Discovery and Structure−Activity Relationship of Antagonists of B-Cell Lymphoma 2 Family Proteins with Chemopotentiation Activity in Vitro and in Vivo

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Development of a rationally designed potentiator of cancer chemotherapy, via inhibition of Bcl-X_L function, is described. Lead compounds generated by NMR screening and directed parallel synthesis displayed sub-μM binding but were strongly deactivated in the presence of serum. The dominant component of serum deactivation was identified as domain III of human serum albumin (HSA); NMR solution structures of inhibitors bound to both Bcl-X_L and HSA domain III indicated two potential optimization sites for separation of affinities. Modifications at both sites resulted in compounds with improved Bcl-X_L binding and greatly increased activity in the presence of human serum, culminating in **73R**, which bound to Bcl-X_L with a K_i of 0.8 nM. In a cellular assay **73R** reversed the protection afforded by Bcl-X_L overexpression against cytokine deprivation in FL5.12 cells with an EC_{50} of 0.47 μM. **73R** showed little effect on the viability of the human non small cell lung cancer cell line A549. However, consistent with the proposed mechanism, **73R** potentiated the activity of paclitaxel and UV irradiation in vitro and potentiated the antitumor efficacy of paclitaxel in a mouse xenograft model.

Introduction

Programmed cell death, or apoptosis, is a highly regulated process used to eliminate defective and unnecessary cells.[1] Disregulation of this process is strongly associated with cancer.[2] Impaired apoptosis has been shown to be a key contributor to various stages of neoplastic progression[3,4] and also provides an innate defense to cytotoxic chemotherapy.[5,6] A group of important players in the apoptotic process is the Bcl-2 (B-cell lymphoma) family of proteins. Members of this family share up to four Bcl-2 homology (BH) domains, and the family is composed of both prosurvival (Bcl-2, Bcl-X_L, Bcl-w, Mcl-1, A1) and proapoptotic members. Proapoptotic proteins are further subdivided into two groups, the Bax subfamily (Bax, Bak, Bok) and the larger group of BH3-only proteins (Bad, Bid, Bim, Bik, Puma, Noxa, and others).[7]

The proapoptotic Bax and Bak are direct mediators of apoptosis, respectively localized in the cytosol and mitochondria under normal conditions. Following multiple death stimuli, both Bax and Bak form aggregates within the mitochondrial outer membrane, releasing cytochrome *c* and triggering the mitochondrial apoptosis pathway.[8] Antiapoptotic Bcl-2 family proteins (e.g. Bcl-2 and Bcl-X_L) inhibit cytochrome *c* release by blocking Bax/Bak activation.[9] The exact mechanism of action of Bcl-2 and Bcl-X_L has not been unambiguously determined. It is known they can form heterodimers with pro-apoptotic Bcl-2 family proteins and that the ratio of pro- to anti-apoptotic proteins is associated with cell survival.[10,11] Bcl-2 and Bcl-X_L do act via sequestration of pro-apoptotic BH3-only proteins.[12] The BH3-only proteins appear to perform different roles than their fully elaborated pro-apoptotic counterparts Bax and Bak.[13] In response to cellular stress, some BH3-only proteins (Bim, Bid) directly activate Bax and Bak, a process which is inhibited by antiapoptotic Bcl-2 family members.[13,14] Other BH3-only proteins, such as Bad, cannot directly activate Bax and Bak but instead bind to antiapoptotic Bcl-2 family members, freeing the BH3-only proteins that are capable of activating Bax and Bak. Thus, members of this subgroup behave as sensitizers rather than direct activators. A small molecule that preferentially interacts with the BH3-binding groove of Bcl-2 or Bcl-X_L would then be expected to mimic the activity of a Bad-like protein. Such a compound would be capable of restoring the inherent cellular potential for apoptotic response, thereby potentiating the effects of existing therapies, improving sensitivity, and overcoming resistance. Cellular data using various BH3-derived peptides are in support of this model.[15,16]

There is a wealth of evidence that overexpression of antiapoptotic Bcl-2 family proteins, especially Bcl-2 and Bcl-X_L, are associated with tumor progression, poor prognosis, and drug resistance. In particular, Bcl-X_L overexpression shows a more consistent correlation with intractability of cancer cell lines than does Bcl-2. As an illustration of this, an informatics study on the NCI 60 tumor cell line panel demonstrated that Bcl-X_L expression exhibited a strong negative correlation with sensitivity to both 122 standard chemotherapeutic agents and a larger set of 1200 cytotoxic agents.[17] This association was p53 independent, was not observed for Bcl-2 or Bax, and was more significant than the correlation between cytotoxicity and p53 mutational status. Our goal was then to specifically target Bcl-X_L activity as a strategy for developing a small molecule that would act primarily as a potentiator in conjunction with standard cancer chemotherapies. We recently disclosed the discovery of ABT-737, which represents the culmination of efforts that began from compound **1** (Figure 1a).[18] Here we show detailed SAR of a portion of that work, which delineates our efforts to

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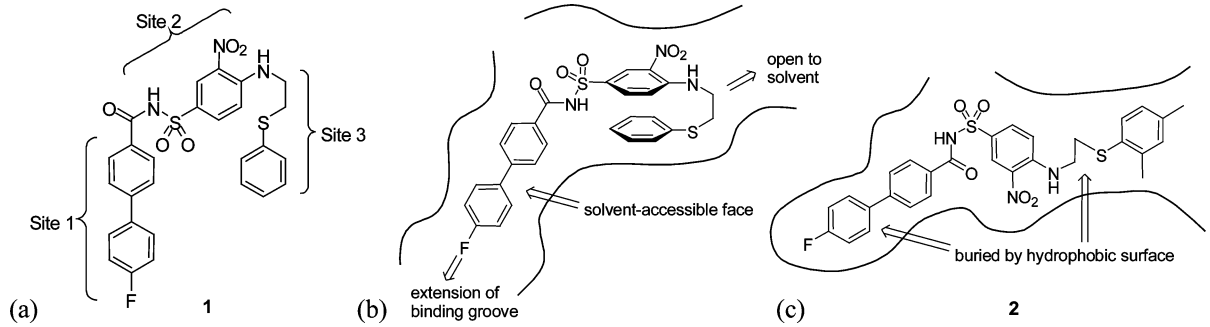


Figure 1. (a) Structure of **1** with substructure nomenclature. (b) Diagram of **1** bound to Bcl-X_L, showing bent-back conformation. Arrows indicate locations of proposed structure modifications to **1**. (c) Diagram of **2** bound in extended conformation to HSA-III, with arrows pointing to structure modification sites.

Table 1. Binding of **1**

assay	μM[a]
Bcl-2 K_i	0.433 ± 0.020*
Bcl-X_L K_i	0.036 ± 0.009*
Bcl-X_L K_i 1% HS	2.50 ± 0.58*
Bcl-X_L K_i 10% HS	1% inhib @ 10 μM

[a] Values with standard error and asterisk if three or more experiments.

Table 2. Deactivation of **1** by Serum Components

added protein	IC_{50} (μM)[a]	deactivation
none	0.093	
1% human serum	>10*	>100
HSA from 1% HS[b]	>10	>100
HSA-III from 1% HS[b]	6.30	68
α_1-AG from 1% HS[b]	0.122 ± 0.006	1.3

[a] Values with standard deviation if two experiments were performed; with standard error and asterisk if three or more experiments. [b] See Experimental Section for concentrations.

uncouple Bcl-X_L and serum affinity, resulting in a phenylpiperidine analogue acting via Bcl-X_L inhibition and constituting the first compound to show efficacy *in* vitro and in vivo.

Rationale

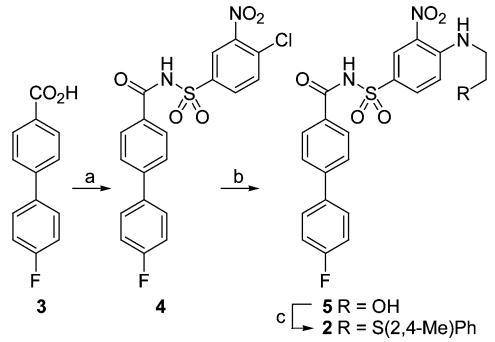
Three-dimensional structures of complexes of Bcl-X_L with Bax-[19] and Bad-derived[20] BH3 peptides have been described. The amphipathic α-helical BH3 domains bind to the long hydrophobic groove formed by the combination of BH1, BH2 and BH3 domains of Bcl-X_L. Compound **1**, generated from a combination of NMR-based screening and directed parallel synthesis, spans most of the length of the BH3-binding groove of Bcl-X_L and interestingly exhibits a bent-back π-stacked structure within the groove, with its phenylthio group tucked under its nitroaryl ring. Data in Table 1 show **1** to bind to Bcl-X_L with a K_i of 36 nM and with moderate activity against Bcl-2. However, Bcl-X_L K_i determination in the presence of 1% human serum provided evidence of tight binding by one or more serum components, resulting in a 69-fold deactivation, and binding in the presence of 10% human serum was almost completely abolished. The aim of the present study was to increase Bcl-X_L affinity in the presence of serum to provide a compound which we could more thoroughly examine in vitro and in vivo.

Given the extreme serum effect on binding, an immediate goal was to determine the exact component or components in serum that were responsible for the deactivation. We began by determining Bcl-X_L/**1** binding affinity in the presence of human serum, and separate serum components known to bind small molecules (Table 2). While 1% human serum deactivated **1** by a factor of over 100, the quantity of human serum albumin (HSA) present in 1% human serum by itself showed a similar deactivation, while the quantity of α_1-acid glycoprotein in 1% serum displayed little effect.[21] We further refined our search by focusing on domain III of HSA (HSA-III). This domain contains the primary binding site within albumin for medium- and long-chain fatty acids and displays high affinity for small anionic aromatic compounds; thus HSA-III was a likely candidate to bind the aryl acylsulfonamide **1**.[22] In the event, the 68-fold deactivation exhibited by the 1% serum equivalent of HSA-III indicated its likely identity as the main driver of serum deactivation of **1**.

We were able to obtain an NMR-derived structure of **2**, a closely related dimethyl analogue of **1**, bound to HSA-III.[20] The protein is populated by **2** at a single site within subdomain IIIA. This subdomain was previously reported to be the primary binding region for the biphenyl carboxylic acid diflunisal[23] and is also the site occupied by two myristic acid molecules in an HSA cocrystal complex.[24] As further confirmation of the importance of this interaction, results of a direct, competitive binding assay measuring affinity to this particular binding site of HSA-III by displacement of a dansyl sarcosine probe provided a K_d of <100 nM for **1**.[25]

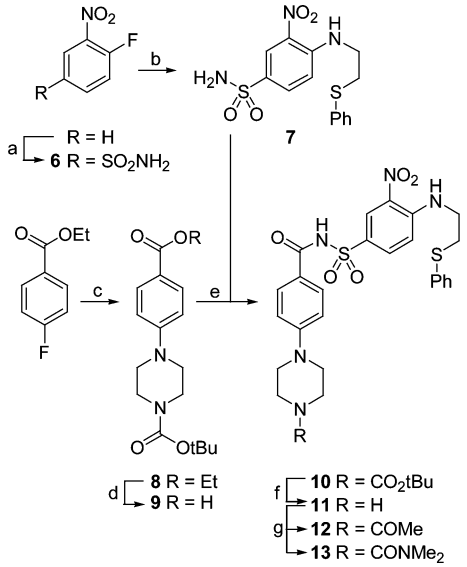
We next attempted to apply structure-based design to decouple affinity for Bcl-X_L from that of HSA-III. The differences in the binding modes of **1** and **2** bound to Bcl-X_L and HSA-III, respectively, suggested two ways in which albumin binding might be reduced (Figure 1).[20] First, in contrast to the bent-back conformation of **1** bound to Bcl-X_L, **2** displays an extended structure within domain III, with the Site 3 phenylthioethyl tail buried by nonpolar residues. This suggested to us the possibility of adding to the structure of **1** by building off of the ethylene group. These substitutions should not be easily accommodated by HSA-III, while Bcl-X_L should more readily tolerate such groups, allowing them to extend into solvent. Moreover, appended groups should optimally be polar, considering the aqueous and hydrophobic environments to be encountered by binding to Bcl-X_L and HSA-III, respectively. A recent chemometric analysis of ligand binding to HSA-III found that various amines, and to a lesser extent polar, uncharged groups such as carbamates, amides, and sulfones, most effectively decreased binding to HSA-III.[26] A second opportunity for modifications was located at the terminal biphenyl end of **1**/**2**. The Bcl-X_L-bound structure is presented with additional space at the fluoro end of the biphenyl and is partially solvent-exposed, while the fluorophenyl in HSA-III is more thoroughly surrounded by nonpolar residues. We thus felt it might be possible to maintain or improve the affinities of our compounds toward Bcl-X_L and reduce affinities toward HSA-III by either increasing the polarity of the Site 1 fragment or by extending the end of the fragment by again appending a polar group. One caveat that should be

Scheme 1[a]



[a] Reagents and conditions: (a) 4-chloro-3-nitrobenzenesulfonamide, EDCI, DMAP, CH_2Cl_2; (b) 2-aminoethanol, dioxane, 80 °C; (c) Bu_3P, ADDP, 2,4-dimethylthiophenol, THF.

Scheme 2[a]



[a] Reagents and conditions: (a) chlorosulfonic acid, 80 °C, then NH_4OH, −78 °C; (b) 2-(phenylsulfanyl)ethanamine, DMSO; (c) BOC-piperazine, K_2CO_3, DMSO, 120 °C; (d) LiOH, THF; (e) EDCI, DMAP, CH_2Cl_2; (f) 4 M HCl, dioxane; (g) AcCl or Me_2NCOCl, pyridine, Et_3N.

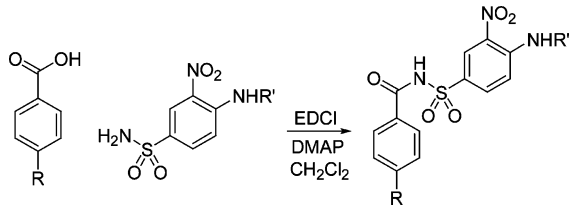
applied to such an analysis is that HSA is notoriously elastic in its binding conformations, and protein−protein binding sites in general involve flexible topology,[27] but the above analysis nevertheless suggested most likely venues for structure modification.

Synthesis

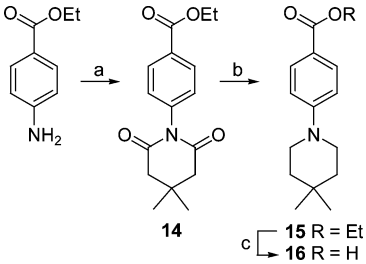
Compound **2** was synthesized as part of an SAR study on thiophenol substitution (Scheme 1). The commercially available acid **3** was coupled to 4-chloro-3-nitrobenzenesulfonamide with EDCI to give the acylsulfonamide **4**. Aromatic substitution of the activated chloride with aminoethanol cleanly gave **5**, which subsequently was subjected to modified Mitsunobu conditions to give **2**.[28]

The new Site 1 compounds featuring replacement of the fluorophenyl ring with a piperazine moiety, were synthesized by a route allowing for late exchange of the terminal nitrogen substituents (Scheme 2). Thus, 2-fluoronitrobenzene was chlorosulfonylated, the deactivated ring requiring neat $ClSO_3H$ at 80 °C, and the resulting sulfonyl chloride was carefully subjected to ammonolysis to produce **6**, with low temperature required in order to not displace the activated fluoro group. Subsequent desired displacement of the fluorine produced the complete Site 2/3 sulfonamide unit **7** in near quantitative yield. The acid **9** was produced via S_NAr reaction on ethyl 4-fluorobenzoic acid to give the ester **8**, followed by LiOH-mediated hydrolysis. The acid and sulfonamide were coupled using EDCI to give the BOC-protected **10** in good yield. The free secondary amine was deprotected with aqueous HCl to give **11**, and **12** and **13** were produced by reaction with the appropriate carbonyl chloride.

Chart 1. General Coupling Protocol



Scheme 3[a]



[a] Reagents and conditions: (a) 3,3-dimethylglutaric anhydride, AcCl, $ClCH_2CH_2Cl$; (b) 2-methoxyethyl ether, $BF_3{\cdot}Et_2O$, $NaBH_4$; (c) LiOH, THF, H_2O.

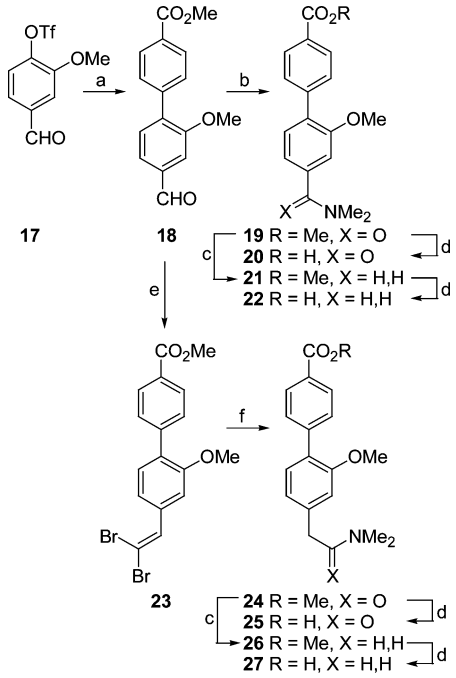
The EDCI coupling to form the acylsulfonamide was a key step in the syntheses of our compounds and, with the exceptions of the cases just discussed, constituted the final step of all syntheses presented here (Chart 1). In rare cases a deprotection step was incorporated into the workup of the coupling reaction (see Experimental Section). Thus, synthetic plans were reduced to targeting the appropriate acids and sulfonamides.

The dimethylpiperidine-containing acid **16** was made in three steps from ethyl 4-aminobenzoic acid (Scheme 3). Condensation with 3,3-dimethylglutaric anhydride gave **14** in 95% yield. The imide was reduced with $NaBH_4$ and $BF_3{\cdot}OEt_2$ to give the ester **15**,[29] which was saponified with LiOH to give **16**.

Schemes 4 and 5 illustrate syntheses of compounds wherein the fluoro group of **1** was replaced by polar tails of varying lengths. The synthetic precursor **17**, the triflate ester of vanillin, is easily made and has been reported.[30] Suzuki coupling of **17** to 4-carbomethoxybenzeneboronic acid cleanly gave **18**. The aldehyde was oxidized to the acid using the Sharpless protocol,[31] and the subsequent acid chloride was reacted with dimethylamine to give **19**. The amide was reduced with borane to the amine **21**, and both **19** and **21** were saponified to the respective acids **20** and **22**. For the one-carbon homologous compounds, **18** was subjected to the two-step aldehyde-to-amide homologation described previously.[32] The aldehyde was cleanly transformed into the dibromoolefin **23**, and hydrolysis with dimethylamine and water in DMF produced **24** in excellent yield. Compounds **25**−**27** were then produced in a fashion analogous to **19**−**21** above.

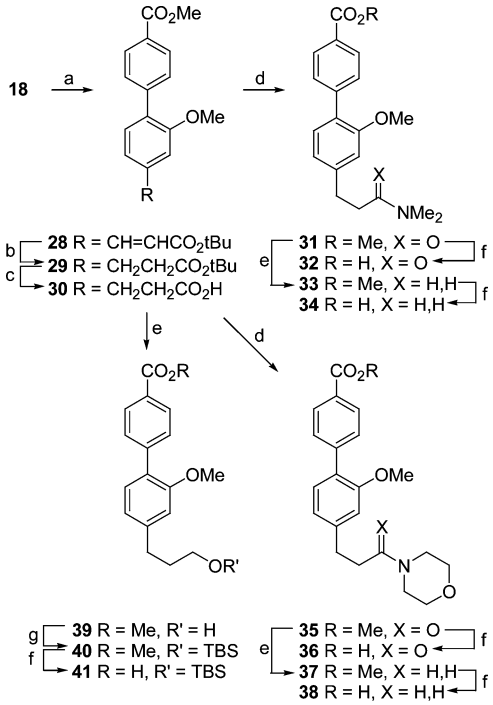
For the two-carbon homologated acids, **18** again served as starting material (Scheme 5). Reaction with the commercially available acetate-derived phosphorane gave **28**. The resulting olefin was hydrogenated with Wilkinson's catalyst to give **29**, and the *tert*-butyl group was removed with TFA/Et_3SiH to quantitatively provide the acid **30**. The amidoester **31** and aminoester **33** and corresponding acids **32** and **34** were then

Scheme 4[a]



[a] Reagents and conditions: (a) 4-(CO_2Me)PhB($OH)_2$, $PdCl_2$(dppf), CsF, dioxane, 90 °C; (b) $NaIO_4$, $RuCl_3$, CCl_4, CH_3CN, H_2O, then $(COCl)_2$, Me_2NH, CH_2Cl_2; (c) BH_3, THF; (d) LiOH, THF, H_2O; (e) CBr_4, PPh_3, CH_2Cl_2; (f) Me_2NH, DMF, H_2O, 80 °C.

Scheme 5[a]



[a] Reagents and conditions: (a) Ph_3P=$CHCO_2$tBu, THF; (b) $RhCl(PPh_3)_3$, H_2, toluene, 60 °C; (c) TFA, Et_3SiH, 50 °C; (d) $(COCl)_2$, CH_2Cl_2, amine; (e) BH_3, THF; (f) LiOH, THF, H_2O; (g) TBSOTf, 2,6-lutidine, CH_2Cl_2.

synthesized as before. The corresponding morpholine-derived compounds **35**−**38** were also produced similarly. Finally, **30** was employed in the synthesis of the hydroxy acid **41**, via borane reduction to **39**, TBS-protection of the resulting alcohol, and saponification.

Sulfonamides with a two-carbon side chain were synthesized as shown in Scheme 6. We began with either antipode of Fmoc-Asp(OtBu)-OH, with the D form leading to compounds with the R configuration throughout, and the L form producing S-sense compounds. Reduction of the acid side chains with $NaBH_4$ via the mixed anhydride gave **42R,S**, typically in excellent yield.[33] This method is known to maintain chirality of N-protected amino acids, and is also mild enough not to remove the Fmoc group. Next, the modified Mitsunobu procedure was again used to install the phenylthio group of **43R,S**. The Fmoc group was removed and the resulting amine displaced the highly activated fluoro group of **6** in one pot at room temperature, resulting in **44R,S**. The *tert*-butyl ester was removed quantitatively with aqueous HCl to give **45R,S**, which were transformed into the amides **46R,S** and **48R,S** using EDCI. The amides were again selectively reduced with borane to provide the amines **47R,S** and **49R,S**, typically in 70−80% yield.

Three-carbon side chain compounds were synthesized as shown in Scheme 7. The hydroxy group of N-BOC-L-serine methyl ester was transformed into the thioether under neutral conditions via conversion to the mesylate and immediate in situ displacement by thiophenol. The resulting ester **50** was reduced to the aldehyde **51** with DIBAL in moderate yield, with separation of small amounts of starting ester and alcohol required. **51** was homologated using the Wittig phosphorane to give the olefin **52**, which was quantitatively both hydrogenated with Wilkinson's catalyst, and saponified to give the saturated acid **53**. The dimethyl amide of **54** was again installed via EDCI coupling, and the combination of BOC-deprotection and S_NAr displacement on **6** was again employed to provide **55**. For **56** we chose to reduce **54** to the amine before deprotection and reaction with **6**, though these steps could likely have been performed in the reverse order, as with the other series of compounds.

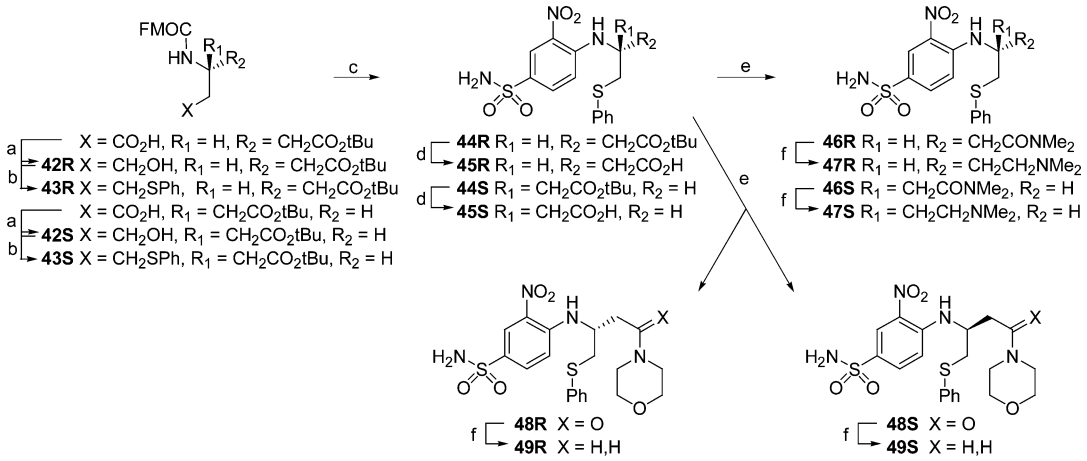
Finally, the four-carbon tail synthesis (Scheme 8) began with reduction of the acid functionality of Fmoc-D-Lys(BOC)-OH, again using the chloroformate/$NaBH_4$ method, giving **57** in excellent yield. The PhS group **58** was installed using the standard disulfide method, though here heating to 80 °C was required.[34] The BOC group was removed and the tertiary dimethylamine **60** formed via reductive amination. The sulfonamides **59** and **61** were produced from **58** and **60** using the one-pot Fmoc-deprotection/addition protocol.

Results and Discussion

Structure−**Activity Relationships**. Compounds were evaluated in a fluorescence polarization assay (FPA) for their ability to displace a Bad-derived peptide from Bcl-X_L, and a Bax-derived peptide from Bcl-2.[35] We tracked Bcl-2 binding, because while we were primarily concerned with activity against Bcl-X_L, the high structural homology of the BH3 binding grooves of Bcl-2 and Bcl-X_L suggested that compounds would possess significant Bcl-2 affinity.[36] We note that the relative importance of these related proteins in a clinical setting is not yet known. More importantly, to track deactivation by HSA-III and other serum components, compounds were also evaluated against Bcl-X_L in the presence of human serum. We initially used a concentration of 1% serum for these assessments.

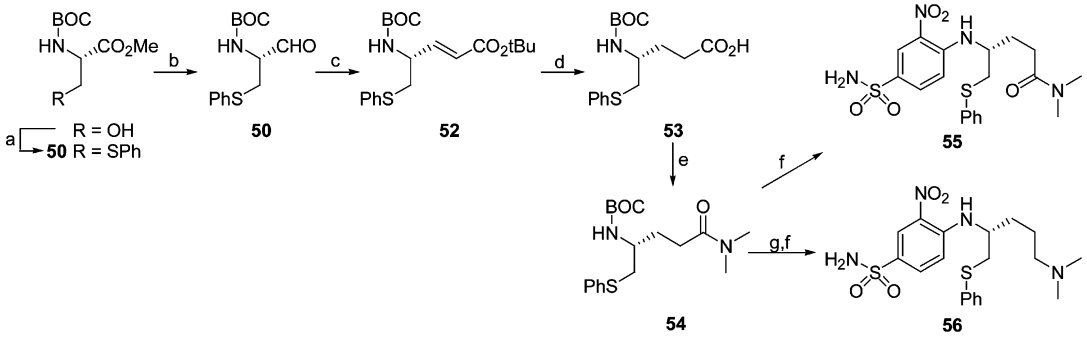
We began our work by assessing Site 1 modification as an approach to decreasing serum deactivation. In the first part of our two-pronged study, a series of polar tails of various lengths, capped primarily by amides and amines, were appended to the end of a methoxy-substituted Site 1 biphenyl (Table 3). The methoxy group came out of a small investigation of ring-substitution effects on binding of biphenyl-containing compounds; the methoxy group increased potency by a small amount

Scheme 6[a]



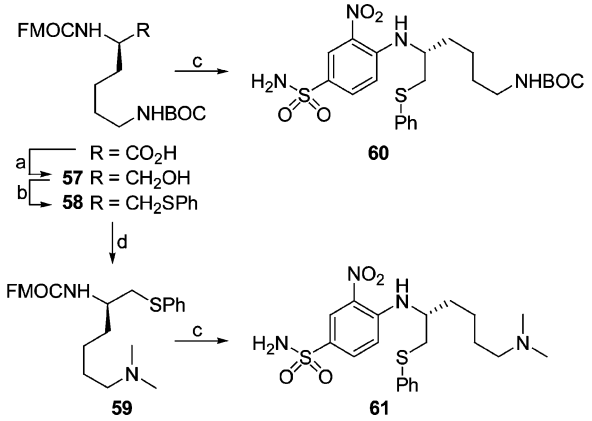
[a] Reagents and conditions: (a) ^{i}BuOCOCl, THF, then $NaBH_4$, MeOH; (b) ADDP, Bu_3P, PhSH, THF; (c) **6**, DIPEA, DMF; (d) 4 M HCl, dioxane; (e) amine, EDCI, DMAP, DMF; (f) BH_3, THF.

Scheme 7[a]



[a] Reagents and conditions: (a) MsCl, DIPEA, CH_2Cl_2, then PhSH; (b) DIBAL, CH_2Cl_2, −78 °C; (c) $Ph_3P{=}CHCO_2tBu$, THF; (d) $RhCl(PPh_3)_3$, H_2, toluene, 50 °C, then LiOH, THF, H_2O; (e) Me_2NH, EDCI, DMAP, DMF; (f) 4 M HCl, dioxane, then **6**, DIPEA, DMF; (g) BH_3, THF.

Scheme 8[a]

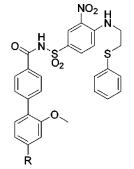


[a] Reagents and conditions: (a) ^{i}BuOCOCl, DME, then $NaBH_4$, H_2O; (b) PhSSPh, Bu_3P, toluene, 80 °C; (c) **6**, DIPEA, DMF; (d) TFA, CH_2Cl_2, then CH_2O, $NaBH_3CN$, THF.

compared to parent (data not shown). A clear length dependence on affinity is immediately evident. One- and two-carbon compounds **62**−**65** proved severely detrimental to Bcl-X_L (and Bcl-2) binding, while three-carbon compounds displayed Bcl-X_L affinity roughly equal to, or in the cases of **68** and **70**, slightly greater than that of **1**. We surmise that the chain lengths of **62**−**65** are too short to deliver the polar groups beyond the end of the flexible hydrophobic BH3-binding site and into more solvent-accessible space. Equally important is that all of the amines and the three-carbon amides **67** and **69** produced a suppression of deactivation by 1% serum. The dimethylamines in particular, fully protonated under physiological conditions, were very effective in this regard, showing no dependence on chain length and reducing deactivation in this assay from 69-fold to from 1.5- to 7-fold, resulting in improved binding over **1** in the 1% HS assay for even **62** and **64**. Nevertheless, combined with intrinsic affinities, the three-carbon compounds, and in particular the amines **66** and **68**, appeared to stand out among this group of compounds. Furthermore, in an FPA with 10% serum present, only **66** and **68** displayed Bcl-X_L activity of less than 10 μM, with K_i values of 5.66 and 4.3 μM, respectively. While the serum K_i data for **68** was clearly driven less by a reduced serum effect than was the data for **66**, the superior intrinsic Bcl-X_L binding led us going forward to focus primarily on the morpholino moiety of **68** from this group. It is also of note that **68** and **70** appeared to have gained some separation between Bcl-X_L and Bcl-2 affinity, driven solely by gains toward Bcl-X_L.

The second aspect of our approach to Site 1 modification emphasized an increase in polarity of the existing framework, as opposed to an explicit lengthening of the Site 1 fragment. Key compounds deriving from this approach are included in Table 4. The piperazines **10**, **12**, and **13** appear similar to **62**−**65** in Table 3, with short, polar appendages leading to significant drops in Bcl-X_L affinity. Compounds **11** and **71**, presenting their polar NH and O groups still closer to the center of the hydrophobic BH3-binding groove, are unsurprisingly further deactivated. While the relative serum deactivation of the polar compounds improved as a group, only the relatively nonpolar **72** showed an affinity comparable to **1**, an indication that any significant increase in polarity in the vicinity of the fluorophenyl

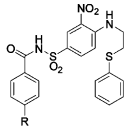
Table 3. Site 1 Biphenyl Tail SAR

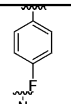
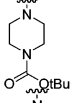
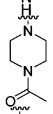
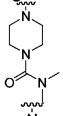


	R	K_i (μM)[a] Bcl-2	Bcl-X_L	Bcl-X_L1%HS
1	F(des-OMe)	0.433 ± 0.020*	0.036 ± 0.009*	2.50 ± 0.58*
62		4.57	0.426	1.00
63		1.98	0.471	>10.0
64		3.26	0.665	0.998
65		>10.0	0.251	>10.0
66		1.23 ± 0.18	0.106	0.73 ± 0.26
67		0.40 ± 0.11	0.0389	1.00 ± 0.46
68		0.402 ± 0.066	0.0104 ± 0.0040	0.58 ± 0.30
69		0.366	0.0579	0.79 ± 0.13
70		0.721	0.0215	2.96

[a] Values with standard deviation if two experiments were performed; with standard error and asterisk if three or more experiments.

Table 4. Site 1 Fluorophenyl Replacement SAR



	R	K_i (μM)[a] Bcl-2	Bcl-X_L	Bcl-X_L1% HS
1		0.433 ± 0.020*	0.036 ± 0.009*	2.50 ± 0.58*
10		2.88	0.396	3.35
11		--	2.52	>10.0
12		>10	0.894	2.97
13		3.54	0.294	0.869
71		>10.0	1.08	>10.0
72		0.192 ± 0.013	0.019	0.652

[a] Values with standard deviation if two experiments were performed; with standard error and asterisk if three or more experiments.

ring-binding region would almost certainly have too detrimental an effect on Bcl-X_L binding to be of interest. **72** also showed a decrease in serum deactivation from **1** of from 69-fold to 34-fold.

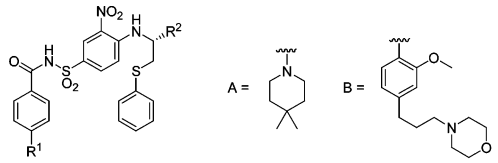
In general, then, the hypothesis that an increase in the polarity of compounds at Site 1 would lead to decreased serum deactivation was validated. However, affinity to Bcl-X_L was in most cases also reduced by an unacceptable amount, underscoring a need for either a minimal polarity increase to the Site 1 core or moving the source of polarity sufficiently near the end of the peptide-binding region. On the basis of these results, we narrowed our choices of modifications to Site 1 as we turned to Site 3 modification, choosing to go forward with the Site 1 fragments from **68** and **72**. We felt, based on the obtained structural information,[20] that the most straightforward point of attachment for Site 3 augmentation with polar groups was at the carbon atom adjacent to the aniline nitrogen. This necessitated creation of a chiral center; however, routes to a variety of compounds arising from a number of commercially available D- and L-amino acid derivatives were readily apparent. Thus we synthesized both enantiomers of selected inhibitors. We again focused primarily on amides and amines, and also focused on a two-carbon chain length because it appeared from the structure that a two-carbon linker would provide a length sufficient to situate the polar groups at the edge of the Bcl-X_L surface. Furthermore, we felt that increasing the chain length of the Site 3 amines might well be neutral toward Bcl-X_L binding but would be more likely to influence binding to HSA-III. Thus, we also synthesized some compounds with chain lengths of three and four carbon atoms.

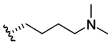
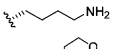
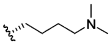
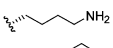
Anticipating some measure of additivity within this study toward reducing serum binding, we evaluated serum deactivation with 10% rather than 1% serum in our binding assay. Cellular EC_{50} data were obtained using Bcl-X_L-transfected FL5.12 cells in both the absence and presence of 3% fetal bovine serum (Table 5). FL5.12 is an IL-3 dependent murine pro-B lymphoid cell line characterized by low-moderate Bcl-X_L expression levels. Transfection with human Bcl-X_L produces cells that express roughly 10-fold higher protein levels as quantitated by Western blot analysis, thus allowing cells to survive in the absence of IL-3.

Table 5 collects results from key compounds of both the **68**- and **72**-derived series. It was immediately evident from the amine pairs **73R,S**, **77R,S**, and **79R,S** that *R*-chirality was preferred. This result fit well with structural information, which suggested that the carbon chains of the *R*-tails would be more likely to maintain close contact with Bcl-X_L, while the *S*-tails would immediately enter solvent. All of the *R*-chiral compounds displayed extremely high, roughly 1 nM affinity to Bcl-X_L. Clearly, the Site 3 modifications as a group markedly improved Bcl-X_L affinity. It is not clear from observation of the structure of complexed inhibitors that the increased affinity comes from a single source; rather, it seems likely that both the carbon chains and the amines contribute to binding. With regard to serum deactivation, the **68**- and **72**-derived groups showed similar patterns. Both the pair of amides **78** and **84** and the pair of morpholines **77R** and **83** were much less effective than the rest of the amines in reducing serum deactivation in the binding assay; thus, it appears that at least at this site, a charged species is particularly effective at reducing serum binding.

Interestingly, all of the dimethylamines as well as the primary amines **76** and **82** showed similar levels of serum deactivation, indicating that variation in chain length not only did little to influence Bcl-X_L binding but also had little effect on serum binding. However, cellular EC_{50} data separated compounds further. The two-carbon dimethylamines **73R** and **79R** were more active both under serum-free conditions and in the presence of 3% FBS than their three- and four-carbon counterparts; this chain-length variation is unexplained. The mor-

Table 5. Combination Substitution



			K_i (μM)[a]			FL5.12 Bcl-X_L EC_{50} (μM)[a]	
	R^1	R^2	Bcl-2	Bcl-X_L	Bcl-X_L10%HS	gelatin	3% FBS
73R	A		0.067 ± 0.006*	0.0008 ± 0.0002*	0.360 ± 0.067*	0.470 ± 0.050*	5.10 ± 0.53*
73S	A		1.26 ± 0.01*	0.252 ± 0.016*	3.85 ± 0.66*	9.50 ± 0.71	16.1 ± 1.3
74	A		0.0921	0.0026	0.728	2.00 ± 0.59*	14. 1 ± 2.8*
75	A		0.073	0.0012	0.174	1.08 ± 0.43*	3.89 ± 0.65*
76	A		0.231 ± 0.001	0.001	0.256	4.13 ± 0.44*	20.8 ± 1.1
77R	A		0.055 ± 0.004	0.0011 ± 0.0008	>10.0	0.368 ± 0.089*	7.0 ± 1.6*
77S	A		0.732	0.075 ± 0.001*	6.05 ± 0.94*	4.2 ± 1.8*	9.31 ± 0.27
78	A		0.075	0.0031	1.79	2.14 ± 0.61*	15.0 ± 1.9*
79R	B		0.116 ± 0.028*	<0.0005	0.148 ± 0.006*	0.399 ± 0.099*	2.08 ± 0.87*
79S	B		2.16 ± 0.06*	0.250 ± 0.019*	1.14 ± 0.24*	32.5 ± 1.8	59.2 ± 4.0
80	B		0.317	0.0009	0.071	3.01 ± 0.33*	8.60 ± 0.58*
81	B		0.0588	<0.0005	0.215	1.20 ± 0.22*	6.0
82	B		0.070	0.0008 ± 0.0002*	0.0296	3.05 ± 0.57*	9.0 ± 1.8*
83	B		0.165	<0.0005	>10.0	0.382 ± 0.055*	2.11 ± 0.51*
84	B		0.121	0.0017	1.00	1.28 ± 0.12*	7.63 ± 0.33*

[a] Values with standard deviation if two experiments were performed; with standard error and asterisk if three or more experiments.

pholines and amides, unsurprisingly, were also less potent than their comparators **73R** and **79R** in the presence of 3% FBS, though both groups showed less deactivation in this cell line than the binding data would have predicted. Ultimately, the dimethylamines **73R** and **79R** emerged from this final group of compounds. We verified that this pair of compounds indeed derived much of their improved binding in the presence of serum to greatly decreased affinity for HSA-III, with K_d values for affinity to the key HSA-III binding site of 13.6 and 94 μM, respectively. We also verified the dominant affinity of **73R** against Bcl-X_L compared to other Bcl-2 family members; affinity to Bcl-w and Mcl-1 were determined to be 0.459 μM and >10 μM, respectively. On the basis of ease of synthesis, prospects for future modifications, and pharmacokinetic profile, we chose to examine **73R** in a further series of *in* vitro and in vivo experiments designed to test its ability to cooperate with standard chemotherapies in combating tumor growth.

Chemo- and Radiopotentiation in Human Tumor Cells. Results from the experiments with IL-3 deprived FL5.12 cells, combined with the currently accepted hypothesis for the role of Bcl-X_L in the apoptotic process, indicated that **73R** would be most effective in the presence of an apoptotic stimulus. We used A549 cells to evaluate the effect of combination treatment. A549 is a human nonsmall cell lung carcinoma line,[37] whose cells express large amounts of Bcl-X_L.[17,38] Cells were treated with serial dilutions of **73R** plus serial dilutions of the costimulus. At 48 h posttreatment, cell viability was measured by the MTS assay and cell viability was normalized to untreated cells. Treatment with **73R** alone at up to 20 μM had little effect on cell viability.

Initially, treatment with UV-C radiation in 10% FBS was chosen in order to eliminate the possibility that potentiation was due to a change in cellular uptake of the apoptotic stimulus. As illustrated in Figure 2a, **73R** induced a leftward shift of the UV-C dose−response curve, driving a two- to 3-fold dose-dependent potentiation of UV-C induced cytotoxicity, as measured by shifts in EC_{50} values. It was also observed that in the absence of **73R**, a plateau was reached in the ability of UV-C irradiation to block cell growth; at a UV-C dose of 16 mJ/cm^2, the fraction of viable cells (35 ± 6%) was not significantly different than at 32 mJ/cm^2 (29 ± 3%). In combination, however, **73R** also induces a concentration-dependent increase in extent of cell kill at high UV-C doses, which was observed at concentrations as low as 2.5 μM. In comparison, the enantiomer **73S**, employed as a mechanistic control compound, shows no effect under the same conditions (Figure 2b).

The ability of **73R** to enhance the effects of a chemotherapeutic agent was evaluated next. Figure 2c shows the result of an experiment run with paclitaxel under serum-free conditions. As was observed with UV-C irradiation, **73R** in a concentration-dependent fashion decreased the fraction of viable cells at the highest paclitaxel dose. Furthermore, the addition of **73R** 48 h after the initiation of paclitaxel treatment resulted in a 21-fold potentiation of paclitaxel cytotoxicity. Again, the enantiomer, **73S**, had no effect on cell viability in similar experiments (data not shown).

In Vivo Evaluation. We next evaluated **73R** in an A549 xenograft tumor model. A549 tumors grow relatively slowly in immunocompromised mice, requiring 35−40 days to reach 1 cm^3 on a *Scid* background. Xenograft studies from numerous

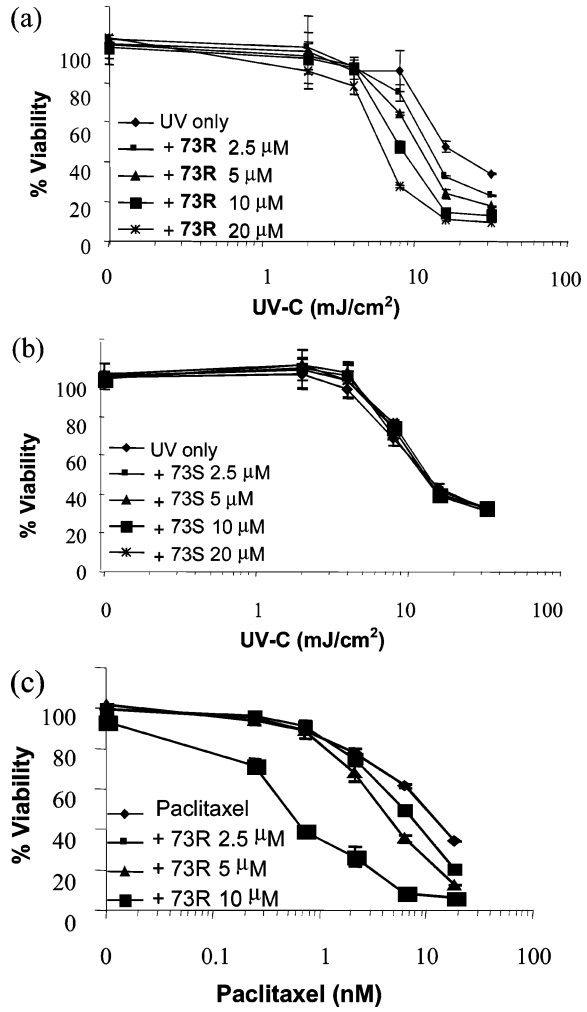


Figure 2. (a) Effect of **73R** in combination with 16 mJ/cm^2 UV-C radiation on A549 human NSCLC cells in the presence of 10% FBS, showing potentiation of radiation. (b) Corresponding graph of **73S** in combination with UV-C radiation, showing no effect potentiation with **73S**. (c) Effect of **73R** in combination with paclitaxel on A549 cells. Experiment run under serum-free conditions. Cells were treated with paclitaxel for 96 h and with **73R** from the 48 to 96 h time points. Viability was compared to untreated samples.

laboratories have demonstrated that this tumor line is resistant to most commonly used cytotoxic agents; however, administration of paclitaxel at the maximum tolerated dose (MTD) can effect a reduction of tumor growth rate of 60−70%.[39] In an established, staged tumor model experiment, **73R** enhanced the antitumor activity of paclitaxel with no overt evidence of increased toxicity (Figure 3). A549 cells were inoculated subcutaneously and allowed to grow to approximately 240 mm^3 (day 15), at which point mice were assigned to treatment groups and therapy was initiated. Paclitaxel was given at its MTD of 30 mg/kg/day on days 1, 5, and 9 with and without cotreatment with **73R** at 75 mg/kg/day for 21 days (see Experimental Section for details). Treatment with the MTD of paclitaxel alone resulted in tumor growth inhibition for two weeks after treatment. In contrast, the combination of paclitaxel plus **73R** caused regression of established A549 tumors during the treatment period, resulting in 75% tumor growth inhibition and significantly enhanced tumor growth delay with a time to 1 cm^3 tumor volume of 182%. We also note that although treatment with **73R** alone was not included in this particular experiment, we analyzed its effect in numerous studies similar to this one, and

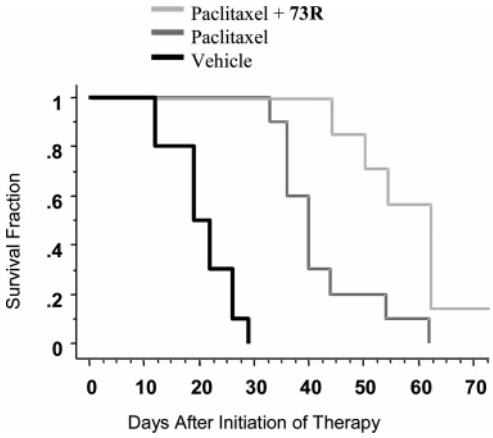


Figure 3. Kaplan−Meier analysis of **73R** plus paclitaxel in the A549 NSCLC model. Treatment with paclitaxel at 30 mg/kg/day (dark gray), paclitaxel at 30 mg/kg/day plus **73R** at 75 mg/kg/day (light gray) or vehicle (black). The %ILS, measured as the median time to 1 cm^3 tumor volume, was 95 for paclitaxel and for the **73R**/paclitaxel combination was 182. See Experimental Section for more details.

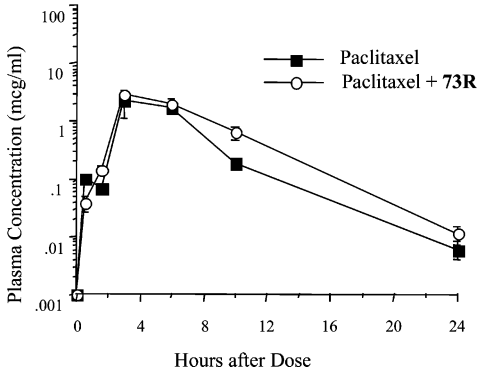


Figure 4. Paclitaxel plasma concentrations after ip dosing (15 mg/kg) alone or in combination with **73R** (100 mg/kg) in *Scid* mouse.

the effect of **73R** monotherapy was always statistically the same as vehicle.

To demonstrate that the effects of combination therapy were due to the interaction of **73R** with Bcl-X_L and not due to enhancement of paclitaxel exposure, we assessed potential pharmacokinetic interactions between the two agents. Studies involving coadministration of **73R** and paclitaxel in *Scid* mice demonstrated that neither the C_{max} nor the AUC of paclitaxel was significantly altered by the presence of **73R** (Figure 4).

Conclusions

Rationally designed modifications to an inhibitor of Bcl-X_L function have been described that directly addressed deleterious binding to serum components, specifically to domain III of HSA. Polarity-driven modification of the core structure at Site 1 maintained affinity to Bcl-X_L and modestly decreased serum deactivation, while combination with modifications to Site 3 produced affinity improvements of at least an order of magnitude, resulting in compounds **73R** and **79R**, with roughly 1 nM affinity for Bcl-X_L. These compounds exhibited 67- and 230-fold lower affinity for Bcl-2, respectively, and **73R** was also shown to have much lower affinity for Bcl-w and Mcl-1. Compounds also displayed cellular efficacy in cells stressed with an apoptotic stimulus. Furthermore, deactivation from serum binding was greatly reduced; in particular, the targeted HSA-III affinity was reduced by over 2 orders of magnitude. **73R** demonstrated the ability to potentiate the activity of UV radiation in vitro and paclitaxel in both in vitro and in vivo models of

human tumor growth, thus verifying the potential utility of a small-molecule BH3-mimetic as an anticancer agent.

Experimental Section

General Methods. All reactions were carried out under inert atmosphere (N_2) and at room temperature unless otherwise noted. Solvents and reagents were obtained commercially and were used without further purification. All reported yields are of isolated products and are not optimized. ^{1}H NMR spectra were obtained on a Varian UNITY or Inova (500 MHz), Varian UNITY (400 MHz), or Varian UNITY plus or Mercury (300 MHz) instrument. Chemical shifts are reported as δ values (ppm) downfield relative to TMS as an internal standard, with multiplicities reported in the usual manner. Mass spectra determinations were performed by the Analytical Research Department, Abbott Laboratories; DCI indicates chemical ionization in the presence of ammonia, ESI indicates electron spray ionization, APCI indicates atmospheric pressure chemical ionization with ammonia. Elemental analyses were performed by Quantitative Technologies, Inc., Whitehouse, NJ. Column chromatography was carried out in flash mode on silica gel (Merck Kieselgel 60, 230−400 mesh). Unless otherwise noted, preparative HPLC samples were purified on a Waters Symmetry C8 column (25 × 100 mm, 7 μm particle size) using a gradient of 10−100% CH_3CN:0.1% TFA over 8 min (10 min run time) at a flow rate of 40 mL/min.

4-Chloro-*N*-(4′-fluorobiphenyl-4-carbonyl)-3-nitrobenzenesulfonamide (4). A solution of **3** (4.54 g, 21.0 mmol), 4-chloro-3-nitrobenzenesulfonamide (4.74 g, 20.0 mmol), EDCI (4.80 g, 25.0 mmol), and DMAP (1.23 mg, 10.0 mmol) in CH_2Cl_2 (60 mL) was stirred for 16 h, diluted with EtOAc (200 mL), washed sequentially with 1 M HCl (50 mL), water (50 mL), and brine (20 mL), dried ($MgSO_4$), filtered, and concentrated. The concentrate was flash chromatographed on silica gel with 50% EtOAc/hexanes to provide 6.5 g (75%) of **4**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.62 (d, J = 2.2 Hz, 1H), 8.25 (dd, J = 8.8, 2.2 Hz, 1H), 8.05 (d, J = 8.4 Hz, 1H), 7.98 (d, J = 8.5 Hz, 2H), 7.79 (m, 4H), 7.33 (dd, J = 8.9, 8.8 Hz, 2H). MS (ESI) m/z 433 (M − H)$^-$.

***N*-(4′-Fluorobiphenyl-4-carbonyl)-4-(2-hydroxyethylamino)-3-nitrobenzenesulfonamide (5)**. A solution of **4** (2.5 g, 5.75 mmol) and 2-aminoethanol (10 mL) in dioxane (10 mL) was stirred at 80 °C for 20 min, taken up in 1 M HCl (100 mL), extracted with EtOAc (3 × 100 mL), washed with 2 × 1 M HCl, water and brine, dried (Na_2SO_4), filtered and concentrated to provide a yellow oil which was flash chromatographed on silica gel eluting with 5% MeOH/EtOAc to provide 2.57 g (97%) of **5**. 1H NMR (300 MHz, DMSO-d_6) δ 8.72 (t, J = 6 Hz, 1H), 8.67 (d, J = 2 Hz, 1H), 7.95 (m, 3H), 7.80 (m, 4H), 7.31 (m, 3H), 5.00 (br m, 1H), 3.65 (t, J = 6 Hz, 2H), 3.51 (dt, J = 6, 6 Hz, 2H). MS (ESI) m/z 458 (M − H)$^-$.

4-[2-(2,4-Dimethylphenylsulfanyl)ethylamino]-*N*-(4′-fluorobiphenyl-4-carbonyl)-3-nitrobenzenesulfonamide (2). A 0 °C solution of Bu_3P (155 μL, 0.62 mmol) and 1,1′-(azodicarbonyl)-dipiperidine (157 mg, 062 mmol) in THF (4 mL) was treated with **5** (149 mg, 0.32 mmol) and 2,4-dimethylthiophenol (50 μL, 0.37 mmol), stirred for 48 h, and concentrated. The concentrate was flash chromatographed on silica gel with 70% EtOAc/hexanes to provide 78 mg (41%) of **2**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.51 (m, 2H), 7.95 (br d, J = 8 Hz, 2H), 7.87 (dd, J = 2, 9 Hz, 1H), 7.73 (dd, J = 6, 9 Hz, 2H), 7.60 (br d, J = 8 Hz, 2H), 7.29 (m, 3H), 6.98 (m, 3H), 3.54 (dt, J = 7, 7 Hz, 2H), 3.18 (t, J = 7 Hz, 2H), 2.27 (s, 3H), 2.22 (s, 3H). MS (ESI) m/z 578 (M − H)$^-$.

4-Fluoro-3-nitrobenzenesulfonamide (6). A mixture of 2-fluoronitrobenzene (141.2 g, 1.0 mol) and chlorosulfonic acid (300 mL) was heated to 95 °C for 18 h, cooled to room temperature, and slowly added over 1 h to a mixture of 2-propanol (3.2 L) and concentrated NH_4OH (800 mL) maintained between −35 and −20 °C. The mixture was stirred an additional 30 min at −35 °C and concentrated HCl was added until the solution was acidic. The resulting slurry was partially concentrated in vacuo, water was added, and the process was repeated to give an aqueous slurry (3L). The solid was filtered, rinsed with 1 M HCl (1L) and water (2 L) and dried at 50 °C to provide 162.4 g (74%) of **6**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.52 (dd, J = 7.0, 2.5 Hz, 2H), 8.19 (ddd, J = 8.8, 4.1, 2.4 Hz, 2H), 7.78 (dd, J = 11.1, 8.7 Hz, 1H), 7.70 (br s, 2H). MS (ESI) m/z 219 (M − H)$^-$.

3-Nitro-4-(2-phenylsulfanylethylamino)benzenesulfonamide (7). A solution of **6** (314 mg, 1.33 mmol), 2-(phenylsulfanyl)-ethanamine (204 mg, 1.33 mmol), and DIPEA (0.5 mL) in DMSO (5 mL) was stirred for 16 h, diluted with EtOAc (100 mL), washed sequentially with 3 M HCl, water, and brine, dried (Na_2SO_4), filtered, and concentrated. The residue was chromatographed on silica gel with 25% EtOAc/hexane to give 450 mg (97%) of **7**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.74 (d, J = 2.4 Hz, 1H), 8.61 (br s, 1H), 7.83 (dd, J = 8.5, 2.4 Hz, 1H), 7.41 (m, 2H), 7.30 (m, 3H), 6.81 (d, J = 9.2 Hz, 1H), 4.76 (br s, 2H), 3.58 (dt, J = 6.6, 5.6 Hz, 1H), 3.22 (t, J = 6.6 Hz, 1H). MS (DCI) m/z 354 (M + H)$^+$.

4-(4-Ethoxycarbonylphenyl)piperazine-1-carboxylic Acid *tert*-Butyl Ester (8). A suspension of 4-fluorobenzoic acid ethyl ester (16.8 g, 100 mmol), piperazine-1-carboxylic acid *tert*-butyl ester (18.6 g, 100 mmol) and K_2CO_3 (20.7 g, 150 mmol) in DMSO (100 mL) was stirred at 120 °C for 10 h. The reaction mixture was cooled to room temperature and poured into water (1 L). The solid precipitate was filtered, washed with water and dried in a vacuum oven at 40 °C for 24 h to provide 13.38 g (40%) of **8**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.79 (d, J = 8.8 Hz, 2H), 6.98 (d, J = 9.2 Hz, 2H), 4.24 (q, J = 7.1 Hz, 2H), 3.45 (m, 4H), 3.29 (m, 4H), 1.42 (s, 9H), 1.29 (t, J = 7.1 Hz, 3H). MS (ESI) m/z 335 (M + H)$^+$.

4-(4-Carboxyphenyl)piperazine-1-carboxylic Acid *tert*-Butyl Ester (9). **9** was prepared from **8** using the procedure for the preparation of **27**. ^{1}H NMR (300 MHz, DMSO- d_6) δ 12.29 (br s, 1H), 7.78 (d, J = 9.2 Hz, 2H), 6.98 (d, J = 9.2 Hz, 2H), 3.43 (m, 4H), 3.29 (m, 4H), 1.42 (m, 9H). MS (ESI) m/z 305 (M − H)$^-$.

General Coupling Procedure. A suspension of acid (1 equiv), sulfonamide (1 equiv), EDCI (2−4 equiv) and DMAP (0.5−1 equiv) in CH_2Cl_2 (20 mL/mmol substrate) was stirred for 24 h. The reaction mixture was diluted with 5−10 volumes CH_2Cl_2 and washed with water. The organic phase was separated, dried ($MgSO_4$), filtered, and condensed. The crude reaction mixture was purified either by HPLC or by silica gel chromatography.

4-{4-[3-Nitro-4-(2-phenylsulfanylethylamino)benzenesulfonylaminocarbonyl]phenyl}piperazine-1-carboxylic Acid *tert*-Butyl Ester (10). A suspension of 9 (246 mg, 0.8 mmol), 7 (284 mg, 0.8 mmol), EDCI (308 mg, 1.6 mmol) and DMAP (98 mg, 0.8 mmol) in CH_2Cl_2 (10 mL) was stirred overnight. The reaction mixture was diluted with CH_2Cl_2 (20 mL) and washed with water (10 mL). The organic phase was separated, dried ($MgSO_4$), filtered, and condensed. The crude reaction mixture was chromatographed on silica gel using 5% MeOH/CH_2Cl_2 to yield 333 mg (65%) of 10. 1H NMR (300 MHz, DMSO-d_6) δ 8.77 (t, 1H), 8.60 (d, 1H), 7.91 (dd, 1H), 7.76 (d, 2H), 7.36 (d, 2H), 7.14−7.29 (m, 4H), 6.94 (d, 2H), 3.66 (q, 2H), 3.37−3.45 (m, 4H), 3.23−3.30 (m, 6H), 1.41 (s, 9H). MS (ESI) m/z 640 (M − H)$^-$. Anal. ($C_{30}H_{35}N_5O_7S_2$) C, H, N.

3-Nitro-4-(2-phenylsulfanylethylamino)-*N*-(4-piperazin-1-ylbenzoyl)benzenesulfonamide (11). A solution of **10** (4.49 g, 7.0 mmol) in CH_2Cl_2 (20 mL) and dioxane (20 mL) was treated with 4 M HCl in dioxane (50 mL) for 3 h. The reaction mixture was condensed and purified by reverse phase chromatography (Sep-pak C18, 0−40% MeCN/water/0.1% HCl) to give 3.31 g (95%) of **11**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.50 (t, J = 5.8 Hz, 1H), 8.47 (d, J = 2.0 Hz, 1H), 7.86 (dd, J = 8.8, 2.0 Hz, 1H), 7.77 (d, J = 8.8 Hz, 2H), 7.40 (m, 2H), 7.31 (m, 2H), 7.20 (m, 1H), 6.96 (d, J = 9.2, 1H), 6.88 (d, J = 8.8 Hz, 2H), 3.59 (q, J = 6.3 Hz, 2H), 3.38 (m, 4H), 3.26 (m, 2H), 3.20 (m, 4H). MS (ESI) m/z 540 (M − H)$^-$. Anal. ($C_{25}H_{27}N_5O_5S_2\cdot$HCl$\cdot$1.5$H_2O$) C, H, N.

***N*-[4-(4-Acetylpiperazin-1-yl)benzoyl]-3-nitro-4-(2-phenylsulfanylethylamino)benzenesulfonamide (12).** To a solution of **11** (54.1 mg, 0.1 mmol) in pyridine (2 mL) and Et_3N (1 mL) was added acetyl chloride (14.3 μL, 0.2 mmol), and the reaction mixture was stirred for 24 h. The reaction mixture was condensed, and the

product was purified by HPLC. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.04 (s, 1 H), 8.77 (t, J = 5.4 Hz, 1H), 8.60 (d, J = 2.0 Hz, 1H), 7.91 (dd, J = 9.2, 2.1 Hz, 1H), 7.76 (d, J = 9.2 Hz, 2H), 7.36 (m, 2H), 7.14−7.28 (m, 4H), 6.95 (d, J = 9.2 Hz, 2H), 3.67 (q, J = 6.5 Hz, 2H), 3.55 (m, 4H), 3.38 (m, 2H), 3.27 (m, 4H), 2.03 (s, 3H). MS (ESI) m/z 582 $(M - H)^-$. Anal. ($C_{27}H_{29}N_5O_6S_2 \cdot 0.5H_2O$) C, H, N.

4-{4-[3-Nitro-4-(2-phenylsulfanylethylamino)benzenesulfonyl-aminocarbonyl]phenyl}piperazine-1-carboxylic Acid Dimethylamide (13). 13 was prepared from **11** and dimethylcarbamoyl chloride using the procedure for the preparation of **12**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.64 (m, 1H), 8.54 (d, J = 2.4 Hz, 1H), 7.88 (dd, J = 9.2, 2.0 Hz, 1H), 7.75 (d, J = 8.8 Hz, 2H), 7.38 (m, 2H), 7.29 (m, 2H), 7.18 (m, 1H), 7.08 (d, J = 9.2 Hz, 1H), 6.89 (d, J = 9.2 Hz, 2H), 3.63 (m, 2H), 3.18−3.34 (m, 10H), 2.77 (s, 6H). MS (ESI) m/z 611 $(M - H)^-$. Anal. ($C_{28}H_{32}N_6O_6S_2 \cdot 0.5H_2O$) C, H, N.

Ethyl 4-(4,4-Dimethyl-2,6-dioxopiperidin-1-yl)benzoate (14). A solution of ethyl-4-aminobenzoate (2.2 g, 13.1 mmol), 3,3-dimethylglutaric anhydride (2.0 g, 13.1 mmol), and 1,2-dichloroethane (33 mL) was refluxed for 4 h. After cooling to room temperature, AcCl (1.9 mL, 27 mmol) was added dropwise, and the reaction was refluxed for 1 h and then cooled to room temperature. The solution was diluted with CH_2Cl_2 (150 mL), washed with water, saturated aqueous $NaHCO_3$, and brine, dried ($MgSO_4$), and condensed to afford 3.64 g (95%) of **14**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.14 (d, J = 8.5 Hz, 2H), 7.16 (d, J = 8.8 Hz, 2H), 4.39 (q, J = 7.1 Hz, 2H), 2.69 (s, 4H), 1.39 (t, J = 7.1 Hz, 3H), 1.22 (s, 6H). MS (DCI) m/z 290 $(M + H)^+$.

Ethyl 4-(4,4-Dimethylpiperidin-1-yl)benzoate (15). **15** was synthesized according a literature procedure.[29] A solution of **14** (900 mg, 3.06 mmol) and 2-methoxyethyl ether (10 mL) was stirred at 0 °C as $BF_3 \cdot Et_2O$ (0.88 mL, 6.8 mmol) was added dropwise. A suspension of $NaBH_4$ (248 mg, 6.48 mmol) in 2-methoxyethyl ether (8.3 mL) was then added slowly. The reaction was stirred for 15 min at 0 °C and for 4 h at room temperature. After cooling to 0 °C, the reaction was quenched by the cautious addition of ice, followed by excess water, and the mixture was stirred at room temperature for 1 h. The solid was filtered, washed with water, and dried to afford 0.61 g (76%) of **15**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90 (d, J = 9 Hz, 2H), 6.86 (d, J = 9 Hz, 2H), 4.32 (q, J = 7.1 Hz, 2H), 3.32 (t, J = 6.1 Hz, 4H), 1.49 (t, J = 6.1 Hz, 4H), 1.36 (t, J = 7.1 Hz, 3H), 0.99 (s, 6H). MS (DCI) m/z 262 $(M + H)^+$.

4-(4,4-Dimethylpiperidin-1-yl)benzoic Acid (16). A solution of **15** (9.5 g, 36.3 mmol), $LiOH \cdot H_2O$ (1.52 g, 36.3 mmol), THF (500 mL), water (125 mL), and MeOH (125 mL) was stirred for 18 h. Solvent was evaporated, and 1 M HCl was added. The resultant solid was filtered, washed with water, and dried to afford 8.1 g (96%) of **16**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.74 (d, J = 8.8 Hz, 2H), 6.93 (d, J = 9.1 Hz, 2H), 3.32 (m, 4H), 1.40 (t, J = 5.8 Hz, 4H), 0.95 (s, 6H). MS(DCI) m/z 234 $(M + H)^+$.

4′-Formyl-2′-methoxybiphenyl-4-carboxylic Acid Methyl Ester (18). A mixture of 3-methoxy-4-trifluoromethanesulfonyloxybenzaldehyde (36.0 g, 127 mmol), 4-methoxycarbonylphenylboronic acid (27.4 g, 152 mmol), $PdCl_2(dppf) \cdot CH_2Cl_2$ (2.93 g, 4.0 mmol), and CsF (39.5 g, 260 mmol) in dioxane (400 mL) was heated to 70 °C, stirred for 16 h, cooled, filtered through a pad of silica gel, and rinsed with ether (250 mL) and concentrated. The concentrate was triturated with EtOAc/hexanes to provide 30.0 g (87%) of **18**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.06 (s, 1H), 8.02 (d, J = 8 Hz, 2H), 7.70 (d, J = 8 Hz, 2H), 7.63 (m, 3H), 3.90 (s, 3H), 3.89 (s, 3H). MS (DCI) m/z 271 $(M + H)^+$.

4′-Dimethylcarbamoyl-2′-methoxybiphenyl-4-carboxylic Acid Methyl Ester (19). A mixture of **18** (1.10 g, 4.07 mmol), $NaIO_4$ (1.75 g, 8.15 mmol), and $RuCl_3$ (20 mg) in CCl_4 (10 mL), CH_3CN (10 mL), and water (15 mL) was stirred for 1 h. The mixture was poured into water (50 mL) and extracted with CH_2Cl_2 (3 × 70 mL), and the extracts were washed with brine, dried (Na_2SO_4), filtered, and concentrated. The crude acid (600 mg, 2.1 mmol) was taken up in CH_2Cl_2 (30 mL), and to the solution was added $(COCl)_2$ (220 μL, 2.5 mmol) and a drop of DMF. The reaction was stirred for 1 h and condensed. The crude acid chloride was taken up in THF (100 mL) and 2 M Me_2NH in THF (7 mL) was added. The reaction was stirred for 30 min and then poured into 1 M HCl (100 mL). The mixture was extracted with ether (2 × 100 mL), and the combined extracts were rinsed with saturated Na_2CO_3 and brine, dried (Na_2SO_4), filtered, and concentrated. The concentrate was flash chromatographed on silica gel with 30% EtOAc/hexanes to provide 626 mg (49%) of **19**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.01 (d, J = 8.5 Hz, 2H), 7.66 (d, J = 8.5 Hz, 2H), 7.40 (d, J = 7.5 Hz, 1H), 7.14 (d, J = 2 Hz, 1H), 7.07 (dd, J = 7.5, 2 Hz, 1H), 3.88 (s, 3H), 3.81 (s, 3H), 3.01 (s, 3H), 2.98 (s, 3H). MS (ESI) m/z 314 $(M + H)^+$.

4′-Dimethylaminocarbamoyl-2′-methoxybiphenyl-4-carboxylic Acid (20). A solution of **19** (622 mg, 2.0 mmol), $LiOH \cdot H_2O$ (336 mg, 8.0 mmol), THF (50 mL), water (15 mL), and MeOH (15 mL) was stirred for 24 h. The mixture was poured into 1 M HCl (100 mL), and the resulting mixture extracted with EtOAc (3 × 100 mL). The extracts were washed with brine, dried (Na_2SO_4), filtered, and concentrated to provide 585 mg (98%) of **20**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.62 (br s, 1H), 7.99 (d, J = 8.8 Hz, 2H), 7.63 (d, J = 8.8 Hz, 2H), 7.39 (d, J = 8.1 Hz, 1H), 7.14 (d, J = 1.4 Hz, 1H), 7.07 (dd, J = 8.1, 1.4 Hz, 1H), 3.81 (s, 3H), 3.00 (s, 3H), 2.98 (s, 3H). MS (ESI) m/z 300 $(M + H)^+$.

4′-Dimethylaminomethyl-2′-methoxybiphenyl-4-carboxylic Acid Methyl Ester (21). **21** was prepared from **20** using the procedure for the preparation of **33**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.06 (d, J = 8.5 Hz, 2H), 7.60 (d, J = 8.5 Hz, 2H), 7.28 (d, J = 7.5 Hz, 1H), 7.01 (s, 1H), 6.96 (d, J = 7.5 Hz, 1H), 3.93 (s, 3H), 3.84 (s, 3H), 3.51 (s, 2H), 2.33 (s, 6H). MS (ESI) m/z 300 $(M + H)^+$.

4′-Dimethylaminomethyl-2′-methoxybiphenyl-4-carboxylic Acid (22). **22** was prepared from **21** using the procedure for the preparation of **27**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.98 (d, J = 8.5 Hz, 2H), 7.62 (d, J = 8.5 Hz, 2H), 7.39 (d, J = 7.5 Hz, 1H), 7.37 (s, 1H), 7.15 (d, J = 7.5 Hz, 1H), 3.82 (s, 3H), 3.75 (s, 2H), 2.59 (s, 6H). MS (ESI) m/z 286 $(M + H)^+$.

4′-(2,2-Dibromovinyl)-2′-methoxybiphenyl-4-carboxylic Acid Methyl Ester (23). A solution of **18** (1.35 g, 5.0 mmol) in CH_2Cl_2 (30 mL) was treated with CBr_4 (1.82 g, 5.5 mmol) and PPh_3 (2.88 g, 11 mmol), stirred for 1 h, treated with hexanes (50 mL), and filtered through silica gel (50 g). The solution was rinsed with 1:1 water/CH_2Cl_2 (100 mL), the layers were separated, and the organic phase was concentrated. The concentrate was flash chromatographed on silica gel with 2−10% EtOAc/hexanes to provide 2.07 g (97%) of **23**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.08 (d, J = 8.5 Hz, 2H), 7.62 (d, J = 8.5 Hz, 2H), 7.52 (s, 1H), 7.34 (d, J = 8 Hz, 1H), 7.22 (d, J = 8 Hz, 1H), 3.93 (s, 3H), 3.83 (s, 3H). MS (DCI) m/z 427 $(M + H)^+$.

4′-Dimethylcarbamoylmethyl-2′-methoxybiphenyl-4-carboxylic Acid Methyl Ester (24). A mixture of **23** (213 mg, 0.5 mmol) and 2 M Me_2NH in THF (1 mL), DMF (1.5 mL), and water (0.25 mL) was heated to 80 °C for 8 h, diluted with EtOAc (100 mL), washed with water (45 mL) and brine (10 mL), dried ($MgSO_4$), filtered, and concentrated. The concentrate was flash chromatographed on silica gel with 2−10% MeOH/CH_2Cl_2 to provide 140 mg (86%) of **24**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.05 (d, J = 6.5 Hz, 2H), 7.59 (d, J = 6.5 Hz, 2H), 7.27 (d, J = 7.5 Hz, 1H), 6.93 (s, 1H), 6.91 (d, J = 7.5 Hz, 1H), 3.93 (s, 3H), 3.81 (s, 3H), 3.76 (s, 2H), 3.06 (s, 3H), 3.00 (s, 3H). MS (ESI) m/z 328 $(M + H)^+$.

4′-Dimethylcarbamoylmethyl-2′-methoxybiphenyl-4-carboxylic Acid (25). 25 was prepared from **24** using the procedure for the preparation of **20**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.87 (br s, 1H), 7.96 (d, J = 7.5 Hz, 2H), 7.59 (d, J = 7.5 Hz, 2H), 7.27 (d, J = 7.5 Hz, 1H), 7.01 (d, J = 1 Hz, 1H), 6.91 (dd, J = 7.5, 1 Hz, 1H), 3.76 (s, 3H), 3.74 (s, 2H), 3.04 (s, 3H), 2.85 (s, 3H). MS (ESI) m/z 314 $(M + H)^+$.

4′-(2-Dimethylaminoethyl)-2′-methoxybiphenyl-4-carboxylic Acid Methyl Ester (26). 26 was prepared from **24** using the procedure for the preparation of **33**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.97 (d, J = 8.4 Hz, 2H), 7.62 (d, J = 8.4 Hz, 2H), 7.24 (d, J = 8.0 Hz, 1H), 7.02 (s, 1H), 6.92 (d, J = 8.0 Hz, 1H), 3.87 (s,

3H), 3.78 (s, 3H), 2.76 (t, $J = 8.2$ Hz, 2H), 2.50 (t, $J = 8.2$ Hz, 2H), 2.22 (s, 6H). MS (ESI) *m/z* 314 $(M + H)^+$.

4′-(2-Dimethylaminoethyl)-2′-methoxybiphenyl-4-carboxylic Acid (27). A solution of **26** (105 mg, 0.335 mmol), $LiOH·H_2O$ (564 mg, 1.35 mmol), THF (20 mL), water (7 mL), and MeOH (7 mL) was stirred for 24 h. 1 M HCl (2 mL) was added, the mixture was poured into saturated NaH_2PO_4 (50 mL), and the resulting mixture was extracted with EtOAc (3 × 50 mL). The extracts were washed with brine, dried (Na_2SO_4), filtered, and concentrated to provide 96 mg (96%) of **27**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.94 (d, $J = 8.5$ Hz, 2H), 7.56 (d, $J = 8.5$ Hz, 2H), 7.23 (d, $J = 8$ Hz, 1H), 7.01 (d, $J = 1$ Hz, 1H), 6.93 (dd, $J = 8$, 1 Hz, 1H), 3.77 (s, 3H), 2.76 (t, $J = 8$ Hz, 2H), 2.53 (t, $J = 8$ Hz, 2H), 2.22 (s, 6H). MS (ESI) *m/z* 300 $(M + H)^+$.

4′-((*E*)-2-*tert*-Butoxycarbonylvinyl)-2′-methoxybiphenyl-4-carboxylic Acid Methyl Ester (28). A mixture of (*tert*-butoxycarbonylmethylene)triphenylphosphorane (2.25 g, 5.5 mmol) and **18** (1.35 g, 5.0 mmol) in THF (20 mL) was stirred for 3 h, diluted with hexanes (30 mL), and filtered through silica gel (50 g). The silica gel was rinsed with 50% CH_2Cl_2/ether, and the combined solutions were concentrated to provide 1.66 g (90%) of **28**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.07 (d, $J = 8.5$ Hz, 2H), 7.61 (d, $J = 8.5$ Hz, 2H), 7.59 (d, $J = 16$ Hz, 1H), 7.34 (d, $J = 8$ Hz, 1H), 7.20 (d, $J = 8$ Hz, 1H), 7.11, (s, 1H), 6.41 (d, $J = 16$ Hz, 1H), 3.94 (s, 3H), 3.85 (s, 3H), 1.55 (s, 9H). MS (ESI) *m/z* 367 $(M - H)^-$.

4′-(2-*tert*-Butoxycarbonylethyl)-2′-methoxybiphenyl-4-carboxylic Acid Methyl Ester (29). A mixture of **28** (20.0 g, 54.0 mmol) and $RhCl(PPh_3)_3$ (2.5 g) in toluene (300 mL) was stirred under a hydrogen atmosphere at 60 °C for 24 h, cooled, and concentrated. The residue was taken up in EtOAc and filtered through a pad of Celite (100 g) and silica gel (100 g), and the pad was rinsed with EtOAc (200 mL) and concentrated to provide 20.0 g (100%) of **29**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.97 (d, $J = 8$ Hz, 2H), 7.61 (d, $J = 8$ Hz, 2H), 7.24 (d, $J = 8$ Hz, 1H), 7.02, (d, $J = 1$ Hz, 1H), 6.91 (dd, $J = 8$, 1 Hz, 1H), 3.88 (s, 3H), 3.77 (s, 3H), 2.78 (t, $J = 7.5$ Hz, 2H), 2.58 (t, $J = 7.5$ Hz, 2H), 1.40 (s, 9H). MS (ESI) *m/z* 371 $(M + H)^+$.

4′-(2-Carboxyethyl)-2′-methoxybiphenyl-4-carboxylic Acid Methyl Ester (30). A solution of **29** (1.75 g, 4.73 mmol) in TFA (20 mL) and Et_3SiH (5 mL) was stirred at 50 °C for 24 h. The solution was condensed and then condensed from heptane (2×) to provide 1.53 g (99%) of **30**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.16 (br s, 1H), 7.97 (d, $J = 9$ Hz, 2H), 7.61 (d, $J = 9$ Hz, 2H), 7.25 (d, $J = 8$ Hz, 1H), 7.03, (d, $J = 1$ Hz, 1H), 6.92 (dd, $J = 8$, 1 Hz, 1H), 3.86 (s, 3H), 3.77 (s, 3H), 2.88 (t, $J = 7.5$ Hz, 2H), 2.60 (t, $J = 7.5$ Hz, 2H). MS (ESI) *m/z* 313 $(M - H)^-$.

4′-(2-Dimethylcarbamoylethyl)-2′-methoxybiphenyl-4-carboxylic Acid Methyl Ester (31). A solution of **30** (500 mg, 1.59 mmol) in CH_2Cl_2 (5 mL) was treated with 2 M oxalyl chloride in CH_2Cl_2 (1 mL) and a drop of DMF, stirred for 1 h, concentrated under vacuum, and dissolved in CH_2Cl_2 (5 mL). The mixture was treated with 2 M Me_2NH in THF (1.0 mL), and the resulting slurry was filtered through silica gel (10 g). The silica gel was rinsed with EtOAc and concentrated. The concentrate was flash chromatographed on silica gel with 30% EtOAc/hexanes to provide 490 mg (91%) of **31**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.05 (d, $J = 8.5$ Hz, 2H), 7.59 (d, $J = 8.5$ Hz, 2H), 7.25 (d, $J = 7.5$ Hz, 1H), 6.90 (d, $J = 7.5$ Hz, 1H), 6.88 (s, 1H), 3.93 (s, 3H), 3.81 (s, 3H), 3.02 (t, $J = 8$ Hz, 2H), 2.98 (s, 6H), 2.67 (t, $J = 8$ Hz, 2H). MS (ESI) *m/z* 342 $(M + H)^+$.

4′-(2-Dimethylcarbamoylethyl)-2′-methoxybiphenyl-4-carboxylic Acid (32). **32** was prepared from **31** using the procedure for the preparation of **20**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.72 (br s, 1H), 7.95 (d, $J = 8$ Hz, 2H), 7.59 (d, $J = 8$ Hz, 2H), 7.23 (d, $J = 8$ Hz, 1H), 7.02, (d, $J = 1$ Hz, 1H), 6.92 (dd, $J = 8$, 1 Hz, 1H), 3.78 (s, 3H), 2.96 (s, 3H), 2.75 (t, $J = 7.5$ Hz, 2H), 2.72 (s, 3H), 2.65 (t, $J = 7.5$ Hz, 2H). MS (ESI) *m/z* 328 $(M + H)^+$.

4′-(3-Dimethylaminopropyl)-2′-methoxybiphenyl-4-carboxylic Acid Methyl Ester (33). A solution of **31** (4.90 g, 12.8 mmol) in THF (30 mL) was treated with 1 M BH_3 in THF (51 mL), stirred for 24 h and slowly quenched at 0 °C with MeOH (8 mL). The mixture was poured into 6 M HCl (200 mL) and stirred for 2 h. The solution was adjusted to pH >10 with solid KOH and water, and the resulting solution was extracted with EtOAc (2 × 400 mL), washed with water and brine, dried (Na_2SO_4), filtered, and concentrated to provide a yellow oil which was flash chromatographed on silica gel eluting with 50% EtOAc/hexanes to provide 3.90 g (82%) of **33**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.97 (d, $J = 8$ Hz, 2H), 7.62 (d, $J = 8$ Hz, 2H), 7.25 (d, $J = 7.5$ Hz, 1H), 6.98 (s, 1H), 6.90 (d, $J = 7.5$ Hz, 1H), 3.87 (s, 3H), 3.78 (s, 3H), 2.63 (t, $J = 7.8$ Hz, 2H), 2.25 (t, $J = 7.3$ Hz, 2H), 2.15 (s, 6H), 1.74 (tt, $J = 7.5$, 7.3 Hz, 2H). MS (ESI) *m/z* 328 $(M + H)^+$.

4′-(3-Dimethylaminopropyl)-2′-methoxybiphenyl-4-carboxylic Acid Methyl Ester. 34 was prepared from **33** using the procedure for the preparation of **27**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.96 (d, $J = 8.5$ Hz, 2H), 7.59 (d, $J = 8.5$ Hz, 2H), 7.27 (d, $J = 8$ Hz, 1H), 7.02 (s, 1H), 6.93 (d, $J = 7.5$ Hz, 1H), 3.79 (s, 3H), 2.88 (m, 1H), 2.75 (m, 1H), 2.62 (dt, $J = 8$, 2 Hz, 2H), 2.56 (s, 6H), 2.01 (m, 2H). MS (ESI) *m/z* 314 $(M + H)^+$.

2′-Methoxy-4′-(3-morpholin-4-yl-3-oxopropyl)biphenyl-4-carboxylic Acid Methyl Ester (35). **35** was prepared from **30** and morpholine using the procedure for the preparation of **31**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.97 (d, $J = 8$ Hz, 2H), 7.60 (d, $J = 8$ Hz, 2H), 7.24 (d, $J = 8$ Hz, 1H), 7.03, (d, $J = 1$ Hz, 1H), 6.93 (dd, $J = 8$, 1 Hz, 1H), 3.87 (s, 3H), 3.78 (s, 3H), 3.51 (m, 4H), 3.44 (m, 4H), 2.78 (t, $J = 7.5$ Hz, 2H), 2.69 (t, $J = 7.5$ Hz, 2H). MS (ESI) *m/z* 384 $(M + H)^+$.

2′-Methoxy-4′-(3-morpholin-4-yl-3-oxopropyl)biphenyl-4-carboxylic Acid (36). 36 was prepared from **35** using the procedure for the preparation of **20**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.94 (d, $J = 8$ Hz, 2H), 7.57 (d, $J = 8$ Hz, 2H), 7.24 (d, $J = 8$ Hz, 1H), 7.02, (d, $J = 1$ Hz, 1H), 6.93 (dd, $J = 8$, 1 Hz, 1H), 3.78 (s, 3H), 3.51 (m, 4H), 3.44 (m, 4H), 2.87 (t, $J = 7.5$ Hz, 2H), 2.69 (t, $J = 7.5$ Hz, 2H), 0.86 (s, 9H), 0.03 (s, 6H). MS (ESI) *m/z* 370 $(M + H)^+$.

2′-Methoxy-4′-(3-morpholin-4-ylpropyl)biphenyl-4-carboxylic Acid Methyl Ester (37). **37** was prepared from **35** using the procedure for the preparation of **33**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.97 (d, $J = 8$ Hz, 2H), 7.61 (d, $J = 8$ Hz, 2H), 7.25 (d, $J = 7.5$ Hz, 1H), 6.99 (s, 1H), 6.89 (d, $J = 8$ Hz, 1H), 3.87 (s, 3H), 3.78 (s, 3H), 3.58 (m, 4H), 3.38 (m, 4H), 2.33 (m, 4H), 1.78 (t, $J = 7$ Hz, 2H). MS (ESI) *m/z* 370 $(M + H)^+$.

2′-Methoxy-4′-(3-morpholin-4-ylpropyl)biphenyl-4-carboxylic Acid (38). **38** was prepared from **37** using the procedure for the preparation of **27**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.4 (br s, 1H), 7.96 (d, $J = 9$ Hz, 2H), 7.59 (d, $J = 9$ Hz, 2H), 7.28 (d, $J = 7.5$ Hz, 1H), 7.03 (d, $J = 1.5$ Hz, 1H), 6.94 (dd, $J = 7.5$, 1.5 Hz, 1H), 3.88 (m, 2H), 3.79 (s, 3H), 3.69 (dt, $J = 13$, 4 Hz, 2H), 2.83 (m, 4H), 2.64 (m, 2H), 2.09 (m, 2H). MS (ESI) *m/z* 356 $(M + H)^+$.

4′-(3-Hydroxypropyl)-2′-methoxybiphenyl-4-carboxylic Acid Methyl Ester (39). A solution of **30** (2.92 g, 9.3 mmol) in THF (10 mL) was treated with 1 M BH_3 in THF (18.6 mL), stirred for 24 h, and slowly quenched at 0 °C with MeOH (5 mL). The mixture was poured into 4 M HCl (200 mL) and stirred for 1 h. The solution was extracted with EtOAc (3 × 150 mL), washed with water and brine, dried (Na_2SO_4), filtered, and concentrated. The crude product was flash chromatographed on silica gel eluting with 50% EtOAc/hexanes to provide 1.51 g (57%) of **39**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.97 (d, $J = 7$ Hz, 2H), 7.62 (d, $J = 7$ Hz, 2H), 7.24 (d, $J = 8$ Hz, 1H), 6.98, (d, $J = 1$ Hz, 1H), 6.89 (dd, $J = 8$, 1 Hz, 1H), 4.50 (t, $J = 5$ Hz, 1H), 3.87 (s, 3H), 3.78 (s, 3H), 3.46 (dt, $J = 7$, 5 Hz, 1H), 2.67 (t, $J = 8$ Hz, 2H), 1.77 (tt, $J = 8$, 7 Hz, 2H). MS (ESI) *m/z* 301 $(M + H)^+$.

4′-[3-(*tert*-Butyldimethylsilanyloxy)propyl]-2′-methoxybiphenyl-4-carboxylic Acid Methyl Ester (40). A solution of **39** (234 mg, 0.66 mmol), TBDMSOTf (165 μL, 0.72 mmol), and 2,6-lutidine (92 μL, 0.79 mmol) in CH_2Cl_2 (10 mL) at 0 °C was stirred for 30 min, and flash chromatographed on silica gel with 50% EtOAc/hexanes to provide 300 mg (97%) of **40**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.92 (d, $J = 8$ Hz, 2H), 7.57 (d, $J = 8$ Hz, 2H), 7.20 (d, $J = 8$ Hz, 1H), 6.91, (d, $J = 1$ Hz, 1H), 6.84 (dd, J

= 8, 1 Hz, 1H), 3.82 (s, 3H), 3.72 (s, 3H), 3.59 (t, J = 7.5 Hz, 2H), 2.62 (t, J = 8 Hz, 2H), 1.76 (tt, J = 7.5, 8 Hz, 2H), 0.84 (s, 9H), 0.00 (s, 6H). MS (ESI) m/z 415 $(M + H)^+$.

4′-[3-(*tert*-Butyldimethylsilanyloxy)propyl]-2′-methoxybiphenyl-4-carboxylic acid (41). **41** was prepared from **40** using the procedure for the preparation of **27**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.93 (d, J = 8 Hz, 2H), 7.57 (d, J = 8 Hz, 2H), 7.23 (d, J = 8 Hz, 1H), 6.94, (d, J = 1 Hz, 1H), 6.86 (dd, J = 8, 1 Hz, 1H), 3.75 (s, 3H), 3.62 (t, J = 7 Hz, 2H), 2.64 (t, J = 8 Hz, 2H), 1.80 (tt, J = 7, 8 Hz, 2H), 0.86 (s, 9H), 0.03 (s, 6H). MS (ESI) m/z 399 $(M - H)^-$.

(*R*)-3-(9*H*-Fluoren-9-yloxycarbonylamino)-4-hydroxybutyric Acid *tert*-Butyl Ester (42R). A solution of Fmoc-D-Asp(OtBu)-OH (9.0 g, 21.8 mmol) and DIPEA (4.6 mL) in THF (100 mL) at −40 °C was treated with isobutyl chloroformate (3.1 mL, 24.1 mmol), warmed to 0 °C over 30 min, cooled to −20 °C, and treated slowly with $NaBH_4$ (1.64 g, 43.6 mmol) and MeOH (10 mL). The reaction was gradually warmed to room temperature over 2 h, diluted with EtOAc (200 mL), washed with water (100 mL) and brine (50 mL), dried ($MgSO_4$), filtered, and concentrated to provide 8.22 g (95%) of **42R**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.77 (d, J = 8 Hz, 2H), 7.59 (d, J = 7.5 Hz, 2H), 7.40 (dd, J = 7.5, 7.5 Hz, 2H), 7.31 (dd, J = 8, 7.5 Hz, 2H), 5.45 (m, 1H), 4.41 (br d, J = 6.8 Hz, 2H), 4.22 (t, J = 6.8 Hz, 1H), 4.03 (m, 1H), 3.72 (m, 2H), 2.56 (m, 2H), 2.34 (m, 1H), 1.45 (m, 9H). MS (ESI) m/z 398 $(M + H)^+$.

(*S*)-3-(9*H*-Fluoren-9-yloxycarbonylamino)-4-hydroxybutyric Acid *tert*-Butyl Ester (42S). **42S** was prepared from Fmoc-L-Asp(OtBu)-OH using the procedure for the preparation of **42R**. MS (ESI) m/z 398 $(M + H)^+$.

(*R*)-3-(9*H*-Fluoren-9-yloxycarbonylamino)-4-phenylsulfanylbutyric Acid *tert*-Butyl Ester (43R). A solution of Bu_3P (7.3 mL, 29.2 mmol) and 1,1′-(azodicarbonyl)dipiperidine (7.35 g, 29.2 mmol) in CH_2Cl_2 (100 mL) was treated with **42R** (9.65 g, 24.3 mmol) and thiophenol (5.0 mL, 48.6 mmol), stirred for 24 h, and concentrated. The concentrate was flash chromatographed on silica gel with 50% EtOAc/hexanes to provide 8.5 g (72%) of **43R**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (d, J = 7.4 Hz, 2H), 7.57 (d, J = 7.1 Hz, 2H), 7.40 (m, 4H), 7.30 (m, 4H), 7.19 (t, J = 7.5 Hz, 1H), 5.48 (m, 1H), 4.35 (br d, J = 7.4 Hz, 2H), 4.19 (t, J = 7.1 Hz, 1H), 4.13 (m, 1H), 3.26 (m, 1H), 3.11 (m, 1H), 2.64 (m, 2H), 1.43 (m, 9H). MS (DCI) m/z 490 $(M + H)^+$.

(*S*)-3-(9*H*-Fluoren-9-yloxycarbonylamino)-4-phenylsulfanylbutyric Acid *tert*-Butyl Ester (43S). **43S** was prepared from **42S** using the procedure for the preparation of **43R**. MS (DCI) m/z 490 $(M + H)^+$.

(*R*)-3-(2-Nitro-4-sulfamoylphenylamino)-4-phenylsulfanylbutyric Acid *tert*-Butyl Ester (44R). A mixture of **43R** (600 mg, 1.23 mmol), **6** (298 mg, 1.34 mmol), and DIPEA (3 mL) in DMF (3 mL) was stirred for 12 h, diluted with EtOAc (100 mL), washed with water (45 mL) and brine (10 mL), dried ($MgSO_4$), filtered, and concentrated. The concentrate was flash chromatographed on silica gel with 30% EtOAc/CH_2Cl_2 to provide 390 mg (68%) of **44R**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.68 (s, 1H), 8.66 (d, J = 7 Hz, 1H), 7.75 (d, J = 7 Hz, 1H), 7.55 (m, 1H), 7.37 (m, 2H), 7.27 (m, 4H), 6.67 (d, J = 9.1 Hz, 1H), 4.83 (s, 2H), 4.17 (m, 1H), 3.20 (d, J = 6.4 Hz, 2H), 2.77 (ddd, J = 10.5, 9, 6.4 Hz, 1H), 1.42 (m, 9H). MS (ESI) m/z 468 $(M + H)^+$.

(*S*)-3-(2-Nitro-4-sulfamoylphenylamino)-4-phenylsulfanylbutyric Acid *tert*-Butyl Ester (44S). **44S** was prepared from **43S** using the procedure for the preparation of **44R**. MS (ESI) m/z 468 $(M + H)^+$.

(*R*)-3-(2-Nitro-4-sulfamoylphenylamino)-4-phenylsulfanylbutyric acid (45R). A mixture of **44R** (2.8 g, 6 mmol) and 4 M HCl (50 mL) in 1,4-dioxane (50 mL) was stirred for 6 h. The solution was concentrated and then concentrated from toluene to provide 2.47 g (99%) of **45R**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.67 (m, 2H), 8.03 (s, 2H), 7.74 (d, J = 9.1 Hz, 1H), 7.37 (m, 2H), 7.16 (m, 3H), 6.67 (d, J = 9.2 Hz, 1H), 4.22 (m, 1H), 3.777 (m, 2H), 3.22 (d, J = 6.1 Hz, 2H). MS (ESI) m/z 410 $(M - H)^-$.

(*S*)-3-(2-Nitro-4-sulfamoylphenylamino)-4-phenylsulfanylbutyric acid (45S). **45S** was prepared from **44S** using the procedure for the preparation of **45R**. MS (ESI) m/z 410 $(M - H)^-$.

(*R*)-*N*,*N*-Dimethyl-3-(2-nitro-4-sulfamoylphenylamino)-4-phenylsulfanylbutyramide (46R). A solution of **45R** (411 mg, 1 mmol), 2 M Me_2NH in THF (1 mL), EDCI (296 mg, 1.5 mmol), and DMAP (10 mg) in DMF (10 mL) was stirred for 16 h, diluted with EtOAc (200 mL), washed sequentially with 1 M HCl (50 mL), water (50 mL), and brine (20 mL), dried ($MgSO_4$), filtered, and concentrated. The concentrate was flash chromatographed on silica gel with 100% EtOAc to provide 245 mg (56%) of **47R**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.77 (d, J = 9.5 Hz, 1H), 8.39 (s, 1H), 7.72 (dd, J = 9.2, 2.3 Hz, 1H), 7.24−7.35 (m, 6H), 7.21 (m, 1H), 7.09 (d, J = 9.5 Hz, 1H), 4.40 (m, 1H), 3.41 (d, J = 6.4 Hz, 2H), 2.94 (m, 1H), 2.91 (s, 3H), 2.79 (s, 3H), 2.72 (m, 1H). MS (ESI) m/z 439 $(M + H)^+$.

(*S*)-*N*,*N*-Dimethyl-3-(2-nitro-4-sulfamoylphenylamino)-4-phenylsulfanylbutyramide (46S). **46S** was prepared from **45S** using the procedure for the preparation of **46R**. MS (ESI) m/z 439 $(M + H)^+$.

4-((*R*)-3-Morpholin-4-yl-3-oxo-1-phenylsulfanylmethylpropylamino)-3-nitrobenzenesulfonamide (48R). **48R** was prepared from **45R** and morpholine using the procedure described for the preparation of **46R**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.70 (d, J = 9.5 Hz, 1H), 8.39 (d, J = 2 Hz, 1H), 7.73 (dd, J = 9.5, 2 Hz, 1H), 7.22−7.36 (m, 6H), 7.18 (t, J = 7 Hz, 1H), 7.09 (d, J = 9.5 Hz, 1H), 4.42 (m, 1H), 3.42−3.56 (m, 4H), 3.39 (m, 4H), 3.30 (d, J = 7 Hz, 2H), 3.00 (dd, J = 16, 7 Hz, 1H), 2.78 (dd, J = 16, 5.5 Hz, 1H). MS (ESI) m/z 481 $(M + H)^+$.

4-((*S*)-3-Morpholin-4-yl-3-oxo-1-phenylsulfanylmethylpropylamino)-3-nitrobenzenesulfonamide (48S). **48S** was prepared from **45S** and morpholine using the procedure described for the preparation of **46R**. MS (ESI) m/z 481 $(M + H)^+$.

4-((*R*)-3-Dimethylamino-1-phenylsulfanylmethylpropylamino)-3-nitrobenzenesulfonamide (47R). A mixture of **46R** (4.06 g, 9.25 mmol) and 1 M BH_3 in THF (20 mL) was stirred for 16 h, treated with MeOH (5.0 mL) and concentrated HCl (2 mL), stirred at 80 °C for 3 h, cooled to room temperature, adjusted to pH 10 with 4 M Na_2CO_3, diluted with EtOAc (150 mL), washed with water (50 mL) and brine (10 mL), dried ($MgSO_4$), filtered, and concentrated. The concentrate was flash chromatographed on silica gel with 20% MeOH/CH_2Cl_2 to provide 3.88 g (99%) of **47R**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.64 (br d, J = 9 Hz, 1H), 8.39 (d, J = 2 Hz, 1H), 7.71 (dd, J = 9, 2 Hz, 1H), 7.31 (m, 4H), 7.27 (m, 2H), 7.19 (m, 1H), 7.08 (br d, J = 9 Hz, 1H), 4.12 (m, 1H), 3.37 (m, 2H), 2.40 (m, 1H), 2.20 (m, 1H), 2.11 (s, 6H), 1.94 (m, 1H), 1.82 (m, 1H). MS (ESI) m/z 425 $(M + H)^+$. $[\alpha]_D^{23.4} = -342°$ (c 0.51, acetone).

4-((*S*)-3-Dimethylamino-1-phenylsulfanylmethylpropylamino)-3-nitrobenzenesulfonamide (47S). **47S** was prepared from **46S** using the procedure for the preparation of **47R**. MS (ESI) m/z 425 $(M + H)^+$. $[\alpha]_D^{23.1} = +334°$ (c 0.42, acetone).

4-((*R*)-3-Morpholin-4-yl-1-phenylsulfanylmethylpropylamino)-3-nitrobenzenesulfonamide (49R). **49R** was prepared from **48R** using the procedure described for the preparation of **47R.** ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.41 (d, J = 9.2 Hz, 1H), 8.40 (d, J = 2 Hz, 1H), 7.73 (dd, J = 9.5, 2 Hz, 1H), 7.22−7.35 (m, 6H), 7.19 (t, J = 7 Hz, 1H), 7.11 (d, J = 10 Hz, 1H), 4.18 (m, 1H), 3.54 (m, 4H), 3.38 (m, 2H), 2.28−2.40 (m, 4H), 2.21 (m, 2H), 2.00 (m, 1H), 1.92 (m, 1H). MS (ESI) m/z 467 $(M + H)^+$.

4-((*S*)-3-Morpholin-4-yl-1-phenylsulfanylmethylpropylamino)-3-nitrobenzenesulfonamide (49S). **49S** was prepared from **48S** using the procedure described for the preparation of **47R.** MS (ESI) m/z 467 $(M + H)^+$.

(*R*)-2-*tert*-Butoxycarbonylamino-3-phenylsulfanylpropionic Acid Methyl Ester (50). A 0 °C solution of *N*-(*tert*-butoxycarbonyl)-L-serine methyl ester (30 g, 137 mmol) and $^i Pr_2NH$ (58 mL, 330 mmol) in CH_2Cl_2 (250 mL) was treated with methanesulfonyl chloride (11.65 mL, 151 mmol), stirred for 20 min, treated with thiophenol (15.5 mL, 151 mmol), warmed to room temperature, stirred for 30 min, and concentrated. The crude material was flash

chromatographed on silica gel with 10% EtOAc/hexanes to provide 25.0 g (59%) of **50**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.35 (m, 5H), 7.24 (m, 1H), 4.10 (m, 1H), 3.60 (s, 3H), 3.35 (dd, J = 14, 6 Hz, 1H), 3.14 (dd, J = 14, 10 Hz, 1H), 1.38 (s, 9H). MS (ES) m/z 310 $(M - H)^-$.

((*R*)-1-Formyl-2-phenylsulfanylethyl)carbamic Acid *tert*-Butyl Ester (51). A solution of **50** (8.1 g, 26.0 mmol) in CH_2Cl_2 at −78 °C was treated with 1 M DIBAL in CH_2Cl_2 (52 mL), stirred for 3 h, quenched with MeOH (20 mL), and poured into saturated NaH_2PO_4. The mixture was extracted with EtOAc (3 × 300 mL), and the combined extracts were dried (Na_2SO_4), filtered, concentrated, and flash chromatographed on silica gel with 30% EtOAc/hexanes to provide 5.0 g (68%) of **51**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.47 (s, 1H), 7.34 (m, 5H), 7.23 (m, 1H), 4.00 (m, 1H), 3.40 (dd, J = 14, 6 Hz, 1H), 3.07 (dd, J = 14, 10 Hz, 1H), 1.39 (s, 9H). MS (ESI) m/z 280 $(M - H)^-$.

(*E*,*R*)-4-tert-Butoxycarbonylamino-5-phenylsulfanylpent-2-enoic Acid *tert*-Butyl Ester (52). **51** (8.4 g, 30 mmol) in THF (25 mL) at 0 °C was treated with a solution of $Ph_3P{=}CHCO_2tBu$, (13.6 g, 36 mmol) in THF (150 mL), warmed to room temperature, stirred for 24 h, treated with hexanes (100 mL), filtered through a pad of silica gel, and concentrated. The concentrate was flash chromatographed on silica gel with 10% EtOAc/hexanes to provide 9.5 g (83%) of **52**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.38−7.49 (m, 4H), 7.21 (m, 2H), 6.76 (dd, J = 16, 6 Hz, 1H), 5.78 (dd, J = 16, 2 Hz, 1H), 4.14 (m, 1H), 3.19 (m, 1H), 3.01 (m, 1H), 1.41 (s, 9H). MS (ESI) m/z 378 $(M - H)^-$.

(*R*)-4-*tert*-Butoxycarbonylamino-5-phenylsulfanylpentanoic Acid (53). A mixture of **52** (5.0 g, 13.2 mmol) and $RhCl(PPh_3)_3$ (1 g) in toluene (125 mL) was stirred under H_2 at 50 °C for 24 h, cooled, filtered through silica gel, and concentrated. The concentrate was dissolved in THF (90 mL), water (30 mL), and MeOH (30 mL), treated with LiOH (2.77 g, 66 mmol), and stirred for 24 h. The mixture was poured into saturated NaH_2PO_4 solution (200 mL) and extracted with EtOAc (3 × 200 mL). The combined extracts were washed with brine, dried (Na_2SO_4), filtered, and concentrated to provide 4.12 g (96%) of **53**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.33 (m, 4H), 7.18 (m, 1H), 6.72 (br d, J = 9.5 Hz, 1H), 3.55 (m, 1H), 2.99 (d, J = 7 Hz, 2H), 2.21 (m, 2H), 1.85 (m, 1H), 1.58 (m, 1H), 1.38 (s, 9H). MS (ESI) m/z 326 $(M + H)^+$.

((*R*)-3-Dimethylcarbamoyl-1-phenylsulfanylmethylpropyl)-carbamic Acid *tert*-Butyl Ester (54). A solution of **53** (10.6 g, 32.6 mmol), 2 M Me_2NH in THF (32.6 mL), EDCI (12.5 g, 65 mmol), and DMAP (4.0 g, 32.6 mmol) in CH_2Cl_2 (10 mL) was stirred for 24 h, diluted with EtOAc (200 mL), washed sequentially with 1 M HCl (50 mL), water (50 mL), and brine (20 mL), dried (Na_2SO_4), filtered, and concentrated. The concentrate was flash chromatographed on silica gel with EtOAc to provide 9.0 g (78%) of **54**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.32 (m, 4H), 7.18 (m, 1H), 6.82 (br d, J = 9.5 Hz, 1H), 3.55 (m, 1H), 3.01 (d, J = 7 Hz, 2H), 2.91 (s, 3H), 2.79 (s, 3H), 2.27 (m, 2H), 1.83 (m, 1H), 1.59 (m, 1H), 1.38 (s, 9H). MS (ESI) m/z 353 $(M + H)^+$.

(*R*)-4-(2-Nitro-4-sulfamoylphenylamino)-5-phenylsulfanylpentanoic Acid Dimethyl Amide (55). A mixture of **54** (1.13 g, 3.22 mmol) in dioxane (50 mL) and 4 M HCl (50 mL) was stirred for 2 h, poured into saturated Na_2CO_3 (400 mL), and extracted with EtOAc (3 × 300 mL). The combined extracts were dried (Na_2SO_4), filtered, and concentrated to provide the pure primary amine. The amine was taken up in DMF (10 mL), **6** (880 mg, 4 mmol), and DIPEA (1 mL) were added, and the reaction was stirred for 4 h. The reaction was poured into water (200 mL) and extracted with EtOAc (3 × 100 mL). The extracts were washed with water (3 × 100 mL) and brine (100 mL), dried (Na_2SO_4), filtered, concentrated, and flash chromatographed on silica gel with 50% EtOAc/hexanes to provide 890 mg (61%) of **55**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.40 (d, J = 2 Hz, 1H), 8.28 (br d, J = 9 Hz, 1H), 7.72 (dd, J = 9, 2 Hz, 1H), 7.14−7.39 (m, 8H), 4.12 (m, 1H), 3.38 (m, 2H), 2.88 (s, 3H), 2.78 (s, 3H), 2.41 (m, 2H), 1.97 (m, 2H). MS (ESI) m/z 453 $(M + H)^+$.

4-((*R*)-4-Dimethylamino-1-phenylsulfanylmethylbutylamino)-3-nitrobenzenesulfonamide (56). A mixture of **54** (9.0 g, 25.5 mmol) and 1 M BH_3 in THF (94 mL) was stirred for 24 h, treated with MeOH (10 mL) and 4 M HCl (100 mL), and stirred for 24 h. The mixture was adjusted to pH > 12 with KOH, extracted with EtOAc (3 × 200 mL), and the extracts were washed with water (200 mL) and brine (200 mL), dried (Na_2SO_4), filtered, and concentrated. The amine was taken up in DMF (100 mL), **6** (5.9 g, 26.8 mmol) and DIPEA (5 mL) were added, and the reaction was stirred for 4 h. The reaction was poured into water (400 mL) and extracted with EtOAc (3 × 300 mL). The extracts were washed with water (3 × 200 mL), dried (Na_2SO_4), filtered, concentrated, and flash chromatographed on silica gel with 1/10/89 TEA/MeOH/EtOAc to provide 9.8 g (88%) of **56**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.39 (d, J = 2 Hz, 1H), 8.27 (br d, J = 9 Hz, 1H), 7.74 (dd, J = 9, 2 Hz, 1H), 7.31 (m, 4H), 7.14−7.25 (m, 4H), 4.09 (m, 1H), 3.33 (m, 2H), 2.15 (t, J = 7 Hz, 2H), 2.05 (s, 6H), 1.74 (m, 2H), 1.47 (m, 2H). MS (ESI) m/z 439 $(M + H)^+$. $[\alpha]_D^{23.7} = -321°$ (c 0.33, acetone).

((*R*)-5-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-6-hydroxyhexyl)carbamic Acid *tert*-Butyl Ester (57). A solution of Fmoc-D-Lys(BOC)-OH (2.102 g, 4.5 mmol) in DME (5 mL) at −15 °C was treated successively with *N*-methylmorpholine (0.56 mL, 5.0 mmol) and isobutyl chloroformate (0.7 mL, 5 mmol), stirred for 2 min, and filtered. The filter cake was washed with DME (3 × 5 mL), and the combined filtrate and washings were cooled to −5 °C and treated with $NaBH_4$ (0.3 g, 7.5 mmol) and water (5 mL) and additional water (250 mL) immediately afterward. The mixture was stirred for 15 min and filtered. The filter cake was washed with water and dried to provide 1.94 g (95%) of **57**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (d, J = 8 Hz, 2H), 7.59 (d, J = 7.5 Hz, 2H), 7.39 (dd, J = 7.5, 7.3 Hz, 2H), 7.31 (dd, J = 8, 7.3 Hz, 2H), 5.07 (m, 1H), 4.58 (m, 1H), 4.41 (d, J = 5.8 Hz, 2H), 4.21 (t, J = 6.8 Hz, 1H), 3.62 (m, 4H), 3.09 (m, 2H), 1.26−1.54 (m, 6H), 1.43 (s, 9H). MS (APCI) m/z 455 $(M + H)^+$.

((*R*)-5-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-6-phenylsulfanylhexyl)carbamic Acid *tert*-Butyl Ester (58). A mixture of **57** (2.0 g, 4.4 mmol), PhSSPh (1.44 g, 6.6 mmol), and PBu_3 (1.65 mL, 6.6 mmol) in toluene (50 mL) at 80 °C was stirred for 18 h and concentrated. The concentrate was flash chromatographed on silica gel with 25% EtOAc/hexanes to provide 1.83 g (76%) of **58**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (d, J = 8 Hz, 2H), 7.52 (m, 4H), 7.25−7.42 (m, 6H), 7.21 (t, J = 7 Hz, 1H), 5.46 (m, 2H), 4.50 (m, 2H), 4.36 (m, 1H), 4.06 (m, 2H), 1.18−1.66 (m, 6H), 1.43 (s, 9H). MS (APCI) m/z 547 $(M + H)^+$.

((*R*)-5-Dimethylamino-1-phenylsulfanylmethylpentylcarbamic Acid 9*H*-Fluoren-9-ylmethyl Ester (59). A solution of **58** (1.36 g, 2.4 mmol) in CH_2Cl_2 (5 mL) and TFA (5 mL) was stirred at room temperature for 30 min, and concentrated. The concentrate was dissolved in AcOH (1 mL) and 37% aqueous formaldehyde (5 mL), treated with 1 M $NaCNBH_3$ in THF (10 mL), stirred for 30 min, adjusted to pH 7 with saturated $NaHCO_3$ solution, and extracted with EtOAc (3 × 100 mL). The combined extracts were washed with water and brine, dried (Na_2SO_4), filtered, and concentrated. The concentrate was flash chromatographed on silica gel with 48:48:4 EtOAc/CH_2Cl_2/MeOH to provide to provide 638 mg (56%) of **59**. ^{1}H NMR (300 MHz, DMSO-d_6, TFA salt) δ 7.89 (d, J = 7.5 Hz, 2H), 7.69 (m, 2H), 7.42 (dd, J = 7.5, 7 Hz, 2H), 7.32 (m, 7H), 7.15 (m, 1H), 6.04 (m, 2H), 3.55 (m, 2H), 3.01 (m, 2H), 1.15−1.60 (m, 6H). MS (ESI) m/z 475 $(M + H)^+$.

((*R*)-5-(2-Nitro-4-sulfamoylphenylamino)-6-phenylsulfanylhexyl)carbamic Acid *tert*-Butyl Ester (60). **60** was prepared from **59** using the procedure for the preparation of **44R**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.69 (s, 1H), 8.38 (d, J = 9 Hz, 1H), 7.74 (d, J = 9 Hz, 1H), 7.36 (d, J = 8 Hz, 2H), 7.14−7.36 (m, 6H), 6.65 (d, J = 9 Hz, 1H), 4.80 (m, 2H), 4.49 (m, 1H), 3.55 (m, 2H), 1.93 (m, 2H), 1.73 (m, 4H), 1.43 (s, 9H). MS (ESI) m/z 523 $(M - H)^-$.

4-((*R*)-5-Dimethylamino-1-phenylsulfanylmethylpentylamino-3-nitrobenzenesulfonamide (61). **61** was prepared from **59** using the procedure for the preparation of **44R**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.39 (d, J = 2 Hz, 1H), 8.26 (d, J = 9 Hz, 1H), 7.74 (dd, J = 9, 2 Hz, 1H), 7.13−7.37 (m, 8H), 4.08 (m, 1H), 3.16 (m,

2H), 2.21 (m, 2H), 2.14 (s, 6H), 1.75 (m, 2H), 1.47 (m, 4H). MS (ESI) *m/z* 453 $(M + H)^+$. $[\alpha]_D^{23.8} = -327°$ (*c* 0.21, acetone).

***N*-(4′-Dimethylaminomethyl-2′-methoxybiphenyl-4-carbonyl)-3-nitro-4-(2-phenylsulfanylethylamino)benzenesulfonamide (62). 62** was prepared from **22** and **7** using the general coupling procedure. ^{1}H NMR (300 MHz, DMSO-d_6, TFA salt) δ 9.45 (br s, 1H), 8.80 (t, $J = 7.5$ Hz, 1H), 8.63 (d, $J = 2.4$ Hz, 1H), 7.94 (dd, $J = 8.8$, 2.4 Hz, 1H), 7.92 (d, $J = 8.4$ Hz, 2H), 7.61 (d, $J = 8.5$ Hz, 2H), 7.38 (m, 3H), 7.27 (m, 4H), 7.17 (m, 2H), 4.31 (d, $J = 4.4$ Hz, 2H), 3.80 (s, 3H), 3.68 (d, $J = 6.8$ Hz, 2H), 3.29 (d, $J = 6.8$ Hz, 2H), 2.78 (s, 6H). MS (ESI) *m/z* 621 $(M + H)^+$. Anal. ($C_{31}H_{32}N_4O_6S_2 \cdot 4.5C_2HF_3O_2$) C, H, N.

2-Methoxy-4′-[3-nitro-4-(2-phenylsulfanylethylamino)benzenesulfonylaminocarbonyl]biphenyl-4-carboxylic Acid Dimethylamide (63). 63 was prepared from **20** and **7** using the general coupling procedure. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.45 (br s, 1H), 8.78 (t, $J = 7.5$ Hz, 1H), 8.63 (d, $J = 2.5$ Hz, 1H), 7.94 (dd, $J = 9.1$, 2.5 Hz, 1H), 7.91 (d, $J = 8.7$ Hz, 2H), 7.62 (d, $J = 8.7$ Hz, 2H), 7.38 (m, 3H), 7.27 (dd, $J = 8.1$, 7.2 Hz, 2H), 7.21 (d, $J = 9.4$ Hz, 1H), 7.18 (t, $J = 7.5$ Hz, 1H), 7.13 (s, 1H), 7.06 (d, $J = 7.8$ Hz, 1H), 3.79 (s, 3H), 3.68 (d, $J = 6.9$ Hz, 2H), 3.29 (d, $J = 6.9$ Hz, 2H), 3.00 (s, 3H), 2.96 (s, 3H). MS (ESI) *m/z* 633 $(M - H)^-$. Anal. ($C_{31}H_{30}N_4O_7S_2 \cdot 0.4H_2O$) C, H, N.

***N*-[4′-(2-Dimethylaminoethyl)-2′-methoxybiphenyl-4-carbonyl]-3-nitro-4-(2-phenylsulfanylethylamino)benzenesulfonamide (64). 64** was prepared from **27** and **7** using the general coupling procedure. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.45 (br s, 1H), 8.79 (t, $J = 7.5$ Hz, 1H), 8.62 (d, $J = 2.5$ Hz, 1H), 7.94 (dd, $J = 9.2$, 1.2 Hz, 1H), 7.91 (d, $J = 8.4$ Hz, 2H), 7.57 (d, $J = 8.7$ Hz, 2H), 7.14−7.40 (m, 7H), 7.07 (s, 1H), 6.96 (d, $J = 7.8$ Hz, 1H), 3.78 (s, 3H), 3.68 (t, $J = 6.5$ Hz, 2H), 3.42 (m, 2H), 3.19 (m, 2H), 3.02 (m, 2H), 2.85 (s, 6H). MS (ESI) *m/z* 635 $(M + H)^+$.

2-{2-Methoxy-4′-[3-nitro-4-(2-phenylsulfanylethylamino)benzenesulfonylaminocarbonyl]biphenyl-4-yl}-*N*,*N*-dimethylacetamide (65). 65 was prepared from **25** and **7** using the general coupling procedure. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.45 (br s, 1H), 8.78 (t, $J = 7.5$ Hz, 1H), 8.62 (d, $J = 2.3$ Hz, 1H), 7.93 (dd, $J = 9.2$, 2.4 Hz, 1H), 7.89 (d, $J = 8.5$ Hz, 2H), 7.58 (d, $J = 8.1$ Hz, 2H), 7.37 (d, $J = 7.1$ Hz, 2H), 7.20−7.28 (m, 4H), 7.19 (t, $J = 7.5$ Hz, 1H), 7.00 (s, 1H), 6.89 (d, $J = 7.8$ Hz, 1H), 3.74 (s, 3H), 3.73 (s, 2H), 3.68 (d, $J = 6.5$ Hz, 2H), 3.28 (d, $J = 6.5$ Hz, 2H), 3.03 (s, 3H), 2.84 (s, 3H). MS (ESI) *m/z* 647 $(M - H)^-$.

***N*-[4′-(3-Dimethylaminopropyl)-2′-methoxybiphenyl-4-carbonyl]-3-nitro-4-(2-phenylsulfanylethylamino)benzenesulfonamide (66). 66** was prepared from **34** and **7** using the general coupling procedure. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.50 (br s, 1H), 8.78 (t, $J = 7.5$ Hz, 1H), 8.63 (d, $J = 2.5$ Hz, 1H), 7.94 (dd, $J = 9.3$, 2.5 Hz, 1H), 7.89 (d, $J = 8.8$ Hz, 2H), 7.58 (d, $J = 8.7$ Hz, 2H), 7.38 (d, $J = 7.2$ Hz, 2H), 7.24 (m, 4H), 7.19 (t, $J = 7.2$ Hz, 1H), 7.01 (s, 1H), 6.92 (d, $J = 6.6$ Hz, 1H), 3.78 (s, 3H), 3.68 (m, 2H), 3.29 (t, $J = 6.9$ Hz, 2H), 3.09 (m, 2H), 2.79 (s, 6H), 2.67 (t, $J = 7.1$ Hz, 2H), 2.00 (m, 2H). MS (ESI) *m/z* 647 $(M - H)^-$. Anal. ($C_{33}H_{36}N_4O_6S_2 \cdot 0.5C_2HF_3O_2$) C, H, N.

3-{2-Methoxy-4′-[3-nitro-4-(2-phenylsulfanylethylamino)benzenesulfonylaminocarbonyl]biphenyl-4-yl}-*N*,*N*-dimethylpropionamide (67). 67 was prepared from **32** and **7** using the procedure for the general coupling procedure. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.50 (br s, 1H), 8.79 (t, $J = 7.5$ Hz, 1H), 8.63 (d, $J = 2.3$ Hz, 1H), 7.94 (dd, $J = 9.2$, 2.4 Hz, 1H), 7.88 (d, $J = 8.5$ Hz, 2H), 7.57 (d, $J = 8.5$ Hz, 2H), 7.37 (d, $J = 7.4$ Hz, 2H), 7.24 (m, 5H), 7.02 (s, 1H), 6.92 (d, $J = 7.8$ Hz, 1H), 3.76 (s, 3H), 3.68 (m, 2H), 3.29 (m, 2H), 2.95 (s, 3H), 2.84 (m, 2H), 2.83 (s, 3H), 2.64 (t, $J = 8.1$ Hz, 2H). MS (ESI) *m/z* 661 $(M - H)^-$. Anal. ($C_{33}H_{34}N_4O_7S_2 \cdot 0.5C_2HF_3O_2$) C, H, N.

***N*-[2′-Methoxy-4′-(3-morpholin-4-ylpropyl)biphenyl-4-carbonyl]-3-nitro-4-(2-phenylsulfanylethylamino)benzenesulfonamide (68). 68** was prepared from **38** and **7** using the general coupling procedure. ^{1}H NMR (300 MHz, DMSO-d_6, TFA salt) δ 9.68 (br s, 1H), 8.80 (t, $J = 7.4$ Hz, 1H), 8.62 (d, $J = 2.4$ Hz, 1H), 7.93 (m, 1H), 7.88 (d, $J = 8.4$ Hz, 2H), 7.58 (d, $J = 8.4$ Hz, 2H), 7.37 (d, $J = 8.1$ Hz, 2H), 7.24 (m, 5H), 7.01 (s, 1H), 6.93 (d, $J = 7.7$ Hz, 1H), 3.99 (m, 2H), 3.78 (s, 3H), 3.67 (m, 2H), 3.51 (m, 4H), 3.29 (t, $J = 6.8$ Hz, 2H), 3.11 (m, 4H), 2.68 (t, $J = 8.4$ Hz, 2H), 2.02 (m, 2H). MS (ESI) *m/z* 691 $(M - H)^-$. Anal. ($C_{35}H_{38}N_4O_7S_2 \cdot 1.5C_2HF_3O_2$) C, H, N.

***N*-[2′-Methoxy-4′-(3-morpholin-4-yl-3-oxopropyl)biphenyl-4-carbonyl]-3-nitro-4-(2-phenylsulfanylethylamino)benzenesulfonamide (69). 69** was prepared from **36** and **7** using the general coupling procedure. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.50 (br s, 1H), 8.79 (t, $J = 7.4$ Hz, 1H), 8.62 (d, $J = 2.4$ Hz, 1H), 7.93 (dd, $J = 9.1$, 2.4 Hz, 1H), 7.88 (d, $J = 8.8$ Hz, 2H), 7.56 (d, $J = 8.4$ Hz, 2H), 7.37 (d, $J = 8.1$ Hz, 2H), 7.24 (m, 5H), 7.02 (s, 1H), 6.92 (dd, $J = 7.8$, 1.4 Hz, 1H), 3.76 (s, 3H), 3.67 (m, 2H), 3.52 (m, 4H), 3.45 (m, 4H), 3.27 (m, 2H), 2.86 (t, $J = 8.5$ Hz, 2H), 2.67 (t, $J = 8.5$ Hz, 2H). MS (ESI) *m/z* 703 $(M - H)^-$. Anal. ($C_{35}H_{36}N_4O_8S_2 \cdot H_2O$) C, H, N.

***N*-[4′-(3-Hydroxypropyl)-2′-methoxybiphenyl-4-carbonyl]-3-nitro-4-(2-(phenylsulfanylethylamino)benzenesulfonamide (70). 70** was prepared from **41** and **7** using the general coupling procedure. The crude product was stirred with 4 M HCl (10 mL) and dioxane (10 mL) for 1 h prior to purification. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.45 (br s, 1H), 8.80 (t, $J = 7.5$ Hz, 1H), 8.63 (d, $J = 2.4$ Hz, 1H), 7.94 (dd, $J = 9.1$, 2.1 Hz, 1H), 7.88 (d, $J = 8.8$ Hz, 2H), 7.58 (d, $J = 8.4$ Hz, 2H), 7.37 (d, $J = 7.1$ Hz, 2H), 7.24 (m, 5H), 6.96 (s, 1H), 6.88 (d, $J = 7.8$ Hz, 1H), 4.50 (br m, 1H), 3.76 (s, 3H), 3.68 (d, $J = 7$ Hz, 2H), 3.44 (t, $J = 6.4$ Hz, 2H), 3.27 (m, 2H), 2.65 (t, $J = 7.6$ Hz, 2H), 1.76 (tt, $J = 7.6$, 6.4 Hz, 2H). MS (ESI) *m/z* 620 $(M - H)^-$. Anal. ($C_{31}H_{31}N_3O_7S_2 \cdot 0.25C_2HF_3O_2$) C, H, N.

***N*-(4-Morpholin-4-ylbenzoyl)-3-nitro-4-(2-phenylsulfanylethylamino)benzenesulfonamide (71). 71** was prepared from 4-(4-morpholinyl)benzoic acid and **7** using the general coupling procedure. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.05 (br s, 1H), 8.75 (t, $J = 5.9$ Hz, 1H), 8.59 (d, $J = 2.0$ Hz, 1H), 7.90 (dd, $J = 9.2$, 2.0 Hz, 1H), 7.77 (d, $J = 9.2$ Hz, 2H), 7.37 (m, 2H), 7.27 (m, 2H), 7.17 (d, $J = 8.8$ Hz, 2H), 6.95 (d, $J = 9.2$ Hz, 1H), 3.63−3.74 (m, 6H), 3.22−3.32 (m, 6H). MS (ESI) *m/z* 542 $(M + H)^+$. Anal. ($C_{25}H_{26}N_4O_6S_2$) C, H, N.

***N*-[4-(4,4-Dimethylpiperidin-1-yl)benzoyl]-3-nitro-4-(2-phenylsulfanylethylamino)benzenesulfonamide (72). 72** was prepared from **16** and **7** using the general coupling procedure. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.98 (br s, 1H), 8.77 (t, $J = 6.1$ Hz, 1H), 8.59 (d, $J = 2.3$ Hz, 1H), 7.91 (dd, $J = 9.1$, 2.0 Hz, 1H), 7.71 (d, $J = 9.1$ Hz, 2H), 7.36 (d, $J = 8.8$ Hz, 2H), 7.17−7.27 (m, 4H), 6.91 (d, $J = 9.2$ Hz, 2H), 3.67 (dt, $J = 6.6$, 6.4 Hz, 2H), 3.25−3.37 (m, 6H), 1.37 (m, 4H), 0.95 (s, 6H). MS (ESI) *m/z* 569 $(M + H)^+$. Anal. ($C_{28}H_{32}N_4O_5S_2$) C, H, N.

4-((*R*)-3-Dimethylamino-1-phenylsulfanylmethylpropylamino)-*N*-[4-(4,4-dimethylpiperidin-1-yl)benzoyl]-3-nitrobenzenesulfonamide (73R). 73R was prepared from **16** and **47R** using the general coupling procedure. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.45 (br s, 1H), 8.46 (d, $J = 2.0$ Hz, 1H), 8.24 (d, $J = 8.8$ Hz, 1H), 7.81 (dd, $J = 8.8$, 2.0 Hz, 1H), 7.72 (d, $J = 9.2$ Hz, 2H), 7.32 (d, $J = 7.2$ Hz, 2H), 7.25 (dd, $J = 7.2$, 7.1 Hz, 2H), 7.17 (t, $J = 7.1$ Hz, 1H), 6.90 (d, $J = 9.5$ Hz, 1H), 6.81 (d, $J = 9.1$ Hz, 2H), 4.07 (m, 1H), 3.37 (m, 2H), 3.21 (t, $J = 5.6$ Hz, 4H), 2.87 (m, 2H), 2.50 (s, 6H), 2.04 (m, 2H), 1.39 (t, $J = 5.6$ Hz, 4H), 0.94 (s, 6H). MS (ESI) *m/z* 640 $(M + H)^+$. $[\alpha]_D^{23.0} = -232°$ (*c* 0.42, DMF). M.p. 217 °C. Anal. ($C_{32}H_{41}N_5O_5S_2$) C, H, N.

4-((*S*)-3-Dimethylamino-1-phenylsulfanylmethylpropylamino)-*N*-[4-(4,4-dimethylpiperidin-1-yl)benzoyl]-3-nitrobenzenesulfonamide (73S). 73S was prepared from **16** and **47S** using the general coupling procedure. ^{1}H NMR (500 MHz, DMSO-d_6, TFA salt) δ 11.97 (br s, 1H), 9.48 (br s, 1H), 8.56 (d, $J = 2.5$ Hz, 1H), 8.29 (d, $J = 9.4$ Hz, 1H), 7.88 (dd, $J = 9.0$, 2.1 Hz, 1H), 7.74 (d, $J = 9.1$ Hz, 2H), 7.14−7.25 (m, 6H), 6.92 (d, $J = 9.4$ Hz, 2H), 4.18 (m, 1H), 3.32−3.41 (m, 6H), 3.14 (m, 2H), 2.75 (s, 6H), 2.14 (m, 2H), 1.37 (t, $J = 5.8$ Hz, 4H), 0.95 (s, 6H). MS (ESI) *m/z* 640 $(M + H)^+$. $[\alpha]_D^{23.8} = +226°$ (*c* 0.33, DMF). Anal. ($C_{32}H_{41}N_5O_5S_2$) C, H, N.

4-((*R*)-4-Dimethylamino-1-phenylsulfanylmethylbutylamino)-*N*-[4-(4,4-dimethylpiperidin-1-yl)benzoyl]-3-nitrobenzenesul-

fonamide (74). 74 was prepared from **16** and **56** using the general coupling procedure. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.95 (br s, 1H), 8.48 (d, J = 2.0 Hz, 1H), 8.18 (d, J = 9.0 Hz, 1H), 7.81 (dd, J = 9.0, 2.0 Hz, 1H), 7.72 (d, J = 8.8 Hz, 2H), 7.29 (d, J = 7.7 Hz, 2H), 7.21 (dd, J = 7.4, 7.1 Hz, 2H), 7.15 (t, J = 7.1 Hz, 1H), 7.03 (d, J = 9.1 Hz, 1H), 6.83 (d, J = 9.1 Hz, 2H), 4.06 (m, 1H), 3.38 (m, 2H), 3.24 (m, 4H), 2.94 (m, 2H), 2.72 (s, 6H), 1.72 (m, 4H), 1.39 (t, J = 5.6 Hz, 4H), 0.94 (s, 6H). MS (ESI) m/z 654 (M + H)$^+$. Anal. ($C_{33}H_{43}N_5O_5S_2\cdot 0.5H_2O$) C, H, N.

4-((*R*)-5-Dimethylamino-1-phenylsulfanylmethylpentylamino)-*N*-[4-(4,4-dimethylpiperidin-1-yl)benzoyl]-3-nitrobenzenesulfonamide (75). 75 was prepared from **16** and **61** using the general coupling procedure. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.46 (d, J = 2.4 Hz, 1H), 8.18 (d, J = 9.1 Hz, 1H), 7.81 (dd, J = 9.2, 2.1 Hz, 1H), 7.72 (d, J = 8.9 Hz, 2H), 7.32 (d, J = 7.1 Hz, 2H), 7.25 (dd, J = 7.2, 7.1 Hz, 2H), 7.16 (t, J = 7.2 Hz, 1H), 6.99 (d, J = 9.5 Hz, 1H), 6.81 (d, J = 9.2 Hz, 2H), 4.03 (m, 1H), 3.37 (m, 2H), 3.25 (m, 4H), 2.95 (m, 2H), 2.69 (s, 6H), 1.77 (m, 2H), 1.57 (m, 2H), 1.39 (m, 4H), 1.35 (m, 2H), 0.94 (s, 6H). MS (ESI) m/z 668 (M + H)$^+$. Anal. ($C_{34}H_{45}N_5O_5S_2\cdot 2.25C_2HF_3O_2$) C, H, N.

4-((*R*)-5-Amino-1-phenylsulfanylmethylpentylamino)-*N*-[4-(4,4-dimethylpiperidin-1-yl)benzoyl]-3-nitrobenzenesulfonamide (76). 76 was prepared from **16** and **60** using the general coupling procedure. The crude product was stirred with 4 M HCl (10 mL) and dioxane (10 mL) for 3 h prior to purification. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.00 (s, 1H), 8.53 (d, J = 2.0 Hz, 1H), 8.31 (d, J = 9.2 Hz, 1H), 7.86 (dd, J = 9.2, 2.1 Hz, 1H), 7.74 (d, J = 9.2 Hz, 2H), 7.69 (br s, 2H), 7.09−7.25 (m, 6H), 6.93 (d, J = 9.2 Hz, 2H), 4.08 (m, 1H), 3.57 (s, 2H), 3.29 (m, 4H), 2.72 (m, 2H), 1.77 (m, 2H), 1.77 (m, 2H), 1.52 (m, 2H), 1.37 (m, 6H), 0.95 (s, 6H). MS (ESI) m/z 640 (M + H)$^+$. Anal. ($C_{32}H_{41}N_5O_5S_2\cdot 0.25CH_2O_2$) C, H, N.

***N*-[4-(4,4-Dimethylpiperidin-1-yl)benzoyl]-4-((*R*)-3-morpholin-4-yl-1-phenylsulfanylmethylpropylamino)-3-nitrobenzenesulfonamide (77R). 77R** was prepared from **16** and **49R** using the general coupling procedure. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.48 (d, J = 2.0 Hz, 1H), 8.35 (d, J = 9.2 Hz, 1H), 7.79 (dd, J = 9.2, 2.0 Hz, 1H), 7.72 (d, J = 8.8 Hz, 2H), 7.30 (d, J = 6.8 Hz, 2H), 7.23 (dd, J = 7.5, 7.1 Hz, 2H), 7.15 (t, J = 7.1 Hz, 1H), 6.98 (d, J = 9.5 Hz, 1H), 6.85 (d, J = 9.2 Hz, 2H), 4.13 (m, 1H), 3.51 (m, 4H), 3.37 (m, 2H), 3.26 (m, 4H), 2.37 (m, 4H), 2.28 (m, 2H), 2.00 (m, 1H), 1.83 (m, 1H), 1.39 (t, J = 5.5 Hz, 4H), 0.94 (s, 6H). MS (ESI) m/z 682 (M + H)$^+$. Anal. ($C_{34}H_{43}N_5O_6S_2\cdot 0.5CH_2O_2$) C, H, N.

***N*-[4-(4,4-Dimethylpiperidin-1-yl)benzoyl]-4-((*S*)-3-morpholin-4-yl-1-phenylsulfanylmethylpropylamino)-3-nitrobenzenesulfonamide (77S). 77S** was prepared from **16** and **49S** using the general coupling procedure. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.50 (d, J = 2.1 Hz, 1H), 8.25 (d, J = 9.1 Hz, 1H), 7.80 (dd, J = 9.1, 2.0 Hz, 1H), 7.71 (d, J = 9.0 Hz, 2H), 7.21 (m, 4H), 7.10 (m, 2H), 6.94 (d, J = 8.7 Hz, 2H), 4.21 (m, 1H), 3.87 (m, 4H), 3.31 (m, 4H), 3.12 (m, 2H), 2.95 (m, 4H), 2.28 (m, 2H), 2.20 (m, 2H), 1.71 (m, 2H), 1.36 (t, J = 5.3 Hz, 4H), 0.94 (s, 6H). MS (ESI) m/z 682 (M + H)$^+$. Anal. ($C_{34}H_{43}N_5O_6S_2\cdot 0.5CH_2O_2$) C, H, N.

(R)-3-{4-[4-(4,4-Dimethylpiperidin-1-yl)benzoylsulfamoyl]-2-nitrophenylamino}-*N*,*N*-dimethyl-4-phenylsulfanylbutyramide (78). 78 was prepared from **16** and **46R** using the general coupling procedure. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.97 (s, 1H), 8.85 (d, J = 9.5 Hz, 1H), 8.54 (d, J = 2.4 Hz, 1H), 7.83 (dd, J = 9.5, 2.4 Hz, 1H), 7.72 (d, J = 9.1 Hz, 2H), 7.25 (d, J = 9.1 Hz, 2H), 7.10−7.19 (m, 4H), 6.92 (d, J = 9.2 Hz, 2H), 4.43 (m, 1H), 3.41 (m, 2H), 3.27 (m, 4H), 2.98 (m, 1H), 2.90 (s, 3H), 2.78 (s, 3H), 2.75 (m, 1H), 1.37 (t, J = 5.6 Hz, 4H), 0.95 (s, 6H). MS (ESI) m/z 654 (M + H)$^+$. Anal. ($C_{32}H_{39}N_5O_6S_2$) C, H, N.

4-((*R*)-3-Dimethylamino-1-phenylsulfanylmethylpropylamino)-*N*-[2′-methoxy-4′-(3-morpholin-4-ylpropyl)biphenyl-4-carbonyl]-3-nitrobenzenesulfonamide (79R). 79R was prepared from **38** and **47R** using the general coupling procedure. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.49 (d, J = 2.2 Hz, 1H), 8.12 (d, J = 9.0 Hz, 1H), 7.90 (d, J = 8.4 Hz, 1H), 7.86 (dd, J = 9.1, 2.0 Hz, 1H), 7.42 (d, J = 8.1 Hz, 2H), 7.30 (d, J = 7.5 Hz, 2H), 7.22 (m, 4H), 7.14 (t, J = 7.1 Hz, 1H), 7.04 (d, J = 9.1 Hz, 1H), 7.00 (s, 1H), 6.89 (d, J = 7.8 Hz, 1H), 4.08 (m, 1H), 3.77 (s, 3H), 3.25−3.42 (m, 6H), 2.95−3.11 (m, 8H), 2.72 (s, 6H), 2.06 (m, 2H), 1.76 (m, 4H). MS (ESI) m/z 760 (M − H)$^-$. Anal. ($C_{39}H_{47}N_5O_7S_2\cdot 2C_2HF_3O_2\cdot H_2O$) C, H, N.

4-((*S*)-3-Dimethylamino-1-phenylsulfanylmethylpropylamino)-*N*-[2′-methoxy-4′-(3-morpholin-4-ylpropyl)biphenyl-4-carbonyl]-3-nitrobenzenesulfonamide (79S). 79S was prepared from **38** and **47S** using the general coupling procedure. ^{1}H NMR (300 MHz, DMSO-d_6, TFA salt) δ 9.70 (br s, 1H), 9.40 (br s, 1H), 8.58 (d, J = 2.3 Hz, 1H), 8.32 (d, J = 9.1 Hz, 1H), 7.92 (d, J = 8.5 Hz, 1H), 7.89 (dd, J = 9.2, 2.4 Hz, 1H), 7.59 (d, J = 8.8 Hz, 2H), 7.12−7.28 (m, 7H), 7.01 (s, 1H), 6.93 (d, J = 7.8 Hz, 1H), 4.19 (m, 1H), 3.96 (m, 2H), 3.77 (s, 3H), 3.64 (m, 2H), 3.40 (m, 4H), 3.12 (m, 6H), 2.75 (s, 6H), 2.68 (t, J = 7.6 Hz, 2H), 2.14 (m, 2H), 2.01 (m, 2H). MS (ESI) m/z 762 (M + H)$^+$. Anal. ($C_{39}H_{47}N_5O_7S_2\cdot 2.5C_2HF_3O_2$) C, H, N.

4-((*R*)-4-Dimethylamino-1-phenylsulfanylmethylbutylamino)-*N*-[2′-methoxy-4′-(3-morpholin-4-ylpropyl)biphenyl-4-carbonyl]-3-nitrobenzenesulfonamide (80). 80 was prepared from **38** and **56** using the general coupling procedure. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.95 (br s, 1H), 8.56 (d, J = 2.4 Hz, 1H), 8.33 (d, J = 9.2 Hz, 1H), 7.91 (d, J = 8.2 Hz, 1H), 7.90 (dd, J = 9.2, 2.1 Hz, 1H), 7.59 (d, J = 8.2 Hz, 2H), 7.26 (m, 4H), 7.13 (m, 3H), 7.01 (s, 1H), 6.93 (d, J = 7.6 Hz, 1H), 4.15 (m, 1H), 3.97 (m, 2H), 3.78 (s, 3H), 3.65 (m, 2H), 3.34 (m, 2H), 3.14 (m, 2H), 3.08 (m, 2H), 2.99 (m, 2H), 2.73 (s, 6H), 2.68 (m, 2H), 2.02 (m, 2H), 1.79 (m, 2H), 1.60 (m, 2H), 1.36 (m, 2H). MS (ESI) m/z 774 (M − H)$^-$.

4-((*R*)-5-Dimethylamino-1-phenylsulfanylmethylpentylamino)-*N*-[2′-methoxy-4′-(3-morpholin-4-ylpropyl)biphenyl-4-carbonyl]-3-nitrobenzenesulfonamide (81). 81 was prepared from **38** and **61** using the general coupling procedure. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.56 (d, J = 2.4 Hz, 1H), 8.33 (d, J = 9.2 Hz, 1H), 7.91 (d, J = 8.2 Hz, 1H), 7.89 (dd, J = 8.8, 2.1 Hz, 1H), 7.59 (d, J = 8.2 Hz, 2H), 7.25 (m, 4H), 7.15 (m, 3H), 7.01 (s, 1H), 6.93 (d, J = 7.6 Hz, 1H), 3.97 (m, 1H), 3.74 (s, 3H), 3.65 (m, 2H), 3.34 (m, 2H), 3.14 (m, 2H), 3.08 (m, 2H), 2.99 (m, 2H), 2.73 (s, 6H), 2.68 (m, 2H), 2.02 (m, 2H), 1.79 (m, 2H), 1.60 (m, 2H), 1.36 (m, 2H). MS (ESI) m/z 788 (M − H)$^-$. Anal. ($C_{41}H_{51}N_5O_7S_2\cdot 2.5C_2HF_3O_2$) C, H, N.

4-((*R*)-5-Amino-1-phenylsulfanylmethylpentylamino)-*N*-[2′-methoxy-4′-(3-morpholin-4-ylpropyl)biphenyl-4-carbonyl]-3-nitrobenzenesulfonamide (82). 82 was prepared from **38** and **60** using the general coupling procedure. The crude product was stirred with 4 M HCl (10 mL) and dioxane (10 mL) for 3 h prior to purification. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.47 (d, J = 2.4 Hz, 1H), 8.14 (d, J = 9.1 Hz, 1H), 7.87 (d, J = 8.5 Hz, 1H), 7.82 (dd, J = 8.8, 2.0 Hz, 1H), 7.39 (d, J = 8.8 Hz, 2H), 7.31 (d, J = 6.7 Hz, 2H), 7.25 (dd, J = 7.5, 7.1 Hz, 2H), 7.18 (d, J = 7.5 Hz, 2H), 6.94 (s, 1H), 6.91 (d, J = 9.5 Hz, 1H), 6.85 (d, J = 7.8 Hz, 1H), 3.97 (m, 1H), 3.74 (s, 3H), 3.58 (m, 4H), 3.34 (m, 2H), 2.73 (t, J = 7.1 Hz, 2H), 2.62 (t, J = 7.5 Hz, 2H), 2.35 (m, 4H), 2.32 (m, 2H), 1.77 (m, 2H), 1.49 (m, 2H), 1.37 (m, 2H). MS (ESI) m/z 760 (M − H)$^-$. Anal. ($C_{39}H_{47}N_5O_7S_2\cdot 3HCl$) C, H, N.

***N*-[2′-Methoxy-4′-(3-morpholin-4-ylpropyl)biphenyl-4-carbonyl]-4-((*R*)-3-morpholin-4-yl-1-phenylsulfanylmethylpropylamino)-3-nitrobenzenesulfonamide (83). 83** was prepared from **38** and **49R** using the general coupling procedure. ^{1}H NMR (500 MHz, MeOH-d_4) δ 8.71 (d, J = 2.2 Hz, 1H), 7.94 (d, J = 9.0 Hz, 1H), 7.91 (d, J = 8.5 Hz, 2H), 7.52 (d, J = 8.1 Hz, 2H), 7.29 (dd, J = 8.1, 1.6 Hz, 2H), 7.23 (d, J = 7.5 Hz, 2H), 7.13 (m, 3H), 6.96 (d, J = 9.5 Hz, 1H), 6.95 (s, 1H), 6.89 (d, J = 7.8 Hz, 1H), 4.14 (m, 1H), 3.97 (m, 2H), 3.78 (s, 3H), 3.65 (m, 2H), 3.34 (m, 2H), 3.14 (m, 2H), 3.08 (m, 2H), 2.99 (m, 2H), 2.73 (s, 6H), 2.68 (m, 2H), 2.02 (m, 2H), 1.79 (m, 2H), 1.60 (m, 2H), 1.36 (m, 2H). MS (ESI) m/z 802 (M − H)$^-$. Anal. ($C_{41}H_{49}N_5O_8S_2\cdot 0.5H_2O$) C, H, N.

(*R*)-3-(4-{[2′-Methoxy-4′-(3-morpholin-4-ylpropyl)biphenyl-4-carbonyl]sulfamoyl}-2-nitrophenylamino)-*N*,*N*-dimethyl-4-phenylsulfanylbutyramide (84). 84 was prepared from **38** and **46R** using the general coupling procedure. ^{1}H NMR (500 MHz, MeOH-

d_4) δ 8.72 (s, 1H), 8.69 (d, J = 9.1 Hz, 1H), 7.94 (d, J = 8.5 Hz, 2H), 7.52 (dd, J = 9.0, 2.3 Hz, 1H), 7.46 (d, J = 8.3 Hz, 2H), 7.34 (d, J = 7.3 Hz, 2H), 7.14−7.21 (m, 4H), 6.92 (s, 1H), 6.87 (d, J = 9.5 Hz, 2H), 4.40 (m, 1H), 3.77 (s, 3H), 3.74 (m, 2H), 3.34 (m, 4H), 2.85 (m, 4H), 2.98 (s, 3H), 2.87 (s, 3H), 2.71 (m, 4H), 1.96 (m, 2H), 1.29 (m, 2H). MS (ESI) m/z 774 $(M - H)^-$. Anal. ($C_{39}H_{45}N_5O_8S_2 \cdot H_2O$) C, H, N.

Protein Preparation. A previously described loop-deleted version of Bcl-X_L which lacked the putative transmembrane helix was employed for NMR studies and biological assays.[18,19] The Bcl-2 protein used was a chimera based on isoform 2 (A96T and G110R) in which residues 35−91 were replaced with residues 35−50 from Bcl-X_L, and the C-terminal end (residues 208−219) was excised.[40] For both Bcl-X_L and Bcl-2, uniformly ^{15}N-labeled and ^{15}N-, ^{13}C-labeled protein was expressed in *E. coli* containing the appropriate plasmid, on minimal media containing ^{15}N-labeled ammonium chloride as the sole nitrogen source with or without ^{13}C-labeled glucose as the sole carbon source. Proteins were purified by affinity chromatography on a Nickel-ProBond column (Invitrogen), concentrated, and exchanged into 40 mM disodium phosphate buffer, pH 7.0, containing either 10% or 100% D_2O plus 5 mM deuterated dithiothreitol. Protein samples for NMR were 0.5−1.0 mM in microcells. Ligands were added to the protein from concentrated (100 mM) stock solutions prepared in DMSO-d_6).

NMR-Based Structural Studies. NMR spectra for structural studies were recorded on Bruker DRX600 and DRX800 spectrometers at 303 K. Resonance assignments for ligand-bound Bcl-X_L were extrapolated from those of the apo protein by comparing two-dimensional ^{13}C- and ^{15}N-HSQC spectra and three-dimensional ^{13}C-edited and ^{15}N-edited NOESY spectra of the liganded to the unliganded protein. Intraprotein NOEs for those residues of Bcl-X_L which line the binding groove were extracted from three-dimensional ^{13}C-edited and ^{15}N-edited NOESY spectra recorded with a mixing time of 80 ms. Protein−ligand NOEs were extracted from three-dimensional ^{13}C-edited, ^{12}C-filtered NOESY spectra recorded with mixing times ranging from 150 to 250 ms.

Fluorescence Polarization Assay. K_i and IC_{50} values were determined using a competitive fluorescence polarization assay as described previously.[20,35] A series dilution of compounds were used to compete the binding of 1 nM f-Bad peptide (NLWAAQRYGRELRRMSDK(FITC)FVD) and 6 nM Bcl-X_L or 1 nM f-Bax peptide (FITC-QDASTKKLSECLKRIGDELDS) and 10 nM Bcl-2. The effects of 1% and 10% human serum, and of separate serum proteins, were detected using 30 nM f-Bad peptide and 60 nM Bcl-X_L. Serum and serum components were added to assay buffer. Concentrations for serum components were as follows: HSA, 0.42 mg/mL; HSA-III, 0.146 mg/mL; α_1-acid glycoprotein, 0.0095 mg/mL. Individual determinations were the result of duplicate values. K_i and IC_{50} values were calculated using Microsoft Excel.

FL5.12 Cellular Assay. Mouse FL5.12 cells transfected with Bcl-xL were cultured under standard conditions in RPMI with 2 mM glutamine, 1% 100 mM sodium pyruvate, 2% 1 M HEPES, 4 μL per liter of β-mercaptoethanol, 1% penicillin−streptomycin, 10% FBS, and 10% WEHI-3B conditioned media (for IL-3). For assaying the compound activity, the cells were exchanged into an IL-3 depleted deprivation media, which was identical to the growth media except for the absence of FBS and WEHI-3B conditional media, for 2 days. Then the cells were exchanged to either gelatin assay media (RPMI with 2 mM glutamine, 2% 1 M hepes, 3.4 mg/mL bovine gelatin (Sigma)) or 3% FBS assay media (RPMI with 2 mM glutamine, 1% 100 mM sodium pyruvate, 2% 1 M HEPES, 4 μL per liter of β-mercaptoethanol, 1% penicillin−streptomycin, 3% FBS). Compounds in series dilutions were added, and the cells were cultured for 24 h. Cell viability was assayed by MTS or cell titerGlo from Premega. Individual determinations were the result of duplicate values.

In Vitro Chemopotentiation. Using a 96-well plate format, A549 cells were seeded in DMEM containing 5% FBS at 5×10^3 cells/well the day prior to the experiment. The following day, medium was removed and a dose range of UV-C (starting energy 32 mJ/cm^2, with 50% reductions each step) was applied prior to treating with **73R** (in DMEM containing 10% FBS). Cell viability was measured by MTS readout at 48 h postexposure and percent viability determined by comparison to untreated samples. For paclitaxel, cells in the same media were pretreated with dilutions of paclitaxel (starting dose 20 nM, 3-fold dilutions) for 48 h prior to a 48 h coincubation with paclitaxel and **73R** (in DMEM containing 0.34% gelatin). The total incubation volume was 100 μL. Cell viability was measured by the MTS (Promega) assay.

In Vivo Tumor Efficacy Model. Animal studies were conducted following the guidelines of the Institutional Animal Care and Use Committee. Immunocompromised male *Scid* mice (C.B-17-*Prkdc*^{scid}) were obtained from Charles River Laboratories (Wilmington, MA) and trials were initiated when mice were 7−10 weeks of age. A-549 NSCLC cells were obtained from the American Type Culture Collection (Manassa, VA). 5×10^6 cells in 50% Matrigel (BD Biosciences, Bedford, MA) were inoculated subcutaneously into the flank. Tumors were allowed to reach an average volume of 250 mm^3 (day 15 post inoculation), and mice were assigned to treatment groups of equal tumor size (N =10 mice per group). Therapy was initiated the following day. Tumor size was evaluated by twice weekly measurements with digital calipers. Tumor volume was estimated using the formula: $V = L \times W^2$. **73R** was administered ip in a vehicle of 5% Tween 80, 20% poly(ethylene glycol), 75% 0.1 M $NaPO_4$, pH ∼ 4.0 at 75 mg/kg/day from day 16−36 Paclitaxel was obtained from the Bristol-Myers Squibb Company (Princeton, NJ) and prepared and administered ip at 30 mg/kg/day on days 16, 20, and 24 according to the manufacturers formulation guidelines.

Paclitaxel/73R Pharmacokinetic Study. **73R** and paclitaxel were administered at doses of 100 and 15 mg/kg, respectively, in the same manner as described in the previous section, to *Scid* mice. A single method was developed for the simultaneous quantitation of **73R** and paclitaxel in plasma samples. The initial analytical method used a protein precipitation with CH_3CN to separate both compounds from the plasma. A plasma aliquot (200 μL, sample or spiked standard) was combined with 50 μL of internal standard and 1 mL of acetonitrile. Following vortexing and centrifugation, the supernatant was transferred to a clean tube. An aliquot (10 μL) of DMSO was added to each tube. Supernatant was evaporated to near dryness with dry nitrogen over low heat, and samples were reconstituted by vortexing with 0.2 mL of mobile phase. Samples were analyzed simultaneously with spiked plasma standards. **73R**, paclitaxel, and internal standard were separated on a 50 × 3 mm Keystone Aquasil 5μm C18 column with a CH_3CN: 0.1% TFA mobile phase (85:15, by volume) at a flow rate of 0.35 mL/min. Analysis was performed on a Sciex API365 Biomolecular Mass Analyzer with a turbo-ionspray interface. Analytes were ionized in the positive ion mode. Detection was in multiple reaction monitoring (MRM) mode at m/z 640.5 → 216.4 for **73R** and m/z 854.4 → 286.1 for paclitaxel. Standard peak areas were determined using Sciex MacQuan software.

The plasma drug concentration of each sample was calculated by least squares linear regression analysis (nonweighted) of the peak area ratio (parent/internal standard) of the spiked plasma standards versus concentration. The method for quantitation for **73R** and paclitaxel, evaluated over the concentration range 0−8 μg/mL, was linear (correlation coefficient > 0.99), with mean accuracy values from 91.7 to 108.5% and 85.5−109.4% of theory, respectively, for the analysis of triplicate standards at seven separate concentrations. The limit of quantitation was estimated to be ∼10 ng/mL from a 0.2 mL plasma sample. The three mice with highest concentrations were averaged to provide the peak plasma concentration (C_{max}) and the time to peak plasma concentration (T_{max}). The mean plasma concentration data were submitted to multiexponential curve fitting using WinNonlin. The area under the mean plasma concentration−time curve from 0 to t hours (time of the last measurable plasma concentration) after dosing (AUC_{0-t}) was calculated using the linear trapezoidal rule for the plasma concentration−time profiles. The residual area extrapolated to infinity, determined as the final measured plasma concentration (C_t) divided by the terminal

elimination rate constant (β), was added to AUC_{0-t} to produce the total area under the curve ($AUC_{0-\infty}$).

Supporting Information Available: A table of combustion analysis and HPLC data for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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