoate (127). A suspension of 0.06 g (0.0023 mol) of 95% NaH in 2.5 mL of DMF at 0 °C under an N_2 atmosphere was treated with 0.9 mL (0.015 mol) of $MeNO_2$ and then stirred for 20 min. A 0.33-g (0.0015 mol) sample of **92**[45] was added, and the mixture stirred at 0 °C for 30 min. The reaction mixture was acidified with HOAc and poured into 100 mL of H_2O. The mixture was extracted thrice with 25-mL portions of EtOAc, and the combined extracts were washed with 25 mL of brine, dried ($MgSO_4$), and concentrated. The residue was purified by flash column chromatography on silica gel (90% Et_2O in hexene) to give 0.24 g (57%) of **127** as a white solid: mp 127−129 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.23 (s, 3H), 3.91 (s, 3H), 4.14 (d, J = 4.2 Hz, 1H), 4.53 (dd, J = 13.8, 3.1 Hz, 1H), 4.81 (dd, J = 13.8, 10.3 Hz, 1H), 5.60 (d, t, J = 10.3, 3.6 Hz, 1H), 7.86 (d, J = 2.0 Hz, 1H), 8.0 (dd, J = 8.6, 2.0 Hz, 1H), 8.23 (d, J = 8.6 Hz, 1H), 9.14 (s, 1H); IR (KBr) 3233, 1705, 1678, 1552, 1515, 1298 cm^{-1}; MS (ES$^+$) 283.1 (M + 1). Anal. ($C_{12}H_{14}N_2O_6$) C, H, N.

Methyl 4-(Acetylamino)-3-(2-nitrovinyl)benzoate (128). A mixture of 2.1 g (0.0075 mol) of **127**, 0.17 g (0.0014 mol) of DMAP, 1 mL (0.011 mol) of acetic anhydride, and 15 mL of CH_2Cl_2 was stirred at ambient temperature for 1.5 h. The precipitate was collected by filtration and recrystallized from EtOH to give 1.1 g (45%) of **128** as a yellow solid: mp 200−

203 °C dec; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.16 (s, 3H), 3.87 (s, 3H), 7.8 (dd, J = 8.6, 5.0 Hz, 1H), 8.04 (dd, J = 8.6, 2.0 Hz, 1H), 8.18 (d, J = 13.4 Hz, 1H), 8.24 (d, J = 13.4 Hz, 1H), 8.36 (d, J = 2.0 Hz, 1H), 10.18 (s, 1H); IR (KBr) 3455, 3269, 3098, 1716, 1665, 1515, 1340, 1281, 1239 cm^{-1}; MS (ES$^-$) 263.2 (M − 1). Anal. ($C_{12}H_{12}N_2O_5$) C, H, N.

Methyl 4-(Acetylamino)-3-(2-nitroethyl)benzoate (129). To a solution of 0.89 g (0.0027 mol) of **128** in 15 mL of EtOH at 0 °C was added dropwise over a 10-min period 0.31 g (0.008 mol) of $NaBH_4$ in 20 mL of EtOH. The mixture was stirred for 1 h at 0 °C and acidified to pH 5.6 using 1 N HCl. The mixture was concentrated, and the residue was diluted with 20 mL of EtOAc and 20 mL of H_2O. The layers were separated, and the aqueous layer was extracted thrice with 20-mL portions of EtOAc. The combined organic layers were washed with 25 mL of brine, dried ($MgSO_4$), and concentrated. The residue was triturated thrice with 100 mL of boiling Et_2O and then recrystallized from EtOH to give 0.31 g (43%) of **129** as a white solid: mp 169−170 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.11 (s, 3H), 3.33 (m, 2H), 3.83 (s, 3H), 4.80 (t, J = 7.2 Hz, 2H), 7.72 (m, 1H), 7.82 (m, 2H), 9.58 (s, 1H); IR (KBr) 3421, 3278, 1717, 1664, 1543, 1293 cm^{-1}; MS (ES$^-$) 265.3 (M − 1). Anal. ($C_{12}H_{14}N_2O_5$) C, H, N.

4-(Acetylamino)-3-(2-nitroethyl)benzoic Acid (130). A mixture of 0.25 g (0.0009 mol) of **129** in 1.8 mL of 1 N NaOH was stirred at ambient temperature for 0.75 h. The mixture was filtered, and the filtrate pH was adjusted to 5−6 with glacial HOAc. The resulting solid was collected by filtration, washed with H_2O, and dried to give 0.18 g (78%) of **130** as a white solid: mp >220 °C dec; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.11 (s, 3H), 3.3 (t, J = 7.2 Hz, 2H), 4.80 (t, J = 7.2 Hz, 2H), 7.66 (m, 1H), 7.81 (m, 2H), 9.57 (s, 1H), 12.84 (br s, 1H); IR (KBr) 3461, 3269, 2996, 1695, 1656, 1556, 1281 cm^{-1}; MS (ES$^-$) 251.2 (M − 1). Anal. ($C_{11}H_{12}N_2O_5$) C, H, N.

4-(Acetylamino)-3-(2-aminoethyl)benzoic Acid (131). A mixture of 0.2 g (0.0008 mol) of **130** and 0.04 g of PtO_2 in 8 mL of MeOH was hydrogenated at 50 psi for 4 h. The reaction mixture was filtered through Celite, and the cake was washed thrice with MeOH. The cake was dissolved in 100 mL of H_2O and again filtered through Celite to remove catalyst. This filtrate was concentrated, and the residue was recrystallized from H_2O−EtOH to give 0.08 g (45%) of **131** as a tan solid: mp 212−213 °C dec; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.09 (s, 3H), 2.94 (m, 4H), 7.58 (d, J = 7.7 Hz, 1H), 7.74 (m, 2H); IR (KBr) 3195, 2990, 1668, 1532, 1380 cm^{-1}; MS (ES$^+$) 223 (M + 1). Anal. ($C_{11}H_{14}N_2O_3 \cdot 0.25H_2O$) C, H, N.

Ethyl 3-Nitro-4-[(methylsulfonyl)amino]benzoate (133). To 10 mL of fuming HNO_3 at 5−10 °C was added portionwise 2.8 g (0.008 mol) of ethyl 4-[(methylsulfonyl)amino]benzoate (**132**)[18] over a 15-min period. The mixture was stirred at 10−15 °C for 0.5 h and then poured into 50 mL of cold H_2O. The precipitate was collected by filtration, washed with H_2O, dried, and recrystallized from EtOAc to give 1.7 g (72%) of **133** as yellow crystals: mp 124 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.42 (t, J = 7.2 Hz, 3H), 3.23 (s, 3H), 4.43 (q, J = 7.2 Hz, 2H), 7.95 (d, J = 8.9 Hz, 1H), 8.31 (dd, J = 8.9, 2.0 Hz, 1H), 8.93 (d, J = 2.0 Hz, 1H), 10.06 (s, 1H); IR (KBr) 3271, 1719, 1624, 1535, 1342, 1173 cm^{-1}; MS (ES$^-$) 287 (M − 1). Anal. ($C_{10}H_{12}N_2O_6S$) C, H, N.

Ethyl 3-Amino-4-[(methylsulfonyl)amino]benzoate (134). A mixture of 1 g (0.0035 mol) of **133** and 0.02 g of PtO_2 in 40 mL of EtOH was hydrogenated at 20 psi overnight. The mixture was filtered through Celite, and the filtrate volume was reduced to 20 mL. A precipitate formed which was collected by filtration, washed with cold EtOH, and dried to give 0.75 g (84%) of **134** as a white powder: mp 151 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.29 (t, J = 7.2 Hz, 3H), 2.90 (s, 3H), 4.26 (q, J = 7.2 Hz, 2H), 5.40 (br s, 2H), 7.14 (dd, J = 8.2, 2.0 Hz, 1H), 7.21 (d, J = 8.2 Hz, 1H), 7.38 (d, J = 2.0 Hz, 1H), 8.94 (br s, 1H); IR (KBr) 3476, 3368, 3277, 1705, 1620, 1523, 1157 cm^{-1}; MS (ES$^-$) 257 (M − 1). Anal. ($C_{10}H_{14}N_2OS$) C, H, N.

Ethyl 3-[(Aminoiminomethyl)amino]-4-[(methylsulfonyl)amino]benzoate (135). A mixture of 2.6 g (0.01 mol) of **134**, 0.84 mL (0.01 mol) of concentrated HCl, 4.2 g (0.1 mol) of cyanamide, and 100 mL of EtOAc was heated at reflux for 4 h. The reaction mixture was cooled, and the resulting precipitate was collected by filtration, washed with cold EtOAc, and dried to give 3 g (86%) of **135** as a white powder: mp 160−164 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.31 (t, J = 7.1 Hz, 3H), 3.13 (s, 3H), 4.32 (q, J = 7.1 Hz, 2H), 7.51 (br s, 4H), 7.66 (d, J = 8.5 Hz, 1H), 7.76 (d, J = 2.0 Hz, 1H), 7.92 (dd, J = 8.5, 2.0 Hz, 1H), 9.40 (br s, 1H), 9.98 (br s, 1H); IR (KBr) 3368, 3179, 1680, 1611, 1340, 1296, 1170 cm^{-1}; MS (ES$^-$) 299 (M − 1). Anal. ($C_{11}H_{16}N_4O_4S \cdot HCl \cdot 0.5H_2O$) C, H, N.

3-[(Aminoiminomethyl)amino]-4-[(methylsulfonyl)amino]benzoic Acid (136). A mixture of 1.8 g (0.005 mol) of **135** and 15 mL of 1 N NaOH was stirred at ambient temperature for 4 h. The mixture was filtered, and the filtrate was neutralized with HCl. The resulting precipitate was collected by filtration, washed with H_2O, and dried to give 1.2 g (81%) of **136** as an off-white powder: mp 200 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.75 (s, 3H), 7.32 (d, J = 9.1 Hz, 1H), 7.51−7.62 (m, 6H); IR (KBr) 3437, 1668, 1601, 1379, 1321, 1157 cm^{-1}; MS (ES$^-$) 271 (M − 1). Anal. ($C_9H_{12}N_4O_2S \cdot 0.5H_2O$) C, H, N.

2-[(Methylsulfonyl)amino]-5-(methoxycarbonyl)benzaldehyde Trimethylenemercaptal (138). To a solution of 5.0 g (0.019 mol) of **137**[45] in 90 mL of CH_2Cl_2 and 4.5 mL (0.055 mol) of pyridine was added 4.3 mL (0.055 mol) of methanesulfonyl chloride dropwise over a 5-min period, and the mixture heated at reflux overnight. The reaction mixture was cooled and washed successively with a 10% HOAc solution followed by H_2O. The organic layer was dried (Na_2SO_4) and concentrated, and the residue was purified by column chromatography on silica gel (1:1 EtOAc−hexane). The appropriate fractions were combined and concentrated to give 5.0 g (77%) of **138** as a yellow solid. An analytical sample, mp 168−168.5 °C, was recrystallized from EtOH: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.90−2.01 (m, 1H), 2.20−2.25 (m, 1H), 2.93−2.98 (m, 2H), 3.05−3.11 (m, 5H), 3.91 (s, 3H), 5.4 (s, 1H), 7.77 (d, J = 8.6 Hz, 1H), 8.00 (dd, J = 8.6, 2.0 Hz, 1H), 8.05 (d, J = 20.0 Hz, 1H), 8.21 (br s, 1H); IR (KBr) 3301, 2905, 1723, 1335, 1162 cm^{-1}; MS (ES$^+$) 348 (M + 1). Anal. ($C_{13}H_{17}NO_4S_3$) C, H, N.

2-[(Methylsulfonyl)amino]-5-(methoxycarbonyl)benzaldehyde (139). A solution of 7.7 g (0.022 mol) of **138** in 4.4 mL of DMF and 39 mL of acetone was added dropwise over a 5-min period to a mixture of 2.1 g (0.026 mol) of CuO and 7.0 g (0.055 mol) of $CuCl_2$ in 174 mL of acetone heated at reflux. The mixture was heated at reflux for 2 h, cooled, and filtered through Celite, and the cake was washed with 10% EtOH in CH_2Cl_2. The filtrate was diluted with 125 mL of H_2O and 150 mL of CH_2Cl_2. The filtrate was shaken, the layers were separated, and the aqueous layer was extracted twice with 100-mL portions of H_2O, dried (Na_2SO_4), and concentrated. The residue was recrystallized from EtOH to give 4.5 g (79%) of **139** as a beige solid: mp 161−163 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.31 (s, 3H), 3.89 (s, 3H), 7.69 (d, J = 8.8 Hz, 1H), 8.23 (dd, J = 8.8, 2.1 Hz, 1H), 8.51 (d,J = 2.1 Hz, 1H), 10.10 (s, 1H), 10.68 (s, 1H); IR (KBr) 3138, 1712, 1664, 1612, 1155, 1137 cm^{-1}; MS (ES$^-$) 256 (M − 1). Anal. ($C_{10}H_{11}NO_5S$) C, H, N.

Methyl 3-(1-Hydroxy-2-nitroethyl)-4-[(methylsulfonyl)amino]benzoate (140). To a mixture of 2.6 g (0.01 mol) of **139** and 1.2 g (0.02 mol) of $MeNO_2$ in 40 mL of EtOH at 0 °C (ice−salt bath) was added dropwise a solution of 1.2 g (0.02 mol) of KOH in 18 mL of EtOH and 2 mL of H_2O over a 20-min period. The mixture was stirred for 0.5 h at 0 °C and then at ambient temperature for 4 h. The mixture was neutralized with 2 N HCl and then poured into 100 mL of H_2O. The mixture was extracted with $CHCl_3$, and the organic layer was dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography on silica gel (1−5% MeOH in $CHCl_3$). The appropriate fractions were combined and concentrated, and the residue was recrystallized from EtOAc−hexane to give 0.8 g (25%) of **140** as an off-white solid: mp 148 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.16 (s, 3H), 3.86 (s, 3H), 4.56 (dd, J = 12.7, 9.8 Hz, 1H), 4.82 (dd, J = 12.7, J = 2.9 Hz, 1H), 5.73 (dd, J = 7.8, 2.9 Hz, 1H), 6.41 (br s, 1H), 7.51 (d, J = 8.42 Hz, 1H), 7.92 (dd, J = 8.4, 2.1 Hz, 1H), 8.16 (d, J = 2.1 Hz, 1H), 9.65 (s, 1H); IR (KBr) 3398, 3275, 1685, 1563, 1442, 1330 cm^{-1}; MS (ES$^-$) 317 (M − 1). Anal. ($C_{11}H_{14}N_2O_7S$) C, H, N.

Methyl 3-(2-Amino-1-hydroxyethyl)-4-[(methylsulfonyl)amino]benzoate (141). A mixture of 0.2 g (0.0006 mol) of **140** and 0.02 g of PtO_2 in 10 mL of EtOH was hydrogenated at 50 psi for 8 h. The mixture was filtered through Celite, and the filtrate was concentrated to 5 mL. The filtrate was diluted with 5 mL of EtOAc and 10 mL of hexane and cooled to 0 °C. The precipitate was collected by filtration, washed with a 1:1 solution of EtOAc−hexane, and dried to give 0.1 g (55%) of **141** as a white powder: mp 172−173 °C dec; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.68 (s, 3H), 2.82 (dd, J = 12.2, 7.8 Hz, 1H), 3.09 (dd, J = 12.2, 4.5 Hz, 1H), 3.75 (s, 3H), 4.92 (dd, J = 4.5, 7.8 Hz, 1H), 7.24 (d, J = 9.3 Hz, 1H), 7.61 (dd, J = 9.3, 2.2 Hz, 1H), 7.81 (d, J = 2.2 Hz, 1H); IR (KBr) 3141, 3021, 1695, 1605, 1494, 1273 cm^{-1}; MS (ES$^+$) 289 (M + 1). Anal. ($C_{11}H_{16}N_2O_5S$) C, H, N.

3-(2-Amino-1-hydroxyethyl)-4-[(methylsulfonyl)amino]-benzoic Acid (142). A mixture of 0.05 g (0.000 17 mol) of **141** and 44 mL (0.000 44 mol) of 1 N NaOH was stirred at ambient temperature for 4 h. The mixture was filtered, the filtrate neutralized with 1 N HCl, and the mixture allowed to stand at ambient temperature overnight. The precipitate was collected by filtration, washed with H_2O, and dried to give 0.038 g (81%) of **142** as a white powder: mp 274−276 °C; ^{1}H NMR (400 MHz, ND_4OD) δ 2.90 (m, 5H), 5.08 (dd, J = 4.6, 7.6 Hz, 1H), 7.23 (d, J = 8.4 Hz, 1H), 7.71 (dd, J = 2.2, 8.4 Hz, 1H), 7.80 (d, J = 2.2 Hz, 1H); IR (KBr) 3264, 1608, 1590, 1530, 1395, 1327 cm^{-1}; MS (ES$^+$) 275 (M + 1). Anal. ($C_{10}H_{14}N_2O_5S\cdot 0.2H_2O$) C, H, N.

Methyl 3-[(Hydroxyimino)methyl]-4-(methylsulfonylamino)benzoate (143). A mixture of 2.6 g (0.01 mol) of **139** and 0.87 g (0.0125 mol) of $HONH_2\cdot HCl$ in 40 mL of EtOH was heated at reflux for 2 h. The mixture was filtered hot, the filtrate was concentrated, and the residue was triturated with H_2O. The resulting solid was collected by filtration, dried, and recrystallized from EtOAc/hexane to give 2.4 g (89%) of **143** as an off-white powder, mp 160 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.25 (s, 3H), 3.85 (s, 3H), 7.62 (d, J = 8.7 Hz, 1H), 7.96 (dd, J = 8.7 Hz and J = 2.1 Hz, 1H), 8.19 (d, J = 2.1 Hz, 1H), 8.50 (s, 1H), 10.59 (s, 1H), 11.84 (s, 1H); IR (KBr) 3361, 1698, 1621, 1341, 1286, 1159 cm^{-1}; MS (ES$^+$) 273 (M + 1). Anal. ($C_{10}H_{12}N_2O_5S$) C, H, N.

3-[(Hydroxyimino)methyl]-4-[(methylsulfonyl)amino]-benzoic Acid (144). A mixture of 0.54 g (0.002 mol) of **143**, 5.0 mL of 1 N NaOH, and 2.5 mL of H_2O was stirred at 40 °C for 16 h. The mixture was allowed to cool to ambient temperature and filtered, and the filtrate was neutralized with HCl. The resulting precipitate was collected by filtration, washed with H_2O, and dried to give 0.4 g (81%) of **144** as a white powder: mp 250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.24 (s, 3H), 7.59 (d, J = 8.6 Hz, 1H), 7.95 (dd, J = 8.6, 2.0 Hz, 1H), 8.16 (d, J = 2.0 Hz, 1H), 8.42 (s, 1H), 10.54 (s, 1H), 11.80 (s, 1H), 13.00 (s, 1H); IR (KBr) 3442, 3033, 1691, 1610, 1343, 1289 cm^{-1}; MS (ES$^-$) 257 (M − 1). Anal. ($C_9H_{10}N_2O_5S$) C, H, N.

Ethyl 4-[(2-Methylpropionyl)amino]-3-nitrobenzoate (146). A 29.4-g (0.125-mol) sample of ethyl 4-[(2-methylpropionyl)amino]benzoate (**145**)[47] was added portionwise to 200 mL of stirred, fuming HNO_3 at 10 °C. After the addition was complete, the mixture was stirred at ambient temperature for 30 min. The reaction mixture was poured into H_2O and extracted thrice with 500-mL portions of EtOAc. The combined extracts were washed with 500 mL of 1% $NaHCO_3$ and then 500 mL of H_2O and concentrated. The residue was purified by column chromatography on silica gel. The appropriate fractions were combined and concentrated to give 17 g (49%) of **146** as a yellow solid. An analytical sample, mp 74−76 °C, was recrystallized from hexane: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.32 (d, J = 7.0 Hz, 6H), 1.42 (t, J = 7.1 Hz, 3H), 2.69 (m, 1H), 4.41 (q, J = 7.1 Hz, 2H), 8.27 (dd, J = 1.8, 8.8 Hz, 1H), 8.89 (d, J = 1.8 Hz, 1H), 8.95 (d, J = 8.8 Hz, 1H), 10.67 (br s, 1H); IR (KBr) 3344, 2986, 1712, 1620, 1508, 1335 cm^{-1}; MS (ES$^+$) 281 (M + 1). Anal. ($C_{13}H_{16}N_2O_5$) C, H, N.

Ethyl 3-Amino-4-[(2-methylpropionyl)amino]benzoate (147). A mixture of 8.0 g (0.029 mol) of **146** and 0.05 g of PtO_2 in 75 mL of EtOH was hydrogenated at 35 psi for 2 h. The mixture was filtered through Celite and the filtrate concentrated. The residue was recrystallized from EtOAc−hexane to give 5.0 g (70%) of **147** as a beige solid. An analytical sample, mp 147−149 °C, was prepared by two additional recrystallizations from EtOAc−hexane: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.29 (d, J = 6.8 Hz, 6H), 1.38 (t, J = 7.2 Hz, 3H), 2.58 (m, 1H), 3.61 (br s, 2H), 4.34 (q, J = 7.2 Hz, 2H), 7.26 (br s, 1H), 7.33−7.53 (m, 3H); IR (KBr) 3428, 3270, 1712, 1653, 1527, 1438 cm^{-1}; MS (ES$^+$) 251 (M + 1). Anal. ($C_{13}H_{18}N_2O_3$) C, H, N.

Ethyl 3-[(Aminoiminomethyl)amino]-4-[(2-methylpropionyl)amino]benzoate (148). A mixture of 1.7 g (0.0068 mol) of **147**, 5.7 g (0.136 mol) of cyanamide, and 0.6 mL (0.0068 mol) of concentrated HCl in 50 mL of EtOAc was heated at reflux for 48 h. The mixture was concentrated, and the residue was purified by column chromatography on silica gel (0−3% MeOH in EtOAc). The appropriate fractions were combined and concentrated, and the residue was triturated with EtOAc. The resulting solid was collected by filtration and dried to give 0.76 g (34%) of **148**. An analytical sample, mp 175−177 °C, was isolated as a white solid by a recrystallization from MeOH−Et_2O: ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.12 (d, J = 6.8 Hz, 6H), 1.32 (t, J = 7.1 Hz, 3H), 2.80 (m, 1H), 4.31 (q, J = 7.1 Hz, 2H), 7.51 (br s, 3H), 7.76 (d, J = 1.9 Hz, 1H), 7.89 (dd, J = 1.9, 8.4 Hz, 1H), 7.95 (d, J = 8.4 Hz, 1H), 9.29 (s, 1H), 9.95 (s, 1H); IR (KBr) 3306, 3153, 1720, 1675, 1587, 1307 cm^{-1}; MS (ES$^+$) 293 (M + 1). Anal. ($C_{14}H_{20}N_4O_3\cdot 0.5H_2O$) C, H, N.

3-[(Aminoiminomethyl)amino]-4-[(2-methylpropionyl)amino]benzoic Acid (149). A mixture of 0.36 g (0.0012 mol) of **148** and 2.4 mL of 1 N NaOH was stirred at 38 °C for 2 h. The mixture was filtered, and the filtrate pH was adjusted to 7 with 1 N HCl. The resulting precipitate was collected by filtration, and then the cake was dissolved in a minimum amount of 1 N NaOH. The pH of this solution was adjusted to 7 with 1 N HCl. The resulting precipitate was collected by filtration, washed with H_2O, and dried to give 0.15 g (52%) of **149** as a white solid: mp 238−239.5 °C; ^{1}H NMR (400 MHz, DMSO-d_6 and CD_3OD) δ 1.17 (d, J = 6.8 Hz, 6H), 2.71 (m, 1H), 7.53 (d, J = 8.3 Hz, 1H), 7.78 (d, J = 1.6 Hz, 1H), 7.86 (dd, J = 1.6, 8.3 Hz, 1H); IR (KBr) 3408, 3207, 1683, 1640, 1538, 1383 cm^{-1}; MS (ES$^-$) 263 (M − 1). Anal. ($C_{12}H_{16}N_4O_3\cdot 0.5H_2O$) C, H, N.

Ethyl 4-(2-Furoylamino)-3-nitrobenzoate (151). A solution of 2.3 g (0.011 mol) of ethyl 4-amino-3-nitrobenzoate (**150**)[48] in 30 mL of pyridine was treated with 1.1 mL (0.011 mol) of 2-furoyl chloride (Aldrich), and the mixture stirred at ambient temperature overnight. The mixture was poured into 500 mL of H_2O, and the resulting precipitate was collected by filtration and recrystallized from EtOAc to give 2.4 g (72%) of **151**. An analytical sample, mp 155−156.5 °C, was isolated as a yellow solid after two additional recrystallizations from EtOAc: ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.35 (t, J = 7.0 Hz, 3H), 4.36 (q, J = 7.0 Hz, 2H), 6.79 (dd, J = 1.8, 3.5 Hz, 1H), 7.42 (dd, J = 3.5, 0.7 Hz, 1H), 8.07 (dd, J = 1.8, 0.7 Hz, 1H), 8.28 (m, 2H), 8.52 (m, 1H), 11.15 (s, 1H); MS (no ionization); IR (KBr) 3323, 1713, 1687, 1587, 1508, 1270 cm^{-1}. Anal. ($C_{14}H_{12}N_2O_6$) C, H, N.

Ethyl 3-Amino-4-(2-furoylamino)benzoate (152). A mixture of 2.0 g (0.0066 mol) of **151** and 0.05 g of PtO_2 in 200 mL of EtOH was hydrogenated at 30 psi for 1 h. The mixture was filtered through Celite, and the filtrate was concentrated to give 1.2 g (67%) of **152** as a yellow solid. An analytical sample, mp 134.5−135.5 °C, was recrystallized from EtOAc−hexane: ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.31 (t, J = 7.1 Hz, 3H), 4.28 (q, J = 7.1 Hz, 2H), 5.26 (s, 2H), 6.70 (dd, J = 1.8, 3.5 Hz, 1H), 7.20 (dd, J = 1.9, 8.2 Hz, 1H), 7.33 (dd, J = 0.8, 3.5 Hz, 1H), 7.39 (d, J = 8.2 Hz, 1H), 7.44 (d, J = 1.9 Hz, 1H), 7.94 (dd, J = 0.8, 1.8 Hz, 1H), 9.62 (s, 1H); IR (KBr) 3360, 1701, 1662, 1432, 1316 cm^{-1}; MS (ES$^+$) 275.1 (M + 1). Anal. ($C_{14}H_{14}N_2O_4$) C, H, N.

3-[(Aminoiminomethyl)amino]-4-(2-furoylamino)benzoic Acid (154). A mixture of 0.52 g (0.0019 mol) of **152**, 1.56 g (0.037 mol) of cyanamide, 10 drops of concentrated HCl, and 100 mL of EtOAc was heated at reflux for 14 h. The resulting precipitate was collected by filtration and dried to give 0.4 g (58%) of ethyl 3-[(aminoiminomethyl)amino]-4-(2-furoylamino)-benzoate hydrochloride (**153**) as an off-white solid.

A mixture of 0.3 g (0.0009 mol) of crude **153** and 2 mL of 1 N NaOH was stirred at ambient temperature for 16 h. An additional 1 mL of 1 N NaOH was added and the mixture heated at 50 °C for 6 h. The mixture was filtered, and the filtrate pH was adjusted to 8 with 1 N HCl. The resulting precipitate was collected by filtration and then dissolved in a minimum amount of NH_4OH. The pH was adjusted to 10 with 6 N HCl, and the resulting precipitate was collected by filtration and dried to yield 0.04 g (20%) of **154** as a tan solid: mp 227−228 °C; ^{1}H NMR (400 MHz, ND_4OD) δ 7.20 (dd, J = 1.8, 3.5 Hz, 1H), 7.78 (dd, J = 3.5, 0.8 Hz, 1H), 8.03 (d, J = 1.8 Hz, 1H), 8.11 (dd, J = 8.5, 1.8 Hz, 1H), 8.25 (dd, J = 0.8, 1.8 Hz, 1H), 8.49 (d, J = 8.5 Hz, 1H); IR (KBr) 3455, 3424, 1693, 1511, 1366 cm^{-1}; MS (ES$^+$) 289.1 (M + 1). Anal. ($C_{13}H_{12}N_4O_4$) C, H, N.

4-(Methoxycarbonyl)-2-nitrobenzoic Acid (156). A mixture of 20 g (0.095 mol) of nitroterephthalic acid (**155**; Aldrich) and 10 mL of H_2SO_4 in 100 mL of anhydrous MeOH was heated at reflux for 1 h. The mixture was concentrated, and the residue was poured into 200 mL of a saturated $NaHCO_3$ solution. The mixture was washed with $CHCl_3$, and then the pH of the aqueous layer was adjusted to 2 with 2 N HCl. The mixture was extracted twice with approximately 200-mL portions of EtOAc, and the combined organic layers were washed with H_2O and brine and dried (Na_2SO_4). The residue was recrystallized from EtOAc−hexane to give 11.8 g (55%) of **156** as cream-colored crystals: mp 131−132 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.93 (s, 3H), 7.98 (d, J = 8.2 Hz, 1H), 8.30 (dd, J = 1.6, 8.0 Hz, 1H), 8.43 (d, J = 1.6 Hz, 1H), 14.23 (br s, 1H); IR (KBr) 3115, 1720, 1545, 1501, 1438, 1358 cm^{-1}; MS (ES$^-$) 224 (M − 1). Anal. ($C_9H_7NO_6$) C, H, N.

Methyl 4-[(Methylamino)carbonyl]-3-nitrobenzoate (157). A mixture of 3.6 g (0.016 mol) of **156**, 2 drops of DMF, and 32 mL of $SOCl_2$ was heated at reflux for 3 h. The mixture was concentrated, and the residue was dissolved in 10 mL of CH_2Cl_2. This solution was added dropwise over a 5-min period to a stirred solution of 2 g (0.026 mol) of 40% aqueous $MeNH_2$ and 5 mL of H_2O. The mixture was stirred for 10 min resulting in the formation of a precipitate. The solid was collected by filtration, washed with H_2O, dried, and recrystallized from EtOAc−hexane to give 2.1 g (55%) of **157** as white needles: mp 155 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.77 (d, J = 4.6 Hz, 3H), 3.92 (s, 3H), 7.76 (d, J = 8.0 Hz, 1H), 8.29 (dd, J = 8.0, 1.5 Hz, 1H), 8.45 (d, J = 1.5 Hz, 1H), 8.73 (d, J = 4.6 Hz, 1H); IR (KBr) 3277, 1727, 1653, 1532, 1294, 1247 cm^{-1}; MS (ES$^+$) 339 (M + 1). Anal. ($C_{10}H_{10}N_2O_5$) C, H, N.

Methyl 3-Amino-4-[(methylamino)carbonyl]benzoate (158). A mixture of 3.8 g (0.01 mol) of **157** and 15.3 g (0.08 mol) of $SnCl_2$ in 80 mL of EtOH was heated at 70 °C for 1 h. The reaction mixture was diluted with 150 mL of H_2O, and a saturated $NaHCO_3$ solution was added to adjust the pH to 8. The mixture was extracted with about 200 mL of EtOAc. The organic layer was washed with H_2O and brine, dried (Na_2SO_4), and concentrated. The residue was recrystallized from EtOAc−hexane to give 2.2 g (64%) of **158** as tan needles: mp 167−168 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.74 (d, J = 4.6 Hz, 3H), 3.82 (s, 3H), 6.59 (s, 2H), 7.04 (dd, J = 1.7, 8.2 Hz, 1H), 7.35 (d, J = 1.7 Hz, 1H), 7.53 (d, J = 8.2 Hz, 1H), 8.35 (m, 1H); IR (KBr) 3464, 3317, 1715, 1637, 1589, 1553 cm^{-1}; MS (ES$^+$) 209 (M + 1). Anal. ($C_{10}H_{12}N_2O_3$) C, H, N.

3-Amino-4-[(methylamino)carbonyl]benzoic Acid (159). A mixture of 0.62 g (0.003 mol) of **158** and 4.5 mL of 1 N NaOH was stirred at ambient temperature for 4 h. The mixture was filtered, the filtrate was neutralized with concentrated HCl, and the pH was then adjusted to 3 with HOAc. The resulting precipitate was collected by filtration, washed with H_2O, and dried to give 0.4 g (69%) of **159** as a pale yellow powder: mp 215−217 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.74 (d, J = 4.5 Hz, 3H), 6.54 (s, 2H), 7.02 (dd, J = 1.6, 8.2 Hz, 1H), 7.32 (d, J = 1.6 Hz, 1H), 7.51 (d, J = 8.2 Hz, 1H), 8.32 (m, 1H), 12.86 (br s, 1H); IR (KBr) 3455, 3423, 1697, 1628, 1589, 1538 cm^{-1}; MS (ES$^+$) 195 (M + 1). Anal. ($C_9H_{10}N_2O_3$) C, H, N.

3-[(Aminoiminomethyl)amino]-4-[(methylamino)carbonyl]benzoic Acid (160). A mixture of 0.19 g (0.001 mol) of **159**, 0.59 g (0.014 mol) of cyanamide, 0.08 g (0.001 mol) of concentrated HCl, and 10 mL of EtOAc was stirred at 37−38 °C for 16 h. The precipitate which formed was collected by filtration, and the cake was mixed with 0.4 g (0.01 mol) of cyanamide and 10 mL of EtOAc. The mixture was stirred overnight at 37−38 °C. The resulting precipitate was collected by filtration, washed with EtOAc, and recrystallized from MeOH−Et_2O to give 0.12 g (40%) of **160** as a white solid: mp >300 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.80 (d, J = 4.6 Hz, 3H), 3.17 (s, 0.75H), 7.72−7.97 (m, 7H), 8.76 (q, J = 4.6 Hz, 1H), 9.87 (s, 1H), 13.38 (br s, 1H); IR (KBr) 3379, 3164, 1719, 1679, 1646, 1544 cm^{-1}; MS (ES$^+$) 237 (M + 1). Anal. ($C_{10}H_{12}N_4O_3\cdot HCl\cdot H_2O\cdot 0.25MeOH$) C, H, N.

Methyl 3-Amino-4-(aminosulfonyl)benzoate (162). A mixture of 0.65 g (0.0025 mol) of methyl 4-(aminosulfonyl)-3-nitrobenzoate (**161**)[49] and 0.12 g of PtO_2 in 20 mL of EtOAc was hydrogenated at 50 psi for 1 h. The mixture was filtered through Celite, and the cake was washed with five 5-mL portions of MeOH. The combined filtrates were concentrated to give 0.49 g (85%) of **162**. An analytical sample, mp 211−213 °C, was obtained as a light orange solid by recrystallization from 95% EtOH: ^{1}H NMR (360 MHz DMSO-d_6) δ 3.83 (s, 3H), 6.07 (s, 2H), 7.12 (dd, J = 1.5, 8.3 Hz, 1H), 7.39 (s, 2H), 7.44 (d, J = 1.5 Hz, 1H), 7.63 (d, J = 8.3 Hz, 1H); IR (KBr) 3453, 3365, 3269, 1709, 1432, 1329 cm^{-1}; MS (ES$^-$) 229 (M − 1). Anal. ($C_8H_{10}N_2O_4S$) C, H, N.

3-[(Aminoiminomethyl)amino]-4-(aminosulfonyl)benzoic Acid (164). A mixture of 0.4 g (0.0018 mol) of **162**, 1.9 g (0.045 mol) of cyanamide, 0.23 mL of concentrated HCl, and 10 mL of EtOAc was heated at reflux for 6 h. The solution was cooled, the resulting solid was collected by filtration, and the cake was washed thrice with 10-mL portions of EtOAc and then dried to give 0.44 g (79%) of methyl 3-[(aminoiminomethyl)amino]-4-(aminosulfonyl)benzoate hydrochloride (**163**) as a white solid.

A mixture of 0.3 g (0.001 mol) of the above crude ester **163** and 5 mL of 1 N NaOH was stirred at ambient temperature for 1 h. The mixture was filtered, and the filtrate pH was adjusted to 6 using 1 N HCl. The resulting precipitate was collected by filtration and dried to give 0.2 g (78%) of **164** contaminated with urea. An analytical sample, mp >320 °C, was isolated as a white solid by slurrying the solid thrice with 5-mL portions of fresh MeOH for 15 min and then drying the solid: ^{1}H NMR (360 MHz, CF_3CO_2D) δ 8.34 (m, 3H); IR (KBr) 3443, 3668, 1698, 1620, 1381, 1332, 1159 cm^{-1}; MS (no ionization). Anal. ($C_8H_{10}N_4O_4S\cdot 0.25H_2O$) C, H; N: calcd, 21.33; found, 21.88.

3-Amino-4-(2-hydroxyethyl)benzoic Acid (166). A mixture of 0.9 g (0.0043 mol) of 4-(2-hydroxyethyl)-3-nitrobenzoic acid (**165**)[50] and 0.05 g of PtO_2 in 20 mL of EtOH was hydrogenated at 30 psi for 3 h. The mixture was filtered through Celite and the filtrate concentrated to give 0.8 g (quantitative) of **166**. An analytical sample, mp 173−174 °C, was isolated as an off-white solid from a recrystallization from EtOH: ^{1}H NMR (400 MHz, DMSO) δ 2.65 (t, J = 6.8 Hz, 2H), 3.62 (t, J = 6.8 Hz, 2H), 4.69 (br s, 1H), 5.12 (br s, 2H), 7.03 (d, J = 7.7 Hz, 1H), 7.09 (dd, J = 7.7, 1.6 Hz, 1H), 7.25 (d, J = 1.6 Hz, 1H), 12.5 (br s, 1H); IR (KBr) 3391, 3225, 2878, 1680, 1581, 1444, 1314, 1019 cm^{-1}; MS (ES$^+$) 182.3 (M + 1). Anal. ($C_9H_{11}NO_3$) C, H, N.

Methyl 3-Amino-4-(2-hydroxyethyl)benzoate (167). To a cooled (ice bath) solution of acidic MeOH (prepared from 50 mL of anhydrous MeOH and 5 mL of acetyl chloride) was added 0.86 g (0.0047 mol) of **166**, and the resulting solution stirred at ambient temperature overnight. A small amount of the hydrochloride, mp 217 °C, had precipitated so it was collected by filtration and used to characterize **167** hydrochloride. The filtrate was concentrated, and the residue was treated with 5 mL of H_2O and 25 mL of EtOAc. The pH was adjusted to 7−8 with concentrated NH_4OH. The layers were separated, and the aqueous layer was extracted thrice with 10-mL portions of EtOAc. The combined organic layers were dried (Na_2SO_4) and concentrated to give 1.0 g (91%) of **167** as a gum: ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.90 (t, J = 6.4 Hz, 2H), 3.75 (t, J = 6.4 Hz, 2H), 7.45 (d, J = 8.0 Hz, 1H), 7.8 (dd, J = 8.0, 1.7 Hz, 1H), 7.98 (d, J = 1.7 Hz, 1H); IR (KBr) 3555, 2956, 2585, 2036, 1723, 1490, 1281 cm^{-1}; MS (ES$^+$) 196.3 (M + 1). Anal. ($C_{10}H_{13}NO_3\cdot HCl$) C, H, N.

Methyl 3-[[[(*tert*-Butoxycarbonyl)amino][(*tert*-butoxycarbonyl)imino]methyl]amino]-4-(2-hydroxyethyl)benzoate (168). To a solution of 0.75 g (0.0039 mol) of **167** in 3.5 mL of DMF were added 1.9 mL (0.014 mol) of Et_3N and 1.1 g (0.004 mol) of *N*,*N*-bis(*tert*-butoxycarbonyl)thiourea.[41] The mixture was cooled to 0 °C and treated with 1.2 g (0.0043 mol) of $HgCl_2$. The reaction mixture was stirred at 0 °C for 0.5 h and then at ambient temperature for 24 h. The mixture was diluted with 30 mL of EtOAc and filtered through Celite. The filtrate was washed thrice with 20-mL portions of H_2O, dried (Na_2SO_4), and concentrated. The residue was purified by flash column chromatography on silica gel (20−30% EtOAc in hexane) to give 1 g (64%) of **168** as a white solid: mp 147−150 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.35 (s, 9H), 1.55 (s, 9H), 2.76 (t, J = 6.3 Hz, 2H), 3.65 (m, 2H), 3.9 (s, 3H), 4.83 (t, J = 4.5 Hz, 1H), 7.45 (d, J = 8.0 Hz, 1H), 7.80 (dd, J = 8.0, 1.6 Hz, 1H), 8.05 (d, J = 1.6 Hz, 1H), 9.98 (s, 1H), 11.55 (s, 1H); IR (KBr) 3559, 3266, 3127, 3011, 2971, 1724, 1650, 1596, 1459, 1291, 1230, 1163, 1127, 1057, 760 cm^{-1}; MS (ES$^+$) 438.6 (M + 1). Anal. ($C_{21}H_{31}N_3O_7$) C, H, N.

3-[(Aminoiminomethyl)amino]-4-(2-hydroxyethyl)benzoic Acid (169). To a solution of 0.9 g (0.002 mol) of **168** in 20 mL of CH_2Cl_2 at 0 °C was added dropwise 3.3 mL (0.04 mol) of TFA, and the mixture stirred at ambient temperature for 32 h. An additional 0.8 mL (0.011 mol) of TFA was added, and the mixture stirred for 1 h. The mixture was filtered, the filtrate concentrated, and the residue chased twice with 10-mL portions of CH_2Cl_2. The residue was stirred with 18 mL of 3 N NaOH for 1 h. The mixture was filtered, and the filtrate pH was adjusted to 5 using concentrated HCl and then readjusted to 7 using 1 N NaOH. The precipitate was collected by filtration, successively washed with small amounts of MeOH and Et_2O, and dried to give 0.29 g (59%) of **169** as a white solid: mp >270 °C dec; ^{1}H NMR (400 MHz, NH_4OD) 3.22 (t, J = 7.0 Hz, 2H), 4.15 (t, J = 7.0 Hz, 2H), 7.75 (d, J = 8.0 Hz, 1H), 7.81 (d, J = 1.7 Hz, 1H), 7.98 (dd, J = 1.7, 8.0 Hz, 1H); IR (KBr) 3413, 3343, 3078, 2870, 1689, 1665, 1533, 1389, 1069 cm^{-1}; MS (ES$^-$) 222.4 (M − 1). Anal. ($C_{10}H_{13}N_3O_3\cdot 0.25H_2O$) C, H, N.

Methyl 4-[(Methylsulfoxy)methyl]-3-nitrobenzoate (170). To 100 mL of concentrated H_2SO_4 cooled to 0−5 °C was added 5.3 g (0.025 mol) of **55**. This suspension was treated in five portions over a 30-min period with 12.6 g (0.125 mol) of KNO_3. The mixture was stirred at 4 °C for 66 h and then poured in 1 L of ice H_2O. The mixture was extracted with five 150-mL portions of EtOAc, and the combined extracts were washed with 100 mL of 10% NH_4OH and 100 mL of brine, dried ($MgSO_4$), and concentrated. The residue was purified by flash column chromatography on silica gel (20−0% hexane in EtOAc) to give 2.7 g (42%) of **170** as a white solid: mp 129−130 °C (EtOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.67 (s, 3H), 3.99 (s, 3H), 4.13 (d, J = 12.6 Hz, 1H), 4.64 (d, J = 12.6 Hz, 1H), 7.63 (d, J = 8.0 Hz, 1H), 8.29 (dd, J = 8.0, 1.7 Hz, 1H), 8.77 (d, J = 1.7 Hz, 1H); IR (KBr) 3411, 3012, 2944, 1714, 1531, 1440, 1270 cm^{-1}; MS (ES$^-$) 256.4 (M − 1). Anal. ($C_{10}H_{11}NO_5$) C, H, N.

Methyl 3-Amino-4-[(methylsulfoxy)methyl]benzoate (171). A mixture of 0.63 g (0.0025 mol) of **170**, 0.12 g of PtO_2, and 25 mL of MeOH was hydrogenated at 50 psi for 1 h. The mixture was filtered through Celite, and the filtrate was concentrated. The residue was purified by flash column chromatography on silica gel (10−30% $CHCl_3$−MeOH−NH_4OH (80:18:2) in CH_2Cl_2) to give 0.2 g (35%) of **171**. An analytical sample, mp 161−162 °C, was obtained as a yellow solid by recrystallization from EtOH: ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.55 (s, 3H), 3.89 (d, J = 13.6 Hz, 1H), 3.90 (s, 3H), 4.19 (d, J = 13.6 Hz, 1H), 7.04 (d, J = 8.2 Hz, 1H), 7.40 (d, J = 1.7 Hz, 1H), 7.42 (s, 1H); IR (KBr) 3441, 3341, 3237, 1700, 1660, 1426; MS (ES$^+$) 228.4 (M + 1). Anal. ($C_{10}H_{13}NO_3S$) C, H, N.

Methyl 3-[[[(*tert*-Butoxycarbonyl)amino][(*tert*-butoxycarbonyl)imino]methyl]amino]-4-[(methylsulfoxy)methyl]benzoate (172). A mixture of 0.57 g (0.0025 mol) of **171**, 5 mL of DMF, 1.2 mL (0.009 mol) of Et_3N, and 0.8 g (0.003 mol) of *N*,*N*′-bis(*tert*-butoxycarbonyl)thiourea[41] at 0 °C was treated with 0.8 g (0.003 mol) of $HgCl_2$. The reaction mixture was stirred at 0 °C for 0.5 h and then at ambient temperature overnight. The mixture was diluted with 50 mL of EtOAc and filtered through Celite. The filtrate was washed successively with 10 mL of H_2O and 10 mL of brine, dried ($MgSO_4$), and concentrated. The residue was purified by flash column chromatography on silica gel (30% hexane in EtOAc) to give 0.63 g (54%) of **172** as a white solid: mp 140−141 °C (EtOAc−hexane); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.32 (s, 9H), 1.51 (s, 9H), 2.53 (s, 3H), 3.87 (s, 3H), 4.07 (d, J = 13.0 Hz, 1H), 4.31 (d,J = 13.0 Hz, 1H), 7.54 (d,J = 8.0 Hz, 1H), 7.87 (dd, J = 8.0, 1.7 Hz, 1H), 8.05 (d, J = 1.7 Hz, 1H), 9.95 (s, 1H), 11.46 (s, 1H); IR (KBr) 3421, 3262, 3122, 1728, 1648, 1591, 1404, 1307, 1232, 1130, 1060 cm^{-1}; MS (ES$^+$) 470.9 (M + 1). Anal. ($C_{21}H_{31}N_3O_7S$) C, H, N.

3-[(Aminoiminomethyl)amino]-4-[(methylsulfoxy)methyl]benzoic Acid (173). A solution of 0.4 g (0.0009 mol) of **172** in 8.5 mL of CH_2Cl_2 was treated dropwise with 1.6 mL (0.021 mol) of TFA and stirred at ambient temperature for 32 h. The solution was treated with 1.3 mL (0.017 mol) of TFA and stirred for 1 h. The mixture was concentrated, and the residue was chased twice with 10-mL portions of CH_2Cl_2. A mixture of the residue and 4.3 mL of 1 N NaOH was stirred at ambient temperature for 2 h. The mixture was filtered, and the filtrate volume was reduced to 2 mL. The pH was adjusted to 10−10.5 with 1 N HCl, and the resulting precipitate was collected by filtration and dried to give 0.13 g (59%) of **173** as a white solid: mp 211−213 °C; ^{1}H NMR (400 MHz, CF_3CO_2D) δ 2.95 (s, 3H), 4.36 (d, J = 13.8 Hz, 1H), 4.73 (d, J = 13.8 Hz, 1H), 7.76 (d, J = 8.1 Hz, 1H), 8.30−8.40 (m, 1H); IR (KBr) 3502, 3356, 3140, 1684, 1640, 1404, 1382, 1012; MS (ES$^+$) 256.2 (M + 1). Anal. ($C_{10}H_{13}N_3O_3S\cdot H_2O$) C, H, N.

Methyl 4-[(Methylsulfonyl)methyl]-3-nitrobenzoate (174). To 50 mL of 90% fuming HNO_3 at 0−5 °C was added portionwise over a 20-min period 4.2 g (0.019 mol) of **56**. The reaction mixture was stirred at 0−5 °C for 0.5 h and at ambient temperature overnight and then poured into 1 L of cold H_2O. The mixture was extracted with four 200-mL portions of EtOAc, and the combined extracts were washed successively with 100 mL of a saturated Na_2CO_3 solution and 200 mL of brine, dried ($MgSO_4$), and concentrated. The residue was purified by flash column chromatography on silica gel (25−0% hexane in Et_2O) to give 3.8 g (75%) of **174** as a light yellow solid: mp 114−115 °C; ^{1}H NMR (400 MHz) δ 3.04 (s, 3H), 3.93 (s, 3H), 5.08 (s, 2H), 7.84 (d, J = 8.0 Hz, 1H), 8.32 (dd, J = 8.0, 1.7 Hz, 1H), 8.49 (d, J = 1.7 Hz, 1H); IR (KBr) 3103, 3019, 2961, 2928, 1727, 1538, 1298, 1141 cm^{-1}; MS (ES$^-$) 272.7 (M − 1). Anal. ($C_{10}H_{11}NO_6S$) C, H, N.

A 1.3-g (25%) sample of the 2-nitro isomer **175** was also isolated as a light yellow solid: mp 115−117 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.99 (s, 3H), 3.33 (s, 3H), 3.87 (s, 2H), 7.86 (dd, J = 8.0, 1.5 Hz, 1H), 7.92 (d, J = 8.0 Hz, 1H), 8.13 (d, J = 1.4 Hz, 1H); IR (KBr) 3010, 1747, 1534, 1306, 1122 cm^{-1}; MS (ES$^-$) 272.1 (M − 1). Anal. ($C_{10}H_{11}NO_6S$) C, H, N.

Methyl 3-Amino-4-[(methylsulfonyl)methyl]benzoate (176). A mixture of 1.2 g (0.004 mol) of **174**, 0.2 g of PtO_2, and 50 mL of MeOH was hydrogenated at 50 psi for 1 h. The mixture was filtered through Celite, and the volume of the filtrate was reduced to 20 mL. The hot solution was slowly cooled to give 0.5 g (48%) of **176** as a tan solid: mp 151−153 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.93 (s, 3H), 3.81 (s, 3H), 4.47 (s, 2H), 5.56 (br s, 2H), 7.14 (dd, J = 7.9, 1.7 Hz, 1H), 7.22 (d, J = 7.9 Hz, 1H), 7.35 (d, J = 1.7 Hz, 1H); IR (KBr) 3447, 3364, 3014, 2929, 1705, 1580, 1435, 1289, 1252, 1133 cm^{-1}; MS (ES$^+$) 244.2 (M + 1). Anal. ($C_{10}H_{13}NO_4S$) C, H, N.

Methyl 3-[[[(*tert*-Butoxycarbonyl)amino][(*tert*-butoxycarbonyl)imino]methyl]amino]-4-[(methylsulfonyl)methyl]benzoate (177). A mixture of 2.3 g (0.0094 mol) of **176**, 20 mL of DMF, 4.6 mL of Et_3N (0.033 mol), and 2.9 g (0.01 mol) of *N*,*N*′-bis(*tert*-butoxycarbonyl)thiourea[41] stirred at 0 °C was treated with 2.8 g (0.01 mol) of $HgCl_2$. The mixture was stirred at 0 °C for 0.5 h and then at ambient temperature overnight. The mixture was diluted with 50 mL of EtOAc and filtered through Celite. The filtrate was washed thrice with 25-mL portions of H_2O and twice with 25-mL portions of brine, dried ($MgSO_4$), and concentrated. The residue was purified by flash column chromatography on silica gel (30−0% hexane

in Et_2O) to give 2.7 g (60%) of **177** as a white solid: mp 145−148 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.44 (s, 9H), 1.55 (s, 9H), 2.92 (s, 3H), 3.93 (s, 3H), 4.40 (s, 2H), 7.60 (d, J = 8.0 Hz, 1H), 7.94 (d, J = 8.0 Hz, 1H), 8.28 (s, 1H), 10.15 (s, 1H), 11.62 (s, 1H); IR (KBr) 3269, 3134, 2983, 1727, 1650, 1403, 1233, 1156, 1132 cm^{-1}; MS (ES$^+$) 486.7 (M + 1). Anal. ($C_{21}H_{31}N_3O_8S$) C, H, N.

3-[(Aminoiminomethyl)amino]-4-[(methylsulfonyl)-methyl]benzoic Acid (178). A solution of 0.49 g (0.001 mol) of **177** in 10 mL of CH_2Cl_2 was treated dropwise with 1.9 mL (0.025 mol) of TFA, and the mixture stirred at ambient temperature for 16 h. An additional 1.9 mL (0.025 mol) of TFA was added, and the mixture stirred for 1 h. The mixture was concentrated, and the residue was chased twice with 10-mL portions of CH_2Cl_2. A mixture of the residue and 5 mL of 1 N NaOH was stirred at ambient temperature for 1.5 h. The mixture was filtered, and the filtrate was concentrated to 1 mL. The pH was adjusted to 6 using HOAc. The resulting precipitate was collected by filtration, washed with a 1:1 solution of 2-PrOH−Et_2O, and dried to give 0.22 g (88%) of **178** as a white solid: mp 261−264 °C; ^{1}H NMR (400 MHz, CF_3CO_2D) δ 3.22 (s, 3H), 4.79 (s, 2H), 7.75 (d, J = 8.0 Hz, 1H), 7.25 (s, 1H), 8.29 (d, J = 8.0 Hz, 1H); IR (KBr) 3538, 3461, 3365, 3163, 1684, 1621, 1404, 1386, 1291, 1133 cm^{-1}; MS (ES$^-$) 269.9 (M − 1). Anal. ($C_{10}H_{13}N_3O_4S\cdot H_2O$) C, H, N.

3,5-Bis(cyanoamino)benzoic Acid (180). To a mixture of 3.8 g (0.025 mol) of 3,5-diaminobenzoic acid (**179**; Aldrich) and 4.1 g (0.05 mol) of NaOAc in 30 mL of a 1:1 solution of HOAc−H_2O at 0 °C was added 6.5 g (0.06 mol) of cyanogen bromide in two portions over a 10-min period. The reaction mixture was stirred at ambient temperature overnight and then poured onto 130 g of ice. The precipitate was collected by filtration, washed with H_2O, and dried. One recrystallization from MeOH gave 2.5 g (50%) of **180** as a gray solid: mp >300 °C dec; ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.80−7.18 (m, 3H), 10.68 (br s, 2H), 13.0 (br s, 1H); IR (KBr) 3173, 2229, 1737, 1617, 1519, 1453 cm^{-1}; MS (ES$^-$) 201 (M − 1). Anal. ($C_9H_6N_4O_2$) C, H, N.

Ethyl 3-Amino-5-[[[(*tert*-butoxycarbonyl)amino][(*tert*-butoxycarbonyl)imino]methyl]amino]benzoate (182). To a mixture of 1.8 g (0.01 mol) of ethyl 3,5-diaminobenzoate **(181**, Pfaltz & Bauer), 8 mL of DMF, 4.9 mL (0.035 mol) of Et_3N, and 2.8 g (0.01 mol) of *N*,*N*′-bis(*tert*-butoxycarbonyl)thiourea[41] stirred at 0 °C was added 3 g (0.011 mol) of $HgCl_2$. The mixture was stirred at 0 °C for 0.5 h and at ambient temperature for 24 h and diluted with 75 mL of EtOAc. The slurry was filtered through Celite; the filtrate was washed with four 15-mL portions of H_2O, dried (Na_2SO_4), and concentrated. The residue was purified by flash column chromatography on silica gel (10−20% EtOAc in hexane) to give 2.5 g (60%) of **182** as a white solid: mp 126−128 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.30 (t, J = 7.0 Hz, 3H), 1.40 (s, 9H), 1.50 (s, 9H), 4.25 (q, J = 7.0 Hz, 2H), 5.51 (s, 2H), 6.89 (s, 1H), 7.00 (s, 1H), 7.35 (s, 1H), 9.92 (s, 1H), 11.35 (s, 1H); IR (KBr) 3577, 3410, 3263, 2981, 1710, 1646 cm^{-1}; MS (ES$^+$) 423.6 (M + 1). Anal. ($C_{20}H_{30}N_4O_6$) C, H, N.

Sodium 3-Amino-5-[(aminoiminomethyl)amino]benzoate (183). To a solution of 1.8 g (0.0043 mol) of **182** in 20 mL of CH_2Cl_2 at 0 °C was added dropwise 3.3 mL (0.043 mol) of TFA, and the solution stirred at ambient temperature for 6 h. An additional 3.3 mL (0.043 mol) of TFA was added, and the mixture stirred overnight. The mixture was concentrated, and the residue was chased twice with 20-mL portions of CH_2Cl_2. A mixture of the residue and 22.5 mL (0.068 mol) of 3 N NaOH was stirred at ambient temperature for 0.75 h, and then the pH was adjusted to 7−8 with 1 N HCl. The solution was filtered, and the filtrate was concentrated to one-half its volume. The filtrate was allowed to stand at ambient temperature for 2 h, and the resulting precipitate was collected by filtration, washed successively with a small amount of MeOH and Et_2O, and dried to give 0.66 g (66%) of **183** as a white solid: mp 198−203 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.2 (s, 2H), 6.45 (s, 1H), 6.9 (s, 1H), 7.05 (s, 1H), 8.05 (br s, 4H), 12.1 (br s, 1H); IR (KBr) 3362, 3216, 3006, 1677, 1629, 1599, 1543, 1395, 1201 cm^{-1}; MS (ES$^+$) 195.3 (M + 1). Anal. ($C_8H_9N_4NaO_2\cdot H_2O$) C, H, N.

3,5-Bis[(aminoiminomethyl)amino]benzoic Acid (184). A mixture of 2.3 g (0.01 mol) of 3,5-diaminobenzoic acid dihydrochloride semihydrate (**179**; Aldrich) and 2.5 g (0.06 mol) of cyanamide in 50 mL of absolute EtOH was heated at reflux for 8 h. The mixture was cooled, the precipitate collected by filtration, and the cake washed with several portions of EtOH and dried to give 2.2 g (73%) of **184** as an off-white powder: mp 280 °C dec; ^{1}H NMR (400 MHz, D_2O) δ 1.20 (t, J = 7.0 Hz, 0.6H), 3.67 (q, J = 7.0 Hz, 0.4H), 7.42 and 7.72 (aromatic protons, 3H); IR (KBr) 3320, 3160, 1672, 1560, 1379 cm^{-1}; MS (ES$^+$) 237 (M + 1). Anal. ($C_9H_{12}N_6O_2\cdot 1.5HCl\cdot 0.2EtOH$) C, H, N.

Methyl 3-Formyl-5-nitrobenzoate (186) and Methyl 3-(Hydroxymethyl)-5-nitrobenzoate (187). To a solution of 1.5 g (0.02 mol) of DMF in 30 mL of CH_2Cl_2 at 0 °C was added 5 mL (0.057 mol) of oxalyl chloride, and the mixture stirred at ice bath temperature for 1 h. The solution was concentrated, and the residue was suspended in 50 mL of THF and 30 mL of MeCN. To this mixture at −30 °C was added dropwise a solution of 4.5 g (0.02 mol) of 3-(methoxycarbonyl)-5-nitrobenzoic acid (**185**)[51] and 1.6 g (0.02 mol) of pyridine in 10 mL of THF over a 10-min period. The reaction mixture was stirred at −30 °C for 1 h and then treated with a suspension of 0.38 g (0.002 mol) of CuI in 10 mL of THF. The reaction mixture was cooled to −70 °C, and 40 mL (0.04 mol) of a 1 M solution in THF of lithium tri-*tert*-butoxyaluminohydride (Aldrich) was added dropwise over a 10-min period. The mixture was stirred for an additional 10 min at −78 °C and then quenched with 50 mL of 2 N HCl. The layers were separated, and the organic layer was washed with a $NaHCO_3$ solution, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography on silica gel (33% EtOAc in hexane) to give 0.25 g (6%) of **186** as an off-white solid: mp 85 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.05 (s, 3H), 8.86 (d, J = 1.5 Hz, 1H), 8.9 (dd, J = 2.2, 1.5 Hz, 1H), 9.09 (dd, J = 2.2, 1.6 Hz, 1H), 8.19 (s, 1H); IR (KBr) 3076, 2962, 2872, 1736, 1703, 1616, 1540, 1355, 1295, 1206, 1184 cm^{-1}; MS (ES$^-$) 209.9 (M − 1, 5). Anal. ($C_9H_7NO_5$) C, H, N.

Other fractions when combined and concentrated gave 2.3 g (55%) of **187** as an off-white solid: mp 77 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.4 (br s, 1H), 3.99 (s, 3H), 4.88 (s, 2H), 8.35 (m, 1H), 8.44 (m, 1H), 8.76 (m, 1H); IR (KBr) 3497, 3096, 2961, 1722, 1528, 1355, 1300, 1216, 1062, 738 cm^{-1}; MS (ES$^-$) (no ionization). Anal. ($C_9H_9NO_5$) C, H, N.

Another run using the exact procedure as above except that only 20 mL (0.02 mol) of a 1 M solution in THF of lithium tri-*tert*-butoxyaluminohydride was added gave 1.4 g (33%) of **186** and 0.8 g (19%) of **187**.

Methyl 3-[(*N*-Hydroxylimino)methyl]-5-nitrobenzoate (188). A mixture of 0.42 g (0.002 mol) of **186** and 0.21 g (0.003 mol) of hydroxylamine hydrochloride in 5 mL of EtOH was heated at reflux for 2 h. The mixture was poured into H_2O, and the resulting precipitate was collected by filtration. The cake was recrystallized from EtOAc−hexane to give 0.22 g (49%) of **188** as an off-white solid: mp 144−145 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.94 (s, 3H), 8.43 (s, 1H), 8.56 (m, 2H), 8.65 (m, 1H), 11.84 (s, 1H); IR (KBr) 3406, 3105, 3067, 2960, 1705, 1531, 1305, 1276, 1221, 978 cm^{-1}; MS (ES$^-$) 223.4 (M − 1). Anal. ($C_9H_8N_2O_5$) C, H, N.

Methyl 3-Amino-5-(aminomethyl)benzoate (189). A mixture of 0.1 g (0.0005 mol) of **188**, 0.02 g of 10% Pd/C, 30 mL of EtOH, and 0.5 mL of concentrated HCl was hydrogenated at 40 psi for 2 h. The mixture was filtered through Celite and the filtrate concentrated. The residue was recrystallized from EtOH−toluene−H_2O to give 0.09 g (76%) of **189** as a white solid: mp 224−226 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 3.85 (s, 3H), 4.01 (m, 2H), 7.30 (s, 1H), 7.58 (s, 1H), 7.69 (s, 1H), 8.47 (br s, 4H); IR (KBr) 3440, 3024, 2577, 1725, 1314, 1228 cm^{-1}; MS (ES$^+$) 181.5 (M + 1). Anal. ($C_9H_{12}N_2O_2\cdot 2HCl\cdot 0.75H_2O$) C, H, N.

Methyl 3-Amino-5-[[[[(*tert*-butoxycarbonyl)amino][(*tert*-butoxycarbonyl)imino]methyl]amino]methyl]benzoate (190). A mixture of 0.76 g (0.003 mol) of **189**, 6 mL of DMF, 2.5 mL (0.018 mol) of Et_3N, and 2.1 g (0.008 mol) of *N*,*N*′-bis(*tert*-butoxycarbonyl)thiourea[41] at 0 °C was treated with 2 g (0.008 mol) of $HgCl_2$. The reaction mixture was

stirred at 0 °C for 0.5 h and at ambient temperature overnight and then diluted with 50 mL of EtOAc. The slurry was filtered through Celite and the filtrate washed successively thrice with 25-mL portions of H_2O and twice with 25-mL portions of brine. The organic layer was dried ($MgSO_4$) and concentrated, and the residue was purified by flash column chromatography on silica gel (30% EtOAc in hexane) to give 0.3 g (25%) of **190** as a white solid. An analytical sample, mp 150 °C, was recrystallized from Et_2O−hexane: ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.39 (s, 9H), 1.48 (s, 9H), 3.80 (s, 3H), 4.40 (d, J = 5.7 Hz, 2H), 5.45 (s, 2H), 6.69 (s, 1H), 7.03 (s, 1H), 7.08 (t, J = 1.8 Hz, 1H), 8.63 (s, 1H), 11.55 (s, 1H); IR (KBr) 3461, 3335, 2983, 1732, 1713, 1648, 1599, 1309, 1229, 1164, 1140 cm^{-1}; MS (ES$^+$) 423.4 (M + 1). Anal. ($C_{20}H_{30}N_4O_6$) C, H, N.

3-Amino-5-[[(aminoiminomethyl)amino]methyl]benzoic Acid (191). A solution of 0.12 g (0.0003 mol) of **190** in 2.8 mL of 6 N HCl was heated at reflux for 1 h. The mixture was concentrated, and the residue was recrystallized from EtOH−Et_2O to give 0.04 g (69%) of **191** as a tan solid: mp 245−247 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.42 (d, J = 6.3 Hz, 2H), 7.14 (s, 1H), 7.35 (br s, 5H), 7.51 (d, J = 6.0 Hz, 1H), 8.19 (t, J = 6.3 Hz, 1H), 13.0 (br s, 1H); IR (KBr) 8415, 3208, 3162, 3056, 2792, 1685, 1644, 1601, 1237; MS (ES$^+$) 209.3 (M + 1). Anal. ($C_9H_{12}N_4O_2$·2HCl) C, H, N.

Methyl 3-[[[(*tert*-Butoxycarbonyl)amino][(*tert*-butoxycarbonyl)imino]methyl]amino]-5-[[[[(*tert*-butoxycarbonyl)amino][(*tert*-butoxycarbonyl)imino]methyl]amino]methyl]benzoate (192). A mixture of 1.2 g (0.0045 mol) of **189**, 15 mL of DMF, 3.2 mL (0.023 mol) of Et_3N, and 2.5 g (0.009 mol) of *N*,*N*′-bis(*tert*-butoxycarbonyl)thiourea[41] at 0 °C was treated with 2.4 g (0.009 mol) of $HgCl_2$. The reaction mixture was stirred at ambient temperature for 16 h, diluted with 150 mL of EtOAc, and filtered through Celite. The filtrate was washed with 60 mL of H_2O, dried (Na_2SO_4), and concentrated. The residue was dissolved in 150 mL of a 7:3 hexane−EtOAc solution and filtered through a 1″ × 2″ silica gel plug. The filtrate was concentrated, and the residue was recrystallized from EtOAc−hexane to give 1.9 g (61%) of **192** as a white solid: mp 114−118 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.51 (m, 36H), 3.91 (s, 3H), 4.67 (d, J = 5.34, 2H), 7.75 (s, 1H), 7.87 (s, 1H), 8.13 (s, 1H), 8.60 (s, 1H), 10.42 (s, 1H), 11.52 (s, 1H), 11.61 (s, 1H); IR (KBr) 2982, 1726, 1643, 1320, 1233, 1155 cm^{-1}; MS (ES$^+$) 664.9 (M + 1). Anal. ($C_{31}H_{48}N_6O_{10}$·0.75H_2O) C, H, N.

3-[(Aminoiminomethyl)amino]-5-[[(aminoiminomethyl)amino]methyl]benzoic Acid (193). A solution of 0.8 g (0.0012 mol) of **192** in 12 mL of 6 N HCl was heated at reflux for 1 h. The mixture was concentrated, and the resulting oil was crystallized from 2-PrOH−H_2O to give 0.09 g (23%) of **193** as a white solid: mp >300 °C dec; ^{1}H NMR (400 MHz, CF_3CO_2D) δ 4.72 (s, 2H), 7.73 (s, 1H), 8.15 (s, 1H), 8.24 (s, 1H); IR (KBr) 3206, 1691, 1938, 1604, 1388 cm^{-1}; MS (ES$^+$) 251.5 (M + 1). Anal. ($C_{10}H_{14}N_6O_2$·HCl·0.75H_2O) C, H, N.

Methyl 3-Amino-5-[(*N*-hydroxylimino)methyl]benzoate (194). A mixture of 2.0 g (0.009 mol) of **188**, 0.25 g of PtO_2, and 150 mL of EtOH was hydrogenated at 30 psi for 0.5 h. The mixture was filtered through Celite, the filtrate was concentrated, and the residue was recrystallized from EtOAc−hexane to give 1.1 g (64%) of **194** as an off-white solid: mp 160−162 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.81 (s, 3H), 5.53 (s, 2H), 7.04 (m, 1H), 7.19 (m, 1H), 7.29 (t, J = 1.4 Hz, 1H), 8.04 (s, 1H), 11.20 (s, 1H); IR (KBr) 3324, 3625, 2957, 3885, 7714, 1603, 1441, 1345, 1239 cm^{-1}; MS (ES$^+$) 194.8 (M + 1). Anal. ($C_9H_{10}N_2O_3$) C, H, N.

Methyl 3-[[[(*tert*-Butoxycarbonyl)amino][(*tert*-butoxycarbonyl)imino]methyl]amino]-5-[(*N*-hydroxyimino)methyl]benzoate (195). A mixture of 0.97 g (0.005 mol) of 194, 10 mL of DMF, 1.8 g (0.018 mol) of Et_3N, and 1.5 g (0.0055 mol) of *N*,*N*-bis(*tert*-butoxycarbonyl)thiourea[41] at 5 °C was treated with 1.5 g (0.0055 mol) of $HgCl_2$. The mixture was stirred at 5 °C for 0.5 h and at ambient temperature for 18 h and then diluted with 40 mL of EtOAc. The mixture was filtered through Celite, and the filtrate was washed with H_2O, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography on silica gel (25−50% EtOAc in hexane) to give 0.7 g (30%) of 195 as a white solid: mp 158−160 °C (EtOAc−hexane); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.40 (s, 9H), 1.51 (s, 9H), 3.87 (s, 3H), 7.92 (s, 1H), 7.96 (s, 1H), 8.22 (s, 2H), 10.10 (s, 1H), 11.27 (s, 1H), 11.48 (s, 1H); IR (KBr) 3455, 3245, 3134, 2983, 1730, 1650, 1636, 1577, 1397, 1320, 1230, 1151, 1104, 769 cm^{-1}; MS (ES$^+$) 436.9 (M + 1, 40). Anal. ($C_{20}H_{28}N_4O_7$) C, H, N.

Other fractions when combined and concentrated gave 0.1 g (11%) of methyl 3-amino-5-cyanobenzoate (**196**) as a yellow solid byproduct: mp 121 °C (EtOAc−hexane); ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.84 (s, 3H), 5.97 (s, 2H), 7.09 (dd, J = 2.3, 1.5 Hz, 1H), 7.35 (dd, J = 2.0, 1.5 Hz, 1H), 7.45 (dd, J = 2.3, 1.5 Hz, 1H); IR (KBr) 3456, 3365, 3251, 2235, 1790, 1704, 1600, 1455, 1440, 1265, 1256, 1158, 770 cm^{-1}; MS (ES$^-$) 174.9 (M − 1). Anal. ($C_9H_8N_2O_2$) C, H, N.

3-[(Aminoiminomethyl)amino]-5-[(*N*-hydroxyimino)methyl]benzoic Acid (197). A mixture of 0.36 g (0.0008 mol) of **195** and 1.5 mL of TFA in 5 mL of CH_2Cl_2 was stirred at ambient temperature for 24 h. The mixture was concentrated, and the residue was chased repeatedly with CH_2Cl_2. A mixture of the residue and 3 mL of 1 N NaOH was stirred at ambient temperature for 6 h. The mixture was filtered, and the filtrate pH was adjusted to 8 with 1 N HCl. The resulting precipitate was collected by filtration, washed with H_2O, and dried to give 0.125 g (64%) of **197** as an off-white solid: mp 248−253 °C dec; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.56 (m, 1H), 7.72 (m, 1H), 7.95 (s, 1H), 8.17 (s, 1H), 8.20 (br s, 4H), 11.29 (s, 1H), 13.01 (s, 1H); IR (KBr) 3339, 3181, 2985, 2364, 2338, 1681, 1653, 1585, 1372, 1328 cm^{-1}; MS (ES$^+$) 222.9 (M + 1). Anal. ($C_9H_{10}N_4O_3$·0.75H_2O) C, H, N.

Methyl 3-Amino-5-(hydroxymethyl)benzoate (198). A mixture of 1.9 g (0.009 mol) of **187,** 0.1 g of PtO_2, and 100 mL of MeOH was hydrogenated at 50 psi for 0.5 h. The mixture was filtered through Celite and the filtrate immediately acidified with concentrated HCl. The mixture was concentrated, and the residue was recrystallized from MeOH−toluene to give 1.3 g (78%) of **198** as a white solid: mp 203−205 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.87 (s, 3H), 4.57 (s, 2H), 7.47 (m, 1H), 7.71 (m, 1H), 7.79 (m, 1H); IR (KBr) 3193, 2860, 2648, 2623, 1710, 1608, 1542, 1328, 1233, 1056 cm^{-1}; MS (ES$^+$) 182.4 (M + 1). Anal. ($C_8H_{11}NO_3$·HCl) C, H, N.

Methyl 3-[[[(*tert*-Butoxycarbonyl)amino][(*tert*-butoxycarbonyl)imino]methyl]amino]-5-(hydroxylmethyl)benzoate (199). A mixture of 2.9 g (0.013 mol) of **198**, 15 mL of DMF, 13 mL (0. 11 mol) of Et_3N, and 4 g (0.016) of *N*,*N*′-bis(*tert*-butoxycarbonyl)thiourea[41] at 0−5 °C was treated with 3.6 g (0.013 mol) of $HgCl_2$. The reaction mixture was stirred at ambient temperature for 16 h and then diluted with 250 mL of EtOAc. The mixture was filtered through Celite; the filtrate was washed with 60 mL of H_2O and concentrated. The residue was purified by column chromatography on silica gel packed in $CHCl_3$ (5% MeOH in $CHCl_3$) to give 4.9 g (86%) of **199** as a cream-colored solid: mp 80−83 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.51−1.54 (m, 18H), 3.91 (s, 3H), 4.74 (s, 2H), 7.79 (m, 1H), 8.02 (m, 3H), 10.48 (m, 1H), 11.53 (2s, 1H); IR (KBr) 3434, 2982, 1727, 1646, 1321, 1233, 1152, 1107 cm^{-1}; MS (ES$^-$) 422.7 (M − 1). Anal. ($C_{20}H_{29}N_3O_7$) C, H, N.

Methyl 3-[[[(*tert*-Butoxycarbonyl)amino][(*tert*-butoxycarbonyl)imino]methyl]amino]-5-formylbenzoate (200). A mixture of 2.5 g (0.006 mol) of **199** and 1.5 g (0.007 mol) of pyridinium chlorochromate (Aldrich) in 25 mL of CH_2Cl_2 was stirred at ambient temperature for 16 h. The mixture was filtered through a plug of Florisil, diluted to 150 mL with $CHCl_3$, and filtered through a plug of silica gel. The filtrate was concentrated, and the residue was recrystallized from EtOH−$CHCl_3$ to give 2.1 g (82%) of **200** as a cream-colored solid: mp 138−141 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.52 (s, 9H), 1.55 (s, 9H), 3.96 (s, 3H), 8.28 (s, 1H), 8.41 (s, 1H), 8.54 (s, 1H), 10.05 (s, 1H), 10.64 (s, 1H), 11.61 (s, 1H); IR (KBr) 2982, 1647, 1318, 1149 cm^{-1}; MS (ES$^+$) 422.3 (M + 1). Anal. ($C_{20}H_{27}N_3O_7$) C, H, N.

Methyl 3-[[[(*tert*-Butoxycarbonyl)amino][(*tert*-butoxycarbonyl)imino]methyl]amino]-5-(1-hydroxy-2-nitroethyl)benzoate (201). To a stirred suspension of 0.28 g (0.011 mol) of 95% NaH in 8.0 mL of DMF under a N_2 atmosphere at 0 °C was added 5.3 mL (0.088 mol) of $MeNO_2$. Stirring was continued at 0 °C followed by the addition of 3.7 g (0.009 mol)

of **200**. The reaction mixture was stirred at 0 °C for 0.5 h, acidified with HOAc, and poured into 250 mL of H_2O. The aqueous layer was extracted thrice with 75-mL portions of EtOAc. The combined organic layers were washed with 75 mL of brine, dried ($MgSO_4$), and concentrated to give 4.4 g (quantitative) of **201** as a white solid. An analytical sample, mp 146−147 °C, was recrystallized from EtOH: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.51 (s, 9H), 1.55 (s, 9H), 3.28 (br s, 1H), 3.93 (s, 3H), 4.58 (m, 2H), 5.52 (dd, J = 9.1, 3.3 Hz, 1H), 7.82 (s, 1H), 8.05 (s, 1H), 8.14 (s, 1H), 10.52 (s, 1H), 11.60 (br s, 1H); IR (KBr) 3534, 3276, 3156, 2990, 1721, 1652, 1560, 1234, 1154 cm^{-1}; MS (ES$^-$) 481.5 (M − 1). Anal. ($C_{21}H_{30}N_4O_9$) C, H, N.

Methyl 3-[[[(*tert*-Butoxycarbonyl)amino][(*tert*-butoxycarbonyl)imino]methyl]amino]-5-(2-nitroethyl)benzoate (202). To a solution of 3.7 g (0.0086 mol) of **201** in 10 mL of EtOH at 0 °C was added dropwise over a 10-min period a solution of 1 g (0.026 mol) of $NaBH_4$ in 20 mL of EtOH. The solution was stirred at 0 °C for 1 h and then the pH adjusted to 5−6 with a 20% HOAc solution. The solution was diluted with 20 mL of H_2O, and then the mixture was saturated with solid NaCl. The mixture was extracted thrice with 25-mL portions of EtOAc, and the combined extracts were dried ($MgSO_4$) and concentrated. The residue was purified by flash column chromatography on silica gel (25−35% Et_2O in hexane). The appropriate fractions were combined and concentrated to give 1 g (26%) of **202** as a white solid: mp 167−169 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.5 (s, 9H), 1.6 (s, 9H), 3.40 (t, J = 7.0 Hz, 2H), 3.90 (s, 3H), 4.70 (t, J = 7.0 Hz, 2H), 7.65 (s, 1H), 7.85 (s, 1H), 8.15 (s, 1H), 10.4 (s, 1H), 11.6 (s, 1H); IR (KBr) 2978, 1726, 1649, 1158, 1111 cm^{-1}; MS (ES$^+$) 467.4 (M + 1). Anal. ($C_{21}H_{30}N_4O_8$) C, H, N.

3-(2-Aminoethyl)-5-[(aminoiminomethyl)amino]benzoic Acid (204). A mixture of 0.23 g (0.0005 mol) of **202**, 0.06 g of PtO_2, and 10 mL of MeOH was hydrogenated at 50 psi for 2 h. The mixture was filtered through Celite, and the filtrate was concentrated. The residue was purified by flash column chromatography on silica gel (10−20% $CHCl_3$−MeOH−concentrated NH_4OH (80:18:2) in CH_2Cl_2) to give 0.11 g (51%) of methyl 3-(2-aminoethyl)-5-[[[(*tert*-butoxycarbonyl)amino][(*tert*-butoxycarbonyl)imino]methyl]amino]benzoate (**203**).

A solution of 0.11 g (0.000 25 mol) of the above **203** in 2 mL of 5 N HCl was heated at reflux for 1 h. The mixture was concentrated, and the residue was successively triturated with four portions of Et_2O and four portions of EtOAc and dried to give 0.03 g (36%) of **204** as a tan, highly hygroscopic solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.99 (t, J = 6.2 Hz, 2H), 3.08 (t, J = 6.2 Hz, 2H), 7.43 (s, 1H), 7.6 (s, 1H), 7.72 (br s, 3H), 7.74 (s, 1H), 8.16 (br s, 3H), 10.36 (br s, 1H); IR (KBr) 3435, 1673, 1631, 1595 cm^{-1}; MS (ES$^+$) 223.3 (M + 1). Anal. ($C_{10}H_{14}N_4O_2$·2HCl·H_2O·0.25EtOAc) C, H, N.

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