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Design, Synthesis, Protein−Ligand X-ray Structure, and Biological Evaluation of a Series of Novel Macrocyclic Human Immunodeficiency Virus-1 Protease Inhibitors to Combat Drug Resistance†

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The structure-based design, synthesis, and biological evaluation of a series of nonpeptidic macrocyclic HIV protease inhibitors are described. The inhibitors are designed to effectively fill in the hydrophobic pocket in the S1′−S2′ subsites and retain all major hydrogen bonding interactions with the protein backbone similar to darunavir (**1**) or inhibitor **2**. The ring size, the effect of methyl substitution, and unsaturation within the macrocyclic ring structure were assessed. In general, cyclic inhibitors were significantly more potent than their acyclic homologues, saturated rings were less active than their unsaturated analogues and a preference for 10- and 13-membered macrocylic rings was revealed. The addition of methyl substituents resulted in a reduction of potency. Both inhibitors **14b** and **14c** exhibited marked enzyme inhibitory and antiviral activity, and they exerted potent activity against multidrug-resistant HIV-1 variants. Protein−ligand X-ray structures of inhibitors **2** and **14c** provided critical molecular insights into the ligand-binding site interactions.

Introduction

HIV/AIDS has become one of the major medical and humanitarian challenges in the 21st century.[1] Highly active antiretroviral therapy (HAART[a]), which combines protease inhibitors along with reverse-transcriptase inhibitors, is currently used to combat this pandemic. HAART therapy has resulted in a significant decline in the number of deaths due to HIV infection in the developed countries.[2] One of the major challenges still faced is the emergence of drug-resistant viral strains rendering the present drug regimens ineffective.[3] There is an urgent need for development of antiretroviral agents with minimal side effects and broad-spectrum activity for current and future management of HIV/AIDS.

Recently, our structure-based design of inhibitors maximizing interactions within the active site protease backbone led to the development of nonpeptide inhibitors (**1**−**2**) that have shown picomolar enzyme affinity and exceptional antiviral activity against both wild-type and drug-resistant HIV-1 strains.[4−6] The X-ray crystallographic studies revealed that the backbone conformation of mutant protease is minimally distorted compared to wild-type HIV-1 proteases. We, therefore, speculated that maximizing "backbone binding" may be an important design strategy to combat drug resistance.[7] Inhibitor **1** (darunavir, TMC-114) was recently approved by the FDA for the treatment of drug-resistant HIV strains.[8] More recently, it has been approved for all HIV/AIDS patients including pediatric AIDS patients.[9] The protein−ligand X-ray structure of darunavir and its analogue **2** (TMC-126) exhibited extensive hydrogen bonding interactions with the backbone atoms throughout the active site of the HIV-1 protease.[10,11]

In our continuing efforts toward the conceptual design of novel PIs, we now plan to design PIs with functionalities that interact with the protein backbone as well as introduce flexible macrocycles involving P1′−P2′ ligands for effective repacking due to side chain mutations. The notion of such macrocyclic design evolved from the observation that certain mutations lead to decreased van der Waals interactions and increased the size of the S1 hydrophobic pocket. The X-ray structure and modeling studies of PI (2*S*,2′*S*)-*N*,*N*′-((2*S*,3*R*,4*R*,5*S*)-3-hydroxy-4-methyl-1,6-diphenylhexane-2,5-diyl)bis(3-methyl-2-(3-methyl-3-(pyridin-2-ylmethyl)ureido)butanamide) (A-77003)[12] indicated that the V82A mutant results in decreased van der Waals interactions with the phenyl rings in both the S1 and S1′-subsites.[13] There was also evidence of repacking of inhibitor side chains and enzyme atoms in the S1-subsite. On the basis of this insight of enzyme flexibility in accommodating alternate packing, we planned to design flexible macrocycles between the P1′ side chain and the P2′ sulfonamide ring to fill in the S1′ and S2′-subsites.[13] In particular, we envisioned

†The PDB accession code for **2**-bound HIV-1 protease X-ray structure is 3I7E and **14c**-bound HIV-1 protease X-ray structure is 3I6O.

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[a] Abbreviations: HIV, human immunodeficiency virus; bis-THF, bis-tetrahydrofuran; PI, protease inhibitor; HAART, highly active antiretroviral therapy; APV, amprenavir; DRV, darunavir; SQV, saquinavir; IDV, indinavir; LPV, lopinavir; DIAD, diisopropyl azodicarboxylate.

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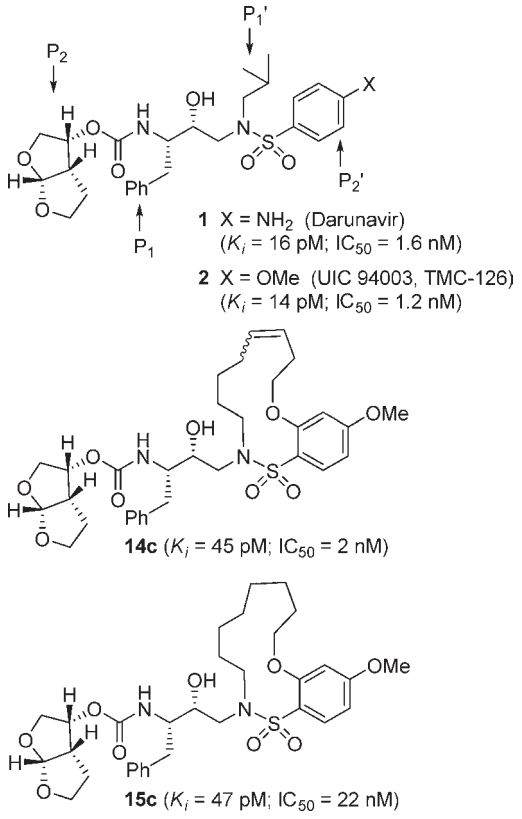
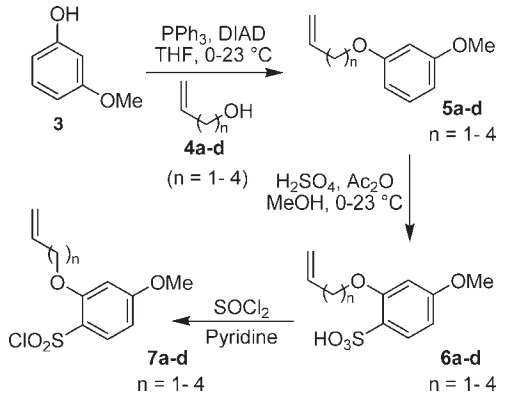


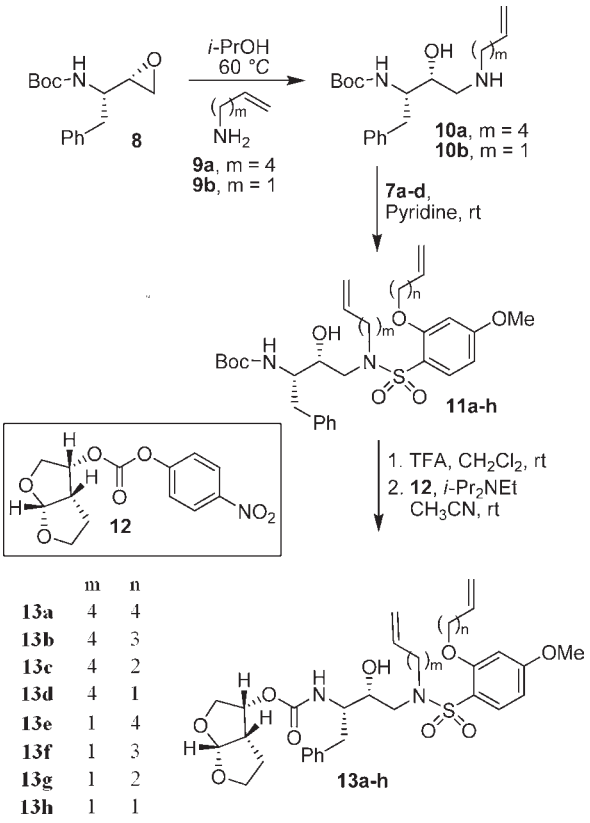
Figure 1. Structure of inhibitors **1**, **2**, **14c**, and **15c**.

Scheme 1. Synthesis of Sulfonyl Chlorides **7a**−**d**



that 11−15-membered saturated and unsaturated macrocycles would effectively fill in the S1′−S2′ hydrophobic pocket of the enzyme active site while retaining all major interactions of PIs **1** and **2** with the protein backbone. Conceivably, such inhibitors will maintain potency against both wild-type and mutant strains. On the basis of this presumption, we designed a series of PIs that incorporated various macrocycles that could effectively fill in the enzyme active site. Herein we report the structure-based design, synthesis, and preliminary biological results of these macrocyclic inhibitors. Among these compounds, **14b** and **14c** were the most potent, with both excellent enzyme inhibitory and antiviral activity. Both inhibitors exerted potent activity against multidrug-resistant HIV-1 variants. Furthermore, protein−ligand X-ray structures of inhibitors **2**- and **14c**-bound HIV-1 protease have revealed important insights regarding ligand-binding interactions (Figure 1).

Scheme 2. Synthesis of Compounds **13a**−**h**



	m	n
13a	4	4
13b	4	3
13c	4	2
13d	4	1
13e	1	4
13f	1	3
13g	1	2
13h	1	1

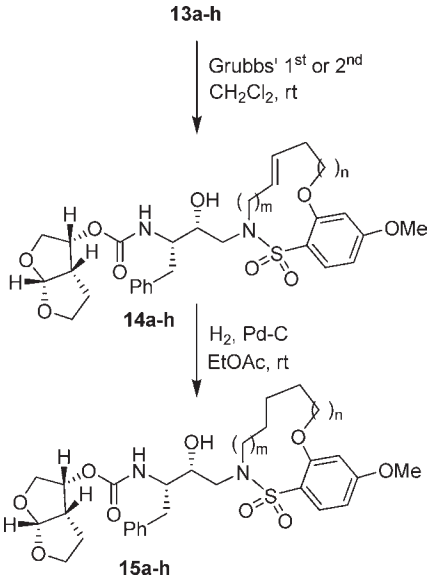
Chemistry

The synthesis of sulfonyl chlorides **7a**−**d** is shown in Scheme 1. A Mitsunobu-type reaction between 3-methoxyphenol and alcohols **4a**−**d** in the presence of triphenylphosphine and diisopropyl azodicarboxylate (DIAD) afforded ethers **5a**−**d**.[14] Electrophilic aromatic substitution of ethers **5a**−**d** with acetic anhydride and concentrated sulfuric acid in methanol furnished a mixture of sulfonic acid regioisomers in a 1:1 ratio that were separated by flash chromatography to give **6a**−**d**. Structural confirmation of the isomers was determined by extensive 2D NMR experiments (NOESY and HMBC). Conversion to the sulfonyl chlorides **7a**−**d** was achieved by reaction of the sulfonic acids **6a**−**d** with thionyl chloride in the presence of pyridine.

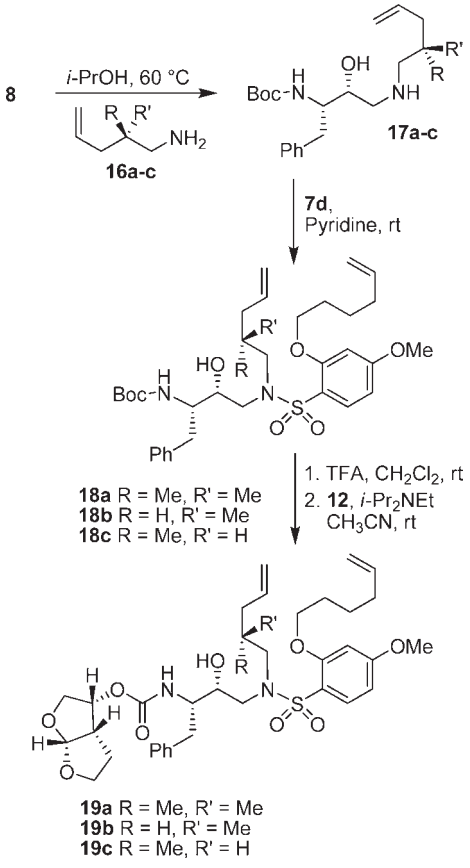
The synthesis of compounds **13a**−**h** is outlined in Scheme 2. Nucleophilic attack of amines **9a** and **9b** on commercially available epoxide **8** in the presence of isopropyl alcohol gave hydroxy amines **10a** and **10b**. The conversion of amines **10a** and **10b** to the sulfonamides **11a**−**h** was realized by coupling with sulfonyl chlorides **7a**−**d** in the presence of pyridine. Removal of the Boc protecting group from sulfonamides **11a**−**h** using 30% trifluoroacetic acid in CH_2Cl_2 furnished the corresponding amines, which were then coupled with activated bis-THF[15] (**12**) to give acyclic inhibitors **13a**−**h**.

The acyclic compounds **13a**−**h** thus obtained were exposed to ring closing metathesis using Grubbs' first- or second-generation catalyst[16] (Scheme 3) to give the unsaturated macrocyclic inhibitors **14a**−**h**. Interestingly, larger ring sizes (15−13) gave a mixture of *E*/*Z* isomers, while in the case of smaller rings (12−9), the *Z* isomer was obtained exclusively. The *E* and *Z* isomers were isolated using reversed-phase HPLC and the stereochemistry established by 2D NMR

Scheme 3. Synthesis of Macrocyclic Inhibitors **14a**−**h** and **15a**−**h**



Scheme 4. Synthesis of Compounds **19a**−**c**



(COSY and NOESY) experiments, allowing their individual biological evaluation. The unsaturated compounds were subsequently reduced using hydrogen and 10% Pd−C as the catalyst to yield inhibitors **15a**−**h**.

A series of selectively methylated inhibitors were prepared in a similar fashion. Nucleophilic attack of amines **16a**−**c** on commercially available epoxide **8** gave hydroxy amines **17a**−**c** (Scheme 4). The conversion of amines **17a**−**c** to the sulfonamides **18a**−**c** was realized by coupling the amines with

Scheme 5. Synthesis of Macrocyclic Inhibitors **20a**−**c**, **21a**−**c**, and **22a**−**c**

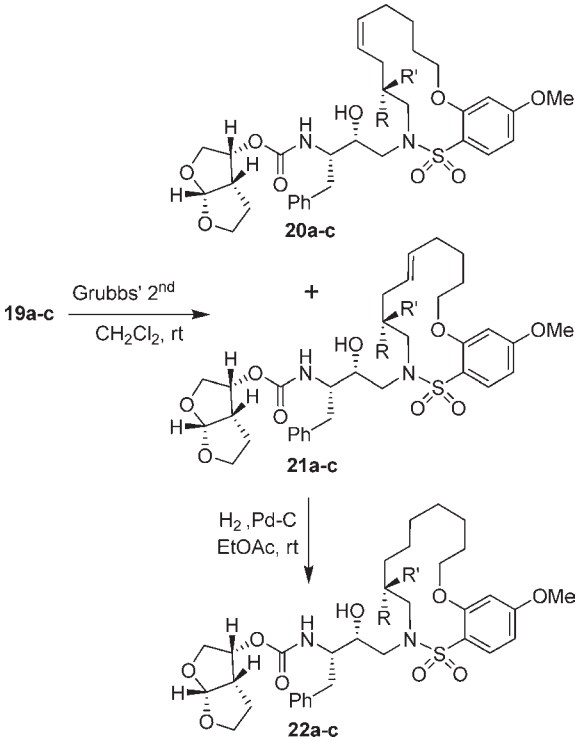
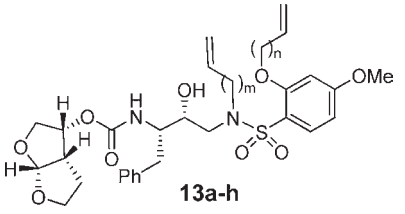


Table 1. Enzyme Inhibitory and Antiviral Activity of Inhibitors **13a**−**h**

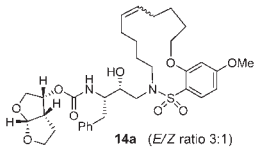
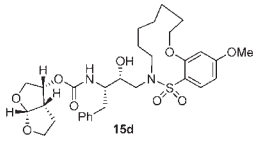
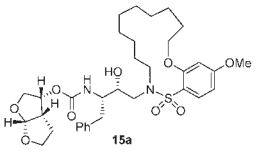
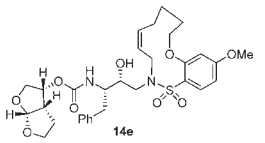
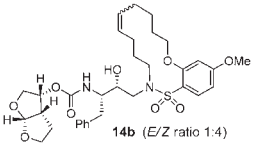
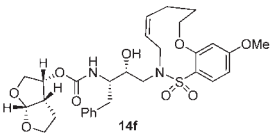
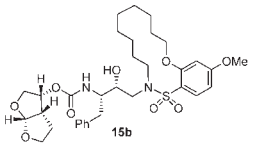
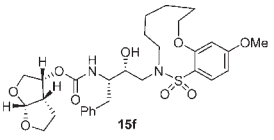
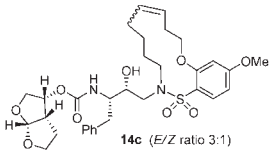
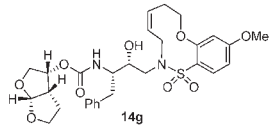
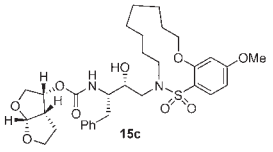
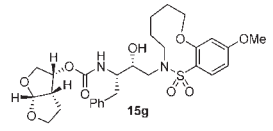
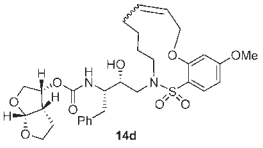
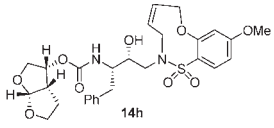
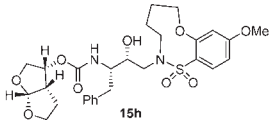


compd	m	n	K_i (nM)	IC_{50} (nM) [a]	ring size by RCM
13a	4	4	16.5 ± 0.5	> 1000	15
13b	4	3	11.5 ± 0.4	> 1000	14
13c	4	2	6.9 ± 0.1	ND	13
13d	4	1	10 ± 2	ND	12
13e	1	4	1.70 ± 0.07	270	12
13f	1	3	1.02 ± 0.08	290	11
13g	1	2	0.63 ± 0.01	ND	10
13h	1	1	0.10 ± 0.01	ND	9

[a] Human T-lymphoid cells, MT-2 cells (2×10^3), were exposed to HIV-1_{LAI} (100 $TCID_{50}$), cultured in the presence of each PI, and IC_{50} values were determined by using the MTT assay. The IC_{50} values of saquinavir (SQV) and amprenavir (APV) tested as reference agents were 16 and 27 nM, respectively. ND: not determined.

sulfonyl chloride **7d** in the presence of pyridine. Removal of the Boc protecting group from sulfonamides **18a**−**c** using 30% trifluoroacetic acid in CH_2Cl_2 furnished the corresponding amines, which were then coupled with the mixed carbonate of activated bis-THF[15] (**12**) to give acyclic inhibitors **19a**−**c**. A ring closing metathesis reaction using Grubbs' second-generation catalyst[16] (Scheme 5) provided a *E*/*Z* mixture of unsaturated macrocyclic inhibitors **20a**−**c** and **21a**−**c**, which were separated by reverse-phase HPLC and identified by 2-D NMR (COSY and NOESY). The unsaturated compounds

Table 2. Enzyme Inhibitory and Antiviral Activity of Inhibitors **14a−h** and **15a−h**

Ring size	Inhibitor structure	K_i (nM)	IC_{50} (nM)[a]	Ring size	Inhibitor structure	K_i (nM)	IC_{50} (nM)[a]
15	 **14a** (*E*/*Z* ratio 3:1)	0.38 ± 0.03	120	12	 **15d**	0.017 ± 0.001	14
15	 **15a**	0.82 ± 0.03	318	12	 **14e**	0.08 ± 0.02	23
14	 **14b** (*E*/*Z* ratio 1:4)	0.082 ± 0.008	4	11	 **14f**	0.077 ± 0.004	20
14	 **15b**	0.67 ± 0.02	49	11	 **15f**	0.15 ± 0.01	95
13	 **14c** (*E*/*Z* ratio 3:1)	0.045 ± 0.005	2	10	 **14g**	0.051 ± 0.004	15
13	 **15c**	0.47 ± 0.002	22	10	 **15g**	0.09 ± 0.01	5.5
12	 **14d**	0.058 ± 0.005	14	9	 **14h**	2.4 ± 0.3	n.t.[b]
				9	 **15h**	1.27 ± 0.03	77

[a] MT-2 human T-lymphoid cells exposed to HIV-1_{LAI}; saquinavir and amprenavir exhibited IC_{50} values of 16 and 27 nM, respectively. [b] n.t. = not tested.

20a−c and **21a−c** were subsequently hydrogenated over 10% Pd−C as the catalyst to yield inhibitors **22a−c**.

Biological Evaluation and Discussion

The inhibitory potencies of acyclic and cyclic inhibitors were measured by the assay protocol of Toth and Marshall.[17] Compounds that showed potent enzyme inhibition K_i values were further evaluated in an antiviral assay. Biological results for the acyclic compounds **13a−h** are shown in Table 1. In general, elongation of the carbon chains resulted in lower enzyme inhibitory activity. Extension of the β-hydroxyl amine chain by three methylene groups resulted in a 10-fold loss in activity (**13a** K_i = 16 nM versus **13e** K_i = 1.7 nM). Similarly, extension of the ether carbon chain by three methylene groups resulted in a 17-fold loss in activity (**13h** K_i = 0.10 nM versus **13e** K_i = 1.7 nM).

Interestingly, conversion of these acyclic inhibitors **13a−h** to their cyclic analogues **14a−h** and **15a−h** resulted in significant improvements of enzyme inhibitory and antiviral activity as shown in Table 2. For example, the 14-membered macrocyclic inhibitors **14b** and **15b** have K_i values of less than 0.7 nM and IC_{50} values less than 49 nM, whereas their corresponding acyclic inhibitor **13b** had a K_i of 11 nM and IC_{50} value of >1000 nM (greater than 15-fold and 20-fold change, respectively). Another trend observed is a preference of the S1′-subsite for macrocylic rings of size 10 and 13. This preference is consistent with our energy-minimized model structure of a saturated 13-membered prototype inhibitor **15c** where a macrocycle is incorporated in place of the P1′-isobutyl group and attached at the ortho-position of the sulfonamide ring of inhibitor **2** as shown in Figure 2. The model of inhibitor **15c** was created based upon the crystal structure of inhibitor **2**-bound HIV-1 protease.

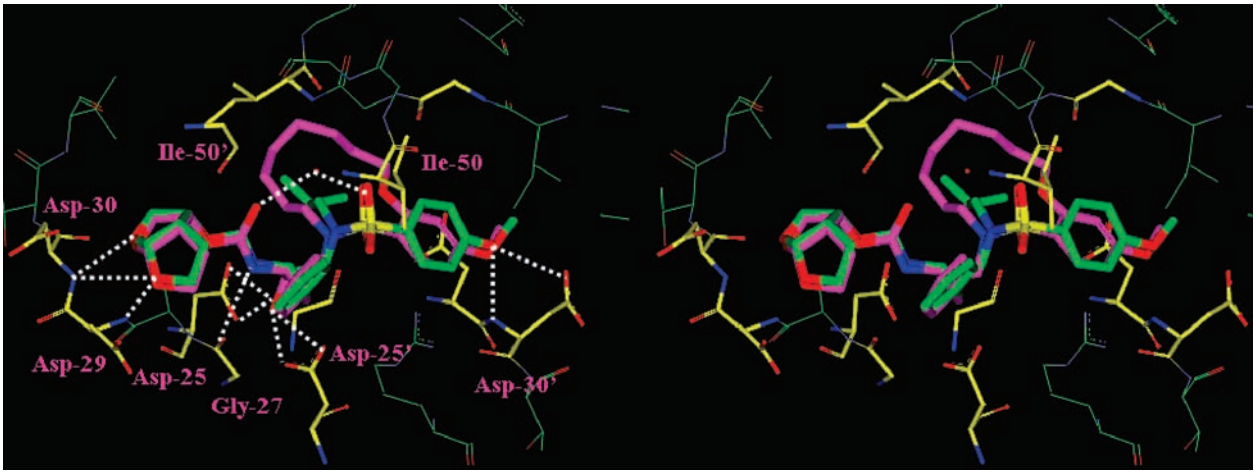
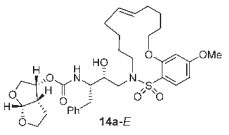
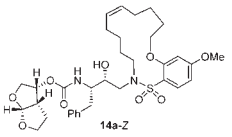
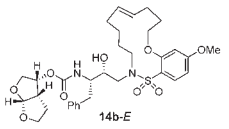
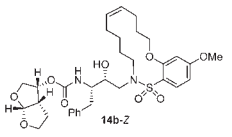
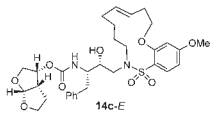
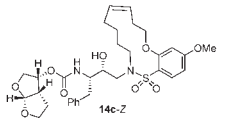


Figure 2. An overlay of energy-minimized macrocyclic inhibitor **15c** (magenta) with the X-ray structure of inhibitor **2** (green)-bound HIV-1 protease.

Table 3. Enzyme Inhibitory and Antiviral Activity of *E* and *Z* Isomers of **14a−c**

Ring size	Inhibitor structure	K_i (nM)	IC_{50} (nM) [a]
15	 **14a-*E***	0.24 ± 0.07	360
15	 **14a-*Z***	0.16 ± 0.04	>1000
14	 **14b-*E***	0.18 ± 0.01	6.6
14	 **14b-*Z***	0.9 ± 0.3	2.9
13	 **14c-*E***	0.06 ± 0.01	7
13	 **14c-*Z***	0.012 ± 0.004	4.6

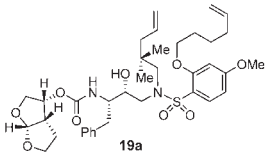
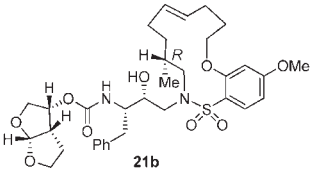
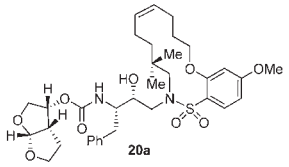
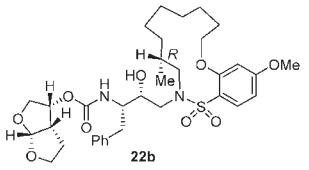
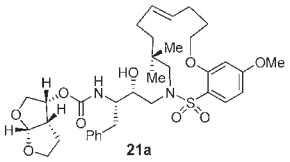
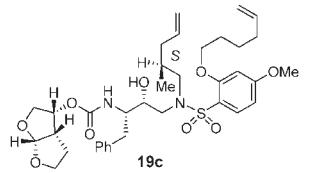
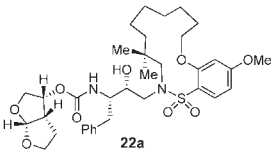
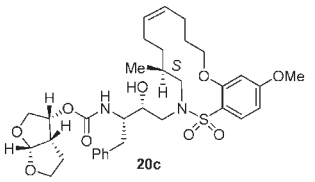
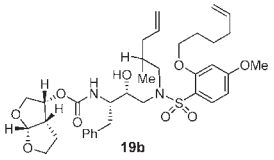
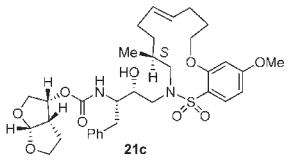
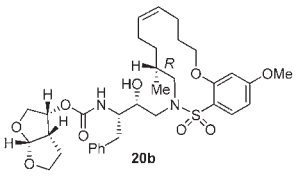
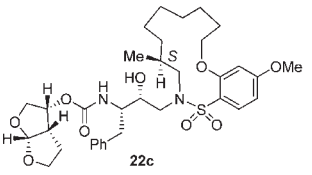
[a] MT-2 human T-lymphoid cells exposed to HIV-1_{LAI}; saquinavir and amprenavir exhibited IC_{50} values of 16 and 27 nM, respectively.

Indeed, the most potent compound of the series, inhibitor **14c** incorporating a 13-membered ring, showed a K_i of 45 pM and IC_{50} of 2 nM. It would appear the 13-membered ring provides an optimally sized macrocyclic ring as increasing the ring size to 14 or 15 as well as decreasing the ring to 12 or 11 resulted in reduced enzymatic inhibitory and antiviral activity. Interestingly, inhibitors incorporating a 10-membered ring (**14g** and **15g**) were also exceedingly potent, displaying a similar activity profile as the 13-membered ring **14c** (**14g** K_i = 51 pM; **15g** K_i = 86 pM and IC_{50} = 5.5 nM).

In comparing the potency of **14c** to its unsaturated analogue **15c**, we observed a dramatic improvement in the K_i and IC_{50} values for the unsaturated cyclic inhibitor **14c**. Furthermore, the effect appeared to remain consistent throughout the variously sized unsaturated macrocycles **14a−h** as compared to their saturated analogues **15a−h**. For the 13-membered ring series, the presence of a double bond resulted in a 10-fold increase in both K_i and IC_{50} values (**14c** K_i = 45 pM and IC_{50} = 2 nM as compared to **15c** K_i = 470 pM and IC_{50} = 22 nM). Similar differences in potency can be seen for the 11, 14, and 15-membered macrocycles, although for the smaller rings 9, 10, and 12, the effect is less pronounced. This effect may be explained by a restriction of conformation in the molecule that results from the presence of the double bond and leads to a better fit in the hydrophobic pocket of the S1′-subsite. These observations led us to take a closer look at the olefinic compounds and evaluate the importance of the stereochemistry of this group. As shown in Table 3, only minor variations in activity (less than 5-fold) were observed between the *E* and *Z* isomers of the 13, 14, and 15-membered macrocycles. For the 13-membered ring system, the *Z* isomer was favored over the *E* configuration.

On the basis of these exciting results for this series of macrocyclic inhibitors, we began to envision possible substitutions that could be made across the macrocyclic ring system in order to further enhance biological activity. The energy-minimized structure of **15b**-bound HIV-1 protease with **2**-bound HIV-1 protease (Figure 2) suggested that a single methyl substitution β to the macrocyclic amine could fill an additional hydrophobic pocket previously filled by darunavir's isobutyl group. To this end, we designed and synthesized a series of mono- and dimethylated 14-membered macrocyclic ring systems and evaluated the impact of this substitution on the biological activity. Geminal dimethyl at this location (**19a** through **22a**, Table 4) significantly decreased (10-fold) the enzyme inhibition and greatly reduced antiviral activity. In most cases, the addition of a single methyl group to the ring also reduced biological activity as compared to **14b** and **15b** although results varied greatly depending upon the stereochemistry of the ring systems. Inhibitors **20c** and **21b** were the most potent compounds from this series with K_i = 0.31 and 2.8 nM and IC_{50} = 9.0 and 6.3 nM, respectively. In general, antiviral potency of cyclic inhibitors was superior to that of their acyclic homologues, unsaturated macrocyclic derivatives

Table 4. Enzyme Inhibitory Activity of **19a−c**, **20a−c**, and **21a−c**

Inhibitor structure	K_i (nM)	IC_{50} (nM)[a]	Inhibitor structure	K_i (nM)	IC_{50} (nM)[a]
 **19a**	82 ± 2	>1000	 **21b**	2.8 ± 0.3	6.3
 **20a**	16 ± 1	>1000	 **22b**	3.7 ± 0.3	650
 **21a**	6.9 ± 0.2	>1000	 **19c**	58 ± 6	>1000
 **22a**	6.8 ± 0.8	>1000	 **20c**	0.31 ± 0.03	9
 **19b**	15 ± 3	380	 **21c**	0.15 ± 0.01	52
 **20b**	6 ± 1	200	 **22c**	0.07 ± 0.02	170

[a] MT-2 human T-lymphoid cells exposed to HIV-1_{LAI}; saquinavir and amprenavir exhibited IC_{50} values of 16 and 27 nM, respectively.

were more potent than the corresponding saturated derivatives, and the addition of methyl substituents tended to decrease potency. The two most potent macrocyclic inhibitors identified were **14c** and **14b** having enzyme inhibitory K_i values of 45 and 82 pM and antiviral IC_{50} values of 2 and 4 nM, respectively.

The inhibitors **14b** and **14c** were then examined for their activity against a clinical wild-type X_4-HIV-1 isolate (HIV-$1_{ERS104pre}$) along with various multidrug-resistant clinical X_4- and R_5-HIV-1 isolates using PBMCs as target cells.[6b] The results are shown in Table 5. As can be seen, the potency of inhibitors **14b** and **14c** against HIV-$1_{ERS104pre}$ (IC_{50} = 7 and 5 nM, respectively) are superior to FDA approved inhibitors IDV, APV, and LPV. It is comparable to saquinavir but nearly 2-fold less potent than darunavir (IC_{50} = 3 nM).[6b] In these studies, both indinavir and lopinavir were unable to suppress the replication of the multidrug-resistant clinical isolates examined that include HIV-$1_{MDR/B}$, HIV-$1_{MDR/C}$, HIV-$1_{MDR/TM}$, HIV-$1_{MRR/MM}$, and HIV-$1_{MDR/JSL}$. Of particular note, lopinavir which is widely used as a first-line agent in HAART treatment regimens, was not active against these multidrug-resistant clinical isolates. Amprenavir displayed a 10-fold or greater reduction in potency except against HIV-$1_{MDR/MM}$, where it showed a 7-fold reduction in potency. Inhibitor **14c**, while less potent than darunavir, maintained 7-fold or better potency over amprenavir against HIV-$1_{MDR/C}$, HIV-$1_{MDR/G}$, HIV-$1_{MDR/TM}$, and HIV-$1_{MDR/ISL}$. It maintained over a 6-fold potency against HIV-$1_{MDR/MM}$. Inhibitor **14b** maintained superior potency against HIV-$1_{MDR/C}$ and HIV-$1_{MDR/G}$ (greater than 12- and 21-fold) compared to amprenavir. It maintained 3-fold or better potency compared to amprenavir against all other multidrug-resistant clinical isolates tested. Both inhibitors **14b** and **14c** have shown low cytotoxicity (CC_{50} values 49 and 33 μM, respectively) in target CD_4^+ MT-2 cells. Furthermore, they prevented the replication of HIV-1_{NL4-3} variants selected against up to 5 μM of saquinavir, lopinavir, and indinavir with IC_{50} values of 20−46 nM. More detailed virologic studies with inhibitors **14c** and **14b** will be published in due course.

Table 5. Antiviral Activity of Macrocyclic Inhibitors against Multidrug Resistant Clinical Isolates in PHA-PBMs[a]

virus	SQV	IDV	APV	LPV	DRV	**14b**	**14c**
HIV-$1_{ERS104pre}$ (wild-type: X4)	0.008 ± 0.005	0.043 ± 0.004	0.030 ± 0.005	0.034 ± 0.002	0.003 ± 0.0002	0.007 ± 0.002	0.005 ± 0.003
HIV-$1_{MDR/B}$ (X4)	0.27 ± 0.073 (34)	> 1 (> 23)	> 1 (> 33)	> 1 (> 29)	0.019 ± 0.012 (6)	0.089 ± 0.016 (13)	0.037 ± 0.016 (7)
HIV-$1_{MDR/C}$ (X4)	0.032 ± 0.002 (11)	> 1 (> 23)	0.37 ± 0.011 (12)	> 1 (> 29)	0.008 ± 0.006 (3)	0.029 ± 0.001 (4)	0.044 ± 0.002 (9)
HIV-$1_{MDR/G}$ (X4)	0.030 ± 0.002 (4)	0.34 ± 0.14 (5)	0.43 ± 0.004 (14)	0.26 ± 0.04 (8)	0.023 ± 0.006 (5)	0.028 ± 0.004 (4)	0.057 ± 0.012 (11)
HIV-$1_{MDR/TM}$ (X4)	0.26 ± 0.04 (33)	> 1 (> 23)	0.32 ± 0.007 (11)	> 1 (> 29)	0.004 ± 0.001 (1)	0.072 ± 0.014 (10)	0.027 ± 0.001 (6)
HIV-$1_{MDR/MM}$ (R5)	0.19 ± 0.05 (24)	> 1 (> 23)	0.21 ± 0.222 (7)	> 1 (> 29)	0.011 ± 0.002 (4)	0.055 ± 0.025 (8)	0.033 ± 0.010 (7)
HIV-$1_{MDR/JSL}$ (R5)	0.30 ± 0.02 (37)	> 1 (> 23)	0.62 ± 0.02 (21)	> 1 (> 29)	0.027 ± 0.011 (9)	0.21 ± 0.032 (30)	0.073 ± 0.07 (15)

[a] Amino acid substitutions identified in the protease-encoding region compared to the consensus type B sequence cited from the Los Alamos database include L63P in HIV-$1_{ERS104pre}$; L10I, K14R, L33I, M36I, M46I, F53I, K55R, I62 V, L63P, A71 V, G73S, V82A, L90M, and I93L in HIV-$1_{MDR/B}$; L10I, I15 V, K20R, L24I, M36I, M46L, I54 V, I62 V, L63P, K70Q, V82A, and L89 M in HIV-$1_{MDR/C}$; L10I, V11I, T12E, I15 V, L19I, R41K, M46L, L63P, A71T, V82A, and L90 M in HIV-$1_{MDR/G}$; L10I, K14R, R41K, M46L, I54 V, L63P, A71 V, V82A, L90M, and I93L in HIV-$1_{MDR/TM}$; L10I, K43T, M46L, I54 V, L63P, A71 V, V82A, L90M, and Q92K in HIV-$1_{MDR/MM}$; L10I, L24I, I33F, E35D, M36I, N37S, M46L, I54 V, R57K, I62 V, L63P, A71 V, G73S, and V82A in HIV-$1_{MDR/JSL}$. HIV-$1_{ERS104pre}$ served as a source of wild-type HIV-1. IC_{50} values were determined by using PHA-PBMs as target cells, and inhibition of p24 Gag protein production by each drug was used as an end point. Numbers in parentheses represent *n*-fold changes of IC_{50} values for each isolate compared to IC_{50} values for wild-type HIV-$1_{ERS104pre}$. All assays were conducted in duplicate or triplicate, and data shown represent mean values (±1 standard deviation) derived from results of three independent experiments.

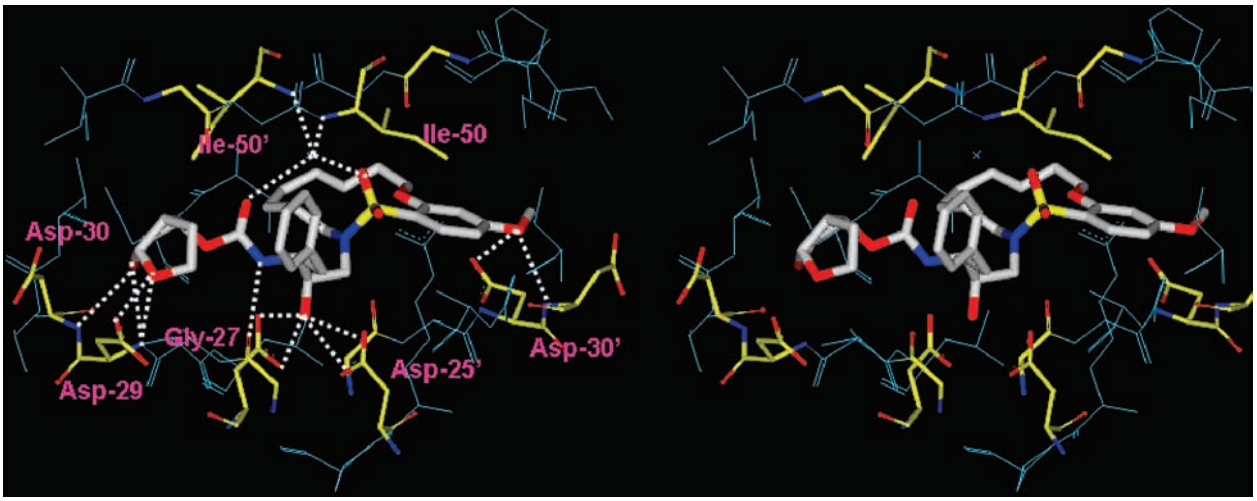


Figure 3. A stereoview of the X-ray structure of macrocyclic inhibitor **14c** (light gray)-bound HIV-1 protease. All strong hydrogen bonding interactions are shown as dotted lines.

The reason why these macrocyclic inhibitors maintained impressive potency against multidrug-resistant clinical isolates is possibly due to their ability to make extensive hydrogen bonds with protease backbone and effectively fill in the hydrophobic pockets in the S1′−S2′-subsites.

X-ray Crystallography

To gain molecular insights into ligand-binding site interactions responsible for the potent antiviral activity of inhibitor **14c**, we have determined an X-ray crystal structure of the inhibitor complexed with wild-type protease. The crystal structure was solved and refined at 1.17 Å resolution with an *R*-factor and R_{free} of 16.0% and 19.4%, respectively. In this high resolution structure, the inhibitor **14c** was bound to the HIV-1 protease active site in the two orientations with a ratio of 1:1. A stereoview of the X-ray structure of **14c**-bound HIV-1 protease is shown in Figure 3. As can be seen, the inhibitor makes extensive interactions involving the P2 to P2′-ligands in the protease active site, most notably through favorable polar interactions including hydrogen bonds and weaker C−H···O interactions in the active site. The transition-state hydroxyl group in **14c** forms asymmetric hydrogen bonding interactions with all four carboxylate oxygen atoms of the Asp25 and Asp25′ with distances of 2.5−3.3 Å. The conserved tetrahedral water molecule forms hydrogen bonds with one of the sulfonamide oxygens, the urethane carbonyl oxygen, and the backbone amide of Ile50 and Ile50′ with distances of 2.6−3.1 Å. These interactions have been observed in a majority of HIV-1 protease complexes with the inhibitors[18] and substrate analogues.[19] The flexible P1′−P2′ macrocyclic ligand nicely packs into the hydrophobic pocket in the S1′-subsite. It also makes weaker C−H···O interactions, which play important roles as we have reported earlier.[20−22] The macrocyclic ring zigzags into a crown shape and fits well in between the S1′ and S2′-pockets. The protein−ligand complex shows three major interactions with the carbonyl oxygen of backbone residues, one with Gly27′ and two with Gly48′, with distances ranging from 3.0 to 3.6 Å. In comparison to the X-ray structures of the protease with **1** and **2**, the P1-phenyl ring in **14c** is rotated about 30° toward Asp 29′ along the backbone. The macrocycle acts more or less like a spring that pushes against the P1-phenyl ring, causing this rotation.

Both P2 and P2′ ligands form five strong N−H···O hydrogen bonds with the protease backbone. Of these, three hydrogen bonds are formed between the P2-bis-THF ring oxygens and the backbone amide nitrogens of Asp29 and Asp30 with distances of 3.1, 3.0, and 3.2 Å. The fourth interaction is between the P2-urethane NH and the carbonyl oxygen of Gly27 with a distance of 3.0 Å. The fifth backbone interaction is between the *p*-methoxy group of the P2′-sulfonamide and the

amide nitrogen of Asp30′, with a distance of 3.0 Å. All of these ligand−backbone interactions are present in the X-ray structure of **2**-bound HIV-1 protease as well. This backbone binding with the main chain atoms of the protease may be responsible for both inhibitors' (**2** and **14c**) abilities to maintain robust potency against multidrug-resistant HIV-1 variants.

Conclusions

In summary, we have designed novel macrocyclic protease inhibitors modifying P1′−P2′ ligands of darunavir-like PIs and investigated their biological activity. The inhibitors were designed to maintain key hydrogen bonding interactions with protease backbone, similar to darunavir. The design of macrocycles involving the P1′−P2′-ligands is based upon the premise that a flexible macrocycle would effectively repack the hydrophobic pocket in the S1′ to S2′-subsites when it is altered by mutations. We have investigated inhibitors containing 9−15-membered macrocycles containing both *E*/*Z* olefins and the corresponding saturated derivatives. Grubbs' metathesis reaction was the key step in building these inhibitors in very good yields. Most remarkably, all macrocyclic inhibitors are significantly more potent than their acyclic counterparts. The saturated inhibitors are in general less active than the corresponding unsaturated derivatives. Our investigation resulted in the identification of inhibitors **14b** and **14c**, which have displayed significantly better antiviral activity than many of the currently FDA-approved inhibitors. Inhibitor **14b** contains a 14-membered ring with a *Z*-olefin, and **14c** contains a 13-membered ring with an *E*-olefin. Both inhibitors exerted potent activity against HIV-1_{LAI}, with IC_{50} values of 4 and 2 nM, respectively. They have maintained excellent potency against multidrug-resistant HIV-1 variants. These inhibitors have shown low cytotoxicity (CC_{50} values 49 and 33 μM, respectively) in target CD_4^+ MT-2 cells. Furthermore, both inhibitors **14b** and **14c** blocked the replication of HIV-1_{NL4-3} variants, selected after exposure of up to 5 μM of saquinavir, lopinavir, and indinavir, with IC_{50} values of 20−46 nM. The protein−ligand X-ray structure of **14c** showed critical ligand-binding site interactions in the protease active site. Particularly, it maintained all key backbone hydrogen bonding interactions similar to darunavir and inhibitor **2**. Also, the conformational flexibility of the P1′−P2′ macrocycle most likely contributed to its impressive activity against multidrug-resistant clinical variants. Further design and optimization of P1′−P2′ macrocyclic ligands are in progress.

Experimental Section

General Experimental Methods. Chemicals and reagents were purchased from commercial suppliers and used without further purification. Anhydrous solvents were obtained as follows: pyridine and dichloromethane were distilled from calcium hydride; tetrahydrofuran and diethyl ether were distilled from sodium with benzophenone as an indicator. All other solvents were reagent grade. All moisture sensitive reactions were carried out in oven-dried glassware under argon. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance ARX-400, Bruker DRX-500, or Bruker Avance-III-800 spectrometer. Chemical shifts are given in ppm and are referenced against the diluting solvent. For chloroform-*d*: ^{13}C triplet = 77.00 $CDCl_3$ and ^{1}H singlet = 7.26 ppm. For methanol-d_4: ^{13}C septuplet = 49.05 and ^{1}H quintuplet = 3.31 ppm. Characteristic splitting patterns due to spin−spin coupling are expressed as follows: br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, sept = septuplet. All coupling constants are measured in hertz. FTIR spectra were recorded on a Mattson Genesis II FT-IR spectrometer or a Perkin-Elmer spectrometer L1185247 using a NaCl plate or KBr pellet. Optical rotations were recorded on a Perkin-Elmer 341 or Rudolph Research Autopol III polarimeter. Low resolution mass spectra were recorded on a FinniganMAT LCQ or Hewlett-Packard Engine mass spectrometer. High-resolution mass spectra were recorded on a FinniganMAT XL95 mass spectrometer calibrated against PPG. Column chromatography was performed with Whatman 240−400 mesh silica gel under low pressure of 3−5 psi. TLC was carried out with E. Merck silica gel 60-F-254 plates. HPLC data was collected using a system composed of an Agilent 1100 series degasser, quaternary pump, thermostatable column compartment, variable wavelength detector, and Agilent 1200 series autosampler and fraction collector controlled by Chemstation software. All chromatographic reagents used were HPLC grade. The reported inhibitors were found to be >95% pure by reversed-phase gradient HPLC (see Supporting Information for specific method conditions).

1-(Hex-5-enyloxy)-3-methoxybenzene (5a). To a stirred solution of 3-methoxy phenol (1.24 g, 10 mmol), 5-hexen-1-ol, **4a** (1.4 mL, 12 mmol), and Ph_3P (3.14 g, 12 mmol) in THF (20 mL) at 0 °C was added DIAD (2.3 mL, 12 mmol) dropwise. After stirring the solution for 30 min at 0 °C, the reaction mixture was warmed to 23 °C and stirred for 3 h. The reaction mixture was concentrated in vacuo, and the residue was subjected to column chromatography (98:2 hexanes:EtOAc) to yield **5a** (1.98 g, 96% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.58−1.66 (m, 2H), 1.80−1.88 (m, 2H), 2.15−2.20 (m, 2H), 3.82 (s, 3H), 3.98 (t, J = 6.4 Hz, 2H), 5.02−5.12 (m, 2H), 5.83−5.92 (m, 1H), 6.52−6.56 (m, 3H), 7.19−7.24 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.3, 28.7, 33.4, 55.1, 67.6, 100.9, 106.0, 106.6, 114.7, 129.8, 138.5, 160.3, 160.8. FT-IR (film, NaCl) ν_{max} = 3075, 2939, 1599, 1493, 1287, 1200, 1152, 1046 cm^{-1}. CI LRMS (m/z): 207.25 $[M + H]^+$.

1-Methoxy-3-(pent-4-enyloxy)benzene (5b). Title compound was obtained from 4-penten-1-ol **4b**, as described for **5a** in 95% yield after flash-chromatography (98:2 hexanes:EtOAc) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.88−1.94 (m, 2H), 2.25−2.30 (m, 2H), 3.81 (s, 3H), 3.98 (t, J = 6.4 Hz, 2H), 5.03−5.13 (m, 2H), 5.85−5.95 (m, 1H), 6.51−6.56 (m, 3H), 7.20 (t, J = 8.1 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.3, 30.0, 55.1, 67.0, 100.9, 106.0, 106.6, 115.1, 129.7, 137.7, 160.2, 160.7. FT-IR (film, NaCl) ν_{max} = 3076, 2940, 1599, 1492, 1287, 1200, 1152, 1048 cm^{-1}. CI LRMS (m/z): 193.25 $[M + H]^+$.

1-(But-3-enyloxy)-3-methoxybenzene (5c). Title compound was obtained from 3-buten-1-ol **4c**, as described for **5a** in 96% yield after flash-chromatography (98:2 hexanes:EtOAc) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.54−2.60 (m, 2H), 3.81 (s, 3H), 4.02 (t, J = 6.7 Hz, 2H), 5.13−5.23 (m, 2H), 5.89−5.97 (m, 1H), 6.51−6.56 (m, 3H), 7.20 (t, J = 8.1 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 33.6, 55.1, 67.1, 100.9, 106.2, 106.6, 116.9, 129.8, 134.4, 160.1, 160.8. FT-IR (film, NaCl) ν_{max} = 3136, 2378, 1644, 1509 cm^{-1}. CI LRMS (m/z): 179.20 $[M + H]^+$.

1-(Allyloxy)-3-methoxybenzene (5d). Title compound was obtained from allyl alcohol **4d** as described for **5a** in 96% yield after flash-chromatography (98:2 hexanes:EtOAc) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.81 (s, 3H), 4.02 (d, J = 6.7 Hz, 2H), 5.13−5.23 (m, 2H), 5.89−5.97 (m, 1H), 6.51−6.56 (m, 3H), 7.20 (t, J = 8.1 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 55.1, 67.1, 100.9, 106.2, 106.6, 116.9, 129.8, 134.4, 160.1, 160.8.

2-(Hex-5-enyloxy)-4-methoxybenzenesulfonic Acid (6a). To **6a** (2 g, 9.7 mmol) was added acetic anhydride (1.4 mL, 14.5 mmol), and the resulting mixture was stirred at 0 °C. To this was then added concentrated H_2SO_4 (1.1 g) followed by methanol (20 mL). The resulting solution was warmed to 23 °C and stirred for 12 h. After this time, the reaction mixture was concentrated in vacuo and the resulting red oil was subjected to column chromatography (88:12 CH_2Cl_2:MeOH) to give **6a** (1.06 g, 38%) as a red waxy solid. ^{1}H NMR (400 MHz, D_2O) δ 1.44

(quintet, J=7.4 Hz, 2H), 1.66−1.73 (m, 2H), 1.99 (q, J = 7.1 Hz, 2H), 3.70 (s, 3H), 3.98 (t, J = 6.5 Hz, 2H), 4.85−4.97 (m, 2H), 5.74−5.92 (m, 1H), 6.52−6.56 (m, 2H), 7.19−7.24 (m, 1H). ^{13}C NMR (100 MHz, D_2O) δ 25.3, 28.7, 33.4, 55.1, 67.6, 100.9, 106.0, 106.6, 114.7, 129.8, 138.5, 160.3, 160.8. ESI (m/z): 285.09 $[M - H]^-$.

4-Methoxy-2-(pent-4-enyloxy)benzenesulfonic Acid (6b). Title compound was obtained from ether **5b** as described for **6a** in 36% yield after flash-chromatography (88:12 CH_2Cl_2:MeOH) as a white solid. ^{1}H NMR (400 MHz, D_2O) δ 1.75−1.82 (m, 2H), 2.10−2.16 (m, 2H), 3.69 (s, 3H), 3.98 (t, J = 6.4 Hz, 2H), 4.88−5.00 (m, 2H), 5.77−5.87 (m, 1H), 6.45 (dd, J = 8.6, 2.2 Hz, 1H), 6.51 (d, J = 2.1 Hz, 1H), 7.58 (d, J = 8.8 Hz, 1H). ^{13}C NMR (100 MHz, D_2O) δ 28.0, 29.9, 56.1, 68.7, 100.5, 104.9, 115.5, 123.7, 130.2, 139.4, 157.8, 163.5. ESI (m/z): 271.07 $[M - H]^-$.

2-(But-3-enyloxy)-4-methoxybenzenesulfonic Acid (6c). Title compound was obtained from ether **5c** as described for **6a** in 30% yield after flash-chromatography (88:12 CH_2Cl_2:MeOH) as a white solid. ^{1}H NMR (400 MHz, D_2O) δ 2.38−2.43 (m, 2H), 3.62 (s, 3H), 3.98 (t, J = 6.8 Hz, 2H), 4.93−5.05 (m, 2H), 5.77−5.87 (m, 1H), 6.37 (dd, J = 8.6, 2.2 Hz, 1H), 6.43 (d, J = 2.4 Hz, 1H), 7.58 (d, J = 8.8 Hz, 1H) ^{13}C NMR (100 MHz, D_2O) δ 33.3, 56.1, 68.8, 100.6, 105.0, 117.5, 123.7, 130.2, 135.4, 157.5, 163.4. ESI (m/z): 257.10 $[M - H]^-$.

2-(Allyloxy)-4-methoxybenzenesulfonic Acid (6d). Title compound was obtained from ether **5d** as described for **6a** in 35% yield after flash-chromatography (88:12 CH_2Cl_2:MeOH) as a white solid. ^{1}H NMR (400 MHz, D_2O) δ 3.71 (s, 3H), 4.58−4.75 (m, 2H), 5.16−5.38 (m, 2H), 5.92−5.99 (m, 1H), 6.47−6.57 (m, 2H), 7.58 (d, J=8.8. Hz, 1H). ^{13}C NMR (100 MHz, D_2O) δ 56.1, 69.7, 101.1, 105.3, 118.0, 123.7, 130.6, 133.2, 157.1, 163.5. ESI (m/z): 243.13 $[M - H]^-$.

2-(Hex-5-enyloxy)-4-methoxybenzene-1-sulfonyl Chloride (7a). To a stirring solution of sulfonic acid **6a** (266 mg, 0.9 mmol) in pyridine (2 mL) was added thionyl chloride (0.2 mL, 2.8 mmol) dropwise. The resulting solution was allowed to stir for 4 h and then the reaction mixture concentrated in vacuo. The resulting residue was purified using column chromatography (5:1 hexanes: EtOAc) to give **7a** (140 mg, 50%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.63−1.70 (m, 2H), 1.87−1.93 (m, 2H), 2.10−2.16 (m, 2H), 3.88 (s, 3H), 4.14 (t, J = 6.2 Hz, 2H), 4.95−5.05 (m, 2H), 5.76−5.86 (m, 1H), 6.51−6.54 (m, 2H), 7.84 (d, J = 9.6 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.9, 28.1, 33.1, 55.9, 69.2, 99.9, 104.6, 114.7, 124.3, 131.7, 138.3, 158.7, 166.8.

4-Methoxy-2-(pent-4-enyloxy)benzene-1-sulfonyl Chloride (7b). Title compound was obtained from ether **6b** as described for **7a** in 48% yield after flash-chromatography (6:1 hexanes:EtOAc) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.96−1.2.03 (m, 2H), 2.31−2.37 (m, 2H), 3.88 (s, 3H), 4.15 (t, J = 6.2 Hz, 2H), 4.99−5.09 (m, 2H), 5.79−5.90 (m, 1H), 6.51−6.54 (m, 2H), 7.84 (d, J= 9.2 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.8,29.7, 55.9, 68.4. 99.9, 104.6, 115.6, 124.3, 131.7, 137.3, 158.6, 166.8.

2-(But-3-enyloxy)-4-methoxybenzene-1-sulfonyl Chloride (7c). Title compound was obtained from ether **6c** as described for **7a** in 52% yield after flash-chromatography (6:1 hexanes:EtOAc) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.62−2.70 (m, 2H), 3.88 (s, 3H), 4.19 (t, J=6.2 Hz, 2H), 5.12−5.25 (m, 2H), 5.91−6.05 (m, 1H), 6.52−6.56 (m, 2H), 7.86 (d, J = 9.2 Hz, 1H).

2-(Allyloxy)-4-methoxybenzene-1-sulfonyl Chloride (7d). Title compound was obtained from ether **6d** as described for **7a** in 58% yield after flash-chromatography (6:1 hexanes:EtOAc) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.88 (s, 3H), 4.72 (d, J = 4.4 Hz, 2H), 5.33 (d, J = 10.6 Hz, 1H), 5.57 (d, J = 17.3 Hz, 1H), 5.99−6.07 (m, 1H), 6.52−6.55 (m, 2H), 7.83 (d, J = 8.7 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.9, 69.7, 100.5, 105.0, 118.1, 124.4, 131.0, 131.7, 158.0, 166.7.

***tert*-Butyl (2*S*,3*R*)-4-(hex-5-enylamino)-3-hydroxy-1-phenylbutan-2-ylcarbamate (10a).** A solution of hex-5-en-1-amine **9a** (297 mg, 3 mmol) and epoxide **8** (263 mg, 1 mmol) was heated to 60 °C in isopropyl alcohol (4 mL) for 4 h. The solvent was then evaporated under reduced pressure, and the resulting residue was purified by silica chromatography (5:95 MeOH:$CHCl_3$) to give **10a** (350 mg, 97%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.34 (s, 9H), 1.39−1.50 (m,4H), 2.05 (q, J = 7 Hz, 2H), 2.55−2.68 (m, 6H), 2.81−2.86 (m, 1H), 2.96 (dd, J = 4.5, 14 Hz, 1H), 3.44−3.49 (m, 1H), 3.79 (br s, 1H), 4.72 (d, J = 8.7 Hz, 1H), 4.92−5.02 (m, 2H), 5.74−5.84 (m, 1H), 7.17−7.28 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.4, 28.2, 29.4, 33.4, 36.5, 49.6, 51.3, 54.1, 70.7, 79.3, 114.5, 126.2, 128.3, 129.4, 137.8, 138.6,155.9. CI LRMS (m/z): 363.55 $[M + H]^+$.

***tert*-Butyl (2*S*,3*R*)-4-(allylamino)-3-hydroxy-1-phenylbutan-2-ylcarbamate (10b).** Title compound was obtained from allylamine **9b** and epoxide **8** as described for **10a** in 99% yield after flash-chromatography (5:95 MeOH:$CHCl_3$) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.35 (s, 9H), 2.60−2.86 (m, 5H), 2.96 (dd, J = 4.5, 14.1 Hz, 1H), 3.16−3.29 (m, 2H), 3.50−3.53 (m, 1H), 3.81 (br s, 1H), 4.73 (d, J=9.1 Hz, 1H), 5.10 (d, J=10.3 Hz, 1H), 5.17 (d, J=17 Hz, 1H), 5.81−5.91 (m, 1H), 7.13−7.32 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.2, 36.5, 50.7, 52.1, 54.1, 70.9, 79.3, 116.2, 126.2, 128.3, 129.4, 136.3, 137.8, 155.9. CI LRMS (m/z) 321.50 $[M + H]^+$.

***tert*-Butyl-(2*S*,3*R*)-4-(*N*-(hex-5-enyl)-2-(hex-5-enyloxy)-4-ethoxyphenylsulfonamido)-3-hydroxy-1-phenylbutan-2-ylcarbamate (11a).** To a stirring solution of **10a** (50 mg, 0.14 mmol) in pyridine (2 mL) was added **7a** (64 mg, 0.20 mmol), and the resulting solution was allowed to stir for 2 h at 23 °C. The reaction mixture was concentrated under reduced pressure, and the resulting residue was purified by flash column chromatography (3:1 hexanes:EtOAc) to yield **11a** (74 mg 84% yield) as a colorless oil. $[\alpha]_D^{20}$ −1.4 (*c* 1.00, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25−1.34 (m, 11 H), 1.47−1.50 (m, 2H), 1.57−1.60 (m, 2H), 1.82−1.90 (m, 2H), 1.97 (q, J=7.1 Hz, 2H), 2.11 (q, J = 7.0 Hz, 2H), 2.92−2.95 (m, 2H), 3.10−3.17 (m, 1H), 3.30 (br s, 3H), 3.76 (br s, 2H), 3.84 (s, 3H), 3.90−3.92 (m, 1H) 4.03 (t, J= 6.7 Hz, 2H), 4.65 (d, J = 7.1 Hz, 1H), 4.89−5.04 (m, 4H), 5.66−5.82 (m, 2H), 6.46 (d, J = 2 Hz, 1H), 6.49 (dd, J = 8.8, 2.3 Hz, 1H), 7.17−7.29 (m, 5H), 7.83 (d, J = 7.8 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.9, 25.7, 27.9, 28.2, 28.3, 33.1, 33.2, 35.1, 49.9, 52.3, 54.5, 55.6, 69.1, 72.2, 79.4, 100.2, 104.1, 114.7, 115.0, 119.4, 126.2, 128.3, 129.5, 133.5, 137.8, 138.1, 138.2, 156.0, 157.6, 164.7. FT-IR (film, NaCl) ν_{max} = 3395, 2935, 1705, 1597, 1325 cm^{-1}. ESI (+) LRMS (m/z): 653.13 $[M + Na]^+$.

***tert*-Butyl-(2*S*,3*R*)-4-(*N*-(hex-5-enyl)-4-methoxy-2-(pent-4-enyloxy)phenylsulfonamido)-3-hydroxy-1-phenylbutan-2-ylcarbamate (11b).** Title compound was obtained from **10a** and **7b**, as described for **11a** in 79% yield after flash-chromatography (3:1 hexanes:EtOAc) as a colorless oil. $[\alpha]_D^{20}$ −1.6 (*c* 1.20, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25−1.37 (m, 12H), 1.43−1.51 (m, 2H), 1.88−1.98 (m, 4H), 2.27 (q, J=7.1 Hz, 2H), 2.86−2.97 (m, 2H), 3.10−3.17 (m, 1H), 3.25−3.31 (m, 3H), 3.76 (br s, 2H), 3.84 (s, 3H), 4.04 (t, J = 6.6 Hz, 2H), 4.66 (d, J = 7.1 Hz, 1H), 4.88−5.10 (m, 4H), 5.65−5.72 (m, 1H), 5.78−5.85 (m, 1H), 6.46 (d, J = 2 Hz, 1H), 6.49 (dd, J = 8.8, 2.0 Hz, 1H), 7.17−7.29 (m, 5H), 7.83 (d, J = 8.7 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.7, 27.9, 28.2, 29.7, 33.1, 35.2, 49.8, 52.2, 54.6, 55.6, 68.4, 72.2, 79.4, 100.2, 104.1, 114.7, 115.7, 119.4, 126.2, 128.3, 129.5, 133.5, 137.0, 137.8, 138.2, 156.0, 157.6, 164.7. FT-IR (film, NaCl) ν_{max} = 3398, 2931, 1706, 1596, 1325 cm^{-1}. ESI (+) LRMS (m/z): 639.06 $[M + Na]^+$.

***tert*-Butyl-(2*S*,3*R*)-4-(2-(but-3-enyloxy)-*N*-(hex-5-enyl)-4-methoxyphenylsulfonamido)-3-hydroxy-1-phenylbutan-2-ylcarbamate (11c).** Title compound was obtained from **10a** and **7c**, as described for **11a** in 50% yield after flash-chromatography (3:1 hexanes:EtOAc) as a colorless oil. $[\alpha]_D^{20}$ +0.6 (*c* 2.00, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.28−1.38 (m, 12H), 1.48−1.52 (m, 2H), 2.00 (q, J=6.7 Hz, 2H), 2.65 (q, J=6.7, 2H), 2.96 (br s, 2H), 3.14−3.18 (m, 1H), 3.30−3.39 (m, 3H), 3.79 (br s, 2H), 3.88 (s, 3H), 4.13 (t, J=7.1 Hz, 2H), 4.68 (d, J=5.1 Hz, 1H), 4.92−4.98 (m, 2H), 5.17−5.24 (m, 2H), 5.68−5.76 (m, 1H), 5.91−5.99 (m, 1H), 6.51 (d, J = 2 Hz, 1H), 6.54 (dd, J = 8.8, 2.0

Hz, 1H), 7.21−7.32 (m, 5H), 7.88 (d, J = 8.8 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 25.9, 28.2, 28.4, 29.9, 33.4, 35.4, 50.1, 52.5, 54.8, 55.9, 68.7, 72.5, 79.7, 100.6, 104.5, 114.9, 118.0, 119.8, 126.5, 128.6, 129.8, 133.7, 133.9 138.1, 138.5, 156.3, 157.7, 165.0. FT-IR (film, NaCl) ν_{max} = 3394, 2931, 1704, 1596, 1325 cm^{-1}. ESI (+) (m/z): 625.05 $[M + Na]^+$.

***tert*-Butyl(2*S*,3*R*)-4-(2-(allyloxy)-*N*-(hex-5-enyl)-4-methoxyphenylsulfonamido)-3-hydroxy-1-phenylbutan-2-ylcarbamate (11d).** Title compound was obtained from **10a** and **7d**, as described for **11a** in 64% yield after flash-chromatography (3:1 hexanes:EtOAc) as a colorless oil. $[\alpha]_D^{20}$ +1.1 (c 2.80, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25−1.34 (m, 11H), 1.39−1.46 (m, 2H), 1.96 (q, J = 7.0 Hz, 2H), 2.89−2.96 (m, 2H), 3.08−3.15 (m, 1H), 3.24−3.30 (m, 3H), 3.77 (br s, 2H), 3.84 (s, 3H), 4.60 (d, J = 5.4 Hz, 2H), 4.66 (d, J=7.2 Hz, 1H), 4.88−4.94 (m, 2H), 5.33 (d, J = 10.4, 2H), 5.43 (d, J = 17.4 Hz, 1H), 5.63−5.73 (m, 1H), 6.00−6.10 (m, 1H), 6.47 (d, J = 1.8 Hz, 1H), 6.54 (dd, J = 8.9, 1.9 Hz, 1H), 7.18−7.29 (m, 5H), 7.85 (d, J=8.8 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.7, 27.8, 28.2, 33.1, 35.2, 49.9, 52.5, 54.5, 55.6, 69.9, 72.2, 79.4, 100.6, 104.4, 114.7, 119.4, 119.7 126.2, 128.3, 129.5, 131.9, 133.5, 137.9, 138.2, 155.9, 157.0, 164.6. FT-IR (film, NaCl) ν_{max} = 3400, 2929, 1704, 1596, 1324 cm^{-1}. ESI (+) LRMS (m/z): 611.03 $[M + Na]^+$.

***tert*-Butyl-(2*S*,3*R*)-4-(*N*-allyl-2-(hex-5-enyloxy)-4-methoxyphenylsulfonamido)-3-hydroxy-1-phenylbutan-2-ylcarbamate (11e).** Title compound was obtained from **10b** and **7a**, as described for **11a** in 77% yield after flash-chromatography (3:1 hexanes: EtOAc) as a colorless oil. $[\alpha]_D^{20}$ −6.6 (c 1.96, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.36 (s, 9H), 1.60−1.66 (m, 2H), 1.87−1.97 (m, 2H), 2.13−2.17 (m, 2H), 2.91−2.98 (m, 2H), 3.27−3.39 (m, 2H), 3.79 (br s, 2H), 3.87 (s, 3H), 3.91−3.99 (m, 2H), 4.08 (t, J= 6.7 Hz, 2H), 4.65 (d, J=7.1 Hz, 1H), 4.98 (d, J=10.1, 1H), 5.04 (d, J = 17.1, 1H), 5.13−5.19 (m, 2H), 5.63−5.73 (m, 1H), 5.78−5.86 (m, 1H), 6.50−6.53 (m, 2H), 7.21−7.31 (m, 5H), 7.88 (d, J = 8.7 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.0, 28.3, 33.3, 35.3, 51.2, 52.2, 54.6, 55.7, 69.2, 71.8, 79.5, 100.3, 104.2, 115.1, 119.0, 119.6, 126.3, 128.4, 129.6, 133.6, 137.9, 138.2, 156.1, 157.7, 164.8. FT-IR (film, NaCl) ν_{max} = 3392, 2932, 1702, 1595, 1324 cm^{-1}. ESI (+) LRMS (m/z): 611.04 $[M + Na]^+$.

***tert*-Butyl-(2*S*,3*R*)-4-(*N*-allyl-4-methoxy-2-(pent-4-enyloxy)phenylsulfonamido)-3-hydroxy-1-phenylbutan-2-ylcarbamate (11f).** Title compound was obtained from **10b** and **7b**, as described for **11a** in 86% yield after flash-chromatography (3:1 hexanes:EtOAc) as a colorless oil. $[\alpha]_D^{20}$ −5.6 (c 1.10, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.32 (s, 9H), 1.94 (quintet, J=7 Hz, 2H), 2.65 (q, J=7 Hz, 2H), 2.86−2.96 (m, 2H), 3.27 (dd, J = 7.4, 14.8 Hz, 1H), 3.34−3.38 (m, 1H), 3.76 (br s, 2H), 3.82 (s, 3H), 3.87−3.92 (m, 2H), 4.04 (t, J = 6.5 Hz, 2H), 4.65 (d, J = 8.2 Hz, 1H), 4.98−5.15 (m, 4H), 5.57−5.63 (m, 1H), 5.75−5.86 (m, 1H), 6.46−6.49 (m, 2H), 7.12−7.27 (m, 5H), 7.83 (d, J=8.5 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.8, 28.1, 29.7, 35.3, 51.0, 51.9, 54.5, 55.6, 68.4, 71.8, 79.3, 100.2, 104.2, 115.7, 119.0, 119.5, 126.1, 128.2, 129.5, 133.4, 137.2, 137.9, 155.9, 157.6, 164.7. FT-IR (film, NaCl) ν_{max} = 3390, 2976, 1710, 1597, 1325 cm^{-1}. ESI (+) LRMS (m/z): 597.13 $[M + Na]^+$.

***tert*-Butyl-(2*S*,3*R*)-4-(*N*-allyl-2-(but-3-enyloxy)-4-methoxyphenylsulfonamido)-3-hydroxy-1-phenylbutan-2-ylcarbamate (11g).** Title compound was obtained from **10b** and **7c**, as described for **11a** in 78% yield after flash-chromatography (3:1 hexanes: EtOAc) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.34 (s, 9H), 2.62 (q, J=7 Hz, 2H), 2.88−2.96 (m, 2H), 3.27 (dd, J = 7.8, 15.1 Hz, 1H), 3.34−3.38 (m, 1H), 3.76 (br s, 2H), 3.85 (s, 3H), 3.87−3.99 (m, 2H), 4.10 (t, J = 6.5 Hz, 2H), 4.65 (br s, 1H), 5.10−5.22 (m, 4H), 5.57−5.63 (m, 1H), 5.87−5.95 (m, 1H), 6.49 (d, J=2.2 Hz, 1H), 6.51 (dd, J=2.3, 8.7 Hz 1 H) 7.18−7.37 (m, 5H), 7.85 (d, J=8.7 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.8, 28.1, 35.3, 51.0, 51.9, 54.5, 55.6, 68.4, 71.8, 79.3, 100.2, 104.2, 115.7, 119.0, 119.5, 126.2, 128.2, 129.5, 133.4, 137.2, 137.9, 155.9, 157.6, 164.7. FT-IR (film, NaCl) ν_{max} = 3390, 2976, 1710, 1597, 1325 cm^{-1}. ESI LRMS (m/z): 582.95 $[M + Na]^+$.

***tert*-Butyl-(2*S*,3*R*)-4-(*N*-allyl-2-(allyloxy)-4-methoxyphenylsulfonamido)-3-hydroxy-1-phenylbutan-2-ylcarbamate (11h).** Title compound was obtained from **10b** and **7d**, as described for **11a** in 80% yield after flash-chromatography (3:1 hexanes:EtOAc) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.33 (s, 9H), 2.83−2.96 (m, 2H), 3.25−3.36 (m, 2H), 3.77 (br s, 2H), 3.83 (s, 3H), 3.87−3.94 (m, 2H), 4.56−4.67 (m, 3H), 5.07−5.14 (m, 2H), 5.33 (d, J=10.5 Hz, 1H), 5.44 (d, J = 17.2 Hz, 1H), 5.57−5.64 (m, 1H), 6.00−6.10 (m, 1H), 6.47 (d, J=1.9 Hz, 1H), 6.51 (dd, J =1.9, 8.9 Hz, 1 H) 7.16−7.28 (m, 5H), 7.85 (d, J = 8.7 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.2, 35.3, 51.3, 52.1, 54.5, 55.6, 69.9, 71.8, 79.3, 100.6, 104.5, 119.0, 119.4, 119.8, 126.2, 128.2, 129.5, 131.8, 133.4, 137.9, 155.9, 157.0, 164.7. FT-IR (film, NaCl) ν_{max} = 3390, 2976, 1710, 1597, 1325 cm^{-1}. ESI (+) LRMS (m/z): 569.06 $[M + Na]^+$.

Compound 13a. To a stirring solution of **11a** (63 mg, 0.1 mmol) in CH_2Cl_2 (2 mL) was added a solution of 30% trifluoroacetic acid in CH_2Cl_2, and the resulting mixture was stirred for 30 min. The solvent was evaporated under reduced pressure, and the residue was dissolved in CH_3CN (2 mL). To this solution was added **12** (31 mg, 0.11 mmol), followed by *i*-Pr_2NEt. After stirring for 24 h, the reaction mixture was concentrated in vacuo and the resulting residue was subjected to flash-chromatography (1:1 hexanes:EtOAc) to give **13a** (36 mg, 53% yield) as a colorless oil. $[\alpha]_D^{20}$ −13.1 (c 1.50, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.28−1.31 (m, 6H), 1.47−1.51 (m, 3H), 1.58−1.61 (m, 3H), 1.84−1.88 (m, 2H), 1.96−1.99 (m, 2H), 2.01−2.13 (m, 2H), 2.79−2.84 (m, 1H), 2.88−2.92 (m, 1H), 3.09−3.15 (m, 1H), 3.24−3.40 (m, 3H), 3.61 (br s, 1H), 3.64−3.72 (m, 2H), 3.83−3.92 (m, 4H), 3.93−3.96 (m, 1H), 4.05 (t, J = 6.7 Hz, 2H), 4.90−5.03 (m, 4H), 5.64 (d, J = 5 Hz, 1H), 5.66−5.82 (m, 2H), 6.46 (s, 1H), 6.51 (d, J = 8.8 Hz, 1H), 7.17−7.26 (m, 5H), 7.83 (d, J = 8.8 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 27.9, 28.2, 28.1, 29.4, 31.7, 32.9, 33.1, 35.1, 45.1, 49.8, 52.1, 54.8, 55.5, 68.9, 69.3, 70.5, 72.0, 73.1, 100.1, 103.9, 109.0, 114.6, 114.9, 118.9, 126.3, 128.2, 129.1, 133.4, 137.4, 137.8, 138.0, 155.2, 157.4, 164.6. FT-IR (film, NaCl) ν_{max} = 3343, 2928, 1721, 1595, 1325 cm^{-1}. ESI (+) HRMS (m/z): $[M + Na]^+$ calcd for $C_{36}H_{50}N_2O_9S$, 709.3135; found, 709.3136.

Compound 13b. Title compound was obtained from **11b** and **12** as described for **13a** in 55% yield after flash-chromatography (1:1 hexanes:EtOAc) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.27−1.32 (m, 3H), 1.41−1.53 (m, 3H), 1.57−1.62 (m, 1H), 1.92−1.99 (m, 4H), 2.27 (q, J=7.05, 2H), 2.80 (dd, J=10, 14 Hz, 1H), 2.86−2.91 (m, 1H), 3.01 (dd, J = 4, 14 Hz, 1H), 3.10−3.15 (m, 1H), 3.27−3.33 (m, 2H), 3.38 (dd, J=8.6, 15.2 Hz, 1H), 3.61 (br s, 1H), 3.64−3.70 (m, 2H), 3.81−3.84 (m, 5H), 3.88−3.95 (m, 2H), 4.05 (t, J=6.6 Hz, 2H), 4.89−5.09 (m, 5H), 5.63 (d, J=5.2 Hz, 1H), 5.65−5.73 (m, 1H), 5.77−5.85 (m, 1H), 6.46 (d, J=2 Hz, 1H), 6.51 (dd, J = 2.1, 8.8 Hz, 1H), 7.17−7.26 (m, 5H), 7.83 (d, J=8.8 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.7, 27.9, 29.7, 33.1, 35.4, 45.1, 49.8, 52.1, 55.1, 55.7, 68.5, 69.5, 70.7, 72.2 73.2, 100.3, 104.2, 109.2, 114.8, 115.7, 119.2, 126.4, 128.4, 129.3, 133.6, 137.1, 137.6, 138.2, 155.4, 157.5, 164.8. ESI (+) HRMS (m/z): $[M + H]^+$ calcd for $C_{35}H_{48}N_2O_9S$, 673.3159; found, 673.3153.

Compound 13c. Title compound was obtained from **11c** and **12** as described for **13a** in 81% yield after flash-chromatography (1:1 hexanes:EtOAc) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.31−1.35 (m, 3H), 1.50−1.54 (m, 3H), 1.62−1.67 (m, 1H), 2.00 (q, J=7 Hz, 2H), 2.65 (q, J=6.7 Hz, 2H), 2.82−2.87 (m, 1H), 2.91−2.94 (m, 1H), 3.06 (dd, J = 4.1, 14.2 Hz, 1H), 3.13−3.19 (m, 1H), 3.30−3.43 (m, 3H), 3.62 (br s, 1H), 3.68−3.74 (m, 2H), 3.85−3.88 (m, 4H), 3.92−3.99 (m, 2H), 4.13 (t, J=6.9 Hz, 2H), 4.93−5.05 (m, 2H), 5.07−5.09 (m, 2H), 5.17−5.24 (m, 2H), 5.66 (d, J=5.1 Hz, 1H), 5.69−5.77 (m, 1H), 5.90−5.99 (m, 1H), 6.52 (s, 1H), 6.55 (dd, J = 2.05, 8.8 Hz, 1H), 7.22−7.30 (m, 5H), 7.87 (d, J = 8.8 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 25.8, 25.9, 28.1, 29.8, 33.3, 35.6, 45.4, 50.0, 52.4, 55.2, 55.8, 68.7, 69.7, 70.8, 72.4, 73.4, 100.6, 104.5, 109.4, 114.9, 118.0, 119.5, 126.6, 128.6, 129.5, 133.5, 137.8, 138.3, 155.9, 157.6, 164.9. FT-IR (film, NaCl) ν_{max} = 3339, 2929, 1719,

1596, 1324 cm^{-1}. ESI (+) HRMS (*m*/*z*): $[M + Na]^+$ calcd for $C_{34}H_{46}N_2O_9S$, 681.2822; found, 681.2812.

Compound 13d. Title compound was obtained from **11d** and **12** as described for **13a** in 55% yield after flash-chromatography (1:1 hexanes:EtOAc) as a colorless oil. ^1H NMR (500 MHz, $CDCl_3$) δ 1.24−1.30 (m, 2H), 1.43−1.51 (m, 3H), 1.57−1.63 (m, 1H), 1.96 (q, J = 7 Hz, 2H), 2.80 (dd, J = 10, 14 Hz, 1H), 2.86−2.91 (m, 1H), 3.01 (dd, J = 4.5, 14.5 Hz, 1H), 3.08−3.14 (m, 1H), 3.25−3.30 (m, 2H), 3.40 (dd, J = 8.5, 15 Hz, 1H) 3.58 (br s, 1H), 3.65−3.72 (m, 2H), 3.80−3.82 (m, 2H), 3.87 (s, 3H), 3.88−3.96 (m, 2H), 4.60 (d, J = 5.5 Hz, 2H), 4.90−4.95 (m, 2H), 4.98−5.06 (m, 2H), 5.33 (d, J = 10 Hz, 1H), 5.45 (d, J = 18 Hz, 1H), 5.64 (d, J = 5.5 Hz, 1H), 5.65−5.72 (m, 1H), 6.02−6.10 (m, 1H), 6.47 (d, J = 2 Hz, 1H), 6.53 (dd, J = 2.5, 9 Hz, 1H), 7.17−7.27 (m, 5H), 7.85 (d, J = 9 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 25.7, 27.8, 33.0, 35.3, 45.2, 49.9, 52.4, 55.0, 55.6, 69.5, 69.9, 70.6, 72.2, 73.3, 100.6, 104.5, 109.2, 114.7, 119.4, 126.4, 128.4, 129.3, 131.9, 133.5, 137.6, 138.1, 155.4, 157.0, 164.7. FT-IR (film, NaCl) ν_{max} = 3339, 1718, 1594, 1324 cm^{-1}. ESI (+) HRMS (*m*/*z*): $[M + Na]^+$ calcd for $C_{33}H_{44}N_2O_9S$, 667.2665; found, 667.2661.

Compound 13e. Title compound was obtained from **11e** and **12** as described for **13a** in 68% yield after flash-chromatography (1:1 hexanes:EtOAc) as a colorless oil. ^1H NMR (400 MHz, $CDCl_3$) δ 1.52−1.61 (m, 3H), 1.81−1.93 (m, 2H), 2.09 (q, J = 7 Hz, 2H), 2.76 (dd, J = 10, 13.7 Hz, 1H), 2.83−2.88 (m, 1H), 3.01 (dd, J = 3.6, 14.2 Hz, 1H), 3.28−3.32 (m, 2H), 3.61−3.69 (m, 2H), 3.78−3.85 (m, 6H), 3.87−3.93 (m, 4H), 4.04 (t, J = 6.5 Hz, 2H), 4.92−5.01 (m, 3H), 5.09−5.16 (m, 3H), 5.60−5.71 (m, 2H), 5.72−5.81 (m, 1H), 6.47−6.51 (m, 2H), 7.14−7.26 (m, 5H), 7.82 (d, J = 8.6 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.9, 25.7, 28.2, 33.2, 35.4, 45.3, 51.0, 52.1, 54.9, 55.7, 69.1, 69.5, 70.7, 71.8, 73.1, 100.3, 104.2, 109.2, 115.0, 119.0, 119.2, 126.3, 128.3, 129.3, 133.4, 133.6, 137.5, 138.0, 155.4, 157.6, 164.8. FT-IR (film, NaCl) ν_{max} = 3368, 1720, 1596, 1325 cm^{-1}. ESI (+) HRMS (*m*/*z*): $[M + Na]^+$ calcd for $C_{33}H_{44}N_2O_9S$, 667.2665; found, 667.2668.

Compound 13f. Title compound was obtained from **11f** and **12** as described for **13a** in 64% yield after flash-chromatography (1:1 hexanes:EtOAc) as a colorless oil. ^1H NMR (400 MHz, $CDCl_3$) δ 1.37−1.43 (m, 1H), 1.54−1.62 (m, 1H), 1.91−1.97 (m, 2H), 2.27 (q, J = 7 Hz, 2H), 2.76 (dd, J = 10.1, 13.9 Hz, 1H), 2.84−2.89 (m, 1H), 3.02 (dd, J = 4, 14.1 Hz, 1H), 3.30−3.37 (m, 2H), 3.54 (br s, 1H), 3.62−3.69 (m, 2H), 3.79−3.87 (m, 5H), 3.88−3.95 (m, 4H), 4.06 (t, J = 6.7 Hz, 2H), 4.96−5.00 (m, 2H), 5.04−5.16 (m, 4H), 5.59−5.67 (m, 2H), 5.76−5.85 (m, 1H), 6.47 (d, J = 1.9 Hz, 1H), 6.50 (dd, J = 2.1, 8.9 Hz, 1H), 7.16−7.26 (m, 5H), 7.82 (d, J = 8.8 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.7, 27.9, 29.7, 35.5, 45.3, 51.0, 52.1, 54.9, 55.7, 68.5, 69.5, 70.7, 71.8, 73.2, 100.3, 104.2, 109.2, 115.7, 119.1, 119.3, 126.4, 128.3, 129.3, 133.5, 137.1, 137.7, 155.4, 157.6, 164.8. FT-IR (film, NaCl) ν_{max} = 3350, 1720, 1596, 1325 cm^{-1}. ESI (+) HRMS (*m*/*z*): $[M + Na]^+$ calcd for $C_{32}H_{42}N_2O_9S$, 653.2509; found, 653.2509.

Compound 13g. Title compound was obtained from **11g** and **12** as described for **13a** in 68% yield after flash-chromatography (1:1 hexanes:EtOAc) as a colorless oil. ^1H NMR (400 MHz, $CDCl_3$) δ 1.39−1.45 (m, 1H), 1.56−1.63 (m, 1H), 1.81 (br s, 1H), 2.61 (q, J = 6.6 Hz, 2H), 2.75−2.80 (m, 1H), 2.86−2.91 (m, 1H), 3.02 (dd, J = 4.1, 14.1 Hz, 1H), 3.32 (d, J = 5.8 Hz, 2H), 3.53 (br s, 1H), 3.64−3.70 (m, 2H), 3.81−3.95 (m, 8H), 4.10 (t, J = 6.7 Hz, 2H), 4.97−5.01 (m, 2H), 5.12−5.20 (m, 4H), 5.62−5.66 (m, 2H), 5.86−5.94 (m, 1H), 6.49 (s, 1H), 6.51 (d, J = 9.1 Hz, 1H), 7.18−7.26 (m, 5H), 7.85 (d, J = 8.8 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.8, 33.2, 35.6, 45.4, 51.2, 52.3, 55.0, 55.8, 68.7, 69.6, 70.8, 71.9, 73.3, 100.5, 104.5, 109.3, 117.9, 119.2, 119.4, 126.5, 128.5, 129.4, 133.5, 133.7, 137.8, 155.4, 157.5, 164.9. FT-IR (film, NaCl) ν_{max} = 3350, 1722, 1596, 1325 cm^{-1}. ESI (+) HRMS (*m*/*z*): $[M + H]^+$ calcd for $C_{31}H_{40}N_2O_9S$, 617.2533; found, 617.2540.

Compound 13h. Title compound was obtained from **11h** and **12** as described for **13a** in 52% yield after flash-chromatography (1:1 hexanes:EtOAc) as a colorless oil. ^1H NMR (400 MHz, $CDCl_3$) δ 1.37−1.43 (m, 1H), 1.54−1.65 (m, 1H), 2.73−2.80 (m, 1H), 2.85−2.90 (m, 1H), 3.02 (dd, J = 2.9, 13.7 Hz, 1H), 3.28−3.39 (m, 2H), 3.51 (br s, 1H), 3.63−3.70 (m, 2H), 3.77−3.95 (m, 10H), 4.62 (d, J = 5.2 Hz, 2H), 4.96−5.06 (m, 2H), 5.10−5.16 (m, 2H), 5.33 (d, J = 10.4 Hz, 1H), 5.45 (d, J = 17.2 Hz, 1H), 5.62−5.66 (m, 2H), 6.01−6.10 (m, 1H), 6.49 (s, 1H), 6.52 (d, J = 8.9 Hz, 1H), 7.18−7.33 (m, 5H), 7.85 (d, J = 8.8 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.7, 35.4, 45.3, 51.4, 52.2, 54.9, 55.7, 69.6, 70.0, 70.7, 71.8, 73.1, 100.7, 104.5, 109.2, 119.1, 119.4, 119.5, 126.4, 128.4, 129.3, 131.8, 133.5, 137.7, 155.3, 157.0, 164.8. FT-IR (film, NaCl) ν_{max} = 3351, 1717, 1596, 1324 cm^{-1}. ESI (+) HRMS (*m*/*z*): $[M + H]^+$ calcd for $C_{30}H_{38}N_2O_9S$, 603.2376; found, 603.2375.

Inhibitor 14a. To stirring solution of **13a** (32 mg, 0.047 mmol) in CH_2Cl_2 (15 mL) was added Grubbs' first-generation catalyst (4 mg, 0.0046 mmol). After stirring at 23 °C for 16 h, the solvent was evaporated under reduced pressure and the residue was subjected to flash column chromatography to yield **14a** (27 mg, 88% yield) as a white solid and *E*/*Z* mixture (3:1, determined by HPLC). The isomers were isolated by reverse-phase HPLC using the following conditions: YMC Pack ODS-A column (250 mm × 100 mm, 5 μm); flow rate = 2.75 mL/min; isocratic 60:40 CH_3CN:H_2O; T = 35 °C; λ = 215 nm; *E* isomer R_t = 16.5 min; *Z* isomer R_t = 14.5 min.

Compound 14a*E*. ^1H NMR (800 MHz, $CDCl_3$) δ 1.39−1.44 (m, 2H), 1.48−1.53 (m, 1H), 1.57−1.64 (m, 2H), 1.68−1.74 (m, 3H), 1.77−1.82 (m, 1H), 1.83−1.88 (m, 1H), 1.94−1.98 (m, 1H), 2.10−2,14 (m, 3H), 2.71 (dd, J=9.6, 14.1 Hz, 1H), 2.86−2.89 (m, 1H), 2.90 (dd, J = 4.3, 14.1 Hz, 1H), 2.91−2.99 (m, 2H), 3.31−3.33 (m, 1H), 3.61−3.64 (m, 1H), 3.65−3.70 (m, 2H), 3.73 (br s, 1H), 3.76−3.78 (m, 1H), 3.79−3.85 (m, 5H), 3.93 (dd, J = 6.6, 9.4 Hz, 1H), 3.96−3.98 (m, 1H), 4.02−4.05 (m,1H), 4.87 (d, J = 9.1 Hz, 1H), 4.97−5.00 (m, 1H), 5.44−5.48 (m, 1H), 5.54−5.56 (m, 1H), 5.63 (d, J = 5.1 Hz, 1H), 6.44 (d, J = 2 Hz, 1H), 6.49 (dd, J = 2.2, 8.8 Hz, 1H), 7.13−7.24 (m, 5H), 7.84 (d, J = 8.8 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 25.1, 25.3, 25.7, 26.8, 29.8, 30.4, 32.5, 35.5, 45.2, 50.6, 51.6, 54.7, 55.6, 68.8, 69.5, 70.7, 71.6, 73.3, 99.9, 103.9, 109.2, 118.1, 126.4, 128.4, 129.3, 130.5, 132.1, 134.0, 137.4, 155.2, 157.9, 164.9. ESI (+) HRMS (*m*/*z*): $[M+Na]^+$ calcd for $C_{34}H_{46}N_2O_9S$, 681.2822; found, 681.2815.

Compound 14a*Z*. ^1H NMR (800 MHz, $CDCl_3$) δ 1.28−1.33 (m, 2H), 1.38−1.47 (m, 2H), 1.52−1.64 (m, 5H), 1.84−1.90 (m, 2H), 2.04−2.08 (m, 2H), 2.16−2.22 (m, 2H), 2.74 (dd, J = 9.5, 14.1 Hz, 1H), 2.88−2.90 (m, 1H), 3.00 (dd, J=4.5, 14.1 Hz, 1H), 3.05−3.14 (m, 2H), 3.15 (dd, J=9.4, 15.1 Hz, 1H), 3.44−3.48 (m, 1H), 3.61 (br s, 1H), 3.66−3.71 (m, 2H), 3.76−3.89 (m, 6H), 3.95 (dd, J = 6.2, 9.6 Hz, 1H), 4.10−4.15 (m, 1H), 4.87 (d, J =9.1 Hz, 1H), 5.00−5.03 (m, 1H), 5.35−5.39 (m, 1H), 5.49−5.52 (m, 1H), 5.63 (d, J = 5.2 Hz, 1H), 6.50 (s, 1H), 6.49 (dd, J = 2.3, 8.8 Hz, 1H), 7.17−7.26 (m, 5H), 7.84 (d, J=8.8 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 25.0, 25.1, 25.7, 26.0, 26.6, 27.8, 29.6, 35.5, 45.2, 48.5, 52.0, 54.7, 55.6, 68.8, 69.5, 70.6, 71.9, 73.3, 100.7, 104.2, 109.2, 117.8, 126.4, 128.4, 129.2, 129.8, 130.0, 134.1, 137.5, 155.2, 158.0, 165.0. ESI (+) HRMS (*m*/*z*): $[M + Na]^+$ calcd for $C_{34}H_{46}N_2O_9S$, 681.2822; found, 681.2819.

Inhibitor 14b. The title compound was obtained from a ring closing metathesis reaction of **13b** using Grubbs' first-generation catalyst as described for **14a**. The crude material was purified by silica gel chromatography (60:40 EtOAc:hexanes) to give the desired product (50% yield) as a mix of *E*/*Z* isomers (27:73 by HPLC). The isomers were isolated by chiral HPLC using the following conditions: Chiralpak IA column (250 mm × 4.6 mm, 5 μm); flow rate = 0.75 mL/min; isocratic 60:40 IPA: hexanes; T=25 °C; λ=215 nm; *E* isomer R_t=7.56 min; *Z*-isomer R_t=8.89 min.

Compound 14b*E*. ^1H NMR (800 MHz, $CDCl_3$) δ 1.60−1.20 (m, 4H), 2.30−1.90 (m, 7H), 3.10−2.80 (m, 5H), 3.32 (m, 1H),

4.00−3.50 (m, 12 H), 4.15 (m, 2H), 4.98 (m, 2H), 5.45 (m, 1H), 5.63 (m, 2H), 6.51 (m, 2H), 7.25−7.15 (m, 5H), 7.76 (d, J = 19.2 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 23.1, 24.8, 25.0, 25.7, 26.2, 26.6, 29.0, 35.5, 45.2, 48.2, 52.0, 54.8, 55.6, 67.1, 69.5, 70.7, 71.6, 73.3, 100.1, 104.0, 109.2, 114.6, 118.4, 126.4, 128.4, 128.9, 129.3, 130.6, 133.8, 137.4, 155.3, 157.7, 164.9.; ESI (+) HRMS (m/z): $[M + Na]^+$ calcd for $C_{33}H_{44}N_2O_9S$, 667.2665; found, 667.2660.

Compound 14b*Z*. ^{1}H NMR (800 MHz, $CDCl_3$) δ 1.60−1.0 (m, 7H), 1.72 (m, 2H), 1.90 (m, 3H), 2.21 (m, 2H), 2.43 (m, 1H), 2.55 (m, 1H), 2.67 (m, 1H), 2.76 (m, 1H), 2.84 (m, 1H), 3.01 (m, 1H), 3.26 (m, 1H), 3.41 (m, 3H), 3.60 (m, 4H), 3.69 (m, 1H), 3.86 (m, 2H), 4.43 (d, J = 15.2 Hz, 1H), 4.83 (m, 1H), 5.24 (m, 1H), 5.33 (m, 1H), 5.51 (d, J=8.8 Hz, 1H), 6.45 (m, 2H), 7.17 (m, 3H), 7.24 (m, 2H), 7.87 (d, J=13.6 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 24.7, 25.7, 27.3, 28.6, 30.9, 32.1, 35.5, 45.2, 49.3, 51.8, 54.8, 55.6, 69.5, 70.7, 71.0, 71.9, 73.3, 101.2, 104.3, 109.2, 119.3, 126.4, 128.4, 128.9, 129.3, 130.8, 130.9, 133.4, 137.5, 155.3, 158.2, 164.5. ESI (+) HRMS (m/z): $[M+Na]^+$ calcd for $C_{33}H_{44}N_2O_9S$, 667.2665; found, 667.2667.

Inhibitor 14c. Title compound was obtained from **13c** and Grubbs' first-generation catalyst as described for **14a** in 89% yield after flash-chromatography (2:3 hexanes:EtOAc) as a white solid and E/Z mixture (3:1, determined by HPLC). The isomers were isolated using reversed-phase HPLC under the following conditions: YMC Pack ODS-A column (250 mm × 100 mm, 5 μm); flow rate = 2.75 mL/min; isocratic 60:40 CH_3CN:H_2O; T = 35 °C; λ = 215 nm; E isomer R_t = 13.43 min; Z isomer R_t = 11.76 min.

Compound 14c*E*. ^{1}H NMR (800 MHz, $CDCl_3$) δ 1.45−1.54 (m, 3H), 1.56−1.64 (m, 2H), 1.68−1.72 (m, 1H), 2.11−2.20 (m, 2H), 2.52−2.62 (m, 2H), 2.79 (dd, J=9.6, 14 Hz, 1H), 2.87−2.90 (m, 1H), 2.96−3.00 (m, 2H), 3.04 (dd, J = 4.2, 14.2 Hz, 1H), 3.40−3.44 (m, 1H), 3.53−3.56 (m, 1H), 3.66−3.70 (m, 3H), 3.82−3.89 (m, 6H), 3.94 (dd, J = 6.3, 9.5 Hz, 1H), 4.07−4.14 (m, 2H), 4.96 (d, J = 9.4 Hz, 1H), 4.98−5.01 (m, 1H), 5.53−5.56 (m, 1H), 5.64−5.68 (m, 2H), 6.52−6.53 (m, 2H), 7.18−7.27 (m, 5H), 7.76 (d, J = 9.4 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 24.4, 25.7, 28.3, 32.3, 32.4, 35.6, 45.2, 49.6, 51.9, 54.9, 55.6, 69.5, 69.9, 70.7, 71.9, 73.2, 101.9, 104.9, 109.2, 119.3, 126.4, 128.0, 128.4, 129.3, 133.4, 133.9, 137.6, 155.3, 158.0, 164.5. ESI (+) HRMS (m/z): $[M + H]^+$ calcd for $C_{32}H_{42}N_2O_9S$, 631.2689; found, 631.2698.

Compound 14c*Z*. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.48−1.52 (m, 3H), 1.57−1.72 (m, 3H), 2.10−2.14 (m, 1H), 2.28−2.32 (m, 1H), 2.47−2.51 (m, 1H), 2.78 (dd, J = 9, 14 Hz, 2H), 2.87−2.91 (m, 1H), 2.98−3.08 (m, 3H), 3.45−3.60 (m, 2H), 3.65−3.71 (m, 3H), 3.80−3.90 (m, 6H), 3.95 (dd, J = 6, 9.5 Hz, 1H), 4.07−4.10 (m, 1H), 4.18−4.21 (m, 1H), 4.95 (d, J = 9.5 Hz, 1H), 4.98−5.02 (m, 1H), 5.44−5.49 (m, 2H), 5.63 (d, J=5.5 Hz, 1H), 6.51 (dd, J = 2.5, 9.8 Hz, 1H), 6.53 (d, J = 2 Hz, 1H), 7.18−7.27 (m, 5H), 7.81 (d, J=9 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 24.7, 24.8, 25.7, 26.3, 27.7, 35.5, 45.2, 46.9, 50.2, 54.9, 55.6, 69.5, 69.9, 70.6, 70.7, 73.2, 101.9, 104.8, 109.1, 120.5, 126.4, 127.6, 128.4, 129.3, 129.6, 132.5, 133.2, 137.6, 155.3, 158.1, 164.6. ESI (+) HRMS (m/z): $[M + H]^+$ calcd for $C_{32}H_{42}N_2O_9S$, 631.2689; found, 631.2706.

Inhibitor 14d. Title compound was obtained from **13d** and Grubbs' first-generation catalyst as described for **14a** in 71% yield after flash-chromatography (2:3 hexanes:EtOAc) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.40−1.47 (m, 1H), 1.58−1.62 (m, 2H), 1.92−1.95 (m, 1H), 2.11−2.15 (m, 1H), 2.28−2.39 (m, 2H), 2.73−2.78 (m, 1H), 2.80−3.05 (m, 6H), 3.64−3.70 (m, 3H), 3.80−3.89 (m, 6H), 3.93−3.96 (m, 2H), 4.12−4.15 (m, 1H), 4.62 (br s, 1H), 4.97−4.99 (m, 1H), 5.11 (d, J = 9.2 Hz, 1H), 5.52−5.54 (m, 2H), 5.61 (d, J = 5.1 Hz, 1H), 6.46−6.49 (m, 2H), 7.18−7.26 (m, 5H), 7.80 (d, J = 9.4 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 22.6, 22.9, 23.4, 25.7, 25.9, 29.6, 35.4, 45.1, 45.2, 47.9, 55.0, 55.6, 62.0, 69.5, 70.7, 73.2, 100.2, 103.6, 109.2, 119.9, 123.5, 126.4, 128.4, 129.3, 132.6, 137.6, 140.4, 155.4, 156.4, 164.3. ESI (+) HRMS (m/z): $[M + H]^+$ calcd for $C_{31}H_{40}N_2O_9S$, 617.2533; found, 617.2534.

Inhibitor 14e. Title compound was obtained from **13e** and Grubbs' second-generation catalyst as described for **14a** in 52% yield after flash-chromatography (2:3 hexanes:EtOAc) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.46−1.51 (m, 1H), 1.60−1.76 (m, 4H), 1.88−1.92 (m, 2H), 2.23−2.37 (m, 2H), 2.79 (dd, J = 9, 14 Hz, 1H), 2.88−3.01 (m, 2H), 3.10−3.13 (m, 2H), 3.57 (br s, 1H), 3.66−3.72 (m, 2H), 3.72−3.89 (m, 5H), 3.92−3.99 (m, 2H), 4.07−4.15 (m, 2H), 4.94 (d, J = 8.5 Hz, 1H), 5.00−5.04 (m, 1H), 5.44−5.54 (m, 3H), 5.64 (d, J = 5.1 Hz, 1H), 6.43 (d, J = 2 Hz, 1H), 6.49−6.51 (m, 1H), 7.16−7.28 (m, 5H), 7.81 (d, J=9 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 25.7, 26.3, 26.5, 27.4, 29.6, 35.5, 43.9, 45.2, 50.7, 54.7, 55.6, 69.5, 69.7, 70.7, 73.3, 100.0, 103.9, 109.2, 117.7, 122.9, 126.4, 128.4, 129.3, 133.7, 135.1, 137.4, 155.3, 157.7, 164.9. ESI (+) HRMS (m/z): $[M + Na]^+$ calcd for $C_{31}H_{40}N_2O_9S$, 639.2352; found, 639.2345.

Inhibitor 14f. Title compound was obtained from **13f** and Grubbs' second-generation catalyst as described for **14a** in 81% yield after flash-chromatography (2:3 hexanes:EtOAc) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.40−1.47 (m, 1H), 1.58−1.62 (m, 2H), 1.92−1.95 (m, 1H), 2.11−2.15 (m, 1H), 2.28−2.39 (m, 2H), 2.73−2.78 (m, 1H), 2.80−3.05 (m, 5H), 3.64−3.70 (m, 2H), 3.80−3.89 (m, 6H), 3.93−3.96 (m, 2H), 4.12−4.15 (m, 1H), 4.62 (br s, 1H), 4.97−4.99 (m, 1H), 5.11 (d, J = 9.2 Hz, 1H), 5.52−5.54 (m, 2H), 5.61 (d, J = 5.1 Hz, 1H), 6.46−6.49 (m, 2H), 7.18−7.26 (m, 5H), 7.80 (d, J = 9.4 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 22.7, 25.1, 25.7, 29.6, 35.5, 41.2, 45.3, 47.8, 54.8, 55.6, 65.9, 69.5, 70.7, 73.2, 101.0, 104.3, 109.2, 118.7, 123.3, 126.4, 128.4, 129.3, 133.4, 133.6, 137.5, 155.4, 157.8, 164.7. ESI (+) HRMS (m/z): $[M + H]^+$ calcd for $C_{30}H_{38}N_2O_9S$, 603.2376; found, 603.2369.

Inhibitor 14g. Title compound was obtained from **13g** and Grubbs' second-generation catalyst as described for **14a** in 81% yield after flash-chromatography (2:3 hexanes:EtOAc) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.47−1.55 (m, 1H), 1.64−1.70 (m, 2H), 2.51−2.54 (m, 1H), 2.73−2.78 (m, 1H), 2.84−3.94 (m, 2H), 3.04−3.11 (m, 2H), 3.19 (d, J=13.6 Hz, 1H), 3.70−3.75 (m, 2H), 3.86 (s, 4H), 3.94−3.99 (m, 4H), 4.07−4.08 (m, 1H), 4.18 (br s, 1H), 4.58 (s, 1H), 5.02−5.06 (m, 1H), 5.12 (s, 1H), 5.61 (d, J = 5 Hz, 1H), 5.76−5.82 (m, 1H), 5.88−5.93 (m, 1H), 6.35 (d, J=1.1 Hz, 1H), 6.51 (dd, J = 1.5, 8.7 Hz, 1H), 7.20−7.31 (m, 5H), 7.79 (d, J = 8.8 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 25.8, 26.7, 35.4, 43.6, 45.3, 47.1, 55.1, 55.7, 67.4, 69.6, 70.5, 70.8, 73.3, 99.4, 104.1, 109.2, 120.5, 126.5, 126.9, 128.5, 129.4, 131.2, 132.0, 137.6, 155.6, 157.4, 164.7. ESI (+) HRMS (m/z): $[M + Na]^+$ calcd for $C_{29}H_{36}N_2O_9S$, 611.2039; found, 611.2040.

Inhibitor 14h. Title compound was obtained from **13h** and Grubbs' second-generation catalyst as described for **14a** in 79% yield after flash-chromatography (2:3 hexanes:EtOAc) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.45−1.51 (m, 1H), 1.60−1.68 (m, 2H), 2.80−2.90 (m, 2H), 3.00−3.12 (m, 3H), 3.53 (br s, 1H), 3.66−3.71 (m, 2H), 3.83 (s, 4H), 3.92−3.95 (m, 3H), 4.25 (br s, 1H), 4.88−5.01 (m, 3H), 5.13 (d, J = 8.3 Hz, 1H), 5.63 (d, J = 5.1 Hz, 1H), 5.72−5.76 (m, 2H), 6.59−6.64 (m, 2H), 7.19−7.26 (m, 5H), 7.74 (d, J=8.7 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 25.7, 29.6, 35.3, 43.9, 45.2, 47.8, 55.1, 55.7, 69.5, 70.7, 71.1, 73.3, 104.5, 107.2, 109.2, 123.4, 126.1, 126.4, 128.4, 129.3, 131.3, 131.5, 137.6, 155.6, 157.7, 164.5. ESI (+) HRMS (m/z): $[M + Na]^+$ calcd for $C_{28}H_{34}N_2O_9S$, 597.1883; found, 597.1887.

Inhibitor 15a. To a stirring solution of **14a** (10 mg, 0.015 mmol) in EtOAc (2 mL) was added 10% Pd on carbon and the reaction was stirred under H_2 atmosphere for 12 h. After this time, the reaction was filtered through a pad of celite and solvent was evaporated under reduced pressure. The residue was then purified by flash-chromatography to give **15a** (9 mg, 93% yield) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.87−0.92 (m, 2H), 1.25−1.28 (m, 8H), 1.38−1.49 (m, 5H), 1.60−1.64 (m, 3H),

1.71−1.83 (m, 4H), 2.32−2.38 (m, 1H), 2.75 (dd, J = 9.5, 13.7 Hz, 1H), 2.86−2.91 (m, 1H), 3.01−3.10 (m, 3H), 3.64−3.71 (m, 2H), 3.85 (s, 4H), 3.94 (dd, J = 6.4, 9.5 Hz, 1H), 4.01−4.04 (m, 1H), 4.10−4.16 (m,1H), 4.94 (d, J = 9.2 Hz, 1H), 5.01−5.15 (m, 1H), 5.67 (d, J = 5.1 Hz, 1H), 6.52−6.54 (m, 2H), 7.19−7.29 (m, 5H), 7.85 (d, J = 9 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 23.8, 24.2, 25.5, 26.3, 26.4, 28.2, 29.1, 29.4, 35.3, 45.1, 51.3, 52.8, 54.6, 55.4, 69.2, 69.3, 69.5, 70.5, 71.9, 73.1, 100.2, 103.9, 109.0, 118.7, 126.3, 128.2, 129.1, 133.5, 137.3, 155.1, 157.9, 164.6. ESI (+) HRMS (m/z): $[M + Na]^+$ calcd for $C_{34}H_{48}N_2O_9S$, 683.2978; found, 683.2984.

Inhibitor 15b. Title compound was obtained from **14b** as described for **15a** in 90% yield after flash-chromatography (2:3 hexanes:EtOAc) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.30−1.47 (m, 8H), 1.57−1.70 (m, 5H), 1.72−1.79 (m, 1H), 1.82−1.87 (m, 2), 2.77 (dd, J = 10, 14 Hz, 1H), 2.86−2.91 (m, 1H),3.00 (dd, J = 4.5, 14 Hz, 1H), 3.05 (d, J = 2.5, 15 Hz, 1H), 3.14−3.23 (m, 2H), 3.56−3.62 (m, 2H), 3.65−3.72 (m, 2H), 3.81−3.90 (m, 6H), 3.94 (dd, J = 6.5, 10 Hz, 1H), 4.05−4.15 (m, 2H), 4.96 (d, J = 9.5 Hz, 1H), 4.99−5.02 (m, 1H), 5.64 (d, J = 5.5 Hz, 1H), 6.49−6.52 (m, 2H), 7.17−7.27 (m, 5H), 7.82 (d, J = 9.5 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 23.2, 24.2, 24.9, 25.2, 25.7, 26.1, 27.6, 28.9, 35.4, 45.2, 49.2, 52.7, 54.8, 55.6, 68.9, 69.5, 70.7, 71.7, 73.2, 100.5, 104.2, 109.2, 118.5, 126.4, 128.4, 129.3, 133.8, 137.5, 155.3, 158.1, 164.8. ESI (+) HRMS (m/z): $[M + Na]^+$ calcd for $C_{33}H_{46}N_2O_9S$, 669.2822; found, 669.2828.

Inhibitor 15c. Title compound was obtained from **14c** as described for **15a** in 90% yield after flash-chromatography (2:3 hexanes:EtOAc) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.35−1.39 (m, 3H), 1.40−1.54 (m, 4H), 1.60−1.66 (m, 6H), 1.85−1.98 (m, 2H), 2.78 (dd, J=9.1, 14 Hz, 1H), 2.88−2.91 (m, 1H),2.97−3.03 (m, 1H), 3.06−3.14 (m, 1H), 3.39−3.43 (m, 1H), 3.50−3.56 (m, 1H), 3.66−3.72 (m, 3H), 3.82−3.85 (m, 6H), 3.95 (dd, J = 6.3, 9.6 Hz, 1H), 4.07−4.10 (m, 2H), 4.95−5.03 (m, 2H), 5.64 (d, J = 5 Hz, 1H), 6.50−6.53 (m, 2H), 7.17−7.27 (m, 5H), 7.82 (d, J=9 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 22.6, 23.5, 24.0, 24.6, 25.3, 25.7, 26.1, 35.5, 44.8, 45.2, 46.5, 51.0, 54.8, 55.6, 69.5, 70.1, 70.7, 70.8, 73.2, 100.9, 104.3, 109.2, 118.5, 126.4, 128.4, 129.4, 133.9, 137.5, 155.2, 158.2, 164.8. ESI (+) HRMS (m/z): $[M + Na]^+$ calcd for $C_{32}H_{44}N_2O_9S$, 655.2668; found, 655.2667.

Inhibitor 15d. Title compound was obtained from **14d** or **14e** as described for **15a** in 94% yield after flash-chromatography (2:3 hexanes:EtOAc) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.33−1.54 (m, 6H), 1.57−1.74 (m, 3H), 1.86−1.94 (m, 2H), 2.77 (dd, J=9.5, 14 Hz, 1H), 2.86−2.90 (m, 1H), 2.96−3.08 (m, 3H), 3.24−3.29 (m, 1H), 3.64−3.72 (m, 2H), 3.74−3.86 (m, 7H), 3.94−4.00 (m, 2H), 4.15−4.22 (m, 2H), 4.92 (d, J = 9.2 Hz, 1H), 4.97−5.02 (m, 1H), 5.62 (d, J = 5.2 Hz, 1H), 6.48 (d, J=2.1 Hz, 1H), 6.52 (dd, J=2, 8.9 Hz, 1H), 7.17−7.27 (m, 5H), 7.87 (d, J = 8.6 Hz, 1H) ^{13}C NMR (125 MHz, $CDCl_3$) δ 23.2, 25.0, 25.6, 26.1, 26.3, 35.4, 42.9, 45.2, 48.5, 54.7, 55.6, 69.3, 69.5, 70.7, 73.3, 99.9, 103.8, 109.1, 117.1, 126.4, 128.4, 129.3, 134.6, 137.4, 155.1, 157.7, 165.2. ESI (+) HRMS (m/z): $[M + Na]^+$ calcd for $C_{31}H_{42}N_2O_9S$, 641.2509; found, 641.2512.

Inhibitor 15f. Title compound was obtained from **14f** as described for **15a** in 93% yield after flash-chromatography (2:3 hexanes:EtOAc) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.33−1.48 (m, 4H), 1.56−1.66 (m, 3H), 1.67−1.71 (m, 2H), 1.83−1.86 (m, 1H), 2.04−2.12 (m, 2H), 2.74 (dd, J = 9.7, 14 Hz, 1H), 2.83−2.99 (m, 4H), 3.18 (br s, 1H), 3.64−3.71 (m, 2H), 3.76−3.85 (m, 6H), 3.89−3.99 (m, 3H), 4.08 (br s, 1H), 4.19−4.23 (m, 1H), 4.96−5.00 (m, 2H), 5.63 (d, J=5 Hz, 1H), 6.45 (d, J=2 Hz, 1H), 6.50 (dd, J=2.5, 9 Hz, 1H), 7.15−7.26 (m, 5H), 7.88 (d, J=9 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 22.2, 24.4, 24.6, 25.7, 25.8, 35.4, 45.2, 46.8, 51.8, 54.7, 55.7, 69.2, 69.6, 70.5, 70.8, 73.3, 99.8, 103.9, 109.2, 117.5, 126.5, 128.4, 129.3, 134.3, 137.4, 155.2, 157.8, 165.3. ESI (+) HRMS (m/z): $[M + Na]^+$ calcd for $C_{30}H_{40}N_2O_9S$, 605.2533; found, 605.2526.

Inhibitor 15g. Title compound was obtained from **14g** as described for **15a** in 90% yield after flash-chromatography (2:3 hexanes:EtOAc) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.46−1.50 (m, 3H), 1.59−1.67 (m, 4H), 1.96−2.04 (m, 2H), 2.81−2.91 (m, 2H), 3.03 (dd, J = 3, 14 Hz, 1H), 3.07−3.12 (m, 2H), 3.56 (br s, 1H), 3.57−3.71 (m, 2H), 3.83−3.91 (m, 6H), 3.93−4.02 (m, 3H), 4.25 (br s, 1H), 4.98−5.02 (m, 2H), 5.63 (d, J = 5 Hz, 1H), 6.40 (d, J = 2 Hz, 1H), 6.49 (dd, J = 2, 9 Hz, 1H), 7.18−7.28 (m, 5H), 7.76 (d, J = 9 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 22.4, 24.4, 25.3, 25.7, 35.4, 43.1, 45.2, 47.3, 54.9, 55.6, 69.5, 70.1, 70.6, 70.7, 73.2, 100.5, 104.2, 109.2, 121.4, 126.4, 128.4, 129.3, 131.5, 137.5, 155.4, 157.7, 164.5. ESI (+) HRMS (m/z): $[M + H]^+$ calcd for $C_{29}H_{38}N_2O_9S$, 591.2376; found, 591.2381.

Inhibitor 15h. Title compound was obtained from **14h** as described for **15a** in 92% yield after flash-chromatography (2:3 hexanes:EtOAc) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.39−1.51 (m, 3H), 1.60−1.68 (m, 3H), 1.72−1.81 (m, 2H), 2.82−2.92 (m, 2H), 3.00−3.06 (m, 3H), 3.66−3.71 (m, 3H), 3.80−3.87 (m, 6H), 3.95 (dd, J=6, 9.5 Hz, 1H), 3.97−4.02 (m, 1H), 4.25 (br s, 1H), 4.98−5.02 (m, 2H), 5.63 (d, J = 5 Hz, 1H), 6.62−6.64 (m, 2 H), 7.18−7.28 (m, 5H), 7.77 (d, J=10.5 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 24.3, 25.0, 25.7, 35.3, 44.5, 45.2, 46.5, 55.0, 55.6, 69.5, 70.6, 73.2, 73.9, 105.6, 107.3, 109.1, 126.4, 128.4, 129.3, 130.9, 137.3, 155.5, 157.4, 164.4. ESI (+) HRMS (m/z): $[M + H]^+$ calcd for $C_{28}H_{36}N_2O_9S$, 577.2220; found, 577.2222.

***tert*-Butyl-(2*S*,3*R*)-4-(2,2-dimethylpent-4-enylamino)-3-hydroxy-1-phenylbutan-2-ylcarbamate (17a).** A stirring solution of amine **16a** (1.58 g, 4.33 mmol) and epoxide **8** (304 mg, 1.15 mmol) was heated to 60 °C for 4 h and allowed to stir at 23 °C overnight. The reaction mixture was concentrated and purified by silica chromatography (3:97 MeOH:CH_2Cl_2) to give 370 mg (98% yield) of product as a clear oil that solidified upon refrigeration. $[\alpha]_D^{20}$ +0.9 (c 0.14, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.90 (s, 6H), 1.36 (s, 9H), 2.01 (d, J = 7.6 Hz, 2H), 2.35 (s, 2H), 2.69 (d, J = 4.8 Hz, 2H), 2.87 (dd, J = 8, 14.8 Hz, 1H), 2.98 (dd, J = 4.8, 9.2 Hz, 1H), 3.45 (m, 1H), 3.82 (m, 1H), 4.79 (d, J = 9.2 Hz, 1H), 5.03 (m, 2H), 5.81 (m, 1H), 7.26 (m, 5H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 25.4, 25.4, 29.6, 34.3, 36.8, 44.5, 52.1, 54.3, 60.1, 70.3, 79.1, 116.9, 126.2, 128.3, 129.4, 135.2, 137.9, 155.8. FTIR (NaCl) ν_{max} = 3349, 3064, 2928, 1693, 1391, 1366, cm^{-1}. ESI LRMS (m/z): 376.06 $[M + H]^+$.

***tert*-Butyl-(2*S*,3*R*)-4-(*N*-(2,2-dimethylpent-4-enyl)-2-(hex-5-enyloxy)-4-methoxyphenylsulfonamido)-3-hydroxy-1-phenylbutan-2-ylcarbamate (18a).** To a mixture of amine **17a** (188 mg, 0.5 mmol) and sulfonyl chloride **7d** (183 mg, 0.6 mmol) at 0 °C under argon atmosphere was added pyridine (8 mL, freshly distilled over KOH), and the reaction was allowed to warm to 23 °C while stirring. The reaction turned orange and was allowed to stir overnight. The reaction was condensed under reduced pressure, washed with saturated $CuSO_4$, and the product extracted with dichloromethane. The organic layer was washed with H_2O, brine, dried over sodium sulfate, and purified by silica chromatography (20:80 EtOAc:hexanes) to give **18a** (180 mg, 56% yield) as a clear oil. $[\alpha]_D^{20}$ +18.3 (c 0.12, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.90 (s, 3H), 0.92 (s, 3H), 1.25 (m, 1H), 1.31 (s, 9H), 1.59 (m, 2H), 1.86 (quintet, J = 8 Hz, 2H), 1.99 (d, J = 7.2 Hz, 2H), 2.12 (q, J = 6.8 Hz, 2H), 2.77 (dd, J=8, 13.2 Hz, 1H), 2.86 (dd, J = 4.8, 14.4 Hz, 1H), 3.20 (m, 4H), 3.66 (m, 1H), 3.80 (m, 1H), 3.83 (s, 3H), 4.00 (m, 2H), 4.40 (d, J = 8.8 Hz, 1H), 5.00 (m, 4H), 5.79 (m, 2H), 6.47 (m, 2H), 7.20 (m, 5H),7.78 (d, J = 8.4 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 25.0, 25.5, 25.6, 28.0, 28,2, 28.4, 33.2, 35.8, 45.2, 54.2, 55.5, 55.6, 62.4, 69.1, 72.0, 79.3, 100.1, 104.2, 115.1, 117.5, 118.5, 126.2, 128.3, 129.4, 134.0, 134.7, 137.6, 138.0, 155.5, 157.5, 164.8. FTIR (NaCl) ν_{max}=3412, 2852, 1701, 1596, 1496, 1456, 1391, 1367 cm^{-1}. ESI (+) HRMS (m/z): $[M + Na^+]$ calcd for $C_{35}H_{52}N_2O_7S$ 667.3393; found, 667.3399.

(3*R*,3a*S*,6a*R*)-Hexahydrofuro[2,3-*b*]furan-3-yl (2*S*,3*R*)-4-(*N*-(2,2-Dimethylpent-4-enyl)-2-(hex-5-enyloxy)-4-methoxyphenylsulfonamido)-3-hydroxy-1-phenylbutan-2-ylcarbamate (19a). To a stirring

solution of **18a** (180 mg, 0.28 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added trifluoroacetic acid (1.5 mL). The reaction was allowed to warm to 23 °C and stirred overnight. Solvents were removed under reduced pressure, and saturated aqueous $NaHCO_3$ and 1N NaOH (1 mL) was added and extracted with ether. Solvents were removed under reduced pressure to afford the crude amine product.

To a solution of above amine (0.28 mmol) in MeCN (20 mL) was added carbonate **12** (92 mg, 0.31 mmol) under argon followed by dropwise addition of *i*-Pr_2NEt (1 mL) and pyridine (1 mL), and the reaction was allowed to stir overnight at 23 °C. After stirring for 2 days, the solvent was removed under reduced pressure and the crude material purified by silica gel column chromatography (50:50 EtOAc:hexanes) to give **19a** (144 mg, 73% yield over two steps) as a white solid; mp = 49−52 °C; $[\alpha]_D^{20}$ +3.9 (*c* 1.03, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.90 (s, 3H), 0.91 (s, 3H), 1.35 (m, 1H), 1.58 (m, 3H), 1.86 (m, 2H), 2.02 (m, 3H), 2.11 (m, 2H), 2.69 (dd, J = 9.6, 14 Hz, 1H), 2.84 (m, 1H), 2.94 (dd, J = 4, 14.4 Hz, 1H), 3.08 (m, 2H), 3.29 (m, 2H), 3.63 (m, 2H), 3.78 (m, 2H), 3.83 (s, 3H), 3.91 (m, 3H), 4.03 (m, 2H), 4.87 (d, J = 9.6 Hz, 1H), 5.02 (m, 4H), 5.60 (d, J = 5.2 Hz, 1H), 5.77 (m, 2H), 6.48 (m, 2H), 7.18 (m, 5H), 7.79 (d, J = 8.4 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 24.9, 25.4, 25.5, 25.7, 28.3, 33.2,35.5, 35.7, 45.1, 45.2, 54.8, 55.6, 55.6, 62.6, 69.2, 69.5, 70.6, 72.3, 73.1, 100.1, 104.3, 109.2, 115.0, 117.6, 118.0, 126.3, 128.3, 129.2, 134.0, 134.5, 137.5, 137.9, 155.0, 157.6, 164.9. FTIR (NaCl) ν_{max} = 1323, 1595, 1722, 2922, 3487 cm^{-1}. ESI (+) HRMS (*m*/*z*): $[M + H]^+$ calcd for $C_{37}H_{52}N_2O_9S$ 701.3472; found, 701.3473.

Inhibitors 20a and 21a. Compound **19a** (100 mg, 0.14 mmol) was dissolved in CH_2Cl_2 (90 mL). Grubbs' second-generation catalyst (12 mg, 0.014 mmol) was added, and the reaction was allowed to stir overnight at 23 °C. Solvent was removed under reduced pressure and the material purified by silica gel column chromatography (50:50 → 75:25 EtOAc:hexanes) to give 92 mg (96% yield) of product as a mixture of stereoisomers (31:69 *Z*/*E* by HPLC) as a white solid. The individual stereoisomers were isolated by reversed-phase HPLC YMC-Pack ODSA (250 mm × 10 mm, 5 μm); flow rate = 1.5 mL/min; isocratic 80:20 MeOH:H_2O; T = 25 °C; λ = 210 nm, R_t *Z* = 17 min, R_t *E* = 18 min).

Compound 21a. $[\alpha]_D^{20}$ −0.8 (*c* 2.36, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 800 MHz) δ 1.05 (s, 3H), 1.12 (s, 3H), 1.24 (t, J = 6.4 Hz, 1H), 1.25 (s, 1H), 1.39 (m, 1H), 1.58 (m, 1H), 1.90−1.72 (m, 4H), 1.94−2.10 (m, 4H), 2.68 (dd, J = 9.6, 14.4 Hz, 1H), 2.86 (q, J = 7.2 Hz, 1H), 2.92 (dd, J = 4, 14.4 Hz, 1H), 2.97 (dd, J = 2.4, 15.2 Hz, 1H), 3.11 (dd, J = 9.6, 15.2 Hz, 1H), 3.64 (m, 1H), 3.68 (m, 1H), 3.71 (dd, J = 6.4, 13.6 Hz, 1H), 3.77 (m, 1H), 3.82 (m, 1H), 3.84 (s, 3H), 4.04−3.88 (m, 4H), 4.71 (d, J = 9.6 Hz, 1H), 4.98 (q, J = 7.2 Hz, 1H), 5.60 (d, J = 5.6 Hz, 1H), 5.62 (d, J = 5.6 Hz, 1H), 5.68 (m, 1H), 6.47 (m, 1H), 6.50 (dd, J = 1.6, 8.8 Hz, 1H), 7.12 (d, J = 7.2 Hz, 2H), 7.18 (t, J = 7.2 Hz, 1H), 7.24 (t, J = 8 Hz, 2H), 7.79 (d, J = 8.8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 25.7, 26.1, 27.6, 29.2, 29.6, 29.7, 35.6, 35.8, 45.3, 46.1, 54.7, 55.7, 56.1, 63.0, 69.6, 69.7, 70.7, 72.2, 73.3, 100.6, 104.4, 109.3, 117.8, 126.5, 128.4, 128.5, 129.3, 133.6, 134.5, 137.5, 155.1, 158.1, 164.9. FTIR (film, NaCl) ν_{max} = 3445, 2926, 1720, 1596, 1575, 1469, 1369 cm^{-1}. ESI (+) HRMS (*m*/*z*): $[M + Na]^+$ calcd for $C_{35}H_{48}N_2O_9S$ 695.2978; found, 695.2989.

Compound 20a. $[\alpha]_D^{20}$ −0.5 (*c* 0.99, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 800 MHz) δ 1.07 (s, 3H), 1.13 (s, 3H), 1.25 (s, 3H), 1.38 (m, 1H), 1.57 (m, 1H), 1.62−1.76 (m, 2H), 1.90 (br s, 2H), 1.98−2.20 (m, 4H), 2.70 (dd, J = 9.6, 14.4 Hz, 1H), 2.86 (m, 1H), 2.91 (dd, J = 0.8, 15.2 Hz, 1H), 2.93 (dd, J = 4, 14.4 Hz, 1H), 3.08 (dd, J = 8.8, 15.2 Hz, 1H), 3.65 (m, 1H), 3.68 (dd, J = 6.4, 9.6 Hz, 1H), 3.79 (m, 1H), 3.82 (dt, J = 2.4, 8.8 Hz, 1H), 3.85 (s, 3H), 3.93 (dd, J = 6.4, 9.6 Hz, 1H), 3.96 (m, 1H), 4.08 (t, J = 4.8 Hz, 2H), 4.74 (d, J = 9.6 Hz, 1H), 4.98 (q, J = 5.6 Hz, 1H), 5.53 (q, J = 8.8 Hz, 1H), 5.62 (d, J = 5.6 Hz, 1H), 5.65 (q, J = 8.8 Hz, 1H), 6.47 (d, J = 2.4 Hz, 1H), 6.51 (dd, J = 2.4, 8.8 Hz, 1H), 7.13 (d, J = 7.2 Hz, 2H), 7.18 (d, J = 7.2 Hz, 1H), 7.23 (t, J = 8 Hz, 2H), 7.81 (d, J = 8.8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 164.9, 157.9, 155.1, 137.5, 134.8, 131.0, 129.3, 128.4, 126.7, 126.5, 117.3, 109.3, 104.1, 100.2, 73.3, 72.6, 70.7, 69.6, 68.8, 64.1, 56.1, 55.7, 54.7, 45.3, 40.5, 36.1, 35.6, 29.7, 27.4, 26.7, 26.1, 25.8, 25.3. FTIR (film, NaCl) ν_{max} = 3344, 2925, 1718, 1595, 1575, 1388 cm^{-1}. $[M + Na]^+$ calcd for $C_{35}H_{48}N_2O_9S$ 695.2978; found, 695.2970.

Compound 22a. The corresponding olefin **20a** or **21a** (22 mg, 0.032 mmol) was dissolved in ethyl acetate (5 mL). Pd/C (10%) was added and the reaction flask flushed with H_2 gas. After stirring overnight, the reaction was filtered over celite and purified by silica chromatography (50:50 → 75:25 EtOAc:hexanes) to give 19 mg (90% yield) of the desired product. $[\alpha]_D^{20}$ −2.9 (*c* 0.70, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 800 MHz) δ 0.97 (s, 3H), 1.03 (s, 3H), 1.18 (m, 1H), 1.27 (m, 2H), 1.38 (m, 3H), 1.45 (m, 4H), 1.54−1.64 (m, 3H), 1.69 (m, 1H), 1.81 (m, 2H), 2.71 (dd, J = 9.6, 14.4 Hz, 1H), 2.87 (m, 1H), 2.98 (dd, J = 4, 14.4 Hz, 1H), 3.01 (dd, J = 1.6, 15.2 Hz, 1H), 3.19 (dd, J = 9.6, 15.2 Hz, 1H), 3.63−3.70 (m, 2H), 3.80 (m, 1H), 3.82 (dt, J = 1.6, 8 Hz, 1H), 3.86 (s, 3H), 3.93 (dd, J = 6.4, 9.6 Hz, 1H), 3.94 (m, 1H), 4.07 (m, 1H), 4.10 (m, 1H), 4.75 (d, J = 9.6 Hz, 1H), 4.98 (q, J = 5.6 Hz, 1H), 5.63 (d, J = 4.8 Hz, 1H), 6.51 (m, 2H), 7.15 (d, J = 7.2 Hz, 2H), 7.18 (t, J = 7.2 Hz, 1H), 7.24 (t, J = 8 Hz, 2H), 7.82 (d, J = 8.8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 19.6, 22.3, 25.0, 25.4, 25.8, 26.2, 27.4, 28.3, 35.7, 35.8, 39.8, 45.3, 54.8, 55.7, 56.1, 61.8, 68.9, 69.6, 70.7, 72.5, 73.3, 100.2, 104.0, 109.3, 117.7, 126.5, 128.4, 129.3, 135.6, 137.5, 155.1, 158.1, 164.9. FTIR (NaCl) ν_{max} = 3449, 2930, 1718, 1596, 1534 cm^{-1}. ESI (+) HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{35}H_{50}N_2O_9S$ 675.3315; found, 675.3318.

***tert*-Butyl-(2*S*,3*R*)-3-hydroxy-4-(2-methylpent-4-enylamino)-1-phenylbutan-2-ylcarbamate (17b and 17c).** A mixture of 2-methylpent-4-en-1-amine (460 mg, 4.6 mmol) and epoxide **8** (361.5 mg, 1.3 mmol) was allowed to stir at 60 °C overnight under argon atmosphere. The crude material was purified by silica gel column chromatography (3:97 MeOH:CH_2Cl_2) to give **17b** and **17c** (470 mg, 95%) as a white solid as a mix of diastereomers (50:50 by NMR); mp 89−90 °C; $[\alpha]_D^{20}$ +5.4 (*c* 2.58, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.91 (d, J = 2 Hz, 3H), 0.92 (d, J = 2.4 Hz, 3H), 1.35 (s, 18H), 1.67 (m, 2H), 1.92 (m, 2H), 2.13 (m, 2H), 2.40 (dd, J = 6.8, 11.6 Hz, 2H), 2.52 (dd, J = 6, 12 Hz, 2H), 2.60−3.01 (m, 10H), 3.45 (m, 2H), 3.59−3.45 (m, 4H), 4.55−4.77 (m, 2H), 5.04 (m, 4H), 5.77 (m, 2H), 7.22 (m, 6H), 7.29 (m, 4H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 17.8, 28.3, 33.2, 36.7, 39.2, 39.2, 51.4, 51.4, 54.1, 55.7, 70.6, 79.3, 116.0, 126.3, 128.4, 129.5, 137.0, 137.9, 155.9. FTIR (NaCl) ν_{max} = 3365, 1683, 1520, 1455, 1391, cm^{-1}. ESI (+) LRMS *m*/*z* (relative intensity): 363.03 $[M + H]^+$.

Pure **17c** was prepared in a similar fashion by using enantiopure (*S*)-2-methylpent-4-en-1-amine. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.99 (d, J = 6 Hz, 3H, 1.32 (s, 9H), 1.99 (m, 2H), 2.12 (m, 1H), 2.70−2.95 (m, 4H), 3.08 (m, 2H), 3.80 (m, 2H), 5.04 (s, 1H), 5.07 (m, 1H), 5.71 (m, 1H), 6.41 (br s, 3H),7.15−7.34 (m, 5H).

***tert*-Butyl-(2*S*,3*R*)-4-(2-(hex-5-enyloxy)-4-methoxy-*N*-(2-methylpent-4-enyl)phenylsulfonamido)-3-hydroxy-1-phenylbutan-2-ylcarbamate (18b and 18c).** A mixture of amines **17b** and **17c** (166 mg, 0.46 mmol) and sulfonyl chloride **7d** (167 mg, 0.55 mmol) were cooled to 0 °C. Pyridine (5 mL) was added and the solution stirred overnight. Solvents were removed by reduced pressure and the crude material purified by silica gel column chromatography (20:80 EtOAc:hexanes) to give a mixture of **18b** and **18c** (148 mg, 51% yield) as a colorless oil. $[\alpha]_D^{20}$ −1.8 (*c* 0.67, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.80 (d, J = 6 Hz, 3H), 0.88 (d, J = 6 Hz, 3H), 1.33 (s, 18H), 1.52−1.65 (m, 4H), 1.70−1.92 (m, 9H), 2.06−2.26 (m, 6H), 3.36−2.80 (m, 12H), 3.73 (br, 4H), 3.84 (s, 6H), 4.06−3.94 (m, 5H), 4.59 (m, 2H), 4.90−5.06 (m, 8H), 5.58−5.87 (m, 4H), 6.44−6.54 (m, 4H), 7.16−7.30 (m, 10H), 7.83 (d, J = 8.7 Hz, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 17.1, 25.0, 28.2, 28.3, 31.7, 31.9, 33.2, 35.3, 38.7, 53.2, 53.3, 54.4, 54.6, 55.6, 56.8, 57.1, 69.1, 2.3, 72.7, 79.4, 100.2, 104.1, 115.1, 116.3, 119.0,

126.3, 128.3, 129.5, 133.7, 136.3, 137.7, 137.9, 138.1, 155.9, 157.6, 164.7. FTIR (NaCl) ν_{max} = 3392, 2929, 1702, 1640, 1445, 1391, 1366, cm^{-1}. ESI (+) HRMS (*m*/*z*): $[M + H]^+$ calcd for $C_{34}H_{50}N_2O_7S$ 631.3417; found, 631.3408.

Pure **18c** was prepared in a similar fashion using pure **17c**. ^1H NMR ($CDCl_3$, 400 MHz) δ 0.80 (d, J = 6 Hz, 3H), 1.34 (s, 9H), 1.59 (m, 2H), 1.74 (m, 2H), 1.87 (m, 2H), 2.12 (q, J = 7.2 Hz, 2H), 2.19 (m, 1H), 2.94 (m, 3H), 3.25 (m, 3H), 3.74 (m, 2H), 3.85 (s, 3H), 3.96 (m, 1H), 4.03 (t, J = 6.8 Hz, 2H), 4.57 (d, J = 7.2 Hz, 1H), 4.93−5.05 (m, 4H), 5.66 (m, 1H), 5.80 (m, 1H), 6.46 (d, J = 2.4 Hz, 1H), 6.50 (dd, J = 2.4, 8.8 Hz, 1H), 7.20 (m, 3H), 7.27 (m, 2H), 7.84 (d, J = 8.4 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 17.1, 25.0, 28.2, 28.4, 31.8, 33.3, 35.2, 38.1, 53.2, 54.5, 55.7, 56.9, 9.1, 72.3, 79.5, 100.2, 104.1, 115.1, 116.3, 119.1, 126.3, 128.4, 129.6, 133.7, 136.4, 137.7, 138.1, 155.9, 157.6, 164.7.

(3*R*,3a*S*,6a*R*)-Hexahydrofuro[2,3-*b*]furan-3-yl (2*S*,3*R*)-4-(2-(hex-5-enyloxy)-4-methoxy-*N*-(2-methylpent-4-enyl)phenylsulfonamido)-3-hydroxy-1-phenylbutan-2-ylcarbamate (19b and 19c). To a stirred solution of **18b** and **18c** (140 mg, 0.22 mmol) in CH_2Cl_2 (4.5 mL) was added TFA (1.5 mL). The resulting mixture was stirred for 4 h and then quenched with saturated aqueous $NaHCO_3$ (1.5 mL). Then 2N NaOH was added until the solution turned basic. The aqueous layer was extracted with ether, washed with brine, and dried over Na_2SO_4. Solvents were removed under reduced pressure to give crude *N*-((2*R*,3*S*)-3-amino-2-hydroxy-4-phenylbutyl)-2-(hex-5-enyloxy)-4-methoxy-*N*-(2-methylpent-4-enyl)benzensulfonamide.

To the above crude amine in MeCN (5 mL) were added (3*R*,3a*S*,6a*R*)-hexahydrofuro[2,3-*b*]furan-3-yl 4-nitrophenyl carbonate **12** (71 mg, 0.24 mmoL) and *i*-Pr_2NEt (0.75 mL) under argon atmosphere and the reaction stirred overnight. Solvents were removed under reduced pressure and the crude material purified by silica gel column chromatography (50:50 EtOAc:hexanes) to give 68 mg (45% yield) of product as a clear oil. $[\alpha]_D^{20}$ −5.7 (*c* 0.17, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz) δ 0.80 (d, J = 6.4 Hz, 3H) 0.90 (d, J = 6.4 Hz, 3H), 1.40−2.24 (m, 22H), 2.70−3.40 (m, 14H), 3.62−4.06 (m, 24H), 4.92−5.05 (m, 12H), 5.60−5.85 (m, 6H), 6.50 (m, 4H), 7.18 (m, 6H), 7.24 (m, 4H), 7.83 (d, J = 8.8 Hz, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 17.1, 25.0, 25.7, 28.4, 31.9, 32.1, 33.2, 35.4, 38.1, 38.7, 45.3, 53.2, 53.4, 54.9, 55.0, 55.7, 56.9, 57.3, 69.2, 69.6, 70.7, 72.3, 72.8, 73.3, 100.3, 104.2, 109.2, 115.1, 116.5, 118.7, 118.8, 126.5, 128.4, 129.3, 133.8, 136.2, 137.5, 137.6, 138.0, 155.3, 157.6, 164.8. FTIR (film, NaCl) ν_{max} = 3436, 2970, 2927, 1718, 1600, 1458, 1374 cm^{-1}. ESI (+) HRMS (*m*/*z*): $[M + Na]^+$ calcd for $C_{36}H_{50}N_2O_9S$ 709.3135; found, 709.3131.

Pure **19c** was prepared in a similar fashion using pure **18c**. ^1H NMR ($CDCl_3$, 400 MHz) δ 0.81 (d, J = 6 Hz, 3H), 1.45−1.90 (m, 8H), 2.12 (m, 2H), 2.23 (m, 1H), 2.80 (dd, J = 9.2, 14 Hz, 1H), 2.93 (m, 3H), 3.15 (dd, J = 2.4, 15.2 Hz, 1H), 3.24 (dd, J = 8, 14 Hz, 1H), 3.38 (dd, J = 9.2, 15.6 Hz, 1H), 3.65−3.75 (m, 3H), 3.77−3.89 (m, 6H), 3.95 (dd, J = 6.4, 9.6 Hz, 1H), 4.05 (t, J = 6.8 Hz, 2H), 4.85−5.05 (m, 6H), 5.62−5.85 (m, 3H), 6.48 (d, J = 2.4 Hz, 1H), 6.52 (dd, J = 2, 8.8 Hz, 1H), 7.20 (m, 3H), 7.26 (m, 2H), 7.84 (d, J = 8.8 Hz, 1H). ESI (+) HRMS (*m*/*z*): $[M + Na]^+$ calcd for $C_{36}H_{50}N_2O_9S$ 709.3135; found, 709.3139.

Inhibitors 20b, 20c, 21b, and 21c. To a solution of carbamates **19b** and **19c** (119 mg, 0.17 mmol) in CH_2Cl_2 (125 mL) was added Grubb's second-generation catalyst (15 mg, 0.02 mmol). The resulting solution was stirred overnight. The solvent was removed under reduced pressure and the crude material purified by silica gel chromatography (50:50 EtOAc:hexanes) to give 110 mg (97% yield) of product as an oil. Reversed-phase HPLC (Waters Sunfire C_{18} 50 mm × 4.6 mm, 5 μm coupled to Agilent Eclipse XDB C_{18} 150 mm × 4.6 mm, 5 μm and YMC-Pack C_8 250 mm × 4.6 mm, 5 μm, flow rate = 0.95 mL/min, λ = 215 nm, T = 30 °C, isocratic 60:40 MeCN:H_2O) was used to isolate the individual isomers: R_t (*RZ*) = 22 min, R_t (*SZ*) = 24 min, R_t (*RE*) = 25.3 min, R_t (*SE*) = 26.8 min.

Compound 20b. $[\alpha]_D^{20}$ −29 (*c* 0.60, $CHCl_3$). ^1H NMR ($CDCl_3$, 800 MHz) δ 1.12 (d, J = 6.4 Hz, 3H) 1.50−1.62(m, 2H), 1.73 (m, 2H), 1.86 (m, 1H), 1.95 (m, 1H), 2.01−2.11 (m, 3H), 2.18 (m, 1H), 2.74 (dd, J = 9.6, 14.4 Hz, 1H), 2.81 (d, J = 15.2 Hz, 1H), 2.87 (m, 1H), 2.99 (dt, J = 4, 13.6 Hz, 2H), 3.16 (dd, J = 9.6, 15.2 Hz, 1H), 3.65−3.75 (m, 5H), 3.79−3.85 (m, 2H), 3.85 (s, 3H), 3.93 (dd, J = 6.4, 9.6 Hz, 1H), 4.11 (m, 2H), 4.25 (m, 1H), 4.84 (d, J = 9.6 Hz, 1H), 4.99 (q, J = 6.4 Hz, 1H), 5.44 (q, J = 9.6 Hz, 1H), 5.57 (q, J = 7.2 Hz, 1H), 5.63 (d, J = 4.8 Hz, 1H), 6.48 (d, J = 1.6 Hz, 1H), 6.50 (dd, J = 2.4, 8.8 Hz, 1H), 7.17 (m, 3H), 7.24 (m, 2H), 7.86 (d, J = 8.8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 17.4, 25.4, 25.8, 26.1, 27.5, 32.2, 32.8, 35.7, 45.3, 53.9, 54.7, 55.7, 59.1, 68.4, 69.6, 70.7, 73.0, 73.3, 100.3, 103.9, 109.3, 118.1, 126.5, 127.2, 128.4, 129.4, 131.1, 134.4, 137.5, 155.2, 157.8, 164.9. FTIR (film, NaCl) ν_{max} = 3467, 2923, 1717, 1595, 1444, 1384, 1256 cm^{-1}. ESI (+) HRMS (*m*/*z*): $[M + Na]^+$ calcd for $C_{34}H_{46}N_2O_9S$ 681.2822; found, 681.2829.

Compound 20c. $[\alpha]_D^{20}$ −30.6 (*c* 0.83, $CHCl_3$). ^1H NMR ($CDCl_3$, 800 MHz) δ 1.13 (d, J = 7.2 Hz, 3H) 1.61 (m, 1H), 1.69 (m, 1H), 1.78−1.90 (m, 4H), 1.94−2.09 (m, 5H), 2.18 (m, 1H), 2.74 (dd, J = 8.8, 13.6 Hz, 1H), 2.89 (m, 2H), 2.96 (dd, J = 3.2, 13.6 Hz, 1H), 3.11 (dd, J = 8, 14.4 Hz, 1H), 3.65−3.74 (m, 3H), 3.78−3.86 (m, 6H), 3.88−4.02 (m, 4H), 4.84 (d, J = 8.8 Hz, 1H), 5.00 (q, J = 5.6 Hz, 1H), 5.52 (m, 1H), 5.64 (d, J = 4.8 Hz, 1H), 5.66 (m, 1H), 6.78 (d, J = 2.4 Hz, 1H), 6.50 (dd, J = 1.6, 8.8 Hz, 1H), 7.17 (m, 3H), 7.25 (m, 2H), 7.83 (d, J = 8.8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 18.4, 25.8, 26.2, 28.6, 29.7, 30.0, 32.3, 35.4, 37.9, 45.3, 54.4, 54.8, 55.7, 58.1, 58.5, 69.6, 70.8, 72.6, 73.4, 100.7, 104.3, 109.3, 118.8, 126.5, 128.1, 128.5, 129.3, 133.3, 134.0, 137.6, 155.3, 158.1, 164.8. FTIR (NaCl) ν_{max} = 3442, 3339, 2924, 1717, 1595, 1495, 1444, 1325, 1256, 1206 cm^{-1}. ESI (+) HRMS (*m*/*z*): $[M + Na]^+$ calcd for $C_{34}H_{46}N_2O_9S$ 681.2822; found, 681.2825.

Compound 21b. $[\alpha]_D^{20}$ +27.6 (*c* 0.36, $CHCl_3$). ^1H NMR ($CDCl_3$, 800 MHz) δ 1.09 (d, J = 6.4 Hz, 3H) 1.50−1.60 (m, 2H), 1.72 (m, 1H), 1.86 (m, 2H), 1.94−2.12 (m, 4H), 2.18 (m, 1H), 2.78 (dd, J = 10.4, 14.4 Hz, 1H), 2.90 (m, 2H), 3.02 (dd, J = 4.8, 14.4 Hz, 1H), 3.12 (dd, J = 4, 14.4 Hz, 1H), 3.17 (m, 2H), 3.46 (m, 1H), 3.64−3.71 (m, 3H), 3.84−3.90 (m, 5H), 3.96 (m, 2H), 4.12 (m, 1H), 4.15 (m, 1H), 4.88 (d, J = 8.8 Hz, 1H), 5.00 (q, J = 8 Hz, 1H), 5.47 (q, J = 8.8 Hz, 1H), 5.57 (q, J = 7.2 Hz, 1H), 5.64 (d, J = 5.6 Hz, 1H), 6.49 (m, 2H), 7.20 (m, 3H), 7.27 (m, 2H), 7.83 (d, J = 8.8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 18.0, 25.3, 25.8, 26.0, 27.3, 32.2, 32.7, 35.4, 45.3, 53.3, 55.1, 55.7, 58.0, 68.4, 69.6, 70.9, 72.3, 73.4, 100.2, 104.0, 109.3, 118.3, 126.5, 127.6, 128.5, 129.3, 130.8, 134.3, 137.7, 155.6, 157.8, 164.8. FTIR (film, NaCl) ν_{max} = 3467, 2927, 1717, 1595, 1456, 1325, cm^{-1}. ESI (+) HRMS (*m*/*z*): $[M + Na]^+$ calcd for $C_{34}H_{46}N_2O_9S$ 681.2822; found, 681.2819.

Compound 21c. $[\alpha]_D^{20}$ +20.5 (*c* 1.17, $CHCl_3$). ^1H NMR ($CDCl_3$, 800 MHz) δ 1.06 (d, J = 6.4 Hz, 3H), 1.47 (m, 1H), 1.63 (m, 1H), 1.70−1.82 (m, 4H), 1.87 (m, 1H), 1.93 (m, 1H), 2.17 (m, 2H), 2.26 (m, 1H), 2.47 (br, 1H), 2.76 (dd, J = 8.8, 14.4 Hz, 1H), 2.91 (m, 2H), 2.98 (m, 2H), 3.55 (m, 1H), 3.69 (m, 2H), 3.75 (m, 1H), 3.80 (m, 1H), 3.85 (m, 4H), 3.88 (t, J = 8.8 Hz, 1H), 3.94 (dd, J = 6.4, 9.6 Hz, 1H), 4.11 (m, 1H), 4.17 (br, 1H), 4.76 (d, J = 9.6 Hz, 1H), 5.00 (q, J = 8 Hz, 1H), 5.53 (m, 1H), 5.64 (d, J = 4.8 Hz, 1H), 5.67 (m, 1H), 6.47 (d, J = 2.4 Hz, 1H), 6.50 (dd, J = 2.4, 8.8 Hz, 1H), 7.14 (d, J = 7.2 Hz, 2H), 7.18 (m, 1H), 7.24 (t, J = 7.2 Hz, 2H),7.83 (d, J = 8.8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 19.0, 25.8, 26.2, 28.1, 29.8, 30.3, 33.6, 35.7, 38.2, 45.3, 54.6, 54.9, 55.7, 58.6, 69.6, 70.8, 71.5, 73.4, 100.5, 104.2, 109.3, 119.0, 126.5, 128.5, 129.4, 129.6, 132.5, 134.2, 137.4, 155.2, 158.1, 164.8. FTIR (film, NaCl) ν_{max} = 3463, 3339, 1717, 1595, 1495, 1387, 1326 cm^{-1}. ESI(+) HRMS (*m*/*z*): $[M + Na]^+$ calcd for $C_{34}H_{46}N_2O_9S$ 681.2822; found, 681.2812.

Compound 22b. To a stirred solution of the corresponding (*R*)-*E* olefin (8 mg, 0.013 mmol) was dissolved into EtOAc (5 mL) a spatula tip of Pd on carbon (10 wt %) and the mixture was stirred under H_2 atmosphere. The reaction stirred overnight.

Solvents were removed under reduced pressure, and the crude material was purified by silica gel column chromatograph (70:30 Et_2O:hexanes → 100% Et_2O) to give **22b** (8 mg, quantitative yield) as a white solid. TLC 100% Et_2O. $[\alpha]_D^{23}$ −7.8 (*c* 0.84, $CHCl_3$). ^1H NMR ($CDCl_3$, 500 MHz) δ 0.92 (d, $J = 7$ Hz, 3H) 1.16−1.84 (m, 15H), 2.74 (dd, $J = 9.5$, 14 Hz, 1H), 2.89 (m, 1H), 3.05 (m, 3H), 3.28 (m, 2H), 3.65−3.87 (m, 9H), 3.94 (m, 1H), 4.10 (m, 1H), 4.21 (m, 1H), 4.84 (d, $J = 9.5$ Hz, 1H), 5.01 (q, $J = 7$ Hz, 1H), 5.64 (d, $J = 5.5$ Hz, 1H), 6.51 (m, 2H), 7.19 (m, 3H), 7.26 (m, 2H),7.88 (d, $J = 9.5$ Hz, 1H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 17.8, 23.4, 23.5, 25.5, 25.8, 26.1, 27.5, 31.8, 31.9, 35.7, 45.3, 52.5, 54.8, 55.2, 55., 68.8, 69.6, 70.7, 71.3, 73.3, 100.4, 104.0, 109.3, 118.0, 126.5, 128.5, 129.4, 134.4, 137.6, 155.2, 158.0, 165.0. FTIR (NaCl) ν_{max} = 3463, 2924, 2853, 1721, 1596, 1323, 1256 cm^{-1}. ESI(+) HRMS (*m*/*z*): $[M + Na]^+$ calcd for $C_{34}H_{48}N_2O_9S$ 683.2978; found, 683.2987.

Compound 22c. To a solution of the corresponding (*S*)-*E* olefin (12 mg, 0.018 mmol) in EtOAc (5 mL) a spatula tip of Pd on carbon (10 wt %) was added and the mixture was stirred under H_2 atmosphere. The reaction was stirred overnight. Solvents were removed under reduced pressure and the crude material purified by silica chromatograph (70:30 Et_2O:hexanes → 100% Et_2O) to give **22c** (9 mg, 78% yield) as a white solid. $[\alpha]_D^{23}$ −6.6 (*c* 0.91, $CHCl_3$). ^1H NMR ($CDCl_3$, 800 MHz) δ 0.90 (d, $J = 6.4$ Hz, 3H) 1.20−1.90 (m, 15H), 2.73 (m, 1H), 2.79 (dd, $J = 8.8$, 13.6 Hz, 1H), 2.90 (m, 1H), 2.96 (m, 1H), 3.08 (m 1H), 3.33 (dd, $J = 9.6$, 15.2 Hz, 1H), 3.47 (m, 1H), 3.61 (m, 1H), 3.70 (m, 2H), 3.85 (m, 6H), 3.95 (m, 1H), 4.10 (m, 1H), 4.25 (m, 1H), 4.86 (d, $J = 8.8$ Hz, 1H), 5.03 (q, $J = 7.2$ Hz, 1H), 5.65 (d, $J = 4.8$ Hz, 1H), 6.52 (m, 2H), 7.18 (m, 3H), 7.25 (m, 2H), 7.87 (d, $J = 9.6$ Hz, 1H). ^{13}C NMR ($CDCl_3$, 200 MHz) δ 18.4, 23.4, 24.3, 25.0, 25.8, 26.5, 27.2, 32.8, 33.1, 35.4, 45.3, 53.5, 54.8, 55.7, 57.0, 69.3, 69.6, 70.8, 72.2, 73.3, 100.6, 104.2, 109.3, 118.0, 126.5, 128.5, 129.4, 134.5, 137.5, 155.3, 158.1, 165.1. FTIR (NaCl) ν_{max} = 3463, 2854, 1717, 1596, 1444, 1323, 1256 cm^{-1}. ESI (+) HRMS (*m*/*z*): $[M + Na]^+$ calcd for $C_{34}H_{48}N_2O_9S$ 683.2978; found, 683.2976.

Determination of X-ray Structures of HIV-1 Protease (wt)−Inhibitor Complexes for Inhibitors 14c and 2. X-ray Structure for 14c. The HIV-1 protease construct with the substitutions Q7K, L33I, L63I, C67A, and C95A to optimize protein stability without altering protease structure and activity was expressed and purified as described.[23,24] Crystals were grown by the hanging drop vapor diffusion method using 1:15 molar ratio of protease at 2.0 mg/mL and inhibitor **14c** dissolved in dimethylsulfoxide. The reservoir contained 5% glycerol, 0.5 M NaI in 0.2 M MES buffer with pH 6.0. Subsequently, crystals were mounted on nylon loops in liquid nitrogen with additional 28% glycerol (v/v) as cryoprotectant. X-ray diffraction data were collected on SER-CAT 22-ID beamline of the Advanced Photon Source, Argonne National Laboratory, with wavelength 0.8 Å under the stream of liquid nitrogen at 90 K. Data were processed by HKL2000,[25] resulting in R_{merge} of 10.8% for **14c**, and the structure was solved by molecular replacement with our previous structure (PDB code 3B7V) using PHASER[26] in CCP4i Suite,[27,28] refined by SHELX-97,[29,30] and refitted manually with the graphic programs O10,[31] and COOT.[32] The inhibitor structure was modeled by PRODRG-2,[33] which also provided restraints for refinement. The crystal structure of HIV-1 protease with inhibitor **14c** was determined at 1.17 Å resolution. The rmsd for Cα atoms in comparison with our wild type protease with DRV (PDB code 2IEN[19]) is only 0.33 Å . Alternate conformations were modeled for the protease residues when obvious in the electron density maps. Anisotropic atomic displacement parameters (*B* factors) were applied for all atoms including solvent molecules. The occupancies of iodine atoms were refined and varied from 0.24 to 0.60. Hydrogen atoms were added at the final stages of the refinement. The final structure includes 2 Na^+ ions, 19 I^- ions, 2 glycerols, and 181 waters in PR-**14c** complex. The *R* factor and R_{free} are 16.0 and 19.4%. The crystallographic statistics are listed in Table 1 (Supporting Information). The crystal structure and structure factors have been deposited in the RCSB Protein Data Bank,[34] with PDB code 3I6O for inhibitor **14c**.

X-ray Structure for 2. The wild-type HIV-1 protease was expressed and purified as described previously.[35] After purification, the protease was concentrated to 4.0 mg/mL. Inhibitor **2**, at a 10-fold molar excess, was mixed with the protease. The mixture was incubated at room temperature for 2 h and then clarified with a 0.2 mm spin filter. The crystallization trials employed the hanging drop method using equal volumes of enzyme−inhibitor and well solution. The reservoir contained 20% $(NH4)_2SO_4$, 200 mM $NaPO_4$/citric acid buffer, pH 5.5. The well solutions also included 10% dimethyl sulfoxide, 30 mM β-mercaptoethanol, and 4% isopropyl alcohol.

Diffraction data were collected at room temperature with an Raxis-IV image plate and integrated and reduced with the HKL program package.[25] The diffraction data set has a resolution of 1.7 Å with an R_{merge} value of 0.052. Data completeness was 95.5%. The space group was determined to be $P2_12_12_1$ with unit cell dimensions of $a = 51.9$ Å, $b = 59.0$ Å, $c = 62.6$ Å, with one dimer in the asymmetric unit. The initial phase was solved by molecular replacement using the program AMoRe.[36] A protease dimer (PDB code 2AID)[37] from a previously determined HIV-1 protease crystal structure was used as the search model. Crystallographic refinement was carried out using X-PLOR 3.1. Molecular graphics program O[31] was used for map display and model building. Water molecules were added to the structure as identified in the $|F_o| - |F_c|$ map contoured at the 3σ level. Data collection and refinement statistics are listed in Table 2 (Supporting Information). The final R_{work} was 21.6% and R_{free} was 28.9% for data between the resolution of 20 and 1.7 Å (Table 1). The rmsd values from ideal bond distances and angles were 0.009 Å and 1.5°, respectively. The average *B* factor was 28.3 and 19.6 $Å^2$ for protein and inhibitor atoms, respectively, and 41.0 $Å^2$ for solvent atoms. The crystal structures and structure factors have been deposited in the RCSB Protein Data Bank,[34] with PDB code 3I7E for inhibitor **2**.

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Supporting Information Available: HPLC and HRMS data of all inhibitors; crystallographic data collection and refinement statistics. This material is available free of charge via the Internet at http://pubs.acs.org.

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