Polycationic Sulfonamides for the Sequestration of Endotoxin

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Lipopolysaccharides (LPS) play a key role in the pathogenesis of septic shock, a major cause of mortality in the critically ill patient. We had previously shown that monoacylated polyamine compounds specifically bind to and neutralize the activity of LPS with high in vitro potency and afford complete protection in a murine model of endotoxic shock. Fatty acid amides of polyamines may be rapidly cleared from systemic circulation due to their susceptibility to nonspecific serum amidases and, thus, would be predicted to have a short duration of action. In a systematic effort to increase the likelihood of better bioavailability properties together with structural modifications that may result in gains in activity, we now report structure−activity relationships pertaining to endotoxin-binding and -neutralizing activities of homologated polyamine sulfonamides.

Introduction

Endotoxins, or lipopolysaccharides (LPS[a]), are outer membrane constituents of Gram-negative bacteria.[1] A pivotal role has been implicated for LPS in the pathogenesis of septic shock, a syndrome of systemic toxicity that occurs as a consequence of serious systemic infections (Gram-negative sepsis).[2] Gram-negative sepsis is the number one cause of deaths in the intensive care unit,[3] accounting for more than 200,000 fatalities in the U.S. annually.[4]

The syndrome of multiple system organ failure, a hallmark of septic shock, appears to be a consequence of a precipitous activation of the innate immune response[5,6] leading to the uncontrolled production of a host of proinflammatory mediators, including cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6), primarily by cells of the monocyte/macrophage lineage,[7,8] as well as reactive oxygen intermediates, eicosanoids, and proteases from neutrophils that, in concert, act to cause severe tissue damage.[9] It has been demonstrated by total synthesis[10−13] that the toxicity of LPS resides in its structurally highly conserved glycolipid component called lipid A.[14,15] Lipid A is composed of a hydrophilic, bisphosphorylated diglucosamine backbone and a hydrophobic domain of six (*E. coli*) or seven (*Salmonella*) acyl chains in amide and ester linkages[16−18] (Figure 1). The incidence of sepsis has risen almost 3-fold from 1979 through 2000,[19] and sepsis-associated mortality has essentially remained unchanged at about 45%.[20] The only available modality for the therapy of sepsis is drotrecogin-alfa (activated recombinant protein C),[21] an antithrombotic agent whose efficacy has recently been called into question.[22−24] The urgent unmet need to develop therapeutic options specifically targeting the pathophysiology of sepsis has spurred a number of approaches, notable among which are E5564, a synthetic lipid A analog[25−28] that acts as an antagonist at the toll-like 4 receptor,[29,30] the principal receptor for LPS, and TAK-242,[31,32] a small molecule inhibitor of cytokine production. Both of these agents are currently in phase III clinical trials.

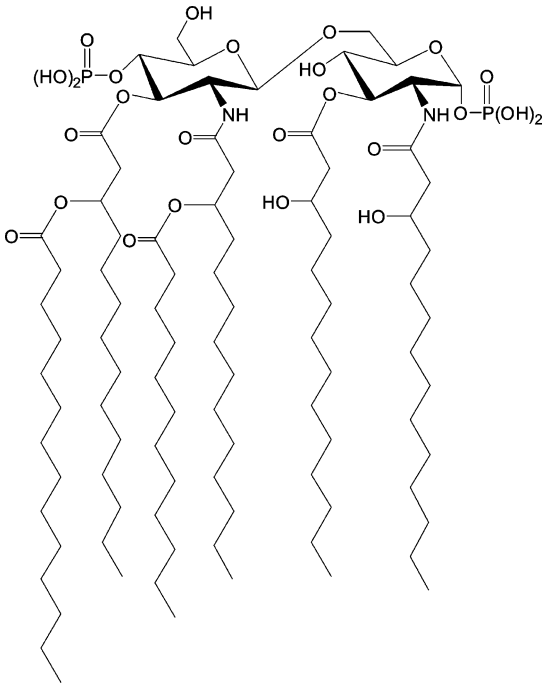

Figure 1. Structure of lipid A, the toxic moiety of bacterial lipopolysaccharide.

One possible approach to therapeutically addressing the problem of Gram-negative sepsis has been to target LPS itself by the use of an agent that would bind to and sequester it. Polymyxin B (PMB) is a membrane-active peptide antibiotic known to sequester LPS and abrogate its toxicity. The pronounced oto- and nephrotoxicity of PMB precludes its systemic use and has led to the development of an extracorporeal hemoperfusion cartridge based on PMB covalently immobilized on a polystyrene-based fiber.[33−35] Approved for clinical use in Japan in late 2000, this provides a clinically validated proof-of-concept of the therapeutic potential of sequestering circulating

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[a] Abbreviations: LPS, lipopolysaccharide; PMB, polymyxin B; IL-1β, interleukin-1β; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; NO, nitric oxide

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LPS. A major goal in our laboratory has been to develop small-molecule analogues of PMB that would sequester LPS with a potency at least equivalent to that of PMB and, importantly, be nontoxic and safe, so that it can be used parenterally for the prophylaxis or therapy of Gram-negative sepsis.

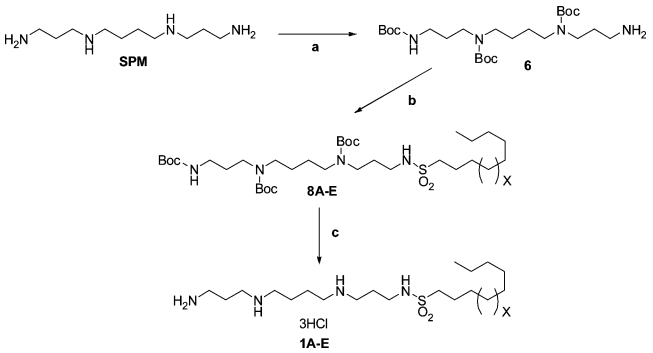
We have, over the past decade, characterized the interactions of lipid A with a number of classes of cationic amphipathic molecules including proteins,[36,37] peptides,[38−42] pharmaceutical compounds,[43,44] and other synthetic polycationic amphiphiles.[45−48] From these studies, we have determined that the pharmacophore necessary for optimal recognition and neutralization of lipid A[44] by small molecules requires two protonatable positive charges so disposed that the distance between them is equivalent to the distance between the two anionic phosphates on lipid A (∼14 Å), enabling ionic H-bonds between the phosphates on the lipid A backbone and the positive charges on the compound. These structural requisites are exemplified in the lipopolyamines, which are of particular interest because they are active in vitro and afford protection in animal models of Gram-negative sepsis, are synthetically easily accessible, and, importantly, are nontoxic, on account of their degradation to physiological substituents (spermine and fatty acid).[46,49−51] Fatty acid amides of polyamines may be rapidly cleared from systemic circulation due to their susceptibility to nonspecific serum amidases and, thus, might be predicted to have a short duration of action. In a systematic effort to increase the likelihood of better bioavailability properties together with structural modifications that may result in gains in activity, we have prepared and characterized the homologated polyamine sulfonamides described in this report.

Results and Discussion

Synthesis of Polyamine Sulfonamides: Synthesis of the N^1-sulfonylated spermine analogs was accomplished most simply by the direct sulfonylation of spermine in CH_2Cl_2 solution.[52] This results in a mixture of mono- and bis-sulfonylated species being generated through reaction with the primary amines of spermine. Evidence supporting predominant primary versus secondary amine reactivity is discussed below. Careful chromatography can provide pure products from this method, but an improvement was enabled through the use of tri-Boc-protected spermine **6**. The synthesis of this useful intermediate[53] has been described by Blagbrough and Geall[54] and subsequently modified by Wellendorph et al.[55] Regiospecific sulfonylation of **6** followed by column chromatography and subsequent HCl-mediated deprotection was used to produce the spermine sulfonamide analogs in Series 1 (SPM; Scheme 1; see Table 1 analogs **1A**−**E**). The homologated spermine analogs (HOMO−SPM) shown in Table 1 were produced via monoalkylation using a Michael reaction between acrylonitrile and **6** (Scheme 2). Boc-carbamate protection of the resulting secondary amine followed by reduction of the nitrile using $Pd(OH)_2$ in CH_3CO_2H gave the desired homologated intermediate **9**[56,57] in pure form following column chromatography. Synthesis of the homologated spermine sulfonamides (HOMO−SPM) belonging to Series 2 was then accomplished by sulfonylation of the primary amine of this intermediate. Chromatography followed by acid-mediated deprotection gave the desired molecules in pure form (Table 1; analogs **2A**−**E**).

Utilization of the di-Boc-spermine **7** product obtained during the synthesis tri-Boc-spermine intermediate **6** production for the synthesis of the Series 3 analogs was accomplished by the route depicted in Scheme 3. The bis-acylonitrile spermine adduct formed from **7** and two equivalents of acrylonitrile was treated directly with Boc_2O, and the resulting tetracarbamate **11** was isolated in pure form by column chromatography in 61% yield. This bis-cyano product was converted to the bis-amino product **12** by catalytic hydrogenation using $Pd(OH)_2$ in CH_3CO_2H in a 71% yield. Standard sulfonylation followed by acid deprotection gave the bis-sub-bis-HOMO−SPM Series 3 analogs **3A**−**E** shown in Table 1.

Scheme 1. Synthesis of Series 1 Compounds (SPM)[a]

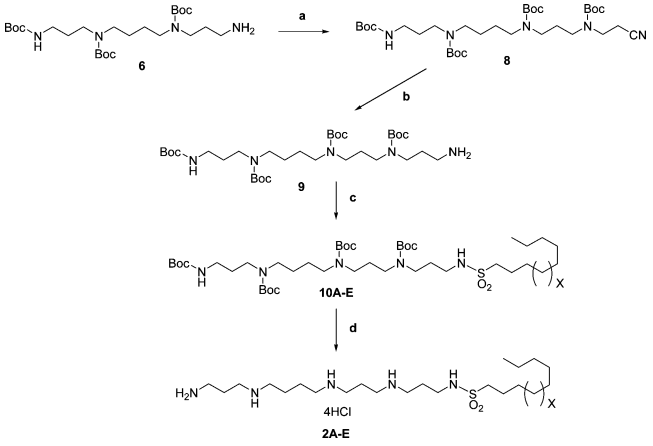


[a] Reagents and conditions: (a) (i) F_3CCO_2Et, MeOH, −78 °C; (ii) Boc_2O; (iii) NaOH aq, 48%; (b) RSO_2Cl, Et_3N, THF, 57%; (c) HCl/CH_3OH, 100%.

Dialkylation of the primary amine of tri-Boc-spermine **6** or the homologated tetra-Boc-carbamate intermediate **9** could be accomplished using an excess of acrylonitrile at an elevated temperature, as shown by Schemes 4 and 5, respectively. A literature report showed that the addition of a catalytic amount of cation exchange resin facilitated the production of the di-adduct.[58] Application of this technique to the present problem helped to drive the conversion to the di-adducts **14** and **17**, which could be obtained following column chromatography in 90% and 46% yields, respectively. Hydrogenation using $Pd(OH)_2$ in CH_3CO_2H again gave the desired product in good yield. Sulfonylation, column chromatography, and HCl-mediated Boc deprotection then gave the branched-SPM (analogs **4A**−**E**) and branched-HOMO−SPM (analogs **5A**−**E**) compounds shown in Table 1 (Series 4 and 5, respectively).

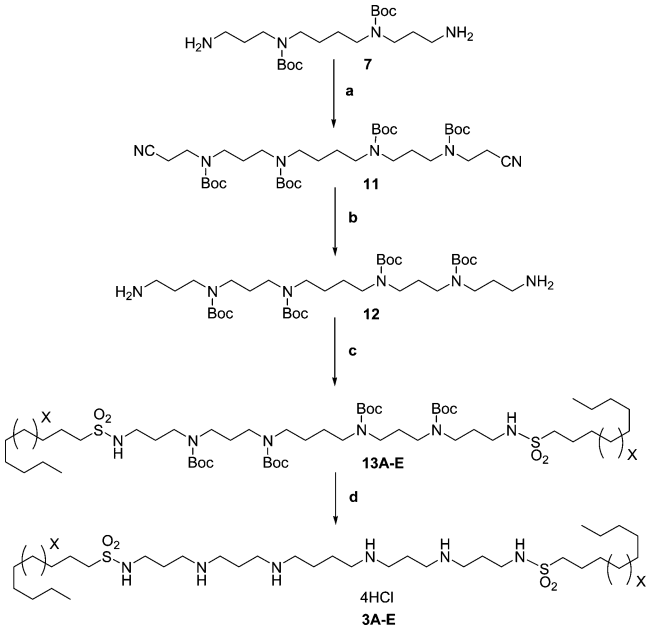
The various analogs shown in Table 2 were synthesized by direct sulfonylation of the modified polyamines using the direct conditions given above. These analogs were purified by column chromatography over silica gel using 80:18:2 CH_2Cl_2/MeOH/concd NH_4OH followed by conversion to their per-HCl salt forms for characterization. Several examples such as **31** and **32** were synthesized using the corresponding acid chloride instead of sulfonyl chloride. The special case of **22** deserves mentioning here. We produced this analog via the regiospecific sulfonylation of the secondary amino group of spermine through the use of previously described N^1, N^{14}-bistrifluoroacetylspermine diTFA salt.[59] Workup, removal of TFA groups using NaOH in MeOH, followed by chromatography gave **22** in pure form. Comparison of this material and its primary regioisomer **1D** (made either by the direct or tri-Boc-spm sulfonylation methodologies) using LC/MS demonstrated that they were distinct species (different retention times, see Supporting Information). This analysis furthermore showed that **1D** synthesized by the direct spermine sulfonylation route contained a minor amount of the secondary sulfonamide impurity. This drop in the regiospecificity may be unique for sulfonylation of spermine because we know of no literature reference to loss of primary/secondary amine selectivity during carbonyl acylation reactions on spermine. Because of this, all biological data presented in the following discussion utilized materials synthesized using the regiospecific, Boc-protected, intermediate routes for the compounds shown in Table

Scheme 2. Synthesis of Series 2 Compounds (HOMO−SPM)[a]



[a] Reagents and conditions: (a) (i) CH_2═CHCN, MeOH, 25 °C; (ii) Boc_2O, 66%; (b) $Pd(OH)_2$, HOAc, H_2, 71%; (c) RSO_2Cl, Et_3N, THF, 65%; (d) HCl/CH_3OH, 97%.

Scheme 3. Synthesis of Series 3 Compounds (Bis-Sub-Bis-HOMO−SPM)[a]

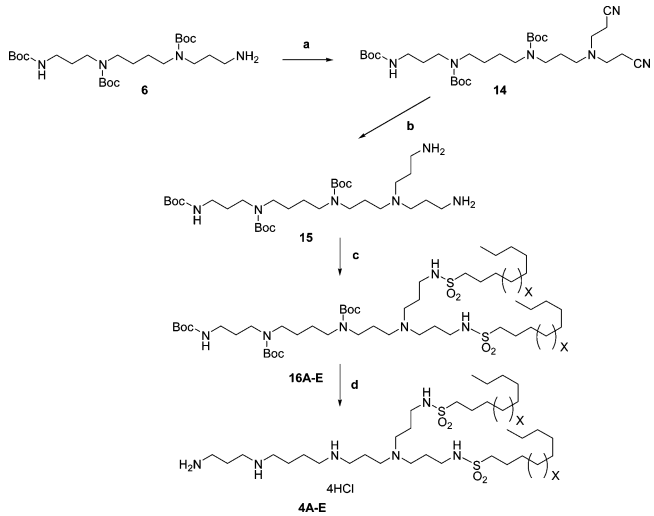


[a] Reagents and conditions: (a) (i) CH_2═CHCN (2 equiv), MeOH; (ii) Boc_2O, CH_2Cl_2, 61%; (b) $Pd(OH)_2$, HOAc, H_2, 71%; (c) RSO_2Cl, Et_3N, THF, 23%; (d) HCl/CH_3OH, 100%.

1. Analyses of these analogs by ^{1}H-NMR, LC/MS, and elemental analysis gave results consistent with their structures.

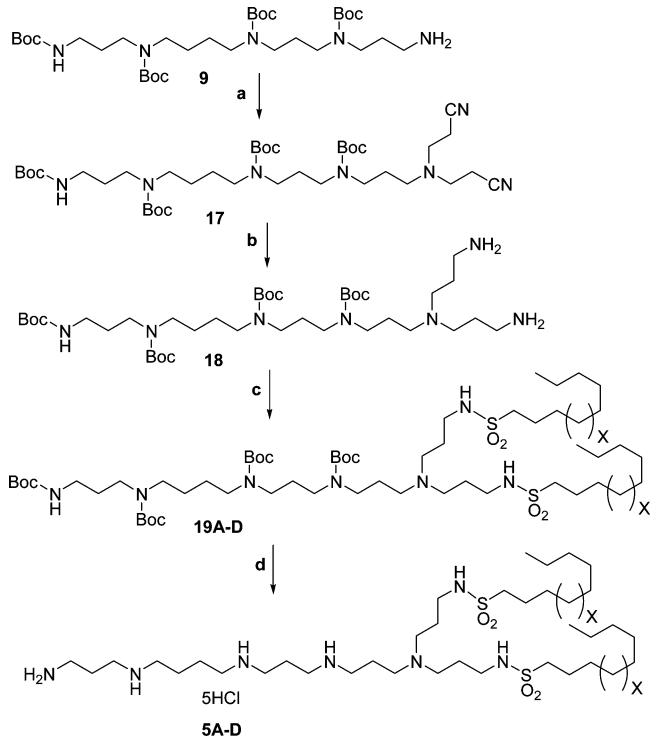
Affinity for Binding to Lipopolysaccharide and In Vitro Endotoxin Neutralizing Activity: All analogs were evaluated for binding affinity to LPS using a high-throughput fluorescent dye displacement assay (ED_{50} values) together with two cell-based assays measuring the inhibition of LPS-induced nitric oxide (NO) and NFκB production. There was a progressive increase in cell-based LPS-sequestering activities between C8 and C16 analogs for the monosulfonamide analogs in Series 1 and 2, although this trend is not paralleled in corresponding enhancements in binding affinities (Table 1 and Figure 2). For example, the SPM analog **1D** (C16) showed high affinity to LPS (ED_{50} value of 3.87 μM) and potent ability to inhibit the cell-based LPS-induced cytokine production (NO IC_{50} value of 0.45 μM; NFκB IC_{50} value of 0.28 μM). Analog **2D** (Series 2; HOMO−SPM; C16) also showed similarly high LPS-neutralizing potency (NO IC_{50} value of 0.12 μM; NFκB IC_{50} value of

Scheme 4. Synthesis of Series 4 Compounds (BRANCHED-SPM)[a]



[a] Reagents and conditions: (a) CH_2═CHCN (excess), MeOH, catalytic Dowex 50W×400 (H^+ form), reflux, 90%; (b) $Pd(OH)_2$, HOAc, H_2, 90%; (c) RSO_2Cl, Et_3N, THF, 15%; (d) HCl/CH_3OH, 75%.

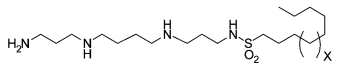
Scheme 5. Synthesis of Series 5 Compounds (Branched-HOMO−SPM)[a]



[a] Reagents and conditions: (a) CH_2═CHCN (excess), MeOH, catalytic Dowex 50W×400 (H^+ form), reflux, 46%; (b) $Pd(OH)_2$, HOAc, H_2, 95%; (c) RSO_2Cl, Et_3N, THF, 62%; (d) HCl/CH_3OH, 96%.

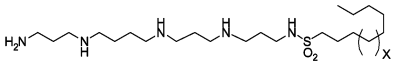
0.20 μM) and an LPS affinity value of 4.71 μM. C8 chain length containing analogs **1A** and **2A** had comparable ED_{50} values of 6.64 and 2.84 μM, respectively, whereas their NO inhibition values (1.44 and 6.47 μM) and NFκB inhibition values (33.3 and 0.816 μM) were significantly diminished in comparison to their more lipophilic counterparts (between a 3- and 54-fold diminished NO inhibition and between a 3- and 166-fold diminished NFκB inhibition). We ascribe the lack of significant change in ED_{50} values (binding affinities) to the fact the fluorescent displacement assay is very sensitive to electrostatic interactions between ligand and LPS, but does not adequately

Table 1. Binding Affinity (BC Displacement; ED_{50}) and Biological Activity (NO Inhibition in Murine J774 Cells; IC_{50}; NF$\kappa\beta$ Inhibition, IC_{50}) of Monosubstituted Spermine-Sulfonamide Analogs (SPM, Series 1), Monosubstituted Homologated Spermine-Sulfonamide Analogs (HOMO−SPM, Series 2), Bis-Substituted Bis-Homologated Spermine-Sulfonamide Analogs (Bis-Sub-Bis-HOMO−SPM, Series 3), Disubstituted Branched Spermine-Sulfonamide Analogs (Branched-SPM, Series 4), Disubstituted Branched Homologated Spermine-Sulfonamide Analogs (Branched-HOMO−SPM, Series 5



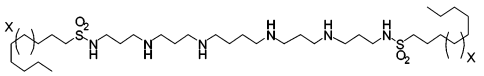
Series 1

MQTS	X	C-Number	ED_{50} (μM)	NO inhibition IC_{50} (μM)	NFκβ inhibition IC_{50} (μM)
1A	0	C8	6.64	1.44	33.3
1B	2	C10	3.7	4.42	0.50
1C	4	C12	2.69	1.34	0.41
1D	8	C16	3.87	0.45	0.28
1E	10	C18	6.01	0.48	0.36



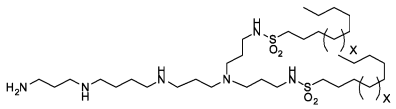
Series 2

2A	0	C8	2.84	6.47	0.816
2B	2	C10	2.89	1.09	0.278
2C	4	C12	2.92	0.39	0.190
2D	8	C16	4.71	0.12	0.200
2E	10	C18	4.45	0.23	1.06



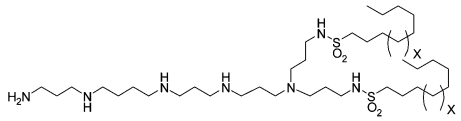
Series 3

MQTS	X	C-Number	ED_{50} (μM)	NO inhibition IC_{50} (μM)	NFκβ inhibition IC_{50} (μM)
3A	0	C8 X 2	2.6	0.577	0.607
3B	2	C10 X 2	13.5	0.692	4.2
3C	4	C12 X 2	55.2	5.00	10.3
3D	8	C16 X 2	132.6	6.3	11.7
3E	10	C18 X 2	5000	4.22	6.49



Series 4

4A	0	C8 X 2	2.17	1.82	0.669
4B	2	C10 X 2	2.40	1.10	5.17
4C	4	C12 X 2	3.98	2.27	13.2
4D	8	C16 X 2	26.4	15.1	4.85
4E	10	C18 X 2	28.3	2330	64



Series 5

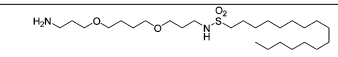
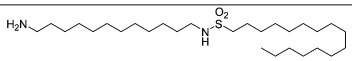
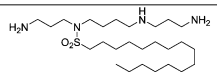
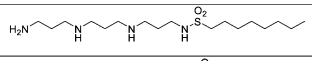
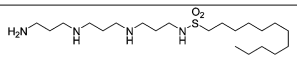
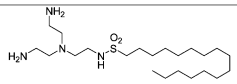
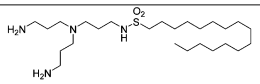
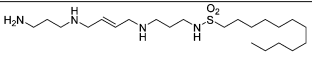
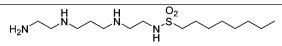
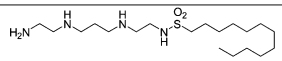
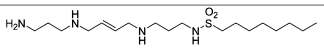
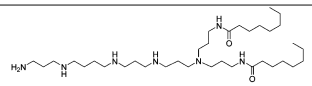
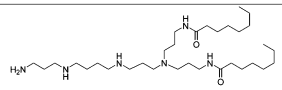
5A	0	C8 X 2	2.62	0.32	0.30
5B	2	C10 X 2	1.16	0.43	2.2
5C	4	C12 X 2	6.82	0.51	16.4
5D	8	C16 X 2	9.31	5.2	2.68

report hydrophobic interactions.[60−62] Furthermore, the cell-based cytokine inhibition data reflects a more complex, multistep process.

In contract to monosulfonamides discussed above, the bis-sulfonamide analogs (Series 3), as well as branched-chain compounds (Series 4 and 5), showed an apparent inverse relationship between carbon lengths and binding affinity and antiendotoxic activity. Maximal affinity and activity was observed with C8 substitutions (**3A**, **4A**, **5A)** and decreased with longer hydrophobic groups. This, however, is not inconsistent with the results obtained with the monosulfonamides for the compounds in Series 3−5 have two hydrophobic groups in sulfonamide linkages, and the net carbon number for the C8 analogs is 16. The additional hydrophobicity gained with longer appendages results in lower aqueous solubility, leading to an apparent decrease in activity, as has been noted in other lipopolyamines we examined earlier.[51,63,64]

To further define the molecular requirements of this inhibition, a variety of analogs shown in Table 2 were produced. The absolute requirement for a poly-charged species is demonstrated by oxa- and carba-analogs **20** and **21**. These nonpolyamine analogs showed much weaker activities, suggesting that the internal secondary amines (H-bond donor atoms) may contribute to the enthalpy of binding via additional H-bonds with the glycosidic backbone of lipid A (see Figure 1). This conjecture is also supported by the observation that **22**, which is substituted on one of its internal secondary amines, shows greatly diminished activity compared to its primary amine-substituted regioisomer **1D**. Additional analogs containing the same number of charges, but with differing spacing relative to spermine in

Table 2. Binding Affinity (BC Displacement; ED_{50}) and Biological Activity (NO Inhibition in Murine J774 Cells; IC_{50}; NF$\kappa\beta$ Inhibition, IC_{50}) of Miscellaneous Backbone-Modified Polyamine-Sulfonamide Analogs (MISC-PA, Series 6)

MQTS	Structure	ED_{50} value (μM)	NO inhibition IC_{50} value (μM)	NFκβ inhibition IC_{50} value (μM)
20		>5000	30.9	22.4
21		---	---	200
22		45.6	13.7	2.68
23		6.7	---	5.56
24		4.27	---	0.363
25		3.54	---	0.379
26		12.3	---	9.19
27		3.83	---	0.522
28		10.6	---	13.8
29		4.77	---	0.686
30		12.45	---	12.72
31		1	2.48	3.17
32		1.32	9.02	9.76

the polyamine backbone, maintain binding affinity and potency. For instance, the norspermine backbone of **24** has a diaminopropane central segment in contradistinction to the diaminobutane fragment in spermine. Analog **27** contains an unsaturated diaminobut-2-ene central core. Both of these compounds displayed good activity.

Two observations were somewhat counterintuitive and are worth noting. The branched carboxamide analogs **31** and **32** (Table 2), despite having good affinity for LPS in the binding assays (1.32 μM and 1.00 μM, respectively), showed greatly diminished biological activity in comparison to their sulfonamide counterparts (**4A** and **5A**); (NFκB IC_{50}: 9.76 μM vs 0.669 μM for carboxamide/sulfonamide branched-SPM pair, and 3.17 μM vs 0.30 μM for carboxamide/sulfonamide branched-HOMO−SPM pair). We do not yet understand the structural basis of this apparent discrepancy but it suggests higher activity in sulfonamide analogs. The observation that the sulfonamides exhibit significantly higher activity in comparison to their carboxamide counterparts is further supported by comparing to some homologated analogs previously reported by this laboratory.[51] The best in the sulfonamide series show low nanomolar NO and cytokine-release inhibition activity (e.g., analog **2D**: 0.12 and 0.20 μM), whereas the comparable carboxamide analog (e.g., **4e** 4.22 and 1.91 μM (in ref 51)) showed 10- to 35-fold lower activities in these assays. These differences may also be attributable to an increased half-life of the sulfonamides versus the carboxamides in these cell-based assays. The other observation was the marked dissociation between **25** and **26** (Table 2). The N^1,N^1-bis(2-aminoethyl)ethane-1,2-diamine motif of **25** appears to correspond to greater affinity and activity than the N^1,N^1-bis(3-aminopropyl)propane-1,3-diamine core structure of **26**. We surmise that this may be a consequence of more favorable multidentate ionic H-bonds[65] formed with the lipid A phosphates[44] by **25**.

Dissociation between NO and NFκB Inhibition Activities: Although not readily apparent from a cursory inspection of Figure 2, we have observed a rather pronounced dissociation between NO inhibition on the one hand and NFκB attenuation on the other. Plots of IC_{50} values of the compounds in the two in vitro assays revealed a distinctly dimorphic distribution, yielding two independent and divergent linear regression fits (Figure 3, top panel). This was unexpected and had not been observed before in several SAR studies on homologous series.[50,60,66,67] Upon close examination of this data from these two cell-based assays, we found that this was attributable to a marked difference in SAR trends between the monosubstituted (Series 1 and 2) compounds and the bis- and disubstituted/branched (Series 3−5) compounds (Figure 3, bottom panels; in these plots it is important to note that the symbol size corresponds to the carbon number of the acyl groups). For the Series 1 and 2 compounds, lengthening the acyl chain length results in a dramatic decrease in the NO IC_{50} ($\sim 10^{-5}$ M to $\sim 10^{-7}$ M), while changes in NFκB IC_{50} values are more subtle (Figure 3, bottom left panel). The converse is true for Series 3−5 analogs in that increases in the acyl chain length result in a pronounced increase in NFκB IC_{50} values, while the NO inhibition constants are less affected. The presence of a secondary target that is differentially affected by each of these two classes of compounds is suggested by this data. We hypothesize that this may be related to the different inhibitory activities of these compounds on calmodulin, given that spermine sulfonamides have been shown to antagonize calmodulin[68,69] and inducible NO synthase activity is Ca^{++}/calmodulin-dependent.[70,71] The effect of these polyamine compounds on calmodulin are currently being examined.

Cytokine Inhibition in Human Blood Ex Vivo: It was important to verify that the endotoxin-sequestering activity of the most active polyamine compounds would be manifested in the milieu of whole human blood, characterized not only by its high ionic strength ($\sim$300 mOsmoles), which attenuates electrostatic interactions,[44] but also by near-millimolar concentrations of albumin. Albumin has been shown to bind both LPS[36,37] as well as acylpolyamines.[72] Furthermore, given the amphipathic nature of both LPS and the polyamine analogs, it is conceivable that substantial partitioning of both LPS and ligand into the lipoprotein constituents could occur. For these reasons, and prior to confirmatory experiments in an animal model, we compared the effects of high-potency hits identified in the NFκB and NO in vitro screening assays, relative to PMB as a reference compound, in ex vivo cytokine release assays using whole human blood. Gratifyingly, all analogs tested inhibited TNF-α, IL-6, and IL-8 production in a dose-dependent manner with the IC_{50} values considerably lower than that of PMB (Table 3), demonstrating that these compounds were fully active under physiological conditions.

Protection against Endotoxin-Induced Lethality in Mice: A well-established murine model of lethal septic shock[46,50,51,64,73] was employed to evaluate the potencies of the three most active test compounds. Cohorts of 10 CF-1 mice per group, sensitized

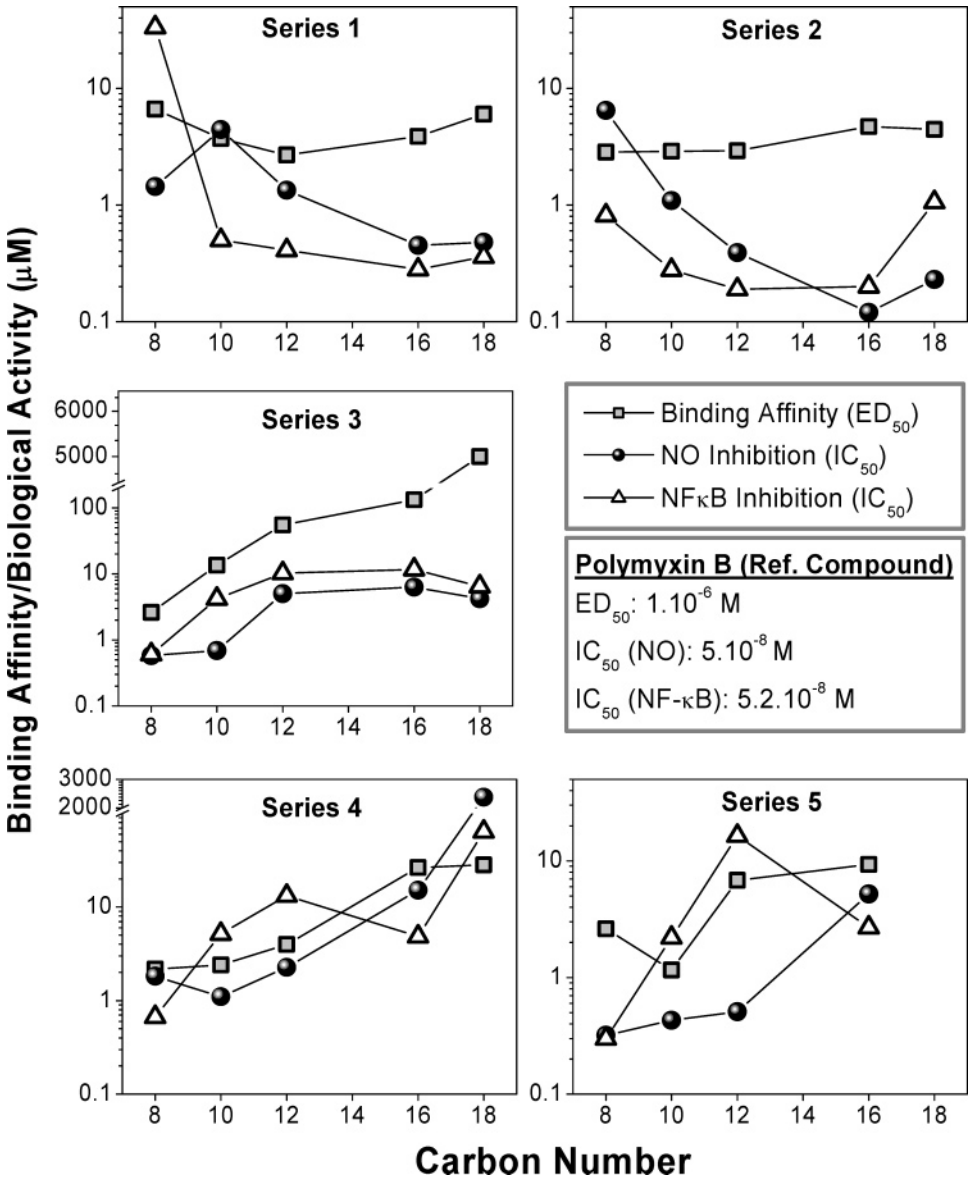


Figure 2. Binding affinities and inhibitory potencies in in vitro NO release and NFκB nuclear translocation assays for Series 1−5.

to the lethal effects of LPS with D-galactosamine, were challenged with a supralethal dose of LPS ($2\times$ LD_{100} dose = 200 ng/animal) administered intraperitoneally (i.p.). This was preceded by a subcutaneous (s.c.) injection of graded doses of **1D**, **2D**, or **5A** given 1 h prior to LPS challenge. These compounds respectively represent the best-in-class of the spermine−sulfonamide Series 1 (SPM), Series 2 (HOMO−SPM), and Series 5 (branched-HOMO−SPM) and specifically contain example(s) of both the linear monosulfonamide and branched bis-sulfonamide. As shown in Figure 4, i.p. administration of **1D** and **2D** afforded a clear, dose-dependent protection from lethality with complete protection being evident at a dose of 200 μg/mouse (8 mg/kg). Compound **5A** displayed toxic effects above doses of 50 μg/animal (2 mg/kg).

Concluding Remarks: A series of novel, homologated spermine sulfonamides were examined for their ability to sequester endotoxin and inhibit the production of downstream cytokine production. A single long-chain (C16−C18) or two moderate-length (C8−C10) aliphatic group(s) are essential for optimal LPS binding and neutralization. Compound **2D**, the principal lead, sequesters LPS with an IC_{50} of 4.2 μM and inhibits LPS-induced NO and NFκB production in cell-based assays at 0.12 and 0.20 μM, respectively. This compound affords dose-dependent protection in a mouse model of septic shock. The questions that remain to be carefully addressed are the following: First, what is the toxicity profile of polyamine sulfonamides such as **2D**? Polyamine analogs have been known to exert cytostatic and antineoplastic activities[74−78] mediated by a variety of mechanisms including the depletion of intracellular polyamine pools,[79] inhibition of ornithine decarboxylase, and the upregulation of spermidine/spermine acetyltransferase activities.[79−82] It would, therefore, not be surprising to find some of these activities with compounds such as **2D**. Indeed, a pharmaprofiling (CEREP screening) of **2D** shows several off-target effects primarily involving amine transporters (data not shown). However, sequestration of LPS in the context of managing Gram-negative sepsis would entail a short-term use, quite unlike the chronic dosing required in antineoplastic regimens. What the consequences of these off-target effects upon short-term administration would be and whether they would be tolerable in the setting of an acute, but frequently fatal syndrome, are questions that are crucial in the additional preclinical

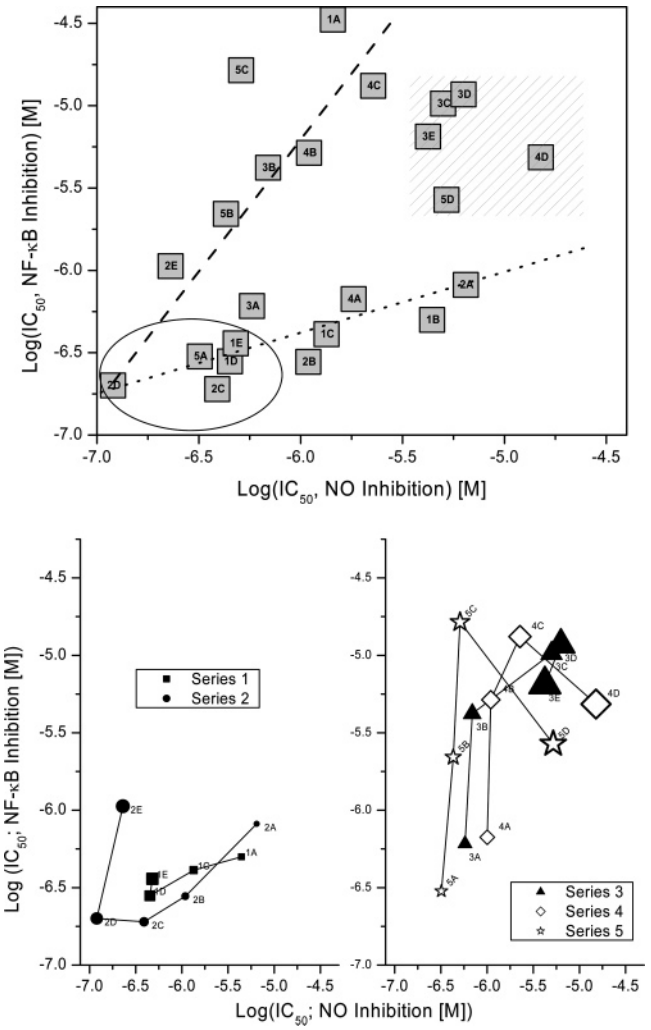


Figure 3. Top: Dimorphic correlation between in vitro NO release and NFκB nuclear translocation IC_{50} values for Series 1−5. The series number is shown in the boxed symbol and the corresponding compound numbers are listed alongside. Compounds in hatched rectangle were insoluble and were, therefore, inactive in either assay. Compounds that were highly active in both screens (in ellipse) were selected for in vivo testing in a murine model of septic shock. Bottom: Inverse relationship between NO and NFκB inhibition activities between monosubstituted analogs in Series 1 and 2 (left panel), and disubstituted/branched compounds in Series 3−5 (right panel). The symbol sizes correspond to the total carbon number of the hydrophobic chain.

Table 3. Secondary Screen IC_{50} Values for Inhibition of LPS (100 ng/mL)-Induced Cytokine Release in Whole Human Blood for Polymyxin B (PMB; Reference Compound) and Compounds That Were Active in Both NO Release and NFκB Translocation Assays[a]

	cytokine inhibition in ex vivo whole human blood: IC_{50} values (μM)		
cmpd	TNF-α	IL-6	IL-8
PMB	22.3	15.0	25.2
1D	22.1	8.17	15.2
1E	18.0	8.80	15.4
2B	32.9	29.4	22.4
2C	30.1	12.1	15.3
2D	14.2	5.92	15.1
5A	11.0	7.77	12.5

[a] See Figure 3.

evaluation of compounds such as **2D.** Careful evaluation of acute and chronic toxicity using escalating dose-regimens as well as a more detailed evaluation of its efficacy in large-animal models of sepsis are being planned. Second, as mentioned earlier,

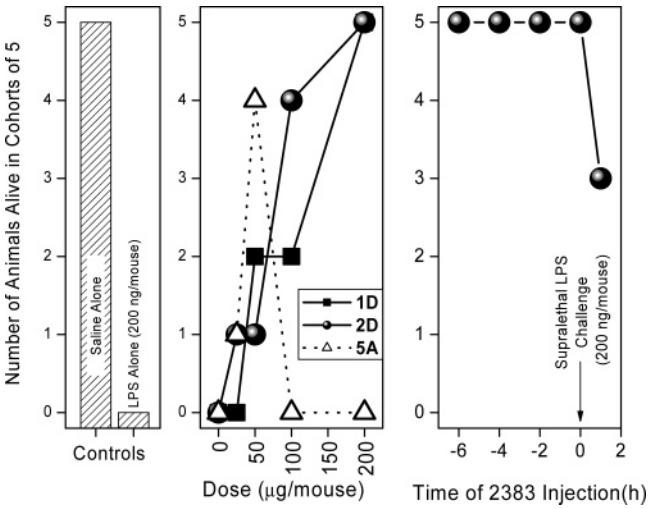


Figure 4. Dose−response of select compounds (left) and time-course of protection afforded by **2D** (right) in a D-galactosamine-sensitized mouse model of septic shock. Dose−response: Cohorts of five mice each were challenged with a supralethal (twice the LD_{100} dose; 200 ng/animal) of LPS. Graded doses of compound were administered concurrently by separate intraperitoneal injection. Time-course: A fully protective dose of **2D** (200 μg/mouse) was given at various times preceding LPS challenge. Lethality was scored at 24 h post-LPS challenge in both experiments.

carboxamide analogs[48,50,53] have an intrinsic advantage over the sulfonamide compounds presented in this work in being degraded to nontoxic metabolites (fatty acid and polyamine). Time-course experiments aimed at understanding the pharmacodynamics of **2D** (Figure 4) do indeed suggest a much longer half-life than some carboxamide analogs that we have previously examined.[48,50,53] This observation needs to be formally examined via pharmacokinetic experiments. Equally important, it remains to be seen whether the potential gain in half-life with the sulfonamides is offset by additional and perhaps unexpected toxicity. Work addressing these issues is currently in progress.

Experimental Section

Chemistry: General Experimental Methods. The sources of all chemical reagents and starting materials were of the highest grade available and were used without further purification. Drying of organic layers was performed using $MgSO_4$. LC/MS analyses were performed using a Hewlett-Packard 1050 system. Detection was by a Finnigan AQA operating in ESI^+ mode (*m*/*z* range 140 to 1600 amu). Gradient elution from 2 to 7 min at 0.2 mL/min was performed using 2% to 100% CH_3CN in H_2O (both with 0.05% TFA) using a Waters XTerra MS C_{18} 2.1 × 150 mm (3.5 μm) column. ^1H NMR spectra were recorded at 300 MHz on a Bruker AV300 spectrometer at the University of Washington, Seattle. ^1H NMR signals were generally multiples, unless otherwise noted, designated as s = singlet, d = doublet, t = triplet, or m = multiplet. Chemical shifts are relative to external 3-(trimethylsilyl)-1-propanesulfonic acid, sodium salt.

Monosubstituted Spermine-Sulfonamide Analogs (SPM)—Direct Route Synthetic Method. Synthesis of N^1-Hexadecylsulfonyl-1,14-diamino-5,10-diazabuta-decane Tris(hydrochloride) Salt 1D. To the clear solution of 200 mg (1 mmol, 10 equiv) of spermine in 10 mL of dry CH_2Cl_2 was added a solution of 32 mg (0.1 mmol, 1 equiv) of hexadecylsulfonyl chloride in 1 mL of dry CH_2Cl_2 dropwise at 25 °C. After stirring for 16 h, the heterogeneous mixture was washed with 5% Na_2CO_3 and brine, dried, and evaporated to give the crude product as a mixture of mono-, di-, and trisubstituted spermine sulfonamides. Column chromatography was performed using 900 mg of silica gel, eluting with CH_2Cl_2 containing 5 to 10% MeOH and 1% NH_4OH. Product-containing fractions were combined and evaporated to give **1D** as a pure oil

in its free base form. This was converted to its trihydrochloride salt form by treatment with and evaporation from MeOH saturated with hydrogen chloride gas. ^{1}H NMR (D_2O, ppm): 3.07 (m, 12H), 2.08 (m, 2H), 1.88 (m, 2H), 1.74 (m, 4H), 1.26 (m, 30H), 0.82 (t, 3H). Elem anal. Calcd for $C_{26}H_{61}Cl_3N_4O_2S$: C, 52.03; H, 10.24; N, 9.33. Found: C, 51.82; H, 10.24; N, 9.37. LC/MS by ESI$^+$ mode analysis observed *m*/*z* 491 at 11.9 min retention time.

Monosubstituted Spermine-Sulfonamide Analogs (Series 1: SPM)—Alternative Synthetic Route Method: Synthesis of *N*^{1}-Hexadecylsulfonyl-1,14-diamino-5,10-diazabutadecane Tris(hydrochloride) Salt 1D: A solution of 0.20 g (0.40 mmol) of tri-Boc-spm **6** in 2 mL of dry CH_2Cl_2 was treated with 0.061 mL of triethylamine (1.10 equiv) followed by 0.143 g of solid hexadecylsulfonyl chloride at 25 °C. The resulting solution was stirred for 16 h when TLC analysis (hexane/EtOAc 2:1) showed the reaction was complete. The reaction solution was diluted in CH_2Cl_2 and washed with ice-cold 0.1 N HCl, H_2O, and then brine, dried with $MgSO_4$, and evaporated to give the crude product as an off-white foam. Chromatography over silica gel using hexane/EtOAc 2:1 gave 0.18 g (57% yield) of the tri-Boc intermediate **8D**. This material was completely dissolved in 5 mL of CH_3OH and treated with 5 mL of 6 N HCl at 25 °C. After 16 h, the solvents were evaporated to give 0.14 g (100%) of **1D** as its trihydrochloride salt as a white solid. Characterization matched that found above. Synthesis of **1A** (C_8), **1B** (C_{10}), **1C** (C_{12}), and **1E** (C_{18}) followed the same procedure except for the use of the appropriate alkanesulfonylchloride.

***N*^{1}-Octanylsulfonyl-1,14-diamino-5,10-diazabutadecane Tris(hydrochloride) Salt 1A (C_8):** ^{1}H NMR (D_2O, ppm): 3.02 (m, 14H), 1.98 (m, 2H), 1.83 (m, 2H), 1.68 (m, 6H), 1.31 (m, 2H), 1.18 (m, 8H), 0.72 (t, 3H). Elem anal. Calcd for $C_{18}H_{45}Cl_3N_4O_2S$: C, 44.30; H, 9.29; N, 11.48. Found: C, 44.19; H, 9.25; N, 11.27. LC/MS by ESI$^+$ mode analysis observed *m*/*z* 379 at 10.7 min retention time.

***N*^{1}-Decanylsulfonyl-1,14-diamino-5,10-diazabutadecane Tris(hydrochloride) Salt 1B (C_{10}):** ^{1}H NMR (D_2O, ppm): 3.04 (m, 14H), 1.99 (m, 2H), 1.82 (m, 2H), 1.67 (m, 6H), 1.33 (m, 2H), 1.19 (m, 12H), 0.76 (t, 3H). Elem anal. Calcd for $C_{20}H_{49}Cl_3N_4O_2S$: C, 46.55; H, 9.57; N, 10.86. Found: C, 46.46; H, 9.50; N, 10.69. LC/MS by ESI$^+$ mode analysis observed *m*/*z* 407 at 10.9 min retention time.

***N*^{1}-Dodecanylsulfonyl-1,14-diamino-5,10-diazabutadecane Tris(hydrochloride) Salt 1C (C_{12}):** ^{1}H NMR (D_2O, ppm): 3.07 (m, 14H), 2.04 (m, 2H), 1.86 (m, 2H), 1.72 (m, 6H), 1.34 (m, 2H), 1.18 (m, 16H), 0.78 (t, 3H). Elem anal. Calcd for $C_{22}H_{53}Cl_3N_4O_2S$: C, 48.56; H, 9.82; N, 10.30. Found: C, 48.40; H, 9.79; N, 10.31. LC/MS by ESI$^+$ mode analysis observed *m*/*z* 435 at 11.2 min retention time.

***N*^{1}-Octadecanylsulfonyl-1,14-diamino-5,10-diazabutadecane Tris(hydrochloride) Salt 1E (C_{18}):** ^{1}H NMR (D_2O, ppm): 3.04 (m, 14H), 2.05 (m, 2H), 1.94 (m, 2H), 1.78 (m, 6H), 1.26 (m, 30H), 0.82 (t, 3H). Elem anal. Calcd for $C_{28}H_{65}Cl_3N_4O_2S$: C, 53.53; H, 10.43; N, 8.92. Found: C, 53.39; H, 10.46; N, 8.75. LC/MS by ESI$^+$ mode analysis observed *m*/*z* 519 at 12.5 min retention time.

Monosubstituted Homologated Spermine-Sulfonamide Analogs (Series 2: HOMO—SPM)—Synthesis of N^1-Hexadecanylsulfonyl-1,18-diamino-5,9,14-triazaoctadecane tetrahydrochloride salt 2D: To the solution of 2.6 g (6.5 mmol) of tri-Boc-spm **6** in 120 mL of dry CH_3OH was added 0.85 mL of acrylonitrile. Following stirring for 18 h, TLC analysis (CH_2Cl_2/MeOH/NH_4OH 90:8:2) showed the reaction was nearly complete. The solvent was evaporated and the oily residue was dissolved in 100 mL of CH_2Cl_2 and treated with 3.06 g (14 mmol, 2.15 equiv) of Boc_2O. After 16 h, the solvents were evaporated and the oily residue was purified by chromatography over silica gel (hexanes/EtOAc 3:2) to give 2.8 g (66%) of monoalkylated product **8** as a colorless oil. This intermediate was dissolved in 30 mL of glacial acetic acid, and 3 g of $Pd(OH)_2$ was added. This mixture was placed under 50 psi of H_2 pressure and shaken for 15 h. The catalyst was removed by filtering over a pad of Celite, and the pad was washed with CH_3OH and the combined filtrates were evaporated to give the crude product as a colorless oil. This was purified over silica gel using $CHCl_3$/MeOH/concd NH_4OH, 92:8:2 to give 2.0 g (71%) of **9** as a colorless oil. This intermediate is also used for the synthesis of branched HOMO-spermine analogs depicted in Table 1 (Series 4). A 0.20 g (0.30 mmol) portion of this material was dissolved in 7 mL of dry CH_2Cl_2 and treated with 0.063 mL (1.5 equiv) of triethylamine, followed by 0.15 g (1.5 equiv) of solid hexadecylsulfonyl chloride at 25 °C. Following 16 h, the reaction was diluted with CH_2Cl_2 and washed with ice-cold 0.1 N HCl, H_2O, and then brine. Drying and evaporation gave the crude product. Chromatography over silica gel using hexane/EtOAc 2:1 gave 0.171 g (65%) of **10D** as a colorless oil. This was dissolved in 5 mL of CH_3OH and treated with 5 mL of 6 N HCl at 25 °C. The resulting colorless solution was stirred for 8 h when evaporation gave 0.121 g (97%) of **2D** as a white solid in its tetrahydrochloride salt form. ^{1}H NMR (D_2O, ppm): 3.05 (br s, 16H), 2.08 (br s, 4H), 1.90 (br s, 2H), 1.73 (m, 6H), 1.22 (m, 28H), 0.78 (br s, 3H). Elem anal. Calcd for $C_{29}H_{69}Cl_4N_5O_2S$: C, 50.21; H, 10.02; N, 10.09. Found: C, 50.17; H, 9.96; N, 10.11. LC/MS by ESI$^+$ mode analysis observed *m*/*z* 548 at 11.7 min retention time. Synthesis of **2A** (C_8), **2B** (C_{10}), **2C** (C_{12}), and **2E** (C_{18}) followed the same procedure except for the use of the appropriate sulfonylchloride.

***N*^{1}-Octanylsulfonyl-1,18-diamino-5,9,14-triazaoctadecane Tetrahydrochloride Salt 2A:** ^{1}H NMR (D_2O, ppm): 3.05 (m, 18H), 2.03 (m, 4H), 1.83 (m, 2H), 1.67 (m, 6H), 1.32 (m, 2H), 1.18 (m, 8H), 0.73 (t, 3H). Elem anal. Calcd for $C_{21}H_{53}Cl_4N_5O_2S$: C, 43.37; H, 9.19; N, 12.04. Found: C, 43.23; H, 9.14; N, 11.85. LC/MS by ESI$^+$ mode analysis observed *m*/*z* 426 at 10.8 min retention time.

***N*^{1}-Decanylsulfonyl-1,18-diamino-5,9,14-triazaoctadecane Tetrahydrochloride Salt 2B:** ^{1}H NMR (D_2O, ppm): 3.02 (m, 18H), 2.02 (m, 4H), 1.81 (m, 2H), 1.68 (m, 6H), 1.31 (m, 2H), 1.22 (m, 12H), 0.74 (t, 3H). Elem anal. Calcd for $C_{23}H_{57}Cl_4N_5O_2S$: C, 45.32; H, 9.42; N, 11.49. Found: C, 45.32; H, 9.45; N, 11.23. LC/MS by ESI$^+$ mode analysis observed *m*/*z* 464 at 10.9 min retention time.

***N*^{1}-Dodecanylsulfonyl-1,18-diamino-5,9,14-triazaoctadecane Tetrahydrochloride Salt 2C:** ^{1}H NMR (D_2O, ppm): 3.02 (m, 18H), 2.02 (m, 4H), 1.83 (m, 2H), 1.72 (m, 6H), 1.37 (m, 2H), 1.22 (m, 16H), 0.74 (t, 3H). Elem anal. Calcd for $C_{25}H_{61}Cl_4N_5O_2S$: C, 47.09; H, 9.64; N, 10.98. Found: C, 47.19; H, 9.68; N, 10.87. LC/MS by ESI$^+$ mode analysis observed *m*/*z* 492 at 11.1 min retention time.

***N*^{1}-Octadecanylsulfonyl-1,18-diamino-5,9,14-triazaoctadecane Tetrahydrochloride Salt 2E:** ^{1}H NMR (D_2O, ppm): 3.08 (br s, 18H), 2.12 (br s, 4H), 1.91 (br s, 2H), 1.74 (m, 6H), 1.34 (m, 2H), 1.23 (m, 28H), 0.74 (t, 3H). Elem anal. Calcd for $C_{31}H_{73}Cl_4N_5O_2S\cdot0.5H_2O$: C, 50.95; H, 10.21; N, 9.58. Found: C, 51.08; H, 10.05; N, 9.32. LC/MS by ESI$^+$ mode analysis observed *m*/*z* 576 at 12.8 min retention time.

Synthesis of Bis-Substituted Bis-Homologated Spermine-Sulfonamide Analogs (Series 3: Bis-Sub-Bis-HOMO—SPM)—Synthesis of *N*^{1},*N*^{14}-Bis[*N*-octanylsulfonyl-3-aminopropyl]-1,14-diamino-5,10-diazabutadecane Tetrahydrochloride Salt 3A: To a solution of 2.6 g (6.5 mmol) of *N*^{5},*N*^{10}-Bis[*t*-butoxycarbonyl]-1,14-diamino-5,10-diazabutadecane (*N*^{5},*N*^{10}-bisBOC-spm) **7** in 35 mL of CH_3OH was added 0.85 mL (2 equiv) of acrylonitrile at rt for 16 h when TLC analysis (95:5:0.2 CH_2Cl_2/CH_3OH/NH_4OH) showed complete consumption of starting material. The solvents were evaporated and the residue was dissolved in 100 mL of CH_2Cl_2 and treated with 3 g (2.15 equiv) of Boc_2O. After 8 h, the solvents were evaporated and the residue was purified by column chromatography over silica gel using 3:2 hexane/EtOAc to give 2.8 g (61%) of **11** as a clear oil. This material was dissolved in 40 mL of glacial AcOH and treated with 3 g of $Pd(OH)_2$ and 50 psi of H_2 pressure. After 4.5 h, the catalyst was filtered off and the filtrate was evaporated. The resulting residue was dissolved in EtOAc and washed with 1 N NaOH and brine, dried, and evaporated to give the crude product as an oil. This material was purified by column chromatography over silica gel using 95:5:0.5 $CHCl_3$/MeOH/NH_4OH to give 2.0 g (71%) of **12** as a clear oil. A 0.20 g (0.28 mmol) portion of this product was dissolved in 5 mL dry CH_2Cl_2 and treated with 0.12 mL (3 equiv) of triethylamine

followed by 0.164 mL (3 equiv) of octanylsulfonylchloride dropwise. The reaction was stirred under argon for 16 h when the reaction was diluted with CH_2Cl_2, washed with 0.1 N HCl and brine, dried, and evaporated. The crude residue was purified over silica gel chromatography using 1:1 hex/EtOAc to give 69 mg (23%) of pure **13A** product. This was directly deprotected using 2 mL of 1:1 CH_3OH/6 N HCl. This solution was allowed to stand for 16 h when evaporation gave 52 mg (100%) of pure **3A** as its tetrahydrochloride salt. ^{1}H NMR (D_2O, ppm): 3.14 (m, 6H), 3.08 (m, 18H), 2.09 (m, 4H), 1.89 (m, 4H), 1.68 (m, 8H), 1.35 (m, 4H), 1.22 (m, 16H), 0.75 (t, 6H). Elem anal. Calcd for $C_{32}H_{76}Cl_4N_6O_4S_2$: C, 47.16; H, 9.40; N, 10.31. Found: C, 46.99; H, 9.40; N, 10.09. LC/MS by ESI$^+$ mode analysis observed *m*/*z* 670 at 12.7 min retention time. Synthesis of **3B** (C_{10}), **3C** (C_{12}), **3D** (C_{18}), and **3E** (C_{20}) followed the same procedure except for the use of the appropriate sulfonylchloride.

***N*^{1},*N*^{14}-Bis[*N*-decanylsulfonyl-3-aminopropyl]-1,14-diamino-5,10-diazabutadecane Tetrahydrochloride Salt 3B:** ^{1}H NMR (D_2O, ppm): 3.10 (m, 24H), 2.11 (m, 4H), 1.92 (m, 4H), 1.72 (m, 8H), 1.37 (m, 4H), 1.23 (m, 24H), 0.78 (t, 6H). Elem anal. Calcd for $C_{36}H_{84}Cl_4N_6O_4S_2 \cdot H_2O$: C, 48.63; H, 9.75; N, 9.45. Found: C, 48.94; H, 9.57; N, 9.34. LC/MS by ESI$^+$ mode analysis observed *m*/*z* 726 at 13.4 min retention time.

***N*^{1},*N*^{14}-Bis[*N*-dodecanylsulfonyl-3-aminopropyl]-1,14-diamino-5,10-diazabutadecane Tetrahydrochloride Salt 3C:** Elem anal. Calcd for $C_{40}H_{92}Cl_4N_6O_4S_2$: C, 51.82; H, 10.00; N, 9.06. Found: C, 51.68; H, 9.99; N, 8.99. LC/MS by ESI$^+$ mode analysis observed *m*/*z* 782 at 15.3 min retention time.

***N*^{1},*N*^{14}-Bis[*N*-hexadecanylsulfonyl-3-aminopropyl]-1,14-diamino-5,10-diazabutadecane Tetrahydrochloride Salt 3D:** Elem anal. Calcd for $C_{48}H_{108}Cl_4N_6O_4S_2$: C, 55.47; H, 10.47; N, 8.09. Found: C, 55.28; H, 10.54; N, 7.81.

***N*^{1},*N*^{14}-Bis[*N*-octadecanylsulfonyl-3-aminopropyl]-1,14-diamino-5,10-diazabutadecane Tetrahydrochloride Salt 3E:** LC/MS by ESI+ mode analysis observed *m*/*z* 950 at 24.8 min retention time.

Disubstituted Branched Spermine-Sulfonamide Analogs (Series 4: branched-SPM)—Synthesis of *N*^{1}-Di[*N*-hexadecylsulfonyl-3-aminopropyl]-1,14-diamino-5,10-diazabutadecane Tetrahydrochloride Salt 4D: To a solution of 2.0 g (4.0 mmol) of triBoc-spm **6** in 100 mL of dry CH_3OH was added 2.7 mL (10 equiv) of acrylonitrile. A catalytic amount of Dowex 50W×400 cation-exchange resin was added (250 mg). The reaction was refluxed, and after 18 h, TLC analysis (CH_2Cl_2/MeOH/NH_4OH 90:8:2) showed the reaction was nearly complete. Following filtration, the solvent was evaporated and the bis-alkylated adduct was purified by column chromatography using 1:1 hexane/EtOAc to give 2.2 g (90%) product as an oil. A 1.1 g (1.81 mmol) portion of this material was dissolved in 20 mL of glacial acetic acid and 1 g of $Pd(OH)_2$ on carbon was added. The mixture was placed under 50 psi H_2 pressure and shaken for 5 h. This was followed by filtration of the mixture over a Celite pad, and the pad was washed twice each with CH_3OH and H_2O. The filtrate was diluted in EtOAc and 1 N NaOH, and the organic layer was removed, dried, and evaporated to give 1.0 g (90%) of **15** as a clear oil. A 0.167 g (0.32 mmol) portion of this triamine was dissolved in 10 mL of dry CH_2Cl_2, and 0.136 mL (0.96 mmol, 3 equiv) of triethylamine was added. To the resulting solution was added 0.171 mL (1.0 mmol, 3.1 equiv) of hexadecylsulfonyl chloride at 25 °C. Following stirring for 18 h, the solvents were evaporated and the residue was purified over silica gel using $CHCl_3$/CH_3OH/concd NH_4OH 98:1.8:0.2 to give 0.058 g (15%) of pure **16D** product. This material was dissolved in 5 mL of CH_3OH and treated with 5 mL of 6 N HCl at 25 °C for 16 h. Evaporation gave 0.038 g (75%) of **4D** as a white solid. LC/MS by ESI$^+$ mode analysis observed *m*/*z* 894 at 18.3 min retention time. Synthesis of **4A** (C_8), **4B** (C_{10}), **4C** (C_{12}), and **4E** (C_{18}) followed the same procedure except for the use of the appropriate sulfonylchloride.

***N*^{1}-Di[*N*-octanylsulfonyl-3-aminopropyl]-1,14-diamino-5,10-diazabutadecane Tetrahydrochloride Salt 4A:** ^{1}H NMR (D_2O, ppm): 3.23 (m, 6H), 3.08 (m, 18H), 2.08 (m, 4H), 1.90 (m, 4H), 1.71 (m, 8H), 1.34 (m, 4H), 1.22 (m, 16H), 0.77 (t, 6H). LC/MS by ESI$^+$ mode analysis observed *m*/*z* 669 at 11.1 min retention time.

***N*^{1}-Di[*N*-decanylsulfonyl-3-aminopropyl]-1,14-diamino-5,10-diazabutadecane Tetrahydrochloride Salt 4B:** ^{1}H NMR (D_2O, ppm): 3.10 (m, 24H), 2.08 (m, 4H), 1.96 (m, 4H), 1.73 (m, 8H), 1.36 (m, 4H), 1.22 (m, 24H), 0.81 (t, 6H). LC/MS by ESI$^+$ mode analysis observed *m*/*z* 726 at 11.9 min retention time.

***N*^{1}-Di[*N*-dodecanylsulfonyl-3-aminopropyl]-1,14-diamino-5,10-diazabutadecane Tetrahydrochloride Salt 4C:** ^{1}H NMR (D_2O, ppm): 3.23 (m, 6H), 3.07 (m, 18H), 2.19 (t, 4H), 2.08 (m, 4H), 1.84 (m, 4H), 1.72 (m, 4H), 1.50 (m, 4H), 1.21 (m, 16H), 0.77 (t, 6H). LC/MS by ESI$^+$ mode analysis observed *m*/*z* 669 at 11.1 min retention time.

***N*^{1}-Di[*N*-octadecanylsulfonyl-3-aminopropyl]-1,14-diamino-5,10-diazabutadecane Pentahydrochloride Salt 4E:** ^{1}H NMR (D_2O, ppm): 3.23 (m, 6H), 3.08 (m, 18H), 2.08 (m, 4H), 1.90 (m, 4H), 1.71 (m, 8H), 1.34 (m, 4H), 1.22 (m, 16H), 0.77 (t, 6H). LC/MS by ESI$^+$ mode analysis observed *m*/*z* 950 at 21.5 min retention time.

Disubstituted Branched Homologated Spermine-Sulfonamide Analogs (Series 5: branched-HOMO−SPM)—Synthesis of *N*^{1}-Bis[*N*-octanylsulfonyl-3-aminopropyl]-1,18-diamino-5,9,14-triazaoctadecane Pentahydrochloride Salt 5A: To a solution of 1.0 g (1.5 mmol) of the tetraBocHOMO-spermine derivative **9** produced in Series 2 (HOMO−SPM) above in 20 mL of dry CH_3OH was added 1.0 mL (10 equiv) of acrylonitrile. A catalytic amount of Dowex 50W×400 cation-exchange resin was added (250 mg). The resulting clear mixture was heated to reflux to give a mixture of mono- and bis-addition products. Following filtration, the solvents were evaporated and the residue was purified by column chromatography using a 1:1 to 2:1 EtOAc/hexane solvent mixture to give 0.53 g (46%) of the bis-addition product **11** as a colorless oil. A 0.52 g portion of this oil was dissolved in 20 mL of glacial acetic acid and treated with 0.5 g of $Pd(OH)_2$ under 50 psi H_2 pressure for 3.5 h. The resulting mixture was filtered over a pad of celite and evaporated to give 0.51 g (95%) of **12** as a clear oil. LC/MS analysis of this product confirmed identity and purity. A 1.0 g portion of this product was dissolved in 20 mL of dry CH_2Cl_2 and treated with 0.54 mL of Et_3N (3 equiv) followed by 0.75 mL (3 equiv) of octanylsulfonylchloride at rt under an argon atmosphere. After stirring for 16 h, the resulting reaction solution was evaporated and the crude residue was partitioned between 75 mL of EtOAc and 50 mL of cold 0.1 N HCl. The organic layer was washed again using cold 0.1 N HCl then dried and evaporated to give the crude, oily product. This was purified by silica gel chromatography using 98:2:0.2 CH_2Cl_2/MeOH/NH_4OH to give 0.90 g (62%) pure **13A** product. This material was dissolved in 15 mL of MeOH and treated with 15 mL of 6 N HCl. The resulting solution was stirred for 16 h when the solvents were evaporated to give 0.70 g (96%) of desired product **5A** in its pentahydrochloride salt as a white solid. Elem anal. Calcd for $C_{35}H_{84}Cl_5N_7O_4S_2 \cdot 3/2H_2O$: C, 44.94; H, 9.37; N, 10.48. Found: C, 44.85; H, 9.33; N, 10.43. LC/MS by ESI$^+$ mode analysis observed *m*/*z* 727 at 12.8 min retention time. Synthesis of **5B** (C_{10}), **5C** (C_{12}), and **5D** (C_{16}) followed the same procedure except for the use of the appropriate sulfonylchloride.

***N*^{1}-Di[*N*-decanylsulfonyl-3-aminopropyl]-1,18-diamino-5,9,14-triazaoctadecane Pentahydrochloride Salt 5B:** ^{1}H NMR (D_2O, ppm): 3.26 (m, 6H), 3.06 (m, 22H), 2.09 (m, 10H), 1.68 (m, 8H), 1.38 (m, 4H), 1.25 (m, 24H), 0.75 (t, 6H). Elem anal. Calcd for $C_{39}H_{92}Cl_5N_7O_4S_2 \cdot H_2O$: C, 47.67; H, 9.64; N, 9.98. Found: C, 47.32; H, 9.59; N, 9.80. LC/MS by ESI$^+$ mode analysis observed *m*/*z* 783 at 11.6 min retention time.

***N*^{1}-Di[*N*-dodecanylsulfonyl-3-aminopropyl]-1,18-diamino-5,9,14-triazaoctadecane Pentahydrochloride Salt 5C:** ^{1}H NMR (D_2O, ppm): 3.10 (m, 28H), 2.09 (m, 10H), 1.70 (m, 8H), 1.40 (m, 4H), 1.25 (m, 32H), 0.75 (t, 6H). LC/MS by ESI$^+$ mode analysis observed *m*/*z* 839 at 14.6 min retention time.

***N*^{1}-Di[*N*-hexadecanylsulfonyl-3-aminopropyl]-1,18-diamino-5,9,14-triazaoctadecane Pentahydrochloride Salt 5D:** LC/MS by ESI$^+$ mode analysis observed *m*/*z* 951 at 20.1 min retention time.

Synthesis of Miscellaneous Analogs. The various miscellaneous analogs shown in Table 2 were synthesized by sulfonylation of the modified polyamine under standard conditions. These analogs were purified by column chromatography over silica gel using 80:18:2 CH_2Cl_2/MeOH/concd NH_4OH. Several examples such as **31** and **32** were synthesized using the corresponding acid chloride instead of sulfonyl chloride. Values (*m*/*z*; ESI^+) and retention times (in minutes) obtained by LC-MS were as follows: **20**, 493/17.9; **21**, 489/18.9; **22**, 492/11.7; **23**, 365/10.8; **24**, 422/11.1; **25**, 436/12.2; **26**, 477/11.9; **27**, 434/11.2 min; **28**, 338/10.8; **29**, 394/11.1; **30**, 377/10.8; **31**, 627/11.0; **32**. ^1H NMR (D_2O, ppm): 3.12 (m, 20H), 2.18 (t, 4H), 2.05 (m, 4H), 1.84 (m, 4H), 1.70 (m, 4H), 1.51 (m, 4H), 1.17 (m, 16H), 0.75 (t, 6H). LC/MS by ESI^+ mode analysis observed *m*/*z* 570 at 11.0 min retention time.

In Vitro Screening Assays: The relative binding affinities of the sulfonamide analogs with LPS were determined using an automated, rapid-throughput fluorescence-based displacement assay, employing BODIPY−TR cadaverine (BC; (5-(((4-(4,4-difluoro-5-(2-thienyl)-4-bora-3a,4a-diaza-*s*-indacene-3-yl)phenoxy)acetyl)-amino)pentylamine hydrochloride) as the probe.[50,51,60,64] Results are reported as the effective concentration displacing 50% of the LPS-bound probe (ED_{50}). The inhibition of NO in murine macrophage J774A.1 cells stimulated with 100 ng/mL LPS was performed using an automated Griess assay, as described previously.[50,51,60,64] The inhibition of induction of NFκB (a key transcripitional activator of the innate immune system was quantified using human embryonic kidney 293 cells cotransfected with TLR4 (LPS receptor), CD14, and MD2 (co-receptors), available from InvivoGen, Inc. (HEK-Blue, San Diego, CA), as per protocols provided by the vendor). Stable expression of secreted alkaline phosphatase (seAP) under control of NFκB/AP-1 promoters is inducible by LPS, and extracellular seAP in the supernatant is proportional to NFκB induction. seAP was assayed spectrophotometrically using an alkaline phosphatase-specific chromogen at 620 nm using a rapid-throughput, automated protocol employing a Bio-Tek P2000 liquid handler. Inhibition of LPS-induced cytokine production was quantified concurrently in ex vivo healthy human blood using a FACSArray multiplexed (TNF-α, IL-1β, IL-6, IL-8, IL-10, and IL-12p70) flow-cytometric bead array system (Becton-Dickinson-Pharmingen, San Jose, CA), as described previously.[51] Data for the above-mentioned three in vitro biological assays are presented as the concentration of compound inhibiting 50% of the response (IC_{50}). All experiments were performed in triplicate, and polymyxin B was included as a reference compound, along with appropriate negative and positive controls.

Mouse Lethality Experiments: Female, outbred, 9- to 11-week-old CF-1 mice (Charles River, Wilmington, MA) weighing 22−28 g were used as described elsewhere.[46,51] The animals were sensitized to the lethal effects of LPS by D-galactosamine.[83−85] The lethal dose causing 100% mortality (LD_{100}) for the batch of LPS used (*E. coli* 0111:B4) was first determined by administering D-galactosamine (800 mg/kg) and LPS (0, 10, 20, 50, 100, 200 ng/mouse) as a single injection intraperitoneally (i.p.) in freshly prepared saline to cohorts of five animals in a volume of 0.2 mL. The expected dose−response profile was observed in two independent experiments with all five mice receiving 100 ng succumbing within 24 h, establishing the LD_{100} dose to be 100 ng/mouse. In experiments designed to test dose−response effects of the polyamine sulfonamides in affording protection against LPS-induced lethality, mice received graded doses of compound diluted in saline, i.p. in one flank, immediately before a supralethal (200 ng) LPS challenge, which was administered as a separate i.p. injection into the other flank. In experiments in which the temporal window of protection was to be examined, a fixed dose of 200 μg/mouse of compound was administered at various times, before or after supralethal (200 ng/mouse) LPS challenge. Lethality was determined at 24 h post LPS challenge.

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Supporting Information Available: Synthetic procedure to secondary amine sulfonamide **22**. LC/MS chromatograms of **1D** made via direct and regiospecific routes compared to **22**. LC/MS chromatographs of analogs **20**−**32**. This material is available free of charge via the Internet at http://pubs.acs.org.

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