Design, Synthesis, and Structure−Activity Relationships of Novel Insulin Receptor Tyrosine Kinase Activators

Robert T. Lum,[†] Mingshan Cheng,[†] Cristina P. Cristobal,[†] Ira D. Goldfine,[§] Joseph L. Evans,*[,#] James G. Keck,[†] Robert W. Macsata,[†] Vara Prasad Manchem,[†] Yukiharu Matsumoto,[‡] Sophia J. Park,[†] Sandhya S. Rao,[†] Louise Robinson,[†] Songyuan Shi,[†] Wayne R. Spevak,[†] and Steven R. Schow[†]

Telik, Inc., 3165 Porter Drive, Palo Alto, California 94304, University of California at San Francisco, Mount Zion Medical Center, San Francisco, California 94115, ReceptorBio, Inc., 1155 Chess Drive, Suite 116, Foster City, California 95051, and Sanwa Kaguku Kenkyusho Co. Ltd., Nagoya 461-8631, Japan

Received May 20, 2008

A novel series of symmetrical ureas of [(7-amino(2-naphthyl))sulfonyl]phenylamines were designed, synthesized, and tested for their ability to increase glucose transport in mouse 3T3-L1 adipocytes, a surrogate readout for activation of the insulin receptor (IR) tyrosine kinase (IRTK). A structure−activity relationship was established that indicated glucose transport activity was dependent on the presence of two acidic functionalities, two sulfonamide linkages, and a central urea or 2-imidazolidinone core. Compound **30** was identified as a potent and selective IRTK activator. At low concentrations, **30** was able to increase the tyrosine phosphorylation of the IR stimulated by submaximal insulin. At higher concentrations, **30** was able to increase tyrosine the phosphorylation levels of the IR in the absence of insulin. When administered intraperitoneally (ip) and orally (po), **30** improved glucose tolerance in hypoinsulinemic, streptozotocin-treated rats. These data provide pharmacological validation that small molecule IRTK activators represent a potential new class of antidiabetic agents.

Introduction

Diabetes mellitus is a serious and growing worldwide medical problem. In 2000, 171 million people were estimated to be affected by the disease, and that number is expected to more than double to 366 million by 2030.[1,2] Two major clinically distinct types of diabetes have been described. Type 1 diabetes is caused by an autoimmune-mediated destruction of the insulin producing β cells of the pancreas leading to an absolute insulin deficiency. It is managed by administering exogenous insulin. Type 2 diabetes is, by far, the most common form of diabetes and accounts for greater than 90% of all cases. This form of the disease is characterized by insulin resistance, which is a reduced sensitivity of the target tissues (liver, skeletal muscle, adipose) to insulin, along with abnormal secretion of insulin.[3] Initially, the body is able to compensate for insulin resistance by secreting more insulin, and this state of hyperinsulinemia is often an early indicator of the disease.[4,5] Eventually, the pancreas will be unable to secrete enough insulin to maintain glucose homeostasis, and consequently oral antidiabetic agents will be needed.[6]

Current oral antidiabetic agents exert their glucose lowering effects by five main modes of action.[7] The sulfonylureas and meglitinides stimulate insulin secretion by the pancreatic β cells.[8,9] Metformin, a biguanide, decreases hepatic glucose production,[10] although it is likely that its ability to stimulate AMP-activated protein kinase also contributes to its antihyperglycemic activity.[11] The α-glucosidase inhibitors reduce gastrointestinal absorption of carbohydrates.[12] The thiazolidinediones increase glucose utilization in muscle and liver.[13] Activation of the peroxisome proliferator-activated receptor-γ (PPARγ[a]) in adipose tissue by this class of drugs directs changes in adipose gene expression including the increased production of adiponectin, which in turn elicits insulin sensitizing effects in the target tissues.[14,15] Mostly recently, inhibitors of dipeptidyl peptidase IV (DPP-IV), a promiscuous enzyme responsible for the abbreviated plasma half-life of glucagon-like peptide-1 (GLP-1), have been developed.[16] These compounds serve to elevate circulating GLP-1 levels, enhance insulin secretion, and improve glycemic control. Unfortunately, these agents do not work in all patients, and some have potentially serious side effects.[7,17−19] For example, the sulfonylureas, meglitinides, and thiazolidinediones all have a tendency to cause weight gain. This is undesirable in a population already at risk for cardiovascular disease.[20,21] In addition, the U.S. Food and Drug Administration removed the thiazolidinedione, troglitazone, from the market because of its liver toxicity. Such problems illustrate the need to develop new treatments for diabetes with novel mechanisms of action.

The insulin receptor (IR) is a large transmembrane glycoprotein found in insulin sensitive target cells (liver, muscle, and fat).[22] It comprises two extracellular α-subunits that contain the insulin-binding domain and two membrane spanning β-subunits that contain a ligand activated tyrosine kinase, which will be referred to as the insulin receptor tyrosine kinase (IRTK). Insulin acts by binding to the extracellular domain of the IR, thus inducing autophosphorylation and activation of the IRTK. A cascade of signaling events is initiated leading to increased

* To whom correspondence should be addressed. Phone: 650-255-5806. Fax: 309-406-8212. E-mail: jevansphd@earthlink.net.
[†] Telik, Inc.
[§] University of California at San Francisco.
[#] ReceptorBio, Inc.
[‡] Sanwa Kaguku Kenkyusho Co. Ltd.

[a] Abbreviations: CKD, cytoplasmic kinase domain; DPP-IV, dipeptidyl peptidase IV; EGFR, epidermal growth factor receptor; GLP-1, glucagon-like peptide-1; GLUT4, insulin-sensitive glucose transporter; HIV, human immunodeficiency virus; IGF-1R, insulin-like growth factor-1 receptor; IR, insulin receptor; IRS-1/2, insulin receptor substrates 1 and 2; IRTK, insulin receptor tyrosine kinase; PI 3-kinase, phosphatidylinositol 3-kinase; PPARγ, peroxisome proliferator-activated receptor-γ; SAR, structure−activity relationship; SEM, standard error of the mean; STZ, streptozotocin; TK, tyrosine kinase; TRAP, target-related affinity profiling.

10.1021/jm800600v CCC: $40.75
Published on Web 09/13/2008

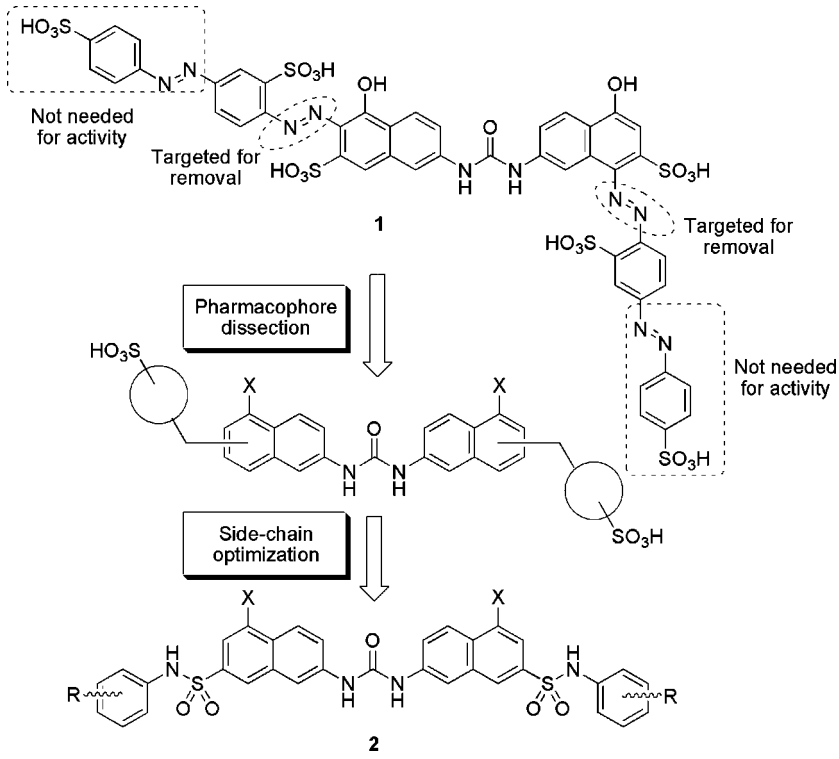


Figure 1. Summary of changes made to compound **1**.

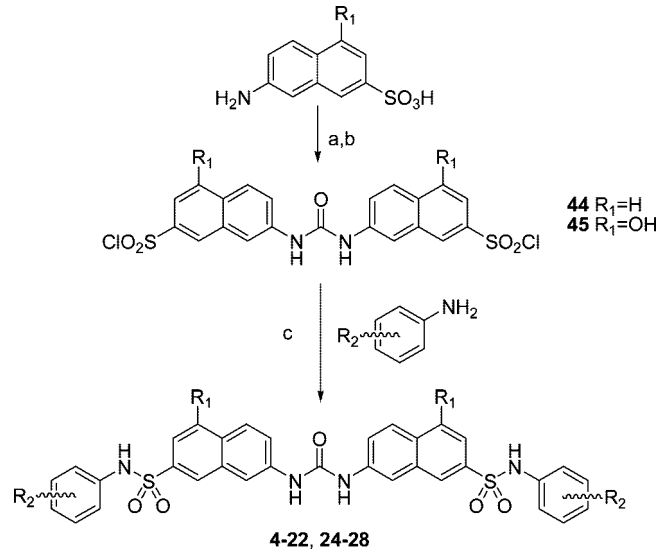
tyrosine phosphorylation of multiple intracellular substrates, including the insulin receptor substrates 1 and 2 (IRS-1/2), and the activation of second messenger systems such as phosphatidylinositol 3-kinase (PI 3-kinase).[23] These pathways act to trigger the translocation of GLUT4 to the cell surface.[24] GLUT4 is one of a family of membrane proteins responsible for glucose uptake in mammalian cells and is the major isoform responsive to insulin stimulation.[25]

Given the essential and early role the IR plays in insulin action, it represents an important and unexploited target for drug development. A family of quinones has been reported as the first selective activators of the IRTK.[26−29] These compounds exhibit antihyperglycemic activity in several animal models of type 2 diabetes[26,27,29,30] and thus provide pharmacological validation that small molecule IRTK activators represent a potential new class of antidiabetic agents. By use of our proprietary drug discovery target-related affinity profiling (TRAP) technology,[31] the azonaphthylurea **1** (TLK16998) was discovered. This compound functions as an IR sensitizer both in vitro and in vivo.[32] Compound **1** increases tyrosine phosphorylation of the IRTK, with subsequent enhancement of downstream signaling events including IRS-1 phosphorylation and GLUT4 translocation, resulting increased in cellular glucose transport. Compound **1** is also efficacious at lowering glucose in two mouse models of type 2 diabetes.[32] Compound **1** is also effective at increasing IRTK in several cellular models of impaired insulin receptor function.[33] A novel series of IRTK activators that are exemplified by general structure **2** (Figure 1) were designed using the azonaphthyl urea **1** as a starting point. The design, synthesis, and SAR of this series of novel antidiabetic agents are reported here.

Chemistry

The naphthylureas, as exemplified by **2**, were prepared by one of two general methods. Method A (Scheme 1) represents the most direct approach and was used for the majority of compounds that were prepared. Reaction of the 7-amino-2-naphthalenesulfonic acids with triphosgene afforded the corresponding ureas, which were converted to the bis-sulfonyl chlorides **44** and **45** on treatment with phosphorus oxychloride. Coupling of these intermediates with the appropriate aniline furnished, after any necessary deprotection steps, the sulfonamides **4**−**22** and **24**−**28** (Table 1). Incomplete reaction at the final stage provided the monoaddition products **31** and **32** (Table 2), which were readily separable from their symmetrical analogues.

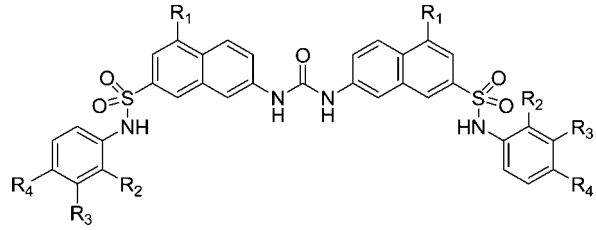
Scheme 1[a]



[a] (a) triphosgene, THF/aqueous NaOH; (b) $POCl_3$; (c) pyridine, THF.

Method B, although requiring an additional synthetic step, was more convenient on a large scale (Scheme 2). In this procedure, the 7-amino-2-naphthalenesulfonic acids were acetylated prior to formation of the sulfonyl chlorides **46** and **47**.

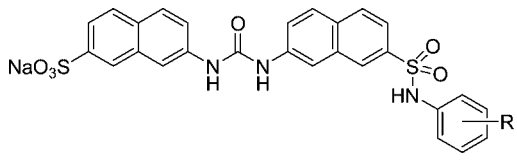
Table 1. Glucose Uptake Data and Experimental Methods for Ureas **3**−**30**[a]



compd	R_1	R_2	R_3	R_4	method	EC_{50} (μM)	no. of assays	*P*
1						90	1	
3	OH	H	SO_3Na	H	B	26 ± 5.6	6	<0.001 vs **1**
4	H	H	SO_3Na	H	A	22	2	ns vs **3**
5	H	H	H	H	A	>200	1	inactive compound
6	H	H	NO_2	H	A	>200	1	inactive compound
7	H	H	CN	H	A	>200	1	inactive compound
8	OH	H	CF_3	H	A	>200	1	inactive compound
9	OH	H	CO_2H	H	A	43	1	<0.05 vs **3**
10	H	H	CO_2H	H	A	24	1	ns vs **4**
11	OH	H	tetrazole	H	A	73	2	<0.012 vs **3**
12	H	H	tetrazole	H	A	54	2	<0.05 vs **4**
13	OH	H	CH_2OH	H	A	>200	1	inactive compound
14	H	H	OH	H	A	>200	1	inactive compound
15	H	H	SO_2NH_2	H	A	>200	1	inactive compound
16	OH	H	CH_2CO_2H	H	A	>200	1	inactive compound
17	H	H	H	SO_3Na	A	94	1	<0.01 vs **4**
18	OH	H	H	CO_2H	A	103	2	<0.001 vs **9**
19	OH	CO_2H	H	H	A	93	2	<0.001 vs **9**
20	H	H	H	CO_2H	A	183	1	<0.001 vs **10**
21	H	CO_2H	H	H	A	41	1	<0.001 vs 10
22	OH	H	CO_2H	Cl	A	18 ± 8.7	3	<0.001 vs **9**
23	H	H	CO_2H	Cl	B	16	2	<0.001 vs **10**
24	OH	H	CO_2H	OH	A	43	2	ns vs **9**
25	H	H	CO_2H	OH	A	9	2	<0.001 vs **10**
26	H	H	CO_2H	NO_2	A	40	1	ns vs **10**
27	H	OH	CO_2H	H	A	150	1	<0.001 vs **9**
28	OH	Cl	CO_2H	H	A	121	1	<0.05 vs **22**
29	H	H	SO_3Na	Cl	B	6 ± 1.0	4	<0.015 vs **4**; <0.05 vs **23**
30	OH	H	SO_3Na	Cl	B	5 ± 1.2	7	<0.002 vs **3**; <0.03 vs **22**

[a] Test compounds were evaluated in each screening assay at four concentrations using triplicate incubations in the presence of 5.6 nM insulin, a submaximally effective concentration, as described in Experimental Section. The EC_{50} value was calculated using nonlinear regression analysis of the best-fit curve of the untransformed data. Values shown are the mean ± SEM [when *n* (number of independent assays) ≥ 3]. Under the conditions employed, the % coefficient of variation for this screening assay was typically ≤15%. For statistical comparison of test compounds when $n \geq 2$, an unpaired *t* test was used to compare the EC_{50} of two compounds. For statistical comparisons of a panel of compounds when $n = 1$, an ANOVA (repeated measures) was used to compare the counts per minute data (four concentrations, triplicate incubations) followed by the Newman−Keuls multiple comparison test. Significance was accepted at $P < 0.05$. Typically, 2-fold differences in EC_{50} values were statistically significant, while differences less than 2-fold were not. All analyses were performed using GraphPad Prism (MS Windows, version 4.03), GraphPad Software, San Diego, CA (www.graphpad.com). ns, not significant.

Table 2. Glucose Uptake Data For Truncated Analogues **31** and **32**



compd	R	EC_{50} (μM)
31	3-SO_3Na, 4-Cl	>200
32	3-CO_2H, 4-OH	>200

Coupling with the appropriate anilines yielded the sulfonamides **48** and **49**, which after deprotection were treated with triphosgene to afford symmetrical ureas **52** and **53**. In this manner, symmetrical ureas **3**, **23**, **29**, and **30** were prepared.

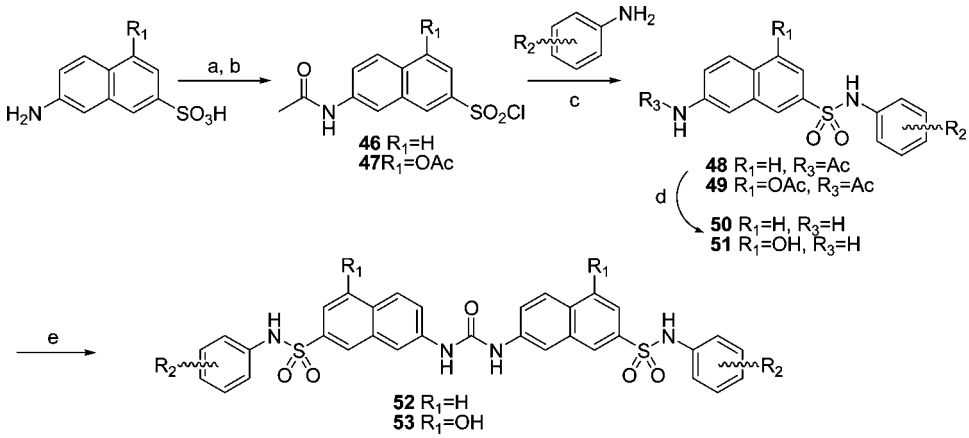
For sulfonic acid containing anilines, it proved convenient to couple them as their sulfonate esters. This allowed for the facile chromatographic separation (method A) or extractive separation (methods A and B) of any unreacted naphthalenesulfonic acid. The *p*-cresolaniline sulfonates **54**, **55**, and **56** were prepared from the corresponding nitrobenzenesulfonyl chlorides by esterification with *p*-cresol followed by reduction of the nitro group (Scheme 3). 3-(1*H*-1,2,3,4-Tetrazol-5-yl)phenylamine **57** was prepared in one step from 3-aminobenzonitrile and sodium azide.[34]

The amides **33** and **34** were prepared according to the procedure described in Scheme 4, starting from the known aminonaphthalenecarboxylic acid **58**.[35] Protection of the amine followed by acid chloride formation and amidation with the appropriate aniline yielded carboxamides **59** and **60**. After Fmoc removal and reaction with triphosgene, protected ureas **61**and **62** were obtained. Basic hydrolysis provided the desired diacids **33** and **34**.

The *N*-methylsulfonamide **35** was simply prepared by methylation of sulfonamide **4** (method A, $R_1 = H$, $R_2 = 3\text{-}SO_3Na$) using potassium carbonate and iodomethane.

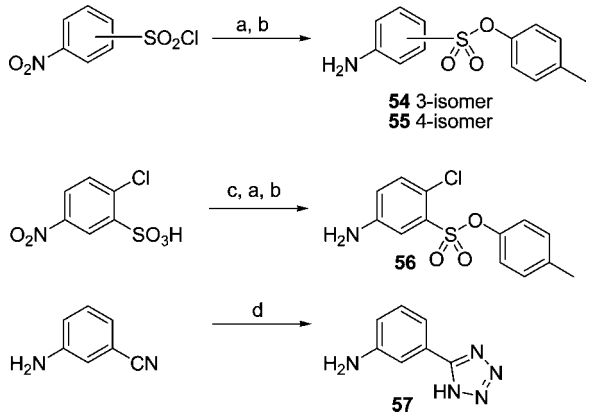
Compounds **36** and **37**, which contain a flexible ethyl linker, and the constrained 2-imidazolidinone **43** were prepared as described in Scheme 5. 1,2-Dibromoethane was alkylated with excess 7-amino-2-naphthalenesulfonic acid, and the nitrogen atoms were then acetylated prior to sulfonyl chloride formation.

Scheme 2[a]



[a] (a) Ac_2O, pyridine; (b) $POCl_3$; (c) pyridine, THF; (d) 5 N NaOH, 60°C; (e) triphosgene, THF, 1 M NaOAc buffer, pH 4.7.

Scheme 3[a]



[a] (a) *p*-cresol, pyridine, THF; (b) $SnCl_2$, HCl; (c) $POCl_3$, DMA; (d) NaN_3, Et_3NHCl, toluene, 100°C.

The resulting sulfonyl chloride **63** was coupled to 5-amino-2-chlorobenzoic acid followed by liberation of the amines by basic hydrolysis to give the ethyl linked analogue **37**. Triphosgene induced cyclization afforded the 2-imidazolidinone **43**.

Scheme 6 shows the synthesis of the *N*,*N*′-dimethylurea **42**. Triphosgene mediated ureidation of 7-amino-2-naphthalenesulfonic acid (method A, Scheme 1) was followed by methylation of the urea nitrogens. Conversion to the bis-sulfonyl chloride, coupling to methyl 5-amino-2-chlorobenzoate, and subsequent ester hydrolysis gave the desired *N*,*N*′-dimethylurea **42**.

The importance of the central urea linker was further explored by the synthesis of thiourea **38** and guanidine analogues **39**−**41** as depicted in Scheme 7. The 7-aminonaphthalene **64** was obtained by esterification of intermediate **51** (method B, Scheme 2, R_2 = 3-CO_2H, 4-Cl). It was then converted to the thioisocyanate **65** using 1,1′-thiocarbonyldiimidazole. This thioisocyanate was treated with 7-aminonaphthalene **64** to afford the thiourea **66**, which was subsequently hydrolyzed to furnish the dicarboxylic acid **38**. The thiourea **66** also gave access to a series of guanidine isosteres by initial S-methylation followed by reaction with the appropriate amine in a sealed tube. Basic hydrolysis of the resulting esters **67**−**69** gave the desired guanidines **39**−**41**.

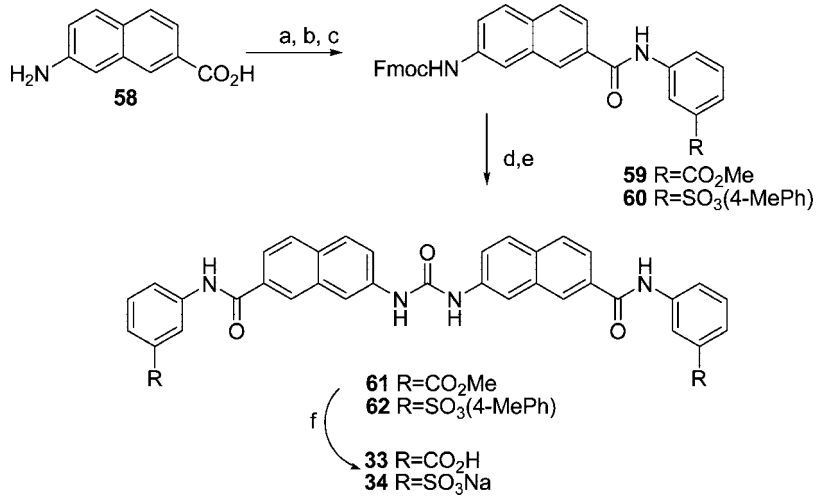
Results and Discussion

The azonaphthylurea **1** contains a number of undesirable features for a drug candidate. In particular, the elimination of the six sulfonic acids and four azo linkages and reduction of the high molecular weight were targeted as early goals for the improvement of our lead structure. The initial SAR work with the azo analogues of **1** focused on the role of the benzenesulfonic acids. It was found that the distal benzenesulfonic acid groups were not required for activity (Figure 1). However, the sulfonic acids on the benzene rings directly attached to the central naphthalene urea core were found to be essential for activity. Replacement of the inner azo linkages of **1** with another type of linker to the requisite benzenesulfonic acid groups was next examined. A subsequent set of compounds using the sulfonic acids on the naphthalenes as a handle for the formation of sulfonamide linkages was explored. Changing the linker eliminated the undesirable azo linkages and incorporated neutral sulfonamides. This strategy provided compounds of the general structure **2** (Figure 1), many of which retained the IRTK activation activity of **1**. In order to establish the SAR of these IRTK activators, an expanded series of analogues was designed and synthesized.

Compounds **1** and **3** were initially compared by their effect on autophosphorylation of the cytoplasmic kinase domain of the human insulin receptor. Both compounds demonstrated a dose dependent activation of the autophosphorylation activity (Figure 2). The biphasic dose−response curve is likely a consequence of incubation conditions associated with the in vitro (enzymatic) assay. Importantly, neither compound (**1** or **3**; data not shown) nor **30** exhibited a biphasic dose−response in a cellular assay measuring insulin receptor tyrosine phosphorylation (Figure 3) or other cellular assays (data not shown).

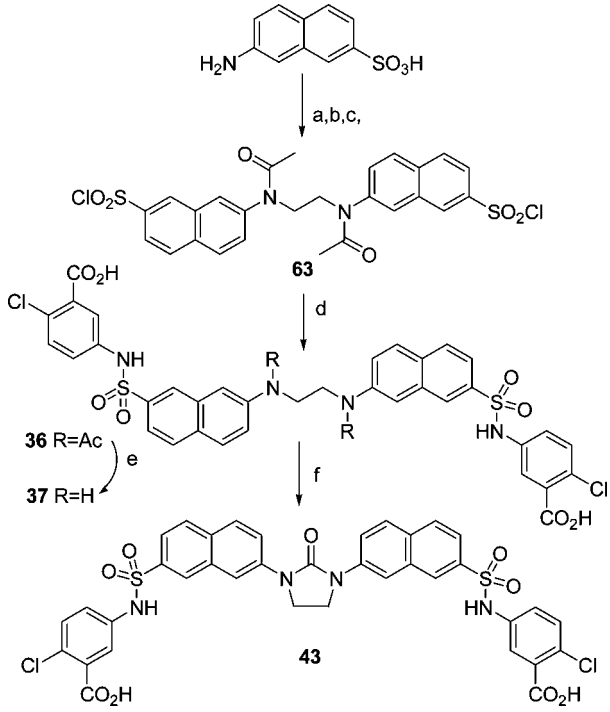
A downstream consequence of IRTK activation in the cell is the stimulation of glucose transport. This whole-cell functional assay provides a measure of the ability of test compounds to activate the insulin receptor and for that activation to lead to a quantifiable signal reflecting the cellular uptake of glucose.[36] This assay uses differentiated mouse 3T3-L1 adipocytes and measures the ability of test compounds to increase (i.e., stimulate) glucose transport in the absence and presence of insulin using 2-deoxy-D-[^{14}C]glucose. Compound **3** stimulated glucose transport (EC_{50} = 26 μM) in the presence of 5.6 nM insulin relative to compound **1** (EC_{50} = 90 μM). The increased potency of **3** in the glucose transport assay agrees with the observed relative potencies of compounds **1** and **3** in the phosphorylation assay. At high concentrations, **3** increased glucose transport in the absence of insulin; this was not observed for compound **1**. Therefore, at higher concentrations, **3** acts as an insulin receptor agonist in 3T3-L1 adipocytes, while at lower

Scheme 4[a]



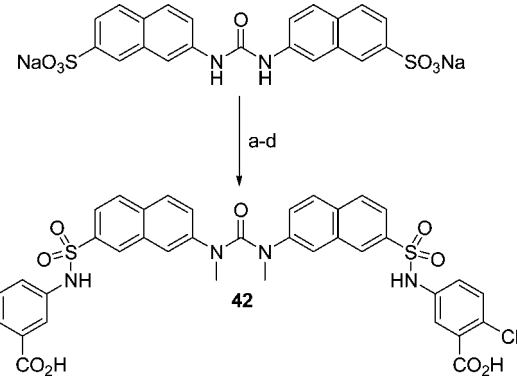
[a] (a) Fmoc-Cl, NaOH; (b) $SOCl_2$, pyridine; (c) methyl 3-aminobenzoate or **54**; (d) piperidine, CH_2Cl_2; (e) triphosgene, THF/aqueous NaOH; (f) NaOMe, THF/H_2O.

Scheme 5[a]



[a] (a) 1,2-Dibromoethane, K_2CO_3, DMF; (b) Ac_2O, pyridine; (c) $POCl_3$; (d) 5-amino-2-chlorobenzoic acid, pyridine; (e) 5 N NaOH; (f) triphosgene THF/aqueous Na_2CO_3.

Scheme 6[a]



[a] (a) NaH, MeI, DMF, 70 °C; (b) $POCl_3$; (c) methyl 2-amino-5-chlorobenzoate, pyridine; (d) NaOH.

concentrations, the presence of insulin is required to observe an increase in glucose transport. The cellular activity of **3** demonstrated that this simplified scaffold was a good starting point to begin SAR studies.

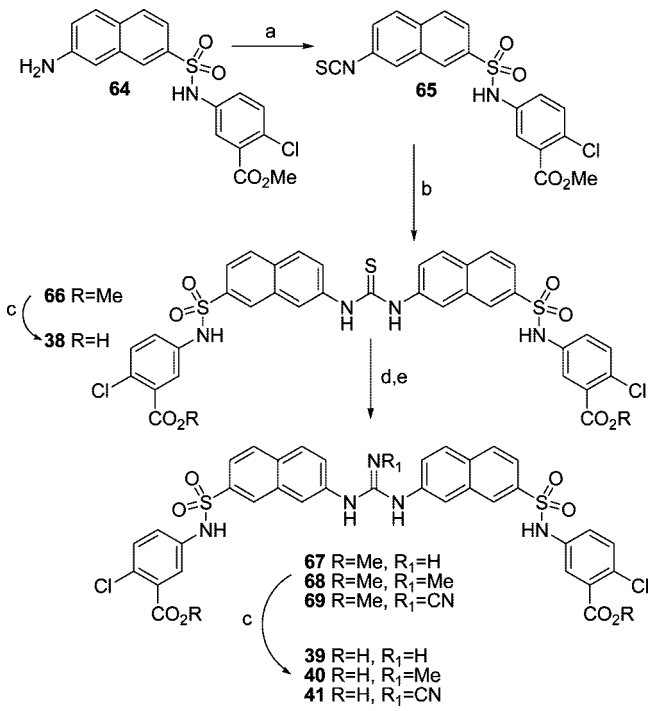
The importance of the naphthalene hydroxyls of **3** on its glucose transport activity was first addressed. The *des*-hydroxyl analogue **4** (EC_{50} = 22 μM) was prepared and was shown to be equipotent to **3** (Table 1), thus showing that these hydroxyl groups are not essential for glucose transport activity.

The need for acidic functionalities on the terminal benzene rings was established as exemplified by compounds **5**−**8** in Table 1, suggesting that the sulfonic acid groups are playing a critical role in activating the IRTK. In order to determine if other acidic groups could take the place of the sulfonic acids, analogues containing carboxylic acids, **9** and **10**, tetrazoles, **11** and **12**, alcohols, **13** and **14**, and sulfonamide **15** at the 3-position of the terminal benzene rings were prepared. The alcohols and sulfonamide were inactive in the glucose transport assay. The carboxylic acids **9** (EC_{50} = 43 μM) and **10** (EC_{50} = 24 μM) were slightly less potent than the corresponding sulfonic acids, and the tetrazoles **11** (EC_{50} = 73 μM) and **12** (EC_{50} = 54 μM) were weaker still. In addition, a derivative in which the terminal rings were phenylacetic acids (**16**) was prepared; this analogue proved to be inactive. As had been observed previously, the naphthalene hydroxyl groups had little effect on activity.

The effect of the position of the acidic groups on the terminal benzene rings was next explored. When the sulfonic acid was moved to the 4-position, **17**, the activity was reduced by 4-fold. Attempts to prepare analogues with the sulfonic acid in the 2-position were unsuccessful, and this was postulated to be due to steric crowding. However, in the case of the carboxylic acid series, both the 2- and 4-substituted analogues **18**−**21** could be prepared. These analogues were again less active (2- to 8-fold) than the corresponding 3-substituted carboxylic acid analogues **9** and **10**.

After establishment of a preference for acidic functionality at the 3-position of the terminal ring, exploration of the effects of additional substituents on that ring was initiated. For synthetic reasons the 3-substituted carboxylic acids **9** and **10** were chosen as models for this series. Incorporation of a chlorine at the

Scheme 7[a]



[a] (a) 1,1′-Thiocarbonyldiimidazole; (b) **64**, CH_2Cl_2; (c) 1 N NaOH; (d) MeI; (e) R_1NH_2, sealed tube, 70−80 °C.

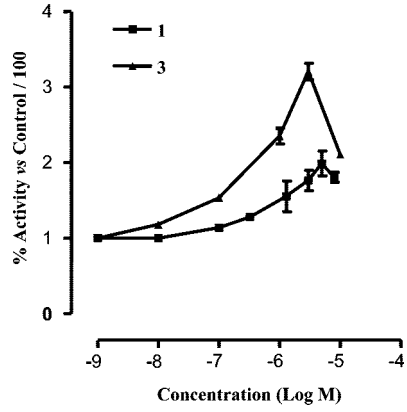


Figure 2. Effects of compounds **1** and **3** on the autophosphorylation of the insulin receptor cytoplasmic kinase domain (CKD). Results are mean ± SEM values of three independent experiments.

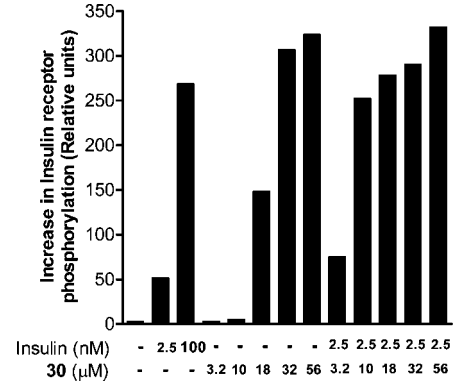


Figure 3. Effect of compound **30** on insulin receptor tyrosine phosphorylation.

4-position of the terminal ring resulted in compounds **22** and **23** with increased potency, and this trend was also observed by the incorporation of a hydroxyl group at the same 4-position giving salicylic acid **25**. The analogous salicylic acid derivative containing the naphthalene hydroxyl groups (**24**) was an outlier in this series, being only equipotent to its analogue **9**. Introduction of a chlorine or hydroxyl substituent between the acidic functionality and the sulfonamide linker gave compounds **27** and **28**; however, both exhibited a reduction in potency. The increased potency observed with chlorine substitution in the 4-position in the 3-benzoic acid series was also observed with the 3-sulfonic acid analogues **29** and **30**. These two compounds had EC_{50} values of 6 and 5 μM, respectively, and are the most potent analogues in this series.

To confirm the requirement for acidic functionalities on the terminal benzene rings, carboxylate and sulfonate ester intermediates of the active compounds were tested in the glucose uptake assay; all were inactive. In addition, two truncated analogues, **31** and **32**, which lack one of the acid functionalized terminal benzene rings were also tested; both showed a dramatic reduction in glucose uptake activity (Table 2).

The 2,7-substitution pattern of the naphthalene rings was found to be critical to activity. A number of analogues containing 2,6-substituted naphthalenes were synthesized, and all proved to have little or no activity.

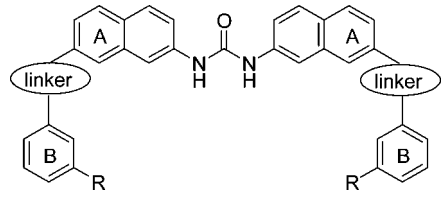
The importance of the sulfonamide linkages was examined next. Amide analogues **33** and **34** both exhibited a loss in activity (Table 3), showing that the sulfonamide linkage could not be replaced by this spatially dissimilar linker. The *N*-methylsulfonamide **35** showed a reduction in activity, perhaps indicating that the sulfonamide proton is also important.

To assess the importance of the central linkage, the urea linker was modified using compound **23** as the comparator (Table 4). Compounds **36** and **37**, which contain a flexible ethyl linker, were both inactive. Replacement of the urea with a thiourea **38**, guanidine **39**, or substituted guanidines **40** and **41** resulted in compounds with vastly diminished or no activity. Methylation of the urea nitrogens gave **42**, which was also inactive. The only modification to the urea linker that retained moderate activity was the cyclic 2-imidazolidinone **43**, which had an EC_{50} of 56 μM. These data suggest that it is not the urea protons that are critical for activity, but perhaps it is the trans, trans conformation[37] adopted by the urea linker that is important. It is known that *N*,*N*′-dimethyldiphenylureas adopt a cis,cis conformation,[38,39] while for thioureas[40] and guanidines[41] the mixed cis,trans conformation may predominate. Since the urea linkage acts as a hinge in the center of the molecule, even subtle changes in orientation can cause large movements in the relative positions of the outer rings.

Compound **30** was chosen for further characterization as a representative example from this series. At this stage, it is unknown with certainty if the pharmacological properties of **30** differed compared to **29**. This is currently under investigation. In mouse 3T3-HIR cells that overexpress the human IR, **30** (10 μM) significantly potentiated the effects of 2.5 nM insulin to increase the tyrosine phosphorylation of the IR (Figure 3). At 3 μM **30** + 2.5 nM insulin, there was a trend toward increasing in IR phosphotyrosine (~75%) vs ~50% with 2.5 nM insulin alone. At 10 μM, **30** plus insulin increased IR tyrosine phosphorylation to a degree similar to 100 nM insulin alone. Compound **30**, at concentrations 18 μM or greater, was able to increase tyrosine phosphorylation of the IR in the absence of insulin.

The effects of **30** on glucose transport has been evaluated in 3T3-L1 adipocytes.[36] In the absence of insulin, 30 and 50 μM **30** produced a significant increase in glucose transport by

Table 3. Glucose Uptake Data for Analogues with Changes to the Sulfonamide Linker[a]



compd	linker (A → B)	R	EC_{50} (μM)	no. of assays	*P*
10	$-SO_2NH-$	CO_2H	24	1	ns vs **4**
4	$-SO_2NH-$	SO_3Na	22	2	ns vs **3**
33	$-CONH-$	CO_2H	>200	1	inactive compound
34	$-CONH-$	SO_3Na	>200	1	inactive compound
35	$-SO_2N(CH_3)-$	SO_3Na	91	1	<0.001 vs **10**; <0.001 vs **4**

[a] Test compounds were evaluated in each screening assay at four concentrations using triplicate incubations in the presence of 5.6 nM insulin, a submaximally effective concentration, as described in Experimental Section. The EC_{50} value was calculated using nonlinear regression analysis of the best-fit curve of the untransformed data. Values shown are the mean ± SEM [when *n* (number of independent assays) ≥ 3]. Under the conditions employed, the % coefficient of variation for this screening assay was typically ≤15%. For statistical comparison of test compounds when $n \geq 2$, an unpaired *t* test was used to compare the EC_{50} of two compounds. For statistical comparisons of a panel of compounds when $n = 1$, an ANOVA (repeated measures) was used to compare the counts per minute data (four concentrations, triplicate incubations) followed by the Newman−Keuls multiple comparison test. Significance was accepted at $P < 0.05$. Typically, 2-fold differences in EC_{50} values were statistically significant, while differences less than 2-fold were not. All analyses were performed using GraphPad Prism (MS Windows, version 4.03), GraphPad Software, San Diego, CA (www.graphpad.com). ns, not significant.

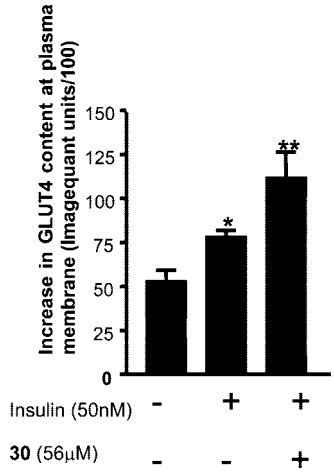


Figure 4. GLUT4 content at the plasma membrane is increased by treatment with compound **30**: (∗) $p < 0.05$; (∗∗) $p < 0.01$.

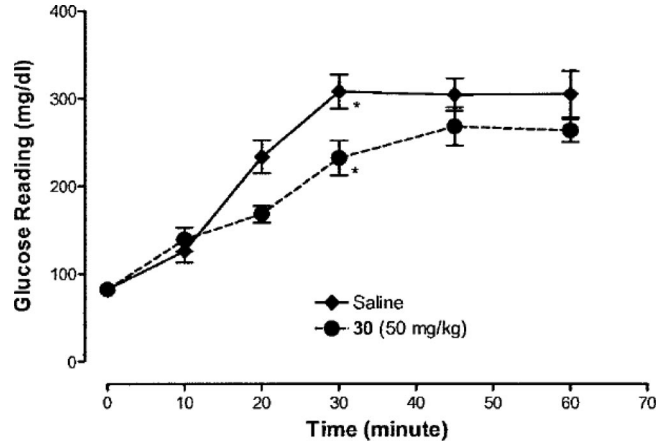


Figure 5. Effect of a single intraperitoneal (ip) dose of compound **30** in an oral glucose tolerance test in STZ-diabetic rats. Glucose load was administered 15 min postdose (50 mg/kg): (∗) $p = 0.014$ (*t* test, unpaired).

approximately 340% and 875%, respectively. Additional experiments evaluating the effects of **30** on insulin-stimulated glucose transport in 3T3-L1 adipocytes were also performed using additional concentrations of insulin. In the presence of 10 and 30 μM **30**, the insulin concentration−response curve was significantly left-shifted, indicative of an IR sensitizing effect.[36] Taken together, these results indicate that at concentrations of ≥30 μM, compound **30** increases glucose transport in the absence of insulin but functions more potently to enhance insulin stimulated glucose transport (i.e., functions as a cellular insulin sensitizer).

Compound **30** also increased plasma membrane GLUT4 content by ∼85% in adipocytes stimulated with 50 nM insulin (Figure 4). This is consistent with the increase in glucose transport activity observed with compound **30** and the expected translocation of GLUT4 from intracellular stores to the cell membrane following IRTK activation.

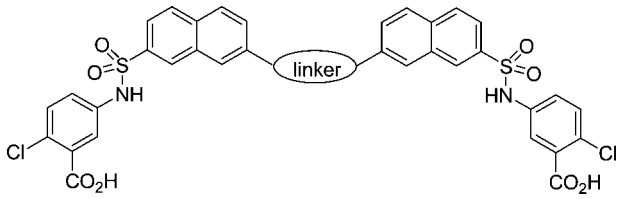
To illustrate the selectivity for the IR, compound **30** was tested for its ability to enhance tyrosine phosphorylation of the related insulin-like growth factor-1 (IGF-1R) and epidermal growth factor (EGFR) receptors. At concentrations up to 30 μM, **30** had no effect on either IGF-1-stimulated tyrosine phosphorylation of the IGF-1R or EGF-stimulated phosphorylation of the EGFR.[36]

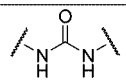
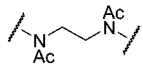
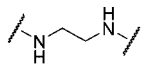
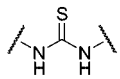
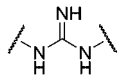
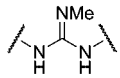
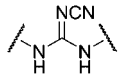
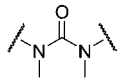
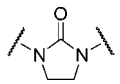
Compound **30** was also tested in vivo for its ability to improve glucose tolerance in an oral glucose tolerance test in hypoinsulinemic rats. A single ip dose of **30** (50 mg/kg) was able to lower blood glucose ($p = 0.014$) by approximately 25% by 30 min after glucose loading (Figure 5).

Compound **30** (1000 mg/kg) was also orally active in this model and produced an approximate 32% reduction in blood glucose ($p < 0.05$) by 30 min after glucose loading (Figure 6).

Protease inhibitor therapy is known to reduce the morbidity and mortality associated with HIV infection. However, protease inhibitors are also associated with metabolic abnormalities including insulin resistance, hyperglycemia, and hypertriglyceridemia.[42,43] In normal rats made insulin resistant by the HIV protease inhibitor indinavir, oral administration of **30** (10 mg/kg) resulted in improved glucose tolerance and reduced plasma insulin levels 30 min after the glucose challenge.[36] Streptozotocin (STZ)-treated rats are hyperglycemic and hypoinsulinemic and are significantly more insulin resistant than indinavir-treated rats. In addition, the hyperglycemia that is a consequence of STZ treatment further exacerbates the insulin resistance, a condition commonly referred to as glucotoxicity. In contrast, indinavir-treated rats are normoglycemic with normal insulin levels. Hence, it is

Table 4. Glucose Uptake Data for Analogues with Changes to the Urea Linker



No.	Linker	EC_{50} (μM)
23		16
36		>200
37		>200
38		162
39		179
40		>200
41		>200
42		>200
43		56

not unexpected that the efficacious dose of **30** was higher in STZ-treated rats compared to indinavir-treated rats.

Conclusion

A series of compounds based upon the symmetrical ureas of [(7-amino(2-naphthyl))sulfonyl]phenylamines were designed and synthesized. The presence of an acidic functionality on the terminal benzene rings was shown to be critical for activity. The relative positioning of these two acidic groups was also important because changes to the central urea linker cause conformation changes in the central hinge of the molecule, and therefore, changes in the relative positions of the two acidic groups invariably gave analogues with little or no activity.

Many compounds in the series stimulated the activity of the IRTK, leading to an increase in glucose uptake in cells. In addition several analogues showed activity in animal models of type 2 diabetes. These data demonstrate that interaction of these small molecules with the IR is able to elicit a similar response both in vitro and in vivo as the interaction of insulin with the IR. Therefore, our data provide pharmacological validation that small molecule IRTK activators represent a potential new class of antidiabetic agent.

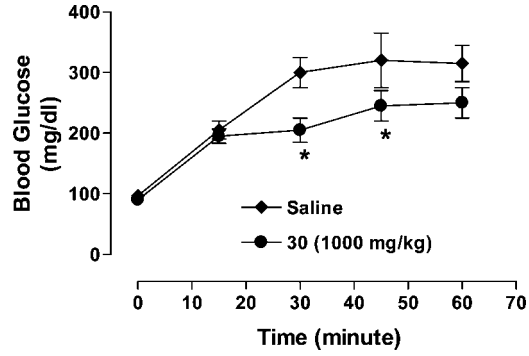


Figure 6. Effect of a single oral (po) dose of compound **30** in an oral glucose tolerance test in STZ-diabetic rats. Glucose load was administered 15 min postdose (1000 mg/kg): (∗) $p < 0.05$ (*t* test, unpaired).

Experimental Section

General. Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. 7-Amino-2-naphthalenesulfonic acid (TCI America) and 7-amino-4-hydroxy-2-naphthalenesulfonic acid (Fluka) were purified prior to use by filtration (0.2 μm nylon filter) of a 1 N NaOH solution followed by precipitation of the zwitterion after acidification with HCl. The silica gel used in column chromatography was 230−400 mesh from EM Science. Reverse-phase HPLC (RP-HPLC) was performed with a C18 stationary phase and a solvent system of CH_3CN/H_2O and 0.5%TFA or CH_3CN/H_2O and NH_4OAc. Melting points (Pyrex capillary) were determined on a Mel-Temp apparatus and are uncorrected. ^1H (300 Mhz) and ^{13}C (75 Mhz) NMR spectra were recorded on a Varian XL-300 spectrometer. Chemical shifts for ^1H spectra are reported as δ values in parts per million (ppm) relative to DMSO at 2.51 ppm, CD_3OD at 3.31 ppm, or TMS at 0.00 ppm in $CDCl_3$. Chemical shifts for ^{13}C spectra are reported as δ values in ppm relative to DMSO at 39.5 ppm or $CDCl_3$ at 77.0 ppm. Coupling constants (*J*) are reported in hertz (Hz). The splitting pattern abbreviations are designated as follows: s = singlet, d = doublet; dd = doublet of doublets, t = triplet, q = quartet, br = broad, m = multiplet, app = apparent. Matrix assisted laser desorption ionization (MALDI) mass spectra were obtained using a Finnigan Lasermat. Electospray ionization (ES) mass spectra were obtained using a Hewlett-Packard series 1100 MSD. Elemental analyses were performed by Quantitative Technologies Inc. (Whitehouse, NJ).

3-{[(4-Hydroxy-7-{[(5-hydroxy-7-{[(3-sulfophenyl)amino]sulfonyl}(2-naphthyl))amino]carbonylamino}-2-naphthyl)sulfonyl]amino}benzenesulfonic Acid, Disodium Salt (3). The title compound was prepared as described for compound **23** from compound **47** and 4-methylphenyl 3-aminobenzenesulfonate (**54**) in 55% overall yield as a tan solid. ^1H NMR (DMSO-d_6) δ 10.83 (2H, s), 10.42 (2H, s), 9.53 (2H, s), 8.13 (2H, d, $J = 1.6$ Hz), 8.07 (2H, d, $J = 9.0$ Hz), 7.74 (2H, s), 7.66 (2H, dd, $J = 1.9$ Hz, $J = 9.2$ Hz), 7.46 (2H, s), 7.13−7.24 (6H, m), 7.01 (2H, d, $J = 1.6$ Hz); ^{13}C NMR (DMSO-d_6) δ 154.2, 152.7, 148.9, 139.1, 138.0, 137.4, 133.9, 128.3, 123.1, 122.1, 121.1, 120.8, 119.6, 117.6, 117.4, 114.3, 102.5; MS (MALDI(−)) *m/z* 835 (M − Na). Anal. ($C_{33}H_{24}N_4Na_2O_{13}S_4$) C, H, N.

General Method A (Scheme 1). To compound **44** or **45** dissolved in THF was added a solution of 2.2 equiv of the aniline as a solution in THF with 5 equiv of pyridine. The mixture was allowed to stir at ambient temperature for 16 h. The products were purified by RP-HPLC, silica gel flash chromatography, or extraction followed by chromatography.

3-{[(7-{[(7-{[(3-Sulfophenyl)amino]sulfonyl}-2-naphthyl)amino]carbonylamino}-2-naphthyl)sulfonyl]amino}benzenesulfonic Acid, Disodium Salt (4). The reaction of **44** with 4-methylphenyl 3-aminobenzenesulfonate (**54**) according to general method A gave 4-methylphenyl 3-[({7-[({3-[(4-methylphenyl)oxysulfonyl]phenyl}amino)sulfonyl]-2-naphthyl}amino)carbonylamino]-2-naphthyl}sulfonyl)amino]benzenesulfonate in 84% yield as an off-white solid. ^{1}H NMR (DMSO-d_6) δ 10.94 (2H, s), 9.23 (2H, s), 8.31 (2H, s), 8.26 (2H, s), 8.05 (2H, d, J = 8.8 Hz), 7.98 (2H, d, J = 9.1 Hz), 7.76 (2H, dd, J = 2.0 Hz, J = 8.9 Hz), 7.63 (2H, dd, J = 1.7 Hz, J = 8.7 Hz), 7.57−7.59 (4H, m), 7.50 (2H, app t, J = 8.2 Hz), 7.35 (2H, d, J = 7.8 Hz), 6.84 (4H, d, J = 8.4 Hz), 6.51 (4H, d, J = 8.6 Hz), 2.12 (6H, s); ^{13}C NMR (DMSO-d_6) δ 152.2, 146.5, 138.8, 138.7, 136.5, 136.3, 135.2, 132.2, 130.4, 130.3, 129.8, 129.0, 128.4, 126.7, 125.2, 123.0, 122.5, 120.9, 119.5, 118.1, 114.4, 19.9; MS (MALDI(−)) m/z 962 (M − H).

This material was hydrolyzed in a 1:1 mixture of aqueous 2 N NaOH and methanol at 50 °C for 6 h. Purification by RP-HPLC gave the title compound in 98% yield as a tan solid. ^{1}H NMR (DMSO-d_6) δ 10.38 (2H, br s), 9.31 (2H, s), 8.28 (2H, s), 8.19 (2H, s), 7.97 (2H, d, J = 8.7 Hz), 7.93 (2H, d, J = 9.2 Hz), 7.77 (2H, dd, J = 1.9 Hz, J = 9.0 Hz), 7.60 (2H, dd, J = 1.7 Hz, J = 8.7 Hz), 7.43 (2H, s), 7.12−7.21 (6H, m); ^{13}C NMR (DMSO-d_6) δ 152.5, 149.1, 138.8, 137.2, 137.1, 132.4, 130.2, 128.9, 128.5, 128.3, 126.6, 122.2, 121.1, 119.9, 119.5, 117.4 114.2; MS (MALDI(−)) m/z 802 (M − Na). Anal. ($C_{33}H_{24}N_4Na_2O_{11}S_4$) C, H, N.

5-{[(7-{[(7-{[(3-Carboxy-4-chlorophenyl)amino]sulfonyl}-5-hydroxy(2-naphthyl))amino]carbonylamino}-4-hydroxy(2-naphthyl))-sulfonyl]amino}-2-chlorobenzoic Acid (22). The title compound was prepared by method A from compound **45** and 5-amino-2-chlorobenzoic acid in 63% yield as a brown solid. ^{1}H NMR (DMSO-d_6) δ 13.41 (2H, br s), 10.84 (2H, s), 10.71 (2H, s), 9.17 (2H, s), 8.15 (2H, s), 8.09 (2H, d, J = 9.2 Hz), 7.75 (2H, s), 7.67 (2H, dd, J = 1.8 Hz, J = 9.1 Hz), 7.55 (2H, d, J = 2.7 Hz), 7.42 (2H, d, J = 8.8 Hz), 7.29 (2H, dd, J = 2.6 Hz, J = 8.7 Hz), 6.97 (2H, d, J = 1.5 Hz); ^{13}C NMR (DMSO-d_6) δ 165.9, 154.2, 152.4, 139.0, 137.1, 136.9, 133.7, 131.7, 131.4, 126.3, 123.04, 123.01, 122.0, 121.4, 120.9, 117.5, 114.4, 101.9; MS (ES(−) m/z 811 (M − H). Anal. ($C_{35}H_{24}Cl_2N_4O_{11}S_2$) C, H, N.

Representative Example of Method B (Scheme 2). 5-{[(7-{[(7-{[(3-Carboxy-4-chlorophenyl)amino]sulfonyl}(2-naphthyl))amino]carbonylamino}(2-naphthyl))sulfonyl]amino}-2-chlorobenzoic Acid (23). To 4.98 g (22.4 mmol) of methyl 5-amino-2-chlorobenzoate hydrochloride was added 50 mL of THF followed by 11 mL (0.136 mol) of pyridine. The stirred suspension was cooled in an ice bath, and then 5.30 g (18.7 mmol) of *N*-[7-(chlorosulfonyl)-2-naphthyl]acetamide (**46**) was added as a suspension in 50 mL of THF. The mixture was allowed to warm to ambient temperature and to stir for 16 h. All volatiles were removed by rotary evaporation, and the resulting residue was extracted with ethyl acetate and 1 N HCl. The organic layer was dried ($MgSO_4$) and filtered and volatiles were removed to provide 6.63 g (82%) of methyl 5-({[7-(acetylamino)(2-naphthyl)]sulfonyl}amino)-2-chlorobenzoate as a faint yellow solid: mp = 165−172 °C; ^{1}H NMR (DMSO-d_6) δ 10.77 (1H, s), 10.31 (1H, s), 8.42 (1H, s), 8.31 (1H, s), 8.01 (1H, d, J = 8.5 Hz), 7.95 (1H, d, J = 8.9 Hz), 7.74 (1H, dd, J = 1.8 Hz, J = 8.9 Hz), 7.63 (1H, dd, J = 1.7 Hz, J = 8.6 Hz), 7.56 (1H, d, J = 2.6 Hz), 7.43 (1H, d, J = 8.7 Hz), 7.32 (1H, dd, J = 2.7 Hz, J = 8.8 Hz), 3.81 (3H, s), 2.12 (3H, s); ^{13}C NMR (DMSO-d_6) δ 168.7, 164.6, 138.4, 136.8, 136.4, 132.0, 131.5, 130.7, 130.2, 129.1, 128.3, 127.1, 126.5, 123.7, 122.7, 121.5, 120.1, 115.7, 52.4, 23.9; MS (MALDI(+)) m/z 457 (M + Na).

To 4.81 g (11.1 mmol) of the above material was added 160 mL of 5 N NaOH and 7 mL of dioxane. The mixture was allowed to stir at 55 °C for 18 h. Then the mixture was diluted with 30 mL of water and filtered through a 0.2 μm nylon filter. The resulting clear filtrate was carefully acidified with 26 mL of concentrated H_2SO_4. The finely divided solid precipitate was collected by vacuum filtration and dried in vacuo. This produced 4.01 g (95%) of 5-{[(7-amino(2-naphthyl))sulfonyl]amino}-2-chlorobenzoic acid as a tan solid: mp = 160−165 °C; ^{1}H NMR (DMSO-d_6) δ 10.63 (1H, s), 8.00 (1H, s), 7.80 (1H, d, J = 8.4 Hz), 7.68 (1H, d, J = 8.8 Hz), 7.53 (1H, d, J = 2.6 Hz), 7.39 (1H, d, J = 8.7 Hz), 7.34 (1H, dd, J = 1.5 Hz, J = 8.7 Hz), 7.27 (1H, dd, J = 2.7 Hz, J = 8.7 Hz), 7.11 (1H, dd, J = 2.0 Hz, J = 8.7 Hz), 6.96 (1H, s); ^{13}C NMR (DMSO-d_6) δ 165.8, 147.4, 136.8, 136.1, 133.5, 131.6, 131.2, 128.9, 128.5, 127.5, 126.2, 124.8, 123.1, 121.4, 121.2, 116.6, 107.0; MS (ES(−)) m/z 375 (M − H).

To 3.90 g (10.4 mmol) of 5-{[(7-amino(2-naphthyl))sulfonyl]amino}-2-chlorobenzoic acid was added 55 mL of 1 M acetate buffer (pH 4.6), 2.1 mL of 5 N NaOH (10.5 mol), and 34 mL of THF. To this reaction solution, a solution of 0.508 g (1.71 mmol) of triphosgene in 2 mL of THF was added slowly over a 1 h period. The reaction was judged incomplete, so another 4.2 mL of 5 N NaOH and 25 mL of water were added followed by the dropwise addition of a THF solution of 0.508 g of triphosgene in 2 mL of THF. The reaction was still incomplete, so another 4.2 mL of 5 N NaOH, 25 mL of water, and 0.508 g of triphosgene in 2 mL of THF (dropwise) were added. The reaction was essentially complete. The volatiles (THF) were removed from the reaction by rotary evaporation, and then the mixture was acidified with concentrated HCl. The resulting solid was collected by vacuum filtration and was washed with water. After drying in vacuo, this provided 3.79 g (93%) of the title compound as an off-white solid: mp = 182−185 °C; ^{1}H NMR (DMSO-d_6) δ 13.42 (2H, br s), 10.73 (2H, s), 9.23 (2H, s), 8.32 (2H, s), 8.25 (2H, d, J = 1.3 Hz), 8.02 (2H, d, J = 8.8 Hz), 7.97 (2H, d, J = 8.8 Hz), 7.76 (2H, dd, J = 1.9 Hz, J = 9.0 Hz), 7.62 (2H, dd, J = 1.6 Hz, J = 8.7 Hz), 7.54 (2H, d, J = 2.7 Hz), 7.41 (2H, d, J = 8.7 Hz), 7.31 (2H, dd, J = 2.6 Hz, J = 8.7 Hz); ^{13}C NMR (DMSO-d_6) δ 165.8, 152.4, 138.8, 136.7, 136.6, 132.3, 131.7, 131.4, 130.3, 129.2, 128.6, 126.8, 126.5, 123.2, 122.6, 121.5, 119.8, 114.5; MS (MALDI(−)) m/z 780 (M − H). Anal. ($C_{35}H_{24}Cl_2N_4O_9S_2$) C, H, N.

5-{[(7-{[(7-{[(3-Carboxy-4-hydroxyphenyl)amino]sulfonyl}-5-hydroxy(2-naphthyl))amino]carbonylamino}-4-hydroxy(2-naphthyl))-sulfonyl]amino}-2-hydroxybenzoic Acid (24). The title compound was prepared by method A from compound **45** and 5-aminosalicylic acid in 5% yield as a white solid. ^{1}H NMR (DMSO-d_6) δ 10.75 (2H, s), 10.07 (2H, s), 9.14 (2H, s), 8.08 (2H, d, J = 8.9 Hz), 8.08 (2H, d, J = 2.1 Hz), 7.66 (2H, dd, J = 2.0 Hz, J = 9.2 Hz), 7.61 (2H, s), 7.52 (2H, d, J = 2.7 Hz), 7.24 (2H, dd, J = 2.8 Hz, J = 8.8 Hz), 6.92 (2H, d, J = 1.5 Hz), 6.84 (2H, d, J = 8.8 Hz); MS (ES(−)) m/z 773 (M − H). Anal. ($C_{35}H_{26}N_4O_{13}S_2$) C, H, N.

2-Hydroxy-5-{[(7-{[(7-sulfo(2-naphthyl))amino]carbonylamino}(2-naphthyl))sulfonyl]amino}benzoic Acid (25). This compound was prepared by a variation of the general method A. To 120 mg (0.78 mmol) of 5-aminosalicylic acid suspended in 4 mL of pyridine was added 100 mg (0.20 mmol) of compound **44** as a solid. The mixture was allowed to stir at ambient temperature for 16 h. Then 100 mL of diethyl ether was added and the resulting solid collected by centrifugation. The title compound was obtained by RP-HPLC purification using a gradient system of CH_3CN/H_2O and 0.5% TFA. This provided 24 mg (20%) of the desired product **25** as a brown solid. ^{1}H NMR (DMSO-d_6) δ 9.73 (1H, s), 9.24 (1H, s), 9.18 (1H, s), 8.15 (1H, s), 8.14 (1H, s), 8.03 (1H, s), 8.01 (1H, s), 7.96 (1H, d, J = 8.0 Hz), 7.94 (1H, d, J = 8.3 Hz), 7.85 (1H, d, J = 8.8 Hz), 7.78 (2H, d, J = 8.3 Hz), 7.67 (1H, dd, J = 1.8 Hz, J = 8.7 Hz), 7.57 (1H, dd, J = 1.4 Hz, J = 8.4 Hz), 7.53 (1H, dd, J = 1.8 Hz, J = 8.7 Hz), 7.46 (1H, d, J = 2.8 Hz), 6.94 (1H, d, J = 8.8 Hz), 6.53 (1H, d, J = 8.8 Hz); ^{13}C NMR (DMSO-d_6) δ 171.4, 159.6, 152.6, 145.8, 138.9, 137.7, 137.3, 132.7, 132.3, 131.3, 129.9, 128.8, 128.5, 128.3, 127.9, 127.6, 126.8, 126.43, 126.36, 124.7, 123.1, 122.1, 121.9, 120.1, 120.0, 116.2, 114.2, 114.1; MS (MALDI(−)) m/z 606 (M − H).

2-Chloro-5-{[(7-{[(7-{[(4-chloro-3-sulfophenyl)amino]sulfonyl}(2-naphthyl))amino]carbonylamino}(2-naphthyl))sulfonyl]amino}benzenesulfonic Acid, Disodium Salt (29). The title compound was prepared as described for compound **23** (method B) from compound **46** and 4-methylphenyl 5-amino-2-chlorobenzenesulfonate (**56**) in 61% overall yield for three steps as a white solid. ^{1}H NMR (DMSO-d_6) δ 10.51 (2H, s), 9.27 (2H, s), 8.29 (2H, s), 8.21 (2H, s), 8.00

(2H, d, J = 8.8 Hz), 7.95 (2H, d, J = 8.8 Hz), 7.80 (2H, dd, J = 1.8 Hz, J = 8.8 Hz), 7.71 (2H, d, J = 2.7 Hz), 7.60 (2H, dd, J = 1.6 Hz, J = 8.7 Hz), 7.22 (2H, d, J = 8.5 Hz), 7.12 (2H, dd, J = 2.7 Hz, J = 8.6 Hz); ^{13}C NMR (DMSO-d_6) δ 152.2, 145.9, 138.4, 137.4, 135.8, 132.1, 130.2, 130.0, 128.4, 128.0, 126.1, 125.2, 122.1, 121.1, 120.6, 119.7, 114.5; MS (ES(−)) m/z 851 (M − H). Anal. ($C_{33}H_{22}Cl_2N_4Na_2O_{11}S_4$) C, H, N.

2-Chloro-5-{[(7-{[(7-{[(4-chloro-3-sulfophenyl)amino]sulfonyl}-5-hydroxy(2-naphthyl))amino]carbonylamino}-4-hydroxy(2-naphthyl))sulfonyl]amino}benzenesulfonic Acid, Disodium Salt (30). The title compound was prepared as described for compound **23** (method B) from compound **47** and 4-methylphenyl 5-amino-2-chlorobenzenesulfonate (**56**) in 29% overall as a white solid. ^{1}H NMR (DMSO-d_6) δ 10.83 (2H, s), 10.48 (2H, s), 9.23 (2H, s), 8.11 (2H, d, J = 2.5 Hz), 8.09 (2H, d, J = 8.7 Hz), 7.75 (2H, d, J = 1.4 Hz), 7.73 (2H, d, J = 2.7 Hz), 7.71 (2H, dd, J = 2.5 Hz, J = 8.7 Hz), 7.22 (2H, d, J = 8.5 Hz), 7.05 (2H, dd, J = 2.7 Hz, J = 8.5 Hz), 6.96 (2H, d, J = 1.4 Hz); ^{13}C NMR (DMSO-d_6) δ 154.1, 152.4, 145.6, 139.0, 137.5, 135.9, 133.7, 130.8, 125.5, 123.0, 122.0, 120.79, 120.76, 120.5, 117.4, 114.3, 102.1; MS (ES(−)) m/z 882 (M − H). Anal. ($C_{33}H_{22}Cl_2N_4Na_2O_{13}S_4\cdot$0.5NaCl) C, H, N, Cl.

3-({7-[(*N*-{7-[*N*-(3-Carboxyphenyl)carbamoyl]-2-naphthyl}carbamoyl)amino]-2-naphthyl}carbonylamino)benzoic Acid (33). To 20 mg (0.022 mmol) of compound **61** was added 1.5 mL of 1.37 M sodium methoxide in methanol, 1 mL of water, and 0.5 mL of THF. The resulting solution was allowed to stir at ambient temperature for 2 days. The reaction was acidified with 1 N HCl (aqueous), and the organic volatiles were removed in vacuo. The solid precipitate was collected by vacuum filtration to afford 13 mg (90%) of the title compound as a tan solid. ^{1}H NMR (DMSO-d_6) δ 13.00 (2H, br s), 10.57 (2H, s), 9.19 (2H, s), 8.54 (2H, s), 8.48 (2H, s), 8.34 (2H, s), 8.12 (2H, d, J = 7.9 Hz), 7.96−8.00 (4H, m), 7.90 (2H, dd, J = 1.5 Hz, J = 8.4 Hz), 7.68−7.71 (4H, m), 7.51 (2H, app t, J = 7.9 Hz); ^{13}C NMR (DMSO-d_6) δ 127.3, 126.0, 113.0, 99.7, 98.3, 93.1, 92.6, 91.6, 90.7, 89.0, 88.5, 87.9, 87.3, 84.7, 82.8, 81.8, 81.5, 74.9; MS (MALDI(−)) m/z 638 (M).

5-({[7-(*N*-{2-[*N*-(7-{[(3-Carboxy-4-chlorophenyl)amino]sulfonyl}(2-naphthyl))acetylamino]ethyl}acetylamino)(2-naphthyl)]sulfonyl}amino)-2-chlorobenzoic Acid (36). To 750 mg (4.37 mmol) of 5-amino-2-chlorobenzoic acid was added 30 mL of THF and 605 μL of pyridine. This solution was cooled in an ice bath, and then 452 mg (0.76 mmol) of the disulfonyl chloride (**63**) was added. The mixture was left to stir and warm to room temperature for 16 h. Then the volatiles were removed by rotary evaporation. The resulting residue was extracted with ethyl acetate and 0.5 N HCl. The ethyl acetate layer was dried ($MgSO_4$) and filtered, and the volatiles were removed by rotary evaporation. The title compound was purified by silica gel column chromatography, eluting with 20:2:1 ethyl acetate/isopropanol/water and then 10:2:1 ethyl actate/isopropanol/water. This provided 156 mg (24%) of the desired product as a white solid. ^{1}H NMR (DMSO-d_6) δ 8.26 (2H, s), 8.01 (2H, d, J = 7.4 Hz), 7.88 (2H, d, J = 8.4 Hz), 7.78 (2H, s), 7.76 (2H, d, J = 7.4 Hz), 7.38 (2H, d, J = 8.4 Hz), 7.26 (2H, s), 7.11 (2H, d, J = 8.5 Hz), 7.02 (2H, d, J = 8.1 Hz), 3.87 (4H, s), 1.70 (6H, s); MS (MALDI(+)) m/z 886 (M + Na).

5-({[7-({2-[(7-{[(3-Carboxy-4-chlorophenyl)amino]sulfonyl}(2-naphthyl))amino]ethyl}amino)(2-naphthyl)]sulfonyl}amino)-2-chlorobenzoic Acid (37). To 156 mg (0.18 mmol) of compound **36** was added 10 mL of 5 N NaOH, and the resulting solution was heated at 70 °C for 16 h. The mixture was cooled and then acidified with 6 N HCl to pH 1. The resulting solid was collected by vacuum filtration to give, after drying, 137 mg (98%) of the title compound as a tan solid. ^{1}H NMR (DMSO-d_6) δ 13.43 (2H, br s), 10.62 (2H, s), 8.08 (2H, s), 7.80 (2H, d, J = 8.4 Hz), 7.68 (2H, d, J = 8.8 Hz), 7.53 (2H, d, J = 2.6 Hz), 7.39 (2H, d, J = 8.7 Hz), 7.35 (2H, dd, J = 1.8 Hz, J = 8.5 Hz), 7.27 (2H, dd, J = 2.6 Hz, J = 8.8 Hz), 7.16 (2H, dd, J = 2.1 Hz, J = 8.8 Hz), 6.95 (2H, s), 6.35 (2H, br s), methylene protons are under HOD peak; MS (ES(−)) m/z 778 (M − H).

5-({[7-({[(7-{[(3-Carboxy-4-chlorophenyl)amino]sulfonyl}(2-naphthyl))amino]thioxomethyl}amino)(2-naphthyl)]sulfonyl}amino)-2-chlorobenzoic Acid (38). To 53 mg (64.3 μmol) of compound **66** was added 5 mL of 1 N NaOH. The resulting solution was allowed to stir for 1 h at ambient temperature, and then it was acidified with 6 N HCl to pH 1. The resulting solid was collected by vacuum filtration. Drying provided 44 mg (86%) of the title compound as a white solid. ^{1}H NMR (DMSO-d_6) δ 13.42 (2H, br s), 10.73 (2H, s), 10.31 (2H, s), 8.38 (2H, s), 8.19 (2H, d, J = 1.4 Hz), 8.07 (2H, d, J = 8.8 Hz), 7.98 (2H, d, J = 9.2 Hz), 7.85 (2H, dd, J = 1.8 Hz, J = 8.9 Hz), 7.69 (2H, dd, J = 1.7 Hz, J = 8.7 Hz), 7.54 (2H, d, J = 2.7 Hz), 7.39 (2H, d, J = 8.8 Hz), 7.29 (2H, dd, J = 2.7 Hz, J = 8.8 Hz); ^{13}C NMR (DMSO-d_6) δ 180.0, 165.6, 138.6, 136.5, 131.8, 131.7, 131.6, 131.2, 131.1, 128.9, 127.7, 127.0, 126.7, 126.4, 123.2, 121.6, 120.9, 120.8; MS (ES(−)) m/z 795 (M). Anal. ($C_{35}H_{24}Cl_2N_4O_8S_3$) C, H, N.

5-{[(7-{2-Amino-1-aza-2-[(7-{[(3-carboxy-4-chlorophenyl)amino]-sulfonyl}(2-naphthyl))amino]vinyl}(2-naphthyl))sulfonyl]amino}-2-chlorobenzoic Acid (39). To 50 mg (62.0 μmol) of compound **67** was added 10 mL of 1 N NaOH. The resulting solution was allowed to stir at ambient temperature for 1 h. The mixture was acidified to pH 1 using 11 mL of 1 N HCl. A white precipitate formed, which was collected by vacuum filtration and was washed with water. The solid was dried in vacuo to provide 45 mg (93%) of the title compound as a white solid. ^{1}H NMR (DMSO-d_6) δ 10.65 (2H, br s), 8.40 (2H, s), 8.08 (2H, d, J = 9.3 Hz), 8.06 (2H, s), 8.02 (2H, d, J = 8.9 Hz), 7.71 (2H, dd, J = 1.5 Hz, J = 8.7 Hz), 7.62 (2H, dd, J = 1.7 Hz, J = 8.7 Hz), 7.43 (2H, d, J = 2.6 Hz), 7.34 (2H, d, J = 8.7 Hz), 7.24 (2H, dd, J = 2.6 Hz, J = 8.7 Hz); ^{13}C NMR (DMSO-d_6) δ 166.7, 152.3, 138.3, 136.7, 136.4, 133.8, 132.2, 131.4, 130.8, 129.0, 128.8, 126.9, 126.0, 125.7, 122.5, 121.3, 120.7, 120.5; MS (MALDI(+)) m/z 779 (M).

5-{[(7-{[*N*-(7-{[(3-Carboxy-4-chlorophenyl)amino]sulfonyl}(2-naphthyl))-*N*-methylcarbamoyl]methylamino}(2-naphthyl))sulfonyl]amino}-2-chlorobenzoic Acid (42). To 300 mg (0.58 mmol) of 7-{[(7-sulfo-2-naphthyl)amino]carbonylamino}naphthalene-2-sulfonic acid, disodium salt (first intermediate in synthesis of **44**) suspended in 40 mL of DMF was added 60 mg (60% dispersion in mineral oil, 1.50 mmol) of NaH. The mixture was allowed to stir for 1 h, and then 90 μL (1.45 mmol) of iodomethane was added. The mixture was allowed to stir for 19 h, and then another 25 mg (0.625 mmol) of NaH dispersion was added followed by 30 μL (0.483 mmol) of iodomethane after 1 h. The mixture was allowed to stir an additional 24 h before precipitation with diethyl ether. The resulting solid was collected by vacuum filtration, and the solid was dissolved in water. The product was precipitated by the addition of brine and was collected by vacuum filtration. Drying provided 7-{*N*-methyl[methyl(7-sulfo(2-naphthyl))amino]carbonylamino}-naphthalene-2-sulfonic acid, disodium salt, as a white solid. ^{1}H NMR (DMSO-d_6) δ 8.01 (2H, s), 7.65 (2H, d, J = 8.8 Hz), 7.63 (2H, s), 7.55 (4H, d, J = 6.8 Hz), 7.17 (2H, dd, J = 1.9 Hz, J = 8.8 Hz), 3.22 (6H, s); MS (MALDI(−)) m/z 521 (M − Na).

To all of the product from step 1 was added 25 mL of phosphorus oxychloride. This stirred suspension was cooled in an ice bath for 1 h and then allowed to warm to room temperature. The suspension was allowed to stir at ambient temperature for 26 h. Then the cloudy suspension was poured onto 1 L of ice and this suspension placed in a second ice bath. After the ice had melted, the precipitate was collected by vacuum filtration and was washed with water. The white solid was dried to provide 247 mg of *N*-[7-(chlorosulfonyl)(2-naphthyl)]{[7-(chlorosulfonyl)(2-naphthyl)]methylamino}-*N*-methylcarboxamide.

To 300 mg (1.62 mmol) of methyl 5-amino-2-chlorobenzoate dissolved in 15 mL of freshly distilled THF was added 100 μL (1.24 mmol) of pyridine. To this solution was added 243 mg (0.452 mmol) of the disulfonylchloride prepared above. The mixture was heated to reflux for 40 h. Then the mixture was partitioned between ethyl acetate and 1 N HCl (aq). The organic layer was further washed with 6 N HCl (2×), 1 N $NaHCO_3$ (2×), water, and brine. The ethyl acetate layer was dried with $MgSO_4$ and filtered, and the volatiles were removed by rotary evaporation. This provided 275

mg (73%) of methyl 2-chloro-5-({[7-({*N*-[7-({[4-chloro-3-(methoxycarbonyl)phenyl]amino}sulfonyl)(2-naphthyl)]-*N*-methylcarbamoyl}methylamino)(2-naphthyl)]sulfonyl}amino)-benzoate as an off-white solid. ^{1}H NMR (DMSO-d_6) δ 10.75 (2H, br s), 8.28 (2H, s), 7.75 (2H, d, J = 8.5 Hz), 7.74 (2H, s), 7.56 (2H, dd, J = 1.6 Hz, J = 8.7 Hz), 7.55 (2H, d, J = 2.6 Hz), 7.45 (2H, d, J = 8.7 Hz), 7.40 (2H, d, J = 9.1 Hz), 7.29 (2H, dd, J = 2.7 Hz, J = 8.7 Hz), 7.22 (2H, dd, J = 2.1 Hz, J = 8.9 Hz), 3.81 (6H, s), 3.24 (6H, s); MS (ES(−)) m/z 835 (M − H).

To 140 mg (0.168 mmol) of the product from step 3 was added 25 mL of 1 N NaOH (aq). After 1 h, the reaction solution was acidified with 6 N HCl (aq) to produce a white precipitate. The solid was collected by vacuum filtration, washed with water, and dried to give 119 mg (88%) of the title compound as a white solid. ^{1}H NMR (DMSO-d_6) δ 13.53 (2H, br s), 10.71 (2H, s), 8.27 (2H, s), 7.74 (2H, d, J = 8.7 Hz), 7.72 (2H, s), 7.55 (2H, dd, J = 1.6 Hz, J = 8.8 Hz), 7.54 (2H, d, J = 2.8 Hz), 7.41 (2H, d, J = 8.8 Hz), 7.35 (2H, d, J = 8.7 Hz), 7.26 (2H, dd, J = 2.8 Hz, J = 8.7 Hz), 7.18 (2H, dd, J = 2.0 Hz, 8.9 Hz), 3.24 (6H, s); ^{13}C NMR (DMSO-d_6) δ 165.7, 159.1, 143.6, 136.5, 136.2, 131.8, 131.7, 131.6, 131.1, 130.9, 128.5, 127.7, 127.0, 126.4, 126.2, 123.1, 121.5, 120.7, 37.7; MS (ES(−)) m/z 807 (M − H).

5-[({7-[3-(7-{[(3-Carboxy-4-chlorophenyl)amino]sulfonyl}(2-naphthyl))-2-oxoimidazolidinyl](2-naphthyl)}sulfonyl)amino]-2-chlorobenzoic Acid (43). To 126 mg (0.16 mmol) of compound **37** was added 10 mL of saturated Na_2CO_3, 10 mL of water, and 10 mL of THF. This formed a clear brown solution. To this solution was added, dropwise over 30 min, a solution of 52 mg (0.18 mmol) of triphosgene in 800 μL of THF. An aliquot was examined by HPLC, and the reaction was determined to be incomplete. Then another solution of triphosgene (53 mg in 800 μL of THF) was added dropwise and the reaction monitored by HPLC. This was repeated six times until all starting amine had been consumed. The reaction solution was acidified with 6 N HCl and then reduced in volume by rotary evaporation. The resulting brown solid was collected by vacuum filtration. The title compound, 73 mg (56%), was obtained by preparative RP-HPLC (CH_3CN/water with 0.5% TFA) as a white solid. ^{1}H NMR (DMSO-d_6) δ 13.45 (2H, br s), 10.75 (2H, s), 8.43 (2H, dd, J = 2.0 Hz, J = 8.8 Hz), 8.40 (2H, s), 8.06 (2H, d, J = 9.3 Hz), 8.05 (2H, s), 8.03 (2H, d, J = 9.3 Hz), 7.66 (2H, dd, J = 1.8 Hz, J = 8.6 Hz), 7.54 (2H, d, J = 2.6 Hz), 7.41 (2H, d, J = 8.7 Hz), 7.30 (2H, dd, J = 2.5 Hz, J = 8.6 Hz), 4.17 (4H, s); ^{13}C NMR (DMSO-d_6) δ 165.6, 154.1, 139.2, 136.6, 136.5, 131.9, 131.7, 131.2, 130.3, 128.9, 128.1, 127.0, 126.5, 123.3, 121.7, 121.3, 121.2, 120.1, 114.4; MS (ES(−)) m/z 805 (M − H). Anal. ($C_{37}H_{26}Cl_2N_4O_9S_2$) C, H, N.

***N*-[7-(Chlorosulfonyl)(2-naphthyl)]{[7-(chlorosulfonyl)(2-naphthyl)]amino}carboxamide (44).** To 5.25 g (0.024 mol) of 7-amino-2-naphthalenesulfonic acid suspended in 80 mL of water was added a solution of 6.5 mL of 10 N aqueous NaOH (0.065 mol) diluted to 30 mL with water and a solution of 3.20 g (0.011 mol) of triphosgene in 30 mL of THF portionwise, alternating such that the pH of the reaction was maintained above 8. After the reaction was complete by TLC (6:2:1 ethyl acetate/isopropanol/water) the pH was lowered to 1 with aqueous HCl and the volatiles were removed by rotary evaporation. The solid product was collected by vacuum filtration and was washed with water. This afforded 3.41 g (63%) of 7-{[(7-sulfo-2-naphthyl)amino]-carbonylamino}naphthalene-2-sulfonic acid, disodium salt, as an off-white solid. ^{1}H NMR (DMSO-d_6) δ 9.04 (2H, s), 8.01 (2H, s), 8.00 (2H, d, J = 2.0 Hz), 7.85 (2H, d, J = 8.8 Hz), 7.77 (2H, d, J = 8.6 Hz), 7.71 (2H, dd, J = 2.0 Hz, J = 8.9 Hz), 7.57 (2H, dd, J = 1.5 Hz, J = 8.5 Hz); ^{13}C NMR (DMSO-d_6) δ 152.6, 145.7, 137.8, 132.7, 128.8, 127.9, 126.9, 123.2, 121.9, 120.1, 114.0; MS (MALDI(−)) m/z 492 (M − Na). To 2.35 g (4.55 mmol) of 7-{[(7-sulfo-2-naphthyl)amino]carbonyl amino}naphthalene-2-sulfonic acid, disodium salt, prepared above was added 116 mL of sulfolane, 25 mL of acetonitrile, 31 mL of phosphorus oxychloride, and 1 mL of dimethylacetamide. The mixture was allowed to stir for 72 h at ambient temperature. This produced a nearly clear solution. The mixture was poured onto 1.5 L of ice, and the flask was placed in an ice bath. After all the ice within the flask had melted, the solid was collected by vacuum filtration and was washed with water. The solid was dried under high vacuum for 24 h. This produced 2.29 g (99%) of the title compound as a white solid. ^{1}H NMR (DMSO-d_6) δ 9.10 (2H, s), 8.00 (4H, s), 7.84 (2H, d, J = 9.1 Hz), 7.77 (2H, d, J = 8.5 Hz), 7.70 (2H, dd, J = 2.0 Hz, J = 8.8 Hz), 7.56 (2H, dd, J = 1.5 Hz, J = 8.5 Hz). This compound was used without further characterization.

***N*-[7-(Chlorosulfonyl)-5-hydroxy(2-naphthyl)]{[7-(chlorosulfonyl)-5-hydroxy(2-naphthyl)]amino}carboxamide (45).** To 10.77 g (0.045 mol) of 7-amino-4-hydroxy-2-naphthalenesulfonic acid dissolved in 45 mL of 1 N aqueous NaOH and 50 mL of water was added 3.70 g (0.045 mol) of sodium acetate. The pH of the solution was above 9. The mixture was cooled to less than 5 °C in an ice−water bath. Then 2.23 g (7.50 mmol) of triphosgene dissolved in 15 mL of THF was added in three portions. The pH of the mixture fell to 4−5 and was readjusted to 7−8 by the addition of 1 N aqueous NaOH. TLC (6:2:1 ethyl acetate/isopropanol/water) indicated the reaction was incomplete. Another 2.20 g (7.50 mmol) of triphosgene in 10 mL of THF was added portionwise with the pH kept above 7 by the addition of 1 N aqueous NaOH. When the reaction was judged complete by TLC, the pH was lowered to 1 with aqueous HCl and the volatiles were removed by rotary evaporation. The solid product was collected by vacuum filtration. This afforded 10.85 g (88%) of 4-hydroxy-7-{[(5-hydroxy-7-sulfo(2-naphthyl))amino]carbonylamino}naphthalene-2-sulfonic acid, disodium salt, as a tan solid. ^{1}H NMR (DMSO-d_6) δ 10.06 (2H, s), 9.22 (2H, s), 8.01 (2H, d, J = 9.25 Hz), 7.96 (2H, d, J = 2.15 Hz), 7.58 (2H, dd, J = 2.15 Hz, J = 9.25 Hz), 7.50 (2H, s), 7.03 (2H, s); ^{13}C NMR (DMSO-d_6) δ 152.9, 152.6, 146.2, 138.1, 134.2, 122.5, 120.3, 118.4, 114.3, 113.5, 104.4; MS (ES(−)) m/z 525 (M − Na).

To 5.00 g (9.11 mmol) of 4-hydroxy-7-{[(5-hydroxy-7-sulfo(2-naphthyl))amino]carbonylamino}naphthalene-2-sulfonic acid, disodium salt, suspended in 70 mL of phosphorus oxychloride was added dropwise 4 mL of dimethylacetamide. The mixture was allowed to stir at ambient temperature for 24 h. Then the mixture was poured onto 1 L of ice, and this flask was placed in an ice bath. After the ice within the flask had melted, the solid was collected by vacuum filtration and was washed with water. The product was dried under high vacuum for 24 h to provide 4.1 g (83%) of the title compound as a tan solid. This compound was not stable to long-term storage and was therefore prepared and used within a few weeks. The compound was used without characterization.

***N*-[7-(Chlorosulfonyl)-2-naphthyl]acetamide (46).** To a mixture of 300 mL each of acetic anhydride and pyridine cooled in an ice bath was added 59.7 g (0.27 mol) of solid 7-amino-2-naphthalenesulfonic acid portionwise, keeping the reaction temperature below 20 °C. After the final addition, the reaction solution was allowed to warm to ambient temperature and stirred for 16 h. The suspension was poured into a mixture of 2 L of diethyl ether and 1 L of THF. The resulting solid was collected by vacuum filtration. The pyridinum salt was dissolved in 300 mL of methanol. Next, a sodium methoxide solution in methanol was prepared by the addition of 7.9 g (0.34 mol) Na(s) to 300 mL of methanol. After all of the Na(s) had reacted, this clear solution was added to the above methanol solution of crude product. A solid began to form and was allowed to sit for 12 h. The product was collected by vacuum filtration and dried in vacuo to afford 64.2 g. The filtrate was reduced in volume to about 400 mL and allowed to sit for an additional 12 h for precipitate formation. Collection of this second crop of product provided 4.8 g. This provided a total yield of 69.0 g (88%) of 7-(acetylamino)naphthalene-2-sulfonic acid, sodium salt, as a white solid. ^{1}H NMR (DMSO-d_6) δ 10.16 (1H, s), 8.16 (1H,s), 7.96 (1H, s), 7.83 (1H, d, J = 8.8 Hz), 7.77 (1H, d, J = 8.4 Hz), 7.68 (1H, dd, J = 2.0 Hz, J = 8.8 Hz), 7.58 (1H, dd, J = 1.5 Hz, J = 8.5 Hz), 2.10 (3H, s); ^{13}C NMR (DMSO-d_6) δ 168.6, 146.0, 137.4, 132.6, 129.4, 127.9, 126.9, 123.5, 122.5, 120.5, 115.7, 24.0; MS (MALDI(−)) m/z 263 (M − H).

To 10.3 g (35.8 mmol) of the above material was added 125 mL of phosphorus oxychloride. The resulting suspension was allowed to cool in an ice bath until an internal temperature of 5 °C was reached. Then 5 mL of dimethylacetamide was added dropwise. The mixture was allowed to stir and warm to ambient temperature for 23 h. Then the suspension was poured onto 4 L of ice. The container of ice was placed in a second container of ice and allowed to melt. When most of the ice had melted, the white solid precipitate was collected by vacuum filtration and washed with additional water. The solid was dried in vacuo to afford 6.67 g (66%) of *N*-[7-(chlorosulfonyl)-2-naphthyl]acetamide (**46**) as an off-white solid. ^{1}H NMR (DMSO-d_6) δ 10.19 (1H, s), 8.18(1H, s), 7.96 (1H, s), 7.84 (1H, d, J = 8.8 Hz), 7.78 (1H, d, J = 8.7 Hz), 7.67 (1H, dd, J = 1.8 Hz, J = 8.8 Hz), 7.58 (1H, dd, J = 1.4 Hz, J = 8.5 Hz), 2.10 (3H, s).

7-Acetamido-4-hydroxynaphthalene-2-sulfonyl Chloride (47). To 93.0 g (0.389 mol) of 7-amino-4-hydroxynaphthalene-2-sulfonic acid was added a solution of 200 mL of acetic anhydride and 200 mL of pyridine. The reaction temperature increased, and so the mixture was was placed in an ice bath for 10 min. The mixture was removed from the ice bath and allowed to stir at ambient temperature for 16 h. The solid was collected by vacuum filtration and was washed with THF. Drying in vacuo provided 155 g (99%) of 7-(acetylamino)-4-acetyloxynaphthalene-2-sulfonic acid, pyridinium salt, as a tan solid. ^{1}H NMR (DMSO-d_6) δ 10.25 (1H, s), 8.84 (2H, d, J = 4.9 Hz), 8.38 (1H, dt, J = 1.5 Hz, J = 7.8 Hz), 8.27 (1H, s), 7.89 (2H, dd, J = 4.9 Hz, J = 7.8 Hz), 7.85 (1 H, d, J = 9.1 Hz), 7.81 (1H, s), 7.71 (1H, dd, J = 1.9 Hz, J = 9.1 Hz), 7.34 (1H, d, J = 1.4 Hz), 2.44 (3H, s), 2.11 (3H, s); MS (ES(−)) m/z 280 (M − CH_3CO).

This was converted to the title compound as described for the synthesis of *N*-[7-(chlorosulfonyl)-2-naphthyl]acetamide (**46**). The compound was used without characterization.

Methyl 3-({7-[(Fluoren-9-ylmethoxy)carbonylamino]-2-naphthyl}carbonylamino)benzoate (59). To 0.504 g (2.70 mmol) of 7-aminonaphthalene-2-carboxylic acid (**58**) was added 10 mL of dioxane, 5 mL of 10% sodium carbonate, and 35 mL of water. To this clear solution was added 0.786 g (3.04 mmol) of 9-fluorenylmethyl chloroformate, portionwise, over 15 min. After 3 h, the mixture was acidified with 1 N HCl and the resulting white precipitate was collected by vacuum filtration. This solid was suspended in diethyl ether and stirred to give a fine precipitate that was collected by vacuum filtration. This provided 0.948 g (86%) of 7-[(fluoren-9-ylmethoxy)carbonylamino]naphthalene-2-carboxylic acid as an off-white solid. ^{1}H NMR (DMSO-d_6) δ 9.93 (1H, br s), 8.25 (1H, s), 8.02 (1H, br s), 7.94 (2H, d, J = 7.3 Hz), 7.86 (1H, d, J = 8.5 Hz), 7.73−7.83 (4H, m), 7.59 (1H, br s), 7.44 (2H, app t, J = 7.1 Hz), 7.36 (2H, app t, J = 6.8 Hz), 4.55 (2H, d, J = 6.0 Hz), 4.35 (1H, t, J = 6.2 Hz); ^{13}C NMR (DMSO-d_6) δ 169.0, 146.3, 142.4, 139.2, 137.2, 137.2, 134.1, 134.0, 128.6, 127.8, 127.2, 126.9, 126.3, 121.5, 120.9, 119.6, 119.0, 109.1, 106.8; MS (MALDI(+)) m/z 431 (M + Na).

To 305 mg (0.75 mmol) of 7-[(fluoren-9-ylmethoxy)carbonylamino]naphthalene-2-carboxylic acid was added 10 mL of chloroform, 3.5 mL of thionyl chloride, and 180 μL of pyridine. The mixture was allowed to stir at ambient temperature for three hours, followed by removal of volatiles by rotary evaporation. The resulting residue was stripped 2 times from chloroform. Then 50 mL of chloroform, 124 mg (0.89 mmol) of methyl-3-aminobenzoate, and 100 μL of pyridine were added. The mixture was allowed to stir at ambient temperature for 16 h. The mixture was extracted twice with 1 N HCl (aqueous) and once with water. The dried organic layer ($MgSO_4$) was filtered, and the volatiles were removed by rotary evaporation. The resulting residue was treated with methanol to form a solid precipitate that was collected by vacuum filtration. This afforded 380 mg (93%) of the title compound as a white solid. ^{1}H NMR (DMSO-d_6) δ 10.59 (1H, s), 10.06 (1H, br s), 8.51 (1H, d, J = 1.7 Hz), 8.50 (1H, d, J = 9.0 Hz), 8.24 (1H, br s), 8.14 (1H, d, J = 9.1 Hz), 7.88−7.98 (5H, m), 7.80 (2H, d, J = 7.3 Hz), 7.72 (1H, d, J = 7.8 Hz), 7.63 (1H, br s), 7.54 (1H, app t, J = 7.9 Hz), 7.44 (2H, app t, J = 7.3 Hz), 7.37 (2H, app t, J = 7.3 Hz), 4.58 (2H, d, J = 6.4 Hz), 4.37 (1H, t, J = 6.3 Hz), 3.89 (3H, s); MS (MALDI(−)) m/z 542 (M − H).

Methyl 3-[(7-{[(7-{*N*-[3-(Methoxycarbonyl)phenyl]carbamoyl}-2-naphthyl)amino]carbonylamino}-2-naphthyl)carbonylamino]benzoate (61). To 380 mg (0.70 mmol) of compound **59** was added 30 mL of dichloromethane, 3 mL of THF, and 1 mL of piperidine. The resulting clear solution was allowed to stir for 3 h. Then the reaction was extracted with ethyl acetate and 1 N HCl (aqueous). The dried organic layer ($MgSO_4$) was filtered, and the volatiles were removed in vacuo. The resulting residue was dissolved in dichloromethane, and 3 mL of 1 N HCl in diethyl ether and 50 mL of diethyl ether were added to form a precipitate that was collected by vacuum filtration. Drying provided 212 mg (85%) of methyl 3-[(7-amino-2-naphthyl)carbonylamino]benzoate hydrochloride. ^{1}H NMR (DMSO-d_6) δ 10.50 (1H, s), 8.51 (1H, s), 8.42 (2H, br s), 8.20 (1H, s), 8.12 (1H, d, J = 7.8 Hz), 7.76 (1H, d, J = 8.4 Hz), 7.70 (1H, d, J = 8.6 Hz), 7.68 (1H, d, J = 8.3 Hz), 7.62 (1H, dd, J = 1.5 Hz, J = 8.6 Hz), 7.52 (1H, app t, J = 7.9 Hz), 7.05 (1H, dd, J = 2.0 Hz, J = 8.7 Hz), 6.96 (1H, s), 3.88 (3H, s); MS (MALDI(+)) m/z 342 (M + Na).

To 305 mg (0.75 mmol) of methyl 3-[(7-amino-2-naphthyl)carbonylamino]benzoate hydrochloride was added 10 mL of chloroform, 3.5 mL of thionyl chloride, and 180 μL of pyridine. The mixture was allowed to stir at ambient temperature for 3 h, followed by removal of volatiles by rotary evaporation. The resulting residue was stripped 2 times from chloroform. Then 50 mL of chloroform, 124 mg (0.89 mmol) of methyl-3-aminobenzoate, and 100 μL of pyridine were added. The mixture was allowed to stir at ambient temperature for 16 h. The mixture was extracted twice with 1 N HCl (aqueous) and once with water. The dried organic layer ($MgSO_4$) was filtered, and the volatiles were removed by rotary evaporation. The resulting residue was treated with methanol to form a solid precipitate that was collected by vacuum filtration. This afforded 380 mg (93%) of the title compound as a white solid. ^{1}H NMR (DMSO-d_6) δ 10.59 (1H, s), 10.06 (1H, br s), 8.51 (1H, d, J = 1.7 Hz), 8.50 (1H, d, J = 9.0 Hz), 8.24 (1H, br s), 8.14 (1H, d, J = 9.1 Hz), 7.88−7.98 (5H, m), 7.80 (2H, d, J = 7.3 Hz), 7.72 (1H, d, J = 7.8 Hz), 7.63 (1H, br s), 7.54 (1H, app t, J = 7.9 Hz), 7.44 (2H, app t, J = 7.3 Hz), 7.37 (2H, app t, J = 7.3 Hz), 4.58 (2H, d, J = 6.4 Hz), 4.37 (1H, t, J = 6.3 Hz), 3.89 (3H, s); MS (MALDI(−)) m/z 542 (M − H).

***N*-[7-(Chlorosulfonyl)(2-naphthyl)]-*N*-(2-{*N*-[7-(chlorosulfonyl)(2-naphthyl)]acetylamino}ethyl)acetamide (63).** To 2.04 g (9.33 mmol) of 7-amino-2-naphthalenesulfonic acid was added 50 mL of dry DMF. The stirred suspension was heated at 110 °C, and then 1.4 g of potassium carbonate was added followed by 400 μL (4.6 mmol) of 1,2-dibromoethane. The mixture was kept at 110 °C for 18 h and at 80 °C for an additional 18 h. Then the mixture was allowed to cool to ambient temperature. The resulting insoluble precipitate was collected by vacuum filtration and washed with methanol. The crude product was purified by silica gel flash chromatography (6:2:1 ethyl acetate/isopropanol/water). This provided 596 mg (14%) of 7-({2-[(7-sulfo-2-naphthyl)amino]ethyl}amino)naphthalene-2-sulfonic acid as a tan solid. ^{1}H NMR (D_2O) δ 7.99 (2H, s), 7.78 (2H, d, J = 8.7 Hz), 7.63 (2H, d, J = 8.8 Hz), 7.51 (2H, d, J = 8.4 Hz), 7.01 (2H, dd, J = 2.1 Hz, J = 8.4 Hz), 7.00 (2H, s), 3.44 (4H, s); ^{13}C NMR (DMSO-d_6) δ 146.7, 145.6, 134.0, 127.7, 126.4, 125.9, 121.9, 119.0, 118.2, 103.3, 42.0; MS (MALDI(−)) m/z 494 (M − H+Na).

To a solution of 25 mL each of acetic anhydride and pyridine was added 2.13 g (4.51 mmol) of 7-({2-[(7-sulfo-2-naphthyl)-amino]ethyl}amino)naphthalene-2-sulfonic acid, and the mixture was allowed to stir at 70 °C for 16 h. The mixture was poured into 400 mL of THF and left to stir for 12 h. The resulting solid was collected by vacuum filtration. Then this solid was dissolved in 35 mL of methanol, and a solution of 0.273 g (11.9 mmol) of sodium in 10 mL of methanol was added. The solution was cooled in an ice bath and diluted with 100 mL of isopropyl alcohol. The resulting solid was collected by vacuum filtration. The solid was redissolved in a minimum of methanol, filtered through a 0.2 μm nylon filter, and then precipitated by the addition of isopropyl alcohol. Drying

in vacuo provided 2.01 g (74%) of 7-(*N*-{2-[N-(7-sulfo-2-naphthyl)-acetylamino]ethyl}acetylamino)naphthalene-2-sulfonic acid, disodium salt, the title compound as a white solid. ^{1}H NMR (DMSO-d_6) δ 8.12 (2H, s), 7.93 (2H, d, J = 7.3 Hz), 7.90 (2H, d, J = 7.9 Hz), 7.89 (2H, s), 7.74 (2H, d, J = 7.4 Hz), 7.32 (2H, d, J = 8.3 Hz), 3.88 (4H, s), 1.73 (6H, s); ^{13}C NMR (DMSO-d_6) δ 169.2, 146.4, 140.4, 132.3, 131.4, 128.8, 126.9, 126.7, 126.1, 124.5, 123.9, 46.3, 22.3; MS (ES(−)) m/z 555 (M − H).

To 511 mg (0.85 mmol) of compound **44** was added 50 mL of phosphorus oxychloride in an ice bath. The mixture was allowed to stir and warm slowly to room temperature. The mixture was allowed to stir for a total of 4 days. Then the mixture was poured onto 600 mL of ice and this suspension placed in a second ice bath. After the ice had melted, the yellow solid was collected by vacuum filtration. This provided 455 mg (90%) of the title compound (**63**) as a white solid. It was used immediately without characterization.

Methyl 5-{[(7-Amino(2-naphthyl))sulfonyl]amino}-2-chlorobenzoate (64). To 4.98 g (22.4 mmol) of methyl 5-amino-2-chlorobenzoate hydrochloride was added 50 mL of THF followed by 11 mL (0.136 mol) of pyridine. The stirred suspension was cooled in an ice bath, and then 5.30 g (18.7 mmol) of *N*-[7-(chlorosulfonyl)-2-naphthyl]acetamide (**46**) prepared above was added as a suspension in 50 mL of THF. The mixture was allowed to warm to ambient temperature and to stir for 16 h. All volatiles were removed by rotary evaporation, and the resulting goo was extracted with ethyl acetate and 1 N HCl. The organic layer was dried ($MgSO_4$) and filtered, and volatiles were removed to provide 6.63 g (82%) of methyl 5-{[(7-amino(2-naphthyl))sulfonyl]amino}-2-chlorobenzoate as a faint yellow solid: mp = 165−172 °C; ^{1}H NMR (DMSO-d_6) δ 10.77 (1H, s), 10.31 (1H, s), 8.42 (1H, s), 8.31 (1H, s), 8.01 (1H, d, J = 8.5 Hz), 7.95 (1H, d, J = 8.9 Hz), 7.74 (1H, dd, J = 1.8 Hz, J = 8.9 Hz), 7.63 (1H, dd, J = 1.7 Hz, J = 8.6 Hz), 7.56 (1H, d, J = 2.6 Hz), 7.43 (1H, d, J = 8.7 Hz), 7.32 (1H, dd, J = 2.7 Hz, J = 8.8 Hz), 3.81 (3H, s), 2.12 (3H, s); ^{13}C NMR (DMSO-d_6) δ 168.7, 164.6, 138.4, 136.8, 136.4, 132.0, 131.5, 130.7, 130.2, 129.1, 128.3, 127.1, 126.5, 123.7, 122.7, 121.5, 120.1, 115.7, 52.4, 23.9; MS (MALDI(+)) m/z 457 (M + Na).

To 4.81 g (11.1 mmol) of methyl 5-{[(7-amino(2-naphthyl))sulfonyl]amino}-2-chlorobenzoate was added 160 mL of 5 N NaOH and 7 mL of dioxane. The mixture was allowed to stir at 55 °C for 18 h. Then the mixture was diluted with 30 mL of water and filtered through a 0.2 μm nylon filter. The resulting clear filtrate was carefully acidified with 26 mL of concentrated H_2SO_4. The finely divided solid precipitate was collected by vacuum filtration and dried in vacuo. This produced 4.01 g (95%) of 5-{[(7-amino(2-naphthyl))sulfonyl]amino}-2-chlorobenzoic acid as a tan solid: mp = 160−165 °C; ^{1}H NMR (DMSO-d_6) δ 10.63 (1H, s), 8.00 (1H, s), 7.80 (1H, d, J = 8.4 Hz), 7.68 (1H, d, J = 8.8 Hz), 7.53 (1H, d, J = 2.6 Hz), 7.39 (1H, d, J = 8.7 Hz), 7.34 (1H, dd, J = 1.5 Hz, J = 8.7 Hz), 7.27 (1H, dd, J = 2.7 Hz, J = 8.7 Hz), 7.11 (1H, dd, J = 2.0 Hz, J = 8.7 Hz), 6.96 (1H, s); ^{13}C NMR (DMSO-d_6) δ 165.8, 147.4, 136.8, 136.1, 133.5, 131.6, 131.2, 128.9, 128.5, 127.5, 126.2, 124.8, 123.1, 121.4, 121.2, 116.6, 107.0; MS (ES(−)) m/z 375 (M − H).

To 10.1 g (0.027 mol) of 5-{[(7-amino(2-naphthyl))sulfonyl]amino}-2-chlorobenzoic acid dissolved in 250 mL of methanol was added 50 mL of 4 N HCl in dioxane. This solution was allowed to stir at ambient temperature for 18 h. The reaction was incomplete, so the solution was heated at reflux for an additional 5 h. Then the volatiles were removed by rotary evaporation and the resulting solid was extracted with ethyl acetate and 0.4 N sodium bicarbonate, water, and brine. The organic layer was dried ($MgSO_4$) and filtered, and the volatiles were removed by rotary evaporation to yield 8.71 g (83%) of the title compound as a tan solid. ^{1}H NMR (DMSO-d_6) δ 10.66 (1H, s), 7.98 (1H, d, J = 1.4 Hz), 7.78 (1H, d, J = 8.7 Hz), 7.66 (1H, d, J = 8.8 Hz), 7.56 (1H, d, J = 2.7 Hz), 7.43 (1H, d, J = 8.7 Hz), 7.31 (1H, dd, J = 1.8 Hz, J = 8.5 Hz), 7.30 (1H, dd, J = 2.8 Hz, J = 8.7 Hz), 7.08 (1H, dd, J = 2.2 Hz, J = 8.8 Hz), 6.91 (1H, d, J = 2.0 Hz), 5.71 (2H, s), 3.82 (3H, s); ^{13}C NMR (DMSO-d_6) δ 164.6, 148.0, 136.9, 135.9, 133.5, 131.3, 130.2, 128.8, 128.3, 127.2, 126.2, 124.7, 123.5, 121.4, 121.0, 116.3, 106.2, 52.2; MS (ES(−)) m/z 389 (M − H).

Methyl 2-Chloro-5-{[(7-isothiocyanato(2-naphthyl))sulfonyl]-amino}benzoate (65). To 4.5 g (11.5 mmol) of compound **64** and 9.01 g (50.6 mmol) of 1,1′-thiocarbonyldiimidazole was added 50 mL of THF. The solution was allowed to stir at ambient temperature for 1.5 h. Then the mixture was poured into 300 mL of ethyl acetate and extracted with 1 N HCl, water, and brine. The organic layer was dried ($MgSO_4$) and filtered, and the volatiles were removed by rotary evaporation to yield 4.77 g (96%) of the title compound as a tan solid. ^{1}H NMR (DMSO-d_6) δ 10.86 (1H, br s), 8.48 (1H, s), 8.27 (1H, d, J = 1.9 Hz), 8.16 (1H, d, J = 8.8 Hz), 8.10 (1H, d, J = 8.8 Hz), 7.80 (1H, dd, J = 1.8 Hz, J = 8.7 Hz), 7.72 (1H, dd, J = 2.0 Hz, J = 8.8 Hz), 7.54 (1H, d, J = 2.6 Hz), 7.44 (1H, d, J = 8.7 Hz), 7.30 (1H, dd, J = 2.7 Hz, J = 8.8 Hz), 3.81 (3H, s); MS (MALDI(−)) m/z 431 (M − H).

Methyl 5-({[7-({[(7-{[(3-Methoxycarbonyl-4-chlorophenyl)amino]sulfonyl}(2-naphthyl))amino]thioxomethyl}amino)(2-naphthyl)]-sulfonyl} amino)-2-chlorobenzoate (66). To 4.0 g (9.26 mmol) of compound **65** dissolved in 200 mL of dichloromethane was added 3.0 g (7.67 mmol) of compound **64**. The mixture was allowed to stir at ambient temperature for 16 h. A white solid had formed, which was collected by vacuum filtration. This provided 5.2 g (82%) of the title compound as a white solid: mp = 157 − 163 °C; ^{1}H NMR (DMSO-d_6) δ 10.80 (2H, s), 10.32 (2H, s), 8.40 (2H, s), 8.20 (2H, s), 8.07 (2H, d, J = 8.8 Hz), 7.98 (2H, d, J = 8.9 Hz), 7.85 (2H, dd, J = 1.9 Hz, J = 8.8 Hz), 7.70 (2H, dd, J = 1.7 Hz, J = 8.7 Hz), 7.57 (2H, d, J = 2.6 Hz), 7.43 (2H, d, J = 8.7 Hz), 7.32 (2H, dd, J = 2.7 Hz, J = 8.7 Hz), 3.80 (6H, s); MS (ES(−)) m/z 823 (M − H).

Methyl 5-({[7-(2-Amino-1-aza-2-{[7-({[4-chloro-3-(methoxycarbonyl)phenyl]amino}sulfonyl)(2-naphthyl)]amino}vinyl)(2-naphthyl)]sulfonyl}amino)-2-chlorobenzoate (67). To 1.0 g (1.21 mmol) of compound **66** dissolved in 50 mL of acetonitrile was added 1.1 mL of methyl iodide (17.7 mmol). The mixture was allowed to stir under an argon atmosphere for 72 h. The volatiles were removed by rotary evaporation, and the resulting yellow solid was extracted using ethyl acetate and 1 M sodium carbonate. The organic layer was washed with 50/50 brine/water followed by brine. The organic layer was separated and dried ($MgSO_4$), and volatiles were removed by rotary evaporation to yield 0.95 g (94%) of methyl 5-{[(7-{[(1*Z*)-2-aza-2-(7-{[(3-methoxycarbonyl-4-chlorophenyl)amino]sulfonyl}(2-naphthyl))-1-methylthiovinyl]amino}(2-naphthyl))sulfonyl]amino}-2-chlorobenzoate as a yellow solid. ^{1}H NMR (DMSO-d_6) δ 9.17 (1H, s), 8.40 (1H, s), 8.28 (1H, s), 8.21 (1H, s), 7.95 (1H, d, J = 9.2 Hz), 7.90 (1H, s), 7.90 (1H, d, J = 9.0 Hz), 7.88 (2H, s), 7.61 (2H, d, J = 8.6 Hz), 7.50 (1H, s), 7.45 (2H, dd, J = 2.1 Hz, J = 10.1 Hz), 7.33 (1H, dd, J = 1.8 Hz, J = 8.5 Hz), 7.30 (1H, app t, J = 8.5 Hz), 7.25 (1H, d, J = 8.8 Hz), 7.20 (1H, dd, J = 2.4 Hz, J = 8.9 Hz), 7.15 (1H, dd, J = 2.4 Hz, J = 8.9 Hz), 3.80 (3H, s), 3.74 (3H, s), 2.42 (3H, s); MS (ES(−)) m/z 836 (M − H).

To 220 mg (0.26 mmol) of the material obtained above was added 10 mL of a 0.5 M NH_3 solution in dioxane. The resulting solution was placed in a sealed tube and heated at 70 °C for 18 h, followed by 24 h at 80 °C. Then additional NH_3 gas was bubbled into the mixture for 4 min. The mixture was sealed, and heating at 80 °C continued for an additional 51 h. Then the reaction temperature was lowered to 65 °C, and the reaction continued for 11 days. At this point, the reaction had reached 70% completion based upon HPLC analysis. The reaction was stopped by removal of the volatiles using rotary evaporation. The product was purified by silica gel column chromatography, eluting with 3% methanol in dichloromethane followed by 5% methanol in dichloromethane. Finally, the product was eluted with 90:2:1 ethyl acetate/isopropanol/water. This provided 117 mg (56%) of the title compound as an off-white solid. This compound exists as a mixture of tautomers: ^{1}H NMR (DMSO-d_6) δ 10.65 (2H, br s), 8.27 (2H, s), 7.98 (3H, d, J = 8.8 Hz), 7.90 (3H, d, J = 8.9 Hz), 7.57 (6H, m), 7.42 (2H, d, J = 8.7 Hz), 7.31 (2H, dd, J = 2.6 Hz, J = 8.8 Hz), 5.30 (3H, br s), 3.80 (6H, s); MS (MALDI(+)) m/z 806 (M).

Autophosphorylation of Human Insulin Receptor Cytoplasmic Kinase Domain (IR CKD). An amount of 200 ng of IR CKD, "β-insulin receptor kinase" which is the entire cytoplasmic domain of the insulin receptor without any extracellular or transmembrane domain residues (Stratagene, Inc., San Diego, CA), was dissolved in 30 μL of 50 mM Tris, pH 7.4, 2 mM $MnCl_2$, 10 mM $MgCl_2$, 50 μM ATP, and [γ-^{32}P]ATP (10 μCi) and combined with different concentrations of either **1** or **3**, and the mixture was incubated for 10 min at 25 °C. For gel analysis, the samples were boiled in SDS−PAGE sample buffer for 5 min, electrophoresed on 10% SDS−PAGE gels, and visualized by autoradiography. Radioactivity was quantified by scintillation counting of the excised, labeled bands. For analysis by blotting, the samples were precipitated with trichloroacetic acid, spotted on a 96-well plate (Multiscreen, Millipore, Bedford, MA), washed three times with cold 75 mM phosphoric acid, air-dried, and quantified by scintillation counting.

Glucose Transport in Adipocytes and GLUT4 Translocation. 3T3-L1 fibroblasts (ATCC no. 173-CL) were cultured in 96-well plates and induced to differentiate into adipocytes by incubation in medium containing 1 μM dexamthasone, 0.5 mM isobutylmethylxanthine, and 1.7 μM insulin for 72 h.[44] The cells were shifted to media containing 1.7 μM insulin but without dexamthasone or isobutylmethylxanthine for 48 h. Finally, the cells were returned to normal supplemented medium for 4 days. The cells were serum-starved in medium containing 0.1% bovine serum albumin for 16 h, stimulated with various concentrations of compound with and without 5.6 nM insulin (a submaximal concentration; 100 nM insulin gives maximal response) for 30 min at 37 °C, and then incubated with 0.5 μCi/mL of 2-deoxy-D-[^{14}C]glucose for 30 min at 37 °C. The cells were rinsed with cold phosphate buffered saline and 20 μM glucose and lysed. The radioactivity was quantified by scintillation counting. The preparation of plasma membrane sheets and GLUT4 translocation was performed as described by Clancy and Czech.[45]

In Vivo Experiments. All animal protocols were approved by the Telik Institutional Animal Care and Use Committee. Outbred male CD IGS rats (250−300 g; Charles River, Wilmington, MA) were administered 60 mg/kg streptozotocin (STZ) by intraperitoneal (ip) injection. Blood samples were taken for glucose measurements 3 days post-STZ injection, and animals were enrolled in glucose tolerance test studies if their fed glucose values were ≥250 mg/dL. To assess effects of test compounds on oral glucose tolerance, animals were used 4 days post-STZ injection and fasted overnight (typically 12−16 h). On the day of the experiment, animals were weighed and blood samples for glucose analyses taken by tail incision before dosing (0 min time point). Animals were divided (using a spreadsheet-based randomization software application) into two groups (n = 12 per group) based on the time-zero glucose reading. Animals were treated with **30** or phosphate buffered saline administered either ip or orally (po). Ten minutes after dosing, animals were given a bolus of standard glucose solution (50% dextrose, 4 mL/kg) by gavage. Blood glucose was measured at the indicated times after glucose loading using a glucometer (Glucometer Elite, Bayer). Data are expressed as mean ± SEM values. Differences between mean values were assessed by the Student's t test. Statistical significance was accepted at $P < 0.05$. All analyses (and graphics) were performed using GraphPad Prism (MS Windows, version 4.0), GraphPad Software, San Diego, CA (www.graphpad.com).

Supporting Information Available: Table of purity of all target compounds along with HPLC tracings and conditions. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Zimmet, P.; Alberti, K. G.; Shaw, J. Global and societal implications of the diabetes epidemic. *Nature* **2001**, *414*, 782–787.

(2) Wild, S.; Roglic, G.; Green, A.; Sicree, R.; King, H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* **2004**, *27*, 1047–1053.

(3) DeFronzo, R. A. Pathogenesis of type 2 diabetes: metabolic and molecular implications for identifying diabetes genes. *Diabetes Rev.* **1997**, *5*, 177–269.

(4) Facchini, F. S.; Hua, N.; Abbasi, F.; Reaven, G. M. Insulin resistance as a predictor of age-related diseases. *J. Clin. Endocrinol. Metab.* **2001**, *86*, 3574–3578.

(5) Reaven, G. M. Role of insulin resistance in human disease. *Diabetes* **1988**, *37*, 1595–1607.

(6) Turner, R. C.; Cull, C. A.; Frighi, V.; Holman, R. R. Glycemic control with diet, sulfonylurea, metformin, or insulin in patients with type 2 diabetes mellitus. Progressive requirement for multiple therapies (UKPDS 49). *JAMA, J. Am. Med. Assoc.* **1999**, *281*, 2005–2012.

(7) Evans, J. L.; Rushakoff, R. J. Oral Pharmacological Agents for Type 2 Diabetes: Sulfonylureas, Meglitinides, Metformin, Thiazolidinediones, α-Glucosidase Inhibitors, and Emerging Approaches. In *Endotext.com: Diabetes and Carbohydrate Metabolism*, 1st ed.; Degroot, L., Goldfine, I. D., Rushakoff, R. J., Eds.; Medtext, Inc.: Burr Ridge, IL, 2007; http://www.medtext.com.

(8) Pfeifer, M.; Halter, J. B.; Beard, J. C.; Porte, D., Jr. Differential effects of tolbutamide on first and second phase insulin secretion in noninsulin-dependent diabetes mellitus. *J. Clin. Endocrinol. Metab.* **1981**, *53*, 1256–1262.

(9) Lebovitz, H. E. Insulin Secretagogues: Sulfonylureas and Meglitinides. In *Diabetes Mellitus: A Fundamental and Clinical Text*, 2nd ed.; LeRoith, D., Taylor, S. I., Olefsky, J. M., Eds.; Lippincott Williams & Wilkins: Philadelphia, PA, 2000; pp 769−778.

(10) Stumvoll, M.; Nurjhan, N.; Perriello, G.; Dailey, G.; Gerich, J. E. Metabolic effects of metformin in non-insulin-dependent diabetes mellitus. *N. Engl. J. Med.* **1995**, *333*, 550–554.

(11) Zhou, G.; Myers, R.; Li, Y.; Chen, Y.; Shen, X.; Fenyk-Melody, J.; Wu, M.; Ventre, J.; Doebber, T.; Fujii, N.; Musi, N.; Hirshman, M. F.; Goodyear, L. J.; Moller, D. E. Role of AMP-activated protein kinase in mechanism of metformin action. *J. Clin. Invest.* **2001**, *108*, 1167–1174.

(12) Bischoff, H. Pharmacology of alpha-glucosidase inhibition. *Eur. J. Clin. Invest.* **1994**, *24* (Suppl. 3), 3–10.

(13) Berger, J.; Moller, D. E. The mechanisms of action of PPARs. *Annu. Rev. Med.* **2002**, *53*, 409–435.

(14) Moller, D. E. New drug targets for type 2 diabetes and the metabolic syndrome. *Nature* **2001**, *414*, 821–827.

(15) Sharma, A. M.; Staels, B. Review: peroxisome proliferator-activated receptor gamma and adipose tissue—understanding obesity-related changes in regulation of lipid and glucose metabolism. *J. Clin. Endocrinol. Metab.* **2007**, *92*, 386–395.

(16) Deacon, C. F.; Ahren, B.; Holst, J. J. Inhibitors of dipeptidyl peptidase IV: a novel approach for the prevention and treatment of type 2 diabetes. *Expert Opin. Invest. Drugs* **2004**, *13*, 1091–1102.

(17) Richter, B.; Bandeira-Echtler, E.; Bergerhoff, K.; Clar, C.; Ebrahim, S. H. Rosiglitazone for type 2 diabetes mellitus. *Cochrane Database Syst. Rev.* **2007**, CD006063.

(18) Devchand, P. R. Glitazones and the cardiovascular system. *Curr. Opin. Endocrinol. Diabetes Obes.* **2008**, *15*, 188–192.

(19) Bolen, S.; Feldman, L.; Vassy, J.; Wilson, L.; Yeh, H. C.; Marinopoulos, S.; Wiley, C.; Selvin, E.; Wilson, R.; Bass, E. B.; Brancati, F. L. Systematic review: comparative effectiveness and safety of oral medications for type 2 diabetes mellitus. *Ann. Intern. Med.* **2007**, *147*, 386–399.

(20) Ginsberg, H. N. Insulin resistance and cardiovascular disease. *J. Clin. Invest.* **2000**, *106*, 453–458.

(21) Kahn, B. B.; Flier, J. S. Obesity and insulin resistance. *J. Clin. Invest.* **2000**, *106*, 473–481.

(22) Ward, C.; Lawrence, M.; Streltsov, V.; Garrett, T.; McKern, N.; Lou, M. Z.; Lovrecz, G.; Adams, T. Structural insights into ligand-induced activation of the insulin receptor. *Acta Physiol. (Oxford)* **2008**, *192*, 3–9.

(23) White, M. F. Regulating insulin signaling and beta-cell function through IRS proteins. *Can. J. Physiol. Pharmacol.* **2006**, *84*, 725–737.

(24) Watson, R. T.; Pessin, J. E. GLUT4 translocation: the last 200 nanometers. *Cell. Signalling* **2007**, *19*, 2209–2217.

(25) Huang, S.; Czech, M. P. The GLUT4 glucose transporter. *Cell Metab.* **2007**, *5*, 237–252.

(26) Zhang, B. B.; Salituro, G.; Szalkowski, D.; Li, Z. H.; Zhang, Y.; Royo, I.; Vilella, D.; Diez, M. T.; Pelaez, F.; Ruby, C.; Kendall, R. L.; Mao, X. Z.; Griffin, P.; Calaycay, J.; Zierath, J. R.; Heck, J. V.; Smith, R. G.; Moller, D. E. Discovery of a small molecule insulin mimetic with antidiabetic activity in mice. *Science* **1999**, *284*, 974–977.

(27) Liu, K.; Xu, L.; Szalkowski, D.; Li, Z.; Ding, V.; Kwei, G.; Huskey, S.; Moller, D. E.; Heck, J. V.; Zhang, B. B.; Jones, A. B. Discovery of a potent, highly selective, and orally efficacious small-molecule activator of the insulin receptor. *J. Med. Chem.* **2000**, *43*, 3487–3494.

(28) Wood, H. B., Jr.; Black, R.; Salituro, G.; Szalkowski, D.; Li, Z.; Zhang, Y.; Moller, D. E.; Zhang, B. B.; Jones, A. B. The basal SAR of a

novel insulin receptor activator. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1189–1192.
(29) Qureshi, S. A.; Ding, V.; Li, Z.; Szalkowski, D.; Biazzo-Ashnault, D. E.; Xie, D.; Saperstein, R.; Brady, E.; Huskey, S.; Shen, X.; Liu, K.; Xu, L.; Salituro, G. M.; Heck, J. V.; Moller, D. E.; Jones, A. B.; Zhang, B. B. Activation of insulin signal transduction pathway and anti-diabetic activity of small molecule insulin receptor activators. *J. Biol. Chem.* **2000**, *275*, 36590–36595.
(30) Strowski, M. Z.; Li, Z.; Szalkowski, D.; Shen, X.; Guan, X. M.; Juttner, S.; Moller, D. E.; Zhang, B. B. Small-molecule insulin mimetic reduces hyperglycemia and obesity in a nongenetic mouse model of type 2 diabetes. *Endocrinology* **2004**, *145*, 5259–5268.
(31) Beroza, P.; Damodaran, K.; Lum, R. T. Target-related affinity profiling: Telik's lead discovery technology. *Curr. Top. Med. Chem.* **2005**, *5*, 371–381.
(32) Manchem, V. P.; Goldfine, I. D.; Kohanski, R. A.; Cristobal, C. P.; Lum, R. T.; Schow, S. R.; Shi, S.; Spevak, W. R.; Laborde, E.; Toavs, D. K.; Villar, H. O.; Wick, M. M.; Kozlowski, M. R. A novel small molecule that directly sensitizes the insulin receptor *in vitro* and *in vivo*. *Diabetes* **2001**, *50*, 824–830.
(33) Li, M.; Youngren, J. F.; Manchem, V. P.; Kozlowski, M. R.; Zhang, B. B.; Maddux, B. A.; Goldfine, I. D. Small molecule insulin receptor activators potentiate insulin action in insulin resistant cells. *Diabetes* **2001**, *50*, 2323–2328.
(34) Koguro, K.; Oga, T.; Mitsui, S.; Orita, R. Novel synthesis of 5-substituted tetrazoles from nitriles. *Synthesis* **1998**, 910–914.
(35) Gray, G. W.; Jones, B. The preparation of 4- and 5-*n*-alkoxy-1-naphthoic and 6- and 7-*n*-alkoxy-2-naphthoic acids. *J. Chem. Soc.* **1954**, *1954*, 678–682.
(36) Cheng, M.; Chen, S.; Schow, S. R.; Manchem, V. P.; Spevak, W. R.; Cristobal, C. P.; Shi, S.; Macsata, R. W.; Lum, R. T.; Goldfine, I. D.; Keck, J. G. In vitro and in vivo prevention of HIV protease inhibitor-induced insulin resistance by a novel small molecule insulin receptor activator. *J. Cell. Biochem.* **2004**, *92*, 1234–1245.
(37) Etter, M. C.; Panunto, T. W. 1,3-Bis(*m*-nitrophenyl)urea: an exceptionally good complexing agent for proton acceptors. *J. Am. Chem. Soc.* **1988**, *110*, 5896–5897.
(38) Dannecker, W.; Kopf, J.; Rust, H. *N*,*N*′-Diphenylurea, $C_{13}H_{12}N_2O$. *Cryst. Struct. Commun.* **1979**, *8*, 429–432.
(39) Ganis, P.; Avitabile, G.; Benedetti, E.; Pedone, C.; Goodman, M. Crystal and molecular structure of *N*,*N*′-diethyl-*N*,*N*′-diphenylurea. *Proc. Natl. Acad. Sci. U.S.A.* **1970**, *67*, 426–433.
(40) Lozanova, C.; Galabov, B.; Ilieva, M.; Vassilev, G. Infrared intensities and conformation of substituted thioureas. *J. Mol. Struct.* **1984**, *115*, 427–430.
(41) Tanatani, A.; Yamaaguchi, K.; Azumaya, I.; Fukutomi, R.; Shudo, K.; Kagechika, H. *N*-Methylated diphenylguanidines: conformations, propeller-type molecular chirality, and construction of water-soluble oligomers with multilayered aromatic structures. *J. Am. Chem. Soc.* **1998**, *120*, 6433–6422.
(42) Carr, A.; Samaras, K.; Chisholm, D. J.; Cooper, D. A. Pathogenesis of HIV-1-protease inhibitor-associated peripheral lipodystrophy, hyperlipidaemia, and insulin resistance. *Lancet* **1998**, *351*, 1881–1883.
(43) Carr, A.; Samaras, K.; Burton, S.; Law, M.; Freund, J.; Chisholm, D. J.; Cooper, D. A. A syndrome of peripheral lipodystrophy, hyperlipidaemia and insulin resistance in patients receiving HIV protease inhibitors. *AIDS* **1998**, *12*, F51−F58.
(44) Frost, S. C.; Lane, M. D. Evidence for the involvement of vicinal sulfhydryl groups in insulin-activated hexose transport by 3T3-L1 adipocytes. *J. Biol. Chem.* **1985**, *260*, 2646–2652.
(45) Clancy, B. M.; Czech, M. P. Hexose transport stimulation and membrane redistribution of glucose transporter isoforms in response to cholera toxin, dibutyryl cyclic AMP, and insulin in 3T3-L1 adipocytes. *J. Biol. Chem.* **1990**, *265*, 12434–12443.

JM800600V